

May the Force Be with You

Paul Martin and Susan M. Parkhurst

Morphogenesis, or the shaping of an embryo, not only depends on specific patterns of gene expression, but also critically involves the tugging, bending, folding, and sculpting of tissues—generally epithelial sheets—to mold the form of the embryo. Just like origami—the Japanese art of paper folding—a series of simple individual steps orchestrated in sequence results in the building of incredibly complex structures such as embryos. The fruit fly *Drosophila* is an excellent model organism with which to explore the genetics of morphogenesis. In a study on page 145 of this issue, Hutson *et al.* (1) use *Drosophila* embryos to get to grips with the forces exerted by tissues during embryogenesis. In addition, their work suggests ways to predict the key cell and molecular events that regulate morphogenetic tissue movements.

A morphogenetic episode that has received much attention is dorsal closure, which takes place late in *Drosophila* embryogenesis. In this process, a dorsally located epithelial hole that initially housed amnioserosa tissue (an extraembryonic membrane) is zippered closed. As this dorsal opening is eye-shaped, the left and right ends where zippering takes place resemble the corners of an eye and so are referred to by the human anatomical term, canthi (see the figure). There are more than 30 *Drosophila* mutants whose embryos fail to exhibit proper dorsal closure. Larval cuticle preparations from these mutant embryos reveal variously sized holes corresponding to regions where the embryonic epithelium is absent and the cuticle has not been laid down. Genes that are essential for dorsal closure encode either elements of the various signaling cascades directing this morphogenetic event or components of the cytoskeletal and adhesion machineries that drive key tissue movements (2). For example, flies with mutations in the *hemipterous* gene, which encodes the signaling molecule Jun kinase kinase, fail to undergo proper dorsal closure (3), as do

flies with certain mutations in the *zipper* gene, which encodes the heavy chain of nonmuscle myosin II (4).

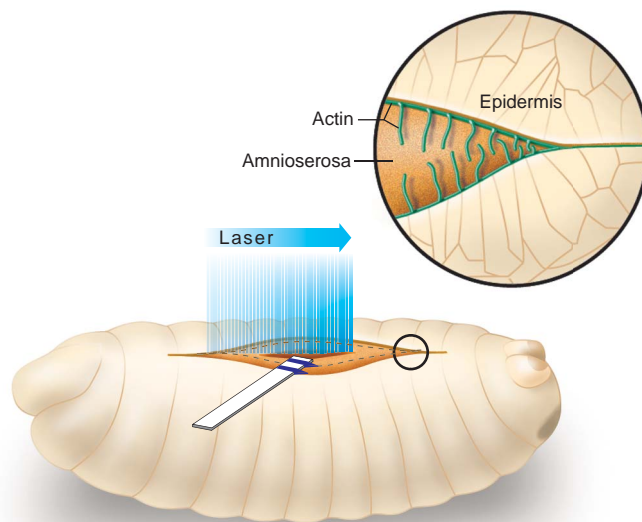
Dorsal closure takes place in one plane, making it relatively easy to observe tissue movements in action with confocal time-lapse movies of fly embryos expressing dorsal closure proteins tagged with green fluorescent protein (5). These studies provide good evidence that cells of the leading epithelial edge become polarized (6). The polarized cells then contribute to the assembly of linked contractile actin cables that act like purse strings drawing the epithelial sheets forward while maintaining tension (and thus organization) within that leading edge (7, 8). As the epithelial fronts meet one another, a second mechanism comes into play. The epithelial fronts are zippered together by interactions between opposing filopodial protrusions (see the figure) (9). The amnioserosa underlying the epithelial fronts appears to be contractile and may tug these epithelial fronts toward one another (10–12).

What has been missing is an understanding of the various tensions and forces within each of the contributing tissues at various times during dorsal closure. It is now possible to measure forces exerted by individual cells (13), but so far this has not been extrapolated to groups of cells, much less to tissues in live organisms. So how can we measure these vital forces? A big step forward in visualizing such forces came from Kiehart and colleagues (10), who revealed the play of tensions within the components of a tissue by cutting them with a laser beam and observing (but not measuring) the consequential gaping of adjacent tissues.

In their new work, Hutson and colleagues (1) have refined these laser microsurgery tools

so that they are able to generate a controlled spot (circular) or incisional (thin line) lesion in any region of the embryo. The precision of these types of lesions combined with some heavy-duty mathematical equations has allowed insights into how, for example, zipper fronts or canthi participate during the closure process. The authors locally ablated one or both of the zipper fronts of fly embryos with a spot lesion. When the zippers reestablished themselves, the authors blasted them with the laser again, and again, and again. Repetitions of this procedure revealed how embryos cope without a functioning zipper.

The investigators showed clearly that dorsal closure can proceed without zippering. The opposing epithelial fronts continue to advance toward one another as though pushed from the rear or tugged from the front, or a combination of both. Of course, this doesn't rule out a role for the zipper during normal unperturbed dorsal closure, but it does imply that other forces may also be operating and that these forces can compensate, at least in part, for the missing zipper machinery. Such work also raises the issue of the identity of these other forces. The authors address this question by making in-



Forcing tissues to meld. A *Drosophila* embryo undergoing dorsal closure. The hole in the upper (dorsal) surface of the embryonic epidermis is in the process of being zippered closed from both the left and right ends (canthi). By passing a laser beam from left to right, a cut is made in the amnioserosa, the exposed tissue in the epithelial hole region (1). A micrometer measures the extent of gaping of the epithelial edge as all tension in the amnioserosa is lost after passage of the laser beam. (Inset) A higher magnification view of the right-end zipper front (canthus) where the two epithelial fronts (cell outlines in shaded zone) become knitted together through filopodial protrusions at their front edges.

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cisional lesions that slice the exposed amnioserosa from canthus to canthus, and then measuring the consequential gaping of the leading-edge epithelium. This epithelium twangs back at a rate about 80 times the rate of its previous forward motion, until it reaches a new equilibrium where the applied tissue forces are again in balance. The quantitative data gathered from these “mechanical-jump experiments” revealed that the amnioserosa and the force-generating mechanisms in the adjacent epithelium make comparable contributions to the advancement of the epithelial leading edge.

These data raise several fascinating issues. For example, it is remarkable that the zipper rate increases in the unperturbed canthus when the opposite canthus is laser-ablated. It is tantalizing to imagine how cells in the embryo might regulate such a response. Presumably, cells at the healthy canthus detect some change in the global stress pattern that directs their compensatory response. Indeed, this sort of mechanism illustrates how the cues “read” by cells during development may often be mechanical, with cells sensing tension, stretch, and so forth, as opposed to diffusible growth factor signals. Indeed, what more ideal signal to announce that one embryonic tissue movement is complete, and the next can commence, than some mechanical cue arising from the completion of the preceding morphogenetic episode.

Important for any mathematical modeling of a developmental process is that it should feed back to the experimental scientist with some predictions about the episode being modeled. As their test case, Hutson and colleagues selected the *myspheroid* fly mutant, which has a defective β_{PS} integrin and fails in dorsal closure. Their model predicts that the failure of dorsal closure in this mutant results from defective zippering as opposed to aberrant epithelial sweeping forces, suggesting unexpectedly that integrins are involved in the zippering process. Time, and some tough future experiments, should tell us whether this is indeed where integrins fit into the dorsal closure story.

As the authors point out, many other fly mutants could do with a similar experimental treatment. A good example might be the *Rho1* mutant, in which the small guanosine triphosphatase Rho is nonfunctional, resulting in failure of the normal assembly of the leading-edge actin cable (7, 8, 14). Surprisingly, these embryos can generally close the dorsal hole, albeit in a rather haphazard fashion. Information about the rejiggered balance of tissue tensions within the leading-edge epithelium and the adjacent amnioserosa would be invaluable in the interpretation of how the embryo compensates

for the loss of Rho and the actin cable. This knowledge would in turn help us to determine more precisely the normal function of Rho and actin cables during embryonic development in vivo.

Can this kind of mathematical modeling be done for other morphogenetic events, and will it be as revealing? Dorsal closure is the morphogenetic episode about which we have the most genetic clues, partly because it takes place late, after the maternal pools of mRNA and protein are diminished. Also, because it occurs superficially (unlike the epithelial invagination events of fly gastrulation) and essentially in one plane, it is particularly amenable to imaging and to experimental manipulations such as laser ablation. But, with some effort, similar approaches will be possible for gastrulation and other tissue movements in the developing fly embryo, such as the amnioserosa-driven germ band retraction episode that precedes dorsal closure (15). These tissue movements are very different from dorsal closure and will reveal other components of the machinery that the embryo uses to shape itself.

The Hutson *et al.* study investigating the forces directing dorsal closure is a good first step toward dissecting the forces governing tissue movements in embryos.

CELL BIOLOGY

Apoptosis—the Calcium Connection

Nicolas Demaurex and Clark Distelhorst

The cells of our body are able to quickly commit suicide in response to genetic or environmental cues, a process termed apoptosis. This process is essential for development, tissue homeostasis, and defense against pathogens. Organized life requires cell death, and execution of cell death relies on the very machinery of life. Mitochondria, the organelles that produce energy through cellular respiration, integrate death signals mediated by proteins belonging to the Bcl-2/Bax family, and kill cells by releasing critical factors such as cytochrome c that activate executioner caspase proteases (1, 2). Calcium ions (Ca^{2+}), the cellular messengers that control every aspect of cell and tissue physiology, can be

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There are almost certainly morphogenetic episodes in human embryogenesis that mirror the events of dorsal closure, for example, closure of the eyelids during mid-gestation. And, as Hutson and colleagues point out, what is true for morphogenetic hole closure may also serve as a good model for damage-triggered hole closure during wound healing (10, 16, 17).

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turned into death signals when delivered at the wrong time and place (3, 4). Mitochondria eventually decide whether Ca^{2+} signals are decoded as life or death signals (5), but it is not clear whether Ca^{2+} is an additional stress factor that “tips the balance” or is an obligatory signal for death. On page 135 of this issue, Scorrano *et al.* (6) demonstrate that the transfer of Ca^{2+} from the endoplasmic reticulum (ER) to the mitochondria is required for initiation of programmed cell death by some, but not all, apoptotic signals (see the figure). Their elegant approach of genetically inactivating crucial proteins and reconstituting them in specific organelles reveals that the Ca^{2+} content of the ER determines the cell’s ability to commit suicide, defining the ER as a new gateway to apoptosis.

By using Ca^{2+} as an intracellular messenger, cells walk a tightrope between life and death. Because of the toxicity of Ca^{2+} ions, a low Ca^{2+} concentration must be maintained in the cytoplasm, and most of the cellular