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Current Opinion in Cell Biology

The reversibility of cellular mechano-activation

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Abstract

The cellular microenvironment is highly heterogeneous and dynamic. Therefore, cells must be equipped with molecular tools to adapt and respond to constantly fluctuating inputs. One such input is mechanical force, which activates signalling and regulates cell behaviour in the process of mechanotransduction. Whereas the mechanisms activating mechanotransduction are well studied, the reversibility of this process, whereby cells disassemble and reverse force-activated signalling pathways upon cessation of mechanical stimulation is far less understood. In this review we will outline some of the key experimental techniques to investigate the reversibility of mechanical signalling, and key discoveries arising from them.

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Current Opinion in Cell Biology 2023, **84**:102229

This review comes from a themed issue on **Cell Signalling**

Edited by **JoAnn Trejo** and **Giorgio Scita**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2023.102229

Keywords

Mechanotransduction, Mechanical memory, Reversibility.

Introduction

Mechanotransduction, the process by which cells convert a mechanical stimulus into a biochemical signal, underpins a myriad of biological functions at all stages of life, from embryo development, homeostasis, aging and disease [1,2]. Cells detect forces from the external environment through a plethora of mechanisms at the molecular and supramolecular level, spread throughout the entirety of the cell. For example, close to the cell membrane, forces trigger conformational changes in individual proteins such as the opening of ion channels [3] or mechanical unfolding of adhesion proteins [4,5]. Whereas at the nucleus, mechanical forces trigger global rearrangements such as the dilation of the nuclear pore complex and the rearrangement of chromatin [6]. Most studies have focused on elucidating the molecular mechanisms that allow cells to sense an increase in the magnitude of force. However, it is not sufficient for forces to merely activate mechanosensitive signalling pathways. For cells to effectively adapt to the dynamic physiological setting, they must also sense and adapt to a decrease in the magnitude of mechanical stimuli, disassembling these mechanically activated pathways in a controlled and timely manner. However, we know comparatively little about mechanisms underpinning the reversibility of mechanotransduction.

The mechanisms and timescales of mechanotransduction reversibility will determine how long a cell maintains a mechanically active phenotype in the absence of force. This includes cell shape, the localisation of mechanosensitive transcription factors or the activation of genes. If these signals persist for long enough, this endows cells with a form of mechanical memory - maintaining the behaviour dictated by the previous mechanical environment, which can persist for orders of magnitude longer than the timescales of cellular signalling. To investigate the reversibility of mechanotransduction, we need techniques capable reversing the steps of mechanotransduction while simultaneously monitoring cellular response. Here, we will review the current approaches for studying mechanical reversibility and some key scientific discoveries arising from them.

Direct mechanical manipulation tools

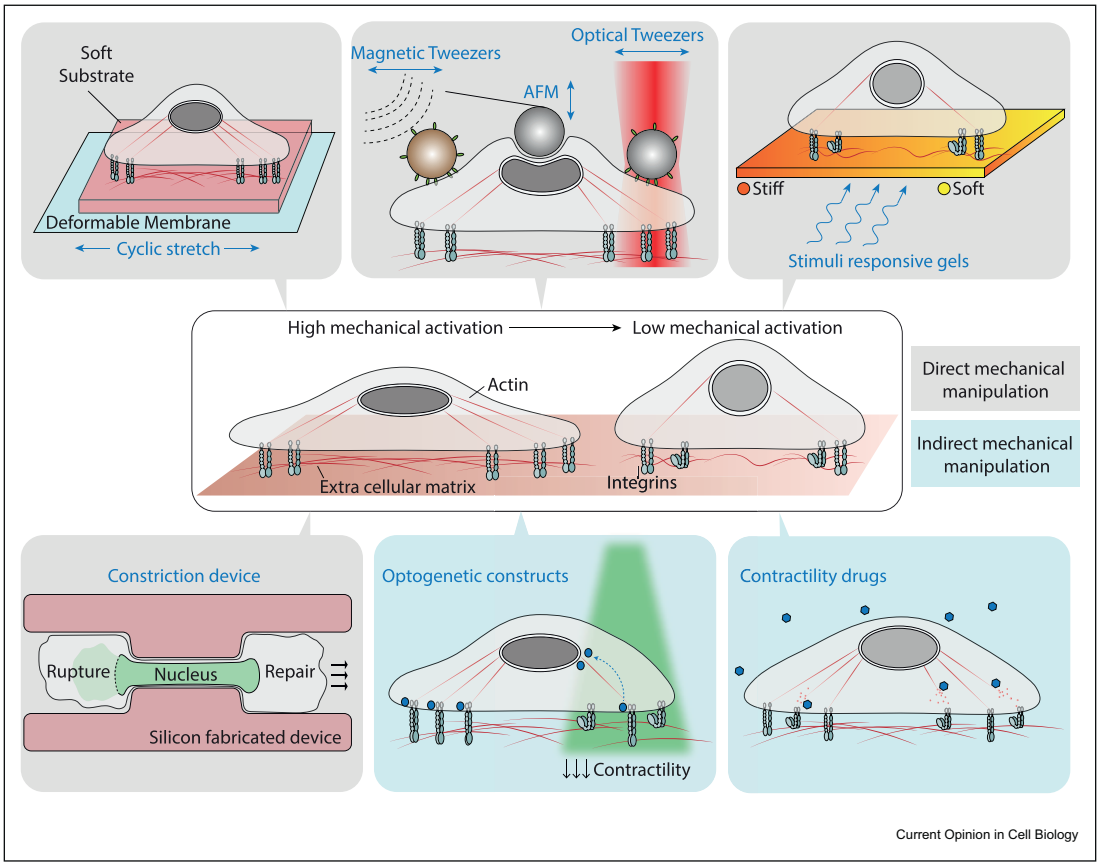
To study the reversibility of mechanotransduction, this necessarily means that cells must first be mechanically activated. This can be achieved using tools that directly apply and remove a mechanical force (Figure 1). For example, the atomic force microscope (AFM) can reversibly apply pushing forces to different regions of the cell. Alternatively optical and magnetic tweezers can reversibly pull or oscillate beads attached to the plasma membrane. Stretch systems can stimulate cells with single or cyclic stretch pulses and then cease the force application, and compression systems can confine and subsequently release the force. These have elicited important mechanisms into how cells respond to both the application and cessation of

mechanical force at different cellular structures from the focal adhesions, the cytoskeletal elements and within the nucleus.

Common markers of mechanotransduction include cell spreading, the maturation of integrin-mediated cell–matrix adhesions (focal adhesions) and of actin stress fibres, and the translocation of mechanosensitive transcription factors (such as YAP and MRTF-A) to the nucleus. These markers are typically absent in cells seeded on soft substrates, and are activated on rigid substrates. However, recent work has shown that cyclic stretching of cells on soft substrates can mimic the effect of a stiff substrate, triggering a mechanically active phenotype [7]. Therefore, upon stopping the stretch, cells must re-adapt to their soft surroundings. Furthermore, this approach allows investigation into how the timescales of activation (duration of stretch) is linked to the timescales of reversibility. Cells on soft nanopillars stretched for 1 h rapidly disassemble the focal adhesions upon the cessation of stretch. However, 4 h stretching resulted in the maintenance of adhesions for 18 h and sustained cell spreading. Furthermore, stopping cyclic stretching after 2 h led to an immediate relocalisation of MRTF-A, whereas 8 h stretching delayed the relocalisation into the cytoplasm [8].

Similarly, the application of force directly to the nucleus using an AFM triggers a significant increase in the nuclear localisation of mechanosensitive molecules. Upon withdrawal of the force, the displaced molecules almost instantaneously exit the nucleus and return to the unperturbed levels. This has been shown for YAP [9] and artificially designed mechano-reporter molecules [10], and reveals a stark contrast in the timescales of nuclear exit compared to the long time-scale stretch experiments. This points to the existence of multiple regulatory mechanisms that distinguish between the type and

Figure 1



An overview of experimental approaches to investigate the reversibility of cellular mechanoactivation. The techniques available to study the reversibility of cellular mechanoactivation can be broadly categorised into two approaches, firstly tools that allow direct application and removal of mechanical forces (direct mechanical manipulation – grey boxes). These include cyclic stretching, force probe approaches (atomic force microscope, optical and magnetic tweezers), stimuli responsive gels, and cell constriction devices. Alternatively, there are tools that target the force-generating components within the cell (indirect mechanical manipulation – blue boxes). This includes optogenetic constructs or pharmacological treatments that trigger a change in function of proteins that control force-mediated processes such as contractility.

duration of force application. Mechanistically, these differences may be partially explained by the remodelling of the actin cytoskeleton that occurs upon cyclic loading. Reconstituted cross-linked actin networks subjected to shear stress exhibit mechanical hysteresis where the modification to the elastic properties is sustained after the removal of stress application [11–13]. This suggests that an irreversible change in cytoskeleton nonlinear elasticity may underpin some aspects of cellular mechano-memory.

Mechanical forces applied at the cell membrane also reach the nucleus, triggering mechanotransduction events in the nucleus itself. However, how nuclear mechanotransduction is retained or dissipated upon cessation of the force is largely unknown. Force application using magnetic twisting cytometry to integrins at the cell surface triggers stretching of the chromatin via the actin cytoskeleton, the LINC complex and LAP2β [14,15]. The application of a short (2–10min) and highly localised 15Pa force pulse at the plasma membrane triggers chromatin to decondense and increases the diffusivity of proteins inside the nucleus. Upon cessation of the force application, this mechanically-activated event persists on the order of minutes, and is mediated by the nuclear pore complex [16]. This is an exciting example of short-term mechanical memory mediated by the nucleus.

Tools to manipulate substrate stiffness

Beyond direct force application, another approach to alter the cell mechanical environment is to modify the rigidity of the cell substrate. This can be achieved by seeding cells on stimuli-responsive hydrogels [17,18], in which their stiffness can be tuned through dynamic rupture and reformation of the cross-linking elements (Figure 1). These systems undergo rapid changes in response to the stimuli, and therefore provide insight into the temporal cell changes upon an abrupt change in the elasticity. pH-responsive gels that switch from a high (∼40 kPa) to low (∼1 kPa) rigidity demonstrated that mouse myoblasts respond to this rapid change in elasticity by reducing spreading area [19]. This is associated with a change in the cell-substrate contact angle and a significant reduction in the order parameter of the actin cytoskeleton [20]. The loss of actin organization upon *in-situ* substrate softening was also observed for fibroblasts, and this occurs in conjunction with a reduction in the nuclear localisation of YAP [21]. Prolonged mechanical activation of fibroblasts leads to myofibroblast conversion, and at short activation timescales this is fully reversible. However, longer activation triggers chromatin condensation via the actin cytoskeleton and the LINC complex, leading to the persistent activation of the myofibroblasts upon *in-situ* gel softening [22]. Thus, mechanical activation can trigger irreversible changes in cell plasticity and behaviour.

Photoresponsive gels have provided invaluable insight into the mechanical memory of mesenchymal stem cells (MSCs). Mechanically priming MSCs on a high elastic modulus gel (∼10 kPa) for days prior to *in-situ* softening (∼2 kPa), reveal an intriguing relationship between priming time and reversibility. Gel softening after 1 day priming, leads to full reversibility with YAP re-localising from the nucleus to the cytoplasm. However, 10 days priming triggers the irreversible activation of YAP [23]. In addition to nuclear YAP retention, mechanical priming leads to elevated microRNA-21 levels which persist upon transfer to soft substrates, and maintains mechanical memory [24]. This gradual mechanical irreversibility can be recapitulated in a mathematical model by introducing a positive reinforcement between mechanically activated signalling and transcription [25]. While most studies have focused on understanding the contribution of only the substrate mechanics, Zhang et al. developed an elegant approach to investigate mechanical memory on static gels based on the interplay between cell–matrix adhesions and cell–cell adhesions. Using gels with a mixture of the integrin binding ligand, RGD, and the N-cadherin ligand, HAVDI, revealed that the memory effects observed with RGD can be reversed by incorporating HAVDI [26]. This is explained by a molecular clutch model whereby the HAVDI ligand disrupts the RGD bound integrins, leading to a reduction in cell generated forces and changes to nuclear morphology. These works demonstrate the complex interplay between timescales, mechanics, ECM- and cell–cell interactions in regulating mechanical memory.

Constriction devices

In migratory cells, microfabricated devices featuring narrow constrictions can be used to impose a deformation on cells as they traverse the constrictions (Figure 1). This offers an easy approach to transiently compress the cell and the nucleus. This allows investigation into the effect of the deformation and the subsequent recovery steps once the cell exits the confinement. This has recently been used to enhance the conversion of fibroblasts to neurons, an effect that persists long after the millisecond compression [27]. Mechanistically, this fleeting nuclear compression is sufficient to trigger a myriad of changes within the nucleus, including disassembly of the nuclear lamina, wrinkling of the nuclear envelope, and changes to histone and DNA methylation levels that all contribute to the irreversible cellular reprogramming.

In extreme cases, mechanical deformation of the nucleus triggers nuclear envelope rupture and the spillage of the nuclear content into the cytoplasm. In this instance, the nuclear envelope is rapidly resealed thanks to the recruitment of the barrier-to-autointegration factor (BAF) protein [28] and the subsequent recruitment of the ESCRT III machinery that localises to the

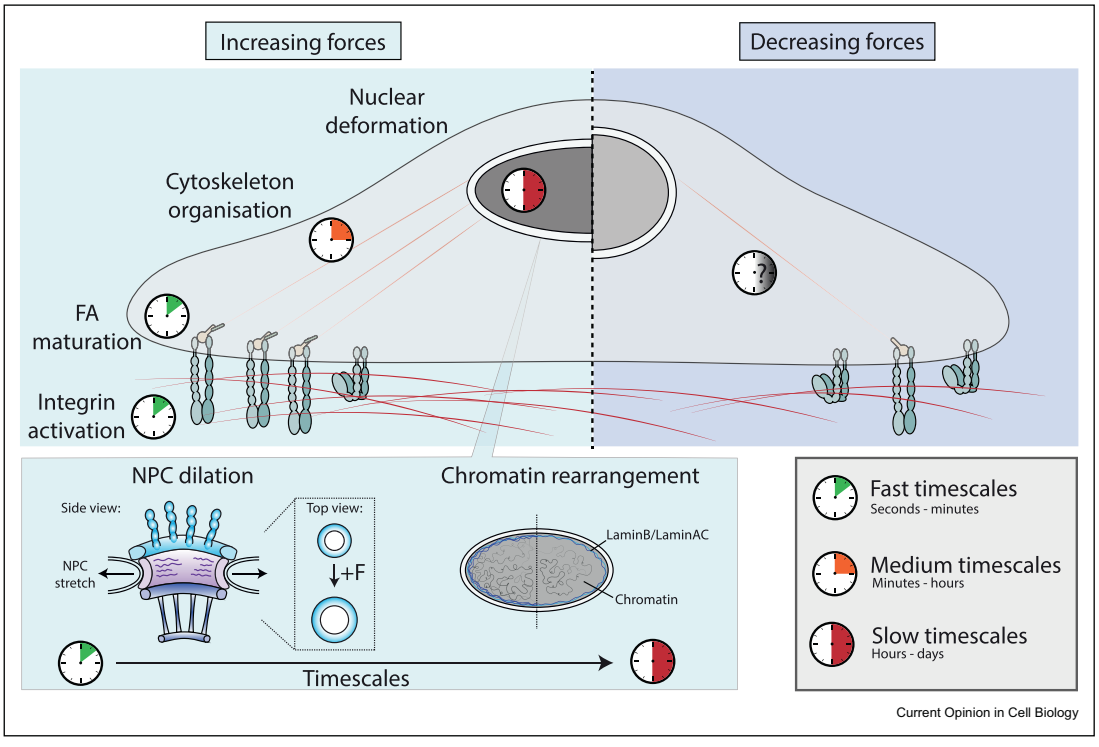
rupture site and reverses the catastrophic mechanically-induced event [29,30]. This nuclear envelope reformation must occur despite considerable cytoskeletal compressive forces. To circumvent this problem, the ESCRT III associated protein BROX causes the ubiquitination of nesprin-2G to reduce the contractile forces of actin close to the rupture site [31].

Tools interfering with the cellular force generating machinery

Mechanotransduction processes are often triggered not by external forces, but by internal cell forces generated by the actomyosin machinery. Therefore, it is possible to mechanically perturb cells by interfering with aspects of the force generating machinery (Figure 1). An easy way to do this is with pharmacological treatments that inhibit different aspects of contractility. For example, the depolymerisation of actin leads to an increase in nuclear deformability [32] and a reduction in nuclear tension, and this can be prevented if the nucleus is physically constrained by the application of pressure, maintaining the morphology and molecular localisation of a mechanically activated nucleus [33]. This approach has also revealed the importance of actin in the maintenance of YAP in sparsely seeded cells [34]. Alternatively, it is possible to investigate reversibility by activating contractile elements and then washing away to reverse the effects. For example, cells seeded on soft gels treated with Mn^{2+} to activate integrin engagement show enhanced spreading, and upon replacement of the media, the cells immediately begin to retract and revert to the previous morphology [35]. While these pharmacological approaches can offer insights into mechanotransduction reversibility, it can be challenging to pinpoint precise molecular mechanisms.

Optogenetic tools are artificially designed proteins that reversibly change their function and/or localisation in response to light illumination. Therefore, if designed to target contractile components, they offer a more controlled and localised approach to switch on or off mechanical activation. The RhoA protein is a key regulator of cell generated forces, controlling the formation of actin stress fibres and actomyosin contractility. RhoA at the plasma membrane increases contractility, and therefore an optogenetic construct that triggers RhoA to localise to the mitochondria causes a reduction in the cell generated forces [36]. This leads to a rapid reduction in cell generated traction forces, the disassembly of focal adhesions, and YAP nuclear exit. All these effects

Figure 2



Timescales of cellular mechanoadaptation. Examples of mechanisms and timescales underpinning cellular adaptation to an increase in the magnitude of mechanical forces. At short timescales is the activation of integrins and the maturation of the focal adhesions (FA) complex. This is followed by a change in the organisation of cytoskeletal elements such as actin. Finally, forces reach the nucleus and trigger changes to the nuclear morphology which is associated with a myriad of signalling changes, including the dilation of the nuclear pore complex (NPC) and changes to chromatin organisation and Lamin A/C levels. By contrast, we are lacking a detailed explanation of the mechanisms and timescales underpinning cellular adaptation to a decrease in the magnitude of forces.

are reversible upon removal of the illumination. Alternatively, OptoMYPT decreases the actomyosin contractile forces by dephosphorylating myosin regulatory light chains on command [37]. There are constantly new optogenetic constructs being developed that target different proteins linked to the cellular mechanomachinery, such as OptoShroom3 which induces cell shape perturbations via apical constriction [38]. Furthermore, optogenetic tools are now being used to investigate nuclear import and subsequent export of mechanical cargos [10,39]. Given the molecular specificity and the dynamic and spatial control of these optogenetic tools, we anticipate that many of them will become powerful approaches to investigating the mechanisms and timescales underpinning the reversibility of mechanotransduction.

Conclusions and future directions

Mechanisms exist to minimise damage upon a sudden and extreme increase in the mechanical load [40,41]. But do mechanisms exist to protect the cell from a decrease in the mechanical load? The work presented in this review highlights the huge variability in mechanical memory depending on the magnitude, directionality, type and timescales (Figure 2) of the force application. This points towards a plethora of different mechanisms that allow cells to adapt and respond to a cessation of forces. However, we are still far from a comprehensive understanding of how cells dissipate mechanical forces and reverse the effects.

Many pathways that allow cells to sense mechanical force are governed by a force-mediated conformational change in the individual force-bearing proteins that leads to altered protein reactivity. For example, talin reversibly unfolds and refolds in the presence and absence of force, respectively [42]. In the unfolded state, vinculin binds, and this locks talin into an unfolded conformation and prohibits folding until vinculin is expelled from the backbone [43,44]. Alternatively, multiple rounds of mechanical unfolding–refolding cycles reveal that individual proteins display folding fatigue [45] or become trapped in non-native configurations [46]. It is tempting to speculate that the folding dynamics of individual proteins may play an important role in the reversibility of focal adhesion mechanoactivation.

CRediT author statement

Amy EM. Beedle: Writing- Original draft preparation. Writing- Reviewing and Editing. Pere Roca-Cusachs: Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

Acknowledgments

AEMB was the recipient of a Sir Henry Wellcome fellowship (210887/Z/18/Z). P.R.-C. acknowledges funding from the Spanish Ministry of Science and Innovation (PID2019-110298 GB-I00), the European Commission (H2020-FETPROACT-01-2016-731957), the Generalitat de Catalunya (2021 SGR 01425), The prize "ICREA Academia" for excellence in research, Fundació la Marató de TV3 (201936-30-31), and "la Caixa" Foundation (Agreement LCF/PR/HR20/52400004). IBEC is a recipient of a Severo Ochoa Award of Excellence from MINCIN.

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