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REVIEW

Principles and regulation of mechanosensing

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ABSTRACT

Research over the past two decades has highlighted that mechanical signaling is a crucial component in regulating biological processes. Although many processes and proteins are termed 'mechanosensitive', the underlying mechanisms involved in mechanosensing can vary greatly. Recent studies have also identified mechanosensing behaviors that can be regulated independently of applied force. This important finding has major implications for our understanding of downstream mechanotransduction, the process by which mechanical signals are converted into biochemical signals, as it offers another layer of biochemical regulatory control for these crucial signaling pathways. In this Review, we discuss the different molecular and cellular mechanisms of mechanosensing, how these processes are regulated and their effects on downstream mechanotransduction. Together, these discussions provide an important perspective on how cells and tissues control the ways in which they sense and interpret mechanical signals.

KEY WORDS: Mechanosensing, Mechanotransduction, Cytoskeleton, Force, Adhesion

Introduction

Cell biology rarely sits still. This is true whether describing our ever-increasing knowledge in the field or describing the components inside cells and organisms. In some sense, cell biology is a study of motion, as it is the spatial and temporal coordination of interactions that allows for complex cellular behaviors to emerge from what would otherwise be chaos. Although this coordination is most often considered in the context of biochemical regulation, it is becoming increasingly clear that it has a comparable role in the context of mechanics. Newton's first law tells us that changes in motion arise from forces. In cells, these forces can be generated internally, such as by myosin motors pulling on actin filaments (Quintanilla et al., 2023), or generated in the extracellular environment by neighboring cells and tissues (Saraswathibhatla et al., 2023). In each case, these forces directly and indirectly influence cell and tissue behavior, beginning at the molecular level.

Since the pioneering work of Alfred Harris, who visualized the forces produced by fibroblasts as wrinkles on an elastic silicone substrate (Harris et al., 1980), there has been impressive development of new tools to visualize and measure forces and mechanical interactions in and around cells (Iskratsch et al., 2014; Lavrenyuk et al., 2021; Liu et al., 2017; Roca-Cusachs et al., 2017). This has allowed an explosion of progress in the field of mechanobiology, and detailed reviews covering such advancements can be found in the following areas: cell–matrix adhesion (Humphrey et al., 2014), cell–cell adhesion (Campàs et al., 2024), development (Petridou et al., 2017), metabolism (Zanotelli et al., 2021), transcription (Dupont and Wickström, 2022), physiology and health (Janmey et al., 2020), disease (Sheetz, 2019), and aging (Phillip et al., 2015). This non-exhaustive list demonstrates the pervasive and important roles of mechanical signaling, and yet we are likely still only scratching the surface. Understanding how mechanical signals are interpreted and how their downstream effects are controlled is therefore of paramount importance. In traditional biochemical signaling, many pathways are regulated via processes like post-translational modification to add additional layers of control. Recent evidence has demonstrated that additional layers of mechanisms to regulate mechanical signaling and mechanosensitivity also exist. In this Review, we present concise and clear definitions for mechanosensing and mechanotransduction, cover examples of the different types of molecular mechanosensing mechanisms and discuss the mechanical and biochemical methods that cells use to regulate these processes.

Mechanosensing versus mechanotransduction

The words 'mechanosensing', 'mechanosensitive' and 'mechanotransduction' are often used interchangeably, which can create confusion when discussing the subtle differences between underlying mechanisms. We therefore find it useful to first suggest definitions for these terms to assist in differentiating between specific phenomena. 'Mechanosensitive' and 'mechanosensitivity' are the broadest, most general terms and describe a process that changes either directly or indirectly in response to a change in applied force (Fig. 1). In contrast, we define mechanosensing as the direct action of changing behavior (i.e. responding) as the result of a force. We further define indirect mechanosensing (i.e. mechanoresponsive) as the action of responding to a change in force that is being applied to another structure – that is, mechanosensing is the first component of mechanotransduction. Using this scheme, a process labeled as mechanosensitive might therefore be a downstream product of the application of force and not actually be involved in direct mechanosensing. As an example, consider the process of cell spreading, which is an intuitive and easy-to-visualize example of how mechanical changes in the environment encountered during development or disease can dramatically impact cellular behavior (Janmey et al., 2020). Under otherwise equal conditions, cells plated on soft substrates tend to be round and spread significantly less than cells on stiff substrates, where they flatten out and typically polarize (Yeung et al., 2005). By our definition, the process of cell spreading is mechanosensitive in that it depends on the mechanical properties of the substrate. The actual mechanosensing that occurs during spreading is achieved by a combination of integrins, which are a diverse family of heterodimers that span the plasma membrane and connect the internal cytoskeleton to the extracellular matrix (ECM) (Hynes, 2002; Luo et al., 2007), and various proteins in focal adhesions (Oakes et al., 2018; Choi et al., 2008; Kanchanawong et al., 2010), both of which

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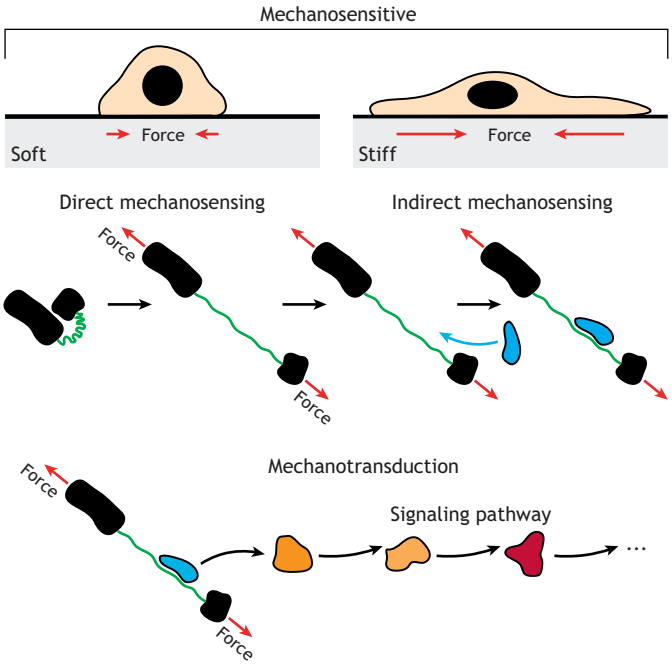

Fig. 1. Defining mechanosensing versus mechanotransduction. Although these terms referring to mechanical interactions in cells are often used interchangeably, they have distinct meanings. 'Mechanosensitive' (top), the broadest term, is a general descriptor for all direct and indirect mechanosensing and mechanotransduction processes. For example, cell spreading, which is dependent on substrate stiffness, is a mechanosensitive behavior that involves both mechanosensing events and downstream mechanotransduction. 'Mechanosensing' (middle) is specifically the action of responding to an applied force, which can be either direct (for example, a protein exhibiting a conformational change in response to applied force; green) or indirect (for example, a protein recognizing a binding site on a different protein uncovered as a result of applied force; blue). Finally, 'mechanotransduction' (bottom) describes the process of converting a mechanical signal into a biochemical signal.

respond to forces generated in the actomyosin cytoskeleton. The functional act of spreading, although clearly mechanosensitive, is thus a downstream product of these mechanosensing actions.

Finally, mechanotransduction is the process of converting mechanical signals into biochemical signals, and thus by definition occurs downstream of a mechanosensing process. Some proteins, such as the focal adhesion proteins talin1 (herein referred to as talin) and vinculin, which connect the actin cytoskeleton to the ECM via integrins, occupy multiple roles and can participate both directly in mechanosensing and indirectly in downstream mechanotransduction. Mechanotransduction itself can involve multiple proteins and signaling events or can be initiated within a single protein. For instance, the LIM domain family protein zyxin uses its C-terminal LIM domains to recognize strained actin filaments in stress fiber tears and initiates their repair through its N-terminal VASP- and α-actinin-binding domains (Smith et al., 2010), making stress fiber maintenance a tightly localized mechanotransduction process. In contrast, the stretching of talin, which occurs at cell adhesions, involves multiple signaling events including changes to interactions between talin, vinculin, Rap1-GTP-interacting adaptor molecule (RIAM, also known as APBB1IP) and their associated downstream partners (Rio et al., 2009; Goult et al., 2013). In each case, however, there is a distinct point at which a force is converted into a change in biochemical signals.

Direct mechanisms of mechanosensing

There are multiple mechanisms of mechanosensing in which proteins respond directly to applied forces. Some proteins undergo changes in conformation, whereas others experience a change in activity. Many proteins combine multiple mechanosensing behaviors and features, as these categories are not mutually exclusive. Before we can understand how cells regulate these processes, it is therefore useful to discuss the underlying molecular mechanisms.

Structural changes

The simplest mechanosensing mechanism occurs when an applied force changes the conformation of a protein. However, not all conformational changes are equal, as changes can vary in magnitude or simply alter activity rather than overall protein structure. Here, we break down these changes into three categories: conformational changes, domain unfolding and signaling changes.

Conformational changes

Most proteins contain unstructured linker regions between domains that give the protein some element of flexibility to adopt different conformations. Mechanosensitive ion channels, which become activated in response to changes in membrane tension, are perhaps the clearest example of force causing a conformational change that leads to a change in protein behavior (Árnadóttir and Chalfie, 2010) (Fig. 2A). The bacterial channel MscL, which helps bacteria regulate turgor pressure, was the first ion channel to be shown to be structurally mechanosensitive, as applying tension was found to result in the displacement of bundles of α-helices within the channel, thereby creating an opening for ions to pass through (Sukharev et al., 2001). Subsequently, the Piezo family of channels were the first mechanosensitive ion channels to be identified in mammals (Coste et al., 2010, 2012), and this family has since been implicated in a wide range of physiological processes (Syeda, 2021). Interestingly, the channel opening mechanism in the Piezo family channels differs from that of MscL and other mechanosensitive channels, such as two-pore domain potassium (K2P)-type channels (Ridone et al., 2019), suggesting that multiple mechanisms of ion channel mechanosensing are likely to have evolved independently (Kefauver et al., 2020). This type of mechanosensitive behavior is not limited to ion channels; for example, aquaporins respond to changes in membrane tension and act as valves to help mediate differences in osmotic pressure (Ozu et al., 2023), and pores in the nuclear envelope respond to membrane deformation, allowing cytosolic phospholipase A2 (cPLA2) to bind to the nuclear envelope, which leads to increased actin and myosin activity in the cytoskeleton (Lomakin et al., 2020; Venturini et al., 2020). Pores in the nucleus can also be deformed in response to cytoskeletal tension allowing proteins like the mechanosensitive transcriptional regulator Yes-associated protein (YAP, also known as YAP1) to translocate to the nucleus and affect transcription in response to mechanical stimuli (Elosegui-Artola et al., 2017).

A number of adhesion proteins also experience structural changes that remove autoinhibition and alter downstream signaling, such as focal adhesion kinase (FAK, also known as PTK2) (Lietha et al., 2007) and vinculin (Cohen et al., 2005), which both possess flexible linker regions that straighten under applied loads. In the case of FAK, unfolding of the interaction between the protein 4.1, ezrin, radixin, moesin (FERM) domain and the kinase domain exposes a centrally located phosphorylation site, which ultimately allows binding of the kinase Src and facilitates additional downstream

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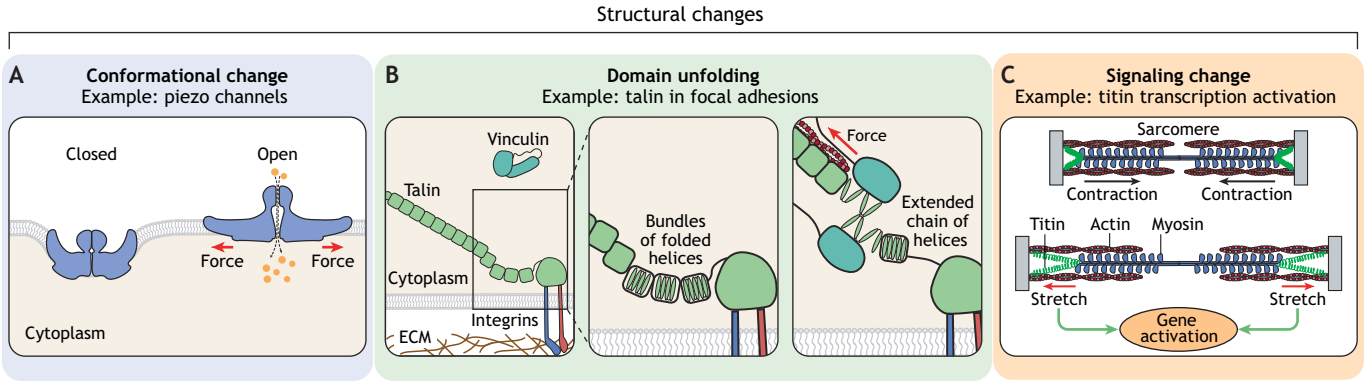

Fig. 2. Examples of direct mechanosensing via structural changes in proteins. (A) Mechanosensitive channels, such as members of the Piezo family, undergo small conformational changes in response to force applied on the plasma membrane, allowing ions to pass through the channel. (B) The focal adhesion protein talin contains multiple domains composed of folded helical rods that undergo unfolding when under tension to reveal hidden vinculin-binding sites. Binding of vinculin to talin leads to additional downstream signaling events that are critical for adhesion formation. (C) Titin, a giant protein found in sarcomeres, contains a kinase domain that is activated as titin is stretched, inducing signaling pathways that lead to downstream transcriptional activation.

signaling (Torsoni et al., 2003; Zhou et al., 2015). Crucially, this conformational change occurs below the threshold of force required to unfold the more structured domains of FAK (Bauer et al., 2019). Similarly, vinculin, in addition to binding to stretched talin, undergoes a mechanically driven conformational change that separates its own head and tail domains, allowing it to bind F-actin more efficiently (Golji and Mofrad, 2013). The use of tension-calibrated fluorescence resonance energy transfer (FRET) modules inserted between the head and the tail domains of vinculin has revealed that individual vinculin molecules experience loads on the order of 1–10 pN within focal adhesions (Grashoff et al., 2010; LaCroix et al., 2018). Interestingly, vinculin appears to be under greater load at the distal tip of adhesions (LaCroix et al., 2018). This is consistent with other tension sensor measurements that suggest that tension is heterogenous within the adhesion, potentially enabling highly localized differences in mechanical responses within a single adhesion (Morimatsu et al., 2013; Chang et al., 2016). For both FAK and vinculin, these conformational changes lead to a number of downstream interactions that demonstrate how mechanotransduction can propagate after the initial mechanosensing event. For instance, recent work has revealed many additional proteins present at adhesions that depend on tensed vinculin for their own recruitment (Tao et al., 2023).

Domain unfolding

Beyond these relatively modest conformational changes that straighten flexible linkers between domains, application of larger magnitudes of force can lead to further unfolding of helical structures that reveal hidden binding sites for other proteins (Beedle and Garcia-Manyes, 2023). A canonical example of this is talin, which consists of a globular head domain followed by 13 α-helical bundles (Goult et al., 2021). Buried within multiple helical bundles are obscured vinculin-binding sites (Ziegler et al., 2008), which become exposed as talin is put under mechanical load (Rio et al., 2009) (Fig. 2B). Stretching of talin is primarily a mechanically driven process, as it does not alter the phosphorylation state of talin (Giannone et al., 2003), and only requires physiological loads (~5–10 pN) to begin unfolding (Yao et al., 2016). Subsequent work has shown that vinculin indeed binds to talin in this stretched state (Margadant et al., 2011; Yao et al., 2014a); however, only 40–70% of talin molecules in an adhesion are loaded at any given time (Ringer et al., 2017). These findings highlight that vinculin can act as a part of a mechanotransduction signaling response in addition to its own direct mechanosensing mechanism. Although vinculin is considered one of the primary mechanosensitive proteins involved in talin-mediated signaling at adhesions, recent work has shown that talin also contains cryptic binding sites for protein kinase A (PKA) that are exposed under tension (Kang et al., 2024).

The unfolding of subdomains in response to force is also a feature of many ECM proteins (Vogel, 2006). For example, fibronectin contains a series of globular domains that can unfold when loaded at physiological force levels (Krammer et al., 1999; Baneyx et al., 2002). In particular, unfolding of the type III domains can reveal cryptic binding sites (Klotzsch et al., 2009) that play important roles in the self-assembly of fibronectin into fibrils (Gao et al., 2003) and interactions with additional ECM proteins like collagen I (Kubow et al., 2015). Similarly, the ECM protein von Willebrand factor (VWF) undergoes a conformational change when under tension that leads to a dramatic increase in protein length (Schneider et al., 2007; Bergal et al., 2022). This increase in length helps to build fibrillar networks required for blood clotting, not only by allowing VWF to interact with other VWF fibrils, but also by revealing cryptic binding sites for the glycoprotein Ib alpha chain (GPIbα, also known as GP1BA) adhesion receptor expressed on platelets (Fu et al., 2017).

It is perhaps unsurprising that many of these proteins that contain cryptic binding sites are found at adhesions or in the ECM, as both these structures are coupled to the actomyosin cytoskeletal network, which is responsible for the majority of contractile forces produced by the cell (Cai et al., 2010). Other examples of mechanosensitive proteins associated with the actin cytoskeleton, junctions and/or the ECM include α-catenin (herein referring to αE-catenin, encoded by *CTNNA1*), a key component in cell–cell junctions that contains a cryptic binding site for vinculin (Yao et al., 2014b), and filamin A, an actin-crosslinking protein that contains a cryptic integrin-binding site (Pentikäinen and Ylänne, 2009). There also exist many additional proteins that are proposed to sense forces via domain unfolding. For example, dystrophin, which links the actin cytoskeleton and membrane proteins in muscle cells, contains multiple spectrin repeat domains that undergo physiological stretching and are thought to act as scaffolds for protein–protein interactions (Le et al., 2018).

Journal of Cell Science (2024) 137, jcs261338. doi:10.1242/jcs.261338

Signaling changes

In addition to causing conformational changes and domain unfolding, tension applied to proteins can also impact their signaling activity. For instance, phosphorylation of the adhesion protein p130Cas (also known as BCAR1) is increased under stretch (Sawada et al., 2006), leading to downstream activation of Rap1 signaling (Tamada et al., 2004). A non-phosphorylatable mutant of p130Cas has minimal Rap1 activation in response to stretch, indicating that the change in phosphorylation state in response to stretch is a key element in this process that induces downstream signaling (i.e. mechanotransduction) (Sawada et al., 2006).

Another example of a change in signaling in response to force is the giant protein titin, which plays a crucial mechanical buffering role during sarcomere contraction and was one of the earliest proteins to be identified to undergo reversible unfolding (Rief et al., 1997). Titin includes multiple signaling domains that are thought to be activated by force (Voelkel and Linke, 2011), including a C-terminal kinase domain that becomes activated through mechanical stretch in the presence of ATP (Puchner et al., 2008). This kinase domain plays a crucial regulatory role in protein turnover and gene expression (Lange et al., 2005) (Fig. 2C), and changes to the amount of force applied to titin can thus impact overall heart health (Krüger and Linke, 2009).

Kinetic changes

Forces applied to proteins can also impact their binding kinetics (Evans and Calderwood, 2007). Here, we group these effects into two categories: catch bonds and tunable interactions.

Catch bonds

The lifetime of protein–protein bonds can significantly impact downstream signaling either by allowing signals to persist longer or by turning them off more quickly. The vast majority of these bonds are slip bonds, for which the lifetime of the bond decreases as more force is applied to it – similar to how a piece of adhesive tape might behave. Catch bonds, however, increase in lifetime as more force is applied, up to a certain threshold at which they ultimately fail – similar to the finger trap toys we may have encountered as children (Thomas et al., 2008) (Fig. 3A). The physical mechanisms underlying these behaviors have been reviewed previously (Prezhdo and Pereverzev, 2009; Guo et al., 2018). In addition to the force magnitude, a key component in catch bond behavior is the loading rate of the applied force, as the timescale over which the force is applied can impact the ability of the protein to respond and maintain the bond (Jo et al., 2024). Integrins were among the first proteins shown to be affected by the rigidity of the cell substrate (Choquet et al., 1997). It has subsequently been shown that many members of the integrin family form catch bonds with their ECM ligands (Kong et al., 2009; Chen et al., 2019; Elosegui-Artola et al., 2016). Increasing the lifetime of integrin–ECM bonds plays a key role in stabilizing adhesions, thereby influencing how cells spread on substrates of different stiffness (Elosegui-Artola et al., 2016). On soft substrates, the deformation of the substrate minimizes the force on the integrins, resulting in a short lifetime of integrin–ECM bonds and poor adhesion formation. On stiff substrates, the force on the integrin–ECM bonds is larger, increasing the lifetime of the bond and allowing adhesion formation to continue to develop. For integrins, this catch bond behavior can be further modulated through the addition of Mn^{2+} (Gailit and Ruoslahti, 1988), which stimulates cells to spread on soft substrates (Oakes et al., 2018).

Multiple other proteins form catch bonds, including the cell adhesion molecules P-selectin (Marshall et al., 2003) and cadherins (Manibog et al., 2014), kinetochore components, which link chromosomes to mitotic spindle microtubules (Akiyoshi et al., 2010), and the bacterial surface receptors FimH (Thomas et al., 2002) and SdrE (Paiva et al., 2023). Interestingly, a subset of such proteins show directional catch bond behavior, including vinculin (Huang et al., 2017), talin (Owen et al., 2022) and α-catenin (Bax et al., 2023). In these proteins, the direction of the applied force can affect the bond lifetimes by up to two orders of magnitude (Owen et al., 2022). A similar directional dependence has also been inferred for integrins (Nordenfelt et al., 2017), suggesting that this might be a prevalent feature of many catch bonds. Intriguingly, all of these directional catch bonds show a preference for forces pointing towards the pointed (−) end of actin filaments (Huang et al., 2017; Owen et al., 2022; Bax et al., 2023; Nordenfelt et al., 2017), which would maximize their effect in response to the forces generated by retrograde flow of the actin cytoskeleton and possibly contribute to clustering of adhesion proteins.

Tunable interactions

Mechanical load can also ‘tune’ the activity of some proteins. The clearest example of this behavior is found in the force dependence of cytoskeletal motors, which convert the energy released by ATP hydrolysis into mechanical movement. This mechanochemical cycle has been observed to exhibit load dependence for myosin (Greenberg et al., 2016; Cheng et al., 2020), kinesin (Visscher et al., 1999) and dynein (Rao et al., 2019), demonstrating that this is a

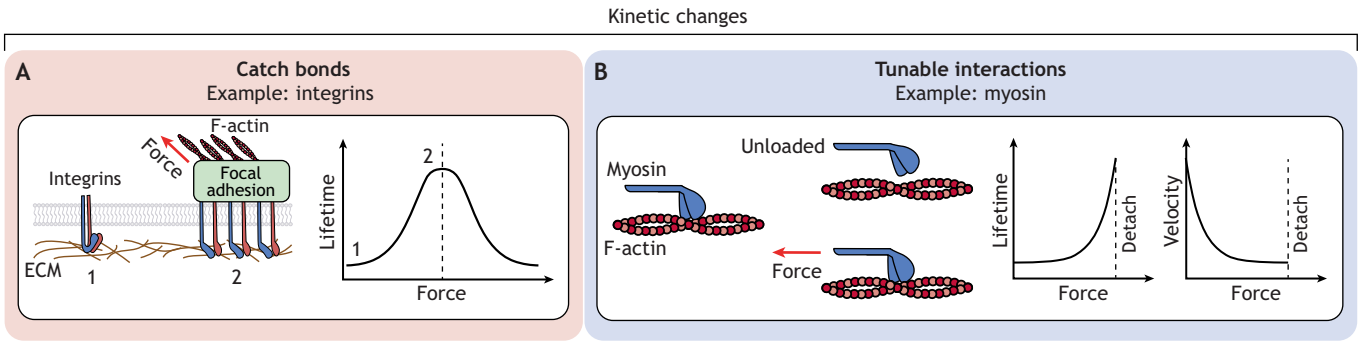

Fig. 3. Examples of direct mechanosensing via kinetic changes in protein binding and activity. (A) Integrins couple the cytoskeleton to the ECM. Interactions between integrins and the ECM behave as catch bonds, a type of protein–protein interaction in which the bond lifetime increases with applied force, up to a point, as illustrated for unloaded integrins (1) and integrins under load (2). (B) Myosin motor proteins use a mechanochemical cycle that converts hydrolysis of ATP into motion. The rate of this motion is load dependent. When a force is applied that resists this motion, the bond lifetime of actomyosin interaction increases and the velocity of the motor decreases, an example of protein–protein interactions that can be tuned by applied force.

broadly conserved property of motor proteins. Generally, increased load on a motor results in an increased duty ratio (i.e. time bound to the substrate) and a longer mechanochemical cycle. For processive motors, which can take multiple 'steps' without dissociating from their 'track', this results in longer binding lifetimes to the 'track' for motors with cargo compared to unloaded motors (Fig. 3B). As an example, myosin V is a processive motor that walks along actin towards its barbed (+) end. When tension is applied on the myosin V molecule towards the pointed (−) end of the actin filament, it significantly slows its catalytic cycle, leading to longer dwell times (Purcell et al., 2005). Similarly, the power stroke of myosin II is smaller and slower under load (Reconditi et al., 2004), and the detachment rate of β-cardiac myosin (also known as MYH7) is reduced with increasing force (Liu et al., 2018). In the case of myosin II, the increased resistive load affects ADP release but not binding of the nucleotide itself (Kovács et al., 2007), though the responses can vary between different isoforms (Greenberg et al., 2016). These tunable changes to the mechanochemical activity of myosin can thus affect the functional roles of myosin in regulating cellular contractility and mediating cargo transport (Hartman and Spudich, 2012).

This behavior is not limited to just cytoskeletal motors, as other molecular motors such as RNA polymerases have also been shown to be load dependent (Abbondanzieri et al., 2005). A number of non-motor proteins also share this behavior. Filamin A, for instance, shows an increased affinity for β7 integrins (encoded by *ITGB7*) when under tension (Ehrlicher et al., 2011), and tension can activate PKA in a manner comparable to its typical allosteric activation by binding of cAMP (Choi and Zocchi, 2006).

Indirect mechanosensing

In the cases discussed above, a force directly acts on the proteins, causing changes to their conformation or biochemical activity. A separate category of mechanosensitive proteins have their activity altered by force that is applied to their binding partner. They thus do not fit the definition of mechanosensing explained above, as they are only indirectly impacted by the change in force. Although these mechanoresponsive events could technically be qualified as mechanotransduction, we believe that these indirect mechanosensing mechanisms warrant their own category of discussion.

Strain sensing

Strain sensing has been most often associated with members of the LIM domain family of proteins, which contains a number of mechanosensors (Smith et al., 2014). LIM domains are composed of two zinc finger motifs that facilitate diverse protein–protein interactions, and many members of this family of proteins contain multiple LIM domains (Sala and Oakes, 2023). The LIM domain family protein zyxin was first identified as a focal adhesion protein that relocates from adhesions to actin stress fibers in response to cyclical stretch (Yoshigi et al., 2005). Subsequent work has revealed that zyxin also transiently relocates to spontaneous stress fiber tears to facilitate their repair (Smith et al., 2010) (Fig. 4A). Specifically, the three LIM domains in the C-terminal half of zyxin recognize strained (i.e. stretched) actin filaments in the stress fiber, and repair is mediated by recruitment of α-actinins and the actin regulatory proteins Mena (also known as ENAH) and VASP, which bind to the N-terminal half of zyxin. Subsequent work has shown that other LIM domain proteins behave similarly, including hydrogen peroxide–inducible clone 5 (Hic-5, also known as TGFB1I1) and cysteine and glycine-rich protein 2 (CRP2, also known as CSRP2) (Kim-Kaneyama et al., 2005), paxillin (Smith et al., 2013), four-and-a-half LIM domains protein 2 (FHL2) (Sun et al., 2020), engima (also known as PDLIM7) (Winkelman et al., 2020), and testin (Sala and Oakes, 2021).

Although the exact mechanism of actin strain sensing remains unknown, LIM domain protein binding in purified systems containing actin and myosin has been demonstrated in studies from two independent groups (Sun et al., 2020; Winkelman et al., 2020; Phua et al., 2024 preprint), albeit not at the same magnitude as observed *in vivo*. These groups also surveyed multiple members of the LIM domain family and have suggested that the strain-sensing behavior of LIM domain proteins requires at least three LIM domains in series (Winkelman et al., 2020) and that there might be a conserved phenylalanine residue in the second zinc finger of mechanosensitive LIM domains (Sun et al., 2020). Shortly thereafter, however, our group showed that the first LIM domain of testin is mechanosensitive on its own, suggesting that these prerequisites are not universally characteristic of mechanosensitive LIM domain proteins (Sala and Oakes, 2021). More recently, computational models have suggested that LIM domains might recognize 'cracks' formed between actin monomers in a tensed filament, but this hypothesis remains to be experimentally validated (Zsolnay et al., 2024).

Although this actin strain-sensing mechanism was previously thought to be the sole provenance of LIM domain proteins, we have recently shown that the intrinsically disordered C-terminal region of the short isoform of xin actin-binding repeat-containing protein 2 (XIRP2), a protein found in stereocilia, also recognizes and relocalizes to sites of strained actin (Wagner et al., 2023). As

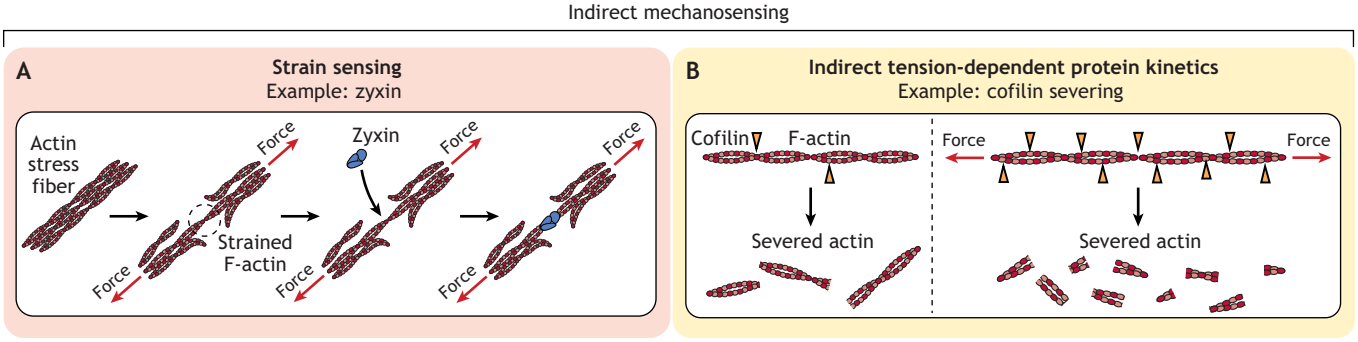

Fig. 4. Examples of indirect mechanosensing. (A) Strain on protein structures such as stress fibers can be recognized through indirect mechanosensing. Zyxin, a LIM domain protein, recognizes strained actin filaments at the sites of stress fiber tears (dashed circle). By relocating to these sites, zyxin facilitates stress fiber repair by recruiting other actin-polymerizing and actin-crosslinking factors. (B) Mechanical changes such as tension or torsion can indirectly impact protein kinetics. For example, tension on an actin filament increases the ability of cofilin proteins to bind and sever the filament.

Journal of Cell Science (2024) 137, jcs261338. doi:10.1242/jcs.261338

these interactions are transient, they are difficult to detect through traditional binding assays, leading us to speculate that there are likely many other examples of strain-sensing proteins and mechanisms waiting to be discovered. Indeed, recent works have shown that LIM domain proteins can recognize strain in proteins beyond actin. The tandem LIM domain proteins LIMK1 and LMO1 have been shown to recognize strain in keratin intermediate filaments (Kim et al., 2024); additionally, FHL2 recognizes and binds to sites in the N2B unique sequence (N2B-us) of titin when this region is stretched (Sun et al., 2024). These findings further illustrate that strain sensing is likely an important feature of many different LIM domain proteins and that the underlying mechanisms are likely specific to each LIM domain protein.

Indirect tension-dependent changes in protein activity

Similar to proteins whose activity or kinetics change directly in response to force, a number of proteins show indirect responses. For example, the actin cytoskeleton undergoes many conformational changes in response to extensile and torsional forces (Bibeau et al., 2023), placing actin firmly in the category of mechanosensing proteins and making it a conduit to indirectly effect the dynamics of other proteins. As an example, both tension and torsion enhance the F-actin-severing ability of cofilin proteins without impacting their ability to bind to actin (McCullough et al., 2011; Wioland et al., 2019) (Fig. 4B). Formins, proteins that facilitate actin polymerization, are similarly sensitive to tension on the actin filament to which they are bound. Interestingly, however, the effects of tension on formin activity depend on the specific formin. The yeast formins Bni1p (Courtemanche et al., 2013) and Cdc12 (Zimmermann et al., 2017) both exhibit reduced polymerization rates when the actin is under tension. In contrast, the polymerization rate of the formin mDia1 (also known as DIAPH1) increases when the actin filaments are pulled on (Jégou et al., 2013; Yu et al., 2017), whereas activity of mDia2 (also known as DIAPH3) remains unaffected (Zimmermann et al., 2017). Tension on actin filaments has also been shown to subtly modulate the actin architecture in a way that alters the binding affinity of α-catenin (Mei et al., 2020).

Whereas these examples are concentrated within actin-related proteins, similar processes are at play elsewhere in the cell. Classical experiments using glass micropipettes showed that tension alters the attachment of microtubules to kinetochores (Nicklas and Koch, 1969). More recent work has determined that tension specifically alters microtubule binding of the kinetochore protein NDC80 via changes in activity of Aurora kinase B (Yoo et al., 2018; Mukherjee et al., 2019). The nuclear envelope is also home to a number of proteins, such as lamins, nesprins and members of the linker of nucleoskeleton and cytoskeleton (LINC) complex, that respond to applied forces (Fedorchak et al., 2014). However, classifying the signaling pathways associated with these proteins as directly or indirectly mechanosensitive is challenging, as it remains difficult to parse exactly how force propagates between the many proteins connecting the cytoskeleton and the nucleus.

Regulation of mechanosensing

A major challenge in interpreting mechanosensing behaviors arises from the fact that multiple mechanosensitive systems are integrated in parallel or obscured by other effects, not least of which are the effects of cell morphology and architecture (see Box 1). Many early experiments exploring mechanosensing compared cells on substrates of different stiffness as a proxy for applied force to show that processes as diverse as migration (Lo et al., 2000) and differentiation (Engler et al., 2006) are mechanosensitive. Many additional processes have also been shown to be sensitive to substrate stiffness, including generation and magnitude of traction stress (Han et al., 2012; Oakes et al., 2014), localization of the transcriptional coactivators YAP and TAZ (also known as WWTR1) to the nucleus (Dupont et al., 2011), phosphorylation of lamin A/C (encoded by *LMNA*) in the nucleus (Swift et al., 2013) and even chromatin remodeling (Walker et al., 2021). Substrate stiffness sensing, however, is mediated by many interactions, including integrin catch bond dynamics and adhesion dynamics, making it difficult to interpret whether downstream effects are the product of differential mechanosensing or simply differences in the capability of cells to spread (Janmey et al., 2020). For instance, we have shown that altering the catch bond dynamics of integrins is sufficient to induce cells to spread on soft substrates in a myosin-independent manner (Oakes et al., 2018). This raises the question: are the various processes mentioned above actually sensitive to substrate stiffness itself, or are they the product of reduced contractility resulting from an inability of the cell to spread?

Hippo signaling, and specifically cytosolic or nuclear localization of the downstream Hippo pathway effectors YAP and TAZ (hereafter collectively referred to as YAP–TAZ), illustrates this issue beautifully. The Hippo pathway regulates processes including cell proliferation and apoptosis to control tissue size and growth. Initial studies showed that limiting cell spreading has the same effect on YAP–TAZ localization as plating a cell on soft substrates: namely, both conditions result in YAP–TAZ remaining cytosolic (Dupont et al., 2011). Follow-up work showed that the critical

Box 1. Architecture sensing versus mechanosensing

Distinguishing between architecture sensing, in which proteins recognize specific higher-order structures, and mechanosensing is challenging because changes in shape are often accompanied by, or the result of, the application of forces (Luciano et al., 2024). Many different proteins, including BAR domain proteins, dynamins and septins, have been found to recognize specific membrane curvatures at organelles or the plasma membrane (Cannon et al., 2017). Some of these proteins clearly respond to force-driven changes. For instance, caveolin-1 detaches from the plasma membrane when it is stretched, allowing cells to adapt to changes in membrane tension (Sinha et al., 2011). Similarly, although BAR domain proteins have long been known to preferentially recognize nanometer-scale membrane curvature, they have also recently been shown to actively contribute to membrane reshaping in response to compression (Le Roux et al., 2021). These two examples demonstrate that these curvature-sensing mechanisms are a product of the protein responding to an applied force and should thus be classified as mechanosensing mechanisms.

Other examples, however, suggest that curvature sensing is independent of force. For instance, the actin-nucleating complex Arp2/3 preferentially branches new F-actin from the convex side of actin filaments (Risca et al., 2012), and the microtubule-binding protein doublecortin binds preferentially to bent microtubules (Bechstedt et al., 2014). In each of these cases, preferential binding is retained when the actin and microtubule filaments are immobilized on a coverslip in these curved conformations, suggesting that Arp2/3 and doublecortin are sensing the architecture of the filament and not responding to an applied force. Similarly, recognition of micrometer-scale curvatures by septins has been shown on rigid, lipid-coated beads (Bridges et al., 2016) and grooved surfaces (Nakazawa et al., 2023) in a manner dependent on their amphipathic helix domains (Cannon et al., 2019) and independent of any applied force. This of course does not preclude these proteins from participating in mechanotransduction, but in these instances, their behavior is more consistent with recognizing a specific architecture rather than being force responsive. However, future research might reveal that these proteins are directly involved in mechanosensing.

regulator of YAP–TAZ localization is actually mechanical tension (Aragona et al., 2013), which is lower both in cells that are constrained from spreading and in cells plated on soft substrates. A proposed molecular regulatory mechanism might lie in the activity of the tension-sensitive LIM domain proteins TRIP6 and LIMD1; when under high levels of force, these proteins bind to and sequester the Hippo pathway kinases LATS1 and LATS2, which phosphorylate YAP–TAZ causing their retention in the cytoplasm (Dutta et al., 2018; Ray et al., 2024). This example illustrates how carefully decoupling the effects of substrate stiffness and cellular contractility when identifying the mechanism of mechanosensing is crucial for understanding how mechanotransduction is regulated. Next, we will discuss types of mechanical and biochemical regulation of mechanosensing.

Mechanical regulation

As mechanosensing is by definition a mechanical process, the most obvious method of regulation is achieved by tuning the magnitude of the applied force. This can be accomplished through active modulation of factors such as cytoskeletal contractility or cell architecture.

Internal contractility

The cytoskeleton is a highly dynamic structure, and the buildup of cytoskeletal contraction coincides with an increase in structural ordering (Aratyn-Schaus et al., 2011; Tee et al., 2015). This allows the cell to respond to both acute (Mitrossilis et al., 2010) and sustained (Buck, 1980) mechanical perturbations. We have previously shown that cells perform a constant amount of mechanical work that scales with their spread area (Oakes et al., 2014), which is consistent with the cellular tensional integrity (tensegrity) models that have been proposed (Ingber, 2003). When pushed away from this contractility setpoint, either by increasing (Oakes et al., 2017) or decreasing myosin activity (Aratyn-Schaus et al., 2011), cells respond by returning to their equilibrium contractile state (Fig. 5A). Importantly, changes in global contractility typically coincide with large changes in cytoskeletal architecture (Ridley and Hall, 1992; Kolega, 2006), which can impact mechanosensitive processes on their own (Choi et al., 2008). Modulating the total tension in the system thus modulates the tension across many mechanosensing proteins, thereby altering their behavior and function.

Architecture and morphology

A similar active response can be seen in how cells reorient and restructure their cytoskeleton in response to changes in tension (Buck, 1980). Specifically, cells typically align their stress fibers perpendicular to the direction of cyclically applied stretch, minimizing the strain on the fibers (Livne et al., 2014); however, there is a dependence on both frequency of stretch and cell type (Liu et al., 2008). Cells will also realign their stress fibers in the direction of shear flow (Conway and Schwartz, 2013) (Fig. 5B). These responses might be dependent on the activity of LIM domain proteins like zyxin and their ability to relocate to stress fibers (Yoshigi et al., 2005; Sun et al., 2020). One hypothesis is that the tensed stress fibers act as a sink to sequester zyxin and FHL2 at the cytoskeleton and keep them out of the nucleus (Sun et al., 2020), where they can act as transcription factors (Wang et al., 2019). Such a mechanism might explain why zyxin-deficient vascular smooth muscle cells fail to respond to applied strain and dedifferentiate from the contractile phenotype to the more motile synthetic phenotype (Ghosh et al., 2015).

Biochemical regulation

In addition to mechanical regulation, cells appear to have various biochemical mechanisms to regulate mechanosensing. We will next describe select examples of this additional layer of control over mechanosensitive cellular processes.

Tuning mechanosensing

Protein–protein interactions and post-translational modifications can affect subsequent downstream signaling events. As an example, cyclin-dependent kinase 1 (CDK1) is a critical regulator of the cell cycle and binds directly to talin (Gough et al., 2021). When bound, CDK1 also phosphorylates talin, reducing the amount of force required to unfold the R7 and R8 domains, which are sites of important interactions between talin and its cytoskeletal and adhesion binding partners. CDK1 thus has the ability to regulate the downstream interactions of talin with integrins, microtubules and the actin cytoskeleton at adhesions in a cell cycle-dependent manner (Fig. 5C). Reciprocally, binding of talin to CDK1 could also potentially provide a pathway to alter the cell cycle in response to mechanical changes in the external environment (Gough et al., 2021).

Similarly, Src-mediated phosphorylation of vinculin has been shown to disrupt the interaction between the head and tail domains of the protein, reducing its ability to bundle actin without affecting its ability to bind actin (Tolbert et al., 2014). This in turn impacts how force is transmitted across the protein. In addition to phosphorylation, other biochemical modifications can impact the structural components tied to mechanosensing. It has recently been shown that the nucleotide state of actin monomers (that is, ATP-, ADP-P_i- or ADP-bound) affects the structural rigidity of the actin filament, thereby altering the ability of cofilins to bind and sever actin (Reynolds et al., 2022). The microtubule cytoskeleton is also susceptible to multiple post-translational modifications that impact microtubule stability and can lead to downstream changes in contractility via, for example, release of the RhoA-activating guanine-nucleotide-exchange factor GEF-H1 (also known as ARHGEF2) (Eshun-Wilson et al., 2019; Seetharaman et al., 2022). Given the abundance of actin- and microtubule-binding proteins, these biochemical changes that alter the mechanical structure of F-actin and microtubules likely have many additional downstream ramifications.

On–off switching

In contrast to mechanisms that finely tune protein mechanosensitivity, mechanosensing can also be regulated in a switch-like manner, in which the response to applied force is either ‘on’ or ‘off’. Our group has recently found that the mechanosensitivity of the LIM domain protein testin, which forms homodimeric complexes, behaves in this way. Specifically, testin typically localizes to the cytoplasm and does not associate with stress fibers, but it contains three LIM domains in its C-terminal half that are highly mechanosensitive, as a truncated construct of the LIM domains alone is sufficient to recognize and localize to actin stress fiber strain sites (Sala and Oakes, 2021). This contradictory behavior of the testin LIM domains compared to the full-length protein suggests that the mechanosensitivity of testin can be regulated. Indeed, mutations to key tyrosine residues in the dimerization region of testin result in the protein becoming mechanosensitive, likely by causing the homodimer to break apart and freeing the LIM domains to interact with strained actin (Sala and Oakes, 2021) (Fig. 5D). Expression of a constitutively active version of RhoA, a master regulator of cytoskeletal activity, also

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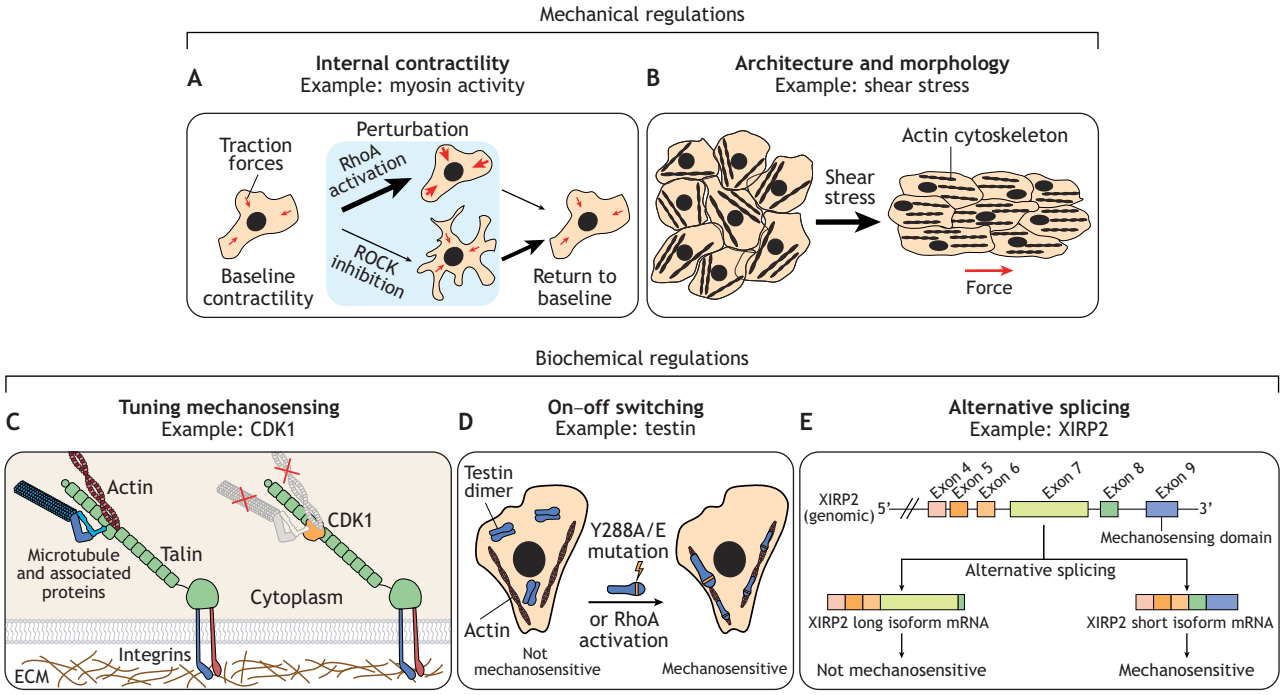

Fig. 5. Mechanisms of regulating mechanosensing. (A,B) Mechanical methods of regulating mechanosensing. (A) Cells actively maintain a baseline cytoskeletal contractility and will return to this state following perturbations. This in turn regulates any downstream components that are sensitive to tension in the cytoskeleton. ROCK, Rho-associated protein kinase. (B) Cells also respond to externally applied forces by changing their internal architecture. For example, when shear stress is applied to a cell monolayer, the actin cytoskeleton reorganizes in the direction of the shear. (C–E) Biochemical methods of regulating mechanosensing. (C) CDK1 can bind and phosphorylate talin. This alters the mechanical stability of talin and disrupts other cytoskeletal interactions in this location, thus tuning the mechanosensitivity of talin. (D) Testin, a LIM domain protein, is typically found in the cytoplasm, where it likely forms homodimers and does not recognize strained actin. However, mutation of tyrosine residues involved in mediating testin dimerization or expression of a constitutively active RhoA results in the protein becoming mechanosensitive and recognizing stress fiber strain sites. Testin is thus able to switch its mechanosensitivity on and off. (E) Lastly, in stereocilia, a short isoform of XIRP2 is expressed that contains a mechanosensitive region in its unstructured C-terminal half. By changing expression levels of this isoform compared to levels of a long isoform lacking the mechanosensitive domain, cells can regulate the mechanosensing activity of XIRP2.

causes testin to become mechanosensitive (Sala and Oakes, 2021). Although the exact mechanism in this case remains unclear, increasing myosin contractility alone is not sufficient to reproduce this response, suggesting that additional biochemical interactions are involved. Similar switch-like behavior has been reported for the protein XIRP2 (Wagner et al., 2023), as detailed in the next section. As revealing this type of regulatory mechanism often requires specific spatial and temporal conditions, we expect that there are likely many more proteins with this ‘hidden’ behavior waiting to be found.

Alternative splicing

An additional method that can be employed to regulate mechanosensitivity is changing gene expression through, for example, alternative splicing. It has recently been shown that auditory hair cells express two splice isoforms of the XIRP2 protein at stereocilia (Wagner et al., 2023) (Fig. 5E). The long isoform contains a C-terminal actin-binding domain, whereas the short splice isoform lacks this domain and instead contains a single LIM domain and a long unstructured region at the C terminus. In stereocilia, the short isoform of XIRP2 has been shown, via immunofluorescence, to localize to breaks in the F-actin cores of stereocilia. When expressed in fibroblasts, the full-length short isoform shows cytoplasmic localization and does not recognize strained actin, whereas a truncated construct consisting of just the unstructured C-terminal region strongly relocates to sites of actin strain. In addition to regulation of mechanosensitive XIRP2 splice isoforms via gene expression, this suggests that there is likely another as-yet-unidentified regulatory mechanism that enables on–off switching of the mechanosensitivity of the short isoform of XIRP2.

Conclusions and perspectives

It is clear that mechanics play a fundamental role in cell physiology. Just as post-translational modifications like phosphorylation represent biochemical mechanisms to control protein function, mechanics represent a physical mechanism to achieve similar ends. Changing cellular and environmental forces can modulate downstream signaling (i.e. mechanotransduction), providing pathways for mechanical signals to influence function from the molecular to the tissue scale. Rather than being perpetually ‘on’, we also must consider that mechanosensing can instead be dynamic and nuanced. Recent work exploring the regulation of mechanosensing associated with talin–CDK1, testin, vinculin and XIRP2 suggest that we have only begun to scratch the surface of how the mechanosensing activity of proteins is biochemically modulated and controlled in cells. Clearly, this ability to biochemically tune mechanosensing offers an additional layer of regulatory control over cellular function and could potentially allow for pleiotropic behaviors in different environments. Identifying the proper conditions to study the function of these types of mechanosensitive proteins, however, remains a significant challenge.

We further believe that the mechanisms discussed above represent just the beginning of our understanding of mechanosensing and

mechanotransduction and that there are undoubtedly additional mechanisms to be discovered. Although we have focused primarily on individual proteins and their response to force, it is entirely possible (and in fact likely) that mechanosensitive complexes requiring multiple components to sense mechanical signals also exist. The complexity of different tissue environments is also likely to affect how proteins experience mechanical signals in ways not yet understood. In particular, the cardiovascular and immune systems are often subject to mechanical changes (for example, stiffening of arteries or inflammation) in response to both physiological and pathological stimuli, making them fertile grounds to identify new mechanosensitive proteins and mechanosensing mechanisms. Similarly, investigating defects in mechanosensing as a function of aging represents an intriguing path to explore, as aging is associated with changes in tissue structure and integrity. Understanding different mechanosensing mechanisms in a wide variety of contexts will thus be vital when developing therapeutic strategies that target mechanosensing pathways.

In conclusion, we stress that the proteins and examples discussed here are in no way exhaustive and that many proteins fall under more than one of the categories we describe. We believe this broad adaptability to be a feature rather than a 'bug' of mechanotransduction. Furthermore, recent findings showing the ability of cells to biochemically tune protein mechanosensing suggest that there are likely many additional mechanosensing proteins that are hiding in plain sight, waiting for researchers to uncover the right spatial and temporal dynamics to reveal their secrets. The future of mechanosensing research is bright with opportunity, and we look forward to seeing where it goes next.

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Competing interests
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