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SPOTLIGHT

Charting the unknown currents of cellular flows and forces

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ABSTRACT

One of the central questions in developmental biology concerns how cells become organized into tissues of the correct size, shape and polarity. This organization depends on the implementation of a cell's genetic information to give rise to specific and coordinated cell behaviors, including cell division and cell shape change. The execution of these cell behaviors requires the active generation of mechanical forces. However, understanding how force generation is controlled and, importantly, coordinated among many cells in a tissue was little explored until the early 2000s. Suzanne Eaton was one of the pioneers in this emerging field of developmental tissue mechanics. As we briefly review here, she connected the quantitative analysis of cell behaviors with genetic assays, and integrated physical modeling with measurements of mechanical forces to reveal fundamental insights into epithelial morphogenesis at cell- and tissue-level scales.

KEY WORDS: *Drosophila*, E-cadherin, Cell packing geometry, Junctional remodeling, Tissue mechanics

Introduction

The interplay between genetics and physics at the cellular level ultimately gives rise to organs of characteristic sizes and shapes. Suzanne Eaton (1959-2019) pioneered an emerging field that aimed to understand this interplay, connecting the genetics of development with the physics of cellular forces. She and her colleagues explored how cell polarity, the cytoskeleton and cell adhesion intersect with material properties and mechanical forces to produce collective behaviors of large cell populations. Throughout her career, and in particular over the past two decades, she defined how state-of-the-art quantitative descriptions of cell dynamics, when combined with genetic manipulation, mechanical perturbations and physical models, can reveal profound insights into morphogenesis at the scale of large tissues.

Packing cells within a tissue

Suzanne's work in the early 2000s on planar cell polarity (PCP) offered an entry point into the analysis of how cells coordinate their behavior within a developing tissue (Fig. 1). PCP refers to the parallel alignment of cellular structures, such as hairs or stereocilia, in the plane of a tissue. At the molecular level, PCP depends on conserved proteins (called PCP proteins), first identified in *Drosophila*, that organize long-range polarity vectors across a cell population by coordinating local polarity between neighboring cells.

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Suzanne's early work on PCP focused on the adult *Drosophila* wing. In this context, several thousand cuticular hairs align to point towards the distal wing margin. During pupal development, hairs are produced at the distal vertices of individual wing epithelial cells. Both the distal and proximal cell junctions are decorated by PCP proteins, and Suzanne's early interest was to understand how PCP proteins are localized and trafficked to these cell junctions (Das et al., 2004; Eaton et al., 1996; Feiguin et al., 2001; Hannus et al., 2002; Paricio et al., 1999). By interfering with endocytic trafficking, Suzanne's group then revealed that this changes not only local patterns of adult wing hair alignment but also the packing geometry of epithelial cells in a critical period during which proximo-distal planar cell polarity is set up (Classen et al., 2005). During this period, the packing geometry undergoes a striking transition with respect to neighbor numbers and cell area. Specifically, a packing geometry characterized by high variation in cell areas and neighbor numbers changes to become a quasi-hexagonal cellular array, where similarly sized cells are in contact with an average of six neighbors. This change in packing geometry strongly correlates with the emergence of proximo-distal planar polarity and is driven by the dynamic remodeling of adherens junctions between neighboring cells (Classen et al., 2005). Junctions shrink and elongate dynamically, occasionally transitioning through a 4-way vertex intermediate (so-called T1 transitions). Thereby, cells lose and gain contact with neighboring cells.

Strikingly, at that time, myosin-driven cell rearrangements involving T1 transitions were also reported by the group of Thomas Lecuit to be essential for tissue elongation during *Drosophila* embryonic germband extension (Bertet et al., 2004). Thus, the remodeling of cell contacts through T1 transitions emerged as a universal driver of epithelial morphogenesis. The dynamics of these cell rearrangements and the resulting changes in packing geometry invoked analogies to the physics of ordered and disordered foams (Hayashi and Carthew, 2004; Zallen and Zallen, 2004). Although preliminary, these analogies opened up the opportunity to apply physical models to the mechanobiology of cell-cell interactions in large epithelial sheets.

Mechanical forces guide cell packing

Consequently, Suzanne connected with Frank Jülicher – a biophysicist who had just moved to Dresden at that time. They embarked on an extraordinary friendship that spanned 15 years and led to multiple landmark studies in which they combined quantitative imaging with genetic analysis and physical modeling to understand tissue-level principles of morphogenesis (Dye et al., 2017; Eaton and Jülicher, 2011; Etournay et al., 2016, 2015; Farhadifar et al., 2007; Iyer et al., 2019; Jülicher and Eaton, 2017; Merkel et al., 2017; Sagner et al., 2012). It was amazing to watch Suzanne and Frank connect and quickly drift off into detailed discussions of physical laws and their mathematical principles.

Their first collaboration demonstrated that epithelial packing geometry displays universal features that are indeed based on the physical properties of cells and the reorganization of junctional

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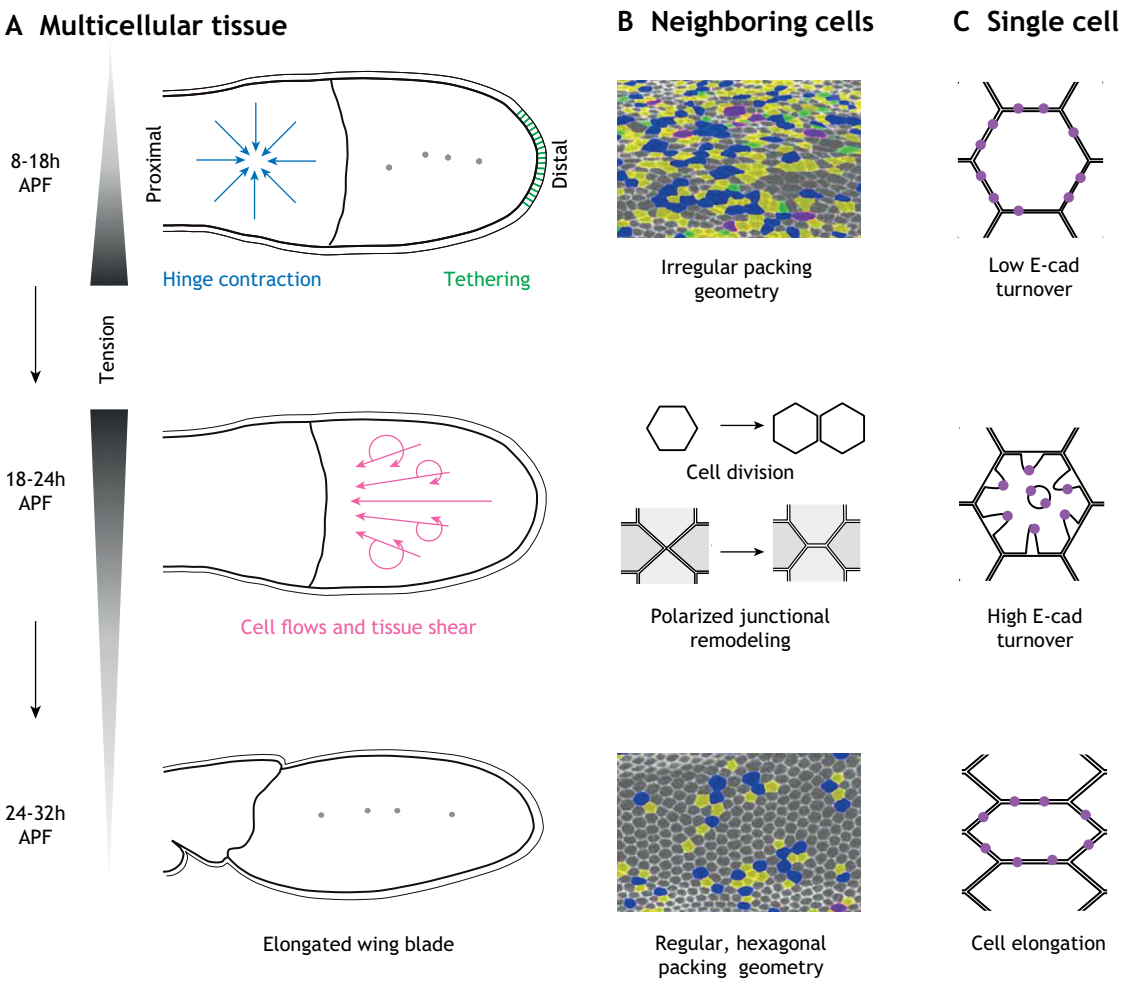

Fig. 1. Charting the unknown currents of cellular flows and forces. The figure illustrates how cell behaviors are coordinated (at the tissue, cellular and single-cell levels) during morphogenesis of the *Drosophila* wing. (A) Top: During early pupal development (8-18 h after puparium formation; APF), the proximal wing hinge contracts (blue arrows), thereby imposing shear stress onto the wing epithelium, which is tethered by Dumpy (green) to the distal cuticle. At this time, sensory organ precursors (gray dots) have a stereotypic position within the blade. Middle: The shear stress then induces a net 'flow' of cells with a proximal and rotational component towards the wing midline, thereby elongating the wing blade. Bottom: The final shape of the wing at 32 h APF is elongated. Larger spatial distances between sensory organ precursors (gray dots) arise from convergent-extension type elongation of the wing field. (B) Top: At the cellular level, the tissue initially assumes an irregular, sub-hexagonal packing geometry (tetragon, green; pentagon, yellow; hexagon, gray; heptagon, blue; octagon, purple). Middle: Cell divisions and the polarized addition of new junctions along the proximal-distal axis of the wing tissue then resolve shear stress. Bottom: The tissue ultimately assumes a regular, hexagonal packing geometry representing a low-energy packing configuration. (C) Top: Before hinge contraction begins and tension in the wing builds, the junctional adhesion molecule E-cadherin (E-cad, purple) displays a low turnover rate. Middle: Then, at time points of high shear stress, E-cadherin turnover increases, allowing cells to viscoelastically respond to tension in the tissue. Bottom: As a result, cells assume an elongated shape. This elongated shape is subsequently resolved by junctional rearrangement to allow the more isotropic shape observed in final hexagonal packing geometries.

networks as a result of cell divisions (Farhadifar et al., 2007). Central to this study was a cell-based mathematical model (a 2D vertex model). In this model, the arrangement of cells is represented by a network of vertices connected by cell junctions for which force-balanced configurations are governed by parameters relating to cell elasticity, actin-myosin contractility and junctional adhesion. Suzanne and her co-workers validated this model with a quantitative description of cell packing geometries in the proliferating *Drosophila* wing disc and, importantly, with a quantitative analysis of contractile forces acting on adherens junctions (Farhadifar et al., 2007). Prior to this study, laser ablation of cell boundaries had been used to demonstrate that adherens junctions are in fact under tension (Kiehart et al., 2000). However, by using laser ablation and analyzing the resulting recoil velocities of the adjoining vertices, Suzanne's work introduced a quantifiable measure of junctional tension to the field of tissue mechanics. Today, junctional recoil velocities are a central experimental parameter used to measure relative forces in epithelia.

Suzanne's combination of approaches was hugely influential on the field, as it established state-of-the-art quantitative read-outs for force inference (cell packing geometry), force measurements (junctional recoil velocity after laser ablation) and force predictions (physical modeling). This work ultimately moved the field towards a quantitative, tissue-level analysis of actomyosin force transmission at adherens junctions and explained how cells arrange themselves in an epithelial tissue as a result of junctional remodeling, cell division and apoptosis.

Pulling forces result in tissue shear and cellular flows

While the field expanded to describe how cell-autonomous forces affect tissue morphogenesis, Suzanne's work in the early 2010s quickly progressed to dissect the contribution of non-autonomous

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forces to tissue size and shape. At that time, PCP proteins were known to be able to locally coordinate planar polarity between neighboring cells but how the robust long-range proximo-distal polarity of the pupal wing was set up remained unclear. In fact, a diffusible morphogen called Factor X had been proposed to form a gradient along the proximo-distal axis but remained elusive (Strutt, 2008).

Suzanne's work provided a stunning solution to this problem by characterizing in detail the cellular events that occur to align pupal wing cells along the proximo-distal polarity axis (Aigouy et al., 2010). Specifically, she and her group demonstrated that cells across the wing blade are subject to anisotropic tension in the proximo-distal axis as a result of the contraction of the proximal hinge field, a large cell population connected to the wing blade. The non-autonomous pulling forces generated by contraction of the hinge create shear within the wing tissue. This shear stress drives local flows of cells in a convergent-extension-like manner and helps to elongate the wing. Importantly, this flow also exhibits a rotational component. Combining a PCP polarity module in the 2D vertex model with experimental validation, Suzanne and colleagues made the unexpected prediction that the rotational component of cell flows reoriented an initial fan-shaped PCP polarity towards a proximo-distal axis (Aigouy et al., 2010).

Suzanne's discussion of these observations in the context of physical parameters outlined the intriguing possibility that shear can reorient polarity along or perpendicular to the shear vector axis. Based on these arguments, shear vectors arising from non-autonomous forces in a tissue may represent the long-sought Factor X, providing long-range polarity cues to align PCP across a tissue. Thus, in line with the emerging understanding at that time that classical morphogen concepts cannot sufficiently explain all aspects of tissue morphogenesis, Suzanne's work highlighted that mechanical signals can drive long-range, self-organizing processes across epithelial sheets.

Molecular anchors resist pulling forces

Subsequently, Suzanne's lab revealed – using beautiful live imaging and mechanical manipulation of hinge and wing margin domains – that shear stress and the resulting cellular flow depends not only on hinge contraction but also on macroscopic anchor points in the distal wing margin (Etournay et al., 2015). This anchoring is mediated by the large apical matrix protein Dumpy, which attaches the distal wing margin to the overlying cuticle and withstands the pulling forces generated by proximal hinge contraction. A tour-de-force of automated image analysis at high spatial resolution provided a description of the specific cell behaviors that occur during and after hinge contraction. Specifically, a detailed quantitative deconstruction outlined the temporal contribution of cell elongation, cell division and junctional rearrangements that occur as shear builds and the force balance is restored to an ordered hexagonal cellular array (Etournay et al., 2015). This work demonstrated, in breathtaking quantitative detail, that wing epithelial cells generate and respond to mechanical stress *in vivo*. Thus, this work highlighted the interplay between cell-autonomous and non-autonomous forces and provided a framework for understanding how this interplay specifies the remodeling dynamics of the junctional network that ultimately creates a wing of the correct size and shape.

Sensing mechanical stress

But how do cells sense mechanical stress at the molecular level? Most recently, work from Suzanne's lab revealed that the cellular basis for mechanosensation of shear in the pupal wing may reside with p120 catenin (Iyer et al., 2019), a protein that associates with E-cadherin at adherens junctions before and after hinge contraction. However, at times of high shear stress (e.g. during hinge contraction), p120 catenin dissociates from adherens junctions and allows for an increase in the rate of E-cadherin turnover. This molecular transition facilitates the viscoelastic behavior of the tissue to relieve shear stress by facilitating cell elongation. Strikingly, p120 mutant wings lose viscoelastic features and behave elastically. As a consequence, they are hyper-elongated, reflecting a failure of wing epithelial cells to resist the shear stresses arising from hinge contraction (Iyer et al., 2019). This study provided the first cell-resolved quantitative description of viscoelastic behavior in epithelial tissues *in vivo* and shed light on the molecular regulation of tissue stress resolution.

Suzanne's personal legacy

Throughout her career, Suzanne approached the science of tissue mechanics with a playful curiosity that was such an inspiration to us. She understood the power that mathematical and physical approaches can bring to biology and effortlessly integrated these approaches into her work. Her work inspired studies on quantitative cell dynamics and physical modeling in many other systems, thus expanding the idea of tissue mechanics into the broader scientific community. Her enthusiasm and generosity in sharing her insights made her ideas accessible and inspired many people to join her efforts in this field. Personally, it was an incredible privilege to have known and have been able to work with her. She always inspired us to move beyond the seemingly obvious, to take an unbiased look at what we see, and to accept the failures of our hypothesis. As Suzanne once said '… because really, the truth is so much more interesting…'.

This article is part of a collection that commemorates the work of Suzanne Eaton. See also Mlodzik (2020), Palm and Rodenfels (2020) and Prince et al. (2020) in this issue.

Competing interests
The authors declare no competing or financial interests.

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