Mechanotransduction: All Signals Point to Cytoskeleton, Matrix, and Integrins

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Mechanotransduction is cellular signal transduction in response to mechanical stimuli. Mechanical stresses regulate a diverse array of physiological functions, ranging from highly specialized sound sensation by the cells of the inner ear, to the detection of fluid shear stress imparted by blood flow across the endothelium. Mechanical cues modulate almost all aspects of cell function, including growth, differentiation, migration, gene expression, protein synthesis, and apoptosis. Mechanical forces also directly affect the form and function of tissues; the effects of compression on bone and cartilage and of tension on muscle and skin are two familiar examples. Thus, uncovering the mechanisms by which living cells sense mechanical stress lies at the core of understanding how they respond and adapt to their physical environments.

Traditionally, investigators distinguished mechanotransduction from other types of signal transduction because they assumed that it occurs independently of ligand-mediated activation of cell surface receptors, and instead relies on converting mechanical stimuli to chemical sequelae through generalized membrane distortion. This assumption led to a search for membrane mechanotransducers, components of the cell surface that could mediate this type of mechanochemical conversion. Indeed, mechanochemical transducing molecules have been identified, such as the ubiquitous stress-sensitive ion channels that are found in cell surface membranes. These channels either increase or decrease ion flux when the membrane is mechanically stressed. However, these transducers normally do not act alone. For example, although members of the Msc family of bacterial stretch-sensitive channels can be activated by mechanical distortion when isolated in pure liposomes, they lack the regulation they normally exhibit in the bacterial membrane (*1*). In *Xenopus* eggs, stress-sensitive ion channels are highly regulated in intact cells, but become hyperreactive when the surface bilayer is physically torn away from the underlying cortical cytoskeleton (*2*). In fact, among the mechanosensitive signaling pathways of most cells, including specialized sensory cells, a common theme emerges: the function of their mechanotransducer molecules depends on linkage to the cytoskeleton.

That the cytoskeleton, the load-bearing architecture of the cell, plays a role in mechanotransduction is not surprising. In living tissues, mechanical stresses are normally distributed to cells through the extracellular matrix (ECM) scaffolds that hold the cells together and provide mechanical support to the tissue. Mechanical signals that propagate from the ECM converge on cell surface adhesion receptors known as integrins. These trans-

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membrane receptors link intracellularly to the actin cytoskeleton within specialized focal adhesion structures that are essentially dynamic "spot-welds" between the cell and the ECM (*3*). Thus, the role of these structural connections in mechanotransduction has become a focus of investigation, and it has yielded fruitful results.

Much of the research on mechanotransduction in eukaryotes has been conducted on specialized cells whose main function is to sense and respond to mechanical stresses. These specialized cells include hair cells of the inner ear and cutaneous touch-sensitive neurons. The identity of the molecules that mediate mechanotransduction in these cells remains elusive. Based on the speed of these cells' depolarizations, however, it is almost certain that they possess cation channels that are mechanically gated independently of second messengers (*4*). Yet none of these channels appear to function independently of connections to the cytoskeleton.

Recently, investigators identified brain sodium channel-1 (BNC-1) as critical for touch sensation in mice (*5*). BNC-1's structure and function correlate well with known *Caenorhabditis elegans* touch-sensitive channels composed of degenerins, because they all belong to the same superfamily of channels, known as the degenerins/epithelial sodium channels (DEG/ENaCs). Degenerins were originally identified in a simple genetic screen for touch-insensitive worms, known as *mec* mutants, and importantly, degenerins require connections to cytoskeleton and ECM proteins to be touch sensitive: MEC-7 and MEC-12 are tubulins; MEC-2 is homologous to stomatin, a membrane protein that associates with cytoskeleton; and MEC-9 is secreted by the sensory neuron and associates with extracellular MEC-5, which resembles collagen (*6*).

In hair cells, although no specific mechanosensitive channel has been identified, much is known about the channel's location and the cytoskeletal proteins involved in its gating and adaptation (*4*, *6*). In particular, the channels are located at the tips of a hair cell's stereocilia, whose core filaments are composed of cross-linked actin. Extracellularly, the channels are linked by an unidentified protein or protein complex known as the tip-link, which is normally under tension; it is "prestressed," much like a tuned violin string. When the stereocilia are deflected in response to sound vibrations, the tip-link (or an associated protein at the base of the tip-link) becomes stretched, and the resulting increase in tension opens the channel. Intracellularly, the channel most likely connects to the actin scaffold through a myosin-based motor, which tends to travel toward the tip of the stereocilium along an actin filament, maintaining basal levels of tension and accounting for a hair cell's adaptation to prolonged deflections. Thus, the prestress or tone in the cell may help to tune the mechanical signaling response.

Given the important role of cytoskeletal force transmission in the rapid detection mechanisms of cutaneous and inner ear

mechanosensory cells, it is not surprising that the function and development of these specialized cells relies on the presence of specific cytoskeletal products. Deafness can be caused by mutations in mDia1(*7*), a protein that binds to the small guanosine triphosphatase (GTPase) Rho, an established regulator of cytoskeletal dynamics and prestress (*3*), or by mutations in espins, actin-binding and cross-linking proteins of the stereocilia (*8*). Maturation of stereocilia, along with hair cell differentiation, is hindered by mutations in various specialized ECM proteins and by the absence of integrin α8β1, which in hair cells is found specifically in stereocilia (*9*).

Although linkage to the cytoskeleton predominates as a theme in mechanisms of specialized mechanosensory cells, responses to mechanical stimuli are also well documented among many different cell types and across many different organ systems. Mechanosensitive osteocytes, osteoblasts, and osteoclasts supervise bone remodeling in response to altered compressive loads. Smooth muscle alters its tone in response to increased pressure in blood vessels, intestines, and airways. Stress stimulates fibroblasts to deposit ECM proteins. Endothelial cells express atheroprotective gene products in response to laminar blood flow. These are all cell types whose primary function is not one of mechanosensation, yet they are mechanoresponsive nonetheless. Moreover, these mechanically triggered responses involve rapid activation of stretch-sensitive ion channels, as well as more complex and slower intracellular signaling cascades that result in transcriptional changes. It is therefore reasonable to expect that their mode of sensing mechanical stimuli may be less specialized than that of hair cells, yet is still coupled to the cytoskeleton. However, the role of cytoskeleton in "generic" mechanotransduction by nonspecialized parenchymal cells has been a matter of contention. Some investigators posit that cortical membrane deformation is the main mediator of cellular mechanotransduction (*10*), whereas others hold that the cell can be treated as a mechanical continuum, like a membrane filled with a viscous fluid cytosol (*11*), in which case cytoskeletal components should bear no more load than any soluble protein.

Our group recently demonstrated that the mechanical behavior of cells is not one of a mechanical continuum or of a tensed cortical membrane, but rather, one in which discrete elements of the cytoskeleton bear compression in cooperation with the ECM in order to prestress and thereby stabilize a network of tension elements (*12*). The intracellular compression elements are primarily microtubules, whereas the tension elements are primarily actin microfilaments and intermediate filaments. In response to a mechanical load, either focused at a point or over the whole cell, subcellular displacements were observed preferentially in cytoskeletal elements and physically associated structures (for example, organelles), and the pattern of deformation was consistent with mathematical predictions based on the tensegrity model of cell architecture in which tensile prestress plays a fundamental stabilizing role (*13*). These findings establish that loads applied to the cell surface are preferentially borne by the cytoskeleton, and most importantly, that the transmission of the load depends on integrin-mediated connectivity between the ECM, the cell surface, and the cytoskeletal lattice.

All adherent cells have integrins that link the internal actin cytoskeleton to the ECM through a group of actin-associated proteins (for example, talin, vinculin, paxillin, and α-actinin) within the focal adhesion. Focal adhesions also harbor many proteins involved in various biochemical signaling cascades [for

example, tyrosine kinases, inositol lipid kinases, ion channels, heterotrimeric guanine nucleotide-binding proteins (G proteins), growth factor receptors] (*14, 15*). Thus, it follows that mechanical forces, either applied at the integrin or transmitted outward from the cytoskeleton, converge with biochemical signals at the focal adhesion. For this reason, researchers have focused on integrins as candidates for transducing mechanical stimuli into biochemical responses inside the cell (*16, 17*). In fact, many studies clearly demonstrate a central role for cell surface integrins in cellular mechanotransduction. For example, the adenosine 3′,5′-monophosphate (cAMP) signaling pathway can be upregulated by applying mechanical stress to integrins using a magnetic twisting cytometer (*18*). In this method, micrometer-sized ferromagnetic beads coated with ligands or antibodies for specific cell surface receptors are allowed to bind to cells and are then twisted by a magnetic field to produce a local shear stress at the interface between the bead and the cell, but only through the ligated receptors. In this manner, different cell surface proteins can be targeted and assessed for their ability to transduce mechanical signals. Importantly, cAMP signaling was up-regulated when mechanical stress was applied to β1 integrins, whereas this response did not occur when the same stress was applied to transmembrane metabolic receptors that do not physically interconnect with the internal (noncortical) cytoskeleton. Although this signal transduction mechanism required the action of G proteins, transduction did not occur if mechanical stress was similarly applied to traditional G protein–coupled receptors. The integrins also had to be in an activated form with their binding sites for RGD (the arginine-glycine-aspartic acid motif found in many ECM proteins) occupied in order to transduce the mechanical signal into a chemical response. This finding is consistent with earlier studies demonstrating that when ligand-activated, integrins increase recruitment of many more signaling molecules to their focal adhesion sites (*14*). Thus, it is possible that G proteins that mediate production of cAMP (Gs_α) may be recruited to these sites as well.

Many other studies also demonstrate that integrins play a central role in cellular mechanotransduction in response to fluid shear flow. Among recent findings, expression of endothelin-1 (ET-1), a potent vasoconstrictor implicated in many cardiovascular diseases, was stimulated in endothelial cells when integrins were manipulated with a variation of magnetic twisting cytometry (*19*). Again, twisting nonadhesion receptors, such as metabolic acetylated low-density lipoprotein receptors or histocompatibility antigens, yielded no change in ET-1 mRNA levels. Furthermore, reducing cytoskeletal tension (prestress) or disrupting actin stress fibers blocked twist-induced ET-1 expression. Blocking stretch-activated channels with gadolinium also prevented this response, thus linking integrins and cytoskeleton once again to these direct mechanochemical transducer molecules.

In another study, fluid shear flow-induced vasodilation of coronary arteries was effectively blocked by either soluble RGD peptides or a blocking antibody against β3 integrin exposed on arterial endothelial cells, both of which compete with ECM proteins for integrin binding (*20*). In osteoblasts, actin disruption, mutant focal adhesion proteins, and mutant Rho all inhibited shear flow-induced expresssion of the enzyme Cox-2 and the transcription factor c-fos (*21*). Fluid shear-induced association of Shc [an adapter protein upstream of the extracellular signal-regulated kinase (ERK) signaling pathway] to integrins depend-

ed on the type of ECM ligand: sheared cells plated on fibronectin accumulated αvβ3-Shc complexes, whereas similarly treated cells on vitronectin accumulated α6β1-Shc complexes (22). The accumulation of these putative signaling complexes under shear flow required dynamic interactions between integrins and the activating ligands; blocking new binding of additional integrins with antibodies prevented the integrin-Shc association. Finally, other investigators have demonstrated that apically applied forces require mDia1-Rho-mediated transmission through the cytoskeleton to stimulate basal focal adhesion growth and activity (23). All of these findings raise the possibility that the mechanical load in fluid shear flow is transmitted from the luminal surface of the cell, through the cytoskeleton, to the basal focal adhesions, and that this is where much of the mechanochemical conversion occurs, rather than on the apical cell surface.

Despite the strong evidence for the role of integrins and the cytoskeleton in mechanotransduction, some researchers argue that certain mechanosensitive events may occur independently of focal adhesion-dependent linkages to the cytoskeleton. For example, fluid shear flow activates G proteins that have been reconstituted in liposomes, suggesting that lipid bilayer distortion, or membrane fluidity, is alone sufficient for activation in the cell (10), and that such a mechanism could account for transient shear-induced ERK activation (24). Membrane transport vesicles called caveolae, which are characterized morphologically by a smooth invagination of the plasma membrane and biochemically by the presence of caveolin, may also be important for mechanotransduction, in that flow induces tyrosine-phosphorylation and recruitment of cytosolic signaling molecules to caveolae (25). A separate study blocked the fluid shear flow-activated ERK pathway by introducing anti-caveolin-1 antibody into the cytoplasm (26). Given the role of caveolae in the compartmentalization and concentration of cell-surface G_q, researchers have also proposed that the G_q-caveolin complex functions as a mechanotransducer (27).

But links are even emerging between integrins and these putative membrane mechanotransducers. For instance, caveolin-1 association with integrin subunits is required for Shc recruitment to integrins, subsequent ERK pathway activation, and adhesion-dependent cell proliferation (28). These findings agree well with the aforementioned independent findings that fluid shear flow induces ERK activation both through caveolin-1 and through activated integrins. As for biological responses to shear flow mediated by G pro-

teins, increasing evidence suggests their connection to integrins through an intermediate linker. Because caveolin-1 associates with both G proteins and integrins, it is a good candidate. Other studies, however, have uncovered a supramolecular complex containing G protein subunits, an integrin-associated protein (CD47), and integrins. This linkage possibility is particularly intriguing in that CD47 (with five transmembrane segments) appears to play the role of an adapter by associating with the two integrin subunits to form a seven-transmembrane spanning complex that resembles a traditional G protein-coupled receptor (29).

The future of the field requires a more detailed view of the connections between activated integrins, other structural components of focal adhesions, and the signal transduction molecules that are involved in mechanosensitive pathways (Fig.
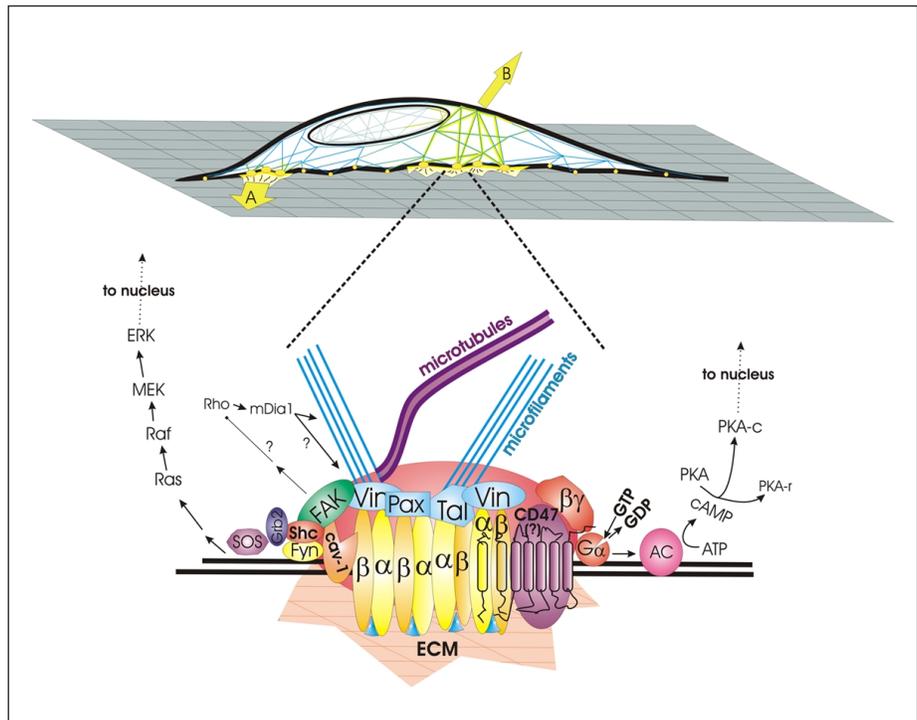
Fig. 1. Schematic diagram of how forces applied through the ECM (**A**) or directly to the cell surface (**B**) travel to integrin-anchored focal adhesions through matrix attachments or cytoskeletal filaments, respectively. Internally-generated tension and forces transmitted through cell-cell contact similarly reach focal adhesions through the cytoskeleton. Forces concentrated within the focal adhesion (magnified at bottom of figure) can stimulate integrin clustering and induce recruitment of additional cytoskeletal linker proteins (Vin, vinculin; Pax, paxillin; Tal, talin) that connect directly to microfilaments and indirectly to microtubules. Forces applied to this specialized cytoskeletal adhesion complex also activate integrin-associated signal cascades. Focal adhesion kinase (FAK) may be involved in Shc recruitment as well as modulation of Rho activity which, in turn, can regulate the force response through mDia1. Caveolin-1 (cav-1) can also recruit Shc to integrins to activate the ERK cascade. CD47 associates with the integrin heterodimer to form a protein complex with seven transmembrane segments that mimics the action of G protein-coupled receptors. In the case shown, when integrins are mechanically stressed, the complex stimulates G_s-mediated up-regulation of the cAMP cascade through adenylate cyclase (AC), resulting in nuclear translocation of the catalytic subunit of protein kinase A, PKA-c. See text for details. MEK, mitogen-activated protein kinase or extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate; ATP, adenosine triphosphate.

1). It is also critical to understand that, even in specialized cells like hair cells, mechanotransduction likely does not occur independently of other signaling pathways. Signals emanating from mechanical stress and classical receptor-ligand interactions may converge into common pathways that enter the nucleus, so that ultimately, mechanical stress modulates cellular response to receptor-ligand interactions, and vice versa. Furthermore, similar to how the prevailing tension in a tip-link modulates a stereocilium's response to further deflection, cellular prestress may govern how nonspecialized cells respond to both mechanical and chemical signals. As an example, growth factors, ECM, and mechanical distortion (cell shape changes) all promote growth, yet cell cycle progression can be blocked under conditions of optimal stimulation by dissipating cytoskeletal prestress using inhibitors of actomyosin-based tension generation (*30*). Finally, at some point the distinction between mechanical responses and traditional ligand responses should disappear, because every ligand-receptor interaction essentially yields a change in physical conformation of the receptor, causing a mechanical "stress" on a much smaller scale than the twist of a bead.

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