Branching morphogenesis: a common theme in organogenesis
(MF lecture 2)

General points

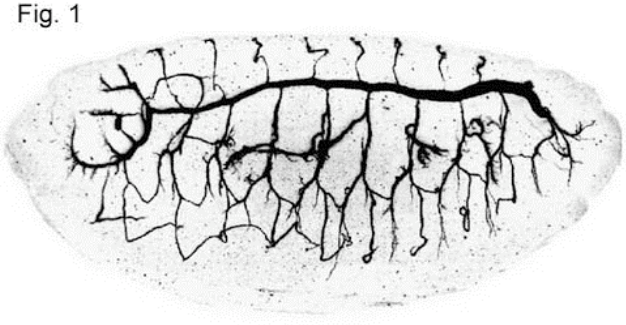
Even though all organisms are 3 dimensional "objects" metabolic exchange processes occur over 2 dimensional surfaces. In single cell organisms the surface of the organism is sufficient for metabolic exchange with the environment. However, in bigger organisms metabolic exchange surfaces have to be much larger. These exchange surfaces are folded and fragmented so they can be packed inside the body of animals (unlike plants). For example, gas exchange in the human lung occurs across the surface of about 300 million alveoli with a total surface of about 100 m^2; nutrient and gas exchange in the human vasculature takes place across the total surface of about 600 m^2. Evolution has favored a branched morphology of such metabolically active surfaces, maximizing the active surface and minimizing transport distances. Lungs, kidneys, mammary glands, salivary glands or the vasculature all display a branched morphology.

How to build branched structures

In principle, a branched structure can be built by the iterative use of a few simple subroutines. These are the initiation of a bud, the extension of a bud and the splitting at the end of a bud. With this simple set of morphogenetic units complex structures can be built. The formation of new buds, creating new centers of growth, is important for the overall morphology of the branched structure. For example in the human lung different modes of bud formation occur during different stages of development. In the early phases of bronchi formation new buds arise at the side of an extended bud, a process termed ‘secondary budding’. At later stages new buds form at the end of an extended bud (‘branching’) and in the final stages during alveoli formation terminal sacs are split in several compartments by invagination (‘splitting’).

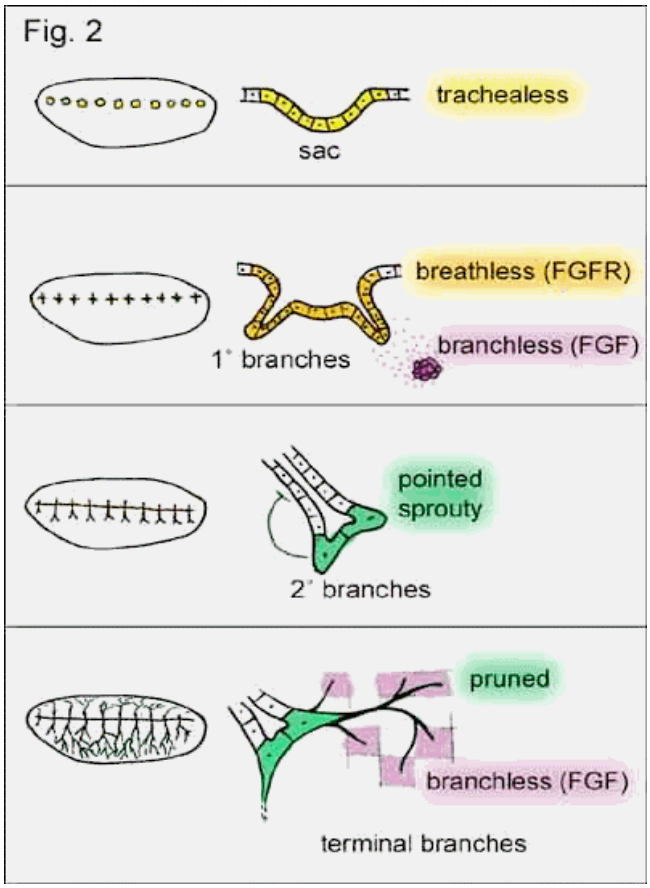
Drosophila tracheal system

Fig. 1



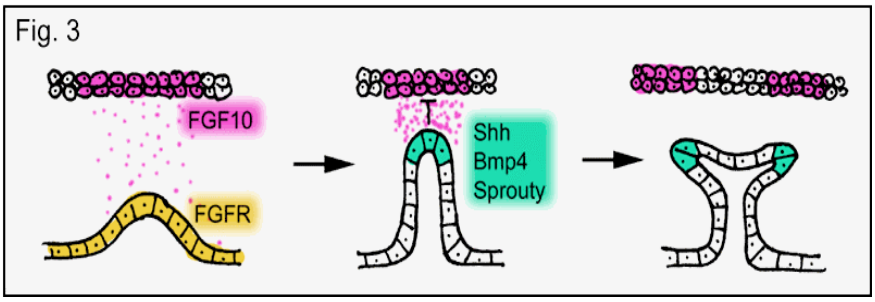
The tracheal system in drosophila larvae is a relatively simple model system for the study of branched structures and has provided some amazing insights into the biology of branching morphology. The system is a tubular network formed by a monolayer epithelium (Fig.1). Oxygen enters through the spiracular openings and diffuses freely to the target tissues. The network has three branching levels: primary branches, secondary branches and blind ending terminal branches. The first two branching levels display a stereotypical morphology whereas the terminal branches are not stereotypical. Terminal branches are very thin cytoplasmic extensions contacting in many tissues almost every single cell. The reproducible pattern of the primary and secondary branches implies a tight morphogenetic program responsible for the development of the network.

Morphogenetic control of the drosophila tracheal system



Genetic screens of drosophila larvae have revealed more than 50 genes involved in the formation of the tracheal system. Some of the genes are used during each generation of branch formation whereas others are specifically involved in early or late stages of the developing network. A gene expressed very early in development is *trachealess*, a transcription factor, which appears in 10 patches bilaterally along the longitudinal axes of the larvae. This initiates tissue locally to form slight invaginations (sacs) and defines cells as future components of the tracheal system. Without it no trachea will form (hence the name). It also induces the expression of *breathless* in these sacs, a drosophila ortholog of the mammalian fibroblast growth factor receptor (FGFR). The ligand for this receptor is *branchless* (drosophila ortholog of FGF) and is dynamically expressed in 6 patches of mesenchymal tissue around each sac. This diffusible factor induces bud formation and bud extension from the sacs resulting in 6 primary branches. Some of these branches fuse forming a longitudinal continues tube (involving a gene called *escargot*). The other branches grow closer to the *branchless* expressing centers exposing the tip of the growing branch to high concentrations of Branchless. This high concentration of Branchless induces the expression of *pointed* (transcription factor) and *sprouty* (antagonist of *branchless*) at the tip of the branch. *Pointed* causes the tip of the branch split (secondary branches) and *sprouty* restricts branching to the tip by inhibiting branching further back along the extended branch. Secondary branches also express *pruned* (transcription factor) which is a prerequisite for the formation of terminal branches. These terminal branches are very thin cellular processes growing towards cells secreting Branchless. However, this time *branchless* is not expressed stereotypical but is induced in any cell experiencing hypoxia. In summary, the diffusible factor Branchless is used three times in the formation of the drosophila tracheal system but at each level in a different context. At the level of primary branches *branchless* induces bud formation and extension, at the secondary level high concentrations of Branchless induces the expression of genes involved in secondary branching and at the level of terminal branches *branchless* mediates the need of oxygen-starved cells for oxygen by promoting and directing the growth of terminal branches.

Mammalian lung



Like most internal organs the lungs develop from the foregut (in the early embryo a ventral longitudinal tube). Initially, two buds extend from the foregut resulting in the left and right bronchus. In the mouse four secondary buds extend from the two initial branches (three on the right-hand side and one on the left) giving rise to four lung lobes. The genetic control of these processes is not completely understood but it has been shown that *Gli* genes are involved in the induction of the secondary buds. It is surprising that also FGF (human ortholog to drosophila branchless) is crucially involved in mammalian lung formation. Mammals possess more than one *FGF* gene. Currently there are more than 20 different FGFs known and many of them have overlapping functions. However, "knock out mice" lacking FGF10 are born without lungs and limbs (limbs are also formed from buds!). The function of *FGF* in lung development is similar to *branchless* in drosophila trachea formation (Fig. 3). The underlying principle is again a mesenchymal-epithelial cell-cell interaction mediated by FGF. Epithelial cells, expressing *FGF receptor*, respond to the secretion of FGF from nearby mesenchyme by bud formation and bud extension towards the FGF source. Exposure of the branch tip to high concentrations of FGF induces the expression of secondary genes in the tip such as bone morphogenetic protein 4 *(BMP4)*, sonic hedgehog *(Shh)* and a mammalian sprouty ortholog *(Sprouty 2)*, thus, turning the tips of the bronchial branches into signaling centers. BMP4 inhibits epithelial cell proliferation limiting branch extension. Shh is proposed to inhibit *FGF10* expression in the mesenchyme near the tip, which splits *FGF10* expression promoting the next round of branching and Sprouty2 (like drosophila *sprouty)* restricts branching to the tip of the branch.

Summary

In order to form a branching structure an iterative process of bud formation, bud extension and branching is required. It is remarkable that FGF-FGFR interactions appear to be a central unit repetitively used to generate the branching morphology in both the drosophila tracheal system and the mammalian lung.

Further reading:

B.L. Hogan, Morphogenesis (1999) Cell 96, 225-233.

R.J. Metzger and M.A. Krasnow, Genetic control of branching morphogenesis (1999) Science 284, 1635-1639.