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Mechanochemistry: A Molecular Biomechanics View of Mechanosensing

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Abstract

Molecular biomechanics includes two themes: the study of mechanical aspects of biomolecules and the study of molecular biology of the cell using mechanical tools. The two themes are interconnected for obvious reasons. The present review focuses on one of the interconnected areas – the mechanical regulation of molecular interaction and conformational change. Recent conceptual developments are summarized, including catch bonds, regulation of molecular interaction by the history of force application, and cyclic mechanical reinforcement. These studies elucidate the mechanochemistry of some of the candidate mechanosensing molecules, thereby providing a natural connection to mechanobiology.

Keywords

single molecule; conformational change; catch bond; cyclic mechanical reinforcement

1. Introduction

The development of molecular biomechanics parallels the development of molecular biology. As biological research moves towards understanding the molecular mechanisms of cellular functions, biomechanics research also moves to smaller and smaller scales from tissues to cells to molecules. In many ways, molecular biology and molecular biomechanics represent similar reductionist approaches that attempt to explain the complex cell by examining its constituent molecules in hope that their assemblies would help elucidate the cellular behavior. The development of molecular biomechanics is also driven, at least in part, by the development of molecular biology. In 1998, Bruce Alberts published an overview in *Cell* entitled: “The cell as a collection of protein machines”¹. In the paper, Alberts advocates a view of the cell “as a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines”. This view calls for mechanical analysis of such protein machines from the working principles to design constraints. At that time, the field of molecular biomechanics was still in its early stage of development with only a small number of investigators. The past 15 years have witnessed a rapid expansion of molecular biomechanics with a significantly greater number of biomedical engineers contributing to the field. A decade after Alberts’ overview, the substantial increase in the publications in this area prompted the Biomedical Engineering Society to establish Cellular and Molecular Bioengineering as an official journal of the society, second after Annals of Biomedical Engineering².

Molecular biomechanics includes two interconnected themes: The first theme is the study of mechanical aspects of biomolecules, including but not limited to molecular deformation³⁻⁶, conformational change⁷, protein unfolding⁸⁻¹¹, mechanical regulation of molecular interactions¹²⁻¹⁷, and force-regulated enzymatic reaction^{18,19}. The second theme is the study of molecular biology of the cell using mechanical tools, methods, and approaches, including properties and functions of molecular motors^{20,21} and conformational regulation of molecular interactions⁷, to name a couple. Naturally the two themes are closely related. A major objective of studying the mechanical aspects of biomolecule is to tackle issues in molecular biology of the cell that require mechanical analysis to understand. Mechanical tools, methods, and approaches are employed to study the molecular biology of the cell because many questions in it include mechanical aspects of biomolecules. One area in which the two themes blend particularly well relates to mechanobiology, which studies biological responses to mechanical signals²². In this paper, some recent progress in molecular biomechanics will be reviewed with a focus on mechanochemistry as related to the mechanical regulation of molecular interaction and protein conformational changes.

2. The initial concept of force-regulated receptor-ligand dissociation

In his 1998 *Cell* overview¹, Alberts argued that any real understanding of the function of a protein machine will require not only its resting structure in atomic detail, but also a knowledge of the kinetics and energetics of each of its reaction intermediates. He pointed out the limited relevance of the traditional physical chemistry treatment of isolated soluble proteins that randomly diffuse, collide and react in a test tube to what actually occurs in a real cell. This is because the chemistry that makes life possible includes much more elaborate and sophisticated interactions within and among protein assemblies, which is highly coordinated, tethered (to other structures of much larger sizes), ordered and regulated. Of the different types of regulations of protein interactions, mechanical regulation is of particular interest to molecular biomechanics for obvious reasons. This type of regulations does not exist for untethered proteins in solution, but is ubiquitous and likely important inside and between cells for proteins that have a load-bearing role in supporting and/or regulating the forms and shapes of cellular and subcellular structures, such as structural, scaffolding and connective proteins. Twenty years prior to the Alberts overview, George Bell had already laid the groundwork in this area of mechanochemistry. He formulated the well-known Bell model, proposing that force exponentially accelerates molecular dissociation²³. This pioneering work is ahead of its time, as not until 1995 was the first experimental test of the Bell model published²⁴. In 1997, Evans and Ritchie developed a theoretical framework for interpreting rupture forces of single molecular bonds in terms of their off-rate²⁵. It follows from the Bell equation that when a ramp force is applied to rupture a molecular bond, the most probable rupture force, identified from the peak of the histogram of a large population of rupture forces measured under identical conditions, depends linearly on the logarithm of the ramp rate. Based on this prediction, the method of dynamic force spectroscopy (DFS) was developed^{12,26,27}, leading to a rapid growth of research that analyzed the force-dependent dissociation of a large number of molecular interactions^{28,29}. The theoretical basis of DFS has also been further extended to include other effects such as those of the elastic potential of the molecular complex and the force probe^{30,31}. The typical information extracted from DFS analysis is the shape of the energy landscape, modeled by a harmonic energy well, and parameterized by its width and the height of the energy barrier at zero force (Fig. 1A)¹⁶. In addition to steady force-ramp DFS, constant force-clamp DFS has also been used where single-bond lifetimes were measured in a range of constant forces¹³. Assuming that off-rate in the first-order irreversible dissociation kinetics equation is a single-valued function of force, off-rate can be expressed in terms of the survival probability and its derivative (i.e., probability density) of rupture

force or bond lifetime¹⁴. This formulation allows off-rate to be evaluated from the measured force or lifetime histograms independent of how force is applied over time^{32,33}.

3. Slip, catch, and ideal bonds

In 1988, Dembo et al. extended Bell's formulation of force-dependent off-rate of molecular dissociation. In addition, these authors proposed a classification of three types of molecular bonds according to how the off-rate depends on force (Fig. 2A)³⁴. The behavior described by the Bell model is called slip bonds, as force shortens bond lifetime. The opposite behavior is called catch bonds, as force prolongs bond lifetime. The third class is ideal bonds that designate the case in which bond lifetime is independent of force. Molecular interactions analyzed by the conventional steady force-ramp DFS give rise to slip bonds. This is not surprising because the theoretical basis of the method is the Bell equation, which by definition describes slip bonds only.

The pioneering paper by Dembo et al. is also ahead of its time because catch bonds were derived from theoretical considerations only³⁴. Initially, whether such unusual and counter-intuitive behavior would exist was hotly debated. Some early experiments were consistent with the existence of catch bonds, although alternative interpretations could not be ruled out. Most noticeably of these are the data of flow-enhanced adhesion of bacteria³⁵, leukocytes³⁶, and platelets³⁷ to surfaces bearing ligands to the respective cell surface receptors. Of significance, Thomas et al. used molecular dynamics (MD) simulations and mutagenesis analysis to pinpoint the cause of shear-enhanced *E. coli* attachment to target cells at the structure of the fimbrial adhesin FimH receptor³⁸. Using constant force-clamp DFS to directly measure single-bond lifetimes to prevent possible new bond formation from complicating data interpretation, Marshall et al. obtained the first definitive experimental demonstration of catch bonds¹³. A decade later, more than a dozen of molecular interactions have been shown to exhibit catch bond behavior. These include both cell surface receptors, such as adhesion molecules, and cytoplasmic proteins, such as structural and motor molecules, with one thing in common: one of their functional roles is to support or transmit force. Cell adhesion molecules shown to form catch bonds include three (P-, L- and E-) selectins interacting with their common and distinct carbohydrate ligands^{13,39,40}, two integrins, $\alpha_5\beta_1$ and $\alpha_L\beta_2$, interacting with their respective ligands, fibronectin and intercellular adhesion molecule 1 (ICAM-1)^{41,42}, platelet glycoprotein Iba (GPIba) interacting with von Willebrand factor (VWF)⁴³, *E. coli* fimbrial adhesin FimH receptor interacting with mannose ligand⁴⁴, and homotypical interaction between E-cadherins⁴⁵. Published catch bonds between intracellular structural and motor proteins include that of actomyosin⁴⁶, kinetochore protein⁴⁷, and actin⁴⁸. In addition, catch bonds have also been found in force-dependent intracellular interaction^{19,42} and enzymatic reaction^{18,19}. More recently, ideal bonds have also been observed⁴⁵. Interestingly, all catch bonds observed to date only exist in a finite force regime beyond which they transition to slip bonds. In all cases, catch bonds are followed by slip bonds. In addition to such biphasic catch-slip bonds (Fig. 2B), triphasic slip-catch-slip bonds have also been observed (Fig. 2C)⁴⁰.

The demonstration of catch bonds prompted theoretical development to account for this unusual and counter-intuitive behavior. Several models for catch bonds have been developed, including one that hypothesized before the demonstration of catch bonds⁴⁹. Different mechanisms have been assumed in these models, such as single⁵⁰ or dual bound states^{51,52} with two dissociation pathways (Fig. 1B), force-regulated dynamic disorder^{53,54}, combined entropic-controlled dissociation and energetic-controlled rupture⁵⁵, deformation^{56,57}, allostery (Fig. 3B)⁵⁸⁻⁶¹, and sliding-rebinding^{62,63} mechanisms. Note that multiple mechanisms may be operative as they are not necessarily mutually exclusive. MD simulations and mutagenesis analysis have been used to elucidate the structural bases of

catch bonds^{38,43,48,64-69}. In all cases so analyzed, catch bonds result from force-induced formation of new non-covalent interactions at the atomic level that are not observed in the crystal structures at the zero-force state. The deformation model assumes that force causes deformation local to the binding interface that strengthens the interaction (Fig. 3A). By comparison, allosteric models assume that the force-induced conformational changes occur in a remote region and propagate along the molecular structure to the binding pocket to change its interaction with ligand (Fig. 3B and C). For the conventional allosteric mechanism, the newly formed or strengthened interactions are still largely confined in the original binding pocket, which binds more tightly upon propagation of force-induced conformational change from a distant site to the binding site (Fig. 3B). This structural mechanism has been proposed to be operative in the catch bonds of FimH receptor with mannose ligand as supported by MD simulations and mutagenesis^{38,70}, of integrin $\alpha_L\beta_2$ with ICAM-1 as observed by crystal structures⁷¹ and by MD simulations^{68,72}, and P-selectin with P-selectin glycoprotein ligand-1 (PSGL-1) as supported by MD simulations⁶⁷ and mutagenesis⁷³. The sliding-rebinding model represents a variant form of allostery in that new interactions can be formed beyond the original binding pocket. The force-induced conformational change tilts the binding interface, allowing the two interacting molecule to slide relative to each other over it, thereby promoting new contacts (Fig. 3C)^{62,63}. This structural mechanism has been suggested by MD simulations and mutagenesis to be operative in the L-selectin-PSGL-1⁶³, P-selectin-PSGL-1⁶⁴, GPIIb α -VWF⁴³, and actin-actin⁴⁸ catch bonds.

4. Force-history dependent molecular dissociation

The mathematical formulation of mechanochemistry resembles that of constitutive equation in mechanics. In conventional mechanics, the change in shape from the initial to final configurations is measured by deformation and its dependence on force is described by a constitutive equation. An example is the Hooke's law for spring where the deformation is proportional to force. In molecular biomechanics, molecular dissociation and conformational change are modeled by chemical reaction kinetics equations based on mass action and conservation laws. The transition from one state or form to another is measured by the rate coefficient and its dependence on force is modeled by the equations of Bell²³, Dembo et al.³⁴, and those describing various catch-slip bonds^{49-58,61,64,68}. In these models, the rate coefficient is assumed to be a single-valued function of force, similar to constitutive laws in the theory of elasticity. In mechanics, however, there are also viscoelastic materials. An example is a damping element connected to a spring, which exhibit hysteresis and memory. The deformation of such materials depends not only on the instantaneous value of force but also on the prior history of force application up to the present time. By the same token, off-rate of molecular dissociation may exhibit force history dependence.

The initial evidence for force history dependence came from the qualitatively distinct characteristics of receptor-ligand dissociation analyzed by different experimental assays. When constant force-clamp DFS was used to measure bond lifetime (Fig. 4A) from which force-dependent off-rate was directly derived, catch bonds at certain force ranges were observed for dissociation of respective counter-molecules from P-selectin¹³, L-selectin³⁹, E-selectin⁴⁰, integrin $\alpha_5\beta_1$ ⁴¹, integrin $\alpha_L\beta_2$ ⁴², and actomyosin⁴⁶. When steady force-ramp DFS was used to measure rupture forces (Fig. 4B) and the analysis was based on the Bell equation to derive model parameters, only slip bonds were observed for the same interactions over the entire force ranges studied^{46,74-79}. Even for unfolding of the VWF A2 domain, constant force-clamp measurements observed catch-slip bonds¹⁹ and steady force-ramp measurements observed slip bonds⁸⁰. These discrepancies could not be explained by potential differences in reagents used in the different laboratories tested by different instruments. Two of these studies were in fact done by the same laboratories in side-by-side

experiments^{14,46}. These discrepancies persisted even when off-rate as a function of force was directly derived from the rupture force histogram, therefore without the *a priori* slip bond assumption implied by the Bell equation¹⁴.

In a systematic study, the *a priori* assumption that off-rate is a function of instantaneous force was invalidated by demonstrating ligand-specific force history effects of L-selectin dissociation⁸¹. The bond lifetime was found to be impacted by the ramp-rate at the force-ramp phase before reaching the clamp force at which lifetime measurement began in a ligand-specific fashion (Fig. 4C). Bonds between L-selectin and PSGL-1 behaved as catch-slip bonds in the force-clamp phase when the ramp-rates at the force-ramp phase were low; but the force range for catch bond progressively left-shifted towards smaller forces and narrowed as the ramp-rate increased and vanished when the loading rate was $>7,000$ pN/s, leaving slip-only bonds (Fig. 5A). By comparison, the catch-slip bonds between L-selectin and 6-sulfo-sialyl Lewis X (6-sulfo-sLe^x) were impervious to ramp-rate changes (Fig. 5B). The effect of the ramp-rate resembled the effect of a point mutation at the L-selectin surface (L-selectinA108H) predicted to contact PSGL-1 but not 6-sulfo-sLe^x (Fig. 5C and D). This similarity suggests that high ramp-rate induced similar structural changes as the mutation. While the A108H substitution in L-selectin eliminated the ramp-rate responsiveness of its dissociation from PSGL-1 (Fig. 5E), the inverse mutation H108A in P-selectin acquired the ramp-rate responsiveness (Fig. 5F). These data were explained by the sliding-rebinding model for catch-slip bonds extended to incorporate the additional force history dependence, with the L-selectin residue A108 playing a pivotal role in this structural mechanism (Fig. 6).

Thus, the discrepancies in the force-dependent off-rate have been reconciled by the concept of force history-dependent off-rate. The new paradigm introduces a generalized form of mechanical regulation of molecular interaction, allowing catch bonds (originally defined based on the concept that off-rate is a function of the present level of force) to be regulated by the ramp-rate (a parameter describing the prior force history). In this new paradigm, the kinetic rate in the mass action model at any given time is a functional of all values of force in the past times up to the present time, not just its present value^{14,81}. The new concept may be understood by considering the long range effect of force on molecular dissociation. Force applied to a multi-domain molecule may induce deformation or conformational changes in other regions and propagate to the binding interface to regulate the kinetic rate. The response of the interfacial structure to force local to the binding pocket may be sufficiently rapid, resulting in the kinetic rate to depend on the instantaneous level of force. But coupled conformational changes between the binding pocket and remote regions may accumulate over time as propagation of conformational changes over distance requires time, giving rise to a force history-dependent kinetic rate. Note that similar considerations should be applicable to protein conformational changes because most contributions to the energy barrier impeding rapid transition in these processes are likely from non-covalent interactions localized in a small region that resembles the binding pocket in the molecular interaction case.

5. Cyclic mechanical reinforcement of molecular dissociation

Forces exerted to and experienced by cells are often dynamic and cyclic. As such, structural, scaffolding and connective proteins that bear and transmit these forces are subject to dynamic loading and unloading cycles. For example, cells exert dynamic tractions to the extracellular matrix (ECM) in cycles of edge protrusion, adhesion and retraction to generate motility^{82,83}, which transmits myosin-generated forces via the retrograde flow of actin filaments to ligand-bound integrins in a repetitive loading-unloading fashion^{84,85}. Thus, cyclic force represents a common type of time course of force application and may regulate molecular interaction and conformational change.

In a recent study, the regulatory effects of different types of cyclic force histories on integrin–ligand dissociation have been demonstrated¹⁵. Loading an integrin via an engaged ligand (but not an antibody) to a peak force, unloading it to a low force, and clamping it at that low force prolonged the bond lifetime by up to two orders of magnitude (Fig. 7A). This observation, termed cyclic mechanical reinforcement, revealed two states of the integrin–ligand bond: a short-lived state and a long-lived state (Fig. 7B). Force could switch the bond from the short- to long-lived state. The probability for this to occur increased with increasing peak force and reached 100% beyond ~20 pN (Fig. 7D), manifesting as an initial increase and a later plateau of the mean bond lifetime (the weighted average of the two subpopulations of short- and long-lived states) with increasing peak force (Fig. 7C).

Mechanical reinforcement and catch bond appears to share some similarities as force strengthens molecular interaction in both cases. However, the two concepts are distinctly different. The lifetime of a catch bond is prolonged by the present level of force, whereas the lifetime of a cyclically mechanically reinforced bond is prolonged by the peak force before arriving at the present level. Catch bonds are strengthened under tension but weakened when tension is released³⁴, whereas mechanically reinforced bonds stay long lived after force removal. Cyclically mechanically reinforced bonds also exhibit other properties beyond the classical catch bond definition³⁴ and models^{62,86,87}. In addition to reinforcement by a single cycle with a high peak force, integrin–ligand bonds were observed to be reinforced by multiple force cycles with a low peak value (Fig. 8A and B). The mean bond lifetime increased with increasing number of cycles initially and reached a plateau beyond a few cycles (Fig. 8C), indicating an accumulation of the likelihood for force to switch the integrin–ligand bond from the short- to long-lived state until reaching a 100% probability (Fig. 8D). Thus, cyclic mechanical reinforcement strengthens the bond, remembers the history of force application, accumulates over repeated cycles, but does not require force to be sustained. The study of Kong et al. also indicates that cyclic mechanical reinforcement strengthens the fibronectin–integrin $\alpha_5\beta_1$ bond through the RGD binding site of the ligand with the synergy binding site greatly facilitating the process. A flexible integrin hybrid domain is important for cyclic mechanical reinforcement. Thus, the long-lived state is hypothetically depicted in Figs. 7D and 8D as an extended integrin with a swing-out hybrid domain engaged with fibronectin in both the RGD and synergy sites, which may represent a ‘trapped’ conformation with slow reversion kinetics. This work has revealed a novel mechanical regulation of receptor–ligand interactions and identified a molecular mechanism for cell adhesion strengthening by cyclic forces. It is possible that cyclic mechanical reinforcement also serve as a mechanism for mechanical regulation of other molecular systems, such as FimH–receptor–mannose ligand, selectin–ligand, and actin–actin interactions. It is also possible that resistance to protein conformational change in some systems can also be mechanically reinforced.

Catch bonds and cyclic mechanical reinforcement are different from mechanical strengthening of adhesion that is triggered by force-dependent signals that activate remodeling of adhesions, which has been more commonly observed. The latter case occurs at the cellular level whereas the former two cases occur at the molecular level. For this reason, mechanical strengthening of adhesion involves mechanobiology whereas catch bonds and cyclic mechanical reinforcement are based on mechanochemistry. An example of mechanical strengthening of adhesion starts with forced-exposure of cryptic binding sites on fibronectin, which results in extracellular matrix remodeling and adhesion reinforcement⁸⁸. Likewise, changes in focal adhesions result from similar exposure of cryptic sites and focal adhesion remodeling^{89,90}.

6. Biological functions

The original proposal of catch bond was based on biophysical considerations. The broad interest in catch bond, however, lies not only in its counterintuitive biophysical properties but also in its biological functions. The same can be said about force history dependence and cyclic mechanical reinforcement of molecular interaction. Several biological functions have been proposed to take advantage of the unusual properties of catch bond¹³, force history dependence⁸¹, and cyclic mechanical reinforcement¹⁵ of molecular interaction. Of these proposals, two kinds of biological functions have received significant in vitro experimental support: 1) reducing cell rolling velocity as fluid shear stress increases and 2) preventing agglutination of platelets in the circulation.

Several catch bond forming receptor–ligand interactions share one thing in common: they mediate tethering of flowing cells to and their rolling on the surface of a fluid containing vessel wall^{91,92}. Selectin–ligand interactions function in this way during inflammatory response, leukocyte trafficking, and lymphocyte homing. GPIb α –VWF interaction functions in this way during hemostatic and thrombotic responses. FimH–receptor–mannose ligand interactions also function in this way during bacterial infection of and colonization in the host. Cell adhesion in these scenarios also share one thing in common: the adhesion is enhanced rather than impeded by increasing flow despite the increase in the dislodging force imposed by the flowing fluid^{35–38,93,94}. Flow enhanced adhesion is important biologically as it allows cells to attach to desirable sites against the opposing flow. Flow-enhanced adhesion also seems physically counter-intuitive. Interestingly, catch bonds provide the molecular mechanism for flow enhanced cell rolling, as demonstrated by dimensional analysis and mutagenesis studies^{43,95}. Thus, mediating flow-enhanced adhesion is one of the biological functions of these catch bond forming receptor–ligand interactions⁹¹.

The molecular interactions that mediate cell adhesion to vascular surface may also mediate agglutination of circulating cells⁹². Catch bonds have been suggested to prevent inappropriate aggregation of leukocytes that express both L-selectin and PSGL-1, of leukocytes and platelets that can be induced to express P-selectin, and of platelets that may be cross-linked by multimeric plasma VWF binding to GPIb α ^{43,63}. In a vessel, the fluid shear stress is highest at the wall where cell arrest takes place but progressively lower toward the center of the vessel where agglutination of flowing cells may occur. Also the shear force is much greater on cells attached to the stationary vessel wall than on cells flowing with the fluids. Therefore, force on bonds connecting a cell to the stationary wall is generally much greater than that linking two flowing cells. Thus, catch bonds may allow high force to strengthen cell attachment to the vessel wall and low force to prevent stable interactions of L-selectin–PSGL-1 between two leukocytes⁶³, P-selectin–PSGL-1 between an activated platelet and a leukocyte, and GPIb α –VWF–VWF–GPIb α between two platelets that are flowing in the blood stream⁴³.

A powerful tool for determining the structure-function relationship of mechanical regulation of molecular interaction is mutagenesis. This has been used to identify the structural bases and biological functions of the catch bonds of L-selectin–PSGL-1⁶³, FimH–receptor–mannose ligand³⁸, GPIb α –VWF⁴³, and actin–actin⁴⁸ interactions, force history dependent of selectin–ligand interactions⁸¹, and cyclic mechanical reinforcement of integrin–ligand interactions¹⁵. Interestingly, some of the mutations in VWF that convert its catch bond with GPIb α to a slip-only bond occur naturally in patients with type 2B von Willebrand disease (VWD), suggesting a possible VWD preventing function of the GPIb α –VWF catch bond⁴³. Similarly, key residues observed in MD simulations that are involved in the formation of actin–actin catch bonds⁴⁸ are related to nemaline myopathy mutations in the human actin

geneACTA1^{63,96-98}. These intriguing correlations should prompt formulation of interesting hypotheses to be tested in future studies.

Some speculated biological functions of mechanically-regulated molecular interactions are based on the mechanical environments where these particular interactions take place. For example, ramp-rates may differ markedly for selectin–ligand bonds formed at the leading and trailing edge of a rolling leukocyte. Ramp-rates may also be affected by local variations in fluid dynamics, as and when a flowing leukocyte uses L-selectin–PSGL-1 bonds to tether to and roll on a leukocyte already arrested on the vascular surface. This might explain in part why force history affects L-selectin interactions with PSGL-1 that is expressed on leukocytes, but not with 6-sulfo-sLe^x on mucins that are expressed on endothelial cells⁸¹. As another example, migrating cells apply cyclic traction through nascent adhesions at the leading edge to the ECM. Switching integrin–ligand bonds from the short- to long-lived state by cyclic mechanical reinforcement may provide a dual function. On the one hand, it may allow the newly formed adhesions to undergo fast turnover for rapid exploration of its environment. On the other hand, it may enable these nascent adhesions to be stabilized by myosin-generated contractile forces for adhesion maturation and downstream signaling¹⁵.

Other speculated biological functions of mechanically-regulated molecular interactions are based on the properties such regulation revealed. For example, the stability of the long-lived state of bonds mechanically-reinforced by cyclic forces may allow the cell to “remember” where forces have been applied previously. Moreover, since the reinforcement effects are cumulative over small repetitive forces, this ability to integrate may allow the cell to “count” the force cycles and therefore sense its physical microenvironment¹⁵. Elucidating how the history of force application regulates receptor–ligand bond-mediated biological functions will be an interesting area of future research.

7. Mechanochemistry in mechanobiology

Mechanobiology is distinct but related to biomechanics. Biomechanics is usually considered as a sub-discipline of mechanics, biophysics, and biomedical engineering. By comparison, mechanobiology is usually considered as a sub-discipline of biology. In recent years, the rapid progress in mechanobiology has revealed and elucidated many effects of force in a wide variety of biological processes from cell proliferation and differentiation to tissue formation and remodeling²². At macro-scales, the studies of biological effects of force associated with physical activities (e.g., heart beating, muscular skeletal movement, blood flow and breathing) may be more closely related to biomechanics at the tissue and organ levels. At the micro- to nano-scales, however, research of mechanical effects on cells and molecules is more closely related to cell and molecular biomechanics. Biomedical engineers have made significant contributions to the field, especially to the development of enabling technologies that allow in vitro experiments that manipulate the mechanical environment experienced by cultured cells to mimic in vivo conditions, measure the forces generated by the cell and applied to the neighboring cells and/or ECM, and analyze the cell’s mechanical and biological responses. For example, sensory neurons convert touching on the skin into electrical signaling⁹⁹. Stem cells can sense mechanical properties of their living environment, converting substrate rigidity into various differentiation signals that leads to different lineage commitment¹⁰⁰. Together with the work of cell and molecular biologists, these studies have generated a vast body of information regarding signaling pathways that transduce mechanical stimuli to activate biological responses. The studies on mechanotransduction employ biochemical, molecular, genetic, and other biological methods in similar ways as analysis of signaling pathways that transduce biochemical stimuli, such as a cytokine. In addition, a number of candidate molecules or molecular assemblies that may sense and/or respond to mechanical stimuli and their sensory mechanisms have been

identified or proposed with increasing supporting evidence. Some of these include: mechanosensitive ion channels (MSCs) that can sense sound waves, blood pressure and touching, adhesive proteins that mediate cell–cell (e.g., cadherin) or cell–ECM (e.g., integrins and fibronectin) adhesions, and cytoskeleton (e.g., actin and microtubules) and its associated motor proteins (e.g., myosin)¹⁰¹.

MSCs are a major family of mechanosensitive molecules that rapidly transform mechanical forces into electrical and biochemical signals. Transient receptor potential cation channel subfamily V member 4 (TRPV4) works as osmotransducer as it can be activated by cell swelling in response to osmolality changes in the environment⁹⁹. Another member of the TRP family of ion channels TRPN1, or NOMPC (no mechanoreceptor potential C), has recently been identified as a mechanotransduction channel for gentle touch¹⁰². Structurally, its N-terminal domain that may indirectly connect to cytoskeleton may serve as tension transmitting module to the pore-forming region⁹⁹. Patch-clamping¹⁰³ and crystallographic¹⁰⁴ studies have revealed that stretching mechanosensitive cation channels (MscCa) or large conductance channel (MscL) by membrane tension can produce conformational changes (e.g., intra-molecular displacement in the gating domain) that alter opening or closing rates to modulate the cation-transporting activity. Recently, piezo proteins have been identified as a new class of mechanosensitive ion channels and can form a pore through tetrameric association^{105,106}. Interestingly, piezo1 activation does not require cytoskeleton but may require lipid bilayer's tension¹⁰⁷.

It has been recognized that almost all cells can sense and respond to their mechanical environment, not just “professional mechanosensing cells”. Professional mechanosensing cells such as the sound-sensing hair cells the inner ear are equipped with sub-cellular structures specialized for sensing mechanical cues and convert mechanical energy to biochemical signals. For example, stereocilia, microvillar projections of hair cells, can deflect by sound vibration and induce opening of mechanically gated ion channels to flux ions to trigger cell to depolarize. Depolarized cell then activate auditory nerve fibers and convey mechanical signals from sound to the brain¹⁰¹. MSCs may be considered as “professional mechanosensing molecules.” However, it is not clear whether all cells are equipped with specialized mechanosensing cells. It is possible that cells not specialized in mechanosensing may use other “nonprofessional mechanosensing molecules” by taking the advantage of the fact that forces alter the conformation, and hence the activity, of virtually any protein. For example, Reinhertz and colleagues showed that the T-cell receptor (TCR), a typical immunoreceptor, behaved as an anisotropic mechanosensor such that tangential (but not normal) force applied on the TCR could trigger T-cell downstream signaling¹⁰⁸. The authors also proposed a triggering mechanism that involved a series conformational changes on TCR/CD3 complex^{109,110}.

To this end, many crucial questions have yet to be answered. In some cases, it is still not known what and where the mechanosensitive molecules are and how mechanosensing is triggered. Another challenge is the identification of physiologically relevant contexts in which forces contribute to biological processes. In this regard, it may be informative to compare similar questions on biochemical sensing. The questions of what the biochemical sensing molecules are and where they are located have been largely answered, as many of such molecules, generally termed receptors, have been identified and their expression patterns characterized. Several reasons may explain such differential progresses in the identity and location questions between biomechanical and biochemical sensory molecules. Biochemical sensory molecules are usually cell surface receptors because the membrane is impermeable to ligands. Receptor–ligand interactions are specific and localized, making them amenable to many well-developed strategies in biological research, such as to alter (delete, overexpress, mutate, etc.) the candidate molecule and observe the consequences. In

contrast, mechanical interaction is nonspecific and can transmit a long range through intracellular structures. As such, mechanosensing may occur not only at the cell surface but also inside the cell. Even when the responses are observed only when force is applied via a specific cell surface molecule or are not observed when this specific molecule is deleted, it can only be identified as being involved in mechanosensing, but it may still be unclear whether its role is sensing or transmitting force¹¹¹.

An example of candidate mechanosensing molecules not directly interacting with extracellular ligand and even not residing at the cell membrane is talin, a cytoplasmic protein associated with the integrin cytoplasmic domain. Using a combination of single-molecule mechanical experiment, single-molecule fluorescence measurement and MD simulations, Sheetz and colleagues demonstrated that stretching talin rod could open cryptic sites to activate vinculin binding⁹⁰. Here, the mechanical force may be transmitted from the cell surface via integrin–ECM binding into the cell but converted into biochemical signal inside the cell. As another example, Vogel and colleagues used microelectronic and micromechanical system (MEMS) force sensors and Förster resonance energy transfer (FRET) probes to demonstrate that mechanical force could unfold fibronectin fibers to expose switchable cryptic sites for cell binding⁸⁸. Here, the candidate mechanosensing molecule is an ECM protein residing outside the cell. Opposing stalling force could inhibit movement of molecular motors, e.g., myosin¹¹², due to force-induced conformational changes that reduce single translocation step. In this example, the mechanical responsive molecules could be dispersed anywhere in the cytoplasm.

Despite that a large number of signal sensing molecules have been identified and extensively studied, the question of how many of them are triggered remains largely unclear. This is the case even for many biochemical sensing molecules. For candidate mechanosensors identified by the elimination of mechanotransducing effect upon their removal, the triggering question includes the magnitude, direction, duration, frequency, and waveform of force needed to trigger and how force-triggered changes couple to biochemistry and signaling cascades. Here we submit that an important reason for this unsolved question may be that many receptor triggering mechanisms involve mechanochemistry. It is the lack of understanding of the mechanochemistry of these receptors and the tools to obtain such understanding that hinders the progress of the field. For example, receptor–ligand interactions are still primarily studied using force-independent solution binding assays, such as surface plasma resonance. Bond properties in these contexts are often completely different from the corresponding properties when the molecular bonds are subjected to force. After all, an important aspect of mechanochemistry is force regulation of receptor–ligand interaction, as discussed in early sections of this review. Furthermore, we suggest that molecular biomechanics has an important role to play in elucidating the mechanochemistry of receptor triggering processes. Moreover, we argue that to identify mechanosensors may require the understanding of how they are triggered. Only through elucidation of how a candidate molecule or assembly of molecules works can we definitively identify it as a mechanosensor. So the various questions regarding mechanosensing are inherently intertwined and may have to be solved together.

Let us look at the receptor triggering question from a mechanical perspective. Most receptors are transmembrane proteins that bind ligands via their extracellular domain above the cell membrane. Upon ligand binding, the biochemical signaling cascade is initiated via phosphorylation of the cytoplasmic domain of either the receptor itself or the signaling chains with which the receptor associates. Note that the phosphorylation site is ~10 nm away from the receptor's ligand-binding site on the other side of the cell membrane. For the chemical event of ligand binding to induce the chemical event of phosphorylation, a mechanical coupling between the two sites (i.e., allostery) is required because no chemical

reaction by itself (i.e., direct binding) can bridge such a wide spatial gap. This coupling between chemical and mechanical events is at the heart of the receptor mechanochemistry. The receptor assembly (itself or together with the signaling chains) may provide the physical structure for mechanical coupling, as many of these protein assemblies contain highly coordinated moving parts. In fact, ligand binding induced receptor conformational changes (with or without dimerization or energy consumption) represents a major hypothetical mechanism of receptor triggering, as in the case of integrin outside-in signaling. Since receptor–ligand interaction and protein conformational change can be regulated mechanically, as discussed in earlier sections of this paper, it seems natural that force (and force history) can regulate the receptor triggering process that converts the information embedded in the extracellular stimuli, be them biochemical or biomechanical, into intracellular signals. This argument suggests that mechanosensing may even include receptors commonly thought of as biochemical sensing molecules, such as the TCR/CD3 complex^{108,110} and the GPIIb-IX-V complex¹¹³. Future research may find that mechanosensing may be more common than we presently appreciate. Central to the above argument is the mechanochemistry of the signal sensing molecule, including mechanosensing molecules. These force-responsive proteins are part of an integrated mechanical and signaling system in cells and tissues. Thus in a broader context, mechanochemistry plays an integral role in the biochemical signaling networks.

8. Concluding remarks

We have reviewed an important area of molecular biomechanics, i.e., the study of mechanical regulation of molecular interaction and conformational change. Since molecular interaction and conformational change are commonly modeled mathematically using chemical reaction equations based on mass action and conservation laws, their mechanical regulation is formulated as the dependence of the chemical reaction rates on mechanical variables such as force and force history. Thus, this area of research is naturally and closely connected to the mechanochemistry of molecules and molecular assemblies. This connection opens a door for molecular biomechanics to contribute to mechanobiology via identifying and elucidating the inner working of mechanosensing and mechanotransducing molecules.

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Figure 1.

Conceptual energy landscapes that govern force regulation of molecular dissociation. A, A single bound state, single dissociation pathway energy landscape of a slip bond depicted as bond energy vs. the projection of dissociation pathway along the direction of an externally applied force f . The corresponding off-rate is described by the Bell equation $k_{\text{off}} = k_{\text{off}}^0 \exp(\Delta x f / k_B T)$ where k_B is Boltzmann constant and T is the absolute temperature. The stress-free off-rate k_{off}^0 is related to the energy barrier height ΔE by $k_{\text{off}}^0 = \exp(\Delta E / k_B T)$ and Δx represents the width of the energy well such that the energy barrier will increase e fold when the work done by force equals $k_B T$. Adapted from Ref. 86. B, An energy landscape of a catch-slip bond that has two bound states and dissociates along two pathways according to the sliding-rebinding mechanism. Starting from the bound-state energy well, the bond may dissociate to the free-state along pathway 1 or slide into a neighboring energy well upon formation of new interactions. From the new interaction energy well the bond may dissociate to the free-state along pathway 2 or rebound back to the bound-state energy well. As the magnitude of force increases, its direction also turns from the direction of pathway 1 to the direction of sliding as a result of force-induced conformational change, such that sliding into the new interaction energy well increases with increasing force from the low to intermediate levels. Sliding into the new interaction energy well and rebinding to the bound-state energy well prolong bond lifetime, thereby giving rise to catch bonds. At high forces all energy barriers are suppressed and dissociation is accelerated despite sliding and rebinding, which transitions the catch bonds to slip slips. Adapted from Ref. 64.

Figure 2.

Different types of bonds. A, Three types of bonds are defined depending on how their bond lifetime depends on whether bond lifetime increases (catch), decreases (slip), or does not change (ideal) with increasing force. B, Catch-slip bond of integrin $\alpha_L\beta_2$ -ICAM-1 interaction³⁶. C, Triphasic slip-catch-slip bond of E-selectin-ligand interaction³⁴.

Figure 3. Conceptual models of catch bond. A, Deformation model. B, Allosteric model. C, Sliding-rebinding model. Adapted from Ref. 86.

Figure 4.

Assays for mechanical regulation of molecular interaction. A, Constant force-clamp DFS for measuring bond lifetime under a constant force. B, Steady force-ramp DFS for measuring rupture force at a constant ramp-rate. C, Two constant force-clamp DFS assays with different ramp rates prior to clamping the force for bond lifetime measurement.

Figure 5.

Regulation of catch bond by force history. A and B, Plots of lifetime of L-selectin bonds with PSGL-1 (A) and 6-sulfo-sLex^x (B) vs. the force ramped to the clamped level at indicated different ramp-rates. The catch-slip bond at low ramp-rate ($r_f \sim 10^3$ pN/s) is converted to slip-only bond by high ramp-rate ($r_f \sim 10^5$ pN/s) for PSGL-1 (A) but not for 6-sulfo-sLex^x (B). C and D, The point mutation A108H converts the catch-slip bond of L-selectin to slip-only bond for 2-GSP-6 (a short N-terminal region of PSGL-1) (C) but not for 6-sulfo-sLex^x (D). E and F, the mutation L-selectinA108H is no longer responsive to ramp-rate increase (E) but the reverse mutation P-selectinH108A becomes responsive to ramp-rate increase (F).

Figure 6.

Modified sliding-rebinding model for the dual-dependence of bond lifetimes on hold-force and ramp-rate. A, Conceptual energy landscape for the interaction between L-selectin (and P-selectinH108A) and PSGL-1 with one or two dissociation pathways that respond in different ways to changes in ramp-rate and hold-force. At low ramp-rates, high hold-force (upper right) applied to the lectin (Lec) domain unbends the hinge (depicted by a coiled spring) with the epidermal growth factor (EGF) domain, causing the selectin ligand (SL) to tangentially slide along the inclined binding interface [state (1,0)]. From here, formation of new interaction(s) with the Lec domain is energetically more favorable than dissociation along pathway 1 (indicated by the relative height between the two energy barriers besides state (1,1), upper right), which dominates at low forces due to the unopened hinge (upper left). Sliding into a new interaction energy well [state (0,1)] and rebinding back to the initial bound state [state (1,1)] would strengthen binding and prolong lifetime, resulting in catch-slip bond behavior. In contrast, high loading rates during ramp phase would open the interdomain hinge angle even at low hold-forces (lower left), allowing the selectin-PSGL-1 complex to slide into state (0,1) and rebound back to state (1,1). Higher hold-forces cannot further enhance the already maximized sliding-rebinding, but would accelerate dissociation

from state (0,1) (lower right). *B*, The Ala to His substitution in L-selectin changes residue 108 from not contacting to contacting the PSGL-1 peptide, abolishes the sliding-rebinding mechanism and reduces the two-dimensional energy landscape to one of a single dimension, resulting in slip bond behavior. Adapted from Ref. 81.

Figure 7.

Effects of peak force of single-cycled mechanical reinforcement on fibronectin (FN)- $\alpha_5\beta_1$ bond lifetimes. A, Representative force-time traces. B, Distributions of bond lifetimes at ~5 pN with indicated peak forces (points) and their theoretical fits by the two-state model (curves). The natural log of the number of events with a lifetime t normalized by the natural log of the total number of events is plotted vs. t . Data for different peak forces (indicated) are separated for the sake of clarity. C, Mean $\pm$ s.e.m. of bond lifetimes increased with increasing peak force. D, Model of single-cycled mechanical reinforcement. Left: Liganded integrin in the short-lived state. Key $\alpha_5\beta_1$ domains are indicated along with the RGD and synergy binding sites on the FNIII 10 and 9 domains, respectively. Middle: Force

applied via the ligand primes the integrin for conformational change in the headpiece. Higher peak force (*Top*) better primes the integrin than lower peak force (*Bottom*). Right: During unloading, the FN- $\alpha_5\beta_1$ bond may be switched to the long-lived state (*Top*) or may return to the short-lived state (*Bottom*). A bond previously primed by a higher peak force is more likely to be switched to the long lived state than a bond primed by a lower peak force, as indicated by the sizes of the arrows. Adapted from Ref. 15.

Figure 8.

Effects of repetitive force cycles on FN- $\alpha_5\beta_1$ bond lifetimes. A, Representative force-time traces showing lifetime measurements at 10 pN with 0.5, 1.5, 2.5, and 3.5 cycles of loading-unloading with a 10 pN peak force. Complete unloading to zero force was used. B, Lifetime distributions of the measurements shown in A. Data for different numbers of cycles (indicated) are separated for the sake of clarity. C, Mean $\pm$ s.e.m. bond lifetime vs. number of repetitive loading-unloading cycles. D, Reinforcing FN- $\alpha_5\beta_1$ bond by multi-cycled small forces. Far-left: FN bound $\alpha_5\beta_1$ in the short-lived state as that in A Left. Mid-left: A loading-unloading cycle has a certain probability to switch the FN- $\alpha_5\beta_1$ bond to the long-lived state

(*Top*) but also a probability not to change the short-lived state (*Bottom*). Mid-right: After another loading-unloading cycle, the bond that is already in the long-lived state stays in the long-lived state (*Top*). However, the bond that remains in the short-lived state has a certain probability to switch to the long-lived state (*Bottom*). Far-right: As the number of cycles increases, the FN- $\alpha_5\beta_1$ bond is more and more likely to be switched to the long-lived state (*Top*) and less and less likely to remain in the short-lived state (*Bottom*), resulting in an accumulating effect. Adapted from Ref. 15.