# Mechanobiology: ubiquitous and useful

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It is my privilege to provide a short introduction for the second annual Forces on and within Cells special issue of *Molecular Biology of the Cell*. For the less familiar, the term mechanobiology is often used to describe the aspects of cell and developmental biology that are intrinsically physical in nature. The relevance of physical forces to cell biology is obvious in the case of nerve cells that sense sound and touch, or cells such as myocytes that respond to mechanical load as an intrinsic part of their physiological function. However, at a deeper level it can be argued that the existence of life itself implies motion: the movement of DNA polymerase along a template DNA strand requires the generation of mechanical force, as does the division of one cell into two. At a larger length scale, embryonic development is subject to intricate layers of mechanical feedback that dictate the shapes that growing tissues assume. Thus, central aspects of life, from the replication of genetic material to the morphogenesis of complex organisms, are quintessentially mechanical in nature.

The ubiquity of mechanobiology is amply demonstrated by the articles presented in this special issue. Yamashiro *et al.* used single-molecule fluorescence imaging to track individual actin molecules in the lamellae of migrating cells. They found that small-molecule inhibition of myosin contractility resulted in an increased rate of F-actin disassembly, suggesting that myosin-generated tension can stabilize the F-actin cytoskeleton. The mechanistic details of how this occurs are not completely understood, though previous in vitro studies demonstrate that mechanical tension can slow F-actin severing by cofilin (Hayakawa *et al.*, 2011). Regardless of the specific mechanism, the study by Yamashiro *et al.* provides evidence that feedback loops help to stabilize at least some F-actin networks under load, exactly as would be required for the cell to efficiently transmit mechanical force between its cytoskeleton and its surroundings.

In a related study, Lee *et al.* used laser microdissection to sever individual stress fibers (SFs) in living cells. Their measurements indicate that different subtypes of stress fibers, termed ventral SFs, dorsal SFs, and transverse arcs, perform mechanically distinct functions in constructing and maintaining the overall architecture of the cell. However, all three SF subtypes are mechanically linked and work together to generate the overall architecture of the cell (Burnette *et al.*, 2014). As the authors point out, relatively few studies to date have examined how SFs may function in three-dimensional environments such as occur in vivo (Owen *et al.*, 2017). Extending the mechanistic insights into cytoskeletal dynamics such as those reported by Yamashiro *et al.* and Lee *et al.* to three-dimensional circumstances represents an exciting challenge for future investigations.

The work by Kelley *et al.* represents one such step toward understanding how cytoskeletal dynamics contribute to physiological function in a fully in vivo context. The authors report the first characterization of the function of myosin light chain kinase (in this study termed MLCK-1) in *Caenorhabditis elegans* and find that this protein is required for the contraction of the spermatheca, the structure in which oocytes are fertilized. Further, they demonstrate that Rho kinase (ROCK), the key effector in a parallel pathway that also activates nonmuscle myosin II, is expressed in a distinct subset of spermathecal cells from MLCK-1 and that both pathways act in concert to coordinate the timing of contraction. This study presents a fascinating counterpoint to the better characterized functions of MLCK and ROCK in regulating the subcellular activity of myosin II in mammalian cells (Totsukawa *et al.*, 2000) and illustrates that we still have much to learn about how evolution has harnessed these parallel pathways in order to control cell- and tissue-level dynamics.

The question of how higher-order biological function arises from the properties of subcomponents is likewise addressed by Parreno *et al.* In their study, the authors examined how the physical resiliency of the eye's lens arises from its substructures, which consist almost exclusively of intricate folded layers of epithelial cells. To do so, they used a combination of mechanical characterization and three-dimensional imaging to determine how mechanical strain propagates in this complex tissue. These measurements provide physical insight into the factors that make the lens physically robust, as well as the specific cellular structures that fail when its mechanical capacity is exceeded.

Articles in the special issue also highlight how the mechanical aspects of cellular function can turn up in unexpected places. A study by Pfiefer *et al.* builds on previous studies (Denais *et al.*, 2016; Irianto *et al.*, 2016, 2017; Raab *et al.*, 2016) to examine how cell migration through small pores can cause both ruptures in the nuclear envelope and DNA damage. Here, the authors report that DNA damage occurred irrespective of the cell's point in the cell cycle and that passage through small, constricting pores suppressed entry into the cell cycle. As the authors point out, their data support the general concept that migrating cancer cells must choose to either "go or grow," namely that migration and proliferation are exclusive events (Giese *et al.*, 1996).

Li *et al.* describe a similarly unexpected role for fluid flow in controlling macrophage differentiation. Macrophages differentiate to adopt the proinflammatory or immunosuppressive M1 and M2 phenotypes, respectively. Unfortunately, macrophages in the tumor

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microenvironment are often predisposed toward the M2 state, contributing to an immune-privileged environment that contributes to cancer progression. In this study, the authors report that the physical cue provided by interstitial flow, such as is generated by the increased pressure within tumors, polarized macrophages toward the M2 state. Further, they observed that macrophages migrate against the flow direction, an effect that could facilitate the enrichment of M2 macrophages in the tumor microenvironment. The studies by Pfiefer *et al.* and Li *et al.* highlight how intrinsically physical factors can regulate cancer cell proliferation and dissemination in unexpected but potentially important ways.

Recent studies have likewise revealed unexpected roles for physical forces in immunological function. Pulling forces transmitted across the immunological synapse play a central role in allowing cells to gauge the specificity of the interaction between the T-cell receptor (TCR) complex and the major histocompatibility complex (MHC) (Liu *et al.*, 2014; Das *et al.*, 2015). In this issue, Pageon *et al.* review how molecular-scale physical forces regulate receptor triggering and immune cell activation. Importantly, they also provide an introduction to the biophysical tools that led to these findings, and that promise to further enrich our understanding of immunological function.

A study by Sorkin *et al.* discusses one such emerging biophysical technique, namely acoustic force spectroscopy (AFS). This tool uses acoustic standing waves to exert precisely calibrated forces on micron-sized objects. In this study, the authors used AFS to pull on silica microspheres attached to immobilized red blood cells. This technique allowed them to characterize the physical characteristics of 10s of cells in parallel, a potential advantage relative to techniques that yield similar data in a serial manner.

At the opposite end of the length scale, Jahed *et al.* used molecular dynamics simulations to examine how atomic-level interactions regulate the assembly of the nuclear LINC complex, which spans the nuclear envelope to link the nucleo- and cytoskeletons. These simulations lend support to detailed proposals for how autoinhibition in the monomeric SUN2 protein may be relieved in order to allow assembly of the LINC complex. This study illustrates the growing power of molecular dynamics simulations to promote understanding, and provoke novel hypotheses, about how complex functions can arise at the molecular level.

Finally, Alimohamadi et al. use a combination of analytical theory and modeling to address a long-standing challenge in cellular biophysics, namely estimating the forces experienced by a lipid membrane based on measurements of its shape. They show that, at least in principle, differences in shape can be used to differentiate between mechanisms of membrane deformation, for example in the context of membrane budding. As discussed by the authors, extensions to their theoretical framework that account for shape asymmetries can potentially result in a useful means of estimating the forces acting on membranes, both in purified systems and, plausibly, in living cells.

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