Local force and geometry sensing regulate cell functions

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Abstract | The shapes of eukaryotic cells and ultimately the organisms that they form are defined by cycles of mechanosensing, mechanotransduction and mechanoresponse. Local sensing of force or geometry is transduced into biochemical signals that result in cell responses even for complex mechanical parameters such as substrate rigidity and cell-level form. These responses regulate cell growth, differentiation, shape changes and cell death. Recent tissue scaffolds that have been engineered at the micro- and nanoscale level now enable better dissection of the mechanosensing, transduction and response mechanisms.

Mechanotransduction
Conformation-dependent biochemical reactions (read-out) that activate intracellular signals (amplification); for example, G-protein activation, Tyr-kinase activation, lipase activation, kinase cascades or Ca^{2+} release.

Rigidity
The compliance (amount of displacement per unit of applied force) of the matrix substrate.

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Throughout the biological kingdom there is a wide diversity of shapes, and this phenomenon has interested physical biologists for a long time (see *On Growth and Form*, D'Arcy W. Thompson[1]). It is still unknown how cells of 10–40 μm in diameter can assemble and reproducibly shape an organism that is metres in size, nor do we know how cells recognize their spatial position within such multicellular systems. During development cellular monolayers curve and contort to create the complex morphology of the different tissues. Extracellular matrices and their neighbouring cells constitute the main signals that any individual cell uses to establish and maintain its shape. However, there is a wonderful diversity in the form of biological systems that is often intimately linked to cellular function. Therefore, cells must sense physical aspects of their environment and respond appropriately over time for proper cell function.

On the basis of extensive analyses, the physical aspects of the cellular environment that are sensed by cells are force and geometry at the nano-to-micrometre level. A multitude of design principles are emerging to describe the mechanosensory elements that are integrated into the structural motifs of various proteins that can be mechanically switched between conformations (reviewed in REFS 2–8). Cellular mechanotransduction systems can then transduce the physical signals into biochemical responses. More complex physical parameters such as matrix rigidity or micrometre-level geometry can be measured by integrated force- and geometry-dependent transduction processes (FIG. 1). Therefore, it is important to differentiate between the primary sensory processes, the transduction processes and the downstream mechanoresponsive pathways that integrate the multiple biochemical signals that are derived from sensing and transduction events over space and time (FIG. 1).

It has also been postulated that cytoskeletal filaments can propagate stresses over long distances[9,10], which would require the existence of other distant mechanosensory and signalling components. Considering the diversity of mechanosensory proteins, it is possible that transduction of mechanosensing into biochemical processes activates many signalling pathways that might interact to produce controlled functional responses. Subsequent cellular-motility responses can elicit additional mechanical signals that will lead to a second cycle of responses. The primary cellular responses to mechanical signals occur in seconds to minutes, therefore hundreds of thousands of stimulus–response cycles might occur over the days that a cell is maintained in culture.

Cell–cell, cell–matrix and flow forces are all sensed in different contexts, from the high forces that are sensed by chondrocytes in the cartilage matrix to the relatively low flow forces that are sensed in kidney tubules[11–13]. The forces that are developed by the cells themselves on matrix or cellular contacts are also important[14]. Cell–cell contacts are dynamic, and cells seem to evaluate the level of force and make adjustments, as the cytoskeleton filaments and their linkages to transmembrane proteins assemble, break down and reassemble.

Sensing of geometry at the sub-cellular level is a crucial component of the cellular sensing of two-dimensional (2D) versus 3D matrices (FIG. 1c). Recent studies have determined that the same matrix protein will elicit a different response when it is organized in filaments from when it is displayed on a flat surface[15–18], and that cells can sense nanoscale surface topographies (reviewed in REF. 19). In the context of a tissue, the cells will need to discriminate formed matrix fibres from the same matrix molecules that are present in a soluble form

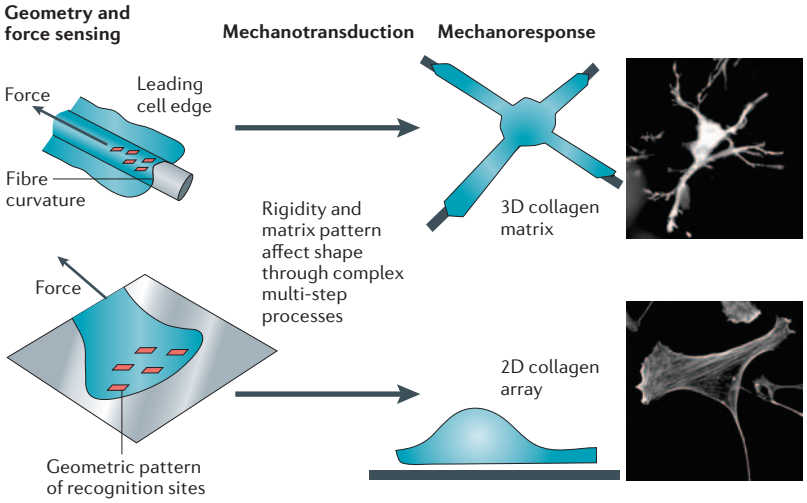

Figure 1 | **Cellular mechanics involves three steps: mechanosensing, mechanotransduction and mechanoresponse.** This figure emphasizes the steps that are involved in the responses of cells to their environment. Local mechanosensing is transduced into biochemical signals that result in cell responses. Force-induced changes in protein conformation and geometry-dependent interactions are locally transduced into biochemical signals that activate various mechanosensitive signalling pathways, ultimately regulating cellular mechanoresponses. As an example we show the dramatic difference in morphology that the same cells will assume after only 4 hours in different matrices. Human fibroblasts project a dendritic network of extensions in collagen matrices but not on collagen-coated coverslips. Fibroblasts were incubated for 4 hours on collagen-coated surfaces (bottom panel) or in collagen matrices (top panel)[18]. Images reproduced, with permission, from REF. 18 © (2003) The American Society for Cell Biology.

or individually attached to a neighbouring cell or a foreign body. Although there are many examples of local geometries that are imposed by the environment, adjacent cells or matrices, we will discuss how fibre curvature can cause membrane curvature and how the spatial distribution of cytoplasmic adhesion proteins could conform to those curved membranes, thereby possibly affecting the adhesion complexes.

The transduction of local mechanostimuli into biochemical signals occurs through several signalling pathways, but many recent examples show that transduction occurs locally at the cell periphery, even though the forces and the biochemical signals propagate throughout the cell[20–23]. Complicated transduction processes such as rigidity responses involve several steps that can combine the transduction of force and/or geometry sensing with time (reviewed in REFS 24,25). Guanine nucleotide-exchange factors, Ca^{2+} ion channels, receptor-like protein tyrosine phosphatases, Src-family kinases and membrane receptors have all been invoked as early steps in force or geometry transduction[23,26–28]. The downstream signals can then involve complex signalling pathways that intersect and modify each other to produce reliable cell responses. One system of interest is the cellular response to the rigidity of the environment[24], which seems to be important in cancerous growth[29]. Recent studies indicate that the loading rate of the force onto the cell can be crucial[30], in addition to the possible matrix stiffening that is caused by the physical activity of cells[31,32]. Integration of different mechanical signals, at different locations and times, occurs through target proteins and signalling pathways that elicit a programmed response to properly shape cells and tissues.

In this article, we focus on the possible cellular mechanisms of mechanosensing and the early signalling events that they induce. Through nanofabrication and other new technologies, the mechanisms of mechanosensing can now be addressed systematically (BOX 1). These techniques can be applied to cells with altered protein content through molecular-biological manipulations to address the molecular bases of cellular responses.

Conversion of force into biochemical signals

Studies of the mechanical properties of single molecules using nanotools[33–35] revealed a diverse set of structural motifs that could change conformation over a range of mechanical forces and could potentially serve mechanosensory functions (reviewed in REFS 2–8) (FIG. 2). These include the force-induced exposure of otherwise cryptic peptide sequences (FIG. 2a), the opening of mechanosensitive ion channels (FIG. 2b), and receptor–ligand interactions that strengthen if strained (FIG. 2c). It is important to note that such force-induced changes are only effective if they produce a change at the biochemical level. Here we focus on a few selected proteins that are part of the physical network through which force is transmitted bidirectionally from the cell exterior to the interior and *vice versa*.

Exposure of cryptic peptide sequences. Most extracellular matrix (ECM) proteins, as well as many proteins that link the integrins to the cytoskeleton, consist of tandem-repeat sequences (these include spectrin-family members, such as α-actinin and dystrophin, as well as talin, titin, fibronectin, cadherins and others; FIG. 2a). Forces can induce the unravelling of modules, which results in alterations in molecular-recognition sites, or the exposure of peptide sequences that are otherwise hidden in the folded modules (reviewed in REF. 8). Differences in the mechanical stability between such repeats can define the sequence in which they unravel[36–38].

The passing of energy barriers to unravel proteins typically coincides with the breakage of force-bearing hydrogen bonds that stabilize their tertiary structure[39,40]. The mechanical stability of the modules therefore depends on how frequently the force-bearing hydrogen bonds can be attacked by free water molecules. An important role of amino-acid side chains in regulating mechanical stability is whether they shield or expose those underlying bonds from electrophilic attack by water molecules[36,41]. Local charges also affect the shielding efficiency of amino-acid side chains and, consequently, pH and ionic strength can both further tune the mechanical stability of proteins[36,42]. Finally, many modules are mechanically stabilized by disulphide bonds and their redox state can be force-sensitive if the disulphide bonds are buried deep in the module[43].

Mechanoresponse
Spatio-temporal signal integration will modify cell motility and contractility. Long-term cellular responses to signals will modify protein expression and the motility systems, and regulate overall cellular behaviour.

Mechanosensing
Force-induced conformational changes or geometry-dependent molecular clustering that can cause changes in biochemical reactions.

Membrane curvature
The radius of curvature along the two principal axes in the membrane (mathematically explained as the sum of 1/r for the two axes).

Mechanosensitive channels
Ion channels that open on the application of matrix forces to cells.

Exploiting hierarchical mechanical stabilities for mechanosensing is particularly powerful if the tandem repeats of a protein have different molecular-recognition sites, which can be functionally switched in response to partial unravelling. Sequential unfolding can signal the magnitude of the stress that is acting on multimodular proteins. The cell-adhesion protein fibronectin, for example, consists of more than 50 modular repeats — which are of special interest with respect to mechanosensing — and is abundant in serum as well as in many matrices. It physically links the ECM to the contractile cytoskeleton through its tripeptide sequence, RGD, which is recognized by integrins. Cell contractility is sufficient to partially unfold fibronectin in matrix fibres[44,45] (FIG. 2a). Although many molecular-recognition sites are present in the loop regions of these β-sandwich motifs, many cryptic binding sites have been identified that are buried within individual fibronectin modules in the folded state (reviewed in REFS 8,46).

Force regulation of protein activities. One way of transducing force into biochemical signals is to alter enzyme activity by directly applying force to either the enzyme or its substrate, or to an activator/inhibitor of the enzyme (reviewed in REFS 47,48). Force is a potent regulator of enzyme activity, as many enzymes can undergo conformational changes during activity[49], which often also involves conformational changes of their substrates[50]. In addition, many motor proteins will experience a decrease in turnover — for example, ATP hydrolysis — with increasing force (reviewed in REFS 47,51). As tension in the cytoskeleton increases, motor activity will decrease, until isometric conditions are reached. The isometric ATPase activity of motors keeps the tension in the cytoskeleton constant, and the recruitment of other motors will then increase the overall tension in the cytoskeleton. Because the cytoskeleton filaments are dynamic and undergo assembly–disassembly cycles on the timescale of seconds to minutes, filament tension will be rapidly lost and must constantly be maintained by motor activity.

But is it possible that conformational strain could increase the activity of some enzymes? The question of whether the activity of some enzymes can be upregulated by strain if they or their substrates are physically integrated into a force-bearing structure is relatively unexplored in the context of mechanosensing. Force might open up enzymatic cleavage sites through partial unravelling. Fibronectin, for example, has a partially cryptic disulphide-isomerase[52] and a cryptic metalloprotease activity[53], but it is not known whether these activities can be regulated by force. Titin contains a module that can show kinase activity and computational studies indicated a mechanical opening of its active site[54]. Furthermore, it is well known that enzymes exert strain on their substrates on binding — thereby catalysing the reaction (reviewed in REF. 7) — and straining the enzymatic substrate could therefore potentially regulate enzyme activity. Recent studies show that the Src substrate is primed (activated) for phosphorylation by mechanical unfolding. Finally, some cytoplasmic proteins, including vinculin[55,56] and Src[57], are allosterically activated when their regulators bind to or unmask sites, thereby opening otherwise autoinhibited conformations. Can force also induce a similar opening of sites in those molecules? The mechanisms by which force can regulate protein function are only gradually emerging because new tools and assays are required to investigate force-induced functional changes.

Mechanosensitive ion channels. The structural diversity of mechanosensitive channels seems to have been driven by the physiological necessity to detect mechanical stimuli — from thermal energy to high pressures — as changes in conductive state (FIG. 2b) (reviewed in REF. 58). Some channels respond to stress in the lipid bilayer, whereas others must be physically connected to the cytoskeleton and/or the extracellular matrix to transmit forces to the channel.

The bacterial mechanosensitive K^+ channel, MscL, represents the first case and has been structurally analysed. Membrane-tension forces are mainly concentrated in the interfacial polar headgroup regions of MscL, thereby inducing helix tilting that opens and wets the pore interior[5,59]. Bacterial channels are relatively force-insensitive and only open at high tensions that are approaching the lytic tensions for the lipid bilayer. Plant channels seem to operate at tensions that are of an order of magnitude lower than bacterial channels, whereas typical membrane tensions in animal cells are a thousand-fold lower than the activating bacterial tensions[60].

Although clearly demonstrated for bacteria, it has not been shown whether either in-plane membrane tension or forces that are normal to the membrane are the primary cause of channel opening in animal cells, but most of the osmotic and other mechanosensitive responses require tethering to force-bearing filaments[4,61]. In the

Box 1 | **Techniques for evaluating mechano- and form-sensing mechanisms**

Cell-generated force measurement
- Deformable substrates[152]
- Microfabricated surfaces and cantilevers[153–155]
- Laser tweezers[114]
- Pillar devices[156]

External application of force to probe cellular responses
- Magnetic tweezers[121]
- Pipettes[157]
- Stretchable substrates[26]
- Laser tweezers[21,23]
- Atomic force microscopy[158]
- Flow[143]

Rigidity manipulation
- Polyacrylamide or PDMS (polydimethylsiloxane)[132,144]
- Laser tweezers[30]
- Oscillatory magnets[135]

Form manipulation
- Curvature (fibre diameters; micro- and nanofabrication)[19,81,85,87,112,159,160]
- Spacing and island shape (micro- and nanofabrication)[82,83,112–114]

case of out-of-plane force-sensing channels, the effects of force are partially understood at the molecular level in only a few specialized cases. In the case of the outer-hair-cell bundle of the ear, the architecture of the linkage is known, but the molecular components have not been identified[62]. In the case of the *Caenorhabditis elegans* touch sensor, the molecular components have been identified through genetic screens, but the architecture of the linkage is not known[63]. Other channels are implicated in the regulation of the mechanically induced propagation of Ca^{2+} waves — probably through tethering to cytoskeletal components[64]. Similarly, ion-dependent mechanosensory phenomena in bone and cartilage[65] are probably responding to out-of-plane forces on channel–cytoskeleton linkages.

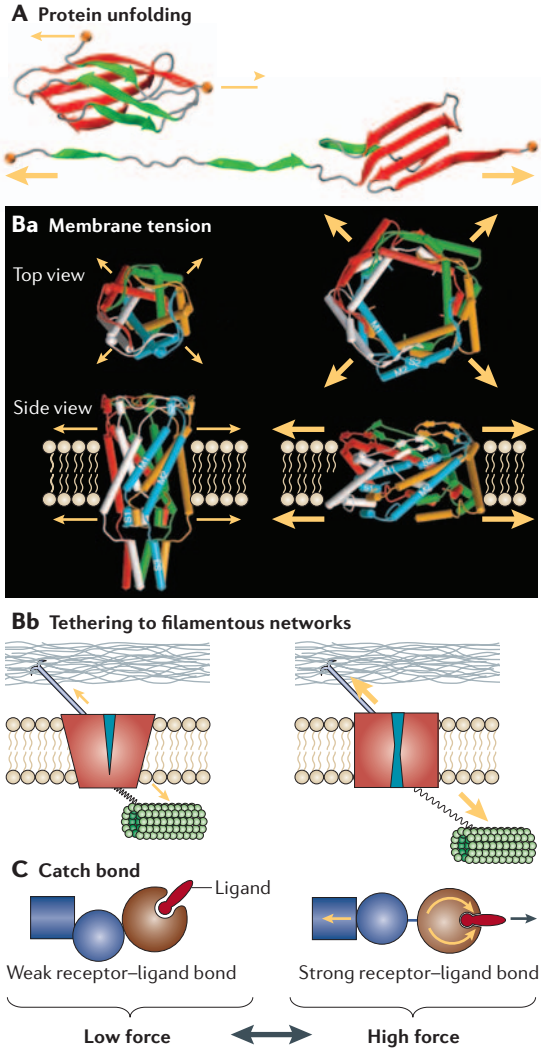

Figure 2 | **Mechanisms of force sensing.** These panels illustrate the three basic mechanisms of force sensing. **A** | Conversion of force into biochemical signals by partial protein unfolding (as shown for the fibronectin module III1; REF. 161). This can result in the gain or loss of binding sites, increased separation between protein domains, or the gain or loss of enzyme function. **B** | The opening of some mechanosensitive ion channels can be regulated by membrane tension[5] (**Ba**), whereas the opening of others requires that their intra- and/or extracellular domains are physically connected to force-bearing filaments (**Bb**). **C** | Stabilizing receptor–ligand bonds by switching them to a long-lived state by force (catch bonds)[76]. Yellow arrows indicate forces. Part **Ba** reproduced with permission from *Nature* REF. 5 © (2005) Macmillan Publishers Ltd.

Stabilizing adhesion sites against force-induced breakage. In the protein networks that couple the ECM to the contractile cytoskeleton, the physical connections are mediated by weak non-covalent bonds and the weakest link will fail first if the protein network is under tension. Because of thermal activation, these bonds have modest lifetimes[66]. In the case of slip bonds, bond lifetimes are progressively shortened when forces act on a protein–protein or protein–ligand complex, as force tilts the energy landscape, thereby accelerating dissociation of the complex. The bond-survival time of the strongest non-covalent bond, which is formed between biotin and streptavidin, is diminished from more than a day under static conditions to about 1 minute or 0.001 second at bond stresses of 5 pN or 170 pN, respectively[67], whereas 5 pN can be exerted onto a protein complex by a single motor protein. Therefore, non-covalent bonds will fail under any level of tensile stress if held for sufficient time periods[67]. Adhesion sites form in response to tensile forces acting on the membrane, and one consequence of the shortened bond lifetimes under force must be high turnover rates of the constituents in newly formed adhesion sites. Turnover rates on the timescale of seconds have been observed for actin filaments and the two focal-contact proteins, paxillin and vinculin, which might serve as signalling molecules after their release from the cell contacts[68–70].

How can cells therefore form force-sustaining adhesion sites? Because force is transmitted across the membrane, not by single integrins, but by integrin clusters that increase in size with time in a force-dependent manner[22,71,72], a single bond-rupture event does not necessarily lead to a failure of attachment. Assuming N identical bonds are loaded in parallel, and the force is distributed equally among the bonds, the force needed to break a cluster of parallel bonds is about a factor of N larger than it is for a single bond under the same rate of loading. And, most importantly, this value has a non-linear and even steeper dependency if rebinding events of single broken bonds can occur[66,73].

To counteract the exponentially decreasing lifetime of bonds with force, are there mechanisms by which the bond lifetime can be increased by force? Although this has been theoretically predicted[74], evidence exists only for two types of receptor–ligand complex that are switched by force from a short-lived to a long-lived state. These include adhesion molecules, such as the bacterial adhesin FimH[75,76], and P- and potentially L-selectins[77–79]. It is unknown at present whether other adhesion proteins, such as integrins or proteins that anchor the cytoskeleton to integrins, can form these so-called catch bonds that strengthen under the influence of force (FIG. 2c), or if they rely on the formation of multiple linked bonds.

Slip bond
Protein–ligand bonds that decrease in lifetime with increasing force for all rates of force application.

Catch bond
Protein–ligand bonds that increase in lifetime with increasing force at high rates of force application.

Geometry sensing

The natural ECM of cells is a complex 3D fibrous meshwork with a wide distribution of fibres and gaps that provide complex biochemical and physical cues, which are very different from uniformly coated 2D surfaces. Cell reactions to 3D matrices are altered from their reaction to 2D matrices of the same material[80]. How do these different physical and chemical properties co-regulate local cell form and ultimately global cell function? Most relationships between cell form and function have been deduced from 2D-cell-culture experiments, but new tools have recently been developed to fabricate patterns, topographies and fibres at the micro- and nanoscale. It is therefore possible to learn more about the differential contributions of various physical and chemical properties that are involved in the regulation of cell function.

We define geometry sensing as the formation of signalling complexes by changes in the spacing of molecular-recognition sites the geometrical shape of the substrates. For example, surface steps as small as 11 nm can lead to contact guidance[81]. Restricting cells to spreading on adhesive micropatterns of various shapes can regulate cell proliferation and cell death[82], or the direction of polarization can control the direction in which the cells move[83]. The size of micropatterns also regulates whether mesenchymal stem cells differentiate into adipocytes or osteoblasts[84]. Because micropatterns confine cell shape, thereby causing an integrated cellular response, we will only consider how cells might sense nanoscale surface features, including the size of nanoscale fibres and topographies, as well as the spatial presentation of molecular-recognition sites.

Sensing substrate curvature. 3D matrix fibres are sensed by cells differently from the same matrix molecules in 2D, perhaps because of the membrane curvature that the fibres induce (FIG. 3). To artificially produce replacement matrices, tissue engineers have fabricated many microporous scaffolds from a large range of materials with limited success. Micro- and nanofibrous scaffolds are now under investigation for bone and soft-tissue engineering applications. It has been shown that astrocytes preferentially adhere and proliferate on carbon fibres that have a diameter above 100 nm and low surface energies[85]. Fibroblasts move on collagen fibres by a specific mode of motility that involves one myosin isoform[86]. The same myosin isoform was shown to be involved in *in vivo*-like organization and morphogenesis of fibroblasts grown on electrospun nanofibrous matrices[87]. It has also been shown that 3D networks of nanofibres presenting the neurite-promoting laminin epitope promoted the selective differentiation of neural progenitor cells[88]. Furthermore, the nanofibre alignment and direction of mechanical strain affect the ECM production[89]. As we progress in appreciating the complexity of the natural ECM, in part through the fabrication of analogous model systems that mimic subsets of selected properties, we can better design new biomaterials and scaffolds for tissue engineering.

BAR domain
(Bin, amphiphysin, Rvs domain). A domain that is found in a large family of proteins. It forms a banana-like dimer, and binds to and tabulates lipid membranes.

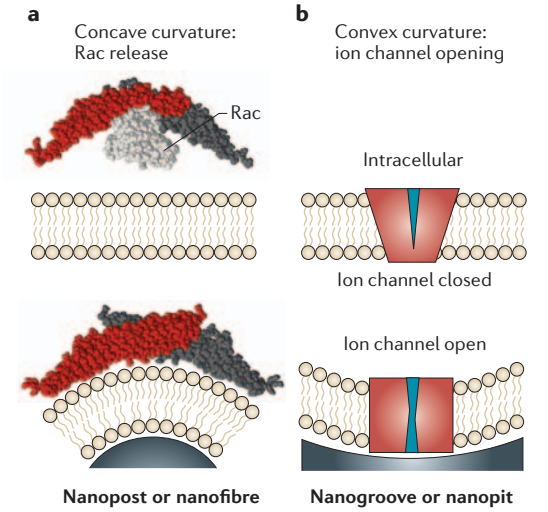

Figure 3 | **Mechanisms of form sensing.** These diagrams illustrate (**a**) how inward curvature of the plasma membrane could cause BAR-domain (Bin, amphiphysin, Rvs domain) proteins to release Rac[98,99] and (**b**) how outward curvature could activate the opening of an ion channel[102]. The soluble form of the BAR-domain protein has bound Rac and would be expected to release it on binding to curved membrane regions, which could stimulate further motility in those regions of the cell. Outward curvature could be induced by filopodial extension or the formation of retraction fibres by the cell pulling away from contacts.

But how can cells differentiate between a concave and a convex membrane curvature? It has been found that cells adhere differently to surfaces that are structured with nanoposts compared with nanopit-covered surfaces. Although some 13-nm high nanoposts increased cell spreading, proliferation and cytoskeletal formation[90], a nanopitted pattern that was embossed into polymethylmethacrylate decreased adhesion relative to flat substrates[91]. In fact, fibroblasts seem to endocytose nanocolumns[92]. As the cell forms adhesions on substrates with micro- or nanoscale surface features, the membrane is forced to follow the external surface contours.

Recent studies of endocytosis show that the BAR-domain (Bin, amphiphysin, Rvs domain) proteins can induce or recognize a concave membrane curvature and recruit small G proteins. The BAR domains of arfaptins were shown to bind to the small GTPases Rac, adenosine-ribosylation factor-1 (ARF1), ARF3 and ARF6, as well as to the ARF-like protein-1 (ARL1; REFS 93–96). Another member of the BAR-domain family, BAP2α/IRSp53 (insulin-receptor substrate protein of 53 kDa) has been shown to be the link between Rac and WAVE (the Wiskott–Aldrich syndrome protein (WASP)-related protein) in regulating membrane ruffling[97]. However, it is still unclear whether GTPase binding and membrane-curvature sensing and induction are common features of all BAR-domain family members (FIG. 3a) (reviewed in REFS 98,99). Several of the BAR-domain proteins have effects on motility, which is consistent with a role in sensing the form of concave matrix-induced membrane

curvature[100,101]. Therefore, the recruitment of BAR proteins to membranes that are bound to curved surfaces can have profound effects on cell function, because the BAR proteins that would assemble on membranes with a concave curvature could locally increase small-G-protein activity.

Another implication of membrane curvature is that the inner and outer membrane leaflets, which have an equal tension in a planar configuration, exhibit differential tensions on bending. Beyond affecting the local lipid composition and, potentially, the redistribution, spatial clustering or segregation of transmembrane proteins, this could also lead to the opening of mechanogated ion channels (FIG. 3b). Certain K^+ channels, for example, are opened by a convex curvature of the membrane[102].

We propose that concave and convex membrane curvatures might therefore be sensed by two different mechanisms. BAR domains might sense the concave membrane curvature that is formed in contact with external posts and fibres, whereas membrane channels might selectively be opened if membranes come into contact with surface indentations. If BAR-domain proteins do indeed bind preferentially to concave surfaces, such as posts or fibres, the local release of Rac and its subsequent activation, for example by integrin-linked kinase[103], might lead to a local enhancement of traction forces. In support of this proposition, Rac activation is known to enhance focal-complex assembly[103–106] and cells adhere more weakly on surfaces with nanopits than on those with nanoposts[90,91].

Protein clustering and spacing. Because membranes are fluid, there can be mechanical or diffusional separation of components over micron distances that can have biochemical consequences[107]. The most dramatic examples are in the formation of immune synapses[108], where there is good evidence that the physical separation of different transmembrane receptors and their spatial patterning regulates T-cell activation[109,110].

Many protein aggregates form at sites of cell–matrix or cell–cell contacts and the size and shape of those aggregates is influenced by the spacing of the extracellular ligands to which they bind. The formation of cell-adhesion sites requires that the RGD peptides are clustered[111] at a distance of less than 73 nm (REF. 112). A mechanism for sensing the spacing was indicated by studies at the single-molecule level, which showed that a single trimer of fibronectin type III domains 7–10 (with spacing of 50–60 nm) was preferentially bound to leading edges of active lamellipodia when 55-nm long talin dimers were present[113,114]. Once spatially organized by crosslinking, either through talin or α-actinin[115–117], these oligomeric sites recruit and activate other components to form focal contacts[14,112,118–120]. The size of those adhesion-site complexes is limited by the size and shape of the external surface contacts[121–123] and focal adhesion kinase (FAK) might regulate their turnover[124–128].

In addition, studies on single adhesive islands revealed that focal adhesions localize asymmetrically along the periphery of the small islands that experienced the highest tensional stress[129].

Force-activated machanoresponses

Considering the large number of mechanosensory motifs, there are potentially several pathways and engineering paradigms by which mechanical signals could be transduced into biochemical signals. In many examples, the mechanotransduction process is position- and time-dependent. The robust behaviour of the mechanical aspects of the development of most organisms belies a signalling system that is highly engineered and remarkably reproducible, despite the fact that many signalling pathways are activated. The final integrated response is often tuned to give an important functional behaviour. Here we consider the cellular response to rigid versus soft surfaces, but other mechanoresponsive pathways that are involved in the integrated responses to overall size and shape of the cellular environment have been at least partially identified[130].

The response of cells to substrate rigidity in cell culture is cell-specific and seems to be correlated with the rigidity of the native environment of the cell, which can vary significantly between different tissues[131,132]. It is still unclear why some cells normally require a rigid surface for growth, whereas others thrive on soft surfaces. At the physical level, transduction of substrate rigidity into a biochemical signal involves either measuring the compliance of the external linkages or measuring how much they are spatially displaced for a given force.

Changing the rate-of-force increase. The rigidity of the substrate might tune binding strength of the cell by changing the rate-of-force increase acting on the receptor–ligand bond. It has been postulated that there is a rate-dependent increase in the time required for receptor–ligand unbinding and therefore a higher matrix rigidity would increase the bond lifetime[66]. In addition to the stiffness of the linkage, some bonds in adhesion complexes might strengthen if strained (catch bonds). Therefore, physical mechanisms could be sensitive to the rate of increase in the force exerted on external ligands, as determined by its tether compliance.

Measurement of displacement for a given force. Alternatively, the binding of an external ligand or a mechanical deformation could initiate the assembly of two different complexes in the cell, one that depends on the force because it is linked to both the cytoskeleton and the external ligand and a second that is only linked to a separate extracellular site or a curved membrane edge (FIG. 4). The first complex can translocate with the rearward-moving actin filaments, whereas the second complex remains stationary. When the cytoskeleton pulls on the ligand, the rigidity of the external ligand linkage will determine how far the cytoskeleton-linked complexes will be displaced in a given time period before reaching isometric conditions and whether the force will be sufficient to activate any of the force sensors through the exposure of otherwise cryptic peptide sequences. For soft substrates, the separation between the fixed and moving components is greater, and might be sufficient to prevent the sensor from interacting with the stationary component (FIG. 4).

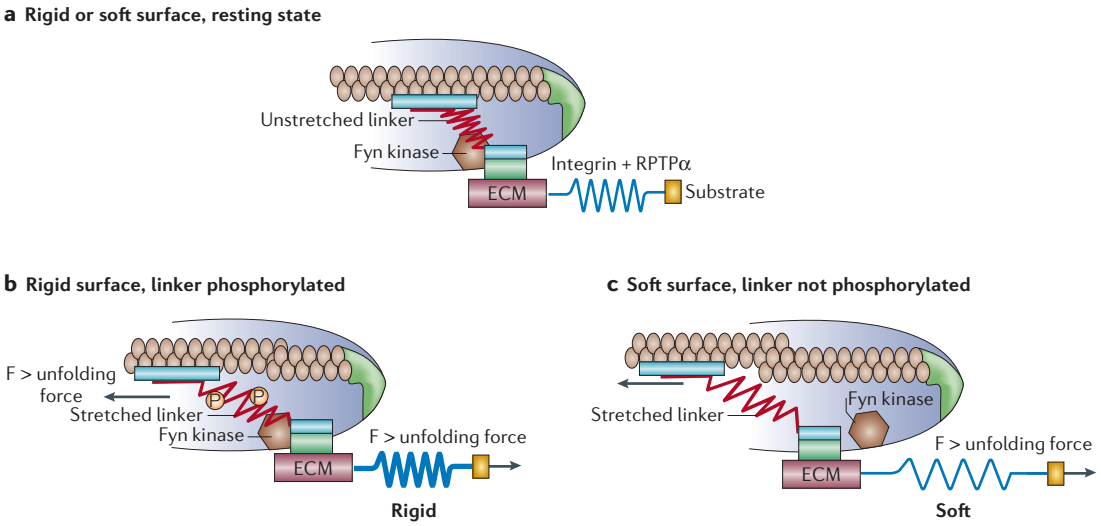

Figure 4 | **Mechanisms of rigidity sensing.** These panels illustrate the position-dependent mechanism of rigidity sensing. **a** | The crucial feature in such a model is that the enzyme, Fyn, and the substrate to be activated by stretch (kinase and substrate in this example) move relative to one another by actin rearward transport. **b** | If the surface is hard, the components would be close enough for modification to occur (causing small displacement). **c** | If the surface is soft, then enzyme and substrate would be separated before force could activate the reaction (in this case phophorylation of the linker; causing large displacement). Contractile activity in a soft tissue could make it seem hard, because an external pull on the integrin before it moves significantly will cause unfolding while the substrate and enzyme are still close together. A time-dependent mechanism would be similar except that activity, rather than position, would be force-dependent with a biphasic response. The velocity of the cytoskeleton contraction would define the time window of the relevant activity in that model. We feel that position-dependent mechanisms are more robust. ECM, extracellular matrix; F, applied force; RPTPα, receptor-like protein tyrosine phosphatase-α. Figure modified, with permission, from REF. 30 © (2006) The Biophysical Society.

Other relative changes in spacing might occur that are due to partial protein unfolding. Based on observations with laser tweezers, it became clear that changes in force (of ~10 pN) over a timescale of 1 second or over a length of 50–100 nm (for example, a molecular level) can give rise to rigidity responses[25,133]. Several observations indicate that the timescale of 1 second is important. For example, the normal rearward transport rate of actin (60 nm second^{-1}; REF. 134) will move sites by 50–100 nm in approximately 1 second. Body motions such as walking, heart rates and other activities that produce tissue contractions are also on the timescale of approximately 1 second. Furthermore, analyses of the reaction of endothelial cells to magnetic beads show dramatic differences with oscillations in force on a frequency of about 1 Hz (REF. 135). We could therefore postulate that oscillations in force on a 1-second timescale can produce a rigidity response and such oscillations *in vivo* could result from the normal contractile activity of tissues and tissue rigidity.

Different cells often have different responses to matrix rigidity[24]. For example, neurons have a preference for soft surfaces, whereas fibroblasts grow better on rigid surfaces[132]. The tyrosine kinase, FAK, has been shown to be involved in the pathway that senses the rigidity of polyacrylamide surfaces that are coated with collagen[136], whereas the Src kinases are involved in sensing rigidity on surfaces that are coated with fibronectin[30] (A. Kostic and M.P.S., unpublished observations). Furthermore, an important substrate for the Src-family kinases, p130Cas, shows a marked increase in phosphorylation on cell or cytoskeleton stretch[137]. Several studies have indicated that tyrosine kinases and phosphatases have a crucial role in the sensing of rigidity. Extracellular signal-regulated kinases (ERKs) and Rho constitute part of an integrated mechanoregulatory circuit that links matrix stiffness, through integrin clustering, to cytoskeletal tension and, ultimately, regulation of tissue phenotype[2,9,84,105,138].

During cell spreading or migration, cells are continually encountering new ligands. As cells stabilize in a tissue, they become less dynamic, however, there is a continuous turnover of contact and cytoskeletal proteins on the timescale of minutes or less[14,69,72,106,120,139]. As cellular contacts are maintained for days and possibly longer, new contacts must form as the older ones are lost. Those new contacts can be tested for rigidity and, therefore, the cells can continually sample the rigidity of their environment. Indeed, during spreading and migration, cells generate periodic contractions of their lamellipodia on rigid, but not soft, substrates[134]. Surprisingly, it has been shown that some cells lose their shape sensitivity to substrate rigidity once they come into contact with other cells[31]. Cell–cell binding can overwrite cell-substrate-induced signalling. In tissues, cellular-level mechanosensing, transduction and response processes can maintain the proper physical homeostasis, which is markedly altered in cancers[29].

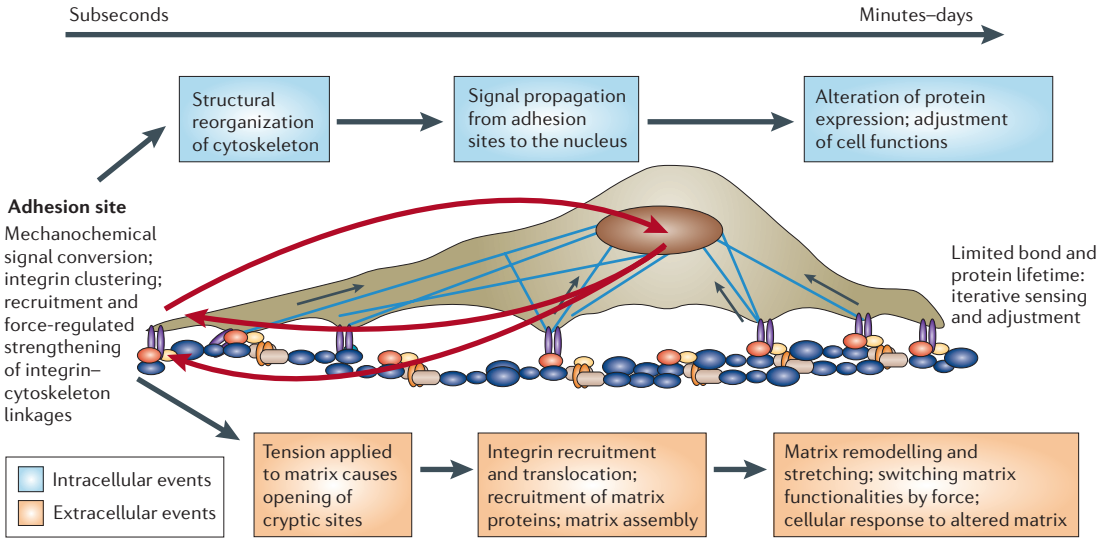

Figure 5 | **Cellular processes of mechanosensing and responses.** This diagram shows the steps in mechanosensing over time that involve periodic testing of the substrate, substrate modification and changes in cellular protein content. Initially, cells will sense the mechanical features of their environment, which will cause rapid motility and signalling responses. As the cell pulls on the environment, it will modify the extracellular matrix and will create new signals, such as those originating from fibronectin unfolding. Intracellular signals will alter the expression pattern of the cell and, over time, the cellular forces and cellularly generated matrices will change the cell shape. At any stage, extracellular signals, such as hormones or external mechanical stimuli, can cause acute changes that will set off a further round of cell and matrix modifications.

Global regulation of cell function

At the cellular level, mechanical signals can have profound effects that lead to cell death, growth or differentiation. Because the effects are extremely important, cells not only constantly monitor the mechanical environment but they also actively remodel their environment. It is therefore not surprising that attempts to engineer tissue scaffolds have often been confounded by adverse cell behaviours, especially at later time points[133,140,141]. It is therefore useful to discuss the overall process, from mechanosensing to protein expression, in terms of associated time-dependencies (FIG. 5).

Initial interaction and mechanosensing events occur on the subsecond to second timescale. Early cell responses take seconds to minutes and involve cytoskeletal redistribution, reinforcement of linkages and changes in cell motility. These changes entail activation of actin-filament extension, the recruitment of myosin molecules to generate cell contractions, consolidation of adhesive contacts by the recruitment of other receptors and cytoplasmic molecules to support the myosin contraction forces, and sometimes a global cellular response, such as a burst in Ca^{2+} signalling or general contraction.

As the cell generates forces on the proteins that link the ECM to the cytoskeleton, the responses of the mechanosensitive sites to the forces and compliance or changes in local curvature will cause a secondary cellular response. Examples of such reiterative processes include cell polarization and the size-dependent phagocytosis of large particles that involves a specific mode of actin-dependent membrane extension that conforms to the shape of the particle being phagocytosed[142]. In the case of cell polarization, uniaxial stretching, fluid shear or rigidity gradients are often sufficient to cause polarization[26,143,144], and the extracellular matrix guides the orientation of the cell-division axis[145]. The assembly of the actin cytoskeleton can be mechanically triggered. Uniaxial stretch suppresses lamellipodia in a directional fashion[26], whereby Rac activation depends on the direction of the tension[26,106]. During these early mechanosensing and response events, there is often insufficient time for the cell to synthesize new proteins.

Although the physical properties of the ECM determine the initial rigidity response, cell-generated forces on matrices align and stiffen them[32] and partially unfold ECM proteins in a continuous feedback loop that is regulated by cell contractility[45,146,147] (M. Antia, G. Baneyx and V.V., unpublished observations). The extent to which the matrix proteins are unfolded should regulate the exposure of their cryptic peptide sequences or alter the configuration of exposed recognition sites, therefore giving the cell the ability to self-regulate, through contractions and signalling processes (reviewed in REFS 8,148). Mechanical stress can also upregulate the production of ECM proteins indirectly, by stimulating the release of a paracrine growth factor, or directly, by triggering an intracellular signalling pathway that activates gene expression[149]. External forces or changes in cell contractility will further change the configuration of exposed sites. A quasi-, although highly dynamic, steady state is often reached that involves cycles of protein expression, modification and finally turnover with further regulation by external chemical and physical stimuli.

What effect does the local response to rigidity and form have on cell shape and gene expression? As noted above, force on peripheral contacts can produce signalling molecules that could translocate to the nucleus. Alternatively, the plasma membrane is physically linked

with the nuclear membrane[150], and it might be possible that physical factors and nuclear deformation could regulate gene expression through an as-yet-unknown mechanism. There has been relatively little discussion of the fact that the forces act on peripheral adhesion complexes, and therefore the associated spatial displacements or the effect on protein unfolding are higher there than at the nuclear membrane. Because forces are dissipated from the sites of local impact at the periphery of the nucleus, it is likely that the mechanism of mechanosensing is amplified more efficiently at the periphery and therefore has the greatest effect on the peripheral contacts.

Transcription factors that are recruited to the adhesion sites could have an important role in translating the physical stimulus that is sensed at the periphery into biochemical signals that alter gene expression. Transcription factors might be modified in a force-dependent manner and transported to the nucleus; for example, paxillin is modified at focal-contact sites and is then transported to the nucleus[70]. This mechanism provides an obvious way to transform force on specific intracellular-adhesion sites to a change in protein expression.

Recent microarray studies confirm that changes in cell shape correlate with several changes in gene expression[19]. Important shape-dependent changes in gene expression occurred for proteins of the cytoskeleton, proliferation, transcription, translation, ECM production and inter- and intracellular signalling complexes. As the cells rounded up, genes encoding a few small G proteins were among those that were downregulated. Tyrosine kinases that interact with G proteins of the Ras family to stimulate cell proliferation and differentiation are also downregulated, as is α-actinin[19]. Many of the tyrosine kinases and phosphatases that have been linked to changes in cell and tissue shape are also linked to the early events of force and rigidity sensing (reviewed in REF. 25). These observations indicate that the overall cell shape that results from the local responses to force and geometry over time, and the integration of those responses, have an important role in the regulation of gene expression. For a synthetic surface, however, it is not just the geometry and rigidity but also its surface chemistry that ultimately controls the composition and conformation of surface-adsorbed proteins and, therefore, the integrin-mediated cell-signalling processes[151].

Conclusions

There is a continual feedback between cell sensing of force, rigidity or form and the cell contractility that, together with biochemical signals, coregulates cell and tissue shape and, ultimately, the shape of the organism. New nanotechnologies will enable us to test the molecular mechanisms of mechanosensing and transduction. Important questions include a further definition of the local responses to mechanical and geometrical forces and the investigation of signal propagation within the cell, including within the nucleus, to control gene expression. Tissue scaffolds that are engineered at the micro- and nanoscale will enable a better understanding of the force- and geometry-sensing mechanisms, which then must be linked with the cellular-response pathways for both short- and long-term morphological changes.

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