Trends in Cell Biology

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Review

Control of Mechanotransduction by Molecular Clutch Dynamics

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The linkage of cells to their microenvironment is mediated by a series of bonds that dynamically engage and disengage, in what has been conceptualized as the molecular clutch model. Whereas this model has long been employed to describe actin cytoskeleton and cell migration dynamics, it has recently been proposed to also explain mechanotransduction (i.e., the process by which cells convert mechanical signals from their environment into biochemical signals). Here we review the current understanding on how cell dynamics and mechanotransduction are driven by molecular clutch dynamics and its master regulator, the force loading rate. Throughout this Review, we place a specific emphasis on the quantitative prediction of cell response enabled by combined experimental and theoretical approaches.

Highlights

By considering the molecular and mechanical properties of actin filaments, myosin motors, adaptor proteins, and integrins/cadherins, the molecular clutch model can quantitatively predict cell response to internal and external mechanical factors.

These factors include cell contractility, matrix rigidity, and the density, nature, and distribution of matrix ligands, and affect cell response largely by controlling the rate of force loading in specific molecules.

Due to its dynamic nature, clutch-mediated mechanosensing requires force application to at least two molecular mechanosensors in series, with differential response to force.

The type of cell responses involved so far in clutch-mediated mechanosensing include cytoskeletal dynamics, the growth of cell adhesions, the nuclear localization of transcriptional regulators, and cell migration.

The Molecular Clutch Hypothesis: A Means to Conceptualize Cell Adhesion Dynamics

Cells in almost any physiological setting, from bacteria infecting a tissue to neurons within the brain, are constantly exerting mechanical forces and transmitting them to neighboring cells and the extracellular matrix (ECM) [1–3]. These forces direct cell functions such as differentiation [4] or migration [5], and drive processes in development [6], cancer [7], and the physiology of the cardiovascular system [8]. Unravelling the mechanisms and implications of these mechanical interactions requires the understanding of how cells exert forces, how those are transmitted to the cell microenvironment, and how they trigger downstream events affecting cell function. In most eukaryotic settings, cells exert forces largely through actin polymerization, and the contraction of the actin cytoskeleton by myosin molecular motors. Once force is exerted to actin, it is transmitted first to a series of **adaptor proteins** (see Glossary) linked to actin, and then to transmembrane proteins linking adaptor proteins to the cell microenvironment (Figure 1). These transmembrane proteins consist mostly of integrins (which bind to the ECM) [9] and cadherins (which bind to neighboring cells) [10], and the molecular assemblies composed of actin, adaptor proteins, and integrins or cadherins are known respectively as cell–matrix or cell–cell adhesion complexes.

From actin to integrins/cadherins, adhesion complexes exhibit a precise spatial molecular organization [11,12], and are responsible for the specific adhesion of cells to their environment, which is otherwise dominated by nonspecific repulsive interactions [13]. A fundamental aspect of adhesion complexes is that they are extremely dynamic. Myosin-powered **contractility,** and actin polymerization pushing against the membrane [14,15], drive a constant flow of actin, generally termed '**retrograde flow**' because it moves from the cell edge where cell–ECM adhesions form towards the cell center [16,17]. This flow, which can be observed for different types of actin structures, from lamellipodia to stress fibers, is only partially transmitted to adaptor proteins and integrins, leading to progressively slower retrograde speeds as the molecules get closer to the ECM [18,19]. Even though they are far less characterized, similar

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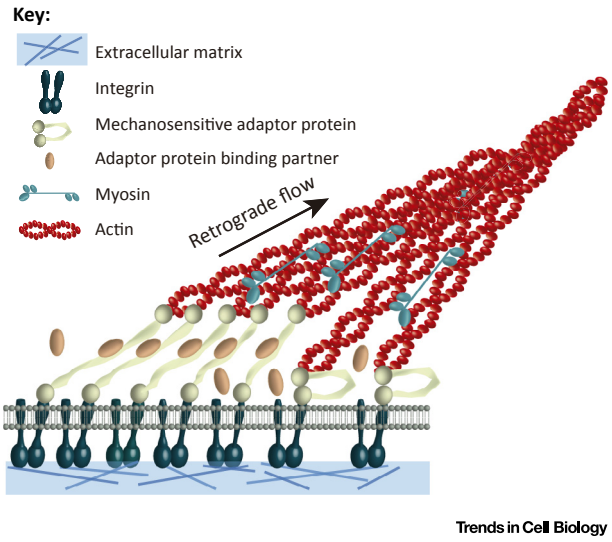


Figure 1. Cartoon Depicting the Serial Connection between the Extracellular Matrix, Integrins, Mechanosensitive Adaptor Proteins, and Actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

flows apply in cadherin-based cell–cell adhesions [20], and even nonactin based systems [21]. This progressively reduced flow points at a dynamic formation and release of bonds between the different molecular elements, which only transmit movement and force when the system is engaged. Consistently, retrograde flows inversely correlate with cell migration speed [14,16,22]. This suggests that when the system is engaged, force transmitted to the ECM counters myosin contractility, slowing actin retrograde flow (as observed in fish keratocytes [23]) and fostering actin protrusion away from the cell center. The dynamic nature of the cytoskeleton–ECM linkage, and its relationship to cell movement, led Mitchison and Kirschner [24] to introduce the term '**molecular clutch**' to describe it, in an analogy to the dynamic linkage between different shafts of a mechanical engine.

Because it regulates both force transmission and cell movement, this molecular clutch between actin and the ECM (or neighboring cells) controls the mechanical balance within a tissue, its remodeling, and the onset of mechanotransduction events. Importantly, because there is significant knowledge on the biochemical and mechanical properties of the molecular elements involved, quantitative modeling can be carried out, and quantitative mechanistic predictions can be obtained. This review focuses on how the molecular clutch concept, and its quantitative predictions, provides a framework to understand how cells respond to mechanical signals like forces or tissue rigidity. Thus, we do not discuss details of the complex molecular regulation of cell–cell and cell–ECM adhesions or the actin cytoskeleton, on which there are excellent recent reviews [25–27]. First, we summarize the molecular pathway that force must follow from actin to integrins/cadherins, and evidence for mechanical tension in the molecules involved. We note that, whereas most of the examples and discussion refer to the better-studied case of integrin-based cell–ECM adhesion, the concepts discussed are generalizable to cell–cell, and potentially almost any type of specific adhesion. Second, we describe the behavior of the clutch model, and how it responds to its main mechanical and molecular parameters. In this regard, we discuss the fundamental notion that molecular clutch response is not driven by forces *per se* (which constantly change due to their dynamic nature) but by the force **loading rate**. Third, we

Glossary

Adaptor proteins: term usually employed to refer to the proteins linking actin to either integrins or cadherins in cell–matrix or cell–cell adhesion complexes, respectively.
Binding/unbinding rates: for a given binding event [such as an integrin–extracellular matrix (ECM) bond], this is the inverse of the average time required to bind/unbind the bond, respectively. Binding occurs at zero force, whereas unbinding rates depend on the force applied to the bond.
Catch bond: more precisely defined as a catch–slip bond, a catch bond is a bond in which unbinding rates decrease with applied force up to a given threshold, and then increase. Catch bonds thus have an optimal stability (minimum unbinding rate) when a specific value of force is applied to the bond. Importantly, this concept can also be applied to molecular events other than unbinding, such as protein unfolding.
Contractility: ability of a cell to contract its actin cytoskeleton via myosin motors. In a situation with very low cell adhesion, contractility would power fast retrograde flows. In a context of high adhesion, contractility is transmitted to the substrate, leading to cell–matrix (or cell–cell) force transmission.
Durotaxis: directional cell migration towards areas of increased substrate rigidity.
Frictional slippage: regime with low cell–matrix adhesion, in which transient clutch engagement is unable to significantly slow retrograde flow.
Load and fail/stick–slip: regime with high cell–matrix adhesion, in which simultaneous engagement of several clutches leads to repeated cycles of progressive buildup of force, followed by complete disengagement and force release.
Loading rate: in units of force/time, rate at which applied force increases for a given clutch or clutch ensemble.
Molecular clutch: link between actin and an ECM ligand (or a neighboring cell) which can be bound (engaged) or unbound. Usually assumed to represent the serial link between actin, an individual

review how clutch mechanics couple to mechanosensitive proteins to enable cell mechanoresponse. Finally, we address these implications in cell migration.

Molecular Pathways of Force Transmission through the Clutch

Despite the molecular complexity of cell–ECM adhesions, the fundamental components of a molecular clutch system can be summarized as: (i) actin filaments, (ii) myosin motors pulling on actin filaments, (iii) adaptor proteins, (iv) integrins/cadherins, and (v) extracellular ligands at the ECM or other cells (Figure 1). Numerous proteins from both the cell–cell and cell–ECM adhesome are potentially involved in force transmission. As to cell–ECM interactions, force is transmitted through: (i) direct interactions between the ECM and integrins [28,29]; (ii) adaptor proteins that directly connect integrins to the actin cytoskeleton, including α-actinin [30], filamin [31], tensin [32], kindlin [33], and talin [34–36]; and (iii) indirect interactions between integrins and actin, mediated by vinculin [34,37–39], FAK, paxillin, and kank [40], among others. Regarding intercellular interactions, an equivalently complex network of adaptors connects cadherins to actin [41]. Recently, some of these adaptors (such as vinculin) have been shown to be shared between cell–ECM and cell–cell interactions [12]. Only a few of the several proteins linking actin to integrins and cadherins have been experimentally verified to be submitted to force, although potentially several more could be. For instance, experiments pulling on integrin–ECM or cadherin–cadherin bonds with magnetic tweezers, or measuring tension on ECM ligands through fluorescence reporters or tension gauges (that dissociate above a given force) have shown that integrins [29,42–47] and cadherins [48–51] withstand forces. Also, fluorescence tension probes have confirmed with piconewton resolution that not only integrins [52,53] and cadherins [54] are under force, but also intracellular proteins like vinculin [37] and talin [35,36] in cell–matrix adhesions and alpha-catenin [55] in cell–cell adhesions.

Regulation of Force Transmission through the Clutch

The fundamental property of the molecular clutch connecting actin to the ECM is its dynamic nature, that is, the more engaged the different components are to each other, the more effectively force will be transmitted. However, the interplay between the different elements leads to interesting nontrivial behaviors, which can be understood through mathematical models [56] that initially emerged inspired by the similar and better-studied system of muscle contraction. These models can be in the form of computational simulations [57–60] or analytical solutions [60–63], and all consider the effect of dynamic bonds between a surface and a sliding filament. In the form proposed by Chan and Odde [57], model response rests on two key properties under force of the molecules involved. First, myosin motors will contract actin filaments at a fixed speed (of about 120 nm/s) if their action is unopposed by force [29,57]. If a force opposes myosin action, its contraction speed will decrease with force until stalling completely if the force applied matches the maximum force that a myosin motor can apply (2 pN) [64]. This inverse relationship between actin speed and force has been widely reported [29,34,57,65], although it is worth noting that a direct relationship has been observed below speeds of 10 nm/s [65], possibly due to changes in myosin density in cell lamellae [66]. Second, as force is transmitted to molecular bonds (actin–adaptor proteins, adaptor proteins–integrins, or integrins–ECM), the lifetime of the bonds will be affected, eventually destabilizing bonds when submitted to sufficiently high forces (see section below for the distinction between **slip bonds** and **catch bonds**). In most models, only one type of bond is considered, which is assumed to correspond to the weakest link in the actin–adaptor protein–integrin–ECM chain. This 'weakest link' has been attributed both to intracellular bonds involving adaptor proteins [57,67], or to the integrin–ECM link [29,68,69]. In any case, the fact that different clutch components show different retrograde flow speeds [18] suggests that all bonds play a role, and that modeled bonds likely reflect an integrated response of the entire clutch rather than a weakest link.

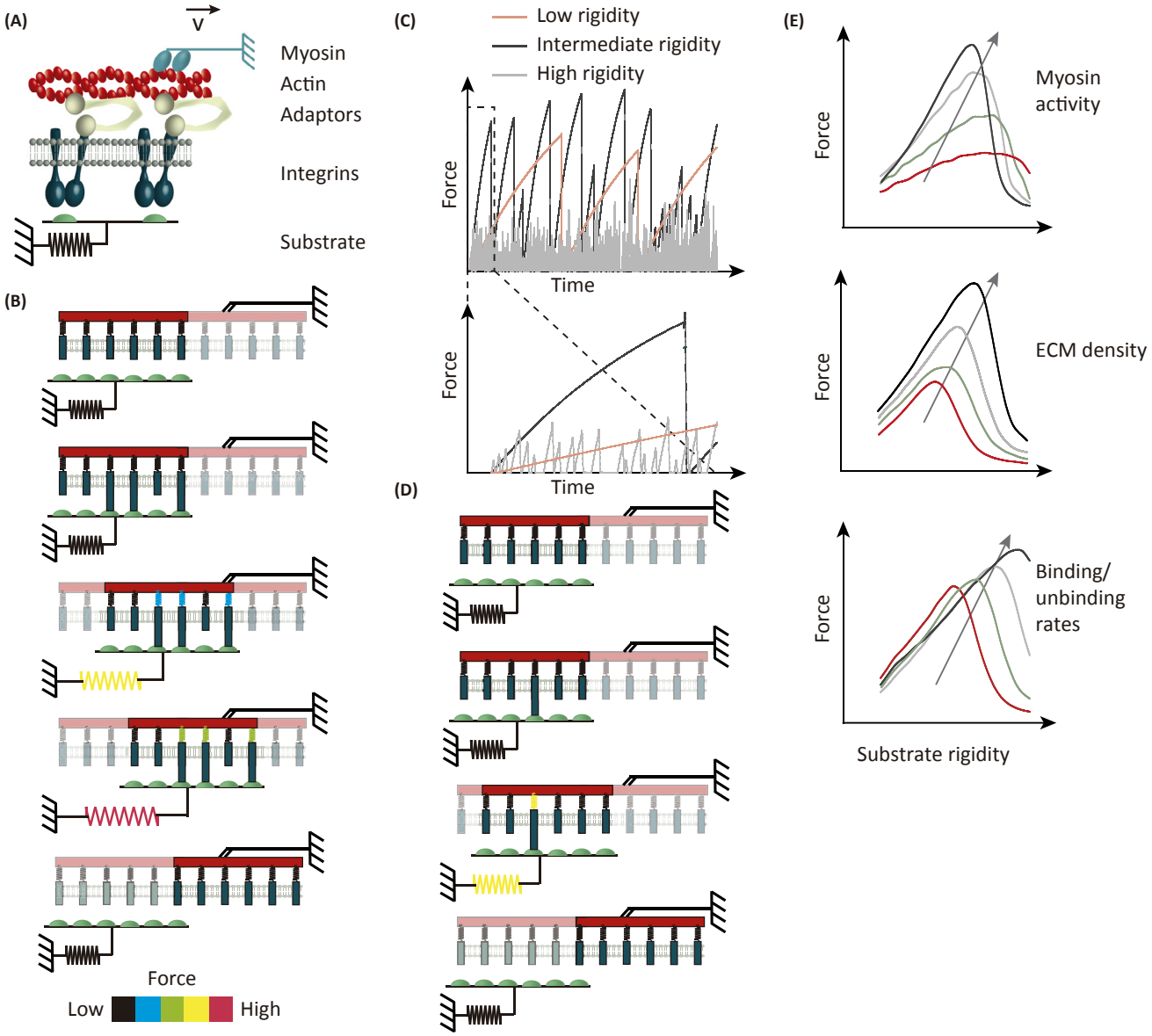
adaptor protein, an integrin, and an ECM ligand.

Molecular mechanosensor: molecule that responds to force application in any way (domain unfolding, unbinding from ligands, conformational changes, or others).

Retrograde flow: movement of actin filaments from the edge towards the center of cells. It can be powered by myosin contractility, actin polymerization, or both. It is important to note that in the context of a migrating cell, the relevant flow that drives force transmission to the substrate is the one measured with respect to substrate (and not cell) position.

Slip bond: bond in which unbinding rates increase monotonically with applied force. Importantly, this concept can also be applied to molecular events other than unbinding, such as protein unfolding.

In a typical molecular clutch simulation, the system begins with myosin freely contracting an actin filament, containing several adaptor protein–integrin complexes (clutches) which are not bound to the substrate (Figure 2A,B). With time, clutches begin binding to the substrate according to a given **binding rate**. Once the system is engaged, myosin contractility pulls



Trends in Cell Biology

Figure 2. Force Transmission through the Molecular Clutch. (A) Cartoon summarizing the fundamental elements of the system. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch (rectangles represent integrins). As clutches bind, myosin contractility deforms the substrate, building force on the substrate and each bound clutch. At some point, force leads to bond destabilization, all clutches disengage, and the cycle starts again. (C) Typical plots of force exerted versus time for molecular clutches on low, intermediate, and high rigidity. (D) From top to bottom, sequence of events in a typical 'frictional slippage' cycle of a molecular clutch, observed on a high-rigidity regime. As a clutch binds, myosin contractility builds force very quickly due to the high rigidity, leading to clutch disengagement before others have time to bind. This limits overall force transmission to the substrate. (E) Clutch model predictions of average force transmission to the substrate as a function of substrate rigidity. Top, middle, and bottom graphs show the changes in the curve induced by increasing myosin activity, increasing ECM ligand density, and simultaneously increasing binding and unbinding rates, respectively. Abbreviation: ECM, Extracellular matrix.

on the substrate, deforming it if it is compliant, and exerting a force which distributes among the different bound clutches. As force keeps on building, bonds eventually fail, leading to a catastrophic event which quickly releases all force and disengages all bonds, allowing the cycle to start again (Figure 2B,C). Such cycles are termed '**load and fail**' or '**stick–slip**' behavior, and have been observed in neuronal growth cones [57], focal adhesions [70], and the leading edge of mouse embryonic fibroblasts [71,72].

Interestingly, the cycles of force generation are finely regulated by the properties of both the molecular players involved and the cell microenvironment, endowing cells not only with exquisite mechanosensitivity but also with the ability to tune it. The fundamental factor driving clutch mechanosensitivity (and response to both cellular and extracellular parameters) is the force loading rate (i.e., the speed at which force in clutches builds once they engage). This is nicely exemplified in the case of cell response to substrate rigidity, a microenvironmental factor that drives cell differentiation [4] or tumor progression [73]. Substrate rigidity directly controls the loading rate, which in simple terms can be understood as the product of the substrate rigidity times the speed of retrograde flow. In clutch models, force transmission is maximized for a specific value of rigidity, or loading rate. Above the optimal rigidity, force in individual clutches loads so fast upon binding that clutches become destabilized and disengage before additional clutches can bind. That is, **unbinding rates** (off rates) become faster than binding rates (on rates), the number of clutches simultaneously engaged drops drastically, and overall force transmission decreases (Figure 2C,D). This is a regime known as '**frictional slippage**', characterized by high retrograde flow, low forces, and no load and fail cycles, and observed, for instance, in neuronal growth cones [57] or the trailing edge of migrating keratocytes [74]. Below the optimal rigidity, force loading becomes so slow that clutches eventually disengage before high forces can be reached. Thus, the molecular clutch model predicts a biphasic relationship between rigidity (loading rate) and force, in which forces first increase and then decrease with rigidity. Such behavior has indeed been observed in neuronal growth cones and glioma cells [57,75], but in several other systems a monotonically increasing rigidity/force relationship has been reported instead [28,76–78]. This discrepancy is due to the fact that in many cases, cells grow focal adhesions above a threshold in rigidity (due to talin unfolding; see below). Large adhesions increase integrin clustering, the effective binding rate of the system, and the number of bound clutches, preventing the entry into the frictional slippage regime and maintaining high force transmission [34].

Other than rigidity, several cellular and extracellular parameters tune the mechanosensitivity of the molecular clutch. In most cases, the effects can also be understood through the regulation of the loading rate. First, reducing myosin contractility lowers the loading rate. Consequently, in myosin inhibition conditions, reaching the optimal loading rate for force transmission requires a higher substrate rigidity. Therefore, whereas myosin inhibition of course reduces overall contractility, there is a specific range of substrate rigidity in which force transmission can be increased (Figure 2E). This counterintuitive prediction, which has been observed experimentally [34], occurs at a rigidity where the loading rate is optimal in myosin-inhibited conditions, but too high and already within the frictional slippage regime in control conditions. Second, decreasing ECM ligand density reduces binding sites and therefore overall force transmission (Figure 2E). However, since myosin contractility is now distributed among less clutches, the loading rate experienced by each molecular clutch increases. In turn, this decreases the substrate rigidity corresponding to the optimal loading rate, and optimal force transmission [34]. Third, altering different parameters at the same time can lead to combined effects that also shift optimal force transmission (Figure 2E). This can be achieved, for instance, by binding to the ECM through different integrin types (with different binding and unbinding

rates) [29], or simultaneously altering the numbers of myosin motors and available clutches [75,79].

While less well characterized, it is tempting to speculate on how different integrin and focal adhesion regulators could impact molecular clutch behavior. For instance, we recently reported [44] that ZO-1, an adaptor protein normally present in cell–cell adhesions but that can also bind α5β1 integrins [80], increases the binding and unbinding rates of α5β1 to fibronectin. This then fosters the formation of adhesions in a manner consistent with molecular clutch predictions [44]. Other adaptor proteins, such as the recently characterized sharpin [81], shank [82], kank [40], or kindlin [33] also regulate integrin properties and could therefore have similar effects. Finally, it is interesting to note that whereas the effect of rigidity has largely been studied with purely elastic substrates, adding a viscoelastic behavior has a significant effect [83]. In this regard, we have recently shown that cell response to purely viscous environments can also be understood through a molecular clutch mechanism driven by force loading rates [84].

Regulation of Force Transduction by the Clutch

Once we understand how the molecular clutch regulates cell–ECM force transmission, the next pressing question is to determine how force then triggers mechanosensing events, (i.e., how cells convert force into biochemical signals that will eventually affect cell function). This process is generally believed to occur through mechanosensing molecules, in which force alters their conformation and biochemical properties. The best known example is that of the actin–integrin adaptor protein talin, which unfolds under force and exposes binding sites to vinculin [85,86]. Other proteins such as α-catenin [87] or filamin [88] also change binding partner affinities under force, and force-induced molecular events include changes in integrin conformation [89], ion channel activity [90,91], or kinase activity [92] (see [3,93] for recent reviews). However, it is important to note that in the context of a continuously contracting cell, none of these **molecular mechanosensors** is sufficient on its own to build an effective cell mechanosensing mechanism. Taking talin as an example, if a given actin–talin–integrin clutch engages to the substrate, myosin contractility will start pulling on it. This will eventually load force sufficiently to induce talin unfolding, regardless of substrate rigidity or any other external mechanical stimulus.

To properly discriminate between different levels of rigidity, a system of at least two mechanosensors with different properties is required. In the case of the actin–talin–integrin–ECM clutch, this is provided by the different properties under force of talin unfolding, and of integrin–ECM binding [34]. Talin unfolding responds to force according to the bell model [13] as a classical slip bond. That is, when a constant force is applied to a single talin molecule, the time required to unfold decreases exponentially with force [86]. By contrast, the binding between α5β1 [94] or αvβ3 [34,89] integrins and the ECM protein fibronectin behaves as a catch bond (or more accurately, a catch–slip bond). That is, the time required to break the bond first increases and then decreases with force. This differential behavior leads to a crossover between the two force/lifetime curves, such that for low forces integrin unbinding is faster than talin unfolding, and for high forces the opposite holds (Figure 3A). Upon integrin unbinding, force would be released and no longer pull on talin, therefore this system effectively triggers talin unfolding only above a force threshold. Talin unfolding then leads to vinculin binding, which in turn triggers focal adhesion growth through mechanisms that are not fully elucidated [38,95].

A relevant nuance is that, for simplicity, we have referred to force to reason on the differential response of the mechanosensors, whereas (as discussed above) a molecular clutch system controls force loading rate rather than force itself. However, the dependency of unfolding/unbinding rates on loading rate can be readily calculated if force/lifetime curves are known [96],

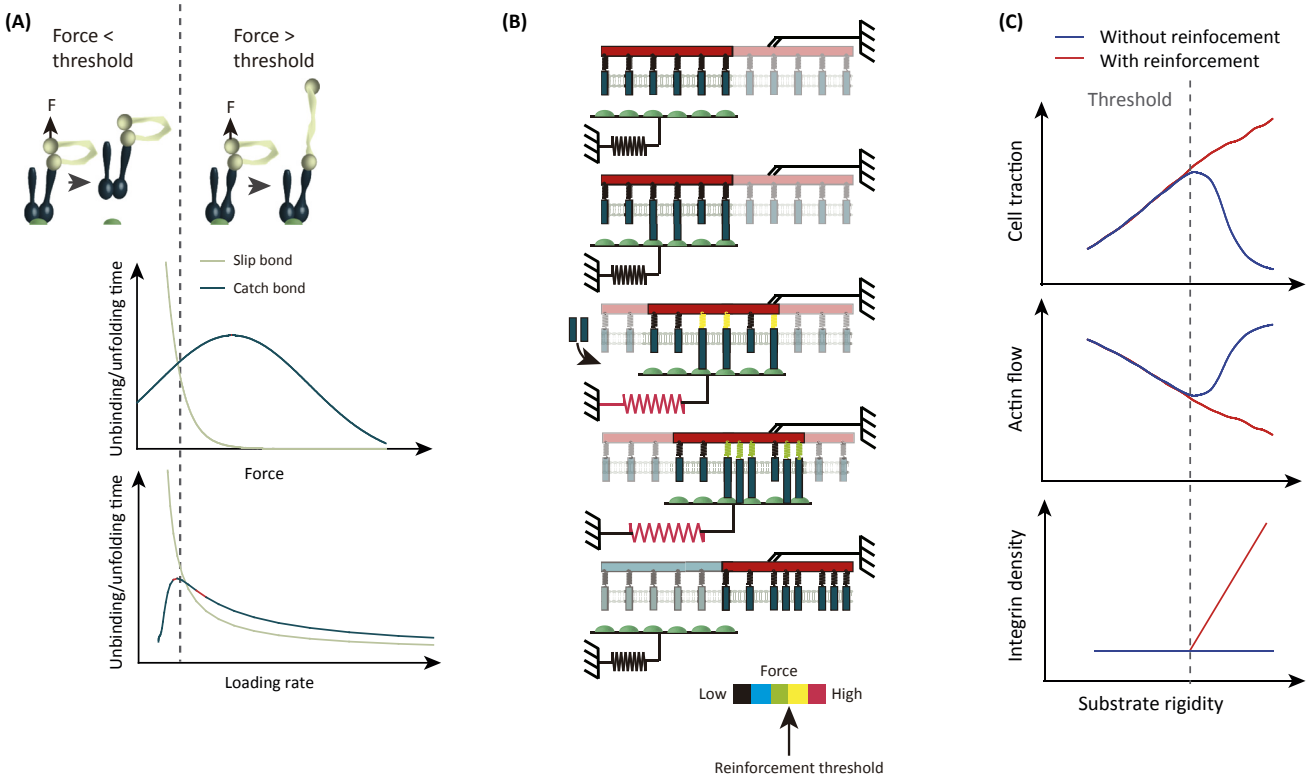


Figure 3. Force Transduction through the Molecular Clutch. (A) Bottom, effect of either a constant force or a constant force loading rate on the average times required for protein unfolding or bond unbinding. Typical curves for a slip or catch bond are shown. Top, expected effect on a system in which force is applied to a serial link between a molecule that unfolds as a slip bond (such as talin) and a bond that unbinds as a catch bond (such as an integrin–fibronectin bond). Unbinding occurs first when force is below the threshold, and unfolding (and subsequent mechanotransduction) occurs first when force is above the threshold. (B) From top to bottom, sequence of events in a typical 'load and fail' cycle of a molecular clutch, including mechanotransduction (reinforcement) events. As clutches bind and force builds, some clutches surpass the threshold force required for mechanotransduction, leading to the recruitment of additional integrins. This increases the number of bound clutches, reducing the force applied per clutch, delaying the failure of the system, and increasing average force transmission. (C) Examples of predicted force/rigidity curves in the presence and absence of reinforcement. Reinforcement only affects force transmission above a threshold in rigidity, which corresponds to the loading rate threshold from (A). Then, the increase in integrin recruitment prevents the reduction in force (and increase in actin flows) normally expected in a molecular clutch system.

leading to the same crossover behavior (Figure 3A). Thus, all the factors described above, controlling the loading rate experienced by individual molecules (substrate rigidity, myosin contractility, ECM coating, integrin binding kinetics) will determine not only force transmission but also force transduction, the activation of downstream signals such as focal adhesion formation, and the nuclear localization of the transcriptional regulator YAP [34,97]. In the case of rigidity, for instance, talin unfolding only occurs above a given threshold. Subsequent focal adhesion growth (reinforcement) then increases the clutch binding rate, simply because there are more integrins to bind to. This then prevents the decrease in force and increase in actin retrograde flows that would be otherwise expected at high rigidities (Figure 3B,C). Below the rigidity threshold, integrin unbinding (rather than talin unfolding) predominates, as supported by experiments using ECM ligands attached to tension gauge tethers [46]. Other than rigidity, we have recently shown that cell sensing of the nano-scale distribution of ECM ligands, and subsequent formation of focal adhesions, can also be explained by a clutch model considering two differential mechanosensors, and the spatial arrangement of ligands [98].

Whereas this clutch-mediated differential mechanosensing mechanism has so far been demonstrated only for the talin unfolding versus integrin–ECM unbinding system, it could apply in several other instances. Potential examples include cadherin–cadherin unbinding versus α-catenin unfolding [87] (in cell adhesions), glycoprotein Ib (GPIb)–von Willebrand factor unbinding versus GPIb unfolding (in platelets) [99], or stretch-induced conformational changes in the actin cross-linker filamin [88], which could add an additional mechanosensor in series with the integrin–talin system. Importantly, the fundamental feature in enabling mechanosensitivity is the crossover between the lifetimes of the two mechanosensors, and not necessarily slip bond/catch bond behavior *per se*. Thus, in principle, mechanosensitivity could also be achieved with two slip bonds, as long as their sensitivities to force were different.

Summarizing, the fundamental parameter that determines the response of a molecular clutch system is the force loading rate, which is sensitive to factors both external (substrate rigidity, ECM, or cadherin ligand density) and internal (myosin contractility, type and clustering of integrins), and varies greatly in different physiological conditions [100]. This endows cells with exquisite mechanosensitivity, which results in regulation of both force transmission and in the activation of mechanosensors. Supporting this hypothesis of the loading rate as the key ingredient, experiments have shown that it controls integrin adhesion [101,102] and focal adhesion formation [103]. Interestingly, this hypothesis also proposes an alternative to an old debate in the field, which is whether cells sense rigidity by applying a given deformation (strain) to the substrate and measuring the resulting force (stress), or vice versa [78,104,105]. Measuring force loading rates may be more optimal than measuring forces or deformations *per se*, for two fundamental reasons. First and as noted theoretically [106,107], if time dependency (and loading rate) is ignored, the magnitude of force that cells can apply depends on their contractility but not necessarily on the mechanical properties of the cell environment, precluding proper mechanosensing. Second, cell-applied forces continuously fluctuate, as observed at scales ranging from cell collectives [108], to focal adhesions [70], to local 100 nm-scale contractions in the leading edge of fibroblasts [109]. In fact, molecular clutch mechanisms driven by loading rates have been proposed to explain force fluctuations at the level of cell collectives [5] and focal adhesions [110]. The mechanics of nano-scale contractions, which are associated with altered response to substrate rigidity [111], and altered activity of receptor tyrosine kinases [112], is less clear. However, both the contractions and the trigger of mechanosensing events affecting kinase activity may also be controlled by the loading rate.

Regulation of Cell Migration by the Clutch

Since the clutch model predicts cell–substrate forces, one could think that this can directly explain cell migration. Yet, cells generate tractions that are orders of magnitude higher than those needed to migrate, and tractions generated by a migratory single cell add up to zero within measurement noise [113]. Tractions should thus not be interpreted as propulsion forces. However, tractions are linked to migration speed through the retrograde flow [14,16,22]. For a given actin polymerization rate, cells exhibiting the slowest retrograde flow, and therefore the highest traction, should be the ones that migrate faster. This relationship is well captured by early clutch models, which focused only on dynamics of the leading edge [57]. A more general formulation of cell migration in terms of clutch models requires taking into account not only the leading edge, but also how all protrusions pull on the cell body. Such a formulation was accomplished by Bangasser *et al*. [75], who showed that a generalized clutch model predicts an optimal rigidity for migration as a function of the number of clutches and motors. These predictions were successfully tested for neurons and glioma cells, which exhibit a biphasic behavior of their migratory properties [75]. We note, however, that these cells do not exhibit

adhesion reinforcement, so the general ability of clutch models to predict a relationship between migration speed and rigidity needs to be further assessed.

Besides contributing to the understanding of single cell migration, clutch models have also been successful at explaining collective **durotaxis**; this is, the ability of groups of cells to follow gradients of rigidity [5]. When a group of epithelial cells was seeded on a substrate with a rigidity gradient, cells moved preferentially towards the stiff area of the substrate. Collective durotaxis was lost when force generation was inhibited with blebbistatin and when cell–cell junctions were abrogated. Traction maps revealed that cells exerted inward forces of the same magnitude but opposite sign only at the two edges of the monolayer. This force pattern implies long range force transmission through cell–cell junctions. To explain collective durotaxis, we modeled the cell monolayer as a contractile continuum adhered to the substrate through two clutches, located at the stiff and soft edges. Force balance implies that cells on soft and stiff areas of the substrate generate the same force, and therefore cell–matrix adhesions are subjected to the same loading rate. The model then predicts that dynamics at both edges are identical but that substrate displacement is larger on the soft edge than on the stiff one. As such, contraction of the monolayer systematically shifts the center of the cell cluster, thereby resulting in durotaxis. This simple model, designed to explain collective durotaxis, is also applicable to single cell durotaxis [114], which is predicted to be more efficient for cells that are large and highly contractile.

Concluding Remarks

The dynamic nature of the cytoskeleton and adhesion complexes has long been acknowledged, and the molecular clutch concept has been demonstrated to be a useful framework to understand the underlying mechanisms. Further, recent developments have shown that quantitative modeling of the different molecular elements in the clutch provides a powerful tool to predict how cells detect cues from their environment, and respond by tuning their migration, but also adhesive and signaling events. However, several outstanding questions remain (see Outstanding Questions). First, how force is transmitted and distributed through the very complex molecular assemblies at cell–matrix and cell–cell adhesions (i.e., which adaptor molecules are directly submitted to force, and to what degree) remains largely unknown. Addressing this question, and understanding the force-induced molecular events involved, will enable the refining of clutch models to predict cell response in a much more general way. Second, it is highly likely that dynamic clutch-like adhesion occurs not only at cell adhesions but also throughout cells, for instance, in cytoskeletal–nuclear coupling. Exploring such events and their implications is also a major area of exploration. Finally, whereas the molecular clutch concept has been largely explored in cells seeded on flat two-dimensional substrates, the interaction between actin structures, myosin, and adhesive complexes is known to be largely affected by the three-dimensional setting found in most physiological conditions. While the effect of this three-dimensional setting in the molecular clutch concept has begun to be explored [19], its implications remain largely uncharted. Addressing these and other open questions is thus likely to lead to new exciting developments in the coming years.

Outstanding Questions

How is force transmitted across the complex molecular assemblies at cell–cell and cell–matrix adhesions, what are the corresponding force-induced molecular events, and how can they be introduced in molecular clutch models?

Do clutch-like adhesive mechanisms take place outside of cell adhesions, such as in nuclear–cytoskeletal links?

How is the molecular clutch concept affected by the three-dimensional distribution of cytoskeletal and adhesive structures in physiological scenarios?

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