TRANSMEMBRANE EXTRACELLULAR MATRIX–CYTOSKELETON CROSSTALK

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Integrin-mediated cell adhesions provide dynamic, bidirectional links between the extracellular matrix and the cytoskeleton. Besides having central roles in cell migration and morphogenesis, focal adhesions and related structures convey information across the cell membrane, to regulate extracellular-matrix assembly, cell proliferation, differentiation, and death. This review describes integrin functions, mechanosensors, molecular switches and signal-transduction pathways activated and integrated by adhesion, with a unifying theme being the importance of local physical forces.

INTEGRINS
A group of heterodimeric transmembrane adhesion receptors for extracellular-matrix proteins such as fibronectin and vitronectin.

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Cell interactions with the extracellular matrix (ECM) and with neighbouring cells trigger numerous responses that have essential roles in the regulation of their behaviour and fate[1,2]. ECM adhesion and assembly affect cells in many ways. As the ECM provides the physical microenvironment in which cells live, it provides a substrate for cell anchorage and serves as a tissue scaffold, guides cell migration during embryonic development and wound repair, and has other key roles in tissue morphogenesis. However, beyond these obvious scaffolding functions, the ECM is also responsible for transmitting environmental signals to cells, which affect essentially all aspects of a cell's life, including its proliferation, differentiation and death. So, selection of the appropriate matrix for experiments using cultured cells is crucial, as it can profoundly affect the cellular response. As will be discussed below, not only is the molecular composition of the ECM important, but also its topography and mechanical properties[3–6]. These features of the ECM are determined both by the cells that produce it and by the cells that grow on or inside the matrix. So, the life of a cell seems to involve intense and complex crosstalk with the matrix.

In this review, we focus on the molecular events that occur at both faces of the cell membrane in cell–matrix adhesions. We describe briefly the molecular organization and complexity of the membrane–cytoskeleton interface in this region, as well as external interactions with the ECM. We show how cells actively shape the matrix around them, and that the entire process is controlled both locally and globally by specific regulatory pathways, with a mechanosensory system and molecular switches acting as pivotal elements. We also consider several puzzles in rapidly developing areas that provide intriguing opportunities for new advances.

Cell–matrix adhesions

Adhesions with the ECM are formed by essentially all types of adherent cell, but their morphology, size and subcellular distribution can be quite heterogeneous. Many of these adhesions, nevertheless, share two common features — they are mediated by INTEGRINS, and they interact with the actin cytoskeleton at the cell interior. The extracellular ligands that anchor these adhesions include fibronectin, vitronectin and various collagens. The best-characterized adhesions are the 'classical' focal adhesions (also termed focal contacts), and variants include fibrillar adhesions, focal complexes and podosomes (FIG. 1 and TABLE 1).

Focal adhesions are flat, elongated structures that are several square microns in area, and are often located near the periphery of cells[7–10]. Focal adhesions mediate

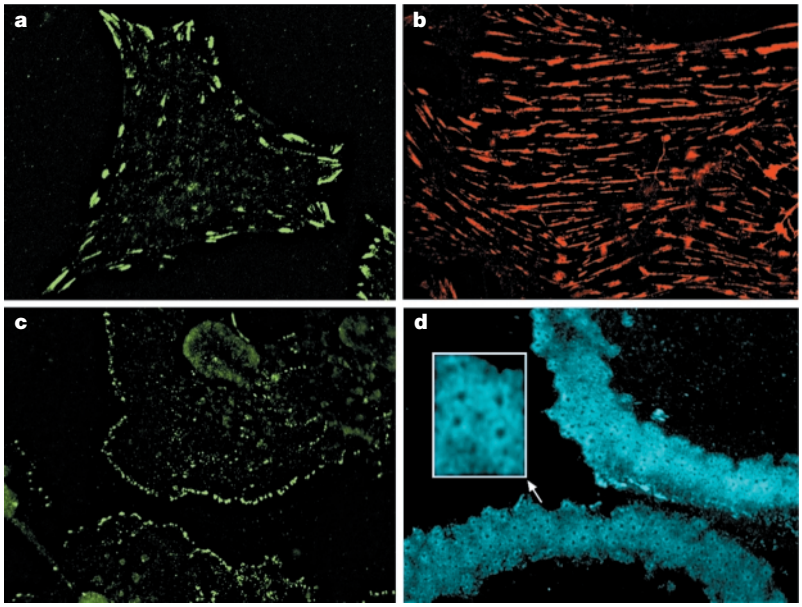

Figure 1 | **Immunofluorescence microscopic localization of the main forms of integrin-mediated matrix adhesions. a** | Human foreskin fibroblast, labelled for phosphotyrosine, displaying mainly 'classical' focal adhesions, which are located primarily at the cell periphery. **b** | Fibrillar adhesions of human foreskin fibroblasts labelled for tensin. These adhesions are typically associated with fibronectin fibrils and are enriched in central regions of the cells. **c** | Human fibroblasts (SV80 line) treated with the Rho-kinase inhibitor Y-27632 and immunolabelled for phosphotyrosine. The labelling is associated primarily with small dot-like structures associated with the lamellipodium, which are identified morphologically as focal complexes. **d** | Paxillin-labelled podosomes formed by a primary rat osteoclast. Individual podosomes consist of a ring containing several 'plaque proteins' (see insert), and an actin-rich central domain. As seen in this picture, podosomes often tend to cluster into large arrays.

strong adhesion to the substrate, and they anchor bundles of actin microfilaments through a plaque that consists of many different proteins. Development of focal adhesions is stimulated by the small GTPase Rho-A, and is driven by actomyosin contractility (see below). Characteristic plaque proteins include vinculin, talin, paxillin and tyrosine-phosphorylated proteins (FIG. 1a). Although by definition, focal adhesions are formed by cultured cells that grow on solid surfaces, structures with similar molecular properties are found *in vivo*. For example, adhesions formed by aortic endothelium cells with the underlying BASEMENT MEMBRANE[11], membrane-bound dense plaques of smooth muscle cells and myotendinous junctions that are formed by skeletal muscle cells[12] are all closely related to focal adhesions.

In more central locations of many cell types are the fibrillar adhesions (previously termed ECM contacts)[13–15], which are elongated or dot-like structures that are associated with ECM fibrils (FIG. 1b). The typical components of fibrillar adhesions are extracellular fibronectin fibrils, the fibronectin receptor $\alpha_5\beta_1$ integrin, and the cytoplasmic protein tensin[14].

Another group of matrix adhesions are focal complexes (FIG. 1c), which are small, dot-like adhesions that are present mainly at the edges of the LAMELLIPODIUM[16–18]. These sites can be associated with cell migration or serve as precursors of focal adhesions. Their formation is induced by the RHO-FAMILY GTPASE Rac[17,18].

A final variety of ECM adhesions are podosomes, which are small (~0.5 μm diameter) cylindrical structures containing typical focal contact proteins — such as vinculin and paxillin — that are found in various malignant cells and in some normal cells, including MACROPHAGES and OSTEOCLASTS[19–21] (FIG. 1d). The characteristic and indispensable proteins of podosomes are gelsolin[22] and membrane invagination-associated dynamin[23].

Although there are some distinctive features of these various forms of adhesions (TABLE 1), it is noteworthy that the molecular and functional differences between them are still poorly defined. Some of these differences will be highlighted below.

The molecular complexity of focal adhesions

When the Emperor Joseph II first listened to *The Abduction from the Seraglio*, he reportedly said, "My dear Mozart, that is too fine for my ears; there are too many notes."

When viewing the dauntingly expanding list of focal-adhesion components (FIG. 2), it is also easy to conclude that there are too many components. However, to continue the story, Mozart replied, "I ask your Majesty's pardon, but there are just as many notes as there should be." Similarly, gene knockout analyses confirm the importance of each focal-adhesion component in animals, although this has not been the case in all cultured cells (for example, for vinculin[24]). Similarly, even though integrins show important overlaps in binding specificity for individual ECM proteins in cultured cells, each is essential *in vivo*[25].

BASEMENT MEMBRANE
A dense, sheet-like, laminated extracellular matrix that separates epithelia, muscle, or other tissues from connective tissue.

LAMELLIPODIUM
A thin, flat extension at the cell periphery, which is filled with a branching meshwork of actin filaments.

RHO-FAMILY GTPASES
A family of monomeric G proteins — comprising Rho, Rac and Cdc42 — that are homologous to Ras. These are important molecular switches, which control cytoskeletal assembly and contraction.

MACROPHAGE
A white blood cell that is specialized for phagocytosis.

OSTEOCLAST
A specialized cell that is involved in active bone resorption.

Table 1 | **Characteristic features of different types of cell–matrix adhesions**

Property/structure	Focal complexes	Focal adhesions	Fibrillar adhesions
Location	Edge of lamellipodium	Cell periphery	Central region of cells
Morphology	Dot-like	Elongated, oval	Fibrillar or beaded
Size (long axis)	1 μm	2–5 μm	Variable: 1–10 μm
Typical constituents	Paxillin Vinculin Tyrosine-phosphorylated proteins	α_V integrin Paxillin Vinculin α-actinin Talin Focal adhesion kinase Tyrosine-phosphorylated proteins	α_5 integrin Tensin
Induced by	Rac	Rho	Rho (?)

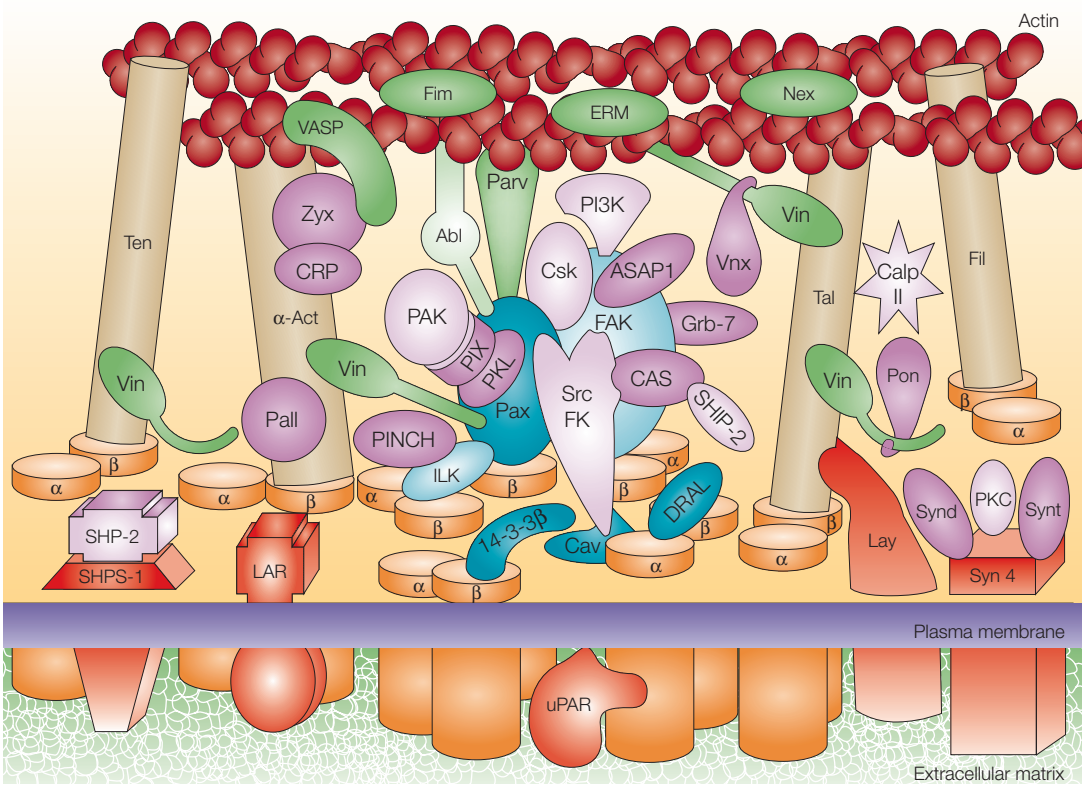

Figure 2 | **Schematic depicting the complexity of the main molecular domains of cell–matrix adhesions.** The primary adhesion receptors are heterodimeric (α and β) integrins, represented by orange cylinders. Additional membrane-associated molecules enriched in these adhesions (red) include syndecan-4 (Syn4), layilin (Lay), the phosphatase leukocyte common antigen-related receptor (LAR), SHP-2 substrate-1 (SHPS-1) and the urokinase plasminogen activator receptor (uPAR). Proteins that interact with both integrin and actin, and which function as structural scaffolds of focal adhesions, include α-actinin (α-Act), talin (Tal), tensin (Ten) and filamin (Fil), shown as golden rods. Integrin-associated molecules in blue include: focal adhesion kinase (FAK), paxillin (Pax), integrin-linked kinase (ILK), down-regulated in rhabdomyosarcoma LIM-protein (DRAL), 14-3-3β and caveolin (Cav). Actin-associated proteins (green) include vasodilator-stimulated phosphoprotein (VASP), fimbrin (Fim), ezrin–radixin–moesin proteins (ERM), Abl kinase, nexillin (Nex), parvin/actopaxin (Parv) and vinculin (Vin). Other proteins, many of which might serve as adaptor proteins, are coloured purple and include zyxin (Zyx), cysteine-rich protein (CRP), palladin (Pall), PINCH, paxillin kinase linker (PKL), PAK-interacting exchange factor (PIX), vinexin (Vnx), ponsin (Pon), Grb-7, ASAP1, syntenin (Synt), and syndesmos (Synd). Among these are several enzymes, such as SH2-containing phosphatase-2 (SHP-2), SH2-containing inositol 5-phosphotase-2 (SHIP-2), p21-activated kinase (PAK), phosphatidyl inositol 3-kinase (PI3K), Src-family kinases (Src FK), carboxy-terminal src kinase (Csk), the protease calpain II (Calp II) and protein kinase C (PKC). Enzymes are indicated by lighter shades. For further details about these and additional focal-adhesion components see REF. 10.

Focal-adhesion components have been identified over the past two decades, primarily by immunofluorescence studies carried out by many laboratories on numerous cell types. As reviewed elsewhere[10], more than 50 different molecules are found either stably or transiently in focal adhesions and other cell–matrix adhesions, and many others can affect these structures without being physically associated with them. For the sake of clarity, we will assign (somewhat artificially) the various constituents of focal adhesions to specific functional subdomains of these adhesions and discuss their function in that context.

The membrane domains of ECM adhesions contain specific integrins, which are heterodimers of α and β subunits that bind to the ECM through a large extracellular domain. They span the membrane and contain a cytoplasmic region through which they interact with plaque proteins[26]. The most common integrins found in focal adhesions and other ECM adhesions are $\alpha_5\beta_1$ (the classical fibronectin receptor) and $\alpha_v\beta_3$ (vitronectin receptor), although others are present on substrates such as collagen.

Somewhat less-characterized exterior molecules are the membrane-bound, non-integrin components of focal adhesions (red-coloured molecules in FIG. 2). They include potentially adhesive molecules, such as syndecan-4 (REF. 27) and the hyaluronan-binding protein layilin[28], as well as the urokinase plasminogen activator receptor (uPAR), which binds integrins and stabilizes their complexes with the membrane protein caveolin[29], and various other molecules whose association with ECM adhesions need further substantiation and characterization. For example, the signalling lipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2), which can regulate actin polymerization[30] and cytoskeleton–plasma membrane interaction[31], might be associated with focal adhesions (REF. 32, but also see REF. 33). In addition, the transmembrane tyrosine phosphatase leukocyte common

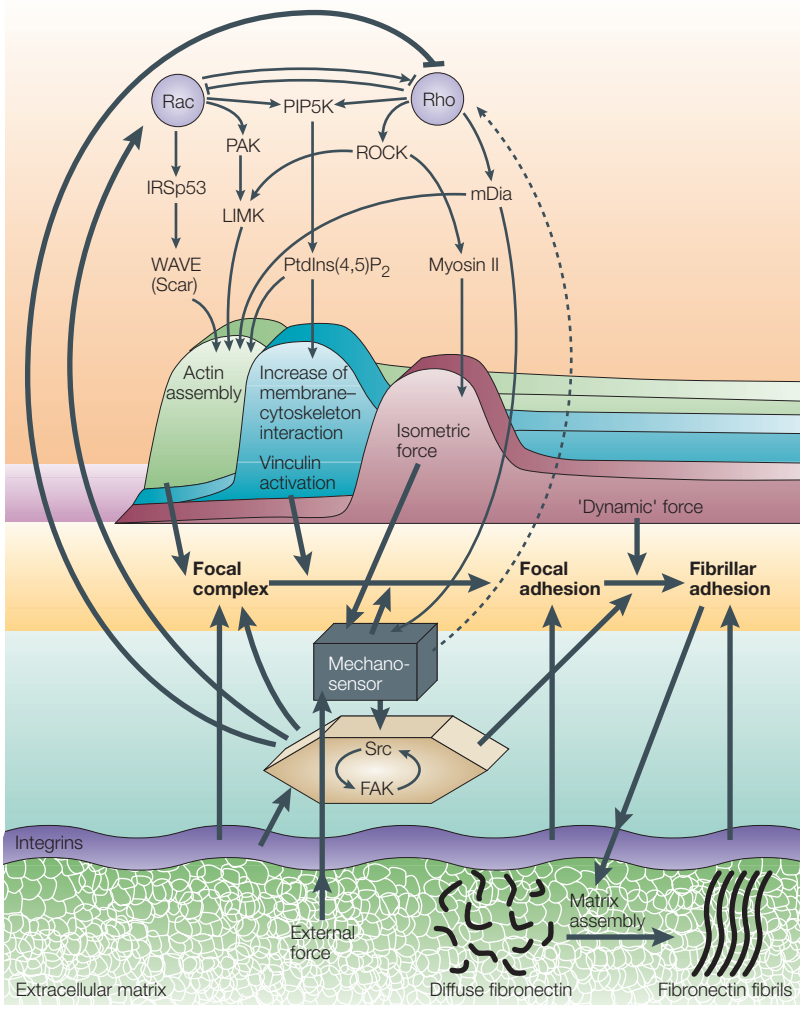

Figure 3 | **Cells probe, respond to, and remodel the extracelluar matrix (ECM) using integrin–actin cytoskeleton adhesion complexes.** A highly schematic representation of local and global events associated with focal-adhesion formation and development. The temporal steps from focal-complex formation to the development of focal adhesions and fibrillar adhesions are listed from left to right (central yellow belt). Just above it, important molecular and physical events that drive focal-adhesion formation are shown, including the polymerization of actin, the activation of association of plaque proteins (such as vinculin) to the membrane, and the increase of tension forces, which drive focal-adhesion and fibrillar-adhesion development. Rac- and Rho-dependent signalling pathways triggering these events are shown above (see REF. 50 for more detail). In adhesions formed with rigid ECM, isometric force develops and stimulates focal-adhesion growth. In interactions with a more pliable matrix, the force (defined here loosely as 'dynamic' force) mobilizes both the matrix and the attached adhesion complex. This process leads to assembly and reorganization of the ECM from a diffuse to a fibrillar matrix (bottom part of the scheme). The transmembrane linkage and regulation of these processes are mediated through integrins, which affect both the local regulatory system (which consists of a putative mechanosensor and the focal adhesion kinase (FAK)–Src phosphorylation system) and a global regulatory system, which consists of Rho, Rac and their targets (upper part of the scheme). PAK, p21-activated kinase; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; ROCK, Rho-associated kinase.

antigen-related receptor (LAR) and transmembrane glycoprotein SHP-2 substrate-1 (SHPS-1) also localize at focal adhesions, and potentially modulate integrin signalling[34,35]. The interplay between these membrane molecules and integrins is still not known.

In the cytoplasm, several integrin-associated multimolecular domains have been identified[10]. A small group of proteins (talin, α-actinin, tensin and filamin; represented by golden rods in FIG. 2) can function as direct integrin–actin linkers bridging between membrane integrins and the cytoskeleton[10,36]. Additional integrin-associated molecules (coloured blue in FIG. 2) do not interact directly with actin, but might bind to the cytoskeleton indirectly through other components of the submembrane plaque. Some of them, such as focal adhesion kinase (FAK), down-regulated in rhabdomyosarcoma LIM protein (DRAL), integrin-linked kinase (ILK) and 14-3-3β (REF. 37), are signalling molecules. Most of these proteins interact with β integrins, although paxillin can also tightly interact with two closely related α integrins (α_4 and α_9). This binding inhibits cell spreading and the formation of focal adhesions[38,39]. Paxillin has an important role in anchoring a host of proteins, which include the FAK regulator of focal adhesions to the membrane.

An additional group of focal-adhesion-associated proteins includes actin-binding proteins, which are not thought to interact directly with integrins (including vinculin, vasodilator-stimulated phosphoprotein (VASP)/Ena and ezrin–radixin–moesin (ERM) proteins — green in FIG. 2). Vinculin has a pivotal role as a universal linker as it interacts with many plaque proteins (including talin, α-actinin, VASP/Ena, ponsin, vinexin and protein kinase C (PKC)), as well as with acidic phospholipids, membranes and actin. A very large group of proteins (too many to list here) consists of adaptor proteins, which apparently interact with actin-bound and integrin-bound components and link them to each other.

These components of cell–matrix adhesions have an unusually wide range of intrinsic activities. Beyond their protein–protein binding specificities, many of these proteins are enzymes (light shading in FIG. 2), including tyrosine kinases (such as members of the Src family and FAK); serine/threonine kinases (such as ILK, PKC and p21-activated kinase (PAK)); tyrosine phosphatases (such as SHP-2 and LAR); inositol 5-phosphatases (such as SH2-containing inositol 5′-phosphatase-2 (SHIP-2)[40]); modulators of small GTPases (such as ASAP1, 180-kDa protein downstream of CRK (DOCK180), PAK-interacting exchange factor (PIX) and GAP for Rho associated with focal adhesion kinase (GRAF)); and other enzymes, such as phosphatidylinositol 3-kinase (PI3K) and the protease calpain II (REF. 10). The true molecular complexity of focal adhesions is probably greater than depicted in FIG. 2, as many of these components represent products of multigene families (for example, ERM, Src-family kinases and even integrins themselves) rather than a single molecule, and as many additional components will probably be found.

A striking characteristic of many focal-adhesion components is that they are multidomain molecules that can interact with several distinct partner molecules (although not necessarily simultaneously). For example, molecules such as vinculin, FAK, Src kinases and paxillin can each bind to more than ten different partners. So, the theoretical number of different combinations of molecular interactions that might be involved in linking integrins to actin is enormous. How, then, do cells actively regulate the proper assembly of the submembrane plaque? The key mechanisms seem to involve integrin

Box 1 | **Cellular mechanosensors**

In order for cells to migrate and organize tissues successfully, they must not only sense chemical signals in their microenvironment, but also physical cues. How such cellular mechanosensors detect forces, sense the physical properties of substrates and convert them to signals is still virtually unknown — they could consist of one–two proteins or be a supramolecular complex. Besides the mechanical stimulations that are described in this review, cells sense and respond to being stretched, as well as to shear stress from flowing liquids (for example, blood over endothelial cells), which might involve either the same or different mechanisms. The mechanosensor for the latter force involves integrins that interact with extracellular matrix (ECM) and activation of signal-transduction pathways, including FAK and two MAPKs[64,111–113]. It is noteworthy that mechanosensors are present in other systems too. These include stretch-activated ion channels, which provide a direct mechanism for converting a force into a signalling response[114], the mechanically gated ion channels that are involved in sound detection through hair cells of the inner ear[115], and the tension-driven modulation of protein phosphorylation at the kinetochore, which allows onset of anaphase only when the chromosome is properly attached to the mitotic spindle[116].

In the formation and enlargement of cell adhesions, a principal cellular response involves the assembly of multimolecular complexes. These responses to mechanosensory input involve what can be termed molecular switches. One class of such molecular switches is well known for signal transduction; for example, covalent modification and activation (or inactivation) of a protein with a phosphate group after phosphorylation by a kinase, such as for activation of FAK or Src (see below). However, other molecular switches use conformational changes in a protein to expose cryptic sites. Opening up of a protein or a protein domain to expose new protein–protein interaction sites occurs for Src family kinases[117], vinculin (reviewed in REF. 10), ERM proteins[118], various targets of small Rho G-proteins, such as mDia, ROCK or WASP[30,51], and fibronectin (see below). This on/off switching can be mediated by enzymatic modification (for example, phosphorylation or dephosphorylation of Y527/530 of Src), signalling molecules (for example, PtdIns(4,5)P_2 for vinculin, Rho for Dia, etc.), or force (stretching of FIBRONECTIN (type III) MODULES). In addition, more complex mechanisms of mechanosensory switching can be predicted[50].

FIBRONECTIN MODULES
Subunits of fibronectin are comprised of repeating structural modules of three types (I, II, III). Each module is encoded by one or two exons with introns that precisely separate repeats. There are 12 type I modules, each around 45 amino-acids long and clustered into three groups; two type 2 modules, each 60 amino acids-long; and 15–17 type III repeats, each about 90 amino-acids long (see FIG. 6).

BDM
(2,3-Butanedione monoxime) An inhibitor of myosin ATPase.

ML-7
(1-(5-iodonaphthalene-1-sulphonyl)-1-H-hexahydro-1, 4-diazepine) A kinase inhibitor thought to be relatively specific for myosin light-chain kinase.

H-7
(1-(5-isoquinolinylsulphonyl)-2-methylpiperazine) A broad-spectrum serine–threonine kinase inhibitor that blocks myosin light-chain kinase, Rho kinase and certain other kinases.

mechanics, mechanosensors, molecular switches and classical signal-transduction pathways (FIG. 3, BOX 1).

Cellular control of focal-adhesion assembly

Initiation of complexes. A chief mechanism for inducing focal-adhesion assembly and signalling involves responses to local mechanical forces. Such triggering of signalling processes is a unique feature of cell adhesions to solid surfaces (ECM or the membrane of other cells), which cannot be mimicked by binding to soluble monovalent ligands. An initial step involves the physical clustering of integrin molecules, which by itself can initiate various signals[41]. Monovalent ligand binding (occupancy) of the integrin alone can sometimes trigger a signalling response but generally not the formation of adhesion complexes. For the successful accumulation of cytoskeletal and many signalling molecules, ligand clustering of the integrin is also required along with occupancy[42].

The first small integrin complexes that are formed at cell contacts with the ECM are commonly termed focal complexes (FIGS. 1 and 3). These structures can be transient or evolve to focal adhesions. It should be noted that the definition of these structures is still rather tentative and potentially confusing. A 'spatial definition' refers to focal complexes as small (~1 μm^2) structures that contain integrins and associated molecules at the edge of the lamellipodium; a 'temporal definition' refers to them as the earliest form of adhesion complexes[43], and a 'signalling definition' indicates that these could be structures that are induced by the small guanine-nucleotide-binding protein Rac[17,18] (FIG. 3). Whether all three definitions refer to exactly the same structure is still unclear.

Although this article focuses specifically on integrin-mediated adhesion, it is noteworthy that additional adhesion systems, for example, adhesion to glycosaminoglycans such as hyaluronan, might be involved in the interactions of a cell with an external surface, providing the first tethering of the cells to the surface (E. Zimmerman, B. G. and L. Addadi, unpublished data).

Sensing of local tension as a mechanical switch. Focal complexes can disappear or evolve into stable focal adhesions. Forces that activate the stabilization and growth of focal adhesions can be generated internally by the intracellular contractile machinery, or applied from the outside. So, the activation of myosin-II-driven contractility by serum stimulation or microtubule disruption leads to a striking enhancement of focal-adhesion formation[44,45]. Alternatively, the ability of external forces to activate focal-adhesion development can be shown by gentle application of a mechanical force to the edge of serum-starved cells[46]. As shown in FIG. 4, local mechanical perturbation (FIG. 4a,c) induces a pronounced local increase in focal-adhesion dimensions (FIG. 4b,d). This treatment transforms small peripheral focal complexes into focal adhesions.

Actomyosin inhibitors, such as BDM, ML-7 or H-7, as well the Rho-kinase inhibitor Y-27632, reduce contractility and produce a rapid loss of focal adhesions[18,44,47,48] (FIG. 1b). When more physiological relaxation is induced by the expression of caldesmon — a natural regulator of actomyosin contractility — cells that grow on an elastic silicone surface show complete relaxation, as manifested by loss of substrate wrinkling (REF. 49; FIG. 4e). The caldesmon-transfected cells also lose their large focal adhesions, leaving only punctate, focal-complex-like structures[49] (FIG. 4f, g). As discussed in detail elsewhere[46,50], the enhancement of focal adhesions by the application of external force does not require cellular actomyosin contractility as might be expected, because local tension is generated by the external intervention. It does, however, require the Rho-target protein Diaphanous (mDia1) (REFS 46, 51; FIG. 3). Experimental relaxation of contractility also induces focal complexes, which indicates that the focal complex-to-focal adhesions transition might be reversible, although the relationship of these drug-induced structures to the early ECM contacts that are involved in motility is uncertain.

The transformation of initial integrin adhesion complexes into cytoskeleton-restrained adhesions was also studied using LASER TWEEZERS to arrest the centripetal transport of fibronectin-coated beads from the LEADING EDGE. Contacts that are established between such beads and the cell surface are initially weak (they can be disrupted by <5 pN force) but can be reinforced within 10 seconds by cytoskeletal interactions if the cell senses local resistance to movement, so that even a force of 60 pN applied by the laser trap cannot disrupt them[52]. A local

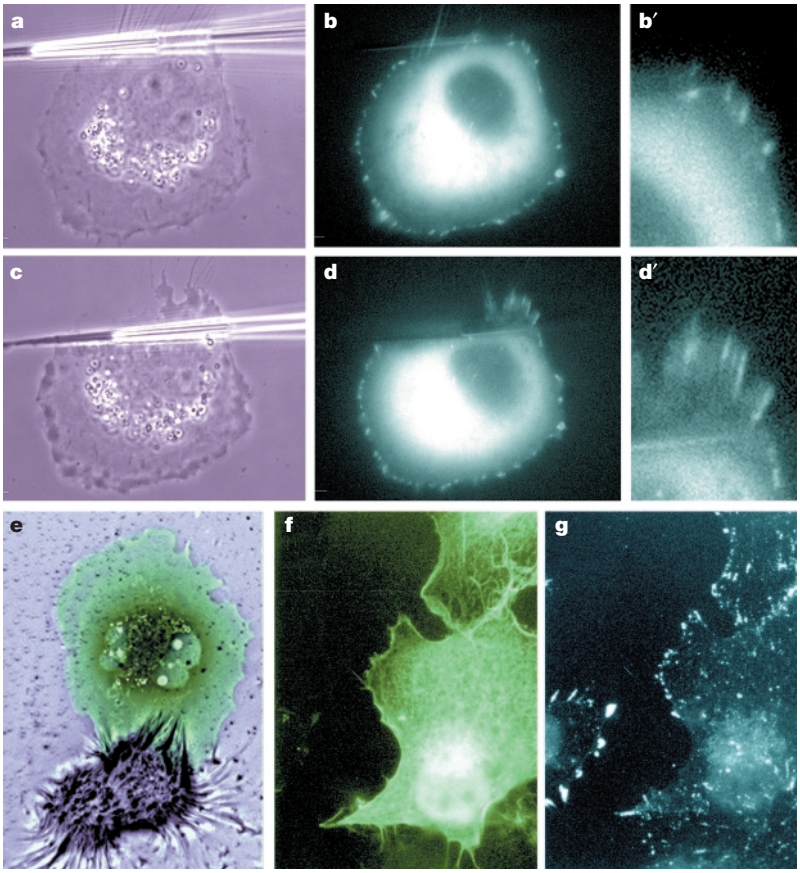

Figure 4 | **Modulation of focal-adhesion assembly by application of external force (a–d) or relaxation of actomyosin contractility (e–g). a–d** | Serum-starved SV80 cells expressing GFP–vinculin were plated on a fibronectin matrix and subjected to local centripetal force applied by a micropipette. **a** and **b** | Phase-contrast and fluorescence images of the cell before application of force. **c** and **d** | The same field as in **a** and **b** 3 minutes after pulling. **b′** and **d′** | Affected cell region before and after pulling. Note the local growth of vinculin-containing focal adhesions in response to force application. Reproduced from REF. 49 by permission of the American Society of Microbiology via the Copyright Clearance Center **e** | Two cells are shown attached to a flexible silicone rubber film. The upper cell was transfected with GFP–caldesmon, a negative regulator of myosin ATPase and therefore of actomyosin contractility. Note that the transfected cell fails to wrinkle the silicone substrate, contrary to the contractile control cell. **f** and **g** | GFP–caldesmon-transfected cells (on the right) display diminished focal adhesions compared with the non-transfected cell on the left (phosphotyrosine labelling in **g**). Reproduced from REF. 46 by copyright permission of The Rockerfeller University Press.

mechanosensor seems to probe for local forces at the attachment site, and if firm, the cellular response is a connection or anchoring to the cytoskeleton. Analogously, when a cell attaches to a solid substrate, it initially forms tiny contacts that contain paxillin but not α-actinin. These contacts depend on $\alpha_5\beta_1$ integrin, although the levels of this integrin might be below the levels of visual detection[43]. Nevertheless, seemingly similar early contacts have been reported to exert exceptionally strong forces that provide the main motive force for cell migration[53]. These puzzling findings indicate mechanisms for rapid local mechanosensing, cytoskeletal linkage, and development of strong locomotory forces that will need molecular explanations.

Focal adhesions as tactile mechanisms of cells

The idea that mechanical force can activate links to the cytoskeleton and then focal-adhesion assembly and signalling implies that a cell can sense the elasticity of the matrix and respond to it. So, a cell might distinguish between soft and rigid matrices (irrespective of their specific molecular composition) by determining the level of tension that can be developed on binding to them, and might then respond with counteracting forces. In this regard, focal adhesions can be considered both as sensors of force and as sites that originate cytoskeletal forces through anchored actin-microfilament bundles.

Although the molecular nature and mechanisms of action of the mechanosensor systems are not yet known, biophysical experiments have provided initial quantification of the response. The local forces that are generated by early integrin–cytoskeleton complexes or at focal adhesions have been measured by several experimental approaches, including CELL-INDUCED SUBSTRATE WRINKLING[54,55], use of a special MICRO-CANTILEVER TILTING DEVICE[56], and the DEFORMATION OF ELASTIC GELS[57–59].

These measurements have yielded intriguing insights into the timing, magnitude and polarity of mechanical force development. As expected, focal adhesions (or focal complexes) are indeed sites at which force is applied to the ECM. Motile cells show strong propulsive traction at the leading-edge area (at apparent early focal complexes), with forces (traction stress) approximately fourfold larger than those associated with mature focal adhesions[53]. In mature focal adhesions, the direction of the force and its total magnitude correspond to the long axis and size of the focal adhesion, respectively[59]. In non-motile cells, a close correlation was found between the local force at a focal adhesion and its size, indicating that the mechanical stress exerted in these sites could be uniform per unit area (~5.5 $nN\mu m^{-2}$). The local concentrations of proteins, such as vinculin and paxillin (as determined by measuring fluorescence intensity of green fluorescent protein (GFP) fusion derivatives of the two proteins), in mature focal adhesions are also largely uniform[59]. These findings also indicate that differential substrate rigidity might control directional motility by reinforcing cell adhesions at the more rigid areas along the ECM. Indeed, cells tend to migrate towards more rigid areas of the matrix[6].

The data described above provide compelling evidence for the existence of mechanosensory systems within focal adhesions and focal complexes. This mechanosensor can transform mechanical perturbation into a signal that effects assembly of focal adhesions and activates integrin-mediated signalling. The downstream targets of the mechanosensor are still poorly defined, although indirect evidence points to the involvement of tyrosine phosphorylation (FIG. 3 and see below) in the process.

Tyrosine phosphorylation in focal adhesions

A well-known mediator and regulator of protein–protein interactions is the binding of proteins that contain Src homology 2 (SH2) domains to tyrosine-phosphorylated partners. Focal adhesions have many SH2-containing components (such as Src kinases, PI3K, SHP-2), as well as many tyrosine-phosphorylated molecules (for

LASER TWEEZERS
Microscope-based device that traps micron-sized particles in a focused laser beam. Can be used to move or to stop such particles.

LEADING EDGE
The leading region of the advancing lamellipodium in a motile cell.

CELL-INDUCED SUBSTRATE WRINKLING
An approach for visualizing cellular contractility that is based on wrinkling of a thin and flexible silicone rubber film on which the cell is cultured.

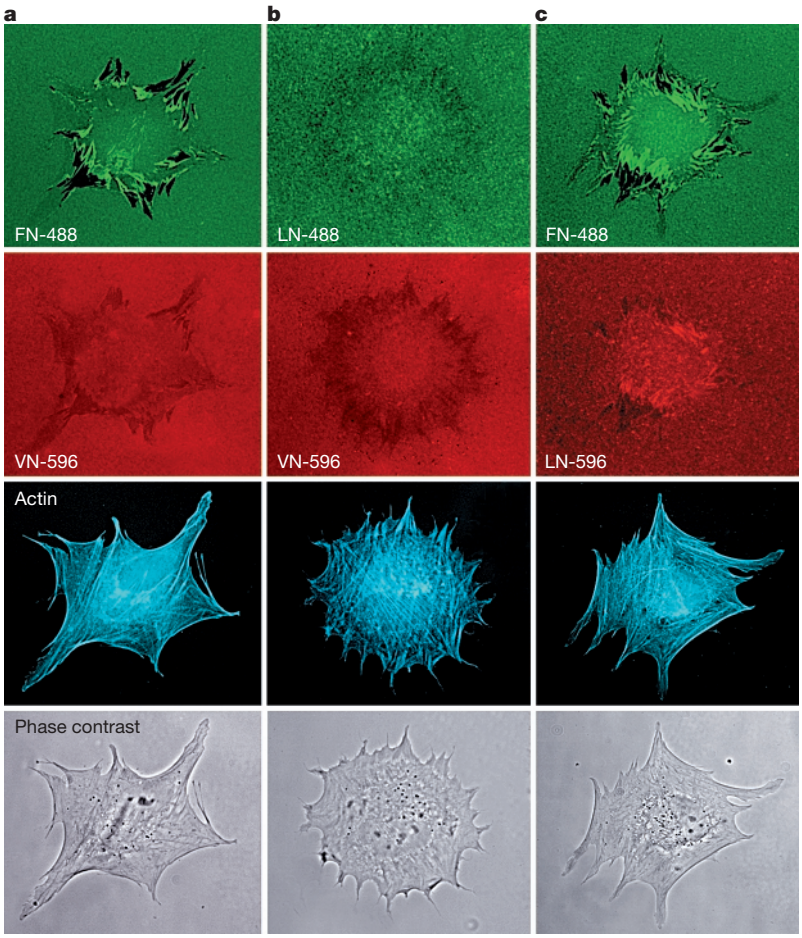

Figure 5 | **Cells reorganize the underlying substrate differentially depending on its composition.** The ability of cells to modify their extracellular environment was tested by plating primary human fibroblasts on coverslips that were coated with mixtures of two different ECM molecules (5 μg ml^{-1} each), which were directly labelled with the green dye Alexa fluor 488 or red Alexa fluor 596. **a** | Fibroblasts markedly reorganize fibronectin (FN)-containing substrates to form aggregates and fibrils. However, these cells have minimal effects on vitronectin (VN)-coated (**a**,**b**) or laminin (LN)-coated (**b**) surfaces. However, some laminin molecules can associate with fibronectin and become co-distributed during fibronectin fibrillogenesis (**c**). The actin cytoskeleton is visualized with phalloidin–CPITC (blue), and overall cell shape is shown by phase-contrast microscopy. Note that the type of substrate also affects the actin cytoskeleton and cell shape.

MICRO-CANTILEVER TILTING DEVICE
A microscopic device in which cells are attached to a surface that consists of arrays of cantilevers. Local forces that are applied to this surface induce tilting of these cantilevers, which can be measured.

DEFORMATION OF ELASTIC GELS
Polymeric elastic gels that either contain impregnated beads or are surface micro-patterned are used as substrates for cultured cells. Local forces that are applied to these substrates can be measured, based on the distortion of these patterns.

example, FAK, paxillin, tensin, CAS, SHPS-1 and caveolin). So, tyrosine phosphorylation of these sites, which is induced by clustering of integrins[42,60,61], growth factor stimulation[62,63], or applying force[64–66] could potentially stimulate focal-adhesion assembly by promotion of intermolecular SH2–phosphotyrosine interactions. Indeed, inhibitors of tyrosine phosphorylation block adhesion-complex formation and recruitment of a large subset of focal-adhesion components[41,61].

Moreover, the phosphotyrosine content in focal adhesions increases on force application before any important changes in focal-adhesion structure are apparent[14], which indicates a potential role for tyrosine phosphorylation in focal-adhesion assembly. Therefore, you might consider the following molecular scenario: FAK might be recruited to the membrane following integrin engagement and become autophosphorylated at tyrosine 397. Src might then be recruited through its SH2 domain to the phosphorylated FAK[67], and both enzymes might phosphorylate various focal-adhesion targets, creating additional docking sites for SH2-containing proteins. Genetic studies using *Src*-null cells indeed indicate that the rate of spreading and focal-adhesion development seems slower[68–71].

However, recent studies, as well as re-examination of earlier findings on Src/FAK function, show a far more complex situation. Although integrin-activated FAK and Src initially seemed to be ideal candidates for the initiation of this cascade of phosphorylation and complex formation, subsequent studies indicated that neither of these components is essential for focal-adhesion assembly; for example, genetic deficiencies of either protein do not prevent focal-adhesion formation[71,72]. By contrast, both FAK and Src seem to have principal roles in promoting the turnover and dynamics rather than assembly of focal adhesions. Cells that are deficient in FAK or Src have enlarged focal adhesions[71,72], and FAK-deficient fibroblasts show defects in migration that are consistent with roles in focal-adhesion turnover, which is needed for locomotion (for review see REF. 67). Moreover, osteoclasts that are deficient in Src lose their characteristic podosomes (adhesions induced in other cells by oncogenic Src) and instead acquire actin microfilament bundles that closely resemble the focal adhesion-dependent cytoskeleton of fibroblasts[73]. Along the same line, the 'reinforcement' response to early focal complex formation that is induced by ECM-coated beads is inhibited by the tyrosine-phosphatase inhibitor phenylarsine oxide[52], indicating a causal role for downregulated phosphotyrosine. Moreover, the linkages between vitronectin-coated beads and the force-generating cytoskeleton are higher in Src-null compared to wild-type cells[70]. So, it seems that tyrosine phosphorylation and, in particular, Src–FAK-mediated phosphorylation, can have dual, and apparently conflicting, roles in regulating focal-adhesion dynamics: on the one hand, it might trigger molecular interactions and facilitate focal-adhesion growth, and, on the other, it is involved in focal-adhesion turnover, which leads to the formation of fibrillar adhesions[71] or even podosomes. The mechanism underlying this effect is not clear, but it might involve suppression of Rho activity[74,75].

Cell adhesions organize the extracellular matrix

Although cell adhesions mediate attachment, migration and signalling on extracellular substrates, they have equally important roles in the creation and organization of the ECM. For example, gene knockout studies have shown the importance of adhesion receptors for correct assembly of basement membranes — β_1, α_3 and α_6 *integrin*-null mice, as well as *dystroglycan* knockouts, all showed basement membrane defects[76,77]. Integrin and dystroglycan receptors cooperate in the process of laminin polymerization on cell surfaces that are associated with actin reorganization and signalling events[78].

Cultured cells can markedly and selectively rearrange ECM proteins, such as fibronectin, on substrates and assemble them into cell-surface fibrils[79,80] (FIG. 5). The

early pattern of organization of collagen fibrils *in vitro* depends on fibronectin[81], the organization of which depends on integrins[82]. Moreover, cells in organized collagen gels generate tension that is transmitted by integrins to reorganize the collagenous matrices[83]. These and many other examples underscore the importance of cell-surface interactions, particularly integrin-mediated adhesion, in organizing the ECM.

Fibronectin fibrils and integrin dynamics

The best-characterized role of adhesions in organizing extracellular matrices is seen in the creation of extracellular fibrils of fibronectin from soluble fibronectin. This complex process of fibrillogenesis is driven by an intriguing cooperation between two distinct types of cell-surface adhesions: the focal and fibrillar adhesions. They cooperate in a process by which integrins and dynamic tension forces seem to unmask cryptic fibronectin assembly sites that mediate this polymerization and generate networks of fibrillar ECM.

Fibronectin–integrin interactions in fibrillogenesis. Fibronectin (FIG. 6) is a large glycoprotein that circulates in blood at high concentrations (0.3 g l^{-1} of plasma) as a dimer in an apparently non-functional closed form. By contrast, fibronectin generally functions in tissues in the form of insoluble ECM fibrils composed of fibronectin multimers. Fibronectin fibrils are prominent in loose CONNECTIVE TISSUE, GRANULATION TISSUE, embryonic basement membranes and on many cells in tissue culture[84–86].

Because accidental conversion of the large quantities of soluble, circulating fibronectin to insoluble aggregates in the bloodstream would have disastrous consequences, the creation and deposition of fibronectin fibrils in the ECM is a tightly regulated, cell-mediated process. Although studied for over a decade, new steps in this process have recently been identified. The first phase, most investigators agree, involves binding of fibronectin to the surface of a cell. This step is mediated primarily by the $\alpha_5\beta_1$ integrin, although a couple of other integrins (for example, $\alpha_V\beta_3$) can support fibronectin-matrix assembly with lower efficiency[82,87–89]. Unidentified non-integrin receptors could also be involved in this initial cell-surface binding[90].

Fibronectin elasticity and role of cell-generated tension. Although necessary, the binding of fibronectin to cells is not sufficient for fibrillogenesis. A crucial step is now thought to be the cell-driven exposure of cryptic self-association sites in fibronectin, which are necessary for polymerization. Sites that are implicated in such fibronectin-matrix assembly are shown in FIG. 6. Although one mechanism for exposing fibronectin cryptic sites could be conformational changes induced by fibronectin binding to integrins[91], additional steps seem necessary to explain the requirement for cellular contractility in fibronectin fibrillogenesis (see below). Interestingly, almost all of these cryptic sites are localized within fibronectin type III repeating modules, which are known to unfold

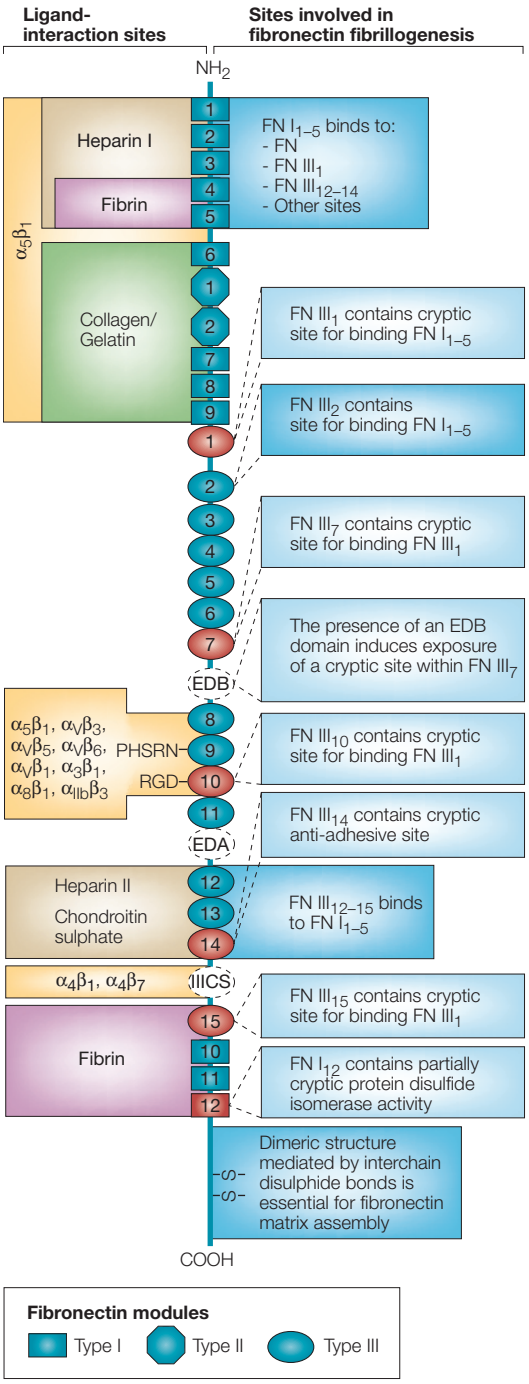

Figure 6 | **Ligand-binding regions and interaction sites of fibronectin.** Fibronectin (FN) is composed of three types of repeating unit, designated type I (rectangles 1–12), type II (octagons 1–2), and type III (ovals 1–15) modules. Dotted ovals indicate units in which alternative splicing of messenger RNA inserts type III modules termed ED-A (EDA) and ED-B (EDB), or portions of the variable IIICS region. The labels along the right side of the molecule indicate exposed or cryptic self-interaction sites involved in fibronectin fibrillogenesis. Modules reported to contain cryptic fibronectin–fibronectin interaction sites are coloured red. The labels at the left indicate regions involved in binding interactions with different members of the integrin family or other ECM molecules. The primary adhesive recognition (RGD) and synergy (PHSRN) sequences within the central cell-binding region of fibronectin molecules are also indicated.

CONNECTIVE TISSUES
Tissues that form the architectural framework of the vertebrate body. In these tissues, the extracellular matrix is plentiful and cells are sparsely distributed within it.

GRANULATION TISSUE
A contractile, myofibroblast-containing tissue formed in wounds.

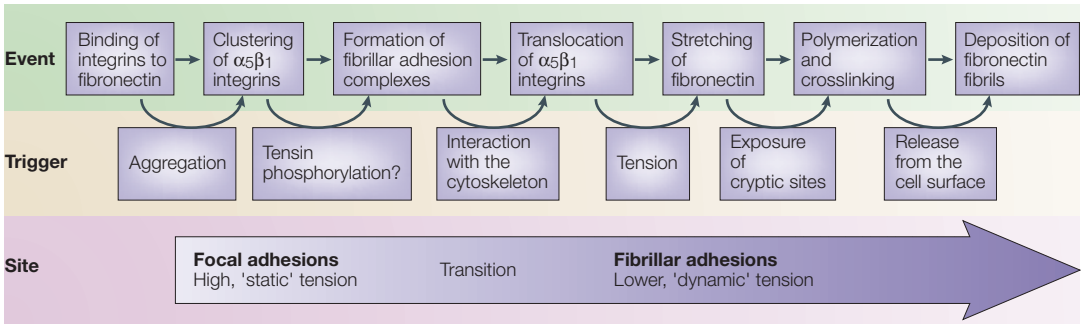

Figure 7 | **Schematic representation of the main steps in fibronectin fibrillogenesis.** The process of fibrillogenesis can be viewed as a series of events (top green band) that sequentially trigger subsequent steps by inducing specific molecular changes, such as intermolecular interactions, tension or altered conformation (central yellow band). The site or structure at which these steps occur shifts from focal adhesions to fibrillar adhesions, driven by centripetal tension from higher, static and isometric tension in focal adhesions to lower and dynamic tension in fibrillar adhesions (bottom pink band). See text for further discussion.

reversibly in response to applied force[92]. So, external tension to unfold fibronectin was proposed to be a trigger for fibronectin polymerization[93,94]. In fact, cell-generated tension is now known to be a prerequisite for fibronectin fibrillogenesis, and loss of cellular contractility by downregulating Rho, myosin inhibitors, or actin-disrupting agents inhibits fibronectin-matrix formation[80,90,94–96].

The fibronectin-unfolding process probably involves both the molecule as a whole and its individual domains. To maintain its relatively globular shape in blood, fibronectin might use intramolecular interactions between $I_{1–5}$ modules in the amino-terminal 70-kDa domain and the III_1 or the III_2 module[91,97]. Besides opening fibronectin from this closed conformation, however, cell-generated tension apparently unfolds individual fibronectin type III modules. This reversible unfolding can explain the known elasticity of fibronectin, which is under tension and is tightly stretched in cell culture[92,98]. In fact, stretching fibronectin on a rubber substrate by 30% exposes a cryptic epitope and induces a sevenfold increase in binding of soluble fibronectin, consistent with the need to unfold fibronectin for matrix assembly[96].

The level of force that is sufficient to unfold various individual fibronectin type III modules is ~3.5–5 pN, and a similar force (5 pN) is sufficient to stretch intact plasma fibronectin fivefold to a length of 860 nm (REF. 92); although interestingly, fibronectin–GFP can be stretched as much as fourfold by living cells[98]. This force level is substantially lower than the force of 10–30 pN needed to disrupt bonds between integrins and fibronectin[92]. Forces of 5 pN per molecule are readily achievable by living cells that normally develop forces in the range of 1–10 nN μm^{-2} (REFS 53,56,57,59). In fact, a single myosin molecule can generate 3–4 pN (REF. 99), indicating that several myosin molecules working together could generate enough force to completely stretch the fibronectin molecule.

Transmission of cell-generated tension

Integrin molecules that connect the actin cytoskeleton to the extracellular matrix are candidates for conveying tension that is generated by the actin cytoskeleton externally to fibronectin. Two structures that might transfer cell-generated tension are focal and fibrillar adhesions.

It was recently established that ligand-bound $\alpha_5\beta_1$ integrins actively translocate from focal adhesions to fibrillar adhesions[80]. This concerted movement provides a potential mechanism for integrins to apply tensile forces to stretch fibronectin and induce fibrillogenesis. $\alpha_5\beta_1$ integrins move from focal adhesions along fibrillar adhesions parallel to small actin-microfilament bundles. This highly directional, escalator-like type of movement of $\alpha_5\beta_1$ becomes activated when this integrin binds fibronectin (see FIG. 7) and is associated with elongation of newly forming fibronectin fibrils. Fibronectin fibrillogenesis is functionally tightly coupled with $\alpha_5\beta_1$ integrin translocation in fibrillar adhesions, with complete correlation under 14 different experimental states, which strongly implies causality[80].

Fibrillar adhesions are rich in tensin, which, as previously described, also translocates markedly[14,15]. Disrupting tensin function with a dominant-negative inhibitor causes fibrillar adhesions to disappear and blocks fibrillogenesis but does not disrupt focal adhesions[80]. Interestingly, vitronectin is also required for integrin translocation and fibronectin fibrillogenesis. Vitronectin is a prime target for the $\alpha_V\beta_3$ integrin of focal adhesions, further supporting the need for cooperation with focal adhesions, which could function as anchors or initiators[80]. Phosphorylation of tensin by $pp60^{c\text{-}src}$ might be the switch that regulates segregation of fibrillar adhesions from focal adhesions[71], which would in turn help to regulate fibronectin-matrix assembly.

Although focal-adhesion complexes act as substrate-anchoring sites for stress fibres and sustain high ISOMETRIC TENSION[59], we have proposed that the complexes that are involved in fibrillar adhesions can transform tension into directed movement along actin filaments[80]. Interestingly, the $\alpha_V\beta_3$ integrin of focal adhesions remains anchored, whereas the $\alpha_5\beta_1$ integrin moves, which indicates that there could be functional differences between these integrins – the former mediating isometric force and the latter 'dynamic' force involved in ECM assembly[15].

Additional insight into focal-adhesion dynamics was recently obtained using time-lapse video microscopy of

ISOMETRIC TENSION
A condition in which contraction of muscle, non-muscle cells or the actomyosin network is opposed by an equal load that prevents net shortening, even though tension increases.

cells that express GFP-tagged components. Such studies showed that focal adhesions and their variant forms are dynamic structures that can exchange components with the soluble cytoplasmic pool and translocate. Using GFP fusion proteins of classical focal adhesions (such as paxillin) and of fibrillar adhesions (tensin), it was found that both adhesion structures are dynamic[15]. Focal adhesions expand, shrink or translocate centripetally[15,100]. GFP–tensin, on the other hand, was continuously displaced from peripheral focal adhesions towards the cell centre[15] in parallel with the translocation of $\alpha_5\beta_1$ and fibronectin during the formation and extension of fibrillar adhesions[80].

The exact mechanism of translocation of the latter structures is not entirely clear. A possible force-generating system responsible for the long-range movements of fibrillar adhesions might be the actomyosin system. This possibility is supported by the capacity of contractility inhibitors such as H-7, ML-7 or the actin-polymerization inhibitor LATRUNCULIN-A, to block the movement of fibrillar adhesions. Interestingly, in contrast to focal contacts, which are strictly tension-dependent structures, the maintenance of fibrillar adhesions does not depend on actomyosin contractility[5,14,15].

An attractive, yet speculative, alternative mechanism for the generation of motile short-range forces might involve a TREADMILLING mechanism. Although possibly coincidental, the velocity of integrin movement in these adhesions ($6.5 \pm 0.7\ \mu m\ h^{-1}$)[80] resembles the rate of actin treadmilling *in vitro*, which has a calculated flux rate of $2\ \mu m\ h^{-1}$ (REF. 101); by contrast, the rate of bulk centripetal movement of actin in cells is an order of magnitude faster[102]. Newly incorporated actin appears first at focal adhesions and much later along stress-fibre bundles[103]. Capping of the BARBED END could lead to treadmilling-driven shortening of the capped filaments, which, together with the release of actomyosin-based isometric tension, might result in active pulling of attached fibrillar-adhesion complexes.

Tensin has an actin barbed-end capping domain together with two actin-binding domains, and it has been proposed that its dimer might embrace the ends of two actin filaments[104]. These features again point to tensin as a potential regulator or mediator of the formation of fibrillar adhesion complexes and might explain why it is necessary for fibrillogenesis. Regardless of the actual mechanism underlying the integrin dynamics in fibrillar adhesions, integrin translocation provides an attractive mechanism for transferring tension generated by the intracellular contractile machinery to extracellular fibronectin for fibrillogenesis and matrix assembly. As pointed out above, this process might be regulated by the FAK/Src system, as *Src*-null cells apparently fail to develop fibrillar adhesions[71].

Tension and crosslinking as potential regulators of fibronectin fibrillogenesis. Besides mediating unfolding, tension might also regulate the fibronectin-polymerization process itself. Different type III fibronectin modules respond differently to external forces. STEERED MOLECULAR DYNAMICS (SMD) SIMULATION shows significant differences in mechanical stability between fibronectin-III_7, fibronectin-III_8, fibronectin-III_9, and fibronectin-III_{10} modules, which might control the sequence in which cryptic self-association sites within fibronectin become exposed and function[105]. Moreover, unfolding of the fibronectin type III_{10} module might also reduce accessibility of the RGD ADHESION SEQUENCE to integrins early in the stretching process[106]. This phenomenon might promote the release of cells from fibronectin or of fibronectin fibrils from cells.

Maturation of fibronectin fibrils involves gradual conversion into a detergent-insoluble, stable matrix[107]. This process is not fully understood but might involve intrinsic disulphide-isomerase activity, surprisingly identified within the type I_{12} module of fibronectin itself[108] (FIG. 6). Interestingly, this activity of fibronectin also seems to be partially cryptic, indicating that conformational changes and tension might also be involved in its control. Covalent crosslinking by disulphides or TRANSGLUTAMINASE makes matrices more rigid. As discussed above, cells respond to a rigid fibronectin matrix with large focal adhesions and loss of fibrillar adhesions. So, rigidification of local areas of matrix by cells would modify the responses of other cells.

It is worth noting that ECM organization might be regulated not only by focal adhesions and fibrillar adhesions, but also by other forms of integrin–actin adhesions, such as podosomes. These structures are prominent in rapidly moving cells that show invasive properties, either physiological (for example, in leukocytes) or pathological (for example, Src-transformed cells)[19,21]. In addition, podosomes are primary adhesion structures of osteoclasts (REF. 20; FIG. 1) and have an important role in bone resorption[22,109]. So, it seems that these adhesions might specifically participate in matrix degradation.

Regulation

Although functionally distinct, the processes of fibronectin-matrix assembly, focal-complex generation and focal-adhesion organization are all regulated by Rho-family GTPases. Stimulating Rho enhances, and inhibiting Rho function strongly inhibits, both the formation of fibronectin fibrils[96] and focal adhesions (reviewed in REF. 50). Rho promotes organization of the actin cytoskeleton and increased contractility as reviewed above (FIG. 3). Conversely, Rac-1 activation promotes the formation of small focal complexes and actin assembly in lamellipodia[17,18] (FIG. 3). An additional possible effect of Rac/Rho signalling — and in particular the generation of $PtdIns(4,5)P_2$ — is the induction of conformational changes in focal-adhesion molecules, such as vinculin, and their targeting to the membrane[10] (FIG. 3). Further discussion of Rho actions on focal adhesions is provided elsewhere[9,50].

An apparent paradox is how Rho and contractility can stimulate both focal-adhesion formation and fibrillogenesis, especially as enhanced focal-adhesion formation on rigid substrates antagonizes fibrillar-adhesion formation and matrix assembly[5]. A possible explanation involves the interplay between 'dynamic' and isometric force/tension as implied by FIGS 3 and 7. Actomyosin

LATRUNCULIN-A
A macrolide that is derived from the Red Sea sponge *Latrunculia magnifica*, which binds and sequesters actin molecules, and thereby prevents the assembly of actin filaments.

TREADMILLING
A special state in polymer dynamics, when monomer addition at one end occurs at the same rate as monomer dissociation at the other end, which keeps the polymer length unchanged.

BARBED END
The fast-polymerizing end of actin filaments (defined by the arrowhead-shaped decoration of actin filaments with myosin fragments).

STEERED MOLECULAR DYNAMICS SIMULATION
A computer simulation method for studying force-induced reactions in biopolymers.

RGD ADHESION SEQUENCE
The primary adhesive motif in many extracellular matrix molecules, which contains the amino-acid triplet, Arg–Gly–Asp.

TRANSGLUTAMINASE
An enzyme (such as factor XIIIa) that helps to crosslink fibronectin and other molecules through isopeptide linkages.

contractility (stimulated by Rho) is essential for focal-adhesion formation, fibronectin fibrillogenesis and formation of fibrillar adhesions, but not for temporary retention of pre-existing fibrillar adhesions[5,15,80]. We propose that although increasing contractility can promote further focal-adhesion assembly, fibrillogenesis might be inhibited beyond a certain threshold. In fact, when faced with a rigid fibronectin substrate, cells retain $\alpha_5\beta_1$ fibronectin receptors in enlarged focal adhesions, presumably under high isometric tension[5], and do not form fibrillar adhesions.

Another uncertainty in the literature is whether Rho activation is purely upstream of integrins or can also be triggered by them. Apparently, substrate adhesion can activate Rho, which together with the mitogen-activated protein kinase (MAPK) can stimulate cell proliferation[110]. Furthermore, integrin ligation can regulate Rho in a biphasic manner, and its activation can be suppressed by Src/FAK signalling[74,75]. So, the interrelationships of integrins, Rho and mechanosensor(s) are probably complex and multidirectional.

Conclusions and perspective

A recurring theme throughout this review has been the importance of local, physical forces in the form of internally generated tension or externally applied force for activating molecular switches. Cells use transmembrane actin–integrin adhesion complexes as mechanosensors to probe the rigidity of the extracellular environment, mediate adhesion, initiate intracellular responses by forming multimolecular complexes, and trigger signalling, but they are also used to remodel or degrade the extracellular matrix. Physical forces induce transitions in the types and functions of these cell–matrix adhesions. Focal complexes become larger focal adhesions through steps that include the response of a mechanosensory system to isometric forces, vinculin activation and increased membrane–cytoskeletal interactions. Focal adhesions can then serve as the source of fibrillar adhesions by using dynamic force to convert cellular contractility into integrin and tensin translocation. Integrin translocation in turn appears to stretch fibronectin molecules, exposing cryptic sites that mediate matrix assembly into extracellular fibrils.

A key mechanism in these transitions seems to be conformational changes that are induced by force or a local reorganization of scaffold or signalling molecules to promote multimolecular assembly; for example, they reveal cryptic assembly sites in fibronectin, form new docking sites at the submembrane plaque by post-translational modifications, or expose new binding sites in vinculin and other cytoskeletal proteins. In another parallel, both intracellular molecular-complex formation and ECM assembly are regulated by Rho-family GTPases. Whereas the integrin-based family of adhesion complexes shows extraordinary molecular complexity in terms of numbers of components, overall regulation by mechanosensors and mechanical switches has a crucial role in detecting tension and responding to it. Such transfers of physical information across the cell membrane might also be simultaneously bidirectional and cooperative.

The overall challenge for the future is to determine the molecular and biophysical mechanisms that underlie these important processes, as well as how they are regulated. As indicated in this review, additional intracellular and external components of the various types of adhesion need to be identified, and many components must still be characterized for morphological and functional specificity. The various focal complex-like structures need careful comparison, as they might have differing roles in cell migration and anchorage. An important challenge will be to determine the molecular nature of cellular mechanosensors that are involved in cell–matrix adhesion, as well as how they activate cytoskeletal and signal-transduction pathways that ultimately regulate growth, differentiation and apoptosis. Other fruitful questions will concern the regulation and functional integration of the various forms of adhesion, which change depending on the state of differentiation, tissue location and local forces on each cell. Other challenges will include exploring the structure and function of cell–matrix adhesions in three-dimensional microenvironments *in vivo* and explaining the roles of complex carbohydrates in cell–matrix interactions. As adhesions represent the interaction interfaces between cells and the ECM, their study will continue to provide exciting insights into the interplay between physical forces and molecular signalling in cell regulation.

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