Research Highlight in Developmental Biology: Tubulogenesis–Microtubules Make the Move

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Abstract
Invagination is a key morphogenetic event in the generation of epithelial tube architectures. The driving force for cell shape changes that enable coordinated invagination is provided by the cytoskeleton. Much has been learned about the dynamics of actomyosin – as drivers of cell shape changes, particularly apical constriction – during invagination. However, the role of microtubules has not been clear. In a recently published study, Booth et al. describe the dynamics of microtubule during morphogenesis of a tubular organ (the salivary glands) in the Drosophila embryo. The authors observed both a 90° change in microtubule orientation relative to the cellular apicobasal axis and a loss of centrosomal attachment coincident with tube formation of cells fated to form the salivary glands. They showed that targeted disruption of the microtubule cytoskeleton leads to a failure of invagination by destabilization of the medial actomyosin network, a driver of apical constriction. The study also suggests that a cytolinker protein – Shot – links the microtubule bundles with the medial actomyosin network.

Key words: Drosophila, epithelium, invagination, morphogenesis, salivary gland

Construction of tubular organs during embryogenesis is integral to a functionally complete metazoan body plan. In the Drosophila embryo, the cells of the salivary glands (SG) undergo a remarkable series of morphogenetic changes as they form a tubular secretory organ (Figure 1). Following specification, the ventrally located secretory epithelial cells of the SG placodes (dense cell clusters) invaginate to form incipient tubes on either side of the embryonic anterior. Cellular shape changes and rearrangements further elongate the tube as the cells collectively migrate dorsally, and continue migration posteriorly to position the gland along the anteroposterior axis (Andrew and Ewald, 2010). The Drosophila SG has become an ideal system in which to explore the molecular biology of epithelial tube morphogenesis since the absence of cell death and cell division allows for the exclusive investigation of the morphogenetic processes that generate a monolayered epithelial tube from cells previously specified for the SG fate (Maruyama and Andrew, 2012). The roles of various genetic elements and their exquisite regulation during the specification and morphogenesis that enable the formation of a polarized tubular epithelium have been studied in the Drosophila SG (Haberman et al., 2003; Kerman et al., 2008).

The first steps in epithelial tube morphogenesis are propelled by a dynamic interplay between cell shape changes and rearrangements effected primarily by the cytoskeletal machinery. Transformation of a two dimensional epithelial sheet into a three dimensional epithelial tube is accomplished by invagination. At the site of invagination (the invagination pit), a cell shape transformation – from columnar to wedge – enabled by apical constriction (AC) occurs as a result of pulsating apicomedial actomyosin network activity that functions like a purse-string (Mason et al., 2013). However, it was not clear whether microtubules (MTs) played a role during invagination, and whether there was an interaction between MTs and actomyosin

networks during cell shape changes. The recently published study by Booth et al. (Booth

et al., 2014) provides intriguing light to the underappreciated role of MTs in SG morphogenesis.

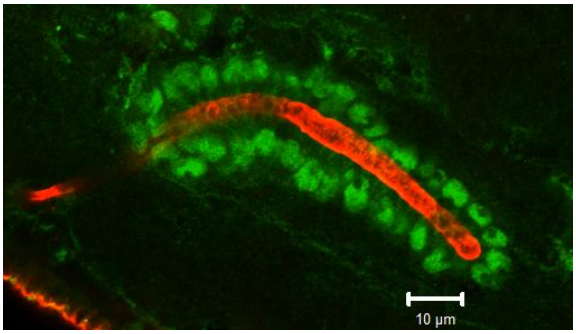

Figure 1: *Drosophila melanogaster* embryonic salivary gland at 11 hours of development (stage 14). In this longitudinal section through the gland, secretory cell nuclei (green) are stained with SAGE, a cell fate marker. The tube lumen (red) is formed by the membrane of the cells stained with SAS, an apical polarity marker. Anterior is to the left.

Using tubulin markers, Booth et al. were able to track a 90° change in orientation of stable MTs prior to AC in invaginating SG placodal cells. Initially the MTs were localized as dense arrays parallel to the apical surface of the placodal cells. However, just prior to invagination, the MT bundles changed their orientation to align perpendicular to the apical surface of placodal cells. Near the basal cell surface, the (+) ends of the MT bundles showed dynamic behavior. Interestingly, the authors also noticed a non-centrosomal localization of MTs coincident with the MT rearrangement, and their analysis of non-centrosomal γ-tubulin distribution suggested the possibility of a non-centrosomal nucleation of MTs at the apical surface of invaginating cells.

Evidence for the critical role of MTs during invagination came from experiments in which Spastin, a MT severing protein, was overexpressed in the SG placode cells in order to deplete the cells of functional MT. Cells in the MT-depleted placodes showed fewer ACs and failed to invaginate. The authors also demonstrate the presence of both a medial actomyosin network in the SG during invagination and the apical apposition of this network with the minus ends of the MT bundles following their orientation perpendicular to the apical cell surface. Also, a significant decrease in the apical medial myosin pool was observed upon depletion of Spastin. These experiments demonstrated that the MTs are necessary for the proper function of the actomyosin network during cellular AC.

Furthermore, using time series analysis of SG invagination in live embryos, Booth et al. were able to show a positive correlation between the AC of placodal cells and periodically fluctuating apical medial myosin intensities. The pulsatile activity of the medial myosin network observed in the controls was compromised in embryos overexpressing Spastin. By analyzing markers of epithelial cell junctional integrity and apicobasal polarity, the authors rule out compromised cell integrity and polarity (secondary to MT depletion) as causal factors leading to invagination failure during Spastin overexpression. Overall, these experiments point to the possibility of a direct role for MTs in association with the actomyosin network activity in driving the apical membrane dynamics that underlie epithelial invagination and tube formation.

In a final set of experiments, Booth et al. explored the possibility that a cytolinker protein, Shot, which consists of both actin and MT binding domains could functionally bridge apical MT ends with the medial actomyosin network. In addition to demonstrating a co-localization of Shot with the apical MT assembly in the SG placode, they expressed the MT binding Gas2 domain of Shot using a strong maternal driver and a SG specific driver. Although the MT stability was comparable to controls, the transgene overexpression had a dominant negative effect on endogenous Shot localization, presumably affecting the coupling of apical medial actomyosin with MT, resulting in aberrant

invagination of SG placodal cells. Thus, Shot localization to the minus ends of MTs in the apical medial compartment of invaginating cells was critical for normal AC of these cells during tubulogenesis.

The study by Booth et al. highlights the role of MTs in cell shape changes during morphogenesis in general. Specifically, it uncovers a role for MTs that is intertwined with the previously described actomysoin dynamics during epithelial invagination. This study raises a number of intriguing questions worthy of future explorations. First, the molecular mechanisms underlying the possibility of γ-tubulin as a source of apically localized non-centrosomal MT nucleation is a fertile area for future experiments. Also, whether the role of MTs extends to effect membrane dynamics during tube elaboration, beyond epithelial invagination, is unclear. For instance, Rab11 mediated vesicle recycling occurs to expand the luminal membrane thereby affecting the apical membrane dynamics during tube elongation (Cheshire et al., 2008; Kerman et al., 2008). Thus, whether the apically localized MT network mediates luminal membrane expansion in association with actomyosin and cortical moesin is an open question. Finally, since SG morphogenesis also involves the dynamics of basal cell membranes for collective migration and organ positioning (Vining et al., 2005), the prospect that a basally oriented MT cluster could participate in organ-scale morphogenetic deformations by coordinating basal membrane movements through a dense tissue interface is intriguing.

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