Ontogenesis of the *corpora pedunculata*: Integral Relay Structures of Chemosensory Content Addressable Memory Networks of Hexapods

—

A Synthesis of Development and Function.

by Christian A. Hehn

Submitted for the Degree of Masters of Science.

University of Glasgow,
Institute of Biomedical and Life Sciences,
Division of Molecular and Cellular Biology,
Laboratories of Biotechnology and Genetics,
Church Street, Glasgow, G11 5JS, Scotland,
United Kingdom of Great Britain and Northern Ireland.

14, Thursday February, 1995

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

The research reported within the appendix of this thesis is either partially or entirely my own work, except otherwise indicated. This work has not been submitted for any other degree. It shall be noted that ideas and research mentioned within the introductory portion of thesis thesis is that of other researcher. The conclusions gathered from their research are entirely my own. For copyright and time reason it was not considered to illustrate this thesis with figures of other researcher's work. I therefore have to refer the reader to the relevant references.*

Christian R. Hehn

**Note: The enhancer trap mutagensis was joint work, which was co-ordinated by Mingyao Yang. For details on in situ hybridisations and breeding schemes, see the forthcoming Ph.D. thesis of Mingyao Yang.*

This thesis was computer typeset using 12 point Times. Headings used were either 24 or 36 point Palatino. The word processing program used was Microsoft Word 5.1a. (Microsoft co-operation, Seattle, Washington, USA, © November, 1992). The plasmid maps were created on MacPlasmap, Version 1.82. Freecopy by Jingdong Liu ©1988-1990. Biol. Dept., Universtiy of Utah, Utah, USA.

Summary

Biological memory is the temporal storage of information as a function of evolution. Several mechanisms have evolved by which memory can be stored. There are two components involved in the storage of memory in metazoan organisms. Innate memory is strictly teleonomically determined, and hence, depends on the phylogenic predisposition of an organisms' ontogenesis. 'Learned' memory is, in contrast, strictly ontogenically determined and, hence, influenced by the organisms environment. Whilst strictly ontogenic determined memory is stored in the spatial arrangement of nerve cells, phylogenic memory is stored in the sequential arrangement of the four components of the DNA. Accordingly, ontogenic memory is lost in subsequent generations, whereas phylogenic memory is passed on and recalled during the course of evolution.

Insects are among the best understood organisms. The fruit fly *Drosophila melanogaster,* for instance, has been widely used as a model to unravel the genetic components of development. Most of the genes that are involved in this process are known. Other insect species have been physiologically and behaviourally well researched. By assembling the information derived from the latest research on *Drosophila melanogaster* and other insect species, I have made the attempt to characterise the different components of molecular memory formation (hereafter referred to as mnemogenesis) in insects.

Chemosensory memory pathways of *Drosophila* are composed of at least two different entities: the morphogenic fields such as the peripheral and the central nervous system. I have concluded that during the ontogenesis of the *Drosophila* chemosensory memory pathways, genes are active that function as modules during this process. Most of the genes which mediate this process are not strictly employed during the morphogenesis of the chemosensory memory pathways. However, they are redeployed to a large extent during development of other germ layers and morphogenic fields, as well. Only certain key genes, which expression is initiated by the several coinciding morphogenic signals, determine the specificity

of the different components of the chemosensory memory pathways. Hence, the specificity of the chemosensory memory pathways of *Drosophila* is determined by the temporally and spatially distinct expression of genes, in addition to the modification of their products. Whilst stage and cell specific gene expression is primarily regulated on the level of chromosome structure and transcriptional activity, the specific function of genes that are expressed in the different regions during different stages of ontogenesis is generated by messenger ribonucleic acid and protein processing. The morphogenic cascades are probably frozen down once the chemosensory memory pathways have reached the state of maturity.

The mature insect has maintained the ability to employ some components of the developmental cascade to modulate its memory in response to environmental stimuli. Imaginal chemosensory memory pathways comprise at least four levels. Chemosensory receptor (level I) cells receive environmental information. Projection neurones (level II) reduce the background noise and transfer the information to diverging memory structures, in addition to the control centres (levels III/i and III/ii). Whereas memory structures modulate chemosensory information, the control centres feed this modulated information into output fibres that link the chemosensory memory networks with the premotor fibres (level IV). The function of the memory structures, which in insects are called the corpora pedunculata, is to compare input information to the information stored intrinsically in these organs. The information that is stored intrinsic to these structures is able to modulate the behaviour of an signal, which exits the chemosensory pathways via the premotor neurones. It has been postulated that the modulation of this information depends on the synaptic configuration within the corpora pedunculata. Hence, the synaptic arrangement is thought to underlie the modulation of the information transfer within the chemosensory memory networks. Long term memory is associated with the alteration of this synaptic configuration, which in turn requires the activity of several genetic circuits. Intriguingly, these genetic circuits are probably identical to those employed during axonogenesis, in addition to other morphogenic events.

Prelude

Foreword

"Science is not a system of certain, or well established, statements; nor is it a system which steadily advances towards a state of finality. Our science is not knowledge (επιστεμε): it can never claim to have attained our truth, or even a substitute for it, such as probability. Yet science has more than mere biological survival value. It is not only a useful instrument. Although it can attain neither truth nor probability, the striving for knowledge and the search for truth are still strongest motives of scientific discovery.

We know: we can only guess. And our guesses are guided by the unscientific, metaphysical (though biologically explicable) faith in laws, in regularities which we can uncover - discover. Like Bacon, we might describe our own contemporary science - 'the method of reasoning which men now ordinarily apply to nature—as 'consisting of anticipations, rash and premature' and of 'prejudices"

From Sir Karl Poppers' "The Logic of Scientific Discovery". Page 278, Routledge London, 1992

"No one is a more ardent enthusiast than the convert; he may be an embarrassment to his own friends; he is likely to become more royal than the king. Perhaps I am in that state with the respect to genetics. For seeking the old and widely held belief that genes are concerned only with certain limited characteristics which geneticists are pleased to study, I have now come to the point where I feel that every feature of the animal has genetic origin. The disciplined geneticist ascribes to the action of genes only those characteristics which segregate (more or less) in breeding experiments."

From Sir Vincent B. Wigglesworth (1961). Insect Polymorphisms- A Tentative Synthesis. *In* Kennedy J.S. (ed.): **Insect Polymorphism.** pp. 104- 111. **London: Symposium of Royal Entomological Society**

"I must first reassure you two matters. First, I am not going to tell you anything original, or anything you know already: you need have no fear that I shall be obscure. (I was once invited by the B.B.C. to prepare a contribution to their Third Programme; but they sent my script back asking whether I could not let them have something more profound. I replied that I could to write a script that was more profound, but if that would be more acceptable, I could easily make it more obscure).

Secondly, it is well known that when we can no longer get our experiments to work, we may turn to philosophy in despair—as the unsuccessful thief may turn thief-taker. I may myself be happy one day to enter that particular pathway to decrepitude—but that is not my intention at the moment. I propose in this address to devote myself not to philosophy but to 'common sense'—a subject that is indeed anathema to the philosopher."

From Sir Vincent B. Wigglesworth (1967). The Religion of Science. **Ann. Appli. Biol.** 60: 1.

Aim

Here, I present an approach that, though incomplete, may hint which aspects are involved in the generation of learning mutant phenotypes. The question of my interest was as to whether mutations associated with learning defects are due to an impaired developmental function, or if just the learning function of the fully differentiated organism is impaired. Two possible approaches were chosen to unravelling this question in *Drosophila melanogaster*.

(a) Enhancer trap lines

Enhancer trap P- element facilitate the study of the lineage of sets of neurones that constitute the mature insect brain. Of particular interest is thereby to elucidate as to when the these neurones or their precursors first express a P-element marker.
A next step would subsequently be to correlate the expression of the P-element marker with the transcription of genes that occur in the vicinity of the P-element insert.

(b) Cell Culture

Last, it would have been feasible to monitor the behaviour of a neurone derived from a known learning mutation. Does the development in *dunce* mutants impair the cellular out growth in those P-element marked cells? And having isolated such marked cells I would have been interested in studying the gene expression in a cell culture system of embryonic neurones/ neuronal precursors.

Acknowledgements

Christian  , would kindly like to thank all the people who helped him with his

thesis:

Dr. Anadurai, Mr. Milligan, Mr. Armstrong, Dr. Harbison, Ms. Duncanson, Dr. Goodwin, Mr. Yang, and Dr. Yu. Thanks also to Dr. P. Fyfe, I.B.L.S., Biochemistry Laboratory of the University of Glasgow, who corrected some parts of this thesis for the correct usage of the English language.

Further, he is particularly indebted to the Department of Genetics Preproom ladies and the crew of Glasgow University Computer services for their great and patient help. Many thanks to the Universities of Edinburgh and Göttingen for kindly making available their library facilities to him. Not to forget the great pieces of advice given to him by Prof. Dübendorfer (University of Zürich, Switzerland), Dr. Bicker (Free University of Berlin, Germany), Dr. Milner (University of St. Andrews), and Dr. Krasnow (University of California, USA). Despite all he wishes to thank everyone who has granted him quit a lot of help during these though times. For the financial bit he is particularly grateful for the crucial financial support given by his father Mr. Klaus Hehn and grandfather ex-rector Friedrich Ehrhardt.

Last, he would particularly like to thank Professors Reichert (Basel, Switzerland) , Menzel (Berlin, Germany), Lancet (Tel Aviv, Israel), Greenspan (New York, USA), Benzer (Pasadena, California, USA), and Tully (Cold Spring Harbour, USA), as well as Drs. Ish- Horowicz (Oxford, U.K.), Ito (Mainz, Germany), Klämpt (Cologne, Germany), Boyan (Munich, Germany), Müller (Berlin, Germany), and Bray (Cambridge, U.K.) for some stimulating discussions. In particular he is grateful to Prof. Tully for giving him some insight into his latest research.

Table of Contents

PART 1: INTRODUCTION AND THEORY 1

Chapter 1: The Hypothesis 1

Chapter 2: The Terminology 3

1. Morphogenesis 3
2. Mnemogenesis 4

Chapter 3: The Theory 7

Chapter 4: Biophysical Considerations 9

1. Thermodynamics of Sensory Modalities 9
2. Thermodynamics of Organisms 12
3. Thermodynamics of Sensory Receptors 13
4. Thermodynamics of Nervous Systems 14
5. Electrochemistry of Neural Signal Transduction 19
6. Specific Features of Neural Coding 21

Chapter 5: Neural Network Considerations 23

Chapter 6: The Evolution of Memory 26

1. The Origins of Life and Genetic Memory (Teleonomy) 26
2. Chemosensory Memory of Escherichia coli 27
3. Evolution of Phylogenic and Ontogenic Memory in Metazoans 30

3.1. Introduction 30
3.2. Phylogeny of Memory in Lower Metazoans 30
3.3. The Memory of *Drosophila melanogaster* 32
3.4. The Mnemogenesis of Aplysia california 35

4. The Modulation of Teleonomic Memory in Higher Metazoan Organisms 38
5. Synthesis 39

PART 2: DISSECTION OF MORPHO- AND MNEMOGENESIS - TOOLS FOR STUDYING THE ANATOMY AND GENETICS OF CHEMOSENSORY MEMORY CIRCUITS IN *DROSOPHILA MELANOGASTER* 40

Chapter 1: Enhancer Detector Analysis to Study the Genomic Identities of Morphogenetic Fields 41

1. Using the Extraordinary Abilities of P-elements for Mutagenesis 43

2. Δ2-3 Transposase a Stable Source of Transposase Enables Deleted P-elements to Transpose 46

3. The Features of Enhancer Trap P-elements 47

4. Reporter Constructs 49

5. Plasmid Rescue 50

6. Genetic Mosaics 51

Chapter 2: Conventional Mutagenesis 52

1. Collisional Ionising Radiation 52

2. Ethyl Methane Sulfonate Induced Mutations 53

3. Genomic Walk 54

4. Genetic Mosaics 54

PART 3: MORPHOGENESIS 55

Chapter 1: General Aspects of *Drosophila* Morphogenesis 57

Chapter 2: The Generation of Embryonic Fields 59

1. Formation of the Cephalon 59

1.1. Maternal Effector Genes 60
1.2. Expression of Cephalon Specific Gap Genes in the *Drosophila* Embryo 64
1.3 . The Specification of Segments in the Cephalic Region 68

2. Segmentation in Thorax and Abdomen 70

2.1 Pair-Rule Segmentation Genes Determine the Boundaries of the Parasegments 71
2.2. Specification of Parasegments by Segment Polarity Genes 74

Chapter 3: The Establishment of Morphogenic Fields - Foundation of the Central Nervous System 79

1. Cytology of *Drosophila melanogaster* Neurogenesis 80

1.1. Overview of Cellular Interactions during the Process of Neurogenesis 80
1.2. Cellular Systematics of the Ventral Neuroectoderm of *Drosophila melanogaster* 81

2. Genetic Circuitry of Neurogenesis 82

2.1. Dorso Ventral Morphogene Activation of Proneural Genes 83
2.2. Characterisation of the Achaete-Scute Complex Genes 84
2.3. The Regulation of *Achaete- Scute* Complex Genes 86
2.4. *AS-C* responsive Genes 88
2.5. Structural Properties of the *NOTCH* Signalling Pathway 90

3. Synthesis 93

4. Midline Formation 97

5. Gliagenesis 99

Chapter 4: The Integration of Neurogenesis into The Segmentation Process 101

1. Neuronal Identity is a Function of the Segmentation in the Ventral Neuroectoderm 101
2. Determination Neuroblast Identity in the Procephalic Region 103

3. Homeotic Neuroblast Identity Genes 106

Chapter 5: Control of Neuroblast Proliferation 109

1. Postembryonic Neuroblast Proliferation 109

2. Mushroom Body Defect Controls the Neuroblast Number 110

3. Anachronism Controls the Temporal Activation of the Cell Cycle in Neuroblasts 110

Chapter 6: Differentiation of Neuronal Fields 112

1. Genes which Control the Differentiation of Neurones 114

2. The Principles Axonogenesis in the Ventral Nervous System 115

2.1. Overview of Axonogenesis 115
2.2. Cytology of Axonogenesis 116
2.3. Factors Involved in Axonogenesis 119
2.4. Mutant Phenotypes 131

3. Synthesis 138

Chapter 7: Synaptogenesis 141

1. The Course of Synaptogenesis 141

2. Molecular Biology of Synaptogenesis 143

Chapter 8 : Control of Neuronal Differentiation by Hormones 145

1. 20-Hydroxyecdysone Induces Hierarchical Gene Transcription Cascades 145

2. 20-Hydroxyecdysone Responsive Elements and Their Involvement in Neuronal Differentiation 146

3. Ecdysone Responsive Genes 148

4. Ecdysone and Apoptosis 150

5. Juvenile Hormone 150

PART 4: MNEMOGENESIS 152

Chapter 1: The Anatomy of Insect Chemosensory Memory Networks 153

1. Organisation of Insect Nervous Systems 153

2. Cytology of the Supraoesophageal Ganglion 154

3. Overview of Anatomy of the Olfactory and Gustatory Memory Networks in Insects 154

4. General Anatomy of Insect Chemosensory Organs 156

5. Anatomy of Chemosensory Memory Pathways in *Apis mellifera* 158

5.1. Odourant Receptor Cells 158
5.2. The Antennal Lobe 159
5.3. The Olfactorio- Globularis Tracts 160
5.4. Anterior - Superior Optic Tract 161
5.5. The Corpora Pedunculata 161
5.6. Extrinsic Fibres 163

6. The Chemosensory Memory Pathways of *Drosophila melanogaster* 168

6.1. Anatomy of the *Drosophila melanogaster* Antenna 168
6.2. The Antennal Lobe s of *Drosophila melanogaster* 169
6.3. The Corpora Pedunculata of *Drosophila melanogaster* 172

7. The Cytology of the Corpora Pedunculata of *Formica lugbris* 172

Chapter 2: Biochemistry and Pharmacology of Insect Chemosensory Memory Pathways 174

1. Pharmacology and Biochemistry of the Insect Peripheral Chemosensory Nervous System 174

1.1. Biochemistry and Organisation of Perireceptor Sensilla Components 177
1.2. Biochemistry of Chemosensory Signal Transduction 180

2. Pharmacology and Biochemistry of Insect Central Nervous System 181

2.1. Acetylcholine 183
2.2. γ - aminobutylic acid (GABA) 183
2.3. Taurine 184
2.4. Serotonin 184

2.5. Octopamine 185
2.6. Pigment Dispersing Hormone (PDFs) 185
2.7. Dromyosuppressin Peptide (TDVDHVFLFR amide) 186

Chapter 3: Biophysics of Chemosensory Memory Pathways in Insects 187

1. Characterisation of a Discrete Giant Lateral Olfactorio- globularis tract Neurone in *Apis mellifera* 188

2. Examination of Odour Processing in the Antennal Lobes of *Schistocerca americana* 189

3. Biophysical Characterisation of the Corpus Pedunculatum Kenyon Neurones of *Schistocerca americana* 192

4. Characterisation of a Recurrent Feedback Neurones in *Apis mellifera* 194

5. Characterisation of an Identified Kenyon Neurone Efferent (Pe-1) of *Apis mellifera* 194

6. Pheromone Stimulated Response of Two Descending Ventral Nerve Cord Neurones in Male *Bombyx mori* 197

7. Synthesis 198

Chapter 4: The Modulation of the Synaptic Plasticity by Biochemical Signalling Pathways in Chemosensory Memory Networks 201

1. Overview 201

2. Mnemogenetic Second Messenger Pathways of *Drosophila melanogaster* 203

2.1. The Adenylyl Cyclase Pathway 203
2.2. The Inositol 1,4,5 Triphosphate (IP_3) Transduction Cascade 204
2.3. The Coincidence Detector Protein Kinase A 205
2.4. Nitric Oxide - A Putative Mnemogenetic Second Messenger 206
2.5. Cyclic Adenylyl Responsive Element- Binding Proteins (CREBs) and Modulators (CREMs) 208

3. Inconsistencies within the Putative Mnemogenetic Second Messenger Pathways 208
4. Synthesis 210

PART 5: CONCLUSIONS AND DÉNOUEMENT 213

Chapter 1: The Theory 214

Chapter 2: Synthesis 216

1. Morpho- and Mnemogenetic Genes 216

2. Correlation between Cellular Diversification and Teleonomic Memory 220

3. Mnemogenesis - Critique de la Raison 227

4. The Scientific Paradox: Triviality and Complexity 234

Chapter 3: The Ultimate Goal 236

1. Sir Karl Popper Says 236

2. Sir Francis Crick Says 236

3. Sir John Eccles Says 238

4. David Hume Says 239

APPENDIX: EXPERIMENTAL OBSERVATIONS 240

Summary 240

Introduction 242

Materials and Methods 244

P-element Mutagenesis 244

Drosophila melanogaster : Strains 244
Drosophila melanogaster: Culture 244
Recipes for *Drosophila* Media 244

Subcloning 245

Bacterial Strains 245
Plasmids 245
Plasmid pBluescript® I SK+/- 245
The plasmid pPNT. 246
The vector pUAST 246
Restriction Digests 246
Agarose Gels 246
Electrophoresis 247
Visualisation of the Fragments on the Gel 247
Competent Bacteria 248
Ligations and Transformations of the Plasmids into E.coli 248
Selection of Transformed Colonies 249
Plasmid Mini Preparation 250
Large Scale Plasmid Preparation 250
Probe preparation 252
Southern blot 253
Sequencing 254

Immunocytochemistry 257

Embryo Immunocytochemistry 257
Crosses 257
Dechorionation and Fixation 257
Preincubation 257
Antibody Incubations 258
Dissection of Brains 259
Dissection of Larval Brains 259
Dissection of Pupal Brains 260

Dissection of Imaginal Brains 260
Preincubation of Brains 260
Confocal Laser Scanning Microscopy 261

Results 1 **262**

1. **Confirmation of the Vector Map pBluescript® SK+/- neo** 262
2. **Subcloning of pUAST-neo** 263
3. **Plasmid Map of pPNT** 264
4. **Probe Preparation** 264
5. **Ligation of the pUAST - neo Construct** 265
6. **Sequence analysis** 267

Results 2 **268**

1. **Genetic Strategies and Enhancer Trap Mutagenesis** 268
2. **Anti-β– galactosidase Immunoreactivity and Confocal Imaging** 269

Discussion 1 **273**

Discussion 2 **276**

1. **Overview** 276
2. **Enhancer Trap Mutagenesis** 277
3. **Development of the Embryonic Precephalic Region with Respect to the Formation of the Corpora Pedunculata** 279
4. **Correlation between Corpora Pedunculata Development and Neuroblast Proliferation Patterns** 280
5. **Rearrangement of Corpora Pedunculata Intrinsic Neurones during Metamorphosis May Reflect the Integration of Extrinsic Neurones** 282
6. **Factors Triggering Metamorphic Rearrangement of Corpora Pedunculata** 284
7. **Sexual Dimorphism** 286
8. **Memory during Metamorphosis** 287

PARTS 1-5 & APPENDIX: REFERENCES **288**

List of Figures

Figure 1. Plasmid map of pBluescript® SK+/-, 245

Figure 2. The plasmid pPNT., 246

Figure 3. pUAST vector, 246

Figure I.1., Restriction digest of pBluescript SK® +/- *neo* 262

Figure I.2., Restriction digest of pBluescript SK® +/- *neo* 263

Figure I.3., Restriction digest of pBluescript SK® +/- *neo* . 263

Figure I.4.a., Restriction digest of the pPNT vector. 264

Figure I.4.b., Restriction digest of the pPNT vector. 264,265

Figure I.5., Restriction digest of the pPNT vector. 264,265

Figure I.6.a., Restriction digest of the recombinant pUAST vectors. 266

Figure I.6.b., Restriction digest of the recombinant pUAST vectors. 266

Figure I.7.a., Southern Blot of pBluescript SK® +/- *neo* digests. 265

Figure I.7.b., Southern Blot of the pUAST recombinant vector digests. 267

Figure 1.8. Sequence of rec. pUAST-*neo* 267

Figure II.1., Stage 9 embryo. Anti- β- galactosidase staining of line 238Y using HRP secondary antibodies. 270

Figure II.2., Dorsal view of a stage 15 embryo (line 238Y). The reporter protein β-galactosidase was detected by immuno peroxidase staining. 271

Figure II.3., Stage 12 embryo. Anti- horse radish peroxidase antibodies recognising specific epitopes, which are expressed on the central nervous system. 270

Figure II.4., Anterior first instar central nervous system (CNS). Lateral view. Staining cells represent the domain of β- galactosidase expression. 271

Figure II.5., Anterior second instar central nervous system. Confocal laser scanning microscope image. 271

Figure II.6., Second instar nervous system (CNS). Dorso- caudal view. Secondary FITC antibody immunoreactivity exhibits several groups of cells in the ventral nerve cord, 271

Figure II.7., Third instar central nervous system (CNS). Dorso- caudal view. Blue chromophore is the product of lacZ activity. The cortices emanate axons into the calyces and continue to run more rostro- ventrally. 271

Figure II.8., Female third instar central nervous system (CNS). Frontal view. Secondary FITC antibody immunoreactivity reveals several groups of cells in the ventral nerve cord. 271

Figure II.9., Male third instar central nervous system (CNS). Dorsal view. Secondary FITC antibody Immunostaining against β– galactosidase. 271

Figure II.10., Dorsal view of a female third instar female central nervous system. 271

Figure II.11. a#1, Male prepupal central nervous system (CNS) (2- 4 hours after puparium formation. Dorsal view. 272

Figure II.11. a#2, Section through the neuropil of the male prepupal corpora pedunculata (2- 4 hours after puparium formation; APF). 272

Figure II.11. b., Male prepupal central nervous system (CNS) (2- 4 hours after puparium formation. Dorsal view. 272

Figure II.12. a., Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. 272

Figure II.12. b., Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. Dorsal view of the entire CNS, focal plane on the neuropil. 272

Figure II.12. c., Female early pupal central nervous system (CNS) (≈4- 6 hours after puparium formation. 272

Figure II.12. d., Female early pupal central nervous system (CNS) (≈4- 6 hours after puparium formation. Frontal view. Focus on the neuropil. 272

Figure II.12. e., Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. Frontal view. Secondary FITC antibody immunoreactivity. Frontal view. 272

Figure II.13. a., Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. Dorsal view. 272

Figure II.13. b., Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. 272

Figure II.13. c., Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. 272

Figure II.14., Female early pupal central nervous system (CNS) (≈8- 10 hours after puparium formation. Dorsal view. 272

Figure II.15. a., Dorsal view of the female pupal corpora pedunculata (ca. 12- 15 hours after puparium formation). 272

Figure II.15. b., Section through the lobes of a pupal corpora pedunculata. The α– lobe has not extended. 272

Figure II.16. a., Female pupal central nervous system (CNS) (≈21- 23 hours after puparium formation. Dorsal view. 272

Figure II.16. b., Female pupal nervous system. 272

Figure II.17., Corpora pedunculata of a female pupae (18- 20 hours after puparium formation). 272

Figure II.18., Section through the lobes of the male pupal corpora pedunculata (22- 25 hours after puparium formation). 273

Figure II.19., Section through the corpora pedunculata neuropil of an male pupae (>25 hours after puparium formation). 273

Figure II.20.a., Configuration of the corpora pedunculata of a male pupa (30 hours after puparium formation). 273

Figure II.20.b., Male pupal central nervous system (CNS) (≈30 hours after puparium formation. Dorsal view. 273

Figure II.21., Female imaginal central nervous system (CNS) (1 hour after eclosion). Dorsal view. 273

PART 1
Introduction and Theory

CHAPTER 1
The Hypothesis

The notion which types of nervous memory function are teleonomically, and which are ontogenetically determined, has been debated for generations by natural scientists (for review see Gould, 1977). New genetic approaches have allowed the research community to gain insight into the complex events underlying the process of memory formation. In this thesis I have researched the notion that learning is the alteration of a cells genetic activity in response to an environmental stimulus. In analogy, I have reasoned that development is the change in a cells genetic activity in response to an innate stimulus. The difference of both processes is, however, based in the specificity by which the cell perceives the signal. In contrast to learning the signals need to be very specific during development and perceived in a spatial and chronological sequence. For the learning process these undirected environmental signals need to be converted into this precise sequence. Both mechanisms result finally in the acquisition of a cells memory.

Genetically spoken both processes are thought to involve identical genetic modules (e.g. second messenger pathways, tissue specific transcription factors) which are differentially expressed during the organisms ontogenesis in response to the combinatorial action of positional and temporal signals. Hence, the developing cell has to make a tissue genetic switch to adopt a specific identity within a embryo. In a 'learning' nerve cell the environmental information is converted into an alteration of transcriptional activity. Such transcriptional alterations finally result in a change of the nerve cells identity. Thus, I have concluded that the mechanisms underlying both processes, which I hereafter wish to call morphogenesis and mnemogenesis are similar. *Drosophila* and other insects make thereby owing to their genetic and morphological simplicity awarding studying objects. In accordance with the aims of our laboratory to dissect learning

and memory in *Drosophila*, this thesis is restricted to an examination of morpho- and chemosensory mnemogenesis in insects. Here, I introduce a new insect the 'Droapschibo', which is a composite of *Drosophila* (*Diptera* [Holometabola]) (the genetic portion), *Apis mellifera* (*Hymnoptera* [Holometabola])(the behavioural, anatomical and electrophysiological portion), *Schistocerca gregaria* (*Orthoptera* [Hemimetabola]) (another electrophysiological portion) as well as, *Bombyx mori* and its relatives [*Lepidoptera*](the world record holders of chemosensory perception) to verify my hypothesis.

CHAPTER 2
The Terminology

1. Morphogenesis

The *Drosophila* embryo (hereafter referred as to type 3 embryo) unlike the embryos of echinoderms and mammals (hereafter referred as to type 1 and type 2 embryos, respectively) makes use of the **syncytial mode** to establish early **embryonic fields** along **gradients**. This has the advantage that upon cellularisation the nuclei are already programmed to accept a specific fate within the embryo, circumventing the use of uneconomic intercellular signalling mechanisms as seen for type 1 and type 2 embryos. The *Drosophila* embryo develops in three **spatially and temporally synergetic dimensions**. All three dimensions share a common developmental plan, whereby boundaries between spatially defined genetic active regions are established. This reorganisation process is regulated by morphogenetic circuits of signalling processes leading to the positive or negative regulation of the expression of distinct classes of genes (St. Johnston and Nüsslein - Volhard, 1992; Davidson, 1993). The first dimension is the establishment of a framework of **parasegments**. Parasegments are confined to the embryo. They divide each segment into posterior and anterior compartments (Lawrence and Morata, 1976; Lewis, 1978). Each segment is a distinct entity which interprets its developmental potential distinctively under the control of omnipresent transcriptional regulators (e.g. Murre et al., 1989). The second dimension is the establishment of **progenitor fields** within the parasegments. According to Spemann (1938) a progenitor field is defined as "a region of the embryo which gives rise to a specific structure". Davidson (1993) extended this definition of a progenitor field to a "region generated by regulatory and signalling functions" which result in the transcription of discrete sets of genes. Thus, in *Drosophila* the cell lineage of a progenitor field is indeterminate. Or, to put it into other words, the expression of genes is <u>not</u> dependent upon program, which is intrinsic to a cell lineage. Rather it is thought that the identity of a cell is determined by its exposure to positional information within a spatial and temporal interval during

embryogenesis. The third dimension is the **cell motility** of the progenitor fields with respect to each other. In *Drosophila* cell migration is predominately a passive event (with the exception of the migration of syncytial nuclei to the cortex) (Irvine and Wieschaus, 1994).

2. Mnemogenesis

Mnemogenesis, a term which will be frequently used in this thesis, is derived form the word mnemon (*ní-món*) [f. Gr. $\mu\nu\eta\mu-\eta$ memory; on= suffix denoting a fundamental particle] an expression shaped by Young (1965). He concluded that memory is based in minimal combinations of brain neurones, hence the mnemons. Cherkin (1966) proposed that a mnemon is a minimum physical change in the nervous system (compare Gould, 1977). Mnemogenesis is characterised by the molecular mechanisms underlying the change of synaptic plasticity in response to several coinciding convergent stimuli (Hyatt, 1897). The corpora pedunculata of *Drosophila* exhibit a large degree of homology to content addressable memory (CAM) networks (Hopfield, 1983). Networks similar to the CAM networks are also found in higher vertebrates. Here, they are, however, electrophysiologically better characterised. The rat hippocampus is the more sophisticated mammalian equivalent of the insect corpora pedunculata. It serves to integrate convergent signals resulting in an alteration of synaptic plasticity. Already one century ago the Spanish physician Ramón y Cajal (1898) stated that learning could be accomplished by a strengthening of the synapses following their intense activity. And indeed Bliss and Lømo (1973) found by recording the excitatory postsynaptic potentials (EPSPs) of hippocampal dentate granule cells after four brief stimulating tetani lasting 15 sec., in 15 sec intervals, that synaptic strengthening must have occurred. Depending on the time span these stimulating tetani are given, one differs between a milliseconds lasting post tetanic potential (PTP), short term potentiation (STP) and long term potentiation (LTP), which is only initiated after a certain threshold. This threshold is dependent upon a high frequency train of action potentials (APs) produced synchronously in a small population of neurones. These LTPs last under certain circumstances for

weeks. The neurotransmitter initiating such an associative LTP is l-glutamate. This neurotransmitter binds to two types of receptors. The AMPA receptor (named after the selective ligand α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate), a receptor of the GluR1-4 family, is responsible for low frequency activation of the postsynaptic neurone. 1- glutamate has, furthermore, the ability to bind to the NMDA postsynaptic receptor (blocked by antagonist of *N*-methyl -D-aspartate). In an inactive state the NMDA receptor is blocked by Mg^{2+}. Following the synchronic excitation of the postsynaptic cell, however, the NMDA receptor is unblocked. Hence two events are required for the activation the NMDA receptor:

- the postsynaptic membrane must be sufficiently depolarised to remove Mg^{2+} from the receptor
- l-glutamate has to bind to the receptor synergistically.

The induction of an LTP depends now upon the simultaneous activity of a population presynaptic neurones. Coinciding presynaptic activities in this population following the arrival of a tetanus elicit an depolarisation in overlapping sets of postsynaptic neurones, and, hence, induce an homosynaptic LTP. GABA could induce a inhibitory postsynaptic potential (IPSP), which is responsible to keep the NMDA receptors blocked after a low EPSP has been induced by the AMPA receptor. NMDA receptors are permeable to Ca^{2+}. Within the postsynaptic cells the Ca^{2+} current is further amplified by triggering the release of Ca^{2+} from intracellular Ca^{2+} /Inositol 1,3,5 triphosphate stores. Alternatively, mGluRs might be able to trigger through G-proteins the activation of phospholipase A_2 and phospholipase C, which in turn generate the second messenger metabolites arachidonate and diacylglycerol. Further downstream adenylate cyclase might modify the titres of cAMP within the cell. Taken together all these messengers may alter the plasticity of the postsynaptic cell. Not only the postsynaptic cell undergoes such changes. There could be a retrograde transmitter in form of nitric oxide or arachidonate which influences the plasticity of the presynaptic cells, since it has been proven that both postsynaptic and presynaptic cells respond to the Ca^{2+} influx into the postsynaptic cells .

Kandel hypothised that out of phase activity of active fibres leads to the weakening of synaptic connections. An LTP stimuli can produce long term depression (LTD) in fibres which are out of phase and, thus, inactivate these fibres. LTD is associated with a reduction in the synaptic number between pre- and postsynaptic neurones. NO may trigger the depression when it is released from inactive or asynchronously active neurones. Thus, all these events bring about changes in the synaptic plasticity (for review, see Bliss and Collingridge, 1993).

CHAPTER 3
The Theory

On the Tenovus Scotland conference Sydney Brenner (1994) stated that there are not enough essential (non-redundant or 'non-junk') genes in the genome of an organism encoding a function for each of the developmental and physiological processes. This limited repertoire would adequately have the consequence that a cell redeploys identical mechanisms for its propagation, differentiation, maturation and function. The systems used during this process, hence, equal modules, which are redeployed in different combinations at any stage during ontogenesis.

Each metazoan organism consist of a mulltitude of cells. Though these cells are genotypically identical, they differ phenotypically. This phenotypical diversity is the result of differential transcription of genes. A cell is first born following the fusion of gametes which function not only to supply a diploid (the exception that proves the rule) genome, but also to deliver the enzymatic components of the germ cell cytoplasm, which initiate the developmental program by differential transcribing the genes of an organisms gene pool. Following, birth the cell forms by mitotic divisions a progeny. Each group of progeny cells shares a common identity and fate within a specific insulated environment: the morphogenetic field. Signal transduction cascades mediate the translation of an extracellular stimulus into a pattern of gene expression within the anlage of the morphogenetic field. Development involves the specification of these cells to generate anlagen which are characterised by the cells common selectivity and response to external signals. Thus, the cells of a morphogenetic field of a developing organism acquire a common identity in terms of gene expression. Hence, differential gene expression within a group of cells demarcates the boundaries of the respective morphogenetic fields.

During development a organism is a isolated system which gradually acquires proportionally to its cellular diversity to receive and process environmental factors. Within the organism each cell is contained within the environment of other cells. Thus, in the first developmental stages cells receive environmental input form neighbouring cells.

With a increasing differentiation level the cell (or its lineage progeny) learns to interpret factors form the extracellular environment. As the maternally supplied embryonic food sources are depleted as soon as the metazoan organism has hatched, it needs to find its food autonomously. Therefore it needs to learn about the quality and the position of a food source. Moreover, it needs to learn to how to propagate to ensure its progeny. Mechanisms for these processes are already supplied during embryogenesis. They just need to become activated. Thus, mechanisms used for embryogenesis may be redeployed by a cell beyond embryogenesis according to the cells' specificity. On the other hand some developmental programs, which are no longer required by the cell, might be halted and a pathway may be called into action that are specific to mature organisms.

The acquisition of memory in a differentiated organism depends, on the quality of a sensor to select signals from the environment and to convert them into cellular language. Dependent on the degree of amplification of the signal by cellular communication pathways, the signal may induce changes in the cellular plasticity. Hence, cellular plasticity 'freezes' a sufficiently amplified signal by comparing its quality to the quality of information stored in a current state network and then decides about the modulation of this network in response to the signal (compare to short term and long term memory). The modulation of a memory network would then in turn involve the alteration of gene transcription. Thus, memory dependent changes of cell plasticity may rely on the same mechanism as neural development.

CHAPTER 4
Biophysical Considerations

Chemical reactions in the entire universe are assumed to follow the laws of thermodynamics. The first law of thermodynamics states that "the internal energy of a system is constant unless it is changed by doing work or by heating". The second law of thermodynamics is defined as the "potential of a system to change spontaneously, whereby this change is called entropy of a system".

A system is thereby a part of the entirety, which according to our definition is separated by a boundary. Hence, a system is a defined unit of the universe, which allows us to define the processes underlying the entirety.

1. Thermodynamics of Sensory Modalities

All systems contain internal energy (U') which is strictly dependent upon the energy of the environment (U). The velocity of a change in energy within a system is a consequence of environmental alterations (Enthalpy, H). It depends on the degree of insulation of a system from the environment. Hence, the resistance (Gibbs energy) of the systems boundary (threshold) determines the work an environmental energy needs to overcome in order to trigger changes within a system. The energy required to induce these changes within a can thereby be positive or negative. Negative energy is thereby associated with a reduction in volume and positive energy is defined as an increase in volume of a system (with disregard of none volume work). The absorption of energy by a system is called endothermic, whereas the release of energy is called exothermic.

$$\Delta U = \left(\frac{\partial U}{\partial V}\right)_T \Delta V + \left(\frac{\partial U}{\partial T}\right)_V \Delta T$$

dU : change of internal energy as a function of changes (partial) (∂) in volume (V) and temperature (T).

(1) After Atkins P.W. : 'Physical Chemistry' (1989)

Is the resistance of the boundaries between the system environment indefinitely high one calls the boundaries adiabatic. Diathermic boundaries in contrast have an indefinite low resistance. The transfer of energy can either be chaotic or organised.

In summary, energy spreads in form of chaotic or organised waves to change a system. Negative and positive waves level another so that the net energy is conserved. The state where no changes in energy occur is called equilibrium. The spontaneous change within an equilibrated system is associated with a change in the systems entropy. Thus, entropy reflects the potential of a system to change.

$$S = k \ \ln \mathrm{W}$$

S: the entropy of a system. k= 1.381×10^{-23}J K^{-1} (Bolzmann Constant), W: work

(2)

The amount work required to change the internal energy of an system in equilibrium can only be added but not withdrawn. Hence, the change of a system is an unilateral process, which depends on the resistance of the boundary (Clausius Inequality)

$$\Delta \mathrm{S} \geq \frac{\Delta q}{T}$$

dq is the energy added to a system; T is the temperature

(3)

To overcome the resistance of the boundary positive entropy in form of work needs to be added (Third law of Thermodynamics). Thus, the entropy of a system can consequently only be changed if energy is added.

Related to the changes in a closed system the change in internal energy reflects the change of entropy with the work accomplished. As work is the force used to overcome a distance, the change in volume is proportional. Thus, $\Delta w = -p\ \Delta \mathrm{V}$. The entropy mirrors the change in temperature within a system , which does not change its composition and, hence its potential: $\Delta q = T\ \Delta \mathrm{S}$.

As the change of internal energy is the sum of the work accomplished and the associated change in energy, are related by the expression:

$$\Delta U = T\,\Delta S - p\,\Delta V$$

(4)

The change of inner energy U of a system is, thus, strictly dependent upon S and V. Or, U is a function of both S and P. A change in enthalpy of a system manifest itself only when the boundary, or hence, the systems entropy is changed. Thus, the deviation between enthalpy and entropy is expressed in the Gibbs equation:

$$\Delta G = \Delta H - T\,\Delta S$$

(5)

This equation assumes that work accomplished is only converted into expansion work. In this case the change in pressure has to be taken into account

$$G_f = G_i + \int_{p_i}^{p_f} V\,\Delta p$$

(6)

This integral reflects the changes in volume over an interval (Carnot cycle) of changes in pressure in response to a change of the state of a system e.g. form solid (i) to liquid (f).

The volume is defined by the motion of a gas and is therefore dependent on the statistical distribution of a gas within the system.

$$n_i = \frac{N e^{-\beta\varepsilon_i}}{\sum_j e^{-\beta\varepsilon_j}}$$

$\beta = 1/kT$, whereby T is the temperature; i: is the state; e: exponent; N: no. of atoms; n_i: the number of Bolzmann distributed molecules[1]

(7)

R the gas constant is proportional to N_A the Avogado's number and the Bolzmann's constant. $R = N_A k$. . Henceforth, the argument follows that one can replace the volume by nRT/p, a term which describes properties of the gas constant (5):

$$G_{(pf)} = G_{(pi)} + nRT \int_{p_i}^{p_f} \frac{\Delta p}{p}$$

(8)

The Gibbs function thus is the ultimate theorem, which I wish to use here to explain the current theory how the thermodynamical memory works.

2. Thermodynamics of Organisms

A living organism reflects exactly what I have mentioned for a thermodynamic system. It has thermodynamic boundaries, the reactions within the organism follow the same laws as the chemical reactions. The problem for an living organism is that it is very volatile. Each organism is in effect more or less isotherm with its environment. An organism is designed to operate under specific conditions. Within these limits the kinetics of the organism is more or less in an equilibrium This equilibrium is maintained in temperatures that range from -1.8°C (the fish *Trematomus*) to 103°C (the eggs of the fresh water crustacean *Triops*) (Schmidt-Nielsen, 1990). *Drosophila* can only subsist in temperatures between 14 and 30°C (Ashburner, 1989). As an organisms boundary with the environment is diathermic it needs to counteract changes in entropy. From the most

[1]For proof and derivation see P.W. Atkins Physical Chemistry (1990). p. 571

primitive archae bacterium to the sophisticated *Homo sapiens sapiens* sensors give the organism feed back about the changes in enthalpy. This is an absolute necessary requisite as an imbalance within the organism would inevitable lead to its death. Furthermore, an organism requires energy for its maintenance. As metazoan organisms can not synthesise their energy reserves autonomously by making use of quantum energy, it needs to wander around and feed on those anabolic organisms of the plant world.

3. Thermodynamics of Sensory Receptors

Environmental energy can be derived from different sources: Thermal energy, light energy, acoustic energy and chemical energy. All these forms of energy are interconvertible by physical laws (Feynman, 1954). The forms of energy which are perceived by an organism as different sensations are called **sensory modalities.**

One type of sensory modality is chemoreception, a process during which chemical energy in the form of a discrete solid, fluid or gaseous chemical substance is coded as a stimulus of a discrete intensity by the nervous system. Chemoreception follows the same physical laws as the perception of other forms of energy. Most of the odorants bind to a specific receptor. The dissociation constant of the receptor for a particular odorant determines the response of the chemosensory cell. Has a particular threshold been exceeded odorant binding triggers a change in the receptor cells free energy which is directly related to ΔS (change in the cells entropy by augmenting the receptor cells thermal motion proportionally to the Boltzmann's constant) (Block, 1994). The higher the thermal energy of the odorant (e.g. ether) the greater ΔS. ΔS needs, thus, to be transformed into a directed response by means of changing the cells free enthalpy ΔG. Hence, the odorant alters the cells ion fluxes and triggers a whole array of electrochemical cascades.

A sensory receptor cell has, henceforth, the extraordinary ability to detect and convert selective changes in enthalpy of the environment because of its discrete entropy

(neural coding). Thus, it can detect even a source of low noise from its surroundings and to converts it into changes of its own entropy.

In the case of chemoreceptory cells of *Bombyx mori* so are these so specialised that they can detect 10^{-12} M of the respective pheromone implicating that they must have an extraordinary binding capacity which can also be expressed in terms of free enthalpy (Kaissling, 1970; for review, see Berg, 1983).

4. Thermodynamics of Nervous Systems

Each metazoan organism has the ability to convert a change in environmental entropy into electrochemical signals. These electrochemical signals have the advantage that they are directed, and not chaotic. These electrochemical signals practically simulate a perceived change in enthalpy and transmit this to the centres, which are able to redirect and to process this change. Electrochemical signals are, hence, directly related to the Gibbs function.

The Gibbs' function can easily be converted into the molar function for an ideal gas.

$$G_m = G_m^{\ominus} + RT \ln \frac{\Delta p}{p^{\ominus}} \tag{10}$$

The standard pressure in thereby assumed to be $p^{\theta} = 1$ bar.

The chemical potential is give as

$$G_m = \mu = \left(\frac{\partial n G_m}{\partial n}\right)_{p,T} \tag{11}$$

Thus, it follows:

$$\mu = \mu^{\Theta} + RT \ln \frac{\Delta p}{p^{\Theta}}$$

(12)

As under standard conditions the pressure is proportional to the molar activity (a), one can write:

$$\mu = \mu^{\Theta} + RT \ln a$$

(13)

To become applicable for electrochemical signal transfer the charge of the molecules, which participate in this reactions needs to be added. The charge has two functions: It determines the direction of the flow information perceived from the environment takes, and synergistically amplifies this signal by the path it takes in the neural circuit. Hence, The electricity canalises an otherwise undirected environmental signal.

The Faradays law takes this fact into consideration. Electrons follow the same gradient, which is expressed by the Gibbs function. They flow from region of surplus of negative charge (Anode) to a region of lack of electric charge to adjust their equilibrium. By definition negatively charged ions are called anions [μ_-], whereas positively charged ions are called cations [μ_+] (referring to univalent ions). Both are present is their respective molar [m] activities [a] in the system

$$\mu = \mu^{\Theta} + RT \ln \frac{[\gamma m]}{[m^{\Theta}]}$$

γ determines the activity of the ions is response to the entropy of the system.

(14)

$$\mu = \mu^{0} + RT \ln \gamma$$

μ^0 chemical potential for a solution at the start.

(15)

Considering that the system described here consists only of anion and cations. It thus, follows that their motility is a direct function temperature, enthalpy and entropy- a de-

pendency given the Gibbs function. The Gibb's function describes therewith the activities of both cations and anions in relation changes in enthalpy and entropy:

$$\mu_+ = \mu_+^0 + RT \ln \frac{[\gamma m_+]}{[m_+^0]}$$

(16)

$$\mu_- = \mu_-^0 + RT \ln \frac{[\gamma m_-]}{[m_-^0]}$$

μ^0 chemical potential at 0; m^0 molarity at 0

(17)

Thus,

$$G = \mu_+ + \mu_-$$

(18)

and,

$$G^0 = \mu_+^0 + \mu_-^0$$

(19)

Taken both concentration equal another

$$m_-^0 = m_+^0$$

(20)

then,

$$G = G^0 + \mu_+ + \mu_- + RT \ln \frac{[\gamma m_+]}{[m_+^0]} + RT \ln \frac{[\gamma m_-]}{[m_-^0]}$$

(21)

yields

$$G = p\mu_+ \; q\mu_-$$

(22)

whereby p the number of cations and q the number of anions or,

$$G = G^0 + pRT \ln \gamma + qRT \ln \gamma$$

(23)

When Δ G is constant, because it cannot exceed a threshold. The enthalpy needed to overcome this threshold is called potential $E.$. The potential $E.$ is also manifested in the energy required to change the entropy of the system, hence Δ G. Thus, the potential $E.$ is proportional to Δ G.

The potential is limited by the volume of a system and the molar mass. Both determine how fast an ion can travel. A high volume means a high resistance, low volume manifest themselves in low speeds. Consequently, the potential is defined by the distance an ion is able to travel following a change in ΔG. The extend of a reaction ξ is the molar function of ΔG. This function neglects a change in temperature or pressure (which in the nervous system are anyway constant). Thus, ξ is proportional to the partial of G.

$$\mu_+ + \mu_+^0 = \left(\frac{\partial G}{\partial \xi}\right)_{p,T}$$

(24)

or

$$\mu_- + \mu_-^0 = \left(\frac{\partial G}{\partial \xi}\right)_{p,T}$$

(25)

gives:

$$\Delta G = \left(\frac{\partial G}{\partial \xi}\right)_{p,T}$$

(26)

ν (quantity) $\partial\xi$ is the amount of ions, which must travel from anode a to cathode. The Avogado number is the product of the gas constant and the Bolzmanns' constant which both give information about the statistical distribution of the ions.

N_A equals $1.38 \times 10\text{-}^{16}$ erg . K^{-1}. The product of $\partial\xi$ and N_A is the Faraday constant (F).

$$F = 96.485 \text{ kC mol}^{-1}.$$

The work, which needs to be accomplished to transfer a charge is $dw = \Delta G \; d\xi$

This is equivalent to the product of charge and potential.

$$dw = E \times \text{-nF } d\xi$$

(27)

If one equates the work then: $\Delta G \; d\xi = E \times \text{-nF } d\xi$

$$\text{Or, } \Delta G = E \times -nF$$

(28)

$$\text{As, } \Delta G = \Delta G^0 + pRT \ln\gamma + qRT \ln\gamma$$

(29)

and assumed that $p = q$

consequently: $\Delta G = \Delta G^0 + nRT \ln \frac{[\gamma m]}{[m^0]}$

(30)

$$E = -\frac{\Delta G^0}{nF} - \frac{RT}{nF} \ln \frac{[\gamma m]}{[m^0]}$$

(31)

As

$$\Delta G^0 = E^0 \times -nF^0$$

(32)

$$E = -E^{0} - \frac{RT}{nF} \ln \frac{[\gamma m]}{[m^{0}]}$$

(33)

The Nernst function is the cardinal equation, which describes the directed fluxes of ions within a neurone. Hence, taking the equations (Bronsteïn and Zhemenzhanev, 1979; Aidley, 1989; Atkins, 1989) applied in thermodynamics, it is therewith proved, how a change in entropy is converted by a closed system into a direct change of ion fluxes, which can, then, be amplified.

The nervous system can be seen as an example of such a closed system. As already mentioned above the sensor, which perceives the environmental information uses the energy contained in it to transform the signal into a change of ion fluxes in the nervous system. The Nernst - equation can, hence, be converted into the Goldman - Katz equation which reflects the ion fluxes within the nervous system (for review, see Rashevsky, 1938).

5. Electrochemistry of Neural Signal Transduction

As I have mentioned above the laws of thermodynamics are the fundaments for the function of the nervous system of metazoan organisms. Neural responses to stimuli manifest themselves in the alteration of neural membrane potentials and synaptic activities.

Uneven ion fluxes, which follow their osmotic potential, generate gradients accross the neural membrane. These gradients are additionally amplified by ion pumps. Thus, in the resting stage the membrane is hyperpolarised, meaning that the interior of the nerve cell is negatively and exterior positively charged. The membrane potential, thus, reflects the activity coefficient of the participating ions: Na^{+}, Cl^{-} and K^{+}, which follows the Goldman equation:

$$V_m = \frac{RT}{F} \times ln \frac{\sum_{k=1}^{n} z_k P_k [X_k]_e + \sum_{l=1}^{m} z_k P_l [Y_l]_i}{\sum_{k=1}^{n} z_k P_k [X_k]_i + \sum_{l=1}^{m} z_l P_l [Y_l]_e}$$

Whereby, X stands for n different types of positively charged ions, Y represents n negatively charged ions. [] is the respective molar concentrations. e means exterior and i means interior. k/l= 1 is the lower border and m/n the upper border of the sum. P is the total permeability and z the charge of an ion. The term RT is the gas constant (8.314 $deg^{-1} \times mole^{-1}$) and T is the absolute temperature. F is the Faraday's constant (96,500 coulombs $mole^{-1}$)

A chemosensory stimulus, which is perceived by a sensory receptor cell, is amplified through a signal transduction cascade. This modulates, in turn, the properties of membrane channels. Once this signal is sufficiently amplified, selected membrane channels open and elicit an influx of positively charged Na^+ ions and an efflux of monovalent K^+ manifesting itself in a characteristic hyperbole as a function of changes in charge across the membrane during a time interval. The propagation of the signal proceeds only unidirectional, as for a moment the membrane potential in the region, where the membrane was depolarised, needs to regenerate. The regeneration is mostly active and proceeds through ion exchange pumps. Once the signal has propagated to the terminus of a nerve fibre, it needs to be abridged to the next nerve cell. Synapses are nanometer small insulated gaps between two nerve cells, which function to transmit the signal to the postsynaptic nerve cell. When a signal is received by the terminus of the presynaptic cell, it triggers the release of a highly diffusible neurotransmitter into the synaptic cleft. The neurotransmitter then binds a receptor, which is confined to the postsynaptic membrane. The receptor in turn either propagates the signal directly by opening the ion channels of the postsynaptic neurone, or it modules the state of the channels through intracellular signalling mechanisms. Hence, it might either be excitatory or inhibitory. Excitatory postsynaptic potentials (EPSPs) may elicit an action potential in the postsynaptic cell through the spatial or temporal summation of single quantum responses mirroring the binding of the neurotransmitter to its receptors. Regions in the postsynaptic cells, which receive synaptic input require a lower threshold than other regions downstream in the neurone indicating that the transmission of an action potential in the

synaptic region enhances the synaptic signal by modulating the state of the ion channels in this region. The action potential is then transmitted orthodromically along the axon.

The inhibitory postsynaptic potential is injected by discrete nerve cell antagonising the actions of neurones with excitatory neurotransmitters. They directly counteract Na^+ by opening selective ion channels for K^+ or Cl^- and thereby hyperpolarising the depolarised postsynaptic axon. The course a signal takes within the organism depends on the networking of the nervous system (for review, see Aidley, 1989).

6. Specific Features of Neural Coding

To understand the theory behind the electrochemical component of the memory process (see Part 4, Chapter 4) one needs to consider the **quality** of the electrochemical signal. As mentioned in Chapter 5 the quality of the signal depends firstly on the intensity of the stimulus, and secondly on the potential of the neural network to modulate this signal.

The **intensity** of a given **stimulus** is thereby directly proportional to the **discharge frequency** of the neurone. The discharge frequency in turn is related to the **dynamics** of the neural network. This dependence is expressed by the **intensity function** which describes the periodic rhymic activity (**phase**) and the **amplitude** of action potentials in response to the intensity of a stimulus. Several periodical recurring bursts are also called **oscillations**. The number of oscillations evoked in a receptor is called the **frequency code** of stimulus intensity (for review, see Trotier, 1994).

Magnitude and intensity of an sensory stimulus also determine the number of neurones which are (a) activated, and (b) logged in phase. Hence, the phasic and synergetic activation of a population of neurones as a result of the stimulus intensity is described by **population code**. For each neurone of a population to respond in the same frquency code the phases of the membrane depolarisation oscillations ought to be reset, a phenomenon known as **stimulus- timing- phase singularity** of a population of neurones. The co-ordination of differntially oscillating neurones is thereby a purely **topological** problem. The **phase velocity** and the phase shift have to be taken into consid-

eration to solve this problem. Neural resposnes are genuiely considered to be **excitatory**. The interior of the neurone becomes positive during an excitatory response. A population of neurones, which has the ability to couple other neurones to its phase by means of excitatory currents is denoted as **phase attractors**. Underlying neural excitation are inhibitory responses, known as **phase repellors**. These counteract excitatory currents and may, thus, tune them in phase with each other. Oscillations of indivual oscillating neural populations become, hence, **coupled**. Any **perturbations** are removed generating a **high gain low noise signal. Synergistically** swinging neurones might then have the potential to induce long term changes to the neural hardware - **mnemogenesis.** It should, however, be noted that coding of different stimuli may call discrete autonomously swinging neural populations into action. These populations might, however, not necessarily be coupled to other neurones (for review see Miller, 1974; for mathematical treatise of the oscillation problem, see Murray, 1989).

CHAPTER 5
Neural Network Considerations

So far I have only mentioned the effects of changes of enthalpy on the organisms sensory cells entropy. Beyond this, however, the information needs to be transmitted to the centres of information processing. The nervous system, serves as a pipeline, which directs environmental signals via ion currents to the respective control centres, which in turn mediate the response. Thus, an environmental 'simulator' is created. The quality of this 'simulator' depends directly on the ability of the underlying neural network to accept environmental information. The neural network needs, thereby, to approximate most accurately the environmental stimuli and should be able to store these. Hence, memory is such a simulator, and the process by which memory is created is here denoted as mnemogenesis.

Neuronal networks are, hence, the requisite for mnemogenesis as they are able to make a switch to direct the signals to the appropriate areas. To operate properly they need to be as efficient and as reliable as possible.

Memory networks of insects (and most of the other creatures) consist of several individual components, the neurones, which are connected sequentially at different levels following an hierarchical plan. Each level shares different functional and organisational features. In the front line are thereby specialised sensory cells which mediate the contact with the environment. They have the task to convert i.e. a chemosensory submodality into the electrochemical energy in a process called neural encoding. Both quantity an quality of the stimulus determine the feature of the neural coding process. Sensory neurones are mostly clustered to form specific structures: the sense organs. Each cluster of sensory neurones consists of two portions or fields dividing each neurone into two portions. The more exterior portion, the receptive field, has the task to detect a given chemosensory stimulus. The more interior portion, the transductory field, translates and propagates the stimulus. The process of translation, hence, depends upon the potential of a chemosensory receptor to convert this stimulus. As seen before

several parameters have to be satisfied before the threshold of neural encoding will be overcome.

First order neurones generally converge onto higher order neurones. These higher order neurones, too, consist of receptive and transductory fields; in this case, however, the fields are associated with post- and presynaptic termini. Synaptic structures may adopt quite complex configurations bearing in mind that they will be distributed across the entire surface of a neurone and will consist of either excitatory or inhibitory components.

Relay neurones are switched in between higher order neurones. They have the task to enhance the resolution (reduction of background noise) of the neural signal by either amplifying or inhibiting a given stimulus. Three modes of action are thereby conceivable: Firstly, these relay interneurones may run parallel to the higher order neurones and converge onto an neurone of a superior level where they modulate the signal. Secondly, relay interneurones may run parallel to a given set of higher order neurones but transmit the signal retrogradely to a lower level of higher order neurones. Thirdly, relay interneurones may connect different higher order neurones of the same level diagonally to modulate their function.

This pattern led Hopfield (1982, 1987) to conclude that sensory memory systems are organised as content addressable memory (CAM) networks. The basic features of CAM networks are to be explained here briefly.

To operate as CAM networks an current state memory vector which is stored at a stochastic level in the neural network is compared to an input vector, the stimulus. The derivation between the given current state vector and the input vector is described in terms of the Hamming distance. The dimension of the Hamming distance is the bit. The CAM memory network, hence, compares stochastically, the current state to the input and abridges the deviation between both vectors to give rise to a novel current state vector (Hopfield, 1984).

The novel state CAM network (gain CAM network) can, hence, be considered as the alteration of the stochastic current state stability triggered by an non-linear input

vector at invariant times (Hopfield et al., 1983). Accordingly, the neural input-output relation is redefined in a stochastic fashion (Hopfield and Tank, 1986).

CHAPTER 6
The Evolution of Memory

1. The Origins of Life and Genetic Memory (Teleonomy)

As mentioned before each organism can be considered as a diathermic chemical reaction, which is influenced by the environment. In the evolutionary sense, one assumes that organisms were created by a chemical reaction in a closed system. It was even demonstrated by Stanley Miller (1987) that under laboratory conditions life could be created. The question, however, arises how did life retain its memory that it is life? Or, how did life become established? Hence, the chemical reaction, which has occurred must have created a memory, which can be recalled in generations. Thus, heredity is evolutionary memory based in molecules. These are deoxyribonucleic acid and ribonucleic acid. The most primitive organisms, which are in their majority composed of the nucleic acids. So for example the bacteriophage lambda. Lambda contains a set of only 137 genes or so - most of which are redundant anyway - which store its genetic information (or teleonomic memory). One type of memory, which lambda has obtained during the evolution is its host specificity as it is only found in bacteria. The second type of memory, which it has acquired is the decision as to whether it should proceed with a lytic or lysogenic life cycle. The decision is ultimately dependent upon the nutritional state of its host. If for instance its host bacterium is staving, it integrates into the genome and proceeds with the lysogenic cycle and waits until the bacterium has recovered until it proceeds with the lysis, whereby it excises from the genome and breaks open the bacterium to find a new host. All these events depend on external signals, which recall the genomic memory stored in its DNA. The factor cI is thereby of cardinal importance as it translates the state of the host into transcriptional activity of genes involved in either life cycle. cI binds to specific operator sequences (sequences controlling binding and transcriptional activity of the host RNA polymerase from phage promoters), which prevent the lytic cycle genes from being transcribed. Instead it directs the expression of the lysogenic genes, which encode recombinases, enabling the

phage genome to become integrated into the host genome. The transcription of the repressor is probably influenced by the host genes *h*igh *f*requency *l*ysogenisation genes *hflA* and *hflB*, which probably dependent upon the hosts physiological state, determine the binding of the cI antagonising factor *cro* to the operator of the lysogenic genes and shut down their transcription in order to activate transcription of the lytic genes. Hence, the memory of the lambda phage is stored in its genome, in particularly in the operator sequences and in the open reading frame for both antagonising repressor proteins (Miller, 1992).

One of the first realm of organisms, which are thought to have evolved are the prokarotes, i.e. the archae bacteria. These bacteria are autonomous, hence, directly exposed to the environment. Purple bacteria have, for instance, acquired the ability to move randomly within a fluid until they encounter a spot of bright light. Once, the have entered this spot they remain there. Thus, underlying this process is a primitive form of photoreceptor, which upon its activation recalls the hosts memory which tells them to rest at a specific wavelength of light and to refuel their energy stores.

Thus, memory of primitive organisms is stored in their genome. An experimental strategy devised to test the purple bacteria if they can find a source of light faster once they have been predisposed to it has yet to be established.

2. Chemosensory Memory of *Escherichia coli*

Chemotaxis is known as a organisms ability to respond with a movement to an odorant. Two substances, attractant and repellent, are known to influence chemotaxis. Chemotaxis of an *Escherichia coli* population within a test tube is reflected by the polarised distribution following the introduction of either attractant or repellent. *E. coli* cells propel themselves using eight flagella, each of which is 7 µm long. Clockwise flagellum movement causes the cells to sprawl. Counter clockwise rotation of the flagella results in forward taxis of the *E. coli* cell. Cells attracted by an odorant in the solution approach the source by random movement within the medium. The cell switches during the 'search' process between counter clockwise and clockwise flagella move-

ment. The closer the cell comes to the odorant source, the more frequent become the sprawling periods. Once it has it reached the odorant source, it switched from counter clockwise flagellum movement towards clockwise movement. Hence, sprawling allows the bacterium to identify the odorant, whereas swimming allows the random taxis within the medium.

Several mutant phenotypes for the pathway underlying chemoreception and chemotaxis in bacteria have identified. Twenty different chemosensors are known. Half of them bind attractants and the other half bind repellents. Odorant perception starts with the binding of an odorant by specific binding proteins, the chemosensor. These occur within the periplasmic space and transfer the odorant to the cytoplasmic membrane. Mutants for either of these genes are denoted 'specifically non-chemotactic'. Receptor molecules such as the glucose receptor presumably accept the odorant form the chemosensor. Chemoreceptors are integral components of the cytoplasmic membrane and transfer the odorant into the lumen of the cell. Generally, these chemoreceptors may have an affinity for various odorant molecules. Mutations in these odorant transporter molecule are, therefore, said to be 'multiply chemotactic'. These mutants can be divided into four complementation groups defining the four genes *tsr*, *tar*, *trg* and *tap*. Receptor molecules activate an intracellular transduction cascade composed of the products of genes *cheA*, *cheB*, *cheR*, *cheW*, and *cheZ*. This signal transduction cascade is activated by all chemoreceptor proteins equally. *CHEW* transfers the signal from the chemoreceptor to *CHEA*. *CHEA* is, in turn, phosphorylated at an histidine residue at position 48. This phosphoryl group is subsequently transferred to either *CHEY* or *CHEB*. *CHEY* interacts directly with the products of the genes *fliG*, *fliM*, and *fliN*. These flagella associated proteins, finally, modulate the response of the flagella. Thus, the presence of an odorant stimulus induces clockwise flagella movements (sprawling). This fact has been confirmed as in mutants deficient for either *cheA* or *cheY* the bacteria swim continuously due to an impaired signal transduction to the flagella associated proteins. The response is terminated by the phosphorylated product of the *cheB* gene. By removing the methyl groups from discrete glutamic acid

residues of the chemoreceptors, it freezes the signalling cascade and induces counter clockwise flagella movement again (swimming).

CHEZ has the ability to undo the phosphorylation of *CHEY* and acts therewith as a safeguard to reinstate the counter clockwise movement of the flagella (swimming).

The key molecule for sensory adaptation is *CHER*. *cheR* is of particularly interest, as mutants for this gene tend to swim continuously. It is well established that *CHER* methylates the *TRG* (i.e. serine) receptor rendering it able to initiate the signal transduction cascade *de novo*. The activity of the receptor can also be fine tuned. Each receptor molecule can accept as many as four methyl groups, which enhance the receptors response. Hence, the higher the concentration of an attractant, the more residues on *TRG* receptor are methylated by *CHER* . By contrast, with decreasing attractant concentration less residues become methylated.

Conversely, an *E.coli* cell has still the ability to sprawl and to swim when it has encountered no odorant at all and very high odorant concentrations, respectively. Low levels of phosphorylated *CHEB* increase the levels of receptor methylation by *CHER*, thus, resulting in the accommodation of cells to high odorant concentrations.

When the bacterium cell encounters, however, a high odorant concentration, mnemogenesis comes into action. The bacteria needs to compare spatial and temporal levels of attractants and respond to it. The concentration of a given attractant is reflected by the methylation of the *TRG* receptor. This methylation serves to abridge temporal and spatial varying attractant concentrations. Once, a threshold has been reached, and the cell has been saturated with attractants, it adapts and does not respond to an attractant stimulus, anymore. This is the result of basic phosphorylation of *CHEA*. *CHEZ* and phosphorylated *CHEB* have the ability to interrupt these sprawling episodes and the cell continues to swim into the same direction. Hence, binding of attractant to chemoreceptor then prevents *CHEA* form being phosphorylated, reducing the sprawling periods. During sensory adaptation following the encounter of high concentrations of attractant, the periods of sprawling become much less frequent. Coincidental movement of a *E.coli* cell towards higher concentrations of attractant reduce the sprawling period even further. Repellents, in contrast, result in increased *CHEA* phosphorylation and induce

sprawling allowing the cell to randomly become detached from the repellent. Underlying this process is *CHER*. In amalogy , low attractant levels result in a basic phosphorylation of *CHEA* causing the celll to sprawl.

In summary, *E.coli* memory is teleonomically determined. Thus, the ontogenic chemosensory component of *E.coli* resides in the differential methylation of the *TRG* receptor. The product of the *cheR* gene determines the sensory adaptation. It is not known as to whether, sensory stimuli result in the recruitment of new genes and, hence, store the memory a *E.coli* cell has acquired im a different behavioural response (Nara et al., 1991; Maddock and Shapiro, 1993; for review see Macnab, 1987)

3. Evolution of Phylogenic and Omtogenic Memory in Metazoans

3.1. Introduction

In prokaryotes memory is strictly teleonomically determined. Here all the information is stored in the genome and is recalled in the offspring. Metazoan organisms have gradually developed the ability to firstly polarise the cellular memory function and to acquire memory from the environment iin these centres. Specific organs have developed, which mimic environmental information and supply it to the respective centres of information processing. Thus, in response to environmental information metazoans are able to modulate their genetic memory. This does not happen by altering the genetic hardware but by making differential use of this hardware. As a consequence the central control organ, the brain is regrouped according to the environmental input it has received.

3.2. Phylogeny of Memory in Lower Metazoans

Hence, the body plan of metazoan organisms is highly heterologous. Specific sensory cells and nervous cells control the function of the remaining cells in a highly specific mode. Though in the sessile *Polifera* and *Coelenterata* the nervous system is very diffuse the more sophisticated bilateralia comtain a polarised nervous system. The region

most anterior to the direction into which the bilateralian organism moves contains and amalgamation of nerve cells. The development of these polarised nervous systems dates back as far as 600- 1000 Million years ago (Algonicium), where the first annelids have evolved. Annelids such as *Neophytes* display a well differentiated supraoesophageal ganglion of 3000- 4000 nerve cells in size. The leech contains in each metameric subunit up to 400 neurones.

The actual diversification of the central nervous system has obviously occurred during the cambium, thus, 590 until 500 Million years ago. The 850 µm long maxillopod *Bredocaris admirabilis*, for example shows already a highly sophisticated nervous system (for review, see Miklos, 1993). The same body plan as found in this species is generally conserved in its evolutionary offspring. So shares the oldest known fossil bee *Trigona prisca* (96- 74 million years ago) not only certain homologies to *Bredocaris admirabilis*, but also to the present day *Apis mellifera* suggesting that not only anatomical details have been retained during the course of evolution, but also their function such as learning, foraging and navigation. The insect order of *Diptera*, which has emerged 300 million years ago displays the same organisational features of the ocular segment throughout the different species as its ancestor. The memory function in several insect species is based to a large extend on the insect brains anatomy rather than its synaptic reorganisation. Thus, memory is a is a function of the teleonomic neural networking. Taking the may fly as an example. This *Ephemeropteraeh* species is only short lived. During this time it has to mate and (dependent upon gender) to lay eggs. Hence, as Gabor Miklos (1993) has put it, it either needs to have 'on board insectronics' to recall the genetic memory, which is reconstructed during development, or it needs to have an extraordinary high capacity to store information during its 6 hour long live span (From studies on *Dipteran* species *Drosophila melanogaster* it has been proven that the latter option is not true).

3.3. The Memory of *Drosophila melanogaster*

As *Drosophila melanogaster* is the main pillar of my thesis I wish to describe the evolutionary implications of its neurogenetic viability in detail. The size of the *Drosophila* genome is estimated to be 165 megabase pairs (Mb). The human genome in contrast exceeds 3,000 Mb. One estimates the *Drosophila* genome numbers in between 5,000 (by virtue of the number of lethal mutations) and 15,000 (by virtue of the number of transcription units) (Merriam et al., 1991). Taken into account that there is a certain redundancy of 60% (genes, which are not used or repeated) within the genome one may reason that the actual number of essential genes lies somewhere in the range of 5,000-6,000 (Ashburner, 1994). Some of these 5,000-6,000 essential and 6,000- 7,000 non-essential genes must participate a least in part in the generation of the 200,000 neurones, which constitute the *Drosophila* brain. This neural diversity can only be generated by combinatorial interactions of the participating genes. In mosaic studies on the photoreceptor, it has been established that about two thirds of the essential genes are employed just to generate this organ (Thaker and Kankel, 1992). In analogy to the memory pathways in *Drosophila* one could consider that the number of genes which participate in this event is at least as high as for the development of the photoreceptor. Thus, it seems highly likely that the cellular diverse networking of the *Drosophila* brain determines the flies behaviour and not the rewiring of neurones once, the pharate imago has emerged. Thus, once fledged the young adult can immediately fly and feed without having to acquire its information from complex learning paradigms (Murphey, 1986). Thus, to take *Drosophila* as a model for synaptic plasticity would be the wrong choice. For studying the general principles of neural networking *Drosophila.* however, is an ideal organism. Additionally, it seems as if there is a high degree of biochemical homology in the *Drosophila* and the mammalian nervous systems. Miller and Benzer (1983) for instance have generated several monoclonal antibody homogenates of *Drosophila* brains half of which displayed cross-immunoreactivity to several epitopes of the human nervous system. Moreover, evidence that *Drosophila* and mammalian species use the same pathways comes from the observation that the homeobox genes of

Drosophila , apart from being highly identical, map to the same organisational regions in the mouse embryo, and occur in the genome in the same order as they are spatially expressed during development (for review, see Krumlauf, 1993). A knock out of the mouse calcium-calmodulin-dependent kinase II (CaM) gene demonstrated that the behavioural sequence of transgenic mutant mice is disturbed (Abeliovich, 1992). Similarly, the electrophysiology of the affected neurones in *Drosophila* mutants for components of the cyclic adenosylate pathway (cAMP) is severely impaired (Zhong and Wu, 1991; Zhong and Wu, 1993). Moreover, several signalling pathways, such as the *Notch, ras* and *Wingless* pathway have been found in mouse and human, too, where they assume the same function their *Drosophila* homologues (Ellisen et al., 1991; Robbins et al., 1992; for review, see Dickson and Hafen, 1994; McMahon and Bradley, 1990). Last, ion channels and several synaptic receptors display high similarities in *Drosophila* and mammals (for review, see Gundelfinger, 1992; Warmke and Ganetzky, 1994).

Additionally, *Drosophila* serves as a good model to study the evolution of the brain. Several mutants have, for instance, been identified, which apart from manifesting themselves in behavioural abnormalities, display an aberrant morphology. Heisenberg and co-workers (1985) isolated, for instance, several learning deficient mutants, which displayed an aberrant morphology. In these mutants -*mushroom body* miniature (*mbm*) *A*, *B*, and *C* - proper axonal outgrowth is impaired. Of particular interest is the mutant *mushroom body defect.*(*mud*) . Here the number of neuroblasts in the brain as well as in the ventral central nervous system is significantly increased. Thus, in this mutant far more neurones are generated as there are normally present. The mutant alleles for *mud* do not affect the viability of the mutant fly. If such a mutation would affect only the brain region, the increase in neural numbers may be associated with the acquisition of new memory storage capabilities. Evidence for this notion comes form the numbers of neuroblasts in the brain of *Schistocerca gregaria.* Usually about 24- 30 neuroblasts are present in each segmental hemineuromere. In the deutocerebrum which is thought to be a segmental neuromere, about houses 32 neuroblasts. Assumed that the protocerebrum comprises two neuromeres - the preantennal neu-

romere and the labral neuromere - the number of neuroblasts in both segment is about 86. Thus, in evolutionary terms the number of large neuroblasts must have been duplicated here (Zacharias, 1993).

Hence, one may argue that the larger the brain in proportion to the body size, the more space there should be for the storage of information? This notion is not necessarily true. Firstly, it is well established that with the size of the brain the body size increases within an insect order. For example two cockroach species, one *Blatella germanica* (1.5cm), the other *Macropanesthia rhinocerus* (7.5 cm) have both the same behavioural repertoires despite there is only a modest increase in brain size in proportion to body size in the latter species (Miklos, 1993). However, in between the two different insect orders, the complexity of the brain decreases/increases markedly. Although *Apis mellifera* can be considered as having the same brain size as *Macropanestia,* it has a much more complex behavioural repertoire. It has, for example, the ability to learn to associate an odorant with a sugar water reward, which is presented for 100 msec. only. It stores this information for 2 sec. in its short term memory and following three repeated trials the information is then converted into an intermediate term memory. This intermediate term memory lasts for approximately 1 day. Following subsequent trials the information is subsequently converted into the long term memory, which lasts until the bees death. Even just prior to its death it will reorient towards the sugar source when perceiving the odorant. The number of neurones present in the worker honey bee brain is impressive, as well: There are 1 million neurones! The problem is that when altering the learning sequence the bee fails to combine the changes and will become apathetic after some trails indicating that it cannot follow really complicated events (Menzel, 1990). Other Hymenoptera such as a small parasitic wasp, in contrast, contain only 10,000 neurones and their behavioural repertoire is restricted to finding a suitable host.

Secondly, it has been proven that there is a great abundance of neurones in some insects. And it has even been established that when surgically ablating the corpora pedunculata in *Periplaneta americana* for instance, the response of the male to female pheromones showed latency, but was not impaired (Drescher, 1960).

3.4. The Mnemogenesis of *Aplysia california*

In the phylum of molluscs, the differences in neural organisation between the orders are even greater than in insects. The sea hare (*Aplysia california*), which is frequently used for studies on memory contains only 20,000 neurones. It shows a variety of responses reflecting all elementary forms of learning but in a rather stereotyped fashion: Habituation, sensitisation and classical conditioning. Its 20,000 central nervous cells enable it to carry out a set of defensive reflexes for withdrawing its tail, gill and siphon a small fleshy spent above the gill used to expel sea water and waste.

Habituation

Habituation is the simplest form of learning. The animal responds to a novel stimulus with a series of orienting reflexes. Synaptic potentials summate and cause the motor neurones to discharge strongly giving rise to an abrupt withdrawal of the gill. When the stimulus persists, and the animal learns to recognise it, the synaptic potentials of the sensory neurones in the interneurones and motor neurones, as well as, in the excitatory interneurones decrease. Provided the stimulus is neither rewarding nor noxious the animal learns to suppress its responses owing to a reduction in neurotransmitter released by any action potential from the presynaptic terminal as a consequence of an inactivation of the N-type Ca^{2+} channel. Hence, with each action potential less Ca^{2+} flows into the terminals and reduces the ability of transmitter vesicles to be transferred into the active zone. The reduction in the synaptic effectiveness is followed by a homosynaptic depression after a long term stimulus has been received associated with a back-formation of certain neural synapses and a change in the cellular plasticity. In habituated animals the incidence of detectable connections between sensory neurones and motor cells is reduced to 30% and this change last for weeks. long term changes, which manifest themselves in the reduction of synaptic connections are induced by the neuropeptide Phe-Met-Arg-Phe amide (FMRF amide). FMRF amide increases the K^+-current and decreases the voltage dependent Ca^{2+}- current. It produces a synaptic depres-

sion, when applied for more than two to a neural cell culture (Schacher and Montarolo, 1991).

Sensitisation

During sensitisation the animal learns about the properties of a noxious stimulus and consequently remembers to respond more effectively to a variety of other stimuli, even innocuous ones. Since a single noxious stimulus to head or tail activates numerous neurones mediating in the gill-withdrawal complex by forming new axo-axonal synapses; one speaks in this context of heterosynaptic facilitation. This event increases the amount of second messenger cAMP in the sensory neurones.

The mediator bringing about all the changes during heterosynaptic facilitation is 5-hydroxytryptamine (5-HT). This neurotransmitter binds to receptors coupled to the GTP binding protein (G_s), which in turn activates cAMP cyclase. cAMP presumably activates PKC, which then phosphorylates a S-type K^+-channel. The repolarisation of the action potential is subsequently reduced allowing N-type Ca^{2+} to be activated for longer. As more Ca^{2+} is able to enter the terminals the transmitter release augments reflecting the enhanced availability of transmitter vesicles (Abrams et al., 1991).

Long term sensitisation is associated with the synthesis of new proteins during the period of 5-HT application. 2-D gels revealed that 15 early proteins and two late proteins are expressed in responses to long term serotonin application. One of these late proteins is Calreticulin. Calreticulin is a major Ca^{2+} binding protein in the lumen of the endoplasmatic reticulum of the perikarya. It acts as a buffer and increases the capacity of the ER to store Ca^{2+} which is associated with inositol 1,4,5 triphosphate (IP_3) Ca^{2+} store. IP_3 releases the store of intracellular Ca^{2+}. The IP_3 receptor is enriched in sensory neurones (Kennedy et al., 1992).

Moreover, 5-HT enhances the number of siphon neurones innervating e.g. the gill motor neurone L7. Every neurone expresses on its surface a variety of *Aplysia* specific Cell adhesion molecules (apCAMs). During the application of learning associated stimuli these are thought to become reorganised. As concluded from studies on cell culture of *Aplysia* neurones, long term facilitation enhances titres of 5-HT, which trig-

gers Ca^{2+} dependent cAMP signal transduction pathway the internalisation of these apCAMs and increases the expression of the light clathrin chain only in the siphon neurones (Mayford et al., 1992). The internalisation occurs at specialised depressions in regions of contact between axonal fascicles, which function as molecular sieves and concentrate apCAMs. The remaining apCAMs on other regions of the neurone remain unaffected. Hence, apCAMs are not constitutively internalised. After the formation such coated pits at the site of membrane apposition, these complex invaginations are separated from the surface membrane to form coated vesicles, which are subsequently endocytosed. In the cell body uncoupling (CURL) vesicles mature in lysosomes, provided that a ligand has bound to the receptor at the cell surface. The degradation occurs at a specific PEST sequence in the cytoplasmic region of the transmembrane form of apCAM. Alternatively, some CURLs may be separated and recycled (Bailey et al., 1992).

Classical Conditioning

During classical conditioning the subject must learn the relationship between two stimuli. An initially weak or ineffective stimulus becomes highly effective in producing a response after it has been paired or is associated with a strong unconditioned stimulus. The gill and siphon withdrawal complexes of *Aplysia* are examples of behaviours, which can be enhanced by both classical conditioning and sensitisation. Only by pairing a stimulus to either the siphon and mantle shelf the withdrawal reflex is elicited. Each of these areas is innervated by a distinct class of sensory neurones. Pairing a stimulus to either class of neurones with an unconditioned stimulus initiates the pathway. There is a time delay between conditioned and unconditioned stimulus of 0.5 sec. Such that there is a convergence received by the motor neurones. This convergence enhances the presynaptic facilitation by the following mechanism: An action potential allows Ca^{2+} to move into the sensory neurone. This Ca^{2+} influx acts through calmodulin which amplifies adenylate cyclase by serotonin an other modulatory transmitters. More cAMP is generated when Ca^{2+} is bound to adenylate cyclase then when it is not (Kaang et al., 1993).

4. The Modulation of Teleonomic Memory in Higher Metazoan Organisms

Thus, *Aplysia* has a range of behavioural patterns, which are, however, more stereotyped and with regard to memory more telonomically rather than ontogenetically determined. Higher molluscs like squids and octopuses display, in contrast to *Aplysia*, much more sophisticated behavioural patterns, which in case of octopuses almost parallels the behavioural diversification of mice. The brain of *Octopus vulgaris* presumably contains 500 million neurones. The genome size of the haploid *Loligo pealei* and *Octopus bimaculautus* exceeds, however, 0.82 and 1.25 times, respectively in comparison to the human genome. The genome of *Loligo* is scattered over 84 and 86 chromosomes. The diversity of mRNAs in the squid brain further levels approximately that of mouse (65%). Interestingly, the brain volume of adult octopuses increases markedly during visual learning. Hence, the evolutionary threshold of onboard developmental memory and acquired memory is overcome. Thus, this class of organisms acquires its memory from the developmental exposure to the environment and hence during its ontogenesis, rather than form a developmentally (teleonomically) innate memory (Young, 1965; for reviews see Young, 1966; Miklos, 1993). Leaving aside the world of the chordate phyla pices, aves, reptilia and amphibia, the mammalian brain is one of the most intensively studied. The principles of learning in the mammalian mouse brain have already been described above. The mouse genome contains, for instance, up to 50,000 gene transcription units 5,000- 10,000 of which are estimated to be essential. Interestingly, half of these transcription units are estimated to be expressed in the nervous system. Moreover, in *Homo sapiens sapiens*, the number of cells is thought to be about 85,000 million, 80% of which are granule cells of the cerebellum. If one considers that each of the neurone may form 1000 synapses than the numbers of synapses may excess 10^{14}. This diversity is generated by only 50,000 transcription units suggesting that alternative splicing/ polyadenylation mechanisms (Boelens et al., 1993; van Gelder et al., 1993; Wassarmann and Steitz, 1993; Gunderson et al., 1994), RNA editing (Sommer et al., 1991) and differential transcription form discrete promoters combined

may generate this monumental diversity. How complex the brain function is, is shown by the fact that the generation of long term memory in *Felis* requires between 5 million and 100 million neurones in distinct cortical regions.

In addtition, the mammalian brain is anatomically highly conserved as 95% of the rat nuclei are also found in the human brain. Furthermore, with the exception of area 17 of the primate visual cortex the neocortices of the mouse, rat, cat and human contain the same number of nerve cells in each volume unit. Though the neocortices are different in these animals, the density of neurones seems to be identical. In contrast to most other phyla, the brain of mammals does not stop to develop but continues to grow postnatally. The role of the hippocampus in the learning process is still disputed as large lesion in the hippocampus prolong the visual navigational learning process but do not impair it. The exact tasks of the different parts of the hippocampus also remains still unresolved (for review, see Miklos, 1993).

5. Synthesis

It is just hoped that the human intelligence stops us from using unethical and trivial methods to study and explain the human brain. Genes are certainly involved in this process, their combinatorial actions determine the complexity of the brain rather than the actions of single genes.

It needs, however, to be pointed out that genetic engineering of primate and human brains should not be started, as otherwise George Orwells' "1984" and the Huxleys' "Brave New World" are the most likely apocalyptic future visions. Instead, we should use our knowledge to aim for a better world. Therefore, the respective researchers selfishness should be restrained. And the competitive science race as a result of lucrative funding from even as selfish multinational companies should be stopped. Moreover, patents for genes should not be given. Last, manipulations on the human genome should be monitored by the society as a whole rather than a few individuals. Here our **con***science* should prevail.

PART 2

Dissection of Morpho- and Mnemogenesis: Tools for Studying the Anatomy and Genetics of Chemosensory Memory Circuits in *Drosophila melanogaster*

To isolate the genes which co-ordinatively participate in this process a variety of strategies have been developed. An extensive mutational survey conducted by Wieschaus and Nüsslein- Volhard (1980) identified a whole array of genes required for the formation of the embryonic axes, segmentation and neurogenesis. The evaluation of the mutant phenotypes by anatomical and molecular means led to the comprehensive model applied today to *Drosophila melanogaster* embryogenesis. Most interesting for developmental biologists is thereby the study on the development of the central nervous system (CNS). From such a study one hopes to gain insight in the anatomy and the function of the CNS in *Drosophila*. A further advantage is that most of the genes which participate in neurogenesis have their mammalian homologues which allow to draw parallels on neurogenesis of more complex creatures such as mice and humans.

With the generation of several neurogenic mutants one has hoped to identify the multitude of genes which function to form the complexity of the adult nervous system (Datta and Kankel, 1992; Seeger et al., 1993). Other mutant screens have been conducted to tackle the question how mutants affect the function of the adult nervous system (Benzer, 1967; Heisenberg and Bohle, 1979). Certainly these broad mutant screens have contributed very much to our current understanding of the processes underlying development and function of the nervous system. However, to assess the development and function of individual subsets of neurones a more refined and combinatorial approach is required.

CHAPTER 1

Enhancer Detector Analysis to Study the Genomic Identities of Morphogenetic Fields

The ability of mobile genomic elements - which are scattered across the entire euchromatic moiety of *Drosophila melanogaster* genome - to induce mutations was subsequently used to track down and mutate genes which are thought to be involved in the nervous function of the eye and the brain, respectively (Kidwell et al., 1977, 1979; Ballinger and Benzer, 1989; Kaiser and Goodwin, 1990). As natural P-elements are, however, poor markers and *in situ* visualisation of the spatial domains of their activity can only be achieved by tedious hybridisation techniques, a new system was devised allowing the detection of active P-elements without any complications in the whole mount tissue.

It has been known for some time that P-element promoters respond to tissue specific enhancers. Hence, P-element specific genes were deleted without affecting the ability of P-elements to transpose and replaced by the bacterial β- galactosidase gene (*lacZ*). The expression of β- galactosidase can easily be scanned for with a chromogenic substrate. Thus, the spatial activity of a nervous system specific enhancer is reflected by the *in situ* activity of β- galactosidase (for review, see Bellen, 1990). Moreover, genes upstream or downstream of the respective Enhancer trap P-element insert can be easily cloned using the plasmid rescue method (Cooley, 1988; Bellen et al., 1989; for reviews see Cooley, 1988; Bellen et al., 1990). These histological markers have several advantages over the conventional approach involving the complicated mapping and cloning of mutant genes. Moreover, monoclonal antibodies which have been deployed to identify the respective gene products might not necessarily identify the correct epitopes, and *in situ* hybridisation techniques only identify RNA species in the endoplasmatic reticulum of the soma without identifying dendritic and axonal processes.

Several P-element mutagenesis screens were carried out to track down genes which are of interest for the process of neurogenesis and neuronal function. A screen by

Bellen et al. (1989) identified about 48% of transposants which express β- galactosidase in the cells of the central nervous system. In analogy Bier et al. (1989) identified 49% of the p[lacW] lines which expressed β- galactosidase in the CNS. 5.14% thereof displayed expression in the brain and only 0.13% in the optic lobes. Moreover, dissection of these lines (Hartenstein and Jan, 1992) classified eight categories of *lacZ* expression in the CNS some of which displayed β- galactosidase activity in cells of the midline and optic lobes. In most case staining was not only confined to the CNS but appeared frequently in other germ layers, as well. Most of the 'background staining' occurred in the sensilla (43%) and epidermis (25%), as well as, the trachea (15%), foregut/ hindgut, midgut (13%), the mesoderm (13-14%), as well as, in the fat body and in the oenocytes. A further screen was conducted to isolate glia cells and their precursors which participate in the formation of the eye (Winberg, 1992). Several other enhancer trap lines that were generated by Spradling and collaborators were assayed particularly for *lacZ* staining in the brain region. In the latter screen 58% of the lines displayed staining in the central nervous system, whereby there was no specific staining in either the procephalic lobe and the ventral nervous system (Datta et al., 1993). Han et al. (1992) used an enhancer trap screen to assay specific staining patterns of cells which are thought to involved in chemosensory learning.

A new generation of enhancer trap elements has even facilitated the histological and molecular study of the central nervous system further. It allows the expression of the yeast transcription factor GAL4 form a stable marker enhancer trap element under control of a genomic enhancer. GAL4 can activate the ectopic expression of reporter genes which are under control of the GAL4 binding site (UAS) of a P-element shuttle vector (pUAST). This, allows the introduction of genes which can be used as phenotypic, selectable (Steller and Pirotta, 1985), mutational (for review, see Kilby, 1993) and ablational (Bellen et al. 1992; Moffat et al., 1992; Kalb et al., 1993) reporter genes. Additionally, other genes such as development and metabolism specific genes can be induced and potential mutations can be rescued easily in particular subsets of cells by complementing the mutant allele with the ectopically expressed wild type allele.

1. Using the Extraordinary Abilities of P-elements for Mutagenesis

P-elements are the most frequent group of mobile elements within the *Drosophila melanogaster* genome. They are either thought to be primitive precursors to viruses or derived from viruses (for review, see Finnegan, 1992). Their 2907 bp genome contains three long open reading frames (ORFs) comprising 714 bp, 792 bp and 654 bp, respectively, whereas the fourth short ORF is less than 400 bp long. The ORFs are flanked to either site by 31 bp long perfect inverted repeats (Laski et al., 1986). The P-element promoter is located 30 nucleotides upstream of the short site extending approximately 15 bp downstream of the initiation site for the transactivating transposase protein, which is required to excise the P-element from a position within the genome and to insert it into a new position (for review, see Rubin et al., 1985; Mullins et al., 1989). Transposase contains a helix-loop-helix motif encoded by ORF and thus is assumed to belong to the family of DNA binding proteins. It recognises a non - palindromic consensus sequence containing a 10 bp AT rich repeat.

To the 5' end transposase binding spans nucleotides 48 - 68 and 2855 - 2871 to the 3' end. The non-palindromic nature of the binding site indicates that transposase does not bind as a dimer. Controversially, ORF 1 contains a leucine rich motive implicated in homo- and heterodimer formation. None of these hypothised dimers have so far been found. There is a considerable overlap between the promoter function based in nucleotides 44- 70 bp and the transposase binding site suggesting that transposase acts autoregulatory (Kaufman et al., 1991). High levels of transposase would accordingly compete with transcription factors for the promoter sequence. Thus, as a consequence the transcription frequency of transposase decreases. The function of the 31 bp perfect inverted repeats in the transposition process remains obscure. The inverted repeat binding protein (IRBP), a host encoded factor, seems to be a good candidate for recognition and interaction with these sequences. Its function is not known to be dependent on transposase (Kaufman et al., 1989; for review, see Rio, 1991).

P-element transposition mechanism is a conservative mechanism associated with gap repair and thus independent of the replication process. The transposition event is

confined to germline cells and is thought to occur either prior or during meiosis. The P-element excises precisely and leaves a double stranded gap behind. This gap is detected and screened by the hosts own DNA repair mechanisms. The gap is widened by exonuclease digestion which removes at least one of the two 8 bp target duplications generated during insertion. A processive search is thought to select homologous DNA. Both ends flanking the gap are preferrentially confronted with the homologous template of the sister chromatid (Engels et al., 1990). But, ectopic homologous DNA may serve as a template for the gap repair process, as well (Engels et al., 1990). Information is then transferred from the homologous template to the gap (Nassif and Engels, 1993).

In association with P-element transposition large deletions in the adjacent DNA have been detected. These were explained with the abortion of the gap repair mechanism, during the onset of the meiotic anaphase I. Alternatively, P-elements which often insert as tandems could take with them adjacent host DNA when transposing. The likelihood for repairing such a large gap completely seems therefore be to reduced (Daniels and Chovnick, 1993).

A P-element is only restored at a gap when the homologous template carries a P-element, as well. Several types of truncated P-elements with internal deletions are thought to be the product of an aborted gap repair, which only fills in the gap partially and deletes predominantly sequences in the middle of the P-element genome.

P-elements are quite fastidious and several authors seem to have established a consensus sequence 5'-GGCCAGAC-3' into which P-elements most frequently insert. Euchromatic DNA is preferred to heterochromatic DNA for P- element insertion (O'Hare and Rubin, 1983; Berg and Spradling, 1991; O'Hare et al., 1992; Zhang and Spradling, 1994). Upon insertion the P-element duplicates a 8 bp stretch of target DNA to either site. When a P-element inserts it needs to cleave the DNA, a function, which is probably carried out by the transacting transposase molecule itself. P-elements transpose preferentially to nearby chromosomal sites. In a screen by Zhang and Spradling (1993) most insertions were found within 6 kb of the start site. The orientation of local insertions occurs non-randomly (Tower et al., 1993). Proximal to the start

site new insertions in the opposite orientation to the start element predominated. Local transposition in females was 100 fold higher than in males (Lemaître et al., 1993).

The P-element transposition is tissue specific. A functional 87 kDa transposase molecule is solely produced by 2907 bp autonomous P-elements in germ line tissues. In pole cells, primordial germ cells and germ cells proper three splicing events have been identified. The first intron (IVSI) between open reading frames O and 1 (ORF0 - ORF1) is spliced between 442 and 501 bp. The splice sites of the second intron (IVS2) between ORF 1 and ORF 2 are located at nucleotides 1222 for the 5' splice site and 1156 nucleotides for the 3' splice site. The ORF2 to ORF 3 splice at position 1947 (5' splice) is confined to germ line cells (Rubin et al., 1985; Laski et al., 1986; Siebel and Rio, 1990; for review, see Rio, 1990; Tseng et al., 1991). This splice removes IVS 3 during the very early stages of oogenesis. In embryos the maximal splicing activity is found 5-6 hours post fertilisation and peaks again after 10 hours in primordial germ cells. Those pole cells which do not take on the fate germ cells cease slicing IVS3. The germ line splice persists throughout development and is confined to the female zygote (Kobayashi et al., 1993).

In somatic cells the IVS3 splice is inhibited by an 97×10^3 kDa transacting protein, which has been implicated to bind specifically to a region in which the IVS3 splice occurs. A truncated 2.5 kDa transcript is synthesised in somatic cells terminating at position 2710 bp. The 2.5 kb transcript therefore encompasses the polyadenylation signal sequence at position 2696 bp. This sequence is required for the binding of the polyadenylation tract during the polyadenylation process. The polyadenylation protein Ui 70K is thought to mediate this process. The 2.5 kDa transcript is translated into a 66 kDa protein, which is regarded as a somatic inhibitor of the transposition event. Though the exact mode of action is not well understood to date, it is widely assumed that it dimerises with the 87 kDa transposase molecule at the leucine zipper motif and therewith represses transposition (Misra and Rio, 1990).

Under laboratory conditions several *Drosophila* strains have been isolated, which exhibit the phenomenon of hybrid dysgenesis, which is associated with genotypic and phenotypic abnormalities including an elevated mutation rate, chromosomal abnor-

malities and sterility due to abnormal gonadal development. Hybrid dysgenesis occurs when a male of a P-strain ('repressor' strain) is crossed to a female of a M-strain ('mutator' strain) which is devoid of P-elements. The ensuing progeny is described as being of M-cytotype whereas the progeny of the reciprocal cross is described as having P cytotype. P-cytotype represses transposition whereas M cytotype permits transposition (Kidwell et al., 1977; Eggleston and Exley, 1992). The P-cytotype represses the P-element promoter in germ line and somatic tissues presumably by encoding either antisense RNA (Rasmusson et al., 1993) or by encoding inhibitory proteins, which compete either with transposase for DNA binding or by dimer formation (for review, see Rio, 1990; Gloor, 1993; Lemaître et al., 1993; Misra et al., 1993).

2. Δ2-3 Transposase a Stable Source of Transposase Enables Deleted P-elements to Transpose

As seen above deleted P-elements, which still contain the sequences spanning the region for the inverted repeat to the second P- element exon, and form the middle of the third exon to the 3' inverted repeat still retain their ability to transpose when they come under control of a trans acting transposase source (Mullins et al., 1989). These elements are called 'mutator elements' for their ability to transpose to another region when under control of a transposase source.

The 'jumpstarter' element, which contains the transposase source needs to have two characteristics in order to direct the mobilising activity of 'mutator' elements.

Firstly, it should be able to express sufficiently transposase in germ line and somatic tissues. Secondly, it needs to be immobilisable to keep it at a stable chromosomal position within the genome to isolate its function during mutagenesis crosses. P[ry$^+$ Δ2-3] (99B) fulfils both requisites. It lacks the third intron enabling the generation of transposase in somatic tissues. Δ2-3 expresses very high rates of transposase presumably as a result of lacking buffering activity of the 5' genomic region, which lies in-between the presumptive enhancer and the P-element. Moreover, Δ2-3 is extremely stable as a result from its position of insertion or predicted changes in its structure (Laski et al., 1986; Robertson et al., 1988). The 'jump starter' line carries on its third Δ2-3 contain-

ing chromosome an dominant amorph allele of the *Drop* [*Dr*] locus as a marker which characterises heterozygous individuals as having an extremely reduced number of facets (Lindsey and Zimm, 1992).

3. The Features of Enhancer Trap P-elements

The ability of P-elements to transpose to different locations of the genome can now be used to trace genes, which are involved in the development of *Drosophila*. To use such P-elements efficiently and to detect them after they have inserted into the host chromosome their genome can be artificially modified. So one can make use of P-elements as a way to induce foreign genes into the *Drosophila* genome, with the precondition that any additional fusion gene does not effect the sequences, which are required for transposition, namely nucleotide 48 and 68 to the 5' end and nucleotides 2855- 2871 to the 3' end of the P-element (Mullins et al., 1989). Moreover, the fusion gene has to have an active promoter, which is whether derived directly from the P-element or an inducible promoter, e.g. the *Drosophila* heat shock gene 70, which encodes a 70 kDa heat shock protein. This promoter has the advantage that it can be turned on at a specific point during development if one exposes the transformant to temperatures of 38-39°C for about one hour.

Another possibility is to place the fusion gene under control of the P-element promoter (44- 70 bp), which overlaps with the transposase binding site.

The open reading frames ORF0 - ORF3 starting at 87 bp can be deleted. The P-element promoter has the advantage that it is turned on, when it comes under control of an nearby enhancer. Moreover, it is continuously active in cells throughout development as seen in the splicing experiments by Laski, Rio and Rubin, so that any spatially regulated expression pattern can be detected after the P-element has come under control of an enhancer. To obtain a cellular detector *lacZ* was fused in frame to the second exon of the P-element. The *lacZ* gene is derived from the *E.coli* lac operon, which encodes β -galactosidase. β -galactosidase cleaves lactose into galactose and glucose. When 5- bromo - 4 chloro - 3 indolyl β -D galactose (X-gal) is added, this is hydrolysed

to yield a blue chromophore, which can easily be detected. Initially the full *lacZ* sequence was inserted into the P-element. The problem was, however, that the nascent β-galactosidase protein contained an N-terminal nuclear localisation sequence that confined it to the nucleus (Bellen et al., 1988). Thus, for effective cytoplasmic distribution the nuclear localisation sequence was deleted enabling *lacZ* expression in axonal processes, as well. As a phenotypic marker O'Kane & Gehring (1987) fused the dominant eye colour gene *rosy*$^+$ gene (Doyle and Bray, 1994) behind the tailing sequences and poly (A) site of the *Drosophila hsp 70*. A more useful dominant eye marker, however, is the mini white gene which has many advantages to *rosy*$^+$. Flies heterozygous for the miniwhite gene in a genetic background null (w^{1118}) for the white locus display generally orange eyes, whereas homozygous for the same elements have red eyes. The eye colour is, hence, dosage dependent which means that it arises from the subthreshold activity of this altered version of the white gene, so that flies hetero- and homozygous to the p[Gal 4,w$^+$] insertion can be rapidly scored (Hazelrigg et al. 1984). In the *rosy* marker, the same eye colour in hetero- or homozygous individuals is yielded. Moreover, the white gene comprises only 7.1 kb and, hence, is 0.9 kb smaller than the *rosy* gene. This should allow the white gene to jump more effectively. Last, the loss of a P-element manifests itself quite quickly and isolation of revertants is easy to accomplish (Datta et al., 1993).

A more versatile P-element enhancer system is based on the yeast transcription activator Gal4. This dual system utilises the ability of Gal4 to bind its yeast target sequence, the Upstream Activated Sequence (UAS), a motif consisting of multiple copies of a 17 bp palindromic sequence, when ectopically expressed in *Drosophila*.

This system consists of two components:

- The marker P-element vector, which is mobilised to the desired locus within the *Drosophila* genome, where it is subsequently stabilised.
- The reporter P-element vector, which serves as shuttle for the ectopic expression of genes that have been cloned into it.

Both P-element vectors are transfected into two different fly lines, which when crossed to each other yield the expression of the relevant reporter gene.

To construct the stable marker P-element enhancer trap vector [pGal4;w$^+$], the *lacZ* gene was replaced by the Gal4 gene. The yeast specific Gal4 gene encodes a 100 kDa protein that binds as a dimer to the the *cis* acting UAS regulatory sequence. This protein contains several zinc fingers, which create a loop structure permitting the local residues to interact specifically with the UAS sites. In yeast the binding of Gal4 then facilitates the transcription of various enzymes needed for the galactose metabolism of this organism.

Transgeneic *Drosophila melanogaster* individuals that contain the pUAST vector should, hence, be responsive to transcriptional activation by Gal4. It contains the five optimised Gal4 binding sites and a *hsp* 70 TATA box. The poly (A) tract was derived from the SV 40 virus small T-intron and the polyadenylation site. A *lacZ* gene encoding β-galactosidase was originaly inserted into the polylinker between the hsp70 TATA box and the poly (A) adenylation site. The *lacZ* gene can, however, be replaced by other genes.

Moreover, the pUAST vector contains the *rosy* (*ry*) gene as a marker. *ry* manifests itself in reddish brown eyes. Homozygotes for the null allele w^{1118} lack xanthine dehydrogenase activity express this marker.

The Gal4 enhancer trap system has a particular impact on the study of development for it allows the expression of developmentally regulated genes in another context in response to a different enhancer, which controls Gal4 expression.

4. Reporter Constructs

To obtain most efficient staining of neurones, the nuclear localisation sequence of the β- galactosidase gene has been deleted. Albeit this modification β- galactosidase still expresses in the somata. Thus, an attempt was made to link β- galactosidase to a antrograde microtubule associated transport molecules, allowing β- galactosidase to be transported into the region of synaptic output inclusive the dendritic fields. *kinesin* is an ATPase, which is composed of two large and two small moieties. The motor moiety forms cross- bridges with the microtubules (Yang et al., 1990; Gho et al., 1992). By

fusing the motor moiety of *kinesin* to β- galactosidase and transforming it into *Drosophila* several central nervous system neurones stained with antibodies against anti β- galactosidase. Alterations on the properties of the neurones were, however, frequent (Giniger et al., 1993b), so that another construct of the *tau* protein was made. *tau* is a microtubule associated protein, which is required for the formation of axonal processes. A fusion *tau* β- galactosidase construct is therefore able to stain axonal processes (for review, see Skoufias and Scholey J.M., 1993).

5. Plasmid Rescue

Once a P-element enhancer trap vector of either category has inserted in the desired genomic locus, and the expression pattern reflects this insertion, one can rescue the P-element insert. Based on the consideration that an *ampicillin resistant* (amp^R) gene renders transfected bacteria resistant, a nuclease digested *Drosophila* genome, which contains the P-element enhancer trap vector with an integral amp^R gene can be used to select for transfected bacteria. Dependent on the position relative to the ampicillin resistance gene genomic sequences either upstream or downstream of an unique restriction endonuclease recognition site can be rescued together with the vector. The circularised and religated fragments of the digested genome are then transfected into bacteria. Usually only one plasmid is accepted by a bacterium, so that when its clone selected for ampicillin resistance, it should survive because it contains the ampicillin gene within a sequence of adjacent *Drosophila* genome. Bacteria transfected with genomic plasmids, which do not contain the ampicillin resistance gene will subsequently die. The plasmid can be isolated from the resistant bacteria colony and probed to a phage library form which the corresponded cDNA can be isolated. Sequencing subsequently reveals the quality of the respective cDNA clone with regard to its putative function. Problematic with plasmid rescue is that the gene which has been rescued due to its proximity to the P-enhancer trap element does not reflect the activity of the P-element enhancer trap. Thus, the transcription of the P- element vector is reflects the activity of a nearby enhancer, which needs not necessarily activate, which flanks the P- element.

6. Genetic Mosaics

In an elegant experiment Golic made use of the yeast recombinational FLP-FRT system. FLP is a recombinase, which recognises a binding site called FRT (FLP recombination targets). The FRT comprises three 13 bp binding elements of which only 8 bp core sequence encompassed by the two asymmetrically arranged binding sites serves as a substrate for FLP tetramers (Dixon and Sadowski, 1993). Once it has recognised a FRT it matches both FRTs and induces a staggered nick in the double strand of FRT to either site of the 8bp sequence and exchanges both paired strands via the transient formation of an Holliday junction (Holliday, 1964). FRT has been put under the control of a hsp 70 promoter and induced into the genome via P-element mediated transformation. w^{1118} was used as a marker to verify the efficiency of transcription from the heat shock promoter (Golic and Linquist, 1989). Of interest would it be to drive the expression of FLP form GAL4 enhancer traps. As GAL 4 expression is induced only by specific tissue specific enhancers, one can make use of this property and drive FLP expression from this the pUAST construct, which as a result several mosaic recombinations are induced into these cells (Golic, 1993). Alternatively, the flanking of cloned genes by FRTs may serve as an aid to create ransom insertions of these genes into allelic FRT sites. Thus, the FLP-FRT mutagenesis system is much more versatile than conventional mutagenesis methods (Golic, 1991; Xu and Rubin, 1993).

CHAPTER 2

Conventional Mutagenesis

1. Collisional Ionising Radiation

Two kind of effects can be observed when DNA is subjected to collisional ionising radiation. One effect is that single stranded breaks are produced. Dependent upon the nature of the break ends, which contain a 3'-OH and a 5' - phosphate tend to be more likely to be repaired. γ- radiation also produces the formation of formamidopyrimidine by ionising N- residues 7 and 9 of the purine ring.

Although the ability of ionising radiation to produce chromosomal breaks is random, the probability for a chromosomal break to occur increases with augmenting doses of ionising radiation. When germ line cells of *Drosophila* are irradiated large translocations in form of inversions or deletions, as well as, asymmetrical or symmetrical interchanges are observed. The production of acentric and dicentric chromosomes is, for instance, associated with large genomic deletions, reflected by the loss of the acentric chromosome during mitosis. Smaller deletions tend to affect only portions within the genome. They arise when the phosphodiester bonds between adjacent nucleotides are hydrolysed. In chromosomal preparations such deletions manifest themselves in that chromosomal interbands are absent, or a loop occurs at the position where these bands should be. These loops arise when both sister chromatids are unable to pair due to a missing DNA segment.

Inversions require the presence of two breaks. DNA repair mechanism then match the sequence and insert it the other way round. Dependent on the scale of such inversions the centromere can be affected, as well. In pericentric inversions, for instance, the position of the centromere has changed whereas the position of the centromere in paracentric inversions does not change. Such chromosomal abnormalities are generally not used for mutational analysis of genes involved in neurogenesis. Inversions affect generally large portions of the genome and can, hence, easily be mapped. If one wishes to test single base pair mutations for their function, one uses these mapped inversions and

complements them with the mutant alleles. Is there an overlap between mutant alleles on homozygous individual should arise. From the complementation analysis of two mutant alleles one can draw conclusions about the position of cis and trans- acting elements as well, as the functional domains of a given gene product, which might be truncated by such a mutation (for review, see Yedvobnick et al., 1985).

2. Ethyl Methane Sulfonate Induced Mutations

EMS is a potent alkylating agent, which attaches a new alkyl group to the starting alkyne of N7 of guanine. The N7 terminal alkylated guanine leads in turn to an unstable quaternary nitrogen which is lost (depurination). Instead a new base will be incorporated. This in turn may yield transitions or transversions. When balanced the heterozygous mutant allele will be complemented to a chromosome characterised by complex inversions designated by a dominant phenotypic marker. These mutations can be balanced and complemented to known mutations to determine potential allelism. The failure to produce phenotypical trans-heterozygous can be used to isolate mutant alleles (Nüsslein -Volhard and Wieschaus, 1980; Nüsslein -Volhard et al., 1984; Jürgens et al., 1984; Wieschaus et al., 1984; Seegers et al., 1993; for review, see Yedvobnick et al., 1985; Noll et al., 1993).

Conditional mutations are mutations, which under certain circumstances are reverted. Amber mutations, for example, are 'non-sense' mutations affecting the stop codons UAA, UAG and UGA. Here the polypeptide chain stops. In mutants for some tRNA species, however, a tyro tRNA is able to recognise the amber codons UAG and UAA to insert a tyrosine residue instead of the stop codon and thereby reverting the ability of a 'non-sense' mutation to manifest itself. Temperature shift conditional mutants are characterised by the ability to manifest themselves in response of a temperature shift from permissive to restrictive temperatures.

3. Genomic Walk

Once, a gene has been mapped to a region bracketed by two known markers, one can now use the sequence of a known terminal marker gene to probe this to a phage library, and, using DNA hybridisation techniques, screen for overlapping phage clones, which span this region.

4. Genetic Mosaics

In genetic mosaics the genetic composition of somatic tissue varies. X-ray induced chromosomal breaks, which are induced during morphogenesis may render cells within an organism phenotypically different from their neighbours. This fact can be used to trace somatic tissue clones throughout development. For example do *Notch* mutant alleles affect, for example, the number of bristles. Hence, when irradiated, somatic tissue patches display an abnormal high number of bristles (hyperplasia). The effects of such mosaics are examined, for instance, by taking a heterozygous mutant for a given allele and expose it to ionising radiation. The emerging adult will display the mutations when both alleles for the given locus have been affected. In addition one could therewith test as to whether a locus is cell autonomous or it obeys to signals form other cells. For example it has been established that *Notch* and *Delta* loci do interact. Taken a mosaic mutant for *Delta,* the phenotype of these cells will be the same as observed in *Notch* mutations. Thus, genetic mosaics are a versatile tool to elucidate the interactions of genes during development in either heterozygous or homozygous lethal mutations without effecting the viability of the mosaic organism as well. Or to put it into toehr words, it allows the making of cell clones in order to trace their fate during morphogenesis (for review, see Yedvobnick et al., 1985).

PART 3
Morphogenesis

The generation of chemosensory memory pathways follows the same principles as applied for neurogenesis in other domains of the embryo. Only the specificity of the process differs substantially form other embryonic domains. Neurogenesis as a whole, is the process during which the fully differentiated neuroderm is generated. This process involves the differentiation of neural precursor cells (neuroblasts) at stereotyped positions of the gastrula stage embryo in response to coinciding dorso-ventral and anterior posterior signals during development. These signals also determine the neuroblasts identity, which it retains during subsequent cell divisions each generating a progeny neuroblast and a ganglion mother cell. Ganglion mother cells are then programmed by postional information to divide symmetrically and to form a progeny of two sibling neurones. These differentiate with respect to their position within the segmented embryo and emanate axonal processes, which navigate over a long distance to synapse their targets

These mechanisms are identical for all neurones, be it either interneurones, motoneurones or sensory cells. The specificity of each neurone depends on its position and its integration within the different embryonic compartments and segments. There they receive segment specific information programming them socialise with the remaining cells within this region. In the procephalic region progenitor proneural cells receive this information prior to cellularisation from gap genes. Each procephalic gap gene is differentially expressed along an anterior posterior gradient of the morphogenes *bicoid*. The differential expression depends on the quality of each gap genes specific promoter which displays a discrete binding affinity and threshold to activate gap gene transcription. The segmentation process of the procephalon then integrates each domain of dynamic gap gene expression to define the specificity of the respective segment. This information is used to determine the differentiation of cephalic neuroblasts and regulates

its proliferation by intervening in the course of the cell cycle. About axonogenesis in the procephalic region hardly anything is known. The gross morphology of the chemosensory memory pathways is created by stage 17. It is subsequently reinforced and sophisticated. Hormones, the essential players of metamorphosis, act as well to regulated the postembryonic development of the *Drosophila* nervous system. Chemosensory memory pathways express their own specific set of receptors for these hormones, which co-ordinate the tissue specific response. It is not known as to whether these hormones recall a program within the neurones and their precursors, or if they induce a program. It is only evident that during this process the proliferation of neuroblasts is greatly enhanced and several new neurones become recruited. During metamorphosis the combinatorial expression of hormone receptors first triggers the degradation of neural processes and subsequently promotes the differentiation again giving rise to the imaginal peripheral chemosensory and central nervous systems. These are finally responsive to gustatory and olfactory information.

CHAPTER 1
General Aspects of *Drosophila* Morphogenesis

In this thesis, I will not confine myself on reporting on the development of *Drosophila* a *Dipteran* insect species solely. Among the many insect species *Drosophila* is by far the most extensive studied insect but its compactness has put severe limits to following certain cellular interactions during development so that I will have to refer to the *Hymnopteran Apis mellifera*, the honey bee, as well as to the *Caeliferan Schistocerca*. *Diptera* and *Hymnoptera* belong to the long germ band type insects. Here the metameric germ band arises by partitions of the blastodermal space into segments without the necessity of cell divisions. *Caelifera* belong to the short germ band insects. Here the metameric germ band is generated by cellular divisions, which bud off processes from the short subterminal zone of the blastoderm (Krause, 1939; Anderson, 1972).

The embryonic development of *Drosophila melanogaster* starts with the formation of a syncytium, which is characterised by nuclei sharing a common cytoplasm. The nuclei, which are initially scattered across the cytoplasm migrate subsequently to the surface, where they form the cortex. By this time the pole cells segregate from the bulk of the syncytial nuclei and migrate to the posterior terminus of the embryo. At the end of the syncytial embryonic stage, the nuclei in the cortex cellularise to from the single layered blastoderm.

At the onset of the gastrula stage shortly after the formation of the cephalic furrow two epithelia arise: the germ layers. The inner layer, the mesoderm, is formed by the involution of cells along the midline. These cells are the precursors of the internal organs such as muscles and the centre part alimentary column (Fullilove et al., 1978). The outer layer, the ectoderm differs from the mesoderm by the expression of the segment polarity gene *engrailed*.

With the exception of the amnioserosa the entirety of cells form the germ band which is composed of gnathal, thoracic and abdominal segments. Shortly after gastrulation the germ band extends posteriorly and bends backwards so that the most poste-

rior end meets the head region of the embryo. During this process no further cells are added by divisions. The change in shape is rather the consequence of cellular rearrangements within the germ band. Cells along the dorsal ventral axis are reallocated along the anterior posterior axis causing a *de facto* stretching of the germ band (Hartenstein and Campos- Ortega, 1985). Forces catalysing this two hour long process are derived from changes of the intracellular adhesiveness in conjunction with the expression of gap genes. The cell intercalations observed during this process depend chiefly on the pair-rule gene *even-skipped* (Irvine and Wieschaus, 1994). The whole process is accompanied by the formation of parasegments dividing the epidermis of the germ band stage embryo into 14 grooves. Each parasegment consist of an anterior and a posterior compartment. These compartments which divide each parasegment into two regions are characterised by a distinct lineage, which becomes prominent during germ band extension (stage 10) (Lewis, 1979).

Prior to the completion of the germ band extension, a third layer the neuroectoderm delaminates from the ectoderm and inserts between the ectoderm and the mesoderm. As the germ band retracts several larval and imaginal structures are formed and the parasegmental pattern is lost and replaced by segments. Embryogenesis is completed 21 hours post fertilisation (at 25°C). The newly hatched first instar larva then feeds before it undergoes its first mould after one day followed by a second moult after a further day. The third instar larva growth tremendously over the following two days. Just prior to puparium formation it starts to wander with its anterior spiracles extended outward. The whole body contracts and a strong pupal case is formed by the larval epidermis. The cuticle remains white for the first four hours during pupal development (prepupal stage) and subsequently dehydrates displaying a brown colour. During metamorphosis larval structures are histolysed and replaced by imaginal structures. Four and a half hours afterwards the imago hatches.

CHAPTER 2
The Generation of Embryonic Fields

Before the actual neurogenesis begins, the embryo has already established a multitude of cells. Their fate is determined by positional information, which they have received during the early stages of embryonic development. Initially the pattern is founded by the uneven distribution of two morphogenes either along the anterior- posterior or the dorso-ventral axis. Both morphogenes mediate the transcriptional activation of several gene cascades. As the result of their activity a segmentally divided embryo emerges. At this time neuroblast arise in several independent domains along the anterior posterior axis probably as the consequence of the action of the dorso ventral gradient. Hence, the overlap of both anterior posterior and dorso ventral signals mediates the transcriptional activity of proneural genes. The structures into which the neuroderm becomes embedded reveals much about the symmetry of the nervous system. The head (cephalic) region is, however, an exception. Here the segmentation is much less evident owing to the lack of morphological markers, which separate each segment in the trunk. Only genetic markers have so far been used to elucidate the segmentation of this region. The research on the procephalic region is further hampered by the S-shaped configuration of the precephalic region.

1. Formation of the Cephalon

Three concepts play a major role in the formation of the cephalon prior to cellularisation into which later during development the brain becomes embedded. The generation of the anterior posterior axis during embryogenesis depends on the formation of a maternally supplied *BICOID* (*BCD*) gradient along the anterior- posterio axis of the embryo. Along this gradient *BCD* binds with different affinities the promoters of *BCD* inducible genes. High concentrations of *bicoid* activate genes, which contain low affinity promoters at the anterior pole. Low concentrations activate the promoters of high affinity genes in the posterior pole of the embryo. Genes with low affinity promoters

are thought to repress the genes with high affinity promoters at the anterior pole, so that a concentration dependent gene activation along the anterior posterior gradient is established. Along this gradient the reiterated segments are generated and segment specific homeobox genes are activated.

The second maternal system active to establish the anterior- posterior axis is the terminal system. The terminal system is ultimately responsible for the generation of the unsegmented acron at the anterior pole. As a representative of the maternal terminal effector genes the torso cascade antagonises the *BCD* mediated expression of gap genes and initiates the expression of the *TORSO* responsive gap genes. Thus, the *TORSO* signalling pathway has the task to limit the transcriptional activity anteriorly.

The third maternal system, the dorso ventral system is centred around the *DORSAL* morphogene. Its asymmetric nuclear distribution, following the binding of *SPÄTZLE* to the *TOLL* receptor, defines the dorso- ventral axis of the embryo. It seems that the dorsal morphogene acts in the cephalic region to mobilise co- repressor genes, which in turn modulate the transcriptional activity of several gap genes along the dorso- ventral axis.

1.1. Maternal Effector Genes

The *Bicoid* (*bcd*) Morphogene Generates the Anterior-Posterior Polarity in the *Drosophila* Embryo

I best start with the oogenesis in the mother because by then factors are supplied to the ovum, which as soon as the egg is laid guarantee the proper initiation of the developmental program. The maternal effect genes of particular interest for the formation of the anterior region are *bicoid* (*bcd*), *torso* (*tor*), *torso- like* (*tsl*) and *trunk* (*tr*). Whereas *bcd* is supplied as mRNA (Driever and Nüsslein- Vollhard, 1988a; Driever and Nüsslein- Volhard, 1988b), *Tor* (Casanova and Struhl, 1989), *Tsl* (Stevens et al., 1990; Martin et al., 1994) and *Tr* are probably secreted as proteins by specific follicle nurse cells in the mother. *bcd* mRNA is prelocalised to the anterior pole of the embryo generating a gradient of the *bcd* transcript (*BCD*) along the anterior- posterior axis of the embryo (Macdonald et al., 1993). It has been proposed that by binding to the stem like 3'UTR region of the *bcd* mRNA, the maternal *STAUFEN* protein may localise the *Bcd*

transcripts in a gradient along the anterior axis. The localisation presumably requires the interaction of the *bcd- STAUFEN* complex with microtubules (Ferrandon et al., 1994). *BCD* protein contains a highly conserved homeobox domain motif, which has been implicated to bind DNA at specific *bcd* boxes and to act as a transcription activator. Its activity depends on its phosphorylation state. Only dephosphorylated *BCD* activates the expression of the subordinate genes along the anterior posterior axis of the embryo in a concentration dependent fashion. The affinity of the *BCD* binding sites is correlated to the threshold concentration required for transcriptional activation (for review, see St. Johnston and Nüsslein- Vollhard, 1992).

Terminal Genes Demarcate the Anterior Cephalic Boundary

In the most anterior 10% of the embryo *BCD* fails to activate the head specific gap genes *hunchback* (*hb*) (Tautz et al., 1987; Tautz, 1988), *orthodenticle* (*otd*) (Finkelstein and Perrimon, 1990), and *empty spiracles* (*ems*) (Walldorf and Gehring, 1992). Here the maternal effector proteins come into action. In *tor*, *tsl* and *tr* loss-of-function mutations the acron fails to form and anterior- posterior ends fail to delineate. *tor* encodes a receptor tyrosine kinase, which is uniformly expressed on the plasma membrane of the cleavage stage ovarium. Its activation requires the presence of a ligand. This ligand is probably supplied by the action of the *tr* and *Tsl* gene products, as both act upstream of *tor*. *tsl* encodes a protein, which is co-localised with the *TOR* activity to the anterior 13% of the embryo. The N- terminus of *tsl* is strongly hydrophobic. A single cysteine residue has been implicated to mediate leucine zipper formation in this protein. Furthermore, a putative peptidase cleavage site has been identified indicative of putative enzymatic function, which may reside in the *TSL* protein. The specific function of this maternal effector is, however, still unknown (Martin et al., 1994).

At this point I have to refer to studies on mammalian cell culture signalling systems as here most of the studies, which have yielded the receptor tyrosine kinase model were undertaken (Biggs et al., 1994 and references therein). Upon activation by a ligand the RTK forms homodimers (Gómez-Skarmeta et al., 1993). Grb2 is an effector protein with a SH3- SH2- SH3 domain (SH means Src homology). The SH2 domain presum-

ably interacts with the phosphotyrosine of RTK. For most efficient binding an asparagine residue is required two amino acids downstream of the phosphotyrosine (Hunter, 1994; for review, see Pawson and Hunter (ed.), 1994). The SH3 domain of Grb2 is required for binding to *Son of sevenless* (*Sos*), which becomes allocated next to the plasma membrane. Shc or IRS-1 are probably phosphorylated in conjunction with Grb2. Shc is thought to link Grb2/Sos to the PTK. *SOS* acts as a GDP releasing factor, which catalyses the exchange of GDP for GTP in the G- protein Ras (Simon et al, 1991; Lu et al, 1993). The GTP bound stage of RAS is crucial for signal transduction as it interacts with the N- terminal domain of it substrate *Raf*. *Raf* then activates the serine/threonine kinase and mitogen activated protein kinase (MAP kinase) activator MEK. In the mammalian cell culture system MEP subsequently activates MAP.

In *Drosophila melanogaster* several homologues of the factors mediating the RTK have been identified. A substrate for phosphorylation by activated RTK is the phosphotyrosine protein *corkscrew* (*csw*) (Perkins et al., 1992). It encodes two SH2 modules, which promote interactions between *Tor* and the *D-RAF* protein a homologue to the mammalian Raf. *D- raf* is encoded by the *l(1) pole hole* gene (Nishida et al., 1988; Ambroso et al., 1989). Also present, but so far only identified in the *sevenless* pathway are the guanine nucleotide release factor *Son of sevenless* (*Sos*) and the Sem5/Grb2 homologue *Downstream of receptor kinase* (*Drk*) (Doyle and Bishop, 1993; Rogge et al., 1991; Olivier et al., 1993; Simon et al., 1993). The *Drosophila* homologues to MEK *Dsor1* and MAP *DmERKA* (which is encoded by the *rolled* locus) have recently cloned though their interactions have not been established (Tsuda et al, 1993; Biggs et al., 1994).

Thus, it might be conceivable that MAP is the enzyme of the *Tor* pathway, which is associated with the phosphorylation of early transcription factors such as *BCD*. Phosphorylated *BCD* may, hence, be rendered inactive at the anterior most pole of the *Drosophila* embryo the region, which is identical with the domain of *Tor* activity. In this case the head specific gap genes *btd, otd* and *ems* are not transcribed (Ronchi et al., 1993) in the anterior most 10% of the embryo the acron region.

Generation of the Dorsoventral Asymmetry in the Cephalic Region of *Drosophila* Embryos

Anterior posterior pattering only determines the second dimension of the embryo. The third dimension is added by the dorso ventral pattering system. The third dimension integrates the generation of cell diversity within each segment. Along the dorsoventral gradient the endoderm, the mesoderm, the neuroderm and finally the ectoderm are formed[2].

The generation of the dorso-ventral asymmetry depends on the ventral activation of the maternally supplied *TOLL* protein by the predicted ligand *Spätzle* protein (Roth et al., 1989; Schneider et al., 1994). Upon its activation (9 minutes postfertilisation) *TOLL* triggers downstream the recruitment of the *PELLE KINASE* protein. *PELLE* protein kinase has then the ability to phosphorylate the *Drosophila* NF-κB homologue *DORSAL* resulting in the dissociation of a complex formed by the *DORSAL* and its counterpart, the I- κB homologue, *CACTUS*. Once dissociated *DORSAL* enters the nucleus, where it activates the *DORSAL* responsive genes in a concentration dependent fashion.. Hence, the asymmetric activation of T1 induces a gradient resulting in the ventral distribution of the bHLH protein dorsal within the blastoderm stage nuclei (Rushlow et al. 1989; Steward, 1989; Jiang et al., 1992; Kidd et al., 1992; Shelton and Wasserman, 1993; Whalen and Steward, 1993). *Dl* binds specific DNA target sites, E- boxes in a concentration depended manner. By direct interactions with helix-loop-helix proteins of the achaete of scute complex it determines the expression of the putative transmembrane receptor *Rhomboid (Rho)* (Ip et al., 1992), which expression coincides with the lateral neuroectoderm formation. The expression of the *Rho* protein in this region is confined by the *Snail* (*Sna*) protein, which binds in competition with the high affinity *Dl* protein to the E-boxes in the mesoderm of the embryo and shuts down *Rho* transcription (Jiang and Levine, 1993; Gonzáles and Levine, 1993). The actions of *dl* in the head region have not been elucidated until now.

---

[2]At this point I wish to briefly mention the geometrical implications for embryogenesis. Taken that the embryo equals an hyperbolic each segment divides this hyperbolic into 17 discs along the anterior posterior axis. Each disc is sliced dorsoventrally into four levels (for calculations see appendix or Bronstein and Zhemendzayev, 1956).

1.2. Expression of Cephalon Specific Gap Genes in the *Drosophila* Embryo

As mentioned above *BICOID* activates several gap genes proportionally to its concentration and activity in the head (procephalic) region. The promoters of each gap gene have discrete affinities for a certain titre of *BICOID*. The domains defined by a certain titre of *BICOID* become later during development the segments. Here gap genes act to induce the expression of the segment polarity genes *engrailed* and *wingless* which define the segmental boundaries. Each domain along the *BICOID* concentration gradient is additionally characterised by the combinatorial expression and interaction of the gap genes, which confers upon each segment its distinct identity. In the procephalic region, the differential expression of gap genes during the syncytial blastoderm stage results in the establishment of six or seven head segment anlagen. The genes, which are thought to be involved in this processes and their possible interaction to define procephalon specific structures are described below:

- *huckebein (hkb)*

  The expression of *huckebein (hkb)* in the anterior most region is triggered by the *torso* pathway (Pignoni et al., 1990). It is assumed that *hkb* transcriptional activation, which is independent of *BICOID* is mediated by the activation of an unknown transcription factor as the result of an active *torso* signalling cascade (Steingrimsson et al., 1991). *hkb* is ultimately involved in the determination of the anterior and posterior limits for the midgut formation (Reuter and Leptin, 1994). Moreover, *hbk* is thought to control the formation of the frontal ganglion, the frontal commissure, the nervus frontalis, the nervus recurrents, the epiphysis, the dorso caudal pharyngeal and the pharyngeal monocolopidial chordotonal organ as well as the expression of the clypeolabral *engrailed* stripe in conjunction with the gap gene *crocodile* (Schmitt-Ott et al., 1993).

- *Crocodile*

  The *Crocodile* transcription factor which is transcribed in the domain of *hbk* expression is associated with the development of the ocular structures in the cephalic region of the embryo.

- *Tailless (Tll)*

  Its expression overlaps with *Tailless (tll)*, a member of the steroid receptor superfamily, is correlated with the formation of the cephalic region of the central nervous system. At the end of the cellular blastoderm stage it is restricted to two discrete dorsolateral patches anterior to the cephalic region. In the stage 8 embryo *tll* is accumulated in the region, which corresponds to the formation of the cephalic neurogenic region. Later, during stage 16 *tll* becomes confined to the optic lobe of the brain (Pignoni et al., 1990). In mutants for *tll* the frontal ganglion, the frontal commissure, the nervous frontalis, the nervous recurrens, the epiphysis cerebri, the dorsocaudal pharyngeal organ, the pharyngeal monoscolopidial chordotonal organ, the labial organ and the optic lobe are absent (Schmitt-Ott et al., 1993). *tll* down regulates the expression of *Krüppel* and *fushi tarazu* in the head region and, thus, represses the formation of parasegments.

- *Hunchback (hb)*

  Hunchback (hb) is spatially restricted to the anterior pole. It acts there to define the boundaries of the head and thorax region (Driever and Nüsslein-Volhard, 1989). *BCD* binds to five consensus binding sites upstream of the *hunchback* gene in a concentration dependent manner.

- *lätzchen*

  The *lätzchen* gene encodes a transcription factor with a *fork head* domain similar to that found in *sloppy-paired.* It has been localised to the procephalic neurogenic region and seems to be regulated by *BCD* (Häcker and Jäckle, 1993).

- *orthodenticle (otd)*

  The *otd* gene codes for a DNA binding protein of the homeobox class (Finkelstsin et al., 1990). The *OTD* protein first appears 2.5 hours post fertilisation during the formation of the cellular blastoderm in a broad circumferential stripe extending from 70-90% egg length (Finkelstein and Perrimon, 1990). The precephalic furrow forms posterior to the domain of *otd* expression. During the extended germ band stage *otd* transcription and expression is confined to the head region. Mutations in the *otd* gene result in disrupted labial, intercalary and gnathal segments. The Bolwig's organ, the optic lobe, the hypopharyngeal organ and probably the dorso-medial papilla are missing in mutations affecting the *otd* gene (Finkelstein et al., 1990). The expression of the segment polarity genes *engrailed* and *wingless* in preantennal and antennal regions is deleted in such mutants. Later during development *otd* is required for the formation of the CNS and the proper establishment of commisures.

- *empty spiracles (ems)*

  ems an other homeobox domain containing protein, appears slightly after the expression of *otd* (Finkelstein and Perrimon, 1990). The *ems* expression domain first overlaps with the deformed stripe of parasegments 0 and 1 which plays a crucial role in determining the identity of the maxillary segment. Subsequent *ems* expression is similar to the *hairy* and *even-skipped* expression domains. *ems* mutants lack *en* expression in the intercalary, the antennal and the preantennal region. In contrast to *bottonhead* mutants the expression of *en* is, however, retained. *ems* and *hunchback* interact either directly or indirectly to form the head anlagen such as the Antennal sense organ, the optic lobe and parts of the head exoskeleton.

- *bottonhead (btd)*

  The SpI like transcription factor *btd* is first expressed in a head stripe of the syncytial blastoderm stage embryo, where it determines the mandibular, intercalary and antennal stripes. Its expression overlaps anteriorly with *ems* and *otd*. During the

onset of gastrulation the *bicoid* stripe is localised at the anterior edge of the cephalic furrow, where is presumably interacts with *Knirps* to define the mandibular segment. In *btd* mutants the segmentation *engrailed* is absent in the intercalary mandibular and antennal segments. *btd* persists until the onset of germ band retraction (Wimmer et al., 1993).

- *Sloppy-paired.*

  The *sloppy-paired* (*slp*) locus consist of two transcription units encoding transcription factors with a fork head domain. Whereas, *slp2* does not have any noteworthy function during head development, *slp1* seems to be a key factor during this process. Its expression its initiated by the combinatorial interaction between *BCD* and the terminal *TORSO* pathway at the 5' terminal promoter in the anterior 30% of embryonic egg length during the syncytial blastoderm cycle 9. Soon afterwards the influence of the *TORSO* pathway seem the repress the transcription of *SLP1*. *SLP1* transcripts reappear at 71- 87% of the egg length, repressed dorsally by ems. Repressor activity of *ems* is presumably affected by the components of the dorsal morphogene cascade. Upon cellularisation during stage 5(2), the expression domain divides into an anterior cap and a posterior stripe. Amorph alleles of *slp1* particularly affect the outcome of head segmentation and fail to complete head invagination. As in the torso, the function of *slp1* is to regulate pair rule genes. The two paired stripes in the gnathal segment primorida are, hencewith, wider in mutants containing any amorph *slp1* alleles. The expression of segment polarity genes impaired as a consequence resulting in missing mandibular *engrailed* and *wingless* expression. The maxillary *engrailed* domain is, in contrast, expanded.

- *runt*

  runt, which encodes a transcription factor with a novel motif, antagonises the *BICOID* activation of *otd* and *ems*. The overexpression of *runt* shifts the *otd* band anteriorly and suppresses *ems* expression almost completely (Tsai and Gergen, 1994).

Implication of Gap Gene Expression for Head Segmentation

With regard to the co- expression with other genes, evidence suggest, that synergistic expression together with other gap genes defines six domains in the pregnathal domain of the head. *otd* expression seems to define the clypeolabral segment. The postulated preantennal segment is divide into an *otd/slp* domain and an *otd*, *slp* and *ems* domain. More posterior expression of *otd*, *slp*, *ems* and *btd* gives rise to the antennal segment. This is followed by the *slp*, *ems* and *btd* expressing intercalary segment. Thus, one may arrive at the view that the pregnathal region comprises altogether 5 distinct domains characterised by the differential expression of head specific gap genes. The preantennal segment is divided according to this scheme into two discrete regions. If this findings have any impact on the boundaries of head segmentation awaits, however, to be elucidated (Grossniklaus et al., 1994).

1.3. The Specification of Segments in the Cephalic Region

Although the procephalon is deprived of superficial grooves which serve as a landmark to define the boundaries of each segment, markers such as *EN*and *WG* may reveal where the segmental boundaries in the procephalic region are to be found. Unlike in the torso, segmentation of the procephalon does not involve the genes *even-skipped, fushi tarazu* and *hedgehog*, indicating that these genes may act to specify the epidermal morphology of the torso rather than the genetic boundary formation between each segment. Instead, it is assumed that in the procephalon *orthodenticle, empty spiracles* and *bottonhead* may either directly or indirectly stimulate the transcription of *engrailed* and *wingless.*

An elegant study by Schmitt- Ott and Technau (1992) correlated the formation of head segments to the domains of *EN* and *WG* expression. Indicative for early metameric units of the presumptive segments of the head region is the anti-*ENGRAILED* immunoreactivity (DiNardo et al., 1985; Diederich et al., 1991; Fleig, 1990, 1994). By embryonic stage 10, five centres of *EN* expression have been identified in the pregnathal (precephalic) regions in *Drosophila melanogaster*:

The *EN* antennal stripe, the *EN* antennal 'blob', the *EN* preantennal (ocular) 'blob' (which divides into the primary and secondary *EN* head 'blob' during stage 13/14), the *EN* intercalary 'blob', *EN* expression in the 'dorsal clypeolabral structure' (labrum) and *EN* expression in the clypeolabrum (Schmitt- Ott and Technau, 1992). The epidermal metameric units, as reflected by the *EN* expression patterns, are arranged in a S- shaped deviation (Technau and Campos- Ortega, 1985).

The most anterior marker is the *EN* labral 'blob'. In stage 10 it is based in the anterior dorsal region of the embryo. Ventrally the clypeolabral *EN* 'blob' is found. Both 'blobs' give rise to head skeletal structures and the forgut. More posteriorly in the anterior dorsal region the preantennal *EN* 'blob' is based destined to become a part of the brain. In between the preantennal and the ventral intercalary *EN* 'blob' the antennal *EN* 'blob' is inserted. The *EN* antennal stripe contributes cells to the antennal *EN* 'blob'. It delaminates during stage 10/11 and becomes a part of the dorsal organ. In mutants for *btd* both the antennal *EN* stripe and the antennal *EN* 'blob' are depleted. The ventral intercalary *EN* 'blob' forms the border between pregnathal and gnathal region. It probably gives rise to tritocerebral structures, as well as, the head skeleton. The intercalary *EN* 'blob' is followed dorsally by the discrete gnathal *EN* stripes: mandibulae, maxillae and labium.

The implications of these findings for the configuration of the pregnathal neuromeres will be discussed at later point.

The notion that the pregnathal region in *mandibulates* comprises four segments as advocated by Schmitt- Ott and Technau (1992) is not shared by other developmental biologists. Strikingly, in the first study on this subject, Diederich and collaborators (1991) failed to detect the preantennal (ocular) segment at all. Comparative studies on *Apis mellifera* and *Leptinotarsa decemlineata* embryos (Fleig, 1990; 1994) confirmed the notion that the number of segments in the gnathal and pregnathal region amounts to six. *Apis* embryos which as *Drosophila* embryos belong to the long germ band type are excluded form head invagination. Moreover, seen in proportion, the onset of segmentation in *Apis* is earlier as in *Drosophila* (Krause, 1939). In *Apis* and *Leptinotarsa* the head does not curve dorsally so that each *EN* 'blob' can be assigned more easily to

each segment. Particularly disputed is the assignment of the anterior most, the dorsal clypeolabral segment, as a segment of the pregnathal region. As Fleig (1994) pointed out this *EN* 'blob' may be identical with the non segmental acron and is therefore not thought to belong to the pregnathal region. In *Leptinotarsa* a rudimentary seventh segmental anlage is amalgamated with the clypeolabrum which led Fleig (1994) to believe that the proposed preantennal segment forms a single segment with the clypeolabrum. Additionally, anti *WG* immunoreactivity could help to demarcate the boundaries of each segment (Baker, 1987; Martínez- Arias et al., 1988; van den Heuvel et al., 1989).

2. Segmentation in Thorax and Abdomen

In the cephalon the seven segment specific gap genes either directly activate the expression of the segment polarity genes *engrailed* and *wingless* or they act through head specific pair rule genes. Therefore, I here refer to the segmentation process in the well studied thoracic and abdominal regions. Studies on the abdominal region have the advantage that owing to the well defined, symmetrical arrangement of the segments, the processes underlying the formation of segmental boundaries is far better defined than the process in the procephalic region. The process of segmentation in the cuticle is of cardinal importance to understand the arrangement of the underlying nervous system as the positioning of neuroblasts and their progeny follows exactly a segmentally reiterated pattern. Thus, neurogenesis and segmentation during gastrulation and germ band extension proceed synergistically.

The parasegments of the thorax and abdomen *Drosophila* embryo become apparent when, during cell cycle 14, the interactions between gap genes and maternal effect genes leads to the expression of the pair- rule genes. Pair- rule genes determine the periodic pattern of the in thorax and abdomen of the embryo before at the end of the blastoderm stage prior to the cellularisation.

One distinguishes three types of pair rule genes according to their chronological order of expression. Following the thirteen's cell division cycle the primary pair rule genes appear. The dynamic expression of *runt*, *hairy*, and *even-skipped* results in the

demarcation of parasegments. Their expression is strictly depended on the ability of several gap genes either to activate or repress their regulatory domains. Following this initial activation of the primary pair rule genes they expression becomes stabilised and results in the activation of the secondary pair rule genes. In between the gradients, which determine the expression of the primary pair rule genes subsequent *fushi tarazu* stripes become intercalated at position predictive of odd numbered segments. Finally, pair rule genes as *sloppy-paired, naked,* and *paired* mediate the expression of segment polarity genes which determine the differentiation of each segment along regulatory gradients. Each parasegment is divided into an anterior *engrailed* (*en*) expression domain and a posterior *wingless* (*wg*) expression domain. The *EN* stripe determines the anterior posterior boundary of each segment. *wg* in contrast mediates the formation of the *naked* cuticle in the posterior region of each segment. Both domains are tightly regulates by several signalling cascades.

2.1 Pair-Rule Segmentation Genes Determine the Boundaries of the Parasegments

Initiation of Pair-Rule Gene Expression

The expression of gap genes proceeds in an irregular pattern, when compared to the subsequent periodic expression of pair rule and segment polarity type segmentation genes. The pattern laid down by the gap genes becomes elaborated when during cell cycle 12 the interaction between gap genes and maternal effector genes results in the activation of pair rule genes (Frasch and Levine, 1987). Initially, the genes *hairy* and *fushi-tarazu* are called into action. Following the appearance of their transcripts during cleavage stage 12 within aperiodic and broad domains, their transcription pattern soon resolves to well defined bands. Whereas the 7 *hairy* stripes become confined to the predictive odd numbered parasegments, each of the *fushi tarazu* (*ftz*)stripes becomes intercalated between these *hairy* stripes. These 2-3 wide *ftz* bands are predictive of the odd numbered parasegments, which are subsequently to be found in the gastrula stage embryo. It appears that the 6th stripe of *hairy* is jointly regulated by the repressor gene *krüppel* and the activator *knirps.* Both genes control *hairy* expression by binding to the

response *hairy* promoter. The ratio of occupancy of this promoter by either *KRÜPPEL* or *KNIRPS*., determines if *hairy* transcription is induced or repressed (Pankratz et al., 1989).

Using mutant analysis, it has been demonstrated that the expression of *ftz* is negatively regulated by the *hairy* encoded bHLH protein. Hencewith, the domain of *ftz* expression is significantly expanded (Ish- Horowicz and Pinchin, 1989). Additionally, ectopic expression of *hairy* from an heat shock promoter results in the extinction of *ftz* in early cycle 14 embryos (Carroll et al., 1986)[3]. The regulatory function of *hairy* resides in the maternally supplied transducin- like protein *GROUCHO* (*GRO*). *GRO* has been advocated to interact with the WPRW motif of the *HARIY* protein rendering it active (Paroush et al., 1994).

Similar opposing effects as those observed for the interaction between *hairy* and *ftz* appear also to be employed for the regulation of the primary pair rule genes *even-skipped* (*eve*)[4] and *runt*.. As for *ftz* the margins of *eve* gene expression are controlled by anterior and posterior terminal systems (Klingler and Gergen, 1993). Determinative for the expression of *eve* are firstly the capacity of its enhancer to enable differential binding of regulatory proteins, and secondarily the availability of these proteins in the different regions of the embryo (Stanojevic et al., 1989,1991; Small 1991, 1992). The outcome of *ftz* and *eve* activation is an metameric pattern of alternating stripes, whereby *ftz* expression appears in odd numbered segments, whilst *eve* is found in even numbered segment (Gaul and Jäckle, 1989). Furthermore, is has been demonstrated that *runt*, a transcription factor containing a novel motif antagonises the expression both *hairy* and *eve* in even numbered segments.

In mutants for *runt eve* appears broadly, whereas in embryos, where *runt* is ectopically expressed *eve* and *hairy* transcription are lost (Tsai and Gergen, 1994).

---

[3]Additionally, the secondary pair rule gene *fushi tarazu* seems to be regulated by the *FTZ*-F1a steroid receptor isoform, which binds specifically to regulatory sequences flanking the *fushi tarazu* gene (F1RE) probably in conjunction with another transcription factor (Ueda et al., 1990; Lavorgana et al., 1993; Ohno et al., 1994).

[4] This classification refers to the temporal expression of the pair- rule genes. With regard to their interaction with *runt* and *hairy*, *eve* has been classified as a primary pair rule gene, whereas, *ftz* as a secondary gap gene. *eve*, *runt* and *hairy* pattering are not altered in *ftz* and prd embryos! (Frasch and Levine, 1987)

Activation of Segment Polarity Genes

Combinatorial interactions between secondary and tertiary pair- rule genes, is subsequently required to lay out the metameric parasegmental pattern of germ band embryos. Parasegments comprise an anterior *wingless* (*wg*) and coinciding with the parasegmental boundary, an posterior *engrailed* (*en*) compartment. It has been proposed that the initiation of *en* transcription requires the activity of the more superior pair-rule gene *ftz* in odd numbered segments and *eve* in even numbered segments (Levine and Hardin, 1989). Consistent with this hypothesis is that at the anterior expression boundary of either genes directly overlaps with each of the 14 germ band stripes of *en* (Ingham et al., 1988; Ish- Horowicz et al., 1989; Manoukian and Krause, 1992. Furthermore, the inhibitory function of *odd-skipped* (*odd*) restricts the expression of *en* within each of the 7 *ftz* stripes to the most anterior *ftz* positive cells.

On the other hand, it has been postulated that the *eve* or *ftz* expression domains define the anterior boundary of the *wg* domain in the anterior compartment of each parasegment (Levine and Hardin, 1989). In addition, the combinatorial interactions between the three transcription factors *odd-paired* (*opa*), *paired* (*prd*), and *sloppy-paired* (*slp*) demarcate the *wg* compartment. The zinc finger DNA binding protein *OPA* and its respective transcripts are found within all segmental primordia (Benedyk et al., 1994). Its task to induce *wg* gene activation within specific compartments requires its spatially restricted activation by upstream second messenger cascades, presumably involving the exterior matrix protein *TENASCIN*^{m} (*TEN*^{m}). It is thought that *TEN*^{m} initiates the signal transduction cascade by binding via its 6 EGF to a putative transmembrane receptor resulting in the downstream activation of *opa* (Baumgartner et al., 1994). By contrast, the fibronectin- like domain might interact with syndecans (Spring et al., 1994).

Also determining the *wg* compartment is the fork head domain containing transcription factor *slp*, which in blastula stage embryos was already recruited to act as a gap gene. Rather than being involved in initiating the expression of *wg*, it has been proposed that *slp* is involved in the maintenance of *wg* expression (Cadigan et al., 1993). By contrast, the expression of *hedgehog*, a further segment polarity genes, is antagonised by *slp* in the anterior region of the posterior *en* compartment. Moreover, as *HH*

is absent in mutants for *eve*, it is thought to be positively regulated by this pair rule gene (Ingham and Hidalgo, 1993). Paradoxically, more posteriorly, at the parasegmental boundary, *slp* seems to mediated the expression of *en* (Cadigan et al., 1994). Evidence suggests that the class three pair rule gene *naked* (*nkd*) suppresses *en* expression in the anterior *wg* compartment, where it promotes the foundation of the naked cuticle (DiNardo et al., 1988). Last but probably not least, a further factor with homology to tenascins (striking homology to TEN^{m}) is encoded by the *odd oz* gene. This transmembrane protein presumably mediates the activation of segment polarity gene. Strikingly, it is the last gene in the pair-rule hierarchy that has been found so far (Levine et al., 1994).

2.2. Specification of Parasegments by Segment Polarity Genes

Determination of the Posterior *engrailed* Domain

WG specifies the development of the naked cuticle for the mutant phenotype of *WG* exhibits that the ventral cuticle is completely covered with denticles (Cabrera et al., 1987). When ectopically expressed *WG* is able to retrieve the mutant phenotype when activated from mid stage 7 to early stage 9. This ectopic *wg* expression causes a mirror like duplication in the region encompassing the each parasegmental groove. Following its activation by *PRD* the *WG* expression is maintained by an autoregulatory loop. Initially this autoregulatory loop is independent of *GOOSEBERRY DISTAL* (*GSB-D*). Instead genes such as *porcupine* (*porc*), *dishevelled* (*dsh*), *fused* (*fu*), *smoothened* (*smo*), and *cubitus interruptus* (*ci*) mediate the autocrine *wg* self regulation (Hooper, 1994). Later the autoregulatory loop involves *gsb-d* (Li and Noll, 1993; Zhang et al., 1994). *GSB-D* expression overlaps with the *WG* stripe in the posterior half and with the anterior most *EN* stripe(Gutjahr et al., 1993). For ectopic *GSB-D* expression causes mirror like duplications, which encompass each parasegmental border, it is thought that *GSB-D* controls the *EN* and *WG* expression, which in turn respecify the anterior portion of the segment (Zhang et al., 1994).

Furthermore, it has been established that in mutants for the pair rule gene *slp GSB-D* expression declines following the reduction in *wg* transcription. Another transcription factor *ETS-2* is co-expressed in ectoderm anterior to parasegmental grooves which overlaps with the domain of *WG* expression suggesting that *ETS-2* is involved in the *wg* pathway (Chen et al., 1992).

As *WG* is secreted extracellularly it needs to be exocytosed. It was proposed that a factor encoded by the *porc* locus aids this process. This notion results from the observation whereafter *WG* is retained within the cell *porc* mutant embryos (Siegfried et al., 1994). Once secreted *WG* acts either in an autocrine or paracrine mode forming a gradient across each parasegment. Concentrations of *WG* in the posterior region of each parasegment are high, whereas titres in the anterior region are low (Sampedro et al., 1993). In the posterior region it presumably binds its own receptor and maintains the expression of *EN*. (There is even considerable evidence that *WG* may bind discrete extracellular matrix domains of the *NOTCH* transmembrane receptor (Couso and Martinez-Arias, 1994)).

Once *WG* has bound its receptor downstrean responses may either be linear or co-linear. Mutant studies indicate that the first intracellular response following:
DSH is thought to mediate the first downstream response following the binding of *WG* to its receptor (Noodermeer et al., 1994; Theissen et al., 1994). Two possible functions are conceivable: From its sequence homology with cell junction like proteins, one may arrive at the conclusion that *dsh* is closely associated with cell junctions. *DSH* may, hencewith, be required for the formation of junctions between cells of different segments at the *EN* border. *WG* would subsequently stimulate the synthesis or enzymatic activation of *DSH*. Alternatively, one may suppose that *WG* could mediate the allocation, relocation or dislocation of *DSH* at the membrane. Either of these processes may be closely correlated to the accumulation of intracellular *ARMADILLO (ARM)* on the cell membranes (Riggleman et al., 1990). One may envisage that this proceeds as follows: Upon activation by the *WG* signal, *DSH* may mediate the contacts between i.e. integrins on the cell membrane that then signal further downstream to inactivate *ZESTE WHITE 3/ SHAGGY KINASE (ZW3)* (Ruel et al., 1993).

The second proposal implies that *DSH* may be a component of a direct signalling cascade. Downstream of *DSH ZW3*, subsequently, transduces the *WG* signal: While in absence of a *WG* signal *ZW3* is active, the arrival of the *WG* signal renders *ZW3* inactive (Diaz- Bejumea and Cohen, 1994).

A possible substrate for *ZW3* is the adhesions junction protein *ARM*.. From cell culture experiments it is evident that *ARM* — the *Drosophila* β- cadherin homologue — interacts with *Drosophila* α-catenin (*Dro* α-catenin) to form Ca^{2+} dependent cell aggregates (Oda et al., 1994). Two models have been proposed to explain the action of *ZW3*. The first model implies that in absence of *WG ARM* is concentrated at cell adhesions junctions. Active *ZW3* then destabilises cytoplasmic *ARM*. The *WG* signal inactivates *ZW3*, which in turn stabilises cytoplasmic *ARM* enabling it to form new adhesions junctions (Riggleman et al. 1990). The second model supposes that catalytic *ZW3* function stabilises *ARM* at the cell adhesions junctions. The *WG* signal then inactivates *ZW3* yielding the degeneration of *ARM* at cell adhesions junctions (Peifer et al., 1994; Siegfried et al., 1994). The assembly or disassembly of cell adhesions junction are an important manifestation of the regional regrouping of cells during germ band retraction. It is highly likely that cells receiving the *wg* signal socialise with others as in mutants for *ARM* the *wg* signalling is impaired indicative of an detachment of cells from their tissue. The stabilisation of cellular complexes may, thus, result in the maintenance of autoregulatory *EN* loop. The autoregulatory *WG* loop, in contrast, does not seem to be involve either *ZW3* or *ARM* (Hooper, 1994).

Determination of the *Wingless* Domain

The domain of *HH* expression identifies with the domains of *EN* expression. The *hh* gene encodes as the *wg* gene a secreted protein. In contrast to *WG* which triggers responses over a distance *HH* acts only locally.

In cells anterior and posterior to its expression domain *HH* enhances the expression of *PATCHED (PTC)*, which shows striking identity to transmembrane proteins with several spanning domains (Hooper and Scott, 1989; Nakano et al., 1989). *PTC* is co- expressed with the integrins PS1 and PS2 in the mesoderm (Leptin et al., 1989) and seems

to be a component of cell adhesions junctions. The fact that the *PTC* protein is endocytosed in vesicles together with *WG* indicates that it may intercept the *WG* protein in *WG* competent cells (Capdeila et al., 1994)[5].In the anterior region *HH* determines the expansion of *WG* expression boundaries presumably by acting through *FUSED KINASE* (*FU*) (Ingham, 1993; Ingham and Hidalgo, 1993; Préat et al., 1990). *FU* may inhibit the suppressor of *wg* transcription *COSTAL- 2* by interacting with *SUPPRESSOR OF FUSED* (*SU(FU)*) gene product which is an activator of *COSTAL-2* (Préat et al., 1993; Forbes and Ingham, 1994). *fU* seems also to be positively regulated by *NKD* (Limbourg- Bouchon et al., 1991). Hence, it seems likely that the different responses to *HH* are mediated by *COSTAL-2*. So it has been proposed that in the anterior regions *COSTAL-2* is activated by *PTC*, which limits the domain of *WG* expression (Préat et al., 1993).

In essence, the regions anterior and posterior to the domain of *HH* expression have different affinities for *HH*, which ultimately regulates the propagation of *WG* expression. High *WG* titres in the posterior region trigger the formation of a seal for its own propagation at the parasegmental boundary anterior to the *EN* stripe. The formation of this seal probably involves the creation of cell adhesions junctions by *PTC*, *DSH* or *ARM*. As shown above ectopic *WG* expression is able to break this seal and to propagate posterior to it creating a mirror like duplication in the region encompassing the parasegmental boundary (Sampedro et al., 1993). In the region anterior to its expression *WG* is distributed along a gradient, which is presumably controlled by *PTC* through *COSTAL-2* or *PTC* activated endocytosis. *HH* is able to either maintain *WG* expression in the posterior region of each parasegment or *PTC* expression in both regions of the parasegment. In the anterior region of each parasegment *COSTAL-2* represses *HH* dependent activation of *WG* through *FU*. *FU* might not necessarily be involved in the deregulation of *PTC*, which in turn is able to control the titres of excreted *WG* in these cells limiting the action of *WG* to the posterior region of each segment (Ingham and Hidalgo, 1993, Ingham, 1993).

---

[5] Yet, conflicting evidence suggests that hh may act as an cleavage factor that upon secretion enzymatically cleaves *ptc*, which in turn becomes endocytosed. This would then lead to enhanced transcription of *ptc* (Ingham pers. comm.)

Alternatively, *PTC* may act as a cell adhesion molecule, which assembles in junctional complexes together with *ARM* and *DSH* at the anterior border of each parasegment as long *WG* is present. Once, *WG* is present, *ptc* may dissociate from the membrane and activate *COSTAL-2* inhibiting further activation of *WG* by *HH* by activating endocytosis (Campdevilla et al., 1994).

CHAPTER 3

The Establishment of Morphogenic Fields: Foundation of the Central Nervous System

The ground plan of the *Drosophila melanogaster* central nervous system is generated in a precisely co-ordinated series of events. Unlike in type I and type II embryos the neuroderm in *Drosophila* arises at different predetermined unconnected positions along the anterior-posterior axis in response to information derived from dorso-ventral gradients. The mechanisms of neurogenesis are the same for the entire neuroderm and are redeployed during later neurogenic events, as well. Neurodermal cells are recruited from common progenitors of both the ectoderm and neuroderm — the region which has the potential to adopt neural fate is called neuroectoderm. As revealed by transplantation studies the ectodermal region outside the neuroectoderm has diverged before neurogenesis and these cells cannot adopt neural fate when under influence of neuroectodermal information. It is not thought that ectodermal and neuroectodermal cells are derived from a common lineage. It is rather their exposure to postional information prior, during and following the cellular blastoderm stage, which renders the syncytial nuclei of the *Drosophila* embryo germ layer specific. Hence, it is the position into which the syncytial nuclei during the blastoderm stage migrate that determines their fate. Thus, the neuroderm is a germ layer in which cells are incoherently founded in response to a spatially and temporal coinciding information.

Though all neuroectodermal cells have the potential to become neuroblasts, again only a specific set of stereotyped positionally prefigured cells finally delaminated as neuroblasts. This process is again gradient specific whereby the putative neuroblast gradually alters the expression equilibrium of genes, which act as protagonist or antagonists during neural differentiation. Proneural genes are thereby the protagonists, whereas neurogenic genes are the antagonists. The shift towards proneural gene expression finally results in the detachment from the neuroectoderm and the associated signals and in the progression with neural differentiation.

1. Cytology of *Drosophila melanogaster* Neurogenesis

1.1. Overview of Cellular Interactions during the Process of Neurogenesis

The ground plan of the insect body reveals that the body is organised into three parts: The cephalon, the thorax, and the abdomen. Each of the three parts is subdivided into segments. Eight abdominal and three thoracic segments can easily be distinguished. The segmentation of the head is more complex. There are genuinely considered to be three gnathal segments: the mandibular, the maxillary and the labial segment. The number and organisation of the pregnathal segments is, however disputed. Recent findings in the embryos of several insect species suggest that the pregnathal region comprises three or four segments: a labial, a preantennal [ocular]?, an antennal and an intercalary, which are arranged in S-shape.

Each of the segments is divided along the midline into two hemisegments. The nervous system follows the epidermal segmentation pattern. Each segment contains a reiterated set of neural cells, which varies from segment to segment. The entirety of neural cells in each segment is called neuromere. During germ band extension neuroblasts delaminate from distinct regions of the ectoderm. The identity of each segregating neuroblast is thereby subordinate to the ectodermal segmentation process. The segment specific differentiation of each neuromere is correlated with the expression of homeotic genes e.g. *Ultrabithorax*. (for review, see Goodman and Doe, 1993). The neuroectoderm comprises three components differing from one another in terms of their location and a specific pattern of gene expression:

The ventral neuroectoderm generates the ventral nerve cord and the suboesophageal ganglion. The dorsal neuroectoderm forms the midline and the procephalic neuroectoderm gives rise to the cerebral hemispheres.

Cells of the three neurogenic anlagen have the potential to either become ectodermal cells, nerve or glial cells. This process proceeds in three steps. Firstly, ectodermal cells acquire the potential to adapt neural fate. Secondly, once a neuroblast is isolated it sends in the third step inhibitory signals to the remaining ectodermal cells. With the

delamination of the neuroblast a new germ layer is formed: the neuroderm, which is sandwiched between the outer ectodermal layer and the inner mesodermal layer (For review see Campos Ortega, 1993).

Cells of the ventral neuroderm are arranged in a ventral bilateral symmetric pattern with the dorsal neuroderm arranged dorso-medially. Each abdominal neuromeres comprises about 30 neuroblasts including glial cell generating glioblast. Eight further cells are seen in the segmental midline cluster.

Three types of precursor cells constitute the insect central nervous system. Glioblasts are the founder cells of glia cells which give structural and logistical support to neurones. Neurones themselves arise from neuroblasts by a series of asymmetric divisions each generating a neuroblast and a ganglion mother cell. The ganglion mother cell then generates during a symmetric cell division two sibling neurones. Neuroglioblasts have the potential to either adopt a neural or a glial fate (Udolph et al., 1993).

1.2. Cellular Systematics of the Ventral Neuroectoderm of *Drosophila melanogaster*

Neuroblasts delaminate in five consecutive pulses from the neuroectoderm in the ventral neuroectoderm. With the start of stage 9 the first pulse S1 generates ten primary neuroblasts from proneural clusters of 5-7 cells (Skeath et al., 1994). The positions in a hemisegment where neuroblasts are formed are best described in an orthogonal matrix, whereby each variable demarcates the putative position of a neuroblast. The matrix comprises 4 longitudinal rows (1-4) and three vertical columns (median [m], intermediate [i] and lateral [l]). Three neuroblasts NB 2-2 [m], 3-2 [i], 2-5 [l] are found in the first row. The second row comprises only the NB MP-2 [m] and NB 3-5 [l], whereas in row three NB 5-2 [m], 5-3 [i] and 5-6 [l] are found. The last row again contains only two NB: 7-1 [m] and 7-4 [l]. With the next generation of SII NBs the symmetric arrangement is lost. I therefore refer the reader to figure which demarcates the positions of the SII and SIII neuroblasts. As during pulse SIV and SV the pattern becomes more chaotic, no group has attempted to trace the lineage of neurones arising from these neu-

roblasts. Each NB forms a discrete lineage in the neuroectoderm (for review see, Goodman and Doe, 1993). The origin of the following identified neurones is given in table 1.:

Neuroblast; Glioblast	Ganglion Mother Cell	Neurone/Sibling Neurone; Gl
NB1-1	?	aCC, pCC
GB-1		A-Glia, 2× B-Glia
MP-1	?	MP-1
?	?	RP-1, RP-3
NB4-2	GMC-1	RP-2/sib

Table 1. Known lineages of neuroblasts in the ventral nervous system of *Drosophila*

2. Genetic Circuitry of Neurogenesis

This neuroblast formation in the ventral neuroectoderm follows the same principles as neurogenesis in the procephalic region. Owing to the symmetrical arrangement of the ventral neuroectodermal region, immunocytological studies are able to identify the laws governing this process much better than in the deflected procephalic neuroectodermal region. The formation of the neuroectoderm is thought to be initiated by dorso- ventral gradients. Their coincidental expression together with genes, which are involved in the formation of the anterior- posterior gradient initiate the expression of proneural genes. These proneural genes confer upon distinct domains of cells the potential to adopt a neural lineage. Antagonising the neural differentiation are the neurogenic genes. Neurogenic genes act to confine the region of cells, which in fact adopt neural identity by preventing the remaining cells to detach form the neuroectoderm, so that they still remain responsive to neuroectoderm specific signals. In the delaminated neuroblast which has become detached form these signals, an equilibrium between proneural and neurogenic gene expression is established. A shift in this equilibrium towards the preferential expression of proneural genes triggers the activation of a neural specific program in the neuroblast. Its identity is thereby directly correlated to segment polarity signals it receives form the superior ectoderm. In response to the combinatorial action of these signals neuroblast specific genes are expressed, which regulate the identity of the neuroblasts. Furthermore, they determine the differentiation of these neuroblasts to

fully functional neurones. In summary, neurogenesis seems to follow a certain hierarchy whereby the positional information derived from the coincidental expression of dorso- ventral and anterio-posterior gradient genes is translated into a program, which defines the both specificity and identity of neural precursor cells.

There are several circuits involved some of which have been better studied than others. To date, only the interactions between the *Notch* and the *Sevenless* pathway have been established. No further information is available about the interaction of other pathways.

2.1. Dorso Ventral Morphogene Activation of Proneural Genes

Consequent to the formation of the dorsoventral gradient by *Dl* during the early blastoderm stage, the *SNA* protein defines the mesoderm, where it is thought to repress the transcription of proneural genes such as *single minded* (*sim*), *achaete-scute complex* (*AS-C*) and *rhomboid.* Conversely, *sna* expression is subsequently found in the neuroectoderm following a pair rule pattern. During the delamination of SI neuroblasts *sna* expression becomes confined to three rows of neuroblasts. The *sna* transcripts then appear in the GMC and the neural lineage of these neuroblasts. It further has been established that the *sna* downstream regulatory region comprises elements, which are specific to mesodermal expression on the one hand and CNS or PNS expression on the other hand (Ip et al., 1994). As revealed by deletion studies, the neuroderm *sna* upstream regulatory elements can be further divided into discrete CNS and PNS specific regions as revealed by deletion studies. The CNS specific element extends from 2.8 to 2.2 kb upstream the *sna* gene whereas the PNS specific region lies 0.9 -0.2 kb upstream of the *sna* gene. In terms of *sna* mRNA splicing and *SNA* protein stability Ip and co-workers suggested (1994) that *sna* might, additionally, be regulated posttranscriptionally during the different stages of neural differentiation. The differential expression of *sna* depends on coinciding dorsoventral and anterioposterior transcriptional activators along the embryonic axes. *sna* itself does, however, not to be a cardinal factor for the development of the neuroectoderm as in mutants for this gene the develop-

ment of the nervous system proceeds phenotypically normal. It has, thus, been suggested that other related Zn- finger transcription factors such as *scratch* are necessary for the proper development of the nervous system.

The *NK-2* homeobox binding protein is initially expressed in a latitudinal stripe of 7 cells width extending from 0- 90% egg length in stage 4 and 5 embryos. The activation of the *NK-2* the depends on the *dsl* protein. A putative *dsl* binding site has been identified in the 5' flanking region of the *NK-2* gene. The nuclei in which *NK-2* is expressed all adopt neurodermal lineage later during development. Additionally, a *Sna* binding site occurs in the 5' upstream region. These show a considerable overlap to at least twenty high affinity and thirteen low affinity binding sites for *NK-2* proper. It is thought that *Sna* represses the transcription of *NK-2* in the mesoderm (Wang et al., 1993). Once, *NK-2* has been activated by *dsl*, its expression is possibly maintained in an autoregulatory loop as long as the expression of *sna* and the mesoderm specific gene *twist* do not interfere with its expression (Nakayama et al., 1993).

2.2. Characterisation of the *Achaete-Scute Complex* Genes

Coincidental expression of pair rule genes and dorso-ventral gradient genes also determine the spatially restricted expression of proneural genes of the *achaete-scute complex* (*AS-C*). *AS-C* genes account for the development of 50% of neuroblast lineages. The expression of the *AS-C* genes confers upon a stereotyped array of neuroectodermal cells the potential to segregate and to adopt a neurodermal lineage. Three of the genes, namely *achaete* (*ac*), *scute* (*sc*) and lethal of scute (*l'sc*) encode just as many discrete transcripts, T5, T4, and T3, respectively, which are arranged sequentially in this locus. A fourth transcription unit *asense,* is not involved in the early neurogenesis (for review, see Cabrera, 1992). Each of the *AS-C* gene products contains a bHLH motive involved in transcriptional activation.

Here, I have to make a brief interlude explaining the geometry of the differential gene expression of neuroectodermal cells. Skeath and colleagues (1992) have made the attempt to allocate prospective neurodermal cells to a co-ordinate within a grid accord-

ing to their differential expression of the *AS-C* genes. Most obvious is that neuroectodermal cells occur in clusters of 5- 7. Three cell clusters are found in each odd numbered vertical row encompassing each hemisegment. The even numbered rows contain only two clusters, whereby the intermediate cluster is absent. Hence, the median and the lateral rows comprise four columns, the intermediate row only two. All cells of the clusters within the odd numbered columns express *l'sc* during stage 8. By stage 9 this expression is reduced to the central most cells within each cluster and is absent in the lateral cluster of the second row. Clusters within the fourth row co-express all three genes during stage 8. Clusters of the second row share the expression of *ac* and *sc*. By stage 9 the expression of both genes is, as well, restricted to the central most cells in the fourth row and in the second row, first column.

Juxtaposed between *ac* and *sc* lies a cis acting regulatory region, which specifies the spatially regulated expression of both genes. This regulatory region contains at least two regulatory elements as identified by mutational analysis. The element 3' proximal to *ac* is decisive for *ac* and *sc* activation in the second row. The 3' distal element determines the expression of both genes in the fourth row (Skeath et al., 1992). The expression of the *l'sc* T3 transcript is driven by five cis acting control elements. It is not known yet as to whether the 5' regions are shared with *ac* or *sc* or if *l'sc* acts on its own to stimulate the expression of *ac* and *sc*. Intriguingly, the latter option was found to hold true for activation of *ac* expression in NB 7-1 (row 4) neuroblasts. However, it needs to be emphasised the transcription of the nuclear protein *l'sc* generally overlaps with the expression of *ac* and *sc* only in a few clusters so that this option can be neglected. Cis acting regulatory elements, which initiate *l'sc* expression belong predominantly to the class of basic helix loop helix protein binding sites. Only one *sna* type E-box has so far been identified in the *AS-C* cluster to encourage binding of bHLH proteins (Martín-Bermudo et al., 1993).

2.3. The Regulation of *Achaete-Scute Complex* Genes

The activation of *ac* and *sc* is mediated by the combinatorial action of pair rule genes and auxiliary genes such as *ventral nervous system condensation defective* (*vnd*) and *extramicroachaete*. There is a strong correlation between the domains of expression of superior pair rule genes and transcriptional activity of *ac*. Mutations in *ftz*, *odd-skipped* (*odd*), *prd* and *eve* impair the activation of *ac* in row 4. *odd-paired (opa)* , *slp*, and *prd* determine the expression of *ac* in row 2. At detailed look at *ac* expression in row 4 of odd numbered segments reveals that the expression domains of *ftz* and *ac* are identical. That of *opa* is, in contrast, conterminous with the *ac* expression domain, suggesting that *ftz* activates *ac* expression whereas *opa* limits the expression of *ac*. In even numbered segments the activation of *ac* is attributed to the coincidental expression of the superior *prd* and *eve* genes.

It is not known as to whether pair rule genes activate the expression of *AS-C* genes directly. They may either form heterodimers with other transcription factors or activate an array of secondary transcription factors, which in turn regulate the *AS-C* genes. Positive and negative of those secondary regulatory transcription factors have been identified. The proneural gene *vnd* is a positive regulatory transcription factor, which requires for the spatially restricted transcriptional activation of *ac*, *sc* and *l'sc* the formation of homo- or heterodimers. It has been demostrated that *vnd* is expressed in the medial column of rows 2 and 4. In mutant alleles for *vnd*, however, abolish the expression of *ac* and *sc* in SI NBs MP2 and NB 7-1, indicating that *vnd* is required for the activation of *ac* and *sc* expression in this region. Additionally, *vnd* seems to be required for initiation of *l'sc* expression in the lateral column of row 4 where the NB 7-4 arises.

The *extramacroachaete* (*emc*) gene is a negative regulator for the transcription of *ac*. Interestingly, the *emc* gene encodes a protein without any DNA binding domain. Instead, a dimerisation motif is found in *EMC*, which probably neutralises the function of all basic loop helix binding proteins (van Doren et al., 1992; Ellis et al., 1993; Cubas et al., 1994). Thus, *emc* may directly interfere with the expression of *AS-C* transcrip-

tion factors by down regulating their transcriptional activity (Singson et al., 1993). Once activated, the genes of the *AS-C* confer upon neuroectodermal cells the potential to segregate as neuroblasts. The products of the *AS-C* locus are, however, not competent to activate transcription alone. To become potent transcriptional activators they require the formation of either homodimers or heterodimers in conjunction with unknown candidate transcription factors.

For one of these candidates, the *Daughterless (Da)* transcription factor, it has recently been demonstrated that it does not interact with the *AS-C* gene products at all. Evidence rather suggests that *Da* seems not to be directly involved in the process of neuroblast segregation. In mutants for *da*, the expression of neural precursor genes in cells committed to become neuroblasts seems to be severely impaired. Hence, *da* is not required for the spatial commitment of a cell to segregate as a neuroblast. Rather it is likely that *da* initiates the hierarchical activation of a set of genes which mediate the cellular events underlying neuroblast precursor formation. One may thus arrive at the view that *da* is not a **selector gene**[6] like the genes of the *AS-C* rather then a **co-ordinator gene** (Vaessin et al., 1994). *da* presumably activates the downstream gene *deadpan* (*dpn*). Pieces of evidence form different systems suggest that in mutants for *da* eliminate the expression of *dpn* in the remaining neuroblasts. *dpn* encodes a DNA binding protein with a motif, which is 42% identical to the *hairy*. The predicted motif is rich in proline residues, which are presumed to bind cognitive DNA. Interestingly, *DPN* is prescibed to act as a negative transcriptional regulator that functions similar to the proteins of the *E(Spl)-C*. It is not known by which mechanism *dpn* is activated but the involvement of maternally supplied *GROUCHO* protein can be taken into consideration (Paroush et al., 1994). Prior to neuroblast formation cells in the proneural clusters express *dpn* transiently. Just prior to their delamination *dpn* expression becomes restricted to the neuroblasts proper. There the expression is maintained until the neuroblasts undergo their first division. This expression reappears during neural outgrowth. Evidence suggests that *dpn* may counteract *AS-C* genes (Bier et al., 1992).

---

[6]Not referred to as in the same context as McGinnes, who means by selector genes homeobox genes that give each structure within a segment its specificity)

2.4. AS-C responsive Genes

The genes of the *AS-C*, hence, determine the positional competence of neuroectodermal cells to assume a neural lineage. This process is tightly regulated for no more than the required number of neuroectodermal cells are recruited to become neuroblasts. According to a recent hypothesis the genes of the *Enhancer of Split Complex E(Spl)-C* regulate the reciprocal interactions during lineage dichotomy (for review, see Campos-Ortega, 1993a,b). The *E(Spl)-C* contains in addition to the twelve structurally related genes, which encode for bHLH transcription factors a single open reading frame coding for a transducin like protein. It is interesting to note that all the genes of the *E(Spl)-C* are differentially transcribed: While the *E(Spl)-C* genes *HLH-mδ/mγ, mβ, m3, m5, m7* and *gro* are essential for the correct segregation of neural precursor cells only the *E(Spl)-C* genes *m4, HLH-m5, HLH-m7, E(Spl), HLH-mβ, HLH-mγ* and *HLH-mβ* seem to be confined particularly to the neuroectoderm (Knust et al., 1992). The seven genes scrutinised in the study by Knust et al. (1992) are expressed serially at distinct times during embryogenesis: *m4, m5, m7* and *E(spl)* appear first in the neuroectoderm. *mβ, mγ* and *mδ* transcripts do not appear until stage 7. Following their transcriptional initiation they are expressed synergistically with the first group of genes. Once the SI neuroblasts have delaminated, the transcripts become confined to banded regions paralleling the midline. With the exception of *m5* and *E(spl)* the expression of these genes is excluded from the segregated neuroblasts (Jennings et al., 1993; Alifragis et al., 1993; Preiss et al., 1993; Jennings et al., 1994). Consequent to the formation of the SI neuroblasts, by stage 9, the distribution of the transcripts becomes scattered over the entire neuroectoderm again but reorganises along the midline at the time when the SII neuroblasts delaminate. The difference is that this time transcripts are present along the entire germ band. Transcriptional activity of the seven genes in the neuroectoderm disappears subsequently by stage 15 (Knust et al., 1992). With the exception of *HLH-m3* which is supplied maternally and is expressed homogeneously in the entire embryo, the actions and the predicted DNA binding motives of the remaining *E(Spl)-C* genes resemble each

other. The distribution of the *E(Spl)-C* transcripts is basically mirrored by the grouping of proteins. Monoclonal antibodies against *Mδ* and *Mγ* detect both proteins during stage 8. The allotment of *Mδ* and *Mγ* during stages 10 and 11 coincides with the segregation of SII and SIII neuroblasts. The expression of the protein ceases, however, soon afterwards (Jennings et al., 1993; Alifragis et al., 1993; Preiss et al., 1993; Jennings et al., 1994).

Downstream of the coding regions for each *E(Spl)-C* helix-loop-helix protein, the *M9/M10* transcription unit of the *groucho* (*gro*) is found — a decisive factor in determining the neuroepidermal lineage dichotomy — which shows some striking similarities to the β- subunit of transducin of mammals. It appears that the *gro* gene is closely regulated in conjunction with the remaining genes of the *E(Slp)-C*. In particular, it has been demonstrated using the yeast interaction trap method that maternally expressed *GRO* directly interacts WRPW motifs of the *hairy* related proteins, hence, those which are encoded by the *E(Spl)-C* , i.e.*E(SPL)*, *Mδ*, *Mγ*, *m7* and *m8*.. The WD 40 motifs contained within *GRO* are thought to mediate protein interactions — so they do at least in homologous proteins. Controversially it has, however, been established that these motifs, which stretch from residues 251- 719 aa are not required for the interaction of *GRO* with the WRPW motif of the *E(SPL)-C* proteins (Schrons et al., 1992; Parouch et al., 1994).

The *E(slp)* and *HLH-5* gene promoter contains binding sites for its activation by *daughterless* or *l'sc* heterodimers (Vaessin et al., 1994), as well as, for the *E(spl)* and *HLH-5* protein. Particularly the regions downstream of *m8* (-136 to +96 kb [N1] for SI NB segregation and -241 to -136 kb [N2] for SII NB segregation) have been identified as autoregulatory enhancers. Hence, the regulation of these *E(Spl)-C* genes is probably determined by two antagonising *E(Spl)*-C regulatory regions. The more proximal E1 box enhances the activation of the genes of the *E(Spl)-C* in the neuroectoderm by either *ac*, *sc* or *l'sc* homo- or heterodimers (Vässin et al., 1994). *HLH-5* and *E(spl)* are thought to regulate each other through a negative autoregulatory feedback loop. This probably involves the binding of both gene products to N1/N2 boxes and thus represses their expression in putative neuroblasts (Kramatschek and Campos Ortega, 1994).

Moreover, it has been proposed that *ac* and *sc* have specific abilities to either activate or to suppress neuroectodermal function (Singson et al., 1993).

big brain (*bib*) a neurogenic locus encodes a 700 aa channel like protein. As *bib* is co-expressed with the genes of the *AS-C*, the Jans' laboratory suggested that *bib* expression might be driven by the products of the *AS-C* genes. In mutants for *bib* the neural cells are enlarged, hence, **hypertrophic**. As mosaic studies have shown *bib* functions cell autonomously. The *bib* channel like protein is probably required to mediate intercellular communication between neurogenic cells (Rao et al., 1992). Evidence for this notion comes from the observation that *bib* is expressed in the neuroectodermal cell prior to the delamination of presumptive neuroblasts from this germ layer. In particular, *bib* seems to be restricted to the basolateral membranes of these cells. Albeit, it has also been detected in vesicles within cells that have acquired the potential to assume a neural lineage. Once, a neuroblast is committed to adopt neural identity the expression of *bib* ceases (Doherty et al., 1993).

Lachesin is a member of the immunoglobin superfamily, which is expressed throughout the neurogenic region similar to the expression of proneural genes. Its expression domains differ from these of either *ac* and *l'sc*. Interestingly *Lachesin* is co-expressed with *Dl* and *Notch* in positions where neuroblasts first form. The expression of *Lachesin* coincides exactly with the chronological sequence of neuroblast generation. It first appears in prospective NB 3-5 and NB 4-1 followed by NB 2-5. In contrast to the expression of *Notch* and *Dl Lachesin* continues to be expressed in neuroblasts while they delaminate. *Lachesin* might act as a adhesion molecule in subsets of cells thereby determining their position with respect to other neural precursors. Later during development *Lachesin* continues to be expressed only in subsets of neurones (Karlström et al., 1993).

2.5. Structural Properties of the *Notch* Signalling Pathway

A well studied example of a signalling pathway, which might be involved in the establishment of neural clusters in the neuroectoderm is the *Delta Notch* system

(Yedvobnick et al., 1985). As mutants for the *E(Spl) -C* genes, mutants for *Notch* and *Delta* are associated with neural **hyperplasia**. The cell surface receptor *Notch* may play a key role in the segregation process by activating a whole array of intrinsic signalling proteins, which have all been implicated to become activated due to the interaction of *NOTCH* with its putative ligands.

The *NOTCH* pathway is initiated by *Delta* (*Dl*) or *serrate* which is expressed homogeneously in the cells of the neuroectoderm. *DL* encodes a ~100 kDa transmembrane surface protein with 9 EGF like repeats in the transmembrane domain.

The *Notch* receptor is a large transmembrane protein. It comprises a extracellular membrane about 1700 amino acids in length including 36 tandem epidermal growth factor (*EGF*)- like motives proceeded by cysteine rich *lin-12/Notch* elements (Wharton et al., 1985; Kidd et al., 1986; Struhl et al., 1993). Repeats 11 and 12 (Rebay et al., 1991) as well as repeats 14 and 29 (Lieber et al., 1992) have been postulated to function as *in vitro* binding sites for *Delta* a potential *Notch* ligand. This domain is followed by two cysteine residues at 1693 and 1696 thought to mediate dimerisation between two *Notch* receptors (Kidd et al., 1989). The 1000 amino acid long cytoplasmic domain encompasses six copies of cdc 10/ ankyrin repeats (Kidd et al., 1994 for publication) known to be involved in protein-protein interactions (for review, see Kidd, 1992). Moreover, structural motives are the *strep/ opa* (*polyQ*) sequence and the *PEST* sequence. Former is related to glutamine rich domains, which might represent transcriptional activators; the latter is involved in high protein turnover. Deletion of the extracellular moiety of the *Notch* protein have revealed that the cytoplasmic domain migrates to the nucleus (Rebay et al., 1993a,b; Lieber et al. 1993). Sequence studies further identified two putative bipartite nuclear localisation sequences in the cytoplasmic domain (Lieber et al., 1993) with the consensus (Chelsky, 1989) KRQR (amino acids 1832 - 1835) and KKAK (amino acids 2202 - 2205). In addition, Lieber et al. (1993) observed that the cytoplasmic domain when expressed ectopically on its own is rapidly degraded within the nucleus. Interestingly, during neuroblast segregation *NOTCH* and *DL* were found together in endocytic vesicles within the neuroblast. A current hypothe-

sis suggests that these sequences are required for recycling the *NOTCH* protein in neuroblasts, once they have delaminated (Kooh et al., 1993).

Moreover, deletion studies and overexpression studies of the *NOTCH* protein have revealed that the cdc10/ankyrin region plays an important role in signal transduction as loss of this domain results in a strong **neurogenic** phenotype indicative of a **dominant loss-of-function mutation** (Lyman and Young, 1993). It may be possible that the intact extracellular domain sequesters it ligands which, thus, results in the neurogenic phenotype. Hence, permanent binding of the ligand results in the activation of the *NOTCH* pathway and the inhibition of other cells to become neuroblasts (Lieber et al., 1993) [fig. 3.13.]. On the other hand deletion of the extracellular domain results in a dominant **gain-of-function mutation**. The resulting **antineurogenic** phenotype is associated with a consecutive activation of the cytoplasmic domain of the receptor. The same results were obtained for deleting the lin 12/ ankyrin repeats and the cysteine residues implicated in dimerisation of *NOTCH* molecules.

NOTCH appears to be a very versatile protein that has the ability to initiate several downstream events , at once. So two substrates have been identified that interact with the cdc10/ ankyrin repeats. Accordingly, the most satisfying interpretation is that two diverging downstream pathways are initiated at *NOTCH*.. Firstly, biochemical analysis revealed that upon binding of *DELTA* the *NOTCH* protein seems to interact with *DELTEX* a transmembrane protein, which is particularly rich in glutamate, histidine and serine residues (Busseau et al., 1994). *DELTEX* may, hence, be the first signal transmitter in the *Notch* pathway. In particular, it has been established that *DELTEX* interacts with the *NOTCH* ankyrin motives (Diederich et al., 1994). Interstingly, mutations is the *Notch* ankyrin motive have the same effect as mutations within the *Deltex* gene.

A second substrate for the *NOTCH* intracellular cdc10/ankyrin motifs is — as established in a screen for enhancers of the *Notch* mutant phenotype — the *SUPPRESSOR OF HAIRLESS* (*SU(H)*) protein. *Su(H)* encodes a homologue of the Epstein Barr virus nuclear antigen 2 (EBNA2), a transactivator protein, which is targeted by the CBF1 protein to the C promoter, where it establishes viral latency. In the search further possible genetic interactions with alleles of the *Su(H)* locus, it had previous been established

that the *SU(H)* gene is halpoinsufficient for the *hairless* locus. The conundrum of *deltex* and *Su(H)* interactions with *Notch* has been solved in cell culture analysis and confirmed with the yeast interaction trap method. So two constructs were made either contianing the *Su(H)* coding region fused to a *cMyc* epitope tag[7] under control of a *hsp 70* gene heat shock promoter, or the *Notch* coding region under control of a *Metallothionine* gene promoter. When both constructs were transfected into S2 cells and either promoter was induced N became localised to the surface and the cytoplasm, whereas the *SU(H)* protein was restricted to the cytoplasm. Upon removal of the *Notch* intracellular cdc10/ ankyrin motifs, it has, however, been found that *SU(H)* becomes restricted to the nucleus. Similarly, the binding of *DELTA* to the extracellular membrane spanning domain induces the localisation of *SU(H)* to the nucleus, suggesting that in an inactive state the *NOTCH* transmembrane receptor sequesters the *SU(H)* protein within the cytoplasm, essentially through its cdc10/ ankyrin motifs. These results have been directly confirmed in the yeast interaction trap assay (Fortini and Artavanis- Tsakonas, 1994).

The *mastermind* (*mam*) gene codes for a nuclear protein, which is expressed in a variety of tissues in early *Drosophila* development. Mutants within the *mam* neurogenic locus are associated with neural hypertrophy. The *MAM* protein is maternally supplied, as well as, synthesised *de novo* in the zygote (Smoller et al., 1990). Its function during neurogenesis still awaits to be solved (Schmid et al., 1993).

3. Synthesis

The coincidental expression of genes, which determine the position dependent transcription of neurogenic genes is the prerequisite for the segregation and isolation of neural cells from the ectoderm. The region, which confers upon ectodermal cells the potential to assume neural lineage is called neuroectoderm. Cells in the neuroectoderm need, hence, to express a specific set of genes: The proneural genes promote the neural commitment, and the neurogenic genes antagonise this commitment. The expression of

[7] The cMyc epitope tag allows protein localisation of this chimeric protein without the need to generate antibodies against Su(H)

both protagonistic and antagonistic genes is probably in an equilibrium at this stage. Once, a cell in this tissue has become committed to become a neuroblast, a threshold needs to be overcome and equilibrium shifted towards the expression of proneural genes. This process might become more complex as new genes and their function are to be found. Thus, I restrict myself to explaining the fundaments of the already known leaving aside the *sna*, *mam* and *NK-2*, as no link has so far been established between their involvement in neurogenesis and the enrolment of the *AS-C E(Spl)-C* circuits. These circuits are closely linked with the pathways of the cell cycle which I refer to later. Both circuits have, hence, a double function: Firstly, they need to select stereotyped cells to become neural cells. Secondly, they need to initiate the program, which allows neuronal cells to express a distinct phenotype.

To specify this model of neurogenesis, I best start with the expression of the pair rule genes. In odd numbered stripes the expression of the bHLH transcription factor *Ac* in row 4 seems to be the consequence of the positive regulation by the homeobox transcription factor *Ftz* and the negative regulation by *Opa.* In analogy the expression of an unspecified dorsoventral morphogene ought to coincide with *Ftz* for it to activate the neural program of the *AS-C* genes in row 4 (Skeath et al., 1992). The gene *vnd* may either be a mediator of the gap genes by acting through the regulatory regions 5' to *ac* to enhance *Ac* expression (Skeath et al., 1994) or it may be a transformer of dorsoventral information. Either notion is worth to be considered, as there are at least two regulatory elements downstream of *ac*, which mediate the position dependent transcription of *ac* by binding region specific transcription factors. The D element, which activates transcription in row 4, may, hence respond to *ftz* and *vnd* to initiate as transcription (Skeath et al., 1994). The antagonist of *ac* expression is *emc*. It probably acts to limit the proneural cell clusters of the neuroectoderm, which express *ac* to a specific region (van Doren et al., 1992; Ellis et al., 1993, Cabrera et al., 1994). At this stage cell clusters of the row 4 have acquired the potential to delaminate as neuroblasts (Skeath et al., 1992). As this region is quite large and not every cell in this cluster may become a neuroblast only types of neuroectodermal cells can be selected, which are ideally posi-

tioned to acquire a neural phenotype[8]. As mentioned above genetic equilibria play a cardinal role in this process. The converter for this positional signalling are the regulatory regions of the *Enhancer of Split Complex* genes. The most proximal region downstream of *m8* is a E-box, which is specifically associated with the binding of *AS-C* transcription factors such as *l'sc*. Overlapping with the E-box another transcription factor binding motive is found: This N-box comprises three units each associated with the sequential binding of *E(Spl)-C* gene products according to the time of neuroblast segregation (e.g. N1 is associated with SI NB delamination, whereas N2 corresponds to SII NB segregation). The HLH proteins *E(spl)* and *HLH-m5* bind the two units N1/N2 during SI neuroblast delamination (Kramatschek and Campos Ortega, 1994).

Hence, products of the *AS-C*, as well as, other unspecified transcription factors (as *AS-C* positive cells only account for 50% of the total neuroectodermal cells) may bind the E-box either as homodimers or as heterodimers and, thus, activate the expression of *E(Spl)-C* genes homogeneously throughout the neuroectoderm. *HLH-m5* and *E(spl)* in turn compete with *AS-C* transcriptional activators and upon successful binding to their respective enhancer regions they downregulate their own expression (Kramatschek and Campos-Ortega, 1994). At this point the *AS-C* and *E(Spl)-C* transcription factors are still in an equilibrium. If the equilibrium of proneural and neurogenic gene products is shifted towards the neurogenic gene products, the transcriptional activity of *E(Spl)-C* genes is shut down. These conlusions are in accordance with the observation that some genes of the *E(Spl)-C* are initially expressed in putative neuroblasts (Kramatschek and Campos Ortega, 1994).

It might well be conceivable that there are other links between proneural genes and neurogenic genes. Latest findings suggest that the genes of the *AS-C* activate expression of *DL* throughout the neuroectoderm (for review, see Campos-Ortega, 1993a,b). Conversely, Jennings and co-workers (1994) found that in mutants for *Notch* expression of the *Mδ* and *Mγ* proteins of the *E(Spl)-C* is severely impaired, suggesting the requirement of maternal and zygotic *Notch* during this process. Furthermore, current

---

[8]Good reading in this context makes 'Neural Darwinism' by the Nobel Laurate Gerald M. Edelman published by Basic Books, New York 1987.

findings using the yeast interaction trap assay indicate that maternally supplied *groucho* is necessary to render the bHLH proteins of the *E(Spl)-C* active by binding to their oligomerisation domains. Hence, *groucho* may be a key modulator of transcriptional repression by the bHLH proteins of the *E(Spl)-C* (Paroush et al., 1994).

Thus, at the time of neuroblast delamination *DL* and *NOTCH* are initially expressed synergistically throughout the entire neuroectoderm (Kooh et al., 1993). It can, hencewith, be assumed that *DL* stabilises the intercellular contacts between the cells in this region by mediating the formation of intercellular junctions with *NOTCH*. Thus *DL-NOTCH* interactions may therefore prevent these cells from adopting neural lineage. Additionally, *BIB* may function as a communication channel between the neuroectodermal cells solidarising against adopting a neural lineage. The genes of the *E(Spl)-C* may, thus, regulate the neurogenicity of these cells in response to common signals exchanged through *BIB* channels (Doherty et al., 1993). Binding of *DL* to the *NOTCH* receptor then mediates through *DELTEX* the activation of downstream events augmenting the transcription of the *E(Spl)-C* proteins *Mγ* and *Mδ* (Diederich et al., 1994; Jennings et al., 1994). As latest information from Artavanis-Tsakonas' lab suggests, the activation of the *NOTCH* pathway by *DELTA* results in the redistribution of the *SUPPRESSOR OF HAIRLESS* (*SU(H)*) protein from the cytoplasm to the nucleus (Furukawa et al., 1992; Fortini and Artavanis-Tsakonas, 1994). As seen before, the shift in the equilibrium between proneural and neurogenic genes results in the delamination of neuroblasts. The trigger for this shift might be *HAIRLESS*. Mutations in *hairless* act as a **suppressors** of **amorph** and **antimorph** *Dl* alleles (Klein and Campos-Ortega, 1992). Additional studies by Maier and Preiss (1993) have identified the *hairless* gene product as an antagonist to neurogenic gene activity. *hairless*, which encodes a nuclear 1076 aa basic protein enriched in serine residues might, thus, be the ultimate switch for neuroblast identity in this cascade. It can be assumed that *hairless* acts to suppress the phenotype of *Dl* either through interaction with *AS-C* genes or by initiating the neuroblasts endocytotic activity in absence of ligand binding. Following the endocytosis of *NOTCH* and *DL* from the surface membrane neuroblast dissociate form the neuroectoderm. As a consequence these neuroblasts become disconnected from neuroectoderm specific in-

formation and enrol their own developmental program. The expression of *AS-C* genes, however, persists. As soon as neuroblasts have become dissociated from the neuroectoderm, the expression of *Dl* reappears probably in response to the actions of the *hairless* protein. The *hairless* protein becomes presumably derepressed by internalised *NOTCH* in absence of *Dl*. Hence, internalised *NOTCH* sequesters the repressor of *hairless SU(H)* into the cytoplasm. *HAIRLESS* may in turn consolidate the NBs neural specification within the reformed neuroderm. Synergistically, *daughterless* may co-ordinate the cellular events underlying this process conferring each neuroblast is specific phenotype (Vaessin et al., 1994). It must, however, be emphasised that the entire process is not associated with the cell cycle. Cell divisions only generate the substrate, the neuroectodermal cells, for neuroblast differentiation. Only the differentiated neuroblasts have the potential to enter the cell cycle again.

4. Midline Formation

The formation of the midline proceeds in three sequential stages, each of which is associated with the expression of stage specific genes. Firstly, the plan for the mesoectoderm is established in blastula and gastrula type embryos (stages 5- 7). Secondly, midline precursor cells arise during stages 8- 11. Lastly, these mature into fully differentiated midline cells (stage 12- 17).

Whilst the components of the dorso- ventral patterning cascade outline the presumptive mesoectoderm, it is the task of the midline specific genes to recruit the relevant midline precursor cells. Some evidence suggests that the different midline specific genes are co-operatively controlled through similar control regions 5' of each gene. The consensus established for these control regions has the consensus 5' -ACGTG- 3', indicative of xenobiotic control elements (XBEs). For each XBE, a consensus of 5' TNGCGTG-3' has been established occurring mostly 5' of the genes responsive for drug and cancer metabolism. It is genuinely understood that this type of consensus sequence serves as a binding motif for basic helix- loop- helix transcription factors, bHLH- PAS such as *dorsal*. The *sim* gene contains, evidently, five of these

consensus motifs downstream of the coding frame within a 2.8 kb stretch of DNA. Among these elements are two different promoters. The *sim* early promoter is first activated during the germband extension. The late promoter seems to be required for autoregulation (Nambu et al., 1991). Similar results were obtained for *Toll* gene: Within, 0.9 kb fragment 5' of the *Toll* gene four of these conserved sites have been found. There is, however, only a single site upstream of the gene *slit.*

Furthermore, deletions within the motif abolish germ band stage mediated transcription of genes that occur downstream of these motifs. However, the number of mutated motifs is determinative for the frequency of transcription. The higher the number of mutated elements, the less frequent is the transcriptional activation. All these findings strongly suggest that bHLH proteins need to form homo- /heterodimers in order to activate the expression of midline specific genes by binding these asymmetric XBE enhancer motifs (Wharton, 1994).

The *sim* bHLH- PAS[9] protein acts to control the events that govern the development of the midline precursor cells (MPCs) (Thomas et al., 1988). Experimental evidence suggests that the midline cells migrate normally to the ventral midline at gastrulation in mutant alleles for *sim.* However, the absence of the synchronised cell divisions in the MPCs in mutants for *sim* indicates that this gene has also the potential to influence the cell cycle events. Evident is also an aberrant phenotype of the *sim* mutant MPCs. Particularly evident is the fusion of longitudianl axon bundles and axonal neuromas (Nambu et al., 1990; 1991).

An other gene, which is critical for the development of at least some subsets of midline cells, is the gene *bottonhead* (*btd*). *btd* is of particular interest for the process of midline formation, because its reading frame codes for an nuclear protein containing a homeobox. About the transcriptional activation of *btd* hardly anything is known but one might expect that transcritpion factors such as the *TWIST* bHLH protein might bind to the 6 consensus binding sites 5'- CTNNAG-3' found within a 300bp region of genomic sequence immediately upstream of the *btd* reading frame. Strikingly the *btd* transcription coincides the appearance of *fushi tarazu* (*ftz*) transcripts at the extended germ

---

[9]PAS stands for *period, arnt,* and *single-minded* domain

band stage 11 ($5\frac{1}{2}$ - 6 hours AEL), whereby each of the *btn* expressing cells - the dorsal median (DMs) cells - is intercalated between each *ftz* stripe at a position predictive of the parasegmental boundary. Studies on mutant alleles for *btd* have conclusively demonstrated that the DM midline cells, which mark the path of the commissures along the segment boundary in wild type embryos, are deleted. Hencewith, DM cells, which in the *Drosophila* embryos serve as a substratum for the latitudinal outgrowth of commissural tracts, rely on the cell specific expression of *btn* to affirm their specificity (Chiang et al., 1994).

5. Gliagenesis

Glial cells fulfil three different functions during the development of metazoan nervous systems. Firstly, they direct the neural cell cycle and differentiation process in an paracrine mode. Secondly, they specify the outgrowth of axons along their prefigured pathways and define the domains where dendritic fields arise. Thirdly, the supply nerve cells with essential ana- and metabolites.

Glial cells of the *Drosophila melanogaster* nervous system have three different origins: The two non-neural types of glia either derive from specific lateral glioblasts (Jacobs et al, 1989) or as the midline glia from mesoectodermal precursors (Crews et al., 1988).

Midline glia cells have been extensively studied with regard to their involvement in the formation of commissural pathways in *Drosophila*. When the three midline cells MGA (anterior glia), MGM (medial glia) and MGP (posterior glia) are born, they adopt a postion within each segment, which is opposite to their final location. Once the commissures are formed MGM migrate anteriorly over MGA and MGPs cross the segmental boundary positioning themselves posterior to MGMs (for review see Goodman and Doe, 1993).

The origin of the glia of the aCC/pCC neurone cluster will be dealt with later. Longitudinal glia derive from a separate type of precursors as the both types of glia described above. Glioblasts (GBs), which generate midline glia, are of distinct genetic

identity. It is likely that both derive from a common neurogenic precursor cell of the peripheral ectoderm during following the 14th mitotic division. GBs are positioned laterally to the NBs. They divide symmetrically to form a pair of progeny cells. Following their 'birth' lateral glia cells migrate anteromedially, where they form two rows. About genetic interactions of gliagenesis hardly anything is known. Midline glia are identified by their expression of the marker *slit* and *sim*. Longitudinal glia express the marker *ftz* , *otd* and *pros* (for review, see Goodman and Doe, 1993).

To study the anatomy of the glia system in *Drosophila* embryos in detail, Nelson and Laughon (1993) fused the *lacZ* gene of *E.coli* to a glial specific promoter, which contains a homeodomain consensus similar to that of the *fftz* allowing both PNS and CNS glia to be marked. The supraoesophageal ganglion is surrounded by a glial sheath, which is arranged in a web-like pattern. The type of glia stained in their study depended upon the orientation of the insert.

CHAPTER 4

The Integration of Neurogenesis into The Segmentation Process

The expression of transcripts and proteins of most proneural and neurogenic genes can be found in the procephalic region, as well. The procephalic region differs, however, in terms of the anatomy. The cerebral ganglion of insects is composed of several amalgamated neuromeres. As seen before the seven pregnathal segments are arranged in a S-like configuration. This arrangement has so far complicated studies on neurone number and axon outgrowth in the procephalon. The identity of the neuroblast is established by pair rule, gap and segment polarity genes in the torso of the *Drosophila* embryo (Doe et al., 1988). With regard to their actions these genes can be divided into three different classes:

Firstly, genes, which are closely linked to the segmentation process in the epidermis. Secondly, genes, which are involved in the segmentation process but which act independently during neurogenesis (Patel et al., 1989b). The third class is formed by genes, which are expressed *de novo* during neurogenesis and therefore do not obey to the segmentation process at all (for review, see Doe and Scott, 1988).

1. Neuronal Identity is a Function of the Segmentation in the Ventral Neuroectoderm

The first class comprises the genes of the *gooseberry* locus, *patched*, *naked* and *engrailed*. *Fushi tarazu*, *even- skipped*, *odd oz*, *wingless* and *runt* for instance are redeployed during neurogenesis following their initial involvement in the segmentation process, and therewith, belong to the second class.

Mutations in the *engrailed* locus predominantly effect the expression of other genes in the region of each parasegmental boundary. Apart from this mutant embryos for *en* only develop a single axonal commissure in each hemisegment (Ouellette et al., 1992). *EN* expression is associated with neuroblast clusters of row 5 and 6 (Gutjahr et al.,

1993). Specifically closely associated with defects in the epidermis are mutations induced in the neuroectoderm by genes of the *gooseberry- zipper* locus. A discrete function can be assigned to each of the two highly homologous transcripts of the *gooseberry* locus. As discussed above the *gooseberry-distal* (*gsb-d*) gene determines in conjunction with *wg* and *en* the formation of the *naked* cuticle (Nüsslein-Volhard and Wieschaus, 1980; Côté et al., 1987; Baumgartner et al., 1987; Gutjahr et al., 1993) and thus acts as a postional cue (Zhang et al., 1993). Its expression begins before gastrulation starting with stripes in odd numbered segments followed by stripes in even numbered segments and cedes with the end of head involution (Gutjahr et al., 1993). In mutants for *wg* and *en* the regions of *gsb-d* expression are considerably reduced. Ectopic *gsb-d* expression leads to a duplication of regions adjacent to the epidermal parasegmental boundary (Zhang et al., 1994).

gooseberry-proximal (gsb-p) expression is restricted to clusters of neural cells situated beneath the parasegmental boundary just anterior to each *EN* domain, and thus acts as a selector gene (Zhang et al., 1993). It corresponds mostly to the neighbouring *WG* expression region. *gsb-p* expression is initiated during stage 10 in a limited number of neuroblasts. With augmenting intensity expression can be detected in each metameric neuromere by stage 11. The *gsb-p* expression domains are situated dorsally to the posterior axon commissure of each neuromere corresponding to neuroblast rows 5 and 6 as well transiently to row 7 (Gutjahr et al., 1993). The midline is excluded from *gsb-p* expression. *gsb-p* is activated in trans by *gsb-d* which, too, is expressed in the neuroectoderm (Gutjahr et al., 1993). Thus, in mutants deficient for *en* and *wg gsb-d* fails to transfer the information to *gsb-p* (Ouellette et al., 1992).

In embryos defective in the *gsb-p* gene the expression of *ac* (***vide infra***) expands posteriorly into *l'sc* expressing neurones resulting in the depletion of the posterior commissure and the CQ neurones. Moreover, the progeny of the NB1-1 (rows 5 and 6), the aCC, pCC, and RP2 neurones is duplicated in these mutants, perhaps as the result of the premature expansion of *Ac* (Skaeth et al., 1992). The EL neurones remain, however, not affected (Patel et al., 1989). By contrast, ectopic expression of *gsb-p* fosters the misexpression of *GSB-P* in adjacent neurones, which usually do not express this

gene. The EL neurones, for example, do not express *GSB-P* in wild type embryos. Ectopic *GSB-P* expression alters their fate, so that EL specific markers fail to recognise them as such. Markers for *GSB-P* positive neurones, in contrast, detect twice as many *GSB-P* positive neurones. Hence, ectopic *gsb-p* or *gsb-d* expression reverses the effect of *gsb* mutations (Zhang et al., 1994).

It has been proposed that cytoplasmic tyrosine kinase may mediate eihter upstream or downstream functions of *gsb-p* as mutants for this enzyme fails to form the posterior commissure, as well (Ouellette et al., 1992). As *gsb-p* and *gsb-d* are highly homologous each protein can substitute the other. Even *paired* with its 87% homology to *gsb* is able to retrieve mutations in the *gsb* locus suggesting a common evolutionary origin of the *paired* type domain (Treisman et al., 1991; Gutjahr et al. 1993; Li and Noll, 1993b). Ouellette and collaborators (1992) further reported that in mutants for the segment polarity gene *naked* adjacent neuromeres are occasionally fused.

2. Determination Neuroblast Identity in the Procephalic Region

ENGRAILED Domains

The segmentation pattern of the brain differs substantially from that of the torso. As in the torso each segment is allocated a distinct neuromere. The anatomy of the procephalic nervous system differs accordingly. The insect brain represents an amalgamation of several neuromeres. The thoracic and abdominal neuromeres are laid out in a simple pattern which anatomy is well understood. Due to the deflected arrangement of neuromeres, symmetries observed in the trunk have so far not been established. Cell diversity of the procephalic region is quite enormous.

The brain morphology of the African desert locust *Schistocerca gregaria* is exceptionally easy to follow as here as in other short germ bad insects segments assume an planar configuration. In each brain hemisphere of the *Schistocerca* embryo 130 large mitotically a active neuroblasts are formed between the 20 and the 45% stages. These neuroblast then migrate passively driven by divisions in this tissue to the founder brain regions. Each neuroblast in the brain of *Schistocerca* produces by asymmetrical cell

divisions a limited set of GMCs. Each GMC forms in turn two sibling neurones. The number of neurones, thus, generated by a single neuroblast amounts to about hundred. Zacharias and coworkers (1993) counted 41 large NB in the main protocerebrum, 25 large NB in the lateral protocerebrum, 32 large NB in the deuterocerebrum, 12 large NB in the tritocerebrum, and finally 20 large NB in the *pars intercerebralis.* The counts of this study reflects, however, only the number of large NBs. The number of small NBs has not been determined and probably exceeds the number of large NBs by far.

en expression (***vide infra***) demarcates each segment of the *Drosophila* brain. Thus, as in the abdomen anti-*EN* immunoreactivity reveals the segmental borders and therefore defines each segmental neuromere. Each neuromere of the three gnathal segments in the *Schistocerca* embryo comprises 24- 30 segmentally homologous NB pairs. The number of neuroblasts in each of the four pregnathal segments, however, remains, obscure.

In *Drosophila*, the neuromere of the labral segment is presumably the most anterior. It probably contributes cells to the anterior ventral part of the brain. The preantennal neuromere, which is found more posteriorly, is followed by the antennal neuromere. The intercalary neuromere forms the border between the procephalic lobe and the mandibular neuromere. The corpora pedunculata neuroblasts of the *Drosophila* brain are thought to derive from the anterior ventral site of the *EN* head 'blob'. Schmitt-Ott and Technau (1992) have attributed the primary and secondary *EN* head 'blob' to the posterior boundary of the preantennal segment. Hence, the corpora pedunculata arise in between the labral *EN* 'blob' and the preantennal *EN* 'blob'. Boyan (pers. comm.) proposed that some smaller neuroblasts within the pars intercerebralis are identical with the corpora pedunculata neuroblasts. These neuroblasts have, however, not been counted in the study by Zacharias and co-workers (1993). Thus, it might be that the pars intercerebralis can be attributed to the labral segment and the corpora pedunculata may arise in *Schistocerca* on the border between the labral and preantennal segment (Zacharias et al., 1993). According to the study by Zacharias et al. (1993), there are altogether 86 neuroblasts in the labral and antennal neuromeres, far more then the anticipated 40 to

60 which are found in the remainder of neuromeres. And this number does not even take the smaller neuroblasts into account.

Moreover, the preantennal segment contains the anlagen for the ocular tract of *Drosophila* (Schmitt-Ott and Techanau, 1992), *Apis mellifera* (Fleig, 1990), *Schistocerca* (Patel, 1989; Zacharias et al., 1993) and *Leptinotarsa* (Fleig, 1994). The antennal neuromere of the *Drosophila* embryo is positioned dorsally of the preantennal neuromere. This neuromere has unambiguously been identified as the origin of the antennal lobes. The number of neuroblasts present in this region in the *Schistocerca* embryo amounts to 32 which nearly corresponds to the average of neuroblasts found in a neuromere of the gnathal region (Zacharias et al., 1993). The *EN* intercalary 'blob' contributes the intercalary neuromere. It is positioned just anterior to the mandibular neuromere.

Gooseberry Domains

The controversy as to whether there are six or seven neuromeres in the gnathal or pregnathal region can probably be solved when comparing the distribution of the *gsb-p* gene product. As seen above each *gsb-p* stripe overlaps with the *wg* and partially with the *EN* expression region. Ouellette and his co-workers (1992) pointed out that in the head region of the stage 12 embryo at the onset of germ band retraction three pregnathal stripes are prominent. Following the genealogy of these cells, then it is evident that a single domain *of gsb-d* expression — the *gsb-d* antennal stripe — is present in the blastula stage embryo at the end of cellularisation. During mid-germ band extension the intercalary *gsb-d* stripe diverges form the *gsb-d* antennal stripe. Subsequently two bilateral symmetric stripes of *gsb-d* expression emerge in the region of the clypeolabrum. Last the preantennal *gsb-d* stripe forms (Gutjahr et al., 1993). A comparison with to Schmitt-Otts' and Technaus' (1992) *EN* expression map reveals that there is a substantial overlap between three procephalic neuromeres: the preantennal (ocular), the antennal, and the intercalary neuromere. The lightly stained intercalary neuromere demarcates the border between pregnathal and gnathal region (Ouellette et al., 1992; Gutjahr et al., 1993). No region of *gsb-p* immunoreactivity corresponds to the labral *EN*

'blob', but *gsb-d* immunoreactivity revealed that the additional seventh segment stains (Gutjahr et al., 1993).

In the precephalic region about 8 neuroblast clusters express the pair rule gene *eve* which here as well, is positively regulated by *runt* (Duffy et al., 1991). I was , however, not able to identify these clusters.

The *WG* preantennal 'blob' becomes incorporated into the brain (Schmitt Ott and Technau, 1992). Interestingly, the expression of the preantennal *WG* 'blob' depends on the gap gene *orthodenticle* (Jürgens and Cohen, 1990). The optic anlagen are excluded from *WG* expression. The *WG* intercalary spot fades by stage 11 and becomes a component of the CNS.

3. Homeotic Neuroblast Identity Genes

Homeotic selector genes of the *antennapedia* and *bithorax* complexes specify the appropriate neural lineages to adopt a discrete fate (Kaufman et al., 1990). The genes of these complexes are arranged sequentially on the third chromosome reflecting their serial expression along the anterior posterior axis. Most anterior lies the *labial* gene which presumably defines the status of the intercalary segment (Chouinard and Kaufman, 1991). *proboscipedia* probably confers the identity upon the maxillary segment of the gnathal region (Pultz et al., 1988). The expression of *deformed* overlaps in the labial and the first thoracic segment (Jack and McGinnis, 1990). For the abdomen it has been demonstrated that *Antennapedia-P2* is responsible for determining the specificity of neurones within thoracic segments. *D-ets-3* is thought to translate the information of selector genes into the specification of the neural identity (Chen et al., 1992). For discussion of the remaining head specific selection genes see before.

How specific the selection process by homeobox genes might be by which certain morphogenetic fields are isolated from neighbouring tissue was shown by Jones and McGinnis (1993). Both identified a homeobox gene, which is exclusively expressed in the ocular region of the developing brain. First, this gene was identified in terms of its

homology to known the known *eve* homeobox, but it was later established that *bsh* also displays a striking similarity to *Antennapedia* like homeobox domains.

The brain *specific homeobox* gene (*bsh*), a realisator class gene, determines the formation of the Bolwig's organ, the primitive eye, in the mid stage 11 embryo. Initially expressed in only two well separated cells in each hemisphere of the anterior ventral procephalic lobe two layers beneath the epidermis, the domains of expression soon extend to other cells so that by stage 15 about 30 cells express this gene. The cells of the more anterior cluster become more embedded into the brain by migrating dorsally during embryogenesis. Once the *bsh* clusters have been established, axons of the Bolwig's nerve extend from the cephlopharygeal region of the embryo through a pathway preformed by glia into the *bsh* cluster which lies next to the presumptive anlagen of the optic lobe. Afterwards *bsh* expression persists until stage 16.

The *ftz eve* and *wg* genes seem to function during the neuroderm formation in a restricted autonomous pattern. *WG* is expressed in row 5 of SII neuroblast during midstage 9. The domain of *WG* expression overlaps directly with the *WG* ectodermal stripes. It seems, however, that the latter has a different function. The secreted neural wg is received by adjacent rows 4 and 6/7. It is received by adjacent rows 4 and 6/7. In conditional mutants for *wg*, the *runt* gene is expressed ectopically in row 4 neuroectoderm. In such mutants the gene expression in row 4 corresponds to that of the neighbouring row 3. When differentiated row 4 GMC loose the identity of row 3 GMCs. The lack of *wg* in the neuroectoderm seems to repress the transcription of the pair- rule gene *even- skipped* (Chu-LaGraff and Doe, 1993). *runt* expression in the neuroectoderm is independent of its function as a pair-rule gene. In neuroblast another lineage the CQ neurone cluster *runt* is co-expressed with *eve.* In contrast to other GMC clusters the *eve* expression in the EL cluster is depleted in conditional *runt* mutants. In this region *runt* is the activator of *eve* but is not required for the maintenance of *eve* (Doe et al., 1988; Duffy et al., 1991).

castor (*cas*) is gene, which is co-expressed with *en*.. It encodes a nuclear zinc-finger transcription factor with a novel domain. Its expression peaks during stage 12, when SIII neuroblast delaminate. Of particular interest is that *cas* seems to be expressed in

the procephalic lobe. Expression of *cas* persists in dividing GMCs (Kambadur et al., 1993).

CHAPTER 5

Control of Neuroblast Proliferation

The proliferation and differentiation is directly correlated with the cell cycle activity of neuroblasts. Factors, which control the chronological regulation of the cell cycle are secreted by other cells and activate via signal transduction cascades the activation of the cell cycle form the G_1- phase in spatially and temporally defined domains (Foe , 1989). Following activation the cell cycle proceeds with the S- phase (Knoblich et al., 1994) during which daughter strands are synthesised (Durino and O'Farell, 1994). The neuroblasts proceeds than with the G_2- phase (Lehner and O'Farell, 1990) during which the relevant molecules, which are required for the cell division are expressed (M-Phase) (Dawson et al., 1993; Neufeld and Rubin, 1994). Mutations, which affect the activation of the transition form G_1- to S- phase either prevent the cell cycle from proceeding or induce permanent activation of the neuroblast cell cycle. With particular eye mark of the procephalic region two genes have been found, which control the resting phase of neuroblasts during the cell cycle. So far these mutants have only been researched in the first instar nervous system. Both genes affect the proliferation of neuroblasts in the ventral nervous system equally. Before I proceed I will have to mention the neuroblast proliferation pattern of the postembryonic cephalic lobe and the neuroblasts, which give rise to the corpora pedunculata in particular (for reviews see Glover, 1991; O'Farell, 1992).

1. Postembryonic Neuroblast Proliferation

During the late embryonic stages the number of neuroblast in the central brain region declines to ten. Each neuroblasts generates in average a progeny of 5 daughter cells: the ganglion mother cells (GMCs). In larvae a set of 4 neuroblasts supply ganglion mother cells to the corpora pedunculata. An isolated neuroblast, which belongs to the deuterocerebrum supplies GMCs to the antennal lobe. The mushroom body neuroblast are of 8-10µm diameter and, hence, are quite large (contrasting with the results ob-

tained by Boyan in *Schistocerca*). They are located beneath the outer surface of the cortex in the postero- dorsal cephalic region. These four neuroblasts as they can be seen in the first instar larva multiply to generate a progeny of 20-30 neuroblasts per cerebral hemisphere by 20 hours after larval hatching. Their number increases to 85 neuroblasts during the late third instar larval stage. This set of neuroblast persist until 20 to 30 hours after puparium formation and subsequently declines to only four neuroblasts. This quartet stops dividing 90 hours after puparium formation (Ito and Hotta, 1992).

2. *Mushroom Body Defect* Controls the Neuroblast Number

The *mushroom body defect* mutation (*mud*) affects the number of neuroblasts in both brain and ventral nerve cord. Here I wish only to refer to neuroblast formation in the procephalic lobe. *mud* is presumably a mutation, which affects the cell cycle in neuroblasts throughout the neuroderm in *Drosophila.* Neuroblast alternate between quiescent and proliferative state. It is assumed that *Drosophila* neuroblast arrest in the G_0 phase. Mitotic events are normally cell cycle dependent controlled. In *mud* the large numbers of reduced size neuroblasts are probably generated because the mitotic arrest is abolished. In *mud* mutant embryos 7 -8 more neuroblasts than in wild type were labelled with the nucleotide analogue BrdU. Similarlar results were obtained for larvae: in 0-4 hour old wild type larva 45- 69 neuroblasts were BrdU labelled, whereas only 49- 120 neuroblasts were detected *mud* mutants. These additional neuroblasts outlast the four neuroblast, which are found the late pupal brain. Instead 43 neuroblasts are still continuing to divide in this stage (Prokop and Technau for publication).

3. *Anachronism* Controls the Temporal Activation of the Cell Cycle in Neuroblasts

Another mutation in the *anachronism* (*ana*) locus is associated with abnormal neuroblast numbers. Whereas the numbers of neuroblasts are still identical in the embryos of wild type and *ana* mutants by 30 hours after larval hatching five times more neuroblasts

were detected in the *ana* mutant larval brain indicating that the onset of neuroblast proliferation is precocious. In contrast to the *mud* mutation the absolute number of neuroblasts of wild type flies is not exceeded. The numbers of precociously generated neurones in *ana* mutants are five times higher than in wild type control. Thus *ana* mutations have an impact on the number of neurones generated due to the premature onset of NB activity but does not affect the quantity of NBs. The *ana* locus encodes an 474 aa endocrine glycoprotein, which is secreted by glia cells and is assumed to retain neurones in the G_0 phase by inhibiting the cell cycle to proceed. In *string* mutations the neuroblasts form normally but are unable to divide and ganglion mother cells are absent in this phenotype. Neuroblast do therefore not require cell division to adapt their fate (Durino and O'Farell, 1994).

CHAPTER 6

Differentiation of Neuronal Fields

Once, neuroblasts have arisen they divide asymmetrically to generate another neuroblast and a ganglion mother cell. The ganglion mother cell then divides symmetrically to form two sibling neurones. Both sibling neurones may then migrate to their final position where they become anchored by glial cells. From there they emanate axonal processes into a direction, which is predetermined by their interaction with other strategically positioned auxiliary cells, such as other neurones or glial cells. Most of the cells an axon encounters during the pathfinding process secrete chemotropic factors, or differentially express cell adhesion surface molecules. These cellular surface molecules serve as a substrate for axon specific recognition molecules. These feed back to the neurone, informing it about the position of its course. The strategical arrangement of the cells guiding the axon to its target is a direct function of the segmentation process. Furthermore, it is established that axonogenesis is closely correlated with germ band retraction, although no studies have so far been undertaken to tackle this mutual depndency (Jacobs, 1993). The polarity of the neurone presumably obeys the same laws and pathways which are used to generate dorso-ventral asymmetry during early embryonic development (Keshishan et al., 1993).

As seen above, glia cells are thought to play an important role during the process of neurogenesis. They provide logistical, strategical and tactical help during this process. Logistical help is thought to be derived form glia, which ensheath the soma. These glia provide a triple function: (i) They supply the soma with nutrients; (ii) they anchor the soma to the cytoplasm and (iii) they control the cell cycle of NBs. The strategy for axonal outgrowth is provided by glia cells, which mark the presumptive pathway of an axon. One may consider the neuropil associated glia as such strategic cells. In *Drosophila* 14- 16 non-static interface associated glia, amongst which several longitudinal glia are found (***vide post***), and 3- 4 midline glia ensheath the neuropil at the neuropil cortex interface. Thus, the glia cells strategic positioning along potential pathways aids axon navigation.

Lastly, the tactical implication. As defined tactics is the detailed direction and control of a movement to achieve an aim. In this respect I would relate the surface associated glia of *Drosophila* with tactical tasks. These 14- 16 subperineural glia are situated dorsally of the perineural sheath and extend their processes along the ventral nerve cord. 6-8 of these surface associated glia divide each segmental neuromere along the midline and form a channel through, which commissures navigate contralaterally into the other hemisegment (Ito and Technau, 1993).

Once a pioneering tract has been established (*Schistocerca*: growth cone extension rate: 8µm/hr; filopodia extension rate:<101µm/hr; filopodia retraction rate:<104µm/hr (Myers and Bastiani, 1993b), other axons follow suit in fascicles and in most cases the pioneering axon degrades. The formation of gap junction with neighbouring cells secures the axonal fascicle within the surrounding tissue. The main axons finally extend to synapse with their target. Highly organised dendritic fields may branch off during this process. These need to recognise their targets within the tissue surrounding the main axon. Genetic circuitry underlying the process of axonogenesis need therefore to interact even more precisely than a clock work. Mutations in one of the many genes may disturb the equilibrium of axonogenesis and will lead in severe cases to the eventual death of the organism. As my thesis is about the ontogenesis of the corpora pedunculata I would have to refer to axonogenesis of these structures. However, the not even the most daring research laboratory has even thought of commencing work on the procephalon. This is partially due to its deflected structure but may also be attributed to the lack of markers. With my three slides I have in hand the proof that some nerve cells of the procephalic lobe may behave as in the ventral neuroectodermal region. Time lapse studies and a vital dye would be quite helpful to trace the process of axonogenesis in the procephalic region. Thus, as I have already mentioned virtually nothing is known about axonogenesis is the procephalon leaving me to having to refer to the ventral nervous system which due to its ideally symmetric arrangement a lucrative system to study. Moreover, the key principles of axon guidance are thought to be the same in the entire CNS. Only the fate of the procephalic neuromeres has changed during evolution. Firstly, there are, for instance, more neuroblasts in each pregnathal segment whereas the

number of mesodermal and ectodermal cells is reduced in this region. One may, thus, reason that the other two germ band layers are therefore severely reduced in favour of the neuroderm.

1. Genes which Control the Differentiation of Neurones

The process of axonogenesis depends on the position specific switches, which are made to give a neurone its identity. Several genes have been implicated in participating in neural identity formation. It is assumed that these genes are expressed along segment polarity gradients and define the specificity of neural subsets according to the position information which they have received.

miti-mere, *pdm1*, *dPOU28* (Yang et al., 1993a,b) and *dPOU19* all belong to the class of these POU homeodomain proteins. In addition to functioning in several developmental programs, this class of proteins acts to define the outcome of GMC cell divisions. *miti* and *pdm1* are found within a common locus. Both share a large degree of sequence homology and are expressed synergistically in GMC-1 and its progeny, the RP-2 neurones and their siblings. Bhat and Schedl (1993, 1994) created an antimorph *miti* allele driven by the hsp70 promoter and transfected this transgene into flies. The transgene lacks the N-terminal portion of the POU protein, which is responsible for the DNA binding specificity and probably functions as a mediator in transcriptional activation. It is assumed that the transgenic protein competes with the wild type *miti* protein for binding to the upstream regulatory sequences and thus, when heat shocked, the expression of *miti* target genes, which is normally confined to the ventral neurogenic region, should be deleted. Experimental evidence suggests that the antimorph *miti* allele prevents GMC-1 from dividing properly only when provided as two copies. Hence, the higher the copy number of the antimorph *miti* allele, the more severe is the mutant phenotype of the RP2 neurones. A genomic duplication of the *miti* gene results in contrast in the generation of replica neurones in the embryo. Thus, it can be assumed that the *miti* POU protein acts to define the type of cell divisions of GMC-1. Upon the presence of high concentrations of *miti-* protein the GMC-1 acquires the potential to divide four

times, generating a progeny of two GMC-1s by a symmetric division, which in turn form four RP-2 and sibling neurones. Lower copy numbers reduce the ability of GMC-1 to divide symmetrically. Instead only one progeny GMCs may be generated together with one sibling neurone. The progeny GMC-1 still has the potential to finally form the RP-2 and a sibling neurone. Hence, the initiation of cell divisions of GMCs depends upon threshold titres of *miti* to determine the type of cell divisions. Thus, *miti* confers upon the GMC-1 the potential to regenerate in a concentration dependent mode. The more diluted the titres of *miti* in each cell, the less becomes the ability of GMC-1 to divide.

The neural identity gene *ming* encodes a putative zinc finger protein which is involved in determining the identity of neuroblasts. The expression of *MING* is restricted to the NB lineages 6-1 and 7-4. Whereas, *ming* in NB 6-1 is already expressed following the onset of neuroblast delamination, in the NB lineage 7-4 it does not appear until the neuroblasts have gone through two divisions whereby two GMCs have been generated. Hence, it is likely the *ming* gene is expressed as a result of the preceding cell cycle events(Cui and Doe, 1992).

The *prospero* locus codes for a protein with a certain sequence homology to the *zeste* protein helix 3 homeodomain required for binding of *zeste* to DNA. It is first expressed in stage 6 and 7 embryos. *pros* transcripts are confined to the neuroectoderm and following neuroblast delamination become concentrated in the neuroblast. *pros* is expressed at its highest levels only in NBs and GMCs, but not in neurones. *pros* expression in LG in stage 13 co-ordinates proper neural outgrowth (Doe et al., 1991; Vaessin et al., 1991).

2. The Principles Axonogenesis in the Ventral Nervous System

2.1. Overview of Axonogenesis

After having described the formation of the fundamental architecture into which the nervous system is embedded this paragraph will deal with the process of spatial arrangement of neural networks within the abdomen. The neurones of the CNS extend their axons in a well co-ordinated manner to innervate their targets precisely. This is

particularly important as aberrant neurite outgrowth impairs neuronal function severely. An axon needs during this process to explore its pathways through the surrounding tissue. During this pathfinding process, the differential expression of many extracellular matrix molecules plays a crucial role in the generation of neural diversity and the temporal and spatial organisation of complex neural networks. It should not be forgotten that these surface molecules found so far represent only a fraction of the potential diversity of these molecules. The recognition and fasciculation with the correct pathways feeds back to the neurone postional information, which it in turn translates into axonal outgrowth and interaction with other cells during this process. The regulation of intracellular events is mediated by several interacting cascades of second messenger pathways, which mutually enhance or inhibit their mutual. It has been universally accepted that these cascades trigger the activation or inhibition of several transcription factors. Once activated these transcription factors co-ordinate the transcription of proteins and enzymes required for metabolic and structural functions during axonal outgrowth. Thus, protagonists, antagonists, as well as, their respective supporters are involved in axon extension and targeting. It is interesting to note that the expression of most of the cell surface receptors with catalytic intracellular domains varies considerably during development owing to differential polyadenylation and splicing activities.

2.2. Cytology of Axonogenesis

Connective Formation

The development of glia associated with the abdominal aCC/pCC cluster of the MP1 pathway have been studied extensively. aCC extends its axon contralaterally into the anterior root of the intersegmental nerve. The pCC neurite runs anteriorly, where it enters the medial section of the ipsilateral connective. The glial cells associated with the aCC/pCC cluster are found just beneath the dorsal and ventral surface of the ventral nerve cord, respectively. Two glia lie dorsally (B-glia) and one glia lies ventrally (A-glia). In proportion to aCC and pCC, B-glia lie anteriorly to aCC and A-glia lie posteriorly to pCC.

Each of the cells in the aCC/pCC cluster descents form a single progenitor cell: NB 1. Thus, NB 1-1 forms both glia and neural lineages and was, thus, denoted as a neuroglioblast. NB1-1 divides sequentially to from NB1-2, NB 1-3 and so forth. The A-glia and two B-glia are among the glial progeny of NB1-1. The neural progeny forms the two sibling neurones aCC and pCC. Following the birth of these neurones both cross the segmental border anteriorly and become located in the posterior neuromere of the anterior segment. Once pCC has reached its final position in the posterior half of the anterior segment, it emanates an axon that migrates anteriorly deviating slightly laterally. It subsequently meets one of the median glial cells (LG5) and fasciculates with vMP2 axons that partially enwrap this neurite. Both extend further anteriorly using glial cells as guide posts. The pCC growth cone subsequently meets the vMP2 axon which it enwraps tightly in order to form a fascicle. During further growth cone extension pCC acts always as the pioneer of this axonal pathway. LG5 serves a guidance post for the anterior migration of the fascicle. Both neurones maintain close contact with the surface of the glia. At the beginning of stage 13, MP1 projects its axon laterally toward the inner surface. It then bifurcates and sends a neurite posteriorly around the aCC axon, which persists for as long as the other branch has not met longitudinal median glia (LMG). Once, this has happened the connection with aCC retracts. It enters the dMP2/pCC pathway at the LMG and fasciculates with dMP2. SP1 joins the pathway medially and extends along these axons. These axonal fascicles do not enter the posterior segments until they have encountered the LG of the neighbouring segment. Once, MP1/dMP2 fascicles have reached the posterior segment, they innervate their homologues in this segment and proceed further posteriorly (for review, see Goodman and Doe, 1993) .

aCC extends its axon into the intersegmental nerve, and hence, leaves the CNS at the exit junction during late stage 12. There it encounters the tracheal placodea. It uses this structure as point of reference and follows it until it fascicles with sensory axons. It subsequently follows these neurones (stage 15) and proceeds behind the main tracheal trunk to synapse with muscle fibre 1 at stage 16. In contrast to the abdominal aCC/pCC neurones cluster the development of the thoracic aCC/pCC cluster precedes different.

Here, NB1-1 does not generate a glial lineage (for review see Goodman and Doe, 1993; Udolph et al., 1993). It would be interesting to know aw to whether the stereotyped growth pattern can be translated into the brain region as well. As I have mentioned before, this region is partitioned into segments as well, and it might be that in this region neurones which in the thorax and abdomen are more widely distributed are amalgamated here(*vide supra*).

Commissure Formation

During the blastoderm stage all three germ layers are generated. Mesoectodermal precursor cells (MEPs) delaminate at this time form two bilaterally arranged columns to either site of the midline. This single wide stripe of MEPs divides both the neuroectoderm and the mesodermal anlage along the midline. Following the invagination of the presumptive neuroectoderm during stage 6, the neurogenic regions arise. At the time when SI neuroblasts delaminate form the neuroectoderm, the MEPs are already internalised dividing each segment into two hemisegments. Hence, four cells from either hemisegment migrate to the midline where they line up to face another. Next, all eight precursors become intercalated to form a column along the midline. The anterior three cells form a glial lineage (MGA, MGM, MGP), whereas the posterior cells MP1, MP2 and four other ventral unpaired median neurones (VUM) form the posterior portion of this column. In contrast to their lateral VNE counterparts MPs form a progeny of only two daughter neurones. MP1 forms two unpaired neurones and MP2 generates two bilateral paired neurones (Klämpt et al., 1991).

VNS interneurones send their projections into the anterior and posterior commissural tracts of each hemisegment from stage 12 onwards. The first posterior commissure pioneers the midline during stage 12/5 in germ band extended embryos. This pioneering commissure explores the anterior most of the VUM cells which in turn triggers the ventrolateral displacement of the anterior pair of the MP1 progeny. Once, this has happened the growth cone loops anteriorly around the VUM cell thereby making use of the gap generated by the displacement of the MP1. It fasciculates with MGA and VUM and then proceeds into the contralateral neuromere.

By stage 12/3 the anterior commissure is pioneered, guided by MGA and VUM growth cones. At this point the VUM extend their projections anteriorly, whereafter they bifurcate laterally. The posterior commissure is at this stage bend with ascending and descending branches going to and leaving the midline. At the bend at the midline it meets the anterior commissure. Both commissures are separated during stage 12/0 by the VUM somata. MGA then provides the substrate for the anterior commissure which is located posteriorly. The separation of both commissures is the result of the posterior shift of MGM ventrally over the MGA to the VUMs and the medial displacement of RP1 and RP2 soma. These cells finally form a cluster, which divides the two commissures. The posterior commissure as the result runs now straight across the midline into the contralateral neuromere. Similar cellular interactions have been observed in *Schistocerca americana.* Here Q1 and Q2 'negotiate their way' through the midline. On several occasions the Q1 growth cone changes its morphology probably reflecting its interaction with other cells (Myers and Bastiani, 1993a).

2.3. Factors Involved in Axonogenesis

Extracellular Matrix and Transmembrane Molecules

Several types of extracellular matrix proteins are expressed predominately during axonogenesis. These cell surface proteins participate in the process of axon guidance by demarcating the pathway an axon chooses. One class of cell surface molecules may either contain or be directly coupled to an intracellular domain, which mediates signal transduction. Other surface molecules may only process a transmembrane domain without bearing enzymatic function in the intracellular domain. A further class may not have any transmembrane domain at all. Instead it might be secreted. One distinguishes between heterophilic and homophilic cell adhesion molecules. Connectin a novel surface molecule, which contains 10 leucine rich motives and lacks an intracellular domain mediates homophilic cell adhesions in subsets of axonal tracts. *FASCICLIN I* is composed of a novel homophilic extracellular cell adhesion sequence differing substantially form other known cell adhesion molecules. It is attached to the membrane via a glycol-

ipid linkage, which lacks a transmembrane domain. Homozygous *fasciclin I* mutants do not effect axonogenesis. By contrast double mutants homozygous for both *fasciclin I* and *Drosophila Abelson kinase* lead to the loss of commissures as exemplified by axon guidance defects in the RP1. Hence, the function of *FASCICLIN I* is predominantly effected by *DROSOPHILA ABELSON KINASE* (Zinn et al., 1988; Elkins et al., 1990). *FASCICIN II* and *NEUROGLIAN* are members of the immunoglobulin superfamily. Both are involved in homophilic cell adhesion processes. Whereas *NEUROGLIAN* is related to vertebrate neural cell adhesion molecules, the three immunoglobulin repeats of *FASCICLIN III* are more heterogeneous (Bieber et al., 1989; Snow et al., 1989). *FASCICLIN IV* has so far only been cloned in *Schistocerca gregaria* It shows striking similarities to the *COLLAPSIN* molecule of chicken neurones (Kolodkin et al., 1992; Luo et al., 1993; for review, see Goddman and Doe, 1993).

fasciclin II

FASCICLIN II acts in the developing nervous system as a *bona fide* homophilic cell surface recognition molecule for the MP1 pathway. The extracellular matrix domain of *FASCICLINII* is composed of five predicted C-2 Ig- like domains and two fibronectin type III -like domains with seven potential N-glycosylation sites as seen in vertebrate neural CAMs. Its transmembrane domain consists of a hydrophobic glycosyl phoshatidyl inositol domain. *FASCICLIN II* is initially expressed in the MP-1 pathway, and than appears in the whole longitudinal pathways. Particularly dominant is *FASCICLIN II* on the growth cones of the developing aCC and pCC axons. In mutants for *fasciclin II* the members of the MP1 pathway MP1, dMP2 and vMP2 growth neurones do not fasciculate to form the MP1 fascicle. In contrast, aCC and the VUMs proceed normally with their development (Grenningloh et al., 1991).

lachesin

Drosophila LACHESIN is a 38 kDa protein with high homology to *Drosophila AMALGAM*. Its one V- and two C2-type immunoglobulin domains classify it as a member of the immunoglobulin superfamily. Its is linked to the membrane by a hydrophobic glycosyl

phoshatidyl inositol anchor in the C-terminal sequence. The *Schistocerca americana* *LACHESIN* homologue is expressed in the embryo first in the brain neuroblasts and extends in the ventral nervous system. Following neuroblast delamination this cell adhesion molecule appears in longitudinal and commissural tracts as well as, in the intra- and intersegmental nerve. All median precursor neurones and Q1, Q2, G and C neurones express *LACHESIN* once they arise. aCC and pCC seem not to express lachesin. Extensive anti- *LACHESIN* immunoreactivity can be detected along the filopodia and growth cones. Later, *LACHESIN* expression becomes constricted to a subset of commissural and longitudinal axon fascicles (Karlström et al., 1993).

pollux

The *pollux* locus encodes a transmembrane 77kDa protein. The extracellular domain of *POLUX* is composed of a specific tripeptide sequence (Arg-Gly-Asp) called RGD, which serves to bind other neurones in analogy to vertebrate fibronectin. It has been suggested following observations on cell culture experiments that *POLLUX* is a homophilic cell adhesion molecule. The transmembrane domain contains a putative leucine - zipper motif indicating that *POLLUX* may form heterodimer. Interestingly, *POLLUX* is selectively expressed in the supraoesophageal commissure as well as in the longitudinal connectives. No expression was observed in the commisures of the ventral nervous system (Zhang et al., 1993).

Tenascins

Three types of tenascins have so far been isolated from *Drosophila melanogaster*. All three have been implicated in participating in neurogenesis. The first, *odd oz* codes for a 300 kDa protein with extensive homology to vertebrate tenascins. The extracellular portion of *ODD OZ* consists of eight *EGF*- like repeats (230- 280 aa) with seven putative glucosaminoglycan attachment sites homologous to that of vertebrate tenascins. The transmembrane domain anchors *ODD OZ* to the membrane. It has been postulated that the COOH terminal portion (1500aa) mediates signal transduction through its 5' consensus sites for tyrosine phosphorylation with no homology to either SH2 or SH3.

It has, however, no been demonstrated that *ODD OZ* is phosphorylated. Mutations in the *odd oz* gene manifest themselves in in an severaly impaired formation of connective an commissures. Addtitionally, the somata are partially missing (Levine etla., 1994).

ten^{a} encodes a transmembrane protein which contains several EGF- like repeats. It contrast to vertebrate tenascins, neither fibronectin type II like repeats, nor domains with fibrinogen homology are prominent. Its expression coincides with neuromuscular synaptogenesis, as well as, axonogenesis (Baumgartner and Chiquet- Ehrismann, 1993).

ten^{m} has been cloned using the tena EGF- like repeats as a probe. Its 7945 bp open reading frame contains five sites indicative of exon intron splice sites. The 281 kDa tenm protein contains towards its N- terminus a stretch homologous to the secretory signal sequence. This stretch is followed by a series of eight type EGF like motifs each of which is ~31 bp in length. C - terminally 11 repeated structures reminiscent to fibronectin type III like domains have been postulated. The remaining 277 amino acids display significant homology to RGD sites which in mammals bind to PS2 integrins and modulates their downstream activities. Unlike *ODD OZ* and *TEN*^{A} there are no indications that *TEN*^{M} contains a transmembrane domain. By contrast, *TEN*^{M} is likely to be a secreted extracellular matrix protein.

As *ODD OZ* and *TEN*^{A}, *TEN*^{M} is expressed during embryonic stages 12 and 13 on the somata of the ventral nervous system neurones. The *TEN*^{M} protein first appears first on the pioneering axons of the anterior commissures. Thereafter, it is found on the anterior commissures and longitudinal connectives. *In situ* analysis revealed that *ten*^{m} mRNAis not only transcribed at spatial prefigured locations of the ventral nervous system but appears also on the components of the tracheal tree. Mutant analysis should reveal more about the function of *ten*^{m} in the development of the central nervous system. It is interesting to note that the transcription of *ten*^{m} persists throughout larval development. *ten*^{m} is in particular confined to the ventral nerve cord, the cerebral ganglion and the imaginal discs. During the pupal stage the *TEN*^{M} protein seems to be involved in the development of the eye: Its expression coincides with the expression of *Drosophila* PS inte-

grins during the development of the eye (Zusman et al., 1993; Baumgartner et al., 1994).

Semaphorin

semaphorin (*sema*) genes in *Drosophila* encode proteins, which show a high degree of similarity to *Schistocerca FASCICLIN IV*. The two proteins encoded by the *sema* genes both share both structural domains. *D-SEMA I* codes for a putative transmembrane protein, whereas *D-SEMA II* encodes a secreted protein of about 400 aa with high similarity with chicken *collapsin*, which provides repulsive cues during growth cone guidance (Luo et al., 1993). Similarly, it was suggested that *FASCICLIN IV* and *semaphorins* may provide repulsive cues during *Drosophila* embryogenesis (Kolodkin et al., 1993).

BL97

BL97 was named after an enhancer trap. It is alternatively spliced but either splicing isoform generates an identical 881 aa transmembrane protein. Its four repeating domains of approximately 150 aa each display some degree of sequence homology to *Drosophila FASCICLINI* but a putative transmembrane domain was not detected. Mutations in *BL97* caused a phenotype almost identical to the hypermorphic *Abruptex* alleles of *Notch* suggesting that *BL97* may act as a negative modulator of *NOTCH* (Hu and Crews, 1993).

Signal Transduction Cascades

neurotactin

The predicted *NEUROTACTIN* heterophilic cell adhesion protein consists of an 120kDa core protein with two N-linked oligosaccharides. It has been proposed that the extracellular domain of *NEUROTACTIN* might be related to acetylcholine esterase (De la Escalera et al., 1990). The cytoplasmic domain of *NEUROTACTIN* can be phosphorylated by orthophosphate *in vitro*, but sequence comparisons revealed that it does not display

homologies to known threonine/serine or tyrosine kinases which in other molecules mediate the phosphorylation function (Jiménez et al., 1993). *NEUROTACTIN* expression predominates at junctions between neurones and other neurones or other cells and overlaps with the distribution of *AMALGAM*, which probably serves as a ligand for *NEUROTACTIN* (Barthalay et al., 1990). Mutations in the *neurotactin* gene display clear defects in axonal pattering.

DER-spitz/dpp

DER is the *Drosophila* homologue to the mammalian epidermal growth factor. It contains two cysteine rich repeats in the extracellular domain and a putative tyrosine kinase in the intracellular domain (Schejter and Shilo, 1989). It is ubiquitously expressed throughout the four germ layers in the *Drosophila* embryo. Its putative ligands are the *Drosophila* TGF-β homologous *decapentaplegic* (*dpp*) and *tolloid* as well as probably *Spitz*, the homologue of TGF-α (Rutledge et al., 1992). *dpp* presumably acts as a dorsalising signal, which is translated by the ventralising *DER* into dorso-ventral axis formation. It was suggested that upon binding of *dpp DER* interacts with *Spitz* group genes *star* and *Spitz* through a signal transduction pathway. Thsi suggestion follows the observation that mutations in the *DER* locus *faint little ball* (*flb*) display the same abnormalities as observed for mutations in the *spitz* group genes (Raz and Shilo, 1993).

Abelson kinase/ Protein Tyrosine Phosphatases

Receptor linked protein tyrosine phosphatases (PTPs) are understood to couple the binding of a ligand to their extracellular receptor to the dephosphorylation of the regulatory tyrosine residue of tyrosine kinases. This dephosphorylation is accompanied by a several fold increase in the catalytic activity of tyrosine kinases (Klausner and Samelson, 1991). It has been proposed that the PTPs are distributed asymmetrically along the growth cone of the axon. They might then dephosphorylate PTK, which prevents the assembly of microtubules in the region of the growth cone until an axonal tract has established proper contacts with adjacent cells (Yang et al., 1991). Thus, the

PTPs substrate in *Drosophila* might be the Abelson tyrosine kinase (D-*abl*) or one of its homologues. interactions may give each axon feedback signals to extend properly (Tian et al., 1991).

So far three homologous PTP genes have been identified by means of homology cloning. These include DLAR, DPTP99A and DPTP10D. All PTPs have in common that they are either alternatively transcribed (e.g. the three embryonic DPTP99A transcripts share exon1 whereas the postembryonic transcripts do not) or differentially spliced and polyadenylated (e.g. the three embryonic protein isoforms of DPTP99A differ in their COOH terminal domain). The extracellular N- terminal domains are commonly assembled of multiple FNIII repeats (numbering from 2- 12 dependent of the gene) with several glycosylation sites which are involved in substrate recognition. These are preceded by a transmembrane spanning domain. The intracellular domain is composed of either one (in DLAR, $DPTP_{10D}$) or two (in $DPTP_{99A}$) tandem repeats with homology to PTPase domains (Streuli et al., 1989; Tian et al. 1991; Yang et al., 1991). Differential posttranscriptional modifications manifest themselves in isotype specific COOH termini (Yang et al., 1991). Thus two $DPTP_{10D}$ isoforms, 185 kDa or 177 kDa , as well as two $DPTP_{99A}$ isoforms, of 137 and 120 kDa, respectively, have been predicted.

All three genes are first expressed, apposed to each other, in the neuroderm of germ band stage embryos. $DPTP_{99A}$ expression during stage 12 encompasses both in commissure generating RP (RP1, RP2, and RP3) cluster, and the connective/ intrasegmental nerve generating aCC/pCC cluster, as well as, in a subset of VUMs. The expression becomes then constricted to aCC and RP2 during stage 13 and 14 (Yang et al., 1991). It is apparent that by stage 14 expression in the connectives is most extensive at the junction with the connectives. Expression in the CNS persists until stage 17 but ceases before in the exit neurones. The transcriptional activity of $DPTP_{99A}$ is in accordance with these findings. pCC and the sibling neurone of aCC are excluded from expression (Tian et al., 1991; Yang et al., 1991). DLAR expression in contrast is more homologous (Streuli et al., 1989). There is a substantial overlap in the expression between $DPTP_{99A}$ and DLAR in both commissures and connectives during stages 13 and 14 of

embryonic development, although Tian and collaborators (1991) proposed a deviation in the expression pattern of both proteins in these domains.

$DPTP_{10D}$ expression is initially confined in each hemisegment to clusters of 8 cells adjacent to the midline. Next, during stages 13/ 14 the expression in connectives is more intense than in commissures. There $DPTP_{10D}$ expression predominates in the anterior commissure and is reduced in the posterior commissure. Prominent during stages 13 and 14 is the distribution of $DPTP_{10D}$ in the junction between anterior commissure and longitudinal axonal tracts. It was suggested (Tian et al., 1991) that the expression of $DPTP_{10D}$ is required for commissural growth cone interaction with MGM and MGA. $DPTP_{10D}$ is, however, not expressed in the inter- and intrasegmental nerve. Expression $DPTP_{10D}$ first fates in the commissures by stage 15 starting in from the midline. In the connective the expression of $DPTP_{10D}$ persists until stage 17. The protein expression patterns correspond to the transcriptional activity of the three genes as confined by in situ hybridisation. In neither case analogies could be drawn that would suggest that transcriptional and translational activity of the PTP genes corresponds to that of other known transmembrane proteins. As a substrate for the $DPTP_{10D}$ a 150kDa transmembrane molecule has been identified. This molecule denoted p150 binds the cytoplasmic domain of $DPTP_{10D}$ and shows similarity in its extracellular domain similarities to connectin and choaptin. Its expression predominates as muscle attachment sites (Tian et al., 1991; 1993).

GTP-binding protein

The function of GTP binding proteins, other than those in the *ras* signalling pathway, in *Drosophila* is not very well researched. Referring to mammalian studies on GTP binding proteins, these have been postulated to mediate changes in the actin cytoskeleton upon binding to an associated receptor (for review, see Nobes and Hall, 1994). Effectors and substrates of the *Drosophila* GTP- binding protein have so far not been found.

The GTP- binding protein isolated by Fredieu and Mahowald (1993) shows homology to the GTP binding regions of two signal recognition particle receptors containing

putative binding sites for the phoshoryl group and chelated Mg^{2+} of guanine ribonucleotide. Further a binding site for the keto and amino group of the guanine ring with a slight deviation from the consensus was predicted (Fredieu and Mahowald, 1993). GTPase was identified due to its expression in association with axonogenesis. Antibodies against the putative GTPase revealed that this antigen is not expressed until stage 9, 7 hours postfertilisation, when expression is restricted to the ventral midline to a region dorsal to the ventral neuroectoderm. Following germ band retraction GTPase appears in cells dorsolateral to the ventral midline with contact to the contralateral neuromere via processes, which are identical to the pioneering axons of the RP1 cluster.

ABELSON TYROSINE KINASE

ABELSON KINASE are related to the Src family of tyrosine kinases. Their src- homology 2 (SH2), src- homology 3 (SH3) and the kinase domains are for instance arranged in the same way as in their Src homologues. In contrast to the members of the Src family, however, they contain extensive COOH terminal domains which in some cases may be involved in nuclear targeting and DNA binding. Furthermore, it has been revealed that the constitutively active enzymatic kinase domain might be controlled by PTPs. Abelson kinases generally function to signal through the *ras* pathway (for reviews see Wang, 1993; Rodrigues and Park, 1994).

The non-receptor linked *Drosophila* Abelson kinase (*D-abl kinase*) displays in its SH2, SH3 and the kinase domains 80% similarity to its mammalian homologue. Heterozygous mutations for *D-abl kinase* lead to defects late during development, which resulted in either pupal or imaginal lethality. In particular, defects in the axonal pattern of the CNS were apparent. A suppressor mutation of *D-abl kinase* is *enabled.* It is possible, and logical that a mutation in the *enabled* gene leads to a permanent activation of the yet uncharacterised *enabled* gene product, which is thought to act downstream of *D-abl kinase* (Gertler et al., 1990).

D-abl kinase mRNA is supplied maternally to the embryo. This mRNA is first translated into a protein after fourth nuclear division in early blastoderm stage embryos. During cellularisation *D-abl kinase* is distributed along the cleavage furrows of the

compartmentalising cells. The protein predominates particularly at the cell junctions of cellular blastoderm stage embryos.

Zygotic *D-abl kinase* is not expressed until the germ band stage. In CNS it appears after stage 12/5 in the axonal tracts. By then *D-abl kinase* is expressed at higher levels in growth cones of the extending neurones. Its location in or near the growth cone determines its activity. The levels of *D-abl kinase* expression in longitudinal tracts are higher than in the latitudinal tracts. It has been suggested that *D-abl kinase* interacts with *disabled* and *fasciclin I* (*fasI*) at the axonal cell membrane.

Homozygous *fas I* and *D-abl kinase* double mutations manifest themselves in defective neural guidance of the commissural tracts during stage 12/3. Particularly evident is the failure of neurone RP1 to pioneer the commissural pathway properly. It has therefore been suggested that the driving force behind *fasI* expression might be the mitogen activated *D-abl kinase* signal transduction cascade (Elkins et al., 1990). Similarly, in homozygous *disabled* and *D-abl kinase* double mutant embryos axons do not appear to fasciculate properly to from commissures or connectives (Gertler et al., 1989). Hence, *D-abl kinase* is required for proper intercellular adhesion during axonal outgrowth. Beyond the embryonic development *D-abl kinase* expression persists in neurones until the late third instar. Though it disappears then temporally, it can be detected 6 hours after pupal formation in the brain again (Bennett and Hoffmann, 1992).

DFR (Drosophila fibroblast growth factor receptor homologue) - SYNDECAN Interactions

The *Drosophila* fibroblast Growth Factor homologue *DFR* contains in its extracellular domain several immunoglobulin repeats with an intracellular tyrosine kinase coupled to it (Shishido et al., 1993). *Drosophila Syndecan* is a hydrophilic heparin sulphate proteoglycan that presumably acts as a substrate for the *Drosophila* fibroblast growth factor tyrosine kinase coupled receptors homologues. *Syndecan* is expressed as three tissue specific isoforms, whereas two *DFR* isoforms where identified. *Syndecan 3* predominates in neural tissues. Its expression is in line with the expression of *DFR1* in

the connectives and *DFR2* in the commissures (Shishido et al., 1993; Spring et al., 1994).

DROSOPHILA PHOSPHOLIPASE C 21 (PLC21)

Phospholipase C is a likely candidate for the activation by receptor linked tyrosine kinases (RTK) or G- proteins. In platelets RTKs mediate the cleavage of *PtdIns(4,5)P2* to produce inositol triphosphate and diacylglycerol which both act downstream as second messengers to increase in intracellular Ca^{2+} levels. (Kim et al., 1991). In *Drosophila melanogaster* the locus *mushroom body miniature* (*mbm*) encodes such a phospholipase C designated *plc21* (Shortridge et al., 1991). Mutations in *mbm* lead to severe defects in the development of the corpora pedunculata. Two *plc21* isotypes have been predicted form the respective putative transcripts. The 7.6 kb transcript is head specific whereas the 5.6 kb transcript is distributed homogeneously in the whole animal. In the larval and imaginal heads the 7.6 kb *plc21* can be detected in the α- and β- lobes, as well as in a portion of the peduncle of the corpora pedunculata. In embryos *plc21* is expressed throughout the CNS (Albert et al., 1994).

Nuclear Proteins

POINTED (PNT)

A well studied locus of the Spitz group is *pointed* (*pnt*). *pnt* accommodates the open reading frames for two related putative transcription factors each containing an ETS motif. The shorter form, *pnt1*, is 623 aa in size, whereas the longer from comprises 718 aa. The C-terminal portion of *pnt1/2* which is encodes by exons 2/3 displays high homology to other related members of the *ETS*- family. The N-terminal domain, however, is unique to each protein. Two trans-acting sequences with either enhancing or silencing effects have been identified though their identity has not been revealed, yet. Mutational analysis of exon 2 revealed that the *ets*-domain is not solely required for transcriptional activation but may function in protein interactions as well. Hence, *ETS* -motif adequate but not essential for the function of both *pnt* proteins. The expression

of either protein is driven from two discrete promoters which are separated by 50 kb. The expression of *pnt1* is initiated form the first promoter during stage 6 in two broad stripes predictive of the neurogenic region in accordance with the pair rule pattern. During gastrulation the *pnt1* expression becomes confined to a three cell wide stripe adjacent to the neuroectodermal cells. The expression becomes further constricted to a two cell wide stripe during germ band extension. During stage 11 *pnt1* appears in the tracheal tree and in the head region. In the LGs *pnt1* expression proceeds until stage 14 whereafter it disappears. *pnt2* expression can be traced back to the anterior most region of the precellular blastoderm stage embryo. The expression of *pnt2* soon ceases and does not reappear until gastrulation where it emerges throughout the mesoderm including the mesoectodermal precursors form which the midline glia and the VUM cluster is derived. Hence, *pnt2* is responsible for the proper separation and segregation of commissures (Klämpt, 1993; Klaes et al., 1994). Homozygous mutants of *pnt2* display a fusion of the commissural tracts. In addition *pnt2* participates in the development of the compound eye where it is activated by MAP kinase at the PLTP motif (Brunner et al., 1994). However, it needs to be emphasised that *pnt1*, in contrast to *pnt2* is constitutively active and does not require the MAP signal transduction pathway for activation (O'Neill et al., 1994). The activation of *pnt2* in the ventral mesoectoderm by the putative *spitz* pathway, which probably involves either *rho* or *DER* awaits to be elucidated (Rut ledge et al., 1992; Raz and Shilo, 1993).

longitudinals lacking (lola)

longitudinals lacking (*lola*) is a mutation, which, as the mutant phenotypes described above, impairs the formation of connectives. *lola* codes for a nuclear protein with two zinc finger motives homologous to those of the known transcription factors tramtrak (62% identity), Broad Complex and GAGA. *lola* is expressed in three different isotypes, named *lola* 3.8, *lola* 4.7 and *lola* 4.9 indicating their relative length in kb. Strikingly, *lola* 3.8 does not contain a zinc finger motif. *lola* transcripts, presumably supplied maternally, are present already in the blastoderm stage embryo and are expressed during embryogenesis in other tissues as well. They accumulate during stages

10-13 predominately in the CNS and PNS, and thereafter only in the CNS (Seeger et al., 1993).

Reversed polarity (*repo*)

Weak alleles of reversed polarity (*repo*) are associated with reverse polarity of currents in the ommatidium of *Drosophila* eye. *repo* encodes a *PAIRED*- type homeodomain which functions as a transcriptional activator which is specifically expressed in longitudinal glia and probably SBCs. In *repo* mutants early development of glia proceeds normally, whereas later during development the longitudinal axon tracts are less condensed and appear thinner during stages 13- 15. During stage 9 *repo* is restricted to glioblasts at margin of each segment behind the segmental border. Two other clusters of *repo* expression appear at posterior and anterior segmental border (Xiong et al., 1994).

24. Mutant Phenotypes

Connectives

Longitudinal axonal pathways are the preformed by the proper positioning of LGs along the anterior posterior axis in the neuroectoderm. As seen before these glia provide the necessary guidance cues for the connectives to form. Proceeding germ band elongation the MP1/dMP2 pathway extends along the anterior axis and establishes longitudinal axonal connections.

In mutants for *pros* the early differentiation of LG proceeds normal. The LGs migrate to the same position as in wild type embryos. Thereafter, during stage 14, the differentiation of these cells is thought to become arrested in a preaxonogenic state. This state corresponds to the expression of *pros* in wild-type embryos. Moreover, LG do not elongate properly and form conspicuous clusters. On the cellular level an extensive rough endoplasmatic reticulum (rER), as well as, a electron lucent cytoplasm are prominent in mutants for *pros*. Last, the affinity for axons of the MP1/dMP2 is

severely impaired so that in *pros* mutants the longitudinal tracts do not form (Jacobs, 1993).

At least two genes of the *polycomb* group play a major part in the development of longitudinal connectives, as well. Genes of these group co-ordinate the spatial regulation of homeobox gene expression. Mutants for *polyhomeotic* (*ph*) display quite significant neuroma-like whorls and lack the ladder-like arrangement of axonal tracts. The development of the LGs follows to the wild type criteria, although no proper elongation of LG was observed in *ph* mutants. As gathered form EM sections axonal growth cones in *ph* mutants do not extend beyond the LG. It is plausible that in wild type the contacts between glia and axonal growth cones may trigger the differentiation of glia, which in turn enwrap the axons. Thus, in *ph* mutants this process might be impaired early during embryogenesis and hence, proper glia-neurone interactions fail to inaugurate axonal tracts (Jacobs, 1993).

In analogy mutants for *posterior sex combs* (*psc*) form neuroma like whorls. Associated with mutations in *Pc* group genes is the absence of the cell adhesion molecule neuroglian in the longitudinal pathways hindering their formation (Jacobs, 1993).

The loss of function of MECs due to their death or improper differentiation in mutants for *sim*, *slit* (*sli*), *rhomboid* (*rho*) and *Star* (*S*) displaces LG to the midline, where they fuse with their contralateral counterparts. As a consequence the longitudinal tracts are displaced medially. Hence, MECs might determine the radius allowed for LG to migrate medially, presumably by exercising an inhibitory function. In all these mutants the anterior-posterior axon elongation, though shifted medially, proceeds normally.

sim and *sli* mutants display complete fusion of the connectives to midline cells. Whereas in *sim* mutants the MEC die, in mutants for *Spitz* group genes, they fail to differentiate properly. In the other two mutants the fusion of connectives at the midline is incomplete. Additionally, in *sli* mutants the number of connectives in each segment is slightly reduced. On the EM level an extensive rER and a lucent cytoplasm are prominent in *sli* mutants. Only longitudinal axons display affinity for LG in these mutants, whereas more contralaterally extending axons do not allowing them to extend more dorsally. These dorsally extending lateral neurones cross the midline beneath the

more ventrally shifted VNE as a result of there lack in adhesiveness to the more ventrally positioned LG. Owing to the dispersion of longitudinal axonal tracts, LG are unable to ensheath the components of these pathways properly.

This versatile protein *pntl* seems to be required for axonogenesis in the longitudinal axonal tracts. *pntl* is probably involved in the direction of connectives, as its expression in LGs during axon formation may reflect the interactions between axons and LGs (Scholz et al., 1993).

The longitudinal tracts are interrupted in mutants for *midline (mid) Longitudinals lacking* (*lola*), *otd*, as well as in mutants, where the process of germ band retraction is impaired e.g. *hindsight* (*hnt*), *u-Shaped* (*ush*), *tail-up* (*tup*), and *serpent* (*srp*). In all these mutants the extension of the connectives may initially be normally, but axonal processes retract at the mid segment and instead extend towards the commissures. Whereas in these mutants the birth and initial development of LG proceeds normally, LGs do not form a proper scaffold, as they are not positioned properly.

In *hnt* mutants the initial scaffolding of axon tracts progresses as in wild-type embryos. As *hnt* mutant embryos do not undergo germ band shortening, the segments are more elongated then in wild-type embryos. Guidance cues such as neuroglian are therefore far more spread and LG fail to from a proper scaffold along the anterio-posterior axis.

mid is associated with the lack of neuroglian in the posterior third of each segment. Hence, no growth cone extends into the areas where neuroglian is absent. In addition, it has been proposed that in mutants for *mid* posterior extending axons fail to travel through the segmental gap into the adjacent segment where they cannot contact the LG.

It has been proposed that *lola* is required for the expression of neural guidance molecules on substratum cells rather then in neurones proper. In neurones only the 'blind' form *lola* 3.8 is expressed (Ginger et al., 1994). In mutants for *lola* the connectives between segmental ganglia are missing. In particular the outgrowth of neurones, which constitute the MP1 pathway seems to be impaired. The pioneering axons pCC, vMP2, and MP1 elongate and fasciculate as normal. The elongation is impaired once LG5 is encountered suggesting that *lola* functions during the interaction between

growth cones and glia. The number of axons, which reaches the neighbouring segment is therefore quite reduced (Seeger et al., 1993). The *lola* gene is presumably a component of the *NOTCH-DL* pathway.

Permissive mutants for *Notch* and *Dl* greatly impair the formation of axonal tracts between the segmental ganglia whilst the growth behaviour of commissures seems only to be modestly effected (***vide supra***). The number of LGs varies in *Notch* mutants from segment to segment. So in some segments there might be twice as many longitudinal glia whilst in adjacent segments these glia are absent. (Giniger et al., 1993).

Peculiar for the *roundabout* (*robo*) mutant phenotype is the enlargement and fuzzy arrangement of commisures in each segment, attributed to a misrouting of longitudinal tract growth cones. The early development and differentiation as well as the initial axon outgrowth of the misrouted longitudinal neurones progresses normally until stage 13. All cells, which participate in the axonal path finding appear to be in their proper position. Following the pathway of pCC in *robo* mutants, reveals that after they have projected anteriorly prior to encountering the LG5 they suddenly turn medially and cross the midline via the anterior commissure to enter the contralateral connective. Similarly MP1 first encounters LG5 and migrates posteriorly across the anterior commissure into the other hemisegment where it proceeds posteriorly along the connective. dMP2 follows basically the same course as pCC once it has projected anteriolaterally. Lateral *fasII* positive longitudinal tracts and the commissural tracts are excluded from any aberrations associated with the mutant phenotype (Seeger et al., 1993).

Commissures

Commissureless (*comm*) is a mutation, which results in the absence of commissural pathways in the embryonic ventral nervous system and cephalic lobe, whereas longitudinal axon pathways, nerve roots, peripheral axon pathways, and peripheral sensory neurones appear not to be affected. The exception that the supraoesophageal commissure is present in *comm* mutants proves the rule. It has therefore been proposed that *comm* mutants are specifically associated with defects in the guidance by midline cells (Seeger et al., 1993a,b). Thus, the absence of commissural tract reflects the failure of MGA,

MGM and MGP to provide the necessary substratum for the guidance of commissural axons. Owing to the absence of glia-neurone interactions, the MGs delocalise laterally. Commissural axons first extend and in the direction of the midline and retract soon afterwards. Axons turn instead rostrocaudally and enter the ipsilateral longitudinal axonal tracts. Additionally, commissural axons express *FASCICLIN II*, albeit the fasciculation process is incomplete in *comm* mutants. Apparently, the RP2 motoneurone, though it does to extend contralaterally, innervates the same muscle ipsilaterally in these mutants .

In Mutants for *Notch* commissural tracts are generally absent and the development of the midline is severely disrupted. Axons rather project into the ipsilateral site where they do not even form connections. Temperature shift experiments on permissive *Notch* mutations revealed that *NOTCH* is required early during development of the midline during the cellular blastoderm stage. After this critical period *NOTCH* appears to be dispensable for the formation of midline cells. Embryos deprived of the maternal *NOTCH* contribution lack MGM whilst MGP are duplicated. These findings lead Menne and Klämpt (1994) to conclude that maternal and zygotic *NOTCH* function is required for the proper activation of *sim*.. It may also be conceivable that apart form *sim* other neural genes such as *l'sc*, *E(spl)* are active during midline cell development.

In *sim* and *slit* mutants the midline cells either die or do not differentiate properly during stage 12/3. In mutations for *sim* the two MP1 progeny, the VUMs and some mesoectodermal cells are missing and both anterior and posterior commissures are absent. As during stage 12/3 the commissures are normally established, it is thought that the lack of contact between the commissural axons and the midline cells leads in *sim* mutants to the death of the midline cells. This proves that for the proper differentiation and survival, interactions between both cell types are absolutely essential. It *slit* mutant embryos the posterior commissure forms on the odd occasion. The ventral displacement of midline cells yields, however, the collapse of initial commissures and thus prevents these from becoming established.

otd is another mutation affecting the formation of commissural pathways. Here the posterior commissures in each segment are missing with the anterior commissure re-

duced. Extension of commissures proceeds normally, the commissures make, however, another pathway choice. Instead of extending contralaterally, in *otd* mutant embryos the posterior commissures extend ipsilaterally in a posterior direction. Anterior commissures, in contrast, innervate the midline directly. Associated with the abnormal commissure formation is that VUM and midline neuroblasts die during 12/3. Thus, the posterior commissure is not able to interact normally with these midline cells and therefore chooses another fate.

Mutations in the three members of Spitz group *spitz*, *Star* and *rhomboid (rho)* generate fused commissures as a result of the failure of MGM to migrate accordingly and to separate both commissures.

In *Star* mutant embryos the onset of the midline glia cell degeneration is retarded to stage 14 as a result of static MGMs. *spitz* encodes a homologue to TGF-α, which binds in vertebrates to the EGF (Rutledge et al., 1992). In *spitz* mutant embryos the arrangement of the midline glia cells is aberrant and therefore the commissures fuse. In *rho* mutant embryos the glia cells seem to be depleted during early development indicating that it might be required during early development.

Particularly well studied is the Spitz group gene *pnt2*. In homozygous mutants for *pnt2* MGM, which normally extend filopodia towards the VUM to 'pull' themselves across the of MGAs, appear not to recognise the surface of the VUMs (Scholz et al., 1993). As the consequence the MG cells become arranged contralaterally along the commissural connections. The recognition of axonal membranes, however, does not seem to be impaired. Thus, as the commissural fibres extend toward the midline they do not become separated into distinct anterior and posterior commissures but form a single fascicle. In addition, it has been demonstrated that strong alleles of *pnt* are associated with reduced expression of the neural antigen 22C10 on both MP2 interneurones at stage 12. SP1 and other neurones are, however, not affected by *pnt* mutants. 22C10 in MP2 neurones is enhanced when *pnt* is ectopically expressed suggesting that new cells have adopted MP2 fate. It has therefore been proposed that midline glia cells, which exclusively exhibit expression of *pnt2* , are responsible for the introduction of 22C10 on neurones (Klaes et al., 1994).

In *ming* mutant embryos the posterior commissure is thinner than in wild type embryos. The *fasciclin II* positive fascicle of this commissure is missing in these mutants.

Intersegmental Nerve

Each neuromere has two peripheral nerve roots where the motoneurones exit the CNS and enter the PNS. The main exit nerve is the intrasegmental nerve (SN) leaves the nervous system by each segment. The intersegmental nerve (ISN), however, exits the central nervous system via the intersegmental nerve root. Two types of glia cells presage the intersegmental nerve root. The intersegmental nerve at the boundary of the CNS is a lucrative system to study axon guidance. Segment boundary cells demarcate the point at which aCC and U growth cones leave the CNS in *Schistocerca* (Bastiani and Goodman, 1986). In *Drosophila* analogous cells have the same task (for review, see Goodman and Doe, 1993). These afferent motoneurones exit the CNS at segment boundary cells and extend and fasciculate along prefigured sets of tracheal cells before they innervate the respective motoneurones in a stereotyped mode. The guidance process of these motoaxones is governed by sets of cell surface molecules, which mark the putative pathway of these neurites. As seen before the ISN courses by stage 13 along the surface of the trachea. The specification of the trajectory intersegmental nerve depends on *DL* and *NOTCH*. The expression of *Dl* is confined to the trachea and the expression of *Notch* to axons. Hence, the expression of on the tracheal cells *DL* defines the path of the ISN axons (Giniger et al., 1993a). Additionally, in the *DL NOTCH* mutant embryos at least four times as many exit glia cells are present.

In *lola* mutants the axons, which exit by the ISN either fail to grow and migrate into neighbouring segments, or defasciculate as in *Dl* or *NOTCH* mutants, which probably either reflects the absence of a cellular substratum for the nerve or a defects in the guidance system (Giniger et al., 1994). The pan-neural gene *pros* regulates the proper outgrowth of aCC an pCC motoneurones. In mutants for *pros* the outgrowth along the segmental or intersegmental nerve track is impaired. It has been proposed that pros coregulates the neural identity of aCC and pCC in conjunction with *eve* and *ftz* (Vaessin et al., 1991; Doe et al., 1991). In some cases the two nerve roots are partially fused in

logo mutants . In other cases one of the nerve roots is even shifted or motor neurones take a different exit from that of wild type embryos (Seeger et al., 1993).

3. Synthesis

Axonogenesis is a very complex process, which requires the extension of axons in the correct position, the proper guidance by auxiliary cells such as glia, other neurones and trachea along preformed pathways and the proper targeting of these axons in a stereotyped mode. Many research groups have selected for neuroma mutants phenotypes in hope to elucidate pathways underlying axonogenesis. Two events, which underlay axonogenesis can be distinguished: Firstly, cells marking the path of axons need to become positioned properly. Secondly, the communicational events between axons and glial need to function appropriately to establish the respective axonal tracts.

The positioning of the cells is a function of the segmentation process. The communicational events use several pathways to feed back postional information to each neurone.

Once a neurone is born, it first needs to position itself appropriately before is extends axonal processes. As described above mutations affecting the neural as well as glial identity have been described before. Depending on the positional cues the neurone decides as to whether it should extend its axons either into the longitudinal connectives or into the horizontal commissures. Two types of positional cues have been identified. The first type is purely chemotrophic. It is provided in form of a secreted polypeptide. *semaphorin* (*sema*) genes encode such polypeptides which may provide repulsive cues during *Drosophila* embryogenesis (Kolodkin et al., 1993). Other guidance cues may be expressed on the surface of the membranes and aid the axonal navigation process. Such guidance cues may be provided by the three *fasiscilins* and *neuroglian.*

Most of the guidance cues molecules do not have any intracellular domains and therefore function to enable signal receptors to find there appropriate substrate by physically matching two cells with another. The cascades initiated by substrate recognition may give the neurone a feed back signal about its course.

The *Notch* pathway as many receptor linked tyrosine phosphatases and tyrosine kinases are assumed to operate as feedback devices, which control the navigation of the axon. The *Drosophila* homologue of non receptor linked *Abelson kinase* (*D-Abl*) is a good candidate for this tyrosine kinase as it activates a downstream signalling cascade, which finally results in transcriptional activation. As the catalytic domain of *D-Abl* kinase is probably like in other members of its family constitutively active, one may reason, that its activity is controlled by *Drosophila Protein Tyrosine Phosphatase* homologues (*DPTP*). Upon binding of a ligand to the extracellular domain, DPTPs are thought to dephosphorylate *D-abl kinase* and, hence, to activate its catalytic domain. As a result it has been proposed *D-abl kinase* might be activated particularly in the growth cones of distinct subsets of neurones. The details of downstream responses have so far not been elucidated, but it is understood from mutant studies that in response to the active *D-abl* signalling pathway the transcription of the extracellular matrix glycoprotein fasciclin I is initiated. The *Drosophila* GTP binding protein might be involved in mediating the downstream response in the *D-Abl* kinase signal transduction cascade.

Of particular interest are the interactions between mesoectodermal precursor cells and commissural growth cones at the midline. Once a growth cone has reached the midline several types of midline cells regroup. There is the possibility that the axonal growth cone triggers the differentiation of the glial cells by activating the appropriate signalling pathways. In ommatidial precursors the *sev* signalling cascade coupled to the MAP kinase activates the ETS like transcription factor *pointed 2* (*pnt2*) through interaction with the *pnt2* MAP kinase phosphorylation site. In analogy it might be conceivable that the same mechanism might be involved in controlling *pnt 2* transcriptional activity in midline glia and the ventral median unpaired (VUM) neurones. In the VUM cells the Star group genes may mediate the differentiation. *Spitz*, for example encodes a EGF transmembrane protein with high homology to TGFα, which may represent a putative ligand for the transmembrane receptor *rhomboid* (*rho*) (Rutledge, 1992; Noll et al., 1994) or *DER* (Raz and Shilo, 1993; Kolodkin et al., 1994). It might be well conceivable that *rho* signals in conjunction with *DER* and *Star* downstream the MAP *ras*

pathway to activate *pnt2*. and thus triggering the transcriptional events that serve to trigger the appropriate midline reorganisation events (Brunner et al., 1994). Moreover, it has been demonstrated that in glia ectopically expressed *pnt1* cDNA triggered the expression of 22C10 antigen on adjacent neurones (Klaes et al., 1994).

In the ommatidium the *NOTCH* pathway is thought to antagonise the *SEVENLESS* pathway. Whereas the *SEVENLESS* pathway seems to induce cellular differentiation, the *NOTCH* pathway counteracts the *SEVENLESS* pathway. During axonogenesis mutations in *NOTCH* mostly affect glial differentiation and have therefore only a secondary impact on axonal navigation. *longitudinals lacking* (*lola*) might be closely linked to the *NOTCH* pathway. *roundabout* (*robo*) and *commissureless* (*comm*), in contrast, may be involved in the differentiation and positioning of glia. The differential expression of surface molecules on these glia may then presage the path for the correct neural growth cone navigation (Seeger et al., 1993).

CHAPTER 7

Synaptogenesis

Synapses can either occur between either several neurones or between neurones and their muscular targets. All synapses have in common that they transfer a charge from a neurone to its target. As synaptic circuits are discussed later I wish to discuss here how synapses are generated. In *Drosophila melanogaster* it is difficult to follow the formation of synaptic connections *in vivo*. This applies particularly to the electrical properties of the synapses during their formation which is a crucial test for their function. An awarding studying system is the formation neuromuscular junction allowing one to draw parallels on the formation of synapses between CNS neurones. The motoneurones aCC, RP1, RP3, and RP4 leave the CNS via the intersegmental nerve root to innervate the discrete syncytial muscle fibres muscle fibres within each hemisegment. Two classes of motoneurones with discrete anatomy innervate the neuromuscular junction have been identified: Type I interneurones are characterised by their stereotyped branch anatomy. Their endings correspond to large- size buttons, which innervate distinct regions generally of only a single muscle fibre. Their motor outputs appear to be tonic and fatigue resistant. The type I neurones use several neurotransmitter. Type II neurones, in contrast, display a variable anatomy. They extend over the surface of multiple muscle fibres surface.

1. The Course of Synaptogenesis

The type II motoneurones evoke fast wide spread muscular contractions and are easily fatiguing. Their small buttons appear not to use synaptic co-transmitters. Several neurotransmitter have been localised to the neuromuscular junction. Most frequently used is, however, glutamate (Johansen et al., 1989). Additionally proloctine and octopamine might co-localise with glutameric synapses (Anderson et al., 1988; Halpern et al., 1991).

As long as motoneurones have not encountered their target muscle, their axonal endings form characteristic growth cones. Once they have found their muscle target, they differentiate into fully functional neuromuscular junctions. Type I motoneurone RP3 sends its projections onto muscle fibre 7 and 6. When it meets its muscle target, it emanates filopodia in stereotyped directions, which then course along and innervate the myotubules in a presaged mode. RP3 first explores the surface of the myocyte anterior and posterior to the target point. The anterior processes retract consequently and instead more processes extend posterior to the target site. Filopodia explore the surface of myotubules 6 and 7 Moreover, thereby extending lateral branches into the region of the presumptive synaptic cleft between 13.5 and 14.5 hrs after egg laying (AEL). Finally only the anterior-medial processes are left, whereas all other processes retract. During the time of filopodial exploration the axonal growth cone expresses the first neurotransmitter (12.5 hrs AEL). Only shortly afterwards the myotubules fuse to generate single cells (13- 13.5 hrs AEL). The glutamate receptors (gluRs) are expressed immediately after myotube uncoupling (12.5 - 13 hrs AEL). They first appear evenly distributed over the entire surface of the myotubule and subsequently cluster at the future synaptic zone (13.25 - 14 hrs AEL) (compare Burden, 1993). Coincidentally, filopodia and neurotransmitter accumulate in the region of the synaptic cleft in the premotor fibre. The filopodial localisation is completed by 14- 14.5 hrs AEL.

A functional neuromuscular junction is characterised by the presence of neurotransmitter and its receptor in the presynaptic and postsynaptic cell, respectively. Hence, the neurotransmitter glutamate and/or the gluR are the rate limiting factor. The activity of a unitary receptor is mirrored by changes in charge in the postsynaptic membrane. To cause a fully borne postsynaptic excitatory junctional potential, the activities of several neurotransmitters receptors (quantum responses) need to coincide. Thus, either the temporal or the spatial summation of postsynaptic potentials result in an excitatory potential.

As in an immature neuromuscular junction (14- 14.5 hrs AEL) the gluR has not sufficiently accumulated, the spatial distribution gluR is the rate limiting factor for the postsynaptic response. As the amplitudes of voltage clamped myotubes reveal in the

14.25 hrs. old embryos, the 10 -15 gluR are already sufficient to trigger an EJP. The time course of the myotubular amplitudes during this stage is other than in mature neurones, however, far more prolonged.

The temporal limit of the quantal response is set by the availability of neurotransmitter. As in immature presynaptic terminals not sufficient glutamate is present, the transmission of 13.5 hr AEL synapses fatigues relatively fast when recorded at a frequency of 0.1 Hz. With maturation of the synapse the fatigue response declines from <5 sec (13.5 hrs AEL) to <60 sec. (15 hrs AEL) due to the enhanced availability of glutamate (Broadie and Bate, 1993a).

2. Molecular Biology of Synaptogenesis

Hence, the innervation pattern of muscle fibre 13 by motoneurone RP1 follows a distinct timetable. This predictable synaptic timetable is, however, only observed in wild type embryos. Mutations, heat shock induced myotubule duplications or laser ablation experiments lead to an aberrant motoneurone innervation pattern. Neuromuscular innervation starts with the expression of adhesive molecules such as *CONNECTIN*. *CONNECTIN* is ubiquitously expressed following the innervation of the muscle by the synapsis. The homophilic cell adhesion molecules *FASCICLIN* I and III are expressed on axonal growth cones and myotubules. RP expresses specifically *FASCICLIN* I, whereas *FASCICLIN* III is concentrated in different subset of muscle fibres. Although single mutants for either *fasciclin* I and *fasciclin* III display no aberrant phenotype, *fasciclin* I and *fasciclin* III double mutants cause severe defects in the outgrowth of motoneurone axons as indicated by increased branching at target and axon defasciculation. In spite these abnormalities, RP3 still innervates fibre 7.

In mutant embryos for *prospero*, however, *FASCICLIN* III is not expressed on the muscle fibre. Moreover, though the expression of the gluR starts normally, the accumulation of gluR at the presumptive neuromuscular junction following nerve- muscle contact is absent in such mutations. Thus, as *prospero* is exclusively expressed on nerve cells, it has been suggested that the nerve fibre directs the expression of *FAS-*

CICLIN III on the synapsis, and Moreover, triggers the accumulation, but not the initial expression, of gluR (Broadie and Bate, 1993b).

Mutations for *rhomboid*, on the other hand, suppress the development of myotubule 7. Because myotubule 7 fibre cannot serve as a target in *rhomboid* mutants, its effector, the motoneurone RP3 targets solely myotubule 6. In line with this observation is the aberrant innervation pattern of RP1 observed when myotubules 13 were duplicated by heat shock treatment of embryos. In this case 3% of abdominal segments innervated myotubule 13, as well as, its duplicate. The innervation pattern for RP3, in contrast, was normal. The mutant phenotype of mutations for *numb* manifests itself in the absence of muscle fibres 7, 12, and 13. Though the target for RP1 was deleted in these mutants, RP3 was able to find and innervate the remaining muscle fibre 6 correctly. As a result of missing targets RP1 innervates myotubule 6 ectopically in 13% of the cases. In agreement with these findings, laser ablation of muscle fibres in third instar nerve yield ectopic innervation of other muscle fibres without affecting the arrangement of native motoneurones on this muscle.

In mutants for the dorso-ventral transmembrane receptor *toll* a sample of 23% RP neurones are depleted and myotubules 7 and 6 are not innervated by RP3. Instead both fibres are ectopically innervated by other fibres. Zygotic *toll* expression is confined to subset of ventral myotubules where it accumulates at the contact sites between muscle fibres. It was thus proposed that *toll* has a putative role in synaptic recognition.

It seems likely that the numbers of synapses are also regulated by their electric activity. To test this notion the injection of the Na^+ channel blocker tetradotoxic (TTX) was injected into embryos, which undergo synaptogenesis. TTX impairs the body wall peristalsis and hatching movements. Its injection into developing embryos during synaptogenesis increases the number of neuromuscular junctions two fold. The innervation pattern of muscle fibre 7 and 6 appears, however to be not affected.

CHAPTER 8

Control of Neuronal Differentiation by Hormones

In the syncytial embryo cells can easily synchronised in mitosis and differentiation by mutual cellular interactions. The regulation of the cell cycle becomes increasingly difficult in the multicellular embryos, larva, pupae and imagoes. It is not know as to whether the system which regulates cellular differentiation events is present already in the embryo, but it certainly is involved in co-ordinating the cellular events associated with the larval moulds and metamorphosis. The best characterised hormone is the steroid hormone ecdysone but yet other hormones such as retinoic acid are assumed to occur in *Drosophila* as well. During metamorphosis, when ecdysone titres are highest this hormone triggers the histolysis of strictly larval structures and promotes the differentiation of imaginal tissues. Particularly, it is thought to prepare the chemosensory memory pathways for their imaginal function.

1. 20-Hydroxyecdysone Induces Hierarchical Gene Transcription Cascades

The observation that ecdysone triggers changes in the puffing pattern of *Drosophila* salivary gland chromosomes led to the assumption that these hormones either directly or indirectly regulate the transcriptional activity of certain subsets of genes in a hierarchical manner (Clever and Karlson, 1960; Ashburner, 1990). Ecdysone seems to be the trigger for wandering behaviour of the third instar larva 72- 80 hours post larval hatching. This behaviour is accompanied by changes in gene transcription (Beckendorf and Kafatos, 1976; Belyaeva et al., 1981; Lepesant et al. al, 1982; Savakis et al., 1986; Georgel, 1991). In *Manduca sexta* the wandering behaviour is initiayted following a small peak of ecdysone after the extensive feeding period (Riddiford, 1986). The synthesis of ecdysone in the ring gland of *Drosophila* depends on the catalytic actions of a cascade of enzymes that use cholesterol as their substrate (Grieneisen et al., 1993). Mutations within this enzymatic pathway induce larval growth arrest, but do not effect the embryonic development. In particular, the pupal head evasion and other metamor-

phosis characteristical processes are suppressed (Sliter and Gilbert, 1992). Responses to the ecdysone peaks are tissue specific and not all tissues respond to ecdysone in the same manner (Andres and Cherbas, 1993). Ecdysteroids bind to a distinct class of receptors called the steroid hormone receptor (*HR*). Binding of hormone to the receptor incudes an allosteric change in the protein and therewith alters the conformation of the receptor for DNA binding (for review see Evans, 1988; Beato, 1989; Segraves, 1991; Karlson et al., 1994). The dimer hormone receptor binds to the specific ecdysone specific receptor element which adopts the form of an imperfect palindronic sequence PuG(G/T)T(C/G)A(N)TG(C/A)(C/A)(C/T)Py in a tissue specific manner (Ozyhar et al., 1991; Antoniewski et al., 1993).

2. 20-Hydroxyecdysone Responsive Elements and Their Involvement in Neuronal Differentiation

The putative ecdysone receptor of *Drosophila* (Koelle et al., 1991) occurs in three splicing isoforms with identical DNA and hormone binding domains but with different in the N-terminal domains. The *EcR* requires the *Drosophila* homologue of the retinoic X receptor ultraspiracle for heterodimerisation (Thomas et al., 1993). Ecdysone may either stabilise the dimerisation event (Koelle et al, 1993) or alternatively may terminate EcR binding to *EcRE* (for review see Cherbas, 1993). Each of these three isotypes have spatially and temporally restricted expression patterns in different tissues. Ecdysone receptor *EcRA* is presumably maternally supplied and its 4 kb *EcRA* mRNA was detected as early as 3 hours after egg laying (Talbot et al., 1993). It is newly synthesised in 12 hour embryos and occurs synergistically with the *EcR-B1* isoform in 18-21 hour embryos. These two isoforms are preferentially expressed in the CNS of embryos larvae and pupae (McNabb and Truman, 1993; Truman et al., 1994). The expression of *EcR-B1* in embryos predominates in glial cells and is thought to be involved in axonal outgrowth (Dübendorfer, 1981; McNabb and Truman, 1993). *EcR* mutations in embryos are, however, not lethal.

The larval and pupal expression of both isoforms strongly correlates with the titres of hydroecdysone(20-OH ecdysone). Both receptor isotypes have different tasks.

Whereas EcR promotes the maturation of tissues, *EcR-B1* is associated with the proliferation. In mushroom body neurones *EcR-B1* can be detected at the onset of metamorphosis. This is exactly the time when the peduncular Kenyon axons of *Drosophila* and the meal beetle *Tenebrio molitor* are assumed to rearrange (Technau and Heisenberg, 1982; Breidbach, 1993; Breidbach, pers. comm.). According to Technau and Heisenberg's findings several larval axonal elements degenerated during the onset of metamorphosis. Their data revealed 'scattered profiles of groups of extremely thin fibres' replacing about 40% of degenerating larval axonal processes 14 hours after puparium formation. Six hours afterwards the this process was completed. These finding contrasted strongly to the observations of Breidbach, pers. comm.) in the beetle *Tenebrio* which also belongs to the holometabolous insects. A neurone of the antennoglomerular tract persisted until adult stage without any significant changes. As Breidbach suggested, the neurones are closely packed into glial scaffold that no such gross anatomical changes are possible. The ecdysone isoform *EcR-B1* is detected in 108 hours after egg laying. It levels peak during puparium formation and disappeared 50 hours after puparium formation. This isoform was assumed to promote axonogenesis during this stage in *Drosophila* and other insect (Oland and Hayashi, 1993; Truman et al., 1994).

In analogy to the corpora pedunculata 20- OH ecdysone the development of the imaginal olfactory system occurs during early puparium formation in holometabolous insects. Sensory axons grow inward the brain - possibly along guiding cues provided by glia cells - where they meet the olfactory interneurones in *Periplaneta americana* (Salecker and Boeckh, 1994). In *Manduca sexta* the formation of the chemosensory tracts is completed by 60% of development (Sanes and Hildebrand 1976 a, b, c; Jan and Jan, 1990). Olfactory binding proteins are normally expressed in *Manduca sexta* 36-40 hours before imago emergence, but when deprived of ecdysteroids the onset of olfactory binding proteins in the antennal sensilla are expressed prematurely. Thus, varying titres of ecdysone seem to have opposing effects of the different tissues that constitute the chemosensory memory pathways (Vogt et al., 1993).

3. Ecdysone Responsive Genes

Ashburner (for review see 1990) proposed a model according to which ecdysone acts in a hierarchical mode to activate several genetic regulatory cascades. The progression of ecdysone induced puffs in salivary gland chromosomes depends on the *de novo* synthesis of proteins. Whereas early puffs persist when the protein synthesis is inhibited, late puffs do not appear. Thus, it can be conceded that the induction of late puffs depends on the production of early proteins, which soon shut down their own transcription. In terms of their response two classes of ecdysone inducible genes can be distinguished. Class I genes (EcR, BR-C and E74 transcripts) are transcribed in response to low but increasing 20-OH ecdysone titres. Class II transcripts (*E75A/B/C*, and *BR-C*) are characterised by their transcription in response to peaking 20-OH ecdysone titres (Karim and Thummel, 1992). The 74EF early puff encodes two different proteins with distinct N-terminal domains and a common C-terminal domain. Both proteins *74A* and *74B* 1/2 contain a common *ets*- related motif in their C-terminal domain. These putative *ets* - motives are thought to bind regulatory sequences of genes that induce cellular differentiation (Chen, 1992). Transcription of*E74A* and *E74B* is differentially initiated from two discrete promoters during the development of the third instar larva. Both proteins persists into the late pupal stage, but whereas the expression of *E74B* ceases prior to eclosion, the expression of *E74A* persists during imaginal development (Burtis et al., 1990). *E74A* transcription is already detectable in the late embryo (16hrs AEL) presumably under control of embryonic tissue specific interactions. Later activation seems to be controlled by ecdysone although it is not known as to whether the *EcR* binds directly to the promoter or other transcription factors similar to those in embryos bind the promoter in the larva. Interestingly, *E74A* transcripts appear in the proliferation centres of the larva brain indicating that they may mediate there the transformation from larval neuropil to an imaginal neuropil (Thummel et al., 1990).

The *Broad-Complex* (*BR-C*) locus, encodes a dozen different transcripts each containing a distinct reading frame as a result of the use of differential promoters and/or discrete splicing activities. Using the arrangement of the C_2H_2 zinc fingers as a crite-

rion, three classes of transcripts can be distinguished which all share a 'core' exon (Dibello et al., 1991). The transcripts of the *BR-C* accumulate and are required during early metamorphosis. In mutants for *BR-C* the central nervous system is not properly remodelled (Restifo and White, 1991). The E75 20-OH ecdysone inducible puff contains three open reading frames of several nested transcription units. All transcripts share a common 3' exon and differ in their N-terminal exons which translate into the N-terminus (Segraves and Hogness, 1990; for review see Segraves, 1991).

Of the variety of genes that are induced by ecdysteroids the ecdysone inducible protein 28/29 has been well studied. *Eip 28/29* encodes two alternatively spliced transcripts of 979 and 967 bp respectively giving rise to *28/29* kDa proteins (Cherbas et al., 1986; for review see Cherbas, 1993). Both transcripts are expressed throughout development in the central nervous system in response to 20-OH ecdysone peaks. Two regions have been identified to act as a down stream enhancer region (KE) located 450 bp apart from the *Eip 28/29* gene. Transcriptional activity further depends upon an auxiliary sequence between -40 and -134 bp. These regions have been identified to contain *EcRE*. (Cherbas et al., 1991). Further *EcRE* have been identified proximal from +2651 to 2681 bp as well as distal from +4433 to +4469. The function of the downstream regulatory region seems to be essential for expression in the nervous system (Andres and Cherbas, 1992). A model predicts that in absence of ecdysone *EcR* inhibits the transcriptional activation *Eip 28/29*. The number of *EcRE* determines therefore with which efficiency ecdysone inducible genes are suppressed (for review see Cherbas, 1993).

The E78 locus of the late early 78C puff codes for two transcripts that are expressed in response to a E75 protein only from early to mid-pupal development. The nested transcription units of this locus share several polyadenylation signals. Whereas the *E78A* transcript retains full DNA binding ability, the shorter *E78B* lacks the DNA binding domain. Both proteins belong to the hormone receptor superfamily (Stone and Thummel, 1993). About its tissue specific interactions nothing is known so far but the genes may mediate tissue specific responses during the onset of pupation (Steven Rusell, pers. comm.).

4. Ecdysone and Apoptosis

Cell death is a prominent feature during development. In the ventral nerve cord of stage 16 *Drosophila* embryos - coinciding with the first occurrence of *EcR-A* - apoptotic cells have routinely been observed (Abrams et al., 1993). In addition Robinow and Truman (1993) observed that of 300 ventral nerve cord neurones that express the ecdysteroid receptor *EcR-A* all but two are apoptosed during metamorphosis. Moreover, both authors have demonstrated that upon application of 20-OH ecdysone the respective neurones do not undergo apoptosis. *EcR-A* can, hence, be assumed to mediate apoptosis in absence of 20-OH ecdysone. Interestingly, the intersegmental muscle of *Manduca sexta* expresses prior to its histolysis ubiquitin. The expression of ubiquitin coincides with a time interval during which the titres of 20 -OH ecdysone are particularly low. Ubiquitin is a highly conserved 79 aa protein which marks certain type of proteins for degradation. Upon binding to the protein which is predetermined to undergo degradation, three other enzymes are activated which under consumption of a ATP connect ubiquitin to the acceptor protein. Once marked the acceptor protein is attacked by a protease complex (Proteasome) which cleaves it into several polypetides or single amino acids (for review see Karlson et al., 1994).

5. Juvenile Hormone

The activation of late ecdysone responsive genes that are responsible for initiating metamorphosis is suppressed during the larval development by the sesquiterpene juvenile hormone (JH) (Richards , 1978) Thus, high titres of juvenile hormones during larval development inhibit metamorphosis (Riddiford, 1974; Riddifird and Truman, 1974). As the synthesis of ecdysone, the production of the three JHs (I, II, III) in *Locusta migratoria migratorioides* R. is regulated by the circadian control centres (Horseman et al., 1994). JHIII is produced ubiquitously during insect larva development (Richard et al., 1989). In the *Drosophila melanogaster* female imago JH III functions to co-ordinate sexual maturation and reproductive physiology (Riddiford, 1974; Riddiford and Truman, 1974; Bownes, 1989). Neurosecretory cells that are

based in the pars intercerebralis release allostatins, allatropins and allatinhibitins. Whereas allatostatin and allatinihibitin inhibit the synthesis of JH III allatotropins mediates the synthesis of JHIII and methyl farnesoate in the corpora allata or the corpus allatum portion of the ring gland (Rembold et al., 1986; Bhaskaran et al., 1990; Donly et al., 1993; Unni et al., 1991; Richard et al., 1990). Electrical stimulation of the nervi corpora allatii inhibits the synthesis of JH III in ovipositioning females (Horseman, 1994).

When released, JH III presumably binds a specific JH binding protein that represses certain actions of 20 OH- ecdysone (Ilan et al., 1972). It has for example been demonstrated that the JH analogue methoprene represses the activation of the small heat shock protein by 20-OH ecdysone (Berger et al., 1992).

Part 4
Mnemogenesis

The function of the imaginal nervous system is it to receive, to transmit and to process information. The majority of these processes seem to be enrolled in the cerebral ganglion of the mature insect. Accordingly, the great P.E.Howse (1974) proposed that three distinct functions can be ascribed to cerebral ganglion:

- the activation of motor centres
- the co-ordination of the nervous activity
- translation of process resulting from one type nervous activity into another.

Chemosensory memory pathways receive chemical signals and convert those into a signal in form of an action potential. Though *Drosophila* is well understood in terms of development and genetics, the anatomical, biophysical and biochemical properties of the protocerebrum are less well researched, because insects such as *Schistocerca* and *Apis mellifera* are much more accessible to electrophysiological and behavioural studies then *Drosophila*, partially due to the fact that both display an much more differentiated behavioural patterns in nature than *Drosophila*. Moreover, the compactness and less sophisticated anatomy make it difficult to conduct electrophysiolocial experiments on *Drosophila*. Last, chemosensory perception, unlike this of several moth species is very primitive in *Drosophila*. I thus have made an attempt to describe the function of the chemosensory pathways in an ideal insect, which assembles all of the extraordinary abilities of other insect species. Though phylogenically incorrect, this evolutionary unified insect serves its purpose to exemplify my theory.

CHAPTER 1

The Anatomy of Insect Chemosensory Memory Networks

1. Organisation of Insect Nervous Systems

The nervous system of the imago consists of the supra- and suboesophageal ganglion, as well as, the ventral nerve cord and the peripheral nervous system. The supra- and suboesophageal ganglion represent an amalgamation between various commissures and connectives in the anterior region of the insect. In the supraoesophageal ganglion three distinct entities can be distinguished: The protocerebrum receives input form the ocular and sensory organs. It in turn comprises several neuropil masses: the optic lobe, the ocellar organs, the central complex (including the protocerebral bridge), the corpora pedunculata and the pars intercerebralis. The deuterocerebrum is chiefly made up of olfactory neurones like the antennal glomeruli which represent the sites of synaptic input from each antenna into commissures connecting both sites. From here fibres of the antenno-glomerular tract run dorso- posteriorly into the corpora pedunculata of the protocerebrum. Furthermore, antennal motor centres are found in this region. The tritocerebrum is located ventrally to the protocerebrum. It projects its fibres into the labrum and into the digestive tract both of which from the stomatogastic nervous system. This part of the supraoesophageal ganglion is also the termination point of connectives, which descend into the ventral nerve cord via the suboesophageal ganglion. Postoesophageal and postural commissure couple both sites.

The ventral nervous system proceeds as a double nerve cord comprising two parallel running longitudinal connectives, which are linked by horizontal commissures to the anterior and the posterior end of each segment. Seen from a developmental perspective, the thoracic (pro-, meso-, and metathoracic) ganglia remain fused whereas the abdominal ganglia become ramified sending branches of axons into each segment where they exit into the peripheral nervous system.

2. Cytology of the Supraoesophageal Ganglion

The whole brain is surrounded by an acellular neural lamina beneath which the cellular perineurium and the nerve cell bodies (somata) are found. The nerve cell bodies themselves are ensheathed by supporting glial cells. The neural lamina is intercalated by cytoplasmic projections from the cellular perineurium held together by desmosomes and tight junctions (Lane and Treherne, 1972). Three types of glial cells have been identified so far. Type I envelope the somata, type II extends along the neuropil and contains several cisterns of endoplasmatic reticulum, whereas type III is smaller than the previous types and is found within the neuropil (Ito and Technau, 1993). The latter is distributed ubiquitously within the brain; the other two types are allocated to distinct areas. Glial cells ramify between the axons of the neuropil providing structural support and marking the region of synapse formation.

3. Overview of Anatomy of the Olfactory and Gustatory Memory Networks in Insects

The various species of the *arthropod* calls *insecta* receive sensory modalities from sensory receptor cells which are located on various parts of the body. Cercii, sensory bristles and other structures for instance are found in the thoracic and abdominal regions. In the head (cephalic) region, however, these sensory organs are particularly abundant. Here, the ocelli, the eyes, the antennae and the gnathal organs are found all of which innervate the different regions of the cerebral ganglion. Although the cerebral ganglion receives nearly all input from the head based sensory organs, the integration of information from the sensory organs in other body part is less understood.

The chemosensory memory networks of most insects have a common plan consting of at least four differnt levels of distinct neural components. At the first level, chemosensory memory networks receive convergent input from **antennal chemosensory receptor cells** and **oesophageal taste receptor cells**. These chemosory receptor organs are specialised to encode different chemosensory submodalities. Chemosensory receptor cells of these organs are divided into an **receptive field**, and into an **transduc-**

tory field. The receptive field is the region in which the stimulus is detected whereas the transductory field is the region in which the stimulus is translated and propagated once a discrete threshold has been overcome.

Chemosensory receptor cells converge in the **antennal lobe** onto second order interneurones. Whereas reception and assimilation of **chemosensory input** form the **antennae** by the **glomeruli** of the antennal lobes has been well demonstrated, the relay of converging **gustatory information** is less well understood. Local interneurones of the antennal lobe connect the different glomeruli to each other. Antennal lobe neurones either project directly onto these neurones, which innervate the motor centres of the suboesophageal gnaglion or onto three tracts of projection neurones, the **olfactorio-globularis tract (OGTs)**. The OGTs relay sensory information to the calyces of the **corpora pedunculata**, as well as, to the **lobulus lateralis protocerbralis (LLP)**.

The corpora pedunculata are two bilateral symmetric neuropilar structures, which display certain homology to content addressable memory networks. In *Apis*, in particular, further input is received form the supraoptic tracts, which, too, converges into the corpora pedunculata. Hence, projection neurones, which receive three sensory modalities converge into the corpora pedunculata of *Apis*. (Mechanical input is derived form the antennae, chemosensory input from the gustatory and olfactory organs, and visual input from the optic lobes). It needs to be mentioned, however, that individual projection neurones of each different group diverge onto the corpora pedunculata. The innervation by afferents is spatially restricted to discrete regions of the calyces. Form the calyces bundles of parallel fibres run anteriodorsally through the pedunculus, whereafter they bifurcate into α- and β- (γ-) **lobes**. The lobes represent the regions of synaptic output. Exit fibres derive their synaptic output from both lobes and transfer it to the LLP. From here the sensory information enters prossumably into the premotor fibres, which diverge either into the motoneurones of the antennae or into the suboesophageal and thoracic ganglia. Direct connections between chemosensory pathways and optomotory and/or circadian networks have so far not been established (for review, see Howse, 1974).

4. General Anatomy of Insect Chemosensory Organs

The chemosensory system of insects comprises two functional components: **Olfactory sense organs,** such as the antennae, are largely implicated in the perception of gaseous chemicals from the air. **Gustatory sensory structures** within the mouthparts and the legs, in contrast, mediate the recognition of solid and liquid substances. Several sensilla, cuticular structures, which are innervated by one, or more, bipolar nerve cells, accumulate at spatially defined regions on the antennae and the mouth parts, in order to form these sense organs. The cuticle is provided with a large number of small pores allowing the contact with the exterior. With regard to its function, the cuticular part of each sensillum adopts at least four different configurations:

- ***Sensilla trichodea*** are long slender hairs which mostly end with a sharp tip.'
- ***Sensilla basiconica*** in contrast have the form of a projecting peg
- ***Sensilla placodea*** are flat plates, which are particularly found on the antennae of *Apis mellifera*.
- ***Sensilla coelonconica*** adopt the shape of short pegs set in a small pit.

It has been proposed that the form of each sensillum is the evolutionary adaptation to their function. Sensilla in form of protruding hairs or pegs are adapted to detect low odorant concentrations. For the detection and absorption of relatively small concentrations of an odorant from the air these olfactory sensilla need to have a high surface to volume ratio. The surface area of the long trichoid sensilla of the male moth *Bombyx mori* is i.e. 600 μm^2. Additionally these sensilla are particularly densely arranged on the male antennae: Whereas the female of *Bombyx* contains only 6,000 of these long trichoid sensilla the number of the male moth nearly exceeds 17,000. Although these sensilla cover only 14% of the entire surface area of the antennae the virtual increase in surface area in the sensilla clusters enables the antennae to capture an maximum number of odorants (Steinbrecht, 1973).

Those sensilla in sunken pits presumably function in conjunction with higher odorant concentrations.

There is an inverse relationship between size of the sensillum and the number of neurones, which innervate it; i.e. whereas olfactory sensilla with a large surface area are innervated by only a few neurones, sensilla with a small volume might be innervated by up to 40 neurones. The long trichoid sensilla of *Bombyx mori* are innervated by two dendrites, which proceed unbranched to the tip. The large basiconic sensilla of the same insect comprises the dendrites of three neurones which ramify across the entire volume of the sensilla

The diameter of the pores within the cuticle of the different sensilla varies from 10 to 100 nm. On trichoid sensilla of male *Bombyx mori* there will be 2- 7 pores per μm^2 of the cuticle surface; on the coeloconic pegs of the same individual there are, in contrast, as many as 20 pores per μm^2 (Steinbrecht, 1973). Some gustatory sensilla, which belong to the class of contact chemoreceptors have, however, only a single pore on their tips.

Chemosensory receptor neurones form the core of each sensillum. To the interior side of the sensillum they emanate large axonal processes, which in case of the antennal receptor cells terminate in the antennal lobe. In the cuticular exterior region each sensory neurone forms a dendritic compartment which accommodates of one or more dendritic processes. These dendritic processes are reinforced by longitudinal microtubules. The sensillum lymph surrounds the dendritic compartment constantly. The dendritic compartment is separated from the soma and axonal compartment by a short ciliary stretch. The ciliary stretch is composed of nine peripherally arranged microtubular doublets. The more interior located soma contains the membrane bound compartments such as endoplasmatic reticulum, mitochondria and other components of the cytoplasm.

The chemosensory receptor cells are ensheathed proximally by several accessory cells. Altogether three types of accessory cells have been identified which are embedded into the epidermis. The accessory cells of each sensillum are linked to eachother, the chemosensory receptor cells, and the epidermal cells by tight junctions. Trichogen accessory cells are located at the base of the sensory receptor neurones. They surround the soma. Here the cuticle of the peg, the scolopale and the pore tubulus originate. Moreover, trichogen cells contain an large indentation, the sensillum lymph cavity,

where the sensillum lymph might be secreted. Tormogen cells secrete the cuticle of the socket and bands the receptor lymph cavity. Both have been implicated in keeping the sensillum fluid separate from the haemolymph which is of a different composition.

The somata and the base of the exterior compartment are tightly ensheathed by the thecogen cells. Further downstream at the exit of the soma the thecogen cells are superseded by glia cells (for review, see Kaissling, 1986).

5. Anatomy of Chemosensory Memory Pathways in *Apis mellifera*

The chemosensory memory pathways of *Apis mellifera* represent a classical example on which the relationship between mnenogenesis and anatomy has been demonstrated. If for example the corpora pedunculata of *Apis* are chilled or even extirpated the behaviour of *Apis* in response to an chemosensory stimulus is severely impaired. This description on the structure of the bee olfactory tracts refers to extensive studies by Schürmann (1974), Mobbs (1982, 1984), Arnold et al. (1985), Bicker (1985), Maulshagen (1993), Hammer (1993) and Rybak and Menzel (1993).

5.1. Odourant Receptor Cells

Cells involved in the perception of chemical signals predominate in the antennae in the labium as well as in the legs and the abdomen. The task of chemoreception is allotted to specific structures called the sensilla. The sensilla are cuticular structures, which contain a large number of small pores. Through these pores a stimulant enters the sensilla and is absorbed by the sensilla lymph. The antennal flagellatum of *Apis mellifera* contains the sensilla placodea the chief chemoreceptors. Each flagellatum comprises about 2600 sensilla made up the 15 to 30 sensory neurones (Esslen and Kaissling, 1976). There are seven times as many pore plates in the drone as there are in other insects. In the worker bee the chemoreceptors of each poreplate project to glomeruli of different antennal lobe regions (Brockmann and Brückner, 1994).

5.2. The Antennal Lobe

The antennal lobe receives its input from the antennal nerve and forwards signals to the median and lateral olfactorio- globularis tracts. Two strands of the antennal nerve innervate the antennal lobe from different directions. A putative mechanosensory strand projects the antennal lobe from the dorsal site. The dorsal strand, which is thought to be chemosensory, enters directly into the glomerular neuropil of the antennal lobe. Three types of projections have been identified to enter diverse areas of the antennal lobe (Mobbs, 1982; Arnold et al., 1985; Flanagan and Mercer, 1989). The first, T_1 projects the lobe centrally and innervates the lobe dorsally. T_2 takes first the same path and synapses with medio-ventral glomeruli. T_3 enters the lobe from the periphery. Chemosensory receptor cells axons predominantly innervate the T_1-T_3 region (Brockmann and Brückner, 1994). Each region contains 70 glomeruli. The ending of each receptor neurones are generally unipolar. T_4 branches off from T_3 and proceeds to the posterior part of the lobe. T_5 and T_6 both enter the dorsal lobe. While T_6 continues and splits into two T_{6I} and T_{6II}, which terminate in the protocerebral posterior slope and the suboesophageal ganglion, respectively, the T_4 **projections** end in the dorsal lobe proper (Maronde, 1990).

The antennal lobe constitutes a macroglomerular neuropil containing approximately 100 neurones. Accordingly, the glomeruli can be subdivided into five types.

Type 1: T_1 glomeruli are branched varicose.

Type 2: Those glomeruli in proximity to the projection of the antennal nerve contain axons, which are finely blebbed.

Type 3: Confined to centrally located areas; more diffuse.

Type 4: Sparsely blebbed dendrites.

Type 5: Occurs in smaller ventrally located regions.

Several interneurones arise within the antennal lobe and enter the antennal glomerular tract. Two types of glomerular interneurones have been observed. The first type is multiglomerular and the second type is uniglomerular. The dendritic fields of these

glomeruli are arranged in a ball like shape. In the rind of the antennal lobe there are the cell bodies of several interneurones, which link the respective glomeruli to each other.

5.3. The Olfactorio- Globularis Tracts

The under surfaces of the calyces are innervated by tracts connecting them to the antennal lobes. There are three **olfactorio- cerebral tracts.** O_1, O_2 and O_3 originate from the antennal lobe and the anterior superior optic tract from the compound eye. The **median olfactorio- globularis tract** O_1 consists of many hundreds of fibres arising in the core of the glomerular neuropil of the antennal lobe, as well as, in the dorsal lobe and the suboesophageal neuropil. The latter fibres may represent chemosensory input from the mouthparts. From their origin in the antennal lobes the O_1 run medially and posteriorly over the surfaces of the β- lobes, continue dorsally and turn sharply outwards to were they meet the median calyx. The median antenno-glomerular tracts continue dorsally, and turn sharply outward, where they encounter median calyx, as well. Within the network of fibres, about the calyx, the **O_2 axons** branch off and send projections into each of the calyces. They then loop laterally and ventrally, and terminate at the base of the **Lobulus Lateralis Protocerebralis (LLP).** Some axons continue ventrally within the lateral antenno-glomerular tract, O_3 and return to the antennal lobe. The **lateral olfactorio- globularis tract (O_3)** axons project posteriorly form the antennal lobe and proceed in fascicles passing the origin of the O_2 neurones and curve laterally. Finally they follow the O_1 tract to the lobula where they join the inner ring tracts (Mobbs, 1982, Arnold et al., 1985). Well studied has been the O_3 neurone **VUMmx1** (Arnold et al., 1985; Hammer, 1993). It originates form a soma based in the dorsal suboesophageal ganglion. Anterior of the oesophagus it bifurcates and sends branches into the protocerebral gustatory sensory cells of the oesophagus. It continues laterally and radiates large dendritic fields into both antennal lobes followed by small field dendrites into the LLP. It finally meets the calyces at the lip and the basal ring where it innervates the intrinsic Kenyon cells (Hammer, 1993; pers. comm.).

5.4. Anterior - Superior Optic Tract

These tracts arise form stratified dendritic fields of the medulla and lobula and course along the cortex to innervate the external fibre plexus of the calyces. When entering the calyces it forms the outer ring tract encircling the outer rings of the calyces. From there dendrites enter the collar.

5.5. The Corpora Pedunculata

Intrinsic Neurones

The highly dimorphic corpora pedunculata of the honeybee *Apis mellifera* are the most extensively studied (Kenyon, 1898; Mobbs, 1982). Worker bees (0.05334 mm^3) and drones (0.04290 mm^3) display significant volume differences (Buitkamp-Möbius, 1975). The number of somata in the worker bee is estimated to be about 340,000 in contrast to the 295,000 in the drones (Witthöfft, 1967).

There are two calyces in *Apis* , which form cup like neuropilar structures: the lateral calyx extends dorso-medially, whereas the median calyx opens dorsally. Each of the calyces is filled with the bodies of Kenyon cells. Fibres, which derive from the calyx extend anterio- posteriorly into the pedunculus which proceeds ventrally and divides to give rise to the α- and β- lobes.

A vertical section through the **Supraoesophageal ganglion** shows that convex cups of the **calyces** are filled dorsally with **Kenyon cell somata**. Three regions form the cup like shaped calycal neuropil; the **lip**, the **collar** and the **basal ring**. The lip region receives mainly optic input, input into the collar is mainly olfactory whereas the basal ring receives both types of afferent fibres and is, hence, said to be multimodular. Only the neuropils of the basal ring are microglomerular, indicating that they are the site of association between postsynaptic K- cells and presynaptic efferent neurones. In the calyces the Kenyon cells become arborised. Dendrites arising in this region are of post-synaptic nature. Small field dendrites have generally low input capacity whereas wide-field dendrites contain numerous spines for synaptic contact (for review, see Schürmann, 1987). The K- cell projections into the α- and β- lobes are highly ordered

and regularly arranged calycal stalks. These K- cell stalks arise in a particular region of the tripartite calyx and extends a projection into the **pedunculus**, which divides into a λ- shape and innervates both α- and β- lobes.

The **corpus pedunculum** in either brain hemishere is composed of several K- cell subclasses:

- **K_a cell bodies** are directly attached to the primary dendritic segment of the calycal arborisations.
- **K_b somata** synapse with a neurone to the peduncular projection in the immediate vicinity of the calycal dendritic field and do not contact the pedunculus directly.

Each type of K-cells corresponds to a specific calycal region from which it arises.

(1) **K I somata** are arranged concentrically around the lip neuropil and descend into a region below the lip of the median and lateral calyces. The morphology of each K I neurone varies slightly. Their spherical dendritic fields are approx. 75µm in diameter. Each dendrite has dense spiny endings. The posterior section of the peduncle is covered with knobs and spines.

(2) **K II neurones** occur exclusively in the collar zone. They display a roughly cylindrical configuration and almost completely innervate the collar neuropil. They correspond to the K_b type. The first 50µm of the KI and K II fibres receive dendritic synapses and spines. Their length and the density of the spines fluctuate considerable.

(3) **K III neurones** arise from the central group of Kenyon cell somata. Their neurites are confined to the basal ring zone. The configuration of the KIII neurones is highly heterogeneous. Their dendritic fields occupy the entire depth of the calycal neuropil and resemble cones or truncated cones.

(4) **K IV neurones** emanate form the central group of K-cell somata or beneath the basal ring neuropil on the base of the calyx. They are either of K_a or K_b type. Their extensions are found exclusively within the basal ring neuropil. Together with K II neurones, K IV neurones produce two stalks innervating the α- /β- lobes.

(5) **K V neurones** constitute K_a as well as K_b cells. They occur both in the basal ring neuropils with cell bodies located either above the calycal neuropil in the central

group of K-cell somata or below it. The K III, K IV and K V cells form four stalks to the corpus pedunculus.

The degree of the dendritic overlap between neurones of K I, K II/ K III type varies between 50 % and does not exceed 90%. KII-V cells are irregularly blebbed with some spines. Each of the three calycal zones generates an equal number of different strata. Further layers are made up of individual K- neurone classes. Branches within the α- lobe are arranged in the same order as in the pedunculus. Hence, K-cell of the lip region generate the ventral part of the α- lobe and the anterior strata of the β- lobe. Collar fibres sent strata into the central regions of both lobes. Basal ring K- cells terminate in the upper α- lobe and the posterior β- lobe, respectively.

5.6. Extrinsic Fibres

The α-lobe is subcompartmentalised into six strata (Mobbs, 1982; Gronnenberg, 1984). Several extrinsic neurones, which enter the α- lobe have been identified in a study by Rybak and Menzel (1993) .

α- Lobe Exit Fibres

Overview

Altogether 7 soma clusters have been identified to project their neurites into the α-lobe of the corpora pedunculata. Only about 400 fibres do so, the remainder continue beyond these structures without actually contacting them. Glial cells act to insulate the K-cell neurites of the α- lobe from interference from other neural pathways (Bicker, pers. comm.). Neurites, which penetrate the α- lobe enter this structure laterally at the α- lobe exit.

(1) The soma of the **A6 cluster** are embedded in the protocerebral lobe ventrally to the lateral calyx. Two types of distinct neurites emanate from this cluster: The **A6-1** and **A6-2 neurites.**

A6-1 neurites project first into the dorsal protocerebral lobe, and course subsequently parallel to the protocerebral calycal tract, whereafter they turn anteriorly. They

subsequently meet the α- lobe dorso-laterally and bifurcate. The main arbour penetrates the α- lobe laterally and fans out into the α- lobe neuropil. The minor branch continues posteriorly. There it innervates the protocerebral lobe and proceeds as a collateral through the dorsal protocerebral lobe into the other brain hemisphere. Here the fibres traverse the other protocerebral lobe and end with bleb like termini in the dorsolateral neuropil surrounding the α- lobe.

A6-2 neurites arise in soma based ventrally to the lateral calyx. They presumably correspond to the **anterior dorsal protocerebral commissure (branch i)**. They send off branches, which loop around the α- lobe. These extend several dendritic fields dorsally. Other neurites run in a W- like configuaration into the other brain hemisphere. There they turn ventrally and arborise at the outer margin of the α- lobe.

(2) The somata of the **A7 cluster** are located in the ventral protocerebrum. Neurones arising from this cluster form the **anterior dorsal protocerebral commissure**. The A7 cluster extends **A7-1** and **A7-2 neurites**. They traverse the β- lobe posteriorly encircle it and turn anteriorly where they enter the β- lobe. **A7.1 neurones** branch on the level of the α-lobe stalks. Their dendritic fields turn vertically and project into the α-lobe. **A7-2 neurites** in contrast enter the β- lobe at the β- lobe exit. They branch off as small dendritic fields into median and dorsal α-lobe. Their termini in the dorsal and lateral protocerebral lobe the are blebbed. They fan out into the median and dorsal α- lobe. At the level of the medial protocerebral lobe, the neurites curve contralaterally and project anteriorly where they parallel the **anterior dorsal protocerebral commissure**.

The **α-lobe to anterior optic tubercle tract** innervates the **anterior optic tubercle**. Its dendritic fields extends into both α- and β- lobe. Neurites ramify into the **lateral** and **median protocerebrum**. Subsets of neurites bypass the ipsilateral α-lobe. There they branch into the **lateral** and **median protocerebrum**. Other subsets parallel the protocerebral tract.

Feedback neurones of the **protocerebral-calycal tract** belong to the recurrent neurones. The **protocerebral- calycal tract** is composed of a heterogeneous population of neurones described by Rybak and Menzel (1993) as A3 bilateral neurone clus-

ters. This tract assembles neurones from the anterior dorsal protocerebral commissure, the anterior lateral protocerebral tract as well as the α- lobe to anterior optic tubercle tract. Neurones of this tract arise in the dorsal and ventral **A3 soma clusters** of the anterior latero-ventral protocerebrum. **Ventral A3** neurites extend posteriorly and penetrate the α-lobe. Efferent neurites of this cluster could in fact correspond to the anterio-lateral protocerebral tract. Their neurites pass along the protocerebral lobe and bifurcate dorso-laterally to the α-lobe. The chief branch runs along the **protocerebral- calycal tract**. It sends arbours into ipsilateral, median and lateral calyces via the **inner ring tracts**. Their fibres end either in the **basal ring** and **lip zones** or **median to the basal ring** and **collar** neuropil. Further fibres proceed into the pedunculus.

(3) The **dorsal A3 cluster** fascicle enters the α- lobe anteriorly to the ventral A3 bundle. They fan out anterio- dorsally into the α-lobe. They subsequently exit the α-lobe form a loop posteriorly to the β- lobe. It enters the β- lobe laterally and sends fine arborisations into this lobe. Several unilateral neurones penetrate the α- lobe.

(4) The **A1** and **A2 clusters** are restricted ipsilaterally to the protocerebrum anterior dorso-medially and dorsally to the α- lobe, respectively. Their fibres meet the α-lobe at its ventro-medial margin, where they bifurcate. Minor branches, which encircle the α-lobe, project small dendritic fields into the anterior protocerebral lobe posterior of this lobe. The termini are not blebbed but of fine appearance. Another minor branch of A1 continue posteriorly and penetrate the α-lobe medially, whereas fibres of the minor branch from the A2 cluster enter the lobe ventro-medially. The neurites emanate large dendritic fields anterio-dorsally within this lobe. The major branches proceeds into the thin layer of the anterior β- lobe where they fan out.

(5) The **A4 cluster soma** within the median rind of the **antennal lobe** emanate neurites, which run dorsally into the protocerebral lobe where they branch ventrally into the α-lobe. The **A4-1** and the **A4-2 subclusters** display specific projection patterns. The A4-1 neurites extend dorso-laterally. Ventral to the α- lobe they bifurcate. One branch enters the α- lobe ventrally and arborises there into large sparsely packed dendritic fields. These projections run parallel to the length of the K-cell peduncular projections. The termini are finely structured without any prominent blebs. The second

branch extends into the **lobulus lateralis protocerebralis (LLP)**. There it enters the α-**lobe to protocerebral lobe tract**. Some fibres cover the dorso-lateral margin of the α-lobe. Others from a triangle shaped neuropil extending either dorsally or ventrally to the **LLP**. The dendritic fields form a shield medially of the **LLP** penetrating this structure only marginally. Other types of A4-1 neurones enter the α- lobe ventro- medially and form dense dendritic fields perpendicularly to the length of intrinsic K-cell axons. Some A4- 1 neurites display a projection pattern, which is almost identical to the first A4-1 type. Branches into the **LLP** are, however, less well developed and a branch off fibres extending medio-dorsally into the β- lobe and proceeding into the anterior parts of **lip** and **collar** neuropil of the median calyx.

The **A4-2 cluster**, which is located more posteriorly sends fibres into the α- lobe. These fibres subsequently branch off and penetrate the ventro lateral margin of the α-lobe. There they form a sparse dendritic field. The other arbour proceeds into the **LLP**. It penetrates the **LLP** and ramifies fibres ventro- and dorso- medially.

(6) **A5 neurones** emanate from large somata in the medio-anterior brain cortex. The **A5-1 neurone** is the largest with primary neurites extending medially across the midline. They enter the α-lobe at the medial exit. Outside the exit small dendritic fields are formed. Some dendrites encircle the α-lobe and extend dendrites into the **LLP**. The chief branches bifurcate first when entering the α-lobe. The first branch extends ventrolaterally. The neurites follow the ventral margin of the α-lobe and ramify to sent branches dorsally. On the level of the α-lobe equator they form fine densely packed dendritic fields. The second branch emanates directly dendrites into this dendritic field. These are connected by dendrites, which run horizontally posteriorly of the dense dendritic fields.

Neurones of the **A5-2 cluster** run medio-ventrally and enter the α- lobe at the medio- ventral exit. Having penetrated the α-lobe they bifurcate and fan out fine and densely packed dendritic fields, each of which are confined to the ventral region of the α-lobe. These dendritic fields open toward the equator of the α- lobe. A minor branch leaves the main tract prior to entering the α- lobe. Side branches surround the α-lobe from the medial and lateral site. A small branch turns around the α- lobe from the me-

dial site contacts the **ventral protocerebral lobe**. Branches encircling the α-lobe laterally are reinforced. They send off branches into the optic tubercle where they form medium dense dendritic fields invading this structure either from the ventral site or from the dorsal site. Further fibres extend into the **protocerebral lobe**. They contact the **LLP** dorsally.

Neurones of the **A5-3 soma cluster** are found in the antero-medial contra lateral protocerebrum. The neurites run ipsilaterally and bifurcate at the level of the medioventral α- lobe. Here branches off collaterals surround the α- lobe. Three main branches, however, proceed into the α- lobe where they arborise forming distinct dendritic fields. Posteriorly dendrites connect these branches.

(7) The **Pe-1 neurone** arises from a soma close to the midline below the anterior cortex of the brain. Its axon (10-15 µm in diameter; depth 140- 200µm from the anterior brain surface) projects across the midline within the median protocerebral lobe and courses back into the brain hemisphere of its origin. There it emanates small dendrites into the median protocerebral lobe. It subsequently penetrates the pedunculus ipsilaterally in proximity to the β exit point. There it extends a dendritic tree into the pedunculus. This dendritic tree diverges several times within the pedunculus, where it presumably receives postsynaptic input. So it has been established that the parallel dendritic arborisations of the Pe-1 neurone might be the regions, where this neurone innervates the Kenyon cells of the pedunculatum (Maulshagen, 1993; Rybak and Maulshagen, 1994). The main branch continues anteriorly. At the level of the α- lobe it branches off fine dendritic ramifications which encircle the α- lobe. Having passed the α- lobe it bifurcates. The first branch terminates in the LLP where it ramifies extensive branches at a depth of 200- 300 µm. The other branch returns to the posterior pedunculus (Maulshagen, 1993; Rybak and Menzel, 1993).

β- Lobe Exit Fibres

There are either large or small efferents penetrating the β- lobe. Some of the large extrinsic fibres form unstratified dendritic fields others form stratified dendritic fields, which extend either veritcally or horizonally to the length of the K-cell peduncular projections. Several β- lobe exit fibre tracts have been described above. Their are, however, some individual β- lobe tracts that are not present from the α- lobe. Most of the tracts are, however, found in both lobes. The β- lobe to central body tract comprises 20-30 neurones. They exit the α- and β- lobe ventro-medially, extend posteriorly along the β- lobe and are assumed to innervate the central complex.

The β-lobe to β-lobe tract is a small U-shaped tract connecting both β- lobes in either brain hemisphere.

6. The Chemosensory Memory Pathways of *Drosophila melanogaster*

6.1. Anatomy of the *Drosophila melanogaster* Antenna

The anatomy of *Drosophila melanogaster* olfactory system is relatively simple in contrast to that of *Apis mellifera.* The third antennal segment, the funiculus, contains the cardinal olfactory apparatus consisting of 500 olfactory hairs (sensilla). There are at least three morphologically distinct types of sensilla on the third antennal segment each adopting a distinct cuticular shape:

- Trichoid sensilla are lengthy in shape and contain at their ends a sharp tip.
- Coeloconic sensilla are relatively small and are of cone shaped morphology.
- Basiconic sensilla are club shaped.

The cuticle is pierced by several thousand pores allowing the penetration of the respective odorant. The one to four neurones which innervate each sensillum (altogether 2,500 neurones innervate the 500 sensilla) encode the electrochemical response for each type of odorant (for review, see Carlson 1991, 1992).

The gross morphology of the *Drosophila melanogaster* funiculus reveals other than in *Bombyx mori* no significant sexual dimorphism. It is likely that in *Drosophila melanogaster* contact chemoreceptors of the prothoracic legs are rather implicated in ol-

factory sexual discrimination (Possidente and Murphey, 1989). In addition to the funiculus several olfactory sensilla are confined to the maxillary palp (Ayer and Carlson, 1992).

6.2. The Antennal Lobes of *Drosophila melanogaster*

The antennal lobe — as the antennae of *Drosophila melanogaster* — displays no sexual significant dimorphism. Such a sexual dimorphism is only prominent in the antennal nerve. The antennal lobes of *Drosophila melanogaster* are composed 35 glomeruli. The five most centrally located glomeruli are the exclusive recipients of ipsilateral sensillum receptor neurones derived form the antennae. The remainder receives its input from both antennae. Input fibres from the maxillary palps innervate distinct glomeruli (Ayer and Carlson, 1992). The glomeruli of the antennal lobe are connected by local interneurones. These small field local interneurones extend only ipsilaterally and do not extend beyond the antennal lobe itself. Of the several fibres which leave the antennal lobe the unilateral interneurones enter the antennal glomerular tracts and innervate either the calyces of the corpora pedunculata or the lobulus lateral protocerebralis. Other unilateral interneurones terminate in the lobulus lateralis protocerebralis only. A giant interneurones which display similarity to the VUMmx1 neurone of *Apis mellifera* extends two bilateral neurites form its soma in the suboesophageal ganglion into both antennal lobes. Several commissural neurones extend contrallaterally into the antennal lobe of the other brain hemisphere. Several unilateral interneurones leave the cerebral ganglion altogether and innervate the thoracic neuropil.

Afferents

The antennal nerve supplies the antennal lobe with chemosensory input. The antennal nerve comprises 1700- 1800 fibres. 1200 of the sensory fibre derive from the funiculus, the third antennal segment. They consist of several unilateral neurones and bilateral neurones, which send fibres into specific types of glomeruli of the antennal lobe. The uni- lateral neurones innervate each of the five postero- lateral glomeruli V, VP_{1-3} and

VL_1 in the ipsilateral lobe once. A glomerulus, V, in this region assembles its input from the basiconic sensilla of the ipsilateral antennal. In the rule, each glomerulus does not gather more than 2- 4 afferents. Another two of the five postero- lateral glomeruli, VP_2 and VP_3, which connect the ipsilateral aristal sensillum, however, were found to receive the terminals of several branches.

The bilateral neurones potentially synapse any of the remaining glomeruli. Each fibre may extend beyond a given glomerulus into the glomerulus in its proximity. Fibres innervating these glomeruli link the basiconic sensilla to glomeruli VM_1, DM_1. Minor input from the basiconic sensilla runs into glomeruli VL_1, VM_1, and DL_2. Receptors from the trichoid sensilla run into glomeruli VL_1, VA_1, and DA_1. Neurones of the coeloconic sensilla terminate in glomeruli VL_1, VM_1, and DL_2.

The remainder represent of afferents represent mechanosensory input of the first and second antennal segment. The latter fibres are confined to a single brain hemisphere.

The antenno- suboesophageal tract derives from maxillary palps. It projects the suboesophageal ganglion via the labial nerve. It subsequently innervates the ipsilateral antennal lobe ventro- laterally. They synapse either the $VA_{2,3}$ and the DM_2 glomeruli.

Intrinsic Neurones

The soma of **local interneurones** are located laterally to the antennal lobe cross linking the glomeruli within this structure. These interneurones are probably involved in the modulation of the synaptic function within glomerulus. The neurotransmitter in these neurones is probably GABA indicating that the local interneurones form a disinhibitory circuit. Both unilateral and bilateral neurones are present (for review, see Christensen and Hildebrand, 1987).

Efferents

The antennal lobes of both hemispheres are connected by the **antennal commissure** which contains approximately 2,500 fibres.

As in *Apis mellifera,* the **olfactorio- globularis tracts** of *Drosophila melanogaster* divide into three and connect the antennal lobe with the protocerebral structures such as the LLP and the calyces of the corpora pedunculata. The number of neurones in the inner olfactorio- globularis tract does not exceed 200. The median olfactorio- globularis tract assembles fibres derived from the inner olfactorio- globularis tract, which terminate in the LLP. Dendrites branch off the median glomerular tracts and parallel the pedunculus. Lateral of the inner glomerular tract exit the outer olfactorio- globularis tract leaves the antennal lobe and projects into the LLP.

Unilateral neurones are confined to a single hemisphere.

Monoglomerular unilateral neurones number about 92. They supply mostly the inner glomerular tracts with fibres, which arise from soma ventro- dorsally or dorsally of the antennal lobe. They extend dense dendritic fields into a single glomerulus. Fibres terminate either in the calyces or in the LLP. Other monoglomerular unilateral neurones project into the median and lateral olfactorio- globularis tracts. These neurones predominantly assemble fibres from ipsilateral glomeruli VL_1 or VP_3.

Somata of oligoglomerular unilateral neurones are positioned ventro- laterally to the antennal lobe. They arborise dendritic fields only into a few glomeruli and run through the median olfactorio- globularis tract mostly into the LLP.

Stocker et al. (1990) further distinguished polyglomerular unilateral neurones. They form glomeruli in the lateral, antero- lateral, posterior medial, in the dorsal and VM_2/VA_2 region of the antennal lobe.

The somata of **bilateral neurone** are predominantly located laterally to the oesophagus. They link two contralateral glomeruli of either brain hemisphere to each other. These bilateral neurones run along the inner olfactorio- globularis tract. Some of the bilateral interneurones display serotonin immunoreactivity. Others pass the oesophagus and terminate in the contralateral LLP.

Giant Symmetric Relay Interneurones (GSRIs) are probably homologues to the VUMmx1 of *Apis mellifera* though they do not seem to extend beyond the antennal lobe. These neurones arise from somata located dorsally to the oesophagus within the tritocerebrum. They extend mirror like dendritic fields into the oesophagus and into

posterior brain region ventrally to the great commissure. Dorsally of the oesophagus they bifurcate and extend two bilateral symmetric branches into either antennal lobe. There they innervate discrete sets of glomeruli. Some fibre even run along the antennal nerve. According to the branching pattern two types of GSRI can be distinguished (Stocker et al., 1990; Ayer and Carlson, 1992).

The **bilateral relay interneurone** and the **thoracic interneurone** extend fibres along the oesophagus. The somata of the first are contained within the antennal lobe proper. The latter arise in VA, DL_1/DA_3 and VP_{1-3} glomerular regions. They then synapses with the thoracic ganglion.

6.3. The Corpora Pedunculata of *Drosophila melanogaster*

Schürmann (1987) counted for *Drosophila melanogaster* cell bodies in either brain hemisphere. A variety of intrinsic fibres do not branch off into the α- lobe of *Drosophila melanogaster* (Technau, 1984). The neuropilar fibre compartments are surrounded by a glial sheath without completely separating those. The corpora pedunculata of *Drosophila melanogaster* are structures of ca. 2500 parallel running interneurones, the Kenyon cells, which are arranged symmetrically in either brain hemisphere. They receive input from the antennal lobe via the antenno glomerular tracts. Kenyon axons are unipolar neurones which somata are located in the dorso posterior region of the insect brain. The fibres which they extend into the calyces proceed anteriorly along the peduncle. Having passed this structure subsets of neurites branch off fibres into the α-lobe. Each K-cell axon then terminates in the β- /γ- lobe (Technau and Heisenberg, 1982; for review, see Heisenberg, 1989).

7. The Cytology of the Corpora Pedunculata of *Formica lugbris*

Several electron microscope studies have focused on the ultrastructural anatomy. of the corpora pedunculata. The corpora pedunculata of the wood ant *Formica lugbris* are enveloped by the perineurium. The Kenyon cell somata are arranged in a cortex emanating neurones into the neuropil (Lane, 1974). There are thought to be four Kenyon

cell types (Goll, 1967). Because in insects the somata are completely ensheathed by glial cells axon-axonal synaptic contacts can only be made in the neuropil. The corpora pedunculata of *Formica* are, however, an exception as here soma-somatic contacts are prominent. These contacts may function as low resistance electrical junctions (Landolt and Ris, 1966). The perikarya display a well developed Golgi body. Endoplasmatic reticulum and the ribosomes are, however, less elaborated. Glial cells which ensheath the perikarya supply the Kenyon cells with metabolites such as glycogen (Wigglesworth, 1960; Landolt, 1965). In the calyces several blebbed pre-synaptic structures have been observed. In the α- and β-lobes small interglobular synaptic contacts as well as contacts between globoli and extrinsic dendrites are formed. Glomeruli in the peripheral parts of the peripheral parts of the peripheral calyces mediate synaptic contacts between antennal glomerular tracts and the corpus pedunculus neuropil (Trujillo-Cenós and Melamed, 1962). The glomerular endings of the antenno-glomerular tract are pre-synaptic and peripheral Kenyon axons are postsynaptic (Landolt and Ris, 1966). Tight junctions in the synaptic knob stabilise the synapse in this region (Steiger, 1967).

Both α- and β- lobe are structurally very similar. Two types of nerve fibres were distinguished. Type one neurones receive axo-axonic synapses resulting from intrinsic neurones. These synapse with type two fibres, which are of extrinsic origin (Frontali and Manicini, 1970).

CHAPTER 2

Biochemistry and Pharmacology of Insect Chemosensory Memory Pathways

The principle features of a neurone is to receive and to transmit information. Whereas sensory cells are clearly divided into an receptive and an transductory field, this classification is not as evident in neurones of the central nervous system.

Chemosensory cells are therefore able to detect either specific or unspecific chemical stimuli of the environment, and to convert them into neural language. Here specific odorant receptors aided by several auxiliary proteins within the sensillum lymph translate a chemosensory stimulus into membrane depolarisation. Each type of chemosensory receptors is expressed of discrete sets of chemosensory receptor neurones. Their spatial arrangement determines the quality of the transduced chemosensory signal within a chemosensory memory network.

In the central nervous system, in contrast, these signals need to be very specific and compatible to the relevant nerve cells. These specific signals serve to reduce the background noise during interneural communication. Thus, in the central nervous system of diverse insect species several low molecular weight neurotransmitters and their respective receptors were identified to mediate electrochemical synaptic signal transduction. Generally, one can distinguish between excitatory and inhibitory neurotransmitters. Moreover, several neuromodulators which bind to specific receptors induce signalling cascades that modulate the activity of the respective ion channels either orthodromically or antidromically.

1. Pharmacology and Biochemistry of the Insect Peripheral Chemosensory Nervous System

Two classes of chemosensory stimuli trigger changes in the electrochemical behaviour of neurones. Olfactory stimuli are primarily gaseous, whereas gustatory stimuli are

solid, and, hence, to perceive the latter stimulus the insect needs to make physical contact with its source.

Whereas the pharmacology of the olfactory system is well studied, relatively little is known about the pharmacology of the gustatory system. The latter will therefore only mentioned marginally. It operates, however, along the same guide lines as the olfactory system.

The olfactory pathway starts in insects with the perception of an odorant (for discourse on odour reception in bees see Getz, 1993; Breed, 1993). Odorant receptor cells in the olfactory sensillum within the antenna perform this task. One distinguishes 'generalist' and 'specialist' receptor cells. 'Generalist' odour receptors respond either simulative or inhibitory to a variety of substances (Schneider, 1969; Vogt et al., 1991). 'Generalist' detector cells detect odorants by the comprehensive response elicited by all receptor cells in the epithelium. 'Specialist' detector cells, in contrast, perceive only a small range of odorants. Well studied were the 'specialist' olfactory receptor cells in the antennae of the male moth *Bombyx mori*.. These receptor sensory cells are contained within the olfactory sensilla. The receptors are particularly responsive to a blend of sex pheromones produced and emitted by female moth. The main compound of the blend is bombykol (but the ratio of all sex hormones is determinative) (Karlson and Butenandt, 1959). When applied to the sensillum bombykol elicits an action potential and triggers the male moth its wings to move (Kaissling and Priesner, 1970; Vogt and Riddiford, 1981).

It has been demostrated that bombykol needs to be captured by the senillum first and has to be transported throught the sensillum lymph to the relevant olfactory receptor cells before it elicits an action potential. This process is aided by the anatomy of the sensilla. The outer coating of the sensillum cuticle is composed of polymerised lipid, which, as electron microscope studies revealed consists of three layers: An outer layer of 2.5 nm envelopes the entire cuticle. This is followed by an 7.5 nm thick electron lucid layer, which extends into the pores. This layer is again followed by an electron dense region. As soon as an odorant impacts the outer layer it is transported to the pores. On *Bombyx mori* trichoid sensilla, is has been estimated that the diffusion of an

odorant to the pore should not take longer than 2 msec. Within another 1 msec. the odorant reaches then the relevant receptor molecule on the dendritic membrane of the olfactory receptor neurone. This process requires the presence of an sophisticated network of perireceptor molecules, which mediate the odorant transport (Steinbrecht, 1973; Vogt et al., 1991).

The gustatory apparatus of the bow fly *Phormia* consists of 240 trichoid sensilla which are arranged outside the labral lobes. Here four physiologically distinct 'generalist' gustatory contact receptors have been identified. Strikingly, all receptors fall into two categories: sugar and salt receptors.

In other insects such as the beetle *Chrysolina brunsvicensis* the host specificity its the feeding plant is determined by the presence of 'specialist' gustatory contact receptors on the tarsi of the legs. These specifically respond to the compound hypercin, which is contained within its host plant (Rees, 1969). The cabbage white butterfly *Pieris brassicae* is particularly attracted by the mustard oils of *brassicae*. When its mouthparts are stimulated by this mustard oil this leads to feeding. The reception of a molecule is similar to that of the olfactory apparatus. Here, however, only a few pores allow the gustatory molecule to penetrate the sensillum. Generally, the molecule diffuses through a mucopolysaccharide cap of the entrance pore(s) at the opening of the scolopale to the receptor neurones. Here, too, is good evidence that the transport of the gustatory molecules is mediated by acceptor proteins. The perception of a chemical depends on its concentration and an its quality. So do compounds with high energy residues and with high ionic mobility have the ability to elicit an action potential much more readily in the bow fly *Phormia* than those which have relatively poor ionic and energetic properties. If the sensillum receives blends of several gustatory compounds, neurones of this sensillum encode each compound differentially, so that in end effect the neural networking of the sensillary receptors is responsible for the resolution of these signals (for reviews, see Dethier, 1963; Städler, 1984).

1.1. Biochemistry and Organisation of Perireceptor Sensilla Components

The insect peripheral olfactory system is organised into clusters of two or more chemosensory receptor neurones which are contained within a cuticular sensillary structures. These neurones are anchored most proximally by three glia like accessory cells into the epidermis. The space between the sensillary cuticle and the chemosensory neurone is filled with the sensilla fluid.

The sensillum lymph of insect olfactory (and gustatory) sensilla is analogous to the vertebrate mucus. Several components within both viscous perireceptor fluids are involved in mediating the process of olfactory coding. In the vertebrate mucus the presence of two classes of olfactory recognition molecules has been proposed. Olfactory binding proteins (OBPs) presumably act as scavengers of specific odorant groups, and thus, preselect chemicals absorbed at the mucus. Olfactory receptor proteins (ORPs) are members of a multigene family that are exclusively found at the receptor membrane. Both types of olfactory molecules are restricted to discrete zones of the vertebrate olfactory epithelium as established by a PCR based detection method and immunohistochemical analysis (Buck and Axel, 1991).

In insects so far only two types of olfactory molecules have been either biochemically or genetically characterised. OBPs are contained within the sensillum lymph, where they presumably function as solubilisers and carriers of lipophilic odorants. Hence, once they bind to the insect OBP they are presumably carried to the dendritic membranes of the chemosensory neurones (Ziegelberger, 1994). Odorant degrading enzymes (ODEs) sequester these odorants shortly afterwards to abort their function. The enzyme aldehyde oxidase is a prime candidate of these ODEs (Vogt et al., 1990).

As many as 20 insect OBPs have so far been characterised from several moths species, as well as, from *Drosophila melanogaster* (though the function of the latter OBPs has not been established, yet). These OBPs proteins divide into at least two different functional classes. All OBPs that have been characterised thus far share several features. They are about 16 kDa in size and occur in concentrations as high as 10mM in the sensilla lymph.

Two subclasses of general olfactory binding proteins (GOBP1 and GOBP2) are associated with 'generalist' chemosensory receptor cells, which equally occur in both sexes of the several insect species. A member of each GOBP subclass has been cloned and from either *Manduca sexta* (Vogt et al., 1991) and *Antheraea polyphemus* (Breer et al., 1990, Vogt et al., 1991).

Pheromone binding proteins (PBPs), in contrast, are associated with 'specialist' olfactory receptor cells. The latter are specifically distributed on the antennae of male moths. Implicated in the perception of female pheromones such as bombykol or bombykal, PBPs play a crucial role in the mating behaviour of several moth specifies (Van den Berg and Ziegelberger, 1991, for review see Breer et al., 1992). PBPs belong to the genetically and biochemically best characterised class of OBPs. They have been cloned and sequenced from *Manduca sexta, Anthreaea polyphemus* and *Drosophila melanogaster* (Gyorgyi et al., 1988; Raming et al., 1990, Pikielny et al., 1993; 1994). Sequence comparisons between different PBP cDNAs revealed no great deal of sequence similarities as hybridisation between the different PBPs cDNAs is mostly absent. Whereas the moth PBP species are almost co-linear (also 30% sequence identity to GOBPs), the putative *Drosophila* PBPs (*DroOBPs*) display quite significant sequence variations. The spacing of the six cysteine residues within the different subclasses of PBPs adopts, however, almost an consensus (Vogt et al., 1991; Pikielny et al., 1994; for review, see Breer et al., 1992). That these cysteine motifs play a crucial role during olfaction comes from biochemical studies on two PBPs species of *Antheraea polyphemus*. To identify their pheromone binding capacity, the *AntPBPs* proteins were separated on an non- SDS PAGE gel. By cross- linking radiolabelled female sex hormone (E,Z)- 6, 11- hexadecadienyl acetate the two candidates for pheromone binding were identified. Both displayed, however, no peptide diversity. Instead their differential migration on the PAGE gel was the result of discrete disulphide bond formation. Hence, differential disulphide bond formation between the different cysteine residue may indicate the physiological stage of both PBP peptides (Ziegelberger, 1994).

Moreover, each of the PBPs contains putative signal peptides towards their N-terminus indicating that each member of the PBP subclass is secreted into the sensillary lymph (Gyorgyi et al., 1988; Raming et al., 1990, Pikielny et al., 1993; 1994).

It is assumed that not more than a dozen different *DroOBP* occur in the *Drosophila melanogaster* olfactory organs (for review see, Carlson, 1991, 1992). Unlike in moths, however, the *DroOBPs* are not distributed in a sexual dimorphic pattern (Pikielny et al., 1993, 1994; Hekmatpanah et al., 1994). Two PCR based differential hybridisation screens of antennal minus head cDNAs by Pikielny et al. (1993; 1994) and Hekmatpanah et al., (1994) isolated altogether seven differentially distributed putative *DroOBPs* few of which display significant sequence similarities with each other. Two *DroOBPs* namely PBP H1/ PBP- H2 (Hekmatpanah et al., 1994) are presumably identical to PBPRP- 3 (Pikielny et al., 1993, 1994) by virtue of the chromosomal location of their genes (both map to 83C/D) and their mRNA distribution. Both $DroOBP^{83C/D}$ transcripts are expressed in a pattern that corresponds approximately to the distribution of the *sensilla trichoidea*. As PBP H1 and PBP H2, which are encoded by the 83C locus share 71% cDNA sequence identity, it can be assumed that they are either derived from a gene duplication in this region or are the products of the alternative splicing process. As most of the olfactory binding protein and olfactory receptor genes of moth and human, respectively, are intronless and the sequences of PBP H1 and PBP H2 cDNAs do not reveal any splice junctions, it is conceivable that both transcripts are derived from a duplication of an ancestry olfactory gene (for reviews, see Lancet, 1994a, b).

As indicated by chromosomal localisation all other *DroOBPs* are encoded by different loci of the second, third and X chromosome, respectively. They are therefore designated according to the locus which contains their reading frames. Whereas $DroOBP^{19D}$ is expressed in the appendages, as well as, in the head, the remainder of the *DroOBPs* are transcribed exclusively in the funiculus of the antennae. $DroOBP^{83C/D}$ and $DroOBP^{84D}$ show, thereby the highest level of expression in this organ. In situ hybridisation analysis of the spatial expression patterns of these *DroOBPs* identified that $DroOBP^{19D}$ mRNA is found close to the sensillary cuticle and not in the vicinity of the sensory nerve cells. It is present in most, but not all of the three types of the sensilla.

DroOBP^{69B} expression coincides with that of *DroOBP*$^{83C/D}$ mRNA, whereas *DroOBP*^{28B} mRNA transcription is restricted to the medial and posterior areas of the funiculus with little overlap to the previous. The last identified putative *DroOBP*, *DroOBP*^{84D} is found only in a limited number of sensilla scattered cross the entire funiculus (Pikielny et al, 1994).

As anticipated, the expression of all these *DroOBPs* is not sexually dimorphic.

In addition several small abundant proteins, all of which display high sequence homology have been isolated from the taste sensilla of different *Dipteran* species (for review see Kaissling, 1994).

1.2. Biochemistry of Chemosensory Signal Transduction

Well studied are the molecular events underlying olfaction of the vertebrate sensory system (for reviews, see Anholt, 1994; Breer, 1994). Insect analogues to vertebrate ORPs have not been identified. So one may only speculate how the exact path chemosensory signal transduction operates in insects. In any event, the presence of i.e. an pheromone elicits an action potential in the chemosensory receptor neurones of the moth *Bombyx mori* indicating that this signal is the sufficient trigger for neural coding. The biochemical events that bring about this membrane depolarisation shall be discussed here. Electrochemical properties of the chemosensory apparatus are discussed later in chapter 3 of this part.

In vitro studies of antennal homogenates have well established that the application of pheromones to *Antheraea polyphemus* leads to an increase of intracellular levels of 1,4,5- triphosphate (IP_3)(Kaissling and Boekhoff, 1993). The same effect was observed, when cDNA of mammalian olfactory receptors were transfected using the baculovirus system into insect cell lines (Raming et al., 1993). Evidence for the involvement of the IP_3 pathway in olfactory signal transduction comes also from the observation that in adult *Drosophila melanogaster* the expression of the IP_3 receptor gene (*dip*) predominates in the antennae. These IP_3 receptor are genuinely considered to be linked to Ca2+ release channels. (Hasan and Rosbash, 1992). In *Heliothis virescens* this

pheromone stimulation IP_3 leads to the phosphorylation of specific proteins (Raming et al., 1994). It is, however, very much debated, as to whether protein phosphorylation results in an altered ionic conductivity (opening of ion channels of mammalian olfactory neurones is modulated as the result of phosphorylation), or as more likely in the inactivation of the putative odorant receptors following stimulation (for review, see Ache, 1994). In cultured odorant receptor cells of the hawk moth *Manduca sexta* inward Ca^{2+} currents are elicited by continuous addition of pheromones (Wegerer, 1992; Stengl, 1993). The pheromone induced depolarisation is terminated when the ion channels close. It is assumed that NO synthase plays an important role in this process. There is a possibility that in *Manduca sexta* the ion influx is regulated by cGMP, which titres might be enhanced by NO (Ewer, 1993; Stengl, 1994). The involvement of messengers of the cAMP pathway is, in contrast to the vertebrate olfactory pathways, not evident from current studies on insect olfactory systems. It needs to be mentioned, as well, that the components of the olfactory cAMP signal transduction cascade are genetically and biochemically quite different from those of the cAMP signal transduction cascade implicated in learning, which is found in hippocampal neurones (for reviews, see Ache, 1994; Anholt, 1994; Breer, 1994).

2. Pharmacology and Biochemistry of Insect Central Nervous System

Few synaptic transmission mechanisms have been implicated in mediating nervous coding in higher level neurones of the insect chemosensory memory pathways. These synapses have the function to co-ordinate electrochemical impulses in the central nervous system. They need therefore be expressed at discrete key positions within the chemosensory memory networks allowing an high signal to background noise ratio to propagate to the centres of these networks. Molecular and histochemical studies have identified a great variety of synaptic neurotransmitters, the enzymes implicated in synthesising these neurotransmitters, and the receptors they bind to. Nearly all of these have been mapped to the distinct set of neurones at different levels of the chemosensory memory pathways of several insect species. It is interesting to note that the expression

domains of these molecules are highly conserved within the different species of the insect world.

So it has, for instance, been demonstrated that inhibitory GABAeric synapses are found in several interneurone homologues of several insect species. Other parallel bundles of neurones, which criss- cross the chemosensory memory networks have highly conserved excitatory synaptic endings such as cholineric synapses, whereas again other specifically expressed synapses are modulatory and release their synaptic content into the haemolymph, where it modulates the function of receptive neurones. In protocerebrum of *Formica* even electrical synapses have found, which operate via gap junctions and do not use any biochemical transmitters, whatsoever. These have, however, not yet been found in the brain of other insect species.

Postsynaptic ion channels of biochemical synapses may either be directly gated and, hence, directly associated with an receptor (i.e. synapses that express the nicotinic Acetylcholine receptor); other ion channels, in contrast, may be indirectly gated and, hence, responsive to second messenger molecules generated by the activation of only a small number of receptor molecules (i.e. synapses which express GABA or serotonin (5 HT) receptors). The latter may have the advantage that they can amplify the impact of a small number of neurotransmitter molecules to evoke an membrane depolarisation in the postsynaptic cells. The variability of the response of this type of indirectly gated postsynaptic ion channel is therefore much greater than that of directly gated ion channels. This indirectly gated ion channels are therefore employed in feed back neurones, which function depends on gradual, rather, than all or none responses as observed for directly gated postsynaptic ion channels. However, directly gated ion channels such as the ones associated with the nicotinic receptor are more commonly used for signal propagation in the insect brain as they operate more precisely than indirectly gated receptors.

2.1. Acetylcholine

The nicotinic acetylcholine receptor of *Drosophila melanogaster* is composed of several subunits capable of mediating several processes in the membrane of the postsynaptic nerve cell following ligand binding (for review, see Gundelfinger, 1992 and references therein). Here it should only briefly mentioned that this receptor consists of five subunits that in their entirety act as ionophoric ion channels for Na^+ and K^+. Amongst the subunits that have been characterised in *Drosophila* are the non- ligand binding subunit ARD and the ligand- binding α- like subunit ALS. Immunohistochemical analysis of the two acetylcholine receptor subunits (ARD and ALS) in *Drosophila melanogaster* revealed that these are prominent in the β- lobe of the corpora pedunculata. Particularly the cap structure of β- and γ- lobes was found to stain intensely. There is some degree of weak immunoreactivity in a distinct region to the posterior end of the calyces (Schuster et al., 1993). There is no immunoreactivity to both acetylcholine receptor subunits in the pedunculi and the α- lobe indicating that Kenyon cell axons arbourising into the α- lobe must be of distinct identity.

Acetylcholine esterase occurs extracellularly in the synaptic cleft of the cholineric synapse. This enzyme cleaves the neurotransmitter acetylcholine into choline and acetate. Acetylcholine esterase terminates therewith the postsynaptic activity of acetylcholine and prepares choline for endocytosis and recycling by the presynaptic cell. Acetylcholine esterase co-localises with ARD to the synaptic cleft between β- and γ- lobe and their efferents.

2.2. γ-aminobutylic acid (GABA)

γ- aminobutylic acid (GABA) binds to a discrete receptor subtype that opens K^+ or Cl^- selective ion channels and therewith hyperpolarises the postsynaptic neurone. In *Apis* feedback neurones that extend from feedback neurones with arborisations from α-lobe back into the calyces show GABA immunoreactivity (Bicker et al., 1985). As seen below when activated through stimulation of the Kenyon neurones in *Schistocerca*

americana , hyperpolarisations induced by feedback from the α- lobe initiate inhibtory postsynaptic potentials (IPSPs).

2.3. Taurine

Taurine (NT1) is ascribed to be a neuromodulator in insects. NT1 reduces the accumulation of calcium into synaptosomes of the locust and affects the subsequent release of radiolabelled transmitters. Moreover, it was demonstrated that NT1 prohibits excitation induced octopamine release into the cerebral coelom associated with an augmented release of GABA. Additionally it derepresses afferent cholineric transmission to the calyces by reducing the release form synaptosomes. NT1 accounts for 16% of the total free aminoacids in the brain of the honeybee. Higher levels of NT1 are found in the majority of mushroom body intrinsic neurones and in the photoreceptor cells of the compound eye and ocelli. Schäfer and colleagues (1987) reported high levels of NT1 in all Kenyon cells of the worker honey bee. In *Locusta migratoria* most intensive immunoreactivity was found in the posterior lateral part of the peduncle as well as in the ventral layers of the adjacent β-lobe suggesting that it localises to the synaptic regions of the GABAeric interneurones (Bicker , 1991; Stevenson et al., 1994).

2.4. Serotonin

Serotonin (5-hydroxytryptamine or 5-HT) is ascribed to be a major neuromodulator in the insect brain . Two different receptors have been identified that either activate cAMP cyclase (*dro1*) or inhibit adenylyl cyclase and stimulates phospholipase C (*dro 2A*) (Witz et al., 1990; Saudou et al., 1992). Serotonin is synthesised from tryptophane through the formation of 5- hydroxytryptophane (Livingston and Tempel, 1983; Vallés and White, 1990). Its immunoreactivity of the *Drosophila* brain follows a developmentally distinctive regulated pattern initiated but not maintained by the transcription factor ELF-1 (Vallés and White, 1988; Johnson et al., 1989; Bray et al., 1989; Lundell and Hirsh, 1994). Both in *Apis mellifera* and in *Manduca sexta* immunoreactive sero-

tonineric neurones have been found in the antennal lobes which emerge from the supraoesophageal ganglion (Kent et al., 1987; Rehder et al., 1987). In *Drosophila melanogaster* 5-HT immunoreactivity is evident in the antennal commissure that connects the antennal lobes contralaterally (Stocker et al., 1990; Vallés and White, 1988). Moreover, it might be possible that giant suboesophageal interneurone displays 5-HT immunoreactivity, as well. Last, the *Drosophila* homologues of the anterior-dorsal commissure are clearly recognisable for their immunoreactivity (Vallés and White, 1988).

2.5. Octopamine

Octopamine is a biogenic monoamide that functions as a neuromodulator. Octopamine like immunoreactivity can be localised to the dorsal unpaired median neurones, which are a part of the pars intercerebralis of *Locusta migratoria* and *Apis mellifera.* Its release is probably the direct consequence of circadian rhythms. Moreover, it is ascribed to participate in the learning and memory process by its binding to G-protein coupled receptors which in turn modulate the activity of several cAMP dependent ion channels. Octopamineric receptors which respond to secreted octopamine are localised with the radiolabelled agonists such as the phenyliminoimidazolidine derivative NC7 and antagonists such as epinastine to the corpora pedunculata of both insects (Orchard, 1982; Degen, 1994).

2.6. Pigment Dispersing Hormone (PDFs)

Neurones immunoreactive for the neuromodulator pigment dispersing factor (PDF) arise from somata positioned to the lateral site of the frontal protocerebrum. They from a wide meshed network in the medulla. Whereas the main axon crosses the midline and innervates the medulla of the opposite hemisphere it arborises dentrites when entering the contralteral median protocerebrum. These in turn encircle the calyces of the corpora pedunculata. Moreover, less densely stained neurones are thought to emanate form soma clusters ventrally to the corpora pedunculata. Fibres do not seem to penetrate the

corpora pedunculata. As the neurones are neurosecretory, synaptic contacts with the corpora pedunculata are not required. PDF neurones are under circadian rhythm control and in crustaceans they mediate the release of chromophores from the sinus gland. Its function as an octapeptide hormone in insects is still obscure, but it might be conceivable that PDF immunoreactive neurones innervate the pars intercerebralis rather than the corpora pedunculata (Dirksen, 1994). More unlikely is, that PDF immunoresponsive neurones modulate the circadian rhythm control of the corpora pedunculata.

2.7. Dromyosuppressin Peptide (TDVDHVFLFR amide)

Though the function of Dromyosuppressin is largely unknown neurones immunoresponsive for this peptide neurotransmitter innervate the calyces and descend down into the central nerve cord (McCormick and Nichols, 1993).

CHAPTER 3

Biophysics of Chemosensory Memory Pathways in Insects

Present studies have focused on characterising the function of corpora pedunculata, as well as, their afferents and efferents. Chemosensory afferents innervating the calyces of the corpora pedunculata arise in the antennal lobe. The *Apis mellifera* antenno-glomerular tract neurone VUMmx1 belongs to the neurones of the lateral antenno-glomerular tract (O_3). It displays a high degree of plasticity during conditioned learning indicating that the corpora pedunculata are not solely implemented in this process (Hammer, 1993). In a second study the population code of antennal local interneurones and neurones of the antenno glomerular tract were scrutinised. Changes in field potentials — alterations in charges of the extracellular fluid — thereby were measured and correlated to the production of action potentials. Evident is, thereby, that the field potential oscillations are phase locked with the oscillations determined by intracellular recordings. The findings of this study were confirmed by research on the recurrent interneurones.

The information that is received in the antennal glomeruli is not simply transmitted to the antenno cerebral tract. The glomeruli serve rather as a processing unit of the information before the information proceeds into the antenno cerebral tract or the suboesophageal premotor fibres (for review, see Homberg et al. 1989; Boeckh and Tolbert, 1992). Information input from receptor neurones is processed by a network of output neurones the uniglomerular projection neurones (Getz and Chapman, 1987; Getz and Page, 1991). Several classes of interneurones mediate cross communication between the glomeruli (Distler and Boeckh, 1994).

The output neurones of the antennal lobe of the *Periplaneta americana* are GABAeric implying that some circuits within the antennal lobe are 'disinhibitory' (Distler and Boeckh, 1994). The olfactorio- globularis tract neurones generally terminate in the calyces of the corpora pedunculata. Several pheromone inducible projection neurones of *Spodptera littoralis* display a considerable sexual dimorphism in their in-

nervation pattern. In males pheromone sensitive projection neurones terminate in the inferior lateral protocerebrum. Female homologues end in the lateral horn of the protocerebrum (Anton and Hanssen, 1994). No such dimorphism has been demonstrated in *Drosophila melanogaster*, yet (Stocker, 1990).

1. Characterisation of a Discrete Giant Lateral Olfactorio- globularis tract Neurone in *Apis mellifera*

That **associative learning** depends on the **frequency code** of neural activity was demonstrated in this elegant study by Hammer (1993). Young bees with no previous foraging experience need to learn how to associate a specific smell with a food source. During feeding on sucrose the honey bee associates the **unconditioned stimulus** (taste) with an **conditioned stimulus** (odorant). The presentation of a conditioned stimulus prior to the presentation of an unconditioned stimulus is known as **forward pairing**. The forager learns to associate the conditioned stimulus with the presentation of an unconditioned stimulus. Does, however, the presentation of a unconditioned stimulus precede the presentation of a conditioned stimulus (**backward pairing**), the young forager does not respond to the unconditioned stimulus. If the order of presentation is reversed again the bee requires an adaptation phase after which it is able to associate the conditioned stimulus with an unconditioned stimulus. Thus other than if it exposed to a novel stimulus (forward pairing) backward pairing seems to be inhibitory to the bees ability to associate the conditioned stimulus with the unconditioned one (Hellstern and Hammer, 1994). The proboscis extension reflex - the duration for which the bee extends its proboscis - is an ideal measure to study the bees learning ability. The proboscis extension reflex in an unconditioned bee during the first visits is 120 seconds of duration. Once the long term memory has been acquired, the duration proboscis extension reflex becomes significantly reduced during subsequent visits of the food source (Greggers and Menzel, 1993).

The VUMmx1 neurone is a component of the lateral olfactorio- globularis tract. It has the task to transfer information orthodromically from the trito- and deuterocerebrum to the calyces of the corpora pedunculata. VUMmx1 converges in the region of gusta-

tory and olfactory input. It may thus receive and co-ordinate input from both sensory pathways. The perception of odorant (carnation) in trained bees triggers only a short phasic response in VUMmx1. When, however, sucrose is applied to the proboscis VUMmx1 responds with a 30 sec long action potential (AP). Hence, the transmission of acquired olfactory information depends on the ability of the VUMmx1 neurone to integrate olfactory and gustatory information. The VUMmx1 neurone behaves, thereby, different in forward and backward paired individuals.

In order to make comparative recordings of the responses of VUMmx1 in backward and forward paired individuals a depolarising current was applied. These voltage clamp studies revealed that there was a remarkable decline the in the spike frequency of the VUMmx1 response (presumably with exponential decrement) in backward paired individuals. Forward pairing, by contrast, increases the phasic response and converts it into high frequency oscillations. In this case responses derived form a conditioned and an unconditioned stimulus (input from gustatory and olfactory sensory cells) are integrated and converted into a long term oscillations.

The presentation of an unknown conditioned stimulus (orange blossom odour) reduces the excitatory response within the VUMmx1 neurone quiet significantly.
The differential responses of the VUMmx1 neurone indicates that changes plasticity occur before the olfactory pathway enters the corpora pedunculata (Hammer, 1993). It might thus be that the corpora pedunculata act as a relay structures and not as the primary information processing centre (Bicker, pers. comm.).

2. Examination of Odour Processing in the Antennal Lobes of *Schistocerca americana*

Thus, the VUMmx1 neurone integrates information of the olfactory and gustatory centres. The quality of the response of this neurone is determined by the temporal sequence of the different olfactory and gustatory stimuli, hence, the frequency code. The coding of olfactory information depends, as mentioned above, also on the number and frequency of activated neurones (**population code**). To allow an insect to discriminate between different odours more or less specialised odorant receptor cells need feed all

the information they have received into the chemosensory networks. The differential coding by the neurones of this network elucidates the signal, which is then received by the central brain. In the locust *Schistocerca americana*, as in *Apis mellifera*, the antennal lobe gathers its converging input (50,000 neurones) from the antennae of both sides of the head. Projection neurones of the antenno glomerular tracts (AGNs) then send their fibres into the calyces of the corpora pedunculata. By contrast, local interneurones (ALIs) connect the glomeruli of the antennal lobe with each other (Laurent and Naraghi, 1994).

To characterise the spatial distribution (population code) of odorant activated neurones of the antenno glomerular tracts and local antennal lobe interneurones of *Schistocerca americana* Laurent and Davidowitz (1994) used two types of recordings:

- Local field potential recordings where derived form the outer membranes of the respective neurones
- Intracellular recordings were made form single neurones after injection of a depolarising current.

Ion fluxes in ultimate proximity of the neural membranes are indicative of neural activity. The local field potential recording technique discloses these outer membrane ionic activities. This method does not only serve to detect ionic fluxes in particular proximity of a particular neurone but also to record ionic fluxes of other neurones in this region.

Intracellular recordings, in contrast, identify only the activity of membrane potentials of discrete single neurones. Recordings in response to odorant stimulation with intracellular recordings reveal that odorant induced oscillations of the antenno glomerular tract neurones result from excitatory and inhibitory postsynaptic potentials. Characteristically, oscillations of both antennal efferents - the local antennal lobe interneurones (ALIs) and the antenno glomerular tract neurone (AGNs) - are phase locked. Other than antenno glomerular tract projection neurones, the ALIs do not produce any overshooting volley of action potentials. Their depolarisation activity correlates, however, with the peak of the field potential oscillations in AGNs. In terms of

their response, it might be possible that the ALIs are the origins of the IPSPs, which underlie the train of excitatory depolarisations in the AGNs.

Hence, populations of AGNs, as well as, ALIs respond with rhythmic firing and membrane potential oscillations. The response of both groups of neurones is dependent upon the chemical quality of the odorant. An identified AGN neurone responds, for example, with an phasic membrane potential depolarisation to cherry or pine odour. Conversely, these rhythmic responses are inhibited by apple and floral odour. Field potential oscillations of the entire neurone population, however, persist in spite of this inhibition. Is a blend of apple and cherry odour applied to the antennae, this identified AGN responds with several IPSPs, which suppress the occurrence of EPSPs.
These differential responses are also observed in ALIs. A pair is of ALIs responds, for instance, with in-phase oscillations, when stimulated by the odour cineole. Stimulations with apple and cherry, on the other hand, result in out-of-phase oscillations.

Moreover, when membrane potential recordings are related to field potential recordings, several populations of overlapping AGNs show harmonising phasic oscillations owing to stimulation with a single odorant. Stimulation with several distinct odorants reveals, in contrast, that a specific AGN respond differentially to each odorant: Whereas, the response to apple is monophasic, the response to either citrus or mint takes the shape of an multiphasic response. Characteristically, two forms of multiphasic responses can be discerned: Firstly, the undulatory membrane depolarisations evoked by the citrus odour are aborted by antagonising inhibitory synaptic currents. Secondly, membrane potential oscillations caused by mint are only temporarily interrupted. The same findings apply to the function of other individual AGNs, as well. Here again multiphasic responses are the result of an antagonism between the inhibitory and excitatory postsynaptic currents during odorant induced membrane depolarisation.

Simultaneous recordings of two AGNs indicated that the sequence of membrane potential oscillations to isoamylacetate and cineole diverges quite significantly. It is also evident that the oscillatory responses can vary quite considerably in a single neurone and may or may not correlate to field potential population responses. The spike phase of an AGNs evoked by mint odour, for instance, is only phase locked to a popu-

lation of neurones sharing the same field potential oscillations during the second portion of its response. By contrast, several neurones have been identified, which correlate with population responses only during the initial portion of their response and become later uncoupled. It can, thus, be envisaged, that AGNs initially respond to an odorant stimulus with out- of- phase action potentials. These are subsequently coupled with population oscillations. As the result the initial responses of individual neural elements become synchronised, an event known as **phase resetting** or **stimulus- timing- phase singularity** (for review, see Miller, 1974; Laurent and Davidowitz, 1994).

3. Biophysical Characterisation of the Corpus Pedunculatum Kenyon Neurones of *Schistocerca americana*

As discussed in paragraph two of this chapter, it is thought that the synchronous 20Hz oscillations in the lateral Kenyon neurones of the corpora pedunculata originate (at least partially) in the antennal lobes. It has, for instance been demonstrated, that, when the corpora pedunculata are extirpated, the membrane depolarising oscillations of the antennal lobe efferents persist. Interestingly, field potential oscillations of Kenyon neurones are generally phase locked -70° out of phase with the membrane potentials of AGNs (Laurent and Davidowitz, 1994).

As seen before, the Kenyon neurones are an integral part of several synaptic circuits in the insect supraoesophageal ganglion. Synaptic input from the olfactorio- globularis tract and synaptic output into the LLP or the lobulus medialis protocerebralis (LMP) is thought to be cholineric. When the sensilla of *Schistocerca americana* are exposed to air puffs, local field potential recordings from Kenyon cell somata dorsal to the calyx neuropil reveal that these air puff induce action potentials (APs).

The application of a specific odorant pulse to the antennae (without the test animal having encountered this odour in the past) evokes oscillatory EPSPs (~20 Hz) in distinct subsets of Kenyon cells. The amplitude of the initial EPSPs declines, however, soon, but is reinstated only shortly afterwards. It is thought that the decline in the initial EPSP amplitude is owing to IPSPs, which are closely associated with the initial EPSPs. These IPSPs are presumably instigated by γ- aminobutylic acid (GABA) containing

presynaptic neurones (***vide ante***). A likely candidate for these GABAeric neurones are the recurrent feedback neurones that connect both lobes to the calyces. With sustained odorant pulses, however, the IPSP diminishes with a gradual decrement and finally disappears (***vide infra***). Synergistically, the amplitudes of the oscillatory EPSPs increase reciprocally, with the oscillations of all recorded Kenyon neurones having generally been in phase. Some out of phase action potentials may occur, however, outwith the periods of odorant presentation.

The presentation of an odour to the antennae, whilst subsets of Kenyon axons aredirect current (DC) voltage clamped, results in an alteration of the spiking activity. At the resting potential level the odorant induces fast phasic oscillations intermitted by a low tonic spiking activity. The subsequent summation of these oscillatory EPSPs following a repeated odorant presentation induces APs within postsynaptic neurones. During odorant presentation the underlying depolarisation tendencies are suppressed. When a depolarising potential reaches a threshold, a large spiking afterpotential follows suit suppressing the oscillations. In accord with local field potential recordings, the initial EPSP of voltage clamp recordings is followed by an massive hyperpolarisation. This suggests that there ought be an underlying hyperpolarising component in form of an IPSP, which initially alters the spiking activity and declines with a perpetuated olfactory stimulus.

The stimulation of antennal nerve interneurones triggers polysynaptic responses in Kenyon neurones. The summation of several EPSPs induces an unitary AP per oscillation cycle in the postsynaptic Kenyon neurones. Two EPSP components in the K- neurone can be distinguished: a **passive EPSP**, which underlies the **active EPSP**. Active EPSPs are induced 15-25 msec. following odorant stimulation of the antennal nerve, whereby each K-neurone responds to a specific presynaptic fibre with an unitary EPSP. As seen before, the induction of these active EPSP components depends upon the availability of receptors in the postsynaptic Kenyon cell membranes. Hence, the initiation of postsynaptic responses requires the temporal or spatial summation of several discrete EPSP quantum units. Or to put it in other words, one can predict that the number of quantal units is proportional to the amplitude of the depolarising current of the antennal

nerve. Is now a hyperpolarising current applied, one can easily discriminate between the different quantal values of each K-neurones by filtering out the responses of neurones that would have otherwise cross-talked with the signal. The number of 8- 10 quantal units as deduced from this study is the result of the summation of excitatory input derived from an equivalent number of interneurones. The amplitude of the recurring depolarisation phase is, hence, proportional to the AP in odour induced responses of hyperpolarised Kenyon-cells. As a result of the hyperpolarisation, it was observed — as predicted — that each AP correlates with the descending phase of the local field potential confirming that the oscillations within a population are synchronous.

4. Characterisation of a Recurrent Feedback Neurones in *Apis mellifera*

Recurrent GABAeric feedback neurones connect the α- and β- lobe exit points with the calyces of *Apis mellifera*. Action potentials (APs) recorded at the α- lobe exit of 2-3 msec duration peaking at 20- 40 mV are elicited by summating EPSPs (increase in frequency ≡ oscillatory velocity). Three phases can be distinguished during postsynaptic EPSPs responses: An initial stimulus is proceeded by a sustained excitation and terminated by a short off response (Grünewald, 1994; pers. comm.). The oscillations peaked 4 -12 mV with an frequency of 100 Hz during the time span. The off responses are either characterised by an increased or decreased spike frequency. Here again, most neurones show odorant specificity and alter their oscillatory response specifically to an odour (Grünewald, 1994; pers. comm.).

5. Characterisation of an Identified Kenyon Neurone Efferent (Pe-1) of *Apis mellifera*

Several diverging efferent fibres receive converging output from Kenyon neurones of each bilateral corpus pedunculatum of *Apis mellifera*. A well characterised efferent neurone is the Pe-1 neurone. It presumably innervates each corpus pedunculatum through the β- exit of the peduncle. Information is presumably transferred to the lobulus lateral protocerebralis where it is further processed.

In an elegant study Juliane Maulshagen (1993, pers. comm.) has correlated the frequency code of this neurone with the process of associative learning. As mentioned above, sensitisation involves the application of an single sensitising stimulus to the antennae and proboscis. Single conditioning, in contrast, is sequential application of an conditioned stimulus (carnation) and unconditioned stimulus (sucrose) to antennae and proboscis, respectively. Differential conditioning reveals the properties of two different odours, i.e. paired (carnation) and unpaired odour (orange blossom) on the bees learning behaviour. The time sequence of the application of each stimulus has thereby an cardinal effect on the outcome of the learned response.

Pe- 1 responds to chemicals, like odorants and gustatory substances, as well as, mechanical stimuli. The response of the Pe-1 neurone to these stimuli is a oscillatory excitation phase, which is not correlated to the duration of an stimulus. Odorant induced responses generally show latencies between 30 and 125 milliseconds. Initially, the oscillation frequency rises considerably and reaches values between 100- 330 Hz. Voltage clamp recordings further identify a direct current potential of 5- 25 mV. The high frequency excitation segment of the response is followed by an decrement during which frequency and amplitude of the oscillation declines. During this time - 60 milliseconds to 1.2 seconds following stimulus onset - pulses occur more and more randomly.

To assay the different segments of the Pe-1 neurone response, Juliane Maulshagen considered it to be necessary to divide each response into intervals. Ideally suited she considered thereby six time intervals - 6 to 100 milliseconds - of different time spans varying between 30 seconds and 5 minutes.

The responses of the Pe- 1 neurone of sensitised and single trial conditioned individuals behaves analogous. In both cases the response consists of an excitatory and an inhibitory segment. During the excitatory segment the phase of the oscillations increases rapidly - the increment of the response. The initial excitatory segment is proceeded by the inhibitory segment during which the oscillatory response declines gradually, the decrement of the response. It is interesting to note that the phases of the oscillations induced by either antennal or proboscis sensitisations differ in sign and are, thus,

not in phase. Conflictingly it has been found that although sucrose stimulation to the antennae is most effective in modulating the neural response, sucrose stimulation to the proboscis is the more dominant. Maulshagen (1993) therefore argued that the initial excitatory segment is initiated at the antennae, whereas the following inhibitory segment originates in the proboscis. In summary, the responses of the Pe-1 neurone of sensitised and associative single trial conditioned bees behave almost identical. Dependent upon whether the antennae or the proboscis have been stimulated, the frequency changes accordingly.

The Pe-1 neurone of differentially conditioned bees responds after 15 to 25 seconds following associative and non- associative stimulation. In untrained bees the spontaneous responses to carnation and orange blossom are identical. Interestingly, in bees trained in an differential condition learning paradigm, the Pe-1 neurone encodes carnation and orange blossom differentially. After the first differential conditioning trial the Pe-1 neurone still responds with an increasing decrement during the second inhibitory segment of the response, indicative of an odorant specific modulation. Four learning trials, however, enhance the frequency of the inhibitory segment quite considerably and therewith reduce the decrement. The course of the signal, hence, mirrors associative changes in the frequency of the oscillations during the falling phase of the response. Maulshagen (1993) speculated that this alteration might be due to the gradual adaptation of the Pe-1 neurone to the differential learning sequence. Conversely, Maulshagen found no difference in the response of Pe-1 to either an conditioned or unconditioned stimulus. Both had the same potency in evoking an attenuated decrement in the second segment of the response.

In direct current voltage clamped Pe-1 the frequency was reduced after the second conditioning trial. During the fifth conditioning trial the frequency increased significantly in comparison to control bees. It can be assumed that these modulations are the consequence of incoming oscillations EPSPs rather than due to the alteration of the Pe-1 neurone itself.

Also scrutinised were in this experiment the responses of the anterior dorsal protocerebral commissure (adpc), which is situated in ultimate vicinity of Pe-1. Neurones of

this commissure respond quite differently from Pe-1. It has, for instance, been established that the amplitudes of the oscillations in these neurones are much higher than those of Pe-1 during spontaneous activity. Additionally, each oscillation is associated with an after potential. The spike duration exceeds approximately >4 milliseconds. Moreover, following intracellular stimulation with an 1 nA depolarising current, adpc neurones respond with low frequency activities (Maulshagen, 1993).

6. Pheromone Stimulated Response of Two Descending Ventral Nerve Cord Neurones in Male *Bombyx mori*

In the male silkworm moth *Bombyx mori* axon that arise in the lobulus lateralis protocerebralis descend within ganglia down into the **ventral nerve cord**. Furthermore, it has been demonstrated that — in *Bombyx* as in other insect species — the neurones of the lateral lobe make connections with another protocerebral region: the **central complex**. The central complex is thought to be a major mediator in the orientation of the insects, and probably receives major input from the visual apparatus (for review see, Homberg, 1987). Hence, the neurones of the ventral nerve cord receive converging input from both the corpora pedunculata and the central complex (for review, see Howse, 1974; Kanzaki et al., 1994). Interestingly, do male *Bombyx*, once they have perceive an pheromone pulse, walk in a zigzag like motion towards the odour source. This aberrant walk seem to be directly controlled by the modulation of the normal walking pattern by the corpora pedunculata. The zig-zag like walk seems to be the consequence of alteration in the normal firing behaviour of two descending interneurones in response to pheromone stimulation analogous to the modulation of an electrical circuit by an electronic flip-flop device. Characteristically, such modulated firing patters integrate two alternating frequencies: One is high, the other is low. The phase of the flip-flop activity may, however, vary within the population of neurones, which constitute the descending connective, as well. In terms of the phase of the pheromone induced oscillations Kanzaki et al. (1994) have categorised the descending nerve cord connectives into two different populations. Whereas ff neurones seems to always be locked in phase with each other, the oscillations of the FF neurones are generally out of phase with the re-

maining neurones of this connective. It is quite likely that these asynchronic oscillating neurones are of distinct morphologies, and transmit the impulse according to their discrete properties. It may, however, be conceivable that similar to AGNs, the neurones, which constitute the descending ventral nerve cord commissure, ought to respond 'in phase' before an efficient downstream impulse can be elicited. In any event, it is not known as too whether the asynchronic firing activity has any implication on the outcome of the walking response (Kanzaki et al., 1994).

7. Synthesis

Olfactory stimuli elicited an neural response once a threshold has been overcome. By unknown mechanisms the signal then enters the chemosensory memory pathways. The transmission of the signals proceeds through several synaptic regions like those found in the antennal lobe, the lobulus lateralis protocerebralis, the calyces, peduncles and lobes of the corpora pedunculata. The synaptic regions are the main modulators of incoming signals. They can either amplify an odour stimulus, or inhibit it.

Odorants are thought to be perceived at discrete topographical zones of the antennae. It remains to be elucidated as to whether receptor cells of each topographic zone project proportionally to each glomerulus, or if the glomerular arrangement is even more complex. Anyway, the signal that is received from functional identical receptors of each topological zone converge onto the antennal lobe. The glomeruli of the antennal lobe play a crucial role during the initial stages of signal coding. They act as a switchboard to relay signals to either the lobulus lateral protocerebralis, the corpus pedunculatum, or the suboesophageal motoneurones. Efferent neurones that innervate the glomeruli consist of local interneurones and several antenno glomerular tract neurones. Whereas, synapses at the interneurones have potential inhibitory capacities, the synapses at neurones of the antennal glomerular tract are probably excitatory. Signals evoked by different odorants in *Schistocerca americana* are encoded by the antennal lobe so that specific populations of neurones are activated. These characteristically 'swing' in phase in response to a specific odorants. Other odorants in contrast may

evoke an attenuation of the phase and, thus, counteract the population code of this odorant. Moreover, frequency changes have been observed in an characterised neurone *Apis mellifera* which are proportional to the sequence by which the different olfactory stimuli are given. This neurone which integrates olfactory and gustatory input answers with high frequency oscillations as long as conditioned and unconditioned stimuli are given in the trained order to antennae and proboscis, respectively. Pairing in the reverse order triggers an decline in frequency.

At the synaptic junction between the afferent olfactorio- globularis tracts and the Kenyon neurones incoming signals are transduced and elicit postsynaptic responses proportionally to the intensity of the presynaptic signal (proportional to the quality of the odorant). These cholineric EPSPs propagate through the peduncle and reach the synaptic terminals of the α- or β- lobe. Here they enter either the inhibitory feedback neurones and oscillate back to the calycal input regions or transcend into premotor fibres. Signals that enter the GABAeric feedback interneurones of the *Apis mellifera* corpus pedunculatum initiate IPSPs, which inhibit the incoming EPSPs in order to synchronise the postsynaptic oscillations. With increasing frequency the oscillatory EPSPs (summating EPSPs) suppress the underlying IPSPs and may finally elicit an action potential. The action potential finally mediates the release of neurotransmitter into the synaptic cleft and elicits responses in the postsynaptic efferents once a certain quantum threshold has been reached in *Schistocerca americana*. In the postsynaptic efferent Na^+ channels might be opened once a threshold is reached. As a secondary response a Ca^{2+} influx might be elicited, which might trigger the activation of the cAMP second messenger pathways. About the identity of these efferents so far hardly anything is known. It might be that these efferent are premotor neurones or the GABAeric feedback neurones that then elicit the activation of the second messenger cascades either intrinsically in the α- or β- lobe or as a result of the feed back activity in the Kenyon neurones.

Contrastingly, an putative peduncle exit fibre shows reduced frequencies in response to olfactory stimuli. Whereas in unlearned bees the responses are generally reproducible, responses of individuals of the different learned groups fluctuated quite considerably. That this neurone is a component of the chemosensory memory path-

ways was established, because its activity was initiated with olfactory stimulation and ceased following the abortion of the stimulus.

CHAPTER 4

The Modulation of the Synaptic Plasticity by Biochemical Signalling Pathways in Chemosensory Memory Networks

1. Overview

Anatomical studies (*vide ante*) on *Apis mellifera* have revealed that chemosensory memory pathways comprise at least four different levels: Sensory neurones, interneurones, corpora pedunculata, lobulus lateralis protocerebralis (LLP) and their respective connectives, as well as, premotor fibres, which serve as efferents, can be distinguished (Mobbs, 1982; Rybak and Menzel, 1993). In *Drosophila melanogaster* the anatomy of the chemosensory memory pathways , although not studied in detail yet, is thought to be similar (Technau and Heisenberg, 1982; Stocker, 1990; for review, see Davis, 1993). The question arises, however, if neurones of each level are involved in mnemogenesis or if the process of mnemogenesis is topographically confined to a discrete level within the chemosensory memory pathways (e.g. corpora pedunculata or LLP; for review, see Davis, 1993). Hence, two possibilities can be considered:

Firstly, it might be conceivable that all neurones within the chemosensory memory pathways are equally involved in the mnemogenic process. Alterations may be specific to certain topological domains, which receive several coinciding stimuli to mediate changes in the neural behaviour.

Secondly, within the chemosensory memory network each group of neurones might be genetically distinct and therefore assume specific tasks. As I will mention in this chapter, presumably both notions hold true.

A second question, which needs to be addressed is the mode by which certain sets of neurones accomplish an alteration in their behaviour. Here again two mutually dependent notions can be considered: Firstly, the state of neural activity within the chemosensory memory networks might be altered as a result of electrical switching between genetically discrete sets of neurones (Hammer, 1993; Laurent and Naraghi, 1994; Laurent

and Davidowitz, 1994; Weismann and Marder, 1994 [in somatogastric nervous system of the shore crab *Cancer borealis*]). Alternatively, the behaviour of electrochemical circuits might be modulated by stimuli, which have been received by genetically predisposed neurones (Bliss and Lømo, 1973; for review, see Bliss and Collingridge, 1993). Finally, one may ask how the topography of the chemosensory memory networks relates to the biochemical memory pathways.

It is well known that mnemogenesis comprises two additive components:
The first the short term memory is thought to result from the alteration of neural electrochemical activity in response a single or several stimuli. In *Drosophila melanogaster* the reception of these stimuli seems to be mediated by the products of the genes *latheo* and *linotte* (Boynton and Tully, 1992; Dura et al., 1993). Several different putative members of the adenylyl signal transduction pathway have been postulated to participate in the formation of short term memory. So it has for instance been reported that genes such as *rutabaga* and *dunce* participate in the formation of short term memory (Drain et al., 1991; Chen et al., 1986; Tully et al., 1994; for reviews see Davis and Dauweiler, 1991; Davis, 1993). The *amnesiac* gene is probably involved in the transformation of short term memory into middle term memory (Tully, 1994).

The second, the long term memory is the consolidated form of short term memory. Form studies on the rat hippocampus it has, for instance, been gathered that the acquisition of long term memory requires the activity of several converging and, hence, additive stimuli received within a discrete time window. There are indications from studies on rat brain slides that strong tetanic stimuli induce transcriptional activity, and therewith the *de novo* synthesis of proteins in genetically and topographically presaged hippocampal neurones, although extracts of these proteins have not yet been purified (Nguyen et al., 1994; and references therein; for review, see Bliss and Colingridge, 1993). In *Drosophila melanogaster* the involvement of several genes, which function in the inositol triphosphate pathway, namely *Drosophila melanogaster* protein kinase C (*plc21*), calmodulin (*CAM*), Calbindin- 32, the *Drosophila* gap 43 homologue (*igloo*), and two *Drosophila* homologues of intracellular Ca^{2+}- release channels are presumably

required for the formation of long term memory (Shortridge et al., 1993; Albert et al., 1994; Kovalick and Beckingham, 1992; Reifgerste et al., 1993; Neel and Young, 1994; Hasan and Rosbash, 1992; Tully pers. comm.). The IP_3 signal transduction cascade probably converges onto the cAMP pathway (Anholt, 1994). There is further evidence that downstream of protein kinase A several cAMP responsive transcription factors are activated (Foster et al., 1984; Yin et al., 1994).

In addition to long term memory the existence of a further form of long lasting memory, the anaesthesia resistant memory (ARM), has been proposed by Tully et al. (1994). Strikingy, ARM persists if the protein biosynthesis is inhibited by 50%. It is, however, abolished in mutants for *radish* (Folkers et al., 1993; Tully et al., 1994).

2. Mnemogenic Second Messenger Pathways of *Drosophila melanogaster*

2.1. The Adenylyl Cydase Pathway

A general problem, which needs to be tackled is how the adenosine 3',5'-cyclic monophosphate (cAMP) pathway is initiated in neurones of the chemosensory memory networks. A likely candidate for the activation of the cAMP pathway of *Drosophila melanogaster*, on the level of the corpora pedunculata seems to be the 5- hydroxy-tryptamine (5- HT) receptor. This hypothesis has excited considerable interest as this receptor is a likely activator of the cAMP pathway in the sea hare *Aplysia california* (Braha et al., 1990). Two putative 5-HT receptors *dro1* and *dro2A* have so far been identified in *Drosophila* (*vide ante*) (Wirtz et al., 1990; Saudou et al., 1992). The ligand induced receptor associated GTP-$G_{s\alpha}$ subunit of the GTPase holoenzyme may then act in conjunction with Ca^{2+}/calmodulin to activate Ca^{2+}/ calmodulin dependent adenylyl cyclase (cAMP cyclase), which in *Drosophila melanogaster* is encoded by the *rutabaga* gene (Levine et al., 1992). The *rutabaga* cAMP cyclase displays a high degree of homology to its vertebrate counterpart. Hence, the activity of *rutabaga* adenylyl cyclase requires the convergence of two effectors to become activated and to catalyse the conversation of ATP to cAMP. In *Drosophila melanogaster*, the notion that cAMP cyclase is involved in this process has been confirmed as in mutants for *rutabaga* the basal levels of cAMP are significantly reduced. The *rutabaga* gene prod-

uct has been localised to the K-cell neuropil and is, thus, correlated to the region of synaptic activity (for review, see Davis, 1993).

The levels of cAMP are directly controlled by cyclic nucleotide phosphodiesterase II (PDEII). This enzyme which does not represent a direct component of the cAMP pathway breaks down abundant cAMP. In *Drosophila melanogaster* PDEII is encoded by the *dunce* locus. In accordance with its function mutations in the alleles of *dunce* act as suppressers of the *rutabaga* phenotype. In accordance with this observation is that null mutations in alleles of *dunce* act as suppressors of the *rutabaga* mutant phenotype. Thus, null mutations for Ca^{2+}/calmodulin dependent cAMP cyclase are associated with depleted cAMP titres due to the actions of cyclic nucleotide phosphodiesterase, in double mutants for cyclic nucleotide phosphodiesterase and Ca^{2+}/calmodulin dependent cAMP cyclase neither the *de novo* synthesis nor depletion of cAMP can take place.

As cAMP cyclase, PDEII is confined topographically to the lobes of the corpora pedunculata (for reviews see Davis, 1993 and references therein. Anholt, 1994). It is thought that cAMP interacts with Ca^{2+}/Calmodulin to activate protein kinase A (Skoulakis et al., 1993).

2.2. The Inositol 1,4,5 Triphosphate (IP_3) Transduction Cascade

The pathway implicated in the formation of long term memory is the 1,4,5 triphosphate (IP_3) signal transduction cascade. This pathway plays strikingly enough a major part in the process of axonogenesis (for discussion ***vide post***). It might be plausible that the *Drosophila* 5- HT receptor homologue *dro2A* acts to activate *PHOSPHOLIPASE C* (*PLC 21*) through the GTPase, which is associated with it. *PLC21* which in *Drosophila* is encoded by the *mushroom body miniature* locus may be the phosphorylating agent of the *Drosophila GAP-43* homologue *igloo* (*igl*) (Shortridge, 1991; Albert et al., 1994, Neel and Young, 1994). *IGL* occurs as two splicing isoforms. The isoform *IGL-L* predominates during preadult development in the central nervous system. The *IGL-S* isoform, in contrast, is abundantly expressed in the adult central nervous system. Whereas *IGL-L* is rapidly phosphorylated by purified PKC *in vitro*, the *IGL-S* isoform is a potent inhibitor

of phosphorylation. Moreover, it has been demonstrated that *PLC 21* may catalyse the hydrolysis of phosphatidyl inositol 4,5- bisphosphate [Ptd Ins $(4,5)P_2$] to yield diacylglycerol and inositol triphosphate (IP_3) either in conjunction with myristoylated *IGL-L* or on its own (Neel and Young, 1994). IP_3 presumably binds to the *Drosophila* homologue of the *Drosophila* IP_3 receptor *dip*, which might act to release Ca^{2+} from intercellular cisterns. It has been assumed that several splicing isoforms mediate different responses in concert with a particular developmental function. In the adult form of *DIP* is particularly expressed in the protocerebrum, as well as, in the deuterocerebrum (Hasan and Rosbash, 1992). DAG might have the same function. One may speculate that Ca^{2+} could bind to calmodulin, which, too, has an developmental function (*vide ante*) (Kovalick and Beckingham, 1992). An intriguing candidate for downstream targets are either protein kinase C or more likely the components of the cAMP pathway (for review, see Anholt, 1994; Tully pers. comm.).

2.3. The Coincidence Detector Protein Kinase A

Downstream of cAMP Ca^{2+}/calmodulin dependent cAMP cyclase, cAMP is thought to activate cAMP dependent protein kinase (PKA). Alternatively it was proposed that PKA may act independently of cAMP in a cGMP responsive mode (for review, see Breer and Shephard, 1993). However, it seems to be more likely that cAMP is the major activator of PKA (Hildebrandt et al., 1994). PKA is composed of four different subunits: two regulatory (R) and two catalytic(C). When the subunits are combined, the tetrameric holoenzyme R_2C_2 is inactive. There are two discrete regulatory subunits of PKA in *Drosophila* each of which contains three functional domains:

- an N-terminal region responsible for binding to its counterpart, which is always the identical isoform.
- an N-terminal region, which is responsible for inhibiting the catalytic subunit
- two similar binding sites for cAMP

cAMP activates cAMP dependent PKA by altering the confirmation of its regulatory subunits, so that they dissociate from the catalytic subunits. Each of the catalytic sub-

pathway, which integrates PKA may be prominent in the calyces, the region of synaptic input (Skoulakis et al., 1993).

The pathway, which is described at here displays, however, another important discrepancy. Firstly, it has not yet been established as to whether NOS- activity occurs in postsynaptic nerve cells or presynaptic cells. NO is an important component of both of the cAMP and the IP_3 pathway. Within the chemosensory memory pathways NOS is ascribed to have two functions. As it is an easy diffusible messenger NO might either act antrogradely or retrogradely. Hence, NO may have two different functions in sensory neurones and higher order interneurones such as the K- neurones of the corpora pedunculata, respectively. This hypothesis has been experimentally substantiated by two findings:

Firstly, biochemical studies on cultured cells, which are derived from the antennae, *Manduca sexta*, indicate that NO acts in these cells in an antrograde mode: Thus, in antennal sensilla neurones, cGMP is thought to stimulate the dose of extracellular cation channels in response to NO. Hence , in antennal sensilla neurones, NO counteracts IP_3, which is responsible for keeping the cation channels open (Henn and Kaup, 1994; Stengl, 1994). In the K- neurones of the corpora pedunculata, it has been proposed that, similar to the pyramidal cells of the mammalian hippocampus, NO acts to enhance the effects of both the IP_3 and the cAMP pathway.

Secondly, *in situ* NDP activity staining exhibits quite different patterns in the antennal lobe and corpora pedunculata, respectively (Elphick, Hildebrandt et al., 1994).

Contrasting sharply with the findings of Stengl (1994) and Hildebrandt et al. (1994) are recently revelations that in sensory neurones of the rat olfactory bulb, NOS, or so to speak its product, NO, might have an unique function as a developmental regulator and exclusively be involved in axonogenesis. CO might instead be involved in the sensory transduction process (Verna, 1993; Raskams et al., 1994, for review see Dawson and Snyder, 1994). CO is, as demonstrated by Verma et al. (1993), produced by haeme oxygenase 2, a enzyme ubiquitously expressed in the mammalian brain, which catalyses the cleavage of the haeme ring into CO and biliverdin. The latter is rapidly reduced

by bilirubin. The electrons for the CO synthesis are derived from cytochrome P-450 reductase.

With disregard of the above mentioned pathways, *amnesiac* a other learning deficient mutant has been shown to be deficient for a pituitary encoded protein. The pituitary is the mammalian neurohaemal organ, which releases peptide hormones. Its analogue in insects is the pars intercerebralis. Thus, the acquisition of long term memory might also be dependent on the action of peptidehormones such as the period controlled *amnesiac* and forskolin (Dudai et al., 1985).

4. Synthesis

Storage of neural information (mnemogenesis) is a process, which is not completed with the end of a behavioural learning sequence. As mentioned above, there are two **components** to the mnemogenesis: Information recorded during the learning process is first entered into the **short term memory**. This information is subsequently transferred to the **long term memory**, which is thought to require the *de novo* synthesis of proteins. A well investigated example of how mnemogenesis in insects may proceed is the postpupal development of the honey bee (*Apis mellifera*). The honey bee *Apis* displays a far more differentiated behaviour then *Drosophila* and the behavioural sequences are far more co-ordinated and seem to follow a distinctive time table . Various stages of the imaginal development of the honey bee can be distinguished starting with an 4-7 week long interval during which the worker bees are deployed to care for the queen and to nurse the brood. This serves to teach the young bees to recognise the features of the hive. After some 4- 7 weeks the young foraging bee leaves the hive. It subsequently learns to interpret the position of the sun through perception of polarised light. The position of the sun is reflected by the degree of polarised light (Petzhold and Labhart, 1994). *Apis* perceives and memorises the amount of polarised light and uses it to define the direction of a food source. When entering the hive the bee translates the polarisation of the sun light into movements along a gravitational axis. This gravitational axis is communicated to its hive mate using the hive dance. A movement 180° upwards

represents a food source in direction of the polarised light. The antennal lobe and the corpora pedunculata as the main olfactory processing circuits play thereby an important role. As seen before they contain the nervous function, which enables a bee to learn to associate an odour (taste) or a location with a particular food source.

Adapting itself from the nursing stage to the foraging stage requires certain changes in the brain anatomy in nurse bees. The olfactory glomeruli and the Kenyon cell cortices, which contain the somata are more voluminous in foragers than their counterparts in bees with foraging experience. The Kenyon cell neuropil, however, increased markedly in size, suggesting that striking changes in the plasticity of Kenyon cell neuropil must occur during the transition from nurse bees to foraging bees. This process is not age dependent but is a direct consequence of the foraging experience! The more voluminous neuropil may reflect the formation of new synapses during the foraging experience (Withers et al. 1993, Greggers and Menzel, 1993).

In *Drosophila melanogaster* Technau (1984) observed that the number of axons within the peduncle decreased significantly when eclosing imagoes were deprived of olfactory or mechanosensory stimuli compared to normal developing controls. Regularly developing control individuals displayed significant increases in pedunculata axon seven days after eclosion, which were reduced in three to four week old flies.

The whole process seems to be correlated with an augmentation in the titres of isoprenoid juvenile hormone. In the house cricket *Acheta domesticus* juvenile hormone may induce the differentiation of putative neuroblast subsets located above the K- neurone cortices. Thus, it may well be the case, that neurogenesis, continues beyond the point Ito and Hotta (1992) regarded as the disappearance of neuroblasts form the *Drosophila melanogaster* Kenyon neurone cortices (Carye et al., 1994). In accordance with my theory changes in the plasticity of the corpora pedunculata may be the consequence of a predetermined developmental program set by the organisms genomic interactions. External factors such as the absence of mature bees in a hive in which young foragers predominate may influence to enrolment of this program by mechanisms, which are not yet understood. For example it was observed that the maturation of the corpora pedunculata proceeds much slower in young bees, when a mixed population of

older and younger bees is present in the hive. The absence of older bees, however seems to enhance the path of mushroom body maturation. It was, thus, suggested, that elder bees have an inhibitory effect on the maturation of the corpora pedunculata in immature foragers (Withers et al., 1993).

PART 5
Conclusions and Dénouement

'It is the task of the natural scientist to search for laws which will enable him to make predictions.' (Sir Karl R. Popper). Thus, I will now resort to putting forward a model explaining the course of events during the process of morpho- and mnemogenesis.

CHAPTER 1
The Theory

Morphogenesis is a direct function of the organisms ontogenesis with respect to its teleonomy, or hence, its genetic content reflects the evolutionary adaptation to its environment (Davis et al., 1990). Thus, one may consider the outcome of morphogenesis as the teleonomic memory, which is stored in the DNA. Mnemogenesis in contrast is ontogenically determined. A current theory is that the mnemogenesis involves changes in neural plasticity within the metazoan organisms teleonomic cerebral framework. During ontogenesis strictly defined developmental regulatory circuits create the metazoan organism. As the metazoan organism becomes autonomous, some of the developmental circuits are redeployed to receive and to process environmental information. Hence, a metazoan organism is not solely individual in terms of its genetic content, but also how it uses the genetic content in response to environmental information. Thus, the differential expression of genes in response to environmental information is stored in the nervous system. Ontogenic nervous memory is a very good example for the functional genetic adaptation of an organism to its environment. This memory is highly dynamic and the phenotype can well be studied in terms of the organisms behavioural adaptation as well as nerve cell plasticity and function. In summary, mnemogenesis is predetermined by neurogenesis (hardware). New information (software) can be entered during ontogenesis, which alters the hard- ware.

Hence, to verify the above mentioned theory, I have chosen the fruit fly as a model. It has the advantage that its genetics (teleonomic memory) is well understood and that its nervous system is not as complex. From studies on the development of the compound eye, for instance, it has been proven that about 3,300 of the essential genes participate in the development of this structure (5,000- 7,500 of all genes) (Thaker and Kankel, 1992), whereas in mammals perhaps twice as many genes, as well as, a multitude of different protein isotypes generated by mRNA splicing, processing and editing participate in this process.

Of particular interest are the chemosensory memory pathways. As demonstrated in a variety of behavioural studies, *Drosophila* shows a degree of learning plasticity in chemosensory learning paradigms. Moreover, with the generation of learning mutants one can identify messenger pathways underlying the synaptic plasticity (for review, see Heisenberg, 1989). As described above the pathways, which impair this component are multimodal, and many pathways (though not all) are used as well during development. According to the temporal activity of a respective pathway three possibilities can be distinguished, how this pathway can affect mnemogenesis. The first possibility is that the pathway is used purely during morphogenesis and is switched off when this process has been completed (class I pathway genes). A second possibility might be that the pathway is active during the entire ontogenesis of *Drosophila* (class II pathway genes). The last possibility is that the pathway becomes only active during postembryonic development (but is still a product of the proper completion of morphogenesis; class III pathway genes).

CHAPTER 2
Synthesis

1. Morpho- and Mnemogenic Genes

The development in *Drosophila* proceeds in several waves of proliferation, differentiation, identification and maturation. Each wave creates thereby a more refined structure. The mechanism deployed each of the developmental processes tend to reiterate spatially and temporally. Hence, the genetic circuits engaged in either process can be considered as modules, which are redeployed during the different stages of the development. The redeployment of such a pathway depends on the information delivered within a spatial and temporal window to which the respective cells are exposed.

Several class I regulatory pathways have been identified, which are redeployed during the different stages of development but are probably not used in an differentiated organism. The regulatory circuits used for differentiation appear to be redeployed during several developmental stages and in several tissues. It has for example been established that members of the achaete- scute complex positively regulate the differentiation of neuroblasts during neurogenesis in germ band staged embryos. The members of the *AS-C* also determine the formation of sensory bristles later during development. Mutations in this complex are associated with the loss of 50% of cells in the nervous system. In an other example differentiation of the R7 photoreceptor cell from ommatidia precursor cells is co-ordinated by the sevenless MAP kinase signalling pathway. The same pathway is also used to mediate the differentiation of acron and tectum at the anterior and posterior tips of early blastoderm stage embryos.

It has been well established that the *NOTCH -DELTA* pathway controls the differentiation of cells. For instance in the neuroectoderm *NOTCH-DELTA* interactions antagonise the commitment of a cell to proceed with a neural lineage. The mechanism is used again during the formation of bristles, eyes, legs and wings later during development. In particular during the development of the compound eye, the *NOTCH* pathway has the ability to block the protagonistic sevenless RTK *ras* pathway in order to prevent the re-

cruitment of more ommatidial precursors than required. Parallels to neurogenesis in the germ band stage embryo await to be elucidated.

As a further example for a class I pathway genes (***vide ante***) I have to mention the *wingless - engrailed - hedgehog* pathway, which co-ordinates through genetic gradients the formation of segmental boundaries in the ectoderm. It also provides positional cues for cells of the underlying neuroectoderm presumably through *gsb-p*. What I have mention in this contexts is that this pathway is applied during leg, and wing formation as well as during later developmental waves.

Interestingly, the class I gene *otd* , which determines the identity of several head specific segments, is active during neuroderm and the ocellar development, as well. In all these cases *otd* seems to be involved in activating *en*.. So in *otd* mutants either the *en* stripes, or *en* positive neurones are absent.

ftz and *eve* are expressed in bisegmental periodicity following cell division cycle 13 (stage 1). Both genes determine the identity of alternating segmental boundaries. In the nervous system *ftz* and *eve* are responsible for conferring identity to defined groups of ganglion mother cells and neurones within each parasegment.

Another class I gene is the *Toll* receptor, which activates the *dorsal* pathway and therewith determines dorso-ventral polarity. In mutants for *Toll,* the embryo assumes a uniform dorso-ventral pattern (Anderson et al., 1985). Additionally, in *Toll* conditional mutants 4% of the RP neurones are absent. It was, thus proposed that *Toll* (either directly or by mechanisms similar to the actions of *Toll* in the early embryo) might participate in the generation of synaptic or neural polarity (Burden, 1993; Kreshishian et al., 1993). It might be interesting to see if *Toll* participates also in the process of mnemogenesis.

The protein kinase A gene may be considered as a class II gene. At the moment it is very much debated which role protein kinase A might have during morphogenesis. Mutants for this gene have been implicated in the learning process (for review, see Davis, 1993). There is clear abundant expression of protein kinase A in the central

nervous system of *Drosophila* embryos suggesting the involvement of protein kinase A in the learning process (Skoulakis et al. 1993).

Particularly, interesting are recent findings on the mutant phenotype of the suppressor of position effect variegation (PEV) locus *Su-var(3)6*. This locus is allelic to the ck1 locus, which codes for a serine-threonine protein phosphatase (PP1) catalytic subunit. The *Su-var(3)6* gene has been implicated in the regulation of the temporal sequence of premitotic events in third instar larval brain neuroblasts. Moreover, it has been established, that several mutant alleles of *Su-var(3)6* show strong suppression of PEV. PP1 has itself a quite broad substrate specificity. As a result it might act in other processes, as well (Baksa et al., 1993). For example, it has been proposed that *Su-var(3)6* participates in visual and olfactory learning of *Drosophila*. In *Su-var(3)6* mutants PP1 activities are quite considerably diminished. It is therefore assumed that hypomorph *Su-var(3)6* alleles fail to provide the heterozygous mutant flies with sufficient PP1 to induce mnemogenesis dependent signalling cascades. Hence, homozygous mutant flies display aberrant olfactory associative learning. The olfactory acuity and sensitivity to an electric shock is, however, not affected. Only the temporal memory seems to decline quite considerably in these mutants though initial memory does not seem to be affected. Visual memory in heterozygous flies, as well, shows a quite considerable decline (Asztalos et al., 1993).

Another class II gene is the *east* gene. east is first transcribed in stage 9 embryos, particularly in the regions involved in neurogenesis. During germ band retraction (stage 12) *east* expression becomes constricted to the abdominal, thoracic and cephalic neuromeres. Strong expression is thereby found in the cerebral hemisphere. Its expression subsequently appears in the peripheral nervous system. In the fully developed embryo *east* is found in the antennomaxillary complex, the epiphysis, the pituitary and the clypeolabrum. In the adult *east* appears in the proboscis, the retina and weakly in the antennal lobes. Of particular interest is thereby the clustering of *east* positive neurones in the sensillum. In mutants for *east*, the imago fails to discriminate between and to respond to a variety of olfactory stimuli reflecting the absence of *east* transcription in the olfactory sensillum (VijayRaghavan et al., 1992).

The *non-transient A* (*nonA*) locus encodes a nuclear protein with RNA binding motifs (RNP). The *NONA* protein probably functions during development by occupying enhancer regions of the salivary gland secretion protein and other genes. No direct DNA *NONA* interaction has, however, been demonstrated. The sequence homology to RNPs suggests that *NONA* may be involved in RNA splicing. This protein is ubiquitously expressed nearly in every cell. *NONA* is first supplied maternally. This maternal protein lasts until 15 hours in the embryo. Mutations in the maternally supplied *NONA* result in embryonic lethality. Here the segment number is reduced, and segment fusion and head defects are prominent. Thus, it is thought that only the maternal *NONA* is required for the early embryonic development. If only the zygotic gene is mutated, embryonic lethality is reversed. The hypomorph zygotic *nonA* mutant alleles results later during development in semilethality, but no phenotypic abnormalities. Generally, it is assumed that *NONA* acts during development to co-operatively interact with other yet unidentified factors to maintain mRNA processing.

In imaginal males *NONA* mutations lead to aberrant courtship and visual behaviour (Stanewsky et al., 1993). About the actual molecular function of *NONA* hardly anything is known, it can, however, be assumed that it either is involved in the formation of the thoracic nervous system, where courtship song behaviour originates, or it may equally effect the production of enzymes, which participate in this process, so that *nonA* can be considered as a class II gene.

DROSOPHILA PHOSPHOLIPASE C 21 (PLC21) is encoded in *Drosophila melanogaster* by the locus *mushroom body miniature* (*mbm*) (Shortridge et al., 1991). Interestingly, *PLC21*. might be a participant of the learning signalling pathways owing to its ability to alter synaptic plasticity. The 7.6 kb head specific transcript may be a component of such a pathway. Alternatively, *plc21* might be involved in the accurate outgrowth of Kenyon neurones in the corpora pedunculata following neural birth. The distribution of *plc21* in the α- and β- lobes, as well as in a portion of the peduncle of the corpora pedunculata confirms its double function (Albert et al., 1994).

Class III genes are those which are expressed postembryonically. In particular, mutations, which affect channel and receptor function in the nervous system belong to this category. The *ether à go-go* (*eag*) gene for example encodes a voltage activated potassium channel. Mutations in this gene only affect the functional nervous system and do not have any morphogenetic implications. Similarly mutations in the *shaker* potassium channel (Pongs et al., 1988) and voltage activated Ca^{2+} channel (Leung and Byerly, 1991) do not have an impact on the outcome of the morphogenesis. As mentioned before injection of the Na^{+} Channel blocker TTX into developing embryos during synaptogenesis, however, increases the number of neuromuscular junctions two fold suggesting that the neural function might well be required for the last stages of morphogenesis (Keshishian et al., 1993).

2. Correlation between Cellular Diversification and Teleonomic Memory

Hence, the genetic circuits I have mentioned above seem to be redeployed in waves during development. The question arises now how these circuits are activated to generate the multitude phenotypes of 200,000 neurones in the *Drosophila* brain during development ?

Cellular differentiation in *Drosophila* embryo may proceed on four levels: transcriptional, posttranscriptional, translational and posttranslational.

Transcriptional control is the cardinal mechanism which ultimately controls every other transaction within a cell. Transcription is a direct function of the cell cycle. The cell cycle is under control of an array of positional factors, which determine the state of the chromosomes in different tissues. The components of the cell cycle are redeployed in different tissues regardless if it is the embryonic syncytium or the eye anlage (O'Farell, 1992). Hence, proliferation of a cell is determined by varying proliferative signals, which control the stereotyped program of the cell cycle. Two loci have been identified, which control the cell cycle and induce proliferation of mushroom body neuroblasts. *anachronism* is a glycoprotein, which is secreted by glia cells, and *mushroom body defect* (*mud*) seems to control the numbers of cell cycles through which a neurob-

last goes. Both factors are probably nervous system specific whereas the cell cycle cascade, which is identical throughout the germlayers.

The chromatin structure depends on the stage of the cell cycle and the cells position within the *Drosophila* embryo. It is crucial for transcriptional activation in the *Drosophila* embryo. Only euchromatic regions contain transcriptionally active genes. Heterochromatin in contrast defines the region of no transcriptional activity. Several factors mediate the transition from facultative heterochromatin to euchromatin and vice versa in response to positional and chronological signals during *Drosophila* embryogenesis (Position Effect Variegation).

Hence, the initial pattern of gene expression, which is laid down during embryogenesis, is stabilised by the actions of epigenetic genes. Each of the epigenetic genes interacts *in trans* with several *cis* -acting heterochromatin associated DNA elements. Several factors have been identified, which are thought to deregulate the facultative heterochromatin portion of the *Drosophila* genome, and therewith allow the transcription to proceed. The *trithorax* group (*trxG*) genes encode several members of these transcriptional regulators. Prominent in each of these genes are several putative zinc finger motives and two acidic domains, indicative of transcriptional activators (Breen and Harte, 1993). Similarly, the *brahma* gene contains a motif with high homology to the yeast SW12/SN2 protein a putative helicase (Tamkun et al., 1991).

A further essential compontent of the heterochromatin deregulator type genes is the GAGA transcription factor. It ensures that the distinctive lineage of a cell is retained in its progeny, whilst it enters the proliferative cycle. Its substrate are several nuclease hypersensitive GA/CT rich DNA binding sequences, which are scattered across the bulk of the heterochromatic portion of the *Drosophila* genome. By binding directly to these regions, it might affect the topological state of the heterochromatin in such a way, that it allows transcription to proceed during all stages of the cell cycle (Raff et al., 1994).

Well characterised are the GA/CT rich region, which brackets the 87A7 heat shock locus. When cloned into a region flanking the *white* gene, it has been demonstrated that two specific structures, scs and scs' are responsible for maintaining the heterochromatin boundary. Moreover, it is thought that the 900 bp GAGA rich core ele-

ment, with the exclusion of the central most A/T rich element, decrease the frequency of promotor and enhancer interactions of the white gene (Vazques and Schedl, 1994). If the GAGA factor has the potential to revert this effect, has not yet been elucidated.

The genes of the *Polycomb* group (*PcG*), in contrast, have been implicated in antagonising the transcriptional activation of such genes as *engrailed* and *acheate* by defining the regions of heterochromatin (Tamkun et al., 1992; Breen and Harte, 1993; for reviews see Paro, 1990; Travers, 1992). Similar to imprinting in mammals (for review, see Antequera and Bird, 1993), the genes of the *PcG* group may act upon specific elements to induce conformational changes in the chromatin, rendering genes inaccessible to the transcriptional machinery (Moazed D. and O'Farrell, 1992). There are several indices that suggest an co-operative model of *PcG* action (Cheng et al., 1994).

According to this model, several non-complementing genes form a multimeric complex with *polyhomeotic* (*ph*). Among these is the X- chromosomal gene *Posterior sex combs* (*Psc*). Interasetingly, both *ph* and *Psc* share a zinc finger motif, which is followed by several homopolymeric peptide stretches (Brunk et al., 1991; Franke et al., 1992). Furthermore, *Pleiohomeotic* (*Pc*) is implicated in participating in this complex. In contrast to the above mentioned *ph* and *Psc*, *Pc* encodes a motif, which is commonly known as a chromo domain (Paro and Hogness, 1991; for review, see Moehrle and Paro, 1994). *polycomb-like* (*pcl*), on the other hand, does not contain any known DNA binding motif at all, and no further homology to the remaining PcG genes (Lonie et al., 1994).

A factor which does not co-localise with the products of the *PcG* genes in *in situ* polythene chromosome preparations, is the *Drosophila* heterochromatin associated chromatin protein -1 (HP-1), which is encoded by the Su(var)^{205} locus. Similar to *Pc* it contains an chromo domain motif (Eissenberg et al., 1992). It seems, however, that other then *ph,* HP-1 is dispensable for targeting proteins to heterochromatin (for reviev, se Moehrle and Paro, 1994). The previously discussed protein phosphatase- 1, which is encoded by the Su-var(3)6^{01} locus might have the task to control the signalling events that possibly lead to the assembly of the PcG gene complex (position effect variation) (Baksa et al., 1993).

Once the genes on a chromosome have become derepressed, their enhancers become accessible to DNA transcriptional binding proteins, which in turn activate the transcription of the respective gene. The gradient theory proposes that the differential distribution of at least two morphogenesis *dorsal* and *bicoid* in the syncytium determine the activation of gap genes in the cellular cortex of the cellularised embryo in a concentration dependent manner. The *hunchback* gene is for instance activated by the binding of dephosphorylated *bicoid* to the *hunchback* promoter 5'-TCTAATCCC-3'. For proper initiation of *hunchback* transcription all three *bicoid* binding sites need to be occupied. The uneven maternal distribution of *bicoid* mRNA by BLE1 might be the cause for different concentrations of *bicoid* resulting in the differential occupation of the *hunchback* promoter sequence. In analogy such a mechanism has been proposed for the generation of the germ layers by the HLH transcription factor *dorsal*. As *bicoid* *dorsal* acts in a concentration dependent manner. Hence, upon a certain threshold in concentration it activates its target gene. On the other hand, its has been demonstrated that *dorsal* is able to form heterodimers with other helix loop helix transcription factors. The *rhomboid* promoter sequence for instance does not contain only targets for *dorsal* binding. Aside form the specific *dorsal* binding sites it contains also HLH specific E-boxes. Thus, the dimer formation with other HLH proteins confers upon *dorsal* a whole repertoire of potential interactions with different promoter sequences along the dorso-ventral axis. Additionally, each of the promoters may also contain low affinity and high affinity *dorsal* binding sites. The threshold required for the activation by *dorsal* may thus depend on the uneven distribution of *dorsal* along its dorso-ventral gradient.

Another mechanism of differential transcriptional activation was found for the regulation of *E(spl)*. This gene processes three downstream regulatory elements, which are probably the targets for either *l'sc* (E1 -box) and *E(spl)* itself (N1/N2 boxes). Both N1 and E-boxes are intercalated. Upon binding of *E(spl)* to the N box for instance the binding of *l'sc* to its E-box is impaired. The activation of the *E(spl)* gene, hence, depends upon the equilibrium of *l'sc* and *E(spl)* in the presumptive neuroblast. High titres

of *E(spl)* shut the transcription of the *E(spl)* gene down and high titres of *l'sc* activate *E(spl)* transcription.

Hence, developmental tissue specificity is regulated by the combinatorial interactions of DNA binding proteins with enhancer elements. The availability and configuration of enhancers may in turn specify the course of transcriptional activation.
Firstly, a single enhancer element may control a whole array of genes (divergent enhancer cascades). As seen for the transcriptional activation of the *AS-C* complex genes *ac* and *sc*, there is the possibility that the transcription of both genes is driven by a single enhancer region. This common control region which comprises two elements is located between both genes.

Secondly, genes may be transcriptionally regulated on an individual basis, by multiple discrete tissue specific enhancer elements (convergent enhancers cascades). For example, it has been established that genes such as *sna* and *dpn* contain two distinct tissue specific promoters, which activate their expression in the CNS and the PNS, respectively. Here, a common tissue specific signal results in the activation of only a single gene.

In summary, one may reason that the combinatorial interactions between transcription factors and the availability of their binding sites along gradients confers upon each cell a unique position dependent phenotype. This tissue specific regulation confirms results obtained form studies on mammalian and avian systems (Grossveld, 1994).

Heterogeneous nuclear RNA (hnRNA) splicing and processing functions are an important mechanism by which protein diversity is created during development. The tissue specifc diversity of *DPTP*s, for example, is generated by the differential use of exons, which encode the C-terminal region. Furthermore, the transcription factor *lola* expresses three different isotypes each of which is expressed during a different developmental stage.

Two different types of factors participate in the splicing process. Small nuclear ribosomal particles or snRNPs are composed of very stable small nuclear RNAs and at least seven polypeptides as well as several snRNP specific polypeptides. They recog-

nise specific binding sides where they hydrolyse the nascent hnRNA. The different fragments are then joined together to for the mRNA.

hnRNA is itself associated with ribonucleoproteins and forms hnRNP. The *Drosophila* homologues of the A (*Hrb98*DE) and B (*Hrb87*F) hnRNP protein complexes have so far been molecularly cloned (Haynes et al., 1990, Hayne et al., 1991). The *HrbDE* gene is expressed following an initial ubiquitous distribution in the entire embryo specifically in the central nervous system.

About mechanisms underlying the development splicing process in *Drosophila* not very much is known.

It has for example been established that P-elements undergo a germ line specific splice of their third intron. Three proteins of 40, 45 and 90 kDa, respectively, were identified in terms of their preferential binding to IVS3 DNA in germ line tissues (Chain et al., 1991). Moreover, it has been proposed that the binding of a 97 kDa protein specifically to the cis- regulatory sequence may inhibit splicing in somatic cells (Siebel and Rio, 1990). The exact mechanisms how this splice may proceed are still relatively unknown. Several homologues to mammalian splicing factors have so far been isolated in *Drososphila.* The *Drosophila* RNA-binding protein RBP1 for instance was localised to transcriptionally active chromosomes. It displays 37- 47% identity and some degree of similarity to the human alternative splicing factor (ASF/SF2). Two further factors, the nuclear phosphoprotein SRp55 and the puff associated gene B52, which are associated with tissue specific splicing have been isolated in *Drosophila.* In mammalian tissue extracts it has been demonstrated that SRp55 can fully complement the functions of ASF/SF2 (Champlin et al., 1991; Maydea, 1992).

Well studied is the sex specific splicing process of *Drosophila.* The *transformer* gene (*tra*) determines the female sex specificity. Mutations in this gene result in the development of solely male flies. *tra* transgenic flies adopt, in contrast, a female phenotype. During larval development the *tra* gene is transcribed equally in both sexes. *tra* hnRNAs contains, however, a translational stop codon within the second exon.

In female tissues this stop codon is converted into a intron and is subsequently spliced out rendering the nascent protein product functional. Similar to the 97 kDa

protein, a nRNA binding factor encoded by the *Sex-lethal* gene recognises the *tra* pre-RNA polypyrimidine tract preventing the activation of the splicosome and thus, resulting in a truncated protein. Hence, mutant alleles in the *Sex-lethal* gene result in the development of female flies, only. Competing with *Sex lethal* is presumably the splicing factor U2AF. It might, thus, be argued that a threshold of *Sex-lethal* determines the sex specification in flies. Functional *tra* acts in conjunction with *tra-2* in the sex specific splicing of *doublesex* (*dsx*) nRNA which is encoded by the sex determination gene *dsx* . The *dsx* gene has two sex specific slicing sites. Whereas the male splicing site does not require any auxiliary splicing factors rather then those found in the splicosome, the binding of the *tra-2* - *tra* complex serves to direct the splicing factors specific binding site in the female.

It has therewith been demonstrated, that the splicing process depends on the relative concentration of RNA binding factors (RNPs) within both sexes. The correctly spliced *tra* protein participates in the second step of the splicing cascade which results in the sex specific splicing of the sex determination protein *double sex* (*dsx*).

It might therefore be conceivable that analogous mechanism are used as well during the differentiation of the nervous system. Several neurone specific RNA binding proteins have been identified. A family of those RNA binding proteins is encoded by the gene *embryonic lethal abnormal visual system* or brief *elav*. The five developmentally and neurone specifically regulated *ELAV* binding proteins function analogues to *TRA* . *ELAV* which is expressed form the birth of a neurone (not present in NBs and GMCs) to its death is obviously crucial for the nervous function and maintenance. It probably determines the neural splice specificity of genes, which are expressed in other tissues as well (for review, see Yao et al., 1993).

rbp 9 another neurone specific gene shares three RNA binding proteins with *ELAV* and co-localises with *ELAV*. Expression is particularly prominent during the mid-pupal stage (Kim and Baker, 1993). Further regulation may proceed on the level of mRNA stability protein translation processing and stability. I do not wish to conider these aspects further in this thesis.

3. Mnemogenesis - Critique de la Raison

Neuronal networks are, hence, the requisite for mnemogenesis as they are able to make switch to direct the signals to the appropriate areas. To operate properly they need to be as efficient and as reliable as possible. In the honey bee a interneurone has been found that integrates two kinds of information and converges this information into memory pathways.

Additionally, the wiring model of the *Drosophila* chemosensory pathways (that contain the corpora pedunculata as an integral part) assumes that an odorant that is perceived by the antennal receptor cells and processed in the glomeruli of the antennal lobes. The stimulus resultant form the different olfactory receptor cells is transmitted according to its input to the respective region of the corpora pedunculata. The corpora pedunculata receive the signal in proportion to the activity of each glomerulus. When the stimulus reaches the corpora pedunculata it is processed there. Known stimuli trigger a response in group of permanent Kenyon fibres without altering their synaptic plasticity. Unknown stimuli are thought to induce alterations in the labile fibres (Heisenberg, 1989). A unconditioned when an conditioned stimulus is presented before a unconditioned stimulus, the information of both stimuli may, hence, diverge into the different groups of Kenyon cells. The divergence is probably accomplished by the enhancement polarisation within the signal (Haykin, 1994). The multitude of the Kenyon neurones serves as a amplifier for the information to generate more bits to store the memory. Thus, by maximising the information storage area the loss of information is minimised. Do to the large number of parallel running Kenyon fibres in proportion to the input fibres, the exit fibres receive additive Gaussian noise. Hence, the more fibres are active the more likely is the occurrence of an error (Hopfield et al., 1983). The convergence of the information into the exit fibres may serve as a feedback to compare conditioned and unconditioned stimulus. Thus, the difference between input (calyces) and output (lobes) is compared by the exit fibres. The function of corpum pedunculatum neural networks remains, however, very sketchy and certainly the lobulus lateralis protocerebralis needs to be taken into account.

The elucidation of the integration of mnemogenesis into the process of neurogenesis will still require a great deal of work. Primarily, one needs to unravel if both processes are similar, identical or only mutually dependent. Current research by Tim Tully, Ron Davis and associates on learning and memory of *Drososphila* does not consider the teleonomic aspect of the insect learning process. And this process does not depend on one two or three of their favourite learning deficiency molecules. It is rather the combinatorial interaction of genes, or, the entirety of the process of neurogenesis that generates a mnemogenic system that particularly in *Drosophila*, as Gabor Miklos (1993) correctly pointed out is based on 'insectronics' rather than on complex behavioural plasticity. It is therefore no wonder that the short lived May fly, contains already once hatched its 'insectronics' enabling it, whilst envisaging death, to rushingly mate and propagate. Bees on the other hand have a sophisticated postmetamorphotic learning pattern during which the bee solely acquired environmental information but the reliability of the bee memory is limited, as well. Controversially, recent findings suggest that memory, which third instar larvae have acquired in an negatively reinforced learning paradigm is retained throughout metamorphosis until a pharate imago has emerged. Thus, do insects have the ability to memorise information prior to pupation (Tully et al., 1994)? Further proof would be required to confirm this notion. Paradoxically, the newly fledged *Drosophila* imago is immediately able to fly, a process that certainly does not depend on potential memory acquired during larval development (for review see Miklos, 1993). Thus, is chemosensory memory an exception? Additionally, the developmental component of *dunce*, *rutabaga* and the remaining molecules with these fancy names has not been researched. A more fundamental approach was adopted by Heisenberg (for review see 1989), who also managed to relate the corpora pedunculata to their onto- and phylogeny.

Moreover, there is conflicting evidence in which region of the chemosensory memory pathways the actual ontogenic chemosensory memory is based. Tully, Davis and associates advocated that the corpora pedunculata are the ultimate regions of chemosensory memory in insects.

Some experimenters even suggested that the responses of thorax and abdomen are autonomous and not related to the brain function. This can easily be demonstrated by beheading a locust. This beheaded animal will still be able to jump for some hours in response to mechanical stimuli. Hoyle (1972) has even demonstrated that headless *Periplaneta americana* is able to react with a co-ordinated movement of a single leg when an electric shock is applied to it. Moreover, the response time to subsequent electric shocks in other legs decreases markedly after a first stimulus has been perceived by the leg. The effects of this learning persists, however, only for a given period and declines thereafter suggesting that this phenomenon is due to short rather than long term memory (Chen, 1970).

It might, however, be also conceivable that in primitive arthropods chemosensory stimuli are already processed on the level of the antennal lobes. It has, for example, been demonstrated that similar products encoded by the *dunce* and *rutabaga* genes participate, as well, in the function of sensory neurones (for review see Breer et al., 1992). In this respect, I wish to consider the antennal sensilla of lobsters. Here, the two antagonising pathways function to mediate neural plasticity. The cAMP pathway which inhibits olfactory signal induced membrane depolarisation by opening potassium channels. These potassium channels counteract the olfactory signal inducted Ca^{2+} influx mediated by the IP_3 cascade. As both systems operate in an equilibrium a threshold following the binding of a specific olfactory molecule needs to be overcome to induce chemoreceptory membrane depolarisation and therewith the propagation of the signal. Hence, habituation to a certain chemosensory stimulus can already be achieved by altering the threshold towards the cAMP pathway in response to common olfactory molecules (Ache, 1994).

Nitric oxide (NO) is as well present on the level of the antennal sensilla as well as on the level of the corpora pedunculata. It is understood that on the level of the antennal sensory cells, NO acts in conjunction with cGMP through IP_3 to close the Ca^{2+} channels, which are opened by the odorant activated cAMP pathway. NO acts here anterogradely and orthodromically (in the direction into which a stimulus propagates)

rather than retrogradely (in this context: transfer of information from the postsynaptic to the presynaptic cleft).

In the mammalian analogue of the insect corpora pedunculata — the hippocampus — it has for instance been established that NO acts as a retrograde indirect transcriptional activator in the presynaptic cells. However, to draw any conclusions from this observation on the function of NO in insects would with our current knowledge certainly be a step too far.

A further complication is, that the integration of NO into the cAMP pathway is also an undissolved issue as one does not know as to whether NO synthase acts downstream and/ or upstream of this pathway (Müller, pers. comm.). Thus, the parallels drawn from studies on mammalian cell culture systems can be taken into consideration, but might not be necessarily applicable to *Drosophila* and *Apis*, as well. In this respect one has to be very cautious not to trivialise the phylogenic diversity of NO action .

Evidence for the action of NO comes from immunohistochemical studies with an antibody raised against NO synthase on the rat olfactory bulb. Here it has been demonstrated that NO synthase is expressed during development of the olfactory bulb and following bulbectomy during the regrowth of the olfactory neurones. Carbon monoxide (CO) an other diffusible neuromodulator, in contrast, presumably mediates signal transduction during olfactory signal transduction (Roskams et al., 1994). CO is thought to repress the activity of NO synthase. In the cerebral cortex, the hippocampus and the corpus striatum of the rat brain anti NO synthase immunoreactivity is localised to 1- 2% of neurones. Hippocampal pyramidal cells lack the expression of NO synthase but several interneurones display unequivocally immunoreactivity for NO synthase, thus, lacking in the postsynaptic neurones implicated in an LTP. It is, however, possible that only minor levels of NO synthase, which are hard to detect suffice already to induce an LTP (for review see Dawson and Snyder, 1994). Hence, NO might be implicated in the rearrangement of synapses in the hippocampal region during mnemogenesis (for review see Kandel, 1992).

The integration of PKA into the cAMP pathway of the corpora pedunculata is rather conflicting, as well. Whereas in antennal sensory neurones PKA co-localises with

cAMP phosphodiesterase and G-protein dependent adenylate cyclase, in the corpora pedunculata PKA is accumulated in the [illegible] lobes and the calyces. The α-, β- and γ - lobes in contrast, which are the sites of cAMP phosphodiesterase and Ca^{2+} calmodulin dependent adenylate cyclase concentration do not display any noteworthy staining of anti- PKA immunoreactivity (Skoulakis et al., 1993; Hildebrandt et al., 1994; for review see Anholt, 1994).

Conflicting evidence has also been found concerning the quality of electrochemical signals in the corpora pedunculata. While some authors pretend that the summating oscillations associated with alterations of synaptic plasticity in *Schistocerca* are confined to the Kenyon neurones, other authors describe for *Apis* that such oscillations are already present in the afferents of the corpora pedunculata (Naraghi and Laurent, 1994; Hammer, 1993).

Alternatively, there might be the possibility that chemosensory memory depends on the diversity of olfactory binding proteins or receptors. Thus, the *Manduca sexta* chemosensory tract contains 250,000 olfactory neurones of which 85,000 are male specific. These are contained in approximately 43,000 pheromone specific sensilla. The remaining 166,000 neurones are distributed among 55,000 general odorant sensilla (Sanes and Hildebrand, 1976a). The *Manduca sexta* female has even 300,000 neurones which, however, all belong to the general odorant receptor cells (Oland et al., 1988). Each sensillum neurone secretes its own specific odorant binding protein (OBP) contained in the sensilla mucus. Sex specific sensilla contain predominately pheromone binding proteins (PBPs), whereas generalists contain general odorant binding proteins (GOBP). Two classes of GOBPs (GOBP1 and GOBP2) were identified (Vogt et al., 1991). It can, hence, be assumed that their spatial expression reflects the specificity of the receptor cells in response to specific odorants. Signals received by different sets of neurones can than be encoded differentially to yield specific nervous responses.

In rat and humans - both which contain a high odorant receptor potential - odorants are perceived by several odorant receptors with different graduated affinities and processed by neural networks. Sensory neurones within the nasal cavity enter the mam-

malian brain and terminate in the olfactory bulb. There they synapse with efferents which course into the central brain. The region where synapses are formed is called a glomerulus. Each glomerulus receives about 105 primary and 102 secondary neurones. Hence, the convergence ratio serves to reduce the background noise (Ngai et al., 1993; Ben- Arie et al., 1994; Chess et al., 1994; for reviews see Lancet, 1994a,b). Research on the diversity of the olfactory receptor cells and their odorant receptor molecules is only in its beginnings. There should be an considerable repertoire of odorant receptor molecules within the chemosensory apparatus of insects, too, to detect the enormous array of chemosensory stimuli.

So far not very much on the diversity of insect olfactory molecules has been elucidated. Thus, cross correlations are required that could make an attractive model of how chemosensory cell diversity in insects is generated. In vertebrates 8 families of olfactory gene sequences have been elucidated. Four of these families are only confined to pices and the other four are found within mammals.

This olfactory receptor gene potential is teleonomically determined. There is no evidence that would suggest that the diversity of olfactory receptor genes is generated by somatic rearrangements or mutations. The diversity is, hence, exclusively germ line based. In the human genome several olfactory receptor gene clusters have been found. This olfactory gene repertoire is thought to have arisen as a result of gene duplications allowing the detection of a high number of volatile odorants. Polymorphisms within the different the odorant receptor genes reside particularly within transmembrane helix motives 3, 4, and 5 which have been attributed to odorant binding. All polymorphisms within the this hypervariable region are the results of single base pair substitutions (Ben- Arie et al., 1994).

Odorant receptors of the cat fish are expressed in specific sensory receptor cells, which are distributed across discrete domains of the olfactory bulb. A specific odorant receptor is expressed mostly at random within the sensory tissue of the nasal cavity. It has, for example, been proved that an olfactory receptor might only be expressed in 1% of neurones indicating that a given olfactory receptor is found uniquely per sensory cell. The mechanism that governs the expression of a unique receptor in a sensory cell is

called idiotype exclusion. Thus, only a single allele of several olfactory gene loci is expressed (for review, see Lancet, 1994a.). The inactivation of the remaining olfactory loci obviously occurs rather early during development. Chess and co-workers (1994) demonstrated in an elegant experiment that an individual olfactory receptor gene is singled out by a mechanism called allelic exclusion. By pooling dissected olfactory bulb neurones of the progeny of a cross between two related mouse individuals (*Muscus musculus domesticus* and *Muscus spretus*) in culture at densities of 200 cells a specific olfactory receptor gene (geneI7) was isolated using reverse transcriptase polymerase chain reaction (RT -PCR). Since, the gene I7 contains a region polymorphic in both mouse species a 24 bp oligonucleotides was synthesised which covered this region. By this exclusion method Chess and his colleagues (1994) hoped to identify the cells where the I7 gene was expressed in either species. Individual plates were assayed for neurone clones which probed positively to either species specific probe. The PCR products of seven cells were further analysed. Five of these were identified as *M. musculus* specific cDNA species, two however, were derived from *M. spretus*. Hence, a given neurone expressed either maternal or paternal genes exclusively. These results were confirmed by cloning the 20 different PCR products from 4 pools into the M13 vector and sequencing them. the analysis of another gene I54 brought about the same results. Of thirty two 400 cell pools five were positive for gene 154. In each case only a single allele was present on either pool. If these results were scrutinised by statistical analysis one may arrive at the view that pools positive for both probes occur are random as advocated by the Gaussian distribution. Although it was established that alleles are inactivated, the possibility still persists that other genes of the same chromosome are still coincidentally expressed. Fluorescent *in situ* hybridisation identified that the olfactory receptor cluster of each of both chromosomes replicated asynchronously. Findings suggest that one of two allelic copies of an entire olfactory receptor gene cluster are inactivated early during development, indicating strongly that locus inactivation probably mediated by certain unidentified cis regulatory elements has taken place. Hence, locus and allelic exclusion mechanisms yield the exclusive expression of a single gene in a given neurone. This process assists the coding of olfactory information significantly as

putative background noise is eliminated. The question arises, however, if in insects, where such olfactory genes have yet not been found at all this mechanism applies ,as well. Moreover, one may want to know as to whether generalists and specialist olfactory receptor cells differ in the number of olfactory receptor genes they express or if the olfactory receptor genes which they express are of a certain specificity.

4. The Scientific Paradox: Triviality and Complexity

Thus, as I have demonstrated in this thesis that mnemogenesis in 'Droapschibo' is firstly more complex than anticipated and is secondly largely dependent on the organisms teleonomy rather than on its ontogeny! Last, to tackle mnemogenesis, one needs to consider the entirety of phylogeny and ontogeny and not a only a part of each as otherwise the complexity of mnemogenesis can not be elucidated.

Last, but not least, I wish to make a remark on memory and intelligence. We have the tendency to classify our biosphere into lower and higher organisms. Organisms of the animal kingdom are categorised in terms of their behavioural diversity which we automatically associate with intelligence. We select for the organisms that have an ultimate benefit for us and neglect or destroy these organisms which has no benefit for us. This is what we call civilisation.

However, it has to be remarked that our conception of our surrounding biosphere might not necessarily approximate the laws of this biosphere, even though we try to understand these laws. To understand these laws we have again resorted to awarding systems and neglect the less awarding systems. Thus, our knowledge is only this what we can understand. The extraordinary diversity and complexity, which we encounter is thereby not considered because we cannot understand how the phenomenons, which we observe can be linked to another. Everything what goes beyond our knowledge becomes belief or politics.

Thus, our ignorance of the biosperic laws has led in the past to horrendous consequences. The reader may just reminded that Darwin's findings resulted indirectly in the atrocious eugenics applied in the Third Reich (for review, see Gould, 1977 and

references therein). Our knowledge has made way to delusion. It is therefore a danger that if we have found a biospheric law that we may applied it with disregard of this context. There is concern that recent findings according to which there are genes which cause aggression or homosexuality may not only be a delusion, but may transcend themselves in the horrific expreinces of our past (for review, see Rose, 1995). So one might interpret these findings according to the way one wishes. Certain individuals can say: "We are more intelligent than others, because our brains are different". Other individuals can say: 'The brains of other individuals are different, and hence, they are less intelligent and prone to misbehaviour and crime." When criticised, these individuals can again say that they should be allowed to make these statements, because, their statements and their behaviour towards other individuals is based in their genes. **IT SHOULD BE WARNED NOT TO INTERPRET THESE FINDINGS IN THE WRONG WAY (for review, see Rose, 1995)!**

It needs to be considered that each organism of our biosphere has an ecological niche and the memory whether inert or learned will survive in this. So we should give each member of or biosphere the chance to survive and learn from the enormous diversity, the large gene- pool of our biosphere. So we can learn from each different ethnic and social group of our society how to respect our biosphere, and how to comprehend the laws of nature!

unit is then free to transfer the γ - phosphoryl group of ATP to the OH-groups of selected serine and threonine residues after having recognized specific amino acid sequences in the substrate proteins. The regulatory subunit serves as a substrate for the Ca^{2+} dependent enzyme calpain, a protease, which generates a truncated regulatory subunit with a reduced affinity for the catalytic subunit. (Aszódi et al., 1991). Hence, the convergence of both cAMP and Ca^{2+} renders PKA more active. Thus, signals need to converge twice to become amplified within the cAMP pathway. Hence, both the NO activated cGMP pathway and a Ca^{2+} stimulus might be the prime candidates for such a convergence.

The function of the PKA is in *Drosophila* still far from conclusive though it has been demonstrated *in vitro* that PKA phosphorylates other proteins (Hildebrandt et al., 1994). A potential substrate may either be cAMP dependent potassium channels (Delgado et al., 1991; Zhong and Wu, 1991; 1993) or putative transcription factors (Yin et al., 1994; for review, see Frank and Greenberg, 1994).

2.4. Nitric Oxide - A Putative Mnemogenic Second Messenger

As gathered from studies on the mammalian hippocampus, it has been proposed that nitric oxide (NO) mediates retrogradely an alteration in the presynaptic nerve cell in response to a long term potentiation (LTP) in the postsynaptic cells. Ca^{2+}/calmodulin dependent NO synthase (NOS) catalyses the production of NO from asparagine. This process requires the presence of NADPH as an electron donor. Additionally, it has been demonstrated that the vertebrate brain NOS activity is controlled by calcium which acts in conjunction with calmodulin.

Recently, NOS has been cloned in *Drosophila*. The *Drosophila* NOS has 40% identity to its vertebrate homologue and carries all putative regulatory domains associated with its function (Regulski and Tully, 1993; Tully pers. comm.). The activity of NOS can be visualised with the NOS co-enzyme NADPH diaphorase (NDP). NDP utilises the chromophore nitro blue tetrazolium as a substrate. Nitro blue tetrazolium is only converted into a blue precipitate in the presence of electrondonor NADPH. As

NDP occurs always in proximity to NOS it can be assumed that NOS activity and nitro blue tetrazolium staining co-localise. NOS catalytic activity, hence, corresponds exclusively to the activity of NDP mirrored by nitro blue tetrazolium staining — provided the fixing conditions are correct (Dawson et al, 1991; Matsumoto et al., 1993a, 1993b). NOS activity in *Apis mellifera* and *Drosophila* predominates in the glomeruli of the antennal lobes (Kirchhof and Bicker, 1992; Müller, 1994). A discrete staining pattern was observed in the Kenyon cell neuropil. There is no staining in the Kenyon cell somata.

It is thought that NO synthase is stimulated by the release of Ca^{2+} form intracellular cisterns following the binding of acetylcholine. As acetylcholine receptors are only present in the postsynaptic cells, NO diffuses to its target either within the postsynaptic cell or in the presynaptic cell, where it binds to the haeme group of guanyl cyclase (Liu et al., 1993), which then acts to increase the titres of intracellular cGMP (Ewer and Truman, 1993).

Nothing has so far been elucidated about the action of NO on the Kenyon neurones. Evidence for the action of NO comes from a study on mammalian neurone culture systems. Here it has been demonstrated that upon converging Ca^{2+} and NO signals, cGMP mediates transcriptional activation and induces therewith the *de novo* synthesis of proteins. To modulate transcriptional activity in these PC12 neurones, the synergetic action of the cAMP pathway is required (Peunova and Enikolopov, 1993; for review, see Bliss and Collingridge, 1993).

It would be false to draw any conclusions from these findings on insects but one may speculate that the cAMP pathway might a good candidate for mediating synaptic plasticity in presynaptic cells in response to NO. One might assume that the action of NO amplifies the signal derived from the converging cAMP and IP_3 pathways to induce such changes.

2.5. Cyclic Adenylyl Responsive Element- Binding Proteins (CREBs) and Modulators (CREMs)

Among the multitude of substrates for PKA, several cAMP responsive binding proteins (CREBs) have been identified. These CREBs bind as dimers to highly conserved enhancer elements (CRE) (Yin et al., 1994; for review, see Frank and Greenberg, 1994). Transcriptional activation of CREBs is antagonised by CRE modulators (CREMs). CREMs can either be generated by alternative splicing or alternatively use of different promoters (de Groot et al., 1994).

The CREB gene of *Drosophila* has, recently, been identified and cloned. The cDNA clone of the *Drosophila* CREB gene shows high homology to mammalian CREBs particularly in the region, which codes for the basic leucine zipper motif.

CREB cDNA is present in two isoforms. The first isoform dCREB2-a acts as a PKA responsive transcriptional activator, whereas the second isoform dCREB2-b - a homologue of the vertebrate CREMs - is a potent transcriptional blocker in cell culture. Moreover, it has been demonstrated that hs-dCREB2-b was a potent blocker of the long term memory formation in transgenic flies similar to the effects seen in individuals where 50% of the protein biosynthesis was inhibited by cyclohexamide. CREB obtains its specific function by the removal of glutamine rich activation domains as a result of alternative splicing (Yin et al., 1994). The expression of CREBs in mammlas is presumably regulated by the mitogen activated protein (MAP) kinase pathway (de Groot et al., 1994).

3. Inconsistencies within the Putative Mnemogenic Second Messenger Pathways

There are, however, several inconsistencies within the proposed pathway: In contrast to *rutabaga* and *dunce*, the catalytic subunit of PKA predominates in the calycal Kenyon cell neuropil. It is only weakly enriched in the lobes suggesting that the PKA and the cAMP signalling pathways are spatially separated. Remember that *dunce* and *rutabaga* are confined to the lobes, the region of synaptic output. A different calcium activated

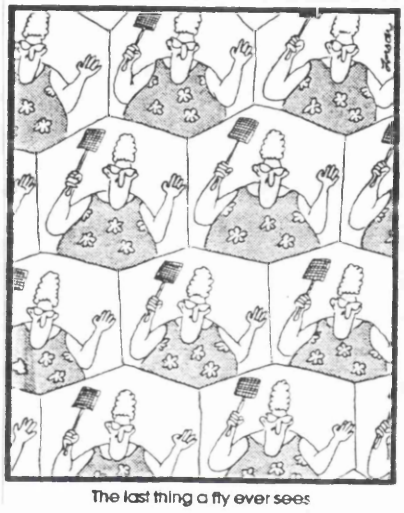


Abbildung 13.1: Die Notwendigkeit rascher Informationsverarbeitung (nach G. LARSON, Copyright © 1988 by Universal Press Syndicate reprinted by permission of Editors Press Service, Inc.)

CHAPTER 3
The Ultimate Goal

1. Sir Karl Popper Says

"The old scientific ideal of επιστεμε (*epistëmë*) - of absolutely certain, demonstrable knowledge - has proved to be an idol. The demand for scientific objectivity makes it inevitable that every scientific statement must be tentative for ever. It may indeed be corroborated, but every corroboration is relative to other statements which, again are tentative. Only in our subjective experiences of conviction, in our subjective faith, can we be 'absolutely certain'.

With the idol of certainty (including that of degrees of imperfect certainty or probability) there falls one of the defences of obscurantism which bar the way o scientific advance. For worship of this idol hampers not only the boldness of our question, but also the rigour and integrity of our tests. The wrong view, that science betrays itself in the craving to be right; for it is not his *possession* of knowledge, of irrefutable truth, that makes the man of science, but his persistent and recklessly critical quest for truth.

Has our attitude, then, to be one of resignation? Have we to say that science only fulfils its biological task; that it merely prove its mettle in practical applications which may corroborate it? Are its intellectual problems insoluble? I do not think so. Science never pursues the illusory aim of making its answers final, or even probable. Its advance is, rather, towards an infinite yet attainable aim: that of ever discovering new, deeper, and more general problems, and of subjecting our ever tentative answers to ever renewed and ever more rigorous tests." (Sir Karl Popper (1934) in the 'Logic of Scientific Discovery').

2. Sir Francis Crick Says

"Reluctantly I must conclude that these models are not really theories but are rather 'demonstrations'. They are existent proofs that units somewhat like neurones can in-

deed do surprising things, but there is hardly anything to suggest that the brain actually performs in exactly the same way as they suggest ...

Part of the troubles with theoretical neuroscience is that it lies somewhat between three other fields. At one extreme we have those researchers working directly on the brain. This is science. It is attempting to discover what devices nature actually uses. At the other extreme lies artificial intelligence. This is engineering. Its object is to produce a device that works in the desired way. The third field is mathematics. Mathematics cares neither for science nor for engineering (except as a source of problems) but only about the relationship between abstract entities. Workers in the brain are thus pulled in several directions. Intellectual snobbery makes them feel they should produce results that are mathematical both deep and powerful and also apply to the brain. This is not likely to happen if the brain is really a complicated combination of rather simple tricks evolved by natural selection. If an idea they conceive doesn't help to explain the brain, the theorists may hope that it may be useful in AI. There is thus no compelling drive for them to press on and on until the way the brain actually works is laid bare ...

Three main approaches are needed to unscramble a complicated system. One can take it apart and characterise all the isolated bits - exactly where each part is located in the system in relation to all the other parts and how they interact with each other. These two approaches are unlikely, by themselves o reveal exactly how the system works. To do this one must also study the behaviour of the system and its components while interfering very delicately with its various parts, to see what effect such alterations have on behaviour at all levels. If we could do all this to our brains we could find out how they work in no time at all.

Molecular and cell biology could help decisively in all these three approaches. The first has already begun. For example, the genes for a number of key molecules have already been isolated, characterised, and their products produced so that they can be more easily studied. A little progress has been made on the second approach, but more is still needed. For example, a technique for injecting a single neurone in such a way that other neurones that are connected to it (and only those) are labelled would be useful ...

The present state of the brain sciences reminds me of the state of molecular biology and embryology in, say, the 1920s and 1930s. Many interesting things have been discovered, each year steady made on may fronts, but major questions are still largely unanswered and unlikely to be with new techniques and new ideas. Molecular biology became mature in the 1960s, whereas embryology is just starting to become a well-developed field. The brain science have still a very long way to go, but the fascination of the subject and the importance of the answers will inevitably carry it forward. It is essential to understand our brains in some detail if we are to assess correctly or place in this vast and complicated universe we see all around us ." (Sir Francis Crick in 'What a Mad Pursuit - A Personal View of Scientific Discovery)

3. Sir John Eccles Says

Realistically it has to be accepted that *Homo sapiens sapiens* will exist in countless generations into the future on this wonderful salubrious home, Planet Earth, which, as Harlow Shapley was fond of declaiming, is a small rocky planet of no exalted status in the material order. Its orbit is not distinctive in the solar system and our sun is a medium sized main sequence star far out in one of the arms of the galaxy with its 100,000 million other stars, one galaxy amongst 100,000 million of other galaxies, all spawned from the Big Bang 17,000 million yBP. Yet on the anthropoid principle our home Planet Earth is the centre of the grand design. Each of us has woken up, as it were, with a human body and brain on the evolutionary origin of *Homo sapiens sapiens*.

We may conclude by saying that the biological evolution transcends itself in providing the material basis, the human brain, for self conscious beings whose very nature is to seek for hope and to enquire for meaning in the quest for love, truth and beauty." (From Sir John Eccles: " The Evolution of the Brain - The Creation of the Self").

4. David Hume Says

"You have probably heard that my friends in Glasgow, contrary to my opinion and advice, undertook to get me elected into that college; and they had succeeded, in spite of the violent and solemn remonstrances of the clergy, if the Duke of Argyle had courage to give me last countenance". (David Hume after having been ***dis***appointed by the University of Glasgow — Edinburgh, Feb. 4th, 1752 to John Clephane)

APPENDIX
Experimental Observations

Summary

The corpora pedunculata are an integral part of chemosensory memory networks of the imaginal cerebral ganglion in insects. Here, olfactory and gustatory information converges, allowing the processing and storage of these signals. In the Dipteran insect species *Drosophila melanogaster* the corpora pedunculata are bilateral structures that comprise approximately 2,500 intrinsic neurones arranged in parallel. These are known as the Kenyon neurones (K- neurones).

The somata of the K neurones are located in the dorso- caudal cerebral ganglion, where they form the cortex. They emanate axons rostro- ventrally into the calyces, — the structures, which are associated with the region of synaptic input. More ventrally the axons of the K- neurones proceed parallel as the peduncles and finally branch off as the α- and β- or γ- lobes.

Here, I report on the development of the corpora pedunculata of *Drosophila melanogaster*.

1500 p[Gal4] enhancer trap lines were generated with the aim to mark cells that constitute the corpora pedunculata. Approximately 30 enhancer trap lines were selected for analysis since they expressed the reporter protein β- galactosidase driven by the marker protein Gal4 predominantly in the corpora pedunculata.

Using confocal microscope imaging of the β- galactosidase immunostained embryonic, larval, pupal and imaginal *Drosophila melanogaster* central nervous system preparations, the expression of β- galactosidase was found to be constitutive during development from embryonic stage 9 onwards.

During the first hours of metamorphosis structures such as the calyces, α-, β- and γ- lobes seemed to become more elaborated.

30 hours after puparium formation no further significant changes were observed.

I propose that the ramification and outgrowth of axonal processes serves as a

means to integrate afferents of the emerging antennal lobe and efferent of the thoracic premotor fibres.

In a second project the *neomycin* phosphotransferase gene (*neo*), a versatile selection marker in prokaryotic, as well as, eukaryotic cell culture systems, was subcloned from a pBluescript SK+/- vector into the pUAST shuttle vector ready to be introduced into the genome of *Drosophila melanogaster* and to be ectopically expressed in the respective GAL positive cell.

Introduction

A major goal of developmental biology is to unravel the complex interactions which govern the generation of a multicellular organism. In this project second generation enhancer trap elements were used to dissect cells, which constitute the corpora pedunculata. The corpora pedunculata of *Drosophila melanogaster* are a bilateral neuropil of 2,500 Kenyon neurones into which olfactory and gustatory information derived from the labrum and the antennae converges. There is considerable evidence that the corpora pedunculata in different arthropod species are involved in chemosensory lerning and memory (for review, see Erber et al., 1987). The somata of the Kenyon neurones are located in the dorsocaudal cortex of the supraoesophageal ganglion, where they are anchored in perineural glial sheath. They emanate their axons anterioventrally into the volume of the supraoesophageal ganglion. In the calyces they puff out to integrate afferents from the antennal lobes. They then course further anterioventrally along the peduncles where they bifurcate into the α- lobe and the β- or γ- lobes. The α- lobe extends rostrally, whereas β- and γ- lobes terminate medially. In the honey bee *Apis mellifera*, three types of efferents can be identified: feedback fibres, which run ipsilaterally to the calyces of the corpora pedunculata, commissural interneurones, which send fibres contralaterally; and neurones which connect the three lobes with other relay structures such as the lobulus lateralis protocerebralis and lobulus medialis protocerebralis (Technau and Heisenberg, 1982; Rybak and Menzel, 1993). Several p[Gal4;w^+] enhancer traps, which marked subsets of Kenyon neurones were selected in terms of their *lacZ* chromophore staining activity. These were in turn screened for embryonic expression. A single line which displayed anti-β galactosidase immunoreactivity in embryos was selected and the developmental expression pattern was assayed using confocal microscopy.

As mentioned above the pUAST vector serves as a good shuttle to introduce genes into the genome of *Drosophila melanogaster* and to ectopically express them in the respective GAL4 positive cell. The *neomycin* phosphotransferase gene (*neo*) is a versatile selection marker in prokaryotic as well as eukaryotic cells. The *neo* gene was

discovered in the *E.coli* transposon Tn5 and confers resistance to the aminoglycosides *kanamycin*, *neomycin* and G418. The aminoglycoside G418 kills non-resistant cells by interacting with the large ribosomal subunit and therewith blocking the initiation of protein biosynthesis in a reversible manner. This selection process is widely applied in mammalian cell culture were it has been successfully used to isolate *neo* transfected cells (Colbière-Garapin et al., 1982). This resistance marker is of particular interest to embryologists. Embryonic stem cells are ideal candidates for transfection experiments. They are pleuripotent, which means that they can differentiate into distinct types of cells. For example, when reintroduced into mouse blastocyte ES cells can continue the differentiation process in response to cues provided by the host blastocyte.

In *Drosophila melanogaster* the *neo* gene has not been studied in cell culture experiments, but Steller and Pirotta (1985) have used this gene successfully to select for transformants of the P-element vector pUChs*neo*. They injected this P-element marker into embryos. The transformants were selected on G418 agar following heat shock treatment which should have induced the expression of the *neo* gene from the heat shock promoter. However, individuals which survived the treatment, displayed phenotypic abnormalities. Especially slow development and a poor level of fitness were prominent. Both balanced (P- element vector insert in vicinity of an active enhancer) and homozygous individuals survived. Non-transformant flies died during the first instar, though unfortunatly the authors did not give any timing. In an other study Tian Xu and Gerald Rubin (1993) used the *neo* gene as a marker to scan for FRT/FLP recombinase induced genetic mosaics. An homologue of the *neomycin* resistance gene, the *hygromycin* resistance gene, was used to select for transformed Schneider 2 cell lines. In analogy to the *neo* gene, *hygro* encodes a phosphotransferase enzyme, which inactivates the aminocyclitol antibiotic *hygromycin* and can, thus, be envisaged as another resistance marker (Cumberledge and Krasnow, 1993). Here I report about a strategy used to subclone the *neo* gene into pUAST.

Materials and Methods

P-element Mutagenesis

Drosophila melanogaster Strains

The relevant *Drosophila melanogaster* strains and markers used for P-element mutagenesis crosses are described in Lindsey and Zimm (1992) or Ashburner (1989). Flies carrying either P-enhancer trap element were denoted following the terminology used by Brand and Perrimon (1993). For genetic strategies and breeding schemes see the results section.

Drosophila melanogaster: Culture

All fly stocks were continuously reared at either 16°C, 18°C or 25°C on Glasgow medium (see Table 1.) in either small Perspex vials or 200mL opaque plastic bottles sealed with a cotton plug. Each fly cross was set up in 6× 3.5× 4 inch fly cages. Flies were amplified in 10× 5× 6 inch large fly cages (made by the Glasgow University Science faculty Workshop). Flies in each cage were fed on grape juice agar plates (Table 2.) coated with a molasses of baker's yeast and water.

Recipes for *Drosophila* Media

Ingredient	Quantity
corn meal	15 g
wheat germ	10 g
soya flour	10 g
treacle	30 g
glucose	30 g
sucrose	15 g
agar	10 g
dried yeast	35 g

Table 1. Glasgow *Drosophila* medium. Made up to one litre of water.

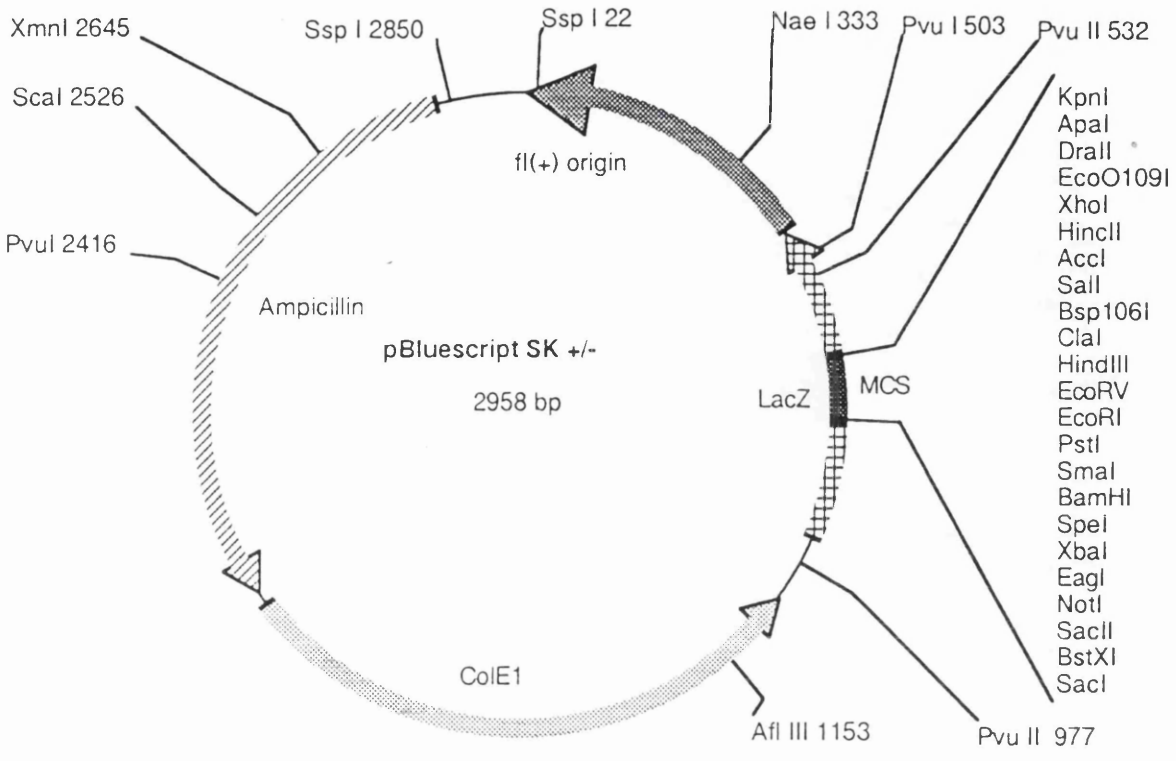


Fig.1 Plasmid map of pBluescript® SK+/-. The pBluescript® SK+/- phagemid is 2958 bp in size. The multiple cloning site contained within the *lacZ* gene, is used for blue/white selection of inserts into this gene. It points in the direction from *Kpn*I and *Sac*I. The *f1* origin of replication is provided to assure autonomous replication within the host. This origin of replication of the phagemid is used to generate single stranded DNA where co-transfected with a helper phage (Alting-Mess et al., 1992).

```
LOCUS       ARBL2SKP     2961 bp    DNA   circular  SYN       23-NOV-1993
DEFINITION  pBluescript II SK(+) vector DNA, phagemid excised from lambda
            ZAPII.
ACCESSION   X52328
KEYWORDS    artificial sequence; cloning vector; expression vector;
vector.
SOURCE      Cloning vector.
  ORGANISM  Cloning vector
            Artificial sequences; Cloning vectors.
REFERENCE   1  (bases 1 to 2961)
  AUTHORS   Thomas,E.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-FEB-1990) to the EMBL/GenBank/DDBJ databases.
Thomas
            E.A., Stratagene Clonin Systems, 11099 North Torney Pines Rd.,
La
            Jolla, CA 92037, USA
  STANDARD  full automatic
REFERENCE   2  (bases 1 to 2961)
  AUTHORS   Short,J.M., Fernandez,J.M., Sorge,J.A. and Huse,W.D.
  TITLE     Lambda ZAP: a bacteriophage lambda expression vector with in
vivo
            excision properties
  JOURNAL   Nucleic Acids Res. 16, 7583-7600 (1988)
  STANDARD  full automatic
REFERENCE   3  (bases 1 to 2961)
  AUTHORS   Alting-Mees,M.A. and Short,J.M.
  TITLE     pBluescript II: gene mapping vectors
  JOURNAL   Nucleic Acids Res. 17, 9494 (1989)
  STANDARD  full automatic
COMMENT     NCBI gi: 58063
FEATURES             Location/Qualifiers
     source          1..2961
                     /organism="Cloning vector"
     misc_feature    1..2961
                     /note="phagemid pBluescriptII SK(+)"
BASE COUNT      746 a    739 c    754 g    722 t
ORIGIN
        1 ctaaattgta agcgttaata ttttgttaaa attcgcgtta aatttttgtt
aaatcagctc
       61 atttttttaac caataggccg aaatcggcaa aatcccttat aaatcaaaag
aatagaccga
      121 gatagggttg agtgttgttc cagtttggaa caagagtcca ctattaaaga
acgtggactc
      181 caacgtcaaa gggcgaaaaa ccgtctatca gggcgatggc ccactacgtg
aaccatcacc
      241 ctaatcaagt tttttggggt cgaggtgccg taaagcacta aatcggaacc
ctaaagggag
      301 cccccgattt agagcttgac ggggaaagcc ggcgaacgtg gcgagaaagg
aagggaagaa
      361 agcgaaagga gcgggcgcta gggcgctggc aagtgtagcg gtcacgctgc
gcgtaaccac
      421 cacacccgcc gcgcttaatg cgccgctaca gggcgcgtcc cattcgccat
tcaggctgcg
      481 caactgttgg gaagggcgat cggtgcgggc ctcttcgcta ttacgccagc
tggcgaaagg
      541 gggatgtgct gcaaggcgat taagttgggt aacgccaggg ttttcccagt
cacgacgttg
      601 taaaacgacg gccagtgagc gcgcgtaata cgactcacta tagggcgaat
tgggtaccgg
      661 gccccccctc gaggtcgacg gtatcgataa gcttgatatc gaattcctgc
agcccggggg
      721 atccactagt tctagagcgg ccgccaccgc ggtggagctc cagcttttgt
tccctttagt
      781 gagggttaat tgcgcgcttg gcgtaatcat ggtcatagct gtttcctgtg
```

tgaaattgtt
841 atccgctcac aattccacac aacatacgag ccggaagcat aaagtgtaaa
gcctggggtg
901 cctaatgagt gagctaactc acattaattg cgttgcgctc actgcccgct
ttccagtcgg
961 gaaacctgtc gtgccagctg cattaatgaa tcggccaacg cgcggggaga
ggcggtttgc
1021 gtattgggcg ctcttccgct tcctcgctca ctgactcgct gcgctcggtc
gttcggctgc
1081 ggcgagcggt atcagctcac tcaaaggcgg taatacggtt atccacagaa
tcaggggata
1141 acgcaggaaa gaacatgtga gcaaaaggcc agcaaaaggc caggaaccgt
aaaaaggccg
1201 cgttgctggc gtttttccat aggctccgcc cccctgacga gcatcacaaa
aatcgacgct
1261 caagtcagag gtggcgaaac ccgacaggac tataaagata ccaggcgttt
cccctggaa
1321 gctccctcgt gcgctctcct gttccgaccc tgccgcttac cggatacctg
tccgcctttc
1381 tcccttcggg aagcgtggcg ctttctcata gctcacgctg taggtatctc
agttcggtgt
1441 aggtcgttcg ctccaagctg ggctgtgtgc acgaacccc cgttcagccc
gaccgctgcg
1501 ccttatccgg taactatcgt cttgagtcca acccggtaag acacgactta
tcgccactgg
1561 cagcagccac tggtaacagg attagcagag cgaggtatgt aggcggtgct
acagagttct
1621 tgaagtggtg gcctaactac ggctacacta gaaggacagt atttggtatc
tgcgctctgc
1681 tgaagccagt taccttcgga aaaagagttg gtagctcttg atccggcaaa
caaaccaccg
1741 ctggtagcgg tggttttttt gtttgcaagc agcagattac gcgcagaaaa
aaaggatctc
1801 aagaagatcc tttgatcttt tctacggggt ctgacgctca gtggaacgaa
aactcacgtt
1861 aagggatttt ggtcatgaga ttatcaaaaa ggatcttcac ctagatcctt
ttaaattaaa
1921 aatgaagttt taaatcaatc taaagtatat atgagtaaac ttggtctgac
agttaccaat
1981 gcttaatcag tgaggcacct atctcagcga tctgtctatt tcgttcatcc
atagttgcct
2041 gactccccgt cgtgtagata actacgatac gggagggctt accatctggc
cccagtgctg
2101 caatgatacc gcgagaccca cgctcaccgg ctccagattt atcagcaata
aaccagccag
2161 ccggaagggc cgagcgcaga agtggtcctg caactttatc cgcctccatc
cagtctatta
2221 attgttgccg ggaagctaga gtaagtagtt cgccagttaa tagtttgcgc
aacgttgttg
2281 ccattgctac aggcatcgtg gtgtcacgct cgtcgtttgg tatggcttca
ttcagctccg
2341 gttcccaacg atcaaggcga gttacatgat cccccatgtt gtgcaaaaaa
gcggttagct
2401 ccttcggtcc tccgatcgtt gtcagaagta agttggccgc agtgttatca
ctcatggtta
2461 tggcagcact gcataattct cttactgtca tgccatccgt aagatgcttt
tctgtgactg
2521 gtgagtactc aaccaagtca ttctgagaat agtgtatgcg gcgaccgagt
tgctcttgcc
2581 cggcgtcaat acgggataat accgcgccac atagcagaac tttaaaagtg
ctcatcattg
2641 gaaaacgttc ttcggggcga aaactctcaa ggatcttacc gctgttgaga
tccagttcga
2701 tgtaacccac tcgtgcaccc aactgatctt cagcatcttt tactttcacc
agcgtttctg

```
    2761 ggtgagcaaa aacaggaagg caaaatgccg caaaaaaggg aataagggcg
acacggaaat
    2821 gttgaatact catactcttc ctttttcaat attattgaag catttatcag
ggttattgtc
    2881 tcatgagcgg atacatattt gaatgtattt agaaaaataa acaaataggg
gttccgcgca
    2941 catttccccg aaaagtgcca c
//
```

```
LOCUS       ARBLSKM      2958 bp    DNA   circular  SYN       23-NOV-1993
DEFINITION  pBluescript SK(-) vector DNA, phagemid excised from lambda
ZAP.
ACCESSION   X52324 S52394
KEYWORDS    artificial sequence; cloning vector; expression vector;
vector.
SOURCE      Cloning vector.
  ORGANISM  Cloning vector
            Artificial sequences; Cloning vectors.
REFERENCE   1  (bases 1 to 2958)
  AUTHORS   Thomas,E.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-FEB-1990) to the EMBL/GenBank/DDBJ databases.
Thomas
            E.A., Stratagene Clonin Systems, 11099 North Torney Pines Rd.,
La
            Jolla, CA 92037, USA
  STANDARD  full automatic
REFERENCE   2  (bases 1 to 2958)
  AUTHORS   Short,J.M., Fernandez,J.M., Sorge,J.A. and Huse,W.D.
  TITLE     Lambda ZAP: a bacteriophage lambda expression vector with in
vivo
            excision properties
  JOURNAL   Nucleic Acids Res. 16, 7583-7600 (1988)
  STANDARD  full automatic
REFERENCE   3  (bases 1 to 2958)
  AUTHORS   Alting-Mees,M.A. and Short,J.M.
  TITLE     pBluescript II: gene mapping vectors
  JOURNAL   Nucleic Acids Res. 17, 9494 (1989)
  STANDARD  full automatic
REFERENCE   4  (bases 1 to 2958)
  AUTHORS   Alting-Mees,M.A., Sorge,J.A. and Short,J.M.
  TITLE     pBluescriptII: multifunctional cloning and mapping vectors
  JOURNAL   Meth. Enzymol. 216, 483-495 (1992)
  STANDARD  full automatic
COMMENT     NCBI gi: 58066
FEATURES             Location/Qualifiers
     source          1..2958
                     /organism="Cloning vector"
     misc_feature    1..2958
                     /note="phagemid pBluescript SK(-)"
BASE COUNT      707 a    755 c    730 g    766 t
ORIGIN
        1 cacctgacgc gccctgtagc ggcgcattaa gcgcggcggg tgtggtggtt
acgcgcagcg
       61 tgaccgctac acttgccagc gccctagcgc ccgctccttt cgctttcttc
ccttcctttc
      121 tcgccacgtt cgccggcttt ccccgtcaag ctctaaatcg ggggctccct
ttagggttcc
      181 gatttagtgc tttacggcac ctcgacccca aaaaacttga ttagggtgat
ggttcacgta
      241 gtgggccatc gccctgatag acggtttttc gccctttgac gttggagtcc
acgttcttta
      301 atagtggact cttgttccaa actggaacaa cactcaaccc tatctcggtc
tattcttttg
      361 atttataagg gattttgccg atttcggcct attggttaaa aaatgagctg
atttaacaaa
      421 aatttaacgc gaattttaac aaaatattaa cgcttacaat ttccattcgc
cattcaggct
      481 gcgcaactgt tgggaagggc gatcggtgcg ggcctcttcg ctattacgcc
agctggcgaa
      541 agggggatgt gctgcaaggc gattaagttg ggtaacgcca gggttttccc
agtcacgacg
      601 ttgtaaaacg acggccagtg aattgtaata cgactcacta tagggcgaat
tgggtaccgg
```

661 gccccccctc gaggtcgacg gtatcgataa gcttgatatc gaattcctgc
agcccgggggg
721 atccactagt tctagagcgg ccgccaccgc ggtggagctc cagcttttgt
tccctttagt
781 gagggttaat ttcgagcttg gcgtaatcat ggtcatagct gtttcctgtg
tgaaattgtt
841 atccgctcac aattccacac aacatacgag ccggaagcat aaagtgtaaa
gcctggggtg
901 cctaatgagt gagctaactc acattaattg cgttgcgctc actgcccgct
ttccagtcgg
961 gaaacctgtc gtgccagctg cattaatgaa tcggccaacg cgcggggaga
ggcggtttgc
1021 gtattgggcg ctcttccgct tcctcgctca ctgactcgct gcgctcggtc
gttcggctgc
1081 ggcgagcggt atcagctcac tcaaaggcgg taatacggtt atccacagaa
tcaggggata
1141 acgcaggaaa gaacatgtga gcaaaaggcc agcaaaaggc caggaaccgt
aaaaaggccg
1201 cgttgctggc gtttttccat aggctccgcc cccctgacga gcatcacaaa
aatcgacgct
1261 caagtcagag gtggcgaaac ccgacaggac tataaagata ccaggcgttt
ccccctggaa
1321 gctccctcgt gcgctctcct gttccgaccc tgccgcttac cggatacctg
tccgcctttc
1381 tcccttcggg aagcgtggcg ctttctcata gctcacgctg taggtatctc
agttcggtgt
1441 aggtcgttcg ctccaagctg ggctgtgtgc acgaaccccc cgttcagccc
gaccgctgcg
1501 ccttatccgg taactatcgt cttgagtcca acccggtaag acacgactta
tcgccactgg
1561 cagcagccac tggtaacagg attagcagag cgaggtatgt aggcggtgct
acagagttct
1621 tgaagtggtg gcctaactac ggctacacta gaaggacagt atttggtatc
tgcgctctgc
1681 tgaagccagt taccttcgga aaaagagttg gtagctcttg atccggcaaa
caaaccaccg
1741 ctggtagcgg tggttttttt gtttgcaagc agcagattac gcgcagaaaa
aaaggatctc
1801 aagaagatcc tttgatcttt tctacggggt ctgacgctca gtggaacgaa
aactcacgtt
1861 aagggatttt ggtcatgaga ttatcaaaaa ggatcttcac ctagatcctt
ttaaattaaa
1921 aatgaagttt taaatcaatc taaagtatat atgagtaaac ttggtctgac
agttaccaat
1981 gcttaatcag tgaggcacct atctcagcga tctgtctatt tcgttcatcc
atagttgcct
2041 gactccccgt cgtgtagata actacgatac gggagggctt accatctggc
cccagtgctg
2101 caatgatacc gcgagaccca cgctcaccgg ctccagattt atcagcaata
aaccagccag
2161 ccggaagggc cgagcgcaga agtggtcctg caactttatc cgcctccatc
cagtctatta
2221 attgttgccg ggaagctaga gtaagtagtt cgccagttaa tagtttgcgc
aacgttgttg
2281 ccattgctac aggcatcgtg gtgtcacgct cgtcgtttgg tatggcttca
ttcagctccg
2341 gttcccaacg atcaaggcga gttacatgat cccccatgtt gtgcaaaaaa
gcggttagct
2401 ccttcggtcc tccgatcgtt gtcagaagta agttggccgc agtgttatca
ctcatggtta
2461 tggcagcact gcataattct cttactgtca tgccatccgt aagatgcttt
tctgtgactg
2521 gtgagtactc aaccaagtca ttctgagaat agtgtatgcg gcgaccgagt
tgctcttgcc
2581 cggcgtcaat acgggataat accgcgccac atagcagaac tttaaaagtg

```
ctcatcattg
    2641 gaaaacgttc ttcggggcga aaactctcaa ggatcttacc gctgttgaga
tccagttcga
    2701 tgtaacccac tcgtgcaccc aactgatctt cagcatcttt tactttcacc
agcgtttctg
    2761 ggtgagcaaa aacaggaagg caaaatgccg caaaaaaggg aataagggcg
acacggaaat
    2821 gttgaatact catactcttc ctttttcaat attattgaag catttatcag
ggttattgtc
    2881 tcatgagcgg atacatattt gaatgtattt agaaaaataa acaaataggg
gttccgcgca
    2941 catttccccg aaaagtgc
//
```

Ingredient	Quantity
glucose	52.2 g
sucrose	26 g
dried yeast	7 g
treacle	30 g
glucose	30 g
grape juice	150 mL
water	800 mL

Table 2. Grape Juice Agar Plates

Subcloning

Bacterial Strains

In all experiments two variants of the gram negative *Enterobacterium Escherischia coli* (*E.coli*) were routinely used (Table 3).

Strain	Genotype	Reference
E.coli Jm 109 (Promega® Ltd., Southhampton, Hamps., England)	*end*A1, *rec*A1, *gyr*A96, *thi*, *hsd*R17, (r_k-, m_k+), *rel*A1, *sup*E44,λ-, Δ (*lac-pro*AB), [F', traD36, proAB, *lac*I^{q}ZΔM15]	Yanish-Perron et al., 1985
E. coli XL1-Blue (Stratagene® Ltd., Cambridge, Cambridges., England)	*end*A1, *rec*A1, *gyr*A96, *thi-1*, *hsd*R17, *rel*A1, *sup* E44, Δ(*lac-pro*AB), [F', proAB, *lac*I^{q}ZΔM15, Tn10 (tet^r), Amy, cam^r]	Bullock, 1987

Table 3.: Bacterial strains

Plasmids

Plasmid pBluescript® I SK+/-

(Stratagene® Ltd., Cambridge, Cambridges., England; Alting- Mees, 1992)

Fig.1 Plasmid map of pBluescript® SK+/-. The pBluescript® SK+/- phagemid is 2958 bp in size. The multiple cloning site contained within the *lacZ* gene, is used for blue/white selection of inserts into this gene. It points in the direction from *Kpn*I and *Sac*I. The *f1* origin of replication is provided to assure autonomous replication within the host. This origin of replication of the phagemid is used to generate single stranded DNA where co-transfected with a helper phage (Alting-Mess et al., 1992).

Fig.2. The plasmid pPNT. (Gift from Anton Berns, Free University of Amsterdam). This vector is used for transfection into cultured mammalian cells. The bacterial *ori* is provided by the pUC ampicillin resistant gene. Two phoshoguanosyl kinase promoters drive the expression of the two selection markers the *neomycin* phophotransferase gene and the HSV thymidine kinase gene.

Fig. 3. The vector pUAST as constructed by Brand and Perrimon (1993).

Restriction Digests

Restriction digests were performed in a total volume of 20µl. 1µg DNA was usually digested with 1µl of the respective restriction endonuclease. Further, to each tube 2µl of the appropriate reaction buffer was added. The volume was adjusted to 20µl with distilled and deionised H_2O (ddH_2O). Restriction digests were carried out in a Techne® (Duxford, Cambridgeshire, England) heated water bath according to the recommendations of the manufacturers. Enzymes were purchased either from Promega® Ltd. (Southhampton, Hamps., England) or Gibco-BRL® (Paisley, Refrewshire, Scotland).

Agarose Gels

The concentration of the gel used depended on the size of DNA fragments to be resolved. Most of the DNA fragments used during the project were of such a size that they resolved in agarose gels with agarose concentrations between 0.8 and 1.0%. Separation of fragments smaller than 0.5 kb required gel concentrations of 1.2 - 1.5%. Multipurpose agarose (Boeheringer® Mannheim, Lewes, East Sussex, England) was weighed into a 400 mL borsilicate bottle and dissolved in 1× TBE buffer (89 mM Tris-Base, 89 mM boric acid, 20mM EDTA) in the microwave (7-9 min.; 340 watts). The agarose was allowed to cool to ≈40°C and poured into the gel caster (either 7× 8 cm or

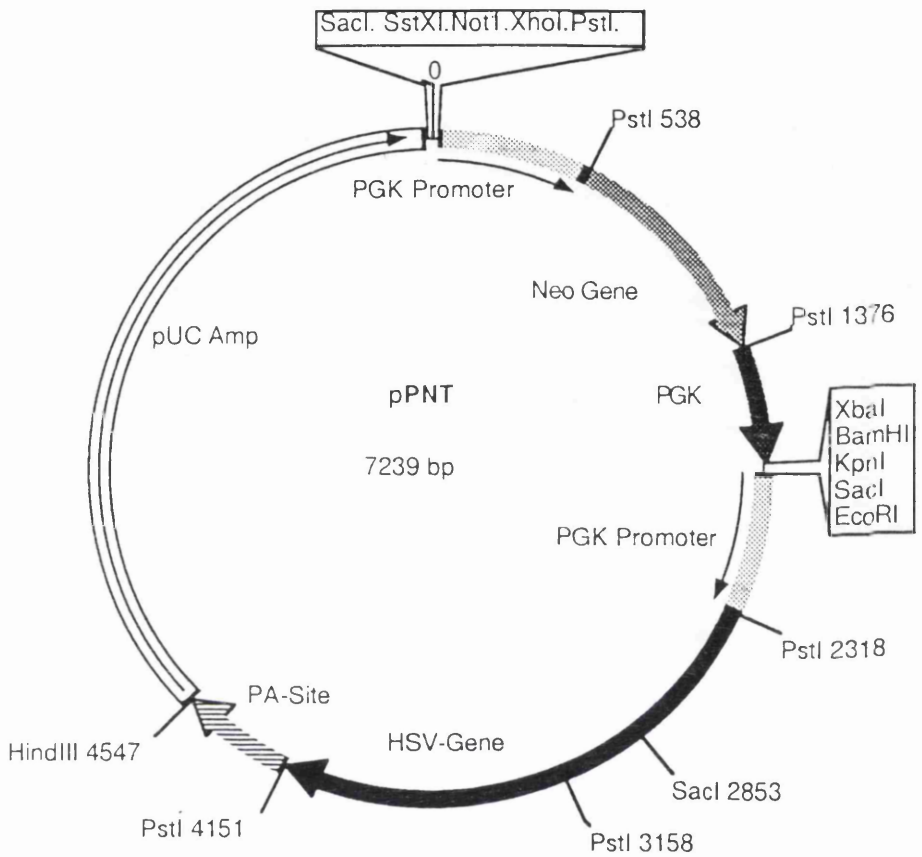


Fig.2. The plasmid pPNT. (Gift from Anton Berns, Free University of Amsterdam). This vector is used for transfection into cultured mammalian cells. The bacterial *ori* is provided by the pUC ampicillin resistant gene. Two phoshoguanosyl kinase promoters drive the expression of the two selection markers the *neomycin* phophotransferase gene and the Herpes virus serine thymidine kinase gene (de Wind et al., 1992).

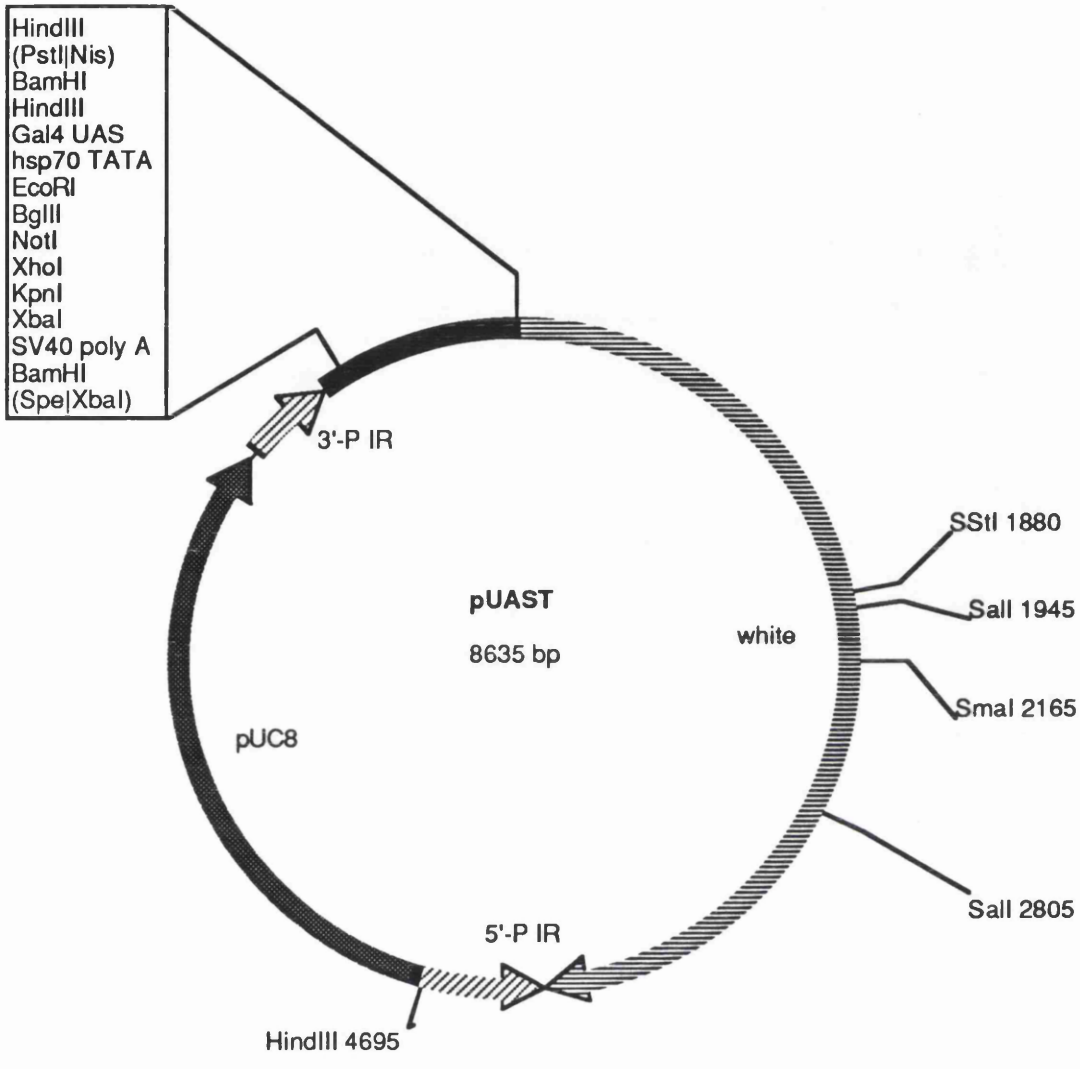


Fig.1.: Map of the pUAST vector. (For description see Brand and Perrimon, 1992). The mini white gene was described by O'Kane and Gehring (1987). The P-element inverted repeats were described by Steller and Pirotta (1986). The region containing the Gal4 UAS, the hsp 70 TATA box, the polylinker and the SV 40 polyadenylation site was described by Brand and Perrimon (1992).

12× 13cm; Hybaid® Ltd., Teddington, Middlesex, England). When the gel was set, 1× TBE buffer was poured carefully into the reservoir of the apparatus until the gel was covered with about 1 mm with 1× TBE buffer. The comb, which formed the sample wells was subsequently removed.

To set up and run a Low Melting Point Agarose (LMP) gel, Ultra Pure™ LMP agarose (Gibco-BRL® Paisley, Refrewshire, Scotland) was dissolved in 1× TAE electrophoresis buffer solution (40mM Tris base; 5mM Sodium Acetate pH 7.8; 0.2mM EDTA). After the gel was poured in the gel apparatus, it was allowed to set for at least 30 min. LMP gels were routinely run at ±4°C.

Electrophoresis

To each restriction digest 10% v/v of tracking dye (0.05% w/v bromphenol blue, 40% w/v sucrose; 0.1 EDTA; 0.5% w/v SDS) were added, prior to loading the gel. The samples were vortexed briefly and spun down, for 5 sec. at 10.000× g. The first well of the electrophoresis gel was loaded with a set of molecular weight marker fragments of DNA (1 kb ladder; Gibco-BRL® Paisley, Refrewshire, Scotland), which calibrated the gel and allowed the determination of fragment sizes in the restriction digest. Additionally, undigested DNA was run as a control next to the digested samples. The samples were electrophoresed at about 50 V× min^{-1} until the tracking dye was about 1.5 cm from the end of the gel.

Visualisation of the Fragments on the Gel

A 200- 400 mL tank of dH_2O was prepared and 0.1µl (100% w/v) ethidium bromide solution for each 1 mL of dH_2O were added. The gel was transferred to the tank and incubated for 30 min. on the shaker. It was, subsequently, rinsed for another 30 min. on the shaker to remove background ethidium bromide.

Thereafter, the gel was examined on the UV transilluminator at (long wave, 302nm) to monitor the separation of DNA fragments in the sample. The gels were photographed using either a Polaroid® camera loaded with 545- or 667- land film and provided with a Kodak® Wratten filter Nº 23A. The photograph of the agarose gel was used to

determine the approximate sizes of the fragments of DNA by restriction with endonucleases. Linear DNA fragments migrated in the agarose gels according to the logarithm of their molecular weight. From the photograph, the distance travelled by each of the bands in the first track (the marker DNA fragments) was measured. A size was assigned DNA fragment band, using the table of marker sizes. The smallest fragments are not necessarily visible on the gel. A graph of log (size of fragment/base pairs) against distance, *d*, migrated in the gel was plotted. From the calibration graph, the approximate sizes of the restriction fragments was determined and compared with the 1 kb size marker, which was run in the right hand side lane. The sizes of the fragments were compared with those predicted from known restriction maps of the ladder were compared (Brown, 1991).

Competent Bacteria

JM 109 was then grown as a 200 mL culture in a 2000 mL Erlenmeyer flask under constant shaking (150 rpm) at 37°C for 3.5 hours until an OD_{600} of about 0.4 - 0.5 was reached. The culture was incubated on ice for a period of 2 hours. The bacteria were collected by centrifugation at 2,500× g for 15 -20 minutes at 4°C in a Beckman® J-4 centrifuge using a JA- 11 rotor. The supernatant was decanted and the bacteria were resuspended in 100 mL of 10 mM $MgCl_2$. Subsequently they were centrifuged at 1,100× g at 4°C. The pellet was resuspended in 50 mL of ice cold 50 mM $CaCl_2$. The bacteria were stored on ice for 30 minutes before they were spun for the last time at 1,100× g at 4°C. The fluid was decanted and 25 mL of fresh 50 mM $CaCl_2$ were added. The now compentent bacteria were kept at 4°C overnight before the transformation was carried out (Sentry pers. comm.).

Ligations and Transformations of the Plasmids into *E.coli*

Both the *neo* gene and the pUAST vector were ligated with 1unit (u.) T4 ligase for 16 hours at 4°C. The ligated plasmids were transfected into the JM 109 *E. coli* strain.

For transformation, 200µl of competent bacteria were transferred to a 1.5mL microcentrifuge tube. Next, 2µl (≡ 150ng) of the ligation mix was added. The bacteria

were incubated for 2 minutes in a 42°C heating block and immediately afterwards chilled on ice for one minute. Finally, the transformation mixture was diluted with 3 mL of prewarmed LB broth and incubated in an test tube under constant shaking (150 rpm) in a 37°C water bath for 3 hours allowing them to express the ampicillin resistance marker β-lactamase (Titus, 1991).

Selection of Transformed Colonies

Agar plates containing the antibiotic ampicillin were prepared by melting to 100°C Luria Bertaini (LB) agar (10g Bacterio-tryptone, 5g Bacto-yeast extract, 5g NaCl) and leaving it to cool down to 40-50°C before the addition of 50μg per 1mL ampicillin (Sigma®, Poole, Dorset, England). The plates were then poured using sterile technique and when the agar solidified stored at 4°C. One hour before plating the bacteria the plates where dried at 37°C to evaporate access water. 100μl of the bacterial broth was streaked onto each plate using the sterile technique. Each plate was incubated for at least 10 hours allowing ampicillin resistant colonies to grow. The colonies were analysed further by plasmid minipreparation (Brown, 1991).

Plasmid Mini Preparation

A test tube containing 10 mL of LB broth and was inoculated with a well separated colony using a sterile toothpick. The culture was grown at 37°C under constant shaking (150rpm) for overnight. Two aliquots of 1.5 mL culture of a single colony were transferred to even as many 1.5 mL microcentrifuge tubes. The bacteria were subsequently sedimented by centrifugation at 14,000× g and the supernatant was removed. To each tube 90μl of Glucose/EDTA/ Tris buffer (25 mM Tris-HCl pH 7.5; 10 mM EDTA pH 8.0; 50 mM glucose) were added. The contents of the tubes were vortexed to disperse the cells. Next, 200μl of SDS/NaOH (200.0 mM NaOH; 3.47 mM SDS) was added to denature DNA and cytoplasmic proteins from lysed cells. After having been left for 5 minutes the lysate was neutralised with 150 μl of ice-cold 3M potassium acetate, pH 4.8. The cell debris were removed by centrifugation at 14,000× g for 10 minutes and the supernatant was pipetted into a new test tube. The supernatant

was subsequently subjected to a 30 minute treatment with RNase I (Sigma®, Poole, Dorset, England). Afterwards the proteins were removed by extraction with 400µl of 1:1 TE saturated phenol/chloroform. The mixture was spun at 14,000× g causing the formation of two layers. The upper layer was transferred to a new 1.5 mL microfuge tube and the process was repeated using 400 µl of chloroform to rid the supernatant of phenol residues. Finally, the plasmid was precipitated with 66% v/v of ethanol. The DNA was sedimented by centrifugation and washed with 400µl 70% v/v ethanol. The DNA was again pelleted by centrifugation and the ethanol was decanted. The tube was inverted over tissue and the DNA allowing the DNA to evaporate. The DNA was redissolved in 50µl of TE buffer (10 mM Tris-HCl, 1mM EDTA (pH 8.0)) (Titus, 1991).

Large Scale Plasmid Preparation

Large scale bacterial cultures for plasmid preparation were grown from a single colony in 2 - 5 mL of LB medium containing 50µg × mL^{-1} (50mg × mL^{-1} stock) for 12- 16 hours at 37°C (*vide ante*). The saturated mini - culture was subsequently diluted 1 : 100 v/v with 500mL of selective ampicillin (50µg× mL^{-1}) LB medium and regrown as an overnight culture until an OD_{600} of 1 - 1.5 (≈1 × 10^9 cells) was reached (*vide ante*). Next, the culture was dispensed into two Nalgene® 500 mL centrifuge tubes (BDH®, Speke, Liverpool, England) and centrifuged at 5,000 × g for 15 min. at 4°C in a Beckman® J-4 centrifuge using a JA- 11 rotor. The cells were resuspended in 2× 6 mL of freshly prepared ice-cold Glucose/EDTA/ Tris Buffer. The lysate was incubated for 20 min. on ice. Subsequently, 2× 12 mL freshly prepared 0.2M NaOH and 1% w/v SDS was added, mixed and incubated on ice water for 10 min. Thereafter, the lysate was provided with 2× 7.5mL of sodium acetate (pH 4.6) and carefully mixed by inversion and incubated in ice water for 10 min.. The bacterial debris and protein were subsequently removed by centrifugation of the lysate at 10,000 × g for 15 min.. Following centrifugation, the supernatant was removed and transferred to a fresh Nalgene® tube, avoiding the white precipitate. RNA was digested by the addition of 50µl of DNase-free RNase A (1mg× mL^{-1} stock; RNase A in 10 mM Tris-HCl, pH 7.5,

15 mM NaCl heated at 100°C for 15 min) to the supernatant. The suspension was incubated for 20 min. at 37°C. Following the digestion of RNA, the DNA was precipitated with 1:1 isopropanol. To precipitate the DNA it was frozen for 30 min. at -70°C and centrifuged in the precooled (- 14°C) Beckman® J2 centrifuge at 14,000× g. The supernatant was discarded, taking care not to disturb the pellet and the tube with the pellet attached to it was inverted on a Kim Wipe® for 5 min. allowing the ethanol to evaporate. For further treatment the precipitated DNA was resuspend in 20 mL TE-buffer and transferred to four 25 mL Nunc® tubes. Proteins still contained in the solution were degraded by phenol: chloroform (1:1 saturated with TE buffer, pH 7-8) treatment for generally 3-5 min.. To obtain immersion of both phases the samples were vortexed for five min.. The organic phase was separated from the aqueous phase by centrifugation at 14,000× g for 10 min. in a Beckman® J2 centrifuge. The upper aqueous layer was transferred to fresh 10 mL Nunc® tubes. This step was repeated at least two times. After the second spin the supernatant was extracted with chloroform. Following the chloroform extraction, the supernatant was transferred to sterile Eppendorf® tubes, precipitated (vide ante) and sedimented by centrifugation at 14,000× g in a microcentrifuge. The pellet formed at the bottom of each tube was dissolved in 1.6 mL of ddH_20. 0.4 mL of 4M NaCl was subsequently dispensed to the tubes and mixed. To remove remaining contaminants, 2 mL 13% (w/v) polyethylene glycol (PEG-8,000; Sigma® Poole, Dorset, England) was added and each tube was incubated in ice water for 60 min.. To recover the precipitated DNA from the samples were centrifuged at 10,000× g for 10 min.. The ethanol was removed using an automatic pipette, taking care not to disturb the DNA at the bottom of the tube and the tubes was inverted on a paper towel and left to dry for 10 min. at room temperature. The pellet was further washed with 70% (v/v) ethanol. The mouth of the tubes were covered with clean parafilm and the samples were placed in the Howe® Gyro Vap (Howe® & Co., Oxon, England), evacuated , and left to evaporate to dryness for 6 min. at 45°C. When the sample was dry, 500µl of Tris-EDTA buffer (TE- buffer), pH 7.5, was added to each tube. The tubes were finally flicked with a finger to assist the dissolution of the DNA (Titus, 1991).

Probe preparation

A 831 bp fragment was cleaved from the pPNT plasmid and run on a 1% (w/v) low melting point agarose gel. A gel slice containing the fragment was excised and subsequently molten on a 65°C heating block for 10 minutes. To the liquidised gel slice 1 mL of Wizard™ resin (Promega® Ltd., Southhampton, Hamps., England) was added and mixed by vortexing briefly. The plunger of a sterile 2 mL disposable syringe was removed. The luer lock™ of this syringe had a minicolumn attached and this was in turn connected to a vacuum manifold (Promega® Ltd., Southhampton, Hamps., England). The DNA resin mixture was pipetted into the syringe and a vacuum applied to the manifold. After the fluid had been drained the column was washed with 2 mL isopropanol. Finally the column was left to dry and 50 µl of 65°C warm TE buffer was applied to it before spinning at 14,000× g for 15 sec..

The sample was concentrated for one hour in the gyro vap to a volume of 10 µl. The concentration was determined by spotting a 1µl sample on a plate with standards corresponding to 250 - 500ng × $1\mu l^{-1}$. 7µl of ddH_2O and 2µl of the DNA (50-100µg) were heated for 2 minutes to 100°C in a boiling water bath and quenched on ice immediately afterwards. The fluid was spun down briefly and 5µl of 4× random primer mix (250mM Tris-HCl pH 8.0; 25 mM $MgCl_2$, 10mM Dithiothreitol (DTT; Sigma®, Poole, Dorset, England), 1 mM N-[2-Hydroxyethyl]piperazine-N'[2-ethanesulphonic acid (HEPES; Sigma®, Poole, Dorset, England)) was added. 5µl of [α-^{33}P]dCTP (50µCi) and 1µl Klenow polymerase were pipetted into the tube. The final volume was adjusted with ddH_2O to an final volume of 20µl. The labelling reaction was incubated at 18°C for overnight.

To remove non-incorporated nucleotides the labelling reaction was applied together with a drop of 7- Hydroxy-8-phenylazo-1,3-naphthalenedisuphonic acid (Orange G; Sigma®, Poole, Dorset, England) to a Sephadex® (Pharmacia® Biosystems Ltd., Milton Keynes, England) column. This column was prepared by pouring the Sephadex® suspended beads up to the 2.5 mL mark of the 2 mL syringe. Subsequently the column was allowed to settle and washed three times with autoclaved TE-buffer. Then the

sample was added. Several one drop fractions of the eluent were collected in 0.5 mL microcentrifuge tubes as soon as the Orange G has reached the bottom of the column. Each fraction was monitored for radioactivity. The sample with the 150 counts per minute was selected (Promega corp. 1993; Titus, 1991).

Southern blot

The subcloned plasmid was digested and run on a 1% agarose gel. The agarose gel was denatured for 25 minutes in 400 mL denaturing solution (1.5 M NaCl; 0.5 M NaOH). The gel was rinsed twice in ddH_2O and afterwards neutralised for 30 minutes in 400 mL neutralising solution (1.5 M NaCl; 0.5 M Tris/HCl (pH 7.2); 1 mM EDTA (pH 8). The gel was subsequently soaked in 20× SSC (3M NaCl, 0.3 M sodium citrate, pH 7.0) and transferred to a glass plate placed over an reserve of 20× SSC on which a filter was placed. A sufficient large piece of Whatman® 3MM filter paper (BDH®, Speke, Liverpool, England) was arranged on top of the glass plate in such a way that both ends were plunged into the reservoir. A piece of a nylon membrane was cut according to the dimensions of the gel. It was briefly soaked in 20× SSC and placed on the top of the gel. The set up was sprinkled with 20× SSC to keep the filter wet. Two layers of Whatman® filter (BDH®, Speke, Liverpool) paper were fitted to the size of the nylon membrane. The gel was surrounded with strips of parafilm. To generate a capillary force by which the DNA is transferred to the nylon membrane it was topped with a stack of paper towels. The Southern blot transfer was allowed to proceed for 16 hours before the filter was removed. The DNA fragments were linked to the membrane using the Stratalinker® set by UV. The membrane was subsequently placed in a Techne® (Duxford, Cambridgeshire, England) hybridisation cylinder. To this hybridisation cylinder 10 mL of prehybridisation solution (1× SSC [0.15 M NaCl, 0.015M sodium citrate, pH 7.0]; Denhardt's solution (2% w/v Bovine Serum Albumin, 2% w/v Ficoll™ 400 [Sigma®, Poole, Dorset, England] and 2% w/v Polyvinylpyrrolidone) solution; 10% w/v SDS) were added. The filters were prehybridised at 68°C for six hours in the Techne® Hybridisation Oven. After this period, 10µl of the probe were added to the prehybridisation solution. The tubes were placed in a hybridisation oven at 68°C

allowing hybridisation of the DNA probe to the DNA on the filter. The hybridisation was allowed to proceed for 16 hours. To wash away the unbound probes, the filters were washed for 20 minutes in 20 mL in 2× SSC at 65°C, followed by 3 subsequent washes at lower stringency (1× SSC, 0.5× SSC, 0.1× SSC) again for 30 minutes each. The filters were finally screened with an Geiger counter to monitor the binding of the DNA to the filter. The filters were subsequently wrapped into Saran wrap™ and arranged on a film cassette to expose a sheet of radiation sensitive Fuji® chrome film. After three hours the film was developed.

Sequencing

An overnight plasmid mini culture was set up. The plasmid was alkaline treated and neutralised (*vide ante*). To the supernatant 1 mL of Promega® Wizard™ resin was added (*vide ante*). The sequencing reaction was started by labelling the complementary strands of the alkaline denatured plasmid by pipetting 7 µl of the denatured double stranded DNA (3-5µg), 2 µl of reaction buffer and 1µl of primer into a 0.5 mL microcentrifuge tube. The contents of the tubes were mixed thoroughly. The tube was heated to 65°C for 2 minutes on a PCR thermocycler. The reaction mix was allowed to adapt slowly to room temperature by switching off the PCR Thermocyler. The fluid was spun to the bottom of the tube and stored on ice.

Meanwhile for each nucleotide 2.5 µl the respective termination mixture was added to a separate test tube. The sequenase® enzyme (Ammersham® International, Little Chalfont, Buckinghams., England) was diluted 5 fold with ddH_2O. Each of the tubes was incubated at 37°C and provided with 10 µl of DNA primer template, 1 µl Dithiothreitol (0.1M, DTT; Sigma®, Poole, Dorset, England), 2 µl diluted labelling mix, 10µCi/µl and 10µM (1000 Ci/mmol) [α-^{35}S]dATP and 2.0 µl diluted sequenase. The labelling reaction was incubated for 2 minutes at room temperature. 3.5µl of the labelling reaction was added to each of the prewarmed termination reaction tubes. The termination reactions were carried out at a temperature of 37°C for 5 minutes in the PCR machine. To discontinue the chain termination reaction, 4µl of stop solution was

pipetted into each tube. Each tube was flicked with the finger and stored on ice until loaded on the sequencing gel.

The sequencing gel was prepared by mixing 5.7% w/v acrylamide, 0.3% w/v N,N'-methylenebisacrylamide (both Biorad® Ltd., Hemel Hempstead, Hertfordshire, England) and 7M urea in 1× TBE (89.2mM Tris Base; 89.0mM Boric Acid; 2.5 mM $Na_2EDTA . 2H_2O$). The polymerisation reaction was initiated by adding 20µl N,N,N'N',-tetramethylethylenediamine (TEMED; Biorad® Ltd., Hemel Hempstead, Hertfordshire, England) and 10% w/v ammonium persulphate. The bottom of the sequencing cell was plugged first using bis- acrylamide. The sequencing gel was poured into the sequencing cell (Sequi-Gen I cell; Biorad® Ltd., Hemel Hempstead, Hertfordshire, England). Aliquots of 30mL were pipetted into the sequencing cell by holding the sequencing cell in a slight angle allowing the bis- acrylamide to run into the corners of the sequencing cell. The comb was inserted the other way round to allow a smooth opening to sequencing cell. The sequencing cell was inserted into its holder and the apparatus was filled to the with buffer 1× TBE. The gel was allowed to set for 4 hours in an almost horizontal position at room temperature. It was subsequently inserted into its holder and the tank was filled with 1× TBE buffer. Next, the gel was connected to the power pack (IBI® Kodak Ltd.) and prerun for an hour. Before the gel was loaded the comb was inserted. The heat denatured samples were then loaded in the following order: A, C, G and T. The gel was run for 3.5 hours and the plates were separated. The gel was covered with a large sheet of Whatman® 3MM™ filter paper. The plate with the gel was turned over and the top plate was carefully removed leaving the gel attached to the Whatman® 3MM™ filter paper. Finally, it was covered with Saran™ wrap and dehydrated on the gel dryer. The dried gel was then transferred to a film cassette to expose radiation sensitive Fuji® NIF RX film. The exposure time was a minimum of 15 hours. The film sheet was developed and analysed on a gel sequencing program (USB, 1993).

Immunocytochemistry

Embryo Immunocytochemistry

Crosses

To set up the crosses 100 p[Gal4, w^+] males and pUAST [*lacZ*; ry^+] 500 virgin females were placed in a small fly cage with a grape agar juice egg plate attached to it. The fly cage was kept at 69-70% relative humidity and exactly 25°C and the flies left to adapt to their new environment for approx. 1 day. The embryos were staged by allowing broods to lay eggs on the yeasted egg plates for a 2-hour period. The egg plates were removed in 1 hr intervals without the use of CO_2 and incubated until further use at 25°C. The flies were left at 16°C overnight allowing the broods to recover.

Dechorionation and Fixation

The first two plates each day were discarded. The egg plates were removed after an hour incubation at 25°C and left for the desired duration at 25°C. The embryos were collected with a brush and distilled water, and squirted into a sieve. The sieve was dipped into an large petri- dish containing ~18% sodium hyperchloride. After 2- 3 minutes the embryos were thoroughly washed with distilled water and transferred to an scintillation vial containing equal volumes of 10 mL *n*-Heptane and 4% w/v paraformaldehyde in 1× PBS (130 mM NaCl, 7 mM Di-sodium orthophosphate, 3 mM Sodium Di-orthophosphate). The embryos were fixed on a roller mixer for 30-45 minutes. Afterwards the paraformaldehyde was discarded and replaced with methanol and 0.05% w/v Ethyleneglycerol-bis(β-aminoethyl Ether) N,N,N'N'- Tetraacetic acid (EGTA; Sigma® Poole, Dorset, England) so that the embryos dropped out of their vitelline membrane and sank to the bottom of the vial. There they were removed with a 1000 µl Eppendorf® pipette.

Preincubation

The embryos were washed two times in 1× PBS and 0.5% v/v Triton™ X-100 (Sigma® Poole, Dorset, England). Afterwards they were incubated for four hours in 10% GNS

(10% v/v Goat normal serum (S.A.P.U. Law Hospital, Caluke, Scotland), 0.5% v/v Triton™ X-100, 1× PBS and 1% w/v Bovine Serum Albumin Fraction V powder (Sigma® Poole, Dorset, England)).

Antibody Incubations

The preincubation solution was removed and replaced with the incubation solution (49.95 mL 1× PBS; 0.1% w/v Bovine Serum Albumin Fraction V powder; 0.3% v/v Triton™-X 100; 10 w/v % goat normal serum) containing rabbit anti β- galactosidase antibodies (Cappel®, Ghent, Belgium). This was at a concentration of 1:1000 for staining with FITC conjugated secondary antibodies or 1:5000 for anti horse radish peroxidase primary antibodies and horse radish peroxidase/ avidin-biotinylated secondary antibodies. The incubation time for the primary antibodies was about 14 hours. Following the incubation with the primary antibodies, the embryos were washed four times in BSA-PBS (49.50 mL 1× PBS; 0.5% w/v Bovine Serum Albumin Fraction V powder ; 0.3% v/v Triton™-X 100).

Anti rabbit horse radish peroxidase conjugated secondary antibody was diluted 1:500 and anti-rabbit FITC conjugated anti rabbit antibody was diluted 1:250 in the incubation solution containing 5% GNS. The secondary antibodies were incubated for 4- 6 hours at room temperature. The FITC stains were washed and mounted immediately in TW-PBS (9.99 mL 1× PBS and 0.1 % v/v Tween™ 20 (Sigma®, Poole, Dorset, England)). The HRP- biotinylated stains were treated for half an hour with the avidin biotin solution (ABC solution; prepared 30 min. prior to use: 1 mL of PTW add 10 mL of solution A, and 10 mL of solution B from the Vectastain® kit.). This was followed by two washes with 1× PBS and 0.5% v/v Triton™ X-100. To prepare the staining solution a Sigma® Fast™ 3,3'- Diaminobenzidine Tablet (Sigma® Poole, Dorset, England) was dissolved together with an urea-H_2O_2 tablet in 1× PBS and 0.5% v/v Triton X-100. The washing solution was replaced by this staining solution. The staining solution was allowed to act for 2 minutes until the characteristic brown staining was observed. The staining solution was immediately drained and the embryos washed five times with 1× PBS to inactivate the peroxidase. The embryos were subsequently

transferred to JB-4A monomer mounting medium. The mounting medium was allowed to penetrate the embryos for one hour. Finally, JB-4 A catalyst and JB-4 B was added. The embryos were transferred to a slide and mounted.

Dissection of Brains

Dissection of Larval Brains

First instar larvae hatch from the eggs usually 21 hours after egg laying at 25°C and 60-70% rel. humidity. The first moult takes place after 27±1 hours, and the second instar lava emerges. It needs another 25±1 hours until the third instar larvae moult takes place. The age of the larvae was determined on the grounds of their anterior spiracles (Bodenstein, 1950). The newly hatched larvae were collected and transferred to a new egg plate. Sexing *Drosophila* in the first instar is not easy but subsequent instars can easily be sexed. The larval body can be divided into 18 segments: 7 head segments (fused), 3 thorax segments and 8 abdominal segments. In the 6th abdominal segment the testis are found. Males and females can be transferred to different egg plates using the presence of testis in the 6 the abdominal segment as the criterion.

Prior to the dissection of the CNS of the different *Drosophila* instars, a petri-dish was prepared into which a slide was placed. The slide was topped with a 22mm and a 18mm circular coverslip. A drop of 4% w/v Paraformaldehyde/1x PBS was transferred with a 200µl Gilson® Pipette to the cover slip. For the dissection a dozen sexed and staged larvae were transferred to a waxed bedded petridish using entomologist forceps. The dish was flooded immediately with 1x PBS (25°C). Under the dissection microscope the larva was held at its pharynx with fine watchmaker forceps (Dumont® Dumoxel # 5 Biologie, Vermont, Switzerland). With the other pair of forceps the posterior part was held and the larvae was pulled slowly apart taking care not to disrupt the CNS. The CNS was usually attached to the pharynx and had to be teased apart using a fine tungsten wire needle (Ø 0.5mm). In third instar larvae the imaginal discs were found to be attached to the CNS. These were removed leaving only the eye disks attached to the brain. Each CNS was then transferred with a 20 µl Gilson® to the drop of 4% w/v Paraformaldehyde.

Dissection of Pupal Brains

To stage pupae, several pupating larvae were collected and transferred to a slide provided with double sided 3M® Scotch™ tape. The slide was incubated in a large fly bottle sealed with a cotton plug at 25°C for 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 hours, respectively (Bodenstein, 1950). The pupal brains were dissected by teasing away the exterior spiracles. The puparium was held with a pair of forceps at the anterior end and the puparium was removed gradually from the posterior cercii. The CNS of the exposed pupa was subsequently dissected with a tungsten wire.

Dissection of Imaginal Brains

Imagoes were etherised with CO_2 and transferred to a wax bed dish. Each fly was fixed with a small insect pin through the thorax to the wax bed dish. The base of the antennae was firmly held with one pair of forceps and with the other, the proboscis was removed. The antennae were subsequently teased out singly. The head capsule was subsequently opened allowing the 4% Paraformaldehyde/1× PBS to penetrate the brain. The fixative was removed after 45 min. and the preparation was washed with TW-PBS. Lastly, the retina was removed and the brain was freed from the head capsule using a tungsten wire needle adopting the form of a tenaculum.

Preincubation of Brains

All brains were immediately transferred to 4% Paraformaldehyde/1× PBS and fixed for 30 minutes. Indigenous peroxidase activity was neutralised by treating the brains with 1% hydrogen peroxide. The brains were washed three times and incubated with avidin B to saturate any surface molecules which could bind the avidin biotin complex later on. The brains were washed two times in 1× PBS and 0.5% Triton™ X-100. Afterwards they were incubated for four hours in 10% GNS (10% Goat normal serum , 0.5% Triton™ X-100, 1× PBS and 1% bovine serum albumin fraction V). The brains were subsequently stained as mentioned above.

Confocal Laser Scanning Microscopy

The preparations were visualised with a Molecular Dynamics® confocal laser scanning microscope Multiprobe 2001 (Sunnyvale, California, USA) attached to a Nikon® (Yokohama, Japan) Inverted microscope. The laser power was adjusted from 0.5 to 3. And the voltage used was in the range between 550- 600 PMT. The pixel size used varied with the specimen. Generally, 10× 10 Pixels were used. The laser detection area used varied from 25μm^2 to 100μm^2. All images were processed on the following programmes: Datamanager™, ImageDisplay™, ImageSpace™, ScanControl™ and VolumeBench™, all Molecular Dynamics®. The programmes were run on a Silicon Graphics® **Indigo2**™ computer (for review, see Wallén et al, 1992).

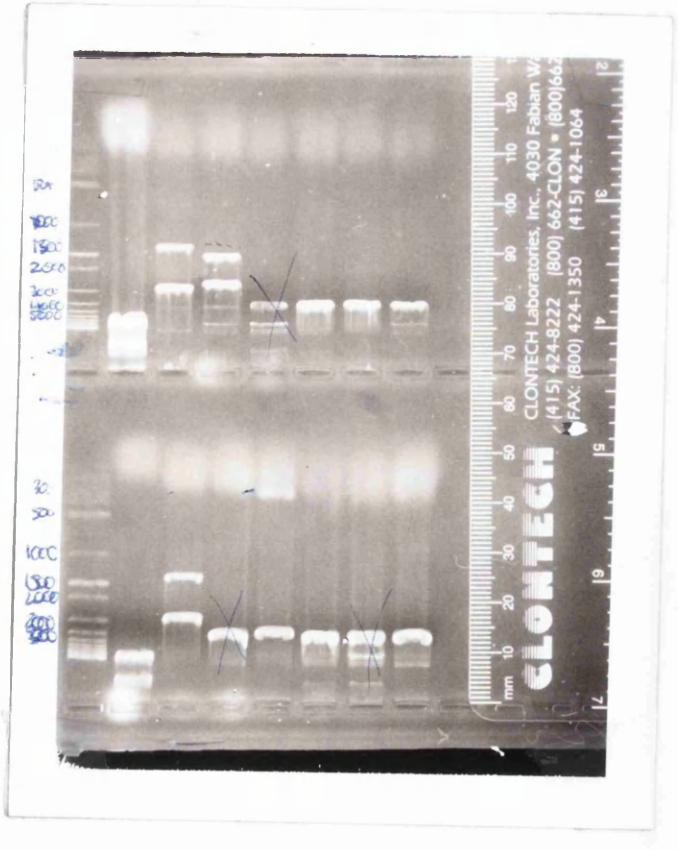
Results 1

The pBluescript® SK +/-*neo* vector was kindly provided by Dr. Dorin (MRC Unit, Western General Hospital, Edinburgh, Scotland). According to the plasmid map it contained a 1.2 kb neomycin gene insert within the 2.96 kb pBluescript® SK +/- vector. The 5' end of the original neomycin sequence was mutated by site selected mutagenesis so that a *Bam*HI instead of an *Bgl*II site was generated. Toward the 3' end a Thymidine kinase polyadenylation site of presumably 200 bp was added. This enables the addition of the polyadenylation tract during processing of the nascent RNA.

1. Confirmation of the Vector Map pBluescript® SK+/- *neo*

First, I considered it would be useful to confirm the parameters of the plasmid. I therefore grew up a 20 mL miniculture and extracted the deoxyribonucleic acid (DNA) with the minipreparation procedure. Each 1 µl (≈250 µg) of DNA was digested with *Hind*III and *Pst*I (lane 2), *Hin*dIII and *Sst*I (lane 4), *Sst*I and *Pst*I (lane 5), *Hin*dIII (lane 6) *Pst*I (lane 7) and *Sst*I (lane 8) [fig.I.1.]. The digests were run on a gel for 2.5 hours at 50 mV× min^{-1}. The fragmented DNA was compared to a size marker (the 1 kb) ladder from Gibco BRL®. The completion of the digest was verified using uncut pBluescript SK® +/- *neo* (DNA) (lane 2)[fig.I.1.]. The digest in the top and bottom lane were carried out with the same enzymes except for these being derived from Promega® (top) and Gibco BRL® (bottom). The *Hind*III and *Pst*I endonucleolytic reaction revealed the linearised 30 kb vector and an 1.6 kb *neo* gene insert (as seen in lane number 3). According to the map there ought to be a single *Hind*III site at the 3' end of the insert. *Pst*I cuts at position 217bp downstream form the *Bam*HI insertion site. The whole insert is assumed to be 1.2 kb in size. Hence, the *Pst*I and *Hind*III endonucleolytic reaction is expected to generate a 100bp fragment. As deduced from the gel lane 6, the size of the presumptive vector is 3.1 kb. The size of the insert, however, exceeds 1400bp and is, thus, 400 bp larger as indicated by the map of Dr. Dorin. The enzyme *Sst*I cleaves its recognition site on the polylinker of pBluescript SK +/- 35bp downstream of

Fig.I.1.: Restriction digest of pBluescript SK® +/- *neo* . The digests were run on the gel for 2.5 hours at 50 mV× min^{-1}. Each 1 µl (≈250 µg) of DNA was digested with *Hind*III and *Pst*I (lane 2), *Hin*dIII and *Sst*I (lane 4), *Sst*I and *Pst*I (lane 5), *Hin*dIII (lane 6) *Pst*I (lane 7) and *Sst*I (lane 8). The fragmented DNA was compared to the 1 kb ladder from Gibco BRL®. The completion of the digest was verified using uncut pBluescript SK® +/- *neo* (DNA) (lane 2). Digests of fig. I.1 a. and b. are identical.



Lane 8
Lane 7
Lane 6
Lane 5
Lane 4
Lane 3
Lane 2
Lane 1
12,216 bp
2,036 bp
1,636 bp
1,018 bp
507 bp
396 bp
298 bp
12,216 bp
2,036 bp
1,636 bp
1,018 bp
507 bp
396 bp
Lane 8
Lane 7
Lane 6
Lane 5
Lane 4
Lane 3
Lane 2
Lane 1

Figure I.1.

the *Bam*HI site. The expected fragment size is exceeded by 400bp giving rise to a 1600 bp fragment. The *Sst*I and *Pst*I cleavage reaction in lane 4 correctly gave rise to a 300bp long DNA fragment. The size of the vector and the excised fragment was, however, 4.2 kb, again 0.4 kb more than expected [fig.I.1.]. I therefore assume that the 0.4 kb of additional sequence is not located at the 5' end the *Pst*I site and does not occur towards the 5' end at the *Bam*HI. The remaining restriction digests serve as controls to verify the ability to cut the plasmid to completion. The restriction reactions of lanes 2 and 5 (top) and in lane 4 (bottom) had not come to completion.

Next, I wanted to know as to whether the 400 bp of additional sequence occurred within the *neo* gene proper or if located upstream towards the 3' end of the coding sequence. I therefore digested the plasmid with *Sma*I, a restriction endonuclease which generates blunt DNA termini, and *Bam*HI. According to the sequence (Beck et al., 1982) it should have provided a 1003 bp fragment as seen on the gel. The remaining vector DNA was 3.5 kb in length, thus 400 bp more as anticipated [fig. I.2.].

2. Subcloning of pUAST-*neo*

The pUAST vector was provided by Andrea Brand. This 8.8 bp vector contained a multiple cloning site downstream of the upstream activated sequence with *Bgl*II and *Kpn*I recognition sites. The pBluescript® SK +/- *neo* was therefore digested with *Bam*HI, *Kpn*I and *Hind*III each linearising the vector (lanes 7- 8) [fig.I.3.]. To insert the *neo* gene into the multiple cloning site pBluescript® SK +/- was digested with *Bam*HI and *Kpn*I (lane 9) and *Bam*HI and *Hind*III (lane10) [fig.I.3.]. Lanes 7- 8 indicate that the vector was linearised to completion and that it corresponds to 4.5 kb in size [fig.I.3.]. Lane 9 clearly show the 2961 and the 1600 bp fragment. As the *Kpn*I and *Hind*III recognition sites are only 38 bp apart, this digest is identical to a digest which would linearise the vector [fig.I.3.]. To ascertain myself that the gene within pBluescript® SK +/- is the correct one, I used an *neo* insert excised from the pPNT vector (kindly provided by Dr. Berns, Amsterdam, Netherlands).

Fig.I.2.: Restriction digest of pBluescript SK® +/- *neo* . The digests were run on the gel for 1 hour at 100 mV× min^{-1}. Lane 2 is the 1 kb ladder from Gibco BRL®. Lane 3 is the pBluescript SK® +/- *neo* vector linearised with *Bam*HI. Lane 4 is the pBluescript SK® +/- *neo* digested with *Bam*HI and *Sma*I. Lane 5 represents uncut pBluescript SK® +/- *neo* vector.

Fig.I.6a.: Restriction digest of the recombinant pUAST vectors. The digests were run on the gel for 1 hour at 100 mV × min^{-1}. Lane 1: Gibco BRL® 1 kb ladder. Lane 2: Clone # 1 uncut. Lane 3: Clone #1 *Bam*HI digest. Lane 4: Clone # 2 uncut. Lane 5: Clone # 2 *Bam*HI digest. Lane 6: Clone # 3 uncut. Lane 7: Clone #3 *Bam*HI digest. Lane 8: Clone # 4 uncut. Lane 9: Clone # 4 *Bam*HI digest. Lane 10: pUAST control uncut. Lane 11: pUAST *Bam*HI digest.

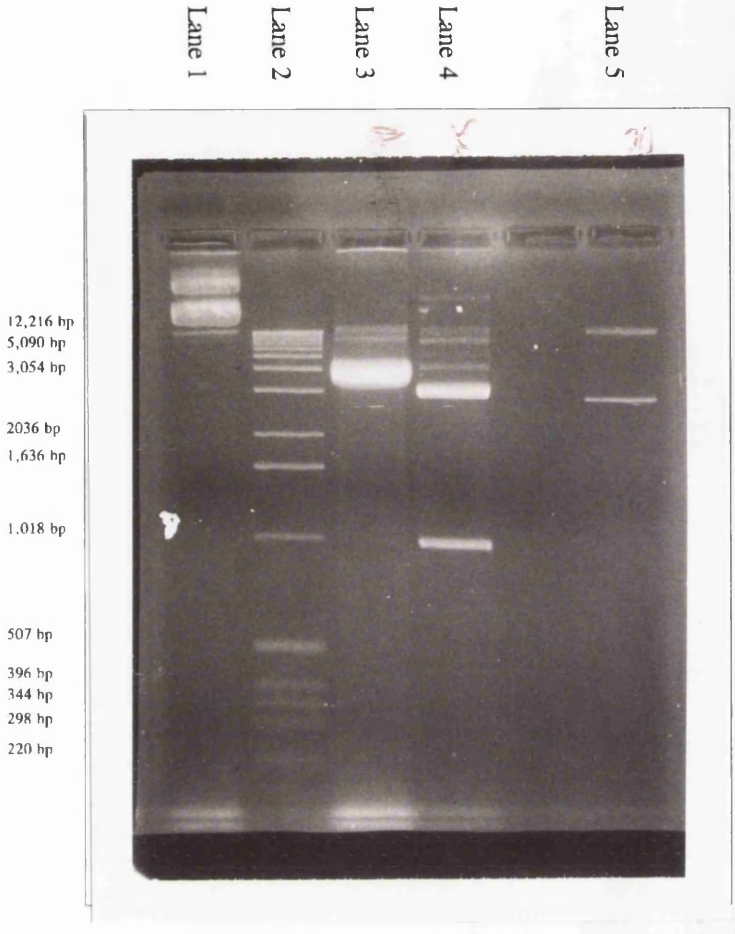


Fig. I.2

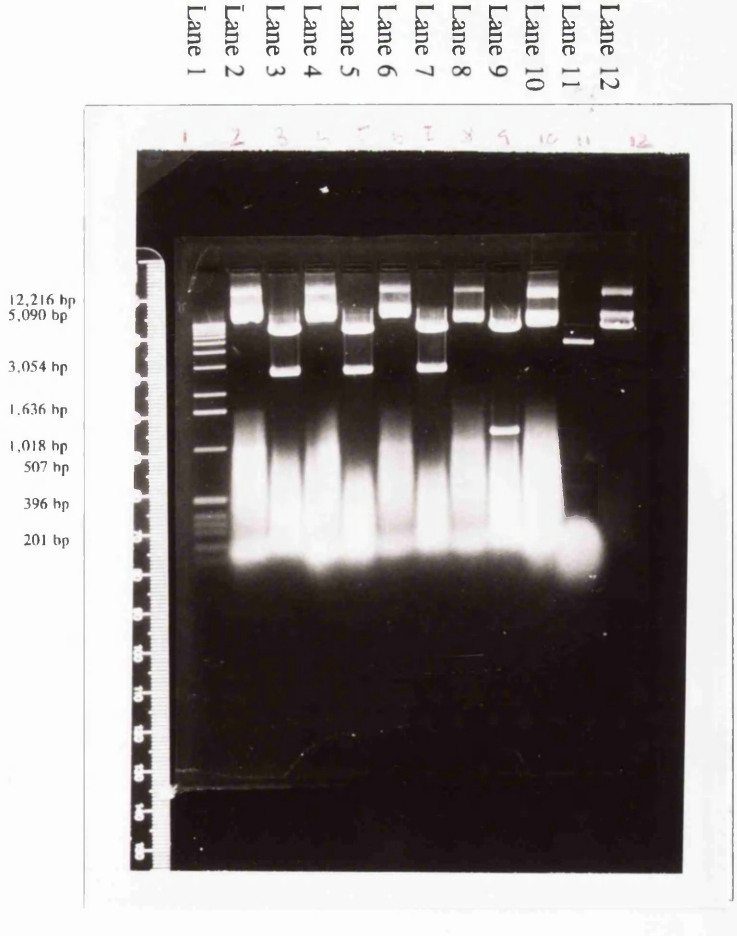


Fig. I.ba

Fig.I.3.: Restriction digest of pBluescript SK® +/- *neo* . The digests were run on the gel for 1 hour at 100 $mV \times min^{-1}$. Lane 1: 1 kb ladder. Lane 2: pBluescript® SK +/- *neo* uncut. Lane 3: pBluescript® SK +/- *neo* digest with *Bam*HI. Lane 4: pBluescript® SK +/- *neo* digested with *Hind*III (did not cut). Lane 5: pBluescript® SK +/- *neo* digest with *Ava*II yielding an 2.0 kb, an 1.5 kb and an 0.3 kb and an 0.2 kb fragment. Lane 6: *Pst* I digest linearises pBluescript® SK +/- *neo* . Lane 6: *Bam*HI and *Kpn*I digest excises the 1600 bp *neo* fragment. Lane 8: *Sac*II linearises the vector.

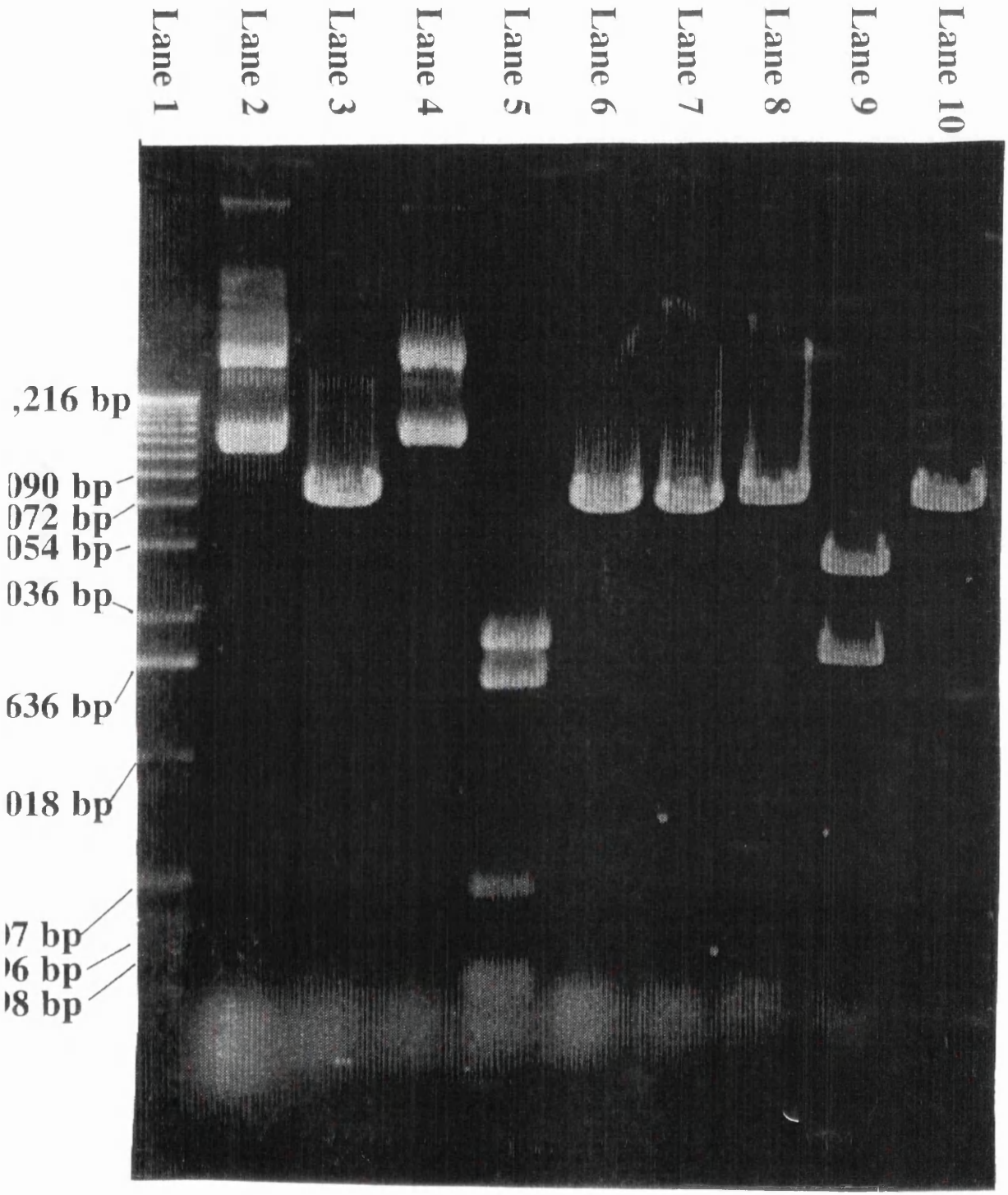
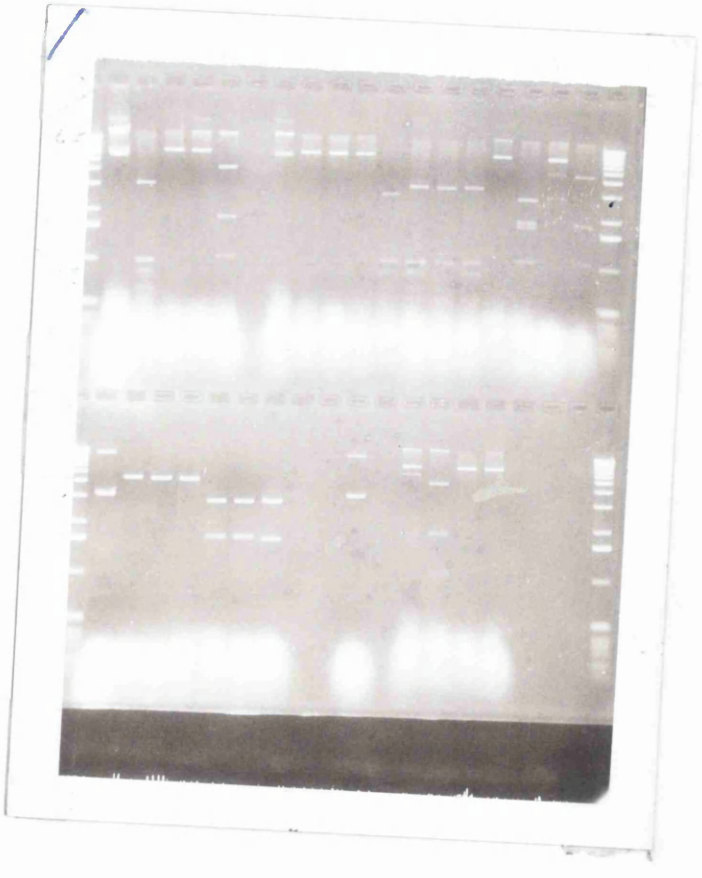

Lane 1
Lane 2
Lane 3
Lane 4
Lane 5
Lane 6
Lane 7
Lane 8
Lane 9
Lane 10
,216 bp
090 bp
072 bp
054 bp
036 bp
636 bp
018 bp
)7 bp
)6 bp
)8 bp

Figure I.3.

Fig.I.4.a: Restriction digest of the pPNT vector. Lane 1 and 20: 1 kb ladder. Lane 2: pPNT uncut. Restriction digests as follows: Lane 3: *Pst* I. Lane 4: *Hind*III. Lane 5: *Xho* I. Lane 6: *Sst*I. Lane 8: *Xba*I. Lane 9: *Bam*HI. Lane 10: *Eco*RI. Lane 11: *Kpn*I. Lane 12: *Pst*I+ *Hind*III. Lane 13: *Pst*I + *Xbo*I. Lane 14: *Pst*I + *Sst*I. Lane 15: *Pst*I + *Xba*I. Lane 16: *Hind*III+ *Xho*I. Lane 17: *Hind*III+ *Sst*I. Lane 18: *Hind* III+ *Xba* I. Lane 19: *Xho*I and *Sst*I.



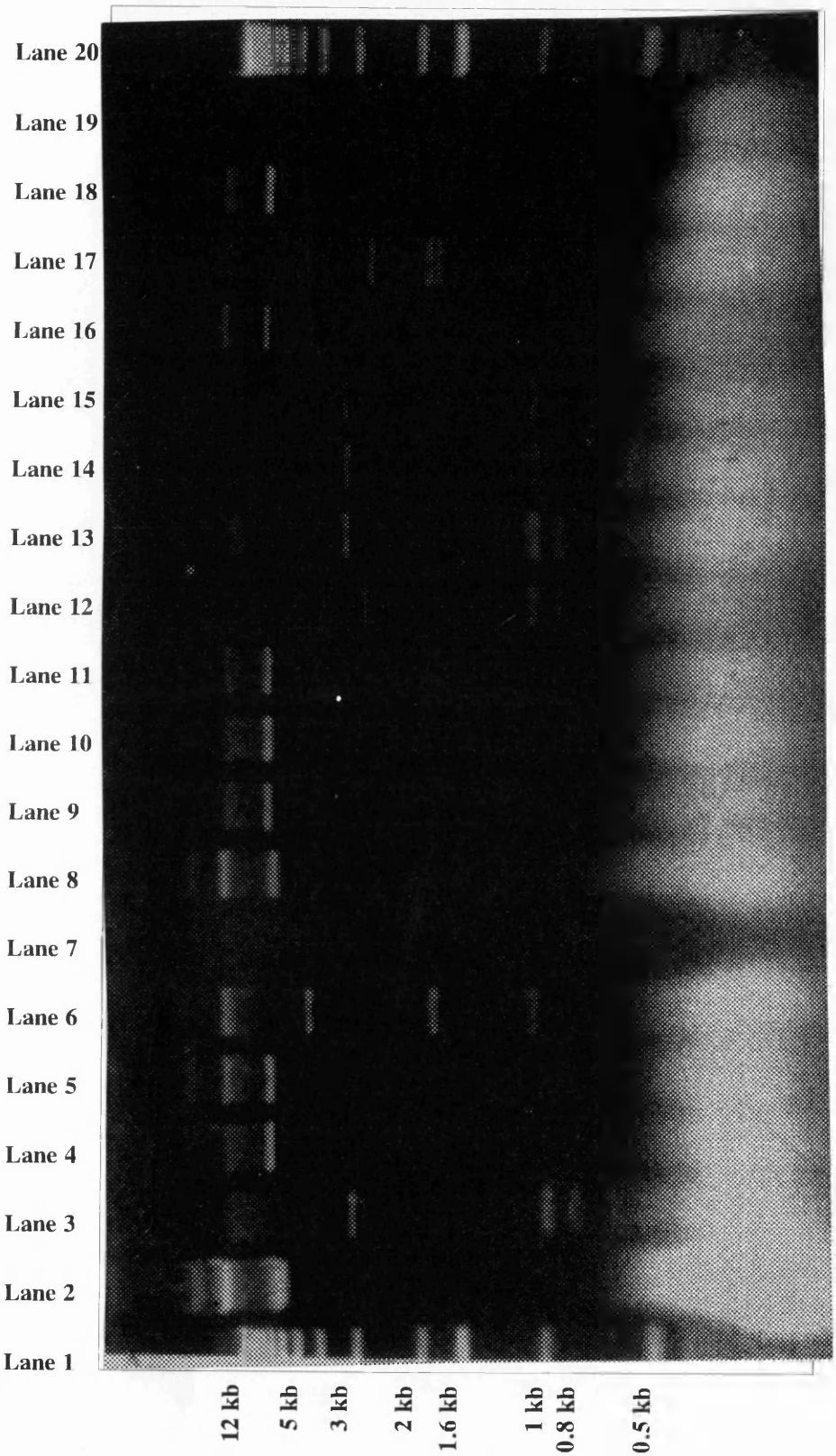

Lane 20
Lane 19
Lane 18
Lane 17
Lane 16
Lane 15
Lane 14
Lane 13
Lane 12
Lane 11
Lane 10
Lane 9
Lane 8
Lane 7
Lane 6
Lane 5
Lane 4
Lane 3
Lane 2
Lane 1
12 kb
5 kb
3 kb
2 kb
1.6 kb
1 kb
0.8 kb
0.5 kb

Fig. I.4a

Fig.I.4.b: Restriction digest of the pPNT vector. Lane 1 and 20: 1 kb ladder. Lane 2: pBluescript® SK +/- *neo* uncut. Restriction digests as follows: Lane 3: pBluescript® SK +/- *neo* *Bam*HI. Lane 4: *Kpn*I. Lane 5: pBluescript® SK +/- *neo* *Hind*III. Lane 6: pBluescript® SK +/- *neo* *Hind*III. Lane 7: pBluescript® SK +/- *neo* *Bam*HI + *Kpn*I. Lane 8: pBluescript® SK +/- *neo* *Bam*HI + *Hind*III. Lane 11: pPNT uncut. Lane 13: pPNT *Xho*I and *Xba*I. Lane 14: pPNT *Sst*I + *Hind*III. Lane 13: pPNT *Sst*I + *Xba*I. Lane 15: pPNT *Bam*HI + *Eco*RI. Lane 16: pPNT *Kpn*I

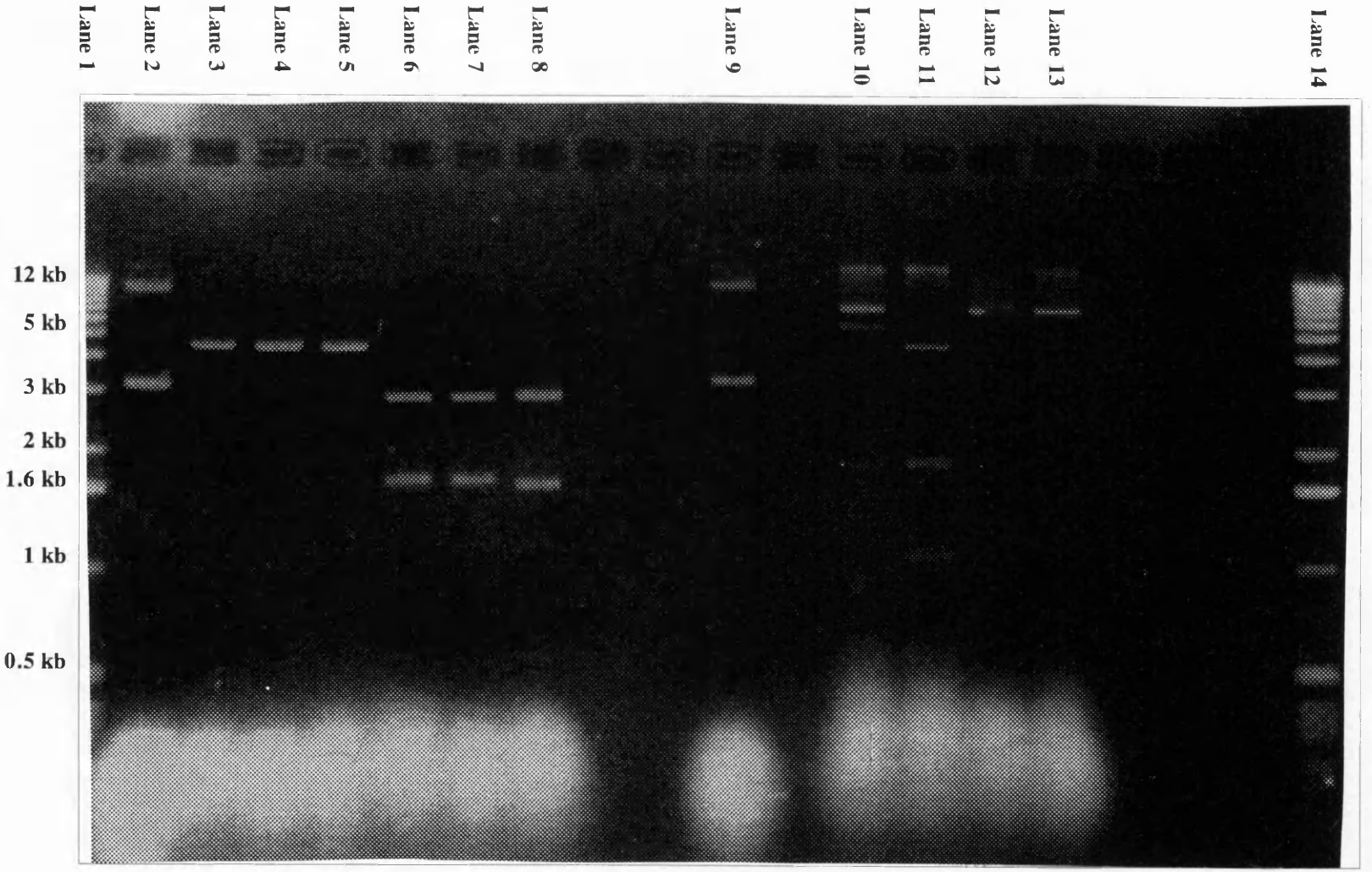
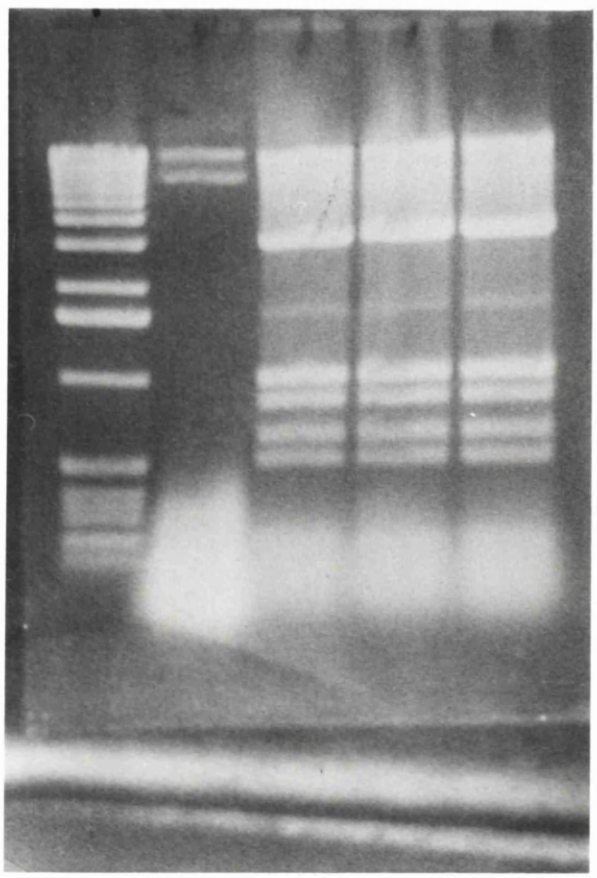

Lane 1
Lane 2
Lane 3
Lane 4
Lane 5
Lane 6
Lane 7
Lane 8
Lane 9
Lane 10
Lane 11
Lane 12
Lane 13
Lane 14
12 kb
5 kb
3 kb
2 kb
1.6 kb
1 kb
0.5 kb

Fig. I.4b

Fig.I.5.: Restriction digest of the pPNT vector. Lane 1: Gibco BRL®1 kb ladder. Lane 2: pPNT uncut. Lane 3: pPNT *Pst*I and *Sst*I. The 800 bp fragment was excised.



3. Plasmid Map of pPNT

pPNT was digested with *Pst*I, *Hind*III, *Xho*I, *Sst*I, *Xba*I, *Bam*HI and *Eco*RI. There were six *Pst*I recognition sites and three *Sst*I sites within the vector. The remaining sites were unique as shown by the single bands—although some digests did not cut to completion. The *Hind*III - *Xba*I digest gave rise to a linearised 7239 bp band as well as a ≈2.8 kb bp and a 4.4 kb band. The *Hind*III - *Sst*I digest gave rise to a 2.8 kb band in addition to a 1.1. kb, a 1.67 and an 1696 band. Thus, *Sst*I cuts the 4.4 bp fragment into three fragments. The *Hind*III *Pst*I restriction reaction resulted in four bands. Again a 2.8kb band, a 1000bp long band, a 900 bp, a 800bp and a 500bp band are visible. An additional 300 bp fragment is not detected because of the contaminating RNA. The *Xba*I - *Pst*I restriction digest indicates that a *Pst*I site lies adjacent to the *Xba*I site.
When pPNT is cleaved with *Sst*I and *Pst*I, it reveals three bands, none of which is a 900 bp fragment. Due to RNA contamination the 300bp, 500 bp fragments can not be seen. All restriction sites correspond to the plasmid map. Further analysis was not pursued as contaminated DNA did not permit it [fig.I.4a and I.4b.].

4. Probe Preparation

The *neomycin* gene is 838bp in length. It was excised with *Pst*I. As there is a second fragment of the same size pPNT was double digested with *Sst*I. The *Sst*I cut generated a 535 and a 305bp fragment instead of a 840bp fragment as expected for a single *Pst*I digest.

The endonucleolytic reaction was run on a 1.2% Gibco BRL® low melting point agarose gel (Ultra Pure™, Paisley , Renfrews., Scotland) in the cold room for 2.5 hours at 50 mV× min^{-1}. As DNA fragments migrate on the gel according to the decadic logarithm of their molecular weight, the 300 bp fragment migrated right to the bottom and was, thus, not detectable as the result of contaminating DNA. However, some fragments were detected of which three were corresponding to the 75- 220 kb size marker of the 1 kb ladder.

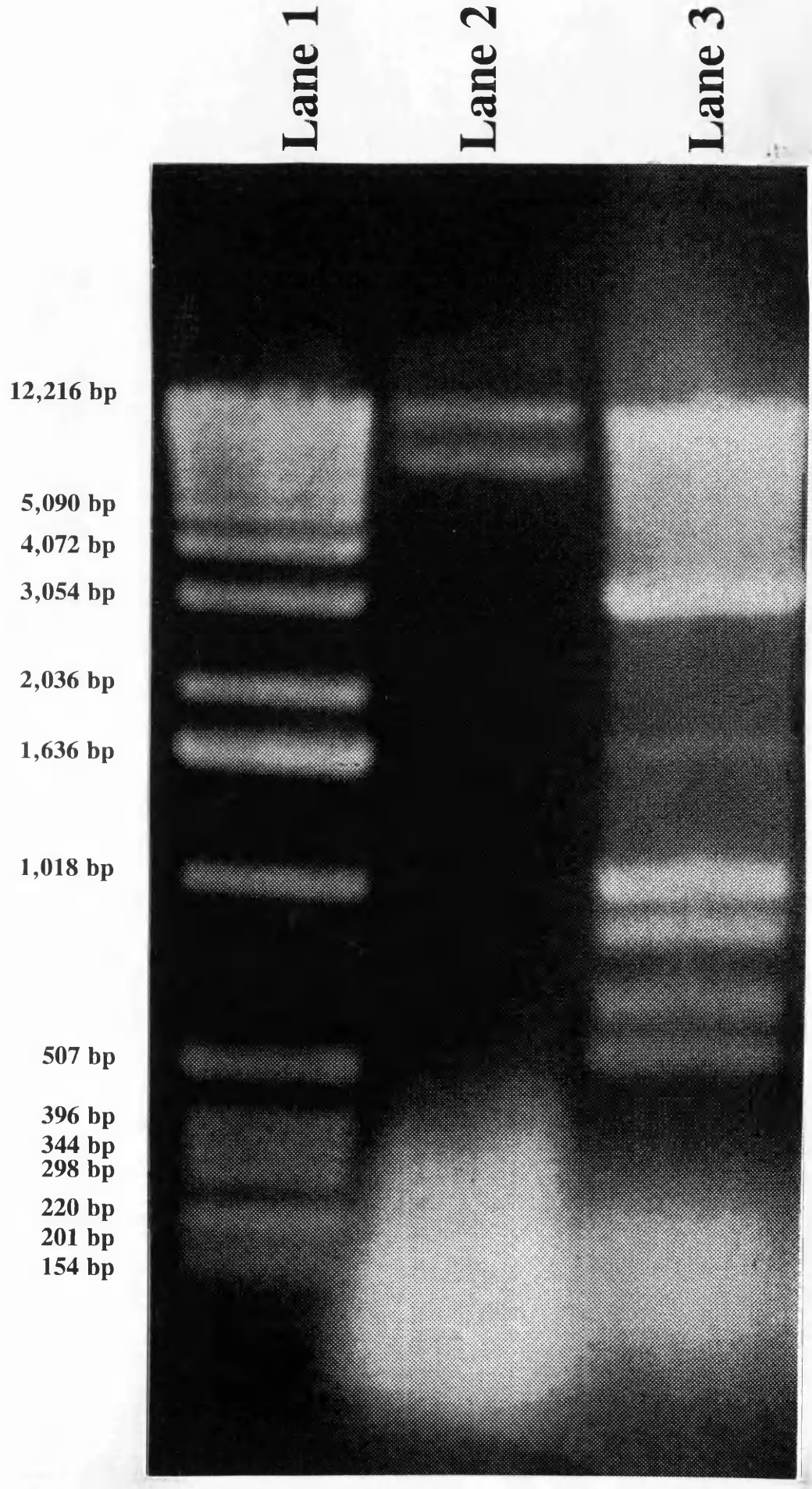

Lane 1
Lane 2
Lane 3
12,216 bp
5,090 bp
4,072 bp
3,054 bp
2,036 bp
1,636 bp
1,018 bp
507 bp
396 bp
344 bp
298 bp
220 bp
201 bp
154 bp

Fig. I.5

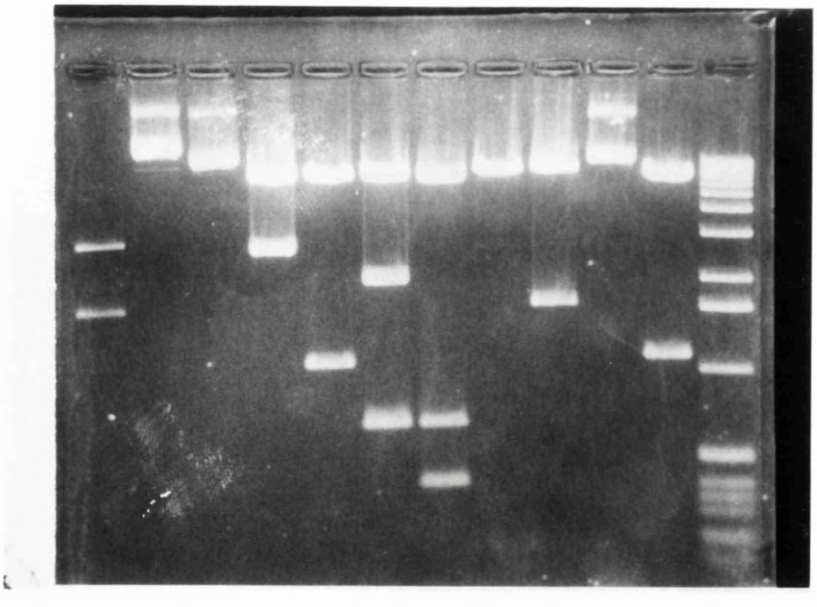
These fragments result from the gaps between the two restriction sites on the polylinker. In comparison to the reference marker, the putative 838bp fragment had a size of 650 bp in size. Obviously, there was a discrepancy of ≈200 bp in size. The fragment was excised, denatured and radiolabelled with the primer extension method using arbitrary primers (Promega®, Titus, 1991). It was probed to the electrophoresed putative *neo* gene [fig. I.4b], which had been transferred to a nitrocellulose filter. Uncut, linearised and excised fragments showed that there was a high sequence homology between the 1600 bp pBluescript® SK +/- *neo* fragment and the pPNT - *neo* probe. Therefore, it was confirmed that the gene in the vector is the correct gene. The pBluescript® SK +/- in lanes 6, 7 and 8 do not appear indicating that its sequence is not homologous to the probed *neo* gene [fig. I.7.].

5. Ligation of the pUAST - *neo* Construct

The pBluescript® SK +/- *neo* vector was digested with *Bam*HI and *Kpn*I. The digest was run on a low melting point agarose gel as described above. The pUAST vector was digested with *Bgl*II and *Kpn*I. The ligations were carried out under standard conditions. *Bam*HI and *Bgl*II digests both generate cohesive ends which are compatible enabling the T4 ligase to catalyse the synthesis of a phosphodiesterbond of randomly fused ends (Murray et al., 1979; for review, see Wilson and Murray, 1991). For the ligation reaction I used a concentration of approximately 400ng of insert and 100ng of vector. The ligated DNA was transfected into bacteria and plated out (see materials and methods). Bacteria transfected the pUAST vector only served as control.

100µl of the culture was spread in different concentrations (1:1, 1:2, 1:4, 1:8 and 1:16) onto 16 agar plates containing the antibiotic ampicillin in a concentration of 50µg× mL^{-1}. The incubated plates were assayed for colonies, I chose a plate onto which the bacterial suspension was plated in a dilution of 1:16 culture to LB medium, where 4 colonies were well separated. I extracted the DNA of the four colonies using the 'miniprep' procedure (Promega® Inc., 1991) to circumvent the use of radioactivity. *Bam*HI is an enzyme which excises the 1200bp Gal4 binding sites, polylinker, and the polyadenyla-

Fig.I.6b.: Restriction digest of the recombinant pUAST vectors. The digests were run on the gel for 1 hour at 100 $mV \times min^{-1}$. Lane 1: pBluescript SK® +/- *neo* *Bam*HI and *Kpn*I digest. Lane 2: Clone # 1 uncut. Lane 3: Clone #4 uncut. Lane 4: Clone # 1 *Bam*HI. Lane 5: Clone # 4 *Bam*HI. Lane 6: Clone # 1: *Bam*HI and *Kpn*I . Lane 7: Clone # 4: *Bam*HI and *Kpn*I. Lane 8: Clone # 4: *Eco*RI and *Kpn*I . Lane 9: Clone # 1 *Eco*RI and *Kpn*I digest. Lane 10: pUAST control uncut. Lane 11: pUAST *Bam*HI digest. Lane 12: Gibco BRL® 1 kb ladder.



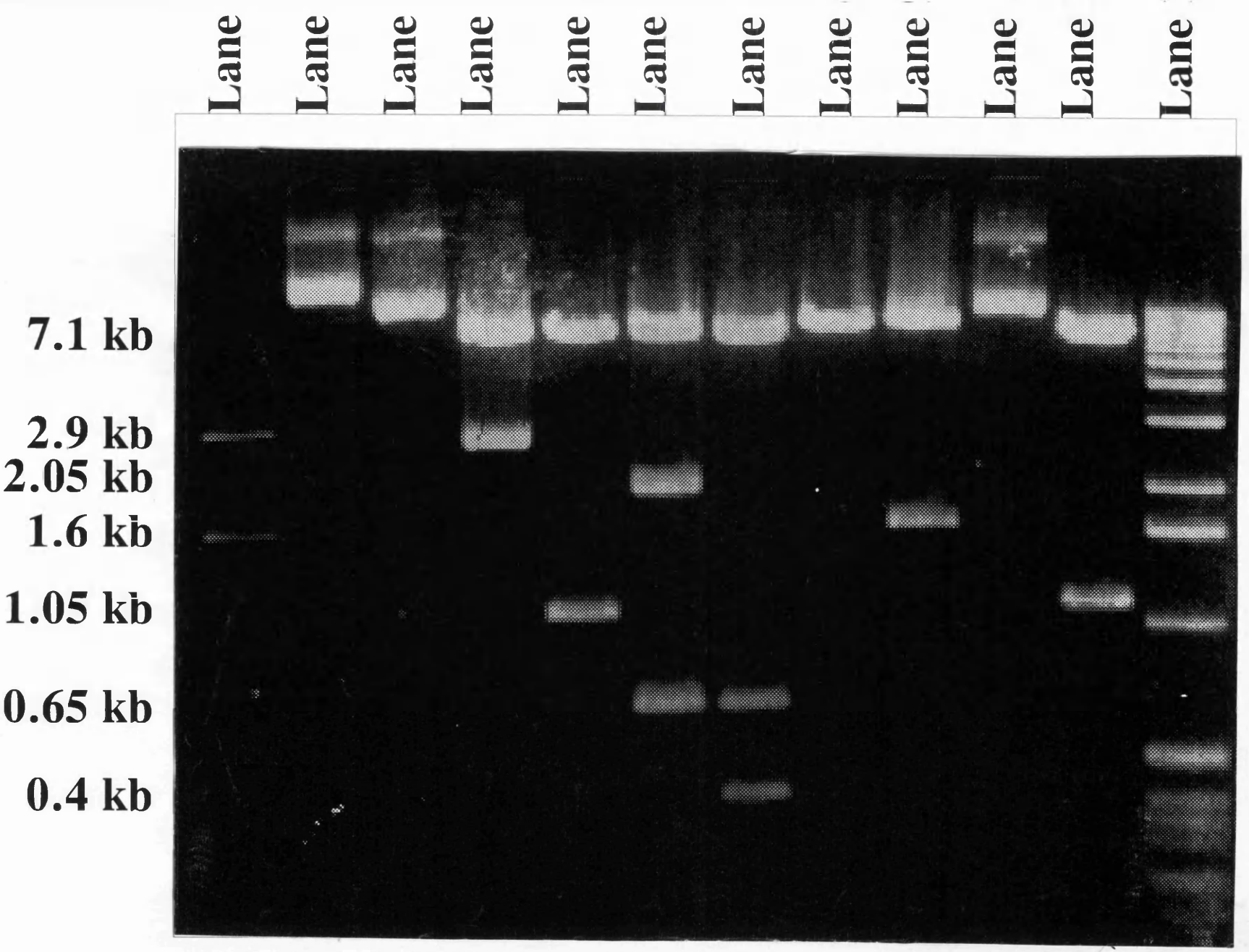

Lane
Lane
Lane
Lane
Lane
Lane
Lane
Lane
Lane
Lane
Lane
Lane
7.1 kb
2.9 kb
2.05 kb
1.6 kb
1.05 kb
0.65 kb
0.4 kb

Fig. I.6b

tion site of pUAST. If the *neo* fragment had been inserted properly, the fragments should have been 3800 bp in size. The remaining pUAST vector sequences are 7.3 kb in size. As deduced from the gel, three colonies contained the recombinant pUAST plasmid. The colony in lane 9 did not contain an insert. The control of lane 12 depicts the undigested pUAST vector which does, hence, not separate as a linearised fragment. To verify if colonies R #1 contained the correct insert, I digested the recombinant plasmid with *Bam*HI and *Kpn*I, *Eco*RI and *Kpn*I. The *Eco*RI site occurs upstream of the *Bam*HI site. The *Bam*HI restriction digest reveals the 3800 bp UAS, polylinker, *neo* insert and polyadenylation site. The corresponding pUAST *Bam*HI cut shows that the insert is again 1200 bp in size [fig. I.6a].

The *Bam*HI *Kpn*I cleavage reaction leaves the 5' *neo* sequence attached to the UAS site but removes all 3' sequences upstream of the *Bam*HI site. The UAS-*neo* SV40 polyadenylation site (polyA$^+$) fragment is 2.6± 0.4 kb in size, whereas the second *Kpn*I - *Bam*HI fragment is 650± 150 bp in size. The control digest of pUAST excised a 300± 100 bp fragment, the putative SV40 polyA$^+$ site. The *Bgl*I insertion site was directly flanked 5' by an *Eco*RI site. Because of the ligation using *Bam*HI and *Bgl*II compatible ends the *Bgl*II and *Bam*HI sites were lost. The *Kpn*I site toward the 3' end was, however, retained. The control digest linearised the pUAST vector. The recombinant plasmid reveals that a 1600 bp *neo* fragment was excised by the digest. This corresponds to the control pBluescript® SK +/- *neo* (lane1). Both are 1600 bp in size [fi. I.6b.].

Using Southern Blot Analyses the vector was probed with the pPNT *neo* fragment. The first row showed the 1600 bp *neo* fragment excised from the pBluescript® SK +/- vector with *Bam*HI and *Kpn*I. It corresponds in size to the band in lane 9 which is the *neo* gene excised by *Eco*RI and *Kpn*I from pUAST *neo*. Both bands in row 2 represented the pUAST *neo* vector uncut. The following row showed the uncleaved pUAST vector which did hybridise to the probe. Row 4 displayed the full size 3800 *Bam*HI band with the insert. There was low background cross- hybridisation of the pUAST vector when the polylinker had been excised using *Bam*HI. The band in row 6 represented the *neo*

Fig.I.7.: Southern Blot of pBluescript SK® +/- *neo* digests. Restriction digests as indicated. Probes hybridised exactly to the *neo* fragments.

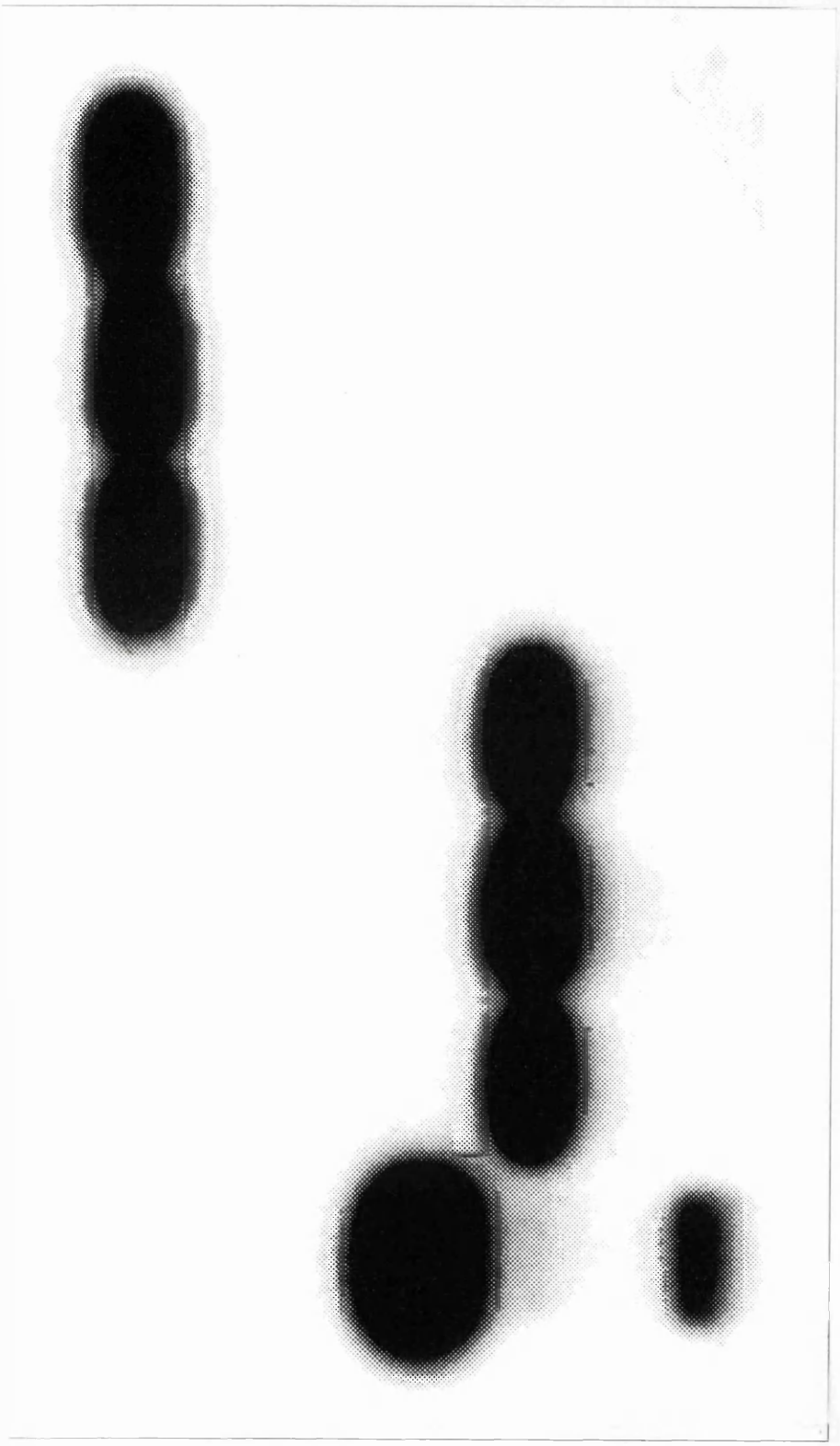
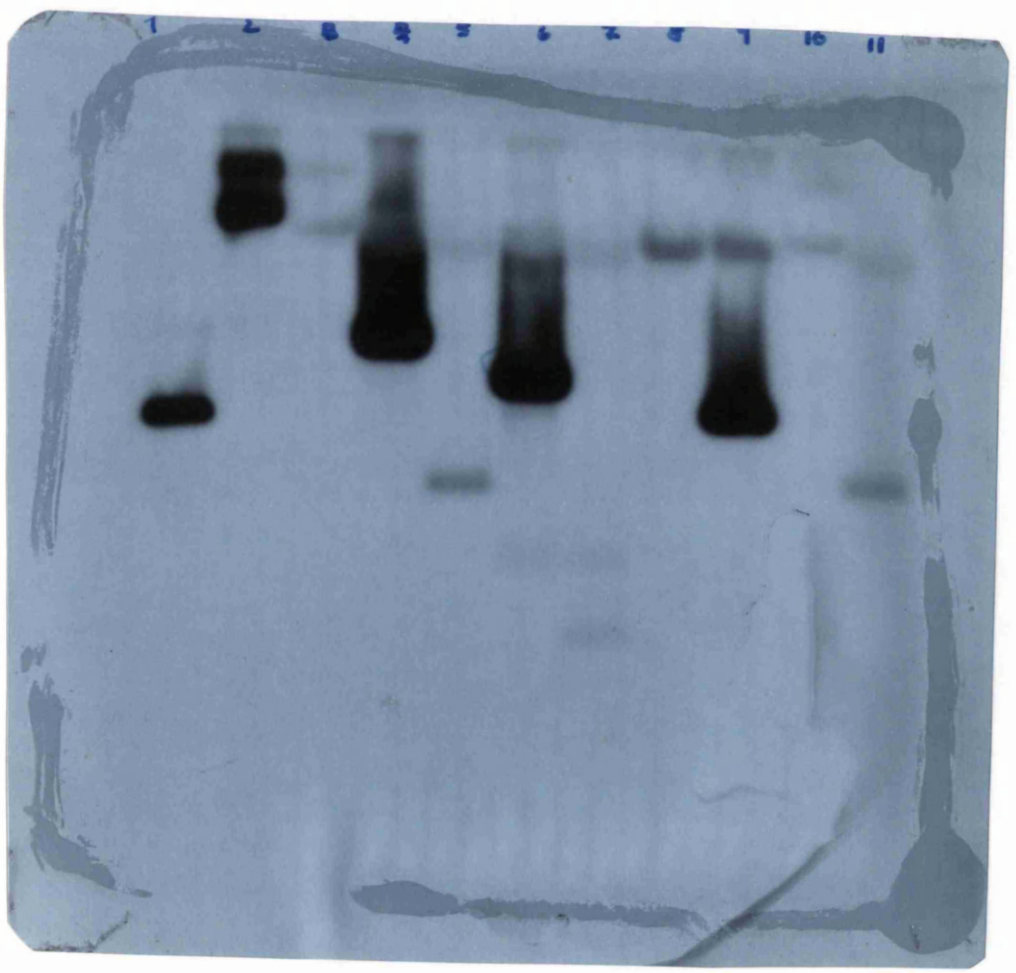

1.6 kb
4.5 kb
BamHI + HindIII
BamHI + KpnI
BamHI + KpnI
HindIII
KpnI
BamHI
UC

Fig. I.7

Fig. **I.7a.:** Southern Blot of the pUAST recombinant vector digests. *neo* probes were derived from the pPNT vector. Lane 1 *neo* fragment of pBluescript SK® +/- *neo*. Lane 2: Clone # 1 uncut. Lane 4: Clone # 1 *Bam*HI. Lane 6: Clone # 1: *Bam*HI and *Kpn*I. Lane 9: Clone # 1: *Eco*RI and *Kpn*I.



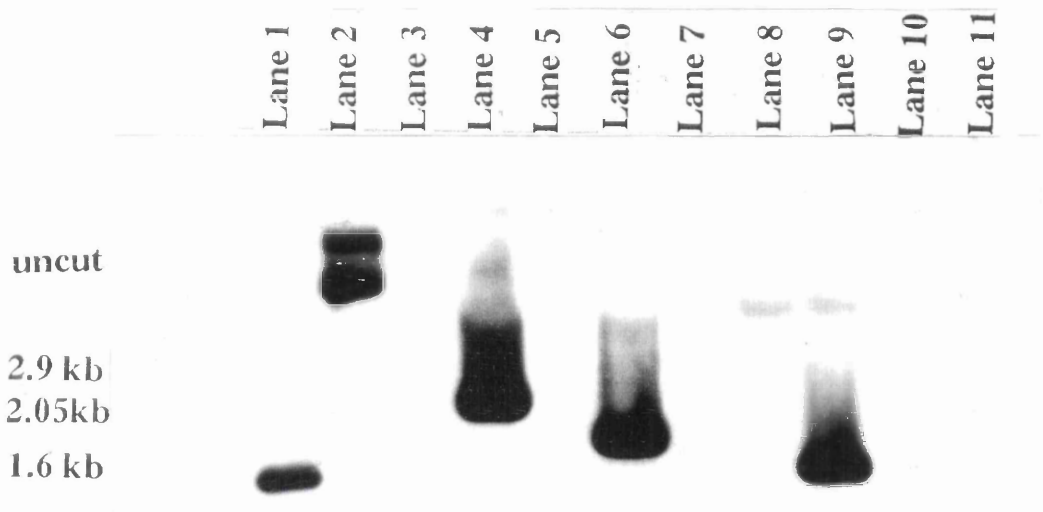

Lane 1
Lane 2
Lane 3
Lane 4
Lane 5
Lane 6
Lane 7
Lane 8
Lane 9
Lane 10
Lane 11
uncut
2.9 kb
2.05kb
1.6 kb

Figure 1.7a.

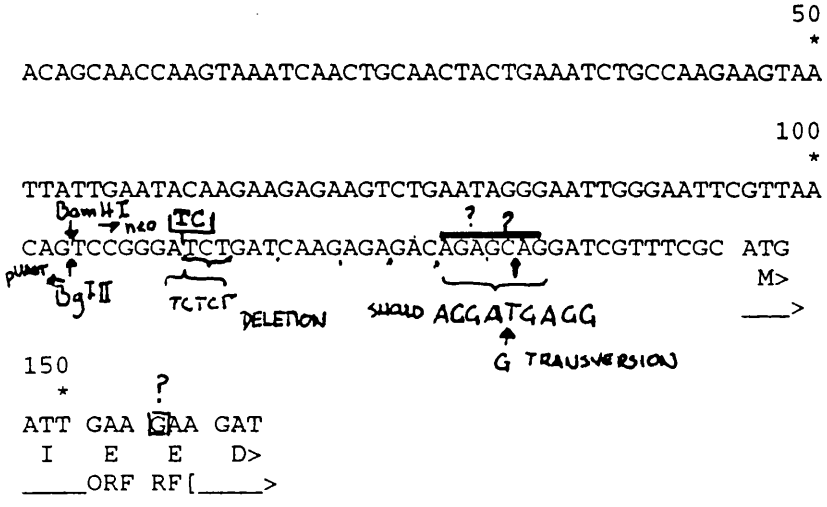
insert excised by *Bam*HI and *Kpn*I leaving the UAS attached to the 5' end of the *neo* gene [fig.I.7b.] .

6. Sequence analysis

The sequencing reactions were performed using Sequenase®, a recombinant T7 polynucleotidekinase. The replication reaction was initiated at a primer specifically designed to the upstream activated binding site of the pUAST. New nucleotides were added by Sequenase® to the 3' end of the primer. During the first run the primer was extended with radiolabelled cytosine triphosphate (^{32}S-CTP). In the proceeding step four separate reactions were set up each containing a different dideoxyribonucleotide - nucleotide analogues which lack the 3' hydroxyl (OH) group. The chain cannot be extended at this residue owing to the missing oxygen atom, which is required for the formation of phosphodiester bonds during the polymerisation reaction. ddXTPs compete with dXTPs to serve as a substrate for T7 polynucleotide kinase and are, hence, integrated randomly. Running these reactions on a high resolution polyacrylamide gel, causes the migration of the fragments on the gel according to the decadic logarithm of their molecular weight. Each reaction was loaded separately, thus allowing comparative reading of the separated bands in each column. By starting at the lowest molecular weight fragment, the sequence was established. The *Eco*RI site of the pUAST polylinker was recognised, as well as, the *neo* insert. There was, however, a deviation within the ATG codon which was obviously mutagenised to AGC indicating that a T to G transversion and a G to C transversion had taken place at residues 131 and 132. Furthermore, other residues of no noteworthy importance were mutated, i.e. residues 110, 127 and 154. As the resolution towards higher molecular weight bands was poor, a stretch of 158 nucleotides was read [fig.I.8.]. The stretch from 100- 158 nucleotides revealed a sequence homology of 91.38 % to the *neo* gene (Beck et al., 1982).

Figure I.8.: Sequence of pUAST TATA box and multiple cloning site with *neo* gene insert. Insertion into the *Bgl* II site of pUAST polylinker. Several mutations can be observed within the sequence: At position 110 of the sequence a double deletion (TC) is prominent. At position 128 an other deletion can be detected, which is followed by an T to G transversion at position 131. At position 132 an G to C transversion is found. At position 154 a C to G transversion can be detected. The original *neo* sequence in which the mutations have been marked is given, as well.

Sequence Range: 1 to 159



```
LOCUS       ISTN5X          1300 bp    DNA             BCT     03-JAN-1995
DEFINITION  Transposon Tn5 fragment encoding neomycin and kanamycin
resistance
            (neo) and a fragment of the reading frame of a further
protein.
ACCESSION   V00618 J01834
KEYWORDS    drug resistance gene; neomycin resistance; transferase.
SOURCE      Escherichia coli.
  ORGANISM  Escherichia coli
            Prokaryota; Bacteria; Gracilicutes; Scotobacteria;
Facultatively
            anaerobic rods; Enterobacteriaceae; Escherichia.
REFERENCE   1  (bases 1 to 1300)
  AUTHORS   Beck,E., Ludwig,G., Auerswald,E.A., Reiss,B. and Schaller,H.
  TITLE     Nucleotide sequence and exact localization of the neomycin
            phosphotransferase gene from transposon Tn5
  JOURNAL   Gene 19, 327-336 (1982)
  STANDARD  full automatic
COMMENT     NCBI gi: 43748
FEATURES             Location/Qualifiers
     source          1..1300
                     /organism="Escherichia coli"
     CDS             151..945
                     /note="NCBI gi: 43749"
                     /codon start=1
                     /product="neomycin phosphotransferase"

/translation="MIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGR

PVLFVKTDLSGALNELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDL

LSSHLAPAEKVSIMADAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDE

EHQGLAPAELFARLKARMPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRY
                     QDIALATRDIAEELGGEWADRFLVLYGIAAPDSQRIAFYRLLDEFF"
     CDS             942..>1300
                     /note="unidentified reading frame;  NCBI gi: 581280"
                     /codon start=1

/translation="MSGTLGFEMTDQATPNLPSRDFDSTAAFYERLGFGIVFRDAGWM

ILQRGDLMLEFFAHPGLDPLASWFSCCLRLDDLAEFYRQCKSVGIQETSSGYPRIHAP
                     ELQEWGGTMAALVDPDGT"
BASE COUNT      245 a    374 c    408 g    273 t
ORIGIN
        1 acagcaagcg aaccggaatt gccagctggg gcgccctctg gtaaggttgg
gaagccctgc
       61 aaagtaaact ggatggcttt cttgccgcca aggatctgat ggcgcagggg
atcaagatct
                  g                          ?
      121 gatcaagaga caggatgaag atcgtttcgc atgattgaac aagatggatt
gcacgcaggt
      181 tctccggccg cttgggtgga gaggctattc ggctatgact gggcacaaca
gacaatcggc
      241 tgctctgatg ccgccgtgtt ccggctgtca gcgcaggggc gcccggttct
ttttgtcaag
      301 accgacctgt ccggtgccct gaatgaactg caggacgagg cagcgcggct
atcgtggctg
      361 gccacgacgg gcgttccttg cgcagctgtg ctcgacgttg tcactgaagc
gggaagggac
      421 tggctgctat tgggcgaagt gccggggcag gatctcctgt catctcacct
tgctcctgcc
      481 gagaaagtat ccatcatggc tgatgcaatg cggcggctgc atacgcttga
tccggctacc
      541 tgcccattcg accaccaagc gaaacatcgc atcgagcgag cacgtactcg
gatggaagcc
```

```
     601 ggtcttgtcg atcaggatga tctggacgaa gagcatcagg ggctcgcgcc
agccgaactg
     661 ttcgccaggc tcaaggcgcg catgcccgac ggcgaggatc tcgtcgtgac
ccatggcgat
     721 gcctgcttgc cgaatatcat ggtggaaaat ggccgctttt ctggattcat
cgactgtggc
     781 cggctgggtg tggcggaccg ctatcaggac atagcgttgg ctacccgtga
tattgctgaa
     841 gagcttggcg gcgaatgggc tgaccgcttc ctcgtgcttt acggtatcgc
cgctcccgat
     901 tcgcagcgca tcgccttcta tcgccttctt gacgagttct tctgagcggg
actctggggt
     961 tcgaaatgac cgaccaagcg acgcccaacc tgccatcacg agatttcgat
tccaccgccg
    1021 ccttctatga aaggttgggc ttcggaatcg ttttccgggg cgccggctgg
atgatcctcc
    1081 agcgcggggg tctcatgctg gagttcttcg cccaccccgg gctcgatccc
ctcgcgagtt
    1141 ggttcagctg ctgcctgagg ctggacgacc tcgcggagtt ctaccggcag
tgcaaatccg
    1201 tcggcatcca ggaaaccagc agcggctatc cgcgcatcca tgcccccgaa
ctgcaggagt
    1261 ggggaggcac gatggccgct ttggtcgacc cggacgggac
//
```

Results 2

1. Genetic Strategies and Enhancer Trap Mutagenesis

The morphological phenotypes of the dominant markers used during P-element mutagenesis Sp (Sternopleural bristles are increased in number at 25°C) and Dr (Drop marks heterozygous individuals as having an extremely reduced number of facets), as well as the respective balancer chromosomes CyO (In Curly flies the wings are curved upward) and TM6B are described by Lindsey and Zimm (1992). p[Δ2-3] was described previously. w^{1118} contains a deletion allele on the X - chromosome (Hazelrigg, Levis and Rubin, 1984).

Heterozygous mutator females w^+/w^+ lines containing a p[Gal4;w^+] insert on the second CyO balancer chromosome above Sp were mated to several w^+/y;+/+; Dr Δ2-3/TM6B jumpstarter males. The resulting G_1 was selected for heterozygous p[Gal4;w^+] CyO/+; Dr Δ2-3/+ males. These were crossed to homozygous female w^{1118} stocks to establish independent excision lines. The resulting G_2 progeny was selected against CyO and Dr Δ2-3 and for w^+/w^+; +/+*; +/+* phenotype. The stars indicate the chromosomes which contained the p[Gal4;w^+] insert (Wilson et al., 1989).

To maintain the new lines, males with red eye colour were repeatedly crossed to female w^{1118} stocks. To localise the expression pattern the respective p[Gal4;w^+] enhancer trap lines were crossed with p[UAS-*lacZ*; ry^+] lines. To select for *lacZ* staining patterns in brain specific neurones, the lines were cryostat sectioned by Yang et al. (in prep.). Several lines were selected in terms of their staining in the corpora pedunculata. None of the lines stained specifically in this neuropil. In addition to β-galactosidase expression in the subsets of neurones, which constitute the corpora pedunculata expression in several ventral neurodermal nerve cell clusters and the optic lobes were observed. Of the thirty lines, which displayed staining in the corpora pedunculata one line 238Y (gift from Yang) was isolated by virtue of anti β-galactosidase immunoreactivity in stage 8 embryos. In this enhancer trap line different

developmental patterns generated by anti β- galactosidase immunoreactivity were monitored in different developmental stages.

2. Anti-β- galactosidase Immunoreactivity and Confocal Imaging

The insect nervous system is arranged in a bilateral symmetrical pattern. Longitudinal neurones, the connectives, run as two separated ganglia along the anterior posterior axis. Both ganglia are connected by two commissures in each segment. The cerebral ganglion represents a fusion of presumably three ganglia. Anti horse radish peroxidase (HRP) immunocytological staining of 10 hours old embryos of the line 238Y revealed a ladder like staining pattern [Fig. II.3]. These results are in accordance with previous experiments and no striking abnormalities within the central nervous system (CNS) were observed (Jan and Jan, 1982). Control experiments using anti HRP immunocytochemistry to Canton-S wild type embryos were not undertaken. The suboesophageal ganglion projects from the remaining central nervous system (←). It is deflected in such as to form a S- like shape. Beneath it the three thoracic segments can be observed followed by seven abdominal segments. As gathered from figure I.3., the last abdominal segment is not covered. Instead, only a sector of the whole embryo was depicted. At each segmental boundary a nerve root exited the central nervous system; each nerve root represented either the inter- and intrasegmental nerve, which innervated some mesodermal structures (muscles etc.) [fig. II.3.].

Immunoreactivity for the reporter protein β- galactosidase in nerve cells reveals the domains of the activity of an enhancer. The enhancer trap line 238Y, which is used in this study reflects the activity of an enhancer during the development of the procephalic region of the embryo. In stage 9 embryos [fig. II.1.] altogether six clusters of bilaterally arranged cells can be detected. The question as to whether these cells represent the actual precursor cells of the corpora pedunculata remains, however, an unresolved issue. It might be that these are the first neuroblast or ganglion mother cells (as neurones are not present during this stage), which supply the corpora pedunculata during embryonic development with the necessary neurones [fig. II.1.]. Interestingly,

VERY IMPORTANT!

Erratum: Fig. II.1.- II.21.! Lobes were mistakenly denoted in a counter clockwise direction (frontal view) contrasting with the terminology used by Technau and Heisenberg (1982). Accordingly the γ- lobe appears first.

Fig. II.1. Stage 9 embryo. Anti- β- galactosidase staining of line 238Y using HRP secondary antibodies. Brown spots representing domains of ectopic β- galactosidase expression (?): Precursors to the Bolwig's organ and the corpora pedunculata (40× air lens).

Fig. II.2.: Dorsal view of a stage 15 embryo (line 238Y). The reporter protein β-galactosidase was detected by immuno peroxidase staining. Note staining in seven widely separated nuclei on both sides of the procephalic lobe. Significant increase of staining cells following germ band retraction in comparison to stage 9 embryos. The bilateral corpora pedunculata (CPs) have extended several axons into the depth of the procephalic lobe. More rostrally, a host like structure, which presumably represents the suboesophageal ganglion. More anteriorly the presumptive optic lobe is found(40× air lens).

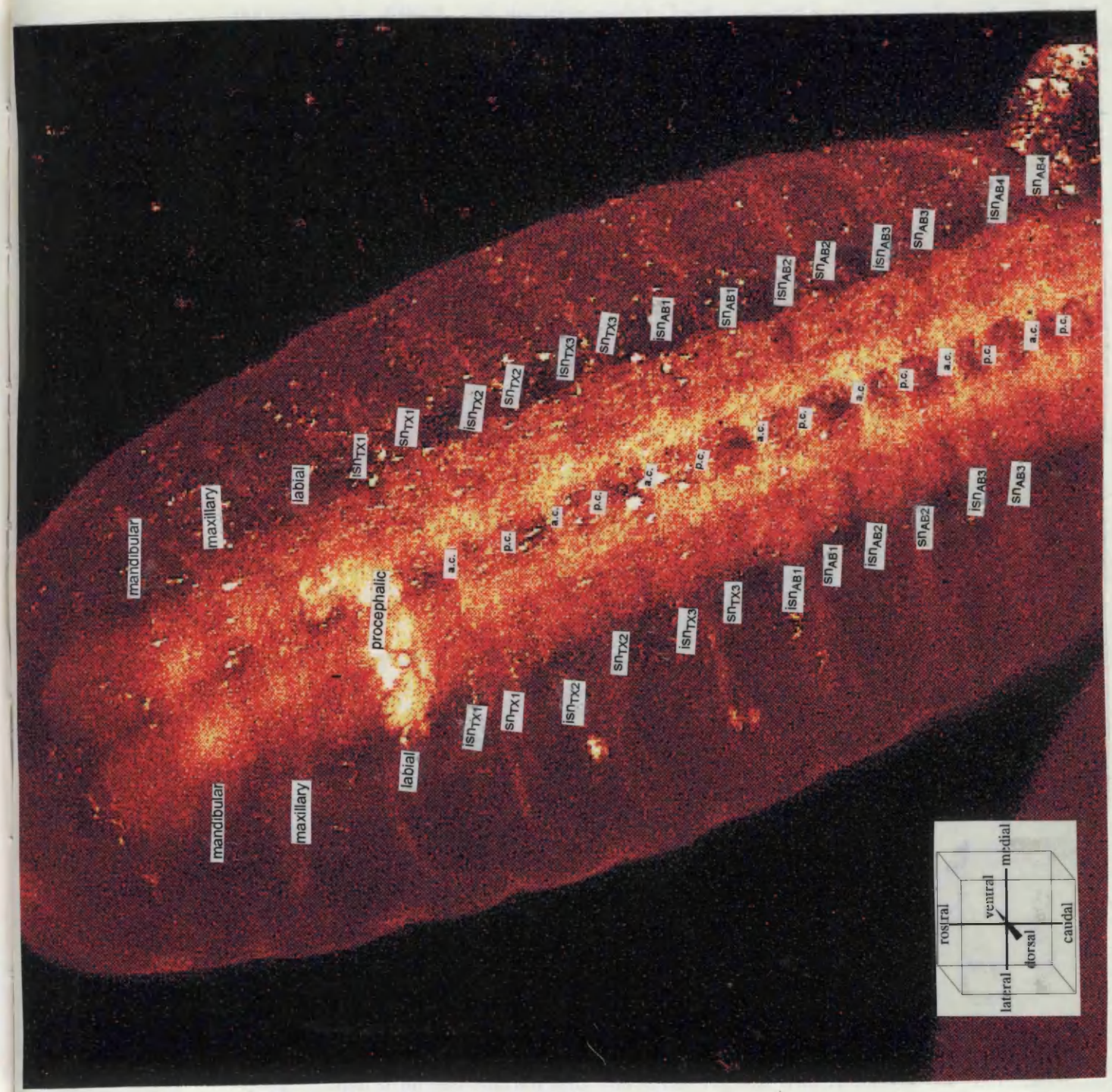
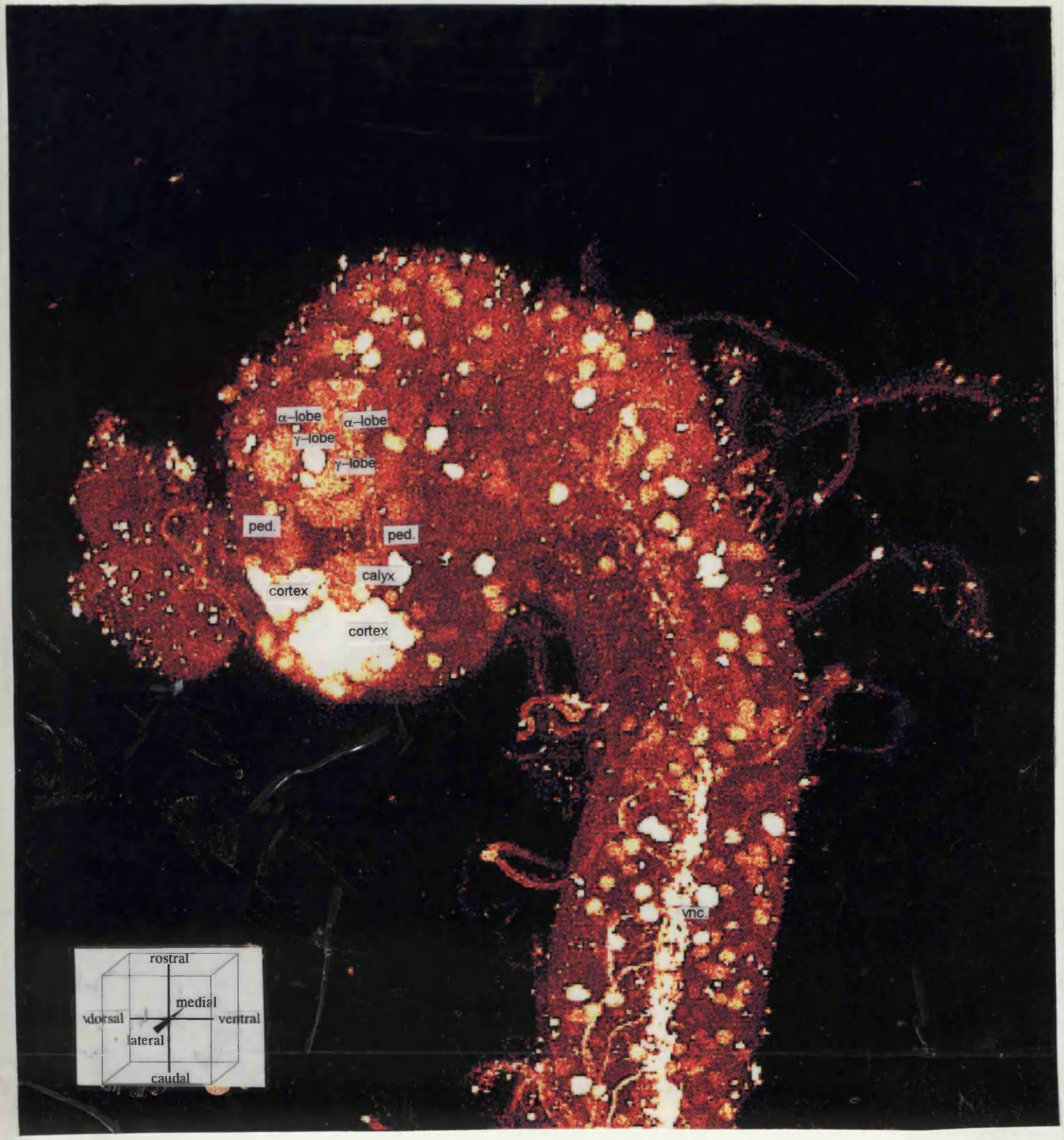


Fig. II.3. Stage 12 embryo. Anti- horse radish peroxidase antibodies recognising specific epitopes, which are expressed on the central nervous system. Ladder like staining pattern represents the organisation of the segmented thoracic and the abdominal ventral nervous system. Two commissures are present in each segment. The anterior commissure (ac.) and the posterior commissure (pc.). The commisures are connected by connectives (ct.), which run horizontally. Two types of fibres exit the central nervous system. The intrasegmental (SN) laves the segmental neuromere; the intrasegmental (ISN) nerve exits the CNS at the segmental boundary (Confocal laser scanning microscope image, 20× air lens, 25 pixel; 625 voxel).

these domains are more sophisticated in a stage 15 embryo. The clusters, which reflect the expression of the β- galactosidase seem to have migrated as a consequence of germ band extension and retraction to their final positions. There they have emanated their axonal processes. Whilst the most posterior β- galactosidase expression domains display some similarity to the imaginal corpora pedunculata, the more anterior region adopts the form of a horse shoe, within which a dark point is found. It might well be that these cells are present in the clusters that are found to stain later in the suboesophageal ganglion. The anterior most spots represent the clypeolabral structures such as the Bolwig's organ or the presumptive optic lobe anlagen. If one imagines the procephalon as a lobe, it is more likely that this structure is the Bolwig's organ, which in the larva functions as a light receptor organ (Fischbach and Technau, 1984).

The corpora pedunculata were first detected in the first instar larvae [fig.II.4.]. The Kenyon cell somata were embedded dorsocaudally in each protocerebral hemisphere. At this stage Technau and Heisenberg (1982) counted approximately 300 peduncle neurones in either hemisphere. The α- lobe is already present. Furthermore, both β and γ- lobes were clearly distinguishable [fig.II.4.]. As depicted is this figure, both α- lobes project rostrally, whilst β and γ- lobe turn rostromedially. In the second instar no changes in the gross anatomy of the corpora pedunculata are observed [fig.II.5., 6]. In some third instar females the somata are arranged in a triangular configuration [fig.II.7, 10.]. In other preparations they are more circular and densely packed [fig. II.9.]. The anterior part of the α- lobe is of spherical shape lying above a thinner base. The β- and γ lobes are well developed [fig.II.9.]. As observed in fig. II.8. — depicting the female third instar larval corpora pedunculata from a frontal perspective — the calyces adopt an ellipsoid configuration indicating that descending K- neurone fibres might receive synaptic input from unidentified afferents in this region. The volume of the peduncles seems to be reduced in this preparation contrasting in shape with the enlarged peduncles seen in fig. II.10. and II.9.. The β and γ - lobes could not be seen from this perspective. (The gap between both cerebral hemispheres was a result of a dissection error). In the male prepupal brain, the configuration of the corpora pedunculata remains largely the same. Noteworthy, however, is the division of the γ- lobe into γ_1 and γ_2- lobe, which



.Fig. II.4. Anterior first instar central nervous system (CNS). Lateral view. Staining cells represent the domain of β- galactosidase expression. Secondary FITC antibody stains reveal several groups of cells in the ventral nerve cord. The cortices emanate axons into the calyces and continue to run more rostro- ventrally. They form short stalks on top of which a spheroid lobe is prominent, which extend rostro- dorsally. Each ipsilateral γ- lobe meets medially. The β- lobe could, however, not be distinguished (Confocal laser scanning microscope image; 20× air lens, 625 voxel).

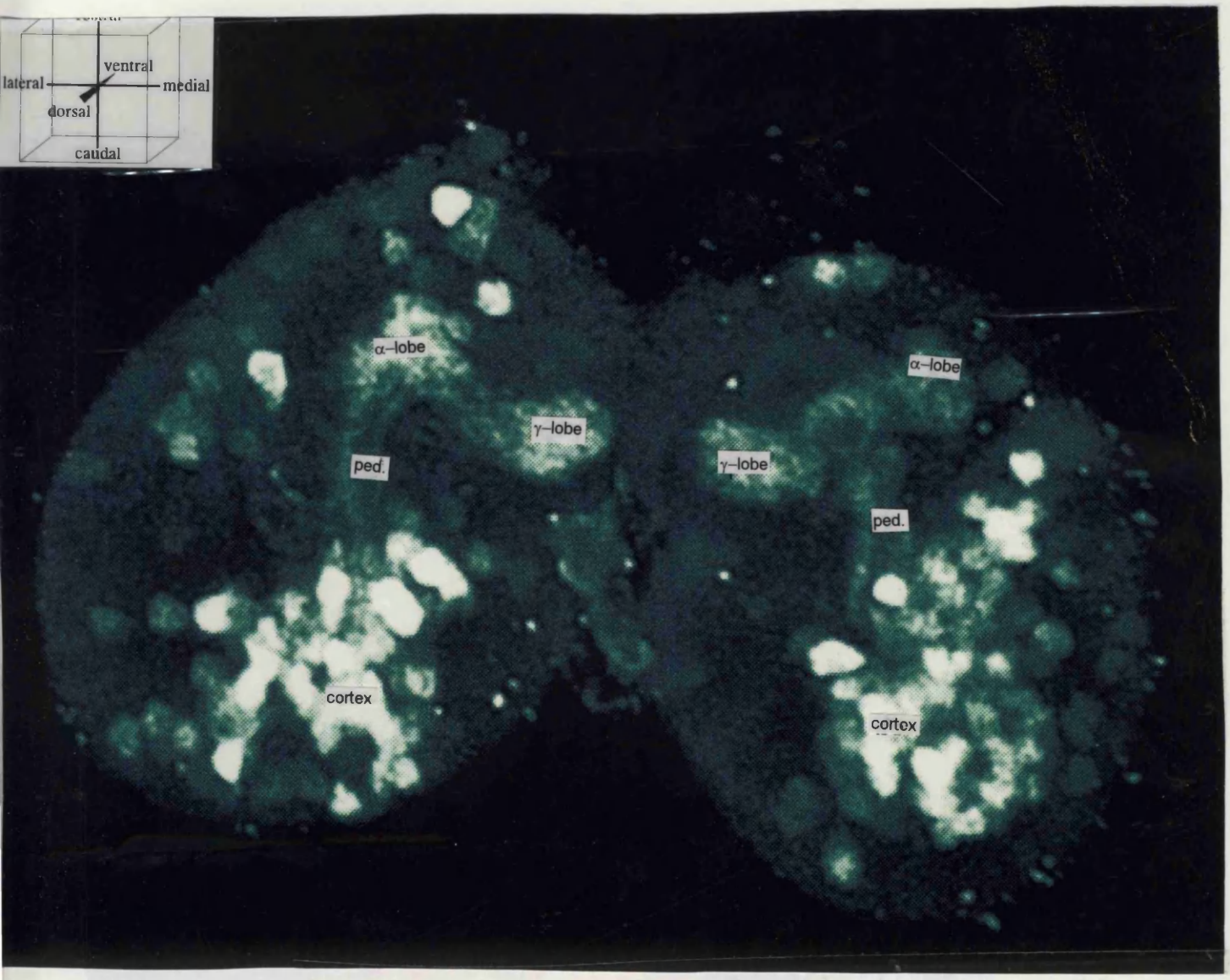


Fig. II.5. Anterior secondary instar central nervous system (CNS). Dorsal view. FITC anti β- galactosidase immunoreactivty of nerve cells. The cortices, which are very loosely arranged, emanate axons onto the calyces. These continue to run more rostro- ventrally where they branch of into the α- lobe and the γ- lobe. Gene al ogically spoken each ipsilateral γ- lobe seems to be present first. The β- lobe could, at least, not be distinguished (Confocal laser scanning microscope image; 20× air lens, 625 voxel).

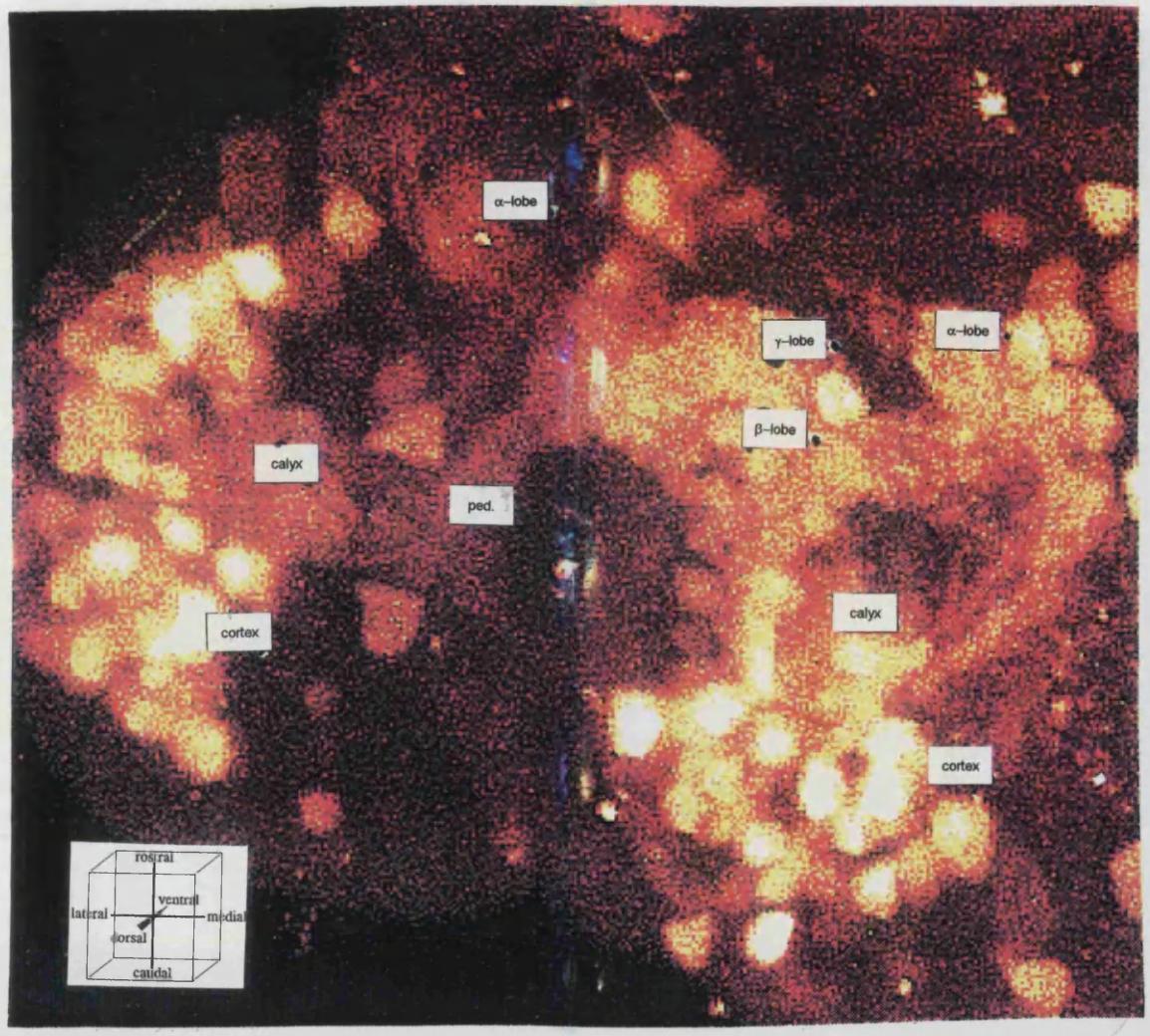


Fig. II.6. Second instar nervous system (CNS). Dorso- caudal view. Secondary FITC antibody immunoreactivity exhibits several groups of cells in the ventral nerve cord. As in the first instar larvae, the cortices emanate axons into the calyces and continue to run more rostro- ventrally. The α- lobe extends slowly. The characteristic spheroid lobe on top of the stalks is clearly visible. The β- lobe, has, however, not divided, yet, into β-/ γ- lobe (Confocal laser scanning microscope image; 20× air lens, 625 voxel).

Fig. II.7. Third instar central nervous system (CNS). Dorso- caudal view. Blue chromophore is the product of lacZ activity. The cortices emanate axons into the calyces and continue to run more rostro- ventrally. The from short stalks on top of which a spheroid lobe is prominent, which extend rostro- dorsally. Two lobes, the β- and γ- lobes run medially and meet their ipsilateral counterpart on the midline (20× air lens, tube mounting technique (Prokop and Technau, 1993))

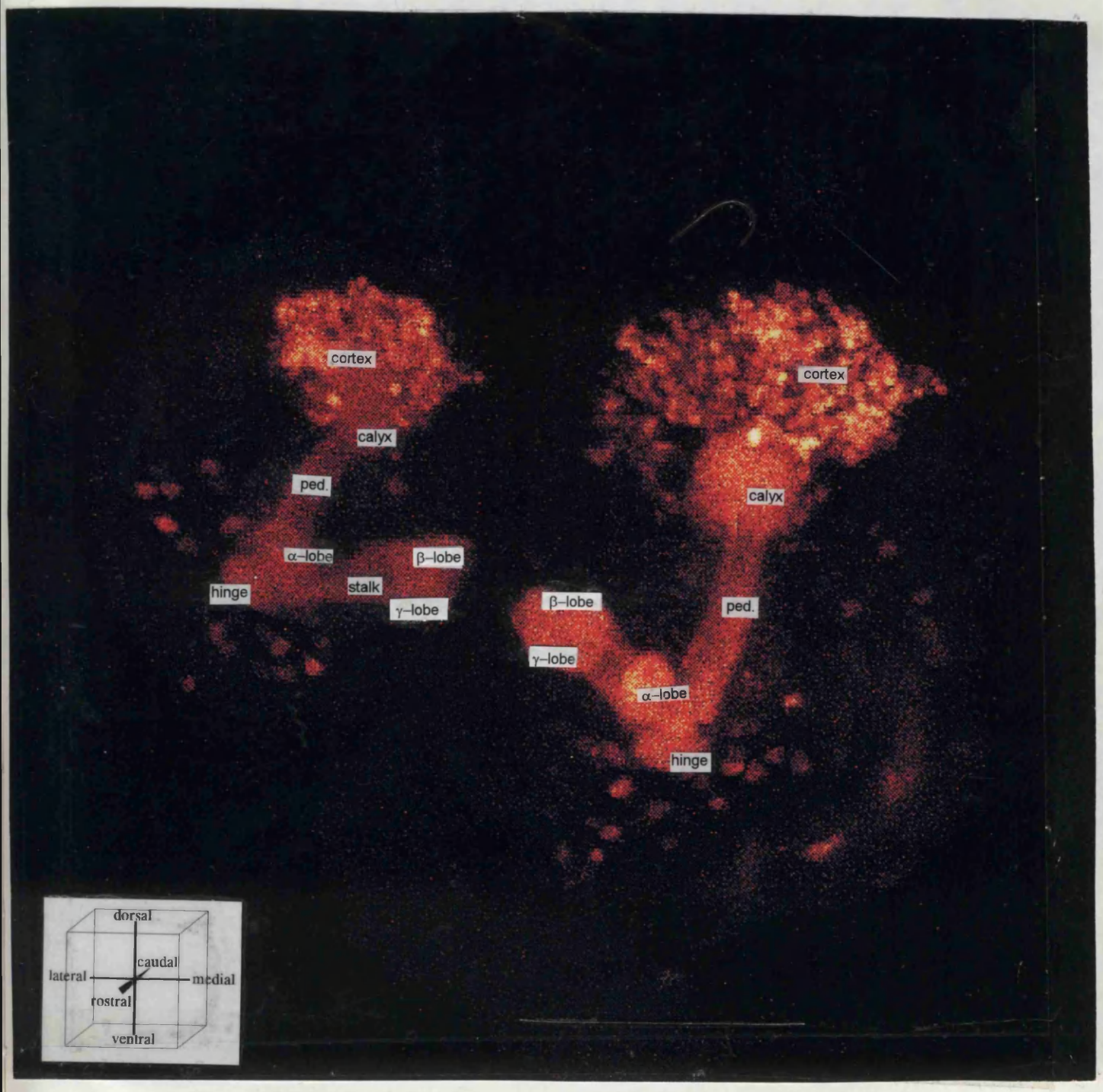


Fig. II.8. Female third instar central nervous system (CNS). Frontal view. Secondary FITC antibody immunoreactivity reveals several groups of cells in the ventral nerve cord. The cortices emanate axons into the calyces and continue to run more rostro- ventrally. The α- lobe extends short stalks rostro-dorsally (in the direction of the readers view) on top of which a spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially. (Confocal laser scanning microscope image; 20× air lens, 1,000 voxel)

Fig. II.9. Female third instar central nervous system (CNS). Dorsal view. Secondary FITC antibody Immunostaining against β- galactosidase. The cortices emanate axons into the calyces and continue to run more rostro- ventrally. The α-lobe extends short stalks rostro-dorsally (into the focal plane) on top of which the spheroid a- lobe is found. Each ipsilateral β-/ γ- lobe meets medially, though no direct contacts between each ipsilateral lobe are seen (Confocal laser scanning microscope image; 20× air lens, 1,000 voxel).

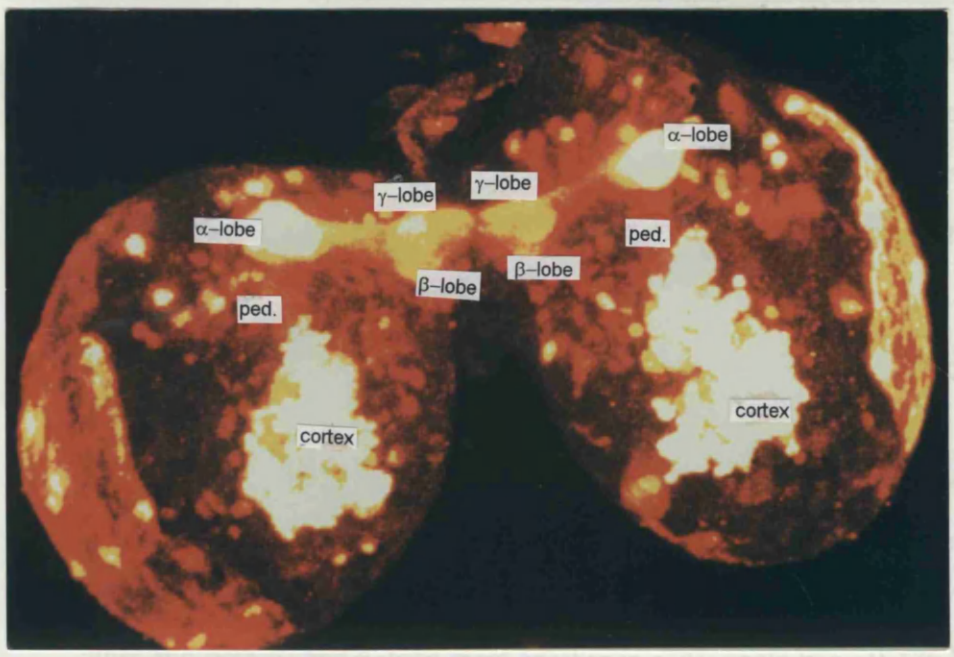


Figure II.10. Dorsal view of a female third instar female central nervous system.

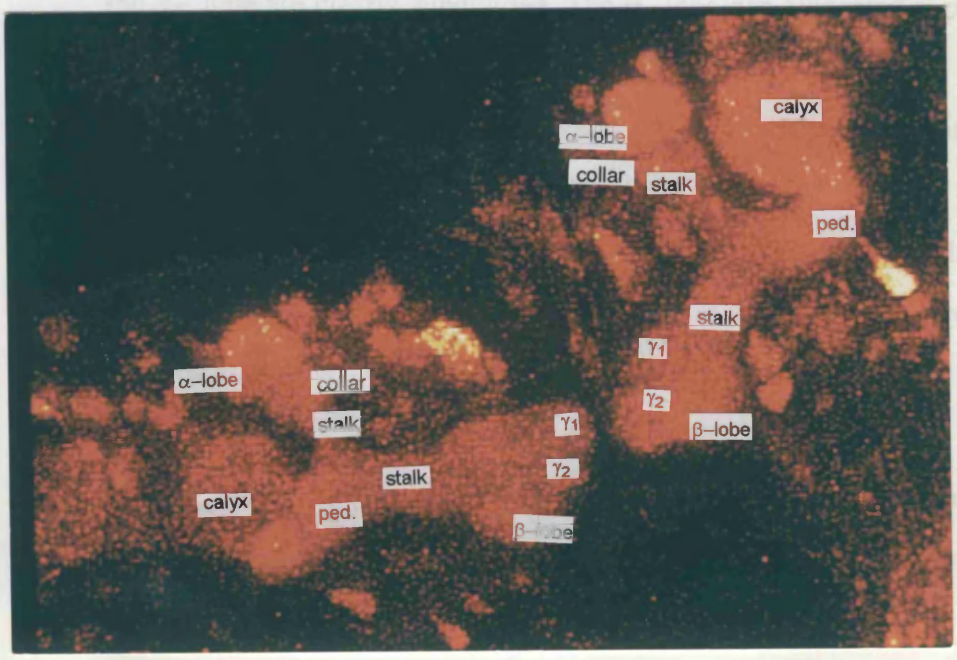


Figure II.11a. Section through the neuropil of the male prepupal corpora pedunculata (2- 4 hours after puparium formation; APF). The γ- lobe is subdivided into γ_1- and γ_2 -lobes. The α- lobe stalk is relatively short. The α- lobe is surrounded by a collar, which disappears with proceeding pupal development.

becomes first apparent during this period. This division persist during each following developmental stage. It remains, however, unclear how these changes reflect the integration of new efferents into the chemosensory memory networks [Fig. II. 11 a#1, a#2. and b.]. No significant changes can be observed during the first 4- 6 hours after puparium formation [Fig.12 a-e.]: The α-lobe is still reduced and the ipsilateral β- and γ- lobes of either cerebral hemisphere seem to meet each other medially, though no direct contact is made [fig. II.12e.]. Approximately 10 hours after the white pupae had been collected, the cerebral ganglion of the male pupa was found to be folded together [Fig. II.13 a.-c.]. This preparation as seen from a dorsal perspective reveals that during this period the ipsilateral peduncles of both hemispheres do not run parallel to each other and both β- and γ- lobe meet another in a 70° angle [fig. II.13b.]. There is still a collar around the sphere of the α-lobe [fig. II.13c.]. The same findings were obtained for a 6- 8 hour old female [II.14.]. In later stages the collar had disappeared and the base of the α lobe shows a considerable enlargement.

In the female corpora pedunculata (14± 2 hours after puparium formation) the γ_1- and γ_2- lobe are clearly subdivided. The α- lobe has elongated significantly, and the collar beneath the α- lobe sphere has almost disappeared [Fig. 15a. and b.]. A photograph depicting the entire female cerebral ganglion reveals that 21± 2 hours after puparium formation the optic lobe has already formed on the retina has fully unfolded (16a.). A more detailed view of the corpora pedunculata indicates that each ipsilateral β-/γ- lobe projects caudally. contrasting with pervious and later developmental stages, in which both lobular structures project medially. Obvious is also the kerb in the α-lobe indicating that the α- lobe is about to divide into α_1- and α_2- lobe. Further, the somata seem to be more densely packed and it looks as if they have multiplied.. Both β and γ -lobes display no changes, although the focal perspective does not allow any further statements to be made [Fig. II.16a. and b.]. In 18- 20 hours old female pupae the stalks of the α-lobes are considerably thinner, but have also extended considerably, though the subdivision into α_1- and α_2- lobe is not evident [fig. II.17.]. In the 22± 2 hour old male pupa, the subdivision into α_1- and α_2- lobe, becomes apparent first, and the stalks of the α - lobe had narrowed considerably. Additionally, the β- and γ- lobe

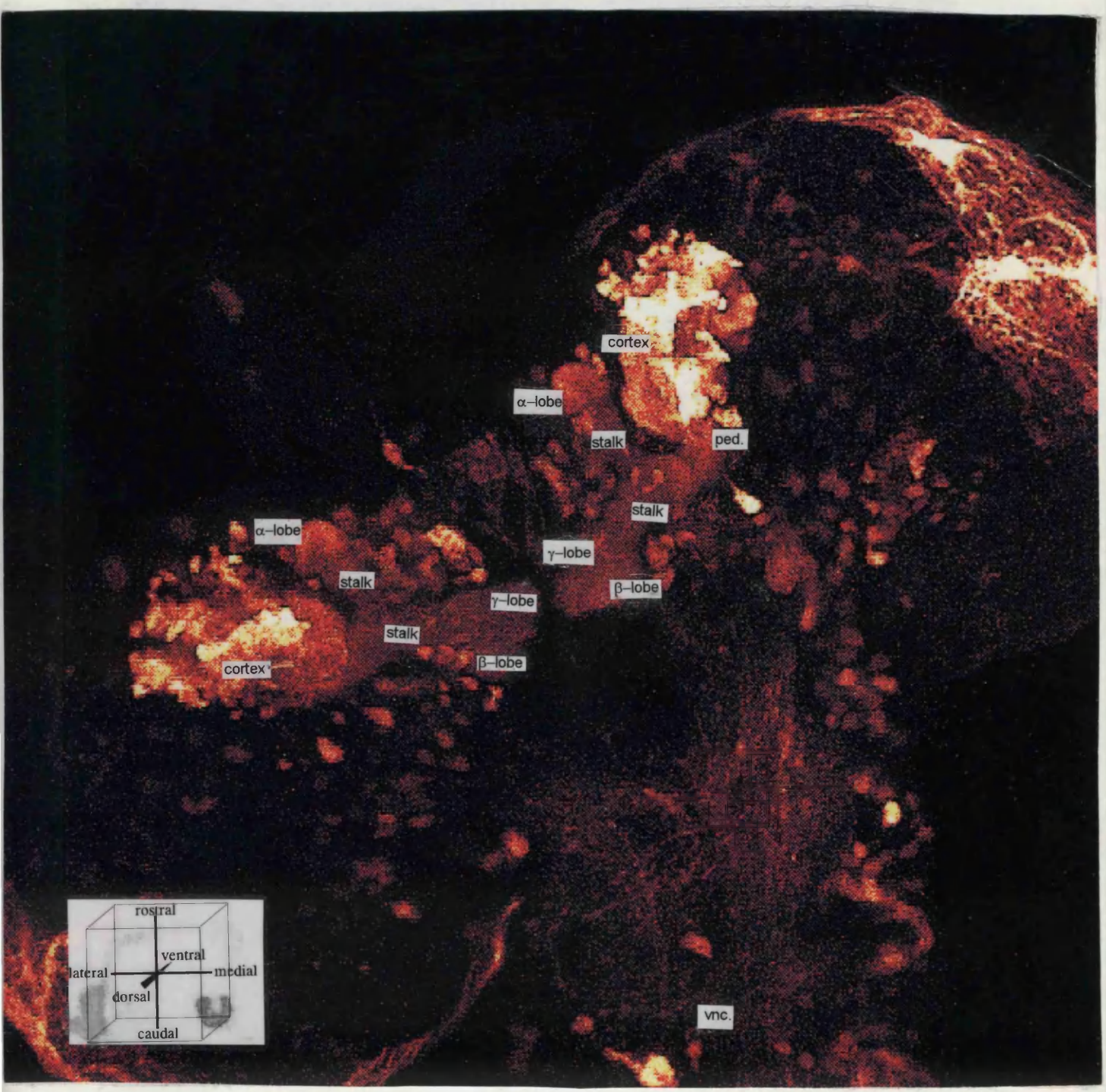


Fig. II.11b. Male prepupal central nervous system (CNS) (2- 4 hours after puparium formation. Dorsal view. Secondary FITC antibody immunoreactivity. Subsets of immunoreactive Kenyon cells are prominent extending their fibres into the neuropil area: The calyx, the peduncle and the lobes and their respective stalks: The α- lobe extends/ short stalks rostro-dorsally on top of which a spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially (Confocal laser scanning microscope image, 20× air lens; 10 µm; 1,000 voxel).

Fig. II.11a. Male prepupal central nervous system (CNS) (2- 4 hours after puparium formation. Dorsal view. Secondary FITC antibody immunoreactivity reveals two domains of β- immunoreactivity. (i) Subsets of cells in the ventral nerve cord and (ii) the corpora pedunculata. The α- lobe extends/ short stalks rostro-dorsally on top of which a spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially (20× air lens).

.

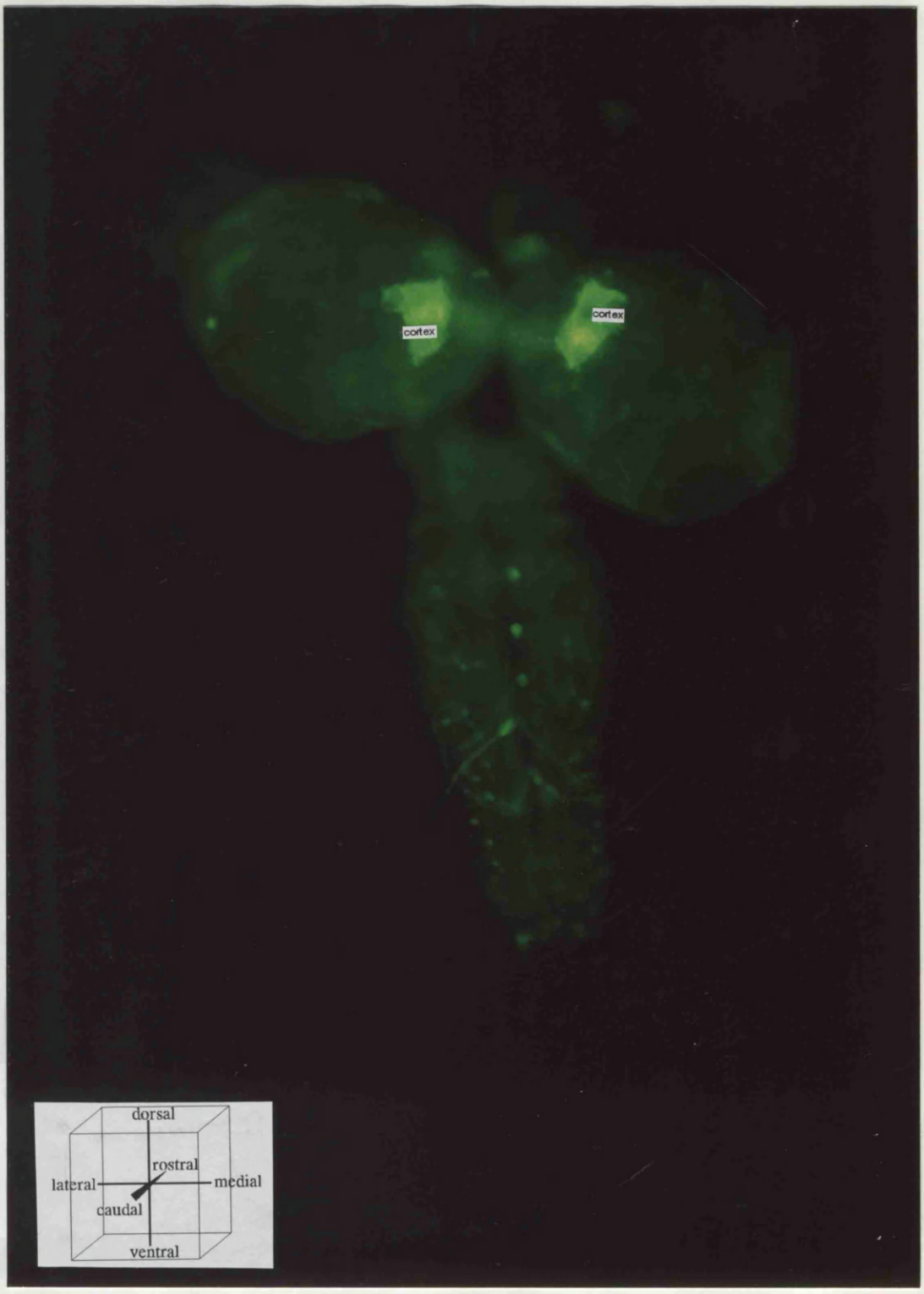


Fig. II.12a. Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. Dorsal view of the entire CNS, focus on the cortices. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata and the ventral nerve cord (20× air lens).

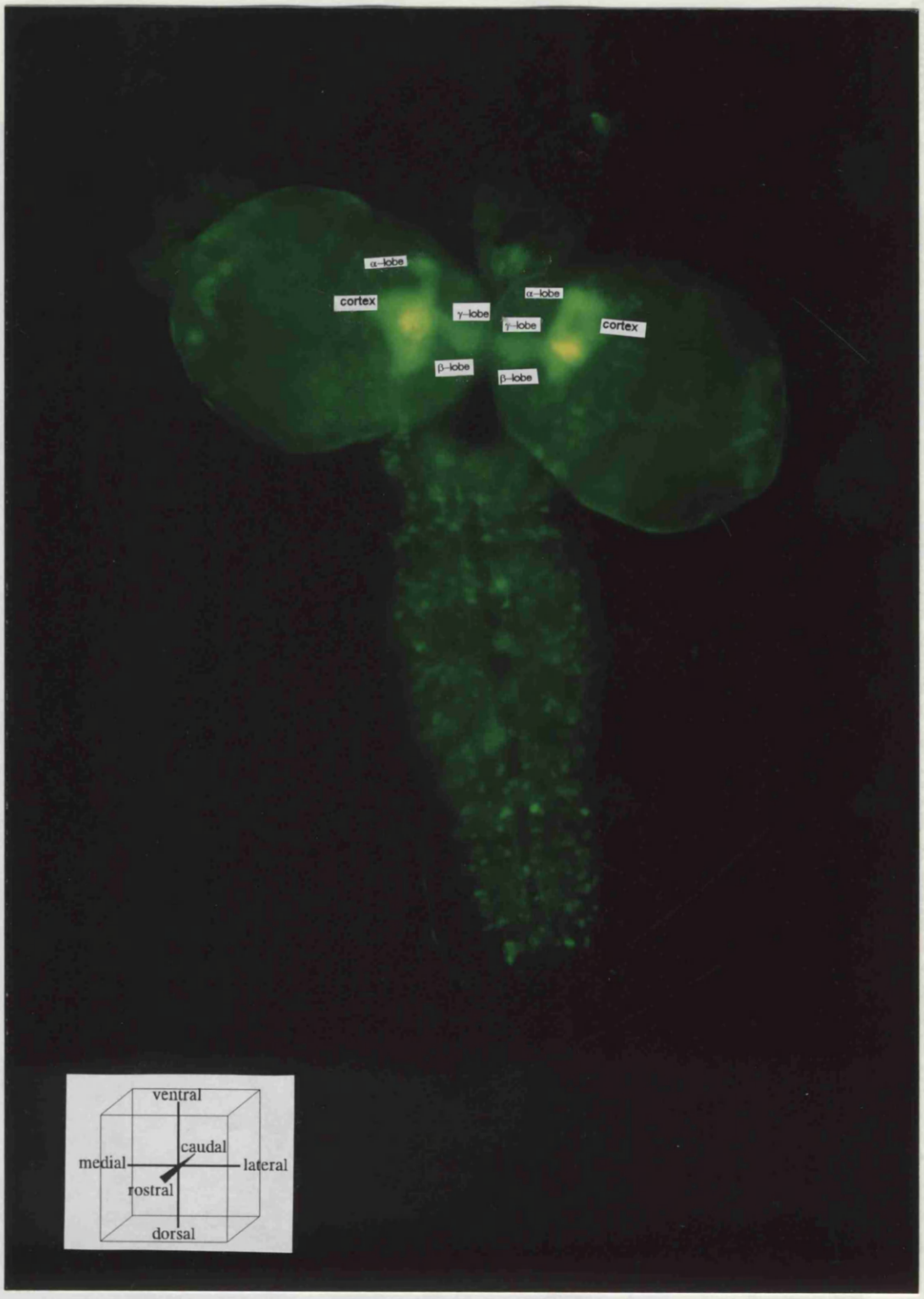


Fig. II.12b. Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. Dorsal view of the entire CNS, focal plane on the neuropil. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata and the ventral nerve cord (20× air lens).

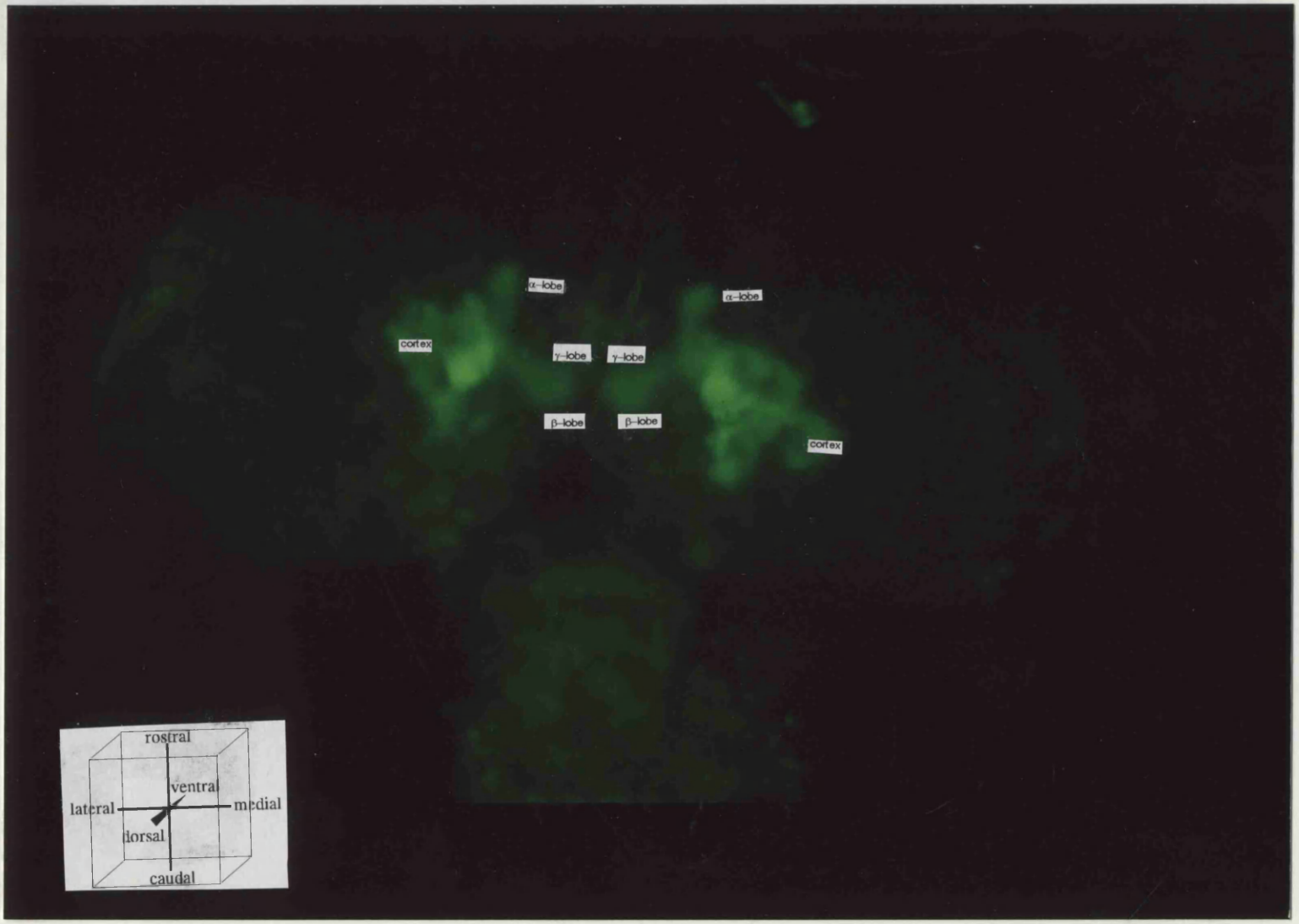


Fig. II.12c. Female early pupal central nervous system (CNS) (≈4- 6 hours after puparium formation. Frontal view. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata. Focus on the cortices. The cortices are diffuse. They form a triangular structure. Beneath the calyces (bright green) appear (40× air lens).

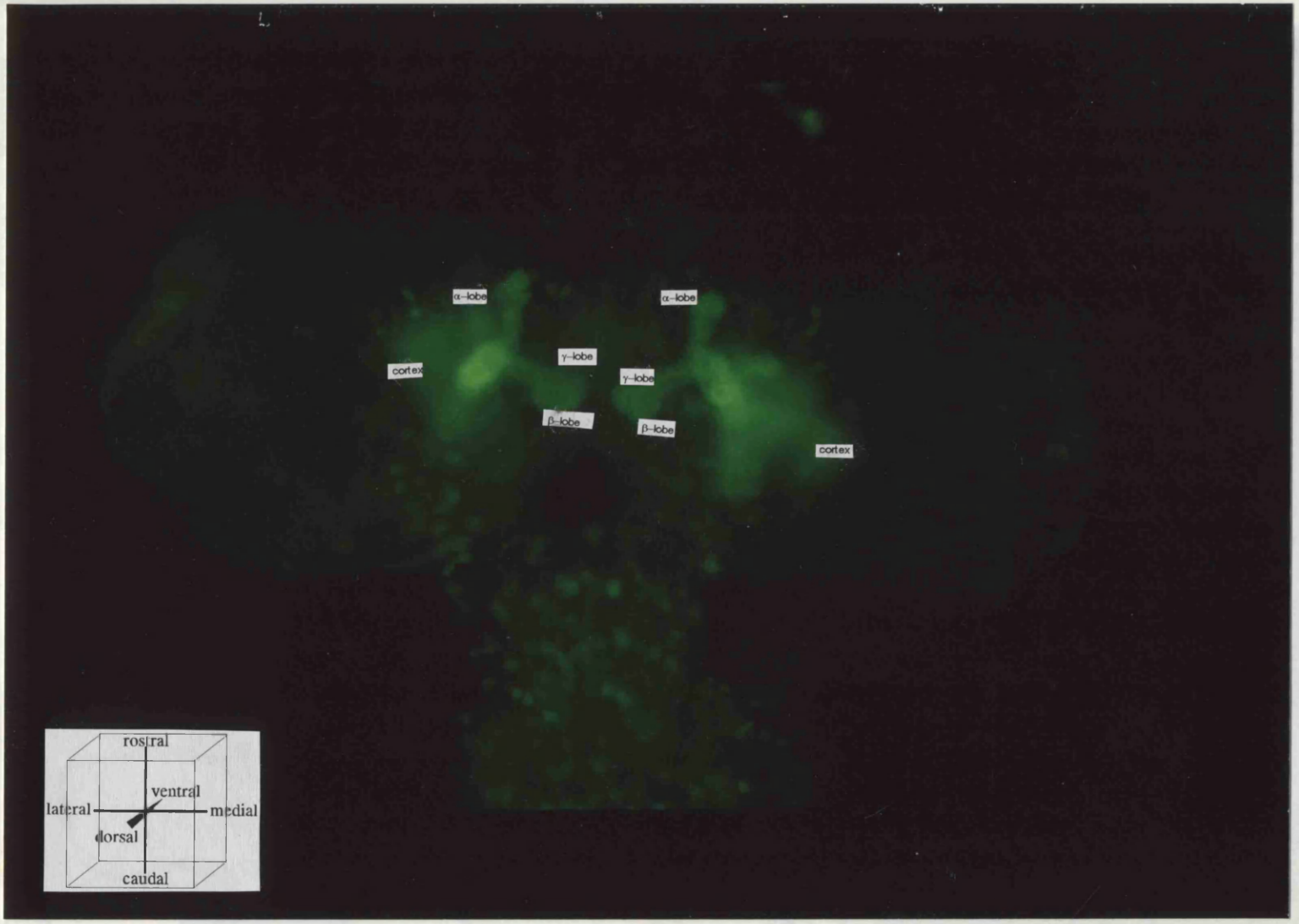


Fig. II.12d. Female early pupal central nervous system (CNS) (≈4- 6 hours after puparium formation. Frontal view. Focus on the neuropil. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata. Focus on the lobes (bright green) appear.(40× air lens).

Fig. II.12e. Female early pupal central nervous system (CNS) (≈4-6 hours after puparium formation. Frontal view. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata. The α- lobe extends short stalks rostro-dorsally on top of which a spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially (Confocal laser scanning microscope, 20× air lens; 1,000 voxel).

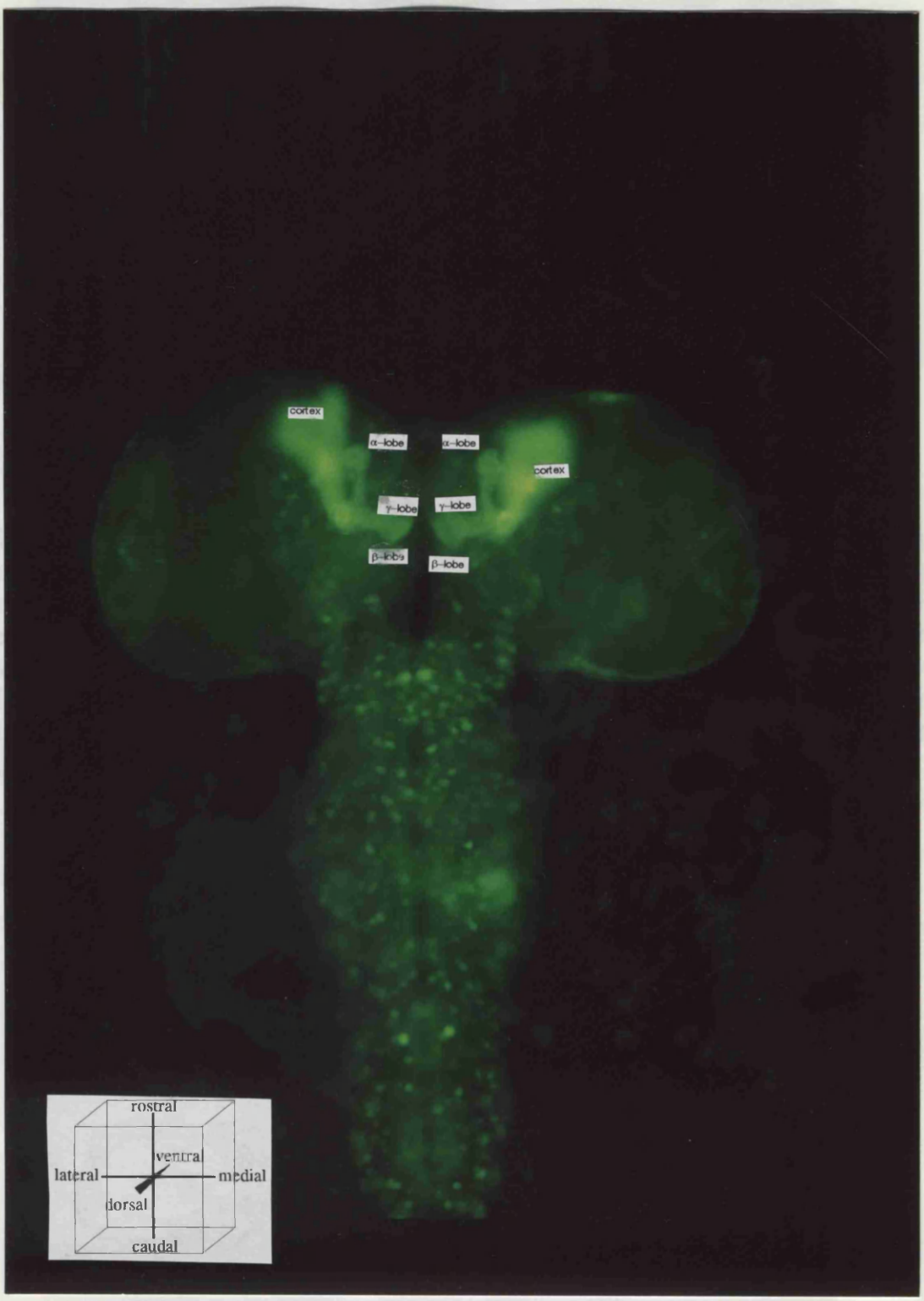


Fig. II.13a. Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. Dorsal view. Several subsets of the nerve cells in the ventral nerve cord as well as in the ventral nerve cord are staining. The nerve cells, which stain in the rostral cephalon are subsets of Kenyon cells. Note that the somata are in a different focal plane. The neuropil structures such as the α-, β-, and γ- lobe are well differentiated. Other somata are found in the suboesophageal ganglion (20× air lens).

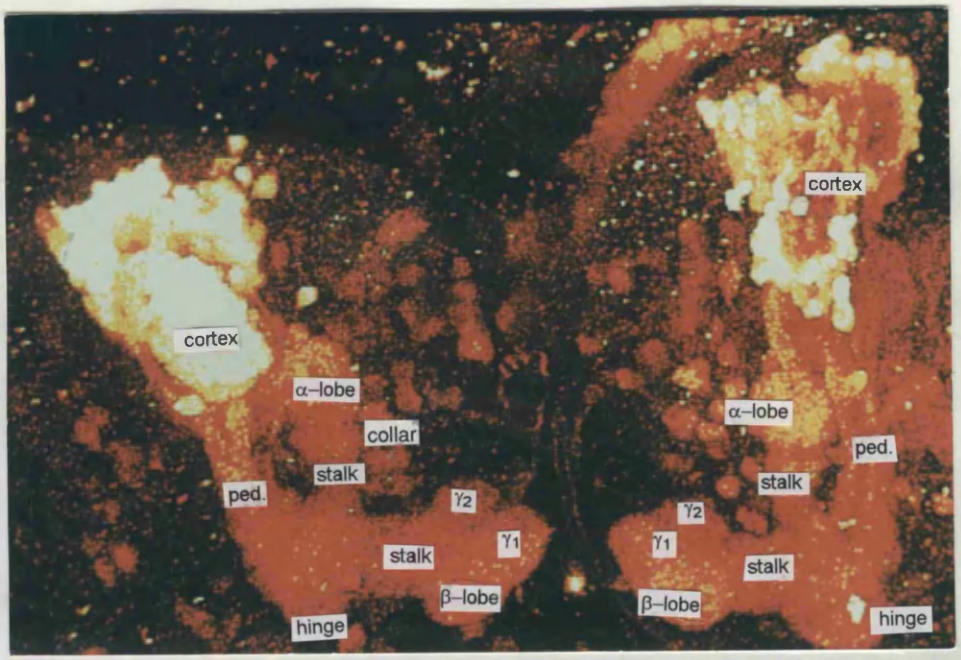


Fig. II.13b. Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. Dorsocaudal view. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata. Figure depicting the entire corpora pedunculata. The somata are tightly packed and extend their axon into the neuropil: The α- lobe extends short stalks rostro-dorsally on top of which a collar and the spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially. The γ- lobe is clearly subdivided into γ_1- and γ_2- lobe. Confocal laser scanning microscope, 20× air lens; 1,000 voxel).

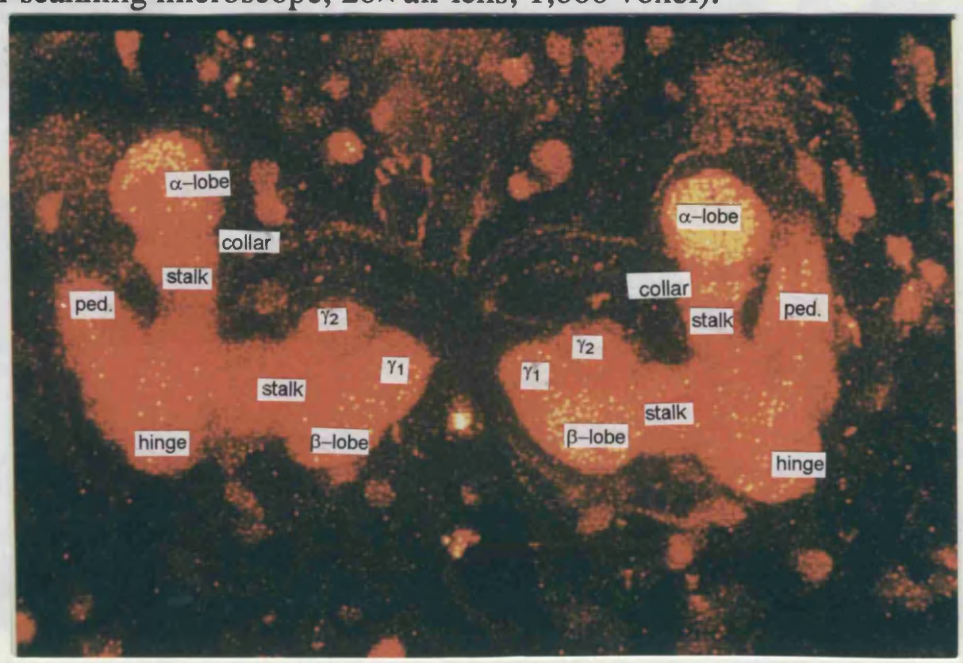


Fig. II.13c. Male early pupal central nervous system (CNS) (≈10 hours after puparium formation. Dorsocaudal view. Secondary FITC antibody immunoreactivity. Staining in subsets of cells in the corpora pedunculata. Horizontal section, without cortices and calyces. The α- lobe extends/ short stalks rostro-dorsally on top of which a spheroid lobe is prominent. Each ipsilateral β-/ γ- lobe meets medially. Severe background staining in trachea observed (Confocal laser scanning microscope, 20× air lens; 1,000 voxel).

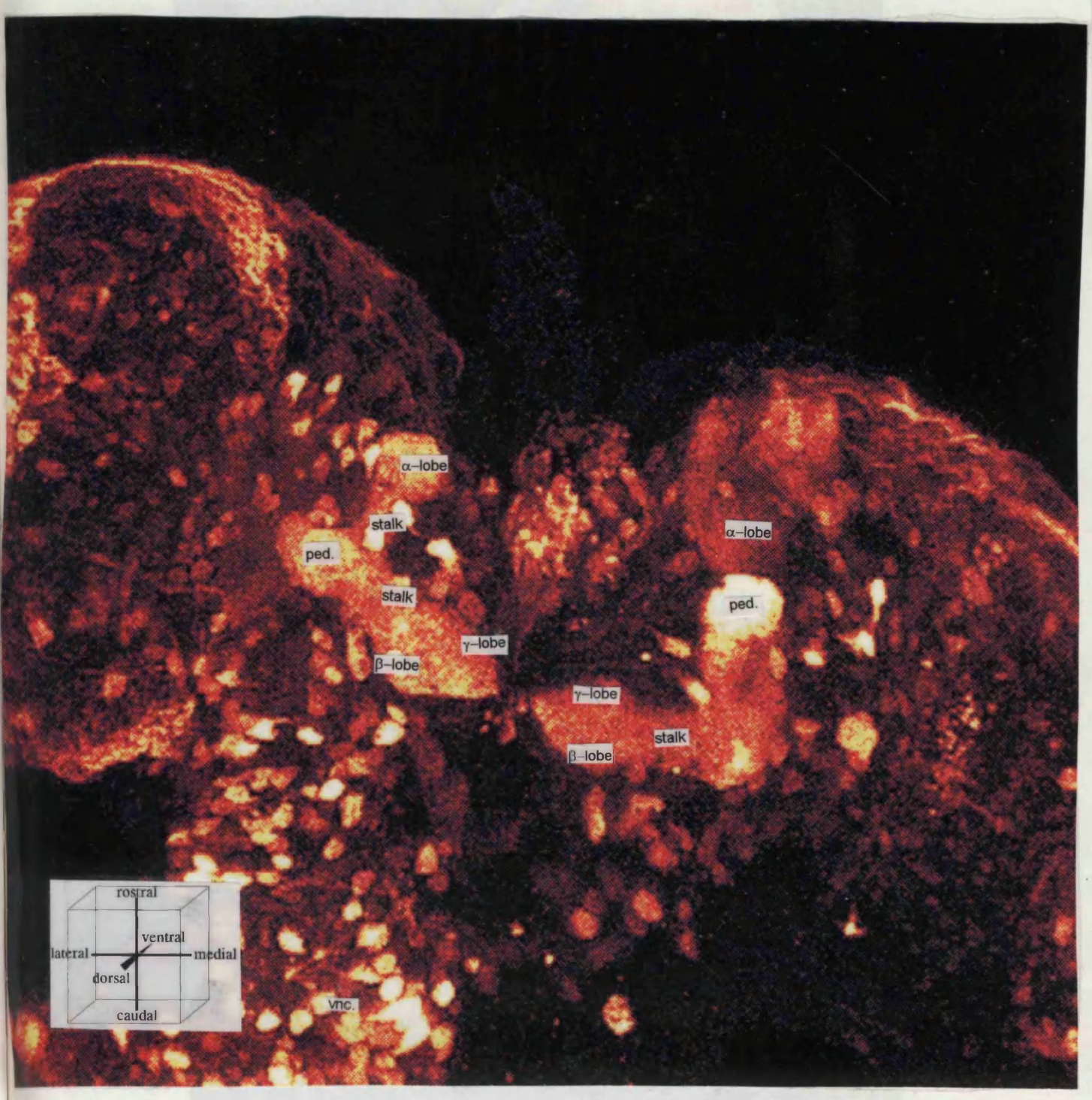


Fig. II.14. Female pupal central nervous system (CNS) (≈ 6-8 hours after puparium formation. Dorsal view. Horizontal section through the neuropil (Confocal laser scanning microscope image; 20× air lens; 1,000 Voxel).

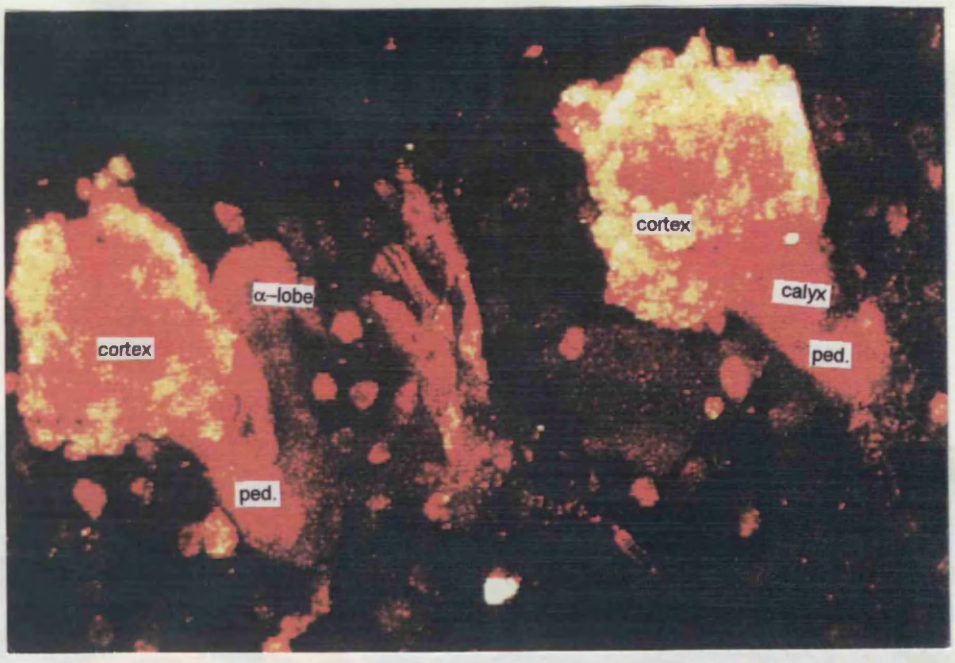


Figure II.15a. Dorsal view of the female pupal corpora pedunculata (ca. 12- 15 hours after puparium formation). The cortices are of integral round shape. Note that the most median Kenyon cells do not stain. The peduncles are reinforced.

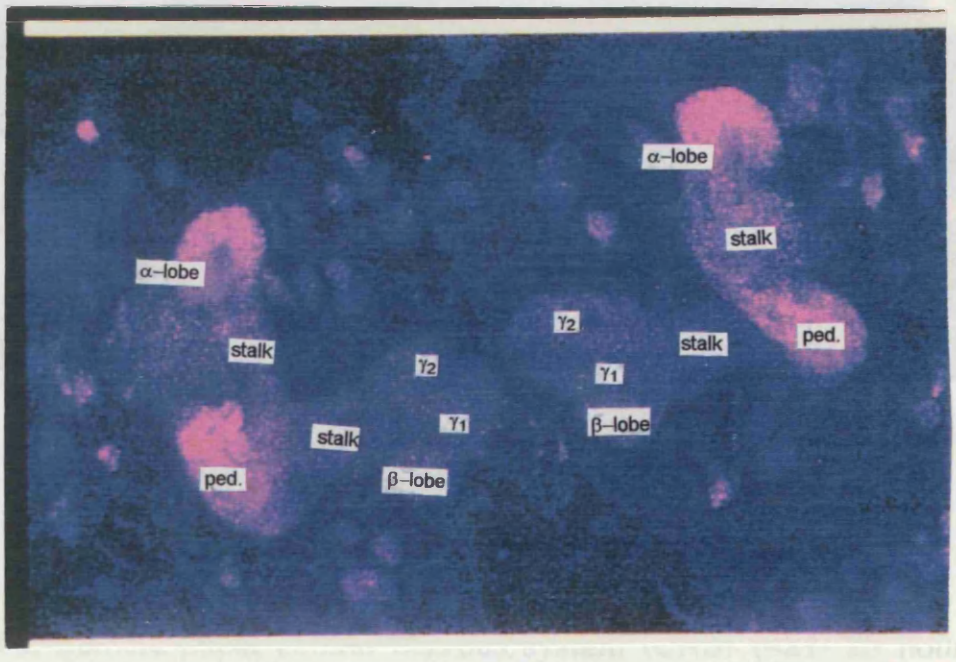


Figure II.15b. Section through the lobes of a pupal corpora pedunculata. The α-lobe has not extended. The collar around the α- lobe is only faintly visible, indicating that in the α- lobe stalks have extended (Confocal microscope image,

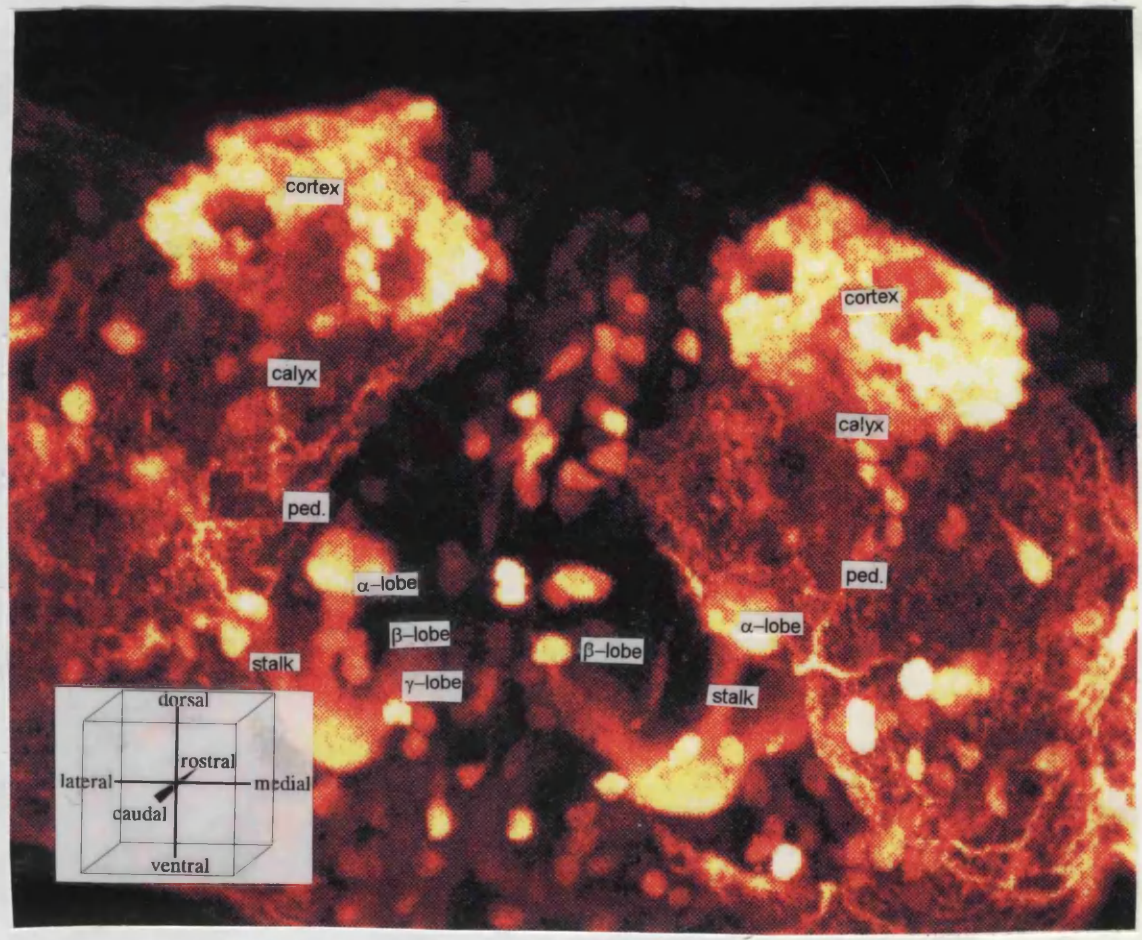


Fig. II.16a. Female pupal central nervous system (CNS) (≈21- 23 hours after puparium formation. Dorsal view. Secondary FITC antibody immunoreactivity. Staining in subsets of the Kenyon neurones of the corpora pedunculata. The a0-lobe has by now extended quite considerably. The kerb seen within the spheroid lobe is predictive of the subdivision into α_1 and α_2 - sublobe, which should be present after ca. 18 hours after puparium formation (Confocal laser scanning microscope image; 20× air lens; 1,000 Voxel).

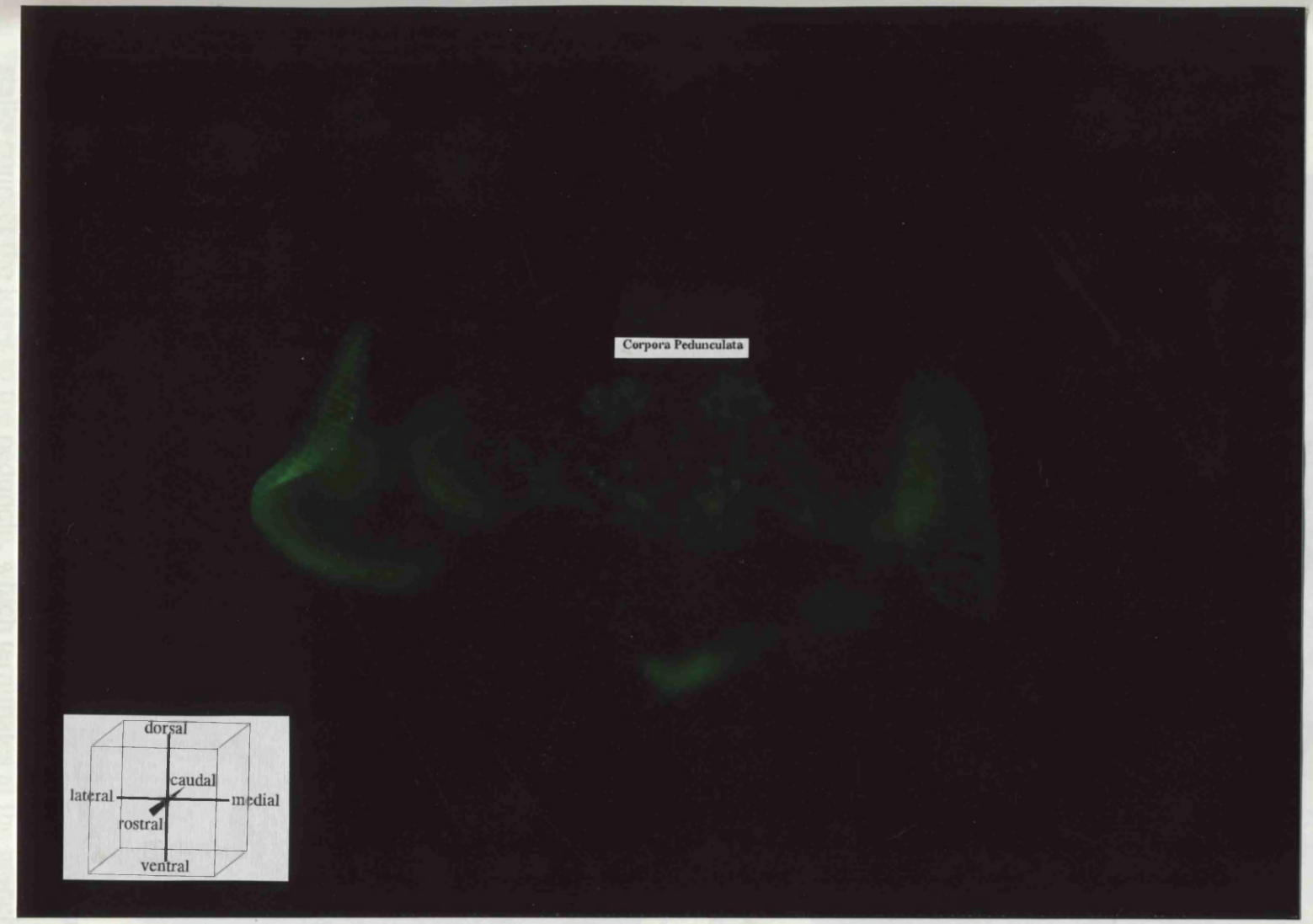


Fig. II.16b. Female pupal nervous system. 20× air lens, fluorescent image. Faintly visible are the elaborated optic lobes. The corpora pedunculata are folded in. The cortices are clearly recognisable (Compare to Kankel and Hall, 1976).

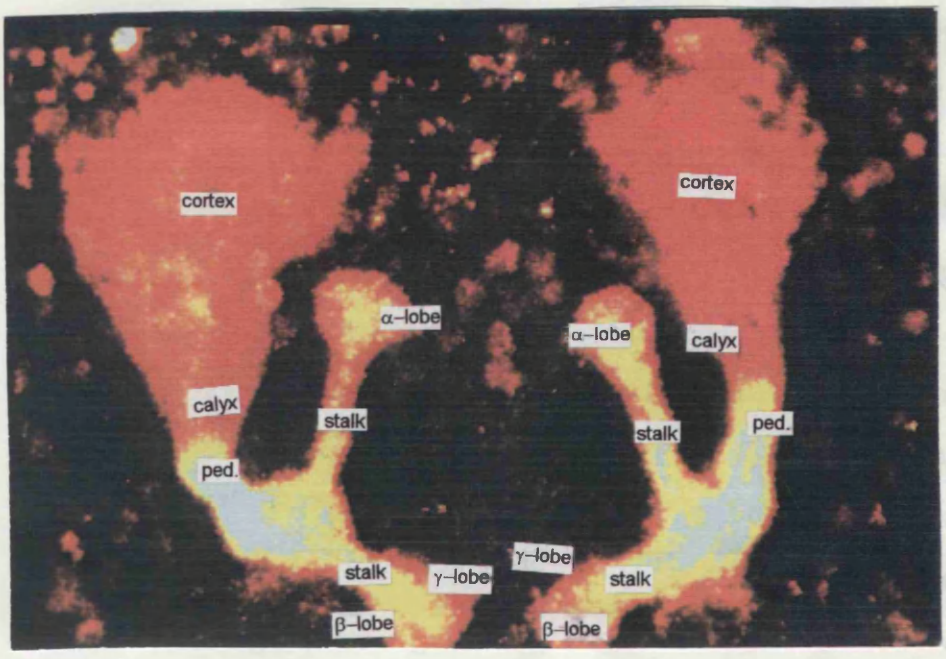


Figure II.17. Corpora pedunculata of a female pupae (18- 20 hours after puparium formation). The stalks have extended considerably. The cortex is folded in.

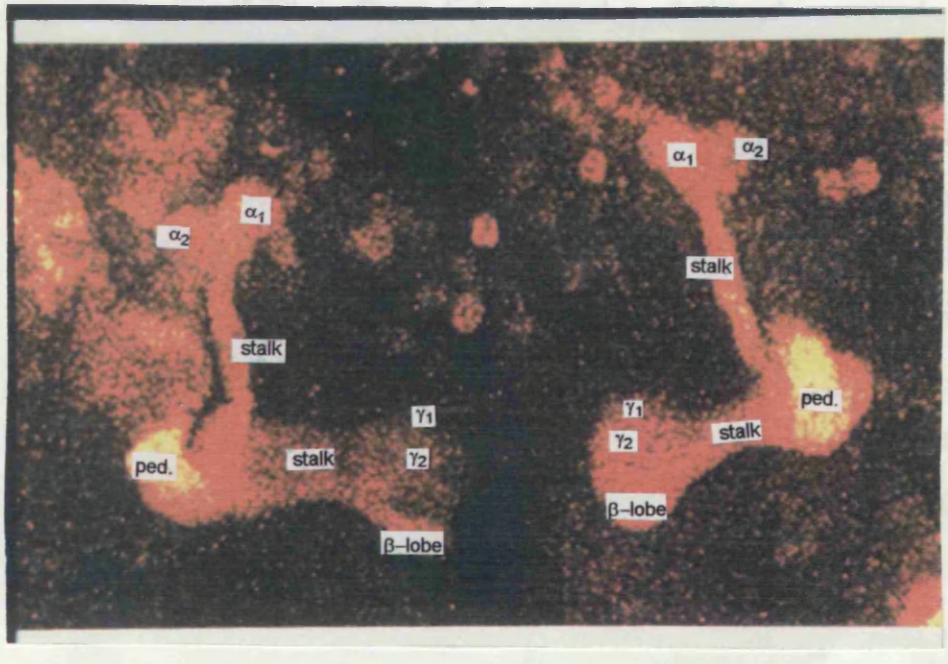


Figure II.18. Section through the lobes of the male pupal corpora pedunculata (22-25 hours after puparium formation). α- lobe is subdivided into α_1- and α_2 lobe. The stalks connect the lobes to the peduncle, which terminates at the branching point. The hinge region is not recognisable. The γ- lobe is subdivided into γ_1- and γ_2 -lobe.

seem to have become more elaborate, indicating that either additional neurones had reached them, or that the neurones within both lobes had undergone an rearrangement yielding a far more sophisticated structure [Fig. II.18.]. About 5± 2 hours later the anti β-galactosidase immunoreactivity in the α- lobes of the male pupal corpora pedunculata[10] had become stronger [fig. II.19.]. The stalks of the α- lobes had ramified forming two arbours. The spherical termini pointed either laterally or medially. It should be pointed out that the β- and γ- lobe were reinforced, in addition. Their stalks had thickened and the division of the γ- lobe into the γ_1- and γ_2- lobe is very apparent. Their stalks are at least three times the size of the peduncles, suggesting that either the peduncular axons must have become more loosely arranged, the stalks had flattened, or the stalks were criss-crossed by extrinsic neurones or glia in this region. This also might be interpretable as an increase in dendrites emanating from the axons in this region, resulting in an augmented volume [II.19.]. However, each neurone of the corpora pedunculata does not express β-galactosidase. This can be seen in the male 30 hour old pupa, where subsets of centrally arranged somata are excluded from staining [Fig.II.20b.]. The radial section through the calyces reveals that the central most region of the calyces does not display anti β-galactosidase immunoreactivity [Fig. II.19.]. Furthermore, a large extent of neurones in the cortices do not show up. This means that only subsets of corpora pedunculata neurones are immunoreactive [Fig.II. 20b.]. The corpora pedunculata in the newly hatched virgin female hardly display changes in anatomy. As seen here, the α-lobe might be split into three sublobes, the stalks of the lobe remain, however, unchanged [fig. II.21.].

In summary, during metamorphosis the corpora pedunculata undergo the major changes 15- 25 hours after puparium formation (Kankel and Hall, 1976).

[10]Note that this brain was initially wrongly labelled as male imaginal brain. However, the entire brain anatomy and the slide on which the brain was incubated reveal that this is a 20- 25 hour old pupa. Furthermore, the optic lobes had not yet formed a pigment (Hofbauer and Campos-Ortega, 1990).

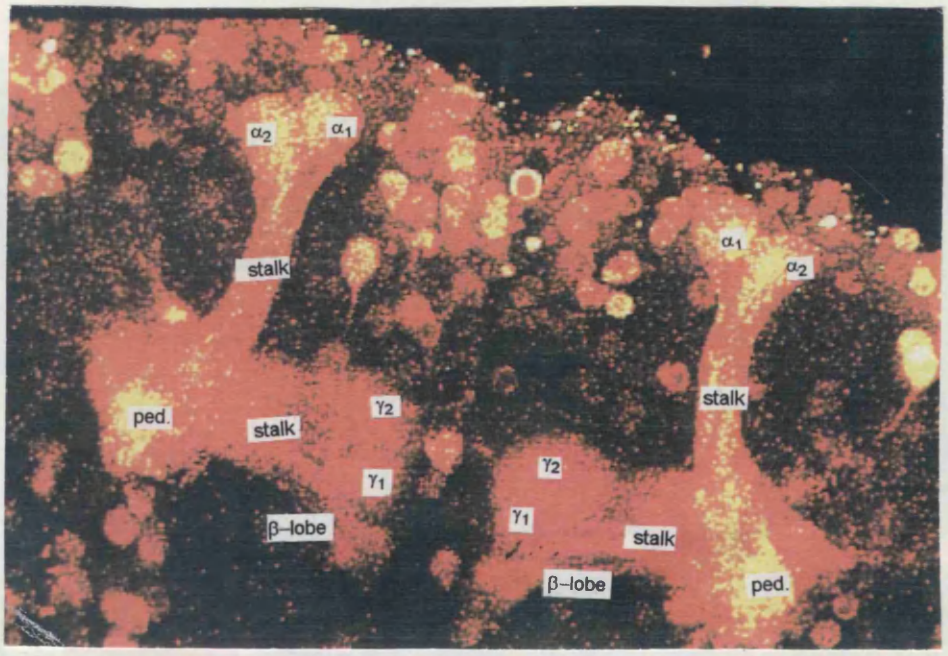


Figure II.19. Section through the corpora pedunculata neuropil of an male pupae (>25 hours after puparium formation). Clearly visible are the divisions of the β- and γ- lobe. The stalks of either the β-/ γ- lobe are reinforced.

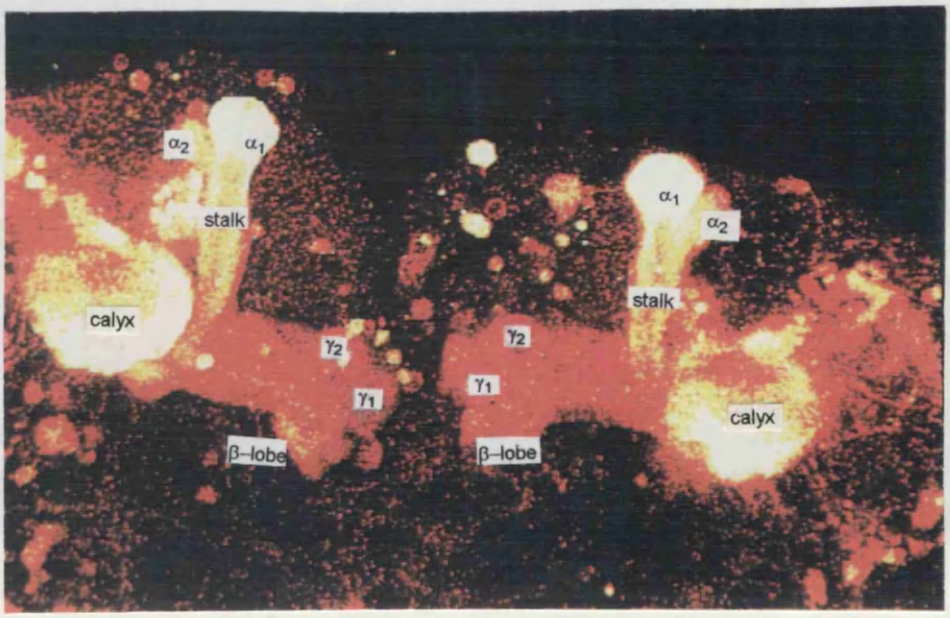


Figure II.20a. Configuration of the corpora pedunculata of a male pupa (30 hours after puparium formation). Clearly visible are the divisions of the β- and γ- lobe. The stalks of either the β-/γ- lobe are reinforced.

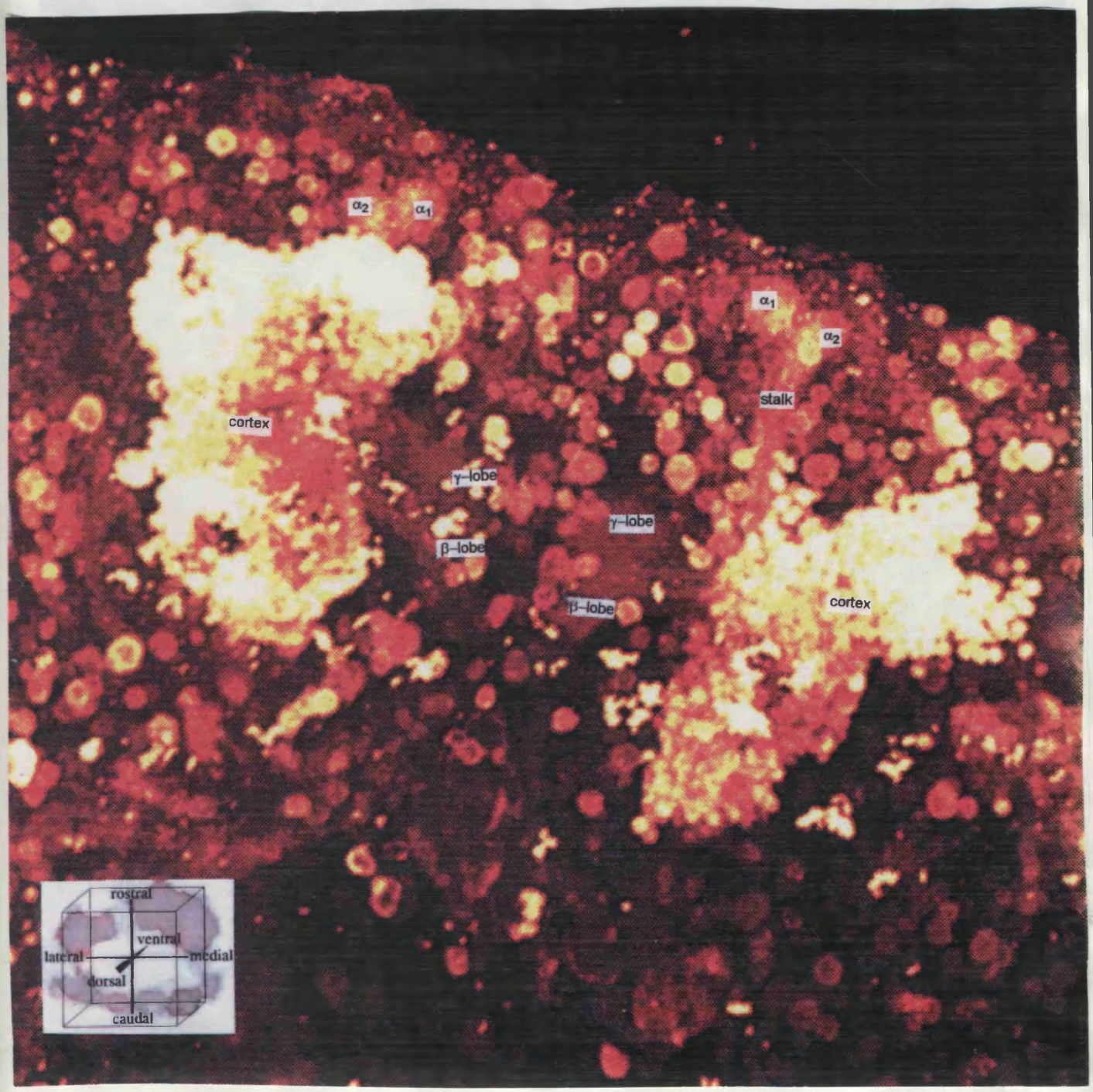


Fig. II.20b. Male pupal central nervous system (CNS) (≈25- 30 hours after puparium formation. Dorsal view. Secondary FITC antibody immunoreactivity. View on the cortices which form a triangular structure. Due to the high background staining the lobes are only faintly visible. The α- lobe, which runs rostro- dorsally is split into an α_1 and α_2 -lobe (Confocal laser scanning microscope image; 20× air lens; 1,000 Voxel).

Fig. II.21. Female imaginal central nervous system (CNS) (1 hour after eclosion). Dorsal view. Secondary FITC antibody immunoreactivity. Horizontal section through the calyces. The α- lobe, which runs rostro- dorsally splits into an α_1, α_2 α_3-lobe. The β- and γ- lobes are clearly distinguishable. The γ- lobe is divided into the γ_1- and the γ_2- lobe (Confocal laser scanning microscope image; 20× air lens; 1,000 Voxel).

Discussion 1

The *neomycin* (*neo*) resistance gene is an important selection marker in mammalian cell culture systems. I have subcloned the *neo* gene from a pBluescript® SK +/- vector into the GAL4 responsive pUAST *Drosophila* P- element vector. The gene was excised with *Bam*HI and *Hind*III giving rise to a 1.6 kb instead of the expected 1.2 kb fragment. An additional 400 bp fragment was found towards the 3' end of the *neo* open reading frame as established by the *BamH*I and *Sma*I digest leaving a 1 kb fragment and a 3.3 kb plasmid. It is, hence, assumed that either additional sequence derived from the bacterial Tn5 transposon is found toward the 3'end or that an additional sequence resulted from the thymidine kinase poly adenylation tract (Zhang et al., 1986). I was unable to elucidate this problem because of a lack in markers. The TK poly (A) tract contained the highly conserved sequence A(A/U)UAAA element which occurs upstream of the cleavage site of the mRNAs (Zarkower et al., 1986). The pre-mRNA is cleaved at this site by cleavage and polyadenylation specific factors (CPSF) (Murthy and Manley, 1992; Bienroth et al., 1991). CPSFs subsequently attract other cleavage and polyadenylation factors (Gilmartin and Nevins, 1991; Bienroth et al., 1993). The poly(A) tail is a homopolymer of nucleotide residues functioning to stabilise the mRNA and to regulate the transcriptional activity.

This polyadenylation site was followed by a *Hind*III site. The *Hind*III site served as a 3' insertion site for pBluescript® SK +/- 3. Downstream of it a *Kpn*I site occurred within the pBluescript polylinker enabling the excision of the 1000bp fragment with *Bam*HI and *Kpn*I and to insert it into the 5' *Bgl*II and the *Kpn*I site within the polylinker of pUAST. *Bgl*II and *Bam*HI ends were compatible so that the *Bam*HI 5' cohesive end fitted directly into the *Bgl*II 3' protruding end. The authenticity of the *neo* gene was established by probing the gene with a known *neo* gene from the pPNT vector. The hybridisations were carried out under high stringency conditions. High stringency conditions favoured a low association rate. Any mismatches in the DNA probe reduced the formation of hybrids between the sequence and its probe, due to thermodynamic

instability. Owing to the high number of putative hydrogen bonds, which might have formed between the pPNT *neo* probe and the *neo* fragment of pBluescript® SK +/-, the hybridisations and washes were carried out at high temperatures (68°C) (MacGinnis et al., 1984). Each wash was performed with successively reduced salt concentrations and increased temperatures. Hence, the hybridisation specificity should have increased under these conditions. The highly specific binding of the probe indicated that there was a high degree of sequence homology between probe and 1600 bp fragment giving rise to the assumption that this fragment contained the correct *neo* gene.

The 1600 bp fragment was excised with *Bam*HI and *Kpn*I to obtain compatible ends for insertion into the 5' *Bgl*II and the 3' *Kpn*I ends of pUAST. Both *Bam*HI and *Bgl*II protruding ends are compatible, allowing perfect ligation of the *neo* fragment.

The ligation efficiency was high: of the four clones that were isolated three contained the *neo* insert, whereas the fourth was devoid of the insert. Thus, the ligation efficiency of 4:1 insert to vector ratio favoured the formation of the correct recombinant vectors. According to my calculations concatemers should not have formed, though a complete restriction digest could not reveal if this was the case (Dugaiczyk et al., 1975). The transformation ratio was calculated as follows: according to these calculations a 1 kb fragment of double stranded DNA corresponded to 6.6×10^5 Daltons. The 8.4 kb pUAST corresponded, hence, to 56.76×10^5 kDa. The insert had a molecular weight of approximately 11×10^5 kDa. Thus, a molar ratio of 4:1 proved to be the correct choice. 75% of the colonies contained the insert. This may not necessarily have been the case for the other plates, however, colonies from these plates were not assayed further. The sequence of the vector was 91.38% identical to that of the *neo* gene as established by Beck (1982). However, several alterations of this sequence were established that primarily resulted from the site selected mutagenesis. For example, it has been established, that at position 110 of the sequence a double deletion (TC) occurs. Moreover, at position 128 an other deletion can be seen. This is followed by an T to G transversion at position 131, whereas at position 132 an G to C transversion is prominent. Last at position 154 a C to G transversion can be detected. It can be assumed that these alterations were induced by Dr. J. Dorin to enhance the translational

efficiency from the second AUG start site. She inserted a synthetic oligopeptide containing this transversion into the nearby restriction sites.

Thus, as gathered from the sequence, the pUAST *neo* vector contained the correct gene. When transfected into the *Drosophila* genome and mobilised to a appropriate location, the optimised 5x 17 bp consensus element (UAS) should allow the binding of GAL4. Upon binding of the GAL4 protein, an 881 amino acid activator protein, the transcription of the adjacent *neo* gene should be initiated. Gal4 contains a helix-loop-helix motif allowing the binding as a dimer to UAS. This factor presumably attracts further transcription factors involved in initiating the transcription of RNA polymerase II in *Drosophila*. The *neo* gene is a potent selection marker. It intercepts the amminoglycoside, G418 in a concentration dependent manner. It has been shown in the past that transformed eukaryotic cell lines can be rendered resistant to G418. In tissue culture G418 induces cell death in cells that are not resistant to this aminoglycoside by reversibly interacting with the large ribosomal subunit (Evans and Kaufman, 1980; Thomas and Cepecchi, 1987). It would be attractive to co-inject the pUAST *neo* resistance marker into *Drosophila* eggs together with *bcl-2* or the baculovirus p35 gene, two highly conserved genes which are widely believed to interact with molecules which induce apoptosis in *Drosophila* (Gagliardini et al., 1994; Hay et al., 1994; Merry et al., 1994).

Discussion 2

1. Overview

The corpora pedunculata represent the main secondary neural processing units for chemosensory stimuli in insects. Input fibres, which arise in the antennal lobe innervate the calyces of the corpora pedunculata bilaterally. In *Hymnopteraen* insect species additional fascicles derived from the optic lobe enter the calyces. Information is presumably processed in this relay structure, however, its detailed function remains obscure. Feedback neurones, which connect the α- and β-lobes, respectively, with the calyces are found in most insect species. In the honey bee *Apis mellifera*, efferents have been identified that extend across the midline to innervate the corpus pedunculatum of the contralateral cerebral hemisphere. (Rybak and Menzel, 1993). In addition, some exit fibres have been observed in *Apis mellifera*, which might, although not completely stained, innervate the central complex (Mobbs, 1982).

The corpora pedunculata of *Drosophila melanogaster* remotely resemble the highly sophisticated corpora pedunculata of *Hymenoptera*. The calyces are severely reduced and not subdivided. The α- lobe forms a very thin stalk and the β- lobe is supplemented by an additional γ- lobe. I have taken a *Drosophila melanogaster* enhancer trap line, which expresses the molecular marker β- galactosidase in a subset of neurones under control of a specific enhancer. Staining with antibody against β- galactosidase was seen in a specific subset of corpora pedunculata neurones as well as other not specified neurones. In my study several K-cell somata of third instar larvae and pupal preparations in the median cortex did not display any staining. Technau and Heisenberg (1982) identified a bundle of very thin fibres within the central peduncle, which behaved differently during metamorphosis from the remainder. In comparison to other neurones no signs of degradation were observed during this process in these fibres, suggesting that those Kenyon cells which did not express β- galactosidase might have been identical to these subsets. Thus, it can be assumed that Kenyon neurones are

of at least two distinct genetic entities. Neurones of the periphery differed from neurones of the central region of the corpora pedunculata. These K- neurones run though the central region of each lobular structure. My aim was to follow the expression of β-galactosidase in these neurones starting with embryonic stage 9 where expression was first detected in cells of the precephalic region .

2. Enhancer Trap Mutagenesis

Enhancer traps are convenient markers to track down specific subsets of neurones in the central nervous system of *Drosophila melanogaster* (for reviews see Bellen et al., 1990; Datta et al., 1993). As Bier et al. (1989) pointed out there was a good correlation between the *lacZ* expression patterns and the expression of known genes. In four out of seven examples a rescued genomic fragment downstream of a P-element insert reflected the β- galactosidase activity of a nearby P- element. 48-49% of these lines expressed *lacZ* in the central nervous system. O'Kane and Gehring (1987) proposed that many P-element insertions were found near promoters in intergenic regions and, hence, may bring the P-*lacZ* fusion under control of an enhancer, which regulates the expression of one or more adjacent *Drosophila* genes. These enhancers have the ability to act at a distance of 5.0 kb or more on the P-element promoter and enable transcriptional read through from *Drosophila* sequences into the P-element giving rise to GAL4 expression. The GAL4 protein subsequently acts upon the UAS sequence of the *lacZ* element.

Hence, pUAST is not dependent upon an enhancer next to its insertion point rather than upon enhancer elements which control the transcription of GAL4 in the corpora pedunculata. Thus, as these enhancers might not necessarily be close to the p[Gal4;w^+], it may be that this P-element has come under the control of different types of enhancers that regulate genes that are predominantly expressed in the corpora pedunculata and other nervous tissues.

These enhancers may occur either upstream or downstream of the respective gene. Similarly, a P-element, which has inserted into the intergenic sequences close to an silent enhancer (non-transcribed regions within euchromatin or facultative/ constitutive

heterochromatin) might be controlled by a different enhancer. Further, there might be the possibility that the P-element transposase promoter is influenced by some but not all regulatory elements of a given gene. Several domains contain enhancers that might clustered in a single region. The genes which respond to these enhancers might be scattered across this region and must not necessarily be located close to the P- elements. In addition, a P- element can come under control of different enhancers. Thus, active P-elements may not necessarily mirror the activity of nearby genes. Rather, the influence of enhancers on P-elements should be seen separately form the influence of enhancers on its subordinate genes (Bier et al. 1989; Datta et al., 1993).

There are, however, several disadvantages to the use of P-elements. In particular, it has been demonstrated that P-element constructs may impair the reading frame of a known gene. Bellen and his colleagues (1989) identified an recessive lethal enhancer trap line which they isolated from female and male sterile mutations on the first and third chromosome. The lethal phenotype manifested itself, when the respective chromosome was balanced. In order to identify if the P-element construct p[lArB] was responsible for lethal mutations, they mobilised this element again to revert the lethality. Most of the insertions in this experiment often excised precisely and are lost in the germ line cell genome when crossed to jump starter males. From a cross of brothers and sisters Bellen and his colleges (1989) recovered viable ry /Sb^+ flies indicating that this insertion was responsible for the recessive lethal phenotype (provided no other P-elements interfered with the results). Since flies reverted successfully they concluded that 83% of their lethal mutations might be due to the p[lARB] insertion within an open reading frame. However, our lines were not balanced and even though an insertion within a genome may have occurred, this recessive phenotype may only be expressed if the lines are homozygous.

On the other hand, P-element regulatory sequences have also been implicated in the ectopic activation of genes when inserted into the promoter region of the respective gene and may therefore not reflect the true domain of genetic function (Ito and Hori, 1993).

Lastly, P-elements though ideally used to track down active tissue specific enhancers do not give any conclusions about tissue specific splicing activity, which is the major criterion to distinguish between several tissue types.

The line isolated in my screen (238Y) seems to express β- galactosidase in identical sets of neurones throughout development. Early expression in the embryonic cephalic lobe is mirrored by the expression of β- galactosidase in the first instar larval brain. It needs, however, to be pointed out that this correlation does not apply for the second anterior (labral) expression cluster of the procephalic lobe. It might be possible that this region corresponds to the presumptive optic lobe. As the expression of β- galactosidase in the optic lobe of other enhancer trap screens seemed to vary quite considerably, I was unable to ascribe the staining pattern of the anterior procephalic region to a specific structure (Datta et al., 1993).

3. Development of the Embryonic Precephalic Region with Respect to the Formation of the Corpora Pedunculata

During this project no experiments were undertaken that confirmed the neuronal nature of the staining cells. Neuronal markers such as horse radish peroxidase (Jan and Jan, 1982) and *embryonic lethal abnormal visual* (*elav*) antibodies (Bandziulis et al., 1989) were not used. It can, thus, only be assumed that β- galactosidase expressing cells were of neuronal nature. Further, no experimental evidence was obtained which might have indicated if the anti β- galactosidase immunoreactive cells were neuroblasts, ganglion mother cells, sibling neurones or single neurones.

Based on anti HRP antibody staining patterns in either hemisphere of stage 9 embryos, three clusters of about five single cells appeared (Campos- Ortega and Hartenstein, 1985). The clusters were arranged bilaterally in either side of the midline of the embryonic procephalic region. Later, during stage 11 a putative axon looped first medially and subsequently posteriorly as seen from a ventral perspective. Following germ band retraction (stage 16) 5 staining clusters were apparent in the procephalic region. The most posterior cluster extended first anteriorly and then turned orthogonally

to meet its contralateral counterpart at the midline. The posterior termini arborised laterally. More anteriorly two further small clusters were seen. Two blobs were traced to either site of the midline. A blob is partially surrounded by a fan shaped stripe. Towards the most anterior end two joist like blobs occurred. These presumably corresponded to the Bolwig's organ or the optic lobe (compare Jones and McGinnis, 1993; Schmucker et al., 1994). Further, staining which might have corresponded to the peripheral nervous system was observed in the clypeolabrum. The most posterior staining patterns of the procephalic region seemed to identify the corpora pedunculata. Hence, by stage 16 the procephalic region adopts its characteristic lobular structure. It fuses to the mesothoracic segment and 'flaps around' that the lobes protrude dorsocaudally to the ventral nervous system.

The axonal processes in the ventral nervous system are fully extended by this stage and have adopted their final position (for review, see Goodman and Doe, 1993). Studies on the neuromuscular junction in *Drosophila melanogaster* embryos revealed that functional synapses were formed 14.5 hours after egg laying, corresponding to stage 16. Thus, synaptic connections of input and output neurones may well have been established in the brain by the end of this stage (for review, see Keshishian et al., 1993). This process prepares the embryo for hatching after which it requires the presence of a functional central nervous system.

4. Correlation between Corpora Pedunculata Development and Neuroblast Proliferation Patterns

The hatching first instar larva displays a fully developed pair of corpora pedunculata which is confined to either brain hemisphere. The K-cell cortex is loosely arranged and the calyces had adopted their characteristic imaginal shape. The α- lobe had not extended to the proportions of the imaginal lobe yet, but was clearly visible in this larval instar. Ito and Hotta (1992) reported that the corpora pedunculata neurones were recruited from neuroblasts in either brain hemisphere. In the locust *Schistocerca gregaria* these neuroblasts are thought to derive from a population of small neuroblasts in the pars intercerebralis (Zacharias et al., 1993; Boyan, pers. comm.). The number of

neuroblasts present in the first instar larva was established as being at least five (Ito and Hotta, 1992). A quartet of neuroblasts generate neurones that form the corpora pedunculata[11]. A single neuroblasts supplied the antennal lobe with neurones. Schmitt-Ott and Technau (for publication) found that this neuroblast quartet was present as early as stage 17 embryos. It was traced rostrally to the K-cells cortex emanating ganglion mother cells (GMC) through a series of unequal cell divisions (Truman and Bate, 1988). This quartet persists until 8 hours of larval development have elapsed, whereafter the number of neuroblasts increases gradually. This gradual increase of neuroblasts is reflected by the reinforcement of K- cells of the cortices. During the third instar the number of peduncular neurites increases from 300 to 2,100 (Technau and Heisenberg, 1982). This increase is closely correlated to the postulated augmentation in the neuroblast number, dorsally of the K-cell cortex to 85. From cell cycle estimates Ito and Hotta (1992) calculated that the number of neurones generated by each neuroblast is 216± 24. Accordingly, the maximum number of neurones generated was estimated as 860 ± 100 in each hemisphere. The number of K-cells in my study was disregarded as the resolution of the air lens was too low. Comparing the volume of the cortices in second instar and late third instar larva, it can be noticed that these increased enormously. The third instar cortices were densely packed. The α- lobe stalk was not yet fully extended. Instead a sphere was observed above the α- lobe stalk. The β- and γ-lobe were more elaborated and were well separated. The more complex lobular structure present in imagoes was still not visible. In the brain of prepupae (2-4 hours after puparium formation) no particular changes in comparison to third instar larvae occurred (Bainbridge and Bownes, 1981). The number of proliferating neuroblasts in the posterior-lateral region above the calyces was described as being constant over the first 20 hours of pupal development. During this stage my findings revealed the corpora pedunculata were reinforced. The expression of β- galactosidase was obviously adopted in the neurones, which are added to the corpora pedunculata during larval development, indicating that the identities of all the β- galactosidase staining Kenyon axons in the

[11] Ito and Hotta (1992) estimated from the actual number of imaginal K-cell fibres that the numbers of neuroblasts ought to have been more than a quartet. Since no further studies have been undertaken in this direction, I refer in my thesis to these neuroblasts as a quartet.

larval, pupal and imaginal brain are the same. β- galactosidase staining in calyces, pedunculus α-, β- and γ- lobes appeared to thicken during the development. The set of 85 neuroblasts generate a proportional number of new neurones which subsequently become integrated into the corpora pedunculata. I did not consider it to be necessary to continue fixing and staining brains beyond the first 20 hours in 2 hour intervals. I only choose a 30 hour old and a 50 hour old pupa to confirm that beyond the first 20 hours no significant changes have occurred (2,100 to 2,500 estimated Kenyon neurones (Balling et al., 1987)). The staining patterns of the imaginal brain shortly after eclosion were similar as in late pupae. By then the 85 neuroblasts in either brain hemisphere decrease to between 20 and 30 after puparium formation rapidly. Subsequently new neurones could only have been generated until 3 hours after the decline in neuroblast number taking into account that the cell cycle for neuroblasts was estimated not to exceed 1.5 hours. I have not found any data conclusively determining the cell division time for ganglion mother cells in the brain region, but this period should equal the temporal sequence of events observed for neuroblasts in the ventral neuroderm. The remaining quartet of neuroblasts should not influence the numbers of neurones present in either brain hemisphere substantially. Strikingly, counts for peduncular neurones in the brain revealed that the imaginal number did not increase substantially between third instar larval and imaginal brain.

5. Rearrangement of Corpora Pedunculata Intrinsic Neurones during Metamorphosis May Reflect the Integration of Extrinsic Neurones

The addition of strictly imaginal tissues such as antennae, ocelli, compound eyes, bristles, legs and wings may further require the innovation of neuronal circuits integrating these tissues. Technau and Heisenberg (1982) found that the number of axonal processes in the pedunculus decreased markedly (40%) in the first 12 hours of pupal development. These findings were not confirmed in my study. Using 2 hour intervals to dissect and fix female and male pupa separately over the first 20 hours I was not able to confirm these results comparing the gross anatomy of the corpora pedunculata. This

does not reflect potential changes that may occur at a microanatomical level. Dendrites and synapses may reform during this process. During this stage strictly larval tissue are hydrolysed and new organs, such as reinforced antennal lobes, labial segments and some *Manduca sexta* ventral nervous commissures mature from pre-existing larval precursor tissue (larval discs). Of particular interest are the output neurones which penetrate the α-, β- and γ- lobe. In *Apis* there are feedback neurones and fibres which run from the α- and β- lobe across the midline into the α- lobe of the other hemisphere. I observed in my preparations that all three lobe structures in *Drosophila melanogaster* become more elaborated with proceeding pupal formation.

Such neurones entering the lobe presumably reassemble during metamorphosis. Particularly evident is that the α- lobe extends and bifurcates into two (three?) sublobes that become visible approximately >20 hours after puparium formation. There is evidence that subsets of fibres constituting the α- lobe were added during this process, however, more work would have been necessary to confirm these findings. The stalks of the α- lobe of the 21± 2 hours old female pupa are extremly fine. In contrast the α-lobe of 22-25 hour female pupal brains were reinforced and appeared more densely stained. The cerebral ganglion in *Drosophila melanogaster* larvae consists of two lobes, which are only fused in the most anterior region. During metamorphosis both hemispheres fuse and connections between both hemispheres are established. Thus, as *Drosophila melanogaster* analogues of the anterior- dorsal protocerebral commissure, and the protocerebral tract might innervate the α- lobe (Mobbs, 1982; Gronnenberg 1987; Rybak and Menzel, 1993). The α- lobe in turn needs to adapt itself to receive fibres of these extrinsic neurones. The fusion of the two brain hemisphere is accomplished 30 hours post puparium formation. It was not easy to distinguish the two hemispheres of the imaginal supra- oesophageal ganglion. Only the oesophageal exclusion between supra- and suboesophageal ganglion demarcates the median between both hemispheres. Information between both hemispheres is exchanged by commissural fascicles connecting the hemispheres. These fascicles link deuterocerebral as well as protocerebral structures to the counterparts in the opposite cerebral hemisphere. Thus, the integration of the corpora pedunculata exit neurones reflect these changes.

6. Factors Triggering Metamorphic Rearrangement of Corpora Pedunculata

Ecdysone is a steroid hormone, which amongst others, triggers changes in insect tissues following each larval moult and metamorphosis. During the development of *Drosophila melanogaster* altogether four ecdysone peaks precede changes in moulting behaviour. These ecdysone peaks trigger the first to second instar and second to third instar ecdysis as well the pupation and eclosion. A minor pre-metamorphic ecdysone peak occurs just prior to the onset of puparium formation (121 hours post fertilisation). This peak triggers the pupation (10- 12 hours post puparium formation). Ecdysone binds a putative ecdysone receptor (*EcR*). The ecdysone receptor occurs in three tissue specific isoforms generated by differential splicing. Each isoform is implemented in a distinct regulation event. The isotype *EcRA* is expressed prior to the onset of the maturation phase in imaginal neurones (Talbot et al., 1993; Truman et al., 1994). It presumably regulates imaginal axono- and synaptogenesis. The N- terminal component is derived from a distinct DNA region. The *EcRB1* receptor is expressed exclusively in proliferative zones. Visualisation of *EcRB2* expression was not accomplished due to sequence similarities between *EcRB1* and *EcRB2* (Truman et al., 1994). In the corpora pedunculata neuropil prior to the extension of the α- lobe, *EcB1* is expressed (108 hours post fertilisation) and peaks during puparium formation (0-6 hours after puparium formation). For high affinity binding to ecdysone receptor responsive elements (*EcRE*) *EcR* forms a dimer with the *Ultraspiracle* protein (*Usp*) (Yao et al., 1993). It is still not known if ecdysone induces or stabilises the dimer formation or perhaps even triggers the DNA binding. Upon the binding of the ecdysone responsive dimer, an array of gene regulatory interactions is initiated leading to the transcription of early genes, their suppression by immediate early genes and the induction of late genes. The late genes are responsible for carrying out selective alterations in a tissue specific manner (for review, see Cherbas, 1993).

Of concern for my thesis is the impact of ecdysone on the transformation of the corpora pedunculata before and during metamorphosis. During this process, as seen

above, strictly larval tissues are hydrolysed and imaginal precursor tissues such as imaginal discs differentiate to form fully functional organs following a minor and a major pulse of ecdysone (Segraves et al., 1993). Substantial alterations in the Kenyon cell neuropil of both female and male brain preparations were observed from 15± 2 to 20± 2 hours after puparium formation coinciding with the lull in ecdysone secretion prior to the onset of the major ecdysone peak, which induces ecdysis. Changes that occurred 30 hours post puparium formation, were confined to the emergence of a third α-sublobe and the reduction in neuroblasts (to a quartet 30 hours post puparium formation) (Ito and Hotta, 1992). Strikingly, the restructuring of the corpora pedunculata is almost completed with the onset of the imaginal ecdysone peak (30 hours post puparium formation) (Truman et al., 1994)! This does not imply that other nervous tissues have stopped to proliferate. The optic lobe and the eye, as well as, presumably a whole variety of interneurones must still find their targets (Hofbauer and Campos- Ortega, 1990). As no detailed analysis of input neurones was performed a statement about any changes in dendritic and synaptic rearrangements was not possible.

The expression of *EcRB1* persists until 50 hours PPF and reappears 85 hours PPF. However, pupation lasts only for 96 hours at 25°C and no further neuroblasts are added to the somata 50 hours after puparium formation. Correlating these finding with the findings of Technau (1984), it might be that the addition of new Kenyon axons is triggered by ecdysone pulses during imaginal development. Present Kenyon axons are supplemented by additional fibres emerging up to seven days after eclosion. If this process is ecdysone driven as well, the appropriate receptor ought to be present in order to trigger the necessary cellular events. However it is not known how the deprivation of olfactory and mechanosensory stimuli may impede the release of ecdysone, which in *Rhodnius prolixus* is under circadian regulation control (eg. Vafopoulou and Steel, 1991).

Further, evidence that the postpupal development of the corpora pedunculata may be under circadian control comes form studies on vertebrates. In song birds like canaries, for example, the volume of the olfactory organ depends on seasonal changes of

titres in the steroid hormone androgen. Androgen, in turn, seems to trigger a increase in dendrites (DeVoogt et al. 1985).

In the study by Truman and coworkers (1994), a subset of central Kenyon neurones continued expressing *EcRB1* throughout metamorphosis. It is not known whether this subset corresponds to the Kenyon cells found by Technau and Heisenberg (1982), which do not undergo alterations during metamorphosis, or to the Kenyon somata of my study that did not stain against β- galactosidase. Additionally in a study by Oland and Hayashi (1993), cultured *Manduca sexta* antennal lobe neurones responded differentially to the application of 20- OH ecdysone. Their response depended on the presence of a preceding ecdysone titre (Truman, 1988).

7. Sexual Dimorphism

As deduced from the gross anatomy of the corpora pedunculata no particulate changes concerning sexual dimorphism were observed in my study. In some of the pupal preparations the cortices in females formed triangular shape, whereas the male cortices adopted a more rounded. Further in female third instar larva the thoracic and abdominal ventral nerve cord was more elongated than that of males (data not shown).

Several steroid hormones in mammals occur and act in a sex specific manner. Ecdysone seems to be expressed in both sexes at equal ratios and not to be involved in any sex specific activity. Juvenile hormone, in contrast, has been implied to mediate female oogenesis (Bownes, 1989 and references therein). Further, it directly regulates the production/ release of ecdysone (i.e. Sakurai et al., 1989; Richard and Gilbert, 1991; Berger et al., 1992). One may thus speculate that the *EcRB1/2* expression may be correlated with sex specific changes in the region of the corpora pedunculata, making these cells receptive to juvenile hormone controlled ecdysone pulses. However, further studies on this topic are necessary to confirm this hypothesis.

8. Memory during Metamorphosis

In a negatively reinforced learning paradigm several normal and mutant third instar larvae were trained on olfactory avoidance. Strikingly, this larval memory is preserved, indicating that memory is retained in the cells of the chemosensory tracts during development. Thus, proposed memory is retained in the synaptic connections of the chemosensory memory pathways and does not change to a great extent during metamorphosis (Tully et al., 1994).

References

Abeliovich A., Gerber D., Tanaka O., Katsuki M., Graybiel A.M., and Tonegawa S. (1992). On Somatic Recombination in the Central Nervous System of Transgenic Mice. **Science** 257: 404- 408.

Abrams J.M., White K., Fessler L.I., and Steller H. (1993). Programmed Cell Death during *Drosophila* Embryogenesis. **Development** 117: 29- 43.

Abrams T.W., Karl K.A., and Kandel E.R. (1991). Biochemical Studies of Stimulus Convergence during Classical Conditioning in *Aplysia*: Dual Regulation of Adenylate Cyclase by Ca^{2+}/Calmodulin and Transmitter. **J.Neurosci.** 11: 2655- 2665.

Ache B.W. (1994) Towards a Common Strategy for Transducing Olfactory Information. **Sem. Cell Biol.** 5: 55- 63. [review]

Adrian E.D., and Zotterman Y. (1926). The Impulses Produced by Sensory Nerve Endings. Part 2. The Response of a Single End Organ. **J. Physiol. (London)** 61: 151- 171.

Aidley D.J. (1989). The Physiology of Excitable Cells. 3rd Edition. **Cambridge: Cambridge University Press.**

Albert S., Heisenberg M., Lerch H.P., McKay R.R., Miller K., Ramsberger V., Schneitz K., Schneuwly S., Shortridge R.D., Twardzik T., and Zillian O. (1994). A New *Drosophila melanogaster* Mutant Allele of *Mushroom Body Miniature* Is a P-element Insert in the Phospholipase C21 Gene. *In* Elsner N. and Breer H. (eds.). **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.792. **Stuttgart- New York: Georg Thieme Verlag.**

Alifragis P., Eastman D., and Delidakis C. (1993). Preliminary Analysis of Protein Interactions Involving Members of the *E(Spl)* Complex. *In* Davis R. and Zipusky L. (eds.): **Neurobiology of *Drosophila*.** p. 19., **New York: Cold Spring Harbor Laboratory Press.**

Alting- Mees M.A., Sorge J.A., and Short J.M. (1992). pBluescriptII: Multifunctional Cloning and Mapping Vectors. **Methods Enzymol.** 216: 483- 495.

Ambroso L., Mahowald A.P., and Perrimon N. (1989). Requirement of the *Drosophila raf* Homologue for *torso* Function. **Nature (London)** 342: 288- 291.

Anderson D. (1972). The Development of Hemimetabolous Insects. *In* Cunce S.J. and Waddington C.H. (eds.): **Developmental Systems: Insects Vol. 1** pp 96- 165. **London: Academic Press.**

Anderson M.S., Halpern M.E., and Keshishian H. (1988). Identification of the Neuropeptide Transmitter Proctolin in *Drosophila* Larvae. Characterisation of Muscle Fibre -Specific Neuromuscular Endings. **J. Neurosci.** 8: 242- 255.

Andres A.J. and Cherbas P. (1992). Tissue-Specific Ecdysone Responses: Regulation of the *Drosophila* Genes *Eip28/29* and *Eip40* During Larval Development. **Development** 116: 865- 876.

Andres A.J. and Thummel C.S. (1992). Hormones, Puffs and Flies: The Molecular Control of Metamorphosis by Ecdysone. **Trends Gen.** 8: 132- 139. [review]

Anholt R.R.H. (1994). Signal Integration in the Nervous System: Adenylate Cyclases as Molecular Coincidence Detectors. **Trends Neurosci.** 17: 37- 41. [review]

Antequera P. and Bird A. (1993). CpG Islands. *In* Jost J.P. and Salutz H.P. (eds.): **DNA Methylation.** Molecular Biology and Biological Significance. pp. 169- 185. **Basel: Birkhäuser Verlag.**

Anton S. and Hanssen B.S. (1994). Central Processing of Olfactory Information in the Antennal Lobe of Male and Female *Spodoptera littoralis* (Lepidotera: Noctuidae).*In* Elsner N. and Breer H. (eds.): **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II** p.420. **Stuttgart- New York: Georg Thieme Verlag.**

Antoniewski C., Laval M., and Lepesant J.-A. (1993). Structural Features Critical to the Activity of an Ecdysone Receptor Binding Site. **Insect Biochem. Molec. Biol.** 23: 105- 114.

Arnold G., Masson C., and Budharugsa S. (1985). Comparative Study of the Antennal Lobes and Their Afferent Pathway in the Worker Bee and the Drone (*Apis mellifera*). **Cell Tissue Res.** 242: 593- 605.

Ashburner M. (1989). *Drososphila*: A Laboratory Handbook. **New York: Cold Spring Harbor Laboratory Press.**

Ashburner M. (1990). Puffs, Genes and Hormones Revisited. **Cell** 61: 1-3. [review]

Ashburner M. (1994). *Drosophila*- Physical Mapping a Small Genome. *In* **The GeneticsS, 1969).tion of European Genetical Societies (FEGS) Inaugural Meeting. Chromosome Stability, Genome Projects and Genetic Regulation.** p. 13. **Swansea, Wales.**

Aszódi A., Müller U., and Friedrich P. (1991). Signal Convergence on Protein Kinase A as a Molecular Correlate of Learning. **Proc. Natl. Acad. Sci. USA.** 88: 5832- 5836.

Asztalos Z., von Wegerer J., Wustmann G., Dombrádi V., Gausz J., Spatz H.-C. and Friedrich P. (1993). Protein Phosphatase 1-Deficient Mutant *Drosophila* Is Affected in Habituation and Associative Learning. **J.Neurosci.** 13: 924 - 930.

Atkins P.W. (1989). Physical Chemistry. 4th Edition. **Oxford- Melbourne- Tokyo: Oxford University Press.**

Ayer R.K. and Carlson J. (1992). Olfactory Physiology in the *Drosophila* Antenna and Maxillary Palp:

acj6 Distinguishes Two Classes of Odourant Pathways. **J. Neurobiol.** 23: 965- 982.

Bailey C.H., Chen M., Keller F., and Kandel E. (1992). Serotonin-Mediated Endocytosis of apCAM: An Early Step of Learning-Related Synaptic Growth in *Aplysia*. **Science.** 256: 645-649.

Bainbridge S. P.., and Bownes M. (1981). Staging the Metamorphosis of *Drosophila melanogaster*. **J. Embryol. Exp. Morphol.** 66: 57- 80.

Baker N.E. (1987). Molecular Cloning Of Sequences from *wingless*, a Segment Polarity Gene in *Drosophila*: The Spatial Distribution of a Transcript in Embryos. **EMBO J.** 5: 1765- 1773.

Ballinger D.G. and Benzer S. (1989). Target Gene Mutations in *Drosophila*. **Proc. Natl. Acad. USA.** 86: 9402- 9406.

Balska K. Morawietz H., Dombrádi V., Axton M., Taubert H., Szabó G., Török I., Udvardy A., Gyurkovics H., Szöör B., Glover D., Reuter G., and Gausz J. (1993). Mutations in the Protein Phosphatase 1 Gene at 87D Can Differentially Affect Suppression of Position - Effect Variegation and Mitosis in *Drosophila melanogaster*. Genetics 135: 117- 125.

Bandziulis R.J., Swanson M.S., and Dreyfus G. (1989). RNA- Binding Proteins as Developmental Regulators. **Genes Dev.** 3: 431- 437.

Barthalay Y., Hipeau-Jacquotte R., de al Escalera S., Jiménez F. and Piovant M. (1990). *Drosophila Neurotactin* Mediates Heterophilic Cell Adhesion. **EMBO J.** 9: 3603- 3609.

Baumgartner S., Bopp D., Burri M., and Noll M. (1987). Genes at the *gooseberry* Locus Related to the Segmentation Gene *paired* and Their Spatial Expression during *Drosophila* Embryogenesis. **Genes Dev.** 1: 1247- 1267.

Baumgartner S. and Chiquet-Ehrismann R. (1993). *Ten*^{a}, A *Drosophila* Gene Related to Tenascin, Shows Selective Transcript Localisation. **Mech. Dev.** 40: 165 - 176.

Baumgartner S., Martin D., Hagios C., and Chiquet-Ehrismann (1994). *Ten*^{m}, A Drosophila Gene Related to Tenascin, Is a New Pair- Rule Gene. **EMBO J.** 13: 3728- 3749.

Beato M. (1989). Gene Regulation by Steroid Hormones. **Cell** 56: 335- 344. [review]

Beck E., Ludwig G., Auerwald E.A., Reiss B., and Schaller H. (182). Nucleotide Sequence and Exact Localisation of the *neomycin* Phosphotransferase Gene From Transposon Tn5. **Gene** 19: 327 - 336.

Beckendorf S.K. and Kafatos F.C. (1976). Differentiation in the Salivary Glands of *Drosophila melanogaster*: Characterisation of the Glue Proteins and Their Developmental Appearance: **Cell** 9: 365- 373.

Bellen H.J., D'Evelyn D., Harvey M., and Elledge S.J. (1992). Isolation of Temperature-Sensitive Diphtheria Toxins in Yeast and their Effects on *Drosophila* Cells. **Development** 114: 787- 796.

Bellen H.J., O'Kane C.J., Wilson C., Grossniklaus U., Kurth Pearson R., and Gehring W. J. (1989). P-Element Mediated Enhancer Detection: A Versatile Method to Study Development in *Drosophila*. **Genes Dev.** 3: 1288- 1300.

Bellen H.J., Wilson C., and Gehring W.J. (1990). Dissecting the Complexity of the Central Nervous System by Enhancer Detection. **BioEsays.** 12: 199- 204. [review]

Belyaeva E.S., Vlassova I.E., Biyasheva Z.M., Kakpokov V.T., Richards G., and Zhimulev I.F. (1981). Cytogenetic Analysis of the 2B3-4-2B11 Region of the X Chromosome of *Drosophila melanogaster*. II. Changes in 20-OH Ecdysone Puffing Caused by Genetic Defects of Puff 2B5. **Chromosoma** 84: 207- 219.

Ben-Arie N., Lancet D., Taylor C., Khen M., Walker N., Ledbettter D.H., Carrozzo R., Patel K., Sheer D., Lehrach H., and North M.A. (1994). Olfactory Receptor Gene Cluster on Human Chromosome 17: Possible Duplication of an Ancestoral Receptor Potential. **Hum. Mol. Genet.** 3: 229- 235.

Benedyk M.J., Mullen J.R., and Di Nardo S. (1994). *odd-paired*: A Zinc Finger Pair- Rule Protien Required for the Timely Activation of *engrailed* and *wingless* in *Drosophila* Embryoes. **Genes Dev.** 8: 105- 117.

Bennett R.L. and Hoffmann M. (1992). Increased Levels of the *Drosophila* Abelson Tyrosine Kinase in Nerves and Muscles: Subcellular Localisation and Mutant Phenotypes Imply a Role in Cell- Cell Interactions. **Development** 116: 953- 966.

Benzer S. (1967). Behavioural Mutants of *Drosophila* Isolated by Countercurrent Distribution. **Proc. Natl. Acad. Sci. USA** 58: 1112-1119.

Berg C.A. and Spradling A.C. (1991). Studies on the Rate and Site-Specificity of P-element Transposition. **Genetics** 127: 515- 524.

Berg H.C. (1983). **Random Walks in Biology. Princeton N.J.: Princeton University Press.**

Berger E.M., Goudie K., Klieger L., Berger M., and DeCato R. (1992). The Juvenile Hormone Analogue, Methoprene, Inhibits Ecdysterone Induction of Small Heat Shock Protein Gene Expression. **Dev. Biol.** 151: 410- 418.

Bhaskaran G., Dahm K.H., Barrera P., Pacheco J.L., Pek K.E., and Muszynska-Pytel M. (1990). Allatinhibin, a Neurohormonal Inhibitor of Juvenile Hormone Biosynthesis in *Manduca sexta*. **Gen. Comp. Endocrinol.** 78: 123- 130.

Bhat K., and Schedl P. (1993) The *Drosophila miti-mere* Gene a Member of the POU family Is Required for the Specification of the RP2/ Sibling Lineage During Neurogenesis. *In* Davis R. and Zipursky L. (eds.). **Neurobiology of *Drosophila*.** p. 21. **New York: Cold Spring Habor Laboratory Press.**

Bhat K. M. and Schedl P. (1994). *The Drosophila miti-mere* Gene, a Member of the POU family, Is Required for the Specification of the RP2/ Sibling Lineage During Neurogenesis. **Development.** 120: 1483- 1501.

Bicker G. (1991). Taurine-Like Immunoreactivity in Photoreceptor Cells and Mushroom Bodies: A Comparison of the Chemical Architecture of Insect Nervous Systems. **Brain Res.** 560: 201- 206.

Bicker G., Schäfer S., Ottersen O.P., and Klingan T. (1985). Mushroom Body Feedback Interneurones in the Honeybee Show GABA-Like Immunoreactivty. **Brain Res.** 360: 394- 397.

Bieber A.J., Snow P.M., Hortsch M., Patel N.H., Jacobs J.R., Traquina Z., Schilling J., and Goodman C.S. (1989). *Drosophila neuroglian*: A Member of the Immunoglobulin Superfamily with Extensive Homology to the Vertebrate Neuronal Adhesion Molecule L1. **Cell** 59: 447- 460.

Bienroth S., Keller W., and Wahle E. (1993). Assembly of a Processive messenger RNA Polyadenylation Complex. **EMBO J.** 12: 585- 594.

Bienroth S., Wahle E., Suter-Creazzolera C., and Keller W. (1991). Purification of the Cleavage and Polyadenylation Factor Involved in 3'-Processing of Messenger RNA Precursors. **J. Biol. Chem.** 22: 19786- 19776.

Bier E., Vaessin H., Shephard S., Lee K., McCall K., Barbel S., Ackerman L., Uemura T., Grell E., Jan L.Y., and Jan Y.N. (1989). Searching for Pattern and Mutation in the *Drosophila* Genome with a P-*lacZ* Vector. **Genes Dev.** 3: 1273- 1287.

Bier E., Vaessin H., Younger-Shepherd S., Jan L.Y., and Jan Y.N. (1992). *deadpan*, an Essential Pan-Neural Gene in *Drosophila*, Encodes a Helix-Loop-Helix Protein Similar to the *hairy* Gene Product. **Genes Dev.** 6: 2137 -2151.

Biggs W.H.I., Zavtiz K.H., Dickson B., van der Straten A., Brunner D., Hafen E., and Zipursky S.L. (1994). The *Drosophila rolled* Locus Encodes a MAP Kinase Required in the *sevenless* Transduction Pathway. **EMBO J.** 13: 1628- 1635.

Bliss T.V.P. and Collingridge G.L. (1993) A Synaptic Model of Memory: Long- Term Potentiation in the Hippocampus. **Nature (London)** 361: 31- 39.

Bliss T.V.P., and Lomø T. (1973). Long-Lasting Potentiation of Synaptic Transmission in the Dentate Area of Anaesthetized Rabbit Following Stimulation of the Perforant Path. **J.Physiol.** 232: 331- 356.

Block S.M. (1994). Biophysical Principles of Sensory Transduction *In* **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I** (Elsner N. and Breer H. eds.). p.91- 117. **Stuttgart- New York: Georg Thieme Verlag.**

Bodenstein, D. (1950). The Postembryonic Development of *Drosophila*. *In* M. Demerec (ed): **Biology of *Drosophila*.** pp.275-367. **New York: Hafner.**

Boelens W.C., Jansen E.J.R., Venrooij W.J., Stripcke R., Mattej I.W., and Gunderson S.I. (1993). The Human U1 snRNP-specific U1A Protein Inhibits Polyadenylation of Its Own pre-mRNA. **Cell** 72: 881- 892.

Bownes M. (1989). The Roles of Juvenile Hormone, Ecdysone and the Ovary in the Control of *Drosophila melanogaster* Vitellogenesis. **J. Insect Physiol.** 35: 409- 413.

Boynton S. and Tully T. (1992). *latheo*, a New Gene Invoved in Associative Learning and Memory in *Drosophila melanogaster*, Identified from *P* Element Mutagenesis. **Genetics** 131: 655- 672.

Braha O., Dale N., Hochner B., Klein M., Abrams T.W., and Kandel E.R. (1990). Second Messenger Involved in the Two Processes of Presynaptic Facilitation that Contribute to Sensitization and Dishabituation in *Aplysia* Sensory Neurones. **Proc. Natl. Acad. Sci. USA.** 87: 2040- 2044.

Brand A. and Perrimon N. (1993). Directed Misexpression of *even skipped* and *Dras 2* Using a Novel System for Targeted Gene Expression. **Development.**

Bray S.J., Burke B., Brown N.H., and Hirsh J. (1989). Embryonic Expression Pattern of a Family of *Drosophila* Proteins that Interact with a Central Nervous System Regulatory Element. **Genes Dev. 3:** 1130- 1145.

Breed M.D. and Julian G.E. (1992). Do Simple Rules Apply in Honeybee Nestmate Discrimination? **Nature (London),** 357: 685- 868.

Breed (1993). Scientific Correspondence. **Nature (London).** 362: 120.

Breen T.R. and Harte P.J. (1993). *trithorax* Regulates Multiple Homeotic Genes in the Bithorax and Antennapedia Complexes and Exerts Different Tissue-Specific, Parasegment-Specific and Promoter-Specific Effects on Each. **Development** 117: 119- 134.

Breer H. (1994). Odor Recognition and Second Messenger Signaling in Olfactory Receptor Neurones. **Sem. Cell Biol.** 5: 25- 32.

Breer H., Boekhoff I., Krieger J., Raming K., Strotman J., Tareilus E. (1992). *In* Corey D.P. and Roper S.D.(eds): **Sensory Transduction.** pp. 94-108. **New York: Rockefeller University Press.**

Breer H., Krieger J., and Raming K. (1990). A Novel Class of Binding Proteins in the Antennae of the Silk Moth *Antheraea pernyi*. **Insect Biochem.** 2: 735- 740.

Breidbach O. (1993). Postembryonic Development of the Insect Brain, The Beetle *Tenebrio molitor I n* Elsner N. and Heisenberg M. (eds.): **Proceedings of the 21st Göttingen Neurobiology Conference 1993. Gene, Brain and Behaviour.** p.121. **Stuttgart and New York: Georg Thieme Verlag**

Broadie K.S. and Bate M. (1993a). Development of the Embryonic Neuromuscular Synapse of *Drosophila melanogaster*. **J.Neurosci.** 13: 144- 166.

Broadie K.S. and Bate M. (1993b). Innervation Directs Receptor Synthesis and Localisation in*Drosophila* neurogenesis. **Nature (London)** 361: 350- 353.

Brockmann A. and Brückner D. (1994). Projections of Antennal Poreplate Sensory Neurones in the Antennal Lobe of the Honey Bee, *Apis mellifera*. *In* Elsner N. and Heisenberg M. (eds.): ***In* Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.416. **Stuttgart- New York: Georg Thieme Verlag.**

Bronstein I.N. and Zhemenzhanev K.A. (1981). Handbook of Mathematics. **Moscow : Nauka Publishers .**

Brown A.L. (1991). Biotechnology Course 3/4 3.1. Manual. **Aberdeen University.**

Brown N.H. and Hartley D.A. (1994). Exploring Signalling Pathways. **Nature (London)** 370: 414- 415. [review]

Brunk B.P., Martin E.C., and Adler P.N. (1991). *Drosophila* Genes *Posterior Sex Combs* and

Suppressor (2) of Zeste Encode Proteins with Homology to the Murine *bmi-1* Oncogene. **Nature (London).** 353: 351- 353.

Brunner D., Dücker K., Oellers N., Hafen E., Scholz H., and Klämpt C. (1994). The ETS Domain Pointed-P2 is a Target of MAP Kinase in the *Sevenless* Signal Transduction Pathway. **Nature (London)** 370: 386- 389.

Buchanan R.L. and Benzer S. (1993). Defective Glia in the *Drosophila* Brain Degeneration Mutant *drop-dead*. **Neuron** 10: 839- 850.

Buchner E., Buchner S., Crawford G., Mason W.T., Salvaterra P.M. and Sattelle D.B. (1986). Choline Esterase Like Immunoreactivity of the Brain of *Drosophila melanogaster*. **Cell Tissue Res.** 246: 57-62.

Buck L. and Axel R.A. (1991). A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odorant Recognition. **Cell** 66: 175- 187.

Burden S.J. (1993). Synapse Specific Gene Expression. **Trends Gen.** 9: 12- 16.[review]

Burtis K.C., Thummel C.S., Jones C.W., Karim F.D., and Hogness D.S. (1990). The *Drosophila* 74EF Early Puff Contains *E74*, a Complex Ecdysone-Inducible Gene That Encodes Two *ets*-Related Proteins. **Cell** 61: 85-99.

Busseau I., Diederich R.J., Xu T., Artavanis-Tsakonas (1994). A Member of the *Notch* Group of Interacting Loci, *deltex* Encodes a Cytoplasmic Basic Protein. **Genetics** 136: 585- 596.

Cadigan K.M., Grossniklaus U., and Gehring W.J. (1994). Localised Expression of *sloppy paired* Protein Maintains the Polarity of *Drosophila* Parasegments. **Genes Dev.** 8: 899- 913.

Cabrera C.V. (1992). The Generation of Cell Diversity During Early Neurogenesis in *Drosophila*. **Development** 113: 893- 901 [review].

Cabrera C.V., Alonso M.C., and Huikeshoven H. (1994). Regulation of *scute* Function by *extramacroachaete* in *vitro* and in *vivo*. **Development** 120: 3595- 3603.

Cabrera C.V., Martínez- Arias A., and Bate M. (1987). The Expression of Three Members of the *achaete-scute* Gene Complex Correlates with Neuroblast Segregation in *Drosophila*. **Cell.** 50: 425- 433.

Cadigan K.M., Grossniklaus U., and Gehring W.J. (1994). Localised Expression of *sloppy paired* Protein Maintains the Polarity of *Drosophila* Parasegments. **Genes Dev.** 8: 899-913.

Campos-Ortega J.A. (1993). Mechanisms of Early Neurogenesis in *Drosophila melanogaster*. **J. Neurobiol.** 24(10): 1305-1327[review].

Campos-Ortega J.A. and Hartenstein V. (1985). The Embryonic Development of *Drosophila melanogaster*. Berlin- New York: Springer Verlag.

Capdevila J., Pariente F., Sampedro J., Alonso J.L., and Guerrero I. (1994). Sublocalisation of the Segment Polarity Protein *patched* Suggests an Interaction with the *wingless* Reception Complex in *Drosophila* e98.

Carlson J.(1991). Olfaction in *Drosophila*: Genetic and Molecular Analysis . **Trends Neurosci.** 14: 520-524.

Carlson J. (1992). Erratum. **Trends Neurosci.** 15: 14.

Carroll S.B., and Scott M.P. (1986). Zygotically Active Genes that Affect the Spatial Expression of the *fushi tarazu* Segmentation during Early *Drosophila* Embryogenesis. **Cell** 45: 113- 126.

Carye M., Strambi C., and Strambi A. (1994). Neurogenesis in an Adult Insect Brain and Its Hormonal Control. **Nature (London).** 368: 57- 59.

Casanova J. and Struhl G. (1989). Localised Surface Activity of *torso*, A Receptor Tyrosine Kinase, Specifies Terminal Body Pattern in *Drosophila*. **Genes Dev.** 3: 2025- 2038.

Chain A.C., Zollmann S., Tseng J.C., and Laski F.A. (1991). Identification of *cis*-Acting Sequences Required for Germ line Specific Splicing of the P-element ORF2-ORF3 Intron. **Mol. Cell Biol.** 11: 1538- 1546.

Champlin D.T., Frasch M., Saumweber H., and Lis J.T. (1991). Characterisation of *Drosophila* Protein Associated with Boundaries of Transcriptionally Active Chromatin. **Genes Dev.** 5: 1611- 1621.

Chen C.N., Denome S., and Davis R.L. (1986). Molecular Analysis of cDNA Clones and the Corresponding Genomic Coding Sequences of the *Drosophila dunce* gene, the Structural Gene for cAMP- Dependent Phosphodiesterase. **Proc. Natl. Acad. USA** 83: 9313- 9317.

Chen T., Bunting M., Karim F.D., and Thummel C.S. (1992). Isolation and Characterisation of Five *Drosophila* Genes That Encode an *ets*- Related DNA Binding Domain. **Dev. Biol.** 151: 176 - 191.

Chen W.Y., Aranda L.C., and Luco J.V. (1970). Learning and Long and Short Term Memory in Cockroaches. **Anim. Behav.** 18: 725- 732.

Cheng N.N., Sinclair D.A.R., Campbell R.B., and Brock H.W. (1994). Interactions of *polyhomeotic* With *Polycomb* Group Genes of *Drosophila melanogaster*. **Genetics** 138: 1151- 1162.

Cherbas L., Lee K., and Cherbas P. (1991). Identification of Ecdysone Response Elements by Analysis of the *Drosophila Eip28/29* Gene, an Ecdysone Inducible Gene. **Genes Dev.** 5: 120-131.

Cherbas P. (1993): The IVth Karlson Lecture: Ecdysone-Responsive Genes. **Insect Biochem. Molec. Biol.** 23(1): 3 -11. [review]

Cherkin A. (1966). Toward a Quantitative View of the Engram. **Proc. Natl. Acad. Sci. USA.** 55: 88- 91.

Chess A., Simon I., Cedar H., and Axel R. (1994). Allelic Inactivation Regulates Olfactory Receptor Gene Expression **Cell** 78: 823- 834.

Chiang C., Patel N.H., Young K.E., and Beachy P.A. (1994). The Novel Homeodomain Gene buttonless Specifies Differentiation and Axon Guidance Functions of *Drosophila* Dorsal Median Cells. **Development** 120: 3581- 3593.

Chouinard S., and Kaufman T.C. (1991). Control of Expression of the Homeotic *labial* (*lab*) Locus of *Drosophila melanogaster*: Evidence for Both

Positive and Negative Autogenous Regulation. **Development.** 113: 1267 -1280.

Christensen T.A. and Hildebrand J.G. (1987). Functions, Organisation and Physiology of the Olfactory Pathways in the Lepidopteran Brain. *In* Gupta A.P. (ed.): **Arthoprod Brain. Its Evolution, Development, Structure, and Function.** pp. 457-484. **New York: John Wiley Sons.**

Chu- LaGraff Q. and Doe C. (1993). Neuroblast Specification and Formation Regulated by *wingless* in the *Drosophila* CNS. **Science.** 261: 1594-1597

Ciu X. and Doe C.Q. (1993). *ming* Is Expressed in Neuroblast Sublineages and Regulates Gene Expression in the *Drosophila* Central Nervous System. **Development** 116: 943- 952

Clark A.G., Silveria S., Meyers W., and Langley C.H. (1994). Nature Screen: An Efficient Method for Screening Natural Populations of *Drosophila* for Targeted P-element Insertions. **Proc. Natl. Acad. USA.** 91: 719- 722.

Clever U. and Karlson P. (1960). Induktion von Puff-veränderungen in den Speicheldrüsenchromosomen von *Chironomus tentans* durch Ecdyson. **Exp. Cell Res.** 20: 623-626.

Cohen S.M. and Jürgens G. (1990). Mediation of *Drosophila* Head Development by Gap-Like Segmentation Genes. **Nature (London)** 346: 482-485.

Cooley L., Berg C., and Spradling A. (1988). Controlling P-element Insertional Mutagenesis. **Trends Gen.** 4: 254- 258. [review]

Cooley L., Kelly R., and Spradling A. (1988). Insertional Mutagenesis of the *Drosophila* Genome with Single P Elements. **Science** 239: 1121-1130.

Coté S., Preiss A. Haller J., Schuh R., Kienlin A., Seifert E., and Jäckle H. (1987). The *gooseberry-zipper* Region of *Drosophila*: Five Genes Encode Different Spatially Restricted Transcripts in the Embryo. **EMBO J.** 6: 2793- 2802.

Cubas P., Mololell J., and Ruis- Gómez M. (1994). The Helix-Loop-Helix *extramacroacheate* Protein Is Required for Proper Specification of Many Cell Types in the *Drosophila* Embryo. **Development** 120: 2555- 2565.

Cumberledge S. and Krasnow M.A. (1993) Intercellular Signalling in *Drosophila* Segment Formation Reconstructed *in vitro*. **Nature (London)** 363: 549-552.

Daniels S.B. and Chovnick A.(1993). P Element Transposition in *Drosophila melanogaster*: An Analysis of Sister-Chromatid Pairs and the Formation of Intragenic Secondary Insertions During Meiosis. **Genetics** 133: 623- 636.

Datta S., and Kankel D.R. (1992). *l(1) trol* and *l(1) delv*, Mutants Affecting the Development of the Adult Central Nervous System in *Drosophila melanogaster*. **Genetics** 130: 523- 537.

Datta S., Stark K., and Kankel D.R. (1993). Enhancer Detector Analysis of the Extend of Genomic Involvement in the Nervous System Development in *Drosophila melanogaster*. **J.Neurobiol.** 24: 824- 841.

Davidson E.H. (1993). Later Embryogenesis: Regulatory Circuitry in Morphogenetic Fields. **Development** 118: 665 - 690.

Davis B.D., Dulbecco R., Eisen H.N. and Ginsberg H. (1990). **Microbiology.** Fourth Edition. **Philadelphia: Lippincott Company.**

Davis R.L. (1993). Mushroom Bodies and *Drosophila* Learning. **Neuron.** 11: 1- 14 [review].

Davis R.L. and Dauweiler B. (1991). The *Drosophila dunce* Locus: Learning and Memory in the Fly. **Trends Genet.** 7: 224- 229. [review]

Dawson I.A., Roth S., Akam M., and Artavanios-Tsakonas S. (1993). Mutations of the *fizzy* Locus Cause Metaphase Arrest in *Drosophila melanogaster* Embryos. **Development** 117: 359-376.

Dawson T.M. and Synder S.H. (1994). Gases as Biologcal Messengers: Nitric Oxide and Carbon Monoxide in the Brain. **J. Neurosci.** 14: 5147-5159. [review]

Dawson V.L., Bredt D.S., Fotuhi M., Hwang P.M., and Snyder, S.H. (1991). Nitric Oxide Synthase and Neuronal NADPH Diaphorase are Identical in Brain and Peripheral Tissues. **Proc. Natl. Acad. Sci. USA.** 88: 7797- 7801.

Degen J., Dyczkowski C., Roeder T., and Gewecke M. (1994). Further Characterisation of Octopamine Receptors From Different Insect Species. *In* Elsner N. and Breer H. (eds.): *In* **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.639. **Stuttgart- New York: Georg Thieme Verlag.**

de Groot R.P., Ballou L.M., and Sassone-Corsi P. (1994). Positive Regulation of the cAMP -Responsive Activator CREM by the p70 S6 Kinase: An Alternative Route to Mitogen-Induced Gene Expression. **Cell.** 79: 81- 91.

Delgado R., Hildalgo P., Diaz P., Latorre R., and Labarca P. (1991). A Cyclic AMP-Activated K^+ Channel in *Drosophila* Larval Muscle Is Persistently Activated in *dunce*. **Proc. Natl. Acad. Sci. USA.** 88: 557- 560.

Dethier V.G. (1963). The Physiology of Insect Senses. **London: Methuen and Sons. [review]**

de Wind N., Domen J., and Berns A.A. (1992). Herpesviruses Encode an Unusual Protein-Serine/Threonine Kinase, which Is Non- essential for Growth in Cultured Cells. **J. Virol.** 66: 5220-5209.

Diaz- Benjumea F.J. and Cohen, S.M. (1994). *wingless* Acts Through the *shaggy/zeste-white3 kinase* to Direct Axis Formation in the *Drosophila* Leg. **Development** 120: 1661- 1670.

Dibello P.R., Withers D.A., Bayer C.A., Fristrom J.W., and Guild G.M. (1991). The *Drosophila Broad-Complex* Encodes a Family of Related Proteins Containing Zinc Fingers. **Genetics** 129: 385- 397.

Dickson B. and Hafen E. (1994). Genetics of Signal Transduction in Invertebrates. **Curr. Opin. Gen.Dev.** 4: 64- 70.

Diederich R.J., Matsuno K., Hing H., Artavanis-Tsakonas S. (1994). Cytosolic Interactions

Between *Deltex* and *Notch* Ankyrin Repeats Implicates *Deltex* in the *Notch* Signalling Pathway. **Development** 120: 473- 481.

Diederich R.J., Pattatucci A.M. and Kaufman T.C. (1991). Development and Evolutionary Implications of *labial*, *Deformed* and *engrailed* Expression in the *Drosophila* Head. **Development** 113: 273- 281.

DiNardo S., Kuner J.M., Theis J., O'Farrell P.H. (1985). Development of Embryonic Pattern in *Drosophila melanogaster* as Revealed by Accumulation of the Nuclear *ENGRAILED* Protein. Cell. 43: 59- 69.

DiNardo S., Sher E., Heermskerk-Jongens J., Kassis J.A., and O'Farrell P.H.(1988). Two-Tiered Regulation of Spatially Patterned *engrailed* Gene Expression During *Drosophila* Embryogenesis. **Nature (London)**. 332: 604- 609.

Distler P. and Boeckh J. (1994). Synapses between Olfactory Receptor Neurons and Uniglomerular Projection Neurons in Antennal Lobe Glomeruli of the Cockroach. *In* Elsner N. and Breer H. (eds): **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.423. **Stuttgart- New York: Georg Thieme Verlag.**

Dixon J.E. and Sadowski P.D. (1993): Resolution of Synthetic c Structures by the FLP Site - Specific Recombinase. **J.Mol. Biol.** 234: 522- 533.

Doe C.Q., Chu- LaGraff Q., Wright D.M., and Scott M.P. (1991). The *prospero* Gene Specifies Cell Fates in the *Drosophila* Central Nervous System. **Cell.** 65: 451- 464.

Doe C.Q. (1992). Molecular Markers for Identified Neuroblasts and Ganglion Mother Cells in the *Drosophila* Central Nervous System. **Development.** 116: 855-863.

Doe C.Q. and Scott M.P. (1988). Segmentation and Homeotic Genes Function in the Developing Nervous System of *Drosophila*. **Trends Neurosci.** 11: 101-106.

Donley B.C., Ding Q., Tobe S.S., and Bendena W.G. (1993). Molecular Cloning of the Gene for the Allatostatin Family of Neuropeptides from the Cockroach *Diploptera punctata*. **Proc. Natl. Acad. Sci. USA.** 90: 8807- 8811.

Doherty D., Jan L.Y. and Jan Y.N. (1993). The Role of the *big brain* Gene in Neurogenesis. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*. . New York Cold Spring Harbor Laboratory Press.**

Doyle H.J., and Bishop J.M.(1993). *Torso*, a Receptor Tyrosine Kinase Required for Embryonic Pattern Formation, Shares Substrates with the *Sevenless* and EGF-R Pathways in *Drosophila*. **Genes Dev.** 7: 633- 646.

Drain P., Folkers E., and Quinn W.G. (1991). cAMP-dependent Protien Kinase and the Disruption of Leaning in Transgeneic Flies. **Neuron** 6: 71- 82.

Drescher W. (1960). Regenerationsversuche am Gehirn von *Periplaneta americana* unter Berücksichtigung von Verhaltensänderung und Neurosekretion. **Ökol. Tiere** 48: 576- 649.

Driever W. and Nüsslein-Volhard C. (1988a). A Gradient of *bicoid* Protein in *Drosophila* Embryos. **Cell** 54: 83-93.

Driever W. and Nüsslein-Volhard C. (1988b). The *bicoid* Protein Determines Position in the *Drosophila* Embryo in a Concentration Dependent Manner. **Cell** 54: 95- 104.

Dübendorfer A. and Eichenberger-Glinz S. (1980). Development and Metamorphosis of Larval and Adult Tissues of *Drosophila in vitro*. *In* Kurstak E., Maramorosch K. and Dübendorfer A.: **Invertebrate Systems *In Vitro*.** pp.169 - 185. **Amsterdam: Elsevier/North-Holland Biomedical Press.**

Dudai Y., Sher B., Segal D., and Yovell Y. (1985). Defective Responsiveness of Adenylate Cyclase to *Forskolin* in the *Drosophila* Memory mutant *rutabaga*. **J. Neurogenet.** 2: 365- 380.

Duffy J.B., Kania M.A., and Gergen J.P. (1991). Expression and Function of the *Drosophila* Gene *runt* in Early Stages of Neuronal Development. **Development** 113: 1223- 1230.

Dugaiczyk A., Boyer H.W., and Goodman H.M. (1975). Ligation of *Eco*RI Endonuclease Generated DNA Fragments into Linear and Circular Strucutres. **J. Molec. Biol.** 96: 171- 184.

Dura J.-M., Preat T., and Tully T. (1993). Identification of *linotte*, A New Gene Affecting Learning and Memory in *Drosophila melanogaster*. **J. Neurogenetics.** 9: 1- 14.

Durinio R.J. and O'Farell P.H. (1994). Developmental Control of a G_1-S Transcriptional Program in *Drosophila*. **Development** 120: 1503 -1515.

Edgar S.J. and O'Farell P.H. (1990). The Three Postblastoderm Cell Cycles of *Drosophila* is Regulated in G_2 by *string*. **Cell** 62:

Eggleston P. and Exley K.A. (1992). Chromosome Interactions in P-M Dysgenic Male Recombinations of *Drosophila melanogaster*. **Genet. Res. Camb.** 60: 165- 174.

Eissenberg J.C., Morris G.D., Reuter G., and Harnett T. (1992). The Heterochromatin- Associated Protein HP-1 Is An Essential Protein in *Drosophila* with Dosage Dependent Effects on Position Effect Variegation. **Genetics** 131: 345- 352.

Ellis H., Burrow M. Comacchio R., and Kocaciatak T. (1993). Spatially Restricted Embryonic Expression of the *extramicroachaete* Gene. In Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*. New York: Cold Spring Harbor Laboratory Press.**

Ellisen L.W., Bird J., West D.C. Soreng A.L., Reynolds T.C., Smith S.D., and Sklar J. (1991). *TAN-1*, the Human Homolog of the *Drosophila Notch* Gene, Is Broken by Chromosomal Translocations in the T-lymphoblastic Neoplasms. **Cell** 66: 649- 661.

Elkins T., Zinn K., McAllister L., Hoffmann F.M. and Goodman C.S. (1990). Genetic Analysis of a *Drosophila* Neural Cell Adhesion Molecule: Interaction of *fasciclin I* and *Abelson Tyrosine Kinase* Mutations. **Cell** 60: 565- 575.

Elphick M.R., Green I.C., and O'Shea M. (1993). Nitric Oxide Synthesis and Action in an Invertebrate Brain. **Brain Res.** 619: 344 -346.

Engels W.R., Johnston-Schlitz D.M., Eggleston W.B., and Sved J. (1990). High-Frequency P Element Loss in *Drosophila* Homolog Dependent. **Cell** 62: 515- 525.

Erber J., Homberg U., and Gronenberg W. (1987). Functional Roles of the Mushroom Bodies in Insects. *In* Gupta A.P. (ed.): **Arthoprod Brain. Its Evolution, Development, Structure, and Function.** pp. 485- 511. New York: John Wiley Sons.

Evans M.J. and Kaufman M.H. (1981). Establishment in Culture of Pluriploral Cells From Mouse Embryos. **Nature (London)** 292: 154- 156.

Evans R.M. (1988). The Steroid and Thyroid Hormone Receptor Superfamily. **Science** 240: 889- 895.[review]

Ewer J. and Truman J.W. (1993). Cyclic-GMC Immunoreactivity in the CNS of Adult *Drosophila* During Specific Behaviors. *In* Davis R. and Zipursky L. (eds.) Neurobiology of *Drosophila*. p. 156. New York: Cold Spring Laboratory Press.

Farbman A.L. (1994). Developmental Biology of Olfactory Sensory Neurones. **Sem. Cell Biol.** 5: 55- 63. [review]

Fehon R.G., Kooh P.J., Rebay I., Regan C.L., Xu T., Muskavitch M.A.T., and Artavanis-Tsakonas S. (1990). Molecular Interactions Between the Protein Products of the Neurogenic Loci *Notch* and *Delta*, Two EGF-Homologous Genes in *Drosophila*. **Cell** 61: 523- 534.

Ferrandon D., Elphick L., Nüsslein- Volhard, and St. Johnston D. (1994). *Staufen* Protein Associates with the 3'UTR of *bicoid* mRNA to Form Particles That Move in a Microtubule- Dependent Manner. **Cell** 79: 1221- 1232.

Finkelstein R. and Perrimon N. (1990). The *orthodenticle* Gene Is Regulated by *bicoid* and *torso* and Specifies *Drosophila* Head Development. **Nature (London)** 346: 485- 488.

Finkelstein R., Smouse D., Capaci T.M., Spradling A.C., and Perrimon N. (1990). The *orthodenticle* Gene Encodes a Novel Homeo Domain Protein Involved in the Development of the *Drosophila* Nervous System and Ocellar Visual Structures. **Genes Dev.** 4: 1516-1527.

Finnegan D.J. (1992). Transposable Elements. **Cur. Opin. Gen. Dev.** 2: 861- 867.

Fischbach K.F., and Technau G.M. (1984). Cell Degeneration in the Developing Optic Lobes of the *Sine Oculis* and Small - Optic - Lobes Mutants of *Drosophila melanogaster*. **Dev. Biol.** 104: 219- 339.

Fjose A., McGinnis W.J., and Gehring W.J. (1985). Isolation of a Homeobox Containing Gene from the *engrailed* Region of *Drosophila* and the Spatial Transcription of its Transcripts. **Nature (London)** 313: 284- 289.

Flanagan D. and Mercer A.R. (1989). An Atlas and 3-D Reconstruction of the Antennal Lobes in the Worker Honey Bee, *Apis mellifera* L. (Hymenoptera: Apidae). **Int. J. Insect Morphol. Embryol.** 18: 145- 159.

Fleig, R. (1990). *Engrailed* Expression and Body Segmentation in the Honey Bee *Apis mellifera*. **Roux's Arch. Dev. Biol.** 198: 467- 473.

Fleig, R. (1994). Head Segmentation in the Embryo of the Colorado Beetle *Leptianotarsa decemlineata* as Seen with Anti *en*- Immunostaining. **Roux's Arch. Dev. Biol.**

Foe V.E. (1989). Mitotic Domains Reveal Early Commitment of Cells in the *Drosophila* Embryos. **Development** 107: 1- 22.

Folkers E., Drian P., and Quinn W.G. (1993). *radish*, A *Drosophila* Mutant Deficient in Consolidated Memory. **Proc. Natl. Acad. USA.** 90: 8123- 8127.

Fortini M.E. and Artavanis- Tskanonas S. (1994). The *SUPPRESSOR OF HAIRLESS* Protein Participates in the *NOTCH* Receptor Signalling. **Cell** 79: 273- 282.

Foster J.L., Guttman J.J., Hall L.M., and Rosen O.M. (1984). *Drosophila* cAMP- Dependent Protein Kinase. **J. Biol. Chem.** 259: 13049- 13055.

Frank D.A., and Green berg M.E. (1994). CREB: A Mediator of Long Term Memory from Mollusks to Mammals. **Cell** 79: 5- 8. [review].

Franke A., DeCamillis M., Zink D., Cheng N., Brock H.W., and Paro R. (1992). Polycomb and Polyhomeotic Are Constituents of a Multimeric Protein Complex in Chromatin of *Drosophila melanogaster*. **EMBO J.** 11: 2941 - 2950.

Frasch and Levine M. (1987). Complementary Patterns of *even-skipped* and *fushi tarazu* Expression Involve Their Differential Regulation by a Common Set of Segmentation Genes. **Genes Dev.** 1: 981- 995.

Fredieu J.R. and Mahowald A.P. (1989). Glia Interactions with Neurones During *Drosophila* Embryogenesis. **Development** 106: 739- 748.

Fredieu J.R. and Mahowald A.P. (1993). Characterisation of a Putative *Drosophila* GTP-binding Protein. **J. Cell Sci.** 105: 81- 91.

Frontali N. and Manicini G. (1970). Studies on the Neuronal Organisation of Cockroach Corpora Pedunculata. **J. Insect Physiol.** 16: 2293- 2301.

Fukukawa T., Maruyama S., Kawaichi M., and Honjo T. (1992). The *Drosophila* Homolog of the Immunoglobulin Recombination Signal - Binding Protein Regulates Peripheral Nervous System Development. **Cell** . 69: 1191- 1197.

Fullilove S.L., Jacobson A.G., and Turner F.R. (1978). Embryonic Development: Descriptive. *In* Ashburner M., and Wright T.R.F.: **The Genetics and Biology of *Drosophila*. Vol. 2c.** pp. 106- 227. **London: Academic Press.**

Gagliardini V., Fernandez P.-A., Lee R.K.K., Drexler H.C.A., Rotello R.J., Fishman M.C., and Yuan J. (1994). Neuronal Death by the *crmA* Gene. **Science** 263: 826- 828.

Gaul U. and Jäckle H. (1989). Analysis of the Maternal Effect Mutant Combinations Elucidates Regulation and Function of the Overlap of *hunchback* and *Krüppt* al., 1989).*hila* Blastoderm Embryo. **Development** 107: 651- 662.

Georgel P., Ramain P., Giangrande A., Dretzen G., Richards G., and Ballard M. (1991). *Sgs-3* Chromatin Structure and *trans* Activation of Developmental and Ecdysone Induction of a

Glue Enhancer Binding Factor, GEBF-I in *Drosophila* Larvae. **Mol. Cell Biol.** 11: 523- 532.

Gertler F.B., Bennett R.L., Clark M.J., and Hoffmann F.M. (1989). *Drosophila abl* Tyrosine Kinase in Embryonic CNS Axons: A Role in Axonogenesis Is Revealed through Dosage Sensitive Interactions with *disabled*. **Cell** 58: 103- 113.

Gertler F.B., Doctor J.S., and Hoffmann F.M. (1990). Genetic Suppression Mutation in the *Drosophila abl* Proto-Oncogen Homolog. **Science** 248: 857- 860.

GetzW.M. (1993). Odour Detection in Bees. **Nature (London).** 362: 119-120.

Gho M., McDonald K., Ganetzky B., and Saxton W.M. (1992). Effects of *Kinesin* Mutations on Neuronal Functions. **Science** 258: 313- 316.

Ginger E., Jan L.Y. and Jan Y.N. (1993a). Specifying the Path of the Intersegmental Nerve of the *Drosophila* Embryo: A Role for *Delta* and *Notch*. **Development** 117: 431- 440.

Ginger E., Wells W., Jan L.Y. and Jan Y.N. (1993b). Tracing Neurons With *Kinesin* β- Galactosidase. **Roux's Arch. Dev. Biol.** 202: 112- 122.

Ginger E., Tietje K., Jan L.Y. and Jan Y.N. (1994). *lola* Encodes a Putative Transcription Factor Required for Axon Growth and Guidance in *Drosophila*. **Development** 120: 1385- 1398.

Gloor G.B., Preston C.R., Johnston-Schlitz D.M., Nassif N.A., Phillis R.W., Benz W.K., Robertson H.M., and Engels W.R. (1993). Type I Repressors of P element Mobility. **Genetics** 135: 81- 95.

Glover D.M., Llamazares S., Girdham C., Maldonado-Codina G., Moreira A., Tavres A., Sunkel C.E., and Gonzalez C. (1991). Cyclical Changes in the Subcellular Essential for Mitosis during Embryogenesis in *Drosophila*. **Cold Spring Harbor Symp. Quant. Biol.** Vol. LVI: 709- 717.

Golic K.G. and Lindquist S. (1989). The FLP Recombinase of Yeast Catalyzes Site - Specific Recombination in the *Drosophila* Genome. **Cell** 59: 499- 509.

Golic K.G. (1991). Site- Specific Recombination Between Homologous Chromosomes in *Drosophila*. **Science** 252: 958- 961.

Golic K. (1993). Generation Mosaics by Site-Specific Recombination. *In* Hartley D.A. (ed.). **Cellular Interactions in Development.** pp. 1- 31. **Oxford: IRL Press/ Oxford University Press.**

Gómez-Skarmeta J.L., Mendez R., de Haro C, Kumar R, and Pulido D. (1993). Signal Transduction by Protein Tyrosine Kinases and Phosphatases During *Drosophila* Neural Development. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.. New York: Cold Spring Harbor Laboratory Press.**

Gonzáles- Crespo S. and Levine M. (1993). Interactions between *dorsal* and Helix-Loop-Helix Proteins Initiate the Differentiation of the Embryonic Mesoderm and Neuroectoderm in *Drosophila*. **Genes Dev.** 7: 1703-1713.

Goll W. (1967). Strukturuntersuchungen am Gehirn von *Formica*. **Z. Morphol. Oekol. Tiere** *59:* 143 -210.

Gould S.J. (1977). Ontogeny and Phylogeny. **Cambridge, Mass. - London: The Belknap Press of Harvard University Press.**

Greggers U. and Menzel R. (1993). Memory Dynamic and Foraging Strategies of Honeybees. **Behav. Ecol. Sociobiol.** 32: 17- 29.

Grenningloh G., Rehm E.J., and Goodman C.S. (1991). Genetic Analysis of Growth Cone *Guidance* in *Drosophila*: *Fasclin II* Functions as a Neural Recognition Molecule. **Cell** 67: 45- 57.

Grieneisen M.L., Warren J.T., and Gilbert L.I. (1993). Early Steps in Ecdysteroid Biosynthesis: Evidence for the Involvement of Cytochrome P-450 Enzymes. **Insect Biochem. Molec. Biol.** 23: 13- 23.

Grossniklaus U., Bellen H.J., Wilson C., and Gehring W.J. (1989). P-Element Mediated Enhancer Detection Applied to the Study of Oogenesis in *Drosophila*. **Development** 105: 35- 52.

Grosveld F.G. (1994). Locus Control Regions. *In* **The Genetics Society Spring Meeting & Federation of European Genetical Societies (FEGS) Inaugural Meeting. Chromosome Stability, Genome Projects and Genetic Regulation.** p. 17. **Swansea, Wales.**

Grünewald B. (1994). Physiology of Feedback Neurones in the Mushroom Bodies of the Honey Bee. *In* N. Elsner and H. Breer (eds):**Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.829. **Stuttgart- New York: Georg Thieme Verlag.**

Gundelfinger E.D. (1992). How Complex Is the Nicotinic Receptor System of Insects? **Trends Neurosc.** 15: 206- 211.

Gunderson S.I., Beyer K., Martin G., Keller W., Boelens W.C., and Mattaj I.W. (1994). The Human U1A snRNP Protein Regulates Polyadenylation via a Direct Interaction with Poly (A) Polymerase. **Cell** 76: 531- 541.

Gutjahr T., Patel N.H., Li X., Goodman C.S., and Noll M. (1993). Analysis of the *gooseberry* Locus in *Drosophila* Embryos: *gooseberry* Determines the Cuticular Pattern and Activates *gooseberry neuro*. **Development.** 118: 21-31.

Gyorgyi T.K., Roby- Shemkovitz A.J., and Lerner M.R. (1988). Characterisation and cDNA Cloning of the Pheromone Binding Protein from the Tobacco Hornworm, *Manduca sexta*: A Tissue Specific Developmentally Regulated Protein. **Proc. Natl. Acad. Sci. USA** 85: 9851- 9855..

Häcker U. and Jäckle H. (1993). The *lätzchen* Gene, Member of a Novel Developmentally Regulated *Drosophila* Gene Family Encoding the Fork Head Domain Is Involved in Head Development. *In* **Abstract for the 13th European *Drosophila* Research Conference,** J 25, Aghia Pelaghia, Crete, Greece.

Halpern M.E., Chibra A., Johansen J., and Keshishian H. (1991). Growth Cone Behaviour Underlying the Development of Stereotypic Synaptic Connections in *Drosophila* Embryos. **J.Neurosci.** 11: 3227- 3238.

Hammer M. (1993). An Identified Neuron Mediates the Unconditioned Stimulus in Associative Olfactory Learning in Honeybees. **Nature (London).** 366: 59-63.

Han P.-L., Levin L.R., Reed R., and Davis R.L. (1992). Preferential Expression of the *Drosophila rutabaga* Gene in Mushroom Bodies, Neural Centres for Learning in Insects. Neuron 9: 619- 627.

Hartenstein A., Rugendorff A., Tepass U., Hartenstein V. (1992). The Function of the Neurogenic Genes During Epithelial Development in the *Drosophila* Embryo. Development 116: 1203- 1220.

Hartenstein V. and Campos- Ortega J.A. (1985). Fate Mapping in wild Type *Drosophila melanogaster*. The spatio-temporal Pattern of Embryonic Cell Divisions. Roux's Arch. Dev. Biol. 195: 210- 221.

Hartenstein V. and Jan Y.N. (1992). Studying *Drosophila* Embryogenesis with P-*lacZ* Enhancer Trap Lines. Roux's Arch. Dev. Biol. 201: 194- 220.

Hasan G. and Rosbash M. (1992). *Drosophila* Homologs of Two Mammalian Intracellular Ca^{2+}- Release Channels: Identification and Expression Patterns of the Inositol 1.4.5- Triphosphate and the Rynodine Receptor Genes. Development. 116: 967- 975.

Hay B.A. Wolff T., and Rubin G.M. (1994). Expression of Baculovirus p35 Prevents Cell Death in *Drosophila* Development 120: 2121- 2129.

Haykin S. (1994). **Neuronal Networks. A Comprehensive Foundation. Toronto: Maxwell Macmillan Canada.**

Haynes S.R., Johnson D., Raychauhuri G., and Beyer A.L. (1991). The *Drosophila Hrb87F* Gene Encodes a new Member of the A and B hnRNP Protein Group. Nucliec Acid. Res. 19: 25-31.

Haynes S.R., Raychauhuri G., and Beyer A.L. (1991). The *Drosophila Hrb98DE* Locus Encodes Four Protein Isoforms Homologous to the AP1 Protein of Mammalian Heterologous Nuclear Ribonucleoprotein Complexes. Mol. Cell Biol. 10: 316- 323.

Hazelrigg T., Levis R., and Rubin G.M. (1984). Transformation of *white* Locus DNA in *Drosophila*: Dosage Compensation, *zeste* Interaction, and Positional Effects. Cell 36: 469- 481.

Heisenberg M., Bost A., Wagner S., Byers D. (1985). *Drosophila* Mushroom Body Mutants are Deficient for Olfactory Learning. J. Neurogenet. 2: 1-30.

Heisenberg M. (1989). Genetic Approach to Learning and Memory (Mnemogenetics) in *Drosophila melanogaster*. *In* Rahmann (ed.): Fortschritte der Zoologie/ Progress in Zoology. Band/Vol. 37. Fundamentals of Memory Formation: Neuronal Plasticity and Brain Function: **Stuttgart- New York: Gustav Fischer Verlag.**

Helfrich- Förster C. and Homberg U. (1993). Pigment-Dispersing Hormone- Immunoreactive Neurons in the Nervous System of Wild- Type *Drosophila melanogaster* and Several Mutants With Altered Circadian Rhythmicality. J. Comp. Neurobiol. 337: 177-196.

Hekmatpanah D.S., McKeena M.P., Gaines P., and Carlson J.R. (1993). Pheromone- Binding Protein Homlogs in *Drosophila*.. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p.150. **New York Cold Spring Harbor Laboratory Press.**

Hellstern F. and Hammer M. (1994). Backward Inhibitory Learning in Honeybees. *In* N. Elsner and H. Breer (eds): **Proceedings of the 22nd Göttingen Neurobiology Conference 1994, Volume II.** p 827. **Stuttgart-New York: Thiemme Verlag.**

Henkemeyer M.J., Bennett R.L., Gertler F.B., and Hoffmann F.M. (1988). DNA Sequence, Structure, and Tyrosine Kinase Activity of the *Drosophila* Abelson Proto-Oncogen Homolog. **Mol. Cell. Biol.** 8: 843- 853.

Henn D.K. and Kaupp U.B. (1994). Analysis of the Transmembrane Topology of cGMP-Gated Ion Channels From Bovine Rod Photoreceptor and *Drosophila melanogaster*. *In* N. Elsner and H. Breer (eds): **Proceedings of the 22nd Göttingen Neurobiology Conference 1994, Volume II.** p. 720. **Stuttgart- New York: Thiemme Verlag.**

Henrich V.C., Szekely A.A., Kim S.J., Bown N.E., Antoniewki C., Hayden M.A., Lepesent J.-A., and Gilbert L.I. (1994). Expression and Function of the *ultraspiracle* (*usp*) Gene during Development of *Drosophila melanogaster*. **Dev. Biol.** 165: 38- 52.

Hildebrandt H., Müller U., and Menzel R. (1994). Chemosensory Stimulation and Nitric Oxide Enhance PKA Activity in the Antennal Lobe of the Honey Bee. *In* N. Elsner and H. Breer (eds): **Proceedings of the 22nd Göttingen Neurobiology Conference 1994, Volume II.** p. 653. **Stuttgart- New York: Thiemme Verlag.**

Hofbauer A., and Campos- Ortega J.A. (1990). Proliferation Pattern and Early Differentiation of the Optic Lobes in *Drosophila melanogaster*. **Roux's Arch. Dev. Biol.** 198: 81- 118.

Holliday R. (1964). A Mechanism for Gene Conversation in Fungi. **Genet. Res.** 5: 282- 304.

Homberg U. (1987). Strucutre and Functions of the Central Complex in Insects. *In* Gupta A.P. (ed.): **Arthoprod Brain. Its Evolution, Development, Structure, and Function.** pp. 347- 363. **New York: John Wiley Sons.**

Homberg U. Christensen T.A., and Hildebrand J.G. (1989). Structure and Function of the Deuterocerebrum in Insects. **Annu. Rev. Entomol.** 34: 477- 501.

Hooper J.E. (1994). Distinct Pathways for Autocrine and Paracrine *WINGLESS* Signalling in *Drosophila* Embryos. **Nature (London).** 372: 461- 464.

Hooper J. E. and Scott M.P. (1989). The *Drosophila patched* Gene Encodes a Putative Membrane Protein Required for Segmental Pattering. **Cell.** 59: 751-765.

Hopfield J.J. (1982). Neuronal Networks and Physical Systems with Emergent Collective Computational Abilities. **Proc. Natl. Acad. Sci. USA.** 79: 2554- 2558.

Hopfield J.J. (1984). Neurons with Graded Response Have Collective Computational Properties Like those of Two- State Neurones. **Proc. Natl. Acad. Sci. USA.** 81: 3088- 3092.

Hopfield J.J. (1987). Learning Algorithms and Probability Distribution in Feed Forward and Feed- Back Networks. **Proc. Natl. Acad. Sci. USA** 84: 8429- 8433.

Hopfield J.J., Feinstein D.I., and Palmer R.G. (1983). 'Unlearning' has a Stabilizing Effect in Collective Memmories. Nature (London). 304: 158- 159.

Hopfield J.J., and Tank T.W. (1986) Computing with Neural Ciruits: A Model. Science 233: 625- 633.

Horseman G., Hartmann R., Virant-Doberlet M., Loher W., and Huber F. (1994). Nervous Control of Juvenile Hormone Biosynthesis in *Locusta migratoria*. Proc Natl. Acad. Sci. USA 91: 2960- 2964.

Howse P.E. (1974). Design and Function of the Insect Brain. *In* Barton Browne L. (ed).: Experimental Analysis of Insect Behaviour. p.115- 138. Berlin- Heidelberg- New York: Springer Verlag.

Hoyle G. (1965). Neurophysiological Studies on "Learning" in Headless Insects. *In* Treherne J. E. and Beament (eds.): The Physiology of the Insect Central Nervous System. pp. 239- 251. London: Academic Press.

Hu S. and Crews S.T. (1993).The *Drosophila* CNS Midline BL97 Gene: Sequence Similarity to *Faciclin I*, Imaginal Disc Expression, and Correspondence to Abrupt Gene. In Davis R. and Zipurski L. (eds.): Neurobiology of *Drosophila*. New York: Cold Spring Habor Laboratory Press.

Hunter T. (1994). Cell Signalling by Receptor Protein- Tyrosine Kinases. *In* Adams R., Anderson P., Goddard J. and Jacobs H. (eds.): Eukaryotic Gene Expression, Eighth Tenovus -Scotland Symposium. p. 20. Glasgow: University Press.

Hyatt A. (1897). Cycle in the Life of the Individual (Ontogeny) and in the Evolution of Its Group (Phylogeny). Proc. Am. Arts Sci. 32: 209- 224.

Ilan J., Ilan J., and Patel N.G. (1972). Regulation of messenger RNA Translation Mediated by Juvenile Hormones. pp. 43- 68. New York: Academic Press.

Ingham P.W.(1988). The Molecular Genetics of Embryonic Pattern Formation in *Drosophila*. Nature (London). 335: 25- 34.

Ingham P.W., Taylor A.M. and Nakamo Y. (1991). Role of the *Drosophila patched* Gene in Positional Signalling. Nature (London). 353: 184- 187.

Ingham P.W. and Hidalgo A. (1993). Regulation of *wingless* Transcription in the *Drosophila* Embryo. Development 117: 283- 291.

Ingham P.W. (1993). Localized *hedgehog* Activity Controls Spatial Limits of *wingless* Transcription in the *Drosophila* embryo. Nature (London). 366: 560- 562.

Ip Y.T., Park R.E., Kosman D., Bier E., and Levine M. (1992). The *dorsal* Gradient Morphogen Regulates Stripes of *rhomboid* Expression in the Presumptive Neuroectoderm of the *Drosophila* Embryo. Genes Dev. 6: 1728- 1739.

Irvine K.D. and Wieschaus E. (1994). Cell Intercalation During *Drosophila* Germband Extension and Its Regulation by Pair-Rule Genes. Development 120: 827- 841.

Ish- Horowicz D., and Pinchin S.M. (1987). Pattern Abnormalities Induced by Ectopic Expression of *Drosophila* Gene *hairy* Are Associated with Repression of the *ftz* Transcription Cell 51: 405- 415.

Ish- Horowicz D., Fichin S.M., Ingham P.W., and Gyurkovics H.G. (1989). Autocatalytic *ftz* Activation and Metameric Instability Induced by Ectopic *ftz* Expression. Cell 57: 223- 232.

Ito H., Hamabata T., and Hori S (1993). Transcriptional Activation of the *Drosophila melanogaster* Glucose- 6-Phosphate Dehydrogenase Gene by Insertion of Defective P-elements. Mol.Gen.Genet. 241: 637- 646.

Ito K. and Hotta Y. (1992). Proliferation Pattern of Postembryonic Neuroblasts in the Brain of *Drosophila melanogaster*. Dev. Biol. 148: 134- 148.

Ito K. and Techanu G.M. (1993). Distribution and Development of Glia in the Ventral Nerve Cord of *Drosophila* Late Embryos and Larvae. *In* Davis R. and Zipursky L.(eds.): Neurobiology of *Drosophila*. New York Cold Spring Harbor Laboratory Press.

Jacobs J.R. (1993). Perturbed Glial Scaffold Formation Precedes Axon Tract Malformation in *Drosophila* Mutants. J.Neurobiol. 24(5): 611- 626.

Jacobs J.R. Hiromi Y., Patel N.P. and Goodman C.S. (1989). Lineage, Migration, and Morphogenesis of Longitudinal Glia in the *Drosophila* CNS as revealed by a Molecular Lineage Marker. Neuron 2: 1625- 1631.

Jack T. and McGinnis W. (1990). Establishment of the *deformed* Expression Stripe Requires the Combinatorial Action of Coordinate, Gap and Pair Rule Proteins. EMBO J. 9: 1187-1198.

Jan L.Y. and Jan N.J. (1982). Antibodies to Horse Radish Peroxidase as Specific Neuronal Markers in *Drosophila* and Grasshopper Embryos . Proc. Natl. Acad. USA. 79: 2700- 2704.

Jan N.J. and Jan L.Y. (1990). Genes Required for Specifying Cell Fates in *Drosophila* Embryonic Sensory Nervous System. Trends Neurosci. 13: 493- 498.

Jennings B., White R.A.H., Delidakis C., and Bray S.J. (1993). What Do the Patterns of Expression of *E(Spl)* Helix-Loop-Helix Proteins Tell Us About Their Function? *In* Davis R. and Zipursky L.(eds.): Neurobiology of *Drosophila*. New York : Cold Spring Harbor Laboratory Press.

Jiang J. and Levine M. (1993). Binding Affinities and Cooperative Interactions with bHLH Activators Delimit Threshold Responses to the *Dorsal* Gradient Morphogen. Cell. 72: 741- 752.

Jiang J., Rushlow C.A., Zhou Q., Small S., and Levine M. (1992). Individual Dorsal Morphogen Binding Sites Mediate Activation and Repression in the *Drosophila* Embryo. EMBO J. 11: 3147- 3154.

Jiménez F., Carmena A., Martín-Bermudo M.D., de la Escalera S., Gómez J. M., and Velasco L. (1993). Cell Adesion and Neural Development in *Drosophila*: Analysis of *NEUROTACTIN*. In Davis R. and Zipursky L. (eds.). Neurobiology of *Drosophila*. New York: Cold Spring Harbor Press.

Johansen J., Halpern M.E., Johansen K.M. and Keshishian H. (1989). Stereotypic Morphology of Glutamateric Synapses on Identified Muscle Cells of *Drosophila* larvae. J. Neurosci. 9: 70- 725.

Johnson W.A., McCormick C.A., Bray S.J., and Hirsh J. (1989). A Neuron -Specific Enhancer of the *Drosophila dopa* Decarboxylase Gene. **Genes Dev.** 3: 676- 686.

Jones B. and McGinnis W. (1993). A new *Drosophila* Homeobox Gene, *bsh*, Is Expressed in a Subset of Brain Cells during Embryogenesis. **Development** 117: 793- 806.

Kaang B.-K., Kandel E.R., and Grant S.G.N. (1993). Activation of cAMP-Responsive Genes by Stimuli That Produce Long Term Facilitation in *Aplysia* Sensory Neurons. **Neuron** 10: 427- 435.

Kaissling K.E. (1986). Chemo- electrical Transduction in Insect Olfactory Receptors. **Ann. Review Neurosci.** 9: 121- 145.

Kaissling K-E. (1994). IP_3 Effects in Moth Pheromone Receptors: Calculations. *In* Elsner N. and Breer H. (eds): **Sensory Transduction Proceedings of the 22nd Göttingen Neurobiology Conference 1994, Vol. I,** p. 95. **Stuttgart- New York: Thiemme Verlag.**

Kaissling K.E. and Boekhoff I. (1993). *In* Wiese K, Gribakin F.G., Popov A.V., and Renninger G. (eds): **Sensory Systems of Arthropods.** pp. 489- 502. **Basel, Boston, Berlin: Birkhäuser Verlag**

Kaissling K.E. and Priesner E. (1970). Riechschwelle des Seidenspinners. **Naturwissenschaften.** 57: 23 -28.

Kalb J.M., DiBenedetto A.J., and Wolfner M.F. (1993). Probing the Function of *Drosophila melanogaster* Accessory Glands by Directed Cell Ablation. **Proc. Natl. Acad. Sci. USA.** 90: 8093 - 8097.

Kanaujia S., and Kaissling K.E. (1985). Interactions of Pheromone with Moth Antennae: Adsorption, Desorption and Transport. **J. Insect Physiol.** 31: 71- 81.

Kankel D.R. and White K. (1993). Developmental Biology of the Fruitfly *Drosophila melanogaster*. **J. Neurobiol.** 24: 699- 700.

Kanzaki R., Ikeda A., and Shibuya T. (1994). Morphological and Physiological Properties of the Pheromone- Triggered Flip-flopping Descending Interneurones of the Male Silkworm Moth, *Bombyx mori*. **J.Comp. Physiol. A.** 175: 1- 14.

Karim F. and Thummel F. (1992). Temporal Coordination of Regulatory Gene Expression by the Steroid Hormone Ecdysone. **EMBO J.** 11: 4083- 40 93.

Karlson P. and Butenandt A. (1959). Pheromones (Ectohormones) in Insects. **Ann. Rev. Entomol.** 4: 39- 58.

Karlson P., Doenecke D., and Koolman J. (1994). Kurzes Lehrbuch der Biochemie für Mediziner und Naturwissenschaftler. 14th Edition. **Stuttgart-New York: Georg Thieme Verlag.**

Karlström R.O., Wilder L.P., Bastiani M.J. (1993). *Lachesin*: An Immunoglobulin Superfamily Protein Whose Expression Correlates With Neurogenesis in Grasshopper Embryos. **Development** 118: 509- 522.

Kaufman P.D., Doll R.F., and Rio D.C. (1989). *Drosophila* P Element Transposase Recognises Internal P Element Sequences. **Cell** 59: 359- 371.

Kaufman P.D. and Rio D.C. (1991). *Drosophila* P-Element Transposase Is a Transcriptional Repressor *in vitro*. **Proc. Natl. Acad. Sci. USA.** 88: 2613-2617.

Kaufman T.C., Seeger M.A., and Olson G. (1990). Molecular and Genetic Organisation of the *Antennapedia* Gene Complex of *Drosophila melanogaster*. **Adv. Genet.** 27: 309- 362.

Kennedy T.E., Kuhl D., Barzilai A., Sweatt J.D., and Kandel E.R. (1992). Long Term Sensitisation in *Aplysia* Leads to an Increase in Calreticulin, a Major Presynaptic Calcium-Binding Protein. **Neuron** 9: 1013-1024.

Kenyon F.C. (1898). The Brain of the Bee. **J.Comp. Neurol.** 6: 133- 210.

Kent K.S., Hoskins S.G., and Hildebrand J.G. (1987). A Novel Serotonin - Immunoreactive Neuron in the Antennal Lobe of the Sphinx Moth *Manduca sexta* Persists Throughout Postembryonic Life. **J.Neurobiol.** 18: 451- 465.

Keshishian H., Chibra A., Chang T.N., Halfon M.S., Harkins E.W., Jarecki J., Wang L., Anderson M.-D., Cash S., Halpern M.E., and Johansen J. (1993). Cellular Mechanisms Governing Synaptic Development in *Drosophila melanogaster*. **J.Neurobiol.** 24: 757 -787.

Kidwell M.G., Kidwell F.J., and Sved J.A. (1977). Hybrid Dysgenesis in *Drosophila melanogaster*: A Syndrome of Aberrant Traits Including Mutation, Sterility and Male Recombination. **Genetics** 86: 813- 833.

Kilby N.J., Snaith M.R., and Murray J.A.H. (1993). Site-Specific Recombinases Tools for Genome Engineering. **Trends Gen.** 9: 413- 421.

Klämpt C., Jacobs J.R., and Goodman C.S. (1991). The Midline of the *Drosophila* Central Nervous System: A Model for the Genetic Analysis of Cell Fate, Cell Migration, and Growth Cone Guidance. **Cell** 64: 801- 815.

Klämpt C. (1993). The *Drosophila* Gene *pointed* Encodes Two ETS-Like Proteins which Are Involved in the Development of the Midline Glial Cells. **Development** 117: 163- 176.

Klausner R.D. and Samelson L.E. (1991). T Cell Antigen Receptor Activation Pathways: The Tyrosine Kinase Connection. **Cell** 64: 875- 878. [review]

Klein T. and Campos-Ortega J.A. (1992). Second-site Modifiers of the *Delta* Wing Phenotype in *Drosophila melanogaster*. **Roux's Arch. Dev. Biol.** 202: 49-60.

Klingler M., and Gergen J.P. (1993). Regulation of *runt* Transcription by *Drosophila* Segmentation Genes. **Mech. Dev.** 43: 3- 19.

Kidd S., Kelly M.R. and Young M.W. (1986). Sequence of the *Notch* Locus of *Drosophila melanogaster* relationship of the Encoded Protein to Mammalian Clotting and growth Factors. **Mol. Cell. Biol.** 6: 3094-3108.

Kidd S. (1992). Characterisation of the *Drosophila cactus* Locus and Analysis of Interactions Between *Cactus* and *Dorsal* Proteins. **Cell** 71: 623- 635.

Kidd S., Baylies M.K., Gasic G.P. and Young M.W. (1989). Structure and Distribution of the *Notch* Protein in Developing *Drosophila*. **Genes Dev.** 3: 1113- 1129.

Kidwell M.G. (1985). Hybrid Dysgensis in *Drosophila melanogaster*: Nature and Inheritance of the P element Regulation. **Genetics** 111: 337- 350.

Kim Y.-J. and Baker B.S. (1993). The *Drosophila* Gene *rbp9* Encodes a Protein that Is a Member of a Conserved Group of Putative RNA Binding Proteins that Are Nervous System Specific in Both Flies and Humans. **J. Neurosci.** 13: 1045- 1056.

Kim Y.-J., Zuo P., Mandley J.L., and Baker B. (1992). The *Drosophila* RNA-Binding Protein RBP1 Is Localised to Transcriptionally Active Sites of Chromosomes and Shows a Functional Similarity to Human Splicing Factor ASF/SF2. **Genes Dev.** 6: 2569- 2579.

Klaes A., Menne T., Stollewrk A., Scholz H., and Klämpt C. (1994). The *Ets* Transcription Factors Encoded by the Drosophila Gene *pointed* Direct Glial Cell Differentiation in the Embryonic CNS. **Cell** 78: 149- 160.

Knoblich J.A., Sauer C., Jones L., Richardson H., Saint R., and Lehmer C.F. (1994). Cyclin E Controls S Phase Progression and Its Down- Regulation during *Drosophila* Embryogenesis Is Required for the Arrest of Cell Proliferation. **Cell.** 77: 107- 120.

Knust E., Schrons H., Grawe F., and Campos-Ortega J.A.(1992). Seven Genes of the *Enhancer of Split Complex* of *Drosophila melanogaster* Encode Helix-Loop-Helix Proteins. **Genetics** 132: 505-518.

Knust E., Tietze K. and Campos-Ortega J.A. (1987). Molecular Analysis of the Neurogenetic Locus *Enhancer of Split* in *Drosophila melanogaster*. **EMBO J.** 6: 4113-4123.

Kobashyshi S., Kitamura T., Sasaki H., and Okada M. (1993) Two Types of Pole Cells Are Present in the *Drosophila* Embryo, One with and one without Splicing Activity for the Third P-element. **Development** 117: 885- 893.

Koelle M.R., Talbot W.S., Segraves W.A., Bender M.T., Cherbas P., and Hogness D.S. (1991). The *Drosophila EcR* Gene Encodes an Ecdysone Receptor, a New Member of the Steroid Receptor Superfamily. **Cell** 67: 59 -77.

Kolodkin A.L., Matthes D.J., O'Connor T.P., Patel N., Admon A., Bentlry D., and Goodman C.S. (1992). *Fasciclin IV*: Sequence Expression, and Function During Growth Cone Guidance in Grasshopper Embryo. **Neuron** 9: 831- 845.

Kolodkin A.L., Matthes D.J., and Goodman C.S. (1993). The *Semaphorin* Genes in *Drosophila* Identify a New Family of Transmembrane and Secreted Growth Cone Guidance. *In* Davis R. and Zipurski L. (eds.): **Neurobiology of *Drosophila*.** p. 18. **New York: Cold Spring Harbor Press.**

Kolodkin A.L., Pickup A., Lin D.M., Goodman C.S., and Banerjee U. (1994). Characterisation of *Star* and Its Interactions with *sevenless* and *EGF Receptor* During Photoreceptor Cell Development in *Drosophila*. **Development** 120: 1731- 1745.

Kooh P.J., Fehon R.G., and Muskavitch M.A.T. (1993). Implications of Dynamic Patterns of *Delta* and *Notch* Expression for Cellular Interactions During *Drosophila* Development. **Development** 117: 493- 507.

Kovalick G.E. and Beckingham K. (1992). Calmodulin Transcription Is Limited to the Nervous System during *Drosophila* Embryogenesis. **Dev. Biol.** 150: 33- 46.

Kramatschek B. and Campos- Ortega (1994). Neurodermal Transcription of the *Drosophila* Neurogenic Genes *E(spl)* and *HLHm-5* is Regulated by Proneural Genes. **Development** 120: 815- 826.

Krause G. (1939). Die Eitypen der Insekten. **Biol. Zentralbl.** 59: 495- 536.

Kreissl S. and Bicker G. (1989). Histochemistry of Acetylcholinesterase and Immunocytochemistry of an Acetylcholine Receptor Like Antigen in the Brain of the Honeybee. **J. Comp. Neurol.** 286: 71- 84.

Krumlauf R. (1993). *Hox* Genes and Pattern Formation in the Branchial Region of the Vertebrate Head. **Trends Genet.** 9: 106- 112.

Lancet D. (1994a). Olfactory Receptors: A Gene Superfamily with a Brain Interface. *In* Elsner N. and Breer H. (eds.): **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I.** pp. 63- 77. **Stuttgart- New York: Georg Thieme Verlag.**

Lancet D. (1994b). Exclusive Receptors. **Nature (London).** 372: 321- 322. [review]

Landolt A.M. (1965). Elektronenmikroskopische Untersuchungen an der Perikaryenschicht der Corpora pendunculata der Waldameise (*Formica lugubris* Zettelmeyer) mit besonderer Berücksichtigung der Neuron-Glia Beziehung. **Z.Zellforsch.** 66: 701- 736.

Landolt A.M. and Ris (1966). Electron Microscope Studies on Soma-Somatic Interneuronal Junctions in the Wood Ant (*Formica lugbris* Zettelmeyer). **J. Cell Biol.** 28: 391- 403.

Lane N. (1974). The Organisation of the Insect Nervous System. *In* Neuberger A. and Tatum E.L. (ser. eds): **North Holland Research Monographs.** Treherne J.E. (ed): **Insect Neurobiology**. pp. 1- 71. **Amsterdam-Oxford: North Holland Publishing Company.**

Lane N.J. and Treherne J.E.(1972). Studies on Perineural Junctional Complexes and the Sites of Uptake of Microperoxidase and Lanthanum in the Cockroach Central Nervous System. **Tissue Cell** 4: 427- 426.

Laski F.A., Rio D.C. and Rubin G.M. (1986). Tissue Specificity of *Drosophila* P-Element Transposition Is Regulated at the Level of mRNA Splicing. **Cell** 44: 7- 19.

Laski F.A. and Rubin G.M. (1989). Analysis of *cis*-Acting Requirements for Germ-Line-Specific Splicing of the P-element ORF2-ORF3 Intron. **Genes Dev.** 3: 720- 728.

Laurent G. and Davidowitz H. (1994). Encoding of Olfactory Information with Oscillating Neural Assemblies. **Science** 265: 1872- 1874.

Laurent G. and Naraghi M. (1994). Odorant- Induced Oscillations in the Mushroom Bodies of the Locust. **J.Neurosci.** 14: 2993- 3004.

Lavorgana G., Karim F., Thummel C.S., and Wu C. (1993). Potential Role for a FTZ-F1 Steroid Receptor Superfamily Member in the Control of *Drosophila* Metamorphosis. **Proc. Natl. Acad. Sci. USA.** 90: 3004- 3008.

Lawrence P.A. and Morata G. (1976). Compartments in the Wing of *Drosophila*: A Study of the *engrailed* Gene. **Dev. Biol.** 50: 321- 337.

Lehner C.F. and O'Farell P.H. (1990). The Roles of *Drosophila* Cyclins A and B in Mitotic Control. **Cell** 61: 525- 547.

Lemaître B., Ronssery S., and Coen D. (1993). Maternal Repression of the P-element Promoter in the Germline of *Drosophila melanogaster*: A Model for the P Cytotype. **Genetics** 135: 149- 160.

Leung H.-T. and Byerly L. (1991). Characterisation of Single Calcium Channels in *Drosophila* Embryonic Nerve and Muscle Cells. **J. Neurosci.** 11: 3047- 3059.

Levine M.S. and Hardin K.W. (1989). *Drosophila*: The Zygotic Contribution. *In* Glover D.M. and Hames B.D.(eds.): **Genes and Embryos**. pp. 39- 94. **Oxford: IRL Press/ Oxford University Press.**

Lepesant J.-A., Levine M., Garen A., Lepesent-Kejzlarvoa J., Rat L., and Somme-Martin G. (1982). Developmentally-Regulated Gene Expression in *Drosophila* Larval Fat Bodies. **J. Mol. Appl. Genet.** 11: 371-383.

Levine A., Bashan-Ahrend, Budai-Hadrian O., Gartenberg D., Menasherow, and Wides R. (1994). oddOZ: A Novel *Drosophila* Pair-Rule Gene. **Cell** 77: 587-698. [israel]

Lewis E.B. (1978). A Gene Complex Controlling Segmentation in *Drosophila*. **Nature (London).** 276: 656- 570.

Li X. and Noll M. (1993). Pole of the *gooseberry* Gene in *Drosophila* Embryos: Maintenance of *wingless* expression by a *wingless- gooseberry* Autoregulatory Loop. **EMBO J.** 12(12): 4499- 4509.

Li X. and Noll M. (1994). Evolution of Distinct Developmental Functions of Three *Drosophila* Genes by Acquisition of Different cis-Regulatory Regions. **Nature (London)** 367: 83- 87.

Lieber T., Kidd S., Alcamo E., Corbin V., and Young M.W. (1993). Antineurogenic Phenotypes Induced By Truncated *Notch* Proteins Indicate a Role in Signal Transduction and May Point to a Novel Function For *Notch* in Nuclei. **Genes Dev.** 7: 1949-1965

Limbourg- Bouchon B., Busson D., and Lamour-Isnard C. (1991). Interactions between *fused*, a Segment Polarity Gene in *Drosophila*, and Other Segmentation Genes. **Development** 112: 417- 429.

Lin H., Song K., Hutcheson G.A., Goutte C.E., and Wolfner M.F. (1991). A Maternally Encoded Nuclear Envelope Protein Required for Embryonic Mitosis in *Drosophila*. **Cold Spring Harbor Symp. Quant. Biol.** Vol. LVI.: 719- 727.

Lindsey D.L. and Zimm G.G. (1992). **The Genome of *Drosophila melanogaster*. San Diego, California: Academic Press**

Liu W., Yoon J., Burg M., Chen L., and Pak W.L. (1993). Receptor-Type Guanylate Cyclase cDNA of *Drosophila*. In Davis R. and Zipursky L. (eds.). **Neurobiology of *Drosophila*. Cold Spring Harbor Laboratory Press.**

Livingstone M.S. (1984). Loss of Calcium/Calmodulin Responsiveness in Adenylate Cyclase of *rutabega*, a *Drosophila* Memory Mutant. **Cell.** 37: 205- 215.

Livingstone M. and Tempel B. (1983). Genetic Dissection of Monoamine Neurotransmitter Synthesis in *Drosophila*. **Nature** 303: 67- 70.

Lonie A., D'Andrea R., Paro R., and Saint R. (1994). Molecular Characterisation of the *Polycomblike* Gene of *Drosophila melanogaster*, a *Trans*- Acting Negative Regulator of Homeotic Gene Expression. **Development** 120: 2629- 2636.

Lundell M.J. and Hirsh J. (1994). Temporal and Spatial Development of Serotonin and Dopamine Neurons in the *Drosophila* CNS. **Dev. Biol.** 165: 385- 396.

Luo Y., Raible D., and Raper J.A. (1993). *Collapsin*: A Protein in Brain That Induces the Collapse and Paralysis of Neuronal Growth Cones. **Cell** 75: 217- 227.

Lyman D.and Young M.W. (1993). Further Evidence for Function of the *Drosophila Notch* Protein as a Transmembrane Receptor. **Proc. Natl. Acad. Sci. USA.** 90: 10395- 10399.

Macdonald P.M., Kerr K., Smith J.L., and Leask A. (1993). RNA Regulation Element BLE1 Directs the Early Steps of *b icoid* mRNA Localisation. **Development** 118: 1233- 1243.

Macnab R.M. (1987). Motility and Chemotaxis. *In* Neidhardt et al. (eds.): ***Escherichia coli* and *Salmonella tryphimurium*: Cellular and Molecular Biology.** pp. 732- 739. **Washington D.C.: American Society for Microbiology Press.**

Maddock J.R. and Shapiro L. (1993). Polar Location of the Chemoreceptor Complex in the *Escherichia coli* Cell. **Science** 259: 1717- 1723.

Maier D. and Preiss A. (1993). Studies on the Structure Function- Relationship and the Distribution of the *Hairless* Gene Product. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 115. **New York Cold Spring Harbor Laboratory Press.**

Manoukian A.S. and Krause H.M. (1992). Concentration - Dependent Activities of the *even-skipped* Protein in *Drosophila* Embryos. **Genes Dev.** 6: 1740- 1751.

Martin J.-R., Raibaud A, and Ollo R. (1994). Terminal Pattern Elements in *Drosophila* Embryo Induced By the *torso-like* Protein . **Nature** 367: 741- 745.

Martín-Bermudo M.D., Gonzáles F., Domínguez M., Rodríguez I., Ruiz-Gómez M., Romani S., Modolell J., and Jiménez F. (1993). Molecular Characterisation of the *lethal of scute* Genetic Function. **Development** 118: 1003- 1012.

Martínez- Arias A., Baker N., and Ingham P.(1988). The Role of Segment Polarity Genes in the Definition and Maintenance of Cell States in the *Drosophila* Embryo. **Development.** 103: 157- 170.

Matsumoto T., Nakane M., Pollock J.S., Kuk J.E., and Förstermann U. (1993a). A Correlation between

Soluble Brian Nitric Oxide Synthase and NADPH- diaphorase Activity Is only Seen After Exposure of the Tissue to Fixative. Neurosci. Lett. 155: 61- 64.

Matsumoto T., Pollock J.S., Nakane M., and Förstermann U. (1993b). Developmental Changes of Cytosolic and Particulate Nitric Oxide Synthase in the Brain. Dev. Brain Res. 73: 199- 203.

Maulshagen J. (1993). Neural Correlates of Olfactory Learning Paradigms in an Identified Neuron in the Honey Bee Brain. J. Neurophysiology. 69: 609- 625.

Mayeda A., Zahler A.M, Krainer A.R., and Roth M.B. (1992). Two Members of Conserved Family of Nuclear Phoshoproteins Are Involved in pre-mRNA Splicing. Proc. Natl. Acad. Sci. USA 89: 1301- 1304.

Mayford M., Barzilai A., Keller F., Schacher S., and Kandel E.R. (1992). Modulation of an NCAM-Related Adhesion Molecule with Long-Term Synaptic Plasticity in *Aplysia*. Science 256: 638- 644.

McCormick and Nichols R. (1993). Spatial and Temporal Expression Identify Dromyosuppressin as a Brain -Gut Peptide in *Drosophila melanogaster*. J. Comp. Neurol. 338: 279- 288.

McKnight S. (1980). The Nucleotide Sequence and Transcript Map of the Herpes Simplex Virus Kinase Gene. Nucl. Acid. Res. 8: 5949- 5964.

McMahon A.P. and Bradley A. (1990). The *Wnt-1* (*int-1*) Proto-Oncogene is Required for the Development of a Large Region of the Mouse Brain. Cell 62: 1073- 1085.

McNabb S. and Truman J.W. (1993). Distribution of Ecdysone Receptors in the Embryonic CNS. *In* Davis R. and Zipurski L. (eds.). Neurobiology of *Drosophila*. p. 47 . New York: Cold Spring Harbor Laborary Press.

Menne T.V. and Kämpt C. (1994). The Formation of Commissures in the *Drosophila* CNS Depends on the Midline Cells and on the *Notch* Gene. Development 120: 123- 133.

Menzel R. (1990). Learning, Memory, and "Cognition" in Honey Bees. *In* Neurobiology of Comparative Cognition. Kesner R.P. and Olton D.S (eds). Neurobiology of Comparative Cognition. pp. 237- 292. Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Merriam J., Ashburner M., Hartl D.L. and Kafatos F.C. (1991). Toward Cloning and Mapping the Genome of *Drosophila*. Science 254: 221- 225.

Merry D.E., Veis D.J., Hickeny W.F., and Korsmeyer S.J. (1994). *bcl-2* Protein Expression is Widespread in the Developing Nervous System and Retained in the Adult PNS. Development 120: 301- 311.

Miklos G.L.G. (1993). Molecules and Cognition: The Latterday Lessons of Levels, Language, and *lac*. Evolutionary Overview of Brain Structure and Function in Some Vertebrates and Invertebrates. J.Neurobiol. 24: 842- 890.

Miller C.A. and Benzer S. (1983). Monoclonal Antibody Cross Reactions Between *Drosophila* and the Human Brain. Proc. Natl. Acad. Sci. USA 80: 7641- 7645.

Miller P.L. (1974). Rhythmic Activities and the Insect Nervous System. *In* Barton Browne L. (ed).: Experimental Analysis of Insect Behaviour. p.115- 138. Berlin- Heidelberg- New York: Springer Verlag.

Miller J.H. (1992). A Short Course in Bacteria Genetics. A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria. New York : Cold Spring Harbor Laboratory Press.

Miller S.L. (1987). Which Organic Compounds Could Have Occurred on the Prebiotic Earth? Cold Spring Harb. Symp. Quant. Biol. LII : 17- 27.

Misra S. and Rio D.C. (1990). Cytotype Control of *Drosophila* P-element Transposition: The 66kd Protein Is a Repressor of Transposase Activity. Cell 62: 269- 284.

Misra S., Buratowski R.M., Ohkawa T., and Rio D.C. (1993). Cytotype Control of *Drosophila melanogaster* P Element Transposition: Genomic Position Determines Maternal Repression. Genetics 135: 785- 800.

Moazed D. and O'Farrell P.H. (1992). Maintenance of *engrailed* Expression Pattern by *Polycomb* Group Genes in *Drosophila*. Development 116: 805- 810.

Mobbs P.G. (1982). The Brain of the Honeybee *Apis mellifera*. I. The Connections and Spatial Organisations of the Mushroom Bodies. Philos. Trans. R. Soc. B (London) 298: 309- 354.

Mobbs P.G. (1984). Neural Networks in the Mushroom Bodies of the Honeybee. J. Insect Physiol. 1: 43- 58.

Moehrle A., and Paro R. (1994). Spreading the Silence: Epigenetic Transcriptional Regulation During *Drosophila* Development. Dev.. Genetics. 15: 478- 484. [review]

Moffat K.G., Gould J.H., Smith H.K., and O'Kane C.J. (1992). Inducible Cell Ablation in *Drosophila* by Cold-Sensitive Ricin A Chain. Development 114: 681- 687.

Müller U. (1994). Biochemical and Histochemical Characterisation of Nitric Oxide Synthase in *Apis mellifera* and *Drosophila melanogaster*. *In* Elsner N. and Breer H. (eds.): Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II. p.654. Stuttgart- New York: Georg Thieme Verlag.

Mullins M.C., Rio D.C., and Rubin G.M. (1989). *Cis*-Acting DNA Sequence Requirements for P-element Transposition. Genes Dev. 3: 729- 738.

Murphey R.K. (1986). The Myth of the Inflexible Invertebrate: Connection and Synaptic Remodelling in the Development of Invertebrate Nervous Systems. J. Neurobiol. 17: 585- 591.

Murray J.D. (1989). Mathematical Biology. New York- Berlin: Springer Verlag.

Murray N.E., Bruce S.A., and Murray K. (1979). Molecular Cloning of the DNA Ligase Gene from Bacteriophage T4. I. Amplification and Preparation of the Gene Product. J. Molec. Biol. 132: 493- 505.

Murthy K.G.K. and Manley J.L. (1992). Characterisation of the Mulltisubunit Cleave-

Polyadenylation Specificity Factor from Calf Thymus. J. Biol. Chem. 267: 14804- 14811.

Myers P.Z. and Bastiani M.J. (1993a). Cell- Cell Interactions During the Migration of an Identified Commissural Growth Cone in the Embryonic Grasshopper. J.Neurosci. 13: 115- 126.

Myers P.Z. and Bastiani M.J. (1993b). Growth Cone Dynamics During Migration of an Identified Commissural Growth Cone. J.Neurosci. 13(1): 127- 143.

Nakano Y.L., Guerrero L., Hidalgo A., Taylor A., Whittle J., and Ingham P. W. (1989). A Protein with Several Possible Membrane Spanning Domains Encoded by the *Drosophila* Segment Polarity Gene *patched*. Nature 341: 508- 513.

Nakayama N., Kim Y., Webber K., Lad R., and Nierenberg M. (1993). Regulation of the NK-2 Homeobox Gene in the Developing Nervous System. *In* Davis R. and Zipursky L.(eds.): Neurobiology of *Drosophila*. New York Cold Spring Harbor Laboratory Press.

Nambu J.R., Franks R.G., Hu S., and Crews S.T. (1990). The *single-minded* Gene of *Drosophila* Is Required for the Expression of Genes Important for the Development of CNS Midline Cells . Cell 63: 63- 75.

Nambu J.R., Lewis J.O., Wharton K.A. Jr., and Crews S.T. (1990). The *Drosophila single-minded* Gene Encodes a Helix-Loop-Helix Protein That Acts as a Master Regulator of CNS Midline Development. Cell 67: 1157- 1167.

Nara T., Lee L., and Imae Y. (1991). Thermosening Ability of the *TRG* and *TAP* Chemoreceptors in *Esdiericia coli*. J. Bacteriol. 173: 1120- 1124.,

Nassif N. and Engels W. (1993). DNA Homology Requirements for Mitotic Gap Repair in *Drosophila*. Proc. Natl. Acad. USA. 90: 1262- 1266.

Neel V. A. and Young M.W. (1994). *igloo*, a GAP-43- Related Gene Expressed in the Developing Nervous System of *Drosophila*. Development 120: 2235- 2243.

Nelson H.B. and Laughon (1993). *Drosophila* Glia Architecture and Development: Analysis Using a Collection of New Cell- Specific Markers. Roux's Arch. Dev. Biol. 202: 341- 354.

Neufeld T.P. and Rubin G.M. (1984). The *Drosophila peanut* Gene Is Required for Cytokinesis and Encodes for Cytokinesis and Encodes a Protein Similar to Yeast Putative Bud Neck Filament Proteins. Cell 77: 371- 379.

Nishida Y., Hata M., Ayaki T., Ryo H., Yamagata M., Shimizu K., and Nishizuka Y. (1988). Proliferation of Both Somatic and Germ Cells Is Affected in the *Drosophila* Mutants of *raf* Protooncogene. EMBO J. 7: 775- 781.

Nobes C. and Hall A. (1994). Regulation and Function of the *Rho* Subfamily of Small GTPases. Curr. Opin. Gen. Dev. 4: 77- 81.[review]

Noll E., Perkins L.A., Mahowald A., and Perrimon N. (1993). Approaches to Identify Genes Involved in *Drosophila* Embryonic Development. J. Neurobiol. 24: 701- 722.

Noll R., Sturtevant M.A., Gollapudi R.R., and Bier E. (1994). New Functions of the *Drosophila rhomboid* Gene During Embryonic and Adult Development Are Revealed by a Novel Genetic Method, Enhancer Piracy. Development 120: 2329- 2338.

Noodermeer J., Klingensmith J., Perrimon N. and Nusse R. (1994). *dishevelled* and *armadillo* Act in the *Wingless* Signalling Pathway in *Drosophila* . Nature (London) 367: 80- 83.

Nüsslein- Volhard C. and Wieshaus E. (1980). Mutations Affecting Segment Number and Polarity in *Drosophila*. Nature. 287: 795- 801.

Nguyen P.V., Abel T., and Kandel E.R. (1994). Requirement of a Critical Perod of Transcription for Induction of Late Phase of LTP. Science 265: 1104- 1107.

Oda H., Uemura T., Harada Y., Iwai Y., and Takkeichi M. (1994). A *Drosophila* Homolog of Cadherin Associated with *Armadillo* and Essential for Embryonic Cell- Cell Adhesion. Dev. Biol. 165: 716- 726.

O'Farrell (1992). Cell Cycle Control, Many Ways to Skin a Cat. TICB 2: 159- 163.

O'Hare K., Driever A., McGrath S. and Johnston- Schlitz D.M. (1992). Distribution and Structure of Cloned P elements From the *Drosophila melanogaster* P strain p_2. Genet. Res. Camb. 60: 33- 41.

O'Hare K. and Rubin G.M. (1983). Structures of P Transposable Elements and Their Sites of Insertion and Excision in the *Drosophila melanogaster* Genome. Cell 34: 25- 35.

O'Kane C. and Gehring W.J. (1987). Detection *in situ* of Genomic Regulatory Elements in *Drosophila*. Proc. Natl. Acad. Sci. USA. 84: 9123- 9127.

Oland L.A., Tiolbert L.P., and Mossman K.L. (1988). Radiation Induced Reduction of the Glial Population during Development Disrupts the Formation of Olfactory Glomeruli in an Insect. J. Neurosci. 8: 353- 367.

Oland L.A. and Hayashi J.H. (1993). Effects of the Steroid Hormone 20-Hydroyecdysone and Prior sensory Input on the Survival and Growth of the Moth Central Olfactory Neurons *in vitro*. J. Neurobiol. 24: 1170- 1186.

Olivier J.P., Raabe T., Henkemeyer M., Dickson B., Mbamalu G., Margolis B., Schlessinger J., Hafen E., and Pawson T. (1993). A *Drosophila* SH2-SH3 Adaptor Protein Implicated in the Coupling of the *sevenless* Tyrosine Kinase to an Activator of Ras Guanine Exchange, Sos. Cell 179- 191.

O'Neill E.M., Rebay I., Tjian R., and Rubin G.M. (1994). The Activities of Two Ets- Related Transcription Factors Required for *Drosophila* Eye Development Are Moulated by the Ras/MAPK Pathway. Cell 78: 137- 147.

Orchard I. (1982). Octopamine in Insects: Neurotransmitter, Neurohormone, and Neuromodualtor. Can. J. Zool. 60: 659- 669. [review]

Ouellette. R.J., Valet, J.-P., and Côté S. (1992). Expression of *goseberry-proximal* in the *Drosophila* Developing Nervous System Responds to Cues Provided by Segment Polarity Genes. Roux's Arch. Dev. Biol. 201: 157-168.

Ozyhar A., Strangmann-Diekmann M., Kiltz H.-H., and Pongs O. (1991). Characterisation of a Specific Ecdysteroid Receptor-DNA Complex Reveals Common Properties for Invertebrate and Vertebrate Hormone- Receptor/ DNA Interactions. **Eur. J. Biochem.** 200: 329- 335.

Pankratz M.J., Hoch M., Seifert E., and Jäckle H. (1989). Gradients of *Krüppel* and *Knirps* Gene Products Direct Pair Rule Stripe Pattern in the Posterior Region of the *Drosophila* Embryo. **Cell** 61: 309- 317.

Paro R. (1990). Imprinting a Determined State into Chromatin of *Drosophila*. **Trends Gen.** 6: 416- 422. [review]

Paro R., and Hogness D. (1991). The *Polycomb* Protein Shares a Homologous Domain with a Heterochromatin-Associated Protein of *Drosophila*. **Proc. Natl.Acad. Sci. USA** 88: 263- 267.

Paroush Z., Finley R.L. Jr., Kid T., Wainwright S.M., Ingham P.W., Brent R., and Ish- Horowicz D. (1994). *Groucho* Is Required for *Drosophila* Neurogenesis, Segmentation, and Sex Differentiation sand Interacts Directly with Hairy- Related bHLH Proteins. **Cell** 79: 805- 815.

Patel N.H., Schäfer B., Goodman C.S., and Holmgren R. (1989b). The Role of Segment Polarity Genes During *Drosophila* Neurogenesis. **Genes Dev.** 3: 890- 904.

Patel N.H., Schäfer B., Goodman C.S., and Holmgren R. (1989). The Role of the Segment Polarity Genes During *Drosophila* Neurogenesis. **Genes Dev.** 3: 955- 968.

Peifer M., Sweeton D., Casey M., and Wieschaus E. (1994). *wingless Signal* and *Zeste-white 3 Kinase* Trigger Opposing Changes in the Intracellular Distribution of *Armadillo*. **Development** 120: 369- 360.

Perkins L.A., Larsen I., and Perrimon N. (1992). *corkscrew* Encodes a Putative Protein Tyrosine Phosphatase that Functions to Transduce the Terminal Signal from the Receptor Tyrosine Kinase *torso*. **Cell** 70: 225- 236.

Petzhold J. and Labhart, T. (1994). Modelling Polarisation-Opponent Interneurons of Insects: Responses to the Polarisation Pattern of the Sky. *In* Elsner N. and Breer H. (eds.): **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II** p.466. **Stuttgart- New York: Georg Thieme Verlag.**

Peunova N. and Enikolopov G. (1993). Amplification of Calcium Induced Transcription by Nitric Oxide in Neuronal Cells. **Nature (London)** 364: 450- 453.

Phillis R.W. and Murphey R.K. (1993). Genetic and Molecular Analysis of *midline uncoordinated*, A Mutation that Alters Coordination and Axon Projection Patterns. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 54. **New York Cold Spring Harbor Laboratory Press.**

Pignoni F., Balderarelli R.M., Steingrimsson E., Diaz R.J., Patapoutian A., Merriam J.R., and Langyie J.A. (1990). The *Drosophila* Gene *Tailless* Is Expressed at the Embryonic Termini and Is a Member of the Steroid Receptor Family. **Cell** 62: 151-163.

Pikielny C.W., Hasan G., Rouyer F., and Rosbash M. (1993). cDNA Clones Present in a PCR Based Subtractive Library Reveal Spatial Organisation of the *Drosophila* Olfactory Apparatus. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 150. **New York Cold Spring Harbor Laboratory Press.**

Pikielny C.W., Hasan G., Rouyer F., and Rosbash M. (1994). Members of a Family of *Drosophila* Putative Odorant- Binding Proteins Are Expressed in Different Subsets of Olfactory Hairs. **Neuron** 12: 35- 49.

Pongs O., Kecskemethy N., Müller R., Krah-Jentgens I., Baumann A., Kiltz H.H., Canal I., Llamazares S., and Ferrus A. (1988). *Shaker* Encodes a Family of Putative Potassium Channel Proteins in the Nervous System of *Drosophila*. **EMBO J.** 7: 1087- 1096.

Possidente D.R. and Murphey R.K. (1989). Genetic Control of Sexually Dimorphic Axon Morphology in *Drosophila* Sensory Neurons. **Dev. Biol.** 132: 448- 457.

Préat T., Thérond P., Lamour-Isnard C., Limbourg-Bouchon B., Tricoire H., Erk I., Mariol M.-C., and Busson D. (1990). A Putative Serine/Threonine Protein Kinase Encoded by the Segment Polarity *fused* Gene of *Drosophila*. **Nature (London)** 347: 87-89.

Préat T., Thérond P., Limbourg-Bouchon B., Pham A., Tricoire H., Busson D., and Lamour-Isnard C. (1993). Segmental Polarity in *Drosophila melanogaster*: Genetic Dissection of *fused* in a *Suppressor of fused* Background Reveals Interaction With *costal-2*. **Genetics** 135: 1047- 1062.

Preiss A., Werner B., Yu Y., Marte B.M., Schäfer W. and Maier D. (1993). Characteristics of Individual Genes in the *E(Spl)- Complex*. p. *In* Davis R. and Zipurski L. (eds.). **Neurobiology of *Drosophila*.** p. 55. **New York: Cold Spring Harbor Laboratory Press**

Prokop A. and Technau G.M. (1993). Cell Transplantation. *In* Hartley D.A. (ed.). **Cellular Interactions in Development.** pp. 33- 57. **Oxford: IRL Press/ Oxford University Press.**

Pultz M.A., Diederich R.J., Cribbs D.L. and Kaufman T.C. (1988). The *proboscipedia* Locus of the *Antennapedia* Complex: A Molecular and Genetic Analysis. **Genes Dev.** 2: 901 -920.

Raff J.W., Kellum R., and Alberts B. (1994). The *Drosophila* GAGA Transcription Factor Is Associated with Specific Regions of Heterochromatin Throughout the Cell Cycle. **EMBO J.** 13: 5977- 5983.

Raming K., Krieger J., Strotmann J., Boeckhoff I., Kubnick S., Baumstark C., and Breer H. (1993). Cloning and Expression of Odorant Receptors. **Nature (London)** 361: 353- 356.

Raming K., Freitag J., Krieger J., and Breer H. (1990). Primary Structure of a Pheromone Binding Protein from *Antheraea pernyi*: Homologies with other Ligand Carrying Proteins. **J. Comp. Physiol. [B]** 160: 503- 509.

Raming K., Freitag J., Krieger J., and Breer H. (1993). Arrestin-Subtypes in Insect Antennae **Cell Siganl.** 5: 69- 80.

Raming K., Krieger J., von Nickisch ., Boekhoff I., Schleicher S., and Breer H. (1994). Molecular Elements of the Olfactory Cascade in Insect Antennae (*Heliothis viscens*).*In* Elsner N. and Breer H. (eds.): **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I.** p.97. **Stuttgart- New York: Georg Thieme Verlag.**

Ramón y Cajal S. (1892). A New Concept of the Histology of the Central Nervous System. Rottenberg D.A. (transl., 1977). *In* Rottenberg D.A. and Hochberg F.H. (eds.): **Neurological Classics in Modern Translation.** p. 7- 29 **New York: Hafner**

Rao Y., Bodmer R., Jan L.Y. and Jan Y.N. (1992). The *big brain* Gene of *Drosophila* Functions to Control the Number of Neural Precursors in the Peripheral Nervous System. **Development** 116: 31-40.

Rashevsky N. (1938). Mathematical Biophysics. **Chicago, Ill.: University of Chicago Press.**

Rasmusson K.E., Raymond J.D., and Simmons M.J. (1993). Repression of Hybrid Dysgenesis in *Drosophila melanogaster* by Individual Naturally Occurring P Elements. **Genetics** 133: 605- 622.

Raz E. and Shilo B.-Z. (1993). Establishment of Ventral Cell Fates in the *Drosophila* Embryonic Ectoderm Requires DER, the EGF Receptor Homolog. **Genes Dev.** 7: 1937- 1948.

Rebay I., Flemming R.J., Fehon R.G., Cherbas L., Cherbas P., and Artavanis-Tsakonas S. (1991). Specific EGF Repeats of *Notch* Mediate Interactions with *Delta* and Serrate: Implications for *Notch* as a Multifunctional Receptor. **Cell** 67: 687-699.

Rebay I., Fortini M.E., and Artavanis-Tsakonas S. (1993a). Analysis of Phenotypic Abnormalities and Cell Fate Changes Caused by Dominant Activated and Dominant Negative Forms of the *Notch* Receptor in *Drosophila* Development. **C.R. Acad. Sci. Paris, Sciences de la vie/Life Sciences** 316: 1111-1123.

Rebay I., Fehon R.G., and Artavanis-Tsakonas S. (1993b). Specific Truncations of *Drosophila Notch* Define Dominant Activated and Dominant Negative Forms of the Receptor. **Cell** 74: 319-329.

Redher V., Bicker G., and Hammer M. (1987). Serotonin-Immunoresponsive Neurones in the Antennal Lobes and Suboesophageal Ganglion of the Honeybee. **Cell Tissue Res.** 247: 59-66

Redkozubow A. (1994) Voltage Clamp analysis of the Electric Circuitry of an Olfactory Sensillum in *Bombyx mori*. *In* Elsner N. and Breer H. (eds.): **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I.** p.92. **Stuttgart- New York: Georg Thieme Verlag.**

Rees C.J.C. (1969). Chemoreceptor Specificity Associated with the Choice of Feeding Site by the Beetle *Chrysolina brunsvicensis* on Its Foodplant *Ayperiucum hirsutum*. **Entomologica Exp. Appl.** 12: 565- 583.

Regulski M., McGinnes N., Chadwick R., and McGinnis W. (1987). Developmental and Molecular Analysis of *Deformed*; A Homeotic Controlling *Drosophila* Head Development. **EMBO J.** 6: 767- 777.

Regulski M. and Tully T. (1993). Cloning of a *Drosophila* Nitric Oxide Synthase. *In* Davis R. and Zipursky L. (eds.): **Neurobiology of *Drosophila*.** p. 126. **New York: Cold Spring Harbor Laboratory Press.**

Reifegerste R., Grimm S., Albert S., Hofbauer A., Lipski N., Oswald P., Pflugfelder G.O., Reichmuth C., and Buchner E. (1993). Calbindin-32: A Novel Calcium- Binding Protein of the Calbindin-Subfamily in *Drosophila*. *In* Elsner N. and Heisenberg M. (eds.): **Proceedings of the 21st Göttingen Neurobiology Conference 1993. Gene, Brain and Behaviour.** p. 824. **Stuttgart and New York: Georg Thieme Verlag**

Rembold H., Schlangenweit B., and Ulrich G.M. (1986). Activation of Juvenile Hormone Synthesis *in vitro* by a Corpus Cardiacum Factor from *Locusta migratoria*. **J. Insect Physiol.** 32: 91- 94.

Restifo L.L. and White K. (1991). Mutations in the Steroid Hormone-Regulated Gene Disrupt the Metamorphosis of the Central Nervous System in *Drosophila*. **Dev. Biol.** 148: 174-194.

Reuter R. and Leptin M. (1994). Interacting Functions of *snail, twist* and *huckebein* During the Early Development of Germ Layers in *Drosophila*. **Development** 120: 1137- 1150.

Richard D., Applebaum S.W., Sliter T.J., Baker F.C., Schooley D.A., Reuter C.C., Henrich V.C., and Gilbert L.I. (1989). Juvenile Hormone Bioxide Biosynthesis *in vitro* by the Ring Gland of *Drosophila melanogaster*: A Putative Juvenile Hormone in Higher *Diptera*. **Proc. Natl. Acad. Sci. USA.** 86: 1421- 1425.

Richard D., Applebaum S.W., and Gilbert L.I. (1990). Allostatic Regulation of Juvenile Hormone Production *in vitro* by the Ring Gland of *Drosophila melanogaster*. **Molec. Cell. Endocr.** 63: 153- 161.

Richards G. (1978). Sequential Gene Activity in the Polytene Chromosomes of *Drosophila melanogaster*. VI. Inhibition by Juvenile Hormones. **Dev. Biol.** 66: 32- 42.

Riddiford L.M. (1974). The Role of Hormones in the Reproductive Behavior of Female Wild Silkmoths. *In* Barton Browne L. (ed).: **Experimental Analysis of Insect Behaviour.** p.115- 138. **Berlin- Heidelberg- New York: Springer Verlag.**

Riddiford L.M., and Truman J.M. (1974). Hormones and Insect Behavior. *In* Barton Browne L. (ed).: **Experimental Analysis of Insect Behaviour.** p.115- 138. **Berlin- Heidelberg- New York: Springer Verlag.**

Riggleman B., Schedl P., and Wieschaus E. (1990). Spatial Expression of the *Drosophila* Segment Polarity Gene *armadillo* Is Post-Transcriptionally Regulated by *wingless*. **Cell** 63: 549- 560.

Rijsewijk F., Schermann M., Wagnaar E., Paren P., Weigel D., and Nusse R. (1987). The *Drosophila* Homolog of the Mouse Mammary Oncogene *int-1* Is Identical to the Segment Polarity Gene *wingless*. **Cell.** 50: 649- 659.

Rio D.C. (1991). Regulation of *Drosophila P* element Transposition. **Trends Gen.** 7: 282- 287.

Robbins J., Blondel B.J., Callahan D., and Callahan R. (1992). Mouse Mammary Tumor Gene *int-3*: a Member of the *Notch* Gene Family Transformants Mammary Epithelial Cells. **J.Virol.** 66: 2594- 2599.

Robertson H.M., Preston C.R., Phillis R.W., Johnston-Schlitz D.M., Benz W.K., and Engels W.R. (1988). A Stable Genomic Source of P-element Transposase in *Drosophila melanogaster*. **Genetics** 118: 461- 470.

Robinow S. and Truman J.W. (1993). The Regulation of Postmetamorphic Programmed Neuronal Death. *In* Davis R. and Zipursky L. (eds.). **Neurobiology of *Drosophila*** . p. 56. **New York: Cold Spring Harbor Laboratory Press.**

Rodrigues G.A. and Park M. (1994). Oncogenic Activation of Tyrosine Kinases. **Curr. Opin. Gen. Dev.** 4: 15- 24.

Rogge R.D., Karlovich C.A., and Banerjee U. (1991). Genetic Dissection of a Neurodevelopmental Pathway: *Son of sevenless* Functions Downstream of the *sevenless* and EGF Receptor Tyrosine Kinases. **Cell** 64: 39- 48.

Ronchi E., Treisman J., Dostatni N., Struhl G., and Desplan C. (1993). Down-Regulation of the *Drosophila* Morphogen *Bicoid* by the *Torso* Receptor-Mediated Signal Transduction Cascade. **Cell** 74: 347- 355.

Rose S. (1995). The Rise of Neurogenetic Determinism. **Nature (London).** 373: 380- 382.

Roskams A.J., Bredt D.S., Dawson T.M., and Ronnett G.V. (1994). Nitric Oxide Mediates the Formation of Synaptic Connections in Developing and Regenerating Olfactory Receptor Neurons. **Neuron** 13: 289- 299.

Roth S., Stein D., and Nüsslein -Volhard C. (1989). A Gradient for Nuclear Localisation of the *dorsal* Protein Determines the Dorso-Ventral Pattern in the *Drosophila* Embryo. **Cell** 59: 1189- 1202.

Rubin G.M., Hazelrigg T., Karess R.E., Laski F.A., Laverty T., Levis R., Rio D.C., Spencer F.A., and Zucker C.S. (1985). Germ Line Specificity of P-Element Transposition and Some Novel Pattern of Expression of Transduced Copies of *white* Gene. **Cold Spring Harbor Symp. Quant. Biol.,** Vol.L: 329- 335.

Ruel L., Pantesco V., Lutz Y., Simpson P., and Bourouis M. (1993). Functional Significance of a Family of Protein Kinases Encoded at the *shaggy* Locus in *Drosophila*. **EMBO J.** 12[4]: 1657- 1669.

Rushlow C.A., Han K., Manley J.L. and Levine M. (1989). The Graded Distribution of the *dorsal* Morphogen Is Initiated by Selective Nuclear Transport in *Drosophila*. **Cell** 59: 1165- 1177.

Rutledge B., Zhang K., Bier E., Jan Y.N., and Perrimon N. (1992). The *Drosophila spitz* Gene Encodes a Putative EGF- Like Growth Factor Involved in the Dorso-ventral Axis Formation and Neurogenesis. **Genes Dev.** 6: 1503- 1517.

Rybak J. and Maulshagen J. (1994). The PE-1 Neuron of the Honeybee - An Efferent Pathway from the Mushroom Bodies to the Protocerebral Lobe. *In* Elsner N. and Breer H. (eds.): **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II** . p.830. **Stuttgart- New York: Georg Thieme Verlag.**

Rybak J. and Menzel R. (1993). Anatomy of the Mushroom Bodies in the Honey Bee Brain: The Neuronal Connections of the Alpha-lobe. **J. Comp. Neurol.** 334: 444- 465.

Salecker I. and Boeckh J. (1994). Influence of Deafferentation on the Formation of Glomeruli in the Antennal Lobes of Cockroach Embryos. *In* Elsner N. and Breer H. (eds.): **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II** . p.421. **Stuttgart- New York: Georg Thieme Verlag.**

Sampedro J., Johnston P., and Lawrence P.A. (1993). A Role for *wingless* in the Segmental Gradient of *Drosophila*. **Development** 117: 677-687.

Sanes J.R., and Hildebrand J.G. (1976a). Structure and Development of the Antennae in a Moth, *Manduca sexta*. **Dev. Biol.** 51: 282- 299.

Sanes J.R. and Hildebrand J.G. (1976b). Origin and Morphogenesis of a Sensory Neurons in an Insect Antenna. **Dev. Biol.** 51: 300- 319.

Sanes J.R. and Hildebrand J.G. (1976c). Acetylcholine and Its Metabolic Enzymes in the Developing Antennae of the Moth, *Manduca sexta*. **Dev. Biol.** 52: 105- 120.

Saudou F., Boschert U., Amlaiky N., Plassat J.-L. and Hen R. (1992). A Family of *Drosophila* Serotonin Receptors with Distinct Intracellular Signalling Properties and Expression Patterns. **EMBO J.** 11: 7- 17.

Savakis C., Ashburner M., and Willis J.H. (1986). The Expression of the Gene Coding for Alcohol Dehydrogenase During the Development of *Drosophila melanogaster*. **Dev. Biol.** 114: 194- 207.

Schacher S. and Montarolo P.G. (1991). Target-Dependent Structural Changes in Sensory Neurons of *Aplysia* Accompany Long Term Heterosynaptic Inhibition. **Neuron** 6: 679- 690.

Schäfer S., Bicker G., Ottersen O.P., and Storm-Mathiessen J. (1988). Taurine-like Immunoreactivty in the Brain of the Honey Bee. **J. Comp. Neurol.** 268: 60- 70.

Schejter E.D. and Shilo B.-Z. (1989). The *Drosophila* EGF Receptor Homolog (DER) Gene Is Allelic to *little faint ball*, a Locus Essential for Embryonic Development. **Cell** 56: 1093- 1104.

Schmid A., Bettler D., Newfeld S., and Yedvobnick B. (1993) Studies on the *Drosophila* Neurogenic Locus *mastermind*. In Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p.58. **New York: Cold Spring Harbor Laboratory Press.**

Schmidt-Nielsen K. (1990). **Animal Physiology** - Adaptation and Environment. 4th Edition. **Cambridge: Cambridge University Press.**

Schmitt-Ott U. and Technau G.M.(1992). Expression of *en* and *wg* in the Embryonic Head and Brain of *Drosophila* Indicates a Refolded Band of Seven Segment Remnants. **Development.** 116: 111-125.

Schmitt-Ott U., González- Gaitán M., Jäckle H., and Technau G.M. (1993). Embryonic Head Phenotype of *Drosophila* "Gap- Gene" Mutants. *In* Elsner N. and Heisenberg M. (eds.): **Proceedings of the 21st Göttingen Neurobiology Conference 1993. Gene, Brain and Behaviour.** p. 132. **Stuttgart and New York: Georg Thieme Verlag**

Schneider D.S., Jin Y., Morisato D., and Anderson K.V. (1994). A Processed Form of the *Spätzle* Protein Defines Dorsal-Ventral Polarity in the *Drosophila* Embryo. **Development** 120: 1243- 1250.

Schneider D. (1969). Insect Olfaction: Deciphering System for Chemical Messages. **Science** 163: 1031- 1037.

Scholz H., Deatrick J., Klaes A., and Klämbt C. (1993). Genetic Dissection of *pointed*, a *Drosophila* Gene Encoding Two ETS-Related Proteins. **Genetics** 135: 455- 468.

Scholz H., Wemmer T., Memme T., and Klämbt C (1993). Genetic Dissection of Glial- Neuronal Interaction in the Embryonic *Drosophila* CNS. *In* Davis R. and Zipurski L. (eds.). **Neurobiology of *Drosophila*.** p. 59 New York: Cold Spring Harbor Laboratory Press.

Schrons H., Knust E., and Campos-Ortega J.A. (1992). The *Enhancer of Split Complex* and Adjacent Genes in the 96F Region of *Drosophila melanogaster* Are Required for Segregation of Neural and Epidermal Progenitor Cells. **Genetics** 132: 481- 503.

Schürmann F.W. (1974). Bemerkungen zur Funktion der Corpora pendunculata in Gehirn der Insekten aus morphogischer Sicht. **Exp. Brain Res.** 19: 406- 432.

Schürmann F.-W. (1987). The Architecture of the Mushroom Bodies and Related Neuropils in the Insect Brain. *In* Gupta A.P. (ed.): **Arthoprod Brain. Its Evolution, Development, Structure, and Function.** pp. 231- 264. **New York: John Wiley Sons.**

Schuster R., Phannavong B., Schröder C., and Gundelfinger E.D. (1993). Immunohistochemical Localisation of a Ligand-Binding and Structural Subunit of Nicotinic Acetylcholine Receptors in the Central Nervous System of *Drosophila melanogaster*. **J. Comp. Neurol.** 335: 149- 162.

Seeger M., Tear G., Ferres-Marco D., and Goodman C.S. (1993a). Mutations Affecting Growth Cone Guidance in *Drosophila*: Genes Necessary for Guidance Toward or Away From the Midline. **Neuron** 10: 409- 426.

Seeger M., Tear G., and Goodman C.S. (1993b). *Commissureless*, A Gene in *Drosophila* Essential For Growth Cone Guidance Towards the Midline. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 60 . **New York Cold Spring Harbor Laboratory Press.**

Segraves W.A. and Hogness D.S. (1990). The *E75* Ecdysone-Inducible Gene Responsible for the 75B Early Puff in *Drosophila* Encodes Two New Members of the Steroid Receptor Family. **Genes Dev.** 4: 204- 219.

Segraves W.A. (1991). Somethings Old, Some Things New: The Steroid Receptor Superfamily in *Drosophila*. **Cell** 67: 225- 228. [review]

Semon R. (1904). **Die Mneme als Erhaltenes Prinzip im Wechsel des Geschehens. Leipzig: Wilhelm Engelmann Verlag.**

Shelton CA. and Wasserman S.A. (1993). *pelle* Encodes a Protein Kinase Required to Establish Dorsoventral Polarity in the *Drosophila* Embryo. **Cell** 72: 515- 525.

Shishido E., Higashijima S., Emori Y., and Saigo K. (1993) 2 FGF- Receptor Homologs Of *Drosophila* - One Is Expressed in Mesodermal Primodium in Early Embryos. **Development** 117: 751- 761.

Shortridge R.D., Yoon J., Lending C.R., Bloomquist B.T., Perdew M.H., and Pak W.L. (1991). A *Drosophila* Phospholipase -C Gene That Is Expressed in the Central Nervous System. **J. Biol. Chem.** 266: 12474- 12480.

Siebel C.W. and Rio D.C. (1990). Regulated Splicing of the *Drosophila* P- Transposable Element Third Intron in vitro: Somatic Repression .**Science** 248: 1200- 1208.

Siegfried E., Wilder E. L., and Perrimon N. (1994). Components of *wingless* Signalling in *Drosophila*. **Nature (London)** 367: 76- 79.

Simon M.A., Bowtell D., Dodson G.S., Laverty T.R., and Rubin G.M. (1991). Ras1 and a Putative Guanine Nucleotide Exchange Factor Perform Crucial Steps in Signalling by the *sevenless* Protein Tyrosine Kinase. **Cell** 67: 701- 716.

Simon M.A., Dodson G.S., and Rubin G.M. (1993). An SH3-SH2-SH3 Protein Is Required for p21Ras1 Activation and Binds to *sevenless* and *Sos* Proteins *in Vitro*. **Cell** 73: 169- 177.

Singson A., Leviten M.W., Bang A., Hua H., and Posakony J.W. (1993). Direct Downstream Targets of *achaete* and *scute* in Proneural Clusters Include Neurogenic Genes. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*. New York Cold Spring Harbor Laboratory Press.**

Skeath J.B., Panaganiban G., Selegue J., and Carroll S.B. (1992). Gene Regulation in Two Dimensions: The Proneural *achaete* and *scute* Genes Are controlled by Combinations of Axis- Patterning Genes Through a Common Intergenic Control Region. **Genes Dev.** 6: 2606- 2619.

Skeath J.B., Panaganiban G., and Carroll, S.B. (1994). The *ventral system defective* Gene Controls Proneuronal Gene Expression at Two Distinct Steps During Neuroblast Formation in *Drosophila*. **Development.** 120: 1517- 1524.

Skoufias D.A. and Scholey J.M. (1993). Cytoplasmic Microtubule- Based Motor Proteins. **Curr. Opin. Cell Biol.** 5: 95 - 104.

Skoulakis E.M.C., Kalderon D., and Davis R. (1993). Preferential Expression in Mushroom Bodies of Catalytic Subunit of Protein Kinase A and Its Role in Learning and Memory. **Neuron** 11: 197- 208.

Sliter T.J. and Gilbert L.I. (1992). Developmental Arrest and Ecdysteroid Deficiency Resulting From Mutations at the *dre4* Locus of *Drosophila* . **Genetics** 130: 555- 568.

Small S., Kraut R., Hoey T., Warrior R., and Levine M. (1991). Transcription Regulation of a Pair Rule Stripe in *Drosophila*. **Genes Dev.** 5: 827-

Small S., Blair A., and Levine M. (1992). Regulation of *even-skipped* Stripe Two in the *Drosophila* Embryo. **EMBO J.** 11: 4047- 4057.

Smoller D., Friedel C., Schmidt A., Bettler D., Lam L., and Yedvobnick B. (1990). The *Drosophila* Neurogenic Locus *mastermind* Encodes a Nuclear Protein Usually Rich in Amino Acid Homopolymers. **Genes Dev.** 4: 1688- 1700.

Snow P.M., Bieber A.J., and Goodman C.S. (1989). *Fasciclin III*: A Novel Homophilic Adhesion Molecule in *Drosophila*. **Cell** 59: 313- 323.

Sohal R.S. and Sharma S.P. (1972). Age Related Changes in the Fine Structure and Number of Neurons in the Brain of the Housefly *Musca domesticus*. **Exptl. Geront.** 7: 243- 249.

Sohal R.S., Sharma S.P., and Couch E.F. (1972). Fine Structure of the Neural Sheath, Glia and Neurons in the Brain of the Housefly, *Musca domesticus*. **Z.Zellforsch.** 135: 449- 460.

Sommer B., Köhler M., Sprengel R., and Seeburg P.H. (1991). RNA Editing in Brain Controls a Determinant of Ion Flow in Glutamate-Gated Channels. **Cell** 67: 11- 19.

Spemann H. (1938). Embryonic Development and Induction. **New Haven: Yale University Press.**

Spring J., Paine-Saunders S.E., Hynes R.O., and Bernfield M. (1994). *Drosophila Syndecan*: Conservation of a Cell-Surface Heparin Sulfate Proteoglycan. **Proc. Natl. Acad. Sci. USA.** 91: 3334 - 3338.

Städler E. (1984). Contact Chemoreception. *In* Bell W.J. and Cardé R.T. (eds.). **Chemical Ecology of Insects.** pp. 3 -35. **London: Chapman and Hall.** [review]

Stanewsky R., Rendahl K.G., Dill M., and Saumweber H. (1993). Genetic and Molecular Analysis of the X Chromosomal Region 14B17-14C4 in *Drosophila melanogaster*: Loss of Function in NONA, a Nuclear Protein Common to Many Cell Types, Results in Specific Physiological and Behavioral Defects. **Genetics** 135: 419- 442.

Stanojevic D., Hoey T., and Levine M. (1989). Sequence Specific DNA- Binding Activities of the Gap Proteins Encoded by *hunchback* and *Krüppel* in *Drosophila*. **Nature (London)** 341: 331- 335.

Stanojevic D., Small S., and Levine M. (1991). Regulation of a Segmentation Stripe by Overlapping Activators and Repressors of the *Drosophila* Embryo. **Science** 254: 1385- 1387.

Steinbrecht R.A. (1973). Der Feinbau olfaktorischer Sensillen des Seidenspinners (*Insecta: Leptidoptera*). **Z. Zellforsch.** 139: 533- 565.

Steingrimsson E., Pignoni F., Liaw G.J., and Lengyel J.A. (1991). Dual Role of the *Drosophila* Pattern Gene *tailless* in Embryonic Termini. **Science** 254: 418- 421.

Steiger U. (1967). Über den Feinbau des Neuropils in Corpus pedunculatum der Waldameise. Elektronenoptische Untersuchungen. **Z. Zellforsch.** 81: 511- 536.

Steller H. and Pirotta V. (1985). A Transposable P Vector that Confers Selectable G418 Resistance to *Drosophila* Larvae. **EMBO J.** 4: 167- 171.

Stengl M. (1993). Intracellular -Messenger-Dependent Calcium Channels Open Before Ca^{2+}-Dependent Cation Channels in Cultured Insect Olfactory Neurons. **Chem Sens.** 18: 633

Stengl M. (1994). Does cGMP Play a Role for Adaptation in *Manduca sexta* Olfactory Receptor Neurons? *In* Elsner N. and Breer H. (eds.): **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I.** p.93. **Stuttgart- New York: Georg Thieme Verlag.**

Stevens L.M., Frohnhöfer H.G., Klinger M., and Nüsslein-Volhart C. (1990). Localised Requirement for *torso*-like Expression in Follicle Cells for Development of Terminal Anlagen of the *Drosophila* Embryo. **Nature (London)** 338: 478- 483.

Stevenson P.A., Pfügler H.-J., Rapus J., and Eckert M. (1994). Colocalisation of Taurine with other Neuromodulators in the Locust. *In* Elsner N. and Heisenberg M. (eds.): *In* **Göttingen Neurobiology Report 1994. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. II.** p.624. **Stuttgart- New York: Georg Thieme Verlag.**

Steward R. (1989). Relocalisation of the *dorsal* Protein from the Cytoplasm to the Nucleus Correlates with its Function. **Cell** 59: 1179- 1188.

St. Johnston R.D. and Nüsslein- Volhard C. (1992). The Origin of Pattern and Polarity in the *Drosophila* Embryo. **Cell** 68: 201- 219.

Stocker R.F., Lienhard M.C., Borst A., and Fischbach K.F. (1990). Neuronal Architecture of the Antennal Lobe in *Drosophila melanogaster*. **Cell Tissue Res.** 262: 9-34.

Stone B.L and Thummel C.S. (1993). The *Drosophila* 78C Late Puff Contains *E78*, an Ecdysone-Inducible Gene That Encodes a Novel Member of the Nuclear Hormone Superfamily. **Cell** 75: 307- 320.

Streuli M., Krüger N.X., and Tsai A.Y.M., and Saito H. (1989). A Family of Receptor- Linked Protein Tyrosine Phosphatases in Humans and *Drosophila*. **Proc. Natl. Sci. USA** 86: 8698- 8702.

Struhl G., Fitzgerald K., and Greenwald I. (1993). Intrinsic Activity of the *Lin-12* and *Notch* Intracellular Domains *In Vivo*. **Cell** 74: 331-345.

Takagaki Y. and Manley J.L. (1994). A Polyadentlation Factor Subunit Is the Human Homologue of the *Drosophila SUPRESSOR OF FORKED* Protein. **Nature (London).** 372: 471- 474.

Talbot W.S., Swyryd E.A., and Hogness D.S. (1993). *Drosophila* Tissue with Different Metamorphic Responses to Ecdysone Express Different Ecdysone Receptor Isoforms. **Cell** 73: 1323- 1337.

Tamkun J.W., Deuring R., Scott M.P., Kissinger M., Pattatucci A., Kaufman T.C., and Kennison J.A. (1992). *brahama*: A regulator of *Drosophila* Homeotic Genes Structurally Related to the Yeast Transcriptional Activator SNF2/SW12. **Cell** 68: 561- 572.

Tautz D.R., Lehman R., Schnurch H., Schuh R., Seifert E., Keinlin A., Jones K., and Jaeckle H. (1987). Finger Protein of a Novel Structure Encoded by

hunchback, a Second Member of the Gap Class of *Drosophila* Segmentation Genes. **Nature (London).** 327: 383- 389.

Tautz D. (1988). Regulation of the *Drosophila* Segmentation Gene *hunchback* by Two Maternal Morphogenic Centres. **Nature (London)** 332: 281- 284.

Technau G. and Heisenberg M. (1982). Neural Reorganisation During Metamorphosis of the Corpora pedunculata in *Drosophila melanogaster*. **Nature (London).** 295 : 405 - 407.

Technau G.M. (1984). Fiber Number in the Mushroom Bodies of Adult *Drosophila melanogaster* Depends on Age, Sex and Experience. **J. Neurogenet.** 1: 113- 126.

Thaker H.M. and Kankel D.R. (1992). Mosaic Analysis Gives an Estimate of the Extent of Genomic Involvement in the Development of Visual Systems in *Drosophila melanogaster*. **Genetics** 131: 883- 894.

Theissen H., Purcell J., Benett M., Kansagara D., Syend A., and Marsh J.L. (1994). *dishevelled* Is Required During *wingless* Signalling to Establish Both Cell Polarity and Cell Identity. **Development** 120: 347- 360.

Thomas H.E., Stunnenberg H.G., and Stewart A.F. (1993). Heterodimerisation of the *Drosophila* Ecdysone Receptor with Retinoid X Receptor and *ultraspiracle*. **Nature (London).** 362 : 471- 475.

Thomas J.B., Crews S.T., and Goodman C.S. (1988). Molecular Genetics of the *single minded* Locus: A Gene Involved in the Development of the *Drosophila* Nervous System. **Cell.** 52: 133- 141.

Thomas K.R. and Capecchi M.R. (1987). Site- Directed Mutagenesis by Gene Targeting in Mouse Embryo- Derived Stem Cells. **Cell.** 51: 503- 512.

Thummel C.S., Burtis K.C., and Hogness D.S. (1990). Spatial and Temporal Pattern of *E74* Transcription during *Drosophila* Development. **Cell** 61: 101- 111.

Tian M. and Maniatis T. (1992). Positive Control of pre-mRNA Splicing *in vitro*. **Science** 256: 237- 240.

Tian S.-S., Tsoulfas P., and Zinn K. (1991). Three Receptor-Linked Protein-Tyrosine Phosphatases Are Selectively Expressed on Central Nervous System Axons in the *Drosophila* Embryo. **Cell** 67: 675 - 685.

Tian S.-S., Hamilton B., Desai C., Kung T.Y., Fashena S., and Zinn K. (1993). Signal Transduction by Axonal Receptor-Linked Protein Tyrosine Phosphatases. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 62. **New York Cold Spring Harbor Laboratory Press.**

Titus D.E. (ed.). (1991). Promega® Protocols and Applications Guide. **Madison, Wiscosin: Promega® Corporation.**

Travers A. (1992). The Reprogramming of Transcriptional Competence. **Cell** 69: 573- 575. [review]

Treisman J., Harris E., and Desplan C. (1991). The *paired* Box Encodes a Second DNA- Binding Domain in the *paired* Homeo Domain Protein. **Genes Dev.** 5:594-604.

Trotier D. (1994). Intensity Coding in Olfactory Receptor Cells. **Sem. Cell Biol.** 5: 47- 54.

Trujillo-Cenós O. and Melamed J. (1962). Electron Microscope Observations of the Calyces of the Insect Brain. **J. Ultrastruct. Res.** 7: 389- 398.

Truman J.W., Talbot W.S., Fahrbach S.E., and Hogness D.S. (1994). A Common Precursor for Glia and Neurones in the Embryonic CNS of *Drosophila* Gives Rise to Segment-Specific Lineage Variants. **Development** 118: 763- 775.

Tsai C. and Gergen J.P. (1994). Gap Gene Properties of the Pair Rule Gene *runt* During *Drosophila* Segmentation. **Development.** 120: 1671- 1683.

Tsuda L, Inoue Y.H., Yoo M.A., Mizuno M., Hata M., Lim Y.-M., Adachi-Yamada T., Ryo H., Masamune Y., and Nishida Y. (1993). A Protein Kinase Similar to *MAP kinase* Activator Acts Downstream of the *raf* Kinase in *Drosophila* **Cell.** 72: 407- 414.

Tully T., Cambiazo V., and Kruse L. (1994). Memory Through Metamorphosis in Normal and Mutant *Drosophila*. **J.Neurosci.** 14: 65- 74.

Tully T., Preat T., Boyton S.C., and Del Vecchio M. (1994). Genetic Dissection of Consolidated Memory in *Drosophila*. **Cell** 79: 35- 47.

Udolph G., Prokop A., Bossing T. and Technau G.M. (1993). A Common Precursor for Glia and Neurons in the Embryonic CNS of *Drosophila* Gives Rise to Segment-Specific Lineage Variants. **Development** 118: 765- 775.

Ueda H., Sonoda S., Brown J.L., Scott M.P. and Wu C. (1990). A Novel DNA Binding Motif Abuts the Zinc Finger Domain of Insect Nuclear Hormone receptor FTZ-F1 and Mouse Embryonal Long Terminal Repeat Binding Protein. **Mol. Cell. Biol.** 12: 5667- 5672.

United States Biochemical® (1993). **Sequenase Version 2.0®. DNA Sequencing Kit.** Step-By-Step Protocols For DNA Sequencing With Sequenase Version 2.0 T7® DNA Polymerase. **Cleveland (Ohio): United States Biochemical® Corporation.**

Unni B.G., Bhaskaran G., Dahm K.H., and Hayes T.K. (1991). Stimulation of Juvenile Hormone Biosynthesis by Analogues of *Manduca sexta* Allotropin: *In Vitro* Studies. **Arch. Insect Biochem. Physiol.** 17: 129- 142.

Vaessin H., Brand M., Jan L.Y., and Jan Y.N. (1994). *daughterless* Is Essential for Neuronal Precursor Differentiation but not for Initiation of Neuronal Precursor Formation in *Drosophila* embryo. **Development.** 120: 935- 945.

Vaessin H., Bremer K.A., Knust E., and Campos-Ortega J. (1987). The Neurogenic Gene *Delta* of *Drosophila melanogaster* is expressed in the Neurogenic Territories and Encodes a Putative Transmembrane Protein with EGF-like Repeats. **EMBO J.** 6: 3431-3440.

Vaessin H., Greil E., Wolff E., Bier E., Jan L.Y., And Jan Y.N. (1991). *prospero* Is Expressed in Neuronal Precursors and Encodes a Nuclear Protein That Is

Involved in the Control of Axonal Outgrowth in *Drosophila*. **Cell.** 67: 941- 953.

Vallés A.M. and White K. (1990). Serotonin-Containing Neurons in *Drosophila melanogaster*: Development and Distribution **J. Comp. Neurol.** 268: 414- 428.

Vallés A.M. and White K. (1990). Serotonin Synthesis and Distribution in *Drosophila Dopa Decarboxylase* Genetic Mosaics. **J.Neurosci.** 10: 3646- 3652.

van den Berg M.J. and Ziegelberger G. (1991). On the Function of Pheromone Binding Protein in the Olfactory Hairs of *Anthraea polyphemus*. **J.Insect Physiol.** 37: 79- 85.

van den Heuvel M., Nusse R., Johson P., and Lawrence P.A. (1989). Distribution of the *wingless* Gene Product in *Drosophila* Embryos: A Protein Involved in Cell-Cell Communication. **Cell** 59: 739 - 749.

van Doren M., Powell P.A., Pasternak D., Singson A., and Posakony J.W. (1992). Spatial Regulation of Proneural Gene Activity: Auto- and Cross-Activation of *achaete* is Antagonised by *extramacroachaete*. **Genes Dev.** 6:2592- 2605.

van Gelder C.W.G., Gunderson S.I., Jansen E.J.R., Boelens W.C., Polycarpou-Schwarz M., Mattaj I.W., and van Venrooij W.J. (1993). A Complex Secondary Structure in U1A pre-mRNA that Binds Two Molecules of U1A Protein Is Required for Regulation of Polyadenlylation. **EMBO J.** 12: 5191- 5200.

Vazquez J. and Schedl P. (1994). Sequences Required for Enhancer Blocking Activity of scs Are Located within Two Nuclease- Hypersensitive Regions. **EMBO J.** 13: 5984- 5993.

Verma A., Hirsch D.J., Glatt C.E., Ronnett G.V., and Snyder S.H. (1993). Carbon Monoxide, a Putative Neural Messenger. **Science** 259: 381- 384.

VijayRaghavan K., Kaur J., Parajape J., and Rodrigues V. (1992). The *east* Gene of *Drosophila melanogaster* Is Expressed in the Developing Embryonic Nervous System and Is Required for Normal Olfactory and Gustatory Responses of the Adult. **Dev. Biol.** 154: 23- 36.

Vogt R.G. and Riddiford L.M. (1981). Pheromone Binding and inactivation by Moth Antennae. **Nature** 293: 161- 163.

Vogt R.G., Riddiford L.M., and Prestwich G.D. (1985). Kinetic Properties of a Pheromone Degrading Enzyme: The Sensillar Esterase of *Anthraea polyphemus*. **Proc. Natl. Acad. Sci. USA.** 82: 8827- 8831.

Vogt R.G., Prestwich G.D., and Lerner M.R. (1991). Odorant-Binding-Protein Subfamilies Associate with Distinct Classes of Olfactory Receptor Neurones in Insects. **J.Neurobiol.** 22: 74- 84.

Vogt R.G., Rybczynski R., and Lerner M.R. (1990). The Biochemistry of Odorant Reception and Transduction. *In* Schild D. (ed.): **Chemosensory Information Processing. NATO ASI Series H, Vol.** 39. p. 33- 76. **Berlin- New York: Springer Verlag.**

Vogt R.G., Rybczynski R., and Lerner M.R. (1991). Molecular Cloning and Sequencing of General Odorant -Binding Proteins GOBP1 and GOBP2 from the Tobacco Hawk Moth, *Manduca sexta*. : Comparisons with other Insect OBPs and Their Signal Peptides. **J. Neurosci.** 11: 2972- 2984.

Vogt R.G., Rybczynski R., Cruz M., and Lerner M.R. (1993). Ecdysteroid Regulation of Olfactory Protein Expression in the Developing Antenna of the Tobacco Hawk Moth, *Manduca sexta*. **J. Neurobiol.** 24: 581- 597.

Wagner-Bernholz J.T., Wilson C., Gibson G., Schuh R., and Gehring W.J. (1991). Identification of Target Genes of the Homeotic Gene *Antennapedia* by Enhancer Detection. **Genes Dev.** 5: 2467- 2480.

Walldorf U. and Gehring W.J. (1992). *Empty spiracles*, a Gap Gene Containing a Homeobox Involved in *Drosophila* Head Development. **EMBO J.** 11(6): 2247- 2259.

Wallén P., Carlson K., and Mossberg K. (1992). **Confocal Laser Scanning Microscopy as a Tool for Studying the 3-D Morphology of Nerve Cells.** *In* Kriete A. (ed.): **Vizualisation In Biomedical Microscopies. 3-D Imaging and Computer Applications.** pp. 109- 144. **Weinheim : Verlagsgesellschaft Chemie.**

Wang J.Y.J. (1993). Abl Tyrosine Kinase in Signal Transduction and Cell Cycle Regulation. **Curr. Opin. Gen. Dev.** 3: 35 - 43 [review].

Wang L.H., Tsao D.H.H., Ferretti J., Chemlik R., and Nirenberg M. (1993). Recognition of DNA by the NK-2 Homeodomain. *In* Davis R. and Zipurski L. (eds.). **Neurobiology of *Drosophila*. New York: Cold Spring Harbor Laboratory Press.**

Warmke J.W. and Ganetzky B. (1994) A Family of Potassium Channel Genes Related to *eag* in *Drosophila* and Mammals. **Proc. Natl. Acad. Sci. USA.** 91: 3438- 3442.

Wassarman K.M., and Steitz J.A. (1993). Association with Terminal Exons in pre-mRNAs: a New Role for the U1 snRNP? **Genes Dev.** 7: 647- 659.

Wegerer J.W., Tareilus E., and Breer H. (1992). Characterisation of Calcium- Dependent Potassium Channels in Antennal Receptor Neurones of *Locusta migratoria*. **J. Insect Physiol.** 38: 237- 248.

Weimann J.M. and Marder E. (1994). Switching Neurones Are Integral Memers of Multiple Ocillartory Networks. **Curr. Biol.** 4: 896- 902.

Whalen A.M. and Steward R. (1993). Dissociation of the *DORSAL- CACTUS* Complex and the Phosphorylation of the *DORSAL* Protein Correlation with the Nuclear Localisation of *DORSAL*. **J.Cell. Biol.** 123: 523- 534.

Wharton K.A. Johansen K.M., Xu T. and Artavanis-Tsakonas S. (1985). Nucleotide Sequence form the Neurogenic Locus *Notch* Implies a Gene Product the Shares Homology with Proteins Containing EGF- like Repeats. **Cell** 43: 567- 581.

Wharton K.A. Jr., Franks R.G., Kasai Y., and Crews S.T. (1994). Control of the CNS Midline Transcription by Asymmetric E- box Like-Elements: Similarity to Xenobiotic Response Regulation. **Development** 120: 3563- 3569.

Wigglesworth V.B. (1960). The Nutrition of the Central Nervous System in the Cockroach *Periplaneta americana*. L. The Role of Perineurium and Glial Cells in the Mobilisation of Reserves. **J. Experimental Biol.** 37: 500- 512.

Wilson C., Pearson K. R., Bellen H.J., O'Kane C.J., Grossniklaus U., and Gehring W.J. (1989). P-element Mediated Enhancer Detection: Isolation and Characterisation of Developmentally Regulated Genes in *Drosophila*. **Genes Dev.** 3: 1301- 1333.

Wilson G.G., and Murray N.E. (1991). Restriction and Modification Systems. **Annu. Rev. Genetics** 25: 585- 627. [review]

Wimmer E.A., Jäckle H., Pfeile C. and Cohen S.M. (1993). A *Drosophila* Homologue of Human Sp1 Is a Head - Specific Segmentation Gene. **Nature** 366: 690-695.

Winberg M.L., Perez S.E., and Steller H. (1992). Generation and Early Differentiation of Glial Cells in the First Optic Ganglion of *Drosophila melanogaster*. **Development** 115: 903- 911.

Withers G.S., Fahrbach S.E., and Robinson G.E. (1993). Selective Neuroanatomical Plasticity and Division of Labour in the Honey Bee. **Nature** 264: 238-240.

Witz P., Amlaiky N., Plassat J.-L., Maroteux L., Borrelli E., and Hen R. (1990). Cloning and Characterisation of a *Drosophila* Serotonin Receptor that Activates Adenylate Cyclase. **Proc. Natl. Acad. USA** 87: 8940- 8944.

Xiong W.-C., Okano H., Patel N., Blendy J.A., and Morell C. (1994). *repo* Encodes a Glial-Specific Homeo Domain Protein Required in the *Drosophila* Nervous System. **Genes Dev.** 8: 981- 994.

Xu T. and Rubin G.M. (1993). Analysis of Genetic Mosaics in Developing and Adult *Drosophila* Tissue. **Development** 117: 1223- 1237.

Yang J.T., Saxton W.M., Stewart R.J., Raff E.C., and Golstein L.S. (1990). Evidence that the Head of Kinesin Is Sufficient for Force Generation and Motility *in vitro*. **Science.** 249: 42- 47.

Yang X., Seow K.T., Bahri S.M., Oon S.H., and Chia W. (1991). Two *Drosophila* Receptor-like Tyrosine Phosphatase Genes Are Expressed in a Subset of Developing Axons and Pioneer Neurons in the Embryonic CNS. **Cell** 67: 661- 673.

Yang X., Yeo S., Dick T., and Chia W. (1993a). The Role of a *Drosophila* POU Homeo Domain Gene in the Specification of Neuronal Precursor Cell Identity in the Developing Embryonic Central Nervous System. **Genes Dev.** 7: 504- 516.

Yang X., Dick T., Bueschner M., Weigman K., and Chia W. (1993b). New Mutations Affecting Specification of Motor Neuron RP2 and Its Precursor Cells. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*.** p. 67. **New York: Cold Spring Harbor Laboratory Press.**

Yanisch-Perron C., VierA J. and Messing J. (1985). Improved M13 PhageCloning Vectors and Host Strains: Nucleotide Sequences of the M13 mp18 and pUC19 Vectors. **Gene** 33:103- 119.

Yao K.-M., Samson M.L., Reevens R., and White K. (1993). Gene *elav* of *Drosophila melanogaster:* A Prototype for Neuronal- Specific RNA Binding Protein Gene Family That Is Conserved in Flies and Humans. **J. Neurobiol.** 24: 723- 739.[review]

Yedvobnick B., Muskavitch M.A.T., Wharton K.A., Halpern M.E., Paul E., Grimwade B.G., and Artavanis-Tsakonas S. (1985). Molecular Genetics of *Drosophila* Neurogenesis. **Cold Spring Harbor Symp. on Quantitative Biol.** Vol. L. p. 841- 854.

Yin J.C.P., Wallach J.S., Del Vecchio M., Wilder E.L., Zhou H., Quinn W.G., and Tully T. (1994). Induction of a Dominant Negative CREB Transgene Specifically Blocks Long- Term Memory in *Drosophila*. **Cell** 79: 49- 58.

Young J.Z. (1965). The Croonian Lecture: The Organisation of a Memory System. **Philos. Trans. R. Soc. B (London)** 163: 18- 91.

Young J.Z. (1966). A Unit of Memory. **New Scientist.** 28: 861- 863.

Zacharias D., Williams L.D., Maier T., and Reichert H. (1993). Neurogenesis in the Insect Brain: Cellular Identification and Molecular Characterisation of Brain Neuroblasts in the Grasshopper Embryo. **Development.** 118: 941- 955.

Zarkower D., Stephenson P., Sheets M., and Wickens M. (1986). The AAUAAA Sequence Is Required for Cleavage and for Polyadenlylation of Simian Virus 40 pre-mRNA *in vitro*. **Mol. Cell Biol.** 6: 2317- 2323.

Zhang F., Denome R.M., and Cole C.N. (1986). Fine-Structure Analysis of the Processing and Polyadenylation Region of the Herpex Simplex Virus Type Thymidine Kinase Gene Using Linker Scanning, Internal Deletion and Insertion Mutations. **Mol. Cell. Biol.** 6: 4611- 4623.

Zhang C.-C., Müller J., Hoch M., Jäckle H., and Bienz M. (1991). Target Sequence for *hunchback* in a Control Region Conferring *Ultrabithorax* Expression Boundaries. **Development** 113: 1171- 1179.

Zhang K., Smouse D., and Perrimon N. (1991). The *crook neck* Gene of *Drosophila* Contains a Motif Found in a Family of Yeast Cell Cycle Genes. **Genes Dev.** 5: 1080 -1091

Zhang P. and Spradling A.C. (1991). Efficient and Dispersed Local P Element Transposition From *Drosophila* Females. **Genetics** 133: 361- 373.

Zhang P. and Spradling A.C. (1994). Insertional Mutagenesis of *Drosophila* Heterochromatin with Single P elements. **Proc. Natl. Acad. Sci. USA.** 91: 3539- 3543.

Zhang S.D., Kassis J.A., Olde B.A., Mellerick D.M., Nazaralu A.J., and Odenwald W.F. (1993). *pullox*, A Novel Membrane Protein, Is Concentrated In A Subset Of Axon Fascicles Within the *Drosophila* CNS. **Soc. Neuroscience. Abstr.** 19(2): 1288[533.13]

Zhang Y., Buenzow D., and Holmgren R. (1993). The *gooseberry* Genes are Required in Cell Fate Specification during *Drosophila* Neurogenesis. *In* Davis R. and Zipursky L.(eds.): **Neurobiology of *Drosophila*. New York Cold Spring Harbor Laboratory Press.**

Zhang Y., Ungar A., Fresquez C. and Holmgren, R. (1994). Ectopic Expression of Either the *Drosophila gooseberry- distal* or *proximal* Gene Causes Alterations of Cell Fate in the Epidermis and Central Nervous System. **Development.** 120: 1151- 1161.

Zhong Y. and Wu C.-F. (1991). Altered Synaptic Plasticity in *Drosophila* Memory Mutants with Defective Cyclic AMP Cascade. **Science** 251: 198-201.

Zhong Y. and Wu C.-F. (1993). Differential Modulation of Potassium Currents by cAMP and Its Long Term and Short Term Effects: *Dunce* and *Rutabaga* Mutants of *Drosophila*. **J.Neurogenetics.** 9: 15- 27.

Ziegelberger, G. (1994). The Odourant Binding Proteins in the Silk Moth *Antherea polyphemus*. *In* Elsner N. and Breer H. (eds.): **Sensory Transduction. Proceedings of the 22nd Göttingen Neurobiology Conference. Vol. I.** p.86. **Stuttgart- New York: Georg Thieme Verlag.**

Zinn K., McAllister L., and Goodman C.S. (1988). Sequence and Expression of Fasciclin I in Grasshopper and *Drosophila*. **Cell** 53: 577- 587.

Zusman S., Grinblat Y., Yee G., Kafatos F.C. and Hynes R.O. (1993). Analyses of PS Integrin Functions during *Drosophila* Development. **Development** 118: 737- 750.

Abbreviations (cont.)

A	adenine/ adenosine/ adenylate
AMP	adenosine 5' - monophosphate
bHLH protein	basic helix- loop - helix protein
bp	base pair(s)
C	cytosine/cytidine/cytidylate
CAT	chloroamphenicol acetyl transferase
cDNA	complementary DNA
Ci	curie
CIP	calf intestinal alkaline phosphatase
CMP	cytidine 5'- monophosphate
c.p.m.	counts per minute
CTP	cytidine 5'- triphosphate
dATP	deoxyadenosine 5'- triphosphate
ddATP	dideoxyadenosine 5'- triphosphate
DEAE	diethyl aminoethyl
DNA	deoxyribonucleic acid
DNaseI	deoxyribonuclease I
EtBr	ethidium bromide
g	gravity
G	guanine/ guanosine/ guanylate
Gal4	transciptional activator protein of the yeast *Gal1- Gal10* divergent promoter
GMP	guanosine 5'- monophosphate
gRNA	guide RNA
GTP	guanosine 5'- triphosphate
H	histone
hn RNA	heterogeneous nuclear RNA
hsp 70	heat shock protein 70
hrs	hours
IPTG	isopropyl- β- D-thiogalactoside
kb	kilobasepair(s)
kDa	kilodalton(s)
kRNA	kinetoplast RNA
K_m	Michealis Menten constant
LCR	locus control region
LDH	lactate dehydrogenase
min.	minutes
mRNA	messenger RNA
mRNP	messenger ribonucleoprotein particle

NDK	nucleoside diphosphate kinase
NES	sodium acetate, EDTA, SDS buffer
NTP	nucleoside 5'- triphosphate
Oct	ocatamer binding protein
ori	origin of replication
PABP	poly(A) binding protein
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PCV	packed cell volume
PEG	polyethylene glycol
PER	pre- edited region
P_i	inorganic phosphate
pit	pituitary
PK	pyruvate kinase
PNV	packed nuclear volume
POU domaine	Pit-Oct-Unc domaine
p.p.m.	parts per million
PVA	polyvinyl alcohol
Poly(A)	polyadenylic acid
Poly(A)$^+$ RNA	polyadenylated RNA
rATP	adenosine triphosphate
rCTP	cytidine triphosphate
rGTP	guanosine triphosphate
RNase	ribonuclease
RNP	ribonucleoprotein particle
rNTP	ribonucleoside 5'- triphosphate
rRNA	ribosomal RNA
sec.	second(s)
SER	spliced exon reopening
SET	salt, EDTA, Tris buffer
SJH	splice junction hydrolysis
snRNA	small nuclear RNA
snRNP	small nuclear riboprotein particle
SSC	standard saline citrate
STE	sucrose, Tris, EDTA buffer
STM	sucrose, Tris, $MgCl_2$ buffer
SV 40	simian virus 40
T	ribothymidine/ribothymidylate
TAE	Tris- acetate-EDTA buffer
TBE	Tris-borate-EDTA buffer

TCA	trichloroacetic acid
TE	Tris- EDTA buffer
TEMED	*N,N,N',N'* - tetramethylendiamine
Tm	melting temperature
TMA	tetramethylammonium chloride
TMN	Tris, $MgCl_2$, NaCl buffer
U	uracil/uridine/uridylate
UDP	uridine 5'- diphosphate
UMP	uridine 5'- triphosphate
V	volts
W	watts
X-	gal 5- bromo-4-chloro-3-indolyl-β-D-galactoside
ψ	pseudouridine

Terminology

e.g.	
Notch	dominant allele of a gene
NOTCH	dominant protein
hedgehog (*hh*)	resessive allelle of a gene
HEDGEHOG (*HH*)	recessive protein