The first "Slit" is the deepest: the secret to a hollow heart

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Tubular organs are essential for life, but lumen formation in nonepithelial tissues such as the vascular system or heart is poorly understood. Two studies in this issue (Medioni, C., M. Astier, M. Zmojdzian, K. Jagla, and M. Sémériva. 2008. *J. Cell Biol.* 182:249–261; Santiago-Martínez, E., N.H. Soplop, R. Patel, and S.G. Kramer. 2008. *J. Cell Biol.* 182:241–248) reveal unexpected roles for the Slit–Robo signaling system during *Drosophila melanogaster* heart morphogenesis. In cardioblasts, Slit and Robo modulate the cell shape changes and domains of E-cadherin–based adhesion that drive lumen formation. Furthermore, in contrast to the well-known paracrine role of Slit and Robo in guiding cell migrations, here Slit and Robo may act by autocrine signaling. In addition, the two groups demonstrate that heart lumen formation is even more distinct from typical epithelial tubulogenesis mechanisms because the heart lumen is bounded by membrane surfaces that have basal rather than apical attributes. As the *D. melanogaster* cardioblasts are thought to have significant evolutionary similarity to vertebrate endothelial and cardiac lineages, these findings are likely to provide insights into mechanisms of vertebrate heart and vascular morphogenesis.

The *D. melanogaster* heart is a comparatively simple structure consisting of two parallel rows of myoendothelial cardioblasts (CBs) enclosing a solitary lumen. As in human heart formation, *D. melanogaster* CBs migrate to the future location of the heart. In a process that requires the well-known Slit–Robo guidance system (for review see Dickson and Gilestro, 2006), CBs organize into two parallel rows that converge at the dorsal midline, just below the epidermis. Near the end of the migratory phase, these initially mesenchymal cells polarize, but, strikingly, they do not establish a typical epithelial polarity. Instead, they establish a unique polarity along the dorsal/ventral axis (Fig. 1). As CBs meet at the midline, they form a tube by apposing their dorsal and ventral edges with the corresponding CB of the opposing row, thus encapsulating a lumen (Fig. 1 A; for review see Tao and Schulz, 2007). This "appositional" mechanism of tube formation is not typically used during epithelial organogenesis, which generally involves deformation of an existing apical surface by invagination or budding, or formation of a new apical (lumenal) surface by cavitation or vesicular fusion (for reviews see Hogan and Kolodziej, 2002; Lubarsky and Krasnow, 2003). Although recent papers have investigated the genes and pathways required for proper migration and organization of CBs into neatly apposed rows (Qian et al., 2005; MacMullin and Jacobs, 2006; Santiago-Martinez et al., 2006) and identified several genes required for lumen formation (Yarnitzky and Volk, 1995; Haag et al., 1999), the present studies are important because they define new molecular mechanisms of tubulogenesis and a lumenal membrane with unique polarity.

As CBs complete migration, the extracellular signaling protein Slit redistributes from a uniform plasma membrane localization on the CBs to specifically decorating the membrane region that faces the apposing CB and that will form the future lumen (Qian et al., 2005; Santiago-Martinez et al., 2006). In this issue, Medioni et al. (see p. 249) and Santiago-Martinez et al. (see p. 241) show that this relocalization is functionally important in that Slit and its transmembrane receptor Robo play central roles in cardiac lumen morphogenesis. Independent of earlier roles of these proteins in CB migration, the loss of Slit or Robo results in a failure to form a lumen or the formation of a small ventrally displaced lumen (Fig. 1 B). Conversely, overexpression of Slit mislocalizes both Slit and Robo outside of the wild-type lumenal domain, producing ectopic lumens.

Why does lumen formation fail when Slit–Robo signaling is compromised? Regulation of cell adhesion is a key factor. Santiago-Martinez et al. (2008) show that loss of Robo leads to a lumenless phenotype in which apposing CBs form an expanded E-cadherin–enriched cell contact. Significantly, this phenotype is mimicked by overexpression of E-cadherin (Fig. 1 B). Similarly, Medioni et al. (2008) found that loss of Slit also causes expansion of the dorsal β-catenin–expressing domain. Thus, lumen formation appears to be blocked because apposing CBs form an extended continuous adhesive surface

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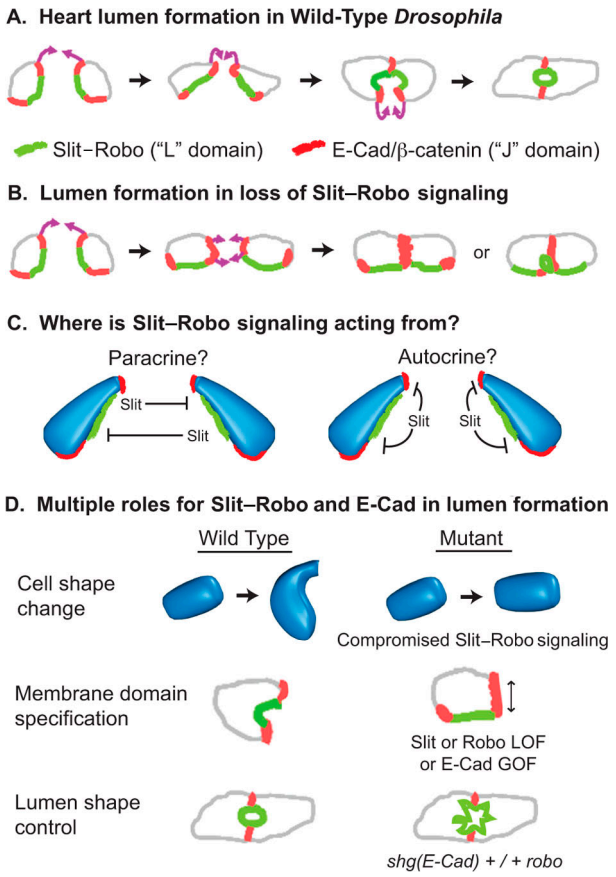

Figure 1. **Slit, Robo, and E-cadherin play key roles in *D. melanogaster* heart tube lumen formation.** (A) Schematic cross section of two wild-type cardioblasts with distinct membrane domains apposing first at their dorsal adhesive/junctional regions ("J" domains) and then ventrally to encapsulate a central lumen bounded by the lumenal membrane ("L" domain) that expresses Slit, Robo, and dystroglycan. (B) When Slit–Robo signaling is compromised, either no lumen or a small mislocalized lumen forms. (C) Because each cardioblast expresses both Slit and Robo, signaling to antagonize E-cadherin–based adhesion may be paracrine, autocrine, or both. (D) Slit and Robo appear to be involved in at least three distinct processes required for a lumen of the correct shape to form at the correct location. E-Cad, E-cadherin; GOF, gain of function; LOF, loss of function.

instead of isolated adhesive membrane patches that would allow encapsulation of a lumen. However, there appears to be a second decisive reason that lumens fail to form in Slit–Robo loss-of-function mutants. Live imaging studies by Medioni et al. (2008) demonstrate that even before apposing CBs have the opportunity to contact each other, they fail to undergo the cell shape changes required to bring the ventral adhesive regions of apposing CBs into contact to complete lumen capture. Therefore Slit–Robo signaling has at least two distinct roles in lumen formation: regulation of cell adhesion and regulation of cell shape (Fig. 1 D).

How do Slit and Robo act to control cell adhesion? Slit–Robo signaling has an extensively studied function in repulsive neuronal axon guidance (for review see Dickson and Gilestro, 2006). Combining this body of knowledge with the findings that Robo and E-cadherin have apparently opposing roles in lumen formation, one possible model would entail a paracrine repulsive role for Slit–Robo signaling. In this scenario, lumens form as Slit–Robo signaling antagonizes E-cadherin–based adhesion specifically at the lumenal domains of apposing CBs (Fig. 1 C, left). By extension, Slit–Robo signaling might not only antagonize adhesive domains but may act positively to specify lumenal characteristics, which would explain the localization of lumenal markers to areas of ectopic Slit localization and the ability of Slit-overexpressing cells to form multiple lumens. However, there are important distinctions between the expression and localization of Slit–Robo in CBs compared with cell guidance systems. Strikingly, each individual CB expresses both Slit and Robo, whereas during migratory processes, Slit is expressed by a signaling cell and Robo by responding cells. The fact that CBs express both Slit and Robo is highly suggestive of autocrine signaling (Fig. 1 C, right). Consistent with this possibility, the live imaging studies of Medioni et al. (2008) show that CB cells commence Slit–Robo–dependent cell shape changes and β-catenin relocalizations even at early time points, when rows of CBs are still distant from each other and separated by amnioserosal cells that could interfere with paracrine Slit–Robo signaling. Further work will establish to what extent the signaling is autocrine versus paracrine.

How do Slit and Robo function to regulate cell shape changes? At present, the answer is unclear, but not only do Slit–Robo regulate cell shape changes leading to lumen formation, in combination with E-cadherin they also appear to have a later and possibly distinct role in controlling lumen shape (Fig. 1 D). Santiago-Martinez et al. (2008) found that, in contrast to *robo* or *shg(E-Cad)* single heterozygotes, which have normal lumen formation and morphologies, CBs in *shg(E-Cad) +/+ robo* transheterozygotes form lumens, but the shape of the lumen is abnormal. This highly penetrant phenotype is counterintuitive because if Slit–Robo and E-cadherin have simple opposing functions, as they appear to in lumen formation, one would predict that the simultaneous loss of one copy of each would have a less rather than more severe effect on lumen formation than the loss of one copy of either E-cadherin or Robo. Thus, Slit–Robo signaling may have no less than three distinct roles in *D. melanogaster* heart lumen morphogenesis (Fig. 1 D).

Beyond defining novel mechanisms of lumen formation, the work of the two groups is noteworthy because their analyses of cell polarity markers show that the membrane domain organization of CBs is radically different than that of epithelial cells, which to date have been the principal focus of investigations of tubulogenesis. In both flies and vertebrates, epithelial cells have distinct apical and membrane domains, with markers such as Crumbs, β_H-spectrin, Bazooka, or aPKC defining the apical domain, and markers such as Discs large (Dlg), Scribble, and Lethal giant larva defining the basal domain. Although the membrane circumscribing the CB lumen has previously been designated "apical," it lacks Crumbs and the other typical epithelial apical markers. But the lumenal membrane domain is not a basal domain because it does not display basal markers such as Dlg. In fact, in CBs, Dlg localizes to the E-cadherin–expressing adherens junction domains. These novelties prompted Medioni et al. (2008) to distinguish between membrane domains by using "L" and "J" for lumenal and junctional domains, respectively, instead of "apical" and "basal," which have come

to have fairly well-defined characteristics in epithelial biology. The use of "L" and "J" appropriately highlights the unique polarity features of CBs and should help avoid confusion arising from using the same terms to describe very different cell membrane domains.

This unique polarity, however, raises questions about the generalizability of a Slit–Robo mechanism of tubulogenesis. Fortunately, although molecular details of polarity in endothelial cells that form vertebrate blood vessels are not well established, current evidence suggests that epithelial and endothelial polarity are markedly divergent and that endothelial lumenal surfaces may in fact have some "basal" epithelial features (Davis and Senger, 2005). Recent evidence suggests that the human cardiovascular system and the fly heart may have common evolutionary origins and that the fly heart is equally closely related to the vertebrate heart myocardium and the vascular endothelium (Hartenstein and Mandal, 2006). Indeed, formation of some of the major blood vessels occurs through an aggregation process reminiscent of *D. melanogaster* heart formation. Overall, *D. melanogaster* heart development is a powerful system for dissecting some fascinating cell biology involving membrane domain specification and cell shape control regulated by Slit–Robo signaling, and offers the potential of contributing important insights into human vascular and cardiac development.

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