# Epithelial polarity and tubulogenesis *in vitro*☆

**Mirjam M.P. Zegers[1], Lucy E. O'Brien[1], Wei Yu[1], Anirban Datta[1] and Keith E. Mostov[2]**

[1]Department of Anatomy, Department of Biochemistry and Biophysics, and Cardiovascular Research Institute, University of California, San Francisco, CA 94143-0452, USA
[2]Room HSE 201P, Department of Anatomy, 513 Parnassus Avenue, San Francisco, CA 94143-0452, USA

**The most fundamental type of organization of cells in metazoa is that of epithelia, which comprise sheets of adherent cells that divide the organism into topologically and physiologically distinct spaces. Some epithelial cells cover the outside of the organism; these often form multiple layers, such as in skin. Other epithelial cells form monolayers that line internal organs, and yet others form tubes that infiltrate the whole organism, carrying liquids and gases containing nutrients, waste and other materials. These tubes can form elaborate networks in the lung, kidney, reproductive passages and vasculature tree, as well as the many glands branching from the digestive system such as the liver, pancreas and salivary glands. *In vitro* systems can be used to study tube formation and might help to define common principles underlying the formation of diverse types of tubular organ.**

Analysis of development *in vivo* has yielded a great deal of information, particularly on the growth factors, receptors, signaling pathways and transcription factors that specify the location, differentiation and patterning of tubular organs [1]. The use of genetically tractable systems has led to great advances, as exemplified by analyses of the formation of the trachea in *Drosophila* [2,3]. Many of the developmental pathways found to be important in flies and worms have been conserved throughout evolution, thereby facilitating analysis of the corresponding genes in vertebrate systems.

During development, tubules can arise from cells in many different starting conditions and configurations, and can be induced by several growth factors. Our premise is that these diverse pathways converge on a smaller number of common downstream mechanisms that are directly responsible for organizing epithelial cells into tubules [3,4]. These mechanisms are still poorly understood and sit at the crossroads of developmental and cell biology. Indeed, tubulogenesis is an exciting new frontier in cell and developmental biology.

Tubulogenesis involves many cellular processes, including differentiation, polarization, shape change, proteolysis, growth, mitosis, death, motility, adhesion, signaling, ion fluxes, cytoskeletal organization and membrane traffic. Although each of these processes has been well studied, we have a less complete picture of how they are coordinated in space and time, and especially how the behaviors of many individual cells are linked together to form tubules. Tubulogenesis is the most complex of several classically described alterations in epithelial tissue architecture, including convergent extension, epiboly, sheet closure and epithelial–mesenchymal transition, and may share some features with these other types of morphogenic movement [5–8].

A principal approach to studying cellular processes is to culture cells *in vitro*. For the most part this has involved plating cells on plastic or glass supports (Fig. 1 and Box 1). For the past 15–20 years, epithelial cells have been cultured frequently on permeable filter supports, which allows much better differentiation of monolayer epithelial cells [9] (Fig. 1b). For example, Madin–Darby canine kidney (MDCK) cells are typically about 3–5 μm tall when grown on solid supports, but about 10–15 μm tall when grown on filters. We refer to cells grown on solid or filter support as two-dimensional (2D) culture. Such 2D cultures on porous supports have been extremely useful for studying many aspects of epithelial cell biology, such as polarized membrane traffic [10].

Here we review the culture and use of epithelial cells in three-dimensional (3D) gels of extracellular matrix (ECM), such as collagen I or Matrigel™ (an extract of the copious ECM produced by Englebreth–Holm–Swarm tumors). Much more '*in-vivo*-like' conditions are produced by 3D cultures than by 2D cultures (Fig. 1 and Box 2). Many epithelial cells, both primary cultures and established lines, form complex epithelial structures when grown in 3D ECM. Three-dimensional cultures have been used by a relatively few investigators for decades, notably in the pioneering work of Bissell and co-workers (reviewed in [11]). Their utility has been greatly increased by several advances, especially confocal microscopy, which permits the facile visualization of tubulogenesis and immunocytochemical localization. Culture in 3D ECM is rapidly becoming more popular, and we anticipate that this technique will have as great an impact on the field as the advent of filter supports has had over the past two decades.

## The 3D–MDCK–HGF system

MDCK cells, which have properties of the kidney distal tubule and collecting duct, and many other epithelial lines

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*Corresponding author:* Keith E. Mostov (mostov@itsa.ucsf.edu).

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## Box 1. Morphogenesis in 2D and 3D culture systems

(a) Cultured epithelial cells are generally passaged by using a combination of protease (typically trypsin) and $Ca^{2+}$ chelator (typically EDTA) to produce a single-cell suspension. The cells can be plated out as non-polarized cells (indicated by the uniform gray shading of cytoplasm) at a subconfluent density in 2D culture on a solid or permeable support.

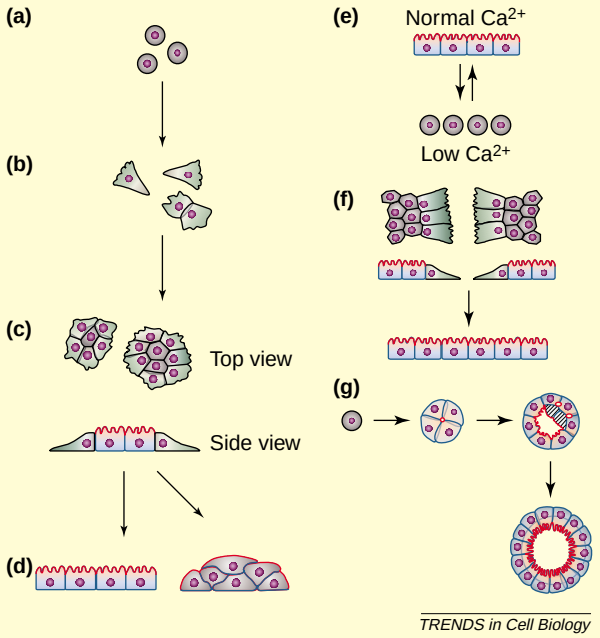
(b) These cells can form cell–cell contacts [58]. The cells migrate and often have a rather fibroblastic appearance and migratory polarity (indicated by the green shading of cytoplasm).

(c) As they migrate and proliferate, the cells form islands. The cells in the center of these islands show greater epithelial differentiation (side view: apical in red, basolateral in blue) than do the cells at the edges, which have still migratory polarity (green).

(d) The islands eventually merge to form a continuous monolayer. Especially when grown on filters, the cells continue to proliferate and become more columnar and more closely packed, although eventually they reach a steady state in which proliferation is balanced by cell death. It is important to note that the cells in the final monolayer show 'contact inhibition', that is, they do not continue to migrate or to proliferate excessively and do not pile up on top of each other. But some experimental manipulations, such as overexpression of a conditionally active mutant of Raf, cause the cells to lose contact inhibition of motility and to depolarize partially, forming a multilayer [59]. It is not clear whether steps (a–d) have a clear *in vivo* correlate; a possibility is that events in steps (a–d) resemble the recovery from diffuse epithelial injury *in vivo* (e.g. in response to a widespread toxic insult that kills many cells).

(e) A second, related type of morphogenesis relies on the manipulation of extracellular calcium by the so-called 'calcium switch' [60]. If a confluent monolayer is placed in medium with an extremely low concentration of calcium (~5 μM), the E-cadherin-dependent cell–cell junctions are disrupted and the cells depolarize. In a variation of this approach, the cells can be initially plated at confluent density but in low calcium medium, so that they never have an opportunity to form cell–cell junctions. Raising the calcium concentration in the medium to a normal level (1.8 mM) results in the rapid formation of cell–cell junctions and the development of cell polarity. Although this manipulation is not physiological, it does allow the synchronous formation of cell–cell junctions, which is particularly useful for biochemical analysis.

(f) A third type of 2D morphogenesis occurs when a confluent monolayer is mechanically wounded. The cells at the edge of the wound migrate into the wound and eventually meet cells coming from the other side. Such cells normally show contact inhibition and restore a confluent monolayer without multilayering or excessive proliferation. The physiological correlate of this *in vitro* assay is probably the healing of a simple mechanical wound. This can be compared to the growth of islands of cells (c), where each island can be considered as surrounded by a wound [61]. (g) In 3D culture, single cells can be plated in an ECM gel, where the cells proliferate. Cells that lack a basal surface will die (hatched cells), whereas cells that lack an apical surface (cells at top right corner) will create apical (red) lumens. Eventually, the cells will form a hollow sphere that is lined by a monolayer of polarized epithelial cells.



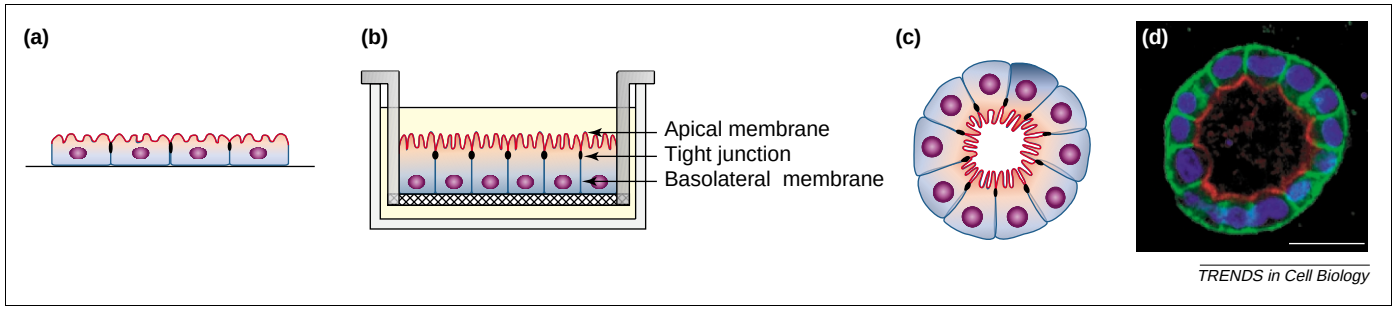




**Fig. 1**. Two-dimensional and three-dimensional culture systems. (a) Like most cultured cells, epithelial cells were classically grown mainly on solid supports, such as coverslips or tissue-culture-treated plastic. Epithelial cells usually obtain most of their nutrition from the basolateral surface (blue), which corresponds to the surface near the blood supply; therefore, tight monolayers of epithelial cells cannot obtain proper nutrition when grown on solid supports. This forces the cells to become partially depolarized and dedifferentiated. Consequentially, epithelial cells, such as MDCK cells, are usually rather flat (squamous) when grown on solid supports. In all panels, the unbroken red and blue lines indicate the apical and basolateral surfaces, respectively. The red and blue shading of the cytoplasm indicates the apical–basolateral polarization of the whole cell. Nuclei are violet. (b) The establishment of techniques to grow epithelial cells on porous filter supports (indicated by cross-hatching) was a major advance [9,54,55]. When grown on porous supports, epithelial cells can obtain nutrients from the basolateral surface and become much better differentiated and polarized as columnar monolayers. The medium in contact with the surfaces of the cell is indicated in yellow. (c) The best *in-vivo*-like conditions for culture of epithelial cells involves growth in 3D gels of ECM. Under such conditions, many well-differentiated epithelial lines form hollow cysts that are lined by a monolayer of cells with the apical surface (red) facing inwards. (d) A single confocal immunofluorescent image of a section through a cyst. The apical surface marker GP135 is stained red, the basolateral marker β-catenin is stained green, and the nuclei are stained blue.

**Box 2. Why 3D culture resembles the *in vivo* situation better than 2D culture**

There are at least six reasons why 3D culture resembles the *in vivo* situation more closely than 2D culture.

(1) Unlike 2D culture, cells in 3D culture do not have the powerful and anisotropic external cue of the artificial support (solid or permeable) and therefore must define their orientation in a relatively isotropic environment. In particular, they must create an apical surface *de novo*.

(2) In 3D culture, the cells are capable of migrating and interacting with the ECM in three dimensions; by contrast, in 2D culture the cells cannot migrate into the support and, if they migrate away from the support, they are forced to crawl on top of other cells without mechanical interaction with the ECM.

(3) In 2D culture the artificial solid or porous support is far more rigid than naturally occurring ECM. Cells can clearly respond to the mechanical (e.g. viscoelastic) properties of their external environment, and this artificial rigidity is likely to affect the cell's behavior considerably [62,63].

(4) The polarized location of certain proteins depends on whether the cells are grown in 2D or 3D culture. For example, Galectin 3 is secreted from the apical surface of 2D cultures [64] but from the basolateral surface of 3D cultures [65]. Galectin 3 promotes the differentiation of epithelial cells, and so its proper polarized localization is likely to be important [66]. There are probably many other proteins whose location changes between 2D and 3D culture.

(5) Cells grown in 3D culture are far more resistant to apoptosis [67].

(6) Cells secrete many proteins, including ECM components (e.g. laminin [41]), growth factors (e.g. transforming growth factor-β) and enzymes (e.g. latent matrix metalloproteinases). These can interact with and be organized by the surrounding ECM, and thereby influence the behavior of the cells.

self-organize into hollow spheres formed by a monolayer of polarized epithelial cells (Fig. 1c). These 'cysts' are reminiscent of the alveoli, acini and follicles found at the end of tubules in many epithelial organs, such as lung, pancreas, mammary and salivary gland. Treating these cysts with hepatocyte growth factor (HGF) causes them to produce branching tubules over a period of several days [12–15,70]. This branching tubulogenesis is reminiscent of the structures found in many epithelial organs; thus, cysts and tubules can be considered as the basic building blocks from which more complex epithelial organs are formed.

Of course, such reductionist culture systems are a gross simplification of true *in vivo* development. The widely used 3D-MDCK-HGF system is not perfectly representative of any *in vivo* developmental process and has a chief limitation: because MDCK cells are derived from the distal nephron of adult and not embryonic kidney, the system might not be especially useful for determining which growth factors are responsible for the very complex steps in kidney morphogenesis [16]. Embryonic kidney cell lines have been developed that are more suitable to analyze these factors [17,18].

The 3D-MDCK-HGF system might be appropriate, however, for studying the growth factors and other signals involved in regenerating adult kidney, such as after acute tubular necrosis, in which HGF has proved beneficial in animal models [19,20]. Given the current interest in using stem cells and other approaches to regenerate epithelial organs, it is important to understand how such regeneration occurs. In addition, the 3D-MDCK-HGF system could be useful for studying the development of tubules in adults, such as mammary and endometrial glands under hormonal control, or submucosal bronchial glands, which proliferate in asthma and hypersecretory diseases of the airway.

Despite its limitations, the simplicity of the 3D-MDCK-HGF system provides advantages for studying the downstream mechanisms underlying tubulogenesis and complements genetic analyses of tubulogenesis *in vivo*. For example, although much *in vitro* and biochemical evidence implicates HGF in kidney development, HGF knockout mice have normal kidneys, presumably owing to genetic redundancy [21–23]. Generally, the mutation of genes whose products carry out very basic cellular functions can be lethal early in development, thereby limiting analyses of later events such as tubulogenesis. In such cases, the 3D-MDCK-HGF system can be valuable by allowing, for example, the conditional expression of toxic dominant-negative mutations.

As described below, the simplicity of 3D cultures also allows the removal of external factors that orientate cell polarity, thereby facilitating a dissection of the orientation mechanism. In addition, *in vitro* culture (especially with MDCK cells) is particularly powerful for analyzing polarized membrane traffic [10], which, as discussed below, is central to understanding epithelial and tubule morphogenesis.

## Concepts from *in vitro* tubulogenesis: monolayer and lumen formation

We focus here on a few principal issues and concepts that are emerging from studies of the cellular and molecular mechanisms of tubulogenesis, primarily from work with the 3D-MDCK-HGF system. The most fundamental feature of internal epithelial structures, both spherical and tubular in shape, is that they are generally lined by a monolayer of epithelial cells [4,24]. These cells are well polarized, with an apical surface facing the central lumen, a lateral surface contacting adjacent cells, and a basal surface adhering to the underlying basement membrane – a specialized type of ECM. The basal and lateral surfaces are not separated by junctions or other diffusion barriers and thus are often considered together as 'basolateral' (Fig. 1b). MDCK cells are by far the most widely used culture system for studying epithelial polarity. They have been particularly useful for analyzing how polarity is modulated during tubulogenesis, which is a main topic of this review.

MDCK and other epithelial cells form spherical structures (cysts) when grown in collagen gels (Fig. 1 and Box 1), which indicates that the cells have the intrinsic ability to coordinate their individual behaviors to form this precise organization. We have previously proposed a simple principle that can explain how cells achieve this spherical arrangement [4]: as it differentiates, each

epithelial cell tries to establish the three types of surface domain characteristic of a polarized epithelial cell – apical, lateral and basal. We call this the 'drive for three surfaces'.

The absence of any of the three surfaces will induce cellular behaviors that will eventually create such a sphere, which is the simplest geometry that can satisfy this 'three surfaces' rule (Box 1). Cells that lack a basal surface – that is, cells that do not contact ECM directly – die [25–27]. (Cells in multilayer epithelia, which are found on external surfaces and in a few internal locations, do not obey this rule.) Cell death is involved in creating cavities in otherwise solid balls of cells, such that the cavity is eventually lined by a monolayer [28]. In thinner cords that are only 2–3 cells thick, however, cell death is not necessary to create a lumen. Instead, cells in cords that lack an apical surface will generate one by creating space between adjoining cells. Cells that lack a lateral surface, and therefore do not touch other cells, can divide to create a neighbor.

Understanding the molecular mechanisms that underlie the formation of these three surfaces is fundamental to our knowledge of epithelial morphogenesis. Formation of the basal and lateral surfaces is the best understood and is largely based on studies of epithelial cells in 2D culture [24]. Epithelial cells express integrin and other receptors for ECM components, which help to define the basal surface. Epithelial cells interact with their neighbors through adhesion proteins (typically E-cadherin) and specialized junctions, such as adherens junctions, tight junctions and desmosomes [29]. This has led to the concept that cell–cell interaction leads to the assembly of a 'targeting patch' [30]. Vesicles carrying proteins from to the basolateral surface are targeted to this patch in a process that uses the exocyst (or Sec6/8 complex) – an eight-subunit complex that was first identified for its role in exocytosis in yeast [31,32].

Formation of the apical surface and the junctions that divide it from the lateral surface is perhaps the most important event that defines epithelial polarization and is unique to polarized epithelia [3,33,34]. Unfortunately, formation of the apical surface and especially formation of the lumen is not as well understood as formation of the basolateral surface. Mainly this is because of the historical reliance on cells in 2D culture for this kind of study. In 2D experimental conditions, the cells are forced from the onset of culture to have one surface in contact with the artificial support (filter, plastic or coverslip) while the other surface is free and in contact with the overlying growth medium. Because the free surface seems to be interpreted by the cell as the apical surface, this system is not suitable for studying how cells create an apical surface and lumen *de novo*.

Work in *Drosophila* and other systems has shown that the formation of apical and basolateral surfaces is dependent on three conserved protein groups that act in a hierarchy [33,35,36]. The Par3/Par6/aPKC complex acts first to define the apical surface; this complex is also involved in polarization of non-epithelial cells and, indeed, Par3 and Par6 were initially discovered through their involvement in polarization of the *Caenorhabditis elegans* early embryo. Downstream of this complex, the Crumbs/Stardust/Discs lost complex promotes apical surface development and acts antagonistically to the Scribble/Discs large complex and its associated Lethal giant larvae protein, which promote basolateral surface development. During development of the *Drosophila* salivary gland, the delivery of membrane vesicles to the apical surface depends on Crumbs and Klarsicht, a regulator of a dynein-like microtubule motor [37]. This result directly ties the establishment of apical polarity to polarized membrane traffic. Understanding the role of Crumbs and how it relates to the other known components of the membrane traffic machinery is an issue that we anticipate might be approached productively through use of cultured cells, such as MDCK cells [38].

## Orientation of polarity

The cells in the monolayer lining a cyst or tubule are all polarized in the same orientation, with their apical surfaces facing the central lumen and their basal surfaces facing the outside of the structure. In 2D culture, this coordination among cells is forced on them by the artificial support, which provides a powerful external cue for placement of the basal surface. Recent work has illuminated how such coordination is achieved in the more physiological context of 3D culture [39]. When MDCK cells expressing a dominant-negative variant of the small GTPase Rac1 (DNRac) were grown in 3D collagen I gel, they formed solid cysts of cells that lacked lumens (Fig. 2). Notably, markers of the apical surface were found around the periphery of the cysts, rather than towards the center, indicating that the orientation of apical polarity of the DNRac cysts was inverted relative to that of control cysts (see Fig. 2d,e). An important conclusion is that the establishment of epithelial cell polarity can be separated experimentally from the orientation of that polarity [4].

The distinction between polarization *per se* and the orientation of polarization has long been appreciated in studies of a different kind of polarization, that of migrating cells such as neutrophils and *Dictyostelium* amoebae [40] (Fig. 2a–c). But this issue had not been previously addressed in epithelial cells, because in 2D cultures the orientation is usually defined by the overwhelming cue from the artificial support. Ordinarily, MDCK cells in culture secrete laminin and assemble a basement membrane at their basal surface. By contrast, the DNRac-expressing MDCK cells do not assemble their secreted laminin correctly into a basement membrane [39].

When the cultures are supplemented with a high concentration of exogenous laminin, which can spontaneously assemble into a network, the DNRac-expressing cells form cysts with central lumens and the apical surface facing the lumen. This suggests that the pathway for orientating the apical surface involves Rac activity, which promotes the assembly of laminin. The assembled laminin then causes the apical surface to be orientated at the opposite pole of the cell. We speculate that in the 3D-MDCK system the assembled laminin functions in a signaling pathway involving an integrin receptor for laminin. We propose that this signal acts upstream of the Par3/Par6/aPKC complex to determine the orientation

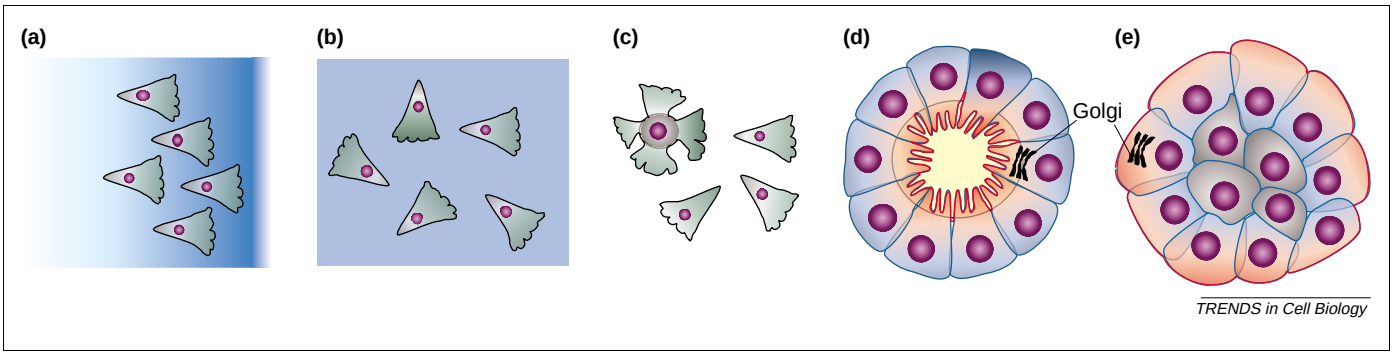



**Fig. 2**. Polarization and orientation of cells. (a) Many migrating cells, such as neutrophils and *Dictyostelium discoideum* amoeba, can move by chemotaxis up a gradient of an external attractant. These cells show a highly polarized morphology, typically with a leading edge in the direction of migration. In all panels, the green shading indicates the polarization of the cell in the direction of migration. (b) When uniformly surrounded by a chemoattractant, however, cells sometimes can be polarized at random [56]. (c) Depending on the situation, cells can show migration and a polarized morphology in a random orientation, or even multiple leading edges suggestive of multipolar polarization [57]. (d) Epithelial cells grown as cysts in 3D culture are polarized and the polarity is coordinated so that the apical surfaces of all the cells are oriented towards the central lumen of the cyst (red). (e) Expression of a dominant-negative mutant of the small GTPase Rac1 causes a partial inversion of polarity. Markers for the apical surfaces of the cells (red) are now on the periphery of the cysts, which lack lumen. The internal organization of the cytoplasm (blue to red shading) is also inverted, such that the Golgi lies beneath the apical surface at the periphery of the cyst.

of polarity. A possible intermediate between the integrin signal and the Par3/Par6/aPKC complex is the small GTPase Cdc42, which associates with the mammalian Par3/Par6/aPKC complex [41–44].

Although much work in *Drosophila* and other systems has been done on the role of cell–cell contact [24,30] and on the three protein groups involved in establishing polarity, these studies have largely not examined how the orientation of epithelial polarity is determined *in vivo*. For example, many studies in *Drosophila* have focused on the initial epithelium that covers the embryo [33,35]. In the covering epithelium the orientation of polarity is most probably determined by the relationship of the epithelial cells to both the underlying organism and the external space surrounding the organism. In this sense, studies of covering epithelium in *Drosophila* have a limitation similar to that of the 2D culture of epithelial cells. Polarity is inverted in *Drosophila* cystic structures resulting from an absence of Lethal giant larvae, Discs large or Scribble activity, but this is apparently due to an expansion of the apical surface rather than a block in the mechanism that orientates polarity [36]. By contrast, in the 3D-MDCK-HGF system the external cues that orientate polarity can be manipulated experimentally, facilitating analysis of the orientation pathway [39].

## Remodeling to form tubes and branches

When MDCK cysts are treated with HGF, they undergo an ordered sequence of events to generate a tubule [45–47] (Boxes 2 and 3). First, some cells send out a large 'extension' of their basolateral surface, although these cells remain in the cyst wall and at this stage retain a small apical surface. This large extension is similar to the large pseudopodia seen in fibroblasts and neurons moving in 3D ECM [48,49]. Extension formation requires phosphatidylinositol 3-kinase and the lipid products of this kinase are concentrated in the plasma membrane of the extension [47]. This is similar to the concentration of phosphatidylinositol 3-lipids in the leading edge of migrating neutrophils and *Dictyostelium* amoebae [40]. Ordinarily, only a few cells in the cyst wall form extensions in response to HGF. Notably, inhibition of Rho kinase causes a tenfold increase in the number of extensions formed, indicating that extension formation is normally limited by the Rho GTPase and its effector, Rho kinase [47].

Next, the cells divide and some migrate out of the wall of the cyst to form 'chains' of cells, which are typically 1–3 cells in length. In the absence of HGF, the cells divide in the plane of the monolayer, such that both daughter cells remain in the cyst wall. On treatment with HGF, however, the orientation of cell division changes such that one daughter leaves the cyst wall and initiates chain formation [47]. Cells in chains have lost their epithelial apical–basolateral polarity. Apical surface marker proteins are undetectable, and basolateral marker proteins such as E-cadherin surround the cell [45–47]. Cells at the end of the chain have a polarity and phenotype that resembles that of a migrating fibroblast or mesenchymal cell.

Finally, the cells divide further to form 'cords', which are about 2–3 cells thick. At this stage, the cells begin to regain epithelial polarity and form small lumens between themselves. Eventually the lumens enlarge, merge and become contiguous with the central lumen of the cyst to form mature tubules.

A key aspect of this sequence of events is that some of the cells undergo a transient loss of epithelial polarization, becoming similar to migrating fibroblasts [47]. We have previously suggested that this is essentially a transient, partial epithelial–mesenchymal transition (EMT) [4,6] and that the cells re-establish the polarized epithelial phenotype to form new tubes. Reversible EMTs occur normally during many stages of development, whereas irreversible EMTs are characteristic of invasive cancers derived from epithelial cells. We suggest that the process of re-establishment of polarity is similar to that used to form cysts and might even involve the same Rac and laminin assembly pathway. Partial EMT, followed by re-establishment of the epithelial phenotype, might be also used to generate branches from an existing tubule [2].

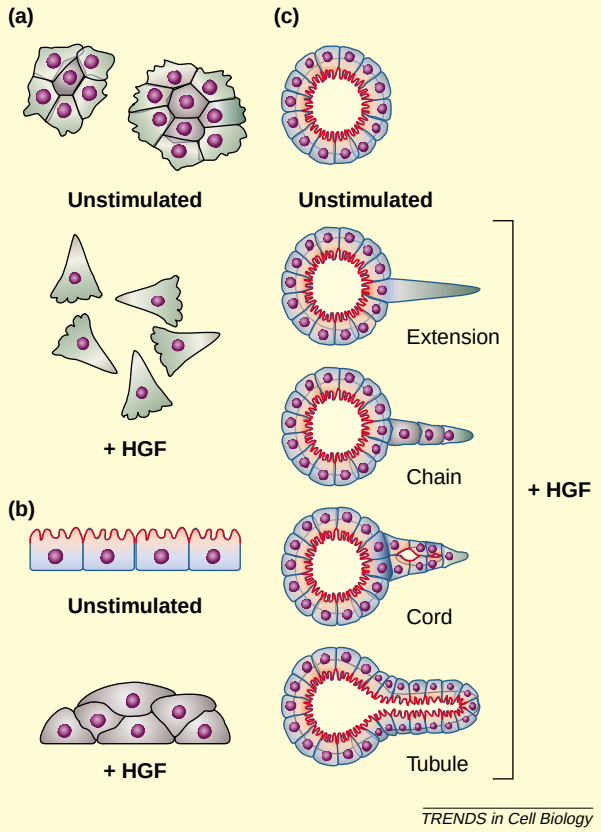
This simple model separates the complex process of tubulogenesis into two chief subprocesses: partial loss of the polarized phenotype to permit remodeling into tubules and more complex shapes, and re-establishment of the polarized phenotype on the basis of the drive for three surfaces.

**Box 3. Morphogenic responses of epithelial cells to HGF in 2D and 3D culture**

(a) When epithelial cells (typically MDCK cells) are grown as small islands in 2D culture and then treated with HGF, the cells respond by spreading, dissociating from each other, and migrating as individual cells. This response is known as 'scattering' and the ability of HGF to cause this led to the alternative name of 'scatter factor' for HGF.

(b) If, instead, a confluent monolayer of MDCK cells in 2D culture is treated with HGF, the cells respond by piling on top of each other and partially losing polarity [68].

(c) If MDCK cells that have formed cysts in 3D culture are treated with HGF, the cells respond by forming branching tubules, as described in the text. MDCK cells also can be plated directly into 3D ECM in the presence of HGF. Under these conditions, the cells form tubules directly without forming a cyst. The cells might go through intermediates that resemble extensions, chains and cords, although this issue has not been clearly resolved [69].




Although tubulogenesis *in vivo* is clearly much more complex, this subdivision provides a first step in understanding tubulogenesis as a sequence of individual subprocesses.

It is important to realize that tubulogenesis involves integrated changes in many different behaviors of the cell, and that experimental perturbations designed to affect only one cellular process can lead to protean changes in many aspects of cyst and tubule formation, and even to unexpected insight into the connections among these processes. For example, the exocytosis of newly made proteins at the basolateral surface involves the exocyst, as noted above. Overexpression of the human Sec10 subunit of the exocyst in MDCK cells leads to increased cyst formation and increased tubulogenesis in response to HGF and also leads to increased synthesis of basolateral membrane proteins and secretory proteins [50]. This is most probably the result of a feedback control between exocytosis and the synthesis of these proteins at the rough endoplasmic reticulum membrane, revealing an unexpected link between cyst and tubule formation, plasma membrane exocytosis and protein synthesis. This example illustrates the importance of an integrated 'systems biology' approach to studying epithelial morphogenesis, as well as the usefulness of reductionist systems for analyzing tubulogenesis.

## Is there a universal mechanism for tubulogenesis?

*In vivo* tubulogenesis occurs by many different mechanisms, which at least superficially seem distinct [1]. Tubulogenesis is such a widespread and fundamental property of epithelial cells, however, that one could imagine an underlying basic program (or small number of programs) for tubule formation, which perhaps might be modified in different organs and taxa [3,4]. In vertebrates, tubulogenesis can be described as occurring by one of two broad mechanistic categories: epithelial sheet deformation, or condensation from non-polarized cells [1,51,52]. In epithelial sheet deformation, the cells move as a coherent sheet that invaginates or evaginates. This sheet can form various shapes, such as a cylinder projecting orthogonal to the original sheet or a long indentation lying parallel to the sheet, as in neural tube formation. In condensation, cells that lack epithelial polarity come together and arrange themselves into a tubule.

The sequence of tubule development from MDCK cysts treated with HGF that we have summarized above has features of both sheet deformation and condensation [46]. The cells in a chain are not moving as part of a sheet, because they have left the monolayer of the cyst wall. But they have not completely dissociated either and retain some cell–cell contact. The existence of an intermediate situation between sheet deformation and condensation suggests that these two mechanisms are not as dichotomous as is usually assumed, but rather that they are likely to be two ends of a continuous spectrum. Indeed, we have suggested previously that some degree of transient EMT is involved in all of these processes [4]. Even cells migrating as part of a sheet extend processes and lose some of their stable epithelial character, which, we propose, represents a limited degree of EMT [53]. Condensation involves the opposite end of the spectrum, where the cells have essentially no epithelial polarization; in this case, the cells might have a non-epithelial origin and simply acquire epithelial polarization in a mesenchymal–epithelial transition.

In addition, MDCK cells can also form tubules in an alternative manner that is much closer to condensation. Instead of first growing cysts in collagen I gels and then treating them with HGF, a dissociated single-cell suspension of MDCK can be cultured in collagen I gels with HGF present from the onset [15]. In this case, the MDCK cells can form tubes directly, without going through an intermediate cyst stage. We suggest that the ability of MDCK cells to use two apparently distinct processes to

form tubules supports the concept that there is an underlying common tubulogenesis program, which can be manifested in several different ways.

## Concluding remarks

The potential value of any reductionist *in vitro* system is the extent to which it can tell us about *in vivo* processes, such as tubulogenesis. The 3D-MDCK-HGF system is the best characterized tubulogenesis system for cellular processes downstream of the inductive signals, and it has already led to the formulation of several simple concepts. These include, first, the drive for three surfaces, which might underlie the formation of polarized monolayers enclosing a lumen; second, the distinction between polarization and orientation of polarization; and third, the use of transient EMT to remodel structures into tubes and branches. These simple concepts can provide a conceptual framework for future analyses of tubulogenesis *in vivo*. Tubulogenesis in animals is much more complex and much more diverse than *in vitro*, and it will be important to see how the basic processes that have been identified through *in vitro* work are implemented and modified *in vivo*.

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