Tetrapod Limb Development

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Tetrapod Limbs

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Limb Patterning

- **Stylopod**
  - Humerus
  - Radius
  - Ulna

- **Zeugopod**
  - Carpals
  - Metacarpals

- **Autopod**
  - Phalanges

**Human arm**

**Chicken wing**

**Chicken leg**

**Orientation**
- Anterior
- Ventral
- Distal
- Posterior
- Dorsal
- Proximal

**Anatomical parts**
- Humerus
- Radius
- Ulna
- Carpals
- Metacarpals
- Phalanges
- Shoulder
- Knuckle
- Palm
- Finger
- Thumb
- Pinkie
Endochondral Bone Development

Epiphyseal cartilage

Compact nodules

Growth plate

Proliferating chondrocytes

Hypertrophic chondrocytes

Osteoblasts

Blood vessel

Proliferating chondrocytes

Secondary ossification center
Somite Formation

- 26-29 h
- 29-33 h
- 33-38 h
- 40-45 h
- 50-53 h
- 55+ h
Limb Buds

Fgf10 expression
Vertebrate Limb Buds

Hox expression determines limb bud location

Hox5
Hox6

Limb bud
Ectoderm

Somites
Lateral plate mesoderm
Mesodermal mesenchyme
Forelimb initiation: - anterior-most point of *Hoxc6* expression
**Tbx Genes Specify Limb Type**

NOTE – *Tbx* genes are not the first step in forelimb/hindlimb specification
- initial step(s) unknown
- *Tbx*’s initiated by Wnts, FGFs
Fgf/Wnt - Limb Bud Initiation

(A) Somitic mesoderm
Intermediated mesoderm

(B) Lateral plate mesoderm
Surface ectoderm

(C) Wnt2b
Fgf10
Wnt8c
Wnt3a
Fgf8
AER

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

Mesoderm

Proximal

Ventral

Posterior

Anterior

Ectoderm

Dorsal

Distal

Shoulder

Knuckle

Pinkie

Palm

Finger
**Apical Ectodermal Ridge (AER)** forms at boundary between dorsal and ventral ectoderm

Lateral plate mesoderm expresses Fgf10
Fgf10 initiates AER via Wnt3a, β-catenin
AER expresses Fgf8, Fgf4; maintains Fgf10 expression
Apical Ectodermal Ridge Manipulation

- **AER removed**
  - Extent of development depends on time of AER removal
  - Limb development ceases

- **Extra AER**
  - Wing is duplicated
  - Distal structures are duplicated

- **Forelimb mesenchyme**
  - Degree of “legness” of wing depends on placement of leg mesenchyme

- **Leg mesenchyme**
  - Leg

- **Nonlimb mesenchyme**
  - Wing

- **AER replaced by FGF bead**
  - Normal wing
  - AER regresses; limb development ceases
**Progress Zone** – mesodermal mesenchyme; receives AER signals:
- promotes proliferation (mitosis)
- prevents differentiation into cartilage
- maintains expression of A/P and D/V-related signals

PZ mesenchyme specifies proximal-distal axis
- transplantation experiments demonstrated that positional information was carried by PZ cells
- PZs conveyed age-appropriate specification instructions
Proximal-Distal Specification Models

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

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

Specifying mechanism - ??
5’ Hox Genes Pattern Limb Elements

Forelimb

Hox paralog group

13
12
11
10
9

Hindlimb

Hox paralog group

13
12
11
10
9

Stylopod

Zeugopod

Autopod
**Anterior-Posterior Specification**

**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

**NOTE** – preaxial polydactyly (PPD) models showed that Shh expression is controlled by a long-range enhancer; located in Lmbr1 locus; ~1Mb away from Shh; Lmbr1 locus contains ZPA Regulatory Sequence (ZRS); ZRS contains core region – Mouse Fish Conserved Sequence 1 (MFCS1)

5’ HoxD proteins bind to ZRS/MFCS1  
- Hoxd8 induces Shh  
- provides regulatory options for Hox proteins
1. dHAND - bHLH transcription factor and Fgf8 from AER stimulate Shh
   - Fgf8 (and Fgf4) maintains Shh expression

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

3. Wnt7a maintains Shh
   Wnt7a determines the size of the AER

Loss-of-function mutants (both Shh and Gremlin1) = syndactyly, loss of digits
Drosophila Hedgehog Pathway

Hedgehog
Patched
Smoothened
Ci protein made activator
Transcription

Cytoplasm
Patched inhibits smoothened

Microtubule
Cos2
Fused
Ci
Repressor
No transcription of Hedgehog-responsive genes

PKA
Smoothened inhibits PKA and Slimb
Slimb

Activation
Transcription of Hedgehog-response genes

CBP

Drosophila
Vertebrate Hedgehog Activity

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

Pathway activity
- SHH absent:
  - Gli3 – proteolytic fragment – acts as a transcriptional repressor (Gli3R)
    - represses e.g. dHAND, Gremlin, Fgf4, Hoxd13
- SHH present:
  - Gli’s retained in long form – acts as a transcriptional activator
    - e.g. Gli1 activates Shh
ZPA Morphogen Gradient

Shh gradient

High [Morphogen] Low

Posterior  Anterior

1  2  3  4  5
ZPA Transplantation

Posterior tissue transplant to anterior = duplicated autopod
  - Note structure: “mirror-image”

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

Retinoic acid operates upstream of Shh
  - implant RA-soaked bead = mirror-image duplication
  - possible Hox gene involvement

Hox Genes in Early Limb Bud

NOTE - SHH binding switches Gli3R (repressor form) to Gli3A (activator form)
- initiates transcription of 5’ Hoxd genes
- Hoxd genes then maintain Shh expression
Shh Specifies Digit Identity

(A) Specifies Digit Identity

(B) Specifies Digit Identity

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

(D) Shh diffusion
Shh descendants
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

Anterior – high Gli3R
Posterior – low Gli3R

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/Gli3 knockouts – multiple digits
#1 or unspecified variants
BMPs Regulate Digital Identity

Shh initiates BMP2 and BMP7 gradients
- BMPs in interdigital mesoderm (webbing) specifies identity of digits anteriorly
- BMP targets unknown

NOTE – BMP effects probably only on tissue “primed” by Shh

Remove interdigital mesoderm

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

Insert BMP antagonist into interdigital webbing

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

**Progress Zone (PZ)**

**Ectoderm**

**Apical Ectodermal Ridge (AER)**

**Zone of Polarizing Activity (ZPA)**

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