



Branching morphogenesis of the lung: new molecular insights into an old problem

Pao-Tien Chuang¹ and Andrew P. McMahon²

¹Cardiovascular Research Institute, University of California, San Francisco, CA 94143, USA

²Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA

It has been known for decades that branching morphogenesis of the lung is mediated through reciprocal interactions between the epithelium and its underlying mesenchyme. In recent years, several key players, in particular members of the major signaling pathways that mediate this interaction, have been identified. Here, we review the genetic and molecular studies of these key components, which have provided a conceptual framework for understanding the interactions of these major signaling pathways in branching morphogenesis. The future challenge is to translate understanding of the signaling cascade into knowledge of the cellular responses, including cell proliferation, migration and differentiation, that lead to the stereotyped branching.*

There have been several recent advances in understanding molecular interactions between major signaling pathways during branching morphogenesis of the lung in mice. It has been known for many years that interaction between the epithelium and its surrounding mesenchyme is the major driving force in inducing lung branching. In the past few years, several key players have been identified that mediate these reciprocal epithelial–mesenchymal interactions. Furthermore, a correlation between combinatorial signaling cascades and positional information that directs lung bud outgrowth has been established. Two general conclusions can be drawn from these studies. First, signaling pathways modulate their own activities by inducing negative regulators. As a result, depending on the threshold level for induction, differential responses are generated in a spatially (and possibly temporally) specific manner. Second, a combinatorial control of multiple signaling pathways in a spatially defined manner directs the processes of cell migration, cell proliferation and differentiation that lead to branching morphogenesis.

Overview of lung branching morphogenesis

The lung primordium arises from the ventral foregut, just anterior to the developing stomach around 9.5 days *post coitum* (*dpc*) during mouse embryogenesis [1–4].

This process coincides with the appearance of another endodermal derivative, the dorsal pancreatic bud primordium, whereas the liver and thyroid bud emerge one day earlier from the ventral foregut endoderm [5]. The lung primordium is composed of two parts: the future trachea and two endodermal buds (primary buds), which give rise to the left and right lobes of the distal lung. Both components are composed of an epithelial layer of endoderm surrounded by splanchnic lateral plate mesoderm cells. Initially the primary buds grow ventrally and caudally, and initiate lateral branches at invariant positions, beginning around 10.5 *dpc*. In this way, five secondary buds are generated, four on the right side and one on the left side, leading to the formation of four right lobes and one left lobe of the mature lung in mice. The number of secondary buds on the left and right sides varies amongst species and is regulated by the pathways that control left–right asymmetry. As lung morphogenesis continues, in addition to lateral branching, dichotomous branching is observed at the tip of each duct. It is the process of dichotomous branching that is responsible for the dramatic expansion of the lung epithelium. Branching outgrowth of an epithelium is a recurring theme in organogenesis, as is the primary role of reciprocal interactions between the epithelium and the underlying mesenchyme in regulating the branching process. The early lung branching pattern does not vary between individuals, indicating that early branching patterns are likely to be regulated by a hard-wired genetic program.

With its relatively few cell types, the availability of organ culture and the stereotyped branching process, the lung is an attractive system to address the molecular mechanism of branching morphogenesis. In the past few years, significant progress has been made in understanding the genetic and molecular control of lung branching. Several key players that mediate epithelial–mesenchymal interactions have been identified [3,4], including members of the Hedgehog (Hh), Fibroblast growth factor (Fgf), Wingless (Wnt) and Transforming growth factor- β (TGF- β) signaling pathways (Table 1). Furthermore, phenotypic and molecular analyses of mice deficient in components of these pathways, combined with *in vitro* organ culture studies, have provided a molecular framework that illustrates how interactions between signaling pathways

Corresponding author: Pao-Tien Chuang (chuang@cvrmail.ucsf.edu).

* This article is the fifth review in our 'Tube Morphogenesis' series that commenced in the August 2002 issue of *TCB*. eds

Table 1. Major signaling factors involved in epithelial–mesenchymal interactions during lung morphogenesis^a

Lung epithelium	Mesenchyme	Comments ^b
Shh	Ptch1; Hip1	Shh signaling to the mesenchyme is mediated through its receptor Ptch1. In addition, Shh signaling is modulated by a second Hh-binding protein, Hip1, which is also expressed in the mesenchyme. Shh signaling appears to modulate mesenchymal Fgf10 signaling and consequently plays an important role in determining sites of lung bud formation.
Fgf9 Fgfr2; Fgfr4	Fgf1; Fgf7; Fgf10	Although multiple Fgfs are expressed in the lung, Fgf10 is the only candidate likely involved in inducing early lung branching. Fgf10 is mediated through Fgfr2, which is expressed in the epithelium. Localization and function of Fgf10 appears to be modulated by Shh and Bmp signaling as well as its own inhibitors, Spry proteins (see text).
Wnt5a; Wnt7b	Wnt2; Wnt5a	At least three Wnt genes are known to be expressed in the developing lungs. The corresponding Frizzled receptor for each Wnt gene has yet to be defined. Wnt2-deficient mice do not have apparent lung defects [34]. By contrast, Wnt5a and Wnt7b are not involved in early lung branching but are involved in later stages of lung development [35,36]. The relationships between Wnt signaling and other major signaling pathways remain to be investigated.
Bmp4; Bmp7 Bmpr1 (low level)	Bmp4 (low level) Bmpr1	Bmp4 is expressed in both the epithelium and mesenchyme, and Bmp4 signaling is mediated through Bmpr1. <i>In vitro</i> evidence suggests that Bmp4 signaling antagonizes Fgf10 signaling to ensure generation of lung buds at proper positions.

^aMajor signaling factors involved or implicated in epithelial–mesenchymal interactions during lung branching morphogenesis and their expression domains. This table identifies the relevant signaling pathways discussed in this review and is not comprehensive. The ligands are either expressed in the epithelium (*Shh*, *Fgf9*, *Wnt5a*, *Wnt7b*, *Bmp4*, *Bmp7*) or the mesenchyme (*Fgf1*, *Fgf7*, *Fgf10*, *Wnt2*, *Wnt5a*, *Bmp4*). Similarly, the receptors are expressed in the epithelium (*Fgfr2*, *Fgfr4*, *Bmpr1*) or the mesenchyme (*Ptch1*, *Bmpr1*). The *Wnt* receptors, members of the *Frizzled* family, are not listed because it has not been documented which *Frizzled* family member(s) is involved in transducing the *Wnt* signal in the lung.

^bAbbreviations: BMP, bone morphogenetic protein; FGF, fibroblast growth factor; Hh, Hedgehog; Hip1, Hedgehog-interacting protein 1; Ptch1, Patched 1; Shh, Sonic hedgehog.

mediate epithelial–mesenchymal interactions to induce lung branching.

Fgf signaling plays a key role in primary bud formation

The molecular mechanism by which the endodermal primordia of the lung buds are specified is not known. Nor is the mechanism understood that septates the endoderm to generate a ventral trachea and dorsal esophagus. Nevertheless, recent evidence indicates that Fgf signaling plays an essential role in directing the outgrowth of the two primary lung buds. Among the Fgf family members, genes encoding *Fgf1*, *Fgf7*, *Fgf9* and *Fgf10* are expressed in the developing mouse lung (Table 1). However, transcripts for *Fgf1* and *Fgf7* are not detected in the mouse lung until 13.5 *dpc*, when extensive lung branching has occurred [6,7]. Although *Fgf9* is expressed in early lung epithelium, phenotypic analysis of *Fgf9*-deficient mice indicates that *Fgf9* is unlikely to play a major role in inducing primary bud formation [8]. *Fgf10* is the only Fgf member whose expression is closely associated with early lung branching. At 9.75 *dpc*, mesenchymal *Fgf10* expression is detected around two small lung buds growing out from the ventral foregut. By 10.5 *dpc*, *Fgf10* expression is restricted to the distal mesenchyme of the two main-stem bronchi generated from the two primary buds [9]. Furthermore, isolated lung endoderm in culture grows towards an FGF10 source at a distance [10]. These results strongly suggest that FGF10 plays a role in inducing primary bud formation *in vivo*. In agreement with this notion, although tracheal development is normal in *Fgf10*-deficient mice, main-stem

bronchial development as well as all subsequent pulmonary branching morphogenesis is completely absent [11,12]. However, it remains possible that *Fgf10* is not required for the initial outgrowth of the primary buds but is necessary for their subsequent growth and extension. It is not well understood whether FGF10 functions as a chemoattractant *in vivo* or is simply required for the proliferation of the lung epithelium during primary bud formation. However, studies performed in lung organ culture with purified FGF proteins indicate that FGF10 could act both as a chemoattractant and a mitogen for distal lung endoderm [9].

The molecular mechanism by which *Fgf10* expression is localized in the mesenchyme to induce the outgrowth of the two primary buds is not known (Fig. 1). It is also not clear whether the positional information that controls mesenchymal *Fgf10* expression is laid down rather early during embryogenesis or is acquired at a later stage through interactions between splanchnic mesenchyme and adjacent tissues such as the notochord and other types of mesoderm. Many transcription factors have been implicated in regionalization of the gut endoderm [5] and are likely candidates for the induction of initial *Fgf10* expression. It is also possible that a signaling cascade directly controls the temporal and spatial expression of *Fgf10*. In this regard, analysis of other major signaling pathways has not yet provided candidates for controlling *Fgf10* expression during primary bud formation. For instance, Hh signaling is not absolutely required for primary bud formation, as revealed by the presence of two primary buds that are smaller in size and fail to grow or branch extensively in the lungs of *Sonic hedgehog* (*Shh*; a Hh family member) mutant

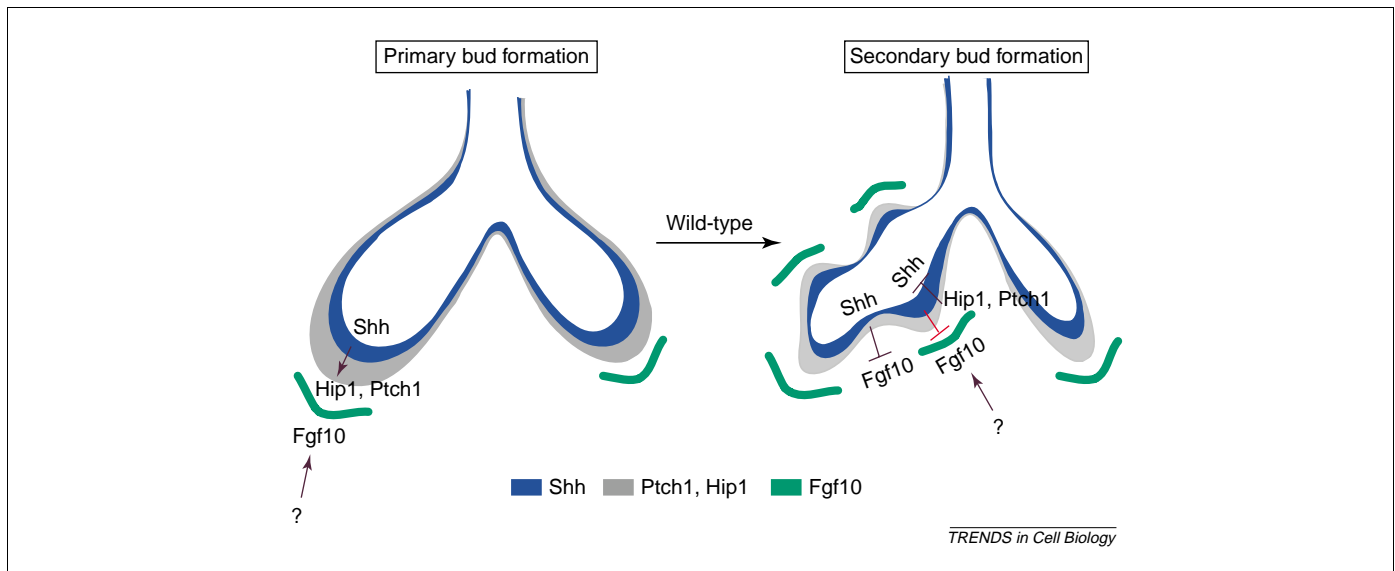


Fig. 1. A model of regulation of Fibroblast growth factor (Fgf) signaling by Hedgehog (Hh) signaling in branching morphogenesis of the lung. During lung branching morphogenesis, the two primary buds initially elongate and later bulge to generate the secondary buds. Localized *Fgf10* expression in the distal mesenchyme induces primary bud formation. The mechanisms by which *Fgf10* expression is initiated and localized are not clear. *Shh* (blue) is expressed in the epithelium and is upregulated at the distal tips of the primary buds. In this model, in the wild-type lung, secondary bud formation is also induced by localized *Fgf10* expression in the mesenchyme corresponding to the future sites of secondary bud formation. It is likely that similar mechanisms (indicated by a '?' in the diagram) are used to localize *Fgf10* to these sites. Epithelial expression of Hh might help restrict *Fgf10* expression to the sites of secondary bud formation by inhibiting *Fgf10* expression. Since *Shh* expression is upregulated in the epithelium of the secondary buds after the activation of *Fgf10*, we propose that this upregulation might not be indicative of more Hh signaling. The Hh pathway transcriptionally activates Hedgehog-interacting protein 1 (*Hip1*; gray) and Patched 1 (*Ptch1*; not shown), repressors of Hh signaling, to downregulate the Hh pathway at sites of bud formation. As a result, the signals that activate *Fgf10* expression overcome the mild inhibition (red 'T' symbol), if any, by Hh signaling. By contrast, in the inter-bud regions, the Hh pathway effectively inhibits *Fgf10* expression.

mice [13,14]. Furthermore, the cellular changes in the epithelium in response to *Fgf10* signaling have not been well characterized in the animals. Nevertheless, the identification of *Fgf10* as a key mesenchymal signal in inducing epithelial branching during early lung development provides an important link to translate the positional information in the mesenchyme into the early branching pattern at the molecular level.

Fgf10 signaling is modulated by Hh signaling in secondary bud formation

Since main-stem bronchial development does not occur in *Fgf10*-deficient mice, it has not been possible to assess the role that *Fgf10* plays in inducing secondary buds or in subsequent branching [11,12]. However, the expression of *Fgf10* is also closely associated with secondary bud formation (Fig. 1). High levels of *Fgf10* expression are detected in the mesenchyme at the very distal tips of the secondary buds [9]. In addition, exogenous FGF10 provided on a coated bead can induce outgrowth of epithelial buds from isolated lungs at a stage when secondary buds are about to form [9,15]. These results suggest that FGF10, in addition to inducing primary bud formation, is likely a key player in secondary bud outgrowth.

While the role of FGF10 appears to be similar in primary and secondary bud formation, signaling by Hh and the TGF- β superfamily member Bmp (Bone morphogenetic protein) is essential for modulating FGF10 activity during secondary bud formation. An interaction between *Fgf10* and *Shh* in inducing secondary bud formation has been revealed through analysis of the lungs from *Shh* mutant mice. *Fgf10* expression is upregulated and delocalized in *Shh* mutant lungs, which consist of two

stunted primary buds [14]. In addition, overexpression of *Shh* in the mouse lung using a transgenic approach leads to reduced *Fgf10* expression, consistent with a negative regulation of *Fgf10* expression by Hh signaling [16]. It is not clear whether *Fgf10* is a direct or indirect target of Hh signaling. The mechanism by which *Fgf10* expression is initiated is also not clear, but localized *Fgf10* expression is essential for secondary bud formation [13, 14]. Hh signaling is unlikely to be responsible for the localized expression of *Fgf10* expression that precedes secondary bud formation as *Shh* expression is uniform at this time except at the tips of the primary buds. Whether *Fgf10* controls Hh signaling has not been clearly demonstrated.

How does Hh signaling facilitate proper *Fgf10* localization during secondary bud formation? One possibility is that Hh inhibition of *Fgf10* expression mainly occurs in the inter-bud regions after mesenchymal *Fgf10* expression has been initiated (Fig. 1). This could potentially be achieved through the induction of negative regulators of Hh signaling that function in a feedback loop to generate regional differences in Hh signaling activity, as described below. Interestingly, *Shh* expression is upregulated at sites of secondary bud formation, which is associated with higher levels of *Fgf10* expression. This creates an apparent paradox as to how *Fgf10* expression could be maintained in the presence of high levels of *Shh* expression (and presumably high levels of Hh signaling) if Hh signaling also inhibits *Fgf10* expression at the sites of secondary buds.

We envision two possible mechanisms by which *Fgf10* expression could be maintained despite high levels of *Shh* expression. In the first, higher levels of *Shh* expression might not reflect higher levels of Shh activity as *Shh* can

activate repressors to modulate its own activity. For instance, Hh-binding proteins, such as Hedgehog-interacting protein1 (Hip1) [17] and Patched1 (Ptch1) [18], are initially induced in the mesenchyme in response to Hh signaling (Fig. 1). Once the amount of Ptch1 exceeds that required for Hh signaling, Ptch1, together with Hip1, sequesters the Hh protein. This quickly leads to decreased Hh signaling. As a result, less Hh signal might be transduced at the buds, as compared with the inter-bud regions of the lung epithelium, despite high levels of *Shh* expression. Thus, at the tips of the lung buds, *Fgf10* expression can be maintained. By contrast, low levels of *Shh* expression in the inter-bud regions might be capable of repressing *Fgf10*, where the Shh inhibitors Ptch1 and Hip1 are expressed at lower levels. The second plausible model supposes that broad Shh signaling from the distal epithelium establishes a general proximal zone of *Fgf10* repression in adjacent mesenchyme. As a result, *Fgf10* activation can only occur at some distance from the underlying epithelium, thereby providing a distant source of ligand to trigger local, directed epithelial outgrowth. Upregulation of *Shh* in the branching epithelium might eventually inhibit the focal sources of *Fgf10* expression as the branching tips approach these groups of cells. To summarize, *Shh* signaling appears to suppress *Fgf10* signaling in a spatially defined manner, resulting in focal *Fgf10* expression, which presumably induces secondary bud formation. Whatever the exact mechanism, the dynamic interactions between *Fgf10* and *Shh* most likely extends beyond the initiation of secondary, lateral branches into later stages of branching morphogenesis.

The induction of negative regulators in response to signaling to modulate signaling activity is a recurring theme in major signaling pathways. In the developing limbs, *Fgf4/fgf8* expression in the apical ectodermal ridge (AER) and *Shh* expression in the zone of polarization activity (ZPA) form a positive-feedback loop, where they require each other for proper expression. Putative positive interactions between Hh and Fgf signaling in lung branching remain to be identified.

The Hh pathway is fairly conserved during evolution, and studies in *Drosophila* indicate that Cubitus interruptus (Ci), the Gli counterpart in fly, is the principle transcriptional effector of Hh signaling. Furthermore, Ci appears to be restricted to the Hh pathway [19]. It is thus unexpected that the foregut endoderm fails to develop into esophagus, trachea and lung in *Gli2*^{-/-} *Gli3*^{-/-} double-knockout mice [20], a phenotype more severe than that observed in *Shh*-deficient mice. It is possible that the Gli proteins in mammals are also involved in other signaling pathways, and it will be of interest to investigate the potential regulation of *Fgf10* expression by the Gli proteins. Alternatively, because Gli proteins also act as negative regulators independent of Hh inputs, the differences in phenotypes between *Gli2*^{-/-} *Gli3*^{-/-} and *Shh* mutant mice might simply reflect the fact that Gli repression is lost in *Gli2*^{-/-} *Gli3*^{-/-} mutants but is intact in *Shh*-deficient mice. These results highlight the importance of further molecular dissection of signaling pathways and their interactions.

Fgf10 signaling is modulated by Bmp signaling in secondary bud formation

Fgf signaling also interacts with Bmp signaling during secondary bud formation. *Bmp4* expression is first detected in the ventral mesenchyme of the developing lung when the primordial lung buds are emerging from the foregut [21]. This mesenchymal expression is maintained until 13.5 *dpc* [21]. Expression of *Bmp4* is also detected in the distal endoderm of the developing lung bud [21]. *Bmp4*-deficient mouse embryos die before 10 *dpc*, precluding analysis of the role Bmp4 plays in lung branching [22]. Insights into the function of *Bmps* came from *in vitro* studies in which cultured lung buds failed to grow towards an FGF10-coated bead when exogenous BMP4 protein was added to the culture medium [10,23]. These results suggest that BMP4 antagonizes the effect of FGF10 in inducing epithelial bud outgrowth (Fig. 2). The molecular mechanism by which BMP4 antagonizes the effect of FGF10 *in vivo* is not known. It is not even clear whether mesenchymal or endodermal BMP4 (or both) is involved in inhibiting FGF10. Nevertheless, mainly based on expression and *in vitro* studies, an attractive model has been proposed in which *Bmp4* functions as an inhibitor of lateral budding [10]. *Bmp4* is induced at the tip of the growing lung buds in response to mesenchymal *Fgf10*. In the presence of high BMP4, FGF10 fails to induce further budding from the growing lung bud, thus ensuring a single extending bud, rather than a cluster of buds. Branching only occurs when the *Fgf10* expression domains shift laterally (Fig. 2), but, as described earlier, the mechanism by which *Fgf10* expression is initiated is unknown. There is also evidence to suggest that *Bmp4* might promote distal lung differentiation [21]. It should also be noted that application of BMP4 to lung explants induces branching [24,25], suggesting that the interactions between FGF10 and BMP4 might be more complicated than simple antagonism of FGF10 by BMP4. Further molecular and genetic studies are required to elucidate the interactions between FGF10 and BMP4 during lung branching.

If *Bmp4* antagonizes the effects of FGF10, there is an apparent paradox as to how *Fgf10* expression can induce bud outgrowth in the presence of high levels of *Bmp4* expression (and presumably high levels of Bmp4 signaling) at the tip of the bud. It is possible that the induction of *Bmp4* expression is gradual and BMP4 will only start to inhibit FGF10 at a specific concentration threshold. As *Fgf10* expression at sites of future bud formation precedes *Bmp4* induction, lung bud outgrowth will occur before *Bmp4* reaches a critical concentration. Alternatively, *Bmp4* signaling could also induce inhibitors to modulate the activity of BMP4, thus allowing FGF10 to exert its effect in inducing bud outgrowth. Interestingly, *noggin*, a Bmp antagonist, is expressed in distal lung mesenchyme [21] and could potentially modulate Bmp signaling at the distal tip (Fig. 2). It is interesting to note that *Fgf10* expression is maintained in the presence of high levels of *Shh* and *Bmp4* expression. Although the molecular mechanism is not yet fully understood, it appears that negative-feedback control of signaling pathways plays an important role in this process.

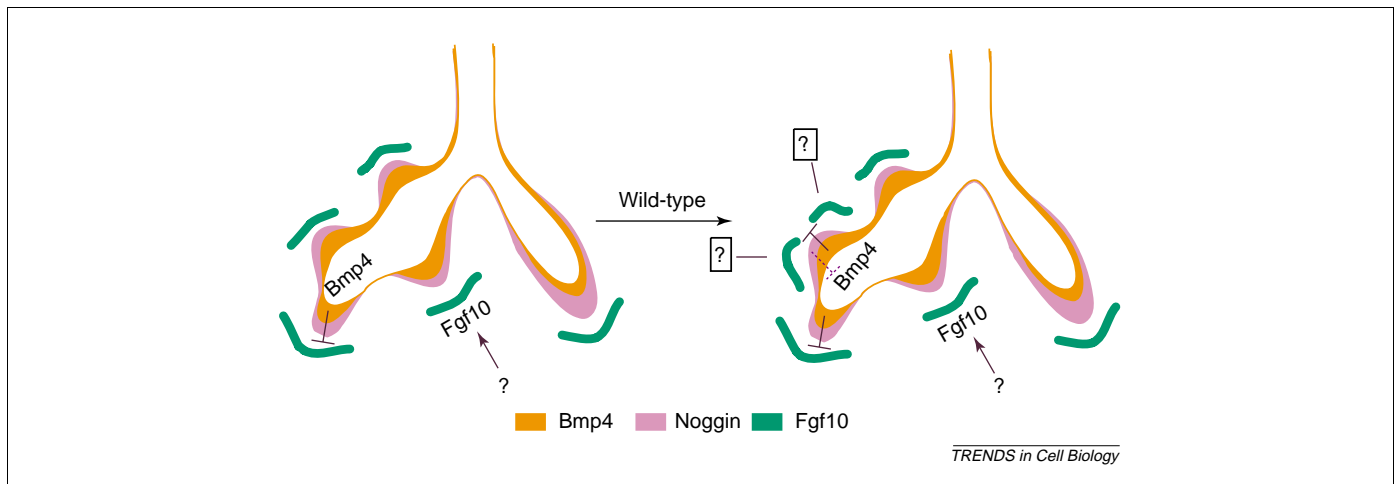


Fig. 2. A model of regulation of Fibroblast growth factor (Fgf) signaling by Bone morphogenetic protein (Bmp) signaling in branching morphogenesis of the lung. During lung branching morphogenesis, *Bmp4* (orange) is induced at the tip of the growing lung buds in response to mesenchymal *Fgf10* (green). Because *Fgf10* expression at sites of future bud formation precedes *Bmp4* induction, lung bud outgrowth will occur before BMP4 reaches a critical concentration sufficient to inhibit FGF10. In addition, *Bmp4* signaling also induces inhibitors (such as Noggin; pink) in the mesenchyme to modulate the activity of BMP4 (purple dotted 'T' symbol), allowing FGF10 to exert its effect in inducing bud outgrowth. In the presence of high BMP4, *Fgf10* fails to induce further budding from the growing lung bud and, as a result, a single extending bud, rather than a cluster of buds, is generated. Branching only occurs when the *Fgf10* expression domains shift (indicated by a split of *Fgf10* expression in the diagram), but the mechanism by which a new *Fgf10* expression domain is initiated is unknown (indicated by a boxed '?' in the diagram).

If both Hh and Bmp signaling regulate *Fgf10* signaling, is there a direct crosstalk between Shh and *Bmp4*? Several lines of evidence suggest that they function independently. In *Shh* mutant lungs, mesenchymal expression of *Bmp4* expression is downregulated, but epithelial expression of *Bmp4* is maintained (perhaps even at a higher level) [13,14]. Furthermore, expression of *Shh* is unaltered in transgenic lungs overexpressing *Bmp4* [26]. These results suggest that Hh and Bmp signaling (at least in the epithelium) probably function independently to regulate *Fgf10*. It is possible that Hh and *Bmp4* regulate *Fgf10* signaling at different steps. Hh signaling might be involved in restricting *Fgf10* expression, whereas *Bmp4* might be involved in regulating the activity of FGF10.

In addition to Hh and Bmp signaling, *Fgf10* could potentially induce its own inhibitor, *Sprouty* (*Spry*), during lung branching. *Spry* was initially identified in a genetic screen for mutations that affect tracheal branching in *Drosophila* [27]. Four mammalian *Spry* genes have been identified, and their expression domains either overlap with, or are immediately adjacent to, the known expression domains of one or more *Fgf* genes [28,29]. In the lung, *Spry1*, 2 and 4 are expressed in the distal epithelial tips of the lung, and *Spry4* is also expressed in the mesenchyme [30]. Expression of *Spry2* is induced by insertion of an FGF10-coated bead into an isolated lung in culture [31]. Furthermore, expression of *Spry2* and 4 is lost in *Fgf8* mutant mouse embryos [29]. These results indicate that the *Spry* genes are targets of Fgf signaling – and likely a primary response as induction of *Spry* gene expression is very rapid [29]. SPRY appears to function as an antagonist of Fgf signaling, as in the fly [27]. However, *Drosophila* studies indicate a more pleiotropic role for *Spry* in antagonizing several receptor tyrosine kinase pathways [32]. Reduction of *Spry2* expression in cultured lungs using an antisense oligonucleotide strategy resulted in an increase in lung branching [31]. Conversely, targeted overexpression of *Spry2* in peripheral lung epithelium

results in a lower level of branching [31]. It is possible that the function of *Spry* overlaps with that of Hh and Bmp signaling in regulating Fgf signaling. Loss-of-function studies in mice will be required to dissect the *in vivo* function of *Spry* family members in regulating Fgf signaling in lung branching. Regulation of *Fgf10* signaling by multiple overlapping mechanisms, such as Hh and Bmp signaling as well as Fgf inhibitors, might ensure a tighter and yet more dynamic control of *Fgf10* signaling.

Branching beyond secondary bud formation

After the secondary buds are specified, the two major modes of branching are lateral sprouting and dichotomous branching. It is likely that a similar mechanism is involved in these two types of branching as the expression patterns of the major signaling molecules that are involved appears to be similar in buds generated from either branching mode. Components of several other major signaling pathways, including the TGF- β , Wnt, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), are also expressed in the lung [1–4]. Functional ablation or overexpression of several of these pathway components perturb lung branching at a later stage after the initial lobation has been established. However, in many cases, overexpression studies rely on available regulatory elements that direct epithelial expression only at later stages of lung development and do not address the potential roles these pathway components play during early lung branching. Understanding the interactions between these pathways at the molecular level will provide insights into the molecular mechanism of branching at later stages (and perhaps at earlier stages as well).

An interesting issue is whether lung branching becomes a stochastic process at later stages. It is possible that the positional information in the mesenchyme is sufficient to generate a fixed number of branches in a spatially specific manner and that the process subsequently becomes random. It seems unlikely that the process of lung branching

is a completely predetermined process. Instead, after a certain number of branchings, this process becomes random and could then be determined by physiological conditions such as local oxygen and nutrient concentration. Consistent with this notion, individual variations in later branching patterns have been observed in humans. Visualization of the branching process in mice using green-fluorescent protein (GFP), as well as physiological studies using new molecular tools, will provide further insights into this issue [33]. This approach will allow a clear description of the branching process, which, in combination with the expression and functional data on signaling pathways, will form the basis of understanding the cellular mechanism of branching morphogenesis.

Concluding remarks

A major challenge in the future is to identify all the key components involved in lung branching, as well as to understand the interactions between signaling cascades at the molecular level and how these interactions regulate the cellular dynamics of epithelial branching morphogenesis. In particular, the molecular mechanisms that pinpoint the precise locations of lung bud formation and the cellular mechanisms responsible for tubule formation during lung bud outgrowth are not clear. The available genomic sequences with predicted open reading frames will undoubtedly lead to the discovery of additional genes involved in lung branching as well as new insights into the underlying molecular mechanism. The ability to manipulate gene expression/function in mice in a temporally and spatially specific manner through transgenesis will allow us to address the major issue of interactions between signaling pathways and their roles in various steps of lung branching. The paradigms established in lung branching will be applicable to other developing systems given the conservation of signaling pathways between species as well as between different tissues.

Acknowledgements

We thank members of the Chuang laboratory for discussion and T'Nay Kawcak, Tony Gerber and Chris Wilson for critical reading of the manuscript. Work in the laboratory of A.P.M. was supported by a grant from the NIH (NS33642). Work in the Chuang laboratory was supported by a grant from the NIH (HL67822).

References

- Hogan, B.L. *et al.* (1997) Branching morphogenesis of the lung: new models for a classical problem. *Cold Spring Harb. Symp. Quant. Biol.* 62, 249–256
- Hogan, B.L. (1999) Morphogenesis. *Cell* 96, 225–233
- Warburton, D. *et al.* (2000) The molecular basis of lung morphogenesis. *Mech. Dev.* 92, 55–81
- Cardoso, W.V. (2001) Molecular regulation of lung development. *Annu. Rev. Physiol.* 63, 471–494
- Wells, J.M. and Melton, D.A. (1999) Vertebrate endoderm development. *Annu. Rev. Cell Dev. Biol.* 15, 393–410
- Fu, Y.M. *et al.* (1991) Acidic fibroblast growth factor in the developing rat embryo. *J. Cell Biol.* 114, 1261–1273
- Mason, I.J. *et al.* (1994) FGF-7 (keratinocyte growth factor) expression during mouse development suggests roles in myogenesis, forebrain regionalisation and epithelial-mesenchymal interactions. *Mech. Dev.* 45, 15–30
- Colvin, J.S. *et al.* (2001) Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. *Development* 128, 2095–2106
- Bellusci, S. *et al.* (1997) Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* 124, 4867–4878
- Weaver, M. *et al.* (2000) Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. *Development* 127, 2695–2704
- Min, H. *et al.* (1998) Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to *Drosophila* branchless. *Genes Dev.* 12, 3156–3161
- Sekine, K. *et al.* (1999) Fgf10 is essential for limb and lung formation. *Nat. Genet.* 21, 138–141
- Litingtung, Y. *et al.* (1998) Sonic hedgehog is essential for foregut development. *Nat. Genet.* 20, 58–61
- Pepicelli, C.V. *et al.* (1998) Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr. Biol.* 8, 1083–1086
- Park, W.Y. *et al.* (1998) FGF-10 is a chemotactic factor for distal epithelial buds during lung development. *Dev. Biol.* 201, 125–134
- Bellusci, S. *et al.* (1997) Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. *Development* 124, 53–63
- Chuang, P.-T. and McMahon, A.P. (1999) Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. *Nature* 397, 617–621
- Goodrich, L.V. *et al.* (1996) Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. *Genes Dev.* 10, 301–312
- Methot, N. and Basler, K. (2001) An absolute requirement for Cubitus interruptus in Hedgehog signaling. *Development* 128, 733–742
- Motoyama, J. *et al.* (1998) Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. *Nat. Genet.* 20, 54–57
- Weaver, M. *et al.* (1999) Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. *Development* 126, 4005–4015
- Winnier, G. *et al.* (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* 9, 2105–2116
- Hogan, B.L. and Yingling, J.M. (1998) Epithelial/mesenchymal interactions and branching morphogenesis of the lung. *Curr. Opin. Genet. Dev.* 8, 481–486
- Bragg, A.D. *et al.* (2001) Signaling to the epithelium is not sufficient to mediate all of the effects of transforming growth factor beta and bone morphogenetic protein 4 on murine embryonic lung development. *Mech. Dev.* 109, 13–26
- Shi, W. *et al.* (2001) Gremlin negatively modulates BMP-4 induction of embryonic mouse lung branching morphogenesis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 280, L1030–L1039
- Bellusci, S. *et al.* (1996) Evidence from normal expression and targeted misexpression that bone morphogenetic protein (Bmp-4) plays a role in mouse embryonic lung morphogenesis. *Development* 122, 1693–1702
- Hacohen, N. *et al.* (1998) sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* 92, 253–263
- de Maximy, A.A. *et al.* (1999) Cloning and expression pattern of a mouse homologue of *Drosophila* sprouty in the mouse embryo. *Mech. Dev.* 81, 213–216
- Minowada, G. *et al.* (1999) Vertebrate Sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. *Development* 126, 4465–4475
- Zhang, S. *et al.* (2001) Expression of Sprouty genes 1, 2 and 4 during mouse organogenesis. *Mech. Dev.* 109, 367–370
- Mailleux, A.A. *et al.* (2001) Evidence that SPROUTY2 functions as an inhibitor of mouse embryonic lung growth and morphogenesis. *Mech. Dev.* 102, 81–94
- Casci, T. *et al.* (1999) Sprouty, an intracellular inhibitor of Ras signaling. *Cell* 96, 655–665
- Srinivas, S. *et al.* (1999) Expression of green fluorescent protein in the ureteric bud of transgenic mice: a new tool for the analysis of ureteric bud morphogenesis. *Dev. Genet.* 24, 241–251
- Monkley, S.J. *et al.* (1996) Targeted disruption of the Wnt2 gene results in placental defects. *Development* 122, 3343–3353
- Li, C. *et al.* (2002) Wnt5a participates in distal lung morphogenesis. *Dev. Biol.* 248, 68–81
- Shu, W. *et al.* (2002) Wnt7b regulates mesenchymal proliferation and vascular development in the lung. *Development* 129, 4831–4842