Model Organisms and
Innovative Approaches
In Developmental Biology

Development of the Lung and Analogous Systems

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DEVELOPMENT OF THE LUNG AND ANALOGOUS SYSTEMS

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RESPIRATORY SYSTEM

I. DESIGN
- principles
- different species

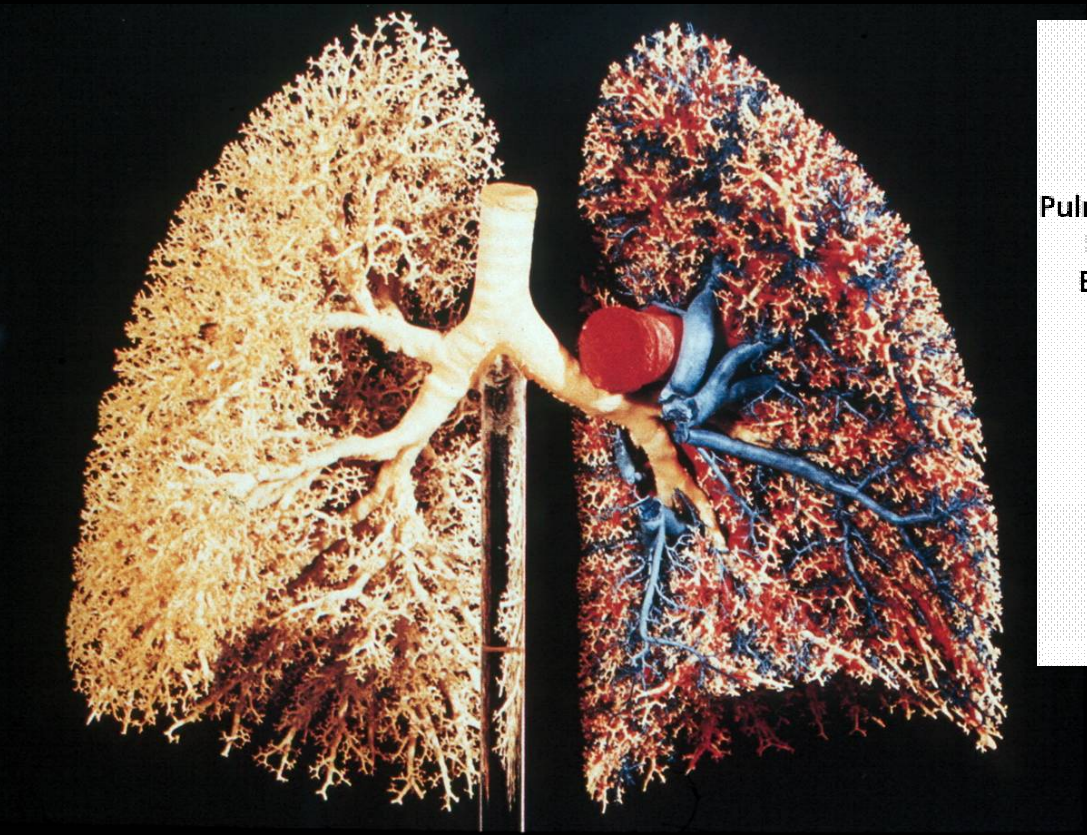
II. EVOLUTION

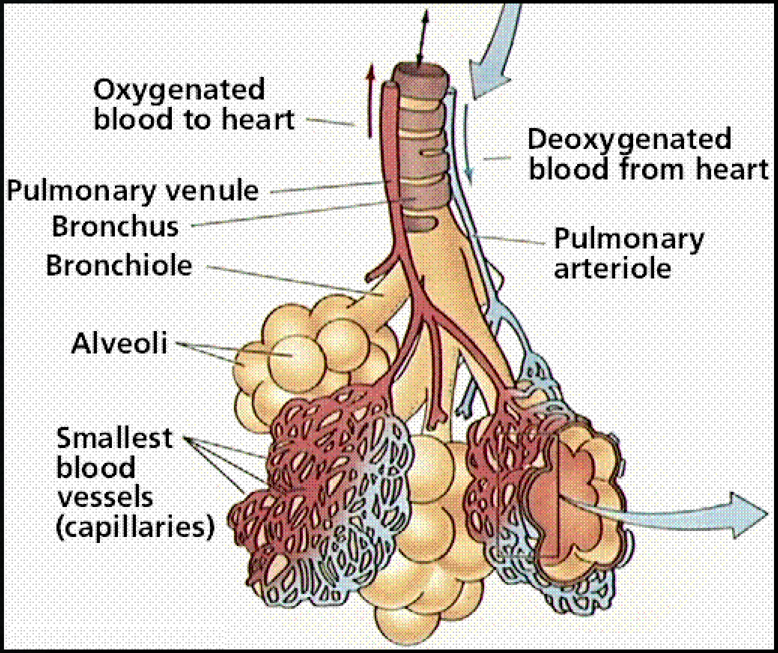
III. DEVELOPMENT
- specification
- tubular morphogenesis

IV. MODEL SYSTEMS

DESIGN AND PRINCIPLES

MAMMALIAN



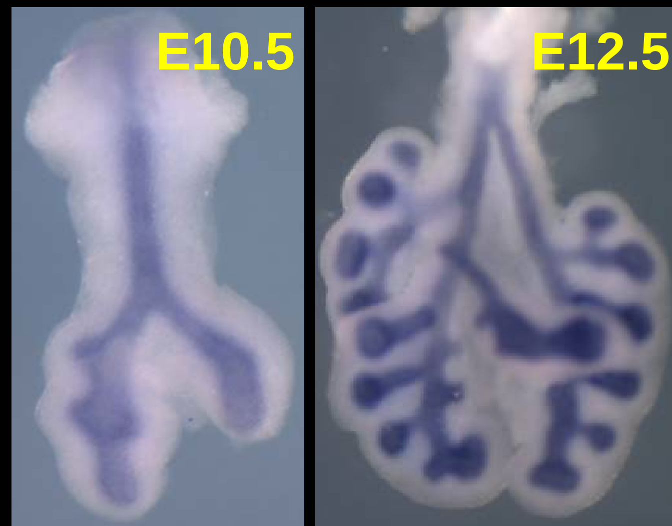


Airways –alveoli
Vascular: arterial, venous

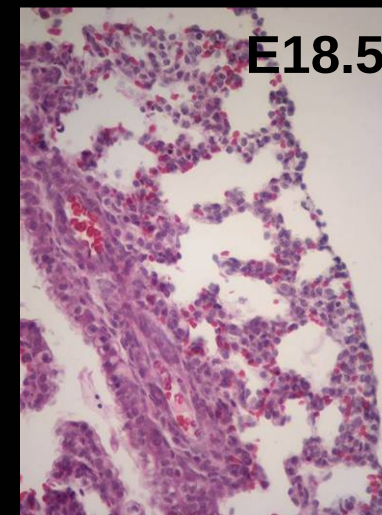
LUNG DEVELOPMENT (MOUSE)

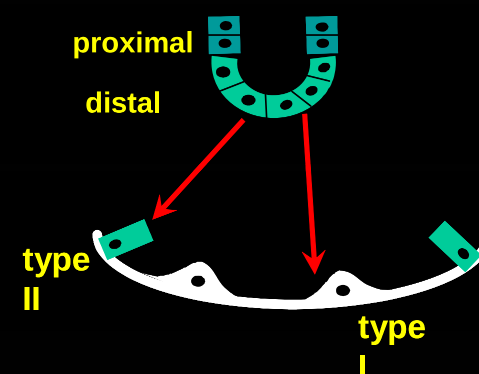
primary budding

branching morphogenesis

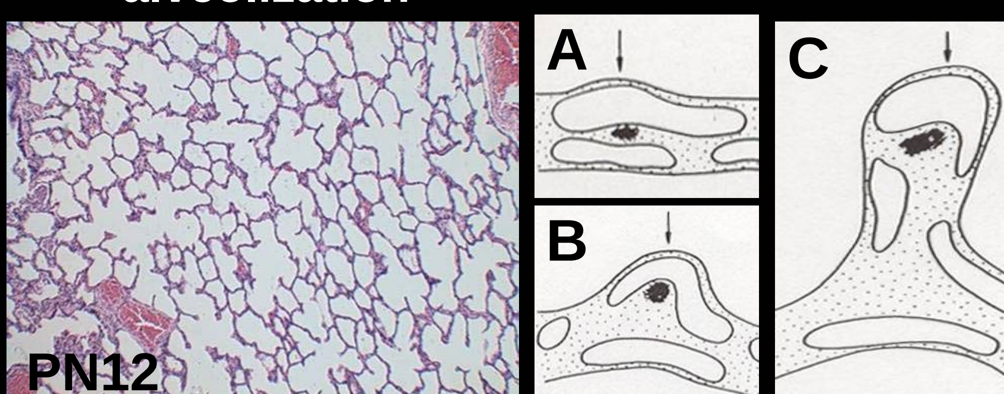


sacculation



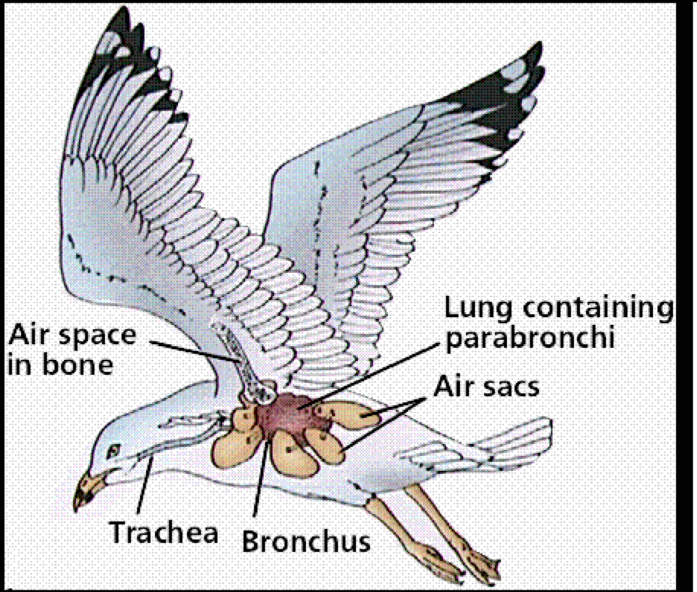


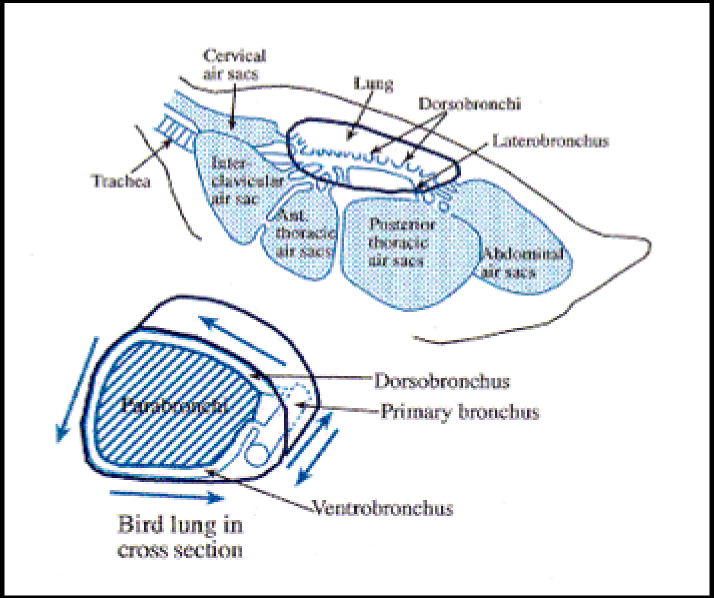
alveolization



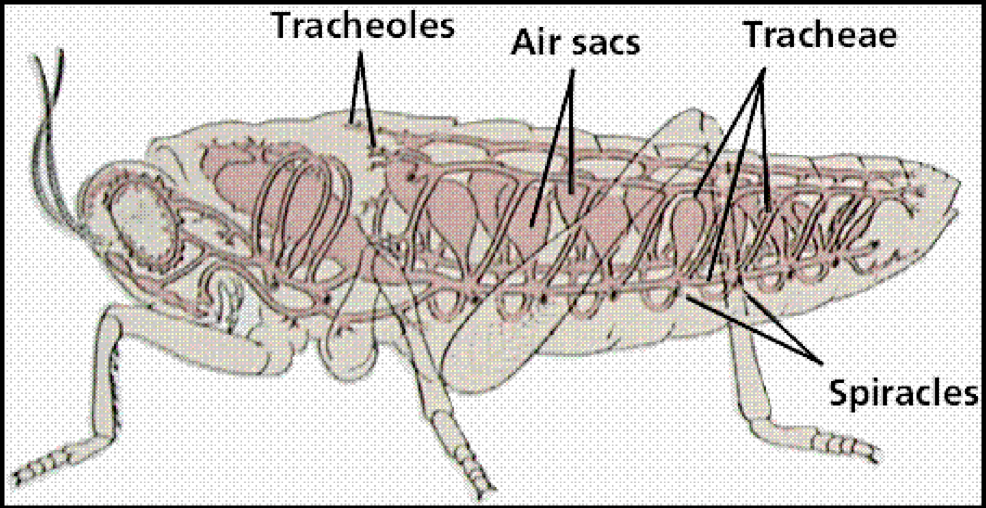
RESPIRATORY SYSTEM DESIGN

BIRDS





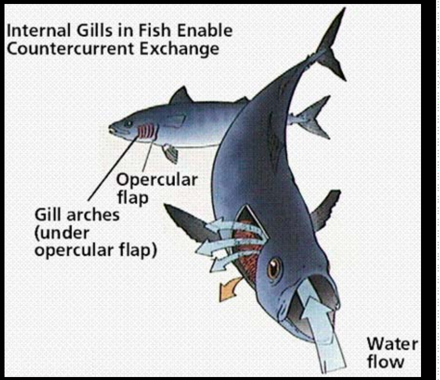
INSECT

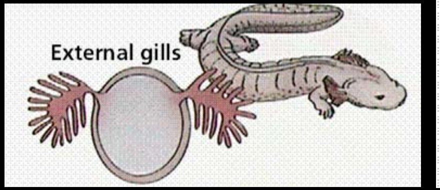


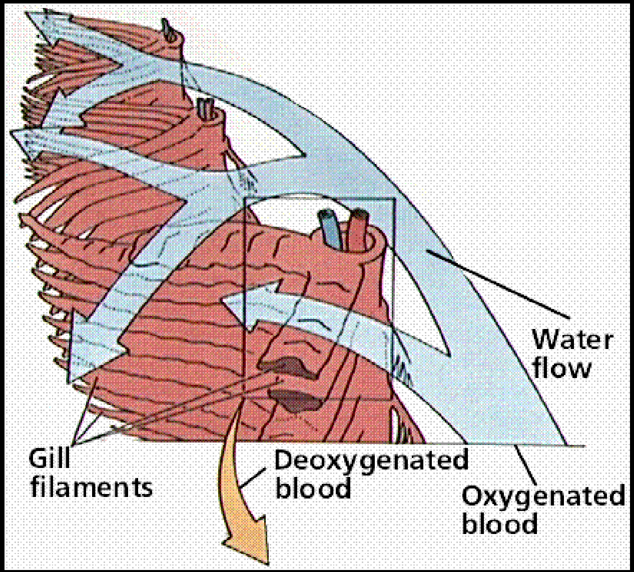
RESPIRATORY SYSTEM DESIGN

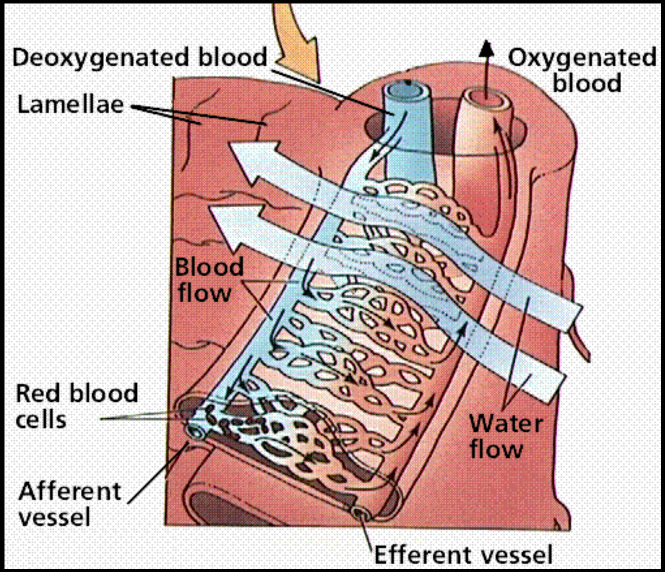
FISH

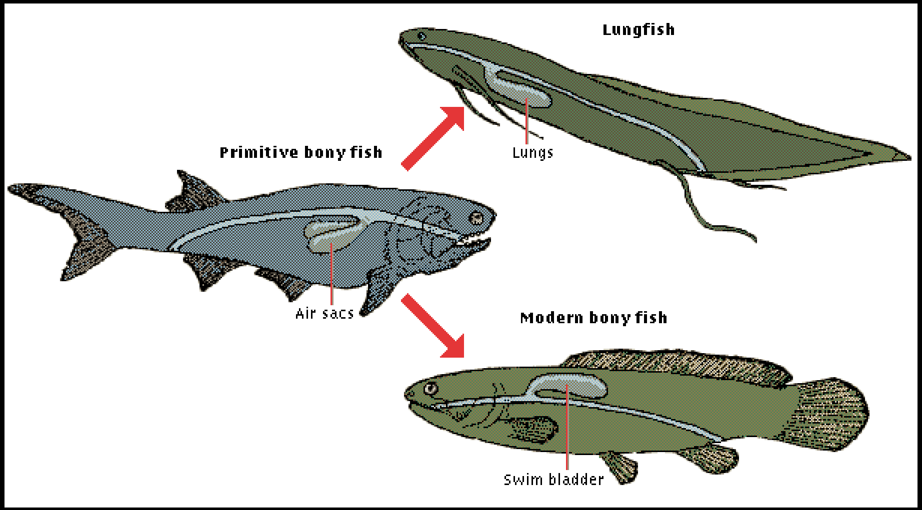
Gills, Lungs & Swimbladder

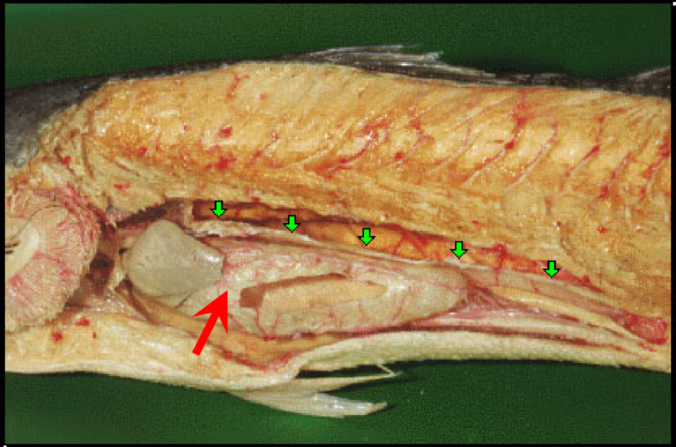






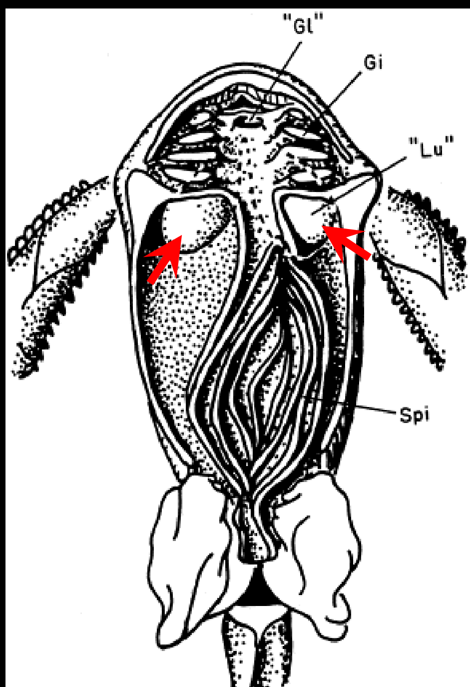






HOW DID THE LUNGS EVOLVE?

THE FIRST LUNG

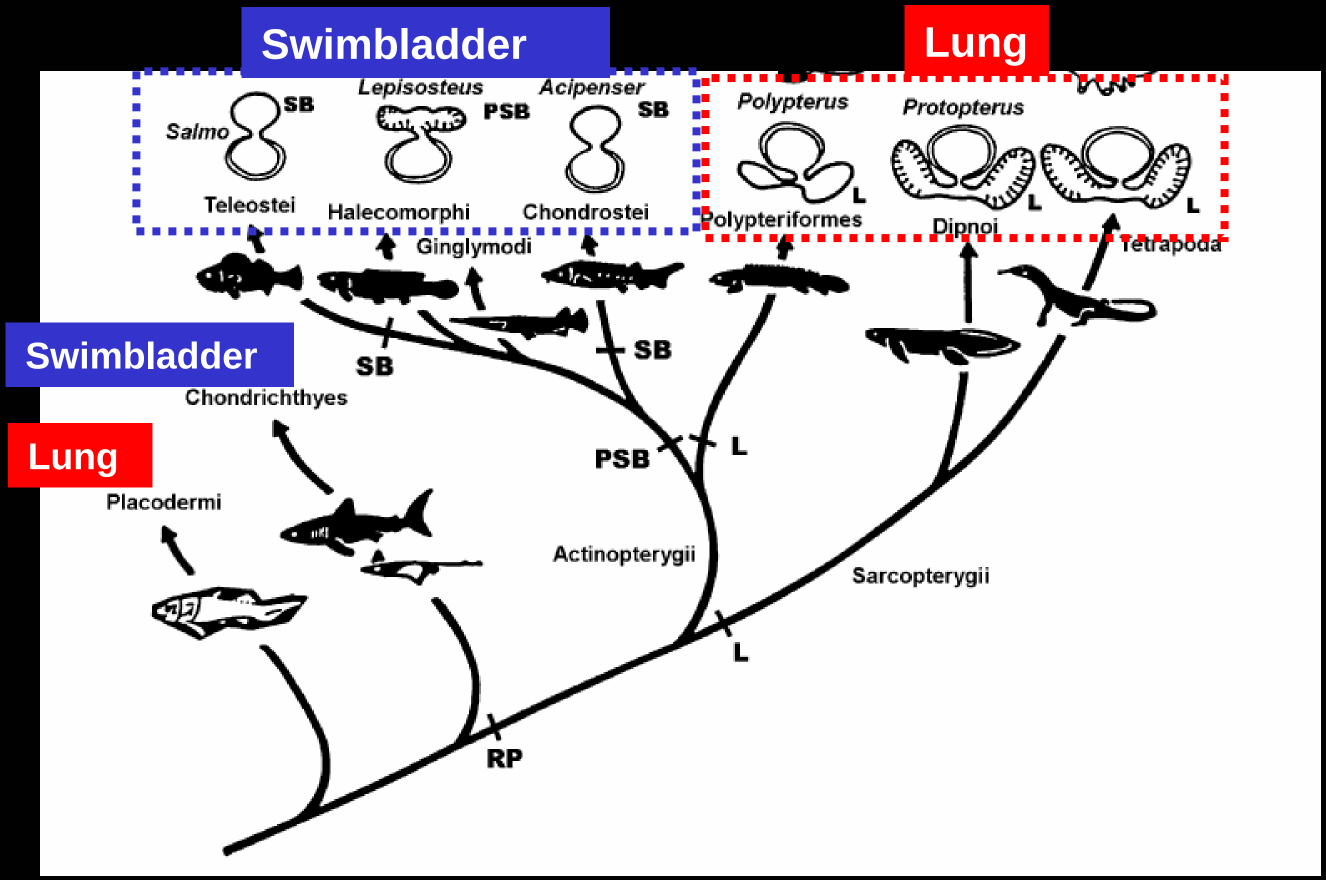


Bothriolepis canadensis

Origin: Devonian (350M years)
- prior to amphibians
Environmental Pressure:
- temperature
- decline in oxygen in water

Which one is the oldest: Swimbladder x lung?

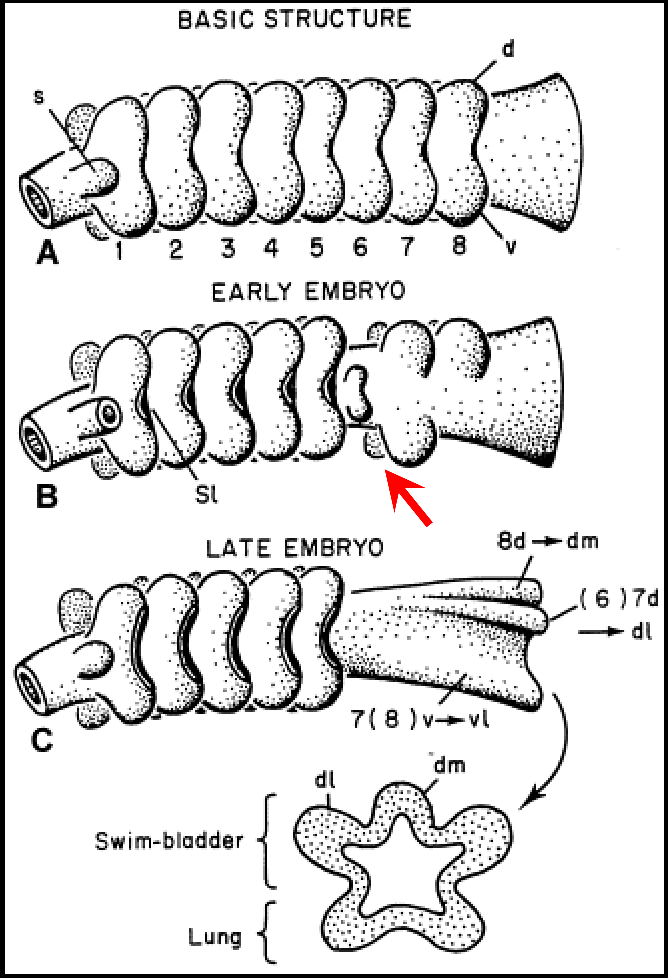
EVOLUTION OF THE LUNG

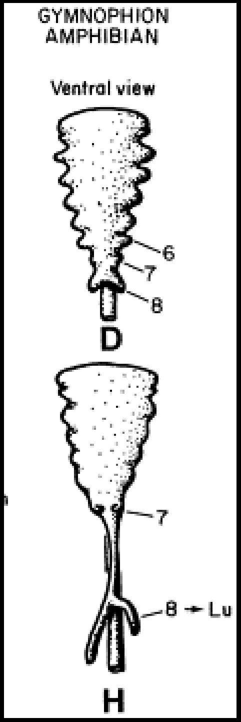


- The swim bladder is found only in one subdivision of the bony fishes, the actinopterygians
- There is no evolutionary advancement with chronology

Perry and Sander, 2004

EVOLUTION OF THE LUNG

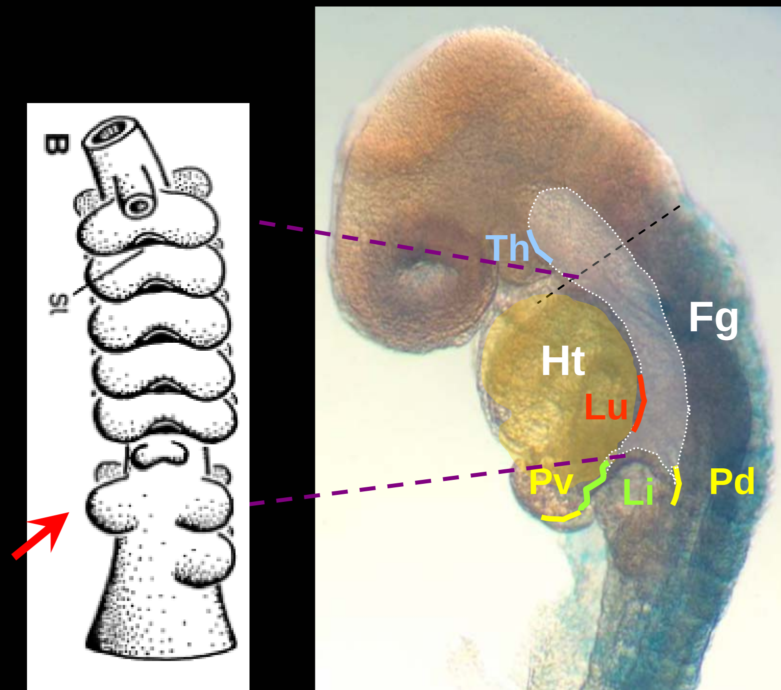




Modification of the posterior pharynx into respiratory pharynx, lungs and swimbladder

Perry and Sander, 2004

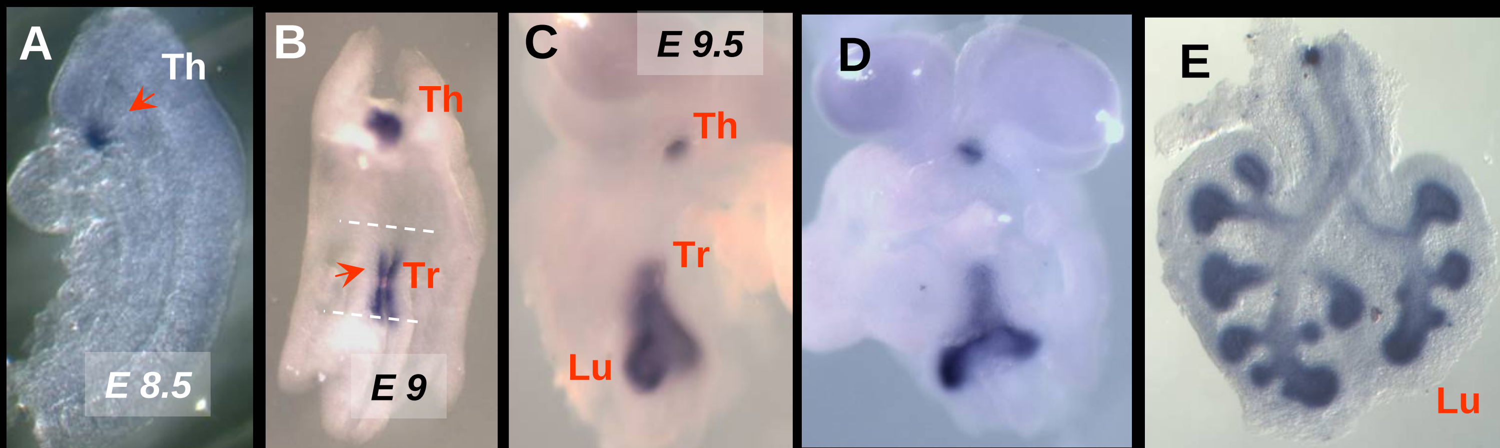
WHICH SIGNALS DID EVOLVE TO SPECIFY AND REMODEL THE POSTERIOR PHARYNX?



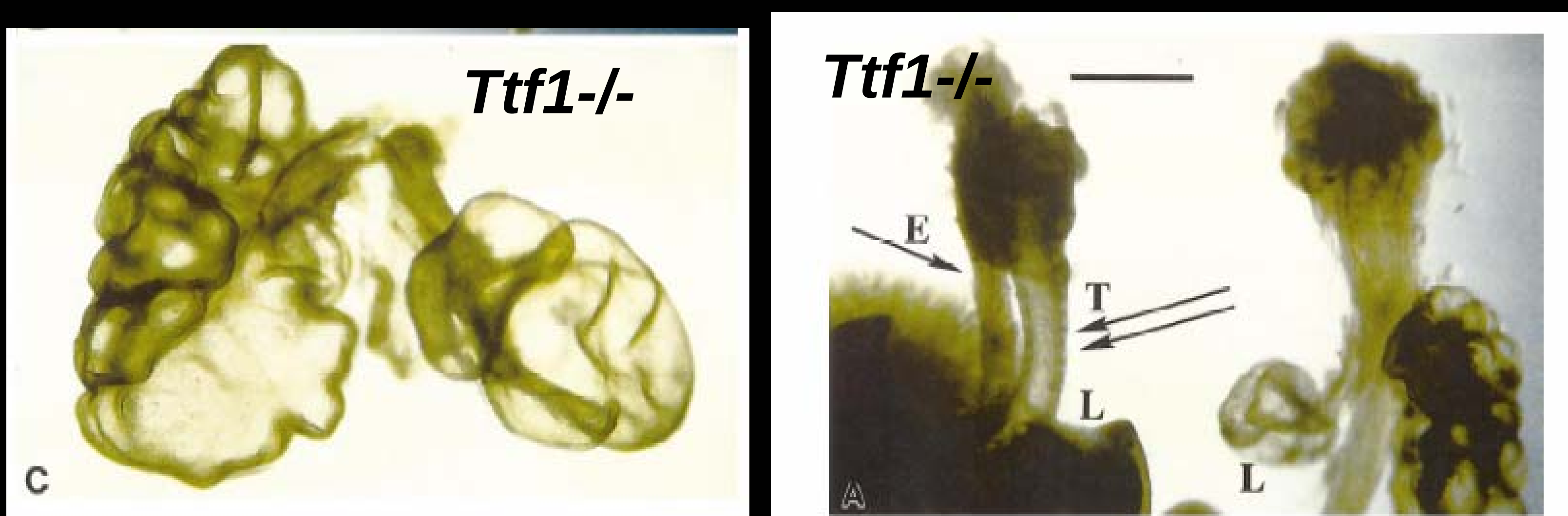
- Anterior-posterior axis (A-P)
- Establishment of lung cell fate

What is the earliest evidence of lung cell fate in the developing foregut?

THYROID TRANSCRIPTION FACTOR 1
(*Ttf1, Nkx2.1*)



Desai et al., 2004



Minoo et al., 1999

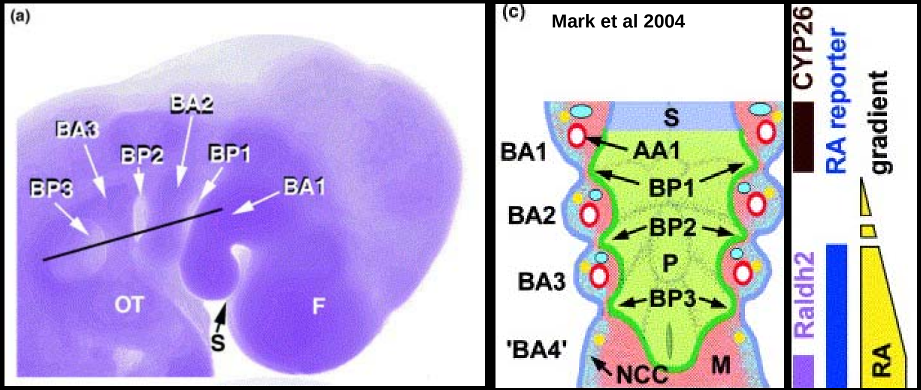
RA AND SPECIFICATION OF THE POSTERIOR FOREGUT

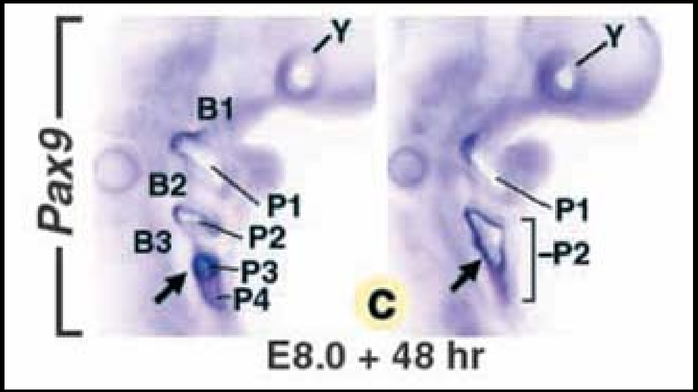
RA regulates A-P patterning of zebrafish foregut (Sttaford & Prince, 2002)

- BMS493, *neckless:* loss of posterior (pancreas, liver)
- RA: anterior expansion of liver, pancreas

RA establishes the posterior limits of pharynx in amphioxus (Schubert et al. 2005)

A-P Patterning of the pharynx by RA in mice-rats- quail

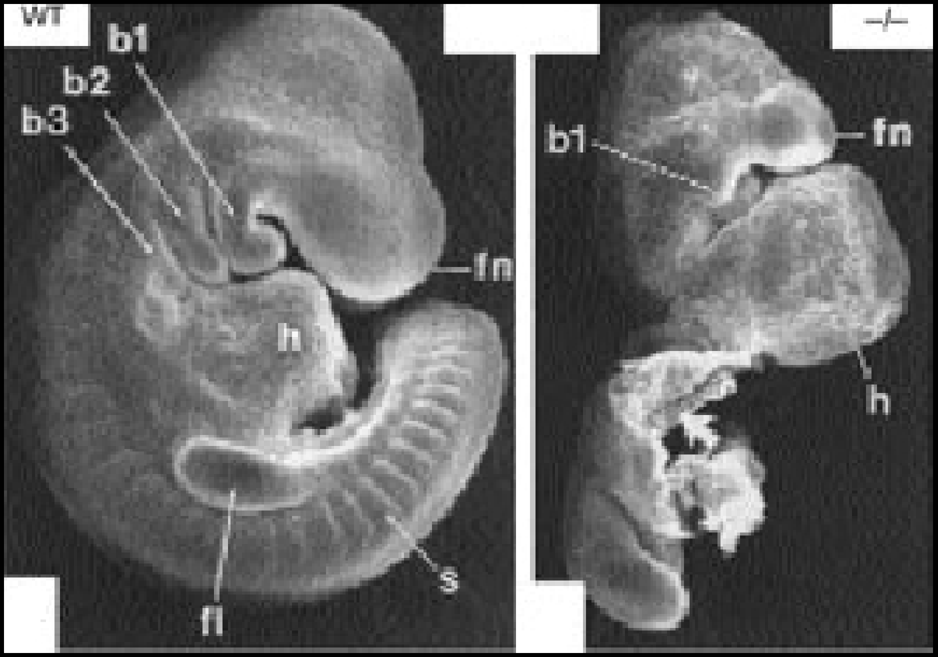


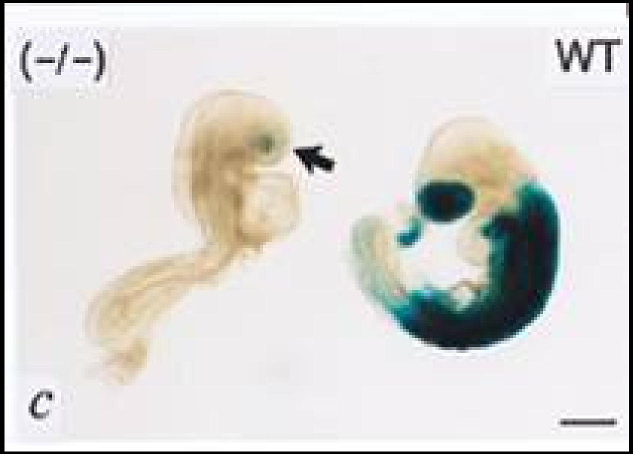


- increasing RA gradient posterior (Niederreither et al., 1999)
- posterior endodermal fates depend on RA (Wendling et al. 2000; Quinlan et al. 2002)
- posterior pouches P3-4 are absent
    - in VAD quail and in *Raldh2 -/-* (Niederreither et al., 1999; Dickson et al.,1997)

Is RA required for lung cell fate specification?

Raldh2-/-

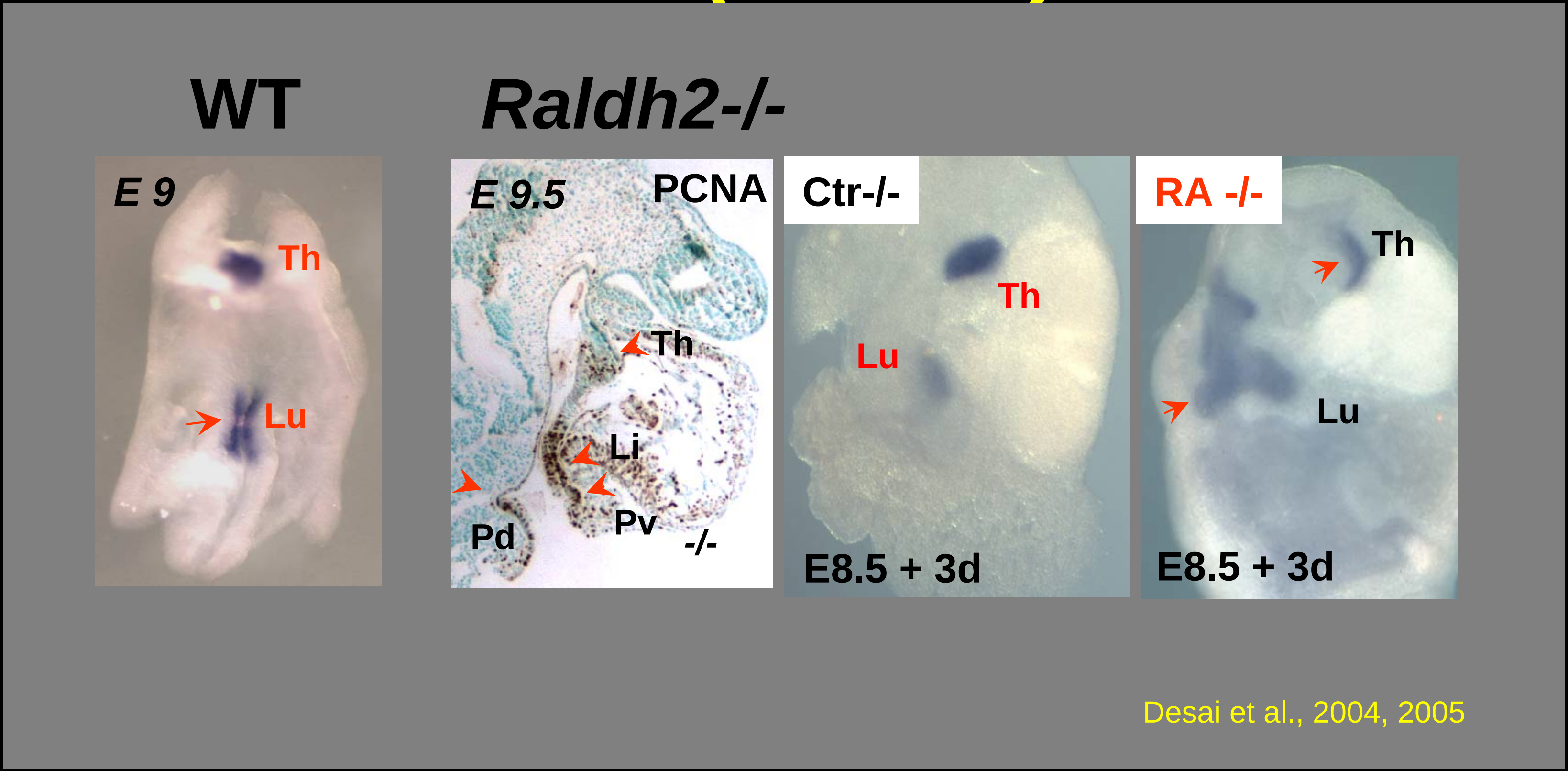




Niederreither *et al*. *Nature Genetics*, 1999

- Death by E10.5
- Axis truncations, cardiovascular defects

Ttf1 (Nkx2.1)

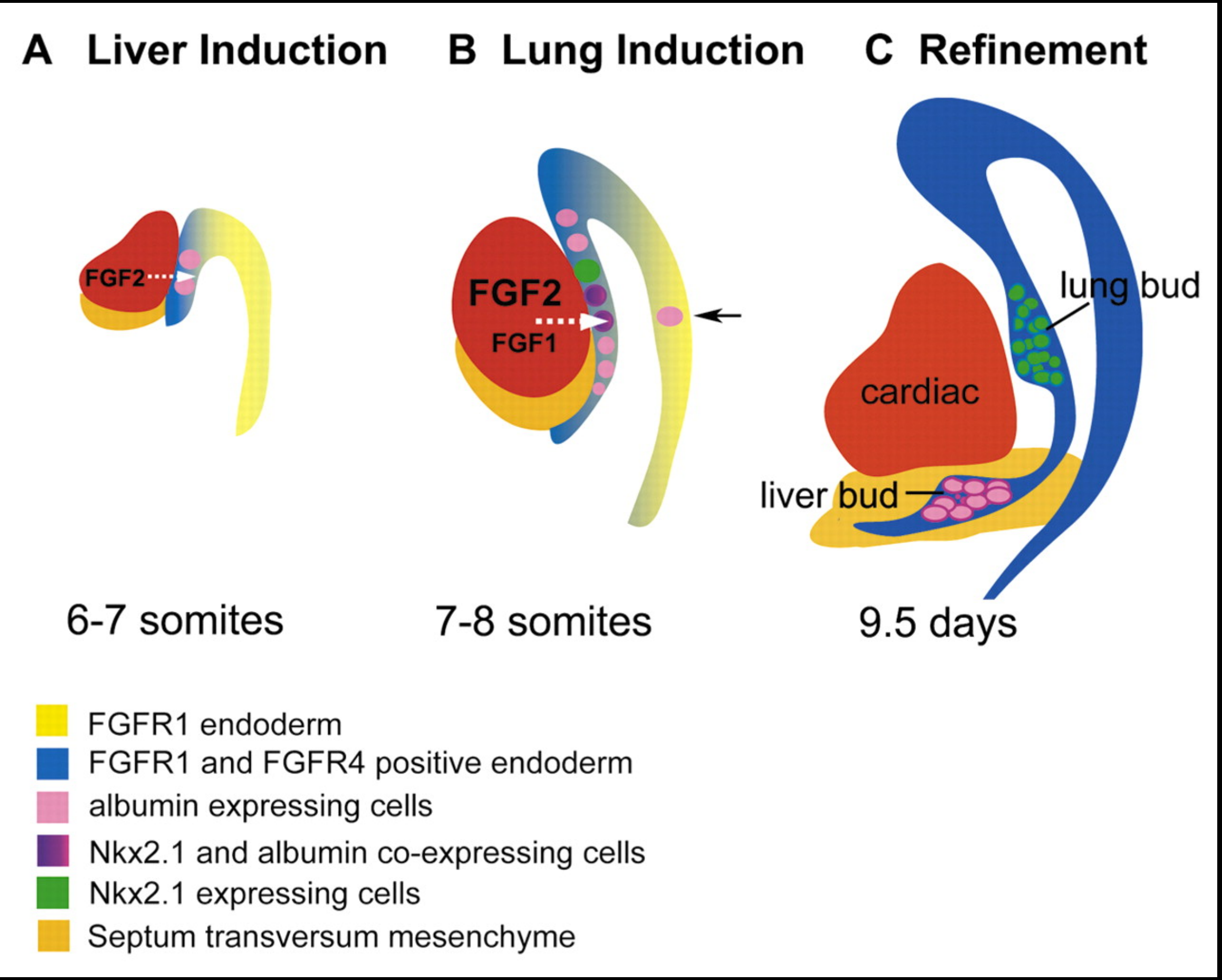


-*Ttf1* is present in the prospective lung endoderm of wild type and *Raldh*2 mutants. No lung buds.

- Lung bud morphogenesis is rescued by RA
- conclusion: initiation/position of lung cell fate in the foregut is not influenced by RA

FOREGUT ORGANOGENESIS

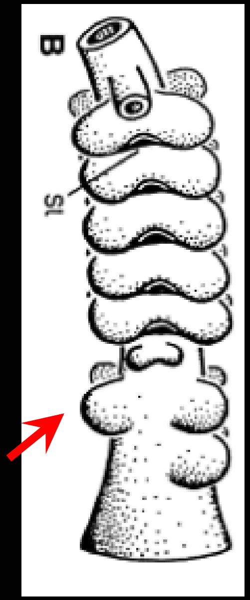
LIVER X LUNG



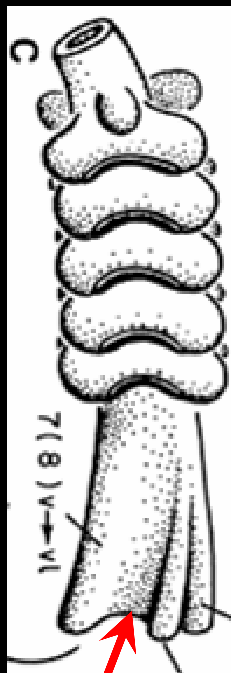
Serls et al 2005

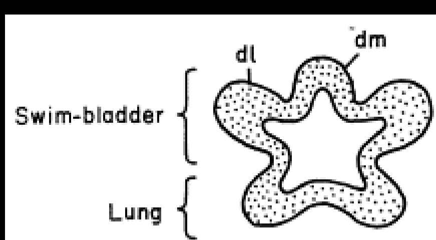
CANDIDATE SIGNALS TO SPECIFY THE LUNG AND REMODEL THE POSTERIOR PHARYNX

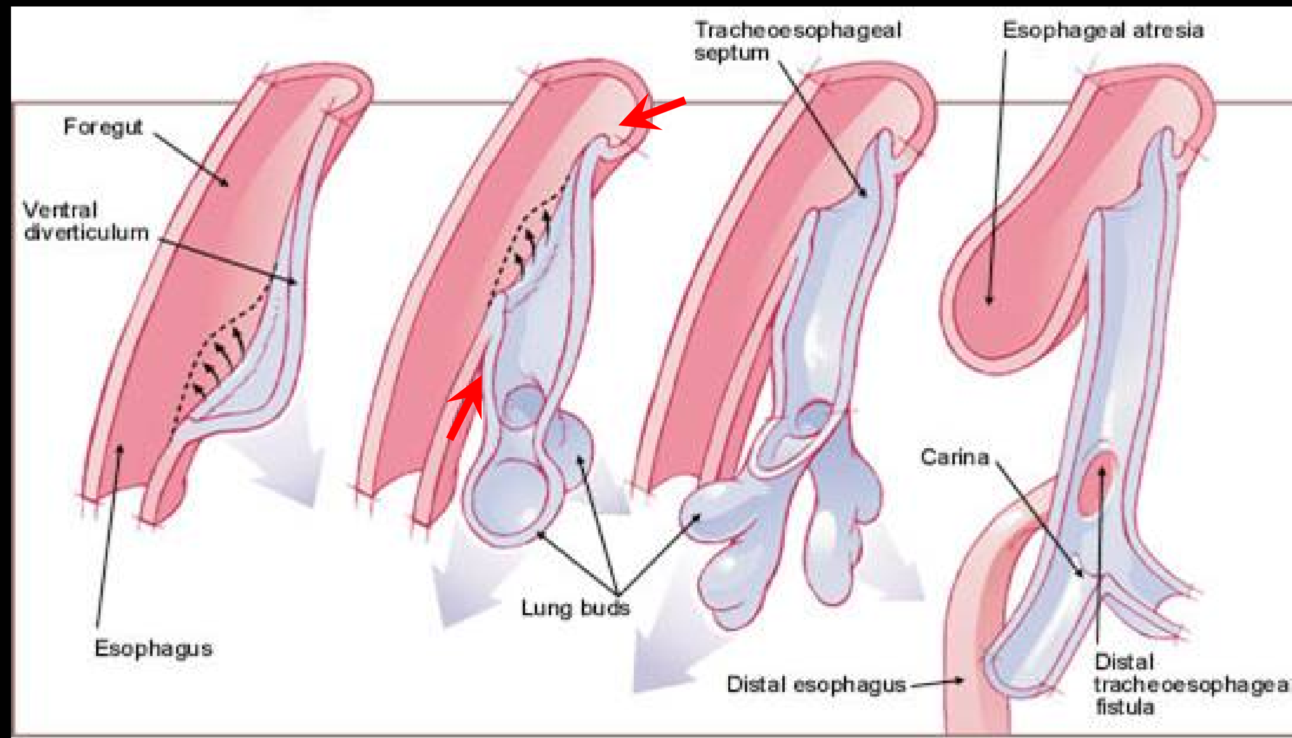
Ttf1 (Nkx2.1) ?
RA: unlike?
Fgf1, 2 ?



D-V PATTERNING OF THE FOREGUT







Clark, 1999

- SEPARATION OF THE RESPIRATORY AND DIGESTIVE TRACTS

Shh (endoderm)

STRATEGIES FOR UNDERSTANDING BASIC MECHANISMS

- Patterning of posterior pharynx & lung
Genetics in zebrafish - Swimbladder ?

McCune and Carlson Zebrafish gas bladder mutants and convergence 249

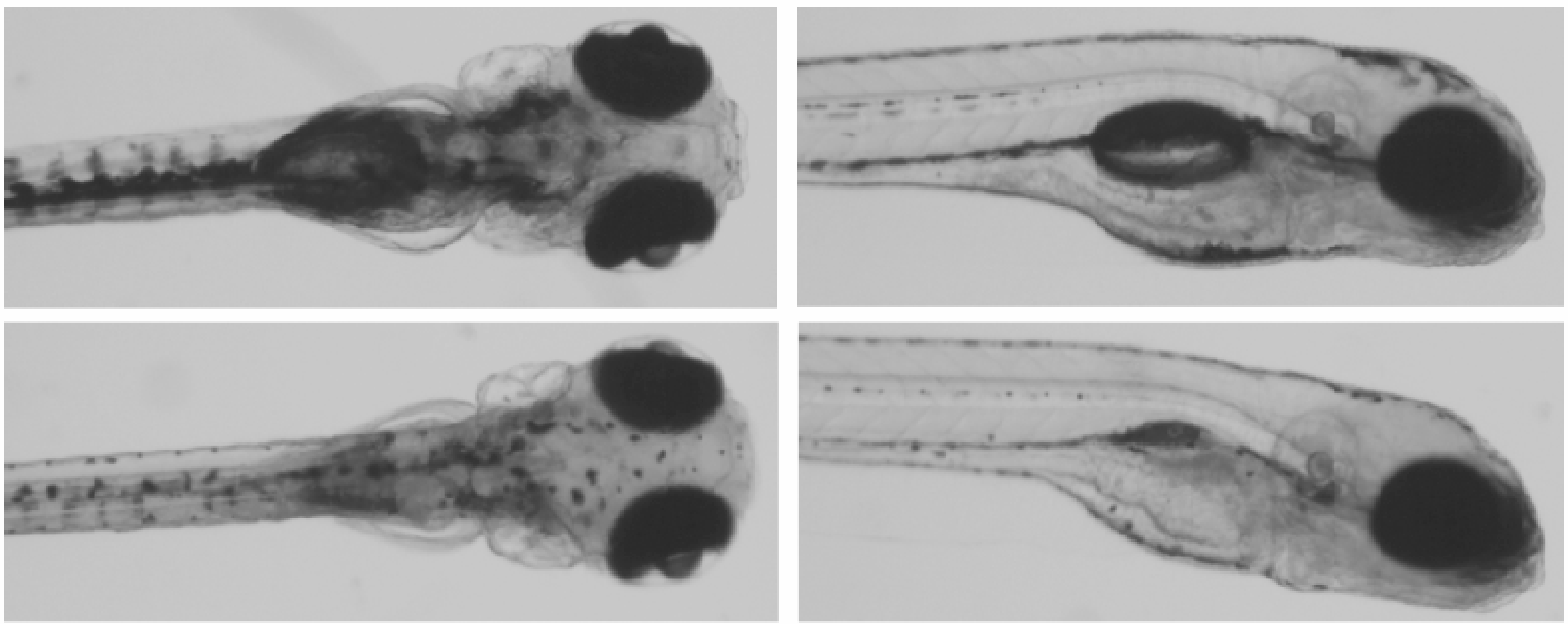


Fig. 2. Example of normal and bladderless individuals from the same clutch. (Left) Dorsal views of a normal phenotype (top) and a bladderless sibling expressing *exb* (bottom). (Right) Side views of the same normal individual (top) and bladderless (bottom) individual expressing *exb*. Magnification, 50×. (Photos by K. McMillan.)

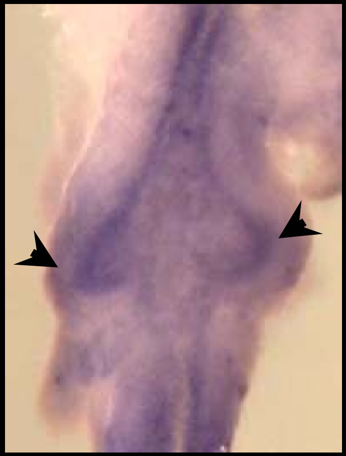
STRATEGIES FOR UNDERSTANDING BASIC MECHANISMS

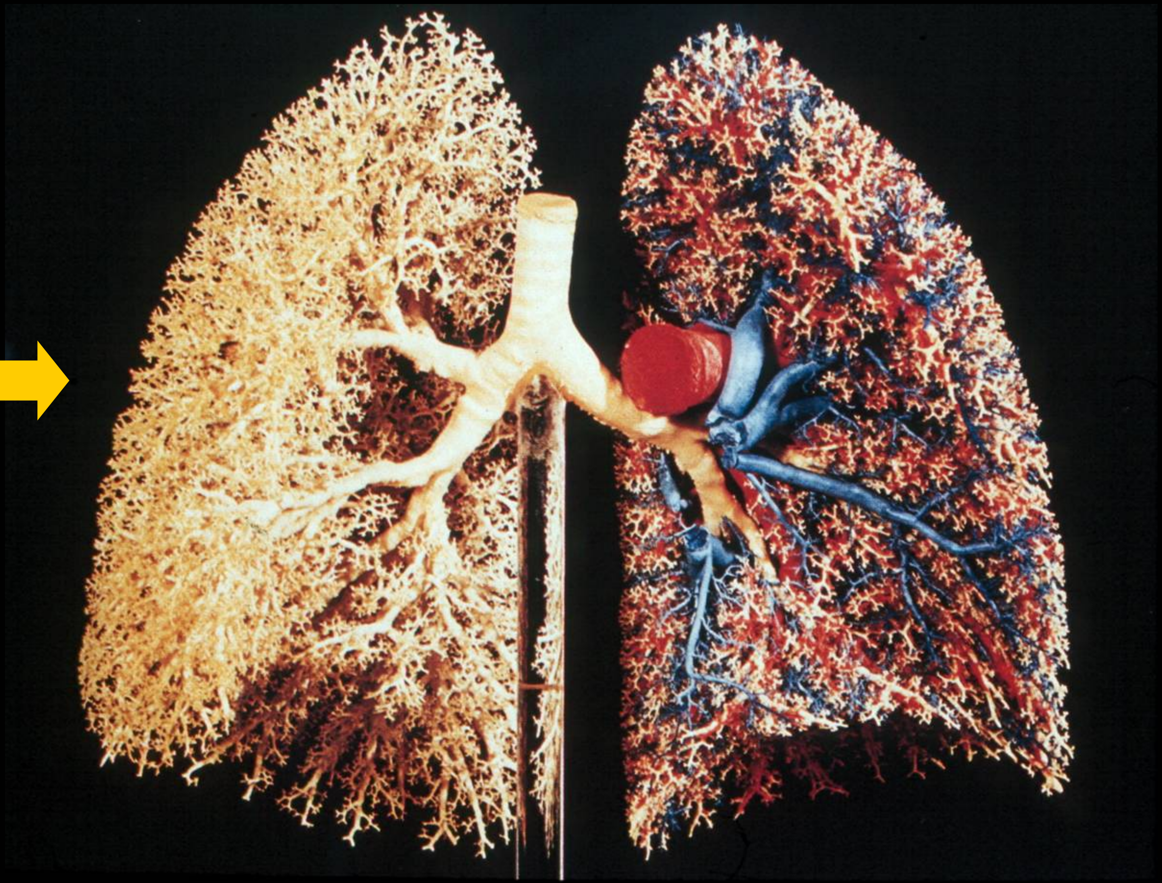
Table 1. Presence/absence of the gas bladder and other phenotypic abnormalities caused by recessive mutations from wild-caught *Danio rerio*

Mutation (abbreviation)	Sibship	Gas Bladder	Other Phenotypic Abnormalities Detected by External Examination and Study of Cleared and Stained Specimens	Day First Seen
anchor (*anc*)	15	Absent	None	~ 4–5
ballast (*bll*)	13	Absent	Lower jaw narrow; notochord wavy	~ 4–5
ballooneyes (*boo*)	1	+	Edema around eyes	5
bent (*ben*)	7	Absent	None	~ 4–5
big bladder (*big*)	10	+	Gas bladder overfilled by day 5 and fish float at surface; by day 9, bladder has resumed normal size; a few big bladder individuals survived beyond day 20	5
bladderless (*bdd*)	9	Absent	Lower jaw narrow; notochord wavy	~ 4–5
bloated (*blt*)	13	+	Extensive edema ventrally, moves only intermittently	6–7
blockhead (*blk*)	13	Absent	Squared off head, eye and body edema	4
bottom heavy (*btt*)	4	Absent	None	~ 4–5
candycane (*cdc*)	14	Absent	Spine curved in candy cane shape; small eyes, heart edema; widespread edema	3
darkguts (*drk*)	1	+	Dark granular yolks; heart, eye, or body edema	1
dividing unevenly (*div*)	14	NA	Cells dividing unevenly (~1.5 h), yolk poking through cluster of cells at 5 h, dead by 24 h, long before gas bladder inflation	0
extra bubbles (*exb*)	14	Absent	None; name refers to transient air bubbles in the gut of mutants during the time that normals are filling their gas bladders	~ 4–5
jaw deformity (*jaw*)	13	Absent	Head stubby, snout does not project anterior to eyes, pronounced lower jaw, upper jaw not clear, lenses do not protrude from eyes, pectoral fins bent; edema around otic capsule	3
jawless eyebulgers (*jey*)	14	Absent	Small head, small eyes with large lenses, extensive eye edema, jaw small or absent, ventral edema, stubby tail	1
kinky (*knk*)	1	Absent	None	~ 4–5
knockout (*kno*)	10	Absent	Eyes small, head knobby anteriorly, lacks jaws, heart edema; lacks pectoral fins	2
knucklehead (*knu*)	3	Absent	Red spot on yolk; heart edema starting day 3; head flat anteriorly, head knobby dorsally, s-shaped spine	2
large lens (*lln*)	5	Absent	Eyes small with lens protruding from eye; jutting jaw develops by day 9	3–4
lead weight (*lwt*)	11	Absent	None	~ 4–5
pseudopunchout (*ppo*)	8	Absent	Eyes small and low on head; head knobby anteriorly; jaws and gill arches poorly defined or absent; s-shaped spine with poor differentiation of spinal column, somites, and muscles; pectoral fins absent; some grainy yolk still present day 5; poor circulation; head and heart edema	2
punchout (*puo*)	11	Absent	Eyes small; head knobby anteriorly; lacks jaws; gut poorly developed; heart and eye edema; head and yolk grainy on day 1	1
sinker (*skr*)	10	Absent	Pigmentation reduced; Meckel's cartilage and ceratohyal bent; notochord wavy; palatoquadrates and ceratohyals too close together	~ 4–5
spirograph (*sph*)	8	Absent	Spine curved, almost circular; moves by spinning in circles; develops severe head edema; pectoral fins absent	3
stiffjaw (*sff*)	14	Absent	Jaw appears to be frozen open; eyes and head slightly reduced; lower jaw curved and projects anterior to upper jaw; pigmentation reduced; some develop edema before death	3
ventral edema (*ved*)	8	Absent	Edema extensive ventrally, moves only intermittently	5
whirly (*whr*)	13	Absent	Almost circular curved spine; moves or spins in circles	2

Note that the gas bladder is present (or inflated) in 4 mutations and absent in 22 mutations. One mutation was lethal before the bladder would normally inflate. Mutants are registered at www.zfin.org, and photographs of many are included in the Zfin database. Fish carrying many of the mutants have been deposited in the Zebrafish International Resource Center at the University of Oregon.

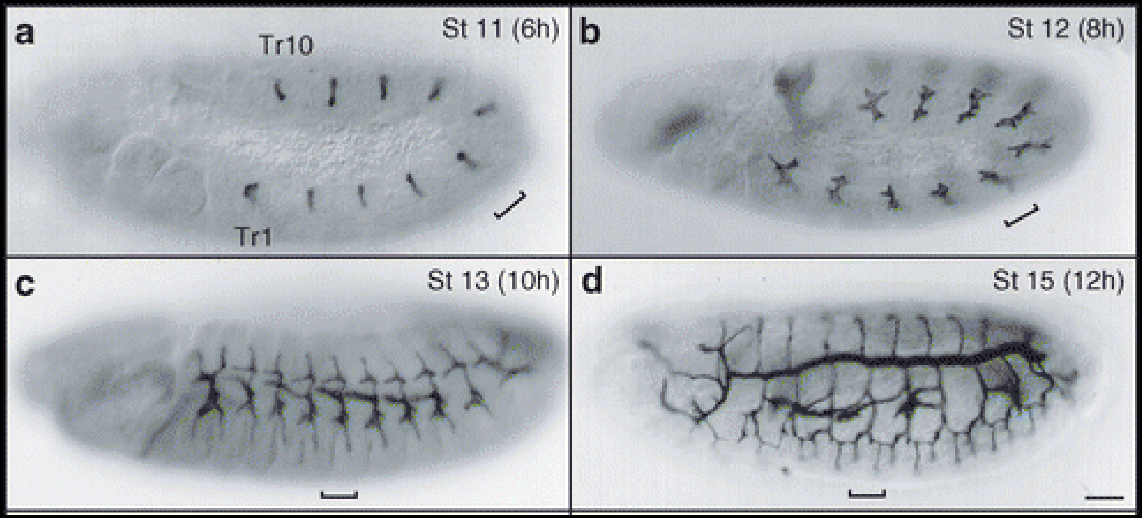
FROM SIMPLE TUBULES TO A COMPLEX BRANCHED ORGAN



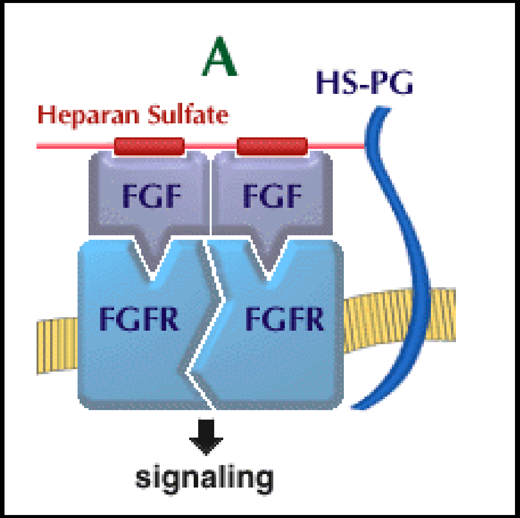


Drosophila: a model system for lung branching morphogenesis

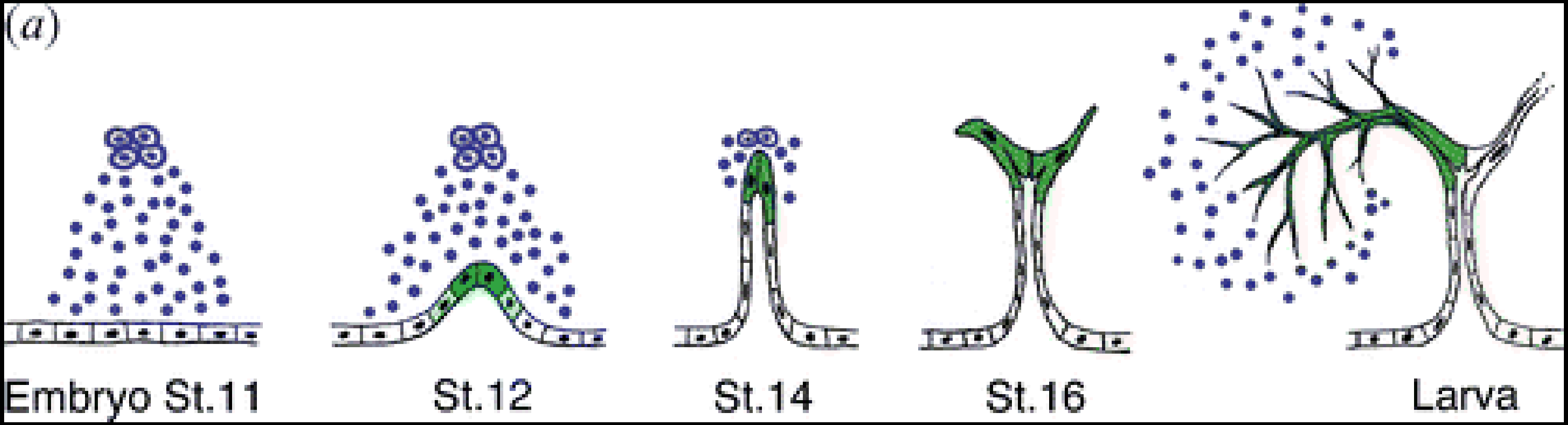
TRACHEAL MORPHOGENESIS DROSOPHILA



FGF SIGNALING

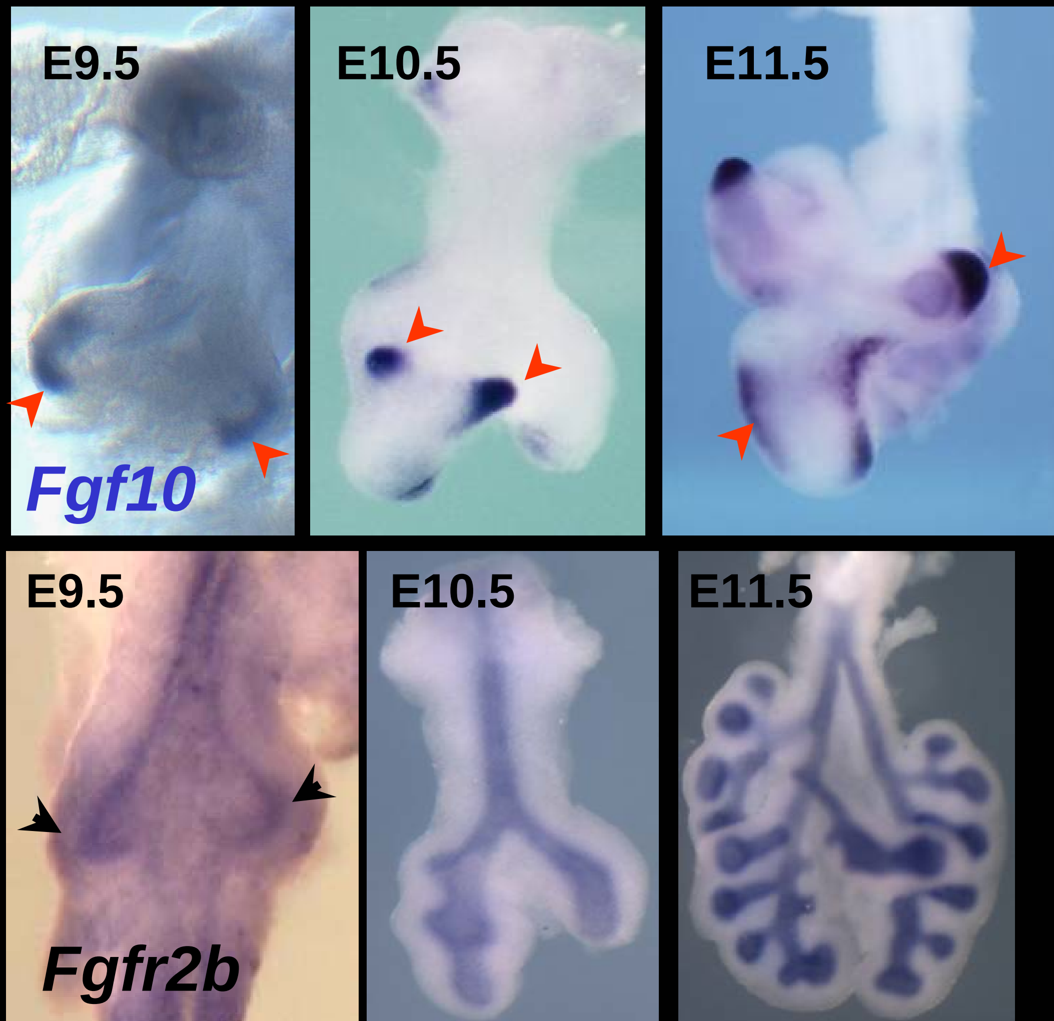


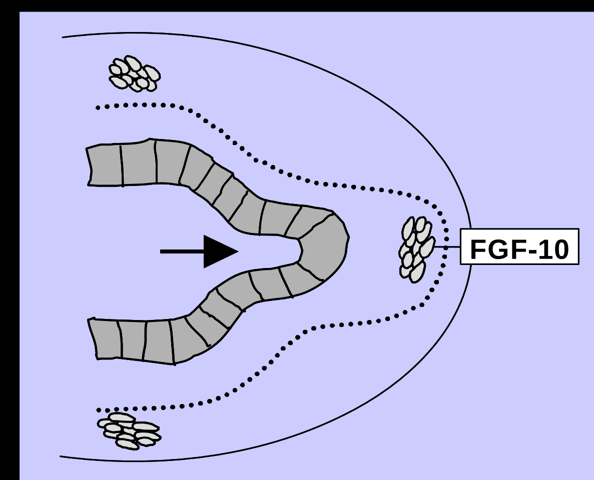
FGF: *branchless*
FGFR: *breathless*



Ghabrial et al., 2004

FIBROBLAST GROWTH FACTOR SYSTEM

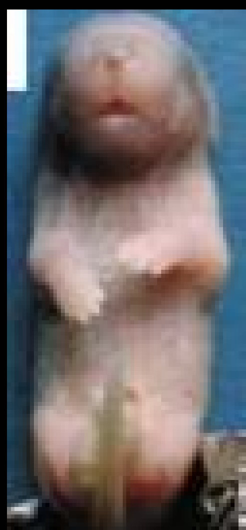




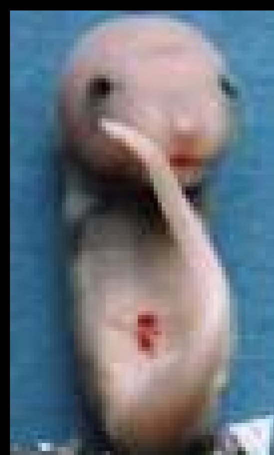
Bellusci et al., 1997 ; Cardoso et al. 1996; Park et al.,. 1998, Weaver et al., 2000

FGF10 IS ESSENTIAL FOR BUDDING

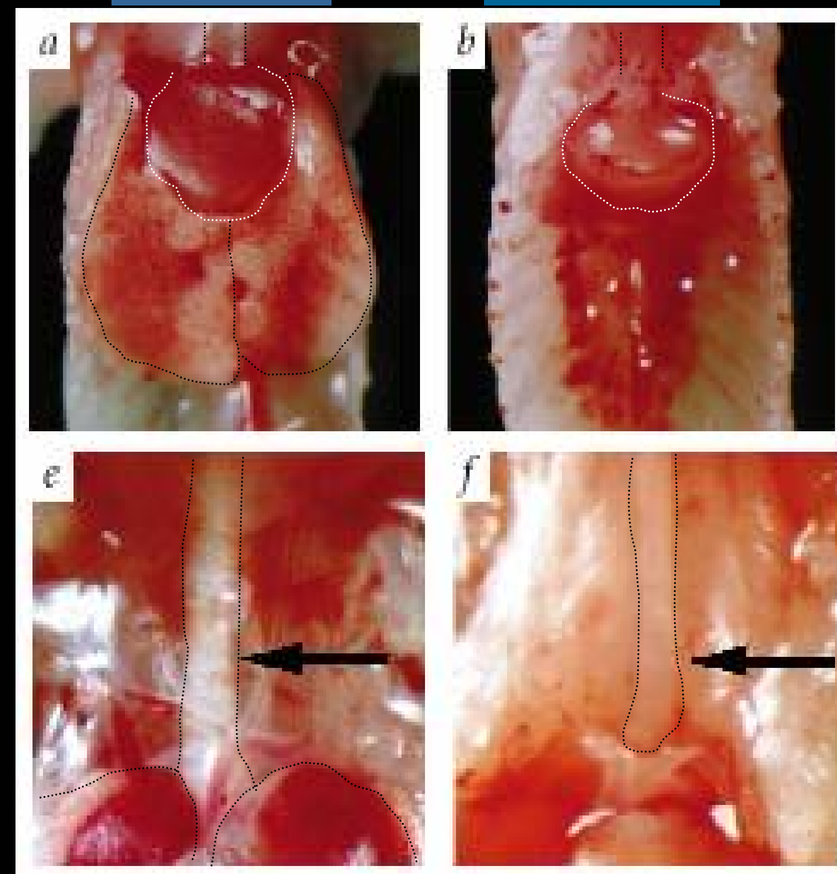
Control



Fgf10 -/-

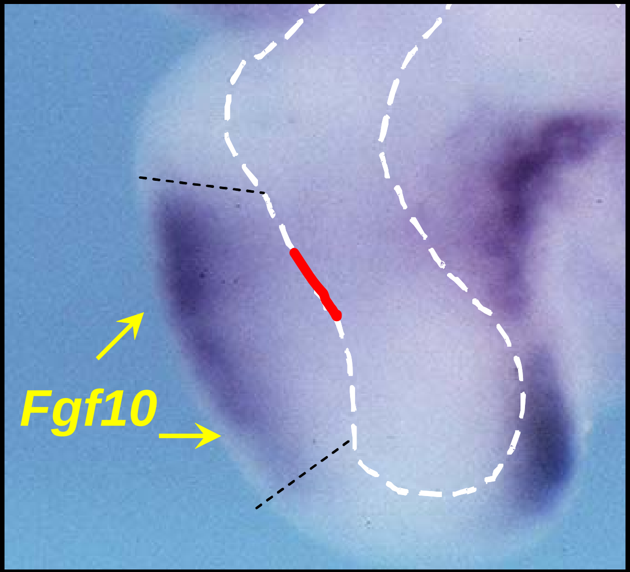


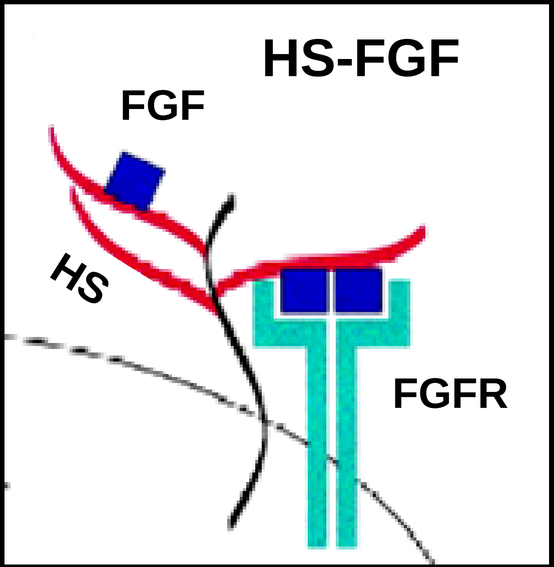
Control | Fgf10 -/-



Sekine et al. 1999

THE FGF MECHANISM IS REFINED BY HSPG

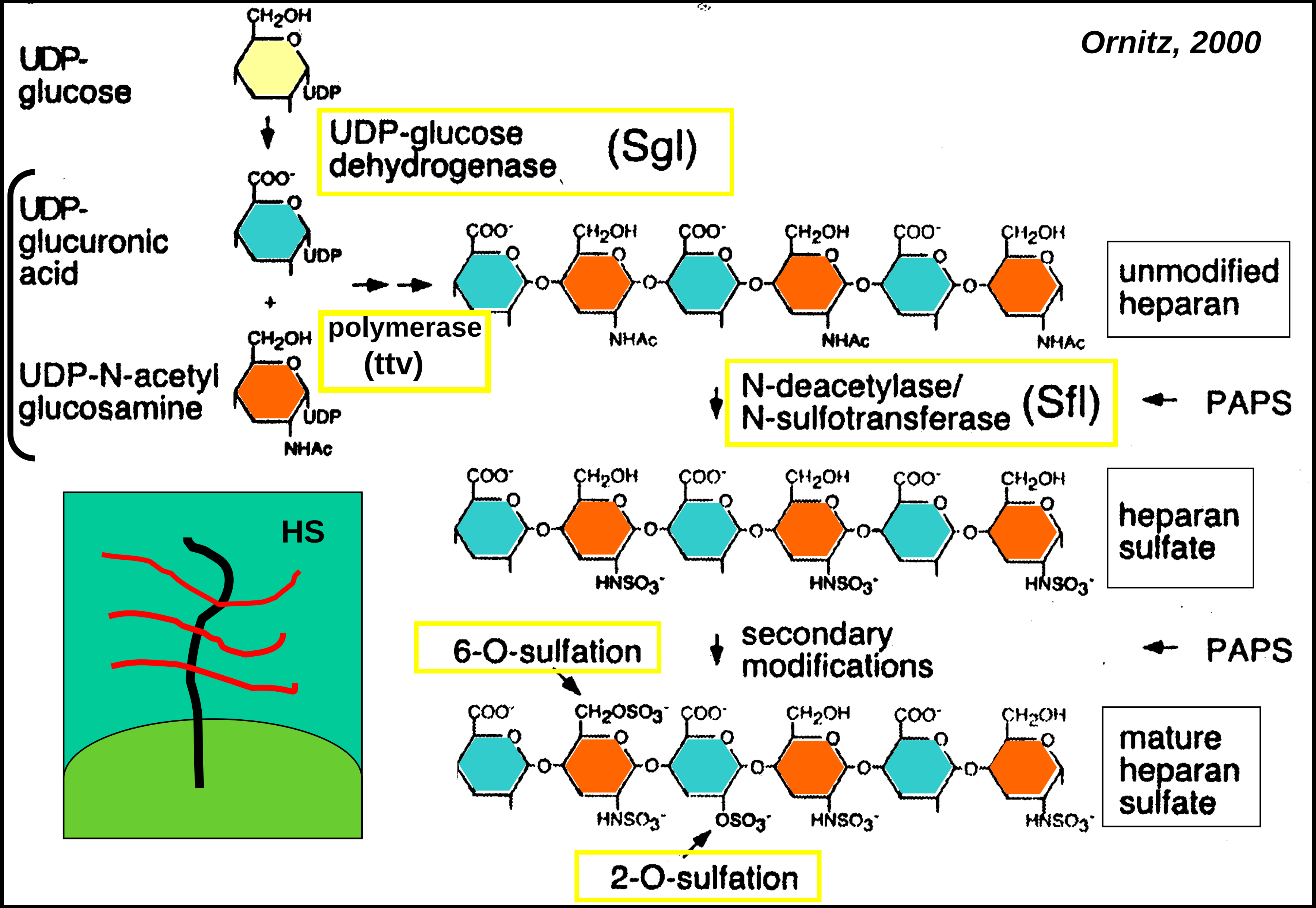




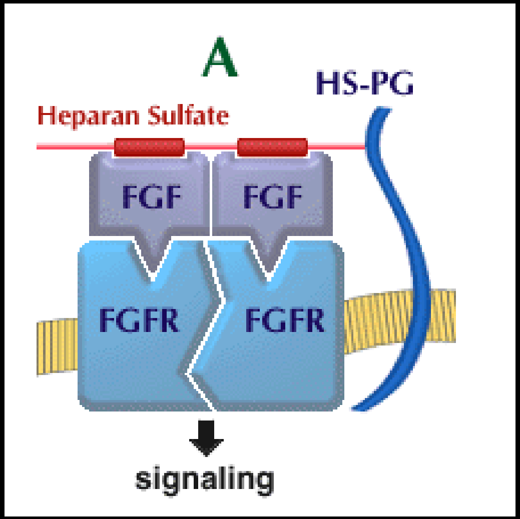
HS modulate FGF distribution, binding and signaling

(Ornitz, 2000; Nugent and Iozzo, 2000, Izvolsky et al., 2003)

HEPARAN SULFATE SYNTHESIS



HS are required for FGF-directed tracheal morphogenesis in *Drosophila*



HS:
- ***sugarless***
- ***sulfateless***
- ***DHs6t***

dHS6ST

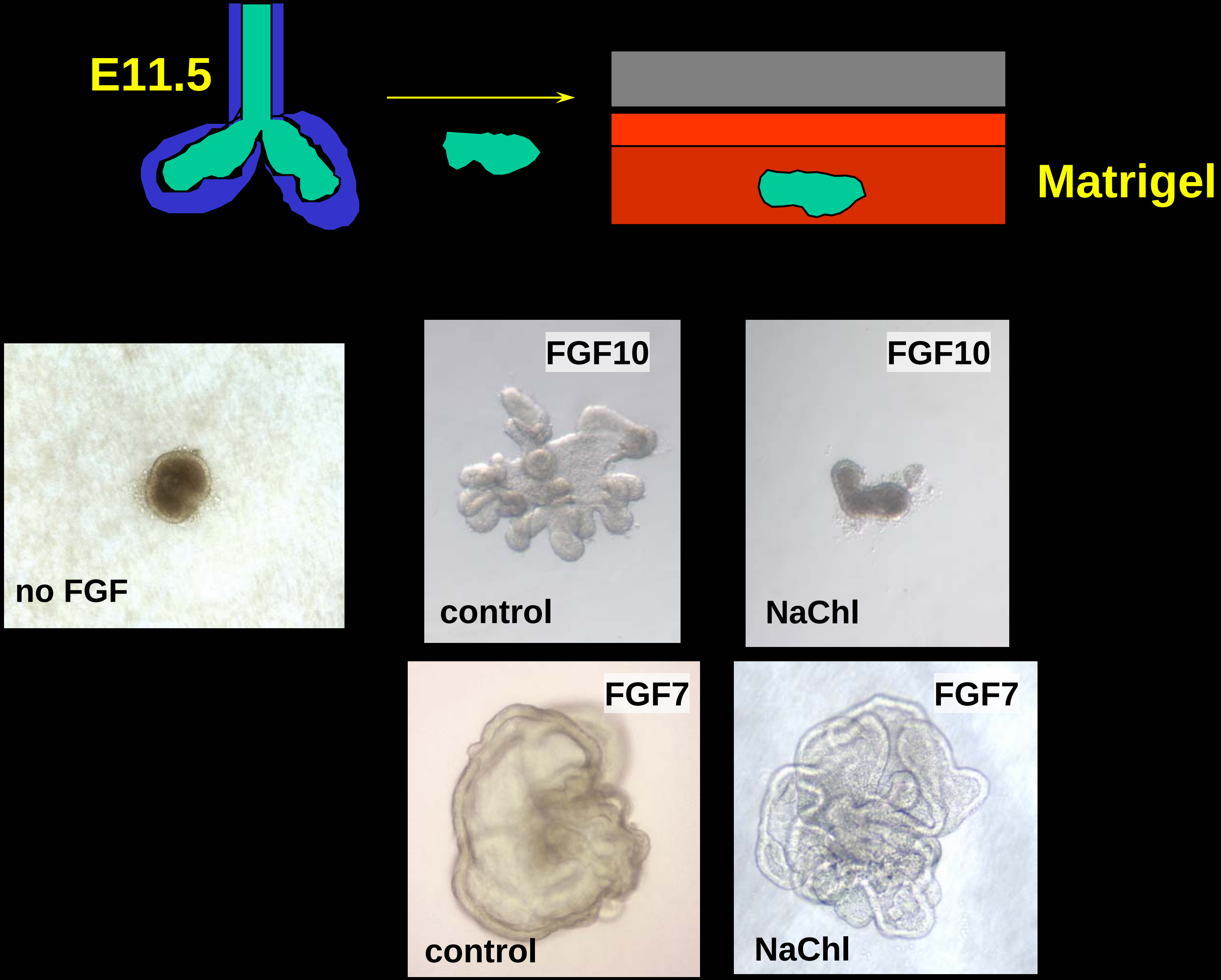
- *RNAi* reduced dHS6ST activity,

-disrupts FGF-dependent MAPK activation , tracheal branching and results in embryonic lethality

- phenotype similar to Fgf (*bnl*) mutant

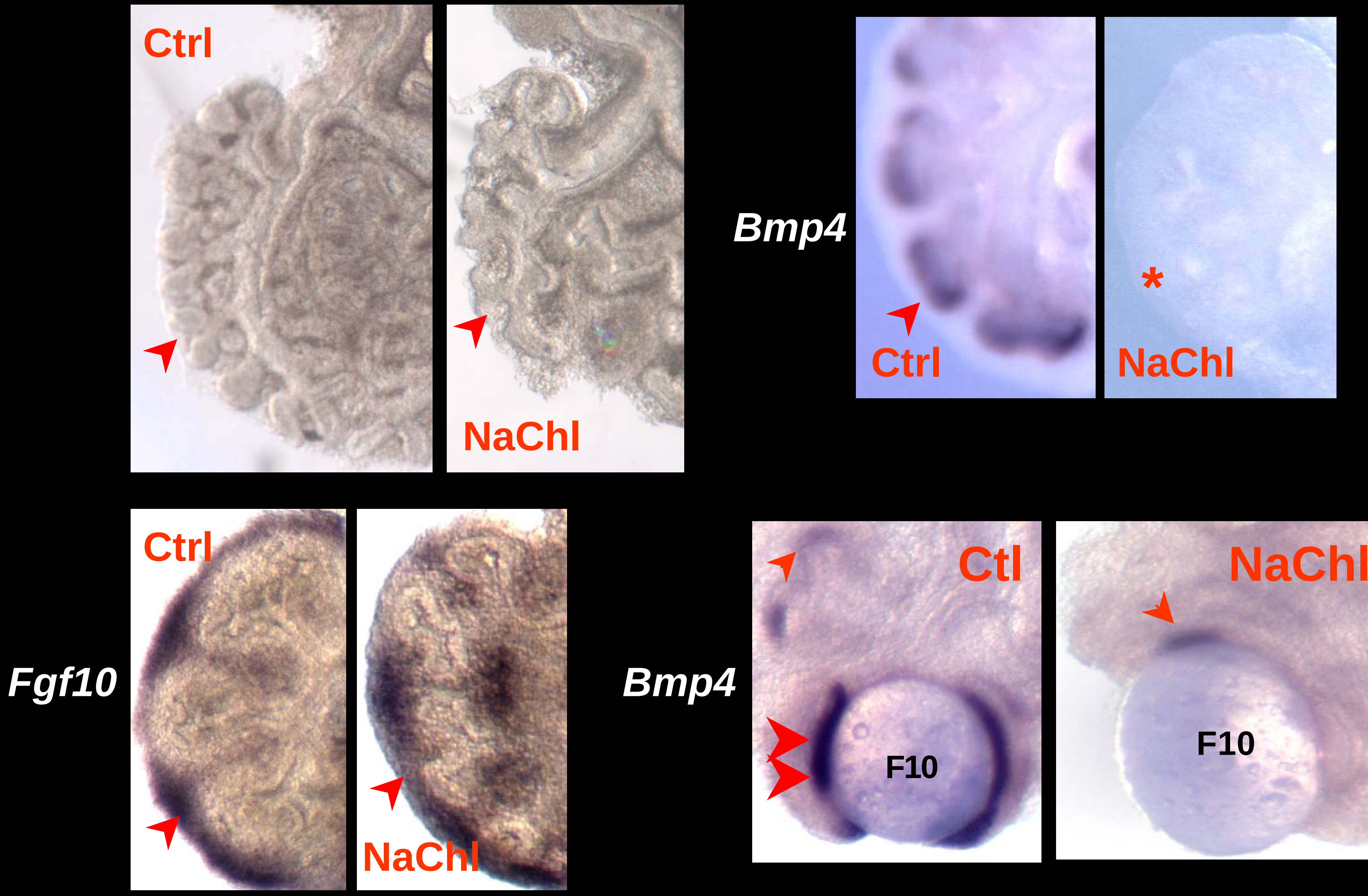
Lin et al., 1999; Toyoda et al., 2000; Kamimura et al., 2000

INTEGRITY OF HS IS REQUIRED FOR FGF10-INDUCED RESPONSES IN LUNG EPITHELIUM



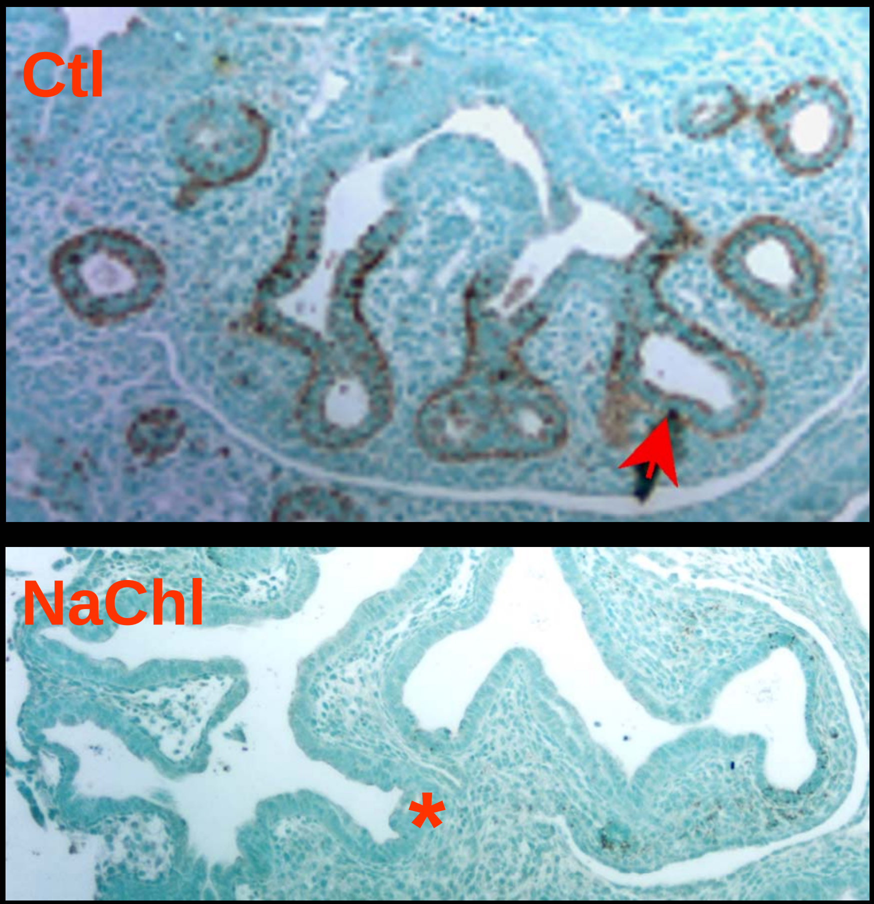
E11.5
Matrigel
no FGF
FGF10
control
FGF10
NaChl
FGF7
control
FGF7
NaChl

HS SULFATION IS REQUIRED FOR BRANCHING AND INDUCTION OF BMP4

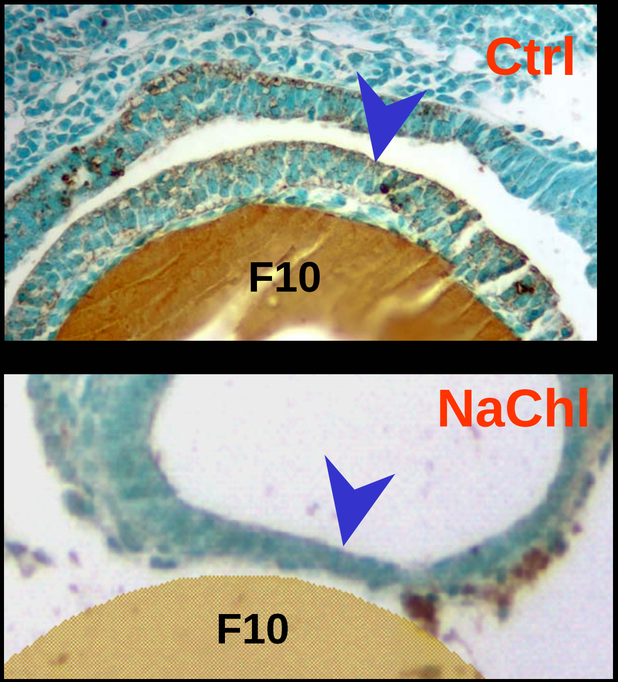

Ctrl
NaChl
Bmp4
Ctrl
*
NaChl
Fgf10
Ctrl
NaChl
Bmp4
Ctl
NaChl
F10
F10

PREVENTING HS SULFATION INTERFERES WITH FGF10 BINDING TO THE LUNG EPITHELIUM

C17

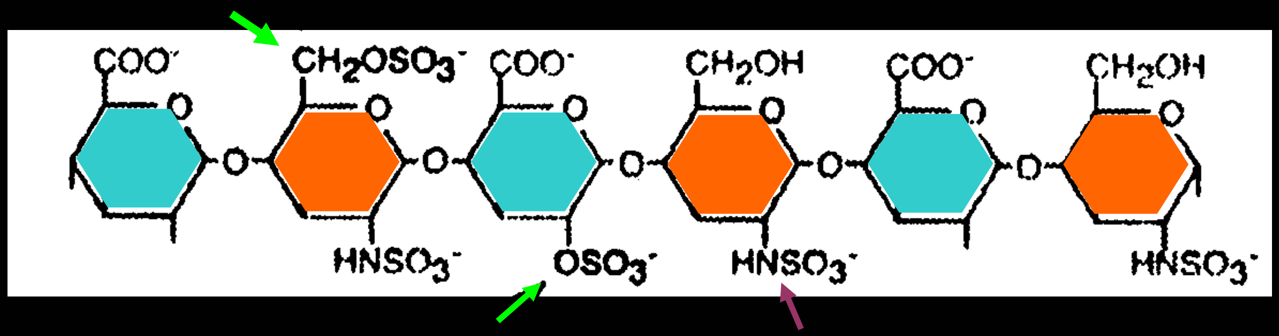


C17



CHEMICALLY MODIFIED HEPARINS

N- versus O-sulfation



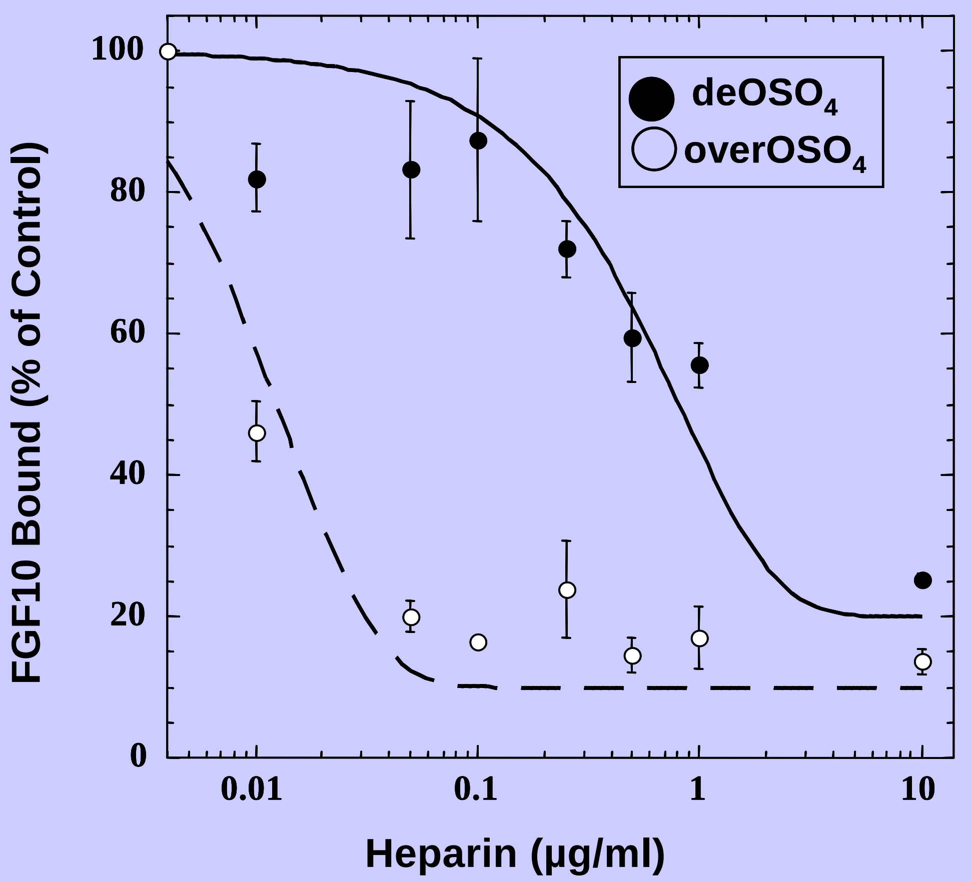
De-O Sulfated Heparin

- all O-SO4 is removed
- N-SO4 remains (amino group)

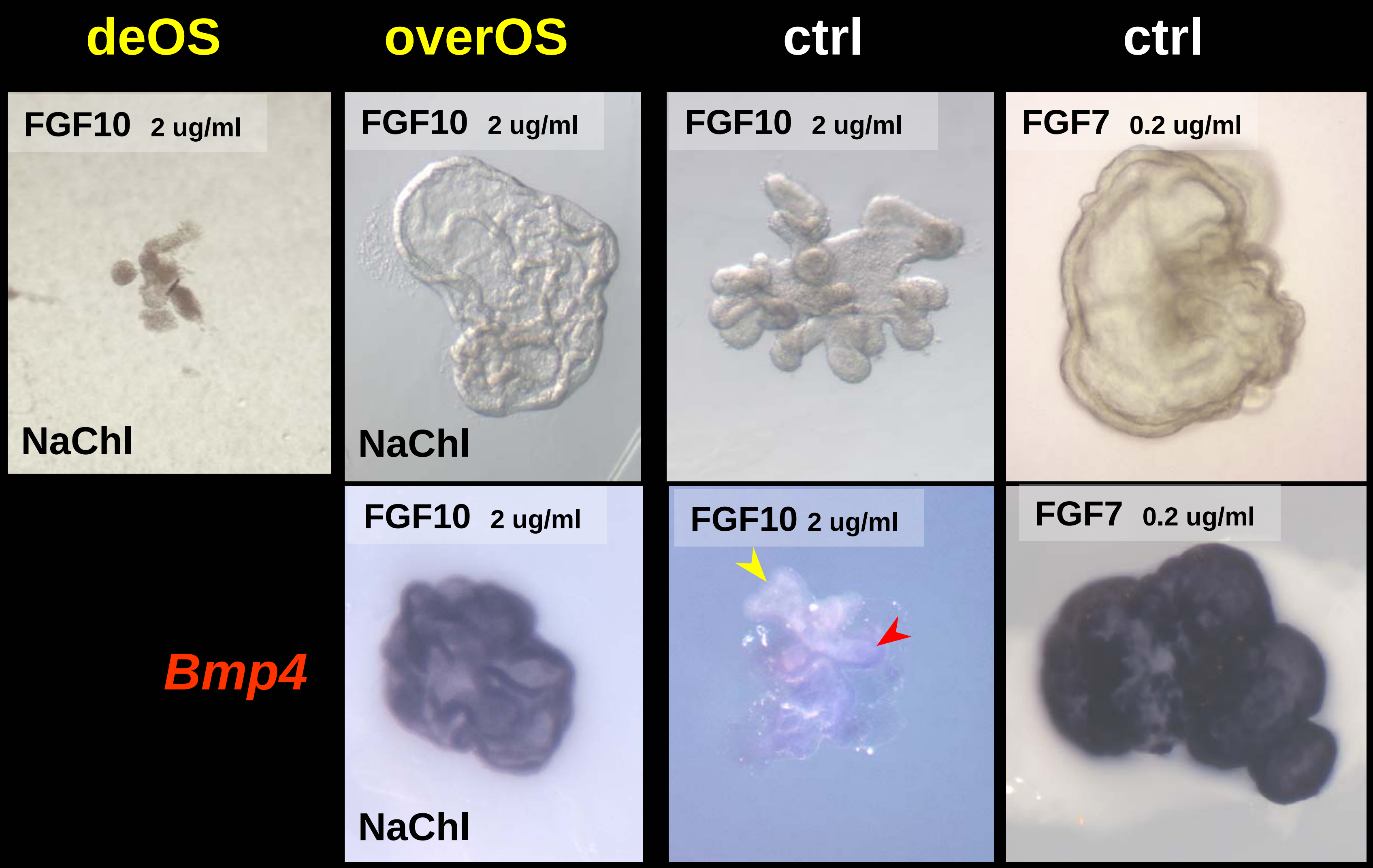
Over-O Sulfated Heparin

- Fully O-sulfated
- all free OH in glucosamine replaced by O-SO4

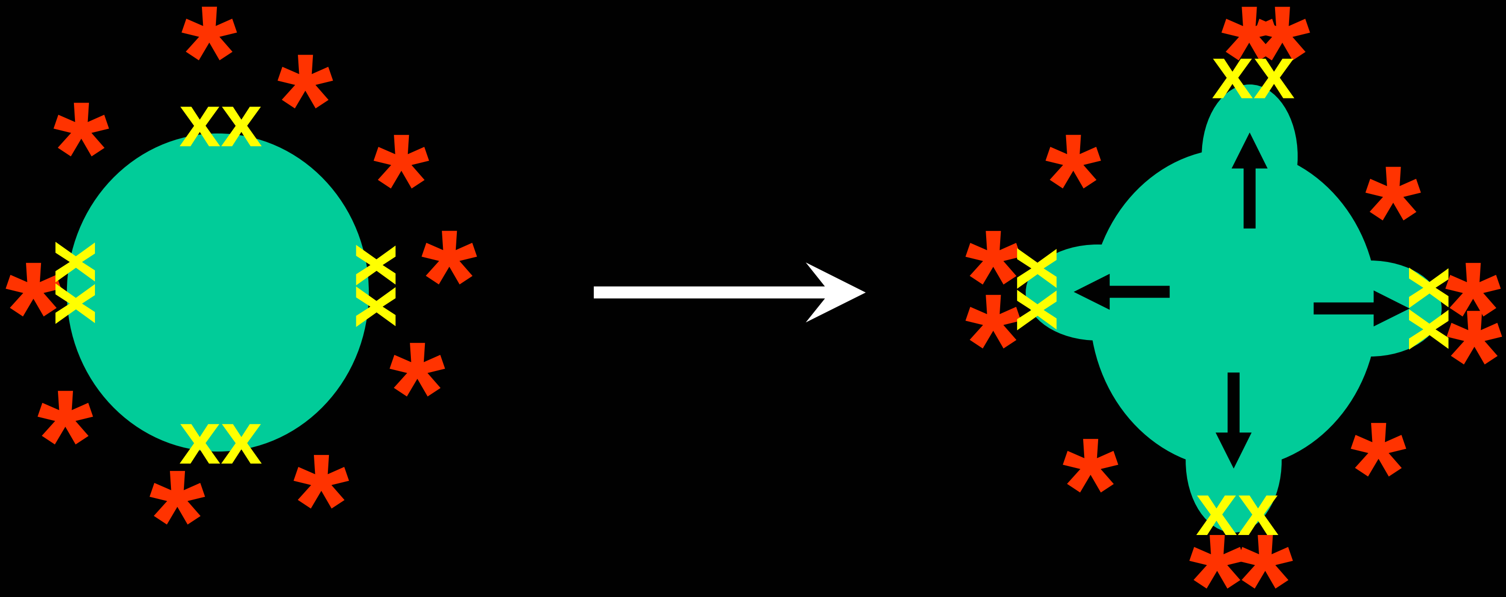
BINDING ASSAYS IN 20-3 EPITHELIAL CELLS



O- BUT NOT N-SULFATES RESCUE FGF10 SIGNALING


deOS
overOS
ctrl
ctrl
FGF10 2 ug/ml
NaChl
FGF10 2 ug/ml
NaChl
FGF10 2 ug/ml
FGF7 0.2 ug/ml
Bmp4
FGF10 2 ug/ml
NaChl
FGF10 2 ug/ml
FGF7 0.2 ug/ml

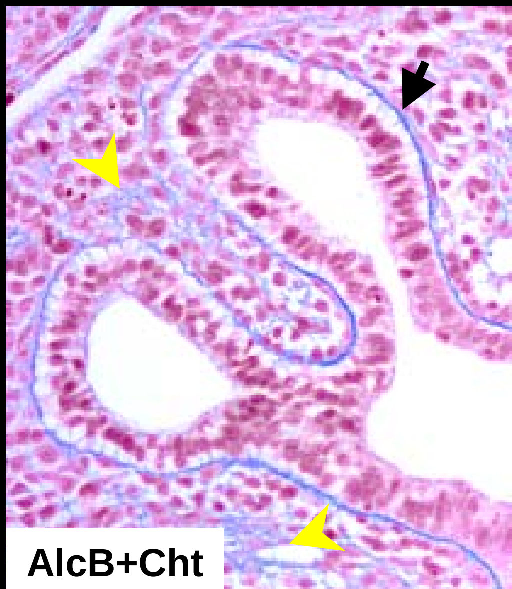
ABILITY OF FGF10 TO INDUCE LOCALIZED EPITHELIAL RESPONSES

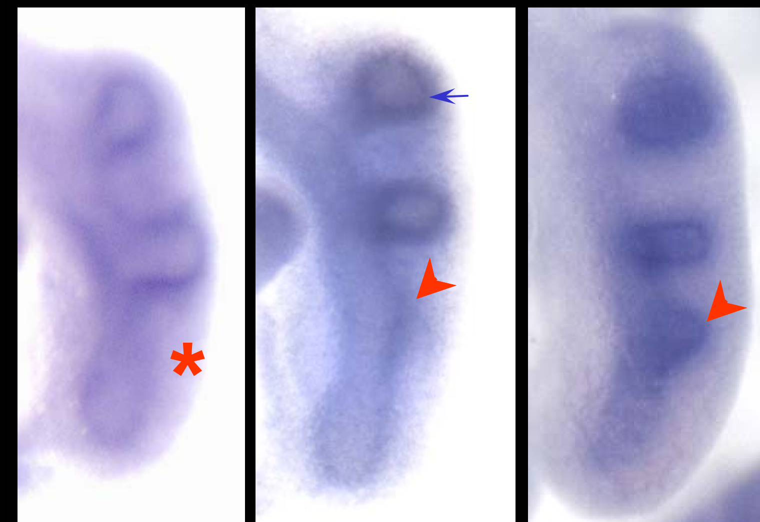


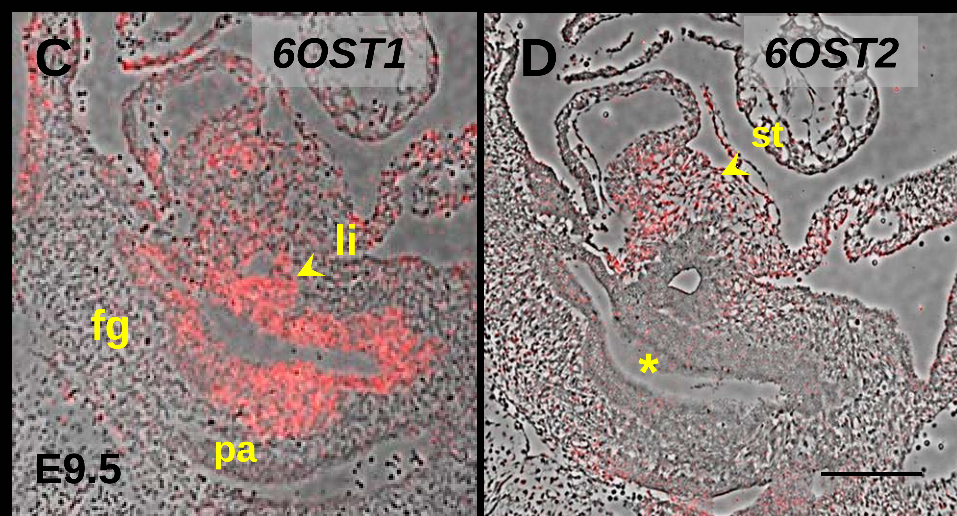
LOCAL GRADIENTS OF ENDOGENOUS SULFATED GAGs

HOW ARE HS EXPRESSED IN THE EMBRYONIC LUNG?

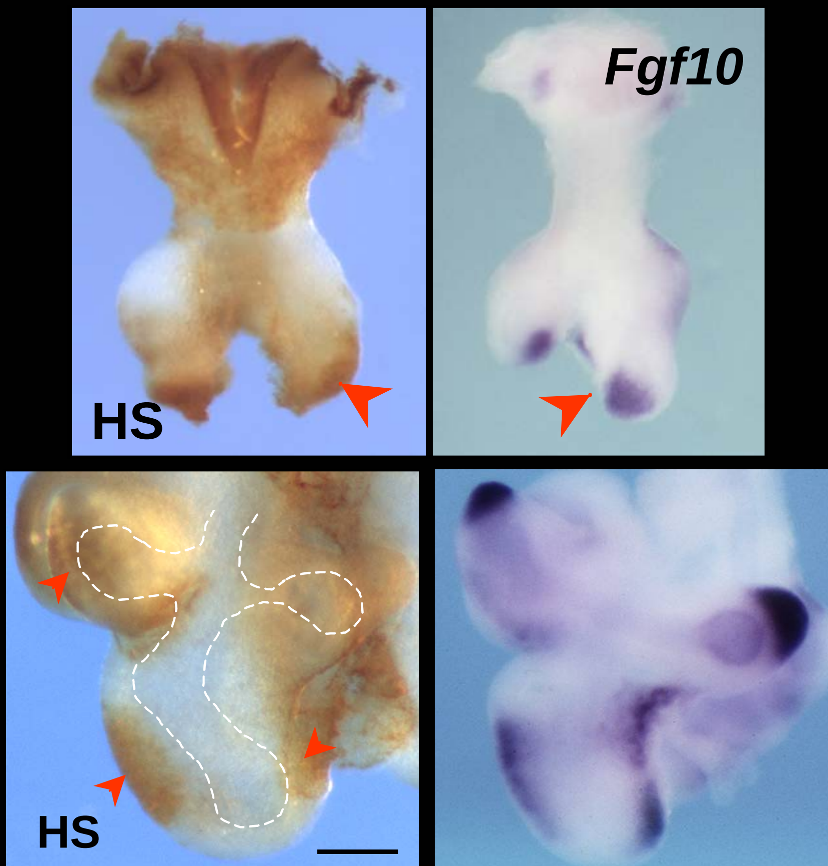
HS - FGF10 INTERACTIONS

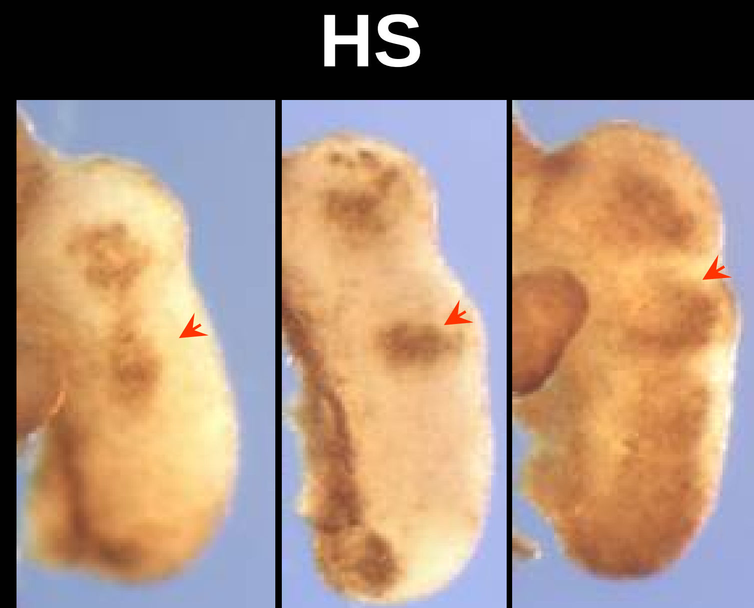




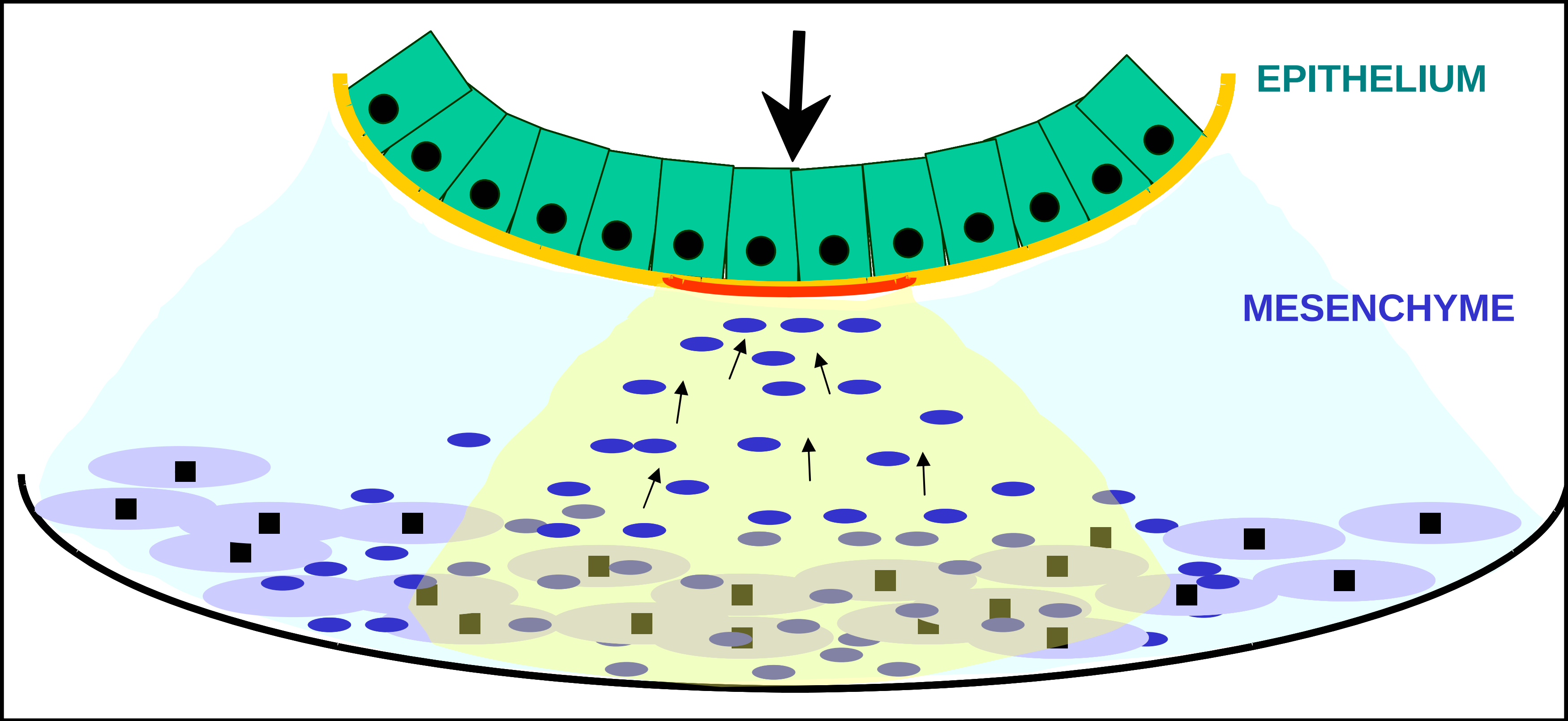


HS 10E4 EPITOPE IS DYNAMICALLY EXPRESSED AT SITES OF Fgf10 EXPRESSION





HS-FGF10 INTERACTIONS DURING BRANCHING



SUMMARY

I. DESIGN

diversity in the respiratory tract as an adaptation for breathing in different environments

II. EVOLUTION & DEVELOPMENT

possibility of studying mechanisms that regulate fate and morphogenesis of the posterior pharynx

III. MODEL SYSTEMS

FGF – HS : branching morphogenesis
Drosophila and mice

University Western Ontario , Canada

Michael Underhill

Columbia University

Cathy Mendelsohn

IGBMC , France

Pascal Dolle, Karen Niederreither, Pierre Chambon