OPINION

Building epithelial architecture: insights from three-dimensional culture models

Lucy Erin O'Brien, Mirjam M. P. Zegers and Keith E. Mostov

How do individual cells organize into multicellular tissues? Here, we propose that the morphogenetic behaviour of epithelial cells is guided by two distinct elements: an intrinsic differentiation programme that drives formation of a lumen-enclosing monolayer, and a growth factor-induced, transient de-differentiation that allows this monolayer to be remodelled.

Multicellularity has its advantages. By functioning as an organized community, cells can exploit resources and carry out functions that would be impossible as single cells. Nature is replete with examples of cell communities, from the simple colonies of the unicellular organism *Volvox* to the highly complex structure of the mammalian kidney, which comprises more than 20 cell types and more than one million nephrons. Reaping the benefits of multicellularity requires that cells assemble into a specific architecture. A single cell in isolation can show many behaviours — proliferation, migration, adhesion, polarization, differentiation and death. But to build a tissue, a population of cells must coordinate these behaviours across space and time.

Little is understood about the mechanisms that orchestrate the actions of single cells during tissue morphogenesis, largely owing to the barriers that are inherent in carrying out such analyses *in vivo*. For epithelia, the alternative approach of three-dimensional (3D) cell culture can provide insight into these issues. Here, we integrate many of the key findings from 3D culture into a working model for how extracellular factors interact with an intrinsic differentiation programme to specify the architecture of epithelial tissues.

Building epithelial architecture

Epithelia are coherent sheets of cells that form a barrier between the body interior and the outside world. These sheets can be one-cell thick in the case of simple epithelia, or many cells thick for stratified epithelia. Some epithelia cover the outside of the organism, whereas others line internal organs. These internal epithelial organs typically contain two types of building blocks, cysts and tubules (FIG. 1). Cysts — also known as acini in the mammary gland, alveoli in the lung and follicles in the thyroid — are spherical monolayers of cells that enclose a central lumen. Tubules are also lumen-enclosing monolayers, but are cylindrical instead of spherical. Combining cysts and tubules can produce complex structures such as the vertebrate lung — a network of branching bronchiolar tubules that terminates in alveolar cysts.

Embryological and genetic studies have yielded much information on the growth factors, growth-factor receptors and transcription factors that control the development of epithelial organs such as the kidney and lung[1–3]. Concomitantly, studies of epithelial cell lines that are grown on two-dimensional filter supports have provided key insights into how the subcellular structure of epithelial cells is established and maintained[4,5]. Between these two approaches lies fertile ground for the cultivation of a cell biological perspective on epithelial development. In particular, how are the actions of individual cells coordinated to form cysts and tubules during morphogenesis?

Three-dimensional culture is a powerful tool for investigating the molecular signals that specify epithelial architecture. The ability of epithelial cells to reproduce a tissue-like organization when grown inside a 3D extracellular matrix (ECM) has been long appreciated, particularly due to the work of Bissell and colleagues[6,7]. For example, Madin–Darby Canine Kidney (MDCK) cells form cysts when embedded in a collagen type I matrix (FIG. 2). Like simple epithelial tissues, MDCK cysts are polarized monolayers that enclose a lumen and are encircled by a basement membrane. Moreover, these cysts develop branching tubules when exposed to mesenchymally derived hepatocyte growth factor (HGF)[8,9], a response that is reminiscent of the epithelial–mesenchymal interactions that stimulate

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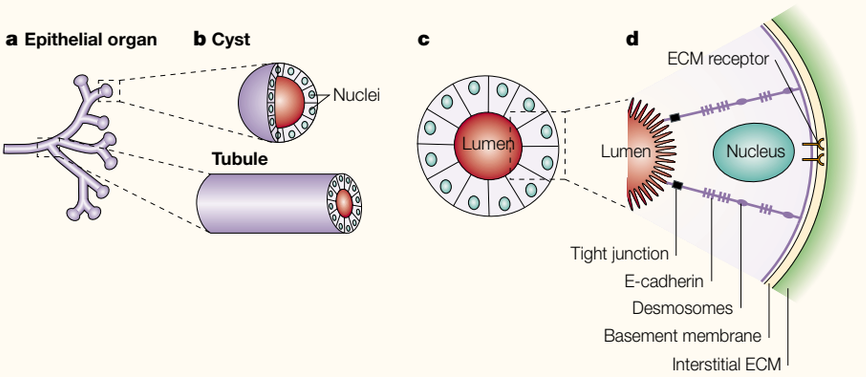

Figure 1 | **The building blocks of epithelial organs.** Internal epithelial organs (**a**) consist of two basic building blocks: cysts and tubules (**b**). **c** | In cross section, these building blocks are lumen-enclosing monolayers of polarized cells. Each cell in the monolayer has a microvilli-rich, free apical membrane domain that faces the lumen, a lateral membrane domain that faces neighbouring cells, and a basal membrane domain that faces the basement membrane (**d**). The basement membrane, a specialized extracellular matrix (ECM) that is produced by epithelial cells, forms a layer between the epithelium and the mesenchymally derived interstitial ECM. Cells attach to ECM through integrin and non-integrin receptors and bind to neighbouring cells through tight junctions, desmosomes and homotypic E-cadherin interactions. Tight junctions also demarcate the boundary between the apical and lateral surfaces.

tubulogenesis *in vivo*. Although 3D culture has limitations — for example, it is not amenable to forward genetic analysis — its relative simplicity and tractability make it ideally suited to studying the morphogenesis of epithelial structure.

Indeed, 3D studies have begun to determine the mechanistic basis of cellular behaviour during cyst and tubule development. Here, we incorporate these findings into a two-part working model for epithelial morphogenesis: first, that the generation of free, lateral and basal surfaces propels the formation of polarized, lumen-enclosing epithelial monolayers; and second, that morphogens such as HGF induce tubular growth by causing cells in these monolayers to transiently de-differentiate. We propose that these concepts could provide insight into the more complex development of epithelial organs.

Three surfaces are the charm

Although differently shaped, cysts and tubules are topologically equivalent, as both are self-enclosed monolayers. Each cell in a fully developed cyst or tubule has three types of plasma membrane surface: a free, apical surface that borders the lumen; a lateral surface that adheres to neighbouring cells; and a basal surface that adheres to the ECM (FIGS 1,2). Because many proteins localize to both the basal and lateral surfaces, these surfaces are often collectively referred to as 'basolateral'. Cells in developing structures often lack one or more surface types; for example, the basal surface might be absent from some cells that are multilayered, whereas other cells might have no free surface because they do not border a lumen (FIG. 3). Cells migrate, proliferate, or form lumens to attain the three surface types; if this is unfeasible, they apoptose. We propose that the pursuit of three surfaces is both intrinsic to epithelial differentiation and fundamental to epithelial architecture. Specifically, the geometric constraint that each cell in a structure has a free, lateral and basal surface results in a self-enclosed monolayer (FIG. 3).

If the pursuit of three surface types drives epithelial morphogenesis, then an intriguing question arises: how does each cell ensure that it has each surface type? We anticipate that at least two components are involved — surface detection and surface generation.

Detecting surfaces. Surface detection requires cells to distinguish three extracellular environments: the ECM, adjacent cells and lumenal fluid. Matrix detection probably requires ECM receptors, such as integrins and dystroglycan. Indeed, abrogating expression of the collagen receptor α2 integrin caused MDCK cysts to apoptose at the one-cell stage, presumably because they could not detect the surrounding matrix[10]. Both integrin- and dystroglycan-receptor complexes contain cytoskeleton-interacting components, which thereby provides a means to signal the presence of ECM to the cell[11,12].

The epithelial junctional complex probably detects neighbouring cell surfaces. This complex comprises tight junctions, adherens junctions and desmosomes, each of which is able to signal through a combination of transmembrane receptors and cytoskeletal linker proteins. Whether one particular junction uniquely detects adjacent cells, or several junctions share this role, is unknown but, at the least, adherens junctions are not solely responsible for lateral surface detection, as MDCK cysts that lack functional cadherins develop normally[13]. The roles of tight junctions and desmosomes in 3D morphogenesis have not yet been directly examined.

In contrast to basal and lateral surfaces, nonadhesive lumenal surfaces seem to be signified by an absence of receptor signalling. The collagen receptor α2β1 integrin is primarily basolateral in MDCK cells, but there is also a small, crucial pool at the free apical surface[14]; the α2β1 integrins are normally inactive here, though, as collagen is absent at free surfaces. Overlaying the free surface with collagen activates the apical integrins and causes cells to create new free surfaces, whereas blocking apical integrins abrogates new lumen formation[14–16]. These findings imply that cells use the activation state of apical integrins as an indicator of free-surface status, and that preventing integrin activation renders cells unable to detect changes at the free surface. A consequence of this idea is that integrin signals that originate from different regions of the cell elicit different responses, as basal integrin activation — unlike apical integrin activation — does not effect lumen formation.

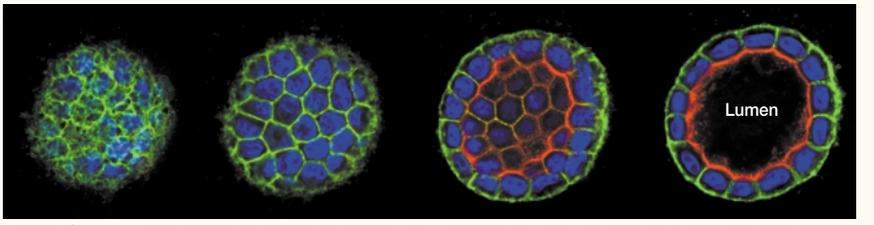

Figure 2 | **MDCK cyst morphology mimics epithelial organization *in vivo*.** Serial confocal sections taken at 5 μm intervals through a cultured MDCK cyst. The cyst is a spherical monolayer of polarized cells that encloses a central lumen. The apical membrane domain (red) is stained for actin, which is enriched in apical microvilli. The basal and lateral domains (green) are labelled using an antibody against p58, a basolateral antigen of unknown function. The nuclei appear blue. MDCK, Madin–Darby Canine Kidney.

Recent evidence might also implicate the Par-1 kinase in free-surface detection. First identified as a polarity regulator in *Caenorhabditis elegans*[17], Par-1 stimulates the formation of multiple, ectopic lumens when overexpressed in MDCK cysts or monolayers (D. Cohen, P. J. Brennwald, E. Rodriguez-Boulan, and A. Müsch, personal communication). Such excessive lumen formation is suggestive of a block in free-surface detection, although whether this phenotype is primary or secondary to Par-1 overexpression remains to be determined.

Generating surfaces. Surface-detecting mechanisms would relay information about the extracellular environment to the cytoplasm, where they must interface with surface-generating mechanisms to induce formation of missing surface types. Classic 3D-culture studies provide compelling evidence for such crosstalk by showing how cysts accommodate changes in their three-surface status through extensive cellular remodelling. When grown in liquid suspension culture, thyroid and MDCK cells form cysts with the free surface on the outside, in contact with the medium[18,19]. The suspension-grown cysts generate basal surfaces *de novo* by creating internal cavities and filling them with basement membrane. If these inverted cysts are then embedded in collagen — which simultaneously eliminates their free surfaces and provides another source of ECM — the cysts create an interior lumenal free space by degrading the centralized basement membrane[18,20]. When this degradation is inhibited pharmacologically, the cysts, undeterred, create free surfaces by forming lumens between adjacent cells[21]. The plasticity of the response of the cysts to changes in their three-surface status implies that there is a hard-wired epithelial programme to monitor and generate surface types.

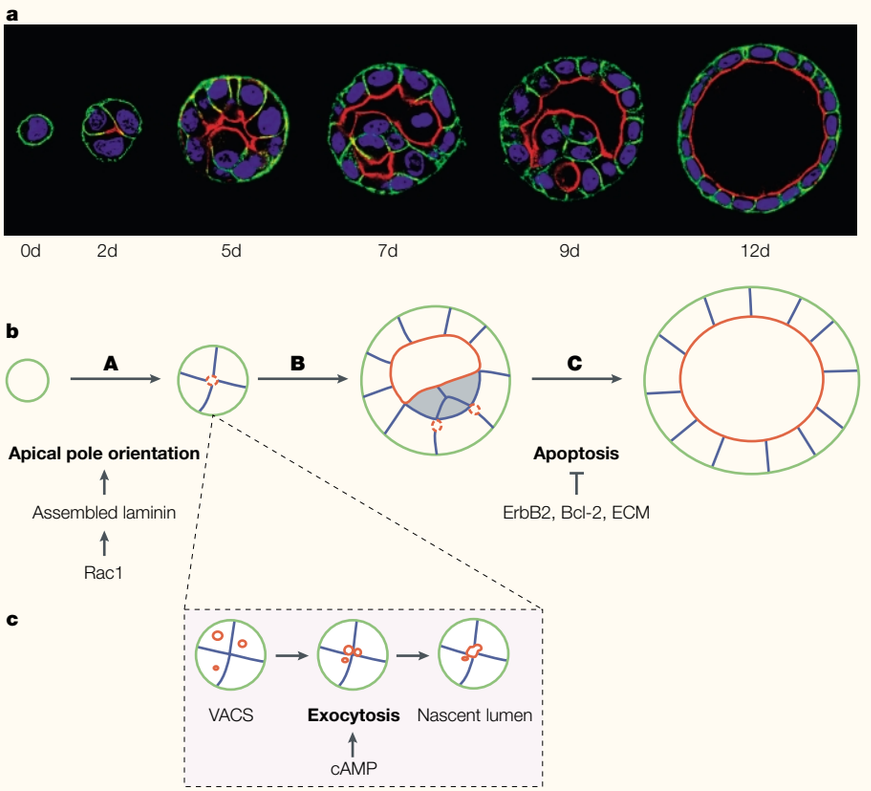

Figure 3 | **Cyst development and the generation of three membrane surfaces. a** | Single epithelial cells that are embedded in extracellular matrix (ECM) proliferate to form clonal cysts. Madin–Darby Canine Kidney (MDCK) cysts at 0, 2, 5, 7, 9, and 12 days (d) of development are shown. The lumenal apical domain is stained for actin (red), basal and lateral domains are stained for p58 (green), and the nuclei appear blue. Although each cell in the mature 12-day cyst has a free, lateral and basal membrane surface, cells in immature cysts often lack at least one surface type. Note the apoptotic nuclei in the lumen of the 7-d cyst. **b** | Proposed model of polarity orientation and lumen formation during cyst development. **A** | Early in cyst development, cell surfaces are predominantly lateral (blue) and basal (green). A nascent free surface (red) is present. Cells assemble laminin in the basement membrane through a Rac1-mediated process. Assembled laminin then directs orientation of the apical domain. **B**,**C** | Cystogenesis continues until each cell has a free, lateral and basal surface. Lumenal expansion involves apoptosis of cells without ECM contact (grey). Apoptosis is inhibited by ErbB2, Bcl-2 or ECM contact. **c** | Exocytosis of vacuolar apical compartments (VACs) might initiate lumen formation through membrane separation.

Creating space

Studies in 2D culture have identified the mechanisms that cells use to create lateral and basal surfaces[22], and similar mechanisms probably operate in 3D culture. Much less is understood about how free surfaces develop, as cells do not form lumens in 2D culture. However, key insights into this essential process have emerged from collagen overlay experiments and, quite recently, from 3D-culture studies.

These studies show that cells create lumenal space through at least two mechanisms: membrane separation and apoptosis (FIG. 3).

Membrane separation. Separation of apposing membranes is sufficient to form intercellular lumens in small colonies of MDCK cells that are overlaid with collagen[23], and also contributes to lumen formation during thyroid-cyst development in 3D culture[24]. Although the molecular events that cause adherent membranes to separate are unknown, membrane detachment might involve steric hindrance of cell–cell adhesion by large transmembrane glycoproteins such as the mucin MUC1. Anti-adhesive proteins might be delivered to the nascent lumenal surface by exocytosis of a specialized organelle, the vacuolar apical compartment (VAC)[25]. VAC membranes resemble the lumenal plasma membrane, complete with microvilli and apical markers, and strong evidence implicates VAC exocytosis in endothelial lumen formation[26,27]. The role of VACs in epithelial lumen formation, however, is more controversial. Epithelial VACs have been observed in culture and *in vivo* under particular circumstances[25,28–30], but their presence is not widely reported in the literature. One proposed explanation for this inconsistency is that apical-surface delivery ordinarily involves short-lived carrier vesicles that are difficult to detect, and that large, easily detected VACs arise through homotypic fusion of these carriers when plasma membrane delivery is blocked. Indeed, certain experimental conditions that are known to interfere with vesicular transport, such as low concentrations of calcium or microtubule disruption, are also known to induce VACs[25,29]. Alternatively, it is possible that VACs are a nonphysiological response to experimental manipulation and

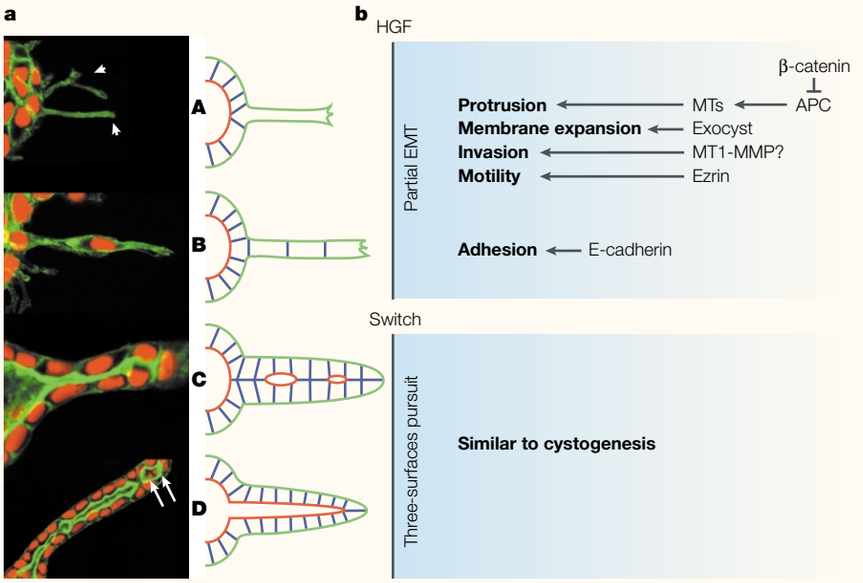

Figure 4 | **Tubulogenetic mechanisms in MDCK cells. a** | MDCK tubules at different stages of development, stained for nuclei (red) and actin (green). In cartoons, blue represents cell–cell contacts, green represents cell–matrix contacts, and red represents free membrane surfaces. **A** | After treatment with hepatocyte growth factor (HGF), new tubules initiate when single cells become invasive and project a long, basal-membrane extension into the extracellular matrix (ECM) (arrowheads). **B** | Cells proliferate to form single-file chains. In addition to being invasive, cells in chains have lost apico–basolateral polarity. Cell–cell contact is dramatically reduced. **C** | Cells form multilayered cords, thereby restoring lateral membrane contacts. Apical polarity is re-established, as shown by intense actin staining at the interface between cell layers, and new lumen formation is initiated. **D** | Lumenal enlargement provides each cell with a free surface. Note apoptotic nuclei in the developing lumen (arrows). Tubulogenesis is complete when each cell has free, lateral and basal surfaces. **b** | Proposed model of cellular regulation during HGF-induced MDCK tubulogenesis. Tubulogenesis involves an initial HGF-dependent phase and a subsequent HGF-independent phase. HGF temporarily suppresses the three-surfaces pursuit and induces a partial epithelial–mesenchymal transition (EMT) that causes cells to form extensions and chains. Selected molecules implicated in HGF-induced EMT are listed on the right. A morphogenetic switch occurs after chain formation, in which HGF signalling is downregulated and cells resume the three-surfaces pursuit. This redifferentiation phase promotes development of chains into cords and mature tubules. In many respects, redifferentiation resembles cyst development, which occurs in the absence of HGF. MDCK, Madin–Darby Canine Kidney.

do not participate in normal lumen formation. Careful work is needed to resolve this issue.

Apoptosis. Larger structures use apoptosis to eliminate cells in the lumenal space. The link between apoptosis and lumen development was first recognized for cavitation of the vertebrate embryo[31] and also applies to cultured cysts[32,33]. Lumenal cells lack a basal surface, as they do not contact ECM; the absence of matrix adhesion probably makes them susceptible to cell death. Recent 3D studies using MCF-10A mammary cells have begun to identify the molecules that control lumenal apoptosis. Specifically, inappropriate activation of the ErbB2 tyrosine kinase receptor in MCF-10A cysts causes repopulation of the lumenal space through resistance to death and increased proliferation[34]. By comparison, overexpression of cyclin D1 or the E7 oncoprotein from Human Papilloma Virus 16 does not effect lumenal filling — despite increased proliferation — because cyclin D1 and E7 do not protect cells from apoptosis (J. S. Debnath, K. Mills, N. Collins and J. S. Brugge, personal communication). Interestingly, suppression of apoptosis through Bcl-2 overexpression delays, but does not eliminate, lumen formation in both MCF-10A and MDCK cysts, which indicates that other space-forming mechanisms are able to compensate[32] (J. S. Debnath, K. Mills and J. S. Brugge, personal communication).

Polarity in a multicellular context

In conjunction with generating three distinct membrane surfaces, cells must target specialized proteins and subcellular structures to the appropriate surface. The polarity of each individual cell must be coupled to the overall organization of the tissue. Cells in epithelial tissues invariably orient their apical poles towards the lumen and their basolateral poles toward the ECM. How is this coordination achieved?

One attractive solution is for pole placement to be guided by the structural features of the tissue. Indeed, the epithelial basement membrane, which has long been recognized to be essential for proper morphogenesis[35–37], seems to direct apical polarization (FIG. 3). The basement membrane is a laminin-rich, polymeric network that is assembled by cells at the cyst–ECM interface. In MDCK cysts, proper apical-pole orientation requires assembled laminin[38]. Disrupting laminin assembly by expressing a dominant-negative form of the small GTPase Rac1 causes a striking inversion of the apical pole, and addition of an excess of exogenous laminin rescues apical polarity.

Although MDCK cells synthesize their own laminin, some epithelia rely on laminin that is provided by other cells. In the mammary gland, myoepithelial cells surround lumenal epithelial cells to create a double-layered acinar structure. The lumenal epithelial cells do not make laminin, but instead use laminin from the myoepithelial cells for basement-membrane assembly and apical orientation[39]. This collaboration between two adjacent cell types compellingly shows the interconnection between tissue structure and polarity orientation.

Tubulogenesis at the cellular level

More than a decade ago, Montesano, Schaller and Orci[9] showed that MDCK cysts form tubules in response to HGF, which established the model that epithelial tubulogenesis involves the inductive activity of mesenchymal growth factors. Many epithelial lines have since been shown to tubulate in 3D culture after treatment with growth factors. Much progress has been made with regard to signalling downstream of the HGF receptor Met and related receptor tyrosine kinases; this field has been reviewed elsewhere[40] and will not be discussed here. By contrast, comparatively little is understood about how these signalling pathways lead to the cellular behaviours that constitute tubulogenesis.

HGF-induced MDCK tubulogenesis is the best understood of the 3D-tubulogenetic systems, yet the precise involvement of HGF during kidney organogenesis is ambiguous. Although the kidneys of *HGF*-knockout embryos seem normal[41,42], this phenotype is at odds with different lines of evidence that implicate HGF in renal development. For example, the temporal and spatial expression patterns of HGF, Met and an HGF-activating protease in the metanephric mesenchyme and

ureteric bud indicate that they might be important[43–46], and HGF-blocking antibodies inhibit morphogenesis of kidney explant cultures[44,45]. Despite the uncertain role of HGF *in vivo*, the HGF–MDCK culture model is a powerful tool for exploring the cell biology of tubule formation. Its strength stems from the use of just one well-characterized cell line and one well-characterized growth factor, which allows a reductionist approach to identify the common principles that probably underlie tubulogenesis in various contexts. More recently, Nigam and colleagues[47] have developed a promising 3D-tubulogenetic system using immortalized ureteric bud cells. It will be interesting to compare morphogenesis of this embryonically derived cell line to that of MDCK cells, which originated from the mature kidney.

Detailed analysis of the HGF–MDCK system has shown that tubule development proceeds through four distinct stages[48] (FIG. 4). In the first stage, a single 'pioneer' cell sends a long cytoplasmic extension into the surrounding matrix. Second, the extension becomes a single-file chain of cells that extends radially from the cyst body. Third, the chain of cells evolves into a multilayered cord. Nascent, discontinuous lumens are often visible in these cords. Finally, cells in the cord migrate, proliferate, expand the lumenal surface and, in some cases, apoptose, until each cell in the tubule has free, lateral and basal surfaces. At this point the tubule is fully mature.

How does HGF transform spherical cysts into elongated tubular networks? As cysts and tubules are topologically equivalent, the three-surface hypothesis proposed earlier would apply equally well to the development of cysts and tubules. HGF must somehow interact with this three-surfaces drive to promote a tubular instead of a spherical architecture. Here, we argue that tubulogenesis comprises two distinct phases: HGF-dependent tubule initiation and HGF-independent tubule maturation. We speculate that HGF initiates new tubule development by inducing a partial, transient epithelial–mesenchymal transition (EMT) that temporarily overrides the drive for three surfaces. EMT would cause cells to migrate out of the cyst wall and invade the surrounding matrix during extension and chain formation. We further predict that, after chain formation, downregulation of HGF signalling causes cells to cease EMT, redifferentiate and resume the three-surfaces pursuit. This switch would allow chains to progress to cords and, ultimately, to mature tubules.

One appeal of this biphasic model is that it offers a simple explanation for how HGF promotes tubular instead of spherical development; namely, by altering the cellular unit that proliferates into a self-enclosed monolayer (FIG. 5). Without HGF, this unit is merely a single cell, which develops into a radially symmetrical cyst. HGF transforms this unit to a linear chain, which develops into a cylindrical tubule. The elongated shape of the tubule is a reflection of the elongated shape of the chain.

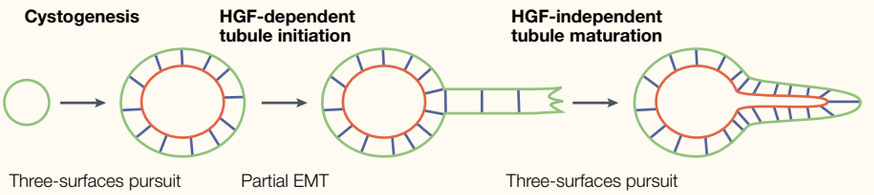

Figure 5 | **The drive for three surfaces and EMT specify epithelial architecture.** A hard-wired epithelial differentiation programme to acquire free, lateral and basal membrane surfaces acts during cystogenesis and later stages of tubulogenesis to determine the formation of a self-enclosed monolayer. Whether this monolayer is spherical or tubular depends on cellular geometry at the onset of the three-surfaces pursuit; single cells give rise to spherical cysts, and elongated chains give rise to cylindrical tubules. Hepatocyte growth factor (HGF) promotes tubular instead of spherical development by inducing chain formation through transient, partial EMT (epithelial–mesenchymal transition).

Transient EMT: getting out there

EMT is a recurring theme in embryogenesis and pathogenesis. To list just a few examples, cells undergo EMT during gastrulation, neural-crest-cell migration and malignant progression of certain epithelial tumours[49]. EMT can occur in response to signalling from growth factors or oncogenes and is marked by the transformation of polarized, noninvasive epithelial cells into nonpolar, invasive mesenchymal cells that break through the basement membrane and invade the interstitial ECM.

In HGF-induced MDCK tubulogenesis, the first manifestation of EMT is the migration of long cytoplasmic extensions into the matrix[9,48]. Extensions can reach several cell diameters in length and are dynamic, repeatedly extending and contracting in a manner that is reminiscent of neuronal growth cones (L.E.O. and K.E.M., unpublished observations) (see online movie). At this point, the extending cell still has three surface types and apico–basolateral polarity is maintained, with the extension being part of the basal domain[48].

The molecular links between HGF signalling and extension formation are beginning to emerge (FIG. 4). One clear downstream target of HGF is the cytoskeleton, which must rearrange dramatically to create extensions. Here, protrusive force might derive from microtubule remodelling by the adenomatous polyposis coli (APC) protein and β-catenin. In particular, β-catenin-deletion mutants that stably bind APC dramatically reduce extension number and length, perhaps by preventing APC from stabilizing microtubules[50,51]. Other cytoskeletal proteins such as ezrin and villin might also be involved (R. Athman, D. Louvard and S. Robine, personal communication)[52]. Another potential target of HGF is the basal plasma membrane, which expands to accommodate the growing extension. This expansion might be fuelled by directing vesicle transport to the site of extension development, which is analogous to the directing of vesicles to the tip of the growing bud in *Saccharomyces cerevisiae*. Intriguingly, the Exocyst (Sec6/Sec8) complex controls targeting to both the yeast bud tip and the MDCK basolateral membrane[53,54], and extension formation is enhanced in MDCK cells that overexpress the Exocyst subunit Sec10 (REF. 55). Matrix invasion probably requires the activity of proteases, such as the membrane-anchored metalloproteinase MT1-MMP[56]. Indeed, overexpression of MT1-MMP confers invasive behaviour to MDCK cells in the absence of HGF, whereas overexpression of soluble MMPs does not[57]. These findings indicate that matrix degradation must be focused to the leading edge of the migrating cell.

Chains represent a more advanced stage of EMT. In addition to being invasive, cells in chains have no free surface and no apico–basolateral polarity[48]. Cell proliferation accompanies chain formation, and it is tempting to speculate that outward growth of the chain involves HGF-induced repositioning of the mitotic spindle. Normally, dividing MDCK cells orient their spindles parallel to the axis of the monolayer[58]. In chains, dividing cells might orient their spindles perpendicularly, such that new daughter cells contribute to chain elongation. Parallel and perpendicular mitotic axes are specified by APC/EB-1 and Par-3–Par-6, respectively, in the embryonic

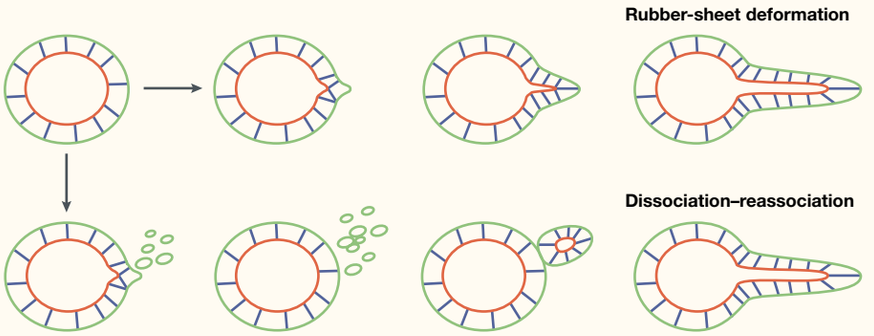

Figure 6 | **Other tubulogenetic mechanisms.** In rubber-sheet deformation (upper), cells in the epithelium maintain free, lateral and basal surfaces, and the coherent sheet remodels though evagination or invagination. During early embryogenesis, the neural tube is created from ectoderm through such a process. In dissociation–reassociation (lower), cells in the epithelium lose all cell–cell contact, migrate to new positions and then reassociate into a tubule. Elements of this model are apparent in the condensation of dissociated cells to form nephrons during kidney development. Rubber-sheet deformation and dissociation–reassociation were traditionally considered to be dichotomous mechanisms of tubule formation. However, the finding that HGF-induced tubulogenesis is the intermediate between these two models implies the existence of a continuous spectrum of tubulogenetic mechanisms; from deformation of an intact monolayer (minimal EMT), to rearrangement without loss of cell–cell contact (partial EMT), to total dissociation (complete EMT). EMT, epithelial–mesenchymal transition; HGF, hepatocyte growth factor; MDCK, Madin–Darby Canine Kidney.

Drosophila melanogaster neuroepithelium[59–61]. Perhaps these proteins specify equivalent mitotic axes in MDCK cells and HGF induces a switch from the parallel APC/EB-1 axis to the perpendicular Par-3–Par-6 axis.

In contrast to cells that undergo complete EMT, cells in chains still adhere to each other. However, the area of contact is much reduced (FIG. 4) and the molecular basis for adhesion is altered. Specifically, the close proximity of tight junctions, desmosomes and adherens junctions in mature epithelia is lost in chains, and each junctional structure relocalizes to a distinct subcellular region[48]. The disparate behaviours of these junctions indicate that selective modulation of cell–cell adhesion might be integral to partial EMT during tubulogenesis. Consistent with this idea, cysts and chains have different requirements for cadherin-mediated adherens junctions; although cells form normal cysts and extensions in the absence of functional cadherins, they are unable to form chains and instead disperse[13].

Restoring order

Cells in chains — migratory, proliferative, nonpolar and without free surface — have strayed far from the epithelial phenotype. In our proposed model, chains are the crucial point at which a morphogenetic switch occurs, causing cells to cease EMT and redifferentiate (FIG. 5). We postulate that the impetus for this switch is an attenuation of HGF signalling, perhaps in part through endocytic downregulation of the HGF receptor Met[62]. Redifferentiation would entail a resumption of the drive for three surfaces.

The idea that the morphogenesis of chains into mature tubules occurs independently of HGF is central to our model. This concept draws support from the morphological resemblance of tubule maturation to two processes that occur without HGF — cyst development and the response of MDCK monolayers to collagen overlay. All three cases show a common morphogenetic progression: cells initially lack free surfaces and apical markers, whereas basolateral markers are present but nonpolar. To generate free surface, cells form multilayers and then create small lumens between the layers. Apical markers localize to these lumenal surfaces, and transiently overlap with the nonpolar basolateral markers. As the lumens expand, basolateral markers polarize to regions of cell–cell and cell–ECM contact. Lumen expansion and cell rearrangements continue until each cell has three surface types[14,16,19,48] (L.E.O. and K.E.M, unpublished observations). The occurrence of this morphogenetic sequence during two HGF-independent processes — cystogenesis and the collagen overlay response — strongly indicates that HGF might not participate in tubule maturation.

The relevance of HGF-induced MDCK tubulogenesis to epithelial organogenesis has yet to be determined. Indeed, the reductionist HGF–MDCK system might not precisely reproduce any specific developmental event. Nonetheless, we anticipate that tubulogenesis in many contexts involves partial EMT/redifferentiation to a greater or lesser extent (FIG. 6). The HGF–MDCK model therefore represents one example in a broad spectrum of morphogenetic possibilities, from rearrangements of intact epithelial sheets to movements of completely dissociated cells.

Conclusions and perspectives

Cell populations must coordinate behaviours such as migration, proliferation and apoptosis over space and time to create a multicellular tissue. For epithelia, 3D-culture models provide a means to explore the potential mechanisms that underlie this orchestration. Here, we have incorporated many of the key findings from 3D-culture systems into a working model for how cellular behaviour is regulated during epithelial morphogenesis (FIG. 5). In essence, we postulate that cells in differentiating epithelia exert a drive for three distinct membrane surfaces. The basic architecture of cysts and tubules, both of which are lumen-enclosing monolayers, would arise as the geometric consequence of the three-surfaces pursuit. We also propose that growth factors such as HGF promote tubular development by inducing a transient, partial EMT that alters the arrangement of cells at the onset of the three-surfaces pursuit.

These ideas provide a conceptual basis for further analysis of cyst and tubule development. Important aspects of our model remain to be tested experimentally. In particular, what exactly are the molecular pathways that allow epithelial cells to distinguish free, lateral and basal surfaces, and how do these pathways interface with surface-generating mechanisms? What controls the extent of HGF-induced EMT, such that cells retain some degree of cell–cell contact during tubulogenesis? How is the switch from EMT back to the three-surfaces pursuit accomplished, and what determines the timing of this switch? Finally, what is the relationship between epithelial morphogenesis in 3D culture and epithelial organogenesis *in vivo*? Organogenesis is a highly complex process, which requires both simultaneous and sequential interactions among several growth factors, cell types and differentiation programmes. From this diversity, common mechanistic themes will probably emerge. The relative simplicity of 3D epithelial culture provides a framework to identify and explore these fundamental themes.

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