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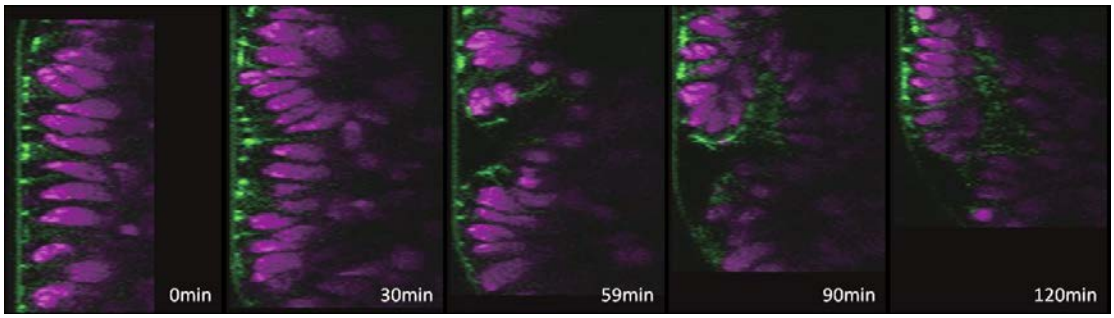
Mitotic cell rounding as a driver of morphogenesis

Jan 25, 2013 – The inward folding, or invagination, of epithelial tissues is an important morphogenetic phenomenon, converting flat sheets of cells into three-dimensional tissues. This process is often, but not always, associated with apical constriction of the actin cytoskeleton and produces indented hollows in epithelial surfaces, such as the placodes that give rise to sensory and other organs. Cells entering mitosis have generally been thought to be incapable of undergoing the intensive cell shape remodeling that underlies these processes, but as some tissues in which invagination takes place show signs of cell cycle activity, leaving open the question of how these two phenomena might interact.

Takefumi Kondo of the Laboratory for Morphogenetic Signaling (Shigeo Hayashi, Group Director) has now shown that a process known as mitotic rounding drives invagination of the tracheal placode in the fruit fly, *Drosophila melanogaster*. This work, published in *Nature*, shows that mitosis plays an unsuspected role in this form of developmental tissue remodeling.



Tracheal invagination begins with a slow phase, later transitioning to a fast phase in which mitotic cells near the center of the internalizing epithelium undergo rounding and drive the formation of an L-shaped tube.

The apical constriction of epithelial cells, in which the apical regions of cells shrinks giving them a roughly conic structure, has long been thought to be the driving force behind invagination. But a number of tantalizing exceptions in which inward folding occurs without such constriction suggested that some other dynamic must also be involved. Using live imaging of the tracheal placode in *Drosophila* embryos, the group sought to answer this question. This placode begins as a flat sheet of about 40 cells, which transforms into an L-shaped tubular structure. Watching the process with an eye to detail, Kondo observed that this invagination played out in two distinct phases. In the first, slow phase, cells in the center of the placode undergo apical constriction, and gradually move toward the embryo interior. A second, fast phase followed in which the tracheal pit grew and cells were more rapidly internalized.

Interestingly, this fast phase was consistently associated with the mitotic entry of cells in the placode center. Immediately prior to entering the cell cycle, cells ball up. In orderly epithelial sheets, this 'rounding' behavior can destabilize the tissue, meaning that its site and timing need to be closely controlled, and indeed studies of mutants have shown that morphogenesis is disturbed when this program is dysregulated. Knowing this, Kondo used mutants of a cell cycle gene (*CycA*), which fail to enter the final stage of embryonic mitosis, and watched for effects on invagination. Although the slow phase proceeded as usual, the pace of the fast phase was appreciably decreased. Despite the delay, however, tracheal invagination did occur, suggesting that other forces might be at work as well.

The FGF signaling pathways is involved in inducing branching morphogenesis in the fly trachea, so Kondo tested next looked for a role for this molecule, but found that in single FGF mutants invagination was unaffected. In double mutants for the cell cycle gene and FGF, however, invagination was slowed and incomplete, suggesting FGF might function as a backup mechanism in this process. In a separate experiment, chemical inhibition of microtubules showed that invagination can take place even when rounding occurs in the absence of mitotic entry, indicating that it is the morphogenetic changes, not the cell division, that are required for the involution of the epithelial sheet.

So how then do cells in the placodal center know when and where to begin invaginating? Kondo turned back to a previous study by the Hayashi lab, in which it was shown that the motor protein

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myosin confers constrictive force to actin fibers in cells peripheral to the tracheal placode center, forming a pressure front that may drive cells at the center inward. As this mechanism relies on the EGF receptor protein (EGFR), the group generated EGFR mutants and watched for effects. When this gene was ablated along with *CycA* and *FGF*, invagination failed completely.

“This work shows how multiple independent mechanisms can play overlapping and complementary roles in epithelial invagination,” says Hayashi. “It is these kinds of elegant, robust programs that enable development to proceed in such as stable fashion. It seems that in order to give rise to the beauty of the animal form, embryos need to follow beautiful rules.”

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