Essence of Shape Formation of Animals

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Although the shapes of multi-cellular animals are diverse, they share the simple unifying principle that each of their bodies is completely enclosed by an envelope of epithelium. In the course of development, the envelope becomes highly convoluted, resulting in the variety of final animal forms. Furthermore, epithelial cells possess an intrinsic ability to form envelopes automatically in the absence of any extrinsic direction or information. This capacity is derived from the behaviors of 'apical vesicles', which are membrane structures created within epithelial cells. Epithelial cells are classified into six cell polarity types, type 0, type 1, type 2, type 3, cup type and tunnel type, which arise from behaviors of apical vesicles.
Key words: Animal Shape, Apical Vesicle, Epithelial, Cell Polarity Type, Self-Construction

1. Introduction

The shape of living organisms is governed by the genome. Mechanism of the genome government is a fundamental problem in biology. Needless to say, multi-cellular organisms consist of cells and cell products. The problem then could be divided into two parts that are rather independent of each other: how the genome determines characteristics of cells, and how the cells construct body shapes based on their characteristics. In the first part, molecular and cellular biology has been elucidating the cell characteristics including cell behaviors. In this paper, the second part, the construction of shapes by cells is discussed.

2. The Animal Body is Completely Enclosed by an Epithelial Sheet

Anatomy and histology textbooks often give the impression that the shapes of multi-cellular organisms are extremely complicated, such that it is difficult to appreciate their overall organization. However, a simple principle underlies these shapes; the body surface of multi-cellular animals is an envelope that completely encloses the body interior (Honda, 1991, 2010).

2.1 An envelope encloses the animal body

Externally the body is bound by the epidermis of the skin, as shown by the outline in Fig. 1. The skin is connected anteriorly to the mouth and posteriorly to the anus. Moreover, the skin is continuous with the internal linings of the mouth, the esophagus, the stomach, and the small and large intestines. Each of these external and internal walls is an epithelium (i.e., a sheet of epithelial cells). Thus, the epithelial sheet extends as a tunnel through the mouth and the alimentary canal to reconnect with the skin at the anus. A tube of the epithelial sheet branches into the lungs at the esophagus where it bifurcates many times, forming a branching system of epithelial tubes terminating in dead ends at the alveoli. Tubular branches of the epithelial sheet also lead into the pancreas and the liver at the duodenum. In addition, the epithelial sheet of the skin surface is invaginated into a tube leading to the kidney. The epithelial sheet, representing the boundary between the inside and the outside of a body, is drawn as a thick black line in Fig. 1. Although the purpose of this diagram is not to portray the internal organs, in fact most organs in a body are automatically delineated. That is, the boundary layer penetrates far into the interior of the body, and compartments topologically equivalent to 'outside spaces' are bound by the epithelial sheet within the interior of the body. Therefore, the extensions of the epithelial sheet provide a convenient basis for understanding the structure and shapes of multi-cellular animal bodies (Honda, 1991, 2010).

2.2 Formation of animal shape by transformation of the envelope

An initial stage in the formation of many animal shapes is an epithelial sheet in the form of a simple hollow sphere (e.g., sea urchin blastula, Fig. 2a). During the development of the sea urchin, a local area on the surface of the ball of cells becomes depressed (b) and the epithelial sheet invaginates, generating a tube (c). The tube then elongates and comes into contact with the opposite wall of the sphere (d). Finally, the tip of the tube unites with the wall and the two epithelial sheets fuse. The fusion creates an opening at the contact point that becomes the mouth, while the initial depression becomes the anus. The process is called the gastrulation, or formation of the primitive gut. Figure 3 shows the process of formation of the neural tube. In this case, the epithelial sheet (ectoderm) invaginates to form a groove (a). The two edges of the groove fuse with each other (b) forming the neural tube (c).

Two types of fusion between two epithelial sheets have been identified (Fig. 4a). In the first, the initial contact is between the outer surfaces of the two sheets (Fig. 4a, left to right). In the other, the initial contact is between the inner

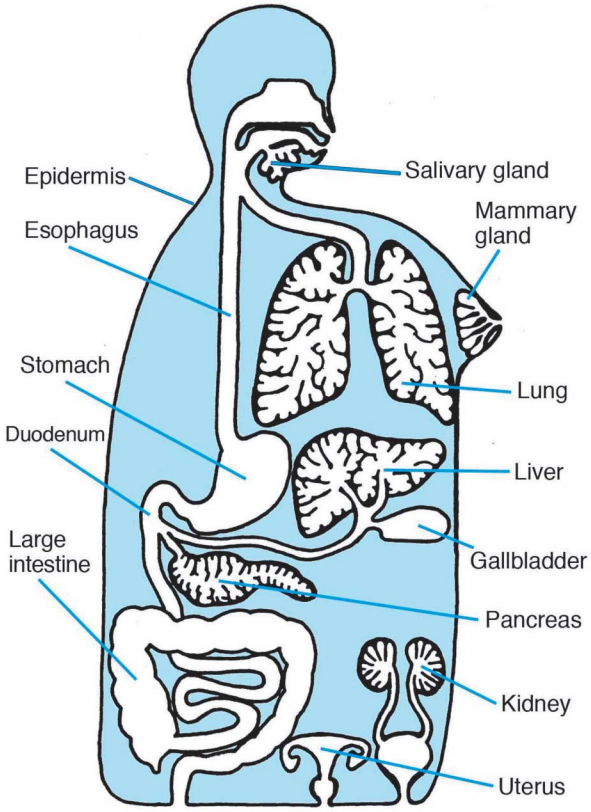

Fig. 1. A body is enclosed by an envelope of the epithelial sheet. The epithelial sheet is shown by solid line. Because the epithelial sheet is continuous, the picture is drawn with a single stroke.

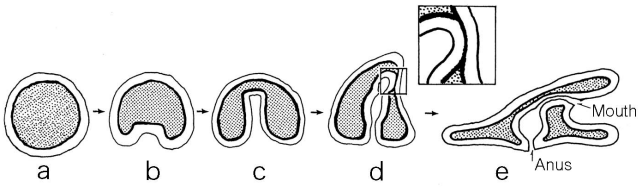

Fig. 2. Formation of the primitive gut in the early development of the sea urchin. Box, Initial contact between the inner surfaces of the epithelial sheet.

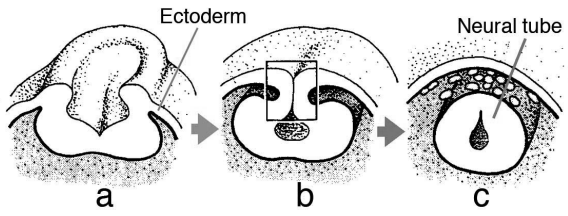

Fig. 3. Formation of the neural tube in the early development of the amphibian embryo. Box, Initial contact between the outer surfaces of the epithelial sheet.

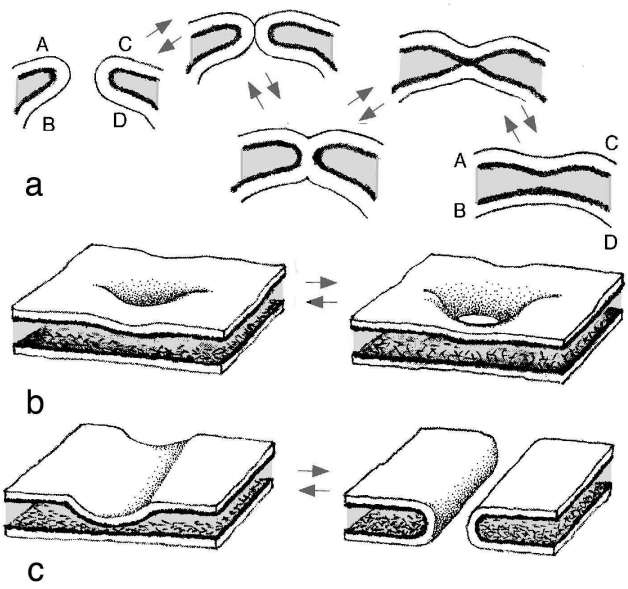

Fig. 4. Fusions between two epithelial sheets. a, Initial contact between the outer (apical) surfaces (left to right) and between the inner (basal) surfaces (right to left). Epithelial sheets are reconnected from A–B and C–D to A–C and B–D (left to right). Fusions form a well (b) and a groove (c).

surfaces of the two sheets (right to left). In each case, the invaginations can form either a well (b) or a groove (c).

Figure 5 shows the formation of the lungs by repeated branching of tubes. The upper part of the esophagus swells and forms a protrusion (a). The protrusion then divides into two (b, c), each of which becomes a new tubular branch (d). Repeated branching then leads to the formation of the two halves of the lung. Thus, we conclude that the formation of animal shapes consists simply of the expanding transformation of the epithelial sheet by a combination of such processes of protrusion, fusion, and branching (Fig. 6). Although these processes are repeated in a complex way, the partitioning between outside and inside compartments by the epithelial sheet is strictly maintained (Honda, 1991, 2010).

Human bodies have two other systems of epithelial sheets (Fig. 7). The circulatory system consists of complicated branching tubular blood vessels. They are lined internally by a category of epithelia called endothelia. The endothelium forms the boundary that encloses the blood fluid and separates it from other interior spaces of the body. A second system consists of body cavities (the coelom) partitioned by the coelomic epithelia. Three main body cavities are the peritoneal cavity surrounding the gut, the pleural cavity containing the lungs, and the pericardial cavities around the heart. The circulatory system and the coelomic cavities are enclosed within the epithelial envelope of the whole body.

The mechanism of transformation of the epithelial sheet to form animal shapes may be briefly summarized as follows (Honda, 2010). Initially the epithelial sheet consists of many equivalent cells. The cells then differentiate into several different types along a concentration gradient of a morphogen that defines the embryo's body axis. Sorting of the cell types divides the epithelial sheet into a number of compartments. Next, cells along the boundaries between the different compartments become specific cells. A number of compartment boundaries appear, and where two boundaries cross, cells at the crossing point become further

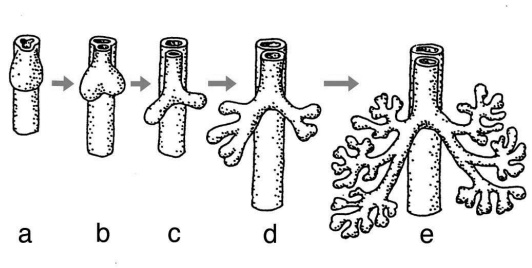

Fig. 5. Formation of the lungs by repeated branching of tubes.

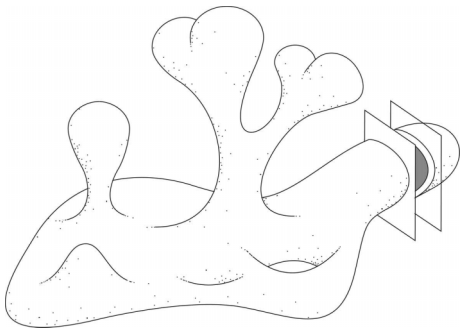

Fig. 6. Transformation of the epithelial sheet by processes of protrusion, fusion, and branching.

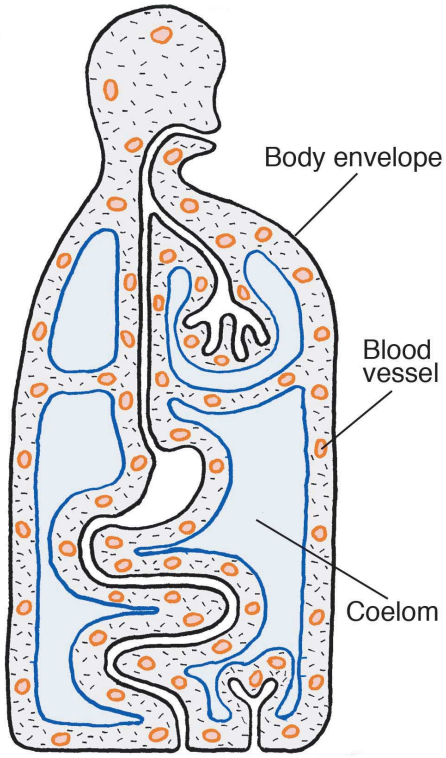

Fig. 7. The epithelial envelope of the whole body encloses the system of blood vessels and the coelomic cavities.

specific cells. These specific cells show various behaviors. For example, frequent cell division may take place, leading to protrusion of the epithelial sheet. These protrusions form appendages such as wings and legs. Embryologists are able to plot points on the blastula surface that develop into particular structures (e.g., points that develop into eyes, mouth, wings, or legs; Fig. 8). The pattern plotted on the blastula is called a 'fate map', which reflects the final body plan of the animal.

2.3 Why does the epithelial sheet completely enclose the animal's body?

The epithelial sheet functions to prevent the body contents from leaking out and to protect the body from invasion by foreign substances. Another important function is to define the boundary of the space under the control of the genome (Honda, 2010). According to Dawkins (1992), the biological world in the process of evolution is an 'examination hall for genomes'. An individual body is the gene's vehicle (Fig. 9) and the genome is the blueprint for body formation. The body that is the product of the genome is examined to evaluate not only the body, but also the genome. If the body survives and produces descendants in the biological world, the genome carried by the body persists into the next generation. Therefore, for rigorous testing of the genome, the body should not contain products of other genomes. The epithelial sheet is the barrier that prevents the penetration of the products of other genomes into the interior of the body.

3. Self-Construction of the Epithelial Envelope

As discussed in Subsec. 2.1, multi-cellular organisms are completely enveloped by an epithelial sheet. Let us consider how this epithelial envelope is formed. In the early stages of development, only the epithelial sheet is present; other tissues (e.g., mesenchyme) are absent from the body of the embryo (e.g., in the sea urchin blastula). During cell division, the cells first form an aggregation, which then transforms into a hollow ball. This process establishes the individuality of the organism. Formation of the shape of the envelope then starts by complex folding of the surface. The formation of a hollow ball seems to be an intrinsic property of the cell aggregate and occurs in the absence of any extrinsic direction or information. In other words, the epithelial cell behaves as if it must inevitably form an envelope (Honda, 2010).

3.1 Computer simulation of the formation of a hollow ball

Mathematical modeling is a useful method to investigate self-construction of cell aggregates (Honda, 2010). After incorporation of some of the cell characteristics into a mathematical model, cell behaviors can be simulated in a computer to investigate the shapes that result.

3.1.1 Equation of motion for cell behaviors Multicellular organisms consist of cells and cell products, and morphogenesis (the development of the shape of multicellular organisms) results from cell behaviors. In contrast to biological systems, analysis of physical materials, such as solid crystals, liquids and gases, consists of atoms or particles. One of the reasons why the physical sciences have made such great advances is that physicists have developed equations of motion for atoms. These equations have led to a better understanding of the properties of physical materials. Thus, development of an equation of motion for cell behaviors would be a powerful tool for understanding cell

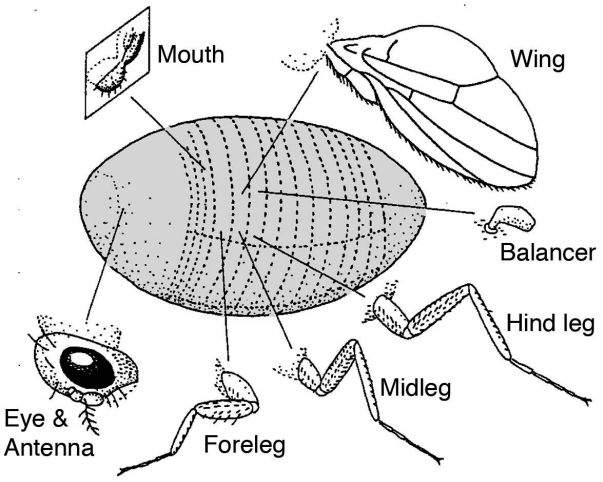

Fig. 8. Fate map plotted on the blastula of the fly embryo.

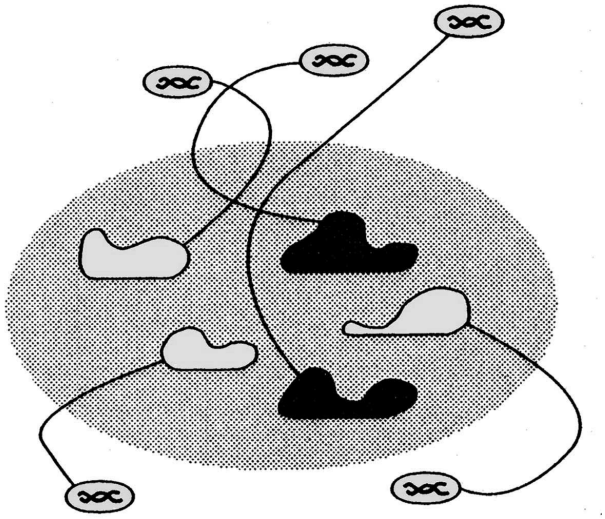

Fig. 9. Individual bodies are the gene's vehicles in the biological world. Each body is produced under the direction of its own genome. If a body survives and produces descendants, the genome carried by the body persists into the next generation.

morphogenesis. The present author has been deriving an equation of motion for cells over many years with collaborators (Masaharu Tanemura and Tatsuzo Nagai) that has been successfully applied (Honda *et al.*, 2004). Our equation of motion for cells allows us to demonstrate how a cell aggregate can automatically become hollow (Honda *et al.*, 2008).

A tissue composed of multiple cells is considered as a 3D space tessellation consisting of polyhedra without gaps or overlaps. When we know the positions of all vertices of an aggregate of polyhedra, the cell interface areas and cell volumes are expressed as x, y and z coordinates of the vertices (Fig. 10). The vertices obey the equation of motion,

$$\eta d\boldsymbol{r}_i/dt = -\boldsymbol{\nabla}_i U \quad (i = 1, \ldots, n_v), \tag{1}$$

where $\boldsymbol{r}_i$ is a 3D-positional vector of vertex i, the nabla $\boldsymbol{\nabla}_i$ is a differential operator of $\boldsymbol{r}_i$ and n_v is number of vertices. The left side of Eq. (1) represents a viscous drag force proportional to the vertex velocity $d\boldsymbol{r}_i/dt$ with a positive constant η (an analog of the coefficient of viscosity). Vertices do not have mass (inertia), so the motion of the vertices is completely damped. The right side of Eq. (1) represents a potential force (driving force), i.e., minus the gradient of the potential U. Change of U with time (dU/dt) is shown to be negative or zero, according to Eq. (1). Then, the vertices move so that U becomes smaller (Honda, 2011). When we put the term of the total surface area into U, the vertices move so that surface energy becomes small (i.e., surface tensions are working). When we put the total elastic terms of the cell volumes ($\Sigma_\alpha (V_\alpha - V_{\mathrm{O}})^2$) into U, the vertices move so that the cells maintain their original volumes, V_{O}. Here V_α and V_{O} are volumes of cell α and the cell volume at the original relaxed state, respectively.

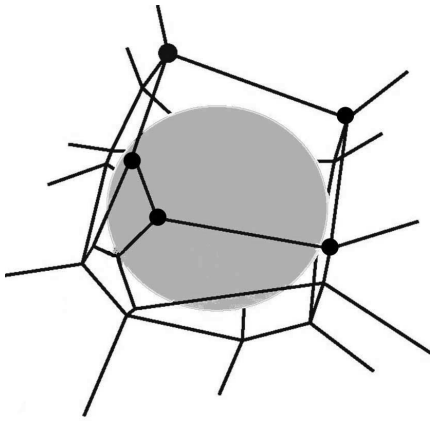

Fig. 10. Shape of polyhedral cells are described by vertex positions.

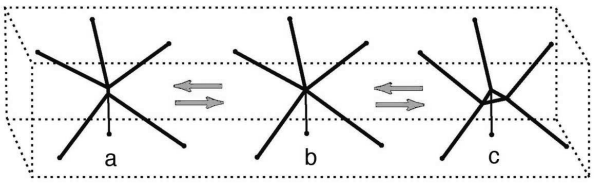

Fig. 11. A short edge (a) and a small triangle (c) are transformed with each other by reconnection of vertices.

During calculation of the equation of motion, the vertices move and sometimes make a short edge or a small triangle. Then, we perform reconnections of vertices as shown in Fig. 11. Transformation takes place between a short edge (a) and a small triangle (c). After the transformation, the potential U decreases further. The transformation changes the shape of polyhedra, e.g., face and edge numbers.

3.1.2 Computer simulation We were then able to apply the equation of motion for cell behaviors to investigate the formation of a hollow ball (Honda *et al.*, 2008). We assumed an aggregate of 40 cells and that the cells are secreting fluid. The increasing volume of the fluid forms a cavity. To simulate the cavity formation, we introduced a new term, $(V_{\mathrm{C}} - V_{\mathrm{CO}})^2$, into U, where V_{CO} is the increasing volume of fluid and V_{C} is the cavity volume. When we increased the volume of liquid V_{CO} with time, the cavity ap-

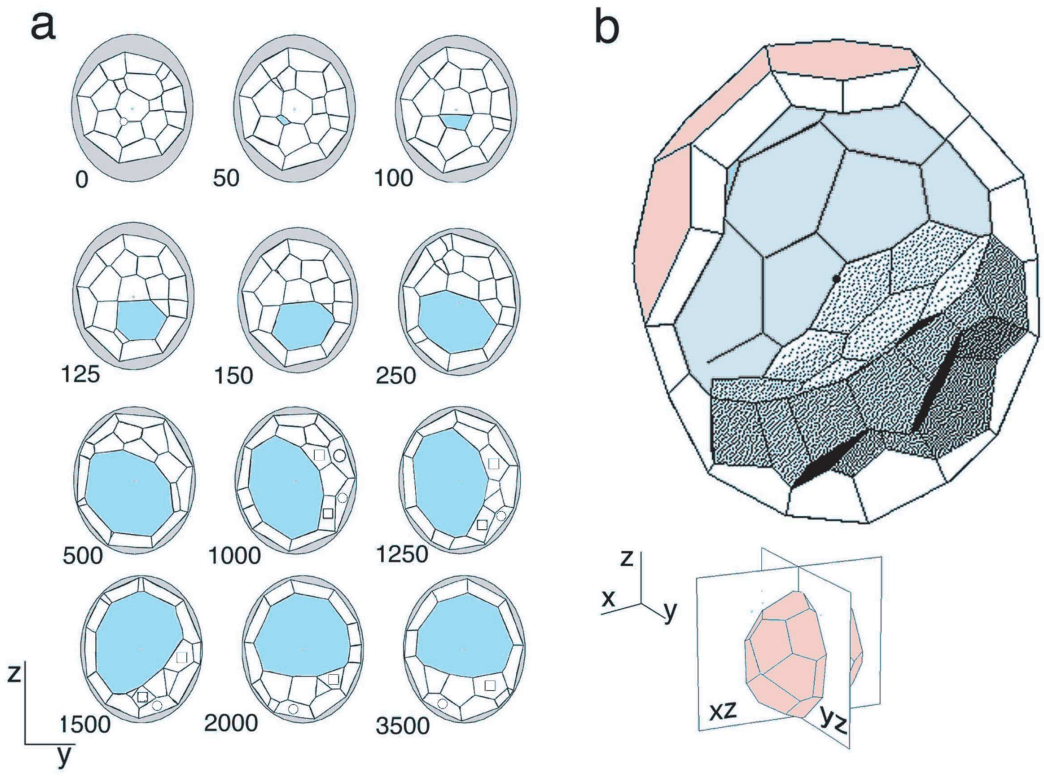

Fig. 12. Computer simulation of formation of the mammalian blastocyst. a, A process of the cavity formation. Sectional views. b, A 3D view of the result.

peared in the cell aggregate and grew, as shown in Fig. 12a. The cell aggregate was transformed into a hollow ball (b). This process modeled in the computer simulation closely resembled the formation of the mammalian blastocyst and shows that it is plausible that cells construct a follow ball by themselves.

3.2 Classification of epithelial cells and derivation of classes

There are several types of epithelial cells in the epithelial sheets that form the envelope of the animal body. List the various types of epithelial cells is given below, and it is proposed that they reflect different behaviors of 'apical vesicles' within cells (apical vesicles are membrane structure and contribute significantly to the self-construction of the envelope, as described in Subsec. 3.3).

3.2.1 Classification of epithelial cells A common type of epithelial cell is shown isolated from the epithelial sheet in Fig. 13a. It possesses an apical membrane domain facing the external medium (fluid or air space), and on the other surface of the cell, the basolateral membrane faces the basement membrane and neighboring cells (b). This type of epithelial cell exhibits apical-basal polarity. The apical domain is encircled by apical junctions (tight junctions and adherens junctions) characterized by range of specific molecules, as shown in Fig. 13c. The apical junctional zone is drawn as a solid line. We will call this type of cell with a single apical domain 'type 1'.

When we examine tubular structures (e.g., blood vessels), most tubes consist of several epithelial cells forming the circumference of the tube (Figs. 14 a–c). These epithelial cells all belong to type 1, as shown in Fig. 14. However, a few of the epithelial cells in the smallest tubes (capillary vessels) possess a tunnel (Kakihara *et al.*, 2008) or a cup-shaped hollow (Figs. 15a and b). The cup type is similar to type 1, but the apical domain is deeply concave. In addition, a number of individual epithelial cells that are buried in collagen gel have an internal vacuole (Fig. 15c). We name these 'type 0'.

In the case of liver tissue, cell arrays are surrounded by material similar to the basement membrane and endothelia (Fig. 16a). Here, two neighboring liver cells form a bile canaliculus between them. The bile canaliculi are the apical domains of the liver cells, and the remaining surface of the liver cells is the basolateral domain. As shown in Fig. 16b and c, a single liver cell may possess two or more apical domains. Thus we designate liver cells as type 2, type 3, and so on.

3.2.2 Derivation of epithelial cell types The different types of epithelial cells are formed systematically by small vesicles of the cell membrane, as shown in Fig. 17 (Honda, 2009). When the epithelial cell contacts collagen molecules or the external cell matrix, the cell begins to produce small vesicles from the cell membrane materials (Gamble *et al.*, 1993). If the epithelial cell is isolated and has no neighbors in the collagen gel, the small vesicles fuse with each other at the center of the cell to form a large vacuole (a, b). This generates epithelial cells of type 0.

If the epithelial cell has neighbors and makes apical junctions with its neighbors, the small vesicles migrate toward the apical junction and their membranes fuse with the outer

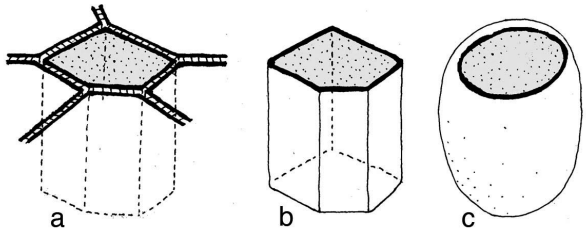

Fig. 13. An epithelial cell in the epithelium (a) has a single apical domain (b, c). The apical domain (dots) is encircled by the apical junction (solid line). This type of epithelial cell exhibiting apical-basal polarity is called type 1.

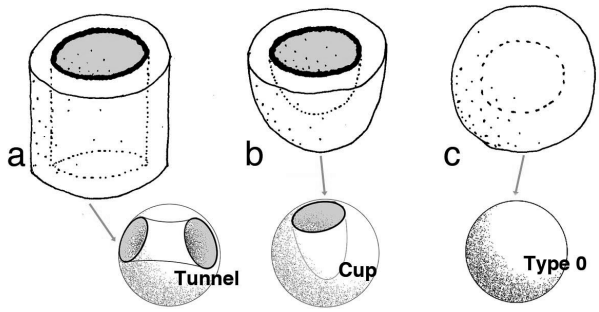

Fig. 15. Epithelial cells having a tunnel (a), a convex face (b) and a vacuole (c). These belong to tunnel type, cup type and type 0, respectively.

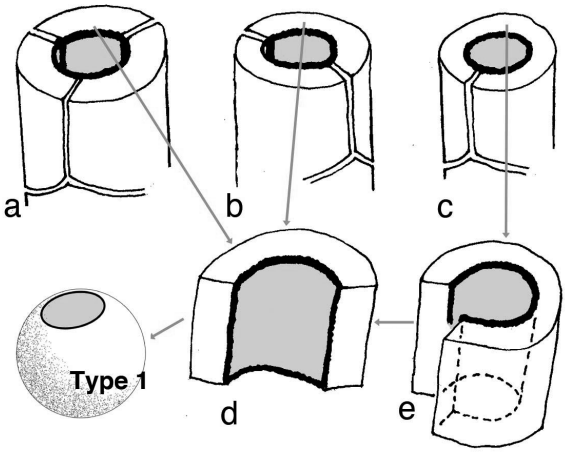

Fig. 14. Epithelial cells in tubes belonging to type 1.

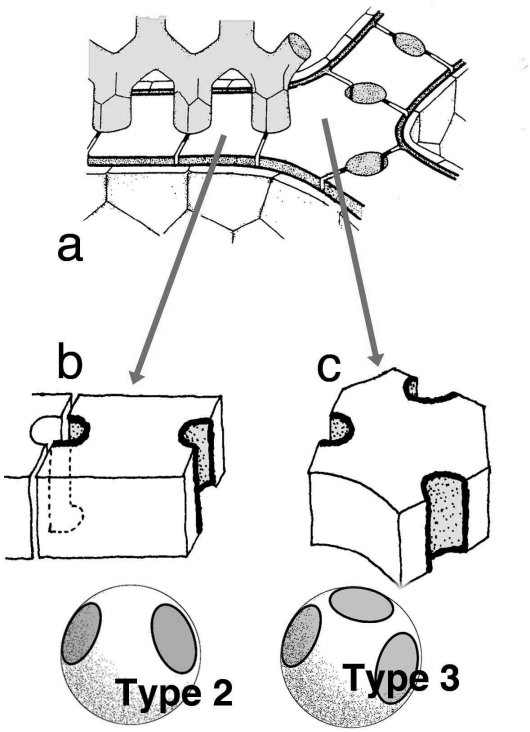

Fig. 16. Liver cells have more than one apical domains. The bile canaliculi consist of the apical domains of liver cells. The liver cells belong to type 2 or type 3.

cell membrane (e). The outer cell membrane is supplied with membrane material from the small vesicle. These apical vesicles correspond to vacuolar apical compartment (VAC, Rodriguez-Boulan and Nelson, 1989). When the epithelial cell has two neighbors, there are two fusion points and, consequently, two apical domains (f, j). When the epithelial cell has three neighbors, it has three apical domains. These epithelial cells correspond to the type 2 and type 3 cells of the liver.

When a cell aggregate faces a space lacking collagen gel, many of the epithelial cells in it face a collagen-free space (top of g). Here one epithelial cell is surrounded by several (about 6) others and makes junctions with them all (k). Apical domains increase their area by supply of membrane materials from the apical vesicles (g, k and l), and finally the apical domains merge together to form a single large apical domain (h and m). The single apical domain faces the collagen-free space. This cell of Fig. 17h and m is a typical epithelial cell of type 1.

A cell of type 0 transforms further (Fig. 17b). The membrane of the large vacuole merges with the outer cell membrane to form a large concave apical domain (c). This 'cup type' cell is topologically similar to type 1 cells, but the surface is concave. If the membrane of large vacuole merges with the outer cell membrane at two points, the cell makes a tunnel (d, Kakihara *et al.*, 2008), i.e., forming a 'tunnel type' cell.

The epithelial cell is described conventionally as possessing apical-basal polarity. However, this is not always appropriate. The type 1 epithelial cell has apical-basal polarity, but as discussed, other types exist. Although the liver cell is epithelial, it usually possesses more than one apical domain. On the other hand, an endothelial cell buried in the collagen gel, although belonging to the category of epithelial cells, does not have an explicit apical domain on its surface. The present author, therefore, propose the adoption of the terms 'type 0', 'type 1', 'type 2', etc., and 'tunnel type' and 'cup type' as the appropriate nomenclature for epithelial cells.

3.3 Epithelial cells inevitably form an envelope

3.3.1 Cell aggregate transforming to a hollow structure It was mentioned in Subsec. 2.2 that the formation of animal shapes occurs by transformation of the epithelial sheet by expansion, fusion and branching. Also during

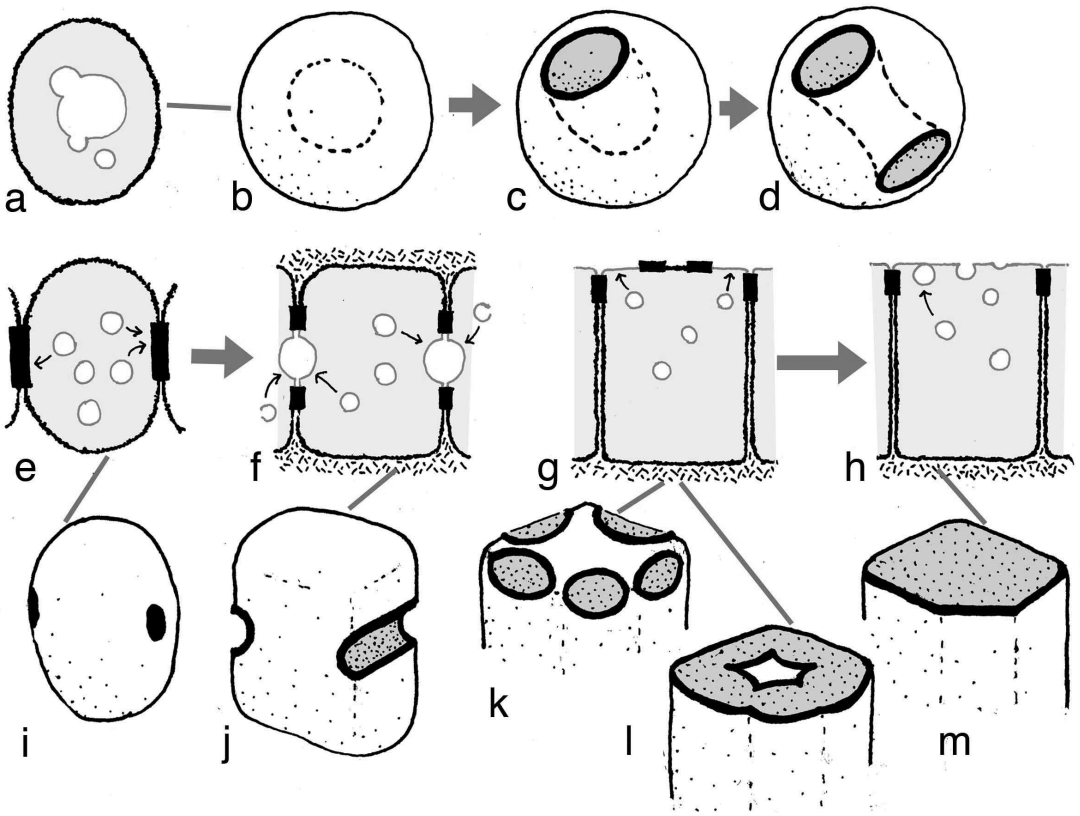

Fig. 17. Activity of the apical vesicles in the epithelial cell forms a variety of cell types. An epithelial cell buried in the collagen gel (a) becomes type 0 (b), cup type (c) and tunnel type (d). An epithelial cell contacting with neighbors in the collagen gel (e, i) become type 2 or type 3 (f, j). An epithelial cell encircled by several neighbors and having a collagen-free space (g, k) becomes type 1 (h, m).

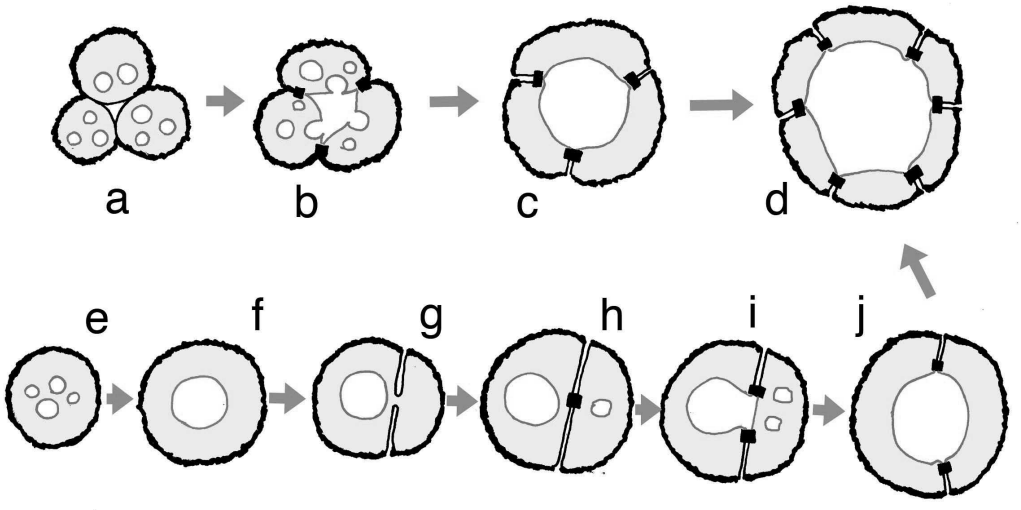

Fig. 18. Two pathways for envelope formation by the epithelial cells. One is originates as a cell aggregate (a–d) and the other is from a single cell that includes a large vesicle (f). The single cell takes place cell divisions (g–i). The envelope consisting of two cells (j) grows by repeated cell division (d).

organogenesis, cell sheets and cell aggregates in the interior of the body form hollow balls or tubes and develop into mature organs. For example, the embryonic small intestine tube is initially packed with cells once, but this mass of cells reforms a tube (Matsumoto *et al.*, 2001). In the development of the branching structure of the kidney, a cell aggregate of metanephric mesenchyme forms a small envelope at first and fuses into the tubular ureter leading to branching structures. The mammalian blastocyst is also formed from a hollow ball, which is in turn formed from a cell aggregate, as described in Sec. 3.1. Thus, epithelial cells typically form hollow balls or envelopes. The ability to form an envelope is considered to be a primary characteristic of epithelial cells.

3.3.2 Formation of a hollow structure by apical vesicles The ability of epithelial cells to form an envelope formation is based on the activities of the apical vesicles within the cells. We have a knowledge of two distinct pathways for envelope formation. The first originates as a cell aggregate (Figs. 18a–d; Nitsch and Wollman, 1980; Folkman and Haudenschild, 1980; Toda *et al.*, 1993). Cells on surface of the aggregate connect with each other and form apical junctions between neighboring cells. Apical vesicles are produced within each cell and migrate to the apical junctions. The apical vesicles construct apical surfaces at the apical junctions. The apical surface is formed on interior side of the cell aggregate, because the outside of the cell aggregate is in contact with collagen gel (a and b). The cell aggregate thus becomes an envelope containing the apical surface within it (Yoshihama *et al.*, 2011). Cells left behind inside the envelope are destined to die through the process of apoptosis (O'Brien *et al.*, 2002; Kim *et al.*, 2007). In the case of the mammalian blastocyst described in Subsec. 3.1, the apical surface is on the outside of the cell aggregate (the morula) and the cells forming the envelope have the basement membrane on the inside. In this case, cells that are left behind inside the envelope during blastocyst formation do not die and form the inner cell mass, because the cells left contact with the basement membrane.

A second pathway to envelope formation is from a single cell that includes a large vacuole of type 0 cell (e, f; Toda *et al.*, 1993). The cell first divides into two cells, a cell containing a vacuole and a cell without a vacuole (g). The vacuole in the cell (h) fuses with the outer membrane that faces the other cell (i), producing an envelope consisting of two cells (j). Therefore, the vacuole that was a cell organelle in a single cell becomes a central space in the cell aggregate. This space should be considered as apical, and the membrane of the vacuole becomes the apical membrane domain. As shown in Fig. 18j and d, the envelope consisting of two cells then grows by repeated cell division.

The first and second pathways to the envelope are observed both in collagen culture dishes of epithelial cells of the thyroid follicle (Toda *et al.*, 1993) and in MDCK cells (Madin-Darby canine kidney cells, O'Brien *et al.*, 2002). The activities of apical vesicles within epithelial cells ensure that the epithelial cells must inevitably form envelopes.

4. Conclusion

Animals share the simple unifying principle that each of their bodies is completely enclosed by an envelope of epithelium. The epithelial cells possess an intrinsic ability to automatically form envelopes in the absence of any extrinsic direction or information. This capacity is derived from the behaviors of 'apical vesicles' which are created within epithelial cells. Animals have such a property vital to their life and reproduction of their species.

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