The Isolation and Characterisation of Y Homologous and XY Homologous Human DNA Sequences.

Submitted by

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in

February, 1989

to

The University of Glasgow

for the degree of

Doctor of Philosophy.

Research conducted in the Department of Medical Genetics, The University of Glasgow.

To Margaret Anne Mitchell

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Acknowledgements

The author would like to thank the following people:-

Dr. Nabeel Affara for the invaluable supervision, guidance and training which he provided throughout the project. Also for the critical reading of this manuscript.

Professor M.A. Ferguson-Smith for creating the environment within which this project was carried out and for providing laboratory space and resources.

Professor J. Michael Connor for help and encouragement with the preparation of this manuscript.

The Medical Research Council for funding the author during the course of the project.

The Staff of the Duncan Guthrie Institute for the provision of essential back-up services.

Jim Galt, Beth Gillard, Doreen Jamieson, Marianna Kalaitsidaki, Kenny Kwok and Lindsay Pirrit for help and comradeship.

Professor Marc Fellous for the provision of the resources necessary for the production of this manuscript.

Dr. Colin Bishop for encouragement and support during the writing of this manuscript.

Dr. Renée Bernstein for providing the Bantu male DNA.

Dr. Joy Delhanty for gorilla male and female, orang-outang male and chimpanzee male fibroblasts.

Dr. B. Weber for orang-outang female, gorilla female, African Green monkey male and female and macaca male DNA.

Dr. S. Malcolm for providing the lymphoblastoid line of MB.

Dr. R. Winter for providing the lymphoblastoid line of DL.

Mrs Margaret Anne Mitchell for making the whole thing considerably more manageable.

My parents for their help and support throughout my "perpetual" studentship.

Summary

The human Y chromosome has been estimated to be around 60Mb in length of which 50-70% is composed of non-functional heterochromatin. Meiotic pairing is observed between the distal end of Xp and Yp. This region was termed the pairing segment although the exact nature of the synapsis was unknown. Karyotype-phenotype correlations have led to the assignment of seven putative Y chromosome genes which include the primary testis determinants, suppressor(s) of the Turner phenotype, and factor(s) involved in the promotion of gonadoblastoma in streak gonads.

The aim of this project was to further characterise the Y chromosome at the molecular level. The basis of the work was the isolation of 47 Y homologous inserts from a flow-sorted Y chromosome specific library. These were identified as Y homologous using the Y-only somatic cell hybrid 3E7. They were further assigned to Yp or to Yq by positive or negative hybridisation, respectively, to DNA from an iso Yp male. Nineteen probes detected Yq specific restriction fragments, eighteen detected Yp specific restriction fragments and nine detected both Yp and Yq restriction fragments. To obtain a finer deletion map of the chromosome these probes were then used in conjunction with a number of other probes to analyse 23 XX males, 8 XY females, 2 XO males and 15 dicentric Yp individuals. These individuals carry varying amounts of the Y chromosome and from this analysis a 30 interval deletion map of the Y euchromatin was created. This map was constructed on the basis that the individuals analysed had terminal deletions of the Y chromosome arising from a single breakpoint. Only five individuals were found not to possess a contiguous piece of Y chromatin based on this consensus ordering of Y sequences.

With the aim of detecting testis transcripts directly thirty of the probes were used in Northern analysis of testis and placenta RNA. Only one probe, GMGY28, gave a distinct hybridisation in the testes track. This probe detects five distinct autosomal bands on Southern analysis and it is therefore possible that the Y sequence isolated is part of a pseudogene of a highly expressed autosomal testes transcript.

Eight of the probes, GMGXY4-GMGXY7, GMGXY9-GMGXY11 and GMGXY19 detected both X and Y restriction fragments. The X fragments were mapped using somatic cell

hybrids, and individuals, carrying deleted X chromosomes or one product of an X:autosome translocation. At least three regions of XY homology were defined between Xp22.3/Yq (GMGXY19), Xq21/Yp (GMGXY4-GMGXY7 and GMGXY9) and Xq27-28/Yp/Yq (GMGXY10). Probes with a similar pattern of hybridisation to the first two homologies have already been described but the GMGXY10 homology has not previously been defined. Five of the eight probes were of the Xq21/Yp class suggesting that such sequences form a large part of Yp. These sequences together with other similarly hybridising probes were mapped to adjacent intervals on Yp.

GMGXY19 and GMGXY3 (the latter isolated by L. Florentin) were used to analyse males with steroid sulphatase (*STS*) deficiency, as a high percentage have deletions of the *STS* gene which also maps to Xp22.3. It was found that 17/23 of the males analysed were deleted for both these probes and one for only GMGXY19. These 18 deletions have been shown to include the *STS* gene. Neither probe detected a transcript in placental RNA indicating that they are not part of the major *STS* transcripts.

The XY homologous probes were used to analyse Southern blots of 59 individuals from five different ethnic origins cut with up to 24 restriction enzymes. This allowed an estimate to be made of the degree of conservation in these regions between the two chromosomes through the XY differences observed at the restriction sites. The Xp22.3/Yq and Xq27-28/Yp/Yq homologies were estimated to be less than 94% conserved while the Xq21/Yp homology was estimated to be 98% conserved. Nine different RFLPs were also revealed for these probes. GMGXY4 detected two different X polymorphisms and the only Y polymorphism found in the whole study. GMGXY5 detected three different X polymorphisms that showed complete linkage disequilibrium while GMGXY9 revealed a four allele X polymorphism. GMGXY7 and GMGXY10 revealed infrequent X polymorphisms with a single enzyme. An estimate of the polymorphic frequency of the X and Y chromosomes revealed that the Y chromosome is as much as 10 times less polymorphic than the X chromosome in these regions.

The recent evolution of the XY homologous probes was studied through the Southern analysis of five primate species; man, chimpanzee, gorilla, orang-outang and African Green monkey. In all species where both a male and a female were tested the probes

could be ascertained as detecting an X homologue. The Xq21/Yp homologous probes only revealed a Y homologue in man. The Xq27-28/Yp/Yq homologous probe detected a Y homologue in all three ape species but not in the monkey species. The Xp22.3/Yq probes detected a Y homologue in chimpanzee and orang-outang but not African Green monkey or gorilla which argues that an independent deletion has occurred of the gorilla Y homologue.

The mapping of the Y chromosome into thirty intervals has provided an excellent basis upon which to localise and clone the genetic functions of the chromosome. Based on this map the testes determinants have been mapped into the most distal non-pseudoautosomal region of Yp defined by a single probe, GMGY3. Based on the analysis of a Yp- XY female the locus suppressing the Turner's phenotype has been mapped to the distal half of Yp while the factor that promotes gonadoblastoma has been mapped to the reciprocal part of the Y chromosome.

For the short arm in particular, it also reveals the rough molecular organisation of the chromosome. It can be divided into six segments running from telomere to centromere as follows: the highly XY homologous pseudoautosomal segment on distal Yp; a region defined by a Y/autosomal homologous probe into which tdf can be localised; a large region of Xq21/Yp homologous probes; a "Y specific" region; a smaller region of Xq21/Yp homologous probes; a small "Y specific" region containing the alphoid repeats. The long arm does not reveal any clear molecular organisation except perhaps that the two Xp22.3/Yq homologous probes map to possibly adjacent segments on proximal Yq.

The results obtained in this study can also be used to draw conclusions about the behaviour and stability of the Y chromatin. Taken together the comparative analysis, the Yp/Yq homologous probes, and the non-consensus individuals in the Y deletion mapping indicate that the Y chromosome may be relatively prone to insertions, deletions, duplications and inversions, when compared to the X chromosome. Contrary to this view of a rapidly mutating Y chromosome are the results of the multiple enzyme analysis which reveals the Y chromosome to be ten times less polymorphic than the X chromosome suggesting it to be perhaps more resistant to point mutations. It is argued that this is at least in part attributeable to the lack of recombination along most of the

Y chromosome's length. The proposed relatively high incidence of DNA rearrangements is concluded to be a result of the Y chromosomes ability to sustain such events because of a relative lack of functional loci.

CHAPTER 1 - INTRODUCTION

1 INTRODUCTION

The human Y chromosome is estimated to be 60Mb in length (Mendelsohn et. al. 1973) of which about half is composed of non-functional heterochromatin. Even if its size is taken into account the Y chromosome appears to be underrepresented for genetic loci since chromosomes 21 and 22, which are of a similar size, have each had 28 loci already assigned to them (Kaplan & Carritt, 1987) whereas the Y chromosome has only seven putative assignments and even some of these are controversial. One of the least controversial assignments is that of the primary testes determinant which presents an excellent opportunity to clone a defined mammalian developmental control gene. The others include a factor involved in spermatogenesis, an oncogene and a cloned pseudoautosomal gene of unknown function (*MIC2*).

Besides its genetic functions the Y chromosome must also carry certain structures essential to its maintenance in mitotic and meiotic cells. Therefore it must also have telomeres, a centromere, one or more origins of replication and a region where it is able to pair with the X chromosome during meiosis. Little is known about the first three structures and they will not be dealt with in detail here. However an XY pairing region has been observed between distal Xp and Yp and the basis of this synapsis has been the subject of much speculation.

The Y chromosomes lack of heritable mutations forces a molecular approach to its further characterisation but the small size of the Y chromosome makes it ideal for such molecular study. In general the approach taken by most groups has been to isolate random stretches of Y chromosomal DNA. These have largely been mapped on the Y chromosome by Southern blot analysis of individuals carrying incomplete Y chromosomes and will provide a deletion map of the chromosome and its genetic functions. From the basis provided by this map the cloning of the genetic loci of the Y chromosome can be undertaken.

The aims of this introduction are first to present a structural and genetic model of the Y chromosome based largely on data from cytogenetic studies. And second to present

the preliminary molecular data and its contribution to the elucidation of the structure and genetic functions of the Y chromosome.

1.1 The Cytogenetic Model of the Y Chromosome

A diagram of this model is presented in figure 1 with the structural aspects of the model on the left and the genetic loci on the right. This model is based on the "Classical Model" proposed by Goodfellow et. al. (1985) but carries some of the more controversial genetic loci. At the structural level the chromosome can be divided into two roughly equal parts; the brightly staining heterochromatin and the non-staining euchromatin. It is into the euchromatin that all the genetic loci are believed to map. The euchromatin is divided into Yp and Yq by the centromere. Most of Yp can be seen to associate with distal Xp during male meiosis and thus is called the pairing region.

Genetically, the primary testes determinants (*TDF*) and a loci which suppresses Turner stigmata were mapped to Yp and a locus involved in spermatogenesis (*AZF*) to Yq. A promoter of gonadoblastoma in streak gonads (*GBY*), a minor histocompatibility antigen *H-Y* and factors affecting stature and tooth size (*GCY*) are assigned to the euchromatin of proximal Yp or Yq.

The structural and genetic aspects of this model will be discussed separately in more detail below.

1.2 Structural Aspects of the Cytogenetic Model

1.2.1 The Heterochromatin

The heterochromatic region of the Y chromosome normally accounts for 50%-70% of the Y chromatin and stains intensely with quinacrine mustard (Zech et.al. 1969). Its length can vary considerably between Y chromosomes in a heritable manner (Bobrow et. al. 1971) and this variation is the major cause of Y chromosome size polymorphism. Its exact function if any is unknown. However it seems unlikely to have any phenotypic effect as normal males occur lacking almost the complete region and a

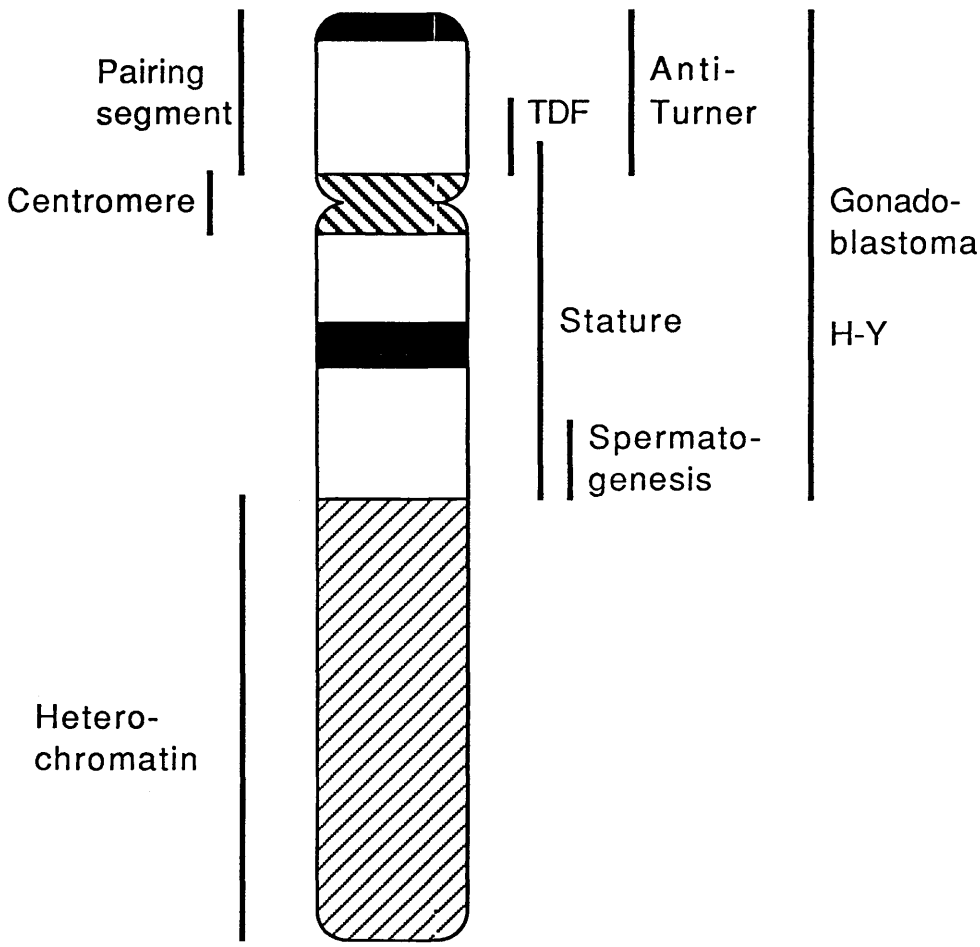


Figure 1: The "Cytogenetic" Model of the Y chromosome (after Goodfellow et al, 1985). Structural loci are shown to the left of the chromosome and genetic loci to the right.

study of 12,942 karyotypes (Cooke et. al., 1979) revealed that an estimated 1 in 3,200 females carry this region on either 13p or 15p.

1.2.2 The X-Y Pairing Segment

The human X and Y chromosomes are observed to pair between distal Xp and distal Yp during male meiosis (Pearson & Bobrow, 1970). This synapsis was estimated by Polani, (1982), from the data of Solari, (1980), to include at least 95% of Yp and and 27% of Xp. However Chandley et al., (1984) found that the pairing region in normal males involved 11.4% to 56.3% of the total Y chromosome and 3.3% to 17.3% of the X chromosome. However it was suggested by Goodfellow et al., (1985) that, as chromosomal pairing can be observed to become less stringent as meiosis progresses, the postulated region of X-Y homology which forms the basis of the synapsis would be much smaller than indicated by the measurements of Polani, (1982) and Chandley (1984). Therefore it is possible that the smallest synapsis observed by Chandley et al., (1984) provides an estimate of the maximum size of the region essential to X-Y pairing. Thus the region can be postulated to be no greater than 4.5Mb.

This observation that the X and the Y chromosomes pair during male meiosis led to the suggestion that there was considerable homology between the two chromosomes involving almost all of Yp (Polani, 1982 and Burgoyne, 1982) and that there was an obligatory cross-over between the chromosomes.

The idea of crossing-over being necessary for the production of viable gametes stems from the observation that chiasmata are always seen between the synapsed chromosomes in a bivalent. It is postulated that the chiasmata are the structures which hold the bivalents together througout prophase I and metaphase I, and that the generation of a chiasma is dependent on a cross-over event between the paired chromatids.

Evidence suggested that in the mouse at least such recombination occurred and indeed was obligatory. This evidence came in part from the elucidation of the dominant sex-reversing mutation sxr which caused XX mice to develop as sterile males. Singh & Jones et al., (1982) showed that a region of the Y chromosome was duplicatively

transferred to the distal end of the Y pairing region in the carrier males. This region which was presumed to carry the mouse testes determinants tdy was then transferred to the X chromosome during male meiosis and was present on one end of the X chromosome in the XX*Sxr* mice. This was shown through in-situ hybridisation studies with a sequence detecting the Bkm satellite repeat which normally hybridises predominately to one end of the mouse Y chromosome and appears to be within the *sxr* region. Evans et al., (1982) later showed the same events by cytogenetic analysis.

From the cross of carrier male with normal female there are equal proportions of four different progeny genotypes: XX, XY, XX*sxr* and XY*sxr*. This ratio is what would be expected for an autosomal dominant gene and suggests that a cross-over is occurring between the X and the Y chromosomes during male meiosis.

Furthermore Winsor et al., (1978) observed a high incidence of X-Y separation at pachytene in XY*sxr* carrier mice. However the observed ratios of progeny genotypes obtained from sxr crosses suggests that those X-Y bivalents which breakdown without recombining do not produce viable sperm. In the mouse at least pairing appears to be crucial for gamete viability.

Further evidence for X-Y homology and recombination came with the conclusive genetic mapping of the steroid sulphatase (*sts*) locus to the murine X chromosome by Keitges et al., (1985). Based on its inheritence pattern this locus had previously been reported as an autosomal locus but the autosome that it mapped to had never been able to be defined. It was concluded that the X borne locus must escape X-inactivation and have a functional Y homologue with which it recombines during male meiosis. Interestingly the human *STS* locus has been mapped close to the pseudoautosomal region at Xp22.3 by Tiepolo et al., (1980) and Ferguson-Smith et al., (1983) and has been shown to escape X-inactivation by somatic cell hybrid analysis of *STS* expression from an inactive X chromosome (Mohandas et al., 1980). However there is no evidence for a functional Y homologue and in man *STS* must lie outwith the pseudoautosomal segment unless it is subject to chromosome-specific control.

Normally in man X-Y recombination would only occur between the regions that lay distal to the loci controlling the expression of *STS* and the X-linked erythrocyte cell

surface antigen *Xg* which are the most distal non-pseudoautosomal Xp loci known. *Xg* like *STS* has been mapped near the pseudoautosomal segment by somatic cell hybrid analysis (Ferguson-Smith et al., 1982) and has also been demonstrated to escape X-inactivation (Gorman et al., 1963 and Ducos et al., 1971). It is detected by serological methods and has two alleles Xga+ and Xga- with which the antiserum, respectively, does and does not react.

Loci in the region of X-Y recombination would appear to be autosomally linked and for this reason this part of the pairing region was termed the pseudoautosomal segment.

Ferguson-Smith, (1965) postulated that the human X and Y chromosomes must share genes that were expressed in normal femals from both X chromosomes to explain the occurrence of the Turner's phenotype in 45, X females but not normal males. Burgoyne, (1982) proposed that these genes may map into the pairing region and that such genes would escape X-inactivation. This was based on the knowledge that STS and Xg which map to this region of the human X chromosome, although not pseudoautosomal, escape X-inactivation. However strong evidence against pseudoautosomal Turner suppressors exists and will be presented in the next section.

1.3 The Genetic Functions of the Y Chromosome

The monosomic nature of the Y chromosome allows certain conclusions to be drawn about the types of functional genes that could reside on it. These fall into three classes :- (1) genes that are involved in the determination or maintenance of the male phenotype, (2) genes which in males effect dosage compensation on any X-borne genes that are expressed in females from both X chromosomes, and (3) genes whose dosage is not crucial to their correct function.

The apparent genetic influence of the Y chromosome in development is relatively easy to detect. However the precise localisation of the Y-borne factors responsible is more difficult. Classical linkage studies on the Y chromosome are excluded through the lack of recombination along most of its length and its small size makes the cytogenetic characterisation of abnormal Y chromosomes difficult. Furthermore detected and

undetected mocaisism may lead to the tissue tested having a different karyotype from the tissue in which the Y-borne factor normally acts.

1.3.1 The Testes Determinants

Until 1959 the key role of the Y chromosome in primary sex determination was not proven. As males have one X chromosome and a Y chromosome while females have two X chromosomes but no Y chromosome it was concluded that either two X chromosomes caused female determination or the Y chromosome caused male determination. This was cleared up when Ford et al., (1959) showed that a female who had Turner's Syndrome 45,X and Jacobs & Strong, (1959) described a male with a 47,XXY karyotype. These cases show that the presence of the Y chromosome rather than the presence of a single X chromosome is the cause of male determination.

A considerable amount of cytogenetic data exists on structural abnormalities of the Y chromosome and their effect on the sexual phenotype. These are reviewed by Davis, (1981) and while mosaicism and the inaccuracies of the cytogenetic techniques used must be borne in mind - particularly prior to the development of banding - a consensus view emerges that the testes determining factors (*TDF*) map to the pericentric region of Yp. The testes determinants can be excluded from the heterochromatic region of Yq12 as there are many males reported who lack this region and many females who bear it on an autosome or the X chromosome.

The localisation to the pericentric region is most strongly argued by measurements of the amount of Yp present in certain 46,Xdic(Yq) males. The region between the centromeres of these chromosomes is believed to be less than the size of Yp. As dicentric Yq chromosomes are assumed to be duplicated for the Yp seqences present it was concluded that *TDF* must lie in the proximal half of Yp. Cases of 46,Xdic(Yq) females are cited which indicate that *TDF* maps to Yp. These apparently non-mosaic cases provide the best evidence for a proximal Yp location for *TDF*.

There is weaker cytogenetic evidence that *TDF* maps to Yq. Ferguson-Smith et al., (1969) reported a Turner's female with some masculinisation of the genital tissues. This patient had an apparent 45,X / 46,X,iso(Yq) karyotype. However as no banding

was performed it is possible that this chromosome is not an iso(Yq) but has Yp sequences present. It could for example be the result of a pericentric inversion. Y:autosome translocations involving at least the heterochromatic portion of the Y have been found in patients with possible signs of masculinisation of the gonads. Bernstein et al., (1978) reported a 46,XX+ with an extended X which appeared to have the fluorescent and part of the non-fluorescent portions of Yq translocated on to Xp. Rary et al., (1979) report a female with both products of a 17:Y translocation and a single X chromosome who had streak gonads and gonadoblastoma. The breakpoint on the Y chromosome is in band Yq11 and it is suggested by the authors that the expression of *TDF* is disrupted as it maps near the breakpoint on Yq11. Alternatively there may have been mosaicism for the translocation products in the early embryo or that there is a deletion of the region bearing *TDF*.

Davis, (1981) attempted to reconcile these contradictions of mapping position by postulating that the location of *TDF* is polymorphic in the population. Those cases that suggest a Yq location for *TDF* are not as numerous as those that suggest a Yp location. This could mean either that *TDF* is more commonly on Yp or else that the configuration of the Y chromosome with *TDF* on Yp is less stable than that which places *TDF* on Yq.

XX Males and XY Females. There are apparent exceptions to the rule that the Y chromosome promotes male determination. These are males with 46,XX karyotypes and females with 46,XY karyotypes.

The XX males were first described by de la Chapelle et al., (1964) and Therkelsen et al., (1964). It was observed that some did not inherit the paternal *Xg* allele and Ferguson-Smith, (1966) postulated that an aberrant terminal exchange between the X and the Y chromosomes during male meiosis transferred the testes determinants to the X chromosome and some X sequences to the Y chromosome. These X sequences would include the *Xg* locus. Thus the father would pass on a deleted X chromosome bearing a region of the Y chromosome containing the testes determinants. This interchange, it was postulated, was facilitated by the pairing of the X and Y chromosomes in this region during male meiosis and was the result of an aberrant recombination event. In seven out of nine informative families the paternal allele was not inherited by the XX male

offspring (Sanger et al., 1977) but the distribution of Xga in XX males initially resembled more that of XXY males than XX females or XY males (Sanger et al., 1971, Sanger et al., 1977). However additional data revealed the XX male alleleic frequencies to be more similar to XY males (X^2=1.31)than XXY males (X^2=8.12) or XX females (X^2=17.11) (Sanger personal communication in Evans et al., 1979). This further backed up the interchange hypothesis as an explanation for the occurrence of most XX males.

Madan et al., (1974) presented evidence in a single XX male that there was extra material, possibly from the Y chromosome, on one of the X chromosomes at the distal end of Xp distal to Xp22. A more extensive study was conducted by Evans et al., (1979) on twelve XX males and they found that eight carried an Xp+ on one of their X chromosomes. They concluded that the extra material was Y in origin and that 70% of XX males arose through X-Y interchange. Magenis et al., (1982) observed extra material on Xp in two out of a total of three XX males and using high resolution banding concluded that the extra material was from the Y chromosome. They described the Xp+ chromosome as a t(X;Y)(p22.3:p11.2) which carried Yp11.2-pter. Magenis et al., (1984) have also reported a 46,XYp- female deleted for Yp11.2-pter. This location is compatible with the other cytogenetic data (Davis et al., 1981) and taken together with it suggested that *TDF* mapped to Yp11.2.

Most pure gonadal dysgenesis 46,XY females appear externally as normal women but internally have streak gonads like Turner females which unlike the Turner streak gonads have a very high predisposition to develop gonadoblastomas. The presence of the full Turner phenotype in in apparent 46,XY females is suspected to indicate the existence of 45,X / 46,XY mosaicism. However there is a small group which have many of the Turner stigmata but have normal stature (Rosenfeld et al., 1979, Magenis et al,. 1984 and two cases by Disteche et al., 1986). These individuals are all reported to be deleted for a portion of the short arm of the Y chromosome. It is assumed that this latter group are deleted for *TDF* and the anti-turner factor(s) while the non-Turner XY females are the result of smaller deletions or other mutations of the Y chromosome

involving just *TDF* or by mutations of genes involved in the male determining pathway elsewhere in the genome.

Ferguson-Smith (1966) hypothesised that the 46,XY females could be the reciprocal of XX males in that they could carry a Y chromosome deleted for *TDF* through X-Y interchange. While it would appear that a small group do indeed carry deleted Y chromosomes the high incidence of Xp+ amongst XX males detcted by Evans et al., (1979) is not reflected by a high incidence of Yp- amongst XY females. This is probably explained by the association of Turner stigmata with XYp- as XO fetuses are known to have a very high level of spontaneous miscarriage.

Thus before the advent of DNA probes it was generally accepted that the testes determinants lay at Yp11.2 and that most XX males were the result of an aberrant interchange between the distal short arms of the X and the Y chromosomes during meiotic synapsis.

1.3.2 Loci which Suppress the Turner Phenotype

Turner's syndrome is primarily observed in females with a 45,X karyotype. It is diagnosed by the presence of a number of characteristic stigmata including gonadal dysgenesis, short stature and webbed neck but can also include peripheral lymphodaema, cubitus valgus, broad chest, coarctation of the aorta, pigmented naevi, and characteristic dermatographs. These symptoms have been suggested (Ferguson-Smith et al.,1965) to be the result of a single dose of X-linked genes which are normally present in a double dose either because they are expressed at a stage in development when both X chromosomes are active or because they escape X-inactivation. From deletions of the X chromosome (reviewed by W ss et al.,1982 and Skibsted et al., 1984) it would appear that in general deletions of the short arm of the X chromosome result in much more severe stigmata than do deletions of the long arm but it is only when a terminal deletion extends to Xp11 that the full syndrome is ever seen. These observations tentatively suggest that there is a major gene involved in the prevention of the Turner phenotype on the proximal half of Xp.

In males the Y chromosome appears to compensate for the presence of only a single X chromosome and it can be put forward that the Y chromosome must carry some factor(s) which prevent the onset of Turner's syndrome.

There have been four 46,XYp- females reported in the literature (Rosenfeld et al., 1979, Magenis et al,. 1984 and two cases by Disteche et al., 1986). All four of these cases had major Turner stigmata but were of normal height and all four had deletions of the short arm of the Y chromosome. This argues that the Y-borne factor which prevents the short stature stigmatum is distinct from the other factors and that these other anti-Turner factor(s) must map on to the short arm of the Y chromosome.

The most obvious location for these factors would be the pseudoautosomal segment. However there are a number of reports of 46,Y,t(X;Y) (p22.3;q11) males (Akesson et al., 1980 , Allderdice et al., 1983, Boyd et al., 1981, Ferguson-Smith et al., 1982, Metaxotou et al., 1983, Tiepolo et al., 1977, 1980, and Van den Berghe et al., 1977). These males, with the exception of that reported by Van den Berghe (1977), have all been reported as deleted for either *Xg* or *STS* which implies that they are also deleted for the X-borne pairing region and have therefore only a single pseudoautosomal region. As the patients lack major Turner stigmata it would appear that the anti-Turner effect of Yp is not mediated by genes of the pseudoautosomal segment which would be expressed in double dose in both males and females. In some of these cases the patient's mother also carries the XY translocation. It is posible that there are some pseudoautosomal sequences present in the translocation product and a detailed analysis of these cases and also the XYp- cases with probes from this region should clarify the situation.

The data from the 46,XYp- females and the 46,Y,t(X;Y) (p22.3;q11) males then allow the factors to be located between the proximal Yp breakpoint of the XYp- females and the pseudoautosomal boundary. Contradictory to this localisation are the reports reviewed in Davis, (1981) of 46,X,i(Yq) females who lack Turner stigmata (e.g. Böok et al., 1973). These cases suggest a Yq location for the anti-Turner factor. A possible explanation of this contradiction is that there was a normal Y or an isoYp in the tissues in which the factors act. If isoYq most frequently arises at an early stage of

development then a population of cells would carry an isoYp. As there are reports of apparently non-mosaic 46,X,i(Yq) females with Turner stigmata (e.g. Magnelli et al., 1974) it would appear that these cases are indeed confused by mosaicism. In contrast if XYp- females do carry the reciprocal t(X;Y) to that present in the XXp+ males which most likely arises during paternal meiosis then, unless this chromosome is unstable, the XYp- females should not be mosaics.

There are three possible models for the action of the Y-borne anti-Turner factor(s). All three models assume that in normal females the X-borne gene is expressed from both chromosomes and that both doses are required for correct development. Two of the models also postulate that the Y-borne factor compensates directly for the presence of only a single dose in males. In one model the Y chromosome does this by producing a homologous product to that produced by the X while in the other the Y produces an up-regulator of the X gene(s). The former model would imply an homologous gene Yp and proximal Xp. The third model postulates that Turner's syndrome is a sex-limited phenotype and that the Y-borne factor is identical with *TDF*. The testes determinant is known to alter development and perhaps on the developmental background of a male determined embryo a single dose of the X-borne factor is sufficient for correct development. The identity of *TDF* and the anti-Turner factor could be combined with the other two models.

1.3.3 Gonadoblastoma Promoter

Both 45,X females and 46,XY females are observed to have streak gonads as the result of gonadal dysgenesis probably caused by the absence of a second X chromosome. However the streak gonads of the XY females exhibit a very high incidence of gonadoblastomas. It would appear that there is a locus on the Y chromosome which when expressed in streak gonads promotes the formation of gonadoblastoma.

Two of the four XYp- females reported (Magenis et al., 1984 and case 2 of Disteche et al., 1986) had developed gonadoblastoma. The other two cases had their gonads removed at anearly when onset was unlikely to have occurred. This maps this locus to either Yq or to the region of Yp proximal to the deletion of the XYp- females.

The apparently non-mosaic 46,X,i(Yq) females as reviewed in Davis, (1981) do not manifest gonadoblastomas (e.g. Böok et al., 1973 and Magnelli et al., 1974) which suggests that the long arm of the Y chromosome does not carry these factors. Furthermore there is a much greater incidence of gonadoblastoma in females with 46,X,dic(Yq) karyotype even though most are mosaics with a 45,X line (Davis, 1981). This indicates that the proximal region of Yp may play a crucial role in the promotion of gonadoblastomas.

These observations do not rule out the possibility that there are factors on both proximal Yp and Yq that are required together to induce gonadoblastomas in the streak gonads.

1.3.4 Factors invovled in Spermatogenesis

The evidence that there is a male fertility factor (*AZY*) on the Y chromosome which maps to the distal Yq euchromatin is reviewed in Davis, (1981). Tiepolo & Zuffardi, (1976) reported six azoospermic males with Yq- Y chromosomes which were non-fluorescent. It was concluded that they were also deleted for the distal non-fluorescent region of Yq and therefore that a factor required for correct spermatogenesis was present in this region of the normal Y chromosome. In only one case was mocaisism detected. This does not imply that there are no other factors on the Y chromosome which are involved in spermatogenesis on either Yp or Yq. However in order to detect these other genes interstitial deletions of Yp or Yq would be required which left both *TDF* and *AZY* intact.

A further complication of using such deleted chromosomes to map factors involved in spermatogenesis is that the deletions may affect the chromosome's ability to pair at meiosis during spermatogenesis. It is strongly implicated from work on the *sxr* mutation in mice that the abnormal Y chromosome carrying the extra copy of the sxr region on its pseudoautosomal segment has an increased level of non-disjunction between the X and Y bivalent (Winsor et al., 1978). Furthermore it is likely that this non-disjunction blocks gamete formation (Burgoyne, 1986). It is therefore possible

that what is being defined in the human is not a spermatogenesis factor but a rearrangement that disrupts meiotic pairing.

1.3.5 H-Y Antigen

The H-Y antigen was initially identified as a minor histocompatibility antigen in mice. It was observed that within an inbred strain grafts from a male mouse to a female mous were rejected. The presence of H-Y can be assayed using cytotoxic T-cells which are histocompatible with the target cells - the cells to be typed. In mice the expression of H-Y antigen measured in this way has been correlated with the presence of the Y chromosome.

It was postulated that the H-Y antigen may be primary testes determinant. However this was shown not to be the case in the mouse. XX*sxr* male mice carry only a small portion of the Y chromosome called the *sxr* region which bears the mouse testes determinants (*tdy*). The *sxr* region was also believed to carry *H-Y* as these mice type H-Y positive. McLaren et al., (1984) described three generations of mice which carry an altered sxr region called *sxr'*. This region while still promoting testes determination in the XX*sxr'* mice fails to promote the expression of H-Y and these male mice type H-Y negative. This clearly shows that the region controlling male determination can be separated from that controlling the expression of H-Y antigen and that male determination and differentiation can occur in the absence of H-Y.

A possible role for *H-Y* has been suggested by Burgoyne et al., (1986) who showed that XO*sxr'* mice have a much more severe spermatogenic failure than do XO*sxr* mice. It therefore appears that *H-Y* has a role not in testes determination but in spermatogenesis. It is however possible that the same event that silenced *H-Y* has also knocked out another gene in the *sxr* region that is involved in spermatogenesis and that H-Y has a quite different function.

In order to avoid the difficulties involved in assaying H-Y by T-cells a serological test for H-Y was developed. It is possible, however, that this test is detecting a different antigen from that defined by graft rejection. Therefore this antigen is termed serologically detectable male (SDM) antigen. The serolgical test is difficult to perform

and to reproduce with the difference between a positive and a negative result being very small (reviewed by Müller, 1982 and Goodfellow & Andrews,1982). Furthermore the groups that perform the assays are each using their own sera and assays which may be detecting different antigens. The murine female anti-male sera is able to detect male specific antigens in all species tested and cell aggregation experiments with testicular and ovarian cells (reviewed by Muller, 1982) suggest that SDM antigen might play a crucial role in the organisation of the testes.

It is likely that the sera will contain antibodies against many antigenic determinants that are present on male but not female cells. These will consist of genes that are induced directly or indirectly by either the presence of a Y chromosome or a single X chromosome. This would explain the SDM positive results in XO mice (Engel et al., 1981) and XO humans (Wolf et al., 1980). It seems likely that the histocompatibility H-Y will be amongst those antigens detected by the SDM anti-sera but that the other specificities complicate and obscure the results obtained.

1.3.6 Height and Tooth Size

It has been reported that the height and tooth size of XX males is close to that of normal women (Alvesalo & de la Chapelle, 1981). Furthermore, the presence of the Y chromosome in the 46,XYp- females with Turner stigmata appears to counteract the short stature normally seen in 45,XO individuals. These examples appear to suggest that the Y chromosome has an effect on stature other than any hormonal effect of male differentiation. The loci involved, termed *GCY*, can be mapped to Yq or proximal Yp beneath the proximal breakpoint of the XXp+ males and the XYp- females.

These few genetic effects that can be ascribed to the Y chromosome are poorly characterised. It is not even possible to be sure that they are all separate loci or even that they are loci at all. However regions of the chromosome have been defined into which these effects map and these provide the starting point for the molecular characterisation of the genetic functions of the Y chromosome.

1.4 The Molecular Analysis of the Y Chromsome

DNA probes detecting Y-specific restriction fragments on Southern analysis of genomic DNA were isolated initially mostly from cosmid libraries made from partially digested DNA from the mouse-human somatic cell hybrid 3E7 (Bishop et. al., 1983 and Wolfe et. al., 1984). This hybrid contains the Y chromosome as its only human chromosome. Human recombinants were identified by screening with total human DNA. However a number of probes were isolated from a cDNA library (Kunkel et. al., 1983), a flow-sorted library of the X chromosome (Koenig et. al., 1984,1985) and a phage library of total human DNA (Page et. al., 1982). All these probes detected restriction fragments specific for both the Y and the X chromosome. Flow-sorted libraries of the Y chromosome as constructed by Cooke et. al., (1983) and Muller et. al., (1983) were not as productive because of contamination from other chromosomes (Muller, 1984 and Cooke, 1985: personal communications in Goodfellow et. al., 1985). The use of such probes has led to the elucidation of some of the structures of the cytogenetic model but has also revealed further structural features at the DNA level.

1.4.1 The Heterochromatin

The molecular analysis of the heterochromatin (reviewed by Cooke et al., 1983) has shown that this region is composed predominately of two repeated elements of 2.47kb and 3.4kb which are present in around 2000 and 4000 copies respectively. Bands of 3.4kb and 2.1kb can be visualised on ethidium bromide staining of *Hae*III digested male DNA after electrophoresis. Partial digestion analysis reveals ladders of fragments that are multiples of the repeat lengths and suggests that the repeats are arranged predominately in tandem arrays.

The discrepancy between the 2.1kb band seen on complete *Hae*III digestion and the actual repeat length of 2.47kb is the result of two close *Hae*III sites in each repeat. Partial *Hae*III or complete *Msp*I digestion on Southern analysis with the 2.1kb repeat gives a ladder of fragments that are multiples of 2.47 in both male and female. The female was measured to have 100 copies of a 2.0kb band which is homologous to the 2.47kb repeat.

The 3.4kb repeat has regular clusters of *Hae*III, *Eco*RI and *Eco*RII sites at 3.4kb intervals along its length. Its copy number as detected by ethidium bromide staining of electophoresed *Hae*III digested DNA is proportional to the length of the heterochromatin observed cytogenetically (McKay et al., 1978). They proposed that the 3.4kb repeat is dispersed throughout the whole region and the variation has evolved through unequal sister-chromatid exchange between the 3.4kb repeats. This is not the case for the 2.47kb repeat which is seen to vary in quantity independently of the 3.4kb repeat. This suggests that at least in the regions where the unequal exchange occurs these repeats are not interspersed with each other. In-situ analysis (Szabo et al., 1980) and restriction enzyme analysis of individuals with deletions of the hetrochromatin (Schmidtke et al., 1980) map the 2.47kb repeats predominately to the distal end of the heterochromatin. The 3.4kb repeat is seen with the low resolution of in-situ hybridisation to map along the entire length of the heterochromatin and also to a lesser extent to the Yq euchromatin. However because the 3.4kb repeat is not seen in Yq- individuals, who appear to be deleted only for the heterochromatin, it is reasonable to assume that almost all of the repeats are situated within the heterochromatic portion of Yq.

Frommer et al., (1984) by sequencing part of a 2.47kb repeat unit showed that it contained a member of the Alu repeat family. It was measured to be 80% homologous to the human Alu consensus sequence.

Comparative mapping in gorilla and chimpanzee with the 3.4kb and the 2.47kb repeats revealed no distinctive Y hybridisation and the hybridisation is largely autosomal. Thus it would appear that the amplification of these repeats on the human Y chromosome has evolved since the divergence of chimpanzee gorilla and man around 5Myr ago.

1.4.2 The Y-Euchromatin

A number of sequences have been isolated and mapped to the Y-euchromatin. Bishop et al., (1984) found that out of 26 Y-homologus cosmids only nine were Y-specific while four were Y/autosomal homolgous and one was X/Y/autosomal homolgous. As

several cosmids contained Y-specific as well as Y/autosomal homologous sequences it was concluded by the authors that these Y/autosomal homologies were the result of small insertion events of autsomal sequences into the Y chromosome. However the remaining twelve were purely XY homologous. This preponderance of probes defining homology between the X and Y chromosomes is discussed in more detail below.

Pseudogenes for actin (Heilig et al., 1984) and argininosuccinate synthetase (Daiger et al., 1984) have been mapped to the Y chromosome.

Wolfe et al., (1984) suggest that the Y-euchromatin is under-represented for Alu sequences. Of their 98 cosmids 27 hybridised with a human Alu-repeat probe but only two of these lay outwith the 2.47kb repeats of the heterochromatin. They also showed that the Alu probe Blur 11 hybridises weakly to 3E7 DNA, which contains a human Y on a mouse background, as compared to "X-only" hybrid DNA.

1.4.3 The Centromeric Region

Members of the alphoid repeat family are found clustered predominately at the centromeric region of all primate chromosomes (reviewed in Singer, 1982). Southern analysis can often reveal a chromosome specific arrangement. Using in-situ hybridisation Willard (1985) showed that, at low stringency a member of the alphoid family from the X chromosome hybridised to the centromeric regions of all chromosomes. Wolfe et al., (1985) have isolated members of the Y-borne alphoid cluster using the X alphoid sequence to screen a Y-cosmid library at low stringency. These cosmids detected a strongly hybridising Y-specific fragment of 5.5kb on Southern analysis of *Eco*RI digested genomic DNA. It was estimated that there were 100 copies of this 5.5kb fragment on the Y chromosome. One of the cosmids isolated contained three tandemly arranged copies of the 5.5kb *Eco*RI repeat and the other only one. Restriction analysis of the inserts revealed an underlying 170bp periodicity and that *Eco*RI, *Pst*I and *Hin*dIII cut every 5.5kb.

The human Y-alphoid sequence revealed no male specific pattern on Southern analysis of genomic DNA from chimpanzee and gorilla. This implies that the 5.5kb

repeat has evolved on the Y chromosome since the divergence of chimpanzee, gorilla and man. It is not possible to conclude that the alphoid repeat plays any role in centromeric function. Its localisation may simply imply that this region of the chromosome has certain properties which are required by this element for its proliferation and maintainence in the genome.

1.4.4 The Pseudoautosomal Region

When Y homologous DNA sequences began to be isolated a high proportion of these sequences detected cross-homologies with the X chromosome. Ironically, these homologies did not map into corresponding regions of Xp and Yp but mapped to Yp/Xq, Yq/Xp and even Yq/Xq (these are discussed in detail in the section "Regions of XY-Homology outwith the Pseudoautosomal Segment"). However eventually such probes were isolated which defined a region of high homology on the tips of Xp and Yp within which recombination occurred between the X and the Y chromosomes.

Two anonymous DNA sequences, isolated independently by Cooke et al., (1985) and Simmler et al., (1985) were the first probes to be definitely confirmed as pseudoautosomal.

Simmler et al., (1985) isolated a cosmid - 113 (DXYS15) - subclones of which were mapped to the X and Y chromosomes. No differences could be detected between the X and Y homologues upon restriction analysis of genomic DNA. One subclone (113D) was highly polymorphic on *Taq*I digests and revealed at least four alleles. As this probe was polymorphic for a number of other enzymes the authors postulated that they arose through an insertion / deletion mechanism. Another subclone (113B) was polymorphic for *Pst*I only which was proposed to be the result of a point mutation. In the cases of both these polymorphisms all the alleles could be found on the X or the Y chromosome. Another subclone called 113F but renamed DXYZ2 defined a repeat element which was estimated to be present in twenty copies.

By hybrid mapping all the homologies detected by these probes were shown to be wholly localised on the X and Y chromosomes and the X homologies were localised to Xp22.3-pter. Mapping by in-situ hybridisation using DXYZ2 confirmed the Xp

localisation and mapped the Y homology to Yp. Family studies showed that the alleles of 113D were exchanged between the X and the Y chromosomes during male meiosis.

Cooke et al., (1985) reported a probe 29CI (DXYS14) which was mapped to within 20kb of the Xp and Yp telomeres by a combination of hybrid mapping, which mapped it to distal Xp and Yp, and Bal31 exonuclease digestion, which defined its proximity to the telomeres. Under controlled conditions Bal31 will progressively degrade the chromosome from its telomeres. If the Bal31 treatment is followed by restriction digestion of the DNA and Southern analysis with a telomeric probe then the hybridisation of that probe will depend on the extent of the Bal31 degradation and the proximity of the probe to the telomere.

DXYS14 was found only on the X and Y chromosomes and was hypervariable in nature. Family studies indicated that as for DXYS15 recombination was occurring in male meiosis between the X and the Y. These data strongly assert that both DXYS14 and DXYS15 are within the pseudoautosomal segment.

More extensive recombinational analysis of male meiosis was performed with these two probes together with a further pseudoautosomal probe -DXYS17 - by Rouyer et al., (1986). A genetic map of the region was drawn up between the three loci and the proximal end of the pseudoautosomal segment, as defined by *TDF*. The genetic distances obtained between each marker were generally consistent throughout the region and no evidence of double recombinants were seen. The telomeric probe DXYS14 was observed to recombine with sex with a frequency of 49.5%. These data suggest that within the region there is a single obligatory cross-over occurring at each male meiosis. This cross-over can occur in different areas of the region and, while only three loci have been used, there is no evidence that these sites are not evenly spread throughout the region.

The recombinational frequencies of male meiosis were compared with those obtained at female meiosis and this indicated that recombination is about 10 times more frequent in this region during male meiosis than during female. This would imply that the extent of the region, rather than being the 50cM measured between DXYS14 and *TDF* in male meiosis, is approximately 5cM. However only two female meiotic

recombination events were scored which means that the difference in recomination frequencies calculated in this way is only a very rough estimate. Furthermore this region may possess novel recombinational properties in female meiosis.

The possibility remains, however, that this region occupies a considerable portion, if not as much as a half, of Yp and this has some implications for the sequence composition of the region. The lack of pseudoautosomal probes in the collection of Y-probes of Geldwerth et al., (1985) suggests that either this region is considerably smaller than 5Mb or that the composition of this region renders much of it difficult to clone perhaps through the presence of tandem repeats or structures which promote recombination. If the region is megabases rather than kilobases long it follows then that those pseudoautosomal cosmids isolated so far may be unrepresentative of the composition of the region as a whole. Physical mapping of this region by pulse-field gel electrophoresis should provide a more accurate measure of its extent.

1.4.5 Pseudoautosomal Functional Loci

MIC2

MIC2 is the locus controlling the expression of a cell surface antigen which is found in abundance on the surface of human leukaemic T-cells but it also found in lesser quantities on all tissues tested. It was originally defined by the monoclonal antibody 12E7 which was raised by immunising mice with lymphocytes from a patient with a T-cell acute lymphocytic leukaemia. The expression of this locus was found to exhibit a quantitative polymorphism linked to the erythrocyte antigen *Xg* (Goodfellow & Tippet, 1981).

The polymorphism detected by 12E7 is linked to Xg in a sex-limited fashion. Females who are Xga+ are high expressors of 12E7 while those who are Xga- are low expressors. However while Xga+ males are high expressors Xga- males can be either high or low expressors (Goodfellow & Tippet, 1981). The authors postulated that there is another locus, other than *Xg* which controls 12E7 expression in males. This is either an autosomal or X-linked control which is only active in males or it is a Y-linked control and therefore only present in males. This controversial locus was called *Yg*.

As the 12E7 monoclonal does not react with rodent cells this locus was able to be mapped using mouse-human hybrids.It was initially shown to be expressed on a hybrid with the X chromosome as the sole human chromosome which mapped it to the X chromosome (Goodfellow & Tippet, 1981). The *MIC2* locus was further mapped on the X using a hybrid (Amir2N) which contained a human t(X;Y)(p22.3;q11) translocation product from a 46,Yt(X;Y) male. This hybrid failed to express the 12E7 antigen and mapped the *MIC2* locus to Xp22.3-qter. The human fibroblast line, however, did express 12E7 and after further hybrid studies the control of the expression was mapped to the Y chromosome between Ypter and Yq11 (Goodfellow et al,. 1983). It was further observed that a hybrid containing only an inactive X chromosome still produced 12E7, though at reduced levels, which suggests that this locus escapes complete X-inactivation in hybrids (Goodfellow et al., 1984).

Therefore despite the lack of a tight localisation to the distal tip of Yp the *MIC2* locus had to be regarded as an excellent candidate pseudoautosomal gene.

The cDNA of *MIC2*, called pSG1, was cloned by using the 12E7 monoclonal to screen a lambda gt11 expression library (Darling et al., 1985). Southern analysis of genomic DNAs, digested with a number of restriction enzymes, from hybrids containing either the X or the Y chromosome as their only human chromosome indicated that the X and Y homologues were highly conserved if not identical. The cDNA was mapped to the X and Y chromosomes and the 12E7 monoclonal was shown to react with a fusion protien partially encoded by pSG1. These findings suggest that 12E7 is detecting the products of the *MIC2* loci and not of a locus controlled by *MIC2*. This probe was used to map the *MIC2* loci on the X and the Y by *in-situ* hybridisation. This showed grains on both distal Xp and Ypter-p11.2 (Buckle et al., 1985) which further suggested its pseudoautosomal localisation.

The *MIC2* cDNA was used to isolate a polymorphic intron of the gene, p19B. This probe revealed a single cross-over event between p19B and *TDF* out of 46 meioses scored. This confirmed that *MIC2* lay within the pseudoautosomal segment but in a more proximal position than the other three loci described (Goodfellow et al., 1986).

Yg.

Tippet et al,. (1986) showed that the locus which controls the Y chromosomal expression of 12E7 is most probably a Y-borne locus. They analysed the 12E7 levels in Xga- sons of Xga- fathers. In all but seven of 250 cases the sons exhibited the same 12E7 levels as their fathers. Only one of the seven was regarded as a "proved" exception and was explained by postulating a rare recombination event between the X and Y chromosomes. If this were a normal event it would argue that *Yg*, the controlling locus for 12E7, is within the pseudoautosomal segment and is distinct from the locus that encodes the structural *Xg*. Goodfellow et al., (1987) postulate that there is a pseudoautosomal locus *XGR* which controls the expression of both *MIC2* and the structural locus for *Xg* - *XGS*. *XGR* would be most likely to map proximal to *MIC2* as recombination events involving *XGR* would result in aberrant inheritence of *Xg* expression which is rarely seen in normal individuals.

1.5 XY-Homology outwith the Pseudoautosomal Segment

Homology between the X and Y chromosomes had been predicted for a long time; homology that would explain the pairing of these two chromosomes during male meiosis. Ironically when the techniques of molecular biology began to be employed the first regions of XY-homology discovered lay in regions of the chromosomes which did not align during male meiosis. These XY-homologous probes defined a number of different regions of homology on the X and the Y chromosome.

1.5.1 Homology between the Y and Xq13-24

There have been a number of reports of probes that define homologies between the Y and Xq (Bishop et al., 1983, 1984, Geldwerth et al., 1985, Koenig et al., 1985, Page et al., 1982, and Wolfe et al., 1984). The first report of such a sequence was from Page et al., (1982), who reported a sequence, DXYS1, isolated at random from a phage lambda total human library. This probe was initially mapped to the Y chromosome and Xp22.3-Xqter. This localisation was narrowed to Yp and Xq13-21 (Page et al., 1983) by in-situ hybridisation and hybrid studies. The initial recombinant was used to isolate

further overlapping clones which allowed a region of 21kb to be restriction mapped. Subsequent restriction analysis of genomic DNA showed that out of forty restriction sites within 36kb there were only four that differed between the X and the Y chromosomes. This implies 99% homology between the X and the Y chromosomes in this region. Comparative studies were also carried out with chimpanzee and it was observed that the chimpanzee carries an X-homologue but not a Y-homologue. As these regions had not diverged greatly between the X and Y it was concluded that this XY-homology had arisen as the result of a recent transposition event from the X to the Y chromosome. The authors invoke this mechanism of duplicative transfer presumeably to account for the fact that the human X chromosome still bears those sequences also found on the human Y chromosome. However in strict terms it is not possible to infer a duplicative transposition event because after the transfer event the X and the Y chromosome involved would have gone into separate gametes. It is extremely unlikely that both were passed on to the next generation and hence the post-transfer structure of this X chromosome is not known.

Bishop et al., (1983 & 1984) and Geldwerth et al., (1985) isolated and characterised forty cosmids nine of which had single-copy regions which were mapped to the Y-euchromatin and Xq13-24 by human-rodent hybrid analysis. Several have been shown to be present in 46,XX males by Bishop et al., (1985) and Vergnaud et al., (1986) (see section i.e.1) which, as these individuals carry Yp sequences, suggests that some at least map to Yp. Wolfe et al., (1984) isolated 98 cosmid clones which hybridised to total female DNA. Out of three cosmids analysed they identified one cosmid which contained two single-copy regions separated by 22kb which mapped to Ypter-Yq1.1 and Xq13-24. Part of this cosmid was reported to be present in some XX males (Goodfellow et al., 1985) which argues its Yp location. Koenig et al., (1985) isolated a similarly mapped XY-homologous probe (st25) from a sorted X chromosome library.

With the exception of the probe of Wolfe et al., (1985) all these probes have been shown, or said, to be highly conserved between the X and the Y chromosomes and to have only an X-homologue in chimpanzees. These facts suggest that they are all a part of the same block of XY-homology defined by Page et al., (1983) with DXYS1. The number of

these probes, in proportion to Y-specific probes argues that this region of XY-homology may form a significant part of Yp.

The findings of Geldwerth et al., (1985) suggest that this region occupies 25% of the Y euchromatin as nine out of forty Y-homologous cosmid clones contained sequences which define this XY-homology. However the cosmid library used was made from 3E7 which contains a Y chromosome with an extra Yp attached to Yqter (Voss, communication in Bishop et al., 1984). This will result in an over-representation of Yp recombinants in the library. Therefore if all these XY-homologous probes map to Yp they are probably two-fold over-represented. While there may be other biases in such cloning techniques it would appear that this region of XY-homology is extensive if not as much as 25% of the Y euchromatin.

1.5.2 Homology between Yq and Xp22.3

There is a smaller group of probes that defines a less conserved region of XY-homology than that between the Y and Xq13-24.

Kunkel et al., (1983) reported a probe detecting homology between Yq and Xp21-pter. This homology was reported to be further localised to Xp21-p22.3 (Mondello & Goodfellow, unpublished observations in Goodfellow et al., 1985). Koenig et al., (1984) also reported a probe with a similar mapping pattern, DXS31, which was isolated from a flow-sorted X-library. Both these probes detect regions that are 80% homologous between the X and Y chromosomes but there is no direct evidence to show that their homologies arose through the same event or that they are part of the same larger XY-homologous block. Koenig et al., (1984) observed on Southern analysis that DXS31 had both an X and a Y- homologue in chimpanzee but in brown lemur had either an XY-similar pattern or was autosomally-linked. Further studies (Koenig et al., 1985) with cercopithicoid species of monkeys showed that in these monkeys DXS31 was X-linked but had no Y or autosomal homologue.

In view of the localisation of the DXS31 X-homologue to Xp22.3 in humans and its X or XY-homologous nature in most of the primate species tested it is attractive to postulate that DXS31 was once within the pseudoautosomal segment. At some point in

evolution, between lemurs and humans, a rearrangement on the Y chromosome carried the Y-homologue out of the segment where it began to diverge from its X-homologue. This is perhaps the same event that removed steroid sulphatase (*STS*) from the pseudoautosomal segment between mouse and man. In the mouse *sts* is pseudoautosomal (Keitges et al., 1985) while in man it maps to Xp22.3 with some diverged Y-homologies that map to Yq (Yen et al., 1987).

1.5.3 Homology between Yq and Xq24-qter

Cooke et al., (1984) isolated an XY-homologous probe from a flow-sorted Y chromosome library. They used this to clone its X-homologue and sequenced both the homologues. Out of 1013 bases sequenced no differences were found. The homologies were mapped to Xq24-qter and Yq1.1-qter but were further localised to Xq26-27 and Yqcen-q11.1 by Bickmore & Cooke, (1987). The high degree of conservation would suggest a pseudoautosomal segment location but the in-situ mapping on human-rodent hybrids puts the homologues at the other ends of both chromosomes. Therefore the conservation suggests that either these sequences have a function or that they are the result of a very recent transfer of sequences between the two chromosomes. The former proposition is rejected by the authors as they do not find a mouse homologue. However the discovery by Yen et al., (1987) that the human STS cDNA is not homologous to the mouse gene implies that this reasoning is not very sound. If the latter proposition is true the event probably occurred more recently than that which gave rise to the Yp/Xq13-24 cross-homology. It may even be the case that it occurred after the evolution of homosapiens and could be observed as an inter-race polymorphism.

The molecular structure of the Y chromosome is dominated by regions of XY homology. These homologies are summarised in figure 2. The region of homology between Xp and Yp, whose presence was predicted to explain the pairing of the two chromosomes during male meiosis, has been shown to exist but the other regions are clearly not involved in XY pairing. The primate studies suggest that the Xq13-24/Yp and Xq24-qter/Yq homologies are also unlikely to be remnants of the homologous pair

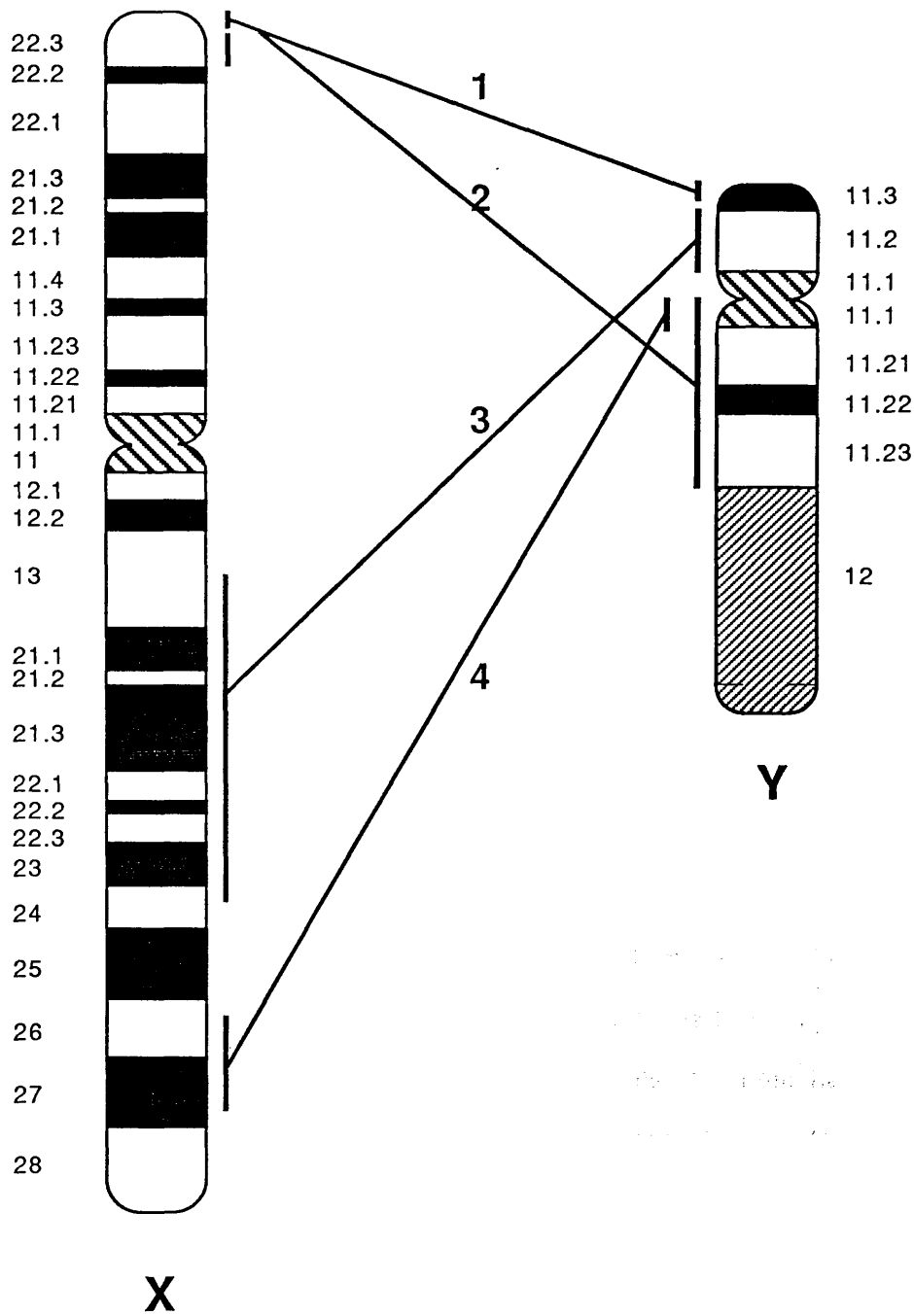


Figure 2: Four regions of XY homology defined by molecular analysis.
1) Pseudoautosomal loci; 100% conserved, (Simmler et al, 1985 & Cooke et al, 1985) 2) Xp22.3/Yq11; ~80% conserved (Kunkel et al, 1983 & Koenig et al, 1984) 3) Xq13-21/Yp; 99% conserved, (Page et al, 1983) and 4) Xq26-27/ Yqcen-q11.1; 100% conserved (Bickmore & Cooke, 1987).

from which the X and Y chromosomes are believed to have evolved (Ohno, 1967). The fact that these cross-homologies are most likely the result of X to Y transfer events argues that they are the consequence of the close association of the sex chromosomes during male meiosis and the ability of the Y chromosome to "accept" rearrangements due to its proposed paucity of genetic loci. On the other hand if the Xp22.3/Yq homology was genuinely of pseudoautosomal origin it may well provide an example of XY homology which is a "remnant" of the original homologous pair.

1.6 A Molecular Map of the Y Chromsome

The way forward to the mapping and cloning of the genes of the Y chromosome is through the analysis of individuals with abnormal Y chromosomes. By defining the Y chromatin present in such individuals and relating this to phenotypic effect it should be possible to create deletion maps of the Y chromosome where genetic effects can be positioned in relation to the probes. The XX males presented an opportunity to deletion map Yp and position the testes determinants in relation to the Yp homologous probes.

1.6.1 Preliminary Molecular Analysis of the XX males

Although it had been predicted for a long time that XX males would carry a region of Yp bearing *TDF* (Ferguson-Smith,1966) this was formally proven by Guellaen et. al. (1984). They showed that three out of a total of four XX males tested by Southern blot analysis were positive for Y-specific restriction fragments. All three had a different but overlapping set of probes. Bishop et. al. (1985) extended this study to 23 XX males of which 15 carried Y sequences. Four different but overlapping sets of probes were revealed. By in-situ hybridisation Magenis et. al. (1985) showed that one of the probes shown to be present in XX males in the above studies was present on Yp in normal males. In XX males it was observed to be present on distal Xp. These data strongly suggested that the presence of Y sequences in XX males was the consequence of a simple terminal transfer from the Y to the X chromosome with heterologous breakpoints on Yp.

If this mechanism is assumed to be the rule rather than more complex rearrangements then a deletion map of Yp can be constructed and *TDF* positioned on it. From this preliminary data it can be concluded that *TDF* must lie to either side of 47c as it is the probe present in most XX males. These results also suggest that *TDF* lies in distal Yp which is in direct contradiction to the cytogenetic data which mapped *TDF* to proximal Yp. However as was discussed earlier it is possible that the pseudoautosomal segment could occupy the distal half of Yp. As *TDF* should lie proximal to the pseudoautosomal region it follows that *TDF* could lie at the distal end of the proximal half of Yp. This would still be in keeping with the cytogenetic data.

1.7 Conclusion

The molecular approach is beginning to elucidate some of the structural features of the model of the Y chromosome based on cytogenetic data. It has also revealed a relationship between the X and Y chromosomes through homologies outwith the pseudoautosomal segment. The further analysis of the regions of XY homology may lead to a greater understanding of the relationship between the sex chromosomes in both evolution and in man.

Furthermore it is allowing the construction of a molecular map of the Y chromosome which will reveal both its sequence organisation and the location of its genetic functions. With more probes and XX males it should be possible to further narrow down the region containing the testes determinants until a small region amenable to chromosome walking is defined. A similar approach using individuals with breakpoints on Yq will permit the construction of a deletion map of the long arm.

Aims

1. To isolate single copy or low copy number DNA sequences from a flow-sorted Y chromosome library.
2. To map these sequences to Yp or Yq using individuals with aberrant Y chromosomes and a somatic cell hybrid carrying an intact Y chromosome.
3. To use the Yp sequences to study XX males, XY females and XO males in order to construct a molecular deletion map of Yp.
4. To use the Yq sequences to analyse dicentric Yp individuals and create a molecular deletion map of Yq.
5. To use these deletion maps to localise and clone genetic loci from the Y chromosome.
6. To identify expressed sequences by Northern analysis of testes RNA and the screening of cDNA libraries.
7. To identify regions of XY homology.
8. To map the X homologies on the X chromosome using somatic cell hybrids, and individuals, carrying a deleted X chromosome or one product of an X:autosome translocation.
9. To study individuals with genetic conditions mapped close to the X homologues of the XY homologous probes.
10. To use the XY homologous probes to screen for restriction fragment length polymorphisms.
11. To compare the X and Y homologues at the DNA level through restriction enzyme analysis.
12. To analyse the evolution of these XY homologous sequences through comparative studies with other primate species.

CHAPTER 2 - MATERIALS AND METHODS

2 Materials and Methods

2.1 Recombinant DNA Manipulations

Most of these manipulations were carried out essentially as described in Maniatis et al., (1982).

2.1.1 The Recombinant DNA Libraries

The probes were isolated from two libraries. The first was a flow-sorted Y chromosome library constructed in the *Hin*dIII site of lambda Charon 21A. This library, provided by Dr. Marvin Van Dilla, was constructed at the Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA, under the auspices of the National Laboratory Gene Library Project, sponsored by the U.S. Department of Energy. The second was a flow-sorted X/7 chromosome library constructed in the *Eco*RI site of lambda NM1149 (Murray 1983). This library was constructed by Mrs Louise Sinclair in our department.

2.1.2 Screening the Libraries

The Y chromosome library was plated out on LE392 bacteria at a density of 300 plaques on a 135mm round petri-dish. An impression was taken using Amersham's Hybond-N precut 135mm circular filters as in the Hybond manual. The filters were applied dry to the plate and and left in contact for one minute. The filter was then laid plaque side up on Whatmann's 3MM paper soaked in 0.5M NaOH, 1.5M NaCl for seven minutes. The filter was then similarly neutralised on 0.5M Tris pH 7.0, 1.5M NaCl. It was then submerged in 2xSSC, air dried and the DNA fixed as for the Southern blots. The filters prepared in this way were hybridised with nick-translated P^{32} labelled total male DNA. Those plaques that failed to hybridise with this probe were picked using a pasteur pipette and the phage particles eluted from the agar into 1ml of SM (100mM NaCl, 10mM $MgSO_4.7H_2O$; 50mM Tris.Cl pH7.5, 0.001% gelatin) at room temperature for four hours. Of this suspension 100ul were plated out with LE392 on to 85mm plates to give confluent lysis. A high-titre stock was prepared from this plate from which DNA was made using the DE52 method.

The X chromosome library was screened as above with the following differences. The library was initially plated to a density of $5x10^5$ palques with NM514 bacteria on a 260mm square petri dish. These plaques were screened with the probe GMGXY3 and the areas where hybridisation was seen were picked. These were replated at densities between 500-1000 plaques per 85mm plate and the screening repeated. One further round was required to obtain pure clones. This was carried out at a density of about 100 plaques per 85mm plate. DNA was then made from a positive clone from these plates.

The recombinant DNA was digested with the appropriate enzyme and then run out on a 1% LMP agarose gel. Of this 3ul was kept aside and frozen for subsequent subcloning into plasmid. After ethidium bromide staining the inserts observed were excised from the gel and some of this DNA was labelled by random primer extension with the Klenow fragment of E. Coli DNA Polymerase 1. These were then hybridised to to mapping panels and those that showed the correct pattern of hybridisation were subcloned into pUC19 or pBluescribe.

2.1.3 Plating out Bacteriophage

An overnight culture of the appropriate bacteria was grown overnight at 37° and then spun down at 2,500g for 10 minutes at 4°. The supernatant was discarded and the pellet resuspended in 10mM $MgSO_4$ to give an $O.D._{600}$ of 2.0. Of this bacterial suspension 100ul were mixed with 100ul of the appropriate dilution of bacteriophage in SM. This was left at 37° for 20 minutes. Then 3ml of molten 0.5% L-broth agar at 45° were added and the tube mixed. This was poured on to an 85mm plate containing 20ml of 1.5% L-broth agar. These plates were incubated inverted at 37° overnight.

2.1.4 Preparation of a Bacteriophage High-Titre Stock.

The phage were plated out at a dilution that would give 10^4-10^5 plaques per 85mm plate. After overnight incubation the top agar was scraped off with a bent pasteur pipette. This agar was placed in a tube with 5ml of SM and 100ul of chloroform and was shaken at 37° for 15 minutes. This tube was then spun at 8,000g for 10 minutes at

20°, the supernatant decanted and chloroform added to 0.3%. These stocks were stored at 4°.

2.1.5 Preparation of Recombinant Phage DNA and Isolation of the Insert

The recombinant phage DNA was prepared after the protocol of Benson & Taylor, (1984). To 600ul of high-titre stock was added 600ul of a DE52 slurry in L-broth. This was inverted 20-30 times and then spun in a centrifuge for 5 minutes at 12,000rpm. The supernatant was removed and respun. To the supernatant 100ul of 5M NaCl and 540ul of isopropanol were added and they were incubated at -20° for 20 minutes. This was spun in the microfuge and washed once in 70% ethanol. The pellet was taken up into 200ul of TE (10mM Tris.Cl pH8, 1mM EDTA pH8) and extracted twice with an equal volume of phenol/chloroform (1/1). The DNA was precipitated with 15ul of 5M NaCl and 2 volumes of 95% ethanol at -20° for 30 minutes. This was spun in the microfuge for 15 minutes and washed with 95% ethanol before being left to air dry. Once dry the pellet was resuspended in 15ul of a reaction mix for restriction enzyme digestion containing buffer, spermidine and enzyme. This was left to digest for 1 hour and then 12ul were run out on a 1% LMP agarose gel from which the insert was excised. The cut-out containing the insert was weighed and a volume of water two times the weight was added. Of this 20ul was used in the oligonucleotide labelling reaction.

2.1.6 Preparation of Plasmid Vector

The plasmid vectors used were pUC19 (Vieira & Messing, 1982) or pBluescript (Stratagene). Plasmid DNA was cut with 2 units of the appropriate enzyme per microgram of plasmid DNA for 1 hour. One unit of calf intestinal phophatase was then added to the reaction which was incubated for a further 30 minutes at 37°. EDTA pH8 was added to 10mM and the reaction heated to 68° for 10 minutes.This was then phenol/chloroform extracted three times and the DNA precipitated with a half volume of 7.5M ammonium acetate and 2.5 volumes of 95% ethanol. The DNA was spun out and washed with 70% ethanol twice, air dried and resuspended to give a concentration of 20ng/ul.

2.1.7 Subcloning from Phage to Plasmid

From the digest of the DE52 DNA preparation 3ul were taken and added untreated to 20ng of suitably digested plasmid vector. These were ligated in 10ul with 1 unit of T4 DNA ligase in 50mM Tris.Cl pH7.4, 10mM $MgCl_2$, 10mM dithiothreitol, 1mM ATP and 100ug/ml BSA. The ligation was incubated at 20° for 2 hours and then 2ul of this was tranformed into JM83 competent cells.

2.1.8 Preparation of JM83 Competent Cells

An L-broth agar plate was streaked out with the bacteria and after overnight at 37° a single colony was used to inoculate an overnight L-broth culture. From this overnight culture 2-5ml were added to 500ml of preheated L-broth in a 2L flask and allowed to grow at 37° until they had reached an $O.D._{600}$ of 0.2. The cells were harvested in pre-cooled 50ml sterile tubes by spinning at 3000g for 10 minutes. The cells were gently resuspended in 250ml of chilled 100mM $CaCl_2$ and left on ice for 15 minutes. The cells were spun down as before and resuspended in 5ml of cold 100mM $CaCl_2$. To this was added 0.5ml of 100% glycerol and the cells were aliquoted into 100ul amounts, frozen in liquid nitrogen and stored at -70°.

2.1.9 Transformation of Competent Cells

The competent cells were thawed at room temperature and 2ul of the ligation was added to 50ul of the cells. This was incubated on ice for 30 minutes and then heat shocked at 42° for 1 minute. The transformation was returned to the ice until plated. It was spread directly on to an L-broth plate containing 50ug/ml of ampicillin which had previously been spread with 40ul of a 20mg/ml solution of 5-bromo-4-chloro-3-indolyl-B-D-galactoside (X-gal) in dimethyl formamide and 40ul of 100mM isopropylthiogalactoside (IPTG). The plate was incubated overnight at 37°. In the pUC19 and pBluescribe plasmids used the cloning site lies within the B-galactosidase gene which if active will cleave X-gal and generate a blue colour resulting in blue colonies. The IPTG induces the expression of this gene. The presence of an insert in the

cloning site disrupts the expression of this gene and thus colonies containing recombinant plasmid remain white. After overnight at 37° DNA was made from the white colonies.

2.1.10 Small Scale Plasmid Preparation

An overnight culture of JM83 containing the plasmid of interest was grown overnight with 100ug/ml ampicillin. At this stage glycerol stocks were made for new subclones by adding glycerol to 25% to a portion of the culture and freezing at -20°. The bacteria were pelleted from 1.5ml of the remaining culture by a 1 minute spin in a microfuge. The pellet was resuspended in 700ul of STET buffer (50mM Tris.Cl pH8, 50mM EDTA pH8, 8% sucrose 5% triton-X100) and 50ul of a freshly made 10mg/ml lysozyme solution was added. This was placed on ice for 5 minutes, boiled for 2 minutes and then spun in a microfuge for 15 minutes. The supernatant was collected and phenol/chloroform extracted twice. A tenth volume 3M sodium acetate pH5.6 together with 0.6 volumes of isopropanol was added. After 20 miutes at -20° the DNA was pelleted in a microfuge for 15 minutes. The pellet was washed once in 95% ethanol, air dried and taken up in 50ul of TE. One fifth of this was digested with the appropriate restriction enzyme and 1ul of RNase A (10mg/ml) was added to remove the contaminating RNA. This was run out on either a 0.8% agarose gel or a 1% LMP agarose gel if the insert were to be labelled. In this case the same procedure was followed as for the phage inserts. In general it was found that an insert prepared thus provided sufficient DNA for 10-15 labellings.

2.2 Radiolabelling

2.2.1 Random Oligonucleotide Primer Extension Labelling

The procedure was carried out as in Feinberg & Vogelstein, (1984). The DNA/agarose mix containing the insert to be labelled was boiled for 5 minutes and left at 37° for 10 minutes. A 50ul reaction was set up with 10ul of 5x reaction buffer, 100ug/ml BSA, 30uCi of P^{32} dCTP, 1 unit of E. Coli Klenow fragment and 20 ul of the

insert DNA. The reaction was left at 20° overnight. The reaction was stopped with 50ul of stop buffer and then run down a G50 column equilibrated with 1xSSC, 0.1%SDS. The first peak was collected, boiled for 5 minutes and then added to 5-10ml of hybridisation buffer.

2.2.2 Nick-Translation P32 Labelling (Rigby et al., 1977)

Nick-translations were carried out on 1ug of DNA with 50uCi of P32 dCTP using the Amersham nick translation kit. They were incubated at 15° for 75 minutes and then stopped with 5ul of 500mM EDTA pH8. They were then treated as the oligonucleotide probes.

2.3 Preparation of Genomic DNA

DNA was prepared from peripheral blood lymphocytes and harvested lymphoblastoid, fibroblast and mouse-human hybrid cell lines based on the protocol described by Kunkel et al., (1977). Blood was taken and mixed with a 1/10 volume of 120mM EDTA pH8, 1M NaCl to prevent coagulation. To 20ml of blood 80ml of lysis buffer (0.32M sucrose, 10mM Tris.Cl pH7.4, 5mM $MgCl_2$, 1% triton X-100) were added. This was incubated on ice for 10 minutes, spun at 3000g for 10 minutes and the supernatant discarded. After this step the preparation from the blood is as for the cell lines. The pellet was resuspended in 10ml of 10mM EDTA pH8, 10mM NaCl by vigorous pipetting and 500ul of 10% SDS and 200ul of 10mg/ml proteinase K. This was gently mixed and incubated at 55° for 1-2hours. This was then phenol/chloroform extracted twice after the addition of a 1/10 volume of 3M sodium acetate pH5.6. Mixing of the two phases was achieved by gentle inversion on a tumbler for 10 minutes. After the extractions two volumes of ethanol were added to the DNA and it was spooled out using a sealed pasteur pipette. The DNA was rinsed in 70% ethanol, air dried and put into 500ul of TE. The DNA was left to dissolve at room temperature for 1 hour. Then the gelatinous DNA lump was broken up by gentle pipetting with a 1ml pipette tip. It was then left to further dissolve at 4° before being used. Typically the final DNA

concentration measured at $O.D._{260}$ was between 500 and 1000ug/ml. For DNA prepared from cell lines $5x10^8$ cells were processed in the volumes described above.

2.4 Digestion of DNA

Digests of eukaryotic genomic DNA were carried out on 5-10ug of DNA in a 40ul reaction volume with 10-30 units of restriction enzyme. All the enzymes were cut in either medium salt conditions (50mM NaCl, 10mM Tris.Cl pH7.4, 10mM $MgCl_2$, 1mM dithiothreitol) or high salt conditions (100mM NaCl, 50mM Tris.Cl pH7.4, 10mM $MgCl_2$, 1mM dithiothreitol) together with a 1/40 volume of 100mM spermidine. All the enzymes were cut at the recommended temperature except TaqI which was cut at 58°. The enzyme was added to the digest last aand the solution pipetted gently to ensure proper mixing of the viscous DNA. The digests were incubated overnight.

Digestions of phage or plasmid DNA were carried out as for genomic DNA with the total volume of the digest varying. In general about 2-5 units of restriction enzyme were used per microgram of DNA and the digests were incubated for 1 hour to overnight.

2.5 Size Separation of the DNA

2.5.1 Gel Electrophoresis of DNA

With the exception of the 1% LMP agarose gels run to obtain inserts for labelling all the gels used in this study were 0.8% gels made of type II, medium EEO agarose. The gels were made and run in 1xTAE buffer (40mM Tris-acetate, 2mM EDTA). For genomic samples gels 13cm wide and 10cm long with 50ul slots were run in a 40cm wide tank. The samples were loaded with a 1/10 volume of loading buffer (10% ficoll type 400, 20% glycerol, 0.25% orange G) and run at 200mA for about 4 hours until the orange G dye front, which runs at about 200bp, had reached the end of the gel. The gel was then stained with ethidium bromide.

Various sizes of gel were run for recombinant DNA but most frequently a 10cm wide by 6.5cm long gel with 15ul slots was used. This gel was run in the 40cm tanks to improve resolution.

2.5.2 Ethidium Bromide Staining of the Gel

After electrophoresis the gel was stained in a 5ug/ml solution of ethidium bromide in 1xTAE for 10 minutes. The gel was then visualised on a U.V. transilluminator (316nm) with Polaroid type 667 film in a Polaroid instant print camera fitted with a Kodak 22A Wratten filter. If necessary, prior to photography, the gel was destained in 1xTAE.

2.5.3 Molecular Weight Markers

Recombinant DNA was sized using the BRL 1kb ladder which has approximate molecular weights of 0.5, 1, 1.6, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12kb. Genomic DNA was sized using λgt11 cut with *Hin*dIII and *Eco*RI. This gave a ladder of approximately 19.6, 9.4, 5.9, 4.3, 3.8 and 0.4 based on the λgt11 map of Young & Davis, (1983).

2.6 Southern Blot Analysis

2.6.1 Southern Transfer (Southern, 1974)

After ethidium bromide staining and photography the gel was depurinated in 240mM HCl for 15 minutes to break up the high molecular weight DNA and facilitate transfer. The DNA was then denatured by two 15 minute washes in 0.5M NaOH, 1.5M NaCl. It was then neutralised by two 15 minute washes in 0.5M Tris.Cl pH7, 1.5M NaCl. All washes were carried out with constant agitation. The gel was then transferred to a platform covered in a 3MM paper wick soaked in the transfer buffer (10xSSC) which dipped into a reservoir of this buffer beneath the platform. A piece of Hybond-N cut to fit the gel exactly was placed dry on to the gel followed by two piecs of 3MM paper pre-wet in 2xSSC. A column of dry paper towels were in turn placed on this followed by a glass plate on which a 750kg weight was placed. The transfer was left to proceed for 18-24 hours. After transfer the filter was removed from the gel and rinsed in 2xSSC for 5 minutes. It was then air dried and baked at 80° for 5 hours to fix the DNA. The filter was pre-wetted in 1xSSC, 0.1%SDS prior to prehybridisation.

2.6.2 Hybridisation of DNA Probes

Prior to the hybridisation with the radioactive probe the filter was incubated at 42° for 18 hours 5ml of the hybridisation buffer (50% deionised formamide, 3x Denhardts, 5x SSC, 25mM sodium phosphate pH6.5, 250ug/ml sheared salmon sperm DNA, 0.2% SDS, 20ug/ml poly(A), 10% dextran sulphate). The labelled probe was added directly to the prehybridised filter down the side of the bag and the bubbles removed before resealing the bag. The bag was returned to 42° for 36 hours. It was found that at least five 10 X 13cm filter could be hybridised in the one bag in 15ml of hybridisation buffer without detrimental effect.

After hybridisation the filters were washed in 1xSSC, 0.1%SDS for 25 minutes at 20° and then at either 1XSSC, 0.5xSSC or 0.1xSSC, 0.1%SDS for 30 minutes at 63° depending on the stringency required. The filters were then dried on 3MM paper until just slightly moist, put between sheets of plastic and autoradiographed at -70° with intensifier screen and Kodak AR-5 film for 24 hours to one week.

2.6.3 Dehybridisation of Filters

After autoradiography the radioactive probe was removed by shaking in 0.4M NaOH for 45 minutes folled by two 15 minute washes in 0.5M Tris.Cl pH7, 1.5M NaCl and one rinse in 1xSSC, 0.1%SDS. The filter was then ready for re-hybridisation with another probe as detailed above.

2.7 RNA Manipulations

2.7.1 Preparation of Total Cellular RNA

The RNA was prepared using a protocol based on that of Auffray & Rougeon, (1980). This involves the selective precipitation of large RNA molecules with LiCl. These large RNAs are insoluble in solutions containing high salt concentrations allowing them to be separated from tRNA, 5SrRNA and small DNA molecules which remain in solution.

The tissue was ground up in a pestle and mortar in the presence of liquid nitrogen. For every 1ml of powdered tissue 8ml of freshly prepared lysis buffer was added (5M

guanidinium isothiocynate, 10mM Tris.Cl pH7.6, 10mMEDTA, 1M B-mercaptoethanol). This was then homogenised until homogeneous, left 20 minutes at room temperature and homogenised a second time. The homogenate was forced through a guage 21 needle 5 or 6 times to break up DNA protein complexes. Freshly prepared 4M LiCl was added to give a final concentration of 3M. This was left overnight at 4° and then spun down at 4° at 6500g for 1.5 hours. The supernatant was discarded and the pellet resuspended in 15ml of 3M LiClper 1ml of original powdered tissue. This was again forced through a guage 21 needle 3 or 4 times. It was next spun at 6500g, 4° for 30 minutes and the pellet resuspended in 5ml of TE, 0.5%SDS per Iml of powdered tissue. This was then extracted with a half volume and then an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1) followed by one extraction with an equal volume of chloroform. This was then precipitated with 1/10 volume of 3M sodium acetate pH5.6 and two volumes of ethanol at 20° for 30 minutes. The RNA was recovered by spinning at 8000g for 20 minutes, the pellet washed in 70% ethanol, 95% ethanol, air dried and taken up in 200ul TE.

2.7.2 Gel Electrophoresis of RNA (Maniatis et al., 1982)

The same moulds, running tanks and agarose as for the genomic DNA were used. The RNA was run in gels containing formaldehyde. The gel runnning buffer was 0.2M morpholino propane sulphonic acid (MOPS) pH7, 50mM sodium acetate, 1mM EDTA pH8 and a 10X stock was made. To prepare the gel the agarose was melted in boiling water, cooled to 60° and 10x running buffer and formaldehyde were added to give final concentrations of 1x and 2.2M respectively. This mix was immediately poured into the gel mould after gentle mixing. A 1% agarose gel was poured. The samples were prepared by mixing 18ug of RNA in 9ul TE with 4ul 10x running buffer, 7ul 37% formaldehyde and 10ul 100% formamide. This mixture was heated at 55° for 15 minutes, 4ul of loading buffer added and the samples loaded onto the gel. The gel was run at 200mA at 20° with constant circulation of the buffer until the dye was 1cm from the end of the gel.

2.7.3 Northern Transfer

After electrophoresis the gel was washed twice in several volumes of 10xSSC for 30 minutes. It was then blotted on to Hybond-N exactly as for DNA and fixed there by 5 hour baking at 80°.

2.7.4 Dehybridisation of Northern Blots

After exposure the radioactive probe was removed by incubation at 90° for 1 hour in TE, 0.1% SDS. The filters were then ready for rehybridisation.

2.8 Cell Hybrids and Cell Lines

Seven different mouse-human hybrids were used in this study: 3E7 and Horl-X which carry a normal human Y and X chromosome respectively as their only human chromosome and Amir2N, NEA9, FNA9, DHTK and WHTK.

Amir2N carries a t(X;Y)(p22.3;q11.2) chromosome together with a number of autosomes. The hybrid is derived from a male with a 46Y, t(X;Y)(p22.3;q11.2) karyotype who is deleted for Xg and STS. His normal mother has a 46X, t(X;Y)(p22.3;q11.2) karyotype (Ferguson-Smith et al., 1982).

NEA9 and FNA9 were derived from females carrying balanced reciprocal translocations involving the X chromosome. NEA9 carries a t(X;11)(q13;p13) translocation product and so effectively carries an X chromosome deleted Xq13-pter. FNA9 bears a t(X;19)(q24;q13) product and thus only carries the Xq24-qter region of the X chromosome. The DHTK line carries Xpter-q27 and is derived from a female with the karyotype 45,X ter rea(X;9)(q27;p23). WHTK was derived from a female with the karyotype 46,X,del(X)(q24-qter) and carries the deleted X chromosome. Karyotypes and further data on these hybrids can be obtained from Florentin, (1987).

Five lymphoblastoid lines were used in this study: WC, ED, MB, Tel26 and DL.

WC was derived from a male carrying a minute metacentric in place of the normal Y chromosome and this was interpreted as an isochromosome for Yp. The male is of normal stature but completely lacks germ cells. There is no evidence of mosaicism (Affara et al., 1986a).

ED was derived from a 48XX, dic(Yq) female with two dicentric Yq chromosome with breakpoints at Yq11.2. The female has secondary amenorrhea (Affara et al., 1986a).

Tel26 was derived from a 46Y, del(X). The deletion has been cytogenetically determined as Xq13-21.3. The male was severely retarded in both mental and motor dvelopment. He had cleft lip and cleft palate. The male was not tested for choroideraemia (Tabor et al., 1983).

MB was derived from a 46Y, del(X)(q21.1-21.3) male. This male had mental retardation and X-linked choroideraemia. He also had a high arched palate (Hodgson et al., 1987).

DL was derived from a female who was 46X, del(X). The abnormal X was cytogenetically determined as having a deletion of Xq27-28. The female appears phenotypically normal and ultrasound revealed a normal uterus and right ovary. However she presented with secondary amenorrhea at age 22 (Winter, R., personal communication).

2.9 The XX Males, XO Males and XY Females

All the XX males used in this study had a 46,XX karyotype with no evidence of mosaicism for a Y bearing line. All had unambiguous male genitalia.

The two XO males used have abnormal karyotypes. JG has a t(Y;5) translocation and has cri du chat syndrome whch is associated with 5p deletions. RW carries a small marker chromosome in 25% of the cells karyotyped. However its origin was not able to be determined cytogenetically. RW manifests some Turner stigmata.

The XY females, with the exception of AM, did not carry cytogenetically detectable deletions of the Yp. AM has been shown to carry a deletion in Yp11.2 (Magenis et al., 1984). AM was also the only XY female to display any Turner stigmata. All had female external genitalia and, with the exception of DM who had no gonads, all had streak gonads. DM has features of testicular regression syndrome and may therfore not be a true pure gonadal dysgenesis XY female. MW and JR are sisters. MW, JR, AM and BK developed gonadoblastomas and SG a calcified fibroma in their streak gonads. No

evidence of mosaicism with a line lacking a Y chromosome was observed cytogenetically in any of these XY females.

2.10 Steroid Sulphatase Deficient Males

All the males used in this study display the symptoms of X-linked ichthyosis and a biochemical assay on their hair roots showed them to be steroid sulphatase deficient. No chromosomal abnormalities were observed cytogenetically.

CHAPTER 3 - RESULTS

3 RESULTS

3.1 Isolation of the Y Homologous Probes

The probes were isolated from a flow-sorted Y chromosome *Hin*dIII library made in lambda Charon 21A (kindly given by Dr M. van Dilla of Lawrence Livermore). The library was screened with nick-translated total human male DNA and 164 plaques which did not hybridise were selected. Seventy of these (43%) contained inserts. These inserts were used in Southern blot analysis of genomic DNA from the members of the panels detailed in figures 3A and 3B inorder to identify Y homologous inserts, to localise the Y homologies to Yp or Yq and to reveal any X or autosomal cross-homologies.

3.2 Regional Localisation

The probes were initially hybridised to *Eco*RI digested mapping panels of genomic DNA from normal male and female, the mouse-human somatic cell hybrids 3E7 and Horl-X which contain the human Y and X chromosomes, respectively, as their only human chromosome, WC a 46,X,i(Yp) male, ED a 47,X,dic(Yq) female and the mouse line A9. This panel is schematically represented in figure 3A and the results obtained shown in figure 4A. Figure 3B shows the panel used in the preliminary mapping of probes which were isolated after some of the XX males had been characterised. This panel consisted of normal male and female, 3E7, Horl-X, XX males GA, AP, RT and A9. Here it was assumed that XX male GA carried most of Yp (see figure 14). The results obtained with this panel are shown in figure 4B. Figure 4A and B shows the hybridisation patterns of most of the probes isolated in this study on the panels detailed in figures 3A and 3B but not that of GMGY5 or the probes that detected both X and Y homologous sequences. GMGY5 is shown in figure 6. The XY homologous probes are shown in figure 9 hybridised to an expanded mapping panel (detailed in figure 8A and B) digested with an enzyme that separates the Y homologous restriction fragments from the X or autosomal cross-homologies.

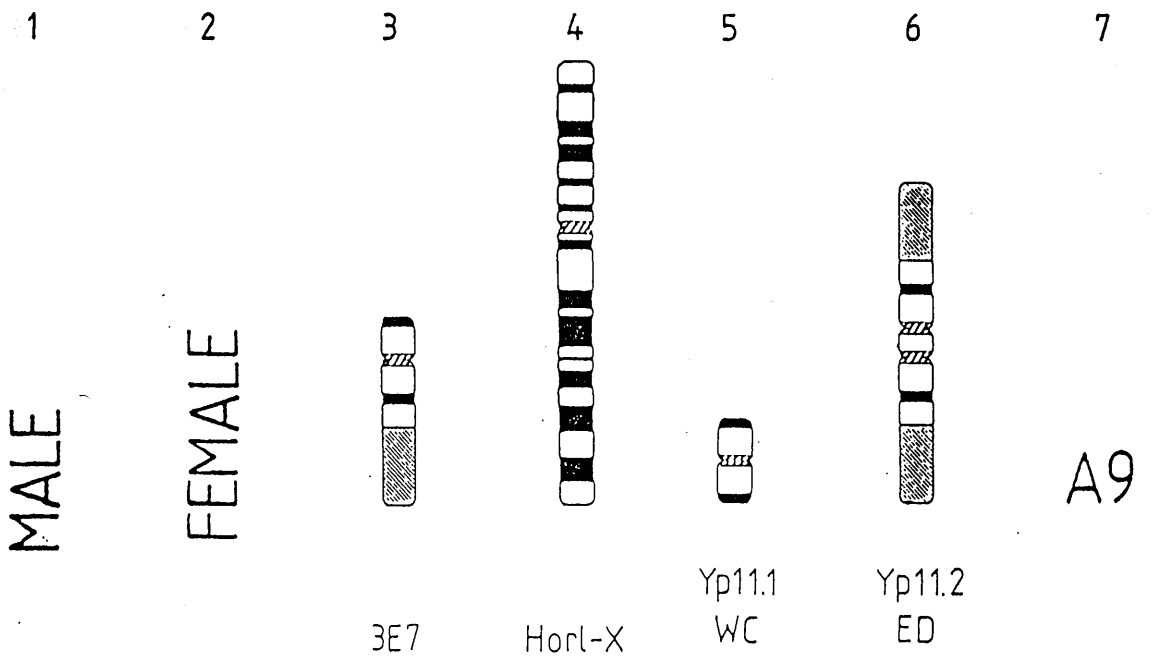


Figure 3A: Schematic representation of the X and Y chromosomes of the members of the panel used in figure 4A. 1: normal male, 2: normal female, 3: 3E7 (Y only) hybrid cell line, 4: Horl-X (X only) hybrid cell line, 5: WC an iso Yp male (Yp11.1), 6: ED a 48,XX female with two dic(Yq) chromosomes (Yp11.2), 7: the hydrids' mouse parent line A9.

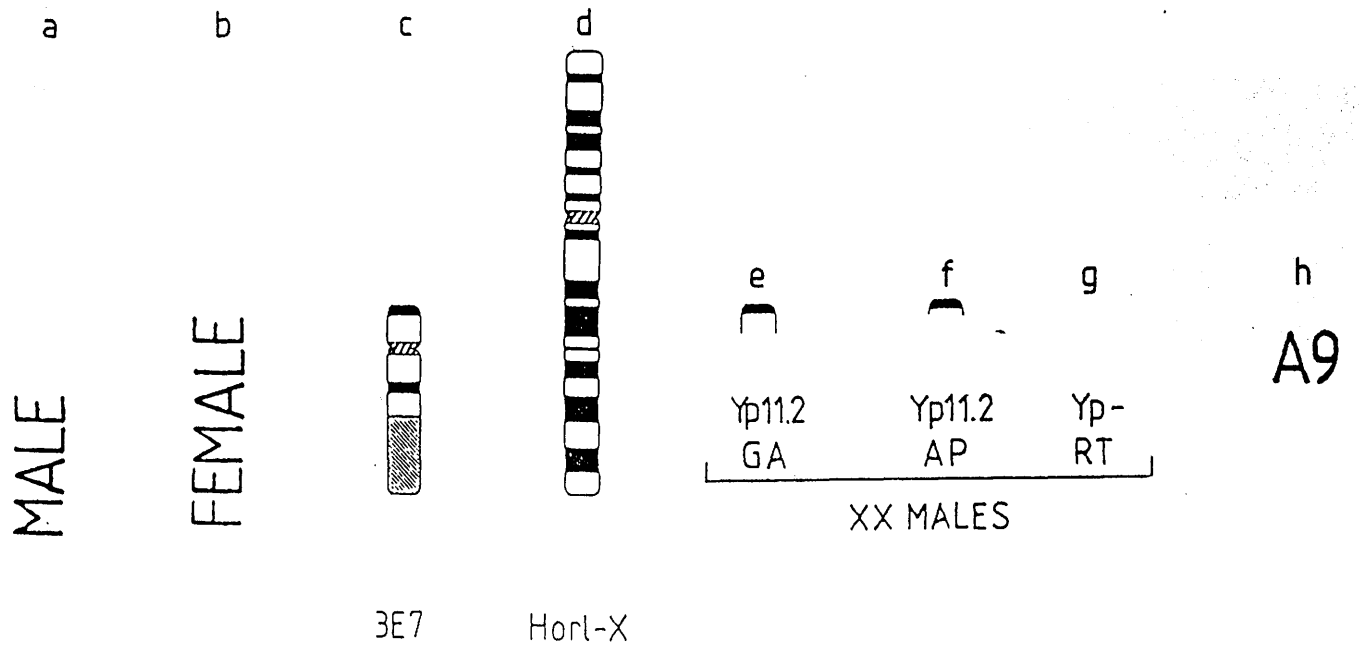


Figure 3B: Schematic representation of the X and Y chromosomes of the members of the panel used in figure 4B. 1: normal male, 2: normal female, 3: 3E7 (Y only) hybrid cell line, 4: Horl-X (X only) hybrid cell line, 5: GA an XXYp+ male carrying most of Yp, 6: AP an XXYp+ male carrying a small portion of Yp, 7: RT an XX male lacking detectable Yp sequences, 8: the hybrids' mouse parent line A9.

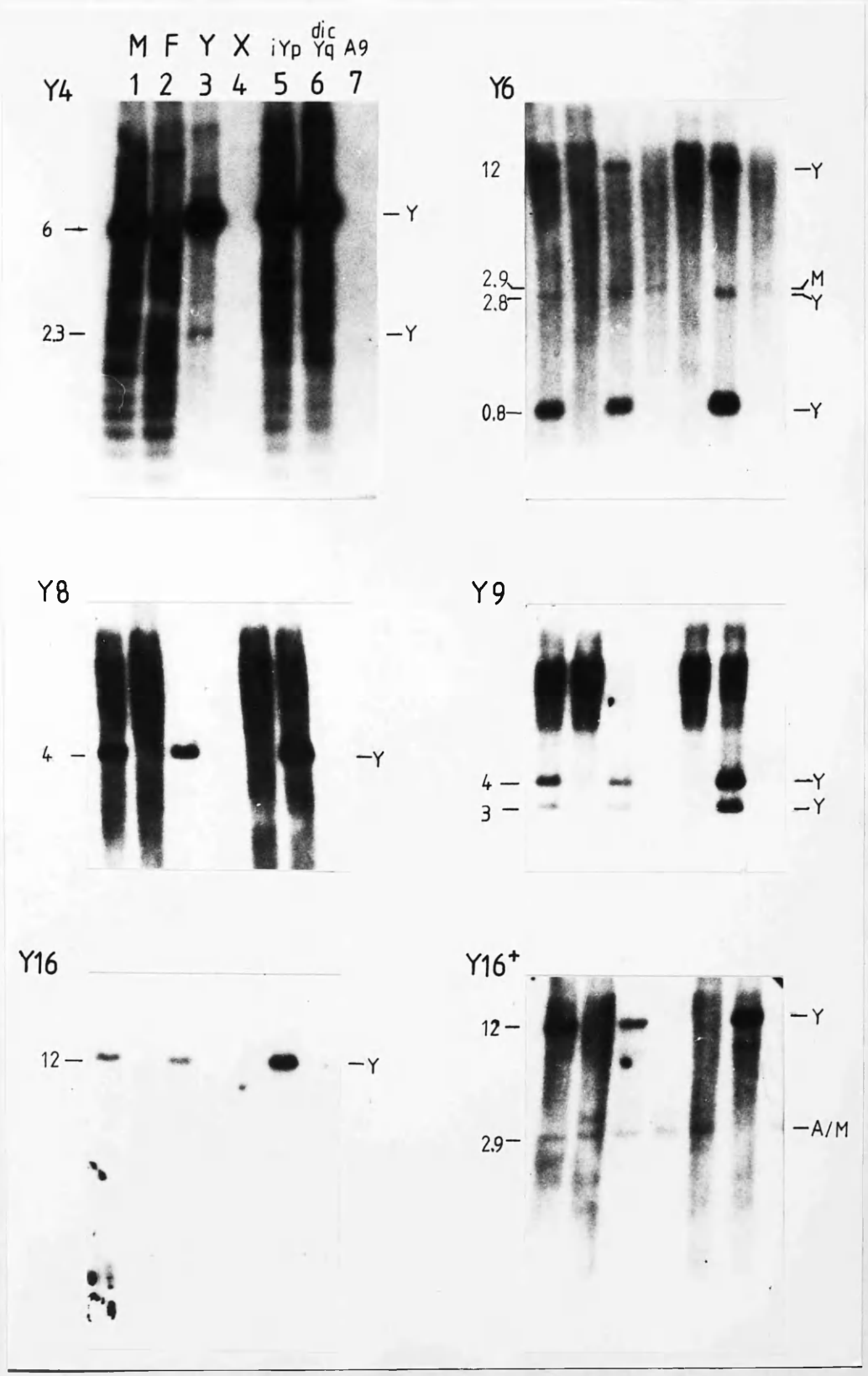


Figure 4A: The results obtained with the Y homologous recombinants on Southern blot analysis of the panels detailed in figure 3A. 1: normal male, 2: normal female, 3: 3E7, 4: Horl-X, 5: WC an iso Yp male, 6: ED a dic(Yp) female, 7: A9. A "+" indicates the low stringency wash. GMGY34 is shown on a panel with Horl-X replaced by the Y hybrid 7631. GMGXY11 is on a panel containing two males, two females, Horl-X, 3E7 and A9. GMGY16 and GMGY21 are shown washed at both high and low stringency.

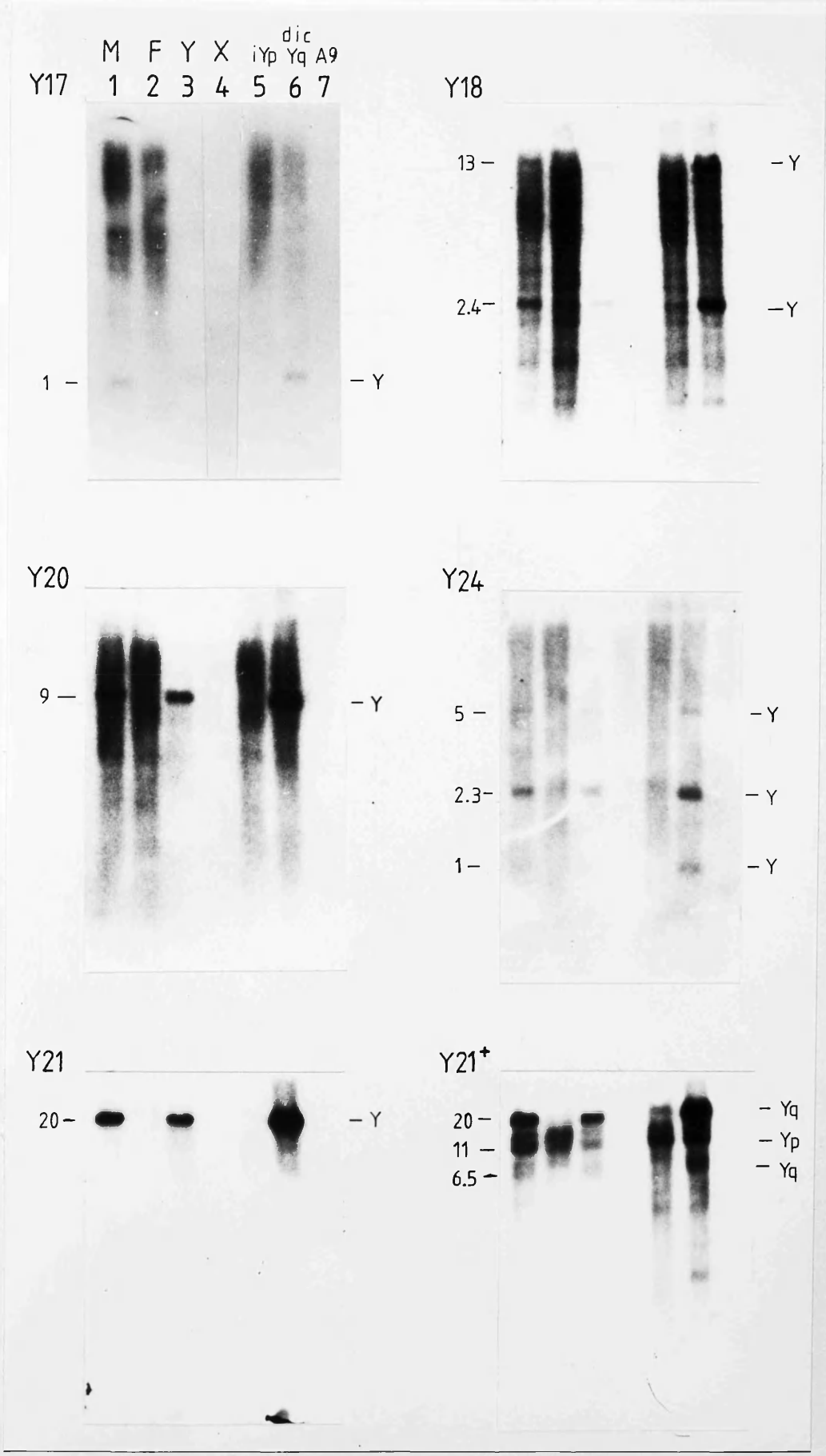


Figure 4A (cont'd)

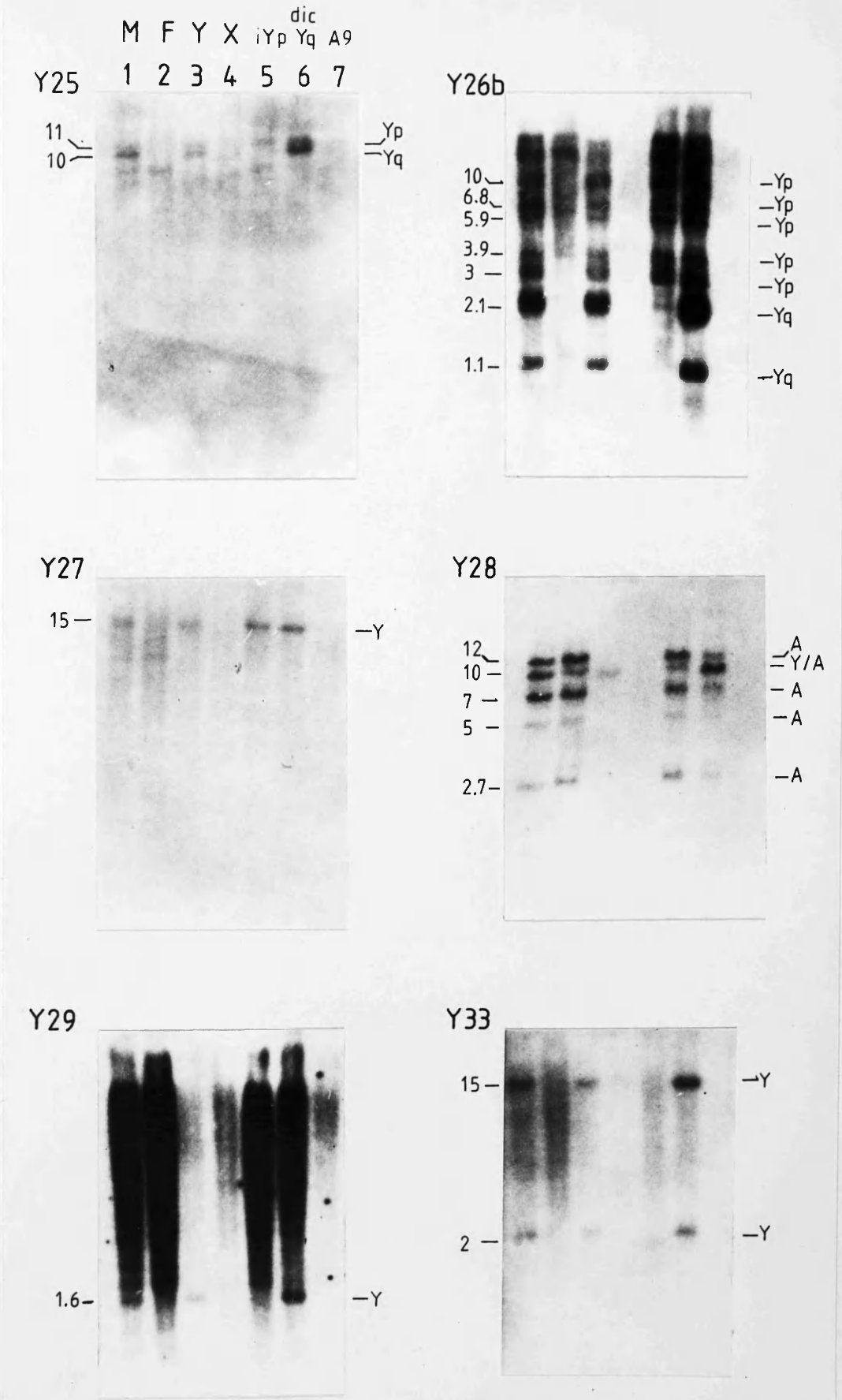


Figure 4A (cont'd)

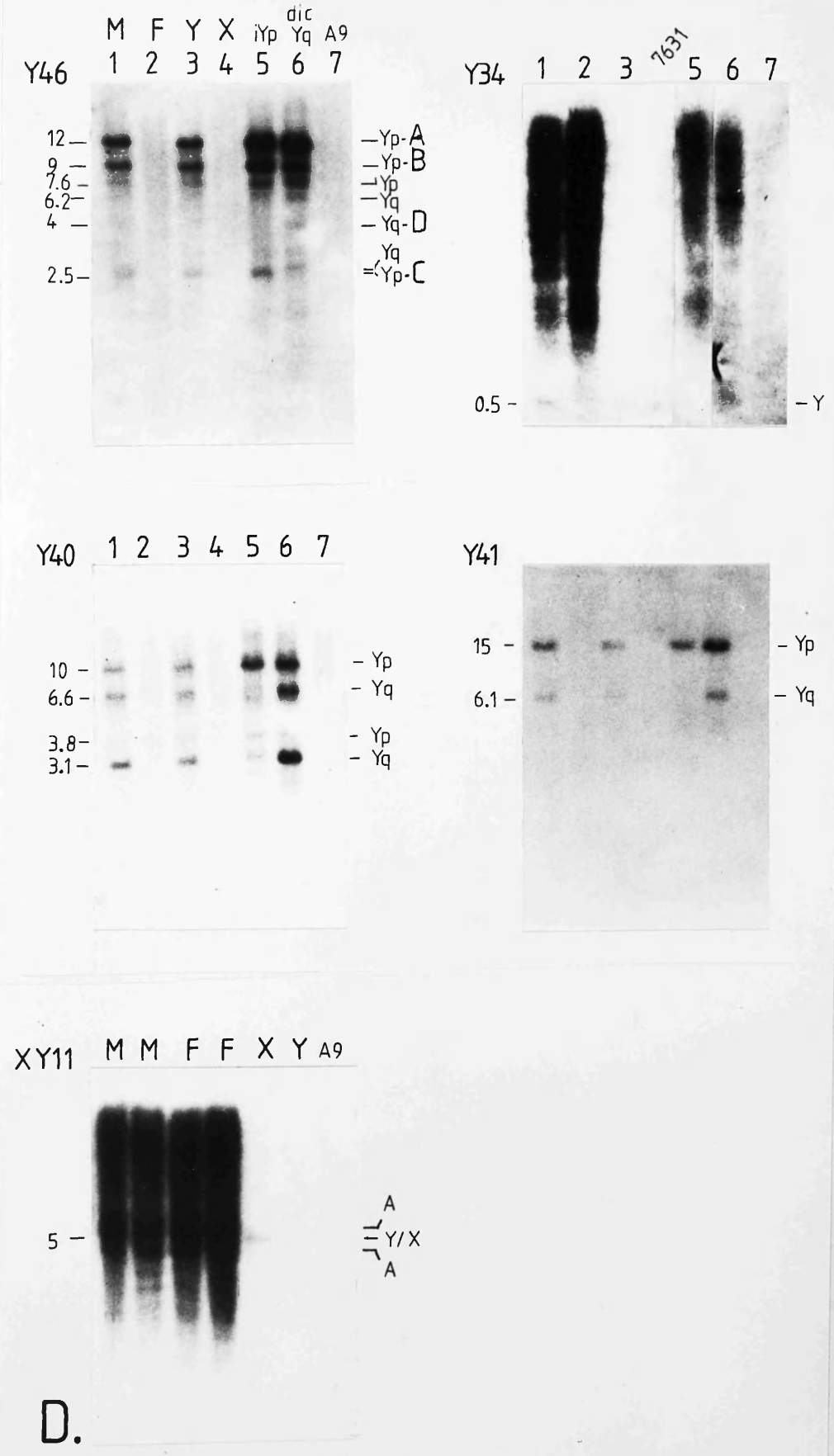


Figure 4A (cont'd)

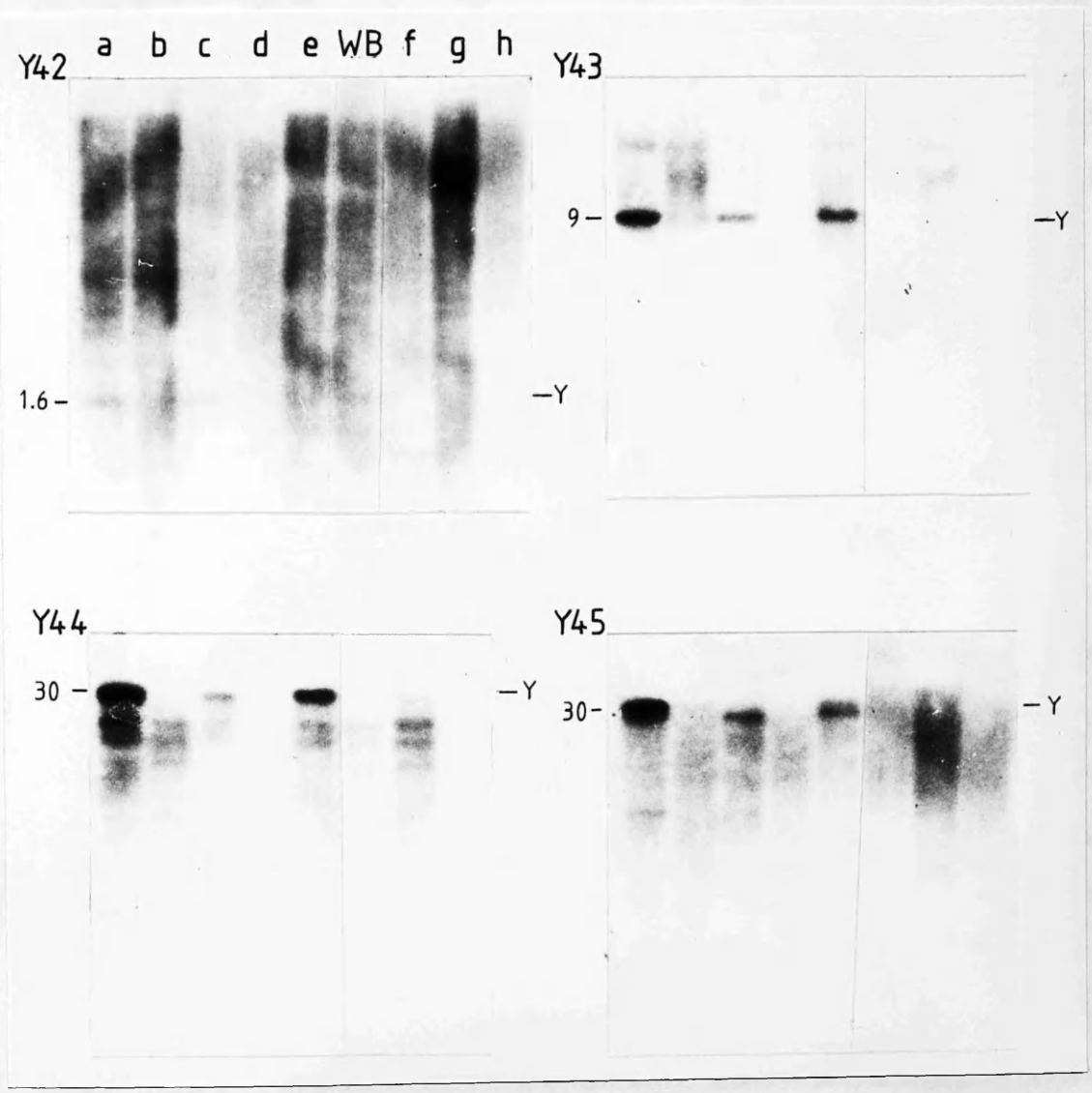


Figure 4B (cont'd)

On the basis of this analysis the Y homologous probes have been divided into three categories based on their hybridisation patterns:

(1) Yp homologous

(2) Yq homologous

(3) Yp/Yq homologous.

All these classes contain probes with X or autosomal cross-homologies.

Each of these classes of probe will be described separately. The mapping data obtained with the XY homologous probes are presented in section 3.3. Owing to constraints on time the autosomal homologies were not further characterised.

3.2.1 Yp Homologous Probes

The assignment of a probe to Yp was on the basis of the presence of a band of the same size in both the 3E7 and WC tracks of panel 3A or the 3E7 and GA tracks of panel 3B. Nineteen Yp homologous probes were assigned in this way. Five of these (data not shown) were judged, on the basis of insert size and pattern of hybridisation, to be homologous to GMGY7, GMGY10 or GMGY22 which had previously been isolated by K. Kwok from the same library. The remaining 13 probes include five XY homologous probes GMGXY4-7 and GMGXY9. GMGY4a, GMGY4b, GMGY4c, GMGY42, GMGY43 and GMGY44 detect autosomal homologies, but GMGY27 and GMGY45 are largely Yp specific.

The GMGY4a, GMGY4b and GMGY4c recombinants have insert sizes of 1.2kb, 1.8kb and 2.3kb respectively. They all detect a strong Y specific band on Southern analysis of *Eco*RI digested male genomic DNA at approximately 6kb along with multiple autosomal bands. There is no hybridisation to the X chromosome which is demonstrated by the lack of signal in the X hybrid track. It therefore seemed possible that these 3 probes shared a common homology. This was determined by blotting the *Hin*dIII digested GMGY4a and b recombinants and probing with GMGY4c. GMGX7 was also blotted as a control. Both Y4a and b inserts lit up strongly while X7 did not. This result is shown in figure 5 and argues that these three probes are defining the same homology present in many copies on the Y chromosome.

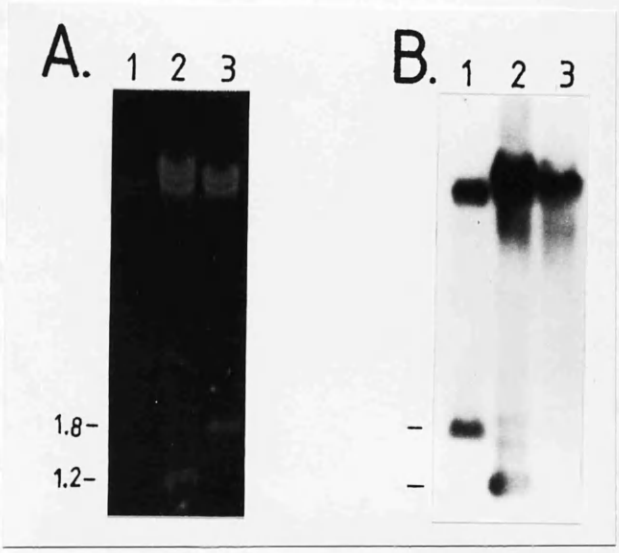


Figure 5: The cross-hybridisation of GMGY4a, b and c. The lanes are 1: GMGY4b, 2: GMGY4a and 3: GMGX7. A:- ethidium bromide stained gel of the recombinants after HindIII digestion and electrophoresis. B:- autoradiograph of the Southern blot of A probed with GMGY4c.

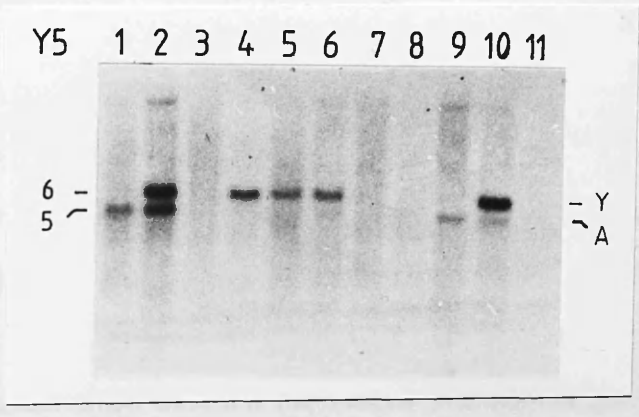


Figure 6: GMGY5 hybridised to a Southern blot of TaqI digested DNA from members of the panel detailed in figure 8A.

3.2.2 Yq Homologous Probes

These probes were assigned on the basis that a given probe detected a band in the 3E7 and ED tracks but not in the WC track of panel 3A or in the 3E7 but not the GA tracks of panel 3B. Although GA does not carry the whole of Yp subsequent analysis of the dicentric Yp individuals confirmed this assignment for two of three probes assigned in this way. The third, GMGY32, was not used in this analysis and so can only be said to more likely map to Yq

There are then 18 Yq homologous probes including one XY homologous probe GMGXY19. Of these GMGY5, GMGY8, GMGY9, GMGY16, GMGY17, GMGY28, GMGY29, GMGY33 and GMGY34 detect autosomal homologies, either as smears or as bands of varying intensities. In the case if GMGY16, GMGY18, GMGY29 and GMGY33 the autosomal homologies are only present at lower stringency (1 x SSC, 0.1%SDS, 65°C). At higher stringency (0.1 x SSC, 0.1%SDS, 65°C) they are removed to leave only a Yq specific pattern of hybridisation. In figure 4A GMGY18 and GMGY29 are shown at low stringency, GMGY33 at high stringency and GMGY16 at both high and low stringency.

Probes GMGY5 and GMGY28 detect three and five autosomal bands respectively. On *Eco*RI digested genomic DNA GMGY5 detects only a single band and *Taq*I digested DNA is required to separate the Y and autosomal bands. GMGY5 was hybridised to an X-hybrid panel as detailed in figure 8A and the hybridisation pattern is shown in figure 6. In the case of GMGY28 the Y and autosomal bands have not been clearly split. However on *Eco*RI digested panels the strong hybridisation at the position of the Y band in ED but not in WC allows a Yq assignment to be made as ED has four copies of Yq and proximal Yp while WC has two copies of Yp. Thus the dosage suggests a Yq location.

Six Yq homologous probes - GMGY6, GMGY20, GMGY24, GMGY30, GMGY31 and GMGY32 - are predominately Y specific. GMGY16 and GMGY6 both detect a 2.9kb band in the mouse A9 track at lower stringency (see figure 4A) which indicates the possibility of conservation between the mouse and human genomes in the regions detected by these probes. It is unlikely that the band detected in the mouse DNA is Y-linked as the Y is unstable in such lines. One of the inserts detected the pattern of the 3.4kb Yq heterochromatic repeat (not shown).

3.2.3 Yp/Yq homologous Probes

Nine probes were isolated which detected at least one band that mapped to Yp and one that mapped to Yq. These probes are GMGY21, GMGY25, GMGY26a, GMGY26b, GMGY40, GMGY41, GMGY46 and the XY homologous probe GMGXY10. GMGY21 only displays this cross-homology at lower stringency and figure 4A shows the pattern of hybridisation at both low and high stringency. At higher stringency GMGY40 detects two Yp and two Yq bands as well as two faint autosomal bands. The Yp bands and the Yq bands both map together on their respective arms. However GMGY46 detects seven Y specific bands. The upper four are present in both WC and ED, suggesting a proximal Yp location. The 4kb band is only in ED which maps it to Yq. The 2.5kb Y band is strong in WC and missing in ED. There is a faint band in ED in this region but it is of slightly higher molecular weight and as this band is not present in the female track it must map to either Yp or Yq. The 2.5 kb band not present in ED must map to the other side of the ED breakpoint and have a more distal Yp location than the other Yp bands. Thus this probe marks three regions of the Y chromosome.

GMGY26a and GMGY26b detect two strong Yq bands of 2.1kb and 1.1kb. Seven Yp bands of higher molecular weight are also picked up, along with a number of autosomal bands. Three probes have been isolated which give this same pattern. Two have insert sizes of 3.5kb (GMGY26a) and the other has a 4.0kb insert (GMGY26b). Although the inserts have not been cross-hybridised these results suggest that these probes define a predominately Yq specific low-level repeat family with some related members on Yp and the autosomes.

Seventy inserts were isolated of which 47 hybridised to the Y chromosome (67%). The remainder showed X or autosomal hybridisation (14 inserts or 20%) or no hybridisation (9 inserts or 13%). However 13 of the Y homologous probes detect Y-borne moderate repeat families. These have either been previously reported - members of the alphoid repeat cluster (GMGY4a, b and c) and the Yq heterochromatic 3.4kb repeat (not shown) - or have been defined as repeats - GMGY7, GMGY10,

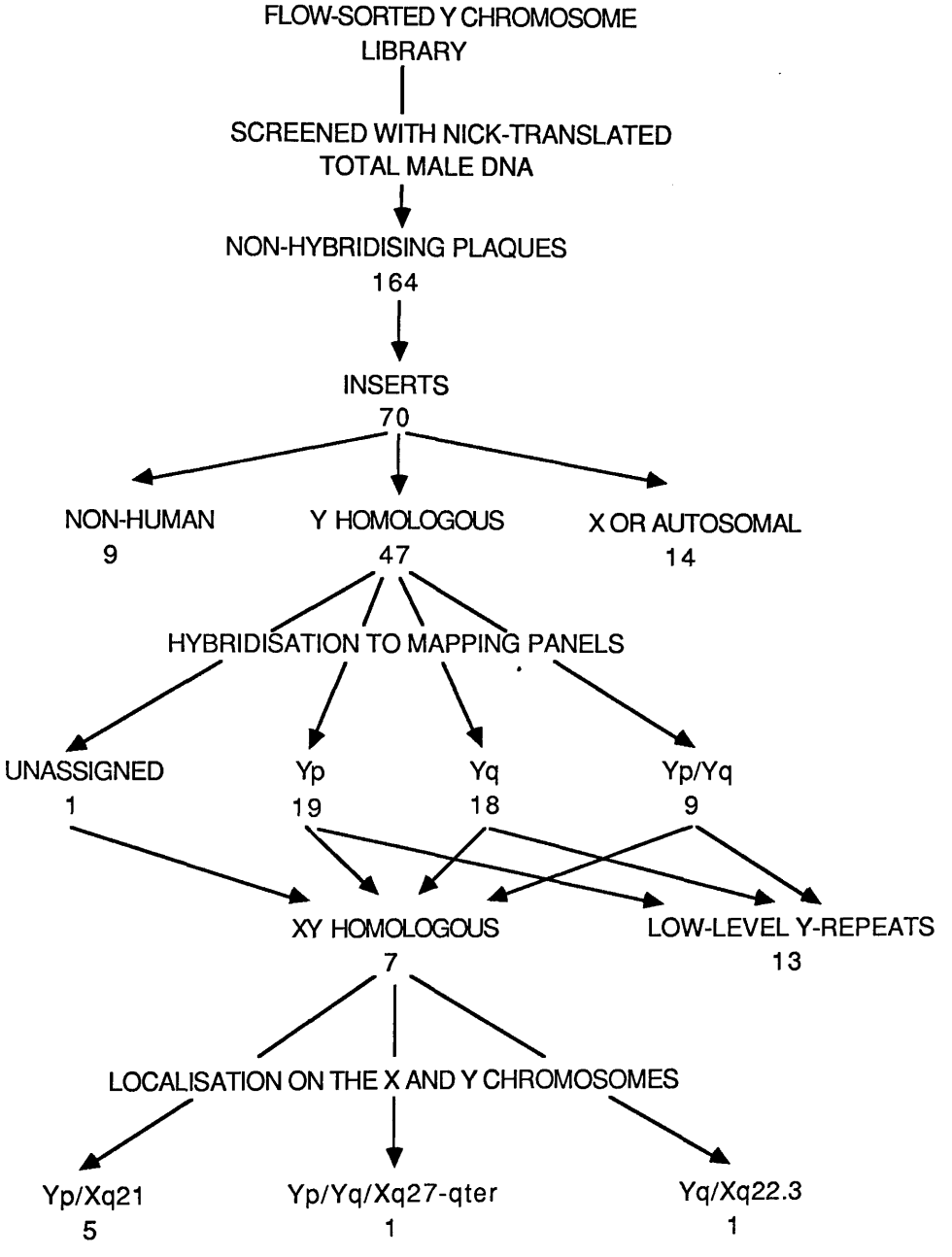


Figure 7: A flow diagram of the isolation of the Y homologous inserts from the Y flow sorted library and their preliminary characterisation.

	No.	% of total	% of inserts	% of Y inserts
Plaques isolated:	164	100	-	-
Inserts:	70	43	100	-
X/autosomal:	14	8	20	-
Non-human:	9	5	13	-
Y homologous:	47	29	67	100
Y repeats:	13	8	18	27
3.4kb	1	-	-	-
GMGY4	4	-	-	-
GMGY7	2	-	-	-
GMGY10	2	-	-	-
GMGY22	1	-	-	-
GMGY26	3	-	-	-

Table 1: The numbers of inserts and insert types isolated in this study.

Yq HOMOLOGOUS PROBES

Probe	HGM symbol	Insert size (Kilobases)
GMGY5	(DYS65)	1.8
GMGY6	(DYS66)	1.8
GMGY8	(DYS67)	1.5
GMGY9	(DYS68)	1.5
GMGY16	(DYS69)	1.5
GMGY17	(DYS76)	1.6
GMGY18	(DYS75)	6
GMGY20	(DYS73)	1.5
GMGY21	(DYS72)	4
GMGY24	(DYS71)	2.7
GMGY25	(DYS70)	2.1
GMGY28	(DYS79)	4.5
GMGY29	(DYS80)	1.6
GMGY30	(DYS84)	3.6
GMGY31	(DYS85)	2.7
GMGY32		1.1
GMGY33		6
GMGY34	(DYS60)	1.1
GMGXY19	(DYS74)	1.5

UNASSIGNED PROBE

Probe	Insert size (Kilobases)
GMGXY11	5

Yp HOMOLOGOUS PROBES

Probe	HGM symbol	Insert size (Kilobases)
GMGY4a	(DYS50)	1.2
GMGY4b	(DYS51)	1.8
GMGY4c	(DYS52)	2.3
GMGY27		1.7
GMGY42		3
GMGY43		4
GMGY44		3.9
GMGY45		2.7
GMGXY4	(DXYS30)	2.3
GMGXY5	(DXYS31)	1.8
GMGXY6	(DXYS32)	2.3
GMGXY7	(DXYS33)	3.5
GMGXY9	(DXYS35)	6

Yp/Yq HOMOLOGOUS PROBES

Probe	HGM symbol	Insert size (Kilobases)
GMGY26a	(DYS77)	3.5
GMGY26b		4
GMGY40		2.6
GMGY41		1.8
GMGY46	(DYS56)	6
GMGXY10	(DXYS37)	4.5

Table 2: List of recombinants isolated in this study divided into groups based on their mapping position on the Y chromosome. The insert sizes are also shown.

GMGY22 and GMGY26 - because they have been isolated more than once from the library used by either the author or K.H. Kwok who independently isolated a series of probes from the same library. Of these only the hybridisation pattern of GMGY26 is shown in figure 2. Thus over a quarter of the Y homologous probes isolated (27%) are repeated on the Y chromosome. Table 1 summarises the recombinant data and figure 7 shows it in diagramatic form. Table 2 shows the insert sizes of all the Y recombinants together with their Human Gene Mapping "D" numbers where they have been assigned.

3.3 XY Homologous Probes

Eight XY homologous probes were isolated in this study. These are GMGXY4, GMGXY5, GMGXY6, GMGXY7, GMGXY9, GMGXY10, GMGXY11, and GMGXY19. The probe GMGXY11 can be seen, from figure 4A to have very strong autosomal homologies. No further characterisation was carried out on this probe.

Further characterisation was carried out on the other probes together with three XY homologous probes not isolated by the author. These three probes were GMGXY8, isolated by K.H. Kwok in our department from the same library as used by the author, GMGXY12, isolated in our department by E.F. Gillard from an X flow-sorted library, and GMGXY3, isolated by L. Florentin in our department from a partial digestion library of a hamster-human hybrid containing the human Y chromosome. GMGXY3 was known to map to Yq and Xp22.3.

GMGXY3 was isolated from a Y chromosomal library and the X band detected is considerably weaker than the Y band. Inorder to improve the X hybridisation the X homologue of this probe was isolated.

3.3.1 Isolation of GMGXXY3 the X Homologue of GMGXY3

GMGXY3 was used to screen 5 x 10^5 recombinants from an amplified *Eco*RI X/7 flow-sorted library made in lambda NM1149 by Louise Sinclair in our department. Three plaques lit up which were purified and DNA was made from the purified recombinants. After *Eco*RI digestion all three were seen to contain a 1.6kb insert. This is the same size as the *Eco*RI XY3 insert which contains a repeat. However when this

insert is further digested with *Hind*III 1.0kb and 0.6kb fragments are generated of which the latter is repeat free. An identical situation was found for one of the recombinants isolated. The other two were not tested. This probe was called GMGXXY3.

3.3.2 Mapping the XY Homologous Probes

The eleven XY homologous probes were used to screen the panels schematically represented in figure 8A and B. The results are shown in figures 9A-D and summarised in table 3. With the exception of GMGXY7 all the probes are shown hybridised to panel DNA digested with an enzyme that revealed an XY difference.

Figure 9A shows representatives of the three different mapping patterns obtained with these eleven probes when hybridised to the hybrid mapping panel of figure 8A and these are described below. The chromosomes used on the panels are described in materials and methods.

GMGXY4

The Y band of GMGXY4 is present in WC, the 46,X,i(Yp) individual, but is missing from ED who carries two dicYq chromosomes. This maps the Y homologue distal to the ED breakpoint on Yp. The X band is present in the hybrid NEA9 but absent from FNA9. Thus it must map between Xq13 and Xq24 which is the region present in NEA9 that is missing from FNA9. Thus GMGXY4 defines a region of homology between Yp and Xq13-24. The other probes which give a similar pattern on this panel are GMGXY5-9, and XY12. The results with these probes can be seen in figures 9B and 9C. The Y band of XY7 has to be mapped to Yp by dosage of the XY common band in WC and ED. Its greater strength in the WC track shows it to map to Yp.

The hybribisation of the GMGXY4 -like probes to the X chromosomes diagrammed in figure 8B is shown in figures 9C and 9D. MB and Tel26 were found to be deleted for all the GMGXY4 -like probes. Cytogenetically Tel26 is believed to have a deletion Xq13-21.3 and MB of Xq21.1-21.3. Thus based on the cytogenetic findings on these two cases the GMGXY4 -like probes can be further localised to Xq21.1-q21.3. The probes were all found to be present in the hybrid DHTK.

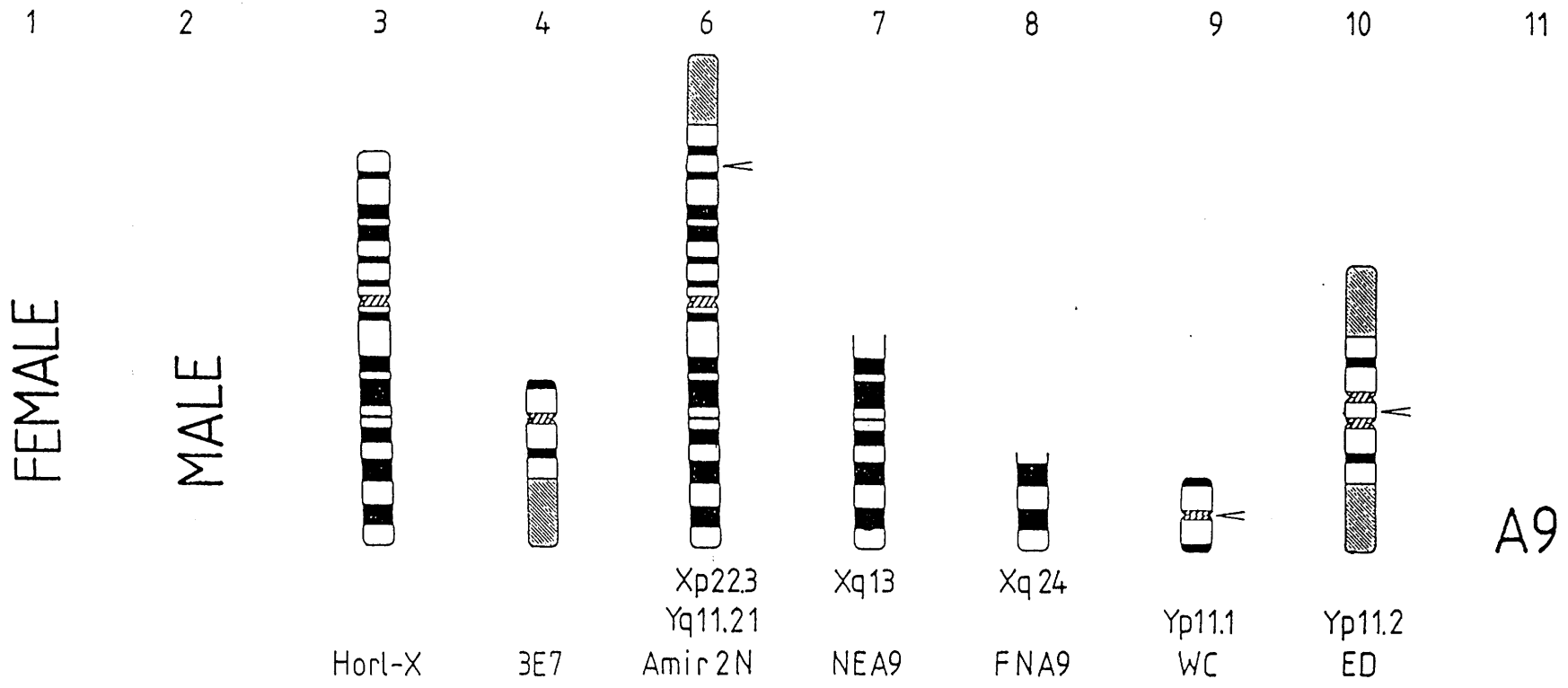


Figure 8A: A schematic representation of the X and Y chromosomes carried by the members of the panel used in figure 9A-C. 1: normal male, 2: normal female, 3: Horl-X (X only) hybrid cell line, 4: 3E7 (Y only) hybrid cell line, 5: 7631 hybrid cell line with Y and some autosomes, 6: mouse-human hybrid Amir2N carrying a human t(X:Y)(Xp22.3;Yq11) chromosome, 7: mouse-human hybrid NEA9 containing Xq13-qter on a human X:11 translocation product, 8: mouse-human hybrid FNA9 containing Xq24-qter on a human X:19 translocation product, 9: WC an iso(Yp) male, 10: ED a 48,XX female with two dic(Yq) chromosomes and 11: A9 the hybrids' mouse parent line.

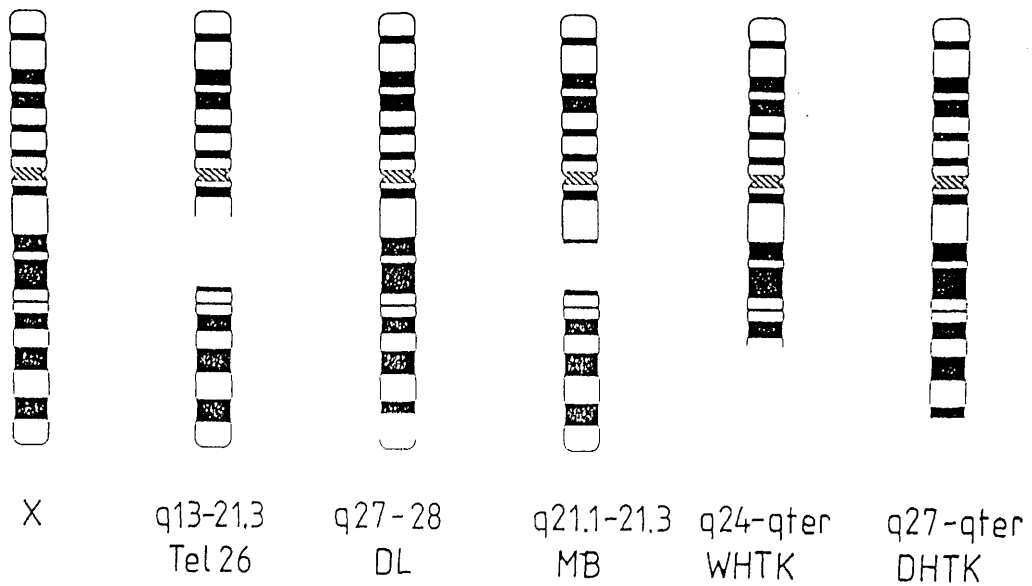


Figure 8B: A schematic representation of the X and Y chromosomes carried by members of the panels used in figure 9C and D. a: Tel 26 a del(Xq13-21.3) male, b: DL a del(Xq27-28) female, c: MB a del(Xq21.1-21.3) male, d: WHTK deleted X hybrid cell line and e: DHTK a hybrid cell line containing Xpter-q27 on a X:9 translocation product.

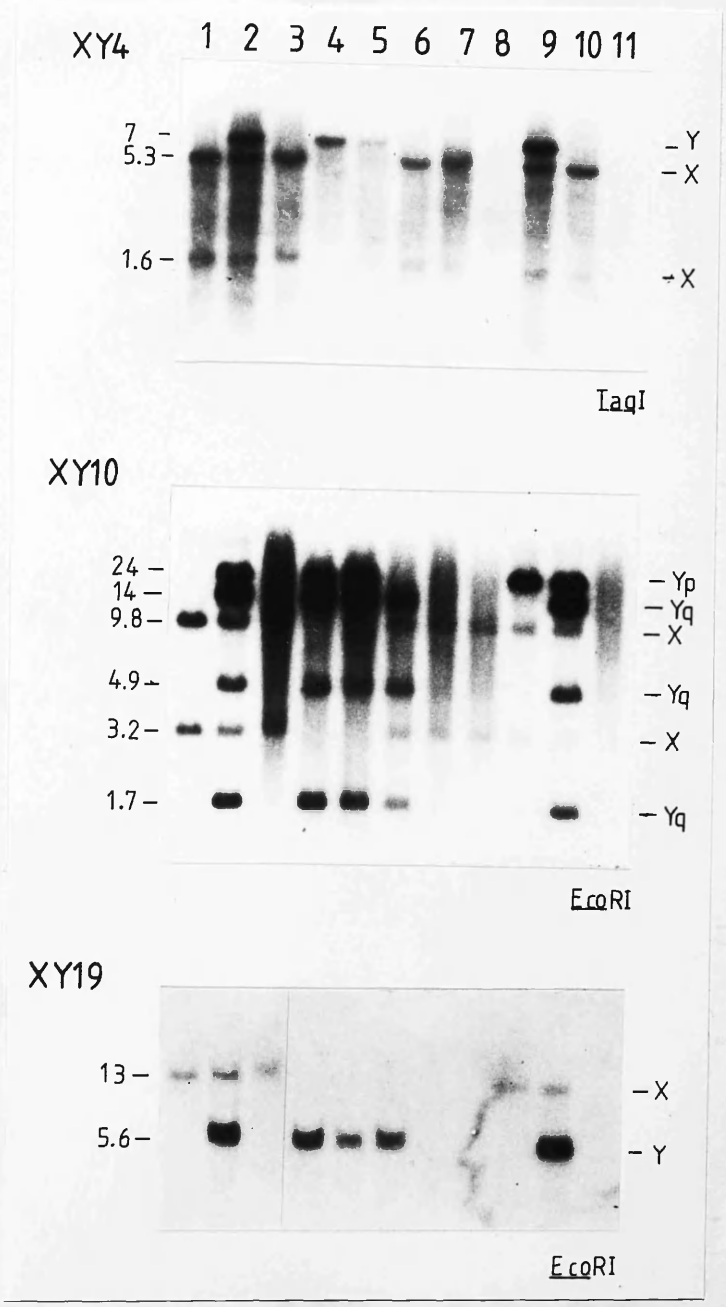


Figure 9A: GMGXY4, GMGXY10 and GMGXY19 hybridised to Southern blots of the panel detailed in figure 8A. This shows the three different patterns of hybridisation obtained with the XY homologous probes.

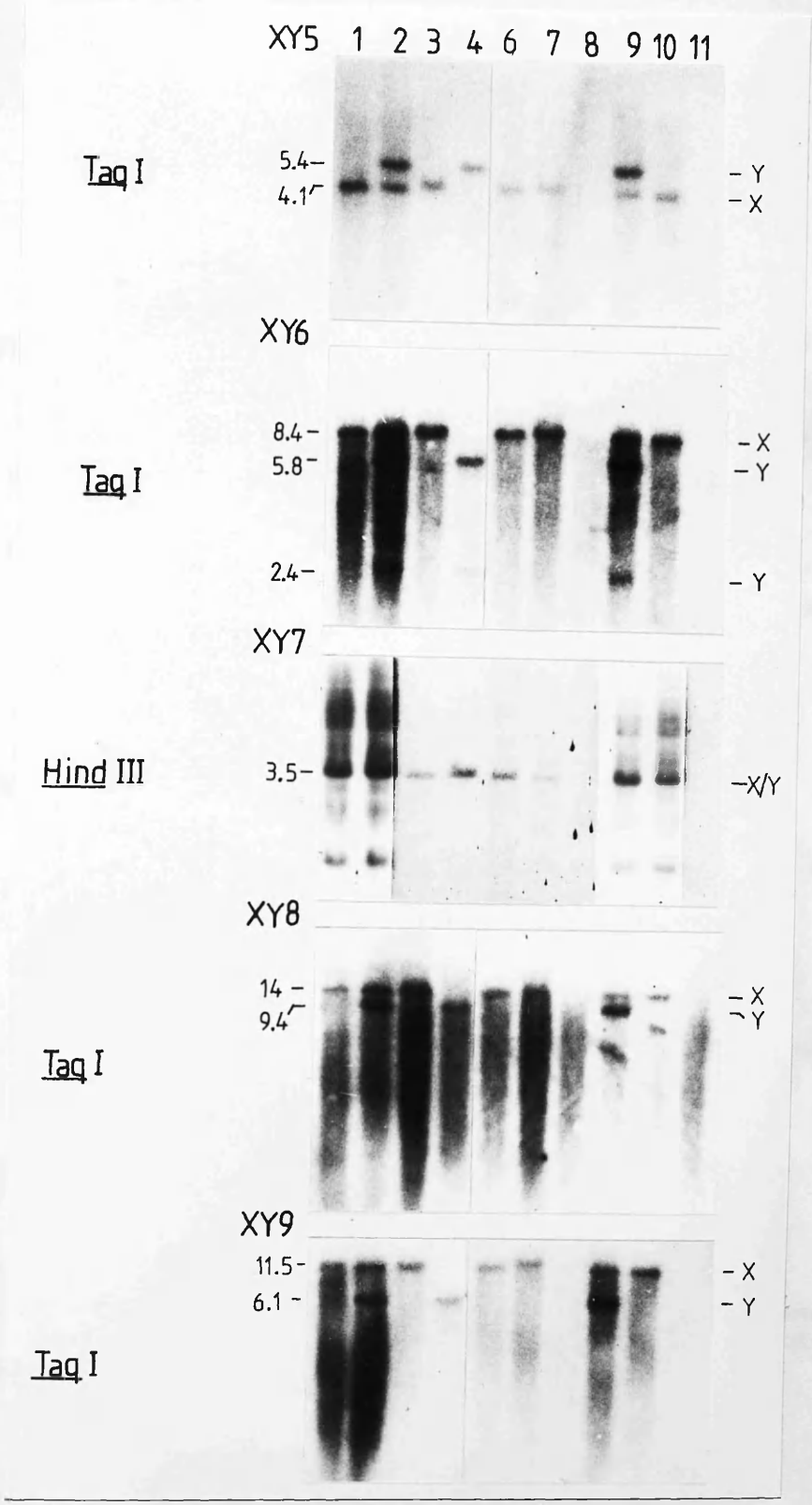


Figure 9B: GMGXY5-XY9 hybridised to Southern blots of the panel as detailed in figure 8A but without the Y hybrid 7631.

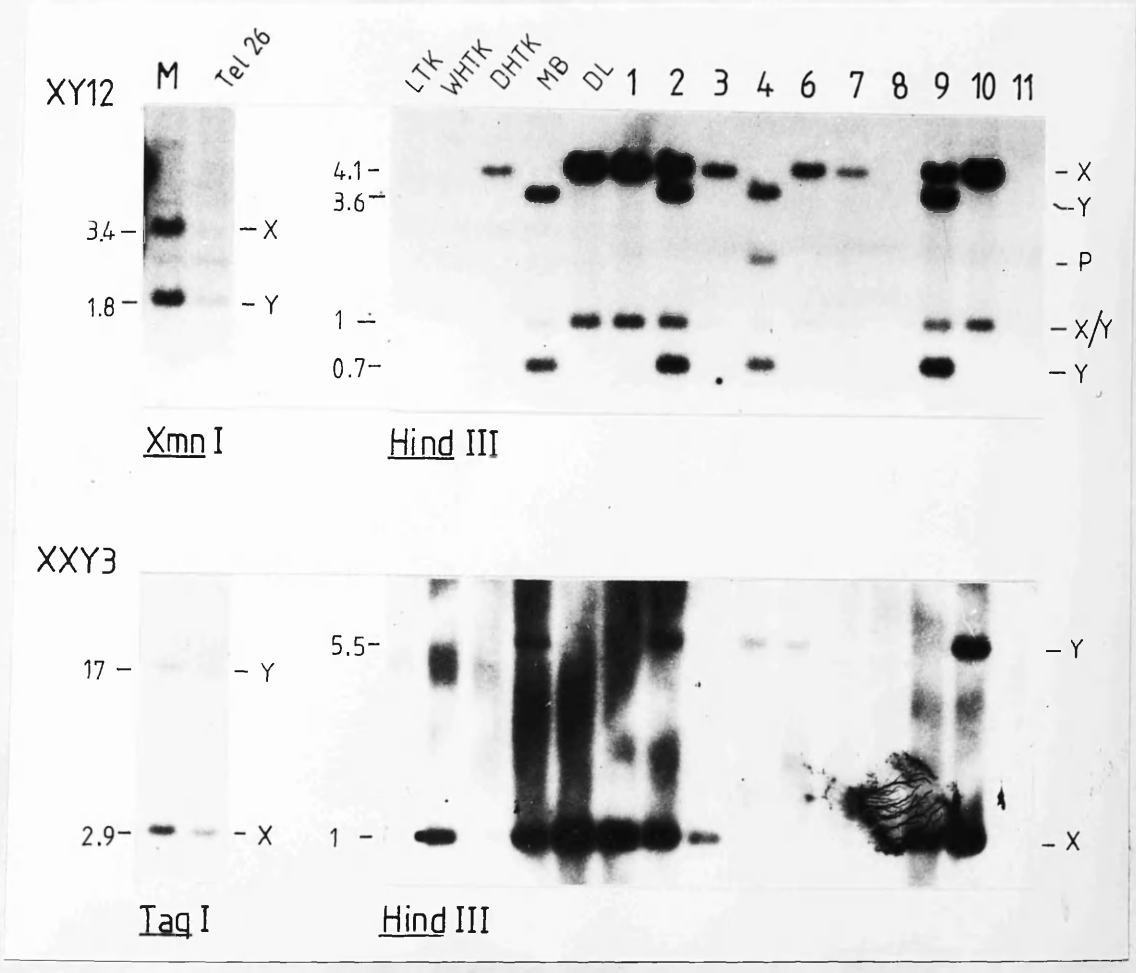


Figure 9C: GMGXY12 and GMGXXY3 hybridised to Southern blots of the panel detailed in figure 8A and the individuals and hybrids detailed in figure 8B. DNAs are digested as indicated. "P" indicates exogenous DNA contamination.

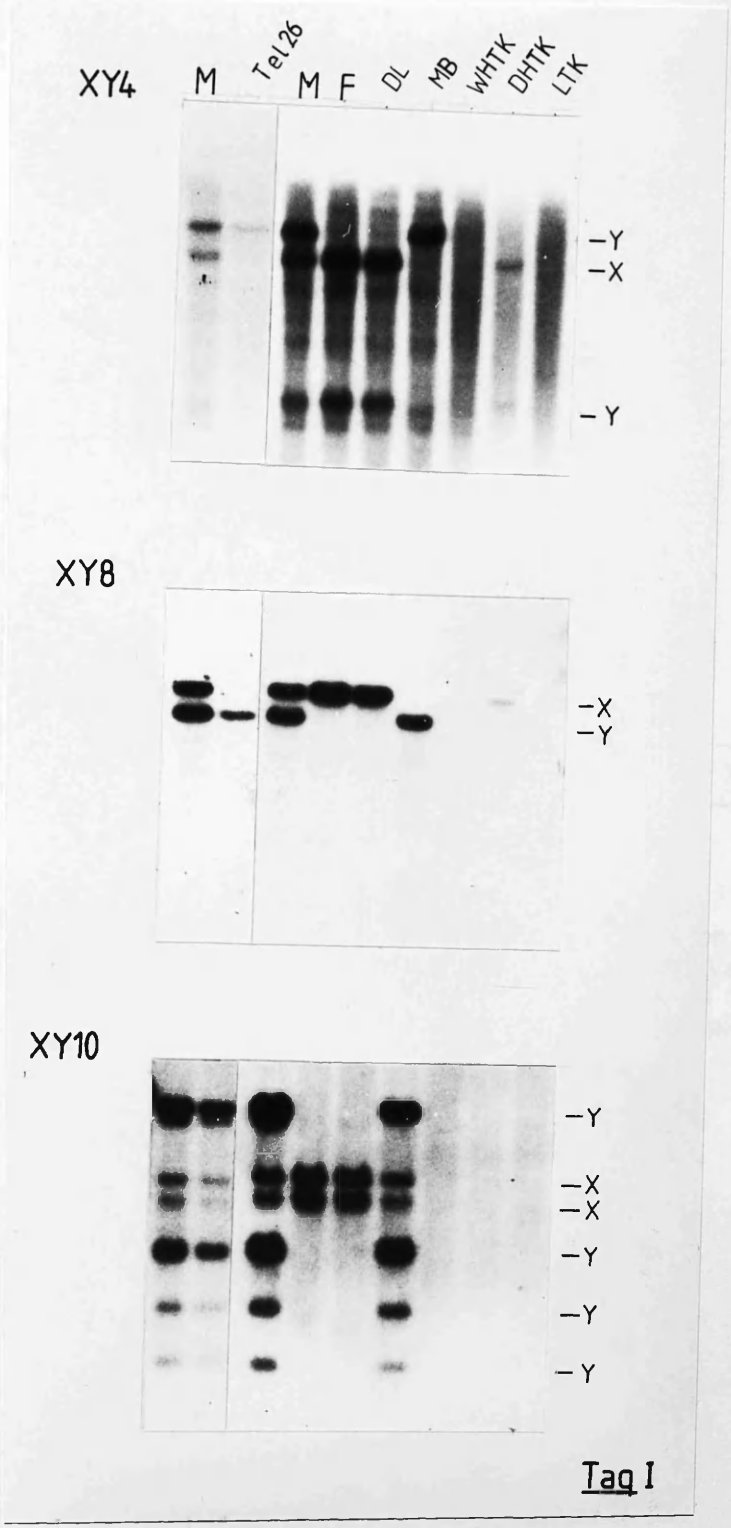


Figure 9D: GMGXY4, GMGXY8 and GMGXY10 hybridised to Southern blots of the individuals and hybrids detailed in figure 8B. M:- normal male and F:- normal female.

Origin of DNA	Region of X deleted	GMGXXY3 GMGXY19	GMGXY4-9 GMGXY12	GMGXY10
Amir2N	Xpter-p22.3	-	+	+
NEA9	Xpter-q13	-	+	+
FNA9	Xpter-q24	-	-	+
Tel26	Xq13-21.3	+	-	+
MB	Xq21.1-21.3	+	-	+
DHTK	Xq27-qter	+	+	-
DL	Xq27-28	+*	+*	+*
Mapping positions :-		Xp22.3	Xq21	Xq27-qter

Table 3: The mapping of the XY homologous probes on the X chromosomal deletion panel detailed in figure 8A and B. The * indicates that the result was obtained through an estimation of the intensity of the X band not its absence or presence.

GMGXY10

From figure 9A it can be seen that the upper Y band is present in both WC and ED which maps it proximal to the ED breakpoint on Yp. However as the other three Y bands are missing in WC but present in ED and Amir2N they must map on Yq distal to the Y breakpoint in Amir2N. The X bands of GMGXY10 are present only in FNA9 and must therefore map to Xq24-qter. Thus XY10 defines homology between Yp, Yq and Xq24-qter. This is the only probe isolated that defines such XY homology.

The mouse-human hybrid DHTK contains a tX:9 product in which most of the X chromosome is present with a cytogenetically determined breakpoint at Xq27-28. Figure 9D shows the X homologue of GMGXY10 to be deleted from this hybrid which argues that this probe can be further localised Xq27-qter. GMGXY10 is present in both MB and Tel26.

GMGXY19

In figure 9A the Y band of GMGXY19 can be seen to map to Yq as it is missing from WC but is present in ED and Amir2N. The X band is absent from Amir2N, NEA9, and FNA9 and as Amir2N is only deleted for Xp22.3-pter GMGXY19 must map into this region. Therefore GMGXY19 detects a region of homology between Yq and Xp22.3-pter. This is the same regions detected by GMGXY3. The pattern of hybridisation obtained with GMGXXY3 - the X homologue - is shown in figure 9C.

The GMGXY19-like probes were found to be present on all the deleted X chromosomes except Amir2N. Inorder to further map them within this region they were used to analyse a number of XY translocations which were cytogenetically the same as Amir2N. These consisted of the hybrids HY21, HY94BTI, HY105E, 817/175 and 445/393 and the individual JH and his mother MH. The hybrid HY105E had a contaminating normal X and so yielded no information about the X bands of the probes. However the Y bands of both probes were present in this hybrid. GMGXY19 is shown hybridised to DNA from HY94BTI, 817/175, 445/393, JH and his mother MH in figure 10A and B. It can be seen that the Y band is present in all the hybrids, JH and MH

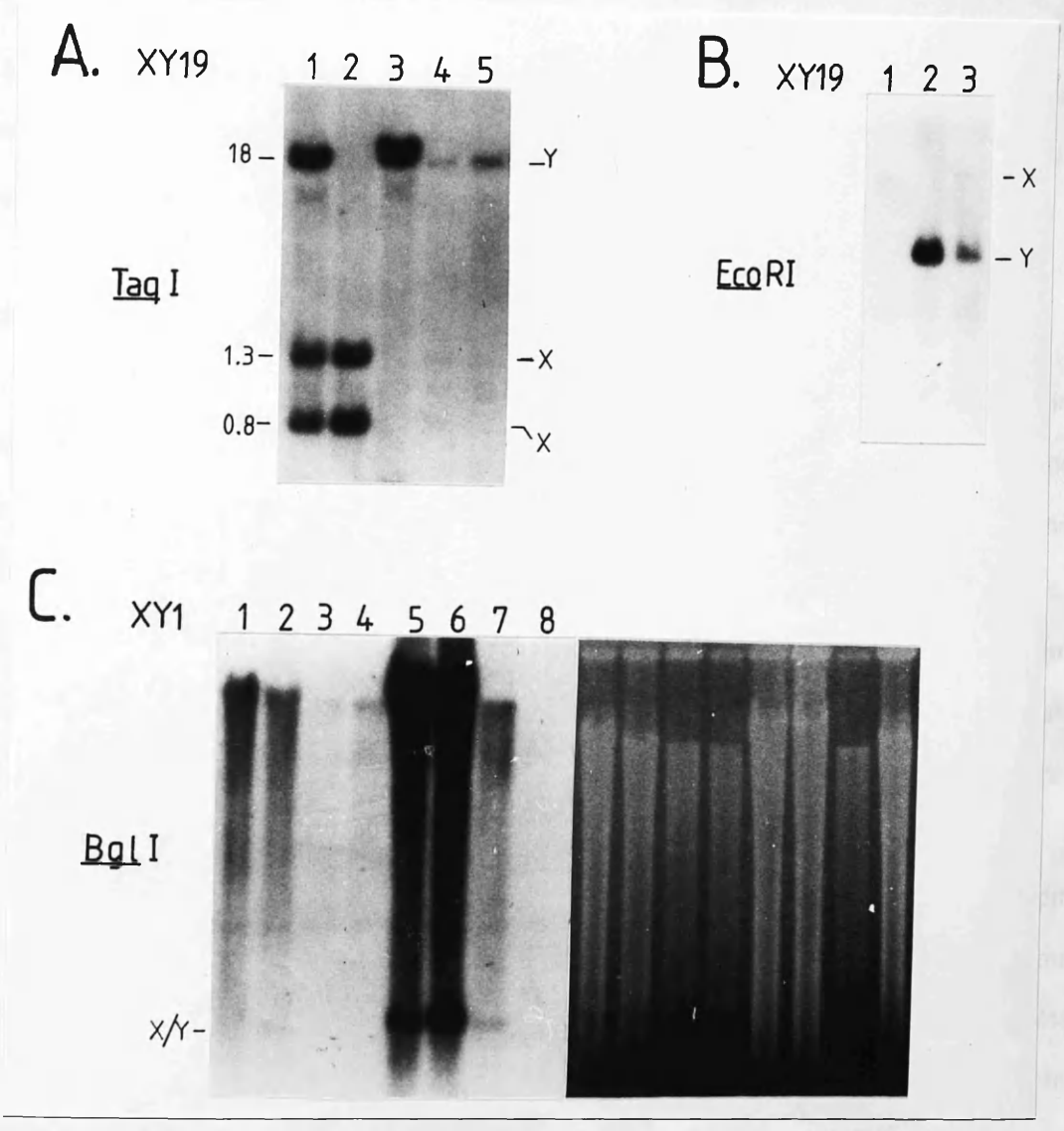


Figure 10: Southern analysis of the t(X:Y)(p22.3;q11.2) translocation products.
A:- GMGXY19 hybridised to 1: normal male, 2: normal female, and three hybrid cell lines 3: HY94BT1, 4: 817/175 and 5: 445/393.
B:- GMGXY19 hybridised to 1: normal female, 2: JH a 46,Y,t(X:Y)(p22.3;q11.2) male and 3: MH the carrier mother of JH.
C: GMGXY1 hybridised to 1: HY94BTI, 2: HY105E, 3: 817/175, 4: 445/393, 5:MH, 6: normal female, 7: normal male and 8: A9. An ethidium bromide stained picture of this gel prior to blotting is also shown.

while the X bands are missing in all but 817/175 and MH who carries a normal X. This same pattern was seen for GMGXXY3. HY21 was deleted for the X bands of both probes but the Y bands were present. This implies that 817/175 has a more distal X breakpoint than the other translocations. Figure 10C shows that 817/175 is deleted for GMGXY1. This probe has been mapped within 5kb of the MIC2 locus (Goodfellow, personal communication) and is therefore known to be at the proximal end of the pseudoautosomal segment. It can be concluded that the breakpoint in this hybrid must lie between the GMGXY19 and XY1. This data is also presented in table 4.

3.3.3 Analysis of the Deleted X Chromosomes of DL and WHTK

None of the members of the three groups were deleted in DL. This was ascertained by dosage comparisons between the hybridisation signals on DL, a normal female and a normal male. The DNA from these three individuals appeared to be of similar intensity on ethidium bromide staining of the gels prior to blotting (not shown). With some of the probes the signal in the DL track is slightly fainter than in the normal female but it is still considerably stronger than the normal male. It was therefore concluded that in these cases the probes were not deleted from DL but that the difference in intensity was caused by small discrepencies in the amount of DNA transferred to the filter.

The hybrid WHTK is cytogenetically interpreted as containing an X chromosome deleted for Xq24-qter. However it was found that while GMGXY10 was deleted as would be predicted the GMGXY4 -like probes were also deleted. The X bands of the GMGXY19-like probes are present in this hybrid. In the light of the results obtained with the other deleted X chromosomes it would seem most likely that the X chromosome of WHTK does not have a simple terminal deletion but has undergone a more complex rearrangement.

Thus based on their mapping positions three classes of XY homologous probe have been defined by the analysis of cytogenetically defined deleted X and Y chromosomes. The GMGXY4 -like group defines homology between Yp and Xq21.1-21.3, GMGXY10

between Yp, Yq and Xq27-qter and the GMGXY19-like probes between Yq and Xp22.3-pter. This mapping data is summarised in table 3.

3.3.4 Analysis of Males with X-Linked Ichthyosis (XLI)

As steroid sulphatase (*STS*) is known to map to Xp22.3, individuals with X-linked ichthyosis (*STS* deficiency) were screened with GMGXXY3 and GMGXY19 which also map into this region. This was done to test these individuals for deletions which could be used to generate a preliminary deletion map of this region.

In total 23 unrelated affected males were analysed with the two probes and 18 were found to be deleted for GMGXXY3. The same 18 together with one further individual not deleted for GMGXXY3 - IB - were found to be deleted for GMGXY19. The Y bands of either of these two probes were found in none of the affected males' mothers or daughters which were tested. Some of the males analysed are shown in figure 11 probed with GMGXXY3 and GMGXY19. The individuals JA, SL, RF, NJ, CK, HL, RM, YM and WR can all be seen to be deleted for both probes while IB is deleted for only GMGXY19. The mother of HL can be seen to lack the Y bands of both probes. Thus from this study it can be concluded that almost 80% of all XLI affected males carry a deletion at Xp22.3.

3.4 Deletion Mapping of the Y Chromosome

The Y sequences isolated presented an opportunity to create a deletion map of the Y chromosome through the analysis of individuals carrying deleted Y chromosomes. To construct such a map for Yp, XX males, XO males and XY females were analysed. Dicentric Yp carrying individuals were used to map Yq. This map could be used to extrapolate both a rough molecular and a genetic map of the Y chromosome.

Most XX males are believed to carry a region of Yp on one of their X chromosomes. Thus assuming this to have arisen by a single XY-interchange event, as opposed to a more complex rearrangement, this portion of Yp should consistently contain tdf and the portions of Yp distal to the testes determining locus. However the Yp breakpoint and therefore the amount of Yp proximal to tdf can vary. A consistent ordering of probes on Yp and their relative mapping position to TDF should therefore be possible by analysing

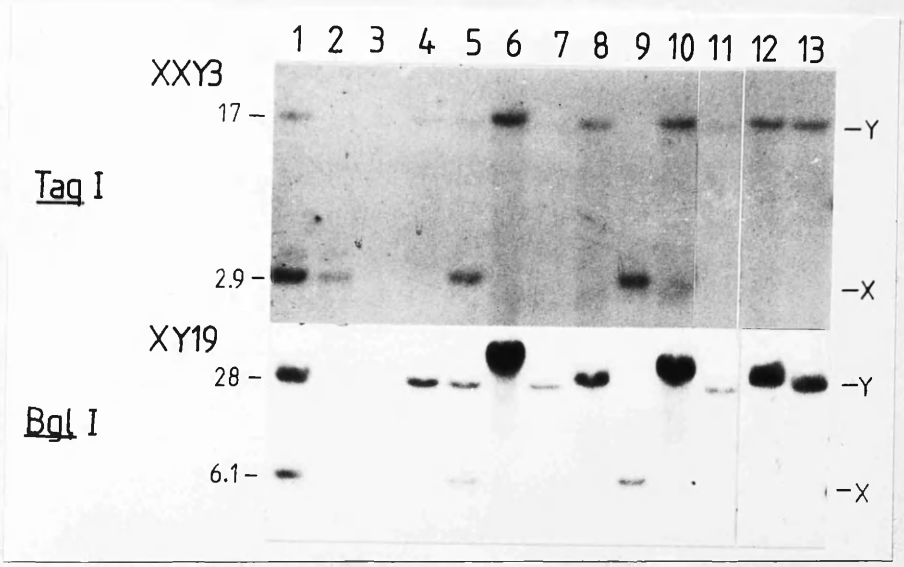


Figure 11: Southern analysis of the STS deficient males and some family members with GMGXXY3 and GMGXY19. Lanes 1: normal male, lanes 2-8 and 10-13 STS deficient males; 2: IB, 3: JA, 4: SL, 5: GB, 6: RF, 7: NJ, 8: CK, 9: mother of CK, 10: HL, 11: RM, 12: YM and 13: WR.

Origin of DNA	GMGXY1	GMGXXY3 GMGXY19
HY105E	+	+
817/175	-	+
Amir2N	-	-
HY94BT1	-	-
HY21	.	-
445/393	-	-
JH	-*	-

Table 4: The results of the analysis of the hybrids and individuals carrying a t(X:Y)(p22.3;q11.2) translocation chromosome with probes mapping to Xp22.3. The * indicates that the result was obtained through an estimation of the dosage of the X band and not by its presence or absence.

such individuals. Similarly the dicYp individuals having Yq breakpoints will have variable regions of Yq which, with the same assumptions as for the XX males, should allow an ordering of the probes on Yq.

While the author assisted in this mapping most of the Yp mapping was done by N. A. Affara and D. Jamieson and most of the Yq mapping by K. Kwok. Besides the probes isolated in this study other probes were also used. Most of these were isolated in this department by K. Kwok or L. Florentin but 47z, 115i, 13d, 118 and 50f2 were obtained from Dr. J. Weissenbach at the Pasteur Institute.

3.4.1 XX Males, XY Females and XO Males

The probes were hybridised to Southern blots of genomic DNA from 23 XX males, 8 XY females and two XO males digested with the appropriate enzymes. Most of the individuals used are shown in figure 12 hybridised with GMGXY10 and a subset of these individuals are shown hybridised to the multiloci probe GMGY46 in figure 13. The Yp bands were scored as positive if present and the results for all the probes are tabulated in table 5. This table was converted into a diagram of Yp with the probes in consensus order along its length (figure 14). Eighteen of the XX males and both XO males were found to be positive for at least one Yp specific restriction fragment but only one XY female was shown to be deleted for Yp sequences. The only Yp restriction fragments not to be present in any XX male are GMGY4, GMGY10A and GMGY10C. Only three XX males - KS, HM and DR - and the XO male RW do not fit into this consensus order while the other 15 Yp+ XX males and the Yp deleted XY female generated a consistent map of Yp. These results were confirmed by repeat testing. The non-consensus individuals were fitted into the map by assuming that a paracentric inversion, as shown in figure 14, was present on the paternal Y chromosome prior to the occurrence of the X-Y interchange. Alternatively there may have been a more complex rearrangement involved in the X to Y transfer.

A number of the probes have not been hybridised to the full panel used to map Yp but can nevertheless be mapped into regions of Yp. The probes GMGY42, GMGY43, GMGY44 and GMGY45 were only hybridised to GA, WB, JM, HM, RT and AP (see figure 4B)

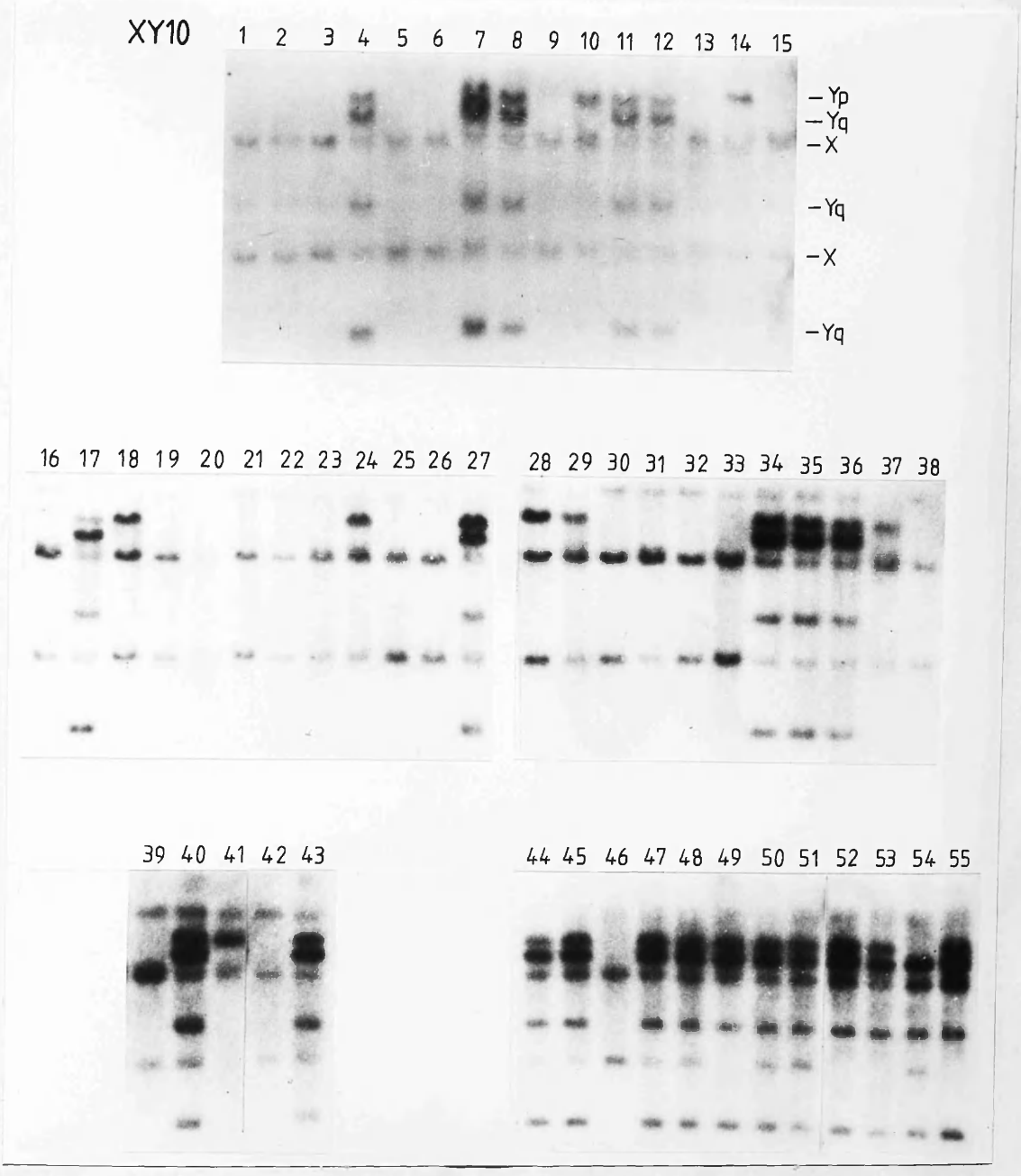


Figure 12: Southern analysis with GMGXY10 of EcoRI digested DNA from the XX males, XY females and XO males used in the Yp deletion mapping. Lanes (XX males) 1: PP, 2: mother of PP, 3: RV, 4: father of RV, 5: mother of RV, 6: HM, 7: father of HM, 8: paternal uncle of HM, 9: sister of HM, 10: JM, 11: father of JM, 12: brother of JM, 13: sister of JM, 14: RH, 15: sister of RH, 16: AP, 17: father of AP, 18: TA, 19: KS, 20,21: sisters of KS, 22: RT, 23: MM, 24: JT, 25: AN, 26: sister of AN, 27: father of AN, 28: GA, 29: WB, 30: OP, 31: TK, 32: GC, 33: DC, 34: father of OP, 35: father of GC, 36: father of DC, 37: AG, 38: NE, (XO males) 39: mother of JG, 40: father of JG, 41: JG, 42: RW, 43: father of RW, (XY females) 44: DM, 45: father of DM, 46: mother of DM, 47: JR, 48: MW, 49: SB, 50: JN (blood), 51: JN (lymphoblastoid), 52: TG, 53: SM, 54: AM, 55: normal male.

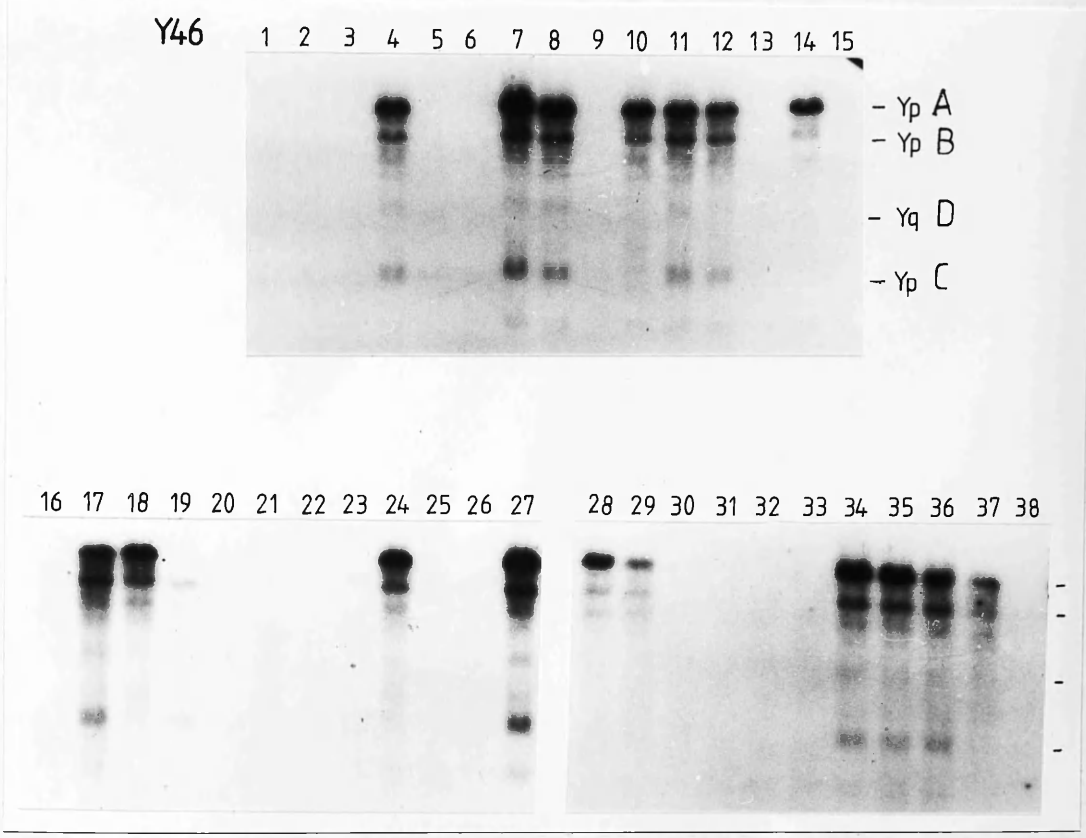


Figure 13: Southern analysis of the XX males with GMGY46. Lanes 1-38 are as for figure 12.

Probe	KS	WB GA JT AG TA JM RH	DR	NI	NE	OP	AP	RS	MM	TK	GC	HM	DC PP RT AN MS
GMGY3	+	+	+	+	+	+	+	+	+	+	-	-	-
47z	+	+	+	+	+	+	+	+	-	-	-	-	-
GMGXY7	+	+	+	+	+	+	+	-	-	-	-	-	-
GMGXY6	+	+	+	+	+	+	+	-	-	-	-	-	-
115i	+	+	+	+	+	+	+	-	-	-	-	-	-
GMGXY4	+	+	+	+	+	+	+	-	-	-	-	-	-
13d	+	+	+	+	+	+	-	-	-	-	-	-	-
GMGXY9	+	+	+	+	+	-	-	-	-	-	-	-	-
GMGXY5	+	+	+	-	-	-	-	-	-	-	-	-	-
GMGXY2	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY10 E"	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY10 E	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY7 A,C,D	-	+	+	-	-	-	-	-	-	-	-	-	-
50f2 A,B	-	+	+	-	-	-	-	-	-	-	-	-	-
118 D,E	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY46 A	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY22	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGXY10	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY41	-	+	+	-	-	-	-	-	-	-	-	-	-
GMGY10 B	-	+	+	-	-	-	-	-	-	-	-	-	-
118 C	-	+	+	-	-	-	-	-	-	-	-	-	-
118 A,B	-	+	-	-	-	-	-	-	-	-	-	-	-
GMGY23	+	+	+	-	-	-	-	-	-	-	-	-	-
GMGY46 B	+	+	+	-	-	-	-	-	-	-	-	-	-
GMGY7 B	+	-	-	-	-	-	-	-	-	-	-	-	-
GMGY7 D'	+	?	?	-	-	-	-	-	-	-	-	-	-
GMGY10 E'	+	?	?	-	-	-	-	-	-	-	-	-	-
GMGY46 C	+	-	-	-	-	-	-	-	-	-	-	-	-
GMGXY8	+	-	-	-	-	-	-	-	-	-	-	-	-
pDP34	+	-	-	-	-	-	-	-	-	-	-	-	-
p2f2	+	-	-	-	-	-	-	-	-	-	-	-	-
50f2 D	-	-	-	-	-	-	-	-	-	-	-	-	-
GMGY10 A,C	-	-	-	-	-	-	-	-	-	-	-	-	-
GMGY4	-	-	-	-	-	-	-	-	-	-	-	-	-
Centromere													
GMGY1	-	-	-	-	-	-	-	-	-	-	-	-	-
50f2 C,E	-	-	-	-	-	-	-	-	-	-	-	-	-
pY3.4	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5: The results of the Southern analysis of the XX males, XO males and XY females with the probes detecting Yp specific restriction fragments. The presence or absence of a fragment is indicated by a "+" or a "-" respectively and a "?" indicates that the presence or absence was unable to be determined. All the individuals are XX males unless otherwise indicated. The XO male JG is shown in table 6 as he has a Yq breakpoint and carries a complete Yp.

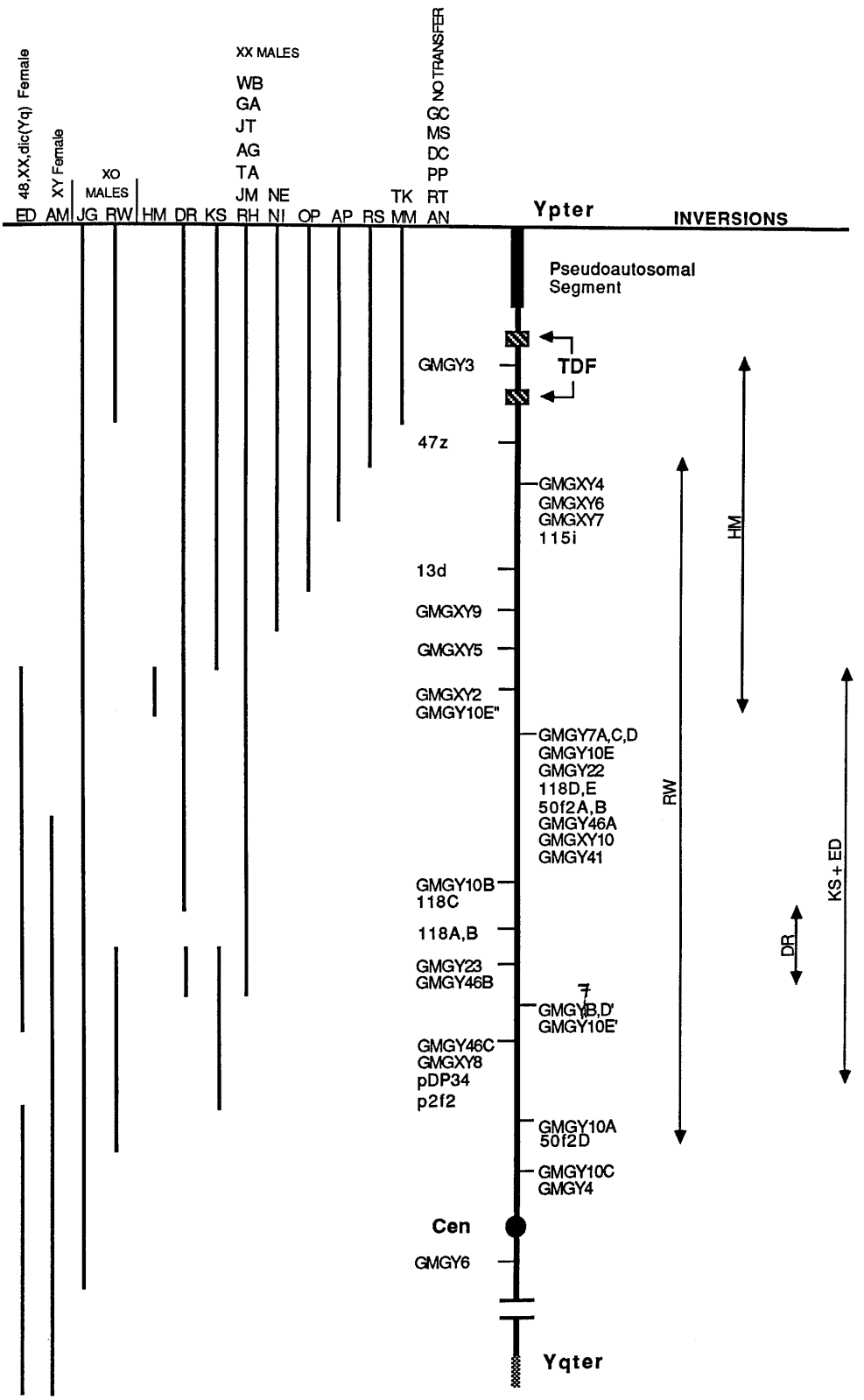


Figure 14: A diagramatic representation of the relative mapping positions of the Yp probes based on the results of the deletion mapping with the XX males, XY females and XO males. The region of the Y chromosome carried by each individual is indicated by a vertical line. The inversions postulated to explain the non-consensus individuals are also shown.

which allows them to be assigned between the breakpoints of AP and GA. The probes GMGY21, GMGY26 and GMGY40 have been across most of the Yp deletion mapping panel but not DR, RW or AM which allows their assignment to between the proximal HM breakpoint and the KS breakpoint which lies between 118A and GMGY23.

This deletion map divides Yp into15 non-pseudoautosomal segments and reveals that the probes define distinct domains based on the nature of the homologies detected. GMGY3 is present in most XX males and is therefore concluded to define the region in which tdf lies. Moving towards the centromere there is a block of restriction fragments with homologues on Xq13-24. Proximal to this lies Y specific clustered low-level repeats - as defined by GMGY7, GMGY10 and GMGY22 - as well as the Yp specific restriction fragments of probes detecting multiple Y loci - GMGY21, GMGY26, GMGY40, GMGY41, GMGY46, 118 and 50f2. GMGY46 can be seen to define three regions within this block as do 118 and GMGY10. GMGY7 defines two loci and the others only one. GMGY46C is not definitely contiguous with this block as it has not been split from GMGXY8, p2f2 and pDP34 which define a further but more proximal Yp/Xq13-24 homologous block. Finally closest to the centromere there are restriction fragments detected by probes GMGY10, 50f2 and the alphoid repeat defining sequence GMGY4.

3.4.2 Dicentric Yp Individuals

The Yq homologous probes were hybridised to Southern blots of genomic DNA from 15 individuals with a dic(Yp) chromosome and a 46,Y,t(X;Y)(p22.3;q11.2) male. Cytogenetically only two different breakpoints can be defined in these individuals distinguished by the presence or the absence of band Yq11.22. Some of these individuals are shown hybridised with GMGXY3, GMGXY19 and GMGY20 in figure 15. The full results are tabulated in table 6. As for the XX males a consensus ordering of restriction fragments defined by the position of the Yq breakpoints in these individuals was worked out and this is diagrammed in figure 16.

There is only one dicentric Yp individual, GC, who does not fit in with the consensus ordering of sequences on Yq. This individual carries GMGY1 and pY3.4b, which map distal, without any of the more proximal mapping sequences. If the 46,

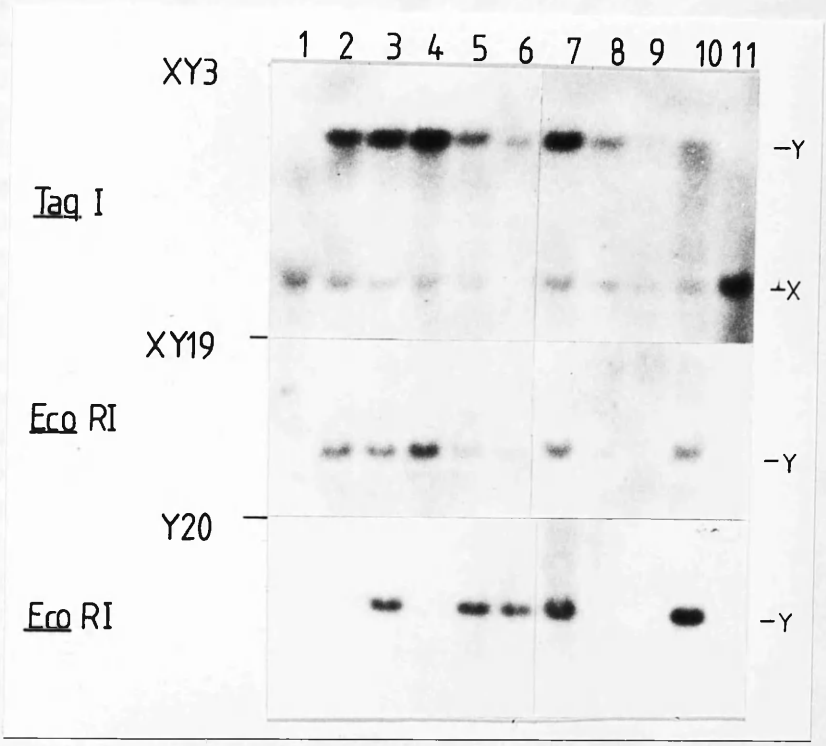


Figure 15: Southern analysis of members of the Yq deletion mapping panel with GMGXY3, GMGXY19 and GMGY20. Lanes 1: WC, 2: FF, 3: GC, 4: IT, 5: FW, 6: DM, 7: AD, 8: Amir2N, 9: HM, 10: KM, 11: PZ, 12: normal male, 13: normal female. All individuals are dicentric Yp males or females with the exception of WC an iso Yp male, AD a 46,XY heterochromatin -ve male and Amir2N.

Probes	WC	PZ	CO	FF	CC	RS KM IT	JC	DM	MN	HM FW	JH JL	GC	AM	JG
GMGXY3	-	+	+	+	+	+	+	+	+	+	+	-	-	·
GMGY6	-	-	+	+	+	+	+	+	+	+	+	-	-	+
GMGY8	-	-	+	+	+	+	+	+	+	+	+	-	-	·
GMGY17	-	-	+	+	+	+	+	+	+	+	+	-	-	·
GMGY35	-	-	+	+	+	+	+	+	+	+	+	-	-	·
GMGY9	-	-	+	+	+	+	+	+	+	+	+	-	+	·
GMGXY19	-	-	+	+	+	+	+	+	+	+	+	-	+	-
GMGY30	-	-	+	+	+	+	+	+	+	+	+	-	+	·
GMGY34	-	-	+	+	+	+	+	+	+	+	+	-	+	·
GMGY37	-	-	-	+	+	+	+	+	+	+	+	-	+	·
GMGY13	-	-	-	+	+	+	+	+	+	+	+	-	+	·
GMGY29	-	-	-	-	+	+	+	+	+	+	+	-	+	·
GMGY15	-	-	-	-	+	+	+	+	+	+	+	-	+	·
GMGY16	-	-	-	-	-	+	+	+	+	+	+	-	+	·
GMGY38	-	-	-	-	-	-	+	+	+	+	+	-	+	·
GMGY33	-	-	-	-	-	-	+	+	+	+	+	-	+	·
GMGY40 B,C	-	-	-	-	-	-	-	+	+	+	+	-	+	·
GMGY12	-	-	-	-	-	-	-	+	+	+	+	-	+	·
GMGY36	-	-	-	-	-	-	-	+	+	+	+	-	+	·
GMGY39	-	-	-	-	-	-	-	-	+	+	+	-	+	·
GMGY14	-	-	-	-	-	-	-	-	+	+	+	-	+	·
GMGY20	-	-	-	-	-	-	-	-	+	+	+	-	+	·
GMGY26	-	-	-	-	-	-	-	-	+	+	+	-	+	·
GMGY31	-	-	-	-	-	-	-	-	+	+	+	-	+	·
GMGXY10 B,D,F	-	-	-	-	-	-	-	-	-	+	+	-	+	-
GMGY18	-	-	-	-	-	-	-	-	-	+	+	-	+	·
GMGY21	-	-	-	-	-	-	-	-	-	+	+	-	+	·
GMGY5	-	-	-	-	-	-	-	-	-	-	+	-	+	·
GMGY1	-	-	-	-	-	-	-	-	-	-	+	+	+	-
pY3.4 B	-	-	-	-	-	-	-	-	-	-	-	+	+	-
GMGY2	-	-	-	-	-	-	-	-	-	-	-	-	+	·
pY3.4 A,C,D	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Table 6: The results of the Southern analysis of the Yq deletion mapping individuals. The presence or absence of a fragment is indicated by a "+" or a "-" respectively and a "·" means not tested. All the individuals are dicentric Yp males or females except WC an isoYp male, AM (Amir2N) and JG an XO male with a Y:5 translocation.

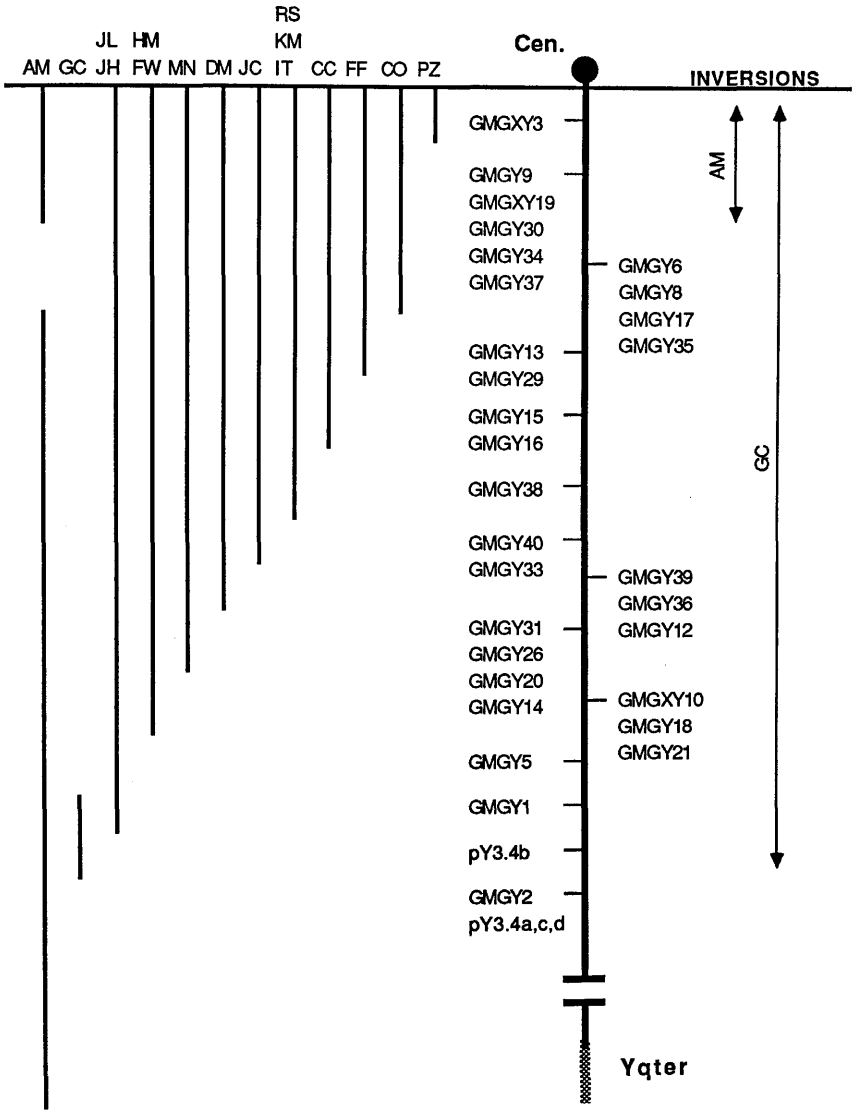


Figure 16: A diagramatic representation of the relative mapping positions of the Yq probes based on the deletion mapping with the dic(Yp) individuals and the hybrid cell line Amir2N. The region of the Y chromosome carried by each individual is indicated by a vertical line. The inversions postulated to explain the non-consensus individuals are also shown.

Yt(X;Y)(22.3;11.2) male AM is taken into account then a further anomaly is created between AM and PZ. If AM is anomalous then the block containing GMGY6, GMGY8, GMGY17 and GMGY35 is interchangeable with the block containing GMGY9, GMGXY19, GMGY30 and GMGY34. However if PZ is anomalous the order would need to be centromere - (GMGY6, GMGY8, GMGY17, GMGY35) - GMGY3 - (GMGY9, GMGXY19, GMGY30, GMGY34).

The deletion mapping of Yq has created a consistent 14 interval map of Yq. Unlike Yp there are no clear molecular domains.

Taken together and including the pseudoautosomal region these deletion maps divide the Y chromosome into 30 regions. Out of the 37 individuals scored to create these maps only six did not fit into the consensus order of the probes along the Y chromosome. TDF has been mapped to the distal Yp between the pseudoautosomal boundary and the Yp/Xq13-24 homologous probe 47z in a region defined by GMGY3.

3.5 Northern Analysis of Testis and Placental RNA

As a step towards identifying Y expressed testis transcripts the sequences isolated in this study were used in the Northern analysis of total RNA from testis.

Thirty of the probes were hybridised to Northern blots of testis and male placental RNA. The probes used were GMGY5, Y6, Y8, Y9, Y16, Y17, Y18, Y20, Y21, Y24, Y25, Y26, Y28, Y31, Y32, Y33, Y40, Y41, XY3, XY4, XY5, XY6, XY7, XY8, XY9, XY10, XY12, XY19 and p2f2. Part of the STS cDNA - p2A4 - was also used to check the placental RNA and its bands of hybridisation were used as molecular weight standards. Figure 17 shows the pattern of hybridisation of GMGY28. This probe hybridises to a 2.3kb band in the testes track but not in the placenta track. The figure also shows the pattern of hybridisation obtained with p2A4 which hybridises only to the placenta RNA and GMGY18 which shows the typical pattern of hybridisation obtained with the other probes used. In all cases except p2A4 the probes hybridised stronger to the testes RNA than to the placenta RNA. Some of the probes which on Southern analysis can be seen to

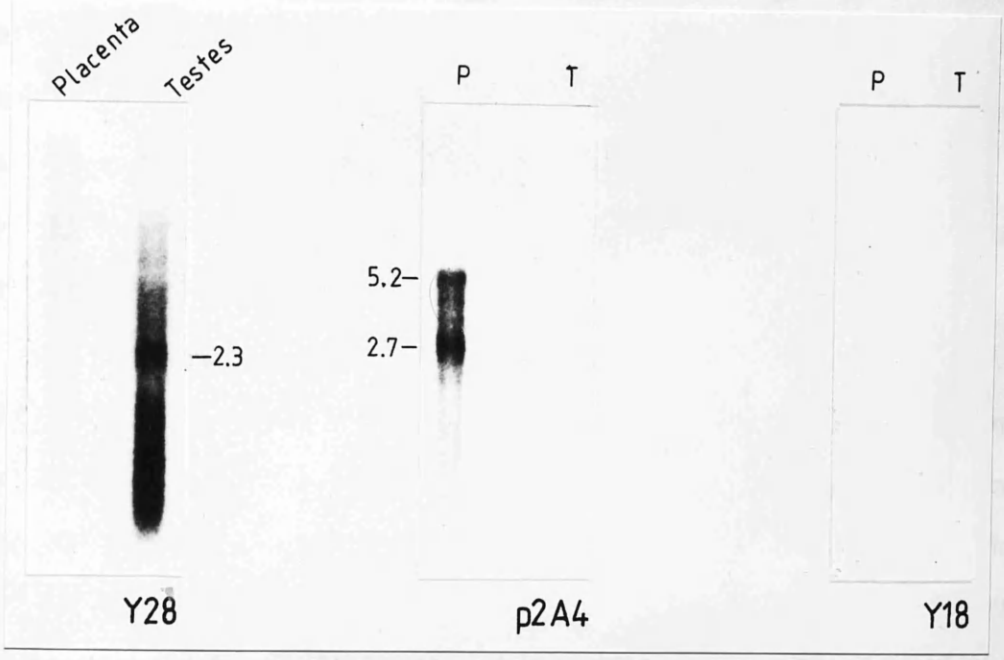


Figure 17: Northern analysis of testis and placenta total RNA. The hybridisation patterns of GMGY28, p2A4 (a partial STS cDNA clone) and GMGY18 are shown. GMGY18 represents the pattern achieved with most of the probes used.

detect repeats - GMGXY9, GMGY5, GMGY8 and GMGY17 - lit up both tracks strongly and were probably detecting transcribed genomic repeats.

3.6 Restriction Enzyme Analysis of Normal Human DNA with the XY Homologous Probes

Inorder to further characterise, at the DNA level, the regions of XY homology defined in this study a multiple restiction enzyme analysis of normal human genomic DNA was carried out. The measurement of restriction site variation between the X and Y chromosomes would allow an estimation of the level of homology between the two chromosomes. This will allow the approximate dating of the events which gave rise to the homologies. The detection of restriction fragment length polymorphisms would allow an estimation of the polymorphic frequencies on the X and the Y chromosome in these regions.

The XY homologous probes GMGXY4 , GMGXY5, GMGXY6, GMGXY7, GMGXY9 and GMGXY12 were hybridised to panels of DNA from seven normal males and eight normal females of Northern European origin digested with 24 different restriction enzymes. The results of this analysis are tabulated in table 7 where restriction site variations between the X and Y chromosomes (XY differences) are shown together with the X polymorphisms detected. GMGXY10 and GMGXY19 were hybridised to 14 of these panels. In most cases all individuals tested were able to be scored but in some cases through partial digestion or poor hybridisation this was not possible.

A further 44 individuals of various ethnic origins were digested with *Taq*I and probed with GMGXY4 , GMGXY5, GMGXY6, GMGXY8, GMGXY9, GMGXY10 and GMGXY19. These panels consisted of 20 Bantu tribesmen, five other African males, four Chinese males, two male and two female Arabs and ten male and two female Indo-Asians.

3.6.1 XY Differences detected by the XY Homologous Probes

Each probe used demonstrated different X and Y specific bands with a number of enzymes. The Yp/Xq21 homologous probes demonstrated a differential pattern with 3-8 restriction enzymes, while GMGXY10 and GMGXY19 showed an XY difference with 13 out of the 14 enzymes used. This argues that the two regions of XY homology defined by

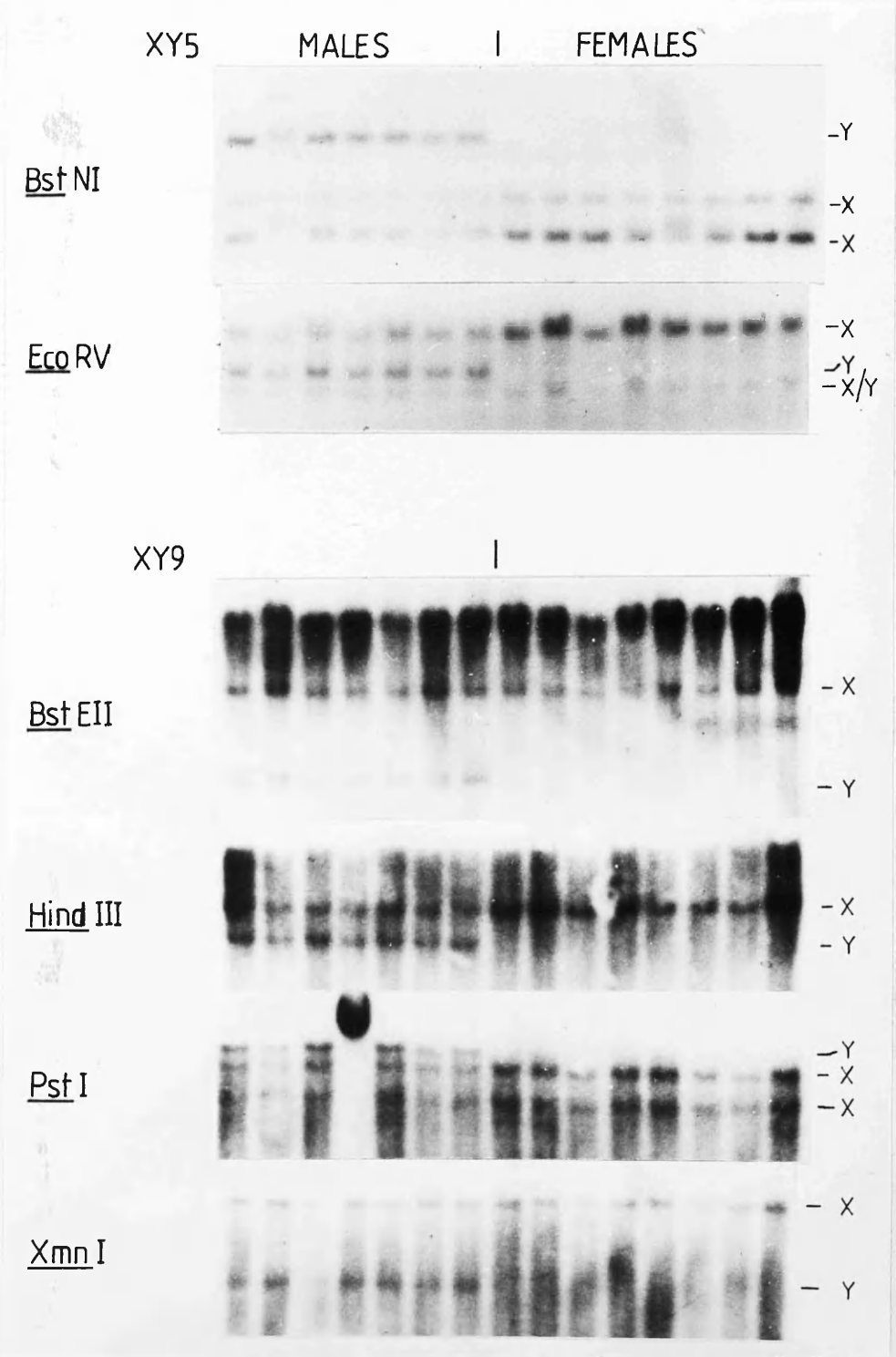


Figure 18: The XY differences of GMGXY5 and XY9. The panels consist of seven normal males and eight normal femles of Northern European origin.

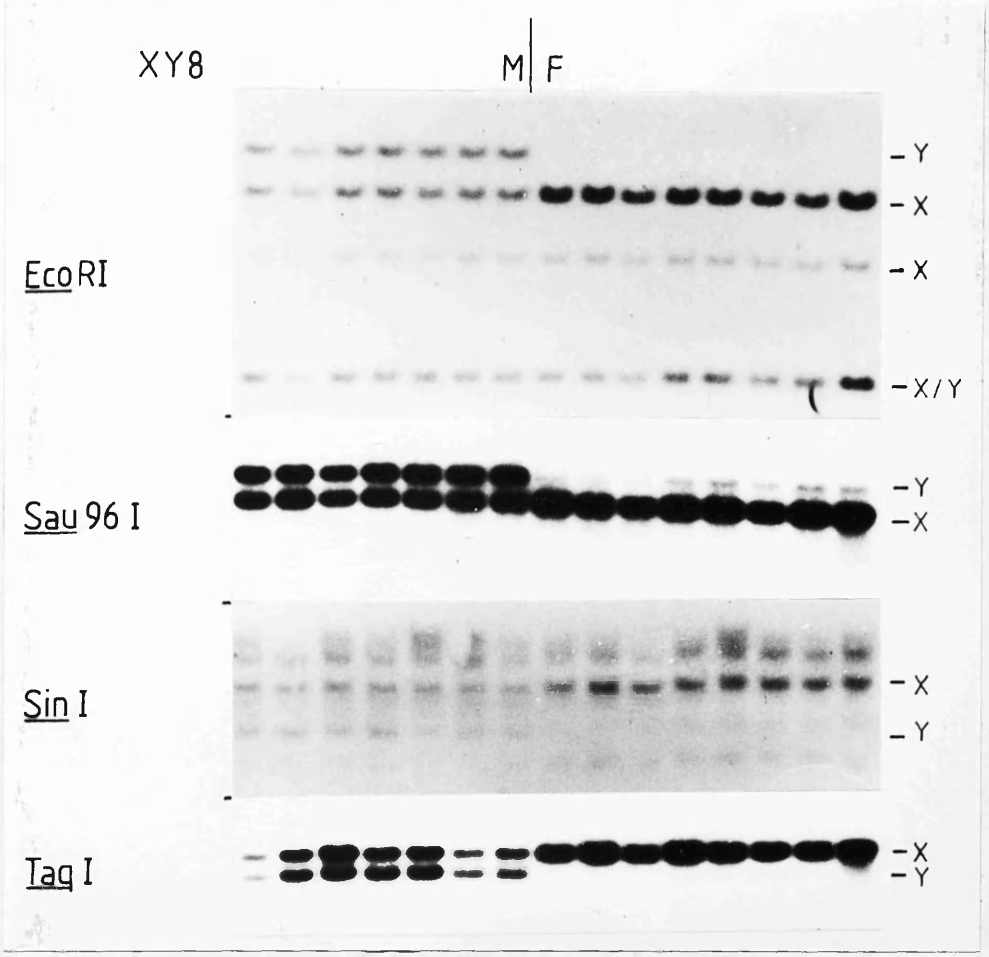


Figure 19: The XY differences of GMGXY8. The panels consist of seven normal males and eight normal femles of Northern European origin.

Probes / Enzyme	GMG-XY4	XY5	XY6	XY7	XY8	XY9	XY12
Alu I	-	-	-	-	-	-	Y-
Bcl I	Y-	-	Y-	-	-	-	-
Bgl I	Y-	-	-	-	-	-	-
Bgl II	-	-	-	Y-	-	-	-
Bst EII	-	-	-	-	-	Y-	-
Bst NI	Y-	Y-	-	-	-	-	-
Dde I	-	-	-	-	-	-	-
Dra I	Y+	-	-	-	-	-	-
Eco RI	-	-	-	-	Y-	-	-
Eco RV	-	Y-	-	-	-	-	-
Hae III	-	-	-	Y-	-	-	-
Hind III	-	-	-	-	-	Y-	Y-
Hinf I	-	+	-	-	-	-	-
Msp I	Y-	-	-	Y-	-	-	Y-
Nde II	-	+	-	-	-	-	-
Pst I	Y-	-	-	-	-	Y-	Y-
Pvu II	-	-	-	-	-	-	-
Rsa I	Y-	+	-	Y-	-	-	Y-
*Sau*96I	-	-	-	-	Y-	Y-	Y-
Sin I	-	-	-	+/-	Y-	-	-
Sst I	-	-	-	-	-	-	-
Stu I	-	-	-	Y-	-	-	-
Taq I	Y-	Y-	Y-	-	Y-	Y+	-
Xmn I	-	-	Y-	Y-	-	Y-	Y-

Table 7: The results of the Southern analysis of DNA from individuals of Northern European origin with the Yp/Xq21 homologous probes. The DNA was digested with 24 different restriction enzymes.

\+ :- polymorphism

\- :- no polymorphism

+/- :- low-level polymorphism

Y :- XY difference

GMGXY10 and GMGXY19 are less conserved than the Yp/Xq21 homologous region. The XY differences on the Northern European panels are shown in figure 18 for GMGXY5 and GMGXY9 and in figure 19 for GMGXY8.

3.6.2 Polymorphisms detected by the XY Homologous Probes

GMGXY4 , GMGXY5, GMGXY7, GMGXY9 and GMGXY10 revealed polymorphisms. These polymorphisms, described below, are summarised in table 8.

GMGXY4

The *Dra*I polymorphism detected by GMGXY4 is manifested in a heterozygous female and a male on the panel. As 20 X chromosomes can be scored from this panel the frequency of the less common allele can be estimated to be approximately 10%. On analysis of *Taq*I digested DNA from the non European individuals GMGXY4 revealed a rare X polymorphism in one Asian and one Bantu male. GMGXY4 also revealed the only Y polymorphism in the whole study in a single Asian male. The GMGXY4 polymorphisms can be seen in figure 20.

GMGXY5

Three frequent polymorphisms are detected by GMGXY5 with the enzymes *Hinf*I (GANTC), *Nde*II (GATC) and *Rsa*I (GTAC). These polymorphisms show complete linkage as can be seen in figure 21A with individuals of Northern European origin and in figure 22 with individuals of non European origins. It is worth noting the similarity of the enzyme sites involved although they are all mutually exclusive. The alleles A1, B1 and C2 always go together as do the alleles A2, B2 and C1. The less frequent allele has a frequency of 43% in the Northern European individuals tested. For *Hinf*I and *Nde*II the Y band can be assumed not to be polymorphic and to run with the less frequent alleles as male homozygotes for the most frequent allele are not observed. In the case of *Rsa*I the Y bands are not coincident with either allele.

GMGXY7

GMGXY7 detects a rare *Sin*I polymorphism which is seen in only one female on the panel shown in figure 21B (marked with a "P"). This implies that its frequency is less than 5%.

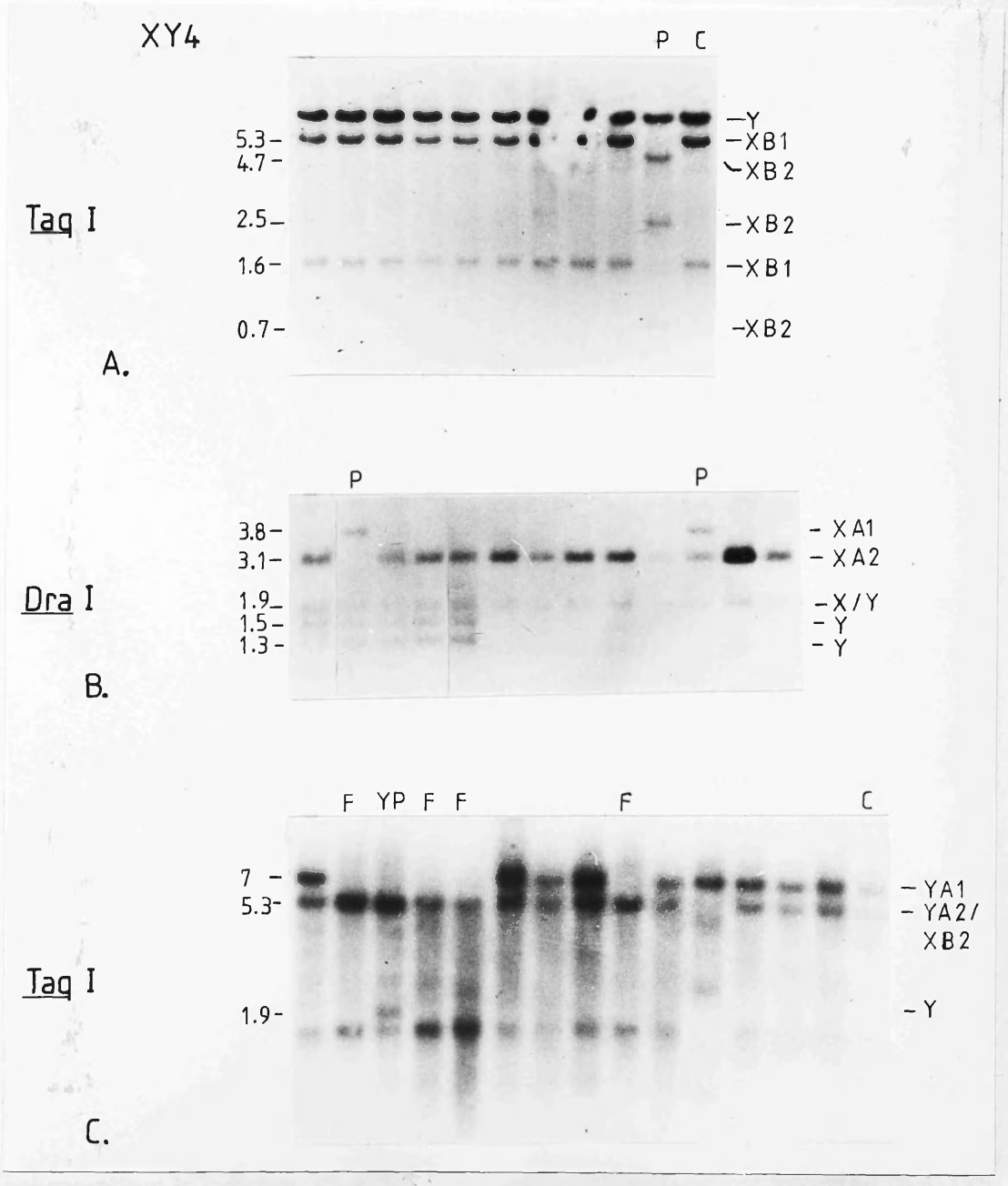


Figure 20: The X and Y polymorphisms of GMGXY4. Individuals showing an X polymorphism are marked with a "P". The male showing the Y polymorphism is marked with a "YP". Panels A:- 10 Bantu tribesmen, B:- 5 males and 8 females of Northern European origin, C:- 4 females and 10 males of Asian origin. F= female, C= control Northern European male.

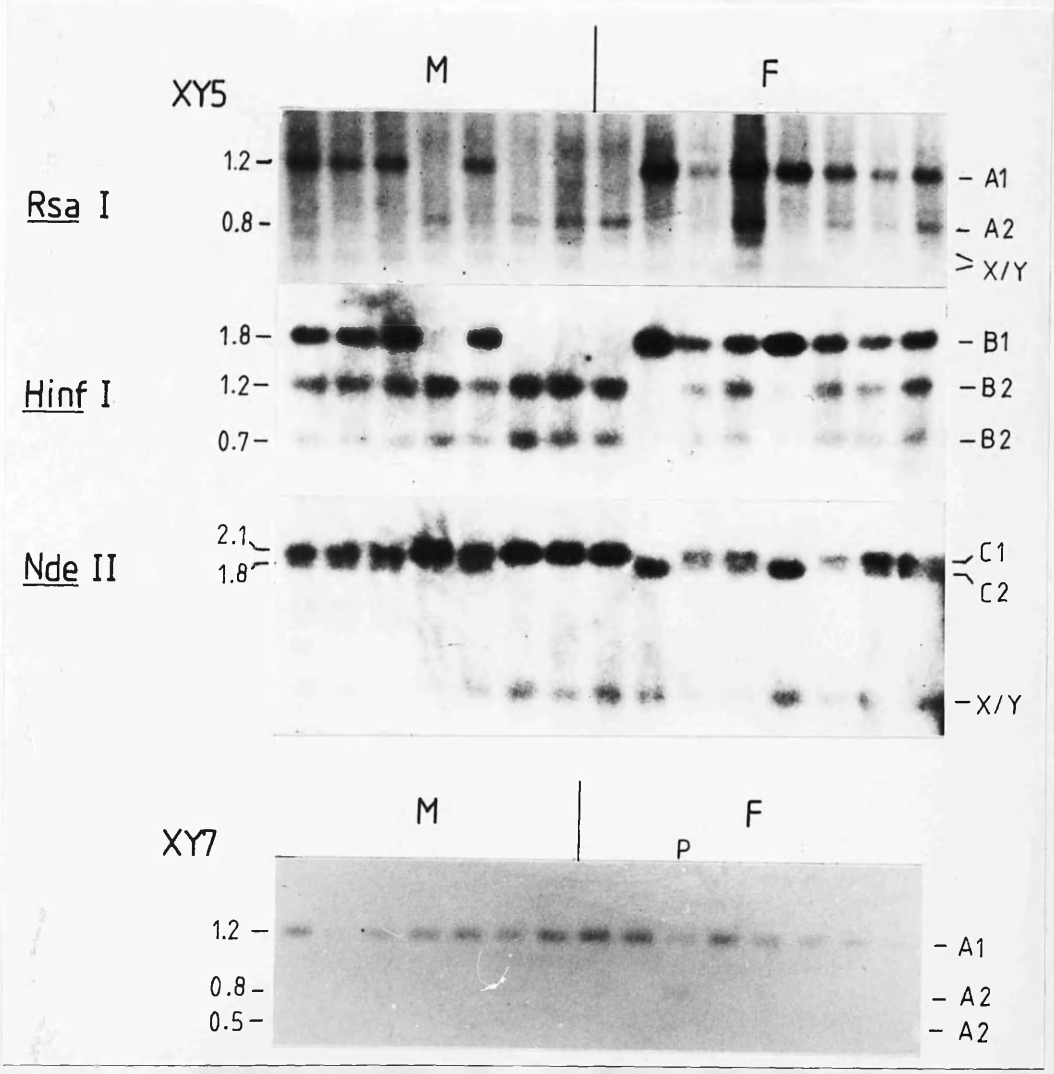


Figure 21: The polymorphisms of GMGXY5 and XY7. The panels all consist of the same 7 males and 8 females of Northern European origin. The individual showing the GMGXY7 polymorphism is indicated by a "P". The linkage disequilibrium of the three GMGXY5 polymorphisms can be seen.

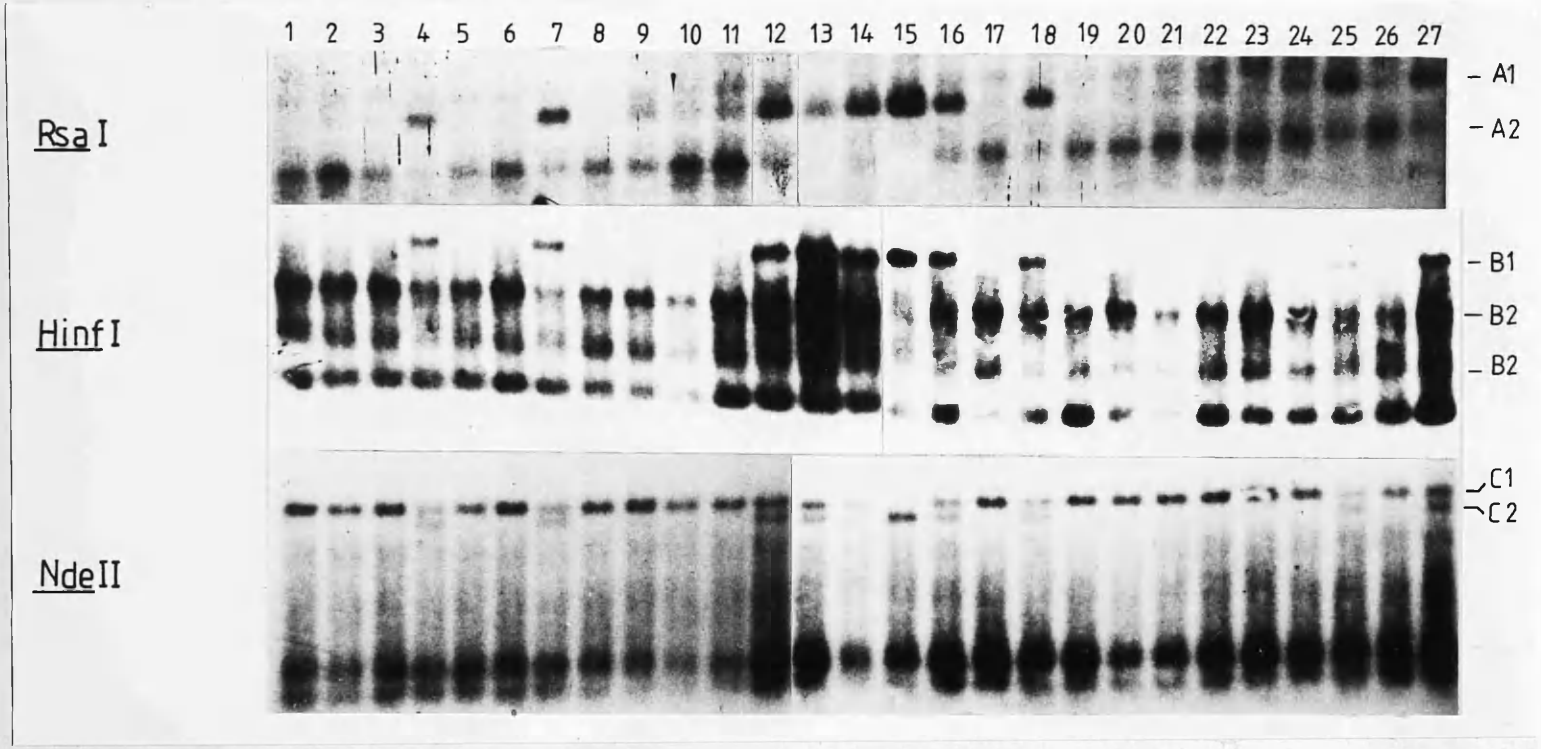


Figure 22: The linkage disequilibrium of GMGXY5 in individuals of non-European ethnic origin. Lanes 1, 3-6, 8-12: Asian males; 2, 7, 25, 26: Asian females; 13, 14: Arab males; 15, 16: Arab females; 17-19: chinese males; 20-24: African males; 27: Northern European female.

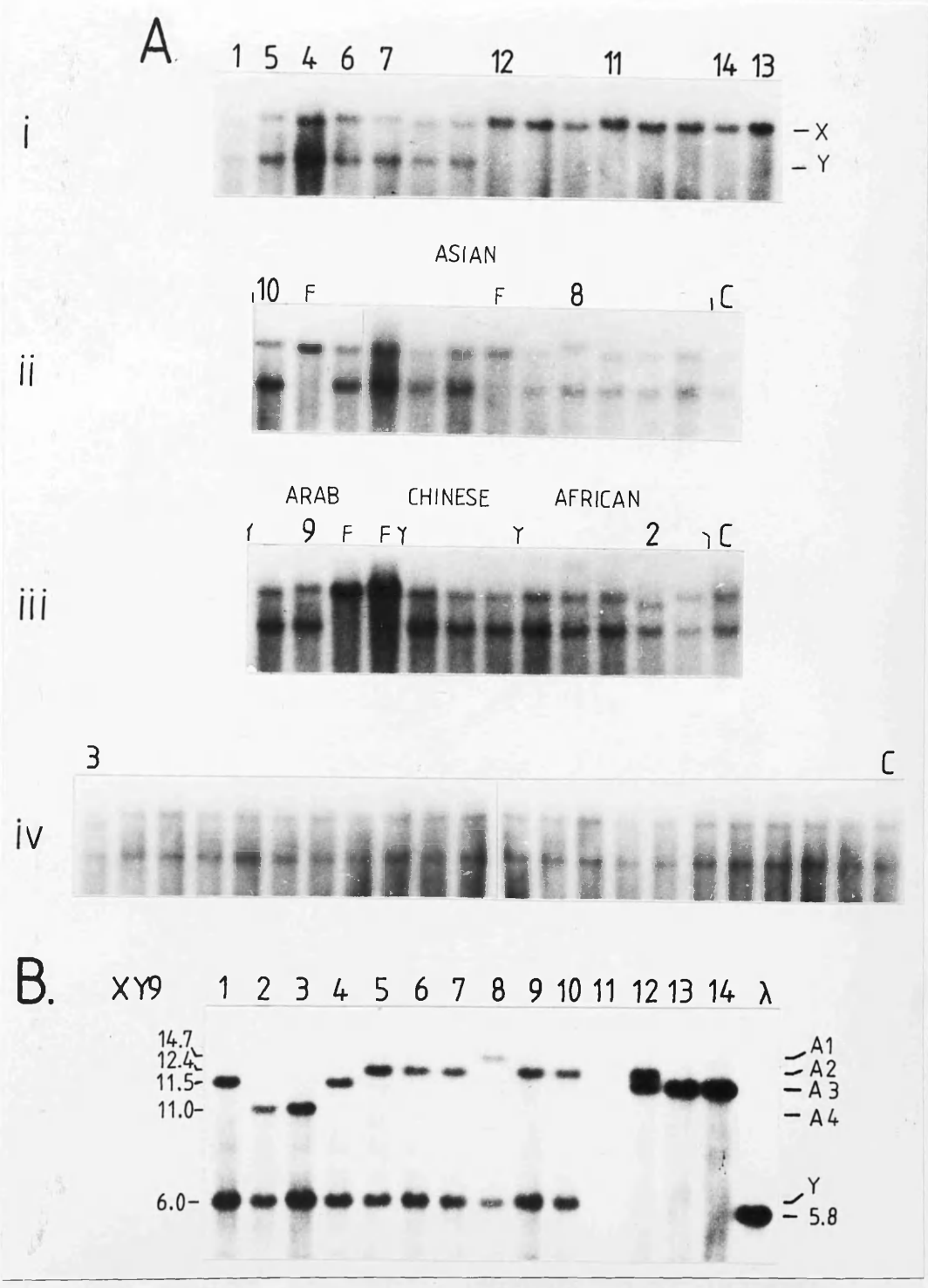


Figure 23: The multi-alleleic *Taq*I polymorphism of GMGXY9.
A:- Hybridisation of GMGXY9 to TaqI digested DNA of individuals from a variety of ethnic origins electrophoresed under standard conditions; i: Northern Europeans, ii: Asians, iii: 4 Arabs, 3 Chineses and 5 Africans, iv: 20 Bantu tribesmen. "C"=Northern European male. The numbers indicate those DNAs that were reprobed after prolonged electrophoresis.
B:- Southern analysis of TaqI digested DNA after prolonged electrophoresis. The numbers correspond to those of figure 23A. All DNAs which appeared to show a variant allele were selected along with some controls which did not. The final lane contains lambda gt11 cut with HindIII/EcoRI as a marker.

Probe	Enzyme	X or Y linkage	Alleles (kilobases)	Allelic frequency
GMGXY4	*Dra* I	X	A1: 3.8	0.1*
			A2: 3.1	0.9*
	Taq I	X	B1: 4.7/2.5/0.7	0.03
			B2: 5.3/1.6	0.97
	Taq I	Y	C1: 7	>0.98
			C2: 5.3/1.9	<0.02
GMGXY5	*Hin*f I	X	A1: 1.8	0.43*
			A2: 1.2/0.7	0.57*
	Nde II	X	B1: 2.1	0.57*
			B2: 1.8	0.43*
	Rsa I	X	C1: 1.2	0.43*
			C2: 0.8	0.57*
GMGXY7	*Sin* I	X	A1: 1.2	>0.95*
			A2: 0.8/0.5	<0.05*
GMGXY9	*Taq* I	X	A1: 14.7	<0.02
			A2: 12.4	0.1
			A3: 11.5	0.85
			A4: 11.0	0.03
GMGXY10	*Eco* RI	X	A1: 9.8/3.7	0.85§
			A2: 9.8	0.15§

Table 8: The restriction fragment length polymorphisms detected by the XY homologous probes. The * indicates that an alleleic frequency was calculated from the Northern European individuals tested. The § indicates that the frequencies were calculated from 30 males. All other frequencies were calculated from the 59 individuals of various ethnic origins used in this study.

GMGXY9

GMGXY9 reveals a moderately frequent polymorphism on *TaqI* digestion of DNA from the Northern European individuals as well as a distinct Y band. In the other ethnic groups used a further two alleles were revealed. Inorder to visualise this polymorphism properly prolonged electrophoresis was required as the alleles were similar in size and of high molecular weight. Figure 23A shows GMGXY9 hybridised to the complete panels of all the individuals tested after normal electrophoresis. Figure 23B shows all the variant alleles, detectable under normal run conditions, after prolonged electrophoresis. The individuals selected for the longer run are indicated on the complete panels in figure 23A and include all the individuals who could be seen to carry a less common allele as well as some of those with the common allele, which were included as controls. It is possible that prolonged electrophoresis of the complete panel may reveal other variants too close in size to manifest themselves under normal electrophoresis conditions. However a lack of time prevented this possibility being investigated. The frequency of the four alleles detected was calculated from all the individuals tested and these are tabulated in table 8. Allele A1 was found only in one Asian while allele A4 was found only in two of the Africans. No other alleles were found in the Africans.

GMGXY10

GMGXY10 detects an EcoRI polymorphism with a minor allele frequency of 0.15. The more frequent allele consists of 9.8kb and 3.7kb bands the less frequent of just the 9.8kb band. Because heterozygotes are difficult to detect for this polymorphism the allelic frequency was calculated from the Southern analysis of 30 males. The polymorphism is shown in figure 12 on the XY female blot where it can be seen that two of the XY females and the control male lack the 3.7kb X band.

3.7 Comparative Studies with the XY Homologous Probes

With the aim of elucidating the origins of the three regions of XY homology defined in this study and the relationships between the X and Y chromosomes during primate

evolution these probes were used in the Southern analysis of a number of primate species.

Initially all the XY homologous probes were hybridised to Southern blots of DNA from a human male control, a human male deleted for Xq13-21.3 (Tel26), a male and female gorilla, two male orang-outangs and a male chimpanzee. The probes were hybridised to *Taq*I digests with the exception of GMGXY7 and GMGXY12 for which *Xmn*I was used. The autoradiographs obtained are shown in figure 24. The bands marked "P" (plasmid) are most likely the result of exogenous DNA contamination in the tracks as the same bands were seen in the same tracks with both GMGXXY3 and another probe (data not shown).

3.7.1 Yp/Xq21 Homologous Probes

As previously documented Tel26 is deleted for the X homologue of all the Yp/Xq21 probes. In the two gorilla tracks no male specific pattern of hybridisation can be discerned with any of these probes. In all cases with the exception of GMGXY9 the hybridisation is stronger in the female track than in the male track. This is despite the latter appearing slightly stronger in the photograph of the ethidium bromide stained gel shown at the top of the second page of figure 24. This strongly suggests that in gorilla at least these probes have no Y homologue only an X homologue.

GMGXY9 is difficult to interpret. The female gorilla has two bands, the lower of which is also present in the male at greater intensity. The two bands in the female could be alleles of an X linked polymorphism which would in part explain the fainter hybridising lower band in the female but as this observation is also compatible with autosomal linkage it is not possible to say that GMGXY9 is definitely X linked in the gorilla. The probes GMGXY4 and GMGXY5 reveal a single band in both gorilla and chimpanzee which is the same size as the human Y band. With *Xmn*I GMGXY12 shows conservation of the human X band in gorilla and chimpanzee and GMGXY7 shows a conserved X band in all three primates tested. These are the only probes in this group to show conservation of band sizes amongst the primates.

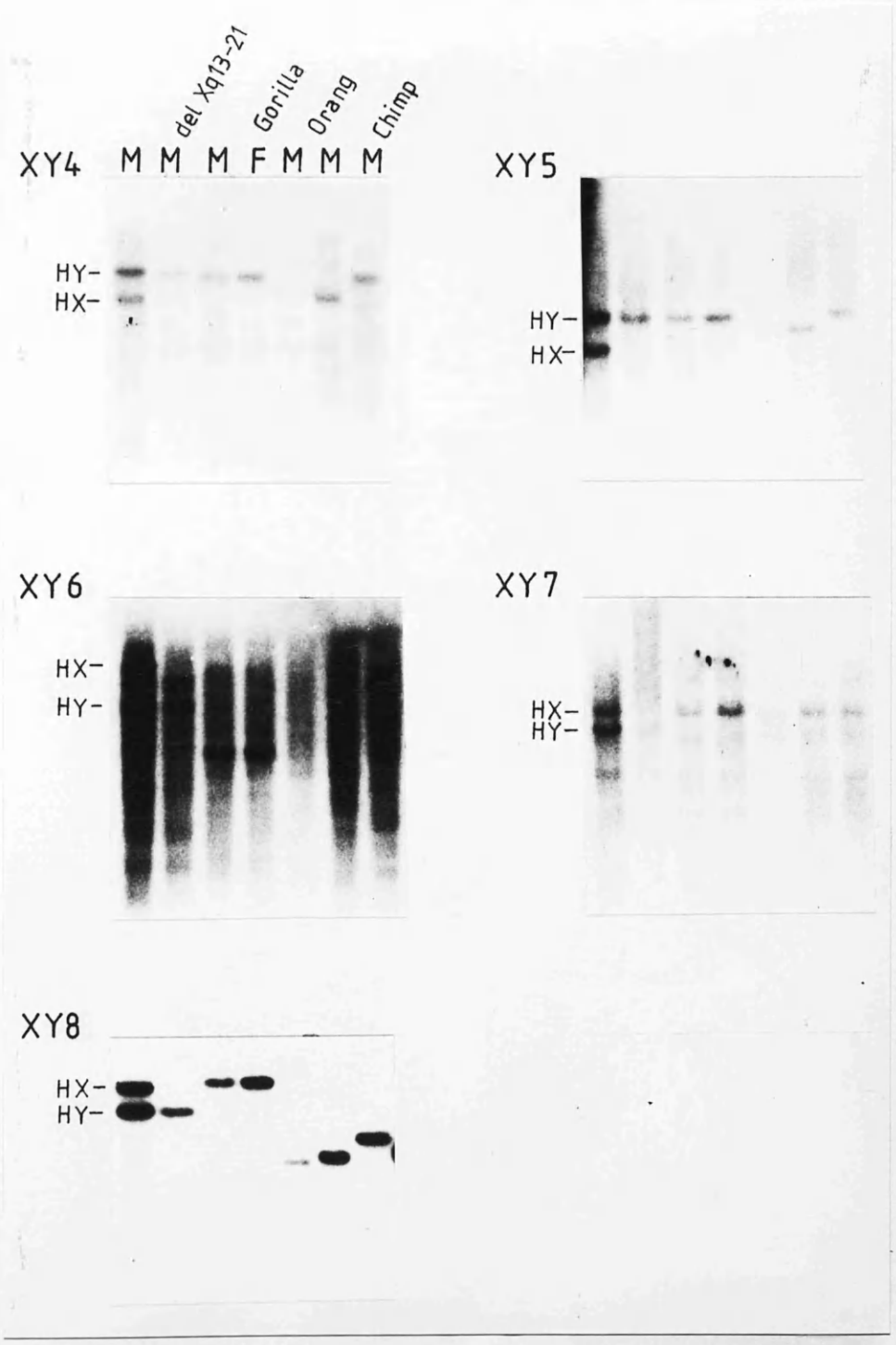
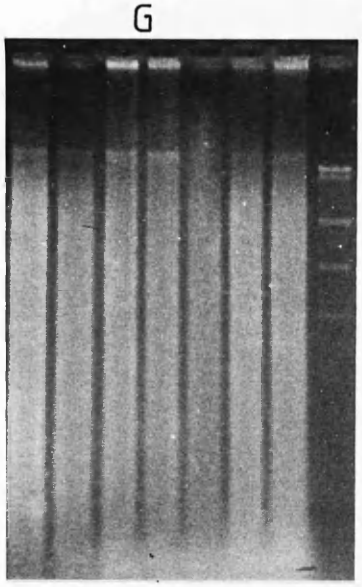
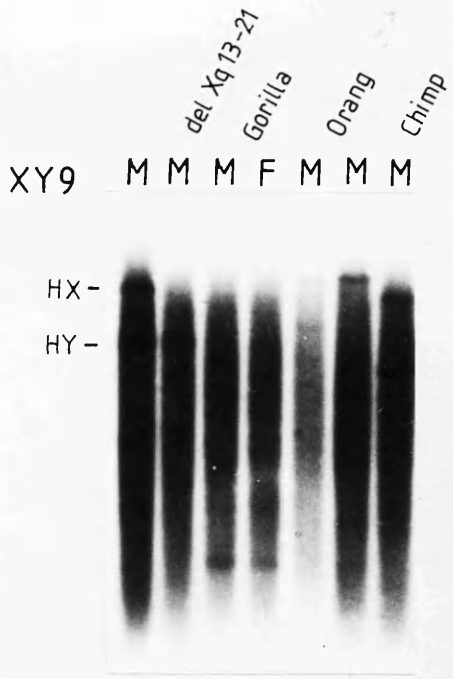
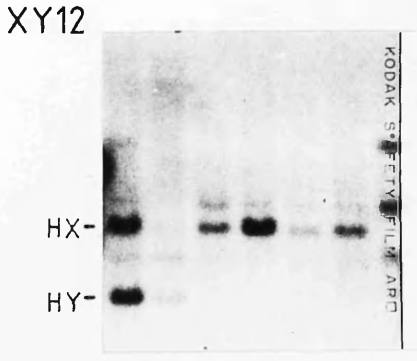
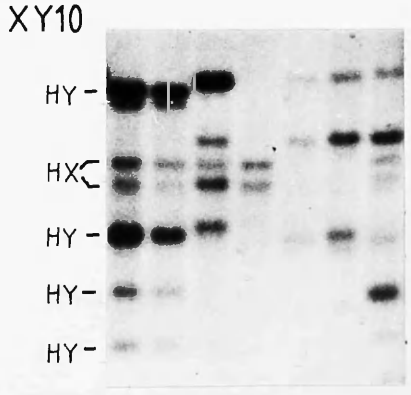


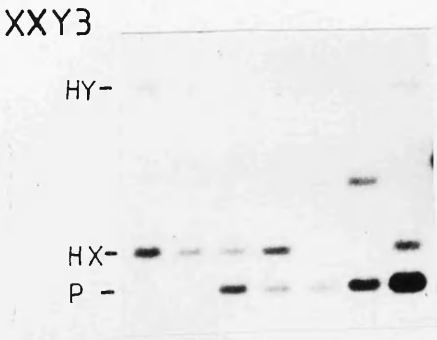
Figure 24: Southern analysis of primate DNA with the XY homologous probes. All blots have identical order of human male, human 46,Y del(Xq13-21) male, gorilla male and female, two male orang-outangs and a male chimpanzee. All the probes are shown hybridised to TaqI digested panels except GMGXY7 and GMGXY12 where XmnI was used. A typical panel prior to blotting is shown stained with ethidium bromide. The gorilla lanes are indicated by a "G". M=male; F=female; HX=human X bands; HY=human Y bands; P=probable plasmid contamination.











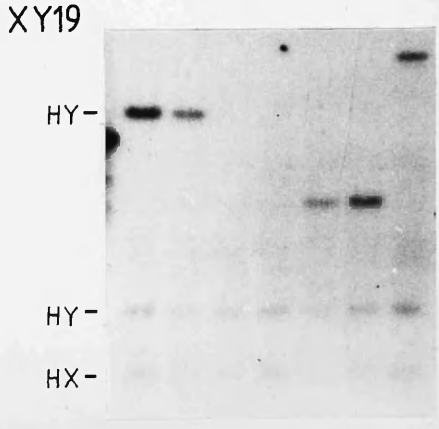


Figure 24 (cont'd)

3.7.2 GMGXY10

In addition to the panel detailed above GMGXY10 was hybridised to an extended panel of a male and female orang-outang, a male and female chimpanzee, a male and female African Green monkey, a male macaca and a human male and female. The results are shown in figures 24 and 25. It can be seen that gorilla, chimpanzee and orang-outang have multiple Y bands as in human and the upper two of these are conserved in the three ape species.

The gorilla and chimpanzee share in common two non Y bands which correspond in size to the human X bands. In the male gorilla the lower non Y band is more intense than in the female. This suggests that there is a Y specific band of the same size. However the increased intensity of the upper band in the female suggests that it at least is X linked. Similarly the chimpanzee female track, in figure 25, can be seen to be more intense than the male for both these bands which argues that they can be assigned to the chimpanzee X chromosome. In the female orang-outang two faint and two strong bands are detected. The upper strong band appears to be overlaid by a Y band in the male but through dosage the lower strong band can be mapped to the X chromosome. The other two weaker bands although slightly more intense in the female than the male do not permit a conclusion to be drawn about their location. The African Green monkey has no male specific bands and by dosage it can be concluded that the single band detected is X linked.

3.7.3 GMGXXY3 and GMGXY19

As for GMGXY10 the results are shown in figures 24 and 25. Both probes can be seen to have male specific bands in chimpanzee and orang-outang but not in gorilla or African Green monkey. Both probes appear, by dosage, to have X linked bands in all the species tested. The X bands of GMGXXY3 and GMGXY19 are conserved in size between human, gorilla and chimpanzee while the X bands of GMGXY19 are also conserved in orang-outang.

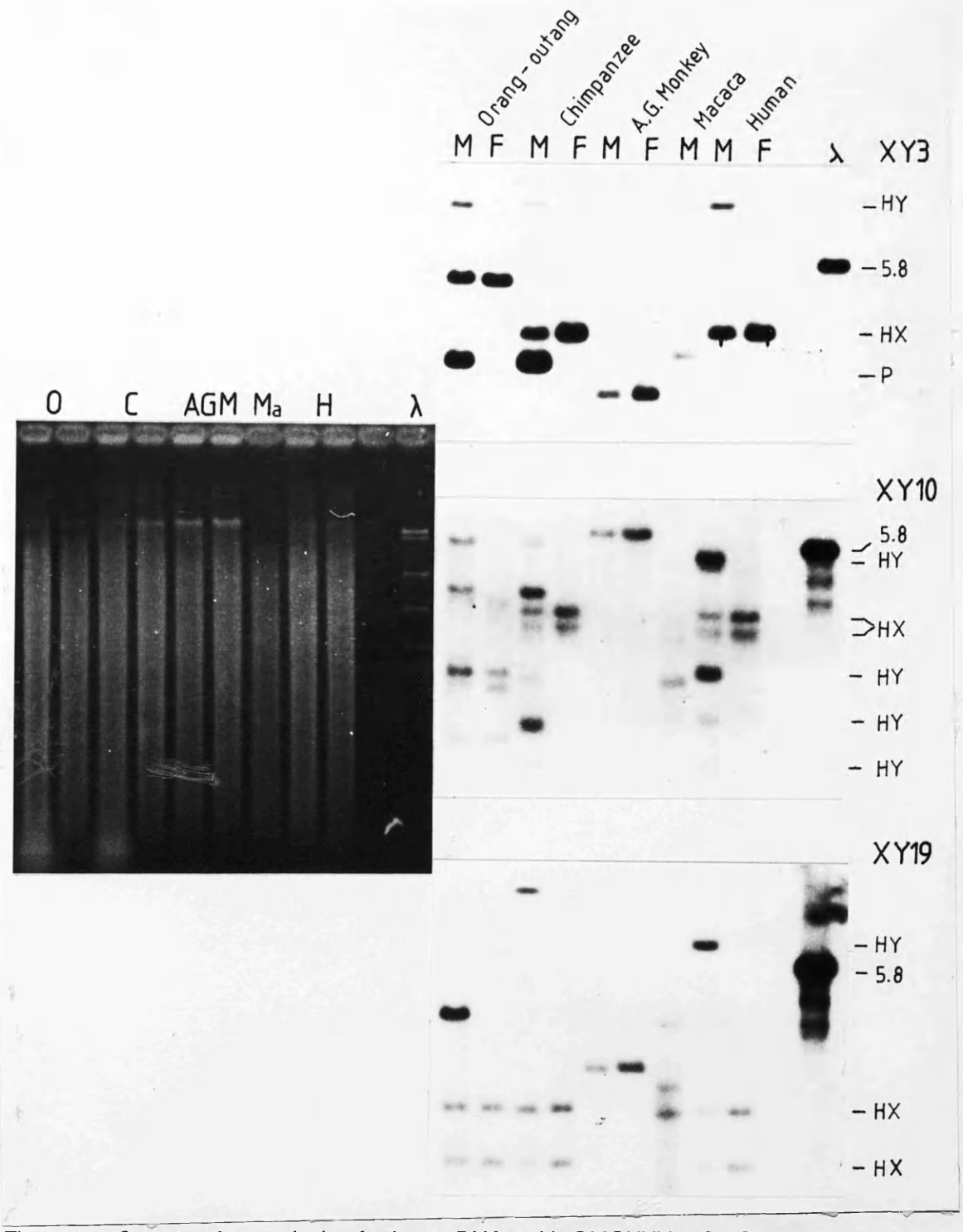


Figure 25: Comparative analysis of primate DNAs with GMGXXY3, GMGXY10 and GMGXY19. The order on the panels is orang-outang male and female; chimpanzee male and female; African green monkey male and female; macaca male; and human male and female. The final lane contains lambda gt11 cut with HindIII/EcoRI as a marker. An ethidium bromide stained gel of the panel prior to blotting is shown on the left. M=male; F=female; HX=human X bands; HY=human Y bands; P=probable plasmid contamination.

The results of the comparative studies with all three probe types are summarised in table 9.

Species / Probe	Human	Chimp-anzee	Gorilla	Orang-outang	African Green Monkey
Yp/Xq21	XY	nt	X	nt	nt
Yq/Xp22.3	XY	XY	X	XY	X
Yp/Yq/ Xq27-qter	XY	XY	XY	XY	X

Table 9: The assignment of the homologies detected by the XY homologous probes to the X and Y chromosomes of five primate species.
nt:- not tested.

CHAPTER 4 - DISCUSSION

4 DISCUSSION

4.1 The Isolation of Y Homologous Sequences

The probes used in this study were mostly isolated from a flow-sorted Y chromosome library constructed in the *Hin*dIII site of lambda Charon 21A. Recombinant phage which did not contain repeats were selected. This method of isolating probes will be discussed and related in theory and in practice to the random isolation of probes from a cosmid library.

4.1.1 The Flow-Sorted Library as a Probe Source

After the initial screening of the library with nick-translated total male DNA it was observed that about 70% of the plaques hybridised with this probe. This 70% should contain only human inserts. However the other 30% as well as containing human inserts will also include empty phage and non-human inserts. It was found that 103 of the 164 plaques picked were either non-recombinant (94) or contained a non-human insert (9). This implies that 86% of the human inserts reacted with the total male probe and that all the probes were isolated from a pool of only 14% of the human *Hin*dIII fragments cloned.

This method of screening will also probably tend to select for the smaller fragments as the larger fragments will be more likely to contain a repeat, and those over 9.1kb will be too large for the vector. The chances of isolating a probe from a given region will depend on the density of repeats and *Hin*dIII sites in that region as well as its extent. The isolation of a large number of probes of the one type that map into the same region of the chromosome, like the Xq21/Yp probes isolated here, thus either indicates a large region, or a region with few repeats in it or many *Hin*dIII sites.

A good example of this potential overrepresentation is seen with GMGY4. Because of its centromeric location, and the strength and pattern of its hybridisation to *Eco*RI digested genomic DNA, this probe has been concluded to be a representative of the alphoid repeat cluster. Four probes were isolated which gave this same hybridisation pattern. This represents about 8.5% of all the sequences isolated from the Y

euchromatin. If cloneability was even throughout this would imply that the alphoid cluster occupies about 2.5Mb of Yp and its 5.5kb repeat unit (Wolfe et al., 1984) would be present in 450 copies. However, Wolfe et al., (1984) estimated there to be only 100 copies of the 5.5kb repeat and so these probes are roughly four times overrepresented in the collection of probes presented here. Other probes were isolated two or three times from the library - GMGY7, GMGY10 and GMGY22 - and gave strong hybridisation to one or two bands on Southern analysis. It is likely that these probes also represent low level repeat clusters. These four probe types together accounted for 45% of all the Yp sequences isolated in this study.

4.1.2 The Cosmid Library as a Probe Source

Cosmid libraries for analysing the Y chromosome have been constructed from the mouse human hybrid 3E7 which contains the Y chromosome as the only human chromosome (Bishop et al., 1983 and Wolfe et al., 1984). Theoretically cosmid libraries minimise the problem of unrepresentative cloning by using partially digested DNA with a frequent cutting restiction enzyme like *Sau*3A. This means that as cosmids can take fragments of over 40kb of DNA it is unlikely that there will be fragments too large to be cloned. Furthermore, as the recombinants are screened with a total human probe, and those that contain human repeats isolated, there is little selection on the basis of the density of high level repeats.

However, in the case of lower level repeats such as the alphoid cluster there is probably a double selection against cloning these regions. Firstly, they may represent long stretches largely lacking in higher level repeats and so only the edges of the clusters will be isolated through surrounding high level repeats. The centre of the cluster will not be isolated. Secondly, the tandemly arranged sequences will probably be unstable in the cosmids leading to a further underrepresentation. This latter effect was clearly seen for cosmids containing regions of the alphoid cluster which were isolated by Wolfe et al., (1985) using the X alphoid repeat. The two cosmids isolated contained inserts of only 10-15kb which is well below the minimum size of insert (30kb) for the cosmid vector used. They therefore must have rearranged. Furthermore

Bishop et al., (1984) did not report a single clustered repeat in their large collection of Y cosmids.

4.1.3 A Comparison of the Two Methods

Apart from the differences outlined above it is interesting to note the similarities between the probe types isolated here and by Bishop et al., (1984) from a cosmid library made from 3E7 DNA. They isolated 40 probes and, as was the experience here, failed to isolate any probes from two regions of Yp. These are the pseudoautosomal segment and the region defined by GMGY3 between the more distal Xq21 homologous block and the pseudoautosomal segment. Because of the different biases in the screening procedures the simplest common explanation for the lack of probes in the GMGY3 region is that this region is very small - perhaps only several hundred kilobases. This is borne out by the physical size of the region which can be estimated from two studies. Page et al., (1987) have walked the proximal 230kb of this region identifying an HTF island near the proximal end of the walk and Pritchard et al., (1987) have identified an HTF island, 100-200kb from the boundary of the pseudoautosomal segment, by pulsed field mapping. These HTF islands are almost certainly the same island as in both cases GMGY3 was mapped to the 100kb immediately distal to the HTF. The region can then be estimated to be only 300-400kb in length. However the lack of probes from the pseudoautosomal segment in both this study and Bishop et al., (1984) is difficult to explain, particularly since the region has been estimated to be 2-3Mb in length by pulsed field electrophoresis (Petit et al., 1988; Brown, 1988).

The other striking similarity between the two collections of probes is the large number of probes detecting homology on Xq13-24. Bishop et al., (1984) isolated nine such probes out of forty while five were isolated here out of forty-seven. This represents a very similar proportion of such probes in both studies if it is taken into account that the cosmid library will be overrepresented for Yp sequences as 3E7 is believed to carry a human Y chromosome with a duplicated short arm, and that the probes isolated here are overrepresented for the low level clustered repeats.

Therefore the "better representation" of the cosmid library is not clearly evident between these two studies. Indeed it is underrepresented for clustered repeats.

However, as a major aim of this project was to isolate probes mapping close to *TDF*, in retrospect a cosmid library would have been a better choice for two reasons. Firstly, the 3E7 hybrid carries a Y chromosome with two short arms - the arm on which *TDF* maps. This would have led to a doubling of the number of Yp probes isolated. Secondly, because no low level cluster defining probes would have been isolated, which accounted for almost half of the Yp sequences isolated in this study. Theoretically, it can be concluded that, if a cosmid library made from 3E7 had been used as a source of probes, half would have been from Yp and none would have been in clusters distant from the *TDF* locus.

4.2 The Mapping of the Y Chromosome

4.2.1 A Molecular Deletion Map of Yp

The probes isolated in this study which detected Y specific restriction fragments mapping to the short arm were used, in conjunction with other such probes, in the Southern analysis of DNA from XY females, XX and XO males with putative breakpoints on Yp. On the basis of these analyses the short arm of the Y chromosome has been divided into 15 regions by 14 different breakpoints in 18 XX males, an XY female AM, an XO male RW and a dicentric Yq female ED. This deletion mapping provides information on the aetiology of the patients used and permits the localisation of genetic functions on Yp. It also allows the extrapolation of a molecular map of Yp based on the mapping positions of the different probe types.

From the data presented here Yp can be divided into six molecular domains. This map is dominated by XY homology. Most distally there is the pseudoautosomal region and then, proximal to a region defined only by GMGY3, there is a region containing a large number of sequences that detect homologues on Xq21. Proximal to this region is the domain that contains the vast bulk of the Y specific Yp sequences. Many of the probes which detect sequences that map into this domain pick up at least two Y loci on Yp or both Yp and Yq and two - GMGXY2 and GMGXY10 - are XY homologous. There are also three probes that detect low level repeats in this domain -GMGY7, GMGY10, and GMGY22. Proximal to this domain lies another region containing sequences with an

Xq21 homologue, but fewer than the more distal region. As these blocks of Xq21 homology are most likely the result of the same recent transposition event from the X chromosome, it would appear that the intervening region carries most of the cloneable "true" Yp sequences. Proximal to the smaller Xq21 homologous block lies a region defined by the alphoid probe GMGY4 and some restriction fragments detected by probes that also detect fragments mapping into the main "truly Y" portion of Yp.

It is inadviseable to draw too many conclusions, about the extent of a region from the number of probes mapping into it, based on the assumption of an evenness of cloneability throughout the chromosome. This must particularly be the case here as no probes were isolated from the pseudoautosomal region which is known to be about 2-3Mb in extent - roughly one third of Yp (Petit et al., 1988; Brown, 1988). However it is tempting to postulate that the regions of Xq21 homology, and the region containing the multiple Y loci detecting probes, form the bulk of the non-pseudoautosomal Yp chromatin. It is possible though that there is another region of the chromosome which, like the pseudoautosomal segment, is difficult to clone and therefore not represented on the map. In this respect, while the high concentration of XX male Yp breakpoints between Y23/Y46B and Y7B/Y7D'/Y10E' may indicate a common breakpoint, it could also be indicative of a large uncloneable region of Yp.

4.2.2 A Molecular Deletion Map of Yq

From the Southern analysis of the dicentric Yp individuals and the 46,Y,tX:Y(p22.3;q11.2) individual (AM), with the Yq homologous probes isolated in this study and elsewhere,13 different breakpoints have been identified and Yq divided into 14 regions. As most of the probes used have been mapped proximal to the heterochromatic repeats defined by pY3.4 it seems likely that they and the intervals they define lie within the euchromatin. Unlike Yp there are no such clear blocks of sequence types. The only possible exception is the two Xp22.3/Yq homologous probes - GMGXY3 and GMGXY19 - which map into the centromeric region of Yq in potentially adjacent intervals. The exact order of the three most proximal intervals of the Yq deletion map is unclear because the sequences present in PZ and AM are inconsistent

with each other. At present any order of these three regions is equally consistent with the data. There is no other evidence of blocks of probes with similar hybridisation properties except the rather tenuous observation that sequences which are detected by probes detecting multiple loci are localised in the distal half of the Yq deletion map.

4.3 The Aetiology of Sex Reversal

4.3.1 XX Males

Of the 23 XX males analysed in this study 18 carried Y sequences as defined by Y specific restriction fragments detected upon Southern analysis. Fifteen of the 18 Yp+ XX males fit into a consensus order of Yp probes arrived at by assuming that the XX males have arisen by a terminal transfer of Yp sequences, including *TDF*, to distal Xp with a single breakpoint on both the X and Y chromosomes. The fact that this assumption produced a consistent order of Yp sequences strongly argues that the terminal loss of Yp sequences from a single breakpoint is the mechanism by which most XX males arise. Furthermore by a variety of techniques it has been shown that in all cases examined the Y sequences have been transferred to the X chromosome (cytogenetically: Evans et al., 1979, Madan et al., 1976, and Magenis et al., 1982; by in-situ hybridisation: Andersson et al., 1986, Buckle et al., 1986, Kalaitsidaki et al., 1987, and Magenis et al., 1985; by measurement of flow-sorted chromosomes: Ferguson-Smith et al., 1987; and by Southern analysis of flow-sorted chromosomes: Affara et al., 1986b). In 14 of the 18 Yp+ XX males presented here there is evidence that the transfer is to the X chromosome. No evidence has yet been found of any other mechanism in the others (Ferguson-Smith et al., 1987). In addition Petit et al., (1987) showed that all informative Yp+ XX males tested carry the complete paternal Y pseudoautosomal region. So it would appear that, as postulated by Ferguson-Smith (1966), most XX males do arise as the result of an XY interchange event which transfers a terminal portion of Yp to the distal end of Xp.

The exact mechanism of transfer in one case has been elucidated by Rouyer et al., (1987). They isolated a cosmid which contained the breakpoint of one of their XX males. Sequencing across the breakpoint revealed the break to lie within an Alu repeat.

Thus in one case the transfer has been mediated by Alu-Alu recombination between the X and Y chromosomes.

There are three XX males - HM, KS and DR - who do not fit in with the consensus order of sequences established here. All three have been explained by postulating the occurence of a paracentric inversion prior to XY interchange (Affara et al., 1986b and 1987). This is the simplest way of explaining these anomalies. However the HM inversion only sufficed if *TDF* mapped distal to GMGY3. The cloning of the Y borne zinc finger domain (*ZFY*) by Page et al., (1987) suggests that *TDF* may map proximal to GMGY3. It would then require two inversions to be invoked to explain HM. HM carries the Y specific restriction fragments detected by GMGXY2 and GMGY10 and these Y sequences are present on one of his X chromosomes (Affara et al.,1986b). It is possible that his father carries an unusual order of Yp sequences but alternatively the father may carry the two Y restriction fragments detected by GMGXY2 and GMGY10 on his X chromosome. This would mean that HM would more rightly belong to the Yp- group of XX males. This could be tested by analysing a sister or paternal grandmother of HM who would carry the father's X chromosome unmasked by a complete Y chromosome. It is also possible that the XY interchange product was unstable in HM and deleted much of the Yp carried after determining sex. In view of this idea it is interesting to note that Ferguson-Smith & Affara, (1988) have reported HM to be negative for the zinc finger domain.

Five of the XX males do not have any detectable Y sequences but it is still possible that they might carry a small region of the Y chromosome bearing *TDF*. However Petit et al., (1987) have shown that a number of similarly Yp- XX males are unlikely to be the result of XY interchange as they carry an X pseudoautosomal region on the paternal X chromosome not the Y pseudoautosomal segment as would be expected if terminal transfer had occurred. This does not mean that all Yp- XX males will be the same, an interstitial transfer from Yp to the X or an autosome is still a formal possibility, but it does suggest that most will completely lack Y sequences. The data presented by Page et al., (1987) suggests that GMGY3 maps distal to *TDF* as it maps 60kb distal to the most distal HTF island in the region of Yp into which *TDF* has been located. This would argue

that in most cases the absence of GMGY3 in an XX male is diagnostic of sex reversal caused by a mutation of a sex determining gene on the X or an autosome. Alternatively, such individuals may have had a Y bearing cell line in the indifferent gonad which does not persist after determining testicular development.

4.3.2 XO Males

The XO male JG is the result of a 5:Y translocation and has been shown to carry a complete Yp and also some of Yq. RW has a 45,X/45,X mar+ karyotype. He is seen to carry Yp sequences including GMGY3 and, although he does not fit in with the general consensus, a single paracentric inversion can again be postulated to explain this anomaly. In-situ hybridisation analysis has shown that the marker chromosome carries GMGY7 (Kalaitsidaki et al., 1987). It is, therefore, likely to be Y derived and carry the other Y sequences shown to be present in RW by Southern analysis. Interestingly, from the order of sequences defined by the XX males it would appear that this marker is acentric as it lacks GMGY4, GMGY10A, GMGY10C and 50f2D which map between the centromere and the proximal RW breakpoint. GMGY4 probably defines the alphoid cluster on the Y chromosome. Clusters of alphoid repeats are known to be associated with centromeres (Willard, 1985) but have not been shown to have a role in centromeric function. However, the marker chromosome of RW is present in 25% of the blood lymphocytes which would argue that it has at least partial centromeric function. GMGY4 and the other centromeric probes have been mapped to Yp because of their presence in WC, the isoYp individual. However they are not present in any XX males and it is possible that they map in proximal Yq. This would mean that WC is in strict terms carrying a dicentric Yp with a small region of proximal Yq. This would allow RW to carry centromeric function while lacking these probes without invoking further rearrangements.

All XO males which have been characterised at the molecular level have been shown to carry Y chromosomal DNA, either in the form of a Y:autosome translocation, a marker chromosome or through the presence of 45,X/46,XY mosaicism. A case of this latter type was originally reported by de la Chapelle et al., (1986) to be Y sequence

negative but further analysis (de la Chapelle et al., 1987) revealed a low level of a Y bearing line. No XO males have been reported which carry Y sequences on the X chromosome.

4.3.3 XY Females

Only one XY female was found to be deleted for Y sequences from nine tested. This XY female, AM, was deleted for all the probes mapped distal to GMGY10B and 118C in the consensus ordering derived from the XX males. This strongly suggests that AM is the result of an XY interchange and carries the reciprocal product to that carried by the XY interchange males. However formal proof that this is the case is lacking as it has not been shown that AM carries a complete paternal X pseudoautosomal region, or any distal Xp sequences, on her deleted Y chromosome. All the XX male causing XY interchanges must have produced a deleted Y chromosome like AM's. However most XY females do not carry Y chromosomes deleted for the sequences present in XX males. The simplest explanation for this paradox stems from the fact that AM, and all the other reported XYp- females, manifest Turner stigmata. It is known that XO foetuses have an extremely high pre-term miscarriage rate. It is therefore likely that most XYp- foetuses do not reach term and thus their occurrence is very rare.

The XY female reported by Page et al., (1987) is atypical of Yp deleted XY females which have previously been reported. This patient carries both products of a reciprocal Y:22 translocation with a breakpoint in the long arm of the Y chromosome. In addition it was revealed that she has a small 160kb interstitial deletion of distal Yp which includes the zinc finger domain and GMGY3. Apart from gonadal dysgenesis this patient does not manifest any other Turner stigmata. Another XY female of similar phenotype has been reported by Rary et al., (1979) who carries both products of a reciprocal Y:17 translocation where the Y breakpoint is again on Yq. Molecular analysis has not been carried out on this individual but it would clearly be of interest to analyse this and any similar cases further. These patients may share a rare mechanism for the generation of XY females.

4.4 Molecular Map to Genetic Map

The two maps of Yp and Yq add up to 28 different breakpoints, including the WC breakpoint around the centromere and allow the division of the chromosome into 30 intervals, counting the pseudoautosomal segment. This map should provide an excellent foundation for the localisation and cloning of some of the genetic functions of the Y chromosome. The extent to which it does this is discussed below.

4.4.1 Localisation of the Testis Determinants

As GMGY3 is present in most of the Yp+ XX males (17 out of 18), and in one is the only Y sequence detected, it can be assumed that *TDF* maps close to this probe on distal Yp. It is not possible from the map, however, to determine to which side of GMGY3 *TDF* lies. It is, therefore, possible that the second most distal probe - 47z - might be closer to *TDF* if GMGY3 lies distal to *TDF*. However, it would appear that the testis determinants must map into the region between 47z and the boundary of the pseudoautosomal segment.

There have been a number of reports of similar deletion mapping of the testis determinants on Yp from our laboratory (Affara et al., 1986b, 1987) and others (Muller et al.,1986a & b, Page et al., 1986 and Vergnaud et al., 1986). All of whom have identified the region between the larger Xq21 homologous block and the pseudoautosomal boundary as that most likely region to contain *TDF*. Page et al., (1987) have identified a zinc finger domain containing gene in this region by walking from an Xq21 homologous probe located proximally in the adjacent interval. Upstream and telomeric of the zinc finger domain lie a further three conserved regions spread over about 50kb. The most telomeric region includes a high density of rare cutting restriction enzyme sites which probably define an HTF island. The finding of three of these four regions in a bovine cDNA clone argues that the four regions are exons of a single gene with an HTF island at its 5' end. This gene lies within a 160kb deletion of an XY female, who presumeably deletes *TDF*, and is within a domain of the Y chromosome implicated by four independent studies as the region to which *TDF* maps. It is,

therefore, a very good candidate gene for *TDF* but direct functional proof of its identity is lacking.

The XY females without detectable deletions of Yp must carry point mutations or small deletions of either *TDF* on the Y chromosome or of other autosomal or X linked genes that normally promote testis determination. The analysis of these patients with the gene defined by Page et al., (1987) will be of extreme interest as mutations of the gene in these XY females would strongly confirm it as the testis determinant . Definitive proof will come from transgenic experiments. It would be expected that an XY embryo, with the true testis determinant mutated, would develop as a female and an XX embryo, with an active testis determinant, would develop as a male.

4.4.2 Localisation of the Factor(s) which Prevent Turner's Syndrome

As was argued in the introduction it is most likely that the factor which prevents the onset of the Turner phenotype is located between the proximal Yp breakpoint of the XYp- females with Turner stigmata and the pseudoautosomal boundary. From the molecular characterisation of the XY female AM in this study they can be placed distal to GMGY10B. Molecular analysis of the pseudoautosomal region in AM (Affara et al., 1987) showed that she carried MIC2 and 29C1 alleles on her Y chromosome. This shows that she has two intact pseudoautosomal regions and argues against the anti-Turner factor being pseudoautosomal. The 45,X/45,X mar+ male RW does not contribute to the mapping of this locus as his Turner stigmata could simply be the result of his 45,XO line.

In strict terms the role of the Y chromosome in preventing Turner stigmata is three fold. The three main features defining Turner's Syndrome were first described as short stature, gonadal dysgenesis and the other phenotypic symptoms exemplified by the webbed neck. The typical stigmata, however, show considerable variation even within non-mosaic cases, and some fertile XO females have even been reported (Kohn et al., 1980). As none of the XYp- females have short stature this argues that this anti-Turner factor is the same as the factor(s) controlling stature on the Y (*GCY*) and must

map to Yq or to Yp proximal to the most proximal breakpoint in the XYp- females. The anti-Turner gene which prevents gonadal dysgenesis could possibly be *TDF*.

Until recently it was possible to postulate that *TDF* also prevented the webbed neck phenotype. However Page et al., (1987) have published an XY female deleted for only 160kb of the region defined by GMGY3, between 47z and the pseudoautosomal boundary. This deletion has been shown to contain a zinc finger containing gene which does not appear to be the factor that prevents the webbed neck phenotype as the female appears phenotypically normal. The deletion in this female extends into the region of Xq21 homologous sequences, which is not likely to house this locus as it is Xp that has been assigned the major role of preventing Turner stigmata (Wyss et al., 1982 and Skibsted et al., 1984). The Y borne locus must map either distal to the zinc finger gene or between GMGY10B and the proximal end of the large block of Xq21/Yp homology. The former region has been estimated by Page et al., (1987), based on the results of Pritchard et al., (1987), to be 95-195kb in length but does not contain an CpG island. However, not all genes have CpG islands (Gardiner-Garden & Frommer, 1987) so this does not totally exclude this interval. It is also a possibility that this female manifests very mild overt Turner stigmata which do not appear abnormal. It is therefore not possible to totally exclude *ZFY* or *TDF* as the factor which prevents the webbed neck phenotype.

4.4.3 Localisation of the H-Y controlling Locus

No information can be obtained directly from the data presented here as to the location of *H-Y*. *H-Y* typing of the individuals used in the deletion mapping is required if this locus is to be positioned on the map. Some XX males have already been typed and all were shown to be H-Y negative (Simpson et al., 1987). This excludes much of Yp and further analysis of cells bearing Y chromatin, from the individuals with breakpoints in regions of the Y chromosome not yet excluded, should in theory localise *H-Y* to a single interval. The probes in that interval can then be used to move towards the gene.

4.4.4 Localisation of the Other Genetic Effects of the Y Chromosome

The genetic effects of the Y chromosome on spermatogenesis, stature, and the induction of gonadoblastoma will be more difficult to localise. In the case of spermatogenesis this is because of the invariable presence of mosaicism in cases with deletions of Yq. It is therefore not possible to be certain that the deletion characterised and not a pairing anomaly causes the spermatogenic failure. Yq deletions with normal spermatogenesis are required but it is unlikely that such patients, being fertile, will be detected. It is therefore not possible to draw any conclusions from the data presented as to the mapping of factors involved in spermatogenesis.

The factor affecting induction of gonadoblastoma has been localised to Yq or to Yp proximal to the Y breakpoint of the XY female AM. The short stature of the XX males and the isoYp male - WC - suggest that the loci affecting stature can be excluded from most, if not all, of Yp. It will be difficult to further localise the gonadoblastoma inducer if this factor maps to Yq as patients will be required with deletions of *TDF* on Yp and deletions of Yq. Perhaps 46,X,ringY carrying females who develop gonadoblastoma could be used although there may well be a problem with mosaicism. However as was pointed out in the introduction the higher incidence of gonadoblastoma in females with a dicentric Yq than in females with an isocentric Yq may indicate that this factor maps to proximal Yp. It should then be possible, by analysing XYp- females with and without gonadoblastomas to define an interval into which it maps. However in practice the awareness of the risk of gonadoblastoma in these XYp- females, who have Turner stigmata and are therefore picked up early, results in the removal of the gonads in which no gonadoblastoma are present. So, it will not be possible to know whether a negative gonad from a young patient could, if untreated, have developed gonadoblastoma in the future.

The mapping of the genetic functions of the Y chromosome are summarised in diagramatic form in figure 26. It would seem that apart from the loci controlling *TDF*, the prevention of Turner stigmata, and *H-Y*, the genetic functions of the Y chromosome will need to be cloned through alternative approaches. These would include cDNA

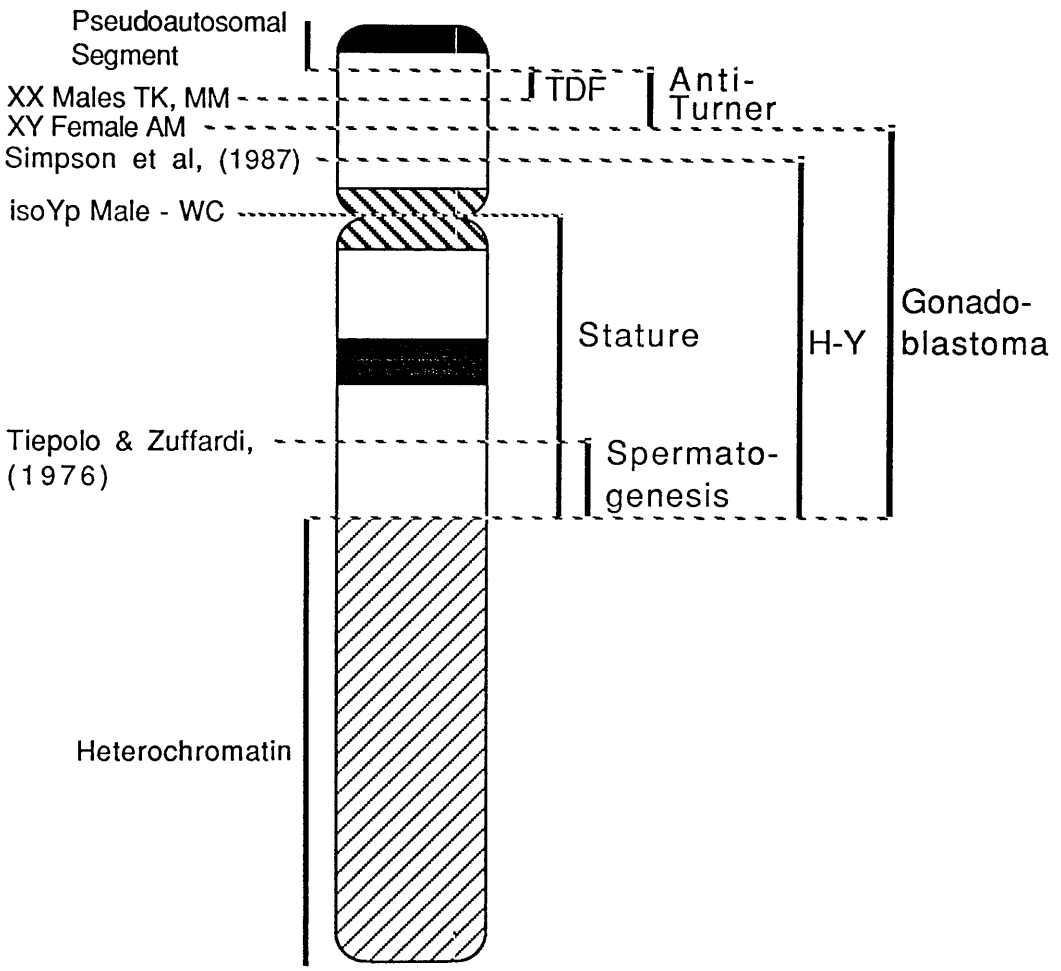


Figure 26: Post deletion-mapping genetic map of the Y chromosome.
The breakpoints or boundaries which permitted a localisation are indicated by dotted lines

library screening with random sequences from the Y chromosome and the identification and isolation of HTF islands as indicators of a region containing a gene. HTF islands could be identified on the Y by the construction of pulsed field maps of regions of the chromosome. Their isolation could be effected through strategies designed to clone fragments containing rare cutting sites such as *Sac*II, *Eag*I, and *Bss*HII which are believed to be strongly associated with HTF islands (Lindsay & Bird, 1987).

4.5 Inversion Polymorphisms on the Y Chromosome

In addition to information about the aetiology of certain Y chromosome anomalies and the location of genetic functions on the Y, the deletion mapping also provides evidence that the Y chromosome may exist in a number of polymorphic forms. These putative polymorphisms would be the result of paracentric inversions on both the long and the short arm.

The individuals who do not fit in with the consensus ordering of Y sequences, obtained with the XX males for Yp and the dicentric Yp individuals for Yq, are most simply explained by the occurrence of a single paracentric inversion. This inversion could either already have been present in the father of the non-consensus individual, or have occurred at the time of the rearrangement. The former explanation would imply that within the population there are polymorphic arrangements of sequences on the Y chromosome, arising chiefly through paracentric - as evidenced here - and pericentric inversions.

Pericentric inversions in the Y chromosome have been detected at low levels in the normal population (reviewed by Verma et al., 1982). These can be transmitted within families and do not confer any apparent selective disadvantage. The estimated incidence of these inverted Y chromosomes is 0.6 : 1000 males. Bernstein et al., (1986) reported a frequency of 56.7 : 1000 males of East Indian origin in South Africa. However, as many of these individuals came from the same region of India, and rarely married outwith their community, it was concluded that the inverted Y chromosomes all arose from a common ancestor, in whom the original inversion had occurred.

The reported variants of Y chromosomes in normal males do not include paracentric inversions but this is, perhaps, only due to the difficulty in seeing such inversions cytogenetically. It will be for the greater sensitivity of the molecular approach, through pulsed field gel electrophoresis, to detect these inversions in the normal male. However, the fact that pericentric inversions of the Y euchromatin can occur at low frequency in the normal population argues that at least within certain, as yet undefined, limits the Y chromosome can support variations in the order of its sequences.

If such order polymorphisms are present on the Y chromosome then it may be difficult to define a standard order of Y sequences along the chromosome. The data obtained from the XX males may, at best, reveal the most common order of Y sequences with some of the more frequent inversions caused by paracentric inversions. At worst, it may represent a Y chromosome organisation that is a minor variant which is highly represented amongst rearrangements of the Y chromosome because of its greater instability. This would imply that such an unstable chromosome could be transmitted through a family giving a higher than normal risk to the male offspring. Evidence that such Y chromosomes exist can perhaps be seen in the familial case where two XX males, related through males to a common great-grandfather, were both shown to carry Yp sequences - the same ones but very few probes were used (Page et al., 1985). It is also noteworthy that the inversion postulated for KS would unite the Yp/Xq21 probes into one block. This order is also seen in the dicentric Yq female ED. Perhaps, then, this is the more common more stable order of sequences on Yp.

This potential for inversion polymorphism should be borne in mind before any long range mapping, walking or jumping is undertaken on the Y chromosome. A probe could be close to the target locus in the individuals used to make the deletion map but in the individual used to construct the jumping library or the pulsed field map it could be a considerable distance away.

4.6 Identification of Potentially Functional Loci

4.6.1 Northern Analysis

In order to determine whether or not any of the probes were expressed in testis, 30 of the probes were used to screen Northern blots of total RNA from testis and male placenta. That most failed to react implies only that they are not abundantly expressed in either tissue. The only single copy Y homologous probe which did react was GMGY28. It produced a band of around 2.3kb only in the testis track. Thus this Y sequence detects an abundant testis transcript.

GMGY28 is seen, on Southern analysis of *Eco*RI digested male genomic DNA, to detect a Y specific band of about 10kb together with five other bands of autosomal origin. Thus if the 2.3kb transcript is produced from the Y chromosome it must be part of a multi-gene family or have spread, by retroposition for example, to a number of different loci throughout the genome. Another explanation is suggested by the similarity of the autosomal pattern of hybridisation to that observed with a cDNA probe (for example see the *STS* cDNA in Yen et al., 1987a). It is , therefore possible that GMGY28 is a pseudogene of an autosomal gene. It was perhaps inserted into the Y chromosome by retroposition after splicing of the transcript had occurred, and each autosomal band will contain all or part of an exon. Mapping of all the autosomal bands to the same autosome will strongly support this theory. The gene is expressed abundantly in testis but not in the same abundance if at all in placenta. The Northern analysis of further tissues will be required to determine whether or not it is testis specific.

On Southern analysis the human Y probe p12f3, isolated by Bishop et al., (1983), detects a similar, but distinct, pattern of hybridisation to that of GMGY28 (a single Y band and multiple autosomal bands). Leroy et al., (1987) showed that in both human and mouse it detects a testis transcript. The murine transcript was localised to a single locus on chromosome 13 and did not detect a Y homology. It was concluded that in the human the Y homologue is a pseudogene. In addition, pseudogenes for argininosuccinate synthetase (Daiger et al., 1982) and actin (Heilig et al., 1984) have been mapped to the Y chromosome. It is postulated that the lack of genes on the Y, together with its

decondensed nature in the germ cells, will allow such insertions of germ cell transcripts to occur and be maintained in the population.

It is possible that there are many such pseudogenes on the Y chromosome. If this is the case then the isolation of the pseudogenes from the Y chromosome might be an efficient method of cloning genes involved in germ cell development. This could perhaps be done by using oligo dT to isolate stretches of Y chromosomal DNA containing stretches of poly dA. These could correspond to the poly A tails of the messenger RNA's which can be found associated with the exonic sequences of pseudogenes.

4.6.2 Mouse-Human Homologous Probes

Another indication that a sequence may be part of a gene is its conservation between widely diverged species. Two Probes - GMGY6 and GMGY16 - detect a restriction fragment after Southern analysis of mouse DNA. These bands are only seen at low stringency (1xSSC, O.1%SDS, 65°c). Both bands are of approximately 2.9kb in size and this raises the possibility that they are simply plasmid contamination. However this band was not seen with any other probes used at low stringency on the same panels.

In the case of GMGY6 the 2.9kb band in the mouse A9 track is also present in the Horl-X track and probably in the 3E7 track although the Y band of 2.8kb obscures it. So if it is plasmid contamination it is in all the mouse DNA containing tracks but not in the human tracks. The pattern of hybridisation of GMGY16 is less clear as although a 2.9kb band is present in A9, 3E7 and Horl-X there are also bands of that size in the male, female and WC tracks but not in ED. The presence in the female argues X or autosomal homology but this band should then also be present in ED.

These probes potential cross-homologies with mouse can easily be tested by probing genomic DNA from male and female mice. GMGY16 should easily give a result as the background in the mouse even at low stringency is very low. Their conservation would argue an important role for these sequences, perhaps a coding function. It is, however, possible that the Y sequences are pseudogenes of an X or autosomal gene because at low stringency both exhibit non-Y hybridisation. Constraints of time did not permit this to be further investigated.

4.7 XY Homologous Sequences

4.7.1 Three Distinct Regions of XY Homology

In this study at least three regions of XY homology have been defined. These homologies are summarised in diagramatic form in figure 27. The homology between Xq21 and Yp defined by GMGXY4-XY7 and XY9 is almost certainly the same as that described by Page et al., (1983), Bishop et al, (1984) and Koenig et al. (1985). The second region of XY homology is that defined by GMGXY19 between Xp22.3 and Yq. Three other probes have been described that define similar homologies, 70-66 (Kunkel et al., 1983), M1A (Koenig et al., 1984) and GMGXY3 which was also used in this study (Affara et al., 1986a). The third region of homology is defined by GMGXY10 as being between Xq27-28 and Yp/Yq. This homology has not been previously described. No probes were isolated from the pseudoautosomal region or the region of XY homology described by Cooke et al., (1984) and Bickmore & Cooke, (1987) between Xq26-27 and Yqcen-q11.1. Although this appears similar to the regions defined by GMGXY10 these probes define two different regions of XY homology for reasons that will be discussed later.

These probes have been used to analyse and compare the X and Y chromosomes in these regions. The primate evolution of these homologies has also been studied. In addition the Xp22.3/Yq homologous probes have been used to analyse mutations of the X chromosome which give rise to X-linked ichthyosis. The findings of these analyses are discussed below.

4.7.2 XY Homology and the Putative Testis Determinant on Yp

Recently it has been shown by Page et al., (1987) that the region of the Y chromosome that is believed to house the testis determinants contains regions of XY homology. These are conserved on the X and Y chromosomes of all mammalian species analysed and as they are homologous to a transcript in bovine testis it is likely that they are the exons of an XY homologous gene. These are the first regions of homology between Xp and Yp which lie outwith the pseudoautosomal segment.

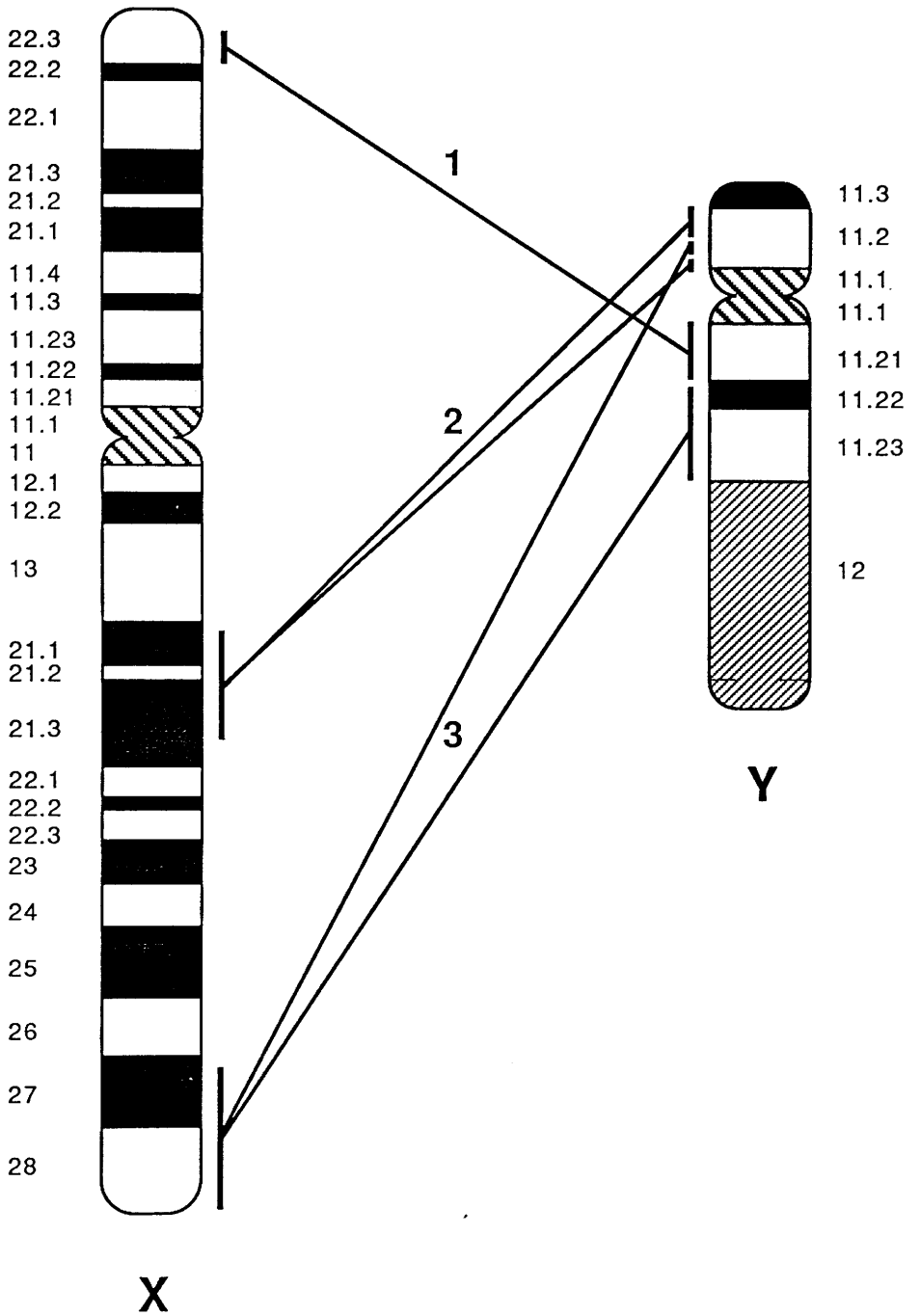


Figure 27: The three regions of XY homology characterised in this study. These are defined by: 1) GMGXY19 & GMGXXY3, 2) GMGXY4-GMGXY9 & GMGXY12 and 3) GMGXY10.

Based on the idea put forward by Ohno, (1967), that the X and Y evolved from an homologous pair of chromosomes differing initially only at a sex-determining locus, it could be postulated that this XY homology is a genuine remnant of the X and Y chromosomes' common ancestor. Furthermore the XY homologous nature of this, the first non-pseudoautosomal gene to be cloned from the Y chromosome, raises the possibility that other genes on the Y chromosome will also have X counterparts. These homologues would have evolved from an ancestral gene separately on the X and Y chromosomes. They perhaps evolved different functions as the result of acquiring mutations at key sites in the gene. Thus, for example, H-Y may have a counterpart H-X.

4.7.3 The Lack of Xp/Yp Homology outside the Pseudoautosomal Segment

It is difficult to explain the lack of Xp/Yp homology outside the pseudoautosomal segment. It is generally held that it is selected against as it allows unequal cross-over to occur between Xp and Yp during male meiosis, when Xp and Yp pair at their distal tips. However, it is hard to imagine such a selection operating when the putative testis determinant itself is Xp/Yp homologous.

It is possible that this apparent lack of Xp homologous sequences on Yp, outside the pseudoautosomal segment, has simply arisen through chance. If one excludes the Xq21 homologous blocks and the pseudoautosomal segment, the amount of Y chromatin in the human Y short arm is considerably less than in the long arm. It is, therefore, more likely that the evidence of random X transfers to the Y chromosome will be seen on Yq. Furthermore as Xq is longer than Xp the X homologies of XY homologous probes are more likely to map to Xq. Thus, there are insufficient regions of XY homology defined to infer a selection against Yp/Xp homologies.

4.8 High Incidence of Deletions in *STS* Deficient Males

The X homologues of GMGXY19 and GMGXY3 were mapped to the region of Xp within which *STS* has been localised. In view of this, males suffering from X-linked steroid sulphatase deficiency were analysed with these probes.

Twenty-three steroid sulphatase deficient males were analysed in this study using the two Yq/Xp22.3 homologous probes - GMGXY3 and GMGXY19. It was found that 18 males were deleted for both probes and one additional male was deleted for just GMGXY19. Thus 83% of such males analysed was deleted for these sequences. These probes have been mapped to Xp22.3, the region of the X chromosome to which *STS* has been mapped, (Ferguson-Smith et al., 1982). This strongly argues that these deletions include the *STS* locus and are the cause of the enzyme deficiency. in these individuals. Similar findings were made by Gillard et al., (1987) with an X specific probe mapping to Xp22.3. Yen et al., (1987a) and Ballabio et al., (1987) using *STS* cDNA clones have shown that in a high percentage of deficient males the gene was indeed deleted. Unpublished studies in our department of the 23 STS deficient males studied here with the *STS* cDNA clone p2A4 (provided by A. Ballabio) showed that this cDNA was deleted in the 19 males deleted for GMGXY19.

It is interesting that with four probes and 19 deletions only one different deletion can be defined. This argues that either the probes are all relatively close together compared to the size of the deletions or that the breakpoints for 18 of the deletions are all in close proximity. By the measurement of flow-karyotypes Cooke et al., (1988) have shown that the deletions carried by the males who are deleted for the probes are between 2-5Mb. This large size perhaps explains why different breakpoints have not been defined with only four probes. It also shows that one can be nullisomic for 2-5Mb of Xp with relatively little effect. The *STS* locus is fairly large at 120kb (Yen et al., 1987b). However in a 2Mb deletion around *STS* there could still be more than ten genes of a similar size.

4.9 Degree of Conservation of XY Homologies

The multiple restriction enzyme analysis of normal human DNA permitted a comparison of the X and Y chromosomes, at the DNA level, in the regions defined by the nine XY homologous probes used.

A measure of the degree of conservation between the two chromosomes was obtained by estimating the total number of base pairs analysed at the restriction sites used per

haploid genome for a given probe type and then dividing this by the number of XY differences observed. A number of assumptions had first to be made. All the probes were assumed to detect only a single X locus. The number of restriction sites detected by a given probe on the X or Y chromosomes was assumed to be equal to the number of X or Y bands plus one. The number of base pairs detected is then equal to the number of base pairs in the recognition site of the enzyme, times the number of bands detected plus one. The XY differences are assumed to be the result of a single base pair change in one of the sites detected.

The seven Xq21/Yp homologous probes were calculated to detect 2181bp at 24 different restriction enzyme sites and revealed 37 XY differences. This gave an estimate of 98% conservation between these two regions or a 2% divergence. For the Xp22.3/Yq homologous probe GMGXY19 an estimated 162 base pairs were analysed at 14 different restriction enzyme sites and 13 XY differences detected. This gives an estimate of 92% conservation. The Xq27-28/Yp/Yq homologous probe GMGXY10 was estimated to detect 222bp at 14 different restriction enzyme sites and revealed13 XY differences which gave an estimate of 94% conservation. In these latter two cases this calculation is clearly inadequate as the lowest percentage conservation it can estimate is about 92% where 14 out of the 14 enzymes used produce an XY difference. It is no longer possible to assume that the XY differences observed are due to a single base pair change. It can then only be concluded that these regions of XY homology are less conserved than estimated, and that they are less conserved than the Xq21/Yp homologies.

GMGXY10 detected a mean of 2 X bands and 3 Y bands with the 14 restriction enzymes used. The other probes detected between 1.1 and 1.8 X and Y bands with an average number of 1.5 bands detected. GMGXY10 is known to detect two Y loci and the average detection of three restriction fragments may indicate the presence of a further locus. Similarly the two X bands detected on average may indicate, as argued to explain the *Eco*RI polymorphism, the presence of two X loci.

4.10 The Nature of the Polymorphisms

Before considering the use of the data generated by the multiple restriction enzyme analysis of normal human genomic DNA in the estimation of the polymorphic frequencies of the X and Y chromosomes the nature of the polymorphisms will be discussed.

4.10.1 The Polymorphisms of GMGXY4 and GMGXY7

The *Sin*I polymorphism of GMGXY7 and the *Taq*I Y polymorphism of GMGXY4 are most likely the result of a loss or gain of a restriction site within the region covered by the probe because the sizes of the two bands of one allele approximately add up to the size of the single band of the other allele. The GMGXY4 X-polymorphism is more difficult to explain as the B1 allele has two bands at 5.3kb and 1.6kb while the B2 allele consists of three bands at 4.7kb, 2.5kb and 0.7kb. One explanation is that the 4.7kb and the 0.7kb bands are the result of a polymorphic *Taq*I site within both the B1 5.3kb fragment and the region defined by the probe, while the B2 2.5kb fragment is the result of the loss of a site, defining the B1 1.6kb fragment, that lies outwith the sequences homologous to the probe. This would imply two polymorphic sites within 2.3kb of each other. The other polymorphisms consist of a shift in molecular weight of a single band. For example the *Dra*I X-polymorphism of GMGXY4 has two alleles, one of 3.1kb and the other of 3.8kb. The simplest explanation for these polymorphisms is the loss or gain of a site that lies outside the region to which the probe hybridises. It is unlikely that they are due to deletions or insertions as the polymorphisms are only seen with one enzyme in all but one case.

4.10.2 Linkage Disequilibrium detected by GMGXY5

The three polymorphisms of GMGXY5 with *Hinf*I, *Nde*II and *Rsa*I show strong linkage disequilibrium. The alleles A1, B1 and C2 always go together as do their reciprocal alleles A2, B2 and C1. The enzyme sites though similar are all mutually exclusive (*Hinf*I:- GANTC, *Nde*II:- GATC and *Rsa*I:- GTAC) and it is not possible that a single point mutation could affect even two of the sites. Furthermore, it is probable that the *Hinf*I

polymorphic site lies within the region detected by the probe while the other two lie outside it. The *Hinf*I polymorphism of GMGXY5 like the *Sin*I polymorphism of GMGXY7 and the *Taq*I Y polymorphism of GMGXY4 is most likely the result of a loss or gain of a restriction site within the region covered by the probe as the sizes of the two bands of one allele approximately add up to the size of the single band of the other allele. The similarity in the base composition of these sites perhaps argues that GMGXY5 lies in a region rich in GATC/GTAC-like sequences which gives rise to a relatively high local concentration of enzyme sites of this base composition. This might increase the chances of finding polymorphic sites for such enzymes.

The linkage disequilibrium was observed in all the individuals tested. This includes 15 Northern European, 13 Asian, 4 Arab, 5 African and 4 Chinese individuals. It is most probable that the close proximity of the sites to each other makes recombination events between them extremely rare. Assuming that the polymorphic *Hinf*I site lies within the region defined by the GMGXY5 probe, while the *Nde*II and *Rsa*I sites lie outside, it can be concluded that the three sites must lie within 2.5kb of each other. Alternatively, the sites could all lie within 100bp of each other.

4.10.3 The Multi-Alleleic Polymorphism of GMGXY9

GMGXY9 detects a *Taq*I polymorphism with four high molecular weight alleles. The most straight forward explanation is that they are the result of gain or loss of *Taq*I sites. However it is also possible that they arose through a deletion/insertion mechanism occurring near the ends of the large *Taq*I fragments detected. These events may not be seen with other restriction enzymes because the restriction fragments generated around GMGXY9 do not extend to the polymorphic region.

It is of interest to note that out of 24 Africans tested the only two variants carried allele A4 which was not found in any other ethnic group. This allele is possibly specific to the African race, but many more individuals would have to be tested before any conclusions could be drawn. In all the races tested the 11.5kb allele A3 was the most common form with allele A2 the most common variant in the Northern European individuals. In 13 Asians one male carried allele A2 and another, unique in the whole

study, carried allele A1. The frequency of allele A2 is higher among the Northern European individuals (5/23 X chromosomes) than the Asian individuals (1/14 X chromosomes). One Arab also carries A2.

This probe exhibits potentially race specific alleles and allelic frequencies. However many more individuals would have to be analysed before any firm conclusions could be drawn.

4.10.4 The X Polymorphism of GMGXY10

The alleles of this polymorphism are A1: 9.8kb/3.2kb and A2: 9.8kb. This makes the polymorphism difficult to score in females, as heterozygotes and A1 homozygotes will be distinguishable only by dosage. On the Northern European males used for the other XY homologous probes only the A1 allele was observed. Sixty males were then used to provide the allelic frequency.

There are two possibilities for the nature of this polymorphism. The first is that the probe hybridises predominately to the 9.8kb band with only 100bp overlapping the 3.2kb fragment. The polymorphic *Eco*RI site would be just beyond the end of the region to which the probe hybridises in the 3.2kb fragment. This would leave a 100bp fragment for the probe to detect which would run off the bottom of the gel. The second possibility is a deletion/insertion mechanism which results in some X chromosomes having both fragments and others having only the 9.8kb fragment. The two fragments would not need to lie adjacent to one another. This could be tested by digesting the males who lack the *Eco*RI 3.2kb band with the other enzymes that also generate two X specific restriction fragments. If these males also lacked one of these X bands this would provide strong support for the latter mechanism. A lack of time prevented this being followed up.

4.11 Restriction Fragment Length Polymorphism Frquency

4.11.1 Polymorphic Frequencies on the X and Y Chromosomes

Inorder to calculate the number of base pairs analysed per haploid genome the same assumptions were made as in section 4.9 (Degree of Conservation of XY Homologies).

Additionally it was assumed that the number of Y restriction sites detected by GMGXY10 was the number of Y bands plus two, as GMGXY10 is known to detect two Y loci. To obtain the total number of base pairs analysed in all individuals tested, the number of base pairs per haploid genome detected by a given probe with a given enzyme, was multiplied by the number of X or Y chromosomes able to be scored for that enzyme. These figures were totalled for all the probes and enzymes. It was calculated that 61023 base pairs of X chromosome DNA and 23577 base pairs of Y chromosome DNA were analysed in total.

Each variant X or Y chromosome was assumed to be the result of a single base pair change at the restriction site of the enzyme used. The alleles already scored for GMGXY9 in the Northern European individuals were not scored in the TaqI analysis of the individuals of other ethnic origins as it was felt that this would biase the results because no other probe showed a frequent *TaqI* polymorphism. The other alleles revealed by GMGXY9 were scored. Two X polymorphic frequencies were estimated one including all the variants detected by the three linked polymorphisms of GMGXY5 and the other, assuming this to be unrepresentative of the general situation on the X chromosome, only scoring one of the polymorphisms.

The following formula suggested by Cooper & Schmidtke, (1984) was used to estimate the polymorphic frequency of the X and Y chromosomes:

$$H_N = 1 - ((a/b)^2 + ((b - a)/b)^2)$$

where H_N is the probability that two homologous DNA sequences will have different base pairs at a given site, "a" is the number of variants (a variant was defined as an X chromosome carrying the less frequent allele of a polymorphism) and "b" is the total number of base pairs analysed. For the X chromosome 45 variants were detected. This was adjusted to 25 when the linked GMGXY5 polymorphisms were treated as a single polymorphism. Only one Y variant was seen.

The calculations reveal that the probability of two homologous sites differing on the X chromosome is 1:1220 with 25 variants and 1:678 with 45 variants. The Y

chromosome is calculated to have a probability of variance at two homologous sites of 1:11,792. This clearly indicates the Y chromosome to be considerably less polymorphic than the X chromosome. From these calculations it appears that the Y chromosome is around ten times less polymorphic than the X chromosome.

The former figure for the X chromosome based on 25 variants is very similar to the probability calculated by Hofker et al. (1986) of 1:1,100. This suggests that the treatment of the GMGXY5 polymorphisms as a single polymorphism is perhaps a more accurate reflection of the situation on the X chromosome.

4.11.2 Variation on the Autosomes, the X and the Y Chromosome

Cooper et al., (1985) suggested that the X chromosome had fewer RFLPs than the autosomes. Hofker et al., (1986) showed this to be a statistically significant observation and estimated that the X chromosome was around three times less polymorphic than the autosomes. Two reasons were proposed for this observation based on the hemizygosity of the X chromosome in males. The first was that the bulk of the X chromosome does not cross-over in male meiosis and proposes recombination as a source of mutation. The second reason was based on the observation that many of the new mutations in hemophilia A and HPRT deficiency can be seen to be paternal in origin. This led to the conclusion that there is more chance of mutation in spermatogenesis than in oogenesis because of the greater number of cell divisions involved. As females have two X chromosomes and males one, two-thirds of X chromosomes are replicated in the less mutagenic environment of oogenesis but autosomes are replicated equally in both.

While the data presented here can be explained by the first reason it is entirely inconsistent with the second. If the second postulate were true then one would expect to see a high number of Y variants, as the Y chromosome always undergoes spermatogenic gametogenesis. It is possible that the Y chromosome, never undergoing oogenesis, is better evolved than the other chromosomes to resist the increased mutagenesis of spermatogenesis, although it is difficult to imagine the selection pressure for this or

the protection evolved. Alternatively the postulated increased mutation rate may only affect recombining regions.

Recombination as a promoter of mutation may well explain the different incidences of RFLPs on the X, Y and autosomes. Recombination can also act as a propagator of mutations spreading them to other chromosomes through gene conversion. This could of course also suppress mutations. Gene conversion can be given further weight in this arguement if it is postulated that strand invasion at the beginning of recombination, which results in three copies of the invaders chromosomal region, is more likely to be initiated in gametogenesis by the chromosomes which have passed through spermatogenesis (the paternal chromosomes). The proposed increased mutation rate of spermatogenesis would then potentially be propogated in all individuals on the autosomes but only in females on the X chromosome and of course not at all on the non-pseudoautosomal Y chromatin.

The observation has been made here that the Y chromosome has a considerably lower polymorphic frequency than the other chromosomes and a number of speculations have been put forward to explain this finding. Whatever the true explanation, this observation should be taken into account when considering the causes of mutation in the germ line.

4.12 The Evolution of Three Regions of Human XY Homology

At least three different regions of XY homology have been described in this study and their recent evolution has been examined by Southern analysis of DNA from a number of primate species. The evolutionary paths of the three regions, as defined by the species used, are distinct from each other. The proposed evolution of these three XY homologies is summarised in figure 28.

4.12.1 The Yp/Xq21 Homology

Most of the probes defining this region of XY homology were shown on the basis of dosage to hybridise to the X chromosome in gorilla but not to the Y chromosome. A male and female of no other species were tested. In the case of GMGXY9 the band in the female

track appears to be fainter than in the male track. However there is also a faint band of higher molecular weight in the female track which may indicate that the female is heterozygous for an X polymorphism at this locus. With this one exception these probes can be argued to define a region which has been transferred from the X chromosome to the Y chromosome, less than 10 million years ago, during the evolutionary time separating man from his common ancestor with the gorilla. The percentage conservation of the X and Y chromosomes in this region has been estimated to be about 98%. The generally accepted rate of divergence of two genetically isolated loci is around 0.24% per million years (Sibley & Alquist, 1987). Thus the divergence of 2% seen here suggests that the event occurred about 8myr ago. These data strongly argue that this region is identical with that defined by Page et al., (1984), Koenig et al., (1985) and Bishop et al., (1984).

From the studies on the XX males this region of XY homology can be seen to be split on Yp by a region containing mostly Y specific probes. This implies that either an inversion or an insertion must have occurred since the transfer. Alternatively there could have been two independant insertion events of sequences from Xq21 into Yp. The simplest explanation is that an inversion has occurred as this involves the fewest chromosome breaks. This possibility is supported by the order polymorphisms observed in the Yp deletion mapping, where two individuals - ED and KS - have a proposed order of Y sequences consistent with the Yp/Xq21 sequences residing in one block in these individuals.

4.12.2 The Yq/Xp22.3 Homology

These probes detect homologues on the X and Y chromosomes of orang-outang and chimpanzee but only an X homologue in gorilla and African Green monkey. (The X homologies were ascertained by the dosage of the proposed X bands in a male and a female). If it is assumed that this homology is the result of an X to Y transfer, one has to postulate that gorilla diverged from the primates' common ancestral line prior to the divergence of orang-outang. This is against current evolutionary theory of primate evolution which argues that the orang-outang lineage diverged first from the common

ancestor of man, then gorilla and finally chimpanzee (Sibley & Alquist, 1987). Thus a more complex explanation is required for the evolution of this region of XY homology.

The facts that the X homologues of these probes map to Xp22.3 and are deleted in STS deficient males who are also deleted for the *STS* locus, argue that they are situated in the same region of the chromosome as the *STS* gene. In the mouse it is known that *STS* is pseudoautosomal (Keitges et al., 1985). However in humans, while still XY homologous, it is no longer pseudoautosomal, mapping to Xp22.3 and Yq (Yen et al., 1987a). The Yq homologue is a non-functional pseudogene with sequences homologous to both the intronic and exonic regions of the X gene. This argues against the Yq homologue being the result of a retroposition event. It appears most likely that the *STS* Y homologue has been transferred out of the pseudoautosomal segment during man's evolution from his common ancestor with mouse.

Further evidence that such an event has occurred in mammalian evolution is provided by the Xp22.3/Yq homologous human probe MIA (DXS31) which in brown lemur exhibits an autosomal, or pseudoautosomal, pattern of hybridisation (Koenig et al., 1984). Thus this XY homology may infact be pseudoautosomal in brown lemur. The brown lemur is believed to have diverged from the human lineage about 70myr ago, 10myr after the divergence of the mouse lineage which means that this event must have occurred less than 70myr ago. Also supporting this idea is the probe CRI-S-232 (DXS278) which detects homologues on Yq and Xp22.3 (Knowlton et al., 1987). This probe detects a hypervariable pattern of hybridisation on the X which is characteristic of probes from the true human pseudoautosomal segment (Cooke et al.,1985 and Simmler et al., 1985 and 1987). These data argue that, by association, GMGXY3 and GMGXY19 were also once in the pseudoautosomal region but were removed during primate evolution. This removal could have been effected by an inversion on the Y chromosome. Alternatively it could have been the result of an insertion into the pseudoautosomal region distal to these loci, which placed them at a distance from the telomere where recombination occurred with insufficient frequency to maintain the homology.

The loss of these Y borne homologues from the pseudoautosomal segment perhaps occurred between the divergence of the brown lemur lineage and the African Green monkey lineage, from their common ancestors with man. One would then need to postulate a further deletion of the Y sequences in the monkey lineage. It is equally possible that this event occurred between the African Green monkey and the orang-outang with the deletion of the Y homologues occurring in the monkey lineage while they were still pseudoautosomal. In either case there must have been an independent deletion of the Y homologues in the gorilla lineage.

Based on the very rough estimate of less than 92% conservation between these loci on the human sex chromosomes one can estimate that they began to diverge more than 32myr ago. This fits with the postulated separation of these sequences at a point between the divergence of the brown lemur and the divergence of the monkey lineages from the human lineage. It does not however fit with the separation occurring between the gorilla and orang-outang lineages divergences.

The idea that such a rearrangement has occurred argues that, barring further rearrangements of the Y chromosome in this region, these loci should all be in close proximity to each other on the human Y chromosome. M1A, *STS* and the probe of Knowlton et al., (1987) have not been mapped on the Yq deletion panel. So, it is not known whether they map into the same Yq intervals as GMGXY3 and GMGXY19, which have been shown to lie in possibly adjacent intervals within the most proximal three intervals of Yq.

These proposals have to resort to invoking two deletions in addition to an inversion to explain the data. This fits with the observations of this region's behaviour in humans, where 80% of mutations of the *STS* gene involve deletions of this region of the X chromosome. Furthermore the pseudogene on Yq is known to be missing many of the exons of the X gene as only two 5' regions of the cDNA detect Y homologies (Yen et al., 1987b). Whether the instability of this region on the X and Y chromosomes evidenced in man reflects a similar instability through primate evolution is of course open to question.

A further point of interest arising from the primate studies is that the restriction fragments detected by both GMGXY3 and GMGXY19 are conserved on the X chromosome between human, gorilla and chimpanzee and those detected by GMGXY19 are also conserved in orang-outang. This may be through chance, although such conservation was only observed with *Taq*I for two Yp/Xq21 probes, GMGXY4 and GMGXY5, whose Y homologues were conserved in chimpanzee and gorilla. In the case of GMGXY19 it is two bands or three *Taq*I sites that are conserved. This perhaps argues a functional role for these sequences.

These sequences are postulated to be ancestral pseudoautosomal sequences which were removed from the pseudoautosomal region on the Y chromosome between the divergence of lemur from the human lineage and that of orang-outang. Two independent deletions are postulated to have occurred in the African Green monkey and gorilla lineages. The Y deletions perhaps echo the instability of this region on the human X chromosome.

4.12.3 The Yp/Yq/Xq27-28 Homology

The probe defining this XY homology - GMGXY10 - is found to have both an X and a Y homologue in gorilla, chimpanzee and orang-outang but is only X linked in African Green monkey. Thus it is simplest to postulate that the Y sequences arose by transfer from the X chromosome between man's common ancestor with African Green monkey and his common ancestor with orang-outang. Sibley & Alquist, (1987) propose that the old world monkeys diverged from their common ancestor with man 25-34Myr ago, while orang-outang diverged 12.2-17Myr ago. Thus it would be predicted that these sequences began to diverge 12.2-34Myr ago. This is consistent with the level of conservation between the X and Y chromosomes which has been estimated to be less than 94%. This suggests that the homologues began to diverge more than 24myr ago.

With many of the enzymes used in the Southern analysis of normal males and females GMGXY10 produced at least three Y bands and two X bands suggesting that it may detect multiple loci on both the X and the Y chromosome. Indeed it has been shown to have a Yp and a Yq homologue. In all three ape species analysed with *Taq*I, multiple Y

bands are observed together with two putative X bands. These latter are conserved between human, chimpanzee and gorilla perhaps indicating functional properties of the sequences. However more enzymes would need to be used on the primates before any conclusions could be drawn.

This region of XY homology has not been previously reported. Cooke et al., (1984) and Bickmore et al., (1987) reported a probe Y2:13 that detected a region of XY homology between Xq26-27 and Yqcen-q11.1 but these homologues were 100% conserved. This region has been extended to 47kb and still no restriction site differences have been observed between the X and Y homologues (Bickmore et al. 1987). Probe pG15 from this region only detects an X homologue in gorilla and chimpanzee. These data suggest a much more recent X to Y transfer than that detected here by GMGXY10. As well as revealing a new region of XY homology GMGXY10 also indicates that duplication events probably occurred on the Y chromosome prior to the divergence of the orang-outang lineage from that of humans.

4.13 The Evolution of the X and Y Chromosomes

The XY homologous probes used in this study allow certain conclusions to be reached about the evolution of the X and the Y chromosomes. These conclusions are drawn from the results of the polymorphism analysis and the comparative studies.

In the analysis of the human genomic DNA with 24 different restriction enzymes using the Yp/Xq21 homologous probes, each probe revealed 2-8 restriction site differences between the X and the Y chromosome. The initial assumption was that as the Y chromosome has few genetic functions these changes will mostly have occurred on the Y chromosome because of a lack of selection pressure. However this assumption ignores the lack of evidence that any of these sequences tested are functional and therefore selectable on the X chromosome. Furthermore, the increased polymorphic frequency of the X chromosome over the Y chromosome suggests that, since the transfer event to the Y chromosome, it is mutations on the X chromosome which account for most of the XY differences seen in this study.

In this particular case of the Yp/Xq21 homologous probes a proviso must be put on to the reasoning involved. A conclusion about the origin of XY differences has had to be drawn from the observed polymorphic frequencies of the X and Y chromosomes. However it is most probable that these two sets of chromosomal changes occurred at different periods in evolution. The transposition event must have occurred between the common ancestor of chimpanzee and man and the evolution of man. It is in this time interval that the differences between the X and Y chromosomes would accumulate. The result of this accumulation would be a homosapien X and a homosapien Y carrying a diverged XY homologous region. As homosapiens began to expand any further changes in these regions would be seen as either X or Y polymorphisms or race specific XY differences. Thus the XY differences would have evolved largely before homosapiens while the polymorphisms arose after homosapiens evolution. It is possible that different pressures were being exerted on these regions at these different points in evolution.

The primate studies revealed that for both GMGXY4 and GMGXY5 the human Y-specific *Taq*I restriction fragment was apparently conserved in size with the X homologue in gorilla. It could, therefore, be that in these two cases the human XY differences are the result of mutations on the X chromosome. On the other hand, the human X-specific *Xmn*I restriction fragments detected by GMGXY7 and GMGXY12 were conserved in size with the X homologue in gorilla. However while these observations show that at some restriction sites the Y chromosome may have evolved slower than the X chromosome a much more extensive primate study, perhaps involving the sequencing of the homologues in each species, will be required before any firm conclusions can be drawn.

In contrast to the indications that the Y chromosome suffers fewer point mutations than the X chromosome, the primate studies reveal that the Y chromosome has suffered many more DNA rearrangements than the X chromosome. None of the sequences analysed were found to be deleted from the X chromosome in evolution and none to be the result of a Y to X transfer. From the data presented here it is not possible to determine whether the X chromosome is as susceptible as the Y to inversions. However, this is

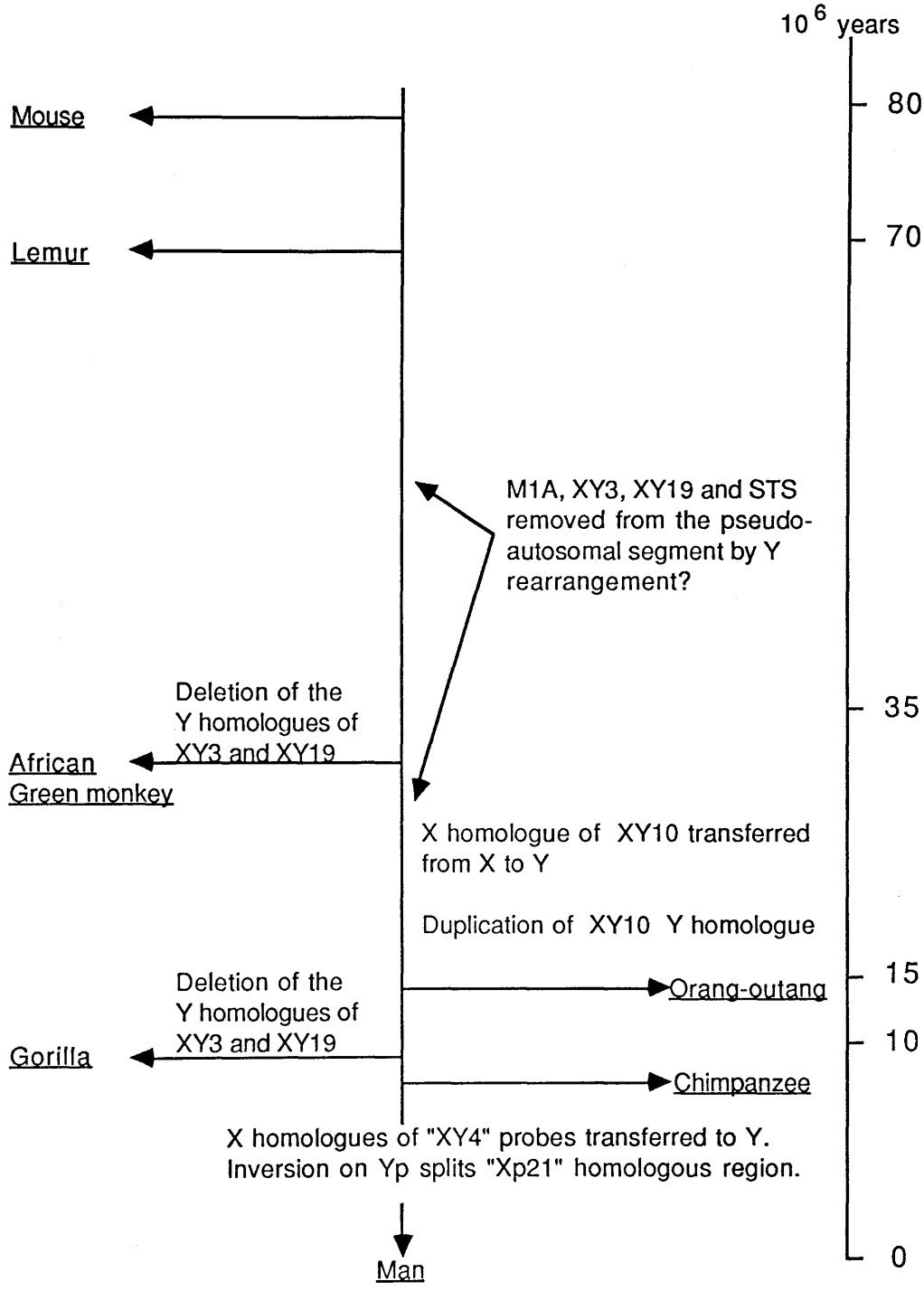


Figure 28: The proposed primate evolution of three regions of human XY homology

argued to be the case by the fact that comparative mapping of the mouse and human X chromosomes has revealed all shared loci to lie in six blocks within which their order is conserved between the two species (Amar et al., 1988). The increased incidence of Y chromosomal rearrangements is most simply explained by the Y chromosomes proposed lack of selectable markers, which allows it to support many more disruptions to its molecular structure than the other "gene dense" chromosomes. In addition, the fact that it does not require to pair or recombine along most of its length probably removes a further selection against the occurrence of such rearrangements.

Thus, by extrapolation of the polymorphism data to the behaviour of chromosomes in mammalian evolution, it is proposed that, for mechanistic reasons, the Y chromosome suffers a much lower point mutation rate than the other chromosomes. However, because of a lack of selection against the disruption of genetic functions and mieotic pairing, it is proposed that the Y chromosome will exhibit a greater fluidity in both the sequences which compose the chromosome and their order on the chromosome.

REFERENCES

References

Affara, N.A., Florentin, L., Morrison, N., Kwok, K., Mitchell, M., Cooke, A., Jamieson, D., Glasgow, L., Meredith, L., Boyd, E. & Ferguson-Smith, M.A. (1986a) Regional assignment of Y-linked DNA probes by deletion mapping and their homology with X chromosome and autosomal sequences. *Nucleic Acids Research*, **14**: 5353-5373.

Affara, N.A., Ferguson-Smith, M.A., Tolmie, J.L., Kwok, K., Mitchell, M., Jamieson, D., Cooke, A. & Florentin, L. (1986b) Variable transfer of Y-specific sequences in XX-males. *Nucleic Acids Research*, **14**: 5375- 5387.

Affara, N.A., Ferguson-Smith, M.A., Magenis, R.E., Tolmie, J.L., Boyd, E., Cooke, A. Jamieson, D., Kwok, K., Mitchell, M. & Snadden, L. (1987) Mapping the testis determinants by an analysis of Y-specific sequences in males with apparent XX and XO karyotypes and females with XY karyotypes. *Nucleic Acids Research*, **15**: 7325-7342.

Akesson, H.O., Hagberg, B. &Wahlstrom, J. (1980) Y-to-X chromosome translocation observed in two generations. *Human Genetics*, **55**: 39-42.

Allderdice, P.W., Aveling, J.V., Eales, B.A., Lewis, M.J., McAlpine, P.J., Ross, J.B. &Simms, R.J. (1983) Familial t(X;Y)(p22.3; q11) associated with short stature in 4 male and 5 female carriers and with X-linked ichthyosis and Anhydrosis in 4 male carriers. *American Journal of Human Genetics*, **35**: 124A.

Alvesalo, L. & de la Chapelle, A. (1981) Tooth size in two males with deletions of the long arm of the Y chromosome. *Annals of Human Genetics*, **45**: 49-54.

Amar, L., Dandolo, L., Hanauer, A., Ryder-cook, A., Arnaud, D., Mandel, J-L. & Avner, P. (1988) Conservation and reorganisation of loci on the mammalian X chromosome: a molecular framework for the identification of homologous subchromosomal regions in man and mouse. *Genomics*, **2**: 220-230.

Andersson, M., Page, D.C. & de la Chapelle, A. (1986) Chromosome Y-specific DNA is transferred to the short arm of the X chromosome in human XX males. *Science*, **233**: 786-788.

Andersson, M., Page, D.C., Pettay, D., Subrt, I., Turleau, C., Grouchy, J. & de la Chapelle, A. (1987) Y;autosome translocations and mosaicism in the etiology of 45,X maleness. *Cytogenetics and Cell Genetics*, **46**: 571.

Ballabio, A., Parenti, G., Carrozo, R., Sebastio, G., Andria, G., Buckle, V., Fraser, N., Craig, I., Rocchi, M., Romeo, G., Tobsisa, A.C. & Persico, M.G. (1987) Isolation and characterisation of a steroid sulphatase cDNA clone: genomic deletions in patients with X-chromosome-linked ichthyosis. *Proc. Natl. Acad. Sci. U.S.A.*, **84**: 4519-4523.

Benson, S.A. & Taylor, R.K. (1984) A rapid small-scale procedure for isolation of phage λ DNA. *BioTechniques*, **May/June**: 126-127.

Bernstein, R., Wadee, A., Rosendorff, J., Wessels, A. & Jenkins, T. (1986) Inverted Y chromosome polymorphism in the Gujerati Muslim Indian population of South Africa. *Human Genetics*, **74**: 223-229.

Bickmore, W.A. & Cooke, H.J. (1987) Evolution of homologous sequences on the human X and Y chromosomes, outside the meiotic pairing region. *Nucleic Acids Research*, **15**: 6261-6271.

Bird, A., Taggart, M., Frommer, M., Miller, O.J. & Macleod, D. (1985) A fraction of the mouse genome that is derived from islands of non-methylated, CpG-rich DNA. *Cell*, **40**: 91-99.

Bishop, C., Guellaen, G., Geldwerth, D., Voss, R., Fellous, M. and Weissenbach, J. (1983) Single-copy DNA sequences specific for the human Y chromosome. *Nature*, **303**: 831-832.

Bishop, C., Guellaen, G., Geldwerth, D., Fellous, M. and Weissenbach, J. (1984) Extensive sequence homologies between the Y and other human chromosomes. *Journal of Molecular Biology*, 173: 403-417.

Bishop, C., Weissenbach, J., Casanova, M., Bernheim, A. & Fellous, M. (1985) DNA sequences and analysis of the human Y chromosome. The Y Chromosome, Part A: Basic characteristics of the Y chromosome, ed. Sandberg, A.A. : 141-176. (Alan R. Liss, Inc.: New York)

Bobrow, M., Pearson, P.L., Pike, M.C., El-Alfi, O.S. (1971) Length variation in the quinacrine-binding segment of human Y chromosomes of different sizes. *Cytogenetics*, **10**: 190-198.

Book, J.A., Eilon, G., Halbrecht, I., Komlos, L. & Shabtay, F. (1973) Isochromosome Y 46,X,i(Yq) and female phenotype. *Clinical Genetics*, 4: 410-414.

Boyd, E., Ferguson-Smith, M.A., Ferguson-Smith, M.E., Jamieson, M.E., Russel, J.E., Aitken, D.A., Tippet, P. & Sanger, R. (1981) A case of X:Y translocation which maps the Xg locus to Xp24-pter. *Journal of Medical Genetics*, **18**: 224.

Brown, W.R.A. (1988) A physical map of the human pseudoautosomal region. *EMBO Journal*, 7: 2377-2385.

Buckle, V., Mondello, C., Darling, S., Craig, I.W. & Goodfellow, P.N. (1985) Homologous expressed gens in the human sex chromosome pairing region. *Nature*, 317: 739-741.

Buckle, V.J., Boyd, Y., Fraser, N., Goodfellow, P.N., Wolfe, J. & Craig, I.W. (1987) Localisation of Y chromosomal sequences in normal and "XX" males. *Journal of Medical Genetics*, **24**: 197-203.

Burgoyne, P.S. (1982) Genetic homology and crossing over in the X and Y chromosomes of mammals. *Human Genetics*, **61**: 85-92.

Burgoyne, P.S., Levy, E.R. & McLaren, A. (1986) Spermatogenic failure in mice lacking H-Y antigen. *Nature*, **320**: 170-172.

Chandley, A.C., Goetz, P., Hargreave, T.B., Joseph, A.M. & Speed, R.M. (1984) On the nature of and extent of XY pairing at meiotic prophase. *Cytogenetics and Cell Genetics*, **38**: 241-247.

Cooke, A., Gillard, E.F., Yates, J.R.W., Mitchell, M.J., Aitken, D.A., Weir, D.M., Affara, N.A. & Ferguson-Smith, M.A. (1988) X chromosome deletions detectable by flow cytometry in some patients with steroid sulphatase deficiency (X-linked ichthyosis). *Human Genetics*, **79**: 49-52.

Cooke, H.J. & Noel, B. (1979) Confirmation of Y/autosome translocations using recombinant DNA. *Human Genetics*, **50**: 39-44.

Cooke, H.J., Fantes, J. & Green, D. (1983) Structure and evolution of human Y chromosome DNA. *Differentiation*, **23**: 548-555.

Cooke, H.J., Brown, W.A.R. & Rappold, G.A. (1984) Closely related sequences on human X and Y chromosomes outside the pairing region. *Nature*, **311**: 259-261.

Cooke, H.J., Brown, W.A.R. & Rappold, G.A. (1985) Hypervariable telomeric sequences from the human sex chromosomes are pseudoautosomal. *Nature*, **317**: 687-692.

Cooper, D.N. & Schmidtke, J. (1984) DNA restriction length fragment polymorphisms and heterozygosity in the human genome. *Human Genetics*, **66**: 1-16.

Cooper, D.N., Smith, B.A., Cooke, H.J., Niemann, S. & Schmidtke, J. (1985) An estimate of unique sequence heterozygosity in the human genome. *Human Genetics*, **69**: 201-205.

Daiger, S.P., Wildin, R.S. & Su, T.S. (1982) Sequences on the human Y chromosome homologous to the autosomal gene for argininosuccinate synthetase. *Nature*, **298**: 683-684.

Darling, S.M., Banting, G.S., Pym, B., Wolfe, J. & Goodfellow, P.N. (1985) Cloning an expressed gene shared by the human sex chromosomes. *Proc. Natl. Acad. Sci. U.S.A.*, **83**: 135-143.

Davies, K.E., Mandel, J-L., Weissenbach, J. & Fellous, M. (1987) Report of the committee on the genetic constitution of the X and Y chromosomes. *Cytogenetics and Cell Genetics*, **46**: 277-315.

Davis, R.M. (1981) Localisation of male determining factors in man: a thorough review of structural anomalies of the Y chromosome. *Journal of Medical Genetics*, **18**: 161-195.

de la Chapelle, A., Hartling, H., Mikko, N. & Wennstrom, J. (1964) XX sex chromosomes in a human male. First case. *Acta Med. Scand. Suppl.* **412**: 25-38.

de la Chapelle, A., Tippet, P.A., Wetterstrand, G. & Page, D.C. (1984) Genetic evidence for X-Y interchange in a human XX male. *Nature*, **307**: 170-171.

de la Chapelle, A., Page D.C., Brown, L., Kaski, U., Parvinen, T. & Tippet, P.A. (1986) The origin of 45, X males. *American Journal of Medical Genetics*, **38**: 330-340.

Disteche, C.M., Casanova, M., Saal, H., Friedman, C., Sybert, V., Graham, J., Thuline, H., Page, D.C. & Fellous, M. (1986) Small deletions of the shoprt arm of the Y chromosome in 46, XY females. *Proc. Natl. Acad. Sci. U.S.A.*, **83**: 7841-7844.

Ducos, J., Marty, Y., Sanger, R. & Race, R.R. (1971) Xg and X chromosome inactivation. *Lancet*, **ii**: 219-220.

Engel, W., Klemme, B. & Ebrecht, A. (1981) Serological evidence for H-Y antigen in XO female mice. *Human Genetics*, **57**: 68-70.

Evans, E.P., Burtenshaw, M.D. & Cattanach, B.M. (1982) Meiotic crossing over between the X and Y chromosomes of male mice carrying the Sxr factor. *Nature*, **300**: 443-445.

Evans, H.J., Buckton, K.E., Spowart, G. & Carothers, A.D. (1979) Heteromorphic X chromosomes in 46, XX males: evidence for the involvement of X-Y interchange. *Human Genetics*, **49**: 11-31.

Feinberg, A.P. & Vogelstein, B. (1984) Addendum: "Oligonucleotide labelling". *Analytical Biochemistry*, **137**: 266-267.

Ferguson-Smith, M.A. (1965) Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. *Journal of Medical Genetics*, **2**: 142-155.

Ferguson-Smith, M.A. (1966) X-Y chromosomal interchange in the aetiology of true hermaphroditism and of XX Klinefelter's syndrome. *Lancet*, **ii**: 475-476.

Ferguson-Smith, M.A. & Affara, N.A. (1988) Accidental XY recombination and the aetiology of XX males and true hermaphrodites. *Phil. Trans. Roy. Soc. Lon.*, B **322**: 133-144.

Ferguson-Smith, M.A., Boyd, E., Ferguson-Smith, M.E., Pritchard, J.G., Yusuf, A.F.M. & Gray, B. (1969) Isochromosome for for long arm of Y chromosome in patient with Turner's syndrome and sex chromosome mosaicism. *Journal of Medical Genetics*, **6**: 422-425.

Ferguson-Smith, M.A., Sanger, R., Tippett, P., Aitken, D.A. & Boyd, E. (1982) A familial t(X;Y) translocation which assigns the Xg blood group locus to the region Xp22.3-pter. *Cytogenetics and Cell Genetics*, **32**: 273-274.

Ferguson-Smith, M.A., Affara, N.A. & Magenis, R.E. (1987) Ordering of Y-specific sequences by deletion mapping and analysis of X-Y interchange males and females. *Development*, **101** (suppl.): 41-50.

Ferris, S.D., Wilson, A.C. & Brown, W.M. (1981) Evolutionary tree for apes and humans based on cleavage patterns of human mitochondrial DNA. *Proc. Natl. Acad. Sci. U.S.A.*, **78**: 2432-2436.

Florentin, L. (1987) D. Phil. thesis, University of Glasgow.

Ford, C.E., Jones, K.W., Polani, P.E., de Almeida, J.C. & Briggs, J.H. (1959) A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*, **i**: 711-713.

Frommer, M., Prosser, J. & Vincent, P.C. (1984) Human satellite I sequences includea male specific 2.47kb tandemly repeated unit containing one ALU family member per repeat. *Nucleic Acids Research*, **12**: 2887-2900.

Gardiner-Garden, M. & Frommer, M. (1987) CpG islands in vertebrate genomes. *Journal of Molecular Biology*, **196**: 261-282.

Gartler, S.M. & Riggs, A.D. (1983) Mammalian X chromosome inactivation. *Annual Reviews in Genetics*, **17**: 155-190.

Geldwerth, D., Bishop, C., Guellaen, G., Koenig, M., Vergnaud, G., Mandel, J.L. & Weissenbach, J. (1985) Extensive DNA sequence homologies between the human Y and the long arm of the X chromosome. *EMBO Journal*, **4**: 1739-1743.

Gillard, E.F., Affara, N.A., Yates, J.R.W., Goudie, D.R., Lambert, J., Aitken, D.A. & Ferguson-Smith, M.A. (1987) Deletion of a DNA sequence in eight of nine families with X-linked ichthyosis (steroid sulphatase deficiency). *Nucleic Acids Research*, **15**: 3977-3985.

Goodfellow, P.N. & Tippett, P.A. (1981) A human quantitative polymorphism related to Xg blood groups. *Nature*, **289**: 404-405.

Goodfellow, P.N. & Andrews, P.W. (1982) Sexual differentiation and H-Y antigen. *Nature*, **295**: 11-13.

Goodfellow, P.N., Banting, G., Sheer, D., Ropers, H.H., Caine, A., Ferguson-Smith, M.A., Povey, S. & Voss, R. (1983) Genetic evidence that a Y-linked gene in man is homologous to a gene on the X chromosome. *Nature*, **302**: 346-349.

Goodfellow, P.N., Pym, B., Mohandas, T. & Shapiro, L.J. (1984) The cell surface antigen locus MIC2X escapes X-inactivation. *American Journal of Human Genetics*, **36**: 777-782.

Goodfellow, P.N., Darling, S. & Wolfe, J. (1985) The human Y chromosome. *Journal of Medical Genetics*, **22**: 329-344.

Goodfellow, P.J., Darling, S., Thomas, N.S. & Goodfellow, P.N. (1986) A pseudoautosomal gene in man. *Science*, **234**: 740-743.

Goodfellow, P.J., Pritchard, C., Tippett, P.A. & Goodfellow, P.N. (1987) Recombination between the X and Y chromosomes: implications for the relationship between MIC2, XG and YG. *Annals of Human Genetics*, **51**: 161-167.

Gorman, J.G., Kire, J., Treacy, A.M. & Cahan, A. (1963) The application of Xga antiserum to the question of red cell mosaicism in female heterozygotes. *Journal of Laboratory Clinical Medicine*, **61**: 642-649.

Guellaen, G., Casanova, M., Bishop, C., Geldwerth, D., Andre, G., Fellous, M. & Weissenbach, J. (1984) Human XX males with Y single copy DNA fragments. *Nature*, **307**: 172-173.

Heilig, R., Hanauer, A., Grzeschik, K.H., Hors-Cayla, M.C. & Mandel, J.L. (1984) Actin-like sequences are present on human X and Y chromosomes. *EMBO Journal*, **38**: 1803-1807.

Hodgson, S.V., Robertson, M.E., Fear, C.N., Goodship, J., Malcolm, S., Jay, B., Bobrow, M. & Pembrey, M.E. (1987) Prenatal diagnosis of X-linked choroideremia with mental retardation, associated with cytologically detectable X-chromosome deletion. *Human Genetics*, **75**: 286-290.

Hofker, M.H., Skaastad, M.I., Bergen, A.A.B., Wapenaar, M.C., Bakker, E., Millington-Ward, A., van Ommen, G.J.B. & Pearson, P.L. (1986) The X chromosome shows less genetic variation at restriction sites than the autosomes. *American Journal of Medical Genetics*, **39**: 438-451.

Jacobs, P.A. & Strong, J.A. (1959) A case of human intersexuality having a possible XXY sex- determining mechanism. *Nature*, **183**: 302-303.

Kalaitzidaki, M., Theriault, A., Boyd, E., Affara, N.A., Cooke, A. & Ferguson-Smith, M.A. (1987) Y-specific sequences located in distal Xp in one of two X chromosomes in twelve X-Y interchange males. *Cytogenetics and Cell Genetics*, **46**: 635-636.

Kaplan, J.C. & Carrit, B. (1987) Report of the committee on the genetic constitution of chromosomes 20, 21 and 22. *Cytogenetics and Cell Genetics*, **46**: 257-276.

Keitges, E., Rivest, M., Siniscalco, M. & Gartler, S.M. (1985) X-linkage of STS in the mouse is evidence for a functional Y-linked allele. *Nature*, **315**: 226-227.

Knowlton, R.G., Nelson, C., Brown, V.A., Page, D. & Donis-Keller, H. (1987) A highly polymorphic locus on Xp with homology to Yq chromosome sequences. *Cytogenetics and Cell Genetics*, **46**: 640.

Koenig, M., Camerino, G., Heilig, R. & Mandel, J.L. (1984) ADNA fragment from the human X chromosome short arm which detects a partially homologous sequence on the Y chromosome's long arm. *Nucleic Acids Research*, **12**: 4097-4109.

Koenig, M., Moisan, J.P., Heilig, R. & Mandel, J.L. (1985) Homologies between X and Y chromosomes detected by DNA probes: localisation and evolution. *Nucleic Acids Research*, **13**: 5485-5503.

Kohn, G., Yarkoni, S. & Cohen, M.M. (1980) Two conceptions in a 45,X woman. *American Journal of Human Genetics*, **5**: 339-343.

Kunkel, L.M., Smith, K.D. & Boyer, S.H. (1977) Human Y chromosome specific reiterated DNA. *Science*, **191**: 1189-1190.

Kunkel, L.M., Tantravahi, U., Kurmit, D.M., Eisenhard, M., Bruns, J.P. & Latt, S.A. (1983) Identification and isolation of transcribed human X chromosome DNA sequences. *Nucleic Acids Research*, **11**: 7961-7979.

Leroy, P., Seboun, E., Mattei, M.G., Fellous, M; & Bishop, C.E. (1987) Testis-specific transcripts detected by a human Y-DNA-derived probe. *Development*, **101** (suppl.): 177-183.

Levy, R., Dilley, J., Fox, R.I. & Warnlee, R. (1979) A human thymus leukaemia antigen defined by hybridoma monoclonal antibodies. *Proc. Natl. Acad. Sci. U.S.A.*, **76**: 6552-6556.

Lindsay, S. & Bird, A.P. (1987) Use of restriction enzymes to detect potential gene sequences in mammalian DNA. *Nature*, **327**: 336-338.

McKay, R.D.G., Bobrow, M; & Cooke, H.J. (1978) The identification of a repeated DNA sequence involved in the karyotype polymorphism of the Y chromosome. *Cytogenetics and Cell Genetics*, **21**: 19-32.

McLaren, A., Simpson, E., Tomonari, K., Chandler, P. & Hogg, H. (1984) Male sexual differentiation in mice lacking H-Y antigen. *Nature*, **312**: 552-555.

Madan, K. & Walker, S. (1974) Possible evidence for Xp+ in an XX male. *Lancet*, **i**: 1223.

Magenis, R.E., Webb, M.J., McKean, R.S., Tomar, D., Allen, L.J., Kammer, H., van Dyke, D.L. & Lovrien, E. (1982) Translocation (X;Y)(p22.33;p11.2) in XX males: etiology of male phenotype. *Human Genetics*, **62**: 271-276.

Magenis, R.E., Tochen, M.L., Holahan, K.P., Carey, T., Allen, L. & Brown, M.G. (1984) Turner syndrome resulting from partial deletion of Y chromosome short arm: localisation of male determinants. *Journal of Paediatrics*, **105**: 916-919.

Magenis, R.E., Brown, M.G., Donlon, T., OLson, S.B., Sheehy, R. & Tomar, D. (1985) Structural aberrations of the Y chromosome, including the nonfluorescent Y: cytological origin and consequences. The Y Chromosome, Part A: Basic characteristics of the Y chromosome, ed. Sandberg, A.A.: 537-574. (Alan R. Liss, Inc.: New York)

Magnelli, N.C., Vianna-Morgante, A.M., Frota-Pessoa, O. & Taboada-Lopez, M.G. (1974) Turner's syndrome and 46, X, i(Yq) karyotype. *Journal of Medical Genetics*, **11**: 403-406.

Maniatis, T., Fritsch, E.F. & Sambrook, J. (1982) Molecular cloning: a laboratory manual. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Mendelsohn, M.L., Mayall, B.H., Bogart, E., Moore, D.H. & Perry, B.H. (1973) DNA content and DNA based centromeric index of the 24 human chromosomes. *Science*, **179**: 1126-1129.

Metaxotou, C., Ikkos, D., Panagiotopoulou, M., Alevizaki, M., Mavrou, A., Tsenghi, C. & Matsaniotis, N. (1983) A familial X/Y translocation in a boy with ichthyosis, hypogonadism and mental retardation. *Clinical Genetics*, **24**: 380-383.

Mohandas, T., Sparkes, R.S., Hellkuhl, B., Greszchik, K.H. & Shapiro, L.J. (1980) Expression of an X-linked gene from an inactive X chromoosme in mouse-human hybrid cells: further evidence for the non-inactivation of the steroid sulphatase locus in man. *Proc. Natl. Acad. Sci. U.S.A.*, **77**: 6759-6763.

Muller, C.R., Davies, K.E., Cremer, C., Rappold, G., Gray, J.W. & Ropers, H.H. (1983) Cloning of genomic sequences from the human Y chromosome after purification by dual beam flow-sorting. *Human Genetics*, **64**: 110-115.

Muller, U. (1982) Identification and function of serologically detectable H-Y antigen. *Human Genetics*, **61**: 91-94.

Muller, U., Donlon, T., Schmid, M., Fitch, N., Richer, C.L., Lalande, M. & Latt, S.A. (1986a) Deletion mapping of the testis determining locus with DNA probes in 46, XX males and in 46, XY and 46, X, dic(Y) females. *Nucleic Acids Research*, **14**: 6489-6505.

Muller, U., Lalande, M., Donlon, T. & Latt, S.A. (1986b) Moderately repeated DNA sequences specific for the short arm of the human Y chromosome are present in XX males and are reduced in copy number in an XY female. *Nucleic Acids Research*, **14**: 1325-1340.

Murray, N.E. (1983) Phage lambda and molecular cloning in "Lambda II", ed, Hendrix, R.W., Roberts, J.W., Stahl, F.W. & Weisberg, R.A.,: 395-432. (Cold Spring Harbor Laboratory)

Ohno, S. (1967) Sex chromosomes and sex-linked genes. Monographs on Endocrinology, ed. Londhart, A., Mann, T., Samuels, L.T. & Zander, J. (Springer-Verlag).

Page, D.C. (1986) Sex reversal: deletion mapping the male-determining function of the human Y chromosome. *Cold Spring Harbour Symposium on Quantitative Biology*, **51**: 229-235.

Page, D.C., Martinville, B., Barker, D., Wyman, A., White, R., Francke, U. & Botstein, D. (1982) Single copy sequence hybridises to polymorphic and homologous loci on human X and Y chromosomes. *Proc. Natl. Acad. Sci. U.S.A.*, **79**: 5352-5356.

Page, D.C., Harper, M.E., Love, J. & Botstein, D. (1983) Occurrence of a transposition from the X chromosome long arm to the Y chromosome short arm during human evolution. *Nature*, **311**: 119-123.

Page, D.C., de la Chapelle, A. & Weissenbach, J. (1985) Chromosome Y specific DNA in related human XX males. *Nature*, **315**: 224-226.

Page, D.C., Mosher, R., Simpson, E.M., Fisher, E.M.C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A. & Brown, L.G. (1987) The sex determining region of the human Y chromosome encodes a finger protein. *Cell*, **51**: 1091-1104.

Pearson, P.L. & Bobrow, M. (1970) Definitive evidence for the short arm of the Y chromosome associating with the X chromosome during meiosis in the human male. *Nature*, **226**: 959-961.

Petit, C., de la Chapelle, A., Levilliers, J., Castillo, S., Noel, B. & Weissenbach, J. (1987) An abnormal terminal XY interchange accounts for most but not all cases of human XX maleness. *Cell*, **49**: 595-602.

Petit, C., Levilliers, J. & Weissenbach, J. (1988) Physical mapping of the human pseudoautosomal region; comparison with genetic linkage map. *EMBO Journal*, **7**: 2369-2376.

Polani, P.E. (1982) Pairing of X and Y chromosomes, noninactivation of X-linked genes and the maleness factor. *Human Genetics*, **60**: 207-211.

Pritchard, C.A., Goodfellow, P.J. & Goodfellow, P.N. (1987) Mapping the limits of the human pseudoautosomal region and a candidate sequence for the male determining gene. *Nature*, **328**: 273-275.

Rappold, G.A., Cremer, T., Cremer, C., Back, W., Bogenburger, J. & Cooke, H.J. (1984) Chromosome assignment of two cloned DNA probes hybridising predominately to the human sex chromosomes. *Human Genetics*, **65**: 257-261.

Rary, J.M., Middleton, A., Mulivor, R.A., Greene, A.E. & Coriell, L.L. (1979) A (Y;17) translocation in a fibroblast culture in a female with 46 chromosomes. *Cytogenetics and Cell Genetics*, **24**: 198.

Rigby, P.W.J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) Labelling DNA to high specific activity invitro by nick-translation with DNA polymerase I. *Journal of Molecular Biology*, **113**: 237-251.

Rosenfeld, R.G., Luzzati, L., Hintz, R.L., Miller, O.J., Koo, G.C. & Wachtel, S.S. (1979) Sexual and Somatic determinants of the human Y chromosome: studies in a 46, XYp- phenotypic female. *American Journal of Medical Genetics*, **31**: 458-468.

Rouyer, F., Simmler, M-C., Johnson, C., Vergnaud, G., Cooke, H.J. & Weissenbach, J. (1986) A gradient of sex linkage in the pseudoautosomal region of the human sex chromosomes. *Nature*, **319**: 291-295.

Rouyer, F., Simmler, M-C., Page, D.C. & Weissenbach, J. (1987) A sex chromosome rearrangement in a human XX male caused by Alu-Alu recombination. *Cell*, **51**: 417-425.

Sanger, R., Tippett, P.A. & Gavin, J. (1971) Xg blood groups and sex abnormalities in people of Northern European ancestry. *Journal of Medical Genetics*, **8**: 417-426.

Sanger, R., Tippett, P.A., Gavin, J., Teesdale, P. & Daniels, G.L. (1977) Xg groups and sex chromosome abnormalities in people of Northern European ancestry: an addendum. *Journal of Medical Genetics*, **14**, 210-211.

Schmidtke, J. & Schmid, M. (1980) Regional assignment of a 2.1kb repetitive sequence to the distal part of the human Y-chromatin. *Human Genetics*, **55**: 255-257.

Sibley, C.G. & Alquist, J.E. (1987) DNA hybridisation evidence of hominoid phylogeny: results from an expanded data set. *Journal of Molecular Evolution*, **26**: 99-121.

Simmler, M-C., Rouyer, F., Vergnaud, G., Nystrom-Lahti, M., Ngo, K.Y., de la Chapelle, A. & Weissenbach, J. (1985) Pseudoautosomal DNA sequences in the pairing region of the human sex chromosomes. *Nature*, **317**: 692-697.

Simmler, M-C., Johnsson, C., Petit, C., Rouyer, F., Vergnaud, G. & Weissenbach, J. (1987) Two highly polymorphic minisatellites from the pseudoautosomal region of the human sex chromosomes. *EMBO Journal*, **6**: 963-969.

Simpson, E., Chandler, P., Goulmy, E., Disteche, C.M. & Page, D.C. (1987) Separation of the genetic loci for the H-Y antigen and for testis determination on the human Y chromosome. *Nature*, **326**: 876-878.

Singer, M.F. (1982) Highly repeated sequences in mammalian genomes. *International Reveiws in Cytology*, **76**: 67-112.

Singh, L. & Jones, K.W. (1982) Sex reversal in the mouse (mus musculus) is caused by a recurrent non-reciprocal crossover involving the X and an aberrant Y chromosome. *Cell*, **28**: 205-216.

Skibsted, L., Westh, H. & Niebuhr, E. (1984) X long arm deletions: a review of non-mosaic cases studied with banding techniques. *Human Genetics*, **67**: 1-5.

Solari, A.J. (1980) Synaptonemal complexes and associated structures in microspread human spermatocytes. *Chromosoma*, **81**: 315-337.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, **98**: 503-517.

Szabo, P., Kunkel, L.M., Yu, L.C., George, D. & Smith, K.D. (1980) Chromosomal distribution of DNA sequences derived from the human Y chromosome in human and higher primates. *Cytogenetics and Cell Genetics*, **25**: 212-213.

Tabor, A., Anderson, O., Lundsteen, C., Niebuhr, E. & Sardemann, H. (1983) Interstitial deletion of the "critical region" of the long arm of the X chromosome in a mentally retarded boy and his normal mother. *Human Genetics*, **64**: 196-199.

Therkelsen, A.J. (1964) Sterile male with the chromosome constitution 46,XX. *Cytogenetics*, **3**: 207-218.

Tiepolo, L. & Zuffardi, O. (1976) Localisation of factors controlling spermatogenesis in the non-fluorescent portion of the human Y chromosome long arm. *Human Genetics*, **34**: 119-124.

Tiepolo, L., Zuffardi, O. & Rodewald, A. (1977) Nullisomy for the distal portion of Xp in a male child with an X/Y translocation. *Human Genetics*, **39**: 277-281.

Tiepolo, L., Zuffardi, O., Fraccaro, M., di Natale, D., Gargantini, L., Muller, C.R. & Ropers, H.H. (1980) Assignment by deletion mapping of the steroid sulphatase X-linked ichthyosis locus to Xp22.3. *Human Genetics*, **54**: 205-206.

Tippett, P.A., Shaw, M-A., Green, C.A. & Daniel, G.L. (1986) The 12E7 red cell quantitative polymorphism is controlled by the Y-borne locus Yg. *Annals of Human Genetics*, **50**: 339-347.

Traub, P. & Nomura, M. (1968) Structure and function of E. Coli ribosome V. Reconstitution of functionally active 30S ribosomal particles from RNA and proteins. *Proc. Natl. Acad. Sci. U.S.A.*, **59**: 777-784.

Van den Berghe, H., Petit, P. & Fryns, J.P. (1977) Y to X translocation in man. *Human Genetics*, **36**: 129-141.

Vergnaud, G., Page, D.C., Simmler, M-C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A. & Weissenbach, J. (1986) A deletion map of the human Y chromosome based on DNA hybridisation. *American Journal of Human Genetics*, **38**: 109-124.

Verma, R.S., Afroza, H; & Dosik, H. (1983) Racial variation of a non-fluorescent segment of the Y chromosome in East Indians. *Journal of Medical Genetics*, **20**: 102-106.

Vieira, J. & Messing, J. (1982) The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene*, **19**: 259-268.

Willard, H.F. (1985) Chromosome-specific organisation of human alpha satellite DNA. *American Journal of Human Genetics*, **37**: 524-532.

Winsor, E.J.T., Ferguson-Smith, M.A. & Shire, J.G.M. (1978) Meiotic studies in mice carrying the sex reversal (Sxr) factor. *Cytogenetics and Cell Genetics*, **21**: 11-18.

Wolf, U., Fraccaro, M., Mayerova, A., Hecht, T., Zuffardi, O. & Hameister, H. (1980) Turner syndrome patients are H-Y positive. *Human Genetics*, **54**: 315-318.

Wolfe, J., Erickson, R.P., Rigby, P.W.J. & Goodfellow, P.N. (1984) Cosmid clones derived from both euchromatic and heterochromatic regions of the human Y chromosome. *EMBO Journal*, **3**: 1997-2003.

Wolfe, J., Darling, S.M., Erickson, R.P., Craig, I.W., Buckle, V.J., Rigby, P.W.J., Willard, H.F. & Goodfellow, P.N. (1985) Isolation and characterisation of an alphoid centriomeric repeat family from the human Y chromosome. *Journal of Molecular Biology*, **182**: 477-485.

Wyss, D., Delozier, C.D., Daniell, J. & Engel, E. (1982) Structural anomalies of the X chromosome: ppersonal observation and review of non-mosaic cases. *Clinical Genetics*, **21**: 145-159.

Yen, P.H., Allen, E., Marsh, B., Mohandas, T., Wang, N., Taggart, R.H. & Shapiro, L.J. (1987a) Cloning and expression of STS cDNA and the frequent occurrence of deletions in STS deficiency: implications for X-Y interchange. *Cell*, **49**: 443-454.

Yen, P.H., Marsh, B., Allen, E., Mohandas, T. & Shapiro, L.J. (1987b) Organisation of the human steroid sulphatase gene and homology between the X and Y chromosomes. *Human Genetics*, **41** (suppl.): A248.

Young, R.A. & Davis, R.W. (1983) Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. U.S.A.*, **80**: 1194-1198.

Zech, L. (1969) Investigation of metaphase chromosomes with DNA binding fluorochromes. *Experimental Cell Research*, **58**: 463-467.