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Exploring the Neighborhood: Adhesion-Coupled Cell Mechanosensors

Minireview

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Here we discuss recent studies addressing adhesion-coupled mechanosensory processes and consider their molecular nature. Are cells using stretch-activated ion channels to explore the extracellular environment surrounding them, or do they use for that purpose the submembrane protein network that interconnects integrin receptors with the actin cytoskeleton?

Throughout their life, cells participate in numerous physical interactions with the world around them: Endothelial cells sense shear stress produced by the blood flow, pressure is applied to cells by neighboring muscular tissues, acoustic waves act on the stereocilia of hair cells, adherent cells are exposed to mechanical perturbation via the extracellular matrix (ECM), and more. Mechanical forces, generated by the cell's own contractile machinery, can also affect cell shape, cytoplasmic organization, and many intracellular processes. A growing body of evidence suggests such mechanical cues play a central role in regulating cell behavior and fate, similar to chemical environmental cues, e.g. cytokines and hormones. Mechanical probing of the immediate environment is considered a critical mechanism controlling such cellular processes as motility, morphogenesis, proliferation, and apoptosis. Consequently, cells possess rather sophisticated mechanosensory devices, which can detect forces and respond to them. The mechanism of action of such "force receptors" is, however, still poorly characterized compared to classical "chemoreceptors." In this article, we address some of the general features of mechanosensory responses, and particularly focus on the involvement of such processes in the regulation of cell adhesion, motility, and adhesion-dependent signaling.

Classical mechanosensory signaling is based on a 5-component transmembrane system consisting of an ion channel, tethered via intracellular and extracellular links to internal (usually cytoskeletal) and external anchors. The ion specificity of the channel and the molecular nature of the links and anchors may vary from one system to the other, but the general mode of action appears to be rather similar. Thus, tension transduced via the linkers to the channel can modulate local ion transduction, which, in turn, may activate a variety of signaling events (for review, see Gillespie and Walker, 2001). In the inner ear, for example, nonselective cation channels, located on stereocilia, are cross-tethered to the actin cytoskeleton and to the membrane of neighboring stereocilia so that sound-induced vibrations stimulate ion (mainly Ca^{2+}) fluxes. Consequently, mutations in a variety of cytoskeletal or putative link proteins can perturb this mechanism and cause deafness. A similar mechanism was found for the touch receptors of *C. elegans*, where the channel is apparently linked to the ECM and to microtubules, the sensory organ of the fly ("bristle receptor"), and more.

Mechanosensitivity is not a unique property of cells in specialized sensory organs but is shared by most or all adherent cells. It has been shown that cells probe the external environment locally by applying actomyosin-driven forces to cell-matrix or cell-cell adhesion sites, and these sites respond to that force by changing their size, dynamics, and signaling activity (Geiger and Bershadsky, 2001). Attempts to characterize the molecular basis for adhesion-dependent mechanosensory processes focused much attention on the subcellular frameworks where such events take place, namely focal adhesions (FA) and related structures.

Focal Adhesions as Mechanosensors

Several lines of evidence implicated integrin-mediated adhesion in a mechanosensory process. Ingber and coworkers (Wang et al., 1993) showed, that twisting of cell-attached magnetic beads, coated with integrin ligands, induces a specific, force-dependent "stiffening response." Upon applied stress, the cells increased their resistance to mechanical deformation (bead twisting). Another seminal experiment (Choquet et al., 1997) was to restrain the centripetal movement of fibronectin-coated beads along the dorsal cell surface by laser tweezers, so that the trapped "mini adhesion" experiences the force produced by the cortical flow. This restraining was shown to increase the force applied to the bead by the cell ("reinforcement"). These experiments suggest that application of force to integrin-mediated adhesions strengthens the cytoskeletal anchorage. Two important features of the "reinforcement" phenomenon are: (1) its dependence on both clustering and occupancy of integrin receptors and (2) the locality of the effect—the fact that reinforcement of one bead does not affect the movement of another bead along the surface of the same cell. More recently, integrin cluster-dependent "reinforcement" of adhesion was recorded in experiments where cell detachment from the substrate coated with clustered RGD peptides was induced by centrifugal force (Koo et al., 2002).

Understanding the molecular basis of force-induced changes in adhesion require some discussion of the molecular complexity, diversity, and dynamics of integrin-mediated adhesions, primarily a subgroup known as focal contacts or FA whose assembly depends on local tension (Geiger and Bershadsky, 2001). These matrix adhesions are multi-molecular complexes consisting of more than 50 different proteins that apparently link ECM-attached integrin to the actin cytoskeleton (Geiger et al., 2001). Recent studies, using fluorescent derivatives of FA components, show that these structures can be highly dynamic and either grow or shrink, depending on mechanical forces applied to them. Small, punctate adhesions at the cell edge (known as "focal complexes") grow in size and transform into "mature" FA following application of local external force either directly to the cell (Riveline et al., 2001), or to the flexible substrate

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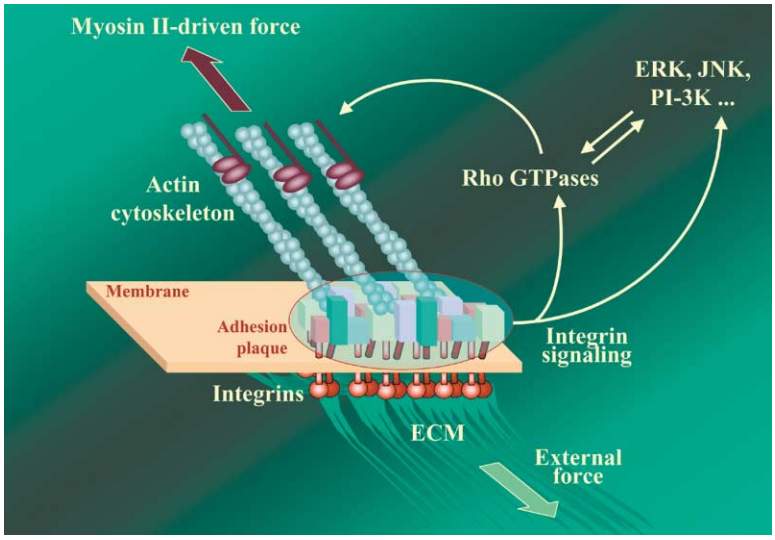

Figure 1. Focal Adhesion (FA) as a Mechanosensor

Focal adhesion is a multi-molecular complex connecting the extracellular matrix with the actin cytoskeleton. Heterodimeric transmembrane integrin receptors (red) bind matrix proteins via their extracellular domains, while their cytoplasmic domains are associated with a dense submembrane plaque containing more than 50 different proteins ("boxes" enclosed in the oval area) including structural elements as well as signal transduction proteins such as FAK, Src, ILK, etc. The plaque, in turn, is connected to the termini of actin filament bundles. The assembly and maintenance of FA depend on local mechanical forces. This force may be generated by myosin II-driven isometric contraction of the actin cytoskeleton, or by extracellular perturbations such as matrix stretching or fluid shear stress. Force-induced assembly of the adhesion plaque leads to the activation of a variety of signaling pathways that control cell proliferation, differentiation, and survival (e.g., MAP kinase and PI 3-kinase pathways) as well as the organization of the cytoskeleton (e.g., Rho family GTPase pathways). Rho, in particular, is an indispensable regulator of FA assembly affecting, via its immediate targets Dia1 and ROCK, actin polymerization and myosin II-driven contractility.

near the cell front (Wang et al., 2001). Tension is essential both for the growth of FA and their maintenance, but not for the assembly of their precursors (focal complexes). Thus, inhibition of myosin II contractility induces rapid disassembly (in a minute range) of fully assembled FA without interfering with the formation of focal complexes along the cell edge (reviewed in Geiger and Bershadsky, 2001). The size and vinculin content of individual FA are proportional to the local force applied to these structures by the contractile system of the cell (Balaban et al., 2001). Altogether, these data suggest that FA act as cellular mechanosensors that respond to changes in applied force by assembly or disassembly in a variety of model systems (Figure 1). What are the physiological contexts in which these mechanosensors might be involved in the regulation of cell behavior?

Cellular Response to Fluid Shear Stress and Substrate Stretching

Cells that are particularly exposed to physiological mechanical stimulation are endothelial cells, which line the interior of the vascular system. These cells experience and respond to extensive fluid shear stress as well as to periodic stretching of the underlying basement membrane, due to systolic-diastolic changes in blood pressure. Simulation of shear stress under culture conditions revealed dramatic effects, manifested by the activation of focal adhesion kinase (FAK), Src, PI3-kinase, and MAP kinases (ERK, JNK, and possibly p38), as well as modulation of the activity of small Rho-family GTPases (Figure 1). It is now becoming increasingly clear that the majority of these responses are triggered by the activation of integrin signaling. This is inferred from comparative studies of responses to the same mechanical stimulus by cells attached to different substrates, interference with integrin-specific antibodies, and direct demonstration of integrin activation in response to mechanical stimulation (see, for example, Li et al., 1997; Tzima et al., 2001, and references therein). It was further shown that in endothelial cells, shear stress-dependent signals are mediated by $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, which localize to FA, and whose growth and orientation are regulated by shear stress (Davies et al., 1994).

Systems simulating changes in the dimensions of the ECM are commonly based on the plating of cells onto flexible substrates, which can be subjected to different regimes of stretching (uniaxial, biaxial, sustained, transitory, periodic, etc.). Plating of cells on pliant substrate (compliance value of about 5×10^{-8} N/μm) does not permit the formation of mature FA, even when the substrate is coated with proper ECM proteins, most likely since cell contractility cannot produce sufficient tension at the adhesion sites to trigger FA assembly (Pelham and Wang, 1997). Stretching of the substrates, however, promotes the assembly of FA (see, for example, Sawada and Sheetz, 2002; Wang et al., 2001) and trigger integrin-dependent signaling, including the activation of MAP kinases via the FAK-Src pathway (see, for example, Aikawa et al., 2002; MacKenna et al., 1998, and references therein). These responses are largely similar to those triggered by fluid shear stress.

Obviously, cell reaction to shear stress and substrate stretching depends on other factors as well, such as stretch-induced secretion of humoral growth factors and effects on cell-cell junctions, some of which might be triggered directly while others are secondary to the adhesion-mediated response. We have focused here on the effects of fluid shear stress and substrate stretching on tension-induced remodeling of integrin-mediated cell-matrix adhesions to illustrate the physiological relevance of mechano-responsive regulation of cell adhesion and adhesion-dependent signaling.

Adhesion-Associated Mechanosensing: Are Stretch-Activated Channels Involved?

The molecular nature of the mechano-responsive element of FA is still unknown. Are cells using the "classical," channel-based mechanosensory system discussed above for probing their immediate environment? Some evidence implicates stretch-activated ion channels in different aspects of cell locomotion, such as the retraction of the cell's trailing edge (Lee et al., 1999). Moreover, recent study shows that gadolinium ions, which inhibit stretch-activated channels, block the formation and maintenance of FA (Yu-li Wang, personal communication). It is not clear, however, whether these channels

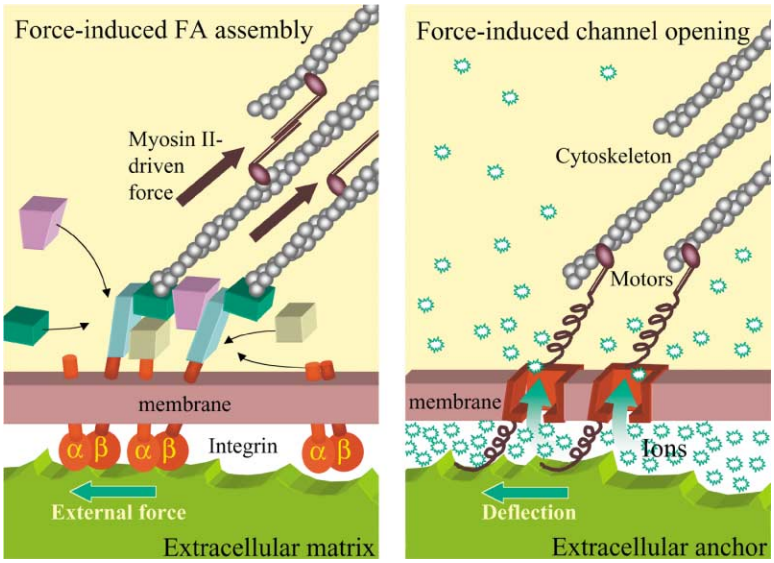

Figure 2. Similarities and Differences between the Putative FA Mechanosensor, and Mechanosensors Based on a Stretch-Activated Channel

Force-induced assembly of FA is illustrated on the left. Myosin II-driven contractile force applied to a cluster of α-β integrin heterodimers associated with the cytoplasmic plaque proteins leads to development of tension if the underlying extracellular matrix is quite rigid. Forces can also be applied externally. The application of force can lead to local and directional process of focal adhesion assembly. The mechanism of this process is, most probably, based on force-induced changes in the conformation or organization of the different plaque proteins. On the right, a "classical," channel-based mechanosensor is depicted (after Gillespie and Walker, 2001). It consists of an ion channel, bound to an extracellular "anchor" via a link protein. Deflection of the anchor relative to the membrane can induce a change in channel conformation and increase of ion flux. The cytoplasmic domain of the channel is anchored to specific cytoskeletal networks (actin-microfilaments or microtubules) via molecular motors that can modulate the forces experienced by the channel. This "device" is extremely sensitive and rapidly responding, but its effects may be less localized, than those triggered by the FA mechanosensors.

are integral and indispensable components of the mechanosensor itself. On the other hand, another line of study strongly suggests that the integrin-mediated FA mechanosensor can respond to applied force even in detergent-treated cells (Sawada and Sheetz, 2002). In these experiments, cells were subjected to stretching after detergent extraction. Following this stimulation, some FA proteins (paxillin, FAK, and p130cas) were selectively incorporated into the stretched cytoskeletons; further experiments with GFP-paxillin confirmed that this protein is recruited to FA following force application in a system where changes in ionic permeability are totally eliminated.

Another, rather indirect argument against a channel-based mechanosensor in FA is related to the spatial resolution of the response. Focal complexes or small FA respond to force in a very localized fashion, and the growth of small adhesions (often measuring only a fraction of a square micrometer) is highly polar, oriented in the direction of the cell center (Riveline et al., 2001). This "high spatial resolution" of the response (FA growth) suggests that the underlying mechanism involves direct macromolecular interactions rather than local changes in the concentration of diffusible ions (see below).

The Mechanosensor at FA: Molecular Reorganization versus Conformational Changes of FA Components

How can FA molecules "sense" mechanical signals? One possibility is that the application of mechanical load alters the relative positions of specific FA components in the three-dimensional protein network forming the submembrane adhesion plaque. Such perturbation might facilitate the incorporation of new components from the soluble cytoplasmic pool. In fact, tension-dependent molecular reorganization of adhesion sites is known to take place. First, transition from nascent "focal complexes" to mature FA is accompanied by a major increase in $\alpha_v\beta_3$-integrin density (Ballestrem et al., 2001). Moreover, fluorescence recovery after photobleaching (FRAP) experiments provide evidence that, within an individual focal adhesion, the β_3-integrin subunit can move in an energy-dependent and myosin II-dependent manner (Tsuruta et al., 2002). An extreme consequence of changes in mutual position of FA components is their "sorting," when myosin-driven force specifically extracts molecular complexes containing $\alpha_5\beta_1$ integrin and tensin from FA. 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, and see below).

Another possible mechanism for force measurement involves conformation changes of specific molecules. Interestingly, several FA proteins exist in two conformations: "closed" (inactive), and "open" (active). Among these are vinculin, ERM proteins, $pp60^{src}$, fibronectin (see below), and even, perhaps, integrin α-β heterodimer. Transition from inactive to active conformation may occur in response to biochemical signals such as binding of PIP2 for vinculin, dephosphorylation of inhibitory phosphotyrosine residue pTyr527 for Src, etc. Since 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 to expose binding sites that can mediate new molecular interactions (Geiger and Bershadsky, 2001). Rough calculation suggests that the mechanical force applied to individual FA molecules is in the range of a few pN, which is sufficient for the induction of such conformational changes (see Balaban et al., 2001).

Changes in either the mutual position of FA proteins or their conformation may affect recruitment and/or function of associated signaling molecules and trigger a cascade of signaling events. In particular, the FAK/Src pathway seems to be involved in the transduction of mechanical signals by FA (Geiger et al., 2001), and FAK null cells are deficient in both force-induced FA

growth and cell response to substrate rigidity (Wang et al., 2001).

One example of how force-induced conformational changes can affect intermolecular interactions is the assembly of extracellular fibronectin fibrils. In a series of recent studies (reviewed in Geiger et al., 2001), it was shown that stretching of fibronectin can unfold the molecule and expose binding sites for another fibronectin molecule, leading to the formation of fibronectin fibrils. Obviously, stretch-induced assembly of fibronectin cannot account for the whole phenomenon of force-induced FA assembly, since cells also form FA on other substrates. However, this example demonstrates that a force-induced assembly process can occur even in a rather simple one-component system and, in a way, serve as an adhesion-dependent mechanosensor.

Some Conclusions

As noted above, it appears highly likely that the adhesion-dependent mechanosensor is localized in the submembrane plaque of FA. The detailed molecular nature of this mechanosensor is largely unknown, yet some of its general features deserve additional discussion. One interesting aspect is related to the differences and similarities between the adhesion-dependent mechanosensory system and channel-based mechanosensors (Figure 2). In both cases, a mechanoresponsive element is linked to the cytoskeleton at the cell's interior and to an extracellular anchor (usually the ECM or the membrane of another cell, or a sensory organelle such as stereocilia). The linkage to the cytoskeleton usually involves a motor protein that might play an important and similar role in the two systems. The motor (myosin II, in the case of a stress-fiber associated FA; myosins Ic and perhaps VIIa in stereocilia, possibly; and microtubule-based motor[s] in *Drosophila* bristle-receptors. [Gillespie and Walker, 2001]) can balance the external force and be instrumental for the adaptation of cells to the external mechanical stimulus. Furthermore, the cytoskeleton-associated motor can generate force, and switch the mechanosensor on, even in the absence of an external perturbation. In the case of the adhesion mechanosensor, such process could be the key for probing the environment. Thus, cells can extend lamellipodia or filopodia, which, in turn, adhere to the matrix and assemble a mechanosensing, actomyosin-linked adhesion site. Pulling action by the attached cytoskeleton will increase the load acting on the mechanosensor, but the exact level of local tension will be strictly determined by the pliability of the ECM. With a stiff matrix, high mechanical stress (up to 5 nN/μm^2, translated into a few pN per individual integrin molecule [Balaban et al., 2001; Geiger and Bershadsky, 2001]) can develop, triggering the growth of the adhesion site or its rupture in the case of development of an excessive force. Interaction with more pliable matrices might lead to a totally different response (Pelham and Wang, 1997). Interestingly, forces that can activate the transduction channels present on stereocilia are apparently orders of magnitude smaller than those "sensed" by individual molecules in FA. It was reported that forces as small as 0.3 pN applied to hair bundles in the bullfrog sacculus can induce a response. Assuming that the response is mediated by $\sim$50 channels operating in parallel, it appears that the average force applied to a single channel can be in the order of a few fN (P. Martin, personal communication; see Martin and Hudspeth, 2001).

Comparing "classical" mechanosensors of specialized organs to the mechanisms operating in FA is mostly speculative at this stage. Nevertheless, it seems that ionic-channel-based mechanosensory "devices" are optimal for activating a cell as a whole, or inducing a general cell polarization, but are less effective in transducing a highly localized response. In the case of FA, the response to mechanical load appears not to depend on ion fluxes, but involves a slower and more localized process of directional assembly of the submembrane plaque. This assembly may lead to the co-clustering of signaling molecules, which might trigger a cascade of downstream signaling events, such as protein phosphorylation and activation of small GTPases. Some of these may act locally on the FA itself, serving as feedback loops controlling FA assembly, while others might have a global effect on the cell, affecting its behavior and fate.

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