Control of mechanotransduction by molecular clutch dynamics

Alberto Elosegui-Artola[1,*], Xavier Trepat[1,2,3,4], and Pere Roca-Cusachs[1,2,*]

1.- Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), 08028 Barcelona, Spain

2.- University of Barcelona, 08028 Barcelona, Spain.

3.- Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

4.- Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina, 28029 Madrid, Spain.

** Correspondence: aelosegui@ibecbarcelona.eu, rocacusachs@ub.edu*

Keywords: Mechanobiology, cell adhesion, focal adhesions, integrins, cadherins.

Abstract

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 the review, we place a specific emphasis on the quantitative prediction of cell response enabled by combined experimental and theoretical approaches.

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], the physiology of the cardiovascular system [8], and several other scenarios in health and disease. 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 (Fig. 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 non-specific 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 flows apply in cadherin-based cell-cell adhesions [20], and even non-actin 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 for instance 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 modelling can be carried out, and quantitative mechanistic predictions can be obtained. This is precisely what this review is focused 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 will not enter in details on 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 will 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 will 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 will describe the behavior of the clutch model, and how it responds to its main mechanical and molecular parameters. In this regard, we will 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 will discuss how clutch mechanics couple to mechanosensitive proteins to enable cell mechanoresponse. Finally, we will address 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 a) actin filaments, b) myosin motors pulling on actin filaments, c) adaptor proteins, d) integrins/cadherins, and e) extracellular ligands at the ECM or other cells (Fig. 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: 1) direct interactions between the ECM and integrins [28, 29], 2) 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 3) indirect interactions between integrins and actin –mediated by vinculin [34, 37-39], FAK, paxillin, and Kank [40], among many others-. As to 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. Besides, 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, i.e., 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 non-trivial behaviors, which can be understood through mathematical models [56] which initially emerged inspired by the similar and better studied system of muscle contraction. Those 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 [57, 64]. 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) [65]. This inverse relationship between actin speed and force has been widely reported [34, 57, 64, 66], although it is worth noting that a direct relationship has been observed below speeds of 10 nm/s [66], possibly due to changes in myosin density in cell lamellae [67]. 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 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, 68], or to the integrin-ECM link [64, 69, 70]. In any case, the fact that different clutch components show different retrograde flow speeds [18] suggests that all bonds play a role, and that modelled bonds likely reflect an integrated response of the entire clutch rather than a weakest link.

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 (Fig. 2A-B). With time, clutches begin binding to the substrate according to a given **binding rate**. Once the system is engaged, myosin contractility pulls 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 (Fig. 2B-C). Such cycles are termed "**load and fail**" or "**stick-slip**" behavior, and have been observed in neuronal growth cones [57], focal adhesions [71], and the leading edge of mouse embryonic fibroblasts [72, 73].

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 which drives cell differentiation [4] or tumor progression [74],

among others. 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 (Fig. 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 [75]. 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, 76], but in several other systems a monotonically increasing rigidity/force relationship has been reported instead [28, 77-79]. 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 (Fig. 2E). This counter-intuitive 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 (Fig. 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 (Fig. 2E). This can be achieved for instance by binding to the ECM through different integrin types (with different binding and unbinding rates) [64], or simultaneously altering the numbers of myosin motors and available clutches [76, 80].

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 [83] that ZO-1, an adaptor protein normally present in cell-cell adhesions but that can also bind α5β1 integrins [84], 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 [83]. Other adaptor proteins, such as the recently characterized sharpin [85], shank [86], 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 [81]. 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 [82].

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 [87, 88]. Other proteins such as α-catenin [89] or filamin [90] also change binding partner affinities under force, and force-induced molecular events include changes in integrin conformation [91], ion channel activity [92, 93], or kinase activity [94] (see [3, 95] 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 their 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 [88]. In contrast, the binding between α5β1 [96] or αvβ3 [34, 91] 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 (Fig. 3A). Because upon integrin unbinding force would be released and no longer pull on talin, 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, 97].

A relevant nuance is that whereas for simplicity we have referred to force to reason on the differential response of the mechanosensors, 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 [98], leading to the same crossover behavior (Fig. 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, and the activation of downstream signals such as focal adhesion formation, and the nuclear localization of the transcriptional regulator YAP [34, 99]. 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 (Fig. 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 [100].

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 [89] (in cell adhesions), glycoprotein Ib (GPIb)/von Willebrand factor unbinding versus GPIb unfolding (in platelets) [101], or stretch-induced conformational changes in the actin crosslinker filamin [90], which could add an additional mechanosensor in series with the integrin/talin system. Importantly, the fundamental feature to enable mechanosensitivity is the crossover between 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 [102]. 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 [103, 104] and focal adhesion formation [105]. 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 [79, 106, 107]. Measuring force loading rates may be more optimal than measuring forces or deformations per se, for two fundamental reasons. First and as noted theoretically [108, 109], 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 [110], to focal adhesions [71], to local 100 nm-scale contractions in the leading edge of fibroblasts [111]. 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 [112]. The mechanics of nano-scale contractions, which are associated with altered response to substrate rigidity [113], and altered activity of receptor tyrosine kinases [114], 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 is 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 [115]. 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 not only taking into account the leading edge, but also how all protrusions pull on the cell body. Such formulation was accomplished by Bangasser et al [76], 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 [76]. 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 understand 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 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 modelled 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 [116], 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 demonstrated to be a useful framework to understand the underlying mechanisms. Further, recent developments have shown that quantitative modelling 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 open (see outstanding questions box). 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. Whereas the effect of this three-dimensional setting in the molecular clutch concept have 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.

References

1. Ng, D. et al. (2016) The Vibrio cholerae Minor Pilin TcpB Initiates Assembly and Retraction of the Toxin-Coregulated Pilus. PLoS Pathog 12 (12), e1006109.
2. Betz, T. et al. (2011) Growth cones as soft and weak force generators. Proc Natl Acad Sci U S A 108 (33), 13420-5.
3. Roca-Cusachs, P. et al. (2017) Quantifying forces in cell biology. Nat Cell Biol 19 (7), 742-751.
4. Engler, A.J. et al. (2006) Matrix elasticity directs stem cell lineage specification. Cell 126 (4), 677-689.
5. Sunyer, R. et al. (2016) Collective cell durotaxis emerges from long-range intercellular force transmission. Science 353 (6304), 1157-61.
6. Bosveld, F. et al. (2012) Mechanical control of morphogenesis by Fat/Dachsous/Four-jointed planar cell polarity pathway. Science 336 (6082), 724-7.
7. Miroshnikova, Y.A. et al. (2016) Tissue mechanics promote IDH1-dependent HIF1alpha-tenascin C feedback to regulate glioblastoma aggression. Nat Cell Biol 18 (12), 1336-1345.
8. Humphrey, J.D. et al. (2015) Role of mechanotransduction in vascular biology: focus on thoracic aortic aneurysms and dissections. Circ Res 116 (8), 1448-61.
9. Roca-Cusachs, P. et al. (2012) Finding the weakest link - exploring integrin-mediated mechanical molecular pathways. J.Cell Sci. 125 (Pt 13), 3025-3038.
10. Ladoux, B. et al. (2015) The mechanotransduction machinery at work at adherens junctions. Integr Biol (Camb) 7 (10), 1109-19.

11. Kanchanawong, P. et al. (2010) Nanoscale architecture of integrin-based cell adhesions. Nature 468 (7323), 580-U262.
12. Bertocchi, C. et al. (2017) Nanoscale architecture of cadherin-based cell adhesions. Nat Cell Biol 19 (1), 28-37.
13. Bell, G.I. (1978) Models for the specific adhesion of cells to cells. Science 200 (4342), 618-627.
14. Lin, C.-H. and Forscher, P. Growth cone advance is inversely proportional to retrograde F-actin flow. Neuron 14 (4), 763-771.
15. Forscher, P. and Smith, S.J. (1988) Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J.Cell Biol. 107 (4), 1505-1516.
16. Wang, Y.L. (1985) Exchange of actin subunits at the leading edge of living fibroblasts: possible role of treadmilling. The Journal of Cell Biology 101 (2), 597-602.
17. Theriot, J.A. and Mitchison, T.J. (1991) Actin microfilament dynamics in locomoting cells. Nature 352 (6331), 126-31.
18. Hu, K. et al. (2007) Differential transmission of actin motion within focal adhesions. Science 315 (5808), 111-115.
19. Owen, L.M. et al. (2017) A cytoskeletal clutch mediates cellular force transmission in a soft, three-dimensional extracellular matrix. Mol Biol Cell 28 (14), 1959-1974.
20. Peglion, F. et al. (2014) Adherens junction treadmilling during collective migration. Nat Cell Biol 16 (7), 639-51.
21. Havrylenko, S. et al. (2014) Extending the molecular clutch beyond actin-based cell motility. New J Phys 16 (10).
22. Lee, J. et al. (1993) Principles of locomotion for simple-shaped cells. Nature 362 (6416), 167-71.
23. Barnhart, E.L. et al. (2011) An adhesion-dependent switch between mechanisms that determine motile cell shape. PLoS Biol 9 (5), e1001059.
24. Mitchison, T. and Kirschner, M. (1988) Cytoskeletal dynamics and nerve growth. Neuron 1 (9), 761-772.
25. Case, L.B. and Waterman, C.M. (2015) Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. Nat Cell Biol.
26. Sun, Z. et al. (2016) Integrin-mediated mechanotransduction. J Cell Biol 215 (4), 445-456.
27. Giannone, G. et al. (2009) Multi-level molecular clutches in motile cell processes. Trends Cell Biol 19 (9), 475-86.
28. Schiller, H.B. et al. (2013) beta1- and alphav-class integrins cooperate to regulate myosin II during rigidity sensing of fibronectin-based microenvironments. Nat Cell Biol 15 (6), 625-36.
29. Elosegui-Artola, A. et al. (2014) Rigidity sensing and adaptation through regulation of integrin types. Nat Mater 13 (6), 631-7.
30. Roca-Cusachs, P. et al. (2013) Integrin-dependent force transmission to the extracellular matrix by alpha-actinin triggers adhesion maturation. Proc Natl Acad Sci U S A 110 (15), E1361-70.
31. Ehrlicher, A.J. et al. (2011) Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. Nature 478 (7368), 260-3.
32. Clark, K. et al. (2010) Tensin 2 modulates cell contractility in 3D collagen gels through the RhoGAP DLC1. J Cell Biochem 109 (4), 808-17.
33. Theodosiou, M. et al. (2016) Kindlin-2 cooperates with talin to activate integrins and induces cell spreading by directly binding paxillin. Elife 5, e10130.
34. Elosegui-Artola, A. et al. (2016) Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. Nat Cell Biol 18 (5), 540-8.
35. Austen, K. et al. (2015) Extracellular rigidity sensing by talin isoform-specific mechanical linkages. Nat Cell Biol 17 (12), 1597-606.

408 36. Ringer, P. et al. (2017) Multiplexing molecular tension sensors reveals piconewton force
409 gradient across talin-1. Nat Methods.
410 37. Grashoff, C. et al. (2010) Measuring mechanical tension across vinculin reveals regulation of
411 focal adhesion dynamics. Nature 466 (7303), 263-6.
412 38. Carisey, A. et al. (2013) Vinculin regulates the recruitment and release of core focal adhesion
413 proteins in a force-dependent manner. Curr Biol 23 (4), 271-81.
414 39. Dumbauld, D.W. et al. (2013) How vinculin regulates force transmission. Proc Natl Acad Sci U S
415 A 110 (24), 9788-93.
416 40. Sun, Z. et al. (2016) Kank2 activates talin, reduces force transduction across integrins and
417 induces central adhesion formation. Nat Cell Biol 18 (9), 941-53.
418 41. Hoffman, B.D. and Yap, A.S. Towards a Dynamic Understanding of Cadherin-Based
419 Mechanobiology. Trends Cell Biol 25 (12), 803-814.
420 42. Liu, Y. et al. (2014) Nanoparticle tension probes patterned at the nanoscale: impact of integrin
421 clustering on force transmission. Nano Lett 14 (10), 5539-46.
422 43. Zhang, Y. et al. (2014) DNA-based digital tension probes reveal integrin forces during early cell
423 adhesion. Nat Commun 5, 5167.
424 44. Gonzalez-Tarrago, V. et al. (2017) Binding of ZO-1 to alpha5beta1 integrins regulates the
425 mechanical properties of alpha5beta1-fibronectin links. Mol Biol Cell 28 (14), 1847-1852.
426 45. Roca-Cusachs, P. et al. (2009) Clustering of alpha(5)beta(1) integrins determines adhesion
427 strength whereas alpha(v)beta(3) and talin enable mechanotransduction. Proc Natl Acad Sci U S A
428 106 (38), 16245-50.
429 46. Rahil, Z. et al. (2016) Nanoscale Mechanics Guides Cellular Decision Making. Integrative
430 Biology.
431 47. Wang, X. and Ha, T. (2013) Defining single molecular forces required to activate integrin and
432 notch signaling. Science 340 (6135), 991-4.
433 48. Bazellieres, E. et al. (2015) Control of cell-cell forces and collective cell dynamics by the
434 intercellular adhesome. Nat Cell Biol 17 (4), 409-20.
435 49. Labernadie, A. et al. (2017) A mechanically active heterotypic E-cadherin/N-cadherin adhesion
436 enables fibroblasts to drive cancer cell invasion. Nat Cell Biol 19 (3), 224-237.
437 50. Andresen Eguiluz, R.C. et al. (2017) Substrate stiffness and VE-cadherin mechano-transduction
438 coordinate to regulate endothelial monolayer integrity. Biomaterials 140, 45-57.
439 51. Muhamed, I. et al. (2016) E-cadherin-mediated force transduction signals regulate global cell
440 mechanics. J Cell Sci 129 (9), 1843-54.
441 52. Morimatsu, M. et al. (2013) Molecular tension sensors report forces generated by single
442 integrin molecules in living cells. Nano Lett 13 (9), 3985-9.
443 53. Chang, A.C. et al. (2016) Single Molecule Force Measurements in Living Cells Reveal a
444 Minimally Tensioned Integrin State. ACS Nano 10 (12), 10745-10752.
445 54. Borghi, N. et al. (2012) E-cadherin is under constitutive actomyosin-generated tension that is
446 increased at cell-cell contacts upon externally applied stretch. Proc Natl Acad Sci U S A 109 (31),
447 12568-73.
448 55. Kim, T.J. et al. (2015) Dynamic visualization of alpha-catenin reveals rapid, reversible
449 conformation switching between tension states. Curr Biol 25 (2), 218-24.
450 56. Schwarz, U.S. and Safran, S.A. (2013) Physics of adherent cells. Rev Mod Phys 85 (3), 1327-
451 1381.
452 57. Chan, C.E. and Odde, D.J. (2008) Traction dynamics of filopodia on compliant substrates.
453 Science 322 (5908), 1687-1691.
454 58. Bangasser, B.L. et al. (2013) Determinants of maximal force transmission in a motor-clutch
455 model of cell traction in a compliant microenvironment. Biophys J 105 (3), 581-92.

59. Escribano, J. et al. (2014) A discrete approach for modeling cell–matrix adhesions. Computational Particle Mechanics 1 (2), 117-130.
60. Sabass, B. and Schwarz, U.S. (2010) Modeling cytoskeletal flow over adhesion sites: competition between stochastic bond dynamics and intracellular relaxation. J Phys Condens Matter 22 (19), 194112.
61. Bangasser, B.L. and Odde, D.J. (2013) Master equation-based analysis of a motor-clutch model for cell traction force. Cell Mol Bioeng 6 (4), 449-459.
62. Sens, P. (2013) Rigidity sensing by stochastic sliding friction. EPL 104 (3), 38003.
63. Srinivasan, M. and Walcott, S. (2009) Binding site models of friction due to the formation and rupture of bonds: state-function formalism, force-velocity relations, response to slip velocity transients, and slip stability. Phys Rev E Stat Nonlin Soft Matter Phys 80 (4 Pt 2), 046124.
64. Elosegui-Artola, A. et al. (2014) Rigidity sensing and adaptation through regulation of integrin types. Nature Materials 13, 631-637.
65. Molloy, J.E. et al. (1995) Movement and force produced by a single myosin head. Nature 378 (6553), 209-12.
66. Gardel, M.L. et al. (2008) Traction stress in focal adhesions correlates biphasically with actin retrograde flow speed. J Cell Biol 183 (6), 999-1005.
67. Craig, E.M. et al. (2015) Model for adhesion clutch explains biphasic relationship between actin flow and traction at the cell leading edge. Phys Biol 12 (3), 035002.
68. Jiang, G.Y. et al. (2003) Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. Nature 424 (6946), 334-337.
69. Aratyn-Schaus, Y. and Gardel, M.L. (2010) Transient frictional slip between integrin and the ECM in focal adhesions under myosin II tension. Curr Biol 20 (13), 1145-53.
70. Ishibashi, M. et al. (2015) Integrin LFA-1 regulates cell adhesion via transient clutch formation. Biochem Biophys Res Commun 464 (2), 459-66.
71. Plotnikov, S.V. et al. (2012) Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. Cell 151 (7), 1513-27.
72. Giannone, G. et al. (2004) Periodic lamellipodial contractions correlate with rearward actin waves. Cell 116 (3), 431-443.
73. Pontes, B. et al. (2017) Membrane tension controls adhesion positioning at the leading edge of cells. J Cell Biol.
74. Levental, K.R. et al. (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139 (5), 891-906.
75. Fournier, M.F. et al. (2010) Force transmission in migrating cells. J Cell Biol 188 (2), 287-97.
76. Bangasser, B.L. et al. (2017) Shifting the optimal stiffness for cell migration. Nat Commun 8, 15313.
77. Ghibaudo, M. et al. (2008) Traction forces and rigidity sensing regulate cell functions. Soft Matter 4 (9), 1836-1843.
78. Califano, J.P. and Reinhart-King, C.A. (2010) Substrate Stiffness and Cell Area Predict Cellular Traction Stresses in Single Cells and Cells in Contact. Cell Mol Bioeng 3 (1), 68-75.
79. Ghassemi, S. et al. (2012) Cells test substrate rigidity by local contractions on sub-micrometer pillars. Proceedings of the National Academy of Sciences of the United States of America 109 (14), 5328-5333.
80. Mekhdjian, A.H. et al. (2017) Integrin-mediated traction force enhances paxillin molecular associations and adhesion dynamics that increase the invasiveness of tumor cells into a three-dimensional extracellular matrix. Mol Biol Cell 28 (11), 1467-1488.
81. Chaudhuri, O. et al. (2015) Substrate stress relaxation regulates cell spreading. Nat Commun 6, 6364.

82. Bennett, M. et al. (2018) Molecular clutch drives cell response to surface viscosity. Proc Natl Acad Sci U S A, In press.
83. González-Tarragó, V. et al. (2017) Binding of ZO-1 to α5β1 integrins regulates the mechanical properties of α5β1-fibronectin links. Mol Biol Cell.
84. Tuomi, S. et al. (2009) PKCε Regulation of an α₅ Integrin–ZO-1 Complex Controls Lamellae Formation in Migrating Cancer Cells. Science Signaling 2 (77), ra32-ra32.
85. Rantala, J.K. et al. (2011) SHARPIN is an endogenous inhibitor of beta1-integrin activation. Nat Cell Biol 13 (11), 1315-24.
86. Lilja, J. et al. (2017) SHANK proteins limit integrin activation by directly interacting with Rap1 and R-Ras. Nat Cell Biol 19 (4), 292-305.
87. del Rio, A. et al. (2009) Stretching Single Talin Rod Molecules Activates Vinculin Binding. Science 323 (5914), 638-641.
88. Yao, M. et al. (2014) Mechanical activation of vinculin binding to talin locks talin in an unfolded conformation. Sci Rep 4, 4610.
89. Yao, M. et al. (2014) Force-dependent conformational switch of alpha-catenin controls vinculin binding. Nat Commun 5, 4525.
90. Ehrlicher, A.J. et al. (2011) Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. Nature 478 (7368), 260-263.
91. Chen, Y. et al. (2016) Force regulated conformational change of integrin αVβ3. Matrix Biology.
92. Coste, B. et al. (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330 (6000), 55-60.
93. Wu, J. et al. (2016) Localized force application reveals mechanically sensitive domains of Piezo1. Nat Commun 7, 12939.
94. Sawada, Y. et al. (2006) Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 127 (5), 1015-1026.
95. Gauthier, N.C. and Roca-Cusachs, P. (2018) Mechanosensing at integrin-mediated cell-matrix adhesions: from molecular to integrated mechanisms. Curr Opin Cell Biol, In press.
96. Kong, F. et al. (2009) Demonstration of catch bonds between an integrin and its ligand. J Cell Biol 185 (7), 1275-1284.
97. Atherton, P. et al. (2016) Mechanosensitive components of integrin adhesions: Role of vinculin. Exp Cell Res 343 (1), 21-27.
98. Dudko, O.K. et al. (2008) Theory, analysis, and interpretation of single-molecule force spectroscopy experiments. Proc Natl Acad Sci U S A 105 (41), 15755-60.
99. Elosegui-Artola, E. et al. (2017) Force triggers YAP nuclear entry by regulating transport across nuclear pores. Cell, In press.
100. Oria, R. et al. (2017) Force loading explains cell spatial sensing of ligands. Nature, In press.
101. Ju, L. et al. (2016) Cooperative unfolding of distinctive mechanoreceptor domains transduces force into signals. Elife 5.
102. Humphrey, J.D. et al. (2014) Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 15 (12), 802-12.
103. Nguyen, N.M. et al. (2017) Characterisation of cellular adhesion reinforcement by multiple bond force spectroscopy in alveolar epithelial cells. Biol Cell 109 (7), 255-272.
104. Jiang, L. et al. (2016) Cells Sensing Mechanical Cues: Stiffness Influences the Lifetime of Cell-Extracellular Matrix Interactions by Affecting the Loading Rate. ACS Nano 10 (1), 207-17.
105. Cui, Y. et al. (2015) Cyclic stretching of soft substrates induces spreading and growth. Nat Commun 6, 6333.
106. Saez, A. et al. (2005) Is the mechanical activity of epithelial cells controlled by deformations or forces? Biophys J 89 (6), L52-4.

552 107. De, R. et al. (2008) Do cells sense stress or strain? Measurement of cellular orientation can
553 provide a clue. Biophys J 94 (5), L29-31.
554 108. Bruinsma, R. (2005) Theory of force regulation by nascent adhesion sites. Biophys J 89 (1), 87-
555 94.
556 109. Schwarz, U.S. et al. (2006) Focal adhesions as mechanosensors: the two-spring model. BioSyst
557 83 (2-3), 225-32.
558 110. Serra-Picamal, X. et al. (2012) Mechanical waves during tissue expansion. Nature Physics 8
559 (8), 628-U66.
560 111. Meacci, G. et al. (2016) alpha-actinin links ECM rigidity sensing contractile units with periodic
561 cell edge retractions. Mol Biol Cell.
562 112. Wu, Z. et al. (2017) Two Distinct Actin Networks Mediate Traction Oscillations to Confer Focal
563 Adhesion Mechanosensing. Biophys J 112 (4), 780-794.
564 113. Wolfenson, H. et al. (2016) Tropomyosin controls sarcomere-like contractions for rigidity
565 sensing and suppressing growth on soft matrices. Nat Cell Biol 18 (1), 33-42.
566 114. Yang, B. et al. (2016) Mechanosensing Controlled Directly by Tyrosine Kinases. Nano Lett 16
567 (9), 5951-61.
568 115. Oliver, T. et al. (1999) Separation of propulsive and adhesive traction stresses in locomoting
569 keratocytes. J Cell Biol 145 (3), 589-604.
570 116. Lo, C.M. et al. (2000) Cell movement is guided by the rigidity of the substrate. Biophys J 79
571 (1), 144-152.

572

573

Figure legends

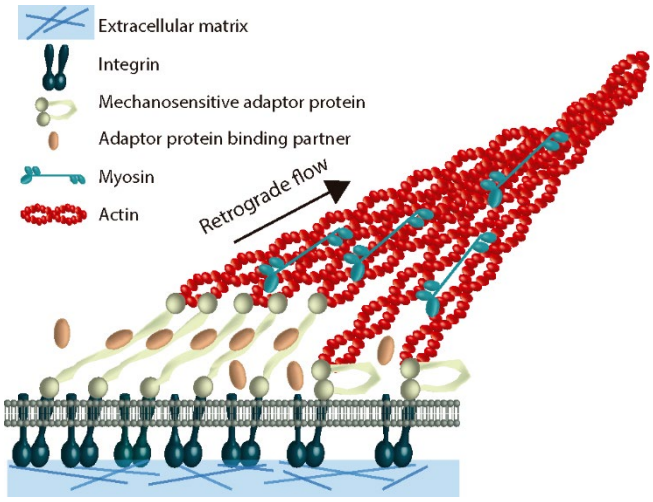

Figure 1. Cartoon depicting the serial connection between the extracellular matrix, integrins, mechanosensitive adaptor proteins, and actin. As myosin pulls on actin filaments, force is transmitted to the different elements, leading to conformational changes in adaptor proteins and affecting unbinding events.

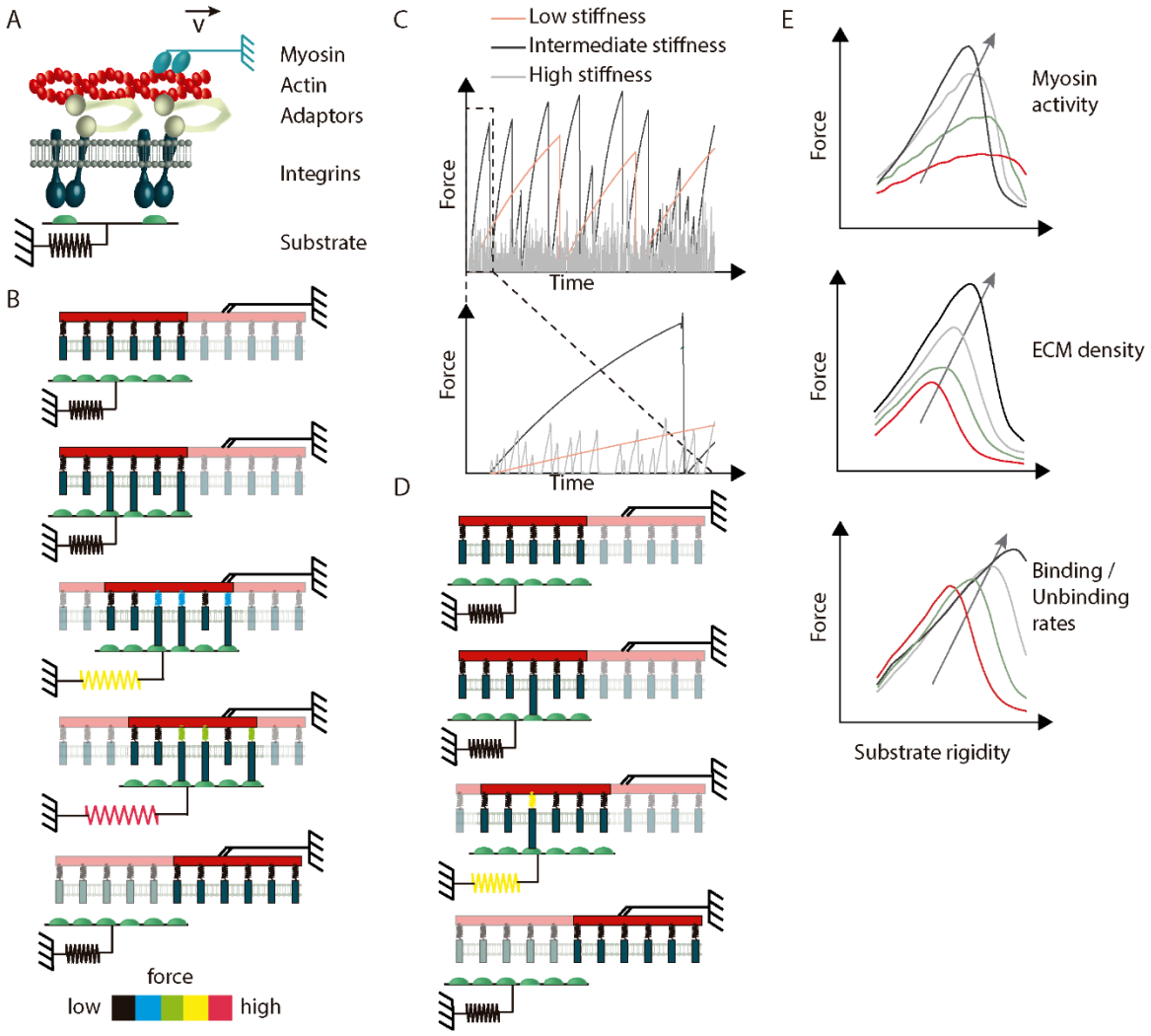

Figure 2. Force transmission through the molecular clutch. A) Cartoon summarizing the fundamental elements of the system. B) From top to bottom, sequence of events in a typical "load and fail" cycle of a molecular clutch (rectangles represent integrins). As clutches bind, myosin contractility deforms the substrate, building force on the substrate and each bound clutch. At some point, force leads to bond de-stabilization, 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.

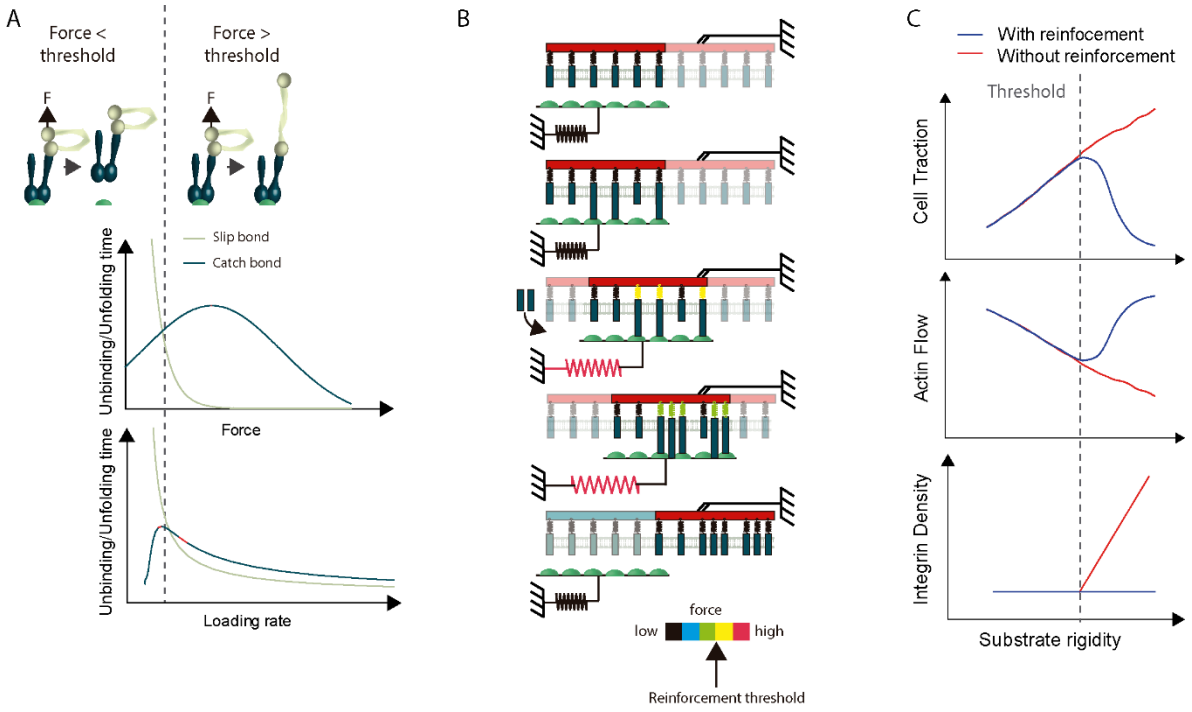

Figure 3. Force transduction through the molecular clutch. A) bottom, effect of either a constant force or a constant force loading rate on the average times required for protein unfolding or bond unbinding. Typical curves for a slip or catch bond are shown. Top, expected effect on a system in which force is applied to a serial link between a molecule that unfolds as a slipbond (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 panel A. Then, the increase in integrin recruitment prevents the reduction in force (and increase in actin flows) normally expected in a molecular clutch system.

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-ECM bond), inverse of the average time required to bind/unbind the bond, respectively. Whereas binding occurs at zero force, 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.

Cell 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 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.

Outstanding Questions Box

- 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 3D distribution of cytoskeletal and adhesive structures in physiological scenarios?

Trends Box

- 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.

- Those 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.