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Dynamic molecular processes mediate cellular mechanotransduction

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Abstract

Cell responses to mechanical forces are crucial in embryonic development, adult physiology, and numerous diseases including atherosclerosis, hypertension, osteoporosis, muscular dystrophy, myopathies and cancer. These responses are mediated by load-bearing, sub-cellular structures, such as the plasma membrane, cell adhesions and cytoskeleton. Recent work has demonstrated that these structures are dynamic, undergoing assembly, disassembly and movement, even when ostensibly stable. An emerging insight is that transduction of forces into biochemical signals occurs within the context of these processes. This framework helps explain how forces of varying strengths or dynamic characteristics regulate distinct signaling pathways.

There is growing recognition that mechanical factors, such as applied forces or the rigidity of the extracellular matrix (ECM), critically influence the form and function of cells and organisms[1–3]. Biological regulation has classically been understood through the concepts of solution chemistry, in which enzyme activities, reaction rates and affinities govern cellular processes. However, mechanotransduction, the conversion of mechanical forces into biochemically relevant information, contributes to numerous developmental, physiological and pathological processes and is a major, rapidly advancing area of current research[1].

In the vasculature, blood flow exerts fluid shear stresses on the endothelial cells that line the vessels, while blood pressure stretches the vessel wall[4]. Shear stress is crucial for remodeling the primitive vascular plexus into a hierarchical vascular tree[5] and for patterning the cardiac outflow tract in developing mouse embryos[6]. Hypertension causes thickening of arterial walls and is a major risk factor for cardiovascular diseases[4]. Atherosclerosis, the chronic cholesterol-dependent inflammation of artery walls, occurs preferentially at regions of disturbed flow such as branch points and areas of high curvature, where both the magnitude and temporal characteristics of the flow are disturbed. The development and pathobiology of bone and muscle also depend strongly on mechanical forces from weight

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and muscle contraction, while lung physiology and pathology are strongly influenced by forces from inflation[1].

Tissue rigidity or stiffness affects many biological processes[7]. Tumors have long been identified by palpation due to local increases in tissue stiffness. Recently, these changes in the mechanical environment have been shown to be causal for tumor progression[8]. Fibrotic lung disease begins with a small change in tissue stiffness, which is sensed by cells to induce more severe and irreversible remodeling[9]. Furthermore, the rigidity of the extracellular environment potently controls the differentiation of mesenchymal stem cells[10] and the self-renewal of hematopoietic stem cells[11]. Developing scaffolds with tunable mechanical properties to control cellular behavior has become a major effort in tissue engineering[12].

While applying forces to cells or altering the rigidity of their environment are clearly distinct processes, the underlying mechanisms of mechanotransduction appear to be similar[2]. A key event in rigidity-sensing is the modulation of cellular contractility. Cells on soft materials exert lower forces than cells on stiff materials, decreasing tension on force-bearing elements. These elements are the same whether forces are generated internally or externally[7], thus, many of the cellular responses to distinct mechanical stimuli are similar. Another unifying principle is that the structures that generate and bear cellular forces are involved in sensing forces[1]. Therefore, cytoskeletal proteins such as actin and tubulin are crucial in mediating mechanical effects in nearly all systems[2,13] (Fig.1a and b). Cellular adhesions, both to the extracellular matrix (ECM) and to other cells, are also key, as these structures mechanically connect cells to their surroundings. Correspondingly, candidate genes for diseases that can be considered “mechanotransduction disorders” such as aortic aneurism, heart failure, hypertension or muscular dystrophy include many proteins of the ECM, adhesion complexes and cytoskeleton[14–17]. There are often drastic changes in the protein composition, dynamics, and mechanics of these structures during metastatic progression[18] and stem cell differentiation[7].

While much progress has been made toward understanding mechanotransduction, a complete picture is lacking. Mechanotransduction is typically depicted as a series of rapid switch-like events, activated in response to step-like applications of force, which eventually lead to cellular responses. This level of detail, however, is insufficient to explain the cellular responses to dynamic mechanical stimuli often found in physiological settings.

In this review, we first outline the basic features of the switch-like model of mechanotransduction. We divide this process into mechanotransmission, mechanosensing and mechanoresponse, and then highlight this model’s limitations. Next, we describe some recent advances in our understanding of the dynamic processes regulating load bearing sub-cellular structures and the behavior of single molecules in response to applied forces. With guidance from mathematical models of adhesion assembly, these examples are used to develop a more complete model of mechanotransduction based on the concept that forces alter the rates of key sub-cellular processes to affect cell function. This perspective allows us to understand how cells respond to multiple, time-varying mechanical stimuli. We end by suggesting that mechanically the cell may function as a multi-bandpass filter where stimuli

with different temporal characteristics activate distinct signaling pathways that affect cell state and disease progression.

Switch-like Models of Mechanotransduction

Descriptions of mechanotransduction typically begin with the forces acting on cellular elements and end with the integrated response from the cell or tissue. These can be divided into three steps:

Mechanotransmission

A force must be transmitted to mechanosensitive elements before it can be sensed. For example, the adhesion receptors that mediate cell-cell and cell-ECM contacts are strongly implicated in mechanotransduction[19]. Equally important are the cytoskeletal structures that adhesion receptors universally connect to, which allow adhesions to resist deformation from applied forces. The cytoskeleton is comprised of filaments, including F-actin, intermediate filaments and microtubules, that are relatively stiff on the micrometer dimensional scale and stable on minute-to-hour time scales[20]. This mechanical continuity allows forces to propagate relatively long distances along filaments in the cell, a process called mechanotransmission. Fluid shear stress, for instance, is exerted on the apical domain of endothelial cells, yet the displacements in vimentin filaments are largest at select areas, often near cell-cell and cell-ECM adhesions[21]. This result correlates with data implicating junctional proteins in responses to shear[22]. Twisting of magnetic beads bound to integrins also revealed long distance effects, which were primarily transmitted by F-actin[23], although some studies have proposed a role for microtubules[24] and intermediate filaments[25]. Using a laser trap to directly apply forces as small as 5.5 pico Newton (pN) to actin stress fibers triggered influx of calcium ions, presumably due to the activation of plasma membrane mechanosensitive ion channels[26]. Mechanotransmission and subsequent responses also can be extremely fast, on the order of 100’s of milliseconds[24,27], consistent with direct mechanical effects.

Mechanosensing

Transmitted forces ultimately impinge upon mechanosensitive macromolecules to alter their conformation and hence their function. While the biological consequences of such events are specific to each system, the underlying physical response is similar; forces promote changes in conformation that accommodate the applied force. The best studied examples at the structural level are bacterial mechanically gated ion channels[28], which open in response to increased lateral tension in the plasma membrane during osmotic swelling (Fig.1c). Similar ion-channels are present in all organisms and are essential for survival under changing osmotic conditions[29].

There is also evidence that unfolding of protein domains under tension mediates responses to applied forces. The first reported instance was fibronectin (FN), which self-assembles into fibrils in the ECM. Formation of FN fibrils requires cell-generated force[30]. Conversely, purified FN undergoes self-association when stretched in vitro[31,32]; fibril assembly is mediated by unfolding of domains revealing cryptic binding sites. Another well studied

example is talin-1, which connects integrins to F-actin, thereby transmitting forces between actomyosin filaments and the ECM[33]. Talin-1 binds vinculin, which also links to F-actin and is recruited to adhesions in response to applied forces. Curiously, many of the vinculin binding sites on talin reside within the interior of bundles comprised of 4–5 α-helices, thus, are inaccessible[33]. Both biochemical and cellular studies provide evidence that tension unfolds these bundles to expose vinculin binding sites, thereby enabling vinculin recruitment[34,35] (Fig.1c). Another protein in the cytoplasmic region of integrin-mediated adhesions is the adapter protein p130Cas. When phosphorylated on tyrosines by src family kinases, p130Cas binds several guanine nucleotide exchange factors (GEFs) that activate small GTPases[36]. Stretching cells enhances phosphorylation of these tyrosines, leading to GEF binding and Rap1 activation[37–40]. Studies with purified proteins showed that stretching increases the susceptibility of p130Cas to phosphorylation without changing the intrinsic activity of src family kinases[40]. Although it is unclear how forces might be transmitted across p130Cas in vivo, these studies illustrate the concept that forces can affect substrate availability through effects on protein conformation.

For physiologically significant mechanosensing, these initial conformation changes must be followed by a second step in which the new conformation triggers downstream events. This step can be fairly direct, as for the mechanosensitive ion channels discussed above. In other instances, changes in protein conformation, especially the opening of domains that contain cryptic sites, leads to binding of new proteins that mediate downstream events. Possibilities include reinforcement of the linkage, as in the case of talin and vinculin[34], or the recruitment and activation of new signaling proteins, as in the case of p130Cas[40]. This general sort of model has been invoked in a wide range of mechanotransduction events in many systems[1]. Understanding in detail how protein domains change conformation under force and how subsequent events transpire has been the major direction in this field.

Mechanoresponse

Ultimately, sensed mechanical signals influence information processing through complex cellular signaling and transcriptional networks that are not specifically force-dependent. In many cases, these responses feed back to alter the mechanosensitive structures that initiated the responses. Both integrin- and cadherin-mediated adhesions enlarge and strengthen in response to tension[19]. Distinct from the very rapid, direct recruitment described above, signaling pathways that occur over minutes (e.g. activation of the small GTPase RhoA stimulates formation of actin stress fibers[41]) and gene expression pathways that operate over hours or days (e.g. induction of vinculin through serum response factor[42,43]) change the composition and structure of adhesions and the cytoskeleton.

Similar principles apply at the tissue level. High blood pressure, for instance, results in thickening of artery walls to bear the increased tension, and hypertrophy of the left ventricle of the heart to enable stronger pumping against high back pressure[44]. Analogously, bone deposition increases under weight-bearing exercise[1]. These integrated responses depend on the intensity and time course of stimulation in ways that differ from the initial responses. For example, a single, brief interval of high blood pressure during exercise will stretch vessel

walls and tax the heart but does not trigger compensatory arterial and cardiac remodeling as does sustained hypertension[44].

Limitations of Switch-like Models

In switch-like models of mechanotransduction, applied forces are instantaneously transmitted to load-bearing sub-cellular structures and induce conformation changes in mechanosensitive proteins. Different forces are sensed largely by conformation changes in protein domains that are stronger or weaker, and thus respond to forces of different magnitudes[45]. This view, however, appears incomplete. For example, the frequency of applied cyclic stretch or compression can have major effects. Steady stretch and cyclic stretch of equivalent magnitude induce distinct genes in endothelial cells[46] and induce differential phosphorylation of sites on FAK in rabbit aortas stretched ex vivo[47]. The frequency of applied cyclic stretch also determines endothelial alignment[48]. In aortic smooth muscle cells, stimulation of integrin activation and subsequent cellular alignment by cyclic stretch depends strongly on stretch duration and frequency[49].

These observations are particularly relevant in the vascular system, where shear stress and circumferential stretch in arteries undergo strong, time-dependent variations during the cardiac cycle. Furthermore, these variations are different in various parts of the vasculature[50] and correlate with location of atherosclerotic lesion formation[4]. Interestingly, recent evidence demonstrates that particular frequencies of mechanical stimulation preferentially activate the inflammatory pathways implicated in atherosclerosis, even when the total power applied to the cell is conserved (Feaver, et. al, submitted). The characteristic time between the peaks in wall stretch and fluid shear stress may also critically regulate endothelial activation[51]. These characteristics of mechanotransduction are not readily captured by switch-like models.

Sub-cellular Structures Are Dynamic

These time-dependent aspects of mechanotransduction can be attributed to the highly dynamic characteristics of the cellular components that bear and respond to force. Cell adhesions to the ECM go through a complicated, force-sensitive maturation process[41]. Nascent cell-ECM adhesions are very small structures (<1μm in diameter) at the edges of lamellipodia that usually disassemble within tens of seconds, or else mature into slightly larger focal complexes (FCs) that persist for only minutes[41]. A fraction of these FCs mature into larger focal adhesions (FAs) that persist for tens of minutes. However, even within stable FAs, proteins constantly exchange, with lifetimes from tens of seconds to at most a few minutes[52]. Thus, even stationary FAs undergo rapid internal dynamics.

Detailed analyses from fluorescence speckle microscopy[53] and other high resolution techniques[54] have revealed complex interplay between cytoskeletal and FA dynamics. Actin filaments constantly polymerize at the leading edge of the cell and flow backwards over the FAs, with the speed of this flow influenced by the nature of the linkages between the cytoskeleton, the integrins and the ECM. Actin flow is faster over areas with few FAs or where the FAs undergo treadmilling toward the center of the cell (so-called sliding FAs); whereas actin flow is slower in areas with stable FAs[53]. These and other results[55] led to the

notion of a “clutch” that controls force transmission between the flowing actin and the integrins (Fig 2). Furthermore, in areas with stable FAs, integrins are immobile and actin flows at 0.1–0.2 μm/min; however, several FA proteins display velocities between these limits[53]. These results indicate the presence of multiple proteins that act as clutches, or force-sensitive linkages, within FAs.

While cadherin-dependent adhesions have not been studied in as much detail, available evidence indicates similarities to FAs[2]. Several studies have shown that cell-cell contacts bear significant forces[56,57] and, like FAs, they undergo dynamic, myosin-dependent elongation[58]. Applied forces[56] and stiffer substrata[59] enhance cell-cell contact assembly indicating that these adhesions also exhibit force-dependent adhesion strengthening. There is also evidence for actin flow along cell-cell contacts[60]. While most of the molecular players are different, vinculin is recruited to both structures in a myosin-dependent manner, which contributes to adhesion strengthening[61,62]. These data suggest that the dynamic properties of cell-cell contacts are regulated by processes physically similar to FA regulation.

Single Molecule Response to Dynamic Forces

Studies of single molecules or pairs of molecules under applied force have rarely revealed simple, switch-like behaviors. Of particular relevance, recent work on the effects of force application on the rates of bond dissociation shows that bonds mediating protein-protein interactions can either decrease or increase their average lifetime in response to applied force, referred to as slip or catch bonds, respectively[63,64] (Box 1). Importantly, both the strength of the force and the rate of application affect the rates of protein conformation changes[63,65] (Box 1). The development of assays involving several molecules (i.e. cross-linking protein adhered to F-actin) have revealed competition between force-activated unbinding and conformation changes[66] (Box 1). A current challenge is determining how these molecular processes are integrated to mediate complex phenomenon like mechanotransduction.

Models of Dynamic FAs

FAs offer a convenient system for understanding the relationship between dynamics and mechanotransduction that may be more generally applicable. From a mechanical perspective, FAs are dynamic, deformable links between an elastic ECM and the force-generating actin cytoskeleton[13,41]. Myosin-generated forces are transmitted through the actin cytoskeleton to FA proteins. The applied forces affect the dissociation rates of the FA proteins from integrin receptors, each other, and F-actin. Forces are then transmitted through bound molecules to deform the ECM (Fig. 2).

Several models that address how cells respond to the mechanical properties of the ECM propose that rigidity determines how quickly forces act upon the integrin-actin linkages[13,67,68]. The models can be classified based on whether rapidly applied forces increase or decrease adhesion turnover by promoting adhesion breakage or strengthening. In all cases, FA kinetics are determined by the balance of protein association and dissociation. On soft substrates or in response to slowly applied forces, the on and off rates of molecules into and out of the adhesions, and the lifetimes of the whole adhesions are moderately fast[68]

(Fig. 2). In models without FA strengthening, exposing cells to large, rapidly applied forces or plating cells on rigid substrata increases dissociation of linker molecules like vinculin or talin from the integrin or the actin (slip bond behavior). However, with large numbers of unoccupied sites, there is also rapid rebinding. This model leads to FAs with faster exchange of linker molecules and shorter whole adhesions lifetimes (Fig. 2)[68]. In models with FA strengthening, applied force either results in decreased protein dissociation (catch bond behavior) and/or a conformation change that induces new protein recruitment[67,69]. Either way, force leads to large, reinforced FAs with slower exchange rates for linker molecules and longer lifetimes (Fig 2).

Interestingly, FA dynamics consistent with both classes of models have been observed[53,68,70]. In some cases, the difference is cell type-dependent. However, there is also evidence for spatial specificity within single cells, such that FA strengthening is restricted to the front of migrating cells[71]. This result makes intuitive sense since, if adhesions always strengthened under force, cells could not migrate. A polarized mechanism that strengthens adhesions at the front while allowing those at the rear to break under tension will produce forward movement when the cell contracts.

The polarized signaling pathways that determine whether adhesions strengthen or weaken under force are unknown. However, a recent study using a novel biosensor that reports the tension across the FA protein vinculin shed some light on the mechanism. It showed that vinculin is under high tension in FAs that assemble, whereas it is under low tension in FAs that disassemble under cellular contractile force in migrating cells[72]. Furthermore, vinculin is required for adhesion strengthening under force. These results suggest that the pathways that determine adhesion strengthening vs. weakening under force regulate whether the force is transmitted across vinculin or across other linkages.

A Dynamic Model of Mechanotransduction

The examples listed above suggest that a dynamic treatment of mechanotransduction is necessary. A key concept is that applied forces can regulate the rates of biochemically detectable processes, such as protein unbinding and protein conformational changes. While switch-like models emphasize the serial nature of the steps of mechanotransduction, a dynamic model reveals a more integrated picture where mechanotransmission, mechanotransduction, and mechanoresponse are intimately related and capable of affecting each other.

Dynamic Mechanotransmission

Broken linkages cannot transmit forces. Thus, the stability of the load-bearing sub-cellular structures dictates paths of force transmission and their duration. On short times (sub-second to tens of seconds), mechanotransmission is governed by the physics of force-activated bond dissociation[63,64]. While some cellular structures may simply be strong enough to bear the relevant forces, others will not. For example, bonds between actin and its cross linking protein α-actinin are slip bonds, and other calponin homology domain actin binding proteins are likely to behave similarly[73]. Other critical linkages in mechanotransduction, such as fibronectin-integrin (α5β1)[74,75] and actin-myosin bonds[76], show catch bond behavior. These

considerations suggest that only certain dynamic, sub-cellular structures may be stabilized in response to applied force to allow force transmission to mechanosensitive areas or molecules.

On larger length scales, the dynamic nature of cytoskeletal protein-protein bonds directly leads to viscoelasticity[77] (Box 2). Applied forces can result in either re-enforcement[23] or fluidization[78] of the cytoskeleton. While the exact mechanisms are still debated, re-enforcement is associated with maintenance of physical linkages, stiffening of the actin network and increased cell contractility[23,79]. Fluidization involves disruption of the cytoskeleton, either from breakage of mechanical linkages[80,81] or force induced, biochemically controlled disassembly[82]. In terms of mechanotransmission, these properties are extremely important as forces will be propagated along re-enforced, elastic filaments, but quickly dissipate in a fluidized, viscous environment. Furthermore, viscoelastic effects can enable certain frequencies of mechanical stimulus to be selectively transmitted over greater distance in cells[83]. The efficiency of transmission for different frequencies is determined by the rates of bond dissociation that cause cellular viscoelasticity. These effects have been observed in force-induced movements of FAs[84] and mitochondria[85]. Mechanical stimuli containing frequencies that are transmitted efficiently are likely to promote greater mechanoresponses.

Dynamic Mechanosensing

The basis of mechanosensing is thought to be force-sensitive changes in the rates of conversion between different protein conformations. These transitions depend on the strength and duration of force application (Box 1). For instance, when forces are applied to talin, 50 pN induces conformational changes within 25 ms, whereas at 20 pN, the same changes require 200 ms[34]. For successful mechanosensing, forces must be transmitted for sufficient time to induce conformational changes and subsequent biochemical detection. However, force also accelerates slip bond breakage, which will terminate the force transmission. Thus, there is competition between conformational change and bond breakage. Transmission pathways with catch bonds will therefore be more sensitive.

The rate at which forces are applied influences force transmission and subsequent signaling. The rate of force application through F-actin to the actin cross-linking proteins α-actinin or filamin determined the relative frequency of dissociation of the actin-linker bonds versus conformational changes[66]. Conformational changes are more likely to occur at higher rates of force application. Other experiments showed that fibronectin coated beads are less likely to dissociate from integrins when forces are applied quickly[86]. The critical effect of rate of force application underscores the importance of these dynamic aspects of mechanotransduction

Dynamic Mechanoresponse

The downstream mechanoresponse pathways are not innately force sensitive but often regulate cytoskeletal and adhesion structures that therefore feed back to influence mechanotransduction. The cytoskeletal protein zyxin, for instance, specifically localizes to areas of strain-induced stress fiber thinning, and recruits α−actinin and VASP, which

promote actin polymerization and stress fiber repair[87,88]. The MAL/serum response factor pathway is activated by actin polymerization in response to force or other stimuli, and regulates expression of numerous cytoskeletal genes including vinculin, filamin and actin[42,43]. Cyclic strain also enhances the expression of ECM proteins and induces the assembly of ECM structures[89]. Thus, on time scales on the order of minutes to days, cells use signaling or transcriptional programs to alter or maintain force transmission pathways.

Cell alignment in response to applied force is a form of adaptation that involves local regulation of dynamic cytoskeletal elements, largely through the regulation of Rho family GTPases[90]. In two-dimensional cultures, uniaxial static stretch ("stretch and hold") induces actin stress fiber and FA alignment parallel to the applied force, consistent with the general notion of adhesion strengthening[90]. By contrast, cyclic stretch induces alignment perpendicular to the applied force[91] in a frequency-dependent manner[49].

A mathematical model recently proposed that forces applied faster than the characteristic rates of remodeling in load bearing sub-cellular structures induce cell alignment perpendicular to the direction of strain to minimize stretching of these elements[92]. By contrast, when forces are applied more slowly than the remodeling rate, cells can internally remodel and align parallel to applied stress. This concept may also explain a fascinating effect in which inhibiting Rho kinase or mDia shifts the direction that cells align under cyclic stretch from perpendicular to parallel[93]. Inhibiting Rho signaling also is known to deplete cells of stable FAs and stress fibers, resulting in more dynamic sub-cellular structures[41]. The switch in direction of alignment may be explained if the higher cytoskeletal remodeling rate now exceeds the characteristic rate of the cyclic stretch, which, according to the mathematical model, would yield alignment in the direction of strain. This model provides insight into how the internal dynamics of the cytoskeleton can determine responses to dynamic mechanical stimuli.

Adhesion Strengthening and Rigidity Sensing

The principles enumerated above can provide at least a first explanation for how cells sense the mechanical properties of their substrata (Fig. 2). A key point is that forces on ECM-integrin-cytoskeletal linkages build up more rapidly in cells on rigid than on compliant surfaces[67,68]. These more rapidly applied forces are more effective at triggering conformational changes in cytoskeletal proteins rather than bond dissociation[66]. Additionally, if these linkages contain catch bonds, whose conversion to high affinity state is predicted to be enhanced at high loading rates[94], they will be further stabilized. As a result, mechanotransmission will be more efficient and longer lived. As a consequence of domain unfolding under force, additional proteins are recruited to support critical linkages. Adhesion strengthening will then occur through the mechanisms we describe above.

Sensing Dynamic, Applied Forces

One set of physiologically important mechanotransduction events involves the stretching of artery walls by blood pressure. Hypertension increases static stretch while cyclic pumping during the cardiac cycle causes time-varying stretch[4], both of which lead to transmission of forces to vascular cells and activation of multiple signaling pathways[95]. However, cyclic

stretch and static stretch of the same amplitude (and likely similar force application rates) induce both common and unique mechanoresponses[46]. For example, 10% cyclic stretch of endothelial cells increases expression of VEGF receptor (VEGFR)2 and the angiopoietin receptor Tie-2, but not VEGFR1. By contrast, 10% static stretch increases expression of VEGFR2 and VEGFR1, but not Tie-2[46]. We propose that these various responses can be explained by the dynamic processes intrinsic to the FAs and the cytoskeleton that sense the applied force (Fig. 3). Responses selectively activated by static stretch are likely mediated by protein conformational changes in strong proteins in relatively stable structures, requiring long applications of force to unfold. Responses selectively activated by cyclic stretch likely involve weaker proteins in dynamic structures that adapt to the statically applied forces but are constantly stimulated by dynamic signals. Responses that are activated by both are likely mediated by weak proteins in relatively stable structures.

Future Perspectives

The ability of mechanical perturbations to influence cellular signaling in a frequency-dependent manner can be conceptualized with mechanotransducers that function as bandpass filters, which selectively transmit specific frequencies. The ability of the cytoskeleton to selectively transmit certain frequencies of mechanical stimuli to sub-cellular structures provides one such mechanism[83–85]. Mechanosensitive elements and mechanoresponse pathways are also rate and frequency sensitive due to their own intrinsic time scales. In this view, the time scale of the applied force must match the critical time scale of a given signaling process in order to affect it. Stimuli that change too quickly are simply averaged, whereas stimuli that vary too slowly are not detected at all. Knowledge of the dynamics of cellular signaling processes could therefore facilitate understanding frequency-dependent cell and tissue responses. As cells contain multiple mechanically sensitive biochemical signaling pathways with a wide variation of critical time scales, they may therefore act as a multi-band pass filters, which pass several ranges of frequencies. These systems would allow cells to distinguish stimuli based on their frequencies or time scales.

Our understanding of mechanical signaling is still slim compared to our understanding of signaling by hormones and growth factors. But the more we learn, the more it seems that mechanical forces can play subtle and precise roles in governing morphogenesis, physiology and disease. We propose that just as conventional signals from soluble regulators act together in regulatory networks where complex temporal and spatial characteristics determine outputs, so mechanical stresses may also convey large amounts of information through precise time and force-dependent modulation. For periodic stimuli, this will take the form of frequency and amplitude features that determine cellular outputs. Elucidating the dynamics of cellular mechanotransduction systems holds the key to understanding these mechanisms.

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Physics of Force-activated Bond Dissociation

The non-covalent bonds that mediate protein-protein interactions have finite lifetimes, ranging from milliseconds to days[63]. Applied force typically shortens the lifetime of a bond (like applying force to remove tape). In a molecular context, these are referred to as slip bonds[63,64]. There are also molecules whose bond lifetimes increase, although not infinitely so, in response to applied forces (like the “finger trap” children’s toy)[64]. These are called catch bonds and, though relatively rare, are often found in critical cytoskeletal and cellular adhesion structures.

Protein conformation changes can be understood as a type of slip bond where the dissociation is internal and due to non-covalent bonds between amino acids in a single protein instead of between two proteins[63,65]. Moreover, both processes can be represented in terms of an energy landscape where two energy minima are separated by a high energy state that slows the rate of transition. Applied force acts both “catalytically” to accelerate rates by lowering the energy requirement for the transition, and by changing the free energy of the states, typically stabilizing the more open conformation or unbound conformations. Thus, the likelihood of a conformation change depends on both the magnitude of the force and its duration[63]. These conformation changes mediate subsequent mechanotransduction events by revealing binding sites for signaling and cytoskeletal proteins. In the diagram, boxes represent binding sites, while circles represent proteins. Also red denotes signaling proteins, while blue represents cytoskeletal proteins.

Furthermore, the bonds mediating protein conformations and protein-proteins interactions tend to have similar affinities and force sensitivities. As protein dissociation will terminate tension application, these processes will effectively compete. This process was recently studied using a single molecule system that contained both a dissociable bond and a protein that could undergo force-dependent conformational opening[66]. Protein dissociation and conformational changes were both observed, but, interestingly, the frequency of conformational changes was enhanced at higher loading rate rates.

Mechanical Properties of the Cytoskeleton

Materials such as rubber or polyacrylamide gels are elastic solids, meaning they deform rapidly in response to applied stress, but spring back to their original shape when the stress ends. By contrast, liquids like water or honey flow in response to applied stress such that deformation increases irreversibly and linearly with time. Many materials, including cells[77] and in vitro mixtures of cytoskeletal components[20], show behaviors between these two limits and are referred to as viscoelastic. Their behavior more closely resembles elastic materials on short time scales and viscous liquids on long time scales. A common example is silly putty, which flows like liquid when slowly squeezed but bounces like an elastic ball when thrown against the floor. On a molecular level, viscoelasticity is due to the presence of stress relaxation, usually through bond dissociation. At times shorter than the dissociation time, stress cannot be relaxed and the materials acts like an elastic solid, while on longer times, the bonds dissociate and the materials flow. The details of viscoelasticity in cytoskeletal networks are still

controversial, but it is likely that dissociation of or conformational changes in cytoskeletal cross-linking proteins are involved[77].

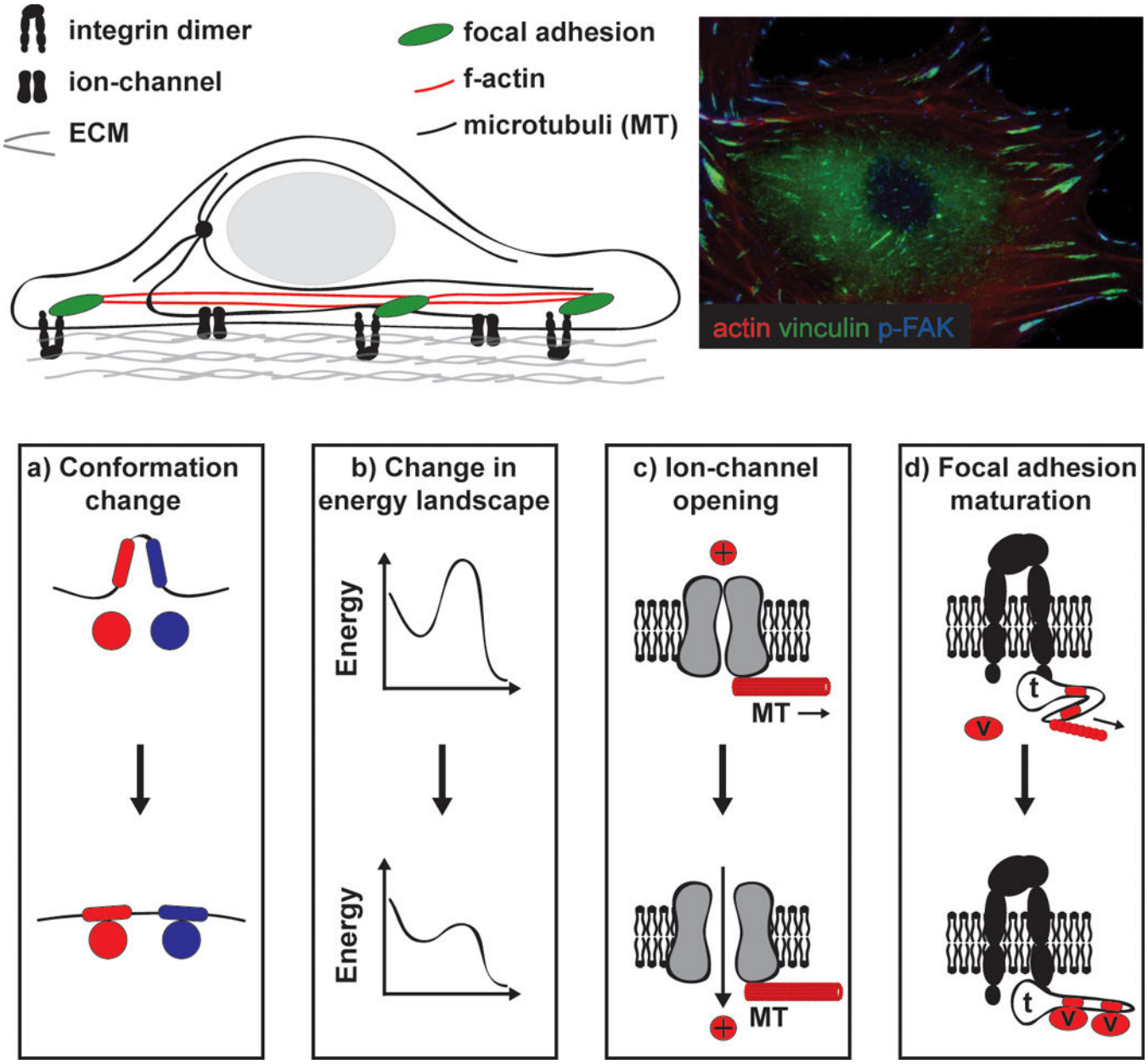

Figure1. Switch-like models of mechanotransduction.

a) Cells are mechanically integrated structures, where the extracellular matrix (ECM) and actin cytoskeleton are connected by integrins and focal adhesion (FA) proteins. Microtubules and many ion channels are also integrated with this network. Forces can be directly applied through the ECM or transmitted through the cytoskeleton to mechanosensitive components, such as FAs, to mediate cellular response to forces. b) Immunostaining of a vascular smooth muscle cell. F-actin filaments (red) link to variably-sized, punctuate FAs visualized by vinculin (green) and phosphorylated focal adhesion kinase (pFAK) staining. The variable amount of pFAK staining in the FAs is indicative of different local signaling environments that are likely linked to distinct mechanical signals. c) A common mechanism of mechanotransduction is force-induced conformational changes. For example, membrane

tension can cause ion channel opening. Also, talin connects the integrin cytoplasmic tail to F-actin; tension on talin reveals cryptic vinculin binding sites, subsequent binding of vinculin reinforces the linkage.

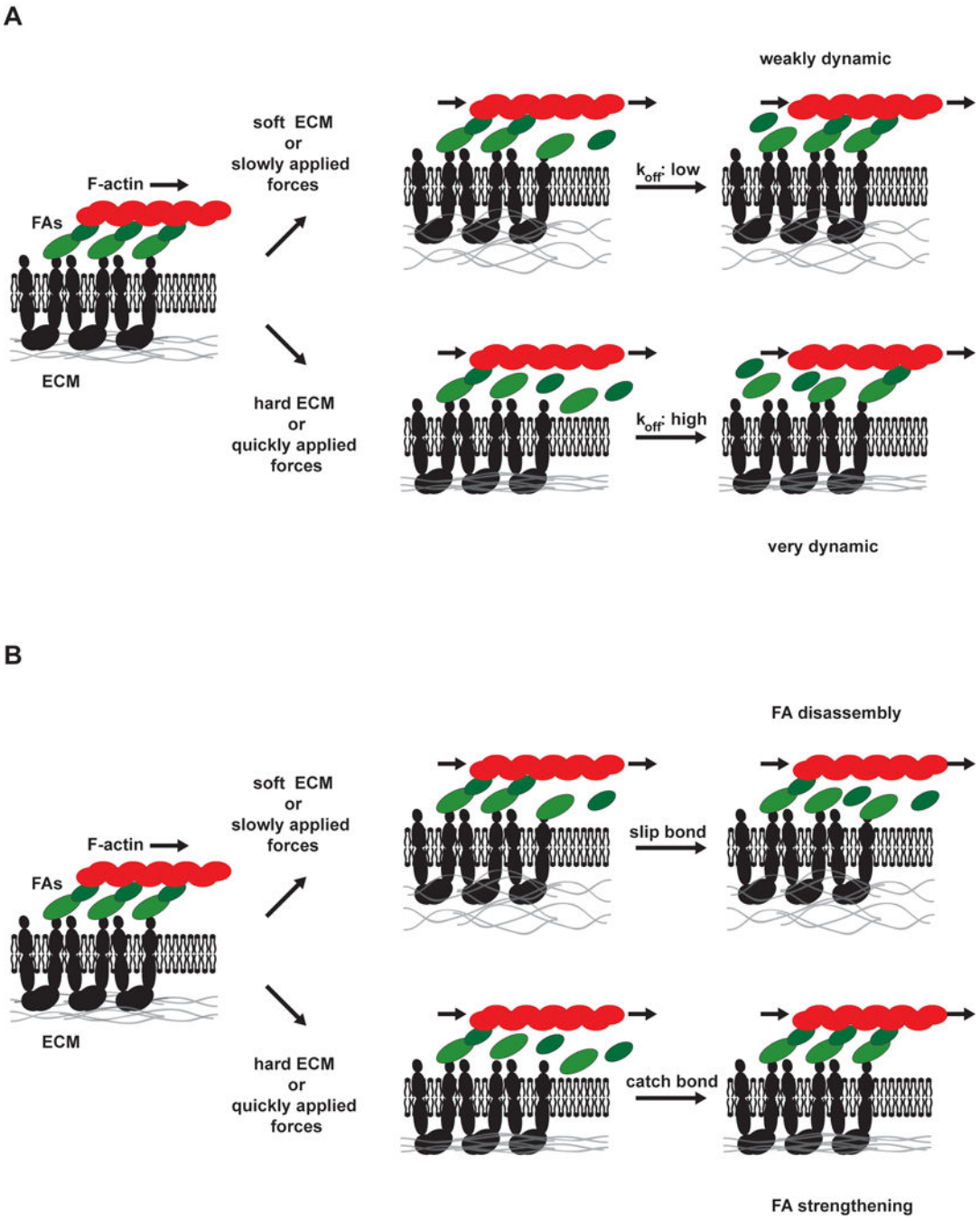

Figure 2. The focal adhesion clutch.

Due to forces from actin polymerization and myosin-dependent contractility, actin filaments flow rearward over FAs toward the nucleus. Through FA proteins that link actin to integrins, force is applied to the ECM. Force-dependent changes in FA exchange rates (represented as green arrows, see Box 1 for details) alter dynamics and size of FAs. On soft surfaces or where external force is applied slowly, on- and off-rates are moderately fast and FAs have moderate lifetimes. However, on stiff surfaces or when external forces are applied quickly, distinct behaviors can be observed. In the absence of FA strengthening, molecular linker

dissociation rates increase (slip bond behavior). However, these proteins are sometimes replaced through re-binding, resulting in large exchange rates and short-lived FAs. FA strengthening is associated with catch bonds that slow FA protein dissociation under force, with exposure of cryptic binding sites to recruit new proteins that reinforce the adhesion, and with conformational changes in FA proteins that activate signaling pathways to recruit additional molecular linkers. Any way, large, long-lived FAs are the result.

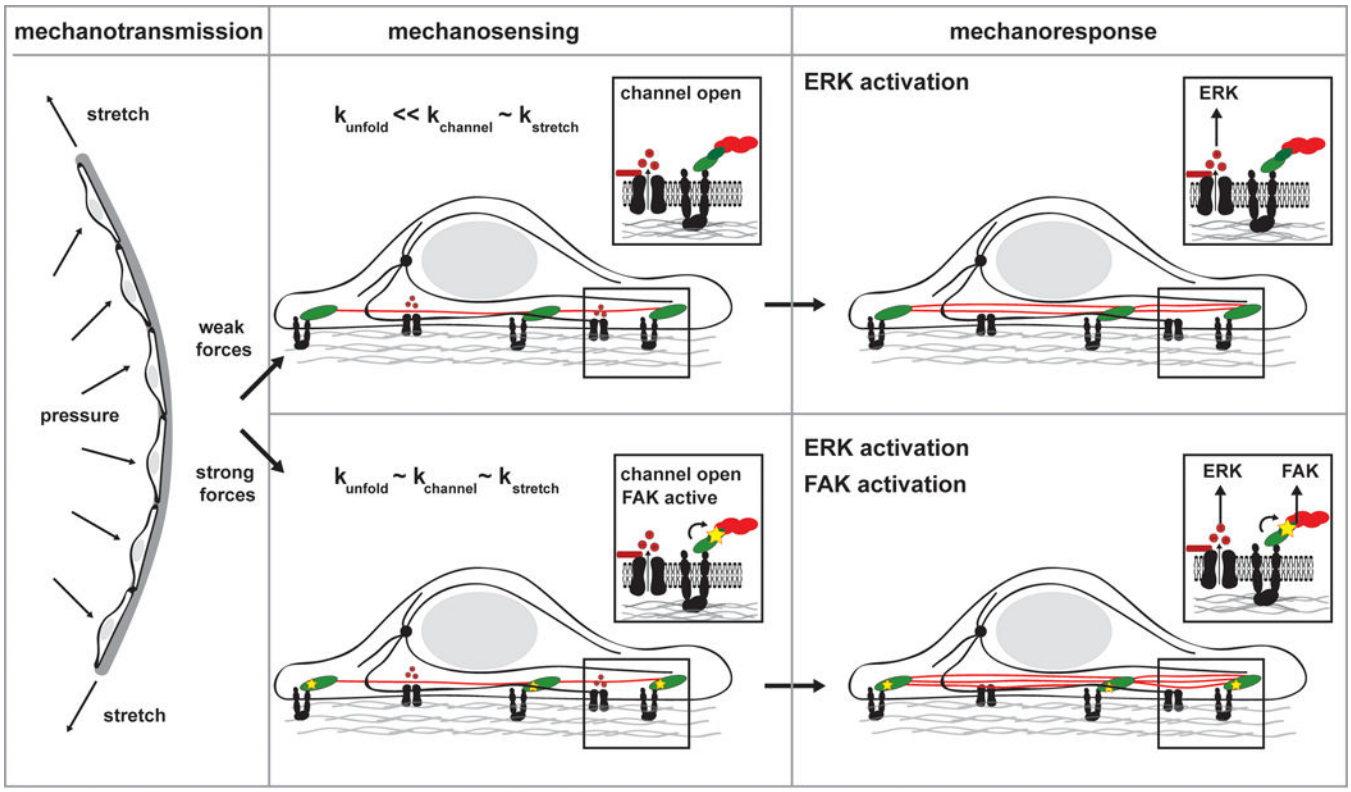

Figure 3. Dynamic aspects of mechanotransduction.

The endothelial cells that line blood vessel walls are subject to both static and cyclic stretch. Even when the signal strengths are matched, dynamically distinct mechanical stimuli can activate common or unique signaling pathways based on the strength of the proteins, specifically resistance to conformation changes, and the dynamic nature of the structures bearing load. In dynamic structures, like nascent adhesions, and stable structures, like mature adhesions and stress fibers, cyclic stretch does not apply forces for sufficient time to induce conformation changes in strong proteins, but weak proteins will signal. In response to static stretch dynamic structures can readily adapt, and there is no long term signaling. In stable structures the long force application causes conformational changes in both weak and strong proteins. Thus signaling pathways that are preferentially activated by cyclic stretch are likely induced by weak proteins that localize exclusively to dynamic structures. Pathways selectively activated by static stretch are expected to contain mechanosensors that are strong proteins in stable structures. And pathways activated by both types of signals are likely due to weak proteins that localize to both dynamic and stable structures. Stars indicate active proteins.