J. Membrane Biol. 174, 85–95 (2000)

The Journal of
Membrane
Biology

Topical Review

Partners in Protection: Interdependence of Cytoskeleton and Plasma Membrane in Adaptations to Applied Forces

K.S. Ko, C.A.G. McCulloch

MRC Group in Periodontal Physiology, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

Received: 8 September 1999/Revised: 15 December 1999

Abstract. In mechanically active environments mammalian cells must cope with potentially injurious forces to survive, but the most proximal mechanosensors are largely unknown. How mechanoprotective responses to applied forces are generated and regulated is still a mystery. We consider recent evidence that suggests cellular mechanoprotective adaptations involve a coordinated remodeling of the cell membrane and the associated cytoskeleton. The plasma membrane "protects" the cytoskeleton by maintenance of intracellular ionic balance and can modulate force-induced cytoskeletal rearrangements by stretch-activated (e.g., Ca^{2+}) ion channels and mechanosensitive enzymes (e.g., Phospholipase A_2 and Phospholipase C). Conversely, the cytoskeleton protects the plasma membrane by providing structural support, reinforcement of the cortical framework at sites of force application, modulation of mechanosensitive ion channels and by potentially contributing to the membrane resealing process after mechanical rupture. We suggest that the plasma membrane and the cytoskeleton are partners in the cytoprotective response to physical forces.

Key words: Mechanoprotection — Cytoskeleton — Ion channels — Membrane

Introduction

For tissues in which the magnitude, direction and frequency of applied physical forces may alter remodeling patterns (e.g., bone trabeculae, Rubin & Lanyon, 1985), the resident cell populations have apparently developed various strategies to sense forces and appropriately generate signals that control the remodeling processes. Across various phyla, species and tissues, these mechanosensory functions exhibit considerable scope, probably because of the wide variation of amplitude and frequency of physical forces that are encountered in different sites and in different environments (Sachs, 1988; Sachs & Morris, 1998; Sackin, 1995). For example, endothelial cells in blood vessels are exposed to shear and stretching forces (Gimbrone, Nagel & Topper, 1997); bone cells are subjected to torque, tension and compression during cyclic loading from gravity and muscular activities (Turner & Pavalko, 1998); chondrocytes in joints are exposed to compressive and shear forces (Guilak et al., 1999); epithelial cells in skin and mucosal linings are periodically stretched (Fuchs & Cleveland, 1998); the cells of some soft connective tissues (e.g., the periodontal ligament) are exposed to very high amplitude of masticatory loading (Berkovitz, Moxham & Newman, 1982). From the standpoint of metabolic regulation, mechanoreception is critical in heart muscle where reciprocal interactions between the electrical and mechanical activities of cardiac cells permit the adjustment of heart rate to changes of mechanical load. But in mechanically active environments cells must also cope with potentially injurious forces to avoid irreversible damage and permit survival. These mechanoprotective adaptations involve cell membranes and associated structures and are the topic of this review.

Collaboration at the Membrane

The widespread and diverse expression of mechanosensory and mechanoprotective functions across phyla has

Correspondence to: K.S. Ko

stimulated considerable interest and hypothesis generation. Some of the central questions include: (i) What mechanosensors are most proximal to the applied force? (ii) Are there direct functional connections between membrane-based mechanosensors and nuclear control of transcription? (iii) Are there logical circuits that enable multiple mechanosensors to function in a collaborative fashion? Perhaps the most pressing challenge for studies of mammalian cells is to determine the molecular identity of even one definitive mechanosensor. Currently, various cellular components have been implicated in mechanotransduction and they include the extracellular membrane "skeleton" (i.e., extracellular matrix molecules); attachment receptors including selectins, cadherins and integrins; ion channels; the plasma membrane phospholipid bilayer; integral membrane proteins; the membrane-associated cytoskeleton. In addition to cell surface mechanoreceptors, intracellular organelles such as flow-sensitive caveolae in endothelial cells (Rizzo et al., 1998) and mitochondria in the filopodia of neurites (Bolanowski et al., 1996) may also be mechanosensitive. While a great deal of detective work has been done in cellular mechanotransduction, as long as the proximal sensors are at large and unidentified, hypotheses will predominate over a mechanistic understanding. In this review, we consider recent evidence that is focused on two prime suspects that may, in fact, be accomplices:

(i) mechanosensitive channels located in the plasma membrane;
(ii) the cortical and subcortical cytoskeleton.

We consider here that the plasma membrane is the lipid bilayer with its integral membrane proteins and that the cell cortex is the specialized layer of cytoplasm on the inner face of the plasma membrane consisting of actin filaments crosslinked into a network by various actin-binding proteins. As there have been several excellent reviews on the role of the cell membrane and the cytoskeleton in mechanotransduction (Sackin, 1995; Ingber, 1998; Janmey, 1998; Sachs & Morris, 1998; Galbraith & Sheetz, 1998), we focus here on an important subset of ideas that are relevant to mechanotransduction: cytoprotective responses to physical forces. The central theme to be explored here is that the cytoskeleton, via its connections to the plasma membrane, contributes to mechanoprotection by protecting the integrity and function of the cell membrane. We propose that the cytoskeleton and the plasma membrane function as an integrated and interdependent unit in the cytoprotective responses of mammalian cells to physical stresses.

Genetic Investigations of Mechanosensors

The search for the prime suspects of mechanosensation has been facilitated by genetic investigations of worms and bacteria. In worms, searches for proximal mechanosensors have lead to the characterization of the *unc-97* and *mec-3* genes (in *C. elegans*) whose gene products contribute to the mechanosensory functions of touch neurons. For example, UNC-97 helps to maintain the structural integrity of muscle adherens junctions (Hobert et al., 1999). A particularly efficient genetic approach for discovery of mechanosensors is available in bacteria since many bacterial species exhibit remarkable adaptation to osmotic changes in their environments. For example, when bacteria such as *E. coli* are subjected to a hypo-osmotic shock, there is an initial rise in turgor pressure caused by an initial inflow of water into the cell. However, normal cell turgor is reestablished through the controlled release of cytoplasmic solutes mainly through different mechanosensitive channels (Berrier et al., 1992). Notably, the mechanosensitive *MscL* gene product was characterized by patch-clamp analysis of purified and reconstituted membrane proteins from *E. coli.* However, the null mutant of the *MscL* gene is without a well-defined phenotype. More recent work has shown that the KefA and YggB gene products, which are located in the cell membrane, are important in regulation of ion balance and turgor pressure but only under conditions that are close to producing irreversible damage (Levina et al., 1999).

The genetic approach to identification of functionally important mechanosensors in mammalian cells has been controversial. For example, the α-subunit of the epithelial sodium channel (α-ENaC) is apparently important in mechanotransduction; some data have suggested that it is mechanosensitive based on reconstitution experiments into purified planar lipid bilayers (Awayda et al., 1995) and in transfected LM(TK-) cells (a null cell for stretch-activated nonselective cation channel activity) (Kizer, Guo & Hruska, 1997). The mammalian neuronal mechano-gated K^+ channels TRAAK (Maingret et al., 1999*a*) and TREK-1 (Maingret et al., 1999*b*) have been transfected into COS cells and found to be sensitive to stretch. These approaches are potentially valuable because of the possibility of identifying mammalian homologues and these in turn could permit functional study of genetically defined gene products. An important caveat of these and related experiments is that the properties of the channels formed by incorporation of the expressed subunits in different cell types are probably not the same as those observed in their native cell type. Indeed in some cases contradictory results have been reported. When the ENaC was expressed in an oocyte system, it was found to be mechano-insensitive at the whole-cell and single-channel levels (Awayda & Subramanyam, 1998). Previous results obtained from α-ENaC reconstitution into planar bilayers could be due to as yet undefined experimental artifacts such as solvent lenses. Therefore, while genetic approaches are potentially use-

ful, their direct application to study of mechanotransduction in mammalian cells may require other structural elements and linkers as are found in the subcortical cytoskeleton. Below we consider structural elements that are most likely involved in mechanical signaling and adaptation.

Mechanosensitive Structures in the Plasma Membrane-Cytoskeleton Complex

Forces applied to a cell can be distributed over various cellular components and at multiple loci including the extracellular matrix, the membrane lipid bilayer, integral membrane proteins and the cytoskeleton. Although the proximal mechanosensors in mammalian cells are poorly characterized, there are many potential candidates that reside in the plasma membrane and the subcortical cytoskeletal complex. These mechanosensitive structures, when modulated by force application to the cell, may interact with each other to protect the cell from physical damage.

A prolonged break in plasma membrane integrity may compromise cell survival in part because of unrestricted ion flow (particularly Ca^{2+}), or because of the resultant loss of lipid and protein domain organization which leads to dissipation of ion gradients and breakdown of signaling systems (McNeil & Steinhardt, 1997). In the context of mechanoprotection, several integral membrane proteins including ion channels (Morris, 1990) and enzymes (e.g., phospholipase A_2, Lehtonen & Kinnunen, 1995; phospholipase C, Kato et al., 1999) are stretch-sensitive. For example, the hydrolytic activity of phospholipase A_2 is affected by membrane stretching induced by osmotic swelling (Lehtonen & Kinnunen, 1995). Maximal phospholipase A_2 activity depends on lipid packing densities; these densities in turn are related to the surface pressure, suggesting that phospholipase A_2 may be a mechanosensor (Lehtonen & Kinnunen, 1995). This notion is supported by the observation that inhibitors of phospholipase A_2 also reduce flow-induced PGE_2 release in osteocytes (Ajubi et al., 1999). Phospholipase C has also been implicated in stretch-induced responses in mesangial cells. Pressure-induced DNA synthesis and the transient formation of inositol 1,4,5-triphosphate are inhibited by the phospholipase C inhibitor 2-nitro-4-carboxyphenyl-N (Kato et al., 1999). The mechanical stimulation of these plasma membrane enzymes leads to activation of signal transduction pathways (such as the phosphatidylinositol pathway) that mediate various cellular responses including cytoskeletal protein rearrangements. In the context of membrane structure and mechanosensitive elements, because the plasma membrane is supported by an underlying membrane skeleton (Viel & Branton, 1996), this skeleton may be well-positioned to regulate various types of ion-permeable channels.

Mechanosensitive Ion Channels

Probably the most extensively studied mechanosensitive structures in the plasma membrane are mechanosensitive channels. The open probability of these channels depends in part on applied stress at the membrane. The mechanosensitive channels include both stretch-activated and stretch-inactivated channels, displacement-sensitive channels and shear-stress-sensitive channels (Morris, 1990). The stretch-activated channels comprise a range of ion channels permeable to anions (e.g., Cl^-), and to cations, such as the stretch-activated K^+ channels (Morris, 1990) and Ca^{2+} channels (Lee et al., 1999). The cytoprotective role of mechanosensitive channels is evident in *E. coli* in which the YggB and MscL genes code for channel proteins that open at a pressure change just below that which would cause membrane disruption from excessive osmotic pressure (Hobert et al., 1999).

The mechanoprotective interactions between the plasma membrane and the cytoskeleton are perhaps best demonstrated by the interdependence of mechanosensitive ion channels and subcortical actin. In *Lymnaea* neurons, depolymerization of subcortical actin does not prevent transmission of mechanical signals (as measured by mechanically evoked whole-cell current); instead the activity of stretch-activated K^+ channels is enhanced (Wan, Juranka & Morris, 1999). Agents that disrupt cytoskeletal integrity (e.g., cytochalasin B) or that tend to decrease cytoskeletal tension (e.g., N-ethylmaleimide) generally enhance mechanosensitivity (Wan et al., 1999). Indeed, increasing evidence supports the notion of cytoskeletal modulation of ion channel activity in the membrane. Cortical actin filaments have been implicated in the regulation of stretch-activated, calcium-permeable channels since sustained force promotes subcortical actin assembly (Pender & McCulloch, 1991) and this assembly response "desensitizes" channels to subsequent force applications. The decrease in stretch sensitivity can be reversed after cytochalasin D treatment (Glogauer et al., 1998). Alterations in the structure of the actin network can also modulate the activity of voltage-gated ion channels in bipolar cells (Maguire et al., 1998).

In addition to the relative abundance of actin filaments, the higher order structures of the actin cytoskeleton may also directly or indirectly regulate mechanosensitive channels. For example, the actin-binding-protein-280 (ABP-280) crosslinks actin filaments to form large orthogonal arrays in the cell cortex which tends to increase membrane rigidity (Hartwig & Kwiatkowski, 1991). ABP-280 would then seem well-suited to regulate ion channel activation. Accordingly, in ABP-280-deficient melanoma cells, osmotically sensitive K^+ channel activation is impaired but regulation can be "rescued" by transfection with ABP-280 cDNA (Cantiello et al., 1993). These results suggest that the actin network modulates the activities of mechanosensitive ion chan-

nels by limiting the deformability of the plasma membrane, probably by increased actin assembly and crosslinking.

The Cortical Cytoskeleton Provides Support and Protection for the Plasma Membrane

The cytoskeleton provides structural support of the plasma membrane, in part to maintain cell integrity during repeated deformations. At one level of structural organization, the plasma membrane is supported by the membrane skeleton, a structural complex which is composed of spectrins, short actin filaments (Picart & Discher, 1999), protein 4.1 and other spectrin-binding proteins (Viel & Branton, 1996). The short actin filaments are largely oriented tangent to the lipid bilayer in the erythrocyte membrane skeleton (Picart & Discher, 1999) and may be important in stretch-resistance of the membrane. Further, the spectrins of the membrane skeleton form a network that is connected to the lipid bilayer through integral membrane proteins or through protein 4.1 via its interaction with transmembrane proteins (Viel & Branton, 1996). Because actin binds to the spectrins directly and indirectly via actin-binding proteins, the membrane skeleton is extensively crosslinked to the cortical cytoskeletal framework.

The membrane skeleton may also protect the plasma membrane from mechanical disruptions. For instance, the spectrin network may facilitate membrane healing after mechanical membrane rupture (Bauman & Grebe, 1998). Another potentially mechanoprotective protein is dystrophin (a member of the spectrin superfamily) which is one of the structural proteins that underlie and support the sarcolemma. Recent evidence from dystrophic mice suggests that dystrophin is responsible for the organization of other membrane skeletal proteins: the lack of dystrophin causes fragility of the sarcolemma and potential tearing of the membrane during contraction of the muscle cell (Williams & Bloch, 1999). Thus the plasma membrane appears to rely on the meshwork of the membrane skeleton (which is crosslinked to the more centrally located cytoplasmic cytoskeleton) to preserve its integrity.

Intermediate filaments have been implicated as mechanical integrators of the whole cytoskeleton and have been shown to support the cytoplasm under high strains (Maniotis et al., 1997). In chondrocytes, intermediate filaments form an interconnected network that surrounds the nucleus and extends to the plasma membrane (Durrant et al., 1999). The importance of intermediate filaments in cytoprotection is evident in epithelial cells from mice with mutated keratins which show skin blistering upon mild mechanical trauma (Vassar et al., 1991). Mutations that compromise the intermediate filament framework increase the risk of cell rupture (Fuchs & Cleveland, 1998). Mice that lack the intermediate filament glial fibrillary protein and vimentin develop less dense scars after injury, and show abnormal healing (Pekny et al., 1999). The absence of vimentin also decreases the flow-induced dilation of arteries in vimentin-null mice (Henrion et al., 1997). The mechanism for mechanoprotection provided by intermediate filaments may arise in part from connections to the plasma membrane via spectrin-associated proteins (Djabali, 1999) and connections to actin via crosslinking proteins. However, in addition to the biophysical and potentially mechanoprotective properties of the individual cytoskeletal polymers, the ability of different classes of cytoskeletal proteins to form interconnected structural networks may have profound implications for the cytoprotective effects of the cytoskeleton.

Interacting Cytoskeletal Polymers

In considering the early cytoprotective response of cells against physical disruptions, we have hypothesized above that the plasma membrane, its associated membrane skeleton and the global cytoskeletal network function as a unit to bring about a coordinated cellular response to applied strain. It has been suggested that microfilaments interact with microtubules and intermediate filaments to form an integrated structure (Ingber & Folkman, 1989). If this concept is correct, an integrated network could be a structural adaptation to physical forces mediated by various cytoskeletal-binding and linker proteins. However it is the extensive interactions among cytoskeletal proteins that make genetic knockout studies of individual cytoskeletal protein difficult to interpret. Further, due to the long time scale in these experiments, reequilibration of the interacting components may induce alterations in other proteins (Campbell & Kahl, 1989) and the results from these experiments cannot be reliably compared to the wild type (e.g., Akinlaja & Sachs, 1998). When exposed to physical forces, cells such as fibroblasts from heavily loaded tissues reinforce locally their connection with extracellular adhesion sites by inducing actin assembly and by recruiting ABP-280 into cortical adhesion complexes (Glogauer et al., 1998). Using ABP-280 as an example, this particular cytoskeletal linker protein potentially reinforces the cytoskeletal network by: (i) mediating actin filament binding to integral membrane proteins including β_1 integrin (Loo, Kanner & Aruffo, 1998); and (ii) crosslinking filaments into a localized, stiff cortical complex. Other actin filament crosslinking proteins such as α-actinin and gelation factor have been shown to exhibit protective responses during osmotic stress (Rivero et al., 1996), suggesting a common role for cytoskeletal polymer crosslinking in response to different types of mechanical stress.

The notion that the interaction and crosslinking of cytoskeletal polymers into higher order structures are potentially important mechanoprotective mechanisms is

Table. Cytoskeletal proteins implicated in mechanoprotection

Protein	Function	Reference
Spectrin	Involved in membrane resealing	Bauman & Grebe, 1998
Dystrophin	Protects membrane from tearing	Williams & Bloch, 1999
ABP-280	Promotes actin assembly at sites of force application	Glogauer et al., 1997
α-actinin	Facilitates cytoprotection against osmotic stress	Rivero et al., 1996
Actin gelation factor	Facilitates cytoprotection against osmotic stress	Rivero et al., 1996
MAP2c	Potentially reinforces actin-microtubules linkages	Cunningham et al., 1997
Coronin	Potentially reinforces actin-microtubules linkages	Goode et al., 1999
BPAG1	Potentially reinforces actin-intermediate filaments linkages	Yang et al., 1996

supported by the wide range of cytoskeleton-binding proteins that facilitate interconnections among the three classes of cytoskeletal polymers (Table). These proteins not only reinforce the structural framework to support the membrane, but they are potentially important in coordinating rearrangements of all three cytoskeletal polymers in response to physical forces (Fig. 1). There are numerous proteins that mediate actin-microtubule and actin-intermediate filament interactions. For example, microtubule-associated protein 2c (MAP2c) reorganizes both microfilaments and microtubules, causes actin gelation and induces formation of microtubule bundles (Cunningham et al., 1997). The actin-binding protein coronin which also promotes the rapid assembly and crosslinking of actin filaments also binds to microtubules, suggesting that it may link these two cytoskeletal systems in yeast (Goode et al., 1999). The mechanoprotective importance of actin-intermediate filament linker proteins is evident in BPAG1 knockout mice that show skin blistering after mechanical stress (Guo et al., 1995). BPAG1 is an intermediate filament binding protein that mediates binding interactions between intermediate filaments and actin (Yang et al., 1996); the lack of this interaction may weaken the cytoskeletal framework and compromise mechanoprotection.

Membrane Tension — A Common Regulator of Cytoskeletal and Plasma Membrane Dynamics

Membrane tension can be defined as in-plane stress that is generated by, for example, osmotic swelling or by adherence of the membrane bilayer to the cytoskeleton when a dragging force is created (e.g., membrane tethers; Sheetz & Dai, 1996). Using micropipette aspiration of single cells, the expansivity modulus of red blood cells was estimated to be 450 dynes/cm (Evans, Waugh & Melnik, 1976). More recent studies using optical tweezers estimated the tension in the plasma membrane of red blood cells to be typically $\geq$7 pN (Sheetz & Dai, 1996). However, despite the application of this method for estimating membrane tension in biological systems, these estimates are derived from formulae that relate membrane geometry to applied forces and are somewhat indirect. In addition, it is very difficult to separate out the various components of membrane-cytoskeletal interactions for the measurement of in-plane membrane tension (Dai et al., 1998). To further complicate data interpretation, the membrane of many cell types is often folded and a direct measurement may not be representative of the whole membrane area. Further, the dynamic nature of the plasma membrane presents significant challenges to obtain accurate estimates of membrane tension. For example, mammalian neurons can regulate membrane tension by forming vacuolelike dilations which appear when cells shrink (Mills & Morris, 1998).

Plasma membrane tension, which is probably a combination of bilayer in-plane tension and the interactions between membrane and cytoskeleton (Dai et al., 1998), is very likely to be an important regulatory factor of both membrane dynamics and cytoskeletal rearrangements in mechanoprotective responses. Indeed membrane tension helps to control plasma membrane area (Mills & Morris, 1998), cell shape (Raucher & Sheetz, 1999) and cellular activities such as endocytosis (Raucher & Sheetz, 1999) and exocytosis (Togo et al., 1999). Membrane transport processes such as endocytosis and exocytosis are regulated by membrane tension and may also depend on cytoskeletal proteins such as actin (Valentijin, Gumkowski & Jamieson, 1999; Niles & Malik, 1999). Membrane regulation also depends on microtubules as membrane trafficking is facilitated by microtubule-based vesicle transport (Hamm-Alvarez & Sheetz, 1998).

Interactions between the plasma membrane and the cytoskeleton in regulation of membrane tension are seen in mechanosensitive channel function. Indeed it has been suggested that membrane tension is transduced by plasma membrane ion channels (Opsahl & Webb, 1994). These channels in turn may be able to modulate cytoskeletal dynamics. Likewise, the neuronal mechanogated K^+ channel TRAAK is activated by a convex curvature of the plasma membrane (Maingret et al., 1999*a*). However, we should note that despite evidence of modulation of ion channel activity by membrane tension, the energy required to activate mechanosensitive ion channels is nearly two orders of magnitude higher than the membrane tension measured in swelling cells. For example, in 50% hypotonic medium, membrane tether

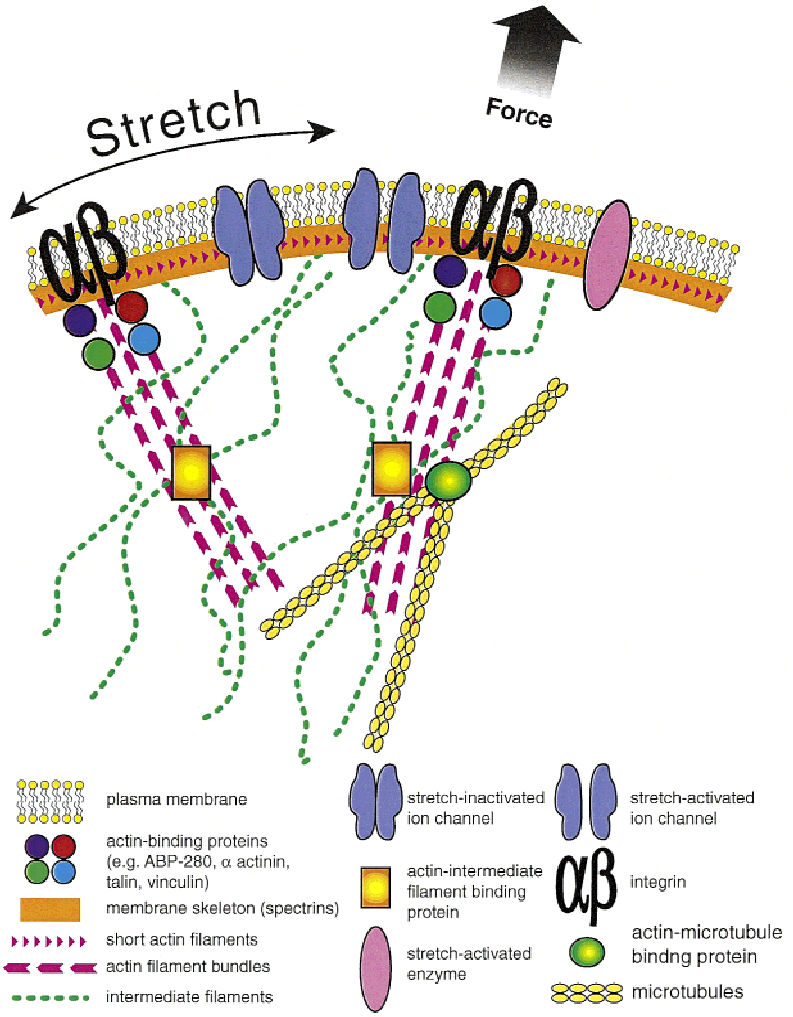

Fig. 1. Schematic diagram showing plasma membrane and cytoskeletal components that are mechanosensitive and potentially contribute to mechanoprotection. Stretch-activated enzymes in the plasma membrane such as phospholipase A_2 and phospholipase C may modulate cytoskeletal remodeling in response to force application which may be delivered through extracellular matrix attachment sites at integrins. Other stretch-activated enzymes such as $pp60^{src}$ (Sai, Naruse & Sokabe, 1999) or myosin light chain kinase (Smith, Tokui & Ikebe, 1995) are located in the cytoplasm and are not shown. The open probability of stretch-activated ion channels is increased while that of stretch-inactivated ion channels is decreased due to stretching of the plasma membrane. The plasma membrane is structurally supported by the membrane skeleton which is comprised of the spectrin family of proteins and short actin filaments. The membrane skeleton is linked to the subcortical actin and the structure of actin filaments is regulated by actin-binding proteins such as ABP-280, α-actinin, talin and vinculin. The subcortical actin framework is reinforced at sites of force application by recruitment of actin and actin-binding proteins such as ABP-280. The membrane skeleton may also be linked to intermediate filaments. The three cytoskeletal polymers are interconnected through linkers proteins such as actin-microtubule-binding proteins (e.g., MAP2c, coronin) and actin-intermediate-binding proteins (e.g., BPAG1).

force increased tenfold in Molluscan neurons (Dai et al., 1998). This discrepancy could be due to the interaction between the plasma membrane and the cytoskeleton, both of which work cooperatively to transduce mechanical signals. Notably, ions such as Ca^{2+} can affect actin assembly by regulating the activity of actin-binding proteins such as gelsolin (Hartwig & Kwiatkowski, 1991). Thus, while the cytoskeleton may be able to modify membrane tension, likewise membrane tension may be a common regulatory factor in controlling the interactions between the cytoskeleton and the plasma membrane.

Despite the evidence for cytoskeletal modulation of ion channel activity described above, certain types of ion channels are sensitive to pressure or tension changes transmitted through the lipid bilayer independent of the cytoskeleton. For example, membrane tension can be transduced by the ion channel protein alamethicin reconstituted into pure lipid membranes without any attached cytoskeletal proteins (Opsahl & Webb, 1994). The conductance of these channels depends on the applied tension and the associated increase of membrane area. The α-subunits of the epithelial Na^+ channel (ENaC) also form mechanosensitive channels when incorporated into planar lipid bilayers (Awayda et al., 1995). Ion channels that are sensitive to pressure transmitted through the lipid bilayer have also been found in a whole-cell system. In neurons, the N-methyl-D-aspartate (NMDA)-activated channel responses can be modulated by deformation of the lipid bilayers produced by the asymmetrical amphiphilic compounds lysophospholipids and arachidonic acids that insert into the plasma membrane. Lysophospholipids can reduce the open probability of NMDA-activated channels similar to that caused by compression, while arachidonic acid potentiated the open probability similar to that caused by stretching (Casado & Ascher, 1998). However the results from experiments that employ amphiphilic compounds should be interpreted with caution as the mode of action (modulation of lipid packing density) of these compounds is only an assumption; their affinity for membrane binding may cause other side effects such as altered bilayer fluidity and activity of intracellular organelles.

If the plasma membrane is normally under a basal level of tension, a break in the membrane caused by mechanical loading should induce a drop in membrane tension and possibly trigger repair processes such as membrane resealing. A decrease in membrane tension effected by a surfactant (e.g., Pluronic F68 NF) facilitates membrane resealing after wounding (Togo et al., 1999), suggesting membrane surface tension regulates membrane repair or resealing. Conceivably, exocytosis of internal membranes and rearrangement of the cytoskeleton following membrane wounding restores membrane tension to a normal level.

Cellular Wound Healing and Force-induced Adaptations

It is evident that mammalian cells possess various mechanosensitive components to sense physical stimuli, respond and adapt. Earlier work using cyclic stretching of endothelial cells for 48 hr showed actual elongation of the cells and perpendicular alignment to the applied strain field (Ives, Skin & McIntire, 1986). Similarly, skeletal muscle cells *in vivo* respond to long-term loading by hypertrophy (Booth et al., 1998). An extreme example of an early cytoprotective response of cells exposed to physical loading is cellular wound healing (McNeil & Steinhardt, 1997), a process that includes plasma membrane resealing and cytoskeletal rearrangements.

Plasma membrane disruptions occur in cells exposed to high amplitude mechanical forces. For example, cells exposed to high amplitude loading (e.g., cardiac myocytes in hypertension) may exhibit membrane "wounding" which causes increased membrane permeability (Fischer et al., 1997). To maintain the barrier function of the plasma membrane, as well as prevention of loss of cytosol and maintenance of physiological ionic balance, cells respond to membrane discontinuities by a process of membrane resealing. Indeed a rapid membrane resealing response is vital for cell survival: living cells normally reseal within seconds to 1 min (Steinhardt, Bi & Alderton, 1994). Cells routinely survive electroporation, a process which creates tears in the membrane and permits large molecules (up to ~66 kDa) to enter cells. Rapid resealing of the plasma membrane helps to preserve membrane integrity (Glogauer & McCulloch, 1992; Meldrum et al., 1999) and indeed there is a very large literature that addresses these protective phenomena in electroporated cells. Membrane resealing thus can be classified as a mechanoprotective response.

The cytoskeleton is instrumental in membrane resealing. After mechanically induced membrane ruptures, biophysical data suggest that the spectrin network plays a role in membrane healing (Bauman & Grebe, 1998). The Ca^{2+} inflow at plasmalemmal lesions induces various membrane structures to form (including endocytic vesicles). Many of these membrane-associated structures interact with each other and the plasmalemma to repair the damage (Eddleman et al., 1998). The repair mechanism may involve a Ca^{2+}-evoked exocytic fusion event in which a replacement patch is formed at the site of plasma membrane disruption (Terasaki, Miyake & McNeil, 1997). As endocytosis and exocytosis are actin-dependent processes, it is likely that the actin cytoskeleton is directly involved in plasma membrane repair after mechanical damage. This is not surprising since the actin network has been implicated in the control of compensatory membrane retrieval following exocytosis in pancreatic acinar cells (Valentijn et al., 1999). Unexpectedly, Cytochalasin D facilitates membrane resealing, possibly due to a reduction in membrane tension (Togo et al., 1999). Membrane resealing also depends on microtubule transport (Steinhardt et al., 1994) probably because many exocytic processes require cytoskeletal tracks to direct the movement of exocytic vesicles (Santella et al., 1999; Rose et al., 1999). Indeed, evidence from studies of membrane dynamics suggests a close cooperation between cytoskeletal and membrane components in membrane resealing. While the cytoskeleton is involved in the transport of membrane vesicles, SNARE proteins that reside on the plasma membrane regulate the membrane fusion process (Niles & Malik, 1999) to complete membrane resealing.

As biological membranes can only expand elastically by < 3% before rupture occurs (Nichol & Hutter, 1996), mechanoprotection of the membrane may involve localized strengthening and remodeling of the subcortical cytoskeleton. Recruitment of subcortical actin to sites of membrane deformation has been visualized in GFP-actin-transfected fibroblasts mechanically stimulated by polylysine-treated needles (Heidemann et al., 1999). The force-induced actin accumulation is not a result of increased integrin clustering or increased number of focal contacts (Glogauer et al., 1997), indicating that the recruitment of actin observed is a force-specific response. The increased subcortical actin at the sites of force application (Glogauer et al., 1998) is correlated with increases of membrane rigidity (Pourati et al., 1998) and dampened calcium influx (Glogauer et al., 1997). These findings relate to the idea that preexisting cytoskeletal tension plays a major role in regulating cell deformability in adherent cells (Pourati, 1998). Thus in addition to limiting the deformability of the membrane, cortical actin may regulate stretch-activated cation permeable channel activity and thereby provides a feedback-controlled desensitization mechanism for cells exposed to repeated long-term mechanical stimuli (Glogauer et al., 1997).

In addition to subcortical actin rearrangement during mechanical loading, more global cytoskeletal responses to applied forces have been observed in some cell types. For example after mechanical stretching, fibroblasts respond by rapid (<10 sec) reductions in actin filament

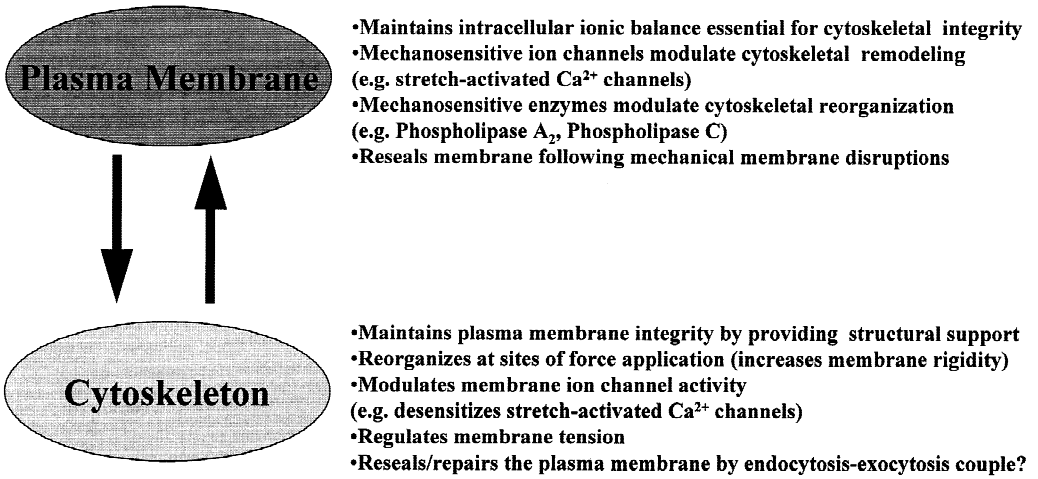

Fig. 2. Partners in protection — schematic diagram showing the functional interaction of plasma membrane and the cytoskeleton in mechanoprotection.

content followed by large increases in cellular actin filament content that are contemporaneous with a change in cell shape. The actin polymerization response to deformation of fibroblast membranes is proportional to the amount of deformation up to a certain threshold level above which there is no further change (Pender & McCulloch, 1991).

Although there are few studies on microtubule rearrangement in response to physical stress, the contribution of microtubules in mechanoprotection may lie in their control of ion channel activities and involvement in membrane transport (Hamm-Alvarez & Sheetz, 1998) which is required for membrane resealing (Steinhardt et al., 1994). Indeed depolymerization of microtubules stimulates calcium channel activities in plant cells (Thion et al., 1998) and changes the activity of voltage-dependent Na^+-channels in oocytes expressing nerve (PN1) or skeletal muscle (SkM) Na^+ channel α subunits (Shcherbatko et al., 1999). Microtubule-based vesicle transport also plays a role in the directed movement of ion channels to and from the plasma membrane (Hamm-Alvarez & Sheetz, 1998).

Although not as extensively studied as actin, in part because of cell-type-specific variations, intermediate filaments also reorganize in response to force applications. For example, chondrocytes in organ culture respond to osmotic swelling by remodeling of the vimentin cytoskeleton while maintaining the actin cytoskeletal structure (Durrant et al., 1999). This reorganization of intermediate filaments probably causes changes of the plasma membrane since intermediate filaments are found closely associated with the plasma membrane (Green & Goldman, 1986).

Conclusions

Cytoprotective responses to a wide range of applied forces require the coordinated interaction of the plasma membrane and the cytoskeleton (Fig. 2). The plasma membrane "protects" the cytoskeleton by maintenance of intracellular ionic balance and modulates force-induced cytoskeletal rearrangements by stretch-activated ion channels and mechanosensitive enzymes. In more extreme situations, the membrane may also maintain the integrity of the cell by resealing after mechanical rupture. Conversely, the cytoskeleton protects the plasma membrane by providing structural support, reinforcing the subcortical framework at sites of force application, regulating membrane tension and desensitizing mechanosensitive ion channels. The cytoskeleton is also essential for membrane transport to enable the membrane resealing process. We suggest that the plasma membrane and the cytoskeleton are in fact partners in the cytoprotective response to physical forces. However, there are still many unsolved mysteries.

Tracking Down the Suspects

As the proximal mechanosensors in mammalian cells are still elusive, there are many missing "downstream" pieces of evidence in the investigation of mechanoprotection. While cytoprotective responses may indeed require membrane-cytoskeletal interactions, several key questions remained unanswered: (i) Does membrane tension modulate eukaryotic mechanosensitive Ca^{2+} channel activities? (ii) Does membrane resealing following mechanical disruption of the plasma membrane depend on an intact cytoskeletal system? (iii) Are stretch-activated Ca^{2+} channels mechanoprotective by regulating apoptosis? (iv) Are intermediate filaments involved in modulation of the ion channels? (v) Are actin-microtubule-binding proteins mechanoprotective? (vi) Are actin-intermediate filament-binding proteins mechanoprotective? To provide some insights into these questions, we suggest some experimental approaches that

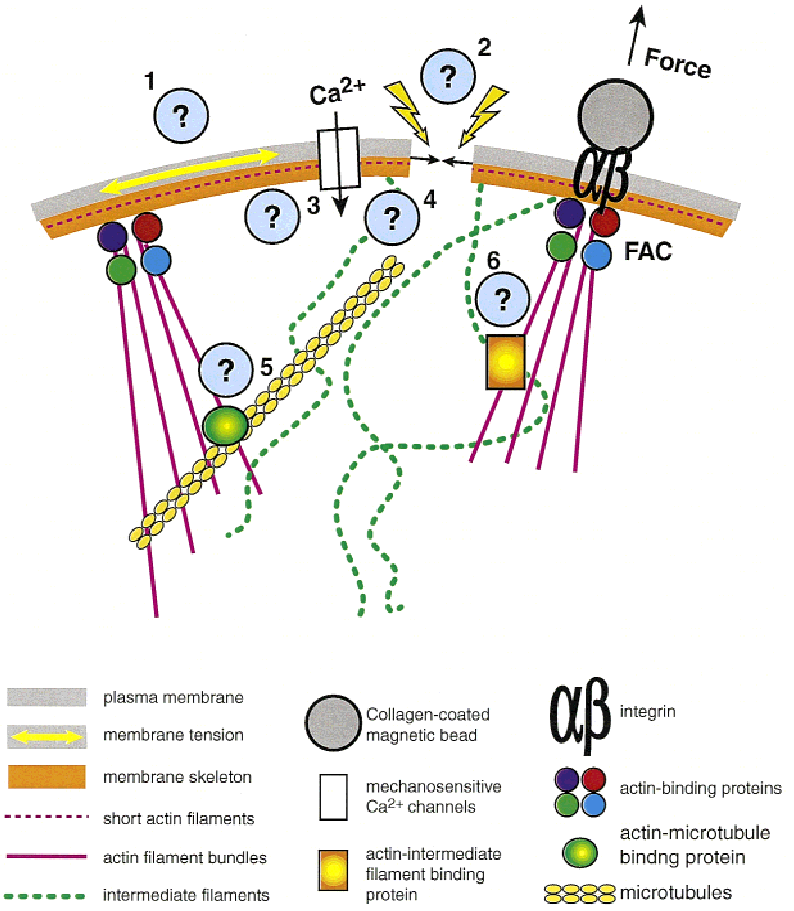

Fig. 3. Hypothesis testing: cytoprotective responses require membrane-cytoskeletal interactions. Cytoprotective responses to applied forces may require membrane-cytoskeletal interactions; several key questions remained unanswered: (1) Does membrane tension modulate eucaryotic mechanosensitive Ca^{2+} channel activities? Previous reports have shown that MscL, a mechanosensitive channel from *E. coli* can be activated by increase in membrane tension (Sachs & Morris, 1998). However, to evaluate the effect of membrane tension on eucaryotic mechanosensitive Ca^{2+} channels (Lee, 1999), stretch-induced Ca^{2+} transients (using magnetic bead force application — Glogauer & McCulloch, 1995) can be measured in the presence of compounds that affect membrane tension by insertion into membrane (e.g., surfactant Pluronic F68 NF, lysophosphatidic acid). Compounds that decrease membrane tension (e.g., surfactant) may desensitize the mechanosensitive Ca^{2+} channels. On the other hand, membrane crenators (e.g., trinitrophenol) may sensitize these channels (Maingret et al., 1999*a*). (2) Does membrane resealing following mechanical disruption of the plasma membrane depend on an intact cytoskeletal system? Electroporation can be used as a model system to induce pore formation in the plasma membrane. Fluorescent molecules of different sizes (e.g., FITC-dextran) could be introduced into cells with this method in the presence of cytoskeletal disrupting agents (e.g., cytochalasin D; intermediate filament disrupting mimetic peptide-Goldman et al., 1996; colcemid). Intensity of intracellular fluorescence by flow-cytometry or quantitative microscopy could be used as a measure of delay membrane resealing. (3) Are stretch-activated Ca^{2+} channels mechanoprotective by regulating apoptosis? Chronic overload of Ca^{2+} increases apoptosis (McConkey & Orrenius, 1997). To delineate whether stretch-induced Ca^{2+} transients are mechanoprotective, cultured cells (e.g., myocardiocytes) exposed to mechanical stretching in medium with or without Ca^{2+} can be compared in apoptotic index. To evaluate the contribution of stretch-activated channels in possible stretch-induced apoptosis, the apoptotic index of mechanically stretched cells can be compared in the presence of absence of mechano-gated channel blockers such as gadolinium chloride, gentamicin and amiloride (Wilkinson, Gao & Hamill, 1998). (4) Are intermediate filaments involved in modulation of ion channel activity? To evaluate the possible involvement of intermediate filaments, Ca^{2+} transient could be measured and compared in control cells and cells electroporated with intermediate filament disrupting mimetic peptides (Goldman et al., 1996). Stretch-induced Ca^{2+} responses can be induced using magnetic bead model of force application. (5) Are actin-microtubule-binding proteins and (6) actin-intermediate-filament-binding proteins mechanoprotective? To evaluate whether actin-microtubule-binding proteins such as MAP2c are mechanoprotective, the magnetic-bead model mentioned above could be used on fibroblasts or other cultured cells from MAP2c-null mice to compare possible inhibition of mechanosensitive Ca^{2+} channels desensitizations after repeated force-applications. To evaluate whether actin-intermediate-filament-binding proteins such as BPAG1 are mechanoprotective, cells from BPAG1-null mice could be used.

could lead to further understanding of how each partner contributes to a mutually protective relationship (Fig. 3).

References

Ajubi, N.E., Klein-Nulend, J., Alblas, M.J., Burger, E.H., Nijweide, P.J. 1999. Signal transduction pathways involved in fluid flow-induced PGE_2 production by cultured osteocytes. *Am. J. Physiol.* **276:**E171–178

Akinlaja, J., Sachs, F. 1998. The breakdown of cell membranes by electrical and mechanical stress. *Biophy. J.* **75:**247–254

Awayda, M.S., Ismailov, I.I., Berdiev, B.K., Benos, D.J. 1995. A cloned renal epithelial Na^+ channel protein displays stretch activation in planar lipid bilayers. *Am. J. Physiol.* **37:**C1450–C1459

Awayda, M.S., Subramanyam, M. 1998. Regulation of the epithelial Na^+ channel by membrane tension. *J. Gen. Physiol.* **112:**97–111

Bauman, M., Grebe, R. 1998. Characteristics of the osmotically induced membrane rupture. *Mol. Mem. Biol.* **15:**193–201

Berkovitz, B.K.B., Moxham, B.J., Newman, H.N. 1982. The periodontal ligament in health and disease. Pergamon Press, Oxford, Toronto

Berrier, C., Coulombe, A., Szabo, I., Zoratti, M., Ghazi, A. 1992. Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. *Eur. J. Biochem.* **206:**559–565

Bolanowski, S.J., Schuyler, J.E., Sulitka, D., Pietras, B. 1996. Mitochondrial distribution within the terminal neurite of the pacinian corpuscle. *Somatosens Mot. Res.* **13:**49–58

Booth, F.W., Tseng, B.S., Fluck, M., Carson, J.A. 1998. Molecular and cellular adaptation of muscle in response to physical training. *Acta. Physiol. Scand.* **162:**343–350

Campbell, K.P., Kahl, S.D. 1989. Association of dystrophin and an integral membrane glycoprotein. *Nature* **338:**259–262

Cantiello, H.F., Prat, A.G., Bonventre, J.V., Cunningham, C.C., Hartwig, J.H., Ausiello, D.A. 1993. Actin-binding protein contributes to cell volume regulatory ion channel activation in melanoma cells. *J. Biol. Chem.* **268:**4596–4599

Casado, M., Ascher, P. 1998. Opposite modulation of NMDA receptors by lysophospholipids and arachidonic acid: common features with mechanosensitivity. *J. Physiol.* **513:**317–330

Cunningham, C.C., Leclerc, N., Flanagan, L.A., Lu, M., Janmey, P.A., Kosik, K.S. 1997. Microtubule-associated protein 2c reorganizes both microtubules and microfilaments into distinct cytological structures in an actin-binding protein-280-deficient melanoma cell line. *J. Cell Biol.* **136:**845–857

Dai, J., Sheetz, M.P., Wan, X., Morris, C.E. 1998. Membrane tension in swelling and shrinking molluscan neurons. *J. Neurosci.* **18:**6681–6692

Djabali, K. 1999. Cytoskeletal proteins connecting intermediate filaments to cytoplasmic and nuclear periphery. *Histol. Histopathol.* **14:**501–509

Durrant, L.A., Archer, C.W., Benjamin, M., Ralphs, J.R. 1999. Organization of the chondrocyte cytoskeleton and its response to changing mechanical conditions in organ culture. *J. Anat.* **194:**343–353

Eddleman, C.S., Ballinger, M.L., Smyers, M.E., Fishman, H.M., Bittner, G.D. 1998. Endocytotic formation of vesicles and other membranous structures induced by Ca^{2+} and axolemmal injury. *J. Neurosci.* **18:**4029–4041

Evans, E.A., Waugh, R.E., Melnik, L. 1976. Elastic area compressibility modulus of red cell membrane. *Biophys. J.* **16:**585–595

Fischer, T.A., McNeil, P.L., Khakee, R., Finn, P., Kelly, R.A., Pfeffer, M.A., Pfeffer, J.M. 1997. Cardiac myocyte membrane wounding in the abruptly pressure-overloaded rat heart under high wall stress. *Hypertension* **30:**1041–1046

Fuchs, E., Cleveland, D.W. 1998. A structural scaffolding of intermediate filaments in health and disease. *Science* **279:**514–519

Galbraith, C.G., Sheetz, M.P. 1998. Forces on adhesive contacts affect cell function. *Curr. Opin. Cell Biol.* **10:**566–571

Gimbrone, M.A. Jr., Nagel, T., Topper, J.N. 1997. Biomechanical activation: an emerging paradigm in endothelial adhesion biology. *J. Clin. Invest.* **99:**1809–1813

Glogauer, M., Arora, P., Chou, D., Janmey, P.A., Downey, G.P., McCulloch, C.A.G. 1998. The role of actin-binding protein 280 in integrin-dependent mechanoprotection. *J. Biol. Chem.* **273:**1689–1698

Glogauer, M., Arora, P., Yao, G., Sokholov, I., Ferrier, J., McCulloch, C.A.G. 1997. Calcium ions and tyrosine phosphorylation interact coordinately with actin to regulate cytoprotective responses to stretching. *J. Cell Sci.* **110:**11–21

Glogauer, M., Ferrier, J., McCulloch, C.A.G. 1995. Magnetic fields applied to collagen-coated ferric oxide beads induce stretch-activated Ca^{2+} flux in fibroblasts. *Am. J. Physiol.* **269:**C1093–C1104

Glogauer, M., McCulloch, C.A. 1992. Introduction of large molecules into viable fibroblasts by electroporation: optimization of loading and identification of labeled cellular compartments. *Exp. Cell Res.* **200:**227–234

Goldman, R.D., Khuon, S., Chou, Y.H., Opal, P., Steinert, P.M. 1996. The function of intermediate filaments in cell shape and cytoskeletal integrity. *J. Cell Biol.* **134:**971–983

Goode, B.L., Wong, J.J., Butty, A., Peter, M., McCormack, A.L., Yates, J.R., Drubin, D.G., Barnes, G. 1999. Coronin promotes the rapid assembly and crosslinking of actin filaments and may link the actin and microtubule cytoskeletons in Yeast. *J. Cell Biol.* **144:**83–98

Green, K.J., Goldman, R.D. 1986. Evidence for an interaction between the cell surface and intermediate filaments in cultured fibroblasts. *Cell Motility and the Cytoskeleton* **6:**389–405

Guilak, F., Jones, W.R., Ting-Beall, H.P., Lee, G.M. 1999. The deformation behavior and mechanical properties of chondrocytes in articular cartilage. *Osteoarthritis Cartilage* **7:**59–70

Guo, L., Degenstein, L., Dowling, J., Yu, Q.C., Wollmann, R., Perman, B., Fuchs, E. 1995. Gene targeting of BPAG1: Abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. *Cell* **81:**233–243

Hamm-Alvarez, S.F., Sheetz, M.P. 1998. Microtubule-dependent vesicle transport: modulation of channel and transporter activity in liver and kidney. *Physiol. Rev.* **78:**1109–1129

Hartwig, J.H., Kwiatkowski, D.J. 1991. Actin-binding proteins. *Curr. Opin. Cell. Biol.* **3:**87–97

Heidemann, S.R., Kaech, S., Buxbaum, R.E., Matus, A. 1999. Direct observation of the mechanical behaviors of the cytoskeleton in living fibroblasts. *J. Cell Biol.* **145:**109–122

Henrion, D., Terzi, F., Matrougui, K., Duriez, M., Boulanger, C.M., Colucci-Guyon, E., Babinet, C., Briand, P., Friedlander, G., Poitevin, P., Levy, B.I. 1997. Impaired flow-induced dilation in mesenteric resistance arteries from mice lacking vimentin. *J. Clin. Invest.* **100:**2909–2914

Hobert, O., Moerman, D.G., Clark, K.A., Beckerie, M.C., Ruvkun, G. 1999. A conserved LIM protein that affects muscular adherens junction integrity and mechanosensory function in caenorhabditis elegans. *J. Cell Biol.* **144:**45–57

Ingber, D.E. 1998. Cellular basis of mechanotransduction. *Biol. Bull* **194:**323–325

Ingber, D.E., Folkman, J. 1989. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro:* Role of extracellular matrix. *J. Cell Biol.* **109:**317–330

Ives, C.L., Skin, S.G., McIntire, L.V. 1986. Mechanical effects on endothelial cell morphology: In vitro assessment. *In Vitro Cell. Devel. Biol.* **22:**500–507

Janmey, P.A. 1998. The cytoskeleton and cell signaling: Component localization and mechanical coupling. *Physiol. Revs.* 78(3):763–781.

Kato, H., Osajima, A., Uezono, Y., Okazaki, M., Tsuda, Y., Tanaka, H., Oishi, Y., Izumi, F., Nakashima, Y. 1999. Involvement of PDGF in pressure-induced mesangial cell proliferation through PKC and tyrosine kinase pathways. *Am. J. Physiol.* **277:**F105–F112

Kizer, N., Guo, X.L., Hruska, K. 1997. Reconstitution of stretch-activated cation channels by expression of the α-subunit of the epithelial sodium channel cloned from osteoblasts. *Proc. Natl. Acad. Sci. USA* **94:**1013–1018

Lee, J., Ishihara, A., Oxford, G., Johnson, B., Jacobson, K. 1999. Regulation of cell movement is mediated by stretch-activated calcium channels. *Nature* **400:**382–386

Lehtonen, J.Y., Kinnunen, P.K. 1995. Phospholipase A2 as a mechanosensor. *Biophys. J.* **68:**1888–1894

Levina, N., Totemeyer, S., Stokes, N.R., Louis, P., Jones, M.A., Booth, I.R. 1999. Protection of *Escherichia coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. *EMBO J.* **18:**1730–1737

Loo, D.T., Kanner, S.B., Aruffo, A. 1998. Filamin binds to the cytoplasmic domain of the beta1-integrin. Identification of amino acids responsible for this interaction. *J. Biol. Chem.* **273:**23304–23312

Maguire, G., Connaughton, V., Prat, A.G., Jackson Jr., R., Cantiello, H.F. 1998. Actin cytoskeleton regulates ion channel activity in retinal neurons. *NeuroReport* **9:**665–670

Maingret, F., Fosset, M., Lesage, F., Lazdunski, M., Honore, E. 1999a. TRAAK is a mammalian neuronal mechano-gated K^+ channel. *J. Biol. Chem.* **274:**1381–1387

Maingret, F., Patel, A.J., Lesage, F., Lazdunski, M., Honore, E. 1999b. Mechano- or acid stimulation, two interactive modes of activation of the TREK-1 potassium channel. *J. Biol. Chem.* **274:**26691–26696

Maniotis, A.J., Chen, C.S., Ingber, D.E. 1997. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA* **94:**849–854

McConkey, D.J., Orrenius, S. 1997. The role of calcium in the regulation of apoptosis. *Biochem. Biophys. Res. Commun.* **239:**357–66

McNeil, P.L., Steinhardt, R.A. 1997. Loss, restoration, and maintenance of plasma membrane integrity. *J. Cell Biol.* **137:**1–4

Meldrum, R.A., Bowl, M., Ong, S.B., Richardson, S. 1999. Optimization of electroporation for biochemical experiments in live cells. *Biochem. Biophys. Res. Commun.* **256:**235–239

Mills, L.R., Morris, C.E. 1998. Neuronal plasma membrane dynamics evoked by osmomechanical perturbations. *J. Membrane Biol.* **166:**223–235

Morris, C.E. 1990. Mechanosensitive Ion Channels. *J. Membrane Biol.* **113:**93–107

Nichol, J.A., Hutter, O.F. 1996. Tensile strength and dilatational elasticity of giant sacolemmal vesicles shed from rabbit muscle. *J. Physiol.* **493:**187–198

Niles, W.D., Malik, A.B. 1999. Endocytosis and exocytosis events regulate vesicle traffic in endothelial cells. *J. Membrane Biol.* **167:**85–101

Opsahl, L.R., Webb, W.W. 1994. Transduction of membrane tension by the ion channel alamethicin. *Biophys J.* **66:**71–74

Pekny, M., Johansson, C.B., Eliasson, C., Stakeberg, J., Wallen, A., Perlmann, T., Lendahl, U., Betsholtz, C., Berthold, C.H., Frisen, J. 1999. Abnormal reaction to central nervous system injury in mice lacking glial fibrillary acidic protein and vimentin. *J. Cell. Biol.* **145:**503–514

Pender, N., McCulloch, C.A.G. 1991. Quantification of actin polymerization in two human fibroblast subtypes responding to mechanical stretching. *J. Cell Sci.* **100:**187–193

Picart, C., Discher, D.E. 1999. Actin protofilament orientation at the erythrocyte membrane. *Biophys. J.* **77:**865–878

Pourati, J., Maniotis, A., Spiegel, D., Schaffer, J.L., Butler, J.P., Fredberg, J.J., Ingber, D.E., Stamenovic, D., Wang, N. 1998. Is cytoskeletal tension a major determinant of cell deformability in adherent endothelial cells? *Am. J. Physiol.* **274:**C1283–C1289

Raucher, D., Sheetz, M.P. 1999. Membrane Expansion Increases Endocytosis Rate during Mitosis. *J. Cell Biol.* **144:**497–506

Rivero, F., Koppel, B., Peracino, Bozzaro, S., Siegert, F., Weijer, C.J., Schleicher, M., Albrecht, R., Noegel, A.A. 1996. The role of the cortical cytoskeleton: F-actin crosslinking proteins protect against osmotic stress, ensure cell size, cell shape and motility, and contribute to phagocytosis and development. *J. Cell Sci.* **109:**2679–2691

Rizzo, V., McIntosh, D.P., Oh, P., Schnitzer, J.E. 1998. *In situ* flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J. Biol. Chem.* **273:**34724–34729

Rose, F., Kurth-Landwehr, C. Sibelius, U., Reuner, K.H. Aktories, K. Seeger, W., Grimminger, F. 1999. Role of actin depolymerization in the surfactant response of alveolar epithelial type II cells. *Am. J. Respir. Crit. Care Med.* **159:**206–212

Rubin, C.T., Lanyon, L.E. 1985. Regulation of bone mass by mechanical strain magnitude. *Calcif. Tissue Int.* **37:**411–417

Sachs, F. 1988. Mechanical Transduction in Biological Systems. *CRC Critical Reviews in Biomedical Engineering* **15:**141–144

Sachs, F., Morris, C.E. 1998. Mechanical transduction in nonspecialized cells. *Revs. Physiol. Biochem. Pharma.* **132:**1–78

Sackin, H. 1995. Mechanosensitive channels. *Annu. Rev. Physiol.* **57:**333–353

Sai, X., Naruse, K., Sokabe, M. 1999. Activation of $pp60^{src}$ is critical for stretch-induced orienting response in fibroblasts. *J. Cell Sci.* **112:**1365–1373

Santella, L., D Rison, L., Gragnaniello, G., Kyozuka, K. 1999. Cortical granule translocation during maturation of starfish oocytes requires cytoskeletal rearrangement triggered by InsP2-mediated Ca^{2+} release. *Exp. Cell. Res.* **248:**567–574

Shcherbatko, A., Ono, F., Mandel, G., Brehm, P. 1999. Voltage-dependent sodium channel function is regulated through membrane mechanics. *Biophys. J.* **77:**1945–1959

Sheetz, M.P., Dai, J. 1996. Modulation of membrane dynamics and cell motility by membrane tension. *Trends in Cell Biol.* **6:**85–89

Smith, P.G., Tokui, T., Ikebe, M. 1995. Mechanical strain increases contractile enzyme activity in cultured airway smooth muscle cells. *Am. J. Physiol.* **268:**L999–L1005

Steinhardt, R.A., Bi, G., Alderton, J.M. 1994. Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science* **263:**390–393

Terasaki, M., Miyake, K., McNeil, P.L. 1997. Large plasma membrane disruptions are rapidly resealed by Ca^{2+}-dependent vesicle-vesicle fusion events. *J. Cell Biol.* **139:**63–74

Thion, L., Mazars, C., Nacry, P., Bouchez, D., Moreau, M., Ranjeva, R., Thuleau, P. 1998. Plasma membrane depolarization-activated calcium channels, stimulated by microtubule-depolymerizing drugs in wild-type Arabidopsis thaliana protoplasts, display constitutively large activities and a longer half-life in ton 2 mutant cells affected in the organization of cortical microtubules. *Plant J.* **13:**603–610

Togo, T., Alderton, J.M., Bi, G.Q., Steinhardt, R.A. 1999. The mechanism of facilitated cell membrane resealing. *J. Cell. Sci.* **112:**719–731

Turner, C.H., Pavalko, F.M. 1998. Mechanotransduction and function response of the skeleton to physical stress: the mechanisms and mechanics of bone adaptation. *J. Orthop. Sci.* **3:**346–355

Valentijin, K.M., Gumkowski, F.D., Jamieson, J.D. 1999. The subapical actin cytoskeleton regulates secretion and membrane retrieval in pancreatic acinar cells. *J. Cell. Sci.* **112:**81–96

Vassar, R., Coulombe, P.A., Degenstein, L., Albers, K., Fuchs, E. 1991. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* **64:**365

Viel, A., Branton, D. 1996. Spectrin: on the path from structure to function. *Curr. Opin. Cell Biol.* **8:**49–55

Wan, X., Juranka, P., Morris, C.E. 1999. Activation of mechanosensitive currents in traumatized membrane. *Am. J. Physiol.* **276:**C318–C327

Wilkinson, N.C., Gao, F., Hamill, O.P. 1998. Effects of mechano-gated cation channel blockers on *Xenopus* oocyte growth and development. *J. Membrane Biol.* **165:**161–174

Williams, M.W., Bloch, R.J. 1999. Extensive but coordinated reorganization of the membrane skeleton in myofibers of dystrophic (mdx) Mice. *J. Cell Biol.* **144:**1259–1270

Yang, Y., Dowling, J., Yu, QC., Kouklis, P., Cleveland, D.W., Fuchs, E. 1996. An essential cytoskeletal linker protein connecting actin microfilaments to intermediate filaments. *Cell* **86:**655–665