Accepted manuscript

Integrin force loading rate in mechanobiology: from model to molecular measurement

Hongyuan Zhang[1], Micah Yang[1], Seong Ho Kim[1], Isaac T. S. Li[1*]

[1] Department of Chemistry, The University of British Columbia, Kelowna, BC, V1V1V7 Canada

[*] Corresponding author: Isaac T. S. Li; email: isaac.li@ubc.ca

Abstract

Integrins are critical transmembrane receptors that connect the extracellular matrix (ECM) to the intracellular cytoskeleton, playing a central role in mechanotransduction—the process by which cells convert mechanical stimuli into biochemical signals. The dynamic assembly and disassembly of integrin-mediated adhesions enable cells to adapt continuously to changing mechanical cues, regulating essential processes such as adhesion, migration, and proliferation. In this review, we explore the molecular clutch model as a framework for understanding the dynamics of integrin–ECM interactions, emphasizing the critical importance of force loading rate. We discuss how force loading rate bridges internal actomyosin-generated forces and ECM mechanical properties like stiffness and ligand density, determining whether sufficient force is transmitted to mechanosensitive proteins such as talin. This force transmission leads to talin unfolding and activation of downstream signalling pathways, ultimately influencing cellular responses. We also examine recent advances in single-molecule DNA tension sensors that have enabled direct measurements of integrin loading rates, refining the range to approximately 0.5 to 4 pN/s. These findings deepen our understanding of force-mediated mechanotransduction and underscore the need for improved sensor designs to overcome current limitations.

Key Words:

integrin, loading rate, molecular clutch, mechanotransduction, DNA-based tension sensor, single-molecule force imaging

Introduction

Cells are constantly exposed to various mechanical cues from their extracellular matrix (ECM) or neighbouring cells (Du *et al.* 2023). Mechanotransduction is the fundamental process by which cells sense, integrate, and convert these physical stimuli into biochemical signals that regulate

This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI.

10.1017/qrd.2024.28

essential cellular functions (Du *et al.* 2023; Huse 2017; Zhang *et al.* 2020). Among the key players in mechanotransduction are mechanosensitive molecules such as integrins (Pang *et al.* 2023; Shen *et al.* 2012), which serve as transmembrane receptors connecting the ECM to the intracellular actin cytoskeleton (Li *et al.* 2016). The integrin family of cell adhesion receptors mediates bidirectional signalling between cells and their surroundings through "inside-out" and "outside-in" pathways. On the one hand, cells actively exert internal actomyosin cytoskeleton forces through integrins to activate integrin binding and deform their surroundings.

On the other hand, ligand binding to integrins transmits external forces from the ECM back to the cell, depending on ECM characteristics such as rigidity (Yi *et al.* 2021), viscosity (Bennett *et al.* 2018), and ligand spacing (Cavalcanti-Adam *et al.* 2007). This bidirectional interaction ultimately influences cellular responses, including cell spreading, retraction, migration, and proliferation, while allowing cells to sense and adapt to their environment. Because it is constantly subjected to the force transmitted between cells and ECM, integrin acts as an ideal biomechanical sensor. Force experienced by integrin mechanically regulates its properties, including ligand-binding kinetics, conformation and activation, clustering and diffusion (Ali *et al.* 2011; Chen *et al.* 2017; Kechagia *et al.* 2019). Upon binding to ECM components like fibronectin and collagen, integrins undergo conformational changes to be activated and cluster at the cell membrane. Following integrin clustering, adaptor proteins such as talin, vinculin, and paxillin are recruited to the adhesion sites to strengthen the integrin-ECM linkage, thus facilitating the formation of focal adhesions. These macromolecular assemblies anchor cells to the ECM and act as signalling hubs (Bauer *et al.* 2019). Focal adhesion kinase and Src are key downstream nonreceptor tyrosine kinases of the formation of focal adhesions. They play a pivotal role in transducing signals from integrins to activate a range of signalling pathways, including the Ras-MAPK and PI3K-Akt pathways, which regulate cellular behaviours such as migration, proliferation, and survival (Bolós *et al.* 2010; Westhoff *et al.* 2004).

Integrin-mediated mechanosensitivity plays a critical role in various biological processes where cells sense and respond to mechanical cues from the ECM (Di *et al.* 2023). First, integrin mediates tissue regeneration and wound healing (Kechagia *et al.* 2019). Connective tissue repair involves fibroblasts, keratinocytes and endothelial cells (Koivisto *et al.* 2014), which express a repertoire of integrins to sense and interact with the ECM. This interaction enables them to migrate toward the wound site and initiate directed migration, re-epithelization, granulation tissue formation and wound contraction. Integrin is also essential for morphogenesis during embryonic development (Molè *et al.* 2021). As embryos develop, cells are sensitive to the mechanical properties of their surroundings. The interaction between integrins and various ECM components dictates the shape and adhesion pattern of stem cells, guiding their differentiation into specific lineages such as muscle, neural or bone tissue (Estrach *et al.* 2024; Lv *et al.* 2015; Yi *et al.* 2021). Moreover, immune cell activation and migration depend on integrin-mediated mechanosensing (Du *et al.* 2023). For example, substrate stiffness modulates a range of T cell behaviours, including migration (Saitakis *et al.* 2017), cytokine secretion (Yuan *et al.* 2021) and cytotoxic function (Saitakis *et al.* 2017; Wang *et al.* 2022b). Lastly, in fibrotic diseases, integrins play a role in excessive ECM deposition, where activated fibroblasts sense increased matrix stiffness, leading to further ECM production and progression of fibrosis (Pang *et al.* 2023; Yang & Plotnikov 2021). Thus, integrin mechanosensitivity is vital for maintaining homeostasis in healthy tissues and can drive pathological changes when dysregulated.

Understanding the mechanical mechanisms at the molecular level is crucial for deciphering these fundamental biological processes. This review highlights the importance of investigating the

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

integrin force loading rate and its biological relevance. We will examine this concept using the well-established molecular clutch model. Finally, we will summarize several recently developed single-molecule techniques for measuring the dynamics of forces, specifically the force loading rates, and discuss current limitations and future aspects.

Dynamics of cell adhesion and the molecular clutch model

The dynamic nature of cell adhesion

Although focal adhesions are robust and stable anchorages, they are dynamic rather than static (Ivaska 2012). Integrins undergo cycles of activation-adhesion and inactivation-detachment, leading to the continuous assembly and disassembly of focal adhesions. This constant remodelling allows cells to firmly attach to the ECM and pull themselves forward during migration by generating traction forces. Integrin-mediated cell adhesion is crucial for directed migration. Cells dynamically assess and sample ECM rigidity by applying variable pulling forces, guiding the process of durotaxis (Plotnikov *et al.* 2012). Real-time traction force microscopy has revealed that cells exhibit tugging traction dynamics in focal adhesions on soft ECMs while they display stable traction on rigid ECMs. Because cells continuously interact with and adapt to ever-changing mechanical cues in their surroundings, understanding cell behaviours in response to their environment within a dynamic context is crucial.

The molecular clutch model

The concept of "molecular clutch" was introduced by Mitchison and Kirschner in 1988 to depict the dynamic linkage between the cytoskeleton and the ECM (Mitchison & Kirschner 1988). Clutches were initially defined as the dynamic linkage between actin filaments and the ECM through focal adhesion proteins and integrins. This concept has evolved and is now used to interpret cellular responses to various mechanical factors within the ECM. Clutches are currently referred to as the dynamic linkage formed by complexes comprising integrins and adaptor proteins (See Fig. 1) (del Rio *et al.* 2009).

Talin is a primary adapter protein that couples integrins to the actin cytoskeleton. When force is transmitted to talin, it unfolds, exposing previously hidden vinculin binding sites. This unfolding allows another adaptor protein, vinculin, to bind to talin with high affinity, further stabilizing the integrin-actin linkage (Atherton *et al.* 2015). In this framework, cells continuously generate forces via myosin, causing contraction of actin filaments and resulting in retrograde actin flow from the cell edge toward the center. When integrins bind to extracellular substrates and couple the actin flow to the ECM, the clutch system engages. As a result, the retrograde flow pulls on the substrate, applying forces and potentially deforming it. Simultaneously, the elastic resistance of the substrate counters myosin contractility, slowing down the retrograde flow and increasing the force loading rate on the clutches (del Rio *et al.* 2009). As force accumulates on talin up to a threshold level, talin unfolds, exposing vinculin binding sites and relieving vinculin's autoinhibition. Vinculin then binds to talin, strengthening the linkage between integrins and the actin cytoskeleton (Wang *et al.* 2021; Yao *et al.* 2014). The interaction between vinculin and the talin-integrin complex drives FA growth and integrin clustering, stabilizing force transmission (del Rio *et al.* 2009; Humphries *et al.* 2007). As more integrins are recruited to the adhesion sites, additional clutches engage. This reduces the force applied to each clutch, preventing the disengagement of the system due to excessive force loading (Elosegui-Artola *et al.* 2018).

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

The integrin-ECM linkage exhibits a catch-slip behaviour, where the bond lifetime initially increases with applied force (catch phase) and then decreases as the force continues to increase (slip phase) (Chen *et al.* 2017; Kong *et al.* 2009). As the force increases, the bond lifetime increases; however, as the force continues to build up, the bond eventually fails and results in the disengagement of the clutch. In contrast, the unfolding behaviour of talin domains follows a Bell-like model, where the unfolding rate increases exponentially with applied force (Bell 1978). To achieve effective mechanotransduction, the force applied to talin must be loaded at an optimal rate that allows talin to unfold within the stable period of the integrin-ECM bond (See Fig. 1).

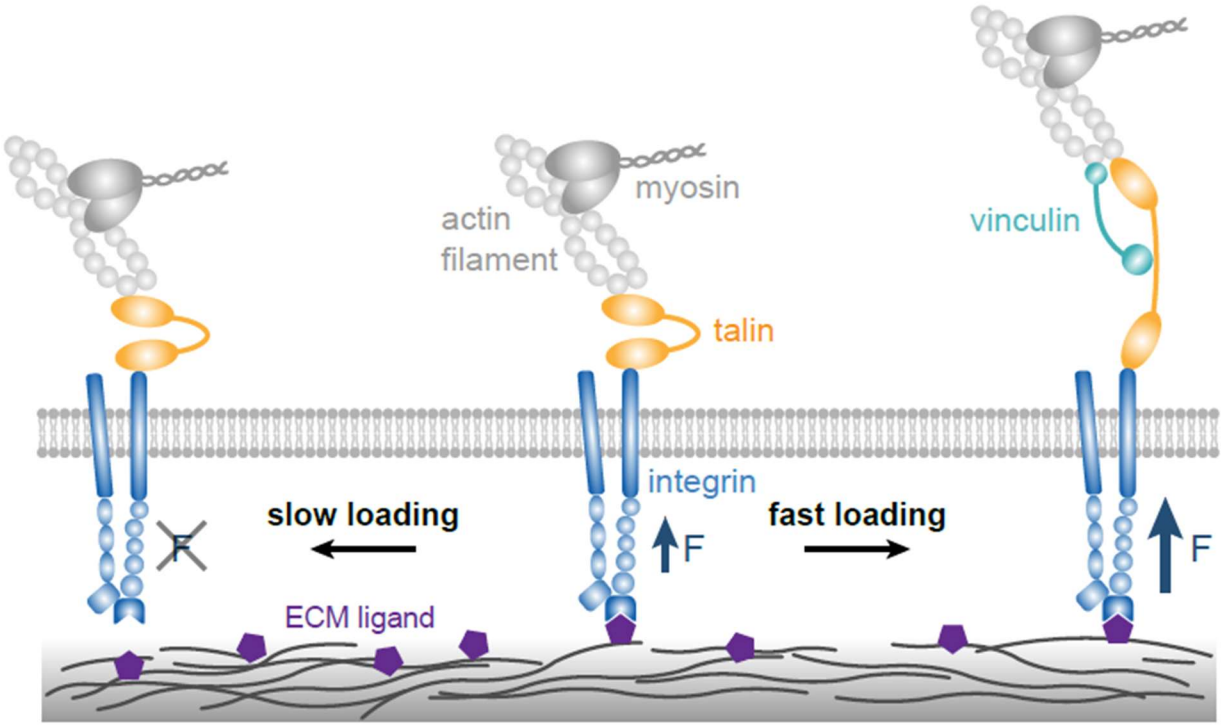

Fig. 1. Schematic of molecular clutch model. The clutch represents the dynamic linkage between integrin and the extracellular matrix (ECM), mediated by adaptor proteins such as talin. Under fast force loading, the force accumulates beyond the threshold required for talin unfolding before the integrin-ECM bond disengages, thereby exposing vinculin binding sites. Vinculin binding reinforces the linkage. In contrast, under slow force loading, the integrin-ECM bond disengages before the force threshold for talin unfolding is reached, preventing vinculin binding. The bond rupture abolishes force transmission.

The force loading rate is a core component of the molecular clutch model (Elosegui-Artola *et al.* 2018), linking cellular mechanosensing to both actively generated forces within the cell and the passive mechanical properties of the ECM (Jiang *et al.* 2016). The internal cellular machinery generates the active forces, mainly through actomyosin contraction. The passive mechanical properties are represented by the effective spring constant (k) of the ECM. This model defines the loading rate as the product of k and actomyosin pulling speed (v) (Jiang *et al.* 2016). From the perspective of loading rate, the molecular clutch model depicts biphasic behaviour in response to the ECM stiffness (Swaminathan & Waterman 2016). On soft substrates, the compliance of the ECM buffers the retrograde movement of actin filaments driven by myosin, slowing the rate at

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

which tension builds on each engaged clutch. When the force is loaded slowly, the integrin-ECM bond is more likely to fail before substantial force is transmitted to talin. In contrast, on rigid substrates, the force is loaded faster, allowing significant force to be transmitted to talin. This rapid force loading leads to talin unfolding, exposing previously cryptic vinculin binding sites and triggering subsequent mechanotransduction pathways.

Thus, the force loading rate is critical in determining whether force transmission through engaged clutches leads to effective mechanotransduction or clutch disengagement. Understanding this rate is essential for comprehending how cells respond to varying ECM stiffness and elucidating the mechanisms underlying cellular processes like migration, differentiation, and tissue development.

Techniques for molecular force measurement

Researchers have developed various techniques to measure the magnitude of cellular forces (Liu *et al.* 2017). These techniques can be broadly classified into three types:

1. Macroscopic deformation: This category includes traction force microscopy and micro-post array detectors, which measure substrate deformations under mechanical forces exerted by cells. While useful, these methods are limited to nanonewton resolution.
2. Instrument-based force spectroscopy: techniques such as atomic force microscopy, optical tweezers, magnetic tweezers, and biomembrane force probes fall under this category. These techniques allow force measurements at the single-molecule level but are limited by low throughput and spatial resolution (Bustamante *et al.* 2021).
3. Molecular tension sensors: this includes tension sensor modules (TSMods) (LaCroix *et al.* 2018), DNA hairpin probes (Zhang *et al.* 2014), and tension gauge tethers (TGTs) (Wang & Ha 2013). These sensors achieve piconewton (pN) resolution with high throughput, providing force readouts through fluorescence signals such as Förster resonance energy transfer (FRET) or fluorescence quenching.

The details of these three types of techniques, including their advantages and disadvantages, were extensively covered in the following excellent reviews (Fischer *et al.* 2021; Liu *et al.* 2017; Tu & Wang 2020), hence we will not discuss them in further details here. We will primarily elaborate on molecular tension sensors. Genetically encoded TSMod incorporates proteins of interest into an elastic FRET module – a flexible peptide linker inserted between two fluorophores. When tension is applied to the protein, the elastic linker extends, decreasing FRET or quenching efficiency. The vinculin tension sensor (VinTS) is specifically designed to measure mechanical forces exerted on vinculin at focal adhesions (see Fig. 2A) (Ayad *et al.* 2022; Grashoff *et al.* 2010). It consists of the head and tail domains of vinculin connected by a 40 amino acid (aa)-long elastomer domain. After calibration, VinTS can reliably report forces within the 1 to 6 pN range, with average forces across vinculin detected at approximately 2.5 pN (Grashoff *et al.* 2010).

Unlike TSMod, which measures intracellular tension directly within the cell, DNA hairpin probes and TGT are typically coated onto substrates like glass coverslips to measure forces transmitted to transmembrane proteins from the extracellular environment. As its name suggests, the DNA hairpin probe consists of a single-stranded DNA sequence that folds back on itself to form a hairpin loop structure (see Fig. 2B) (Zhang *et al.* 2014). The end of the hairpin is bioconjugated with a specific recognition motif, allowing cells to bind and interact with the sensor. When a cell exerts tension on the hairpin, the stem unfolds, separating the fluorophore and quencher. Due to its reversible folding and unfolding in response to mechanical forces, the DNA hairpin probe can

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

monitor real-time tension forces and capture temporal oscillations of integrin tension force (Zhang *et al.* 2014). These sensors can detect forces as low as 4.7 pN up to about 19 pN, tunable by sequence.

TGTs consist of double-stranded DNA modified to bind to cells and measure mechanical forces through fluorescence (see Fig. 2C) (Wang *et al.* 2015; Wang & Ha 2013). TGTs record irreversible rupture events when cells produce sufficient tension to rupture them. The tension tolerance (T_{tol}), a metric describing the strength to resist mechanical rupture in TGT, is defined as "*the lowest force that ruptures the DNA within 2 seconds if the force is applied at a constant level*" (Wang & Ha 2013). Using TGT, researchers have revealed a close interplay between the magnitude of force and mechanotransduction. The integrin tension forces in CHO-K1 cells were reported to be able to rupture TGT with T_{tol} ranging from 12 – 56 pN (Wang & Wang 2016). The growth of focal adhesions correlates positively with integrin tension (Chang Chien *et al.* 2022; Wang *et al.* 2015). Specifically, the sizes of focal adhesions increased from 1 to 6 μm as cells were seeded onto TGT surfaces with increasing tension tolerances (T_{tol} = 43 – 56 pN). Additionally, the translocation of yes-associated protein (YAP), a mechanosensitive transcription factor, from the cytoplasm to the nucleus occurs only when forces across integrins are steadily transmitted on higher T_{tol} TGT (T_{tol} = 50 – 54 pN).

It is important to note that cellular forces quantified by the molecular tension sensors require careful interpretation. The magnitude of the force transmitted by cells is greatly impacted by the mechanical properties of ECM (Humphrey *et al.* 2014). For example, it has been reported that T cells can engage T-cell receptors (TCRs) on hard coverslips with forces sufficient to rupture TGTs with T_{tol} = 12-19 pN (Liu *et al.* 2016). However, on gel-phase supported lipid bilayers (SLBs), the rupture force imposed by TCR was approximately 5 pN (Göhring *et al.* 2021). On the fluid-phase SLBs, the force was further reduced to 1.9 pN.

Furthermore, the reported T_{tol} of TGTs cannot be directly interpreted as the actual force magnitude exerted by cells. Physiologically, cells likely apply forces over longer durations and dynamically in response to the various environments (Gardel *et al.* 2010; Gjorevski *et al.* 2015), while T_{tol} is calibrated within 2 seconds at a constant loading rate. Similarly, the value of $F_{1/2}$ of DNA hairpin probes requires careful calibration to reduce folding/unfolding hysteresis to report more accurately the dynamic and variable force loading experienced by cells in physiological environments (Yasunaga *et al.* 2019).

Despite advancements in the development of first-generation molecular tension sensors, these tools often suffer from limited dynamic ranges or provide only binary outputs, indicating whether a specific force threshold has been exceeded. Such limitations make it challenging to accurately measure the dynamics of molecular tension, particularly the loading rate.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

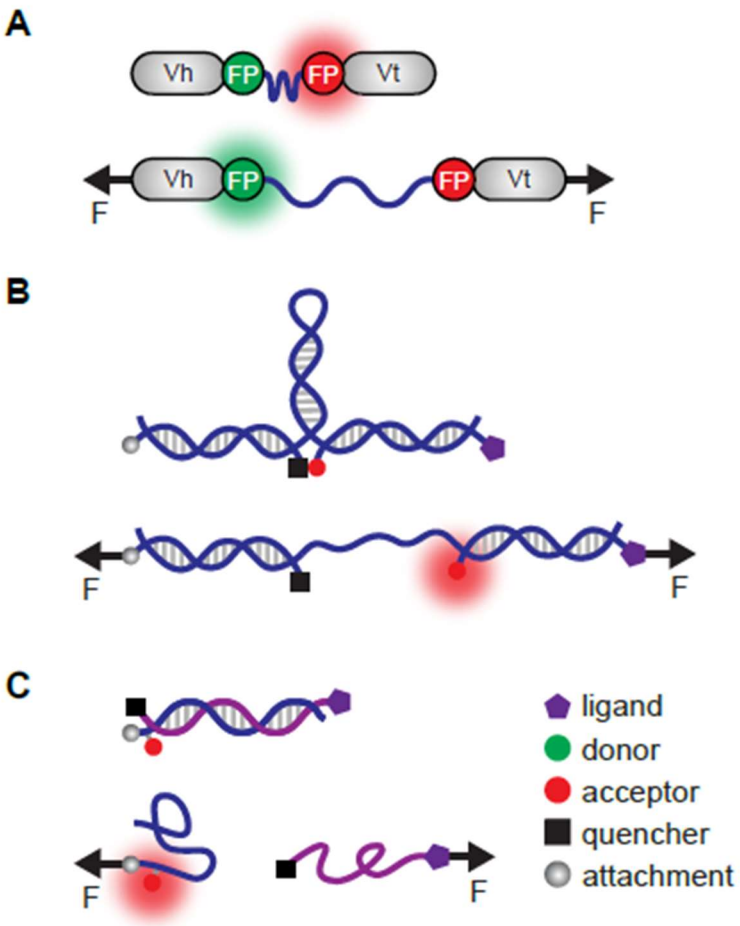

Fig. 2. Schematic representations of various molecular force sensors. **(A)** VinTS comprising head (Vh) and tail (Vt) domains connected by an elastomeric peptide (blue) and a fluorescent protein (FP) FRET pair (red and green), with FRET signal decreasing upon peptide extension under tension; **(B)** DNA hairpin probe, where a fluorophore is quenched in the absence of tension but becomes fluorescent when the hairpin opens under sufficient tension, increasing the distance from the fluorophore to the quencher beyond its quenching range; **(C)** TGT, where a DNA duplex remains quenched when intact, and fluorescence occurs upon dissociation of the strand attached to a ligand (purple) from the surface-bound strand (blue) under applied tension.

Measuring molecular loading rate

Focusing solely on force magnitude overlooks the dynamic nature of cellular responses and the complexity of ECM mechanics. The concept of force loading rate fills this gap by accounting for how quickly the force is applied to molecular bonds, which directly influences whether bonds like integrin–ECM linkages can transmit sufficient force to mechanosensitive proteins before disengaging. This understanding is crucial for deciphering cellular behaviours responding to different mechanical environments.

Rupture force and bond lifetime depend on the loading rate

The magnitude of the force exerted by cells is a critical parameter in mechanotransduction.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

However, focusing solely on force magnitude overlooks the dynamic nature of cellular responses to mechanical stimuli and the complexity of ECM mechanics. The concept of force loading rate fills this gap in understanding dynamic cell behaviours. It deciphers the complex ECM mechanics and translates mechanical signals into biochemical signals to mediate subsequent cellular responses. For instance, integrins have a lower loading rate on soft substrates than stiffer substrates, leading to lower integrin rupture force (Jiang *et al.* 2016). It has long been recognized that force loading rate plays a significant role in molecular adhesion events like bond lifetime and rupture forces, thereby regulating related mechanosensing (Andreu *et al.* 2021). Different loading rates can dramatically change the rupture forces of adhesion proteins, either abolishing or promoting mechanotransduction across the same set of protein-ligand interactions (Huang *et al.* 2017; Liu *et al.* 2014a; Ma *et al.* 2022). This change can be exaggerated depending on the shape of the force-dependent lifetime curve of the bond in question.

Slip bonds, which decrease in lifetime with tension, remain stable at low force but break more readily at high forces. Thus, a slip bond experiencing a particular loading rate will sustain tension initially, with rupture probability increasing as force increases. In this case, a slower loading rate decreases the most probable rupture force; more time spent at a lower force increases the probability of rupture occurring at that force.

The effect is far more dramatic for catch bonds, which have a region where bond lifetime increases with force. A catch bond has a short lifetime at low forces, so at sufficiently slow loading rates, it cannot maintain tension. The loading rate must be fast enough to reach a stabilizing force before the catch bond ruptures. Several adhesive or mechanosensitive proteins, such as certain integrins (Chen *et al.* 2010; Kong *et al.* 2009), cadherins (Manibog *et al.* 2014; Rakshit *et al.* 2012), selectins (Barkan & Bruinsma 2024; Evans *et al.* 2004), actin (Guo & Guilford 2006; Huang *et al.* 2017), actin-binding domain of talin (Owen *et al.* 2022), and T-cell receptors (Liu *et al.* 2014a; Ma *et al.* 2022) have been found to exhibit catch-bond behaviour. Therefore, loading rate, in addition to force magnitude, is critical for a complete understanding of mechanotransduction.

Force loading rate bridges ECM mechanics to mechanotransduction.

While numerous studies have explored the role of matrix stiffness in mediating stem cell behaviour (Chen *et al.* 2010; Manibog *et al.* 2014; Rakshit *et al.* 2012), much less is known about the mechanism by which matrix stiffness leads to changes in cell morphology, adhesion, proliferation and differentiation. Considering that the loading rate is the product of the effective spring constant of the ECM and the actomyosin pulling speed, changes in mechanical properties significantly affect the loading rate applied by cells and thus influence subsequent cellular behaviour (Jiang *et al.* 2016). Force loading rate plays a vital role in translating substrate rigidity into intracellular signalling to regulate cell differentiation.

Mesenchymal stem cells tend to differentiate into neurogenic lineages on soft substrate, whereas they differentiate into osteogenic (bone) lineages on stiff substrate (Wang *et al.* 2022a). Soft substrate limits the force cells apply to the substrate, thus modulating subsequent transcriptional activities. Mesenchymal stem cells on soft substrates exhibit less maturation of focal adhesions, reduced F-actin assembling, and more relaxed nuclei. Andreu and colleagues showed that the loading rate is a driving parameter of mechanosensing (Andreu *et al.* 2021). They manipulated the loading rate by changing the substrate stiffness or the external stretching frequency. Their results demonstrated that increasing the loading rate leads to two major mechanosensitive events: talin-

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

mediated adhesion growth and reinforcement and YAP translocation from cytosol to the nucleus.

A higher force loading rate ensures the force is transmitted to talin and induces its unfolding before the integrin-ligand bond disengages. When talin unfolds, it exposes binding sites for vinculin, which strengthens the connection between talin and F-actin, enhancing force transmission by recruiting additional actin filaments (Li *et al.* 2016). The forces generated at focal adhesions can be transmitted to the nucleus, stretching nuclear pores and facilitating the entry of YAP into the nucleus (Elosegui-Artola *et al.* 2017). Once inside, YAP interacts with TEA domain (TEAD) transcription factors to regulate gene expression. The YAP-TEAD complex promotes cell proliferation and inhibits apoptosis by controlling the expression of target genes (Kwon *et al.* 2022).

In the context of osteogenesis, YAP plays a complex role alongside the transcriptional coactivator with PDZ-binding motif (TAZ) (Pan *et al.* 2018; Wang *et al.* 2023a). TAZ actively promotes osteogenesis by coactivating runt-related transcription factor 2 (RUNX2) genes, which are critical for bone development. On the other hand, YAP has a dual role: it can inhibit RUNX2-mediated transcription, thereby downregulating osteogenesis while stabilizing β-catenin to enhance β-catenin-mediated osteogenesis (Pan *et al.* 2018).

In summary, ECM mechanics, such as stiffness, regulate the force-loading rate onto the cell via integrin. The loading rate determines whether sufficient force can be transmitted to critical mechanosensitive proteins like talin, leading to their activation and triggering downstream signalling pathways and cell behaviours before the integrin-ECM linkage disengages.

Methods to quantify integrin loading rate

While molecular tension sensors allow quantification of force magnitude at the pN level, they do not measure the loading rate of integrin tension. Moore and colleagues estimated the force loading rate of a single integrin by measuring the deformation of the elastomeric substrate, reporting values from 0.007 to 4 pN/s (Moore *et al.* 2010). While this method provided rough estimation, direct measurements at the single-molecule level were needed. In light of this deficiency, three groups recently developed dual DNA tension sensors that directly reported force loading rates at the single-molecule level.

The Ha group developed an overstretching tension sensor (OTS) based on stretching-induced oligonucleotide dehybridization (see Fig. 3A) (Jo *et al.* 2024). They connected two OTSs with distinct dehybridization forces of 16 and 30 pN, labelled with different fluorophores (Atto674N and Cy3). By recording the time interval between the two fluorescence signals when each threshold force was reached, they calculated the loading rate as the force difference divided by this time interval. Using OTSs, they reported that the integrin loading rate ranged from 0.5 to 4 pN/s.

The Salita group developed an loading rate probe (LR probe) that incorporated two oligonucleotide strands, each of which undergoes a conformational change at different force thresholds and reports unique fluorescence signals (see Fig. 3B) (Combs *et al.* 2024). A lower force threshold at 4.7 pN leads to hairpin unfolding, and as force increases, a duplex TGT (with a T_{tol} of 56 pN) gets sheared. The results showed the median loading rate of integrin-mediated force as 1.3 pN/s.

The Liu group designed a ForceChrono probe consisting of two DNA hairpins labelled with distinct fluorophores, each unfolding at different force thresholds (Hu *et al.* 2024). They developed two versions of ForceChrono probes to cover broader mechanical ranges, one for 7-19 pN and another for 17-41 pN forces (see Fig. 3C). The average loading rates derived from these two

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

ForceChrono probes were 0.6 and 1.5 pN/s, respectively. Their single-molecule trajectories revealed a spatio-temporal heterogeneity in the dynamics of integrins where the integrin–talin–actin linkages are initially (first 20 mins) unstable with faster loading rates (~0.9 pN/s) and shorter force durations (~45 s). After 8 hours, as focal adhesions stabilized, the loading rate decreased (~0.5 pN/s), and force duration increased (~100 s). This feature was consistent with the previously discussed cell dynamics observed by traction force microscopy, where cells showed tugging traction force on a soft substrate but exhibited stable traction force on a rigid substrate (Plotnikov *et al.* 2012).

Collectively, the measured loading rates in these three studies overlapped significantly, and the researchers managed to refine this measurement to a much more precise range.

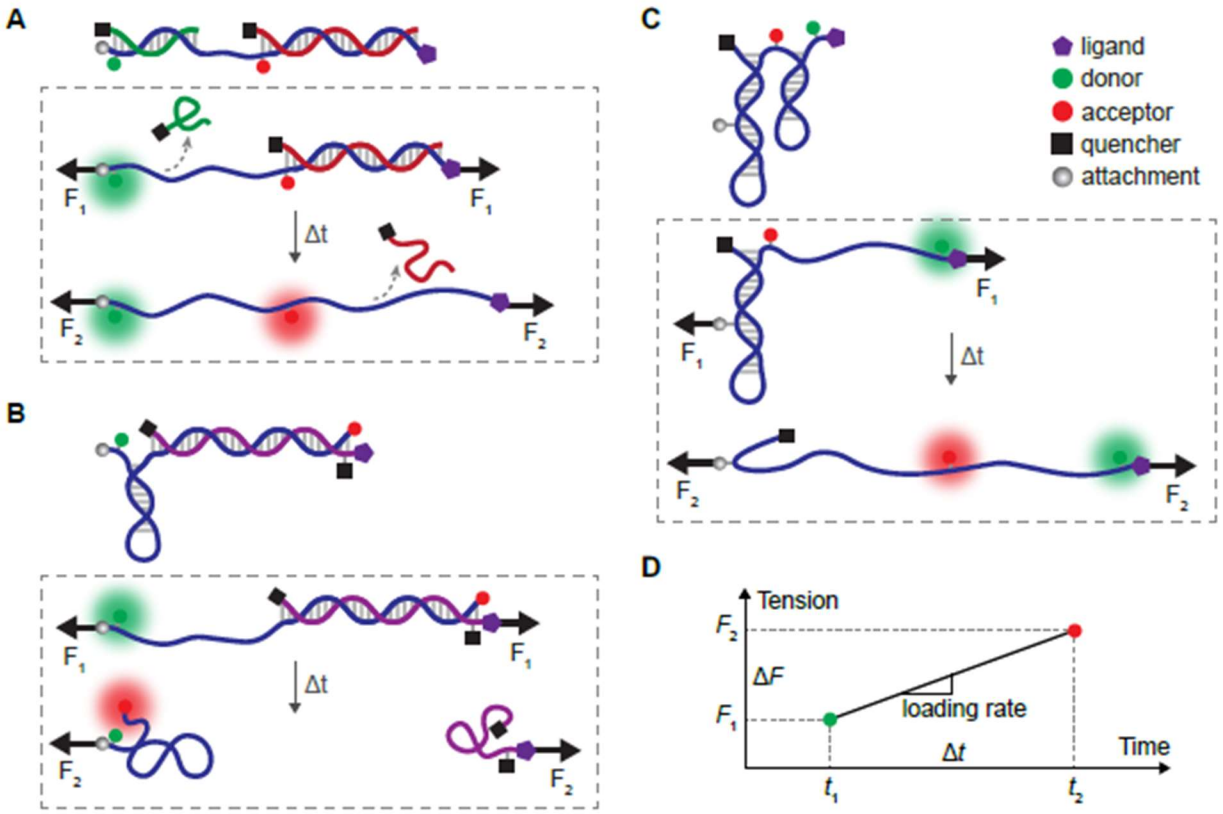

Fig. 3. Schematic of three recently developed force-loading rate sensors. (A) OTS, where forces exceeding F_1 and F_2 sequentially displace two DNA duplexes (green and red), unquenching their corresponding fluorescence signals (green and red) in order; **(B)** LR probe, consisting of a DNA hairpin that opens at force F_1, connected to a TGT designed to rupture at a higher force F_2, detecting two sequential events, with the final event causing the surface attached DNA to recoil and a high-FRET (red) signal; **(C)** ForceChrono probe, utilizing two DNA hairpins with distinct attachment geometries that open sequentially as force increases from F_1 to F_2, resulting in the sequential appearance of red and green fluorescence signals. **(D)** Given the designed force difference (ΔF) and time difference (Δt) between the two events, the loading rate can be determined, assuming linear force ramp between the two events.

Consideration, challenges, and future perspectives

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

Effects of substrate rigidity on loading rate

Rigidity is an essential characteristic of ECM properties. Physiological rigidity varies significantly across tissues—from soft brain tissue (1–4 kPa) to stiff bone tissue (1000–1500 kPa) (Handorf *et al.* 2015). While current studies are performed on hard coverslips to quantify in vivo integrin loading rates (Combs *et al.* 2024; Hu *et al.* 2024; Jo *et al.* 2024), these coverslips are much stiffer than tissues. This could potentially take advantage of the method from Hu and colleagues. They were able to monitor molecular tension at different substrate stiffness by coating DNA tension sensors on soft hydrogels (Wang *et al.* 2023b). They fabricated a series of hydrogels with different moduli ranging from 1 kPa to 80 kPa and coated DNA tension sensors on the soft surface through golden nanoparticles. Their results demonstrated that cells recruit more force-bearing integrins and adjust their interaction dynamics with the ECM to form stronger, more mature focal adhesions on rigid substrates, which is consistent with what the molecular clutch model suggests (Elosegui-Artola *et al.* 2018). Combining this methodology with some advancement in single-molecule imaging in 3D would be very interesting to see how the substrate stiffness alters the loading rate on integrins.

Influence of ligand density on loading rate

Ligand density is also a crucial factor in the ECM environment, affecting cellular adhesion structures and force-mediated mechanosensing (Liu *et al.* 2014b; Oria *et al.* 2017). Schvartzman and colleagues demonstrated a significant increase in cell spreading efficiency when clusters of at least 4 liganded integrins were within ∼60 nm – a spacing within physiological ranges of 10 to 200 nm (Le Saux *et al.* 2011; Schvartzman *et al.* 2011). Considering force balance at the interface, ligand spacing plays a significant role in measuring the loading rate *in vivo*. As integrin binds to ligands to engage the clutch system, the force transmitted to ECM counters myosin contractility, thereby decreasing actomyosin pulling speed (v) (Barnhart *et al.* 2011; Elosegui-Artola *et al.* 2018). Given a constant and optimal rigidity, increasing ligand density increases the number of clutches engaged, thereby slowing down the pulling speed and resulting in a lowered loading rate, which is the product of the effective spring constant of the substrate (k) and actomyosin pulling speed (v). Hu and colleagues investigated the impact of ligand density on integrin loading rates. They found that at lower ligand spacing (40 nm), the average loading rate was slower (~0.3 pN/s) and force duration longer (~180 s) compared to higher ligand spacing (100 nm), where the loading rate was faster (~1.25 pN/s) with shorter force duration (~90 s). These results were consistent with molecular clutch model: higher ligand density allows force to be more stably exerted and distributed over more adhesion points, strengthening integrin–talin–actin linkages. Conversely, lower ligand density leads to less stable force distribution, resulting in instability and frequent bond ruptures. Given there are differences due to integrin density, a systematic investigation of how this affects the loading rate could shed light on the different biological processes that can be controlled entirely by the ligand density.

Interpreting readout from loading rate sensor

While current molecular tension sensors have provided initial insights into the force-loading rates of integrins, there is significant room for improvement. Current techniques for measuring integrin loading rates possess inherent observation biases that must be carefully considered during data interpretation.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

All current techniques rely on the sequential detection of two fluorescent events: the first occurs at t_1, indicating the opening of DNA duplex d_1 at force F_1; the second occurs at t_2, indicating the opening of DNA duplex d_2 at force F_2. The sequence of these events is crucial because F_1 is designed to be lower than F_2. Thus, the only data traces that contain both signals in the correct order are interpretable.

This reliance introduces the first bias that events that do not reach F_1 are undetected, and events that do not reach F_2 are discarded (Fig. 4B). This introduces a bias of only representing the loading rates of events that ultimately reached sufficiently high tension. This limitation is particularly problematic when measuring catch bonds (Fig. 4B), which many mechanosensitive receptors are. Catch bonds have a characteristic double rupture force distribution. The higher force rupture peak is dominant at a high loading rate, but at a low loading rate, the low rupture force events dominate. Due to this, catch bonds with a slow loading rate may not be observed, meaning a potentially large subset of functionally important behaviours are underrepresented if not entirely missing. Therefore, the nature of the adhesion interactions (i.e. catch vs. slip) must be considered when designing the loading rate sensor.

Furthermore, interpreting the data involves assuming a constant loading rate between t_1 and t_2 within the force range between F_1 and F_2. This assumption rests on two key premises: (1) the force difference (ΔF) between F_1 and F_2 remains constant, and (2) that force loading is constant over the time interval (Δt) (Fig 3D, 4A). The first assumption must be carefully designed or accounted for in subsequent analysis because DNA nanomechanics are sensitive to temperature, salt concentration, molecular crowding, and force loading rate. A well-designed loading rate sensor should utilize d_1 and d_2 duplexes that are either equally affected by or insensitive to these factors – ensuring that ΔF remains constant even if the absolute values of F_1 and F_2 change (Hu *et al.* 2024). This minimizes the impact of varying conditions on the loading rate measurement.

While the current designs have addressed the first assumption to some extent, the second assumption presents a greater challenge with current loading rate sensors. Because the sensors report discrete events, they inherently miss the force dynamics between t_1 and t_2. Therefore, the shorter the Δt, the more likely a linear approximation of force loading reflects the underlying reality. For longer Δt, the linear approximations become less accurate due to the time scale of tension dynamics (tens of seconds) (Puklin-Faucher & Sheetz 2009) and the possibilities of many force trajectories that pass through both F_2 at t_1 and F_2 at t_2 (Fig. 4A). One approach to improve the accuracy of data interpretation for loading rate sensors is to decrease Δt or ΔF, albeit at the expense of dynamic range, and multiplex these sensors to obtain a comprehensive picture of loading rates across a broader force range. Alternatively, increasing the number of discrete duplexes that rupture at different forces within the same construct can refine force detection.

Similarly, an analog tension sensor with a large force dynamic range may achieve better temporal resolution. The design of loading rate sensors can also exclude behaviours which violate the second assumption: In the case of reversible constructs with minimal unfolding/refolding hysteresis, one can ensure that the force remains above F_1 while waiting to reach F_2, eliminating oscillating force trajectories, as well as unbinding/rebinding of different ligands. For irreversible constructs, there is no guarantee that the force remains above F_1 before F_2 appears. Current loading rate sensor designs cannot exclude force plateaus, leading to a potential underestimation of the loading rate; this is an opportunity for new, innovative designs moving forward.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

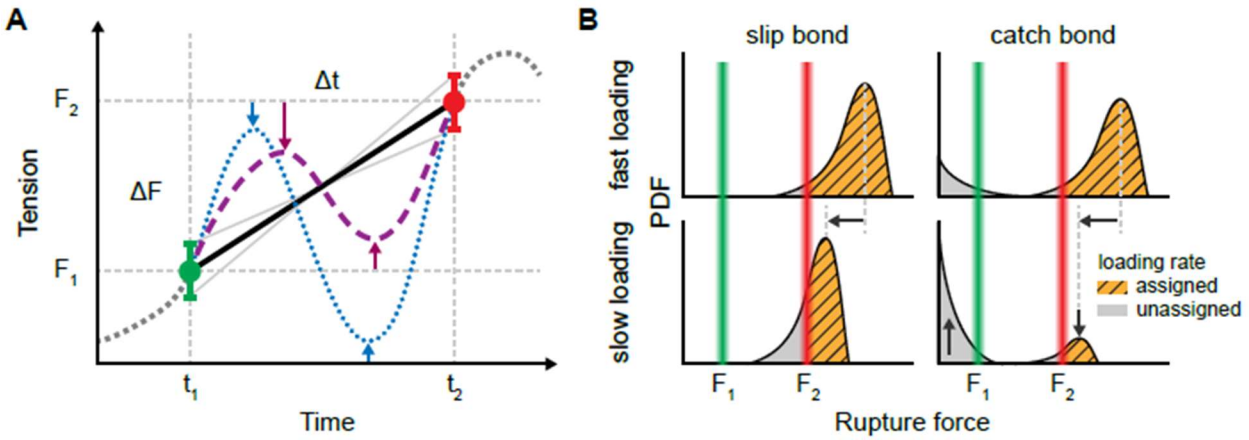

Fig. 4. Potential challenges in interpreting data from current loading rate sensors. (A) Due to the stochastic nature of bond rupture, rupture forces have distributions around F_1 and F_2 (illustrated by error bars) and may be dependent on the loading rate, introducing potential inaccuracies in the assumed linear loading rate. Additionally, different force trajectories (blue dotted line and purple dashed line) can produce identical observed signals. In reversible sensors (purple dashed line) that emit a green signal at F_1, the force range is confined between F_1 and F_2. In contrast, for irreversible sensors (blue dotted line) generating a green signal, the force is only constrained by an upper bound at F_2, while it can decrease toward zero before rising again to F_2 to produce a red signal. As a result, assuming a linear force ramp may be an oversimplification, especially if the duration of events is long. (B) The nature of catch or slip bonds under varying loading rates can obscure certain events. The graphs depict catch or slip behaviours at fast and slow loading rates. The green and red lines represent the sensor rupture forces at F_1 and F_2, respectively. The striped yellow and gray regions under the rupture force distributions represent the populations of native events where the loading rate can (striped yellow) and cannot (gray) be assigned. Receptor-ligand rupture events below F_2 cannot be assigned a loading rate, which biases loading rate observations toward events that occur above F_2. This is particularly problematic for catch bonds, where the bimodal distribution of rupture forces includes a low-force component that dominates at low loading rates.

Conclusion

Accurately measuring the force loading rate is crucial for understanding how cells convert mechanical cues from their environment into biochemical signals that regulate vital functions. Recent advances in single-molecule tension sensor technology, particularly dual DNA tension sensors, have significantly enhanced our ability to measure integrin loading rates with high precision. Combining these advanced measurement techniques with systematic studies of ligand density and substrate stiffness while addressing current methods' limitations can further refine our understanding of integrin-mediated mechanotransduction and its role in cellular functions.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

References

Ali, O., Guillou, H., Destaing, O., Albigès-Rizo, C., Block, M. R., & Fourcade, B. (2011). Cooperativity between Integrin Activation and Mechanical Stress Leads to Integrin Clustering. *Biophysical Journal*, **100**(11), 2595–2604.

Andreu, I., Falcones, B., Hurst, S., … Roca-Cusachs, P. (2021). The force loading rate drives cell mechanosensing through both reinforcement and cytoskeletal softening. *Nature Communications*, **12**(1), 4229.

Atherton, P., Stutchbury, B., Wang, D.-Y., … Ballestrem, C. (2015). Vinculin controls talin engagement with the actomyosin machinery. *Nature Communications*, **6**(1), 10038.

Ayad, M. A., Mahon, T., Patel, M., … Boustany, N. N. (2022). Förster resonance energy transfer efficiency of the vinculin tension sensor in cultured primary cortical neuronal growth cones. *Neurophotonics*, **9**(2), 025002.

Barkan, C. O., & Bruinsma, R. F. (2024). Topology of molecular deformations induces triphasic catch bonding in selectin–ligand bonds. *Proceedings of the National Academy of Sciences*, **121**(6), e2315866121.

Barnhart, E. L., Lee, K.-C., Keren, K., Mogilner, A., & Theriot, J. A. (2011). An Adhesion-Dependent Switch between Mechanisms That Determine Motile Cell Shape. *PLOS Biology*, **9**(5), e1001059.

Bauer, M. S., Baumann, F., Daday, C., … Lietha, D. (2019). Structural and mechanistic insights into mechanoactivation of focal adhesion kinase. *Proceedings of the National Academy of Sciences*, **116**(14), 6766–6774.

Bell, G. I. (1978). Models for the Specific Adhesion of Cells to Cells. *Science*, **200**(4342), 618–627.

Bennett, M., Cantini, M., Reboud, J., Cooper, J. M., Roca-Cusachs, P., & Salmeron-Sanchez, M.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

(2018). Molecular clutch drives cell response to surface viscosity. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(6), 1192–1197.

Bolós, V., Gasent, J. M., López-Tarruella, S., & Grande, E. (2010). The dual kinase complex FAK-Src as a promising therapeutic target in cancer. *OncoTargets and Therapy*, **3**, 83–97.

Bustamante, C. J., Chemla, Y. R., Liu, S., & Wang, M. D. (2021). Optical tweezers in single-molecule biophysics. *Nature Reviews Methods Primers*, **1**(1), 1–29.

Cavalcanti-Adam, E. A., Volberg, T., Micoulet, A., Kessler, H., Geiger, B., & Spatz, J. P. (2007). Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands. *Biophysical Journal*, **92**(8), 2964–2974.

Chang Chien, C.-Y., Chou, S.-H., & Lee, H.-H. (2022). Integrin molecular tension required for focal adhesion maturation and YAP nuclear translocation. *Biochemistry and Biophysics Reports*, **31**, 101287.

Chen, W., Lou, J., & Zhu, C. (2010). Forcing Switch from Short- to Intermediate- and Long-lived States of the αA Domain Generates LFA-1/ICAM-1 Catch Bonds *. *Journal of Biological Chemistry*, **285**(46), 35967–35978.

Chen, Y., Lee, H., Tong, H., Schwartz, M., & Zhu, C. (2017). Force regulated conformational change of integrin αVβ3. *Matrix Biology*, **60**, 70–85.

Combs, J. D., Foote, A. K., Ogasawara, H., … Salaita, K. (2024). Measuring Integrin Force Loading Rates Using a Two-Step DNA Tension Sensor. *Journal of the American Chemical Society*, **146**(33), 23034–23043.

del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J. M., & Sheetz, M. P. (2009). Stretching Single Talin Rod Molecules Activates Vinculin Binding. *Science (New York, N.Y.)*, **323**(5914), 638–641.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

Di, X., Gao, X., Peng, L., … Luo, D. (2023). Cellular mechanotransduction in health and diseases: from molecular mechanism to therapeutic targets. *Signal Transduction and Targeted Therapy*, **8**(1), 1–32.

Du, H., Bartleson, J. M., Butenko, S., … Butte, M. J. (2023). Tuning immunity through tissue mechanotransduction. *Nature Reviews Immunology*, **23**(3), 174–188.

Elosegui-Artola, A., Andreu, I., Beedle, A. E. M., … Roca-Cusachs, P. (2017). Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores. *Cell*, **171**(6), 1397-1410.e14.

Elosegui-Artola, A., Trepat, X., & Roca-Cusachs, P. (2018). Control of Mechanotransduction by Molecular Clutch Dynamics. *Trends in Cell Biology*, **28**(5), 356–367.

Estrach, S., Vivier, C.-M., & Féral, C. C. (2024). ECM and epithelial stem cells: the scaffold of destiny. *Frontiers in Cell and Developmental Biology*, **12**. doi:10.3389/fcell.2024.1359585

Evans, E., Leung, A., Heinrich, V., & Zhu, C. (2004). Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. *Proceedings of the National Academy of Sciences*, **101**(31), 11281–11286.

Fischer, L. S., Rangarajan, S., Sadhanasatish, T., & Grashoff, C. (2021). Molecular Force Measurement with Tension Sensors. *Annual Review of Biophysics*, **50**(1), 595–616.

Gardel, M. L., Schneider, I. C., Aratyn-Schaus, Y., & Waterman, C. M. (2010). Mechanical Integration of Actin and Adhesion Dynamics in Cell Migration. *Annual Review of Cell and Developmental Biology*, **26**, 315–333.

Gjorevski, N., S. Piotrowski, A., Varner, V. D., & Nelson, C. M. (2015). Dynamic tensile forces drive collective cell migration through three-dimensional extracellular matrices. *Scientific Reports*, **5**(1), 11458.

Göhring, J., Kellner, F., Schrangl, L., … Schütz, G. J. (2021). Temporal analysis of T-cell receptor-imposed forces via quantitative single molecule FRET measurements. *Nature Communications*, **12**, 2502.

Grashoff, C., Hoffman, B. D., Brenner, M. D., … Schwartz, M. A. (2010). Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature*, **466**(7303), 263–266.

Guo, B., & Guilford, W. H. (2006). Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. *Proceedings of the National Academy of Sciences*, **103**(26), 9844–9849.

Handorf, A. M., Zhou, Y., Halanski, M. A., & Li, W.-J. (2015). Tissue Stiffness Dictates Development, Homeostasis, and Disease Progression. *Organogenesis*, **11**(1), 1–15.

Hu, Y., Li, H., Zhang, C., … Liu, Z. (2024). DNA-based ForceChrono probes for deciphering single-molecule force dynamics in living cells. *Cell*, **187**(13), 3445-3459.e15.

Huang, D. L., Bax, N. A., Buckley, C. D., Weis, W. I., & Dunn, A. R. (2017). Vinculin forms a directionally asymmetric catch bond with F-actin. *Science (New York, N.Y.)*, **357**(6352), 703–706.

Humphrey, J. D., Dufresne, E. R., & Schwartz, M. A. (2014). Mechanotransduction and extracellular matrix homeostasis. *Nature Reviews Molecular Cell Biology*, **15**(12), 802–812.

Humphries, J. D., Wang, P., Streuli, C., Geiger, B., Humphries, M. J., & Ballestrem, C. (2007). Vinculin controls focal adhesion formation by direct interactions with talin and actin. *The Journal of Cell Biology*, **179**(5), 1043–1057.

Huse, M. (2017). Mechanical forces in the immune system. *Nature Reviews Immunology*, **17**(11),

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

679–690.

Ivaska, J. (2012). Unanchoring integrins in focal adhesions. *Nature Cell Biology*, **14**(10), 981–983.

Jiang, L., Sun, Z., Chen, X., … Yang, C. (2016). Cells Sensing Mechanical Cues: Stiffness Influences the Lifetime of Cell–Extracellular Matrix Interactions by Affecting the Loading Rate. *ACS Nano*, **10**(1), 207–217.

Jo, M. H., Meneses, P., Yang, O., Carcamo, C. C., Pangeni, S., & Ha, T. (2024). Determination of single-molecule loading rate during mechanotransduction in cell adhesion. *Science*, **383**(6689), 1374–1379.

Kechagia, J. Z., Ivaska, J., & Roca-Cusachs, P. (2019). Integrins as biomechanical sensors of the microenvironment. *Nature Reviews Molecular Cell Biology*, **20**(8), 457–473.

Koivisto, L., Heino, J., Häkkinen, L., & Larjava, H. (2014). Integrins in Wound Healing. *Advances in Wound Care*, **3**(12), 762–783.

Kong, F., García, A. J., Mould, A. P., Humphries, M. J., & Zhu, C. (2009). Demonstration of catch bonds between an integrin and its ligand. *The Journal of Cell Biology*, **185**(7), 1275–1284.

Kwon, H., Kim, J., & Jho, E. (2022). Role of the Hippo pathway and mechanisms for controlling cellular localization of YAP/TAZ. *The FEBS Journal*, **289**(19), 5798–5818.

LaCroix, A. S., Lynch, A. D., Berginski, M. E., & Hoffman, B. D. (2018). Tunable molecular tension sensors reveal extension-based control of vinculin loading. *eLife*, **7**, 1–36.

Le Saux, G., Magenau, A., Gunaratnam, K., … Gaus, K. (2011). Spacing of Integrin Ligands Influences Signal Transduction in Endothelial Cells. *Biophysical Journal*, **101**(4), 764–773.

Li, Z., Lee, H., & Zhu, C. (2016). Molecular mechanisms of mechanotransduction in integrin-mediated cell-matrix adhesion. *Experimental Cell Research*, **349**(1), 85–94.

Liu, B., Chen, W., Evavold, B. D., & Zhu, C. (2014a). Accumulation of Dynamic Catch Bonds

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell*, **157**(2), 357–368.

Liu, Y., Blanchfield, L., Ma, V. P.-Y., … Salaita, K. (2016). DNA-based nanoparticle tension sensors reveal that T-cell receptors transmit defined pN forces to their antigens for enhanced fidelity. *Proceedings of the National Academy of Sciences*, **113**(20), 5610–5615.

Liu, Y., Galior, K., Ma, V. P.-Y., & Salaita, K. (2017). Molecular Tension Probes for Imaging Forces at the Cell Surface. *Accounts of Chemical Research*, **50**(12), 2915–2924.

Liu, Y., Medda, R., Liu, Z., … Salaita, K. (2014b). Nanoparticle Tension Probes Patterned at the Nanoscale: Impact of Integrin Clustering on Force Transmission. *Nano Letters*, **14**(10), 5539–5546.

Lv, H., Li, L., Sun, M., … Li, Y. (2015). Mechanism of regulation of stem cell differentiation by matrix stiffness. *Stem Cell Research & Therapy*, **6**(1), 103.

Ma, V. P.-Y., Hu, Y., Kellner, A. V., … Salaita, K. (2022). The magnitude of LFA-1/ICAM-1 forces fine-tune TCR-triggered T cell activation. *Science Advances*, **8**(8), eabg4485.

Manibog, K., Li, H., Rakshit, S., & Sivasankar, S. (2014). Resolving the molecular mechanism of cadherin catch bond formation. *Nature Communications*, **5**(1), 3941.

Mitchison, T., & Kirschner, M. (1988). Cytoskeletal dynamics and nerve growth. *Neuron*, **1**(9), 761–772.

Molè, M. A., Weberling, A., Fässler, R., Campbell, A., Fishel, S., & Zernicka-Goetz, M. (2021). Integrin β1 coordinates survival and morphogenesis of the embryonic lineage upon implantation and pluripotency transition. *Cell Reports*, **34**(10), 108834.

Moore, S. W., Roca-Cusachs, P., & Sheetz, M. P. (2010). Stretchy Proteins on Stretchy Substrates: The Important Elements of Integrin-Mediated Rigidity Sensing. *Developmental Cell*, **19**(2), 194–206.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

Oria, R., Wiegand, T., Escribano, J., … Roca-Cusachs, P. (2017). Force loading explains spatial sensing of ligands by cells. *Nature*, **552**(7684), 219–224.

Owen, L. M., Bax, N. A., Weis, W. I., & Dunn, A. R. (2022). The C-terminal actin-binding domain of talin forms an asymmetric catch bond with F-actin. *Proceedings of the National Academy of Sciences of the United States of America*, **119**(10), e2109329119.

Pan, J.-X., Xiong, L., Zhao, K., … Xiong, W.-C. (2018). YAP promotes osteogenesis and suppresses adipogenic differentiation by regulating β-catenin signaling. *Bone Research*, **6**(1), 1–12.

Pang, X., He, X., Qiu, Z., … Cui, Y. (2023). Targeting integrin pathways: mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy*, **8**(1), 1–42.

Plotnikov, S. V., Pasapera, A. M., Sabass, B., & Waterman, C. M. (2012). Force Fluctuations within Focal Adhesions Mediate ECM-Rigidity Sensing to Guide Directed Cell Migration. *Cell*, **151**(7), 1513–1527.

Puklin-Faucher, E., & Sheetz, M. P. (2009). The mechanical integrin cycle. *Journal of Cell Science*, **122**(2), 179–186.

Rakshit, S., Zhang, Y., Manibog, K., Shafraz, O., & Sivasankar, S. (2012). Ideal, catch, and slip bonds in cadherin adhesion. *Proceedings of the National Academy of Sciences*, **109**(46), 18815–18820.

Saitakis, M., Dogniaux, S., Goudot, C., … Hivroz, C. (2017, June 8). Different TCR-induced T lymphocyte responses are potentiated by stiffness with variable sensitivity. doi:10.7554/eLife.23190

Schvartzman, M., Palma, M., Sable, J., … Wind, S. J. (2011). Nanolithographic Control of the Spatial Organization of Cellular Adhesion Receptors at the Single-Molecule Level. *Nano*

Letters, **11**(3), 1306–1312.

Shen, B., Delaney, M. K., & Du, X. (2012). Inside-out, outside-in, and inside-outside-in: G protein signaling in integrin-mediated cell adhesion, spreading, and retraction. *Current Opinion in Cell Biology*, **24**(5), 600–606.

Swaminathan, V., & Waterman, C. M. (2016). The molecular clutch model for mechanotransduction evolves. *Nature Cell Biology*, **18**(5), 459–461.

Tu, Y., & Wang, X. (2020). Recent Advances in Cell Adhesive Force Microscopy. *Sensors*, **20**(24), 7128.

Wang, H., Yu, H., Huang, T., Wang, B., & Xiang, L. (2023a). Hippo-YAP/TAZ signaling in osteogenesis and macrophage polarization: Therapeutic implications in bone defect repair. *Genes & Diseases*, **10**(6), 2528–2539.

Wang, L., Zheng, F., Song, R., … Li, L. (2022a). Integrins in the Regulation of Mesenchymal Stem Cell Differentiation by Mechanical Signals. *Stem Cell Reviews and Reports*, **18**(1), 126–141.

Wang, M. S., Hu, Y., Sanchez, E. E., … Huse, M. (2022b). Mechanically active integrins target lytic secretion at the immune synapse to facilitate cellular cytotoxicity. *Nature Communications*, **13**(1), 3222.

Wang, W., Chen, W., Wu, C., … Liu, Z. (2023b). Hydrogel-based molecular tension fluorescence microscopy for investigating receptor-mediated rigidity sensing. *Nature Methods*, **20**(11), 1780–1789.

Wang, X., & Ha, T. (2013). Defining Single Molecular Forces Required to Activate Integrin and Notch Signaling. *Science*, **340**(6135), 991–994.

Wang, X., Sun, J., Xu, Q., … Ha, T. (2015). Integrin Molecular Tension within Motile Focal

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press

Adhesions. *Biophysical Journal*, **109**(11), 2259–2267.

Wang, Y., & Wang, X. (2016). Integrins outside focal adhesions transmit tensions during stable cell adhesion. *Scientific Reports*, **6**(1), 36959.

Wang, Y., Yao, M., Baker, K. B., … Yan, J. (2021). Force-Dependent Interactions between Talin and Full-Length Vinculin. *Journal of the American Chemical Society*, **143**(36), 14726–14737.

Westhoff, M. A., Serrels, B., Fincham, V. J., Frame, M. C., & Carragher, N. O. (2004). Src-Mediated Phosphorylation of Focal Adhesion Kinase Couples Actin and Adhesion Dynamics to Survival Signaling. *Molecular and Cellular Biology*, **24**(18), 8113–8133.

Yang, S., & Plotnikov, S. V. (2021). Mechanosensitive Regulation of Fibrosis. *Cells*, **10**(5), 994.

Yao, M., Goult, B. T., Chen, H., Cong, P., Sheetz, M. P., & Yan, J. (2014). Mechanical activation of vinculin binding to talin locks talin in an unfolded conformation. *Scientific Reports*, **4**(1), 4610.

Yasunaga, A., Murad, Y., & Li, I. T. S. (2019). Quantifying molecular tension—classifications, interpretations and limitations of force sensors. *Physical Biology*, **17**(1), 011001.

Yi, B., Xu, Q., & Liu, W. (2021). An overview of substrate stiffness guided cellular response and its applications in tissue regeneration. *Bioactive Materials*, **15**, 82–102.

Yuan, D. J., Shi, L., & Kam, L. C. (2021). Biphasic response of T cell activation to substrate stiffness. *Biomaterials*, **273**, 120797.

Zhang, X., Kim, T.-H., Thauland, T. J., … Li, S. (2020). Unraveling the mechanobiology of immune cells. *Current Opinion in Biotechnology*, **66**, 236–245.

Zhang, Y., Ge, C., Zhu, C., & Salaita, K. (2014). DNA-based digital tension probes reveal integrin forces during early cell adhesion. *Nature Communications*, **5**(1), 5167.

https://doi.org/10.1017/qrd.2024.28 Published online by Cambridge University Press