SELECTION AND CHARACTERISATION OF TRANSFORMED BHK21 CELLS ALTERED IN RESPONSE TO FIBRONECTIN

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To my parents who made it possible

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List of abbreviations

A-CAM	Adherens junction-specific cell adhesion molecule
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CAM	Cell adhesion molecule
cDNA	Copy DNA
CHO	Chinese hamster ovary cells
Con A	Concanavalin A
DNA	Deoxyribonucleic acid
EC	Embryonal carcinoma cells
ECM	Extracellular matrix
EDTA	Ethylene-diamine tetra acetic acid
Fab	Antigen binding fragment (of antibody)
GAG	Glycosaminoglycan
HGPRT	Hypoxanthine guanine phosphoribosyl transferase
I-CAM	Intercellular adhesion molecule
IgG	Immunoglobulin G
IRM	Inteference reflection microscopy
Kd	Kilodaltons
L-CAM	Liver cell adhesion molecule
LFA-1	Lymphocyte function associated antigen
Mab	Monoclonal antibody
MNNG	N-methyl-N' -nitro-N' -nitrosoguanidine
MSA	Mean spread area
N-CAM	Neural cell adhesion molecule
NaDOC	Sodium deoxycholate
Ng-CAM	Neuro-glia cell adhesion molecule
PAGE	Polyacryamide gel electrophoresis
PEI	Poly ethyleneimine
PHA	Phytohaemagglutinin

PMN	Polymorphonuclear (Neutrophil)
PMSF	Phenyl methyl sulphonyl fluoride
PRPP	Phosphoribosyl pyrophosphate
RNA	Ribonucleic acid
RSV	Rous sarcoma virus
SAM	Substratum attachment material
SDS	Sodium dodecyl sulphate
TAME	p-Tosyl-1-arginine methyl ester-HCl
TCA	Trichloro acetic acid
TG	6-thioguanine
TGFs	Transforming growth factors
TS	Thrombospondin
UV	Uvomorulin
VLA	Very late antigen
WGA	Wheat germ agglutinin

Summary

I found that selection of cell lines which do not respond to fibronectin was easy from polyoma transformed cells, but yielded no such cell lines from Schmidt-Ruppin transformed cells. Mixing of Py3 mutants (F2) with SR-WT cells showed that Py3 mutants could be recovered when present at about $1:10^6$ and higher inputs, while the frequency of SR mutants was zero.

Cells with a genetic marker (resistance to 6-thioguanine) were isolated from polyoma transformed cells. Two colonies were selected (TG1, TG2). From one of these lines (TG2), two different low adherent lines both deficient in response to fibronectin, were selected and recloned on soft agar. Variant 1 (TG2F1) has compact colonies of rounded cells while variant 2 (TG2F2) has scattered colonies with a few slightly spread cells. Both are resistant to 6-thioguanine and have undetectable HGPRT-ase activity.

Revertants of two different morphologies were isolated from the compact colony Py3 mutant (F2). Variant 1 (F2R1) has well spread cells while cells of variant 2 (F2R2) are less well spread. Both revertants were found to attach and spread on fibronectin-coated surfaces. A third type of revertant has been observed several times, which had extremely well spread cells, better spread than wild type. Attempts to isolate this type of revertant were unsuccessful.

TG mutants (TG2F1 & TG2F2) were found to spread on Con A but not on WGA-coated surfaces. On poly L-lysine, both wild type and mutants adhere, but none of them spread. Wild type but not mutants spread fully on poly L-lysine in presence of 1% serum. Mutants did not spread on serum or fibronectin.

Mn^{2+} ions were effective in inducing the spreading of TG2-WT and both variants. 10^{-6} M Mn^{2+} was 50% effective in inducing the spreading of TG-WT on fibronectin while 10^{-3} M was 50% effective on haemoglobin. Higher concentrations such as 10^{-2} M were required for TG2F1 cells on both surfaces. 10^{-4} M was effective with TG2F2 cells on fibronectin while 10^{-3} M was effective on

haemoglobin. Mg^{2+} at 10^{-2} M was as effective as Mn^{2+} with parental cells on fibronectin but not as effective as Mn^{2+} with variants on either surfaces. Co^{2+} was less effective at 10^{-2} M with all cell lines compared to Mn^{2+} or Mg^{2+}.

Analysis of lectin binding proteins associated with the detergent soluble extracts of Py3 (NaDOC-solubilised membranes and Triton extracts) failed to reveal differences between wild type, mutants and revertants.

The molecular basis for the differences between wild type and mutants could be in the fibronectin receptor, or in signal transduction required to induce the spreading response, but remains unidentified.

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Chapter one

INTRODUCTION

CELL ADHESION

Adhesion is applied in the biological context to the interaction of cells with each other and with surfaces, and includes both specific and non-specific phenomena. Adhesion phenomena are implicated in a wide range of processes, involving prokaryotes and eukaryotes, and both unicellular and multicellular organisms.

BIOLOGICAL SIGNIFICANCE

There is no subject more fundamental for the morphology and physiology of multicellular organisms than cell adhesion, because the adhesion that cells make with one another lies at the very basis of multicellularity. The form and functioning of all creatures that consist of more than one cell depend on these cells adhering firmly to one another and often to the extracellular materials that intervene (Trinkaus, 1984). When such adhesions break down or are modified, as in malignancy, the life of the individual is in danger. Cell adhesion appears to be a central phenomenon in such processes as morphogenetic movements, cell locomotion, anchorage dependence, contact inhibition of locomotion, tumour metastasis, and the interaction of blood cells with non-circulating cells.

1) Morphogenetic movement

Morphogenesis in all animal tissues seems to be dependent on a limited number of primary processes, one of which is cell adhesion, including both cell to cell and cell to substratum adhesion. In all solid tissues, cells adhere to each other and to the extracellular matrices.

Adhesive events are instrumental both in embryonic development and in the maintenance of tissue architecture (Ekblom et al., 1986). During development of an organism, cells are created by cell division, are brought together by a variety of motions, and are reduced in number by cell death. The groups of cells that are formed and modified in this fashion act as the units of morphogenesis. In some

locations, individual cells cohere for a time and then disperse, in others, sheets of cells migrate, spread and fold, forming tissues and organs of mixed origins.

A common factor in all of these processes is the cohesion of the cell populations as functional units through the process of cell adhesion. Analysis of the molecular events involved in the control of adhesion of cells is therefore critical for understanding the formation, maintenance, and function of multicellular structures during development. For example, during morphogenetic movements in young embryos, both cell-cell and cell-substrate adhesion play an important role in allowing the transformation of the blastula into a three layered embryo, and also contribute to the induction and shaping of the nervous system (Thiery et al., 1985).

During amphibian gastrulation, in some species, the primary mesenchyme cells break loose from the ectoderm at the gray crescent of the blastula and then crawl over the inner face of the wall of the blastula by putting out contractile pseudopodia with adhesive tips which can firmly attach to the ectodermal wall (Trinkaus, 1984). Studies in the chick showed that major cell-cell junctions (tight and gap junctions) (see later) are lost by neural crest cells, but this is described as occurring well before migration actually happens. In neurulation, Trinkaus (1984) found that the folding of the neural plate into a tube may require the increase in adhesion of the neural tube cells with one another and a decrease in adhesion to the mesenchymal cells.

In the chick embryo, crest cells detach from the neural tube during and after its closure and migrate to various sites where they undergo differentiation. The behaviour of crest cells must depend on the control of adhesion, both between the cells, and to the extracellular matrix. From this point of view, the natural history of crest cells can be described as a series of phases involving different adhesive systems (Thiery et al., 1985).

In order for single cells to migrate, the adhesive forces between individual cells and between cells and the substratum must be weak to enable them to move quickly,

without which morphogenetic movement cannot happen.

Neural cells are believed to adhere to each other by the action of a cell adhesion molecule called N-CAM (see later). During the onset of migration N-CAM has been shown to disappear from the migrating cells, presumably resulting in a weak intercellular adhesion, which enables the cells to migrate on their substrates.

A large number of reports have suggested that the neural cell adhesion molecule N-CAM has a role in embryogenesis (see later). It is perhaps not surprising that effective cell migration requires a depletion in N-CAM surface density.

The migration of neural cells on substrata during morphogenesis has been found to require fibronectin, which also supports the migration of these cells in vitro (Duband et al., 1985; 1986; Goodman et al., 1985; Thiery et al., 1985) and in vivo (Hynes and Yamada, 1982; Rovasio et al., 1983; Johnson, 1986).

When part of the blastocoelic roof is inverted, mesodermal cells avoid the area that now lacks an extracellular matrix (Thiery et al., 1985). Microinjection of monovalent anti- fibronectin antibodies into the blastocoelic cavity of late bastulae or early gastrulae blocks gastrulation (Boucaut et al., 1984 a). Similarly, when Arg-Gly-Asp-Ser containing peptides are injected, gastrulation is arrested (Boucaut et al., 1984 b). In Drosophila, injection of such peptide (Naidet et al., 1987) or anti-fibronectin antibodies (Gratecos et al., 1988) into embryos also inhibit gastrulation . [This peptide sequence, which is contained in the cell- binding site of fibronectin, is directly involved in the mechanism of binding of cells to fibronectin (Pierschbacher and Ruoslahti, 1984 a; Yamada and Kennedy, 1984)] (see later).

The fibronectin receptor (a 140 kd glycoprotein, see later) may also be important for embryonic cell migration. This complex interacts with fibronectin to induce spreading, which can then be polarised and leads to cell motility which is necessary for morphogenetic movement.

The only evidence, to date, that the 140 kd complex plays an important role in morphogenesis comes from an antibody inhibition study on neural crest cell migration that involves fibronectin/mediated adhesion (Bronner-Fraser, 1985).

Albini et al. (1987) showed that RGDS-containing fibronectin peptide (GRGDSP) and the 140 kd cell surface receptor for fibronectin (see later) mediate the migratory response of 3T3 and SV3T3 cells to fibronectin and fibronectin fragment. Duband et al. (1986) suggested that the migration of crest cells from the neural tube could be correlated with an increase in the fibronectin receptors, but only in the head. In the trunk, an increase in labelling for fibronectin receptors on crest cells was detectable only long after their departure from the neural tube.

2) Role of adhesion in the mechanism of cell movement

Cell adhesion not only occurs during tissue cell locomotion, it is also necessary for it. A cell must adhere to a substratum strongly enough to gain the traction necessary for its movement. And, of course, it must also be able to detach from that same substratum in order to move, otherwise, it would be immobilised.

Various authors have postulated in general terms that adhesion to the substrate following extension of the cell margin provides an anchorage against which contractile proteins in the cell could pull the cytoplasm forward (Ingram, 1969; Harris, 1973; Huxley, 1973).

As fibroblasts begin to move in culture, they form broad areas at their margins called lamellae. The very thin advancing edge which is not yet adherent to the substratum, whose protrusive activity is an essential act in the movement of the cell, is referred to as the lamellipodium. It seems that adhesion of the lamellipodium is an important step for fibroblast locomotion. Once the lamellipodium adheres to the substratum, retraction of the rear of the cell follows, which leads eventually to cell movement. Without adhesion, retraction will not happen, so there will be no movement.

Two distinct types of contact in fibroblasts where the plasmalemma are close enough to the substratum for adhesion to the substrate, were identified by interference reflection microscopy. These were, first; focal contacts, separated by 10-15 nm

from the substrate, and located under the peripheral regions of the leading lamellae, and other extended non- spreading regions of the cell margin; and close contacts, consisting of broad areas of uniform cell-to-substrate separation (30 nm) and typically present under the peripheral regions of spreading lamellae (Izzard and Lochner, 1976).

Abercrombie and Dunn (1975) have discussed evidence for the adhesive properties of the focal contacts of chick heart fibroblasts, namely that the focal contacts remain stationary relative to the substrate as the cell moves (Lochner and Izzard, 1973), that the attachment points of retraction fibers coincide with the focal contacts in the spread cell, and perhaps most significantly that focal contacts are not separated" by one cell passing " between another cell and its substrate (Armstrong and Lackie, 1975).

Because of the close association of focal contacts with adhesion plaques and microfilament bundles (Heath and Dunn, 1978; Wehland et al., 1979), considerable attention has been given to their possible role in cell motility. However, Couchman and Rees (1979), working with fibroblasts, and Keller et al.(1979) working with neutrophil granulocytes, have noted that focal contacts are more abundant under cells that are less motile. Furthermore, Izzard and Lochner (1980) reported that the extension of the leading edge of a fibroblast occurs only in the presence of a close contact with or without focal contacts.

Other evidence presented by Kolega et al. (1982) supported this view. Working with fibroblasts and epithelial cells, they found that broad close contacts are associated with rapidly moving cells while focal contacts are associated with slowly moving or stationary cells. The authors suggested that for cells in vitro, focal contacts are not associated with rapid cell translocation, whereas close contacts are. These results suggest that close contacts, rather than focal contacts, are the cell-substratum appositions important for cell locomotion.

Using interference reflection microscopy, Izzard and Lochner (1980) studied the formation of close and focal contacts during the movement of chick heart

fibroblasts on planar substrates. They proposed that the close contact provides the adhesion required to transmit to the substrate the forces involved in the forward movement of the marginal cytoplasm.

Another view considered that both contacts are important in cell motility. The continual formation of focal contacts at the leading edge and development of stress fibers from the new focal contacts, together with the loss of both behind the leading edge, are consistent with the requirement to transfer tension in the cell progressively to the advancing margin and with the role of the stress fibers in drawing the cell forward (Izzard and Lochner, 1980). The authors think specifically in terms of close contacts functioning in this role during the marginal spreading of fibroblasts, as distinct from the role of focal contacts in drawing the bulk of the cell forward.

Epithelial cells, unlike fibroblasts and leukocytes, do not normally move individually but often move as a coherent sheet (Kolega, 1981). Only cells at the margin of the sheet are actively motile and the attachment of the sheet to a substratum seems to be at the edge. Protrusive activity occurs at the margin and appears to be basically similar to the mechanism in fibroblasts (Middleton, 1982). Epithelia seem to form hemispherical bulges at the leading edge rather than flat ruffled sheets or microspikes. The mechanical integrity of epithelial sheets depends upon specialised cell junctions, particularly desmosomes (see later). Radice (1978) reports that focal contacts and microfilament bundles are seen only in retracting or slowly advancing epithelial cells cultured from the epidermis of Xenopus tadpoles, and suggests that the close contact in these cells is sufficient to support movement.

A somewhat different example of cell movement is that of leukocytes, such as the neutrophil granulocytes of mammals. These are primarily phagocytic cells which will move into tissues in response to inflammatory stimuli (see later). These cells do not have the clearly differentiated leading lamella which characterises the fibroblast. They have small protrusions with no microfilament bundles except in the tail (Wilkinson and Lackie, 1979), and the only examples of focal adhesions may be

small contact areas at the ends of tail fibers. The tail fibers seem to restrain forward movement and are seen most clearly when the cells are moving on a rather adhesive substratum.

Another example of cell movement dependent on adhesion is seen in the nerve growth cone. As a neurite extends over the substratum, it adheres to it in the region of its leading edges, often at the tips of the microspikes (Bray, 1979). The tip pulls itself along by adhesion of the ends of the microspikes to the substratum, followed by the contraction of their microfilaments.

It has been found that specific substrate-adsorbed molecules such as fibronectin stimulate the locomotion of different cell types in vitro (Couchman et al., 1982; Newgreen et al., 1982; Rovasio et al., 1983). Evidence that the fibronectin receptor is actually involved in cell motility is provided by perturbation experiments (Duband et al., 1986). Blocking the interaction of the receptor with fibronectin inhibits both cell spreading and cell migration. The authors found that the migratory cells were characterised by non-organised microfilament bundles and numerous fibronectin receptors organised diffusely on the membrane. The receptors may be mobile, and a number of them are not bound to fibronectin. This results agrees with the previous idea that close contacts are necessary for cell movement rather than focal contacts.

In contrast, in stationary cells, fibronectin receptors were concentrated in clusters close to focal contact sites and ends of microfilament bundles. They are linked directly or indirectly both to the cytoskeleton and fibronectin fibers. These receptors appear to have poor membrane mobility, and most of them are bound to fibronectin fibers.

If we accept that close contacts are required for cell locomotion, fibronectin may support cell movement through its promotion of these contacts. Norton and Izzard (1982) reported that fibronectin promots the formation of close contacts of the adhesion defective mutant AD6 but not focal contacts. Immobilised peptides

with the sequence RGDS were also found to promote the formation of these contacts in fibroblasts (Beyth and Culp, 1984).

3) Anchorage dependence

The proliferation of most kinds of cells in culture is strongly dependent on attachment to a solid surface. This regulatory mechanism has been called anchorage dependence (Stoker et al., 1968). Because normal cells are unable to grow in suspension (unless transformed by tumour viruses or other carcinogens) they must be provided with a solid surface in order to attach and grow.

When cells are not anchored they are rounded, but when cells are adherent they are more flattened, a shape change which may be important in anchorage dependence.

When cells are placed in suspension culture or plated onto a non adherent surface, anchorage dependent fibroblasts reduce their rates of macromolecular biosynthesis (Ben-Ze'ev et al., 1980) and arrest growth predominantly in the G1 phase in the cell cycle (Otsuka and Moscowitz, 1975). Contact with a substratum is necessary for protein synthesis, while nucleic acid biosynthesis requires a well spread morphology (Ben-Ze'ev et al., 1980).

In culture when cells are grown on artificial substrata, attachment proteins such as fibronectin adsorbed to these substrata, are responsible for inducing cells to attach and spread (Grinnell, 1978). Specific properties of the matrix- coated surfaces could be important. Attachment to certain specific components may promote survival and proliferation even without alteration of the area of the spread cell (O'Neill et al., 1979). Smith (1981) suggested that matrix components can adsorb the molecules of growth factors and the contact of cells with these adsorbed molecules partially could account for the effects of matrix.

Several investigators have shown that the phenomenon is related in some special way to malignant transformation and tumourigenicity (Freedman and Shin, 1974; Shin et al., 1975; Wright et al., 1977; Vasiliev and Gelfand, 1982).

Non-transformed fibroblasts and epithelial cells suspended in semisolid gels made of agar or methylcellulose do not form colonies, while neoplastic cells do (Macpherson and Montagnier, 1964).

Absence of anchorage dependence was observed in experiments with bovine aorta endothelium (Laug et al., 1980) and bovine granulosa cells (Bertoncello et al., 1982); the reasons for the exceptional behaviour of these cells are not known.

Experiments of O'Neill et al. (1986) showed two different mechanisms of anchorage stimulation in freshly explanted cells and 3T3 Swiss mouse fibroblast, the first depending on their exposed surface area, whereas the second had in addition a specific requirement for contact with the substratum. The growth stimulus of substratum contact seen in 3T3 cells must be due to some more subtle effect of contact, involving changes in cell structure.

It seems that anchorage dependence is related in some way to the shape changes (flattening) associated with spreading. The rate of exchange of materials which diffuse through the diffusion boundary layer around a cell is greatly increased when the cells are spread. The main finding of O'Neill et al. (1979) was that anchorage dependence was seen only when attached hamster mouse fibroblasts had a greater area than suspended cells. When the area of the upper surface of attached cells was the same as the total surface area of suspended cells, both cells grew at the same rate. This result suggested that the growth in both suspended and attached states is determined by serum factors whose access to the surface is limited by a diffusion boundary layer. In fact, both substratum and growth factors produce similar effects on metabolism, proliferation, and survival of cells and transforming growth factors (TGFs) added to normal cells allow them to grow in suspension.

4) Contact inhibition of locomotion

When chick heart fibroblasts moving on a surface in tissue culture collide with each other, they tend to show contact inhibition of locomotion. When the collision

is between the leading lamella of one fibroblast and that of another, further movement in the direction of contact stops almost immediately in both fibroblasts, any ruffling of the leading lamellae stops, and eventually a retraction occurs and the leading edges separate. This phenomenon was first described in detail by Abercrombie and Heaysman (1954). When the cells collide, they tend to stop locomoting in that direction. This reaction is a direct response to cell-cell contact and apparently involves adhesion and contraction (Abercrombie, 1970).

Several theories have been proposed to explain the phenomenon of contact inhibition. They have been reviewed in detail by Abercrombie (1970) and Harris (1974) and can reasonably be described as falling into two main groups. According to the first group, a cell might stop upon collision with another cell either because it is more firmly attached to the substrate than to the other cell, or because the upper surface of the opposing cell is non adhesive. The phenomenon would therefore be an example of differential adhesion, as argued by Steinberg (1970).

However, when cells come into contact with a substrate which is less adhesive than the one on which they are moving, although their forward locomotion inhibited, the ruffling of the leading lamella is not (Abercrombie et al., 1970). In a collision between two leading lamellae, the ruffling of both cells is inhibited. There is inhibition not just of overlapping, but of underlapping as well. Thus the idea that contact inhibition is due to differential adhesion is unlikely to be correct.

The second group consider contact inhibition to involve direct inhibition of the cellular machinery which produces cell locomotion, brought about upon collision, either as a result of the transmission of a signal to the cytoplasm or by the interaction of certain properties of the two contacting surfaces.

Heaysman and Pegrum (1973 a) described an area of specialisation of the cortical cytoplasm developing at points of close apposition of the unit membranes of colliding cells and have suggested a correlation between the changes occurring at these points of contact and the sequence of events characteristic of contact

inhibition. These specialisations appear within 20 seconds during contact. Within 60 seconds microfilaments appear associated with these areas, and the cell surfaces become distorted in the area of close opposition. After two minutes retraction probably begins.

The formation of these contact specialisations seems to be fundamental to the process of contact inhibition, thus adhesion may play a central role in this phenomenon. In collisions between chick heart fibroblasts and S180 tumour cells, in which neither cell is inhibited, such contact specialisations were not produced (Heaysman and Pegrum, 1973 b). Transformed cells are generally thought to show reduced contact inhibition with normal cells. However, there is considerable variability in the contact behaviour exhibited by tumour cells, and in some cases the tumour cells appear normal in their response. When transformed 3T3 and BHK cells make adhesive contacts with each other, cell movement, protrusive activity of the lamellipodia, and ruffling are all inhibited, just as in contact inhibition of normal cells (Bell, 1977). Guelstein et al. (1973) working with normal mouse fibroblasts and cells transformed by Moloney mouse sarcoma virus, found no dificiency in contact inhibition between transformed cells, or between transformed and normal cells, while Vesely and Weiss (1973) showed that neoplastic rat fibroblast cells had a high degree of homologous contact inhibition.

5) Tumour metastasis

Metastasis is a complicated phenomenon that depends on the ability of tumour cells to break loose from the primary tumour mass, invade normal tissues, penetrate blood and lymph channels, and be transported long distances in them. When they reach lymph nodes on capillary beds they may adhere to the walls of the vessels, invade the wall of the vessel and work their way out into the surrounding tissue. Thus a metastasis is founded (Trinkaus, 1984; Liotta, et al., 1986).

In this regard, in vitro studies of cell adhesion have been of interest, since cell adhesion may be important in determining a variety of properties important in the

metastatic process. For example, cells must be able to detach from the primary tumour mass (Weiss and Ward, 1983) and once released, they may undergo interactions with normal cells during transport in the lymph or blood. Ludford (1932) and Cowdry (1940) first suggested that membranes of tumour cells had lower general adhesive properties than normal cells.

The invasive properties of tumour cells have been attributed, in part at least, to their reduced adhesiveness, which could facilitate their separation from each other and from the substratum and allow cells with increased motility to invade normal tissue. It has been proposed, for example that a loosening or disappearance of desmosomes could provide the condition for invasive activity in epithelial cells. Early evidence supporting the idea of low adhesiveness came from the experiments of Coman (1944; 1961) who found that the attachment of certain cancer cells to each other and to the glass surface of the culture preparation was more easily disrupted than of certain normal cells. Subsequently some investigators concluded that virus-transformed cells are less adhesive than normal cells (Urishihara et al., 1977; Whur et al., 1977; Edwards et al., 1979; Tanaka and Kaji, 1980; Dennis et al., 1982; Brackenbury et al., 1984) while others found the opposite (Halpern et al., 1966; Wright et al., 1977; 1981). Winkelhake and Nicolson (1976) found that there was little differences in adhesive properties between untransformed and transformed 3T3 cell lines.

Coinjection of fibronectin peptide (GRGDS) with $7x10^4$ B16-F10 murine melanoma cells resulted in a marked reduction of melanotic colonies 14 days later in the lungs of C57BL/6 mice (Humphries et al., 1986 a). RGD-containing peptide was found also to inhibit the invasion of human melanoma cells in vitro (Gehlsen et al., 1988). Vollmers et al. (1985) have recently produced a series of functionally active monoclonal antibodies against melanoma B16 cells which induce more normal behaviour (morphology) of tumour cells in vitro, and reduced the number of growing lung tumours of melanoma cells B16 in mice by 70-90% when injected three days after the tumour cells in vivo.

6) The adhesive interactions of blood cells with non-circulating cells

Many pathological processes depend in one way or another on adhesion (cell-cell or cell-substratum). Adhesion plays an important role in the repair and maintenance of damaged tissues. For example when a blood vessel is damaged, platelets adhere to each other and to the subendothelium in order to plug the defect. In wound healing fibroblasts move separately into the wound area and restore the connective tissue matrix, whereas epithelial cells move as a sheet and join with each other.

Platelets under normal circumstances circulate in the blood and do not normally adhere to any surface. When the endothelial layer is damaged platelets will stick firmly to the exposed sub-endothelial material. Platelets then adhere to each other and form a platelet plug, thus filling any breach in the vessel wall preventing blood loss. Also under abnormal conditions platelets can adhere to each other in the process called thrombosis. For that reason platelet adhesion is vitally important because blood flow under normal conditions must not be interrupted by platelets sticking to each other or to the vascular lining (Gordon, 1980).

Aggregation of platelets during injury is induced by a wide range of biological stimuli such as ADP, serotonin and some prostaglandins. Provision of a fibronectin-coated surfaces to platelets in vitro results in firmer adhesion to the substrate, causing platelet spreading, the extension and thinning of platelets that accompanies full adhesion.

It is likely that fibronectin is also necessary in vivo for full platelet adhesion (Hynes, 1986). Ginsberg et al.(1985) have presented evidence that platelets, once they have been activated by factors released by other platelets at the clotting sites, develop specific receptors for fibronectin.

The low adhesiveness of lymphocytes in vitro reflects their behaviour in vivo. Lymphocytes must interact selectively with the substrata encountered during circulation. Peripheral blood lymphocytes adhere to the high endothelium of postcapillary venules and move between these cells, but probably do not adhere to

normal vascular endothelium appreciably.

After injury leukocytes, probably mostly neutrophils, begin to adhere to the endothelium of post-capillary venules. The adhesion of leukocytes may continue until the walls of the vessel are completely lined with leukocytes and is shortly followed by the emigration of leukocytes from the vessel. Leukocytes move over the endothelium and push their way between endothelial cells. The movement of emigrating cells towards the focus of infection may well involve chemotaxis, a phenomenon which leukocytes demonstrate in vitro (Wilkinson and Lackie, 1979). Brown and Lackie (1981) reported that fibronectin inhibits the adhesion of neutrophil granulocytes to clean glass.

Patel et al. (1985) found that the bone marrow precursors of erythrocytes interact with fibronectin and that loss of a fibronectin receptor is associated with release of the cell into the circulation.

The role of adhesion in a repair process which does not involve suspended cells was described by Radice (1980) who investigated the mechanism of wound closure in Xenopus tadpoles. He showed that cells begin migrating within seconds after wounding, and spread rapidly and continuously until wound margins meet. Cells in the basal layer, both at the margin and within the sheet, seem to spread actively whereas cells in the outer layer are passive, pulled by the activity of the basal cells. In agreement with this result, Trinkaus (1984) suggests that it is due apparently to the firm adhesions of cells to those of the basal layer beneath, by means of numerous desmosomes.

During wound healing, cells travel across blood clots, which are composed largely of fibrin but also contain much fibronectin, which binds to the fibrin as the clots form. Grinnell (1984 b) found that fibroblasts and other cells adhere to the clot and migrate over it only when fibronectin is cross-linked to the fibrin, and that additional fibronectin speeds the repair process.

Donaldson et al. (1987) found that RGDS (50 μg/ml) severely inhibited epidermal cell migration from skin explants in dishes coated with newt plasma and

migration over pieces of newt plasma-coated plastic placed under one edge of a skin wound. Increasing the RGDS concentration to 500 μg/ml resulted in almost total suppression of wound closure. Wounds exposed to the same concentration of Lys-Gly-Asp-Ser peptide closed normally.

MEASUREMENT OF CELL ADHESION

Cell adhesion can be measured by the following classes of method:

1) Measuring the force required to separate cells from each other or from a surface, or measuring the probability they separate under standardised force.

2) Applying a standard force which generate cell-cell or cell-substratum collisions, and measuring the probability of adhesion.

Direct measurement of cell-cell adhesion was reported first by Coman (1944) who separated cells from one another in culture by pulling glass microneedles. The absolute force of separation was calibrated by measuring with small weights the degree to which a needle bent during the separation. Weiss (1967) separated cells by measuring the shear force required to dislodge cells from various materials.

To measure the adhesion of cells to their substrata, cells have been detached with a stream of fluid or blown off with a blast of air, or simply shaken apart with different degrees of agitation. Recently, Francis et al. (1987) measured the force required to detach human red blood cells and Dictyostelium discoideum from glass surface. The force is applied via a flexible glass micropipette, attached by suction to the cell under study, and is calculated directly from the measured pipette deflection.

With the separation approaches, it is difficult to measure the exact force between cell to cell and cell to substrate because when cells are pulled away from glass, they leave antigenic material (protein) behind. So it is still not understood what is happening morphologically at the cell surface when cells are separated from each other or from a noncellular substratum. Another difficulty is a theoretical one. Steinberg (1964) stated that even if the force required to pull cells apart is the same as

that holding them together, measurement of the force alone would not be sufficient. The work required to pull cells apart or a cell away from its substratum can be determined only by integrating the force over the distance through which the force is exerted. Thus, the amount of force required would vary with the manner in which a cell is pulled from its substratum.

Aggregation kinetics should provide a more effective method of investigating cell adhesion because it is possible to obtain absolute quantitative measurements of adhesiveness uncomplicated by side-effects such as sudden changes in adhesiveness or rate limitation by factors other than the adhesiveness of the cells (Curtis, 1973). This technique is based on the shaking or agitation of cell suspensions so that the cell make collisions. If the cell are adhesive, a proportion of the collisions will result in the formation of adhesions. This technique of preparing adhesions was introduced by Gerisch (1960) and popularised by Moscona (1961), but it was not used to follow aggregation kinetics until Curtis and Greaves (1965) reported the course of aggregation in terms of the number of cells which were unaggregated or in two cell aggregates or in large ones.

The use of collecting aggregates as an assay of cell surface adhesiveness was introduced by Roth and Weston (1967). They found that aggregates in isotypic combinations collected many more labelled cells than did aggregates in heterotypic combinations.

Using a monolayer of cells on the bottom of small glass or plastic wells as the collecting surface has also proved to be effective in that a large proportion of homologous suspended cells adhere and show specificities of adhesion similar to those of cellular aggregates (Walther et al., 1973).

Another example of the second method to measure cell substratum adhesion was reported recently. Forrester and Lackie (1984) developed an adhesion assay system by which they can measure the adhesion of the cells, by passing a suspension of cells through a parallel plate chamber. Their adhesiveness can be

assessed by scoring the number of cells trapped on the lower plate, and the fluid shear stress can be defined for given flow rates. Since the adhesiveness of the cell at the instant of collision must exceed the distractive shear if the cell is to stop, the kinetics of cell accumulation provide a measure of the adhesiveness of the cells and the adhesive interaction can be quantified.

A somewhat different technique which might measure cell adhesion is that of Philips and Steinberg (1969). They centrifuged cell aggregates and determined the degree to which they flatten when subjected to a given centrifugal force.

General versus specific adhesive bonding

Because most cells have net negative charge on their surfaces, they will tend to repel each other electrostatically. It is necessary to postulate the existence of attracting forces in order to account for adhesion (Curtis, 1973). Electrostatic forces of attraction will occur if two surfaces have opposite sign of charge. The author proposed that the long range force which is most likely to operate in adhesion of cells is the London dispersion force. However, this idea does not require the existence of specific cell adhesion molecules. Alternatively, cells could adhere to each other by a bridging agent which combines in some way with both surfaces and thus links them together. Recently, many reports have discussed the discovery of cell adhesion molecules (CAMs) such as the liver (L-CAM) and neural (N-CAM) cell adhesion molecules which are thought to be responsible for adhesion between cells, and of various receptors involved in adhesion to substrates such as the receptors for fibronectin, vitronectin, laminin and collagen (see later).

Cell-cell adhesion

Specific cell-cell adhesive interactions are thought to be responsible for morphogenesis during embryogenesis and adult life (Moscona, 1980). It is even possible that some stem cells might be committed according to the adhesive interactions in which they are involved. For example, such a type of determination

has been proposed to explain blastocyst formation (Tarkowski and Wroblewska, 1967). Two different classes of cell surface molecules seem to mediate the adhesiveness of differentiating cells, CAMs, and molecules associated with the formation of cell junctions.

Components associated with junctions such as desmosomes are good candidates for mediators of cell adhesion (Thiery et al., 1985). To promote various interactions between cells, regions of the cell surface become specialised for intercellular contact. These surface specialisations, termed intercellular junctions, are vital for development and normal functioning of tissue. There are four major types of cell junction found in vertebrate cells, desmosomes (maculae adhaerentes), tight junctions (zonulae occludentes), gap junctions and intermediate junctions.

Desmosomes

One of the reasons for the tight mechanical adhesion of epithelial cells is believed to be the presence of adherent junctions, or desmosomes between them. Desmosomes have long been believed to be regions of enhanced adhesion between the cell membranes of epithelial cells (Farquhar and Palade, 1963; Overton, 1975; Cowin et al., 1984 a), being possessed by almost all epithelia, with a few exceptions such as the pigmented retinal epithelium (Docherty et al., 1984). They are found also in myocardium, but they are absent from skeletal muscle, connective tissue and other non-epithelial cells.

In the epidermis, desmosomes are oval or circular areas of enhanced intercellular adhesion, ranging from 0.1 to 1.0 μm in diameter. The membrane at the junction is modified by an accumulation of material, seen both within the intercellular space and the peripheral cytoplasm and by the presence of clusters of up to several thousand membrane associated particles within the bilayer itself (Breathnach, 1975). The intercellular material at the desmosome is approximately 30-40 nm in width. The cytoplasm of the desmosomes appears to have bundles of filaments of approximately 8 nm in diameter, termed tonofilaments, which often

run from the inside of one desmosome to the inside of another one in the same cell.

Desmosomes were isolated from bovine nasal epithelium by Skerrow and Matoltsy (1974). Many investigators have since reported the isolation of various specific proteins from desmosomes, in some cases raising monoclonal antibodies which react with them.

Those desmosomal glycoproteins which possess extracellular domains may play an important role in cell-cell adhesion. Thus, an adhesive function has been proposed for DG II (120, 130 kd), DG III (100,115 kd) (Cowin et al., 1984 b; Miller et al., 1987; Penn et al., 1987; Skerrow et al., 1987) and 125 kd (Jones, 1988). Cowin et al.(1986) suggested that an 83 kd glycoprotein isolated from MDBK desmosomes may also play a role in the adhesion of these cells.

Tight junctions

Tight junction are specialised cell membrane domains at contact regions of neighbouring epithelial cells. They provide a region of intimate contact that completely encircles each cell (Staehelin and Hull, 1978). In some situations, there appears to be no space whatsoever between the two plasma membranes at the site of contact. Because tight junctions are found frequently in epithelial tissues, where the cells are tightly adherent, they are presumed to provide part of the basis for the adhesion.

The main function of tight junctions, however is believed to be to block the passage of materials from one side of an epithelial cell sheet to the other between the cells. Recent work (Darnell et al., 1986) suggests that tight junctions also block the lateral diffusion of membrane proteins and lipids and maintains the separation of the apical and baso-lateral regions of membrane of the epithelial cells.

Intermediate junctions

In the intermediate junction (sometimes known as the belt desmosome), a contractile bundle of actin filaments running along the junction plays an important role in morphogenesis, for example, the folding of epithelial cell sheet into tubes (Staehelin and Hull, 1978). Other types of adhesion plaques including focal adhesions (see later) are found where bundles of actin-containing microfilaments pass through the cytoplasm and meet the plasma membrane, which in this respect are similar to intermediate junctions. In both, adhesion plaques and intermediate junctions, vinculin (130 kd) and alpha actinin are localised to the end of microfilament bundles (Geiger, 1979). In addition to the intracellular proteins above, various extracellular proteins have been found associated with these junctions.

In mouse epithelia a 120 kd protein, the cell adhesion molecule uvomorulin (UV), has been localised in these junctions (Boller et al., 1985). The authors suggest that this molecule may play a role in the adhesive function of this motile system. It might serve as an adhesive factor that provides a counter-force against the tension of the contractile ring, thereby helping to preserve the integrity of the epithelial sheet. In chick epithelia, Liver cell adhesion molecule (L-CAM) (perhaps homologous to uvomorulin in mouse cells) (see later) is found associated with these junctions. These findings support the role of this junction in adhesion between cells.

A 135 kd membrane glycoprotein specifically associated with intercellular adherens junctions has also been identified by Volk and Geiger (1986 a; b). This protein was found along the junctional membrane, and monovalent antibodies reactive with this protein can inhibit both cell-cell interaction and the formation of intercellular adherens-type junctions, suggesting that this protein is involved in the intercellular interaction in adherens junctions. The authors proposed the name A-CAM, namely adherens junction-specific cell adhesion molecule.

A-CAM is similar to other CAMs such as UV, L-CAM, CAM 120/80, E-Cadherin and Arc-1 (see later) in mediating cell- cell adhesion which is Ca^{2+}-

dependent. However, A-CAM is apparently absent from polarised epithelia of intestine and liver and it is expressed in cardiac muscle, brain, and cultured kidney cells (Volk and Geiger, 1984), while UV and L-CAM have been identified from mouse embryonal carcinoma cells and liver cells respectively (Hyafil et al., 1980). A difference between A-CAM and UV is manifested by the effect of antibodies on junction integrity. In contrast to lack of the effect of anti-A-CAM, the junctions mediated by the UV-related molecules (L-CAM, E-Cadherin, CAM 120/80, Arc-1) were disrupted by the intact antibody (complete IgG) used (Damsky et al., 1983; Shirayoshi et al., 1983; Behrens et al., 1985).

Gap junctions

Gap junctions form variable area of contact at which adjacent membranes approach each other closer than 2-3 nm. These structures are thought to be the basis for cell-cell communication in many tissues. Because the gap junction has a remarkable resistance to mechanical forces and to chemical agents such as acetone and EDTA, Goodenough and Revel (1970) suggested that gap junctions, in addition to being possible mediator of electrical coupling, they may play a role in intercellular adhesion. Gap junctions are generally considered to be the sites of ionic and thus of electrical conductance and of metabolic coupling between cells (Warner et al., 1984; Lee et al., 1987).

It has been proposed that gap junctions play a role in the control of cellular growth and differentiation (Loewenstein, 1987). It has been demonstrated that disruption of gap junctional communication can have a substantial effect on embryonic development. Antibodies raised against a gap junctional 27 kd protein, disrupted the junctional communication at early stages and resulted in specific developmental defects (Warner et al., 1984).

Gap junction components have been isolated by several investigators and numbers of different proteins (30, 27, 45, 29, 18 kd) have been reported (Kensler and Goodenough, 1980; Hertzberg and Skibbens, 1984; Page and Manjunath,

1985; Finbow et al., 1985).

The four principal type of junctions, desmosomes, tight junctions, intermediate junctions and gap junctions are illustrated in figure 1.

Identification of cell adhesion molecules

Many workers believe that cell-cell adhesion is initiated and maintained by membrane glycoproteins called cell adhesion molecules (CAMs) (See Edelman, 1983; 1984 a; b; c; 1985 a; b; Damsky et al., 1984; for extensive reviews). The presently known mammalian CAMs have been mainly detected by generating monovalent antibodies (Fabs) that can block adhesion in a functional cell- aggregation assay, thus the functional role of CAMs is mostly defined indirectly via antibodies (Ekblom et al., 1986).

Functionally active anti-adhesion antibodies (Fabs) were first successfully used to detect adhesive cell surface determinants in Dictyostelium (Gerisch, 1977), and with the use of a similar approach, several CAMs have now been characterised in vertebrates.

The known CAMs can be divided into two groups on the basis of metal ion requirement, calcium dependent CAMs which include the liver cell adhesion molecule (L-CAM) and uvomorulin require calcium for adhesion, and in some cases calcium also protects these CAMs from proteolysis (Takeichi et al., 1983) with one exception (Hatta et al., 1985). Calcium independent CAMs include the neural cell adhesion molecule (N-CAM) and neural-glia cell adhesion molecule (Ng-CAM).

Liver cell adhesion molecule (L-CAM)

The liver cell adhesion molecule (L-CAM) is a cell surface glycoprotein that appears in a distinct pattern at a variety of inductive embryonic sites as well as in adult tissues (Thiery et al., 1984; Gallin et al., 1985; Edelman, 1986). L-CAM was found to mediate calcium-dependent adhesion between cells of chicken liver epithelium (Bertolotti et al., 1980; Gallin et al., 1983). The importance of L-CAM

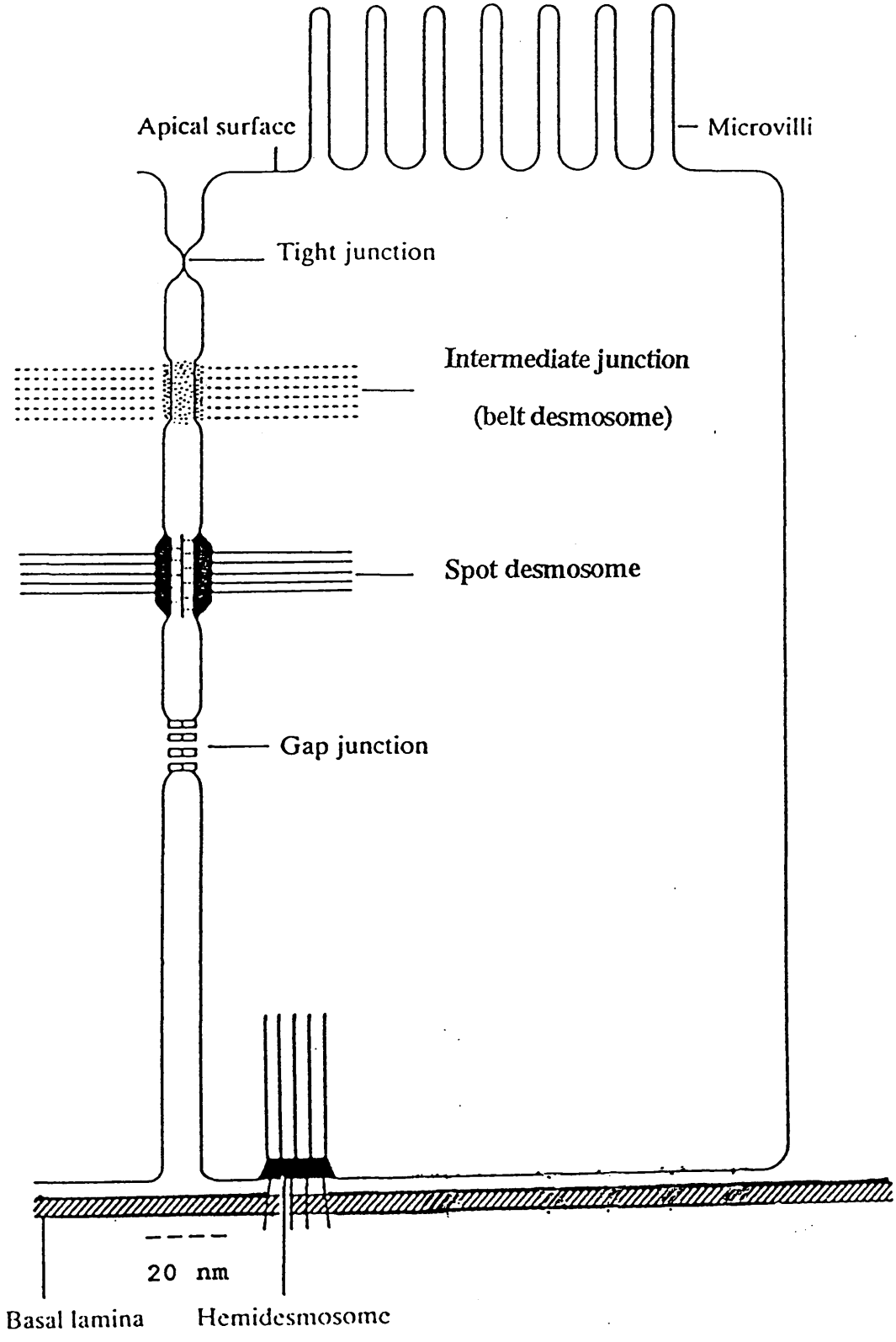


Figure.1 Type of junctions as found in a vertebrate epithelium.

in embryonic development and in the formation of epithelia is suggested by studies in which L-CAM mediated adhesion was blocked by antibodies (Gallin et al., 1987).

An antigenically active L-CAM derivative can be released from the cell surface as a fragment of 81 kd by proteolysis with trypsin in the presence of calcium. By raising a monoclonal antibody against this tryptic fragment, an intact 124 kd component was revealed. Because detergent or proteases were required to solubilise the 124 kd species, Gallin et al. (1983) suggested that L-CAM is an intrinsic membrane protein.

Antibodies (Fabs) that prevent the formation of tight junctions in MDCK cells react with L-CAM rather than components of the tight junction themselves, indicating that the formation of some junctional complexes is dependent on cell-cell adhesion via L-CAM (Gumbiner and Simons, 1986).

Recently, the sequence of a cDNA clone that contains almost the entire L-CAM mRNA including the complete amino acid sequence was reported by Gallin et al.(1987). The sequence includes a hydrophobic segment of 31 amino acids, supporting the conclusion that L-CAM is an intrinsic membrane protein.

Several other calcium-dependent adhesion molecules, including Uvomorulin (Hyafil et al., 1980), E-cadherin (Yoshida-Noro et al., 1984), cell CAM 120/80 (Damsky et al., 1983), and Arc-1 (Imhof et al., 1983) have been isolated from different epithelial tissues or cell lines, and these molecules have biochemical properties (Cunningham et al., 1984) and tissue distributions (Edelman, 1983; Thiery et al., 1984) similar to those of L-CAM. Therefore, Gallin et al .(1987) and Ringwald et al. (1987) suggested that it is likely that all of these molecules are mammalian homologues to L-CAM.

The mouse glycoprotein uvomorulin (UV) is a cell surface molecule that is involved in the compaction of preimplantation mouse embroys (Vestweber and Kemler, 1984) [and the calcium-dependent aggregation of embryonal carcinoma

(EC) cells]. UV was identified by producing rabbit anti-EC antibodies that interfere with the compaction of the preimplantation embryos and the aggregation of EC cells (Kemler et al., 1977).

Purification of a molecule that reverses the antibody effect lead to the identification of an 84 kd glycoprotein, a trypsin fragment of UV (Hyafil et al., 1980). A rat monoclonal antibody against the trypsin fragment (Hyafil et al., 1981) shows that the native UV molecule is a 120 kd glycoprotein. (Peyrieras et al., 1983).

The molecular mechanism of cell adhesion mediated by UV is still not well understood. The search for a functional adhesive site on UV has recently been facilitated by the use of three independently selected anti-UV monoclonal antibodies, each of which disturbs cell-cell contacts (Ekblom et al., 1986). The fact that the epitopes of these monoclonal antibodies are in a 26 kd fragment suggested that this fragment could contain an adhesive domain of UV (Vestweber and Kemler, 1985).

Recently, comparison of the amino-terminal nine residues of N-Cadherin, a calcium-dependent adhesion molecule isolated from chicken neural tissue, and E-cadherin (mouse L-CAM) suggested a striking similarity between them (Shirayoshi et al., 1986). Duband et al. (1987) demonstrated that N-Cadherin and N-CAM are involved in cell adhesion during somitogenesis and suggest that the regulation of N-Cadherin expression might play a key role particularly in the formation and disruption of the somitic epithelium.

Hyafil et al. (1980) found that the conformation of uvomorulin depends upon Ca^{2+}. Ca^{2+} also protects the 84 kd tryptic fragment of UV from further proteolysis by trypsin and this fragment can bind the monoclonal antibody DEI only in presence of Ca^{2+}.

Neural cell adhesion molecule (N-CAM)

N-CAM was the first vertebrate CAM to be identified and described in structural and functional terms (Edelman, 1983). It was originally isolated from chicken retinal cells. N-CAM appears to be an integral membrane protein that can be removed from membrane fractions only by detergent extraction (Hoffman et al., 1982; Hoffman and Edelman, 1983).

N-CAM-mediated adhesion has been shown to play a central role in several developmental events including the orderly outgrowth of axons (Fraser et al., 1984; Silver and Rutishauser, 1984; Chang et al., 1987), and nerve-muscle interaction (Rutishauser et al., 1983).

In mouse, three immunologically related N-CAM polypeptides of approximately 120, 140 and 180 kd have been described (Gennarini et al., 1984). Chicken N-CAM is composed of proteins of 170 and 140 kd that arise by alternative splicing of mRNA from a single N-CAM gene (Murray et al., 1986 a; b). An adult form of N-CAM contains less sialic acid, and it has been suggested that the sialic acid contents of N-CAM influence the adhesiveness of the molecule (Hoffman and Edelman, 1983). Application of anti-N-CAM antibodies blocks the formation of neurite bundles and disrupts histogenesis in cultured chick retina (Buskirk et al., 1980; Rutishauser, 1984).

The sequence of one cDNA clone for chicken N-CAM, which codes for part of the extracellular domain and putative transmembrane and cytoplasmic domains, and represents the largest N-CAM mRNA, has been reported (Hemperly et al., 1986). The nucleotide sequence of the cDNA clone encodes 964 amino acids from the carbohydrate and cell associated domains of the large N-CAM polypeptide (followed by 664 nucleotide of 3' untranslated sequence). The protein sequence contains attachment sites for poly-sialic acid- containing oligosaccharides. The carboxyl terminal cytoplasmic domain includes a block of 250 amino acids present in the larger but not in the smaller N-CAM polypeptide. These have been designated ld (large domain) polypeptide and sd (small domain) polypeptides. The

authors suggest that the intracellular domains of the ld and sd polypeptides are likely to be critical for cell-surface modulation of N-CAM by interacting in a different fashion with other intrinsic proteins or with the cytoskeleton.

Recently, the sequence of the coding region for a mouse N-CAM protein derived from three overlapping cDNA clones has also been reported (Barthels et al., 1987). The deduced amino acid sequence predicts a 79 kd polypeptide which lacks a typical transmembrane segment and may represent N-CAM 120 kd.

Comparison of the nucleic acid sequence and the protein sequences of L-CAM and the largest (ld) chain of N-CAM indicated that there was no homology between the two molecules. L-CAM and N-CAM do not bind to each other and are immunologically distinct (Brakenbury et al., 1981), they show different cleavage patterns and sugar structures (Edelman, 1984 c). At present, there is no evidence that L-CAM and N-CAM are related or derived evolutionarily from a common precursor.

Several other neural cell adhesion molecules have been isolated. A glycoprotein named D2-CAM originally demonstrated in rat neural plasma membrane and brain cell surface protein 2 (BSP-2) have been found to be evolutionairly related to N-CAM (Jorgenson et al., 1980; Hirn et al., 1983; Edelman, 1983).

In neural crest cells, N-CAM appears early in the notochord during neurulation then disappears during migration of the cells on fibronectin, and reappears at sites where ganglia are formed (Thiery et al., 1982; Thiery, 1985; Edelman 1984 b). In regions of the nervous system, particularly those related directly to the neural tube, the N-CAM molecule is stained at all stages. These results raise the possibility that adhesion mediated by N-CAM plays a primary role in early embryogenesis as well as in later histogenesis (Thiery et al., 1982).

Neuron-glia cell adhesion molecule (Ng-CAM)

Grumet et al. (1984 b) first reported the isolation of this adhesion molecule.

Neural-glial interactions were quantitated by measuring the binding of neural membrane vesicles to glial cells. Monoclonal antibodies as Fabs were then obtained that inhibited adhesion in this assay and were shown specifically to recognise a neuronal protein called the neuronal-glial cell adhesion molecule (Ng-CAM). This is a molecule of 135 kd (Grumet and Edelman, 1984) present on the surface of neurons. It directly or indirectly mediates the adhesion of neurons and glial cells (Thiery et al., 1985).

Preincubation of Fab fragments of specific rabbit antibodies to Ng-CAM, or of monoclonal antibody 10F6 (Grumet et al., 1984 a) with affinity purified Ng-CAM, neutralised the inhibition by these antibody fragments of the binding of neural membrane vesicles to glial cells. Neurons but not glial cells were recognised by the monoclonal antibody (Grumet and Edelman, 1984). Since the function of this CAM is Ca^{2+} - independent, it might be evolutionarily related to N-CAM (Edelman, 1984 b).

Ng-CAM has been found to resemble a neural cell adhesion molecule (L1) from chicken (Grumet et al., 1984 a). Chang et al.(1987) suggested that Ng-CAM may play a role in cell migration in chick embryos.

I-CAM

Recently, an intercellular adhesion molecule called I-CAM (60 kd) has been identified (Dustin et al., 1986). I-CAM is a cell surface glycoprotein originally defined by a monoclonal antibody (MAb) that inhibits phorbol ester-stimulated leukocyte aggregation. This molecule is also expressed on fibroblast, endothelial cells, macrophages, and T-lymphocytes. I- CAM could be a cell surface molecule that interacts with lymphocyte association antigen (LFA-1) (see later) on other cells (Marlin and Springer, 1987). This molecule is important in leukocyte adhesion and is regulated in a manner consistent with its being an important molecule in inflammatory and immune responses.

A variable number of as yet unknown adhesion molecules may exist, and the

stabilisation of tissue form may require further components in addition to the presently known CAMs. Nevertheless, it can be concluded that the CAMs characterised to date represent major adhesive components for organogenesis.

CELL SURFACE INTERACTION WITH EXTRACELLULAR MATRICES

The attachment and spreading of cells on extracellular matrix components undoubtedly involves very complex interactions which include not only specific receptors (Takeichi, 1977; Brown and Juliano, 1985; Giancotti et al., 1985; Pytela et al., 1985 a) on the surface of the membrane but also cytoskeletal components beneath it (Lark et al., 1984). An example of a structure associated with cell substratum adhesion which illustrates this is the focal adhesion, which involves cytoskeletal proteins (Lark et al., 1985) and proteoglycans (Garner and Culp, 1981; Lark and Culp, 1984) as well as gangliosides (Cheresh et al., 1984 a, b; Okada et al., 1984).

Cell substratum adhesion

Cell substratum adhesion, particularly of fibroblastic cells in vitro, has been studied intensively over the past decade. Fibroblasts adhere to a substratum in vitro via specialised regions of their ventral surfaces. Two types of specialisation, close contacts and focal contacts, have been distinguished (see earlier).

Using electron microscopy, Chen and Singer (1982) have identified additional sites of cell substratum interactions called extracellular matrix (ECM) contacts, which occur in regions where the cell surface comes in close contact with substantial aggregations of extracellular material.

The relationship of ECM molecules, in particular fibronectin, to the submembranous cytoskeleton at these adhesion specialisations has been closely studied. It has been found that both fibronectin and the submembranous actin-containing microfilament bundles become arranged in very similar fibrillar patterns in well spread cells, and terminate in the focal contacts.

Some studies suggest that, in cultured fibroblasts, the fibrillar fibronectin

pattern is colinear with the termini of microfilament bundles at focal adhesions as identified by IRM and immunofluorescence using anti-vinculin (Hynes and Destree 1978, Singer, 1979, 1982). This conflicts with other results (Birchmeier et al., 1980; Chen and Singer, 1980; 1982; Fox et al., 1981) which suggest that fibronectin is distributed adjacent to the focal adhesion sites in areas of close contacts.

A recent study suggests, however, that cellular fibronectin may be required for the final coordinated development of focal contacts and well organised microfilament bundles (Damsky et al., 1984). Other adhesion proteins may substitute for fibronectin, since Couchman et al. (1982) reported that human fibroblasts can attach and spread on laminin coated substrates and can form microfilament bundles and focal contacts when plated on laminin in fibronectin-depleted serum even if they were pretreated with cycloheximide and are plated in the presence of cycloheximide and anti-fibronectin. Thus, under these conditions, neither cellular nor plasma fibronectin seems to be required for the formation of the focal contact and microfilament bundle arrays, although cellular fibronectin may in some way promote their formation.

However, Virtanen et al. (1982) suggested that although fibronectin is not needed for fibroblast adhesion, endogenous fibronectin does seem to be required for the development of focal adhesion sites, bundle of microfilaments and plaques of vinculin.

More recently, using IRM, Singer et al. (1987) found that a peptide corresponding to the cell attachment recognition site of fibronectin promotes focal contact formation during early fibroblast attachment and spreading. By 1h, 72-78% of the normal rat kidney and Nil 8 cells plated on substrates derivatised with small synthetic peptides containing the RGDS sequence had focal contacts without accompanying fibronectin fibers, while fibroblasts on control peptide lacked focal contacts. The 75 kd fibronectin fragment had a similar effect. Streeter and

Rees (1987) also found that RGDS promotes the formation of focal adhesions, but these occurred only at the outermost cell margins, in contrast to their distribution in cells spread on fibronectin. These results differ from these of Izzard et al. (1986) and Woods et al. (1986), in which human or Balb/c3T3 mouse fibroblast, developed focal contacts on substrates coated with intact fibronectin, but failed to form focal contacts on glass coated with the purified cell binding domain of fibronectin.

Normal fibroblasts in vitro, when presented with an appropriate surface (Grinnell, 1978), will secrete adhesion molecules in the course of attachment and spreading and with time will produce matrix material containing fibronectin, collagen, glycosaminoglycans (GAGs), and smaller amount of several other proteins (Carter, 1982; Lehto et al., 1981; 1983). An interpretation which differs from that of Grinnell is that of Curtis and McMurray (1986) who found that exposure of BHK cells to serum components prevents cell attachment if cycloheximide or fibronectin tetrapeptide (RGDS) is present. If leupeptin is used cell adhesion and spreading occur even when all fibronectin synthesis is suppressed by cycloheximide inhibition, or fibronectin binding by tetrapeptide competition. The authors suggest that fibronectin could act as an activator for cell adhesion rather than as a bonding molecule. This idea of activation has recently been discussed further by Curtis (1987).

Damsky et al. (1979) showed that cytoskeleton-membrane interactions play an important role in the maintenance of adhesive contacts between cells and substrata. This interaction may act as a transducer to couple intercellular responses to extracellular changes (Folkman and Moscona, 1978; Ben-Ze'ev et al., 1980). In culture rounded cells bind to fibronectin adsorbed on the substratum. This interaction triggers a cytoskeletal reorganisation that drives spreading and acquisition of a flat morphology (Grinnell, 1978). Ultimately this process results in the organisation of well-developed microfilament bundles ending at adhesion plaques.

Correlated IRM and immunofluorescence have shown that the microfilament associated proteins alpha actinin (Wehland et al., 1979; Geiger et al., 1980),

vinculin (Geiger, 1979; Burridge and Fermisco, 1980), fimbrin (Bretscher and Weber, 1980), talin (Burridge and Connel, 1983), and the src gene product $pp60^{v\text{-}src}$ in transformed cells, as well as the src gene product $pp60^{c\text{-}src}$ in normal cells (Rohrschneider, 1980; Rohrschneider et al., 1982) are concentrated where microfilament bundles terminate at sites of focal contacts (Hynes, 1981 a; b; Heath, 1982).

Vinculin has been localised closely with focal adhesions (Geiger, 1979). This 130 kd cytoskeletal protein was originally found as a contaminant during the purification of alpha actinin from the smooth muscle of chick gizzard. Vinculin may also be involved in bundling (Jockusch and Isenberg, 1981). It was claimed to bind to the ends of actin filaments (Burridge and Fermisco, 1980) perhaps indicating an attachment role, but this is now believed to have been due to an impurity (Wilkins and Lin, 1986). Electron microscopic studies showed that vinculin to be closer to the plasma membrane than alpha-actinin in a number of locations (Chen and Singer, 1982).

Attempts to identify cell surface species associated with focal adhesions were at first unsuccesful. However, analysis of cells spread on substrata coated with covalently cross-linked fibronectin yielded fluorescence patterns in which fibronectin receptors revealed a needle eye pattern at the termini of stress fibers, suggesting colocalisation of the 140 kd fibronectin receptor (see later) and focal adhesion sites (Damsky et al., 1985). Therefore, this receptor may be important in focal adhesion formation. Other investigators have also reported that the 140 kd fibronectin receptors are associated with focal adhesion sites (Chen et al., 1985; Giancotti et al., 1986; Grinnell, 1986). In contrast, other results showed that fibroblasts were observed to remove fibronectin from beneath focal adhesion (Avnur and Geiger, 1981), and immunoelectron microscopic studies showed that fibronectin is absent from these sites (Chen and Singer, 1982).

The carbohydrate of membrane glycoproteins may be important in the formation of focal adhesions, since focal adhesions, but not close contacts, seem

to be defective in the 3T3 variant, Ad6 which have defective glycoprotein synthesis (see page 54) (Pouyssegur and Pastan, 1977).

From observations obtained using monoclonal antibodies against fibronectin, vinculin, and alpha actinin, Chen and Singer (1982) suggested that focal adhesions, close contacts, and extracellular matrix contacts occur both at fibroblast cell-substratum adhesion and cell-cell interfaces. Using the frozen thin section technique (Tokuyasu and Singer, 1976) and double label immunofluorescence, Chen and Singer (1982) found that alpha-actinin is present near all types of contact sites, whereas vinculin and fibronectin have a complementary distribution. Vinculin is present at focal contacts and a subset of ECM contacts, while fibronectin is found at close contacts and the subset of ECM contacts lacking vinculin.

Talin, a 220 kd protein, was purified from chicken gizzard smooth muscle. Antibodies against this protein localised it in fibroblasts to adhesion plaques, to regions underlying cell surface fibronectin, and to ruffling membranes (Burridge and Connell, 1983). The authors suggest that this protein has some function in the organisation of actin filaments at or close to regions of actin membrane attachment. Recently, Beckerle et al. (1986) demonstrated that talin is homologous to a 235 kd protein on platelets. Another study showed that the antigen (CSAT) (Neff et al., 1982) (see later) which has been identified in chick cells by Mabs as a plasma membrane receptor for fibronectin (Horwitz et al., 1985), has a binding domain for talin.

The protein fimbrin (68 kd) has been identified by antibodies as one of the few major proteins in the microfilament bundles of the microvilli present on intestinal epithelial cells (Bretscher and Weber, 1980). Fimbrin is found particularly prominently in membrane ruffles, microspikes, and microvilli.

An additional protein that has been localised in the adhesion plaque is $pp60^{v\text{-}src}$, the protein kinase coded for by the transforming gene of Rous sarcoma virus (RSV) (Rohrschneider, 1980). This location of $pp60^{v\text{-}src}$ could be related to the

disruption of the actin cytoskeleton which is a prominent feature of RSV-transformed cells.

Recently an 82 kd protein has been identified in focal contacts by use of a nonimmune rabbit serum in cultured chicken cells (Beckerle, 1986). This protein is immunologically distinct from other known adhesion plaque proteins such as vinculin, talin, alpha actinin, and fimbrin. The 82 kd protein is ubiquitous in chicken embryo tissue. However, it appears to be more abundant in fibroblasts and smooth muscle than in brain or liver. The author suggest that this protein is involved in linking actin filaments to the plasma membrane at sites of substrate attachment or regulating these dynamic interactions.

Role of divalent cations

Cells can attach to a clean glass or plastic surface in the absence of divalent cations, as long as a serum-free medium is used (Taylor, 1961; Takeichi, 1971). Once the surface of culture dish is coated with serum proteins or cellular products, however, divalent cations become necessary for cell-to-substrate adhesion (Weiss, 1960). A close relationship exists between the stimulation of cell-substrate adhesion by cations and their ability to promote cell spreading (Takeichi and Okada, 1972), thus enhanced adhesion in the presence of cations may be a secondary effect due to increased cell spreading activity, which causes an increase in the amount of surface in contact through cellular locomotory activity (see Weiss, 1964).

Yasuda (1974) studied the effect of various divalent cations on fibroblast spreading. The minimum concentration at which spreading-enhancing activity was recognised was 10^{-6} M for Ca^{2+} ,10^{-7} M for Mg^{2+}, and 10^{-9} M for Mn^{2+}. Co^{2+} was less effective than the others.

It was shown that Mn^{2+} was able to promote the attachment and spreading of Sarcoma I cells in serum free medium (Rabinovitch and DeStefano, 1973). A similar observation was later made with BHK cells (Maroudas, 1975). Evans and Jones

(1982) proposed that Mn^{2+} might be an especially effective cofactor for the adhesion-promoting activity of secreted or added fibronectin.

A similar result was obtained later by Grinnell (1984 a), who found that in high Mn^{2+} BHK cells attach and spread on substrata without an added adhesion factor such as fibronectin. Mn^{2+}-dependent adhesion also occurred when the substratum was coated with protein, such as albumin, haemoglobin, bovine gamma globulin or ovalbumin, or dried collagen film. The effect of Mn^{2+} was found to be reversible. Addition of anti-WGA-R antibodies that inhibited ligand-mediated cell adhesion, inhibited adhesion of cells in Mn^{2+} -containing media and caused rounding of cells previously attached and spread in the presence of Mn^{2+}.

Recently, Edwards et al. (1987) found that the adhesion and spreading of BHK21 cells on adsorbed bovine and foetal bovine serum require addition of divalent cations to the medium. The order of effectiveness for adhesion was Mn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}. In another study (Klebe et al., 1977 b), Ca^{2+} and Mg^{2+} were required for the attachment of CHO cells on collagen in presence of fibronectin.

In epithelial cells, Ca^{2+} was found to be the essential cation for cell adhesion (Vasiliev and Gelfand, 1981; Trinkaus, 1984), however, Mg^{2+} was also found to be effective (Fritsch et al., 1979). In guinea pig epidermal cells Stenn and Core (1986) have recently found that Mn^{2+} supports the spreading of these cells in the absence of specific spreading protein. It supported spreading in BSA-containing media very rapidly at low concentrations, although Mg^{2+} and Ca^{2+} were found to antagonise the Mn^{2+} effect.

In an earlier study (Shadle and Barondes, 1982) enhanced adhesion of platelets to collagen substrates in the presence of Mg^{2+} was observed. Recently, Santoro (1986) found that divalent cation dependent platelet adhesion does not exhibit an absolute specificity for Mg^{2+}, since other divalent cations such as Mn^{2+}, Fe^{2+}, Cu^{2+}, Ca^{2+} and Zn^{2+} also support adhesion. A 160 kd platelet membrane protein has been identified, that binds to collagen in presence of Mg^{2+}.

^{125}I-fibronectin binding to IIb-IIIa complex (see later) is enhanced much

more in the presence of Mg^{2+} than in Ca^{2+} (Plow et al., 1985 a). The enhancement appears to be due to an increase in affinity and total number of binding sites for fibronectin.

A dependence of adhesiveness of polymorphonuclear leukocytes (PMNs) upon divalent cations was demonstrated (Garvin, 1968). Added singly Mg^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Cu^{2+} or Cd^{2+} were found to be effective whereas Ca^{2+}, Sr^{2+} and Ba^{2+} were ineffective. Hoover et al. (1980) also found that Mg^{2+} is more effective than Ca^{2+} in enhancing the adhesion of PMNs to endothelial cells, while Beesley et al. (1978) found the opposite. Adherence of PMNs to plastic that had been coated previously with albumin also was Mg^{2+} but not Ca^{2+} dependent. It is likely that Mg^{2+} directly influences PMN adhesiveness rather than the function of endothelial cells.

Several possible mechanisms of actions for divalent cations, including Mn^{2+} have been considered. Evans and Jones (1982) suggested that Mn^{2+} promotes fibronectin secretion and /or acts as a more efficient cofactor for fibronectin than Mg^{2+} or Ca^{2+}.

Another possible explanation for the Mn^{2+} effect is that Mn^{2+} promotes cell substratum adhesion regardless of the substratum. There is an electrostatic barrier between cells and the substratum that must be overcome by a contact process (Weiss, 1975). More recent evidence, however, was interpreted as suggesting that divalent cations were required for cytoskeletal function rather than as adhesion bonds per se (Grinnell, 1978).

Many investigators of divalent cation effects on adhesion and spreading of tissue cells have argued that a specific divalent cation-binding site in a protein is likely to be involved (Garvin, 1968; Klebe et al., 1977 b; Stenn and Core, 1986; Edwards et al., 1987).

Recently, models were presented by Edwards et al.(1987) to explain the effect of divalent cations on cell spreading in presence of RGD proteins such as fibronectin

and vitronectin. The authors proposed that divalent cations bind to the receptors producing a conformation change which allows binding of the receptors to the RGD-containing proteins. The divalent cation itself could be part of the RGD binding site, perhaps through an interaction with the oxygen of the aspartic acid residue.

Cell attachment proteins and their receptors

It is now clear that interactions of cells with extracellular materials are often mediated by a class of high molecular weight glycoproteins that are involved both in these interactions and in the actual structure of extracellular matrices. The most intensively studied of these glycoproteins is fibronectin, but there are many other proteins involved, such as fibrinogen, vitronectin, laminin, collagen, chondronectin, and thrombospondin.

Fibronectin

Fibronectins are large glycoproteins that have been, implicated in a wide variety of cellular phenomena, particularly those involving the interactions of cells with extracellular material. These phenomena include cell adhesion, morphology, cytoskeletal organisation, migration, differentiation, oncogenic transformation, phagocytosis, and haemostasis (Hynes and Yamada, 1982; see also Akiyama and Yamada, 1987).

There are at least two types of fibronectin, termed plasma and cellular fibronectins. Plasma fibronectin (0.3 g/L) may synthesised by fibroblasts and hepatocytes (Foidart et al., 1980; Owens and Cimino, 1982; Tamkun et al., 1983), although endothelial cells (Saba and Jaffe, 1980; Hynes and Yamada, 1982) and macrophages (Alitalo et al., 1980) could also contribute. Cellular fibronectin is produced by a wide variety of cells including fibroblasts, myoblasts, chondrocytes, and endothelial cells (reviewed by Furcht, 1983).

Fibronectin can mediate cell attachment and spreading on collagens, fibrin, and

artificial tissue culture substrates (Vaheri and Mosher, 1978; Hughes et al., 1980; Hynes, 1986).

Fibronectin binds to collagen type I, II, III, IV and V (Klebe, 1974; Engvall and Ruoslahti, 1977; Engvall et al., 1978). The collagen binding domain of fibronectin is approximately 30-40 kd (Ruoslahti et al., 1979; 1981; Ruoslahti and Hayman, 1979; Yamada et al., 1980; Hayashi and Yamada, 1981; Ruoslahti et al., 1981).

Fibronectin also binds to fibrin and slightly to fibrinogen, the fibronectin can be cross linked to fibrin or fibrinogen by factor XIIIa transglutaminase (Mosher, 1975; 1976). Finally fibronectin also binds to the sulphated glycosaminoglycans heparin and heparan sulfate (Yamada et al., 1980; Garner and Culp, 1981) and to the glycosaminoglycan hyaluronic acid (Ruoslahti and Engvall, 1980). Chondroitin sulphate proteoglycan can also interact with fibronectin (Knox and Wells, 1979). The binding of fibronectin to these ligands seems to be important to cell interactions with extracellular materials.

Fibronectins are composed of similar polypeptide subunits of 220-250 kd that are linked by disulphide bonds into dimers and higher polymers. The cellular and plasma forms of fibronectin are very similar in structure and function (Yamada et al., 1977; Yamada and Kennedy, 1979), but not identical (Yamada and Kennedy, 1979).

Recent results showing that a single fibronectin gene can give rise to several different mRNAs by alternative splicing suggested an explanation for some of this diversity of fibronectin subunits (Tamkun et al., 1984; Hynes, 1985; Kornblihtt et al., 1985; Schwarzbauer et al., 1985; Borsi et al., 1987; Patel et al., 1987).

The binding of fibronectin to the cell surface requires a specific region of the molecule, termed the cell binding region. A monoclonal antibody, 3E3 was used to isolate an 11.5 kd pepsin-derived fragment of fibronectin (Pierschbacher et al., 1981; Pierschbacher and Ruoslahti, 1984 a; b) which directly promoted the

attachment and spreading of fibroblastic cells in vitro.

Further studies using direct amino acid sequencing identified the active region of the cell attachment promoting domain as containing the amino acid sequence arginyl-glycyl- aspartyl-serine (RGDS) (Pierschbacher and Ruoslahti, 1984 a; 1987; Ruoslahti and Pierschbacher, 1987).

This recognition sequence, or a closely related sequence, is present in a number of proteins other than fibronectin, that interact with cells. These proteins include vitronectin, fibrinogen, thrombin, collagen, a bacterial surface protein, and two viral proteins, as well as discoidin-I, a protein implicated in the aggregation of Dictyostelium discoideum. The RGDS sequence is also found in rat testis specific basic protein, and human complement factor B (Auffray and Norotny, 1986).

Yamada and Kennedy (1984) reported that a high level of soluble fibronectin (5-10 mg/ml) could inhibit the spreading of fibroblastic cells on substrates coated with 3 μg/ml of plasma fibronectin. Inhibition was concluded to be competitive in nature and similar inhibitory effects were also reported using RGDS containing peptides (Pierschbacher et al., 1982; Pierschbacher and Ruoslahti, 1984 a; b; Akiyama and Yamada, 1985 b; Yamada and Kennedy, 1984; 1985; 1987).

The interaction of fibronectin with fibroblastic cells has been examined using intact fibronectin (Akiyama and Yamada, 1985 a), synthetic peptides derived from the fibronectin primary structure (Pierschbacher et al., 1982; Pierschbacher and Ruoslahti, 1984 a; b; Yamada and Kennedy, 1984; 1985) and fibronectin-coated beads (McAbee and Grinnell, 1983), the binding of fibronectin to the cells indicated the presence of a minimum of 10^6 fibronectin receptors per cell, suggesting that such a molecule is a major cell surface component. The location of fibronectin binding sites are illustrated in figure 2.

Fibrinogen

Fibrinogen (340 kd) is a blood plasma protein which when proteolysed by the action of the enzyme thrombin is converted into an insoluble protein, called fibrin.

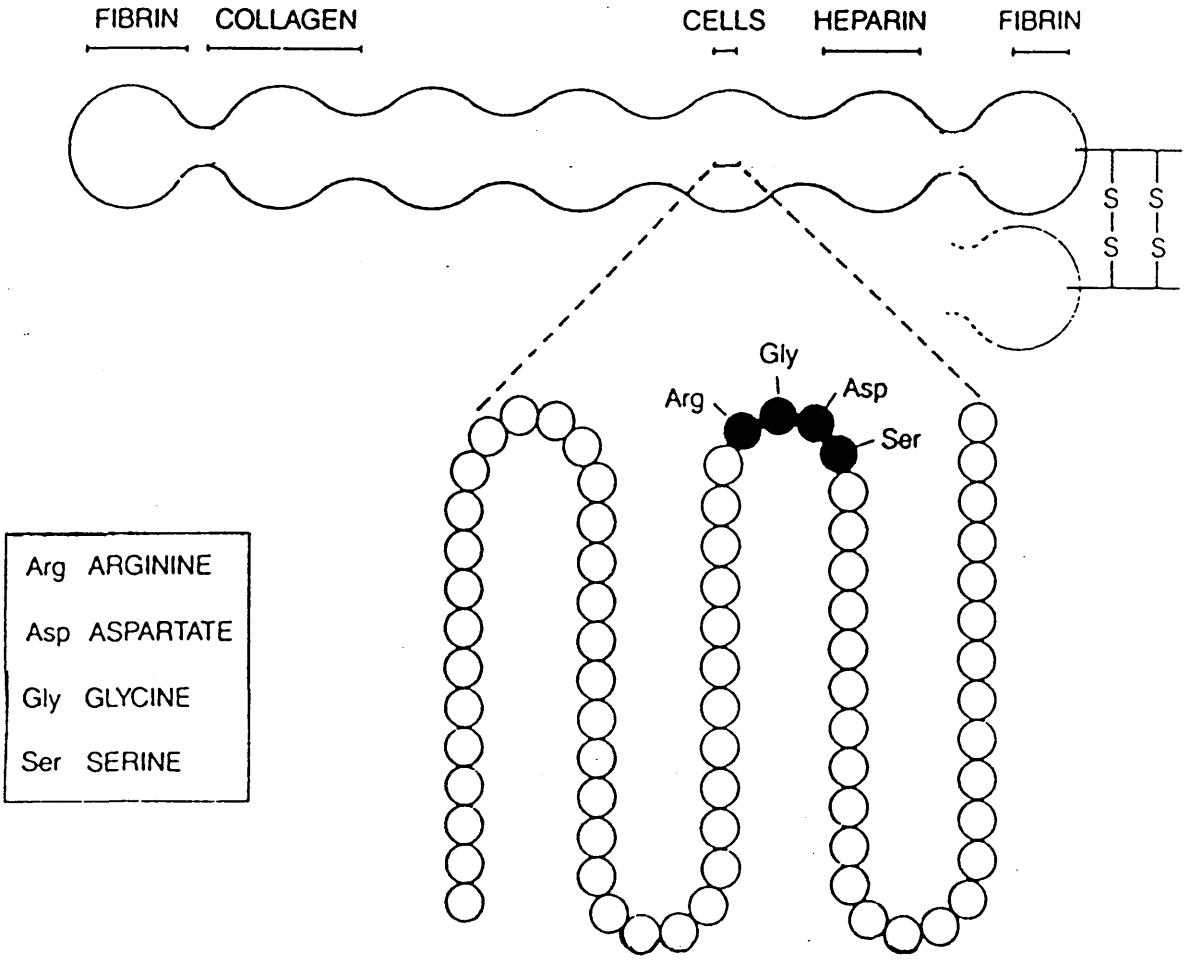


Figure.2 Fibronectin molecule ; its binding sites and the amino acid sequence of the active region which bind to cells

Blood plasma fibrinogen is synthesised in the parenchymal cells of the liver. Fibrinogen occurs, however, also on the platelets, and this form of the protein seems to be synthesised in the megakaryocytes of the bone marrow (Henschen and McDonaga, 1986).

The fibrin(ogen) interacts with fibronectin, thrombospondin, collagen and with different cell types such as platelets, fibroblasts, and endothelial cells. The peptide sequence RGD occurs twice in fibrinogen (Pierschbacher et al., 1985).

Dejana et al. (1987) have reported that substratum-bound fibrinogen, is able to promote in vitro endothelial cell adhesion, spreading, organisation of chick microfilament bundles, and formation of focal contacts, including vinculin. This phenomena is prevented by the presence of RGD peptide sequence in the medium. Fibrinogen supports also the migration of endothelial cells (Kadish et al., 1979; Dang et al., 1985). However, Grinnell et al. (1980) reported that BHK cells did not attach to substrata composed of purified fibrinogen, but that adhesion was supported when plasma fibronectin was covalently cross-linked to fibrinogen.

Fibronectin and fibrinogen receptors

Several types of plasma membrane components have been proposed to serve the function of fibronectin receptors. Photoaffinity cross-linking experiment revealed that chondroitin sulfate proteoglycans (Perkins et al., 1979) and a 47 kd glycoprotein (Aplin et al., 1981) are associated with fibronectin at the cell surface. Membrane glycolipids are also implicated in cell fibronectin interaction. Sialic acid rich gangliosides inhibit cell attachment (Kleinman et al., 1979; Yamada et al., 1981). Several investigators have shown that antibodies that interfere with cell-adhesion recognise cell surface glycoproteins in the 140 kd range (Reviewed by Damsky et al., 1984). Recently, the cell binding domain of fibronectin was used as an affinity matrix to identify putative receptors in human MG-63 osteosarcoma cells and normal rat kidney cells (Pytela et al., 1985 a).

(a) Photoaffinity cross linking experiments

A 47 kd glycoprotein was initially identified by chemical cross-linking experiments designed to identify membrane proteins located very close to substrate-attached fibronectin (Aplin et al., 1981). This protein was reported to be a ricin-binding glycoprotein located on the external surface of the cell. It was labelled poorly by lactoperoxidase-mediated iodination. The authors suggest that this protein is present at the interface between the BHK cell underside and the substrate-attached fibronectin mediating attachment and spreading. It is not secreted from the cell.

The detection of the 47 kd component at sites apposed to the fibronectin-coated substratum requires active cell spreading. It is possible that substantial amounts of this antigen may be intercellular (Hughes et al., 1981), although this point remains to be examined further. In further experiments where fibronectin was replaced by lectins such as Con A, the same 47 kd species became uniquely linked as for fibronectin, despite the known ability of these lectins to bind to many different glycoproteins of BHK 21 cells (Rauvala et al., 1981).

A cell surface glycoprotein of similar size (48 kd) which binds WGA is protected from proteolysis by Ca^{2+} ions and was claimed by Oppenheimer-Mark and Grinnell (1984) to be important in fibronectin-mediated cell adhesion. Proteolytic treatment that cleaves this protein also affects the ability of cells to attach and spread on fibronectin-coated substrates (Oppenheimer-Marks and Grinnell, 1984). This relatively protease-resistant molecule has a similar molecular weight to the protein identified by cross linking, but it may be not identical. A 48 kd glycoprotein was also reported to be retained by cytoskeletons of BHK fibroblasts and embryonic chicken cells after extraction with non-ionic detergent (Docherty et al., 1981).

(b) Gangliosides

A second candidate molecule for a receptor function is the class of ganglioside. It has been suggested that gangliosides serve as functionally important molecules in cell substratum interaction. The recent development of specific monoclonal antibodies directed against the carbohydrate moieties of gangliosides (Kleinman et al., 1979; Yamada et al., 1981; Perkins et al., 1982; Blackburn and Schnaar, 1983; Okada et al.,1984; Cheresh et al., 1986) will aid understanding of biological functions of these membrane-embedded surface-exposed molecules.

Recently Cheresh et al. (1984 a) described monoclonal antibodies directed against the disialogangliosides GD2 and GD3 in human melanoma cells. The epitopes recognised by these Mabs are known to involve the terminal sialic acid moiety since either neuraminidase treatment or mild periodate oxidation abolishes antibody reactivity (Cheresh et al., 1984 b). Using these Mabs, the authors localised GD2 and GD3 in adhesion plaques and on the surface of human melanoma cells. Moreover, they showed that both GD2 and GD3 were indeed heavily concentrated at the interface between these cells and their substratum, suggesting that these molecules may play a role in melanoma cell attachment (Cheresh et al., 1984 a).

Initial evidence indicating gangliosides involvement in cell attachment came from a number of studies that demonstrated that exogenous addition of gangliosides to cells in culture inhibited their attachment to fibronectin (Kleinman et al., 1979; Yamada et al., 1981; Perkins et al., 1982).

Yamada et al. (1981) found that purified solubilised gangliosides inhibit fibronectin mediated haemagglutination, cell spreading, and restoration of a normal BHK cell morphology to transformed cells. The most effective ganglioside inhibitors generally had the most sialic acid. Matyas et al. (1986) however, found no simple relation to sialic acid content, since the ganglioside GD3 in rat liver plasma

membrane which contains two sialic acid residues bound fibronectin only poorly. The result presented by Yamada and his workers supported the hypothesis that the receptor for fibronectin on the cell surface consists either of certain gangliosides or other negatively charged lipids (Yamada et al., 1985).

Reports by Yamada et al. (1983) and Spiegel et al. (1984) demonstrated that gangliosides added to cells caused retention of fibronectin in cell surface-associated fibrillar networks. The observation that gangliosides can mediate the retention of fibronectin at the surface of the ganglioside-deficient mouse L cell variant (NCTC 2071A) has renewed speculation that gangliosides are in some way involved in the interaction of cells with fibronectin (Yamada et al., 1983; Spiegel et al., 1985; 1986). These result are in agreement with Fishman et al. (1981), Mugnai and Culp (1987) and Mugnai et al. (1988) but conflict with the result of Griffiths et al. (1986), who found that variants of BALB/c 3T3 cells which lack complex gangliosides retain a fibronectin matrix and spread normally on fibronectin-coated substrates. This result clearly demonstrated that complex gangliosides are not essential for retention of a fibronectin matrix or for spreading on fibronectin-coated substrates.

Waite et al. (1987) described a second cell-binding domain in plasma fibronectin capable of promoting neurite extension of human neuroblastoma cells. This lies outside the region of plasma fibronectin represented by the 120 kd chymotryptic cell-binding fragment and may in fact be a ganglioside-binding domain (Thompson et al., 1986).

Immunoelectron microscopy and biochemical studies showed that the vitronectin receptor on the surface of the M21 melanoma cells was associated with the diganglioside GD2 only in the presence of Ca^{2+} and that binding of the divalent cation is necessary for the existence of the complex (Cheresh et al., 1987). The fibronectin receptor is probably also associated with gangliosides.

(c) Antibody experiments

This method is used to identify molecules involved in cell substrate adhesion using antibodies or Fabs that inhibit the adhesion of the cells to the substrata.

In chickens, Monoclonal antibodies (CSAT) (Neff et al., 1982; Decker et al., 1984), JG22 (Greve and Gottlib, 1982) were used to identify a 140 kd glycoprotein as the fibronectin receptor [since named integrin (Tamkun et al., 1986)] which consists of at least three glycoproteins with an average molecular weight of 140 kd (Knudsen et al., 1985). The monoclonal antibody CSAT was found to inhibit cellular adhesion of chicken myoblasts to either fibronectin or laminin (Neff et al., 1982). It seems that integrin recognises fibronectin and laminin in an RGD-dependent manner (Akiyama et al., 1986). Other laminin receptors are not members of the RGD receptor family (see later).

Integrin is reported to bind to talin (see earlier), a molecule thought to be important in linking elements of the cytoskeleton with the cell surface. This talin-integrin interaction involves a site of integrin different from that which binds fibronectin and laminin (Horwitz et al., 1986). Buck et al. (1986) have produced a monoclonal antibody that allows the fractionation of integrin subunits. Thus they were able to show that the heterotrimeric (bands 1,2,3) or heterodimeric (bands 1,3 and 2,3) forms are required for binding to fibronectin, laminin, and talin.

Brown and Juliano (1985) obtained a monoclonal antibody to a 140 kd cell surface glycoprotein in fibroblasts that inhibits the adhesion of hamster fibroblasts to fibronectin coated-substrata but does not inhibit adhesion to substrata coated with vitronectin, laminin or serum. The same glycoprotein was identified from rodent and human cells (Brown and Juliano, 1986).

Akiyama et al. (1986) have used a monoclonal antibody that binds to the 140 kd complex and specifically inhibits the direct binding of ^{3}H-labelled 75 kd fibronectin cell binding fragment to chicken embryo fibroblasts in suspension.

The results indicate that the 140 kd complex can bind directly to fibronectin and is likely to be the fibronectin receptor for chicken cells.

Giancotti et al.(1985) purified a 135 kd membrane glycoprotein from BALB/mouse fibroblasts involved in cell attachment and spreading on a fibronectin coated substratum. Further work showed that this glycoprotein is critical for adhesion and is concentrated at close contacts and adhesion plaques (Giancotti et al.1986).

Oppenheimer-Marks and Grinnell (1982) raised antibodies and Fabs against WGA-binding glycoproteins of BHK cells. These antibodies reacted with polypeptides of 48, 61, 83, 105, 165, and 210 kd, and were found to interfere with the spreading of BHK cells on fibronectin coated-substrata.

Antibodies used against rat hepatocytes recognised a 135/200 kd complex (Johansson et al., 1987). Spreading of liver endothelial cells on fibronectin, but not on laminin or collagen, was inhibited by monovalent Fab fragments of the antibodies, suggesting that the 135/200 kd complex is a specific fibronectin receptor for these cells.

A protein related to the IIb-IIIa complex of platelets (see later) was found to be implicated in the adhesion of endothelial cells (Dejana et al., 1988) and in leukocyte adhesion (Burns et al., 1986). A 135 kd glycoprotein was identified by monoclonal antibodies from human platelets (Giancotti et al., 1987). The authors suggest that this protein is immunologically related to the fibronectin receptor but is distinct from the IIb-IIIa complex. Various related molecules have been identified in leukocytes by raising antibodies. These include antigens called Mol and also known as Mac-1 (Hickstein et al., 1987), and lymphocyte-function associated antigen (LFA-1) (Gallin, 1985). All these glycoproteins appear to play a role in the adhesion of neutrophils. It has been found that Mac-1 sequences are related to the alpha chain sequences of fibronectin, vitronectin receptor and the platelet glycoprotein IIb-IIIa (Pytela, 1988). Monoclonal antibodies have been used to identify fibronectin receptors on monocytes (Hosein and Bianco, 1985) and lymphoid cells (Liao et al.,1987), such as the VLA family (Hemler et al., 1987; Hemler, 1988).

(d) Affinity chromatography

Many of the cell-substrate receptors identified by this method seem to belong to a group of receptors that recognise the sequence Arg-Gly-Asp (RGD) in their ligand protein (Leptin, 1986; Ruoslahti and Pierschbacher, 1986; Pytela et al. 1987). This group includes the fibronectin receptor (Pytela et al., 1985 a), vitronectin receptor (Pytela et al., 1985 b), collagen receptor (Dedhar et al., 1987 a) and the IIb-IIIa glycoproteins of platelets (Hagen et al., 1980). Another family of receptors is the Drosophila-specific (PS) antigens found to be similar to these receptors in vertebrates (Leptin et al., 1987).

This approach is based on the use of a large cell attachment-promoting fragment of fibronectin as an affinity matrix, and specific elution by synthetic peptides containing the RGD sequence (Pytela et al., 1985 a). A 140 kd protein was bound by the affinity matrix from an octylglucoside extract of MG-63 human osteosarcoma cells and specifically eluted with the synthetic peptide Gly-Arg-Gly-Asp-Ser-Pro.

The 140 kd protein bound to WGA, and could be incorporated into liposomes at a high efficiency, suggesting that it might be a membrane-embedded cell surface glycoprotein directly involved in the initial step of cell adhesion to fibronectin substrates. Subsequent data such as the amino acid sequence of the human fibronectin receptor confirm these suggestions (Argraves et al., 1986; 1987).

Applying the same technique, Patel and Lodish (1986) identified a 140 kd glycoprotein from mammalian erythroid precursor cells as a fibronectin receptor. With similar approach, Cardarelli and Pierschbacher (1987) identified two polypeptide components, having apparent molecular weight of 160 kd from T-lymphocytes. 135 kd and 115 kd glycoproteins were identified from human endothelial cells as members of the adhesion receptor family implicated in the attachment of human endothelial cells on fibrinogen and von Willebrand factor (Cheresh, 1987).

An affinity matrix (Pytela et al., 1986) made of an insolubilised heptapeptide containing the Arg-Gly-Asp sequence selectively binds the platelet membrane glycoproteins IIb-IIIa from detergent extracts of platelets. When incorporated into liposomes, the isolated protein (124/108) kd confers on them the ability to bind to a surface coated with fibrinogen, fibronectin and vitronectin but not to surfaces coated with thrombospondin or albumin.

This receptor is related to the previously identified fibronectin and vitronectin receptors (see below) in that it recognises the RGD sequence but differs from the other receptors in its wider specificity toward various adhesive proteins. The RGD peptide was found to inhibit platelet adhesion to fibronectin, vitronectin, fibrinogen and von Willebrand factor (Haverstick et al., 1985; Plow et al., 1985 b; 1987; Santoro, 1987). However, it has been found that synthetic peptides corresponding to the carboxyl terminus of the fibrinogen gamma chain inhibit the binding of fibrinogen, fibronectin, and von Willebrand factor to platelets, yet the active peptide sequence (His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val) has only been found in fibrinogen to date (Lam et al., 1987). The authors suggest that RGD and this peptide interact with the same receptor, the IIb-IIIa complex.

It has been reported that IIb-IIIa glycoprotein is sufficient to form a receptor for fibronectin or fibrinogen (Gardner and Hynes, 1985; Parise and Phillips, 1985; 1986; Parise et al., 1987; Phillips et al., 1988). It was found that a glycoprotein resembling the IIb-IIIa complex of platelets mediates endothelial cell attachment on fibronectin, vitronectin, and fibrinogen (Charo et al., 1987).

Vitronectin and its receptor

Human plasma and serum contain a protein immunologically distinct from fibronectin, often referred to as serum spreading factor and having adhesive properties similar to those of fibronectin (Holmes, 1967; Barnes et al., 1980). It has a molecular mass of about 70 kd (Barnes et al., 1980; Hayman et al., 1982). Under appropriate conditions this attachment factor is capable of affecting the attachment,

spreading, growth, differentiation, and migration of a range of cell types in vitro (Barnes and Sato, 1979; Barnes et al., 1980; 1981; 1983; 1984; Barnes and Slinutzer, 1983; Knox and Griffiths, 1982; Hayman et al., 1983; Knox, 1984).

Vitronectin has a heparin binding site distinct from its cell attachment promoting site. In addition, a serum protein adhesive for chondrocytes has also been reported to be a 70 kd glycoprotein (Hewitt et al., 1980). Adhesion of cells to vitronectin is also inhibited by RGD containing peptide (Hayman et al., 1985 a; Silnutzer and Barnes, 1985).

Recently, the vitronectin receptor has been isolated in a similar way to the fibronectin receptor. Pytela et al. (1985 b) have used affinity chromatography to identify a 125/115 kd protein as the vitronectin receptors from human osteosarcoma MG-63 cells.

Suzuki et al. (1985; 1986) found that vitronectin contains the RGD sequence. This result suggests that the same receptor might be responsible for the adhesion of cells to both fibronectin and vitronectin. However this receptor is different from the fibronectin receptor in molecular weight and binding specificity toward fibronectin and vitronectin, although it shares with the fibronectin receptor the ability to recognise the RGD containing peptide. The vitronectin receptor is also thought to be an integral membrane protein because it can be incorporated into liposomes (Pytela et al., 1985 b).

The vitronectin receptor is a glycoprotein composed of a 125 kd alpha and 115 kd beta chain. The alpha subunit sequence shows strong homology with the alpha subunit of fibronectin receptor (Suzuki et al., 1986; 1987). Moreover, the NH_2-terminal sequence of the 125 kd chain was homologous with the NH_2-terminal sequences of two other cell surface proteins, LFA-1 and Mac-1 (see earlier), which have been implicated as receptors for adhesion proteins of leukocytes.

The structures of the fibronectin receptor and IIb-IIIa glycoprotein are similar to

those of the vitronectin receptor. The vitronectin receptor and IIb-IIIa glycoprotein are especially closely related to each other with regard to the molecular size of the subunit (Pytela et al., 1986). Recently, it has been reported that the amino-terminal sequence of IIIa glycoprotein and the beta subunit of vitronectin receptor were identical, while the alpha subunit and the IIb glycoprotein were different, but showed 33% identity (Ginsberg et al., 1987).

Laminin and its receptor

Laminin is a multifunctional protein of the basement membrane with diverse biological activities. Immunological studies showed that laminin is located in the basement membrane of epithelial cells (Damsky et al., 1984). Like fibronectin it can influence cell adhesion (Johansson et al., 1981; Rao et al., 1982; Yamada, 1983) cell growth and differentiation (Manthorpe et al., 1983; Alder et al., 1985), morphogenesis (Spiegel et al., 1983), cell migration (McCarthy et al., 1983; McCarthy and Furcht, 1984) and cancer metastasis (Terranova et al., 1982).

Laminin is able to bind either to the cell surface or to collagen, although laminin appears to be most effective as an attachment protein for epithelial cells when bound to type IV collagen. Primarily, laminin affects cells of ectodermal origin, Hepatocytes and hepatoma cells have been reported to adhere in vitro directly to collagen (Rubin et al., 1981), laminin (Johnsson et al., 1981) or fibronectin (Hook et al., 1977).

Some fibroblasts do not recognise laminin and will not survive if excess laminin is present. Schwann cells can recognise both laminin and fibronectin (Palm and Furcht, 1982). These cells presumably have receptors for both proteins. Endothelial cells secrete laminin (Birdwell et al., 1978) as well as fibronectin.

Laminin was first isolated by Timpl et al. (1979) by extracting the extracellular matrix of a mouse chondrosarcoma. SDS-PAGE showed components of 220 kd (alpha subunit) and 400 kd (beta subunit) which are similar in amino acid

composition (Sakashita et al., 1980). Rao et al. (1982) have isolated the alpha subunit of laminin, the beta subunit was removed by thrombin. The beta subunit of laminin was found to mediate the attachment of human squamous carcinoma cells to type IV collagen. Two distinct cell-binding domains in laminin called E8 (140 and 80 kd) and E1-4 (250 kd) have been found to promote the adhesion and spreading of nonneuronal cells such as myoblast (Goodman et al., 1987).

It is presently unknown whether laminin contains an RGD sequence, however, a nonapeptide (CDPGYIGSR) derived from a laminin B1 chain sequence has recently been shown to inhibit the attachment of epithelial cells to intact laminin (Graf et al., 1987). This peptide appears to compete for the binding of a high affinity 68 kd laminin binding protein to laminin. A recent study reported that this peptide was able to promote B16 melanoma cell migration as well as adhesion (Iwamoto et al., 1988).

Using affinity chromatography techniques and monoclonal antibodies, numbers of glycoproteins (67-120 kd) have been identified as laminin receptors from different cell lines (Lesot et al., 1983; Manlinoff and Wicha, 1983; Liotta et al., 1985; Smalheiser and Schwartz, 1987; Yannariello-Brown et al., 1988).

Finally, laminin and 140 kd fibronectin receptors have been colocalised in epithelial cells (Kleinman et al., 1983; Chen et al., 1985; 1986). However, the in vivo functional role of binding between 140 kd complex and laminin is not known. Chen et al. (1986) found that there is a relationship between the appearance of laminin and the disappearance of 140 kd complex and fibronectin in the differentiation of epithelial cells. Recently, Krotoski et al. (1986) reported that CSAT antigen (140 kd) is distributed along with fibronectin and laminin in the early avian chick embryo. The authors suggested that 140 kd glycoprotein is a receptor for laminin as well as for fibronectin.

Using different synthetic fibronectin-related peptides containing the RGD sequence, Silnutzer and Barnes (1985) found that peptides (Gly-Asp-Ser-Pro, Arg-Gly-Asp-Ser, Thr-Gly-Arg-Gly, Gly-Arg-Gly-Asp-Ser-Pro) which inhibit the

spreading of Hela human carcinoma, human fibroblast, and rat glioma cells promoted by vitronectin, were also capable of inhibiting to a lesser extent cell spreading on laminin coated substrata. A recent study by Yamada and Kennedy (1987) showed that the inverted peptide SDGR was active to inhibit BHK cell spreading on laminin while RGDS was less effective.

Chondronectin

Chondronectin is a glycoprotein isolated from chicken serum that specifically mediates the attachment of chondrocytes to cartilage type II collagen in vitro (Hewitt et al., 1980; 1982 a).

The attachment of chondrocytes to a collagen substrate is stimulated by serum but not fibronectin. The active material in serum was partially purified and was shown to be a protein by its sensitivity to trypsin. This protein of 180 kd, which was named chondronectin, is distinct from fibronectin and does not stimulate fibroblast attachment (Hewitt et al., 1980). Yamada (1983) suggests that chondronectin may be a chondrocyte-specific attachment protein.

Unlike fibronectin and laminin, chondronectin is a compact molecule containing two or possibly three disulfide-linked chains (Hewitt et al., 1982 a) and the cell binding domain resists tryptic digestion. Immunohistological studies suggest that chondronectin is not uniformly distributed in cartilage but is restricted to the regions around the cells at the interface between the chondrocyte membrane and the matrix (Hewitt et al., 1982 a; 1982 b).

Collagen and its receptor

Collagen, the most abundant protein in mammals, is a major component of the extracellular matrix (Kleinman et al., 1981; Yamada, 1981).

Collagen is a trimeric glycoprotein of about 100 kd subunit. Ten distinct collagen types have been characterised from various tissue (Martin et al., 1985). Each type can

be distinguished by the amino acid sequence, the amount of hydroxyproline and hydroxy-lysine, and the degree of glycosylation.

Hayman et al. (1985 b) have shown that cell adhesion to collagen type I is not inhibited by RGDS-containing peptides, while the inverted peptide SDGR was active in inhibiting cell spreading on collagen (Yamada and Kennedy, 1987). Similarly, Nagata et al. (1985) showed that collagen non-competitively inhibited cell spreading but not cell attachment to fibronectin. These data strongly suggest that cell adhesion to collagen is mediated by a receptor distinct from the fibronectin receptor.

The existence of collagen receptors on the cell surface was first suggested several years ago from binding studies of radiolabelled collagen molecules and their peptides to both platelets (Chiang et al., 1977) and fibroblasts (Chiang et al., 1978). A 31 kd collagen binding protein was isolated from purified chick chondrocyte surface membranes by affinity chromatography on type II collagen Sepharose (Mollenhauer and Mark, 1983). This glycoprotein binds to native chick collagen types I, II, III, and V. Since Fab antibody prepared from 31 kd glycoprotein inhibited the attachment of chondrocytes to type II collagen, Mollenhauer et al.(1984) have suggested that anchorin CII (31 kd) plays an important role in mediating the interaction of chondrocytes with collagen.

A set of gelatin binding glycoproteins (47-65) kd have been isolated from mouse embryo parietal endoderm cells (Kurkinen et al., 1984). Only the 47 kd protein (colligin) binds to native type IV collagen.

Recently a 160 kd glycoprotein has been purified from platelets (Santoro, 1986). A single radiolabelled polypeptide of 160 kd was found to bind to collagen in the presence of Mg^{2+}. The authors suggest that this protein mediates the initial divalent cation dependent adhesion of platelets to collagen.

Using affinity chromatography, three polypeptides 250, 70, 30 kd were identified from osteosarcoma MG-63 cells as collagen binding cell surface proteins (Dedhar et al., 1987 a). The authors suggest that this polypeptide complex behaves as

a cell receptor for type I collagen by interacting with it through the Arg-Gly-Asp tripeptide adhesion sequence.

Thrombospondin

Thrombospondin (TS) (420) kd is a multifunctional glycoprotein first described as a secretion product of thrombin stimulated platelets (Baenziger et al., 1972). TS is a component of the extracellular matrix in a wide variety of tissues. Immunohistological studies showed that TS is localised in basement membranes and vessel walls (Wight et al., 1985). TS is also secreted by several cultured cell lines including endothelial cells, smooth muscle cells, fibroblasts, macrophages, monocytes, and tumour cells (Lawler, 1986; Frazier, 1987).

Like fibronectin and laminin, TS binds to different macromolecules and mediates cell adhesion in vitro (Roberts et al., 1985; Lawler and Hynes, 1986; Varani et al., 1986).

The primary structure of TS contains a series of potential calcium-binding sites adjacent to each other. Chymotryptic digestion of TS in the absence of calcium produces a 210 kd trimeric structure (Lawler and Hynes, 1986). This fragment has been shown to bind type V collagen, fibronectin, fibrinogen, and laminin (Mumby et al., 1984)

Recently, two regions of the TS molecule (140 kd and the amino terminal heparin binding site) were found to be involved in the attachment and spreading of human G361 melanoma cells on surfaces coated with TS (Roberts et al., 1987). RGD peptides do not inhibit tumour cell attachment or spreading on TS, although TS contains an RGDA peptide sequence.

Monoclonal antibodies to the IIb-IIIa complex inhibit the binding of TS to platelets (Plow et al., 1985 a). Heparin is a potent inhibitor of the binding of radiolabelled TS to human fibroblasts (Mckeown-Longo et al., 1984), suggesting that binding to these cells may occur via the heparin-binding domain (Murphy-Ullrich and

Mosher, 1987).

Relation between receptors

From recent sequence data, Kishimoto et al. (1987) have suggested that there may be two subfamilies of RGD receptors, each with distinct beta subunits that can associate with multiple alpha subunits. One subfamily consist of chicken integrin, the human fibronectin receptor and human VLA antigens, initially identified on T lymphocytes (Hemler et al.,1985; 1987). An immunological comparison has indicated that the fibronectin receptor is VLA-5 (Takada et al., 1987 b) and this result is confirmed by the identity of the amino-terminal amino acid sequence of VLA-5 alpha subunit (11-14 residues) (Takada et al., 1987 a) with the amino acid sequence deduced for the alpha subunit cDNA clones of the fibronectin receptor (Argraves et al., 1987).

The second subfamily consists of the human vitronectin receptor (Suzuki et al., 1986; 1987) and the platelet IIb-IIIa protein , which share a beta subunit of 90 kd (Ginsberg et al., 1987). The N-terminal sequence of this beta subunit is non homologous to band III of integrin, and is antigenically distinct from the beta subunit of the human fibronectin receptor (Charo et al., 1986; Ginsberg et al., 1987).

USE OF ADHESION ALTERED VARIANTS TO INVESTIGATE CELL SUBSTRATUM ADHESION

Selection for cells poorly adherent to various substrata could be a powerful approach to understand the molecules involved in cell substratum adhesion and the mechanism behind it.

Unfortunately, in many of the variants used to date, the nature of the defect is unknown or the defect affects a biosynthetic pathway such as protein glycosylation and therefore affects many other molecules besides those involved in adhesion (Damsky et al., 1984). Another problem is that cells of such low adhesiveness may

be at a disadvantage for growth if selected from anchorage dependent parental cells (Edwards et al., 1985).

Two types of selection have yielded cell lines with altered adhesiveness to substrates. First, the direct selection of variants with altered adhesion ability to substratum such as adsorbed serum, fibronectin (Klebe et al., 1977 a, Harper and Juliano, 1980; Oppenheimer-Mark et al., 1984) and collagen (Mauch et al., 1986; Paye and Lapiere, 1986). Second, it has been found that cells selected for resistance to certain lectins, often show low adhesiveness to substrates (for review, see Stanley, 1980; Briles, 1982).

A) Selection of poorly adhesive cells

Pouyssegur and Pastan (1976) isolated two mutants of Balb/c 3T3 cells defective in adhesion to tissue culture polystyrene in the presence of serum. Mutagenised cells were preincubated with prostaglandin E1 and methyl-3-isobutylxanthine, a treatment which raises intracellular cyclic AMP and thereby maximises the adhesion of wild type cells.

Poorly adherent cells were then enriched by detachment with a minimal exposure to trypsin. The final selection was made by taking only those cells which were detached by gentle shaking in the absence of trypsin. Two clones isolated by this procedure which appear to be less well spread than the wild type were designated AD6 and AD8

Both mutants have decreased adhesion to their substrate. AD6 has increased numbers of microvilli and agglutinability by low concentrations of Con A, but is not tumourigenic in animals. AD8 showed much less fibronectin labelled by lactoperoxidase catalysed iodination than wild type, whereas AD6 was normal in this respect.

Subsequent studies (Pouyssegur and Pastan, 1977) on AD6 showed that the cells incorporated exogenous D-glucosamine into macromolecules much more slowly than

wild type, and accumulated non acetylated glucosamine 6-phosphate, rather than UDP-N-acetylhexosamines. The enzyme responsible for the acetylation step, glucosamine phosphate acetyl transferase, was successfully assayed in wild type cells but was undetectable in AD6, whereas subsequent enzymes of the pathway were normal (Neufeld and Pastan, 1978). The relation between this enzymic defect and the low adhesive phenotype was shown by supplying the cells with N-acetylglucosamine which restored the normal morphology and adhesion of mutants.

Yates and Izzard (1981) examined contacts between AD6 cells and their substrate using IRM. Both AD6 and the wild type cells formed close contacts, in which the cell surface is believed to be separated from the substrate by about 30 nm, while only 10% of AD6 versus 92% of Balb/c 3T3 cells formed focal contacts and they were very small. Norton and Izzard (1982) showed that N-acetylglucosamine restores the ability of AD6 cells to form focal contacts, suggesting a potential role of glycoproteins in focal contact formation.

A variant of CHO cells defective in adhesion to fibronectin on serum coated collagen was isolated by Harper and Juliano (1980). Both wild type and adhesion variant AD^{V} clones could adhere, spread, and attain a normal morphology on substrata coated with Con-A and poly L-lysine. In addition, the adhesion variants could attach to substrata coated with substratum- attached material derived from wild type CHO cells. Brown and Juliano (1985) prepared monoclonal antibodies PB1-PB2 to the 140 kd fibronectin receptor. PB1 did not block the adhesion of $AD^{V}F11$ to SAM while the adhesion of the CHO wild type to fibronectin was blocked. However, AD^{V} seemed to have normal cytoskeletal and metabolic capacities that allow them to attach and spread on substrata coated with other ligands.

Cheung and Juliano (1985) showed that the adhesion defect in some AD^{V} (CHO) clones can be corrected by raising intracellular c-AMP levels. A recent report (Cheung et al., 1987) strongly indicates that AD^{V} cells have an altered type I c-AMP dependent protein kinase (cAdpk) with lower affinity for cAMP. At normal cAMP levels this

enzyme fails to phosphorylate one or more critical protein substrates; but by raising internal cAMP levels, the defect can be overcome. The authors suggest that type I cAdpk seems to play an important role in the regulation of fibronectin-mediated cell adhesion, cell aggregation, and endocytosis.

Another cell attachment mutant of CHO cells was isolated with the aid of an enrichment procedure (Klebe et al., 1977 a). The mutant was isolated by enriching a wild type CHO population with non attaching cells that did not adhere to collagen. After 1.5 h. the culture medium (Dulbecco's with 10% foetal calf serum) containing non-attaching mutants and contaminating wild type cells, was removed and the cells cultivated. The procedure was repeated seven times over a period of two months at which time a mutant population become obvious. This mutant was found to be defective in its attachment to collagen. Its adhesion could be restored by adding cell attachment protein cCAP (presumably fibronectin) or 100% serum to the medium.

CHO cells with increased adhesion were selected by Atherly et al. (1977). The mutant was obtained from a mutagenised population of CHO cells following ten trypsinisation selection steps in which cells with increased adherence to a substratum (resistance to trypsinisation) were sought. It was found that hyaluronic acid is deficient in this variant.

Benoni and Bradley (1986) isolated mutants of SV40-transformed mouse fibroblasts that have greatly increased cell-substratum adherence. The mutants yielded 2.5-10 fold more substratum attachment material (SAM) than the parental cell lines, and the SAM deposited by mutants was able to mediate attachment of transformed cells to a much greater degree than was the SAM from the parental cell lines. The mutation, which appears to control the generation of SAM "footpads" was shown to cosegregate with resistance to the drug 6-thioguanine, which suggests X-linkage.

A variant with higher adhesion to fibronectin-related peptide has also been isolated. Dedhar et al. (1987 b) reported that culturing MG-63 human osteosarcoma cells in increasingly high concentrations of a synthetic peptide, Gly-Arg-Gly-Asp-

Ser-Pro (GRGDSP), results in selection of cells capable of attaching and spreading in culture in presence of this peptide at 0.85-5.0 mM. These cells overproduce fibronectin receptors but not vitronectin receptors and are morphologically different from the parental MG-63 cells.

Briles and Haskew (1982) isolated three variants of a rat hepatoma cell line termed Ad-3, Ad-4, Ad-5 with altered attachment to collagen, but normal attachment to fibronectin. The rat hepatoma cell line HTC was grown on collagen-coated dishes and incubated at 37° C for 90 minutes. The supernatant fluid, containing non-adherent cells was transferred to culture flask supplemented with growth medium. After several successive rounds of selection, clones were isolated and examined for their ability to attach to collagen. This selection procedure seems to be similar to that of Klebe et al. (1977 a).

Recently, a number of variants defective in their attachment to collagen have been reported. Mauch et al. (1986) reported isolation of a cell line from dermatosparactic fibroblasts defective in adhesion to collagen but not to fibronectin or laminin. It is important to point out that dermatosparactic cells were not selected in vitro but are a cell line from animals with a disease found in sheep and calves. Subsequent study (Mauch et al., 1988) showed that this cells lack a collagen binding protein of 34 kd. In order to study the potential involvement of fibronectin in the adhesive functions of epithelial cells, Paye and Lapiere (1986) selected a transformed epithelial cell line defective in attachment to type I collagen. Like the dermatosparactic fibroblasts, the cells retain the ability to attach to collagen in presence of exogenous fibronectin.

Oppenheimer-Marks et al. (1984) isolated BHK cell variants with an altered adhesion ability. BHK cells were mutagenised with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 4 h at 37° C. The cells were subjected to selection by an enrichment procedure to obtain a population of non-attaching cells. The cells were incubated with growth medium for 10 minutes and rapidly attaching cells were removed. The selection was carried out a total of 15 times until 95% of the cell

population was unable to attach to tissue culture flasks in MEM plus 10% serum.

The variant Fn-1 had decreased ability to attach and spread on fibronectin-coated dishes compared with the parent cells and was unable to phagocytose plasma fibronectin-coated beads, but was able to attach normally and spread partially on substrata coated with polycationic ferritin, Con-A, or anti-BHK cell surface antibody. The authors suggested that the fibronectin receptor function of Fn-1 cells is defective.

Properties of variant mouse fibroblasts selected for poor adhesion to growth substratum have been described by Aplin and Foden (1985). The wild type cells were grown to confluence in tissue culture flasks and subcultured using trypsin/EDTA. Cells were reseeded into the same flask, grown back to confluence and then variants were selected by gentle tapping to dislodge loose cells. These were grown back to confluence and the same procedure repeated until rounded cells had developed.

These variant cells adhere to plastic tissue culture flasks and grow normally to confluence in the presence of serum. After subculture and reseeding onto the same surface the cells initially adhere, but after 2 days of growth they retract into aggregates and detach. If the aggregates are dispersed onto the same surface they remain rounded. However, if the same cells are added back to plastic tissue culture flask they adhere and grow normally. The authors suggest that this variant had a deficiency in the late type of cell substratum adhesion occurring on surface containing accumulated subcellular matrix.

Variants of Polyoma transformed BHK 21 cells unresponsive to fibronectin have been isolated by Edwards et al. (1985). These variants are poorly adhesive both to fibronectin and to the mixed protein film adsorbed to plastic from serum. The variants showed altered morphology in culture, and they were unable to spread on fibronectin, serum and other proteins tested.

B) Selection of lectin resistant cell surface variants

It has been proposed that oligosaccharides moieties serve as specific recognition elements for cell adhesion (Ruavala and Hakomori, 1981). It is therefore of interest that some of lectin-resistant mutants turn out to be adhesion defective (Hughes et al., 1980). These cells should be useful for analysing the role of glycosylation in cell adhesion.

Many cell lines have been selected for ability to grow in the presence of plants lectins [such as wheat germ agglutinin (WGA), and phytohaemagglutinin (PHA), Concanavalin A (Con A) and ricin] (Meager et al., 1976).

Ricin, one of the most toxic substances known, is composed of two, non identical, disulfide bonded subunits, A and B, and is transported into the cell by endocytosis (Olsnes et al., 1974). It binds to cell surface carbohydrate through the B subunits, while once inside the cell, subunit A interacts catalytically with 60s ribosomal subunits to inhibit protein synthesis (Olsnes et al., 1974). The mechanisms of cytotoxicity of the other lectins commonly used as selective agents are not clear. It is possible that they exert their toxicity at the cell surface by inhibiting membrane mobility or the transport of specific molecules.

Stanley and her colleagues have isolated a broad panel of CHO cells resistant to ricin, WGA, and PHA lectins, several of which are well characterised (Stanley, 1980; Stanley, et al., 1975; 1980). Certain of these variants lack an enzyme, N-acetylglucosaminyl transferase which is responsible for the addition of a branch N-acetylglucosamine to the core of asparagine-linked oligosaccharides of glycoproteins.

Ricin and PHA-resistant mutants are poorly spread and weakly adhesive to culture substrates. It seems that the poor adhesion of PHA variants resembles that of other variants like AD6 or other lines with glycosylation defects. WGA-resistant mutants seem generally to be well spread and adhesive to the culture substrates. Briles et al. (1977) isolated two WGA-resistant CHO cells. Both mutants exhibit glycosylation defects decreasing the addition of sialic acid.

Meager et al. (1976) isolated a series of ricin-resistant variants of BHK21 cells in a single step selection after mutagenesis, most of which showed weak adhesion to the culture dishes. Edwards et al. (1976) examined in some detail the adhesive properties, both cell to cell and cell to substrate of 8 of these lines. The authors found 6/8 were strikingly less adhesive to substrates than parental wild type.

Dennis et al. (1984) have isolated WGA-resistant mutants of the highly metastatic tumour cell line MDAY-D2. The mutant cells attach more readily to fibronectin and type IV collagen-coated surfaces than the wild type. Neuraminidase treatment of the wild type cells increased their attachment to the same substrates indicating a possible role for unsialylated glycoconjugates in cell attachment which may in turn influence metastatic capacity.

WGA-resistant mouse melanoma cell lines with increased attachment properties have been described by Finne et al. (1982). These cells show an increase in a specific fucosyltransferase activity resulting in an increase in fucose and decrease in sialic acid. Other WGA-resistant cells in which there is a correlated decrease in metastatic ability, are the PG 19 melanoma cells (Bramwell and Harris, 1978), and the B16 melanoma cells (Tao and Burger, 1977).

Other mutants selected with other lectins such as ricin 1, leucoagglutinin PHA (L), and Con-A have shown either no reductions in their metastatic potential or even an increase (Reading et al., 1980; Tao and Burger, 1982). The ability of ricin resistant cells to spread through the lymphatic channels and their growth in the lymph nodes was not greatly reduced (Reading et al., 1980).

It has been shown that ricin-resistant BHK mutants are deficient in binding of fibronectin (Pena and Hughes, 1978) but these cells have the ability to attach and spread at higher concentration of fibronectin and incorporate to some extent endogenous or exogenous fibronectin into their cell layer (Hughes and Mills, 1986). Pena and Hughes (1978) found that two ricin-resistant BHK21 lines, fail to retain normal levels of surface bound fibronectin, although they appear to synthesise and

secrete normal amounts of active fibronectin into the culture medium and spread on exogenous fibronectin. The adhesion of AD6 3T3 cells was also reverted by exogenous fibronectin (Pouyssegur and Pastan, 1976).

The ricin-resistant BHK cell lines are an interesting model system for studying the functional roles of normal protein glycosylation in properties such as cell-cell and cell-substratum adhesion. These cell lines have been shown to exhibit multiple defects in adhesive behaviour.

The effect of neuraminidase on intercellular adhesion and the low adhesiveness of these cells may be explained by supposing that the form of glycosylation required for cells to be adhesive requires a complex-type asparagine-linked oligosaccharide in which one or more of the antennary galactose residues is not sialylated.

The underglycosylation in variant cells, in some cases involving the peripheral " antennae " of complex-type N-linked glycosylation, causes a major reduction in cell-substrate adhesion of these cells and probably affects intercellular adhesion also. Since adhesion to substrates of these mutants can often be restored by exogenous fibronectin, either binding to cells is not affected, or it is shifted towards weaker binding.

At present, after the isolation of different matrix receptors such as for fibronectin and for vitronectin, one could ask about the function of these adhesion receptors in these lectin-resistant cells. Rees et al. (1978) suggested that the oligosaccharides influence physical properties of, the receptors and thus interactions between individual glycoprotein molecules which control clustering at points of adhesion. Presumably the receptors became more mobile around the cells rather than concentrating in patches opposite the matrix, thus giving weak adhesion. Given that the receptors seem to be dimers perhaps underglycosylation affects the association of alpha and beta subunits resulting in weaker or no adhesion.

Alternatively, the oligosaccharides may sometimes be required for the delivery of matrix receptors to the cell surface, or their stabilisation from cleavage by protease.

Edwards (1983) suggested that both of these possibilities are unlikely, because there is clear evidence that many underglycosylated proteins are secreted normally or expressed normally at the cell surface.

THE AIMS OF MY RESEARCH

The use of adhesion deficient variants has produced some interesting results over the last several years, and is considered a powerful approach to study the mechanism by which cells attach to non cellular substrates (Briles, 1982; Damsky et al., 1984). It was thought that variants unresponsive to fibronectin might be receptor deficient, and might thus aid identification of the fibronectin receptor. Since this work was started, however the receptors which mediate the interaction of cells with fibronectin have been identified by other means (Pytela et al., 1985 a).

Recently, Edwards et al. (1985) isolated adhesive defective variants from BHK21 Polyoma virus-transformed cells. To see whether low adherent mutants can be obtained from other cell types, I set up selection experiments to isolate such mutants from Rous sarcoma virus-transformed cells. All attempts to isolate such mutants were unsuccessful. I next isolated two clones of Polyoma transformed BHK21 cells resistant to 6-thioguanine (6-TG) and from them I isolated two different clones unresponsive to fibronectin. These cells could be used in future fusion experiments with other resistant cells such as thymidine kinase-deficient cells. This type of experiment could show whether the mutation in TG2F1 and TG2F2 is dominant or recessive in the hybrid lines. The TG marker also allows non-adherent cell lines to be monitored for contamination (e.g. by hybrid cells). These variants were used to investigate in some details the aspects of the interaction between these cells both on RGD and non RGD proteins in presence of various divalent cations.

The other part of this project consists of the isolation of two revertants from the non-adherent polyoma cells. These cells showed higher adhesion and spreading on fibronectin compared to the non-adherent parental cells.

Chapter two

MATERIALS AND METHODS

Materials

Cell line

BHK21, clone 13 cells (Stoker and Macpherson, 1964) derived by transformation by Schmidt-Ruppin strain of avian sarcoma virus or polyoma virus were grown as previously described (Edwards et al., 1979) and recloned in soft agar.

Media

The media which have been used mostly in this work are described below:

Bicarbonate buffered Eagles Medium (ECT)

Sterile water	134 ml
X10 Concentrated Glasgow-modified Eagles medium (Flow laboratories).	16 ml
GPSA [Glutamine (200 mM), Penicillin (200,000 units/ ml), Streptomycin (200,000 units/ml), Amphotericin B: Flow laboratories]	5 ml
7.5% $NaHCO_3$	6 ml
Calf serum (Gibco)	20 ml
Tryptose Phosphate Broth (TPB) (Difco)	20 ml

147.5 g TPB (Difco) dissolved in 5 L double-distilled water and pH adjusted to 7.4 .

Cells grown in this medium were supplied with a gas phase of 95% air, 5% CO_2 mixture for buffering purposes.

Ham's F10

20 mM Hepes water pH 7.5	180 ml
X10 concentrated Ham's F10 (Flow)	20 ml
7.5% $NaHCO_3$	1 ml

GPSA [Glutamine (200 mM), Penicillin (200,000 units/ml), Streptomycin (200,000

units/ml), Amphotericin B: Flow laboratories]	5 ml
Foetal calf serum (FCS)(Gibco)	20 ml
Tryptose phosphate Broth (TPB):	20 ml

HEPES Saline (HS)

For 5 L:

NaCl	40 g
KCl	2 g
D-glucose	5 g
Phenol red (1/2%)	10 ml
HEPES	11.9 g

pH adjusted to 7.2 with NaOH.

HEPES is N-2hydroxethyl piperazine-N'-ethane sulphonic acid (Cambridge Research Biochemicals).

Low Calcium HS

The same recipe for HEPES saline was followed except water was distilled from quartz vessel (QDW) to give less than ~10^{-5} M of Ca^{2+}.

Hanks HEPES (HH)

For 5 L:

NaCl	40 g
KCl	2 g
D-glucose	5 g
HEPES	11.92 g
$CaCl_2.2H_2O$	0.93 g
$MgCl_2.6H_2O$	1 g

pH adjusted to 7.2.

Solutions for detaching cells from culture surface

A) Trypsin: 0.25% w/v (Difco 1:250) in Tris-saline. pH 7.4 .

B) Versene: 0.55 mM EDTA (ethylene diamine tetra acetic acid) in phosphate-buffered saline. pH adjusted to 7.4, 1 volume of solution A added to 4 volumes of solution B.

Media for freezing cells for storage

ECT	13 ml
FCS (Gibco)	6 ml
Glycerol (BDH Ltd)	1 ml

40-50x10^6 cells were suspended in 10 ml aliquotted to 1 ml for stock purposes and stored at -71° C.

Phosphate Buffered Saline (PBS)

For 1 L:

NaCl	9.86 g
KCl	0.25 g
Na_2HPO_4 (dry)	1.44 g
KH_2PO_4 (dry)	0.25 g

pH adjusted to 7.2.

Buffered Formalin

For 1 L:

Formaldehyde (40%)	100 ml
NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2PO_4	0.2 g

pH adjusted to 7.2 .

Culture Dishes

35 and 90 mm diameter culture dishes were obtained from Sterilin, 25 and 75 cm^2 culture bottles from Falcon plastics Ltd. Glass bottles for roller culture had an area of about 800 cm^2.

Materials for protein extraction

1) Hanks HEPES (HH)

2) Hanks HEPES containing the following protease inhibitors: 2 mM phenyl methyl sulphonyl fluoride (PMSF) (Sigma). 1 mg/ml p-Tosyl-1-arginine methyl ester-HCl (TAME) (Sigma).

3) Hanks HEPES containing the following:

1% Triton X-100 [polyethylene glycol (9-10) p- t-octyl phenol] (Sigma).

2 mM PMSF

1 mg/ml TAME.

Con A-Sepharose packed into column

1) Tris-Saline

2) 0.01 M Tris, 0.025 M Sucrose, 0.001 $MgCl_2$ pH 7.6. (TSM).

3) 0.01 M Sodium chloride, 0.0015 M $MgCl_2$, 0.01 M Tris, pH 7.4 and included Trasylol (50 units/ml) (Miles-Yeda Ltd) as a general protease inhibitor. (SMT).

4) 1% Sodium deoxycholate (1% NaDOC) (BDH Ltd), 0.05 M Tris,

pH adjusted to 8.3.

5) Con-A-Sepharose (Pharmacia Fine Chemicals, Batch number 95 F-0251).

6) 0.1 M alpha-Methyl Mannoside (Sigma)

1% Sodium Deoxycholate

0.05 M Tris

pH adjusted to 8.3.

Concanavalin A (Con-A) was obtained from Miles-Yeda Ltd.

Con-A Sepharose used "batchwise"

1) 0.1 ml Con A beads (Sigma).

2) Hanks HEPES with 1% Triton X-100.

3) Hanks HEPES with 1% Triton and 100 mM alpha-Methyl Mannoside.

For Wheat germ agglutinin (WGA) beads binding-protein, 100 mM N-acetyl Glucosamine was used instead of alpha-Methyl Mannoside.

Polyacrylamide Gel Electrophoresis (PAGE)

1) Reagents

All reagents were 'electrophoresis' grade and were obtained from the following sources:

Acrylamide and bis acrylamide from Koch-light Lab. Sodium dodecyl sulphate (SDS) from JMC. Ammonium persulfate, Bromophenol blue, and N, N, N'N', tetra methylethylene diamine (TEMED) from Bio-Rad. Tris (hydroxylmethyl) aminomethane (Tris) and glycine from Sigma.

2) Stock solutions

30% acrylamide:

Acrylamide	28.5 g
Bis acrylamide	1.5 g
Distilled water	100 ml

Running gel buffer:

Tris	18.15 g
SDS	0.4 g

dissolved in 50 ml distilled water, pH adjusted to 8.9 with HCl, and made up to final volume of 100 ml with distilled water.

TEMED:

N, N, N', N', Tetra methyl ethylene diamine (10 µL).

Ammonium persulfate:

10% W/V in distilled water freshly made up before use.

Stacking gel buffer:

Tris	5.9 g
SDS	o.4 g

dissolved in 50 ml distilled water, pH adjusted to 6.7 with HCl, and made up to 100 ml with distilled water.

Upper tank buffer:

Prepared as a 5x concentrate, diluted for use in distilled water.

Tris	31.6 g
Glycine	20 g
SDS	5 g

In one liter of distilled water.

Lower tank buffer:

Prepared as a 5x concentrate, diluted for use in distilled water.

Tris	60.5 g
SDS	5 g

pH adjusted to 8.1 with HCl and made up to a final volume of one liter with distilled water.

Protein solubilising medium (boiling mixture):

Stacking gel buffer	2 ml
B-mercaptoethanol (Koch-Light)	2.5 ml
SDS	1 g
Glycerol (BDH Ltd)	2 g
Bromophenol blue	10 mg

Made up to 10 ml with distilled water.

After addition of the boiling mixture, samples were placed in a boiling water bath for 3 minutes, used or stored at -20° C. Used in a ratio of 4 parts sample to 1 part boiling mixture.

Molecular weight standards:

The following proteins were used at a concentration of 1 mg/ml.

Fibronectin (bovine serum) (Gibco)	220 k
B-galactosidase (Sigma)	130 k
Phosphorylase a (Sigma)	125 k
Catalase (Sigma)	60 k
Ovalbumin (Sigma)	45 k
Concanavalin A (Sigma)	22 k

Cell staining

Cells were stained with Kenacid Blue from BDH.

for 0.1% Kenacid Blue:

Methanol	250 ml
Distilled water	250 ml

Acetic acid glacial	35 ml
Kenacid blue	0.5 g

Fibronectin

Fibronectin was isolated from bovine serum on a gelatin Sepharose column using the method of Engvall and Ruoslahti (1977). Eluted with 8M urea, its purity was checked by SDS-PAGE. This protein was used (25 μg/ml) for coating plastic dishes or glass coverslips.

Vitronectin

Serum spreading factor was partially purified from calf serum previously depleted of fibronectin on gelatin-Sepharose, by adsorption on a glass bead column, and elution with 0.06 M $KHCO_3$, 0.2 M K_2CO_3 as described by Barnes and Silnutzer (1983) for human serum. Eluted material was dialysed against Hepes Saline. Though not purified to homogenity, this material had higher specific activity in spreading than purified fibronectin (Edwards et al., 1987). Since the low concentrations of this preparation necessary to induce spreading were insufficient to block access of cells to naked glass or plastic, in experiments with this material coverslips coated with vitronectin or other proteins were further incubated with a blocking solution of haemoglobin (0.5 mg/ml for 30 minutes), a protein chosen for its homogeneity and inactivity in spreading.

Haemoglobin, Bovin serum albumin, Concanavalin-A, Wheat germ agglutinin, and 6-thioguanine were obtained from Sigma. Manganese, magnesium, and cobalt salts, were purchased from BDH LTD.

Radioactive materials

^{3}H-thymidine (specific activity 185 GBq/mmol), ^{3}H-hypoxanthine (specific activity 229 GBq/mmol), and ^{51}Cr (specific activity 9.25-18.5 GBq/mg) were obtained

from Amersham International Ltd, Bucks.

Scintillation fluid

Aquasol was obtained from New England Nuclear and dispensed in 10 ml aliquots.

Methods

Cell culture

Cell lines were grown for 2-3 days in tissue culture bottles in Ham's F10 supplemented with 10% foetal calf serum and 10% tryptose phosphate broth at 37° C. Attached cells were subcultured when reaching 2/3 confluency. The medium was poured off, and the cell monolayer washed twice with Hepes saline (HS). 5 ml (1/4) trypsin/versene mixture was added for 30 seconds. This was poured off and the cells left for a further 5-10 minutes. The trypsin activity was stopped by adding 5 ml fresh medium, the cell suspension transferred to a universal container, aspirated, counted in a haemocytometer and replated at the required density. Cells were used in experiments for 5 weeks then discarded.

Unattached cells (mutants) were subcultured by shaking the culture bottle, aspirating the cells and replating directly for the next culture.

Selection and cloning

1) Selection of non-adherent mutants

BHK21 cells transformed by Rous Sarcoma virus or polyoma virus were first cloned in soft agar, and different colonies were chosen. The cells underwent several passages in (non selective, attached) culture since recloning in soft agar.

Both mutagenised and non-mutagenised cells were used in selection. To mutagenise cells, MNNG (Meager et al., 1975) at 0.05-1 μg/ml was added to 2/3 confluent cultures in ECT or Ham's F10 medium for 24 hours. Then the medium was changed and the cells were grown for a further 2-3 days. About 30-50 million cells from this stage were cultured overnight in a roller culture (800 cm^2) in growth medium.

On the second day, cells which had not attached during roller culture or which

could be released by a gentle rinse with growth medium, were recovered by centrifugation and resuspended in 20 ml growth medium. The cells were distributed between 2 75 cm^2 plastic culture flasks, the growth surface of which had been previously coated with 25 μg/ml bovine plasma fibronectin. After 5-6 hours the unattached cells were transferred in 30 ml growth medium to another single 75 cm^2 flask with or without fibronectin (in different experiments) and incubated at 37° C overnight.

The same procedure was repeated on day 3 and 4. The cells unattached after 5-6 hours on day 4 were plated on dishes for colony counts and inspection of morphology. At each stage, cells were counted and plated on 90 mm^2 tissue culture plastic dishes.

After one week, the unattached cells (which were obtained from polyoma transformed BHK 21 cells only), were recovered and recloned by dilution and in soft agar. An agar underlay was prepared in 90 mm bacterial grade plastic petri dishes with Ham's medium containing 10% foetal calf serum, 10% TPB and 0.5% Noble agar. This was overlaid with 1.5 ml of Ham's containing 0.3% Noble agar and cells in a range of 50-500 per dish were plated. The dishes were incubated at 37° C until colonies developed.

2) Selection of cells resistant to 6-thioguanine

Mutagenised polyoma transformed BHK21 cells, were grown in roller bottles and the attached cells were trypsinised. $1x10^7$ of mutagenised and non-mutagen treated cells were transferred to 75 cm^2 tissue culture plastic flasks in Ham's medium containing 10 μg/ml thioguanine.

The cells were fed with fresh 6-thioguanine in growth medium for 7-10 days until colonies of resistant cells appeared. (The sensitive cells were washed away with warm Hanks Hepes). Resistant cells were cloned in soft agar and used to select variants unresponsive to fibronectin.

3) Selection of revertants

From freshly recloned non-adherent BHK-Py3 mutant cells (F2, see table 1), $1x10^7$ cells were plated in 165 cm^2 tissue culture flasks. After 3 days the floating cells washed away and the attached cells washed 3 times with warm Hanks Hepes. Warm Ham's medium was added to the flask, and the cells further cultured for 4-7 days. This process was repeated 4 times within 7-10 days until attached clones appeared.

Clones were recovered using cloning rings, transferred to multiwell dishes, then to tissue culture flasks. Cells were recloned at 100, 250, and 500 per dish on soft agar for 7-10 days.

Determination of chromosome number

TG2-WT resistant cells and both variant cells (TG2F1 and TG2F2) were grown to 2/3 confluence in Ham's medium and incubated at 37° C for 24 hours in growth medium containing 10 μg/ml colcemide (Gibco). Cells were trypsinised and suspended in graduated, conical-tipped centrifuge tubes containing growth medium, recovered by centrifugation for seven minutes at 1000 rpm, then resuspended in 10 ml preheated hypotonic medium (ECT 1: Distilled water 4) for 20 minutes at 37° C. Cells were recovered by centrifugation, resuspended in 2 ml hypotonic medium for 4-5 minutes, and the volume made up to 10 ml with Carnoy's fixative (ethanol 2: glacial acetic acid 1). Fixative and cell suspension were mixed gently using parafilm to cover the tubes.

2-3 drops of cell suspension were placed onto chilled coverslips, one edge of the coverslips was drained, then the opposite edge, to spread the cells. The coverslips were dried over the pilot flame of a bunsen burner, moving them to avoid cracking. Coverslips were stained for 20 minutes in Giemsa (1: 10 in PBS), washed twice in PBS, air dried and then mounted on clean slides with Clear-Mount. The chromosomes were examined and counted using X100 oil-immersion objective.

Fluorochrome staining for mycoplasma

Py3, F2, F2R1, F2R2, TG2, TG2F1, and TG2F2 cells were grown overnight on 13 mm coverslips in a sectored box containing Ham's medium. Next day, cells were fixed for 2 minutes with (methanol 3: glacial acetic acid 1). The fixative was removed, and the cells were incubated for a further 5-10 minutes with more fixative. The coverslips were rinsed twice in distilled water. 2 ml of bis benzimide fluorochrome "Hoechst 33258" ($5x10^{-5}$ mg/ml) stain was added and incubated at 37° C for 30 minutes. The coverslips were rinsed thoroughly in distilled water, mounted on a glass slide with PBS/glycerol 1:1 and examined using the fluorescence microscope.

Incorporation of ^{3}H-Hypoxanthine into TG-Cells

Py3, F2, TG2-WT, TG2F1, and TG2F2 cells were grown overnight on 13 mm glass coverslips in a multiwell plastic box. Next day, the cells were incubated with 2 μCi/well ^{3}H-hypoxanthine for 0, 1.5, 3, and 4.50 hours at 37° C. Coverslips were washed twice with Hepes saline and then twice with 5% TCA, transferred to scintillation phials, and incubated at 37° C for 30 minutes with 1 ml of 0.3N NaOH. 15 ml of water-compatible scintillation mixture (Aquasol) was then added, and samples counted after standing overnight to reduce chemiluminescene background.

For unattached cells, after labelling, they were collected on a Millipore filter, washed on the filter with 5 ml of 5% TCA and the filters transferred to scintillation phials.

Measurement of TG-resistance

Py3, TG2-WT, TG2F1, and TG2F2 cells were grown in Ham's medium containing different concentration of TG (0-10 μg/ml) for seven days at 37° C. After this period attached cells were trypsinised and counted, while non adherent cells were shaken off and counted by haemocytometer.

Determination of HGPRT-ase specific activity

The standard assay condition for the hypoxanthine phosphoribosyltransferase (HGPRT-ase, EC 2.4.2.8) was as follow: 50 mM Tris-HCl, pH 7.2; 5 mM $MgSO_4$; 1 mM 5-phosphoribosyl-1-pyrophosphate (PRPP); 0.1 mM ^3H hypoxanthine (850 μCi/mmol). Cells were harvested by centrifugation at 3000 g at 4° C for 10 minutes, the pellets were suspended in 2 ml Tris-HCl buffer and lysed by sonication for two periods of 15 seconds.

The assay mixture has, a final volume of 100 μL and contained an amount of extract sufficient to catalyse nucleotide synthesis at a linear rate for the period of incubation. The reaction mixture was routinely preincubated with PRPP at 37° C for 5 minutes before the reaction was initiated by the addition of ^3H-hypoxanthine. A control was run without PRPP. Incubation was at 37° C for one hour and the reaction was terminated by heating the reaction mixture in a boiling water bath for 3 minutes.

The resultant precipitated protein was removed by centrifugation at 2100 xg for 5 minutes and aliquots (10 μL) of the supernatant were spotted onto polyethyleneimine (PEI) cellulose F precoated sheets in the presence of 10 nmol of both unlabelled substrate and product as carriers and the reactants separated by ascending chromatography in distilled, deionised water (Rf values: hypoxanthine, 0.45; IMP, 0.0).

The carrier spots were visualised with ultraviolet light, cut out and counted for radioactivity using a scintillation cocktail containing 0.35% (w/v) 2, 5-diphenylloxazole, 0.005% (w/v) 1,4-di2 (5phenylloxazolyl)-benzene and 3% (v/v) Triton x100 in toluene. One unit of enzyme activity was defined as the amount of enzyme that catalyses the conversion of 1 nmol of substrate to product per minutes. Protein concentrations were estimated by the method of Lowry et al. (1951).

Preparation of coverslips for adhesion/spreading assay

13 or 22 mm glass coverslips were cleaned in an acid bath (sulphuric acid/nitric acid 1:1) for 30 minutes, rinsed thoroughly with tap water then rinsed 3 times in distilled water. Glass coverslips to be coated from solutions of fibronectin or other proteins were placed in 35 mm culture dishes and 2 ml of the protein solution at the desired concentrations added and left for one hour at room temperature, then rinsed twice with Hepes saline or Hanks Hepes.

In some spreading experiments the coated coverslips were further incubated with a solution of 0.5 mg/ml haemoglobin to block free adsorption sites. The coverslips were then rinsed twice in Hanks Hepes or Hepes saline. Some spreading assays were carried out in 35 mm plastic petri dishes.

Adhesion assay

Py3, F2 mutants, and revertants (F2R1 and F2R2) cells were labelled with 2 μCi/ml ^{3}H-thymidine for three days at 37^{o} C. TG2-WT and TG variants (TG2F1 and TG2F2) from 2/3 confluent cultures were labelled with ^{51}Cr (500 μCi in 10 mls Ham's medium) overnight at 37^{o} C. Cells then trypsinised by trypsin/EDTA.

Parental cells and variant cells were washed three times in Hanks Hepes by centrifugation at 1000 rpm for 7-10 minutes and resuspended in Hanks Hepes at concentration of $5x10^{4}$ cells /ml. 2 ml labelled cells of each cell type were plated on 13 mm glass coverslips for 30 or 60 minutes at 37^{o} C, previously coated with 25 μg/ml fibronectin or 10% serum. The coverslips were washed twice with warm Hanks Hepes, then counted as appropriate.

For ^{51}Cr labelled cells, cells were solubilised with one ml 0.3N NaOH for 30 minutes at 37^{o} C, and counted in Gamma counter test tubes. To measure specific radioactivities, $1x10^{5}$ cells were filtered through Millipore filter. Percentage of adhesion was expressed as ratio of counts per minute of labelled cells attaching to glass, to counts per minute in $1x10^{5}$ labelled cells multiplied by 100.

Spreading assay

2 ml cells at $25x10^3$/ml were incubated at 37° C for two hours either on 22 mm glass coverslips or in 35 mm plastic tissue culture dishes previously coated for 30 minutes with fibronectin, vitronectin, laminin, poly L-lysine, Con-A, or WGA, fixed in formal saline, washed three times with distilled water, stained with Coomassie Blue for 20 minutes, rinsed three times with distilled water, air dried, and mounted in Clear Mount.

When divalent cations such as Mn^{2+}, Mg^{2+}, and Co^{2+} were used, cells were suspended three times in Hepes saline or in low calcium Hepes saline (~10^{-5} M) and plated on appropriate surfaces.

Mean spread area (MSA) was measured as described by Edwards et al. (1987). To determine the extent of cell spreading I measured the projected area of ditigised images of fixed and stained cells. Coverslips (22 mm square) were prepared as for spreading assays, but to obtain evenly distributed cells coverslips with fixed and stained cells were placed in 35 mm tissue culture plastic dishes. Images were obtained using a 25x objective of a Leitz Ortholux microscope equipped with a Hamamatsu Vidicon C1000 camera, with M1438 gain expansion and zero offset module. The images were digitised for input to the screen memory of a BBC microcomputer using a Data Harvest video interface (Data Harvest, Leighton Buzzard, UK).

The captured images were in three shades plus background at a resolution of 160/256. The areas of 100 cells per coverslip were measured by counting for each cell the total of 8-connected pixels darker than background using specially written software by Chris Edwards (Edwards et al., 1987). Grey shade detection was standardised by measurement of a reference field of cells. With the 25X objective, 1 pixel corresponded to 2.1 μm^2. In conditions where induction of spreading is very low (e.g. on haemoglobin in Ca^{2+} below 10^{-6} M) the spreading assay can over-estimate the (very small) increase in mean spread area (MSA), as a result of selective loss of rounded cells. The increase in MSA (percentage of control) was calculated by

the following normalisation:

$$\frac{\text{experimental MSA value-minimum MSA value (rounded cells)}}{\text{maximum MSA value (fully spread cells)- minimum MSA value}} \times 100$$

Procedure for protein extraction

For membrane extraction, a protocol similar to that of Crumpton et al. (1978) was used. Py3, F2 mutants, and both revertant (F2R1 and F2R2) cells (100 million) were grown in roller culture bottles. Cells (except the F2 mutants) were washed twice with Hepes saline, and scraped off into 10 ml TSM containing Trasylol at 50 units/ml. The mutant cells were shaken off and spun twice with Hepes saline on the Mistral 4L for 5 minutes. All cell lines were spun for 5 minutes at 1500 rpm, resuspended in 8 ml solution containing 1 mM SMT and PMSF, homogenised (Potter) and centrifuged in a 10x10 ml (Titanium) angle-head rotor on a MSE super speed 65 centrifuge for one hour at 100 kg.

The pellets were resuspended in 7 ml of 1% NaDOC 0.05 Tris pH 8.3 and incubated at 37^{o} C for 30 minutes. The extract was centrifuged for one hour as above. The supernatant was collected, care being taken not to disturb the pellet. The supernatant was applied to a Con-A Sepharose column (bed volume of column used 5ml) which had been previously washed with 0.1 M alpha-methyl mannoside in 1% NaDOC and equilibrated with 1% NaDOC/Tris. The column was washed with 1% NaDOC/Tris until the absorbance at 280 mm returned to its original level. The column was then eluted with 0.1 M alpha-methyl mannoside in 1% NaDOC, 2.5 ml fractions collected, and small aliquots run on SDS-PAGE gels..

Microscale isolation of Con-A and WGA binding proteins

1) Py3, F2 mutants and both revertant were grown in Ham's medium in 75 cm^2 tissue culture flask instead of 800 cm^2 roller culture bottles for 3 days.

2) Cell monolayers were washed twice with Hanks Hepes.

3) Hanks Hepes with inhibitors PMSF and TAME was then added and incubated with the cells for 15 minutes.

4) Hanks Hepes with 1% Triton and protease inhibitors PMSF and TAME was added and incubated for 10 minutes. Mutants were resuspended in the solution above.

The Triton extract was added to 0.1 ml Con-A or WGA Sepharose beads in 1.5 ml polypropylene conical microcentrifuge tubes. (The beads had been previously washed 3 times in Hanks Hepes with 1% Triton/alpha methyl mannoside and 3 times in Hanks Hepes with 1% Triton). The extract was incubated with lectin-Sepharose constantly agitated in an Emscope rotating shaker for 30 minutes at room temperature.

The lectin-Sepharose beads were resuspended 3 times in Hanks Hepes with 1% Triton for 2 minutes and eluted with 100 mM alpha- methyl mannoside. The supernatant was centrifuged for another two minutes and a small further pellet discarded. On WGA-Sepharose, N-acetylglucosamine was used for elution instead of alpha-methyl mannoside. Elution carried out with 0.5 ml Hank Hepes containing 1% Triton and 100 mM N-acetylglucosamine.

In both methods, lectin-binding proteins in the supernatant was diluted 4: 1 with boiling mixture, and boiled for three minutes in a water bath before being analysed on SDS-PAGE gels.

Method for silver staining PAGE gels

The method used was similar to that of Okley et al.(1980). Due to the sensitivity of the stain, clean glassware, and the wearing of vinyl gloves were all essential. The procedure gave good staining for slab gels of 140x140x 1 mm dimensions.

The gel was fixed with 50% methanol, 10% acetic acid for 30 minutes, rinsed 3 times with 15% methanol for 10 minutes each, and fixed with 5% freshly prepared glutaraldehyde for 30 minutes. The gel was then rinsed 3 times with 15% methanol for 20 minutes each.

Ammoniacal silver solution was made up immediately before use, as follows: 1.4 ml of NH_4OH was added to 21 ml of 0.36% NaOH. Under mild agitation 4 ml

of 19.4% $AgNO_3$ was added. A brown precipitate formed but disappeared on shaking. The volume was then made up to 100 mls with double-distilled water. A container slightly larger than the gel was used and allowed the gel to float freely in the solution. The gel was stained for 12 minutes with constant agitation. After staining the gel was thoroughly washed with double distilled water for 10 minutes.

Developing the stain: all the solutions were made immediately before use. A solution of 0.005% citric acid and 0.019% formaldehyde [5 ml of 1% citric acid stock and 0.5 ml of 38% formaldehyde solution (BDH Ltd) in a liter] was used. The proteins became visible 5-10 minutes after the developer was added. The gel was removed from the developer when the background became too dark. Gels were kept in double-distilled water until photographed for a permanent record.

Chapter three

RESULTS

SELECTION OF ADHESION VARIANTS

A) Selection of non-adherent mutants

Edwards et al. (1985) reported the isolation of polyoma transformed variants unresponsive to fibronectin. To see if mutants with similar properties could be selected from BHK21 cells transformed by different agents, a selection procedure was set up using BHK-SR (BHK21 cells transformed by Schmidt-Ruppin virus, a strain of Sarcoma virus, an RNA virus). Cells were plated on fibronectin-coated tissue culture plastic flask for 6 hours. At the end of this period the unattached cells were transferred to fresh medium and re-plated for 18 hours in serum containing medium, in some experiments on fibronectin-coated plastic. The unattached cells were resuspended and replated on fibronectin for 6 hours, and this cycle continued 3 times. The effect of these times should be to avoid passaging wild type cells detached at mitosis. No variants were isolated. The same experiment was repeated several times using cells treated with the mutagen MNNG (0-1 μg/ml), using different media (Eagles and Ham's), and different initial numbers of cells (30-50 millions). At the end of each of these experiments, the unattached cells were transferred to 90 mm tissue culture dishes, grown to yield colonies which were then transferred to Linbro wells using cloning rings, or cloned in soft agar for 7-10 days. All clones appeared to be wild type in response to surfaces, i.e. they showed no sign of reduced adhesion on fibronectin. The experiment was repeated with two different clones of recloned SR-parental cells, with the same result. The same procedure used with Py3 cells yielded mutants successfully.

Adhesion defective mutants from Py3 cells, F1 and F2, (Table 1) had already been isolated in this laboratory. So if a corresponding phenotype of BHK-SR cells exists, it must occur at very low frequency. To confirm this, the same selection was carried out starting with co-cultures of F2 cells with SR cells at 0, $1{:}10^6$, $1{:}10^4$, and

Table (1). Nomenclature of all cell lines used and selected in this work.

CELL LINE	MORPHOLOGY	ORIGIN
WT-CELLS ON FIBRONECTIN		
1.BHK-SR	spread cells	Macpherson (1965, 1966)
2.BHK-Py3	spread cells	Stoker&Macpherson (1964)
3.TG1 (This work)	spread cells	Py3
4.TG2 (This work)	spread cells	Py3
NON-ADHERENT MUTANTS		
1.F1 *	rounded loosely attached cells in compact colonies	Edwards et al.(1985)
2.F2	"	"
3.TG2F1 (This work)	"	TG2
4.TG2F1 (This work) *	"	TG2
5.TG2F2 (This work)	rounded cells in scattered colonies with occasional slight spreading	TG2
REVERTANTS		
1.F2R1 (This work)	spread cells	F2
2.F2R2 (This work)	partially spread cells	F2

* Mutants selected without the use of mutagen (MNNG) and have not been used for adhesion and spreading experiments in this work.

$1:10^2$. F2 cells were recovered at $1:10^6$ and higher inputs, while recovery of SR mutants was again zero (Table 2).

Therefore, the frequency of occurrence of SR mutants similar to F2 mutants must be less than $1:10^6$. SR cells have a flatter morphology than Py3 cells. The mean spread area of SR cells is higher compared to C13 and TG2 cells (see later) (Fig. 3). This may be relevant to the failure to isolate non adherent mutants from SR cells.

B) Selection of cells resistant to 6-thioguanine

To obtain drug resistant mutants, I next isolated Py3 cells with the genetic marker resistant to 6-thioguanine. Py3 recloned in soft agar, were grown in Ham's medium containing 10 μg/ml 6-thioguanine. Two of the largest clones (TG1, TG2) were selected for use as parental cells in selection experiments. 50 million of TG2 cells were plated on fibronectin coated surfaces. After the usual 3 days recycling, the non adherent cells were plated on tissue culture dishes. In addition to colonies similar to the parental cells, a large number (20 colonies/dish on 10 dishes) of colonies of two different morphologies from TG2 were found (Fig. 4 a). Variant 1 (TG2F1) has rounded loosely attached cells in compact colonies (Fig. 4 b) and is morphologically indistinguishable from the variants (F1, F2) isolated by Edwards et al. (1985). Variant 2 (TG2F2) grow as scattered colonies of mostly rounded cells, which attach weakly but spread little on tissue culture flasks (Fig. 4 b). Table 3 shows the number of unattached and easily detached TG-cells to fibronectin during a single cycle of selection (6 hours on fibronectin). In this cycle TG2F1 enrich 47 times relative to wild type. Although more adherent than TG2F1, TG2F2 cells nevertheless enriched 21 times relative to wild type. To ensure that these variants still had a low activity of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT-ase), Py3,F2, TG1, TG2, TG2F1, and TG2F2 were tested for ability to incorporate ^{3}H-hypoxanthine during an incubation of up to 4.30 hours. Fig.5 shows that TG-cells

Table (2) Mixing experiment of BHK21-SR and Py3 mutants (F2).

	SR		Py3 mutants (F2)	
Initial cells (total)	$50x10^6$		50	
	WT		**mutants**	
	colony/dish	+ SD -	colony/dish	+ SD -
Cycle 1 Fn	637	35	12	4
Cycle 2 Fn	214	31	11	3.5
Cycle 3 Fn	113	25	6	1.2
Cycle 4 Fn	13	8	4	1

F2 cells were mixed with BHK-SR cells in ratio 1: 10^6 in a selection experiment. Colonies were fixed and stained with Kenacid Blue and the number of SR-WT cells and F2 mutants colonies were scored as follows: A 3 mm grid was ruled on a transparency acetate and placed under the 90 mm petri dish. Cells in SR-WT colonies were attached firmly and spread on the surface while the non-adherent Py3 mutants have small rounded morphology with no spreading. (Five dishes).

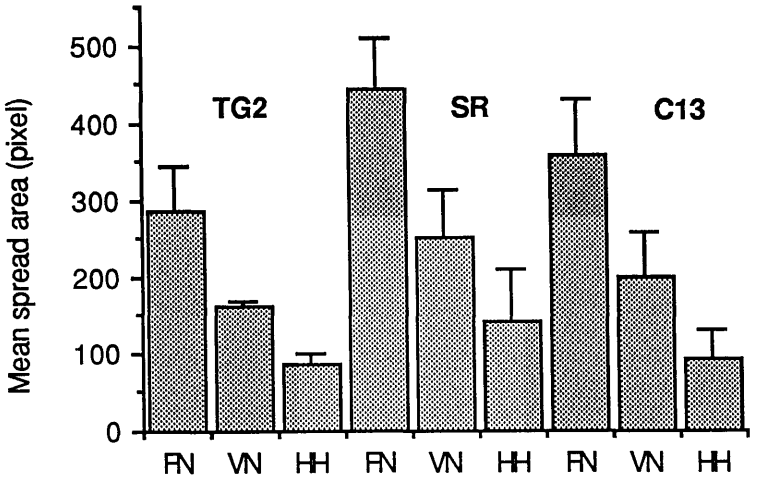


Fig. 3 Effect of fibronectin and vitronectin on the spreading of BHK C13, SR and TG2 cells

FN Fibronectin

VN Vitronectin

HH Hanks Hepes

$25x10^3$ cells/2 ml were plated on coverslips previously coated from solutions of 25 µg/ml fibronectin, or 10 µg/ml vitronectin (partially purified, see methods), coverslips were post-incubated with 0.5 mg/ml haemoglobin for 30 minutes to block non-specific adsorption. The cells were incubated for 2 hours at 37^{o} C and the mean spread area measured (Three experiments).

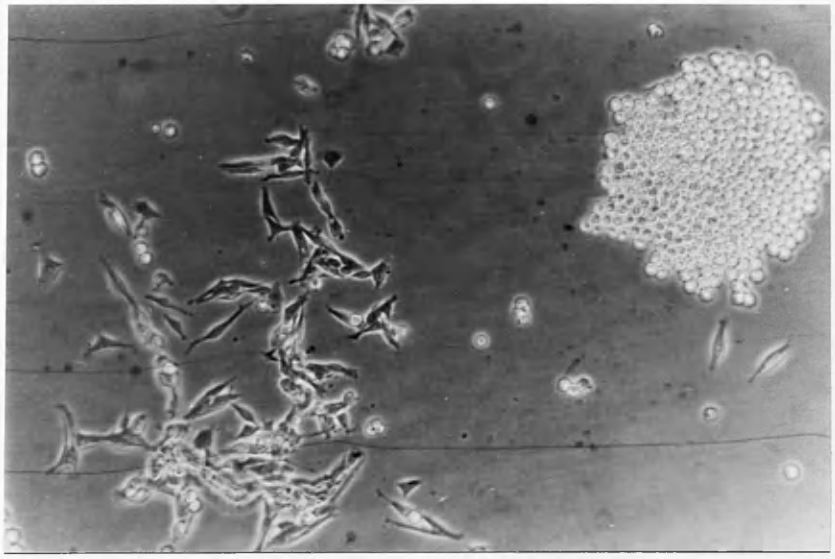
* Error bar in all figures represent the actual variation between separate experiments unless otherwise specified.

Fig. 4 (a) Clonal morphology of variants during selection.

1. Left, TG2 parental cells
   Right: TG2F1 cells
   Phase contrast
   Scale bar : 100 μm

2. Left, TG2 parental cells
   Right: TG2F2 cells
   Scale bar : 100 μm

1



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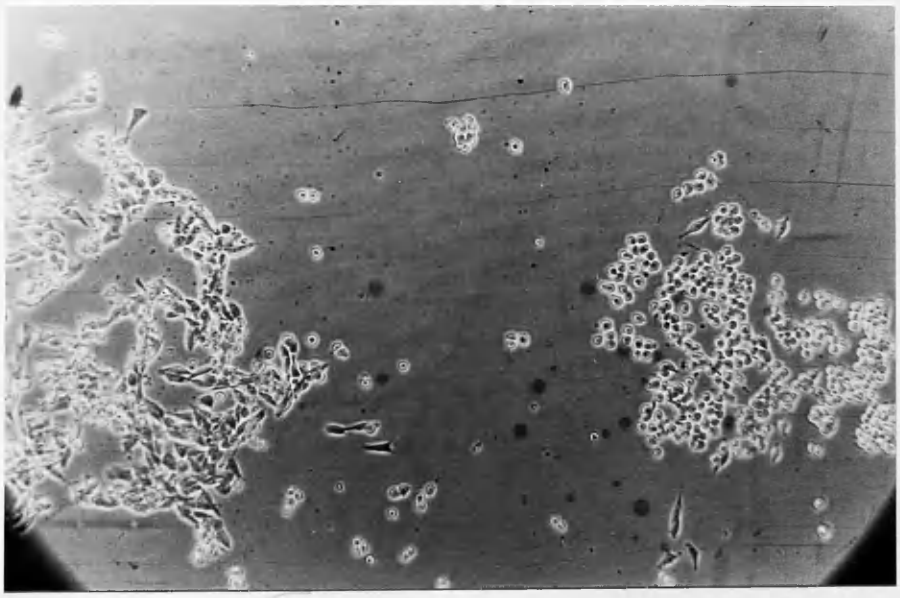

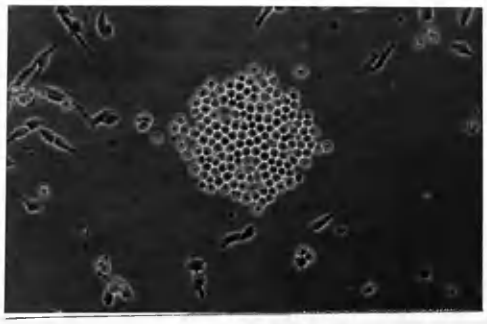

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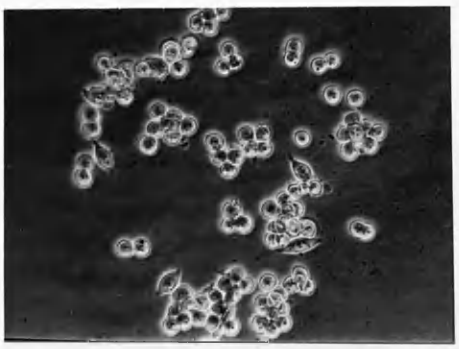

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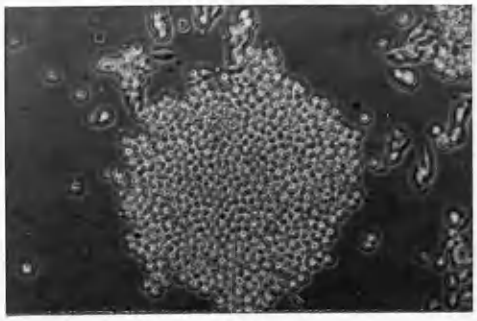

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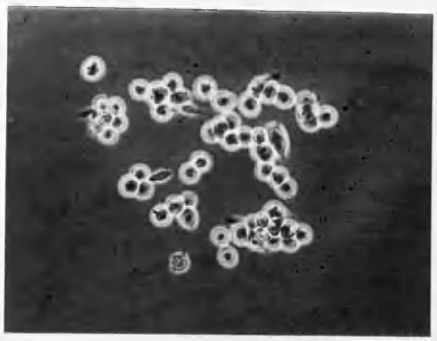

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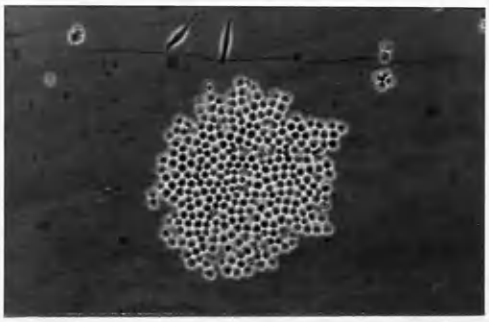

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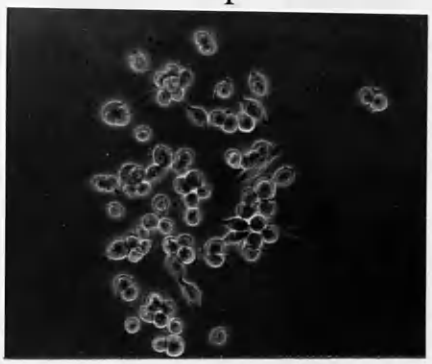

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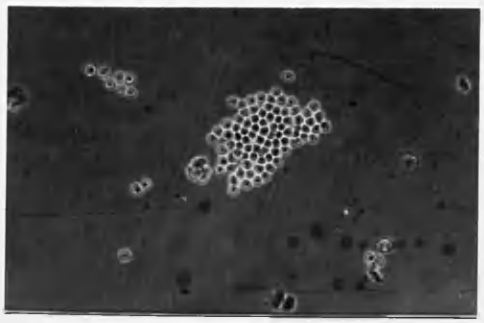

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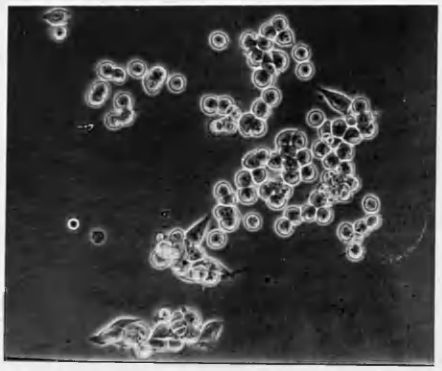


H

Fig. 4 (b) Clonal morphology of variants (Four different colonies of each type)

A,B,C,D TGF1, rounded cells in compact colony
Phase contrast
Scale bar : 100 μm

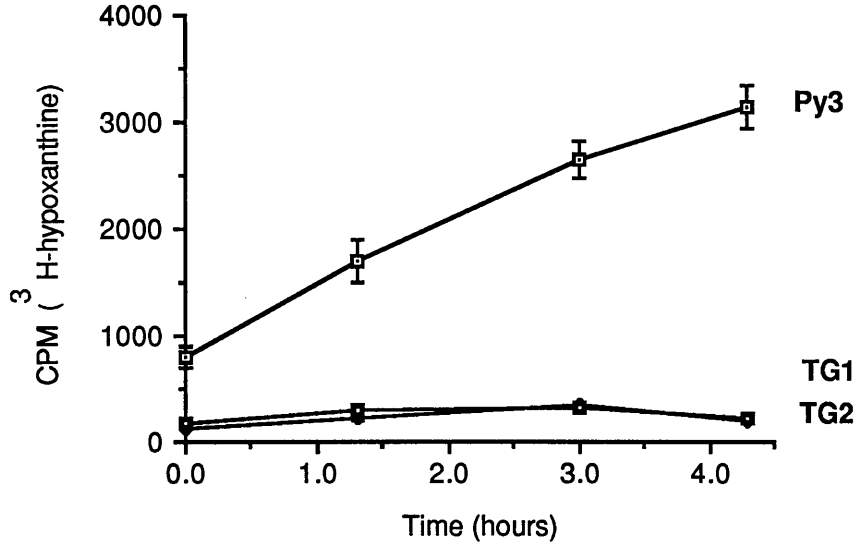
E,F,G,H TG2F2, rounded cells in scattered colony with occasional slight spreading
Phase contrast
Scale bar : 100 μm

Table (3). Enrichment experiment showing the number of unattached cells during 6 hours cycle of TG-cell lines on fibronectin.

	Number of floating cells	Number of easily detached cells	Total
TG2 (WT)	$56x10^3$*	$8x10^3$	$64x10^3$
TG2F1	$2900x10^3$	$56x10^3$	$2956x10^3$
TG2F2	$1300x10^3$	$65x10^3$	$1365x10^3$

* Mean of two samples

$2x10^6$ TG2 , TG2F1, and TG2F2 cells were grown for six hours on fibronectin coated surfaces (25 μg/ml). unattached cells were collected and counted using haemocytometer. Culture surfaces were washed with medium to collect loosely attached cells which were also counted.



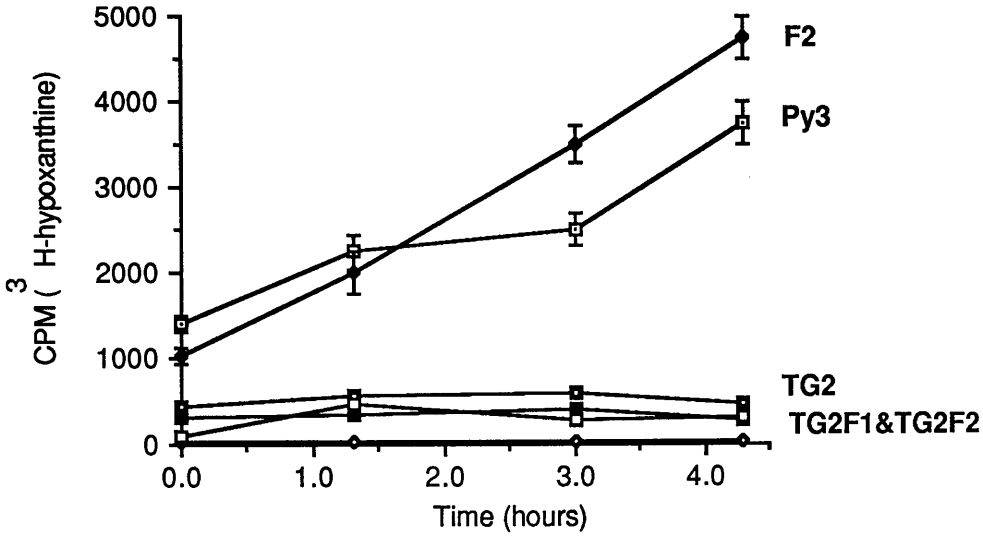


Fig.5 Labelling of Py3, TG1, TG2, TG2F1, and TG2F2 cells with ^{3}H-hypoxanthine

$0.25x10^6$ cells of Py3, F2, TG2-WT, TG2F1, and TG2F2 were grown overnight on glass coverslips which were placed in plastic multiwell dishes (Non adherent variants were grown on Poly L-lysine). The cells were incubated with ^{3}H-hypoxanthine at 2 μCi/well for 0-4.30 hours at 37° C. At the times indicated, coverslips (Four samples) were washed twice in Hanks Hepes, then transferred to scintillation vials. (Five experiments). (Error bar=standard deviation)

either completely lack or have greatly reduced ability to utilise hypoxanthine compared to the parental Py3 cells.

To confirm that TG cells have altered HGPRT-ase, another assay was carried out to measure the specific activity of HGPRT-ase in these cells (see methods). HGPRT-ase specific activity for all TG cells was very low or undetectable (Table 4) while the HGPRT-ase specific activity for Py3 and F2 cells was high and measurable. To check that TG cells are still resistant to 6- thioguanine and have the ability to grow in TG media, Py3, F2, TG2-WT, TG2F1, and TG2F2 were grown in different concentrations of TG medium for 7 days (Fig. 6). The result showed that TG cells were indeed still resistant to 6-thioguanine while Py3 cells were unable to survive in such media.

Time-lapse video-tape recording of TG2-WT, TG2F1, and TG2F2 cells in culture were carried out. Cells were seeded in 25 cm^2 tissue culture flasks in Ham's medium. The behaviour of the cells was recorded on video-tape using a x10 objective, and phase-contrast optics. On seeding, TG2 showed some movement by extending small processes and then spreading. During mitotic division, the cells became rounded, daughter cells separated, moved apart from each other and then respread (Fig. 7).

TG2F1 cells remained rounded, and after division, daughter cells stayed in close contact (Fig.8). A few cells from TG2F2 spread temporarily (Fig.9), but also stayed very close after mitotic division. Both variants showed slight marginal ruffling activity.

TG2F1 cells were subcultured after detachment from their culture surface by a sharp tap (without the use of trypsin/ EDTA) while TG2F2 cells were subcultured by using trypsin/EDTA as for wild type. The wild type and both variant cells can be maintained in continuous culture for at least 5 weeks without changing their respective phenotypes.

Table (4). Measurement of hypoxanthine guanine phosphoribosyl transferase specific activity for different cell types.

Cell type	HGPRT-ase specific activity nmol/min/mg protein
F2	2.57
Py3	1.93
TG2	< 0.0083
TG2F1	< 0.011
TG2F2	< 0.088

10^7 TG2, TG2F1, and TG2F2 cells were centrifuged, the pellets suspended in 2 ml Tris-Hcl buffer, and lysed by sonication. The extracts were incubated with PRPP at 37° C for 5 minutes before the reaction was initiated by the addition of ^{3}H-hypoxanthine (10 µCi/mmol). The precipitated protein was removed by centrifugation and aliquots (10 µL) of the supernatant were spotted onto polyethyleneimine (PEI) cellulose F precoated sheets in the presence of 10 nmol of both unlabelled substrate. Product and the reactants were separated by ascending chromatography. The carrier spots were visualised with ultraviolet light. The spots of the chromatogram containing the purine were cut out and counted for radioactivity (see methods).

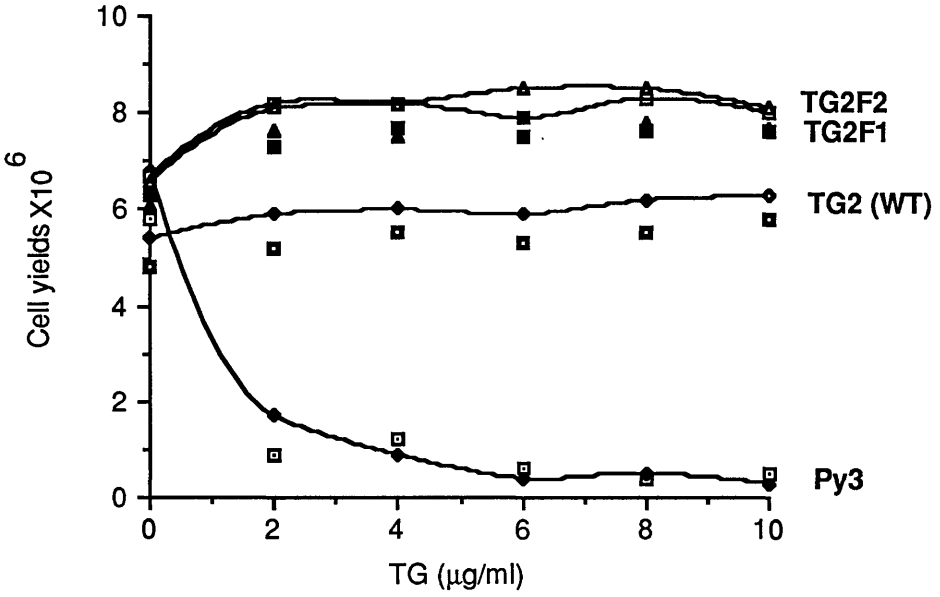


Fig. 6 Effect of 6-thioguanine on the growth of Py3, TG2 (WT) , TG2F1, and TG2F2 cells

10^5 cells of Py3, TG2-WT, TG2F1, and TG2F2 were grown in 90 mm tissue culture dishes for 7 days at 37° C in Ham's medium containing 6-thioguanine (TG) (0-10 μg/ml). To measure cell yields, attached cells were washed twice with Hepes Saline then either trypsinised with 2 ml trypsin/EDTA for 10 minutes (WT), or for mutants, cells were detached by a sharp tap. Cells were collected and counted by Coulter counter. The total cell yield was counted using a haemocytometer (Two experiments).

Fig.7 Video tape recording sequences of TG2 parental cells in culture.

A. Attached cell

B. Two daughter cells extending processes (9 hr.)

C, D, E, F, G, H Partially and fully spread cells

Time: 25, 31, 36, 40, 46, 49 hours.

Scale bar : 100 μm

A

B

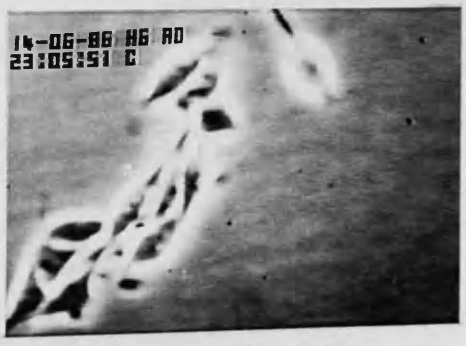
C

D

E

F

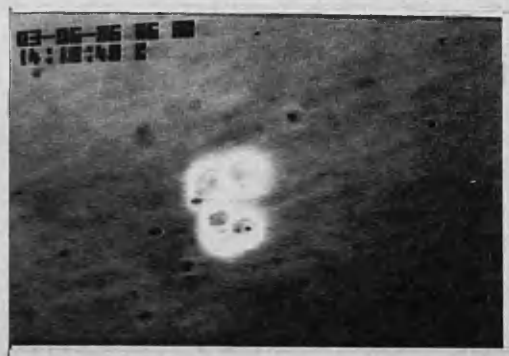
G



H

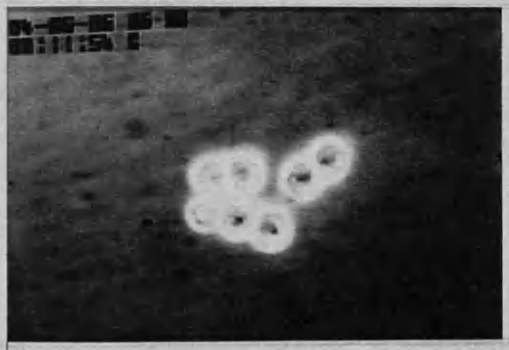
A

B



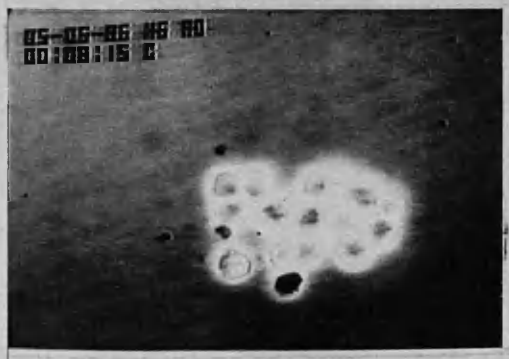
C

D



E

F



G

H

Fig.8 Video tape recording sequences of TG2F1 cells in culture.

A. Three attached cells

B. Two of the above

C. Four daughter cells appeared (14 hr.)

D, E, F, G, H. Small colony growing from 7 to about 20 cells

Time: 23, 24, 35, 36, 41 hours.

Scale bar : 100 μm

Fig.9 Video tape recording sequences of TG2F2 cells in culture.

A, B. Attached cell

C. Cell dividing

D. Four daughter cells appeared (35 hr.)

E, F, G, H. Small colonies formed from 8 or 21 cells. Note transient spreading typical of TG2F2 in E.

Time: 36, 47, 62, 72 hours.

Scale bar : 100 μm

A


08-06-86 H6 AD
09:50:38 C

B


08-06-86 H6 AD
13:55:59 C

C


09-06-86 H6 AD
12:59:14 C

D


09-06-86 H6 AD
13:58:03 C

E


10-06-86 H6 AD

F


10-06-86 H6 AD
19:06:12 C

G


11-06-86 H6 AD

H

C) Selection of revertants

During the passaging of F2 cells, occasional revertant cells which spread on the tissue culture flask were noticed. In order to select these revertants, I set up a selection experiment in which freshly recloned F2 cells were grown in large numbers (10^7) (see methods) in tissue culture flasks for 7-10 days.

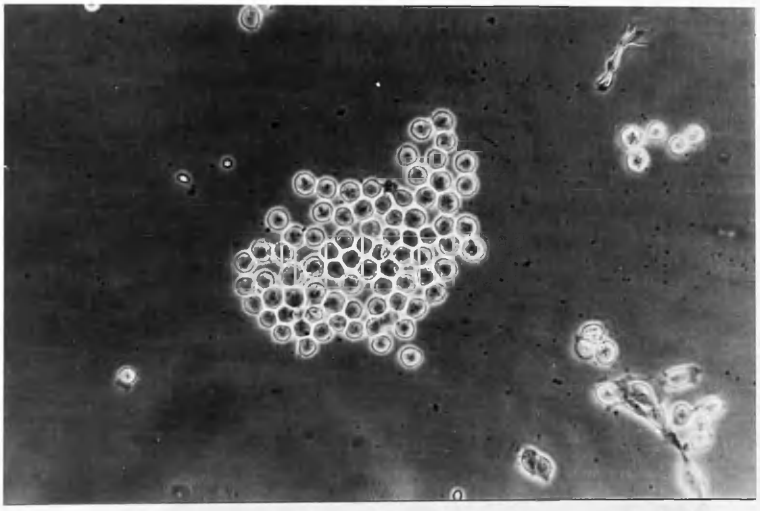
After washing away the non-adherent cells, several clones were transferred and two of different morphology (F2R1, F2R2) were chosen and recloned in soft agar. F2R1 was fully spread in attached culture whilst F2R2 was less spread (Fig. 10 a). Both variant phenotypes were stable in culture during several passages using trypsin/EDTA. Furthermore, a third type of revertant was noticed several times (Fig. 10 b). This clone had fully spread cells similar to that of C13, but the isolation of these cells in Ham's and conditioned media was unsuccessful. By counting attached clones and the number of F2 cells, the frequency of these revertant was estimated to be about 1- $1.4:10^5$.

Chromosome number

To rule out the possibility that the clones of these variant cells arose from cross contamination of BHK-Py3 with other cell lines maintained in this laboratory, and to test whether variants might be derived by chromosome loss from the parental cells, karyotype determination were carried out.

It was found that the chromosome number of variant cells (TG2F1 and TG2F2) was the same as parental (TG2) (about 42), the chromosomes appeared to be telocentric and metacentric (Fig. 11). Therefore, the chromosome counts showed that the variants are derived from TG2 and are not a contaminant with other non-adherent cell lines such as mouse myeloma cell lines.

A



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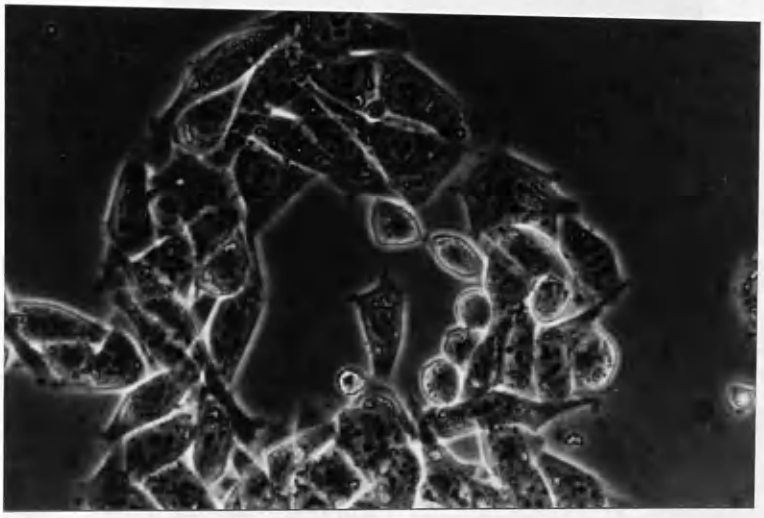

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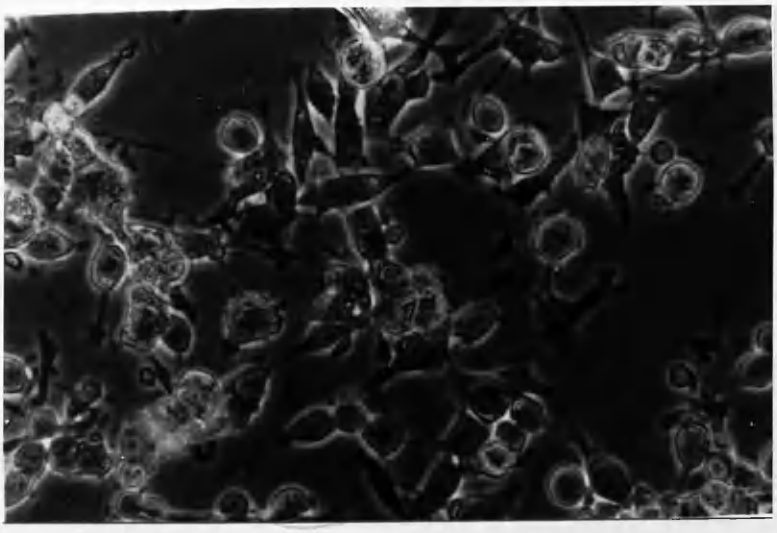


Fig. 10 (a) Morphology of cells in culture

A. F2 mutants (parental cells)
Note the rounded morphology
Scale bar : 100 μm

B. F2R1 cells (very well spread cells)
Scale bar : 132 μm

C. F2R2 cells (partially spread cells)
Scale bar : 132 μm

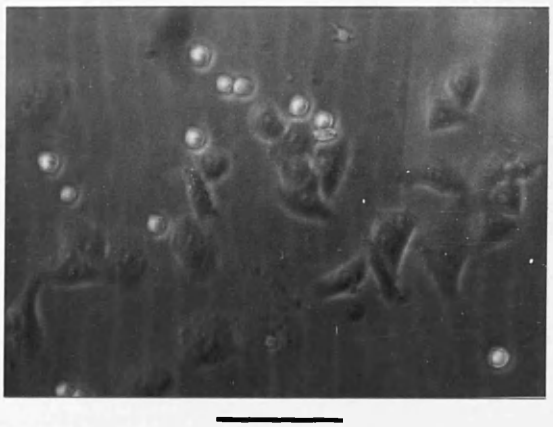
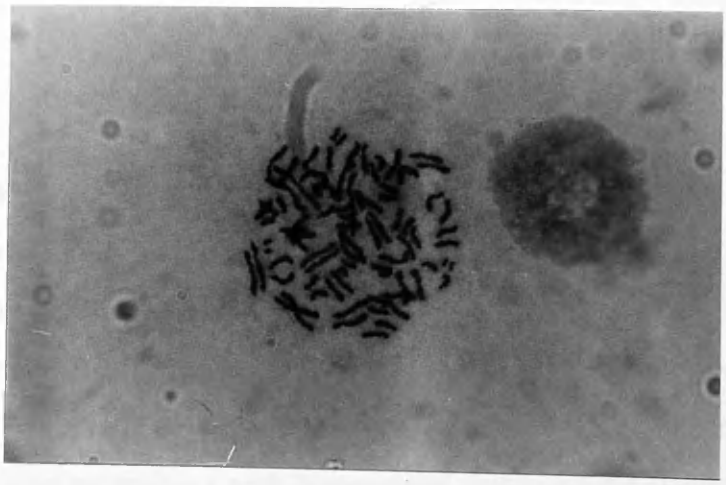


Fig. 10 (b) Morphology of type three revertant

Note that cells are fully spread.

Scale bar : 100 μm

1



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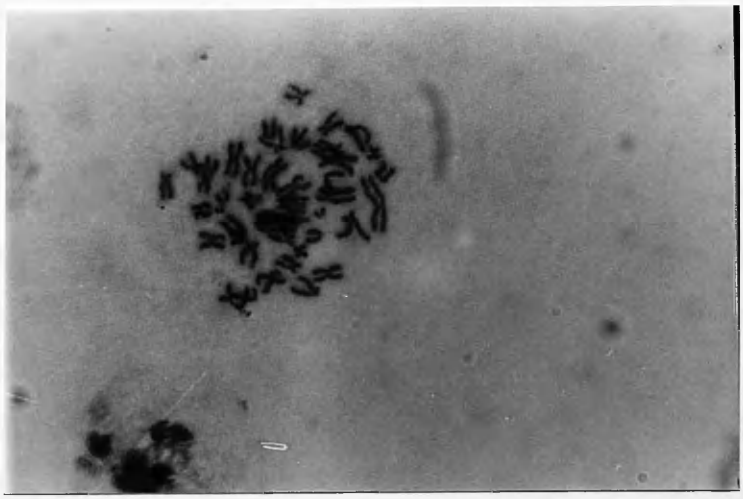

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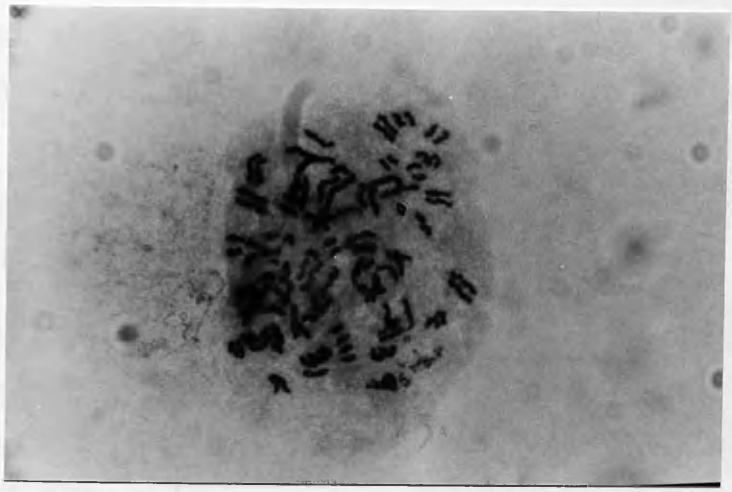


Fig. 11 Karyotyping

Cells were treated with colcemide, fixed and stained (see methods).

1. TG2-WT chromosome number is about 42

2. TG2F1 chromosome number is about 42

3. TG2F2 chromosome number is about 42

Scale bar : 20 μm

Fluorescent technique for detecting mycoplasmas

Mycoplasma sometimes contaminate tissue cultures. Many mycoplasma contaminants grow slowly and do not destroy their host cells, but they can alter their metabolism and behaviour, and could perhaps alter cell spreading. To make sure that all cell lines which were isolated are not affected by these micro-organisms, they were tested by staining with bisbenzimid fluorochrome (Hoechst 33258). Py3, F2, TG2, TG2F1, TG2F2, F2R1, and F2R2 cells were found negative. This test proved that the phenotype of all variants is not due to contamination by mycoplasma (Fig. 12 a,b).

Cell adhesion assay

To compare the ability of the various cell lines to attach to different surfaces, radioactively labelled cells were used. For the TG group labelling was with ^{51}Cr overnight, while Py3, F2, F2R1, and F2R2 were labelled with ^{3}H-thymidine for three days. Cells were resuspended with rinses in Hanks Hepes three times, kept in ice and used very shortly afterwards in this assay. Cells were allowed to attach for 30, or 60 minutes on fibronectin or serum coated plastic or glass coverslips.

Both TG-variant cells differed from WT in response to serum and fibronectin (Fig. 13). 19% of TG2 cells attached to serum after 60 minutes while the percentage of variant cells attached to the same surface was only 4%. Incubation of TG2 cells in plastic wells coated with fibronectin resulted in attachment of most of the cells (84%), while TG2F1 and TG2F2 showed 10% and 60% respectively. Thus, although TG2F1 was very poorly adherent to both surfaces, TG2F2 adhered quite well on fibronectin.

A different adhesion response was found between F2R1, F2R2 and F2 cells on fibronectin while there were very marked differences in the adhesion on serum coated surfaces (Fig. 14). Serum supported the adhesion of Py3, F2R1, and F2R2 weakly and F2 even less.

Fig. 12 Cells stain for DNA with Hoechst 33258

A. TG-cell lines

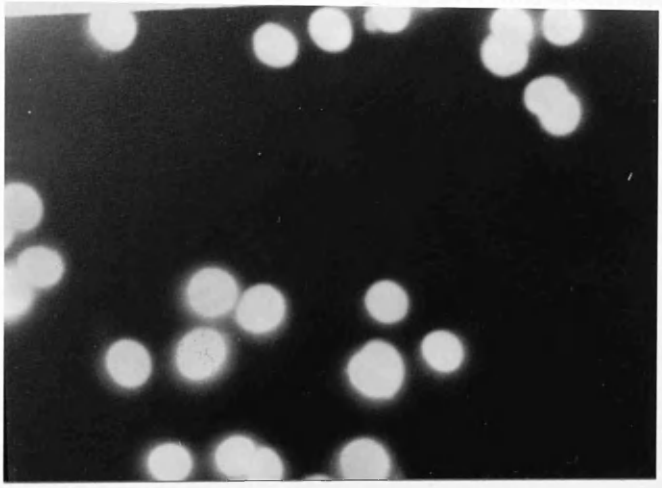
1. Nuclei of TG2-WT cells
   Interphase nuclei clearly seen
   Scale bar : 20 μm

2. Nuclei of TG2F1 cells
   Scale bar : 20 μm

3. Nuclei of TG2F2 cells
   Scale bar : 20 μm

These micrographs show that the cultures used during selection and characterisation of TG-cell lines were not contaminated with mycoplasma.

1



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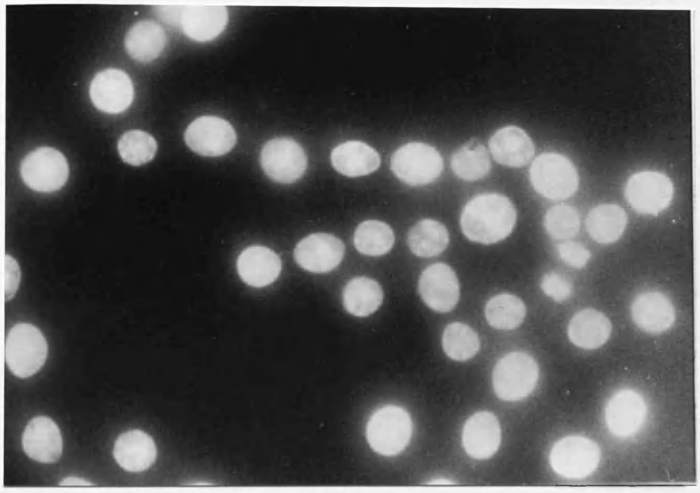

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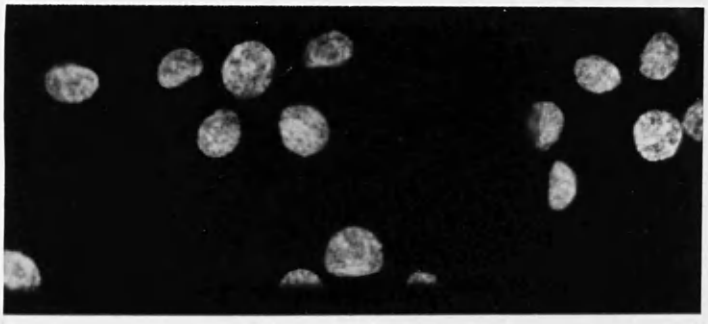

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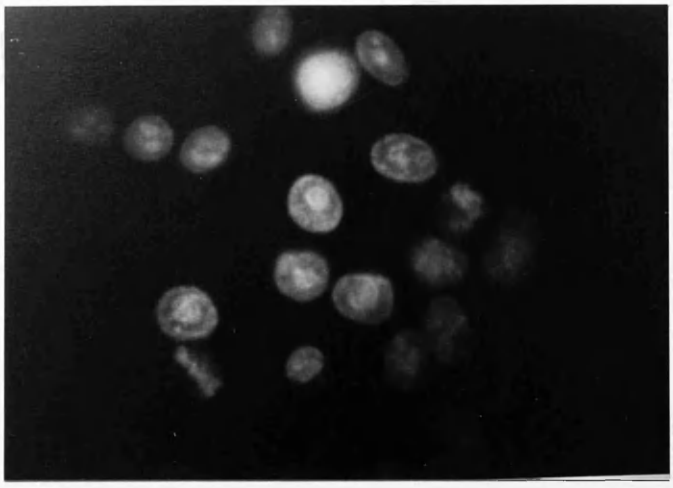

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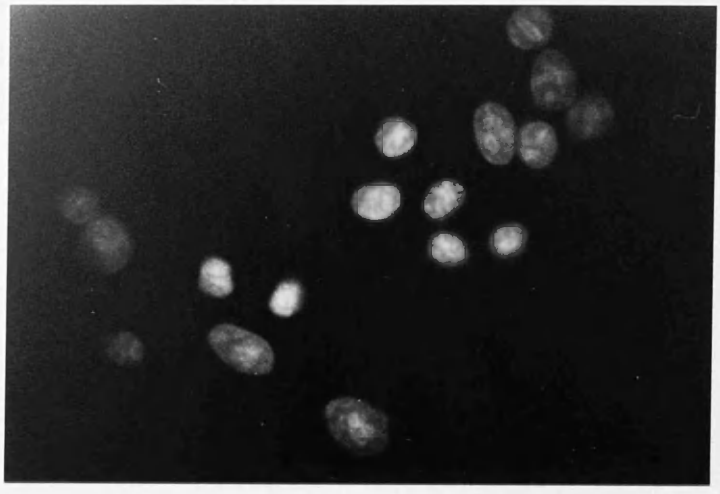

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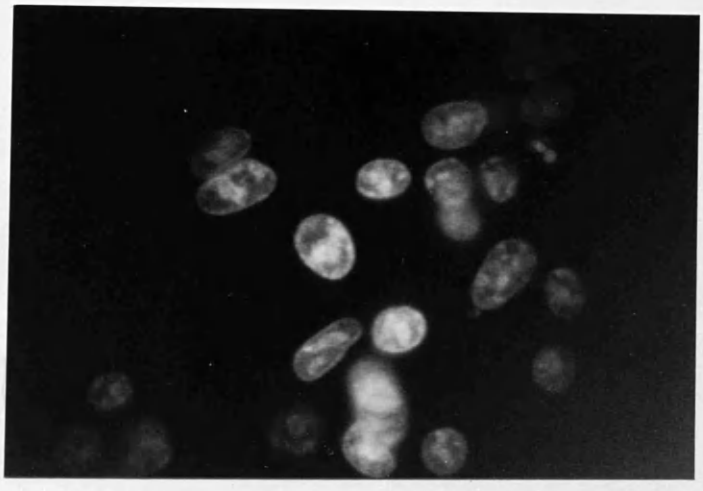


B. Revertants

1. Nuclei of F2 mutants
   Scale bar : 20 μm

2. Nuclei of F2R1 cells
   Scale bar : 20 μm

3. Nuclei of F2R2 cells
   Scale bar : 20 μm

These micrographs show that the cultures used during revertant selection and the characterisation of these revertants were not contaminated with mycoplasma.

TG2-WT, TG2F1, and TG2F2 cells were labelled with ^{51}Cr (500 μCi/ 10 ml Ham's medium) overnight at 37° C. Cells were resuspended three times in Hanks Hepes, diluted to 10^5 /ml, then plated for two hours at 37° C on plastic multiwells (4 replicates) coated previously from solutions of 25 μg/ml fibronectin or 10% serum. After 30 or 60 minutes cells were washed twice with warm Hanks Hepes, then solubilised with 1ml 0.3N NaOH for 30 minutes at 37° C, transferred to Gamma counter 2001 sample tubes, and counted. Percentage of adhesion was calculated (see methods) (Two experiments).

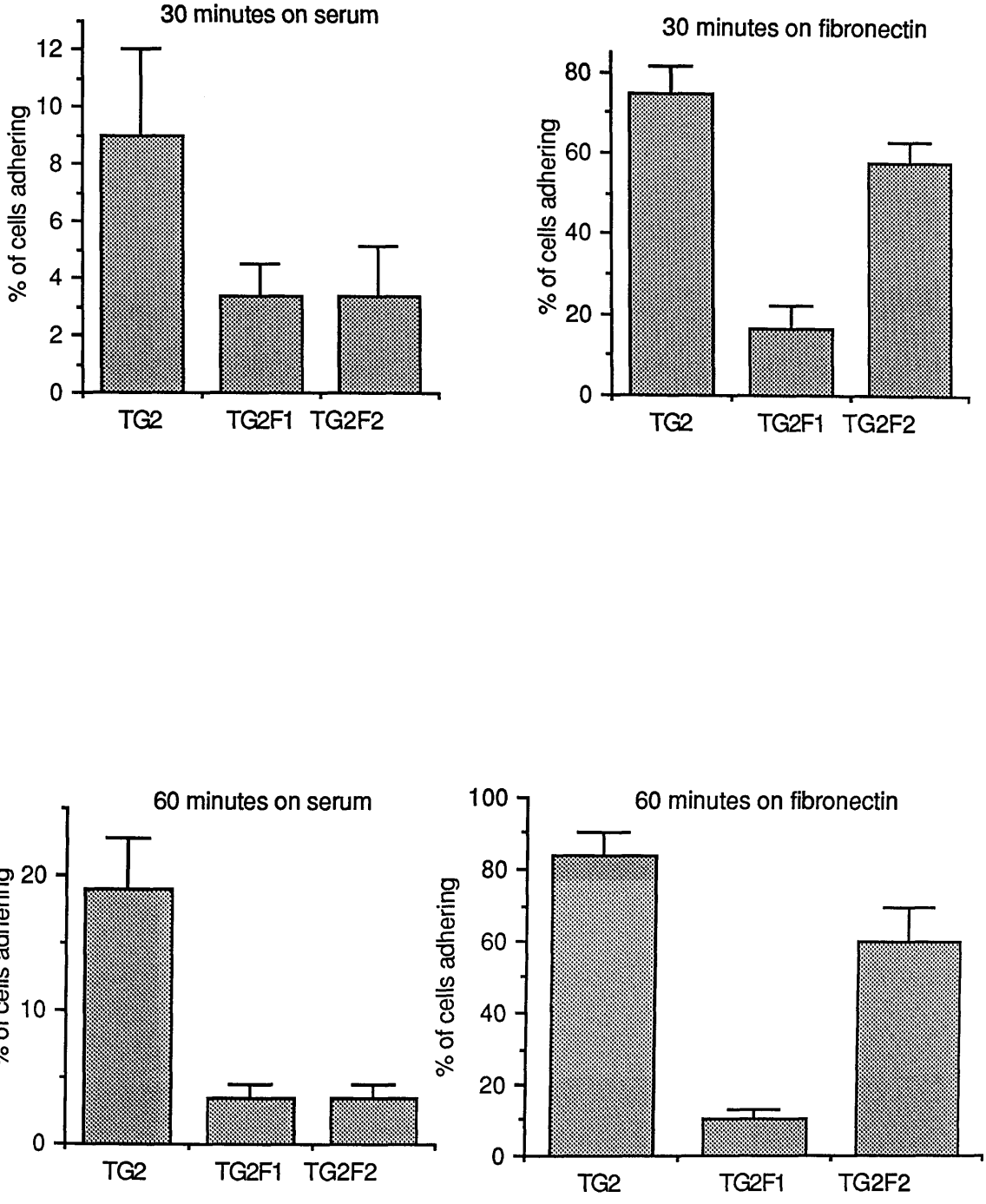


Fig. 13 Effect of fibronectin and serum on the adhesion of TG2, TG2F1, and TG2F2 cells

Py3, F2 mutants and both revertants (F2R1, F2R2) were labelled with ^{3}H-thymidine at 2 μCi/ml for three days. Cells were resuspended three times in Hanks Hepes, 10^{5} cells were plated for 30 or 60 minutes at 37° C on plastic wells previously coated from solutions of 25 μg/ml fibronectin or 10% serum. The radioactivity was counted (see methods) (Three experiments).

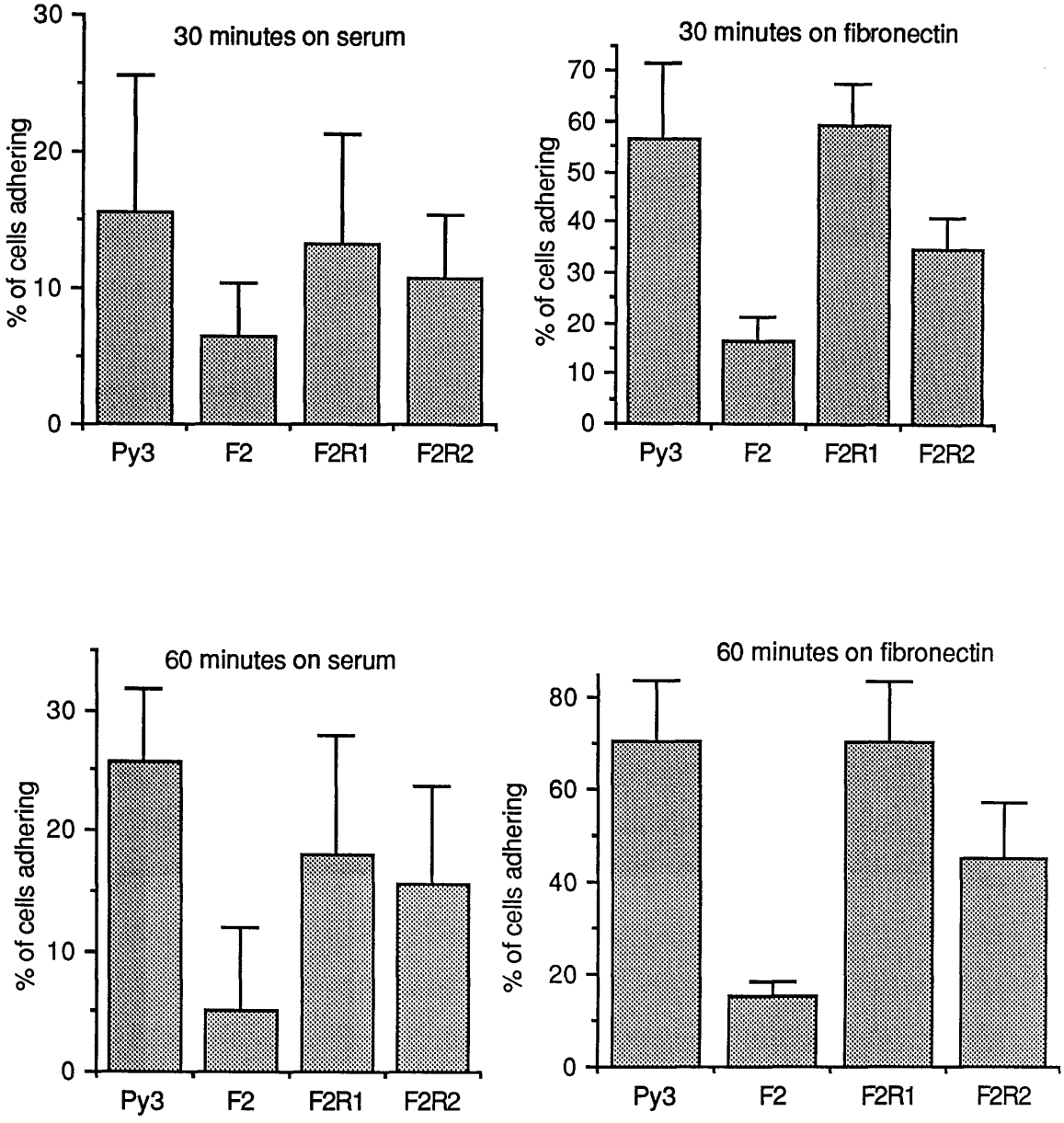


Fig. 14 Effect of serum and fibronectin on the adhesion of Py3, F2, F2R1, and F2R2 cells

F2R1 have the same percentage of adhesion as Py3-WT cells on fibronectin (70%) after 60 minutes incubation while there is a marked difference in the adhesion between F2R2 and Py3 cells on both fibronectin and serum coated surfaces. F2 remained at very low percentage of adhesion on both surfaces (5%, 15.5%). F2R1 is thus more completely reverted than F2R2.

In general all cell lines have much lower percentage of adhesion on serum compared to fibronectin. Cells were partially spread or still rounded after 60 minutes on serum, whilst except F2 cells they were fully spread on fibronectin.

Cell spreading assay

To investigate the effect of fibronectin and Poly L-lysine on the spreading of TG cells and the revertants, cells were resuspended three times in Hanks Hepes and then allowed to spread for 2 hours at 37^{o} C on glass coverslips and plastic tissue culture dishes previously coated by adsorption from a solution containing 25 μg/ml fibronectin or 10 μg/ml poly L-lysine (14 kd).

The ability of revertants and of parental cells to spread on surfaces coated with fibronectin and Poly L-lysine was compared (Fig. 15). Revertants (F2R1,F2R2) and Py3 cells were well spread on fibronectin. F2R1 had the highest increase in mean spread area (100%) while F2 remained rounded (0%). F2R2 showed 71% increase in MSA (Fig.15, 16 a). F2R1, F2R2, and Py3 spread equally on Poly L-lysine (60%, 53%, 50.6%). The spreading of these cells was less than on fibronectin. Although F2 adhere very well on Poly L-lysine they were unable to spread on it (0%) and remained rounded although some cells had fine processes (Fig. 16 b).

TG2-WT cells spread to greater area (510.3 μm^2) on fibronectin coated surfaces than variants, which showed 6% and 18% increase in mean spread area in relation to WT respectively (Fig. 17,18 a). Most TG2F1 cells washed away after incubation on fibronectin. The figure shows also that although TG-WT and both variants attach very well to Poly L-lysine coated surfaces, none of them spread except that small

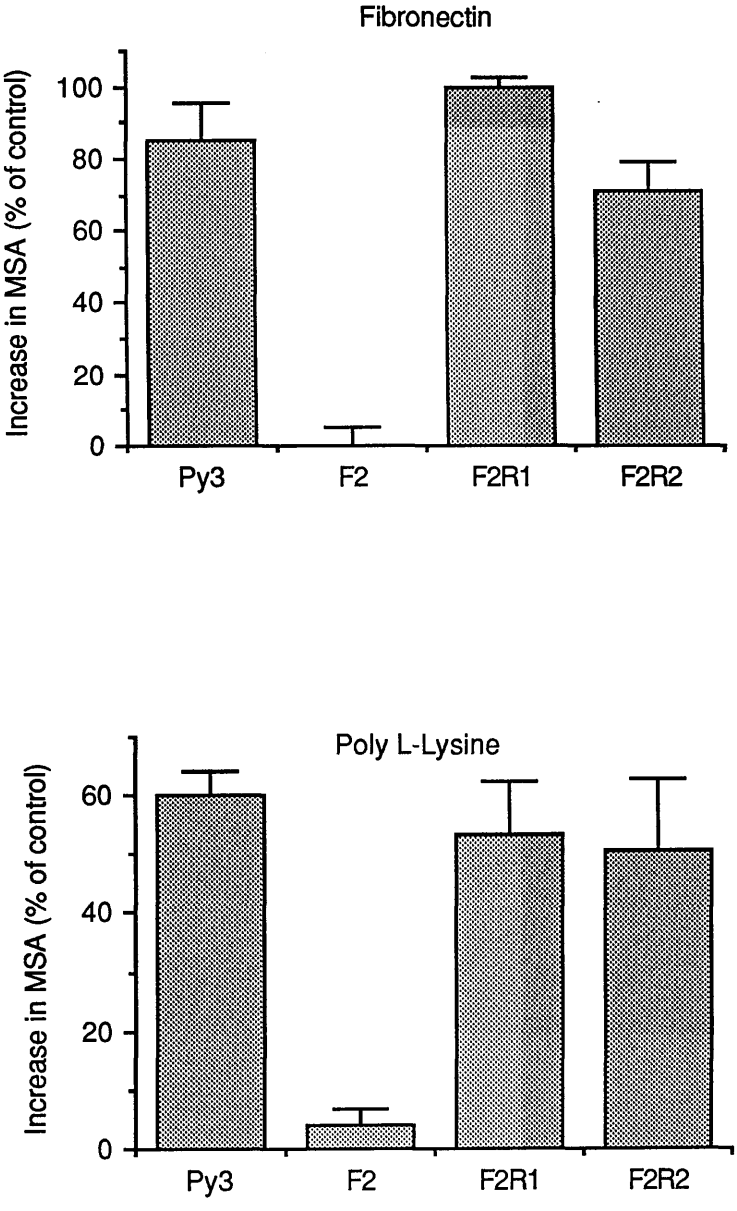


Fig.15 Effect of fibronectin and poly L-lysine on the spreading of Py3, F2, F2R1, and F2R2 cells

2 ml cells at $25x10^3$ /ml were plated for 2 hours at 37° C on coverslips previously coated from solutions of 25 μg/ml fibronectin or 10 μg/ml Poly L-lysine. Coverslips were fixed and stained with Kenacid Blue. Increase in mean spread area measured (Normalisation 115.5-620.3 μm^2, see methods) (Three experiments).

Fig. 16 Cell spreading assay

A. On fibronectin

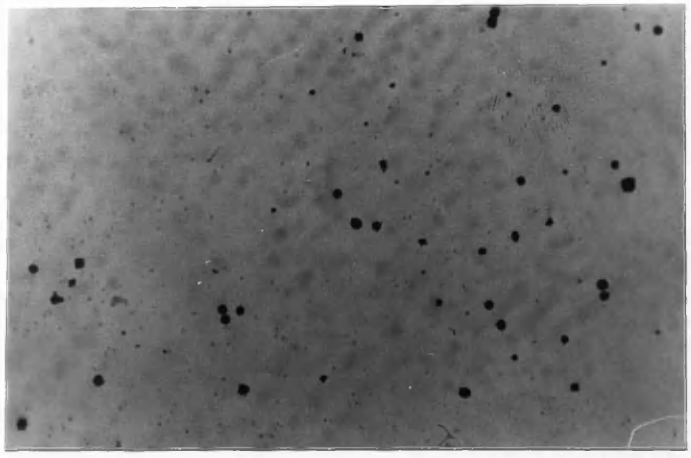
Coverslips were coated from a solution of 25 µg/ml fibronectin in Hanks Hepes. 2 ml cells at $25x10^3$ /ml were plated and incubated for 2 hours at 37° C, fixed and stained with Kenacid Blue.

1. F2 mutants (cells remained rounded)
   Scale bar : 100 µm

2. F2R1 cells (cells were well spread)
   Scale bar : 100 µm

3. F2R2 cells (spread cells)
   Scale bar : 100 µm

1



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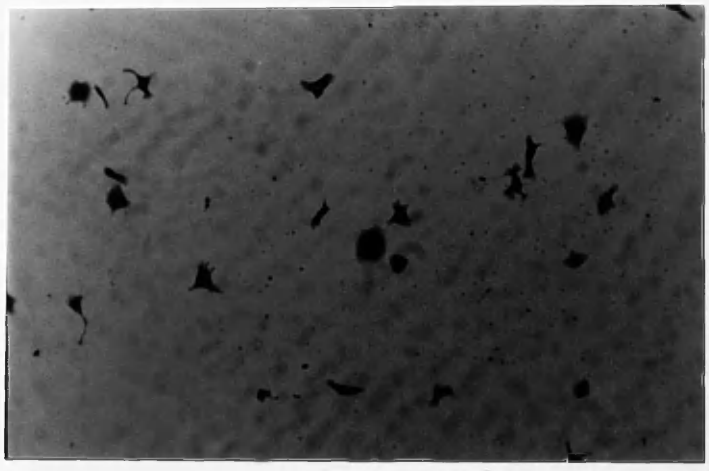

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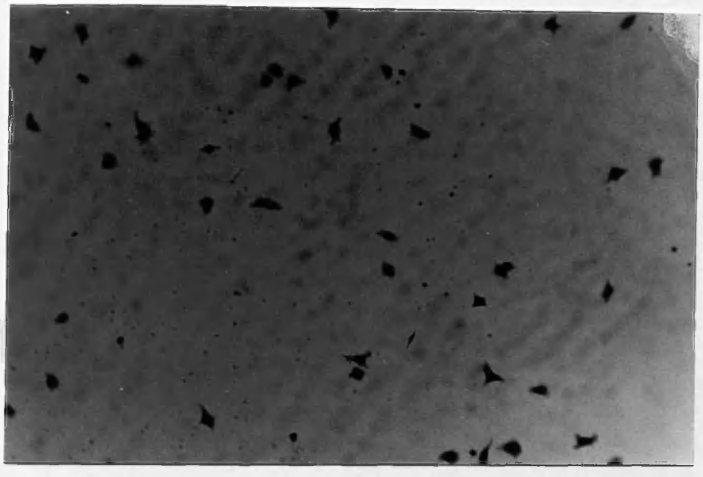

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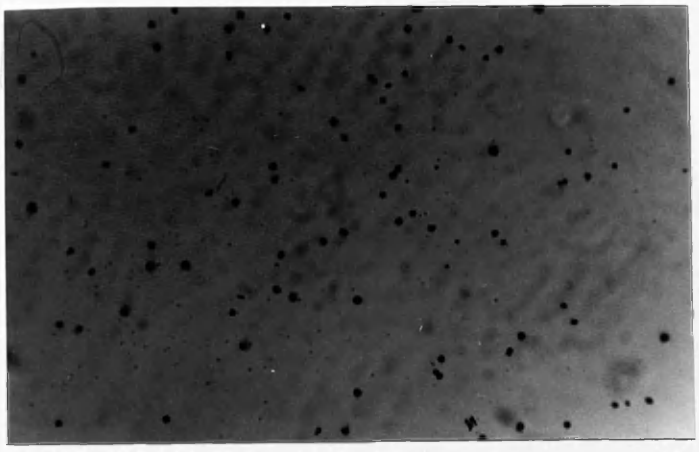

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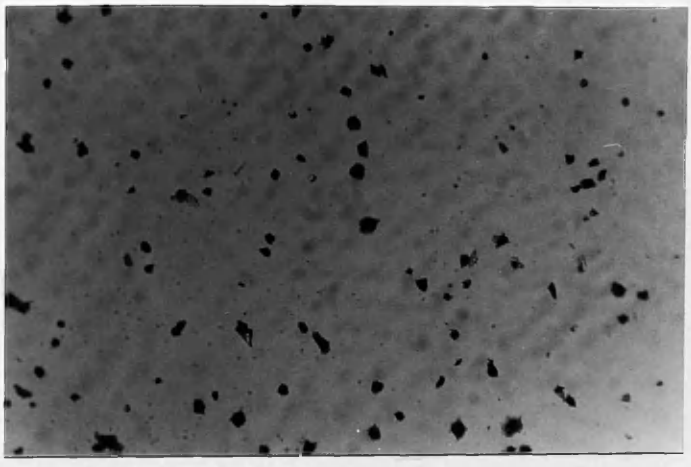


B. On poly L-lysine

Coverslips were coated from a solution of 10 µg/ml poly L-lysine in Hanks Hepes. 2 ml cells at $25x10^3$ /ml were incubated for 2 hours at 37° C.

1. F2 mutants
   Many cells attached , very few partially spread.
   Scale bar : 100 µm

2. F2R1 cells
   Scale bar : 100 µm

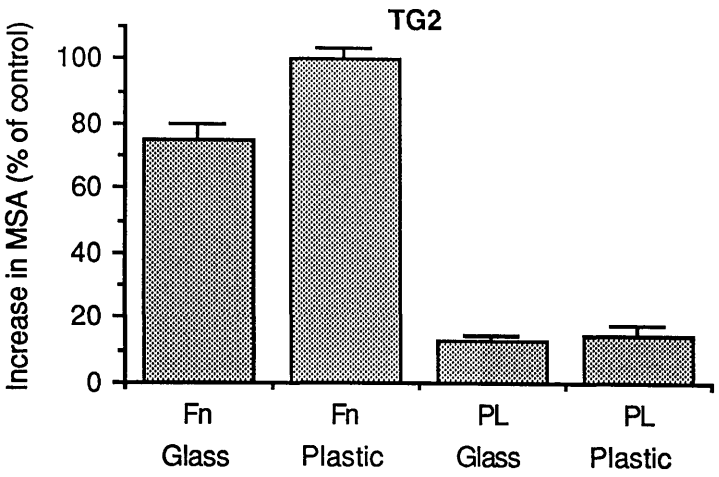
3. F2R2 cells
   Scale bar : 100 µm

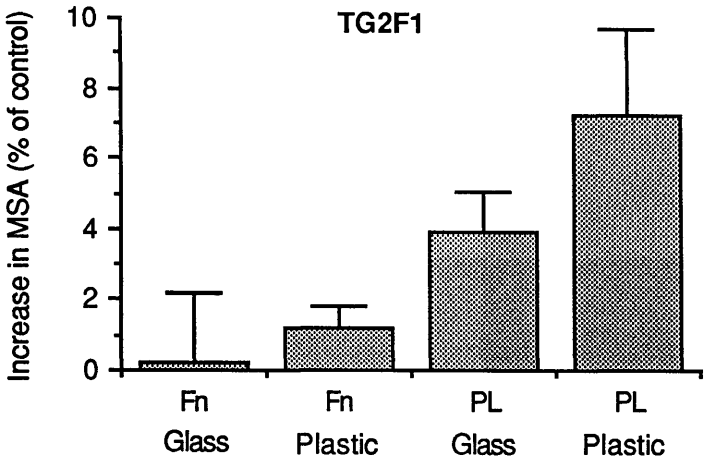
Cells stained with Kenacid Blue.

TG2-WT , TG2F1, and TG2F2 cells were resuspended three times in Hanks Hepes. 2 ml cells at $25x10^3$ /ml were plated on glass coverslips and plastic petri dishes previously coated from solutions of 25 μg/ml fibronectin or 10 μg/ml poly L-lysine. Cells were incubated for 2 hours at 37° C, fixed and stained with Kenacid Blue. Increase in mean spread area measured (normalisation 111.3-510.3 μm^2, see methods) (Two experiments).

Fn Fibronectin

PL Poly L-lysine





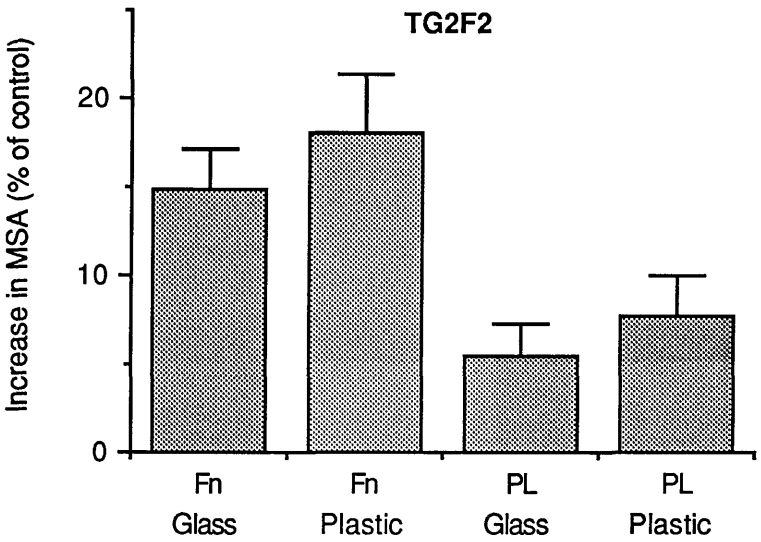


Fig. 17 **Effect of fibronectin and poly L-lysine on the spreading of TG2, TG2F1, and TG2F2 cells**

Fig. 18 (a) Spreading assay on fibronectin and poly L-lysine.

A. On fibronectin

Glass coverslips or plastic dishes were coated from a solution of 25 µg/ml fibronectin in Hanks Hepes for 30 minutes. 2 ml cells at $25x10^3$ /ml of TG2-WT, TG2F1 and TG2F2 were incubated for 2 hours at 37° C. Cells were fixed and stained with Kenacid Blue.

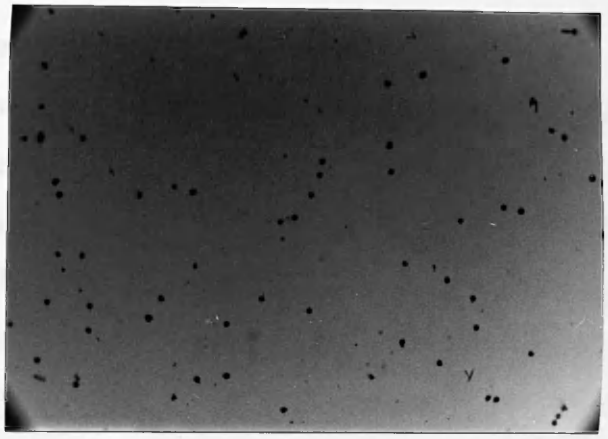
1. TG2-WT cells were well spread
   Scale bar : 100 µm

2. TG2F1 cells were rounded
   Scale bar : 100 µm

3. TG2F2 cells, few cells partially spread
   Scale bar : 100 µm

1

2



3

processes were noticed with the TG2 cells (Fig. 18 b).

TG2-WT cells seem to spread on Poly L-lysine only in the presence of serum. These cells spread very well in presence of a film of serum adsorbed onto Poly L-lysine from 1% solution (Fig. 19, 20). In this experiment the spreading ability of these cells on fibronectin was normal (682.5 μm^2 increase in MSA), while the increase in MSA was 170.6 μm^2 on Poly L-lysine. Another batch of Poly L-lysine (47 kd) was tested and the failure of WT cells to spread was confirmed (Fig. 21). Revertants were spread well on Poly L-lysine compare to TG cell lines.

Effect of various macromolecules on cell spreading

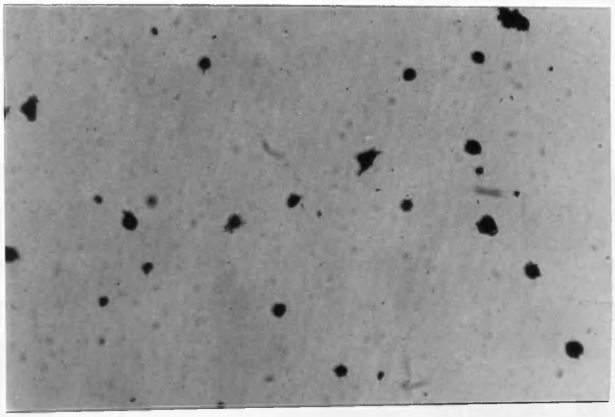
To examine the effect of different adsorbed proteins on the spreading of parental and variants, cells were plated on coverslips coated with an adsorbed layer of Concanavalin A, wheat germ agglutinin, Poly L-lysine, fibronectin, vitronectin (partially purified), and laminin. To prevent any non specific binding, coverslips with the proteins above were post-incubated in a solution of 0.5 mg/ml haemoglobin for 30 minutes. Cells were resuspended with rinses in Hanks Hepes three times and plated for 2 hours at 37° C.

In previous experiments fibronectin induced spreading of parental cells but of neither variant. Interestingly, among all these different proteins, Con A was effective in inducing both variant cells to spread (Fig. 21). TG2 cells showed greater spreading response to Con A (85% increase MSA). TG2F1 showed 55% increase in MSA while TG2F2 showed 65%. WGA and Poly L-lysine did not support the spreading of any cell type.

Vitronectin induced spreading of TG2 (60%) but not the variants. Laminin was found relatively ineffective with all of these cells. The increase in MSA was 30%, 2.5%, 4% for TG2, TG2F1, and TG2F2 respectively (Fig. 22).

To investigate in more detail the effect of Con A, parental and variant cells were incubated for two hours on glass coverslips coated with Con A (0-50 µg/ml) and

1



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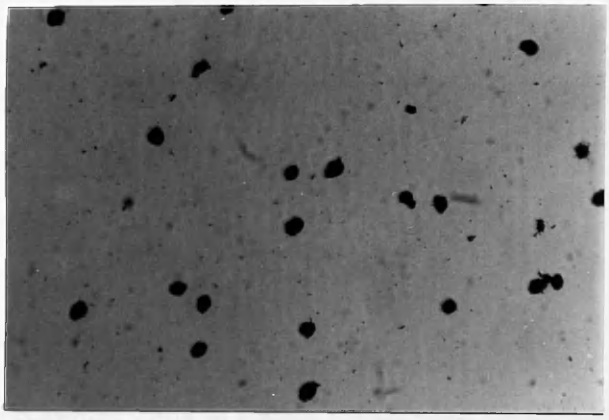

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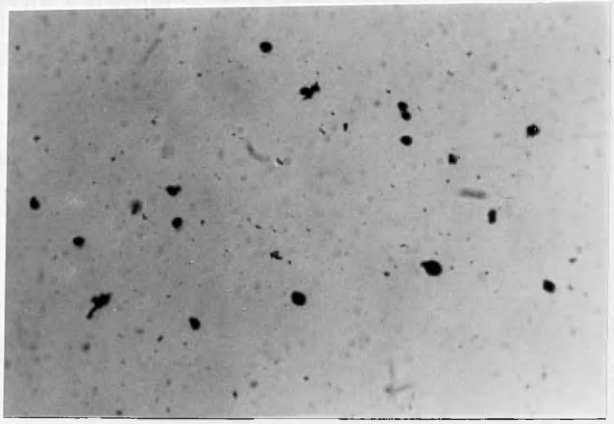


B. On poly L-lysine

Glass coverslips or plastic dishes were coated from a solution of 10 μg/ml poly L-lysine in Hanks Hepes for 30 minutes. Cells were then incubated for 2 hours at 37° C, fixed and stained with Kenacid Blue.

1. TG2-WT cells, few cells partially spread
   Scale bar : 100 μm

2. TG2F1 cells, cells attach to the surface but
   with little spreading.
   Scale bar : 100 μm

3. TG2F2 cells, attached cells.
   Scale bar : 100 μm

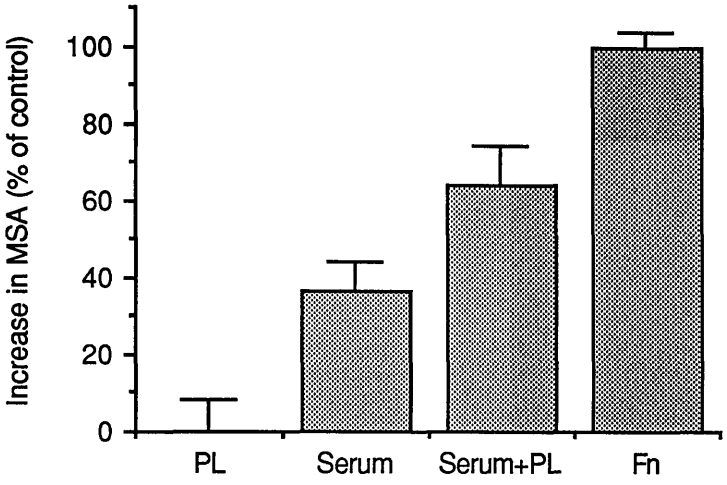


Fig. 19 Effect of Poly L-lysine, serum, and fibronectin on the spreading of TG2 (WT) cells

PL Poly L-lysine

Fn Fibronectin

TG2 parental cells in Hanks Hepes were plated for two hours at 37^{o} C on plastic dishes coated previously with the following solutions:fibronectin (25 μg/ml), 1% serum, Poly L-lysine (10 μg/ml) and Poly L-lysine with serum and the mean spread area was measured (normalisation 170.6-682.5 μm^2). Note that cells do not spread on poly L-lysine but they do in presence of 1% serum coated on poly L-lysine surface (Two experiments).

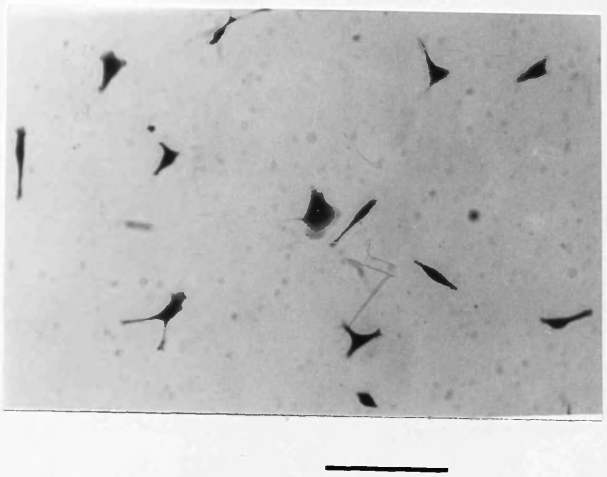


Fig. 20 Spreading assay

Plastic dishes were coated first from solutions of 10 µg/ml poly L-lysine for 30 minutes then from 1% serum for 30 minutes, the surface was washed twice with Hanks Hepes then TG2 cells were incubated for 2 hours at 37^{o} C. Cells were fixed and stained with Kenacid Blue.

Scale bar : 100 µm

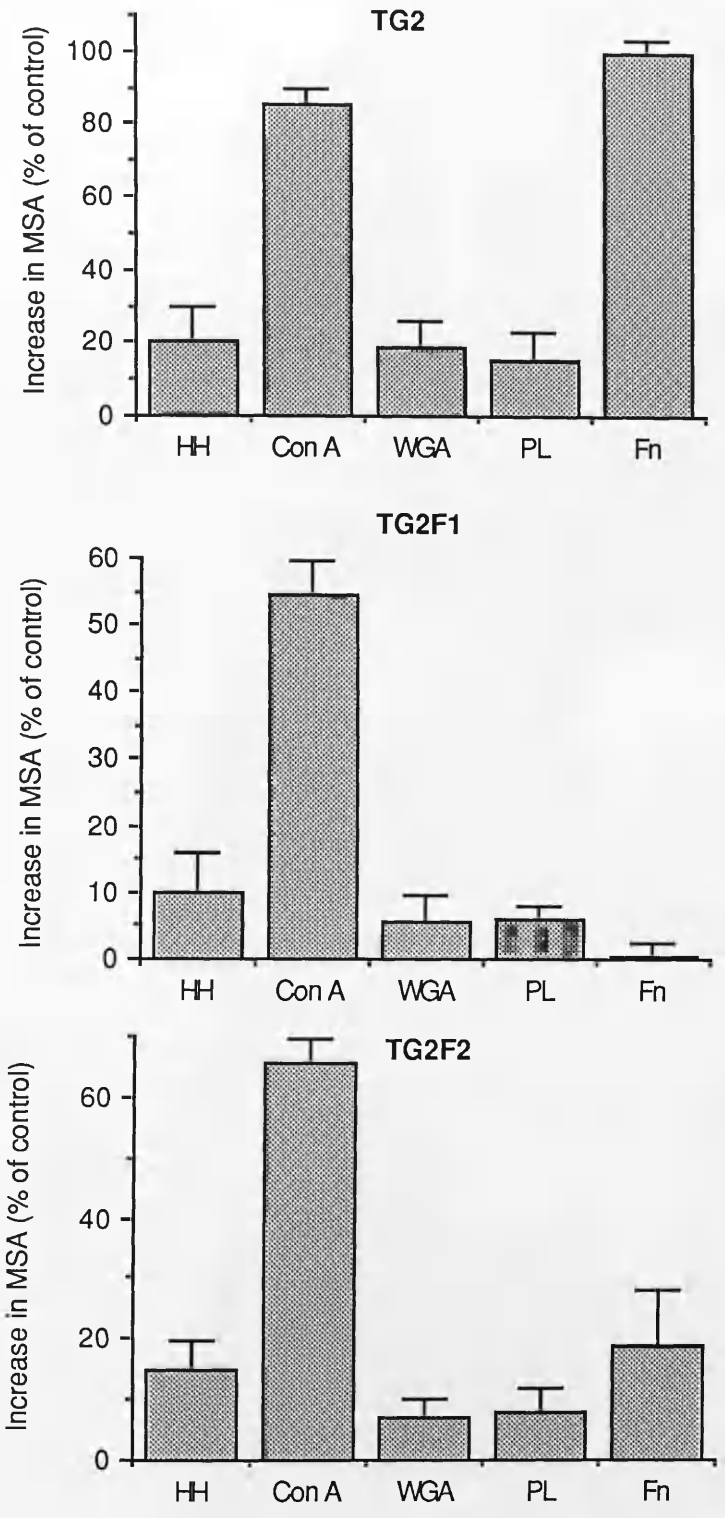


Fig. 21 Effect of various macromolecules on the spreading of TG2, TG2F1, and TG2F2 cells

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hanks Hepes. 2 ml cells at $25x10^3$ /ml were plated on 22 mm glass coverslips previously coated from the following solutions: 40 µg/ml Concanavalin A, 40 µg/ml wheat germ agglutinin, 10 µg/ml poly L-lysine, or 25 µg/ml fibronectin. All surfaces were blocked with 0.5 mg/ml haemoglobin. Cells were incubated for two hours at 37^o C, fixed and stained with Kenacid Blue, and the mean spread area measured (normalisation 132.3-503.4 μm^2) (Two experiments).

HH	Hanks Hepes
Con A	Concanavalin A
WGA	Wheat germ agglutinin
PL	Poly L-lysine
Fn	Fibronectin

2 ml of TG2-WT, TG2F1, and TG2F2 cells in Hanks Hepes at $25x10^3$ /ml were plated for 2 hours at 37^o C on glass coverslips previously coated from solutions of 25 μg/ml fibronectin or 10 μg/ml vitronectin (partially purified). Surfaces were post-incubated with 0.5 mg/ml haemoglobin for 30 minutes,the mean spread was measured (normalisation 111.09-686.7 μm^2) (Two experiments).

HH Hanks Hepes
FN Fibronectin
VN Vitronectin
LN Laminin

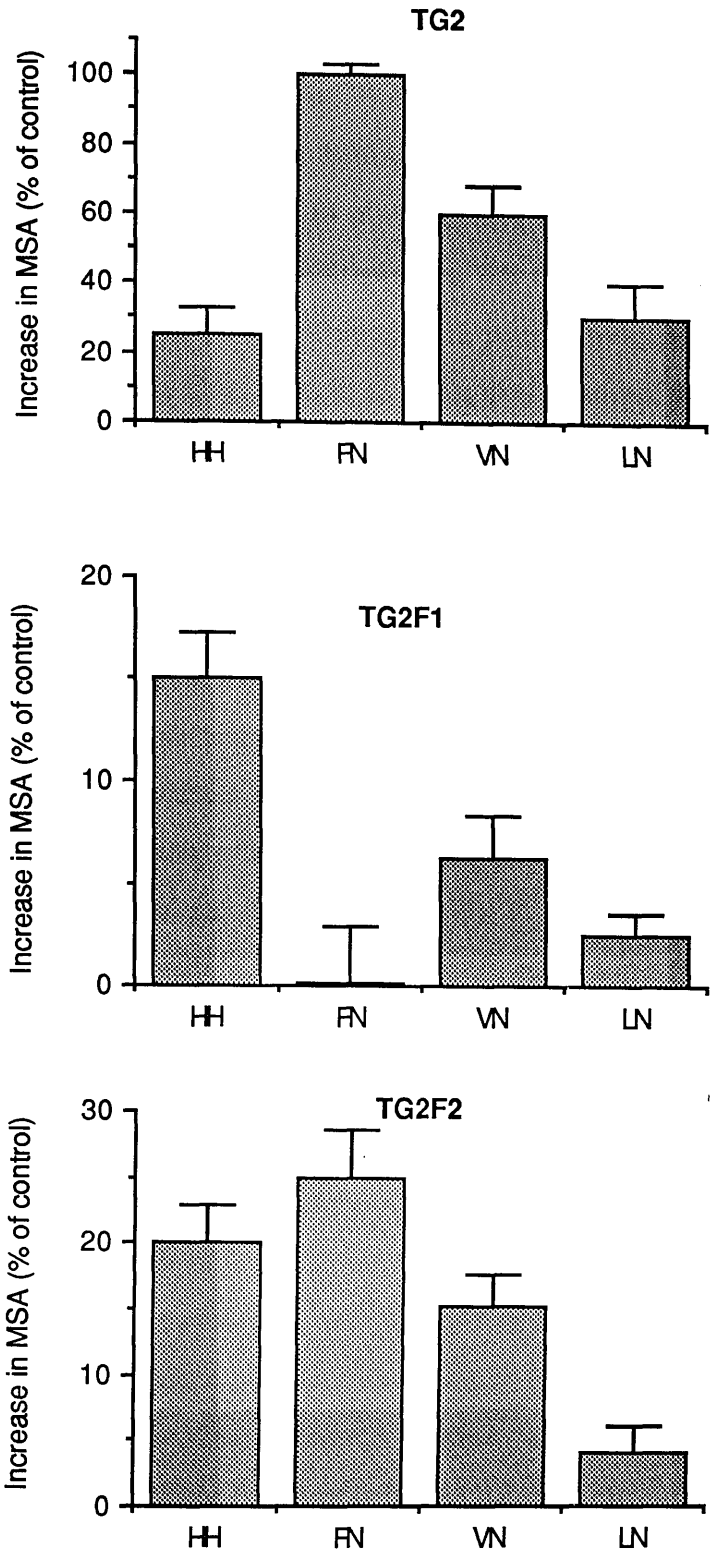


Fig. 22 Effect of various macromolecules on the spreading of TG2, TG2F1, and TG2F2 cells

post-incubated with haemoglobin. Con A again induced the spreading of TG2F1 and TG2F2, and spreading of both parental and variant cells was inhibited completely in presence of 0.1 M alpha-methyl mannoside (Fig. 23). Since the variants spread on Con A-coated surfaces, the internal apparatus necessary for spreading appears to be normal in these cells, which produced their processes very effectively on Con A (Fig. 24 a, b, c) while they remained rounded on the other protein surfaces tested.

Induction of spreading of variants by Mn^{2+} and other divalent cations

Several workers have shown that Mn^{2+} ions support the adhesion and spreading of different types of cells on substrates which are not adhesive in physiological divalent cations. In particular, Grinnell (1984 a) found that BHK cells attach and spread on substrata without added adhesion factors such as fibronectin. This effect may provide a useful tool to investigate the events which take place in cell substratum adhesion (Rabinovitch and DeStefano, 1973).

These authors found that Sarcoma I mouse cells, a cell line which was never observed to spread in Ca^{2+}/Mg^{2+} could be induced to spread on serum-coated surfaces by Mn^{2+}. I used Mn^{2+} in an attempt to determine if the spreading of variant cells TG2F1 and TG2F2 (which were selected for low adhesion to fibronectin in Ca^{2+}/Mg^{2+} medium) could be induced by this ion.

TG2-WT and both variant cells were resuspended 3 times in Hepes saline, then allowed to spread in presence of 10^{-4} M Mn^{2+} or 10^{-2} M Mg^{2+} for 2 hours on two different surfaces, fibronectin (which contains an RGD sequence) and haemoglobin (which does not) (Fig. 25). TG2 cells in Mn^{2+} on fibronectin showed the most spreading of all conditions (increase in MSA 105-581.7 μm^2) (the increase in MSA for the other cells and surfaces is expressed relatively to this value). With Mg^{2+}, TG2 cells spread very well on fibronectin (88.7% increase in MSA), almost as much as in Mn^{2+}. However, on haemoglobin, TG2 in Mn^{2+} spread (53.8%), while

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hanks Hepes. 2 ml cells at $25x10^3$ /ml were plated on 22 mm glass coverslips previously coated from a solution of Concanavalin A (0-50 µg/ml) and in presence of 0.1 M alpha-methyl mannoside. Coverslips were post-incubated with 0.5 mg/ml haemoglobin. Cells were incubated for two hours at 37° C. (normalisation 128.7-640.5 μm^2) (Two experiments).

Con A	Concanavalin A
∝ mm	Alpha methyl mannoside

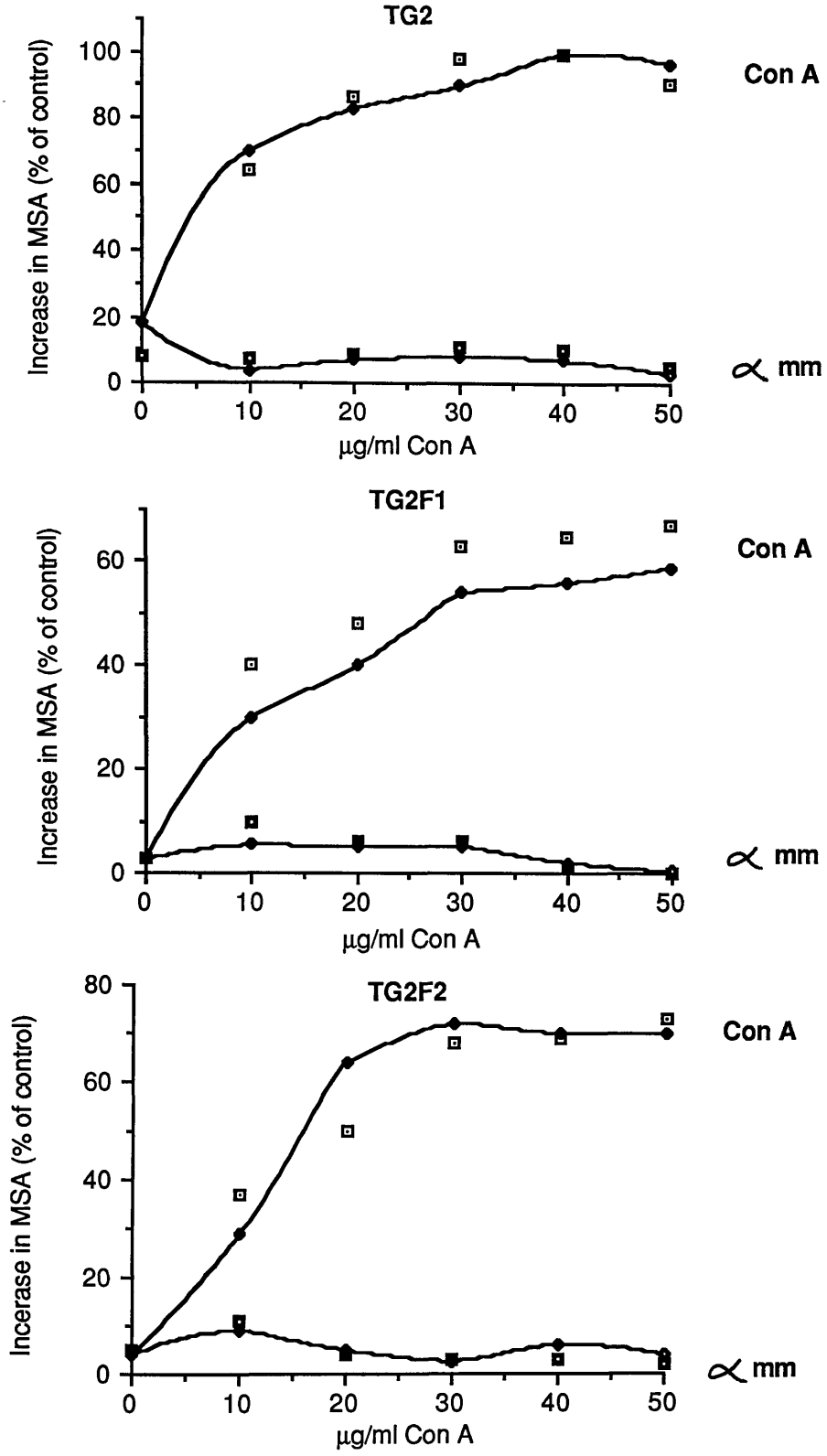


Fig. 23 Induction of spreading of TG2, TG2F1, TG2F2 cells by Concanavalin A

Fig.24 Spreading assay on Concanavalin A.

Cells in Hanks Hepes were plated for two hours at 37° C on 22 mm coverslips previously coated from a solution of Concanavalin A, post-incubated with 0.5 mg/ml haemoglobin. Cells were fixed and stained with Kenacid Blue.

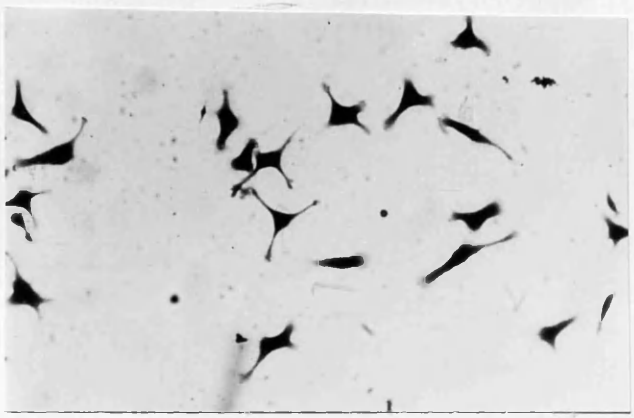
A) TG2 parental cells

1) On Con-A, well spread cells

2) In presence of alpha-methyl mannoside

Scale bar : 100 μm

1



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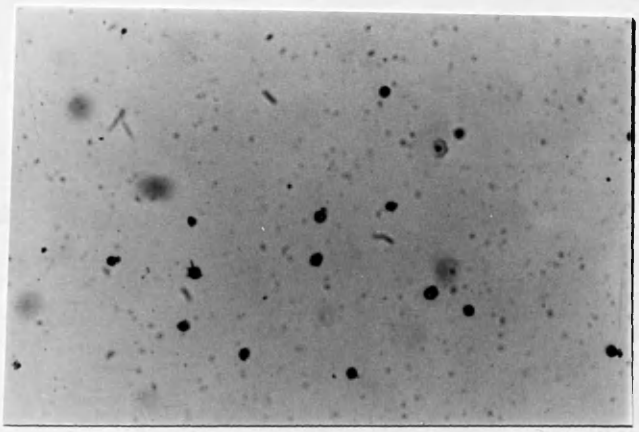
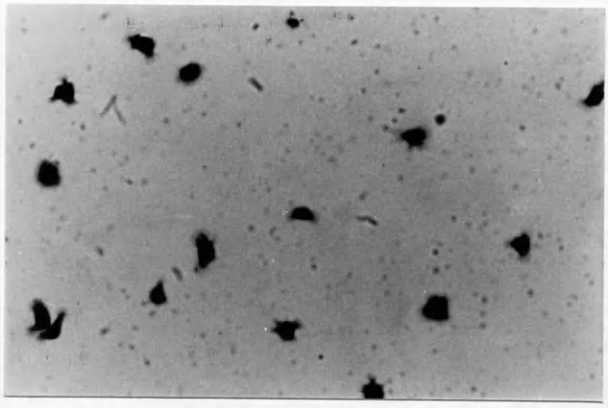
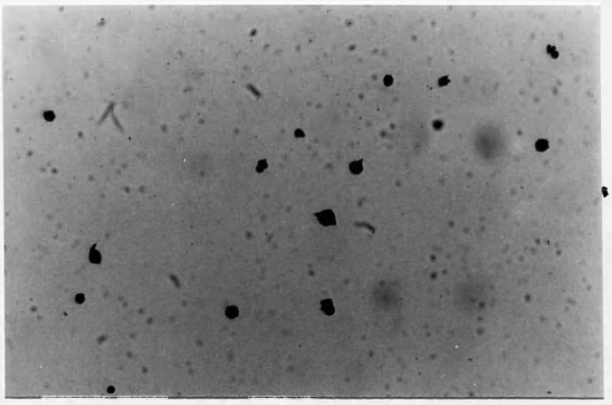




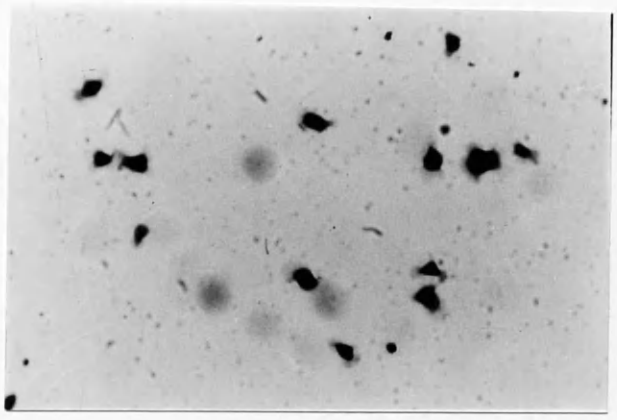
1. On Con A, most cells were spread



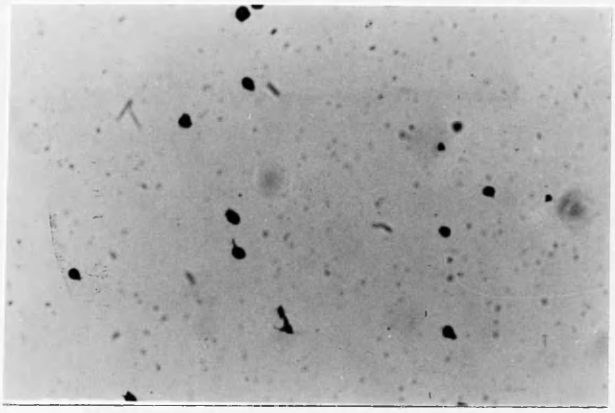
2. In presence of alpha-methyl mannoside

B. TG2F1 cells

Scale bar : 100 μm



1. On Con A, most cells were spread



2. In presence of alpha-methyl mannoside

C. TG2F2 cells

Scale bar : 100 μm

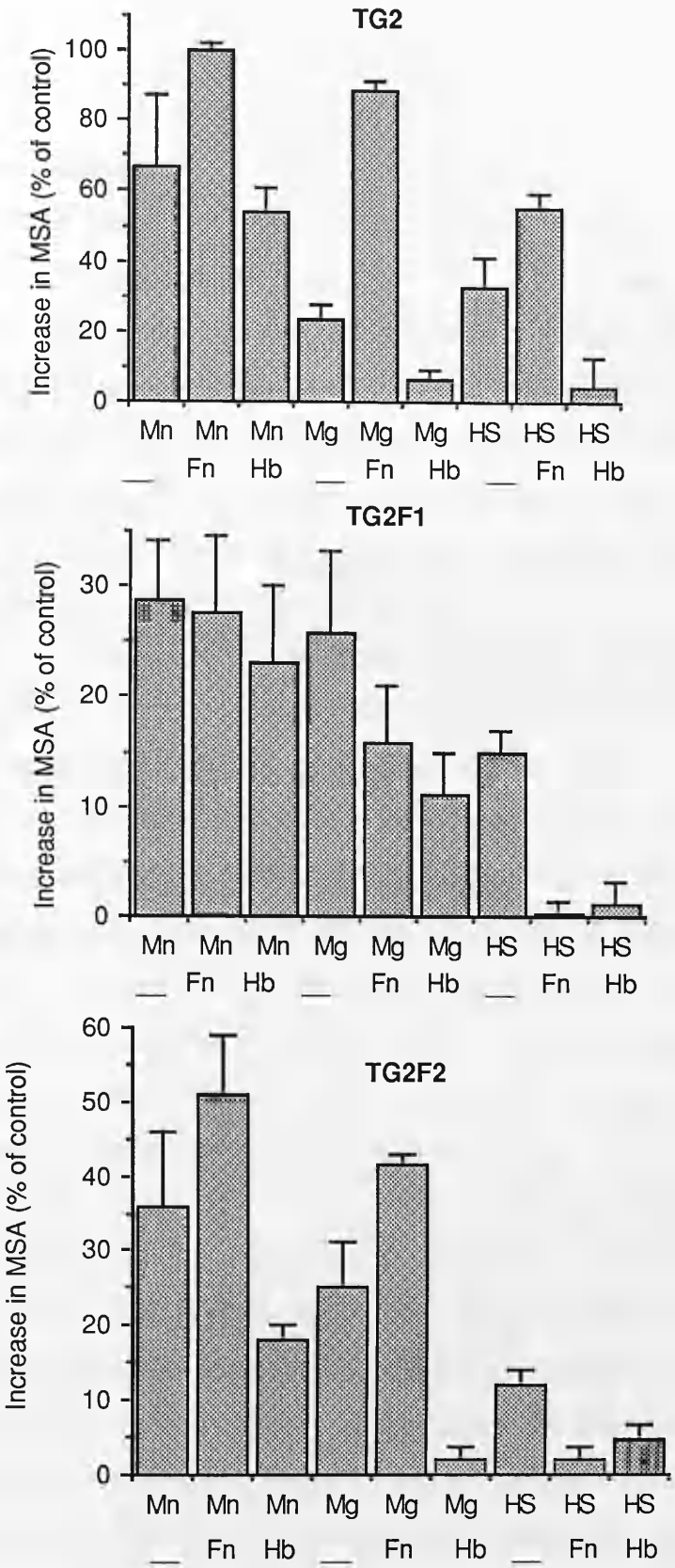


Fig. 25 Induction of spreading of TG2, TG2F1, and TG2F2 cells by 10^{-4} M Mn2+ and 10^{-2} M Mg2+ on different surfaces

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hepes saline. 2 ml cells at $25x10^3$ /ml were plated for two hours at 37^o C on 35 mm plastic tissue culture dishes previously coated from solutions of 25 μg/ml fibronectin or 0.5 mg/ml haemoglobin. Cells were incubated in presence of 10^{-4} M Mn^{2+} or 10^{-2} M Mg^{2+}. Cells were fixed and stained with Kenacid Blue and the increase in mean spread area measured (normalisation 105-581.7 μm^2, see methods) (Seven experiments) (Error bar=standard deviation).

Fn Fibronectin

Hb Haemoglobin

HS Hepes Saline

Mg^{2+} was ineffective on the same surface (6.2%). Thus, Mn^{2+} was found more effective than Mg^{2+} on both surfaces, but Mg^{2+} showed specificity toward fibronectin. On clean plastic, cells spread less (61.9%) than on fibronectin in presence of Mn^{2+} and much less (22%) in presence of Mg^{2+}. These results are in agreement with Grinnell (1984 a) and Stenn and Core (1986) in which the authors found that Mn^{2+} was able to induce cell spreading in the absence of proteins containing the Arg-Gly-Asp tripeptide. TG2 cells spread (55.5%) on fibronectin in the absence of added divalent cations, presumably due to Ca^{2+} background (~ 10^{-5} M) as explained by Edwards et al. (1987).

Important results occurred with variant cells in which Mn^{2+} induced substantial spreading. The increase in MSA of TG2F1 cells was 27.5% & 23% in the presence of Mn^{2+} on fibronectin and haemoglobin respectively. Mg^{2+} was also effective in inducing the spreading of TG2F1 on clean plastic (25.7%) and there was slight spreading, on fibronectin (15.9%) and on haemoglobin (11%). It seems that divalent cations induced some spreading of this variant but fibronectin had no effect.

In Mn^{2+}, TG2F2 cells spread (51%) on fibronectin but less (18%) on haemoglobin. Similarly, Mg^{2+} was also effective in inducing the spreading of the cells on fibronectin (41%) but not on haemoglobin (1.5%). On clean glass, in presence of Mn^{2+} cells showed 25.1% increase in MSA.

In summary, Mn^{2+} seems to co-operate with fibronectin in inducing the spreading of TG2, and to a lesser extent TG2F2, while in Mn^{2+} TG2F1 cells seems to spread equally on clean plastic, fibronectin, and haemoglobin. Throughout, Mn^{2+} was found more effective in inducing the spreading of cells than Mg^{2+}.

To examine the effect of Mn^{2+} in more detail, the assay was repeated with a range of Mn^{2+} concentrations. Figure 26 shows the effect of various concentrations of Mn^{2+} (10^{-8}-10^{-2} M) on cell spreading on fibronectin and haemoglobin coated plastic surfaces. Cells were resuspended three times in Hepes saline, then in low calcium Hepes saline (~ $5x10^{-6}$ M) (Edwards et al., 1987). In respect of TG2-WT

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hepes saline, then allowed to spread in low calcium Hepes saline, (normalisation 114.45-576.45 μm^2).

1) TG2 parental cells (Five experiments)
(Error bar=standard deviation)

2) TG2F1 cells (Two experiments)

3) TG2F2 cells (Two experiments)

FN Fibronectin
HB Haemoglobin

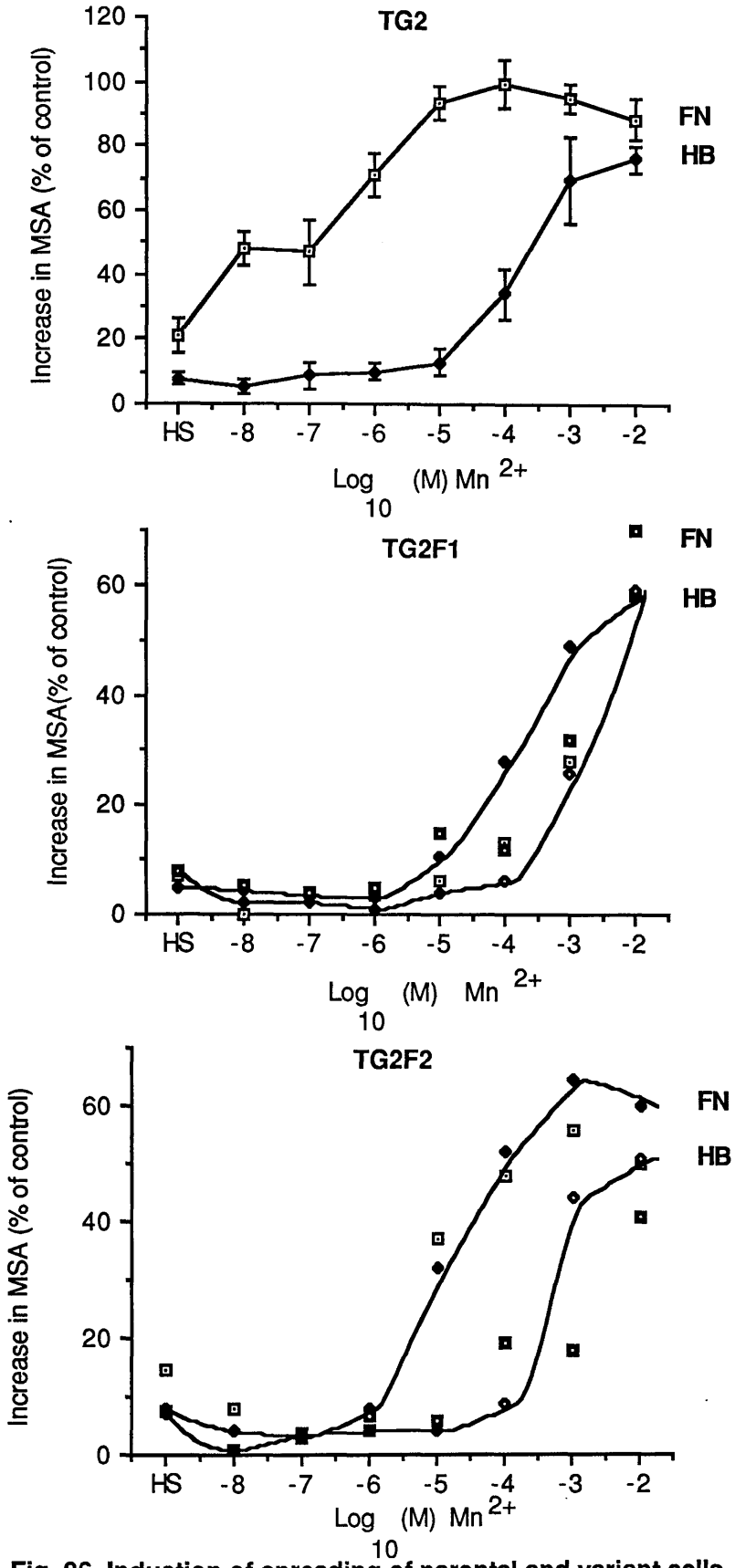


Fig. 26 Induction of spreading of parental and variant cells by Mn2+ on different surfaces

cells, Mn^{2+} was found effective at 10^{-6} M, around 1000x lower concentration on fibronectin (72%) than on haemoglobin (79.8%), at 10^{-3} M.

As for TG2, TG2F1 cells showed a similar increase in MSA on both surfaces in presence of 10^{-2} Mn^{2+} (58.2%, 59.6%). In this case, however, the concentration of Mn^{2+} required on fibronectin was only about 10x lower than on haemoglobin. Mn^{2+} was found effective in inducing the spreading of TG2F2 at 10^{-5} M on fibronectin (60.2%) and at 10^{-3} M on haemoglobin (51%). In this case, the concentration of Mn^{2+} required on fibronectin was about 100x lower than on haemoglobin. TG2-WT and TG2F2 cells showed specific spreading ability (i.e. Fn > Hb) on fibronectin while TG2F1 showed non-specific behaviour (Fn $\doteq$ Hb). Thus, Mn^{2+} at high concentrations was found effective not just on an RGD-containing protein such as fibronectin, but also on haemoglobin in inducing the spreading of these cells while lower concentrations of Mn^{2+} were effective only on fibronectin. Figure 27 shows the morphology of TG2 and TG2F2 when maximally spread on fibronectin in presence of 10^{-4} M and 10^{-3} M Mn^{2+} respectively. The morphology of the cells was similar, especially at higher concentration of Mn^{2+} (10^{-2} M), on both surfaces (Fig 28 a, b, c).

From the previous data, 10^{-3}-10^{-2} M Mn^{2+} was found fully effective in inducing cell spreading. To find out the most effective concentrations within that range, another experiment was carried out. Figure 29 shows the effect of Mn^{2+} (10^{-3}-10^{-2} M) on the spreading of parental and variant cells. TG2 cells spread almost equally with all concentrations used, presumably these concentrations achieve saturation. In case of TG2F1, concentrations above 5 mM Mn^{2+} gave higher increases in MSA on both surfaces compared to 1 mM, while concentrations from 1-3 mM produce similar effect on spreading. This may be due to the saturation of receptors at 5 mM Mn^{2+}. These results are in agreement with the earlier finding in figure 25. The effect of Mn^{2+} concentrations used on the spreading of TG2F2 was similar to TG2-WT cells.

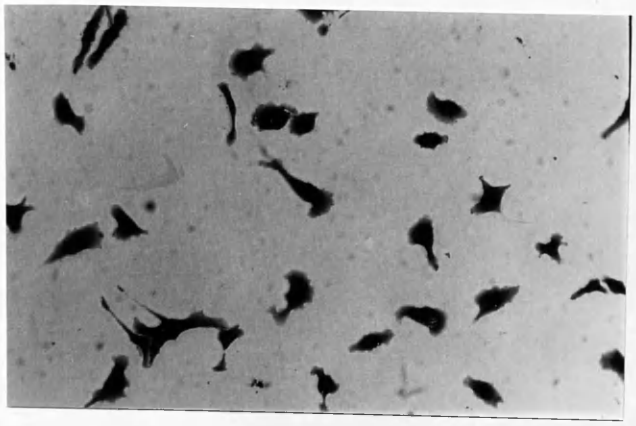
Fig. 27 Spreading assay on fibronectin in presence of Mn^{2+} .

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hepes saline, then allowed to spread in low calcium Hepes saline for 2 hours at 37° C on plastic dishes previously coated from a solution of 25 µg/ ml fibronectin in presence of Mn^{2+} .

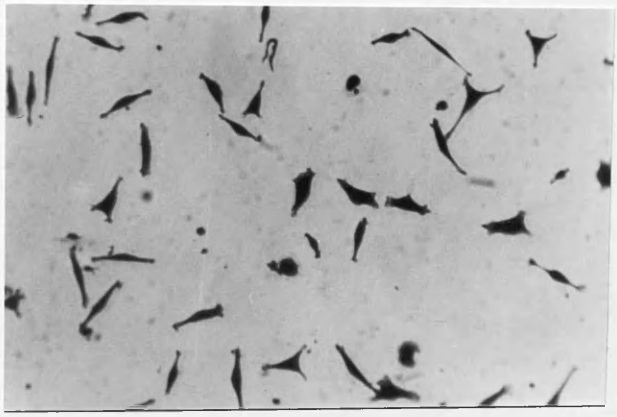
A) Parental cells in presence of 10^{-4} M Mn^{2+} .
Scale bar : 100 µm

B) TGF2 in presence of 10^{-2} M Mn^{2+}.
Scale bar : 100 µm

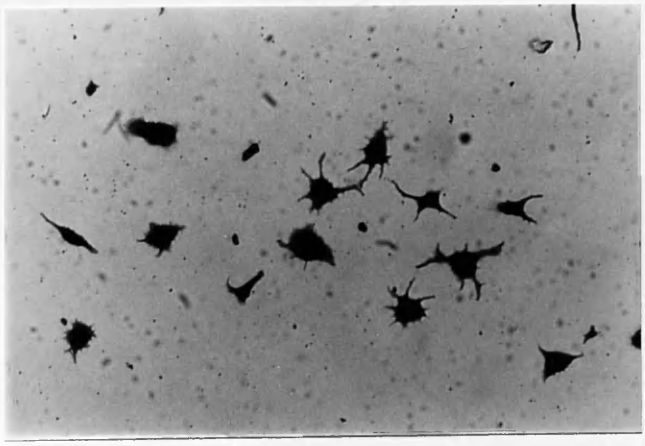
A



B



1



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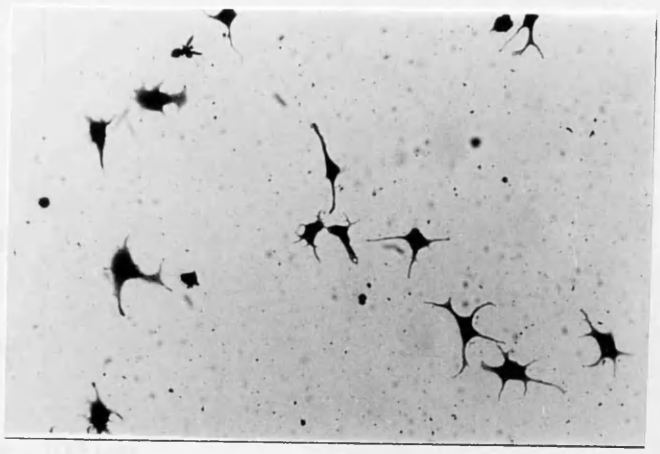


Fig. 28 (a) Cell spreading assay in presence of 10^{-2} M Mn^{2+} .

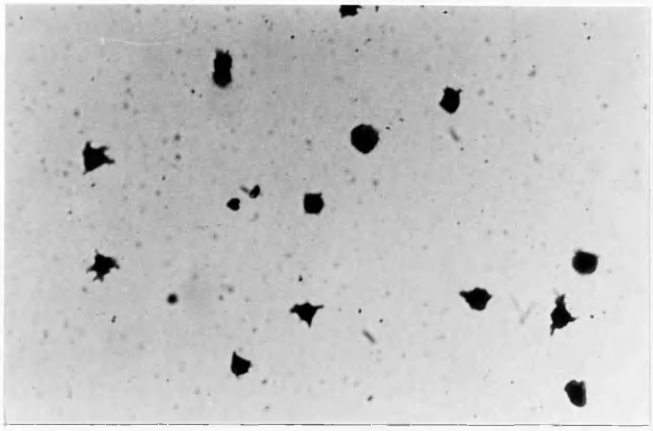
A. TG2 parental cells

1) On fibronectin

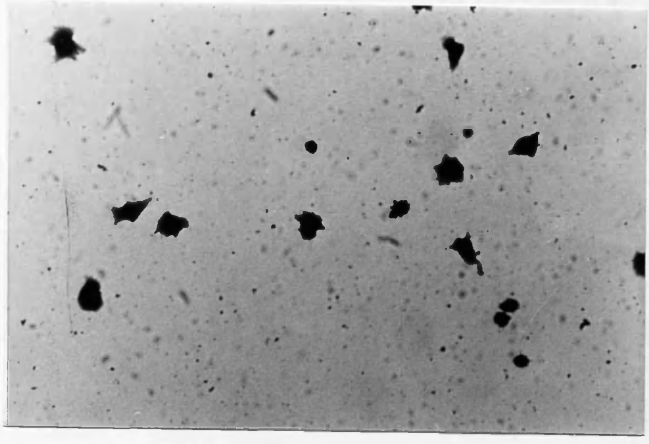
2) On haemoglobin

Cells were well spread on both surfaces.

Scale bar : 100 μm



1. On fibronectin



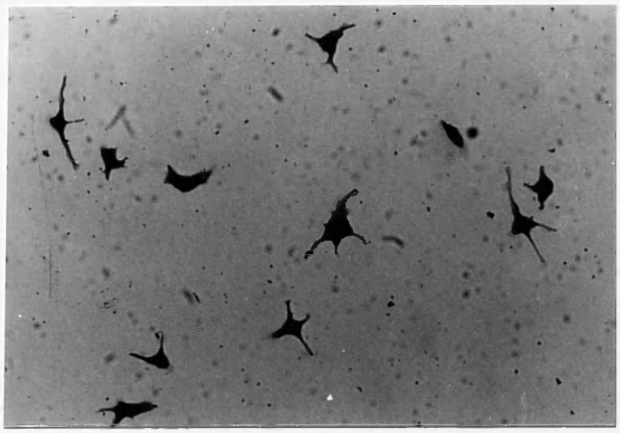
2. On haemoglobin

B. TG2F1

Cells spread on both surfaces.

Scale bar : 100 μm

1. On fibronectin



2. On haemoglobin

C. TG2F2

Cells spread on both surfaces.

Scale bar : 100 μm

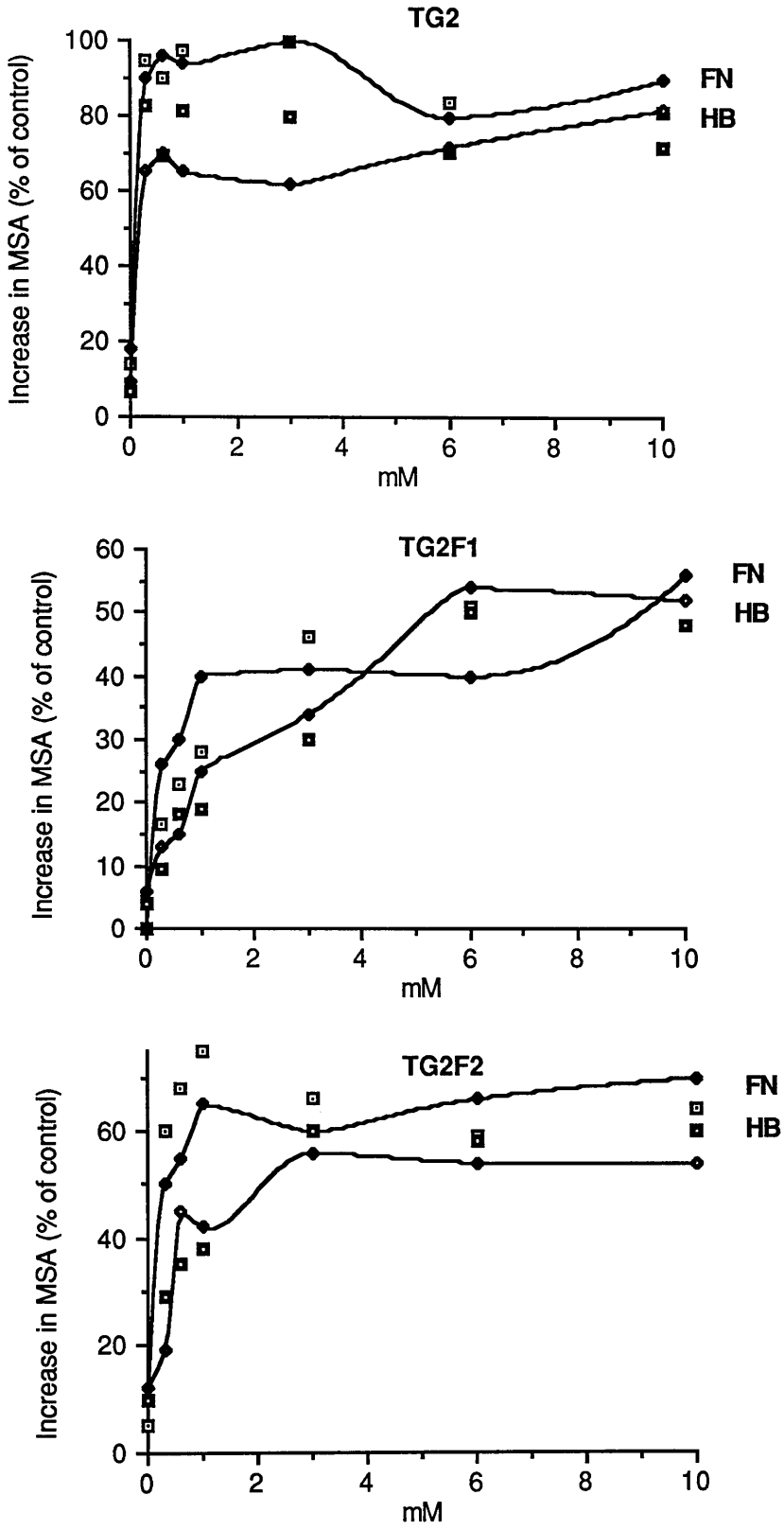


Fig. 29 Effect of Mn2+ on the spreading of TG2, TG2F1, and TG2F2 cells on different surfaces

TG2, TG2F1, and TG2F2 cells were resuspended three times in Hepes saline, then in low calcium Hepes saline on plastic dishes previously coated from solutions of fibronectin or haemoglobin in presence of 10^{-3}-10^{-2} M Mn^{2+}. Mean spread area was measured (normalisation 116.55-594.3 μm^2) (Two experiments).

FN Fibronectin

HB Haemoglobin

Another experiment was carried out to see whether the cytoskeleton participates in the spreading of these cells in Mn^{2+}. Cells were resuspended 3 times in Hepes saline then allowed to spread in low calcium Hepes saline for 2 hours at 37° C on fibronectin and haemoglobin coated surfaces in presence of 10^{-2} M Mn^{2+} and also in presence of 1 μg/ml cytochalasin D (Fig. 30). It was found that the inhibitor decreased the spreading of TG2-WT by about 40% on fibronectin and 50% on haemoglobin. The spreading of TG2F1 cell was inhibited by about 33% on both surfaces while the spreading of TG2F2 was inhibited by about 38% on fibronectin and 45% on haemoglobin in comparison to untreated cells. Mn^{2+} induced spreading is just as sensitive to cytochalasin D on fibronectin as on haemoglobin.

Effect of Co^{2+} on cell spreading

It has been reported that Co^{2+} does not induce the adhesion and spreading of BHK cells in medium containing serum albumin (Grinnell, 1984 a). On the other hand Edwards et al. (1987) found that Co^{2+} was effective in supporting the initial adhesion of these cells to serum-coated glass. To investigate this further, TG2-WT and both variants were seeded in plastic dishes coated from solutions of fibronectin, haemoglobin, or bovine serum albumin for 2 hours at 37° C in presence of 10^{-3} M and 10^{-2} M Co^{2+} (Fig. 31). TG2 cells showed substantial spreading (117.39-394.8 μm^2 increase in MSA) on fibronectin, while the cobalt was less effective on haemoglobin (75.2%) and much less effective on bovine serum albumin (37.5%). TG2F1 showed 25.3% increase in MSA on fibronectin, 19.7% on haemoglobin and 15.4% on BSA-coated surfaces. TG2F2 showed 35% increase in MSA on fibronectin, 22.8 % on haemoglobin and 15.4% on BSA-coated surfaces.

In general, Co^{2+} at 10^{-2} M was found more effective than 10^{-3} M to induce cell spreading. Fibronectin was found to be the best surface to promote cell spreading in presence of Co^{2+}.

To compare the effect of Mn^{2+}, Mg^{2+}, and Co^{2+}, cells were allowed to

TG2-WT, TG2F1, and TG2F2 cells were resuspended three times in Hepes saline, then allowed to spread in low calcium Hepes saline for 2 hours at 37° C on plastic dishes previously coated from solutions of fibronectin (25 μg/ml) or haemoglobin (0.5 mg/ml) in presence of 10^{-2} M Mn^{2+} with and without 1 μg/ml cytochalasin D. Since the cytochalasin D was dissolved in dimethylsulfoxide (DMSO), cells were also allowed to spread in presence of 0.25 v/v% DMSO as control. (normalisation 126-579.6 μm^2) (Two experiments).

Fn Fibronectin
Hb Haemoglobin
HS Hepes Saline
DO Dimethylsulfoxide
CD Cytochalasin D

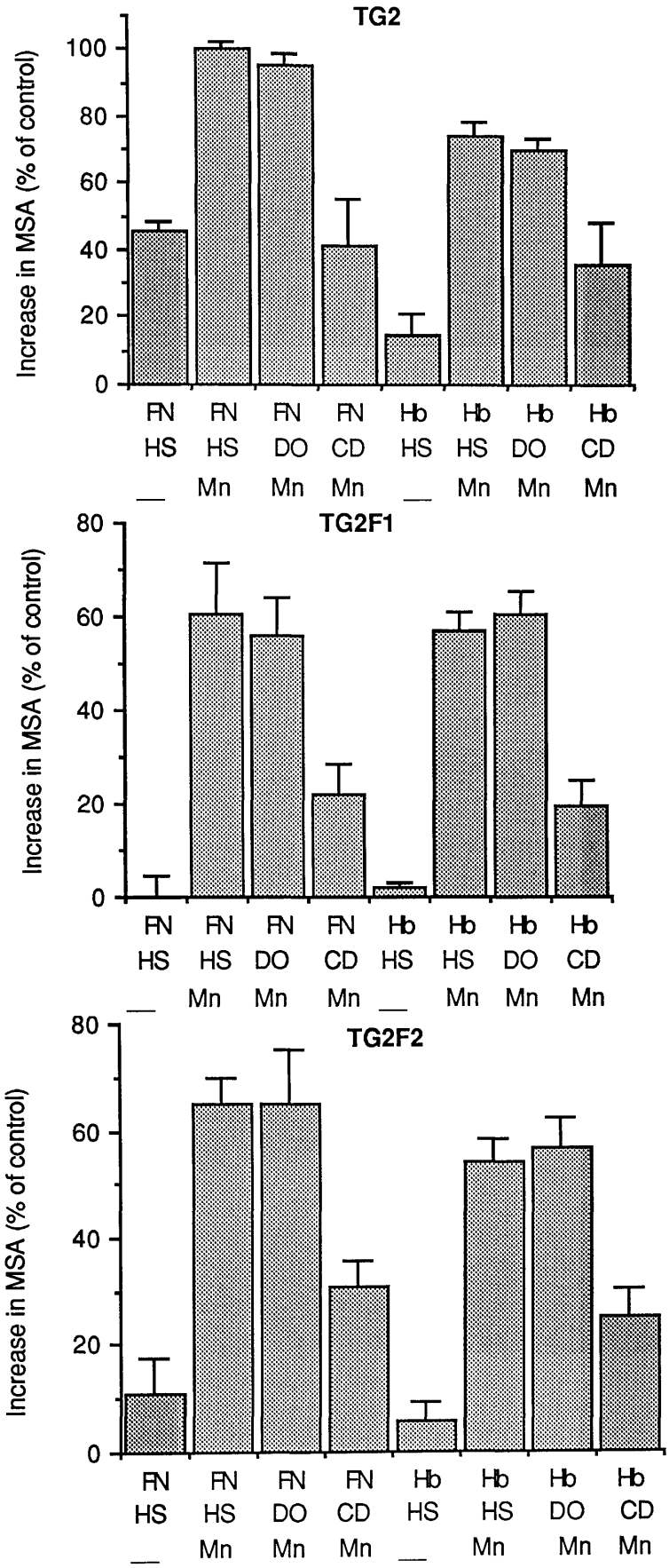


Fig. 30 Effect of cytochalasin D on the spreading of TG2, TG2F1, and TG2F2 cells on different surfaces

2 ml of TG2-WT, TG2F1, and TG2F2 cells at $25x10^3$/ml in low calcium Hepes saline were plated for 2 hours at 37^{o} C on plastic dishes previously coated from the following solutions: 25 μg/ml fibronectin, 0.5 mg/ml haemoglobin, or 100 μg/ml bovine serum albumin in presence of 10^{-3} -10^{-2} M Co^{2+}. (normalisation 117.39-394.8 μm^2) (Two experiments).

FN Fibronectin
HB Haemoglobin
BSA Bovine serum albumin
HS Hepes Saline

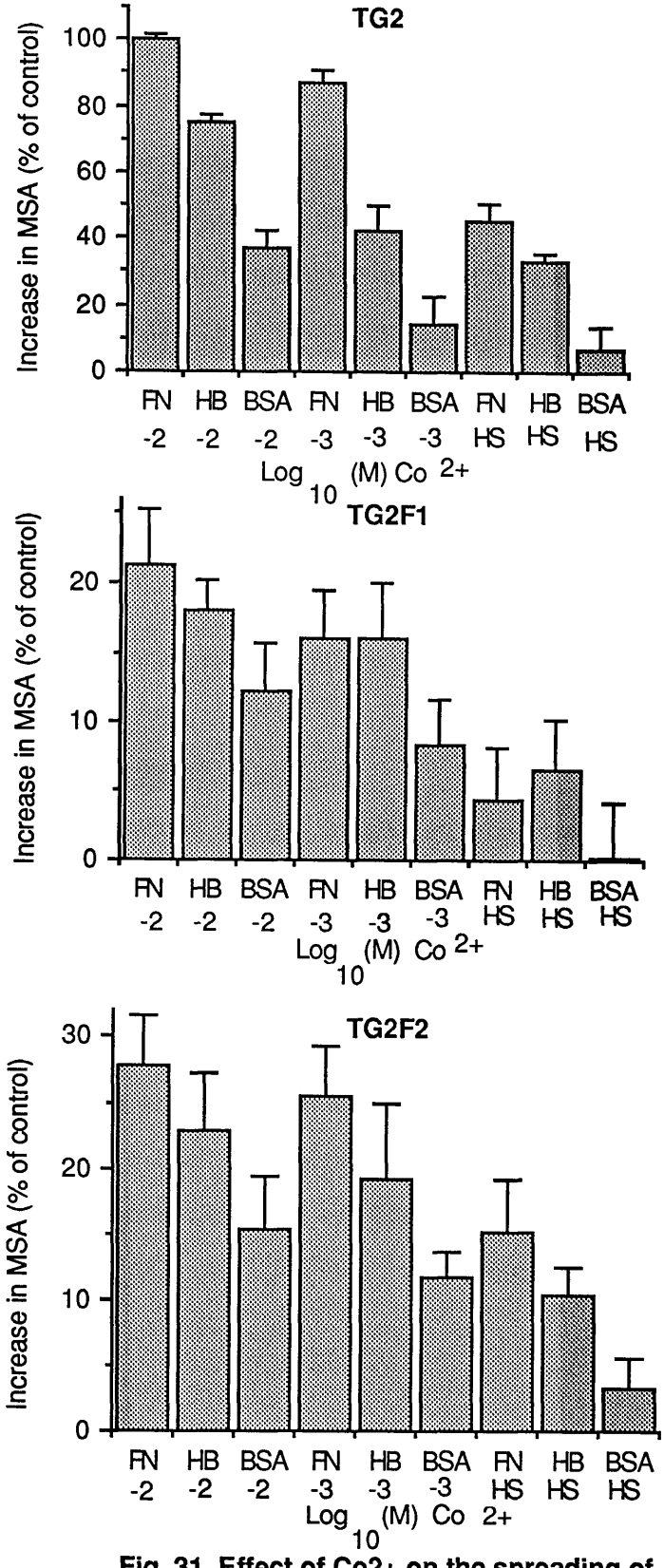


Fig. 31 Effect of Co2+ on the spreading of TG2, TG2F1, and TG2F2 cells on various surfaces

spread on fibronectin in presence of each of these divalent (Fig. 32).

Mn^{2+} was found the most effective divalent in inducing the spreading of parental and both variant cell. 10^{-3} M Mn^{2+} was effective in inducing the spreading of TG2 and TG2F2 while higher concentrations such as 10^{-2} M were effective with TG2F1. Mg^{2+} at 10^{-2} M was as effective as Mn^{2+} with parental cells on fibronectin (94.5%) but not as effective as Mn^{2+} with variants on either surfaces (22.4%, 42.5%). Co^{2+} at 10^{-2} M was less effective with TG2, TG2F1 and TG2F2 compared to Mn^{2+} and Mg^{2+} (70%, 21%, 31%).

Search for differences in proteins between parental and variants

1) Con-A binding proteins

Many of the proteins thought to play an important role in cell adhesion are glycoproteins (see earlier sections). To look for differences in glycoproteins between parental cells and variants, crude membrane fractions of Py3, F2, F2R1, and F2R2 cells were solubilised by 1% NaDOC and the extract subjected to affinity chromatography on Con-A Sepharose (see methods).

A silver stained gel (Fig. 33 a & b) of the fractions from a Con A-Sepharose column eluted with 0.1 M alpha-methyl mannoside reveals numerous glycoproteins. No repeatable differences between cell lines were observed. In particular there were none in the region of about 120-140 kd where the fibronectin receptor might be expected.

2) Neutral detergent extraction

An alternative to use of crude membranes was direct extraction with non-ionic detergent. Cells were treated in vitro with Triton X-100. The residues contain mainly cytoskeletal proteins, and matrix glycoproteins. The Triton soluble material was bound to a small volume (0.1 ml) of Con-A or WGA coupled to Sepharose and then

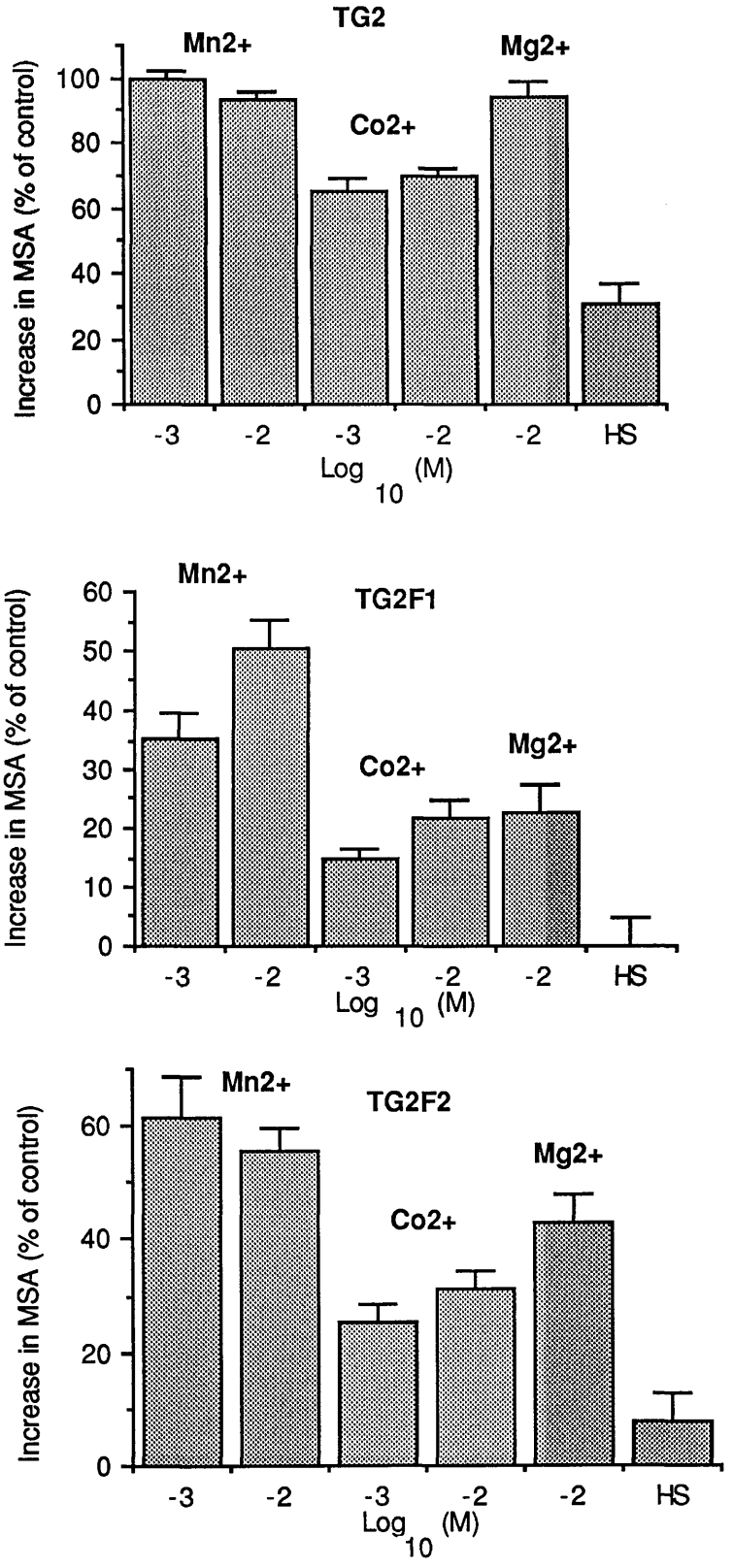


Fig. 32 Effect of different divalent cations on the spreading of TG2, TG2F1, and TG2F2 cells on fibronectin

TG2-WT, TG2F1, and TG2F2 cells in low calcium Hepes saline were plated for two hours at 37° C on plastic dishes previously coated from a solution of fibronectin (25 μg/ml) in presence of 10^{-3}-10^{-2} M Mn^{2+}, 10^{-3}-10^{-2} M Co^{2+}, or 10^{-2} M Mg^{2+}. (normalisation 99.96-615.3 μm^2) (Two experiments).

HS Hepes Saline

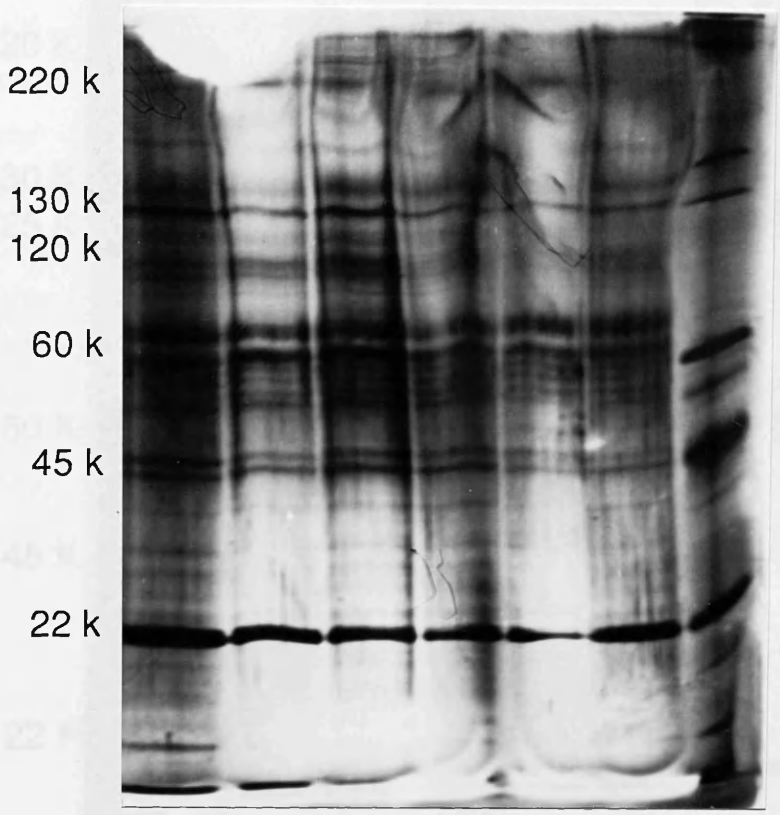
Fig. 33 (a & b) Con-A-binding proteins isolated from NaDOC-solubilised membranes of Py3, F2 mutants, and both revertant cells (F2R1&F2R2).

S Standard

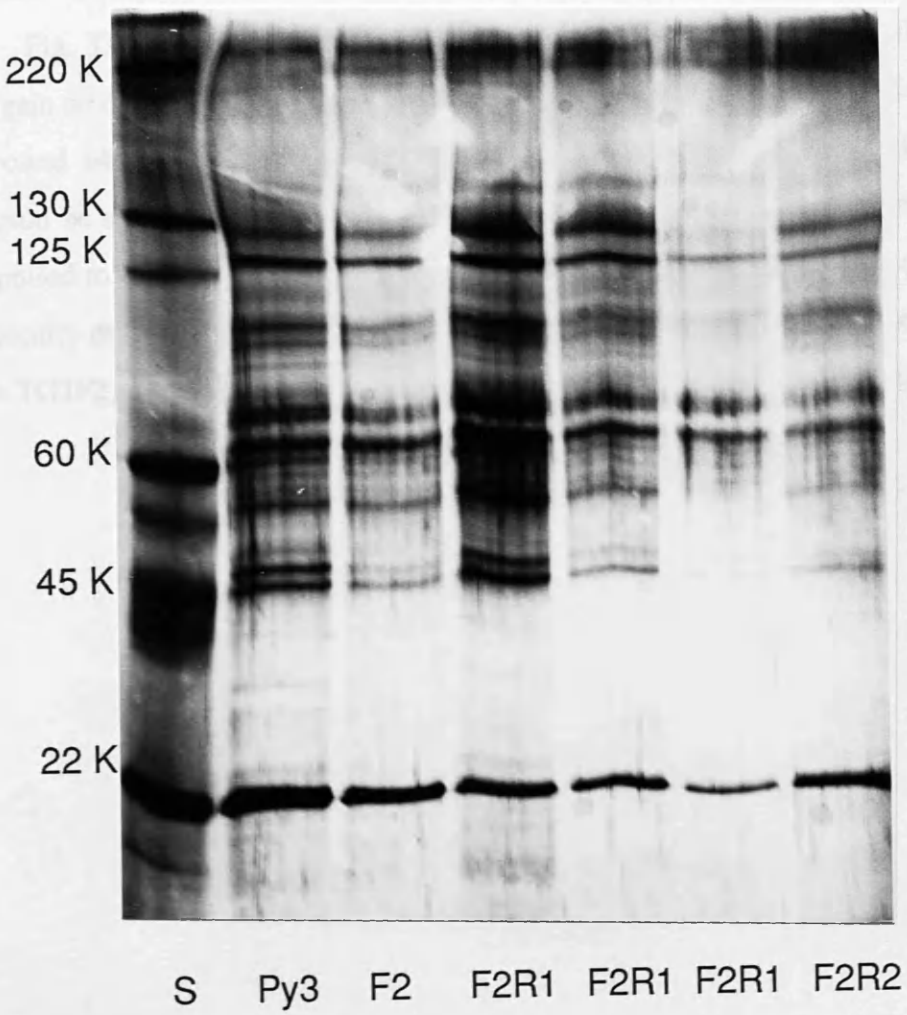
Cells were fractionated by ultracentrifugation to yield a crude membrane preparation and the membranes were homogenised and extracted in 1% NaDOC (see methods). The detergent-solubilised extract was bound to a Con-A Sepharose column, fraction eluted with alpha-methyl mannoside, then mixed with boiling mixture.

Gel 6-10%
Silver stained.

(A)



(B)



eluted with appropriate sugar. The specifically eluted material was then analysed on SDS-PAGE gels.

Fig. 34 shows that there were no differences in the gel pattern between the membrane protein around the area which is believed to be important in cell adhesion (140 kd) in Py3, F2 and F2R1, and F2R2 cells. Figure 35 & 36 also shows the comparison between the NaDOC extracts of membranes and Triton extract of the cells.

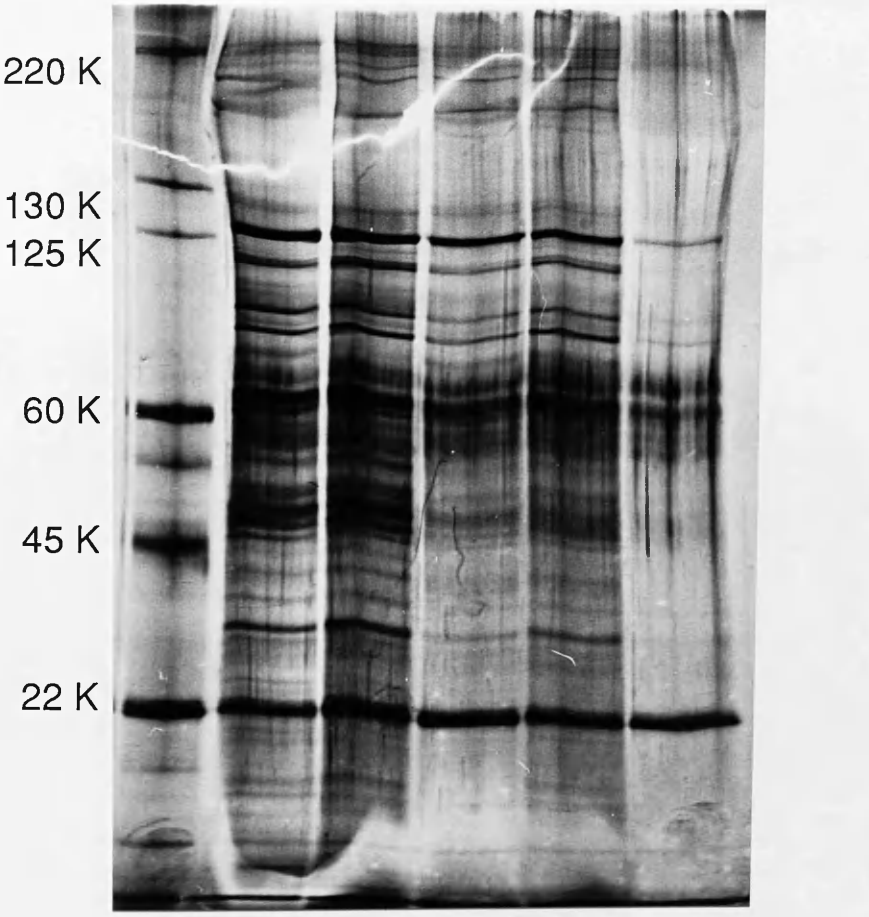
Fig. 37 shows WGA-binding proteins of Triton-soluble extracts of all cell lines. Again no differences in the gel patterns were seen. This gel was clearer in the region around 140 kd than the previous, and all the corresponding bands in the variants could be seen as in parental cells. From the data above, these different methods applied to Py3, F2 mutants and revertants revealed the same results. An attempt to identify differences in glycoproteins gel patterns between TG2 and variants (TG2F1 & TG2F2) using similar methods was also unsuccessful (data not shown).

Fig. 34 Silver stained SDS-PAGE gel of Triton soluble Con-A-binding proteins from Py3, F2 mutants, F2R1, and F2R2 cells.

S Standard

Cell monolayers were washed twice with Hanks Hepes containing protease inhibitors (see methods) for 15 minutes. Cells were then incubated in 1% Triton also containing protease inhibitor for 10 minutes. The Triton extract was then incubated with 0.1 ml Con-A Sepharose beads for 30 minutes. The beads were resuspended 3 times in Hanks Hepes containing Triton and eluted with 100 mM 1% alpha-methyl mannoside.

Gel 6-10%


220 K
130 K
125 K
60 K
45 K
22 K
S
Py3
F2
F2R1
F2R2
Con-A control

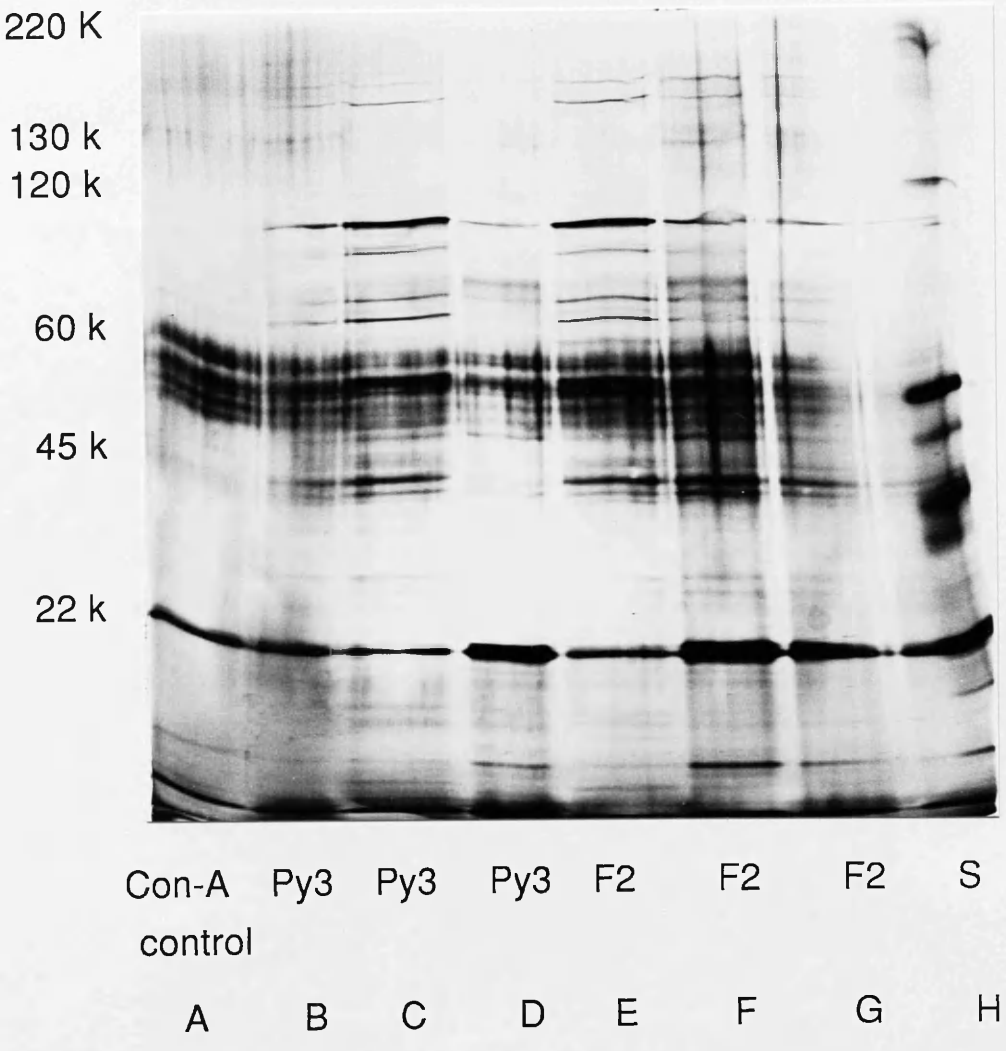

220 K
130 k
120 k
60 k
45 k
22 k
Con-A
control
Py3
Py3
Py3
F2
F2
F2
S
A
B
C
D
E
F
G
H

Fig. 35 Silver stained gel of Con-A-binding proteins of NaDOC solubilised membranes and Triton soluble extracts of Py3 and F2 mutants analysed by SDS-PAGE gels.

Track A	Con A control
Track B	Py3
Track C	Py3
Track D	Py3
Track E	F2
Track F	F2
Track G	F2
Track H	standard

Proteins in track B, C, E were solubilised from membranes in 1% NaDOC while in D, F, G were extracted directly from cells with Triton.

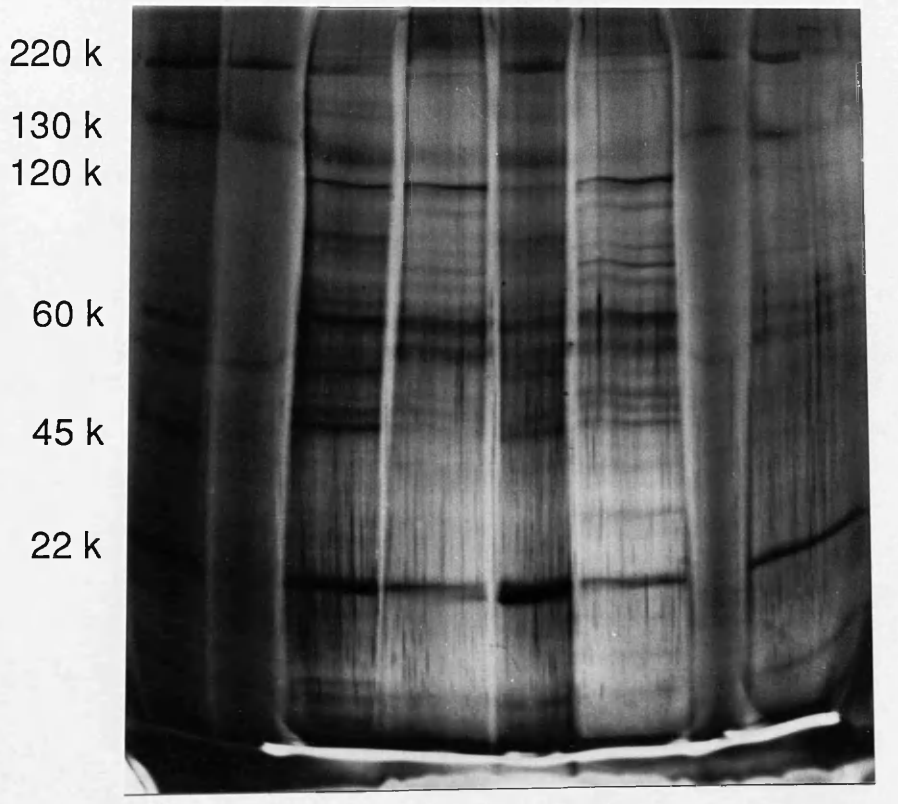
Gel 6-10%

Fig. 36 Silver stain gel of Con-A-binding proteins of NaDOC solubilised membranes and Triton soluble extracts of F2R1 and F2R2 cells analysed by SDS-PAGE gels.

Track A	Standard
Track B	F2R1
Track C	F2R1
Track D	F2R2
Track E	F2R2
Track F	Con A control

Proteins in track b, d were solubilised from membranes with NaDOC while in track C, were extracted directly from cells with Triton.

Gel 6-10%
Silver stained


220 k
130 k
120 k
60 k
45 k
22 k
S
F2R1
F2R1
F2R2
F2R2
Con-A control
A
B
C
D
E
F

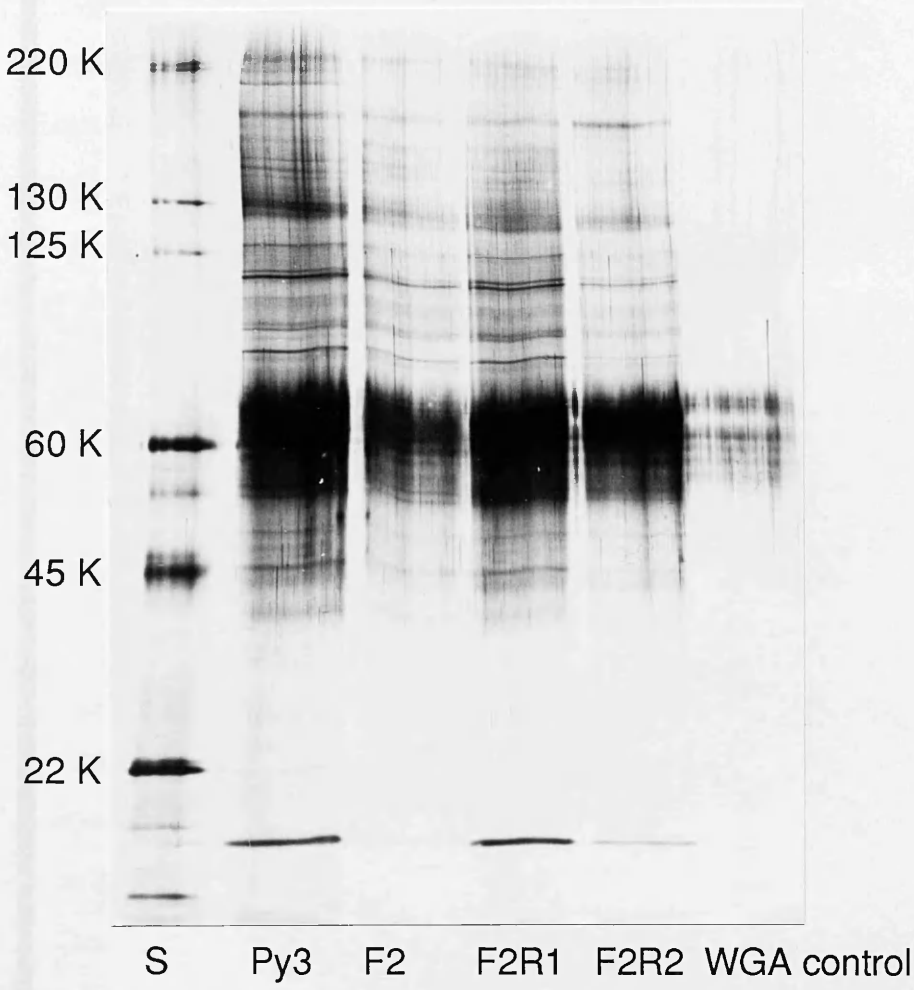

220 K
130 K
125 K
60 K
45 K
22 K
S
Py3
F2
F2R1
F2R2
WGA control

Fig.37 WGA-binding proteins of Triton soluble extracts of Py3, F2 mutants, F2R1, F2R2 cells analysed by SDS-PAGE gel.

S Standard

Cells were washed and extracted as described in fig. 34. The Triton extracts was bound to 0.1 ml WGA Sepharose beads for 30 minutes, then the beads were resuspended 3 times in Hanks Hepes containing Triton and then eluted with 100 mM N-acetyl glucosamine.

Gel 6-10%
Silver stained

Chapter four

DISCUSSION

SELECTION OF VARIANTS

1) Failure to obtain mutants from SR cells

For selection of variants, the BHK21 cells used in this work were transformed by two different tumour viruses:

1. polyoma virus (a DNA virus).
2. Schmidt-Ruppin virus (a strain of avian retrovirus, an RNA virus).

Such transformed cells might seem unpromising for the isolation of low adhesive variants because they are poorly adherent to culture substrates. They produce almost no fibronectin (Hynes, 1976), but as has been shown for many types of transformed cells (Yamada et al., 1976; Ali et al., 1977; Willingham et al., 1977), adhesion of these cells can be greatly increased by adding fibronectin to cultures. So it was against this response that the selection was carried out. These cells are also anchorage independent. Therefore, they can grow without the need to flatten on a solid surface (Stoker et al., 1968). Thus non-adherent mutants would not be at a disadvantage for growth, because both wild type and mutants could grow equally well in suspension.

At the start of this work, variants unresponsive to fibronectin had already been isolated from BHK-Py3 in this laboratory (Edwards et al., 1985) (see table 1). Therefore, to see if similar phenotype could be obtained from other cell lines such as BHK-SR cells, I carried out selection experiments using these cells.

With and without use of a mutagen, no such variant was obtained from any of several different clones tested. Because the frequency of this mutant might be very low, I tried using the unattached cells from 5 roller culture bottles instead of one, but found the same result. Moreover, repeating the selection in parallel with Py3 cells, yielded adhesion-defective variants from Py3 cells but not from SR cells. Therefore, the selection works repeatedly with Py3 cells but not with SR cells. In some selection experiments with SR cells, the frequency of SR wild type colonies in final selection

stages was zero. This happens because the number of wild type cells unattached after fibronectin cycles is very small due to the high percentage of attachment on plastic dishes.

If an SR-mutant with the same phenotype as the Py3 mutants is present, it must be at very low frequency. An attempt to measure a maximum value was made by mixing SR wild type cells with non-adherent Py3 mutants (F2, see table 1) already isolated (see earlier). The frequency of mutants with similar properties to F2 from SR was zero, while F2 mutants were recovered at $1:10^6$ and higher inputs. Therefore, the frequency of occurrence of SR-mutants with similar properties to Py3 mutants must be less than $1:10^6$.

The only known difference between the two cell lines is that Py3 cells are transformed by action of polyoma middle T antigen, while SR cells are transformed by the src gene product $pp60^{v\text{-}src}$.

An endogenous analogue of the viral $pp60^{v\text{-}src}$ has been detected within normal vertebrate cells and by most criteria appears to be very similar to the viral transforming protein (Rohrschneider et al., 1979). This endogenous protein is designated $pp60^{c\text{-}src}$ and probably performs some critical functions within the normal cells. Transformation by $pp60^{v\text{-}src}$ has been proposed to result from the perturbation of the normal cellular functions carried out by $pp60^{c\text{-}src}$.

It has been found that antibody-complexes containing the polyoma middle-T antigen contain the protein $pp60^{c\text{-}src}$ (Courtneidge and Smith 1983; 1984). In this form $pp60^{c\text{-}src}$ has an extra tyrosine phosphate and higher specific enzymic activity. The authors suggests that middle-T antigen itself is not a protein kinase but rather that $pp60^{c\text{-}src}$ phosphorylates middle T-antigen. It is possible that the transforming activity of middle-T antigen is exerted by modification of the $pp60^{c\text{-}src}$ function (Magnusson, 1985).

One could assume either that middle-T antigen makes $pp60^{c\text{-}src}$ identical in activity to $pp60^{v\text{-}src}$ or that it acts in some other way. There are several possible

assumptions about where the mutations of Py3 which yield F1 and F2 might have occurred:

1) The mutation could be in the viral DNA, coding for the middle-T antigen. The previous mutants (F1) were shown by collaborative work at ICRF (J. Wilson and M. Fried, unpublished work) to express polyoma-T antigens of identical mobility in gels to wild type and middle-T antigen with similar kinase activity.

2) The mutation may occur in the cellular DNA. In this case we should expect to obtain mutants with equal frequency in both RSV and polyoma transformed cells.

3) A cellular mutation may have occurred which is expressed only in presence of middle-T antigen. It is still has to be explained why middle-T antigen works but not $pp60^{v\text{-}src}$, two possible explanations may be involved:

(a) $pp60^{v\text{-}src}$ interacts with more than one primary single target, different targets then giving rise to various phenotypes (Weber et al., 1983). $pp^{60c\text{-}src}$ activated by middle-T antigen may interact with a particular protein which leads to the non-adherent phenotype, whereas $pp60^{v\text{-}src}$ does not.

The degree of phosphorylation of various targets may be responsible for generating various transformed phenotypes. Weber et al. (1983) suggested that $pp^{60v\text{-}src}$ of RSV mutants (these mutants affect fibronectin secretion, anchorage dependence, and adhesion of transformed cells) phosphorylate some cellular targets well and others poorly. It is possible to correlate the degree of phosphorylation of some cellular targets with the expression of various transformation characters.

(b) The SR virus, (isolated from birds) may generate relatively low levels of $pp60^{v\text{-}src}$ in hamster cells, a level too low to generate the phenotype.

During selection experiments, transferring 10 million SR and Py3 separately to grow in roller culture bottles for 1-2 days, yielded 20 million unattached Py3 cells, while the number of unattached SR cells was 4 million. Moreover, spreading assays on fibronectin and vitronectin coated surfaces, showed that the percentage increase in mean spread area of SR cells was higher compared to TG2 cells and

C13 cells (see fig. 3). These result may explain in some way the difficulty in isolating a non-adherent mutant from SR cells on fibronectin-coated surface.

4) The virus DNA could be interfere with particular sites on the cellular genes i.e. act as a mutagen.

2) Reversion of F2

I found that Py3 mutants undergo spontaneous reversion at frequency of 1 in 10^5 which it is rather higher than expected for a point mutation. Two clones were isolated which grew as monolayers on tissue culture flask. F2R1 was uniformally flat while F2R2 was less flattened. It is evident that these two revertants lost the non-adherent rounded morphology and low adhesion but retained some of the characteristics of Py3 cells e.g. the ability to grow well in soft agar.

A third phenotype was observed. F2 mutants several times yielded extremely spread cells (very flattened, see fig. 10 b). These cells are morphologically similar to BHK-C13 cells. F2 may revert to an adherent phenotype by loss of middle-T antigen. Unfortunately, attempts to isolate this variant were unsuccessful.

From the reversion frequency, there is no deletion in genes for proteins essential for spreading. If the third type of these revertants was indeed untransformed (i.e. loss of middle-T antigen expression) this would be consistent with explanation 3.

HGPRT-ase in wild type and mutants

Two variants (TG1&TG2) were selected after mutagenesis with MNNG, then variants (TG2F1&TG2F2) unresponsive to fibronectin were selected from TG2. All these cells are deficient in HGPRT- ase activity. Resistance to TG and labelling with ^{3}H-hypoxanthine showed that the variants either completely lack or have very low ability to utilise hypoxanthine. Cells with mutations that inactivate HGPRT-ase grow in media containing purine analogs that kill cells with normal HGPRT-ase levels. These mutants survive due to their ability to synthesise the required purines

by a de novo pathways (Bradley et al., 1981).

This genetic marker has already been useful to show that TG2F1 and F2 were not contaminants picked up in selection on fibronectin. In the future, TG cells could be used in fusion experiments with thymidine kinase deficient both transformed and non-transformed cells.

Effect of Con A, WGA and poly L-lysine on cell adhesion and spreading

Hughes et al. (1979; 1980) and Grinnell and Hayes (1978 a, b) have shown that morphological changes induced by fibronectin, present as a surface coating in assays in vitro, can be mimicked by other proteins known to interact with cell surfaces, such as Con A.

In this study, TG2 showed 85% increase in MSA; TG2F1, 55.75% and TG2F2 65.5% on Con A, compared with fibronectin. Wheat germ agglutinin (WGA), on the other hand induced less spreading of mutants and wild type than haemoglobin control (see fig. 21). The present findings disagree with those of Aplin and Hughes (1981) and Oppenheimer-Marks and Grinnell (1982) who found that cells spread on WGA. This variation may be due to the cell lines used, the amount of endogenous fibronectin being very low in the present study. Alternatively, the different results obtained could be due to the different technique used by Aplin and Hughes (1981) in which WGA was covalently coupled to glass coverslips. The amount of WGA which adsorbs to the plastic surface could be very low. Rauvala et al. (1981) have found that the adsorption of WGA to plastic surfaces was much less than that of Con A. In this case TG cell lines did not respond to WGA presumably because of the small amount of WGA bound to the plastic. Various other adhesion defective variants were able to spread on Con A coated-surfaces: The CHO mutant Ad^{V} (Harper and Juliano, 1980), and the 3T3 mutant AD6 (Norton and Izzard, 1982), the ricin resistant BHK cells (Aplin and Hughes, 1981) spread on such substrates, and FN-1 BHK mutant also spread partially on Con A (Oppenheimer-Marks et al., 1984). Other mutants

such as the hepatoma variants (Briles and Haskew, 1982) were not tested for spreading on Con A.

Recently, Edwards et al. (1985) isolated variants of polyoma-transformed BHK 21 cells unresponsive to fibronectin. Con A was unable to support the spreading of these variants (Salama, 1986). In the present study, Con A did induce the spreading of both TG variants. These variants were also selected from a clone of polyoma BHK21, and TG2F1 is morphologically similar to F2. The differences in the spreading result may be due to differences in technique or in measurement of spreading in the earlier work. Alternatively, TG2F1 is a distinct phenotype. This needs further investigation, using both variants under the same spreading conditions.

The relation between induction of spreading by lectins such as Con A and by fibronectin is still unclear. Several authors e.g. Grinnell (1983) believe that binding of any plasma membrane glycoproteins to substrates can induce cell spreading. A different theory is that lectin-induced spreading, as with fibronectin involves specific matrix receptors. Aplin and Hughes (1981) showed that, as for fibronectin, a threshold density of lectin is required to promote either attachment or spreading of cells. Although, interactions with lectins may be as numerous as in cells attached to a fibronectin matrix at equivalent densities, sufficient interactions with glycoproteins leading to spreading are not formed. Hence, much higher densities of lectins are needed to satisfy the minimum requirements for active cell spreading. In wild type, one could assume that fibronectin binds to its receptor and triggers changes in the cytoskeleton which lead to cell spreading, perhaps accompanied by changes in other factors such as Ca^{2+} or c-AMP. Binding of Con A to cell membranes could also cause clustering of such receptors leading to cell spreading. In the variant cells, which have little ability to adhere and spread on fibronectin, binding of Con A may cause the clustering of other members of the matrix receptor family. Alternatively, the interaction between the receptors and the cytoskeleton could occur via Con A bridging the receptor to an unknown protein which is bound to the cytoskeleton.

Other investigators (Painter et al., 1982; Wheeler et al., 1985) have shown that Con A induces an interaction between the platelet IIb-IIIa glycoproteins and the Triton insoluble cytoskeleton. Painter et al. (1982) concluded that this association between the fibrinogen receptor glycoproteins and the actin cytoskeleton was caused by clustering of IIb-IIIa glycoprotein molecules, and that the polyvalency of Con A mediated such clustering. In agreement with this, Isenberg et al. (1987) suggested that the cytoskeleton does not direct receptor clustering in platelets, but rather, that ligand- induced clustering of IIb-IIIa glycoprotein, mediated by receptor occupancy alone, promotes subsequent association with the actin cytoskeleton.

Yamada and Kennedy (1984) found that Con A mediated spreading was not inhibited by a synthetic decapeptide containing the RGD sequence in an experiment in which fibronectin-mediated spreading was inhibited completely. The opposite result was found by Silnutzer and Barnes (1985) who found that an RGD containing peptide could inhibit the spreading of human fibroblasts on a Con A-coated surface but there was no inhibition with Hela or C6 rat glioma cells on the same surface. A difficulty with the idea that lectins cause spreading by binding to the fibronectin receptor is that the fibronectin receptor from human cells binds well to WGA, whereas it does not bind to Con A (Pytela et al., 1985 a).

Poly L-lysine has been used to attach many different type of cells, e.g. live myoblasts, and amoeba, firmly to surfaces used in microscope observation (Mazia et al., 1975). This is believed to be accomplished through adsorption of poly L-lysine molecules to the surfaces, followed by electrostatic interaction between cationic sites on the attached molecules and anionic sites on the surface of the cell (Mckeehan and Ham, 1976).

Early in this study, I found that Py3 and both revertant cells spread on poly L-lysine (14 kd) whilst F2 did not. However, in later work, the TG parental cells did not spread either. Repeating the experiment with 47 kd poly L-lysine gave the same results. Even C13 cells were unable to spread on this surface but these cells adhered

to it and remained rounded (data not shown). Previously it had been shown that Py3, F2R1, and F2R2 spread on this surface (60%, 53%, 50.6% increase in MSA). These variable results may have occurred because in the earlier work with Py3, the cells were inadequately washed to remove serum. Subsequent experiments with TG-WT showed a requirement for serum for spreading on poly L-lysine, appreciable spreading was obtained on 1% serum. The failure of cells to spread on poly L-lysine has been repeated frequently by other workers in this laboratory. These results are in agreement with Aplin and Hughes (1981) and Pierschbacher and Ruoslahti (1984 a) who found that poly L-lysine was ineffective in promoting the spreading of BHK cells and other fibroblasts respectively. I suggest that spreading of cells on poly L-lysine in presence of serum may be due to the interaction of cells with a trace of vitronectin or fibronectin. If this is true, the RGD peptide should inhibit the spreading of cells on poly L-lysine.

There may be an interaction between the fibronectin receptor and poly L-lysine charges, but this interaction is not enough to trigger spreading. It is not clear why binding to Con A should be more effective.

Both TG variants are similar to the variants isolated by Edwards et al. (1985) in which all variants did not spread on poly L-lysine. Harper and Juliano (1980) found their WT as well as CHO mutant AD^{V} did spread and attain a normal CHO morphology on substrates coated with poly L-lysine. Either the differences in the spreading occurred because the cell type is different or due to the differences in the technique used such as use of higher concentration of poly L-lysine (1 mg/ml) with CHO mutants. Other mutants have not been tested for ability to attach on such surfaces (Klebe et al., 1977 a; Pena and Hughes, 1978; Briles and Haskew, 1982; Norton and Izzard, 1982; Oppenheimer-Marks et al., 1984).

EFFECT OF DIVALENT CATIONS ON CELL SPREADING

Mn^{2+} was found to be effective in inducing spreading of TG2-WT at micromolar level on fibronectin and at millimolar level on haemoglobin. Mg^{2+} was found almost as effective as Mn^{2+} on fibronectin. In respect of TG2F1, the concentration of Mn^{2+} required on fibronectin was only about 10x lower than on haemoglobin, while it was about 100x lower on fibronectin than on haemoglobin in case of TG2F2. The effect of Mn^{2+} on cell substratum adhesion could be explained as follows:

1) Mn^{2+} might promote fibronectin secretion and/or act as a more efficient cofactor for fibronectin than Mg^{2+} or Ca^{2+} (Evans and Jones, 1982). Against this however, all the TG lines (being transformed cells) presumably secrete very low amount of fibronectin. More importantly, millimolar Mn^{2+} was effective on proteins other than fibronectin and with the variants which do not respond to fibronectin.

2) Higher (mM) Mn^{2+} promotes the cell substratum contact process regardless of the substratum. There is an electrostatic barrier between cells and the substratum that must be overcome by a contact process (Curtis, 1964; Weiss, 1975). The possibility must therefore be considered be that Mn^{2+} reduces electrostatic repulsion between the cell and the substratum. However, there should be no such repulsion on poly L-lysine.

3) mM Mn^{2+} activates cell surface components which induce spreading on all of the surfaces (Grinnell, 1984 a).

Recently, the importance of the adhesion receptor recognition signal Arg-Gly-Asp (RGD) in the interaction between the cell binding domains of proteins like fibronectin with their cellular receptors has been demonstrated (Ruoslahti and Pierschbacher, 1986), and another peptide Arg-Glu-Asp-Val (REDV) has also been implicated in the adhesion of melanoma cells on fibronectin (Humphries et al., 1986 b).

Edwards et al. (1987) proposed that divalent cations induce spreading by binding to RGD receptors. It has recently been shown from cDNA sequences that several RGD receptors contain several possible Ca^{2+}-binding sites, similar to those of

calmodulin (Argraves et al., 1987). These sequences provide specific candidates for sites at which divalent cations may bind to activate adhesion and spreading.

The fact that bound fibronectin receptor, vitronectin receptor, and GP IIb-IIIa can all be eluted from ligand-Sepharose affinity columns using EDTA also indicates that divalent cations, e.g. calcium, are generally required for function of these adhesion receptors (Pytela et al., 1987).

It seems likely that at lower concentrations, Mn^{2+} acts at sites normally occupied by Ca^{2+} or Mg^{2+}, in the fibronectin receptor (Edwards et al., 1987). It is possible also that high Mn^{2+} induces spreading by binding to the Ca^{2+}/Mg^{2+} site of Arg-Gly-Asp receptors. On fibronectin, micromolar Mn^{2+} could replace Ca^{2+} or Mg^{2+}, while on haemoglobin higher concentrations are known to be required. To explain the very different concentrations involved with and without fibronectin, Edwards et al. (1988) have suggested that the binding of fibronectin to its receptor cooperatively increases the affinity of the receptor metal sites for the ions, and that bound ions turn the receptor on in presence or absence of fibronectin.

In the variants which have lost their response to fibronectin, Mn^{2+} may act on the other members of the adhesion receptor family.

Edwards et al. (1987) have presented a model, in which the binding of the metal ion at the receptor site, rather than binding of Arg-Gly-Asp, induces or signals spreading. On haemoglobin, Mn^{2+} at higher concentrations (10^{-2} M) could bind to the receptor and trigger spreading without the requirement for the RGD sequence. On fibronectin, RGD could increase the affinity of binding of Mn^{2+} to the receptor.

Oppenheimer-Marks and Grinnell (1984) reported that Ca^{2+} protects 48 kd cell surface WGA-R (fibronectin receptor) against proteolysis. Later, Akiyama and Yamada (1985 a) concluded that the putative fibroblast-fibronectin receptor is resistant to trypsin in the presence of divalent cations but is susceptible to trypsin in the presence of 5 mM EDTA. This suggests that divalent cations protect fibronectin receptors in some way by altering the conformation of this receptor. This

change could alter the interaction of alpha with beta subunits. Fujimura and Phillips (1983) found that Ca^{2+} regulates the association and dissociation of the glycoprotein IIb-IIIa complex in human platelet membranes. Mn^{2+} also caused an amount of glycoprotein IIb-IIIa complex formation similar to that caused by Ca^{2+}, while Mg^{2+} was less effective. It seem that TG2F1 lacks any response to RGD while TG2F2 has some lower response to RGD.

4) Divalent cation effects on Arg-Gly-Asp binding could act through the binding of these divalents directly to the matrix proteins (Edwards et al., 1987). Lawler and Hynes (1986) found that thrombospondin contains possible calcium-binding sequences near the Arg-Gly-Asp tripeptide in this adhesive protein.

COMPARISON OF THE PRESENT VARIANTS WITH OTHER ADHESION DEFECTIVE VARIANTS

Due to the lack of information and to the different methods used to characterised numbers of adhesion defective variants, it is difficult to compare results from other laboratories with the present variants.

From table 5, TG2F1 and TG2F2 are similar in most respect to Ad^{v} (Harper and Juliano, 1980) and CHO $^{att-}$ (Klebe et al., 1977 a). All these variants do not respond to fibronectin. The adhesion of CHO $^{att-}$ mutants could be partly restored in presence of very high Ca^{2+}, Mg^{2+}, or Mn^{2+}. The biochemical defects of one clone of AD^{v} variants has recently been shown to be an alteration of type-I c-AMP protein kinase activity. It is not clear why this defect affects adhesion and spreading on fibronectin, but not other surfaces. It is also not known whether the TG variants can be induced to spread on fibronectin by treatments which elevate c-AMP.

Various mutants which have defects in glycosylation e.g. AD6 (Pouyssegur and Pastan, 1976; 1977) and several ricin resistant mutants (Pena and Hughes, 1978) do spread on fibronectin, although in ricin mutants higher fibronectin concentrations than the wild type were required. It is unlikely, therefore, that TG2F1 and TG2F2 have

Table 5. Spreading of different cell lines on various surfaces

Cell type	Fn	Vn	S	Ln	Coll	Con A	WGA	PL	Induction by D. C	Biochemical Defects
Ad6 (Pouyssegur & Pastan, 1976, 1977)	+					++		++		Block of N-acetylation of glucosamine
ADv (Harper & Juliano, 1980)	--					++		++	--	Altered dependent protein kinase type-1 cAMP
CHO att- (Klebe et al., 1977a, b)	--				--				++ 100 mM Ca^{2+} or 10 mM Mg^{2+}	Unknown
Ad3, Ad4, Ad5 (Briles & Haskew, 1982)	++				--					Unknown
Fn-1 (Oppenheimer-Marks et al., 1984)	--					+				Unknown
Several ricin-resistant BHK (Pena & Hughes, 1978)	+					++				Various glycosylation defects
F1 & F2 (Edwards et al., 1985)	--	--	--	--	--	--	--	--	+ 10^{-4} M Mn^{2+}	Unknown
TG2F1 & TG2F2 (this work)	--	--	--	--		++	--	--	++ 10^{-5} - 10^{-2} M Mn^{2+}	Unknown
Dermatosparactic fibroblasts (Mauch et al., 1986, 1988)	++				+					Lack of 34 kD collagen-binding protein
Sarcoma I (Rabinovitch & DeStefano, 1973)			--						++ Mn^{2+} 50-100 µM	Unknown

Fn, fibronectin; Vn, vitronectin; S, serum; Ln, laminin; Coll, collagen; Con A, concanavalin A; WGA, wheat germ agglutinnin; PL poly-L-lysine; DC, divalent cations; ++, fully spread; +, partially spread; --, do not spread.

glycosylation defects.

Fn-1 mutants selected from BHK cells (Oppenheimer-Marks et al., 1984) are similar to the present variants in lack of response to fibronectin. Comparing TG2F1 and TG2F2 with Fn-1 is difficult because of the lack of data and or illustrations in Oppenheimer-Marks report.

Other cell lines, Ad3, Ad4, Ad5 (Briles and Haskew, 1982) and the dermatosparactic sheep fibroblasts (Mauch et al., 1986; 1988) have been found unresponsive to collagen but have a normal response to fibronectin. Sarcoma I cells are similar to the present variants in their response to Mn^{2+}.

The variants F1 and F2 (Edwards et al., 1985) are similar to the present variants in other respects, but unlike TG2F1 and TG2F2, F1 and F2 were originally described as unable (or poorly able) to spread either on Con A or on fibronectin in high Mn^{2+} (Salama, 1986).

Studies of membrane protein extraction

In the present study, SDS-PAGE gel revealed the presence of many polypeptide and lectin-binding proteins associated with the detergent soluble extracts of Py3 and all cell lines by various techniques, but no repeatable differences were seen. Possibly two dimensional protein mapping could reveal such a difference.

CONCLUSION

During the adhesion of cells to substrata, the initial attachment process is followed by distinctive changes in morphology, resulting in a flattened and spread appearance, and the re-organisation of actin filaments. Therefore, the cytoskeletal system seems to play an integral role in the change in morphology (Willingham and Pastan, 1975).

In the present study, the TG variants are able to spread on fibronectin and on haemoglobin, in presence of high Mn^{2+}, and also on a Con A-coated surface. The spreading of cells in high Mn^{2+} was inhibited by cytochalasin D. The defect in these lines cannot be in whatever internal mechanisms (e.g. alteration in cytoskeleton) are involved in spreading. The defect could be in one or more cell surface receptors which are involved in spreading on fibronectin and probably on vitronectin.

The fibronectin receptor is believed to be one member of a family of receptors each of which contain alpha and beta subunits. Some evidence showed that different alpha subunits are involved in the spreading of cells on different proteins. One could suggest that in the variants some of the alpha subunits are absent or inactive. Alternatively the interaction of these subunits with beta subunits may be inefficient in inducing spreading. Mn^{2+} could activate spreading on fibronectin on these inactive alpha subunits or could activate other members of the receptor family for which the substrates are unknown, in a way that produces signal transduction.

FUTURE WORK

The nature of both mutants and revertants could be investigated by transfection of untransformed C13 cells with the polyoma DNA coding for middle T-antigen cloned from these mutants. If the resulting transfected cells were negative for spreading on fibronectin, then it would be very important to isolate and determine the DNA sequence of T-antigens of both variants compared with that of parental cells.

The variants isolated in the present study, which are resistant to 6-thioguanine, should be useful in somatic cell genetics particularly cell fusion experiments. These cells could be fused with attached cells (including human) lacking thymidine kinase.

The fibronectin and other RGD receptors of both variants and parental cells could be investigated by various approaches such as affinity chromatography, use of antibodies, and the use of appropriate cDNA probes. Antibodies could be used also to inhibit Mn^{2+}-induced adhesion. Applying these methods, changes in the glycoproteins involved in cell substratum adhesion could be identified.

Furthermore, one could study the morphology of the cytoskeleton, focal and close contacts on fibronectin and haemoglobin in the presence of Mn^{2+}. This could be done using IRM and immunofluorescence staining of actin and vinculin and other components.

It is important to know whether the defect in the variants on fibronectin can be corrected by treatments which elevate cyclic-AMP. It is also important to isolate and characterise the third type of revertant (possibly untransformed) which might help explain my failure to obtain the non-adherent phenotype from SR cells.

Chapter five

REFERENCES

Abercrombie, M. (1970). Contact inhibition in tissue culture. In Vitro **6**, 128 142.

Abercrombie, M. & Dunn, G.A. (1975). Adhesion of fibroblast to substratum during contact inhibition observed by interference reflection microscopy. Exp. Cell Res. **92**, 57-62.

Abercrombie, M. & Heaysman, J. E. M. (1954). Observations on the social behaviour of cells in tissue culture. II. Monolayering of fibroblasts. Exp. Cell Res. **6**, 293-306.

Abercrombie, M., Heaysman, J. E. M.& Pegrum, S. (1970). The locomotion of fibroblasts in culture. I. Movements of the leading edge. Exp. Cell Res. **59**, 393-398.

Akiyama, S. K. & Yamada, K. M.(1983). Fibronectin and disease. In Connective Tissue and Diseases of connective tissue, B.W. Wagner, and R. Fleischmajer (eds.). Baltimore, Williams & Wilkins.

Akiyama, S. K. & Yamada, K. M. (1985 a). The interaction of plasma fibronectin with fibroblastic cells in suspension. J. Biol. Chem. **260**, 4492-4500.

Akiyama, S. K. & Yamada, K. M. (1985 b). Synthetic peptides competitively inhibit both direct binding to fibroblasts and functional biological assays for the purified cell binding domain of fibronectin. J. Biol. Chem. **260**, 10402-10405.

Akiyama, S. K. & Yamada, K. M.(1987). Fibronectin. Adv. Enzymol. **59**, 1-59.

Akiyama, S. K., Yamada, S. S. & Yamada, K. M. (1986). Characterisation of a 140-KD avian cell surface antigen as a fibronectin-binding molecule. J. Cell Biol. **102**, 442-448.

Albini, A., Allavena, G., Melchiori, A., Giancotti, F., Richter, H., Comoglio, P.M., Parodi, S., Martin., G.R. & Tarone., G. (1987). Chemotaxis of 3T3 and SV3T3 cells to fibronectin mediated through the cell-attachment site in fibronectin and a fibronectin cell surface receptor. J. Cell Biol. **105**, 1867-1872.

Alder, R., Jerdan, J. & Hewitt, A. T. (1985). Responses of cultured neural retinal cells to substratum-bound laminin and other extracellular matrix molecules. Develop. Biol. **112 (1)**, 100-114.

Ali, I. U., Mautner, V., Lanza, R. & Hynes, R. O. (1977). Restoration of normal

morphology adhesion and cytoskeleton in transformed cells by addition of a transformation-sensitive surface protein. Cell **11**, 115-126.

Alitalo, K., Hovi, T. & Vaheri, A. (1980). Fibronectin is produced by human macrophages. J. Exp. Med. **151**, 602-613.

Aplin, J. D. & Foden, L. J. (1985). Defective adhesion to extracellular matrix leads to altered social behaviour in cultured fibroblasts. J. Cell Sci. **76**, 199-211.

Aplin, J. D. & Hughes, R. C. (1981). Cell adhesion on model substrata: threshold effects and receptor modulation. J. Cell Sci. **50**, 89-103.

Aplin, J. D., Hughes, R. C., Jaffe, C. L. & Sharon, N. (1981). Reversible cross-linking of cellular components of adherent fibroblasts to fibronectin and lectin-coated substrata. Exp. Cell Res. **134**, 488-493.

Argraves, W. S., Pytela, R., Suzuki, S., Millan, J. L., Pierschbacher, M. D. & Ruoslahti, E. (1986). cDNA sequences from the alpha subunit of the fibronectin receptor predict a transmembrane domain and a short cytoplasmic peptide. J. Biol. Chem. **261**, 12922-12924.

Argraves, W. S., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M. D. & Ruoslahti, E. (1987). Amino acid sequence of the human fibronectin receptor. J. Cell Biol. **105**, 1183-1190.

Armstrong, P.B. & Lackie, J. M. (1975). Studies on intracellular invasion in vitro using rabbit peritoneal neutrophil granulocytes (PMNS). Role of contact inhibition of locomotion. J. Cell Biol. **65**, 439-462.

Atherly, A. G., Barnhart, B. J. & Kraemer, P. M. (1977). Growth and biological characteristics of a detachment variant of CHO cells. J. Cell. Physiol. **90**, 375-385.

Auffray, C. & Norotny, J. (1986). Speculations on sequence homologies between the fibronectin cell-attachment site, major histocompatibility antigens, and a putative AIDS virus polypeptide. Human Immunol. **15**, 301-390.

Avnur, Z. & Geiger, B. (1981). Substrate-attached membranes of cultured cells : Isolation and characterisation of ventral cell membranes and the associated cytoskeleton. J. Mol. Biol. **53**, 361-379.

Baenziger, N. L., Brodie, G. N. & Majerus, P. W. (1972). Isolation and properties of a thrombin-sensitive protein of human platelets. J. Biol. Chem. **247**, 2723-

2731.

Barnes, D. W, Bosch, V. D. , Masuitl. J., Miyazaki, K. & Sato, G. (1981). The culture of human tumour cells in serum-free medium. Methods Enzymol. **79**, 368-391.

Barnes, D. W., Mousetis, L., Amos, B. & Silnutzer, J. (1984). Glass bead affinity chromatography of cell attachment and spreading promoting factors of human serum. Ann. Biochem. **137**, 196-204.

Barnes, D. W. & Sato, G. H. (1979). Growth of a human mammary tumour cell line in a serum-free medium. Nature (Lond.) **281**, 388-389.

Barnes, D. W. & Silnutzer, J. (1983). Isolation of human serum spreading factor. J. Biol. Chem. **258 (20)**, 12548-12552.

Barnes, D. W., Silnutzer, J., See, C. & Shaffer, M. (1983). Characterisation of human serum spreading factor with monoclonal antibody. Proc. Natl. Acad. Sci. USA. **80**, 1362-1366.

Barnes, D. W., Wolfe, R., Serrero, G. & McClure, D. G. (1980). Effects of serum spreading factor on growth and morphology of cells in serum-free medium. J. Supramol. Struct. **14**, 47-63.

Barthles, D., Santoni, M-J., Wille, W., Ruppert, C., Chaix, J-C., Hirsch, M-R., Fontecilla-Camps, J-C. & Goridis, C. (1987). Isolation and nucleotide sequence of mouse N-CAM cDNA that codes for a Mr 79000 polypeptide without a membrane-spanning region. EMBO J. **6**, 907-914.

Beckerele, M. C. (1986). Identification of a new protein localised at sites of a cell-substrate adhesion. J. Cell Biol. **103**, 1679-1687.

Beckerele, M. C., O' Halloran, T. & Burridge, K. (1986). Demonstration of a relationship between talin and p235, a major substrate of the calcium-dependent protease in platelets. J. Cell. Chem. **30**, 259-270.

Beesley, J., Pearson, J., Carleton, J., Hutchings, A. & Gordon, J. (1978). Interaction of leukocytes with vascular cells in culture. J. Cell Sci. **33**, 85-101.

Behrens, J., Birchmeier, W., Goodman, S. L. & Imhof, B. A. (1985). Dissociation of MDCK epithelial cells by monoclonal antibody anti-arc-1 : Mechanistic aspects and identification of the antigen as a component related to uvomorulin.

J. Cell Biol. **101**, 1307-1315.

Bell, P. B. (1977). Locomotory behaviour, contact inhibition, and pattern formation of 3T3 and polyoma virus-transformed 3T3 cells in culture. J. Cell Biol. **74**, 963-982.

Ben-Ze' ev, A., Farmer, S. & Penman, S. (1980). Protein synthesis requires cell-surface contacts while nuclear events respond to cell shape in anchorage-dependent fibroblasts. Cell **21**, 365-372.

Bennoni, D. & Bradley, W. E. C. (1986). Highly adherent mutants of SV40-transformed mouse fibroblasts: genetic and biochemical characterisation. J. Cell.Physiol. **126**, 174-180.

Bertolotti, R., Rutishauser, U. & Edelman, G. M. (1980). A cell surface of embryonic liver cells. Proc. Natl. Acad. Sci. USA. **77**, 4831-4835.

Bertoncello, I., Bradley, T.R., Chamley, W.A. & Hodgson, G. S. (1982). The characteristics of an anchorage-independent clonal agar assay for primary explanted bovine granulosa cells. J. Cell. Physiol. **113**, 224-230.

Beyth, R. J. & Culp, L. A. (1984). Complementary adhesive responses of human skin fibroblasts to the cell-binding domain of fibronectin and the heparan sulfate-binding protein, platelet factor-4. Exp. Cell Res. **155**, 537-548 .

Birchmeier, C., Kreis, T. E., Eppenberger, H. M., Winterhalter, K. H. & Birchmeier, W. (1980). Corrugated attachment membrane on WI-38 fibroblasts: Alternating fibronectin fibers and actin-containing focal contacts. Proc. Natl. Acad. Sci. USA. **77**, 4108-4112.

Birdwell, C. R., Gospodarowicz, D. & Nicolson, G. L. (1978). Identification, localisation and role of fibronectin in cultured bovine endothelial cells. Proc. Natl. Acad. Sci. USA. **75**, 3273-3277.

Blackburn, C. C. & Schnaar, R. L. (1983). Carbohydrate-specific cell adhesion is mediated by immobilised glycolipids. J. Biol. Chem. **258**, 1180-1188.

Boller, K. Vestweber, D. & Kemler, R. (1985). Cell adhesion molecule uvomorulin is localised in the intermediate junctions of adult intestinal epithelial cells. J. Cell Biol. **100**, 327-332.

Borsi, L., Carnemolla, B., Castellani, P., Rosellini, C., Vecchio, D., Allemanni, G., Chang, S. E.,Taylor-papadimitrion, J., Pande, H.& Zardi, L. (1987).

Monoclonal antibodies in the analysis of fibronectin isoforms generated by alternative splicing of mRNA precursors in normal and transformed human cell. J. Cell Biol. **104**, 595-600.

Boucaut, J. C. , Darribere, T., Boulekbache, H. & Thiery, J-P. (1984 a). Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos. Nature (Lond.) **307**, 364-367.

Boucaut, J. C., Barribere, T., Poole, T.J., Aoyama, H., Yamada, K. M. & Thiery, J. P. (1984 b). Biologically active synthetic peptides as probes of embryonic development: A competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cells migration of avian embryos. J. Cell Biol. **99**, 1822-1830.

Brackenbury, R., Greenberg, M. E. & Edelman, G. M. (1984). Phenotypic changes and loss of N-CAM mediated adhesion in transformed embryonic chicken retinal cells. J. Cell Biol. **99**, 1944-1954.

Brackenbury, R., Rutishauser, U. & Edelman, G. M. (1981). Distinct calcium-independent and calcium-dependent adhesion systems of embryo cells. Proc. Natl. Acad. Sci. USA. **78**, 387-391.

Bradely, M. O., Bhuyan, B., Francis, M. C., Langenbach, R., Peterson, A. & Huberman, E. (1981). Mutagenesis by chemical agents in V79 chinese hamster cells: A review and analysis of the literature. Mutation Res. **87**, 81-142.

Bramwell, M. E. & Harris, H. H. (1978). An abnormal glycoprotein associated with malignancy in a wide range of different tumours. Proc. R. Soc. Lond. B. Biol. Sci. **201**, 87-106.

Bray, D. (1979). Mechanical tension produced by nerve cell in tissue culture. J. Cell Sci. **37**, 391-410.

Breathnach, A. S. (1975). Aspects of epidermal ultrastructure. J. Invest. Dermatol. **65**, 2-15.

Bretscher, A. & Weber, K. (1980). Fimbrin, a new microfilament associated protein present in microvilli and other surface structures. J. Cell Biol. **86**, 335-340.

Briles, E. B. (1982). Lectin resistant cell surface variants of eukaryotic cells. Int. Rev. Cytol. **75**, 101-165.

Briles, E. B. & Haskew, N. B. (1982). Isolation of cloned variants of a rat hepatoma cell line with altered attachment to collagen, but normal attachment to fibronectin. Exp. Cell Res. **138**, 436-441.

Briles, E. B., Li, E. & Kornfeld, S. (1977). Isolation of wheat germ agglutinin-resistant clones of chinese hamster ovary cells deficient in membrane sialic acid and galactose. J. Biol. Chem. **252**, 1107-1116.

Bronner-Fraser, M. E. (1985). Alteration in neural crest migration by a monoclonal antibody that affects cell adhesion. J. Cell Biol. **101**, 610-617.

Brown, P. J. & Juliano, R. L. (1985). Selective inhibition of fibronectin mediated cell adhesion by monoclonal antibodies to a cell-surface glycoprotein. Science (Wash. DC) **228**, 1448-1450 .

Brown, P. J. & Juliano, R. L. (1986). Expression and function of a putative cell surface receptor for fibronectin in hamster and human cell lines. J. Cell Biol. **103**, 1595-1603.

Brown, A. F. & Lackie, J. M. (1981). Fibronectin and collagen inhibit cell-substratum adhesion of neutrophil granulocytes. Exp. Cell Res. **136**, 225-231.

Buck, C. A., shea, E., Duggan, K. & Horwitz, A. F. (1986). Integrin (the CSAT antigen): functionally requires oligomeric integrity. J. Cell Biol. **103**, 2421-2428.

Burns, G. F., Cosgrove, L., Triglia, T., Beall, J. A., Lopez, A. F., Werkmeister, J. A., Begley, C. G., Haddad, A. P., d' Apice, J. F., Vadas, M. A. & Cawley, J. C. (1986). The IIb-IIIa glycoprotein complex that mediates platelet aggregation is directly implicated in leukocyte adhesion. Cell **45**, 269-280.

Burridge, K. & Connell, L. (1983). A new protein of adhesion plaques and ruffling membranes. J. Cell Biol. **97**, 359-367.

Burridge, K. & Feramisco, J. (1980). Microinjection and localisation of 130 kd protein in living fibroblast: a relationship to actin and fibronectin. Cell **19**, 587-595.

Buskirk, D. R., Thiery, J-P., Rutishauser, U. & Edelman, G. M. (1980). Antibodies to a neural cell adhesion molecule disrupt histogenesis in cultured chick retinae. Nature (Lond.) **285**, 488-489.

Cardarelli, P. M. & Pierschbacher, M. D.(1987). Identification of fibronectin

receptors on T lymphocytes. J. Cell Biol. **105**, 499-506.

Carter, W. G. (1982). The cooperation role of the transformation-sensitive glycoproteins gp 140 and fibronectin in cell attachment and spreading. J. Biol. Chem. **257**, 3249-3257.

Chang, S., Rathjen, F. G. & Raper, J. A. (1987). Extension of neurites on axons is impaired by antibodies against specific neural cell surface glycoproteins. J. Cell Biol. **104**, 355-362.

Charo, I. F., Bekeart, L. S. & Phillips, D. R. (1987). Platelet glycoprotein IIb-IIIa-like proteins mediate endothelial cell attachment to adhesive proteins and the extracellular matrix. J. Biol. Chem. **262**, 9935-9938.

Charo, I. F., Fitzgerald, L. A., Steiner, B., Rall. Jr. S. C., Bekeart, L. S. & Phillips, D. R. (1986). Platelet glycoproteins IIb and IIIa: evidence for a family of immunologically and structurally related glycoproteins in mammalian cells. Proc. Natl. Acad. Sci. USA. **83**, 8351-8355.

Chen, W-T., Chen, J-M. & Mueller, S. C. (1986). Coupled expression and colocalisation of 140 k cell adhesion molecules, fibronectin, and laminin during morphogenesis and cytodifferentiation of chick lung cells. J. Cell Biol. **103**, 1073-1090.

Chen, W-T., Greve, J. M., Gottlieb, D. & Singer, S. J. (1985). Immunocytochemical localisation of 140 kd cell adhesion molecules in cultured chicken fibroblasts, and in chicken smooth muscle and intestinal epithelial tissue. J. Histochem. Cytochem. **33**, 576-586.

Chen, W-T. & Singer, S. J. (1980). Fibronectin is not present in the focal adhesions formed between normal cultured fibroblasts and their substrata. Proc. Natl. Acad. Sci. USA. **77**, 7318-7322.

Chen, W-T. & Singer, S. J. (1982). Immunoelectron microscopic studies of the sites of cell-substratum and cell-cell contacts in cultured fibroblasts. J. Cell Biol. **95**, 205-222.

Cheresh, D. A. (1987). Human endothelial cells synthesise and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc. Natl. Acad. Sci. USA. **84**, 6471-6475.

Cheresh, D. A., Harper, J. R., Schulz, G. & Reisfeld, R. A. (1984 a). Localisation of the gangliosides GD2 and GD3 in adhesion plaques and on the surface of human melanoma cells. Proc. Natl. Acad. Sci. USA. **81**, 5767-5771.

Cheresh, D. A., Pierschbacher, M. D., Herzig, M. A. & Mujoo, K. (1986). Disialogangliosides GD2 and GD3 are involved in the attachment of human melanoma and neuroblastoma cells to extracellular matrix proteins. J. Cell Biol. **102**, 688-696.

Cheresh, D. A., Pytela, R., Pierschbacher, M. D., Klier, F. G., Ruoslahti, E. & Reisfeld, A. (1987). An Arg-Gly-Asp-directed receptor on the surface of human melanoma cells exists in a divalent cation-dependent functional complex with the diganglioside GD2. J. Cell Biol. **105**, 1163-1173.

Cheresh, D. A., Reisfeld, R. A. & Varki, A. P. (1984 b). O-Acetylation of diganglioside GD3 by human melanoma cells creates a unique antigenic determinant. Science (Wash. DC) **225**, 844-846.

Cheung, E., Brown, P. J. & Juliano, R. L. (1987). Altered type I protein kinase in adhesion defective CHO cell variants. J. Cell. Physiol. **130**, 118-124.

Cheung, E. & Juliano, R. L. (1985). cAMP induced phenotypic reversion of adhesion defective CHO cell variant. J. Cell. Physiol. **124**, 337-343.

Chiang, T. M., Beachey, E. H. & Kang, A. H. (1977). Binding of collagen alpha 1 chains to human platelets. J. Clin. Invest. **59**, 405-411.

Chiang, T. M., Postlethwaite, A. E., Beachey, E. H., Seyer, J. M. & kang, A. H. (1978). Binding of chemotactic collagen-derived peptides to fibroblasts. J. Clin. Invest. **62**, 916-922.

Coman, D. R. (1944). Decreased mutual adhesiveness, a property of cell from squamous cell carcinomas. Cancer Res. **4**, 625-629.

Coman, D. R. (1961). Adhesiveness and stickness: Two independent properties of the cell surface. Cancer Res. **21**, 1436-1438.

Couchman, J.R. & Rees, D. A. (1979). The behaviour of fibroblasts migrating from chick heart explants: changes in adhesion, locomotion and growth, and in the distribution of actinomycine and fibronectin. J. Cell Sci. **39**, 149-165.

Couchman, J. R., Rees, D. A., Green, M. R. & Smith, C. G. (1982). Fibronectin has a dual role in locomotion and anchorage of primary chick fibroblasts and

can promote entry into the division cycle. J. Cell Biol. **93**, 402-410.

Courtneidge, S. A. & Smith, A. E. (1983). Polyoma virus transforming protein associates with the product of the c-src cellular gene. Nature (Lond.) **303**, 435-439.

Courtneidge, S. A. & Smith, A. E. (1984). The complex of polyoma virus middle-T antigen and pp60$^{c\text{-}src}$ EMBO J. **3 (3)**, 585-591.

Cowdry, E. V. (1940). Properties of cancer cells. Arch. Pathol. **30**, 1245-1274.

Cowin, P., Kapprell, H-P., Franke, W. W., Tamkun, J. & Hynes, R. O. (1986). Plakoglobin: A protein common to different kinds of intracellular adhering junctions. Cell **46**, 1063-1073.

Cowin, P., Mattey, D. & Garrod, D. (1984 a). Distribution of desmosomal components in the tissues of vertebrates, studies by fluorescent antibody staining. J. Cell Sci. **66**, 119-132.

Cowin, P., Mattey, D. & Garrod, D. (1984 b). Identification of desmosomal surface components (desmocollins) and inhibition of desmosome formation by specific Fab. J. Cell Sci. **70**, 41-60.

Crumpton, M. J., Snary, D., Walsh, F. S., Barnstable, J., Goodfellow, P. N., Jones, E. A. & Bodmer, W. F. (1978). Molecular structure of the gene products of the human HLA system: Isolation and characterisation of HLA-A, -B, -C, and Ia antigen. Proc. R. Soc. B. **202**, 159-175.

Cunningham, B. A., Leutzinger, Y., Gallin, W. J., Sorkin, B. C. & Edelman, G. M. (1984). Linear organisation of the liver cell adhesion molecule L-CAM. Proc. Natl. Acad. Sci. USA. **81**, 5787-5791.

Curtis, A. S. G. (1964). The adhesion of cells to glass: a study by interference reflection microscopy. J. Cell Biol. **19**, 199-215.

Curtis, A. S. G. (1973). Cell adhesion. In A. J. V. Butler and D. Noble (eds.), Progress in Biophysics and Molecular Biology, vol.27. Pergamon press, Inc., Elmsford, N. Y., pp. 315-386.

Curtis, A. S. G. (1987). Cell activation and adhesion. J. Cell Sci. **87**, 609-611.

Curtis, A. S. G. and Greaves, M. F. (1965). The inhibition of cells aggregation by a pure serum protein. J. Embryol. Exp. Morph. **13**, 309-326.

Curtis, A. S. G. & McMurray, H. (1986). Conditions for fibroblast adhesion without fibronectin. J. Cell Sci. **86**, 25-33.

Damsky, C. H., Knudsen, K. A. & Buck, C. A. (1984). Integral membrane proteins in cell-cell and cell-substratum adhesion. In The Biology of Glycoproteins. R. J.Ivatt (ed.). Plenum, New York. pp 1-64.

Damsky, C. H., Knudsen, K. A., Bradley, D., Buck, C. A. & Horwitz, A. F. (1985). Distribution of the cell substratum attachment (CSAT) antigen on myogenic and fibroblastic cells in culture. J. Cell Biol. **100**, 1528-1539.

Damsky, C. H., Richa, J., Solter, D., Knudsen, K. & Buck, C. A. (1983). Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. Cell **34**, 455-466.

Damsky, C. H., Wylie, D. & Buck, C. A. (1979). Studies on the function of cell surface glycoproteins. II. Possible role of surface glycoproteins in the control of cytoskeletal organisation and surface morphology. J. Cell Biol. **80**, 403-415.

Dang, C. V., Bell, W. R., Kaiser, D. & Wong, A. (1985). Disorganisation of cultured vascular endothelial cell monolayers by fibrinogen fragment D. Science (Wash. DC) **227**, 1487-1490.

Darnell, J. Lodish, H. & Baltimore, D. (1986). Molecular cell biology. Scientific American Books. pp.606.

Decker, C., Greggs, R., Duggan, K., Stubbs, J. & Horwitz, A. (1984). Adhesion multiplicity in the interaction of embryonic fibroblasts and myoblasts with extracellular matrices. J. Cell Biol. **99**, 1398-1404.

Dedhar, S., Ruoslahti, E. & Pierschbacher, M. D. (1987 a). A cell surface receptor complex for collagen type I recognises the Arg-Gly-Asp sequence. J. Cell Biol. **104**, 585-593.

Dedhar, S., Argraves, W. S., Suzuki, S., Ruoslahti, E. & Pierschbacher, M. D. (1987 b). Human Osteosarcoma cells resistant to detachment by an Arg-Gly-Asp-containing peptide overproduce the fibronectin receptor. J. Cell Biol. **105**, 1175-1182.

Dejana, E., Colella, S., Languino, L. R., Balconi, G., Corbascio, G. C. & Marchisio, P. C. (1987). Fibrinogen induces adhesion, spreading, and microfilament organisation of human endothelial cells in vitro. J. Cell Biol.

104, 1403-1411.

Dejana, E., Languino, L. R., Colella, S., Corbascio, G. C., Plow, E. D, Ginsberg, M. & Marchisio, P. C. (1988). The localisation of a platelet GP IIb-IIIa-related protein in endothelial cell adhesion structures. Blood **71** (3), 566-572.

Dennis, J. W., Carrer, J. P. & Schachter, H. (1984). Asparagine-linked oligosaccharides in murine tumour cells: Comparison of a WGA-resistant (WGAr) non metastatic mutant and a related WGA-sensitive (WGAs) metastatic line. J. Cell Biol. **99**, 1034-1044.

Dennis, J., Waller, C. & Timpl, R. (1982). Surface sialic acid reduces attachment of metastatic tumour cells to collagen type IV and fibronectin. Nature (Lond.) **300**, 274-276.

Docherty, R. J., Dysart, J. Mck. & Edwards, J. G. (1981). 48-kilodalton glycoprotein associated with cytoskeletons of BHK-21 and chick embryonic cells. Cell Biol. Int. Rep. (suppl. A) **5**, 12.

Docherty, R. J., Edwards, J. G., Garrod, D. R. & Mattey, D. L. (1984). Chick embryonic pigmented retina is one of the group of epithelioid tissues that lack cytokeratins and desmosomes and have intermediate filaments composed of vimentin. J. Cell Sci. **71**, 61-74.

Donaldson, D. J., Mahan, J. T. & Smith, G. N. (1987). Newt epidermal cell migration in vitro and in vivo appears to involve Arg-Gly-Asp-Ser receptors. J. Cell Sci. **87**, 525-534.

Duband, J-L., Dufour, S., Hatta, K., Takeichi, M., Edelman, G. M. & Thiery, J-P.(1987). Adhesion molecules during somitogenesis in the avian embryo. J. Cell Biol. **104**, 1361-1374.

Duband, J. L., Rocher, S., Chen, W-T. & Yamada, K. M. (1986). Cell adhesion and migration in the early vertebrates embryo: Location and possible role of the putative fibronectin receptors complex. J. Cell Biol. **102**, 160-178.

Duband, J. L., Tucker, G. C., Poole, T. J., Vincent, M., Aoyama, H. & Thiery, J.P. (1985). How do the migratory and adhesive properties of the neural crest govern ganglia formation in the avian peripheral nervous system? J. Cell. Biochem. **27**, 189-203.

Dustin, M. L., Rothlein, R., Bham, A. K., Dinarello, C. A. & Springer, T. A.

(1986). Induction by IL 1 and interferon-gamma: Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol. **137**, 245-254.

Edelman, G. M. (1984 a). Cell-adhesion molecules: A molecular basis for animal form. Sci. Am. **250**, 80-91.

Edelman, G. M. (1984 b). Modulation of cell adhesion during induction, histogenesis, and perinatal development of the nervous system. Ann. Rev. Neurosci. **7**, 339-377.

Edelman, G. M. (1984 C). Cell adhesion and morphogenesis. Proc. Natl. Acad. Sci. USA. **81**, 1460-1464.

Edelman, G. M. (1985 a). Cell adhesion and the molecular processes of morphogenesis. Ann. Rev. Biochem. **54**, 135-169.

Edelman, G. M. (1985 b). Expression of cell adhesion molecules during embryogenesis and regeneration. Exp. Cell Res. **161**, 1-16.

Edelman, G. M. (1986). Cell adhesion molecules in the regulation of animal form and tissue pattern. Ann. Rev. Cell Biol. **2**, 81-116.

Edwards, J. G. (1983). The biochemistry of cell adhesion. Progress in surface science (S. G. Davison, ed). **13 (2)**, 125-196.

Edwards, J. G., Campbell, G. & Salama, N. (1985). Variants of Polyoma-transformed BHK21 cells unresponsive to fibronectin. Cell Biol. Int. Rep. **9**, 737-745.

Edwards, J. G., Dysart , J. M., Edgar, D. H. & Robson, R. T. (1979). On the reduced adhesiveness of virally transformed BHK21 cells. J. Cell Sci. **35**, 307-320.

Edwards, J. G., Dysart, J. M. & Hughes, R. C. (1976). Cellular adhesiveness reduced in ricin-resistant hamster fibroblast. Nature (Lond.) **264**, 66-68.

Edwards, J. G., Hameed, H. & Campbell, G. (1988). Induction of fibroblast spreading by Mn^{2+}: a possible role for divalent cations in receptors for proteins containing Arg-Gly-Asp. J. Cell Sci. **89 (4)**, 507-513.

Edwards, J. G., Robson, R. T. & Campbell, G. (1987). A major difference between serum and fibronectin in the divalent cation requirement for adhesion and

spreading of BHK21 cells. J. Cell Sci. **87**, 657-665.

Ekblom, P., Vestweber, D. & Kemler, R. (1986). Cell-matrix interactions and cell adhesion during development. Ann. Rev. Cell Biol. **2**, 27-47.

Engvall, E. & Ruoslahti, E. (1977). Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. Int. J. Cancer **20**, 1-5.

Engvall, E., Ruoslahti, E. & Miller, E. (1978). Affinity of fibronectin to collagens of different genetic types and to fibrinogen. J. Exp. Med. **147**, 1584-1595.

Evans, P. M. & Jones, B. M. (1976). Manganese induced aggregation of human platelets in vitro. Cytobios. **13 (49)**, 37-44.

Evans, P. M. & Jones, B. M. (1982). Mn^{2+} stimulated adhesion and spreading of ehrlich ascites cells are separate processes. Cell Biol. Int. Rep. **6**, 681-685.

Farqar, M. G. & Palade, G. E. (1963). Junctional complexes in various epithelia. J. Cell Biol. **17**, 375-412.

Finbow, M. E., Eldridge, T., Buultijens, J., Kame, E., Schultleworth, J. & Pitts, J. (1985). Comparison of the protein components present in vertebrate and arthropod gap junction preparations. In Gap Junctions. M. V. L. Bennett and D. C. Spray (eds.). Cold Spring Harbor Lab, Cold Spring Harbor, New York, 77-89 .

Finne, J., Burger, M. M. & Prieels, J-P. (1982). Enzymatic basis for a lectin-resistant phenotype: increase in a fucosyl-transferase in mouse melanoma cells. J. Cell Biol. **92**, 277-282.

Foidart, J-M., Berman, J. J, Paglia, L., Rennard, S. R., Abe, S., Perantoni, A. & Martin, G. R. (1980). Synthesis of fibronectin, laminin, and several collagens by a liver-derived epithelial line. Lab. Invest. **42**, 525-532.

Folkman, J. & Moscona, A. (1978). Role of cell shape in growth control. Nature (Lond.) **273**, 345-349.

Forrester, J. V. & Lackie, J. M. (1984). Adhesion of neutrophil leukocytes under conditions of flow. J. Cell Sci. **70**, 93-110.

Fox, C. H., Cottler-Fox, M. H. & Yamada, K.M.(1981). Distribution of fibronectin in attachment sites of chick fibroblasts. Exp. Cell Res. **130**, 477-481.

Francis, G. W., Fisher, L. R., Gamble, R. A. & Gingell (1987). Direct measurement of cell detachment force on single cells using a new

electromechanical method. J. Cell Sci. **87**, 519-523.

Fraser, S. E., Murray, B. A., Cunningham, C-M. & Edelman, G. M. (1984). Alteration of the retinotectal map in Xenopus by antibodies to neural cell adhesion molecules. Proc. Natl. Acad. Sci. USA. **81**, 4222-4231.

Frazier, W. A. (1987). Thrombospondin: A modular adhesive glycoprotein of platelets and nucleated cells. J. Cell Biol. **105**, 625-632.

Freedman, V. H. & Shin, S. I.(1974). Cellular tumourigenicity in nude mice: correlation with cell growth in semi-solid medium. Cell **3**, 355-359.

Fritsch, P., Tappeiner, G. & Huspek, G. (1979). Keratinocyte substrate adhesion is manganese-dependent and calcium independent. Cell Biol. Int. Rep. **3**, 593-598.

Fujimura, K. & Phillips, D. R. (1983). Calcium cation regulation of glycoprotein IIb-IIIa complex formation in platelet plasma membrane. J. Biol. Chem. **258**, 10247-10252.

Furcht, L. T. (1983). Structure and function of the adhesive glycoprotein fibronectin. In Modern Cell Biology (B.H. Satir, e.d.). Alan R. Liss, Inc., New York. **Vol. 1**, 53-117.

Gallin, J. I. (1985). Leukocyte adherence related glycoproteins LFA-1, MOl, and P150, 95: A new group of monoclonal antibodies, a new disease, and a possible opportunity to understand the molecular basis of leukocyte adherence. J. Infect. Dis. **152**, 661-664.

Gallin, W. J., Edelman, G. M. & Cunningham, B. A. (1983). Characterisation of L-CAM, a major cell adhesion molecule from embryonic liver cells. Proc. Natl. Acad. Sci. USA. **80**, 1038-1042.

Gallin, W. J., Sorkin, B. C., Edelman, G. M. & Cunningham, B. A. (1987). Sequence analysis of a cDNA clone encoding the liver cell adhesion molecule, L-CAM. Proc. Natl. Acad. Sci. USA. **84**, 2808-2812.

Gardner, J. M. & Hynes, R. O. (1985). Interaction of fibronectin with its receptor on platelets. Cell **42**, 439-448.

Garner, J. A. & Culp, L. (1981). Aggregation competence of proteoglycans from the substratum adhesion sites of murine fibroblasts. Biochemistry **20**, 7350-7359.

Garvin, J. E. (1968). Effects of divalent cations on adhesiveness of rat polymorphonuclear neutrophils in vitro. J. Cell. Physiol. **72**, 197-212.

Gehlsen, K. R., Argraves, W. S., Pierschbacher, M. D. & Ruoslahti, E. (1988). Inhibition of in vitro tumour cell invasion by Arg-Gly-Asp-containing synthetic peptides. J. Cell Biol. **106**, 925-930.

Geiger, B. (1979). A 130 k protein from chicken gizzard: its localisation at the termini of microfilament bundles in cultured chicken cells. Cell **18**, 193-205.

Geiger, B., Tokuyasu, K. T., Dutton, A. H. & Singer, S. J. (1980). Vinculin, an intracellular protein localised at specialised sites where microfilament bundles terminate at the cell membranes. Proc. Natl. Acad. Sci. USA. **77**, 4127-4132.

Gennarini, G., Rougon, G., Deagostini-Bazin, H., Hirn, M. & Goridis, C. (1984). Studies on the transmembrane disposition of the neural cell adhesion molecule N-CAM. A monoclonal antibody recognises a cytoplasmic domain and evidence for the presence of phosphoserine residues. Eur. J. Biochem. **142**, 57-64.

Gerisch, G. (1960). Zellfunktionen und zellfunktionswechsel in der entwicklung von Dictyostelium discoideum: I, Arch. EntwMech. **151**, 632-654.

Gerisch, G. (1977). Univalent antibody fragments as tools for analysis of cell-cell interactions in Dictyostelium. Curr. Top. Dev. Biol. **14**, 243-270.

Giancotti, F. G., Comoglio, P. M. & Tarone, G. (1986). A 135000 molecular weight plasma membrane glycoprotein involved in fibronectin-mediated cell adhesion. Exp. Cell Res. **163**, 47-62.

Giancotti, F. G., languino, L. R., Zanetti, A., Peri, G., Tarone, G. & Dejana, E. (1987). Platelets express a membrane protein complex immunologically related to the fibroblast fibronectin receptor and distinct from GP IIb-IIIa. Blood **69**, 1535-1538.

Giancotti, F. G., Tarone, G., Knudsen, K., Damsky, C & Comoglio, P. M. (1985). Cleavage of a 135 kd cell surface glycoprotein correlates with the loss of fibroblast adhesion to fibronectin. Exp. Cell Res. **156**, 182-190.

Ginsberg, M. H., Loftus, J., Ryckwaert, J-J., Pierschbacher, M. D., Pytela, R., Ruoslahti, E. & Plow, E. F. (1987). Immunological and amino-terminal sequence comparison of two cytoadhesions indicates they contain similar or identical beta subunits and distinct alpha subunits. J. Biol. Chem. **262**, 5437-

5440.

Ginsberg, M. H., Pierschbacher, M. D., Ruoslahti, E., Margueria, G. & Plow, E. (1985). Inhibition of fibronectin binding to platelets by proteolytic fragments and synthetic peptides which support fibroblast adhesion. J. Biol. Chem. **260**, 3931-3936.

Goodenough, D. M. & Revel, J. P. (1970). A fine structural analysis of intercellular junctions in the mouse liver. J. Cell Biol. **45**, 272-290.

Goodman, S. L., Deutzmann, R. & Mark, K. V. D. (1987). Two distinct cell-binding domains in laminin can independently promote non-neuronal cell adhesion and spreading. J. Cell Biol. **105**, 589-598.

Goodman, S. L., Vollmers, H. P. & Birchmeier, W. (1985). Control of cell locomotion: perturbation with an antibody directed against specific glycoproteins. Cell **41**, 1029-1038.

Gordon, J. L. (1980). Mechanisms regulating platelet adhesion: In cell adhesion and motility, A. S. G. Curtis, & J. D Pitts (eds.), Cambridge University Press. Cambridge, London, New York, New Rochelle, Melbourne, Sydney.pp. 199-233.

Graf, J., Iwamoto, Y., Sasaki, M., Martin, G. R., Kleinman, H. K., Robey, F. A. & Yamada, Y. (1987). Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis and receptor binding. Cell **48**, 989-996.

Gratecos, D. Naidet, C., Astier, M., Thiery, J. P. & Semeriva, M. (1988). Drosophila fibronectin: a protein that shares properties similar to those of its mammalian homologue. EMBO J. **7 (1)**, 215-223.

Greve, J. M. & Gottlieb, D. I. (1982). Monoclonal antibodies which alter the morphology of cultured chick myogenic cells. J. Cellular Biochem. **18**, 221-230.

Griffiths, S. L., Perkins, R. M., Streuli, C. H. & Critchley, D. R. (1986). Variants of BALB/c3T3 cells lacking complex gangliosides retain a fibronectin matrix and spread normally on fibronectin-coated substrates. J. Cell Biol. **102**, 469-476.

Grinnell, F. (1978). Cellular adhesiveness and extracellular substrata. Int. Rev.

Cytol. **53**, 65-143.

Grinnell, F. (1983). Cell attachment and spreading factors. In: G. Guroff (ed.). Growth and maturation factors. John Wiley and Sons, New York. pp. 267-292.

Grinnell, F. (1984 a). Manganese-dependent cell-substratum adhesion. J. Cell Sci. **65**, 61-72.

Grinnell, F. (1984 b). Fibronectin and wound healing. J. Cellular Biochem. **26**, 107-116.

Grinnell, F.& Hayes, D. D. (1978 a). Cell adhesion and spreading factor. Similarity to cold insoluble globulin in serum. Exp. Cell Res. **115**, 221-229.

Grinnell, F. & Hayes, D. D. (1978 b). Induction of cell spreading by substratum adsorbed ligands directed against the cell surface. Exp. Cell Res. **116**, 275-284.

Grinnell, F., Feld, M. & Minter, D. (1980). Fibroblasts adhesion to fibrinogen and fibrin substrata: requirement for cold-insoluble globulin (plasma fibronectin). Cell **19**, 517-525.

Grinnell, F. (1986). Focal adhesion sites and the removal of substratum-bound fibronectin. J. Cell Biol. **103**, 2697-2706.

Grumet, M. & Edelman, G. M. (1984). Heterotypic binding between neuronal membrane vesicles and glial cells is mediated by a specific neuron-glia cell adhesion molecule. J. Cell Biol. **98**, 1746-1756.

Grumet, M. , Hoffman, S., Choung, C-M. & Edelman, G. M. (1984 a). Polypeptide components and binding functions of neuron-glia cell adhesion molecules. Proc. Natl. Acad. Sci. USA. **81**, 7989-7993.

Grumet, M., Hoffman, S. & Edelman, G. M. (1984 b). Two antigenetically related neuronal cell adhesion molecules of different specificity mediate neuron-neuron and neuron-glia adhesion. Proc. Natl. Acad. Sci. USA. **81**, 267-271.

Guelstein, V. I., Ivanova, O. Y., Margolis, L. B., Vasiliev, J. M. & Gelfand, I. M. (1973). Contact inhibition of movement in the cultures of transformed cells. Proc. Natl. Acad. Sci. USA. **70**, 2011-2014.

Gumbiner, B. & Simons, K. (1986). A functional assay for proteins involved in establishing of epithelial occluding barrier: Identification of an uvomorulin-like polypeptide. J. Cell Biol. **102**, 457-468.

Hagen, I., Nurden, A., Bjerrum, O. J., Solum, N. O. & Caen, J. (1980). Immunological evidence for protein abnormalities in platelets from patients Glanzmann' s thrombasthnid and Bernard Soulier syndrome. J. Clin. Invest. **65**, 722-731.

Halpern, B. B., Pejsachowicz, H. L., Febrre, H. L. & Barski, G. (1966). Differences in pattern of aggregation of malignant and non-malignant mammalian cells. Nature (Lond.) **204**, 157-159.

Harper, P. A. & Juliano, R. L. (1980). Isolation and characterisation of chinese hamster ovary cell variants defective in adhesion to fibronectin coated collagen. J. Cell Biol. **87**, 755-763.

Harris, A. K. (1973). Cell surface movements related to cell locomotion. In P. Porter & D. W. Fitzsimons (eds.). Locomotion of Tissue Cells. Ciba foundation symposium 14. Elsevier/ North-Holland publishing company, Amesterdam, pp. 3-26.

Harris, A. K. (1974). Contact inhibition of cell locomotion. In Cell Communication, R. P. Cox, (ed.). New York, John Wiley & Sons.pp, 147-185.

Hatta, K., Okada, T. S. & Takeichi, M. (1985). A monoclonal antibody disrupting calcium-dependent cell-cell adhesion of brain tissue: possible role of its target antigen in animal pattern formation. Proc. Natl. Acad. Sci. USA. **82**, 2789-2793.

Haverstick, D. M., Cowan, J. F., Yamada, K. M. & Santoro, S. A. (1985). Inhibition of platelet adhesion to fibronectin, fibrinogen, and von Willebrand factor substrates by a synthetic tetrapeptide derived from the cell binding domain of fibronectin. Blood **66**, 946-956.

Hayashi, M. & Yamada, K. M. (1981). Differences in domain structures between plasma and cellular fibronectins. J. Biol. Chem. **256**, 1292-1300.

Hayman, E. G., Engvall, E., A' Hearn, E., Barnes, D., Pierschbacher, M. D. & Ruoslahti, E. (1982). Cell attachment on replicas of SDS/polyacrylamide gels reveals two adhesive plasma proteins. J. Cell Biol. **95**, 20-23.

Hayman, E. G., Pierschbacher, M. D., Ohgren, Y. & Ruoslahti, E. (1983). Serum spreading factor (vitronectin) is present at the cell surface and in tissue. Proc.

Natl. Acad. Sci. USA. **80**, 4003-4007.

Hayman, E. G., Pierschbacher, M. D., Suzuki, S. & Ruoslahti, E. (1985 a). Vitronectin a major cell attachment promoting protein in foetal bovine serum. Exp. Cell Res. **160**, 245-258.

Hayman, E. G., Pierschbacher, M. D., & Ruoslahti, E. (1985 b). Detachment of cells from culture substrate by soluble fibronectin peptides. J. Cell Biol. **100**, 1948-1954.

Heath, J. P. (1982). Adhesion to substratum and locomotory behaviour of fibroblastic and epithelial cells in culture, in : Cell Behaviour (R. Bellairs, A. S. G. Curtis, & G. Dunn, eds.), Cambridge University Press, London. pp. 77-103.

Heath, J.P. & Dunn, G. A. (1978). Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflection and high voltage electron-microscope study. J.Cell Sci. **29**, 197-212.

Heaysman, J. E. M. & Pegrum, S. M. (1973 a). Early contacts between fibroblasts. An ultrastructural study. Exp. Cell Res. **78**, 71-78.

Heaysman, J. E. M. & Pegrum, S. M. (1973 b). Early contacts between normal fibroblasts and mouse Sarcoma cells-an ultrastructural study. Exp. Cell Res. **78**, 479-481.

Hemler, M. E. (1988). Adhesive protein receptors on hematopoietic cells. Immunology Today. **9 (4)**, 109-113.

Hemler, M. E., Huang, C. & Schwarz, L. (1987). The VLA protein family. J. Biol. Chem. **262**, 3300-3309.

Hemler, M. E., Jacobson, J. E. & Strominger, J. L. (1985). Biochemical characterisation of VLA-1 and VLA-2. J. Biol. Chem. **260**, 15246-15252.

Hemperly, J. J., Muarry, B. A., Edelman, G. M. & Cunningham, B. A. (1986). Sequence of a cDNA clone encoding the polysialic acid-rich and cytoplasmic domains of the neural cell adhesion molecule N-CAM. Proc. Natl. Acad. Sci. USA. **83**, 3037-3041.

Henschen, A. & McDonagh, J. (1986). Fibrinogen, fibrin and factor XIII in Blood Coagulation, R. F. A. Zwaal and H. Hemker, (eds.) Elsevier Amsterdam, New York, Oxford.pp, 171-241 .

Hertzberg, E. L. & Skibbens, R. V. (1984). A protein homologous to the 27000 dalton liver gap junctions protein is present in a wide variety of species and tissues. Cell **39**, 61-69.

Hewitt, A. T., Kleinman, H. K., Packer, J. P. & Martin, G. R. (1980). Identification of an adhesion factor for chondrocytes. Proc. Natl. Acad. Sci. USA. **77**, 385-388.

Hewitt, A. T., Varner, H. H., Silver, M. H., Dessau, W., Wilkes, C. M. & Martun, G. R. (1982 a). The isolation and partial characterisation of chondronectin, an attachment factor for chondrocytes. J. Biol. Chem. **247** (5), 2330-2339.

Hewitt, A. T., Varner, H. H., Silver, M. H. & Martin, G. R. (1982 b). In Limb Development and Regeneration.part B, R. O. Kelley, P.F. Goetinck & J.B. MacCabe (eds.), New York; Liss, pp, 25-33 .

Hickstein, D. D., Ozols, J., Williams, S. A., Baenziger, J. U., Locksley. R. M. & Roth, G. J. (1987). Isolation and characterisation of the receptor on human neutrophils that mediates cellular adherence. J. Biol. Chem. **262**, 5576-5580.

Hirn, M., Ghandour, M.ZS., Deagostini-Bazim, H. & Gordis, C. (1983). Molecular heterogeneity and structural evolution during cerebellar ontogeny detected by monoclonal antibody of the mouse cell surface antigen BSP-2. Brain Res. **265**, 87-100.

Hoffman, S. & Edelman, G. M. (1983). Kinetics of homophilic binding by E and A forms of the neural cell adhesion molecule. Proc. Natl. Acad. Sci. USA. **80**, 5762-5766.

Hoffman, S., Sorkin, B. C., White, P. C., Brackenbury, R., Mailhammer, R., Rutishauser, U., Cunningham, B. A. & Edelman, G. M. (1982). Chemical characterisation of a neural cell-adhesion molecule purified from embryonic brain membrane. J. Biol. Chem. **257**, 7720-7729.

Holmes, R. (1967). Preparation from serum of an alpha-one protein which induce the intermediate growth of unadapted cells in vitro. J. Cell Biol. **32**, 297-308.

Hook, M., Rubin, K., Oldberg, A., Obrink, B. & Vaheri, A. (1977). Cold-insoluble globulin mediates the adhesion of rat liver cells to plastic petri dishes. Biochem. Biophys. Res. Commun. **79**, 726-733.

Hoover, R., Folger, R., Haering, W., Ware, B. & Karnorsky, M. (1980). Adhesion

of leukocytes to endothelium: role of divalent cations, surface charge, chemotactic agents and substrate. J. Cell Sci. **45**, 73-86.

Horwitz, A. F., Duggan, K., Buck, C., Beckerle, M. C. & Burridge, K. (1986). Interaction of plasma membrane fibronectin receptor with talin a transmembrane linkage. Nature (Lond.) **320**, 531-533.

Horwitz, A., Duggan, K., Greggs, R., Decker, C. & Buck, C. (1985). The CSAT antigen has properties of a receptor for laminin and fibronectin. J. Cell Biol. **101**, 2134-2144.

Hosein, B. & Bianco, C. (1985). Monocyte receptors for fibronectin characterised by a monoclonal antibody that interferes with receptor activity. J. Exp. Med. **162**, 157-170.

Hughes, R. C., Butters, T. D. & Aplin, J. D. (1981). Cell surface molecules involved in fibronectin mediated adhesion. A study using specific antisera. J. Cell Biol. **26**, 198-207.

Hughes, R. C. & Mills, G. (1986). Functional differences in the interactions of glycosylation-deficient cell lines with fibronectin, laminin, and type IV collagen. J. Cell. Physiol. **128**, 402-412.

Hughes, R. C., pena, S. D., Clark, J. & Dourmashkin, R. R. (1979). Molecular requirements for the adhesion and spreading of hamster fibroblasts. Exp. Cell Res. **121**, 307-314.

Hughes, R. C., Pena, S. D. & Vischer, P. (1980). In Cell Adhesion and Motility, A. S. G. Curtis & J. D. Pitts (eds.) London: Cambridge University Press. pp, 329-356.

Humphries, M. J., Olden, K. & Yamada, K.M. (1986 a). A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. Science (Wash.DC) **233**, 467-470.

Humphries, M. J., Akiyama, S. K., Komoriya, A., Olden, K. & Yamada, K. M. (1986 b). Identification of an alternatively spliced site in human plasma fibronectin that mediates cell type-specific adhesion. J. Cell Biol. **103**, 2637-2647.

Huxley, H. E. (1973). Muscular contraction and cell motility. Nature (Lond.) **243**,

445-449.

Hyafil, F., Babinet, C. & Jacob, F. (1981). Cell-cell interaction in early embryogenesis: A molecular approach to the role of calcium. Cell **26**, 447-454.

Hyafil, F., Morello, D., Babinet, C.& Jacob, F. (1980). A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. Cell **21**, 927-934.

Hynes, R.O. (1976). Cell surface proteins and malignant transformation. Biochem. Biophys. Acta **458**, 73-107.

Hynes, R. O. (1981 a). Fibronectin and its relation to cellular structure and behaviour. in: Cell Biology of Extracellular Matrix (E. D. Hay,ed.), Plenum Press, New York, pp. 295-334.

Hynes, R. O. (1981 b). Relationships between fibronectin and the cytoskeleton, in: Cytoskeletal Elements and Plasma Membrane Organisation, G. Poste & G. L. Nicolson, (eds.), Elsevier/North-Holland, Amesterdam, pp, 100-137.

Hynes, R. O. (1985). Molecular biology of fibronectin. Ann. Rev. Cell Biol. **1**, 67-90.

Hynes, R. O. (1986). Fibronectin. Sci. Am. **254**, 32-41.

Hynes, R. O. & Destree, A. T. (1978). Relationship between fibronectin (LETS protein) and actin. Cell **15**, 875-886.

Hynes, R.O. & Yamada, K.M. (1982). Fibronectins: Multifunctional modular glycoproteins. J. Cell Biol. **95**, 369-377.

Imhof, B. A., Vollmers, H. P., Goodman, S. L. & Birchmeier, W. (1983). Cell-cell interaction and polarity of epithelial cells: Specific perturbation using a monoclonal antibody. Cell **35**, 667-675.

Ingram, V. M. (1969). A side view of moving fibroblasts. Nature (Lond.) **223**, 641-644.

Isenberg, W. M., McEver, R. P., Phillips, D. R., Shuman, M. A. & Bainton, D. F. (1987). The platelet fibrinogen receptors: an immunogold-surface replica study of agonist-induced ligand binding and receptor clustering. J. Cell Biol. **104**, 1655-1663.

Iwamoto, Y., Graf, J., Sasaki, M., Kleinman, H. k., Greatorex, D. R., Martin, G. R., Robey, F. A. & Yamada, Y. (1988). Synthetic pentapeptide from the B1

chain of laminin promotes B16F10 melanoma cell migration. J. Cell. Physiol. **134**, 287-291.

Izzard, C.S. & Lochner, L.R. (1976). Cell to substrate contacts in living fibroblasts: an interference reflection study with an evaluation of the technique. J. Cell Sci. **21**, 129-160.

Izzard, C. S. & Lochner, L.R. (1980). Formation of cell-to-substrate contacts during fibroblast motility: an interference-reflection study. J. Cell Sci. **42**, 81-116.

Izzard, C. S., Radinsky, R. & Culp, A. (1986). Substratum contacts and cytoskeletal reorganisation of BALB/3T3 cells on a cell-binding fragments and heparin-binding fragments of plasma fibronectin. Exp. Cell Res. **165**, 320-336.

Jockusch, B. M. & Isenberg, G. (1981). Interaction of alpha-actinin and vinculin with actin: opposite effect on filament network formation. Proc. Natl. Acad. Sci. USA. **78**, 3005-3009.

Johansson, S., Gustafson, S. & Pertoft, H. (1987). Identification of a fibronectin receptor specific for rat liver endothelial cells. Exp. Cell Res. **172**, 425-431.

Johansson, S., Kjellen, L., Hook, M. & Timpl, R. (1981). Substrate adhesion of rat hepatocytes: A comparison of laminin and fibronectin as attachment proteins. J. Cell Biol. **90**, 260-264.

Johnson, K. E. (1986). Transplantation studies to investigate mesoderm-ectoderm adhesive cell interactions during gastrulation. J. Cell Sci. **82**, 99-117.

Jones, J. C. R. (1988). Characterisation of a 125 k glycoprotein associated with bovine epithelial desmosomes. J. Cell Sci. **89**, 207-216.

Jorgensen, O. S., delouvee, A., Thiery, J-P. & Edelman, G. M. (1980). The nervous system specific protein D2 is involved in adhesion among neurites from cultured rat ganglia. FEBS. Lett. **111**, 39-42.

Kadish, J. L., Butterfield, C. E. & Folkman, J. (1979). The effect of fibrin on cultured vascular endothelial cells. Tissue & Cell **11**, 99-108.

Keller, H.U., Barandun, S. Kistler, P. & Ploem, J. S. (1979). Locomotion and adhesion of neutrophil granulocytes. Effects of albumin, fibrinogen and gamma globulins studied by reflection contrast microscopy. Exp. Cell Res. **122**, 352-362.

Kemler, R., Babinet, C., Eisen, H. & Jacob, F. (1977). Surface antigen in early differentiation. Proc. Natl. Acad. Sci. USA. **74**, 4449-4452.

Kensler, R. W. & Goodenough, D. A. (1980). Isolation of mouse myocardial gap junctions. J. Cell Biol. **86**, 755-764.

Kishimoto, T. K., O' Connor, K., Lee, A., Roberts, T. M. & Springer, T. A. (1987). Cloning of the beta subunit of the leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family. Cell **48**, 681-690.

Klebe, R. J. (1974). Isolation of a collagen dependent cell attachment factor. Nature (Lond.) **250**, 248-251.

Klebe, R. J., Rosenberger, P. G., Naylor, S. L., Burns, R. L., Novak, R. & Kleiman, H. (1977 a). Cell attachment to collagen: isolation of a cell attachment mutant. Exp. Cell Res. **104**, 119-125.

Klebe, R. J., Hall, J. R, Rosenberger, P. & Dickey, W. D. (1977 b). Cell attachment to collagen: the ionic requirements. Exp. Cell Res. **110**, 419-425.

Kleinman, H. K., Klebe, R. J. & Martin, G. R. (1981). Role of collagenous matrices in the adhesion and growth of cells. J. Cell Biol. **88**, 473-485.

Kleinman, H. K., Martin, G. R. & Fishman, P. H. (1979). Ganglioside inhibition of fibronectin-mediated cell adhesion to collagen. Proc. Natl. Acad. Sci. USA. **76**, 3367-3371.

Kleinman, H. K., McGarvey, M. L., Hassell, J. R. & Martin, G. R. (1983). Formation of a supramolecular complex is involved in the reconstitution of basement membrane components. Biochemistry **22**, 4969-4974.

Knox, P. (1984). Kinetics of cell spreading in the presence of different concentrations of serum or fibronectin-depleted serum. J. Cell Sci. **71**, 51-59.

Knox, P. & Griffiths, S. (1982). The abnormal morphology of Polyoma-transformed baby hamster kidney cells is due to a failure to respond to 70 k spreading factor. J. Cell Sci. **55**, 301-306.

Knox, P. & Wells, P. (1979). Cell adhesion and proteoglycans. I. The effect of exogenous proteoglycans on the attachment of chick embryo fibroblasts to tissue culture plastic and collagen. J. Cell Sci. **40**, 77-88.

Knudsen, K. A., Horwitz, A. F. & Buck, C. A. (1985). A monoclonal antibody

identifies a glycoprotein complex involved in cell-substratum adhesion. Exp. Cell Res. **157**, 218-226.

Kolega, J. (1981). The movement of cell clusters in vitro: morphology and directionality. J. Cell Sci. **49**, 15-32.

Kolega, J., Shure, M.S., Chen, W-T. & Young, N. D. (1982). Rapid cellular translocation is related to close contacts formed between various cultured cells and their substrata. J. Cell Sci. **54**, 23-34.

Kornblihtt, A. R., Umezzawa, K., Vibe-Pederson, K. & Baralle, E. (1985). Primary structure of human fibronectin: Differential splicing may generate at least 10 polypeptides from a single gene. EMBO J. **4**, 1755-1759.

Krotoski, D. M., Domingo, C. & Bronner-Fraser, M. (1986). Distribution of putative cell surface receptor for fibronectin and laminin in the avian embryo. J. Cell Biol. **103**, 1061-1071.

Kurkinen, M., taylor, A., Garrels, J. I. & Hogan, B. L. M. (1984). Cell surface-associated proteins which bind native type IV collagen or gelatin. J. Biol. Chem. **259** (9), 5915-5922.

Lam, S. C. T., Plow, E. F., Smith, M. A., Andrieux, A., Ryckwaert, J-J., Marguerie, G. & Ginsberg, M. H. (1987). Evidence that Arginyl-Glycyl-Asparatate peptides and fibrinogen Gamma chain peptides share a common binding site on platelets. J. Biol. Chem. **262**, 947-950.

Lark, M. W. & Culp, L. A. (1984). Multiple classes of heparan sulfate proteoglycans from fibroblast substratum adhesion sites. J. Biol. Chem. **259**, 6773-6782.

Lark, M. W., Lattera, J. & Culp, L. A. (1985). Close and focal contact adhesion of fibroblasts to a fibronectin-containing matrix. Fed. Proc. **44**, 394-403.

Laug, W. E., Tokes, Z. A., Bendict, W.F. & Sorgente. (1980). Anchorage independent growth and plasminogen activator production by bovine endothelial cells. J. Cell Biol. **84**, 281-293.

Lawler, J. (1986). Review: The structural and functional properties of thrombospondin. Blood **67**, 1197-1209.

Lawler, J. & Hynes, R. O. (1986). The structure of human thrombospondin, an adhesive glycoprotein with multiple calcium-binding sites and homologues with

several different proteins. J. Cell Biol. **103**, 1635-1648.

Lee, S., Gilula, N. B., & Warner, A. E. (1987). Gap junctional communication and compaction during preimplantation stages of mouse development. Cell **51**, 851-860.

Lehto, V-P., Vartio, T. & Virtanen, I. (1981). Persistence of a 140,000 Mr surface glycoprotein in cell-free matrices of cultured human fibroblasts. FEBS. Lett. **124**, 289-292.

Lehto, V-P., Vartio, T., Bradley, R. A. & Virtanen, I. (1983). Characterisation of a detergent-resistant surface lamina in cultured human fibroblasts. Exp. Cell Res. **143**, 287-295.

Leptin, M. (1986). The fibronectin receptor family. Nature (Lond.) **321**, 728.

Leptin, M., Aebersold, R. & Wilcox. M. (1987). Drosophila position-specific antigens resemble the vertebrate fibronectin-receptor family. EMBO J. **6**, 1037-1043.

Lesot, H., Kuhl, U. & Mark, K. V. D. (1983). Isolation of a laminin-binding protein from muscle cell membranes. EMBO J. **2 (6)**, 861-865.

Liao, N-S., John, J. S., DU, Z. J. & Cheung, H. T. (1987). Adhesion of lymphoid cell lines to fibronectin-coated substratum: Biochemical and physiological characterisation and the identification of a 140-KDa fibronectin receptor. Exp. Cell Res. **171**, 306-320.

Liotta, L. A., Hand, P. H., Rao, C. N., Bryant, G., Barsky, S. h. & Schlom, J. (1985). Monoclonal antibodies to the human laminin receptor recognise structurally distinct sites. Exp. Cell Res. **156**, 117-126.

Liotta, L.A., Rao, C. N. & Wewer, U. M. (1986). Biochemical interactions of tumour cells with basement membrane. Ann. Rev. Biochem. **55**, 1037-1057.

Lochner, L. & Izzard, C.S. (1973). Dynamic aspects of cell-substrate contact in fibroblast motility. J. Cell Biol. **59**, 199 a.

Loewenstein, W. R. (1987). The cell-to-cell channel of gap junctions. Cell **48**, 725-726.

Lowry, O. H., Rosebrough, N. J., Farr, A. L & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265-275.

Ludford, R. J. (1932). Differences in the growth of transplantable tumours in plasma

and serum culture media. Proc. R. Soc. Lond. B. Biol. Sci. **112**, 250-263.

Macpherson, I. A. & Montagnier, L. (1964). Agar suspension culture for the selective assay of cells transformed by polyoma virus. Virology **23**, 291-294.

Macpherson, I. A. (1965). Reversion in hamster cells transformed by Rous sarcoma virus. Science, N. Y. **148**, 1731-1733.

Macpherson, I. A. (1966). Malignant transformation and reversion in virus transformed cells. Recent Results in Cancer Research. 6. Berlin: Springer-Verlag.

Magnusson, G. (1985). Recent progress in studies of polyoma virus tumour antigens. Exp. Cell Res. **157**, 1-7.

Malinoff, H. & Wicha, M. (1983). Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. J. Cell Biol. **96**, 1475-1479.

Manthorpe, M., Engvall, E., Ruoslahti, E., Longo, F., Davis, G. & Varon, S. J. (1983). Laminin promotes neuritic regeneration from cultured peripheral and central neurons. J. Cell Biol. **97**, 1882-1890.

Marlin, S. D.& Springer, T. A. (1987). Purified intercellular adhesion molecule-1 (I-CAM-1) is a ligand for lymphocyte function associated antigen (LFA-1). Cell **51**, 813-819.

Maroudas, N. G. (1975). Adhesion and spreading of cells on charged surfaces. J. Theor. Biol. **49**, 417-424.

Martin, G. R., Timpl, R., Muller, P. K. & Kuhn, K. (1985). The genetically distinct collagens. TIBS **10**, 285-287.

Matyas, G. R., Evers, D. C., Radinsky, R. & Morre, J. (1986). Fibronectin binding to gangliosides and rat liver plasma membranes. Exp. Cell Res. **162**, 296-318.

Mauch, C., Aumailley, M., Paye, M., Lapiere, C. M., Timpl, R. & Krieg, T. (1986). Defective attachment of dermatosparactic fibroblasts to collagens I and II. Exp. Cell Res. **163**, 294-300.

Mauch, C., Mark, K. V. D., Helle, O., Mollenhauer, J., Pfaffle, M. & Krieg, T. (1988). A defective cell surface collagen-binding protein in dermatosparactic sheep fibroblasts. J. Cell Biol. **106**, 205-211.

Mazia, D., Schatten, G. & Sale, W. (1975). Adhesion of cells to surfaces coated

with polylysine. Applications to electron microscopy. J. Cell Biol. **66**, 198-200.

McABee, D. D. & Grinnell, F. (1983). Fibronectin-mediated binding and phagocytosis of polystyrene latex beads by baby hamster kidney cells. J. Cell Biol. **97**, 1515-1523.

McCarthy, J. B. & Furcht, L. T. (1984). Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells in vitro. J. Cell Biol. **98**, 1474-1480.

McCarthy, J. B., Palm, S. L. & Furcht, L. T. (1983). Migration of haptotaxis of a schwann cell tumour line to the basement membrane glycoprotein laminin. J. Cell Biol. **97**, 772-777.

Mckeehan, W. L. & Ham, R. G. (1976). Stimulation of clonal growth of normal fibroblasts with substrate coated with basic polymers. J. Cell Biol. **71**, 727-734.

McKeown-Longo, P. J., Hanning, R. & Mosher, D. F. (1984). Binding and degradation of platelet thrombospondin by cultured fibroblast. J. Cell Biol. **98**, 22-28.

Meager, A., Ungkitchanukit, A. & Hughes, R. C. (1976). Variants of hamster fibroblasts resistants to Ricinus communis toxin (Ricin). Biochem. J. **154**, 113-124.

Meager, A., Ungkitchanukit, A., Nairn, R. & Hughes, R. C. (1975). Ricin resistance in baby hamster kidney cells. Nature (Lond.) **257**, 137-139.

Middleton, C. A. (1982). Cell contacts and the locomotion of epithelial cells. In cellular behaviour, R. Bellairs, A. S. G. Curtis & G. Dunn, (eds.). 159-182. Cambridge: CUP.

Miller, K., Mattey, D., Measures, H., Hopkins, C. & Garrod, D. (1987). Localisation of the protein and glycoprotein components of bovine nasal epithelial desmosomes by immunoelectron microscopy. EMBO J. **6**, 885-889.

Mollenhauer, J., Bee, J. A., Lizarbe, A. & Mark, K. V. D. (1984). Role of anchorin CII, a 31,000-mol-wt membrane protein, in the interaction of chondrocytes with type II collagen. J. Cell Biol. **98**, 1572-1578.

Moscona, A. A.(1961). Rotation mediated histogenetic aggregation of dissociated

cells. Exp. Cell Res. **22**, 455-475.

Moscona, A. A. (1980). Embryonic cell recognition: cellular and molecular aspects. Prog. Clin. Biol. Res. **42**, 171-188.

Mosher, D. F. (1975). Cross-linking of cold-insoluble globulin by fibrin stabilising factor. J. Biol. Chem. **250**, 6614-6621.

Mosher, D. F. (1976). Action of fibrin-stabilising factor on cold-insoluble globulin and alpha 2-macroglobulin in clotting plasma. J. Biol. Chem. **251**, 1639-1645.

Mugnai, G. & Culp, L. A. (1987). Cooperativity of ganglioside-dependent with protein dependent substratum adhesion and neurite extension of human neuroblastoma cells. Exp. Cell Res. **169**, 328-344.

Mugnai, G., Lewandowska, K., Choi, H. U., Rosenberg, L. C.& Culp, L. A. (1988). Ganglioside-dependent adhesion events of human neuroblastoma cells regulated by the RGDS-dependent fibronectin receptor and proteoglycans. Exp. Cell Res. **175**, 229-247.

Mumby, S. M., Raugi, G. J. & Bornstein, P. (1984). Interactions of thrombospondin with extracellular matrix proteins: selective binding to type V collagen. J. Cell Biol. **98**, 646-652.

Murphy-Ullrich, J. E. & Mosher, D. F. (1987). Interactions of thrombospondin with endothelial cells: Receptor-mediated binding and degradation. J. Cell Biol. **105**, 1603-1611.

Murray, B. A., Hemperly, J. J., Prediger, E. A., Edelman, G. M. & Cunningham, B.A. (1986 a). Alternatively spliced mRNA code for different polypeptides chains of the chick neural cell adhesion molecule (N-CAM). J. Cell Biol. **102**, 189-193.

Murray, B. A., Owens, G. S., Prediger, E. A., Crossin, K. L., Cunningham, B. A. & Edelman, G. M. (1986 b). Cell surface modulation of the neural cell adhesion molecule resulting from alternative mRNA splicing in a tissue-specific developmental sequence. J. Cell Biol. **103**, 1431-1439.

Nagata, K. Humphries, M. J., Olden, K. & Yamada, K. M. (1985). Collagen can modulate cell interactions with fibronectin. J. Cell Biol. **100**, 386-394.

Naidet, C., Semeriva, M., Yamada, K. M.& Thiery, J-P. (1987). Peptides

containing the cell-attachment recognition signal Arg-Gly-Asp prevent gastrulation in Drosophila embryos. Nature (Lond.) **325**, 348-350.

Neff, N. T., Lowrey, C., Decker, C., Tovar, A., Damsky, C., Buck, C. & Horwitz, A. F. (1982). A monoclonal antibody detaches embryonic skeletal muscle from extracellular matrices. J. Cell Biol. **95**, 654-666.

Neufeld, E. J. & Pastan, I. (1978). A mutant fibroblast cell line defective in glycoprotein synthesis due to a deficiency of glycosamine phosphate acetyltransferase. Arch. Biochem. Biophys. **188**, 323-327.

Newgreen, D. F., Gibbins, I. L., Sauter, J. Wallenfels, B. & Wutz, R. (1982). Ultrastructural and tissue-culture studies on the role of fibronectin, collagen and glycosaminoglycans in the migration of neural crest cells in the fowl embryo. Cell Tissue Res. **221**, 521-549.

Norton, E. K. & Izzard, C. (1982). Fibronectin promotes formation of the close cell-to-substrate contact in cultured cells. Exp. Cell Res. **142**, 463-467.

Okada, Y., Mugnai, G., Bremer, E. G. & Hakomori, S. (1984). Glycosphingolipids in detergent-insoluble substrate attachment matrix (DISAM) prepared from substrata attachment material (SAM): their possible role in regulating cell adhesion. Exp. Cell Res. **155**, 448-456.

Okley, B. R., Kirsch, D. R. & Morris, N. R. (1980). A simplified ultrasensitive silver stain for detaching proteins in polyacrylamide gels. Ann. Biochem. **105**, 361-363.

Olsnes, S., Refsnes, K. & Pihl, A. (1974). Mechanisms of action of the toxic lectins abrin and ricin. Nature (Lond.) **249**, 627.

O'Neill, C., Jordan, P. & Ireland, G. (1986). Evidence for two distinct mechanisms of anchorage stimulation in freshly explanted and 3T3 swiss mouse fibroblast. Cell **44**, 489-496.

O'Neill, C.H., Riddle, P. N. & Jordan, P.W. (1979). The relation between surface area and anchorage dependence of growth in hamster and mouse fibroblasts. Cell **16**, 909-918.

Oppenheimer-Marks, N. , Border, B. & Grinnell, F. (1984). BHK cell variant with defective fibronectin receptor function. Cell Biol. Int. Rep. **8**, 171-178.

Oppenheimer-Marks, N. & Grinnell, F. (1982). Inhibition of fibronectin receptor

function by antibodies against baby hamster kidney cell wheat germ agglutinin receptors. J. Cell Biol. **95**, 876-884.

Oppenheimer-Marks, N. & Grinnell, F. (1984). Calcium ions protect cell-substratum adhesion receptors against proteolysis. Exp. Cell Res. **152**, 467-475.

Otsuka, H. & Moskowitz, M. (1975). Arrest of 3T3 cells in G1 phase in suspension culture. J. Cell Physiol. **87**, 213-220.

Overton, J. (1975). Experiments with junctions of the adhaerens type. Curr. Top. Develop. Biol. **10**, 1-34.

Owens, M. R. & Cimino, C. D. (1982). Synthesis of fibronectin by the isolated perfused rat liver. Blood **59**, 1305-1309.

Page, E. & Manjunath, C. K. (1985). Biochemistry and structure of cardiac gap junctions: Recent observations. In Gap Junctions. M. L. V. Bennett, and D. C. Spray, (eds.). Cold Spring Harbor Lab, Cold Spring Harbor, New York, 49-56.

Painter, R. G., Ginsberg, M. & Jaques, B. (1982). Concanavalin A induces interactions between surface glycoproteins and the platelet cytoskeleton. J. Cell Biol. **92**, 565-573.

Palm, S. L. & Furcht, L. T. (1982). Production of laminin and fibronectin by schwannoma cells: cell-protein interactions in vitro and protein localisation in peripheral nerve in vivo. J. Cell Biol. **96**, 1218-1226.

Parise, L. V. & Phillips, D. R. (1985). Reconstitution of the purified platelet fibrinogen receptor. J. Biol. Chem. **260**, 10698-10707.

Parise, L. V. & Phillips, D. R. (1986). Fibronectin-binding properties of the purified platelet glycoprotein IIb-IIIa complex. J. Biol. Chem. **261 (30)**, 14011-14017.

Parise, L. V., Helgerson, S. M., Steiner, B., Mannizzi, L. & Phillips, D. R. (1987). Synthetic peptides derived from fibrinogen and fibronectin change the conformation of purified platelet glycoprotein IIb-IIIa. J. Biol. Chem. **262**, 12597-12602.

Patel, V. P., Ciechanover, A., Orah, P. & Lodish, H. F. (1985). Mammalian reticulocytes lose adhesion to fibronectin during maturation to erythrocytes. Proc. Natl. Acad. Sci. USA. **82**, 440-444.

Patel, V. P. & Lodish, H. F. (1986). The fibronectin receptor on mammalian

erythroid precursor cells: Characterisation and developmental regulation. J. Cell Biol. **102**, 440-456.

Patel, R. S., Odermatt, E., Schwarzbaure, J. E. & Hynes, R. O. (1987). Organisation of the fibronectin gene provides evidence for exon shuffling during evolution. EMBO J. **6**, 2565-2572.

Paye, M. & Lapiere, C. M. (1986). The lack of attachment of transformed embryonic lung epithelial cells to collagen I is corrected by fibronectin and FXIII. J. Cell Sci. **86**, 95-107.

Pena, S. D. J. & Hughes, R. C. (1978). Fibronectin plasma membrane interactions in the adhesion and spreading of hamster fibroblast. Nature (Lond.) **176**, 80-83.

Penn, E. J., Hobsen, C., Rees, D. A. & Magee, A. I. (1987). Structure and assembly of desmosome junction: biosynthesis, processing, and transport of the major protein and glycoprotein components in cultured epithelial cells. J. Cell Biol. **105**, 57-68.

Perkins, R. M., Ji, T. H. & Hynes, R. O. (1979). Cross-linking of fibronectin to sulfated proteoglycans at the cell surface. Cell **16**, 941-952.

Perkins, R. M., kellie, S., Patel, B. & Critchley, D. R. (1982). Gangliosides as receptors for fibronectin. Exp. Cell Res. **141**, 231-243.

Peyrieras, N., Hyafil, F., Louvard, D., Ploech, H. L. & Jacob, F. (1983). Uvomorulin: A non-integral membrane protein of early mouse embryo. Proc. Natl. Acad. Sci. USA. **80**, 6274-6277.

Phillips, H. M. & Steinberg, M. S. (1969). Equilibrium measurements of embryonic chick cell adhesiveness. I. Shape equilibrium in centrifugal fields. Proc. Natl. Acad. Sci. USA. **64**, 121-127.

Phillips, H. M., Charo, I. F., Parise, L. V. & Fitzgerald, L. A. (1988). The platelet membrane glycoprotein IIb-IIIa complex. Blood **71(4)**, 831-843.

Pierschbacher, M. D., Hayman, E. G. & Ruoslahti, E. (1981). Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell **26**, 259-267.

Pierschbacher, M. D., Hayman, E. G. & Ruoslahti, E. (1982). Synthetic peptide

with cell attachment activity of fibronectin. Proc. Natl. Acad. Sci. USA. **80**, 1224-1227.

Pierschbacher, M. D., Hayman, E. G. & Ruoslahti, E. (1985). The cell attachment determinant in fibronectin. J. Cell Biochem. **28**, 115-126.

Pierschbacher, M.D. & Ruoslahti, E. (1984 a). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature (Lond.) **309**, 30-33.

Pierschbacher, M.D. & Ruoslahti, E. (1984 b). Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Proc. Natl. Acad. Sci. **81**, 5985-5988.

Pierschbacher, M.D. & Ruoslahti, E. (1987). Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. J. Biol. Chem. **262 (36)**, 17294-17298.

Plow, E. F., McEver, R. P., Coller, B. S., Woods, V. L. & Marguerie, G. A. (1985 a). Related binding mechanisms for fibrinogen, fibronectin, von Willebrand factor, and thrombospondin on thrombin-stimulated human platelets. Blood **66**, 724-727.

Plow, E. F., pierschbacher, M. D., Ruoslahti, E., Marguerie, G. A. & Ginsberg, M. H. (1985 b). The effect of Arg-Gly-Asp-containing peptides on fibrinogen and von Willebrand factor binding to platelets. Proc. Natl. Acad. Sci. USA. **82**, 8057-8061.

Plow, E. F., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G. & Ginsberg, M. H. (1987). Arginyl-Glycyl-Aspartic acid sequences and fibrinogen binding to platelets. Blood **70**, 110-115.

Pouyssegur, J. M. & Pastan, I. (1976). Mutants of BALB/c 3T3 fibroblasts defective in adhesiveness to substratum: evidence for alteration in cell surface proteins. Proc. Natl. Acad. Sci. USA. **73**, 544-548.

Pouyssegur, J. M & Pastan, I. (1977). Mutant of mouse fibroblasts altered in the synthesis of cell surface glycoproteins. J. Biol. Chem. **252**, 1639-1646.

Pytela, R. (1988). Amino acid sequence of the murine Mac-1 alpha chain reveals homology with the integrin family and an additional domain related to von Willebrand factor. EMBO J. **7 (5)**, 1371-1378.

Pytela, R., pierschbacher, M. D. & Ruoslahti, E. (1985 a). Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptors. Cell **40**, 191-198.

Pytela, R., Pierschbacher, M. D. & Ruoslahti, E. (1985 b). A 125/115-KDa cell surface receptor specific for vitronectin interacts with the arginin-glycine-aspartic acid adhesion sequence derived from fibronectin. Proc. Natl. Acad. Sci. USA. **82**, 5766-5770.

Pytela, R., Pierschbacher, M. D., Argraves, S., Suzuki, S. & Ruoslahti, E. (1987). Arg-Gly-Asp adhesion receptors. Methods Enzymol. **144**, 475-489.

Pytela, R., Pierschbacher, M. D., Ginsberg, M. H., Plow, E. F. & Ruoslahti, E. (1986). Platelet membrane glycoprotein IIb-IIIa: Membrane of family of Arg-Gly-Asp-specific adhesion receptors. Scince (Wash. DC) **231**, 1559-1562.

Rabinovitch, M. & DeStefano, M. J. (1973). Manganese stimulates adhesion and spreading of Sarcoma ascites cells. J. Cell Biol. **59**, 165-176.

Radice, G. P. (1978). Analysis of the migration of epithelial cells in vivo and in vitro. J. Cell Biol. **79**, 270 a (abstract).

Radice, G. P. (1980). Locomotion and cell substratum, contacts of Xenopus epidermal cells in vitro and in situ. J. Cell Sci. **44**, 201-223.

Rao, C. N., Margulies, I. M. K., Tralka, T. S., Terranova, V. P. & Liotta, J. A. (1982). Isolation of a subunit of laminin and its role in molecular structure and tumour cell attachment. J. Biol. Chem. **257**, 9740-9744.

Rauvala, H., Carter, W. G. & Hakomori, S-I. (1981). Studies on cell adhesion and recognition I. Extent and specificity of cell adhesion triggered by carbohydrate-reactive proteins (Glycosidase and lectins) and by fibronectin. J. Cell Biol. **88**, 127-137.

Rauvala, H. & Hakomori, S. I. (1981). Studies on cell adhesion and recognition. III. The occurrence of alpha-mannosidase at the fibroblast cell surface, and its possible role in cell recognition. J. Cell Biol. **88**, 149-159.

Reading, C. L., Belloni, P. N. & Nicolson, G. L. (1980). Selection and in vivo properties of lectin attachment variants of malignant murine lymphosarcoma cell lines. J. Natl. Cancer Inst. **64**, 1241-1249.

Rees, D. A., Badley, R. A., Lloyd, C. W., Thom, D. & Smith, C. G. (1978). Glycoproteins in the recognition of substratum by cultured fibroblasts. In Cell-Cell Recognition, S. E. B. Symp. 32, A. S. G. Curtis (ed.), Cambridge University Press, pp, 241.

Ringwald, M., Schuh, R., Vestweber, D., Eistetter, H., Lottspeich, F., Engel, J., Dolz, R., Jahnig, F., Epplen, J., Mayer, S., Muller, C. & Kemler, R. (1987). The structure of cell adhesion molecule uvomorulin. Insights into the molecular mechanism of Ca^{2+}- dependent cell adhesion. EMBO J. **12**, 3647-3653.

Roberts, D. D., Haverstick, D. M., Dixit, V. M., Frazier, W. A., Santoro, S. A. & Ginsberg, V. (1985). The platelet glycoprotein thrombospondin binds specifically to sulfatides. J. Biol. Chem. **260**, 9405-9411.

Roberts, D. D., Sherwood, J. A. & Ginsberg, V. (1987). Platelet thrombospondin mediates attachment and spreading of human melanoma cells. J. Cell Biol. **104**, 131-139.

Rohrschneider, L. R. (1980). Adhesion plaques of Rous sarcoma virus-transformed cells contain the src gene product. Proc. Natl. Acad. Sci. USA. **77**, 3514-3518.

Rohrschneider, L. R., Eisenman, R. N. & Leitch. (1979). Identification of a Rous sarcoma virus transformation-related protein in normal avian and mammalian cells. Proc. Natl. Acad. Sci. USA. **76**, 4479-4483.

Rohrschneider, L. R., Rosok, M. & Shriver. K. (1982). Mechanism of transformation by Rous Sarcoma virus. Events within adhesion plaques. Cold Spring Harbor Symp. Quant. Biol. **46**, 953-965.

Roth, S. and Weston, J. A. (1967). The measurement of intracellular adhesion. Proc. Natl. Acad. Sci. USA. **58**, 974-980.

Rovasio, R. A., DeLouvee, A., Yamada, K. M.,Timpl, R. & Thiery, J.P. (1983). Neural crest cell migration: Requirement for exogenous fibronectin and high cell density. J. Cell Biol. **96**, 462-473.

Rubin, K., Hook, M., Obrink, B. & Timpl, R. (1981). Substrate adhesion of rat hepatocytes: Mechanism of attachment to collagen substrates. Cell **24**, 463-470.

Ruoslahti, E. & Engvall, E. (1980). Complexing of fibronectin glycosaminoglycans and collagen. Biochem. Biophys. Acta **631**, 350-358.

Ruoslahti, E. & Hayman, E. G. (1979). Two active sites with different characteristics in fibronectin. FEBS Lett. **97**, 221-224.

Ruoslahti, E., Hayman, E. G. & Engvall, E. (1981). Alignment of biologically active domains in the fibronectin molecule. J. Biol. Chem. **256**, 7277-7281.

Ruoslahti, E., Hayman, E. G., Kuu, P., Shively, J. E. & Engvall, E. (1979). Isolation of a tryptic fragment containing the collagen-binding site of plasma fibronectin. J. Biol. Chem. **254**, 6054-6059.

Ruoslahti, E. & Pierschbacher, M. D. (1986). Arg-Gly-Asp: A versatile cell recognition signal. Cell **44**, 517-518.

Ruoslahti, E. & Pierschbacher, M. D. (1987). New perspective in cell adhesion. Science (Wash. DC) **238**, 491-497.

Rutishauser, U. (1984). Developmental biology of a neural cell adhesion molecule. Nature (Lond.) **310**, 549-554.

Rutishauser, U., Grumet, M. & Edelman, G. M. (1983). Neural cell adhesion molecule mediates initial interactions between spinal cord neurons and muscle cells in culture. J. Cell Biol. **97**, 145-152.

Saba, T. M. & Jaffe, E. (1980). Plasma fibronectin (opsonic glycoprotein): Its synthesis by endothelial cells and role in cardiopulmonalgy integrity after trauma as related to reticuloendothelial function. Am. J. Med. **68**, 577-594.

Sakashita, S., Engvall, E. & Ruoslahti, E. (1980). Basement membrane glycoprotein, laminin, binds to heparin. FEBS Lett. **116**, 243-246.

Salama, N. M. (1986). Isolation and characterisation of variants of polyoma transformed BHK21 cells defective in adhesion to fibronectin. A Ph.D thesis, University of Glasgow.

Santoro, S. A. (1986). Identification of a 160, 000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell **46**, 913-920.

Santoro, S. A. & Lawing, W. J. (1987). Competition for related but nonidentical binding sites on the glycoprotein IIb-IIIa complex by peptides derived from platelet adhesive proteins. Cell **48**, 867-873.

Schwarzbauer, J. E., Paul, J. I. & Hynes, R. O. (1985). On the origin of species of

fibronectin. Proc. Natl. Acad. Sci. USA. **82**, 1424-1428.

Shadle, P. J. & Barondes, S. H. (1982). Adhesion of human platelets to immobilised trimeric collagen. J. Cell Biol. **95**, 361-365.

Shin, S, I, Freedman, V.H., Risser, R. & Pollack, R. (1975). Tumourigenicity of virus-transformed cells in nude mice is correlated with anchorage-dependent growth in vitro. Proc. Natl. Acad. Sci. USA. **72**, 4435-4439.

Shirayoshi, Y., Hatta, K., Hosoda, M., Tsunasawa, S., Sakiyama, F. & Takeichi, M. (1986). Cadherin cell adhesion molecules with distinct binding specificities share a common structure. EMBO J. **5**, 2485-2488.

Shirayoshi, Y., Okada, T. S. & Takeichi, M. (1983). The calcium-dependent cell-cell adhesion system regulates inner cell mass formation and cell surface polarisation in early mouse development. Cell **35**, 631-638.

Silnutzer, J. & Barnes, D. W. (1985). Effect of fibronectin-related peptide on cell spreading. In Vitro. Cell Develop. Biol. **21(1)**, 73-78.

Singer, I. I. (1979). The fibronexus: a transmembrane association of fibronectin-containing fibers and bundles of 5 nm microfilaments in hamster and human fibroblasts. Cell **16**, 675-685.

Singer, I. I. (1982). Association of fibronectin and vinculin with focal contacts and stress fibers in stationary hamster fibroblasts. J. Cell Biol. **92**, 398-408.

Singer, I. I., Kawka, D. W., Scott, S., Mumford, R. A. & Lark, M. W. (1987). The fibronectin cell attachment sequence Arg-Gly-Asp-Ser promotes focal contact formation during early fibroblast attachment and spreading. J. Cell Biol. **104**, 573-584.

Skerrow, C. J., Hunter, I. & Skerrow, D. (1987). Dissection of the bovine epidermal desmosome into cytoplasmic protein and membrane glycoprotein domains. J. Cell Sci. **87**, 411-421.

Skerrow, C. J. & Matoltsy, A. G. (1974). Isolation of epidermal desmosomes. J. Cell Biol. **63**, 515-523.

Sliver, J. & Rutishauser, U. (1984). Guidance of optic axons in vivo by a preformed adhesive pathway on neuroepithelial endfeet. Develop. Biol. **106**, 485-499.

Smalheiser, N. & Schwartz, N. B. (1987). Cranin: A laminin-binding protein of cell membranes. Proc. Natl. Acad. Sci. USA. **84**, 6457-6461.

Smith, J.C. (1981). Growth factor and pattern formation. J. Embryol. Exp. Morphol. **65**, 187-207.

Spiegel, E., Burger, M. & Spiegel, M. (1983). Fibronectin and laminin in the extracellular matrix and basement membrane of sea urchin embryos. Exp. Cell Res. **144**, 47-55.

Spiegel, S., Schlessinger, J. & Fishman, P. H. (1984). Incorporation of fluorescent gangliosides into human fibroblasts: mobility, fate, and interaction with fibronectin. J. Cell Biol. **99**, 699-704.

Spiegel, S., Yamada, K. M., Hom, B. E., Moss, J. & Fishman, P. H. (1985). Fluorescent gangliosides as probes for the retention and organisation of fibronectin by ganglioside-deficient mouse cells. J. Cell Biol. **100**, 721-726.

Spiegel, S., Yamada, K. M., Hom, B. E., Moss, J. & Fishman, P. H. (1986). Fibrillar organisation of fibronectin is expressed coordinately with cell surface gangliosides in a variant murine fibroblast. J. Cell Biol. **102**, 1898-1906.

Staehelin, L. A. & Hull, B. G. (1978). Junction between living cells. Sci. Am. **238**, 141-152.

Stanley, P. (1980). Surface carbohydrate alteration of mutant mammalian cells selected for resistance to plant lectin, in The Biochemistry of Glycoproteins and Proteoglycans. W. J. Lennarz (ed.), Plenum, New York, pp, 161-189 .

Stanley, P., Caillibot, V. & Siminovitch, L. (1975). Selection and characterisation of eight phenotypically distinct lines of lectin-resistant chinese hamster ovary cells. Cell **6**, 121-128.

Stanley, P., Sudo, T. & Garver, J. P. (1980). Differential involvement of cell surface sialic acid residues in wheat germ agglutinin binding to parental and wheat germ agglutinin-resistant chinese hamster ovary cells. J. Cell Biol. **85**, 60-69.

Steinberg, M. S. (1964). The problem of adhesive selectivity in cellular interactions, in Cellular Membranes In Development, M. Locke, (ed.), Academic press, New York.pp, 34-66.

Steinberg, M. S. (1970). Does differential adhesiveness govern self-assembly processes in histogenesis? Equilibrium configurations and the emergence of a

hierarchy among populations of embryonic cells. J. Exp. Zool. **173**, 395-434.

Stenn, K. S. & Core, N. G. (1986). Cation dependence of Guinea Pig epidermal cell spreading. In Vitro Cell Develop. Biol. **22 (4)**, 217-222.

Stoker, M. & Macpherson, I. (1964). Syrian hamster fibroblast cell line BHK21 and its derivatives. Nature (Lond.) **203**, 1355-1357.

Stocker, M., O' Neill, C.H., Berryman, S. & Waxman, V. (1968). Anchorage and growth regulation in normal and virus-transformed cells. Int. J. Cancer **3**, 683-693.

Streeter, H. B. & Rees, D. A. (1987). Fibroblast adhesion to RGDS shows novel features compared with fibronectin. J. Cell Biol. **105**, 507-515.

Suzuki, S., Argraves, W. S., Arai, H., Languino, L. R., Pierschbacher, M. D. & Ruoslahti, E. (1987). Amino acid sequence of the vitronectin receptor alpha-subunit and comparative expression of adhesion receptor mRNAs. J. Biol. Chem. **262**, 14080-14085.

Suzuki, S., Argraves, W. S., Pytela, R., Arai, H., Krusius, T., Pierschbacher, M. D. & Ruoslahti, E. (1986). cDNA and amino acid sequences of the cell adhesion protein receptor recognising vitronectin reveal a transmembrane domain and homologies with other adhesion protein receptors. Proc. Natl. Acad. Sci. USA. **83**, 8614-8618.

Suzuki, S., Oldberg, A., Hayman, E. G., Pierschbacher, M. D. & Ruoslahti, E. (1985). Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. EMBO J. **4 (10)**, 2519-2524.

Takada, Y., Strominger, J. L. & Hemler, M. E. (1987 a). The very late antigen family of heterodimers is part of a superfamily of molecules involved in adhesion and embryogenesis. Proc. Natl. Acad. Sci. USA. **84**, 3239-3243.

Takada, Y., Huang, C. & Hemler, M. E. (1987 b). Fibronectin receptor structures are included within the VLA family of heterodimers. Nature (Lond.) **326**, 607-609.

Takeichi, M. (1971). Changes in the properties of cell substrate adhesion during cultivation of chicken fibroblasts in vitro. Exp. Cell Res. **68**, 88-97.

Takeichi, M. (1977). Functional correlation between cell adhesive properties and

some cell surface proteins. J. Cell Biol. **75**, 464-474.

Takeichi, M. & Okada, T. S. (1972). Roles of manganese and calcium ions in cell-to-substrate adhesion. Exp. Cell Res. **74**, 51-60.

Takeichi, M., Yoshida-Noro, C., Ogou, S., Shirayoshi, Y., & Okada, T.S. (1983). A cell adhesion molecule involved in embryonic cellular interactions as studied by using teratocarcinoma cells. Teratocarcinoma stem cells. Cold Spring Harbor, Mass: Cold Spring Harbor Lab, 163-171 .

Tamkun, J. W., Desimone, D. W., Fonda, D., Patel, R. S., Buck, C., Horwitz, A. F. & Hynes, R. O. (1986). Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. Cell **46**, 271-282.

Tamkun, J. W., Schwarzbaure, J. E. & Hynes, R. O. (1983). Plasma fibronectin is synthesised and secreted by hepatocytes. J. Biol. Chem. **258**, 4641-4647.

Tamkun, J. W., Schwarzbaure, J. E. & Hynes, R. O. (1984). A single rat fibronectin gene generates three different mRNAs by alternative splicing of a complex exon. Proc. Natl. Acad. Sci. USA. **81**, 5140-5144.

Tanaka, A. & Kaji, A. (1980). Aggregation properties of chondrocytes infected with a temperature-sensitive mutant of Rous Sarcoma virus. J. Cell Sci. **43**, 407-417.

Tao, T. W. & Burger, M. M. (1977). Non metastasising variants selected from melanoma cells. Nature (Lond.) **270**, 437-438.

Tao, T. W. & Burger, M. M. (1982). Lectin-resistant variants of mouse melanoma cells. I. Altered metastasising capacity and tumourigenicity. Int. J. Cancer **29**, 425-430.

Tarkowski, A. K. & Wroblewska, J. (1967). Developmental of blastomeres of mouse eggs isolated at the 4 and 8 cell stage. J. Embryol. Exp. Morph. **18**, 155-180.

Taylor, A. C. (1961). Attachment and spreading of cells in culture. Exp. Cell Res. Suppl. **8**, 154.

Terranova, V. P., Liotta, L. A., Russo, R. G. & Martin, G. R. (1982). Role of laminin in the attachment and metastasis of murine tumour cells. Cancer Res. **42**, 2265-2269.

Thiery, J-P., Delouvee, A., Gallin, W. J., Cunningham, B. A. & Edelman, G. M.

(1984). Ontogenetic expression of cell adhesion molecules: L-CAM is found in epithelia derived from three primary germ layers. Dev. Biol. **102**, 61-78.

Thiery, J-P., Duband, J. L., Rutishauser, U. & Edelman, G. M. (1982). Cell adhesion molecules in early chicken embryogenesis. Proc. Natl. Acad. Sci. USA. **79**, 6737-6741.

Thiery, J-P., Duband, J. L. & Tucker, G. C. (1985). Cell migration in the vertebrate embryo: Role of cell adhesion and tissue environment in pattern formation. Ann. Rev. Cell Biol. **1**, 91-113.

Thompson, L. K., Horwitz, P. M., Bentley, K. L., Thomas, D. D., Alderete, J. F. & Klebe, R. J. (1986). Localisation of the ganglioside-binding site of fibronectin. J. Biol. Chem. **261**, 5209-5214.

Timpl, R., Ronde, H., Roby, P. G., Rennard, S. I., Foidart, J. M. & Martin, G. R. (1979). Laminin-a glycoprotein from basement membranes. J. Biol. Chem. **254**, 9933-9937.

Tokuyasu, K. T. & Singer, S. J. (1976). Improved procedures for immunoferritin labelling of ultra-thin frozen sections. J. Cell Biol. **71**, 894-906.

Trinkaus, J., P. (1984). Cells into organs : The forces that shape the embryo. Prentice-Hall Inc., Englewood Cliffs, N. J.

Urushihara, H., Ueda, M. J., Okada, T. S. & Takeich, M. (1977). Calcium dependent and independent adhesion of normal and transformed BHK cells. Cell Struct. Funct. **2**, 289-296.

Vaheri, A. & Mosher, D. F. (1978). High molecular weight, cell surface-associated glycoprotein (fibronectin) lost in malignant transformation. Biochem. Biophys. Acta **516**, 1-25.

Varani, J., Dixit, V. M., Fligiel, S. E. G., Mckeever, P. E. & Carey, T. E. (1986). Thrombospondin-induced attachment and spreading of human squamous carcinoma cells. Exp. Cell Res. **167**, 376-390.

Vasiliev, J. M. & Gelfand, I. M. (1981). Neoplastic and normal cells in culture. Cambridge: Cambridge University Press. pp, 13.

Vasiliev, J. M. & Gelfand, I. M. (1982). Possible common mechanism of morphological and growth-related alterations accompanying neoplastic

transformation. Proc. Natl. Acad. Sci. USA. **79**, 2594-2597.

Vesely, P. & Weiss, R. A. (1973). Cell locomotion and contact inhibition of normal and neoplastic rat cells. Int. J. Cancer **11**, 64-76.

Vestweber, D. & Kemler, R. (1984). Structural and functional aspects of the cell adhesion molecule uvomorulin. Cell Differentiation **15**, 269-273.

Vestweber, D. & Kemler, R. (1985). Identification of a putative cell adhesion domain of uvomorulin. EMBO J. **4**, 3393-3398.

Virtanen, I., Vortio, T., Badley, R. A. & Lehto, V. P. (1982). Fibronectin in adhesion, spreading and cytoskeletal organisation of cultured fibroblast. Nature (Lond.) **298**, 660-663.

Volk, T. & Geiger, B. (1984). A 135 kd membrane glycoprotein of intercellular adherens junctions. EMBO J. **3**, 2249-2260.

Volk, T. & Geiger, B. (1986 a). A-CAM: A 135-kd receptor of intercellular adherens junctions.I.immunoelectron microscope localisation and biochemical studies. J. Cell Biol. **103**, 1441-1450.

Volk, T. & Geiger, B. (1986 b). A-CAM: A 135-kd receptor of intercellular adherens junctions. II. Antibody-mediated modulation of junction formation. J. Cell Biol. **103**, 1451-1464.

Vollmers, H. P., Imhof, B. A., Wieland, I. Hiesel, A. & Birchmeier, W. (1985). Monoclonal antibodies NORM-1 and NORM-2 induce more normal behaviour of tumour cells in vitro and reduce tumour growth in vivo. Cell **40**, 547-557.

Waite, K. A., Mugnai, G. & Culp, L. A. (1987). A second cell binding domain of fibronectin (RGDS-independent) for neurite extension of human neuroblastoma cells. Exp. Cell Res. **169**, 311-327.

Walther, B. T., Ohman, R. & Roseman, S. (1973). A quantitative assay for intercellular adhesion. Proc. Natl. Acad. Sci. USA. **70**, 1569-1573.

Warner, A. E., Guthrie, S. & Gilula, N. B. (1984). Antibodies to gap junctional protein selectively disrupt junctional communication in the early amphibian embryo. Nature (Lond.) **311**, 127-131.

Weber, M. J., Nakamura, K. D. & Martinez, R. (1983). Cultured cells transformed by Rous sarcoma virus: A genetically defined model and its phenotype. Prog. Clin. Biol. Res. **115**, 201-216.

Wehland, J., Osborn, M. & Weber, K. (1979). Cell to substratum contacts in living cells: a direct correlation between interference-reflection and indirect-immunofluorescence microscopy using antibodies against actin and alpha-actinin. J. Cell Sci. **37**, 257-273.

Weiss, L. (1960). The adhesion of cells. Int. Rev. Cytol. **9**, 187-225.

Weiss, L. (1964). Cellular locomotive pressure in relation to initial cell contacts. J. Theor. Biol. **6**, 275-281.

Weiss, L. (1967). The cell periphery, metastasis and other contact phenomena. Frontiers of Biology, vol, **7**. Elsevier/North-Holland Publishing Company, Amesterdam, pp, 388.

Weiss, L. (1975). Some biophysical aspects of cell contacts in metastasis. In Cellular Membrane and Tumour Cell Behaviour (M. D., Anderson, ed.), Tumour Inst, Baltimore: Williams and Wilkins, pp, 361-382.

Weiss, L. & Ward, P. M. (1983). Cell detachment and metastasis. Cancer Metastasis Reviews **2**, 111-127.

Wheeler, M. E., Gerrard, J. M. & Carroll, R. C. (1985). Reciprocal transmembraneous receptor-cytoskeleton interactions in Concanavalin A-activated platelets. J. Cell Biol. **101**, 993-1000.

Whur, P. H., Koppel, C. & Williams, D. C. (1977). Quantitative electronic analysis of normal and transformed BHK21 fibroblast aggregation. J. Cell Sci. **23**, 193-209.

Wight, T. N., Raugi, G. J., Mumby, S. M. & Bornstein, p. (1985). Light microscopic immunolocalisation of thrombospondin in human tissue. J. Histochem. Cytochem. **33**, 295-302.

Wilkins, J. A. & Lin, S. (1986). A re-examination of the interaction of vinculin with actin. J. Cell Biol. **102**, 1085-1092.

Willingham, M. C. & Pastan, I. (1975). Cyclic AMP and cell morphology in cultured fibroblasts. J. Cell Biol. **67**, 146-159.

Willingham, M. C., Yamada, K. M., Yamada, S. S., Pouyssegur, J. & Pastan, I. (1977). Microfilaments bundles and cell shape are related to adhesiveness to substratum and are dissociable from growth control in cultured fibroblasts. Cell

10, 375-380.

Wilkinson, P. C. & Lackie, J. M. (1979). The adhesion, migration and chemotaxis of leukocyte in inflammation. In Inflammatory Reactions, H. Z. Movat, (ed.), Springer. pp, 47-88 .

Winkelhake, J. L. & Nicolson, G. L. (1976). Determination of adhesion properties of variant metastatic melanoma cells to Balb/c 3T3 cells and their virus-transformed derivatives by a monolayer attachment assay. J. Natl. Cancer Inst. **56**, 285-291.

Woods, A., Couchman, J. R., Johansson, S. & Hook, M. (1986). Adhesion and cytoskeletal organisation of fibroblasts in response to fibronectin fragments. EMBO J. **55**, 665-670.

Wright, T. C., Ukena, T. E., Campbell, R. & Karnovsky, M. J. (1977). Rates of aggregation, loss of anchorage dependence and tumourigenicity of cultured cells. Proc. Natl. Acad. Sci. USA. **74**, 258-262.

Wright, T. C., Underhill, C. B., Toole, B. P. & Karnovski, M. J. (1981). Divalent cation-independent aggregation of Rat-1 fibroblasts infected with a temperature sensitive mutant of Rous Sarcoma virus. Cancer Res. **41**, 5107-5113.

Yamada, K. M. (1981). Fibronectin and other structural proteins, in : Extracellular Matrix (E. D. Hay, ed.), Plenum press, New York, pp, 95-114.

Yamada, K. M. (1983). Cell surface interactions with extracellular materials. Ann. Rev. Biochem. **52**, 761-799.

Yamada, K. M. & Kennedy, D. W. (1979). Fibroblast cellular and plasma fibronectins are similar but not identical. J. Cell Biol. **80**, 492-498.

Yamada, K. M. & Kennedy, D. W. (1984). Dualistic nature of adhesive protein function: Fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. J. Cell Biol. **99**, 29-36.

Yamada, K. M. & Kennedy, D. W. (1985). Amino acid sequence specificities of an adhesive recognition signal. J. Cell Biochem. **28**, 99-104.

Yamada, K. M. & Kennedy, D. W. (1987). Peptide inhibitors of fibronectin, laminin, and other adhesion molecules: Unique and shared features. J. Cell. Physiol. **130**, 21-28.

Yamada, K. M., Akiyama, S. K., Hasegawa, T., Humphries, M. J., Kennedy, D.

W., Nagata, K., Urushihara, H., Olden, K. & Chen, W-T. (1985). Recent advances in research on fibronectin and other cell attachment proteins. J. Cell. Biochem. **28**, 79-97.

Yamada, K. M., Critchley, D. R., Fishman, P. H. & Moss, J. (1983). Exogenous gangliosides enhance the interaction of fibronectin with ganglioside-deficient cells. Exp. Cell Res. **143**, 295-302.

Yamada, K. M., Kennedy, D. W., Grotendorst, G. R. & Momoi, T. (1981). Glycolipids receptors for fibronectin? J. Cell. Physiol. **109**, 343-351.

Yamada, K. M., Kennedy, D. W., Kimata, K. & Pratt, R. M. (1980). Characterisation of fibronectin interactions with glycosaminoglycans and identifications of active proteolytic fragments. J. Biol. Chem. **225**, 6055-6063.

Yamada, K. M., Schlesinger, D. H., Kennedy, D. W. & Pastan, I. (1977). Characterisation of a major fibroblast cell surface glycoprotein. Biochemistry **16**, 5552-5559.

Yamada, K. M., Yamada, S. S. & pastan, I. (1976). Cell surface protein partially restore morphology, adhesiveness and contact inhibition of movement to transformed fibroblast. Proc. Natl. Acad. Sci. USA. **73**, 1217-1221.

Yannariello-Brown, J. Weber, U., Liotta, L. & Madri, J. A. (1988). Distribution of a 69-KD laminin-binding protein in aorta and microvascular endothelial cells: modulation during cell attachment, spreading, and migration. J. Cell Biol. **106**, 1773-1786.

Yasuda, K. (1974). Studies on the factors affecting cellular spreading upon culture-substrate. 1. The effects of divalent cations and conditioned medium on cellular spreading. J. Cell Biol. **15**, 269-278.

Yates, G. R. & Izzard, C. S. (1981). Cell-to-substrate contacts in an adhesion defective mutant of BALB/c 3T3 cells. J. Cell Sci. **52**, 183-196.

Yoshida-Noro, C., Suzuki, N.&Takeichi, M. (1984). Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody. Dev. Biol. **101**, 19-27.