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Mechanotransduction through protein stretching

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Abstract

Cells sense and respond to subtle changes in their physicality, and via a myriad of different mechanosensitive processes, convert these physical cues into chemical and biochemical signals. This process, called mechanotransduction, is possible due to a highly sophisticated machinery within cells. One mechanism by which this can occur is via the stretching of mechanosensitive proteins. Stretching proteins that contain force-dependent regions results in altered geometry and dimensions of the connections, as well as differential spatial organization of signals bound to the stretched protein. The purpose of this mini-review is to discuss some of the intense recent activity in this area of mechanobiology that strives to understand how protein stretching can influence signaling outputs and cellular responses.

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Stretching proteins as a mechanism to convert mechanical signals into chemical signals

Mechanotransduction describes the mechanisms by how cells can sense and respond to physical forces and convert them into changes in cell behavior. There are multiple pathways for mechanotransduction, including membrane receptor activation, reorganization of the nucleus, mechanosensitive transcription factors to mention just a few. Much of this mechanotransduction occurs at sites of adhesion between the cell and its immediate surroundings, the extracellular matrix (ECM), and neighboring cells. Here we briefly introduce the cellular architectures that allow protein stretching to be effectively used to transduce mechanical cues into chemical signals.

Mechanical linkages

One paradigm of protein stretching relates to the proteins that couple the cytoskeleton to the plasma membrane [1,2]. Here, these molecules mediate tension-bearing linkages, coupling adhesion receptors to the cytoskeleton, and as a result, they are held under tension. These proteins serve structural and scaffolding roles, but positioned within mechanical linkages, they have emerged as major mechanosensors. As an example, the cell couples to the ECM via the integrin ECM-receptors on the cell surface that when activated lead to formation of large integrin adhesion complexes. The major coupling of the integrin cytoplasmic tail to the actin and microtubule cytoskeletons is via the protein talin, which mediates mechanical coupling to the actin cytoskeleton directly, and indirectly via the protein vinculin. Most molecules in these connections have emerged as containing mechanosensitive features. Our definition of 'mechanosensitive features' is quite broad and includes: 1) force-bearing structural domains that unfold at higher forces and refold at lower forces, 2) force-bearing disordered regions which assume a more extended conformation at higher forces and a more compact, randomly coiled conformation at lower forces, 3) the force-dependent rate of dissociation of a force-bearing protein-protein interface, 4) force applied to an exposed binding site may influence its binding affinity with binding partners, 5) force applied to a force-bearing structural domain that bears a cryptic binding site may release the autoinhibition of the binding.

Catch bonds, bonds where the lifetime is short until force is applied to them

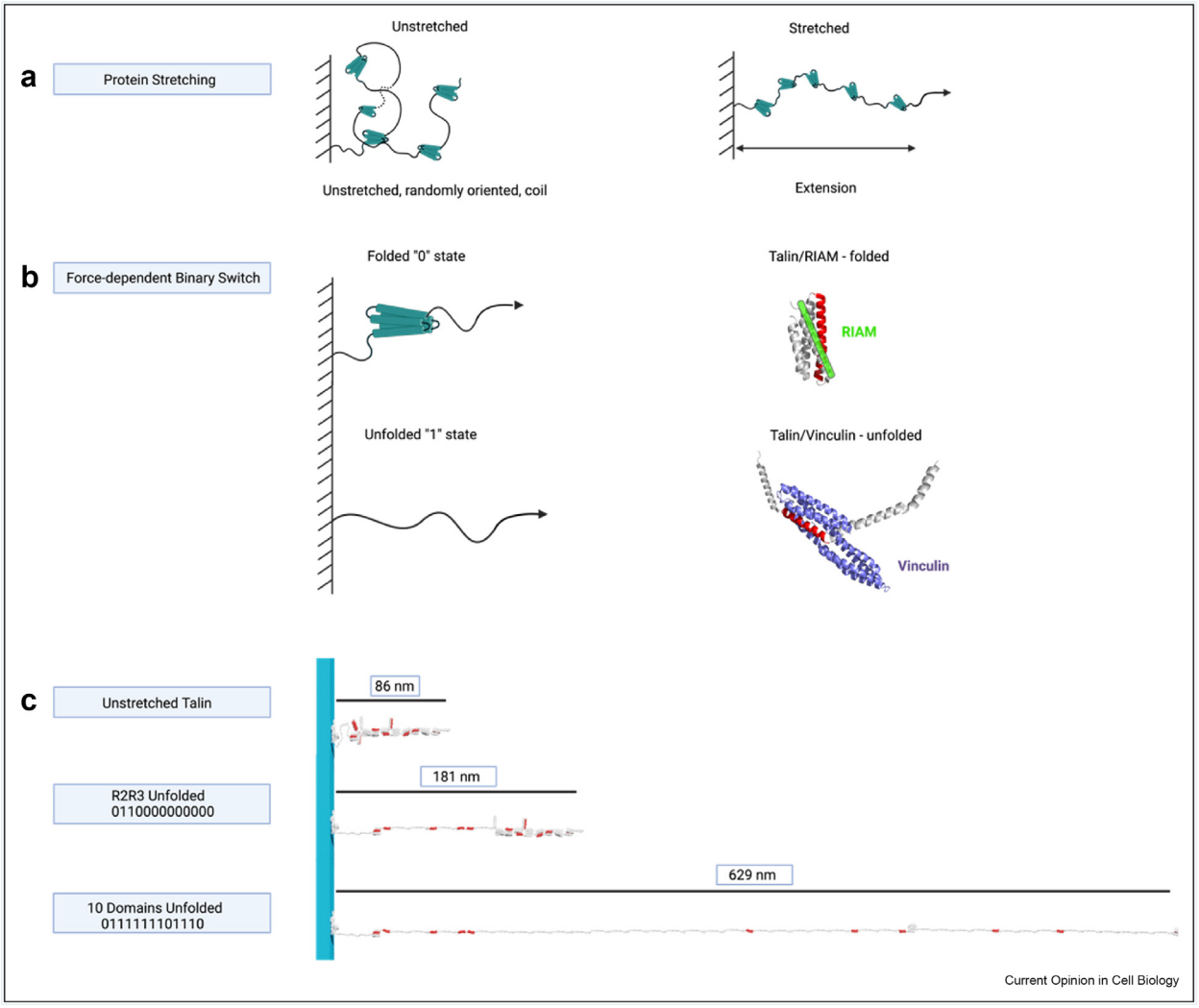
Many of the bonds that mediate the mechanical linkages in cells exhibit catch bond behavior. This is where, in the absence of force, the lifetime is short, but once in a

mechanical linkage, it can be significantly longer. An earlier study on this was on the mechanical linkages at cell-cell junctions, where the stability between the cadherin-catenin complex and F-actin is weak in solution but is stronger when tension is applied [3]. This catch bond behavior is also present in the integrin-adhesion complexes, and each link in the ECM-integrin-talin-vinculin-actin chain, ECM-integrin [4], vinculin-actin [5,6] and talin-actin [7]. The talin-integrin bond has slightly different behavior as it is mechanically weak but a recent study showed that addition of kindlin converts this bond into a force-independent ideal bond [8], in this way kindlin is crucial for the mechanical linkage formation that ultimately allows talin stretching.

Force-dependent binary switches in proteins

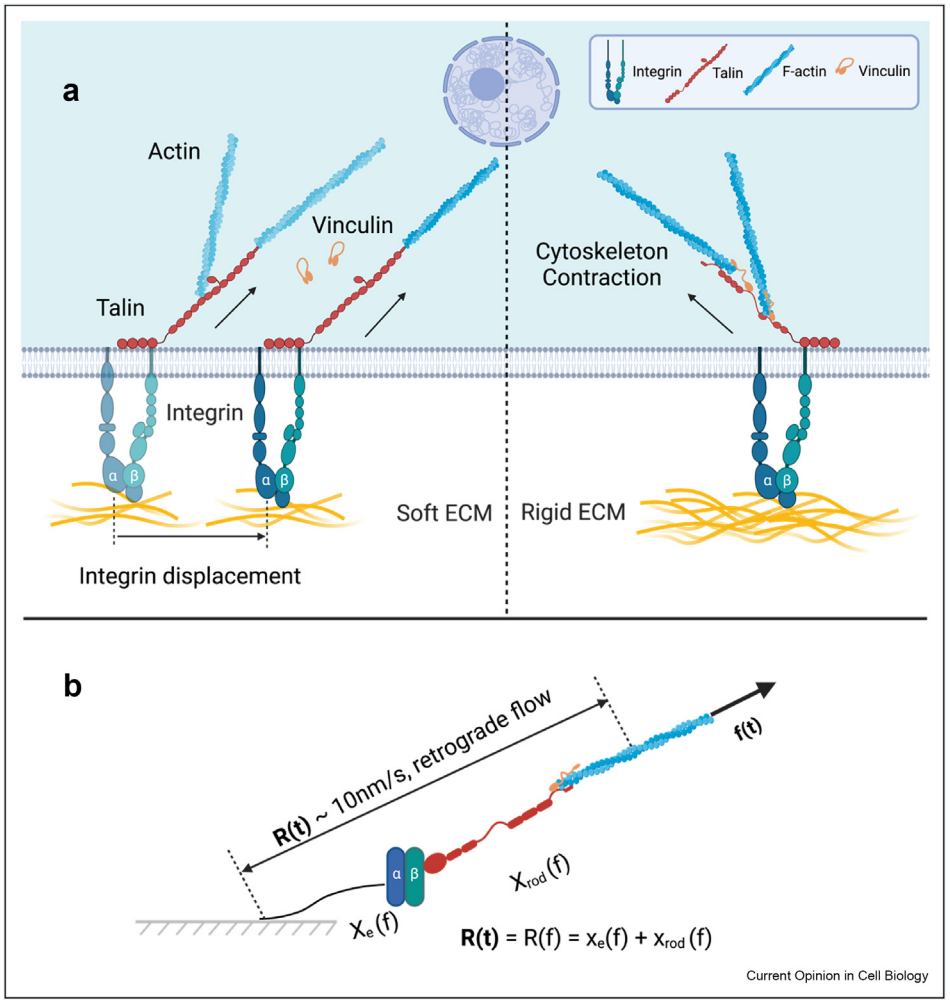
Once a protein is coupled in a mechanical linkage it will experience stretching (Figure 1a,2b). When proteins are stretched, the constituting domains and polypeptide linkers are orientated toward the direction of stretching, resulting in extension. Further, many proteins in these linkages, including talin and vinculin, contain helical

Figure 1



Protein stretching. a) Tension applied to a protein will lead to it being stretched in the direction of the force. Mechanosensing proteins contain many different force-sensing elements including i) intrinsically disordered regions and ii) folded domains that undergo force-dependent structural transitions. **b)** Helical bundles in the force transmission pathway serve as force-dependent binary switches. In the absence of force, or below the force threshold for that domain, the folded "0" state will persist, but unfolding will open the domain to the unfolded "1" state, increasing the length of the protein and altering the recruited signaling molecules. As an example, talin R3 binds RIAM in its folded state while it binds vinculin in its unfolded state (only one vinculin is shown bound but R3 can bind up to two vinculins). **c)** Talin contains 13 of these binary switches, and so its mechanical response is complex. Shown are three states of talin drawn to scale, top) all 13 domains are folded, middle) two domains, R2 and R3, are unfolded, bottom) 10 domains are unfolded. The dimensions of the protein and the mechanical linkage it is part of will change dramatically as a result of protein stretching.

Figure 2



Mechanical linkages in cells. a) Cartoon of a mechanical linkage in cells, here the cell-ECM linkages are shown. Catch bonds form between the integrin-ECM, the talin-actin, and the vinculin-actin interactions to form the mechanical linkage. Tension on the linkage leads to the proteins being stretched in the direction of the force. The protein stretching leads to opening of switch domains and altered dimensions but also mechanotransduction. **b)** The mechanical linkages can be considered as physical systems that can be modeled mathematically and explained by simple physical and biophysical rules. Box 1 details experimental approaches to study these linkages *in vitro*.

bundles that serve as force-dependent binary switch domains. This switch-like behavior arises because folded structural domains, such as alpha-helical bundles or Ig domains, store a large folding energy that prevents spontaneous unfolding by thermal fluctuation. To thermally unfold such domains, a non-physiologically relevant higher temperature would be needed. However, a small force of a few pN applied to such domains is often sufficient to unfold them. If a structural domain binds other factors, then the two force-dependent states (folded or unfolded) can bind different factors, making it a binary switch regulated by force. Due to the large folding energy stored in such domains, the fold change of the binding affinity between the folded and unfolded states can be several orders of magnitude. As these linkages exist under basal tension, these helical bundles exhibit switch-like behavior, as both folded and unfolded states are thermodynamically stable, and can be switched between the two states by small changes in mechanical force/tension.

Talin is a particularly complex and sophisticated example as its rod region contains 13 helical bundles, R1-R13, that are in the force transmission pathway and subjected to mechanical stretching. These helical bundles serve dual purposes: they not only transmit force but also undergo dynamic, force-dependent transitions between folded and unfolded states (Figure 1b,c). Under physiologically relevant force-loading rates (a few pN per second) that are exerted by retrograde actin flow

at speeds of tens of nm per second [9], these helical bundles unfold at forces ranging from approximately 5 to 30 pN [10,11]. This mechanical unfolding endows talin with vital functions. First, it acts as a tension buffer within a narrow force range, averaging less than 15 pN but accommodating large extensions up to 600 nm [11] (Figure 1c) (This shock-absorbing property of multiple switches in series was recently used to generate shock-absorbing hydrogels that harness talin's mechanical properties [12]). Second, it serves as a string of mechanical switches for binding partners, thereby facilitating mechanotransduction. Finally, the switch-like behavior of helical bundles dramatically alters the dimensions of the protein, a typical helical bundle is ~5 nm in length, but unfolding of a domain unpacks ~40–50 nm in contour length that is extended in the direction of stretching [10,13]. These unfolding events lead to the spatial organization of proteins via changes in the extension of proteins when they are stretched (Figure 1c).

Force-dependent switching in binding partners

Nine of the talin rod domains contain vinculin-binding sites which require the domain to unfold to bind to vinculin [10,14–16]. The discovery of signaling molecules, such as RIAM, that bind on the outside of the folded rod domain [17,18] further expanded the notion of these domains being switches. For example, the R3 domain serves as a force-dependent binary switch, binding to RIAM when in the folded "0" state, and unfolding to bind vinculin in the "1" state (Figure. 1b). As the switch patterns are reversibly changing between folded and unfolded states as a function of force, this switches on and off different cellular processes.

The rod helical bundles are rich in binding sites and can accommodate more than a dozen talin-binding proteins [19]. While most of these proteins bind to the folded helical bundles, some, like vinculin [10,11,20] and PKA [21], bind only to unfolded talin rod helical bundles when cryptic binding sites become exposed. The mechanical stability of these rod domains determines the threshold for their mechanical activation. Supporting this notion, a cassette exon in the *Tln1* gene was recently identified that introduces 17 amino acids after Gln665 which results in a reduced force requirement for unfolding its R1 and R2 domains and enhanced vinculin binding [22].

Many studies have now been conducted on the talin R3 domain, on the wildtype, the 'IVVI' mutant that stabilizes the hydrophobic core of R3 and makes the domain more mechanically stable [10,17,23] and mutations that destabilize R3 [24]. Multiple other studies on R3 provide insight into how mechanochemical switch domains behave and how they can be studied experimentally [25–28].

Collectively, these studies on the mechanical responses of talin-1 domains offer invaluable insights into the mechanisms underlying the mechanical activation of talin and its role in ECM rigidity sensing.

Many other proteins contain binary switches

Like talin rod domains, both vinculin [29] and the α-catenin [30] family of proteins that regulate cell-cell adhesions, also contain a linear array of force-bearing structural domains that bear binding sites. Notably, α-catenins contain multiple switch domains, and one also contains a cryptic vinculin-binding site in the folded structure [31,32], which can be exposed by a few pN forces [30,33].

The mechanical stability of vinculin

When bound to a mechanically activated vinculin-binding site on talin or α-catenin, the liberated vinculin tail domain interacts with actin filaments. This interaction induces several α-helical bundles between the D1A subdomain and Vt, as well as the intrinsically disordered linker between the D4 and Vt domains, to undergo mechanical stretching. A recent study has shown that at physiological loading rates, vinculin's structural domains unfold under forces ranging from 5 to 15 pN and refold when these forces drop below 5 pN [29]. Consequently, vinculin serves as an additional force-buffering element, and its domains have the capacity to act as force-dependent molecular switches, like those in talin and α-catenin. Vinculin contains a flexible unstructured linker region which is a hotspot for vinculin's binding partners. Due to the different force-extension curves of the vinculin linker between its unbound and bound states, the force is predicted to have a significant impact on the affinity between vinculin linker and its binding partners.

The mechanical stability of dystrophin

Dystrophin is another large rod-like protein that serves as a mechanical link between the intracellular cytoskeleton of a myofiber and the surrounding ECM via the cell membrane, or sarcolemma. Dystrophin plays a pivotal role in stabilizing the sarcolemma during muscle contractions. Its central rod domain contains 24 spectrin repeats (SRs), three-helix bundles [34], which constitute the primary force-bearing region of the molecule. Recently, force-dependent unfolding and refolding dynamics of all 24 SR domains were explored at physiologically relevant low pN/s loading rates [35], showing similarities in unfolding and refolding behaviors to those observed in talin and vinculin α-helical bundles. Measurement of the force-dependent unfolding and refolding rates of these 24 SRs, indicate that the central domain can maintain forces below 25 pN, even while undergoing significant length changes of up to ~800 nm at physiological loading rates. In addition to its role as a mechanical buffer, dystrophin's SRs also facilitate

cellular signaling and various signaling proteins are linked to dystrophin through syntrophins, which were initially shown to bind to the C-terminal region [36] but have also been more recently discovered to bind to SR16 and SR17 [37]. Consequently, the SRs in dystrophin's central region likely serve as a signaling scaffold that is modulated by mechanical stretching.

Force-dependent regulation of autoinhibition

Full-length vinculin behaves very different to truncated forms. This has been known from cell [38] and animal work, truncated constitutively active vinculin is harmful and induces lethality in flies [39]. However, the ability of full-length vinculin to autoinhibit itself, adds dynamic regulation to these linkages. Autoinhibition represents another type of regulatory axis that is controlled by protein stretching [40], as stretching a protein so that its autoinhibitory domains are held apart locks the protein in an open conformation. Reciprocally autoinhibition is a force-dependent process for proteins in mechanical linkages, as tension stabilizes the open conformation. Evidence that autoinhibition is mechanosensitive has been shown recently, where relief of autoinhibition of talin or vinculin results in force-independent complex assembly [41,42] and formation of complexes and phenotypes that require tension in the wildtype proteins. The interactions between talin and vinculin are allosteric as mechanical exposure of VBS can activate vinculin [20] and conformational changes in vinculin enhance the interaction [43]. Many other factors contribute to the activation states of these proteins, including phosphorylation as evidenced by a recent study on vinculin phosphorylation coordinating vinculin activity by stabilizing the closed conformation [44]. Amazing nuance and complexity can be encoded in the interactions between these two proteins.

Binary switches in proteins can also be mediated by interdomain interactions. Detailed investigations into filamin revealed a new method of binary switching and another type of autoinhibition. Filamins serve as large, elongated, homodimeric proteins capable of cross-linking actin filaments into orthogonal networks. They also mediate physical connections between actin networks and membrane receptors, including integrins and GPCRs. Every filamin monomer features an N-terminal actin-binding domain, succeeded by 24 immunoglobulin (Ig) repeats in its elongated, force-bearing rod region. Specifically, two of the rod domains display increased compactness, attributed to interactions between domains. This compact architecture masks binding sites for several key proteins including integrins [45,46], making them accessible only when mechanical forces on the order of a few piconewtons are applied [47]. This binary switching mechanism was also found in filamin C, a heart-specific isoform, which also demonstrated a high degree of mechanical stability in its rod domains, akin to that observed in filamin A [48]. Consequently, the binary switching mechanism in filamin A represents a conserved feature across all filamin isoforms. Occlusion of binding sites via adjacent folded domains is likely a common mechanism in myriad other systems as well, another example of this mechanism is seen in talin where the R1 and R2 domains interact and separation of them reveals a cryptic binding site for ARPC5L [49].

There are numerous force-regulated proteins involved in adhesion signaling that do not contain binary switch domains. Much of this article has focused on binary switches, but it is important to mention that many proteins respond to force in focal adhesions. For instance, paxillin serves a central role as a hub for mechanotransduction [50–52] due to its multiple binding sites being connected via large unstructured regions that can get repositioned via force.

The mechanical stability of force-bearing protein-protein interfaces

It is not just proteins that get stretched, there are many protein-protein interactions that are maintained under tension, such as the examples covered in the earlier catch bond section, where each connection is maintained under tension. However, there are many additional interactions that tether to the linkages that will also experience tension. Measuring protein-protein interactions under force is challenging as when the bond breaks the tether is lost; however, approaches have been developed to measure this. For example, the interaction between talin and KANK1 which mediates the link between the focal adhesion and the cortical microtubule stabilizing complex, this linkage is under tension from the movement of two large complexes relative to each other. Using a sophisticated design of incorporating a large loop between the talin R7 and the KANK1 KN-domain ligand, a single molecule study of this interaction could be performed [53] which showed the complex force-dependence of this interaction. Another notable example is the force-bearing interfaces between vinculin and exposed vinculin-binding sites in talin and alpha-catenin, which are surprisingly strong, resisting forces over physiologically relevant pN range over a long duration in the order of 10^3 seconds [54].

Mechanically operated signaling scaffolds

It is becoming appreciated that for each example of proteins in mechanical linkages above, that large signaling machineries assemble on top of them, serving as solid-state signaling centers. As these proteins are stretched they undergo changes in switch patterns leading to altered signaling, serving as mechanosensitive signaling hubs [55], with the changes in recruited signals and the spatial organization of signals dictated by the localization of the binding sites along force transmission pathways [13]. Furthermore, the enzymatic coupling whereby enzymes are recruited to certain

switch patterns at adhesion sites indicates a way for changing signaling outputs as a function of switch state.

For example, cyclin-dependent kinase 1 (CDK1) binds to R8 only when in the folded "0" state [56]. Similarly, talin was recently discovered to be an A-Kinase anchoring protein (AKAP) for recruitment of protein kinase A (PKA). PKA binds to R9 only when the domain unfolds to the "1" state as it binds a single helix (helix 41) which is an AKAP helix [21]. This coupling of mechanical and chemical signaling goes one step further, as there are now well-characterized examples of enzymatic alterations in the switch patterns of talin. For instance, CDK1 phosphorylates talin R7 destabilizing the R7R8 domains and leading to them unfolding at lower forces. PKA also phosphorylates the talin scaffold, although the consequences of these chemical modifications remain to be determined.

Finally, the mechanical signaling of these scaffolds can be altered via TGF-Beta signaling which controls alternate splicing of talin1. Alternative splicing of talin introduces an extra exon into the R1-R2 switches that alter their mechanosensitivity, globally altering mechanotransduction pathways in cells [22].

Force-dependent binding constants

Part of the exquisite force sensitivity of integrin adhesion complexes is the dramatic changes in affinity, from essentially no interaction to sub-nanomolar-binding constants as a function of force. For instance, the affinity of vinculin binding to talin changes by 1000s of folds as a function of force [20]. This changing affinity as a function of force highlights how many interactions in cells have force-dependent binding constants, this concept is described more elsewhere [57].

Measuring protein stretching in cells

There are now excellent tools for visualizing where proteins are being stretched in cells and the link between protein stretching and mechanotransduction is actively studied in cells and *in vivo*. The first genetically encoded, FRET-based tension sensor was introduced into vinculin and this provided a powerful tool for studying vinculin tension in cells [58]. More recently these tools have been applied to talin [59–61] and many other proteins. More recently, the vinculin sensor has been coupled with fluorescent localization of other proteins to correlate which proteins are recruited to FAs in response to tension [62]. Novel tension sensor design strategies are being made, including the recent STReTCh (sensing tension by reactive tag characterization) probe by the Dunn Lab which can report on forces transmitted by the cytoskeleton by introducing cryptic SpyTag into proteins that are revealed by tension [63]. Furthermore, chemogenetic and optogenetic approaches allow two halves of talin or desmoplakin to be engineered that can be connected, using either chemical [64] or optogenetic [65,66] means to investigate mechanical linkages.

Protein stretching in mechanical memory

The work of Boris Hinz and others has clearly demonstrated how mechanosensing by cells can lead to epigenetic and persistent gene expression changes that can persist for weeks, providing cells with mechanical memory [67–69]. Protein stretching provides another layer of mechanical memory. Force-dependent mechanical switches in proteins exhibit mechanical hysteresis, meaning they unfold at a higher force than what they refold at [11], this imparts the molecules with mechanical memory, where when maintained in a mechanical linkage the switch patterns have persistence. This mechanical memory of these essential scaffolds indicates a way for information to be written in a binary format into the shape of the molecules [19,70] and future work looking into the concept of mechanomemory in coordinating cellular processes should focus on these mechanical linkages. Recent studies provide strong evidence of talin's role in mechanical memory, in the heart [71], and in the talin-DLC1 interaction [72]. The changes in architecture that result from the altered switch patterns [13] can be measured in cells [73] indicating how cellular signaling can be dynamically regulated by protein stretching as a function of prior events.

Future directions

All the studies given in this short review have been focused on the adhesion proteins as major sites of protein stretching. However, there is a similar set of mechanosensitive linkages at the nucleus, and a detailed description of protein stretching at, and in, the nucleus should be the focus of another review. However, Nesprin [74], Sun proteins, and YAP/TAZ nucleus localization [75] all have been reported to respond to protein stretching, and Nesprin switches have been reported [74]. Having switches at both the adhesion and the nuclear ends of these mechanical linkages indicates a way to synchronize both compartments [76].

Many new techniques are being developed including microfluidic-based approaches for visualizing many molecules under force [26] and novel designs of temperature-regulated magnetic tweezers [77] (see Box 1). Major goals for future work will be measuring the lifetimes of these linkages in cells; since they are formed by non-covalently bound proteins and so have a finite lifespan that is sensitive to both tension and the assemblies that form on the linkages. Other immediate goals should be developing methods for modifying mechanotransduction by altering the stability of protein switches, and visualizing localized enzymatic activity as a function

Box 1. Different approaches to measure protein stretching.

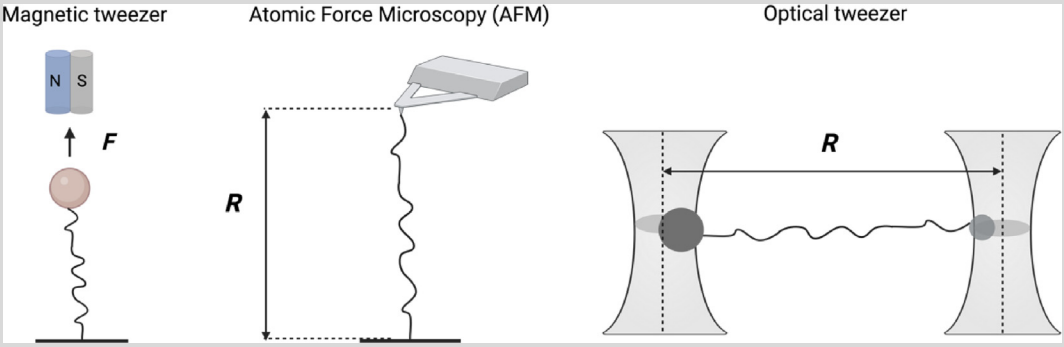
Advanced manipulation techniques precisely quantify force-dependent processes like protein domain unfolding/refolding and protein-protein dissociation at the single-molecule level. These methods measure biomolecular extension changes with nanometer resolution, and the kinetics of the force-dependent structural changes of domains and dissociation of protein-protein complexes. The basic principles of these techniques are outlined here.

Magnetic Tweezers exert force on a biomolecule connected between a superparamagnetic bead and a surface via an external magnetic field. Typically, a pair of magnets generate this force, which is modulated by adjusting the magnet-microbead distance. Biomolecular extension changes are indicated by the change of the bead height from the tethered surface along the force direction.

Atomic Force Microscopy *(AFM)* stretches a biomolecule tethered to a surface using a cantilever's sharp tip. The resulting molecular tension bends the cantilever, which quantifies the tension. Typically, rigid cantilevers are used in AFM, hence changes in biomolecular extension are indicated by the controlled changes in the tip-surface distance.

Optical Tweezers use focused laser beams to stretch biomolecules, tethered to polystyrene microbeads. The laser creates an energy minimum "trap" for the bead. Biomolecular tension displaces the bead from its equilibrium, allowing tension quantification.

These methods enable force measurements from sub-piconewtons to hundreds of piconewtons. They can also record data for a wide range of durations, from seconds to hours or even days.



of switch pattern. Reading the shapes of molecules within cells and visualizing multiple proteins simultaneously interacting with a protein as it is stretched will lead to a deeper understanding of the information content of these proteins. Lastly, cataloging the different chemical modifications (post-translational modifications) that imprint on the scaffolds and stabilize/alter the switch patterns will begin to integrate the chemical and mechanical pathways. This is an exciting time in the field of protein stretching and as the effects on mechanotransduction begin to be realized, and how altered mechanosensing links to disease (discussed in this editorial [78]), our understanding of how cells use physical forces to instruct them will expand rapidly.

Author statement

YG, JY and BTG all were involved in the Writing – original draft; Writing – review & editing.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

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