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ADHESION-DEPENDENT CELL MECHANOSENSITIVITY

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■ **Abstract** The conversion of physical signals, such as contractile forces or external mechanical perturbations, into chemical signaling events is a fundamental cellular process that occurs at cell–extracellular matrix contacts, known as focal adhesions. At these sites, transmembrane integrin receptors are associated via their cytoplasmic domains with the actin cytoskeleton. This interaction with actin is mediated by a submembrane plaque, consisting of numerous cytoskeletal and signaling molecules. Application of intrinsic or external forces to these structures dramatically affects their assembly and triggers adhesion-mediated signaling. In this review, we discuss the structure-function relationships of focal adhesions and the possible mode of action of the putative mechanosensor associated with them. We also discuss the general phenomenon of mechanosensitivity, and the approaches used to measure local forces at adhesion sites, the cytoskeleton-mediated regulation of local contractility, and the nature of the signaling networks that both affect contractility and are affected by it.

CONTENTS

FORCES AT THE INTERFACE BETWEEN CELLS AND THEIR ENVIRONMENT: AN OVERVIEW

Within the tissue environment, cells are constantly exposed to mechanical perturbations evoked either by the cell's own contractile machinery or by a variety of environmental factors. These forces, regardless of their precise origin, act at the interface between cells and their neighbors or between cells and the extracellular matrix (ECM). Studies in recent years have shown that these forces are sensed by cells and their application triggers a variety of physiological responses affecting cell behavior and structure. The response to mechanical perturbations includes local structural changes in adhesion sites and the attached cytoskeleton, alterations in cell motility, proliferation, and survival. The central role of mechanical stimulation of cells is broadly recognized, and the function of force-activated ionic channels has been partially deciphered at the molecular level (Gillespie & Walker 2001, Hamill & Martinac 2001). Yet, the mechanism underlying the conversion of physical signals in adhesion sites into chemical ones is still largely obscure. In this review we discuss recent studies of adhesion-coupled mechanosensory processes. We address the source of forces applied by cells to the surrounding environment at adhesion sites and the nature of mechanical perturbations applied to cells from the outside. Different aspects of the cellular machinery responsible for sensing these forces are described, and we consider the mechanisms underlying the cellular responses to these forces.

It should be emphasized that the mechanical interactions discussed here are not unique to specialized sensory cells but are shared by essentially all adherent cells that are continually exposed to changing physical perturbations. For example, endothelial cells sense shear stress produced by blood flow, pressure, and stretching forces applied to cells by neighboring muscular tissues; hydrostatic pressure inside blood vessels and lymphatics or in different glands affects the adjacent endothelial or epithelial cells; and pulling and pushing forces are applied through adherens junctions to neighboring cells (Davies 1995, Epstein & Davis 2003, Gillespie & Walker 2001, Ingber 2002). Forces produced by the cell itself are primarily generated by the different cytoskeletal networks that span the cytoplasm. These forces can be quite diversified, generated by different molecular interactions. The most prominent force-generating process is actomyosin contractility, driven by the motor protein myosin II and triggered (in nonmuscle and smooth muscle cells) by the phosphorylation of the myosin II regulatory light chain (Bresnick 1999, Somlyo & Somlyo 2000). Other types of myosin motors, such as myosin VIIa (Kussel-Andermann et al. 2000) and myosin X (Cox et al. 2002), may also apply force to adhesion structures. In addition, forces generated by actin polymerization per se can be applied to membranes, organelles, and other cytoskeletal systems, thereby leading to their deformation (e.g., protrusion of the lamellipodium) or translocation (e.g., organelle transport) (Carlier et al. 2003, Mogilner & Oster 2003). Motor-driven and polymerization-dependent forces can also be mediated by other cytoskeletal systems such as microtubules. Examples of the involvement of

these diverse processes in formation and regulation of cellular forces are discussed below.

Cell Adhesions—Major Sites of Force Application

The sub-cellular sites to which cytoskeletal and external forces are applied are adhesion sites to neighboring cells and to the ECM. Any attempt to understand the molecular basis for cellular mechanosensitivity should take into consideration the detailed structure of these sites. A review of this topic in any detail is way beyond our scope here but can be found in several recent review articles (Jamora & Fuchs 2002, for cell-cell junctions; Geiger et al. 2001, Zamir & Geiger 2001, for cell-matrix adhesions). Nevertheless, we briefly outline the major molecular characteristics of one type of mechanoresponsive ECM adhesion sites, namely focal adhesions or focal contacts. In these regions, specific heterodimeric (α-β) transmembrane receptors of the integrin multigene family link the ECM to the cytoskeleton. This transmembrane interaction is mediated by a network of adapter or anchor proteins (more than 50 known to date) that form a submembrane plaque. The cytoplasmic domains of β-integrin subunits play a direct role in the establishment of these connections, and at least four proteins, talin, α-actinin, filamin and tensin, could directly link them with the actin filaments (Geiger et al. 2001, Liu et al. 2000, Zamir & Geiger 2001). In addition to these direct linkers, there are tens of potential connections consisting of two or more links (Geiger et al. 2001, Zamir & Geiger 2001), and the list of newly discovered components is still expanding (Tu et al. 2003).

Recent studies revealed a characteristic feature of integrin interaction with cytoplasmic proteins (Calderwood et al. 2003, Garcia-Alvarez et al. 2003). The β-integrin cytoplasmic domains contain one or two conserved NPxY or NPxF motifs (single-letter amino acid code; x could be arbitrary). Via these motifs, integrins can interact with proteins that contain phosphotyrosine-binding (PTB) or PTB-like modules. It was shown that a majority of integrin β-tails interact with many PTB domain-containing proteins through this structurally conserved mechanism. The focal adhesion proteins talin and tensin belong to this group (Calderwood et al. 2003). Some PTB domain-containing β-integrin partners can function as negative regulators of integrin anchorage. For example, integrin cytoplasmic domain-associated protein 1α (ICAP-1α) induces a rapid disruption of focal adhesions, which may result from the ability of this protein to inhibit the association of β1A integrin with talin (Bouvard et al. 2003). It is worth noting that tyrosine phosphorylation of NPxY motif is not always required for PTB domain-mediated β-integrin recognition, but in some cases it can strongly affect (promote or inhibit) integrin interaction with its cytoplasmic partners (Calderwood et al. 2003).

Phosphorylation and other signaling events are executed in the adhesion plaques by several types of enzymes, including protein tyrosine kinases [e.g., focal adhesion kinase (FAK), Src, Fyn, etc.] (Geiger et al. 2001, Zamir & Geiger 2001); protein tyrosine phosphatases [e.g., receptor-like tyrosine phosphatase α (RPTP-α)] (Von

Wichert et al. 2003); and serine-threonine kinases [e.g., integrin-linked kinase (ILK)] (Sakai et al. 2003) or PAK (Sells et al. 2000). Moreover, many components of focal adhesions, including integrins themselves, are substrates of these enzymes. It was recently shown that type I-γ isoform of phosphatidylinositol phosphate kinase, an enzyme that generates phosphatidylinositol-4,5-bisphosphate (PIP_2), is concentrated in the focal adhesion, bound to talin (Di Paolo et al. 2002, Ling et al. 2002). PIP_2, in turn, promotes transition of talin and vinculin (another important focal adhesion component) from non-active closed to active open conformation (Gilmore & Burridge 1996, Martel et al. 2001). Finally, the focal adhesion–specific protease, calpain, may participate in both turnover and activation of focal adhesion components (Bhatt et al. 2002, Glading et al. 2002). Altogether these signaling events determine the dynamics of focal adhesion and most likely play a role in adhesion-mediated signaling. It is conceivable that mechanically evoked signals also originate in this plaque.

Focal adhesions evolve from small (less than 1 μm in diameter) dot-like adhesion sites termed focal complexes: nascent integrin-mediated adhesions formed during lamellipodial protrusions (Geiger & Bershadsky 2001, Rottner et al. 1999) (Figure 1). Apparently, focal complexes and focal adhesions are associated with different sub-domains of actin cytoskeleton. Protruding lamellipodia contain a dense, rapidly polymerizing branching network of actin filaments. Both filament polymerization and branching depend on a molecular complex known as Arp2/3, which nucleates filament growth and mediates their attachment to pre-existing filaments (Borisy & Svitkina 2000, Pollard & Borisy 2003). Focal complexes (but not mature focal adhesions) contain Arp2/3, and a transient molecular interaction between Arp2/3 and vinculin was reported (DeMali et al. 2002). Transition of the focal complexes into focal adhesions is accompanied by transition of the associated actin mesh into densely packed straight bundles of filaments known as stress fibers (Heath & Dunn 1978). Stress fibers contain many actin-associated proteins, including myosin II in an active form (with phosphorylated light chain) (Matsumura et al. 1998), and are contractile structures (Katoh et al. 2001) that apparently apply tension to the membrane-bound adhesion plaque. Via integrins, these tension forces are transmitted to the extracellular matrix and can be measured.

Forces the Cell Exerts Via Focal Adhesions— A Physical Insight

Measurement of forces, generated or sensed at adhesion sites, is not a simple mission. The small dimensions of individual adhesion sites, their spatial proximity, and the small magnitude of the forces involved, make it challenging to accurately measure and map these sites. Recently, several works have succeeded in measuring traction forces and relating them to focal adhesion assembly. The various techniques for the measurement of cellular forces applied to the substrate have been reviewed recently (Beningo & Wang 2002, Roy et al. 2002) and are considered here only briefly. In general, such forces are measured using specially designed substrates, whose distortion can be visualized under the microscope, an idea

suggested by Harris et al. (1980) more than two decades ago. These distortions of flexible substrates can be visualized by wrinkling thin elastic substrates (Burton et al. 1999, Harris 1984), or using spatial markers such as embedded beads or micro-lithography patterns (Balaban et al. 2001, Beningo et al. 2002, Oliver et al. 1999). A second step involves computation of the mechanical cellular forces on the basis of the local distortions measured by the different devices. Substrates with discrete deflectable elements such as micro-cantilevers (Galbraith & Sheetz 1997) or, more recently, elastic micro-posts (Tan et al. 2003) have the advantage of direct linear relations between substrate distortion and force applied at each element. The proportionality constant is determined using calibrated micropipettes in situ. However, the special topography of these substrates might affect adhesion and spreading and limit the spatial resolution of force detection. For the continuously patterned elastic surfaces, more sophisticated algorithms are needed to solve the inverse problem of translating deflections to local forces (Dembo & Wang 1999, Munevar et al. 2001, Schwarz et al. 2002). The algorithms rely on elasticity theory and use a regularization scheme to solve the ill-posed inverse problem. The regularization requires additional assumptions such as the smoothness of the force field (Dembo & Wang 1999, Munevar et al. 2001) or the restriction of force generation to the locations of focal adhesions (Schwarz et al. 2002). The latter enables the estimation of the force exerted at each focal adhesion.

In general, different cell types produce different traction forces and develop matrix adhesions of varying sizes. Therefore, it is most surprising that the forces per unit of the adhesion area (stress) exerted by different cell types appear to be very similar, even though they were estimated by different methods (Table 1). In two

TABLE 1 Forces exerted by cells at focal adhesions

Reference	Stress ($nN/\mu m^2$)	Cell type	Method of measurement
Galbraith & Sheetz 1997	3 nN/focal adhesion	CEF (fibroblasts)	Micro-cantilevers
Balaban et al. 2001	5.5	HFF (fibroblasts)	Patterned elastomer (PDMS[a])
Balaban et al. 2001	2–5	Cardiac myocytes	Patterned elastomer (PDMS)
Beningo et al. 2001	2–10	CAR (fibroblasts)	Polyacrylamide gel with embedded beads
Munevar et al. 2001	2–10	3T3 (fibroblasts)	Polyacrylamide gel with embedded beads
Tan et al. 2003	4.8	BPASMC (smooth muscle)	elastomeric (PDMS) posts
Burton et al. 1999	2–15	Fish Keratocytes	Wrinkling silicone rubber film
Oliver et al. 1999	0.2–1	Fish Keratocytes	Silicone rubber with embedded beads

[a]PDMS-Polydimethylsiloxane.

studies (Balaban et al. 2001, Beningo et al. 2001), focal adhesions were specifically labeled using transfection with GFP derivatives of their typical components (vinculin, paxillin, zyxin), which made it possible to carefully examine the relationship between sizes of the focal adhesions and the forces transmitted through them. In stationary, non-motile fibroblasts and cardiac cells, clear linear dependence between these two values was found with a slope 5.5 nN/μm^2 (Balaban et al. 2001) (Figure 2). In motile fibroblasts, in addition to focal adhesions with sizes proportional to the local forces, a subpopulation of small zyxin-containing contacts near the cell's leading edge transmitted non-proportionally high forces (Beningo et al. 2001). Recent measurements of adhesion-mediated forces by micro-post deflection also revealed that typical focal adhesions exert forces that are proportional to their sizes (slope 4.8 nN/μm^2), while some small (less than 1 μm) adhesions may produce non-proportionally high forces (Tan et al. 2003) (Figure 2).

The mechanism underlying the high force development at the leading edge-associated adhesions is not clear. One hypothesis is that these contacts are specifically designed to resist high forces essential for pulling forward the cell body of migrating cells (Beningo & Wang 2002, Munevar et al. 2001). Another (not necessarily alternative) possibility is that these adhesion sites, localized immediately behind the lamellipodia, could experience forces produced by Arp2/3-dependent branching actin polymerization, which pushes in a centripetal direction, adding some substantial value (see estimation in Abraham et al. 1999) to the myosin II-driven contractile force. All in all, the measurements summarized above demonstrate that integrin-mediated matrix adhesions transmit forces generated by the actin cytoskeleton and that for a major subclass of such adhesions (classical focal contacts), the transmitted forces are proportional to the size of the adhesion site. The significance of this proportionality is discussed below.

Mechanical Forces Regulate Focal Adhesion Assembly and Stability

When two variables are proportional to each other, the natural question is, which one depends on which. Strange as it may appear, focal adhesion assembly, growth, and maintenance depend on mechanical forces applied to them. This is manifested by the dramatic effect of inhibitors of myosin II-driven contractility (e.g., chemical inhibitors of myosin light chain kinase such as ML-7 and KT5926; myosin ATPase inhibitors such as BDM, or overexpression of caldesmon, a protein that inhibits actin-dependent myosin II ATPase activity) on the formation of new focal adhesions, and the stability of existing ones (Balaban et al. 2001, Chrzanowska-Wodnicka & Burridge 1996, Helfman et al. 1999). Decrease of myosin II-driven tension brings about a rapid decrease of the focal adhesion size, so that the ratio between the two values does not change (Balaban et al. 2001); block of contractility leads to complete dissolution of focal adhesions (Chrzanowska-Wodnicka & Burridge 1996, Helfman et al. 1999). Focal complexes, on the other hand, do not disassemble following inhibition of myosin II contractility (Riveline et al. 2001,

Geiger & Bershadsky 2001). Thus focal adhesions somewhat resemble cats' claws, which are displayed only when the cat tightens special muscles in order to increase its grip and are retracted upon their relaxation. "This may be a small anatomical quibble, but it is important for the cat" (Morris 1997). A seemingly paradoxical dependence of focal adhesion formation on forces that pull them away from the substrate is probably quite important for the cell.

The forces applied to adhesion sites are not determined exclusively by the cellular contractile machinery. An additional important factor is the mechanical nature of the underlying substrate. When cells are attached to a soft flexible substrate, which can be easily deformed, the tension acting on the adhesion plaques may be smaller than the force needed to sustain the adhesion site and the attached stress fibers. Consequently, the typical dimensions of focal adhesions formed with such substrates are considerably smaller than those formed following attachment to a rigid surface (Pelham & Wang 1997). This ability to discriminate between soft and rigid substrate enables cells to become oriented when they sense a gradient in substrate rigidity and move along the substrate in the direction of higher rigidity (a phenomenon known as durotaxis) (Lo et al. 2000).

The forces stimulating focal adhesion formation and stability can be generated not only by the cell's own contractile system but also can be applied from the outside (Kaverina et al. 2002, Riveline et al. 2001). Indeed, external force applied to focal adhesions can effectively substitute for cell-generated forces so that focal adhesion may be stimulated to grow even in relaxed cells treated by BDM or transfected with caldesmon (Riveline et al. 2001). Mechanical force can also be applied to micron-sized beads coated with fibronectin or other extracellular matrix proteins and attached to the dorsal cell surface. Without application of external force, these beads move centripetally, driven by retrograde actin flow (Cramer 1997). However, trapping of a bead with laser tweezers directs the force produced by the flow to the integrin-mediated contact at the interface between the bead and the cell surface. This force was shown to strengthen the transmembrane link between the bead and the actin cytoskeleton, which further increases the flow-driven force applied to the bead and eventually allows it to escape from the trap (Choquet et al. 1997). This process, termed reinforcement, seems analogous to the process of focal adhesion assembly and similarly includes recruitment of the new vinculin molecules into the adhesion plaque (Galbraith et al. 2002).

A variation on the same theme is provided by the cellular response to fluid shear stress. It is now well established that a plethora of shear stress-induced responses of endothelial cells is mediated by integrin-dependent signaling (for a review see Shyy & Chien 2002). Recent studies strongly support the decentralized distribution of luminally imposed forces throughout the endothelial cell (Davies et al. 2003, Helmke & Davies 2002). Therefore, it is reasonable to suggest that the focal complexes and focal adhesions of endothelial cells under shear stress experience some of the externally applied forces. In addition, shear stress was shown to activate myosin light chain kinase (Watanabe et al. 1998), which may increase endogenous myosin II-driven contractility and further augment the forces applied

to the focal adhesions. It was demonstrated that shear stress elicits remodeling of focal adhesions in the endothelial cells (Davies et al. 1994, 1997), which might be a mechanism underlying shear stress-induced activation of the integrin signaling (Shyy & Chien 2002).

Thus a focal adhesion functions as a typical mechanosensory device (Geiger & Bershadsky 2002). It increases in size upon application of force (either cell-generated or external) and shrinks upon the relaxation of force. As a faithful gauge, it keeps the size proportional to the applied tension. What are the molecular mechanisms that could explain this behavior?

Which Mechanisms are Involved in Focal Adhesion Mechanosensitivity?

When it comes to molecular mechanism of any mechanosensory event, the possible analogy to stretch-activated ionic channels comes to mind because the vast majority of known cellular mechanosensors are based on different modifications of this basic mechanism (Gillespie & Walker 2001, Hamill & Martinac 2001). In fact, some preliminary studies indicate that channel inhibition may modify the assembly and dynamics of the focal adhesions (Y. Wang, personal communication). It is worth noting, however, that focal adhesions respond to force in a localized fashion, and the growth of micron-sized adhesions is highly polar, oriented in the direction of applied force (Riveline et al. 2001). This high-spatial resolution seems to be incompatible with the mechanism based on local changes in the concentration of diffusible ions. Nevertheless, in another preliminary study, a local entry of calcium in close proximity to focal adhesion was registered upon mechanical stimulation of the cell (Hayakawa et al. 2002). These studies need to be continued to elucidate the role of stretch-activated channels.

Another line of studies strongly suggests that the integrin-mediated focal adhesion mechanosensor can respond to applied force even in detergent-treated cells (Sawada & Sheetz 2002). In these experiments, cells were plated on a flexible substrate, extracted with Triton X-100, and subjected to stretching in the presence of biotinylated cytoplasmic proteins. Following this stimulation, some of focal adhesion proteins (paxillin, FAK, and p130cas) were selectively incorporated into the stretched cytoskeletons; further experiments with GFP-paxillin confirmed that this protein is recruited to focal adhesions following force application in a system where changes in ionic permeability are totally eliminated.

How does stretching of molecular ensemble affect incorporation of the new components from the soluble cytoplasmic pool? One possibility is that the application of mechanical load simply alters (in a highly ordered way) the relative positions of specific focal adhesion components in the three-dimensional protein network forming the submembrane adhesion plaque. In fact, tension-dependent molecular reorganization of adhesion sites is known to take place. First, transition from nascent focal complexes to mature focal adhesions is accompanied by a major increase in $\alpha_v\beta_3$-integrin density, as revealed by experiments with GFP-β_3-integrin

(Ballestrem et al. 2001). Moreover, fluorescence recovery after photobleaching (FRAP) experiments provides evidence that within an individual focal adhesion, the β_3-integrin subunit is capable of moving in an energy-dependent and myosin II-dependent manner (Tsuruta et al. 2002). An extreme consequence of changes in mutual position of focal adhesion components is their sorting, i.e., when myosin-driven force specifically extracts molecular complexes containing $\alpha_5\beta_1$ integrin and tensin from focal adhesions (Pankov et al. 2000, Zamir et al. 2000). These tensin-enriched adhesion sites, known as fibrillar adhesions, then move centripetally and participate in the formation of fibronectin fibrils outside the cell, transmitting the myosin-driven tension forces to fibronectin molecules (Geiger et al. 2001) (see below).

The observation that relaxation of tension by a variety of myosin II inhibitors leads not only to cessation of growth, but also to a rapid disassembly of focal adhesions suggests that these structures are intrinsically dynamic: They continuously lose old subunits and incorporate the new ones in a tension-dependent fashion. FRAP measurements, indeed, revealed a high turnover rate (Ballestrem et al. 2001). Among other factors, the protease calpain specifically localized at the focal adhesions might be involved in their rapid disassembly (Bhatt et al. 2002).

Another possible mechanism for force sensing involves alterations in the conformation of specific molecules. A striking example is provided by recent structural studies of integrin itself, showing that this molecule can exist in two distinct conformations, one with low affinity for extracellular matrix ligands (inactive), and another with high affinity (active). Binding of the extracellular ligand to the extracellular head of the $\alpha-\beta$ integrin dimer induces gross conformational changes that affect the transmembrane parts of the molecule, inducing separation of the α and β cytoplasmic tails and exposing them for interaction with different intracellular partners such as talin (Giancotti 2003, Hynes 2002). The transition from inactive to active conformation can be triggered by binding of a ligand to the extracellular part of the integrin dimer and also by binding of an intracellular partner to the cytoplasmic portion of the molecule (Calderwood et al. 1999). Interestingly, several focal adhesion plaque proteins also exist in two conformations: closed (inactive) and open (active). Among these proteins are vinculin (DeMali et al. 2002, Gilmore & Burridge 1996), talin (Martel et al. 2001), pp60src (Bjorge et al. 2000), and the matrix protein fibronectin (see below). Transition from inactive to active conformation may occur in response to biochemical signals such as binding of PIP_2 for vinculin and talin, and dephosphorylation of inhibitory phosphotyrosine residue pTyr527 for Src.

However, because the activation of these molecules can be achieved by long-range structural transitions (e.g., opening), one can envisage a scenario where these or analogous conformational changes can be produced by mechanical force, thereby exposing binding sites that can mediate new molecular interactions (Geiger & Bershadsky 2001, 2002). Rough calculation suggests that the mechanical force applied to individual molecules in a focal adhesion is in the range of a few picoNewtons, which is sufficient for the induction of such conformational changes (see Balaban et al. 2001).

One example showing how force-induced conformational changes can affect intermolecular interactions is the assembly of extracellular fibronectin fibrils. In a series of studies summarized in Geiger et al. (2001) and in more recent reports from Baneyx et al. (2001, 2002) and Krammer et al. (2002), it was shown that stretching of fibronectin can unfold the molecule and expose binding sites for integrin as well as for self-association sites, thus leading to the formation of fibronectin fibrils. Obviously, stretch-induced assembly of fibronectin cannot account for the whole phenomenon of force-induced focal adhesion assembly because cells also form focal adhesions on substrates other than fibronectin. However, this example demonstrates that a force-induced assembly process can occur even in a rather simple one-component system and, in a way, can serve as an adhesion-dependent mechanosensor.

Changes in either mutual position of focal adhesion proteins or in their conformation may then affect recruitment and/or function of signaling molecules associated with focal adhesions and trigger a cascade of signaling events. In particular, the FAK/Src pathway seems to be involved in the regulation of focal adhesion turnover (Geiger et al. 2001), and FAK-null cells are deficient in both force-induced focal adhesion growth and cell response to substrate rigidity (Wang et al. 2001). Recently, receptor-like tyrosine phosphatase α (RPTP-α), which is involved in activation of Src family kinases, in particular Fyn, was shown to be necessary for the force-induced enhancement (reinforcement) of $\alpha_v\beta_3$-integrin-cytoskeleton connections (Von Wichert et al. 2003). Future studies might clarify the relationship between structural and signaling events in the functioning of the mechanosensory complex.

Cytoskeletal Regulation of Contractility and Focal Adhesions: Role of Microtubules

Mechanosensory response at focal adhesions, as characterized above, is a local event that depends on only the force applied directly to this miniature molecular device. It is also a self-accelerating process because the growth of the focal adhesion is accompanied by an increase in number of filaments forming the stress fiber associated with the adhesion plaque, which, in turn, increases the applied tension and promotes further focal adhesion growth. How can such local autonomy allow for spatially and temporally regulated processes such as cell motility to take place? For example, how does a cell avoid the formation of huge, unlimitedly growing focal adhesions produced stochastically at the cell margin by the local autocatalytic mechanism described above? A clue to answering these questions is provided by observations of cells treated with microtubule-disrupting drugs (colchicine, colcemid, nocodazole, etc.) (Peterson & Mitchison 2002). Apparently, many cell types treated with such drugs demonstrate the phenotype with exaggerated focal adhesions described above (Small et al. 2002). This augmentation in focal adhesion formation is accompanied by increase in integrin signaling (Bershadsky et al. 1996), yet it is incompatible with directional migration owing to lack of polarization (Vasiliev et al. 1970). This observation suggests that microtubules (among their

other assignments) suppress, in a highly regulated fashion, the growth of focal adhesions and thereby promote the establishment of polarized cell shape.

The mechanism underlying this regulatory process involves two features of microtubules: their ability to suppress contractility and their ability to locate and target focal adhesions. The fact that microtubules can suppress cell contractility was demonstrated long ago (Danowski 1989), and the generality of this process for many different cell types was established (Elbaum et al. 1999, Small et al. 2002). Microtubule disruption can trigger rhythmic oscillations of the contractile actomyosin system (Bornens et al. 1989, Pletjushkina et al. 2001), actomyosin-driven process retraction in nerve cells (Ahmad et al. 2000, Solomon & Magendantz 1981), and increase of actomyosin-based cortical flow rate in *Xenopus* oocytes (Canman & Bement 1997).

However, in spite of its apparent universality, the mechanism of microtubule-mediated suppression of contractility is not clearly understood. Microtubules could be involved in the delivery or removal of some regulatory molecules that affect contractility (signaling model) (Small & Kaverina 2003, Wittmann & Waterman-Storer 2001) or could provide direct mechanical resistance to actomyosin contractility (tensegrity model) (Ingber 2003). There is also some evidence that microtubule-based motors (kinesin-1 in fibroblasts; Krylyshkina et al. 2002, Rodionov et al. 1993)(dynein in nerve cells; Ahmad et al. 2000) play a role in such regulation; however, whether these motors mechanically compete with the actomyosin system or participate in the transport of regulatory factors is not yet clear. Among microtubule-associated signaling molecules, GEF-H, an exchange factor for the small GTPase Rho, is potentially interesting. Its Rho-stimulating activity is low when it is in microtubule-bound state and high when it is free (Krendel et al. 2002). This could possibly explain why microtubule disruption can activate Rho (Krendel et al. 2002, Ren et al. 1999). Future studies will clarify whether this mechanism is indeed responsible for microtubule-dependent actomyosin regulation.

Microtubule-dependent regulation of focal adhesions apparently acts upstream of myosin II contractility (Bershadsky et al. 1996, Helfman et al. 1999). Moreover, local application of myosin II inhibitors by micropipette restores directional migration ability of cells lacking microtubules, most probably by local suppression of focal adhesions (Kaverina et al. 2000). This experiment suggests that microtubule-mediated contractility inhibition is coordinated in time and space with the localization of focal adhesions. Indeed, observations of microtubule dynamics in living cells revealed that application of force to regions of cell-substrate attachment promotes rapid microtubule extension into that region, followed by targeting of the local adhesion sites (Kaverina et al. 2002, Small & Kaverina 2003, Suter & Forscher 2000). This extension of microtubules triggers the arrest of focal adhesion growth and often their disassembly (Kaverina et al. 1999). These studies suggest that microtubules are somehow attracted to focal adhesions, locally suppress the tension forces applied to these structures, and thereby interrupt the positive feedback loop described above (for a more detailed discussion, see Small et al. 2002).

Coordination by Rho-Family GTPases

Which factors coordinate the processes of tension-dependent growth of focal adhesions? To put this question in the context of signaling, it is important to note that the formation and maintenance of classical focal adhesions depends on the activity of the small GTPase Rho (Nobes & Hall 1995, Ridley & Hall 1992, Rottner et al. 1999). Recently, two targets of Rho were shown to be necessary and sufficient to mediate Rho's function in this process: Rho-associated kinase (known also as Rho kinase or ROCK) and the formin homology protein, Dia1 (Nakano et al. 1999, Tominaga et al. 2000, Watanabe et al. 1999). ROCK is known to activate myosin II (Fukata et al. 2001, Kimura et al. 1996); our experiments revealed that the function of ROCK in focal adhesion formation is primarily associated with the activation of myosin II-driven contractility (Riveline et al. 2001).

The function of the second Rho target, Dia1, appears to be more complex. Under conditions of external force application, Dia1 is necessary and sufficient to mediate the Rho-signal, inducing focal adhesion assembly (Riveline et al. 2001). Recent studies demonstrated that the yeast homolog of Dia1, known as Bni1p, is a potent nucleator of actin polymerization (Pruyne et al. 2002, Sagot et al. 2002). Unlike Arp2/3 complex, Bni1p promotes formation of nonbranching linear actin filaments. In addition, our recent findings suggest that Dia1 can participate in the regulation of microtubule dynamics and organization. In cells expressing constitutively active Dia1, both microtubule plus and minus ends are stabilized, and targeting of the plus ends to focal adhesions is enhanced (Ballestrem et al. 2002).

How is the complex system of interactions among contractile actin cytoskeleton, mechanosensory focal adhesions, and contraction-suppressing microtubules coordinated by the small GTPases and, in particular, by Rho and its two targets? A key question here is how are the activated, GTP-bound GTPases distributed in the cell. Whereas direct visualization of active Rho has not been reported yet, there are some interesting data concerning the distribution of activated Rac and Cdc42 based on various modifications of the fluorescence resonance energy transfer (FRET) technique (Gardiner et al. 2002, Itoh et al. 2002, Kraynov et al. 2000). Rac activation can be stimulated by integrin signaling (Price et al. 1998), and there is good evidence for cross talk between Rac activity and formation of new cell-ECM adhesions at the leading edge (Del Pozo et al. 2002, Tzima et al. 2002). Integrin signaling can also activate Rho, although the mechanism responsible for this process is less clear; this activation appears to be integrin-type specific (Danen et al. 2002, Miao et al. 2002) and biphasic (activation is preceded by a decay in activity) (Ren et al. 1999). Nevertheless, it is reasonable to suggest that integrin-mediated signaling induced at focal adhesions can, at some time point, activate Rho, and hence the gradient of activated Rho (and consequently of ROCK and Dia1 too) would depend on focal adhesion distribution. Rho, via ROCK, might coordinate the entire process of focal adhesion maturation by inducing myosin II-driven tension

that activates the focal adhesion mechanosensor. Triggering actin polymerization via Dia1 may provide a complementary mechanism supporting focal adhesion assembly. Furthermore, Dia1-mediated changes in microtubule dynamics and organization may be responsible for focal adhesion downregulation. Elucidation of this complex molecular cross talk and its detailed regulation will be the key for understanding the mechanisms underlying fundamental cellular processes such as cell adhesion and motility and the spatial and temporal relationships between them.

CONCLUSION

In this review, we have considered mechanosensitivity in the context of one particular biological system, integrin-mediated focal adhesion. The focal adhesion mechanosensor is probably not a unique molecular machine but a prototypic device, which can have analogs in other cellular systems such as adherens junction complex (Ko & McCulloch 2001) and the Z-disc-complex in striated muscle (Epstein & Davis 2003, Knoll et al. 2002). We have highlighted features that allow focal adhesions to act as mechanosensors and thereby probe the physical properties of the environment and activate specific signaling pathways within the cells. This capacity to convert physical perturbations into discrete signaling cascades is based on the molecular properties of the focal adhesion-stress fiber complex, a structure that is constantly maintained under certain level of stress, and disintegrates upon relaxation. The broad interest in mechanosensitivity and in adhesion-dependent signal transduction, in general, has resulted in the accumulation of vast information (still, mostly indirect) about these systems, part of which is outlined here. However, some of the very basic features of the mechanosensory apparatus are still poorly defined and await further research. The most obvious missing information concerns the specific molecular target of the applied force. Is it one particular molecule that undergoes conformational change when exposed to mechanical forces? Does tension modify the organization of multi-molecular complexes in focal adhesions or is the response to force mediated by force fields that affect the membrane itself? Equally challenging is the exquisite coordination of local events, such as induction of focal adhesion, formation of extension and global signals, that affect cell polarization and motility. There is no doubt that microtubules and small GTPases play a central role in regulating focal adhesions, but exactly how they perform this mission and maintain the spatial and temporal precision needed to coordinate motile events is yet to be elucidated. Nevertheless, it is hoped that new experimental tools and approaches developed in recent years, including the use of intrinsically fluorescent proteins for live cell monitoring of molecular dynamics, RNA silencing for determining the role of specific proteins in the adhesion process, and different physical approaches (e.g., FRET) for measuring molecular interaction in vivo, will shed light on the mechanisms underlying cellular mechanosensitivity.

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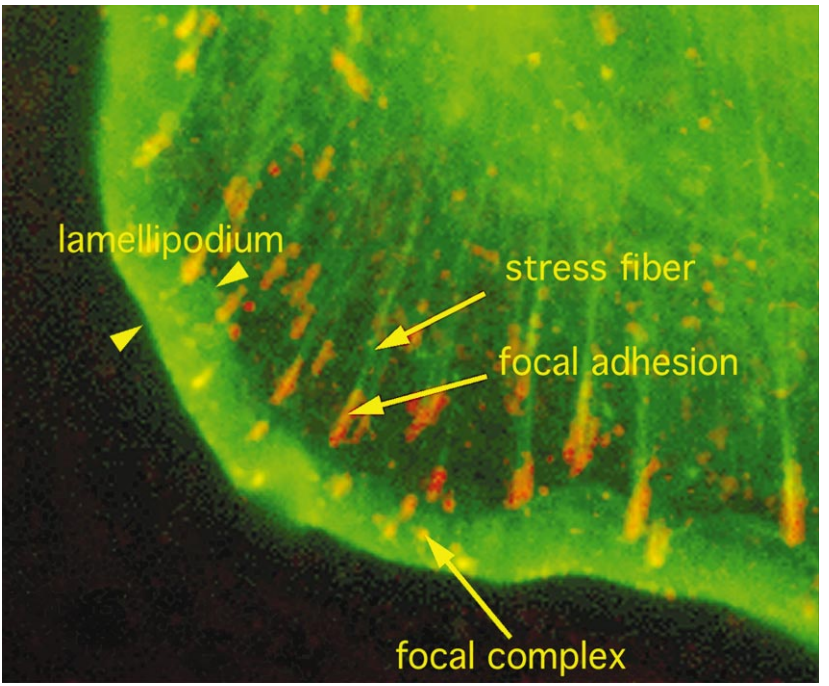

Figure 1 Association of matrix adhesions with the actin cytoskeleton. Part of the leading lamella of actively spreading T47D cell (stimulated by neuregulin) (Chausovsky et al. 2000) is shown. Actin is visualized by phalloidin staining (*green*), and adhesion structures are visualized with anti-phosphotyrosine antibodies (*red*). The margins of the lamellipodium contain a dense actin network, marked by arrowheads. Arrows indicate dot-like focal complex, elongated mature focal adhesion, and associated actin bundle (stress fiber). The photograph was provided by K. Arnold (The Weizmann Institute of Science).

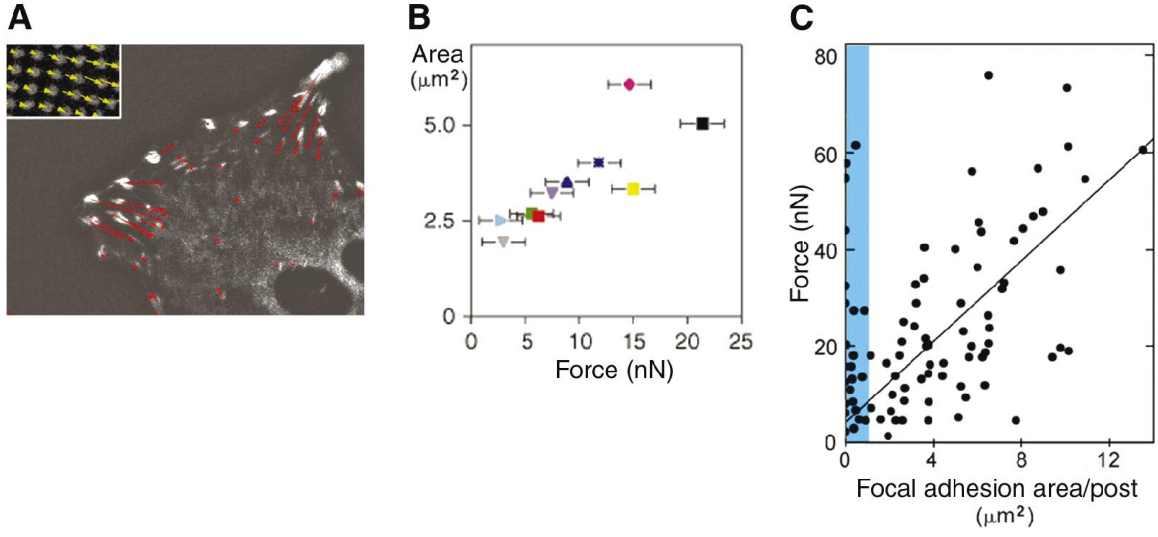

Figure 2 Correlation between forces and focal adhesion dimensions. (*A*) Fluorescence image of a human foreskin fibroblast expressing GFP-vinculin, which localizes to focal adhesions. The cell is attached to an elastic substrate with a pattern of dots, and the displacement of the dots is quantified (inset). The red arrows marks the direction and magnitude of the force transmitted at individual focal adhesions (calculated from displacements of dots). (*B*) A graph showing the correlation between transmitted force and the area of single focal adhesions. Each symbol corresponds to individual focal adhesion shown in (*A*). (From Balaban et al. 2001, with permission.) (*C*) Bovine pulmonary artery smooth muscle cells (BPASMC) were plated on a substrate consisting of elastic posts. Plot of the force generated on the post as a function of area of focal adhesion staining per post (visualized by anti-vinculin antibody staining) is shown. The shaded region (*blue*) indicates the adhesions smaller than 1 μm^2. (From Tan et al. 2003, with permission.)

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