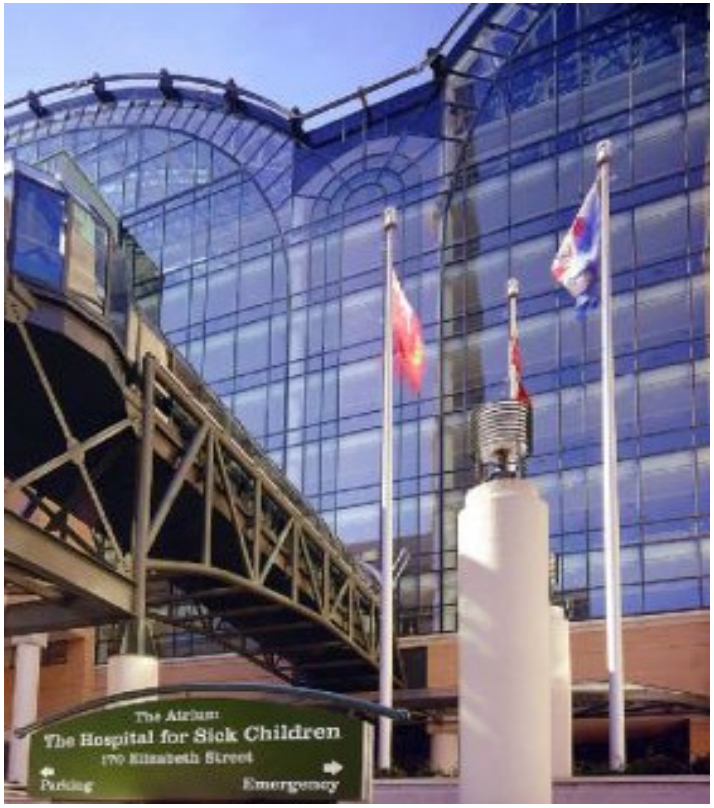
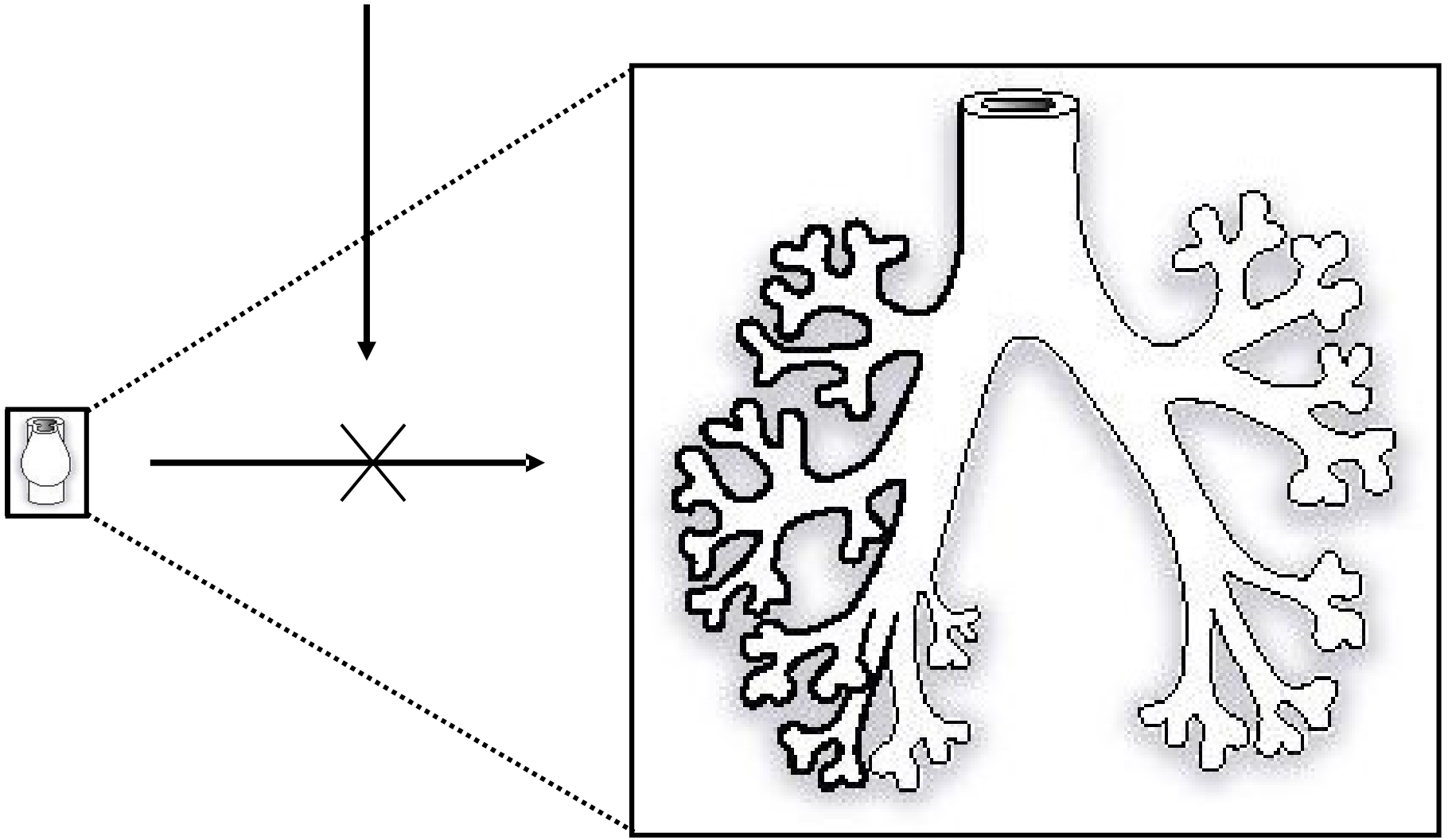
Cells and Maturation of the Lung



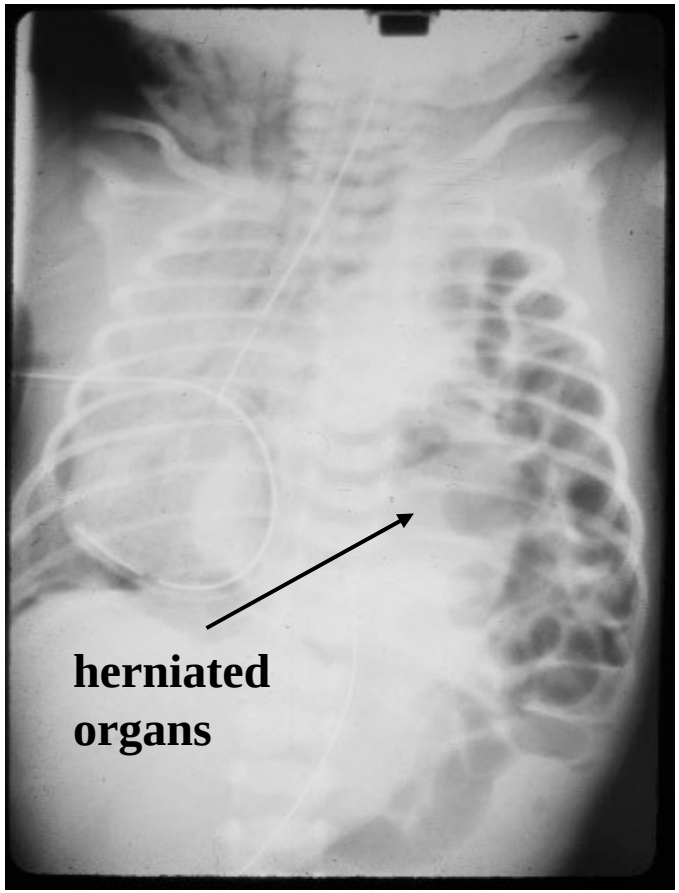
CHI R Group in Lung Development
Hospital for Sick Children, Toronto

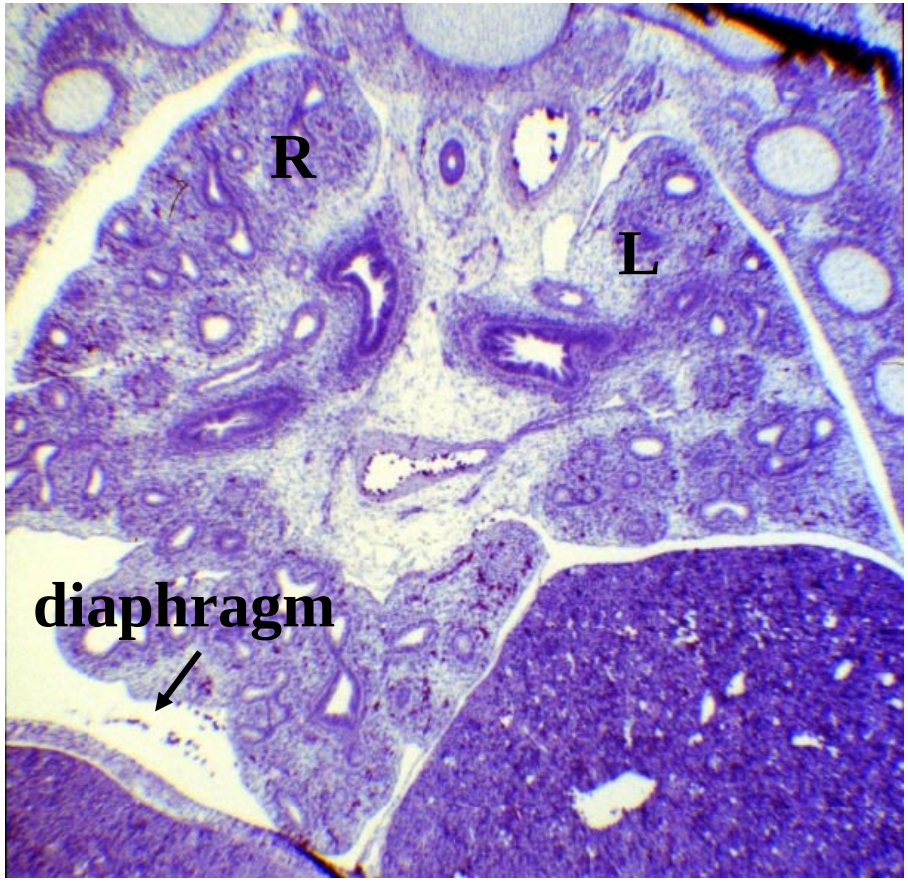
5th World Congress on Pediatric Critical Care, Geneva

Congenital malformations



Congenital Diaphragmetic Hernia

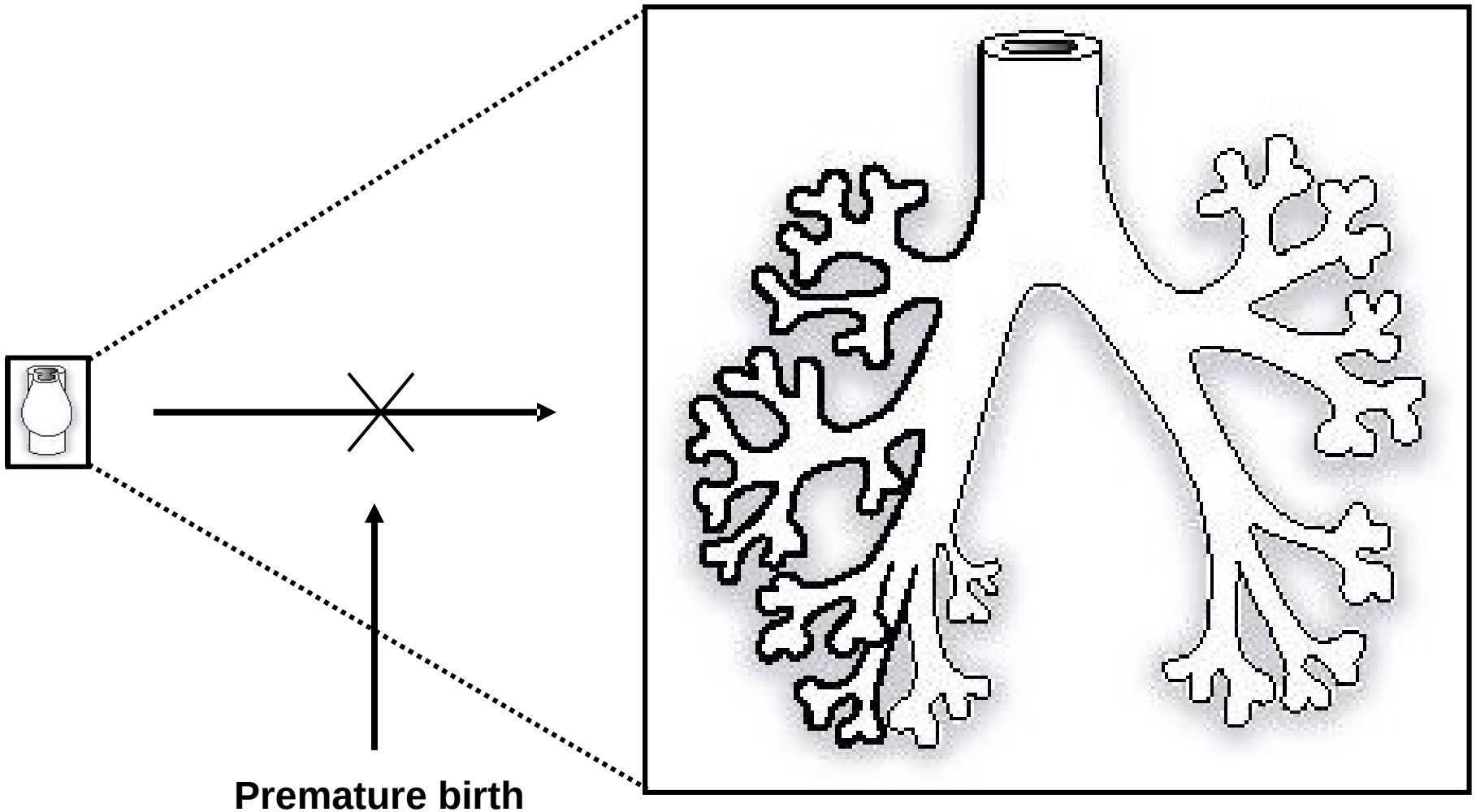




Congenital Diaphragmatic Hernia

Lung Characteristics

- Hypoplasia
- Reduced airway branching
- Diminished vascularization
- Reduced alveolar number



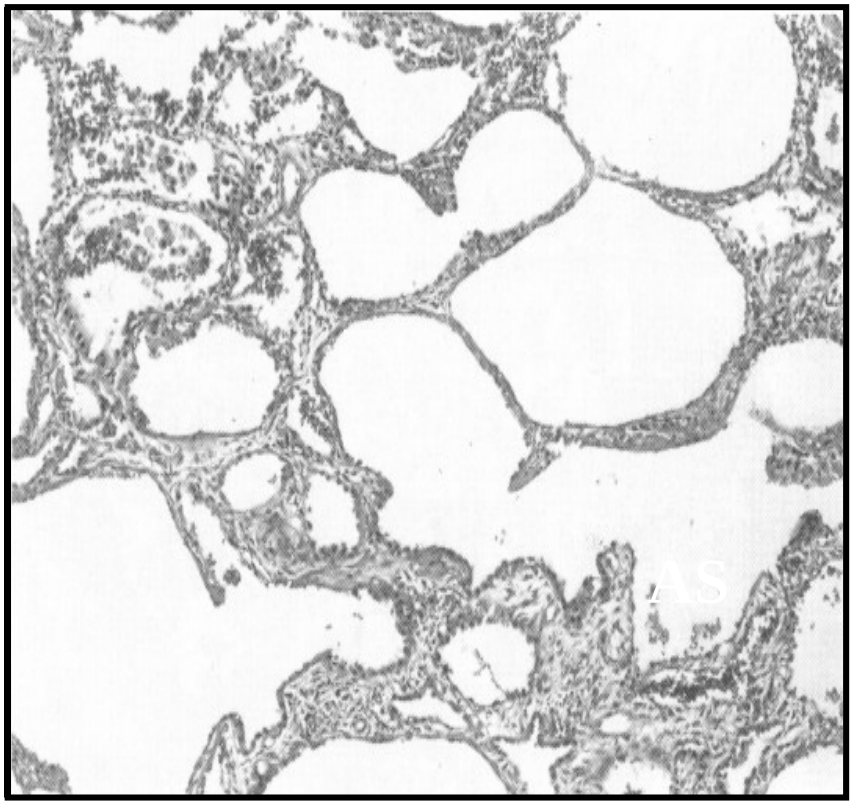
Premature birth
Intrauterine inflammation
Ventilation/oxygen

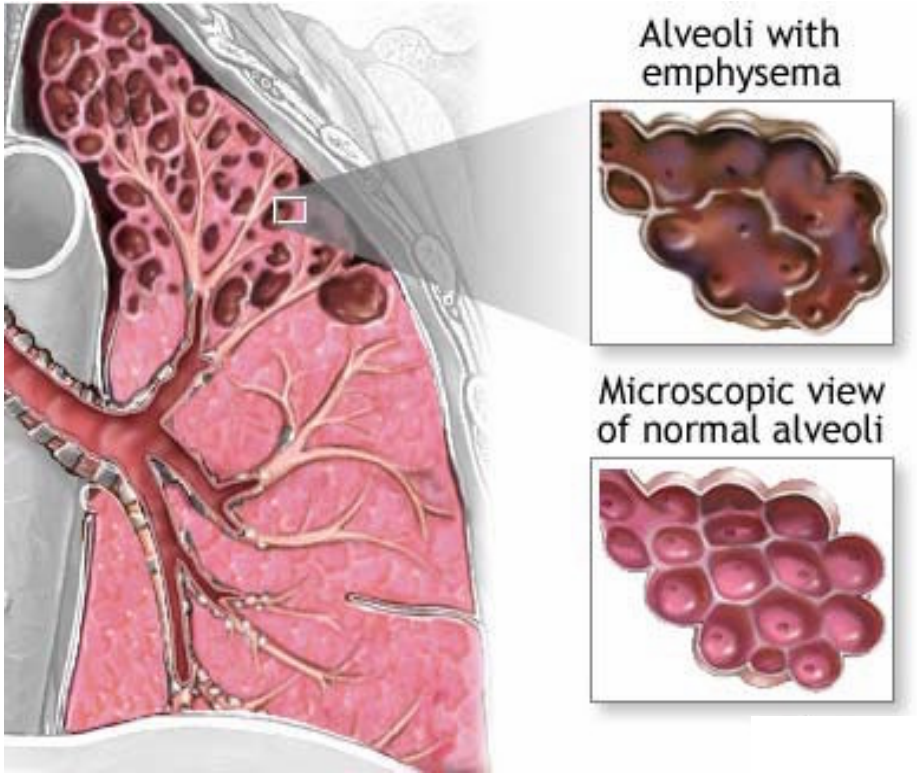
Prematurity

- **5-10% of all births**
- **75% of neonatal deaths**
- **85% of all neonatal complications**

Consequence: Bronchopulmonary Dysplasia

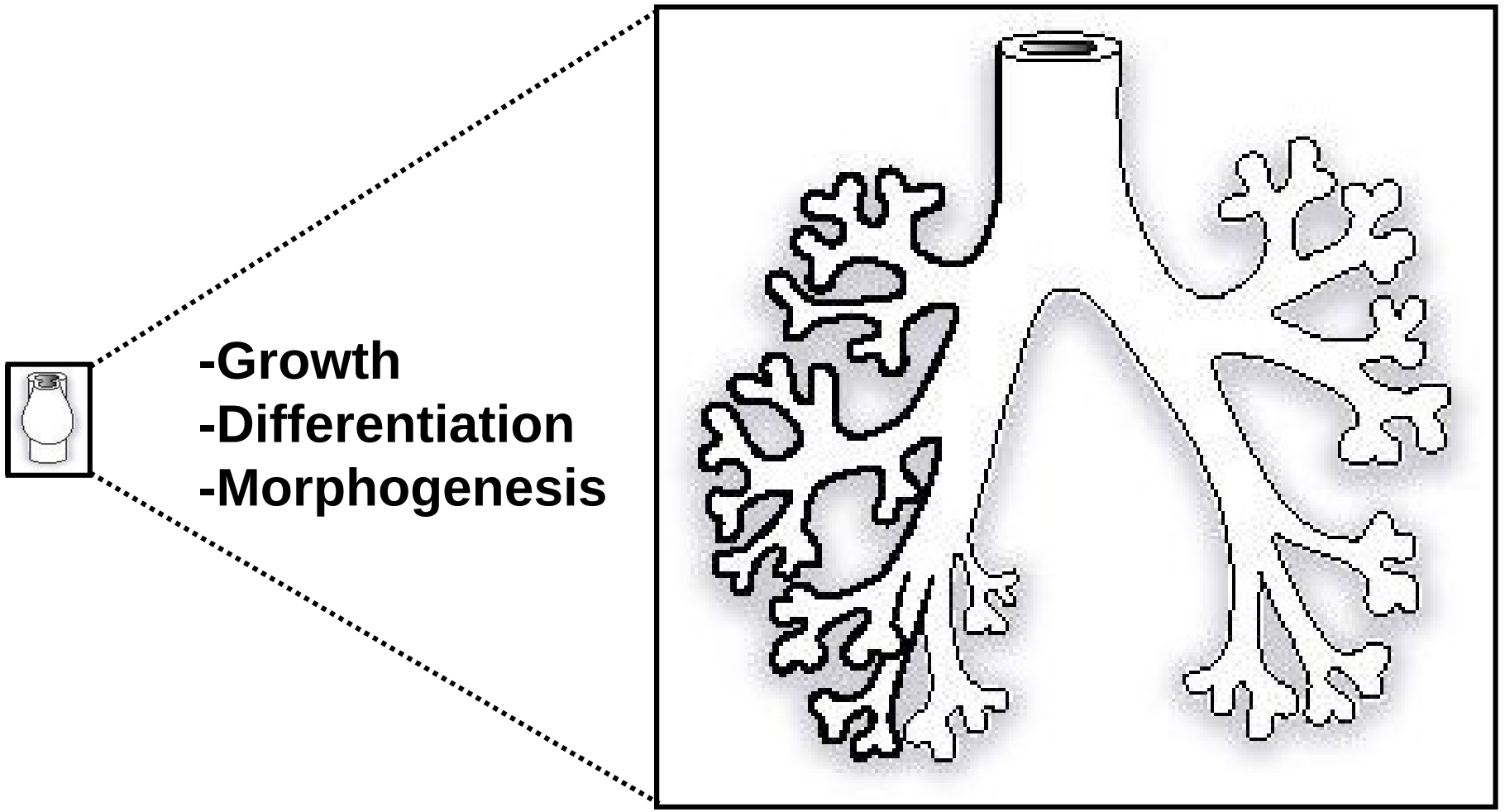
Lung Characteristics





Born: 26 wk; Biopsy: 7 mth

Requirements of Lung Development

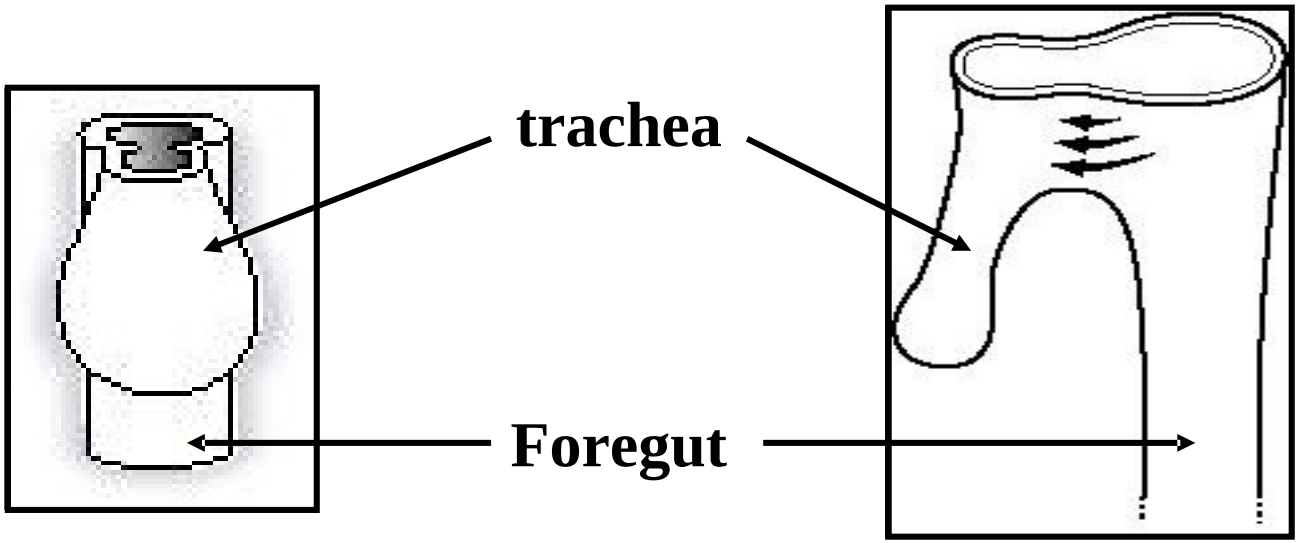


-Growth
-Differentiation
-Morphogenesis

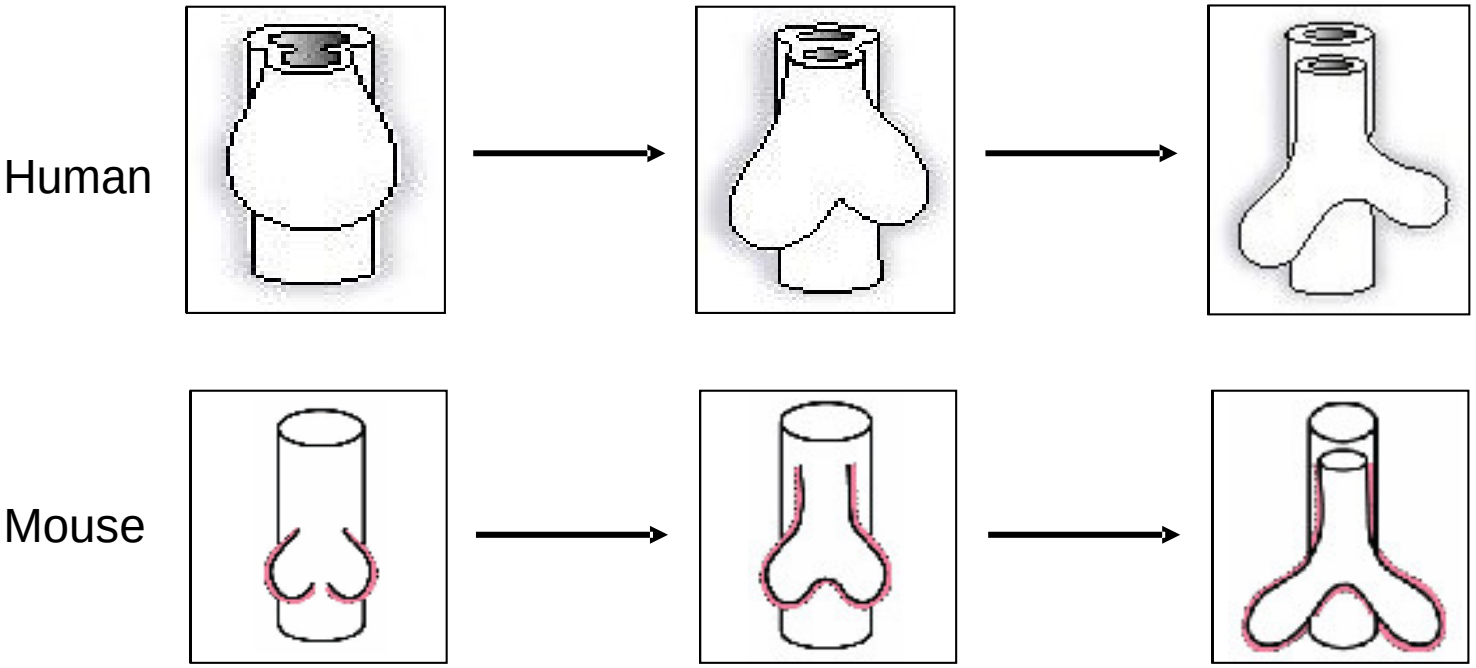
Endodermal Lung Specification

Factors

Foxf2a, TTF-1, GATA-6, Gli2 and Gli3, RA/RAR



Tracheal Outgrowth and Bronchial Bud Formation



Transcription Factors

Nkx2.1(TTF-1)

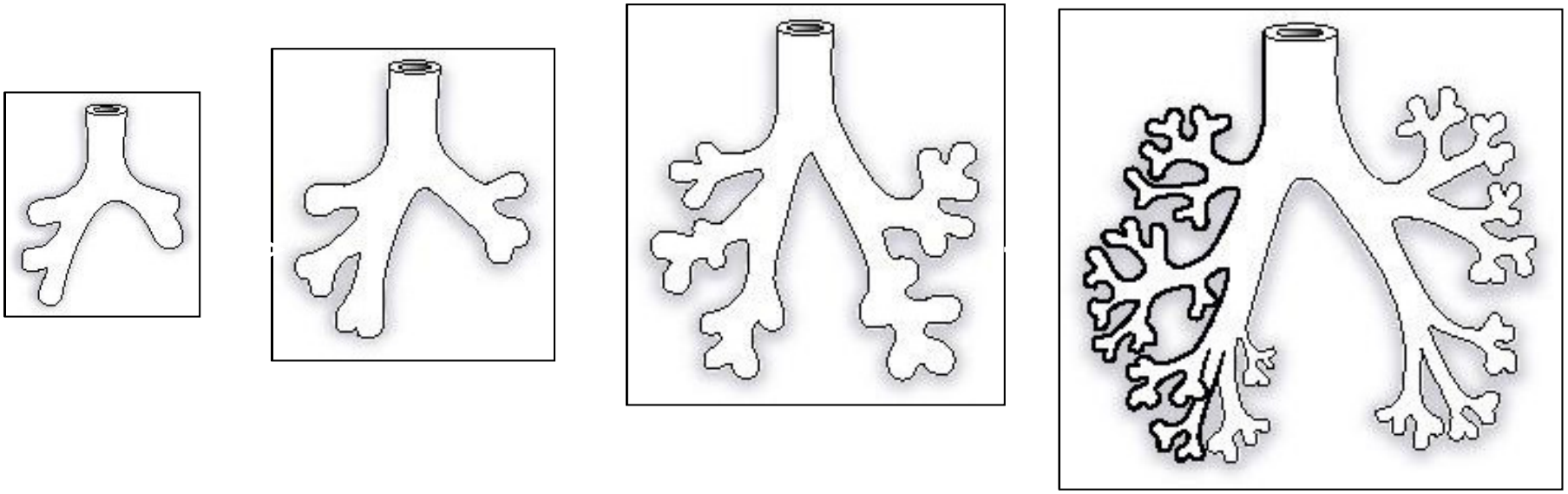
Hoxa5

Growth Factors

FGF10/FGFR2-IIIb

Pulmonary Branching

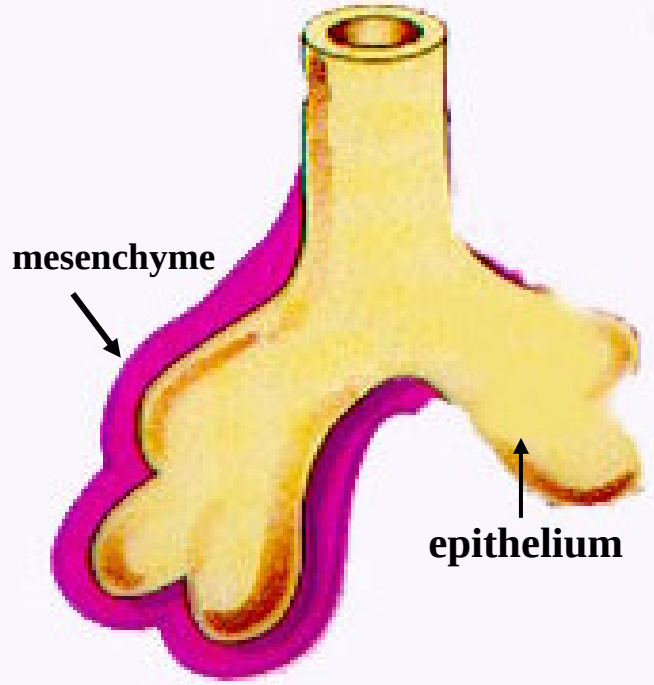
(Conducting airways)

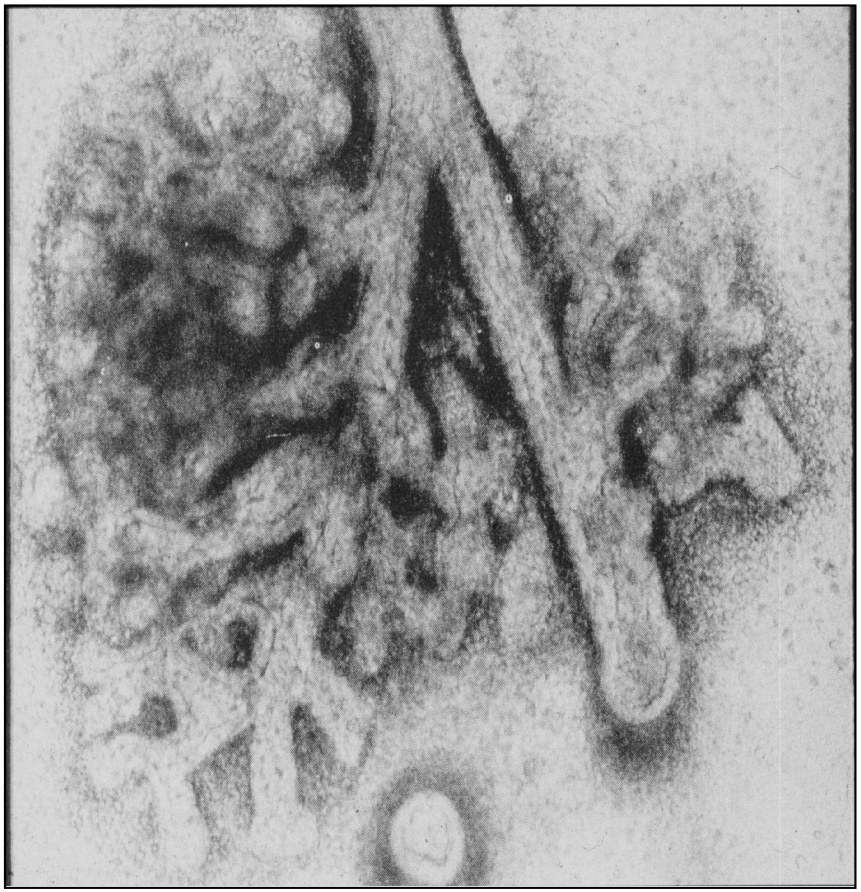


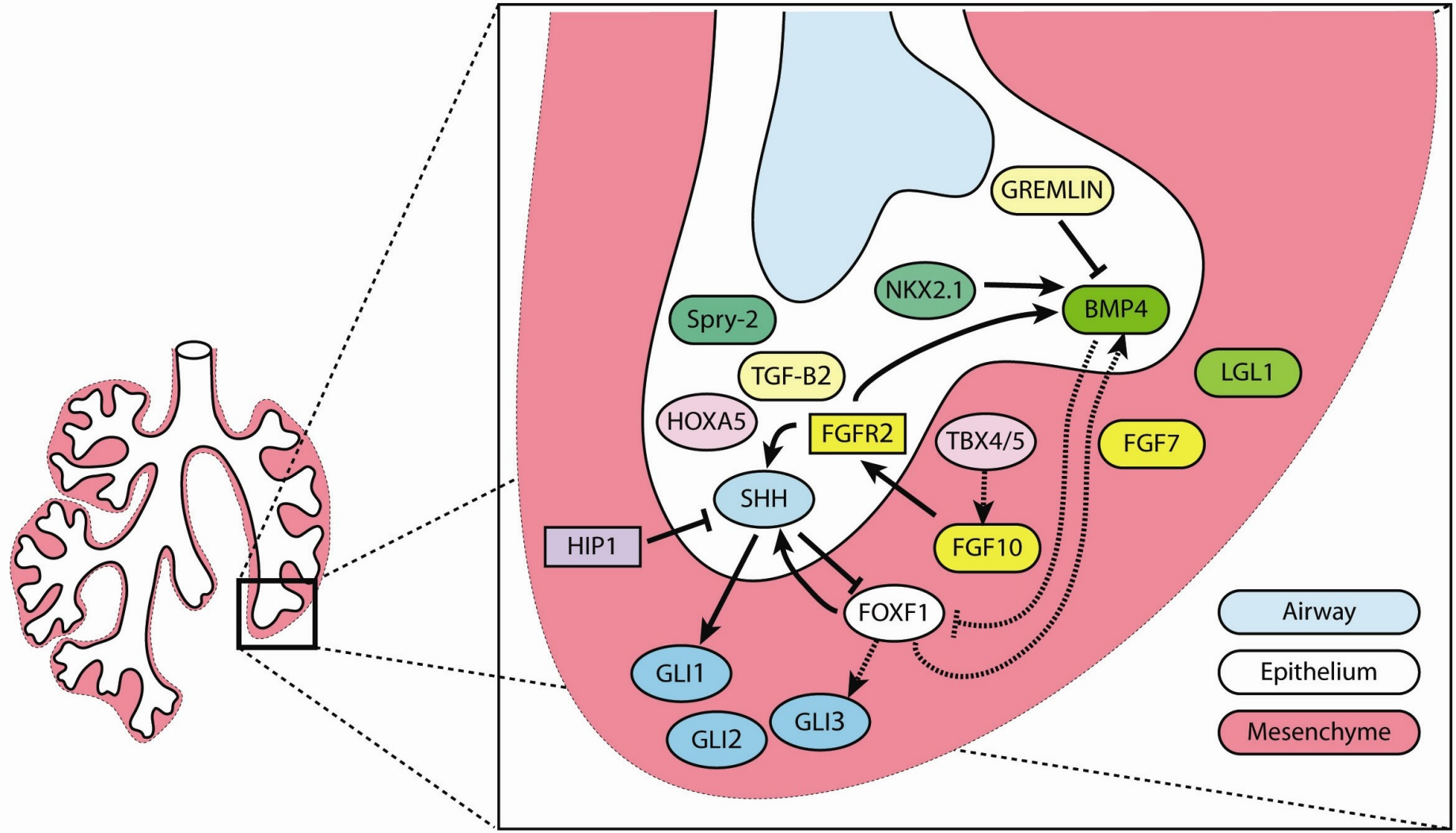
Question: What guides branching morphogenesis?

MESENCHYMAL-EPITHELIAL INTERACTIONS

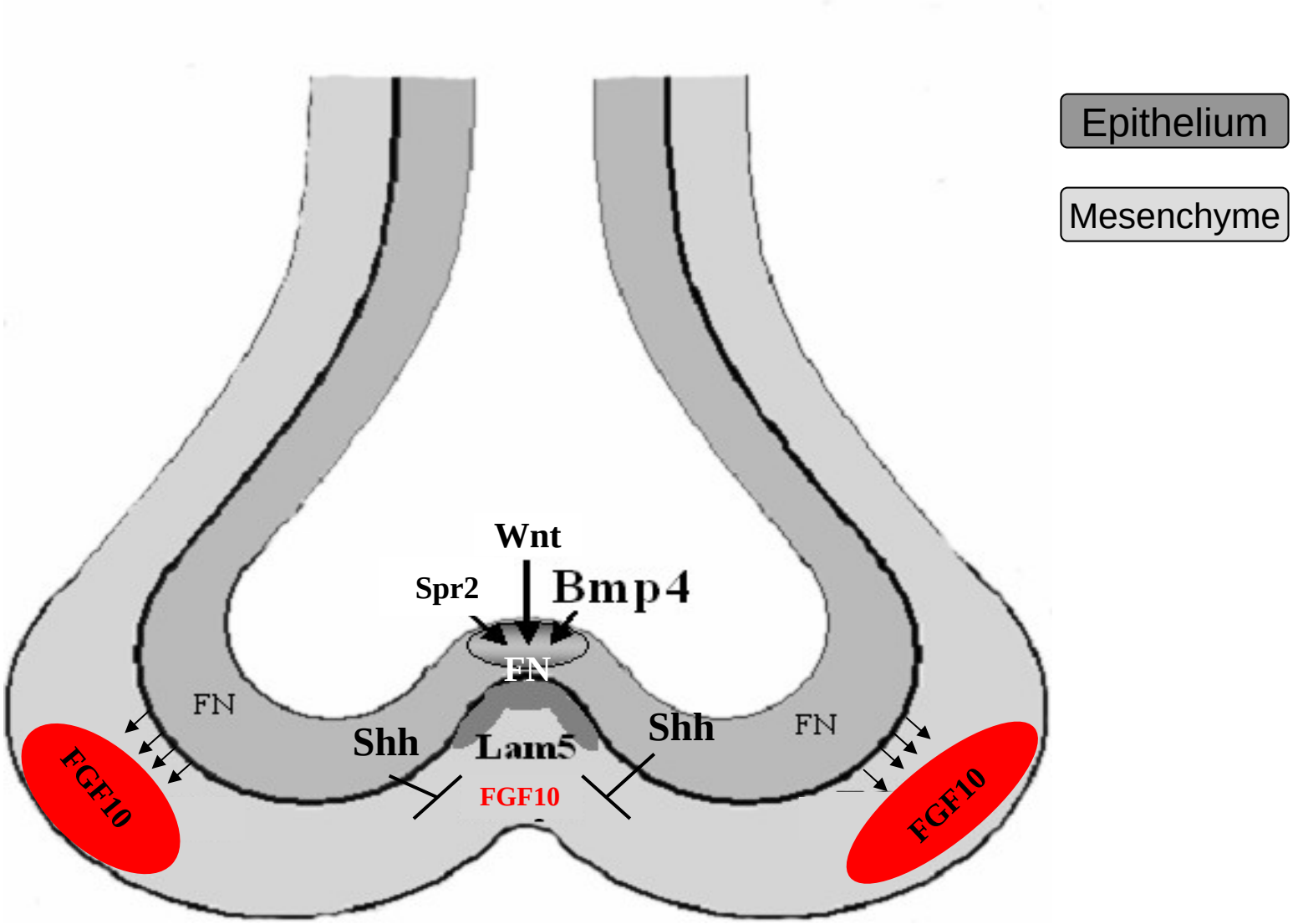
•Removal of **mesenchyme**



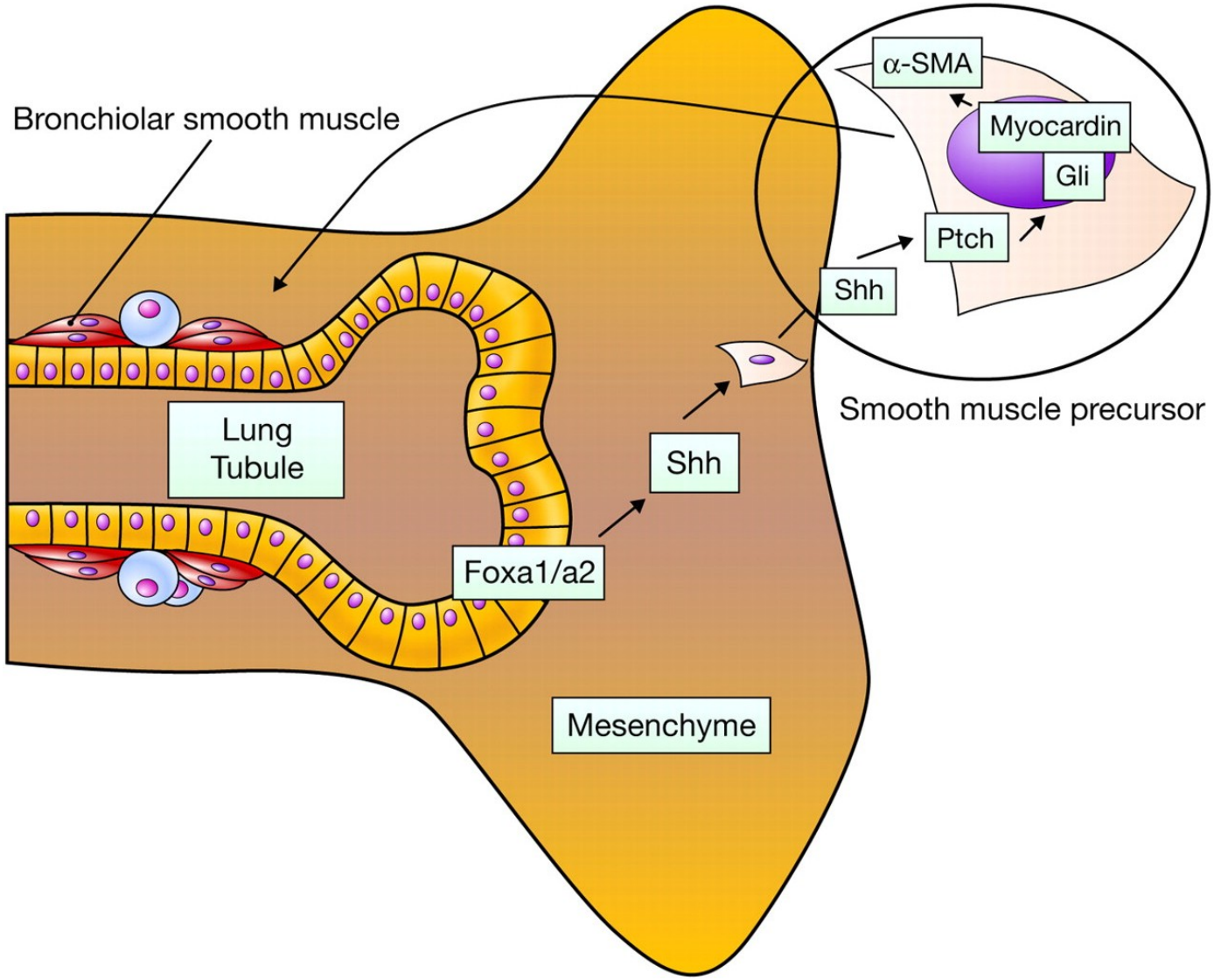



GREMLIN
NKX2.1
BMP4
Spry-2
TGF-B2
LGL1
HOXA5
FGFR2
TBX4/5
FGF7
SHH
HIP1
FGF10
FOXF1
GLI1
GLI3
GLI2
Airway
Epithelium
Mesenchyme

Bud branching simplified



FOXA transcription factors regulate SHH required for branching morphogenesis and smooth muscle cell differentiation



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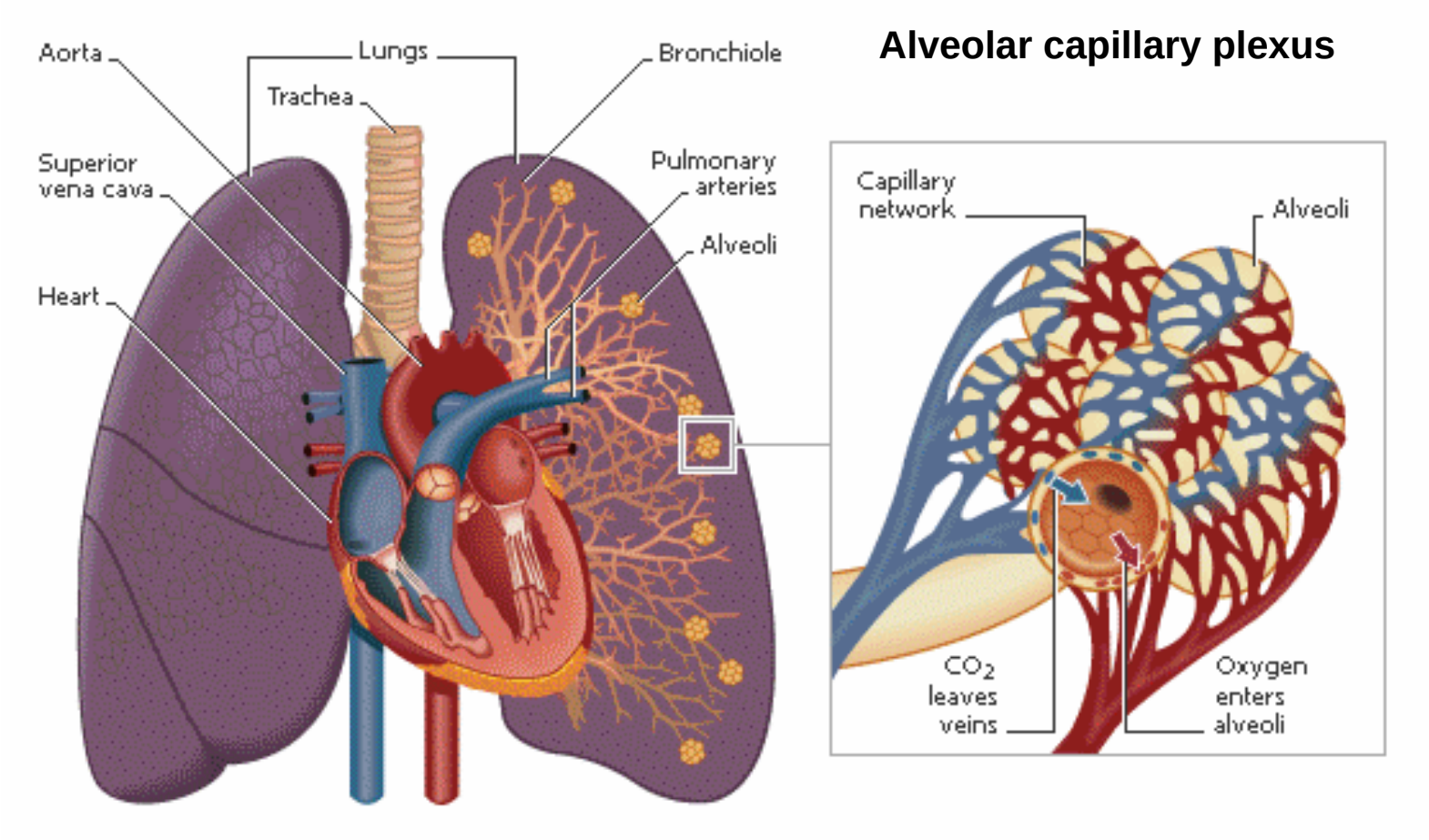
Pulmonary Vascular System

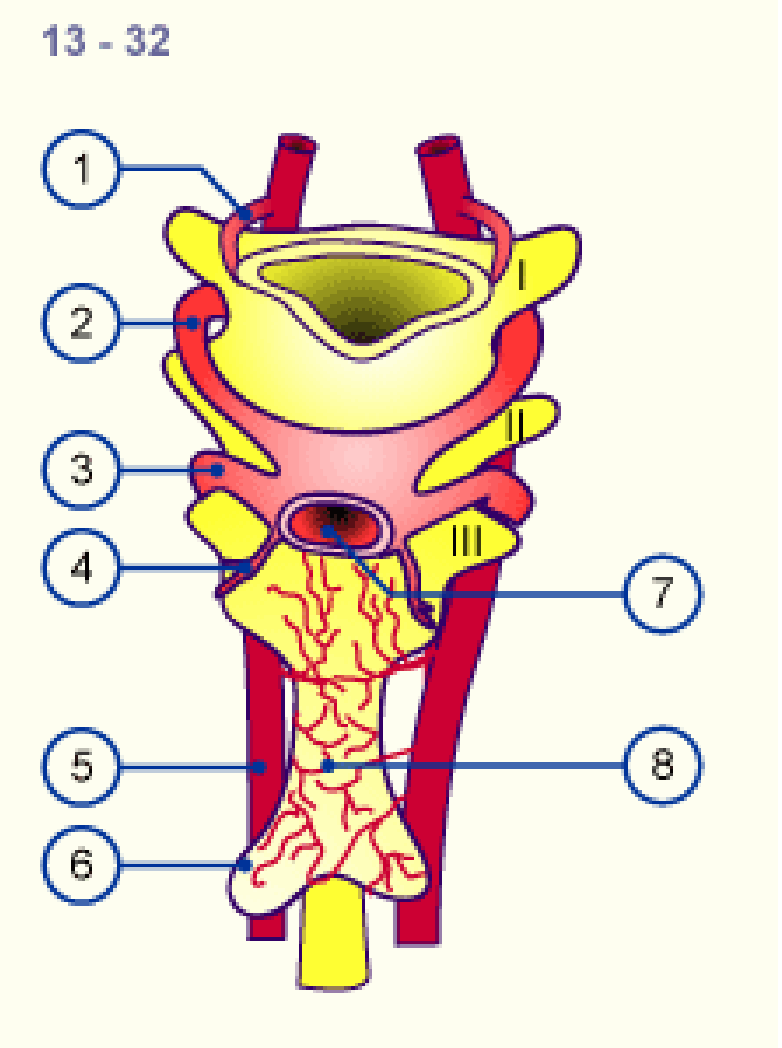


How does vascular system develop?

Development of the pulmonary vessels

From the aortic sac a vessel plexus arises around the lung anlagen that with the lung buds extends caudally (32 days)

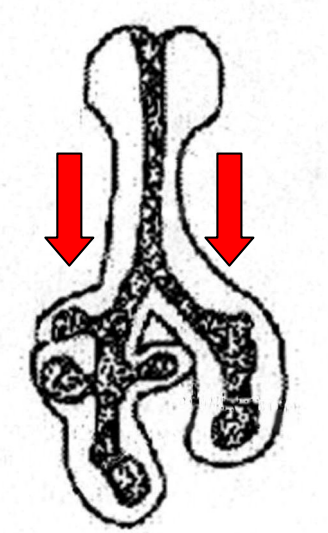
Pharyngeal Arches



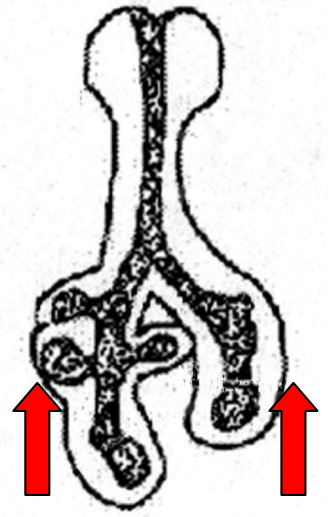
1. First aortic arch
2. Second aortic arch
3. Third aortic arch
4. Fourth aortic arch
5. Dorsal aorta
6. Lung buds
7. Aortic sac
8. Pulmonary plexus

Vascular development of the lung

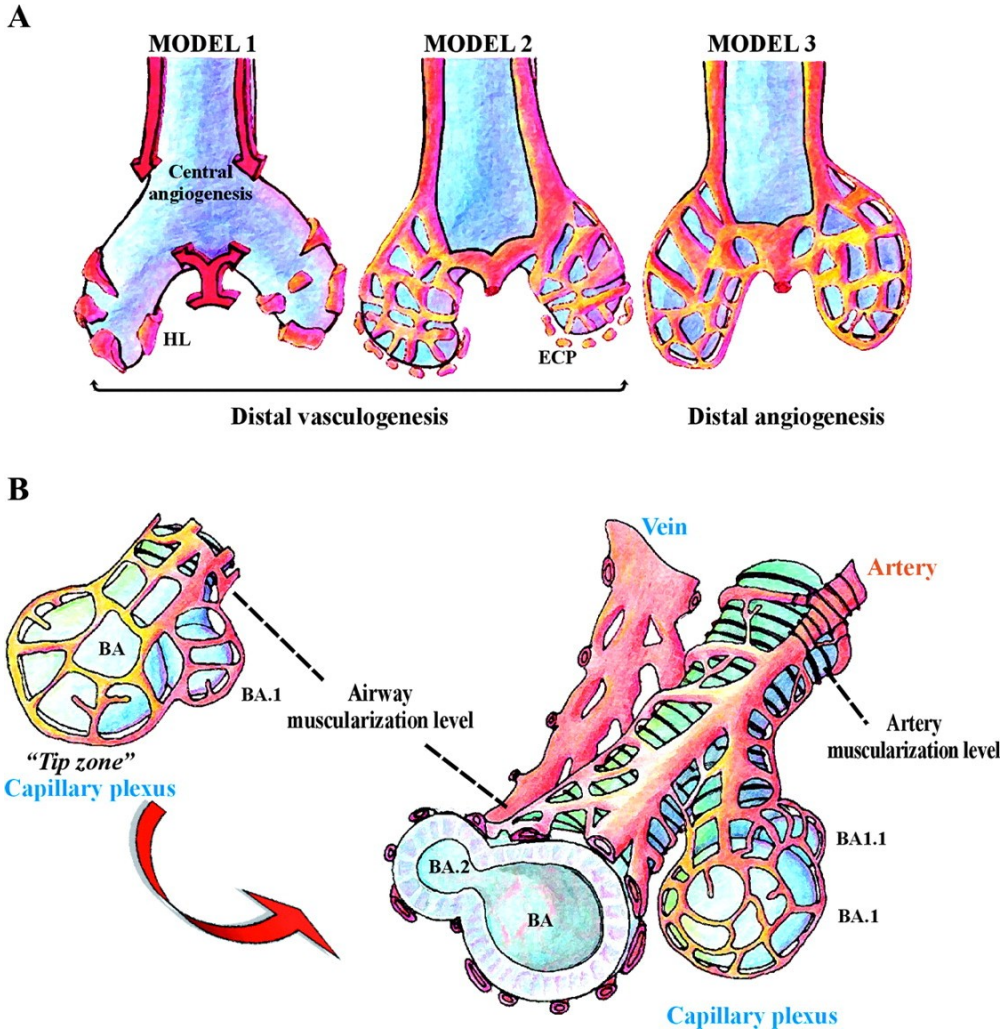
- Angiogenesis: the branching of new vessels from preexisting ones → central



- Vasculogenesis: the development of blood lakes that transform into vessels → peripheral



Lung vascular morphogenesis models



Parera, M. C. et al. Am J Physiol Lung Cell Mol Physiol 288: L141-L149 2005

Vascularization: In Utero Environment

Intervillous space opens around 10-12 wks gestation

pO_2:15-20 mM Hg<10-12 wks > $pO_{2:}$55 mm Hg

Ductus arteriosus shunts 90% of blood from the fetal lung

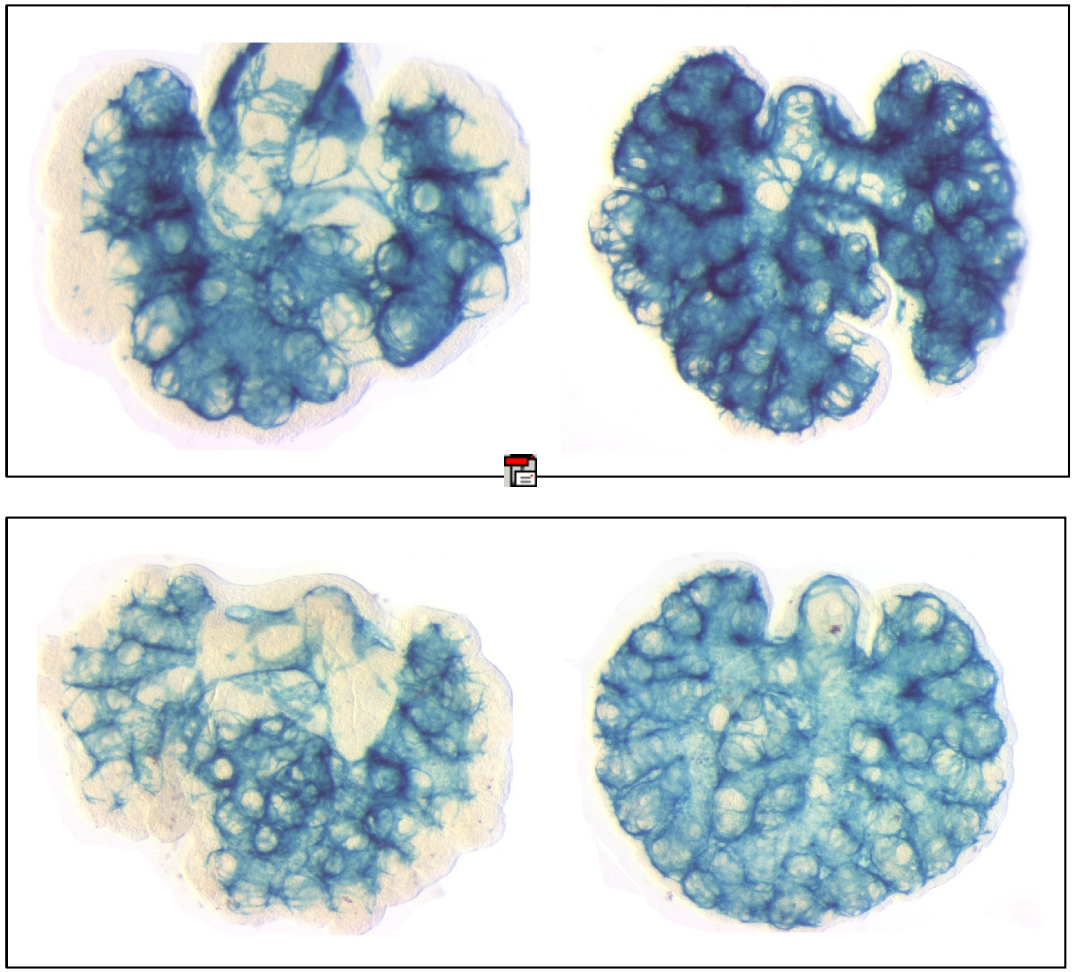
Consequence: Fetal lung develops in relative Low Oxygen environment

Low Oxygen Tension Promotes Vascular Development

(D11 lung explants of Tie-LacZ mice)

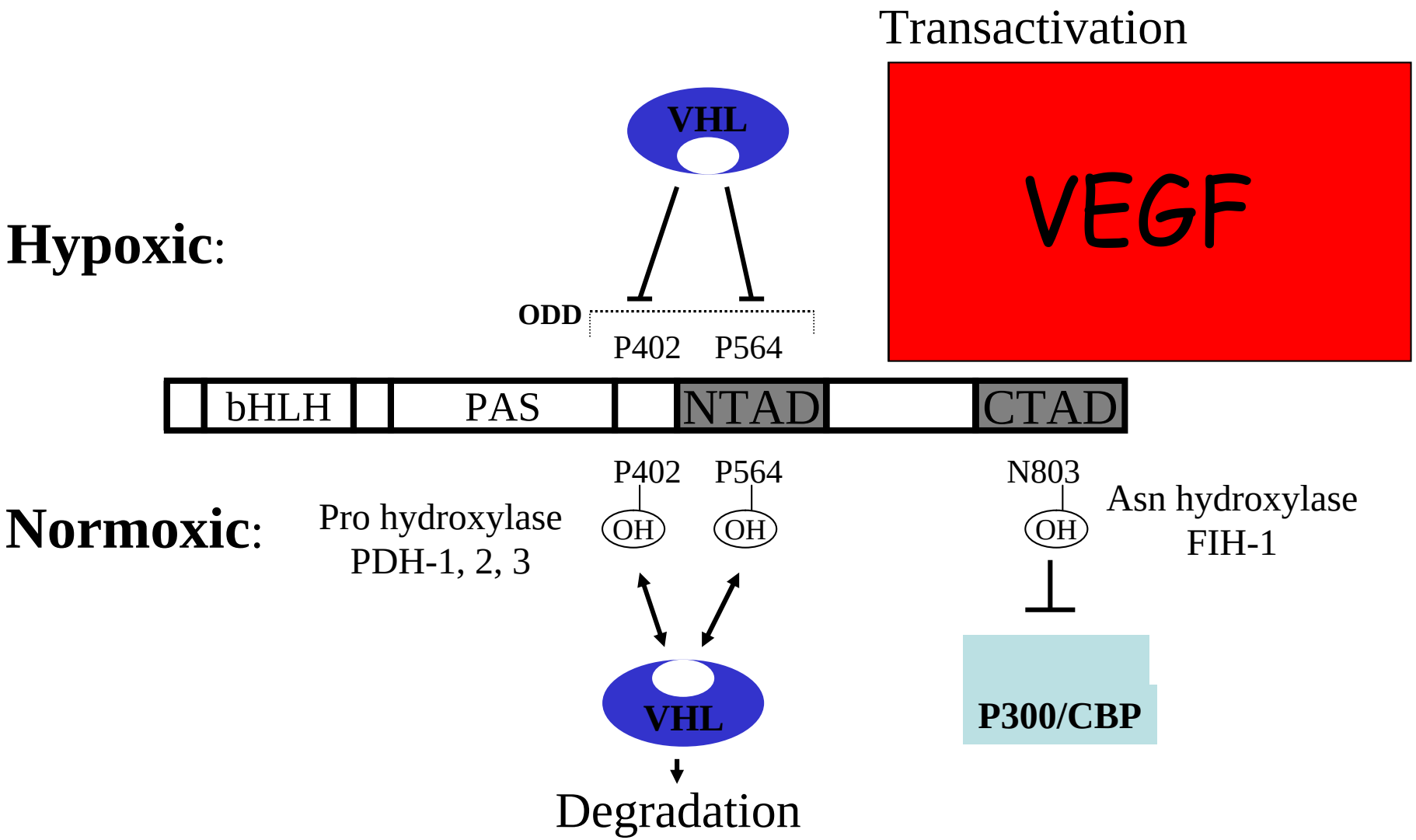
48h

72h



20% **3%**

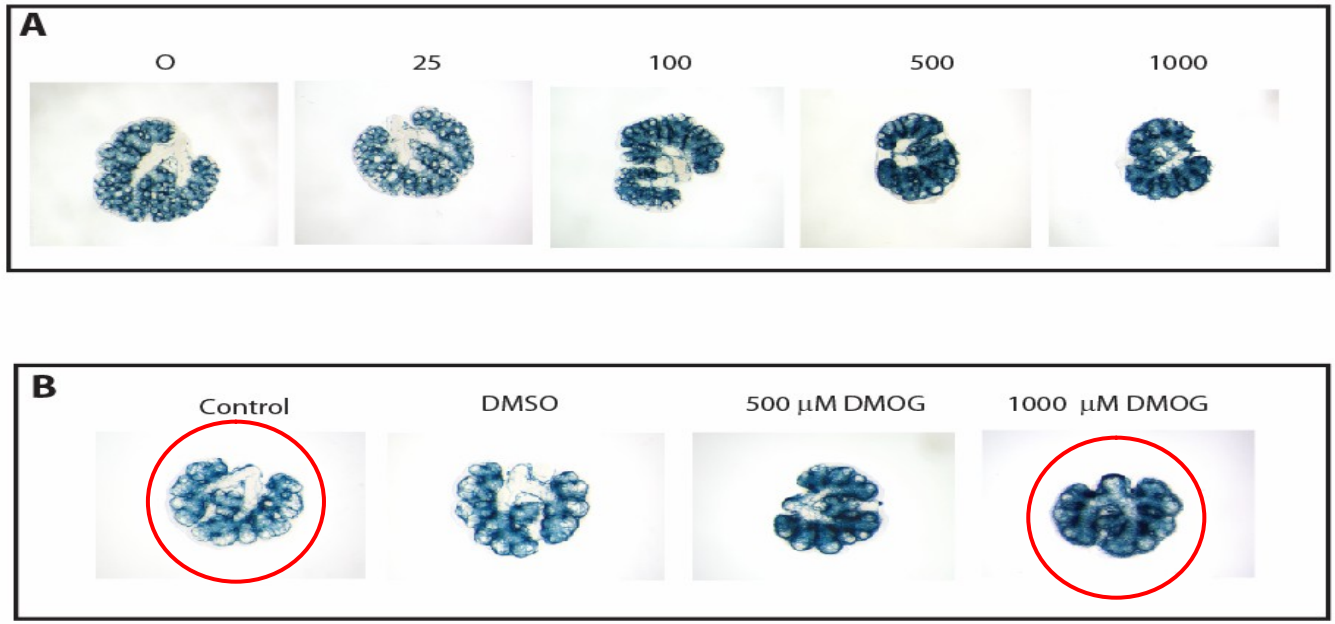
Oxygen concentration



Oxygen-sensing via HIF-1α protein

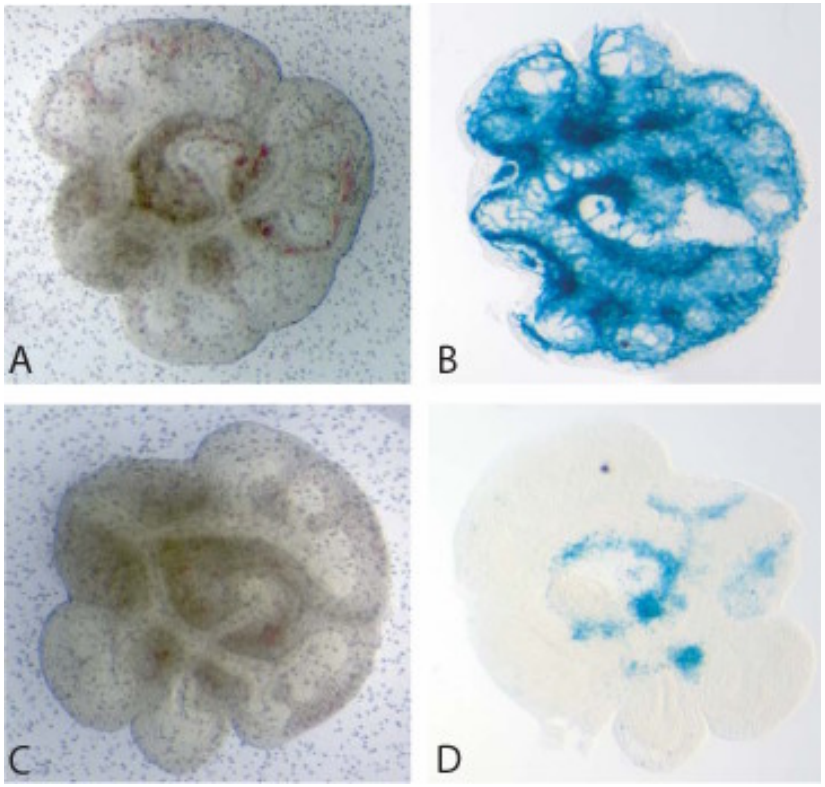
(Semenza: PNAS, 99:11570-11572, 2002)

Stabilization of HIF- 1 with DMOG Stimulates Vessel formation



EFFECT OF INHIBITION OF VEGF SIGNALING ON EARLY LUNG DEVELOPMENT

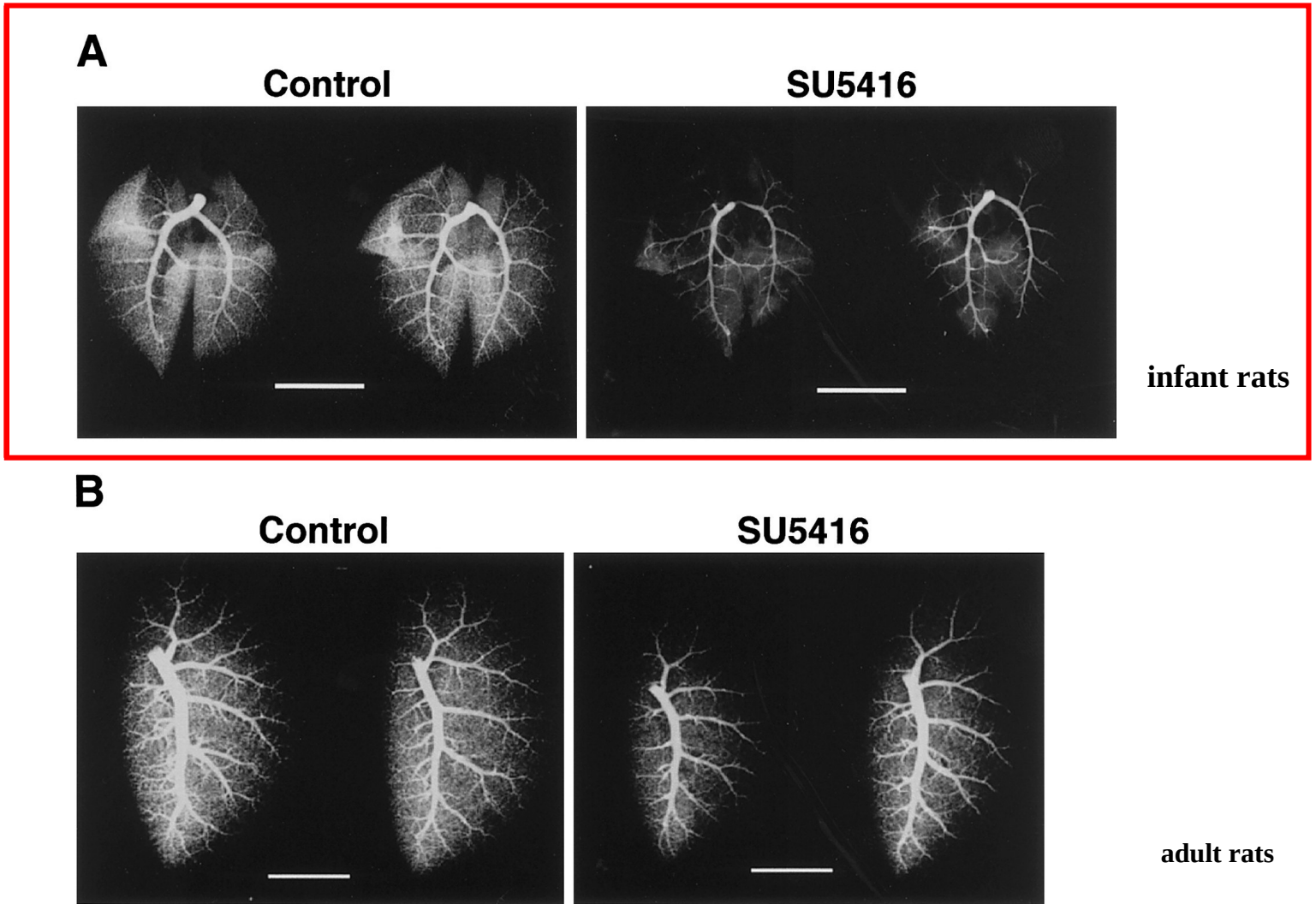
Control



VEGFR2 inhibitor
SU5416

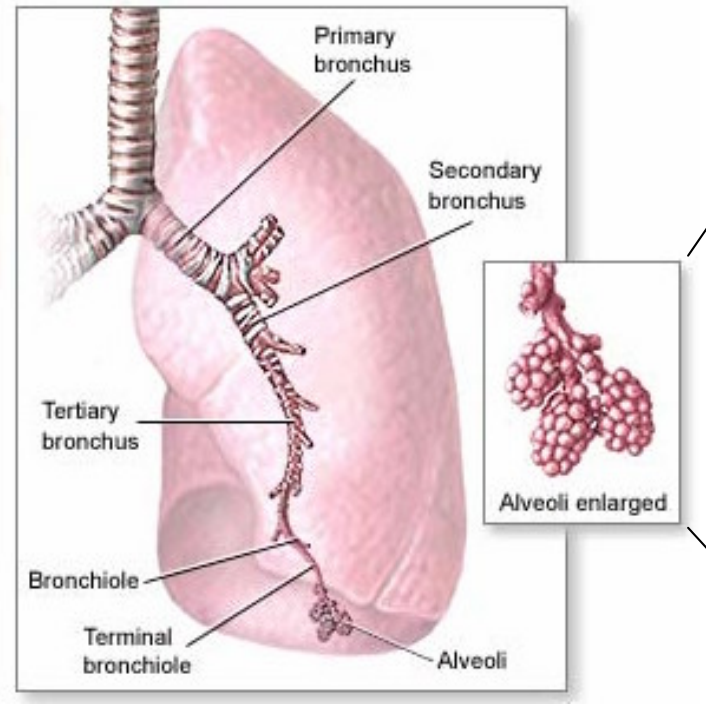
Treatment of newborn rats with a VEGF receptor inhibitor

(barium angiograms)

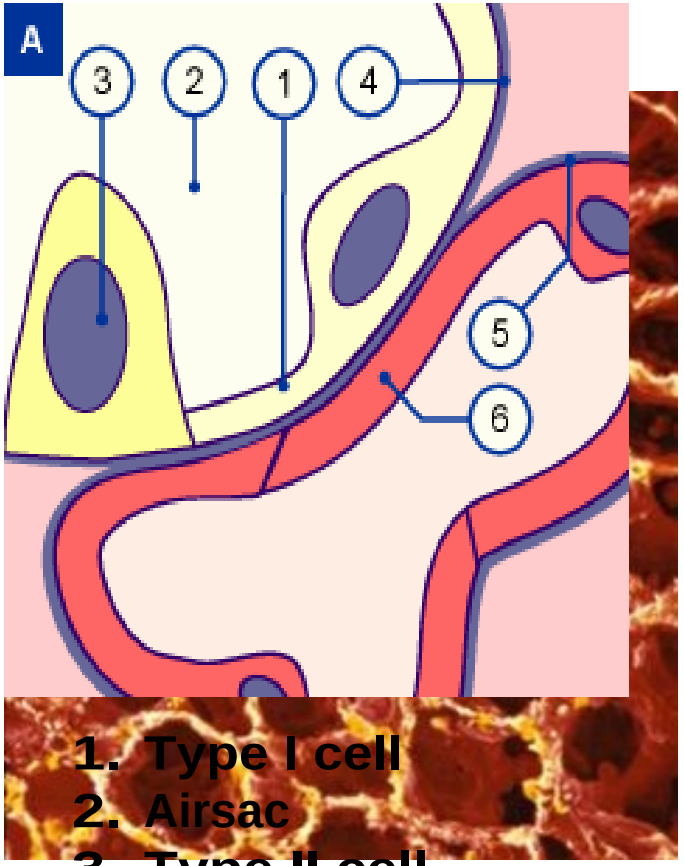


Le Cras, T. D. et al. Am J Physiol Lung Cell Mol Physiol 283: L555-L562 2002

Alveolar Morphogenesis



Blood–air barrier



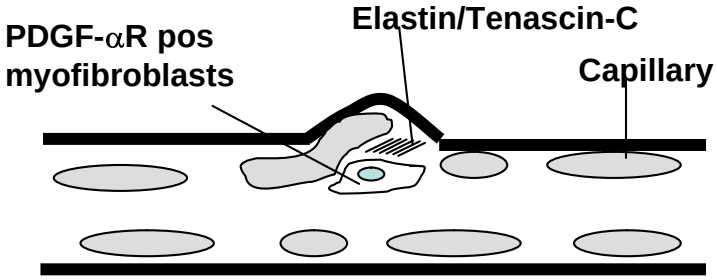
1. **Type I cell**
2. **Airsac**
3. **Type II cell**
4. **Basal lamina of airsac**
5. **Basal lamina of vessel**
6. **Endothelium of the vessel**

Alveolarization

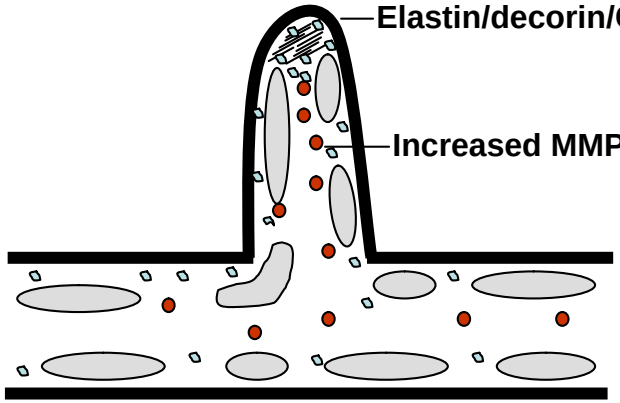
Processes involved

1. Cell growth
2. Septation
3. Microvascularization
4. Apoptosis

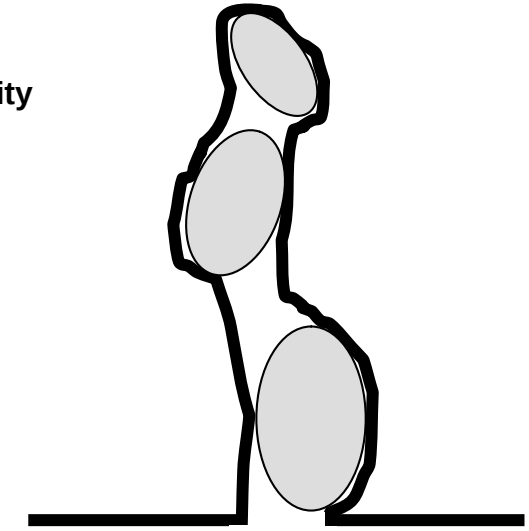
Alveolar septal formation (simplified)



I. Elastin deposition in primary septa



II. New secondary septa with double capillary layer



III. Microvascularization with fusion of capillary layer in single medial layer and thinning of interstitium

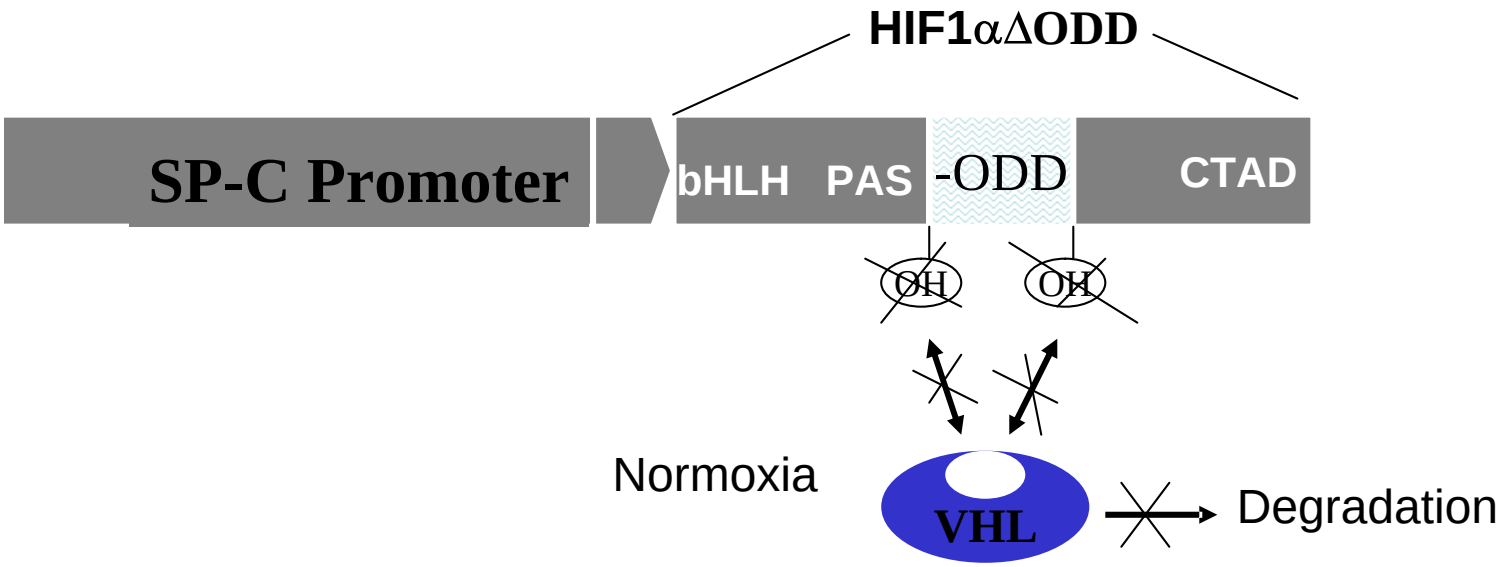
Alveolarization

Processes involved

1. Cell growth
2. Septation
3. Microvascularization
4. Apoptosis

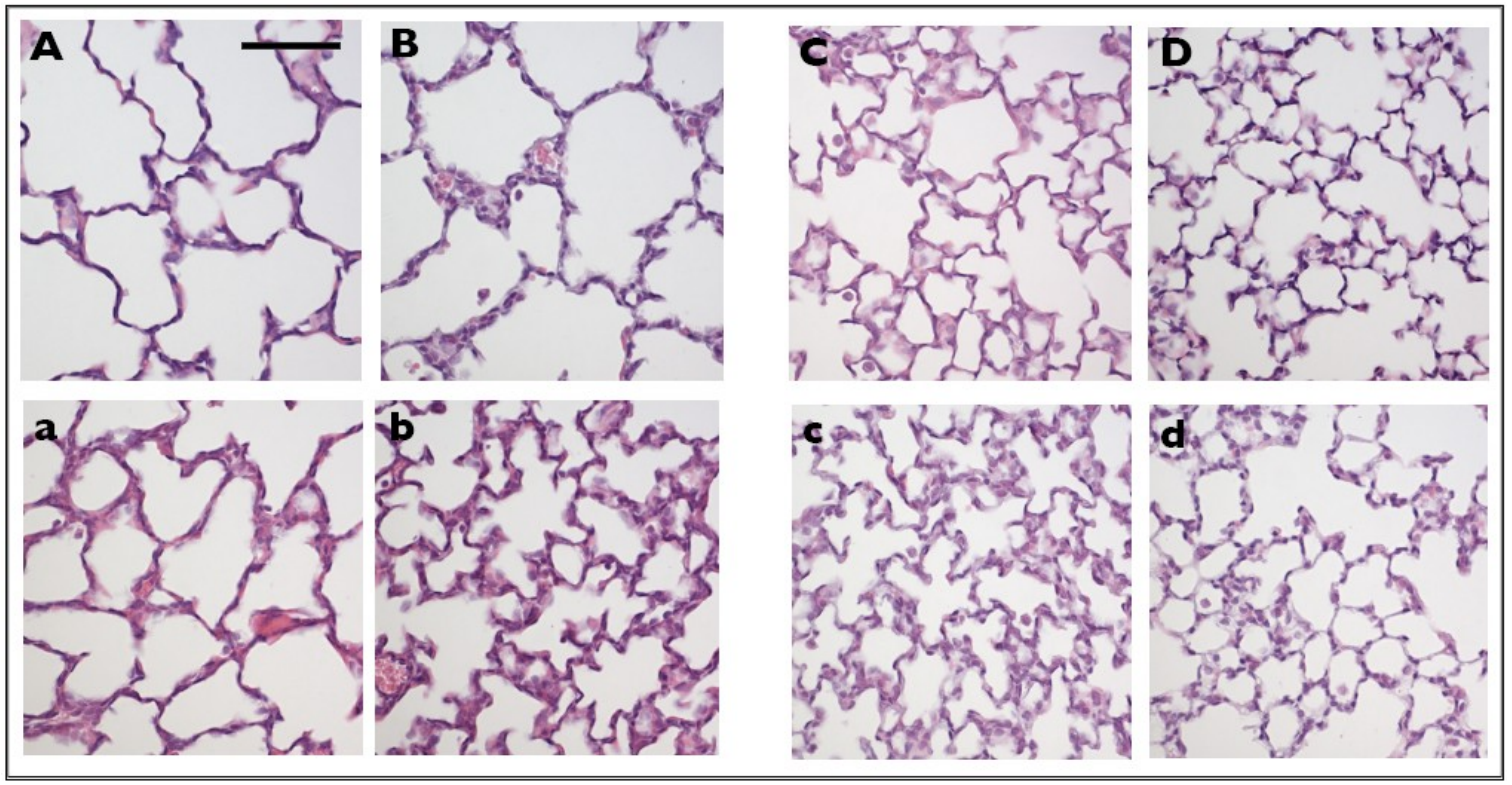
Question ?

Epithelial Overexpression of Oxygen Insensitive HIF-1α

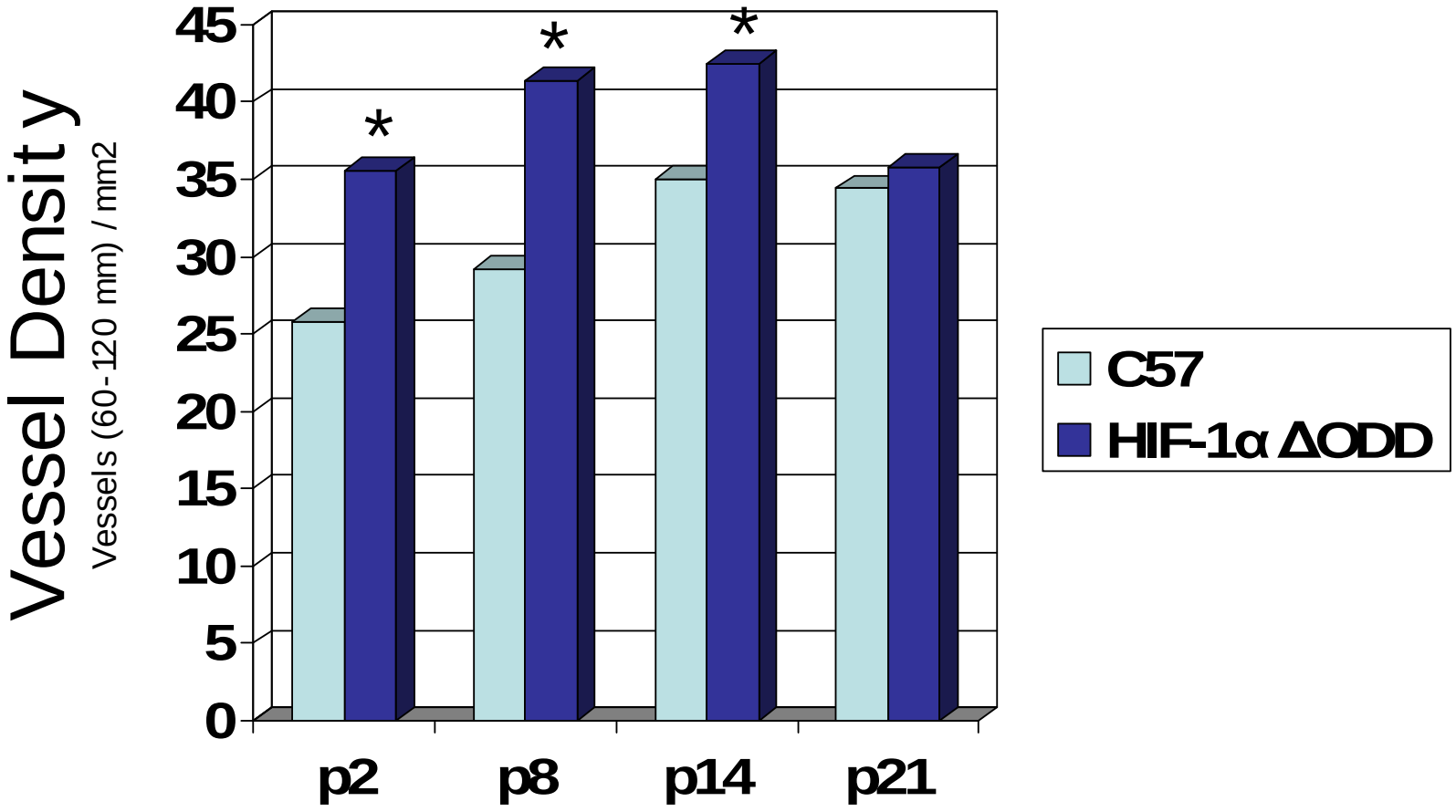


Does overexpression postnatally affect VEGF expression, vessel formation and alveolar formation?

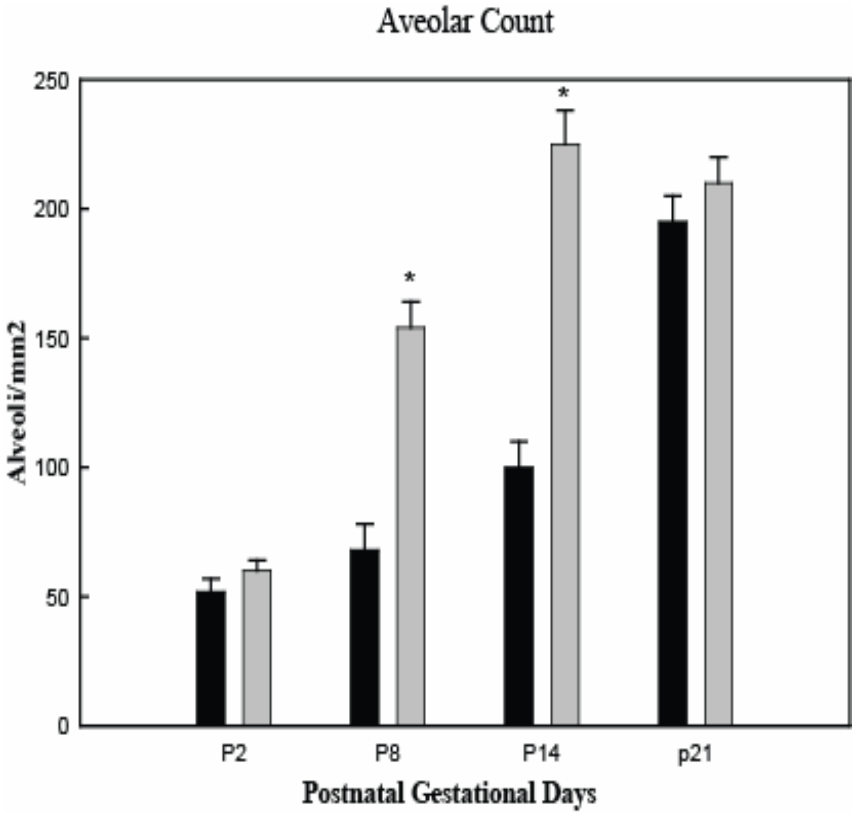
Postnatal Lung histology of C57 wild-type pups and HIF-1α ΔODD transgenic pups

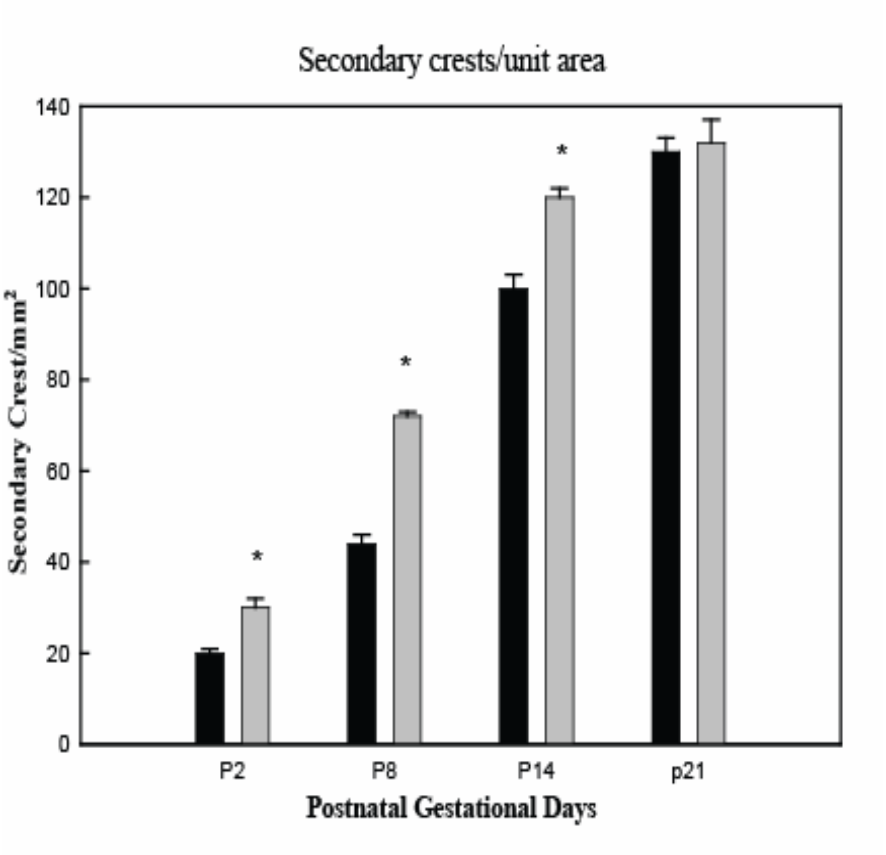


Overexpression of HIF-1α ΔODD Increases Postnatal Peripheral Vessel Number



Overexpression of HIF-1α ΔODD Increases Postnatal Septal Formation and Alveolar Number





FASEB J. 2006 Jun 28; [Epub ahead of print]

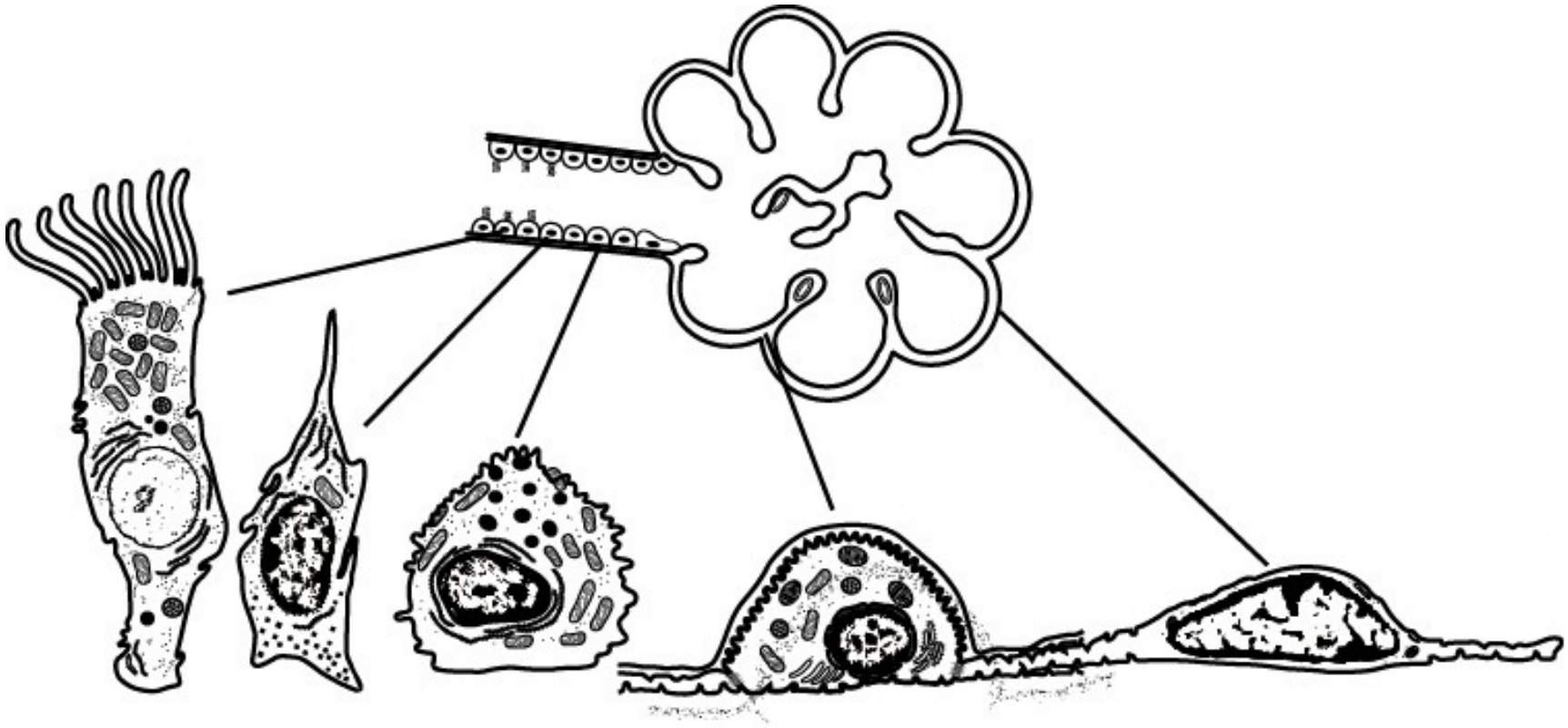
Improved lung growth and function through hypoxia-inducible factor in primate chronic lung disease of prematurity

Tiina M. Asikainen, Ling-Yi Chang, Jacqueline J. Coalson, Barbara K. Schneider, Nahid S. Waleh, Machiko Ikegami, John M. Shannon, Vicki T. Winter, Peter Grubb, Ronald I. Clyman, Bradley A. Yoder, James D. Crapo, and Carl W. White

Bronchopulmonary dysplasia (BPD), a chronic lung disease affecting preterm neonates, is associated with significant childhood and adult health problems. Histopathologic features of BPD include impaired vascular and distal airway development. We previously showed that activation of hypoxia-inducible factors (HIFs) by inhibition of prolyl hydroxylase domain-containing proteins (PHDs) is feasible and that it stimulates vascular endothelial growth factor (VEGF) -dependent angiogenesis in vitro. We tested the hypothesis that enhancement of angiogenesis by activation of HIFs improves lung growth and function in prematurely born neonates in vivo. Preterm baboons (125 day+14 day pro re nata O2 model, corresponding to 27 human gestational weeks) were treated for 14 days with intravenous (i.v.) FG-4095, a PHD inhibitor. Notably, 77% of diminished total alveolar surface area in untreated controls was recovered by FG-4095 treatment. Functional significance of the structural changes was indicated by improved oxygenation and lung compliance in FG-4095-treated newborns. Surfactant proteins B and C and saturated phosphatidylcholine were unchanged. Incidence of spontaneous ductus arteriosus closure was increased, likely contributing to lower ratio of pulmonary to systemic blood flow in FG-4095 group. These findings indicate that HIF stimulation by PHD inhibition ameliorates pathological and physiological consequences of BPD

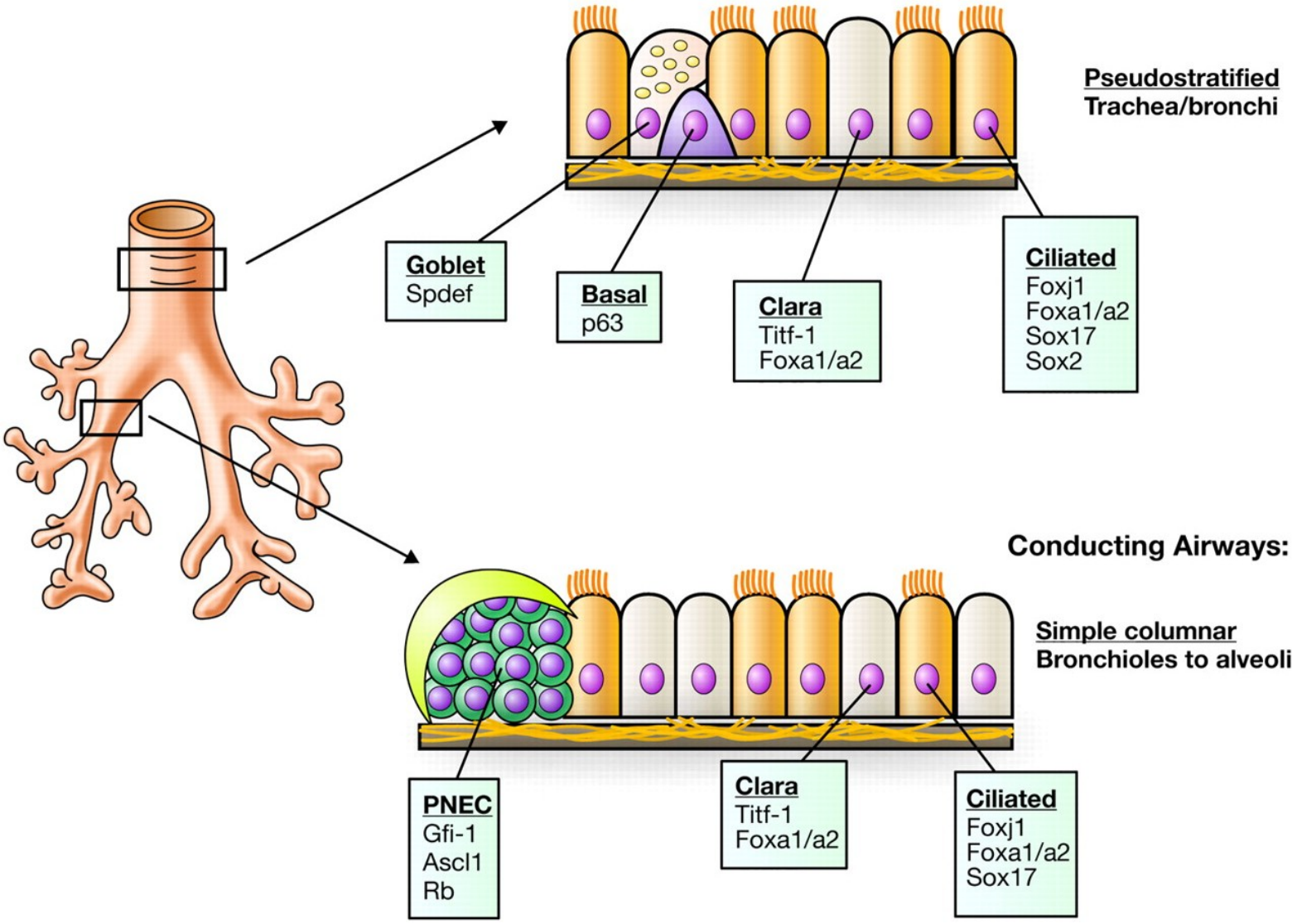
Epithelial Differentiation

Question: Which pathway(s) regulate(s) epithelial cell patterning along the anterior-posterior axis in the lung?



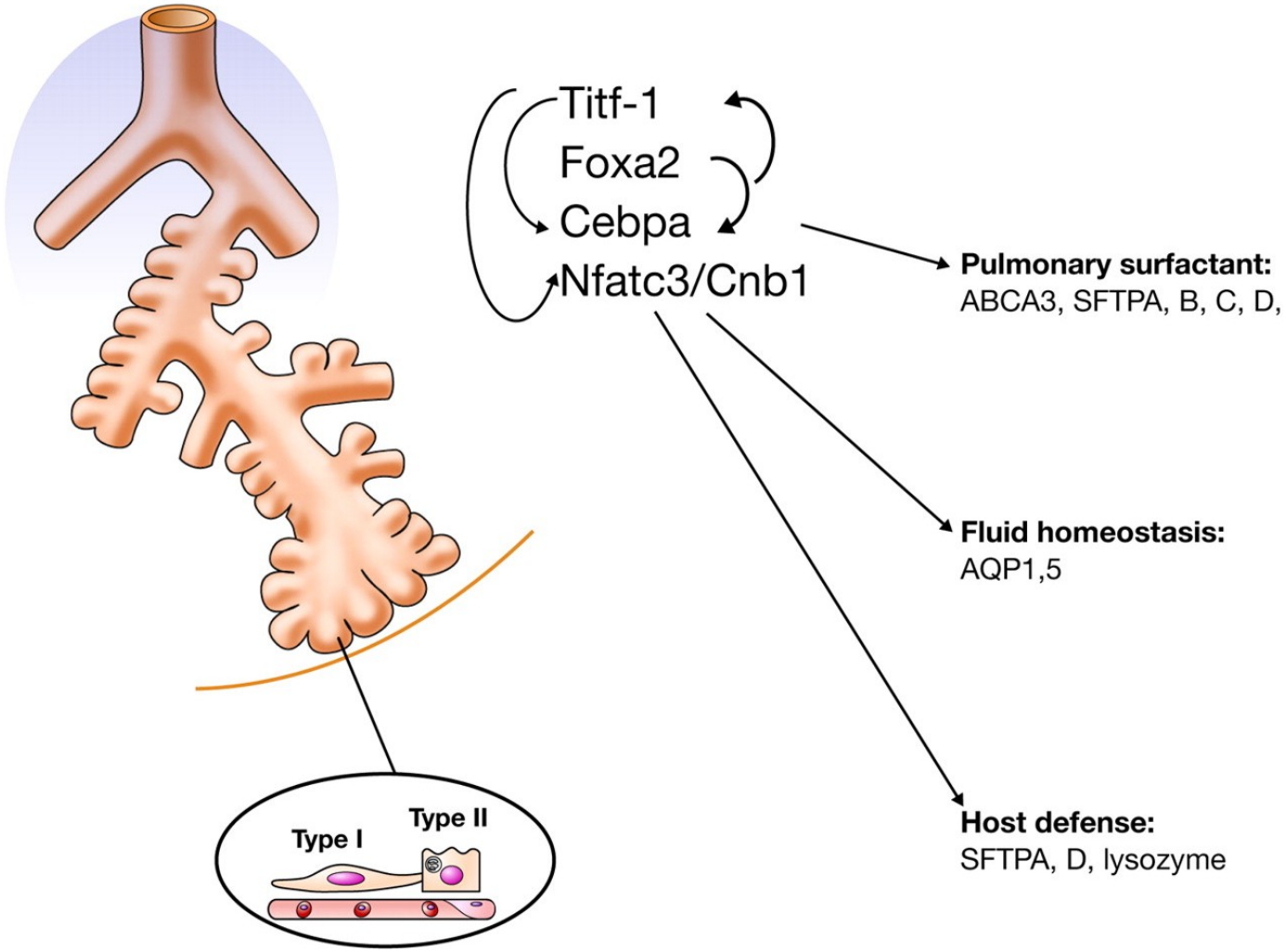
Ciliated Cell PNEC Clara Cell Type II cell Type I cell

Selective expression of transcription factors in the respiratory epithelium



Maeda, Y. et al. Physiol. Rev. 2007

TTF-1, FOXA2, NFATC3, and C/EBPα participate in a network regulating perinatal lung maturation and adaptation to airbreathing at birth



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Overall Conclusion

Formation of the lung is dependent on a myriad of interactions of signaling and receiving molecules controlling proliferation and differentiation

Remaining Question: How are the different pathways integrated and coordinated at the cellular and molecular level and can we built a lung in vitro?

Contributors

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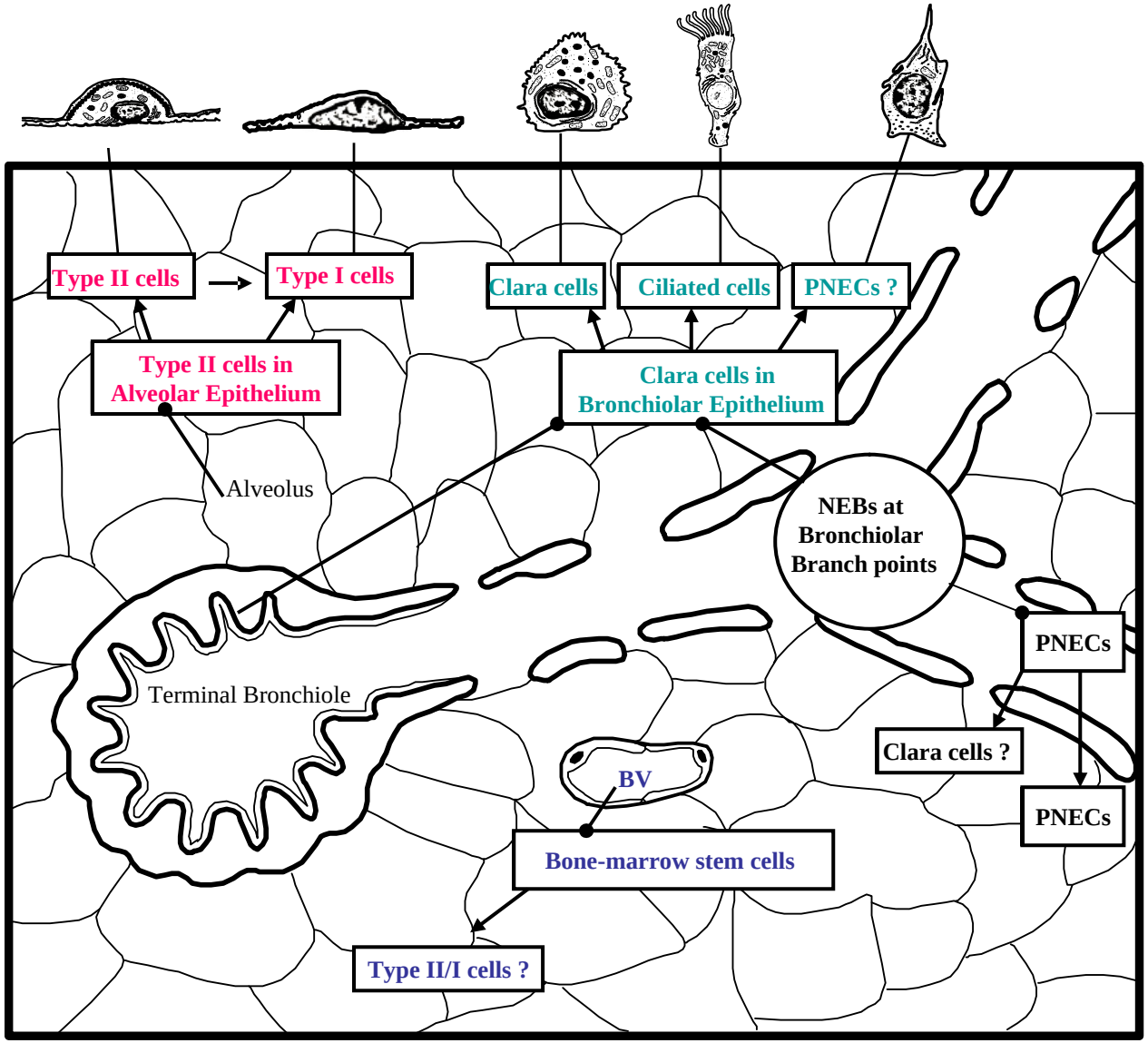
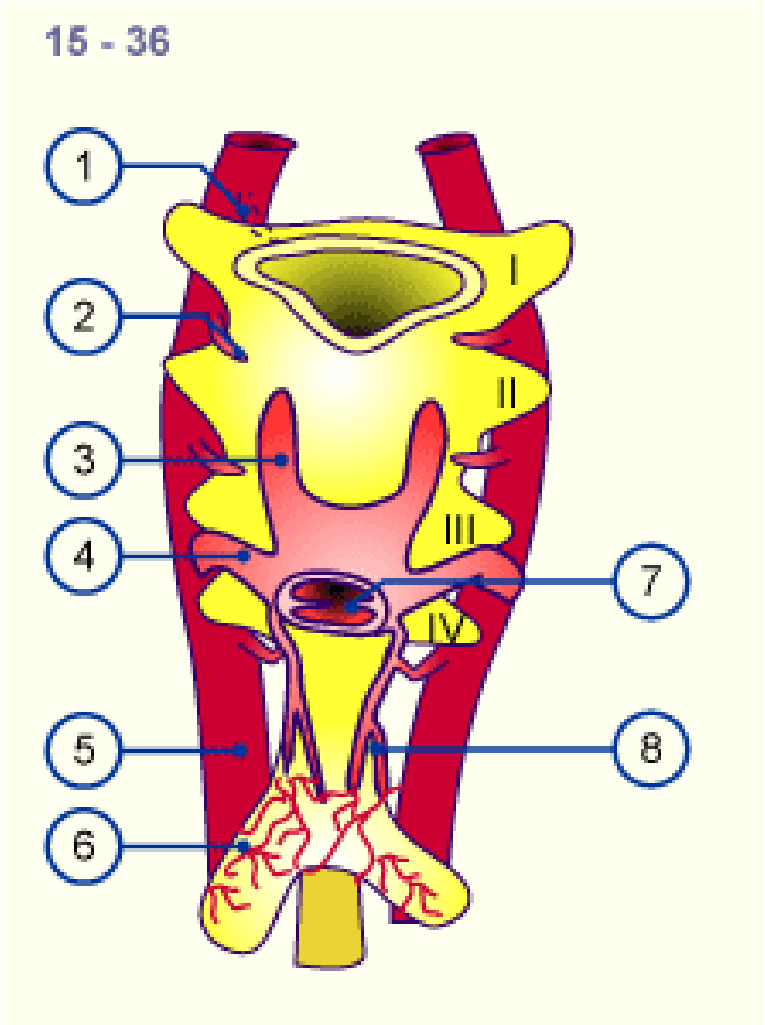
CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Lung Tissue Stem Cells in Lung Regeneration

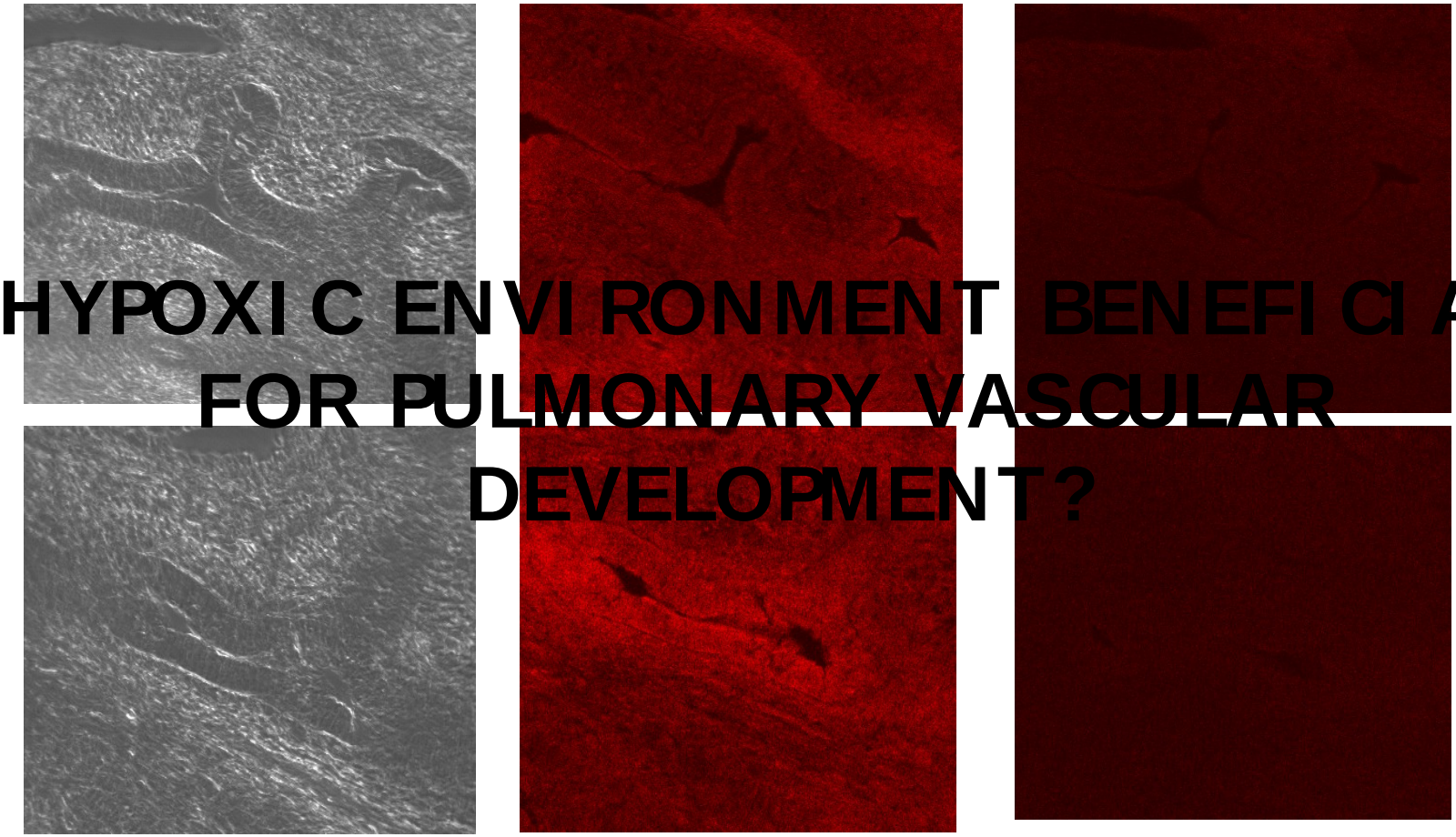


Development of the pulmonary vessels (2)

Also from the dorsal aorta a vessel plexus forms that communicates with the ventral one and thus creates a connection between the ventral aortic sac and the dorsal aorta (36 days).



1. First aortic arch
2. Second aortic arch
3. Third aortic arch
4. Fourth aortic arch
5. Dorsal aorta
6. Lung buds
7. Aortic sac
8. Pulmonary plexus



HYPOXIC ENVIRONMENT BENEFICIAL FOR PULMONARY VASCULAR DEVELOPMENT?

Pregnant mice were injected at E9.5 with nitroimidazole hypoxia marker EF5 and analyzed at E10.5. Developing lung structures were visualized by EF5 antibody and red colour is indicative of an hypoxic environment.