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Regulation of early lung morphogenesis: questions, facts and controversies

Wellington V. Cardoso* and Jining Lü

During early respiratory system development, the foregut endoderm gives rise to the tracheal and lung cell progenitors. Through branching morphogenesis, and in coordination with vascular development, a tree-like structure of epithelial tubules forms and differentiates to produce the airways and alveoli. Recent studies have implicated the fibroblast growth factor, sonic hedgehog, bone morphogenetic protein, retinoic acid and Wnt signaling pathways, and various transcription factors in regulating the initial stages of lung development. However, the precise roles of these molecules and how they interact in the developing lung is subject to debate. Here, we review early stages in lung development and highlight questions and controversies regarding their molecular regulation.

Introduction

The basic design of the mammalian respiratory system, referred to here as the trachea and lung, is that of a tree of epithelial tubules in which air is cleaned, humidified and delivered to numerous alveolar units closely apposed to blood vessels, where the circulating blood is oxygenated.

The respiratory system arises from the ventral foregut endoderm. The process initiates with the establishment of respiratory cell fate in the primitive foregut. This is followed by the development of a tree-like system of epithelial tubules and vascular structures (see Fig. 1), which ultimately gives rise to the mature airways and alveoli. The foregut endoderm differentiates into various epithelial cell types, which line the inner surface of the developing lung and trachea. The lung mesenchyme originates from the lateral plate mesoderm and gives rise to multiple components of the lung, including its connective tissue, endothelial cell precursors, the smooth muscle that surrounds airways and blood vessels, the cartilage of the trachea, the lymphatics, and the mesothelial cells that cover the outer surface of the lung, the pleura. The lung vasculature forms, in part, by migration of blood vessels from the aortic arches and from the left atrium to the lung (angiogenesis). Blood vessels also develop by vasculogenesis in the lung mesenchyme near developing epithelial buds; a rudimentary capillary network initially forms and expands, and later connects to the larger vessels to give rise to the lung vasculature (Wood et al., 1997; Demello et al., 1997; Gebb and Shannon, 2000). There is evidence that, during organogenesis, blood vessels serve as a source of inductive signals to the epithelium (Lammert et al., 2001; Matsumoto et al., 2001). However, the role of the vasculature in epithelial patterning has still to be clearly demonstrated in the developing lung.

The mechanisms that control respiratory system formation have been the subjects of an increasing number of studies. This review focuses on our current knowledge of the molecular regulation of early lung development. Topics discussed here include lung endodermal specification, lung primordium formation, and the regulation of the initial stages of branching morphogenesis and differentiation in the embryonic lung. We address questions such as 'when and how is respiratory cell fate established?', 'how do lung buds form?', 'how are stereotypical patterns of airway branching and cellular diversity generated in the developing lung?' and 'which pathways and targets are key to these processes?'. Most of what is described refers to mouse lung development because of the genetic data available (Table 1). Lung vascular development and later events, such as sacculation and alveoli formation, are not discussed in this review (for reviews, see Pauling and Vu, 2004; Williams, 2003; Bourbon et al., 2005).

From gut to lungs: the origin of the respiratory system

Foregut morphogenesis and establishment of endodermal cell fate

Following gastrulation (embryonic day E7.5 in mice), the definitive endoderm undergoes complex morphogenetic movements that ultimately lead to the formation of the primitive gut tube. The foregut represents the most anterior (cranial) region of this tube, while the midgut and hindgut are located at progressively more posterior regions, towards the caudal end of the embryo (Wells and Melton, 1999). Transcription factor genes such as *Foxa1*, *Foxa2*, *Gata4* and *Gata6*, which are expressed early in the endoderm, are crucial for the survival, differentiation and morphogenesis of the foregut (Kuo et al., 1997; Morrisey et al., 1998; Ang and Rossant, 1994; Wan et al., 2005). By E8.0-9.5, the local expression of transcription factors along the anteroposterior (AP) axis of the gut endoderm marks organ-specific domains (or fields; see Fig. 1A). For example, the homeodomain protein gene *Nkx2.1* [also known as thyroid transcription factor 1 (*Titf1*) or *T/EBP*] is expressed in the thyroid and respiratory fields (Kimura et al., 1996), *Hex* (hematopoietically expressed homeobox) is expressed in the thyroid and liver fields (Martinez Barbera et al., 2000), and the *Pdx1* (pancreas-duodenal-associated homeobox gene) is expressed in the pancreatic and duodenal fields (Offield et al., 1996). In addition, morphogenetic movements foster dynamic interactions between the endoderm and neighboring structures, such as the heart, notochord or the septum transversum (the mesodermal cells that give rise to the diaphragm). Exposure of the endoderm to diffusible signals from these structures at crucial developmental windows is essential for endodermal cell fate specification (Kumar and Melton, 2003; Bort et al., 2004).

Fibroblast growth factor 4 (Fgf4), bone morphogenetic protein 2 (Bmp2) and retinoic acid (RA) are among the signals that confer AP identity to the early endoderm. They render the endoderm competent to respond to signals from the adjacent mesoderm or from nearby structures to initiate morphogenesis (Tiso et al., 2002; Stafford and Prince, 2002; Wells and Melton, 2000). In zebrafish, disrupted RA signaling during gastrulation results in the loss of liver and pancreatic (posterior) fates, while thyroid and pharynx (anterior) fates remain unaltered. Conversely, excess RA induces hepatic and pancreatic cell fates at more anterior domains (Stafford and Prince,

Pulmonary Center, Boston University School of Medicine, Boston, MA 02118, USA

*Author for correspondence (e-mail: wcardoso@lung.bumc.bu.edu)

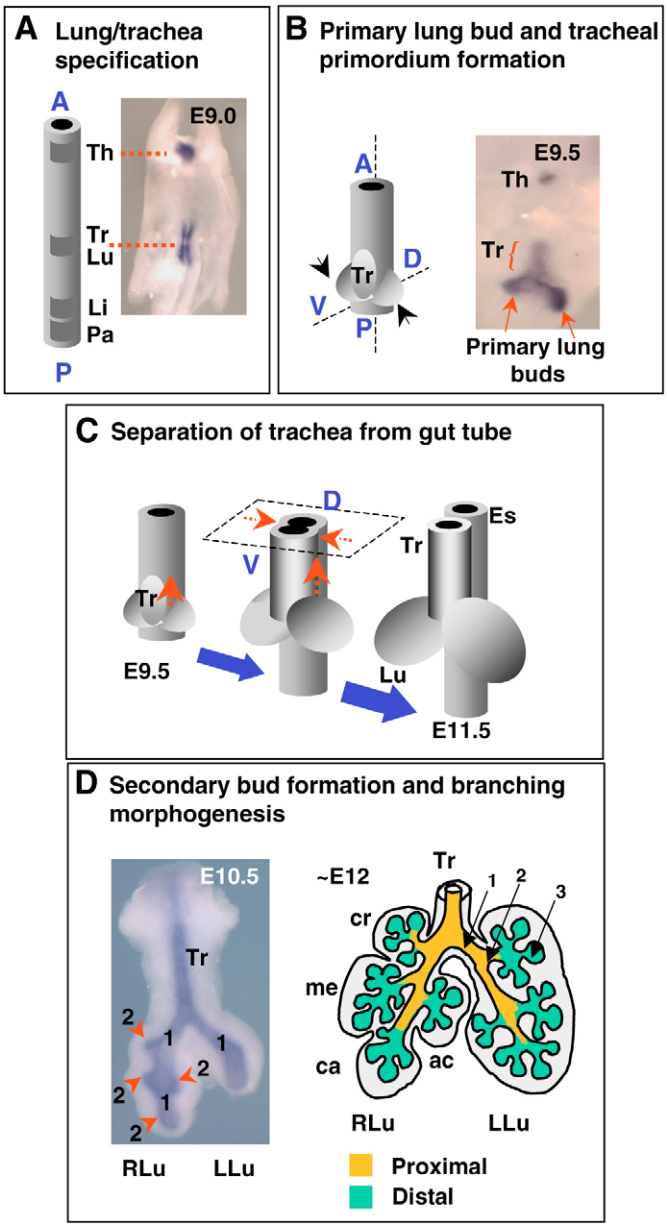


Fig. 1. Key events during early development of the mouse respiratory system. (**A**) The foregut (gray tube) is initially specified into organ-specific domains along its anteroposterior (AP) axis, which later give rise to the thyroid (Th), thymus, trachea (Tr), lung (Lu), liver (Li) and pancreas (Pa). The respiratory progenitors (Tr, Lu) arise from the ventral foregut endoderm, which is posterior to the thyroid but anterior to the liver and to the pancreatic fields. Lung and tracheal progenitors are identified collectively at E9.0 by *Nkx2.1* expression (purple), which also labels the thyroid. (**B**) At E9.5, two endodermal lung buds (black arrows) are induced from the ventral-lateral aspect of the foregut, which then invade the adjacent mesoderm and elongate to form the primary buds (red arrows) of the right and left lung (V-D, ventral-dorsal axis). (**C**) With primary lung bud formation, the tracheal (Tr) primordium forms ventrally and separates from the dorsal foregut, the primitive esophagus (Es), in a poorly understood process that is possibly initiated by growth of an ascending tracheoesophageal septum or by fusion of endodermal ridges from the lateral walls of the foregut (Zaw-Tun, 1982; Sutliff and Hutchins, 1994; Ioannides et al., 2002). (**D**) At ~E10.5 (left), secondary buds arise as outgrowths from the primary lung buds at specific positions (red arrowheads; the epithelium is labeled by *Fgfr2b*). In the right lung (RLu), these buds later develop into separate lobes. At E11.5-12.0 (right), the left lung (LLu) has one lobe and RLu has four: the cranial (cr), medial (me), caudal (ca) and accessory (ac) lobes. From E10.5 to E16.5-E17.0, the epithelium undergoes branching morphogenesis, which involves bud outgrowth and elongation, dichotomous subdivisions and cleft formation at branching points. The process is reiterated over several generations of branches to form the respiratory (bronchial) tree. As this occurs, a proximodistal axis is established in the developing lung. Proximal regions (where buds are initially generated, yellow) stop branching and differentiate into proximal airways (bronchi), while distal regions (green) continue to branch and later give rise to the alveolar region of the lung. Numbers depict primary, secondary and tertiary generations of buds.

2002). In mice and rats, RA signaling initiates soon after gastrulation (Rossant et al., 1991), but does not seem to be as crucial for foregut AP identity as it is in the zebrafish.

When and how is lung cell fate specified in the foregut?

The specification of the liver and pancreas has been extensively investigated (Rossi et al., 2001; Kumar and Melton, 2003). By contrast, relatively fewer studies have focused on the lung. Lineage analysis suggests that the progenitor cells of the trachea and proximal lung differ in origin from those that will form the distal region of the lung (Perl et al., 2002). Precisely when respiratory cell fate is established in the foregut endoderm is still unclear. Respiratory progenitors are first visualized by in situ hybridization as a group of *Nkx2.1*-expressing endodermal cells in the prospective lung/tracheal region of the foregut, at ~E9 (Minoo et al., 1999). *Nkx2.1* transcripts have been reported by RT-PCR to be present in the foregut as early as E8-8.5 (eight-somite stage) (Serls et al., 2005). These signals, however, probably originate from the thyroid primordium, as the thyroid starts to develop at least 1 day before the lung (Lazzaro et al., 1991). The surfactant-associated protein C (*Sftpc*) gene is the most specific marker of lung epithelial cell lineage, but its expression is consistently detected only by E10-10.5, after the primary buds form (Wert et al., 1993).

Studies in mouse foregut cultures strongly indicate that Fgfs emanating from the adjacent heart influence the AP fate of the ventral foregut endoderm in a concentration-dependent manner. A model of foregut specification has been proposed, in which different thresholds of Fgfs pattern the endoderm into different foregut derivatives, including the liver and lung (Fig. 2A). If cultured alone, the endoderm adopts a ‘default’ pancreatic fate; adding increasing amounts of cardiac mesoderm or Fgf2 to the endoderm results first in induction of liver, and then of lung or thyroid fates (Rossi et al., 2001; Serls et al., 2005; Jung et al., 1999). In this system, the induction of lung cell fate appears to involve Fgfr4 signaling (Serls et al., 2005). Lung development, however, is apparently normal in *Fgfr4*-null mice (Weinstein et al., 1998). Currently, there is no evidence that sonic hedgehog (Shh) or Bmps, which are present in the foregut, are involved in lung endoderm specification. In summary, lung specification is likely to depend on signaling molecules, such as Fgfs from the heart, and local transcription factors that have yet to be identified.

Nkx2.1 and distal lung development

Although *Nkx2.1* is the earliest known marker of the presumptive respiratory region, *Nkx2.1*-null mutant mice do have lungs (Minoo et al., 1999). These lungs, however, are highly abnormal and

Table 1. Examples of mutations in mouse giving a reported lung and/or tracheal phenotype

Gene symbol	Gene name	Expression pattern	Phenotype	Reference
Signaling molecule				
Egfr	Epidermal growth factor receptor	Epithelium and mesenchyme	Impaired branching and deficient alveolization	Miettinen et al. (1997)
Fgf18	Fibroblast growth factor 18	Mesenchyme	Deficient alveolization	Usui et al. (2004)
Fgf9	Fibroblast growth factor 9	Epithelium and pleura	Impaired branching, reduced mesenchyme	Colvin et al. (2001)
Grem1	Gremlin 1	Epithelium and mesenchyme	Deficient alveolization	Michos et al. (2004)
Hip1	Huntingtin-interacting protein 1	Mesenchyme	Impaired branching	Chuang et al. (2003)
Shh	Sonic hedgehog	Epithelium	Impaired branching, tracheoesophageal fistula	Litingtung et al. (1998)
Tgfb3	Transforming growth factor, β3	Epithelium and pleura	Impaired branching	Kaartinen et al. (1995)
Wnt7b	Wingless-related MMTV integration site 7B	Epithelium	Vascular defect, reduced mesenchyme	Shu et al. (2002)
Catnnb1	β-Catenin	Epithelium	Impaired branching, proximal/distal specification	Mucenski et al. (2003)
Ltbp4	Latent transforming growth factor β binding protein 4	Not reported	Pulmonary emphysema	Sterner-Kock et al. (2002)
Wnt5a	Wingless-related MMTV integration site 5A	Mesenchyme and epithelium	Increased branching, tracheal defect	Li et al. (2002)
Fgf10	Fibroblast growth factor 10	Mesenchyme	Lung agenesis	Sekine et al. (1999)
Fgfr2b	Fibroblast growth factor receptor 2b	Epithelium	Lung agenesis	De Moerlooze et al. (2000)
Fgf8	Fibroblast growth factor 8	Not reported	Right pulmonary isomerism	Fischer et al. (2002)
Acvr2b	Activin receptor IIB	Not reported	Right pulmonary isomerism	Oh and Li (1997)
Nodal	Nodal	Not reported	Right pulmonary isomerism	Lowe et al. (2001)
Lefty1	Left right determination factor 1	Not reported	Left pulmonary isomerism	Meno et al. (1998)
Traf4	Tnf receptor associated factor 4	Not reported	Tracheal defect	Shiels et al. (2000)
Fgfr3/Fgfr4	Fibroblast growth factor receptor 3/4	Epithelium and mesenchyme	Deficient alveolization	Weinstein et al. (1998)
Nog	Noggin	Mesenchyme	Lobation defect	Weaver et al. (2003)
Transcription factor				
Cebpa	CCAAT/enhancer binding protein (C/EBP), α	Epithelium	Hyperproliferation of type II cells	Sugahara et al. (2001)
Foxa1/Foxa2	Forkhead box A1/A2	Epithelium	Impaired branching, reduced smooth muscle	Wan et al. (2005)
Foxf1a	Forkhead box F1a	Mesenchyme	Impaired branching, lobation defect	Lim et al. (2002)
Hoxa5	Homeobox A5	Mesenchyme	Impaired branching, tracheal defect	Aubin et al. (1997)
Klf2	Kruppel-like factor 2 (lung)	Not reported	Impaired sacculation	Wani et al. (1999)
Mycn	Neuroblastoma myc-related oncogene 1	Epithelium	Impaired branching	Moens et al. (1992)
Trp63	Transformation-related protein 63	Epithelium	Tracheobronchial defect	Daniely et al. (2004)
Titf1	Thyroid transcription factor 1	Epithelium	Loss of distal lung fate, impaired branching, tracheoesophageal fistula	Kimura et al. (1996)
Nfib	Nuclear factor I/B	Epithelium and mesenchyme	Sacculation defect	Steele-Perkins et al. (2005)
Sox11	SRY-box-containing gene 11	Epithelium	Hypoplastic lung	Sock et al. (2004)
Tcf21	Transcription factor 21 (Pod1)	Mesenchyme	Impaired branching	Quaggin et al. (1999)
Rarb/Rara	Retinoic acid receptor α/β	Epithelium and mesenchyme	Left lung agenesis and right lung hypoplasia	Mendelsohn et al. (1994)
Pitx2	Paired-like homeodomain transcription factor 2	Mesenchyme	Right pulmonary isomerism	Lin et al. (1999)
Foxj1	Forkhead box J1	Epithelium	Left-right asymmetry, loss of ciliated cells	Brody et al. (2000)
Gata6	GATA-binding protein 6	Epithelium	Impaired sacculation	Yang et al. (2002)
Gli2/Gli3	GLI-Kruppel family member GLI2/GLI3	Mesenchyme	Lung agenesis	Motoyama et al. (1998)
Ascl1	Achaete-scute complex homolog-like 1	Neuroendocrine cells	Loss of neuroendocrine cells	Ito et al. (2000)
Others				
Eln	Elastin	Mesenchyme	Deficient alveolization	Wendel et al. (2000)
Lmnb1	Lamin B1	Epithelium and mesenchyme	Deficient alveolization	Vergnes et al. (2004)
Lama5	Laminin α5	Epithelium and pleura	Defective lobation	Nguyen et al. (2002)
Pcaf	p300/CBP-associated factor	Epithelium and mesenchyme	Defective proximal and distal epithelial cell differentiation	Shikama et al. (2003)
Adam17	A disintegrin and metallopeptidase domain 17	Epithelium	Impaired epithelial differentiation, impaired branching	Zhao et al. (2001) Peschon et al. (1998)
Crh	Corticotropin releasing hormone	Epithelium	Defective epithelial and mesenchymal maturation	Muglia et al. (1999)
Pthlh	Parathyroid hormone-like peptide	Epithelium	Deficient alveolization	Rubin et al. (2004)
Itga3	Integrin α3	Epithelium	Impaired branching	Kreidberg et al. (1996)
Cutl1	Cut-like 1	Epithelium	Impaired epithelial differentiation	Ellis et al. (2001)

DEVELOPMENT

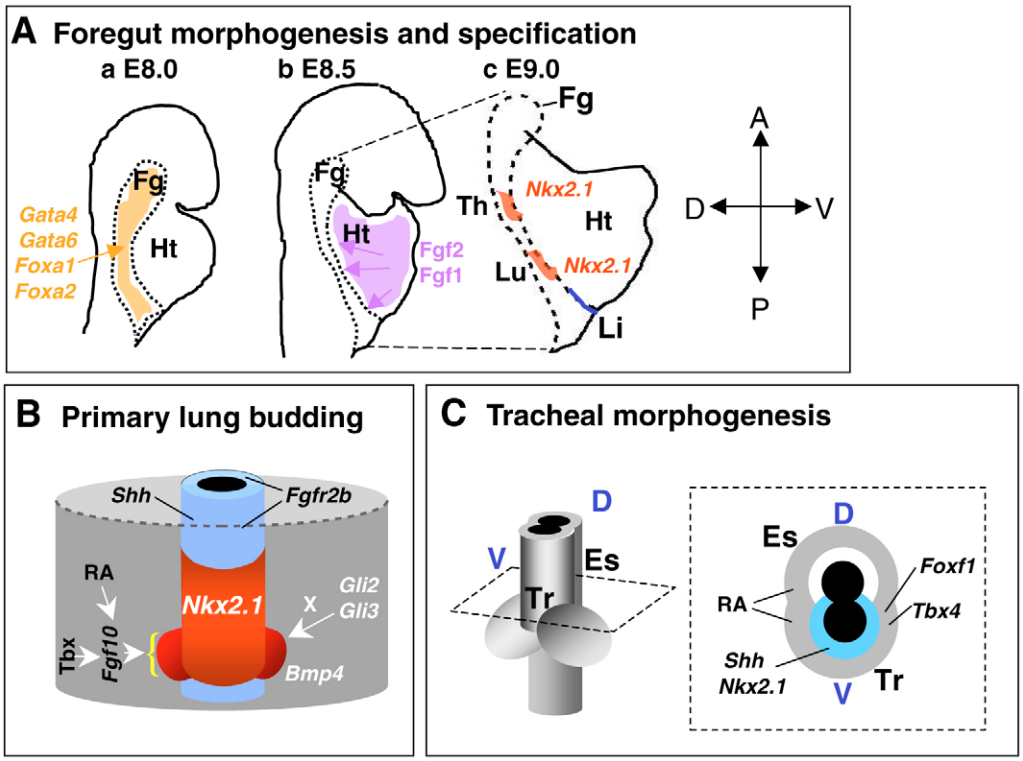


Fig. 2. Molecular regulation of initial events in lung and tracheal development. (**A**) The developing mouse foregut from embryonic day (E) 8.0 to E9.0. (a) The Foxa and Gata transcription factors genes (yellow) are involved in early events, such as foregut (Fg) tube closure and establishing endodermal competence. (b,c) A model of foregut specification, in which increasing thresholds of Fgfs (purple), emanating from the heart (Ht), specify the ventral foregut endoderm into liver (Li) (blue line) or into lung (Lu) and thyroid (Th) (red, *Nkx2.1*-expressing endoderm). [See text and Serls et al. (Serls et al., 2005) for details.] (**B**) Regulation of primary lung bud formation, based on data from mouse and chick (see text for details). Foregut mesoderm is shown in gray, endoderm in blue, and the endoderm of the prospective trachea and lung in red. Lung budding (red) results from mesodermal induction of *Fgf10* and from activation of Fgfr2b signaling in the endoderm (indicated by a yellow bracket). Retinoic acid (RA) and Tbx genes (*TBX4* in chicks) regulate *Fgf10* expression. *Gli2* and *Gli3* are both required for primary lung bud formation, presumably via an unknown intermediate factor (X). *Bmp4* is expressed in the ventral mesoderm at the lung field, where its role is unknown. (**C**) Trachea (Tr) formation from the ventral foregut and its separation from the dorsal gut tube (Es, primitive esophagus). A cross-section through the foregut shows dorsoventral (DV) differences in gene expression that probably influence this process. For example, mice deficient in *Shh* or *Nkx2.1*, which are normally present in the ventral foregut endoderm, show tracheoesophageal fistula (incomplete separation of the respiratory and digestive systems) (Minoo et al., 1999; Litingtung et al., 1998). This defect has been also associated with deficiencies in *Foxf1* (Lim et al., 2002), *Tbx4* (Sakiyama et al., 2003) and RA (Dickman et al., 1997).

consist of two main bronchi, which give rise to cystic structures, lined by columnar cells with scattered cilia – features that are reminiscent of those found in proximal airways. Strikingly, marker analysis shows that the epithelium fails to express any of the surfactant-protein genes typically found in the normal distal lung. Whether distal lung progenitors are specified but not maintained in these mice cannot be determined without the identification of additional early markers of lung cell fate. Besides *Nkx2.1*, no other early marker is currently available. The presence of relatively preserved features of proximal differentiation in *Nkx2.1*-null mutants suggests that *Nkx2.1* may not be crucial for the developmental program of progenitor cells of the proximal lung (Minoo et al., 1999).

Why the lack of *Nkx2.1* has such a negative effect on branching morphogenesis is still unclear. It is possible that, normally, Nkx2.1 controls the expression of molecules that are important for epithelial-mesenchymal interactions. Indeed, collagen type IV and several integrins, which are required for epithelial-mesenchymal interactions, are absent or greatly reduced in the lung epithelium of *Nkx2.1*-null mice (Yuan et al., 2000).

These studies suggest that Nkx2.1 is essential for the developmental program of epithelial cells of the distal lung and that Nkx2.1 is required for expression of several lung markers, such as *Sftpc* (Kelly et al., 1996). Although the promoter region of *Nkx2.1* has been studied, little is known about the cis-active regulatory sequences that direct *Nkx2.1* expression to the lung (Pan et al., 2004).

Primary lung bud formation

Lung bud morphogenesis: the role of Fgf10 and Fgfr2b

In mice, lung primordial buds form at E9.5 (~25-somite stage; Fig. 1). As determined by studies in *Drosophila*, the budding of the developing tracheal system is initiated by the expression of a Fgf ligand (*branchless*) at prospective sites of budding; this is followed by the local activation of a Fgf receptor (*breathless*) in the endoderm to induce budding (Sutherland et al., 1996). In mammals, signaling by Fgf10 and Fgfr2b is crucial for lung bud formation. Fgf10 is a chemotactic and proliferation factor for the endoderm (Bellusci et al., 1997b; Park et al., 1998). Deletion of either *Fgf10* or *Fgfr2b* in mice results in lung agenesis and multiple

abnormalities (De Moerlooze et al., 2000; Min et al., 1998; Sekine et al., 1999). The overlapping features of *Fgf10*- and *Fgfr2b*-null mutants confirm Fgfr2b as the major receptor for Fgf10. Interestingly, unlike *Fgf10*-null mutants, *Fgfr2b*-null mice form an underdeveloped lung bud that soon undergoes apoptosis (De Moerlooze et al., 2000). This has been attributed to Fgf10-mediated activation of Fgfr1b, a receptor that also binds to Fgf10, but with much lower affinity (Lu et al., 1999). It is thus not able to maintain lung epithelial survival and the lung morphogenetic program that is normally carried out by Fgf10 and Fgfr2b signaling. Although tracheal morphogenesis has been reported to be normal in *Fgf10*-null mice at birth, a recent analysis of *Fgf10* heterozygous mice has revealed that the size and number of tracheal submucosal glands are significantly reduced (Rawlins and Hogan, 2005). These structures develop postnatally from the tracheal epithelium and probably recapitulate the Fgf-dependent program of budding and branching seen in the embryonic lung.

What controls lung primordium positioning in the foregut tube?

Little is known about the genes that control the positioning of the lung primordium in the foregut or the boundaries of the *Fgf10* domain in the foregut (Fig. 2B). There is evidence, however, that *Fgf10* expression and bud formation in the lung field are crucially dependent on RA (Desai et al., 2004). RA synthesis and use are prominent throughout the E8.5-9.5 mouse foregut (Malpel et al., 2000). Yet, disruption of RA signaling in the foregut affects the lung most dramatically and leads to several abnormalities, including lung agenesis (Wilson et al., 1953; Mollard et al., 2000). Culturing E8.5 foregut explants in the presence of a RA receptor antagonist prevents lung buds from forming. In this model, RA selectively regulates *Fgf10* where the lung and neighboring stomach form (Desai et al., 2004). This seems to involve signaling by RA receptor β in the mesoderm (Desai et al., 2006).

Gli and T-box (Tbx) transcription factors have been also implicated in the formation of the lung primordium. Gli1, Gli2 and Gli3 are transcriptional effectors of the Shh signaling pathway that are present in the foregut mesoderm and later in the lung mesenchyme (Hui et al., 1994). In *Gli2/Gli3* double-null mice, lung and tracheal primordium never form; other foregut derivatives develop but are smaller than normal, and most embryos die by E10.5 (Motoyama et al., 1998). This phenotype is intriguing, as it is more severe than that of *Shh*-null mutants (Pepicelli et al., 1998), and also because there is no evidence that during development these Gli proteins are preferentially expressed in the lung field. Presumably Gli2 and Gli3 induce a currently unidentified mesodermal diffusible signal that is required for bud formation.

In chick embryos, *Tbx4* and *Fgf10* are co-expressed in the foregut mesoderm in the lung field in a domain that coincides with that of *Nkx2.1* in the endoderm, except in its most anterior portion. Studies in ovo show that misexpression of *Tbx4* induces ectopic *Fgf10* expression and ectopic buds that express *Nkx 2.1* mRNA. Tbx4 and Fgf10 are not required to initiate *Nkx.2.1* expression, but appear to play a role in defining the posterior boundary of *Nkx2.1* and the lung primordium (Sakiyama et al., 2003). However, the genetic inactivation of *Tbx4* in mice does not prevent lung bud formation (Naiche and Papaioannou, 2003). The overlapping expression of *Tbx2*, *Tbx3*, *Tbx4* and *Tbx5* in the developing foregut and lung mesoderm suggests that these genes may have a redundant role in foregut and lung morphogenesis (Chapman et al., 1996).

RA, Fgf10, Gli2, Gli3, Tbx2, Tbx3 and Tbx4, discussed here, all have in common expression in the foregut mesoderm at the onset of lung development and a potential, or demonstrated, involvement in primary lung bud induction.

Making the respiratory tree

From E10.5-E17.0, the lung epithelium undergoes branching morphogenesis to form the respiratory (or bronchial) tree. This process has been extensively studied in the lung (see Table 1), but many questions and controversies about its molecular regulation remain unresolved.

Fgf10 acts as the signal that triggers secondary and subsequent budding, as it does during primary budding (Fig. 3A). This, however, remains to be rigorously tested by conditional inactivation of *Fgf10*. At least during the initial generation of branches, lung buds arise in a highly stereotypical fashion. It has been proposed that the expression pattern of *branchless* in the developing *Drosophila* trachea or *Fgf10* in the lung is invariant within a three-dimensional grid and could be set by global regulators of axis formation, such as the Hox genes (Metzger and Krasnow, 1999). Indeed, several Hox family members are expressed in partially overlapping domains along the AP axis of the mouse developing lung (Bogue et al., 1996; Volpe et al., 1997; Aubin et al., 1997). However, the lack of dramatic changes in the AP axis of the lung in single or double Hox-null mutants suggests that the role of these genes in lung patterning is still unclear.

An intriguing, dynamic pattern of expression of the Sry-like HMG box transcription factor *Sox2* in the developing lung and thyroid epithelium has led to the hypothesis that local downregulation of *Sox2* may be required for commencement of bud morphogenesis. During lung branching morphogenesis, *Sox2* is associated with the epithelium that is less morphogenetically active, and expression is lost at sites where nascent buds arise (Ishii et al., 1998). The role of Sox2 in the lung remains to be demonstrated; mice deficient in Sox2 die prior to organogenesis because of the inability of the stem cells to proliferate (Avilion et al., 2003).

Left-right asymmetry and branching

Left and right lungs are asymmetric, as is apparent by their distinct patterns of branching and by the different number of lobes on each side (lobes are morphological units of the lung that are covered by the visceral pleura). The number of lobes varies in different species. Murine lungs characteristically have one lobe on the left and four lobes on the right (see Fig. 1D). Asymmetry of the lung is dependent on left-right (LR) determinants. The process is part of an early global program of axis specification that is regulated by several Tgfb-related molecules, such as activin receptor 2, Lefty1, Lefty2 and growth differentiation factor 1, and by the bicoid type homeobox gene *Pitx2* (Oh and Li, 1997; Meno et al., 1998; Kitamura et al., 1999; Rankin et al., 2000; Lin et al., 1999). Disruption of these genes results in laterality defects in multiple organs. In the lung, these defects commonly manifest as isomerism, the presence of equal numbers of lobes (with either right or left pattern) on both sides (Table 1). Interestingly, most of these genes are expressed in the mouse foregut mesoderm only transiently (E8-8.5). Except for *Pitx2*, expression of LR determinants ceases by the time the lung primordium forms (E9.5). The relatively late appearance of *Pitx2* has led to the hypothesis that this gene acts as an executor of early genetic programs that control asymmetry in different structures of the embryo (Kitamura et al., 1999). Among the three isoforms (a, b, c), only *Pitx2c* is asymmetrically expressed in the left lung. *Pitx2*-null mice show right pulmonary isomerism (four-lobed lungs bilaterally) (Kitamura et al., 1999). Thus, during normal development, Pitx2

could presumably influence gene expression in the left lung, allowing budding only at specific sites. By doing so, Pitx2 would generate a simpler pattern of branching (and lobation), characteristic of the left lung. How Pitx2 exerts its functions, and whether *Pitx2* and *Fgf10* interact in the lung mesenchyme, are unknown. Only a limited number of *Pitx2* targets have been reported, and they shed little light on these issues (Ganga et al., 2003).

Control of budding by sprouty and Shh

The exchange of signals between the growing bud and the surrounding mesenchyme establishes feedback responses that control the size and shape of the bud during branching. This is illustrated by the mechanisms that control Fgfr2b activity or *Fgf10* expression by the sprouty (Spry) or the Shh pathways, respectively (Fig. 3B-D).

Spry genes encode a family of cysteine-rich proteins (there are four members in mice) that interact with crucial elements of the receptor tyrosine kinase Rtk-Ras-Erk/Mapk cascade and interfere with the intensity or timing of Rtk signaling by ligands such as Fgf and Egf (Kim and Bar-Sagi, 2004). In the mouse lung epithelial cell line MLE15, Spry2 inhibits Fgf10-Fgfr2 signaling by binding to Frs2 (fibroblast growth factor receptor substrate 2), Grb2 (growth factor receptor bound protein 2) and Raf (v-raf-leukemia viral oncogene 1), and by disassociating from Gap1 (GTPase-activating protein 1) and Shp2 (Src homology 2-containing phosphotyrosine phosphatase) (Tefft et al., 2002).

In the developing E11-12 mouse lung, *Spry2* is expressed at the tips of the growing epithelial buds, while *Spry4* is present in the surrounding distal lung mesenchyme. *Spry2* is one of the earliest targets to be induced in the lung epithelium in response to Fgf10 (Mailleux et al., 2001). By acting as a Fgf10-dependent inhibitor of Fgfr2b activity, Spry2 limits the proliferation or migration of the lung epithelium when buds are forming. This could be part of a mechanism to control the size of the bud or to stop bud formation and, ultimately, to inhibit branching morphogenesis. Consistent with this role, reducing *Spry2* activity in lung organ cultures results in increased branching (Tefft et al., 1999), as also reported for the tracheal system of *Drosophila* in *Spry* mutants (Hacohen et al., 1998). Conversely, overexpression of *Spry2* or misexpression of *Spry4* in the distal lung epithelium of transgenic mice severely impairs branching (Mailleux et al., 2001; Perl et al., 2003).

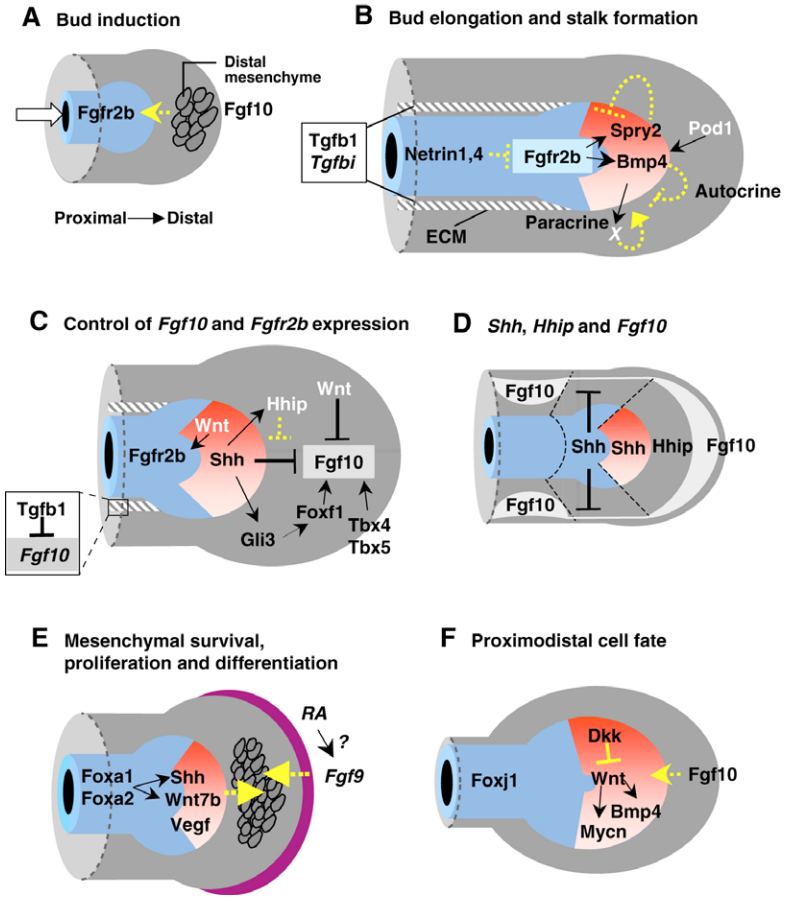


Fig. 3. Models of bud formation and proximodistal patterning in the developing lung. A developing lung bud during branching morphogenesis. Mesenchyme is depicted in gray and the epithelium in blue or red (distal bud). (**A**) Branching initiates with local *Fgf10* expression in the distal mesenchyme. Fgf10 diffuses (yellow arrow) and binds locally to Fgfr2b (expressed throughout the lung epithelium) to activate signaling and induce a bud (white arrow). (**B**) As the bud elongates, Fgfr2b signaling induces expression of *Spry2* (which negatively regulates Fgf signaling and inhibits budding, broken yellow line) and *Bmp4* in the distal epithelium. Bmp4 possibly also inhibits distal budding through autocrine signaling from the epithelium (Eblaghie et al., 2006) (broken yellow line) and can also enhance budding in a paracrine fashion (broken yellow arrow), via an unidentified mesenchymal signal (X). Mesenchymal Pod1 (Tcf21) (indirectly) and epithelial Wnt signaling regulate Bmp4 (see F). Mechanisms that might inhibit ectopic budding in stalk regions include: netrin-mediated Fgfr2b signaling inhibition (broken yellow line); Tgfb activation in the epithelium by Tgfb1 from the subepithelial mesenchyme; Tgfb1-induced synthesis of extracellular matrix (ECM) components, such as collagen and fibronectin, and Tgfbi in stalk mesenchyme. (**C**) Control of *Fgf10* and *Fgfr2b* expression. Canonical Wnt signaling activates *Fgfr2b* expression in the lung epithelium; mesenchymal Wnt (alone or with epithelial Wnt) inhibits *Fgf10*. Positive regulators of *Fgf10* include Foxf1, Tbx4 and Tbx5. Tgfb1 signaling in stalk mesenchyme may prevent *Fgf10* expression in the proximal mesenchyme (box in C). Shh signaling in the distal mesenchyme inhibits *Fgf10* expression, but via Gli3 also controls availability of Foxf1, a positive regulator of *Fgf10*. Shh induction of *Hhip* expression inhibits Shh signaling (broken yellow line) to allow *Fgf10* expression. (**D**) At the bud tips, high Shh (distal epithelium) and Hhip (distal mesenchyme) levels result in overall less Shh signaling and more *Fgf10* than in the immediately adjacent regions, where Shh signaling is unopposed by Hhip. Low Shh levels in more proximal bud regions allow *Fgf10* expression in the adjacent mesenchyme, resulting in later induction of lateral buds. (**E**) The proliferation of multipotent mesenchymal progenitors while the lung grows depends on Shh and Wnt7b signals from the distal epithelium and on Fgf9 from the pleura (purple). Foxa1 and Foxa2 regulate *Shh* and *Wnt7b* expression. Vegf regulates endothelial cell differentiation. RA (from the pleura) may regulate *Fgf9* expression but this remains to be shown. (**F**) A model of proximodistal cell fate regulation in the lung bud epithelium. Mycn and Fgf10 (via Fgfr2b epithelial signaling) maintain the proliferation of progenitor cells of the distal lung epithelium. *Bmp4* prevents distal epithelial cells from assuming a proximal phenotype. Wnt signaling regulates the timing of their differentiation (presumably by controlling *Bmp4* and *Mycn* expression) and is negatively regulated by dickkopf 1 (Dkk1). Foxj1 induces differentiation of proximal epithelium into ciliated cells. See text for references and Eblaghie et al. (Eblaghie et al., 2006).

DEVELOPMENT

Bud formation can be also controlled by diffusible signals originating from epithelial cells at the tips of nascent buds, which may alter levels or distribution of *Fgf10* in the mesenchyme, and ultimately influence Fgf signaling in the epithelium. Shh is highly expressed in the distal epithelium, from where it diffuses to activate signaling in the corresponding mesenchyme via patched (Ptch1)/smoothened (Smo) and their transcriptional effectors Gli1, Gli2 and Gli3 (Bellusci et al., 1997a). Data from lung organ culture and in vivo studies support the idea that Shh negatively regulates *Fgf10* expression in the lung (Bellusci et al., 1997a; Lebeche et al., 1999). These findings have led to the proposal that Shh at the bud tips progressively downregulates *Fgf10* expression as a bud grows towards the *Fgf10*-expressing mesenchyme, thus limiting further bud outgrowth. By doing so, Shh would contribute to controlling bud size. Interestingly, Shh may also control the shape of the bud by preventing the widespread expression of *Fgf10* in the mesenchyme and the generalized activation of Fgfr2b in the distal epithelium. In lungs from *Shh*-null mice, *Fgf10* transcripts are found diffusely expressed in the lung mesenchyme. Instead of forming typical buds, the epithelium develops as large cystic structures and branching morphogenesis is severely disrupted (Pepicelli et al., 1998). Precisely how Shh helps to control the spatial pattern of *Fgf10* expression is unknown. One potential way involves Shh induction of the hedgehog interacting protein *Hhip1* in the distal mesenchyme. Hhip1 inhibits Shh signaling by ligand sequestration and, thus, releases Shh-mediated repression of *Fgf10* locally (see Fig. 3D). In the lungs of *Hhip1*-null mice, branching is inhibited because of increased Shh activity and the nearly complete repression of *Fgf10* expression in the developing lung (Chuang et al., 2003).

Bmp and Wnt signaling: positive or negative regulators of branching?

There have been apparently conflicting reports about how Bmp and Wnt signaling influence lung branching morphogenesis. Among the Bmp ligands present in the embryonic lung, Bmp4 is the best studied. *Bmp4* is transcribed in the lung mesenchyme from its earliest stages, but it is not present in the epithelium until branching initiates. A $Bmp4^{lacZ}$ reporter mouse reveals a striking distribution of *Bmp4* in the ventral foregut mesoderm at the prospective lung region as early as E9 (Weaver et al., 1999). The biological significance of this finding remains to be determined. By E11-12, *Bmp4* transcripts are found in the distal lung epithelium and in the proximal mesenchyme (Weaver et al., 1999; Weaver et al., 2003). The Bmp4 receptor (type I, or Alk3), and the Bmp transducing Smad1 protein are present both in the epithelium and mesenchyme of the embryonic lung (Bellusci et al., 1996; Chen et al., 2005). A detailed analysis of Bmpr2 distribution in the early embryonic lung is not currently available. The patterns above suggest that Bmp4 signaling can be activated both in an autocrine fashion (in the epithelium) and in a paracrine fashion (in the mesenchyme).

The precise role of Bmp4 in the developing lung in vivo remains unclear, in part because of the early embryonic death of *Bmp4*-null mice (Winnier et al., 1995). It has been previously proposed that, during branching, Bmp4 is induced and activated in the epithelium of distal buds to limit Fgf10-mediated bud outgrowth. This model is supported by the following observations (Lebeche et al., 1999; Weaver et al., 2000). Analyses of E11.5 lungs undergoing branching morphogenesis show that *Bmp4* expression in the lung epithelium is highest in distal buds, near *Fgf10*-expressing cells. *Bmp4* is not induced in the epithelium of E11.5 lungs during bud initiation but appears later, once the bud is elongating. In mesenchyme-free lung epithelial cultures, recombinant Fgf10 induces budding and *Bmp4* expression, while recombinant Bmp4 inhibits the Fgf10-mediated budding in these cultures.

Paradoxically, when recombinant Bmp4 is administered to intact lung explants in which the epithelium and mesenchyme are present, branching is enhanced (Bragg et al., 2001). An alternative model has been proposed to explain how Bmp4 can have both positive and negative effects in the lung. The model predicts that the mesenchyme influences the ability of the epithelium to respond to Bmp4. When Bmp4 signaling is activated in the epithelium in an autocrine fashion, proliferation is inhibited (but see Eblaghie et al., 2006). In the intact lung, however, Bmp4 present in the distal epithelium may also activate Bmp signaling in the adjacent mesenchyme (paracrine fashion). Bmp4, then, induces a currently unidentified distal mesenchymal signal that enhances proliferation of distal epithelial buds (Bragg et al., 2001). In this way, negative or positive effects on branching would depend on whether Bmp signaling is activated via an autocrine or a paracrine mechanism (Fig. 3B). Regulation of *Bmp4* in the epithelium is complex and dependent on signals such as Wnt (see below), Fgf10 and Pod1 (Tcf21), a transcriptional factor present in the mesenchyme (Quaggin et al., 1999).

The role of Wnt signaling in lung branching morphogenesis has also been debated. Several Wnt ligands, frizzled receptors and components of the Wnt canonical pathway, such as β-catenin, and Tcf/Lef transcription factors (see http://www.stanford.edu/~rnusse/wntwindow.html) are present in the developing lung (Bellusci et al., 1996; Lako et al., 1998; Zakin et al., 1998; Tebar et al., 2001). Activation of canonical Wnt signaling can be monitored by detection of nuclear translocated β-catenin, and by analysis of a Wnt responsive reporter mouse (TOPGAL), in which *lacZ* is expressed where the β-catenin-Lef1/Tcf complex activates the transcription of Wnt targets (Nelson and Nusse, 2004; DasGupta and Fuchs, 1999). In the E11-13 lung, β-catenin is expressed throughout the entire lung epithelium. However, nuclear-localized β-catenin, Tcf/Lef transcripts and *lacZ*-TOPGAL expression are increased in the distal lung epithelium, the sites that are actively branching (Okubo and Hogan, 2004; De Langhe et al., 2005). Disruption of canonical Wnt signaling at these sites by targeted deletion of β-catenin, or by targeted expression of the Wnt inhibitor dickkopf 1 in vivo, prevents distal lung buds from forming and markedly interferes with branching morphogenesis. The defect appears to result, at least in part, from failure to induce proper levels of *Fgfr2b* in the distal lung epithelium where Wnt/β-catenin signaling is inhibited (Mucenski et al., 2003; Shu et al., 2005). It has been pointed out, however, that although β-catenin deletion is a good method for disrupting all Wnt canonical signaling, other β-catenin functions, not necessarily related to Wnt signaling, may be also affected (Dean et al., 2005). Indeed, β-catenin is also found in cell membranes in a cadherin-bound form that regulates cell adhesion. Thus, it is possible that the branching defect reported in the models in which β-catenin was deleted from the epithelium could have resulted from changes to both its Wnt and non-Wnt functions (Dean et al., 2005).

Interestingly, the results above conflict with that of two other models that show increased branching morphogenesis as a consequence of disrupted Wnt signaling. In one model, lung explants were treated with morpholino oligonucleotides against β-catenin; the other is a genetic model in which mice lack the *Wnt5a* gene (a non-canonical Wnt, normally present in the lung epithelium and mesenchyme) (Li et al., 2002; Dean et al., 2005). In both cases, *Fgf10* expression was locally increased in these lungs. It is possible that this discrepancy in results is due to the fact that in the morpholino and *Wnt5*a models, Wnt signaling was inactivated in

both the epithelium and mesenchyme. In addition, canonical and non-canonical Wnts (and even different Wnt family members) may have distinct functions (Fig. 3C,E,F). Further studies are required to clarify these issues.

The discussion in this section underscores the complexity of the Bmp4 and Wnt signaling during branching, and the importance of taking into account the overall balance of these signals in the epithelium and mesenchyme. In addition, both Bmp4 and canonical Wnt are required for the establishment of distal epithelial cell fate in the lung (see below).

Tgfb signaling as a negative regulator of branching morphogenesis

Tgfb1, Tgfb2 and Tgfb3, members of the Tgfb subfamily, have also been implicated in the control of lung branching morphogenesis. These Tgfb ligands, their receptors (Tgfbr1 and Tgfbr2) and transducing proteins Smad2 and Smad3, are expressed in the developing mouse lung. Many of the biological activities of these Tgfb proteins differ only in the intensity of their effects (reviewed by Massague, 2000). During lung branching morphogenesis, *Tgfb1* is transcribed in the mesenchyme adjacent to the epithelium, without an obvious proximodistal gradient (Lebeche et al., 1999). However Tgfb1 protein accumulates in stalks and in regions in between buds, where extracellular matrix components collagen I, collagen III and fibronectin are also present (Heine et al., 1990). *Tgfb2* is expressed in the distal lung epithelium, while *Tgfb3* is present in the proximal lung epithelium, mesenchyme and pleura (Pelton et al., 1991). Analysis of *Tgfb2*- or *Tgfb3*-null mice reveals that lung branching morphogenesis is affected by the lack of *Tgfb3*, but not by *Tgfb2* deficiency (Sanford et al., 1997; Kaartinen et al., 1995).

Exogenous Tgfb1 inhibits branching morphogenesis, growth and differentiation in cultured mouse embryonic lungs (Serra et al., 1994; Zhao et al., 1996). This effect has been also reported in transgenic mice misexpressing *Tgfb1* in the distal epithelium (Zhou et al., 1996). Interestingly, *Tgfb1*-null mice show no obvious structural lung defect and die perinatally of a diffuse inflammatory syndrome (Letterio et al., 1994). Whether Tgfb1 is dispensable for lung morphogenesis has been debated, as there is evidence that the phenotype may have been rescued by maternal transfer of Tgfb1. Studies in NIH3T3 fibroblasts, lung and prostate organ cultures indicate that activation of Tgfb1 signaling in mesenchymal cells markedly inhibits *Fgf10* expression (Beer et al., 1997; Lebeche et al., 1999; Tomlinson et al., 2004). In the developing lung, Tgfb1 may be part of a mechanism that prevents *Fgf10* from being expressed in the mesenchyme of bud stalks or in more proximal regions of the lung. At these sites, Tgfb1 could also induce synthesis of extracellular matrix components and prevent budding locally (Fig. 3B).

Refining patterning and preventing ectopic budding

Branching patterns can be further refined by mechanisms that regulate bud formation at specific locations in the developing lung. For example, the transcription factor *Foxf1* is expressed throughout the mesenchyme of both lungs from the earliest developmental stages. However, analysis of *Foxf1* heterozygous mice at E10-11 suggests that Foxf1 is required for proper gene expression and budding selectively in the cranial, middle and accessory lobes of the right lung. These mice show low levels of *Fgf10* and *Gli3* mRNAs, and display altered lung bud orientation and ectopic budding in these lobes, among other abnormalities (Lim et al., 2002; Mahlapuu et al., 2001). The presence of lung defects that resemble those from *Gli3*-null mutants suggest a genetic interaction between *Foxf1* and Shh signaling via Gli3 (Fig. 3C). *Foxf1* expression is in part regulated by Shh signaling through Shh-dependent processing of Gli3 (Li et al., 2004).

Other genes are dynamically expressed at the stalks of branching tubules in a pattern that suggests a role in preventing local budding (Fig. 3B). This is the case of the axon guidance molecules netrin 1 and netrin 4, and the extracellular matrix protein Tgfb induced (*Tgfbi* or *βig-h3*) in the E11-13 mouse lung. There is evidence that netrins and their receptors Unc5b and Dcc (deleted in colorectal cancer) are present in the lung epithelium at these stages. Moreover, exogenous netrin inhibits Fgf-mediated Erk phosphorylation and bud formation in mesenchyme-free lung epithelial explants (Liu et al., 2004). These observations favor the hypothesis that, during branching morphogenesis, netrin expression in stalks prevents buds from forming in these region by interfering with local activation of Fgf signaling (Liu et al., 2004). This hypothesis, however, has not been confirmed in vivo, as genetic inactivation of netrins or other axon guidance molecules, such as semaphorins (or Slit and Robo proteins) in mice has not resulted in obvious defects in lung branching morphogenesis (Hinck, 2004). Recent evidence demonstrating that Slit2 and netrin 1 act synergistically in mammary gland ductal morphogenesis suggests that deciphering the role of axon guidance molecules in the lung will require simultaneous inactivation of more than one member of these functionally related families of genes (Strickland et al., 2006).

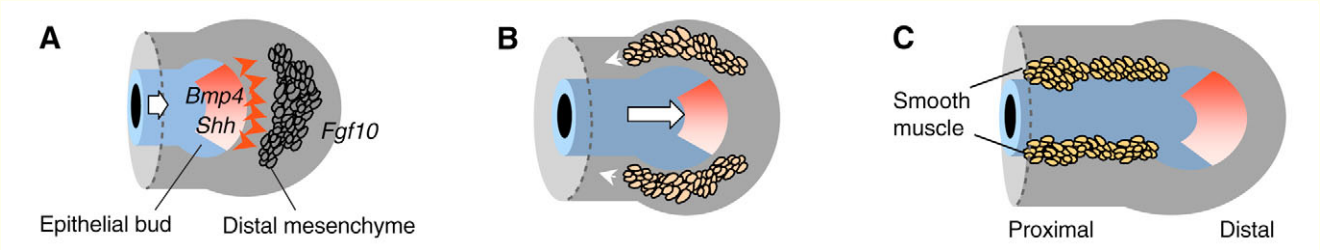
Tgfbi, another molecule dynamically expressed in stalks and in interbud regions of the developing lung, is present in the mesenchyme, at sites traditionally associated with Tgfb-induced deposition of extracellular matrix and decreased epithelial cell proliferation (Serra et al., 1994; Zhao et al., 1996; Heine et al., 1990; Lu et al., 2004). *Tgfbi* is known to be induced by Tgfb1 and to mediate Tgfb responses in some cell systems by a mechanism that is still unclear (Kim et al., 2003). Tgfbi binds to a variety of matrix molecules, such as fibronectin and collagen (Billings et al., 2002). Whether Tgfbi restricts bud formation in stalks during lung branching morphogenesis is currently unknown.

What is downstream of Fgf signaling during lung bud formation?

There is still little understanding of the cellular mechanisms of lung bud formation and of the targets of Fgf10 in this process. In *Drosophila,* Fgf signaling activation causes dynamic actin-based filopodial extensions at the tip of primary branches, leading to epithelial migration. This is in part promoted by the induction of *pointed* (*Spdef* – Mouse Genome Informatics) an Ets transcription factor (Samakovlis et al., 1996; Sutherland et al., 1996). In the E11.5 mouse lung, two Ets family members, Pea3 and Erm (Etv4 and Etv5, respectively), have been identified in the distal epithelium. Although both are downstream of the Fgfr2b pathway, they do not seem to be required for branching, as shown by expression of a dominant-negative *Erm* targeted to the lung in vivo (Liu et al., 2003).

A microarray-based screen to identify Fgf10 targets in mesenchyme-free lung epithelial buds has shown that the initial stages of budding are characterized by upregulation of genes traditionally associated with: cell rearrangement and cell migration [*Tm4sf3*, transmembrane 4 superfamily member 3 (*Tspan8*); the Notch signaling antagonist *Numb*; and *Lmo7*, LIM domain only 7]; inflammatory processes (annexins); lipid metabolism [*HSL*, hormone-sensitive lipase (*Lipe*)]; proteolysis (cathepsin H, *Timp3*); and metastatic behavior (*Tacstd2*, tumor-associated calcium signal transducer 2), but not cell proliferation (Lu et al., 2005a). This

Box 1. Myogenesis and bud morphogenesis



Airway smooth muscle starts to form relatively early in the developing mouse lung. A smooth muscle layer adjacent to the epithelium of the trachea and primary lung buds is present at E11 and continues to form around proximal, but not distal, epithelial tubules as branching proceeds (Tollet et al., 2001). Interestingly, airway smooth muscle appears partly to originate from a pool of progenitor cells in the distal lung mesenchyme that undergoes a program of differentiation that initially depends on Shh and Bmp4, signals expressed mostly by distal epithelial buds. How can these distal signals influence differentiation of a proximal mesenchymal derivative? A model has been proposed to resolve this apparent paradox (see figure) (Mailleux et al., 2005; Weaver et al., 2003). (**A**) Initially, lung epithelial buds (blue) are induced by Fgf10 from the distal mesenchyme, which, in turn, is exposed to high Bmp4 (induced in the epithelium by Fgf10) and Shh levels that diffuse from the tips of growing buds (red region, A-C). (**B**) This triggers a myogenic program in distal mesenchymal cells that continues while they are relocated to more proximal regions by continued bud outgrowth (large white arrow, B) and/or by active mesenchymal cell migration (small white arrows, B). (**C**) The myogenic program completes once these progenitor cells are located along proximal airways. This model is supported by analysis of an *Fgf10*^{lacZ} reporter mouse, which labels *Fgf10*-expressing distal mesenchymal cells and airway smooth muscle (Mailleux et al., 2005). Furthermore, a hypomorphic *Fgf10* mouse mutant, in which low Fgf10 levels result in reduced *Bmp4* expression and epithelial branching, showed decreased smooth muscle differentiation. Smooth muscle cell differentiation also depends on physical stretch, serum response factor, laminin 2, tension-induced proteins such as Tip1 and on Wnt signaling-mediated induction of fibronectin (Yang et al., 2000; De Langhe et al., 2005; Jakkaraju et al., 2005).

observation is consistent with previous studies that show local changes in proliferation are not the triggering event that initiates lung budding (Nogawa et al., 1998).

Patterning the mesenchyme

Integrating epithelial and pleural signals

Lung mesenchyme development is crucially influenced by signals from the epithelium and the pleura (Fig. 3E, Box 1, Table 1) that, in concert, appear to maintain a balance of differentiated and proliferating multipotent progenitors while the lung grows (Weaver et al., 2003). Epithelial buds are a source of trophic or differentiation signals for the mesenchyme, such as *Shh, Vegf* and *Wnt7b*, among others. Lungs from *Shh*-null mice show inhibited mesenchymal cell proliferation and smooth muscle differentiation, and severe hypoplasia, a condition characterized by abnormal small lungs (Pepicelli et al., 1998). *Wnt7b*-null mouse mutants also have defective vascular smooth muscle differentiation and are also severely hypoplastic (Shu et al., 2002). Vegf is crucial for several aspects of vascular development, and for epithelial-endothelial interactions during branching morphogenesis (Pauling and Vu, 2004; Del Moral et al., 2006).

The transcription factors *Foxa1* and *Foxa2* are present in the lung epithelium and have been shown to influence both epithelial and mesenchymal programs by regulating the levels of expression of *Shh* and *Wnt7b*. The conditional deletion of *Foxa1* and *Foxa2* in the distal lung epithelium of transgenic mice inhibits *Shh* and *Wnt7b* expression and results in disrupted branching morphogenesis and smooth muscle differentiation (Fig. 3E) (Wan et al., 2005). It has been proposed that the pleura controls proliferation and the differentiation status of the distal mesenchyme (Weaver et al., 2003). Indeed, mesothelial cells of the pleura express a number of signaling molecules such as *Fgf9, Tgfb3* and RA, which can mediate these activities (Colvin et al., 2001; Bragg et al., 2001; Malpel et al., 2000). Mice in which these pathways have been inactivated have pulmonary hypoplasia. Fgf9 (also present in the distal epithelium), probably signaling via Fgfr1c, acts as a trophic factor for the distal mesenchyme. *Fgf9* mutants have a markedly reduced number of mesenchymal cells, which results in less *Fgf10* being available overall to induce normal epithelial branching (Colvin et al., 2001). Fgf9 prevents Shh-induced differentiation of the lung mesenchyme into smooth muscle in vitro (Weaver et al., 2003).

The RA synthesizing enzyme *Raldh2* (Niederreither et al., 1999) is co-expressed with *Fgf9* in the pleura, and it is possible that *Fgf9* expression is regulated by RA in mesothelial cells (Malpel et al., 2000; Colvin et al., 1999). In the developing heart, RA from mesothelial cells of the epicardium induces *Fgf9*, which then activates an Fgf pathway that is essential for cardiomyoblast expansion (Lavine et al., 2005). As in *Fgf9*-null mice, lung hypoplasia has been also associated with disruption of RA signaling (Wilson et al., 1953).

Generating cell diversity

Mechanisms influencing proximal-distal cell fate and epithelial differentiation

The mature respiratory epithelium consists of multiple cell types, including ciliated, neuroendocrine and secretory cells present in proximal regions of the respiratory system, and type I and type II cells that are typical of the distal alveolar region of the lung. Cell fate is established along the proximodistal axis of the respiratory epithelium as lung buds form and branch, and seems to depend on a distal signaling center in which Bmp4 and Wnt canonical signaling are crucial. When Bmp signaling is inhibited in transgenic mice by targeting a dominant-negative Bmp receptor (*dnAlk6*) or Bmp antagonists (noggin, gremlin) to the distal lung epithelium, development of the distal epithelium is severely impaired and the lung becomes ‘proximalized’ (Weaver et al., 1999; Lu et al., 2001). In the model proposed by the authors of these studies, disrupted Bmp signaling makes lung epithelial cells from distal buds acquire a proximal phenotype and stop branching. Proximalization also results from the targeted deletion of β-catenin in the distal lung epithelium (Weaver et al., 1999; Mucenski et al., 2003; Lu et al., 2001). Thus, a gradient of Bmp4 and Wnt signaling, with the highest

levels of activation in distal epithelial buds, is thought to prevent distal cells from assuming a proximal phenotype (Fig. 3F). At subsequent stages, other molecules contribute to the differentiation of the epithelium into specific cell types. For example, in the proximal lung, the forkhead box transcription factor *Foxj1* is required to form ciliated cells (Chen et al., 1998).

Disrupted distal lung development is also seen in transgenic mice in which RA signaling is constitutively activated in the distal lung epithelium throughout branching morphogenesis (Wongtrakool et al., 2003). Endogenous RA signaling is active during primary lung bud formation, but is downregulated in the epithelium once secondary budding and branching initiates (Malpel et al., 2000). Interestingly, in the transgenic model above, RA signaling persists in the distal epithelium, as during early developmental stages. As a result, distal lung progenitors are present but do not undergo further differentiation and remain immature (Wongtrakool et al., 2003). Thus, in the developing lung, RA seems to act as a developmental switch. RA is initially 'on' to activate an early developmental program, but later it has to be turned 'off' to allow subsequent stages of this program to take place in the distal lung.

Maintaining progenitor cells while differentiating

As epithelial cells in branching airways continue to differentiate, it is crucial to maintain and expand a pool of uncommitted progenitor cells for continued growth. It has been proposed that this pool resides in the distal lung, as a population of proliferating immature epithelial cells that expresses high levels of the proto-oncogene *Mycn*. Targeted disruption of *Mycn* expression in distal lung epithelial cells of mutant mice inhibits distal lung proliferation and induces premature differentiation (Okubo et al., 2005). Inhibition of Wnt canonical signaling in mutant mice causes a similar lung phenotype and shows that Wnt controls levels of *Mycn* and *Bmp4* expression in the distal lung (Shu et al., 2005) (Fig. 3F). There is also evidence that activation of Fgf10 and Fgfr2b signaling in the developing pancreas, tooth, skin and the lung may be required to expand or maintain a pool of epithelial progenitor cells during organogenesis (Harada et al., 2002; Bhushan et al., 2001; Norgaard et al., 2003).

Cell plasticity: reprogramming cell fates

There is accumulated evidence in vitro and in vivo that developmental programs can be altered in cells that have initially embarked on a specific lineage pathway, simply by changing the type or amount of signals in the local environment. This has been documented in tissue recombination experiments in vitro. Reprogramming of tracheal epithelium or ureteric bud by lung mesenchyme has been demonstrated by the induction of a lung-specific pattern of branching and differentiation that is not normally present in tracheal or ureteric progenitor cells (Lin et al., 2003; Shannon, 1994). These observations are consistent with the idea that local inductive signals from the mesenchyme confer novel position-specific information that radically changes epithelial cell fate. Fgf proteins, collagen XVIII, Wnt2, Shh and transferrin are some of the molecules that have been implicated in the in vitro reprogramming of the lung epithelium (Lin et al., 2003; Hyatt et al., 2004; Ohtsuka et al., 2001). Lung epithelial reprogramming has also been shown in vivo in transgenic mice harboring a *Sfptc*-driven constitutively active β-catenin/Lef fusion protein construct (Okubo and Hogan, 2004). Remarkably, these lungs lack differentiated lung cell types and show a hyperproliferative epithelium that expresses *Cdx1, Atoh1* and other genes involved in the establishment of intestinal cell lineages. Although it is not clear exactly when the transgene starts to act, the successful targeting of the transgene to *Sftpc*-expressing cells suggests that increased Wnt signaling leads lung progenitor cells to change their fate into an intestinal secretory cell fate. Whether Wnt signaling influences the initial specification of the lung field in the primitive foregut, remains to be investigated.

Conclusion

Overall, the studies so far suggest that the major events in early lung morphogenesis are controlled by a relatively limited group of molecules (Fgfs, Tgfb, Shh, Wnt proteins). Novel insights will be gained by exploring the different ways by which expression or activation of these molecules is controlled; these include gene methylation, endogenous microRNAs and proteolysis, among other mechanisms (Lu et al., 2005b; Harris et al., 2006; Li et al., 2004). A crucial role for heparan and chondroitin sulfate proteoglycans as modulators of growth factor distribution and signaling in organogenesis has been well documented in several developing systems. In *Drosophila*, integrity and proper sulfation of heparan are essential for Fgf signaling and tracheal morphogenesis (Kamimura et al., 2001). There is evidence that this is also true for the mammalian lung, but the mechanisms remain to be understood (Izvolsky et al., 2003; Shannon et al., 2003).

The lack of early markers of lung progenitor cells represents a clear gap of knowledge in the field. Because *Sftpc* expression cannot be identified prior to the emergence of primary buds, *Nkx2.1* is the only early marker currently available for these cells. A confounding issue is that this gene is also expressed by the thyroid. Laser capture microdissection approaches and detailed gene profiling analysis of the developing foregut will be useful to find other markers of lung progenitor cells. Also crucial will be the development of tools for targeting genes to these early progenitors in the foregut in future functional studies.

Still relatively little is known about the changes that are induced in the milieu around the nascent lung bud and in the bud itself when Fgf10 activates Fgfr2b. How do cells rearrange to form new buds? These questions require powerful image analysis systems and an array of markers, which may be already available.

A rather more complex problem is the understanding of how the coordinates that set up the three-dimensional pattern of morphogens, such as Fgf10, are established in the lung. Finally, there is the much debated issue of stem cells. What are these cells? Where are their niches in the developing lung? How can they be identified? Tackling these issues will provide insights into the molecular and cellular mechanisms by which the lung develops.

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Note added in proof

A recent report by Eblaghie et al. (Eblaghie et al., 2006) provides genetic evidence that Bmpr1a mediates an autocrine signaling required for distal lung epithelial cell proliferation and survival.

References

Ang, S. L. and Rossant, J. (1994). HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* **78**, 561-574.

Aubin, J., Lemieux, M., Tremblay, M., Berard, J. and Jeannotte, L. (1997). Early postnatal lethality in Hoxa-5 mutant mice is attributable to respiratory tract defects. *Dev. Biol.* **192**, 432-445.

Avilion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N. and Lovell-Badge, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* **17**, 126-140.

Beer, H. D., Florence, C., Dammeier, J., McGuire, L., Werner, S. and Duan, D. R. (1997). Mouse fibroblast growth factor 10, cDNA cloning, protein characterization, and regulation of mRNA expression. *Oncogene* **15**, 2211-2218.
Bellusci, S., Henderson, R., Winnier, G., Oikawa, T. and Hogan, B. L. (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.
Bellusci, S., Furuta, Y., Rush, M. G., Henderson, R., Winnier, G. and Hogan, B. L. (1997a). Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. *Development* **124**, 53-63.
Bellusci, S., Grindley, J., Emoto, H., Itoh, N. and Hogan, B. L. (1997b). Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. *Development* **124**, 4867-4878.
Bhushan, A., Itoh, N., Kato, S., Thiery, J. P., Czernichow, P., Bellusci, S. and Scharfmann, R. (2001). Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* **128**, 5109-5117.
Billings, P. C., Whitbeck, J. C., Adams, C. S., Abrams, W. R., Cohen, A. J., Engelsberg, B. N., Howard, P. S. and Rosenbloom, J. (2002). The transforming growth factor-beta-inducible matrix protein (beta)ig-h3 interacts with fibronectin. *J. Biol. Chem.* **277**, 28003-28009.
Bogue, C. W., Lou, L. J., Vasavada, H., Wilson, C. M. and Jacobs, H. C. (1996). Expression of Hoxb genes in the developing mouse foregut and lung. *Am. J. Respir. Cell Mol. Biol.* **15**, 163-171.
Bort, R., Martinez-Barbera, J. P., Beddington, R. S. and Zaret, K. S. (2004). Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. *Development* **131**, 797-806.
Bourbon, J., Boucherat, O., Chailley-Heu, B. and Delacourt, C. (2005). Control mechanisms of lung alveolar development and their disorders in bronchopulmonary dysplasia. *Pediatr. Res.* **57**, 38R-46R.
Bragg, A. D., Moses, H. L. and Serra, R. (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.
Brody, S. L., Yan, X. H., Wuerffel, M. K., Song, S. K. and Shapiro, S. D. (2000). Ciliogenesis and left-right axis defects in forkhead factor HFH-4-null mice. *Am. J. Respir. Cell Mol. Biol.* **23**, 45-51.
Chapman, D. L., Garvey, N., Hancock, S., Alexiou, M., Agulnik, S. I., Gibson-Brown, J. J., Cebra-Thomas, J., Bollag, R. J., Silver, L. M. and Papaioannou, V. E. (1996). Expression of the T-box family genes, Tbx1-Tbx5, during early mouse development. *Dev. Dyn.* **206**, 379-390.
Chen, C., Chen, H., Sun, J., Bringas, P., Jr, Chen, Y., Warburton, D. and Shi, W. (2005). Smad1 expression and function during mouse embryonic lung branching morphogenesis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **288**, L1033-L1039.
Chen, J., Knowles, H. J., Hebert, J. L. and Hackett, B. P. (1998). Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. *J. Clin. Invest.* **102**, 1077-1082.
Chuang, P. T., Kawcak, T. and McMahon, A. P. (2003). Feedback control of mammalian Hedgehog signaling by the Hedgehog-binding protein, Hip1, modulates Fgf signaling during branching morphogenesis of the lung. *Genes Dev.* **17**, 342-347.
Colvin, J. S., Feldman, B., Nadeau, J. H., Goldfarb, M. and Ornitz, D. M. (1999). Genomic organization and embryonic expression of the mouse fibroblast growth factor 9 gene. *Dev. Dyn.* **216**, 72-88.
Colvin, J. S., White, A. C., Pratt, S. J. and Ornitz, D. M. (2001). Lung hypoplasia and neonatal death in Fgf9-null mice identify this gene as an essential regulator of lung mesenchyme. *Development* **128**, 2095-2106.
Daniely, Y., Liao, G., Dixon, D., Linnoila, R. I., Lori, A., Randell, S. H., Oren, M. and Jetten, A. M. (2004). Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. *Am. J. Physiol Cell Physiol.* **287**, C171-C181.
DasGupta, R. and Fuchs, E. (1999). Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* **126**, 4557-4568.
De Langhe, S. P., Sala, F. G., Del Moral, P. M., Fairbanks, T. J., Yamada, K. M., Warburton, D., Burns, R. C. and Bellusci, S. (2005). Dickkopf-1 (DKK1) reveals that fibronectin is a major target of Wnt signaling in branching morphogenesis of the mouse embryonic lung. *Dev. Biol.* **277**, 316-331.
De Moerlooze, L., Spencer-Dene, B., Revest, J., Hajihosseini, M., Rosewell, I. and Dickson, C. (2000). An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. *Development* **127**, 483-492.
Dean, C. H., Miller, L. A., Smith, A. N., Dufort, D., Lang, R. A. and Niswander, L. A. (2005). Canonical Wnt signaling negatively regulates branching morphogenesis of the lung and lacrimal gland. *Dev. Biol.* **286**, 270-286.
Del Moral, P. M., Sala, F. G., Tefft, D., Shi, W., Keshet, E., Bellusci, S. and Warburton, D. (2006). VEGF-A signaling through Flk-1 is a critical facilitator of early embryonic lung epithelial to endothelial crosstalk and branching morphogenesis. *Dev. Biol.* **290**, 177-188.
Demello, D. E., Sawyer, D., Galvin, N. and Reid, L. M. (1997). Early fetal development of lung vasculature. *Am. J. Respir. Cell Mol. Biol.* **16**, 568-581.
Desai, T. J., Malpel, S., Flentke, G. R., Smith, S. M. and Cardoso, W. V. (2004). Retinoic acid selectively regulates Fgf10 expression and maintains cell identity in the prospective lung field of the developing foregut. *Dev. Biol.* **273**, 402-415.
Desai, T. J., Chen, F., Lü, J., Qian, J., Niederreither, K., Dollé, P., Chambon, P. and Cardoso, W. V. (2006). Distinct roles for retinoic acid receptors alpha and beta in early lung morphogenesis. *Dev. Biol.* **291**, 12-24.
Dickman, E. D., Thaller, C. and Smith, S. M. (1997). Temporally-regulated retinoic acid depletion produces specific neural crest, ocular and nervous system defects. *Development* **124**, 3111-3121.
Eblaghie, M. C., Reedy, M., Oliver, T., Mishina, Y. and Hogan, B. L. (2006). Evidence that autocrine signaling through Bmpr1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. *Dev. Biol.* **291**, 67-82.
Ellis, T., Gambardella, L., Horcher, M., Tschanz, S., Capol, J., Bertram, P., Jochum, W., Barrandon, Y. and Busslinger, M. (2001). The transcriptional repressor CDP (Cutl1) is essential for epithelial cell differentiation of the lung and the hair follicle. *Genes Dev.* **15**, 2307-2319.
Fischer, A., Viebahn, C. and Blum, M. (2002). FGF8 acts as a right determinant during establishment of the left-right axis in the rabbit. *Curr. Biol.* **12**, 1807-1816.
Ganga, M., Espinoza, H. M., Cox, C. J., Morton, L., Hjalt, T. A., Lee, Y. and Amendt, B. A. (2003). PITX2 isoform-specific regulation of atrial natriuretic factor expression: synergism and repression with Nkx2.5. *J. Biol. Chem.* **278**, 22437-22445.
Gebb, S. A. and Shannon, J. M. (2000). Tissue interactions mediate early events in pulmonary vasculogenesis. *Dev. Dyn.* **217**, 159-169.
Hacohen, N., Kramer, S., Sutherland, D., Hiromi, Y. and Krasnow, M. A. (1998). sprouty encodes a novel antagonist of FGF signaling that patterns apical branching of the Drosophila airways. *Cell* **92**, 253-263.
Harris, K. S., Zhang, Z., McManus, M. T., Harfe, B. D. and Sun, X. (2006). Dicer function is essential for lung epithelium morphogenesis. *Proc. Natl. Acad. Sci. USA* **103**, 2208-2213.
Harada, H., Toyono, T., Toyoshima, K., Yamasaki, M., Itoh, N., Kato, S., Sekine, K. and Ohuchi, H. (2002). FGF10 maintains stem cell compartment in developing mouse incisors. *Development* **129**, 1533-1541.
Heine, U. I., Munoz, E. F., Flanders, K. C., Roberts, A. B. and Sporn, M. B. (1990). Colocalization of TGF-beta 1 and collagen I and III, fibronectin and glycosaminoglycans during lung branching morphogenesis. *Development* **109**, 29-36.
Hinck, L. (2004). The versatile roles of 'axon guidance' cues in tissue morphogenesis. *Dev. Cell* **7**, 783-793.
Hui, C. C., Slusarski, D., Platt, K. A., Holmgren, R. and Joyner, A. L. (1994). Expression of three mouse homologs of the Drosophila segment polarity gene cubitus interruptus, Gli, Gli-2, and Gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development. *Dev. Biol.* **162**, 402-413.
Hyatt, B. A., Shangguan, X. and Shannon, J. M. (2004). FGF-10 induces SP-C and Bmp4 and regulates proximal-distal patterning in embryonic tracheal epithelium. *Am. J. Physiol. Lung Cell Mol. Physiol.* **287**, L1116-L1126.
Ioannides, A. S., Chaudhry, B., Henderson, D. J., Spitz, L. and Copp, A. J. (2002). Dorsoventral patterning in oesophageal atresia with tracheo-oesophageal fistula: Evidence from a new mouse model. *J. Pediatr. Surg.* **37**, 185-191.
Ishii, Y., Rex, M., Scotting, P. J. and Yasugi, S. (1998). Region-specific expression of chicken Sox2 in the developing gut and lung epithelium: regulation by epithelial-mesenchymal interactions. *Dev. Dyn.* **213**, 464-475.
Ito, T., Udaka, N., Yazawa, T., Okudela, K., Hayashi, H., Sudo, T., Guillemot, F., Kageyama, R. and Kitamura, H. (2000). Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. *Development* **127**, 3913-3921.
Izvolsky, K. I., Zhong, L., Wei, L., Yu, Q., Nugent, M. A. and Cardoso, W. V. (2003). Heparan sulfates expressed in the distal lung are required for Fgf10 binding to the epithelium and for airway branching. *Am. J. Physiol. Lung Cell Mol. Physiol.* **285**, L838-L846.
Jakkaraju, S., Zhe, X., Pan, D., Choudhury, R. and Schuger, L. (2005). TIPs are tension-responsive proteins involved in myogenic versus adipogenic differentiation. *Dev. Cell* **9**, 39-49.
Jung, J., Zheng, M., Goldfarb, M. and Zaret, K. S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* **284**, 1998-2003.
Kaartinen, V., Voncken, J. W., Shuler, C., Warburton, D., Bu, D., Heisterkamp, N. and Groffen, J. (1995). Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. *Nat. Genet.* **11**, 415-421.
Kamimura, K., Fujise, M., Villa, F., Izumi, S., Habuchi, H., Kimata, K. and Nakato, H. (2001). Drosophila heparan sulfate 6-O-sulfotransferase (dHS6ST)

gene. Structure, expression, and function in the formation of the tracheal system. *J. Biol. Chem.* **276**, 17014-17021.
Kelly, S. E., Bachurski, C. J., Burhans, M. S. and Glasser, S. W. (1996). Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. *J. Biol. Chem.* **271**, 6881-6888.
Kim, H. J. and Bar-Sagi, D. (2004). Modulation of signalling by Sprouty: a developing story. *Nat. Rev. Mol. Cell Biol.* **5**, 441-450.
Kim, M. O., Yun, S. J., Kim, I. S., Sohn, S. and Lee, E. H. (2003). Transforming growth factor-beta-inducible gene-h3 (beta(ig)-h3) promotes cell adhesion of human astrocytoma cells in vitro: implication of alpha6beta4 integrin. *Neurosci. Lett.* **336**, 93-96.
Kimura, S., Hara, Y., Pineau, T., Fernandez-Salguero, P., Fox, C. H., Ward, J. M. and Gonzalez, F. J. (1996). The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. *Genes Dev.* **10**, 60-69.
Kitamura, K., Miura, H., Miyagawa-Tomita, S., Yanazawa, M., Katoh-Fukui, Y., Suzuki, R., Ohuchi, H., Suehiro, A., Motegi, Y., Nakahara, Y. et al. (1999). Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. *Development* **126**, 5749-5758.
Kreidberg, J. A., Donovan, M. J., Goldstein, S. L., Rennke, H., Shepherd, K., Jones, R. C. and Jaenisch, R. (1996). Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development* **122**, 3537-3547.
Kumar, M. and Melton, D. (2003). Pancreas specification: a budding question. *Curr. Opin. Genet. Dev.* **13**, 401-407.
Kuo, C. T., Morrisey, E. E., Anandappa, R., Sigrist, K., Lu, M. M., Parmacek, M. S., Soudais, C. and Leiden, J. M. (1997). GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. *Genes Dev.* **11**, 1048-1060.
Lako, M., Strachan, T., Bullen, P., Wilson, D. I., Robson, S. C. and Lindsay, S. (1998). Isolation, characterisation and embryonic expression of WNT11, a gene which maps to 11q13.5 and has possible roles in the development of skeleton, kidney and lung. *Gene* **219**, 101-110.
Lammert, E., Cleaver, O. and Melton, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science* **294**, 564-567.
Lavine, K. J., Yu, K., White, A. C., Zhang, X., Smith, C., Partanen, J. and Ornitz, D. M. (2005). Endocardial and epicardial derived FGF signals regulate myocardial proliferation and differentiation in vivo. *Dev. Cell* **8**, 85-95.
Lazzaro, D., Price, M., de Felice, M. and Di Lauro, R. (1991). The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. *Development* **113**, 1093-1104.
Lebeche, D., Malpel, S. and Cardoso, W. V. (1999). Fibroblast growth factor interactions in the developing lung. *Mech. Dev.* **86**, 125-136.
Letterio, J. J., Geiser, A. G., Kulkarni, A. B., Roche, N. S., Sporn, M. B. and Roberts, A. B. (1994). Maternal rescue of transforming growth factor-beta 1 null mice. *Science* **264**, 1936-1938.
Li, C., Xiao, J., Hormi, K., Borok, Z. and Minoo, P. (2002). Wnt5a participates in distal lung morphogenesis. *Dev. Biol.* **248**, 68-81.
Li, Y., Zhang, H., Choi, S. C., Litingtung, Y. and Chiang, C. (2004). Sonic hedgehog signaling regulates Gli3 processing, mesenchymal proliferation, and differentiation during mouse lung organogenesis. *Dev. Biol.* **270**, 214-231.
Lim, L., Kalinichenko, V. V., Whitsett, J. A. and Costa, R. H. (2002). Fusion of lung lobes and vessels in mouse embryos heterozygous for the forkhead box f1 targeted allele. *Am. J. Physiol. Lung Cell Mol. Physiol.* **282**, L1012-L1022.
Lin, C. R., Kioussi, C., O'Connell, S., Briata, P., Szeto, D., Liu, F., Izpisua-Belmonte, J. C. and Rosenfeld, M. G. (1999). Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphogenesis. *Nature* **401**, 279-282.
Lin, Y., Zhang, S., Tuukkanen, J., Peltoketo, H., Pihlajaniemi, T. and Vainio, S. (2003). Patterning parameters associated with the branching of the ureteric bud regulated by epithelial-mesenchymal interactions. *Int. J. Dev. Biol.* **47**, 3-13.
Litingtung, Y., Lei, L., Westphal, H. and Chiang, C. (1998). Sonic hedgehog is essential to foregut development. *Nat. Genet.* **20**, 58-61.
Liu, Y., Jiang, H., Crawford, H. C. and Hogan, B. L. (2003). Role for ETS domain transcription factors Pea3/Erm in mouse lung development. *Dev. Biol.* **261**, 10-24.
Liu, Y., Stein, E., Oliver, T., Li, Y., Brunken, W. J., Koch, M., Tessier-Lavigne, M. and Hogan, B. L. (2004). Novel role for Netrins in regulating epithelial behavior during lung branching morphogenesis. *Curr. Biol.* **14**, 897-905.
Lowe, L. A., Yamada, S. and Kuehn, M. R. (2001). Genetic dissection of nodal function in patterning the mouse embryo. *Development* **128**, 1831-1843.
Lu, J., Qian, J., Izvolsky, K. I. and Cardoso, W. V. (2004). Global analysis of genes differentially expressed in branching and non-branching regions of the mouse embryonic lung. *Dev. Biol.* **273**, 418-435.
Lu, J., Izvolsky, K. I., Qian, J. and Cardoso, W. V. (2005a). Identification of FGF10 targets in the embryonic lung epithelium during bud morphogenesis. *J. Biol. Chem.* **280**, 4834-4841.
Lu, J., Qian, J., Chen, F., Tang, X., Li, C. and Cardoso, W. V. (2005b) Differential expression of components of the microRNA machinery during mouse organogenesis. *Biochem. Biophys. Res. Commun.* **334**, 319-323.
Lu, M. M., Yang, H., Zhang, L., Shu, W., Blair, D. G. and Morrisey, E. E. (2001). The bone morphogenic protein antagonist gremlin regulates proximal-distal patterning of the lung. *Dev. Dyn.* **222**, 667-680.
Lu, W., Luo, Y., Kan, M. and McKeehan, W. L. (1999). Fibroblast growth factor-10. A second candidate stromal to epithelial cell andromedin in prostate. *J. Biol. Chem.* **274**, 12827-12834.
Mahlapuu, M., Enerback, S. and Carlsson, P. (2001). Haploinsufficiency of the forkhead gene Foxf1, a target for sonic hedgehog signaling, causes lung and foregut malformations. *Development* **128**, 2397-2406.
Mailleux, A. A., Tefft, D., Ndiaye, D., Itoh, N., Thiery, J. P., Warburton, D. and Bellusci, S. (2001). Evidence that SPROUTY2 functions as an inhibitor of mouse embryonic lung growth and morphogenesis. *Mech. Dev.* **102**, 81-94.
Mailleux, A. A., Kelly, R., Veltmaat, J. M., De Langhe, S. P., Zaffran, S., Thiery, J. P. and Bellusci, S. (2005). Fgf10 expression identifies parabronchial smooth muscle cell progenitors and is required for their entry into the smooth muscle cell lineage. *Development* **132**, 2157-2166.
Malpel, S., Mendelsohn, C. and Cardoso, W. V. (2000). Regulation of retinoic acid signaling during lung morphogenesis. *Development* **127**, 3057-3067.
Martinez Barbera, J. P., Clements, M., Thomas, P., Rodriguez, T., Meloy, D., Kioussis, D. and Beddington, R. S. (2000). The homeobox gene Hex is required in definitive endodermal tissues for normal forebrain, liver and thyroid formation. *Development* **127**, 2433-2445.
Massague, J. (2000). How cells read TGF-beta signals. *Nat. Rev. Mol. Cell Biol.* **1**, 169-178.
Matsumoto, K., Yoshitomi, H., Rossant, J. and Zaret, K. S. (2001). Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* **294**, 559-563.
Mendelsohn, C., Lohnes, D., Decimo, D., Lufkin, T., LeMeur, M., Chambon, P. and Mark, M. (1994). Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. *Development* **120**, 2749-2771.
Meno, C., Shimono, A., Saijoh, Y., Yashiro, K., Mochida, K., Ohishi, S., Noji, S., Kondoh, H. and Hamada, H. (1998). lefty-1 is required for left-right determination as a regulator of lefty-2 and nodal. *Cell* **94**, 287-297.
Metzger, R. J. and Krasnow, M. A. (1999). Genetic control of branching morphogenesis. *Science* **284**, 1635-1639.
Michos, O., Panman, L., Vintersten, K., Beier, K., Zeller, R. and Zuniga, A. (2004). Gremlin-mediated BMP antagonism induces the epithelial-mesenchymal feedback signaling controlling metanephric kidney and limb organogenesis. *Development* **131**, 3401-3410.
Miettinen, P. J., Warburton, D., Bu, D., Zhao, J. S., Berger, J. E., Minoo, P., Koivisto, T., Allen, L., Dobbs, L., Werb, Z. et al. (1997). Impaired lung branching morphogenesis in the absence of functional EGF receptor. *Dev. Biol.* **186**, 224-236.
Min, H., Danilenko, D. M., Scully, S. A., Bolon, B., Ring, B. D., Tarpley, J. E., DeRose, M. and Simonet, W. S. (1998). Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev.* **12**, 3156-3161.
Minoo, P., Su, G., Drum, H., Bringas, P. and Kimura, S. (1999). Defects in tracheoesophageal and lung morphogenesis in Nkx2.1(–/–) mouse embryos. *Dev. Biol.* **209**, 60-71.
Moens, C. B., Auerbach, A. B., Conlon, R. A., Joyner, A. L. and Rossant, J. (1992). A targeted mutation reveals a role for N-myc in branching morphogenesis in the embryonic mouse lung. *Genes Dev.* **6**, 691-704.
Mollard, R., Ghyselinck, N. B., Wendling, O., Chambon, P. and Mark, M. (2000). Stage-dependent responses of the developing lung to retinoic acid signaling. *Int. J. Dev. Biol.* **44**, 457-462.
Morrisey, E. E., Tang, Z., Sigrist, K., Lu, M. M., Jiang, F., Ip, H. S. and Parmacek, M. S. (1998). GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. *Genes Dev.* **12**, 3579-3590.
Motoyama, J., Liu, J., Mo, R., Ding, Q., Post, M. and Hui, C. C. (1998). Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. *Nat. Genet.* **20**, 54-57.
Mucenski, M. L., Wert, S. E., Nation, J. M., Loudy, D. E., Huelsken, J., Birchmeier, W., Morrisey, E. E. and Whitsett, J. A. (2003). beta-Catenin is required for specification of proximal/distal cell fate during lung morphogenesis. *J. Biol. Chem.* **278**, 40231-40238.
Muglia, L. J., Bae, D. S., Brown, T. T., Vogt, S. K., Alvarez, J. G., Sunday, M. E. and Majzoub, J. A. (1999). Proliferation and differentiation defects during lung development in corticotropin-releasing hormone-deficient mice. *Am. J. Respir. Cell Mol. Biol.* **20**, 181-188.
Naiche, L. A. and Papaioannou, V. E. (2003). Loss of Tbx4 blocks hindlimb development and affects vascularization and fusion of the allantois. Development **130**, 2681-2693.
Nelson, W. J. and Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* **303**, 1483-1487.
Nguyen, N. M., Miner, J. H., Pierce, R. A. and Senior, R. M. (2002). Laminin alpha 5 is required for lobar septation and visceral pleural basement membrane formation in the developing mouse lung. *Dev. Biol.* **246**, 231-244.
Niederreither, K., Subbarayan, V., Dolle, P. and Chambon, P. (1999).

Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. *Nat. Genet.* **21**, 444-448.
Nogawa, H., Morita, K. and Cardoso, W. V. (1998). Bud formation precedes the appearance of differential cell proliferation during branching morphogenesis of mouse lung epithelium in vitro. *Dev. Dyn.* **213**, 228-235.
Norgaard, G. A., Jensen, J. N. and Jensen, J. (2003). FGF10 signaling maintains the pancreatic progenitor cell state revealing a novel role of Notch in organ development. *Dev. Biol.* **264**, 323-338.
Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L. and Wright, C. V. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**, 983-995.
Oh, S. P. and Li, E. (1997). The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev.* **11**, 1812-1826.
Ohtsuka, N., Urase, K., Momoi, T. and Nogawa, H. (2001). Induction of bud formation of embryonic mouse tracheal epithelium by fibroblast growth factor plus transferrin in mesenchyme-free culture. *Dev. Dyn.* **222**, 263-272.
Okubo, T. and Hogan, B. L. (2004). Hyperactive Wnt signaling changes the developmental potential of embryonic lung endoderm. *J. Biol.* **3**, 11.
Okubo, T., Knoepfler, P. S., Eisenman, R. N. and Hogan, B. L. (2005). Nmyc plays an essential role during lung development as a dosage-sensitive regulator of progenitor cell proliferation and differentiation. *Development* **132**, 1363-1374.
Pan, Q., Li, C., Xiao, J., Kimura, S., Rubenstein, J., Puelles, L. and Minoo, P. (2004). In vivo characterization of the Nkx2.1 promoter/enhancer elements in transgenic mice. *Gene* **331**, 73-82.
Park, W. Y., Miranda, B., Lebeche, D., Hashimoto, G. and Cardoso, W. V. (1998). FGF-10 is a chemotactic factor for distal epithelial buds during lung development. *Dev. Biol.* **201**, 125-134.
Pauling, M. H. and Vu, T. H. (2004). Mechanisms and regulation of lung vascular development. *Curr. Top. Dev. Biol.* **64**, 73-99.
Pelton, R. W., Johnson, M. D., Perkett, E. A., Gold, L. I. and Moses, H. L. (1991). Expression of transforming growth factor-beta 1, -beta 2, and -beta 3 mRNA and protein in the murine lung. *Am. J. Respir. Cell Mol. Biol.* **5**, 522-530.
Pepicelli, C. V., Lewis, P. M. and McMahon, A. P. (1998). Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr. Biol.* **8**, 1083-1086.
Perl, A. K., Wert, S. E., Nagy, A., Lobe, C. G. and Whitsett, J. A. (2002). Early restriction of peripheral and proximal cell lineages during formation of the lung. *Proc. Natl. Acad. Sci. USA* **99**, 10482-10487.
Perl, A. K., Hokuto, I., Impagnatiello, M. A., Christofori, G. and Whitsett, J. A. (2003). Temporal effects of Sprouty on lung morphogenesis. *Dev. Biol.* **258**, 154-168.
Peschon, J. J., Slack, J. L., Reddy, P., Stocking, K. L., Sunnarborg, S. W., Lee, D. C., Russell, W. E., Castner, B. J., Johnson, R. S., Fitzner, J. N. et al. (1998). An essential role for ectodomain shedding in mammalian development. *Science* **282**, 1281-1284.
Quaggin, S. E., Schwartz, L., Cui, S., Igarashi, P., Deimling, J., Post, M. and Rossant, J. (1999). The basic-helix-loop-helix protein pod1 is critically important for kidney and lung organogenesis. *Development* **126**, 5771-5783.
Rankin, C. T., Bunton, T., Lawler, A. M. and Lee, S. J. (2000). Regulation of left-right patterning in mice by growth/differentiation factor-1. *Nat. Genet.* **24**, 262-265.
Rawlins, E. L. and Hogan, B. L. (2005). Intercellular growth factor signaling and the development of mouse tracheal submucosal glands. *Dev. Dyn.* **233**, 1378-1385.
Rossant, J., Zirngibl, R., Cado, D., Shago, M. and Giguere, V. (1991). Expression of a retinoic acid response element-hsplacZ transgene defines specific domains of transcriptional activity during mouse embryogenesis. *Genes Dev.* **5**, 1333-1344.
Rossi, J. M., Dunn, N. R., Hogan, B. L. and Zaret, K. S. (2001). Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. *Genes Dev.* **15**, 1998-2009.
Rubin, L. P., Kovacs, C. S., De Paepe, M. E., Tsai, S. W., Torday, J. S. and Kronenberg, H. M. (2004). Arrested pulmonary alveolar cytodifferentiation and defective surfactant synthesis in mice missing the gene for parathyroid hormone-related protein. *Dev. Dyn.* **230**, 278-289.
Sakiyama, J., Yamagishi, A. and Kuroiwa, A. (2003). Tbx4-Fgf10 system controls lung bud formation during chicken embryonic development. *Development* **130**, 1225-1234.
Samakovlis, C., Hacohen, N., Manning, G., Sutherland, D. C., Guillemin, K. and Krasnow, M. A. (1996). Development of the Drosophila tracheal system occurs by a series of morphologically distinct but genetically coupled branching events. *Development* **122**, 1395-1407.
Sanford, L. P., Ormsby, I., Gittenberger-de Groot, A. C., Sariola, H., Friedman, R., Boivin, G. P., Cardell, E. L. and Doetschman, T. (1997). TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development* **124**, 2659-2670.
Sekine, K., Ohuchi, H., Fujiwara, M., Yamasaki, M., Yoshizawa, T., Sato, T., Yagishita, N., Matsui, D., Koga, Y., Itoh, N. et al. (1999). Fgf10 is essential for limb and lung formation. *Nat. Genet.* **21**, 138-141.
Serls, A. E., Doherty, S., Parvatiyar, P., Wells, J. M. and Deutsch, G. H. (2005). Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung. *Development* **132**, 35-47.
Serra, R., Pelton, R. W. and Moses, H. L. (1994). TGF beta 1 inhibits branching morphogenesis and N-myc expression in lung bud organ cultures. *Development* **120**, 2153-2161.
Shannon, J. M. (1994). Induction of alveolar type II cell differentiation in fetal tracheal epithelium by grafted distal lung mesenchyme. *Dev. Biol.* **166**, 600-614.
Shannon, J. M., McCormick-Shannon, K., Burhans, M. S., Shangguan, X., Srivastava, K. and Hyatt, B. A. (2003). Chondroitin sulfate proteoglycans are required for lung growth and morphogenesis in vitro. *Am. J. Physiol. Lung Cell Mol. Physiol.* **285**, L1323-L1336.
Shiels, H., Li, X., Schumacker, P. T., Maltepe, E., Padrid, P. A., Sperling, A., Thompson, C. B. and Lindsten, T. (2000). TRAF4 deficiency leads to tracheal malformation with resulting alterations in air flow to the lungs. *Am. J. Pathol.* **157**, 679-688.
Shikama, N., Lutz, W., Kretzschmar, R., Sauter, N., Roth, J. F., Marino, S., Wittwer, J., Scheidweiler, A. and Eckner, R. (2003). Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation. *EMBO J.* **22**, 5175-5185.
Shu, W., Jiang, Y. Q., Lu, M. M. and Morrisey, E. E. (2002). Wnt7b regulates mesenchymal proliferation and vascular development in the lung. *Development* **129**, 4831-4842.
Shu, W., Guttentag, S., Wang, Z., Andl, T., Ballard, P., Lu, M. M., Piccolo, S., Birchmeier, W., Whitsett, J. A., Millar, S. E. et al. (2005). Wnt/beta-catenin signaling acts upstream of N-myc, BMP4, and FGF signaling to regulate proximal-distal patterning in the lung. *Dev. Biol.* **283**, 226-239.
Sock, E., Rettig, S. D., Enderich, J., Bosl, M. R., Tamm, E. R. and Wegner, M. (2004). Gene targeting reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. *Mol. Cell. Biol.* **24**, 6635-6644.
Stafford, D. and Prince, V. E. (2002). Retinoic acid signaling is required for a critical early step in zebrafish pancreatic development. *Curr. Biol.* **12**, 1215-1220.
Steele-Perkins, G., Plachez, C., Butz, K. G., Yang, G., Bachurski, C. J., Kinsman, S. L., Litwack, E. D., Richards, L. J. and Gronostajski, R. M. (2005). The transcription factor gene Nfib is essential for both lung maturation and brain development. *Mol. Cell. Biol.* **25**, 685-698.
Sterner-Kock, A., Thorey, I. S., Koli, K., Wempe, F., Otte, J., Bangsow, T., Kuhlmeier, K., Kirchner, T., Jin, S., Keski-Oja, J. et al. (2002). Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Dev.* **16**, 2264-2273.
Strickland, P., Shin, G. C., Plump, A., Tessier-Lavigne, M. and Hinck, L. (2006). Slit2 and netrin 1 act synergistically as adhesive cues to generate tubular bi-layers during ductal morphogenesis. *Development* **133**, 823-832.
Sugahara, K., Iyama, K. I., Kimura, T., Sano, K., Darlington, G. J., Akiba, T. and Takiguchi, M. (2001). Mice lacking CCAAt/enhancer-binding protein-alpha show hyperproliferation of alveolar type II cells and increased surfactant protein mRNAs. *Cell Tissue Res.* **306**, 57-63.
Sutherland, D., Samakovlis, C. and Krasnow, M. A. (1996). branchless encodes a Drosophila FGF homolog that controls tracheal cell migration and the pattern of branching. *Cell* **87**, 1091-1101.
Sutliff, K. S. and Hutchins, G. M. (1994). Septation of the respiratory and digestive tracts in human embryos: crucial role of the tracheoesophageal sulcus. *Anat. Rec.* **238**, 237-247.
Tebar, M., Destree, O., de Vree, W. J. and Have-Opbroek, A. A. (2001). Expression of Tcf/Lef and sFrp and localization of beta-catenin in the developing mouse lung. *Mech. Dev.* **109**, 437-440.
Tefft, J. D., Lee, M., Smith, S., Leinwand, M., Zhao, J., Bringas, P., Jr, Crowe, D. L. and Warburton, D. (1999). Conserved function of mSpry-2, a murine homolog of Drosophila sprouty, which negatively modulates respiratory organogenesis. *Curr. Biol.* **9**, 219-222.
Tefft, D., Lee, M., Smith, S., Crowe, D. L., Bellusci, S. and Warburton, D. (2002). mSprouty inhibits FGF10-activated MAP kinase by differentially binding to upstream target proteins. *Am. J. Physiol. Lung Cell Mol. Physiol.* **283**, L700-L706.
Tiso, N., Filippi, A., Pauls, S., Bortolussi, M. and Argenton, F. (2002). BMP signalling regulates anteroposterior endoderm patterning in zebrafish. *Mech. Dev.* **118**, 29-37.
Tollet, J., Everett, A. W. and Sparrow, M. P. (2001). Spatial and temporal distribution of nerves, ganglia, and smooth muscle during the early pseudoglandular stage of fetal mouse lung development. *Dev. Dyn.* **221**, 48-60.
Tomlinson, D. C., Grindley, J. C. and Thomson, A. A. (2004). Regulation of Fgf10 gene expression in the prostate: identification of transforming growth factor-beta1 and promoter elements. *Endocrinology* **145**, 1988-1995.
Usui, H., Shibayama, M., Ohbayashi, N., Konishi, M., Takada, S. and Itoh, N.

(2004). Fgf18 is required for embryonic lung alveolar development. *Biochem. Biophys. Res. Commun.* **322**, 887-892.
Vergnes, L., Peterfy, M., Bergo, M. O., Young, S. G. and Reue, K. (2004). Lamin B1 is required for mouse development and nuclear integrity. *Proc. Natl. Acad. Sci. USA* **101**, 10428-10433.
Volpe, M. V., Martin, A., Vosatka, R. J., Mazzoni, C. L. and Nielsen, H. C. (1997). Hoxb-5 expression in the developing mouse lung suggests a role in branching morphogenesis and epithelial cell fate. *Histochem. Cell Biol.* **108**, 495-504.
Wan, H., Dingle, S., Xu, Y., Besnard, V., Kaestner, K. H., Ang, S. L., Wert, S., Stahlman, M. T. and Whitsett, J. A. (2005). Compensatory Roles of Foxa1 and Foxa2 during Lung Morphogenesis. *J. Biol. Chem.* **280**, 13809-13816.
Wani, M. A., Wert, S. E. and Lingrel, J. B. (1999). Lung Kruppel-like factor, a zinc finger transcription factor, is essential for normal lung development. *J. Biol. Chem.* **274**, 21180-21185.
Weaver, M., Yingling, J. M., Dunn, N. R., Bellusci, S. and Hogan, B. L. (1999). Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development. *Development* **126**, 4005-4015.
Weaver, M., Dunn, N. R. and Hogan, B. L. (2000). Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. *Development* **127**, 2695-2704.
Weaver, M., Batts, L. and Hogan, B. L. (2003). Tissue interactions pattern the mesenchyme of the embryonic mouse lung. *Dev. Biol.* **258**, 169-184.
Weinstein, M., Xu, X., Ohyama, K. and Deng, C. X. (1998). FGFR-3 and FGFR-4 function cooperatively to direct alveogenesis in the murine lung. *Development* **125**, 3615-3623.
Wells, J. M. and Melton, D. A. (1999). Vertebrate endoderm development. *Annu. Rev. Cell Dev. Biol.* **15**, 393-410.
Wells, J. M. and Melton, D. A. (2000). Early mouse endoderm is patterned by soluble factors from adjacent germ layers. *Development* **127**, 1563-1572.
Wendel, D. P., Taylor, D. G., Albertine, K. H., Keating, M. T. and Li, D. Y. (2000). Impaired distal airway development in mice lacking elastin. *Am. J. Respir. Cell Mol. Biol.* **23**, 320-326.
Wert, S. E., Glasser, S. W., Korfhagen, T. R. and Whitsett, J. A. (1993). Transcriptional elements from the human SP-C gene direct expression in the primordial respiratory epithelium of transgenic mice. *Dev. Biol.* **156**, 426-443.
Williams, M. C. (2003). Alveolar type I cells: molecular phenotype and development. *Annu. Rev. Physiol.* **65**, 669-695.
Wilson, J. G., Roth, C. B. and Warkany, J. (1953). An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. *Am. J. Anat.* **92**, 189-217.
Winnier, G., Blessing, M., Labosky, P. A. and Hogan, B. L. (1995). Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* **9**, 2105-2116.
Wongtrakool, C., Malpel, S., Gorenstein, J., Sedita, J., Ramirez, M. I., Underhill, T. M. and Cardoso, W. V. (2003). Down-regulation of retinoic acid receptor alpha signaling is required for sacculation and type I cell formation in the developing lung. *J. Biol. Chem.* **278**, 46911-46918.
Wood, H. B., May, G., Healy, L., Enver, T. and Morriss-Kay, G. M. (1997). CD34 expression patterns during early mouse development are related to modes of blood vessel formation and reveal additional sites of hematopoiesis. *Blood* **90**, 2300-2311.
Yang, H., Lu, M. M., Zhang, L., Whitsett, J. A. and Morrisey, E. E. (2002). GATA6 regulates differentiation of distal lung epithelium. *Development* **129**, 2233-2246.
Yang, Y., Beqaj, S., Kemp, P., Ariel, I. and Schuger, L. (2000). Stretch-induced alternative splicing of serum response factor promotes bronchial myogenesis and is defective in lung hypoplasia. *J. Clin. Invest* **106**, 1321-1330.
Yuan, B., Li, C., Kimura, S., Engelhardt, R. T., Smith, B. R. and Minoo, P. (2000). Inhibition of distal lung morphogenesis in Nkx2.1(–/–) embryos. *Dev. Dyn.* **217**, 180-190.
Zakin, L. D., Mazan, S., Maury, M., Martin, N., Guenet, J. L. and Brulet, P. (1998). Structure and expression of Wnt13, a novel mouse Wnt2 related gene. *Mech. Dev.* **73**, 107-116.
Zaw-Tun, H. A. (1982). The tracheo-esophageal septum–fact or fantasy? Origin and development of the respiratory primordium and esophagus. *Acta Anat. (Basel)* **114**, 1-21.
Zhao, J., Bu, D., Lee, M., Slavkin, H. C., Hall, F. L. and Warburton, D. (1996). Abrogation of transforming growth factor-beta type II receptor stimulates embryonic mouse lung branching morphogenesis in culture. *Dev. Biol.* **180**, 242-257.
Zhao, J., Chen, H., Peschon, J. J., Shi, W., Zhang, Y., Frank, S. J. and Warburton, D. (2001). Pulmonary hypoplasia in mice lacking tumor necrosis factor-alpha converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung branching morphogenesis. *Dev. Biol.* **232**, 204-218.
Zhou, L., Dey, C. R., Wert, S. E. and Whitsett, J. A. (1996). Arrested lung morphogenesis in transgenic mice bearing an SP-C-TGF-beta 1 chimeric gene. *Dev. Biol.* **175**, 227-238.