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Tube Morphogenesis: Making and Shaping Biological Tubes

Review

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Many organs are composed of epithelial tubes that transport vital fluids. Such tubular organs develop in many different ways and generate tubes of widely varying sizes and structures, but always with the apical epithelial surface lining the lumen. We describe recent progress in several diverse cell culture and genetic models of tube morphogenesis, which suggest apical membrane biogenesis, vesicle fusion, and secretion play central roles in tube formation and growth. We propose a unifying mechanism of tube morphogenesis that has been modified to create tube diversity and describe how defects in the tube size-sensing step can lead to polycystic kidney disease.

Tubes are a fundamental unit of organ design. Most of our major organs including the lung, kidney, and vasculature are composed primarily or exclusively of tubes. They serve as the body's plumbing, transporting critical gases, liquids, and cells from one site to another. These biological pipes are almost invariably composed of living cells, usually attached to one another to form an epithelium (sheet of cells) that is wrapped into a tube with the apical epithelial surface lining the lumen, in contact with the transport medium, and the basal surface facing outward (Figure 1A). Some tubes modify the transport fluid, whereas others act as passive conduits. Despite significant progress over the past decade in understanding how tubular organs are patterned during development, we are only now beginning to understand how cells assemble into tubes and how tube size and shape is regulated. A detailed mechanistic understanding of these processes is important for medicine as well as biology, because many human diseases such as polycystic kidney disease and atherosclerotic heart disease are essentially plumbing defects. A molecular understanding of tubulogenesis could lead to new ways of diagnosing and treating these conditions.

A glance through a comparative physiology textbook reveals the tremendous structural diversity of biological tubes—different tube sizes, shapes, and connecting patterns, each tailored to its specific transport fluid and function. Tube sizes range over six orders of magnitude, from 0.1 micron or less in diameter for the smallest insect tracheal tubes to greater than 20 cm for the gut of an adult elephant (Shoshani, 1982). Their cellular architectures also differ. One difference is the number of cells in each cross-section of the tube, which generally scales with tube diameter. Large bore tubes are composed of hundreds or thousands of cells per cross-section, whereas small diameter tubes can be composed of just a single cell (Figures 1B and 1C). Tubes also differ in the presence or absence of cell junctions. Junctions serve as cell attachment sites that seal the epithelial layer to paracellular leakage, and they delineate the border between apical and basolateral membrane domains. Multicellular and most unicellular tubes contain such junctions (Figures 1A and 1B), whereas some unicellular tubes lack junctional structures (Figure 1C). Most tubes secrete specialized matrices at their apical or basal surface, or contain additional cell layers, but the simplest tubes are epithelial monolayers without any surrounding support structures.

How do such diverse tubes form, and how do they grow to achieve their mature sizes and shapes? Do they use completely different cellular and molecular mechanisms, or are there common underlying mechanisms of tubulogenesis? Tubular organs develop in a wide variety of ways, and many cellular processes have been implicated. But recent investigations of tube formation and growth in a number of systems, from cell culture systems to genetic model organisms to human tubulogenesis diseases, point to critical roles for apical membrane biogenesis, vesicle fusion, and secretion. Although the full process is not well understood for any system, by drawing together results from different systems, a general model for tube morphogenesis can be formulated. This model accommodates a variety of organ and tube-specific variations that tailor tube structure to function and shows how different tube sizes and shapes are created in nature and how defects in the process cause human disease.

Morphological Processes of Tube Formation

Classical descriptions of embryonic development showed that tubular organs develop in many different ways. Indeed, at a gross level, it appears that nearly every tubular organ forms in its own distinct manner, some deploying multiple tube forming mechanisms. Some of this morphogenetic diversity, however, derives from differences in events leading up to or following tube formation, such as the ways cells reach their proper positions, or the means by which extra cells are eliminated or additional cell layers are recruited around the developing tubes. If one considers only the events that occur just as the cells form tubes, then most tubulogenesis processes can be grouped into five general categories (Figure 2; see also Bard, 1990).

Wrapping occurs when an epithelial sheet curls until its edges meet and seal, forming a tubular structure. Typically, this involves only a portion of the epithelium, with the tube-forming cells first invaginating to form a crevice in the epithelium, then sealing off and separating from the rest of the epithelium, as during neural tube formation in many vertebrates (Colas and Schoenwolf, 2001). This generates a tube that runs parallel to the plane of the epithelium from which it derives. In *budding*, cells extend out from the epithelium in a direction orthogonal to the epithelial plane, forming a tube as the

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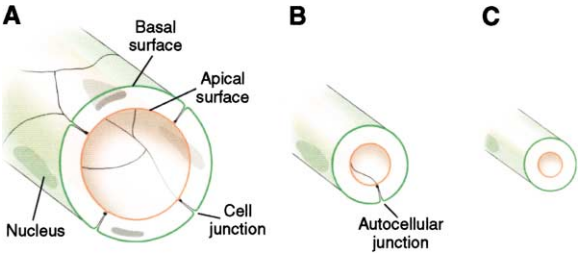

Figure 1. Types of Simple Epithelial Tubes

Tube walls are formed by polarized epithelial cells with their apical membrane surface (red) facing inward toward the lumen space, and their basal surface (green) exposed to the extracellular matrix.

(A) A multicellular tube with four curved cells in the cross-section of the tube.

(B) A unicellular tube formed by a single cell, rolled up to enclose the lumen, and sealed with an autocellular junction.

(C) A unicellular tube with the lumen in the cytoplasm of the cell. There is no autocellular junction; the tube is "seamless."

bud grows. This is how tubes arise during branching morphogenesis of many organs, including the mammalian lung and the major branches of the *Drosophila* tracheal (respiratory) system (Metzger and Krasnow, 1999; Hogan and Kolodziej, 2002). New branches bud from the walls of an existing branch, and the lumen of the new branch is a direct extension of the lumen of the parental branch.

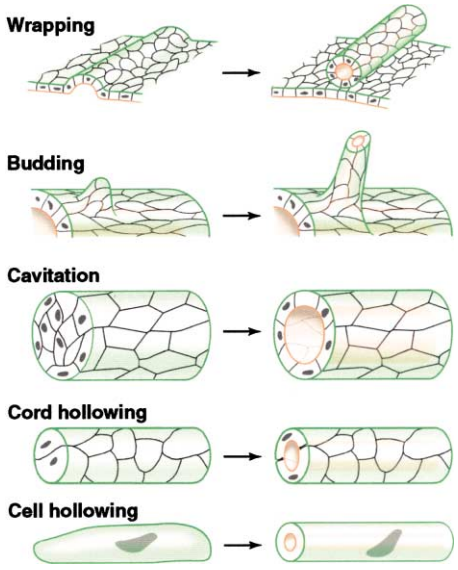

Figure 2. Morphological Processes of Tube Formation

Wrapping: a portion of an epithelial sheet invaginates and curls until the edges of the invaginating region meet and seal, forming a tube that runs parallel to the plane of the sheet.

Budding: a group of cells in an existing epithelial tube (or sheet) migrates out and forms a new tube as the bud extends. The new tube is a direct extension of the original tube.

Cavitation: the central cells of a solid cylindrical mass of cells are eliminated to convert it into a tube.

Cord hollowing: a lumen is created de novo between cells in a thin cylindrical cord.

Cell hollowing: a lumen forms within the cytoplasm of a single cell, spanning the length of the cell.

In both of these mechanisms, tubes arise from a polarized epithelium. By contrast, in the mechanisms described below, tubes arise from clusters of cells or individual cells that are not epithelial, and the cells polarize and/or establish junctions as the tubes form.

During *cavitation*, cells organized in a thick cylindrical mass create a central cavity by eliminating cells in the center of the mass, as occurs during salivary gland morphogenesis (Borghese, 1950; Melnick and Jaskoll, 2000) and formation of the proamniotic cavity (Coucouvanis and Martin, 1995) in vertebrate embryos. In *cord hollowing*, cells assembled in a thin cylindrical cord create a lumen between cells, without cell loss. Examples include the *Caenorhabditis elegans* gut (Leung et al., 1999), the *Drosophila* heart (Rugendorff et al., 1994), and Madin-Darby canine kidney (MDCK) cultured cells (Pollack et al., 1998). *Cell hollowing* is distinct from the other processes in that it involves one cell rather than a group of cells. For instance, some capillary endothelial cells form a membrane-bound lumen within the cytoplasm that spans the length of the cell and opens to the exterior at both ends (Wolff and Bar, 1972).

Cellular Events in Tube Formation

Initial investigations of the cell biology of tubulogenesis suggested that the cellular mechanisms of tube formation might be as diverse as the gross differences in morphogenesis implied. Each system examined seemed to highlight a different cellular event (Hogan and Kolodziej, 2002). One of the earliest and most intensively studied systems, primary neural tube formation in chicks, pointed to the importance of cell shape changes (reviewed in Schoenwolf and Smith, 1990). The first morphological event is elongation of cells in the apicobasal dimension, with subsequent apical narrowing and basal expansion to create wedge shape cells that promote epithelial bending and wrapping. This and other investigations of epithelial morphogenesis led many to focus on cytoskeletal and cell shape changes in tubulogenesis. However, studies of other systems suggested a role for cell death in creating a luminal cavity (Glucksmann, 1951; Coucouvanis and Martin, 1995), and early studies of thyroid follicle cells (Remy et al., 1977) and blood vessel endothelial cells (Folkman and Haudenschild, 1980) noted a possible role for cytoplasmic vesicles in lumen formation (see below). Tubulogenesis processes appeared to have little in common mechanistically.

This view began to change over the past few years as modern cell biological tools were applied to cell culture systems in which the cellular events in tube formation could be carefully analyzed. Results from a number of systems, like the MDCK and endothelial cell culture systems described below, converged on a critical role for apical membrane biogenesis and vesicle fusion in creating a lumen. During this same period, studies of tube growth and maturation in several invertebrate and vertebrate organs, including the respiratory and renal organs discussed below, also directed attention to morphological and molecular events at the apical surface.

Hollowing a Cord of MDCK Cells: Cell Polarization and Exocytosis in Lumen Formation

MDCK cells grown on the surface of a thin ("two-dimensional") collagen substrate have long been recognized

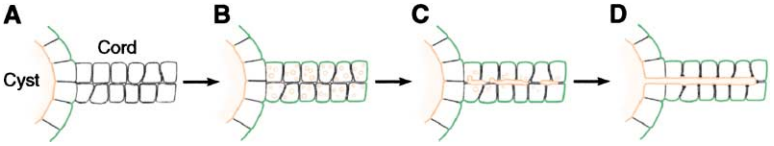

Figure 3. MDCK Cell Tube Formation

(A) Polarized cells in an MDCK cell cyst migrate out from the cyst and form a long multicellular cord (shown in longitudinal section). During their migration, apicobasal polarity is lost.

(B) After an external cue triggers repolarization, cell surfaces in contact with the extracellular matrix accumulate basal markers (green) as cytoplasmic vesicles carrying apical membrane components (red circles) target the developing apical (luminal) surfaces.

(C) The apical vesicles fuse, creating pockets of lumen at the apical surface.

(D) Continued delivery of apical vesicles expands the lumen pockets until they merge and form a complete lumen connecting to the cyst cavity.

as a premier system for studying the establishment of apicobasal polarity of epithelia (Yeaman et al., 1999). But if these cells are grown suspended in a thick ("three dimensional") collagen gel and hepatocyte growth factor (HGF or scatter factor) is added, they form epithelial tubes instead of a planar epithelium (Montesano et al., 1991a, 1991b).

Before addition of HGF, the cells aggregate and form a cyst consisting of a polarized epithelial monolayer surrounding a fluid-filled cavity. HGF stimulates groups of cyst cells to migrate away from the central cavity, forming solid cords of cells that extend out from the cyst (Figure 3). As the cells migrate away from the central cavity, they lose apicobasal polarity (Pollack et al., 1998). The apical membrane protein gp135 and the basolateral protein desmoplakin are lost from the plasma membrane and appear in the cytoplasm. Also, cell junctions break down and the adherens junction protein E-cadherin redistributes uniformly throughout the plasma membrane.

As the cell cord lengthens it also thickens, generating a solid cord two to three cells thick. Then, at various positions along the cord, cells begin to reestablish apicobasal polarity (Pollack et al., 1998). Apical markers appear along the central axis of the cord, and there the cell surfaces separate and pockets of fluid-filled lumen appear. These pockets of lumen expand and coalesce, eventually becoming continuous with the cyst lumen. The key finding in these studies is that lumen formation is tightly coupled to the reestablishment of apicobasal polarity.

This work also highlighted the importance of apical membrane biogenesis in lumen formation (O'Brien et al., 2002). The apical membrane may derive from preformed intracellular stores distributed in cytoplasmic vesicles referred to as the vacuolar apical compartment, or VAC (Vega-Salas et al., 1987). VACs are found in MDCK cells maintained under conditions that prevent cell adhesion, such as low cell densities or very low calcium concentrations. Upon addition of calcium and juxtaposition of two cells, VACs rapidly move to the region of cell-cell contact and fuse to it, creating an apical surface and a luminal space between the cells (Vega-Salas et al., 1988). This finding, and the localization of exocyst secretory machinery components at points of new membrane incorporation during MDCK tubulogenesis (Lipschutz et al., 2000), suggests that targeting of exocytotic vesicles to the plasma membrane is an important step in creating the luminal surface.

Thus, HGF-activated MDCK cells in 3D collagen gels may be poised to rapidly reestablish apicobasal polarity and generate a lumen, awaiting only the correct polarization cue. The trigger is unknown but could be as simple as attachment to a partner cell and exposure of the pair to a uniform circumferential basolateral cue that sets up a radially oriented axis of polarity. Polarization and lumen formation would then be initiated anywhere that the cord of MDCK cells is two cells thick and surrounded by a uniform collagen matrix.

Endothelial Cell Hollowing: Creating a Lumen by Vesicle Coalescence

The MDCK cell tubulogenesis mechanism requires a cell cord two or more cells thick, but some narrow gauge tubes, like the finest capillaries of the vertebrate vascular system, are formed by individual cells or chains of cells only one cell thick. Such cells create an intracellular lumen spanning the length of the cell (Wolff and Bar, 1972). This clearly requires a different tubulogenesis mechanism than the one used to form an extracellular lumen by MDCK cells, yet cytoplasmic vesicles appear central to both.

Cytoplasmic vesicles, reminiscent of VACs of MDCK cells, are a prominent feature of endothelial cells during angiogenesis in vivo and were noted over a half-century ago (Clark and Clark, 1939). The number of vesicles diminish during capillary growth and maturation, suggesting that they are consumed in the process (Wolff and Bar, 1972; Dyson et al., 1976). In vitro studies of angiogenesis in three-dimensional collagen or fibrin matrices provide evidence that formation and coalescence of vesicles is an early and essential step in endothelial tube formation (Folkman and Haudenschild, 1980; Davis and Camarillo, 1996). Inhibiting this process with antibodies to specific integrins or toxins, or by expressing dominant negative mutants of Cdc42 and Rac1 GTPases, blocks lumen formation (Davis and Camarillo, 1996; Bayless and Davis, 2002). A model of endothelial tube morphogenesis incorporating these results is shown in Figure 4. Endothelial cells assembled into long chains polarize and generate apical membrane vesicles. The vesicles then coalesce into an elongate vacuole-like structure spanning the length of the cell, which fuses with the plasma membrane to open to the exterior and establish luminal continuity with the next cell in the chain (Folkman and Haudenschild, 1980; Davis and Camarillo, 1996).

If this model of endothelial tube morphogenesis is correct, then formation and targeting of apical membrane vesicles lie at the heart of both the cord and cell hollowing mechanisms of tube formation. The major difference would be in target site selection: MDCK cell vesicles target the plasma membrane contacting an-

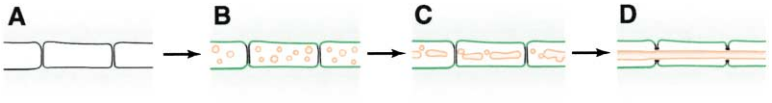

Figure 4. Endothelial Cell Tube Formation

Endothelial cells assembled in a chain (A, shown in longitudinal section) generate cytoplasmic vesicles (B). The vesicles coalesce, forming large, elongate vesicles (C), and ultimately a complete lumen that spans the length of the cell and connects to the lumen of the neighboring cells (D).

other cell, forming a new apical surface. In contrast, endothelial cell vesicles target a central position in the cell and organize an internal apical compartment. Perhaps endothelial cells have the ability to respond to a uniform basolateral cue by establishing a radial polarity axis with an internal apical compartment.

The ability to create an intracellular lumen is even more dramatically manifest by specialized cells like the excretory (renal) cell in *C. elegans*, and terminal cells of the *Drosophila* tracheal system. These individual cells form small tubular networks by extending long, branched cytoplasmic projections and then organizing a lumen within each projection. Although the mechanism of intracellular lumen formation has not been established in these systems, they too may involve the generation and coalescence of apical membrane vesicles aligned along the central axis of the cell and each cytoplasmic projection (Keister, 1948; Manning and Krasnow, 1993; Buechner, 2002; see below).

Apical Membrane Biogenesis and Secretion during Respiratory Tube Growth

Creating a lumen is the critical step in tube morphogenesis, but other important steps follow. Tubes are generally very small when they form and must grow by one or two orders of magnitude to achieve their mature size and shape. The expansion process can begin as soon as the lumen forms or can occur at specific times later in development. Although this aspect of tube morphogenesis has received less attention than tube formation, an analysis of the growth of the major branches in the *Drosophila* tracheal system suggests that regulation of the apical surface is also important for tube expansion (Beitel and Krasnow, 2000).

Primary tracheal branches form by budding morphogenesis during mid-embryogenesis. Although the cellular events that create the lumens are not known, the tubes are all small and of similar diameter ($\sim$2 μm) when they form. Several hours later, however, they undergo a programmed expansion that can triple their diameter. Just before expansion begins, multiple vesicle-like structures carrying an apical membrane antigen appear in the cytoplasm (Figure 5). These are presumably membrane vesicles, like those of MDCK and endothelial cells, which target and fuse to the apical surface, driving the apical membrane expansion and changes in surface antigen expression that occur during lumen growth. As the apical surface expands, the basal surface changes little if at all, demonstrating that lumen expansion is not a process of generalized cell growth but rather a specific growth and remodeling of the apical surface. Two additional expansions later in development increase lumen diameter up to 40 times its original size. Remarkably, expansion is not accompanied by cell proliferation, only by a large increase in apical surface area and dramatic thinning of the cells. Experiments with cell cycle mutants that alter cell number further establish that tracheal tube size is independent of cell number (Beitel and Krasnow, 2000), and classical studies of heteroploid salamanders with increased or decreased cell number support a similar conclusion about vertebrate renal tubules (Fankhauser, 1945). These results argue that it is not general cell cycle regulation, but rather specific regulation of the apical cell surface that governs tube expansion.

Although apical membrane biogenesis is required for both lumen formation and expansion, it is not sufficient. There must also be a mechanism that drives apical membranes apart to keep the lumen open. Liquid secretion into the lumen may play such a role. Although tracheal tubes function in oxygen transport, liquid accumulates in the lumen when the tubes first form and during each expansion, only to be cleared after each expansion cycle so that oxygen can flow (Manning and Krasnow, 1993). That liquid secretion occurs during formation and growth of tubes, including tubes like the trachea and mammalian lung that lack liquid when mature (Jost and Policard, 1948), supports the notion that liquid secretion is an essential step in tube formation and expansion. Indeed, studies of the developing sheep lung suggest that liquid secretion by the pulmonary epithelium drives tube growth (Alcorn et al., 1977). Manipulations that increase the volume of fetal lung liquid increase lung growth and speed maturation, whereas depleting the liquid has the opposite effect. Secretion in the developing lung appears to be mediated by chloride ion transport across the pulmonary epithelium, which provides an osmotic gradient that draws water into the lumen (Olver and Strang, 1974; Hooper and Harding, 1995). As water accumulates and the tubes distend, epithelial stretch may stimulate cell proliferation and tube growth (Hooper and Harding, 1995). In this way, secretion could

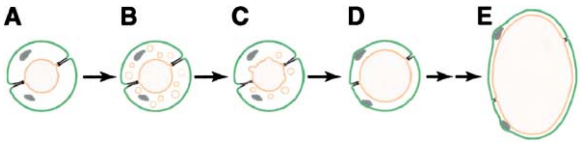

Figure 5. Expansion of *Drosophila* Tracheal Tubes

Primary tracheal branches (A, shown in cross-section) accumulate vesicles carrying apical markers (B, red circles) just before the apical tube expansion. Vesicles are thought to fuse, driving apical membrane growth and lumen expansion (C, D). Note that the cells become thinner because the basal membrane surface does not expand. Later in development, the process of apical membrane biogenesis and lumen expansion repeats, without cell division (E). When the apical surface approaches the size of the basal surface the cells become extremely thin, and the basal surface begins to grow at a similar rate as the apical surface.

drive lumen expansion, just as it could drive the initial opening of a lumen.

Tube expansion is tightly regulated. In the *Drosophila* tracheal system, tube diameter increases only at three brief periods of development, during which the branches expand to their characteristic sizes (Beitel and Krasnow, 2000). These sizes are genetically programmed, and some of the genes in the program have been identified (Beitel and Krasnow, 2000; Hemphala et al., 2003). One idea is that the program dictates branch size by controlling the amount of apical membrane biogenesis and secretion for each branch. The program could also control events at the end of the expansion period, when expansion ceases and stiff cuticle is deposited at the apical surface to prevent lumen collapse after liquid clearing. This expansion arrest is not permanent, though. Tracheal tubes remain capable of expanding again during the next expansion cycle by detaching from the cuticle and repeating the process of apical membrane biogenesis, secretion, and cuticle deposition.

The tight control of tube expansion suggests that tubes can sense their size and inhibit expansion when a specified size is reached. Although the sensor and output pathway have not been identified in the *Drosophila* tracheal system, several components of renal tube size regulation pathways appear to have been found by genetic studies in *C. elegans* and mammals.

Identification of Apical and Luminal Proteins that Restrict Renal Tube Expansion in *C. elegans*

The *C. elegans* excretory system is a network of fine tubules 1–2 μm wide and 100–1000 μm long that function as the worm's renal system, regulating tissue osmolarity and passing excess fluid along the tubules and out a valve-like opening to the exterior called the excretory duct (Nelson and Riddle, 1984; Buechner, 2002). The tubules are formed by a single cell, the excretory cell, which is the largest mononucleate cell in the animal and one of the most extraordinary in structure and function. During development, it extends pairs of thin cytoplasmic projections both anteriorly and posteriorly along the embryonic body wall, creating an elongate H-shaped cell that spans the length of the animal (Nelson et al., 1983). As the cytoplasmic projections grow, a fluid-filled lumen forms within each projection, creating a single-cell tubular network that opens into the excretory duct. Although the mechanism of lumen formation has not been described, it is thought to involve coalescence and fusion of apical membrane vesicles (Buechner, 2002), similar to the process described above for endothelial cell hollowing.

As the tubules mature, the lumen undergoes a structural transition that can be visualized by electron microscopy (Buechner et al., 1999). Initially, the lumen is filled with an amorphous material. Later, the luminal material disappears and the apical cytoplasm underlying the luminal membrane darkens. The mature lumen constitutes a distinct structural entity within the cell, maintaining its size and shape even when the basal cytoplasm and cell surface are perturbed (Buechner, 2002). These results suggest that events at the apical surface and apical cytoskeleton may be important for tube maturation.

Genetic and molecular studies of tube growth and maturation also focus attention on the apical surface and underlying cytoskeleton. Mutations in a dozen genes have been found that affect the size and shape of the excretory canals (Buechner et al., 1999). All of the mutations are recessive and result in enlarged tubules, implying that the wild-type gene products are required to constrain lumen growth. Some of the mutations result in enormous dilatations, swelling to fill almost the entire width of the animal and pushing aside other organs as they expand. All mutants that affect tube diameter have associated defects in the apical cytoskeleton and/or the luminal matrix (Buechner et al., 1999). Furthermore, the three genes that have been cloned all appear to encode apical or luminal proteins. *sma-1* encodes β_{Heavy}-spectrin, a protein that links the actin cytoskeleton to the apical plasma membrane at adherens junctions (McKeown et al., 1998). *exc-5* encodes a homolog of mammalian FGD1 and Frabin, guanine nucleotide exchange factors (GEFs) that regulate Rho family GTPases, regulators of the actin cytoskeleton (Suzuki et al., 2001). An EXC-5 GFP fusion protein localizes apically in the excretory cell. *let-653* encodes a putative mucin-like protein (Jones and Baillie, 1995) that, like vertebrate mucins, may line the lumen surface. Although the specific functions and interactions of these proteins in tube morphogenesis are not yet known, the results imply that proteins at the apical cytoskeleton and luminal surface stabilize lumen structure and limit its growth.

Polycystic Kidney Disease Genes: Identification of a Tube Size Sensor?

One of the most fascinating but poorly understood aspects of tube growth control is the size sensing mechanism. How does a tube measure its diameter so it knows when to stop growing? Insights into the sensing mechanism have begun to emerge from an unexpected direction—the characterization of human disease genes associated with altered renal tube morphogenesis.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic form of polycystic kidney disease and, with a prevalence of ~1 in 800 births (Dalgaard, 1957), ranks among the most common human monogenetic disorders. Mutations in at least three different genes can cause ADPKD. Two of them, *PKD1* and *PKD2*, have been mapped and cloned (E.P.K.D. Consortium, 1994; Mochizuki et al., 1996), as has a gene associated with an autosomal recessive form of polycystic kidney disease (Onuchic et al., 2002; Ward et al., 2002). Individuals with the autosomal dominant form of the disease are plagued with cysts that arise along the proximal and distal tubules and collecting ducts of the kidney (Evan and McAteer, 1990; Gabow, 1993).

Despite their dominant Mendelian inheritance pattern, ADPKD mutations are loss of function alleles and are recessive at the cellular level (Qian et al., 1996; Wu et al., 1998). An acquired somatic mutation that inactivates the wild-type copy of an ADPKD gene in a cell of a heterozygous individual eliminates gene function in that cell and its progeny (Figures 6A–6C). Such clones of mutant renal tubule cells form focal expansions of the tubule. Dilated regions continue to expand and often separate from the tubule, creating large fluid-filled cysts. The expanding cysts impinge on neighboring regions of

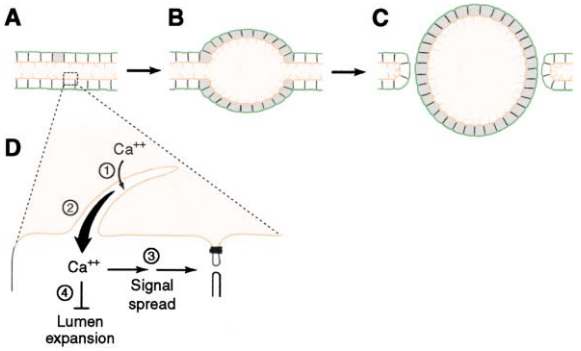

Figure 6. A Model for Pathogenesis of Cysts in Autosomal Dominant Polycystic Kidney Disease

(A) A longitudinal section through a normal renal tubule in a person heterozygous for a loss of function mutation in a PKD gene. A single cilium extends from the apical surface of each tubule cell. One of the cells (gray) acquires a somatic mutation that inactivates the wild-type copy of the PKD gene in that cell.

(B) The mutant cell and its daughters (gray) form a focal expansion of the renal tubule.

(C) The affected region continues to expand and often seals off from the rest of the tubule, creating a cyst. Continued growth and secretion into the lumen enlarges the cyst.

(D) Model for PKD protein function as a ciliary sensor of lumen growth. A close up of the cilium and apical surface of a renal cell is shown. The wild-type products of the PKD1 and PKD2 genes form a Ca^{2+} channel localized within the cilium. Movement or stretching of the cilium during tube growth, perhaps due to its attachment to the neighboring cell or matrix, triggers channel opening and Ca^{2+} influx (1). This is amplified by release of Ca^{2+} from intracellular stores (2), and leads to spread of the signal to neighboring cells (3). The Ca^{2+} signal blocks lumen expansion (4) by suppressing apical membrane biogenesis, secretion, and cell proliferation. If the cilium is disrupted or the PKD1-PKD2 Ca^{2+} channel is missing, the lumen continuously expands.

the kidney and disrupt global renal architecture and function, causing a progressive loss of renal function that leads to end-stage renal failure in about half of patients by age 60 (Gabow, 1993). Mouse knockouts of *Pkd1* or *Pkd2* are homozygous embryonic lethal, and renal tubules in the mutants form normally but then begin to dilate late in embryogenesis (Lu et al., 1997; Wu et al., 2000).

The phenotype of mouse *Pkd1* and *Pkd2* mutants demonstrates that ADPKD genes are not necessary for renal tube formation but are required to regulate subsequent tube growth. How they regulate tube growth is widely debated. The cysts in human patients are characterized by fluid accumulation and cell proliferation and are often accompanied by necrosis, immune cell infiltration, and fibrosis (Evan and McAteer, 1990), making it difficult to distinguish primary from secondary effects of loss of gene function. One theory is that ADPKD genes are tumor suppressor genes, and the primary effect of gene inactivation is increased cell proliferation (Watnick and Germino, 1999). Mutant cells, however, maintain many other aspects of their differentiated state and there is no evidence that they progress as tumors. If ADPKD genes are tumor suppressors, they must be highly selective in their action. The tumor suppressor model is also difficult to reconcile with the result described above that shows cell number is not a critical determinant of renal tube size, at least in salamanders (Fankhauser, 1945). A more appealing model is that ADPKD genes are part of a program that regulates renal tube size, a program inferred from the highly stereotyped sizes and shapes of renal tubes that arise during normal development (Gallagher et al., 2000). According to this model, loss of ADPKD gene function inactivates a part of the program that normally limits tube growth so that mutant cells are unconstrained and form ever larger tubes and cysts.

Molecular characterization of ADPKD gene products and their homologs in other organisms suggest they could function as part of a sensor in the tube size control program. *PKD1* and *PKD2* encode large interacting trans-membrane proteins that are expressed in renal epithelial cells (I.P.K.D. Consortium, 1995; Mochizuki et al., 1996; van Adelsberg, 1999). PKD2 is the prototype of a subclass of the TRP superfamily of Ca^{2+} channel proteins, and co-expression of PKD1 and PKD2 in CHO cells produces a Ca^{2+} channel with ion selectivity similar to other TRP family members (Hanaoka et al., 2000; Somlo and Ehrlich, 2001). The proteins accumulate to high levels in the primary cilium that extends from the apical surface of renal epithelial cells (Figure 6A) (Yoder et al., 2002a). Such solitary non-motile cilia have been known to exist on a variety of mammalian epithelial and endothelial cells for over a century (Zimmermann, 1898; Wheatley, 1982), but their function remained obscure until it was demonstrated recently that renal cell cilia could sense apical surface and luminal events (Schwartz et al., 1997; Praetorius and Spring, 2001). Bending a cilium triggers Ca^{2+} influx, presumably through a ciliary channel (Figure 6D). The Ca^{2+} influx triggers Ca^{2+} release from IP_3-sensitive stores, and the signal spreads to neighboring cells through gap junctions. The PKD1-PKD2-dependent calcium channel is likely a part of this ciliary signal transduction process. Two other mouse genes that cause polycystic kidney disease, *Tg737/polaris/orpk* and *cystin/cpk*, also encode ciliary proteins, and *Tg737* has been shown to be required for cilium formation (Hou et al., 2002; Yoder et al., 2002b). PKD genes appear to comprise a conserved ciliary sensing and signal transduction system, as *C. elegans* homologs of *PKD1* (*lov-1*), *PKD2*, and *Tg737* (*osm-5*) all colocalize in cilia of specific sensory neurons and are required for ciliary morphogenesis or function (Barr et al., 2001; Haycraft et al., 2001; Qin et al., 2001).

The genetic and molecular data suggest that PKD1 and PKD2 may be part of a mechanosensitive Ca^{2+} signaling pathway in renal cell cilia that inhibits renal tube growth. During normal development, renal tube expansion probably proceeds by apical membrane biogenesis and liquid secretion (Grantham, 1993), as described above for other tubes, and is accompanied by proliferation of renal epithelial cells. Cilia protruding from the apical surface could monitor the expansion process, for example by attaching to neighboring cells or the apical matrix. Expansion of the apical cell surface would stretch the cilium, activating the Ca^{2+} signaling pathway and downstream events that inhibit further expansion. If ciliary structure or function is disrupted, the signaling pathway would not be activated by tube expansion, and expansion would continue unabated as in human PKD patients and mouse PKD mutants (Calvet, 2002). This

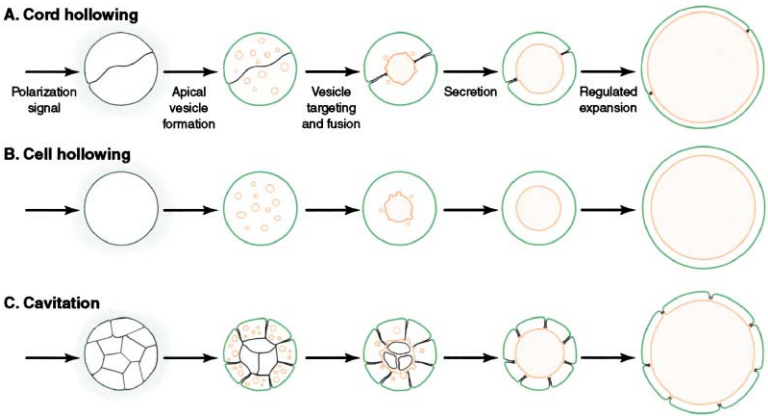

Figure 7. A Common Pathway of Tubulogenesis

Cells about to undergo cord hollowing (A), cell hollowing (B), or cavitation (C) follow a similar series of cellular events to create tubes. After receiving a polarization signal (green shading) that sets apicobasal polarity, the cells establish a basal surface (green) and generate vesicles carrying apical membrane antigens (red circles). The vesicles are targeted to the prospective apical region, where they fuse with existing membrane or each other to form a lumen. Continued vesicle fusion and apical secretion expands the lumen. Expansion is a regulated process that continues until it is inhibited by a tube size sensor set to trigger at a specific value for each tube. The sensor feeds into apical membrane biogenesis and secretory controls, as well as mechanisms for building apical structural support. During cavitation, interior cells fail to receive the polarizing signal and are left within the developing lumen and subsequently eliminated by cell death.

mechanism may be used in other tubes too, as morphologically similar cilia are found on other epithelial and endothelial cells (Wheatley, 1982), some of which also express PKD genes and show inappropriate expansions in PKD mutants, including hepatic and pancreatic cysts and blood vessel aneurysms (Dalgaard, 1957; Lu et al., 1997; Kim et al., 2000; Wu et al., 2000).

A Common Pathway of Tubulogenesis

Despite the wide variety of tubulogenesis mechanisms, emerging descriptions of the cellular and molecular events of tubulogenesis in a number of diverse systems have converged on apical membrane biogenesis, secretion, and other events at the apical surface as critical steps in tube formation and growth. Although the full process is not understood for any system, and the genes and molecules involved are just beginning to be characterized, the available data allow us to piece together a general mechanistic framework for tubulogenesis (Figure 7).

First, a uniform circumferential signal specifies apicobasal polarity of the cell or group of cells, with basal out and apical in. This signal can be as simple as collagen or some other uniformly distributed component of the extracellular matrix surrounding the cells, and it can occur long before lumen formation or immediately precede it as in the MDCK cell system. Second, specialized cytoplasmic vesicles are generated and targeted to the specified apical position. There they fuse, supplying the membrane that forms and enlarges the apical surface. These specialized vesicles also contain membrane proteins or other molecules that insure patency of the lumen, such as anti-adhesive factors to prevent apical surfaces from sticking, or ion pumps to generate osmotic forces that fill and expand the luminal cavity with fluid. There is also a sensor to measure the expansion force or some other parameter of lumen size. In many systems, this could be a ciliary sensor like the PKD1-PKD2-dependent Ca^{2+} channel, but there are probably other types of sensors as well (Lange, 1999) because some tubes lack cilia (e.g., *Drosophila* trachea) or are not affected by mutation of PKD genes (e.g., mammalian lung, *C. elegans* renal tubules). The sensor functions to halt expansion when the lumen reaches a specified size. Expansion arrest could be brought about by inhibiting the expansion force or by activating constraining forces in the apical cytoskeleton or cell surface that fix lumen size. The sensor must be adjustable so that tubes can grow to different sizes and achieve different shapes. If tubes are to grow again, the sensor must also be capable of resetting so the arrest mechanism can reverse and a new cycle of apical membrane biogenesis, secretion, and lumen expansion can begin.

Figure 7 illustrates three of the major morphologic mechanisms of tubulogenesis and shows how the mechanistic framework fits for each. Although each is accommodated, there are clear differences. One difference is that multicellular tubes contain cell junctions that form as the cells polarize (Figure 7A), whereas no junctions form during cell hollowing (Figure 7B). Another difference is the involvement of cell proliferation. Growth of some tubes occurs solely by increasing the apical surface and stretching the cells whereas for others, like in the kidney and lung, cells proliferate as the tubes grow. However neither cell size, shape, or number appears to be a primary determinant of tube diameter, but instead these parameters seem to adjust to accommodate changes dictated by apical surface expansion and secretion. A third difference is the involvement of cell death. Although cell death is observed in most systems that undergo cavitation and was hypothesized to be a critical step in the process, it was recently found that lumens still form in these systems when the apoptotic pathway is blocked (Lin et al., 1999; Debnath et al., 2002). Cell polarization, apical membrane biogenesis, and secretion may be the essential events in lumen formation by cavitation (Figure 7C), similar to the other systems we described. Cell death then eliminates residual cells left inside the luminal cavity.

Differences in Steps Leading up to and Following Tubulogenesis Create Additional Diversity

In addition to the variations just described in the general mechanism, there can also be dramatic differences between systems in the steps leading up to tubulogenesis, which can obscure similarities in the tubulogenesis process itself. For example, the *Drosophila* heart may form by a cord hollowing mechanism fundamentally similar

to that described above for MDCK cells in culture. However, whereas the MDCK cell cord arises by extending from a cyst of polarized cells, the cell cord that gives rise to the *Drosophila* heart forms from a chain of cardiac progenitor cells that originates on each side of the body and migrates dorsally until the two chains meet side-by-side and coalesce at the dorsal midline (Rugendorff et al., 1994). Classically, these examples would be viewed as fundamentally different tubulogenesis mechanisms, but they may simply be variants in an early, preparatory step in the common tubulogenesis process.

There can also be significant differences in steps following tubulogenesis. For example, some newly formed tubes send out signals to recruit additional cell types or cell layers to surround the tube (Lindahl et al., 1997; Benjamin et al., 1998). Also, other mechanisms besides apoptosis are used to eliminate cells left in the interior of a developing tube. In the developing zebrafish gut, as outer cells in the primordium polarize and form the gut wall, cells left in the interior apparently rearrange and integrate into the outer cell layer (Horne-Badovinac et al., 2001). Vascular development in blood islands of the vertebrate yolk sac uses yet another strategy. As outer cells in the mass form blood vessel walls, interior cells differentiate into hematopoietic precursors (Houser et al., 1961). Thus, cavitation systems may all use the same basic mechanism of tubulogenesis (Figure 7C) but deploy different strategies for dealing with residual cells.

While the general model and the variants described above account for a wide variety of tubulogenesis processes, the model does not readily accommodate them all. Although apical membrane biogenesis and secretion usually accompany budding morphogenesis, it is not known if they are crucial. However, recent data suggest that apical membrane biogenesis is important for the budding of the *Drosophila* salivary gland (Myat and Andrew, 2002). It is also not clear if apical membrane biogenesis and secretion are critical to primary neural tube formation in amphibians and chickens, which has traditionally been thought to be driven primarily by constriction of the apical cytoskeleton and related cell shape changes (Schoenwolf and Smith, 1990). Interestingly though, apical secretion of matrix material has been proposed as a mechanism for sea urchin gastrulation, which involves epithelial sheet invagination widely believed to be induced by apical cytoskeletal constriction similar to neural tube formation (Lane et al., 1993). Furthermore, in teleost fish and in posterior regions of most vertebrates including frogs, chickens, and humans, the neural tube forms by cavitation rather than by epithelial wrapping, and appears to proceed by the general mechanism outlined above involving apical membrane biogenesis and secretion (Townes and Holtfreter, 1956). At the junction between the anterior and posterior regions of the chicken central nervous system both cavitation and wrapping are used, and the two lumens that form subsequently coalesce into a single neural tube (Townes and Holtfreter, 1956; Criley, 1969). Perhaps budding and wrapping have more in common with the other tubulogenesis mechanisms than is apparent from our current understanding.

Diversification of Tubulogenesis Mechanisms in Evolution

One of the surprising findings of early descriptions of tubular organ development is that, like chick neural tube formation, the tubes comprising some organs are not all created in the same way. The best known example is blood vessel development, where some vessels arise by the cord hollowing mechanism described above and others form by budding from the walls of existing vessels or by extensive remodeling of existing vessels (Risau, 1997). Similarly, in the mammalian kidney, the collecting ducts and distal tubules arise by sprouting from existing tubes or sacs, whereas proximal tubules arise from condensations of mesenchymal cells that assemble into tubes de novo and connect to the existing network (Saxen, 1987). In the *Drosophila* tracheal system too, different mechanisms are employed at different stages of branching to generate the tubular network (Samakovlis et al., 1996). If each of these mechanisms of tubulogenesis is not fundamentally different but represents a distinct variation on the general tubulogenesis process, then it is easier to envision how such tubular networks evolved: by the emergence of new variants of the tubulogenesis pathway that create tubes of specific sizes or shapes or different functional characteristics within a network. And, if it is easy to generate such variants during evolution, this would help explain how differences arose among related species in the tubulogenesis processes that form the neural tube and other organs such as the heart.

Although we are beginning to pinpoint some of the critical cellular and molecular events of tubulogenesis and can formulate a speculative general model for the process, we expect rapid progress in this area from a large number of systems using a variety of experimental approaches. A field of tube morphogenesis is emerging that not only seeks to answer the fundamental questions of how tubes are made and shaped, but also how these processes go awry in human diseases associated with tube structural defects. Further investigations of the model systems and exploration of the human diseases will continue to provide insights into the molecules and mechanisms of tube morphogenesis. These may in turn suggest ways to repair tubular defects or engineer better tubes.

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