HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Semin Cell Dev Biol. 2017 July ; 67: 123–131. doi:10.1016/j.semcdb.2016.05.006.

Time to make the doughnuts: Building and shaping seamless tubes

Meera V. Sundaram[*] and **Jennifer D. Cohen**

Dept. of Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia PA 19104

Abstract

A seamless tube is a very narrow-bore tube that is composed of a single cell with an intracellular lumen and no adherens or tight junctions along its length. Many capillaries in the vertebrate vascular system are seamless tubes. Seamless tubes also are found in invertebrate organs, including the *Drosophila* trachea and the *C. elegans* excretory system. Seamless tube cells can be less than a micron in diameter, and they can adopt very simple "doughnut-like" shapes or very complex, branched shapes comparable to those of neurons. The unusual topology and varied shapes of seamless tubes raise many basic cell biological questions about how cells form and maintain such structures. The prevalence of seamless tubes in the vascular system means that answering such questions has significant relevance to human health. In this review, we describe selected examples of seamless tubes in animals and discuss current models for how seamless tubes develop and are shaped, focusing particularly on insights that have come from recent studies in *Drosophila* and *C. elegans*.

1. What are seamless tubes?

Organs are composed of tubes with different sizes and shapes that are specialized for their particular functions [1]. Most tubes are composed of polarized epithelial or endothelial cells that have an apical surface facing the lumen and a basal surface facing other tissues, and that are linked together by adherens junctions and tight junctions. Branched organs, such as the mammalian vascular system, lung, and kidney, typically consist of centrally located, larger-bore tubes that transport fluids or gas over long distances, and progressively narrower tubes at the periphery that exchange nutrients and waste with nearby tissues.

Seamless tubes are very narrow-bore tubes that are only one cell in diameter, such that the apical domain and lumen are intracellular (Fig. 1). As their name implies, such tubes do not have adherens or tight junctions along their length, although they do have such junctions where they connect to other tubes. Seamless tubes are found in the vertebrate vascular system (Fig. 1A, B) [2] and in many invertebrate organs and glia [3–7], as well as in plants

[*]author for correspondence: sundaram@mail.med.upenn.edu, (215) 573-4527.

(e.g. pollen tubes [8]). Many insights into how seamless tubes are built and shaped have come from studies of such tubes in the *Drosophila* trachea (Fig. 1C) and *C. elegans* excretory system (Fig. 1D), where imaging is possible at the necessary single-cell resolution, and the power of forward genetics has been applied to identify relevant molecular mechanisms.

2. Examples of seamless tubes

2.1. Seamless tubes in the vertebrate vascular system

Unicellular seamed and seamless tubes make up a sizeable proportion of the capillaries in the vertebrate microvasculature. Decades ago, serial section transmission electron microscopy (TEM) studies of tissues from rats and other mammals revealed many capillaries with only a single cell surrounding the lumen [2, 9, 10]. Some such capillaries had an autocellular junction or “seam” that sealed the tube, but others had no darkly staining junctional material between the lumen and the plasma membrane, and were termed “seamless” (Fig. 1A). Many of these tubes were found at anastomoses or branch points in the capillary network. More recently, seamless tubes have been observed by confocal microscopy in the mouse and zebrafish vasculature (Fig. 1B) [11, 12]. In zebrafish, where live imaging has been possible, some seamless tubes are transient, developmental precursors to larger multicellular tubes or to pruned vessels [13–16]. However, in mammals, TEM studies suggested that as many as 30% – 50% of the tubes in capillary beds of adults can be seamless tubes [10].

Not surprisingly, capillary defects are implicated in various cardiovascular diseases. These include common ailments such as hypertension [17], coronary microvascular disease (MVD), a leading cause of heart attacks, especially in women [18] and cerebral Small Vessel Disease (SVD), a leading contributor to age-related dementia and stroke [19]. Loss or abnormal structure of capillary beds is also observed in Mendelian syndromes such as Hereditary Hemorrhagic Telangiecstasia (HHT) [20], Cerebral Cavernous Malformation (CCM) [21], and familial SVD syndromes like CADASIL [19] (Table 1). Genes involved in these Mendelian syndromes point to dysregulation of TGFβ signaling, vesicle trafficking, cytoskeletal organization and extracellular matrix (ECM) as key factors in capillary malfunction (Table 1).

Due to their small size and density, mammalian capillaries are difficult to visualize *in vivo.* Therefore it is not known if the pathology of these diseases specifically involves seamless tubes. However, some aspects of capillary development can be modeled using human umbilical cord endothelial cells (HUVECs) grown in a 3D collagen matrix [22]. CCM1 and CCM2 are required for intracellular lumen formation in such assays [23, 24]. A zebrafish model of CCM1 [23] and invertebrate models of CCM3 [25, 26] also show seamless tube defects, supporting a connection between capillary diseases and seamless tubes.

2.2. Seamless tubes in the *Drosophila* tracheal system

The *Drosophila* trachea is a branched respiratory organ with ~1600 cells and several different types of tube topologies [6, 27]. The largest tubes are multicellular. Smaller

branches contain seamed unicellular tubes, called stalk cells. At the tips of most branches are two seamless tube types: fusion cells and terminal cells (Figs. 1C, 2A). Fusion cells are very short, simple toroids that undergo anastomosis to connect different branches in the network [28, 29]. Anastomosis fuses the apical membrane but does not remove junctions, so fusion cells have ring-shaped adherens junctions at both ends, where they connect to stalk cells and to each other (Fig. 1C). Terminal cells form complex, highly branched structures with narrow, lumenized processes that are closed at their tips and contact target tissues for gas exchange [6, 30] (Fig. 1C). A terminal cell has a ring-shaped adherens junction at its base, where it connects to a stalk cell, and sometimes a small stretch of autocellular junction (Fig. 2B).

Fibroblast Growth Factor Receptor (FGFR) signaling promotes tip cell vs. stalk cell identity and terminal cell identity [31, 32] (Fig. 2A). Wnt signaling promotes fusion cell identity [33] (Fig. 2A). Signaling leads to many changes in cytoskeletal organization [34–38] (Fig. 2A), and these cytoskeletal changes may be important for the ultimate formation of seamless tubes.

2.3. Seamless tubes in the *C. elegans* excretory system

The excretory system of *C. elegans* is a simple osmoregulatory organ that contains just three tandemly-connected unicellular tubes, two of which are seamless [4, 39] (Fig. 1D). The H-shaped canal cell has four long, seamless, lumenized branches or “canals” that are closed at their tips [40]. The canals merge within the cell body in the head of the worm, where the cell makes a ring-shaped junction to the duct. The duct is a smaller seamless tube with an asymmetric and looping shape [41]. The duct empties into the seamed pore tube, which connects to the outside environment for fluid excretion. The duct has ring-shaped apical junctions at both ends, where it connects to the canal cell and pore.

Epidermal Growth Factor Receptor (EGFR) signaling promotes duct vs. pore cell identity [42], and specifically promotes duct tube seamlessness via upregulation of the fusogen AFF-1 [41] (F. Soulavie and M. Sundaram, unpublished data) (Fig. 3A). Notch signaling and a series of asymmetric cell divisions promote canal cell identity [43].

3. Mechanisms of Seamless Tube Formation

3.1. Polarization

Seamless tube formation requires the cell to establish an unusual pattern of apical-basal polarity, with the apical domain inside the cell. The initial cues that establish such polarity are still unclear. Basal ECM factors may provide cues, since growing HUVEC cells in a 3D collagen matrix promotes intracellular lumen formation, and this requires integrin-ECM interactions [44]. Although integrins can affect terminal cell and canal tube shape [45, 46], they are not essential for polarization and intracellular lumen formation. *In vivo*, seamless tubes form in contexts where a cell makes ring-shaped adherens junctions on either one or two sides (Figs. 1,2A, 3A, 4C), so neighboring tubes might provide a polarizing cue. Several apical polarity proteins localize to apical domains of developing seamless tubes [47–51].

PAR-6 and PKC (but not Bazooka/PAR-3 or Crumbs) are required for lumen growth in the terminal cell [48, 52, 53].

Once polarity has been established to nucleate lumen formation, intracellular vesicle trafficking must direct apical membrane and other relevant molecules to the appropriate region for lumen growth. Both endocytic and exocytic trafficking pathways have been implicated in seamless tube formation and morphogenesis, as discussed below.

3.2. Pinocytosis

One mechanism for seamless tube formation involves pinocytosis ("cell drinking") or macropinocytosis ("cell gulping"), related types of membrane ruffling-associated endocytosis in which the cell internalizes basal plasma membrane to form internal vesicles [54]. These vesicles are proposed to coalesce to form the intracellular apical domain, which then connects to other tubes via anastomosis [11, 22, 44, 55]. This mechanism of tube formation is sometimes called "cell hollowing" (Fig. 4A).

The pinocytic model is based primarily on observations of cultured HUVEC or other mammalian endothelial cells [22]. The cells form large intracellular vacuoles that can be labeled with an exogenously applied dextran-fluorescein tracer, indicating some form of fluid phase uptake [44]. Vesicles have been observed moving from a basal site of origin to the intracellular vacuole [22]. Molecules required for vacuole formation include several regulators of macropinocytosis, including Rac and Pak [56, 57].

In vivo evidence to support the pinocytic model has been more limited. Various large intracellular vacuoles or multivesicular bodies (MVBs) have been detected near the growing lumen of vertebrate capillaries [9, 11], *Drosophila* terminal cells [55] and the *C. elegans* canal cell [4, 58], and have been speculated to derive from pinocytosis and contribute to the apical membrane. However, the origin of these vacuoles has not been determined definitively. Apical markers are generally not observed in any large or abundant vesicular compartments in wild-type animals, suggesting that the seamless lumen does not form from the coalescence of pre-established apical compartments [35, 59]. Rather, membrane may acquire apical characteristics only at late stages of vesicle targeting to the lumen.

Molecular regulators of endocytosis have varied effects on seamless tube growth *in vivo*. Rab11, a marker of recycling endosomes [54], promotes lumen growth in the terminal and canal cells [26, 48]. Early endocytic regulators, such as Shibire/Dynamin, Rab5, Vps45 and Rabenosyn-5, negatively regulate apical growth in terminal cells [53].

3.3. Wrapping and auto-fusion

A second mechanism for seamless tube formation is wrapping and auto-fusion (Fig. 4B). In this mechanism, a cell first forms a seamed tube of defined lumen diameter by wrapping around ECM [60] or a cellular scaffold [5] and forming an autocellular junction. The tube then eliminates its seam by membrane fusion. This mechanism is used by *C. elegans* pharyngeal valve cells [5] and the excretory duct (Fig. 3A) [41]. Conceptually, wrapping and auto-fusion is similar to macropinocytosis, except that it involves fusion rather than fission of a large segment of membrane, an extracellular scaffold, and the formation of a transient

adherens junction; it is not known if similar molecular controls underlie the two mechanisms.

Discovery of this mechanism in *C. elegans* was enabled by identification of the relevant fusogens [61, 62]. EFF-1 and AFF-1 are transmembrane proteins with structural, but not primary sequence, homology to viral class II fusogens [63, 64]. Homotypic interactions between fusogens on apposing plasma membranes mediate cell-cell fusion to generate many syncytial tissues in the worm [62, 65]. In *eff-1* or *aff-1* mutants, normally seamless tubes such as the duct now have a seam, revealing that these fusogens can also mediate tube auto-fusion [5, 41].

Wrapping and auto-fusion also generate some seamless tubes in the zebrafish vasculature [15] and in mammalian Madin-Darby Canine Kidney (MDCK) epithelial cells grown around silicone pegs [66], suggesting this mechanism may be widely used. MDCK auto-fusion requires cytoskeletal and trafficking regulators such as Rac, Rho, Cdc42 and the Arp2/3 complex [67], but the relevant fusogen is unknown.

3.4. Membrane invagination and apically-directed exocytosis

A third mechanism for seamless tube formation is membrane invagination coupled with apically-directed exocytosis (Fig. 4C). Evidence for this mechanism comes from studies of *Drosophila* fusion and terminal cells, the *C. elegans* canal cell, and anastomosing zebrafish vascular cells. In all of these tube types, lumen formation initiates at an intercellular junction with another tube (Figs. 2A, 3A) [13, 14, 41]. Lumen then grows inward in a manner dependent on F-actin and/or microtubules (MTs), which appear to provide routes for motor-directed vesicle trafficking [35, 50, 59, 68–70]. Where examined, lumen growth requires the exocyst [47, 48, 71, 72], a complex that tethers secretory vesicles to the plasma membrane for subsequent SNARE-dependent fusion [73]. Models for cytoskeletal organization and trafficking in the terminal cell and canal cell are shown in Figs. 2B–C, 3C–D. See [29] for a recent review of fusion cell formation

A current challenge is to elucidate the specific pathways and classes of vesicles that deliver apical membrane and luminal cargos. A striking feature of the canal cell is the presence of abundant 50–100 nm tubulo-vesicles, termed canaliculi, which fill the canal cytoplasm adjacent to the lumen (Fig. 3C–D) [4]. The origin of canaliculi is unknown, but canaliculi can dock with the apical membrane and may contribute membrane and/or water to drive lumen expansion (Fig. 3D) [47, 59, 74]. Going forward, live imaging will be an important tool for understanding the origin and role of canaliculi and the various other classes of vesicles that have been observed.

3.5. Combinatorial models

Although different seamless tubes may form by different mechanisms, the three major models discussed above are not mutually exclusive. It is likely that both endocytic and Golgi-derived exocytic vesicles contribute to the growing apical domain of most seamless tubes, although their relative importance could vary.

Within a single tube cell, initial lumen nucleation and subsequent lumen growth could also proceed by different mechanisms. For example, the excretory duct tube continues to grow extensively after initial wrapping and auto-fusion (Fig 1D, 3A) [41]. Several trafficking-related genes are required only for later outgrowth and maintenance of the terminal cell or canal cell, but not for initial tube formation [25, 26, 75, 76]. Terminal cells might nucleate lumen via a wrapping-like mechanism, since some have a small stretch of auto-junction near the cell's base (Fig. 2B), which usually disappears as the cells mature [6, 25, 52].

4. Seamless tube shaping and maintenance

Seamless tubes exhibit varied shapes, including very elongated and branched shapes, as exemplified by terminal cells and the canal cell. The absence of junctions along seamless tubes presents special challenges for tube shaping and maintenance. In multicellular tubes and planar epithelia, junctions play important roles in polarity, trafficking, cytoskeletal organization and in generating and transmitting forces for tissue shaping [77, 78]. In seamless tubes, junctions are likely important during initial tube formation (see above), but as the tubes elongate further away from the junction, they must rely on other, junction-independent mechanisms for shaping and strength.

4.1 Apical vs. basal trafficking

The amount of apical vs. basal outgrowth in a seamless tube will affect its shape. For example, fusion cells generate relatively little apical membrane compared to basal membrane [68]; as a result, the lumen and its associated cytoskeleton may be under tension and exert a pulling force on the attached stalk cells [29], leading to a "finger in a balloon" topology [27]. On the other hand, duct apical outgrowth surpasses basal outgrowth, leading to the characteristic looped path of the lumen in the cell (Fig. 1D) [4, 41]. Molecular mechanisms that restrain or promote apical vs. basal growth in these cells are not yet known.

In the terminal and canal cells, apical and basal outgrowth are closely coordinated; the basal domain grows out first, and the apical domain follows shortly behind [35, 74]. Actin and MT filaments at the leading edge appear to link the apical and basal membranes to coordinate trafficking activity and hold the lumen in place [35, 45, 70, 79]. Defects in branch outgrowth and lumen outgrowth are often coupled, but some mutations disrupt this coordination, and lead to long, convoluted lumens out of register with the basal membrane, or to multiple separate and disorganized lumens. Such mutants affect cytoskeletal organization and/or specific trafficking regulators, such as Rab35 or Cdc42, that may direct vesicles to appropriate locations for membrane fusion [38, 50, 70, 75, 80–82].

Continuous endocytosis and recycling of apical membrane are important for lumen shaping and maintenance. In terminal cells, mutations in early endocytic regulators or in the CCM3-STRIPAK complex cause an over-accumulation of apical membrane markers and result in focal dilations and cysts [25, 53]. In the canal cell, CCM3-STRIPAK and the EXC-1/5/9 pathway both regulate CDC-42 activity to affect recycling endosome trafficking for apical membrane maintenance [26, 75].

Puzzlingly, CCM3 promotes apical growth in the canal cell [26], but inhibits apical growth in terminal cells [25]. Another discrepancy is in the role of the vacuolar ATPase (V-ATPase). The V-ATPase is a proton pump involved in vesicle acidification; it plays multiple roles in vesicle trafficking, fusion and lysosomal degradation [83]. Both *C. elegans* and *Drosophila* V-ATPase mutants accumulate excess MVBs, consistent with a trafficking defect, but the V-ATPase restrains lumen growth in the canal cell [55, 81, 82, 84], yet promotes lumen growth in terminal cells [52, 55]. The mechanistic basis for these discrepancies is not known, but they could reflect differences in MT orientation (Figs. 2C, 3D), differences in junction organization at the cell base [25], or differences in the types of vesicles that contribute to the lumen of each cell [55].

4.2. The apical cytoskeleton and the apical ECM

The cytoskeleton is a major determinant of cell shape, and some effects of manipulating actin or MTs in seamless tubes may be due to changes in structural properties of cytoskeletal support networks [40]. The canal apical cytoskeleton also contains intermediate filaments [74], which generally provide mechanical strength to tissues [85].

Most developing tubes secrete a mix of fibril-forming and gel-forming molecules into their lumens, and studies in the fly and worm showed that this apical ECM influences lumen shape in tubes of all sizes [49, 86–88]. Zona Pellucida (ZP)-domain containing proteins are common constituents of these matrices [89]. Interestingly, the capillary disease-associated ZP protein HHT1/endoglin (Table 1) is a component of the endothelial luminal matrix [90, 91]. The apical ECM can affect signaling [92] and may be physically linked to the cytoskeleton [93–96], but its mechanisms of action and effects on seamless tubes are just beginning to be explored.

In the fly trachea, a transient luminal ECM containing chitin and ZP proteins is present throughout tube formation and morphogenesis (Fig. 2B, C), but is then cleared during cuticle maturation (reviewed in [86, 87]). Mutants for Expansion, a gene required for chitin deposition, revealed large luminal dilations in terminal cells, but not fusion cells, suggesting differential importance of the luminal ECM in shaping these two seamless tube types [97, 98]. Mutations in the receptor tyrosine phosphatases Ptp4E and Ptp10D [99] or the lipid transport protein Mtp [100] result in similar terminal cell cysts, suggesting signaling and lipids might also affect matrix organization.

In the *C. elegans* excretory duct and pore cells, a transient luminal ECM is also present throughout tube formation and morphogenesis, but then cleared during cuticle maturation [49, 101]. One component of the luminal ECM is the secreted ZP protein LET-653 [102] (Fig. 3B), which is critical for maintaining duct lumen integrity during elongation (H. Gill, J. Cohen and M. Sundaram, unpublished data). Mutants for the lipocalin LPR-1 [41], or for the extracellular leucine-rich repeat only (eLRRon) transmembrane proteins LET-4 or EGG-6 [49], exhibit duct defects very similar to those of *let-653* mutants, and may also affect matrix organization (Fig. 3B).

The developing canal cell also contains a luminal matrix of unknown composition and function [40] (Fig 3C). The canal cell does not make cuticle and does not express LET-653

(H. Gill, J. Cohen and M. Sundaram, unpublished data), but does express a different ZP protein, DYF-7 [103].

4.3. The seamless-seamed tube junction

Seamless tubes often connect to seamed tubes at their origin (Fig. 4C). At such a connection point, a ring-shaped junction shared by two different cells is contacted by a linear autocellular junction. The topology of this junction is similar to that of a tricellular junction, in which three linear junctions converge at a single point. Specific proteins localize to, and stabilize, tricellular junctions [104, 105]. Whether these proteins localize to or play a role in the stability of seamless-seamed tube junctions remains to be determined. Interestingly, a number of mutations, including those affecting *Drosophila* CCM3-STRIPAK, specifically affect lumen shape and integrity near a seamless-seamed tube junction [25, 41, 49](H. Gill, J. Cohen and M. Sundaram, unpublished data), suggesting this region is particularly fragile, and that the pathology of human capillary diseases might involve such junctions.

5. Summary and future challenges

Seamless tubes are found in many multicellular organisms, including mammals. The formation and maintenance of these tiny tubes present unique challenges. Recent studies in zebrafish, flies and worms have made great strides in visualizing the processes that build seamless tubes and in identifying specific genes required for their shaping and integrity. Cells can form seamless tubes through macropinocytosis, wrapping and auto-fusion, or apically directed exocytosis, but many questions remain about these mechanisms. What cues trigger inside/outside polarity in seamless tubes? Do endocytic and/or exocytic vesicles nucleate and expand the lumen? What are the relevant trafficking pathways involved? How are apical and basal growth coordinated for tube shaping? Through what mechanisms does the luminal ECM affect tube formation, shaping and maintenance? Finally, why do seamless tubes exist? Are they stronger and less leaky than seamed tubes of comparable diameter? Does the absence of junctions free them to adopt more complex shapes?

Ultimately, a very important challenge is to relate the findings in model systems to human microvascular diseases. Do such diseases specifically disrupt the formation or maintenance of seamless tubes or do they affect narrow tubes more generally? In model systems, it is possible to identify genetic manipulations that ameliorate unicellular tube defects; could such manipulations suggest strategies for treating microvascular disease?

Acknowledgments

We thank Amin Ghabrial, John Murray, Janis Burkhardt and members of our laboratory for helpful discussions and comments on the manuscript. M.S. was supported by grants from NIH (R01GM58540) and NSF (1257879).

References

1. Lubarsky B, Krasnow MA. Tube morphogenesis: making and shaping biological tubes. Cell. 2003; 112:19–28. [PubMed: 12526790]
2. Wolff JR, Bar T. ‘Seamless’ endothelia in brain capillaries during development of the rat’s cerebral cortex. Brain Research. 1972; 41:17–24. [PubMed: 5036034]

3. Freeman MR. Drosophila Central Nervous System Glia. Cold Spring Harbor perspectives in biology. 2015
4. Nelson FK, Albert PS, Riddle DL. Fine structure of the C. elegans secretory-excretory system. J Ultrastruct Res. 1983; 82:156–71. [PubMed: 6827646]
5. Rasmussen JP, English K, Tenlen JR, Priess JR. Notch signaling and morphogenesis of single-cell tubes in the C. elegans digestive tract [see comment]. Developmental Cell. 2008; 14:559–69. [PubMed: 18410731]
6. Samakovlis C, Hacohen N, Manning G, Sutherland DC, Guillemin K, Krasnow MA. Development of the Drosophila tracheal system occurs by a series of morphologically distinct but genetically coupled branching events. Development. 1996; 122:1395–407. [PubMed: 8625828]
7. Shaham S. Glial development and function in the nervous system of Caenorhabditis elegans. Cold Spring Harbor perspectives in biology. 2015; 7:a020578. [PubMed: 25573712]
8. Geitmann A. How to shape a cylinder: pollen tube as a model system for the generation of complex cellular geometry. Sexual plant reproduction. 2010; 23:63–71. [PubMed: 20165964]
9. Dyson SE, Jones DG, Kendrick WL. Some observations on the ultrastructure of developing rat cerebral capillaries. Cell Tissue Res. 1976; 173:529–42. [PubMed: 791503]
10. Bar T, Guldner FH, Wolff JR. “Seamless” endothelial cells of blood capillaries. Cell & Tissue Research. 1984; 235:99–106. [PubMed: 6697387]
11. Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM. Endothelial tubes assemble from intracellular vacuoles in vivo. [see comment]. Nature. 2006; 442:453–6. [PubMed: 16799567]
12. Ehling M, Adams S, Benedito R, Adams RH. Notch controls retinal blood vessel maturation and quiescence. Development. 2013; 140:3051–61. [PubMed: 23785053]
13. Herwig L, Blum Y, Krudewig A, Ellertsdottir E, Lenard A, Belting HG, et al. Distinct cellular mechanisms of blood vessel fusion in the zebrafish embryo. Curr Biol. 2011; 21:1942–8. [PubMed: 22079115]
14. Lenard A, Ellertsdottir E, Herwig L, Krudewig A, Sauteur L, Belting HG, et al. In vivo analysis reveals a highly stereotypic morphogenetic pathway of vascular anastomosis. Dev Cell. 2013; 25:492–506. [PubMed: 23763948]
15. Lenard A, Daetwyler S, Betz C, Ellertsdottir E, Belting HG, Huisken J, et al. Endothelial Cell Self-fusion during Vascular Pruning. PLoS Biol. 2015; 13:e1002126. [PubMed: 25884426]
16. Yu JA, Castranova D, Pham VN, Weinstein BM. Single-cell analysis of endothelial morphogenesis in vivo. Development. 2015; 142:2951–61. [PubMed: 26253401]
17. Murfee WL, Schmid-Schonbein GW. Chapter 12. Structure of microvascular networks in genetic hypertension. Methods Enzymol. 2008; 444:271–84. [PubMed: 19007669]
18. Dean J, Cruz SD, Mehta PK, Merz CN. Coronary microvascular dysfunction: sex-specific risk, diagnosis, and therapy. Nature reviews Cardiology. 2015; 12:406–14. [PubMed: 26011377]
19. Haffner C, Malik R, Dichgans M. Genetic factors in cerebral small vessel disease and their impact on stroke and dementia. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism. 2015
20. Govani FS, Shovlin CL. Hereditary haemorrhagic telangiectasia: a clinical and scientific review. European journal of human genetics: EJHG. 2009; 17:860–71. [PubMed: 19337313]
21. Draheim KM, Fisher OS, Boggon TJ, Calderwood DA. Cerebral cavernous malformation proteins at a glance. J Cell Sci. 2014; 127:701–7. [PubMed: 24481819]
22. Sacharidou A, Stratman AN, Davis GE. Molecular mechanisms controlling vascular lumen formation in three-dimensional extracellular matrices. Cells, tissues, organs. 2012; 195:122–43. [PubMed: 21997121]
23. Liu H, Rigamonti D, Badr A, Zhang J. Ccm1 regulates microvascular morphogenesis during angiogenesis. Journal of vascular research. 2011; 48:130–40. [PubMed: 20926893]
24. Whitehead KJ, Chan AC, Navankasattusas S, Koh W, London NR, Ling J, et al. The cerebral cavernous malformation signaling pathway promotes vascular integrity via Rho GTPases. Nature Medicine. 2009; 15:177–84.

25. Song Y, Eng M, Ghabrial AS. Focal defects in single-celled tubes mutant for Cerebral cavernous malformation 3, GCKIII, or NSF2. Dev Cell. 2013; 25:507–19. [PubMed: 23763949]
26. Lant B, Yu B, Goudreault M, Holmyard D, Knight JD, Xu P, et al. CCM-3/STRIPAK promotes seamless tube extension through endocytic recycling. Nature communications. 2015; 6:6449.
27. Uv A, Cantera R, Samakovlis C. Drosophila tracheal morphogenesis: intricate cellular solutions to basic plumbing problems. Trends Cell Biol. 2003; 13:301–9. [PubMed: 12791296]
28. Samakovlis C, Manning G, Steneberg P, Hacohen N, Cantera R, Krasnow MA. Genetic control of epithelial tube fusion during Drosophila tracheal development. Development. 1996; 122:3531–6. [PubMed: 8951068]
29. Caviglia S, Luschnig S. Tube fusion: making connections in branched tubular networks. Semin Cell Dev Biol. 2014; 31:82–90. [PubMed: 24662893]
30. Jarecki J, Johnson E, Krasnow MA. Oxygen regulation of airway branching in Drosophila is mediated by branchless FGF. Cell. 1999; 99:211–20. [PubMed: 10535739]
31. Ghabrial AS, Krasnow MA. Social interactions among epithelial cells during tracheal branching morphogenesis. Nature. 2006; 441:746–9. [PubMed: 16760977]
32. Schottenfeld J, Song Y, Ghabrial AS. Tube continued: morphogenesis of the Drosophila tracheal system. Curr Opin Cell Biol. 2010; 22:633–9. [PubMed: 20739171]
33. Caviglia S, Luschnig S. The ETS domain transcriptional repressor Anterior open inhibits MAP kinase and Wingless signaling to couple tracheal cell fate with branch identity. Development. 2013; 140:1240–9. [PubMed: 23444354]
34. Cheng YL, Andrew DJ. Extracellular Mipp1 Activity Confers Migratory Advantage to Epithelial Cells during Collective Migration. Cell reports. 2015
35. Gervais L, Casanova J. In vivo coupling of cell elongation and lumen formation in a single cell. Curr Biol. 2010; 20:359–66. [PubMed: 20137948]
36. Lebreton G, Casanova J. Specification of leading and trailing cell features during collective migration in the Drosophila trachea. J Cell Sci. 2014; 127:465–74. [PubMed: 24213534]
37. Okenve-Ramos P, Llimargas M. Fascin links Btl/FGFR signalling to the actin cytoskeleton during Drosophila tracheal morphogenesis. Development. 2014; 141:929–39. [PubMed: 24496629]
38. Ukken FP, Aprill I, JayaNandanan N, Leptin M. Slik and the receptor tyrosine kinase Breathless mediate localized activation of Moesin in terminal tracheal cells. PLoS One. 2014; 9:e103323. [PubMed: 25061859]
39. Nelson FK, Riddle DL. Functional study of the C. elegans secretory-excretory system using laser microsurgery. J Exptl Zool. 1984; 231:45–56. [PubMed: 6470649]
40. Buechner M, Hall DH, Bhatt H, Hedgecock EM. Cystic canal mutants in Caenorhabditis elegans are defective in the apical membrane domain of the renal (excretory) cell. Developmental Biology. 1999; 214:227–41. [PubMed: 10491271]
41. Stone CE, Hall DH, Sundaram MV. Lipocalin signaling controls unicellular tube development in the Caenorhabditis elegans excretory system. Dev Biol. 2009; 329:201–11. [PubMed: 19269285]
42. Abdus-Saboor I, Mancuso VP, Murray JI, Palozola K, Norris C, Hall DH, et al. Notch and Ras promote sequential steps of excretory tube development in C. elegans. Development. 2011; 138:3545–55. [PubMed: 21771815]
43. Moskowitz IP, Rothman JH. lin-12 and glp-1 are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in Caenorhabditis elegans. Development. 1996; 122:4105–17. [PubMed: 9012530]
44. Davis GE, Camarillo CW. An alpha 2 beta 1 integrin-dependent pinocytic mechanism involving intracellular vacuole formation and coalescence regulates capillary lumen and tube formation in three-dimensional collagen matrix. Experimental Cell Research. 1996; 224:39–51. [PubMed: 8612690]
45. Levi BP, Ghabrial AS, Krasnow MA. Drosophila talin and integrin genes are required for maintenance of tracheal terminal branches and luminal organization. Development. 2006; 133:2383–93. [PubMed: 16720877]
46. Hedgecock EM, Culotti JG, Hall DH, Stern BD. Genetics of cell and axon migrations in *Caenorhabditis elegans*. Development. 1987; 100:365–82. [PubMed: 3308403]

47. Armenti ST, Chan E, Nance J. Polarized exocyst-mediated vesicle fusion directs intracellular lumenogenesis within the C. elegans excretory cell. Dev Biol. 2014; 394:110–21. [PubMed: 25102190]
48. Jones TA, Nikolova LS, Schjelderup A, Metzstein MM. Exocyst-mediated membrane trafficking is required for branch outgrowth in Drosophila tracheal terminal cells. Dev Biol. 2014; 390:41–50. [PubMed: 24607370]
49. Mancuso VP, Parry JM, Storer L, Poggioli C, Nguyen KC, Hall DH, et al. Extracellular leucine-rich repeat proteins are required to organize the apical extracellular matrix and maintain epithelial junction integrity in C. elegans. Development. 2012; 139:979–90. [PubMed: 22278925]
50. Schottenfeld-Roames J, Ghabrial AS. Whacked and Rab35 polarize dynein-motor-complex-dependent seamless tube growth. Nat Cell Biol. 2012; 14:386–93. [PubMed: 22407366]
51. Waaijers S, Ramalho JJ, Koorman T, Kruse E, Boxem M. The C. elegans Crumbs family contains a CRB3 homolog and is not essential for viability. Biology open. 2015; 4:276–84. [PubMed: 25661870]
52. Francis D, Ghabrial AS. Compensatory branching morphogenesis of stalk cells in the Drosophila trachea. Development. 2015; 142:2048–57. [PubMed: 25977367]
53. Schottenfeld-Roames J, Rosa JB, Ghabrial AS. Seamless tube shape is constrained by endocytosis-dependent regulation of active Moesin. Curr Biol. 2014; 24:1756–64. [PubMed: 25065756]
54. Doherty GJ, McMahon HT. Mechanisms of endocytosis. Annu Rev Biochem. 2009; 78:857–902. [PubMed: 19317650]
55. Nikolova LS, Metzstein MM. Intracellular lumen formation in Drosophila proceeds via a novel subcellular compartment. Development. 2015; 142:3964–73. [PubMed: 26428009]
56. Bayless KJ, Davis GE. The Cdc42 and Rac1 GTPases are required for capillary lumen formation in three-dimensional extracellular matrices. Journal of Cell Science. 2002; 115:1123–36. [PubMed: 11884513]
57. Koh W, Mahan RD, Davis GE. Cdc42- and Rac1-mediated endothelial lumen formation requires Pak2, Pak4 and Par3, and PKC-dependent signaling. Journal of Cell Science. 2008; 121:989–1001. [PubMed: 18319301]
58. Berry KL, Bulow HE, Hall DH, Hobert O. A C. elegans CLIC-like protein required for intracellular tube formation and maintenance. [see comment]. Science. 2003; 302:2134–7. [PubMed: 14684823]
59. Khan LA, Zhang H, Abraham N, Sun L, Fleming JT, Buechner M, et al. Intracellular lumen extension requires ERM-1-dependent apical membrane expansion and AQP-8-mediated flux. Nat Cell Biol. 2013; 15:143–56. [PubMed: 23334498]
60. Jazwinska A, Ribeiro C, Affolter M. Epithelial tube morphogenesis during Drosophila tracheal development requires Piopio, a luminal ZP protein. Nature Cell Biology. 2003; 5:895–901. [PubMed: 12973360]
61. Mohler WA, Shemer G, del Campo JJ, Valansi C, Opoku-Serebuoh E, Scranton V, et al. The type I membrane protein EFF-1 is essential for developmental cell fusion. Developmental Cell. 2002; 2:355–62. [PubMed: 11879640]
62. Sapir A, Choi J, Leikina E, Avinoam O, Valansi C, Chernomordik LV, et al. AFF-1, a FOS-1-regulated fusogen, mediates fusion of the anchor cell in C. elegans. [see comment]. Developmental Cell. 2007; 12:683–98. [PubMed: 17488621]
63. Perez-Vargas J, Krey T, Valansi C, Avinoam O, Haouz A, Jamin M, et al. Structural basis of eukaryotic cell-cell fusion. Cell. 2014; 157:407–19. [PubMed: 24725407]
64. Zeev-Ben-Mordehai T, Vasishtan D, Siebert CA, Grunewald K. The full-length cell-cell fusogen EFF-1 is monomeric and upright on the membrane. Nature communications. 2014; 5:3912.
65. Podbilewicz B, Leikina E, Sapir A, Valansi C, Suissa M, Shemer G, et al. The C. elegans developmental fusogen EFF-1 mediates homotypic fusion in heterologous cells and in vivo. Developmental Cell. 2006; 11:471–81. [PubMed: 17011487]
66. Sumida GM, Yamada S. Self-contact elimination by membrane fusion. Proc Natl Acad Sci U S A. 2013; 110:18958–63. [PubMed: 24191042]

67. Sumida GM, Yamada S. Rho GTPases and the downstream effectors actin-related protein 2/3 (Arp2/3) complex and myosin II induce membrane fusion at self-contacts. J Biol Chem. 2015; 290:3238–47. [PubMed: 25527498]
68. Gervais L, Lebreton G, Casanova J. The making of a fusion branch in the Drosophila trachea. Dev Biol. 2012; 362:187–93. [PubMed: 22178247]
69. Phng LK, Gebala V, Bentley K, Philippides A, Wacker A, Mathivet T, et al. Formin-mediated actin polymerization at endothelial junctions is required for vessel lumen formation and stabilization. Dev Cell. 2015; 32:123–32. [PubMed: 25584798]
70. Shaye DD, Greenwald I. The disease-associated formin INF2/EXC-6 organizes lumen and cell outgrowth during tubulogenesis by regulating F-actin and microtubule cytoskeletons. Dev Cell. 2015; 32:743–55. [PubMed: 25771894]
71. Jiang L, Rogers SL, Crews ST. The Drosophila Dead end Arf-like3 GTPase controls vesicle trafficking during tracheal fusion cell morphogenesis. Developmental Biology. 2007; 311:487–99. [PubMed: 17919535]
72. Kakihara K, Shinmyozu K, Kato K, Wada H, Hayashi S. Conversion of plasma membrane topology during epithelial tube connection requires Arf-like 3 small GTPase in Drosophila. Mech Dev. 2008; 125:325–36. [PubMed: 18083504]
73. Liu J, Guo W. The exocyst complex in exocytosis and cell migration. Protoplasma. 2012; 249:587–97. [PubMed: 21997494]
74. Kolotuev I, Hyenne V, Schwab Y, Rodriguez D, Labouesse M. A pathway for unicellular tube extension depending on the lymphatic vessel determinant Prox1 and on osmoregulation. Nat Cell Biol. 2013; 15:157–68. [PubMed: 23334499]
75. Mattingly BC, Buechner M. The FGD homologue EXC-5 regulates apical trafficking in C. elegans tubules. Dev Biol. 2011; 359:59–72. [PubMed: 21889936]
76. Ghabrial AS, Levi BP, Krasnow MA. A systematic screen for tube morphogenesis and branching genes in the Drosophila tracheal system. PLoS Genet. 2011; 7:e1002087. [PubMed: 21750678]
77. Guillot C, Lecuit T. Mechanics of epithelial tissue homeostasis and morphogenesis. Science. 2013; 340:1185–9. [PubMed: 23744939]
78. Rodriguez-Boulan E, Macara IG. Organization and execution of the epithelial polarity programme. Nat Rev Mol Cell Biol. 2014; 15:225–42. [PubMed: 24651541]
79. Oshima K, Takeda M, Kuranaga E, Ueda R, Aigaki T, Miura M, et al. IKK epsilon regulates F actin assembly and interacts with Drosophila IAP1 in cellular morphogenesis. Curr Biol. 2006; 16:1531–7. [PubMed: 16887350]
80. JayaNandanan N, Mathew R, Leptin M. Guidance of subcellular tubulogenesis by actin under the control of a synaptotagmin-like protein and Moesin. Nature communications. 2014; 5:3036.
81. Liegeois S, Benedetto A, Garnier JM, Schwab Y, Labouesse M. The V0-ATPase mediates apical secretion of exosomes containing Hedgehog-related proteins in Caenorhabditis elegans. Journal of Cell Biology. 2006; 173:949–61. [PubMed: 16785323]
82. Liegeois S, Benedetto A, Michaux G, Belliard G, Labouesse M. Genes required for osmoregulation and apical secretion in Caenorhabditis elegans. Genetics. 2007; 175:709–24. [PubMed: 17179093]
83. Cotter K, Stransky L, McGuire C, Forgac M. Recent Insights into the Structure, Regulation, and Function of the V-ATPases. Trends Biochem Sci. 2015; 40:611–22. [PubMed: 26410601]
84. Hahn-Windgassen A, Van Gilst MR. The Caenorhabditis elegans HNF4alpha Homolog, NHR-31, mediates excretory tube growth and function through coordinate regulation of the vacuolar ATPase. PLoS Genet. 2009; 5:e1000553. [PubMed: 19668342]
85. Lowery J, Kuczmarski ER, Herrmann H, Goldman RD. Intermediate Filaments Play a Pivotal Role in Regulating Cell Architecture and Function. J Biol Chem. 2015; 290:17145–53. [PubMed: 25957409]
86. Luschnig S, Uv A. Luminal matrices: an inside view on organ morphogenesis. Exp Cell Res. 2014; 321:64–70. [PubMed: 24075963]
87. Zuo L, Iordanou E, Chandran RR, Jiang L. Novel mechanisms of tube-size regulation revealed by the Drosophila trachea. Cell Tissue Res. 2013; 354:343–54. [PubMed: 23824100]
88. Bulik DA, Robbins PW. The Caenorhabditis elegans sqv genes and functions of proteoglycans in development. Biochim Biophys Acta. 2002; 1573:247–57. [PubMed: 12417407]

89. Plaza S, Chanut-Delalande H, Fernandes I, Wassarman PM, Payre F. From A to Z: apical structures and zona pellucida-domain proteins. Trends Cell Biol. 2010; 20:524–32. [PubMed: 20598543]

90. McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat Genet. 1994; 8:345–51. [PubMed: 7894484]

91. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Archiv: European journal of physiology. 2007; 454:345–59. [PubMed: 17256154]

92. ten Dijke P, Goumans MJ, Pardali E. Endoglin in angiogenesis and vascular diseases. Angiogenesis. 2008; 11:79–89. [PubMed: 18283546]

93. Dong B, Hannezo E, Hayashi S. Balance between apical membrane growth and luminal matrix resistance determines epithelial tubule shape. Cell reports. 2014; 7:941–50. [PubMed: 24794438]

94. Brodu V, Baffet AD, Le Droguen PM, Casanova J, Guichet A. A developmentally regulated two-step process generates a noncentrosomal microtubule network in Drosophila tracheal cells. Dev Cell. 2010; 18:790–801. [PubMed: 20493812]

95. Ray RP, Matamoro-Vidal A, Ribeiro PS, Tapon N, Houle D, Salazar-Ciudad I, et al. Patterned Anchorage to the Apical Extracellular Matrix Defines Tissue Shape in the Developing Appendages of Drosophila. Dev Cell. 2015; 34:310–22. [PubMed: 26190146]

96. Tarbell JM, Simon SI, Curry FR. Mechanosensing at the vascular interface. Annual review of biomedical engineering. 2014; 16:505–32.

97. Iordanou E, Chandran RR, Yang Y, Essak M, Blackstone N, Jiang L. The novel Smad protein Expansion regulates the receptor tyrosine kinase pathway to control Drosophila tracheal tube size. Dev Biol. 2014; 393:93–108. [PubMed: 24973580]

98. Moussian B, Letizia A, Martinez-Corrales G, Rotstein B, Casali A, Llimargas M. Deciphering the genetic programme triggering timely and spatially-regulated chitin deposition. PLoS Genet. 2015; 11:e1004939. [PubMed: 25617778]

99. Jeon M, Zinn K. Receptor tyrosine phosphatases control tracheal tube geometries through negative regulation of Egfr signaling. Development. 2009; 136:3121–9. [PubMed: 19675131]

100. Baer MM, Palm W, Eaton S, Leptin M, Affolter M. Microsomal triacylglycerol transfer protein (MTP) is required to expand tracheal lumen in Drosophila in a cell-autonomous manner. J Cell Sci. 2012; 125:6038–48. [PubMed: 23132924]

101. Page AP, Johnstone IL. The cuticle. Wormbook. 2007:1–15.

102. Jones SJ, Baillie DL. Characterization of the let-653 gene in Caenorhabditis elegans. Molecular & General Genetics. 1995; 248:719–26. [PubMed: 7476875]

103. Heiman MG, Shaham S. DEX-1 and DYF-7 establish sensory dendrite length by anchoring dendritic tips during cell migration. Cell. 2009; 137:344–55. [PubMed: 19344940]

104. Furuse M, Izumi Y, Oda Y, Higashi T, Iwamoto N. Molecular organization of tricellular tight junctions. Tissue barriers. 2014; 2:e28960. [PubMed: 25097825]

105. Byri S, Misra T, Syed ZA, Batz T, Shah J, Boril L, et al. The Triple-Repeat Protein Anakonda Controls Epithelial Tricellular Junction Formation in Drosophila. Dev Cell. 2015; 33:535–48. [PubMed: 25982676]

106. Gervais L, Casanova J. The Drosophila homologue of SRF acts as a boosting mechanism to sustain FGF-induced terminal branching in the tracheal system. Development. 2011; 138:1269–74. [PubMed: 21385762]

107. Jiang L, Crews ST. Dysfusion transcriptional control of Drosophila tracheal migration, adhesion, and fusion. Mol Cell Biol. 2006; 26:6547–56. [PubMed: 16914738]

108. Lee M, Lee S, Zadeh AD, Kolodziej PA. Distinct sites in E-cadherin regulate different steps in Drosophila tracheal tube fusion. Development. 2003; 130:5989–99. [PubMed: 14597571]

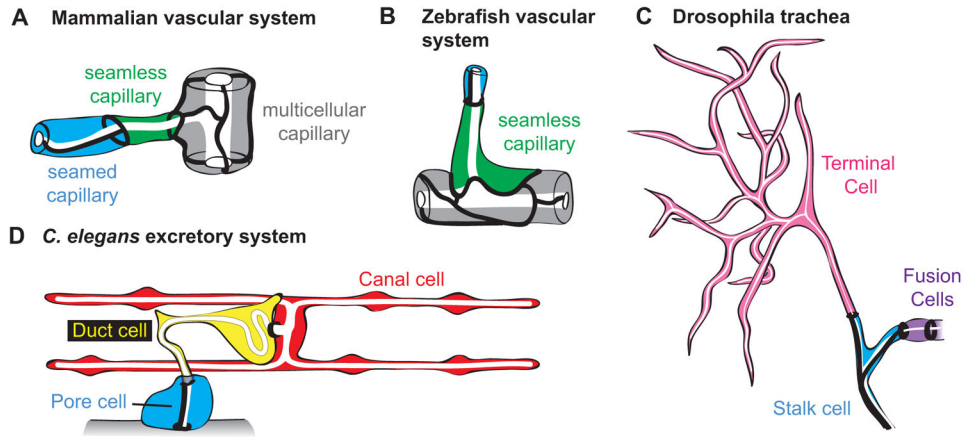

Figure 1. Examples of seamless tubes

(A, B) Vertebrate capillaries are a mix of multicellular, seamed unicellular and seamless tubes. Drawings of adult rat and embryonic zebrafish capillaries based on [2] and [15]. (C) The *Drosophila* trachea contains two seamless tube types, terminal cells and fusion cells. Drawing of third instar larval branch tip based on [31]. (D) The *C. elegans* excretory system contains two seamless tube types, the canal cell and duct. Drawing of early L1 larval excretory system based on [41] and [84].

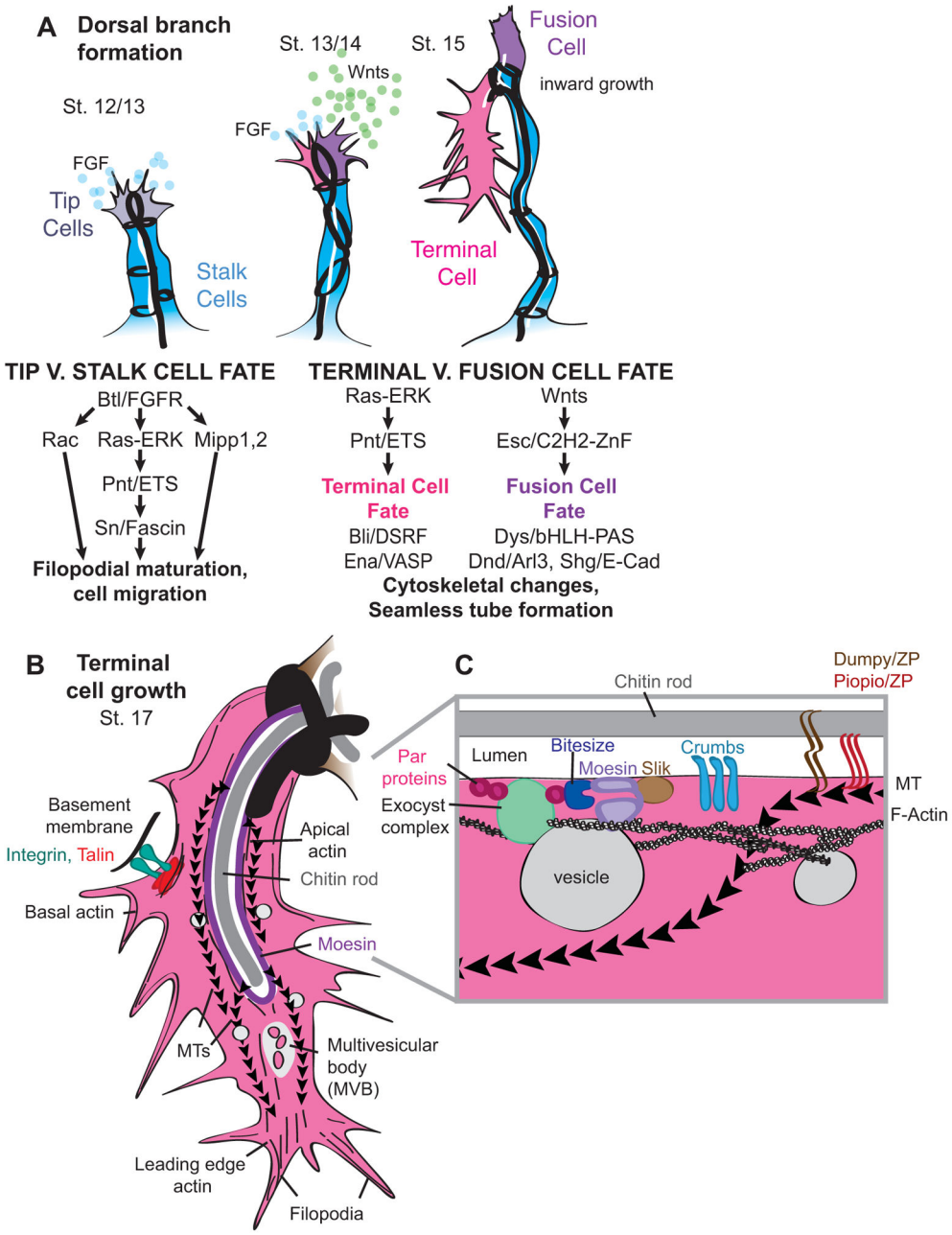

Figure 2. Seamless tube formation and growth in the *Drosophila* tracheal system

(A) Developmental timeline for one dorsal branch [6] and roles of FGF and Wnt signaling. Heavy black lines represent junctions. FGF signaling promotes tip cell identity (gray) [31] and expression or activity of Singed/Fascin [37], Mipp 1 and 2 [34], and Rac [36], which promote filopodia maturation leading to tip cell migration and outgrowth. The FGFR target Pointed (Pnt) promotes terminal cell identity (pink) [6, 31] and expression of the DSRF transcription factor for tube morphogenesis [106]. DSRF upregulates Enabled (Ena) to promote actin filament elongation [35]. Wnt signaling promotes Escargot (Esc) expression and fusion cell identity (purple) [33]. Fusion cells express the transcription factor Dysfusion

(Dys) and upregulate Deadend (Dnd)/Arl3 and Shotgun/E-cadherin to promote anastomosis [71, 72, 107, 108]. The terminal cell turns ventrally and builds lumen starting at the stalk cell interface [35]. The fusion cell extends filopodia dorsally in search of its partner fusion cell [28]. (B) Structure and organization of a growing terminal cell. Multivesicular bodies are present near the leading edge at later stages [55]. Actin is found along the apical and basal membranes and at the leading edge [35, 79]. Microtubules are nucleated along the apical membrane, and extend toward the leading edge [35, 50, 94]. The (−) end directed MT motor dynamin is required for lumen growth [50]. Moesin organizes actin at the apical membrane [80]. Integrins link the basal cell membrane to the basal ECM [45]. (C) Model for terminal cell apical domain organization. Bitesize and Crumbs recruit Moesin to the apical membrane [53, 80], where it is phosphorylated and activated by Slik [38]. Moesin then recruits actin to promote apical-directed trafficking. PAR proteins recruit the exocyst complex for vesicle docking [48]. A chitin rod determines lumen diameter [86, 87, 97, 98]. The ZP proteins Dumpy (Dpy) and Piopio (Pio) may link the chitin rod to the apical membrane and cytoskeleton [60, 93–95].

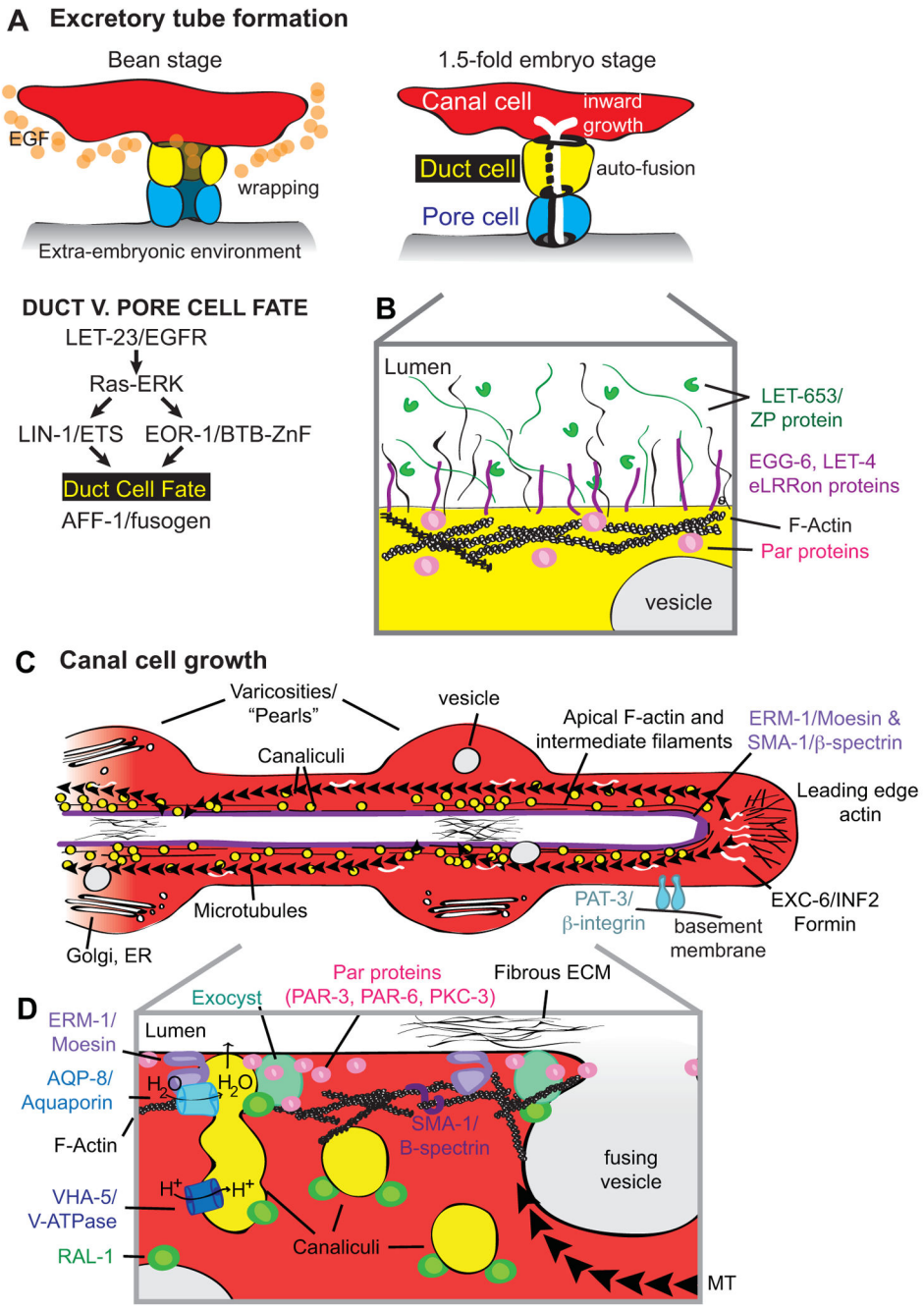

Figure 3. Seamless tube formation and growth in the *C. elegans* excretory system

(A) Developmental timeline and duct fate specification by EGF signaling [42]. The canal cell expresses LIN-3/EGF. EGF-Ras-ERK signaling acts via LIN-1/Ets and EOR-1/BTB-Zinc finger transcription factors to promote the duct fate and a top, canal-proximal position. The other cell adopts the pore fate. The duct and pore cells epithelialize and wrap themselves into tubes. Both the duct and pore initially form auto-junctions, but the duct expresses AFF-1 and auto-fuses [41]. The canal cell lumen originates at the duct-canal junction and grows inward. (B) Model for luminal matrix organization in the duct cell.

During embryogenesis, the duct lumen is filled with a fibrous ECM containing the ZP and mucin-like protein LET-653 (H. Gill, J. Cohen and M. Sundaram, unpublished data). The eLRRon proteins LET-4 and EGG-6 may interact with one or more components of this matrix [49]. (C) Structure and organization of a growing canal arm. Canalicular vesicles are plentiful along the apical region; other vesicle types are present more sparsely [4, 59]. Cytoplasmic swellings, or varicosities, form periodically along the canal arms during growth or recovery from osmotic stress, and canaliculi preferentially dock to the apical membrane in these regions [74]. There are two major pools of actin: apical and at the leading edge [59, 70]. Intermediate filaments also line the apical membrane [74]. MTs are nucleated primarily at the leading edge, where they are linked to actin via the formin EXC-6 [70], and extend back toward the cell body. The kinesin motor promotes lumen growth [70]. ERM-1/moesin and SMA-1/β-spectrin recruit and stabilize actin at the apical membrane [40, 59]. Integrins link the basal cell membrane to the basal ECM [46]. A fibrous apical ECM is sometimes observed [40]. (D) Model for apical membrane addition and lumen growth in the canal cell [47, 59, 74]. Canalicular vesicles contain the V-ATPase and the aquaporin AQP-8; they dock at the apical membrane to allow water influx and, potentially, membrane addition. Other vesicles may also contribute apical membrane. ERM-1 recruits an actin coat to vesicles and binds AQP-8 to promote canaliculi docking. RAL-1 on vesicles promotes fusion by interacting with apical PAR proteins to recruit the exocyst.

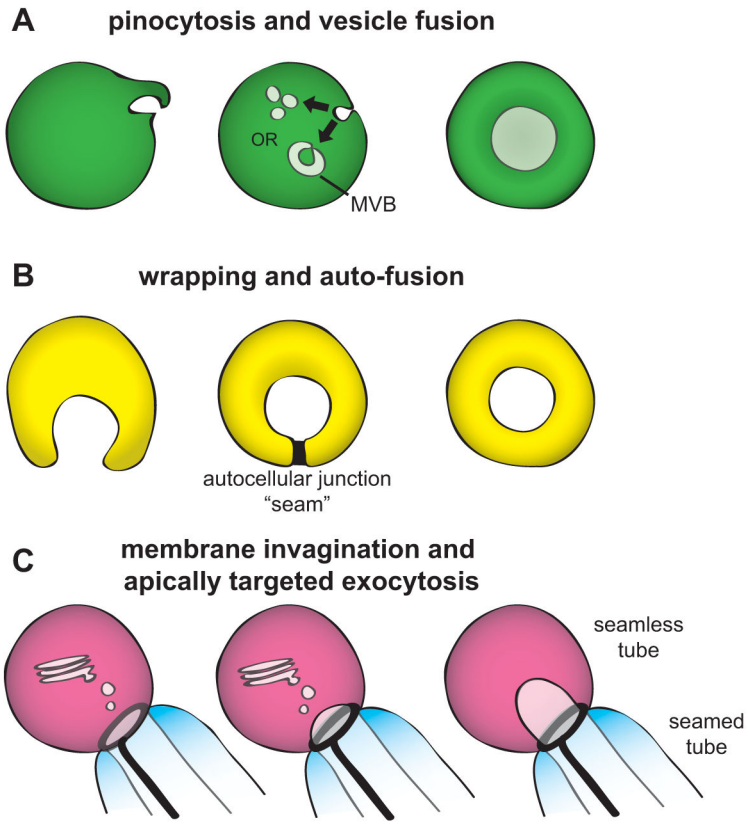

Figure 4. Models for seamless tube formation

(A) Pinocytosis. (B) Wrapping and auto-fusion. (C) Membrane invagination and apically directed exocytosis. See text for details and references.

Table 1

Mendelian diseases associated with capillary abnormalities

Disease	Affected Gene Product(s)	OMIM entries
Cerebral cavernous malformation (CCM)	CCM1/Krit1 CCM2/malcavernin CCM3/PDCD10	OMIM: 116860; 603284; 603285
Hereditary Hemorrhagic Telangiecstasia (HHT)	HHT1/Endoglin HHT2/ALK1/ACVRL1 HHT5/GDF2/BMP9	OMIM: 187300; 600376; 615506
Juvenile Polyposis with Hereditary Hemorrhagic Telangiecstasia (JP/HHT)	SMAD4	OMIM: 175050
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	Notch3	OMIM: 125310
Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)	HTRA1 serine protease	OMIM: 600142
COL4A1/A2-related angiopathies	COL4A1 COL4A2	OMIM: 607595; 614519
Retinal vasculopathy with cerebral leukodystrophy (RVCL)	TREX1 exonuclease	OMIM: 192315