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Introduction

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1.1 Mechanotransduction – Historical Development

Julius Wolff, a nineteenth-century anatomist, first observed that bone will adapt to the stresses it experiences and is capable of remodeling if the state of stress changes. This became known as Wolff's Law and stands today as perhaps the earliest recognized example of the ability of living tissues to sense mechanical stress and respond by tissue remodeling (see Chapter 17 for a detailed historical review). More recently, the term "mechanotransduction" has been introduced to represent this process, often including the sensation of stress, its transduction into a biochemical signal, and the sequence of biological responses it produces. Here we use mechanotransduction in a somewhat more restricted sense, and specifically use it for the process of stress sensing itself, transducing a mechanical force into a cascade of biochemical signals.

Since Wolff's early insight, the influence of mechanical force or stress has become increasingly recognized as one of the primary and essential factors controlling biological function. We now appreciate that the sensation of stress occurs at cellular or even subcellular scales, and that nearly every tissue and every cell type in the body is capable of sensing and responding to mechanical stimuli. Another manifestation of mechanotransduction is known as Murray's Law [1, 2], which states that the flow rate passing through a given artery scales with the third power of its radius. This has been widely recognized to be a response of the arterial endothelium and the smooth muscle cells to remodel the arterial wall to maintain a nearly constant level of hemodynamic shear stress (at $\sim$ 1 Pa), leading to the third power relationship.[1] One aspect of this response is the alignment of endothelial cells in the direction of stress, first observed in studies of arterial wall morphology [4], and later vividly demonstrated in controlled *in vitro* experiments [5]. Other biological factors, such as soft tissue remodeling [6], changes in the thickness of the arterial wall in response to circumferential stress [7], calcification in the heart valve tissue in response to pathological solid and fluid mechanical patterns, and bone loss in microgravity [8, 9], have all been found to be influenced by mechanical stress.

[1] Although accepted for years, recent evidence casts doubt on the validity of Murray's Law and suggests instead that flow rate varies as the vessel radius to the second power [3].

Mechanotransduction is also instrumental in our other senses, touch and hearing in particular [72]. Hearing, for example, is recognized to be mediated by the tension produced in a small filament, termed a tip-link, connecting adjacent stereocilia that project from the surface of the inner and outer hair cells in the form of a conical bundle of fine filaments and can be in direct contact with the tectorial membrane. Oscillations of the membrane cause the stereocilia to slide relative to one another, inducing tension in the tip-link. As a result of this tension, a channel is activated that leads to an increase in calcium ion concentration, initiating a signal transmitted to the brain and heard as sound.

Not only are cells exquisitely sensitive to *externally* imposed stress, but they also *generate* stresses internally, by actomyosin contractions, for example, that allow a cell to probe its mechanical environment, presumably through the response of the surrounding extracellular matrix to these internally generated forces. This is likely an important factor in biological development, guiding cells through a series of mechanical cues (in addition to the more widely studied biochemical ones), and influencing cellular differentiation (see Chapter 17). Stresses have recently been shown to guide the differentiation of stem cells (see Chapter 17); mesenchymal stem cells will differentiate into an osteogenic phenotype when subjected to low levels of strain [10], but into a cardiovascular lineage at higher strains [11]. Other types of cell behavior are also influenced by the stiffness of the matrix on which they are grown, and it is becoming clear that cells can sense their mechanical environment and respond accordingly [12]. Phenomena such as these give rise to the concept of mechanical signaling, both outside-in and inside-out, discussed again later in this chapter and this book.

1.2 Role of Mechanotransduction in Disease

Aside from its central role in a variety of normal, even essential, biological functions, mechanotransduction has a dark side, in that it has also been demonstrated to be a major factor in many pathological processes. We have known for many years that thickening and calcification of the arterial wall associated with atherosclerosis occurs predominantly at localized sites in the circulation of "disturbed flow" – regions prone to complex flow patterns, or low and possibly reversing hemodynamic shear stress. Studies over the past 30 years have led to an increasing appreciation of the central role played by the arterial endothelium, in the initial thickening of the arterial wall intima [13], to the recruitment and activation of circulating monocytes [14, 15], to the changes in endothelial permeability [16, 17], all of which contribute to disease progression. Other studies have demonstrated a link between mechanotransduction and arthritis [18], damage to articular cartilage [19], asthma [20, 21], other types of pulmonary diseases and lung injuries [22], and polycystic kidney disease [23]. (See [24] for a recent review.) These processes are mediated by a host of signaling cascades that are initiated by shear stress, and these are discussed in Chapter 2.

In this context, it is useful to discuss the magnitude of the mechanical stimuli that elicit a biological response. For example, in the vascular system, mean values of shear

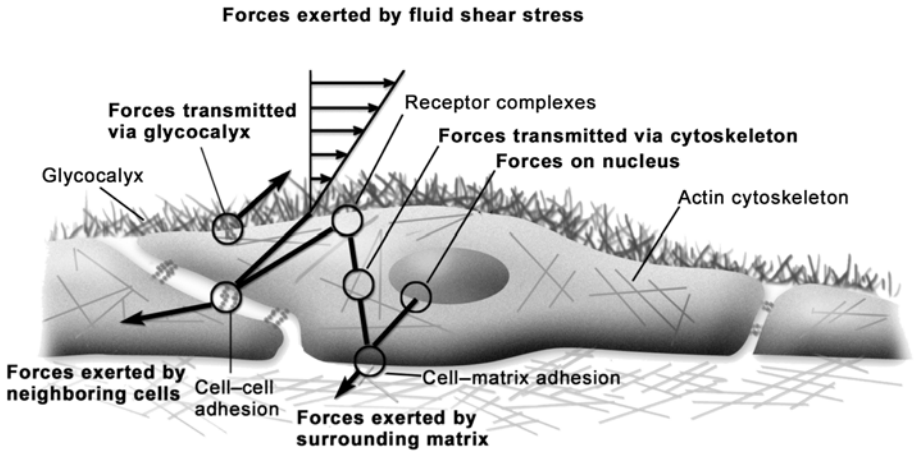

Figure 1.1. Forces experienced by endothelial cells lining a vessel wall (in bold) and the structures, both intra- and extracellular, that transmit these forces (nonbold).

stress associated with arterial blood flow can range from 0.1 to 10 Pa, with even wider variations observed instantaneously during the cardiac cycle; ample evidence exists to suggest that endothelial cells sense this level of stress and regulate their behavior accordingly. Similarly, changes in internal pressure give rise to circumferential strains in the arterial wall in the range of 2 to 18% [25] (see also Chapter 16). Strains of comparable magnitude occur in the lung, so all the cell types contained either in the lung or arterial wall are subjected to these levels of deformation. Airway epithelial cells have also been shown to be responsive to transepithelial pressures in the range of those induced by airway smooth muscle activation, about 1 to 4 kPa [26], and alveolar epithelium has been demonstrated to be stretch sensitive [22]. At the other extreme is bone, where the strains are much smaller, on the order of 1000 με [73]. Even these minute strains, however, are known to be sensed by resident cells, and it has been suggested that bone possesses a special mechanism to enhance sensitivity [27].

1.3 *In Vitro* Tests of Mechanosensation

Its critical role in disease has led investigators to develop a wide variety of experimental tools to probe the effects of mechanotransduction, both *in vivo* and *in vitro*. And because they enable closer control of the various factors, *in vitro* experiments have proven to be particularly informative. These can be categorized in terms of the nature in which force is applied, as illustrated in Figure 1.1, and are discussed in more detail in Chapter 16.

Shear Stress. One of the first observed manifestations of force on cell function was the alignment of endothelial cells subjected to shear stress (Figure 1.2(a)), so it is not surprising that shear stress was one of the first methods used to elicit a response *in vitro*. Several geometries have been utilized including simple unidirectional flow chambers, where the cells, grown on one surface of a rectangular channel, are

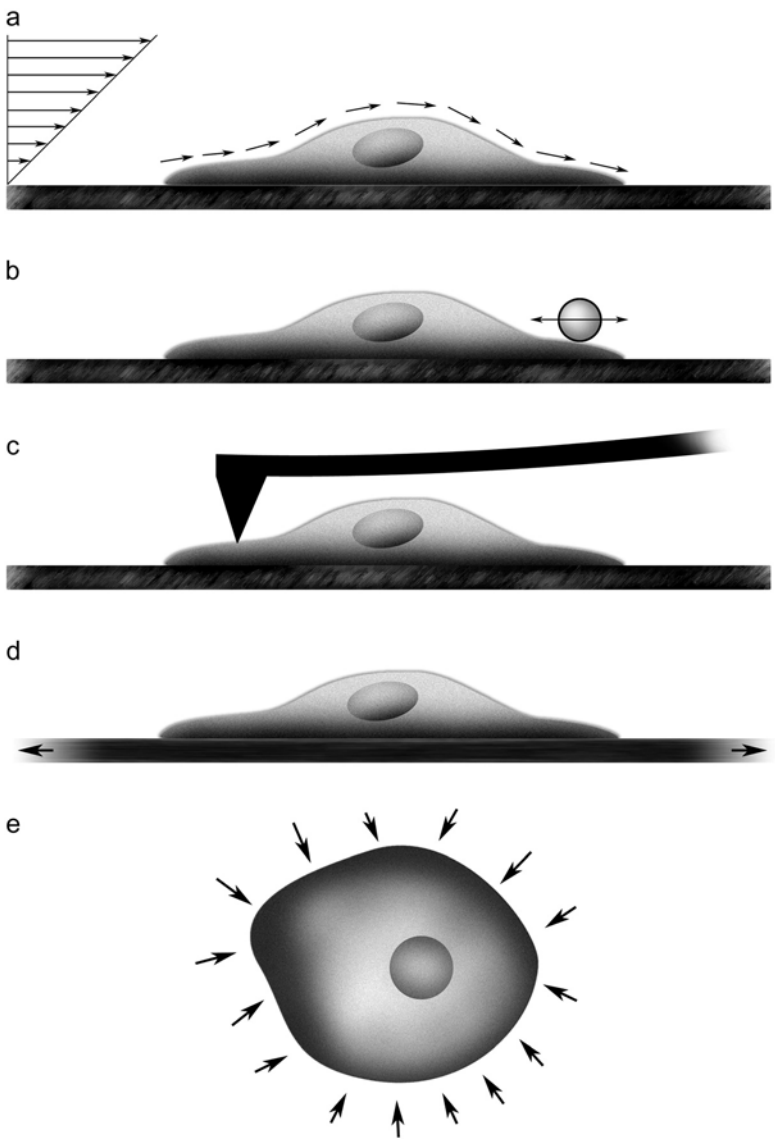

Figure 1.2. Various methods used to apply force to cells either *in vitro* or *in vivo*. (a) Fluid shear stress. (b) Forces applied to microbeads that are tethered to the cell via membrane receptors. (c) Indentation by an atomic force microscope probe. (d) Substrate stretch. (e) Hydrostatic pressure.

exposed to the shear associated with a fully developed flow of medium. Advantages of this technique are its simplicity and, provided an adequate entrance length is used to produce fully developed flow, a uniform shear stress that can be either steady or unsteady, depending only on the ability to produce time-dependent flow waveforms. An alternative system that also produces a well-defined, time-dependent, and spatially uniform shear stress distribution is the cone-and-plate rheometer [5]. By controlling the rotation of the cone, any time-varying shear stress existing in the

circulation can be reproduced. One complicating factor, however, is that the shear stress acting on a given cell is nonuniform due to its uneven surface contour, and even the average shear stress can vary from cell to cell in a given monolayer, due to surface variations [28]. In addition, transmission of shear stress to the cell is known to be mediated by the glycocalyx, a surface glycoprotein layer that coats the apical surface of most endothelial monolayers (see Chapter 2). Not surprisingly, the presence or absence of a glycocalyx has been found to be a major determinant of the cell's response to shear stress [29].

Bead Forcing. At times, it can be useful to apply force in a more localized manner, and with improved force or displacement control or measurement accuracy. To meet these objectives, many have turned to the use of micron-sized beads or microspheres that can be tethered to the cell's membrane receptors by using coatings of an appropriate ligand and manipulated using either magnetic or optical traps (Figure 1.2(b)). In a magnetic trap, paramagnetic or ferromagnetic beads are used, and force is generated by an externally imposed magnetic field. Either linear force or rotational torque can be applied, while the bead's motion is monitored optically. With an optical trap or tweezers, forces are produced that draw the bead toward the center of a focused laser beam, and displacements are, once again, measured optically. In either of these cases, interpretation of the force-displacement data is subject to a number of uncertainties (e.g., strength of attachment to the cell, assumptions concerning the relative importance of membrane and cytoskeleton, active response of the cell to forcing), however, and these methods have been criticized on the basis that they are nonphysiological. Interestingly, endothelial cells exhibit a definitive response to bead forcing at a force level of about 1 nN, roughly corresponding to the shear stress of 1 Pa integrated over the surface area of a typical cell, suggesting that similar processes may be responsible for an endothelial cell's response to blood flow [30].

Atomic Force Microscopy (AFM). Similar experiments can be conducted using the tip of an AFM, either in its normal configuration or with a microbead attached to the cantilever in place of the pyramidal tip (Figure 1.2(c)). An advantage of the AFM is its excellent spatial resolution, but among its disadvantages are that it is difficult to simultaneously apply force to the cell and observe its response.

Substrate Stretch. Cells in the heart, the walls of arteries and veins, and in the lung are all subjected to cyclic strain or stretch, which also influences their behavior. Experimentalists have developed a variety of methods and devices to simulate these effects. In most, cells are grown on a flexible membrane and the membrane is stretched either by mechanical or hydraulic/pneumatic actuation (Figure 1.2(d)). Stretch can be uniaxial or biaxial, and either static or oscillatory. It can also act in a synergistic manner with shear stress, as discussed in Chapter 15. Cells in three-dimensional matrices or gels can also be subjected to strains, for example, by unconfined compression of the gel, which better mimics the environment of chondrocytes, for example.

One of the often ignored limitations of all of these experiments, and virtually all *in vitro* experiments, in fact, is that they almost universally are limited in duration to several days. Some *in vivo* studies have examined changes that occur over longer periods of time; these rarely look at the mechanism of mechanotranduction, but rather, the long-term remodeling that occurs in response to mechanical stimuli. These time-scales need to be compared to those of disease progression, where the changes generally occur over many years. For example, cartilage or joint damage from sports injuries often leads to degeneration and arthritis later in life. Apparently mechanical trauma initiates a sequence of biological events that ultimately lead to deterioration of the cartilage. While short-term experiments are obviously enormously useful and provide important insights into the longer term processes from studies of the initial event, some degree of speculation regarding the detailed connections between the two is always necessary, and represents an important area of ongoing research.

Often, the response to strain is complex. Of particular interest is that stem cells have been shown to change their differentiation pathway depending on the *level* of strain they experience, differentiating into an osteogenic lineage under low strains [10] but a vascular or muscle lineage at higher strains, greater than about 5% [11].

Hydrostatic Pressure. In most instances of mechanotransduction, there is a clear and measurable deformation that occurs in connection with the applied force, so most of the mechanisms described in the following are possible. With hydrostatic pressure (Figure 1.2(e)), however, especially at normal physiological loads, the amount of deformation experienced by the cell is minute, corresponding essentially to the compressibility of water, so the mechanisms of force sensation are less obvious. Numerous studies have been published, however, showing cellular responses to changes in hydrostatic pressure as small as 0.4 kPa [31–33], and it has been postulated that the response may be associated with a corresponding change in membrane-free volume and membrane fluidity, and consequently in the mobility of membrane-associated proteins [34]. Since the mechanism remains unclear, this continues to be an active area of investigation.

1.4 A Focus on Basic Mechanisms

Numerous reviews have been written addressing the signaling pathways that become activated by mechanical stress, the second messengers that convey these signals, and the changes in biological function that occur due to changes in gene expression, protein synthesis, and post-translational processing (see, e.g., [35–37]). In this collection of chapters, we focus instead on the fundamental mechanisms by which a cell senses and transduces mechanical force. That is, we address the factors that activate the various signaling pathways, ultimately leading to the observed biological response, and refer the reader to these other excellent sources for illumination of the detailed pathways that lie downstream of these initiating events. Each of these basic mechanisms is discussed in at least one chapter in this book, and we provide here just a brief summary of the concepts described in much more detail in later chapters.

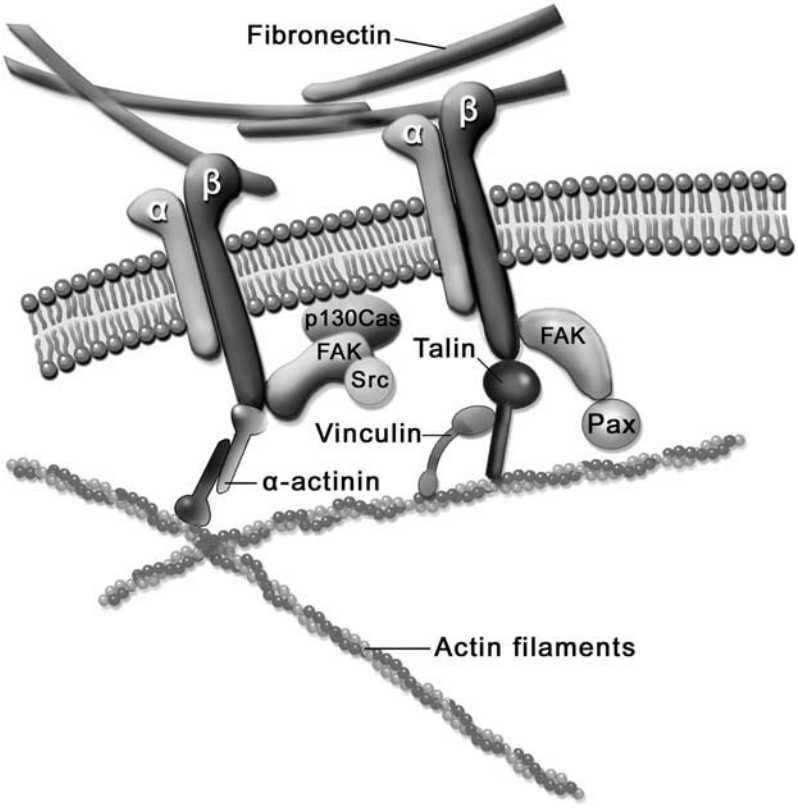

Figure 1.3. Some of the proteins within a focal adhesion that transmit forces from the extracellular matrix, across the cell membrane, to the cytoskeleton. Any of these force-transmitting proteins are candidates for mechanosensation through force-induced conformational change.

In the simplest of terms, mechanotransduction can be viewed as any force-induced process that initiates a biochemical response. That response could be as simple as changing the binding affinity of one protein to another or altering the phosphorylation state of a protein; or the response could be more complex, such as initiating a signaling pathway with a range of downstream consequences including changes in gene expression, protein synthesis, or change in cellular phenotype.

In the quest for mechanosensors, it seems logical to look first at those sites where forces might be amplified. Some of these are obvious, such as the sites where the cell anchors itself to its environment, either at focal adhesions (Figure 1.3) or cell–cell junctions. Others are less evident, since we know that the cell has the capability to focus forces at locations remote from the site of force application (e.g., [38]) and that forces such as shear stress, for example, tend to be distributed over large regions of the cell. In this section, we discuss evidence for a variety of transduction mechanisms, some requiring localized force and others for which the forces are spatially dispersed.

Stretch-Activated Channels. An enormous variety of ion and water channels have been identified and characterized, a small subset of which has been identified or

proposed to be mechanosensitive (e.g., see Chapter 6). Some of these have been well characterized, and a few have even been modeled in a way that provides some insight into the transduction mechanism involved, two of which will be mentioned here. Even these, however, as well as others that are much less well understood, remain the subject of considerable debate.

One channel that has received considerable attention is found in the stereocilia of hair cells in the inner ear, which initiate the signals that are ultimately transmitted to the central nervous system, allowing us to hear. The mechanics of this system are fascinating, especially with regard to the mechanism of activation, which has largely been discovered. A cone-shaped collection of stereocilia is located on the top of a hair cell in the inner ear. These communicate at their tips with the tectorial membrane, which is in contact with the fluid of the inner ear and oscillates due to the propagation of waves in the chochlea [74]. As the membrane oscillates, the stereocilia move back and forth, and as they do, the tension in a small filament that connects the tip of one cilium with the side of its neighbor varies. Although the detailed arrangement is still being elucidated, a stretch-activated channel is known to be located near the attachment point of the filament on the lateral side of the cilium. As force is applied by the connecting filament, the channel's conductance changes, giving rise to a transient rise in Ca^{2+} concentration. Although the details are less clear, there also appears to be a mechanism to adjust the resting level of stress acting on the channel, and this provides a potential means of ''tuning'' its sensitivity (see Chapter 6).

A second channel, the mechanosensitive channel of large conductance (MscL), is found in bacteria but is notable because its crystal structure is known and it can therefore be studied by molecular dynamics. The MscL is known to be activated at levels of tension of $\sim$ 10 mN/m [39]. Two groups have investigated the change in conformation of MscL when membrane stress is applied [40, 41], showing that an initial conformational change can be induced by tensions of this magnitude. However, full activation was not achieved in this simulation, suggesting that this may be the initiating event in a sequence that ultimately leads to the observed change in conductance.

Shear stress or flow is also known to give rise to changes in the channel conductance of, for example, Na^+, K^+, or Ca^{2+}; however, the mechanisms are less well understood and could result from force interactions of the channel either with membrane lipids or the cortical matrix [42–44], and in the case of membrane interactions, either membrane tension or membrane curvature has been implicated [42, 44]. Whatever the mechanism, these channels are exquisitely sensitive, being affected by shear stresses as low as 0.01 Pa. Given the low energy levels required for shear activation (as low as $\sim$ 0.01 kT), it seems unlikely that flow is the direct effector of channel activation in the presence of thermal noise [45]. What role the cortical cytoskeleton or glycocalyx plays in this process remains a subject of debate [46].

Membrane Mechanotransduction. Other membrane-associated proteins have also been implicated in mechanotransduction, in particular G-proteins, G-protein coupled receptors, and various proteins that are found in focal adhesion complexes or the cytoskeleton. These mechanisms are discussed in detail in Chapter 4.

Experiments on G-proteins isolated in lipid vesicles and subjected to shear can become activated by shear [47], showing convincingly that G-proteins by themselves can act as mechanosensors, and that these effects appear to be associated with changes in membrane viscosity.

Changes in membrane fluidity have also been proposed as an initiating event. These ideas arose from early work by Butler [48], demonstrating that membrane fluidity increased in response to shear stress. They hypothesized that this could initiate a mechanoresponsive event through one of several mechanisms. First, increased membrane fluidity implies an increase in diffusivity of the transmembrane, or membrane-associated proteins, and lipids. If the reaction is diffusion limited, then an increase in fluidity would be expected to increase the likelihood of protein interaction [49]. G-proteins and G-protein complexes have been the focus of much attention, since G-protein hydrolysis is diffusion dependent and G-proteins are often implicated in mechanosensation.

Membrane stress, either in tension or in bending, can also directly influence the conformation of transmembrane proteins, or consequently could influence their tendency for activation or interaction with other proteins. This is especially relevant to structures such as calveoli [77], where a relatively minor increase in membrane tension could produce large changes in membrane curvature. This has recently been reported in connection with MAPK activation [50]. Other potential sites that might be influenced by membrane stress include lipid rafts, where G_i-proteins (Chapter 4), frequently implicated in mechanotransduction, are often found. Stress might also alter the thickness of the lipid bilayer, which, due to the complex hydrophobic interactions within the membrane and their influence on protein conformation, could also influence protein function. The primary mechanisms for these effects remain largely unknown.

Mechanotransduction in Focal Adhesion Complexes. A considerable amount of evidence has been reported regarding the role of focal adhesion proteins in transduction events (see Chapter 5). In one set of experiments, cells were first grown on a compliant substrate, and then the cell membranes were removed by application of a detergent, Triton X. The cells were then stretched in the presence of a variety of cytoskeletal proteins that contained a photocleavable botin tag [51]; by comparing the newly bound proteins to stretched and nonstretched cells, those proteins that preferentially bind to stretched cytoskelatal networks could be identified. From these experiments, binding of paxillin, focal adhesion kinase, p130Cas, and PKB/Akt were all found to be enhanced under 10% stretch, providing convincing evidence that mechanotransduction is not simply a membrane-mediated process, and that the focal adhesion complex contains a variety of proteins whose binding affinities are influenced by stretch.

Another vivid demonstration of this can be found in experiments by Wang et al. [52], who developed an assay for activation consisting of phosphorylation of a domain taken from a cSRC subtrate, p130Cas. Activation, in this case, produced a conformational change that could be observed by FRET and exhibited a wave of activation emanating from the site at which a tethered bead applied force to the cell.

In another recently developed technique, the fact that cysteines, which are generally buried in the protein core due to their hydrophobicity and inaccessible, become exposed by the application of force to a cell or single protein [53] is used as a means of detecting these changes in conformation. Introduction of a thiol-reactive fluorescent dye (IAEDANS) to the cell creates a fluorescent signal indicative of cysteine exposure and binding to the IAEDANS. This has been used to explore unfolding in spectrin, nonmuscle myosin IIA, and vimentin, but the method has the potential to be applied to a wide variety of proteins.

Role of the Glycocalyx in Mechanotransduction. Recently, it has been increasingly recognized, especially in the context of endothelial mechanotransduction, that the lipid bilayer is rarely subjected to fluid shear stress directly, but that stresses are instead transmitted via the glycocalyx, the glycoprotein layer that coats most endothelial cells *in vivo* and *in vitro*. Studies have convincingly demonstrated that many of the known responses of the endothelial cells to fluid shear are dramatically influenced by whether or not this layer is intact [54, 55]. This dependence seems a logical consequence of the fact that forces transmitted via the glycocalyx connect with different intracellular structures than forces applied directly to the bilayer. Studies are now attempting to determine which membrane or intracellular structures ultimately bear the load from the glycocalyx, and how these forces are subsequently distributed throughout the cell [29].

Cytoskeletal Transduction. Since the cytoskeleton is the primary pathway for force or stress transmission through the cell, some have suggested that one or more of its component proteins might serve as mechanosensors (see, e.g., Chapters 7, 8, and 10). Some direct evidence for this already exists. Several cytoskeletal proteins have been suggested as mechanosensors, including some actin cross-linking proteins [75] as well as microtubules [56]. Again, these seem likely candidates due to their role in force transmission. For example, forces sufficient to bend or possibly break a microtubule can influence the rate of filament growth or the binding of microtubule-associated proteins [56]. Similarly, forces acting through actin cross-linking proteins can rupture the bond or cause domain unfolding, either of which can lead to cytoskeletal remodeling or changes in the actin microstructure.

Direct Effects of Force on Gene Expression. It has been demonstrated in various ways, both *in vivo* and *in vitro*, that forces are transmitted to the nucleus via the surrounding cytoskeleton, causing changes in the nuclear shape [57, 58]. Just as forces acting on cytoskeletal proteins can change their conformation, DNA can be unwound under applied force to expose a transcription sequence. Pulling on a single strand of DNA can cause histone release and nucleosomal disruption [59], so it is not unreasonable to hypothesize that force could also influence gene expression and replication. Although it has been demonstrated that forces are, indeed, transmitted to the nucleus, evidence for direct control of gene expression by these transmitted forces has not yet been reported. Forces might also be generated internal to the nucleus, since it contains