Tetrapod Limb Development

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Limb Development - Overview

Patterning
Early development
Specification
Establishing limb axes
Morphogenic models
Cross-talk / regulation
Limb Patterning

- Stylopod
- Zeugopod
- Autopod

Human arm
- Humerus
- Radius
- Ulna
- Carpals
- Metacarpals
- Phalanges

Chicken wing
- Shoulder
- Finger
- Pinkie
- Palm
- Knuckle
- Thumb

Chicken leg
- Proximal
- Distal
- Anterior
- Dorsal
- Ventrax

Human arm
- Proximal
- Distal
- Anterior
- Dorsal
- Ventrax

Chicken wing
- Proximal
- Distal
- Anterior
- Dorsal
- Ventrax

Chicken leg
- Proximal
- Distal
- Anterior
- Dorsal
- Ventrax
Limb Bud Formation

- Epaxial myotome bud
- Central dermatome
- Hypaxial myotome bud
- Limb muscle precursors
- Limb bud
- Limb skeletal precursors
- Lateral plate mesoderm
- Myotome
- Sclerotome
- Spinal cord
- Notochord
- Pronephron
- Endoderm
Hoxc6 Specification of Limb Buds

Mouse
Hoxa
Hoxb
Hoxc
Hoxd

Mouse embryo (12 days)

Neural tube
Somites

Chick
Cervical
Thoracic
Lumbar
Sacral
Coccygeal
Vertebrae
Somites

Mouse
Cervical
Thoracic
Lumbar
Sacral
Caudal

Occipital Cervical Thoracic Lumbar Sacral Caudal
Tbx Genes Specify Limb Type

Stage 14/15 (early day 3)

(A) Normal

Forelimb bud

Wing

Hindlimb bud

Leg
Fgf/Wnt - Limb Bud Initiation

(A) 48 h
- Somitic mesoderm
- Intermediate mesoderm
- Fgf10
- Wnt8c

(B) 50 h
- Lateral plate mesoderm
- Fgf10
- Wnt2b

(C) 54 h
- Surface ectoderm
- Fgf10
- Wnt3a
- Fgf8
- AER

Legend:
- Fgf8
- Fgf10
- Fgf10 (stabilized)
- Wnt2b/8c
- Wnt3a
Limb Bud Axes

- Proximal
- Distal
- Anterior
- Posterior
- Dorsal
- Ventral
Apical Ectodermal Ridge (AER) forms at boundary between dorsal and ventral ectoderm.
Apical Ectodermal Ridge Manipulation

- AER removed: Limb development ceases
- Extra AER: Wing is duplicated
- Leg mesenchyme: Leg
- Nonlimb mesenchyme: Wing
- AER replaced by FGF bead: AER regresses; limb development ceases

Forelimb mesenchyme
Progress Zone

Apical Ectodermal Ridge (AER) establishes Progress Zone

- Fgf8
- Fgf4

~200 μm

Progress Zone (PZ)
**Proximal-Distal Specification Models**

**Progress zone model**: Identity established by residence time in PZ

**Early allocation and progenitor expansion**: Elements specified early

Specifying mechanism - ??
Zone of Polarizing Activity (ZPA)

**Shh** necessary and sufficient for establishing ZPA

(Note – Shh not necessary for polarity of stylopod)

Shh induced by **dHAND** and **Hoxb8**

ZPA maintained by feedback loop with AER
1. dHAND (transcription factor) and Fgf8 (from AER) stimulate Shh
   - Fgf8 (and Fgf4) maintains Shh expression

2. Shh up-regulates Gremlin1 in posterior mesenchyme
   - Grem1 antagonizes BMP ligands
   - BMPs repress Fgf expression in AER

3. Wnt7a maintains Shh
   Wnt7a determines the size of AER

Loss-of-function mutants (both Shh and Grem1) = syndactyly, loss of digits
ZPA Transplantation

Posterior tissue transplant to anterior = duplicated autopod

Mirror-image duplication effects can be replicated by transplanting Shh bead

Retinoic acid operates upstream of Shh
- implant RA-soaked bead = mirror-image duplication

"new" posterior

Shh Specifies Digit Identity

(A) Digit 1: Shh-independent
(B) Digit 2: Shh concentration
(C) Digit 3: Shh time of expression and concentration
(D) Digits 4–5: Shh time of expression

Legend:
- Shh diffusion
- Shh descendants
Drosophila Hedgehog Pathway

Hedgehog
Patched
Smoothened
Ci protein made activator
Transcription

Cytoplasm
Patched inhibits smoothened
Microtubule
PKA
Cos2
Fused
Ci
Slimb

Repressor
No transcription of Hedgehog-responsive genes

Activation
Transcription of Hedgehog-response genes

Sonic hedgehog (vertebrates)
Smoothened inhibits PKA and Slimb
PKA
Cos2
Fused
Ci
Slimb

Gli1, 2, 3 (vertebrates)
Vertebrate Hedgehog Activity

Vertebrate homologues:
- Hh - Sonic hedgehog (Shh), plus others
- Ci – Gli (Gli1, Gli2, Gli3)

Without SHH:
- Gli3 – proteolytic fragment – acts as a transcriptional repressor (Gli3R)
  - represses e.g. dHAND, Gremlin, Fgf4, Hoxd13

With SHH signal:
- Gli’s retained in long form – acts as a transcriptional activator
  - e.g. Gli1 activates Shh

$Gli3^{-/-}$ = polydactyly; (~)8 digits; unpatterned
$Shh^{-/-}$ = 1 digit; Gli3R prevails

SHH main function may be to relieve Gli3R repression in posterior region
Shh/Gli3 Knockouts

**Shh digit specification:**

- 5,4 - autocrine
- 3 – autocrine and diffusion (paracrine)
- 2 – diffusion (paracrine)
- 1 – not Shh-dependent

**Shh knockout** – one digit

**Shh**<sup>−/−</sup>

Shh/Gli3 knockouts – multiple digits

#1 or unspecified variants

**Shh**<sup>−/−</sup>/Gli3<sup>−/−</sup>
Hox Genes in Early Limb Bud

(A) ELCR Anterior

(B) Hox1 (repressor)

(C) Initial ZPA

ELCR

Hox1

Hox11

Hox12

Hox13

GCR Posterior

Gli3

dHand

Shh
5’ Hox Genes Pattern Limb Elements

Forelimb

Hox paralog group 13

Hox paralog group 12

Hox paralog group 11

Hox paralog group 10

Hox paralog group 9

Hindlimb

Hox paralog group 13

Hox paralog group 12

Hox paralog group 11

Hox paralog group 10

Hox paralog group 9

Stylopod

Zeugopod

Autopod
**BMPs Regulate Digital Identity**

Shh initiates BMP2 and BMP7 gradients

- BMPs in interdigital mesoderm specifies identity of digits anteriorly
  - BMP targets unknown

**NOTE**

- Fgfs from AER control phalange development; Shh bead inserted between digits can add phalange; Shh sustains Fgf signal; Fgf inhibitor = lack of phalange

**Remove interdigital mesoderm**

**Insert BMP antagonist into interdigital webbing**

**Noggin – BMP antagonist**
**Dorsal-Ventral Specification**

- **Zone of Polarizing Activity (ZPA)**
- **Apical Ectodermal Ridge (AER)**
- **Progress Zone (PZ)**

**Ectoderm**

- **Wnt7a** – necessary and sufficient to dorsalize limb bud
  - **Wnt7a** knockouts = ventral footpads on both surfaces
  - induces **Lmx1** in dorsal mesenchyme
  - **Lmx1** knockouts = ventralized phenotype
Apoptosis in Limb Primordia

(A) DUCK LEG PRIMORDIUM
Minimal cell death

(B) CHICK LEG PRIMORDIUM
Extensive cell death

BMP signals