Research Highlight in Developmental Biology

Tube Morphogenesis—THE EXTRAcellular DIMENSION

Rajprasad Loganathan
Department of Cell biology
The Johns Hopkins University School of Medicine
Email: rlogana2@jhmi.edu

The human body is replete with tubular organs that are essential for the secretion, transport, exchange, and storage of vital fluids. These organs sustain human physiology by virtue of the salient features of a select group of cells that support tubular architectures. Hence, a fundamental question in developmental biology concerns how cells interact with each other to form tubular structures during early embryogenesis. Increasing evidence suggests that in addition to the role played by the cells themselves, the extracellular matrix (ECM) also contributes to tube morphogenesis.

Tube morphogenesis – the generation of a tubular form – is made possible not only by the cells that constitute the tubular organ, but also the ECM that interacts with those cells. The Drosophila embryo has proved to be a valuable system for the investigation of epithelial tube morphogenesis (reviewed in Maruyama and Andrew, 2012). In particular, the formation of the embryonic trachea (Figure. 1), a branched tubular network that supports oxygen transport and exchange, exemplifies the morphogenetic signaling that occurs between the luminal ECM and the epithelium which is necessary to construct functional tubes.

Tracheal network formation is initiated at approximately hour four of embryogenesis and continues well into the larval stages. In order to function properly, the embryonic tracheal epithelium needs to form tubes of the appropriate length and radius. In the polarized epithelial cells of the trachea, the luminal surface is provided by the apical cell membranes. Chitin-rich ECM is secreted into the lumen during tube assembly, and is

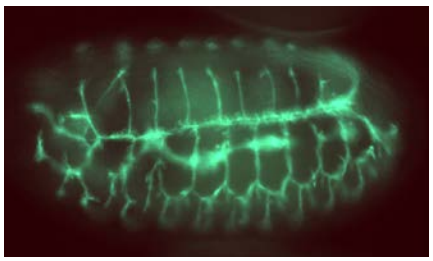

Figure 1. The Drosophila embryonic tracheal network. Orientation of the embryo with its anterior to the left, and dorsal surface facing up.
Courtesy: C. Samakovlis

composed of two structurally distinct components (Devine et al., 2005). A transient luminal cable, made of chitin fibrils, provides initial support, and is cleared from the tubes prior to larval hatching to enable gas filling. Meanwhile, a complex matrix of chitin and its associated luminal proteins persist to make up the taenidia that reinforces the larval tracheal network. In the absence of cell division and cell death, morphogenesis of the tracheal network is accomplished almost exclusively by developmentally regulated structural changes of the cells and the apically-secreted chitinous matrix.

In the work, published in PLOS ONE, by Tiklova et al. (Tiklova et al., 2013), the authors investigate two chitin-binding proteins, Obstructor-A (Obst-A) and Gasp, that are secreted into the tracheal lumen. Both *obst-A* and *gasp* belong to the obstructor multigene family. Embryos mutant for either of these

genes demonstrated tube dilation defects, implying that both Obst-A and Gasp contribute to diametric tube expansion. In addition to these morphogenetic defects, the mutant first instar larvae showed decreased body length without any significant effects on the body width. The phenotypic changes in the double mutants were additive, thus demonstrating synergism between the actions of Obst-A and Gasp. Not surprisingly, cuticular bloating was also observed in the double mutants suggesting a role for these genes in chitin fibril assembly and exoskeleton integrity.

As mentioned, the taenidia provides structural support for the trachea. The larval tracheal network in double mutants showed collapsed tubes that failed to fill with air, thus demonstrating compromised tube structure and function respectively. The authors ruled out compromised epithelial integrity as a possible cause of tube collapse in mutants, since the intensity and localization of epithelial structural markers were comparable to wild type (WT). Instead, they hypothesized that these defects were due to alterations in the ECM leading to aberrant taenidial formation. A direct verification of compromised taenidial assembly in the mutants was made possible by the finding that intraluminal chitin staining intensity was decreased/irregular compared to that of WT embryos. At the ultrastructural scale, the mutant taenidial folds were grossly irregular compared to WT. Taken together, these results suggest a direct role for Obst-A and Gasp in tubular apical ECM assembly and maintenance.

Tiklova et al. also quantified the diametric tubular expansion defect in the mutant trachea by measuring the lumen diameter along the dorsal trunk, a major tracheal conduit spanning the anterior-posterior axis. The mutant trunks showed a decrease in diameter compared to the WT. The tube length, however, was unaffected in mutants. Finally, the authors also verified that the targeted overexpression of Obst-A and Gasp leads to tracheal tube dilation. Together, these findings indicate a role for Obst-A and Gasp in diametric expansion during tube morphogenesis. A surprising finding of this study was the identification of Gasp as the unknown antigen recognized by the antibody mAb 2A12, a luminal marker that has been in use for nearly twenty years.

The findings by Tiklova et al. demonstrate that chitin binding proteins are essential players in the assembly of apical ECM in tubular organs. Without the participation of the luminal ECM, epithelial tube morphogenesis is incomplete. These results also provide additional evidence for both the dynamic role of apical ECM in tube morphogenesis, and the idea that the ECM is an active ingredient of morphogenetic processes (Aleksandrova et al., 2012). Indeed, morphogenesis is not driven solely by the signaling between cells of the developing structure, but includes the synergistic interactions between the cells and the ECM. While this study highlights the importance of ECM, the dynamics of cell-ECM signaling and its regulation during morphogenesis requires further exploration.

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