Vertebrate Pattern Formation and Limb Development
Retinoic Acid Gradient

- Posterior high \(\Rightarrow\) Anterior low
- Retinoic acid production at posterior end
Cdx genes

- Posterior end
  - Activated by retinoic acid
- Drosophila caudal gene homologue
Hox Genes

• Homeotic gene paralogues
• Gene duplication caused multiple copies to form
• Expressed along dorsal axis
  – Neural tube, neural crest, paraxial mesoderm, surface ectoderm
  – Anterior boundary of hindbrain to end of tail
Control of Hox Genes Expression

- Partially controlled by retinoic acid and Cdx genes
  - Transcriptional control
Hom and Hox Gene Paralogues
How does Hox gene expression effect body pattern?

• Three types of experiments on Hox gene expression
  – Gene “knockout” (deletion)
  – Retinoic acid teratogenesis
  – Comparative anatomy
Comparative Anatomy of Bird and Mouse Vertebrae
Mice exposed to retinoic acid

- A, C Normal; B, D Retinoic acid
- Defects in facial and cranial skeleton
- Missing vertebrae
- Deformed pharyngeal arches
Hoxc-8 Deletion in Mouse

- First lumbar vertebra transformed into thoracic
Pharyngeal pouch development in Hoxa3 mutant mouse

- Pouch 3 thymus, pouch 4 parathyroid
- Left wild type, right Hoxa3 deletion
- Neural crest cells develop abnormally
Limb Field

• Forms limb bud
• Only part of limb field required
  – All parts of the limb field have the capacity to produce a limb
  – Transplant experiments
Limb Disc surrounded by Limb Field

Somites

Pronephric kidney

Gills

Peribrachial flank tissue

Free limb

Shoulder girdle
Limb Bud Formation

• Mesenchyme cells from the lateral plate mesoderm proliferates and migrates toward limb bud location to form the skeleton

• Myotome cells follow to form the musculature
Mesenchyme induction

• Lateral plate mesoderm cells that migrate start expressing Fgf10 (arrows)
  – Paracrine factor
  – Activates limb forming genes in ectoderm and mesoderm
Nematode induced limb duplication in frogs
How does the limb bud know where to form?

• Hox gene expression
How is limb type determined?

- Tbx4 expressed in hindlimbs (somite 25)
- Tbx5 expressed in forelimbs (somite 17)
Stage 14/15 (early day 3)

(A) Normal

Forelimb bud

Wing

Hindlimb bud

Leg

(B) FGF induced

FGF

Wing

Chimera

Leg

Tbx5

Tbx4
Pattern Formation in the Vertebrate Limb.
- Induction plays a major role in **pattern formation**.
- **Positional information**, supplied by molecular cues, tells a cell where it is relative to the animal's body axes.

Homeobox-containing (*Hox*) genes play a role in specifying the identity of regions of the limb, as well as the body as a whole.
Limb Disc surrounded by Limb field
What happens when limb disc is removed?
Mutual Induction
Effect of AER on Limb Development

- Forelimb mesenchyme
- AER
- Extra AER
  - Leg mesenchyme
  - Wing is duplicated
  - Leg
- Nonlimb mesenchyme
  - Wing
- AER replaced by FGF bead
  - Normal wing
  - Limb development ceases

- AER removed
  - Limb development ceases
AER is necessary for limb development
Mutual Induction

Limb Bud stage

Somatic mesoderm $\rightarrow$ Ectoderm to become AER
AER $\rightarrow$ mesoderm proximal to distal growth

- **Apical ectodermal ridge (AER).**
- Secretes fibroblast growth factor (FGF) proteins.
  - Required for limb growth and patterning along the proximal-distal axis.
Sonic Hedgehog Expression

(A) Thumb region

(B) Extra digits
   Thumb region

(C) Wild-type
   GCTTGTGTTTTTGGCCACTGATGATCCATAA

(D) Hx mutant
   GCTTGTGTTTTTGGCCACTAATGATCCATAA
Hox Gene Expression

(A) ELCR Anterior

(B) Initial ZPA

(C) ELCR Posterior

- Gli3
- dHand
- Shh

Images courtesy of Wnt7a, dHAND, Shh, Fgf10, Fgf8.
Feedback between AER and ZPA

Stage 16:
- Fgf8 induced by Fgf10
- Fgf10
- Anterior
- Posterior
- Hoxb8
- Somites
- Intermediate mesoderm

Stage 17:
- Proliferation maintained by Fgf8
- Shh induced by Fgf8
- Lateral plate mesoderm

Stage 18:
- Proliferation maintained by Fgf8 + Fgf4
- Shh maintained by Fgf8 + Fgf4
- Fgps maintained by Shh
- Surface ectoderm

AER
Digit formation
Shhh gene expression

BMP concentration
Inhibition of cell death by inhibiting BMP
Which tissue controls Programmed Cell Death?

Meso  Ecto
Duck leg + Chicken leg → ?

Chick leg + Duck leg → ?

What triggers Programmed Cell Death?
(A) Tail tip transplanted to trunk

(B)
Thyroxine

Type II deiodinase

Tri-iodothyronine

Type III deiodinase