Transcription factors in mouse lung development and function

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Costa, Robert H., Vladimir V. Kalinichenko, and Lorena Lim. Transcription factors in mouse lung development and function. Am J Physiol Lung Cell Mol Physiol 280: L823–L838, 2001.—Development of the mouse lung initiates on day 9.5 postcoitum from the laryngotracheal groove and involves mesenchymal-epithelial interactions, in particular, those between the splanchnic mesoderm and epithelial cells (derived from foregut endoderm) that induce cellular proliferation, migration, and differentiation, resulting in branching morphogenesis. This developmental process mediates formation of the pulmonary bronchiole tree and integrates a terminal alveolar region with an extensive endothelial capillary bed, which facilitates efficient gas exchange with the circulatory system. The major function of the mesenchymal-epithelial signaling is to potentiate the activity or expression of cell type-specific transcription factors in the developing lung, which, in turn, cooperatively bind to distinct promoter regions and activate target gene expression. In this review, we focus on the role of transcription factors in lung morphogenesis and the maintenance of differentiated gene expression. These lung transcription factors include forkhead box A2 [also known as hepatocyte nuclear factor (HNF)-3β], HNF-3/forkhead homolog (HFH)-8 [also known as FoxF1 or forkhead-related activator-1], HNF-3/forkhead homolog-4 (also known as FoxJ1), thyroid transcription factor-1 (Nkx2.1), and homeodomain box A5 transcription factors, the zinc finger Gli (mouse homologs of the Drosophila cubitus interruptus) and GATA transcription factors, and the basic helix-loop-helix Pod1 transcription factor. We summarize the phenotypes of transgenic and knockout mouse models, which define important functions of these transcription factors in cellular differentiation and lung branching morphogenesis.

MOUSE LUNG BUD FORMATION INITIATES on day 9.5 postcoitum (pc) from the laryngotracheal groove and involves mesenchymal-epithelial cell interactions, which include paracrine growth factor stimulation that induces cellular proliferation, migration, and differentiation (74). Lung branching morphogenesis involves migration of foregut endoderm-derived epithelial cells into the surrounding splanchnic mesoderm, resulting in formation of the respiratory bronchioles and the terminal alveolar sacs, which integrate with the endothelial capillary bed (74). During mouse lung development, the pseudoglandular stage (days 9.5–16.6 pc) is characterized by formation of the bronchial and respiratory bronchiole tree, which is lined with undifferentiated epithelial cells juxtaposed to the splanchnic mesoderm (130). By day 12 pc of mouse lung development, branching of the bronchial buds gives rise to the left lung lobe and the four lobes of the right lung. There is extensive branching of the distal epithelium and mesenchyme during the canalicular stage (days 16.6–17.4 pc), resulting in formation of terminal sacs lined with

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epithelial cells integrating with the mesoderm-derived vasculature. The terminal sac stage (day 17.5 to postnatal day (PD) 5) of lung development is characterized by a coordinated increase in terminal sac formation and vasculogenesis in conjunction with the differentiation of alveolar epithelial type I and II cells. The alveolar stage (PD5 to PD30) of postnatal lung development features maturation of the terminal respiratory sacs into alveolar ducts and sacs. At the end of this developmental process, the mature respiratory system is lined with epithelial cells possessing distinct pulmonary functions, which vary in their distribution from the proximal to distal airway (130). Squamous epithelial cells line the larynx, and the upper airways are populated by a mixture of ciliated columnar and mucus-secreting goblet cells with foci of pulmonary neuroendocrine cells, whereas Clara cells predominate in the lower airways. The distal alveolar sacs are populated with surfactant protein (SP) A- and SP-C-secreting type II epithelial cells and type I epithelial cells that form tight junctions with the pulmonary endothelial cells, facilitating gas exchange with the circulatory system.

Branching morphogenesis of the lung involves mesenchymal-epithelial signaling that induces cellular proliferation, migration, and subsequent transcriptional activation of lung-specific genes. The lung mesenchyme possesses growth factor receptors, which respond to protein ligands secreted by the adjacent endoderm or epithelial cells. Sonic hedgehog (Shh)-deficient (−/−) mice exhibit fusion of the tracheoesophageal tube, loss of asymmetry of the lung (appears as one lobe), and diminished expansion of the alveolar region (69). Activation of the Gli transcription factors through the Shh signaling transduction pathway plays an important role in lung morphogenesis (38, 86, 93, 110, 136). Transgenic mouse studies in which the SP-C gene-regulatory region was used to increase distal epithelial cell expression of either Shh (6) or keratinocyte growth factor (119) resulted in over-proliferation of lung mesenchyme, leading to defects in branching morphogenesis. Appropriate expression of bone morphogenetic protein-4 (BMP-4) (8, 132), hepatocyte growth factor (HGF) (87), and fibroblast growth factor (FGF)-10 (7) is critical in regulating pulmonary epithelial cell proliferation, migration, and branching morphogenesis of the lung. Fgf10(−/−) mice die immediately after birth due to disruption of pulmonary branching morphogenesis, and this phenotype is coincident with severe reductions in the expression of Shh and BMP-4 (lung endoderm) and mesenchymally derived Wnt2 (114). In combination with in vitro lung culture studies (114, 131), analysis of Fgf10(−/−) mice revealed that FGF-10 is involved in the induction of Shh, BMP-4, and Wnt2 signaling molecules, all of which are essential for lung development. Moreover, transgenic mouse studies (95, 125) have provided further evidence that the FGF signaling pathway is critical for airway branching and pulmonary epithelial differentiation. These studies identified several growth factors and signaling molecules that play important roles in lung morphogenesis during mouse embryonic development.

Vascular endothelial growth factor (VEGF) is expressed in the endoderm or ectoderm and acts in a paracrine fashion on adjacent mesoderm tissue to induce proliferation, cell migration, and angioblast differentiation toward endothelial cell lineage (17, 105). This developmental process is important for the formation of new blood vessels de novo (vasculogenesis) or from preexisting vessels (angiogenesis). Targeted disruption of the VEGF gene produces mutant embryos that display impaired blood island formation and delayed endothelial cell differentiation, leading to abnormal blood vessel development (20, 35). VEGF is the ligand for Fms-like receptor tyrosine kinase [Flt-1; VEGF receptor-1 (VEGFR-1)] and receptor tyrosine kinase Flk-1 (VEGFR-2), which are expressed in the primitive endothelium derived from mesoderm (105). Ablation of the Flk1 gene inhibits vasculogenesis and formation of angioblast cells in the blood islands (115), whereas disruption of the Flt1 gene allows the formation of angioblasts but inhibits their assembly into functional blood vessels (36). Other receptor tyrosine kinases involved in blood vessel formation include the Tie1 and Tie2 (TEK) genes, which are expressed in the lateral and extraembryonic mesoderm of the developing embryo (34, 58). Mice containing targeted Tie1 gene disruption have defects in endothelial cell function and blood vessel formation, leading to pulmonary edema and hemorrhage (99, 112). Targeted ablation of the Tie2 (TEK) gene leads to defects in endothelial cell proliferation and migration, which cause inhibition of angiogenesis (112). Ligand stimulation of the endothelial receptors Tie-1 and Tie-2 therefore plays an important role in vascular remodeling (140). In the lung, overexpression of VEGF in the respiratory epithelium stimulated vasculogenesis in transgenic mouse lungs, but its elevated expression resulted in aberrant vessel formation and increased expression of Flk-1 and Tie-1 (146). Identification of lung mesenchymal transcription factors mediating expression of these receptor tyrosine kinases will therefore provide insights regarding pulmonary vasculogenesis and angiogenesis during embryonic development or after acute lung injury.

The major function of these signaling pathways is to potentiate the activity or expression of mesenchyme- or endoderm-specific transcription factors in the developing lung. These, in turn, bind cooperatively to distinct promoter regions and activate target gene expression. The dynamic changes in gene expression during lung development are critical to mediate lung morphogenesis, which involves extensive cellular proliferation, migration, and establishment of appropriate positioning of respiratory epithelial cells with the mesenchymally derived endothelial cells. The molecular events involved in the process of lung morphogenesis have been reviewed recently by Warburton et al. (130). We focus our review on the role of transcription factors in lung morphogenesis and the maintenance of differentiated gene expression. We include forkhead box (Fox) A2 [FoxA2; hepatocyte nuclear factor (HNF)-3β], FoxF1
[HNF-3/forkhead homolog (HFH)-8; forkhead-related activator (FREAC)-1], and FoxJ1 (HFH-4) transcription factors; Nkx2.1 homeodomain or thyroid transcription factor (TTF)-1; homeodomain box (Hox) A5; the zinc finger Gli transcription factors (related to Drosophila cubitus interruptus); the basic helix-loop-helix (bHLH) Pod1; and GATA transcription factors. We summarize the phenotypes of transgenic and knockout mouse models, which define important functions of these transcription factors in mouse lung development.

IDENTIFICATION OF HNF-3 IN REGULATING LUNG EPITHELIAL CELL-SPECIFIC TRANSCRIPTION

Functional analysis of the regulatory regions of hepatocyte-specific genes served as an important model system and determined that hepatocyte-specific gene transcription is dependent on the recognition of multiple DNA binding sites by distinct families of HNFs as well as by widely distributed transcription factors (21). These studies also revealed that detectable promoter activity required synergistic interactions among multiple HNF proteins and that this requirement plays an important role in maintaining cell-specific gene expression (22, 27–32, 37, 45, 82, 91, 107, 108). The HNF-3α, -3β, and -3γ proteins were originally identified as mediating transcription of hepatocyte-specific genes (28, 64, 65) and sharing homology in the winged helix/forkhead DNA binding domain (25, 73). The HNF-3α and HNF-3β proteins share 93% amino acid homology in the winged helix DNA binding domain, bind to the same DNA consensus sequences (Fig. 1), and are potent transcriptional activators (64, 65, 89). The HNF-3 proteins possess a conserved NH2-terminal transcriptional activation domain that is critical for mediating protein interactions with other HNF transcription factors (41). An essential HNF-3 transcriptional activation domain resides within the COOH-terminal 100-amino acid residues (Fig. 1), which contain the functionally important conserved region II and III sequences (90, 100). Interestingly, a recent study (129) demonstrated that the region II sequences in the HNF-3α promoter bind to the winged helix DNA binding domain, which is a general feature displayed by other forkhead transcription factors (9, 43).

Functional analysis of the regulatory region of lung-specific genes also demonstrated that normal promoter activity required synergistic interaction of multiple cell-specific transcription factors in conjunction with inducible and widely expressed transcription factors (10, 14, 15, 113, 122). These promoter studies (10, 11, 14, 42, 49, 113) demonstrated...
an important role for HNF-3α and HNF-3β proteins in regulating transcription of SP and Clara cell secretory protein (CCSP) genes required for bronchiolar and type II epithelial cell function (Table 1). A transfection study (108) demonstrated that Hnf3β gene expression is stimulated by interferon (IFN)-γ through promoter recognition by the IFN regulatory factor-1 protein (108). Subsequent CCSP promoter studies indicated that IFN-γ induction of CCSP gene transcription involves promoter activation by the HNF-3β and signal transducer and activator of transcription (STAT) proteins (70). Furthermore, HNF-3β regulates promoter expression of the Nkx homeodomain transcription factor TTF-1 (47), which, in turn, regulates transcription of the SP genes (14, 19, 39, 139).

Hnf3β and Hnf3α genes display overlapping expression patterns during lung morphogenesis (Table 1), but Hnf3γ is not expressed in the developing lung (83, 149). Because of the functional redundancies of the HNF-3β and HNF-3α proteins in pulmonary epithelial cells, the function of these transcription factors during lung morphogenesis was not elucidated from analysis of mice containing targeted disruptions of the Hnf3 genes. The Hnf3α(−/−) mice display hypoglycemia due to reduced pancreatic expression of glucagon, but they exhibit normal lung development (50, 117). Likewise, the in vivo role of HNF-3β in lung morphogenesis remains unknown because homozygous null Hnf3β mouse embryos die in utero 9.5 days pc before lung morphogenesis (1, 134). Hnf3β(−/−) embryos exhibit defects in the formation of the node, notochord, foregut endoderm, visceral endoderm, and neurotube (Table 2). With the availability of mice containing the LoxP-targeted HNF-3β locus (123), the use of Cre recombinase technology for generating pulmonary epithelium-specific targeted disruption of the Hnf3β gene will allow examination of the role of HNF-3β in lung morphogenesis with either wild-type or Hnf3α(+/-) mouse backgrounds.

During lung development, HNF-3β protein is expressed at higher levels in epithelial cells lining the proximal airways and at lower levels in the distal type 2 epithelial cells (149). Transgenic SP-C-Hnf3β mice were generated that express high levels of HNF-3β in the distal airway epithelial cells, which disrupted the normal decreasing gradient of HNF-3β in these cells during lung development (148). In the most severe phenotype, the embryonic lungs consisted of primitive tubules, which were lined with undifferentiated columnar epithelial cells that intensely stained positive for the HNF-3β protein (Table 2). Increased expression of HNF-3β in the distal respiratory epithelium caused a striking inhibition in branching morphogenesis and vasculogenesis of the lung, which is coincident with diminished expression of E-cadherin and VEGF in these cells (148). These transgenic mouse studies indicated that maintaining precise levels of HNF-3β is of critical importance in normal branching morphogenesis of the lung.

### Expression of Forkhead Box Transcription Factors in the Lung

Rodent HNF-3 (28, 64, 65) and *Drosophila* homeotic forkhead proteins (133) were the first identified members of an extensive family of transcription factors that share homology in the winged helix DNA binding domain (25). The HNF-3/winged helix/forkhead proteins are a growing family of transcription factors that play important roles in cellular proliferation and differentiation (55) and have recently been renamed as the forkhead box (Fox) family (51). With PCR amplification of rodent organ cDNA with primers made to conserved amino acid sequences in the winged helix DNA binding domain, a number of new Fox family members were isolated from a variety of different mouse tissues (26, 52, 98). Several Fox genes that are expressed in the mouse lung were isolated (Table 1), including HNF3β/forkhead homolog-8 (Hfh8; also known as Freac1 or Foxf1), which is expressed in the mesenchyme of the developing and adult mouse lung (71, 97); forkhead 6 (Fkh6; also known as Fox1), the expression of which is observed in embryonic lung mesenchyme (48); Hfh4 (Fox1), which is expressed in the ciliated epithelial cells of the developing and adult lung (12, 18, 24, 126), and Hfh11 (Foxm1, Trident, Win), the expression of which is restricted to proliferating cells of the embryonic lung (143) and is also reactivated after lung injury (54).

### Mesenchymal Expression of Hfh8 (Foxf1) in Developing Lung

To identify the cellular expression pattern of the Hfh8 (Freac1 or Foxf1) gene during mouse embryonic development, in situ hybridization of mouse embryo paraffin sections was performed with 35P-labeled antisense Hfh8 RNA probe (97). After hybridization, stringent washes and autoradiography, dark-field microscopy was used to visualize HFH-8-expressing cells in the tissues (Fig. 2, B, D, F, H, and J). These studies demonstrate that HFH-8 expression initiates during mouse gastrulation on day 7 pc in the extraembryonic mesoderm, the allantois, and the lateral mesoderm that arises from the primitive streak region (See Table 1). Abundant HFH-8 expression continues in the lateral mesoderm-derived somatopleuric and splanchnopleuric mesoderm (Fig. 2, A and B), which contribute to endothelial cell formation in the embryo proper (92). At the onset of organogenesis at 9.5 days pc, HFH-8 expression is restricted to the splanchnic mesoderm contacting the embryonic gut and presumptive lung bud (Fig. 2, C and D), suggesting that it may participate in the mesenchymal-epithelial induction of lung and gut morphogenesis (71, 97). HFH-8 expression continues in lateral mesoderm-derived tissue throughout mouse development and includes the mesenchymal cells of the oral cavity, esophagus, trachea, lung, gut, dorsal aorta, and intersomitic arteries (Fig. 2, E–H) but not of the head (71, 97). In day 18.5 pc embryos, HFH-8 is restricted to the distal mesenchyme of the lung and the muscle layer of the bronchus, but its signals are absent.
<table>
<thead>
<tr>
<th>Gene</th>
<th>DNA Binding</th>
<th>Mouse Embryonic Expression Pattern</th>
<th>Adult Expression Pattern</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hnf3β (Foxa2)</td>
<td>Winged helix</td>
<td>Gastrulation (6.5–8.5 days pc): Definitive and foregut endoderm and visceral endoderm of yolk sac, notochordal mesoderm, and floorplate of neurotube Organogenesis (9–18 days pc): Endoderm-derived epithelial cells of lung, liver, pancreas, gallbladder, intestine, stomach, esophagus, tongue, trachea, floorplate, and midbrain</td>
<td>Bronchiolar and alveolar type II epithelial cells of the lung and epithelial cells of the liver, thyroid gland, pancreas, stomach, and large and small intestine (cryp)</td>
<td>2, 49, 83, 104, 106, 109, 111, 121, 143, 149</td>
</tr>
<tr>
<td>Hnf3α (Foxa1)</td>
<td>Winged helix</td>
<td>Gastrulation (7.5–8.5 days pc): Similar to Hnf3β except Hnf3α expression initiates 1 day later Organogenesis (9–18 days pc): Similar to Hnf3β but also found in the epithelial cells of intestinal villus, renal pelvis, prostate, and urinary tract</td>
<td>Similar to Hnf3β but also found in the epithelial cells of intestinal villus, renal pelvis, prostate, and urinary tract</td>
<td>2, 49, 57, 83, 96, 106, 109, 143</td>
</tr>
<tr>
<td>Hfh8 (Freac1, Foxf1)</td>
<td>Winged helix</td>
<td>Gastrulation (6.5–9 days pc): Extraembryonic mesoderm of yolk sac and allantois Lateral mesoderm including the somatopleuric and splanchnopleuric mesoderm Organogenesis (9–18 days pc): Splanchnic mesenchyme of distal lung and mesenchyme of oral cavity (including teeth, tongue, oropharynx, epiglottis, esophagus, and trachea), stomach, intestine, intersomitic arteries, and dorsal aorta</td>
<td>Alveolar endothelial cells and smooth muscle surrounding pulmonary bronchioles Endothelial cells and smooth muscle of small intestine Low levels in prostate, bladder, and placenta Ciliated bronchiolar and choroid plexus epithelial cells and stage-specific spermatocytes of testis</td>
<td>79, 97, 12, 24, 40, 68, 94, 126, 138</td>
</tr>
<tr>
<td>Hfh4 (Foxj1)</td>
<td>Winged helix</td>
<td>Gastrulation (7–7.5 days pc): Monociliated node cells Organogenesis: (days 14.5–15.5 pc): Ciliated epithelial cells of the proximal airways (trachea, bronchi, bronchioles), nose and paranasal sinuses, ovaries, testis and kidneys (Day 11 pc): Ciliated choroid plexus and ependymal cells</td>
<td>Ciliated bronchiolar and choroid plexus epithelial cells and stage-specific spermatocytes of testis</td>
<td>5, 13, 128</td>
</tr>
<tr>
<td>Fkh6 (Foxl1)</td>
<td>Winged helix</td>
<td>Gastrulation (8–9 days pc): Posterior mesoderm Organogenesis: Mesenchyme of the kidney, lung, gut, tongue, teeth, temporal bones, nasal cavity and nasal septum, otic and optic capsule</td>
<td>Stomach, small and large intestine, and kidney</td>
<td>48</td>
</tr>
<tr>
<td>Ttf1 (T/ebp, Nkx2.1)</td>
<td>Nkx homeodomain</td>
<td>Organogenesis: Presumptive and developing lung and thyroid gland in the endoderm-derived epithelial cells Expression continues in the distal airway and thyroid epithelial cells</td>
<td>Epithelial cells of the pulmonary bronchioles, alveolar epithelial cells, and thyroid gland</td>
<td>67, 121, 149</td>
</tr>
<tr>
<td>Hox</td>
<td>Homeodomain</td>
<td>Organogenesis: Presumptive lung bud expresses Hoxa5, Hoxb3, Hoxb4, and Hoxb5 (10.5–14.5 days pc): Hoxb3 and Hoxb4 are expressed in the mesenchyme of the trachea, bronchi, and distal lung, whereas Hoxa5, Hoxb2, and Hoxb5 are restricted to the distal lung mesenchyme</td>
<td>Expression of Hoxb genes in the developing lung decreases as embryo reaches maturity</td>
<td>5, 13, 128</td>
</tr>
<tr>
<td>Gata6</td>
<td>Zinc finger</td>
<td>Gastrulation (6.5–9 days pc): Reichert’s membrane, allantois, primitive streak mesoderm, and embryonic mesoderm of head fold region (cardiogenic plate) Organogenesis: Epithelial cells of gut and pulmonary bronchioles Developing heart, arterial smooth muscle (including pulmonary arteries), urogenital ridge, and musculature of bladder</td>
<td>Bronchiolar epithelial cells of lung Epithelial cells of stomach and small intestine Cardiac muscle of heart and smooth muscle cells of aorta and bladder</td>
<td>84</td>
</tr>
<tr>
<td>Gata5</td>
<td>Zinc finger</td>
<td>Gastrulation (6.5–9 days pc): Allantois and embryonic mesoderm of head fold region (cardiogenic plate) Organogenesis: Mesenchyme of the lung and urogenital sinus and smooth muscle cells of pulmonary airways and bladder Transient expression in developing heart (expression extinguished by day 16.5) Epithelial cells of gut</td>
<td>Smooth muscle cells of large airways of the lung and bladder Epithelial cells of stomach and small intestine</td>
<td>85</td>
</tr>
</tbody>
</table>

Hnf, hepatocyte nuclear factor; Fox, forkhead box; Hfh, Hnf3/forkhead homolog; Ttf, thyroid transcription factor; T/ebp, thyroid-specific enhancer-binding protein; Hox, homeodomain box; pc, postcoitum.
Table 2. Phenotype of lung transcription factor homozygous null and TG mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>General Phenotype of Homozygous Null and TG Mice</th>
<th>Lung Phenotype of Homozygous Null and TG Mice</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hnf3β (Foxa2)(−/−) mice</td>
<td>Hnf3β(−/−) embryos die in utero by day 10 because they fail to undergo gastrulation; Lack proper formation of node, notochord, visceral endoderm, foregut, and midgut endoderm and exhibit defects in neurotubes</td>
<td>Unknown because of Hnf3β(−/−) early embryonic lethal phenotype</td>
<td>1, 33, 134</td>
</tr>
<tr>
<td>SP-C (Hnf3β, Foxa2) TG mice</td>
<td>TG mice were created that used the −3.7-kb human SP-C promoter to increase expression of Hnf3β in developing respiratory epithelial cells of the embryonic lung</td>
<td>Disruption in respiratory epithelial cell differentiation, branching morphogenesis and vasculogenesis</td>
<td>148</td>
</tr>
<tr>
<td>H/h4 (Foxj1)(−/−) mice</td>
<td>Arrested differentiation of epithelium</td>
<td>Decreased expression of E-cadherin and vascular endothelial growth factor</td>
<td>124</td>
</tr>
<tr>
<td>SP-C (Hhf4, Foxj1) TG mice</td>
<td>TG mice were created that used the −3.7-kb human SP-C promoter to ectopically express Hhf4 in the developing respiratory epithelial cells of the embryonic lung</td>
<td>Atypical columnar cells lined the distal airway, expressing ciliated cell marker β-tubulin IV protein, TTF-1, and HNF-3β but displayed no expression of SP-B, SP-C, and CCSP</td>
<td>56, 75, 144</td>
</tr>
<tr>
<td>Ttf1 (T/ebp, Nkx2.1)(−/−) mice</td>
<td>Perinatal lethal phenotype Disruption in lung branching morphogenesis and thymus and pituitary gland development Failure in septum formation between trachea and the esophagus (tracheoesophageal tube) Defects in ventral forebrain</td>
<td>Inhibition in lung branching morphogenesis: lungs are arrested at early pseudoglandular stage</td>
<td>38</td>
</tr>
<tr>
<td>Hoxa5(−/−) mice</td>
<td>Perinatal lethal phenotype: improper tracheal and lung morphogenesis leading to tracheal occlusion and diminished surfactant expression Improper maturation of intestinal epithelial cells</td>
<td>Reduction in lung branching morphogenesis and thickening of the alveolar walls Disorganization of proximal and distal respiratory airways Reduced expression of SP, TTF-1, HNF-3β, and c-Myc</td>
<td>4, 5, 81</td>
</tr>
<tr>
<td>Gata6(−/−) mice</td>
<td>Early embryonic lethal phenotype at 5.5 days pc due to defect in extraembryonic tissue</td>
<td>Unknown because of Gata6(−/−) early embryonic lethal phenotype</td>
<td>62</td>
</tr>
<tr>
<td>Gata5(−/−) mice</td>
<td>Homozygous mutants were viable and fertile, but females exhibited vaginal and uterine defects</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Gli2(−/−) mice</td>
<td>Perinatal lethal phenotype with severe skeletal and neuronal defects Hypoplastic trachea and esophagus (zinc finger transcription factor related to Drosophila cubitus interruptus)</td>
<td>Decreased lung proliferation results in formation of only one right lung lobe instead of the normal 4 lobes Diminished expression of the Shh receptor Patch and Gli1</td>
<td>38, 93</td>
</tr>
<tr>
<td>Gli3ΔN3 mice</td>
<td>Analysis of Gli3ΔGli3Δ mice, a naturally occurring mutation in the Gli3 gene</td>
<td>Defects in the right medial, right caudal, and accessory lobes of lungs increase Shh expression</td>
<td>4</td>
</tr>
<tr>
<td>Gli1zf1zfd1zfd1 mice</td>
<td>Deletion of the zinc finger DNA binding domain exons</td>
<td>Lungs are smaller in Gli1zf1zfd1zfd1,Glilzf1zfd1+ but not in Gli1zf1zfd1zfd1zfd1+ mice</td>
<td>93</td>
</tr>
<tr>
<td>Gli2(−/−),Gli3(+/−) mice</td>
<td>No phenotype in Gli1zf1zfd1zfd1 mice</td>
<td>Lung defect is more exacerbated: day 16.5 lungs were more hypoplastic and they failed to separate into left and right lobes</td>
<td>77, 86</td>
</tr>
<tr>
<td>Gli2(−/−),Gli3(−/−) mice</td>
<td>Esophageal atresia with tracheoesophageal fistula and a severe lung phenotype</td>
<td>Lack formation of the lung, trachea, and esophagus More severe phenotype than Shh(−/−) mice that exhibit inhibition of lung branching morphogenesis</td>
<td>101</td>
</tr>
<tr>
<td>Pod1(−/−) mice</td>
<td>Perinatal lethal phenotype: defects in mesenchymal induction of lung and kidney (no glomeruli) morphogenesis (basic helix-loop-helix protein)</td>
<td>Hypoplastic lungs that lack alveolar region and are inhibited in lung branching morphogenesis and epithelial cell differentiation</td>
<td>101</td>
</tr>
</tbody>
</table>

SP, surfactant protein; TG, transgenic; (−/−), homozygous null; BMP-4, bone morphogenetic protein-4; CCSP, Clara cell secretory protein; Shh, sonic hedgehog; zfd, zinc finger DNA binding domain.
in the epithelial cells and mesenchyme of large vessels (Fig. 2, I and J).

In more recent studies, we generated a targeted disruption of the mouse Hfh8 gene in which the winged helix DNA binding domain was replaced by an in-frame insertion of a nuclear-localizing β-galactosidase gene (Kalinichenko VV, Lim L, Whitsett JA, Clark J, and Costa RH, unpublished observations). Expression of the β-galactosidase gene was under the control of the HFH-8 DNA-regulatory sequences, and thus staining for β-galactosidase enzyme activity allowed identification of HFH-8-expressing cells. They were found to be colocalized with platelet endothelial cell adhesion molecule-1-positive alveolar endothelial cells and with α-smooth muscle actin-positive peribronchiolar smooth muscle cells, but pulmonary blood vessels lacked detectable HFH-8 staining (54). These expression studies with adult Hfh8(+/−) lungs demonstrate that HFH-8 expression is restricted to the alveolar endothelial cells and the smooth muscle surrounding the bronchioles. However, our immunohistochemical staining data cannot rule out the possibility that HFH-8 is also expressed in alveolar myofibroblasts. Consistent with the early expression pattern in the extraembryonic and lateral mesoderm-derived tissues, Hfh8(−/−) mice die in utero (Kalinichenko VV, Lim L, Whitsett JA, Clark J, and Costa RH, unpublished observations). Moreover, Hfh8(+/−) mice exhibit defects in lung morphogenesis and function, suggesting that wild-type levels of HFH-8 are necessary for normal lung development.

We determined the DNA binding consensus sequence of HFH-8 using recombinant HFH-8 protein and PCR-mediated DNA site selection (97). Identification of this DNA binding consensus sequence revealed that HFH-8 binding sites are present in the promoter regions of genes critical for lung morphogenesis, mesenchyme proliferation, mesenchymal-epithelial signaling, and angiogenesis or vasculogenesis (see Table 3 for potential Hfh8 target genes). The HFH-8 protein therefore potentially regulates mesenchymal expression of the platelet-derived growth factor receptors (118, 120), which are required for alveolar structure formation (16), and receptor tyrosine kinase Tie-1, VEGFR-1 (Flt-1), and vascular endothelial (VE)-cadherin, which are required for endothelial cell function and assembly of endothelial cells into functional blood vessels (36, 58, 99, 112, 127); HFH-8 binding sites were also found in the promoter regions of mesenchyme-signaling Hgf and Bmp4 genes, the expression of which is critical for
Table 3. Putative HFH-8 target genes in mesenchymal cells of lung and intestine

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession No.</th>
<th>Position, bp</th>
<th>Sequence</th>
<th>HFH-8 BA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung morphogenesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat HGF</td>
<td>L23078</td>
<td>-447/-459</td>
<td>ATGTGTTTATTcTa</td>
<td>ND</td>
</tr>
<tr>
<td>Human HGF</td>
<td>M75967</td>
<td>-407/-419</td>
<td>ATGTGTTTATTcTa</td>
<td>ND</td>
</tr>
<tr>
<td>Mouse BMP-4</td>
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HGF, hepatocyte growth factor; PDGF, platelet-derived growth factor; NOS, nitric oxide synthase; eNOS, endothelial NOS; ECE, endothelin-converting enzyme; Flt-1, fms-like receptor tyrosine kinase; VE-cadherin, vascular endothelial cadherin; MAdCAM-1, murosal addressin cell adhesion molecule-1; uPA, urokinase-type plasminogen activator; MMP-1, matrix metalloproteinase-1; IL, interleukin; MCP-1, monocyte chemotractant protein-1; BA, binding affinity. Lowercase letters, nucleotides that deviate from HFH-8 consensus sequence. HFH-8 BA was determined with an electrophoretic mobility shift assay and is summarized as strong (+++), high (++), moderate (+), weak (+), or not done (ND). HFH-8 DNA binding consensus was determined with PCR-mediated site selection of partially randomized oligonucleotides as described by Peterson et al. (97). V is not T; N is any nucleotide; D is not C or G; Y is C or T.

l lung morphogenesis (8, 87) and extracellular matrix protease genes (Table 3, urokinase-type plasminogen activator, matrix metalloproteinase-1, and collagenase), which are involved in cell migration or cellular repair from injury. Interestingly, HFH-8 may regulate expression of the lung mesenchymal transcription factors homeodomain Hoxa5 and Hoxb5 genes, and they share overlapping embryoic expression with HFH-8 in the mesenchyme of the distal tips of the developing lung (5, 13). Moreover, Hoxa5(−/−) mice show respiratory tract defects attributed to decreases in pulmonary epithelial cell expression of TTF-1, HMF-3β and N-myc (5), suggesting that HFH-8 may also regulate mesenchyme-mediated lung epithelial cell development through regulation of the Hoxa5 gene.

Functional analysis of the HFH-8 protein with co-transfection assays with the Hfh8-dependent reporter gene identified a cell type-specific activation domain (Fig. 1A) that resides in the COOH-terminal region of the protein (71, 97). Interestingly, this HFH-8 transcriptional activation domain also exhibits homology with FoxF2 (FREAC-2; Lun), a Fox transcription factor that is expressed in pulmonary epithelial cell lines and also shares amino acid identity with the HFH-8 winged helix DNA binding motif (44, 76). A second activation domain that included conserved regions II and III was shown to activate transcription in undifferentiated cell lines but did not function in differentiated lung cell lines (44, 71).

Cytokines stimulate the cell type-specific expression of the P-selectin gene, the expression of which mediates cell adhesion of leukocytes to the endothelium and their subsequent extravasation to the underlying injured tissue (135). Cotransfection studies suggest that HFH-8, which is constitutively expressed in alveolar endothelial cells and peribronchiolar smooth muscle cells, may participate in cell type-specific activation of P-selectin in response to cytokines (97). Moreover, HFH-8
and Hfh4-deficient node cells, HFH-4 expression is re-initiating gastrulation. Although cilia were present on the node, which plays an important role in orchestrating morphogenesis, the monociliated cells of the proximal bronchiolar epithelial cells of the mouse embryonic lung 15.5 days pc, before the appearance of ciliated epithelial cells. Expression of HFH-4 mRNA was also observed in the ependymal cells and choroid epithelia on day 11 pc, which is several days before detectable HFH-4 levels in the mouse embryonic lung (68). Later in lung development, immunohistochemical staining demonstrates that the HFH-4 protein colocalizes with β-tubulin IV-positive ciliated epithelial cells of the proximal airways (bronchioles, bronchi, and trachea; see Table 1) (12, 126). HFH-4 protein levels were also observed in ciliated epithelial cells of the esophagus, nose paranasal sinuses, ovaries, testis, and developing kidneys and in the ependymal cells lining the spinal chord and ventricles of the brain (12, 94, 126). In the adult mouse, HFH-4 expression continues in the ciliated epithelial cells of the lung and respiratory system, in the choroid plexus, and in stage-specific spermatocytes of the testis.

Consistent with the role of HFH-4 in mediated formation of ciliated epithelial cells during mouse embryogenesis, Hfh4(−/−) mice lacked staining for left-right dynein protein and 9+2 microtubules (motile type of cilia) in proximal epithelial cells of the lung and in the ventricles of the brain (18, 24). The Hfh4(−/−) mice displayed perinatal lethality because they were deficient in ciliated epithelial cells lining the pulmonary bronchioles and ventricles, leading to defects in lung function and hydrocephalus (Table 2). HFH-4 is also transiently expressed in the monociliated cells of the node, which plays an important role in orchestrating gastrulation. Although cilia were present on the Hfh4-deficient node cells, HFH-4 expression is required for left-right asymmetry of the internal organs and Hfh4(−/−) mice exhibit randomized situs inversus (internal organs display either left or right asymmetry). These genetic studies underscore the importance of HFH-4 in mediating differentiation of the ciliated epithelial cell lineage and in left-right asymmetry decisions during embryonic development.

A transgenic mouse study (124) in which HFH-4 protein was ectopically expressed in the distal respiratory epithelial cells resulted in defects of lung branching morphogenesis. Moreover, the distal airways were lined with atypical cuboidal or columnar epithelial cells. In support of the role of HFH-4 in mediating differentiation of ciliated epithelial cells, the atypical columnar cells that expressed high levels of the Hfh4 transgene stained positive for β-tubulin IV, a marker for ciliated epithelial cells (Table 2). Although these transgenic pulmonary epithelial cells still expressed the transcription factors TTF-1 and HNF-3β, they no longer expressed nonciliated epithelial marker genes including SP-C, SP-B, and CCSP. Ectopic expression of HFH-4 in the developing mouse lung therefore promoted differentiation toward the ciliated epithelial cellular lineage and inhibited expression of nonciliated epithelial marker genes.

HFH-4 protein is a potent transcriptional activator, with two activation domains at the NH2 terminus and COOH terminus of the protein (Fig. 1), and deletion of the conserved region II sequences caused a 25% reduction in transcriptional activity (68). HFH-4 binds to a consensus DNA binding sequence that is distinct from other Fox transcription factors (Fig. 1) in that it prefers guanine residues instead of purines in the core sequence and is less tolerant of nucleotide changes at the 3’-end of the binding site (68). It is interesting to note that although different Fox transcription factors bind to a similar core consensus sequence, slight nucleotide changes either 3’ or 5’ to this core sequence can alter binding specificity between different forkhead family members (89). Although the Fox proteins show strong amino acid identity in the recognition helix (helix 3), each transcription factor is capable of interacting with distinct DNA binding sites (Fig. 1). Structure-function studies with chimeric winged helix recombinant proteins demonstrated that a 20-amino acid region adjacent to the recognition helix can alter DNA binding specificity (see Fig. 1B), and these amino acid sequences are the most divergent within the winged helix DNA binding domain (89). Moreover, nuclear magnetic resonance structure analysis of the HFH-2 winged helix domain demonstrates that this DNA specificity region is able to fold into a fourth helical structure and functions to reposition the recognition helix into the DNA major groove (73).

**EXPRESSION OF FKH6 (FOXL1) TRANSCRIPTION FACTOR IN THE LUNG**

During mouse embryogenesis, Fkh6 expression overlaps with that of HFH-8 in the mesenchyme of the developing lung, stomach, gut, tongue, and teeth (48). The expression pattern of Fkh6 differs from HFH-8 in that it is also expressed in the mesenchyme of the kidney, temporal bones, nasal cavity, and otic and optic capsules. In adult mice, Fkh6 expression is extinguished in the lung, but its expression continues in the kidney, stomach, and small and large intestines. Despite the Fkh6 expression pattern in developing lung mesenchyme, the Fkh6(−/−) mice exhibit no defects in lung morphogenesis or function (53). Fkh6(−/−) mice die postnatally at 1 mo because of defects in the intestinal villus or crypt structures resulting from diminished mesenchymal expression of transforming growth factor-β/activin family members (BMP-2 and BMP-4).
EXPRESSON OF HFH-11B (FOXM1B) IS INDUCED AFTER ADULT LUNG INJURY

The human Fox transcription factor HFH-11B (also known as Trident and FoxM1b) is a proliferation-specific transcription factor that shares 39% amino acid homology with the HNF-3 winged helix DNA binding domain (59, 141, 143). HFH-11B contains a potent COOH-terminal transcriptional activation domain that possesses several phosphorylation sites for M phase-specific kinases as well as proline-glutamic acid-serine-threonine (PEST) sequences (Fig. 1A) that mediate rapid protein degradation (59, 141, 143). HFH-11B is expressed in proliferating embryonic cells (including the lung), but its levels diminish postnatally during terminal differentiation (59, 143). Although HFH-11 expression is markedly induced during cellular proliferation, its promoter region displays only a marginal fourfold stimulation in response to serum, suggesting that increased HFH-11 mRNA stability also plays a role in its increased levels during proliferation (60, 143). Furthermore, HFH-11B function is regulated by nuclear translocation because transgenic HFH-11B protein remains cytoplasmic in quiescent liver and proliferative signals induce HFH-11B nuclear localization (142).

HFH-11 expression is essential for normal embryonic development as evidenced by the perinatal lethal phenotype exhibited by Hfh11/Trident(--/--) mice (61). Consistent with a role in mediating cell cycle progression, Hfh11/Trident-deficient embryos display an abnormal polyploid phenotype in embryonic hepatocytes and cardiomyocytes (day 13 pc), suggesting that HFH-11 expression is required to link DNA replication to mitosis (61). Reactivation of hepatic HFH-11B levels during liver regeneration occurs at the G1/S transition of the cell cycle, and its levels remain elevated throughout the period of proliferation (143). A liver regeneration study with transgenic mice (142) that prematurely expressed hepatic levels of HFH-11B revealed that the mice displayed an 8-h acceleration of hepatocyte entry into the S phase, resulting from earlier expression of cell cycle-regulatory genes.

Expression of HFH-11B is also reactivated by proliferative signals in the adult rat lung after intratracheal administration of keratinocyte growth factor (143). A more recent mouse lung injury study (54) has demonstrated that butylated hydroxytoluene (BHT) lung injury also reactivates expression of HFH-11B in epithelial and mesenchymal cells during the period of lung replication and repair (54). In a manner similar to that described for liver regeneration, this study determined that BHT-mediated lung injury stimulates expression of the HFH-11B transcription factor, suggesting that HFH-11B participates in cellular proliferation during lung injury repair. In the same BHT lung injury model, epithelial expression of HNF-3β remained unchanged, whereas a 65% reduction in HFH-8 mRNA levels was observed during the period of mesenchymal cell proliferation repair (54).

THE HOXBOX TRANSCRIPTION FACTOR NKX 2.1 (TTF-1) IS REQUIRED FOR LUNG MORPHOGENESIS

The Nkx homeodomain transcription factor TTF-1 (also known as Nkx2.1 and thyroid-specific enhancer-binding protein) is expressed in the endoderm-derived epithelial cells of the presumptive and developing lungs and thyroid glands as well as in the embryonic diencephalon (39, 67). Immunohistochemical staining of developing mouse lungs reveals TTF-1 staining in the proximal and distal airway epithelia and, at later stages of lung development, in the distal alveolar epithelial cells (149). Functional analysis of promoter and enhancer regions of the SP, T1α, and CCSP genes has implicated TTF-1 as critical for their transcriptional activation (14, 19, 39, 103, 137, 139, 147). Interestingly, cotransfection assays have demonstrated that TTF-1 promoter activity is stimulated by both the HNF-3β (47) and GATA-6 (116) transcription factors, which may play an important role in stimulating Ttf1 gene expression during lung morphogenesis.

Consistent with the role of TTF-1 in lung development, Ttf1(--/--) mice display severe impairment in branching morphogenesis of the lung and in development of the thymus and pituitary glands (56). They also exhibit pronounced defects in ventral forebrain formation. The Ttf1-deficient lungs develop only into the main stem bronchi and lack development of the distal alveolar region, suggesting that their formation is arrested in the early pseudoglandular stage of lung development (56, 75). These Ttf1-deficient pulmonary epithelial cells fail to express nonciliated marker genes, including differentiated SP-B, SP-C, and CCSP (Table 2), and display reduced BMP-4 levels, which may contribute to the defect in lung branching morphogenesis (75). More recent examination of Ttf1(--/--) mice demonstrated that they lacked formation of septa between the trachea and the esophagus, leading to a common tracheoesophageal tube connecting the pharynx with the stomach (75). Furthermore, the developmentally arrested Ttf1-deficient lungs are connected to the atypical tracheoesophageal tube through the bronchi, which resembles the human pathological phenotype termed the tracheoesophageal fistula (75). This inhibition in tracheoesophageal septum formation is similar to that observed with the cubitus interruptus Gli2(--/--) mouse in a heterozygous Gli3 background (86) and the Shh(--/--) mouse (69). These studies indicate the importance of TTF-1 in the development of the lungs and the respiratory system.

HOX TRANSCRIPTION FACTORS IN LUNG MORPHOGENESIS

A number of distinct Hox transcription factors are expressed in the presumptive lung during mouse embryogenesis, and their expression levels decrease as the mouse embryo reaches gestation (5, 13, 128). The
*Hox* genes share homology in the helix-turn-helix motif but vary among family members in their ability to bind to DNA as either a monomer or dimer (23, 72). The *Hoxb3* and *Hoxb4* genes are expressed in the mesenchyme of the trachea, bronchi, and distal lung, whereas *Hoxa5*, *Hoxb2*, and *Hoxb5* are restricted to the distal lung mesenchyme, suggesting a role in branching morphogenesis (Table 1). Consistent with an important role in mesenchymal-epithelial interactions, the *Hoxa5*(*−/−*) mice display improper tracheal formation and impaired lung branching morphogenesis, leading to tracheal occlusions, diminished surfactant expression, and thickening of alveolar walls (5). Loss of mesenchymal expression of the *Hoxa5* gene caused a disruption in mesenchymal-epithelial signaling, leading to decreases in TTF-1, HNF-3α, and N-myc expression in the pulmonary epithelial cells (Table 2). Future mouse gene targeting studies will allow determination of the role of the *Hox* genes in branching morphogenesis and mesenchymal-epithelial signaling during lung development.

**ROLE OF THE ZINC FINGER GATA TRANSCRIPTION FACTORS IN LUNG MORPHOGENESIS**

The GATA transcription factors were first identified as regulating hematopoietic genes and share homology in their DNA binding domains that contain two zinc finger motifs (reviewed in Refs. 78, 88). In situ hybridization studies (3, 66) demonstrated that GATA-4 is expressed in the heart, gut endoderm, intestinal epithelium, liver, testis, and ovaries. GATA-4 expression is induced by retinoic acid differentiation of F9 cells into visceral or parietal endoderm and in embryoid body-induced differentiation of embryonic stem cells into visceral endoderm (3). Consistent with this embryonic expression pattern, *Gata4*(*−/−*) embryos die shortly after gastrulation and exhibit defects in heart morphogenesis and in foregut endoderm and visceral endoderm formation (63, 79). This phenotype suggests that GATA-4 expression is required for foregut endoderm specification and may play an early role in the development of foregut endoderm-derived organs (145). Future studies involving tetraploid rescue of the visceral endoderm defect will allow examination of the role of GATA-4 in gut endoderm morphogenesis and possibly in lung morphogenesis.

The GATA-5 and GATA-6 transcription factors display nonoverlapping expression patterns in the developing lung; GATA-6 expression is restricted to the bronchiolar epithelial cells of the lung (84), whereas GATA-5 is expressed in the smooth muscle cells of the large airways (85). The GATA transcription factors display similar expression patterns in developing heart, allantois, and gut epithelial cells, but at later stages of heart development, GATA-5 levels diminish. Their expression patterns differ in that GATA-6 expression is found in the primitive streak mesoderm and in Reichert’s membrane (Table 1). Targeted disruption of the *Gata5* gene leads to vaginal and uterine defects in females, but the mice display no defects in lung morphogenesis (81). *Gata6*(*−/−*) embryos die during gastrulation from defects in extraembryonic tissue, and, therefore, its role in lung development remains unknown. GATA-6 may likely play a role in lung morphogenesis given the fact that it regulates expression of TTF-1 (116), which is essential for lung formation. Future experiments with Cre/LoxP technology will allow cell type-specific ablation of the GATA-6 gene and determination of its role in lung morphogenesis and function.

**CUBITUS INTERRUPTUS GLI TRANSCRIPTION FACTORS IN LUNG MORPHOGENESIS**

The zinc finger Gli transcription factors are homologs of the *Drosophila* segment polarity gene *cubitus interruptus* and mediate transcriptional induction in response to Shh signaling (38, 46). Gli3<sup>−/−</sup>/Gli3<sup>−/−</sup> mice, a naturally occurring mouse mutation in the Gli3 gene, exhibit defects in the right medial, right caudal, and accessory lobes of the lungs (38). Targeted disruption of the Gli2 gene results in a perinatal lethal phenotype with diminished lung proliferation and branching, leading to fusion of the four right lung lobes into one lobe (77, 86). This lung phenotype is coincident with diminished expression of the Shh receptor Patch and the isofrom Gli1 (Table 2). Gli2(*−/−*) mice also display severe skeletal and neuronal defects, including hypoplastic trachea and esophagus (77). By contrast, no defects were observed in the transcriptionally inactive Gli1<sup>zfd/zfd</sup> mouse mutation, which deleted the exons encoding the zinc finger DNA binding domain (*zfd*) (93). Interestingly, Gli1<sup>zfd/zfd</sup>/Gli2<sup>zfd/+</sup> mice, but not Gli1<sup>zfd/zfd</sup>/Gli3<sup>zfd/+</sup> mice, die soon after birth and have multiple defects, including development of smaller lungs, suggesting that the Gli1 and Gli2 genes have redundant functions (Table 2). Gli1<sup>zfd/zfd</sup>, Gli2<sup>zfd/zfd</sup> double-mutant mice have more severe lung defects that are similar to those found with the Shh<sup>−/−</sup> mice in which the lung develops but displays inhibition of branching morphogenesis (69).

A more severe lung defect is observed with the Gli2 gene deficiency analyzed in a Gli3 heterozygous background (86). Gli2(*−/−*)/Gli3<sup>+/−</sup> embryonic mouse lungs are more hypoplastic, and the right and left lobes fail to separate (86). These mice have defective tracheoesophageal septum formation and possess a single tracheoesophageal tube, which connects the pharynx with the stomach, resembling the phenotype observed with either the Shh<sup>−/−</sup> (69) or Tnf1 (<sup>Nkx 2.1</sup>)<sup>−/−</sup> mice (75). The most severe phenotype is exhibited by the Gli3(*−/−*)/Gli2(*−/−*) mice, which display a complete absence of lung, trachea, and esophagus and smaller stomach, liver, and pancreas (86). Interestingly, the phenotype of Gli3(*−/−*)/Gli2(*−/−*) mice is more severe than that in Shh<sup>−/−</sup> mice (69), suggesting that these Gli transcription factors are not only regulated by Shh but may also be controlled by other signal transduction pathways.
THE BASIC HELIX-LOOP-HELIX POD1 TRANSCRIPTION FACTOR IN LUNG MORPHOGENESIS

Pod1 is a bHLH transcription factor in which the helix-loop-helix domain mediates protein association, allowing formation of either homodimeric and heterodimeric proteins, which then interact with DNA though the basic amino acid region (80). Pod1 is abundantly expressed in the mesenchyme of developing organs of the mouse embryo, including the lung, kidney, gut, and heart and in glomerular visceral epithelial cells (podocytes) (101, 102). Pod1(−/−) mice exhibit a perinatal lethal phenotype, displaying hypoplastic lungs that lack development of the alveolar region and kidneys that are deficient in mature glomeruli (101). Although Pod1 is exclusively expressed in the mesenchyme and podocytes, major defects are observed in the adjacent epithelia and include abnormalities in epithelial cell differentiation and branching morphogenesis (Table 2). Pod1 therefore appears to be essential for regulating genes involved in mesenchymoepithelial interactions, which are critical for the morphogenesis of the lung and kidney.

We thank Pradip Raychaudhuri and Francisco Rausa for critically reading this review.

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