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Abstract

Since the emergence of mechanobiology, mechanical signals have been shown to influence almost every process in biology. Cells transduce mechanical signals into biochemical signaling pathways, adjust their behavior and/or phenotype before transmitting these signals to neighboring cells. Mechanical signals thus appear as information, which can be "written" by cells in the surrounding extracellular matrix, "transmitted" through it and "read" by other cells. This brief review summarizes our current understanding of the mechanisms regulating the tensional state of cells and tissues subjected to mechanical perturbations, before examining existing or potential experimental approaches to study these mechanisms.

1. Introduction

Since the emergence of mechanobiology, mechanical signals have been shown to influence almost every processes in biology. Macroscopic signals such as physical exercise, breathing or heart pumping have thus been suspected to regulate tissue development more than a century ago [1], while the impact of microscopic mechanical signals, such as cell-generated forces or blood flow, on cell adhesion or differentiation are only evidenced since the 1980's [2]. Step by step, and in close correlation with the advent of technologies to measure and manipulate mechanical signals *in vitro* and *in vivo*, scientists have indeed progressively unraveled some of the mysteries surrounding how cells generate, perceive, transmit and regulate mechanical signals [3]. Extensive work and excellent review articles have been dedicated to the mechanisms of generation, perception and transmission of mechanical signals in biology [2,4]. Here this brief review focuses on this dynamic regulation of mechanical signals in cells and tissues submitted to mechanical perturbations.

In the cytoplasm, the highly dynamic cytoskeleton is the key player in generating and regulating mechanical signals, through its scaffolding architecture composed of actin, intermediate filaments and microtubules. Briefly, the perpetual polymerization/depolymerization of those constituents, as well as the myosin II-induced movement of actin filaments, are responsible for the production of active forces. These forces are directly applied either on the nucleus, through the linker of the nucleoskeleton and cytoskeleton (LINC) complex [5], on the membrane and the focal adhesions, through integrin receptors which transmit them across the cell membrane and deform the ECM [3], or to adherens junctions, where cadherin receptors convey these forces to the neighboring cells [6]. Further along the line, these mechanical signals, propagated through direct cell-cell contact or via the deformation of the ECM mesh, follow the opposite direction, transit through focal adhesions or adherens junctions and deform nearly every intracellular organelle thanks to the connected architecture of the cytoskeleton.

As a result, cells amplify, propagate and transduce this back-and-forth mechanical signaling into intracellular, biochemical signaling pathways and adjust their behavior and/or phenotype, a process called mechanotransduction [7]. A bidirectional coupling can then be identified between the external physical cues and the corresponding cellular response. Overall, mechanical signals thus appear as information, which can be “written” by cells in the surrounding ECM, “transmitted” through it and “read” by other cells. And as any system processing information, the stability and reproducibility of this information is crucial to the system’s stability. The mechanotransduction process is thus equipped with numerous positive and negative feedback loops that generate a highly complex and dynamic mechanical regulation of this information (Fig. 1). Here we summarize our current understanding of the mechanisms regulating the tensional state of cells and tissues subjected to mechanical perturbations, before examining existing or potential experimental approaches to study these mechanisms. We finally examine future directions in this blooming field.

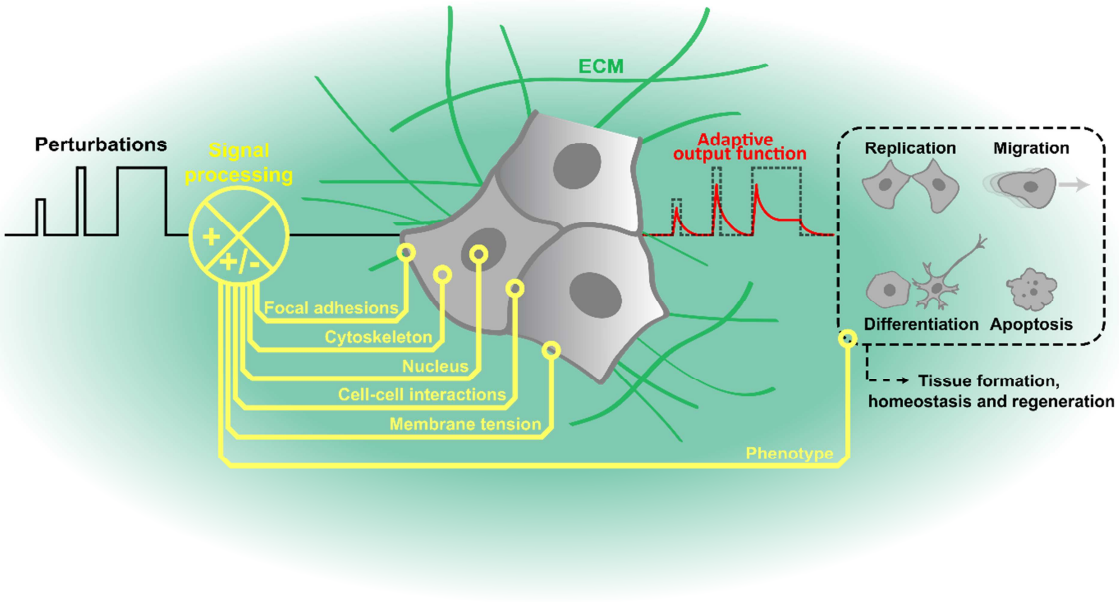

Figure 1: Spatiotemporal regulation of a cell's tensional state through positive and negative feedback loops fed by information coming from various probes, depending on the amplitude, speed and frequency of the perturbations.

2. Regulation of cellular forces in mechanobiology

In order to exert their functions, cells and tissues need to maintain their mechanical integrity by sustaining optimal tensional values and mechanical equilibrium. A collapse of this integrity or abnormal transduction of mechanical information can indeed lead to defective morphogenesis [8,9] or pathophysiological dysfunctions such as fibrosis, atherosclerosis or cancer [10,11]. This state of equilibrium has been linked to the ability of cells and tissues to maintain mechanical tension at a preferred set-point value when faced with external mechanical stimuli, and thus maintain proper physiological functions.

Many definitions have been proposed to address the mentioned biological balance. Following the ideas presented by Claude Bernard back in 1865 regarding internal equilibrium, coined later as homeostasis by Walter Cannon in "The Wisdom of the Body" in 1932, Eastwood *et al.* introduced in 1998 the concept of tensional homeostasis as: "the control mechanism by which fibroblasts establish a tension within their extracellular collagenous matrix and maintain its level against opposing influences of external loading" [12]. In the early 1980's, and in accordance with the homeostatic principles of Cannon, Bornstein and Bissel discussed the concept of dynamic reciprocity, a more refined term that introduced the idea of dynamic

feedback loops between cells and extracellular matrix: “the influence of extracellular matrix on the cell, both during the developmental process and in established tissues, appears to evolve continually” [13,14].

Since then, the ability of cells to maintain a constant level of tension has been challenged by inducing different perturbations in their environment. It is beyond the scope of this review to give a universal definition to the internal tensional balance observed in cells and tissues. The debate this work aims at is how cells adapt their force production to perturbations which occur at different time and space scales, ranging from milliseconds to hours and from the subcellular to the tissue level, and how to experimentally address this phenomenon. Most of the current approaches to perturb and analyze the dynamic regulation of the cell’s behavior are based on either (i) biological perturbations (*e.g.* pharmacology, genetics, inducible promoters) or (ii) physical perturbations (*e.g.* fluid flow, AFM-indentation, geometrical and adhesive constraints). While these techniques helped furthering our understanding of the mechanisms behind the ability of cells to mechanically adapt their behavior to their environment, these biological and physical stimulations are strongly limited by their spatial and temporal resolutions. For example, most drugs can only be applied to the whole cell or tissue at once and takes minutes to induce a noticeable relaxation of cell forces. If one looks for periodical stimulations, the drug needs to be sequentially added and washed, with a temporal resolution no faster than several minutes. As a consequence, most investigations aim at probing more of a steady state of the cell or tissue than dynamical processes, while this dynamic regulation of the cell tensional state has been shown to be key in major cellular processes, such as migration or division. As an example, Théry et al. demonstrated that despite strong heterogeneities in shape and size, normal and transformed MCF10A epithelial cells exhibited a robust tendency relating cell mechanical and motile properties, with a negative correlation between contractile forces and cell speed [15]. On the dynamics of cell division, Trepat and colleagues recently established that the entire cell cycle was regulated by temporal mechanical patterns and showed that tension and mechanical energy could be better predictors of the duration of the G1 phase [16]. Both mentioned results highlight the potential of biomechanical cues in the dynamic regulation of cell processes.

Several recent studies have investigated the dynamic regulation of the cell tensional state. Stamenović and colleagues demonstrated that isolated human endothelial cells exhibited unstable and erratic traction

fields [17]. By applying dynamic mechanical stimulation through the deformation of an elastic substrate to which cells are attached, they also observed that this traction field reoriented transversely to the axis of a periodical stretch, but that tractions remained highly unstable long after their reorientation, suggesting the absence of tensional homeostasis in isolated endothelial cells, as defined by Eastwood *et al.* [12]. Lately, they evidenced that the ability of isolated cells to maintain this tensional homeostasis was dependent upon cell type and that the dynamic fluctuations of the traction field decreased with cell density, suggesting that tensional homeostasis may require multicellularity for specific cell types [18,19]. Several authors probed the dynamic regulation of the tensional state of multicellular assemblies. The total elastic strain energy generated by epithelial cell colonies was thus shown to increase linearly with the size of the colony [20,21], similarly to the active tension of an expanding epithelial monolayer [22]. During wound healing, Murrell *et al.* measured the linear decrease of elastic strain energy with the perimeter of the wound [23]. Overall, these results demonstrate the conservation of the average energy density at a constant level.

In parallel, Fletcher *et al.* used a feedback-controlled atomic force microscope to evidence that single NIH 3T3 fibroblasts do not exhibit tensional homeostasis in the strictest sense, *i.e.* they do not maintain a single, inherent tensional state [12], but instead generated different levels of forces in response to mechanical displacement in a strain-rate-dependent manner [24]. Fletcher proposed the concept of tensional buffering, *i.e.* rather than maintain a constant mechanical state, cells may transition between different tensional states depending on how they are perturbed, permitting distinct responses to slow deformations during morphogenesis and rapid deformations associated with injury.

On the other hand, Weng *et al.* demonstrated the stability, over time and upon mechanical perturbation, of cytoskeletal tension and focal adhesion size in single rat embryo fibroblasts, human mesenchymal stem cells and human skin fibroblasts [25]. They showed this single-cell mechanical homeostasis to be an emergent phenomenon collectively driven by the graduated dynamics of cytoskeletal tension and focal adhesions, the latter a more sensitive gating mechanism for the maintenance or exit of single-cell homeostasis.

Overall, these studies evidenced the existence of a system-dependent regulatory process actively promoting the equilibrium of tension and the conservation of a mechanical function. This mechanism relies

on feedback loops fed by information coming from various probes with intrinsic spatial and temporal scales (Fig. 1). The system will try to adapt to an external perturbation, depending on its amplitude, speed and frequency, which is key to the preservation of healthy and functional cells and tissues. A large body of work thus remains to be done exploring the spatiotemporal range of perturbation within which biological systems can resist and/or adapt to the perturbation. To this end, novel tools will be necessary to mechanically stimulate biological systems, with an independent spatial and temporal control.

3. How to study the regulation of cell tensional state in mechanobiology?

Three key elements are required for designing the dream experiment to study the regulation of cell tensional state in mechanobiology: (i) a time- and space-resolved quantification of the mechanical and biological responses of the system; (ii) a method for dynamically perturbing the system of interest (cell or tissue), with spatial and temporal control; and (iii) a high enough throughput screening in order to improve the experiment's statistics and highlight small intercellular variabilities.

Although no experiment can currently fulfill all these requirements, several recent studies presented promising technologies. The spatiotemporal quantification of the mechanical response of cells or tissues is indeed already well established, mostly by measuring the deformation of a soft environment by cell forces. Since the late 1990's, several approaches have been developed and advanced for quantifying cell forces on 2D substrates [26–28] or in 3D matrices [29–31], and few techniques emerged to assess intercellular forces [32–38]. However, most of these approaches require manual steps that limit the throughput to a 10-50 cells or tissues in a typical experiment, with the exception of a very recent work by Di Carlo and colleagues who used micropatterns of fluorescently labeled-ECM proteins, integrated in a multi-well plate format, to obtain highly parallelized time-course studies of single cell forces [39]. At the tissue scale, Asmani *et al.* extended the work developed by Legant *et al.* [31] by creating microtissue arrays in a multi-well plate format, thus enabling multi-parameter, phenotypic analysis of drug candidates with a throughput higher than conventional assays [40].

Several solutions are thus advanced enough to allow for high throughput quantification of the tensional state of cells and tissues, whereas only very few techniques exist for perturbing them with a spatiotemporal

control and a high enough throughput. As presented before, stretchable substrates are of major interest for studying the impact of dynamic perturbations, but do not allow for a spatial control of local perturbations [17,25]. Of note, using soft lithography, Michielin *et al.* recently engineered a microfluidic-based cell-stretching device which was used to analyze the impact of local, dynamic mechanical perturbations on cytoskeletal remodeling and membrane permeability of healthy and diseased human skeletal muscle cells [41]. By combining this approach with microcontact printing, Xue and colleagues were later able to demonstrate the impact of ectopic mechanical stimulation on the embryonic patterning of neuroectoderm [42]. Although these locally stretchable substrates allow for dynamically perturbing parts of a tissue, they do not enable cell-scale perturbations and are not compatible with a spatial and temporal quantification of the tissue's tensional state yet.

Alternatively, synthetic materials that are capable of responding to external or internal stimuli represent a very exciting emergent area of scientific interest [43]. While the spatial and temporal resolution of some external stimuli such as heat, electrical or magnetic fields may be insufficient for mechanobiology studies, light sensitive materials could be powerful tools for analyzing the dynamic response of cells to well-defined perturbations. The Garcia's team thus developed a general strategy to temporally and spatially control the presentation of cell-adhesive peptides via light exposure [44], while Shou *et al.* engineered surfaces where topography changes upon light exposure [45]. Although not reversible yet, this kind of approach seems promising for studying resilience in mechanobiology, owing to the high degree of spatiotemporal control afforded by light.

Based on the similar idea that light is an excellent candidate for perturbing biological systems, optogenetics has recently emerged as a very potent approach for spatiotemporally controlling the tensional state of cells. By combining optogenetic control of RhoA, live-cell imaging and traction force microscopy, Oakes *et al.* have demonstrated that local activation of RhoA stimulated local recruitment of actin and myosin, and increased cell forces. This perturbation in the cell's tensional state rapidly propagates across the cell via stress fibers and drives increased actin flow [46]. Similarly, Trepat and colleagues engineered an elegant optogenetic system for activating RhoA either at the plasma membrane or at the mitochondrial membrane [47]. They showed that the optogenetic activation of RhoA at the plasma membrane caused a

rapid, local and reversible increase in cellular traction, intercellular tension and tissue compaction. By contrast, the translocation of RhoA at the mitochondria membrane induced opposite results. Using this tool, they were able to quantify the almost immediate impact of contractility variation on the nuclear localization of the transcriptional regulator YAP. In addition, this optogenetic approach allows to locally modify cellular tension, thus opening the way to modify the forces generated by a cell or a group of cells while quantifying the effect of these cell-generated forces on neighboring cells. Trepat *et al.* thus demonstrated that the forces exerted on cells by other cells induced tissue deformation [47] and regulated the duration of mitosis [16]. In this case, the light-induced mechanical response of a cell is also the mechanical stimulus that perturb the neighboring cells. Herein, using a cell as a biological actuator thanks to optogenetics allows for mimicking physiological mechanical stimuli.

Altogether, these recent results combining spatiotemporal control and measurement of cellular contractility pave the way to the study of subcellular, cellular and multicellular processes with micrometer resolution and seconds timescale, opening an exciting avenue for the study of the regulation of cell tensional state in mechanobiology.

4. Future directions.

While the mechanobiological studies described in this review have provided important information on the spatiotemporal regulation of cell tensional state, several key questions remain unanswered. For instance, the intra- and intercellular stability of the cell contractility remain under debate. As stated above, a precise determination of the spatiotemporal range of perturbation within which this regulation is achieved will be crucial to clarify this question. Similarly, while organs maintain optimal tensional values and mechanical equilibrium [48], it is still unclear how this maintenance capacity emerges from the assembly of single cells exhibiting very dynamic, at times erratic, mechanical behavior. This multicellular stability may thus require a higher level of organization than that of a single cell, suggesting that future studies should consider cross-signaling, positive and negative feedback mechanisms within the cell, between neighboring cells as well as between cells and the ECM.

The ECM appears indeed to be a largely neglected player in this spatiotemporal regulation of cell contractility, as most studies are done on plastic or synthetic gels. The ECM is key to architecture in the tissue across multiple length scales, ranging from the organization of ligands and growth factors at the nanoscale to the cell shape and connectivity at the microscale and larger [49]. As the composition and architecture of the ECM can be modified or stabilized by the cells, thus perpetuating either a physio- or a pathological behavior, it is highly probable that the ECM is key to the dynamic regulation of cell tensional state. As such, the engineering of dynamically and locally tunable fibrillary synthetic and natural matrices will be required to bring to light the hidden feedback mechanisms regulating cell/ECM tensional state [50].

Overall, there is thus a strong need for experimental techniques to spatiotemporally control force-responsive molecules in cells. The ability to reversibly activate individual focal adhesions or molecular motors, for instance, similarly to a geneticist modifying individual genes, would be crucial to defining the relevant timescales and amplitudes of perturbations in mechanobiology, ensure robust parameter estimation and identify mechanical dose response curves. In a similar way to genomics or proteomics, such comprehensive quantification of the spatial and temporal regulation of cell mechanics would allow the emergence of mechanomics, *i.e.* a spatiotemporal mapping of how forces are generated, transmitted, transduced and regulated in cells and tissues, dysregulation of which has been associated with pathophysiological conditions in developmental disorders and diseases [51,52].

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