Regulation of protrusive and contractile cell-matrix contacts

Josephine Clare Adams
MRC Laboratory for Molecular Cell Biology and Department of Biochemistry and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK
(e-mail: dmcbjca@ucl.ac.uk)

Summary

The extracellular matrix is vital for tissue organisation in multicellular organisms. Cells attach to the extracellular matrix at discrete points on the cell surface, termed cell-matrix contacts. In general molecular terms, these contacts are assembled from large multiprotein complexes. However, many forms of matrix contacts can be distinguished by microscopy or by biochemical criteria, and these fulfil a diverse range of roles associated with cell adhesion, guidance, migration, matrix assembly, differentiation and survival. Two major functional categories are the protrusive and contractile matrix contacts. I describe contexts for the formation of protrusive or contractile contacts and discuss recent information on the molecular processes by which these contacts are specified, coordinated and regulated at a cellular level.

Key words: Cell adhesion, Extracellular matrix, Cytoskeleton, Signaling, Cell structure

Introduction

Cell-matrix contacts are specialised points on the cell surface where cells are attached to the extracellular matrix (ECM) by specific adhesion receptors that are also linked intracellularly to the cytoskeleton and signalling pathways. Such contacts are fundamental aspects of cell and tissue organisation and are present throughout evolution, even in the most primitive of metazoan organisms. For example, specific cell recognition in Porifera is mediated by cellular interactions with ECM complexes (Fernandez-Busquets and Burger, 1999). Formation of the body wall of Hydra depends on contacts between two layers of epithelial cells and a prototypic basement membrane, the mesoglea (Sarras et al., 2001). The adhesion systems of vertebrates involve far more complex sets of matrix macromolecules (Hynes, 1999), and research on cells from vertebrate tissues has led, over the years and with increasing technological sophistication, to the documentation of numerous types of matrix contacts according to their morphology, ultrastructure, biochemical composition or a combination of these criteria.

The first matrix adhesion receptors, integrins, were cloned in 1986. Subsequent investigations at the molecular level have revealed a multiplicity of roles for matrix-integrin interactions in the regulation of cell behavior. Cell-matrix interactions maintain and regulate cell adhesion and motility, initiate ECM assembly at cell surfaces, act as sites for transmission of mechanical force and elastic recoil between cells and ECM, localise and activate signaling molecules that regulate cell proliferation, behavior and survival and regulate gene expression (reviewed by Schwartz et al., 1995). Specificity of these activities is apparent with regard to individual matrix components and differentiated cell lineages (De Fougerolles et al., 2000; Danen et al., 2000). It is now appreciated that transmembrane proteoglycans and integrin-associated proteins also participate in these roles (Woods and Couchman, 2000; Hemler, 1998). However, there remains a gap in integrating this molecular information to the evident diversity of matrix-contact structures and their 3D forms in intact cells. What are the molecular requirements for formation of different types of matrix contacts? What factors determine the subcellular distribution and dynamics of individual matrix contacts? How does matrix specificity operate at the level of assembly of contact structures? How do single cells coordinate or polarise the formation of different types of matrix contacts? How is the assembly of different forms of contacts important for transduction of information from the ECM?

There are many reasons why answers to such questions are of fundamental importance. In addition to the inherent fascination of biological patterning, routes to manipulate cell phenotype or motile behavior, without gross perturbations of cell-matrix adhesion that would compromise cell viability, are desirable goals for biological therapies of acute and chronic human diseases. An understanding of how matrix contacts are assembled and coordinated by cells could open up new and elegant strategies to manipulate cell locomotion, matrix assembly, or cell interactions in tissue engineering applications. In this review, I briefly describe the various types of matrix contacts that have been identified by morphological or biochemical criteria and discuss recent advances in understanding the molecular processes by which matrix contacts are coordinated and regulated by cells.

Types of cell-matrix contacts

Four general structural elements constitute a functional cell-matrix contact (Fig. 1A). On the extracellular face of the contact, specific matrix-adhesion receptors bind to their cognate matrix ligands on the extracellular face of the contact. This extracellular interaction is sufficient to support high affinity ligand binding and static cell attachment. However,
more complex activities depend on the transduction of mechanical force and signaling information through the intracellular coupling of adhesion receptors to the cytoskeleton (Orlando and Cheresh, 1991; Burridge and Chzanowska-Wodnica, 1996; Huang and Ingber, 1999). In most types of matrix contact, this connection is made to the actin microfilament system. Typically, the physical coupling is achieved through receptor clustering and the binding of receptor cytoplasmic domains to intracellular proteins to form large multiprotein scaffolds that interact, directly or indirectly, with cytoskeletal polymers and also connect to signaling pathways. The group of intracellular proteins that is located between the adhesive receptors and the cytoskeletal filaments is termed a linker complex in this article.

A rational scheme for grouping the most extensively studied matrix contacts into three functional categories, protrusive, contractile and mechanically supportive, is presented in Figure 1B. Within this diversity of contacts, distinct, although to some extent overlapping, sets of proteins make up each of the structural elements (reviewed by Adams, 2001). Protrusive contacts are those in which dynamic extensions of the plasma membrane make contact with the matrix. These regions can be very localised and of highly defined shape, as in filopodia, spikes or podosomes. Alternatively, they can occupy broad, irregular regions of the plasma membrane as in the case of lamellipodia and associated ruffles, invadopodia or pseudopodia. Protrusive contacts are considered to be needed as membrane extensions by which cells make transient adhesions, sample the surrounding environment beyond the cell body and maintain a polarised leading edge during cell locomotion.

Contractile contacts, exemplified by focal adhesions and the various types of matrix assembly sites, make physically closer and/or more prolonged matrix attachments than protrusive contacts. By engaging actomyosin-based cellular contractility, these contacts transmit the isometric tension between cells that is needed for matrix assembly, the maintenance of stable cell-matrix adhesion and the cyclical traction and release of the cell body during cell locomotion (Lauffenburger and Horwitz, 1996; Zamir et al., 2000; Riveline et al., 2001). Hemidesmosomes (Borradori and Sonnenberg et al., 1999) and retraction fibers (Chen, 1981; Lin et al., 1996) form mechanically supportive contacts that maintain matrix adhesion and stabilise the plasma membrane under conditions of varying mechanical force or stabilise the rear end of cells against tail release during cell crawling.

**Cellular coordination of matrix contacts**

Matrix contacts such as hemidesmosomes and podosomes are specific to particular differentiated cell lineages. Many cell types assemble other matrix contacts, such as filopodia, spikes, lamellipodia and matrix assembly sites (Fig. 1B). The mechanisms of contact assembly have principally been studied in fibroblasts and endothelial cells using 2D surfaces coated with matrix molecules. Under these conditions, newly attaching cells undergo a sequential interconversion from protrusive to contractile matrix contacts. Initial cell tethering and exploration by filopodia and spikes is followed by lamellipodia-mediated spreading and ruffling membrane activity and, as cells spread fully, by the development of contractile focal adhesions (Fig. 2A-D). With time, the endogenous matrix is secreted by the cells, and matrix assembly sites predominate (Fig. 2E) (Mosher et al., 1992; Zamir et al., 2000; Riveline et al., 2001). These non-polarised transitions of spreading cells correspond in many ways to the polarised and cyclical reorganisation of protrusive and contractile contacts that are crucial for cell locomotion on 2D matrix surfaces and are widely accepted as generic cell behaviours (Lauffenburger and Horwitz, 1996).

However, other cell types, such as keratinocytes, leukocytes and monocytes, typically form few focal adhesions even in tissue culture. In the case of monocytes and leukocytes, adhesion and migration on a planar matrix is achieved through formation of podosomes (Marchisio et al., 1987; Marchisio et
Regulation of matrix contacts

Images of fibroblasts, leucocytes or embryonic cells in a 3D matrix, where the form of the ECM is fibrillar and discontinuous rather than sheet-like and rigid, reveal a striking topography in which localised protrusive contacts and matrix assembly sites predominate (e.g. Heath and Peachey, 1989; Friedel et al., 1998). Time-lapse movie sequences show that fibroblasts in collagen gels, continuously but slowly, extend and retract filopodial and pseudopodial projections (Roy et al., 1999). This behavior may relate to, or depend on, the tactile sensing properties that enable selective alterations in fibroblast adhesion and migration in response to surface rigidity or microtopography (Lo et al., 2000). A similar reliance on protrusive contacts is apparent in embryonic cell migrations, for example, in the movement of neural crest cells in amphibian embryos (Davidson and Keller, 1999) or the migration of primary mesenchymal cell in sea urchin blastulae (reviewed by McClay, 1999). Cancer cells have increased propensities to form lamellipodia, spikes and specialised protrusive contacts, termed invadopodia, which localise matrix-degrading proteases and are thought to contribute to invasive cell motility (reviewed by Chen and Wang, 1999).

Notwithstanding these indications of rich diversity, understanding of the formation and functions of protrusive contacts has remained patchy. Reliance on morphological characterisations and static images of these dynamic and non-stereotypical structures, plus a lack of robust experimental systems in which to study particular structures under well controlled conditions, has resulted in a multiplicity of interpretations and parallel nomenclatures. An increasing knowledge of biochemical markers that are specific to particular matrix contacts, in combination with advances in imaging technologies, is now beginning to dispel these problems, to foster an upsurge in interest in all forms of matrix contacts and to provoke new questions about the mechanisms by which contact assembly and turnover are attuned to cell behavior.

Mechanisms of contact regulation

The regulation of matrix contacts in intact cells is highly complex and involves both the turnover of single contacts and the integration of assembly and function of different types of contacts with the status of cell activity. I discuss below recent information on molecular processes that are involved in contact initiation, turnover and the integration of protrusive and contractile contacts.

Initiation and spatial segregation of contacts

To assemble a functional matrix contact, the association of all the necessary molecular components and an appropriate spatial placing of the contact is needed (Fig. 3). These processes are best understood for integrin-mediated adhesions. To bind to ligand, integrin αβ heterodimers must be in an active conformation (Fig. 3b). Ligand binding in itself alters integrin conformation and affinity and, in the case of multivalent ligands, integrin clustering. The conformational change in the extracellular domain of integrin induces activation of the αβ cytoplasmic domains, which then coordinate de novo assembly of the linker complex in a process termed outside-in signaling (reviewed by Calderwood et al., 2000). Many integrins are constitutively active, whereas others, principally the β2 fibrillar matrix (H). Under conditions of high adhesion, retraction fibres are often associated with contractile contacts (J). (K.L) In glycosaminoglycan/proteoglycan-mediated adhesion, filopodia, spikes and lamellipodia are the predominant contacts. (L) A cell stained with TRITC-phalloidin. Bar in J, 4 μm; K.L, 5 μm. Panels A-C reproduced from Rajaraman et al (Rajaraman et al., 1974), D-H from Witkowski and Brighton (Witkowski and Brighton, 1971), J.K from Laterra et al. (Laterra et al., 1983) with permission of Academic Press.
integrins of leukocytes and αIIbβ3 of platelets, require cellular activation signals. In both cases, ligand-binding capacity is further modulated or activated by cell-type specific, inside-out signals, which act either on the linker complex or the integrin subunit cytoplasmic domains to bring about a conformational shift in the extracellular ligand-binding site. These processes are termed integrin inside-out signaling (reviewed by Woodside et al., 2001). In vitro, activation of extracellular ligand binding can be achieved by a spatial separation of the intracellular, juxtamembrane regions of the heterodimer subunits (Takagi et al., 2001).

The cellular localisation of active integrins has been analysed with an engineered Fab moiety that acts as a specific reporter of unoccupied, active αvβ3 integrin (Pampori et al., 1999). During endothelial cell spreading on fibrinogen, active αvβ3 integrins localise at the margins of lamellipodia before they become ligand occupied and concentrated in focal adhesions. The activation of αvβ3 depends on phosphatidylinositol-3-kinase, whereas spatial restriction depends on the small GTPase Rac (Kiosses et al., 2001). These observations focus attention on the subcellular distribution of active integrins as an early determinant for the spatial

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**Fig. 3.** Mechanisms of matrix contact assembly and remodelling (A) in a podosome and (B) in a focal adhesion. The inset in B shows the positive and negative actions of protein complexes formed on paxillin subsequent to phosphorylation of residues Y31 and Y118 by focal adhesion kinase. The green circle and yellow oblong represent proximal components of the focal adhesion linker complex such as talin and filamin. Asterisks indicate activated forms of c-Src, Pyk2 and Pak.
positioning of integrin-dependent matrix contacts. Also of interest are the mechanisms for the local sequestration and activation of signaling molecules that coordinate this process (Fig. 3B). At the biochemical level, matrix adhesion stimulates a membrane association of Rac that is needed for activation of its effector, p21-activated kinase (PAK) (Del Pozo et al., 2000).

Whether spatially localised activation of adhesion receptors and downstream signals occur during the initiation of other types of matrix contacts is a question of great interest. The assembly of a cell protrusion has special additional requirements for localised polymerisation of F-actin, concentration of actin-crosslinking proteins and expansion of the plasma membrane to mediate the outward extension of the contact. Delivery of mRNAs for actin and actin-crosslinking proteins to lamellipodial margins and spikes may also facilitate extension of the membrane and cytoskeleton by translation of essential molecules at their sites of function (reviewed by Bassell and Singer, 1997). In general, actin nucleation occurs at the edges of lamellipodia and the tips of filopodia and is coordinated by the activities of the Arp2/3 complex to produce Y-branched actin filaments (reviewed by Borisy and Svitkina, 2000). Lamellipodia are also sites of enhanced membrane trafficking, and the localised delivery of vesicles may assist rapid increases in local membrane surface area and the maintenance of polarity in protrusive contacts (reviewed by Mellman, 2000). Localisation of vesicle docking also depends on cytoskeletal interactions; for example, the GTPase dynamin, which is active in endocytic vesicle trafficking, also localises at membrane ruffles where its interaction with the actin-binding protein cortactin has consequences for cell shape (McNiven et al., 2000). Other recent studies place dynamin within the podosomes of osteoclasts (Ochoa et al., 2000).

Although transmembrane proteoglycans, exemplified by syndecan-4, have traditionally been considered as coreceptors with integrins in contractile contacts (reviewed by Woods and Couchman, 2000), there is growing evidence for early roles of glycosaminoglycan-based interactions and proteoglycans in the formation of initial protrusive contacts. Glycosaminoglycan-dependent attachment of cells has long been associated with the formation of microspikes and lamellipodia (Fig. 2K). The stable formation of fascin microspikes upon cell adhesion to thrombospondin-1 requires the transmembrane proteoglycan syndecan-1 and is dependent upon intracellular coupling by the syndecan-1 cytoplasmic domain (Adams et al., 2001) (Fig. 2L). Layilin, a transmembrane protein that is concentrated in the ruffles of spreading or migrating cells, binds to hyaluronan, a glycosaminoglycan that contributes to the hydration and fluidity of pericellular matrix. Layilin binds to talin intracellularly and is proposed to mediate transient matrix adhesions in lamellipodia (Bono et al., 2001). It would be interesting to know how the roles of proteoglycans in protrusive contacts relate to those of active, unoccupied integrins at lamellipodial margins.

Self-attenuating protein complexes

A second key aspect of regulation is that, once assembled, matrix contacts can be turned over to enable their repositioning or interconversion. This is important for maintaining adhesion and spreading during changes in cell shape, locomotion or physiological activity and for enabling cell rounding during mitosis (Fig. 2; Fig. 3). Information emerging from studies of podosomes and focal adhesions demonstrates that proteins within the linker complex ensure that contact growth is self limited. This is elegantly achieved by their participation in self-attenuating protein complexes. In osteoclasts, podosome formation depends on signaling from the αβ3 integrin, with Src and RhoA as key mediators (Chellaiah et al., 2000) (Fig. 3A). Thus Src-null mice develop osteopetrosis owing to poor osteoclast function (Soriano et al., 1991). In culture, Src-null osteoclasts initially form podosomes, but these are replaced within hours by focal-adhesion-like structures. The cells migrate poorly and do not undergo proper formation of the sealing-ring structures that are needed for bone resorption (Sanjay et al., 2001). The molecular mechanism that underlies these abnormalities in matrix contacts depends on Pyk2 (proline-rich tyrosine kinase 2), which is expressed at particularly high levels in osteoclasts and is activated proximally in αβ3-initiated signaling. In normal osteoclasts, this leads to formation of a protein complex that contains autophosphorylated Pyk2, c-Cbl and activated Src; Pyk2 and c-Cbl both bind to Src. c-Cbl binds through its PTB domain to the autophosphorylation site of Src (residue Y416), thus acting, in the complex, to inhibit Src kinase activity and αβ3-mediated cell attachment (Sanjay et al., 2001). These findings place the Cbl-Src interaction as a feedback mechanism, which achieves the rapid turnover of podosomes by ensuring that Src kinase activity and thereby Src-dependent inside-out activation signals to the αβ3 integrin are time limited (Fig. 3A). Without this process, podosome turnover, and therefore osteoclast migration and bone resorption, are impaired (Sanjay et al., 2001).

Within the linker complex of focal adhesions, paxillin has emerged as an important coordinator of multiple protein complexes that make regulatory contributions to the assembly and disassembly of focal adhesions and to cell motility (reviewed by Turner, 2000). Paxillin binds to multiple structural and signaling molecules through its N-terminal domain, which contains four LD motifs, and through the C-terminal region, which contains four LIM (Lin-11, Isl-1, and Mec-3) domains. One protein complex is initiated by the binding of FAK to the second LD motif and the subsequent phosphorylation of residues Y31 and Y118 of paxillin by FAK. These newly created SH2-binding sites bind to Crk proteins, which recruit the Src substrate p130cas after its phosphorylation by Src. P130cas is also the major substrate of PT-PEST (protein tyrosine phosphatase-PEST), which becomes recruited onto LIM domains 3 and 4. The presence of PT-PEST within the linker complex may provide a mechanism for focal-adhesion disassembly by dephosphorylation of p130cas. Paxillin residues Y31 and Y118, and PT-PEST are also involved in a second set of binding interactions that recruit Csk kinase into focal adhesions. Csk phosphorylates and downregulates Src kinase activity and thus may, in a manner analogous to the activity of c-Cbl in podosomes, initiate local attenuation of contact assembly processes (Fig. 3B).

A third protein complex, which is formed around the LD motif 4 of paxillin, has a separate role in localising and regulating the activities of PAK (p21-activated kinase). PAK is an important downstream effector of the small GTPases Cdc42 and Rac, which has roles in cytoskeletal organisation and cell migration (Kiosses et al., 1999). A novel protein, p95PKL (paxillin-kinase linker), acts as adaptor between LD motif 4
and the Cdc42/Rac guanine nucleotide exchange factor, PIX. PIX binds directly to PAK, and PAK brings Nck into the complex (Turner et al., 1999). This complex supports PAK kinase activity, and experimental perturbations of the complex on LD motif 4 have inhibitory effects on lamellipodia formation and cell migration (Turner et al., 1999). In particular, expression of an LD4-deleted form of paxillin in fibroblasts alters the parameters of spreading, such that cells have enlarged and persistent lamellipodia and retraction fibers as well as increased random migration on fibronectin (West et al., 2001). These effects correlate with sustained Rac activation, are Rac-dependent and are phenotypically similar to the effects of a mutant form of p95PKL that does not bind to paxillin (West et al., 2001). Assembly of the paxillin-PKL complex thus marks a contribution of paxillin to the assembly of protrusive contacts, perhaps by localising PAK activity, as well as to the turnover of focal adhesions (Fig. 3b).

Cellular integration of contact remodeling

The regulatory processes I have discussed so far act from within a matrix contact or localised membrane area. Mechanisms for the integration, balance and remodeling of matrix contacts are also needed at the whole cell level and, in this regard, molecular processes involving the small GTPase Rho, microtubules and regulated proteolysis are all emerging as significant.

The activities of Rho

Rho GTPase has a key role in stimulating focal adhesion assembly through the activation of cell contractility (reviewed by Ridley, 1997). Conversely, inhibition of Rho activity by C3 exotranserase or by overexpression of RhoGAP (GTPase activating protein) or by Rho GDI (GDP dissociation inhibitor) reduces cell spreading and focal contact organisation and promotes the formation of unusual arborised cell projections (Noren et al., 2000; Anastasiadis et al., 2000). However, initial cell spreading and formation of protrusive contacts on fibronectin depend on the activities of Cdc42 and Rac and not on Rho (Clark et al., 1998; Price et al., 1998). During initial spreading on fibronectin, cellular Rho activity is decreased (Ren et al., 1999). The stable formation of cortical fascin spikes and lamellipodia by cells adherent on thrombospondin-1 also depends on Cd42 and Rac and is not inhibited by dominant-negative versions of Rho. Interestingly, Rac and Cdc42 activities are sustained over longer times in cells on a TSP-1 matrix than in matched cells on fibronectin (Adams and Schwartz, 2000). Inclusion of tenasin-C in a mixed fibronectin-fibrinogen provisional matrix also results in decreased Rho activity (Wenk et al., 2000). The existence of similar biochemical effects in different matrix contexts suggests that modulation of Rho activity could participate in the integration of protrusive and contractile matrix contacts. This is a nascent area of study, and it is evident that there are multiple molecular pathways by which Rho can be inhibited.

In suspended cells treated with the GRGDSP peptide, a monomeric ligand that activates integrins but does not induce integrin clustering, downregulation of cellular Rho activity depends on the phosphorylation and activation of p190RhoGAP by Src, leading to an increased rate of RhoGTP hydrolysis (Arthur et al., 2000). Analyses of adherent cells have highlighted novel activities of p120 catenin (p120ctn), a cadherin-binding armadillo repeat protein that is also a substrate of Src, in suppression of Rho. Overexpression of p120ctn in fibroblasts or epithelial cells leads to formation of filopodia, branched cellular processes, lamellipodia and a major reduction in focal adhesions. This activity is a cadherin-independent property of cytoplasmic p120ctn, which is suppressed when p120ctn associates at the membrane with E-cadherin (Anastasiadis et al., 2000; Noren et al., 2000; Grosheva et al., 2001). Regions within the N-terminal domain, armadillo repeats 3-5 and the C-terminus of p120ctn are each important in the Rho inhibitory properties of p120ctn (Grosheva et al., 2001), and several molecular interactions have been defined that contribute to Rho inhibition. One is a specific GDI activity of p120ctn for RhoGDP (Anastasiadis et al., 2000; Noren et al., 2000). A second process appears to depend on activation of Cdc42 and Rac by p120ctn, in that overexpression of p120ctn increases the active pools of both GTPases and the morphological effects of p120ctn are blocked by dominant-negative versions of Cdc42 and Rac (Noren et al., 2000; Grosheva et al., 2001). This mechanism appears to depend on the physical association and activity of the Rac/Cdc42 GEF (guanine nucleotide exchange factor), Vav2, with p120ctn (Noren et al., 2000).

Microtubule targeting

Microtubule depolymerisation in adherent cells results in an increased size and number of focal adhesions, increased integrin signaling and decreased lamellipodial protrusion and cell migration (Bershadsky et al., 1996). These effects depend on Rho GTPase activity (Liu et al., 1998). Time-lapse videomicroscopy of cells colabelled for tubulin and focal adhesion components has now revealed that focal complexes and focal adhesions are actively and transiently targeted by the distal (plus) ends of individual microtubules and that this process results in the stasis or disappearance of contacts (Kaverina et al., 1999). In the absence of microtubules, cells develop large retraction structures; thus negative regulation by microtubules probably also contributes to focal-adhesion disassembly during retraction of the rear edge of locomoting cells (Ballestrem et al., 2000).

The molecular basis for selection of particular focal adhesions by microtubules and the mechanism of contact dissolution both remain to be established. Proteins that specifically associate with the distal ends of microtubules could conceivably regulate microtubule dynamics to facilitate targeting of focal adhesions. The identification of paxillin as a novel tubulin-binding protein raises the possibility that paxillin could act as a focal adhesion recognition protein (Herreros et al., 2000). Tubulin dimers bind to Rac GTP and thus might disassemble the contacts by a direct Rac-dependent inhibition of Rho activity (Saunder et al., 1999). High-resolution methods to localise active Rho are needed to examine this possibility.

The principle of negative regulation by microtubules does not apply to other types of matrix contacts. Lamellipodia characteristically contain few microtubules, and the growth or taxol-stabilisation of microtubules activates Rac and leads to lamellipodial protrusion (Waterman-Storer et al., 1999). However, microtubules themselves are not essential for
protrusion of lamellipodia and ruffles (Ballestrem et al., 2000). In complete contrast, the formation of podosomes in macrophages is microtubule dependent (Linder et al., 2000). It would be interesting to know if this process also involves dynamic physical targeting of podosomes by microtubules. The relationship of microtubule targeting to specific matrix contexts has not been explored; for example, it is not known whether focal adhesions nucleated on different integrin heterodimers have equivalent sensitivities to microtubule targeting or whether proteoglycan-dependent contacts are also physically regulated by microtubules.

Regulated proteolysis

A third mode of contact turnover is provided by regulated proteolysis (Fig. 3D). Intracellular components of focal adhesions, which include the β3 integrin subunit cytoplasmic domain and talin, are substrates of the calcium-dependent protease, calpain (Beckerle et al., 1987; Du et al., 1995; Bialkowska et al., 2000). Calpain-mediated proteolysis contributes to focal-adhesion turnover and is needed for the release and retraction of the tail end of migrating cells and for the complex regulation of αIIbβ3 integrin activity in platelets (Huttenlocher et al., 1997; Schoenwaelder et al., 2000). Calpain also acts in the formation of filopodia and lamellipodia, presumably by facilitating rapid reprocessing of cytoskeletal structures, although the critical target substrates in these structures remain to be identified (Potter et al., 1998). The possible role of proteolysis in regulation of other types of matrix contacts deserves attention.

Future prospects

To unravel and harness the cellular processes that coordinate matrix contacts, a clear molecular definition and an understanding of all types of matrix contacts are first necessities. It is not obvious that the full range of matrix contacts formed by cells is known. Careful studies of normal differentiated cell types or the activities of less-studied, non-ubiquitous matrix components could reveal new types of matrix contacts or create new paths to rational understanding of currently obscure cell-surface features. New techniques are needed to facilitate study of cells in 3D matrices, which is an appropriate context for protrusive contacts and matrix-assembly sites; these are now being developed. However, the power and convenience of 2D matrix surfaces are such that 2D matrix conditions to initiate assembly of protrusive contacts offer important experimental advantages. Methods that measure protein activity and interactions in time and space are needed to elucidate the mechanisms that govern contact placement or contact interconversions. Such methods are now being developed as FRET-based (fluorescence resonance energy transfer) technologies in adherent cells (Ng et al., 1999; Kranyov et al., 2000), and these are likely to expand knowledge of the processes that determine the location and 3D form of matrix contacts. Other technological developments, including the use of beads embedded in flexible matrices, provide precise measurements of cellular contractile forces (Lo et al., 2000). The application of defined mechanical forces to cells on malleable surfaces provides information on the responses of cells to semi-physiological matrix conditions (Parsons et al., 1999). For both 2D and 3D matrices, tracking matrix-contact components as fluorescently tagged proteins over time is a valuable approach to monitoring the turnover and interrelationships of contacts (e.g., Zamir et al., 2000). Matrix contacts are unique as cellular structures in that their function depends on the assembly of multiprotein complexes on both sides of the plasma membrane. At the cell or tissue level, a view of matrix contacts as organised and dynamic functional entities should facilitate further study of cell adhesion systems as effectors of morphoregulatory processes in development, homeostasis and disease.

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References


