To Thine Own Self Be True: Self-Fusion in Single-Celled Tubes

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In this issue of *Developmental Cell*, Rasmussen et al. investigate the morphogenesis of the *Caenorhabditis elegans* pharynx. Their results highlight the usefulness of this system for investigating the molecular mechanisms behind the unusual cell behaviors that underlie the formation of single-celled tubes during animal development.

Tubular structures are found throughout the animal kingdom, from the alimentary canal of bilaterians to the spectacularly complicated branched structures associated with gas exchange and the circulatory system (Hogan and Kolodziej, 2002; Lubarsky and Krasnow, 2003). The ability to precisely define where and when tubular structures form involves a complicated choreography of differentiation and morphogenesis, perhaps best investigated in the *Drosophila* tracheal system, where the genetic regulation of tubulogenesis has been studied in detail (Uv et al., 2003; Myat, 2005). In the case of single-celled tubes, this precision must extend not merely to the level of single cells, but to specific regions within single cells. In the case of *Drosophila* tracheal cells, single-celled tubes form via autocellular junctions, i.e., connections between adjacent regions of the same cell mediated by the same cell-cell adhesion complexes normally used in intercellular adhesion (Samakovlis et al., 1996). In other cases tracheal cells are ramified, and in still other cases they form true toroids, possibly through fusion of intracellular vesicles (Samakovlis et al., 1996; Uv et al., 2003). Such single-celled tubes present an interesting topological challenge, as cells must undergo remarkable contortions to create a subcellular domain that surrounds a central lumen. The solution to this problem is not merely of interest to abstract mathematicians, but is crucial for the formation of the narrowest tubes in tubular networks in animals.

The paper by Rasmussen et al. (2008) in this issue of *Developmental Cell* presents an analysis of a new system for studying single-celled tube formation: the pharynx of *C. elegans*. The pharynx of *C. elegans* is structurally elaborate, and it has been described in impressive detail (Mango, 2007). Moreover, because the development of *C. elegans* is so reproducible from embryo to embryo, this system may provide some advantages for understanding how single-celled tubes form, including how specific domains of single cells participate in this process.

Rasmussen and colleagues show that two cells at the junction between the pharynx and the intestine, known as pm8 and vpi1, must move ventrally across the central axis of the pharyngeal lumen, where they each encircle the lumen by the time the process is over (Figure 1A). Each of these cells essentially forms a cellular "washer" or "gasket," connecting other pharyngeal cells that form a multicellular tube with intestinal cells that form another tube. pm8 forms a conical toroid, and abuts vpi1, which adopts a flatter, cylindrical shape (Figure 1B). To adopt their final configurations, pm8 and vpi1 must each extend across the midline of the pharyngeal lumen. In addition, each cell must fuse with itself, converting each cell from a folded, C-shaped cell, like those that form some single-celled tubes in the *Drosophila* tracheal system, into true toroids (Figure 1C).

There are several steps that are required for each of these cells to adopt their correct positions and shapes. First, the valve cell precursors must differentiate properly, a process which, in the case of pm8, requires upstream Notch signaling. Second, once differentiated properly, pm8 and vpi1 must translocate across the midline of the pharyngeal lumen. Rasmussen et al. detect a potential physical template that may serve as a guide for these cells as they move across the luminal midline: a tract of laminin seems to appear at the site where they migrate. Moreover, pm8 migration is defective in laminin and integrin mutants, which suggests that the laminin substratum is functionally important during migration across the luminal midline.

There is a third step that truly toroidal cells such as pm8 and vpi1 must go through, and it is arguably the most interesting and challenging. These cells must regulate their self-fusion while explicitly avoiding fusion with other nearby cells. Here again *C. elegans* provides some advantages, since components of the cell-cell fusion machinery have been identified. Two key proteins mediate two different types of cell-cell fusion in *C. elegans*: the novel transmembrane proteins EFF-1 (Mohler et al., 2002) and the recently described AFF-1 (Sapir et al., 2007). Rasmussen et al. find that EFF-1 is normally expressed in vpi1 and that loss of *eff-1* function blocks the ability of vpi1 to fuse with itself. In contrast, pm8 does not normally express EFF-1, but instead expresses AFF-1, and *aff-1* loss of function blocks its fusion. By using two different fusion machines, the self-fusion events that must occur in the two adjacent cells remain restricted to their own membranes.

Rasmussen et al. go on to show that Notch signaling is involved in the fusion process. In Notch signaling mutants, pm8 can ectopically fuse with vpi1. If EFF-1 is not normally expressed in pm8, how does Notch signaling regulate self-fusion versus heterologous fusion between pm8 and vpi1? pm8, but not vpi1, expresses a Notch receptor (LIN-12). In Notch mutant embryos pm8 can express

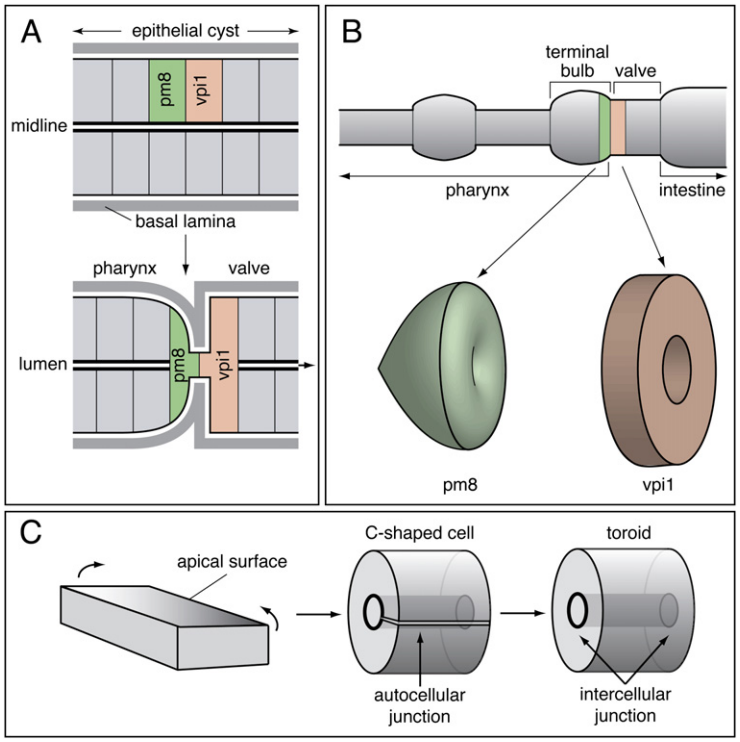

Figure 1. Making Tubes from Single Cells in *C. elegans*

(A) Two cells, pm8 and vpi1, are initially dorsal to the midline of the pharyngeal lumen (top). They cross the lumenal midline and then self-fuse to form the connection between the posterior pharynx and the anterior intestine (bottom).

(B) The final arrangement of the cells pictured schematically and in a 3D model. Based on figures in Rasmussen et al. (2008).

(C) Single-celled tubes can form in several ways from flat precursors, two of which are shown: they can fold into a "C" shape, a shape that can be stabilized by autocellular junctions, such as in some single-celled tubes of the *Drosophila* tracheal system, or they can go on to self-fuse to create a genuine toroid, or "doughnut" shape, as in the *C. elegans* pharynx.

EFF-1, and lose AFF-1 expression. Loss of *eff-1* repression downstream of Notch signaling provides an explanation for the ectopic fusion phenotype: when pm8 inappropriately expresses EFF-1, it now becomes competent to engage in cell-cell fusion with vpi1. Such a mechanism of EFF-1 repression is presumably needed, because EFF-1 mediates fusion between membranes only when *both* membranes contain it (e.g., see Podbilewicz et al., 2006). By repressing EFF-1 expression in pm8, EFF-1's fusogenic activity is restricted to vpi1.

Although the studies by Rasmussen et al. are an important first step in unraveling the processes that lie behind single-celled tube formation involving true single-celled toroids, there are several unanswered questions. First, pm8 requires AFF-1 for its self-fusion. By analogy to vpi1 and EFF-1, is a reciprocal repression of the AFF-1 fusion machinery in vpi1 also necessary? Second, what pathways within pm8 and vpi1 control their polarized migration? Understanding how these cells move and self-fuse may lead to further insights into the cell migratory mechanism underlying formation of single-celled tubes. Whatever the answers to these questions turn out to be, the pharynx of *C. elegans* is a welcome addition to the list of systems in which tubulogenesis can be studied at the cellular and genetic levels, and should help to elucidate common themes in formation of cellular tubes across the animal kingdom.

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