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VIEWPOINT

Mechanotransduction by Membrane Proteins

# Extracellular mechanotransduction

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**We highlight the force-sensing function of extracellular matrix and present a complementary mechanotransduction paradigm.**

## Introduction

Force-sensitive proteins are the mechanistic basis for mechanotransduction, the process by which cells convert mechanical stimuli into biochemical signals. In this Viewpoint, we present evidence that force-sensitive proteins in the extracellular matrix orchestrate a complementary form of mechanotransduction in which matrix is given center focus. Termed "extracellular mechanotransduction," this concept represents a distinct yet integrated addition to current paradigms in tissue homeostasis regulation—cellular mechanotransduction and bidirectional cell–matrix communication (dynamic reciprocity). Extracellular mechanotransduction thus expands on previous perspectives in which matrix biophysically senses, integrates, and encodes mechanical homeostatic information, by describing matrix as a tissue-level interface that integrates cellular and environmental forces.

## Cellular mechanotransduction

In the current paradigm, resident cells maintain tissue homeostasis by sensing and altering the mechanical state of the extracellular matrix through specialized cell–matrix connections (e.g., integrins; Humphrey et al., 2014). These interactions form crucial links in the mechanical chain that connects the inside of the cell to the outside environment. External tissue forces and internal cellular forces thus cross through common membrane associated structures. These structures enable cells to convert mechanical forces into biochemical signals, information subsequently used to deduce and evaluate the mechanical state of the cell and the local microenvironment.

For example, cellular forces generated by the actin cytoskeleton pull against resisting forces of matrix fibers, causing conformational changes in integrins, associated linker proteins (e.g., talin), and other mechanically linked structures (e.g., the nucleus). Force-sensing domains within these structures function as mechanical switches, triggering biochemical events that initiate intracellular signaling (e.g., MAPK, RHO-ROCK; Wang et al., 2009; Humphrey et al., 2014). These signaling pathways activate genetic programs, enabling cells to mount evolved responses to mechanical stimuli, thereby establishing cell-regulated feedback loops that maintain tissue homeostasis.

In this classical view, mechanotransduction occurs within the cell and is dependent on force-sensitive cellular proteins. Many of these proteins are cytoskeletal related, acting as interfaces between cells and the local microenvironment (i.e., matrix), although other non-adhesion force sensors also exist (e.g., Piezo1, LIM). Membrane proteins and their interactions with matrix are thus of critical importance to mechanotransduction (Martino et al., 2018).

## Bidirectional cell–matrix communication

Implicit in the cell-regulated feedback loops that maintain tissue homeostasis is a two-way exchange of information between cells and matrix. Indeed, it is well accepted that matrix contains important mechanical and biochemical cues that modulate resident cell behavior—proliferation, differentiation, migration, and survival (Roskelley and Bissell, 1995; Engler et al., 2006). Likewise, cells can manipulate the information encoded within matrix, thereby modulating neighboring cells indirectly, by secreting growth factors, enzymes, cytokines, and other matrix components. Extracellular matrix is thus not an inert scaffold, but rather a dynamic repository—a local hard drive for cells to "read" and "write" information. Regulation of complex tissue-level processes, including mechanical homeostasis, is thus maintained through this two-way exchange of information termed bidirectional cell–matrix communication (dynamic reciprocity). In this paradigm, cellular mechanotransduction represents one of several information processing modalities cells utilize to read extracellular signals.

For example, matrix stiffness is perceived by cells through integrin-mediated connections, connections dependent on specific biochemical motifs embedded within matrix (e.g., RGD

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domains on fibronectin; Kapp et al., 2017). Activation of cellular mechanotransduction pathways and subsequent changes in cellular behavior thus depends on extracellular cues interpreted through cell–matrix connections. Likewise, changes to matrix composition, fiber orientation, pre-stress, and stiffness initiated by cells similarly depends on cell–matrix connections. These manipulations enable cells to alter the mechanical state of matrix, thereby changing the mechanical cues neighboring cells perceive. The matrix thus forms a type of shared memory populations of cells can organize around and communicate through to establish and maintain tissue homeostasis.

### Extracellular mechanotransduction

Although bidirectional cell–matrix communication includes key roles for both cells and matrix in tissue homeostasis regulation, the central theme of cells as the ultimate sensors and processors of mechanical force remains. Extracellular mechanotransduction moves past this limitation, embracing the expanded viewpoint that matrix can sense and integrate mechanical stimuli *independent of direct cellular action*. This concept is distinct from the "mechanical integration" matrix stiffness and fiber orientation contribute to cellular mechanotransduction. Instead, we expand on the idea that matrix can sense, integrate, and encode mechanical information into extracellular cues using distinct biophysical mechanisms.

The idea that matrix can autonomously integrate and encode information separately from cells is not without precedent. For example, traumatic injuries to blood vessels exposes collagen fibers that activate the intrinsic coagulation cascade. This extracellular process results in remodeled matrix (i.e., a clot) that initially occurs largely independent of the accompanying altered cell behavior. Although coagulation may seem a "special case" distinct from mechanotransduction, analogous mechanisms of autonomous mechanical regulation exist. Indeed, previous perspectives have called attention to these mechanisms, highlighting key examples in which the chemical display of matrix responds to cell generated forces (Vogel, 2018). However, the ability of these mechanisms to integrate internal cellular forces with external environmental forces has not been given due attention in our opinion. Extracellular mechanotransduction thus bridges this gap by centering matrix to integrate cellular and environmental forces (Fig. 1).

A key challenge in rethinking mechanotransduction to include matrix is the current focus on cells as actors and matrix as a regulated target with specific properties (e.g., stiffness; Ma et al., 2013). Extracellular mechanotransduction is founded in the nanoscale understanding that many matrix proteins contain force-sensitive domains, which alter their molecular properties under force in ways analogous to force-sensitive membrane proteins. This novel paradigm thus expands on previous perspectives by adding specialized "integration" functions to the "memory" functions already attributed to matrix, thereby integrating memory with logic in describing how matrix responds to both cellular and environmental forces.

### Force-sensitive matrix proteins

Numerous matrix molecules exhibit force-sensitive properties. Here, we present three examples that highlight important information integrating mechanisms. As a first example, collagen fibers stretched under tension resist proteolytic degradation by matrix remodeling enzymes (e.g., matrix metalloproteinase; Saini et al., 2020). Stress-aligned collagen fibers thus gain a survival advantage during matrix turnover, establishing a quasi-Darwinian paradigm for tissue remodeling based on mechanically biased enzymes kinetics (Fig. 2 A). Indeed, fibrin and Von Willebrand factor further support this concept (Zhang et al., 2009; Li et al., 2017). Fibrin, the fibrous component in blood clots, exhibits decreased lysis under tension analogous to collagen in mature tissues. Conversely, Von Willebrand factor, a large multimeric protein crucial to platelet adhesion, is broken down by mechanically biased proteolysis modulated by blood flow force induced unfolding of buried ADAMTS13 cleavage sites in the A2 domains. Indeed, mechanically biased proteolysis is also a well-established mechanism for Notch receptor activation in cellular mechanotransduction (Lovendahl et al., 2018). While Notch receptor activation is switch-like, a continuous spectrum of proteolytic susceptibility in collagen fibers appears important to matrix remodeling. Together, these examples highlight two key points: (1) matrix proteins share force-sensing mechanisms analogous to membrane proteins; and (2) matrix can use these mechanisms to directly convert and encode mechanical stimuli into extracellular cues important to tissue homeostasis regulation.

As a second example, TGF-β (TGFB) demonstrates how matrix can communicate extracellularly integrated mechanical information to cells without direct mechanical connections. Initially sequestered in matrix in an inactive latent form, TGFB is released by either enzymatic or mechanical activation (Fig. 2 B); TGFB can then activate cell surface receptors to promote matrix remodeling (e.g., increased collagen expression via SMAD signaling; Hinz, 2015). To this end, it is an emerging concept that fibrillin-1, a core extracellular regulator of TGFB, functions as a force-sensitive signaling hub regulating TGFB bioavailability via mechanically biased protein–protein interactions (Sengle and Sakai, 2015; Haller et al., 2020). This is particularly interesting, as mutations in fibrillin-1 cause Marfan syndrome, a connective tissue disorder associated with abnormal TGFB signaling and matrix remodeling. Fibrillin-1 thus offers a prototypical example for how dysfunctional extracellular mechanotransduction may cause human disease. Indeed, force-sensitive signaling hubs are a well-established concept in cellular mechanotransduction, as highlighted by talin-integrin signaling activation (Goult et al., 2018).

Finally, as a third example, fibronectin stretched under tension exposes cryptic integrin-binding domains that have pleiotropic effects on local cells (Kubow et al., 2015). This example, the first to show extracellular protein unfolding by cell generated forces, demonstrates that force-induced conformational changes in matrix proteins can encode new signals into the extracellular space that modulate resident cell behavior though established cell–matrix connections (Fig. 2 C; Baneyx et al., 2002). This concept implies a more prominent role for matrix in pre-processing mechanical stimuli by controlling qualitatively and quantitatively the types of cell–matrix connections able to form. Indeed, while cells control the types of integrin receptors and matrix components expressed in tissue, extracellular forces

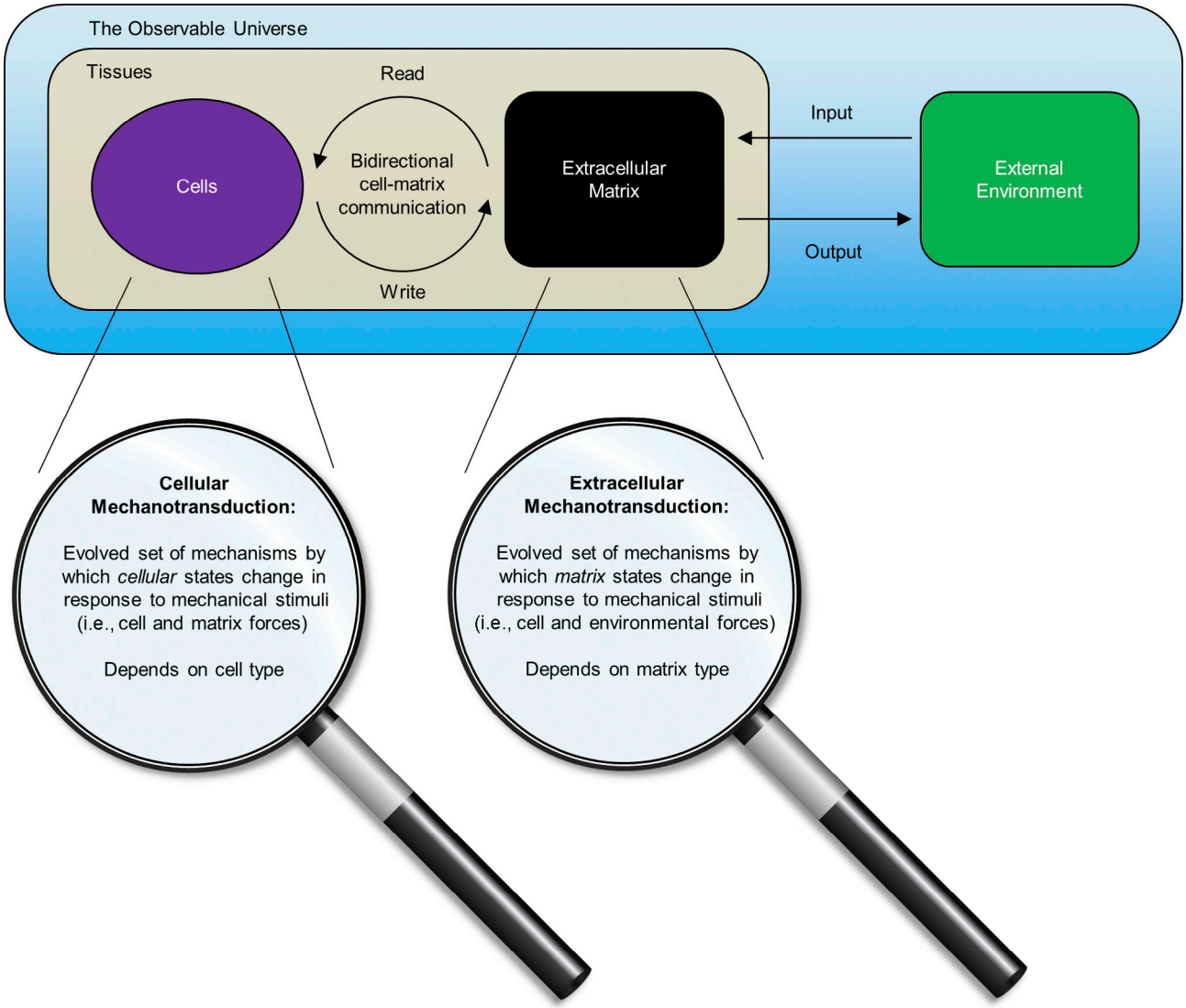


Figure 1. **High-level illustration of cellular and extracellular mechanotransduction placed within current paradigms.** Matrix is centered to integrate cellular and environmental forces.

may control the combinations of integrin–matrix interactions permitted to form under specific mechanical contexts.

To this end, fibronectin and TGFB are particularly interesting because they are potentially responsive to both extrinsic tissue forces and intrinsic cellular forces. It is therefore intriguing to consider that cells may encode force-sensitivity into matrix by pre-tensioning specific matrix components to trigger extracellular signaling networks at calibrated force thresholds. Consistent with this idea, TGFB activation is increased by residual matrix tension (Hinz, 2015). Extracellular matrix thus appears to integrate mechanical information from cells and the environment to regulate TGFB bioavailability. This concept offers a unique advantage over traditional cellular mechanotransduction, as mechanical information could be exchanged between matrix and cells biochemically without direct mechanical connections. This generalizes the types of cell–matrix connections important to mechanical homeostasis regulation past direct connections like integrins. Indeed, many membrane receptors crucial to mechanical homeostasis may not be load bearing, but instead operate through biochemical ligands regulated by matrix tension.

## Why extracellular mechanotransduction?

Over the past 30 yr, systems biology has greatly expanded our understanding of cellular information networks using genomic, proteomic, and metabolomic approaches. Mechanical information networks have likewise been described, but largely from this cellular perspective, with a focus on membrane proteins as force sensors (i.e., cellular mechanotransduction). While recent articles call attention to extracellular mechano-dynamics (Hynes, 2009; Hoffmann et al., 2019), some going as far to define autonomous mechanosensitive roles for matrix in response to cellular forces (Vogel, 2018), they do not go far enough in our opinion to bridge the deep paradigmatic chasm that separates cells from matrix in external force sensation. Extracellular mechanotransduction offers its primary advance by centering matrix at the interface

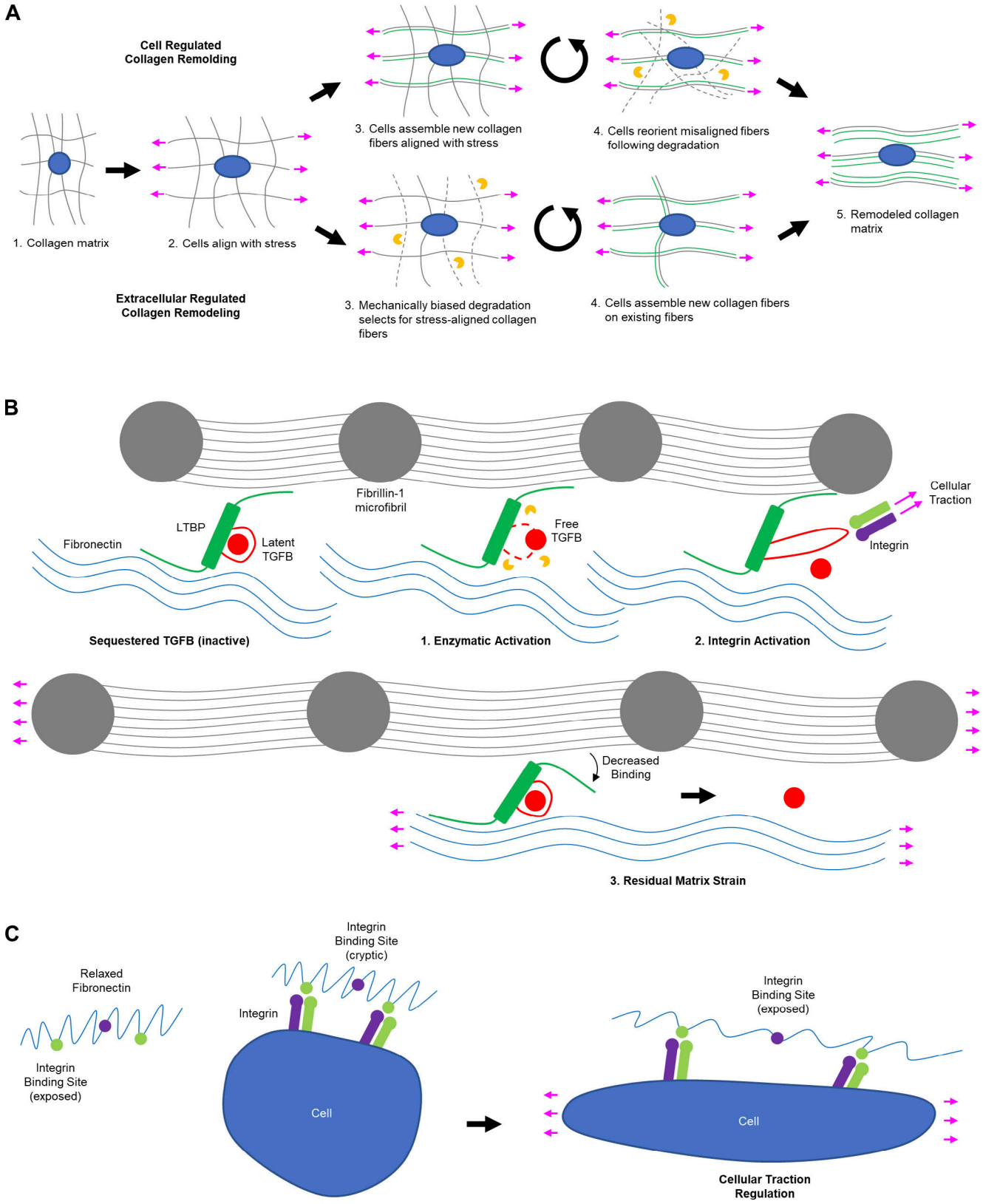


Figure 2. **Extracellular mechanotransduction mechanisms. (A)** Collagen fiber remodeling based on a cell regulated paradigm (top) and an extracellular regulated paradigm (bottom). Note: These mechanisms are not mutually exclusive. **(B)** Latent TGFB, bound by latent TGFB binding protein (LTBP), is initially sequestered in matrix by fibrillin-1 microfibrils and bound by other matrix components (e.g., fibronectin). Free TGFB capable of interacting with cell surface receptors can be activated following: (1) enzymatic cleavage; (2) integrin-mediated cellular traction; and (3) residual matrix strain. Note: The proposed mechanism for residual matrix strain is decreased protein–protein interactions between fibrillin-1 microfibrils and the latent TGFB complex. **(C)** Fibronectin is initially secreted with both exposed and cryptic integrin binding sites. Cellular integrin-mediated forces unfold fibronectin such that cryptic integrin binding sites become exposed.

between cells and the environment. This captures the distinct yet integrated perspective of matrix as both the target being regulated by cells and the sensor signaling its own regulation from cells, while proposing an explicit role for matrix in integrating cellular and environmental (i.e., extramatrix) forces.

Specifically, extracellular mechanotransduction proposes that bidirectional cell–matrix communication links intracellular and extracellular force-sensitive signaling networks. These extracellular networks relieve cells from having to sense and integrate all mechanical information internally. Distributed force sensation thus takes a new meaning, expanding from its current focal distribution amongst cells to include a more continuous distribution across matrix. Indeed, the evolution of extracellular matrix as a shared appendage marks a distinct opportunity for mechanotransduction to have expanded from single-cell based mechanics. While bidirectional cell–matrix communication presently exploits the role of matrix to organize multicellular behavior as a common memory pool, the specific mechanisms through which matrix can generate and encode homeostatic information as an autonomous integrator is largely ignored, particularly in response to mechanical stimuli from the external environment. Extracellular mechanotransduction thus attempts to fill this gap by removing the cell-centric bias commonly ascribed to mechanotransduction.

### Conclusions

The co-existence of analogous force-sensing mechanisms in matrix provides a strong rationale for extracellular mechanotransduction. Elucidating novel force-sensitive signaling networks in matrix thus represents a distinct direction form cellular mechanotransduction to pursue, albeit one with unique challenges. Because these networks presumably interface with cells through specific membrane receptors, cells may be unaffected by crucial intermediary signals for which receptors do not exist or are not expressed. While extracellular encapsulation offers a biological advantage, as different tissues could implement unique networks that communicate with cells through common receptors, it does make studying such networks from a cellular perspective limited. Even experiments aimed at cell–matrix communication could miss the hidden mechanisms in matrix responsible for generating these signals. Extracellular signals that directly influence cell behavior may thus represent only the tip of the iceberg. Future mechanotransduction studies should thus be aimed at matrix as an autonomous force sensor and integrator if all the mechanisms for how tissues, and not just cells, respond to mechanical forces are to be understood. However, before we embark on these experiments, we first need a theoretical framework that agrees that intracellular and extracellular dynamics mark two sides of the same coin. We therefore propose a new 21st century definition for mechanotransduction that includes the full spectrum of cellular and extracellular mechanisms that convert mechanical stimuli into biochemical signals.

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