Respiratory embryology

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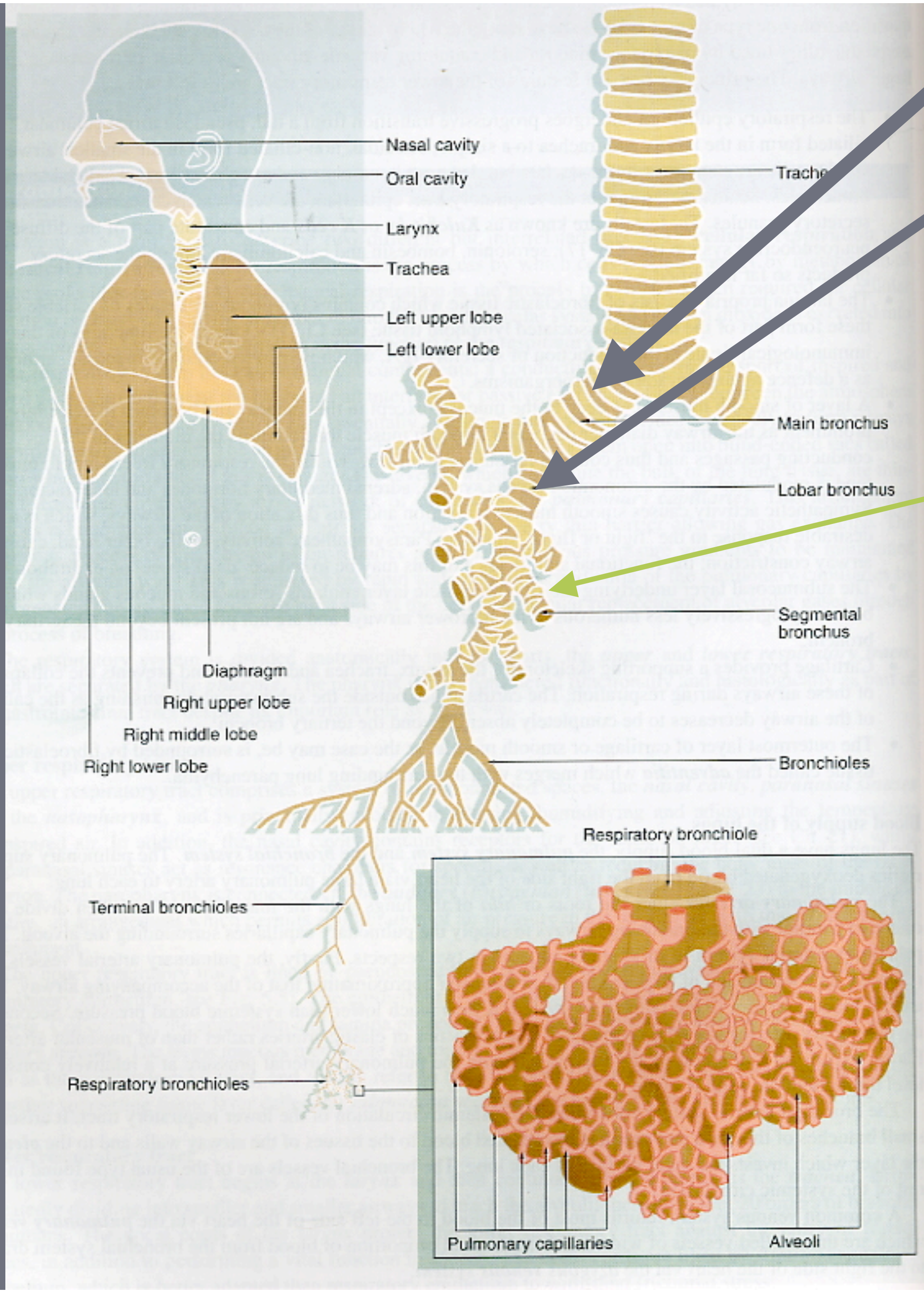
Lung histology

- Cast of Characters
  - Airways
    - Conducting
    - Respiratory
  - Vessels
    - Arteries, arterioles - pulmonary and bronchial
    - Capillaries
    - Veins/Venules and Lymphatics
  - Pleura- visceral and parietal



- Main stem bronchus
- Lobar bronchus (5 lung lobes)
- Segmental bronchus (10 bronchopulmonary segments on right, 9 on left
- Branching continues as airways become bronchioles, then at terminal bronchioles airways transition into respiratory bronchioles
- About 20 branch generations from beginning to end

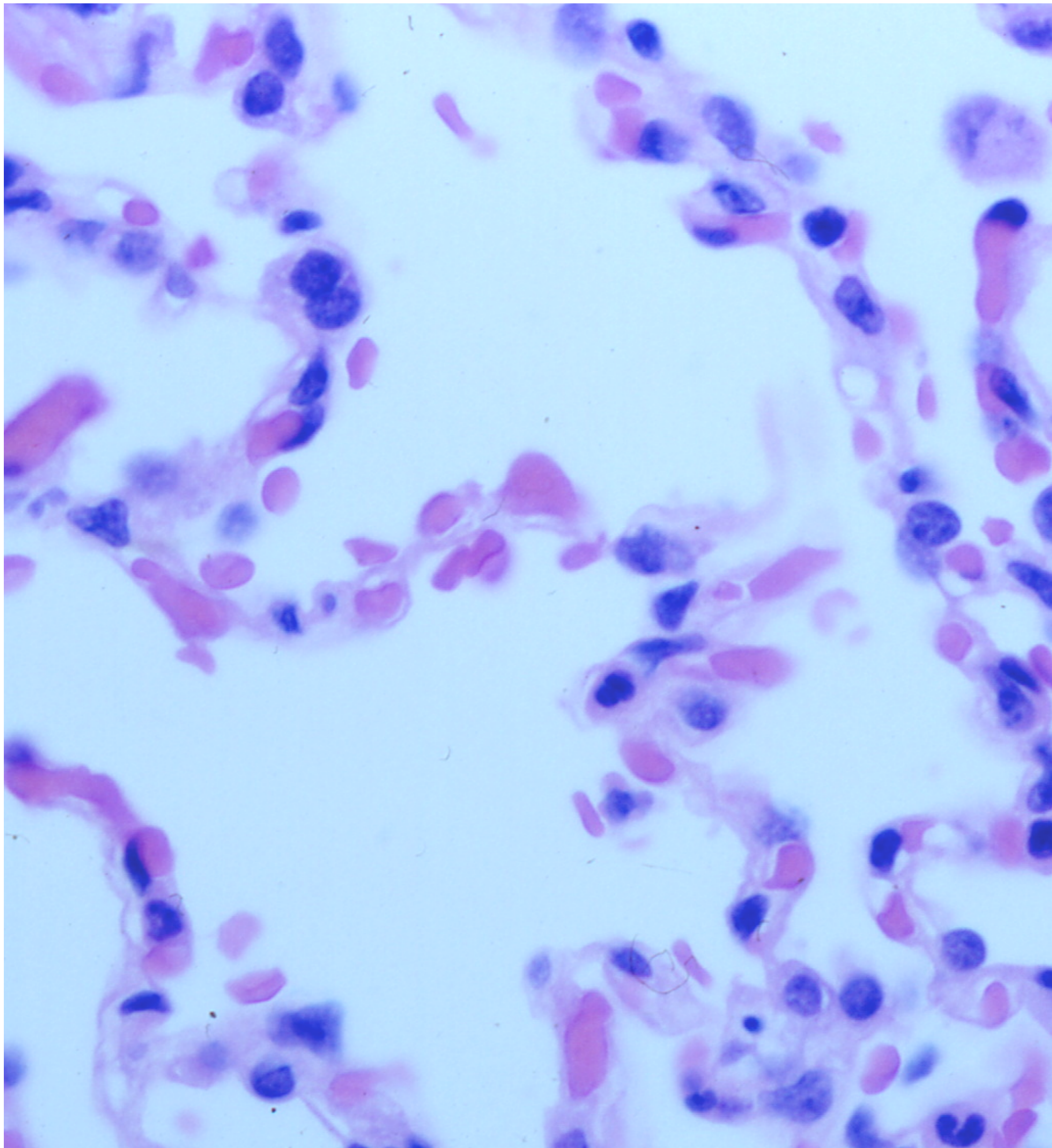
Lung Histology: Conducting zone

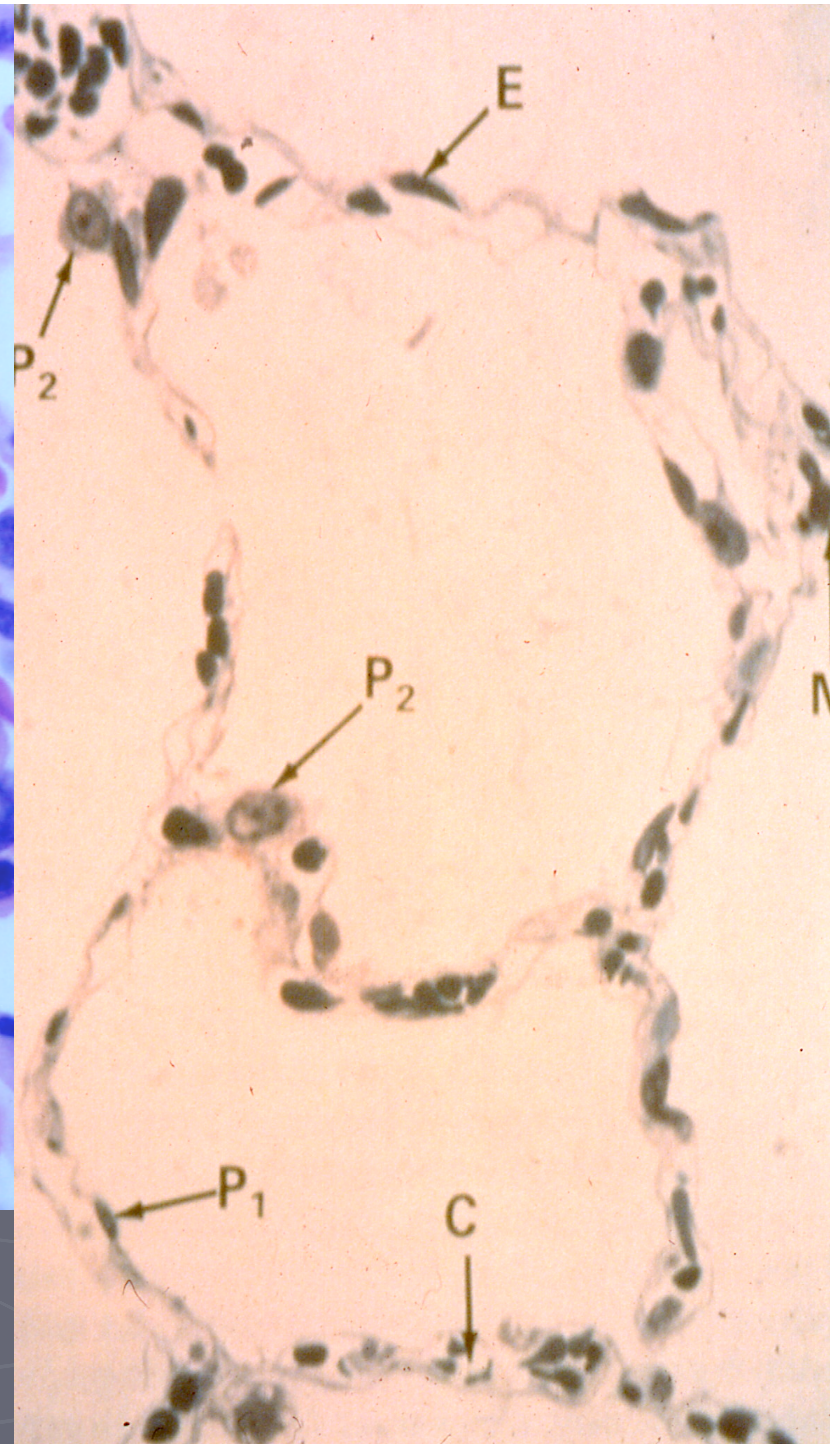
- Airways Conducting Zone
  - Trachea
  - Bronchi - ciliated and goblet cells, elastic tissue, smooth muscle, glands, cartilage
  - Bronchioles - (1 mm) - No cartilage or bronchial glands, ciliated lining,no goblet cells, smooth muscle

- Cell types
  - **CILIATED CELL** - beating of cilia contribute to mucociliary elevator
  - **GOBLET CELL** - Mucus secretion
  - **BASAL CELL** - reserve cell
  - **KULCHITSKY CELL** - neuroendocrine cells.

Pulmonary Histology

- Airways Respiratory Zone
  - Terminal bronchiole to Respiratory bronchiole - lined by ciliated cells and **CLARA CELLS; by transitional zone to RB, all Clara cells.**
  - Alveolar ducts/sacs
    - **Type I cells** 90% of alveolar surface
    - **Type II cells**
- Cell types
  - **CLARA CELLS** - produce a component of surfactant and are the bronchiolar reserve cell
  - **TYPE I CELLS** - Thin lining cell for gas exchange
  - **TYPE II CELLS** - surfactant and alveolar reserve cell





E
P_2
P_2
P_1
C

Laryngeal development

- Week 4
  - Respiratory primordium arises from distal/caudal pharynx
    - Laryngo-tracheal groove
  - Endodermal derivative of epithelium of larynx trachea and bronchi
  - Connective tissue, smooth muscle and cartilage from splanchnic mesenchyme surrounding the foregut

Laryngeal development

- LT groove evaginates and forms LT diverticulum
- This becomes invested with splanchnic mesoderm to form lung bud
- This maintains a laryngeal inlet

The septum that forms by folds and fusion keeps a septate inlet that becomes trachea and esophagus

Epithelium of the larynx

- Endoderm of proximal/cranial end of LT tube and cartilage from neural crest origin
- Formation of proximal larynx – cranial tube
  - Arytenoid swellings grow towards tongue
  - Airway gets closed off, eventually recanalizes
- Laryngeal webs – Incomplete recanalization
- Laryngeal atresia – ascites, hydrops and lungs do not properly form.

Trachea

- Endoderm of distal LT tube
  - Epithelium of trachea and lung
- Splanchnic mesenchyme
  - Connective tissue
- 4th week
  - If esopahgeal separation from LT tube is incomplete, develops into TE fistula

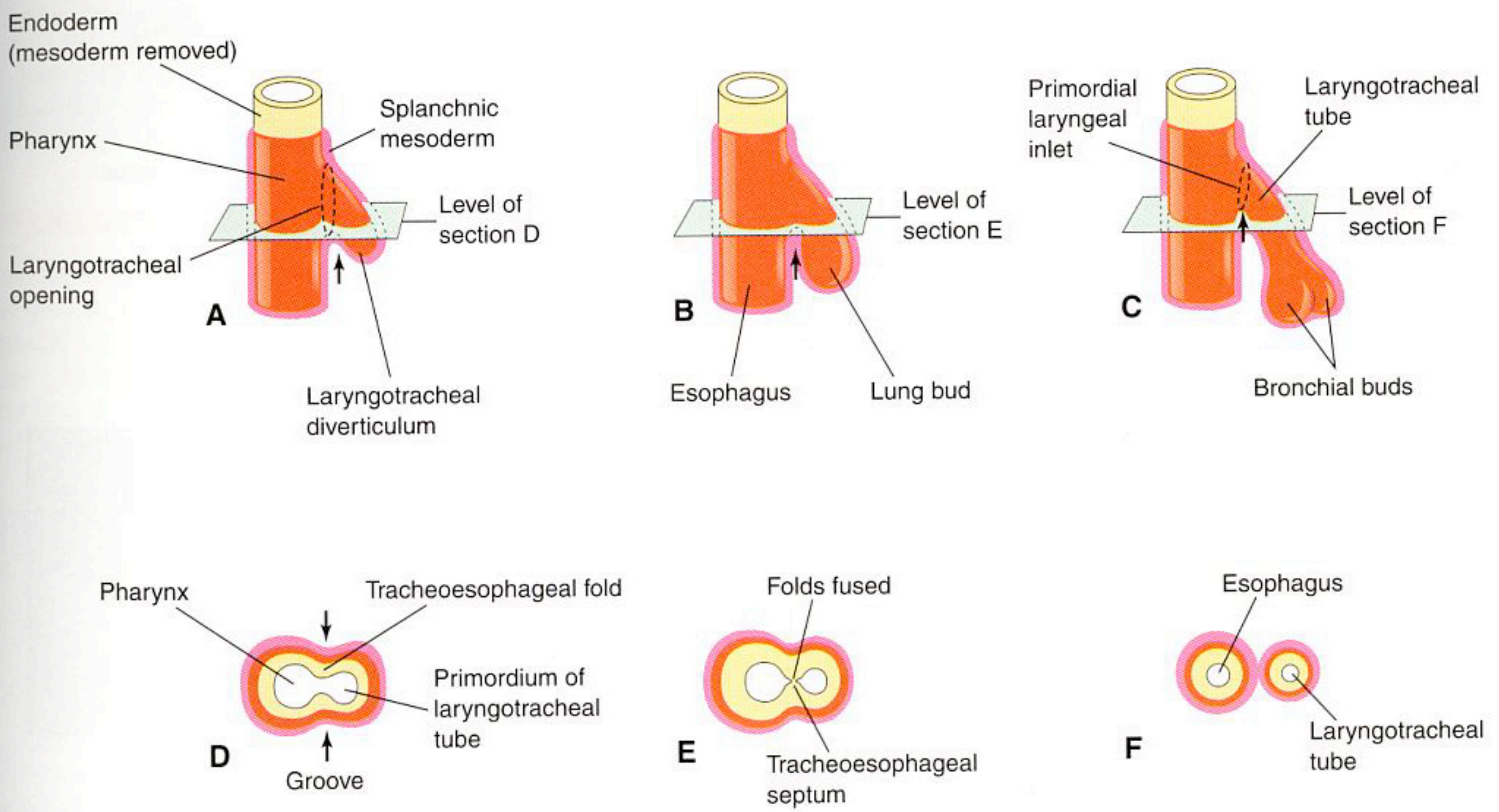


Figure 11–2. Drawings illustrating successive stages in the development of the tracheoesophageal septum during the fourth and fifth weeks. *A*, *B*, and *C*, Lateral views of the caudal part of the primordial pharynx showing the laryngotracheal diverticulum and partitioning of the foregut into the esophagus and laryngotracheal tube. *D*, *E*, and *F*, Transverse sections illustrating formation of the tracheoesophageal septum and showing how it separates the foregut into the laryngotracheal tube and esophagus.

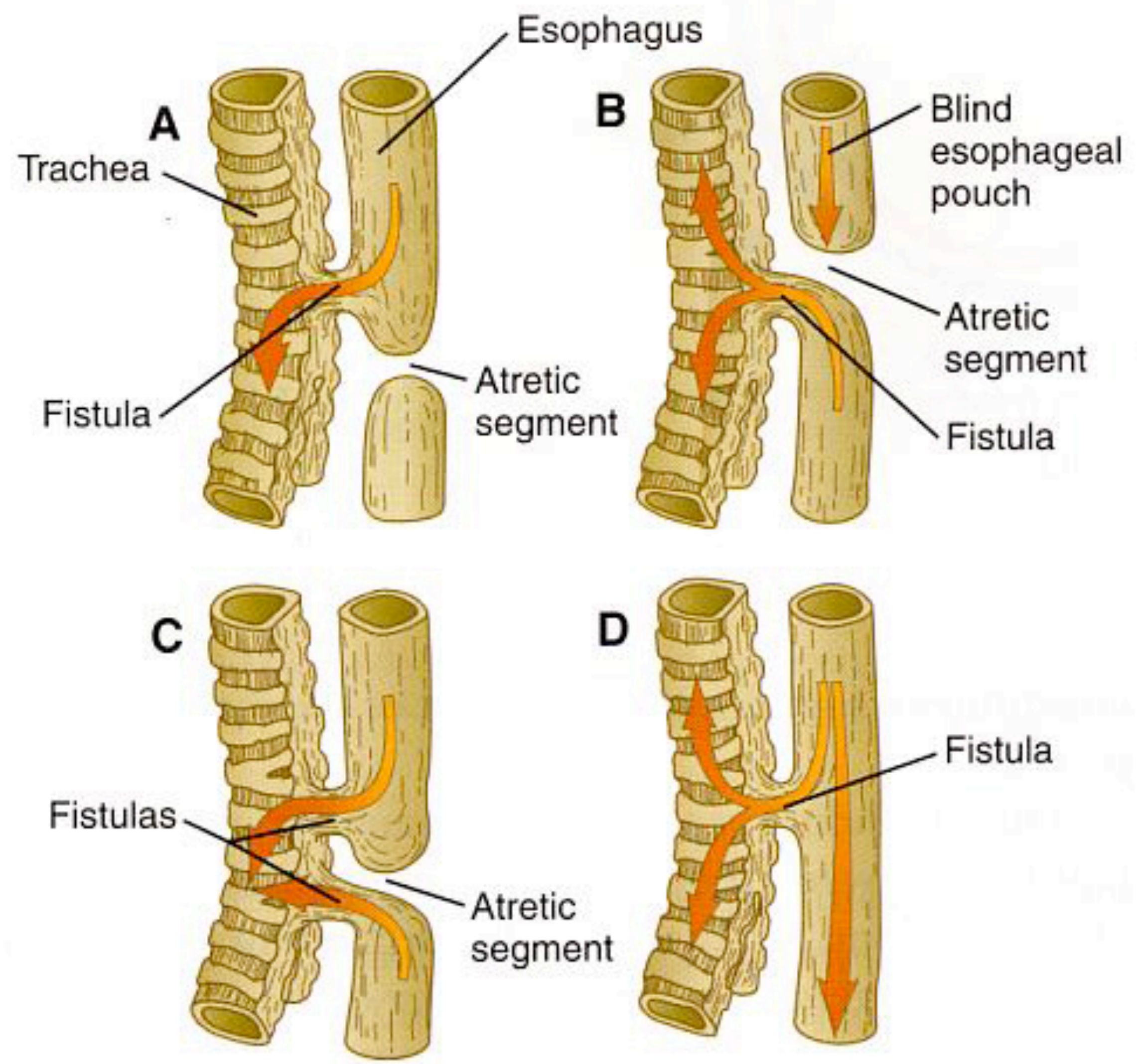
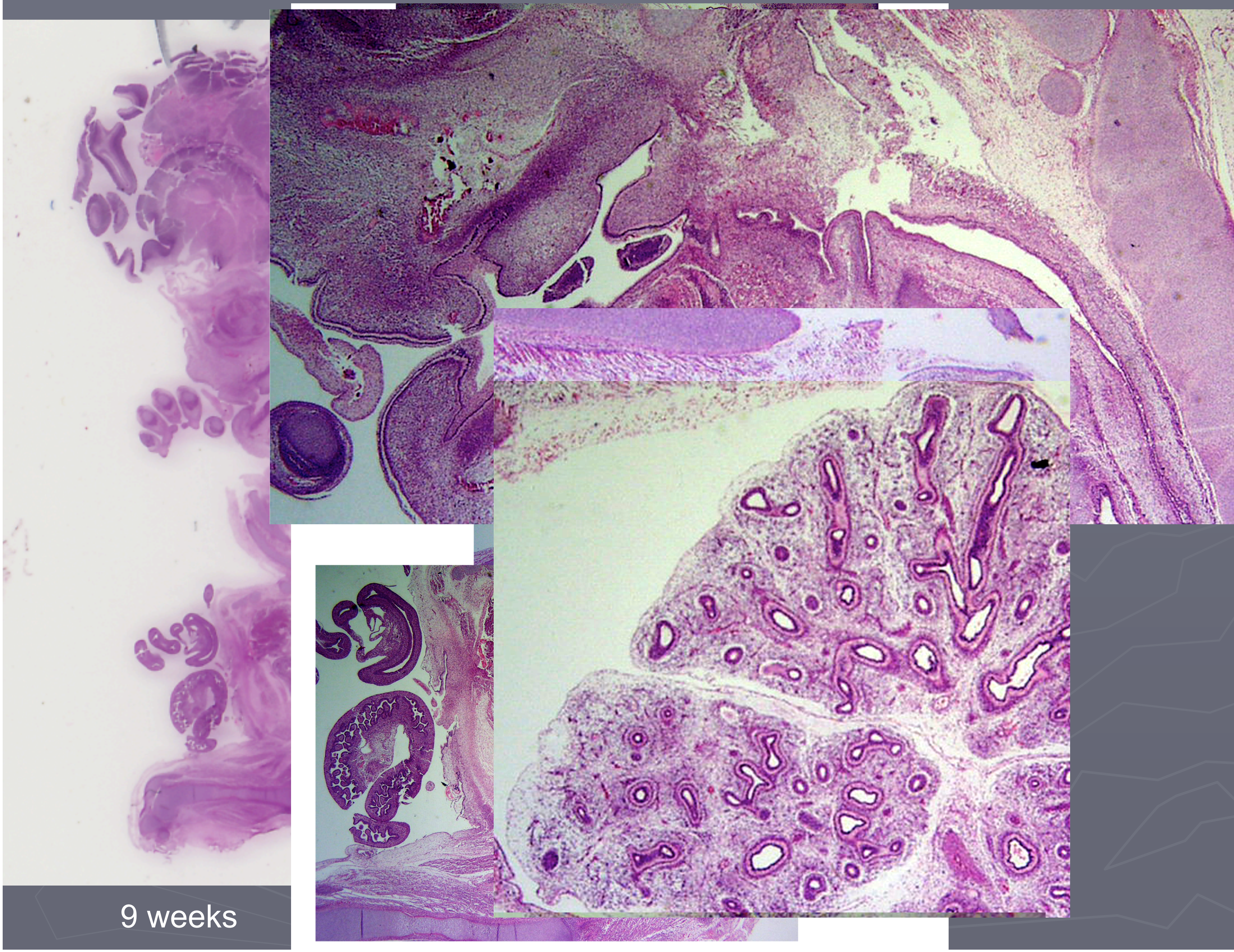


FIGURE 15-28 Varieties of tracheoesophageal fistulas. **A,** Fistula above the atretic esophageal segment. **B,** Fistula below the atretic esophageal segment. **C,** Fistulas above and below the atretic esophageal segment. **D,** Fistula between the patent esophagus and the trachea.

- Mouse models - Mutants in SHH and TTF1 (Nkx2.1) and Gli 2/3 (downstream from SHH) develop absence of TE septation
- Less well understood in humans
  - Aspiration of food
  - In utero, inadequate swallowing of amniotic fluid leads to polyhydramnios (excess intrauterine amniotic fluid)

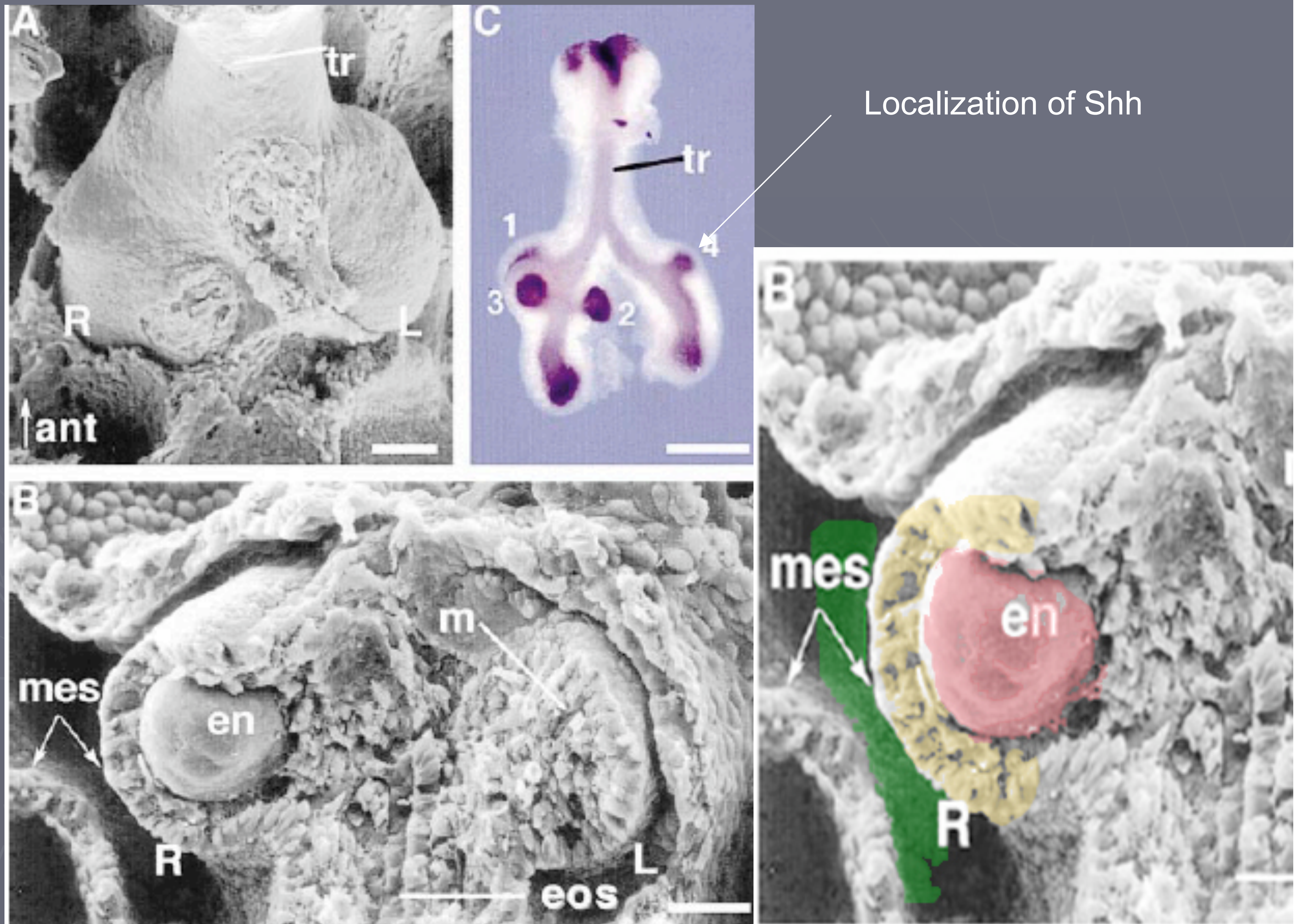


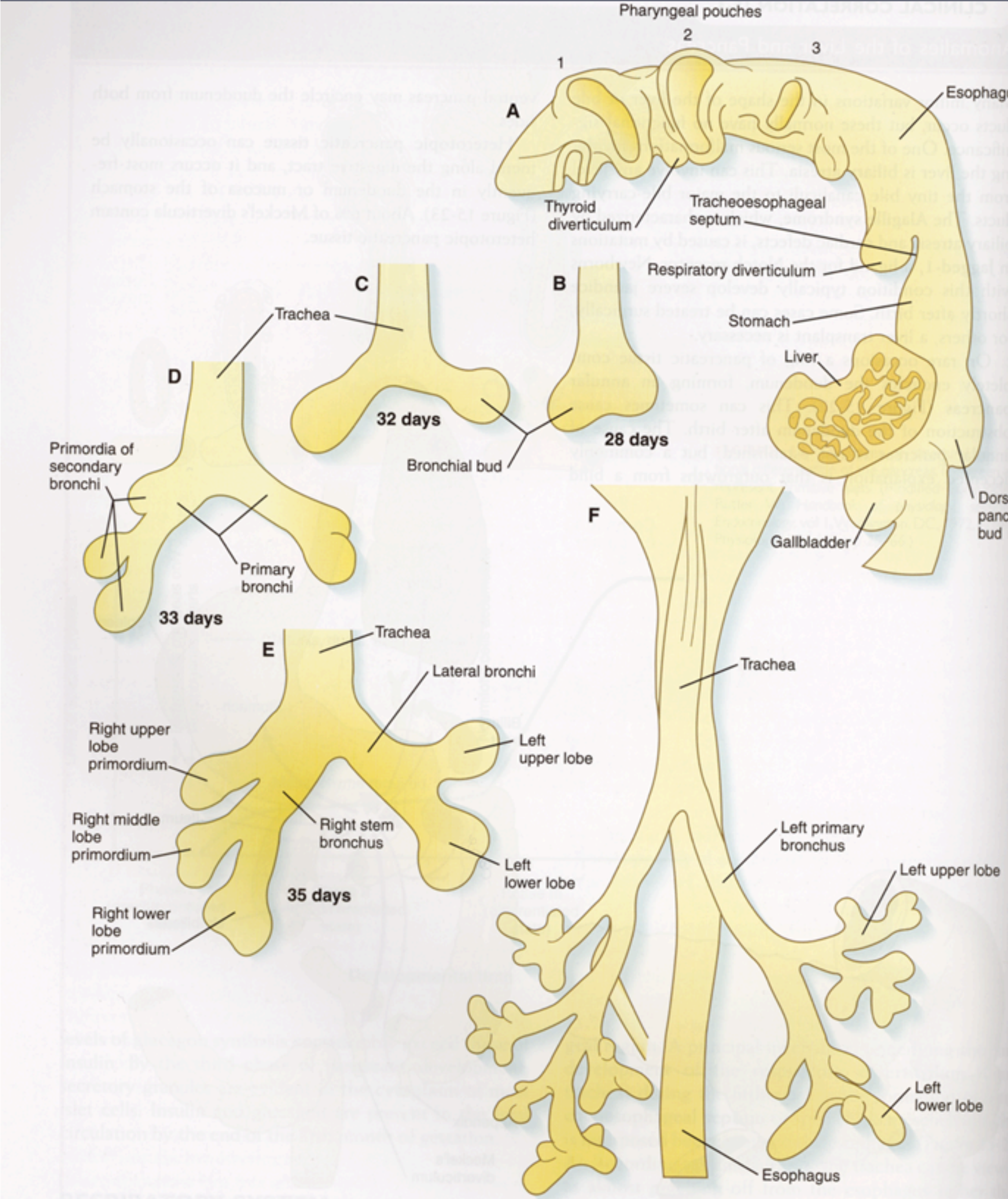
9 weeks

Bronchi/lungs

- By 28 days – endodermal buds grow along with splanchnic mesenchyme
- By 35 days – Second degree bronchi, upper middle and lower on right, upper and lower on left
- By 42 days – Tertiary bronchopulmonary segments, 10 on the right and 8-9 on the left.

Localization of Shh

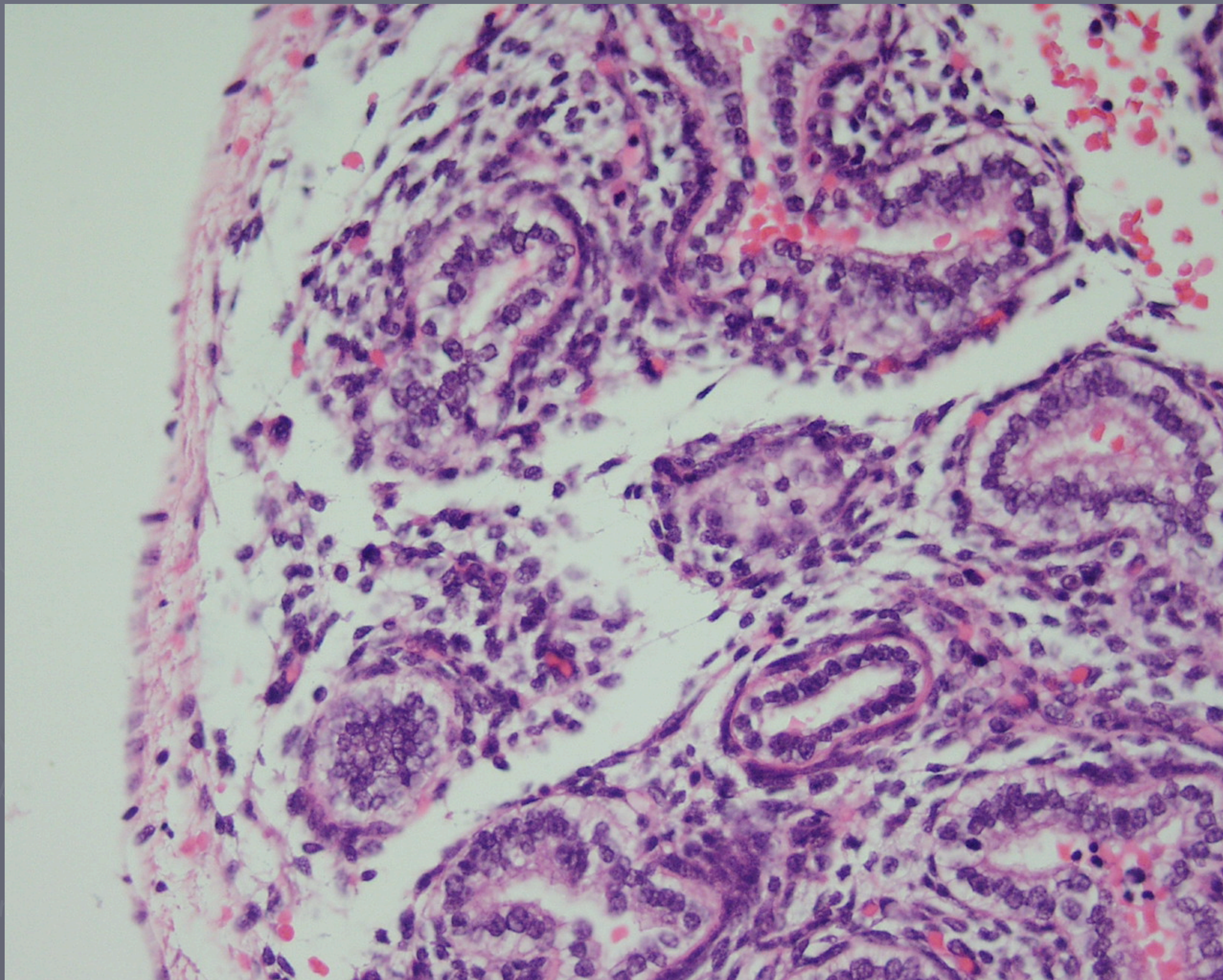




Pharyngeal pouches
1
2
3
A
Esophagus
Thyroid diverticulum
Tracheoesophageal septum
Respiratory diverticulum
Stomach
Liver
Dorsal pancreatic bud
Gallbladder
B
28 days
C
32 days
Trachea
Bronchial bud
D
33 days
Primordia of secondary bronchi
Primary bronchi
E
35 days
Trachea
Lateral bronchi
Right upper lobe primordium
Right middle lobe primordium
Right lower lobe primordium
Right stem bronchus
Left upper lobe
Left lower lobe
F
Trachea
Left primary bronchus
Left upper lobe
Left lower lobe
Esophagus

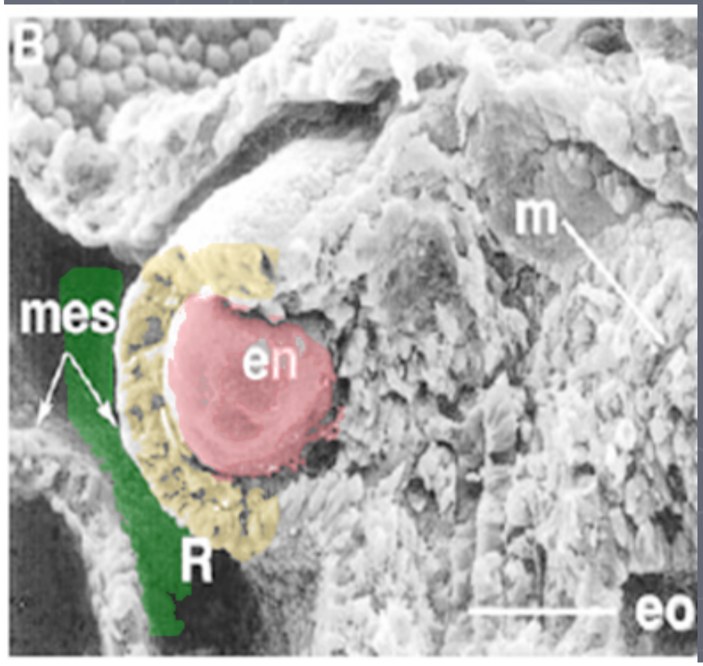
Branching morphogenesis

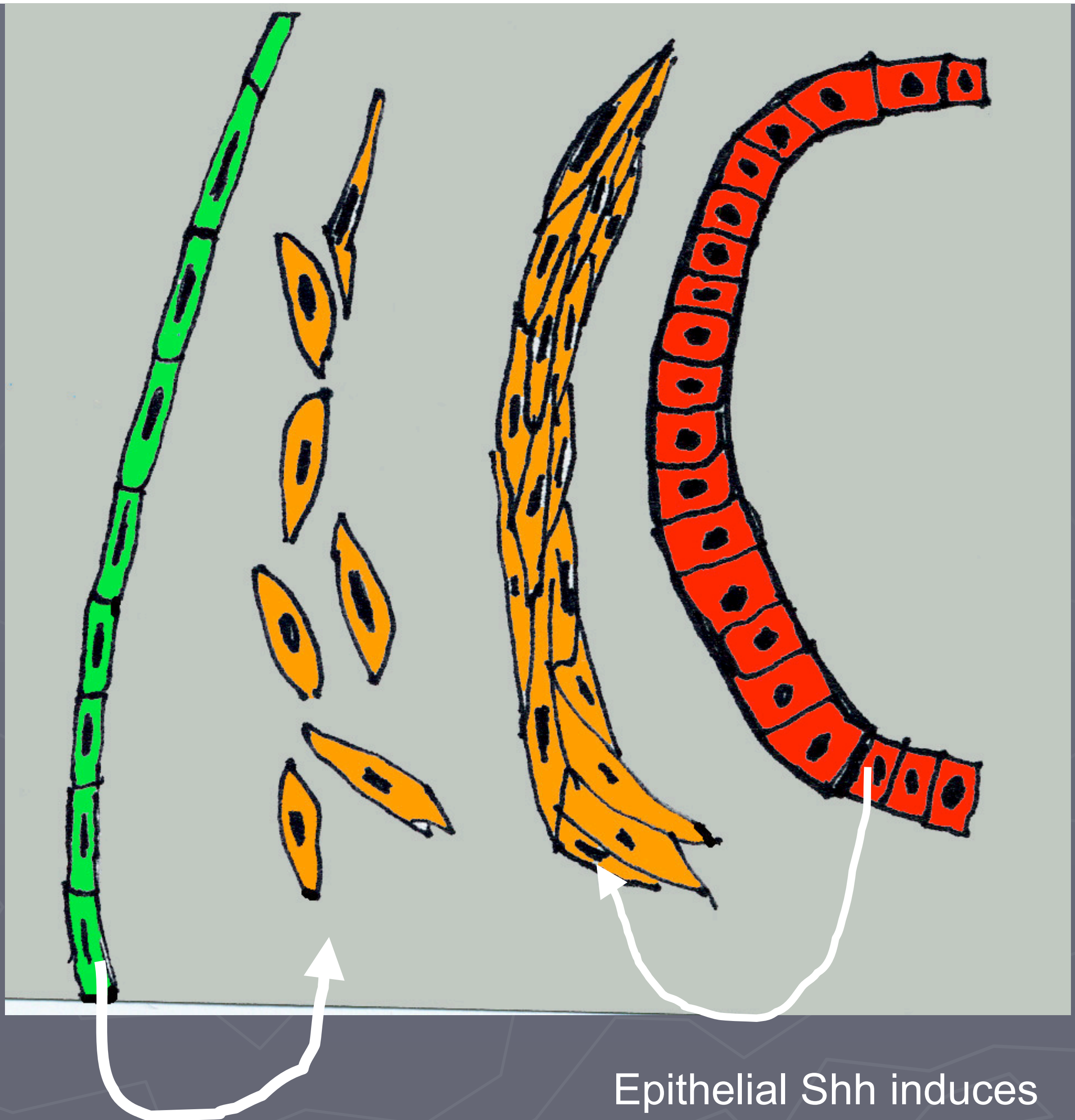
- By 24 weeks, 17 orders of bronchi and respiratory bronchioles (7 more after birth)
- Lungs grow to pleura – visceral pleura from splanchnic mesenchyme and parietal pleura from somatic mesoderm.



When is it done?

This allows for continued
Differentiation and growth
Towards mesothelium
until balanced effect
between FGF9 and Shh
is reached.





FGF9 from mesothelium
Supports mesenchymal
pluripotency

Epithelial Shh induces
Mesenchyme
proliferation
And differentiation

Table 1. Lung development (human) and regulatory factors

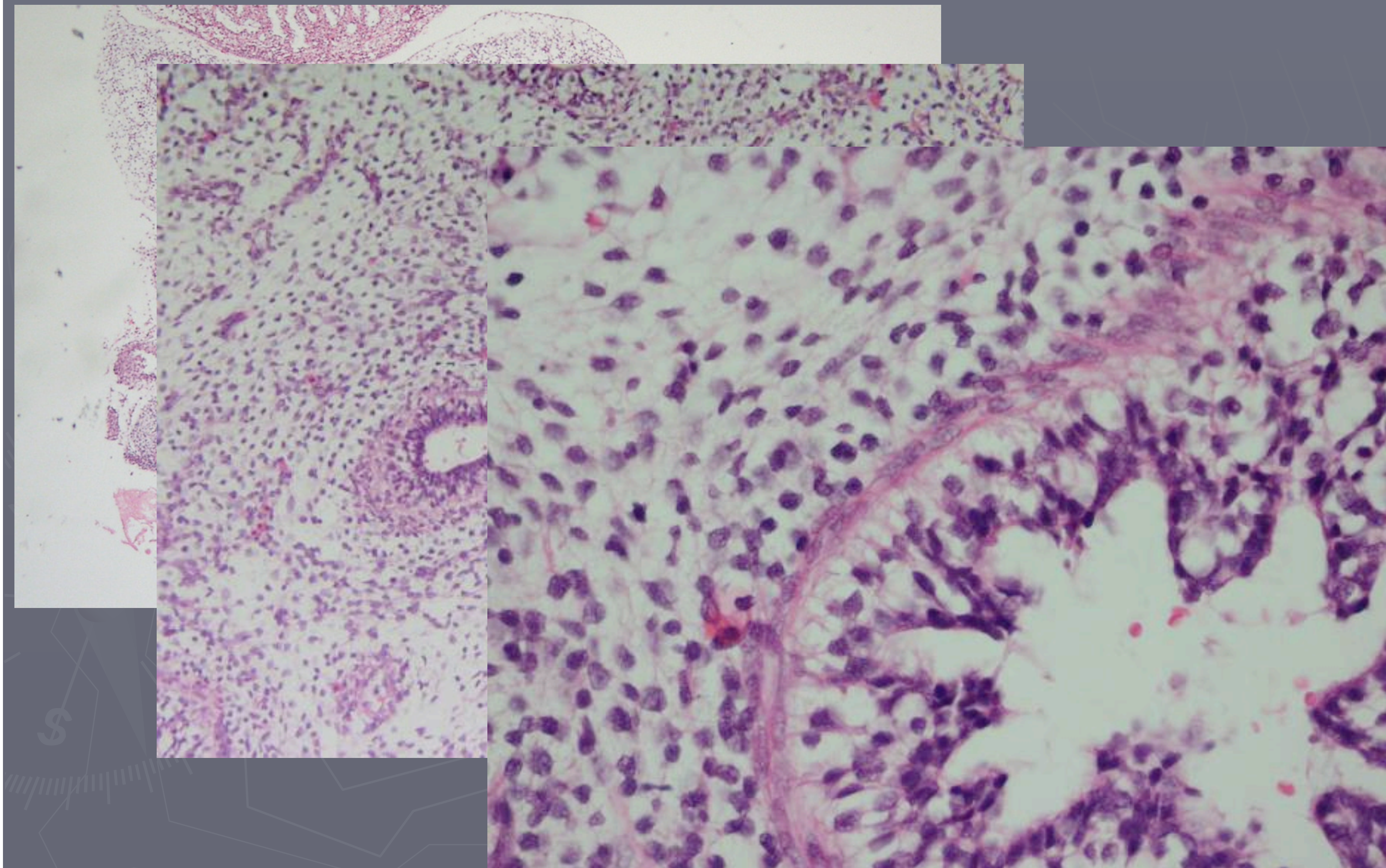
Stage	Duration	Characteristic events	Major molecular mediators
Embryonic	4–7 weeks	Outgrowth of trachea, right and left main bronchi and major airways	HNF-3β, TTF-1, RA, RAR, Shh, Ptch, Gli2, Gli3, FGF-8, FGF-10, HNF-4, N-cadherin, activin-β, activin-β-R IIA, lefty-1/2, nodal, Pitx-2
Pseudoglandular	5–17 weeks	Formation of bronchial tree up to a preacinar level	GATA-6, N-myc, PDGF, PDGF-R, EGF, EGF-R, FGF, TGF-β, Shh, Ptch, VEGF, BMP-4, RA, RAR
Canalicular	16–26 weeks	Formation of the pulmonary acinus and of the future air-blood barrier; increase of capillary bed; epithelial differentiation; first appearance of surfactant	GATA-6, TTF-1, HNF-3β, Mash-1, VEGF
Saccular	24–38 weeks	Formation of transitory air spaces	HNF-3β, TTF-1, NF1, VEGF, VEGF-R
Alveolar	36 weeks to 2 postnatal years	Alveolarization by forming of secondary septa	PDGF, PDGF-R, FGF, FGF-R, VEGF, VEGF-R, angiopoietins, ephrins, RA, RAR
Microvascular maturation	Birth to 3 postnatal years	Thinning of interalveolar walls; fusion of the capillary bilayer to a single layered network	VEGF, VEGF-R, PDGF, PDGF-R, angiopoietins, ephrins

Kleiner Roth and Post Biol Neonate, 2003

Lung Maturation

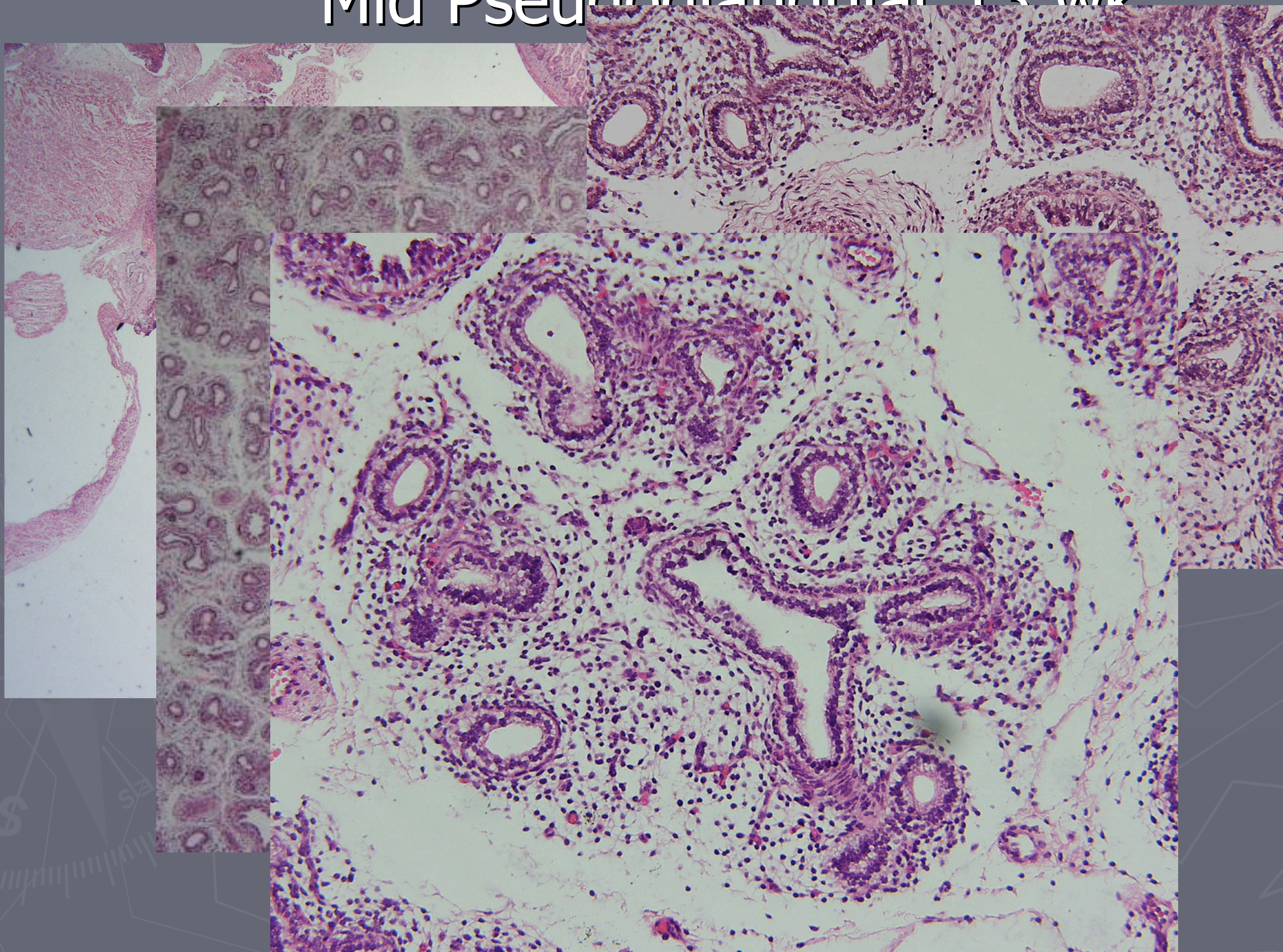
- Pseudoglandular (5-17 weeks)
  - No gas exchange zones
  - Lung resembles an exocrine gland
- Canalicular (17-25 weeks)
  - Terminal bronchioles enlarge and branch 2-3 respiratory bronchioles then 3-6 alveolar ducts. Terminal sacs begin to form
    - VAscularized – caudal slower than cranial
- Terminal sac (25 weeks to 34 weeks) – blood flow and surfactant
  - Epithelium thins to become type I like
  - Capillaries grow in
  - Blood air barrier forms
    - Type I and type II cells
      - Surfactant reduces surface tension allowing expansion.
- Alveolar period (late fetal to childhood)
  - Surfactant
  - Gas exchange
  - Pulmonary vs systemic circulation
  - Alveoli mature from age 3-8. Numbers increase from 50 million at birth and 300 million at age 8 (adult number)

Early pseudoglandular 8 wk

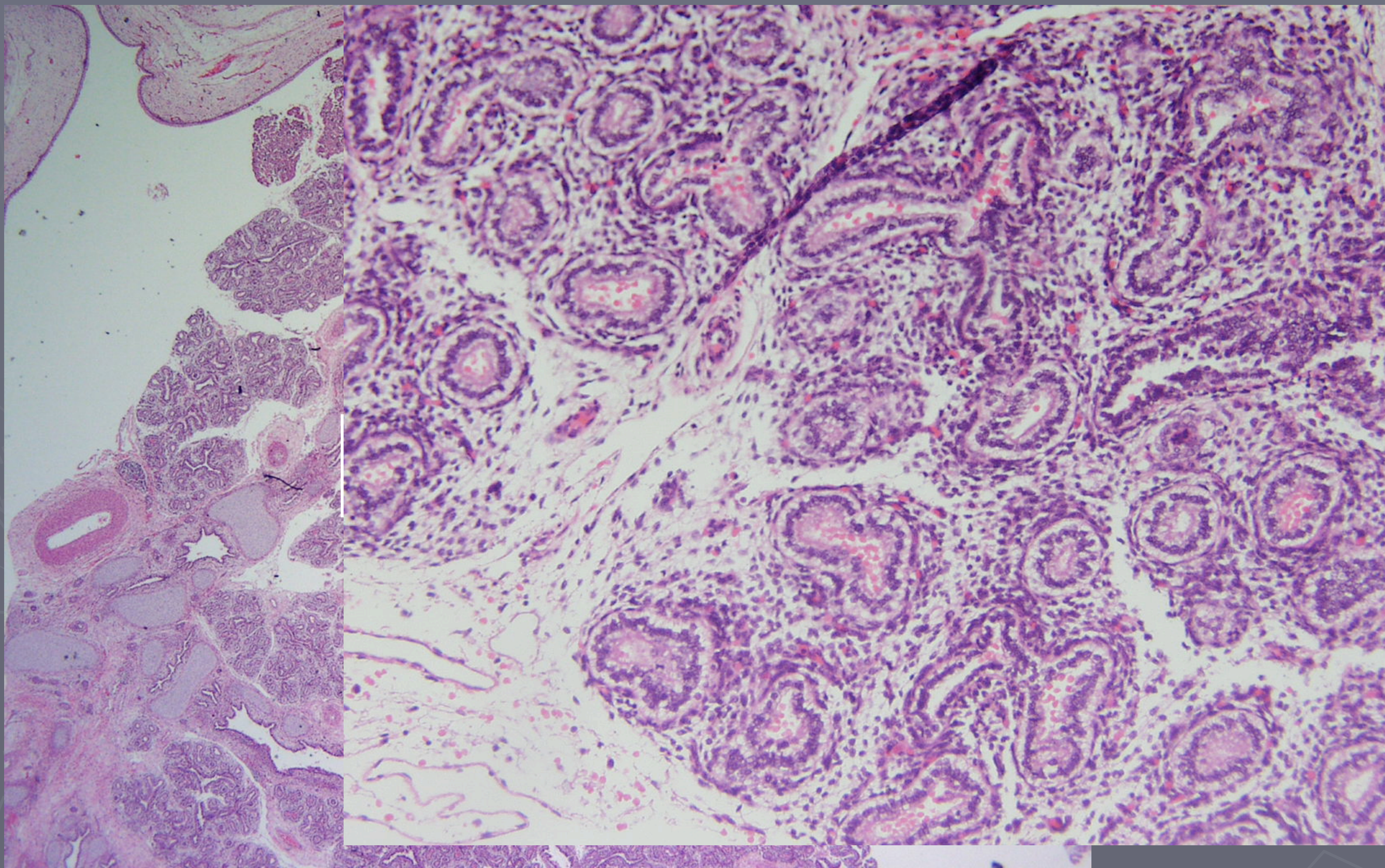


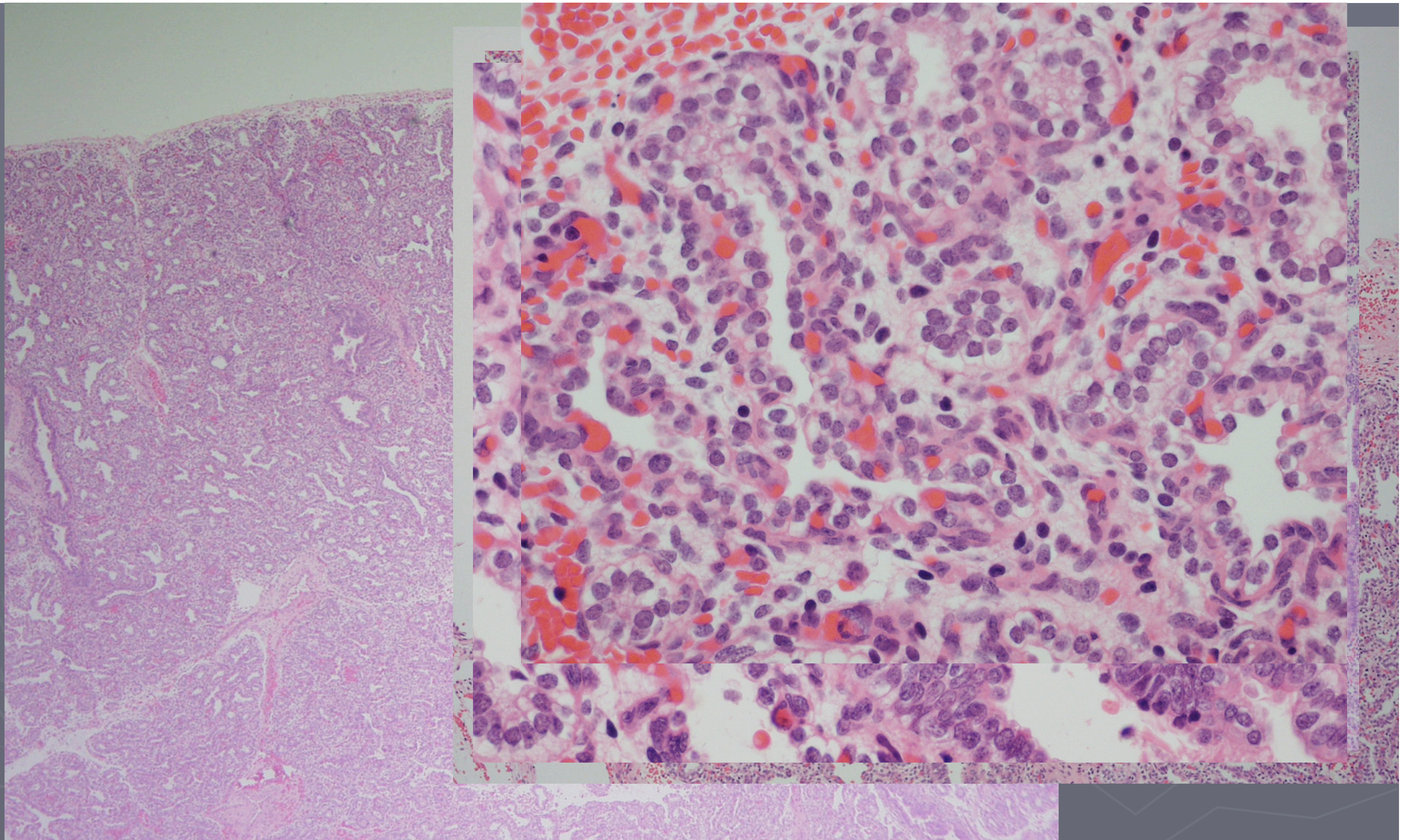
13 wk

Mid Pseudoglandular 13 wk

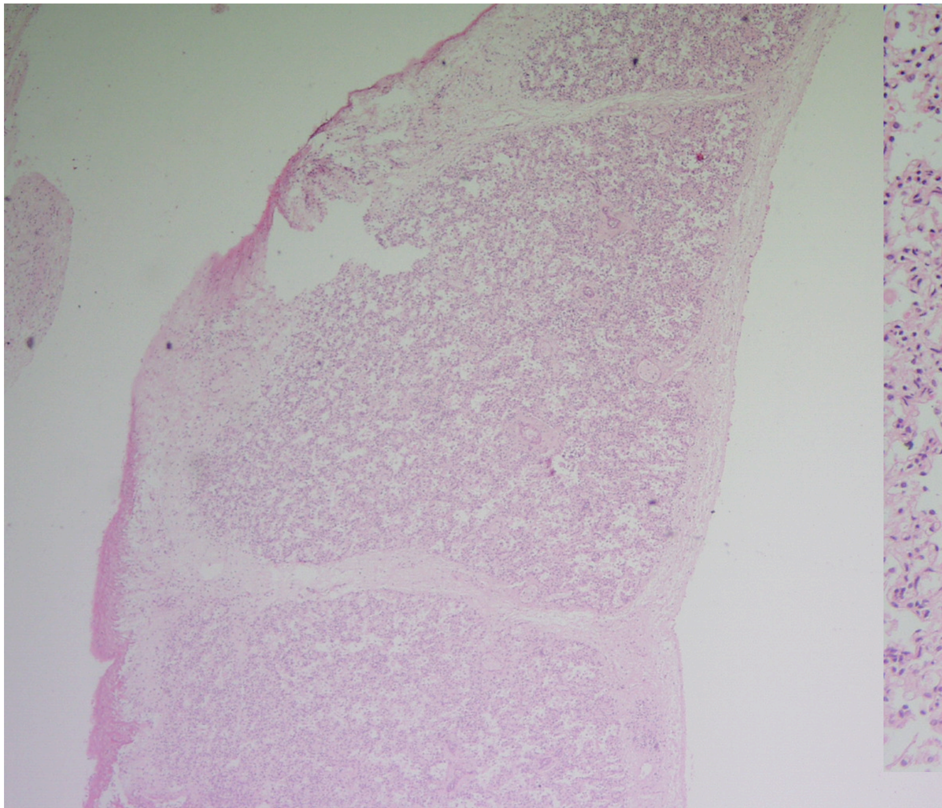


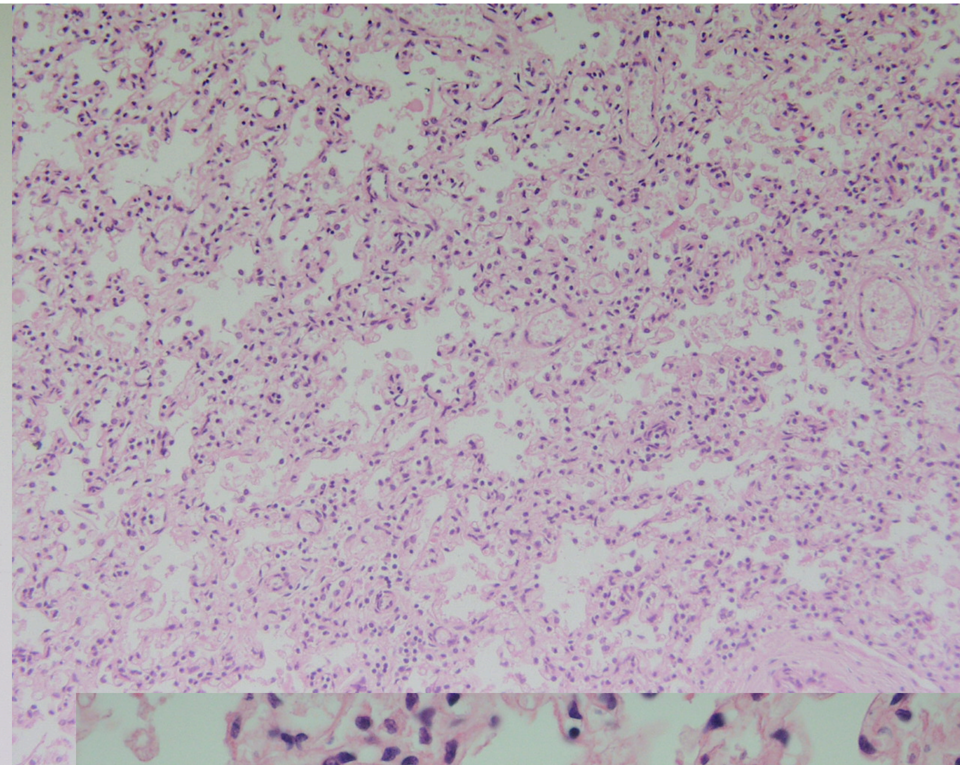
Late pseudoglandular-16 weeks

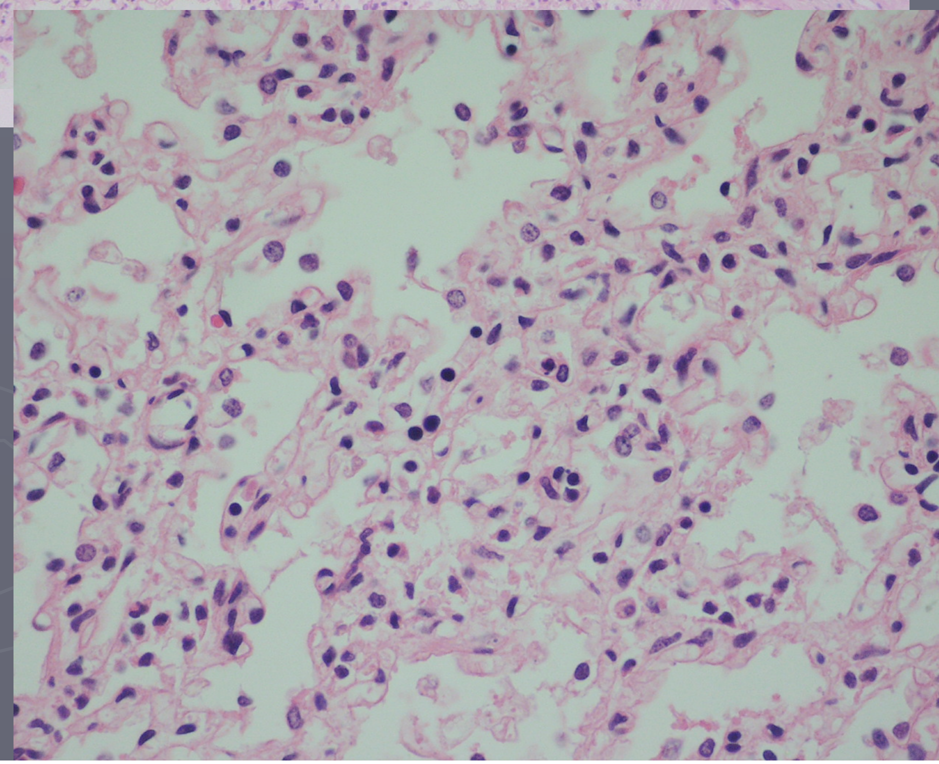




Mid-canalicular - 22 weeks

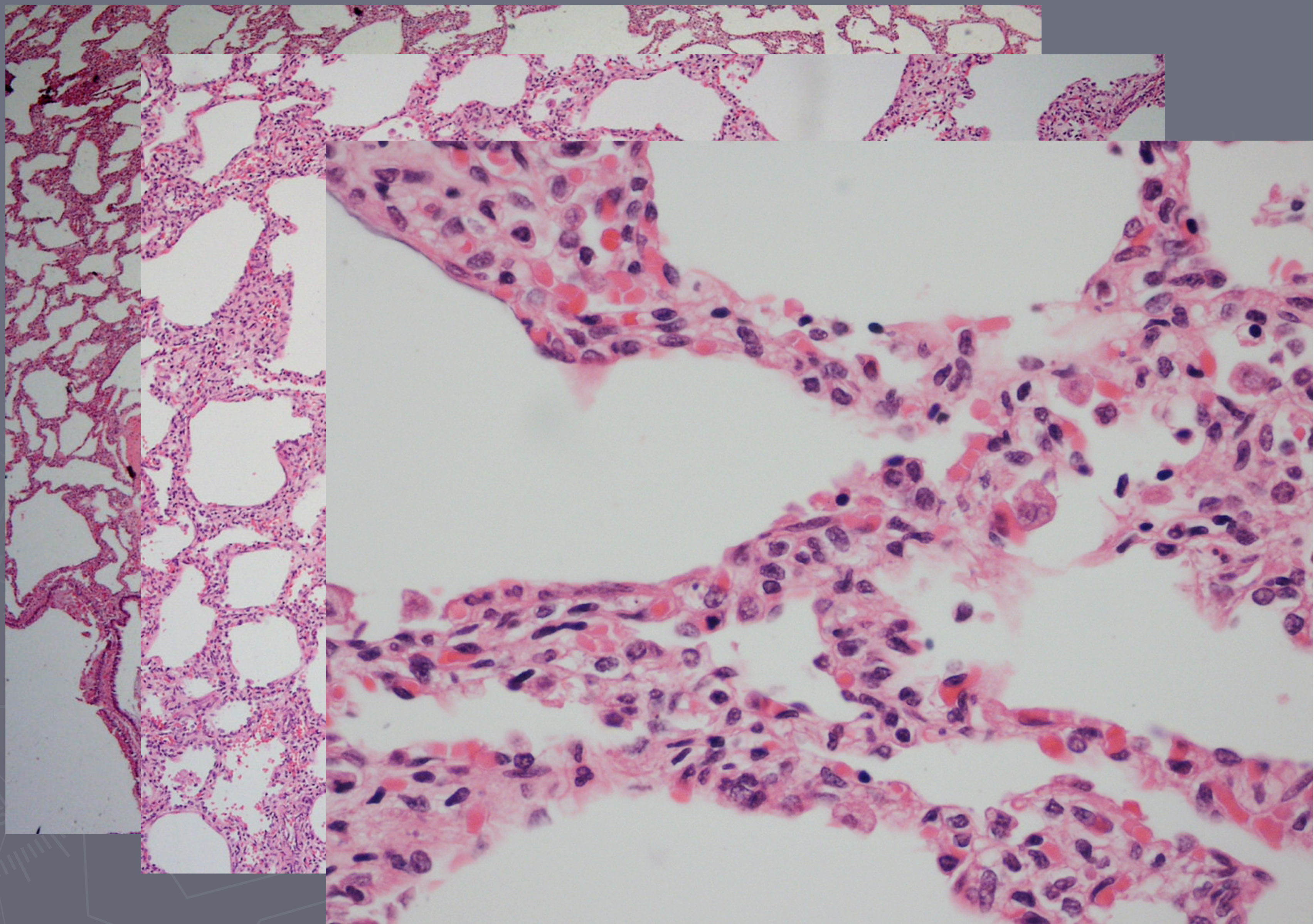




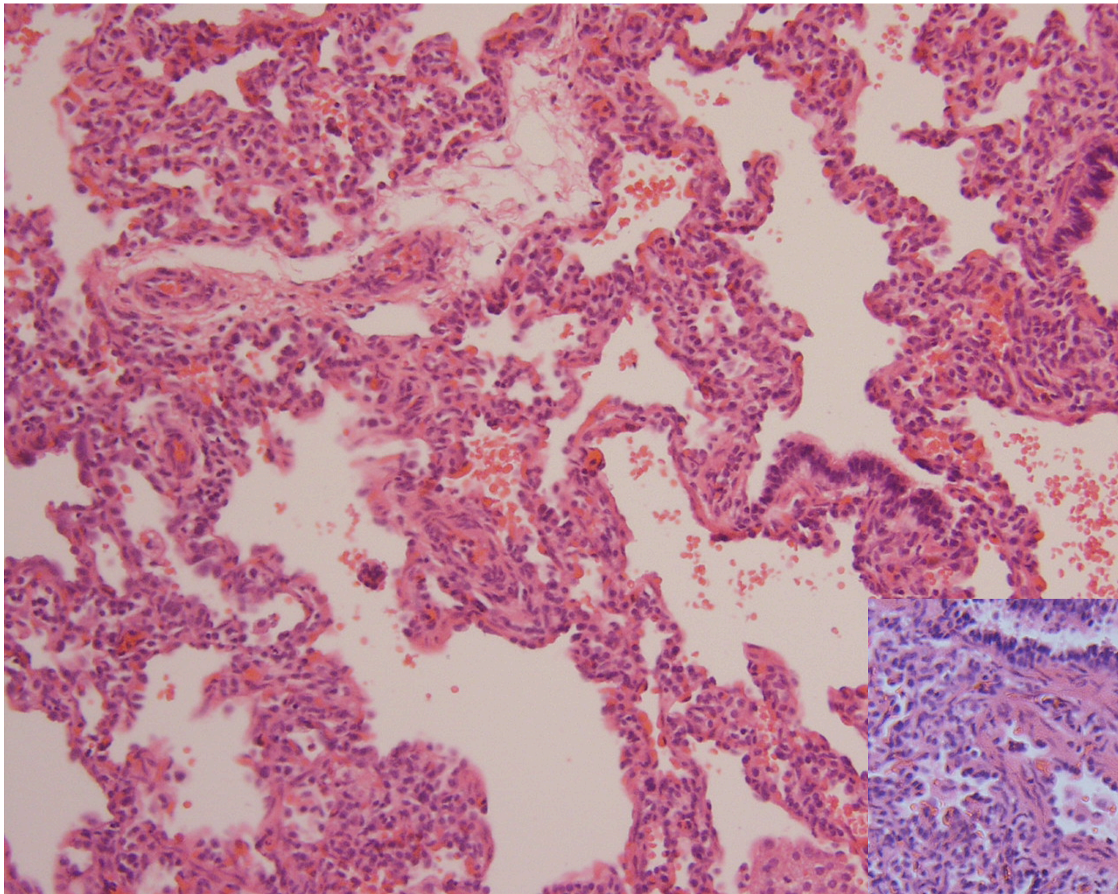


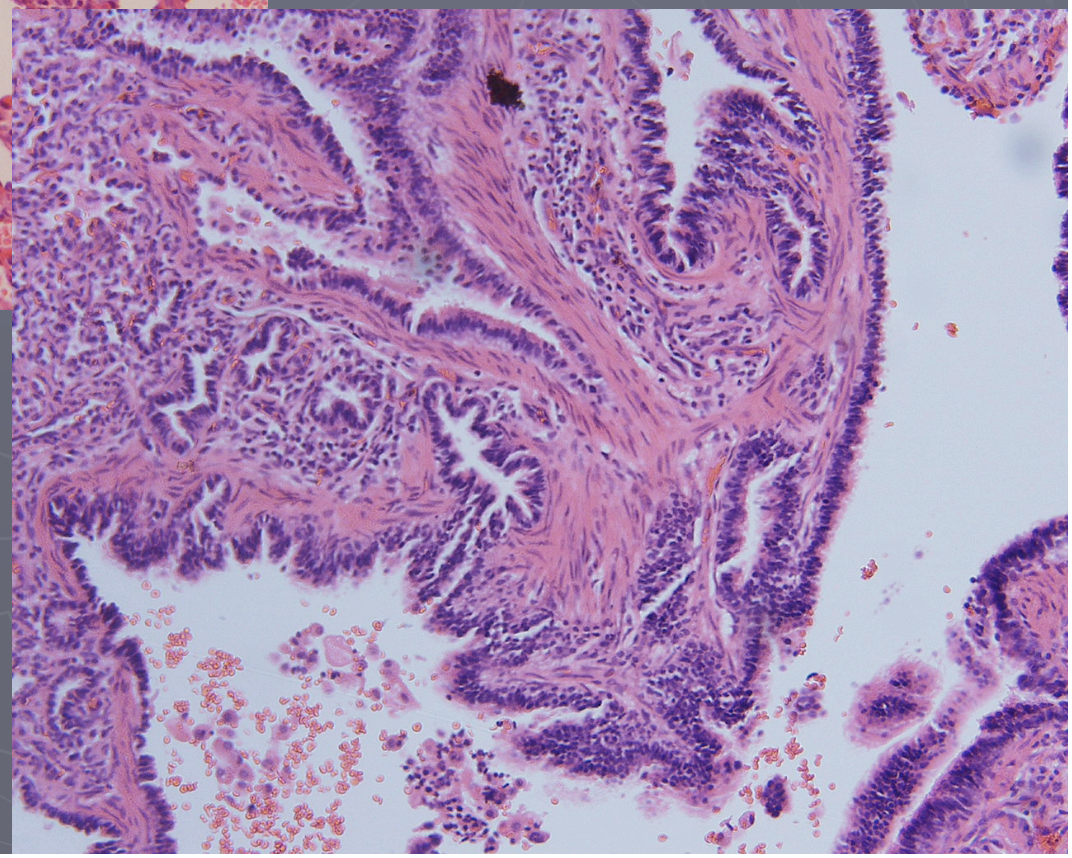
SACCULAR 31 weeks

Term lungs



Term and CCAM



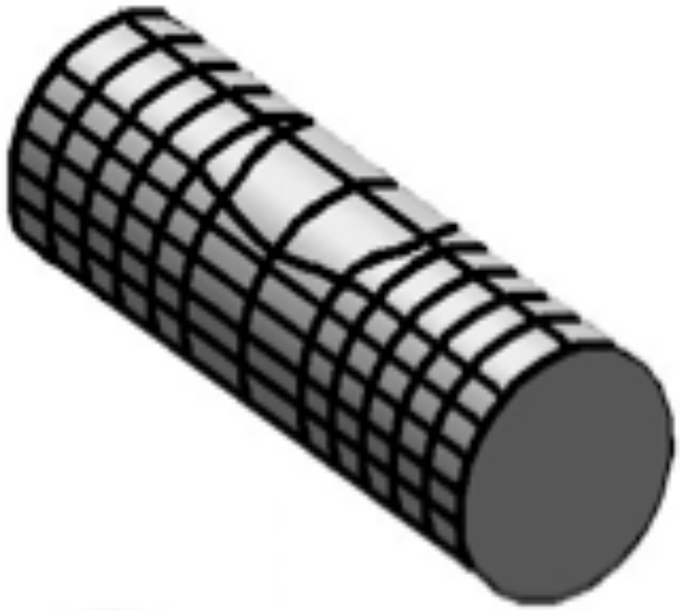


Congenital malformations

- Cystic adenomatoid malformations
  - Maturation arrest in lung segments

- Azyous lobe
  - Superior apical bronchus grows medially instead of laterally; vein is at bottom of superior lobe fissure
- Sequestration –
  - Accessory piece of lung that becomes disconnected from tracheobronchial tree and parasitizes systemic circulation from diaphragm.

Donald Ingber MDPh



Breathing exercise

- Begins pre-natally, allows branching to continue
  - Fluid is expelled from lungs at birth by vaginal pressure into capillaries and lymphatics
- Fluid is needed for proper lung development
  - Insufficient fluid – decreased lung development
  - Insufficient breathing movements – decreased lung development (neurological)

Causes of Lung hypoplasia – diminished lung development

- Oligohydramnios – insufficient amniotic fluid
- Compression
  - Congenital diaphragmatic hernia – intestinal contents compress left hemithorax (usually)
  - Intrathoracic fluid or thoracic wall abnormality

Expansion of the lung activates a transcriptional program.

- Stretching of myofibroblasts induces a transcriptional program that contributes to completion of distal proliferation and differentiation (TGF-B decrease)
- Lung expansion in utero by fluid is critical to proper lung development.

RDS-Respiratory distress syndrome

- Low surfactant – Respiratory distress syndrome – usually due to pre-maturity, rarely due to surfactant protein deficiency (genetic cause)
  - Surfactant is critical to reduce surface tension and allow lung expansion at the air fluid interface.
    - Inadequate surfactant leads to alveolar collapse on expiration of air, and difficulty re-inflating
    - Damage to the alveolus leads to cellular injury and exudation of proteins known as hyaline membranes (Hyaline membrane disease)
      - Continued injury from ventilation of immature lungs can lead to chronic injury known as bronchopulmonary dysplasia.
  - Steroids accelerate lung development and surfactant production
    - Surfactant can also be administered

Pulmonary vasculature

- At birth, fetal lung circulation is a high pressure that must convert to a low pressure circulation.
- As air enters the lung with the first breath, oxygen tension rises.
  - Increased nitric oxide production increases arterial vasodilation, reducing pulmonary arterial pressure.

Molecular determinants of branching morphogenesis

- Much of this data is derived from transgenic animals. Knockout of genes and gain of function mutants
- Also experiments displacing mesenchyme and epithelium to new sites have been critical in understanding the crosstalk between epithelium and mesenchyma

Branching determinants

- Removal of mesenchyme halts branching
- Non lung mesenchyme does not support branching
- Lung mesenchyme placed in trachea or salivary gland induces specific branching
- Proximal vs distal mesenchyme induces site appropriate epithelial cell development
- Mesenchymal factors are diffusable across membranes – No contact needed, but gradients are very local.
- Epithelial factors "crosstalk" to determine mesenchymal growth and differentiation.

FGF-fibroblast growth factor

- Loss of epithelial FGF receptor leads to loss of branching
- FGF10 in mice from mesenchyme binds to FGFR2 on epithelial cells.
- Epithelial Shh (sonic hedgehog) shuts off FGF10 from mesenchyme, stopping growth. New buds form in Shh negative areas that have persistent FGF10 production
- Other FGF (e.g. FGF7) and other FGFR (e.g. FGFR3 and 4) may play a similar role in later stages of distal/alveolar lung development.

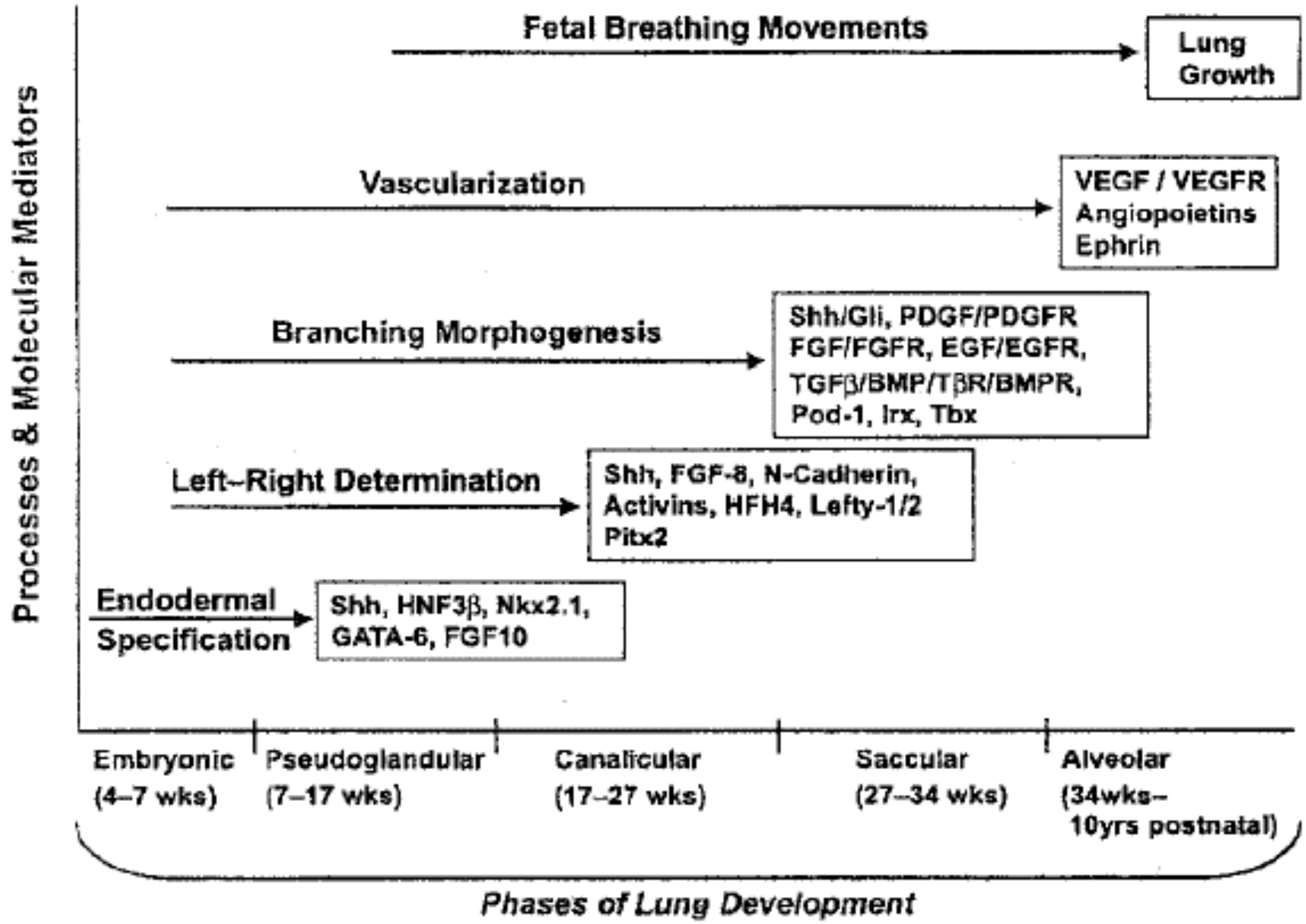
Factors in branching morphogenesis

- Proliferation
  - Growth factors, factors that promote pluripotency
- Differentiation
  - Transcription factors and downstream effectors leading to different cell types
  - Proximal and distal differences (e.g cartilage, type II cells)
- Inhibition of proliferation
  - Growth eventually stops, also a key to branch points
- Apoptosis
  - Structures must be remodeled in second wave of growth.
  - May deal with local proliferation excess.
- Coordinated growth of different cell populations – "crosstalk"
  - Elements may be intracellular pathways, cellular receptor specificity, as well as diffusable factors with very local gradients
- Differential adhesion.
  - Cell to cell, cell to matrix.
  - Adhesion molecules may be expressed reducing cellular fluidity, fostering attachment of epithelium to mesenchyme via common extracellular matrix proteins

Branching parameters

- BMP4 may prevent proximal type differentiation, allowing cells to accept signals for distal development.
- TTF1 and HNF3B may promote differentiation towards surfactant producing cells.
- Shh may induce proliferation and differentiation in mesenchyme and inhibit epithelial proliferation signals (FGF10).
- TGF-B may induce extracellular matrix production, which in turn may further anchor and stabilize epithelial cells.

I. COPLAND AND M. POST

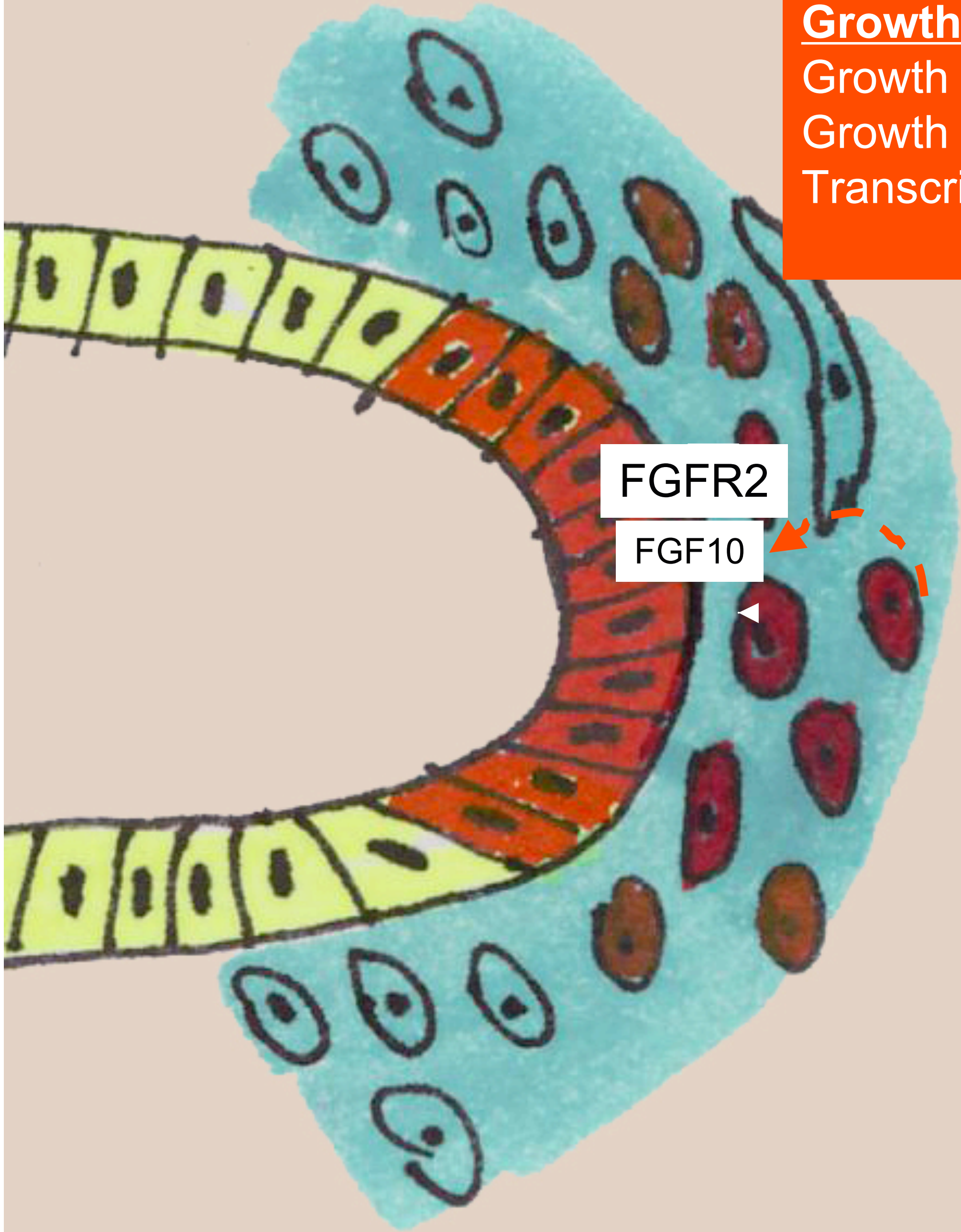


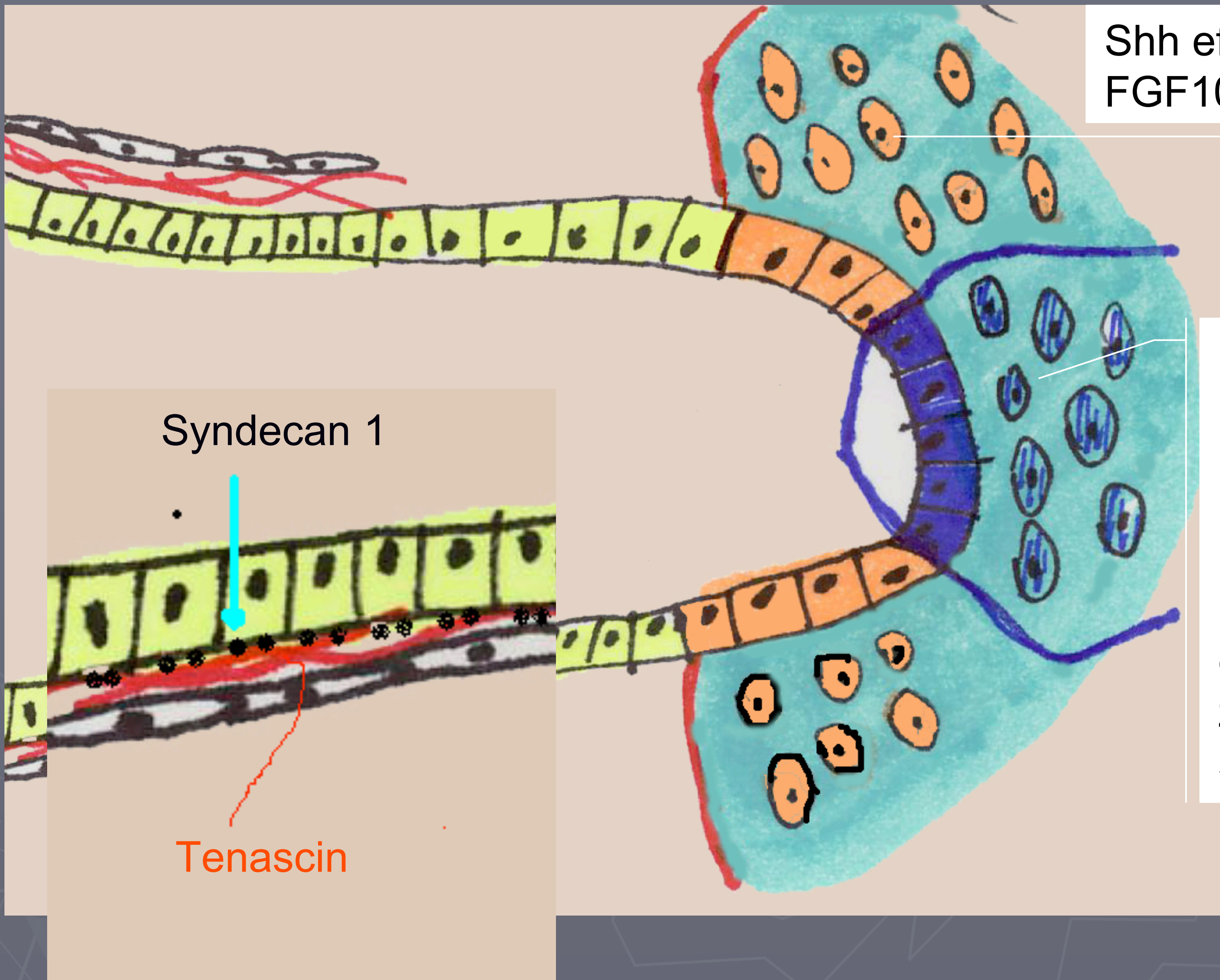
Growth zone –

Growth factor -FGF10 from mesenchyme

Growth factor receptor - FGFR2 on epithelium

Transcription factor TTF1 – epithelial, necessary at this stage

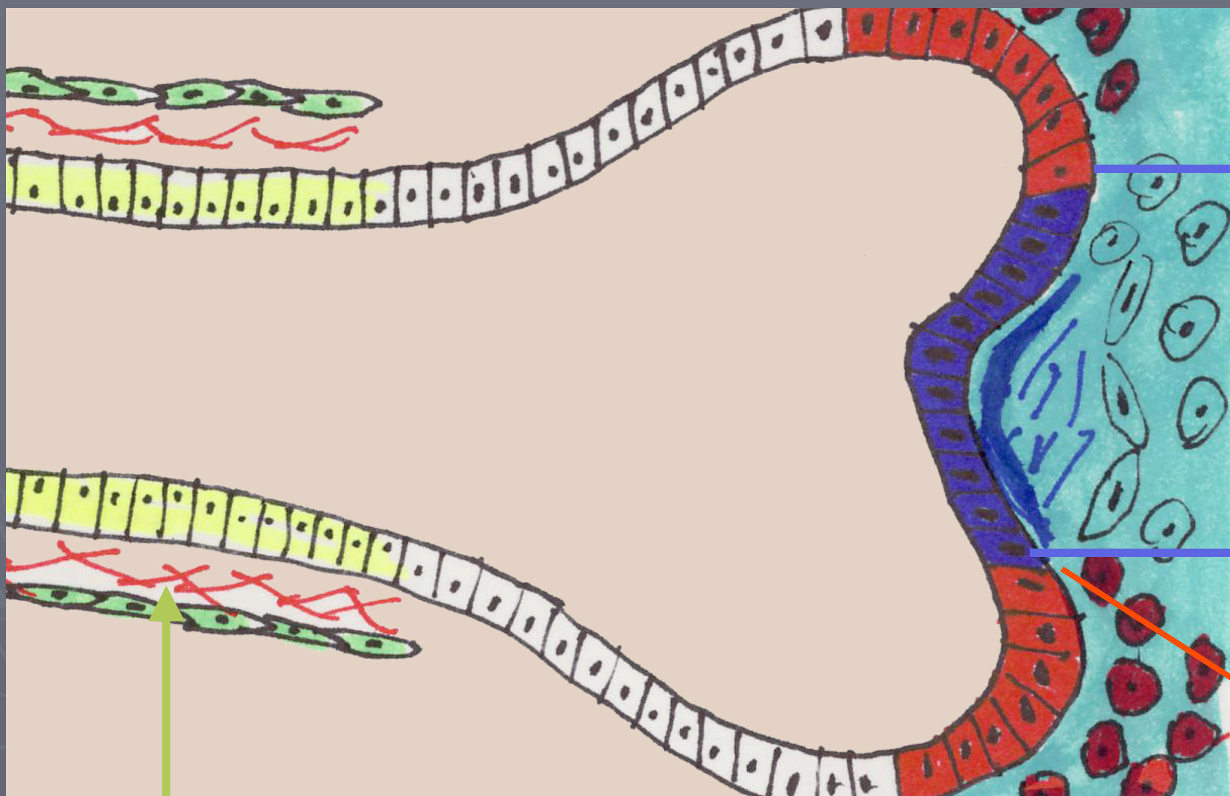




Shh effect is localized, FGF10 less inhibited

Inhibition of FGF10 by epithelial Shh; BMP4 also needed, Shh induces mesenchymal proliferation and differentiation – zone growth stabilized.

Greater stabilization by mesenchymal differentiation, extracellular matrix and cell to matrix adhesion



Stabilized zone, further
stabilized by TGF-B
inhibition of FGF10
TGF B induces
production of matrix –
fibronectin and collagen

Growth zone – FGF10
continues to promote
Growth outside of stabilized
Zone, creating a branch.

More mature zone is fully
Stabilized by differentiation, loss of
Proliferation signals, matrix and anchoring