Dispatches

Developmental Biology: A Growing Role for Computer Simulations

Keeping cells separated in well-defined domains is essential for development. A new computational–experimental study elucidates the physical mechanisms that establish and maintain the dorsal-ventral compartment boundary in the *Drosophila* wing disc and demonstrates the increasing value of computer simulations in developmental biology.

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A fundamental aspect of developmental biology is the ability of tissues to establish domains with distinct cell populations. Such separation requires the establishment and maintenance of smooth boundaries between different cell populations. Different from non-lineage boundaries, where cells switch fate as they cross the boundary, lineage or compartment boundaries separate cells whose fates have been already determined by the activation of selector genes [1]. It is presently unclear, however, how such compartment boundaries can be robustly maintained in the face of the dramatic rearrangements that accompany tissue development, for example, during phases of rapid cell proliferation, increased cell migration or large-scale tissue deformations. In this issue of *Current Biology*, Aliee *et al.* [2] present a combined computational–experimental study to systematically identify the physical mechanisms that maintain the dorsal-ventral compartment boundary in the *Drosophila* wing disc. As the wing disc is rapidly growing, cell proliferation could lead to a rough dorsal-ventral boundary or cell mixing (Figure 1A). The study by Aliee *et al.* [2] now confirms earlier suggestions that the dominant mechanism that prevents this happening is an increase in actomyosin-generated tension at the compartment boundary. Equally important, it also shows that this effect is complemented by oriented cell division and global tissue elongation, and that decreased cell proliferation rates at the boundary are not significant.

The wing disc of *Drosophila melanogaster* is an ideal model system to study the dynamics of compartment boundaries. Due to its flat, quasi two-dimensional shape, it can be imaged with optical microscopy over its developmental timescale, i.e. a few days. Moreover, powerful methods like genetic mosaics are available to systematically perturb the system. As the wing disc develops, two mutually orthogonal compartment boundaries emerge that divide the tissue into four distinct quadrants. The anterior-posterior boundary derives from early polarization in the embryo, while the dorsal-ventral boundary arises during mid-larval development of the wing. Each compartment boundary is associated with corresponding morphogen gradients, for example, Decapentaplegic (Dpp) along the anterior-posterior axis and Wingless (Wg) along the dorsal-ventral axis. An earlier computational–experimental study of the anterior-posterior boundary has shown that it is predominantly maintained by increased tension in the cell junctions forming the compartment boundary [3]. This increased tension results from local upregulation of the actomyosin system by biochemical signals localized to the boundary.

Turning to the dorsal-ventral boundary, it has been noted before that here too the actomyosin system is upregulated [4]. This would suggest that a similar mechanism of increased cellular bond tension at the interface could be responsible for establishing the boundary in this case. Other possible mechanisms do, however, exist, including oriented cell division, controlled proliferation rates or anisotropic stresses external to the boundary. To test these hypotheses in a controlled and quantitative manner, Aliee *et al.* [2] used a computational approach based on a vertex model (Figure 1B). Vertex models were introduced into biology by Nagai and Honda [5] and since then have become increasingly popular for modeling a range of epithelial systems (for example [6–9]). In particular, a vertex model has also been used before to study the dorsal-ventral boundary [10]. If cell walls are imaged with fluorescence constructs for proteins that localize to the plasma membrane (e.g. the cell–cell adhesion receptor cadherin), a polygonal network of ‘cell bonds’ becomes visible. A vertex model explicitly simulates the dynamics of these bonds by defining an energy function depending on their spatial organization. Cell proliferation is introduced by doubling the area of one cell, dividing it and then relaxing the network to mechanical equilibrium. Vertex simulations thus accurately capture the foam-like dynamics of epithelial systems where interfacial energies dominate.

In contrast to their earlier work on cell-bond tension at the anterior-posterior boundary [3], for the dorsal-ventral boundary the authors now consider a larger range of possible mechanisms, each of which is tested computationally for its effectiveness in establishing the boundary (Figure 1C). In order to systematically compare theory and experiment, the same quantitative measure of boundary roughness is applied to both the imaging data and the simulated configurations. Following a concept originating from the mathematical characterization of fractals, boundary roughness is defined as the average deviation from a straight line as a function of distance along the boundary (alternatively, one could have used measures based on local boundary curvature). Using this approach, the authors show that only an increase in cell-bond tension (Figure 1C, case I) or reduction in cellular proliferation near the dorsal-ventral boundary (Figure 1C, case II) would be sufficient by themselves to generate both smooth boundaries and no cell mixing. Looking at the experimental data, however, they

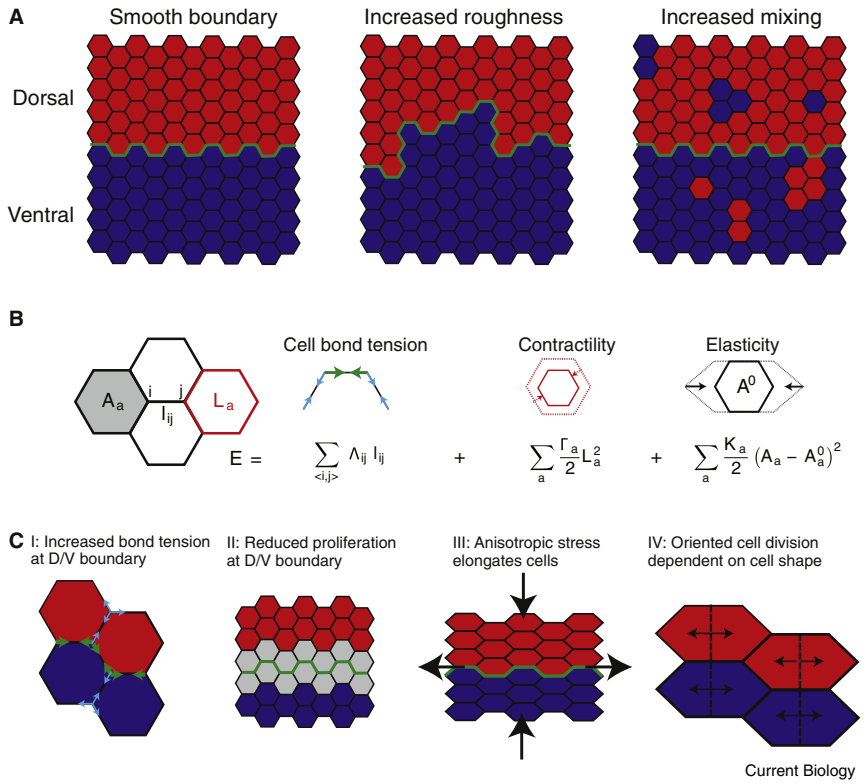

Figure 1. Cell bond tension in conjunction with oriented cell division and stress anisotropy maintain dorsal-ventral compartment boundaries in the *Drosophila* wing disc.

(A) Schematic of dorsal (red) and ventral (blue) compartments separated by the dorsal-ventral boundary (green). After the boundary is established, it becomes increasingly smooth (left). Under the action of rapid proliferation and in the absence of stabilizing forces, the boundary can become rough (middle), or pockets of cells can be left isolated, generating mixing of the two compartments (right). (B) Vertex models lead to foam-like structure and dynamics. An effective energy or work function E is ascribed to each two-dimensional configuration, characterized for each cell a by the length l_{ij} between nodes i and j, cell area A_a and cell contour length L_a. The work function is then minimized after each change to the system, for example due to cell division. Three main effects are taken into account. The first term models bond tension, as it reduces bond length. The second term models cell contractility, as it reduces contour length. The third term models elasticity, as it implements a preferred cell area. (C) Possible physical mechanisms for establishing and maintaining the dorsal-ventral compartment boundary: increased bond tension (case I), locally reduced proliferation rates (case II), anisotropic stress (case III) and oriented cell division (case IV).

observe that cell proliferation is reduced at the compartment boundary only relatively late in development. Increasing the proliferation in this region to the level away from the boundary by co-expressing the cell-cycle regulators *string* and *cyclin E* had no effect on the observed roughness of the boundary. Therefore, for the situation under investigation, cell proliferation can be ruled out as the dominant mechanism, suggesting a scenario where bond tension dominates.

Experimentally, the amount of tension in the cellular bonds can be determined using laser-cutting experiments, where bonds are severed and the maximal distance of retraction is measured (alternatively, one can measure initial speed of retraction). These experiments demonstrate the existence of increased tensional forces at the compartment boundary, but with only a 2.5–3-fold increase, which in the computer simulations is insufficient to explain the observed smoothness of the boundary. By introducing each of the other mechanisms (Figure 1C, cases III and IV) systematically into the simulation and comparing with experimental data, the authors show that the system actually is dependent on a combination of physical factors for its smoothness and robustness. Surprisingly, oriented cell divisions, but only in combination with anisotropic stresses, are required to complete the picture. The anisotropic stress elongates the cells parallel to the dorsal-ventral boundary, thus guiding the oriented cell divisions in the 'right' direction. This supports a previous observation that elongated cells at the dorsal-ventral boundary will result in oriented cell divisions that smooth the compartment boundaries [10].

The present study strongly suggests that locally increased tension is a fundamental mechanism for ensuring smooth compartment boundaries. This strengthens the emerging view that mechanical tension might play an equally important role as differential adhesion in separating cells [11]. Increased tension in compartment boundary cells has been suggested to be generated through local upregulation of the actomyosin system [4]. Aliee *et al.* [2] explore the contribution of actomyosin by examining mutants in which myosin activity is perturbed and they find that roughness dramatically increases. More importantly, however, the study shows that, in general, increased bond tension is supplemented by additional mechanisms that 'top up' this effect. This study also demonstrates the importance of combining computer simulations and experiments, particularly in cases where different mechanisms do not simply superimpose, but instead act synergistically. For example, although the simulation indicates that reduced proliferation can lead to boundary smoothing, experimental data show that this potential mechanism is not in fact used and instead a combination of other mechanisms is favored. One of the driving forces behind the new usefulness of computer simulations to developmental biology is the extraction of appropriate quantitative measures from experimental data. Such quantitative tissue analysis is now an important part of many studies in developmental biology.

Despite the large success of vertex models in understanding the physical mechanisms underlying development of the *Drosophila* wing, it has to be noted that many situations in development are less accessible to such simulations. Vertex models are appropriate if interfacial tension dominates the cell dynamics. This situation dramatically changes when no cell walls are present (as in the syncytium) or if cells become very

motile. It is also much more difficult to deal with non-flat geometries (as in gastrulation). In considering this diversity of physical effects in developmental biology, we will thus have to draw on a range of simulation frameworks, from simulations based on interacting elastic bodies [12] to continuum approaches [13]. Additionally, we face the need to accurately incorporate the wealth of subcellular genetic and biochemical detail being discovered into multi-scale descriptions. Thus, if one thinks about achieving a systems-level understanding of all stages of development, the challenge is now to achieve the same synergy between simulations and experiments in other model cases, as demonstrated so well by Aliee *et al.* [2] for the wing disc.

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Taste: Unraveling Tomato Flavor

New research integrating genetics, chemistry and psychophysics has led to a model for tomato flavor intensity comprising sugars and acids plus six volatile molecules, providing a blueprint for improving the flavor of what has become an iconic symbol of the declining quality of fresh fruits and vegetables.

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For those of us over forty (or fifty) the memory of how tomatoes used to taste is vivid and tomato flavor now seems to be a cherished 'lost virtue' of a recent but bygone era. I have heard, anecdotally, that younger consumers do not have the memory or even the notion that tomatoes were once so flavorful that you could take one in your hand and eat it straight away just like we regularly eat apples or peaches.

The chemical composition of tomatoes has been generally known for some time and a few important determinants of taste and aroma have been characterized. For example, several studies have pointed to an overriding significance of sugars and acids, and in particular to the sugar:acid ratio as a major determinant of tomato flavor [1]. High sugar levels also contribute to the efficiency of tomato processing and, not surprisingly, this trait has been a frequent target for tomato breeders [2]. In addition to the importance of sugars and acids, the characterization of a set of volatiles with concentrations that exceeded their odor threshold pointed to a set of 16 volatiles that have been widely cited as conferring the major tomato aroma [3]. The complexity of volatile composition has, understandably, discouraged tomato breeders and there are relatively few examples of genetic improvement programs targeted towards enhancing the profile or quantity of tomato fruit volatiles [4]. Indeed, one explanation for the decline in tomato flavor is that intensive breeding for production traits, such as yield, disease resistance and sugar content, in the absence of selection for flavor, has allowed the latter trait to progressively decline. In addition to the genetic drift in flavor characteristics, the normal practice of harvesting tomato fruit at the green stage followed by the induction of ripening by ethylene application has also been pointed to as a practice that degrades both sugar and volatile levels with consequent effects on flavor [5].

A study by Tieman *et al.* [6], reported in this issue of *Current Biology*, does not reduce the complexity of tomato flavor determinants, nor does it lead to the perfect-tasting tomato, but it has revealed important insights into the molecular basis of tomato flavor and provides some leads as to what it could become, again. A surprising early result of the analysis was the identification of 68 potentially significant volatiles, some with over 3,000-fold concentration differences between varieties, in spite of the well-known narrow genetic base of cultivated tomatoes. The wide variation in volatile constituents provided an opportunity to develop a quantitative assessment of the determinants of flavor and, more importantly, determinants of preference or 'liking' — in other words to characterize a good-tasting tomato at the molecular level. The experiments integrated tomato genetics to drive fruit chemical diversity, analytical chemistry to identify a diverse array of constituents, and psychophysics to provide a robust scaling methodology that allowed for normalizing across individual tasters and across seasons.