New and Notable

Mechano-Chemical Coupling Drives Cell Area Oscillations during Morphogenesis

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The fact that morphogenesis, the set of tissue movements and deformations that generate amazing and complex forms during embryonic development, results from the tight interplay between biochemical and mechanical processes, has only recently started to be assimilated by the developmental biology community. Although it is now accepted that the material properties of cells and the mechanical stresses generated inside them are important parameters for embryonic development, it remains largely unknown how and at what time- and length-scales the interplay between mechanical and biochemical activity takes place (1,2). It is increasingly evident that answering this question requires a multidisciplinary effort that combines the more-classical approaches of developmental biology with quantitative measurements and physical modeling techniques.

In a recent issue of *Biophysical Journal*, Wang et al. (3) go a big step in this direction by presenting a model of dorsal closure, a morphogenetic process of the *Drosophila* embryo, where the interplay between biochemical and mechanical inputs generates several features of the cell and tissue behavior (reviewed in Gorfinkiel et al. (4)). Dorsal closure (DC) is a very well-studied morphogenetic process whereby interactions between two tissues, the amnioserosa (AS) and the

epidermis, close a discontinuity at the dorsal side of the embryo to generate a continuous epidermis. Using innovative approaches, more than 10 years ago Dan Kiehart and colleagues uncovered the main tissue-level forces involved in this process: the resistive force of the epidermis is mainly countered by an AS contractile force, which is in turn aided by a supracellular actin cable formed at the interface between the two tissues that generates tension in the direction of closure (5,6). In the last few years, work from several labs has started to unravel, with an unprecedented quantitative precision, the molecular and cellular mechanisms underlying the generation of such forces. Importantly, it has been shown that AS cells exhibit oscillations in their apical surface area driven by the transient activity of the actomyosin cytoskeleton. The frequency of these oscillations increase as DC progresses until it becomes undetectable, and this correlates with an increase in the rate of contraction of these cells (7–9).

Models of epithelial organization have successfully been applied to the understanding of how cell mechanical properties generate stable epithelial configurations and the formation of well-defined boundaries between cell populations (see, for example, Aliee et al. (10) and Farhadifar et al. (11)). In this type of approach, it is generally assumed that mechanical properties and other cell behaviors such as nonuniform cell proliferation, anisotropic cell division, and cell elongation, are previously set by signaling molecules operating in these cells. The model presented by Wang et al. (3) moves a step forward in the understanding of how the interplay between mechanical and cellular activity takes place and thus makes an important contribution to the field of epithelial morphogenesis.

The model tackles two of the most relevant questions in the field. The first question refers to the nature of cell area and actomyosin oscillations exhibited by apically contracting cells.

Such oscillatory behavior has also been observed in other tissues and in other organisms, suggesting it represents a fundamental property of cytoskeletal systems (12). The main innovation of the model is that oscillations emerge from the coupling of two different timescales: the timescale of myosin turnover, driven by a signaling molecule (itself oscillatory due to a myosin-dependent degradation rate), and the viscoelastic relaxation timescale of the cells due to their intrinsic viscoelasticity. It is this tight mechano-chemical coupling that generates sustained oscillations in certain regions of the parameter space, which, in the model presented by Wang et al. (3), are chosen such that oscillations do not occur at the single cell level but emerge from neighbor-neighbor interactions.

The second question that the authors explore is the mechanism underlying the net contraction of AS cells. The existence of a ratchetlike mechanism underlying apical cell contraction has been previously suggested in the literature, with two possible scenarios for the AS: an extracellular ratchet provided by the supracellular actin cable and an internal ratchet for each individual cell (7,9,13). Wang et al. (3) explore both these scenarios. The internal ratchet is implemented via the stepwise reduction of the rest length of cell edge and radial viscoelastic elements when the cell area reaches its minimum at each oscillatory cycle, thus providing a bona fide ratchet mechanism for the rest length around which fluctuations occur. Similarly, the external ratchet is realized by adding an elastic spring along the outer boundary of the simulated AS and by decreasing its restlength each time the tissue area reaches a minimum. The theoretical analysis of these two ratchet mechanisms leads the authors to predict that the intracellular ratchet makes the more significant contribution to contraction.

Models are a tool to explore possible mechanisms behind a specific process and, for experimentalists, models are a useful tool if they make predictions than can be tested. This is the case for the model presented by Wang et al. (3). One of the main predictions of the model is the existence of an oscillating signaling molecule, upstream of myosin activity. If this is the case, it is highly improbable that cell area and myosin oscillations arise from oscillations in the expression of this molecule, given the timescale of the oscillations. In fact, in the model by Wang et al., the signaling molecule is produced at a constant rate but its degradation depends on active myosin concentration, so that its levels oscillate. Whether it is the total levels or the active form of the molecule that oscillates, this is an important prediction of Wang's model. An interesting possibility is that the activity of proteins of the Rho GTPase family, which control cytoskeletal dynamics in various systems, is oscillating. In migrating cells, it has been found that the activity of RhoA and Rac/Cdc42, measured through live Förster resonance energy transfer sensors, increases and decreases in synchrony with the protrusion and retraction cycle of the migrating cell (14), raising the possibility that oscillations in any of these proteins could in turn be driving myosin oscillations.

However, at the molecular level, oscillations in cytoskeletal activity have also been reported and modeled using a different framework which invokes a dynamic instability in the force-velocity relationship of a collection of motors moving along a filament (15). More recently, oscillations in cell shape and cytoskeletal activity during cytokinesis have also been modeled by means of coupling the turnover of the actin cortex with the viscoelastic relaxation timescale of the cell (16). The latter approach assumes a mechano-sensory role for the actin cytoskeleton, for which there is still no clear evidence. We expect interesting times ahead trying to solve the origin of such oscillations.

Another important prediction of the model is the existence of an intracellular ratchet allowing net contraction to occur. In Wang's model, this is simulated by a stepwise decrease in the rest-length of the radial spokes and edges of the cells that increases in strength as cells go through the different phases of DC. The authors suggest a plausible mechanism for this based on results from in vitro reconstituted networks that involves the buckling of cross-linked actin filaments onto which actomyosin foci move and the subsequent removal of the extra material (17). Whether such a mechanism underpins the establishment of the ratchet will require careful quantitative experiments measuring the levels of myosin and actin in AS cells as DC progresses through its distinct phases, as well as the function of putative actin cross-linkers.

Of course the model cannot account for all the biological processes underlying DC, nor is this the intention of the authors. An important question that remains unexplored is that of the mechanisms driving the flow of actomyosin foci. Although some actomyosin foci coalesce and soon disassemble without exhibiting any significant movement, several actomyosin accumulations flow across the apical surface of AS cells, exhibiting a preferential direction of movement along the medio-lateral axis of cells. In the *Caenorhabditis elegans* oocyte, a gradient of contractility underlies such actomyosin flows (18). In epithelial cells, it has been suggested that a mechanical imbalance generated by the planar localization of ECadherin is at the basis of the actomyosin flows (19), but AS cells do not show any signs of planar polarization of adhesion proteins. Despite this, it is likely that adhesion complexes have an important role both in the oscillatory behavior and in the effective contraction of AS cells. Adherens junctions are active complexes connecting neighboring cells and transmitting the forces generated inside the cells across the tissue. Several reports show that adhe-

rens junctions feel the tension and can change accordingly, suggesting they may have an important role in feedback loops that increase tension both inside the cells and across the tissue (20,21). In the model of Wang et al., cells are connected passively to each other, but future models of DC and other morphogenetic processes will probably incorporate this essential property of epithelial cells.

Finally, although DC has been, until now, approached as a two-dimensional process, it is evident that cells live in three dimensions and there is some evidence showing that as cells contract, they become columnar (22). Although it is generally assumed that volume is conserved during the process, precise measurements of both changes in cell shape and of cytoskeletal activity in three dimensions are still lacking. It is likely that future models of DC will incorporate the third dimension.

The model by Wang et al. (3) makes an important contribution not only to the field of dorsal closure but also to the young multidisciplinary field emerging at the intersection between developmental biology and the other physical sciences. Lastly, it demonstrates that experiment and theory can be engaged in a feedback loop that advances our understanding of the basic principles underlying biological processes.

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