electro-dewetting, in contrast to electrowetting and, therefore, EWOD.

Unlike EWOD, in which the dielectric layer blocks any electric current, electro-dewetting relies on passing a current through the liquid and a nanometre-thin silicon oxide layer to an underlying electrode. Depending on the direction of the current, charged surfactants are transported either towards or away from the solid surface, inducing surfactant adsorption or desorption, respectively. The authors demonstrate that this technique can be applied to a remarkably wide range of liquids and surfactants, as long as the concentration of these molecules is within a specific range of conveniently low values. Efficient droplet manipulation is also shown for some highly saline buffer solutions that are commonly used in biotechnology.

Li and colleagues use electro-dewetting in conjunction with patterned electrodes, and demonstrate lateral movement of droplets and the basic droplet operations of lab-on-a-chip systems. They find that these manipulations can be carried out even more easily than when using EWOD, despite somewhat slower response times for the droplets and a smaller range of accessible contact-angle variations.

The main promise of the authors' approach is to deliver a robust and versatile droplet-manipulation platform. Although the results presented show a remarkable degree of versatility, challenges remain. For instance, the surfactants tend to adsorb to the solid surface and increase the contact angle even without an applied electric current. But Li *et al.* show that this adverse effect can be suppressed by adjusting the liquid's composition (for example, its pH) depending on the type of surfactant that is used. Given the wide range of surfactants that are available, it seems plausible that suitable material combinations can be found that maximize the electro-dewetting efficiency and that minimize possible interference from other solutes such as proteins, for many applications.

Another challenge is that the required electric current will drive electrochemical reactions that could gradually degrade the droplet-manipulation platform and the associated liquids. Stringent tests will need to be carried out after hundreds, thousands or even millions of adsorption–desorption cycles to fully evaluate the robustness and versatility of electro-dewetting.

Li and colleagues' work might also have implications for fundamental research. Standard wetting theories[9] are equilibrium theories that are based on energy minimization. However, the need for a permanent electric current in electro-dewetting demonstrates that the microscopic origin of this mechanism requires some intrinsically non-equilibrium processes that remain to be identified. This concept could therefore offer opportunities for controlling interfacial adsorption even beyond wettability alteration. ■

Frieder Mugele *is at the MESA+ Institute for Nanotechnology, Physics of Complex Fluids, University of Twente, Enschede, 7500 AE, the Netherlands.*
e-mail: f.mugele@utwente.nl

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DEVELOPMENTAL BIOLOGY

Genetics and mechanics guide gut formation

An analysis of gut formation in the fruit fly has revealed how gene expression and mechanical forces are coordinated in adjacent populations of cells. The findings highlight the tissue-level control of embryonic development. SEE ARTICLE P.467

KRISTEN A. PANFILIO

During embryonic development, generating the correct 3D body form, a process called morphogenesis, requires extensive tissue remodelling. Sheets of cells fold and alter their geometry, undergoing changes equivalent to the paper-folding intricacies of origami. In an early embryo, the cells that will form muscle tissue (termed the mesoderm) and gut tissue (the endoderm) move inwards, and the cells of the outer layer form the skin. On page 467, Bailles *et al.*[1] report a previously unknown aspect of how cells internalize, as revealed by studies of the fruit fly *Drosophila melanogaster*.

Investigations into mesoderm internalization in *D. melanogaster* have established molecular links between cell identity and the physical changes that cells undergo during development. The protein Twist regulates gene expression to confer muscle cell identity, and cells that express Twist constrict their outer (apical) surfaces while maintaining contact with neighbouring cells[2]. The tissue buckling that results from this apical constriction drives cell internalization. Internalization is thus hardwired in the mesoderm, but it also generates forces that affect neighbouring, non-mesodermal tissues[3]. How does mesoderm internalization compare with other examples of cell internalization during development, particularly when many events of morphogenesis occur simultaneously? Bailles and colleagues studied endoderm internalization to investigate this.

Endodermal cells internalize as the entire endoderm tissue — a circular patch of about 15 rows of cells — migrates towards the head region of the early embryo. Using a live-cell imaging microscopy approach and experimental methods supported by mathematical modelling, the authors reveal that there are two distinct regions of the endoderm that differ in their internalization mechanism.

The part of the endoderm that the authors call the primordium region is the first to internalize. Like mesoderm internalization[2], this occurs through a process that is directly regulated by gene expression. The expression and activity of the protein Fog results in an increase in the proteins non-muscle myosin II (MyoII) and Rho1 in the apical region of cells, leading to apical constriction by remodelling of the cells' cytoskeleton (a filament-like internal scaffold in the cytoplasm)[4]. Bailles and colleagues observed that this local Fog activity led to the simultaneous contraction and internalization of all cells of the primordium region (Fig. 1).

In the other part of the endoderm, which the authors term the propagation region, internalization occurred progressively, one row of cells at a time. Bailles and colleagues made the surprising discovery that if transcription was suppressed or the local source of Fog protein was lost, internalization of the propagation region still occurred if the primordium region had already started to contract. Modelling the rate of internalization of the propagation region compared with the maximum estimated speed of Fog diffusion allowed the authors to rule out Fog diffusion as an explanation for how this internalization process is controlled.

Bailles and colleagues then examined whether mechanical influences might have a role. For this, they physically impeded tissue movement, used genetic approaches to alter embryo geometry or used a drug that inhibited MyoII. Their experiments revealed that internalization of the propagation region proceeds by a mechanical positive-feedback

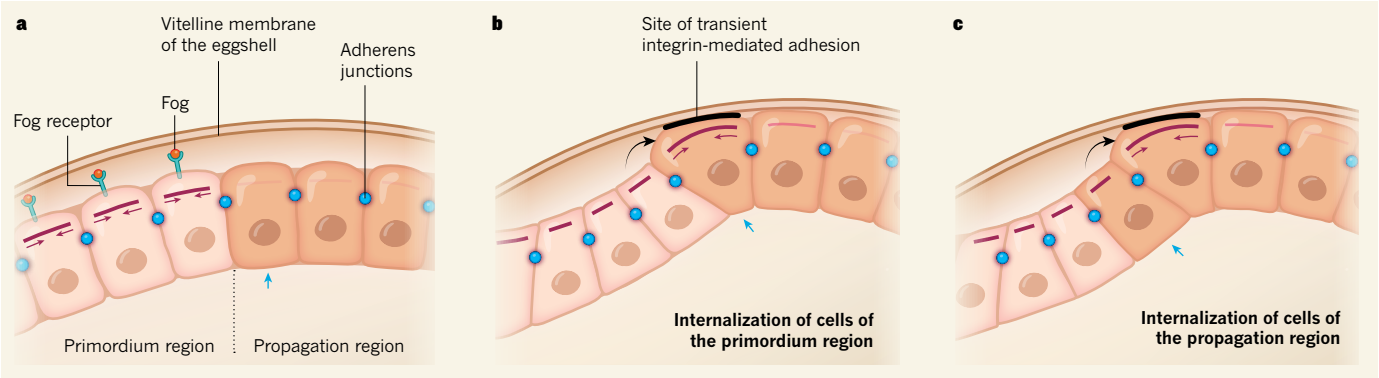

Figure 1 | The internalization of endodermal cells. **a**, During embryonic development of the fruit fly *Drosophila melanogaster*, gut-forming endodermal cells internalize, moving away from the inner layer of the eggshell (the vitelline membrane). Bailles *et al.*[1] reveal how two endodermal regions, termed the primordium region (pink cells) and the propagation region (brown cells), internalize. Only a few cells of these regions are shown, in a cross-sectional view through the middle of the tissue, where each cell visible represents one row of cells that is perpendicular to this field of view. A cell of the propagation region is indicated by a blue arrow to aid tracking of its internalization across the panels. Cells of the primordium region secrete the protein Fog, which binds to its receptor, triggering the accumulation and activation of the protein non-muscle myosin II (pink lines, with darker shades corresponding to greater accumulation and activation). This helps to generate contractile forces (pink arrows). Structures called adherens junctions enable mechanical coupling and the transmission of force between cells. **b**, The cells of the primordium region simultaneously contract their outer (apical) surfaces and internalize. The primordium region transmits mechanical stress (black arrow) to cells in nearby rows of the propagation region. The cells of the adjacent row of the progagation region, only one cell of which is shown, then extend upwards towards the eggshell and transiently adhere there in a process mediated by integrin proteins. The cells of this row accumulate non-muscle myosin II. **c**, The first part of the propagation region internalizes after contraction of its apical surface. The entire process is repeated sequentially across this region.

system. Initial, mild cellular deformations from extrinsic physical stress, transmitted to a cell by a neighbour that is undergoing internalization, triggered MyoII accumulation in the apical region of the non-internalized cell. This accumulation drove cell shape changes that led to a further rise in physical stress and further accumulation of MyoII until cellular contraction reached a level that caused the cell to internalize. Mechanical coupling between neighbouring cells, mediated by protein complexes called adherens junctions that connect cells, ensured that forces were transmitted across the tissue, driving the progressive internalization of the propagation region.

Mechanical regulation of development has been described in other experimental systems[5]. However, it is often difficult to convincingly prove whether mechanical forces acting on cells are the cause or a consequence of a developmental process. Although it is challenging to assess mechanical inputs *in vivo*, Bailles and colleagues' work in the context of a whole embryo strengthens the growing body of evidence for mechanical force as a direct regulator of development, even as it raises new questions for research.

The cells in both endodermal regions have acquired the molecular hallmarks of endodermal identity before internalization occurs. Despite this similarity, why do these regions use distinctive internalization mechanisms? Maybe it is because, if the entire endoderm contracted simultaneously, embryo geometry would be impaired. Another possibility is that differences in mechanical sensitivity between these regions provide a buffer for coping with extrinsic forces that arise from other, concurrent developmental events.

Perhaps ironically, the identification of mechanical regulation as having a crucial role in the internalization of the propagation region provides a reason to examine the role of local gene expression further. How different, genetically, are the primordium and propagation regions? For example, if Fog levels were lower than normal, would this unmask the ability of cells in the primordium region to internalize by the mechanism associated with the propagation region?

It is useful to consider how the egg in which an embryo resides also defines the physical context for morphogenesis. Indeed, the eggshell was recently shown to have a major role in a tissue internalization event during the development of the beetle *Tribolium castaneum*[6]. Building on this work, Bailles and colleagues investigated whether, in fruit flies, the eggshell (a layer called the vitelline membrane) affected internalization of the propagation region. They observed that interactions between the embryo and the vitelline membrane provided a source of mechanical force. A cell on the cusp of internalizing first extended upwards towards the eggshell, then moved abruptly downwards towards the interior, and, just as a wave ripples through a sports-stadium crowd, this pattern of movements was repeated, one cell row at a time, by neighbouring cells of the propagation region (Fig. 1). During the upward movement, each cell transiently adhered to the eggshell by means of a protein called an integrin, which was expressed by the endodermal cell. This interaction caused the endodermal cell's apical surface to spread out, which seemed to provide a small amount of resistance against internalization, possibly generating a force that provided further positive feedback to boost MyoII levels and thus the efficiency of cellular contraction and internalization.

Dynamic adhesion has a crucial role in diverse examples of morphogenesis[7,8], and the specific mechanism of integrin-mediated adhesion to the eggshell is now shown, from the work of Bailles *et al.* and others[6], to be relevant in multiple developmental contexts. In *T. castaneum*, it is the mesoderm that expresses integrins, and these function not to augment mechanical stress but rather to limit tissue displacement, when the entire embryo undergoes progressive internalization relative to its non-embryonic outer protective tissues[6].

As future research improves our understanding of morphogenesis at the molecular and cellular levels, this should help to reveal how commonly interplay occurs between mechanical and genetic regulation. Such knowledge will provide a more complete picture of the factors that govern embryonic form across different tissues and species. ■

Kristen A. Panfilio *is at the School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK.*
e-mail: kristen.panfilio@alum.swarthmore.edu

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