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SPOTLIGHT

Arc spreads Crumbs: Spatial restriction of tissue invagination to form a thin epithelial tube

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In this issue, Kim et al. (https://doi.org/10.1083/jcb.202409078) report that the scaffold protein Arc acts through Crumbs to spatially restrict where actomyosin-based apical constriction occurs across the invaginating *Drosophila* salivary gland. This restriction is needed for a long, thin tube to form.

From the branching of the lung to the looping of the gut tube, tubular organs develop complex architectures important for animal physiology (1, 2). Malformations of tubular organs are associated with various congenital diseases (3, 4, 5). Although much has been learned about the cell and developmental biology underlying the morphogenesis of such organs, many fundamental questions remain. For example, how does an initially flat sheet of epithelial cells remodel into a thin, elongated tube? A common first step is the apical constriction of initially columnar epithelial cells (6). This apical constriction is driven by the localized assembly and contraction of actomyosin networks, and results in wedge-like cell shapes. Cell–cell adhesion through apicolateral adherens junctions coordinates the individual cell shape changes and results in inward tissue bending. But how does the invaginating tissue form an extended tube rather than a more spherical structure? A similar challenge is overcome during glass blowing. Starting from similar blobs of molten glass, a variety of structures can be formed, including spheres and tubes. Application of air pressure is needed for all the structures, but distinctive shapes arise from additional manipulations of a skilled artisan. Analogously, Kim and colleagues from Deborah Andrew's laboratory report that inward bending of an epithelial sheet by actomyosin-based apical constriction is spatially restricted by a separate molecular pathway for a long, thin epithelial tube to develop, the *Drosophila* salivary gland (7).

Drosophila salivary gland development provides an excellent model of how inductive genetic instructions can be coupled with downstream epithelial morphogenesis for organ tubulogenesis (8, 9). Each gland internalizes from the embryo surface, starting as a flat patch of epithelial cells that express a winged-helix FoxA family transcription factor called Forkhead. Resulting gene expression in the patch distinguishes it from the surrounding surface epithelium. A supracellular actomyosin cable assembles around the perimeter of the patch, and actomyosin networks assemble over apical cell surfaces and along cell–cell junctions within the patch. Contractile activities of these cytoskeleton networks elicit changes to cell shapes and cell–cell interactions, but rather than the overall patch bending inward to produce a wide invagination, invagination is restricted to one end of the patch and occurs step by step to form a relatively long and thin epithelial tube. How the internalization is restricted to one end of the patch was not fully understood. Kim and colleagues discovered that a key downstream effect of Forkhead is the expression of a cytoplasmic scaffold protein called Arc that helps restrict where myosin accumulation and tissue invagination occur in the patch.

How did the Andrew laboratory implicate Arc in the control of salivary gland morphogenesis? First, expression screens comparing wild-type and *forkhead* mutant embryos revealed that Arc is expressed in the salivary gland with dependence on Forkhead. To test the function of Arc, knockout *arc* mutants were then generated. In these mutants, the internalizing salivary gland was abnormally short and wide. The total numbers of cells in the abnormal invaginations were unaffected, but more cells formed the circumferences of the tubes. Thus, the authors concluded that the abnormal tube morphology was due to defective changes to cell shapes and interactions during the internalization process. Remarkably, the overexpression of Arc had the opposite effect, resulting in salivary gland tubes with typical cell numbers but with excessively long and thin morphologies.

How does Arc affect cell shapes and interactions during the internalization process? Across the internalizing epithelial patch, Kim and colleagues quantified gradients of both apical cell constriction and associated actomyosin networks, with myosin-based apical constriction highest where the restricted localization of tissue invagination normally occurs (as also reported in reference [10]). In *arc* mutants, however, these gradients were significantly flattened, with actomyosin-based apical constrictions occurring broadly across the patch. Next, the authors turned to Crumbs, an apical transmembrane protein known to

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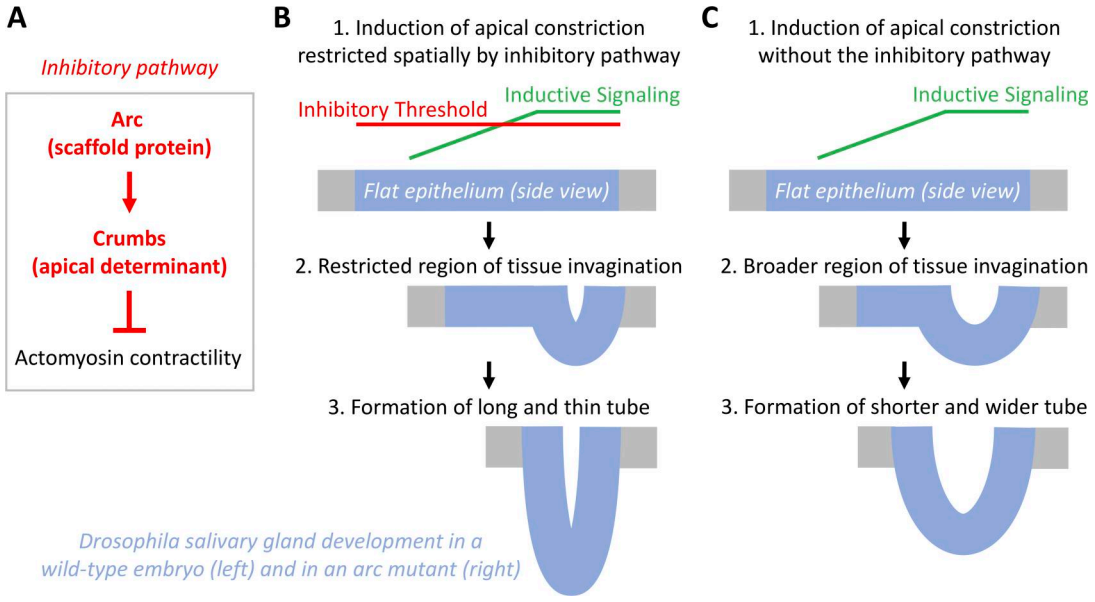

Figure 1. **Spatial restriction of actomyosin-based tissue invagination to form a long and thin epithelial tube. (A)** Molecular components of the inhibitory pathway. **(B)** Formation of a long and thin epithelial tube when localized, inductive signaling for apical constriction (green line) is combined with an inhibitory threshold (red line) across the developing salivary gland of a wild-type embryo. **(C)** Formation of a shorter and wider epithelial tube when localized, inductive signaling for apical constriction (green line) has abnormally broad effects in an *arc* mutant embryo lacking the inhibitory threshold.

modulate myosin during salivary gland internalization (11, 12). In *arc* mutants, normal enrichment of apical Crumbs in the internalizing epithelial patch was significantly reduced. To test whether Arc controls the distribution of myosin via Crumbs, further genetic analyses were conducted. Consistent with Crumbs inhibiting myosin, its overexpression reduced the levels of myosin. Moreover, the overexpression of Arc both expanded the apical distribution of Crumbs and reduced the levels of myosin. Indicating that Arc acts through Crumbs to control myosin, the depletion of myosin with Arc overexpression was reversed when Crumbs levels were genetically reduced. These experiments indicated that Arc affects Crumbs to restrict where actomyosin-based apical constriction occurs in the epithelial patch.

How does Arc affect Crumbs? The two proteins were found to colocalize in apicolateral domains of the internalizing patch's epithelial cells, with Arc additionally localizing to cytoplasmic puncta. Comparisons of Crumbs–GFP fluorescence recovery after photobleaching between wild-type and *arc* mutant samples indicated that Arc primarily affects Crumbs recruitment to the apicolateral domain. Consistent with this model, *arc* mutants displayed excessive cytoplasmic Crumbs puncta partially colocalizing with recycling endosomes. Although the two proteins could not be isolated together in stable complexes, colocalizations of Arc and Crumbs were promoted by one of Arc's two PDZ domains, and Arc's other PDZ domain had functional relevance to salivary gland internalization. Thus, Arc activity as a scaffold protein appears to recruit Crumbs to the apical domain, thereby restricting where actomyosin-based tissue invagination occurs.

Overall, Kim and colleagues identify a molecular mechanism that restricts the distribution of actomyosin-based apical constriction across the internalizing salivary gland tissue such that invagination occurs step by step at a subdomain of the tissue to form a long, thin tube (Fig. 1). Their findings also raise various questions. What is the interplay between the signaling that promotes the actomyosin-based apical constriction (10, 13) and the Arc–Crumbs pathway that inhibits it? Similar to other patterning systems (14), the coupling of local positive signaling with longer range inhibitory signaling seems to dictate the stereotypical location of the invagination site, but the effectiveness of the inhibitory signaling is presumably lost over time to allow all salivary gland cells to eventually internalize at the site. Kim and colleagues additionally show that Arc expression affects the development of another tubular organ of the *Drosophila* embryo. This raises the question of whether varying balances of the positive and inhibitory signaling mechanisms contribute to the natural variety of organ architectures, both within *Drosophila* and across species? With specific respect to the inhibitory mechanism, how exactly does Arc affect Crumbs, what other proteins does Arc integrate with, and what vertebrate scaffold proteins are the functional equivalents of Arc? There is still much to learn about how tubular organs form for their various functions in animals.

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