Mechanisms of Mechanotransduction

Review

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Essentially all organisms from bacteria to humans are mechanosensitive. Physical forces regulate a large array of physiological processes, and dysregulation of mechanical responses contributes to major human diseases. A survey of both specialized and widely expressed mechanosensitive systems suggests that physical forces provide a general means of altering protein conformation to generate signals. Specialized systems differ mainly in having acquired efficient mechanisms for transferring forces to the mechanotransducers.

The conversion of physical force into biochemical information is fundamental to development and physiology. It provides a simple means by which cells and organisms can ensure structural stability, as well as a way to regulate morphogenetic movements to generate precise three-dimensional structures. In the vascular system, pressure and shear stress from pumping blood influence the morphology and pathology of the heart and vasculature. Bone is shaped by forces from gravity and muscle contraction. Hearing and touch are based on neural responses to pressure. Inflation and deflation of the lungs regulate their physiology. Coordinated growth of tissues is guided by mechanical forces, and failure of these mechanisms contributes to cancer. Mechanosensitivity in one form or another appears to be a property shared by all cells of the body and by all phyla from mammals to bacteria.

Unifying Principles
The remarkable breadth of mechanosensitive events makes it unlikely that one or even a few mechanotransducers can account for all of these events. This conclusion is not surprising when one considers that any cellular component that transmits or resists physical forces will be acted upon by those forces. Resultant changes in conformation can modify function to transduce information. In some cases, transduction of physical forces appears to occur through changes in protein conformation. Protein folding in general favors the conformation that yields the lowest free energy; physical forces that modify the energy landscape will therefore directly alter protein folding. Just as protein phosphorylation or other posttranslational modifications mediate signal transduction in large part through changes in protein

conformation, force-induced effects on conformation represent a general mechanism by which enzymatic activity or protein interactions can be modified to mediate signaling.

Stretch-sensitive channels provide the best-studied example of this behavior (Martinac, 2004). Increasing tension within the lipid bilayer from 10–12 to 20 dyn/cm (Evans et al., 1976) increases the channel-opening probability (Martinac and Hamill, 2002). If the open state occupies a greater area within the bilayer, membrane tension will result directly in lower free energy (Figure 1A). Protein unfolding under tension represents a one-dimensional instance of the same principle (Oberhauser et al., 1998), where unfolding lowers the free energy (Figure 2).

Specialized transduction systems in touch, hearing, and flow seem to be finely tuned to transduce small forces that would minimally affect the more widely expressed systems. The force of flowing blood, for example, represents less than 1% of typical traction forces exerted by the cytoskeleton (see Table 1). Urine flow is much weaker still, yet plays an important role in regulating kidney morphogenesis (Serluca et al., 2002; Du et al., 2004). We hypothesize that in specialized transduction systems, efficient transmission of forces to the mechanosensitive elements enhances sensitivity. These mechanisms usually involve linkages to the cytoskeleton and/or extracellular matrix (ECM) that amplify small forces by transmitting displacements of large structures to the transducers (Alenghat et al., 2004; Han et al., 2004b; You et al., 2001). We therefore suggest that specialized tissues employ the same general type of transducers as other cells but increase sensitivity by conveying displacements of larger structures to the primary transducers.

Early Development
Tissue movements such as gastrulation and neurulation involve dramatic rearrangements of cell adhesions, cell movement, and changes in cell shape (Alberts et al., 2002). Because, according to Newton's laws, physical force drives all movements, feedback mechanisms in which forces guide cell behavior would be an attractive mechanism to enhance the robustness of these processes. Mechanotransduction in development has been proposed based on effects of stretching embryos in vitro and altering mechanical forces in vivo (Beloussov, 1980; Beloussov et al., 1988). During vertebrate cardiovascular development, remodeling of the primitive vascular plexus and correct formation of the heart is blocked by a number of interventions or mutations that interfere with blood flow, including mutations of genes expressed outside the target tissue (Hove et al., 2003; discussed in Jones et al., 2004).

Mechanical tension has been proposed to regulate serum response factor-dependent transcription in migrating *Drosophila* border cells (Somogyi and Rorth, 2004). In this system, MAL-D, a transcriptional cofactor for SRF, translocates from the cytoplasm to the nucleus in cells that appear to be stretched. Mutation of *slbo*, a gene required for migration and elongation, blocked

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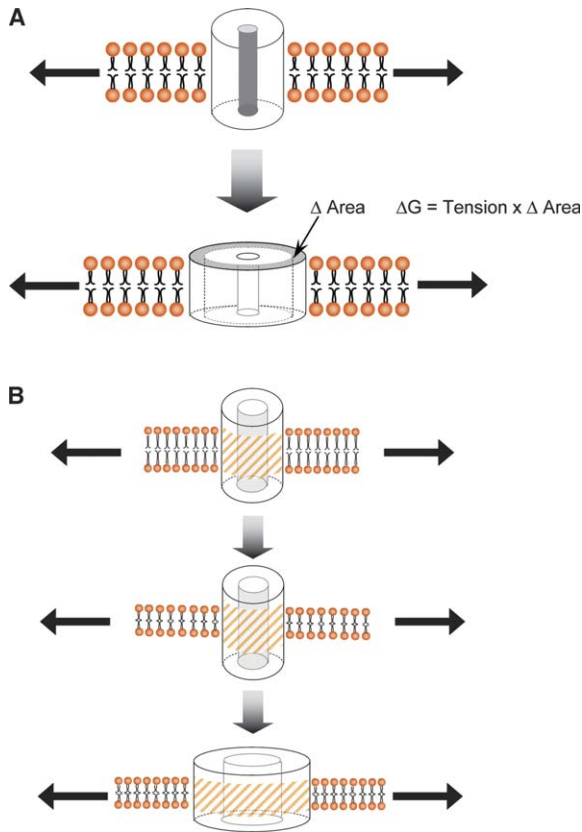Faculty/Schwartz.cfm

Figure 1. Stretch-Activated Channels

Two likely mechanisms by which membrane tension can trigger channel opening include the following.
(A) For any channel where the open state occupies greater area in the membrane than the closed state, the free energy of the open state will be lower according to $\Delta G = \Delta area \times tension$.
(B) Tension causes thinning of the lipid bilayer; if the hydrophobic transmembrane domain is thinner in the open state, channel opening will be favored to avoid the energetic cost of hydrophobic mismatch.

nuclear translocation. In animals containing mixtures of wild-type and *slbo* mutant cells, migrating normal cells adjacent to the mutant cells appeared to pull on the mutants, which rescued MAL-D nuclear translocation in the mutants. These data fit well with results with mammalian MAL, where nuclear translocation and transcriptional activity are regulated by actin polymerization (Miralles et al., 2003). There is also good evidence for mechanical induction of the transcription factor Twist in *Drosophila* (Farge, 2003). In this system, expression of Twist in the anterior foregut and stomodeal primordial appears to be triggered by force applied to these tissues by germ band extension. Additionally, experimentally applied force triggers ectopic Twist expression. Taken together, these data suggest that mechanical forces act through the actin cytoskeleton and associated intercellular junctions to regulate key transcriptional events during development.

Mechanotransduction may regulate the formation of left-right asymmetry in mammals through fluid flow within the ventral node. Specialized motile cilia in the center of the node generate leftward flow of extraembryonic fluid, and left-right asymmetry is first detectable by the
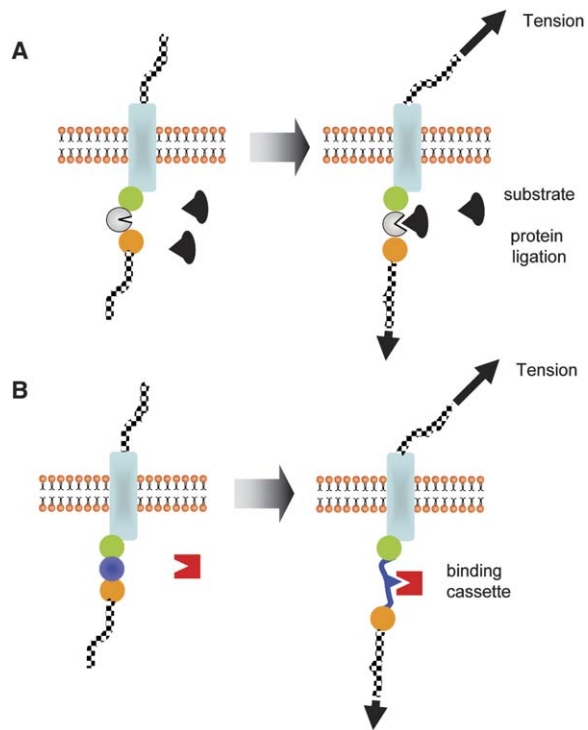

Figure 2. Tension-Induced Protein Conformation Changes
Force could trigger exposure of enzymatic activities or binding sites through changes in protein conformation (A) or folding (B).

polarized expression of *nodal*, *lefty*, and *pitx2* on the left side of the embryo. Mutations in both kinesin and dynein motor proteins disrupt both nodal flow and left-right asymmetry (Nonaka et al., 1998; Supp et al., 1999). In vitro application of flow to nodes of isolated embryos can rescue asymmetry in dynein mutants, whereas applying flow in the opposite direction reverses left-right asymmetry in wild-type animals (Nonaka et al., 2002). A mechanosensing model for transducing nodal flow was recently proposed (McGrath et al., 2003), in which flow induced by motile cilia is sensed by nonmotile cilia in the periphery of the node that contain the putative mechanosensory ion channel polycystin-2. In wild-type animals, intracellular calcium is higher on the left side of the node and both dynein and polycystin-2 mutant embryos lose this asymmetry (McGrath et al., 2003). One problem with this model is that nodes are closed structures and flow rates are proposed to be similar across the node (Cartwright et al., 2004). The mechanosensory model therefore implies that nonmotile cilia can transduce flow directionality, though there is little experimental evidence to support this idea. A second hypothesis is that nodal flow establishes a morphogen concentration gradient. Mathematical modeling suggests that such gradients can exist, and experiments showed that nodal flow induced gradients of externally applied proteins (Cartwright et al., 2004; Okada et al., 2005). Recent work has provided evidence that flow causes movement of vesicles carrying sonic hedgehog and retinoic acid, which fragment at the left side of the node to create gradients of these morphogens (Tanaka et al., 2005). These models are neither definitively established nor mutually exclusive; more work is needed

Table 1. Estimated In Vivo Magnitudes of Mechanical Stimuli on Cells

Physical Profile	Mechanical Stimulus	Typical Values	References
Arterial blood flow	Fluid shear stress	1–3 N/m^2	Davies, 1995
Cell migration	Traction stress	3.0–5.5 kN/m^2 (normal); 1 kN/m^2 (cancer)	Balaban et al., 2001; Munevar et al., 2001
Proximal tubule flow	Fluid shear stress Fluid drag force Bending torque	0.3 N/m^2 0.0074 pN/microvillus 0.016 pN-μm/microvillus	Guo et al., 2000
Stretch-activated channels	Membrane tension	0.012 N/m	Evans et al., 1976; Martinac and Hamill, 2002; Sukharev et al., 1999
Outer hair cell stereocilia	Compression stiffness Force/Δ membrane potential	0.001 N/m 0.1–20 pN/mV	Hallworth, 1995; Spector et al., 1999
Osteocyte processes (bone canaliculi)	Fluid shear stress Fluid drag force Tissue strain	0.8–3.0 N/m^2 20 × shear force 0.03%–0.1%	Weinbaum et al., 1994; You et al., 2001

to determine their relative contribution to left-right asymmetry.

Polycystins (PKD) 1 or 2 have been proposed to participate in mechanotransduction in cilia elsewhere in development and physiology. Polycystins localize to the primary cilium of renal tubule epithelial cells (Delmas, 2004), where PKD1 forms a complex with PKD2. PKD1 is thought to be a regulatory and/or anchoring subunit for mechanically gated calcium channel activity of PKD2, which is homologous to transient receptor potential (TRP) channels. Although the predicted flow rate in proximal tubules imparts a fluid shear stress on the epithelium of only ~3 dyn/cm^2, fluid drag forces that bend the microvilli in the direction of flow are 360–580 times larger (Table 1) (Guo et al., 2000). Fluid drag force near the microvillus tips induces a bending moment, or torque, of 0.016 pN-μm on the microvillus, and the water flux across the tubule epithelium is proportional to the torque magnitude (Du et al., 2004). Bending of the microvilli by proximal tubule flow triggers calcium transients in these cells, which are absent in mutant cells (Nauli et al., 2003). It has been proposed that urine flow triggers signals that modulate kidney tubule growth such that loss of these pathways leads to cystic growth (Delmas, 2004). Interestingly, PKD proteins are also found at cell-cell junctions and in other cell types, and PKD mutations lead to cardiovascular and skeletal defects as well as kidney and left-right asymmetry abnormalities. These molecules might therefore transduce forces in other systems.

Hearing and Touch

Hearing, balance, and touch result from cellular transduction of mechanical stimuli into electrochemical signals that are then transmitted to the brain. A variety of genetic and functional studies strongly implicate ion channels. Mechanically sensitive channels belonging to the degenerin/epithelial sodium channel (DEG/ENaC) family appear to underlie mechanotransduction in both auditory and tactile mechanotransduction (Eberl et al., 2000; Hamill and Martinac, 2001; O'Hagan et al., 2005). Interestingly, these mechanically gated channels show structural homology to bacterial stretch-activated channels involved in osmoregulation. The bacterial channels such as MscL are tuned such that probability of opening is 50% at a resting membrane tension of 12 dyn/cm, and open probability is sigmoidally related to membrane tension around this value (Sukharev et al., 1999). A change in channel area due to pore opening of 6.5 nm^2 leads to a decrease in free energy (the product of tension × area change) of 19 k_BT ≈ 80 pN-nm per channel. Interestingly, these channels do not require links to submembrane cytoskeleton for their mechanosensitivity, but it is unclear whether the mammalian sensory channels also respond to membrane tension or to tension applied through cytoskeletal links or extracellular tethers.

TRP channels form a second, widely expressed group of eukaryotic, mechanically gated channels (Hamill and Martinac, 2001; Maroto et al., 2005). This family includes TRPC1, which has been shown to respond to membrane tension. TRP family members have been implicated in mechanotransduction in sensory systems and in osmoregulation, cell migration, and muscle function. In addition to the free energy model described above (Unifying Principles) (Figure 1A), alternative models have proposed that as membrane tension increases from 12 to 20 dyn/cm, lipid bilayer thinning by 0.15 nm (5%) triggers channel opening (Figure 1B) (Martinac and Hamill, 2002). The open state decreases the thickness of the channel's hydrophobic transmembrane region to minimize hydrophobic mismatch. The open state thus lowers free energy through the hydrophobic effect.

In hair cells, sound waves or head movements oscillate stereocilia bundles containing actin filaments held under prestress by myosin-1c. Their stiffness under passive compression is of order 0.1 dyn/cm, but the actin-myosin interactions also contribute to tension through active mechanisms. Electrophysiological recordings indicate that ion channels localized to the tips of the stereocilia regulate influx of Ca^{2+}, K$^+$, and/or Na$^+$. Membrane depolarization induces force generation in the range of 0.1–20 pN/mV (Hallworth, 1995), resulting in contractile shortening estimated at 6.8 pN/mV/μm (Spector et al., 1999). The tips of the stereocilia are

connected by a "tip link" filament that contains *cadherin 23* (*CDH23*), a gene implicated in deafness and blindness in human Usher's syndrome (Siemens et al., 2004; Sollner et al., 2004). Absence of the tip link correlates with disorganized bundling of the stereocilia and deafness. Although it is not proven that the tip link serves to transmit force to regulate ion channel opening, such a force transmission mechanism has recently been proposed to explain touch in a *Caenorhabditis elegans* model (O'Hagan et al., 2005).

Various TRP family proteins are implicated in hearing and touch sensitivity in flies, zebrafish, and mice (Sidi et al., 2003; Corey et al., 2004). Interestingly, the cytoplasmic domain of the mammalian hair cell homolog, TRPA1, contains 17 ankyrin repeats, suggesting that they could interact with the submembrane actin cytoskeleton. Cytoskeletal linkage might serve to transmit actomyosin-dependent tension or simply to anchor the channel to permit mechanosensation in response to stereocilia displacement. It is not clear, however, whether physical tethers to TRP channels or responses to membrane tension represent the primary mechanism.

In *C. elegans*, null mutations in the genes *mec-2*, *mec-4*, and *mec-6* eliminate rapidly activating mechanoreceptor currents recorded in touch receptor neurons (O'Hagan et al., 2005). MEC-4 is homologous to amiloride-sensitive sodium channels of the DEG/ENaC family, and MEC-2 and MEC-6 associate with MEC-4 channels to enhance channel activity. Interestingly, mechanoreceptor currents were not completely eliminated in MEC-7/β-tubulin null mutants, indicating that microtubules are not required for mechanosensation. Because the MEC-4 complex must be intact and functional in these mutants, another mechanism of force transmission to the channel complex must exist. One possible mechanism is through physical links to the extracellular matrix (analogous to tip links in stereocilia or osteocyte processes in bone canaliculi), but this hypothesis remains to be tested.

Cell Cycle Control and Cancer
In nonhematopoietic mammalian cell types, cell cycle progression requires adhesion and spreading on a solid substratum. Loss of this requirement for anchorage correlates closely with tumorigenicity in vivo and is related to the ability of cancer cells to invade and metastasize into other tissues (Huang and Ingber, 1999). Anchorage dependence clearly involves a mechanical component, because cells spreading on a solid substratum exert tension on the surface and inhibitors of myosin phosphorylation inhibit cell cycle progression (Roovers and Assoian, 2003). Compliant substrata lead to lower tension between the cell and the ECM and also inhibit growth of anchorage-dependent cells, whereas tumor cells grow well on compliant surfaces (Wang et al., 2000). Additionally, externally applied strain that increases tension across cytoskeleton-ECM linkages stimulates cell cycle progression (Huang and Ingber, 1999). Studies on intact tissues also demonstrate increased cell cycle progression with increased tension.

Though cancer cells show lower requirements for anchorage and tension, tumor tissue usually has higher stiffness than normal tissues. Indeed, this property is the basis for both manual screens and ultrasound imaging, where tissue stiffness determines energy transmission (Paszek et al., 2005). Interestingly, higher stiffness may contribute to the disruption of normal tissue architecture in breast cancer. ECM whose compliance is similar to that of normal tissue induces mammary cells to form normal looking hollow cysts (Paszek et al., 2005). In contrast, stiff ECM similar to tumor tissue induces filling of the cystic lumens and disruption of normal epithelial polarity, similar to mammary carcinoma. Thus, changes in the mechanical properties of the tissue can contribute to altered tissue architecture in tumors.

It has been proposed that tension is transmitted from the extracellular matrix through the cytoskeleton to the nucleus, and deformation of the nucleus mediates changes in gene expression that regulate cell cycle progression (Huang and Ingber, 1999). Mutations in the *lamin C* gene lead to alterations in both the mechanical properties of the nuclear lamina and stretch-induced gene expression, supporting this idea (Lammerding et al., 2004b). Integrin-mediated adhesions, however, appear to be the primary site of tension sensing. Focal adhesion formation requires isometric tension between the cell and its substratum (Burridge and Chrzanowska-Wodnicka, 1996). Traction stresses of 3–5.5 $nN/\mu m^2$ have been measured (Balaban et al., 2001; Munevar et al., 2001). In contrast, mean traction stress against the substrate in transformed cells was to $\sim$1 $nN/\mu m^2$. Inhibitors of tension disrupt the clustering of integrins in focal adhesions and reduce phosphorylation of focal adhesion components including focal adhesion kinase. Through this effect, inhibitors of actin polymerization or myosin activity decrease the ability of growth factors to stimulate sustained elevation of Erk MAP kinase activity, which is required for cyclin D1 expression and cell cycle progression (Roovers and Assoian, 2003). Importantly, artificially clustering integrins restores sustained Erk activity and cyclin D1 in cells where myosin activity is inhibited. These results indicate that the clustering of integrins within focal adhesions is the major tension sensor that mediates cell cycle progression in anchorage-dependent cells.

Vascular Smooth Muscle and Cardiac Muscle in Hypertension
Blood pressure is carefully regulated through multiple mechanisms to ensure precise control of blood flow under conditions from strenuous exercise to complete rest (Beevers et al., 2001). The myogenic response, a mechanism intrinsic to arterial smooth muscle, regulates local flow on a time scale of seconds, shielding capillary beds from acute changes in pressure. Baroreceptor neurons innervating the aortic arch and carotid sinus affect blood pressure on a longer time scale by controlling vasoconstrictor release and kidney function, which regulate arterial smooth muscle contraction and blood volume, respectively. Over longer times, elevated blood pressure leads to artery remodeling, cardiac hypertrophy, and, if unchecked, eventually to cardiac failure.

The myogenic response to acute pressure changes is mediated partly through calcium signaling (Davis et al., 2001). Stretching vascular smooth muscle cells opens nonspecific cation channels, resulting in membrane depolarization, and this response is blocked by the stretch-activated channel inhibitor gadolinium. Depolarization

then induces calcium entry through L-type calcium channels, which regulate basal tone and myogenic constriction. ENaC and TRP ion channels have both been implicated in the myogenic response (Drummond et al., 2004; Earley et al., 2004; Welsh et al., 2002), as has increased sensitivity to calcium (Davis et al., 2001).

When arteries are stretched by elevated blood pressure, transmission of the mechanical strain to cells occurs to a large extent through integrins. These receptors are thought to participate in both rapid myogenic responses and in the slower remodeling of arteries in response to sustained hypertension. Many studies have characterized effects of strain on integrin pathways, though molecular mechanisms have proved elusive (Katsumi et al., 2004). Stretch induces structural reinforcement of integrin adhesions and stimulates integrin-associated signaling, such as activation of FAK and MAP kinases. Furthermore, some stretch-induced signals such as JNK activation are dependent on specific ECM proteins, consistent with known integrin signaling specificities (Katsumi et al., 2004). One means by which stretch appears to induce integrin signaling is through modulating integrin affinity. Stretch in vitro enhances $\alpha v\beta 3$ integrin affinity, which leads to increased ECM binding; preventing new integrin ligation abrogated stretch-induced JNK phosphorylation (Katsumi et al., 2005). Relevant to the myogenic response, RGD peptides induce a transient vasoconstriction followed by vasodilation, although it is unclear whether this is due to enhanced integrin signaling or diminished integrin adhesion to endogenous matrix (Davis et al., 2001). Antibodies and peptides that bind integrins have complex effects on vasoregulation, with individual integrins differentially modulating L-type channels. However, the extent to which these effects are mechanical is unclear.

Sensing of force by cardiac muscle involves activation of the angiotensin II receptor in both cardiac hypertrophy and responses to strain. Both increased release of angiotensin (Sadoshima et al., 1993) and angiotensin-independent activation of the receptor (Zou et al., 2004) have been reported, but the molecular mechanisms are poorly understood. Stretch responses in cardiac myocytes, like vascular smooth muscle cells, appear to involve stretch-activated ion channels and integrin/ECM-dependent signals, including activation of Src, FAK, JNK, and ERK (Lammerding et al., 2004a). Melusin, a muscle-specific focal adhesion protein, is involved in cardiac mechanical responses. Melusin knockout mice show normal cardiac tissue architecture and function but pressure overload-induced Akt and GSK3b activation and cardiac hypertrophy are impaired (Brancaccio et al., 2003). Proteins of the cardiac myocyte Z disc, a structure that shares many features with focal adhesions, have been proposed to participate in mechanotransduction based on mutations that increase susceptibility to dilated cardiac myopathy (Knoll et al., 2002). However, rather than act upon signal transduction per se, these defects may affect cellular mechanics, which can affect mechanotransduction indirectly.

Fluid Shear Stress and Atherosclerosis
Fluid shear stress, the frictional drag on the endothelium from blood flow, is a major determinant of vascular physiology and pathology (Davies, 1995). On short time scales, increased flow triggers arteriolar smooth muscle relaxation through endothelial release of nitric oxide (NO). On longer time scales, high flow causes outward remodeling to enlarge the vessels. Arterial flow patterns induce expression of artery-specific genes, which are thought to contribute to the arterialization of vein grafts after bypass surgery (Dai et al., 2004; Garcia-Cardena et al., 2001). Atherosclerosis occurs selectively at vessel branch points and regions of high curvature that introduce disturbances into the normally unidirectional, laminar flow. In vitro studies have shown that long exposure to laminar flow is atheroprotective, inducing expression of genes that inhibit oxidative stress and inflammatory pathways (Gimbrone et al., 2000). Disturbed flows, modeled in vitro by turbulence, oscillatory flow, or step flows with regions of high shear gradients and flow reversal, induce oxidative stress and expression of proinflammatory genes. These opposite effects of laminar versus disturbed flow are believed to play a major role in the site selectivity of atherosclerosis.

A large number of responses of endothelial cells to fluid shear stress have been described (Davies, 1995; Takahashi et al., 1997). Within seconds, acute onset of laminar flow stimulates activation of an inward rectifying potassium channel, release of prostacyclin, and phosphorylation of the PECAM-1 intracellular domain. Intracellular signaling components activated within minutes to tens of minutes include Ras, Erk, JNK, Rho family GTPases, and tyrosine kinases (Davies, 1995; Takahashi et al., 1997). Over minutes to several hours, changes in cytoskeletal organization lead to cell elongation, alignment of the actin cytoskeleton, and orientation of the microtubule organizing center in the direction of flow. Although many of these are restricted to endothelial cells, nonendothelial cells show some responses to fluid flow, including orientation and directional migration (Lee et al., 2005), induction of monocyte chemotactic factor 1 (Shyy et al., 1994), activation of glucocorticoid receptors (Ji et al., 2003), and elevation of intracellular calcium through ATP secretion and increased mass transport (Grierson and Meldolesi, 1995). Thus, conserved and specialized mechanisms coexist.

Some of the pathways activated transiently by onset of laminar flow are activated in a sustained manner by disturbed flow. These include NF-κB, which promotes inflammation in atherosclerosis (Mohan et al., 1997), and tissue factor, a potent thrombotic stimulus (Houston et al., 1999; Mazzolai et al., 2002). These observations lead to the hypothesis that disturbed flow promotes continual signaling through the pathways induced transiently by onset of laminar shear. In laminar flow, cells eventually adapt and downregulate these pathways, whereas in disturbed flow, continual changes in flow magnitude and direction lead to sustained signaling.

Shear stress is by definition applied to the apical cell surface, which is covered by a glycocalyx estimated to be 300–400 nm thick in vivo (Smith et al., 2003; Vink and Duling, 2000). This layer forms a barrier to flow such that shear stress at the apical plasma membrane is essentially zero. Thus, force transmitted through the glycosaminoglycan chains to their transmembrane anchors has been proposed to mediate mechanotransduction (Weinbaum et al., 2003). Enzymatic digestion of carbohydrate chains inhibits NO production in

response to flow (Florian et al., 2003; Mochizuki et al., 2003); however, these enzymes could also affect signaling at the basal cell surface. Thus, a clear role for the glycocalyx remains to be established.

Heterotrimeric G proteins at the apical surface have also been proposed to directly transduce shear stress. G protein activation in response to flow onset occurs within 1 s, consistent with direct mechanotransduction (Frangos et al., 1999). Purified brain heterotrimeric G proteins reconstituted into phospholipid vesicles, ostensibly without membrane receptors, were activated by shear. These results might be linked to membrane fluidity. Onset of flow triggered an increase in lateral diffusion in the upstream portion of the apical plasma membrane, again within seconds (Butler et al., 2001). Whole-cell measurements also showed increased fluidity (Haidekker et al., 2000). G protein activation was stimulated by artificially increasing membrane fluidity and inhibited by decreasing fluidity (Frangos et al., 1999). The plasma membrane phospholipid bilayer itself might therefore transduce force. Along a similar line, caveolae have been hypothesized to mediate flow-dependent responses, possibly by acting as sensors of tension within the membrane (Park et al., 2000; Rizzo et al., 1998). One caveat is that caveolin is required to suppress eNOS activity and production of NO (Bernatchez et al., 2005). Blockade of caveolin could therefore constitutively activate eNOS and possibly other pathways as well, interfering with flow signaling in a dominant manner. In summary, plasma membrane tension is an intriguing candidate mechanosensor but evidence to date is not conclusive.

Forces from the apical surface must be transmitted through the cytoskeleton to points of attachment that resist shear stress and anchor the cell in place (Davies, 1995). Both cell-ECM and cell-cell adhesions have been implicated in shear stress sensing. A direct role for integrins in transduction is not excluded, but integrins are more strongly implicated as intermediates that are activated downstream of PECAM-1. In this pathway, force from flow is transmitted through the cytoskeleton to PECAM-1, which cooperates with VE-cadherin and VEGFR2 to stimulate PI 3-kinase (Jin et al., 2003; Tzima et al., 2005). Importantly, direct application of force to PECAM-1 stimulates relevant signaling events (Osawa et al., 2002; Tzima et al., 2005). PI 3 lipids trigger an increase in integrin affinity, leading to increased integrin binding to the subendothelial ECM. Newly occupied integrins then stimulate changes in activity of multiple signaling pathways, which lead to cell and cytoskeletal alignment in the direction of flow and changes in gene expression (Jalali et al., 2001; Tzima et al., 2001, 2002, 2003). VE-cadherin is required for these effects but does not appear to be a direct mechanotransducer (Shay-Salit et al., 2002; Tzima et al., 2005). Interestingly, imaging studies show that shear-induced strain within the cytoskeleton is not uniform; small regions of high strain are evident, commonly at cell-cell junctions (Helmke et al., 2003). The endothelial cytoskeleton may therefore focus forces from flow on junctional mechanoreceptors.

Bone Mechanotransduction and Osteoporosis
Mechanical loads generated by gravity and locomotion stimulate local bone remodeling to maintain optimal me-chanical performance. Reduced mechanical stimulation associated with sedentary lifestyle, limb paralysis, or space flight results in net bone loss through reduced bone formation and increased bone resorption. Osteoporosis is affected by diet and endocrine status but mechanical stimulation plays a major role (Burger and Klein-Nulend, 1999; Raisz, 1999).

Bone cells are exposed to a uniquely dense matrix composed primarily of mineralized type I collagen. As osteoblasts deposit this matrix, they become trapped and differentiate into postmitotic osteocytes, widely believed to be the major mechanotransducing cell in bone. Osteocytes exist in pockets of unmineralized matrix termed lacunae and connect with adjacent osteocytes and bone-lining cells through slender protrusions termed canaliculi. The osteocyte protrusions interact through gap junctions forming an interconnected network of cells throughout the bone. Mechanically stimulated osteocytes express multiple paracrine factors that stimulate osteoblast function and bone formation, including NO, prostaglandin E2 (PGE2), and insulin-like growth factor. Osteoclast activity is decreased in mechanically stimulated bone through both positive and negative factors released by osteocytes, though little is known about these factors (Raisz, 1999).

Unlike most mechanical systems, the forces acting on bone cells are controversial. The dense bone matrix resists compressive forces, hindering the transfer of forces to the cells. Physiological strains are ∼0.04%–0.3% (Fritton et al., 2000). Cells in vitro require strains more than an order of magnitude higher (1%–10%) to induce responses (Murray and Rushton, 1990; Neidlinger-Wilke et al., 1995). The unmineralized matrix around the osteocytes is more permeable than mineralized bone, forming canals through which interstitial fluid can pass for nutrient and waste exchange. Compressive forces generate pressure gradients that stimulate flow of interstitial fluid through these lacuno-canalicular porosities (Burger and Klein-Nulend, 1999). Surprisingly, this interstitial fluid flow has been predicted to generate shear stresses comparable to vascular wall shear stress (8–30 dyn/cm^2) (Weinbaum et al., 1994). Flow is the most widely accepted model of osteocyte mechanotransduction. Numerous studies have shown that osteocytes and osteoblasts sense shear stress in vitro, inducing several osteocyte responses associated with bone load in vivo, such as decreased apoptosis and production of NO and PGE2 (Burger and Klein-Nulend, 1999). The geometry of the lacuno-canalicular porosity suggests that flow will be preferentially sensed by the osteocyte processes, not the cell body (Anderson et al., 2005). However, due in part to the difficulty of culturing postmitotic osteocytes, most studies have used osteoblasts, which lack these processes, making these results difficult to interpret.

Recent revisions to the flow hypothesis propose that flow of interstitial fluid through the lacuno-canalicular porosity is resisted by the nonmineralized osteocyte pericellular matrix. Mathematical models predict that shear applies drag forces to the matrix that are 20 times larger than fluid shear forces on the cell surface, resulting in strain amplification at the cellular level by 10- to 100-fold (Han et al., 2004b; You et al., 2001). Furthermore, these models predict that flow induces a drag force in the matrix surrounding an osteocytic process. This force

results in a "circumferential hoop strain," similar to the force applied by pulling a ring off a finger. This strain is an order of magnitude higher than the forces generated by shear stress on the cell, suggesting that strain effects may dominate over shear. Osteocyte stretching in vitro results in a gadolinium-sensitive increase in intracellular calcium, consistent with the involvement of stretch-activated channels (Burger and Klein-Nulend, 1999). It will be interesting to follow the development of this model and the potential interplay between shear and shear-induced strain in the osteocyte process.

As in other tissues, putative mechanotransducers in bone include mechanosensitive ion channels and integrin adhesions. Shear stress stimulates rapid and transient increases in intracellular calcium, leading to the production of NO and PGE2, which are implicated in mechanical stimulation of bone formation in vivo (Burger and Klein-Nulend, 1999; Raisz, 1999). All of these events are blocked by the mechanosensitive channel blocker gadolinium (Burger and Klein-Nulend, 1999).

Application of shear stress to osteoblasts also triggers signals associated with focal adhesions, including association of integrin $\alpha v \beta 3$ with Shc (Weyts et al., 2002). Furthermore, soluble RGD peptides inhibit flow-induced PGE2 release in osteoblasts (Ponik and Pavalko, 2004).

Lung Mechanics

From development to maturation, cyclical mechanical deformations that continuously distend and contract the lung are critical to its development, growth, and metabolism (Liu and Post, 2000; Wirtz and Dobbs, 2000). Mechanical forces applied to lung epithelium in vitro and in vivo trigger the synthesis and secretion of surfactant proteins by type II epithelial cells that lower the surface tension at the air-liquid interface and prevent collapse during expiration (Gutierrez et al., 2003; Wirtz and Dobbs, 2000). In addition, lung injury associated with mechanical ventilation likely results from overinflation of bronchioles and alveoli.

One proposed mechanotransduction mechanism involves effects of strain on the actin cytoskeleton that results in c-Src activation (Han et al., 2004a). The actin filament-associated protein (AFAP-110), which is abundant in lung epithelial cells and fibroblasts, can bind and activate c-Src. AFAP-110 could therefore transduce force-induced changes in the actin cytoskeleton to activate c-Src tyrosine kinase (Han et al., 2004a). Another mechanism has been hypothesized in which force is not sensed by a molecule or molecular complex, but by the intercellular space (Tschumperlin et al., 2004). EGFR is activated in both fetal and adult epithelial type II cells in response to cellular contraction, triggering activation of ERK MAP kinases, which leads to induction of surfactant proteins (Correa-Meyer et al., 2002; Sanchez-Esteban et al., 2004). Activation of ERK1/2 via EGFR activation in response to a change in transmural pressure depends on shedding of heparin binding-epidermal growth factor (HB-EGF) into the intracellular space. Pressure decreases the volume of the intercellular space, so that even if shedding were constant, the concentration of HB-EGF would increase, leading to an increase in EGFR activation (Tschumperlin et al., 2004).
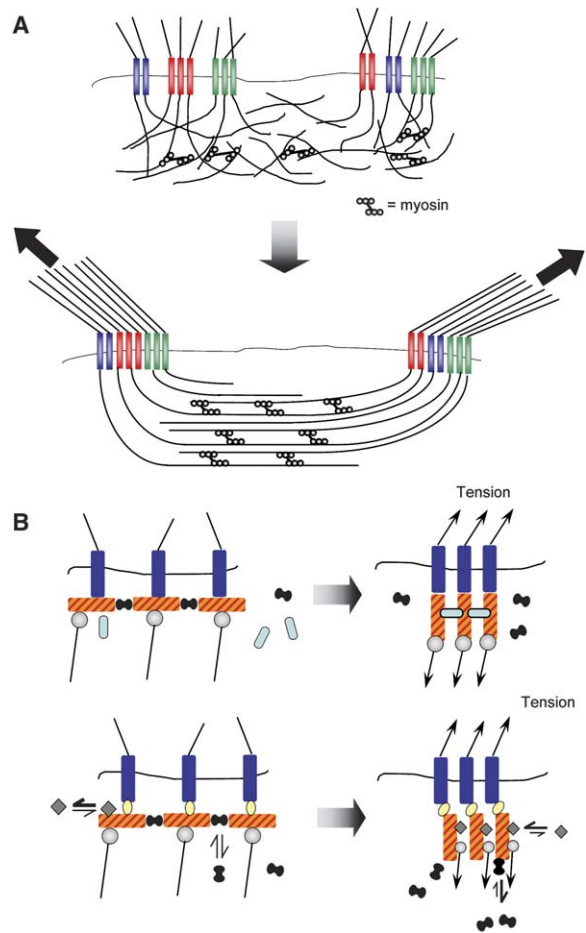

Figure 3. Cooperative Transduction within Multiprotein Complexes

Using focal adhesions as a model, tension could trigger (A) alterations in clustering of receptors and cytoskeletal structures or (B) changes in accessibility of binding sites for other proteins.

Conclusions

These examples highlight the remarkable breadth of mechanosensitive behavior. Returning to the unifying notions discussed at the beginning, we note that protein unfolding under tension has been observed directly by atomic force microscopy (Oberhauser et al., 1998) and can alter protein function. Stretching fibronectin revealed a cryptic binding site and enhanced self-association (Zhong et al., 1998). Stretching permeabilized cells on an elastic substratum increases binding of paxillin, suggesting a similar mechanism (Sawada and Sheetz, 2002). It seems likely that this sort of general mechanism (Figure 2) is used in other instances. For molecular motors such as myosin, higher loads slow the rate of ATP hydrolysis and release. Myosin provides a special case because catalytic activity is directly linked to force production; however, other mechanically coupled enzymes that undergo conformation changes may also show changes in enzymatic activity.

A related type of mechanism involves assemblies of proteins, such as focal adhesions or other cytoskeletal structures. In these cases, there may be no single protein that specifically transduces force. Rather, force may result in changes of the proteins relative to one

another, altering clustering or accessibility of binding sites for other molecules (Figure 3).

Regarding specialized, highly sensitive transduction systems, we have proposed that sensitivity is enhanced by linking the mechanotransducers to larger elements that convey force. For example, in touch and hearing, there is evidence that mechanosensitive channels must be linked to specific cytoskeletal or ECM structures to function properly. In the kidney and elsewhere, primary cilia bend in response to flow. In the ear, the tip link is hypothesized to transmit small displacements of stereocilia to mechanically sensitive channels. In vascular endothelial cells, transmission of forces to cell-cell junctional receptors may serve a similar purpose. In bone, conversion of compression to flow and perhaps flow into strain amplifies the forces on cells that are otherwise insulated by the rigid mineralized matrix. By contrast, integrin-dependent adhesions appear designed for high forces. They do so in part by sharing loads among multiple linkages and in part by strengthening the adhesions in response to force, the latter both by recruiting cytoskeletal elements and by recruiting new integrins to bear the load. In this manner, a common set of molecular mechanisms may underlie the remodeling of an elephant's leg bone, regulation of morphogenesis by the minute flow inside a kidney tubule, or the ability to hear a whisper in the dark.

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