Human airway has a tree-like structure. This structure is generated via repeated tip splitting during development [Fig. 1a, (7)]. This experimental system has been relatively well studied. Organ culture system has been established, and effect of various growth factors has been identified using this system (4). Complex epithelial–mesenchymal interaction is required for this pattern formation (3). It is also known that isolated lung epithelium is shown to retain an ability to form branches when it is cultivated in Matrigel with FGF (5).

In physics and chemistry, there are various non-living materials which show branching morphogenesis. For example, snow crystals exhibit beautiful branches that have anisotropy (6). Or in the field of fluid dynamics, when less viscous fluid is pressed into more viscous fluid, the interface between these two fluids form branched structure. This phenomenon is called viscous fingering (7). Pattern formation of bacterial colony is also well studied by the physicists and applied mathematicians (8).

All these phenomena have one common rule—‘protrusion grows faster’ tendency. In case of crystal growth, protruded region has more tendency to lose heat or to get supersaturation, resulting in faster interface growth. In case of viscous fingering, pointy interface have more tendency to invade into more viscous fluid because pressure gradient is steeper. In case of bacterial colonies, protruded region is exposed to higher concentration of nutrition because it is away from other nutrient-consuming regions, resulting in faster growth. This tendency results in amplification of initial minute fluctuation of interface shape, resulting in formation of branches. In these systems, movement of interface is coupled with the field (heat, solute, pressure, or nutrient distribution) that satisfies diffusion equation (\( \dot{u} = d\nabla^2 u \)) to generate interface instability, hence called diffusion-limited growth. If the dynamics of diffusion equation is fast and under equilibrium (\( \nabla^2 u = 0 \)), this equation is called Laplace’s equation and the phenomenon is called Laplacian growth.

Recent studies try to incorporate these models to understand the mechanism of lung branching morphogenesis. For example, we have done several modelling studies to show that the pattern formation can be described as diffusion-limited growth (9, 10). However, recently several models are proposed that are not simple diffusion-limited growth. In the present review, we focus on modelling studies of lung branching morphogenesis.

Molecules Involved in Lung Branching Morphogenesis

Various molecules are reported to be involved in lung-branching morphogenesis (3). To understand the behaviour of the system using mathematical models, we need to concentrate on the most important factor to reduce the number of variables. To estimate the importance of these molecules, we undertook PubMed searches with ‘lung + branching’ and gene name listed in the review (3) to estimate which molecules is regarded as important in the community (Fig. 1c).

According to the survey, the most important molecule for the early stage of branching morphogenesis is regarded as fibroblast growth factor 10 (FGF10) (Fig. 1c). Targeted deletion of FGF10 results in agenesis of lung (11). Local application of FGF10 protein induces chemotactic response to lung epithelium (12). Moreover, isolated lung epithelium shows branching morphogenesis only when FGF is added in the culture medium (3). Major ligand for FGF10 is FGFRIIb during lung branching morphogenesis (13). Therefore, FGF10 is regarded as the most important morphogen in lung branching morphogenesis.

There are several other diffusible factors that modulate branching morphogenesis. Sonic hedgehog (SHH) is expressed at the tip of the distal airway epithelium.
Targeted inactivation of SHH results in upregulation of FGF10 at the lung mesenchyme and failure of lung branching (15). Bone morphogenetic protein 4 (BMP4) is expressed at the distal lung epithelium and inhibits branching morphogenesis induced by FGF10 (16). Molecular interactions of these key molecules are reviewed in Fig. 1c (3, 17).

Models of Lung Branching Morphogenesis

Recent models try to include key molecular interactions described in the previous section. The models can be classified into several categories. Here we briefly review these studies.

**Diffusion-limited growth by FGF**

The model assumes that the geometry change of epithelial tissue induce interface instability by local inhibition of FGF10 (Fig. 2). We formulated this type of model in 2002 utilizing mesenchyme-free culture system (9). In this model, growth of tissue is proportional to the local ‘concentration’ of FGF, which focus on mitogenic activity of FGF (Fig. 2a). We also proposed a model in which the interface growth is proportional to the local ‘gradient’ of FGF10, which focus on chemotactic activity of FGF (10, 19, 20). These models were used to examine the cyst—branch difference in avian lung (21). The mechanism of pattern formation in the models is basically diffusion-limited growth via depletion of FGF10. Lung epithelium has an ability to decrease the concentration of FGF10 around their source, both in *in vitro* (consumption of FGF) and *in vivo* (inhibition of production via SHH).

This type of model is also used to understand *in vivo* situation. Clément et al. (18) used interface equations to model the geometry change of the epithelial and mesenchymal tissues. They model the distribution of FGF10 (u) by assuming $\Delta u = 0$ in mesenchyme tissue, which means FGF diffuses and reaches steady state. They also set the boundary condition at the mesothelial tissue and surface of epithelial tissue to determine the actual distribution of FGF10. They use direct front tracking to numerically solve interface equations, and reproduce branched structure (Fig. 2b).

**Laplacian growth by pressure (mechanical instability)**

There is an attempt to model the pattern formation phenomena from a purely mechanical viewpoint (22, 23) (Fig. 3). Their model uses physical phenomenon called viscous fingering. When less viscous fluid is pressed into more viscous fluid, the interface between two fluids becomes unstable and form pattern. After the discovery of FGF10, this type of model became less influential. However, with recent revival of mechanics-based models, this mechanical model is again treated as important hypothesis. For example, cytoskeletal tension mediated by Rho signalling plays a role during cleft formation in lung branching (24). Apical

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**Fig. 1 Lung branching morphogenesis.** (a) Development of human lung (1). Lung airway epithelium undergoes tip bifurcation via complex epithelial—mesenchymal interactions. (b) Three patterns of 3D branching (2). (c) Core molecules involved in branching morphogenesis. Four most cited genes are selected from list of the genes involved in lung branching (3), and their interactions are drawn schematically. Sonic Hedgehog in lung epithelium inhibits the expression of FGF10 in mesenchyme, and FGF10 induce epithelial growth or chemotaxis by regulating expression of other signalling molecules like BMP4.
constriction of lung epithelium may be involved in the formation of new bud in chick lung by increasing local curvature (25).

**Turing instability in mesenchyme tissue**

Turing instability, with which two interacting molecules generate periodic pattern spontaneously (27, 28), has been used in many biological systems (29) (Fig. 4). Recently, there is a different attempt to use Turing instability at the lung mesenchyme tissue. Menshykau et al. (26) has proposed that Patched (a receptor of SHH) and SHH consist activator-inhibitor pair in mesenchyme tissue, and as a result inhomogeneous distribution of FGF10 spontaneously arises. The authors try to explain the formation of lateral branches by this mechanism.

**Mixed-type branching model**

In 1976, Meinhardt proposed a model to generate branched structure by modifying his Gierer-Meinhardt model (31) (Fig. 5). The model consists of four variables—activator, inhibitor, tissue
Fig. 3 Mechanics-based model. In this model, we define interstitial fluid region and mesenchyme region, and the interface between these two regions is defined as epithelial sheet. Flow speed and pressure distribution is defined, and interface instability is generated by viscous fingering mechanism (22). \( \tau \) represents surface tension of the epithelium.

Fig. 4 Turing instability-based model. In this model, Sonic Hedgehog act as an inhibitor and its receptor Patched acts as an activator, and this interaction generate periodic pattern of FGF in the mesenchyme. \( \rho \) represents maximum rate of production and \( \delta \) represents decay rate. The model is used to explain the formation of side branches (26).
differentiation state and substrate. Activator and inhibitor in this system show Turing instability, and substrate can lead to interface instability by diffusion-limited growth. Therefore, the system is a mixture of these two mechanisms. Similar system has been used to generate branched structure in a more mathematically sophisticated way to elucidate pattern formation of bacterial colonies (8). Recently, 3D version of this Meinhardt model has been used to lung branching (32, 30).

**Unsolved Problems**

As described in the previous section, various models are proposed to explain lung branching morphogenesis. Therefore, concerning the origin of instability, there are very attractive hypotheses that remain to be verified experimentally. However, there remain several unsolved problems in both experimental and theoretical point of view.

**Relationship between spontaneous pattern formation and stereotypical branch structure formation**

Although lung branching seems to be stochastic at the distal end, proximal branches seem to be more or less stereotypical. For example, left main bronchus has two branches while right main bronchus has three, and this initial branch pattern is common in most individuals (1). It has been believed that left main bronchus has less branches because of spatial limitation by left-sided heart, but studies of ‘situs ambiguus’ (randomization of left-right asymmetry) and lung branch pattern seems to be contradictory to this hypothesis (33). Each bronchus-specific gene may exist, and the mechanism how the stereotypical pattern is generated remains to be elucidated.

Metzger (2) has shown from experimental observations that 3D branch pattern of mouse lung can be classified into three categories—planar bifurcation, orthogonal bifurcation and domain branching (Fig. 1b).
Among these patterns, planar bifurcation and orthogonal bifurcation can be easily reproduced by modifying the shape of the domain in computational models. However, domain branching pattern has not been fully reproduced three-dimensionally. Similar pattern was reproduced in crystal growth (34), but in this case, anisotropy of growth is necessary. It seems the branching pattern of domain branching is more complex than the other two. Guo et al. (32) claimed that they can reproduce domain branching by 3D simulation of the model.

**Diffusion dynamics of morphogens in lung development**

Since the key factor to determine the pattern formation, FGF10, is already known, the next step should be the quantitative measurement of the dynamics of the factors. In fact, in case of Turing instability, after qualitative discovery of the molecular circuit, quantitative measurement of diffusion dynamics of activator and inhibitor became a major topic (35). In the models, the diffusion range of morphogen determines the size of each bud. Diffusion range of morphogen is approximately

\[
\sqrt{d/e}
\]

where \(d\) is diffusion coefficient of the molecule and \(e\) is the degradation rate. Recently, direct measurement of morphogen diffusion and degradation becomes possible using fluorescent proteins (35). Whether diffusion coefficient of FGF10 in lung mesenchyme is within a reasonable order to form pattern remain to be elucidated.

**Alveolus differentiation**

Alveolus, a most distal part of airway tree that undertake gas exchange, is generated by a different mechanism. During later stage of lung development, mesenchyme cells which surrounds airway epithelium gradually disappears, and the distal tip of airway shows dilatation. After birth, septum was formed in the dilated cyst to form alveoli. The septum formation is known to be regulated by platelet-derived growth factor (PDGF) (36, 37). PDGF is known to regulate development of smooth muscle cells, and the septum formation is correlated to the local smooth muscle cell accumulation at the cleft. Since the size of the alveoli seems to be regular, there should be some mechanism to generate periodic expression of PDGF and accumulation of smooth muscle cells.

**Branching in other organ systems**

There are various tree-like structures in biological systems. Many glands [lacrimal gland (38), salivary gland (39), mammary gland (40) etc.] have tree-like structures. Some organs like kidney and liver show branched structure during developmental process. Blood vessels show branched structure as they run from proximal to distal. Although the morphology and molecules involved in pattern formation in these systems are well described, the mechanism how these structures are generated remains to be elucidated.

**Reconstruction of branched structure from cell line**

Due to the progress in recent modelling effort, we have several mechanisms of lung branching morphogenesis that is consistent with most of the experimental result. However, to directly show that the mechanism is valid, we need synthetic biology approach—if we truly understand the system, we should be able to construct the system with known molecules ‘from scratch’. For example, it is known that Madin–Darby canine kidney (MDCK) cell form a cyst when cultured in collagen gel (41). We may use this as a starting point and FGF10-based mechanism seems to be very plausible if we could generate branched structure by introducing several ligands and receptors in the cell line.

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**Conflict of Interest**

None declared.

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