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Dispatch

Drosophila Morphogenesis: The Newtonian Revolution

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Recent quantitative modeling of dorsal closure in the fruitfly *Drosophila* has revealed how multiple forces drive sealing of the two symmetrical epithelial sheets. A predictive model based on the new data allows gene function to be linked to the forces that drive tissue movement.

The ability of cells to change their relative positions is a fundamental process in metazoan development. Many studies have identified genes that are essential for cell and tissue movement, but their specific roles are not well understood, and the description remains mostly qualitative. This complex biological problem requires novel approaches directed at quantitative analysis. In a pioneering study, Hutson *et al.* [1] have combined quantitative modeling and laser microsurgery to describe the forces that drive dorsal closure of the *Drosophila* embryo [2,3]. During this morphogenetic event, two symmetrical epithelia move towards each other in a lateral-to-dorsal direction (Figure 1A). Within two hours, the dorsal borders of the moving epithelia — the leading edges — meet and fuse at the dorsal midline. During closure, the amnioserosa, a transient dorsal tissue, is progressively covered by the incoming lateral epithelia. When viewed from the top, the leading edges draw an eye shape, where the corners — known as 'canthi' — act as zippers. At the cellular level, zipping between approaching edges at the canthi is promoted by active actin protrusions which interlace at the seam [4,5].

In the initial phase of dorsal closure, a prominent actin cable is assembled in the edges, leading to the possibility that a 'purse string' mechanism would provide a major force for closure [6]. In order to evaluate the forces in the tissues that participate in dorsal closure, laser microsurgery and mechanical jump experiments have been used on wild-type embryos [7]. This simple approach indicates that the amnioserosa contributes positively to the movement, as its ablation induces ventralward relaxation of the leading edges. An incision in the lateral epithelium has the opposite effect, revealing a force that resists dorsal closure. The effect of cutting the actin cable, on the other hand, highlights the existence of a tension which is tangent to the leading edge.

From these observations and the application of Newton's second law, it is possible to draw a diagram of the three main forces present at the leading edge symmetry point (Figure 1B): two forces of contraction, from the amnioserosa (σ_{AS}) and the cable ($T\kappa$, where T is the tension in the actin cable and κ is the curvature), which are opposed to the force of resistance from the lateral ectoderm (σ_{LE}). To simplify the calculations, the model focuses on describing the forces at the cable symmetry point only, and takes advantage of the fact that, during mid-to-late stages, the dorsal surface nearly lies in a plane (Figure 1A).

Video recording showed that, following initiation of dorsal closure, the velocity of the leading edge is constant, at 12 ± 1.5 nanometres per second. This striking observation indicates that, once initiation is achieved, the forces driving dorsal closure are in equilibrium — that is, the sum of the applied forces, $\sigma_{LE} - \sigma_{AS} - T\kappa$, is constant and balanced by a drag force. As it is not possible to establish the absolute force values at a given point and time, experiments involving a series of laser-induced mechanical jumps were carried out at different stages of dorsal closure to assess the relative contribution of the amnioserosa and of the tension in the cable. From these data along with simple mathematical arguments and simplifying hypotheses (see [1] for details), the contributions of the three forces (σ_{LE}:σ_{AS}:$T\kappa$) relative to the effective force driving closure (taken as 1) were bracketed between the ratios 120:80:40 and 160:80:80. Note that the magnitude of σ_{LE} can be as much as 160 times bigger than that of the force driving closure.

Two conclusions can be drawn from this determination of the force balance. First, although the tension in the cable, T, is the strongest force, its contribution to closure ($T\kappa$) is comparatively small — because the curvature, κ, is modest — showing that, contrary to common sense, the 'purse string' is not contributing the major force for closure. Second, during mid-to-late closure, the largest individual force is negative, originating from the resistive force generated by the lateral ectoderm.

In addition to providing a quantitative basis of the relative forces shaping dorsal closure, Hutson *et al.* [1] have developed a model to describe the geometry of closure. The rationale is to provide a mathematical model that is predictive, not only for wild-type embryos, but also for mechanically or genetically perturbed ones. For this purpose, the leading edges are viewed as two intersecting circular arcs (Figure 1C), the geometry of which can be described in a time-dependent manner using an empirical rate equation (for details on the mathematical formalism, see [1]). In this model, the changes in shape of the leading edges are due to fractional contributions coming from zipping at the canthi (f_z) and from forces acting on the cable (f_c). During normal dorsal closure, f_z is ~1/3 and f_c is ~2/3 ($f_c = 1 - f_z$). Strikingly, the geometric model not only describes the behavior of leading edges of wild-type embryos, but also of embryos in which f_c or f_z have been strongly affected by laser ablations.

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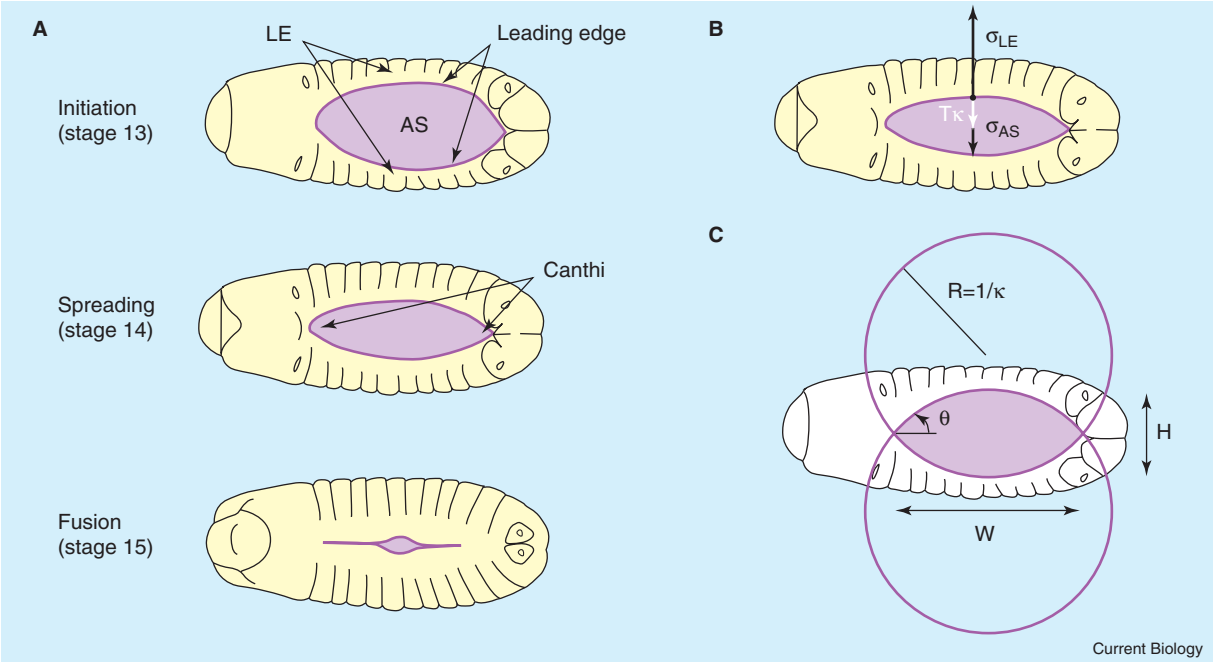

Figure 1. Forces and geometric modeling of dorsal closure in *Drosophila*.

(A) Dorsal views of a fly embryo at representative stages during dorsal closure. The model describes mid-to-late phases — the spreading and fusion stages. LE, lateral ectoderm; AS, amnioserosa. (B) Diagram of the forces driving closure at the symmetry point. Tκ, force induced by the tension in the actin cable; σ_{AS}, force of contraction of the amnioserosa on the leading edge; σ_{LE}, force of retraction of the lateral ectoderm. (C) Geometric modeling of the leading edges by two intersecting circular arcs. W, width of the amnioserosa along the dorsal midline; H, total height; θ, angle between the dorsal midline and the leading edge at the canthus; R, radius of curvature; κ, curvature.

How well does the model work with mutant embryos, and how predictive is it in elucidating a particular gene's function in dorsal closure? Hutson *et al.* [1] applied their model to embryos mutant for the *myospheroid* (*mys*) gene [8], which encodes a cell adhesion protein of the β-integrin family. Interestingly, *mys* embryos proceed towards closure but the edges rip off at the final stage, a phenotype that, in a first approximation, could be interpreted as a strict suture defect. From fitting the equation to the observed actin cable geometry of living *mys* embryos, a previously unknown function of *mys* in dorsal closure was predicted. Indeed, both the reduction in the velocity and the alteration of the specific actin cable geometry indicate that *mys* embryos should have a strong defect in zipping (the model predicts that f_z is reduced to ~1/10). Consequently, compensatory changes have to be invoked to explain the dorsal closure of *mys* embryos. According to the model, this compensation should arise through either a reduction of the total contribution of forces from the amnioserosa and the lateral ectoderm ($\sigma_{LE} - \sigma_{AS}$) or an increase in T.

Unlike *mys* embryos, the vast majority of dorsal closure mutants do not advance very far in closure, making quantitative analyses of their dynamics difficult to study. A major improvement of the current model would be to refine it to describe the local geometric behavior of the leading edge induced by spatially restricted genetic perturbations (for example, after the expression of dominant-negative forms in specific patterns). Although it involves certain simplifications, the quantitative model proposed by Hutson *et al*. [1] should prove to be an invaluable new tool for linking gene function to the dynamics of tissue closure during development, and also during wound-healing to which dorsal closure is very similar [9]. As illustrated with *mys* embryos, the model can be predictive, thereby inspiring biologists to design novel experiments and interpret complex phenotypes.

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