Mechanobiology: forcing the second act

Valerie M. Weaver*
Center for Bioengineering and Tissue Regeneration, Department of Surgery, Departments of Radiation Oncology and Bioengineering and Therapeutic Sciences, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, and UCSF Helen Diller Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94143-0456

Welcome to *Molecular Biology of the Cell*'s 5th special mechanobiology issue of "Forces on and within cells." This special issue presents exciting new work from multiple investigators representing diverse fields and broad geographical regions that summarizes interesting new developments in this exciting field of mechanobiology. The articles in this special issue range from work summarizing new insights regarding membrane topology and ion channels involved in cellular mechanosensing to how the actin cytoskeletal network is regulated to process and integrate mechanical cues from the membrane cytoskeleton and how these forces regulate cellular responses through physical interactions with the nucleus that evoke transcriptional responses. At the cell and tissue levels the articles discuss findings pertaining to the role of force-dependent epithelial cell transdifferentiation, the classification of tumors by mechanical criteria, the impact of mechanics on vascular integrity, and how mechano dysfunction can compromise cardiac homeostasis and regulate tumor cell invasion.

All cells experience force from the molecular and cellular through to the tissue and organismal levels. Nanoscale forces regulate discrete processes such as spindle-dependent cytokinesis that control the fidelity of cell division and talin and vinculin unfolding that regulate focal adhesion assembly and integrin-dependent signaling and actin dynamics. At the mesoscale, force influences the direction of cell migration and tissue processes such as branching morphogenesis while macrolevel forces govern cardiomyocyte function to influence cardiac integrity and endothelial junctions to regulate blood flow dynamics. As the novelty of studying mechanobiology has diminished and more researchers embrace a physical sciences perspective to clarify cellular behavior, the field increasingly demands a more-in-depth understanding of how a cell senses, translates, and integrates mechanical cues to regulate its behavior at both the single cell and multicellular levels. A critical component of these studies is the application of quantitative methods to measure and manipulate force and approaches to image the response of molecules and cellular behaviors to mechanical cues. This shift toward a more-in-depth understanding of mechanobiology is evident in the assorted articles published in this special issue of "Forces on and within cells."

To begin with, while there are abundant published data reporting on the role of ion channel gating and adhesion receptor structure function in sensing and responding to external force as well as modifications in actomyosin-mediated tension on cell phenotype, our appreciation of the impact of physical changes linked to osmolarity has remained rudimentary. In an interesting article, Akella and colleagues report on the role of WNK kinases as key regulators of electroneutral cotransporters controlled by osmotic stress and chloride. Their findings are particularly relevant for clarifying the impact of molecular crowding on cell phenotype. Their results were made possible because the authors applied small-angle x-ray scattering of WNK3 and were thus able to observe an intriguing conformational equilibrium between the inactive and active forms of WNK3 that occurs in response to the level of its molecular hydration and bound water. The work presents a far-more-nuanced appreciation of the impact of osmolytes on cell behavior and osmosensing that certainly merits further investigation. Consistent with a more-fine-grained understanding of how cells sense and translate mechanical cues, Ghilardi and colleagues summarize a series of studies that clarify the interplay between ventral stress fibers and plasma membrane deformation. Using human fibroblasts, the authors identified a novel actin–membrane bending topology where the membrane is deformed outward rather than being pinched inward to form what they define as cytosolic pockets induced by ventral actin stress fibers. The findings expand the role of actin to incorporate localized changes in membrane topology and raise the intriguing possibility that these membrane protrusions create local membrane proximal regions that could participate in cell signaling. Elaborating further on the interplay between actin and membrane protrusions, Wubshet, Bashirzadeh, and Liu summarize their findings on the importance of fascin-induced actin interactions as a molecular mechanism that can temper membrane protrusions. They applied a reconstitution approach that encapsulated bundled and dendritic actin networks inside of giant unilamellar vesicles termed GUVs to study how various actin-binding proteins are able to compete and cooperate to form distinct actin networks. Their studies revealed that, while membrane-bound Arp2/3 nucleation forms an actin cortex, by contrast fascin mediates the formation of actin bundles that are able to protrude out of GUVs. However, Arp2/3 and fascin can cooperate to generate polarized dendritic aggregates that reduce membrane protrusions regardless of membrane associations. The findings

DOI:10.1091/mbc.E21-07-0343

*Address correspondence to: Valerie M. Weaver (Valerie.Weaver@ucsf.edu).

provide insight into how actin-binding proteins can generate complex actin structures. Integrating force in actin organization, Sala and Oakes exploited a photoactivated RhoA to illustrate the critical role of LIM domain proteins as important mechanoresponsive cytoskeletal elements capable of sensing strain in the cytoskeleton. Toward clarifying the role of actin and its binding proteins in force production, Kollimada and colleagues use an alternative traction-force microscopy (TFM) assay that employs a combination of hydrogel micropatterning to define cell adhesion and shape and an intermediate fixation/immunolabeling step to characterize strain energies and to monitor endogenous protein content at the single cell level. Not surprisingly, the authors determined that the biochemical composition of the actomyosin network "tunes" the magnitude of cellular traction forces in the cell. Specifically, they observed that traction-force magnitude is dictated by the relative amounts of molecular motors and cross-linkers per actin filament, rather than the amounts of an individual component of the cytoskeletal network. What is emerging from this area of intense study is how force integrates and collaborates with molecular constituents to modulate the actin cytoskeletal network and membrane topology to influence cell phenotype.

The relevance of detailed cell biology observations is underscored by the work of Rey-Suarez and colleagues, whose article summarizes their studies exploring the interplay between actomyosin and the microtubule cytoskeleton in the formation of the immunological synapse (IS) in T-cells with antigen-presenting cells. The authors employed high-resolution fluorescence microscopy to show that microtubule growth dynamics in the peripheral actin-rich region in the T-cell are distinct from those in the central actin-free region. They determined that these differences arise from differential involvement of Arp2/3 and formin-nucleated actin structures modulated by integrin engagement. The data provide much-needed insight into how cytoskeletal dynamics influence T-cell activation. Cellular mechanobiology similarly regulates CD4+ T-lymphocyte migration and response to exogenous force. This concept was nicely illustrated by a series of experiments reported by Kim and Hammer, who explored how CD4+ T-lymphocytes interact with distinct tissue microenvironments with a range of mechanical properties. Building on prior studies, the authors tested their prediction that the T-cell receptor LFA-1 is a mechanosensor. Consistently, they determined not only that CD4+ T-cells migrate faster when ligating ICAM-1 as a function of increasing substrate stiffness but that the cells also migrate under flow irrespective of substrate stiffness. Their findings confirm that indeed LFA-1 is a mechanoresponsive molecule that is able to respond to diverse mechanical stimuli.

The impact of the actin cytoskeleton and its binding proteins on cell migration extends beyond force transmission to the extracellular microenvironment, as is demonstrated for cancer cells migrating within nonadhesive confined microenvironments through a series of studies reported by Adams and colleagues. Tumor cell invasion into the parenchyma is a hallmark of invasive carcinoma, and migration through the interstitial extracellular matrix and extravasation into the vasculature herald metastatic spread. Not surprisingly, much effort has been directed toward clarifying the molecular mechanisms regulating this phenotype with the ultimate goal of identifying anti-metastatic drugs. To this end, an assortment of migratory phenotypes have been described, including mesenchymal, amoebic, and more recently, confinement-induced, nonadhesive leader bleb–based migration (LBBM). While the cancer community has diligently studied the molecular mechanisms regulating mesenchymal and amoebic tumor cell migration, LBBM migration is less well understood. LBBM is morphologically characterized by a long bleb that points in the direction of the cellular movement that is separated from the cell body by a contractile neck. Using melanoma and osteosarcoma cells, Adams and coworkers determined that actin assembly factors localize toward the leader bleb tip, whereas contractility regulators and cross-linkers primarily localize in the cell body cortex and neck, with only a diffuse amount localizing in the leader bleb. Gain-of-function and loss-of-function studies causally linked the actin cross-linkers filamin-A and fascin-1 to LBBM-mediated tumor cell migration, implicating cortical tension and pressure in confined tumor cell invasion and migration. The findings highlight the role of intracellular force in tumor cell migration and provide a readout for anti-tumor screening. The importance of the cytoskeleton in malignancy is further emphasized by the studies reported by Rabie and colleagues, who examined the impact of substrate stiffness on tumor cell aggression and genomic instability by examining the abscission and multinucleation during cytokinesis that occurs following an epithelial-to-mesenchymal transition (EMT). In their intriguing studies, the authors determined that when tumor cells interact with a stiff extracellular microenvironment, they engage beta 1 integrin and recruit the scaffolding function of integrin-linked kinase (ILK) to promote the multinucleation and aberrant genomic integrity of the cells.

Although force is ultimately sensed and translated at the molecular level, its impact manifests at the cellular and tissue levels and exerts profound effects at the organismal level. This concept is elegantly illustrated by two sets of studies published in this issue. In the first set of studies, Barrick and coworkers explored the molecular impact of a troponin T variant that has been linked to pediatric dilated cardiomyopathy. The authors used in vitro assays to study the molecular- and cellular-level impact of troponin T variant R134G on cardiomyocyte behavior. They used stopped-flow and steady-state fluorescence measurements to determine that the R134G mutant decouples calcium binding by troponin from the closed-to-open transition of the thin filament and decreases the cooperativity of myosin binding to regulated thin filaments. The net effect of this defect is that there is a reduced force per sarcomere such that cardiomyocytes with this mutation are hypocontractile. Consequently these mutant cardiomyocytes demonstrate a profound calcium insensitivity as illustrated by reduced motility at the single cell level and as presented morphologically as disorganized sarcomeres and cellular hypertrophy. Echoing this approach, Trujillo and colleagues used *Drosophila melanogaster* to clarify the molecular impact of the S532P myosin heavy chain mutation in cardiomyocytes in dilated cardiomyopathy. The myosin mutation underlying this disease, which is located at the actomyosin interface, reduced the rate of actin-dependent ATPase activity and actin binding and increased the rate of actin detachment. The authors determined that the result of this molecular defect is a depression of myosin function that reduces the number of cross-bridges during active wing beating, decreases the power output of indirect flight muscles, and consequently compromises flight ability. Furthermore and importantly, the authors reported a dose-dependent phenotype associated with this mutation that recapitulates human disease. These two cardiomyocyte findings emphasize the utility of multiscale studies to clarify how molecular mechanosignaling impacts cell and tissue behavior. The studies herald a new integrated approach to linking mechanobiology to precision medicine.

The vascular system is highly responsive to force and is precisely regulated by blood flow. Perhaps not surprisingly, cardiovascular disease results from compromised vascular integrity and loss of mechanical homeostasis. In an interesting set of studies that also

crosses length scales, Miroshnikova and colleagues explored the impact of cyclic mechanical stretch on vascular integrity by studying the acute and adaptive impact of cyclic stretch on the transient remodeling of the VE-cadherin–based adherens junction and its associated actomyosin cytoskeleton. The authors applied time-resolved proteomic profiling that revealed that this remodeling is driven by calcium influx through the mechanosensitive Piezo channel. They were able to quantify a simultaneous increase in the stiffness of the endothelial cell layer mediated through filamin that permits the mechanoadaptation needed to restore junctional integrity even in the presence of chronic stretch. The intimate role of mechanoadaptation to tissue homeostasis is also discussed by the article by Denis and colleagues that assesses the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex in endothelial cell adhesion and adaptation to shear stress and cyclic stretch. The LINC complex anchors the nucleus to the actin, microtubule, and intermediate filament cytoskeleton and consequently plays a key role in facilitating cellular mechanointegration and -adaptation. Denis *et al*. determined that in the absence of functional interactions between the cytoskeleton and the nucleus, induced through the expression of a dominant negative KASH protein, endothelial cells assemble compromised cell–cell adhesion and fail to generate appropriate barrier function that corrupts their ability to adapt to shear stress and cyclic stretch. In the absence of functional nuclear cytoskeletal interactions, endothelial cells also exhibited impaired collective cell migration following wound healing and inappropriate angiogenesis. The findings once again underscore the intimate role of mechanotransduction in tissue structure and function and illustrate the importance of clarifying the molecular basis of mechanical homeostasis in tissue homeostasis.

In summary, the articles published in this 5th special mechanobiology issue go far to establish the durability of this important area of study. Clearly, we are only just beginning to clarify the role mechanics plays in cell and tissue biology, and I genuinely look forward to participating in this exciting research area for many years to come.