CMLS, Cell. Mol. Life Sci. 58 (2001) 371–392
1420-682X/01/030371-22 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Cell-matrix contact structures

J. C. Adams

MRC Laboratory for Molecular Cell Biology and Department of Biochemistry and Molecular Biology, University College London, Gower Street, London, WC1E 6BT (UK), Fax: +442076797805, e-mail: dmcbjca@ucl.ac.uk

Received 7 September 2000; received after revision 4 October 2000; accepted 6 October 2000

Abstract. Cell-extracellular matrix contacts are points on cell surfaces where adhesion receptors tether cells to matrix and are linked intracellularly to cytoskeletal components. These structures integrate cell organisation within tissues, support cell motility and specialised activities of differentiated cells, and transduce extracellular signals. Current characterisations of matrix contacts are based on morphological and biochemical criteria, yet the levels of definition of different contact types are very varied. Some contacts are surprisingly little-studied given their likely importance in vivo. Here, I describe the general features of matrix contacts, review the functions and molecular composition of major types of transient and stable matrix contacts, and discuss the information that is emerging on contact integration and dynamics in single cells.

Key words: Extracellular matrix; cell adhesion; filopodia; podosome; focal adhesion; matrix assembly; signalling.

Introduction

Cell-extracellular matrix contact structures are spatially restricted sites on the cell surface at which specific adhesion receptors bind extracellular matrix (ECM) and link intracellularly to cytoskeletal components. These contacts are fundamental features of cells in metazoan organisms. Cell-matrix contacts serve several major purposes: to anchor elements of the ECM at the cell surface; to form the continuous physical linkage between ECM and elements of the cytoskeleton that is needed for cell adhesion and locomotion; to act as localised sites for transmission of mechanical force and elastic recoil between cells and extracellular matrix, and to act as sites for localised activity of signalling molecules. They are thus key components in the integration and organisation of cellular and acellular elements within tissues. Currently, characteristions of contacts are built on morphological and biochemical criteria. Although information on the molecular make-up and regulation of certain types of contacts is substantial, others are less well defined. We also lack a coherent view of how cells regulate and integrate different types of contacts, as three-dimensional structures and according to cell-functional needs.

There are numerous types of matrix contact structures assembled according to matrix context, differentiated cell type and cell activity status. In this review, I will outline the general structural elements of a matrix contact and then discuss the different types of contacts and their molecular components. The contacts are grouped as transient or stable contacts, although in reality this is not an absolute distinction. I will also indicate how contacts that have been defined in cell culture systems are related to cell-matrix contacts within tissues. The final sections discuss mechanisms by which contact formation is coordinated and dynamically regulated within single cells and indicate areas of future research interest.

General features of cell-matrix contacts

The principal structural elements of a cell-matrix contact are shown in Figure 1. On the extracellular face, matrix macromolecules bind to specific adhesive receptors

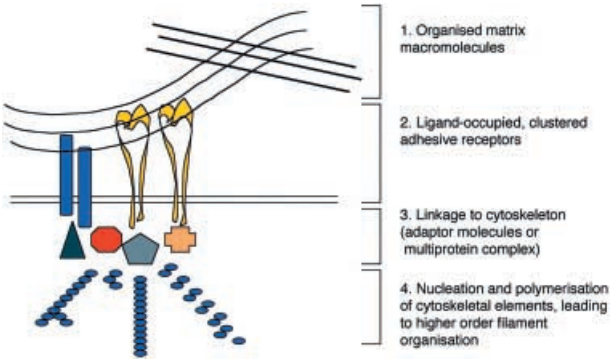

Figure 1. Schematic representation of the general structure of matrix contacts. Not to scale.

which are typically transmembrane or glycosylphosphatidylinositol-linked glycoproteins. On the intracellular side of the plasma membrane, the cytoplasmic domains of receptor molecules interact with either (i) cytoplasmic proteins which provide linkage to cytoskeletal filaments, or (ii) larger sets of cytoplasmic proteins which cluster by protein-protein interactions to form submembranous junctional complexes which then interface with cytoskeletal filaments.

The scale of organisation, stability and elaborateness of these essential elements has determined the accessibility of the contact to characterisation by morphological criteria. Widely used criteria have included descriptions of phase-dark structures detected by phase contrast or interference reflexion light microscopy (IRM), electron-dense and 'organised' structures detected by transmission electron microscopy, and descriptions of cell surface topography by scanning electron microscopy (SEM). These techniques have enabled characterisation and analysis of the largest and most stable types of contacts which include focal adhesions, focal contacts or adhesion plaques, fibrillar adhesions and hemidesmosomes (table 1, fig. 2). These structures have remained a focus of attention, not only because they are obvious specialisations at the plasma membrane, but also because they form recognisable sites where elements of the matrix coalign with elements of the cytoskeleton: the morphological features thus fulfill the experimentalist's intuitive expectations of a structure which anchors cells in place. These expectations have been upheld by recent functional, biochemical and genetic investigations of these contacts.

Other contact structures have been less accessible to identification by these criteria because they are smaller, transient, or of restricted distribution. These contacts include filopodia, spikes, lamellae, podosomes and pseudopodia (table 1, fig. 2). Although the first descriptions and namings of transient contacts and projections can be found in mid-twentieth-century cell biology literature [e.g. ref 1], for the most part, the inherent variability of these structures has made them harder to analyse. Their study, and an appreciation of their contact roles, has progressed according to recent advances in experimental methods, in particular the availability of appropriate immunological rea-

Table 1. Characterisation of cell-matrix contact structures.

Contact type	Dimensions	IRM Image/separation from substratum	Characteristic associations
Close contact	(associated with lamellipodium)	grey in IRM 30–50 nm from substratum	submembranous densities parallel to F-actin meshwork at plasma membrane
Filopodium	20–200 µm long 0.2–0.5 µm diameter	grey in IRM	core bundle of F-actin, integrins, syndecans
Focal contact/ focal adhesion/	0.25 µm wide 1.5 µm long	black in IRM 10–15 nm from substratum	at termini of microfilaments, contain integrins, syndecan-4, low tensin content
Hemidesmosome	plaque ca. 0.15 µm by 0.04 µm	–	connect to intermediate filaments, contain $\alpha 6\beta 4$ integrin, plectin, BP230
Matrix assembly sites/ fibronexus/ fibrillar adhesions	ca. 3–5 µm long	white in IRM 100 nm from substratum	ECM cables align parallel with microfilaments, contain $\alpha 5\beta 1$ and tensin
Podosomes	0.2–0.4 µm diameter	dark in IRM	core bundle of actin perpendicular to substratum; in macrophages contain $\beta 2$ integrins, fimbrin
Spike or microspike	2–10 µm long 0.2–0.5 µm diameter	grey in IRM	core bundle of F-actin, contain fascin

ECM, extracellular matrix; IRM, interference reflexion microscopy; See text for details of molecular associations.

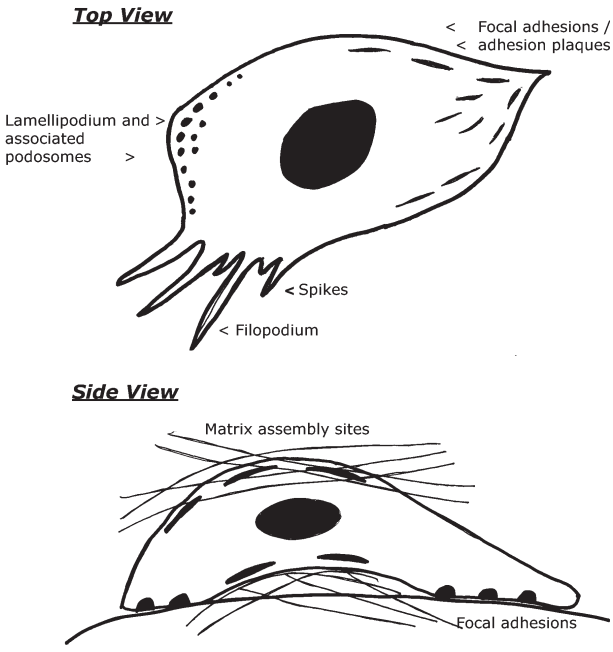

Figure 2. Schematic representation of the major contact types formed by tissue culture cells. See table 1 for details of contact characteristics. Not to scale.

gents, development of experimental conditions that optimise the formation of these structures, and the use of green fluorescent tracking (GFP) tracking of molecules in conjunction with time-lapse and real-time digital imaging in living cells to obtain a coherent view of dynamic structures.

Transient matrix contact structures

Cells in culture undergo continuous dynamic shape changes [2]. In particular, major changes occur during cell division, phagocytosis or directed cell locomotion. In the latter case, cells develop a clear front and rear polarity and undergo cyclical changes in shape [reviewed in refs 3, 4]. However, even stationary cells show activity at points on the cell surface. Many of these changes are associated with the formation and remodelling of matrix-contact structures, and include protrusion of filopodia, spikes and lamellae, lateral or apical ruffles, or pseudopodia. Given their dynamic nature, it is fortunate for experimentalists that a number of these matrix contacts are formed in a temporal sequence after the initial contact of a suspended cell with glass, fibronectin, vitronectin or other matrix components [5–7]. Other adhesion conditions, such as thrombospondin-1, subdomains of fibronectin, or other types of adhesive surface, stabilise the formation of spikes, lamellae and ruffles and so amplify stages within the sequence [8–12]. Certain differentiated cell types have a high propensity to form a particular type of contact, for example the formation of filopodia on nerve cell growth cones [13]. These experimental contexts have opened up new possibilities for studying transient contacts in depth. Here, I discuss the contacts in the sequence in which they would be formed by a newly attaching cell.

Filopodia

Fine extensions at cell surfaces have been noted since cell morphology and behaviour in tissue culture were first studied by light microscopy [1]. These structures have been given a variety of names which seek to capture their essence: fibrous or finger-like projections, spikes, threads, microfibrils, microextensions, or filopodia. Here, the term filopodia (thread feet) is used to refer to actin-containing extensions of around 0.2 μm diameter which extend for between tens of and several hundred microns from the cell surface (table 1, fig. 2). Filopodia are particularly prominent during the initial spreading of fibroblasts on two-dimensional matrix. As fibroblasts attach on glass, filopodia are extended over the substratum around the cell body [5–7]. Use of glass and gold- patterned substrata revealed that filopodia first transiently scan the substratum and then form substratum attachments at their tips. Broadening and branching of filopodia tips give rise to localised adhesion structures termed footpads, where fibronectin, proteoglycans and other matrix components are deposited [9, 14]. These adhesion events are followed by cell spreading mediated by extension of the cell membrane at footpads, or by protrusion of a cytoplasmic sheet, termed a lamella or lamellipodium, between adherent filopodia [7, 9]. These observations led to the proposal that filopodia might primarily function in sensory guidance, adhesive selection and the establishment of cell polarity [7].

Filopodia are also formed in abundance on growth cones, and neuronal cells have been widely adopted as model systems in which to study the dynamics of filopodia and their contacts with extracellular matrix. Neurite outgrowth is stimulated by cell attachment to many matrix components, including laminins, thrombospondin-1, tenascin, fibronectin and heparin-binding growth-associated molecule (HB-GAM) [15–17]. Experiments with patterned matrix surfaces demonstrate roles for filopodia in guidance, selective adhesion and motility. Ablation of filopodia affects growth cone navigation and motility [15, 16]. The ability of cells to form filopodia is strongly influenced by the matrix context and thus presumably by the engagement of particular adhesion receptors. At defined matrix boundaries, for example between tenascin and fibronectin or laminin, preferential extension and adhesion of filopodia mediates growth cone attachment and turning [18, 19]. Other matrix components, in particular chondroitin sulphate proteoglycans, repel growth cones by inhibiting the attachment of filopodia [20, 21].

Filopodia attach to matrix at their tips, or at points along their length. Once attached, they form additional discrete adhesions termed point contacts that may enhance motility by resisting cell contractility [22, 23]. On laminin matrix, these contacts are enriched in β1 integrins, vinculin and phosphotyrosine-containing proteins and have structural and signalling components in common with focal contacts and podosomes [24, 25]. Each filopodium contains a core of unipolar actin filaments that are nucleated at the filopodial tip and bundled along their length by proteins such as fascin and filamin [26–28]. The actin-associated protein Mena localises at filopodial tips and the assembly of filopodia depends critically on the activities of the small guaninine 5′-triphosphatases (CTPases) Rac and Cdc42, and their effectors cofilin and p21-activated kinase 4 (PAK4) [29–34]. Of these regulators, Cdc42 promotes actin nucleation through the actin-related protein 2 and 3 complex (Arp2/3 complex) [35]. Actin filament elongation is also needed to produce filopodia and neural Wiskott-Aldrich syndrome (WAS) protein (N-WASP) and profilin may participate in this process [36, 37]. To initiate a filopodium, these general processes of actin assembly must be under tight spatial regulation and closely coupled to filament cross-linking. Adhesion receptors are obvious candidates to provide such spatial regulation, but how they are connected to regulatory molecules to generate the precise shape of a filopodium is unclear. Point contacts which contain β1 integrins are also regulated by GTPases [31, 34]. Syndecan-3, which binds HB-GAM, is localised on neurites and filopodia and has a filopodial-stimulating activity that might involve the src-cortactin pathway [38].

A specialised form of filopodial-like structure, termed the cytoneme, is present on the cells of *Drosophila* wing imaginal discs [39]. These actin-containing processes, 0.2 µm in diameter and several hundred micrometres in length, extend from lateral cells towards the centre of the disc in the region of the border between the anterior and posterior compartments. The outgrowth of cytonemes from disc cells is stimulated in culture by *Drosophila* fibroblast growth factor and, within the disc, is dependent upon expression of hedgehog protein. Diffusibility of these morphogens is limited in vivo by their interactions with ECM, thus the discovery of cytonemes provides an important clue as to how morphogens could exert long-range effects in disc tissue. Cytonemes, by presenting localised concentrations of adhesive or growth factor receptors, may also act as signalling centres that could relay morphogenetic cues to cells outside the region of immediate cell-cell contact [39].

Filopodial structures of varying lengths have been described in invertebrate and vertebrate embryos which could serve similar adhesive and signalling functions. Thin, long, highly dynamic filopodia are present on many cell types in sea urchin gastrula and may contribute to cell positioning and orientation with respect to the fibrillar matrix [40, 41] . Thoracic closure in *Drosophila* embryos involves filopodia-dependent crawling of imaginal cells [42]. Time-lapse microscopy of zebrafish embryos provides vivid views of filopodial dynamics and substratum sampling activities by migrating neural crest cells or neurons within the developing nervous system [43, 44].

Spikes and microspikes

Spikes or microspikes are short, 2–10 µm, unbranched projections of the cell surface which are distinguished from filopodia on the basis of their length (table 1, fig.2). Microspikes were identified as phase-dark projections at the margins of well-spread cells [45]. Spikes are also protruded with filopodia from the cell surface during initial matrix attachment and are seen in many of the same in vivo contexts as filopodia [5–7, 9, 46]. The main criteria by which spikes and microspikes have been identified in different experimental systems is by morphology under light or electron microscopy or by the presence of an F-actin core [46, 47]. Static, phalloidin-stained images of growth cones or well-spread mesenchymal cells show that the distal ends of the F-actin cores of filopodia and spikes end within the cortical cytoplasm or lamellipodium. This region also contains radial actin bundles, termed ribs, that are not associated with obvious membrane projections [48] (fig. 3 A). Time-lapse studies *of Aplysia* growth cones under polarised light microscopy have revealed a sequence of events in which filopodia initially protrude from the leading edge and the core actin bundle subsequently extends down into the growth cone. These events are associated with dynamic, individual filopodial movements and a general lateral movement of filopodia under retrograde flow [49]. If these findings should also apply to filopodium formation and dynamics in other cell types, it is plausible that the complexity presented by static images corresponds to different morphological states of a single type of dynamic bundle-based structure.

At present, spikes have been studied as specific structures in several experimental systems, but the markers used to characterise them have not been systematically correlated between systems or cell types. It is thus unclear whether there is a single biochemical type of microspike, or whether there are subgroups which either contain different adhesive receptors or actin-bundling proteins, or are restricted to particular cell types. With these points in mind, each system is discussed separately here. It is also noteworthy that short, actin-containing projections, termed dendritic spines, are formed on the postsynaptic membrane of neurons and are implicated in synapse reception. Although they bear a candidate matrix adhesion receptor, syndecan-2 [50], the evidence that spines have a matrix adhesion role is currently ambiguous and they will not be described further here.

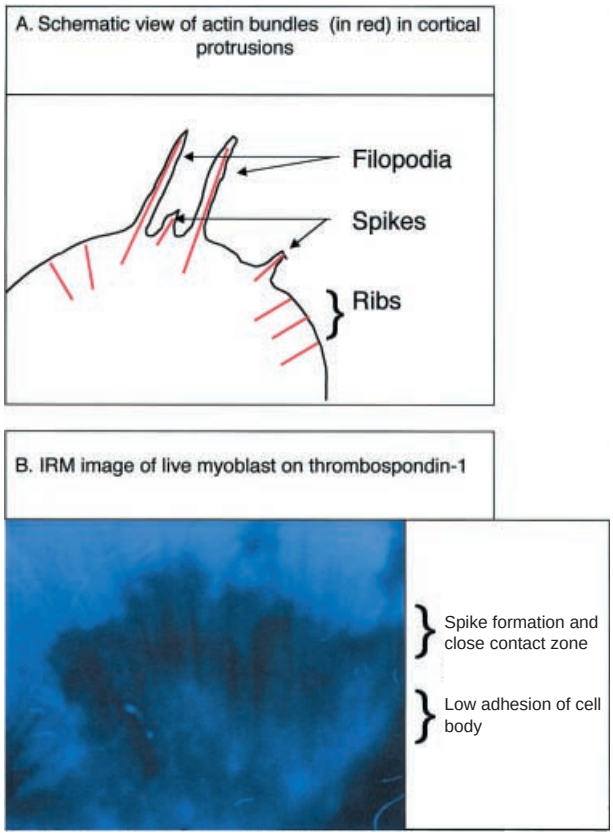

Figure 3. (A) Spatial relationships of F-actin bundles as documented in neuronal growth cones and matrix-adherent fibroblasts. Bundles within filopodia and spikes and actin ribs within the lamellipodium are shown in red. (B) IRM image of the cortex of a living myoblast adherent for 1 h on thrombospondin-l. The lamellar region presents a dark-grey image, indicative of close contact with the matrix. The whiter cell body indicates looser adhesion. Dark stripes within the lamellar region probably correspond to the tightly packed bundles of actin and fascin. This image was prepared with the laboratory of David Gingell at UCL.

Spike formation by thrombospondin-1. Spikes that contain the actin-bundling protein fascin are formed during cell spreading on thrombospondin-1. In this context, cells form stable, flattened lamellae that contain fascin ribs and protrusive fascin spikes and do not contain focal contacts [8, 51]. The lamellae and spikes correspond to areas of close contact which mediate adhesion to the thrombospondin matrix (fig. 3B). Formation of fascin spikes depends upon engagement of syndecan-1 by thrombospondin-1 [J. C. Adams, N. Kureishy and A. Taylor, unpublished data] and requires the activities of the Rac and Cdc42 GTPases for assembly of spatially organised lamellae, actin bundles and spikes [52]. Spike stability is also regulated by the activity of protein kinase C-α (PKC-α). Fascin is maintained in the non-phosphorylated, actin-bundling form throughout adhesion to thrombospondin-1 and thus remains competent to assemble stable, matrix-adherent spikes [53]. In contrast, fascin is phosphorylated by PKC-α on serine-39 during early spreading on fibronectin. This decreases actin-binding activity and results in loss of spikes during spreading and a switch in adhesion mechanism to focal contact-based adhesions [53]. Fascin spikes are also formed by cells adherent to tenascin-C and are found at the leading edge of migratory myoblasts, fibroblasts, epithelial cells and neuronal growth cones where they are functionally involved in cell migration [28, 51, 54, 55].

Spike formation by NG2 proteoglycan. Studies of surface antigens on a malignant human melanoma cell line identified a transmembrane chondroitin sulphate proteoglycan (melanoma chondroitin sulphate proteoglycan; MCSPG) which is spatially localised to microspikes. In these cells, the structures termed microspikes are pointed or branching and of various lengths [56]. MCSPG corresponds to the NG2 proteoglycan, first identified as a proteoglycan of astrocytes in the developing rat [57–61]. In fact, NG2 has widespread tissue distribution that also includes microvascular endothelial cells, smooth muscle and skeletal muscle. The NG2 core protein binds collagen Vl and causes it to accumulate at cell surfaces [62, 63]. The formation of NG2-containing spikes and branched projections is regulated by adhesive context: the projections are not formed during initial spreading on fibronectin, are apparent upon collagen VI matrix, but are strongly enhanced by adhesion to poly-L-lysine. NG2-positive spikes contain β-actin, but otherwise appear to constitute a distinct type of structure, in that fascin and myosin are not present, and vinculin and phosphotyrosine-proteins are concentrated behind the lamellae at the edge of the cell body [64]. Interestingly, cell attachment to an antibody reactive with an epitope in the ligand-binding domain of NG2 also specifically promotes spike organisation. This effect requires the cytoplasmic domain of NG2 [12].
Studies of NG2/MCSPG in melanoma cells have focused on its cooperative role in $\alpha 4\beta 1$ integrin-mediated adhesion to fibronectin. In this context, joint ligation of $\alpha 4\beta 1$ with NG2/MCSPG stimulates the formation of a different type of matrix contact, the focal contact [59, 65].

$\alpha 6\beta 4$ integrin-positive spikes, filopodia and carcinoma migration. The adhesion and migration of certain human carcinoma cells on Engelbreth Holm-Swarm laminin involves the formation of actin-containing filopodia, microspikes and associated lamellae. The adhesive receptor involved is $\alpha 6\beta 4$ integrin, which localises in small patches corresponding to sites where filopodia contact the substratum. The assembly of these spikes also depends on the interaction of $\alpha 6\beta 4$ with F-actin [66]. Although the molecular mechanisms by which $\alpha 6\beta 4$ physically associates with actin have yet to be defined, the formation of these spikes depends on signalling via Rac and phosphatidylinositol-3-kinase, RhoA, and activation of cAMP-specific phosphodiesterase [67–69].

The $\alpha 6\beta 4$ integrin is typically an adhesive receptor of hemidesmosomes in stratified epithelia and the above reports provided the first view of a functional association with F-actin structures. The particular function of these spikes appears to be in cell motility. In cells which normally assemble hemidesmosomes, motogenic stimuli such as epidermal growth factor (EGF) or scrape wounding of cell monolayers trigger relocalisation of $\alpha 6\beta 4$ to filopodia and spikes. This effect depends on serine phosphorylation of the $\beta 4$ subunit [70, 71]

Lamellae and ruffles

In cells newly plated on two-dimensional matrix, initial attachment by filopodia is followed by the extension of matrix-adherent cytoplasmic sheets, termed lamellae or lamellipodia, at lateral cell margins. This process mediates the spreading and flattening of the cell body [7, 9]. As spreading proceeds, dynamic, fan-like extensions often form parallel to the protrusive margins of the lamellipodia, then lift vertically, undergo dynamic movements, or fall backwards. These structures are termed ruffles [72]. In cells adherent on planar substrata, ruffles are visible by phase contrast light microscopy as phase-dark zones. Under SEM, lamellae appear as thin, flattened areas of the cell cortex, with ruffles as elaborate three-dimensional zones at cell margins. Under IRM, the lamellar sheet forms a zone of close contact, separated by approximately 30 nm from the substratum, whereas the ruffling margin forms a ring of tighter adhesion to the substratum (table 1), [73, 74]. In freshly adherent cells spreading on glass or fibronectin, these large, active ruffles are formed along the entire cell margin [5–7, 75, 76]. In migratory fibroblasts, the lamellipodium with associated ruffles and spikes is protruded at the leading edge, and this polarity provides directionality for cell locomotion [3, 4]. Spreading lamellae are also sites of β-actin mRNA accumulation [77]. In stationary, well-spread cells, small ruffles are dynamically turned over in a non-polarised manner at points on cell margins where lamellae have been locally protruded [5–7].

A number of stimuli enhance ruffling in preadherent cells. Ruffling activity in fibroblasts and epithelial cells is quantitatively increased by many growth factors including EGF, hepatocyte growth factor (HGF), insulin, insulin-like growth factor I, platelet-derived growth factor (PDGF) and transforming growth factor-α [78–81]. The AB and BB isoforms of PDGF, and HGF, cause the formation of particularly persistent ruffles which sweep centripetally up over the dorsal surface of cells to form elaborate circular 'crowns' [78, 80]. Ruffling correlates with stimulation of the Na/H exchanger, localisation of certain ion exchangers to ruffles and increased macropinocytosis [80, 81]. Basophilic leucocytes form large ruffles upon antigenic stimulation which may be involved in histamine secretion [82]. Certain cell types display long-lived zones of ruffled membrane which are central to their biological roles. Thus, the ventral ruffled membrane of stationary osteoclasts [83] and the ruffled border of ameloblasts [84] provide enlarged surface areas for matrix contact that are required in controlled bone degradation and enamel mineralisation, respectively.

The formation of ruffles upon matrix contact is intimately linked with the protrusion of lamellipodial sheets at cell margins and is inversely correlated with membrane tension [85]. The dynamics of lamellipodia formation are modulated according to matrix context. Secretion of matrix metalloproteinases is associated with protrusion of lamellae and cell motility [86, 87]. Cell adhesion to fibronectin involves transient formation of lamellae and ruffles during cell spreading, followed by conversion to focal contact adhesions in the later stages of spreading [75, 76]. In contrast, cell adhesion to the central cell-binding domain of fibronectin results in formation of long-lasting lamellae and ruffles [10, 88].

Glycosaminoglycan-dependent attachment of cells, mediated by the binding of cell surface heparan sulphate to platelet factor 4 or the C-terminal heparin-binding domain of fibronectin (fibronectin type III repeats 12–14), also promotes cell spreading and the stable formation of ruffing lamellae in which cortical F-actin is concentrated in ruffles or bundled into ribs [74, 88, 89]. The major heparin-binding site of fibronectin is contained in type III repeat 13 [90]. Cell adhesion to high molar concentrations of this module alone also results in formation of spikes and ruffling lamellae [11].

These features of actin organisation have similarities to the fascin spike-dependent matrix contacts formed during cell spreading on thrombospondin-1. The involvement of glycosaminoglycans in promoting stable formation of lamellae suggests that transmembrane heparan sulphate proteoglycans (HSPGs) such as syndecans may serve to couple matrix-dependent formation of lamellae and ruffles. This role is most evident on matrices which promote stable lamellae. However, heparitinase treatment also delays cell spreading on intact, full-length fibronectin [89]. All these observations suggest that HSPG receptors could be active in adhesion during the initial, transient protrusion of spikes and lamellae on fibronectin.

During adhesion to intact fibronectin, integrin receptors become clustered in ruffles and at lamellipodial edges, along with talin, which binds to the $\beta 1$ integrin subunit cytoplasmic domain, zyxin, vasodilator-stimulated phosphoprotein (VASP), ERM (ezrin, radixin, moesin) proteins and layilin, a talin-binding transmembrane glycoprotein of unknown function [91–95]. The molecular basis of coupling of integrin receptors to actin organisation in lamellae is not well understood and the process depends on actin polymerisation and requires the activities of signalling molecules, in particular the small GTPase

Rac and phosphatidylinositol-3-kinase [75, 76]. Fibronectin adhesion also regulates the coupling of Rac to its effector molecule, PAK, by an effect on the membrane association of Rac [96]. Elucidation of the mechanism involved should shed light on the process of contact assembly in ruffles.

Podosomes

Podosomes, invadopodia and pseudopodia have molecular components in common with those of focal contacts, but by the criteria of structure, shape, localisation, cell type specificity and kinetics of formation are distinct entitics. Podosomes are characteristically formed by monocytes, osteoclasts and virally transformed fibroblasts [97–99]. Invadopodia were first described as 'rosette contacts' which were induced upon viral transformation of cultured fibroblasts [100] and have continued to be characterised as a distinguishing feature of transformed cells. Pseudopodia were first described as dynamic surface features of motile, matrix-adherent tumour cells and fibroblasts in three-dimensional matrix gels. Their relationship to invadopodia or podosomes has not been clarified and so they are treated here as separate structures.

Podosomes (cell feet) were first described in detail in virally transformed avian fibroblasts as short, 0.4-µm-diameter protrusions of the ventral cell surface, which were assembled during the first hour of adhesion to fibronectin [99]. The structures were formed upon transformation by the Rous or Fujinami sarcoma viruses, but not upon transformation by the Abelson leukaemia virus, Kirstein or Snyder-Thelien sarcoma viruses, polyoma or SV40 DNA tumour viruses [99]. In IRM images, these structures correspond to areas of close membrane-substratum contacts (table 1, fig. 2). Podosomes are assembled by freshly adherent cells more rapidly than focal contacts and, unlike focal contacts, their formation is not influenced by serum and does not depend on protein synthesis or secretion. Podosomes may be important in the altered adhesive properties of transformed cells [99]. In monocytes and osteoclasts, podosomes are important for cell physiological activities which require migration across ECM barriers (monocytes), or periodic migratory activity and degradation of bone matrix (osteoclasts).

Podosomes in monocytes/macrophages. The immunoprotective role of monocytes depends on their ability to undergo dynamic alterations in adhesion, migration, chemotaxis and phagocytosis in response to extracellular cues. The β2 integrins are major mediators of these functions [101] . Podosome formation is important for monocyte adhesion and directed motility and is regulated by PKC. Normal monocytes have an inherent capacity to attach and form podosomes on glass and this process is further stimulated by phorbol ester treatment to activate PKC. Macrophage adhesion to laminin matrix also requires intracellular activation by a PKC-dependent process and correlates with the formation of small punctate actin structures which probably correspond to podosomes [67]. Matrix attachment and podosome assembly by malignant monocytes or B lymphocytes also depends on activation of PKC [102, 103].

Several subgroups of integrins are involved in podosome assembly. Within each podosome, a central core of actin filaments bundled by fimbrin is surrounded by a juxtamembrane ring enriched in vinculin, talin, α-actinin, p60src and with high phosphotyrosine content. In monocytes, the αXβ2 integrin is recruited to podosomes, whereas in B chronic lymphocytic leukaemia cells, the β1 integrin subunit colocalises with the tip of the actin core of the podosome and β2 integrins are located at the vinculin ring [102–104].

The dynamics of podosome assembly have been studied most intensively in macrophages. Although fimbrin is known as an actin-bundling protein, it also binds non-polymeric vimentin, probably vimentin tetramers, within newly formed podosomes. This association is transient and may participate in the establishment of the adhesive contact [105]. Podosome assembly also requires intracellular coupling by Cdc42, WASP and the Arp2/3 complex which all colocalise with the F-actin cores of podosomes [106, 107]. Macrophages from WAS patients, which lack normal WASP, do not form podosomes [106, 108]. Overexpression of constitutively active Cdc42 or a putative constitutively active fragment of WASP in wild-type macrophages disrupts podosome assembly [106]. In a chemotactic gradient, WAS macrophages form non-polarised arrays of filopodia and are defective in generating directed motility. These studies clarify a physiological role for podosomes in macrophages [107, 108].

Podosomes in osteoclasts. The biological activities of osteoclasts depend on their ability to switch between migratory and resorptive activities, according to extracellular cues that stimulate the cells to recognise and remain stationary at bone matrix sites which need to be degraded [109, 110]. Osteoclasts express the α2β1, αVβ1 and αVβ3 integrins [111, 112] of which αVβ3 mediates Arg-Gly-Asp-dependent (RGD-dependent) attachment to osteopontin, bone sialoproteins and collagen [112–114]. In migratory osteoclasts, attachment to bone matrix results in the formation of peripheral podosome-type contacts in which perpendicular actin filaments are bundled by T- and L-fimbrins and associated with actinin, gelsolin and vinculin [98, 115]. These podosomes are highly dynamic and turn over within 2–12 min during osteoclast motility [116].

For bone resorption to take place, podosome contacts must become reorganised into an elaborate circumferen-

tial sealing zone around the periphery of the osteoclast. This strong and tight adhesive contact permits the formation of a contained environment beneath each osteoclast into which acid and cathepsin K are secreted to degrade collagen and other components of the bone matrix. These activities can be reconstituted experimentally by culture of osteoclastic cells on dentine slices. The intracellular face of the sealing zone contains a unique organisation of the submembranous cytoskeleton which consists of a dense circumferential band of actin filaments surrounded by a double ring containing vinculin and talin [115]. Proline-rich tyrosine kinase 2 (PYK2) and pl30cas colocalise with F-actin in the central band [117–119]. Although there is much evidence for a major regulatory role of $\alpha V\beta 3$ integrin in the terminally differentiated functions of osteoclasts, $\alpha V\beta 3$ does not localise at the sealing zone, thus this tight adhesion might depend on matrix attachments made by other osteoclast integrins [120, 121].

Formation of the sealing zone contact also involves adhesion-activated intracellular signalling events, which likely participate in the reorganisation of actin and associated proteins from individual podosomes into the continuous band. Organisation of the F-actin ring is inhibited by tyrosine kinase inhibitors and correlates with tyrosine phosphorylation of pl30cas and PYK2. PYK2 and pl30cas are in a stable physical association in osteoclasts and are substrates for c-src kinase. Notably, mice null for c-src tend to develop osteopetrosis due to poor osteoclast function [122] and osteoclasts derived from c-src –/– mice are deficient both in pl30cas phosphorylation and in ring formation [117–119, 123]. The assembly and function of the sealing zone also depends on the activities of phosphatidylinositol 3-kinase and RhoA and requires the actin-binding protein gelsolin [123–125].

Invadopodia

Subsequent to the identification of podosomes in cells adherent to planar fibronectin substrata, similar-shaped structures were characterised in transformed cells seeded onto three-dimensional matrix. In this context, the structures were designated invadopodia (invasive feet) because the protrusions formed on the ventral surfaces of cells and penetrated downwards into the matrix [126]. The original invadopodia assay involved plating Rous sarcoma virus transformed chick embryo fibroblasts onto a layer of ECM produced by chick embryo fibroblasts. Later studies used fluorescein-fibronectin coated onto cross-linked gelatin films or matrix-coated porous beads. In the fibronectin assay format, the invadopodia first generate fluorescein-negative spots in the fibronectin layer and then, within 6 h, cells move off the surface and into the matrix. Invadopodia are thus specifically associated with cell motility [127, 128]. Invadopodia are not restricted to virally transformed fibroblasts and have also been documented in invasive melanoma cells and metastatic breast cancer cells [128, 129] .

The motility-promoting activity of invadopodia very likely depends on the localised secretion of ECM-degrading proteases that act on fibronectin, collagen type I, type IV and laminin [130]. These proteases include gelatinase A, seprase and membrane type 1 matrix-metalloproteinase [128, 131], which become localised to invadopodia through matrix-dependent association with integrins. For example, the $\alpha 3\beta 1$ integrin becomes physically associated with seprase at invadopodia upon adhesion to collagen I [132]. Invadopodia are characterised by a high phosphotyrosine content of proteins that include v-src, paxillin, cortactin and pl90Rho GTPase-activating protein (pl90RhoGAP). Their phosphorylation status depends on $\beta 1$ integrin activation and is closely correlated with invasive behaviour [133–135]. In invasive breast cancer cells, a complex of cortactin, paxillin and PKCμ is also enriched in invadopodia [136]. Interestingly, accumulation of phosphotyrosine-containing proteins in invadopodia is inhibited by genistein under conditions in which cell-matrix attachment, spreading and focal contact organisation are not disrupted. Injection of antibodies to p190RhoGAP caused a tenfold decrease in melanoma cell invasiveness, without affecting cell attachment. These findings support the notion that invadopodia are independently regulated motility structures [134] .

Pseudopodia

Pseudopodia (false feet) were identified as broad, flattened protrusions of the cell membrane of tumour cells in tissue culture. Their formation also correlated with viral transformation of cultured fibroblasts [see for example refs 137, 138]. Comparative studies by light and electron microscopy showed that whereas the anterior edges of normal fibroblasts tended to form flattened lamellae with many microspikes, the edges of transformed fibroblasts lacked microspikes and formed broad, lobular pseudopodia [138, 139]. In three-dimensional matrix, normal fibroblasts also form pseudopodial structures which appear as cylindrical protrusions or extensions which bear spikes or small ruffles at their margins and tips [140] (fig. 4). Although the structures formed by tumour cells likely correspond to the invadopodia formed in three-dimensional matrix, the term pseudopodia has been more widely used in cancer cell biology. Pseudopodia formed on two-dimensional matrix may also correspond with the footpads or lamellipodial extensions of spreading cells [9, 141]. However, no definitive comparison of the various structures has been carried out to clarify their relationships, and so pseudopodia are discussed here separately. In vivo, pseudopodial structures have been described on early blastoderm cells, regenerating stratified epithelia and extravasating leucocytes as well as invading tumour cells [142–144].

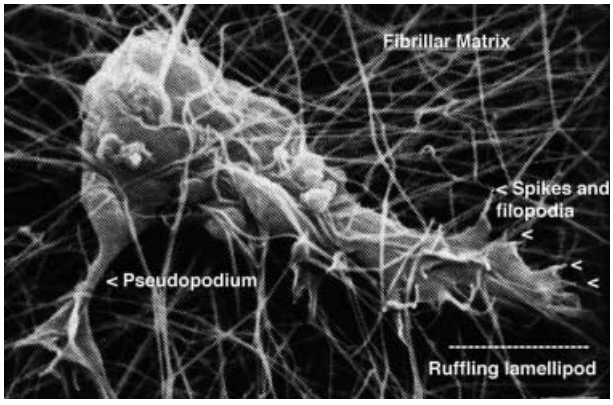

Figure 4. Scanning EM image of a motile fibroblast in three-dimensional matrix. This image illustrates the complex relationships between transient matrix contact structures formed within a fibrillar matrix. Bar, 2.5 µm. (Adapted, with publisher's permission, from Heath and Peachey, Cell Motil. Cytoskeleton **14**, Copyright 1989 John Wiley and Sons. Reprinted by permission of Wiley-Liss Inc., a subsidiary of John Wiley and Sons, Inc.)

Pseudopodia are enriched in integrin receptors and contain prominent F-actin networks [145]. There is evidence that the hyluronan-binding molecule, CD44, has a role in tumour cell motility, but it is unclear whether this receptor is specifically localised in pseudopodia [146]. Motogenic peptides strongly increase pseudopodia formation by cells adherent on two-dimensional matrix, and in cells undergoing directed migration, pseudopodia become polarised at the leading edge. As with the extension of filopodia and spikes, pseudopodial extension exerts little tension on the matrix [147] and thus the structures are believed to have an exploratory role in forming transient adhesions during migration. Why this particular structure should be preferentially formed by tumour cells remains unclear.

Stable matrix contacts

After a few hours in culture, cells develop increased cellular contractility and begin to elaborate secreted matrix components into an assembled matrix. These events coincide with the development of several types of longer-lived matrix contacts, which not only provide continued scope for cell adhesion and migration, but also enable cells to resist deformation by the contractility of the actin cytoskeleton.

Focal contacts/focal adhesions

Focal contacts or focal adhesions were first studied by electron microscopy and IRM in stably adherent cells. Electron microscopical images showed that the leading lamellae of cultured fibroblasts contained plaques of amorphous material associated with the termini of actin microfilament bundles in regions where the ventral plasma membrane was closely opposed to the substratum [141]. In living cells viewed by IRM, focal contacts were defined as phase dark patches under the lamellae where the separation from the substratum was only 10–15 nm [73, 141], (table 1, fig. 2). Direct correlation of interference-reflexion and electron microscopy was used to demonstrate that the focal contacts coincided with the distal ends of microfilament bundles and the plaque structures [149]. In newly adherent cells, these adhesion plaques form after the protrusion of lamellae and continue to mature as cells spread fully and develop isometric tension. Further correlative studies identified actin-associated proteins, including vinculin, α-actinin and talin, within the submembranous plaque and thus showed it to be a multiprotein complex [92, 150, 151].

Since these initial characterisations, the focal contact has become the most intensively studied type of matrix contact in cultured cells. This drive has been fuelled, in large part, by the relative accessibility of these contacts for study, the finding that the major category of adhesion receptors in focal adhesions are the integrins and an appreciation of the importance of focal adhesions in cell signalling and cell contractility under culture conditions. Many excellent reviews have focussed on focal adhesions and so they will be only briefly discussed here [152–154].

An important aspect of the de novo formation of focal contacts is the activation and clustering of ligand-occupied integrins. Specific integrins are recruited according to matrix context: on fibronectin, the major integrin is $\alpha 5\beta 1$, on vitronectin, the major receptor is $\alpha V\beta 3$ [155]. In the case of fibronectin, concurrent adhesion to both the central cell-binding domain (that contains the RGD site and synergy adhesion sites) and the C-terminal heparin-binding domain is required for full assembly of focal adhesions. The activity of the heparin-binding domain is contained within fibronectin type III repeat 13 and is coupled by recruitment of syndecan-4 to focal adhesions [10, 156]. On the intracellular side, focal adhesions contain a large set of structural and signalling molecules. The complexity of interactions and regulatory signals between integrins, adhesion plaque components and actin organisation is a current focus of interest in the area. However, interactions with the cytoplasmic domain of the $\beta 1$ or $\beta 3$ integrin subunits are sufficient to mediate integrin clustering in focal adhesions [157–159], whereas the α subunit cytoplasmic domains exert regulatory influences [160, 161].

The clustering of integrin β subunit cytoplasmic domains provides binding sites for the proximal intracellular components of focal adhesions. Direct binding of talin, α-actinin and filamin to β subunit cytoplasmic domains has been reported. Additional new proteins have been identified in yeast two-hybrid screens [reviewed in refs 162,

163]. The interaction with talin is to some extent constitutive in that active, non-ligand-occupied integrins show association with talin. This may also explain why integrins and talin are prominently colocalised in ruffles. A second step in the assembly of focal contacts appears to be the recruitment and unfolding of vinculin. Vinculin monomers exist in a head-to-tail folded conformation which mask its binding sites for actin and talin [164]. The binding of phosphatidylinositol bis-phosphate (PIP2), a signalling molecule present at increased levels during cell adhesion to fibronectin [165], to vinculin likely serves to unfold the protein and expose the talin-binding site, thus recruiting vinculin into nascent focal contacts and presumably propagating the assembly of other focal adhesion components by inter-molecular interactions [162, 163]. Multiple actin-binding proteins are present in focal contacts and definition of the mechanism(s) by which actin filaments are recruited is an important current goal. Focal adhesions also contain many signalling molecules, most notably PKC isoforms, focal adhesion kinase, c-src and c-abl tyrosine kinases, the activities of which are regulated by matrix adhesion [154, 166] .

As the closest matrix attachment sites for cells on two-dimensional matrix, focal adhesions are also instrumental in the development of isometric tension. Actomyosin-dependent intracellular contractile force is resisted by the connection of integrins to the cytoskeleton and the anchored extracellular matrix. This process depends on the activity of Rho GTPase, which also provides an intracellular pathway for focal adhesion assembly [153, 167]. The equivalents of focal contacts in vivo remain to be identified. The myotendinous junction of skeletal muscle is a much larger structure that provides matrix attachment and has a similar biochemical composition. Activated, spread platelets form vinculin-containing contacts which resemble focal contacts, as do endothelial cells under shear stress. However, leucocytes, macrophages and most epithelial or connective tissue cells do not show focal contact-type adhesions [152].

ECM contacts/matrix assembly sites

Fibrils of collagen and fibronectin and elastic microfibrils are major and ubiquitous components of the matrix of mesenchymal cells in tissues. In cultured cells, ECM contacts were first defined by electron microscope criteria as regions where the ventral plasma membrane had a 100-nm separation from the substratum, yet was connected to the matrix by large fibrils or cables of ECM that included fibronectin and collagen [168–171]. Matrix assembly sites also form on the dorsal surface of cultured fibroblasts several hours after plating (table 1, fig. 2) [172]. Thus, the formation of these contacts is subsequent to the assembly of focal contacts. At the assembly sites, individual collagen or fibronectin molecules are first dynamically organised into fibrils and then laterally aggregated into higher order bundles and filaments by extracellular self-assembly mechanisms, protein-protein interactions or enzymatic cross-linking. Similar contacts between mesenchymal cells and their matrix are found in tissue samples [173, 174].

Collagen fibril assembly sites. Many types of collagens assemble into fibrils; however, the development of collagen fibrils as a cell-mediated process has been studied principally with regard to type I collagen. In tendon fibroblasts, this is a complex process which leads to the orderly assembly of large arrays of fibrils. The process begins with organisation of type I procollagen molecules into oligomers within the endoplasmic reticulum. These electron-dense aggregates then translocate through the Golgi stack to the cell surface [175] . Secretion and cleavage of the N-terminal non-helical domains is coordinated at the cell surface with the extracellular organisation of fibrils by end-to-end association of monomers and the lateral assembly of fibrils into bundles and macroaggregates. The latter events are regulated within narrow channel-like specialisations formed by arrays of thin cytoplasmic fingers at the fibroblast cell surface. Analysis of tendon fibroblasts by electron microscope serial sections has shown that these recesses penetrate deep into the perinuclear region of the cells and may in fact be contiguous with the Golgi stack. Each channel contains a small number of collagen fibrils and formation of bundles of 6–200 fibrils appears to correlate with the fusion or merging of adjacent channels. Larger bundle macroaggregates of 200–400 fibrils are found extracellularly, in association with the cell surface or within the matrix [174, 176].

Cell surface binding of collagens including collagen I is mediated by β1 integrins, predominantly $\alpha1\beta1$ and $\alpha2\beta1$. These interactions depend on a recognition site within the collagen triple-helical structure [177]. Integrin binding and cell adhesion to collagens I or IV depend on recognition of a specific triple-helical peptide motif, GFOGER, by the insertion (I) domains of the α1 or α2 integrin subunits [178–182]. On a planar, monomeric collagen surface in culture, these interactions stimulate the formation of focal adhesions. In the context of a collagen gel or three-dimensional matrix, where polymeric collagen fibrils predominate, fibroblasts develop multiple types of adhesive contacts, including filopodia, spikes, lamellae, ruffles and pseudopodia which dynamically engage with the matrix fibrils (fig. 4) [183]. The coupling of integrins to the cytoskeleton at these matrix contacts enables fibroblasts to exert mechanical force against their matrix. The contractile activity of fibroblasts in collagen gels is mediated by the $\alpha1\beta1$ or $\alpha2\beta1$ integrins and requires the α2 subunit cytoplasmic domain [184–186]. In collagen gels, single fibroblasts develop force in the order of 2.65 µN/

cell [187, 188]. This tensile force is sufficient to contract unattached collagen gels or wrinkle flexible rubber sheets and is thought to be important for wound closure in vivo [189].

Fibronectin matrix assembly sites/fibrillar adhesions. Fibronectin matrix, as visualised by electron microscopy or by immunofluorescent staining, consists of fibrils between 10 nm and 1 μm in diameter. A striking feature of the pattern of fibronectin fibril deposition around cultured mesenchymal cells is the coalignment of fibrils with focal contacts and microfilament bundles [190]. This is particularly noticeable in polarised, migratory cells, where fibronectin fibrils are prominently localised around the lateral margins and retracted tail region of the cells. The development of a GFP-tagged fibronectin revealed that the fully assembled fibronectin matrix shows elastic movements. Thus, fibronectin fibrils can respond in an integrated manner with contractions or protrusions of cell surfaces, or to tension transmitted indirectly through other cell-binding molecules which associate extracellularly with fibronectin fibrils [191]. The development of fibrillar fibronectin around newly adherent cells takes many hours. Early in the process, small patches of fibronectin colocalise with focal contacts [155]. Later, the codistribution of fibronectin with focal contacts becomes less complete as newly secreted fibronectin dimers undergo specific and saturable binding, become unfolded, multimerise and elongate. These fibrils mature into a detergent- and sodium dodecyl sulphate-insoluble form that surrounds cells and aligns parallel to actin microfilaments [192–195]. In dense cultures of fibroblasts or MG-63 cells, fibronectin matrix incorporation is spatially localised at filopodial-like projections at cell margins [195]. With time in culture, these sites become the dominant type of cell-fibronectin contact for most mesenchymal cells [172]. Originally characterised as ECM contact sites, matrix assembly sites, or the fibronexus, the contacts have recently been redesignated fibrillar adhesions [171, 196] .

Fibril formation requires both the central cell-binding domain and the amino-terminal type I repeats 1–5 of fibronectin [191, 197]. The integrins $\alpha5\beta1$ and $\alpha V\beta3$ are important in this process, although in cells where both receptors are expressed, the $\alpha5\beta1$ integrin is dominant. Antibody inhibition of $\alpha5\beta1$ perturbs both fibronectin matrix deposition and the organisation of focal and ECM contacts, as determined by IRM or immunostaining approaches [198, 199]. In cells null for the $\alpha5$ or $\beta1$ integrin subunit, $\alpha V\beta3$ substitutes in matrix assembly [200, 201]. Whether fibril assembly is driven by extracellular self-assembly after integrin binding and unfolding, or whether additional types of adhesion receptors are involved is not fully understood. Certainly domains of fibronectin which do not contain the classical integrin-binding sites participate in matrix assembly. The N-terminal heparin-binding type I repeats 1–5 can be independently incorporated into matrix [197]. Other modules, the type III repeat I, are cryptic in the fibronectin dimer but participate after unfolding [173, 197]. These processes may involve transmembrane coupling by HSPGs, such as syndecan-2 [202]. One model is that, after initial tethering by integrins, the amino-terminal domain binds a separate fibronectin assembly site which serves to concentrate fibronectin molecules appropriately for self-assembly [173].

Recent in-depth characterisation by immunochemical and protein tagging techniques has shed new light on the formation of fibrillar adhesions and the role of $\alpha5\beta1$ integrin. Relative to focal contacts, fibrillar adhesions have a thin, elongated shape, contain little phosphotyrosine, have high levels of the actin-binding protein tensin and accumulate $\alpha5\beta1$ integrin [196, 203]. Early studies of ECM contacts had shown that $\alpha5\beta1$ integrin specifically relocates from focal contacts into newly assembled fibronectin matrix attachment sites [155]. The new studies demonstrate that the organisation of fibrillar adhesions depends crucially on the ability of $\alpha5\beta1$ integrin to reorganise secreted fibronectin into fibrils [203]. This process is evident 4 h after plating cells on fibronectin and is mediated by active, F-actin-dependent translocation of $\alpha5\beta1$ along microfilament bundles towards the perinuclear region [204]. During this period, tensin is also centripetally translocated into the fibrillar adhesions by an actomyosin-dependent process [205]. Tensin is functionally significant in the cytoskeletal coupling of fibrillar adhesions because over-expression of the actin homology 2 region of tensin blocks both $\alpha5$ integrin translocation and fibronectin fibril formation [204].

The importance of cytoskeletal coupling in the formation and function of fibrillar adhesions is also supported by many findings which demonstrate that cell contractility is required for fibronectin matrix assembly. Blockade of integrin-cytoskeletal linkage, either by expression of a cytoplasmic-domain deleted $\beta1$ subunit, or by cytochalasin D treatment, inhibits fibronectin matrix assembly [206]. Conversely, binding of the 70 kDa amino-terminal fragment of fibronectin to cells and fibronectin oligomer assembly are enhanced in the presence of serum or lysophosphatidic acid (LPA), which promote cell contractility through Rho GTPase activity [195, 207]. This relationship between fibronectin matrix and contractility appears to operate independently of cell adhesion. Addition of soluble FNIIIc-1 peptide (one of the cryptic modules implicated in matrix assembly [173, 197] to preadherent cells activates Cdc42 GTPase, promotes the formation of actin-containing cellular protrusions and down-regulates matrix assembly. During these changes, cells remain adherent and phosphorylation of FAK and paxillin are not affected [208].

Fibrillin microfibrils. Microfibrils were first identified morphologically by electron microscopy as filaments of 10–12 nm diameter within the ECM of connective tissue, vascular walls and in association with the basement membrane of the epidermis and other epithelia. Filaments in this size range contain various components, including collagens, proteoglycans or fibronectin, and the term is now restricted to fibrils that contain fibrillin-1 or fibrillin-2 as a core component. Fibrillin microfibrils are widely distributed in most tissue matrices throughout life and are assembled in two forms: with fibrillin as the sole major component in non-elastic tissues, or as an assembly of fibrillin microfibrils with polymerised tropoelastin to form the elastic fibres which impart elastic recoil and resilience to the walls of the vascular system, myocardium, dermis and lung [209–211].

Fibrillin is a large, rod-like molecule that contains clusters of EGF-like repeats separated by so-called 8-cysteine repeats. Microfibrils extracted from tissue or cell culture matrices contain highly disulphide-bonded fibrillin molecules and have a characteristic beads-on-a-string appearance with variable spacing between the beads [212–214]. This beaded appearance can be varied in situ according to applied tension and has thus been modelled as a source of the elastic properties of the filaments [212].

The extracellular assembly of elastic microfibrils is a multistep process which involves the multimerisation and cross-linking of fibrillin molecules, which then serve as a template for the deposition and alignment of tropoelastin monomers and their subsequent cross-linking into elastin polymers [215–217]. The key cell contact interaction in tethering the microfibrils at the cell surface is thus made with fibrillin. Indeed, fibrillin microfibrils are adhesive for vascular smooth muscle cells [213]. Various cell types also attach and spread on purified or recombinant fibrillin and for most of these, attachment is mediated by binding of $\alpha V\beta 3$ integrin to the RGD sites of fibrillin-1 or fibrillin-2 [218–220]. However, attachment of fetal chondroblasts is only partially inhibited by RGD peptide or anti-integrin antibodies, raising the possibility of a second, non-RGD-dependent attachment site [219].

At the ultrastructural level, the points of contact between microfibrils and the plasma membrane resemble focal contacts, with many coincident actin microfilaments that terminate on the intracellular side of the junction. Indeed, cell adhesion to fibrillin correlates with the localisation of the $\beta 3$ integrin subunit to focal contact-type structures [219]. Given the well-established observation that $\alpha V\beta 3$ integrin is retained in focal contacts and does not transit to fibrillar adhesions, it is intriguing to speculate that the development of cell contractility and transmission of mechanical force via $\alpha V\beta 3$ to microfibrils at focal contacts may be an important function of focal contact-type structures in elastic tissues.

Whilst more detailed studies of fibrillin-binding contacts and their role in initial microfibril assembly are needed to resolve these questions, microfibrils clearly have crucial roles in tissue integrity and vascular function. Mutations in tropoelastin predispose to cutis laxa, rupture of blood vessels and other pathologies. Over 200 individual mutations in fibrillin-1 are known to result in Marfan syndrome, a weakening disorder of connective tissue which affects the skeletal, cardiovascular and ocular systems [221, 222].

Dystroglycan-mediated contacts

Although not associated with large, morphologically distinctive contact structures, the dystroglycan-mediated contact has emerged as a biochemically unique type of cell-matrix contact which has important roles in the organisation of basement membranes in skeletal muscle and epithelia. The dystroglycan adhesion receptor consists of the proteolytically cleaved products of the dystroglycan gene, α-dystroglycan, a peripheral membrande protein, and β-dystroglycan, a transmembrane protein. The receptor is linked to ECM by the binding of α-dystroglycan to agrin, perlecan and the G domains of laminins [223–225]. In skeletal muscle, the intracellular interaction of β-dystroglycan with dystrophin, an actin-binding protein, links the complex to the actin cytoskeleton. Additional transmembrane proteins, sarcoglycan and sarcospan, also form part of the complex. In skeletal muscle, the whole complex functions throughout life to stably link muscle fibres to the epimysial basement membrane and to maintain fibre integrity under conditions of varying mechanical tension. Mutations in any single component of the complex (laminin-2, sarcoglycans, dystroglycan or dystrophin) are associated with various forms of muscular dystrophy [226, 227].

The dystroglycan complex also has a major role in basement membrane assembly in non-muscle tissues. In these tissues, α-dystroglycan binds to different members of the laminin family and is linked to the cytoskeleton by a protein complex that contains utrophin rather than dystrophin [228]. Dystroglycan is localised at the basal surfaces of many epithelia [227] and dystroglycan-null mice show major defects in basement membrane organisation early in embryogenesis and do not develop beyond E10.5 [229]. Furthermore, embryoid bodies formed by dystroglycan-null embryonic stem (ES) cells lack basement membranes and show abnormal, amorphous distributions of laminin-1, perlecan and collagen IV. The normal interactions of laminin and perlecan with dystroglycan thus appear to be needed as nucleation sites for self-assembly of laminin molecules at the cell surface [230].

The matrix assembly role of the dystroglycan complex in epithelial cells thus appears analogous to the integrin-dependent fibronectin or collagen matrix assembly sites of

fibroblasts. $\alpha 2\beta 3$ and $\alpha 3\beta 1$ integrins also act as laminin receptors and in skeletal muscle, $\alpha 7\beta 1$ is a major laminin receptor. Experiments with receptor-null muscle cells demonstrate that $\beta 1$ integrins function jointly with dystroglycan for basement membrane assembly in skeletal muscle. Thus, the extent of laminin polymerisation on skeletal myoblasts depends on the expression of both $\alpha 7\beta 1$ integrin and the dystroglycan complex, and involves reorganisation of $\beta 1$ integrins, dystroglycan and actin into discrete patches that coincide with laminin networks [231, 232].

Hemidesmosomes

Hemidesmosomes are the major contacts which maintain stable attachment between the basal surfaces of stratified epithelial cells and their underlying basement membrane. These contacts were named for their appearance under the electron microscope as electron-dense cytoplasmic plaques, which resembled half of a desmosome. Advances made over the last 10 years in understanding the molecular components of hemidesmosomes, in conjunction with studies of human skin blistering diseases and genetically engineered mice, have brought together an unusually full understanding of their essential structure and functions in vivo.

The principal adhesion receptor of the hemidesmosome is the $\alpha 6\beta 4$ integrin, which binds laminin-5 and other laminins within the basement membrane and is essential for the assembly of hemidesmosomes (fig. 5). A second transmembrane protein, BP180/BPAG2, is located in hemidesmosomes, interacts with $\alpha 6\beta 4$ and might also contribute to matrix adhesion. The cytoplasmic domain of BP180 binds the intermediate filament-binding protein BP230/BPAG1 within the hemidesmosomal plaque, and the cytoplasmic domain of the integrin $\beta 4$ subunit is also linked to keratin filaments through its interaction with HD1/plectin/IFAP300 [232–234].

Hemidesmosomes thus have a distinctive molecular composition and location in epithelia in comparison to other contacts which contain $\beta 1$ integrins (fig. 5). The essential and separate role of hemidesmosomes in epithelial matrix adhesion has been clearly demonstrated by the phenotypes of mice null for the $\alpha 6$ or $\beta 4$ integrin subunit genes. Both types of mice die soon after birth with severe skin blistering and detachment of internal stratified epithelia, and ultrastructurally show a complete absence of hemidesmosomes [235, 236]. This phenotype resembles that of a set of human autosomal recessive disorders, the epidermolysis bullosas (EBs), which show epidermal blistering of varying severity in conjunction with separate phenotypes in other tissues. Mutations involved in EB have been mapped to multiple components of the hemidesmosome, including laminin-5, BPAG1e, plectin, keratins 5 and 14 and the integrin $\alpha 6$ or $\beta 4$ subunits [232–234]. Thus, the integrity of the hemidesmosome matrix contact is required in structuring the epidermis, for maintaining adhesion of basal keratinocytes to their basement membrane. Although $\alpha 6\beta 4$ binds laminin-5, a role in basement membrane assembly has not been described for $\alpha 6\beta 4$ integrin. Indeed, typically structured regions of basement membrane are found in the epidermis of $\beta 4$ knockout mice [235]. In epidermis and other epithelia, this role appears to be fulfilled by $\beta 1$ integrins such as $\alpha 2\beta 1$ or $\alpha 3\beta 1$, or the dystroglycan complex [230, 237]. Furthermore, an exogenous laminin-rich matrix is sufficient to induce hemidesmosomes in cultured epithelial cells [238].

Studies of cell culture models and mutant mice have also demonstrated roles for $\alpha 6\beta 4$ in epithelial cell proliferation and survival. Epidermal keratinocytes that lack integrin $\beta 4$ show an increased propensity to undergo apoptosis [232]. Ligand binding by $\alpha 6\beta 4$ leads to tyrosine phos-

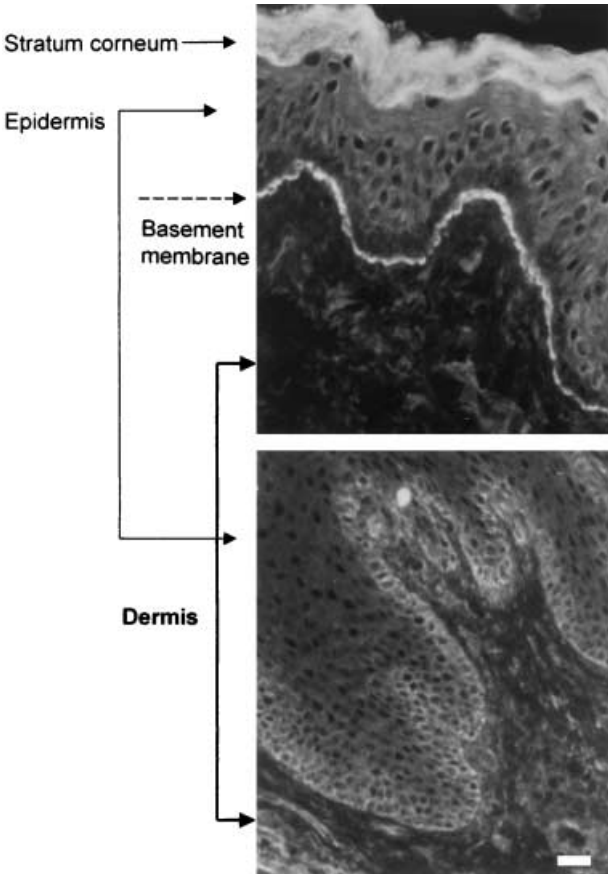

Figure 5. Spatial relationships of integrin adhesion receptors within skin tissue. The $\alpha 6\beta 4$ integrin, the major adhesive receptor of hemidesmosomes, is specifically localised at the basal face of keratinocytes adjacent to the basement membrane (top panel). Bright fluoresence in the stratum corneum is due to second-antibody cross-reactivity. In contrast, $\beta 1$ integrins, which are found in both transient and stable matrix contact structures, are located on dermal fibroblasts, cells of blood vessel walls and around basal keratinocytes (lower panel). Bar 20 µm.

phorylation of the β4 subunit, recruitment of the signalling adaptor proteins Shc and Grb2, activation of the MAPK pathway and cell proliferation. Distinct tyrosine phosphorylation sites on the β4 subunit are involved in cytoskeletal organisation at hemidesmosomes [239]. Transgenic mice expressing a cytoplasmic domain-deleted version of β4 also die at birth due to extensive skin detachment due to cell rupture at the plasma membrane. Thus, although the mutant integrin still binds laminin-5, coupling to the keratin cytoskeleton is ineffective. Interestingly, the mice also show proliferation defects within the epidermis. These results indicate joint roles for $\alpha 6\beta 4$ integrin in cell cycle regulation and cytoskeletal coupling of adhesion [240].

Coordination nnd integration of matrix contact assembly and function

As described above, experiments in which cells are plated on single ECM components or fragments enable contact types to be studied and dissected on an individual basis. Yet, in vivo, adherent cells are always exposed simultaneously to many matrix components and form multiple contacts with appropriate polarity and dynamic regulation. An obvious hypothesis is that cells possess regulatory mechanisms to integrate and coordinate the assembly of different types of matrix contacts. To test this hypothesis effectively, clear definitions of matrix contact types are a first necessity. However, a number of recent advances provide examples of how such putative regulation can work.

One mechanism for directing different contact types is by regulated secretion and deposition of matrix components. There are many examples of regulated alterations in matrix composition during development, cell differentiation and wound healing [241]. In cell culture, alterations in the ratio of matrix components can indeed affect contact types. Surfaces coated with different mixtures of thrombospondin-1 and fibronectin produce graded alterations in the assembly of fascin spikes and focal contacts [51]. Presentation of soluble thrombospondin-1 or tenascin-C to cells adherent on fibronectin also downregulates focal contacts and alters cortical F-actin organisation and fascin localisation [54, 242]. The addition of tenascin-C into a three-dimensional fibronectin and fibrin matrix promotes formation of actin-containing projections [243]. High-resolution microscopical studies are needed in tissue samples to identify matrix niches around cells and to determine if compositional alterations correlate with the dynamics of contact formation.

Matrix composition is also indirectly altered by the regulated activities of matrix metalloproteases. By cleaving matrix molecules at the cell surface, proteases break existing matrix contacts, alter the spatial relationship of cells to matrix and, as exemplified by fibronectin, generate matrix fragments that have different properties with regard to matrix contact assembly [10, 77, 86, 87]. Breakage of existing contacts can also be mediated intracellularly through the activity of calpain in mediating focal contact turnover or cleaving cytoskeletal linker proteins such as ezrin [244, 245].

The profile of adhesion receptors expressed by cells will also affect their ability to bind to particular molecules and the types of contacts they are competent to assemble. Hemidesmosome and dystroglycan-mediated contacts are specific to differentiated cell types which express the appropriate adhesion receptors and intracellular coupling molecules. Most cells express arrays of integrins and proteoglycans which provide a level of redundancy in their matrix-binding specificities and which may cooperate in, or modulate, adhesive activities [160, 241]. Certain integrins, $\alpha 3\beta 1$ and $\alpha 4\beta 1$, are typically excluded from focal contacts and it would be interesting to know how their distributions relate to the formation of transient, protrusive contacts.

The assembly of matrix contacts is also regulated according to biophysical effects on cells. The application of mechanical force to cells of connective tissue leads to alterations in matrix composition [246]. In endothelial cells, application of fluid shear stress that mimics blood flow stimulates the formation and stabilisation of focal adhesions that align in the direction of flow [247].The application of tension to cells, for example in anchored collagen gels or by twisting beads coated with integrin ligands in a magnetic field, promotes focal contact assembly and cytoskeletal reorganisations [187, 248].

Recent advances in understanding the mechanisms by which actin organisation is coordinated provide clues as to how different inputs from ECM are integrated within cells to model the appropriate contacts. The Rho family of small GTPases have general effects on actin cytoskeletal organisation. Over-expression of the GTPases Cdc42, Rac or Rho in adherent fibroblasts stimulates the formation of filopodia, lamellipodia or focal adhesions, respectively [249]. In macrophages, over-expression of Cdc42 causes a major shift from podosomes to filopodia [107, 108]. These results imply that, under normal conditions, the balance and regulation of GTPases and their effectors is likely to be important in regulating the assembly of different contact types.

A second important aspect of normal regulation would be not only to provide global switches between one type of contact and another, but also to provide coherent regulation within single cells. The need for spatial and temporal regulation of assembly of different contacts is particularly evident in migrating cells, but is also required for the formation of specialised contacts, as in osteoclasts and epithelial cells [4, 83, 110, 227, 233]. To achieve these cyclical processes, the activities of GTPases and their

effectors are probably compartmentalised in time and space. Appropriate reporter reagents are now needed to answer this question. However, recent findings indicate that modulation of protrusive, transient contacts in conjunction with contractile focal adhesions involves regulation of Rho GTPase activity. Inhibition of Rho by the activities of Cdc42 and Rac has been reported in several cell types. These effects correlate with the formation of cellular projections or downregulation of focal adhesion assembly [29, 208, 243, 250]. Thus, an understanding of mechanisms which suppress or sequester Rho GTPase might identify a key balance point in the integration of contact assembly.

Conclusions and future prospects

Research into cell-matrix contacts is entering an exciting phase. Developments in experimental methodologies and imaging techniques over the last several years have awakened appreciation of the roles of protrusive cell contacts in adhesion and motility, and are fostering an awareness of the interconnections between different contact types. If, as many studies indicate, protrusive contacts and matrix assembly sites have widespread roles in cell interactions in tissue matrices, a full understanding of the molecular makeup of these contacts is a priority. Identification of molecules specific to these certain types of contacts would enable them to be identified unambiguously, defined in detail and studied as intensively as focal adhesions. Preparation of samples for biochemical analysis from three-dimensional matrix is arduous and two-dimensional matrices that enhance the formation of filopodia, spikes and lamellae will be important in these studies.

To understand the dynamic interconnections between different types of matrix contacts, the beauty of form apparent in scanning electron micrographs need to be linked to the actions of individual molecules. Real-time tracking of fluorescently tagged molecules will continue to be important in understanding contact dynamics and offers a direct approach to investigate relationships between contact types in single cells. Used as an expression library screening method, this approach has the potential to identify novel components of transient contacts. The current definitions of transient contacts depend, in large part, on distinctions made on the basis of morphological features or actin organisation. More sophisticated methods to define the dynamics of filopodia, spikes, lamellae and pseudopods will be important in clarifying whether the present categorisation of contact types is accurate. Advances in microscopical methods and digital image processing should provide new ways to analyse contact organisation in three-dimensional matrix.

A second major set of questions relate to the cell surface coupling of contact formation. Similar sets of signalling molecules (small GTPases, PKC, phosphatidylinositol 3-kinase) regulate the organisation of many different contact types. Thus it seems most likely that the specification to assemble a particular type of contact is made at the cell surface. Major recent advances have been made in understanding the protein complexes that regulate actin filament nucleation; however, even with respect to the well-studied focal contacts, there is a gap in understanding the mechanisms by which activated or clustered receptors physically link to actin nucleation complexes and define the spatial form of the resultant contact.

Furthermore, if the same sets of regulatory molecules are needed in the build-up of different types of contact, how are these contact coherently organised by cells? One model is that activated adhesion receptors, by binding specific cytoskeletal linker proteins such as talin, define an "assembly patch" that locally activates regulatory molecules and recruits actin nucleation complexes. Use of reporters of activation or molecular proximity measurements (e.g., phospho-epitope or conformation-specific antibodies, fluorescence energy transfer methods) would provide insight into the formation and composition of nascent contacts and the relationship of active signalling effector molecules to different types of contact.

The integration of cell organisation within tissues must also depend on intercellular regulation of cell-matrix contacts. Recent advances in understanding the actions of morphoregulatory receptor tyrosine kinase systems indicate their effects on cell-matrix contacts as a new area of potential importance, which should also provide more insight into the coordination of contact types. The ephrin/Eph receptor tyrosine kinase family mediate cell contact-dependent interactions that regulate repulsive guidance by collapse of filopodia in the developing nervous system, and modulate integrin-mediated adhesion in the nervous system and vasculature [251, 252]. In the vascular system, vascular endothelial growth factors and their receptors and the angiopoietins and their receptors, Tie1 and Tie2, act to promote vascular development and stabilise the adult vasculature. At the cellular level, these growth factors promote endothelial cell migration, sprouting and adhesion to smooth muscle and matrix [251]. Elucidation of the molecular mechanisms of these effects, which very likely involve cell-matrix contacts and their cytoskeletal connections, is now an emergent area of great interest.

An exciting possibility for biomedical applications is that, by manipulating the formation of contacts involved in motility and contractility, cell guidance behaviour could be altered whilst maintaining normal matrix adhesion. Such strategies could be used to manipulate angiogenesis, to promote wound healing or tissue regeneration, or to limit the metastatic spread of tumours. The fibroblast in figure 4 vividly illustrates the complexity of cell-matrix interactions. An understanding of contacts as inte-

grated entities should make this fluid topography both comprehensible and regulable.

Acknowledgements. I gratefully acknowledge the financial support of the Wellcome Trust (Senior Fellowship 038284).

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