Forces, Growth and Form: an Editorial introduction

Alpha S. Yap*
Division of Cell and Developmental Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Brisbane 4072, Australia

ABSTRACT **Welcome to this Fourth Special Issue of *Molecular Biology of the Cell* on Forces on and within Cells. As with our other Special Issues, the journal's goal here is to focus attention on a major new direction in cell biology. In this case, it is the field of mechanobiology, which endeavours, broadly, to understand how mechanical forces are harnessed to drive cellular function and how force can also be a mode of biological information that regulates cell behavior. The collection of papers that we have in this issue reflects many current efforts to address these questions. While each of these papers is a distinct creative effort of its authors, I would like to draw your attention to a number of themes that emerge across these diverse studies.**

ACTIVE FORCES AND ORGANELLE POSITIONING

Cells are active materials whose cytoskeleton can generate forces that are directed to serve many different biological functions. Several papers in this Issue ask how cytoskeletal forces contribute to positioning organelles, with a particular focus on microtubule (MT)-based forces. Kimura and Kimura focus on the sperm-derived pronucleus/centrosome complex (SPCC) in the *Caenorhabditis elegans* zygote, whose position ultimately specifies the anterior–posterior axis of the embryo. Interestingly, these investigators show that after sperm entry the SPCC can move within the zygote before adopting its final position that presages the step of symmetry breaking. This movement is driven by a kinesin-1–dependent flow, known as meiotic cytoplasmic streaming. Thus, just as actomyosin-based cortical flows are known to influence polarization once symmetry has broken (Munro *et al.*, 2004), the work of Kimura and Kimura shows that MT-dependent cytoplasmic flow can exert a prior effect to determine where symmetry will be broken. In another example, Nunes *et al.* examine how during mitosis centrosomes are positioned on the shortest nuclear axis to ultimately define the orientation of the mitotic spindle. Centrosome positioning reflected movement both of the centrosomes themselves and of the nucleus, driven by different mechanisms. Dynein-generated forces acting on the nuclear envelope helped support nuclear orientation, while Arp2/3 influenced centrosomal movement, by indirectly regulating MT dynamics.

These first two experimental studies are complemented by the work of Manhart *et al.*, who asked how nuclei are positioned and dynamically rearranged during muscle development. This is a challenging problem because although nuclear positioning is known to be MT dependent, the plethora of proteins that are involved (and gaps in our knowledge of precise molecular details) make it difficult for biological intuition to yield a reliable mechanism for this process. Instead, Manhart *et al.* applied computational screens to identify macroscopic models for combinations of coarse-grained forces (e.g., repulsion between nuclei) that realistically fit experimentally observed nuclear patterns. This allowed them to then develop a microscopic model, where the choice of MT orientation and motors was guided by the computational screen, that was consistent with both the experimental data and the macroscopic models.

DIFFERENT PATHS TO THE NUCLEUS

Mechanotransduction, the process by which mechanical forces are detected and converted into biochemical signals, works on several different length- and timescales. The longest-lived cellular changes occur when mechanical information alters transcription. This is exemplified by the YAP/TAZ transcriptional regulators, which can integrate mechanical inputs such as tension and extracellular rigidity (Halder *et al.*, 2012). But how these mechanical factors are transduced to engage YAP/TAZ signaling is beginning to look as varied as the mechanical inputs themselves. For example, Hoffman *et al.* found that Yap1 accumulated in nuclei when fibroblasts were subjected to cyclic stretch, and this coincided with the coassembly of closely apposed linear arrays of LINC (Linkers of the Nucleoskeleton to the Cytoskeleton) proteins on the nuclear envelope and actin stress fibers. This suggested that coupling of the nuclear envelope to the cytoskeleton might mediate the response to oriented patterns

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*Address correspondence to: Alpha S. Yap (a.yap@uq.edu.au)

of force. Silver *et al*. also found that Yap1 signaling Is activated when epithelial cells experience enhanced tissue stresses. Interestingly, this coincided with a tissue gradient in the transmembrane electrical potential difference (membrane voltage), cells being more depolarized in regions of stress. This difference in membrane was attributable to connexin-43 hemichannels, whose activation was necessary for YAP/TAZ signaling and induction of proliferation. Therefore, bioelectrical signals may be another way for mechanical stresses to be transduced to YAP/TAZ signaling.

Nor are transcriptional regulators the only potential mechanosensitive pathways to the nucleus. Todorovski *et al*. report that nuclear paraspeckles, RNA-protein granules containing the long noncoding RNA NEAT1, are sensitive to substrate rigidity, increasing in number when cells are grown on soft, compared with more rigid, substrata. The functional significance of this observation has yet to be determined, but it is interesting to note that it was documented in cancer cells, where paraspeckles are emerging as regulators of gene expression.

THE MECHANICAL IMPACT OF CELLULAR ENVIRONMENT

A major theme in mechanobiology is how the mechanical properties of the cell's environment can condition its behavior. This has often been studied by probing the interaction between isolated cells and their extracellular matrix (ECM), but several studies in this issue demonstrate how this also applies in tissues. Moyle *et al*. found that niche around skeletal muscle stem cells stiffens during regeneration and show that this influences the orientation of cell division. Planar cell orientation, which promotes symmetric divisions that expand the stem cell pool, was enhanced in rigid rather than soft environments. Thus, environmental mechanics may condition the balance between stem/progenitor regeneration and differentiation. The impact of the ECM was also demonstrated in a novel model system by Madhu *et al*., who report that extracorporeal epithelial tubes in the sea squirt *Botryllus schlosseri* undergo dramatic tissue reorganization when the stiffness of their ECM is experimentally altered.

Neighboring cells form another dimension of the cellular environment within solid tissues. Here, cytoskeletal forces applied to cell–cell junctions serve to drive morphogenetic events, such as epithelial folding and invagination during development (Guillot and Lecuit, 2013). In this issue, Ko *et al*. report how the balancing of forces influences morphogenesis in the *Drosophila* embryo. Building from the observation that mitosis is suppressed when the presumptive mesoderm invaginates to form the ventral furrow, they show that premature mitotic entry inhibits the medial-apical actomyosin networks that are necessary for apical constriction to drive furrowing. Presumably, then, mitosis is developmentally suppressed during mesoderm invagination to ensure effective contractility. Interestingly, mitosis caused ectopic furrows to appear in tissues where cell contractility was experimentally enhanced. Strikingly, invagination affected the nonmitotic cells that flanked the mitotic domains. The authors suggest that in this case furrowing reflected the imbalance of forces between the less-contractile mitotic cells and their hypercontractile neighbors. Together, these highlight how force balance across a tissue critically influences morphogenesis.

Although cells often use adhesion molecules to detect the mechanics of their environment, Nekimken *et al*. remind us that this is not obligatory (Reversat *et al*., 2020). They show that touch-sensitive neurons in *C. elegans* respond to mechanical strain. However, this response persisted even in animals mutant for genes necessary for proper cell–ECM attachments. This suggested that the bulk mechanical properties of the tissue might be sufficient to transduce mechanical force from the skin of the animal to these mechanosensors.

THE POWER OF NEW TECHNOLOGIES

In truth, one could reasonably argue that mechanobiology is an old field that we have recently rediscovered. After all, D'Arcy Wentworth Thompson's *On Growth and Form*, first published in 1917, argued for the seminal role that physical factors (mechanics, geometry) play in biological form and development. Why, then, did this problem fall into decline in the latter half of the 20th century, and what has reinvigorated it in the 21st century? Much of the answer to both these questions lies in the technological and intellectual tools that have been available. In particular, the molecular genetic revolution allowed us to identify molecular mechanisms with a facility and predictive power that was not readily available for students of physical biology.

This has now changed. We now have a variety of tools that allow us to characterize mechanical properties of cells and tissues and their environment. These range from genetically encodable tension sensors to the application of biophysical assays, such as atomic force microscopy (AFM). And new methodologies continue to be introduced into experimental biology. For example, Lee *et al*. applied optical flow, a computer vision algorithm used in robotics and navigation control, to develop an unbiased automated approach that could quantify actin waves in cells. Because optical flow is based on changes in pixel intensity, it may be very well suited to evaluate dynamic process within amorphous structures that do not have clear fiduciary features. Moreover, disparate tools can now be readily combined to suit particular problems. In this issue, Rianna *et al*. combined AFM with fabricated microchannels to show that cancer cells soften when forced to migrate under confinement, and Hobson *et al*. used light sheet microscopy to measure how the nucleus deforms when compressed with an AFM tip. We can anticipate that the introduction of new assays and instruments will continue to increase the repertoire of technologies that can be "mixed and matched" to suit the problem at hand.

AND WE NEED THEORY

The latter-day blossoming of mechanobiology also reflects the application of physical theory and modeling to biological problems. In this issue, Agrawal and Lele develop a computational model to examine how the geometry of the nuclear envelope may condition its stiffness, and, as we have already mentioned, Manhart *et al*. show how computational screens can be used to understand organelle positioning.

But, as well, many of the tools that we can now apply to characterize mechanics require modeling for their interpretation, and indeed can prompt the application of new models to explain the data. For example, Chaubet *et al*. show that a model where mechanics are conditioned by the unbinding kinetics of a dominant cross-linker aligned well with the rheology of the cytoplasm that they measured using an optical trap. This further allowed them to identify α-actinin-4 as one candidate cross-linker whose action became apparent when cellular contractility was inhibited. Hobson *et al*. report that a two-component model could best explain the dynamic response of nuclear morphology to compression, and this led them to identify distinct contributions of chromatin and nuclear lamins to change in nuclear volume and surface area, respectively.

BUT NOT THE END

One last innovation for *MBoC*. This issue gives us the opportunity to welcome Janet Isawa to the journal as our Visualization Editor. Janet created the striking cover image for this issue, which depicts

different types of force acting on and within fibroblasts and their environment. In closing, I should say that we invited several other manuscripts to be revised for this Special Issue, but the authors were unable to complete their work before the production deadline. Of course, in this extraordinary year so many of us have faced unprecedented challenges to our work. Accordingly, we aim to publish a second part of this special edition later in 2020, that we hope will include many of these other studies.

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