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ADHESION PLAQUES: SITES OF TRANSMEMBRANE INTERACTION BETWEEN THE EXTRACELLULAR MATRIX AND THE ACTIN CYTOSKELETON

KEITH BURRIDGE, LESLIE MOLONY AND THOMAS KELLY

Laboratories for Cell Biology, Department of Cell Biology and Anatomy, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA

SUMMARY

In this paper we review what is known about the organization of adhesion plaques, the regions where cells in culture adhere most tightly to the underlying substratum. These specialized areas of the plasma membrane serve as attachment sites for stress fibres. A major objective has been to determine how microfilament bundles are anchored at such regions. In their morphology and composition adhesion plaques resemble the adhesions fibroblasts make to the extracellular matrix. Some extracellular matrix components have been identified on the outside face of adhesion plaques. Within the plasma membrane of adhesion plaques, extracellular matrix receptors, such as the fibronectin receptor (integrin), have been identified. This transmembrane glycoprotein complex has been shown to bind the cytoplasmic protein talin, which, in turn, associates with vinculin. These proteins establish a transmembrane chain of attachment between the extracellular matrix and the cytoskeleton, although how the actin filaments interact with these components remains to be determined. Besides having a structural function, adhesion plaques may also be regions where regulatory signals are transmitted across the membrane. Consistent with this idea has been the finding that various tyrosine kinases and a calcium-dependent protease are concentrated at the cytoplasmic aspect of adhesion plaques. Furthermore, several adhesion plaque proteins become phosphorylated during cell transformation by Rous sarcoma virus. In future work it will be important to determine how such modifications affect the interactions of these proteins and the stability of adhesion plaques.

INTRODUCTION

In the fourth paper of their landmark series on the locomotion of fibroblasts in culture, Abercrombie and his colleagues observed electron-dense plaques on the ventral surfaces of cells (Abercrombie *et al.* 1971). The plaques were associated with filaments and corresponded to regions where the plasma membrane came closest to the underlying substratum. On the basis of their observations they suggested that these regions were involved in cell adhesion and were linked to the cells' filamentous system. Subsequent work has confirmed both ideas. The plaques noted by Abercrombie and coworkers have become known variously as adhesion plaques, focal contacts or focal adhesions. We will use these terms synonymously. These structures have generated much interest, both because they are the sites of attachment of stress fibres to the plasma membrane and because they are regions where the cell interacts with the substratum or extracellular matrix. In this paper we will discuss briefly the organization and biology of adhesion plaques. We wish to emphasize that not only are

they sites of mechanical linkage between the cytoskeleton, the plasma membrane and the extracellular matrix, but that they are also regions of communication between the external environment and the cell. For a more detailed review of adhesion plaque organization and function, the reader is referred to Burridge (1986).

ADHESION PLAQUES, STRESS FIBRES AND THE EXTRACELLULAR MATRIX

The adhesion plaques first observed by Abercrombie *et al.* (1971) were identified by electron microscopy. Although this continues to be a useful technique for examining their ultrastructure, the study of adhesion plaques has been facilitated by various light-microscopic techniques such as interference reflection and immunofluorescence microscopy. Used with increasing frequency, interference reflection microscopy (IRM) gives an indication of the separation of the cell from the substratum, the most adherent regions appearing black or dark grey (Curtis, 1964). Izzard & Lochner (1976) classified the ventral surface of fibroblasts into three types of region depending on their IRM images. The darkest images, the adhesion plaques or focal contacts, corresponded to a separation of about 10–15 nm between the cell and the substratum. These regions are frequently surrounded by broad, grey areas, which they have named 'close contacts'. Finally, there are regions appearing white by IRM, indicating a separation from the substratum of 100 nm or more, where adhesion appears to be minimal. All three types of region are seen in the cell in Fig. 1.

Many cells in tissue culture develop adhesion plaques. Abercrombie *et al.* (1971) considered they were probably involved in cell migration, but subsequent work has shown that they are more prominent in cells displaying little or no motility (Couchman & Rees, 1979; Kolega *et al.* 1982). Highly motile cells reveal regions of close contact by IRM. Stress fibres, the large bundles of microfilaments anchored at adhesion plaques, similarly, seem to have little to do with generating cell movement (Couchman & Rees, 1979; Herman *et al.* 1981). At first this was a surprising observation given that stress fibres are reminiscent of muscle myofibrils and contain many of the same proteins. It has been suggested that their prominence in cultured cells reflects a very tight adhesion to an inflexible substratum and a resulting isometric tension generated by the contractile proteins within the stress fibres (Burridge, 1981). Cells that migrate rapidly lack stress fibres and adhesion plaques, and appear to have a less highly ordered array of microfilaments.

When studying the migration of cells from an explant, Couchman and colleagues observed that initially the cells lacked both stress fibres and focal adhesions (Couchman & Rees, 1979; Couchman *et al.* 1982). With time both of these developed and there was a decrease in motility. These changes were paralleled by an increase in surface fibronectin. It was found that the appearance of stress fibres and focal adhesions could be induced earlier by addition of fibronectin to the cells. Fibronectin addition can also have dramatic effects on the cytoskeletal organization of virus-transformed cells. As will be discussed below, transformed cells typically have reduced levels of fibronectin and lack both adhesion plaques and stress fibres. When

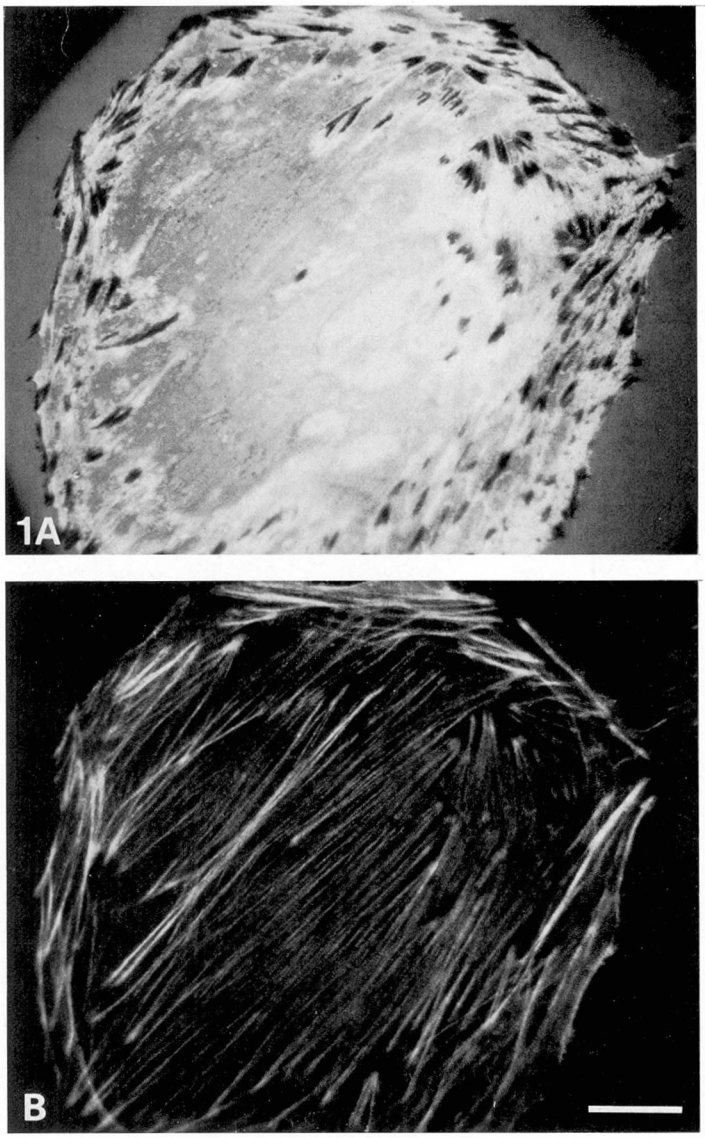

Fig. 1. A cultured BSC-1 cell photographed to show adhesion plaques (A) and stress fibres (B). IRM was used to view the cell in A. The same cell was fixed, permeabilized and stained with rhodamine–phalloidin to reveal the distribution of actin filaments (stress fibres) by immunofluorescence microscopy (B). The darkest regions in the cell in A are adhesion plaques and correspond to the ends of stress fibres seen in B. Large grey areas seen in A are the 'close contacts'. Bar, 20 μm. (Micrographs were kindly provided by C. A. Huff.)

fibronectin is added back to some transformed cells a more normal morphology is restored and the cells develop adhesion plaques and stress fibres (Ali *et al.* 1977; Willingham *et al.* 1977).

Table 1. *Structural proteins in adhesion plaques*

Extracellular face	Transmembrane	Cytoplasmic face
Fibronectin ±	Fibronectin receptor (Integrin)	Actin
		α-Actinin
Heparan sulphate proteoglycan ±	30B6	Fimbrin
	FC1	Vinculin
	HSV D glycoprotein	Talin
		HA1
		$200\times10^3 M_r$
		$82\times10^3 M_r$

The presence of fibronectin depends on the conditions of cell culture. Heparan sulphate proteoglycan has been detected in some adhesion plaques, but how widespread this occurrence is has not been determined. For references see the text.

Stress fibres and adhesion plaques are generally absent from most cells *in situ*, although similar structures are seen at specific locations. For example, the dense plaques of smooth muscle resemble the adhesion plaques of cultured cells, being sites of attachment of actin filaments and regions of adhesion to the extracellular matrix. Adhesion plaques also resemble some types of cell–cell junction, such as the adherens junctions found in epithelial tissues and in cardiac muscle. Analysing the components at these sites, however, indicates that adhesion plaques resemble more closely adhesions to the extracellular matrix than the adhesions made between cells (Geiger *et al.* 1985). For example, both vinculin and talin are found in adhesion plaques and in adhesions to the extracellular matrix, whereas talin appears to be absent from certain cell–cell adhesions, such as the zonula adherens of epithelia and the fascia adherens of cardiac muscle (Geiger *et al.* 1985). When cells in tissue culture interact with glass or plastic substrata, these surfaces are usually covered with adsorbed extracellular matrix components such as fibronectin or vitronectin, and the presence of these appears very important for the formation of adhesion plaques (Woods *et al.* 1986). From these observations we interpret the adhesion plaque of cultured cells as usually being an adhesion to specific extracellular matrix components. In support of this view is the finding that receptors for extracellular matrix components are clustered in adhesion plaques (see below). From this point on, we will consider the adhesion plaque as very closely related, if not equivalent, to an adhesion to the extracellular matrix. Future work may reveal that adhesions to the extracellular matrix can be classified into different types, reflecting the presence of different components. Such a level of analysis has not been achieved.

ORGANIZATION OF ADHESION PLAQUES

We have listed in Table 1 the structural elements concentrated in adhesion plaques. These have been categorized according to their location, on the extracellular face of the plasma membrane, within the membrane or at its cytoplasmic face. A major objective in this area of research has been to identify the critical components

and to determine how those on the cytoplasmic side link up with elements in the membrane and thence to the extracellular matrix.

Extracellular components of adhesion plaques

A major component of the extracellular matrix is fibronectin; its relationship to adhesion plaques has been controversial. Some investigators have found it in these structures (Grinnell, 1980; Singer & Paradiso, 1981; Singer, 1982), whereas others have noted it to be absent (Birchmeier *et al.* 1980; Chen & Singer, 1980; Badley *et al.* 1980). The presence or absence of fibronectin in adhesion plaques appears to reflect the conditions of growth: those cells grown in low serum have fibronectin within their adhesion plaques, whereas those grown in high serum clear it from these regions. In part this is due to the fibronectin being less tightly adsorbed to the substratum in the presence of other proteins (Grinnell, 1986).

Many cells in culture secrete fibronectin and, in addition, it is usually present in soluble form in the serum added to cell cultures. Cultured fibroblasts normally develop extensive fibrillar networks of fibronectin on both their dorsal and ventral surfaces (Chen *et al.* 1976). Frequently, when it is on the cell surface, fibronectin is found to co-align with cytoplasmic adhesion plaque proteins, such as vinculin and talin (Burridge & Feramisco, 1980; Singer & Paradiso, 1981; Burridge & Connell, 1983). This supports the idea that adhesion plaques and the adhesions to fibronectin are closely related, if not equivalent, structures.

The properties of fibronectin and its effect on cells have been extensively reviewed (Hynes & Yamada, 1982; Yamada, 1983). For most cells it increases adhesion to the substratum and promotes flattening and the formation of adhesion plaques and stress fibres. When cells are grown in the absence of serum, fibronectin is often added to promote cell adhesion, which is necessary for normal growth (Orley & Sato, 1979; Rizzino & Crowley, 1980; Wolfe *et al.* 1980; Rockwell *et al.* 1980). Although the increased adhesion resulting from added fibronectin is often associated with reduced motility (Couchman *et al.* 1982), in some cases addition of fibronectin has been found to enhance cell migration (Ali & Hynes, 1978). This apparent discrepancy may reflect the properties of different cell types or the fact that adhesion is necessary for migration, but if adhesion becomes excessive it is inhibitory.

Fibronectin has been biochemically dissected into several domains with distinct properties. One of the domains contains a cell-binding sequence that interacts with a specific plasma membrane receptor (see below). The behaviour of cells plated on coverslips coated with this cell-binding domain has been studied (Woods *et al.* 1986). Somewhat surprisingly this fragment of fibronectin will promote cell attachment and even spreading but is not sufficient to induce the formation of adhesion plaques. However, cells plated on the intact fibronectin molecule will form these adhesions, implying that another region in fibronectin is required. This additional region has been identified as a distinct domain that will bind to heparin. Adding a fragment containing both domains or even addition of the two domains as separate fragments will induce formation of adhesion plaques (Woods *et al.* 1986). These results suggest that the induction of adhesion plaques involves the participation of a proteoglycan

and requires more than a single type of surface receptor. Related to this, Woods *et al.* (1985) have also demonstrated the presence of a heparan sulphate proteoglycan in the adhesion plaques of some cells. It will be interesting to learn whether this or related proteoglycans are always present in adhesion plaques.

Integral membrane components in adhesion plaques

Although many of the effects of fibronectin on cells have been known for some years, a cellular receptor for fibronectin eluded identification until recently. The discovery of the cellular fibronectin receptor came about from research in two different directions. In the one approach, the cell-binding domain of fibronectin was pared down progressively to a smaller and smaller region until a three-amino-acid sequence (Arg-Gly-Asp) was obtained (Pierschbacher & Ruoslahti, 1984; Yamada & Kennedy, 1984). This short sequence would compete with fibronectin for binding to cells and would detach cells that had been plated on a fibronectin substratum. Affinity chromatography on immobilized fibronectin was used to identify a receptor that could be released by peptides containing this cell-binding peptide (Pytela *et al.* 1985*a*). With mammalian cells this approach revealed a glycoprotein complex with an apparent polypeptide molecular weight of 140 000 on SDS–polyacrylamide gels under reducing conditions. In non-reducing conditions two distinct polypeptide bands were identified. This same approach has been used to identify the receptors for other extracellular matrix proteins (e.g. vitronectin) (Pytela *et al.* 1985*b*), many of which also contain the same Arg-Gly-Asp sequence in their cell-binding domains (reviewed by Ruoslahti & Pierschbacher, 1986).

The fibronectin receptor was also identified independently in several laboratories, using a different strategy employing monoclonal antibodies (Mabs). These Mabs, raised against whole cells, were found to affect cell adhesion (Neff *et al.* 1982; Greve & Gottlieb, 1982). They were shown to interact with a glycoprotein complex with subunits of about 140 000 M_r (Chapman, 1984; Horwitz *et al.* 1984; Knudsen *et al.* 1985; Hasegawa *et al.* 1985; Brown & Juliano, 1985). Isolated by affinity chromatography on these Mabs, the glycoprotein complex was shown to bind to fibronectin in solution (Horwitz *et al.* 1985; Akiyama *et al.* 1986). Most work has been performed using two Mabs, CSAT and JG22E, both of which are specific for the avian fibronectin receptor. Whereas the mammalian receptor appears to be a heterodimer by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), the avian receptor reveals three bands on non-reduced SDS–PAGE (Knudsen *et al.* 1985; Hasegawa *et al.* 1985). The reason for this difference is not clear, but the avian receptor isolated on these antibody columns might be a mixture of two heterodimers that share a common polypeptide recognized by the antibodies. The avian receptor differs from the mammalian in that the same receptor binds not only fibronectin but also several other extracellular matrix components, such as laminin, vitronectin and some types of collagen (Horwitz *et al.* 1985). With mammalian cells distinct receptors generally appear to bind the different extracellular matrix components,

although in the case of platelets one receptor, glycoproteins IIb/IIIa, binds several extracellular matrix ligands (Gardner & Hynes, 1985). Cloning and sequencing the genes for some of these receptors indicates a large family of related proteins (reviewed by Hynes, 1987). The name 'integrin' has been proposed for this family of proteins (Tamkun *et al.* 1986; Hynes, 1987; and see Buck & Horwitz, this volume).

The Mabs CSAT and JG22E tend to label migratory cells diffusely, but cells that are more stationary show staining of the adhesion plaques with these antibodies (Damsky *et al.* 1985; Chen *et al.* 1985). By immunofluorescence microscopy the staining of the adhesions is uneven, with most of the stain being concentrated at the periphery, giving rise to an image like the eye of a needle (Fig. 2A). In our laboratory we have raised polyclonal antibodies against two smooth-muscle glycoproteins that appear to be members of the integrin family (Kelly *et al.* 1987). Immunologically these antibodies cross-react with the antigens recognized by the CSAT Mab. Unlike CSAT or JG22E, however, these polyclonal antibodies stain adhesion plaques much more evenly (Fig. 2B). The explanation for this difference may be that the Mabs recognize a single epitope on the proteins that is at or close to the ligand binding site for these receptors. At the centre of adhesion plaques these receptors may be occupied and sterically inaccessible, resulting in a staining pattern that is concentrated at the adhesion plaque periphery. On the other hand, the polyclonal antibodies recognize multiple epitopes on these proteins, particularly in fixed and permeabilized cells. Because most of the antigenic sites on the receptors are not blocked by the presence of extracellular matrix components, these antibodies reveal the presence of the receptors throughout the adhesion plaques.

It is striking that these receptors are clustered within adhesion plaques of chicken embryo fibroblasts even when these plaques lack fibronectin. Since in the avian system this receptor can interact with ligands besides fibronectin, this observation suggests that other extracellular matrix components may be present and important in organizing adhesion plaques. Vitronectin is a possible candidate. It has not been listed in Table 1 because it is not concentrated within these substratum adhesions, but under most culture conditions where serum is used, vitronectin is adsorbed uniformly across the glass or plastic substratum (Neyfakh *et al.* 1983; Hayman *et al.* 1985).

Mab 30B6 binds to another membrane glycoprotein in adhesion plaques of avian cells (Rogalski & Singer, 1985). This glycoprotein shares some characteristics with the avian fibronectin receptor, but the Mab 30B6 stains adhesion plaques more evenly than the Mabs CSAT or JG22E. From our results cited above, however, this would be consistent with 30B6 binding to a different epitope on the same receptor. Further work will be needed to determine whether 30B6 is identifying a novel component in focal contacts.

Two other membrane proteins have been identified in focal contacts, the herpes simplex viral glycoprotein D (Norrild *et al.* 1983) and a component recognized by the Mab FCI (Oesch & Birchmeier, 1982). Since little is known about these they will not be discussed.

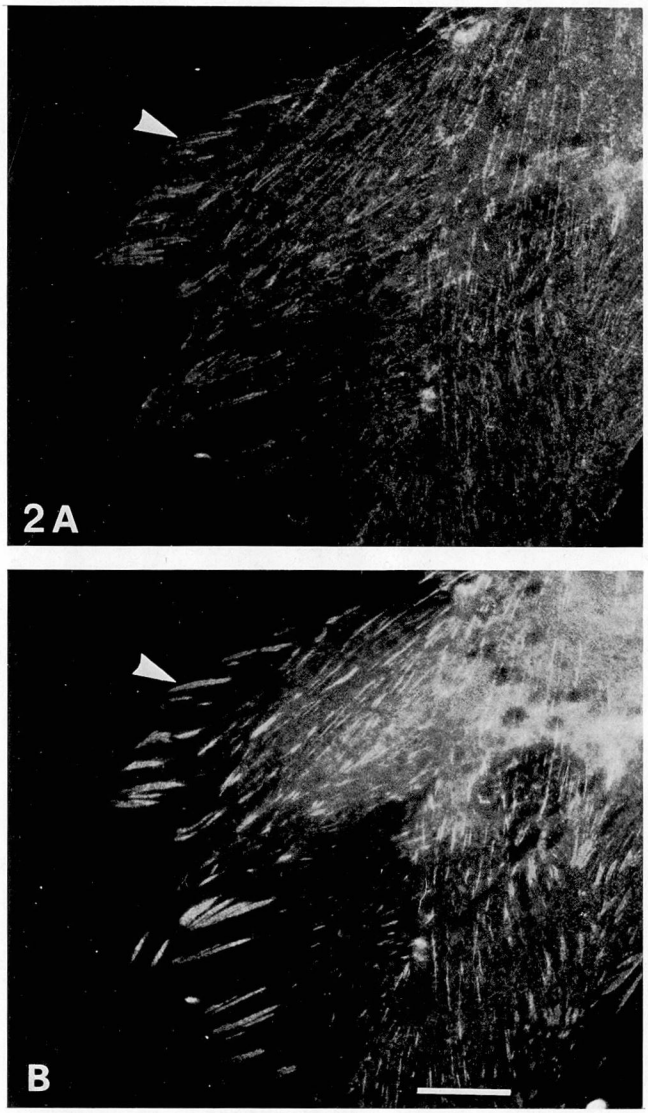

Fig. 2. Distribution of the fibronectin receptor (integrin) in a chicken embryo fibroblast. In A the cell was stained live with the CSAT Mab; in B the same cell was stained after fixation and permeabilization with a polyclonal antibody that binds to the same antigen. Note that in A the antibody labels the adhesion plaques unevenly, concentrating at the periphery, whereas in B the adhesion plaques are more uniformly stained (arrowheads). Bar, 20 μm.

Proteins at the cytoplasmic face of adhesion plaques

Much of the attention focused on adhesion plaques has been aimed at trying to determine how the bundles of actin filaments are attached to the plasma membrane.

This quesion has not been resolved, but several proteins have been identified as candidates for a role in this function. Two proteins that bind to actin filaments, and which are concentrated in adhesion plaques, are α-actinin (Lazarides & Burridge, 1975; Wehland *et al.* 1979) and fimbrin (Bretscher & Weber, 1980). Both proteins crosslink actin filaments *in vitro* and may be important in stabilizing the filaments within a stress fibre. Since they bind to actin they may also function in attachment of the filaments to the membrane. This has been suggested periodically for α-actinin, although evidence against this has also been presented (Burridge & McCullough, 1980). Supporting a possible membrane attachment role for α-actinin are recent experiments indicating that the protein can interact with specific lipids (Burn *et al.* 1985) and also that α-actinin can interact with vinculin (Craig, 1985), itself a protein thought to have a function in attachment to the membrane. If further work substantiates an attachment role for α-actinin, it will be important to explore how this is regulated, since only a fraction of the cell's α-actinin is found in these adhesions and much of the protein is distributed along the stress fibres.

Vinculin and talin are two proteins that have generated much interest as potential links between actin and the adhesion plaque plasma membrane (Geiger, 1979; Geiger *et al.* 1980; Burridge & Feramisco, 1980; Burridge & Connell, 1983; and see Geiger *et al.*, this volume). These two proteins interact (Burridge & Mangeat, 1984) and are generally found co-distributed in fibroblasts, in both adhesion plaques and underlying bundles of fibronectin on the cell surface. How these proteins associate with actin filaments has not been determined, but one mode of interaction with the membrane has been identified. Talin binds to the cytoplasmic domain of the fibronectin receptor (Horwitz *et al.* 1986). It seems probable that vinculin may also interact with membrane proteins directly and this is supported by the fact that in some cells, such as epithelia and cardiac muscle, vinculin is found associated with regions of the plasma membrane in the absence of talin. In some circumstances talin may function independently of vinculin; for example, Kupfer *et al.* (1986) have shown that talin, but not vinculin, concentrates in cytotoxic T lymphocytes at the site of adhesion to a target cell.

For a time vinculin was thought to interact directly with actin, capping the ends of actin filaments or inducing the filaments to form bundles (Jockusch & Isenberg, 1981; Wilkins & Lin, 1982; Burridge & Feramisco, 1982). Further work established that the apparent effects of vinculin on actin were due to contaminants in vinculin preparations (Evans *et al.* 1984; Rosenfeld *et al.* 1985; Schroer & Wegner, 1985; Otto, 1986; Wilkins & Lin, 1986). Because these contaminants flow through a hydroxyapatite column in the first fraction, Wilkins & Lin (1986) have referred to these as the HAI components. Several relatively low molecular weight bands are responsible for this activity, but antibodies raised against these components cross-react with higher molecular weight proteins in immunoblots of whole cells or tissues, suggesting that the low molecular components are proteolytic fragments derived from these larger proteins (Wilkins *et al.* 1986). These antibodies also localize the proteins to adhesion plaques. This result, together with earlier data indicating an

interaction with the ends of actin filaments, suggests that the HAI proteins may have a critical role in attachment of actin to the adhesion plaque membrane. In future work it will be important to characterize these proteins and their interactions in detail.

Even less is known about some of the other components that have been identified in adhesion plaques. For example, Maher & Singer (1983) raised an antibody against a $200\,000 M_r$ component from cardiac fascia adherens and this antibody stains adhesion plaques. This protein has not been purified. While screening rabbit sera, Beckerle (1986) discovered one serum that labelled fibroblast adhesion plaques. This serum bound to an $82\,000 M_r$ protein in immunoblots. Preliminary work has indicated that this is a relatively minor component compared with vinculin or talin, possibly indicating a regulatory role rather than a structural one for this protein.

We are confident that many more proteins remain to be identified in adhesion plaques and many of those that have been identified are poorly characterized. Conscious of these deficits, we have tried to represent the better-characterized adhesion plaque components and their interactions in a simple diagram (Fig. 3). On the outside of the plasma membrane, fibronectin is shown interacting with the fibronectin receptor (integrin). The shape of this receptor complex is based on electron microscopy (Molony, unpublished results) and on parallel studies of the platelet fibronectin receptor, glycoprotein IIb/IIIa (Carrell *et al.* 1985). The receptor is shown spanning the membrane and then binding on the cytoplasmic side to talin (Horwitz *et al.* 1986; Tapley *et al.* unpublished data). In turn, talin is shown interacting with vinculin. The shapes of talin and vinculin are based on platinum-shadowed images of these molecules (Milam, 1985; Molony *et al.* 1987). These have revealed that talin is an elongated, flexible molecule at physiological ionic strengths, whereas vinculin has two domains with a globular head and a short extended tail. Although our work has indicated that the head region of vinculin binds to talin, the binding site on talin has not been defined. Recently, we have discovered that talin will dimerize at concentrations above about $0{\cdot}7\ \mathrm{mg\ ml^{-1}}$ (Molony *et al.* 1987). This has not been indicated in the diagram because we do not know whether this dimerization involves side-to-side or end-on association of the talin molecules. Although not shown, the dimerization would be expected to crosslink the proteins to which talin binds, such as vinculin and the fibronectin receptor, and this may contribute to the stability of adhesion plaques.

In Fig. 3, we have indicated an unidentified protein that may link vinculin to the membrane independently of talin. As mentioned earlier, the existence of such a protein is suggested by finding vinculin associated with some membranes without talin. It is also supported by experiments that showed that fluorescent vinculin would bind back to adhesion plaques in permeabilized fibroblasts and that this was not dependent on talin (Ball *et al.* 1986). Also shown in the diagram is the component(s) HAI capping the end of an actin filament. The shape of this molecule has not been determined and it will be important to discover with what other components it interacts.

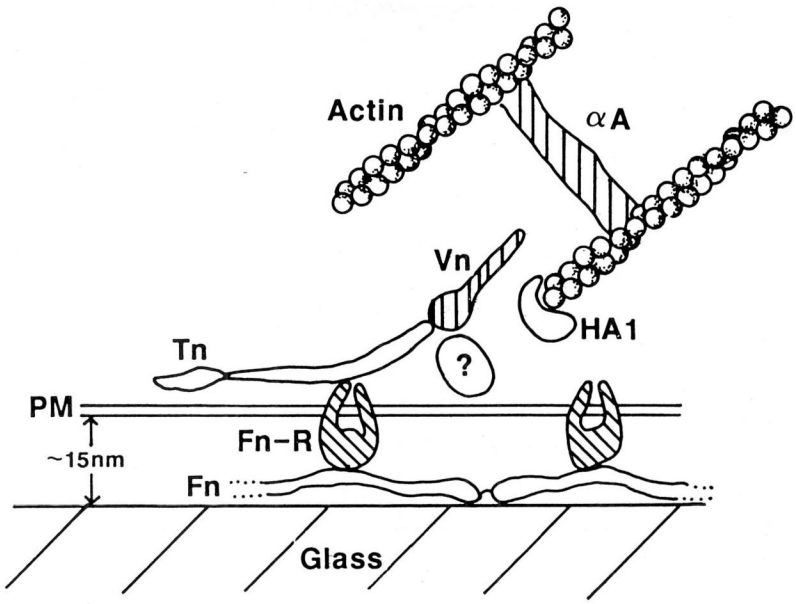

Fig. 3. A diagram of some of the proteins identified in adhesion plaques. The lengths of the proteins are drawn approximately to scale, with the separation of the plasma membrane bilayer (PM) being about 15 nm from the glass substrate. Part of a fibronectin (Fn) dimer is shown adhering to the glass. It should be noted that fibronectin is frequently absent from adhesion plaques although other extracellular matrix components may be present. The fibronectin receptor (Fn-R) (integrin) spans the plasma membrane and binds to talin (Tn) on the cytoplasmic face. In turn, talin binds vinculin (Vn). The shapes of the fibronectin receptor, talin and vinculin are derived from electron microscopy of isolated molecules (Milam, 1985; Molony *et al.* 1987; Molony, unpublished data). A constriction in the talin molecule indicates the site of cleavage by the calcium-dependent protease II. An unidentified protein (?) is shown linking vinculin to the membrane independently of talin. The actin filaments are shown crosslinked by α-actinin (αA) and being capped by the HA1 component(s). The shape of the latter has not been determined. How the actin filaments link to the transmembrane components remains a major unresolved question.

ADHESION PLAQUES AND THE REGULATION OF CELLULAR ACTIVITIES

It is our contention that the adhesion plaque is more than just a structural link between the cytoskeleton, the plasma membrane and the extracellular matrix. Adhesion plaques are sites of communication between the cell and the extracellular environment. It has been known for some time that adequate adhesion is necessary for the growth of many cells in culture. Paradoxically, many agents that promote cell growth and proliferation, such as various hormones, growth factors, tumour promoters and transforming viruses, result in a disruption of these adhesions (reviewed by Burridge, 1986). The reasons for this are not clear. Not only does adhesion permit the growth of many cells, but the type of surface to which the cells adhere can have marked effects on the growth properties, morphology and behaviour

Table 2. *Regulatory proteins in adhesion plaques*

$p60^{src}$ (1) $p120^{gag\text{-}abl}$ (2) $p90^{gag\text{-}yes}$ (3) $p80^{gag\text{-}yes}$ (3)	Tyrosine kinases
Calcium-dependent protease II (4)	

(1) Rohrschneider (1980); Nigg *et al.* (1982); Krueger *et al.* (1984); (2) Rohrschneider & Najita (1984); (3) Gentry & Rohrschneider (1984); (4) Beckerle *et al.* (1987).

of these cells. Again this implies the communication of information across the plasma membrane at adhesions to the substratum or extracellular matrix.

Several potential regulatory enzymes have been identified at the cytoplasmic face of adhesion plaques (Table 2). A number of these are tyrosine kinases, the products of various viral oncogenes. Generally, transformed cells are more rounded with few if any adhesion plaques. However, in cells transformed by the appropriate virus, immunofluorescence microscopy has revealed the presence of these tyrosine kinases in those cells sufficiently adherent and spread to retain adhesion plaques (Rohrschneider, 1980; Rohrschneider & Najita, 1984; Gentry & Rohrschneider, 1984; Nigg *et al.* 1982; Krueger *et al.* 1984). These viral oncogenes have normal cellular homologues, but these are expressed at too low a level in fibroblasts for their distribution to have been detected by immunofluorescence microscopy. Elevated levels of phosphotyrosine, however, have been found in the adhesion plaques of non-transformed fibroblasts (Maher *et al.* 1985), suggesting that tyrosine kinases are normally concentrated within these structures.

What are the substrates for these tyrosine kinases within adhesion plaques? Vinculin was the first adhesion-plaque protein found to contain elevated phosphotyrosine levels in cells transformed by Rous sarcoma virus (RSV) (Sefton *et al.* 1981). This result, combined with the localization of the RSV oncogene product, $pp60^{src}$, within adhesion plaques (Rohrschneider, 1980), led to a model that envisaged the phophorylation of vinculin as being a major event in the disruption of adhesion plaques and stress fibres in transformed cells. Appealing though this model was, it has not been supported by subsequent work. A number of groups have examined the level of vinculin phosphorylation in cells infected with viral mutants of RSV that do not result in the transformed phenotype. In several of these studies the level of vinculin phophorylation on tyrosine residues has been found to be elevated, but this has not been accompanied by a loss of stress fibres or adhesion plaques (Rohrschneider & Rosok, 1983; Iwashita *et al.* 1983; Antler *et al.* 1985; Nigg *et al.* 1986; Kellie *et al.* 1986). At present it is not clear whether vinculin phosphorylation has any significance. The level of vinculin phosphorylation is low at best and it is perhaps an inconsequential event reflecting a fortuitous proximity to the kinase.

Recently, talin has also been found to contain phosphotyrosine and to have elevated levels in cells transformed by some of the viruses carrying oncogenes encoding tyrosine kinases (Pasquale *et al.* 1986; DeClue & Martin, 1987). DeClue & Martin (1987) investigated the level of tyrosine phosphorylation in talin in cells

infected by several RSV mutants that do not induce a fully transformed morphological phenotype. In these partially transformed cells talin was found to contain elevated phosphotyrosine at levels close to those found in cells fully transformed by the wild-type virus. These authors concluded that elevated phosphotyrosine in talin did not correlate with loss of fibronectin from the cell surface, loss of stress fibres or a rounded morphology (DeClue & Martin, 1987).

The avian fibronectin receptor (integrin) also contains elevated levels of phosphotyrosine in cells transformed by RSV, and certain other viruses (Hirst *et al.* 1986). Two of the receptor polypeptides become phosphorylated. One of these polypeptides has been cloned and sequenced (Tamkun *et al.* 1986) and it was noted that a region of homology existed in the presumptive cytoplasmic domain with a sequence in the epidermal growth factor (EGF) receptor. Interestingly, this sequence in the EGF receptor contains the tyrosine that is autophosphorylated in response to EGF binding. It is this related sequence in the fibronectin receptor that is phosphorylated in cells transformed by RSV. In addition, a synthetic decapeptide corresponding to this region of the cytoplasmic domain has been found to inhibit the binding of talin to the fibronectin receptor in solution (Tapley *et al.* unpublished data; and see Buck & Horwitz, this volume). The fibronectin receptor purified from RSV-transformed cells is phosphorylated on this tyrosine residue and has reduced binding of both talin and fibronectin (Tapley *et al.* unpublished data). It has not been determined, however, whether the decreased affinity for talin and fibronectin is due to this phosphorylation or to some other modification that has not been identified. It is easy to envisage how various modifications of the receptor could destabilize the adhesion plaque and promote its disassembly. It will be important, however, to determine whether phosphorylation of the receptor correlates with the transformed phenotype using the viral mutants mentioned above. In transformed cells there are other important events, which undoubtedly affect the integrity of adhesion plaques. In many transformed cells, for example, there is an increased secretion of proteolytic enzymes and a consequent reduction in fibronectin and other extracellular matrix proteins (Unkeless *et al.* 1973; Chen *et al.* 1984). That this reduction in fibronectin affects adhesion plaques and cytoskeletal organization has been shown by adding fibronectin back to transformed cells. In many cases this addition of exogenous fibronectin will temporarily reverse the transformed phenotype, restoring adhesion plaques, stress fibres and a more normal morphology. It is probable that the disruption of adhesion plaques in transformed cells is due to multiple events and it will be important to determine whether the phosphorylation of any of the focal contact proteins contributes to this process.

One of the isoforms of the calcium-dependent protease (CDPII), another potential regulatory enzyme, has also been identified in adhesion plaques (Beckerle *et al.* 1987). Talin has been found to be a particularly good substrate for this enzyme. Following platelet activation cleavage of the platelet form of talin (P235) occurs as a result of calcium-dependent proteases (Fox *et al.* 1985). The significance of this fragmentation of talin in platelets has not been determined, but it raises the possibility that proteolysis of talin by CDPII in fibroblasts may be important in the

disassembly or reorganization of adhesion plaques. We have found that cleavage of talin by CDPII does not separate the vinculin binding site from the fibronectin–receptor binding site, and that this cleavage occurs asymmetrically in the molecule (O'Halloran *et al.* 1985; Beckerle *et al.* 1986; Horwitz *et al.* 1986). The approximate position of the cleavage site in talin is indicated in the diagram, in Fig. 3, by a constriction in the molecule. Although this proteolysis of talin does not appear to affect the binding to vinculin or to the fibronectin receptor, it may affect other talin interactions. We are currently investigating whether it prevents talin dimerization, which might have major consequences on the ability of talin to crosslink and stabilize components in adhesion plaques.

FUTURE DIRECTIONS

In this brief overview we have summarized much of what is known about the structural elements found within adhesion plaques. There are many holes in our current model, indicating that critical components remain to be identified. One of the major questions continues to be: how are the actin filaments of a stress fibre linked to the adhesion plaque membrane? We would not be surprised to find that the filaments are anchored at these sites by more than one set of linker proteins functioning in parallel. It should be remembered that even in the model system of the erythrocyte plasma membrane, actin is linked to the membrane through two different sets of proteins. In the adhesion plaque one transmembrane linkage has been identified to date. This involves the fibronectin receptor, which in avian cells can interact on the outside with several types of extracellular matrix protein besides fibronectin, and which binds talin on the cytoplasmic face of the membrane. We anticipate, however, that this is just the first of several transmembrane links that will be discovered to operate in adhesion plaques.

Several potential regulatory enzymes have been identified in adhesion plaques and we have discussed these enzymes in terms of their modification of various cytoskeletal and membrane proteins. These actions may be involved in regulating the reorganization and disassembly of adhesion plaques and stress fibres; for example, during mitosis or in response to growth factors or transforming viruses. This may be just a small part of the regulatory activity that occurs at these sites. Little is known about the larger question of the signal transduction that occurs at adhesion plaques, and which may also involve enzymes such as the tyrosine kinases that have been identified in these regions. Cells respond in dramatic ways when cultured on different extracellular matrices and cell substrata. Cells that need one set of growth factors when cultured on one substratum may lose this requirement or require different growth factors when cultured on a different substratum (reviewed by Burridge, 1986). Elucidating the signals and second messages generated at adhesion plaques in response to specific extracellular matrix components is an exciting prospect for the future.

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