Epithelial topology

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Summary

It is universally accepted that genetic control over basic aspects of cell and molecular biology is the primary organizing principle in development and homeostasis of living systems. However, instances do exist where important aspects of biological order arise without explicit genetic instruction, emerging instead from simple physical principles, stochastic processes, or the complex self-organizing interaction between random and seemingly unrelated parts. Being mostly resistant to direct genetic dissection, the analysis of such emergent processes falls into a grey area between mathematics, physics and molecular cell biology and therefore remains very poorly understood. We recently proposed a mathematical model predicting the emergence of a specific non-Gaussian distribution of polygonal cell shapes from the stochastic cell division process in epithelial cell sheets; this cell shape distribution appears to be conserved across a diverse set of animals and plants.(1) The use of such topological models to study the process of cellular morphogenesis has a long history, starting almost a century ago, and many insights from those original works influence current experimental studies. Here we review current and past literature on this topic while exploring some new ideas on the origins and implications of topological order in proliferating epithelia. *BioEssays* 30:260–266, 2008. © 2008 Wiley Periodicals, Inc.

Introduction

Perhaps the most abstract expression of emergence in biological systems is the notion of cellular automata, where relatively simple rules expressed by propagating 'cells' can generate large-scale spatial patterns of incredible visual complexity.(2,3) Cellular automata represent a purely theoretical exercise, but tangible, real-world examples of emergence in biology exist as well. The cobblestone pattern of cells in epithelial sheets, for example, has fascinated scientists for over a century because of its resemblance to other cellular structures that arise from relatively simple physical processes. These include honeycombs,(4) soap froths,(5) crack structures in thin ceramic glazes,(6,7) drying mud and even geological features of extraterrestrial lunar surfaces.(8) In each of these instances, a complex large-scale pattern emerges from the sum effect of numerous and unrelated local events. In the early 1900s, D'Arcy Wentworth Thompson's *On Growth and Form* presented a detailed argument of how the combined forces of minimal surface energy at the cell level and close packing at the cell aggregate level could result in predominantly hexagonal two-dimensional epithelial topologies and predominantly 14-sided polyhedral cells in three-dimensional aggregates.(4) Thompson's work presented extensive examples of complex cellular structures from studies of honeycombs to metallurgy, spawning an interest in mathematical properties of biological pattern that has persisted for nearly a century. More recently, Weaire and Rivier (1984) reviewed the remarkable variety of related cellular structures in the natural world and their universal properties.(9) This style of comparative analysis has slowly bridged between disciplines and significantly impacted the treatment of cellular structures in physics, geology and even the biology of the fruit fly, *Drosophila melanogaster*.

In the case of simple (monolayer) epithelial sheets, cells assemble adhesive junctions with their neighbors, resulting in an irregular lattice-like planar configuration of cell boundaries.(10) Strikingly, a simplified two-dimensional diagram of epithelial cell edges is difficult to distinguish from comparable nonliving systems, such as a simplified map of basalt columns in the Giant's Causeway in Northern Ireland (Fig. 1A–C).(9) The two-dimensional lattice structure of adherent epithelial cells indicates a tight connection between *topology* (cell–cell connectivity) and *geometry* (cell shape). Simply put, a cell with *n* adherent neighbors is an *n*-sided polygon. Of course, overall cell shape incorporates many other geometric features such as area, internal angles and side lengths, such that an *n*-sided cell can be quite irregular in form. Nevertheless, the number of sides of a polygonal cell is determined solely by the number of neighbors that it contacts, and to change its polygonal shape the cell must gain or lose a neighbor in some way. We can thus understand the polygonal shape of epithelial cells simply by reasoning about dynamic changes in cell–neighbor contacts. Further, the topological impact of such changes (such as those resulting from cell migration, intercalation or division) can be interpreted without necessarily considering molecular events at the level of cytoskeletal regulation, junctional biogenesis or other processes. This abstraction, i.e. topological models of physical cellular structures, has been a useful tool both historically and in contemporary literature.

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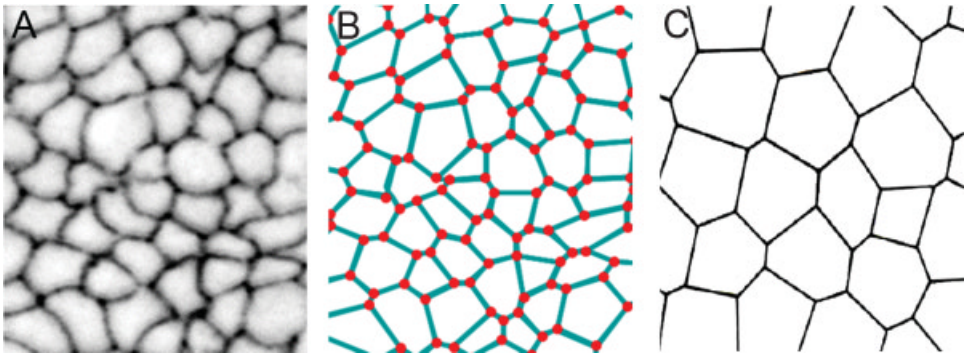

Figure 1. Living and nonliving surface topologies. **A:** Confocal micrograph of cell–cell junctions reveals the characteristic polygonal topology of the *Drosophila* wing imaginal disc. **B:** Diagram of the extracted polygonal network from A. **C:** Diagram of the extracted topology of the Giant's Causeway geological formation in Northern Ireland. The apparent visual similarity between B and C is striking, although the actual polygon distributions are quite different. Panel C is reproduced with permission from: ''Soap, Cells and Statistics: Random patterns in two dimensions.'' Contemporary Physics Vol. 25, No. 1, pp. 59–99 (1984). Taylor and Francis Publishing, London, UK.[9]

Epithelial topology in the fruitful fly field

Recent advances in live imaging, combined with the use of transgenic *Drosophila* strains expressing fluorescently tagged fusion proteins, have led to a series of studies making use of topological arguments to understand epithelial morphogenesis in vivo.[10–15] These studies illustrate the remarkable variety of strategies employed by biological systems to effect the rearrangement of cells within an epithelium. Retinal cells in the fly eye, for example, achieve an optically optimal packing by expressing adhesion molecules in a simple pattern and then allowing free energy minimization to effect the final conformation of cells.[11] In this context, the arrangement of cells is directed by the same physical processes that dictate packing of soap bubbles under similar conditions. In contrast with free-energy minimization mechanisms for determining epithelial topology, a biologically directed Myosin II-dependent junctional transition underlies changes in cell topology during gastrulation of the fly embryo. Here, relatively simple spatial patterns of Myosin II distribution cause locally polarized neighbor-exchange events that drive global elongation of the germ band (Fig. 2A).[12] Simultaneously during *Drosophila* gastrulation, multicellular rosettes form along the embryonic dorsoventral axis and then resolve in alignment with the embryonic anteroposterior axis, illustrating a second mechanism of tissue reorganization during convergent extension (Fig. 2B).[13,14] Later in fly development, similarly directed events transform epithelial topology in the developing pupal wing epithelium. In this case, the irregular topology of the proliferating disc re-sorts into a predominantly hexagonal lattice through polarized cell-junction remodeling governed by the planar cell polarization pathway (Fig. 2C).[15] In sum, these examples illustrate how fairly simple cell behaviors at the local level can manifest in vastly more complex morphogenetic properties of the entire cell sheet. Presumably, however, each mechanism for the directed re-arrangement of cells is constrained by the initial topological conditions. An important question thus becomes how the default epithelial topology is determined, and how initial conditions might constrain or potentiate different cellular rearrangements.

An irregular order: proliferation-dependent epithelial topology

In contrast with directed mechanisms that control epithelial topology by modulating specific cell–cell contacts, predictable frequencies of cellular polygons emerge in epithelia as a simple consequence of cell division.[1,16] Based on ex-vivo time-lapse movies of cell division in the *Drosophila* wing imaginal disc, we developed a simple stochastic model to capture how mitotic division alters the topology of an epithelial cell network (Fig. 3). A central theoretical finding was that, in the absence of cell sorting or migration, stochastic cell division processes will intrinsically converge to an irregular yet predictable global topology regardless of the initial tissue state. Hence, even starting with all hexagons or all nonagons, the distribution of cellular polygons will eventually reach equilibrium at 28.9% pentagons, 46.4% hexagons, 20.8% heptagons, and lesser numbers of other polygon types (Fig. 3). Emergence of this equilibrium distribution is a consequence of the balance between the probability of losing sides (i.e. neighbors) through division and the probability of gaining sides through neighboring cell division events (Fig. 3). Confirming the generality of this concept, we observed the same predicted distribution of polygon types in proliferating epithelia from three divergent animal phyla. Thus, even the simple process of local cell division can lead to the emergence of a predictable default topology at the global tissue level.

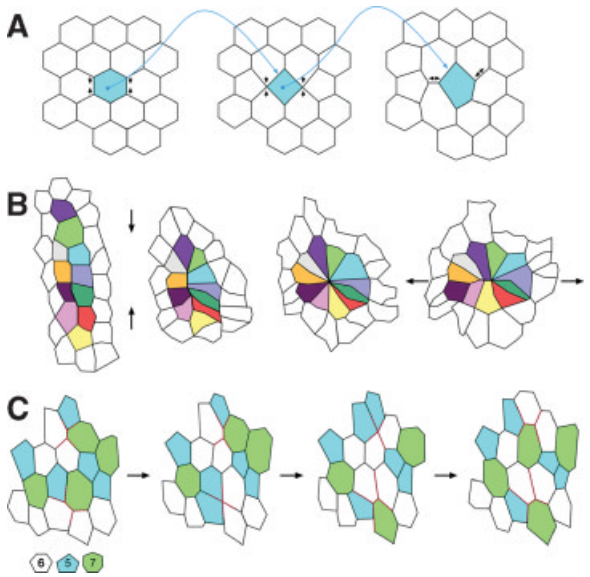

Figure 2. Directed cellular mechanisms underlying changes in epithelial topology. **A:** Planar cell intercalation during germ band extension in the developing *Drosophila* embryo (adapted from Ref. 12). Active mechanisms, dependent upon polarized localization of myosin II, lead to the disassembly of junctions along the embryonic dorsoventral axis and expansion along the anteroposterior axis. The net effect of these movements contributes to a global process of convergent extension and axis elongation.[12] **B:** The formation of multicellular rosettes during *Drosophila* gastrulation (adapted from Blankenship et al. 2006). At a local level within the *Drosophila* blastoderm epithelium, multicellular rosettes form as a consequence of asymmetries in the distribution of F-actin, Myosin II and junctional complexes along the embryonic dorsoventral axis. Resolution of the rosette conformation proceeds by F-actin and E-Cadherin accumulation near the rosette vertex, followed by Bazooka recruitment and expansion of cell interfaces along the anteroposterior axis. In theory, local processes of spatially coordinated rosette formation and resolution will drive global elongation of an epithelium along one axis and contraction on the other.[14] **C:** Hexagonal cell packing during pupal development in the *Drosophila* wing (adapted from Classen et al. 2005). Much like the simple rearrangements described in **A**,[12] pupal wing cells utilize the planar polarization pathway to collapse some junctional interfaces and expand others (*red dashed lines*). The net effect of this local behavior is reorganization of the epithelial lattice toward a more regular hexagonal array. While some of the molecular machinery behind this process has been defined, it remains unclear what targets the remodeling of specific cell contacts to achieve the quasihexagonal end state lattice.[15]

It is notable that a similar connection between epithelial topology, proliferation and cell shape was made in plants almost a century ago by F.T Lewis.[17,18] Drawing on the literature of his time, Lewis cited the first edition of Thompson's *On Growth and Form* as the inspiration for his own study of such properties in plants. Working with proliferating cucumber epidermis, Lewis reported many deep and quantitative observations that still remain poorly understood. For example, Lewis determined the polygonal cell shape distribution for 1000 interphase epidermal cells as well as the polygonal shape distribution for mitotic cells. The results indicated that the global distribution of cells was bounded (no three-sided cells or ten-sided cells), asymmetric (25% five-sided but only 22% seven-sided), and the majority were hexagons (47%). Dividing cells, on the other hand, seemed to have a similarly shaped distribution, but shifted over by one such that the majority of mitotic cells were seven-sided. These findings are strikingly similar to what we reported in the *Drosophila* wing disc. Not only is the distribution almost identical, but both systems also display the same upward shift in the average sidedness of mitotic cells (Fig. 3).[1] Lewis made other interesting empirical observations about the relationship between topology and geometry, including the fact that the average area of a cell varied linearly with the number of sides (now known as Lewis's Law) which, not surprisingly, also holds for the *Drosophila* wing disc cells (RN, AP and MG, unpublished data). Tissue organization and the cellular and genetic mechanisms for division differ significantly between plants and animals. Nonetheless, the fact that both share extensive tissue-level similarities hints at a possible universal linkage between cell proliferation and epithelial topology throughout multicellular life.

Mathematical models for epithelial topology in proliferating epithelia

Lewis' empirical observations led to the conclusion that topology was in fact a strong proxy for many aspects of geometry. But how is tissue topology, with its majority of hexagons, created through cell division? Lewis correctly conjectured that, unlike soap froths where units rearrange to minimize energy, tissue topology is primarily determined by cell division, at least in the absence of directed cell rearrangements. Together with his mathematician colleague, W.C Graustein, Lewis demonstrated that the average number of cell sides in an epithelial sheet must be six. They came to this conclusion by considering how cell division can change a hexagonal lattice and noticed that any division of a cell preserves the average of six sides by creating both smaller and larger cells. They also immediately noted that a theoretical average of six sides does not necessitate that any cells in the sheet be six-sided. Graustein even conjured up a diagram of a lattice with a 14-sided cell and many three-sided cells to demonstrate the striking degree of disorder that is possible while still maintaining an average of six sides.[19]

Lattices drawn on two-dimensional surfaces have some universal mathematical characteristics, regardless of whether they are tissues or soap bubbles, or whether they are created by cell division, death, birth or rearrangement. For example, the average of six sides holds for any planar lattice with mostly

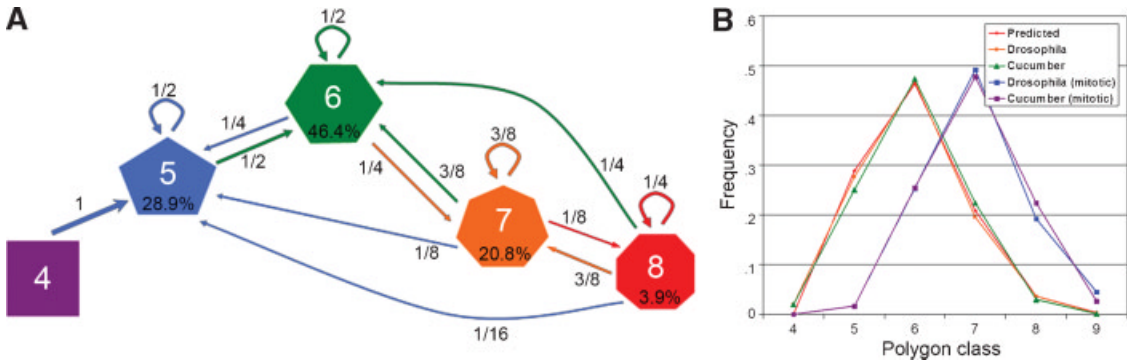

Figure 3. The Markov model for epithelial topology (Adapted from Ref. 1). **A:** A graphical depiction of the stochastic Markov model of cell division. Shapes represent possible polygonal states of a cell, while arrows represent possible transitions between states corresponding to division. Arrows are labeled with transition probabilities P_{nm} and color-coded according to the polygonal end state. **B:** Empirical polygon distributions in plant and animal epithelia. The theoretical equilibrium distribution of polygons predicted to exist in proliferating epithelia (*red line*) is in close agreement with both the *Drosophila* wing (*orange*) and the cucumber epidermis actual cellular topologies (*green*). In the entire cell sheet, cells possess an average of approximately six sides. In both *Drosophila* and cucumber epidermis, mitotic cells have more sides than the general population, exhibiting a distinctly shifted distribution with an average of nearly seven sides (*blue* and *purple* lines, respectively).

tricellular junctions (assuming negligible boundary conditions) as a simple consequence of Euler's theorem. Nevertheless, despite the universal mathematical properties of planar lattices, the actual distribution of cell shapes can vary significantly from system to system while still maintaining an average of six sides. The Giant's Causeway geological formation is composed of 40% pentagonal cells,[17] while the *Drosophila* pupal wing epithelium exhibits almost 80% hexagonal cells.[15] To more completely understand the specific distribution of cell shapes in each case, it is first necessary to define the local mechanism controlling cell topology and then model the emergent consequences of that local mechanism for global structure.

Previously, the distribution of polygonal shapes in proliferating lattices has been modeled using continuous rate equations with several adjustable parameters.[20,21] This approach captured the rate of division, the possible dependence of division rate on cell shape, the probability that division is symmetric (daughters have identical shapes), and the probability that the polygon class of a given cell increases as a function of neighbor divisions. Some parameters were estimated from empirical measurements of various physical systems and minimum entropy arguments, while other free parameters were used to explore the space of possibilities. In particular, these models examined the notion that the cell-shape distribution could be controlled by varying the extent to which the rate of division depended on polygonal cell shape. Intriguingly, as one increases the probability of division of cells with more sides at the expense of cells with fewer, the percentage of six-sided cells continually increases and deviation of cell shape from this mean decreases. This kind of system can in fact produce a wide range of equilibrium distributions, not just ones with peaks at hexagonal cells.

Our recent mathematical model shares many similarities with this approach, including separate terms to represent the probabilities for cell shapes created through self-division versus neighboring cell divisions.[1] One key difference is that our abstract model was designed to capture specific, empirically derived properties of the developing *Drosophila* wing disc. For example, we assume the existence of discrete mitotic cycles such that cells have roughly equal rates of division. Although the cells may divide asynchronously, the population as a whole undergoes rounds of expansion. We also focused on analytical models of division symmetry and neighbor effects, rather than using adjustable or fitted parameters. We derived division symmetry based on the irregular junction distributions and variable division orientations observed in vivo.[1,22] We also used an analytical model (Box 1) to approximate the effect of neighbor divisions, one that may in retrospect hide some geometric correlation between neighbor orientations. This modeling approach employs fairly simple concepts, using topology as a proxy for geometry, to capture the process of cell division. At the same time, it allows us to take advantage of well-developed mathematics for understanding whether an equilibrium steady state will emerge as the result of a stochastic process.

As described above, the Markov chain mathematical model predicts that the cell division process should drive epithelial sheets towards an invariant steady-state distribution of polygonal cell shapes, regardless of the initial tissue topology. In fact, the quantitative equilibrium distribution (featuring a predominance of hexagonal cells) can be calculated directly from the model. Importantly, this result does not involve any

Box 1. Stochastic model of cell division. Here we briefly review the stochastic model of cell division described in Gibson et al. (2006). The model comprises two main components. First, we consider cell-autonomous polygon state transitions resulting from mitotic division. Second, we consider the cell-non-autonomous polygon state transitions that result from neighbor division events.

We model a cell with n sides as an n-sided polygon with n tricellular junctions. Cell division results in: (1) the allocation of κ contiguous junctions to one daughter; (2) allocation of the remaining n-κ junctions to the other daughter and (3) the creation of two new tricellular junctions corresponding to the new interface between the two daughters. Since three-sided cells are rarely seen empirically, we define $\kappa > 2$. With these constraints in mind, we now specify the random algorithm by which cleavage planes are chosen, or equivalently, the distribution of the random variable κ. Assume that the n tricellular junctions of an n-sided cell are distributed uniformly at random around the cell's periphery, which is rounded up prior to cell division. A cleavage plane is chosen uniformly at random to bisect the rounded cell's area, while making sure that no three-sided cells can form. This is mathematically equivalent to requiring that the fate of four of the n junctions is fixed (two to one daughter, and two to the other), while the fate of the other n-4 junctions is random (each has a 1/2 probability of ending up in either daughter). Thus the probability of a daughter getting κ of the n junctions is:

$$D_{nk} = \binom{n-4}{k-4}\left(\frac{1}{2}\right)^{n-4}$$

Having considered autonomous polygonal state transitions as a cell divides, we next take into account how cells can gain sides (junctions) as a result of neighboring division events (see Fig. 4 for diagram). Assume there are C cells in the epithelium. A single generation of cell divisions adds $2C$ junctions to the epithelium (two per dividing cell), which implies an *average* gain of $2C/2C = +1$ junctions (sides) per cell from neighbor divisions. Of course, this is an approximation; in reality, some cells may gain multiple sides from neighbor divisions while others gain none. All together, the probability of an n-sided cell becoming m-sided after a division is:

$$P_{nm} = \binom{n-4}{m-5}\left(\frac{1}{2}\right)^{n-4}$$

The model is depicted as a network in Fig. 3, where the nodes represent a cell's state (its number of sides or junctions) and directed arrows represent the transition probabilities P_{nm}. In the end, it is the statistical balance between losing sides by division and gaining sides by neighbor divisions that results in the equilibrium distribution p^*.

consideration of active rearrangement, optimal packing or free energy minimization. Instead, the equilibrium distribution is an emergent property of cell division in an adherent epithelial sheet. Intuitively, one can reason about how this equilibrium arises. First, when a cell divides, both daughters on average possess fewer sides than the mother. Subsequently, these daughter cells tend to gain additional sides as their neighbors cleave along shared interfaces (Fig. 4). Furthermore, the number of cells expands exponentially with successive rounds of division. As a result, the global topology is increasingly dictated by the statistics of the division process rather than the initial distribution of cell shapes. This is similar to the impact of flipping an unbiased coin and counting 'heads' and 'tails'. The longer the string of coin tosses, the more likely the percentage of heads will be close to 50%. Change the bias of the coin, and the equilibrium will necessarily change as well. Mathematically, the Perron-Frobenius theorem tells us that regardless of the initial state (such as all pentagonal cells) our Markov chain model of cell division will converge to a specific distribution of polygonal cell shapes. Indeed, the predicted distribution and the empirical distribution are remarkably similar in the *Drosophila* wing imaginal disc. Both show less than 50% hexagonal cells, more five-sided cells than seven-sided cells, and no cells with more than nine sides. Almost all cell counts match to within a few percent, and even individual *Drosophila* imaginal discs appear to follow this distribution closely. What is perhaps more unexpected is that proliferating epithelia in diverse organisms appear to show almost identical distributions: *Hydra* epidermis, *Xenopus* tadpole tail epidermis and even Lewis's classical data from the cucumber.[1,17]

From model to experiment

The most-intriguing implication of these results is that the process of division alone, operating without cell rearrangement, can robustly generate a predictable tissue-level topology. This may help explain how epithelial layers can accommodate rapid proliferation while maintaining uniform structural properties and strong adhesion between cells. Secondly, the model provides a quantitative framework for understanding what might happen when cells behave abnormally. Differential growth is an obvious example; within a tissue, homogeneous division rates will result in equilibrium between sides lost and gained. It follows that global increases or decreases in the rate of cell division will still generate the predicted cell shape distribution. In contrast, if a small region of cells proliferates faster than its neighbors, a hypothetical "fault line" should form at the border where slow-dividing cells gain too many sides from their faster-dividing neighbors and, conversely, fast-dividing cells lose too many sides through division without gaining sides through the effect of neighbor divisions. This boundary effect would create a structural inhomogeneity in the otherwise uniform epithelial surface, with unknown biomechanical consequences. Conceivably, gener-

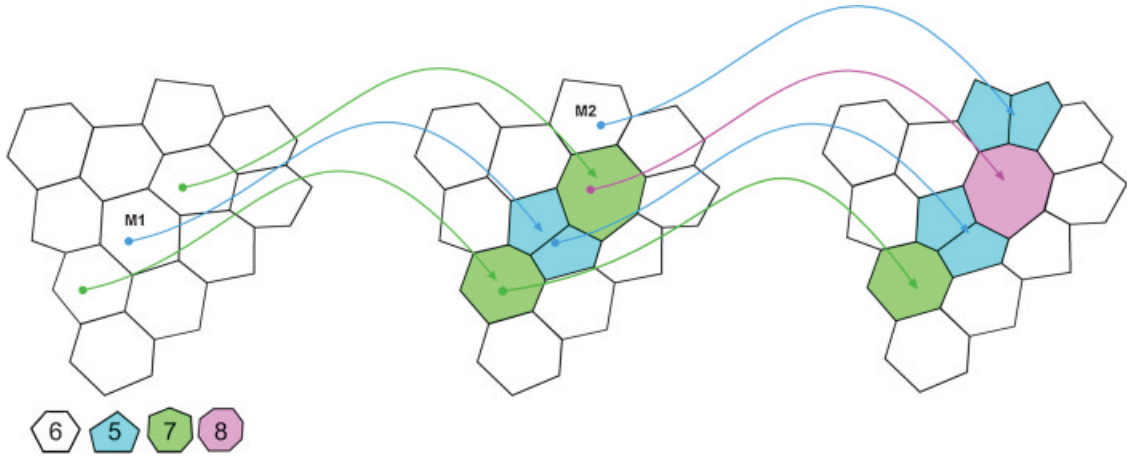

Figure 4. Cell-proliferation-dependent changes in epithelial topology. Two successive cell divisions (M1 and M2) in a hexagonal lattice of adherent epithelial cells can generate a range of polygonal cell types. This diversity results from the tendency of daughter cells to have fewer neighbors than their mother cell combined with the tendency of division events to increase the sidedness of two adjacent cells upon which the cleavage plane impinges. Over many stochastic division events and in the absence of cell sorting, the gain and loss of cell sides increases topological heterogeneity, but also reaches equilibrium at 46.4% hexagons, 28.9% pentagons, 20.8% heptagons and lesser numbers of other polygon types.[1]

ation of these topological fault lines could be a normal process during epithelial folding or invagination, or perhaps an abnormal feature of epithelial tumors. To directly test the effect of localized proliferation on sheet topology, we experimentally created fast-growing cell clones in non-dividing epithelium. As expected, a significant shift in the distribution of polygonal shapes was observed at the clone periphery, including the presence of triangular cells, which were never observed in proliferating epithelia under control conditions.[1] Production of aberrant cell shapes near regions of differential proliferation (such as triangular or 12-sided cells) could conceivably result in cell stress and death, and could also play an unforseen role in cell competition, a process by which slow-dividing cells are eliminated by their fast-dividing neighbors.[23–25] Looking forward, determining precisely how localized topology changes might influence cell or tissue behaviors will become a central experimental question.

Epithelial topology: more problems than paradigms

While successful in predicting the default topology of proliferating epithelia, our model leaves open several key questions. First: Are there other plausible models of cell division that can produce the same cell shape distribution observed in the *Drosophila* wing disc? The conserved cell shape distribution that we observed in multiple species could be due to the conserved effect of randomly oriented cell divisions on epithelial topology. Alternatively, in different tissues or species, distinct cell division patterns may somehow generate identical topologies.

Theoretically, a cell division model distinct from that observed in *Drosophila* could lead to the same effective transition probabilities, or a different matrix of transition probabilities could produce the same equilibrium distribution. Currently, there are many aspects of epithelial cell division that our current model either simplifies or does not directly incorporate. For example, we assume that there are no historical correlations between cell shapes (the transition from a six-sided to a five-sided cell is a fixed probability regardless of how that six-sided cell came about), which makes it difficult to represent mother–daughter correlations or spatial correlations between neighboring cells. The model also assumes equal division rates among all cells, although cell-shape-dependent division rates might be another mechanism for constraining global topology.[20] Finally, there may be external spatial correlations that are set up by morphogen gradients,[22] which are also difficult to capture in a purely topological framework. In short, understanding the topological implications of alternative cell division possibilities presents a formidable theoretical challenge for the future. To understand this local-to-global or cell-to-tissue level property, it is now critical to develop more expressive ways to model, simulate and analyze the system.

A second and related question is: do distinct spatial mechanisms for cell division generate unique and distinguishable cell shape distributions? If so, the cell division process at work could be directly inferred from a simple tissue-level observation. Even if many cell division models produced the same signature, one could quickly identify candidate cell division mechanisms and eliminate other hypotheses. Many

epithelial tissues do not show the default topology predicted to emerge passively from cell division;[13,15] many more epithelial tissues have never been quantified in this light. We still have much to learn about the extent of topology regulation that is possible by cell division alone and the range of mitotic cell behaviors that may exist in nature.

A third question for future research is whether there are limited topological forms that are achievable through cell division alone. For example: is there an existing cell division strategy that might generate a purely hexagonal cell sheet? An intriguing observation is that, while there are some remarkably regular epithelia in nature, these seem to result from complex strategies that involve active cell rearrangement and cell death. The pupal wing of *Drosophila* is a particularly interesting case, since it is a hexagonal lattice that forms directly from the irregular distribution observed in the proliferative larval stages. During pupal morphogenesis, cell division is relatively limited and there is a rapid transition from topological irregularity (~50% hexagons) to a highly regular cell pattern (80% hexagons).[15] This transition appears to be driven almost entirely through active cell rearrangements at the local level (Fig. 2C). Some of the genetic mechanisms involved in the polarized remodeling of cell adhesions to direct the neighbor exchanges have recently been illuminated. Nonetheless, what orients these local cell rearrangements, and why it is functionally important to increase topological regularity remains poorly defined. The fact that this system exhibits a dramatic shift from a phase where topology is purely determined by proliferation to a non-proliferative, directed rearrangement phase underscores the potential incompatibility of cell division with topological regularity. In the broad spectrum of epithelial systems, directed cellular rearrangement (as opposed to spatially oriented cell division or free energy minimization) may be the most feasible mechanism for increasing topological regularity.

The next step: from form to function

In closing, it is worth grappling with the most difficult question of all. Is cellular topology, be it regular or irregular, of any adaptive significance at the tissue level? We currently have no empirical understanding of how differing epithelial topologies might inform the differing physical properties of cell layers, or how aberrations in cell topology might affect pattern-formation processes or other aspects of epithelial morphogenesis. From plants to animals, the universal convergence to a predictable distribution of cell shapes will invariably generate epithelial surfaces with uniform spatial and structural properties. This uniformity may be necessary for maintaining physical and material properties of tissues, such as stiffness or planarity in the face of rapid proliferation or external stress. Alternatively, a quasi-ordered cell pattern may be necessary for the precision of the cellular patterning events that occur simultaneously with proliferation during most of development. Still, despite the potential role of epithelial topology in these processes, questions about the developmental or mechanical implications of different sheet topologies are only now coming into focus. Answering such questions will likely require an entirely new set of biophysical models and experiments to complement progress made to date. As a result, only one thing remains certain about future work, significant insights will continue to emerge from the wonderfully grey zone between mathematics, physics and biology.

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