Molecular Mechanisms of Epithelial Polarity: About Shapes, Forces, and Orientation Problems

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In a variety of organs, epithelial cells assemble into networks of cysts and tubules. Such structures can be reproduced in vitro. Here the importance of plasma membrane compartmentalization and forces that drive morphogenetic events during cystogenesis are discussed.

Organs are elaborate cell communities that perform specialized functions. They generally adopt complex structures, which are reproduced from generation to generation. As their smallest units, cells play an important role in shaping organs. The ability of a cell to control its own shape and contacts with its neighbors is central in organogenesis. The oldest organs, epithelia, developed to serve as specialized interfaces between the organism and the outside world. Mammalian epithelial organs, such as kidney, breast, and lung, consist of cysts and tubules. The shape of each structural unit is crucial for the function of the organ and is dictated by the shape of the individual cells.

A cyst is a spherical cell aggregate consisting of a single layer of polarized epithelial cells enclosing a liquid-filled lumen. All cyst cells organize the apical surface facing the cyst lumen. The lateral surface is in contact with the neighboring cells and the basal one with the extracellular matrix (ECM). In mammals several kinds of cysts exist, all of which are the basis of organogenesis because they either remain important functional units (acinus) or initiate the formation of tubules (nephrogenesis). Tubules are also constituted of epithelial cells with these three surfaces. However, tubules form lumen-containing elongated structures enabling efficient transport of substances into and out of tissues.

The functional unit of the mammalian kidney is the nephron. A nephron is a tubular structure with a lumen where waste products are concentrated and is separated from the inside of the organism by a single layer of epithelial cells. These cells constitute a selective barrier and pump waste products into the lumen. The lateral surface is in contact with the neighboring cells and the ECM. Membrane polarity is reflected by the internal architecture of the cell. The nucleus localizes basally underneath the Golgi complex, microtubules run along the basoapical axis, and actin filaments assemble apically to direct microvilli formation.

In this review, I will summarize how during organogenesis communities of cells reorganize their contacts and polarize to form cysts, i.e., define luminal cavities separated from the internal body parts.

Cells come together to form structural units of organs

What is the origin of an epithelial monolayer? In the case of the nephron, the epithelial monolayer is the product of cell proliferation starting from an amorphous set of undifferentiated cells, the nephronic mesenchyme (Fig. 2). The conversion of the nephronic mesenchyme into nephron epithelium is divided into two stages. During the induction period mesenchyme condenses in response to signals, and during the subsequent morphogenetic period the actual generation of a polarized cell takes place (17). Single mesenchymal cells come together to form a primary condensate. Later this condensate splits into two small cell aggregates, referred to as pretubular aggregates. The smallest detectable pretubular aggregates consist of four to six cells. These cells proliferate and differentiate into epithelial cells. If the aggregates consist of ~30 cells, the first signs of cell polarization can be seen: laminin becomes polarized to the external surface of the aggregate. Subsequently, the mature aggregate undergoes full differentiation into columnar cells. Cells of the pretubular aggregate reorganize themselves first into a comma-shaped, lumen-containing structure, which is subsequently modified into a shaped structure. Cells of the S-shaped structure continue to proliferate and differentiate into glomerulus, juxtapglomerular apparatus, and proximal and distal tubules. The lumen generated in the comma-shaped structure will be extended as the tubular structure of the nephron evolves. Finally, the tubule-lumen is connected with the lumen of the urine collecting duct and is therefore in direct contact with the exterior of the organism.

Importantly, before the formation of the comma-shaped structure each of the aggregate cells had at most two surfaces, basal and apical. A lumen between the cells did not exist.
Lumen formation, a very important morphogenetic event, is first recognizable in the comma-shaped structure. Each cell of this structure is in contact with neighboring cells, a liquid-filled lumen, and the ECM and exhibits therefore three different surfaces. It is critical that all cells of the aggregate orient themselves concertedly in the same direction. Therefore, it seems that each of these cells aims at forming three surfaces (13), which is a prerequisite and likely driving force for lumen formation. We know little of how cells define the center of the cell aggregate to create a cavity only in this location.

Cystogenesis in vitro

Madin-Darby canine kidney (MDCK) cells are dog kidney epithelial cells and constitute a well-characterized model cell line used in studies of cellular polarization. When grown on permeable filter support they differentiate into polarized epithelial cells, establishing a two-dimensional cell layer. MDCK cells have been used for the development of important concepts such as asymmetry of the plasma membrane domains and polarized membrane trafficking. MDCK cells can form cysts and tubules. During cystogenesis the basal surface is present first, followed by a lateral one; the apical surface is formed last. This order of surface generation is also detectable during acinus formation in breasts or formation of the comma-shaped structure during nephrogenesis (Fig. 2), which is relevant for several organogenetic programs in vivo. Therefore, I will discuss the morphogenetic progresses during cystogenesis of MDCK cells (Fig. 3).

When single MDCK cells are seeded in a collagen matrix, they divide and, after several days, form a cyst. A single cell embedded in ECM exhibits only one type of surface, a basal surface. After the first division, the two cells do not really separate but generate one novel contact area (Fig. 3B). Whether contact formation occurs during cytokinesis or shortly afterward is unresolved. The newly generated contact surface enlarges over time, which causes a change of cell shape in each of the two cells. Further divisions lead to a cell aggregate where most of the cells exhibit two surfaces, a lateral and a basal surface (Fig. 3, C and D). Whether cells in the inner part of the aggregate exist that exhibit only a lateral surface is unresolved.

At a certain time point, an apical surface in each of these aggregate cells and thus one central lumen is observable. It remains to be analyzed if the central lumen was directly established or if it is the fusion result of several small lumina that were established at lateral sites more in the periphery of the cell aggregate, as is the case during gut formation in zebrafish (2). Cell proliferation continues, and the lumen generated by the apical surfaces becomes liquid filled through directed...
water transport. This dramatic volume extension has to be compensated in the ECM.

The described cystogenesis has several aspects allowing direct comparison with a part of nephrogenesis (Fig. 2, B and C vs. Fig. 3). The basal surface is present before the generation of a lateral surface, which matures to allow the formation of the apical surface. In the following section, I place known molecular fragments into a sequence of surface generation occurring during cystogenesis.

**Molecular description of surface generation**

**Composition of the lateral surface.** The mature lateral membrane is characterized by several junctional complexes (Fig. 1). TJ are in the most apical position within the lateral surface. More basal are the AJ, which, together with the desmosomes, cause intercellular adhesion. GJ are relevant for the intercellular communication of the cells.

Adhesion is mainly mediated through cis- and trans-complexes of E-cadherin, which is associated on the cytoplasmic side with many regulatory proteins to create a link to the actin cytoskeleton (1). A lateral surface is observable at the end of the first division of the single embedded cell (Fig. 3B). Detailed analysis of the generation of first contacts between epithelial cells support the following “zippering model” for the formation of this initial basal surface. In primary mouse keratinocytes, neighboring cells penetrate each other with filopodia, which carry at their tips clusters of E-cadherin complexes. This generates a two-rowed zipper of embedded puncta. The filopodia tips encounter the cadherin complexes at the neighbor cell and initiate immature contact points. The rate-limiting step for adhesion formation is the anchoring of the cadherin complexes to the cortical actin cytoskeleton, promoting the clustering and stabilization of AJ proteins to form a punctum. Several puncta assemble and subsequently form a continuous belt of E-cadherin-mediated contact region. Thus the actin polymerization-driven filopodia formation brings cells in close contact and mends cells together (15). This mechanism closely resembles what is observed during dorsal closure in *Drosophila*.

The family of nectins, which are Ca\(^{2+}\)-independent immunoglobulin-like molecules, are ubiquitous transmembrane proteins that also constitute a link to the actin cytoskeleton. They localize in AJ, and their presence clearly aids the E-cadherin-mediated adhesion as well as the formation of TJ. E-cadherin-independent functions of Nectin are not well understood so far. Vezatin is a ubiquitous novel transmembrane protein, also localizing to AJ. There it interacts with myosin VIIA and the cadherin complex. Thus myosin VIIA tethered via vezatin to the cadherin complex creates a tension force between AJ and the actin cytoskeleton that is expected to strengthen cell-cell adhesion. This molecular system allows the exertion of forces along the lateral surface.

TJ are multimolecular complexes arranged in a belt-like structure (9). The formation of functional TJ is dependent on adhesion mechanisms bringing cells together. However, in neurons, polarized cells without TJ, a membrane diffusion barrier was demonstrated, which is generated by cross-linking membrane proteins to actin (11). So far it has not been analyzed whether such a fence formation might also occur in epithelial cells. Analyzing this experimentally is very challenging. If true, however, it would restrict the function of TJ gatekeepers regulating the extracellular exchange between two compartments.

The role of desmosomes in the establishment of plasma membrane surfaces was neglected until recently. With the help of peptides binding to desmosomal cadherins, it was demonstrated that desmosomes also function in epithelial morphogenesis. Ca\(^{2+}\)-dependent heterophilic interaction between desmocollins and desmogleins, the desmosomal

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**FIGURE 3.** Schematic representation of in vitro cystogenesis. Single Madin-Darby canine kidney (MDCK) cells (A) embedded in a collagen gel develop within 10–12 days into an epithelial cyst (E). After the first cell division, the cells stay together and form a first lateral surface (B). Morula-like cells (C) change shape in a process called compaction (D; the existence of morula-like cells and the compaction process are speculations). Continued cell divisions cause a volume increase of the cell aggregate and further cell shape changes. Finally, a liquid-filled lumen is surrounded by polarized epithelial cells, each with a basal, lateral, and small apical surface (E).
cadherins, contribute to cell-cell adhesion and organization of the histoarchitecture in the mammary gland. Desmoglein 4 also functions in the differentiation of hair follicle cells (5). It seems that protein kinase C (PKC) activity increases the adhesion of epithelial cells mediated through desmosomes. Also, erbin regulates, via plakophilin-4/p0071, the maintenance of cell-cell contacts. Hence, desmosomal proteins are crucial for epithelial polarity. Interestingly, atypical PKC (aPKC) and scribble (erbin is a scribble-related protein) are constituents of two conserved polarity complexes that will be discussed below. This might indicate that these polarity complexes regulate desmosomes.

GJ are relevant for intercellular communication and might therefore be critical junctions to ensure that all cells in a cell aggregate react at a similar time and with the identical orientation. GJ are channels across the plasma membranes of neighboring cells composed of subunits of proteins encoded by the connexin (Cx) multigene family. Remarkably, zonula occludens-1 (ZO-1), a TJ-associated protein, was found to interact with two different connexins, Cx43 and Cx45. Furthermore, Cx32 formation and/or Cx32-mediated intercellular communication induces expression and function of TJ in a hepatocyte-like cell line. Remarkably, inhibition of gap junction-mediated communication inhibited cadherin function in Xenopus (14).

In summary, E-cadherin-mediated cell adhesion causes the formation of GJ, and these support the formation of TJ. Desmosome formation depends on E-cadherin-mediated contacts and can cause TJ formation. Recent results using mammary cells, however, also support former results (19) that GJ, desmosomes, and TJ can coalesce in the absence of AJ (16). Also, MDCK cells lacking functional E-cadherin develop normal cysts (18). Thus it seems that E-cadherin-mediated adhesion is necessary but not sufficient for the formation of GJ, desmosomes, and TJ. It will be of interest to analyze the maturation of the lateral surface in a larger context, taking into account mechanophysical parameters and the interplay of the cell junctions like desmosomes and GJ.

Maturation of the lateral surface. Recent progress revealed that polarization of epithelia depends on three conserved protein complexes: The Par3-Par6-aPKC complex, the crumbs (Crb)-starburst (Std)-discs lost (Dlt) complex, and the discs large (Dgl)-scribble (Scrib)-lethal giant larvae (Lgl) complex. These complexes are conserved throughout metazoa and function in a genetic hierarchy (6). The localization of the Par3-Par6-aPKC complex defines the apical surface. The formation of this complex is dependent on E-cadherin-mediated contacts. The positioning of the complex is likely to be determined by Par3, which binds to the TJ protein, junction-associated membrane protein, and nectin. Thus this complex can be positioned in adhesion puncta and in apical TJ. Downstream of the Par3-Par6-aPKC complex are the Crb-Std-Dlt and Dlg-Scrib-Lgl complexes. The Crb-Std-Dlt complex is responsible for the formation of the apical surface and inhibits the basal imprinting by the Dlg-Scrib-Lgl complex. Conversely, Dlg-Scrib-Lgl complex localizes in AJ and antagonizes the Par3-Par6-aPKC complex, inhibiting the apical surface identity. It is unresolved how the localization of the Par3-Par6-aPKC complex and TJ components is determined. In other words it is not understood how cells orient these complexes along the lateral surface of the cell. One can speculate that Cdc42, a conserved Rho-like GTPase and regulator of basolateral polarity (7), directs their positioning. Mechanophysiological restrictions might also influence the localization of the complexes.

Mechanical forces are exerted by the cell aggregate as a whole as it increases its volume due to proliferation. The development of blastocysts, liquid-filled lumina surrounded by polarized epithelial cells and an unpolared inner cell mass derived from a fertilized egg, is a variation of in vivo cystogenesis. During blastocyst formation compaction occurs, which is based on the contractility of actomyosin tethering the lateral plasma membrane. It is unresolved whether compaction is followed or accompanied by the formation of apical surface. Apical spectrin can drive apical constriction in Drosophila (20). Interestingly, Crb, a component of one of the conserved polarity complexes, interacts with apical spectrin (10). Through this, link coordination between shape changes and lateral surface maturation is possible. It becomes evident that shape changes and domain formation are tightly linked to epithelial cells. This connection is best expressed with the term tensegrity, which describes a system that gains mechanical stability from continuous tension and local compression (1). Rules, such as energy minimization, topological constraints, structural hierarchies, and autocatalysis, underlying a tensegrity system might guide hierarchical self-assembly processes such as cystogenesis.

Generation of the apical surface. In the three-dimensional culture system of MDCK cells, the apical polarity depends on signals from the basal surface of the cells. The small GTPase Rac1 orients epithelial apical polarity through effects on basolateral laminin assembly. Rac1 influences proper laminin assembly, likely through regulation of the presence of α3-integrins in the basal surface (12). The apical surface will be generated opposite to homogeneously assembled laminin, which is in contact with the basal plasma membrane surface. Thus aggregate cells assemble their own ECM, to which they then respond in an oriented morphogenetic reaction. This study demonstrated the temporal and spatial coordination of several cells with the generation of correctly placed apical surfaces. It is likely that the oriented laminin assembly is in response to the previously surrounding ECM. How do the cells determine...
the plasma membrane region that is opposite to the basal surface? During maturation of the lateral surface, two polarity complexes mark the apical identity. The localization of these complexes itself seems dependent on mechanical constraints. Thus it is tempting to speculate that the cell recognizes the area “opposite to laminin” as a surface area that is under tension. This is the region during the compaction process that experiences the strongest actomyosin contraction. Information is missing concerning the reason for the positioned constriction to explain the generation of the basoapical axis.

The apical surface generation occurs concomitantly or follows lumen formation and is oriented opposite the basal surface. Two mechanisms will be discussed that allow lumen formation: membrane separation and apoptosis (13). First several aspects of membrane separation are presented, and finally some considerations about apoptosis are given.

Membrane separation means that a space is created between certain regions of the surface of contacting neighbor cells. Using fluorescence microscopy, one initial small lumen is observable in the center of the aggregate. This small lumen will extend with time and decrease the lateral surface area. In the following model, the beginning of cell-cell separation is described (Fig. 4): the very starting point to create small lumina could either be the localized secretion of proteins, which are very large and hinder sterically, or the removal of local adhesiveness by expression of an antiadhesive protein. Both processes, however, create only a microlumen of nanometer dimensions. The microlumen might be enlarged through the reversal of the filopodia-mediated epithelial adhesion (4). This means that filopodia form at membrane surfaces that will separate and extend into the microlumen, where cell-cell adhesion was abolished. Actin polymerization could cause the elongation of the filopodia. This might push the opposing membranes apart. In a secondary mechanism, the initial membrane separation will have to be stabilized and the primary microlumen gradually extended. The lumen-facing plasma membrane region constitutes the apical surface.

Apoptosis of inner cells, the removal of their cell debris, could lead, together with a fixation of the plasma membrane shape of the cells surrounding the apoptotic cell, to a lumen. Apoptosis of cells in the inner region of cell aggregates could cause a force-independent means to generate a lumen. Indeed, apoptosis can be observed; however, the suppression of apoptosis by overexpression of Bcl-2 does not eliminate lumen formation (8). This indicates that at least one additional space-forming mechanism besides apoptosis exists.

Conclusion

Organogenesis can start with the formation of liquid-filled spaces surrounded by polarized cells, a cyst. Formation of this multicellular three-dimensional structure goes along with the generation of three different plasma membrane surfaces. The ECM immobilizes single cells, which are unpolarized. Due to a close proximity of cells in the ECM, a lateral surface will be generated that matures over time. The hierarchy of junction formation in the lateral surface is not resolved but is likely linked to cell shape changes. Cell shape changes are the result of contractions or expansions, supporting the generation of apical surfaces in the center of the cell aggregate. Thus it will
be of interest to follow in the future the interdependencies of cell junction formations and contraction mechanisms. Thus analysis of mechanophysical constraints during the development of cell polarity represents a promising field of research for the future.

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References


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