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The world of epithelial sheets

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Short running title: The world of epithelial sheets

Abstract

An epithelium is a layer of closely connected cells covering the body or lining a body cavity. In this review, I address several fundamental questions regarding the epithelium. (1) While an epithelium functions as barrier against the external environment, how is barrier function maintained during its construction? (2) What determines the apical and basal sides of epithelial layer? (3) Is there any relationship between the apical side of the epithelium and the apical membrane of an epithelial cell? (4) Why are hepatocytes (liver cells) called epithelial, even though they differ completely from column-like shape of typical epithelial cells? Keeping these questions in mind, I considered multiple shapes of epithelia, extracted a few of their elemental processes, and constructed a virtual world of epithelia by combining them. I also classified epithelial cells into several types based on the number of apical domains of each cell. In addition, I introduced an intracellular organelle within epithelial cells, the vacuolar apical compartment (VAC), which is produced within epithelial cells surrounded by external cell matrix (ECM). The VAC interacts with areas of cell-cell contact of the cell surface

membrane and is converted to apical membrane. The properties of VACs enable us to answer the initial questions posed above. Finally, I discuss the genetic and molecular mechanisms of epithelial morphogenesis.

Key words: apical–basal polarity, closed envelope, epithelial cell, epithelium, vacuolar apical compartment

Introduction

Epithelia are sheet-like structures that function as barriers to protect animal bodies against the external environment (Honda, 1991; 2010; 2012). In light of the multiple intriguing properties of epithelia, I sought to address the following issues. (1) *Formation of envelopes without leakage*: Usually, an epithelium forms an enclosed envelope. How does it develop without leakage of contents, even when it is under construction? (2) *Determination of apical and basal sides*: An epithelial sheet has apical and basal surfaces facing fluid and solid, respectively. The determination of these surfaces must conform to the overlying architecture of animal bodies. How are these surfaces distinguished and defined? (3) *Relationship between the two apical surfaces*: Epithelial sheets consist of epithelial cells. Epithelial cells have cell membranes, which also function as barriers separating the cytoplasm from the extracellular space. Is there any relationship between the apical surface of epithelial tissues and the apical cell membranes of epithelial cells? (4) *Structural difference between prism-like and hepatic epithelial cells*: While typical epithelial cells are squamous, cuboidal or columnar in

shape (called prism-like generally; a prism is a polyhedron whose two ends are similar and parallel polygons and whose sides are parallelograms), hepatocytes (liver cells) have a different, complex structure; nonetheless, they are still classified as epithelial cells. How should we consider the structural differences between these types of epithelial cells?

I began by considering numerous shapes of epithelia (e.g., Krstic, 1978), and extracted a few elemental processes, based on which epithelial morphogenesis occurs, elongation, invagination (apical surface is concave), protrusion (apical surface is convex) and fusion/reconnection of epithelial sheets. Virtual combinations of these elemental processes yielded various theoretical epithelia, some of which correspond to real epithelia observed in nature. Thus, I constructed a virtual world of epithelial sheets as a group of shapes generated by combinations of the elementary processes. Furthermore, I pointed out that epithelial cells could be classified into several types according to the number of apical domains that epithelial cells have. On the other hand, most epithelia form closed envelopes, and I came to recognize that this is not an optional feature. These characteristic properties of epithelial cells and tissues can be

understood by considering an intracellular organelle, the vacuolar apical compartment (VAC), whose behavior is controlled by apico-basal polarity. At last the genetic and molecular mechanism of this control is described.

The world of epithelial sheets

Epithelia, which are sheets consisting of epithelial cells, adopt various organs in animal bodies and early embryonic bodies, guts, neural tubes, lung branches, vascular networks, and so on. (I do not consider cellular sheets of plant in the present paper, except for *Volvox* mentioned later, because plant sheets generally have openings and partitions of the space are not perfect, e.g., stomata of leaves.) An epithelial sheet functions as a partition that divides a space into two parts. Usually these two spaces are occupied by fluids (liquid or air) and solid materials, respectively (Fig. 1A). Therefore, each epithelial sheet has two sides, the apical and basal surfaces. The partition function requires that epithelial sheet does not have a peripheral edge; otherwise, the materials in the two spaces could escape and mingle with each other along the edge. Therefore, it

naturally follows that an epithelial sheet has no choice but to form a closed envelope (Fig. 1A right). This is a remarkable property of epithelial sheets. The main purpose of the review is to elucidate the autonomous mechanism by which closed envelopes form.

During many morphogenic processes, epithelial sheets deform and change shape. We will extract a few processes from such morphogenesis, as shown in Fig. 1: specifically, elongation (ELG), invagination (IVG) and protrusion (PRT) of epithelial envelopes (Fig. 1B–E). In addition, sometimes two epithelial sheets reconnect to alternate topology via fusion (Fig. 1F right and left). We know two cases of such reconnections: fusion between two apical surfaces that face each other (aREC), and fusion between two basal surfaces that face each other (bREC). We will refer to these events as “elemental processes” of epithelial sheets.

It might be appropriate to add another item to the list of elemental processes, namely, inversion (IVS in Fig. 1G). We know two types of inversions. First, the sponge embryo performs an inversion that takes place within a mother body (*Calcareous sponge*. e.g., Dan, 1987). Initially the outside of epithelial envelope of the sponge embryo is basal. A slit occurs on the envelope, and the sheet goes out through the slit,

whereby the sheet performs a concave–convex transformation; thus, the envelope is turned inside-out. The physical mechanism underlying this inversion remains unclear. A cellular contractile force localized along the periphery of the epithelial sheet has been observed in Madin-Darby canine kidney (MDCK) epithelial sheets (Imai et al., 2015). Such a contractile force may play a role in the late stage of inversion of the sheet of the sponge embryo. The inversion of the embryonic epithelial envelope in the mother body of the sponge may reflect an adaptation to the sponge life cycle, as the inverted embryo is protected against fusion with its mother's epithelial sheet (Dan, 1987). We know of a similar case in algae. *Volvox* performs a similar inversion during its reproduction (Nishii and Ogihara, 1999). Although it is not animal, it should be mentioned. Because it may be important for study of the inversion mechanism to notice such behavior of algae's. In the other type of inversion, which is observed in thyroid follicles (Miyagawa et al., 1982) and in envelopes consisting of MDCK cells (Wang et al., 1990), the closed envelopes are suspended in culture medium and invert the apical–basal polarity of their epithelial cells. Such inversion takes place within cells by reconstruction, redistribution, and rearrangement of intracellular components such as apical junctional apparatus,

Golgi apparatus, and so on. The culture conditions that determine outside–inside polarity have been investigated in detail (Yonemura, 2014), and the genetic and molecular mechanisms of the inversion have also been investigated (O'Brien et al., 2001; Yu et al., 2005; Yu et al., 2008).

Let us now consider combinations of these elemental processes. First, we will consider an envelope in which the apical surface faces outward. I will mention results of application of elemental processes first, then I will consider the correspondences between each result of applications and actual epithelia. Application of [ELG] to a spherical envelope produces an elongated envelope, and application of a combination of elemental processes, [ELG+bREC] (i.e., sequential application of [ELG] followed by [bREC]), produces two separate spheres (Fig. 2A). Outside of artificial reproduction, we cannot find examples of two envelopes generated by [ELG+bREC]. One process in which an envelope could form two isolated envelopes is division of a multicellular body, which does not occur in nature. We sometimes obtain clones via proliferation of a single cell, but not by dissection of a multicellular body. Application of elemental process,

protrusion [ELG] to a spherical envelope produces a sphere having a protrusion (Fig. 2B). Its actual example is a limb bud or a wing bud (Fig. 2B right).

The combination of [IVG+aREC] produces two envelopes nested within one another (Fig. 2C), which would correspond to formation of a neural tube (in section) and the lens (Fig. 2C right). The combination [IVG+bREC] forms an invaginated envelope (Fig. 2D). The bottom of the concave structure faces the basal surface of the same envelope and fuses between the two basal surfaces (rectangle in Fig. 2D). Reconnection between the concave sheet and outer envelope creates a penetrating tube (a torus), forming a gut.

I will now consider the elemental processes that occur between two epithelial envelopes. Between two envelopes of independent individual bodies, [aREC] is prohibited because it would form a chimera body, which cannot maintain its lineage over the course of evolution. However, [aREC] of two epithelia within an individual body can take place. For example, in craniofacial development, the left and right palatal shelves fuse with each other (Fig. 2E right). Another example is the adhesion of mesothelia in the abdominal cavity, where the fusion between peritoneum and

mesothelium of an organ. [bREC] between two envelopes nested within one another takes place in experimental reconstruction of starfish embryos (Fig. 2F), in which the dissociated cells associate into two envelopes that nest within one another (Dan-Sohkawa et al., 1986). In this model, the inner envelope ultimately fuses with the outer envelope to form a gut. This is a process of inversed direction of [IVG+aREC] (Fig. 2C).

Next, I will consider epithelial structures which are included by another larger envelope as shown in Fig. 2C (middle) and Fig. 2F (left). The basal surface of these epithelial structures faces outward (Fig. 3). The large envelops covering the epithelial structures are not shown in Fig. 3 (for example, mesothelium or upper layer of chorion is not shown). I will also consider morphogenesis of the gut and the trachea here, which is not included completely by a larger envelope, but main morphogenesis takes place inside of the larger envelope (villi, folds and crypts of the intestine and branching of the lung, salivary glands and mammary glands). In combination of elemental processes [PRT+aREC], which is the same topology of [ELG+aREC], two protruded folds in the outflow duct of the embryonic heart fuse and reconnect with each other. Then two

arteries form, which correspond to formation of the aorta and the pulmonary artery (Fig. 3A). Here conotruncal swelling ridges fuse with each other to make the conotruncal septum that forms the aorta and the pulmonary artery (e.g., Schoenwolf et al., 2009). Elemental process [PRT] to the intestine tube corresponds to formations of villi or folds (Fig. 3B left). I do not know an obvious case corresponding to [PRT+bREC] (Fig. 3B right). A new envelope is nested within the previous envelope. This is secondary nesting, because the previous envelope has been made by nesting ([IVG+aREC], Fig. 2C). Its situation is similar to a mammalian embryo that is enclosed by blood fluid in the placenta, but it should be examined in detail. A case corresponding to [PRT+aREC] is not known (Fig. 3C). It seems to be a torus within the connective tissue. Its apical-basal polarity is opposite of the early embryo (Fig. 2D). Application of [IVG] to an envelope corresponds to a case of formation of crypts of the intestine (Sato et al. 2011). Iterative invagination causes branching of a cavity, which gives rise to the structure of the lung (Fig. 3D right), the salivary glands and the mammary glands. When considering invagination as sprouting, a new sprout from an existing capillary forms branching structure. Furthermore, additional [bREC] causes fusion to create an anastomosing

network. Thus, [IVG + bREC] corresponds to formation of angiogenesis in capillary networks (Fig. 3E right). An example of an elemental process between two epithelial envelopes is [bREC] of the amnion cavity and the yolk sac in the mammalian embryo (Fig. 3F right), followed by formation of an isolated embryo between them.

The results are summarized in Fig. 4. This is the world of epithelial sheets. Initially, simple epithelial envelopes form, and then several elemental processes operate sequentially on the envelope to produce an epithelial sheet of various shapes, which is suitable for life of living organisms.

An epithelial envelope is variously deformed through combinations of the elemental processes under the condition that it is not separated with each other (Fig. 2). However, it can become two envelopes through nesting, which make further complex morphogenesis (Fig. 3). However, we do not know a few of structures as real, for example, inverted torus in connective tissues (Fig. 3C) and an envelope nested in higher order (nested after nested) (Fig. 3B right).

Classification of epithelial cells

Epithelial sheets consist of epithelial cells, which are prism-like in shape and packed in a two-dimensionally extended monolayer sheet. Each typical epithelial cell has top and bottom surfaces. One of these surfaces is apical, the contact-free surface. The apical surface of a cell is surrounded by apical junctional complexes, which are composed of tight junctions and the adherens junction (Fig. 5A). We can say that each epithelial cell has one apical domain.

Other types of epithelial cells exist (Honda, 2010; 2012). Tubes such as the trachea and vascular or lymph vessels are epithelia. As shown in Fig. 5B–D, most epithelial cells in tubes are a types of cells that have one apical domain. Cells close to the tip of tube, however, are of different types (Fig. 5E and F). When a tip of capillary touches another capillary, and the two capillaries link together (Wolff and Bar, 1972), the tip cell (Fig. 5F) of the capillary becomes a tunnel cell (Fig. 5E). The tunnel cell has a penetrated aperture, but it is distinct from a seamed cell, which is made by seaming two edges of a single cell (Fig. 5D). The formation of tunnel cells (i.e., seamless cells) has been investigated in detail in *Drosophila* trachea and zebrafish intersegmental

vessels (Tanaka-Matakatsu et al., 1996; Kamei et al., 2006; Kakihara et al., 2008). On the other hand, a tip cell has one apical domain that is concave (Fig. 5F).

Next, we will consider the apical surface of epithelial cells. In particular, we will carefully evaluate which surface of the cells is apical during morphogenesis. Figure 6A shows the process of capillary angiogenesis. Cell proliferation adds an endothelial cell to a capillary, and fusion of cell membranes takes place twice (arrows in Fig. 6A). Then, a vacuole in the added cell becomes linked to the lumen of blood capillary (Fig. 6A, bottom). Because the inner surface of the blood lumen is apical, according to this schematic, it is very likely the membrane of the vacuole in the added cell is apical (Fig. 6A, top). This idea has been supported by experiments showing that an antibody specific for the apical membrane becomes localized at the intracellular vacuole in a polarized epithelium (Le Bivic et al., 1988). Therefore, we can conclude that an epithelial cell contains its apical membrane on a vacuole. This addresses issue (3) raised in the Introduction: “Relationship between the two apical surfaces”.

Next, we will consider other types of epithelial cells (Honda, 2010; 2012). Hepatocytes are classified as epithelial cells, but their structures are not simple (Fig. 6B).

In the liver, a hepatocyte has multiple apical surfaces of bile canaliculi, through which bile is secreted. Thus, each hepatocyte has more than one apical domain. Taking this into account, we compiled a classification table of epithelial cells (Fig. 6C). All epithelial cells have one or more than one apical domains of cell membrane. Epithelial cells that have apical domains inside are Type 0. Type 1 cells have one apical domain on the outer surface. Epithelial cells of Type 1 include not only prism-like cells, but also cells that have a concave apical domain (cup type) and a tunnel (seamless) type cell. Hepatic type cells have more than one apical domain. We define Type *n* cell as a cell which has *n* apical domains. This addresses issue (4) raised in the Introduction, "Structural difference between hepatic and prism-like cells".

Vacuolar apical compartment (VAC) in epithelial cells

Above, we pointed out that epithelial sheets have no choice but to make a closed envelope, and classified epithelial cells in several types (Fig. 6C). To understand the mechanisms that cause epithelia to have these properties, we must introduce an

organelle within epithelial cells, the vacuolar apical compartment (VAC). The VAC, an intracellular organelle found in MDCK cells (Fig. 7A), exhibits apical biochemical markers and microvilli and excludes basolateral markers (Vega-Salas et al., 1987; 1988). Exocytosis of VAC occurs on cell surface near areas of cell–cell contact. VAC exocytosis mediated by cell–cell contact plays a role in establishing epithelial cell polarity.

Considering the role of the VAC enables us to understand the classification of epithelial cells (Honda, 2010; 2012). As shown in Fig. 7C (left), when an epithelial cell is surrounded by collagen gel, the VACs in the central part fuse with each other to form a large vacuole (Type 0). As shown in Fig. 7C (right), fusion of the vacuole with the outer cell membrane produces a concave apical domain (Type 1, cup-type). Successive fusion of the concave part with the outer cell membrane leads to penetration of the concave part through the cell, producing another sort of Type 1 cell (tunnel or seamless). In aggregates of epithelial cells within collagen gels, VACs near areas of cell–cell contact fuse with the cell membrane, i.e., apical membrane is supplied to the cell surface at sites of cell–cell contact (Fig. 7D). In this case, the epithelial cells become

hepatic type cells (Types 2, 3, …). In aggregates of epithelial cells on the surface of collagen gel, VACs near areas of cell–cell contact also fuse with the cell membrane (Fig. 7E). Because the cell membrane is free from collagen gel, the fused VAC membranes spread on the outer cell surface, and ultimately become the large apical domain of a prism-like epithelial cell (Fig. 7E). It is noteworthy that hepatic-type cells are produced first, whereas typical prism-like cells are produced at the end. Indeed, prism-like and hepatic-type cells are interchangeable, and the conversion is controlled by PAR1 (Cohen et al., 2004). A transition from the depolarized state to apico-basal polarity through hepatic polarity has been reported in an experiment using a calcium switch model, in which calcium induces reassembly of apical junctions and F-actin reorganization (Ivanov et al., 2005).

Several times in this review, we have emphasized that an epithelial sheet has no choice but to make a closed envelope. This seems mysterious because the epithelial sheet is behaving as if it has a mind of its own. When we introduce VACs to our model of morphogenesis, however, we can understand how an epithelial sheet can autonomously form a closed envelope (Honda, 2010; 2012). As shown in Fig. 7B top, in

aggregates of epithelial cells surrounded by collagen gel, VACs migrate to areas of cell–cell contact, fuse with the cell membrane, and produce an apical space (lumen) in the cell aggregate. Following repeated cell divisions, such an aggregate with a lumen becomes a closed envelope (cyst or follicle) (Nitsch and Wollman, 1980; Folkman and Haudenschild, 1980; Toda et al., 1993). As shown in Fig. 7B (bottom), a single cell in collagen gel also grows to be a closed envelope with a lumen via several stages: enlarged vacuole, cell divisions, and fusions of the cell membrane. This addresses issue (1) in the Introduction, “Formation of envelopes without leakage”. It should be noted that the closed envelope of a cyst or follicle is surrounded by a basement membrane and contains no cells in its lumen. Cells within the lumen are eliminated by apoptosis (Debnath et al., 2002; Martin-Belmonte et al., 2008; Yamamoto et al., 2015). By contrast, within the closed envelope of mammalian blastocyst, the cells of the inner cell mass remain alive. The blastocyst is surrounded by apical surface and its inside is basal space, because the inner surface is lined with basement membrane. Indeed, previous work has demonstrated the presence of basement membrane at the surface of the trophectoderm cells facing the blastocyst cavity (Klaffky et al., 2001).

Genetic and molecular mechanism of establishment of epithelial properties.

We have described a variety of epithelia, and understood formation of the epithelial cell types and formation of the closed envelope of epithelia via the function of a characteristic organelle, the VAC. In addition, we have discussed how the apical and basal surfaces (i.e., apicobasal polarity) are related to the behavior of VACs. We have also touched on deformation of epithelial sheets such as elongation and branching. Now, we attempt to explain these epithelial properties in terms of genes and molecules.

Formation of apicobasal polarity

Apicobasal polarity (AB polarity) is essential for epithelial cells, and is deeply related to the apical and basal surfaces of epithelial sheets (Bryant and Mostov, 2008). Here are epithelial cells (MDCK cells) on external cell matrix (ECM) (Fig. 8A). Laminin molecules in the ECM contact the cells. Rac1 (RAS-related GTP binding protein) in the cells control extracellular laminin assembly, and laminin in turn activates

Rac1, forming an autocrine pathway. These molecules interact with integrins via actin filaments, and establish the basal side of the epithelial cell (O'Brien et al., 2001; Yu et al., 2005). On the other hand, atypical protein kinase C molecule (aPKC) forms a complex with PAR6 (scaffold protein having PDZ domain). When the aPKC–PAR6 complex is close to the membrane area of cell–cell contact, it interacts with PAR3 (scaffold protein having PDZ domains) (Horikoshi et al., 2009). PAR3 and aPKC–PAR6 activate Cdc42 through PTEN-PtdIns(4,5)P2–Annexin II pathway, resulting in cytoskeletal rearrangement (PTEN, Phosphatase and tensin homolog geleted from Chromosome 10; PtdIns(4,5)P2, Phosphatidylinositol 4,5-bisphosphate). PAR3 and aPKC-PAR6 cause the area of cell–cell contact to develop into an apical junctional complex. Here, the apical side is established. A pair of apical and basal sides forms AB polarity. VACs are produced in an integrin-dependent manner at the basal side (Davis Camarillo, 1996; Kamei et al., 2006). Then, the VAC forms a complex with aPKC–PAR6 and moves to the apical side (Suzuki and Ohno, 2006; Horikoshi et al., 2009). Thus, ECM attached to cells defines the location of the basal region, and the area

of cell–cell contact provides cues that promote development of the apical region. This addresses issue (2) raised in the Introduction, “Determination of apical or basal sides”.

Deformation of an epithelial envelope – formation of tubes and tube-networks

Epithelial cells autonomously form cysts (closed envelopes) in ECM. The closed envelope further deforms to be elongated forming tubes and branching networks. The molecular mechanism underlying tube formation has been elucidated (Fig. 8B) (Matsumoto et al., 2014). Treatment of epithelial cells (rat intestinal epithelial cell 6, IEC6) with Wnt3a and EGF results in production of a network of tubes (EGF, Epidermal growth factor). Wnt3a and EGF signals, mediated through the β-Catenin and MAPK pathways, induce expression of Arlc4 (a small G-protein, ADP-ribosylation factor–like c4) and cell proliferation. (The Wnt3a signal suppresses the reduction pathway of β-Catenin, and the resultant increase in β-Catenin promotes the transcription of Arlc4 in the nucleus.) Arlc4 activates Rac1 and suppresses RhoA, resulting in rearrangement of actin filaments, which in turn causes the cell surface to be flexible and promotes cellular deformation. On the other hand, the EGF signal in the MAPK

pathway promotes cell proliferation (MAPK, mitogen-activated protein kinase). The Arlc4 activation and the cell proliferation promote the envelope to grow and become a tube. The tip cell of the tube is also deformed, which causes YAP/TAZ in the cytoplasm of the tip cell to translocate into the nucleus. Then the tip cell proliferates (Fig. 8C), where YAP (Yes-Associated Protein) and TAZ (Transcriptional co-Activator with PDZ-binding motif) are transcriptional activators of the Hippo pathway. On the other hand, Wnt3a and EGF also induce expression of $P2Y_2$ receptor (G-protein coupled purinergic receptor), which breaks bonds between integrin and fibronection and binds integrin. Consequently, the cells detach from the basement membrane, making it easier to form a tube (Fig. 8C) (Ibuka et al., 2015).

Discussion

Genes are thought to make important contributions to morphogenesis. Accordingly, we wish to make a bridge between genes and morphogenesis. Between the two is the world of epithelial sheets, in which cells are actively engaged in elemental processes. These cellular actions are mechano-physical, e.g., cell elongation, cell shortening, cell rotation,

cell locomotion, cell deformation, cell division, and sometimes cell death. We have to investigate two stages, the first stage from genes to the cellular properties that determine cell actions, and the second stage from cell actions to elemental processes (e.g., tissue elongation, invagination, protrusion, and so on). These cell actions are based on cell properties, e.g., cell polarity, anisotropic mechanical properties and so on. The first stage is elucidating using genetic and molecular methods. For example, formation of AB polarity from ECM to cell–cell contact, as well as modification of cell properties by Wnt3a/EGF signals to promote tube formation, as described in the previous section. Planar cell polarity (PCP)-related molecules contribute to the first stage, that is, asymmetric distribution of Dachsous/Four-jointed directs the asymmetrical localization of PCP core proteins such as Frizzled (Ayukawa et al., 2014). Also, the PCP-regulating cadherin Celsr1 introduces anisotropic contraction of apical cell boundaries to promote neural tube formation (Nishimura et al., 2012). On the other hand, mathematical investigations using mechano-physical models have contributed to our understanding of the second stage from cell action to elemental processes. Examples include computer simulations of blastocyst formation (Honda et al., 2008a), tube elongation by cell

intercalation (Honda et al., 2008b), formation of epididymal tubes (Hirashima, 2014), neural tube formation (Inoue et al., 2016), and so on. Thus, the world of epithelial sheets plays a role as a linker between the two stages from genes to cell properties and from cell actions to elemental processes.

Conclusion

The answers to the questions raised in Introduction are summarized here. (1) Epithelial envelopes form from single epithelial cells or an epithelial cell aggregate (Fig. 7B). Epithelial cells make VACs within themselves in response to stimulation by ECM. In a single epithelial cell surrounded by ECM, VACs fuse with each other to make a large vacuole, and the cytoplasm divides. Repeated division forms an epithelial envelope consisting of many cells. In the case of an initial cell aggregate in ECM, VACs in cells attach to areas of cell–cell contact and fuse to outer cell membrane, where VAC membrane is converted to be apical cell membrane. Ultimately, a lumen forms within the cell aggregate. Consequently, there is no risk of leakage of contents during envelope formation. (2) Surroundings around an epithelial cell makes the AB cell polarity (Fig. 8A). ECM induces the basal region, and cell–cell contacts induce the apical region

within each epithelial cell. (3) Epithelial cell membrane consists of apical and basal membranes. An epithelial cell within ECM has basal membrane facing out, whereas the apical membrane is derived from membrane of vacuoles and VACs within the cell. An ordinary epithelial cell has apical and basal membranes on its surface and the apical membrane is enclosed by a boundary of apical junctional complexes (Fig. 5A). The apical surface of epithelial sheets coincides with the apical membrane of cells. (4) An epithelial cell is defined as a cell with apical domains on its cell surface. We classify such cells according to the number of apical domains. The hepatic type of cell has more than one apical domain on its surface, whereas prism-like epithelial cells have only one (Fig. 7D, E).

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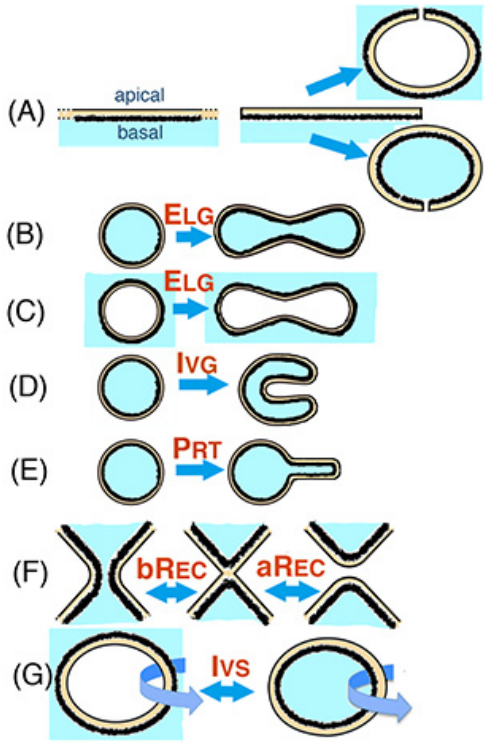

Fig. 1.

Properties of an epithelial sheet and elemental processes of its deformation.

A, Epithelial sheets have two surfaces, apical and basal. A sheet must form a closed envelope. Elemental processes include elongations of envelopes (ELG, B and C), invagination (IVG, D), protrusion (PRT, E), reconnection to alternate topology via fusion (apical surfaces face each other, aREC; basal surfaces face each other, bREC, F), and inversion (IVS, G). The elongation has two cases, elongations of the envelope, outer surface of which is apical (B) and basal (C), respectively.

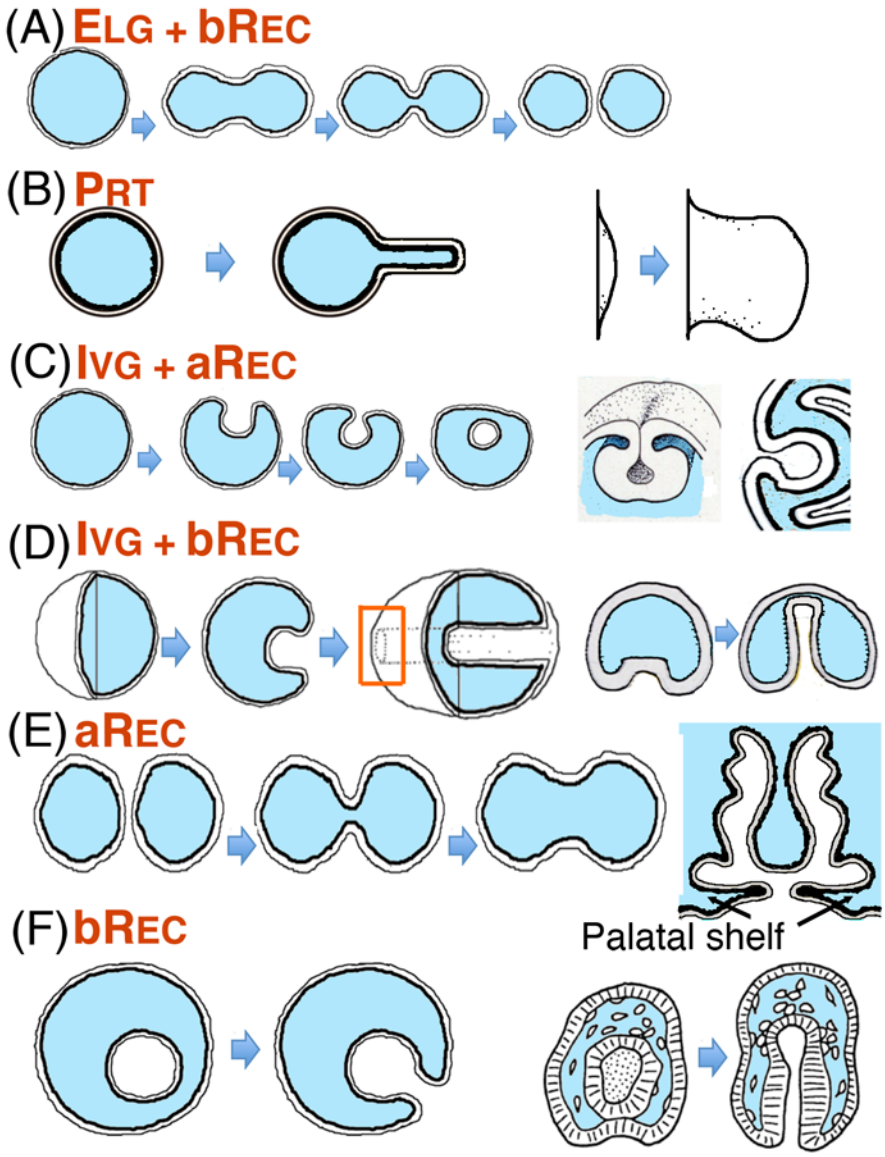

Fig. 2.

Combinations of elemental processes in epithelial envelopes in which the apical surface faces outward (left). Products are shown at right. A, ELG + bREC. B, PRT: protrusion of an envelope, forming a limb bud or wing bud. C, IVG + aREC: formation of neural tube and lens (Honda, 1991). D, IVG + bREC: formation of gut, a torus (doughnut-shape)

(Honda, 1991). Facing of two basal surfaces indicated by rectangle. E, aREC to two envelopes: two apical surfaces of the palatal shelves fuse and reconnected during facial formation. F, bREC to two nested envelopes: gut formation in an experimental reconstruction of the starfish embryo (Honda, 1991). Abbreviations of elemental processes, see the legend of Figure 1.

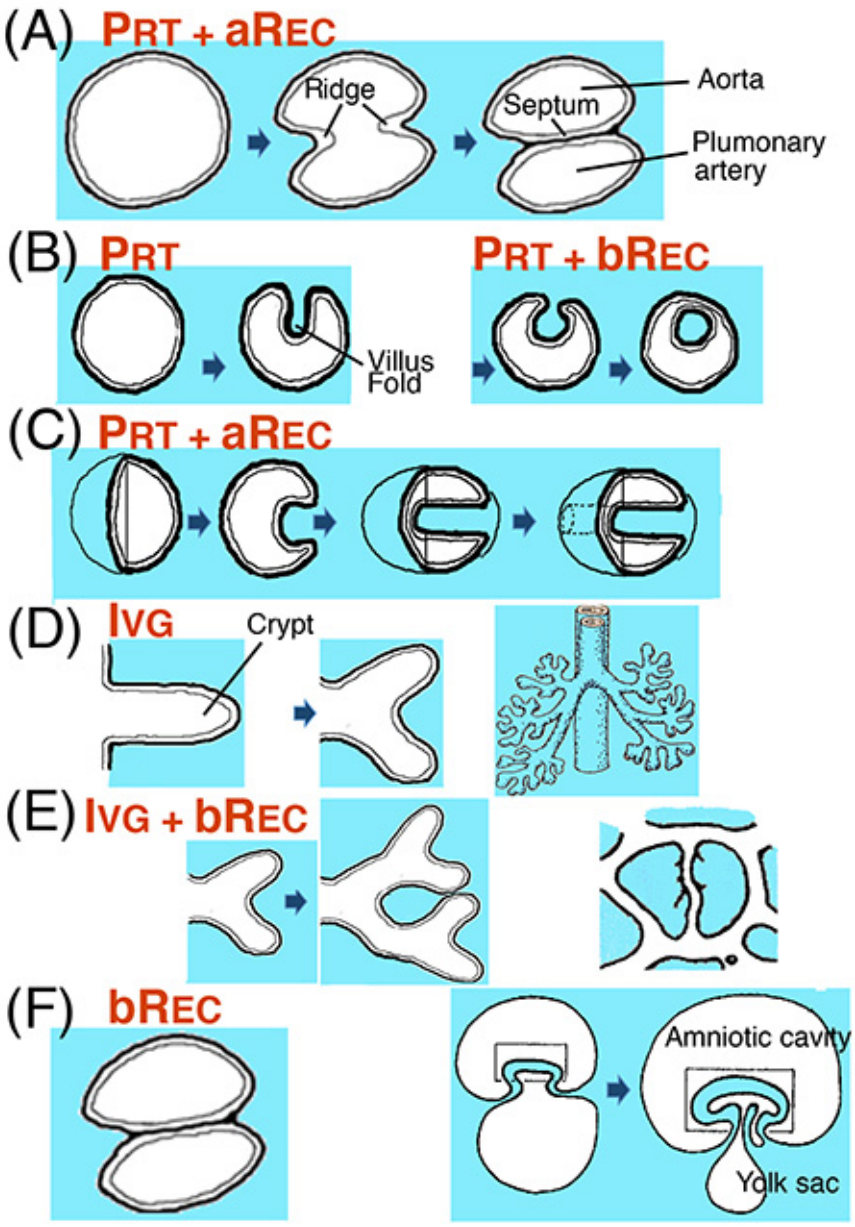

Fig. 3.

Combinations and iteration of elemental processes in epithelial envelopes in which the basal surface faces outward. These structures are almost covered by epithelial sheets (mesothelium or chorion). Products are shown at right. A, PRT + aREC: formation of the

aorta and pulmonary artery. B, PRT: protrusion produces villus or fold of the intestine. PRT + bREC: formation of a closed envelope with apical outer surface, which does not correspond to actual structures. C, PRT + aREC: a torus (doughnut-shape) inverted apical-basal, which does not correspond to actual structures. D, IVG: formation of crypts. Iterated IVG: branching of a cavity, formation of the lung (Honda, 1991). E, IVG + bREC: angiogenesis forming capillary networks (Honda, 2010). F, bREC to two envelopes: formation of a mammalian embryo between the amniotic cavity and the yolk sac. Outer layer of chorion is not shown (Honda, 2010). Abbreviations of elemental processes, see the legend of Figure 1.

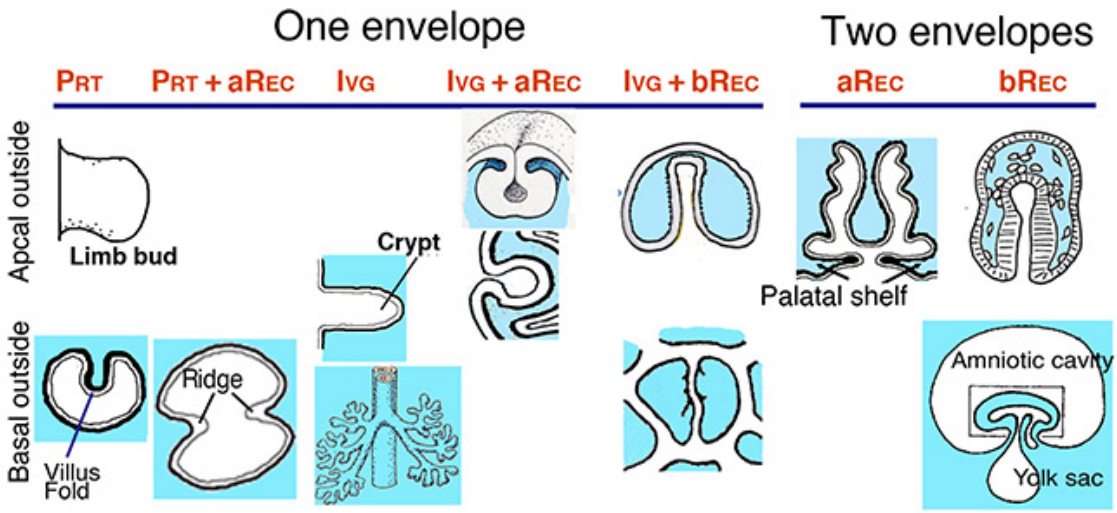

Fig. 4.

The world of epithelial sheets consists of members produced by application of combinations and iteration of elemental processes shown in Fig. 1 to one or two closed envelopes. There are two types of envelopes, outer surface of which is apical and basal (apical outside and basal outside). Figures are taken from Figs. 2 and 3. Abbreviations of elemental processes, see the legend of Figure 1.

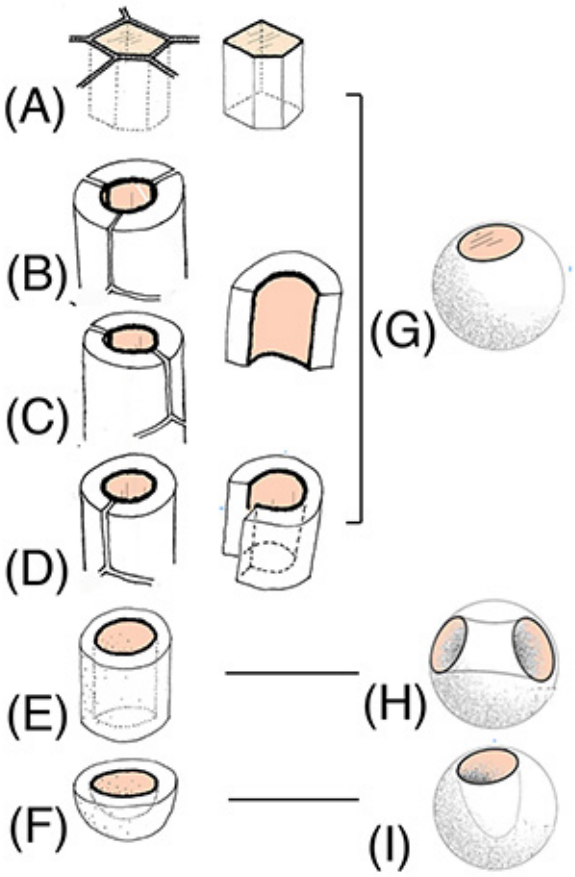

Fig. 5.

Epithelial cells in tissues with one apical domain. Typical prism-like cells (A) and cells that generate a tube (B–D) belong to Type 1 (G). A cell (E) close to the tip of a tube is Type I (tunnel or seamless, H). A tip cell (F) of a tube is Type I (cup, I) (Honda, 2010; 2012).

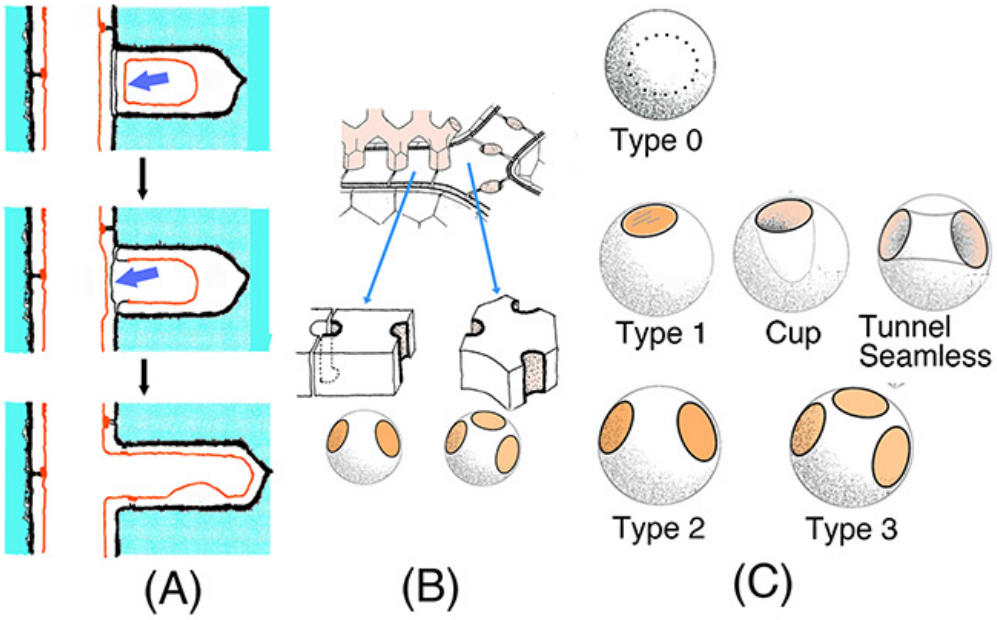

Fig. 6.

Consideration of apical domains and classification of epithelial cells. A, Process of capillary angiogenesis. A cell is added to a capillary by cell proliferation, and fusion of cell membrane takes place twice (arrows); ultimately, the capillary branches. The vacuole in the added cell is considered to be enclosed by apical membrane (Honda, 2010). B, Hepatocytes of liver form bile canaliculi, and they have more than one apical domain (Honda, 2012). C, Classification of epithelial cells. Type 0, epithelial cells with apical domains inside. Type 1, epithelial cells with have one apical domain, includes-cup type and tunnel (seamless) type. Types 2, 3, …, epithelial cells have two, three, or more apical domains (Honda, 2012).

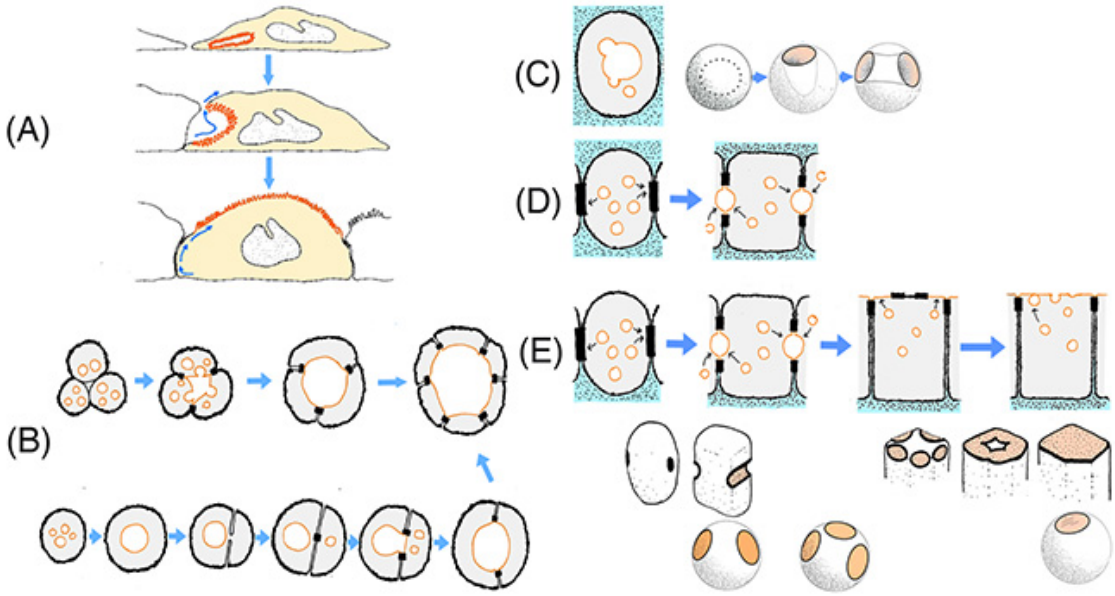

Fig. 7.

Behavior of the VAC (vacuolar apical compartment) and its contribution to epithelial morphogenesis. A, Behavior of VAC (red). VAC that is close to areas of cell–cell contact and fuses to form apical membrane. Reproduced from Vega-Salas et al. (1988). B, formation of a closed-envelope structure from a cell aggregate (top) or a single cell (bottom) (Honda, 2010). C, Formation of Type 1 (tunnel or seamless) cells (Honda, 2012). D, Formation of hepatic type cells (Types 2, 3, …) within ECM (Honda, 2012). E, Formation of a prism-like cell, Type 1 on ECM surface (Honda, 2012).

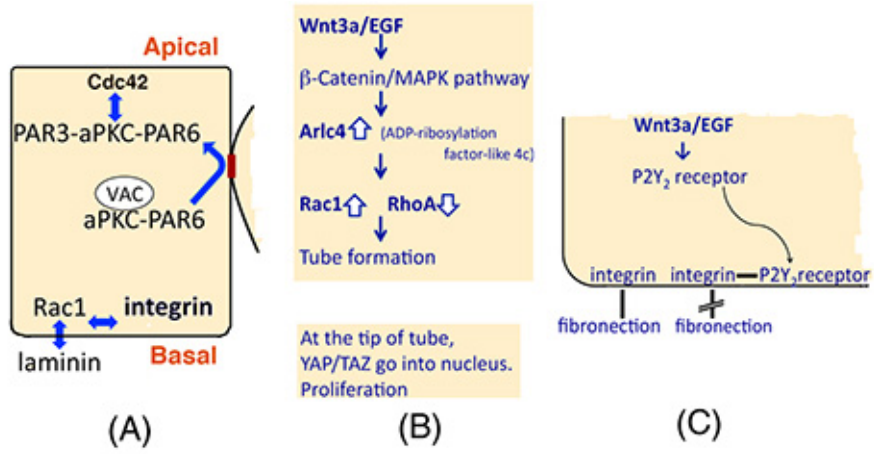

Fig. 8.

Genetic and molecular mechanism of formation of apicobasal polarity and tube formation. A, Formation of apicobasal polarity. See text. Cdc42, Rho family GTP-binding protein; PAR3, PDZ-domain-containing scaffold protein; PAR6, PDZ-domain-containing scaffold protein; aPKC, atypical protein kinase C. Based on Suzuki and Ohno (2006) and Horikoshi et al. (2009). B, Tube formation. See text. Wnt3a, secreted signaling protein; MAPK, mitogen-activated protein kinase; Arlc4, ADP-ribosylation factor-like 4c; Rac1, Rho family GTP-binding protein; RhoA, Rho family GTP-binding protein; YAP, Yes-associated protein; TAZ, transcriptional co-activator with PDZ-binding motif. Based on Matsumoto et al. (2014). C, Tube formation via integrin modification. See text. $P2Y_2$ receptor, G-protein coupled purinergic receptor. Based on Ibuka et al. (2015).