Development 140, 2851-2855 (2013) doi:10.1242/dev.070680

Tubulogenesis

M. Luisa Iruela-Arispe[1,*] and Greg J. Beitel[2,*]

Summary

Metazoans require epithelial and endothelial tubes to transport liquids and gasses throughout their bodies. Although biological tubes may look relatively similar at first glance, there are multiple and distinct mechanisms by which tubes form and even more regulatory events driving the cell shape changes that produce tubes of specific dimensions. An overview of the current understanding of the molecular processes and physical forces involved in tubulogenesis is presented in this review and the accompanying poster.

Key words: Tubulogenesis, Endothelia, Epithelia, Morphogenesis, Lumen

[1]Department of Molecular, Cell and Developmental Biology, Molecular Biology Institute, Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA 90095, USA. [2]Department of Molecular Biosciences and Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Evanston, IL 60208, USA.

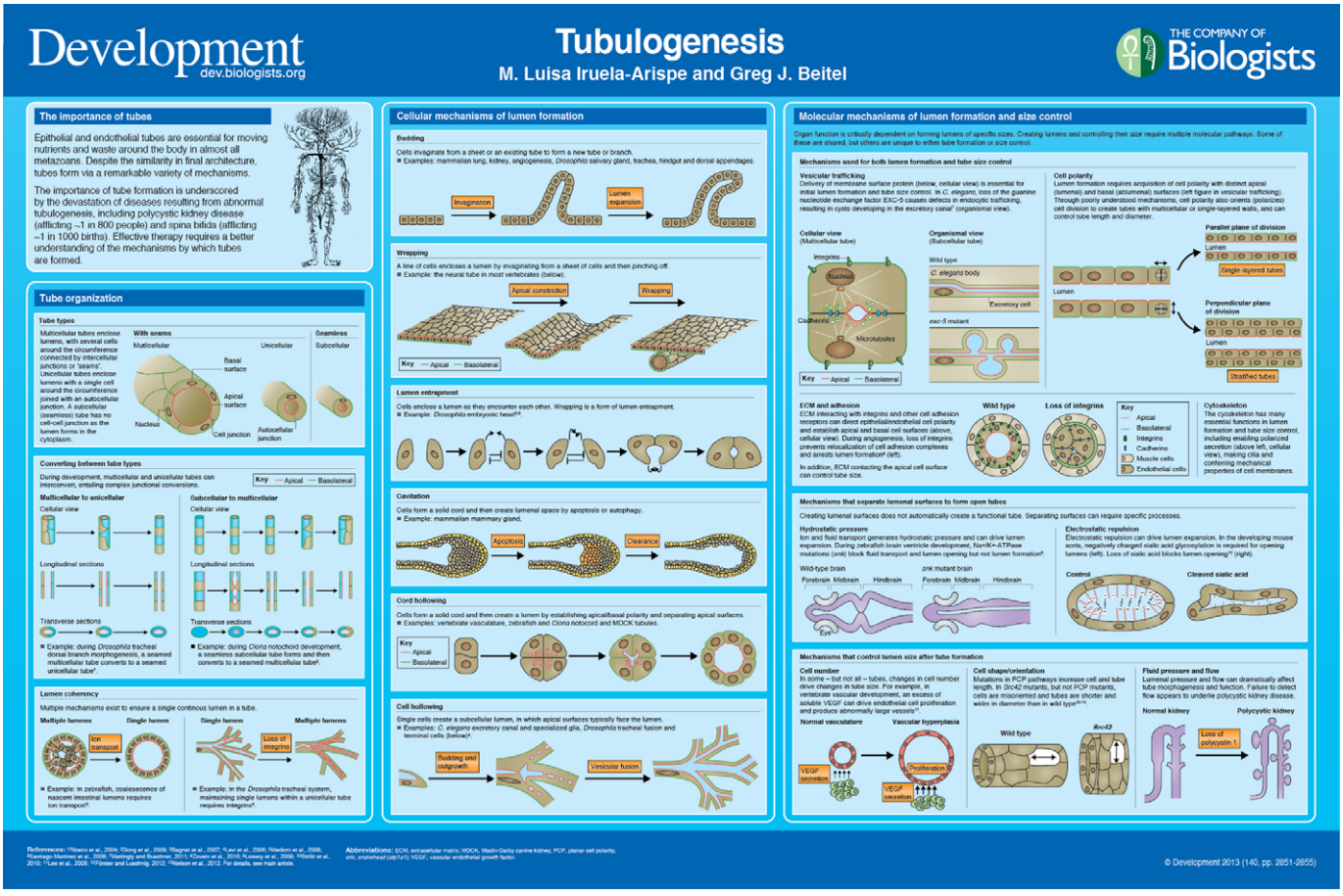
*Authors for correspondence (arispe@mcdb.ucla.edu; beitel@northwestern.edu)

Introduction

Tubes are the fundamental structural unit of many organs, including the vascular system, exocrine glands, digestive tract, lung and kidney. As metazoans evolved into creatures larger than small clusters of cells, tubes became essential for transporting and distributing metabolites. Given the complexity of the processes that create tubes of specific sizes and shapes, it is not surprising that their development and maintenance can go awry and cause devastating diseases.

Organization of tubes: which cells and how are they put together?

Biological tubes come in a remarkable diversity of sizes and range of complexity. They can be small, such as the unicellular tubes in small capillaries or in the *C. elegans* kidney cell. They can be large, but with relatively simple organization, as demonstrated by the massive multicellular tube of the intestine of a blue whale. They can also form organs of extreme complexity, such as the human lung, which includes some 10^5 conducting and 10^7 respiratory airways. Tubes can be composed of single or multiple cell layers



(See poster insert)

with the additional incorporation of other tissues, such as connective tissue and muscle (e.g. the vascular system and esophagus).

Many tubular organs are ultimately contiguous with the external environment and develop from polarized epithelial cells of ectodermal, endodermal or mesodermal origin. However, irrespective of their cellular origin they exhibit one of two types of organization. The large majority are tubes with 'seams' that in transverse section contain a lumen enclosed by one or more cells connected by auto- or intercellular junctions (Lubarsky and Krasnow, 2003). Less common are 'seamless' tubes with no cellular junctions in the circumference of the lumen. Examples of such seamless tubes include the sinusoidal blood vessels in the mammalian kidney (Bär et al., 1984), the *C. elegans* kidney cell (Buechner, 2002) and the *Drosophila* tracheal system (Maruyama and Andrew, 2012). Importantly, an organ can contain multiple tube types and junctional organizations (Buechner, 2002; Herwig et al., 2011; Lubarsky and Krasnow, 2003). Furthermore, there are examples of tubes converting between the seamed and seamless types during development, which involves complex cell surface rearrangements to introduce or eliminate cell-cell junctions and interfaces (Dong et al., 2009; Ribeiro et al., 2004).

A common topological feature of both seamed and seamless tubes is the presence of a single lumen. Whereas a single lumen arises naturally when a tube forms by invagination (see 'budding' below), this outcome is not obvious when a tube forms by the coalescence of vesicles or compartments. Consistent with active processes establishing and maintaining a single lumen in a tube, mutations have been identified that disrupt lumen coherency in the zebrafish gut and *Drosophila* tracheal system, resulting in tubes with multiple or discontinuous lumens (Bagnat et al., 2007; Levi et al., 2006).

Cellular mechanisms of lumen formation

Although seamed tubes have basically the same organization by the end of development, there are at least five cellular mechanisms for creating what is essentially a sheet of cells rolled into a tube.

A common mechanism is termed 'budding', in which one or more cells invaginate from an existing sheet or tube of cells to create a short new tube that is subsequently extended by cell migration and/or cell division (Chung and Andrew, 2008). A classic example of budding is angiogenic sprouting, in which a new blood capillary sprouts or buds off an existing vessel (Iruela-Arispe and Davis, 2009).

Alternatively, in 'wrapping' an entire row of cells can invaginate and pinch off from a sheet of cells to immediately create a longer tube (Lubarsky and Krasnow, 2003; Sawyer et al., 2010). A highly relevant example of wrapping is neural tube formation in mammals, where incomplete closure of the developing tube results in spina bifida. An important distinction between budding and wrapping is that the lumen of a bud is contiguous with the space from which the bud invaginated, whereas wrapping typically isolates a lumen from an existing space.

Lumenal space is also captured to form a tube by cells that are not in a contiguous sheet or tube through the strategy of 'lumen entrapment'. The best defined *in vivo* example of this is *Drosophila* heart formation in which cells migrating in from opposite sides of the embryo converge and touch at their opposing edges, but repel at their opposing centers, thus enclosing a lumen (Medioni et al., 2008; Santiago-Martínez et al., 2008). Lumen entrapment is also used by human endothelial cells in culture to form capillary-like tubes. Single cells exocytose a large vacuole and then 'capture' the former vacuolar space by resealing the opening with autocellular junctions (Davis et al., 2002).

A common theme of budding, wrapping and lumen entrapment is that an existing space is extended or captured. However, a lumen can also be formed by opening a space inside a cord or group of cells. 'Cavitation' creates space by eliminating cells that reside inside a cluster and replacing them with fluid. For example, in mammary gland development, lumenal space is created by apoptosis or autophagy of cells inside the gland (Mailleux et al., 2007). Alternatively, in 'cord hollowing' adjoining cells in a cord establish apposing apical surfaces and secrete fluid and matrix into the lumen. Examples of cord hollowing include zebrafish intestinal and mammalian dorsal aorta development (Herwig et al., 2011; Martin-Belmonte and Rodriguez-Fraticelli, 2009), as well as Madin-Darby canine kidney (MDCK) cell tubulogenesis in a three-dimensional matrix. Notably, there are some similarities between lumen capture by vertebrate endothelial cells and cord hollowing by MDCK cells in that apical surface is created *de novo*. However, there is an important topological distinction in that during cord hollowing the new lumenal space is never contiguous with the space exterior to the developing organ, whereas lumen capture converts exterior space into lumenal space.

Formation of seamless tubes proceeds analogously to cord hollowing, except that the creation of the lumenal surface occurs entirely intracellularly and thus constitutes a distinct mechanism termed 'cell hollowing'. Well-characterized examples of cell hollowing are the development of the *C. elegans* excretory system (Buechner, 2002) and the *Drosophila* tracheal terminal and fusion branches (Levi et al., 2006).

Regulatory molecules in lumen formation and size control

Tubulogenesis and tube size regulation require the coordinated orchestration of multiple molecular pathways including cell surface receptors, cell matrix, adhesion molecules, the cytoskeleton and vesicular transport. Small GTPases play a central role in regulating many of these processes. Here we highlight some of the emerging mechanisms that regulate tubulogenesis.

Both epithelial and vascular lumens rely on the molecular crosstalk between cell-cell and cell-matrix adhesion pathways (Iruela-Arispe and Davis, 2009). These connections provide the information required for the acquisition of cell polarity, a crucial prerequisite for lumen formation. Polarization results in the segregation of cell-cell contacts to the lateral aspects of the cell, while associations with the extracellular matrix (ECM) become exclusive to the basal side. The distribution of these cell surface molecules is concurrent with polarization of the centrosomes and, through an as yet unclear mechanism, regulates the orientation of cell division (Taylor et al., 2010; Zovein et al., 2010). This is crucial because cell divisions in the plane of the lumen wall result in stratification, whereas division orthogonal to this plane will lead to enlargement of the tube.

Although the complete molecular framework responsible for triggering cell polarity during lumen formation is yet to be uncovered, a few key molecules have been identified. Not surprisingly, as key mediators of cell-matrix interactions, integrins are central regulators of polarity and lumen formation. Genetic or pharmacological blockade of integrins results in arrest of vascular tubulogenesis and, although the specific mechanism by which integrins control lumen formation is unclear, both the orientation of cell division and the distribution of cell-cell adhesion molecules are impaired (Drake et al., 1992; Zovein et al., 2010). For example, in the endothelium, inactivation of β1 integrin yields stratification of the endocardium due to inappropriate orientation of cell division,

as well as persistent lumenal expression of cadherins and other cell surface proteins (Zovein et al., 2010). These abnormally localized cell-cell adhesion molecules engage in interactions with neighboring cells, blocking the emergence of a lumen (Zovein et al., 2010).

Cell polarity can also play a decisive role in controlling the dimensions of a tube once a lumen has formed. Changes in the activity of conserved apical-basal polarity complexes that include conserved proteins such as Crumbs and Scribble (Kerman et al., 2008; Laprise et al., 2010), or the activity of planar cell polarity (PCP) complexes (Chung et al., 2009), can change tube size by more than 50%.

Cytoskeletal proteins and their associated GTPases constitute a crucial set of lumen regulators. Both the actin cytoskeleton and microtubules participate in lumen formation by regulating vesicular trafficking and by enabling changes in cell shape that promote lumen enlargement or reduction. Dominant-negative mutants for Rac and Cdc42, which are small GTPases that modulate actin assembly, impair endothelial lumen formation *in vitro* (Bayless et al., 2000; Koh et al., 2008), while in mouse models endothelial deletion of Rac1, as well as its downstream effector Wave2 (also known as Scar2, Imd2 or Wasf4), results in defects in vascular development (Tan et al., 2008; Yamazaki et al., 2003). More recently, genetic inactivation of Rasip and Arhgap29, which are both regulators of Cdc42 and Rac, was shown to lead to lumen collapse in the vertebrate dorsal aorta (Xu et al., 2011).

Mechanical properties of the cytoskeleton also play a key role in tubulogenesis, as revealed by mutations in the BTB protein Ribbon, which increase apical cytoskeletal stiffness several fold and markedly reduce lumen elongation (Cheshire et al., 2008).

Finally, matrix metalloproteinases have also been shown to contribute to lumen formation. In particular, the polarized expression of specific membrane-tethered matrix metalloproteinases in vascular sprouts facilitates activation of Cdc42 and progressive invasion through the degradation of ECM molecules (Sacharidou et al., 2010).

Separating lumenal surfaces

Topologically, a lumen develops when a cell or cells delineate membrane surfaces that enclose a space. However, this does not necessarily mean that a usable tube is created because intercellular adhesion and/or intercellular matrix between apposing cells can prevent a lumen from enlarging to usable dimensions. At least two distinct mechanisms can provide the force needed to separate cell surfaces and generate a lumen between closely apposed cell surfaces. In zebrafish brain ventricle development, the Na^+/K^+-ATPase containing the Snakehead alpha subunit (Atp1a1) is required to 'inflate' the ventricle, presumably through ion transport generating hydrostatic pressure to separate cell surfaces (Lowery et al., 2009). During development of the mouse aorta, by contrast, electrostatic repulsion of heavily sialylated proteins is required to separate the lumenal faces of the aortic cells (Strilić et al., 2010).

Controlling lumen size

Once an open tube is formed, organ function is dependent on lumen size, as this dictates its flow properties. Several obvious mechanisms for tube size control have been identified, including regulation of cell number, cell shape, cell orientation, lumenal matrix, membrane trafficking and pressure/flow. Importantly, although there is overlap between the molecular mechanisms that form tubes and those that regulate tube size, there are also mutations that exclusively target tube size without affecting the initial process of tube formation (see below). The extent to which this separation reflects the differential deployment of similar mechanisms at distinct development times, as opposed to fundamental differences in the formation versus maintenance of an organ, remains to be determined.

Perhaps the simplest strategy for controlling lumen diameter is cell number: add more cells around a tube and it should increase in diameter (or length); reduce cell number and the tube should get narrower (or shorter). The question of control then becomes a problem of coupling cell division and death to a system that senses lumenal size. In the mammalian vascular system, increased levels of soluble growth factors, such as vascular endothelial growth factor (VEGF), can drive increased vessel diameter or length (Lee et al., 2005). However, tube size can be controlled independently of cell number. Classical experiments with salamanders demonstrated that tube diameter (and length) could be maintained despite an approximately fivefold change in cell number (Fankhauser, 1945). Similarly, approximately fourfold changes in cell number in the *Drosophila* tracheal system did not dramatically alter the size of tracheal tubes (Beitel and Krasnow, 2000).

Changes in the organization of cells within a tube can also affect its size. Intercalation can increase the length but decrease the diameter of a tube. Developmental examples of this include the *Drosophila* Malpighian tubules (Jung et al., 2005) and hindgut (Lengyel and Iwaki, 2002). Changing the shape and orientation of cells also alters tube size. For example, in the *Drosophila* tracheal system, mutations in *Src42* do not change the rectangular shape of tracheal cells but instead cause cells to expand in the circumferential rather than axial direction of the tube, resulting in shorter but wider tubes (Förster and Luschnig, 2012; Nelson et al., 2012). Mutations in lumenal ECM proteins have been shown to increase the length of tracheal cells and tubes (Araújo et al., 2005; Swanson and Beitel, 2006). In the mammalian vascular system, rapid changes in diameter do not involve changing endothelial cell number but are instead controlled by contraction and relaxation of smooth muscle cells that surround the vessels.

Given that most lumens are bounded by the apical surfaces of the enclosing epithelial or endothelial cells, it is unsurprising that regulation of vesicular trafficking can drastically affect lumen size. In the *C. elegans* renal tubule, vesicular trafficking defects cause significant dilation of the tubules (Buechner et al., 1999; Mattingly and Buechner, 2011), while apical delivery of the polarity protein Crumbs, and of other apical membrane factors, controls lumen diameter in the *Drosophila* salivary gland and trachea (Kerman et al., 2008; Myat and Andrew, 2002).

Physical forces and tubulogenesis

The ability of tubes to contend with and regulate their size is illustrative of the interplay of physical forces and tubes in biological systems. As a vital function of biological tubes is to transport liquids or gasses, pressure and flow are two physical parameters that cells can sense to regulate lumen size. These physical forces, although often dispensable during the initiation of lumen formation, are essential for keeping the lumen open and also seem to regulate lumen diameter (Jones et al., 2006). Endothelial and epithelial cells can be exquisitely sensitive to shear stress, and loss of the ability to sense flow in the kidney can result in the grossly expanded lumens of mammalian polycystic kidney disease (Wilson and Goilav, 2007). By contrast, blockade of flow in a vascular segment leads to its regression, whereas an increase in pressure results in remodeling of the vessel. Interestingly, recent work has indicated that lymph vessel size is regulated not by flow but by mechanical stress created by

the swelling of tissues that are accumulating fluid (Planas-Paz et al., 2012).

Lessons learned and future directions

At first glance, tubes in biological systems appear to be relatively simple. Sheets of cells enclosing a lumen: how complicated could these be to make? In fact, they are surprisingly complex. Research to date has revealed a plethora of cellular and molecular mechanisms that create and maintain these tubes. It seems almost as though no two tubes are the same even within a given organism or tissue. The complexity of tubulogenesis makes it difficult to draw broad conclusions about the process. Generalizations are further complicated by the fact that even in the most studied systems there is still a very limited understanding of the molecular mechanisms of tubulogenesis. Thus, although there currently appears to be little commonality between the specific mechanisms of lumen formation in different contexts, it is likely that we are at the stage of the proverbial blind men trying to describe an elephant. Every system presents a different entry point into the problem and we are unable to see how the threads weave together.

Nonetheless, a few common elements of tubulogenesis are evident. First, polarity is a key prerequisite for lumen formation. Establishment of molecularly distinguishable basal, lateral and apical sides is essential for the changes that underlie lumen morphogenesis. Second, the molecular mechanisms involve the same broad group of players, including the cytoskeleton, cell adhesion, junctional complexes and cell-matrix interactions. Yet their use and regulation are surprisingly distinct. The diversity of solutions employed in making seemingly simple structures clearly underscores the evolutionary importance of developing tubes in biological systems.

Acknowledgements

We apologize to authors whose work we could not directly cite owing to space limitations.

Funding

The work was supported by funding from the National Institutes of Health to M.L.I.-A. Deposited in PMC for release after 12 months.

Competing interests statement

The authors declare no competing financial interests.

Development at a Glance

A high-resolution version of the poster is available for downloading in the online version of this article at http://dev.biologists.org/content/140/14/2851.full

References

Araújo, S. J., Aslam, H., Tear, G. and Casanova, J. (2005). mummy/cystic encodes an enzyme required for chitin and glycan synthesis, involved in trachea, embryonic cuticle and CNS development – analysis of its role in Drosophila tracheal morphogenesis. *Dev. Biol.* **288**, 179-193.

Bagnat, M., Cheung, I. D., Mostov, K. E. and Stainier, D. Y. (2007). Genetic control of single lumen formation in the zebrafish gut. *Nat. Cell Biol.* **9**, 954-960.

Bär, T., Güldner, F. H. and Wolff, J. R. (1984). "Seamless" endothelial cells of blood capillaries. *Cell Tissue Res.* **235**, 99-106.

Bayless, K. J., Salazar, R. and Davis, G. E. (2000). RGD-dependent vacuolation and lumen formation observed during endothelial cell morphogenesis in three-dimensional fibrin matrices involves the alpha(v)beta(3) and alpha(5)beta(1) integrins. *Am. J. Pathol.* **156**, 1673-1683.

Beitel, G. J. and Krasnow, M. A. (2000). Genetic control of epithelial tube size in the Drosophila tracheal system. *Development* **127**, 3271-3282.

Buechner, M. (2002). Tubes and the single C. elegans excretory cell. *Trends Cell Biol.* **12**, 479-484.

Buechner, M., Hall, D. H., Bhatt, H. and Hedgecock, E. M. (1999). Cystic canal mutants in Caenorhabditis elegans are defective in the apical membrane domain of the renal (excretory) cell. *Dev. Biol.* **214**, 227-241.

Cheshire, A. M., Kerman, B. E., Zipfel, W. R., Spector, A. A. and Andrew, D. J. (2008). Kinetic and mechanical analysis of live tube morphogenesis. *Dev. Dyn.* **237**, 2874-2888.

Chung, S. and Andrew, D. J. (2008). The formation of epithelial tubes. *J. Cell Sci.* **121**, 3501-3504.

Chung, S., Vining, M. S., Bradley, P. L., Chan, C. C., Wharton, K. A., Jr and Andrew, D. J. (2009). Serrano (sano) functions with the planar cell polarity genes to control tracheal tube length. *PLoS Genet.* **5**, e1000746.

Davis, G. E., Bayless, K. J. and Mavila, A. (2002). Molecular basis of endothelial cell morphogenesis in three-dimensional extracellular matrices. *Anat. Rec.* **268**, 252-275.

Dong, B., Horie, T., Denker, E., Kusakabe, T., Tsuda, M., Smith, W. C. and Jiang, D. (2009). Tube formation by complex cellular processes in Ciona intestinalis notochord. *Dev. Biol.* **330**, 237-249.

Drake, C. J., Davis, L. A. and Little, C. D. (1992). Antibodies to beta 1-integrins cause alterations of aortic vasculogenesis, in vivo. *Dev. Dyn.* **193**, 83-91.

Fankhauser, G. (1945). Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adjustment of cell number and cell shape. *J. Exp. Zool.* **100**, 445-455.

Förster, D. and Luschnig, S. (2012). Src42A-dependent polarized cell shape changes mediate epithelial tube elongation in Drosophila. *Nat. Cell Biol.* **14**, 526-534.

Herwig, L., Blum, Y., Krudewig, A., Ellertsdottir, E., Lenard, A., Belting, H. G. and Affolter, M. (2011). Distinct cellular mechanisms of blood vessel fusion in the zebrafish embryo. *Curr. Biol.* **21**, 1942-1948.

Iruela-Arispe, M. L. and Davis, G. E. (2009). Cellular and molecular mechanisms of vascular lumen formation. *Dev. Cell* **16**, 222-231.

Jones, E. A., le Noble, F. and Eichmann, A. (2006). What determines blood vessel structure? Genetic prespecification vs. hemodynamics. *Physiology (Bethesda)* **21**, 388-395.

Jung, A. C., Denholm, B., Skaer, H. and Affolter, M. (2005). Renal tubule development in Drosophila: a closer look at the cellular level. *J. Am. Soc. Nephrol.* **16**, 322-328.

Kerman, B. E., Cheshire, A. M., Myat, M. M. and Andrew, D. J. (2008). Ribbon modulates apical membrane during tube elongation through Crumbs and Moesin. *Dev. Biol.* **320**, 278-288.

Koh, W., Mahan, R. D. and Davis, G. E. (2008). Cdc42- and Rac1-mediated endothelial lumen formation requires Pak2, Pak4 and Par3, and PKC-dependent signaling. *J. Cell Sci.* **121**, 989-1001.

Laprise, P., Paul, S. M., Boulanger, J., Robbins, R. M., Beitel, G. J. and Tepass, U. (2010). Epithelial polarity proteins regulate Drosophila tracheal tube size in parallel to the luminal matrix pathway. *Curr. Biol.* **20**, 55-61.

Lee, S., Jilani, S. M., Nikolova, G. V., Carpizo, D. and Iruela-Arispe, M. L. (2005). Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. *J. Cell Biol.* **169**, 681-691.

Lengyel, J. A. and Iwaki, D. D. (2002). It takes guts: the Drosophila hindgut as a model system for organogenesis. *Dev. Biol.* **243**, 1-19.

Levi, B. P., Ghabrial, A. S. and Krasnow, M. A. (2006). Drosophila talin and integrin genes are required for maintenance of tracheal terminal branches and luminal organization. *Development* **133**, 2383-2393.

Lowery, L. A., De Rienzo, G., Gutzman, J. H. and Sive, H. (2009). Characterization and classification of zebrafish brain morphology mutants. *Anat. Rec. (Hoboken)* **292**, 94-106.

Lubarsky, B. and Krasnow, M. A. (2003). Tube morphogenesis: making and shaping biological tubes. *Cell* **112**, 19-28.

Mailleux, A. A., Overholtzer, M., Schmelzle, T., Bouillet, P., Strasser, A. and Brugge, J. S. (2007). BIM regulates apoptosis during mammary ductal morphogenesis, and its absence reveals alternative cell death mechanisms. *Dev. Cell* **12**, 221-234.

Martin-Belmonte, F. and Rodriguez-Fraticelli, A. E. (2009). Chapter 3: acquisition of membrane polarity in epithelial tube formation patterns, signaling pathways, molecular mechanisms, and disease. *Int. Rev. Cell Mol. Biol.* **274**, 129-182.

Maruyama, R. and Andrew, D. J. (2012). Drosophila as a model for epithelial tube formation. *Dev. Dyn.* **241**, 119-135.

Mattingly, B. C. and Buechner, M. (2011). The FGD homologue EXC-5 regulates apical trafficking in C. elegans tubules. *Dev. Biol.* **359**, 59-72.

Medioni, C., Astier, M., Zmojdzian, M., Jagla, K. and Sémériva, M. (2008). Genetic control of cell morphogenesis during Drosophila melanogaster cardiac tube formation. *J. Cell Biol.* **182**, 249-261.

Myat, M. M. and Andrew, D. J. (2002). Epithelial tube morphology is determined by the polarized growth and delivery of apical membrane. *Cell* **111**, 879-891.

Nelson, K. S., Khan, Z., Molnár, I., Mihály, J., Kaschube, M. and Beitel, G. J. (2012). Drosophila Src regulates anisotropic apical surface growth to control epithelial tube size. *Nat. Cell Biol.* **14**, 518-525.

Planas-Paz, L., Strilić, B., Goedecke, A., Breier, G., Fässler, R. and Lammert, E. (2012). Mechanoinduction of lymph vessel expansion. *EMBO J.* **31**, 788-804.

Ribeiro, C., Neumann, M. and Affolter, M. (2004). Genetic control of cell intercalation during tracheal morphogenesis in Drosophila. *Curr. Biol.* **14**, 2197-2207.

Sacharidou, A., Koh, W., Stratman, A. N., Mayo, A. M., Fisher, K. E. and Davis, G. E. (2010). Endothelial lumen signaling complexes control 3D matrix-specific

tubulogenesis through interdependent Cdc42- and MT1-MMP-mediated events. *Blood* **115**, 5259-5269.
Santiago-Martínez, E., Soplop, N. H., Patel, R. and Kramer, S. G. (2008). Repulsion by Slit and Roundabout prevents Shotgun/E-cadherin-mediated cell adhesion during Drosophila heart tube lumen formation. *J. Cell Biol.* **182**, 241-248.
Sawyer, J. M., Harrell, J. R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M. and Goldstein, B. (2010). Apical constriction: a cell shape change that can drive morphogenesis. *Dev. Biol.* **341**, 5-19.
Strilić, B., Eglinger, J., Krieg, M., Zeeb, M., Axnick, J., Babál, P., Müller, D. J. and Lammert, E. (2010). Electrostatic cell-surface repulsion initiates lumen formation in developing blood vessels. *Curr. Biol.* **20**, 2003-2009.
Swanson, L. E. and Beitel, G. J. (2006). Tubulogenesis: an inside job. *Curr. Biol.* **16**, R51-R53.
Tan, W., Palmby, T. R., Gavard, J., Amornphimoltham, P., Zheng, Y. and Gutkind, J. S. (2008). An essential role for Rac1 in endothelial cell function and vascular development. *FASEB J.* **22**, 1829-1838.
Taylor, S. M., Nevis, K. R., Park, H. L., Rogers, G. C., Rogers, S. L., Cook, J. G. and Bautch, V. L. (2010). Angiogenic factor signaling regulates centrosome duplication in endothelial cells of developing blood vessels. *Blood* **116**, 3108-3117.
Wilson, P. D. and Goilav, B. (2007). Cystic disease of the kidney. *Annu. Rev. Pathol.* **2**, 341-368.
Xu, K., Sacharidou, A., Fu, S., Chong, D. C., Skaug, B., Chen, Z. J., Davis, G. E. and Cleaver, O. (2011). Blood vessel tubulogenesis requires Rasip1 regulation of GTPase signaling. *Dev. Cell* **20**, 526-539.
Yamazaki, D., Suetsugu, S., Miki, H., Kataoka, Y., Nishikawa, S., Fujiwara, T., Yoshida, N. and Takenawa, T. (2003). WAVE2 is required for directed cell migration and cardiovascular development. *Nature* **424**, 452-456.
Zovein, A. C., Luque, A., Turlo, K. A., Hofmann, J. J., Yee, K. M., Becker, M. S., Fassler, R., Mellman, I., Lane, T. F. and Iruela-Arispe, M. L. (2010). Beta1 integrin establishes endothelial cell polarity and arteriolar lumen formation via a Par3-dependent mechanism. *Dev. Cell* **18**, 39-51.