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

Oxygenated blood to heart
Deoxygenated blood from heart
Pulmonary venule
Bronchus
Bronchiole
Alveoli
Smallest blood vessels (capillaries)

Pulmonary arteriole
LUNG DEVELOPMENT (MOUSE)

- **Primary budding**: E9.5
- **Branching morphogenesis**: E10.5, E12.5
- **Sacculation**: E18.5

**Alveolization**: PN12
RESPIRATORY SYSTEM DESIGN

**BIRDS**
- Trachea
- Bronchus
- Air space in bone
- Lung containing parabronchi
- Air sacs

**INSECT**
- Tracheoles
- Air sacs
- Tracheae
- Spiracles
RESPIRATORY SYSTEM DESIGN

FISH

Gills, Lungs & Swimbladder

- Internal Gills in Fish Enable Countercurrent Exchange
- Gill arches (under opercular flap)
- External gills
- Gill filaments
- Deoxygenated blood
- Oxygenated blood
- Water flow
- Lamellae
- Red blood cells
- Afferent vessel
- Efferent vessel
- Deoxygenated blood
- Oxygenated blood

- Lungfish
- Primitive bony fish
- Modern bony fish
- Swim bladder
- Air sacs
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?

*Bothriolepis canadensis*
- 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
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-/-**

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

Niederreither et al. *Nature Genetics*, 1999
- *Ttf1* is present in the prospective lung endoderm of wild type and *Raldh2* 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

A Liver Induction  B Lung Induction  C Refinement

6-7 somites  7-8 somites  9.5 days

- FGFR1 endoderm
- FGFR1 and FGFR4 positive endoderm
- albumin expressing cells
- Nkx2.1 and albumin co-expressing cells
- Nkx2.1 expressing cells
- Septum transversum mesenchyme

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

- SEPARATION OF THE RESPIRATORY AND DIGESTIVE TRACTS

Shh (endoderm)

Clark, 1999
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>
<thead>
<tr>
<th>Mutation (abbreviation)</th>
<th>Shibaip</th>
<th>Gas Bladder</th>
<th>Other Phenotypic Abnormalities Detected by External Examination and Study of Cleared and Stained Specimens</th>
<th>Day First Seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>anchor (an)</td>
<td>15</td>
<td>Absent</td>
<td>None</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>ballast (bl)</td>
<td>13</td>
<td>Absent</td>
<td>Lower jaw narrow; notochord wavy</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>ballissoneyes (boc)</td>
<td>1</td>
<td>+</td>
<td>Edema around eyes</td>
<td>5</td>
</tr>
<tr>
<td>bent (bou)</td>
<td>7</td>
<td>Absent</td>
<td>None</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>big bladder (bg)</td>
<td>10</td>
<td>+</td>
<td>Gas bladder overfilled by day 5 and fish floats at surface; by day 9, bladder has resumed normal size; a few big bladder individuals survived beyond day 28</td>
<td>5</td>
</tr>
<tr>
<td>bladderless (blh)</td>
<td>9</td>
<td>Absent</td>
<td>Lower jaw narrow; notochord wavy</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>bloated (bl)</td>
<td>13</td>
<td>+</td>
<td>Extensive edema ventrally, moves only intermittently</td>
<td>6-7</td>
</tr>
<tr>
<td>blockhead (bh)</td>
<td>13</td>
<td>Absent</td>
<td>Squared off head, eye and body edema</td>
<td>4</td>
</tr>
<tr>
<td>bosom heavy (brn)</td>
<td>4</td>
<td>Absent</td>
<td>None</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>candycone (cck)</td>
<td>14</td>
<td>Absent</td>
<td>Spine curved in candy cane shape; small eyes, heart edema; widespread edema</td>
<td>3</td>
</tr>
<tr>
<td>darkeyes (eph)</td>
<td>1</td>
<td>+</td>
<td>Dark granular yolks; heart, eye, or body edema</td>
<td>1</td>
</tr>
<tr>
<td>dividing unusually (dv)</td>
<td>14</td>
<td>NA</td>
<td>Cells dividing unusually (~1.5 h), yolks peaking through cluster of cells at 5 h, dead by 24 h, long before gas bladder inflation</td>
<td>0</td>
</tr>
<tr>
<td>extra bubbles (eb)</td>
<td>14</td>
<td>Absent</td>
<td>None; refers to transient air bubbles in the gut of mutants during the time that normals are filling their gas bladders</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>jaw deformity (jdn)</td>
<td>13</td>
<td>Absent</td>
<td>Head stubby, snout does not project anterior to eye, pronounced lower jaw, upper jaw not clear, lenses do not protrude from eye, pectoral fins bent, edema around otic capsule</td>
<td>3</td>
</tr>
<tr>
<td>jawless eyestalks (jes)</td>
<td>14</td>
<td>Absent</td>
<td>Small head, small eyes with large lenses, extensive eye edema, jaw small or absent, ventral edema, stubby tail</td>
<td>1</td>
</tr>
<tr>
<td>kinky (knk)</td>
<td>1</td>
<td>Absent</td>
<td>None</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>knockout (kno)</td>
<td>10</td>
<td>Absent</td>
<td>Eyes small, head knobby anteriortly, lacks jaws, heart edema, lacks pectoral fins</td>
<td>2</td>
</tr>
<tr>
<td>knockhead (kha)</td>
<td>3</td>
<td>Absent</td>
<td>Radial spot on yolk, heart edema starting day 3, head flat anteriortly, head knobby dorsally, s-shaped spine</td>
<td>2</td>
</tr>
<tr>
<td>large lens (lh)</td>
<td>5</td>
<td>Absent</td>
<td>Eyes small with lens protruding from eye, jawing jaw develops by day 9</td>
<td>3-4</td>
</tr>
<tr>
<td>lead weight (lw)</td>
<td>11</td>
<td>Absent</td>
<td>None</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>pseudeyeless (psy)</td>
<td>8</td>
<td>Absent</td>
<td>Eyes small and low on head; head knobless anteriortly, jaws and all arches poorly defined or absent; s-shaped spine with poor differentiation of neural tube, somites, and muscles; pectoral fins absent; some grainy yolk still present day 5; poor circulation; head and heart edema</td>
<td>2</td>
</tr>
<tr>
<td>pseudeyeless (psu)</td>
<td>11</td>
<td>Absent</td>
<td>Eyes small, head knobless anteriortly, lacks jaws; gut poorly developed; heart and eye edema; head and yolk granular on day 1</td>
<td>1</td>
</tr>
<tr>
<td>zither (zhe)</td>
<td>10</td>
<td>Absent</td>
<td>Pigmentation reduced; Meckel's cartilage and ceratohyal bent; notochord wavy; pectoral fins and mouth edema similar to normal</td>
<td>~ 4-5</td>
</tr>
<tr>
<td>spiograph (sph)</td>
<td>8</td>
<td>Absent</td>
<td>Spine curved, almost circular, moves by spinning in circles; severe head edema; pectoral fins present</td>
<td>3</td>
</tr>
<tr>
<td>stiffjaw (stj)</td>
<td>14</td>
<td>Absent</td>
<td>Jaw appears to be frozen open; eyes and head slightly reduced, lower jaw curved and projects anteriorto upper jaw; pigmentation reduced; some develop edema before death</td>
<td>3</td>
</tr>
<tr>
<td>ventral edema (ved)</td>
<td>8</td>
<td>Absent</td>
<td>Edema extensive ventrally, moves only intermittently</td>
<td>5</td>
</tr>
<tr>
<td>windy (wil)</td>
<td>12</td>
<td>Absent</td>
<td>Almost circular curved spine moves or spins in circles</td>
<td>2</td>
</tr>
</tbody>
</table>

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.zfro.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 DROSOFLULA

FGF SIGNALING

FGF: *branchless*
FGFR: *breathless*

Ghabrial et al., 2004
FIBROBLAST GROWTH FACTOR SYSTEM

Belluscí et al., 1997; Cardoso et al. 1996; Park et al., 1998, Weaver et al., 2000
FGF10 IS ESSENTIAL FOR BUDDING

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

Ornitz, 2000

UDP-glucose dehydrogenase (Sgl)

polymerase (ttv)

UDP-glucose

COO-

CH2OH

UDP

COO-

CH2OH

UDP

COO-

N-HAc

UDP-N-acetyl glucosamine

N-deacetylaselase/ N-sulfotransferase (Sfl)

unmodified heparan

PAPS

heparan sulfate

6-O-sulfation

secondary modifications

2-O-sulfation

PAPS

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

- **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
HS SULFATION IS REQUIRED FOR BRANCHING AND INDUCTION OF BMP4
PREVENTING HS SULFATION INTERFERES WITH FGF10 BINDING TO THE LUNG EPITHELium

C17

C17

Ctrl

F10

NaChl

F10
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

![Graph showing FGF10 Bound as a function of Heparin concentration for deOSO₄ and overOSO₄.](image)
O- BUT NOT N-SULFATES RESCUE FGF10 SIGNALING
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

AlcB+Cht

6-OST1

E 11.75

6-OST1

E 14.5

ht li

C 6OST1

E9.5

fg pa

D 6OST2

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