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From cells to organs: building polarized tissue

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Abstract

How do animal cells assemble into tissues and organs? A diverse array of tissue structures and shapes can be formed by organizing groups of cells into different polarized arrangements and by coordinating their polarity in space and time. Conserved design principles underlying this diversity are emerging from studies of model organisms and tissues. We discuss how conserved polarity complexes, signalling networks, transcription factors, membrane-trafficking pathways, mechanisms for forming lumens in tubes and other hollow structures, and transitions between different types of polarity, such as between epithelial and mesenchymal cells, are used in similar and iterative manners to build all tissues.

The defining feature of metazoa is that their cells are organized into multicellular tissues and organs. Although almost every eukaryotic cell is spatially asymmetric or polarized, polarity must be coordinated in space and time for individual cells to form a tissue¹. Cell polarity involves the asymmetric organization of most of the physical aspects of the cell, including the cell surface, intracellular organelles and the cytoskeleton^{2,3}. Analysis of the polarization of unicellular eukaryotes, such as yeast, has yielded enormous insights into the mechanisms that underlie the polarity of individual cells³. Formation of a tissue, however, requires an ensemble cast; the emergent properties of the tissue result from the combined roles of the individual cells that are involved. Accordingly, several biological processes, including cell division, cell death, shape changes, cell migration and differentiation, must be coordinated with the polarity requirements of a tissue to form an organ⁴.

Evolutionarily, epithelia are the most archetypal polarized tissues in metazoa, with ~60% of mammalian cell types being of epithelial or epithelial-derived origin⁵. Accordingly, the best studied polarized tissue is the simple epithelium of the mammalian intestine and kidney, the cells of which are columnar in shape (that is, they are taller than they are wide). The apical surfaces of these cells provide the luminal interface and are specialized to regulate the exchange of materials, such as nutrients from the intestine. The lateral surfaces of these cells contact adjacent cells and have specialized junctions and cell–cell adhesion structures^{3,6} (FIG. 1a). The basal surfaces of these cells contact the underlying basement membrane, extracellular matrix (ECM) and, ultimately, underlying blood vessels. The basal and lateral surfaces are fairly similar in composition and organization and are often referred to together as the basolateral surface. The apical and basolateral surfaces, however, have very different

DATABASES

UniprotKB: <http://ca.expasy.org/sprot>

aPKC | CDC42 | claudin-15 | Crb | Dlg | ERBB2 | PAR-3 | PAR6 | PATJ | RAC1 | RhoA | ROCK1 | Scrib | Snail | ZEB1

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compositions. In vertebrates, tight junctions (TJs) are found at the apical-most portion of the lateral surfaces, where the TJs form barriers both between the apical and basolateral surfaces and between adjacent cells, limiting paracellular permeability⁷ (FIG. 1a).

Many epithelial organs make use of interconnected tubular networks, although the basic design principles (as defined by Rafelski and Marshall⁸) are the same: a series of tubes terminates in a spherical ending or cap, which is referred to as an acinus, end bud, alveolus or cyst in different tissues. Tubular networks can either arise independently and then become interconnected, or can be branching trees that form via new sprouts from existing tubes. Many conserved morphogenetic processes give rise to these structures, including mechanisms of lumen formation and expansion, tubulogenesis, branching morphogenesis, mesenchymal–epithelial transitions (MET) and epithelial–mesenchymal transitions (EMT).

Cellular specialization through polarization occurs in almost all cell types. Neural synapses have specialized sites for neurotransmitter release and uptake⁹ (FIG. 1b). The apical membranes of photoreceptor epithelium undergo light-sensing activity, whereas the basal surfaces connect to underlying neurons (FIG. 1c). Migrating cells, such as neutrophils or *Dictyostelium discoideum* amoebae, exhibit asymmetric front–back polarity as they move towards attractive cues¹⁰ (BOX 1). With a core requirement for cellular asymmetry in biological function, understanding how cells polarize and coordinate this process to form a tissue is a central question.

Box 1

Polarity complexes: conserved regulators of great plasticity

Conserved, core protein complexes are involved in the generation and maintenance of all types of polarization. Three major polarity complexes, the PAR (CDC42–PAR3–PAR6–aPKC), Crumbs (Crb–PALS–PATJ) and Scribble (Scrib–Dlg–Lgl) complexes function in such diverse contexts as asymmetric cell division, epithelial and neuronal polarization, chemotactic migration and cell proliferation^{2,17} (see figure). Although these complexes perform seemingly different functions in different cell types and contexts, some overarching themes can be drawn.

Polarity complexes distribute asymmetrically in cells, promoting the expansion of the membrane domain they associate with. In epithelial cells (panel a), the PAR and Crumbs complexes promote apical polarity, whereas the Scribble complex operates at the basolateral surface. The PAR complex can also be divided into two subcomplexes: apical CDC42–PAR6–aPKC and tight junction (TJ)-localized PAR3–aPKC, which recruits the lipid phosphatase PTEN (BOX 2). Polarity complexes can be mutually antagonistic (panel b)^{2,107}, a design principle that allows the establishment of axes of asymmetry⁸. Inappropriate movement of the Scribble complex into the apical domain is antagonized by the phosphorylation of Lgl by aPKC: phosphorylation of Lgl dissociates the protein from the cell cortex¹⁰⁸. Similar reciprocal exclusion mechanisms between apical and basolateral complexes maintain this asymmetry¹⁷, allowing apical and basolateral regions to become discrete, non-overlapping domains — a possible example of zero-order ultrasensitivity¹⁰⁹. Several signalling receptors that disrupt or promote the formation of polarized adhesion appear to do so by modulating this balance^{68,110,111}. Asymmetric polarity complex distribution also polarizes other cell types² (for example, migratory cells; panel c), although the Crumbs complex is apparently specific to epithelia and epithelial-derived cell types, such as neurons.

Despite the integral involvement of polarity complexes in morphogenesis, the mechanisms through which they promote asymmetry are still largely unclear. Recent evidence reveals that these complexes are central organizing platforms that modulate the

microtubule cytoskeleton, membrane traffic and phosphatidylinositol-phosphate regulation² (BOX 2), in part by controlling Rho GTPase activation through guanine nucleotide-exchange factors and GTPase-activating proteins^{2,39,60}. Disruption of polarity complexes also has marked effects on cellular proliferation, revealing that these complexes have key roles in tumour suppression^{67,112}.

Although many biological processes contribute to the formation of an organ, we will focus on how cell polarity is controlled and contributes to morphogenesis in the context of whole tissues. We discuss the molecular control of tissue polarization in *in vivo* organs and in *in vitro* organotypic models, including the establishment, transcriptional control and molecular regulation of tissue polarization, control of polarity orientation, and regulation of polarity by ECM and Rho GTPase signalling. We emphasize the role of epithelial lumen and tube formation and expansion, as epithelial tissues have provided many fundamental insights into how polarity is coordinated at the cellular, tissue and organ level.

Forming polarized tissue

The organization of cells into tissues involves the concerted integration of polarizing cues from various interdependent biological processes. First, cells must sense their environment, including where they are in relation to their neighbours. This can be mediated by direct interaction of cells with the ECM through various receptors, such as integrin, dystroglycan and proteoglycan molecules^{11,12}. Cells can sense and modify the chemical composition, assembly, stiffness and other mechanical properties of the ECM^{13,14}. Cells can also communicate with other cells through an array of adhesion molecules, such as cadherins¹⁵, and through the sensing of diffusible factors, such as morphogens, chemoattractants and chemorepellants¹⁶. These combined cues provide instructions that enable cells to orientate their polarity and begin to assemble into groups. Second, cells in a forming tissue must coordinate the asymmetrical distribution of polarity complexes¹⁷ to establish and enforce the generation of an axis of asymmetric organization (BOX 1). Concurrently with this second step, the cytoskeleton and membrane-trafficking systems organize asymmetrically². These basic steps allow individual cells to become asymmetrically polarized (see the reviews by Bornens and by Nelson and Mellman in this issue). Examples of polarized epithelial and migratory cell polarity and of the formation of polarity complexes are presented in BOX 1.

An important design principle is that polarization must be coordinated between all cells in a tissue. For example, although the organization of polarity complexes, the cytoskeleton, membrane-trafficking events and adhesive junctions must be asymmetric in a single cell, the orientation and organization of this asymmetry must be coordinated between neighbouring cells. In addition to apico–basal polarity, some behaviours, such as cell division and migration, can also be polarized in an orthogonal axis; that is, in the plane of the tissue. Although this planar cell polarity (PCP) is extremely important in tissue formation — for example, it regulates the expansion of a tissue along a particular axis — we will only briefly touch on it here as it has been reviewed excellently elsewhere^{18,19}.

Although the signalling mechanisms to induce and regulate morphogenetic movement during tissue formation are well characterized, how these processes are regulated at the cellular level has only recently started to become clear. The identification of conserved, core polarity-regulating complexes that operate in various contexts has given many insights into these processes (BOX 1). Recent progress in understanding the orientation of tissue polarity, the molecular regulation of epithelial lumen generation and the moulding of biological tubes is discussed below. In addition, key roles for phosphatidylinositol-phosphates (PtdInsPs; BOX 2) and Rho-family GTPases in many of the aforementioned processes have recently become clear, and their contributions to cell and tissue polarity are discussed below.

Box 2

Phosphatidylinositol-phosphates specify membrane polarity

Phosphatidylinositol-phosphates (PtdInsPs) are phospholipids that are singly or multiply phosphorylated on the 3, 4 and/or 5 positions on an inositol head group¹¹³. PtdIns(3,4,5)P₃ can be generated from PtdIns(4,5)P₂ by a family of PtdIns3-kinases (PI3K), and PtdIns(4,5)P₂ can be generated from PtdIns(3,4,5)P₃ by PTEN (a 3-phosphatase). The balance between these two lipids is crucial to polarity homeostasis, and recent evidence reveals that PtdInsP and associated proteins have core roles (see figure, panels a–c) in membrane identity and polarity generation.

Asymmetric PtdIns(4,5)P₂:PtdIns(3,4,5)P₃ distribution occurs in various cell types, including migrating neutrophils¹¹⁴, polarized kidney epithelia⁴⁶ and *Drosophila*

melanogaster photoreceptors¹¹⁵. In polarized MDCK cells (panel **b**), PtdIns(3,4,5)P₃ localizes exclusively at the basolateral membrane, whereas PtdIns(4,5)P₂ is enriched apically^{46,116}, like in *D. melanogaster* epithelia^{117,118}. The PAR complex (PAR3–aPKC) modulates asymmetric PtdIns distribution by recruiting the phosphatase PTEN to tight junctions (TJs)^{118–120}, potentially restricting PtdIns(3,4,5)P₃ from moving across the TJ into the apical membrane. Asymmetry of PtdIns(4,5)P₂:PtdIns(3,4,5)P₃ can also occur without TJs, such as in migrating neutrophils (panel **a**), where the PtdInsP and associated proteins (G12, G13, EBP50, phospho-ERM (pERM)) control ‘backness’ and ‘frontness’¹¹⁴. PtdInsP distribution can also be asymmetric in a single membrane domain, such as in the apical membrane of *D. melanogaster* ommatidia (panel **c**), where PTEN controls levels of PtdIns(3,4,5)P₃¹¹⁵.

Asymmetric PtdIns(4,5)P₂: PtdIns(3,4,5)P₃ distribution is fundamental to the maintenance of cell polarization. Addition of exogenous PtdIns(3,4,5)P₃ to basal membranes results in their expansion into the surrounding matrix; apical addition induces rapid loss of apical identity, transcytosis of basolateral membrane to the apical surface and projections from the apical surface¹²¹. Addition of PtdIns(4,5)P₂ to the basal surface results in rapid redistribution of apical proteins towards the basal membrane⁴⁶. The opportunistic pathogen *Pseudomonas aeruginosa* takes advantage of at the this process by inducing ectopic PtdIns(3,4,5)P₃ at the apical membrane, converting it into basolateral-like membrane and facilitating entry into the epithelium from the luminal surface¹²². Actin-regulatory proteins and membrane-trafficking pathways that are influenced by PtdInsP, such as the vesicle-regulating exocyst complex¹²³, may therefore require PtdIns(4,5)P₂:PtdIns(3,4,5)P₃ asymmetry to ensure correct vesicle targeting, cytoskeletal organization and maintenance of polarity. ZA, zonula adherens.

Tissue morphogenesis by MET–EMT

One design principle underlying morphogenesis is that cells can switch between different types of polarity. In EMT, epithelial cells switch their polarity to that of mesenchymal cells (FIG. 2a). EMT occurs in many developmental processes, such as during gastrulation and during formation of the neural crest and primitive streak, whereby well-polarized epithelial sheets convert to motile mesenchymal cells and give rise to another tissue type²⁰. Loosely defined, EMT involves the disruption of polarized adhesion, such as epithelial (E)–cadherin-based junctions, disruption of apico–basal polarity, reorganization of the cytoskeleton, altered basement-membrane composition and organization, and adoption of motile behaviours and invasion into surrounding tissue. As many aspects of EMT are reminiscent of tumour formation, inappropriate recapitulation of EMT has received much attention as a mechanism for metastasis of epithelial cells (this has been excellently reviewed elsewhere^{20,21}).

Conversely, MET occurs through condensation of mesenchymal cells into tightly adhesive groups, generation of apico–basal polarity, adoption of epithelial characteristics and transition to a polarized epithelial tissue²¹ (FIG. 2a). MET contributes, for example, to certain sections of the developing kidney²².

The exact definition of EMT is not clear-cut, perhaps because different forms of morphogenesis may use some, but not all, aspects of complete EMT. It is also unclear whether EMT involves only changes in motility, adhesion and polarity, or whether EMT involves changes in cell fate and differentiation^{20,23}. There are examples of partial EMT (pEMT) whereby epithelial cells become motile, but do not completely lose adhesion and polarity^{24–28} and do not seem to lose epithelial differentiation and fate. It is important, therefore, to emphasize that cells undergoing different forms of EMT do not lose all

polarity; rather, they may simply substitute epithelial polarity and characteristics for mesenchymal polarity. Many migratory cells, such as neutrophils or *D. discoideum*, display strong front–back asymmetry and exhibit polarized migration towards attractive cues¹⁰. Some of the core polarity complexes that modulate apico–basal polarity (the PAR complex; see BOX 1) are also involved in the organization of front–back asymmetry². Morphogenesis of polarized tissues, which involves the contribution of both stable and motile phases during formation, can therefore be seen as movement along a continuum of MET–EMT stages. Such transitions between polarity states are fundamental for shaping many metazoan tissues, and alteration to polarity provides a mechanism to change cell behaviour without necessarily changing cell fate.

Transcriptional control of polarity in EMT and MET

Asymmetric morphogen gradients can provide instructive cues for a tissue to undergo morphogenesis, for example, by inducing transcription factors (TFs) to drive these processes¹⁶. The Snail and ZEB families of TFs, for example, are potent inducers of, and are often required for, EMT events both *in vivo* and *in vitro*²⁹. How these transcriptional modulators induce cellular outputs to regulate tissue polarity has only recently started to become clear.

The traditional view is that the loss of E-cadherin and of other junction proteins induces altered cellular polarity and EMT²¹. Recent evidence, however, suggests that some EMT-associated TFs also control morphogenesis by directly repressing transcription of molecules that are involved in polarity complexes^{30,31}, membrane-trafficking systems³², the cytoskeleton³² and the basement membrane³³ (FIG. 2b). For example, Snail and ZEB1 repress key components of both the scribble and Crumbs polarity complexes^{30,31}, and re-expression of scribble and Crumb complex proteins partially rescues epithelial polarization. Although loss of apico–basal polarity is an inherent part of the definition of EMT, loss of E-cadherin is not always induced by or correlated with expression of some of these TFs³². In these cases, alterations to polarity complexes or to the ECM may suffice to allow for altered cellular morphogenesis. Indeed, during metastasis of colon carcinoma, invasive fronts of cells only occur at sites of lost basement-membrane integrity³³, showing that ECM signalling is a key regulator of EMT and thus of tissue polarity.

Stimulation of epithelial cells with various cytokines, such as hepatocyte growth factor (HGF) or transforming growth factor- β (TGF β), can induce expression of Snail- or ZEB-family members^{21,29}. Snail expression can induce co-expression of ZEB factors³⁴, which further enables additional TGF β expression, initiating a positive-feedback loop²¹. Interestingly, this EMT-inducing module is also under the control of a negative-feedback loop that involves endogenous microRNAs (miRNAs)^{35,36} (FIG. 2b). The miR-200 family of miRNAs promotes epithelial differentiation via downregulation of ZEB1. Loss of the miR-200 family is found in tumour samples that have lost epithelial polarity, and forced re-expression of the miR-200 cluster restores epithelial polarization and differentiation. This downregulation, however, is also reciprocal; miR-200 members are targets for direct transcriptional repression by ZEB1. Whether cells become polarized into an epithelial tissue (MET) or become motile (EMT) therefore depends on a balance between mutually antagonistic miRNA and TF modules, which controls epithelial polarization, adhesion and the ECM. Unravelling the factors that promote the expression of the miR-200 family will be key to understanding the control of epithelial polarization, and may involve the local signalling microenvironment, such as the ECM.

Orientating polarity: ECM and GTPase signalling

Cells in tissues are often surrounded by ECM. Recent studies have revealed another design principle: the ECM, more than providing a structural scaffold, can define positional information and differentiation cues for tissues, ultimately influencing tissue polarity¹³. Transduction of these cues via ECM receptors, such as integrins, dystroglycan and proteoglycans, can ultimately lead to changes in cell polarity and shape through various mechanisms. Modulation of the cytoskeleton and signalling through Rho-family GTPases appears to be key to regulating ECM-directed polarity specification^{12,13,37,38}.

Orientation and maintenance of tissue polarity

A fundamental design principle for forming a polarized tissue is that cells must interpret an initializing cue to polarize. When isolated cells, such as neutrophils or *D. discoideum*, are stimulated with a chemoattractant, they typically polarize and migrate towards the source of the molecule¹⁰. This orientation of polarity is set up by an asymmetric gradient of the initiating cue, such as a bacterium or chemokine. The PAR complex (BOX 1) is involved in such front-back polarity in migratory cell types, in part by controlling asymmetric orientation of the microtubule cytoskeleton³⁹. What governs the orientation of apical and basolateral polarity in epithelial tissues, on the other hand, is less clear, partially because of the systems that are used to study this process. Polarized epithelial cells that are cultured on an artificial support, such as a dish or filter, receive strong, asymmetric initiating cues for polarization — the rigid substratum provides a cue, while the ‘free’ medium provides another — and there is consequently an axis from which the cells can collectively orientate their polarity^{1,40}.

How is the orientation of polarity defined when cells are completely surrounded by ECM, such as *in vivo*? Studies using epithelial cells grown in 3D culture have provided some answers (BOX 3). Normally, MDCK (Madin–Darby canine kidney) cyst structures form as a simple epithelium surrounding a central luminal space⁴⁰. Inhibition or loss of $\beta 1$ -integrin or the downstream GTPase RAC1 results in inversion of this orientation such that the apical surface becomes orientated towards the matrix^{12,37}. A similar inversion of glandular epithelial polarity also occurs in a subset of invasive ductal breast carcinomas⁴¹, demonstrating that inversion of polarity can also occur *in vivo* during tumorigenesis. By contrast, inversion of polarity orientation is promoted by, and dependent on, the inappropriate activation of the RhoA GTPase and its downstream effectors, ROCK1 and myosin II³⁸. Despite this inversion, cell–cell junctions form and cells maintain some level of polarity; that is, proteins and lipids are asymmetrically distributed. This suggests that the establishment and correct orientation of polarity are not obligate partners and can be molecularly uncoupled. It further suggests that signalling at the cell–ECM interface is a primary determinant of the axis along which epithelial cells orientate their polarity.

Box 3

In vitro and *in silico* modelling of epithelial morphogenesis

Organotypic three-dimensional (3D) culture of epithelial cells in extracellular matrix (ECM; matrigel, a basement membrane-like tumour product, or COLI gels) provides a way to model epithelial morphogenesis *in vitro*. Many types of epithelial cells form organoid structures in 3D culture^{24,40,124–126}, recapitulating varying levels of tissue polarity and architecture. For MDCK cells, at least, this is analogous to a mesenchymal–epithelial transition (MET). Motile cells that are embedded in matrix either migrate together and adhere, or proliferate from an individual precursor to form stable adhesive complexes. Cells then undergo morphogenesis and polarization to eventually form simple

epithelial cysts that surround a hollow, fluid-filled lumen. Stimulation with growth factors, particularly hepatocyte growth factor (HGF), induces branching and tubulogenesis of structures, modelling some aspects of embryonic kidney development^{127,128}. Notably, the tubules produced in this system closely resemble the morphogenesis of the mouse embryonic nephric duct (C. Mendelsohn, personal communication). 3D culture has proved to be indispensable for examining aspects of cell polarization, lumen formation, oncogene function and the influence of the ECM on tissue organization, which remain technically challenging to manipulate *in vivo*. Notably, mutants in many polarity regulators or oncogenes show vastly different phenotypes in 3D culture than in traditional 2D culture^{1,24,37,46}, even in instances where no phenotype is observable in 2D culture.

On the basis of 3D culture, researchers have established a simple set of rules that epithelial cells follow during polarization^{1,40}. Each cell strives to have three types of surface: a basal surface, which contacts the ECM, a lateral surface, which contacts other cells, and an apical surface, which faces the lumen. A cell that does not contact the ECM undergoes apoptosis, whereas a cell that lacks an apical surface will generate a lumen at a region of contact with other cells, or even within itself. Notably, such parameters can be incorporated into an *in silico* model of epithelial morphogenesis and can yield remarkably life-like ‘cysts’ during simulations¹²⁹. Computational and systems biology approaches are likely to play an increasingly important role in the development and analysis of *in vitro* tissue systems, such as in regenerative medicine or stem-cell-derived differentiation of epithelial tissue types, in which induction and maintenance of a polarized epithelium will be crucial.

Maintenance of polarization and migration of neutrophils towards a chemoattractant requires the continued sensing of the polarizing cue, such as by G-protein-coupled receptor-mediated activation of PtdInsP signalling cascades¹⁰. Analogously, maintenance of epithelial polarity in tissues may require the continued sensing of polarizing cues, such as detecting the ECM through integrins and detecting cell neighbours through cadherins. Notably, both of these receptors can control the generation of PtdIns(3,4,5)P₃^{12,42–44}, which has a fundamental role in generating and maintaining basolateral membrane identity (BOX 2), at least in MDCK cells. Cadherin and integrin receptors can signal through small GTPases, such as to the Rho and Arf families, which can function both upstream and downstream of PtdInsP. Receptor–GTPase–PtdInsP signalling modules (whether the receptor is integrins, cadherins or a G-protein-coupled receptor) may therefore be key to both the generation and maintenance of tissue polarity in diverse contexts.

Rho GTPase and PAR complex crosstalk

The three prototypical Rho GTPases, RhoA, RAC1 and CDC42, play integral roles in cytoskeletal arrangement, membrane-trafficking pathways and ECM interactions, and all of these roles are crucial for cell polarization⁴⁵. CDC42 function is crucial for epithelial lumen formation^{46–48} (see below and BOX 1), whereas RAC1 is associated with both integrin and cadherin signalling^{12,44} and controls the orientation of polarity in epithelial and migrating cell types^{12,49}. RhoA is associated with both apical and basal membranes in epithelial cells⁵⁰ and at the rear of migrating cells⁵¹ and appears to regulate cell shape in some systems⁴⁵.

Various connections between the PAR complex and Rho GTPases have recently emerged, emphasizing their key roles in cell polarity. Unique functions of Rho GTPases occur at discrete subcellular locales, regulated by guanine nucleotide-exchange factor (GEF) and GTPase-activating protein (GAP) proteins. For instance, binding of CDC42 to PAR6 is

required for proper function of this apical determinant⁵². In *C. elegans* embryos undergoing radial polarity generation, CDC-42 targets the atypical protein kinase C (aPKC)–PAR-3–PAR-6 polarity complex to non-junctional membranes⁵³. This requires a GAP (PAC-1) to exclude CDC-42 from junctions and a (as yet unidentified) GEF protein to activate CDC-42 at apical membranes. Deletion of this GAP causes ectopic mistargeting of polarity complexes and polarity defects. PAR-3 binds the Rac GEF TIAM-1 to regulate cell–cell junctions. TIAM-1, through aPKC, apparently controls microtubule organization, providing a mechanism for the PAR complex to regulate asymmetric cytoskeletal organization³⁹. In certain *Drosophila melanogaster* and chick embryonic tissues, GEF-mediated activation of RhoA at the apical surface, coupled to GAP-mediated inactivation of RhoA at the basal surface, allows apical cell constriction and remodelling of the epithelium while maintaining apico–basal polarity^{54–59}.

The PAR6–aPKC subcomplex also regulates RhoA signalling through direct interaction with and modulation of p190A RhoGAP activity⁶⁰. RhoA–ROCK signalling can conversely disrupt aPKC–PAR6–PAR3 interaction and function by direct phosphorylation of PAR3 (REF. ⁶¹). This emphasizes the emerging notion that PAR–Rho GTPase complexes act as discrete multimeric signalling ‘hubs’ in different regions of the cell, controlling various aspects of asymmetric cellular organization and polarity. Identifying unique functions for Rho GEFs and GAPs will be crucial to understand Rho GTPases and PAR complex function at such discrete locales. As PAR3–TIAM1 complexes can control microtubule organization³⁹, and as the PAR6–CDC42 subcomplex controls recycling from endocytic compartments⁶², both the cytoskeleton and membrane trafficking systems may be direct targets of such complexes. Similarly, given the key roles of Rac, CDC42 and Rho in cell polarity, it will be important to dissect overlapping or potential novel roles of the remaining Rho GTPase family members in tissue formation and polarity. For a more extensive discussion of the role of GTPase signalling in cell polarity, see the review by Iden and Collard in this issue.

Putting in the plumbing: tube formation

During the morphogenesis of an epithelial tissue, cells often organize into biological tubes. Such tubes provide the basic plumbing that is crucial for organ and organismal function, and their formation is therefore a fundamental event in the generation of diverse tissues during metazoan development⁶³. For example, vascular tubes allow for transport of O₂ and nutrients throughout the body, the digestive system lumen allows absorption of food and mammary tubes allow the secretion of milk⁶⁴. Although classic embryology has shown that there is an enormous diversity of mechanisms for tube formation, some common themes and molecular regulators have emerged.

Making use of polarity: forming lumens

The paramount requirement for a biological tube is that a lumen must form, and the lumen must be enclosed and unobstructed. Many tubes form by rearrangement of existing epithelial sheets. Such sheets can be deformed by evagination, invagination or similar folding to give rise to tubes that bud off⁶⁴, such as the branching of the ureteric bud during embryonic kidney development. A variant of this occurs when an epithelial sheet folds or rolls up along an axis that is parallel to the plane of the sheet, such as in the neuroepithelium of the developing chick. By merging the epithelium only at specialized contact points, a tube with radial tissue symmetry and a central lumen can be formed⁶³. As such morphogenetic movements have been reviewed elsewhere^{50,63,64}, we concentrate here on more recent developments in the different lumen- formation mechanisms of cavitation, hollowing and membrane repulsion. TABLE 1 lists the molecular machinery that is currently implicated in these processes. In these contexts, groups of poorly polarized cells can begin to tightly

adhere to one another and generate lumens *de novo* (see below; FIG. 3). For cavitation and hollowing, such adherence is essentially a MET event and occurs, for example, as part of the condensation of the metanephric mesenchyme during kidney development²².

Cavitation occurs when a group of cells proliferate in an adhesive, but initially only moderately polarized, manner (FIG. 3a). The selective apoptosis of cells that are not in contact with the ECM gives rise to an outer epithelial layer surrounding a now hollow lumen. This process occurs, for example, in three-dimensional (3D) models of mammary acini, and in *in vivo* mouse mammary end buds^{24,65,66}. In these situations, pro-apoptotic BCL2-family factors have key roles in luminal cell apoptosis, although additional mechanisms, such as autophagy, appear to contribute to luminal clearance in such tissues^{65,66}. The PAR, scribble and Crumbs complexes have important roles in suppressing cell proliferation in *D. melanogaster* tissues⁶⁷, and both the PAR (aPKC–PAR6) and scribble complexes promote apoptosis of luminal cells during cavitation⁶⁸, thus contributing to the formation of a polarized tubular epithelium.

During hollowing of rapidly polarizing groups of cells, intracellular vesicles (varying in size between systems) are delivered to the cell surface at a coordinated point between two closely apposed cells, creating a luminal space *de novo* (FIG. 3b). These vesicles are thought to contain fluid that is taken up by endocytosis, and to contain apical proteins that are destined for delivery to the lumen. Their movement to the cell surface results in the generation of space between two (or more) polarized cells, and concomitant cell-surface delivery of the apical, luminal membrane. The surrounding cells now exhibit apico–basal polarity and are orientated around a lumen, and the whole tissue is subsequently expanded in a highly polarized manner. This mechanism has been observed in 3D organotypic models of kidney and vascular development, as well as in blood vessels *in vivo*^{46,69,70} (although its observation in blood vessels is controversial⁷¹).

A molecular understanding of this process has recently become clear⁴⁶ and involves concerted integration of PtdInsP, Rho-family GTPases and the PAR polarity complex. Signalling from the ECM, through integrin receptors, initially orientates apico–basal epithelial polarity in newly adhering groups of cells (see below). Enrichment of PtdIns(4,5)P₂ at the apical plasma membrane by the lipid phosphatase PTEN results in the apical recruitment of the small GTPase CDC42 via the PtdInsP-binding protein annexin-2. Activated CDC42 in turn binds the PAR6–aPKC polarity complex, thereby ensuring targeting to the apical membrane (FIG. 3b; BOX 1). Scaffolding of this complex to nascent Lumina is required to efficiently generate apico–basal polarity and, consequently, a single polarized lumen.

Interestingly, when vacuolar exocytosis is inhibited, lumens eventually form by cavitation⁴⁷, which emphasizes the robust drive of epithelial cells to form a hollow lumen. The CDC42–PAR6–aPKC polarity complex appears to be a master regulator of lumen formation; its disruption results in either multiple small lumens or in the accumulation of apoptotic cells in the lumen^{46,72,73}. Interestingly, in 3D organotypic culture of mammary acini, activation of the ERBB2 oncoprotein, which promotes cell survival and lumen filling in human cancers, also results in a lumen-filling phenotype by inducing uncoupling of PAR3 from the PAR6–aPKC complex⁶⁸. The identities of the downstream effectors of this complex in lumen formation are still unclear, although regulation of both glycogen synthase kinase-3 β (GSK3 β) and microtubules have recently become good candidates^{39,73}. Thus, the CDC42–PAR6–aPKC complex appears to regulate lumen polarity, formation and maintenance in diverse tissue contexts.

During *D. melanogaster* heart-tube formation, membrane repulsion appears to regulate lumen formation. Here, two rows of myoendothelial cells line up along the midline and extend membrane process towards the mirroring cell on the other side of the midline. In epithelial cells, this would normally result in adhesion between cells along the entire lateral contact. In these cells, however, junctions occur only at the dorsal- and ventral-most regions, resulting in the formation of an enclosed luminal tube between the rows of cells (FIG. 3c). Interestingly, Slit–Robo signalling, a ligand–receptor coupling that governs repulsive signalling in other cellular contexts, occurs at the site where the future lumen will form^{74,75}, excluding cadherin complexes from the region and promoting luminal development between cells. As cells must convert adhesive regions between cells into nascent lumens during hollowing, it will be important to determine whether repulsive forces or anti-adhesive molecules (such as gp135 (also known as podocalyxin)⁷⁶) play analogous roles in other tissues. Likewise, although the fly homologue of PAR3 localizes to adhesive interfaces in developing lumens, whether any of the PAR, scribble or Crumbs polarity complexes play a role in this mechanism of lumen formation is currently unclear.

Tissue polarity during lumen and tubule formation

Once rudimentary luminal structures are formed, tubes must lengthen and become interconnected networks. In some systems, such as the mammalian lung and mammary ducts, this is achieved primarily through budding of new sprouts and branches⁶³. This design principle can make repeated use of such budding mechanisms, with the branches following simple patterns⁷⁷. Many rounds of such iterative branching give rise to an extensive tree. In other networks, such as the developing *D. melanogaster* trachea, different parts of the tubular network arise in an initially unconnected manner and sprouting branches extend towards each other, fusing and creating an anastomosing network⁷⁸. Complex rearrangements of apical polarity and cell–cell adhesion must occur when two branches of a network eventually undergo fusion to maintain lumen integrity and generate an interconnected lumen⁷⁹. In some cases, lumens form in single cells, although these often connect up to lumens between multiple cells (FIG. 4). In *D. melanogaster* airways, for example, this occurs via switching between intercellular (between two or more cells) and autocellular junctions⁸⁰ (in a single cell). specialized adhesion molecules, luminal matrix proteins and modulation of endocytic recycling pathways are involved in such rearrangements^{80–83}.

Most, if not all, morphogenetic mechanisms involve alterations in cell polarity at some level, although how polarity is regulated and remodelled during morphogenesis is poorly understood. In simple epithelia, the PAR, scribble and Crumbs polarity complexes specify apico–basal polarity (BOX 1). In pseudo-stratified or multilayered epithelia, or in Lumina in single cells, organization of the tissue, such as location of cell–cell junctions and biogenesis of the apical membrane, is more complex, and how polarity is controlled in these situations remains unclear. For example, budding of the mammary gland tubular tree has recently been shown to involve an intermediate step whereby the buds are filled with multilayered cells⁸⁴; the polarization of multilayered cells is different from and much less well understood than that of simple (monolayered) epithelia. When MDCK cysts are treated with HGF to form tubules, cells undergo a pEMT, passing transiently through an intermediate stage of chains and cords of cells, which have some properties of migrating mesenchymal cells, before returning to complete epithelial polarity^{25,26}. Such chains and cords can also be viewed as small regions of multilayered cells, somewhat analogous to the multilayering seen in mammary gland buds. The polarity requirements of different regions of a tissue, therefore, changes during morphogenesis; cells can switch, even transiently, between polarized states.

Getting it just right: lumen length and diameter

Once a lumen is formed, how is it expanded to the appropriate physiological diameter? Several diseases are caused by tubes that are either too wide⁸⁵ (for example, polycystic kidney disease (PKD)) or too narrow⁸⁰ (for example, vascular stenosis)⁸⁶. In the cavitation model of lumen formation, proliferation of the entire tissue to the required dimension followed by regulated cell death of inner cells allows for the generation of appropriate lumen diameter^{24,65}. In other circumstances, such as in lumens formed by hollowing, sheet wrapping or folding, various mechanisms for lumen expansion exist. In the embryonic zebrafish gut, multiple small lumens are remodelled into a single tube in a process that is dependent on the accumulation of luminal fluid, regulated by certain pumps (Na^+/K^+ -ATPase) and modulated by the ion permeability of TJs (claudin-15)⁸⁷. Similar regulation of lumen expansion is also observed in MDCK cysts and in thyroid 3D cultures where chloride ion secretion is involved in determining lumen diameter^{87,88}. Notably, inhibition of the CFTR chloride channel inhibits lumen overexpansion in both MDCK cyst and mouse models of PKD, suggesting that chloride transport may be a key regulator of lumen size⁸⁹. Moreover, coordinated regulation of the Na^+/K^+ -ATPase and septate junctions (the invertebrate equivalent of TJs) also appears to be key for appropriate lumen expansion in the *D. melanogaster* trachea^{90,91}. Interestingly, this function of the Na^+/K^+ -ATPase appears to be independent of pump activity⁹⁰, suggesting that there are some differences in the mode of actions between species or tissues. Whether multiple small lumens occur as obligatory precursors to a single lumen and whether fluid accumulation drives lumen expansion in other biological tubes remain to be determined.

At least two additional processes account for changes in lumen diameter in the tracheal tube and salivary gland of *D. melanogaster* (FIG. 4a). Initially, the apical surfaces of cells on opposite sides of the tracheal tube are close together, and the lumen is narrow. A rapid burst of membrane traffic to the apical surface and secretion into the lumen occurs, concomitant with lumen expansion^{92,93}. In the trachea, this corresponds to an increase in lumen diameter but not in length (which is apparently controlled by other mechanisms), whereas in the salivary gland both the lumen diameter and length are increased⁹². At roughly the same time an intra-luminal ECM, comprised of fibrils of the Polymer chitin, is assembled in the trachea. The synthesis and subsequent modification of chitin fibrils is thought to provide a mould over which the precise dimensions of the tube can be modelled (reviewed in REF.⁹⁴). The burst of exocytic traffic around this time controls some of the chitin-regulating enzymes, and thus likely controls some of the lumen-expansion mechanism. Subsequently, and in the case of the trachea before gas enters into the lumen, the lumen is rapidly cleared via endocytosis at the apical surface of tracheal cells⁹³. Perturbation of exocytosis or endocytosis halts the processes of lumen expansion and clearance, demonstrating that there is an integral role for membrane trafficking in modulating lumen morphogenesis. It remains unknown whether trafficking of chitin-modifying enzymes or of additional lumen-destined cargo is the most crucial contributor to lumen expansion. However, these studies raise the possibility that rather than responding to a pre-existing apical ECM, tubular epithelial cells may transiently generate their own apical matrix, which may act to regulate lumen expansion.

As there is no homologue of chitin in vertebrates, the extent to which these principles can be directly extended to mammalian tubular systems is unclear. Instead, secretion of other ECM molecules, such as proteoglycans into lumens, which is observed, for example, in the *D. melanogaster* retina, may play an analogous role^{82,83,95} (FIG. 4b). The potential participation of an apical matrix in lumen formation and expansion thus remains an attractive concept. Similarly, membrane-trafficking pathways to the apical surface will likely play a key role in lumen formation and tissue polarity.

Transcriptional control of lumen formation

Although we have begun to understand how TFs can promote the loss of polarized epithelial characteristics, how do transcriptional regulators promote the expression of genes that induce polarization of tissues? Some insight has come from studying *D. melanogaster* tubular epithelia, such as salivary glands, in which a network of TFs, including hairy, huckebein and ribbon, control expression of genes involved in apical polarity and luminal development, among other targets. These targets include components of the Crumbs complex (BOX 1), as well as the apical transport machinery, such as the molecular motor dynein and certain luminal ECM-modifying enzymes^{96,97}, all of which have varied roles in creating the apical luminal structure. Similarly, in the developing zebrafish gut, the TCF2 TF regulates the expression of certain TJ proteins and ion pumps⁸⁷, which regulate apical lumen expansion.

During branching of the *D. melanogaster* airways, the transcription factor Spalt (induced by Wnt–Wingless signalling) promotes expression of RAB11 and RIP11, two members of a membrane-recycling pathway that is involved in surface delivery of *D. melanogaster* E (DE)- cadherin complexes. RAB11 and RIP11 promote inter-cellular adhesion and the formation of lumens between two or more cells. Interestingly, repression of Spalt by the repressor Knirps (induced by TGF β –decapentaplegic signalling) in adjacent regions of the network results in downregulation of this cadherin-recycling pathway, promoting the formation of autocellular junctions and lumens in single cells⁸⁰. Notably, the RAB11–RIP11 complex is also involved in *D. melanogaster* retina development⁹⁸ (FIG. 4c), and recycling from these endosomes is regulated by the PAR6–CDC42 complex⁶², suggesting that diverse lumen-formation contexts are a core requirement for this pathway. Thus, in addition to transcriptional promotion or repression of junction proteins, polarity complexes and basement-membrane proteins, traditional ‘fate inducing’ signalling molecules, such as Wnt and TGF β , can induce tissue formation through the regulation of membrane-trafficking pathways. Further examination of the transcriptional control of membrane traffic, of which little is known, should yield enormous insights into the regulation of cell polarity and morphogenesis during tissue formation.

Conclusions and future prospects

Although much has been learned about how the polarity of individual cells is established and maintained, we are still in the early days of understanding how polarized cells are put together to make tissues. Communication between cells, both through cell–cell contact and via the ECM and diffusible factors, and using both chemical and mechanical signals, is at the heart of polarized tissue and organ formation.

Over 85% of fatal malignancies in adults in the USA arise from epithelial tissues⁹⁹, and loss of polarity is a hallmark of increased malignancy. The mechanistic bases for this connection are rapidly being elucidated, as is described in several recent reviews^{20,67,100}. Acute injury of major epithelial organ systems is collectively one of the most important causes of death worldwide^{101–103}. Understanding polarization of epithelia, therefore, is important in analysing the response of a tissue to acute injury and in generating prospects for regenerative medicine. Many organs, such as the kidney, lung and liver, can recover from even severe or acute injury, provided that the patient survives the initial insult. In the case of the kidney, at least, this involves the local proliferation of epithelial cells, which replace their dead neighbours in denuded regions of the tubules through a process that appears to involve a pEMT^{104,105}. Repeated injury, however, leads to a permanent EMT, whereby epithelial cells become fibroblastic and contribute to a fibrotic response, which ultimately destroys organ function¹⁰⁶. Learning how to improve the response to acute injury, as well as

how to avoid fibrosis and EMT after chronic injury, such as by controlling the polarity state of cells, offers enormous possibilities to enhance human health. As we begin to understand how polarization occurs and is controlled at the tissue level, we move closer to being able to translate such research potential into medical reality.

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Glossary

Basement membrane	A thin extracellular matrix layer that specifically lines the basal side of epithelial sheets, and certain other tissues, to which cells are attached. Also referred to as the basal lamina
Extracellular matrix	An extracellular scaffolding gel that consists of fibrous structural proteins, complex sugars, fluid and signalling molecules
Tight junction	A diffusion barrier-forming junction at the apical-most region of the lateral membrane of vertebrate epithelial cells
Design principle	A simple rule that increases the likelihood of the proper assembly and function of a system
Mesenchymal–epithelial transition	The <i>de novo</i> acquisition of epithelial characteristics, such as apico–basal polarity and epithelial-type junctions, by mesenchymal cells
Epithelial–mesenchymal transition	The transition of epithelial cells to a mesenchymal state by complete loss of apico–basal polarity, epithelial-type junctions, basement membrane and the adoption of migratory behaviours
Front–back polarity	A morphological characteristic, particularly in migratory cells, wherein the front (leading edge) and the back (uropod) show morphological and functional asymmetry
Polarity complexes	Conserved, multimeric protein complexes that promote and modulate the formation of asymmetric cellular architecture in diverse tissue types and organisms
Planar cell polarity (PCP)	The polarization of epithelial cells along the plane of the epithelium, orthogonal to the apico–basal axis, directing the orientation of cell shape, division, movement and differentiation. Non-epithelial cells can also exhibit PCP
Zero-order ultrasensitivity	A reversible system, such as phosphorylation, where modifying enzymes can become saturated with regard to the protein being modified, resulting in a switch-like movement of the substrate between modification states
Condensation	An event wherein non-adherent or loosely adherent cells can move together and tightly adhere to one another
Partial EMT	The transient adoption of some mesenchymal characteristics by epithelial cells without complete or permanent loss of the epithelial phenotype

Exocyst	A highly conserved, octameric protein complex that regulates vesicle docking and delivery to the cell surface
Hepatocyte growth factor (HGF)	A multipotent ligand, also known as scatter factor, for the c-Met receptor. HGF induces proliferation, scattering motility and branching morphogenesis in many epithelia
Transforming growth factor- β	Cytokine ligand that induces strong epithelial–mesenchymal transition in many epithelial cells and tissues
MDCK	Madin–Darby canine kidney cells. A polarized epithelial cell line that is commonly used for studies of polarity, membrane trafficking and cell adhesion
Guanine nucleotide-exchange factor	A protein that catalyses the exchange of GDP for GTP on GTPase proteins, thereby ‘activating’ the GTPase
GTPase-activating protein	Protein that catalyses hydrolysis of GTP to GDP on GTPase proteins, thereby ‘inactivating’ the GTPase
Evagination	The deformation of an epithelial sheet, without the loss of apico–basal polarity, such that part of the sheet extrudes into the extracellular matrix
Invagination	The deformation of an epithelial sheet, without the loss of apico–basal polarity, such that part of the sheet folds into the lumen of the tube
Radial tissue symmetry	The complimentary arrangement of cell polarity in a symmetric manner around a central line, such as the apical surfaces of cells in a biological tube
Cavitation	The formation of a lumen between a group of cells by apoptosis of inner cells that are not in contact with the extracellular matrix
Hollowing	The trafficking of vesicles containing apical membrane to a space between cells, or in a single cell, to form a lumen <i>de novo</i>
Membrane repulsion	Activation of a signalling cascade that promotes membranes between cells to de-adhere or that inhibits any attraction between membrane regions
Mammary end bud	The spherical end of a mammary tubule; referred to as an acinus when fully enclosed in 3D culture
COPI and COPII	Coatomer protein complexes that regulate anterograde (COPII) and retrograde (COPI) membrane transport between the endoplasmic reticulum and through cisternae of the Golgi complex
Autocellular junctions	The formation of junctional complexes in a single cell. They can be used to form a lumen in a single cell
Chain	The extension of one or more cells that have lost apico–basal polarity from an epithelial sheet into the extracellular matrix without losing cell–cell adhesion or becoming multilayered
Cord	Similar to the formation of a chain, but comprised of multilayering of cells that may contain some disconnected luminal structures. Can build on successful chain extension

Polycystic kidney disease	A group of diseases that cause focal dilation of kidney tubules resulting in the formation of large cysts and severely compromised renal function
Vascular stenosis	A pathological vascular condition that involves the narrowing of blood vessels and that results in hypoperfusion of tissues
Septate junction (SJ)	An invertebrate cell–cell junction, localized to the mid-lateral membrane region. Like vertebrate tight junctions (TJs), SJs provide a paracellular diffusion barrier. Unlike TJs, SJs contain basolateral, rather than apical, polarity determinants
Chitin	A polysaccharide that consists of <i>N</i> -acetylglucosamine, the polymer of which is a primary component of insect cytoskeletons

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Figure 1. Cell polarization in diverse tissue types

a | Epithelial tubes are comprised of tightly adhering cells that display strong apico–basal polarity. Lateral membranes possess desmosomes, adherens junctions and tight junctions (TJs), providing cell–cell adhesion and diffusion barriers. Basal membranes interact with underlying basement membrane and extracellular matrix (ECM). Apical membranes are specialized for absorption and secretion, such as for electrolytes, milk or O_2 . **b** | Neurons polarize to form a soma (cell body), an axon shaft, an axon terminal and dendrites. Neural synapses contain adhesion molecules for stabilization of the interaction between cells. The synapse provides a specialized region for neurotransmission to occur, through polarized targeting and uptake of neurotransmitters. **c** | The *Drosophila melanogaster* retina contains ommatidia made up of tubular neuroepithelia surrounding a central lumen (or

interrhabdomeral space (IRS)). Cells are not radially symmetric in the tube but nevertheless follow a defined, polarized pattern. They have typical epithelial junctions (zonula adherens at the most apico–lateral region in *D. melanogaster*) and a subapical actin-dense network (the rhabdomere terminal web), but their apical surfaces are specialized into two domains; the stalk membrane and the light-sensing rhabdomeres, which are specialized microvilli.

Figure 2. EMT and MET in tissue morphogenesis

a | Epithelial cells from tubes undergoing epithelial–mesenchymal transition (EMT) lose apico–basal polarity, downregulate cell–cell adhesion, change their cytoskeleton composition and invade into the extracellular matrix (ECM) at areas where the basement membrane has broken down²¹. As cells adopt a mesenchymal state, they may also be polarized, displaying front–back polarization and Golgi orientation towards the leading edge (front) during migration^{112,116}. Conversely, during mesenchymal–epithelial transition (MET), cells develop apico–basal polarity, express epithelial-specific proteins, form stable adhesions and generate luminal structures. **b** | Transforming growth factor- β (TGF β) (and other morphogens) can induce EMT by inducing Snail and the ZEB family of transcription factors (which can also crosstalk)²⁹. Snail and ZEB directly repress the expression of numerous proteins that are involved in epithelial polarization, including polarity complexes^{30,31}, cell–cell junctions²⁹, the ECM³³ and the cytoskeleton³². Snail-1 can also alter membrane trafficking, such as by direct repression of RAB25 (REF. ³²), a small GTPase that is involved in apical membrane trafficking¹³⁰ (although, paradoxically, RAB25 is overexpressed in some tumour types¹³¹). The miR-200 family of microRNAs promotes epithelial polarity and can induce MET by inhibiting translation of ZEB1, thereby blocking induction of EMT^{35,36}.

Figure 3. Cavitation, hollowing and membrane repulsion as lumen-forming mechanisms

a | During cavitation, groups of cells proliferate to form a cell mass. Apoptosis of non-extracellular matrix (ECM)-contacting inner cells results in lumen and polarized tube or cyst formation. Correct lumen formation requires the PAR and Scribble complexes, which modulate cell proliferation⁶⁸. Apoptosis requires BCL2-family members (BCL2 and BIM) and is inhibited by proliferation-inducing oncoproteins (ERBB2, cyclin D1 and HPV16 E7), causing luminal filling^{24,65,66}. **b** | During hollowing, polarity establishment and orientation requires laminin-β1-integrin to signal RAC1 (REFS^{12,37}), and is inhibited by RhoA-ROCK1 signalling³⁸. Intracellular vesicles (varying in size in different systems⁷¹) containing apical membrane components and endocytosis- and/or *trans*-Golgi-derived material are delivered to regions between cells^{46,69,70}. This delivery depends on PTEN-mediated segregation of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) to nascent apical regions, recruiting annexin-2 and, in turn, the CDC42-aPKC-PAR6 complex^{46,73}. Once rudimentary lumens are formed (there may be multiple such lumens), tight junctions^{87,132,133}, pump proteins^{87,134}, the Crumbs complex^{133,135}, the exocyst (Sec10 (REF.¹³⁶)) and possibly ezrin^{137,138} promote formation of a single, expanded lumen. **c** | During *Drosophila melanogaster* cardiac tube formation, two rows of myoendothelial cells line up along the midline. Membrane processes extend and join between cells on either side of the midline, first at the ventral-most and then at the dorsal-most regions between cells. Resultant junctions containing PAR3 (Bazooka (Baz)), DE-cadherin (Shotgun (Shg)), β-catenin (Armadillo (Arm)) and Ena allow an enclosed lumen. Slit signalling to Robo at lumen surfaces, apparently regulated by dystroglycan (Dg), prevents extended adhesive contacts between cells (membrane repulsion), allowing lumens to form^{74,75}.

Figure 4. Membrane traffic and apical extracellular matrix secretion during lumen formation and expansion

a | In the embryonic *Drosophila melanogaster* trachea an initially narrow lumen is expanded during a rapid burst of Molecular Cell Biology secretion at the apical membrane, dependent on the COPI and COPII vesicle transport complexes^{92,93}. Chitin fibrils in the lumen (made by chitin synthetases) signal to underlying cells to organize luminal diameter before being subsequently remodelled into tracheal cuticle (reviewed in REF. ⁹⁴). Clathrin-dependent endocytic activity at the apical surface directs luminal material to early endosomes (EE) and then to late endosomes (LE) for degradation, thereby clearing the lumen for gas entry⁹³. Correct luminal secretion and expansion requires functional septate junctions⁹⁴. **b** | Part of the *D. melanogaster* airway is divided into the dorsal trunk and dorsal branches, which sprout from the trunk. Wingless (Wg) signalling in the trunk induces the Spalt (SAL) transcription factor, which promotes DE-cadherin recycling through induction of the RIP11–RAB11 membrane-trafficking complex; lumens are formed by intercellular junctions between multiple cells. Decapentaplegic (Dpp) signalling in branches inhibits SAL expression, through the repressor Knirps (KNI), downregulating the RIP11–RAB11 complex and leading to formation of autocellular junctions and lumens in individual cells⁸⁰. **c** | During ommatidium formation in the *D. melanogaster* retina, a tripartite complex of RAB11, RIP11 and the motor protein myosin V (MyoV) regulates transport of cargo to the rhabdomere, and is required for correct ommatidia organization⁹⁸. RAB11 interacts with the exocyst complex, which also regulates apical transport to the rhabdomere¹³⁹. Luminal secretion of the proteoglycan eyes shut (EYS) is required for expansion of the inter-rhabdomeral space (IRS), apparently occurring in an exocyst-independent manner⁹⁵. ZA, zonula adherens.

Table 1

Selected components involved in apical polarity and lumen formation

Component	Function	Model system	Loss-of-function phenotype	Refs
Polarity components				
PAR complex (PAR6–aPKC)	AP polarity	Zebrafish gut; MDCK cyst	Multiple lumens; accumulation of luminal apoptotic cells	46,73,87
Crumbs complex (Crumbs-3–PATJ)	AP polarity	MDCK cyst; <i>Dm</i> salivary gland	Multiple lumens, disrupted apico–basal polarity; distended luminal size	97,133,135
Scribble	BL polarity	MCF-10A cyst	Lumen filling phenotype	68
Rho GTPases				
CDC42 (also ECT2)	Rho GTPase CDC42 GEF;	Embryoid body; MDCK cyst	Disrupted polarity; multiple lumens	46,48,140
RAC1	Rho GTPase	MDCK cyst	Inverted apico–basal polarity	12,37
Apical transport				
Annexin-2 or -13	Apical transport	MDCK cyst	Multiple lumens	46,133
ARL3	Arl GTPase	<i>Dm</i> trachea	Inability to fuse lumens between branches	141
Eyes-closed (EYC)	SNARE-complex modulator	<i>Dm</i> photoreceptor	No lumen expansion	141
Exocyst (SEC5–SEC6)	Vesicle transport	<i>Dm</i> photoreceptor	Abnormal apical transport	139
FAPP2	Apical transport	MDCK cyst	Abnormal lumen expansion	142
RAB8	Apical transport	Mouse intestine, <i>Ce</i> intestine	Gross defect in apical organization	143
RAB11 complex (RAB11–RIP11–MyoV)	Vesicle recycling	<i>Dm</i> photoreceptor; MDCK cyst	Abnormal apical transport, ectopic rhabdomere formation; multiple lumens	98,144
Syntaxin-2 or -3	t-SNARE(s)	MDCK cyst	Disrupted apico–basal polarity, small cysts	133,145
VIP17, galectin-3	Apical transport	MDCK cyst	Multiple lumens	133
Apical ECM/cytoskeleton				
Chitin synthetases and deacetylases	Luminal ECM	<i>Dm</i> trachea	Distended luminal size	94
EPS-8	Actin regulation	<i>Ce</i> intestine	Distended luminal size	146
Ezrin	Membrane–actin crosslinker	<i>Ce</i> intestine; mouse intestine	Distended luminal size; multiple lumens	137,138
Eyes shut (EYS)	Proteoglycan	<i>Dm</i> photoreceptor	No lumen expansion	95
Piopio	Luminal protein	<i>Dm</i> trachea	Inability to fuse lumens between branches	83
Slit–Robo– dystroglycan	Repulsive complex	<i>Dm</i> heart tube	No lumen formed	74,75
Junction proteins				
E-cadherin	Cell adhesion	MDCK cyst	Lumen filling, abnormal lumens	144,147
Na ⁺ /K ⁺ -ATPase	Ion pump	<i>Dm</i> trachea; zebrafish gut	Distended luminal size; multiple lumens	87,90

Component	Function	Model system	Loss-of-function phenotype	Refs
TJs (APG2, claudins, JAM-A, ZO-1)	TJ components	Zebrafish gut; <i>Dm</i> trachea; MDCK cyst	Multiple lumens; disruption to lumen dimensions; disrupted apico-basal polarity	87,132,148,149
Other				
β 1-integrin	ECM signalling	MDCK cyst	Inverted apico-basal polarity	12
BIM, BCL2	Apoptosis	Mouse mammary gland, MCF-10A cyst, MDCK cyst	Accumulation of cells in lumen	47,65,66
CFTR	Chloride channel	Mouse model of PKD, MDCK cyst	Reduced lumen size	89
PTEN	Lipid phosphatase	<i>Dm</i> photoreceptor; MDCK cyst	Abnormal rhabdomere morphogenesis; multiple lumens	46,115
Podocalyxin	Sialomucin	MDCK cyst	No lumen expansion	76

AP, apical; BL, basolateral; *Ce*, *Caenorhabditis elegans*; CFTR, cystic fibrosis transmembrane conductance regulator; *Dm*, *Drosophila melanogaster*; ECM, extracellular matrix; GEF, guanine nucleotide-exchange factor; MDCK, Madin-Darby canine kidney; PKD, polycystic kidney disease; TJ, tight junction.