

# Limb Formation

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**WEEKLY WORLD NEWS**  
March 17, 1992 75¢/80¢ CANADA 18258

## DOLPHIN GROWS HUMAN ARMS!



**OFFICIAL GOVT. PHOTO!**

**Creature loves humans and uses sign language, say marine scientists**

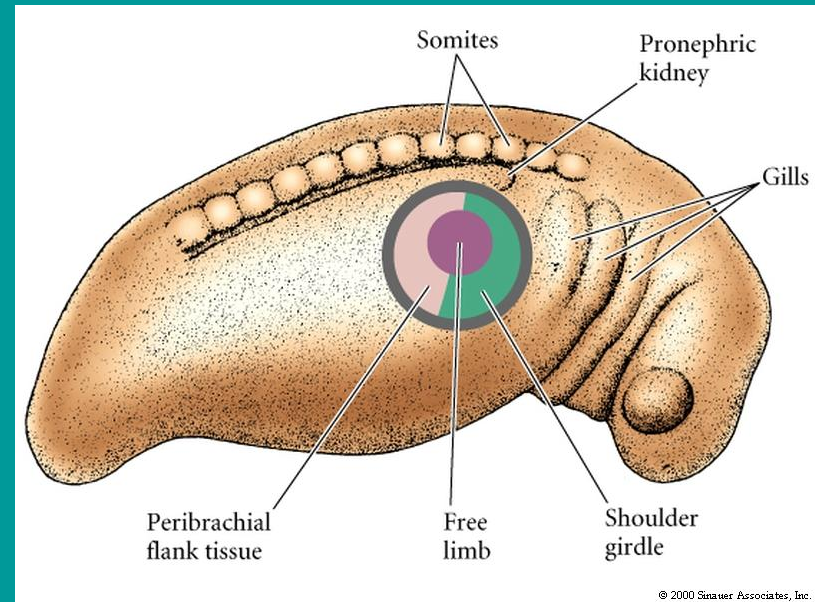
**WORLD'S BIGGEST HOROSCOPE!**



**DEAD HUBBY'S GHOST HAUNTS TOILET, SWEARS TEXAS WIDOW**

# What is a Field?

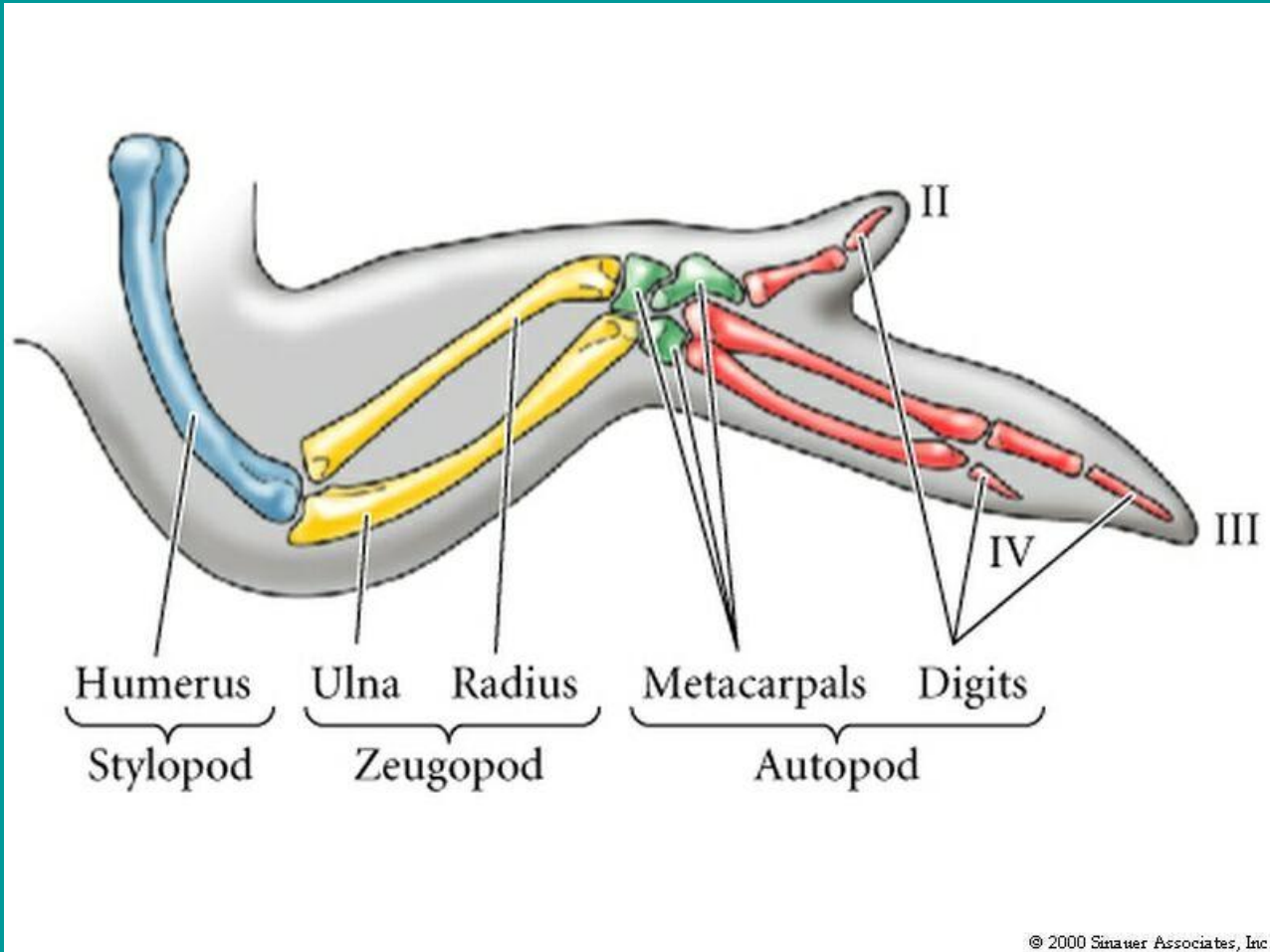
- Group of cells whose position and fate are determined
- Limb Field defined
  - remove → no limb
  - transplant → ectopic limb



# What Characterizes the Vertebrate Limb Axes?

- Proximal (humerus-femur)-Mid (radius/ulna-tibia/fibula)-Distal (carpals-fingers/tarsals-toes)
- Anterior (front-thumb)-Posterior (back-pinky)
- Dorsal (knuckles)-Ventral (palm)
- Positional information

# Chick Wing

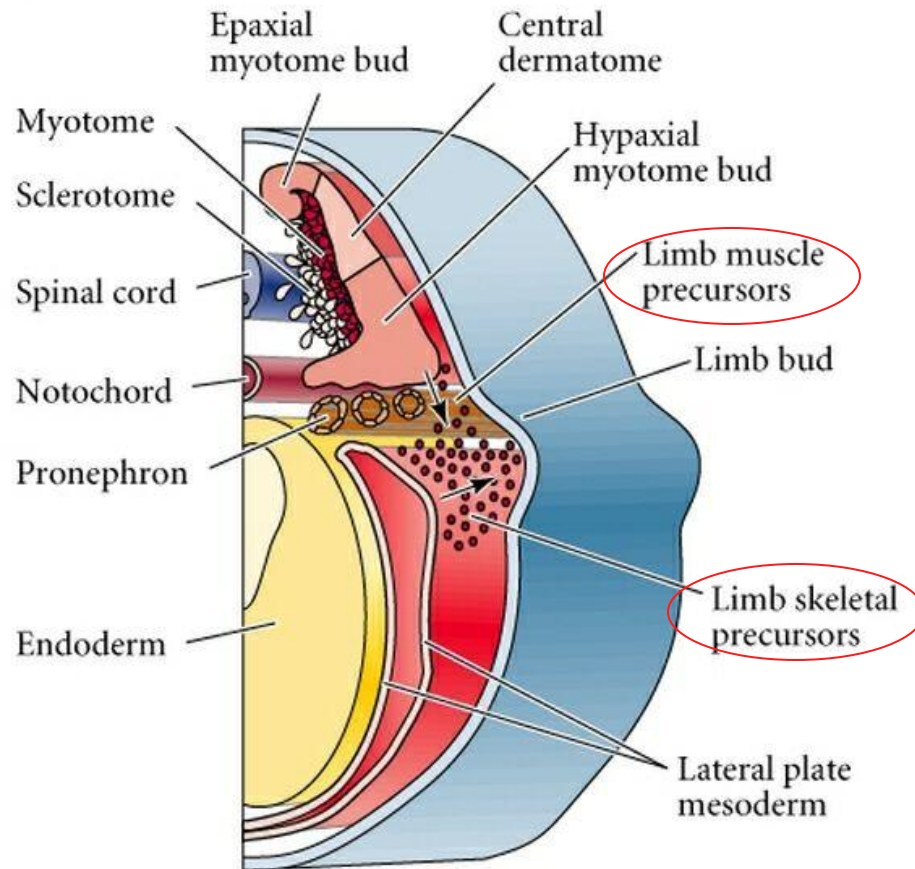


# How Does Limb Bud Form?

- Chick 2-somite stage
- Mesenchyme from lateral plate mesoderm
  - condenses under influence of spinal chord
- Forms limb bud skeleton
- Muscle precursors from myotome

# Limb Bud Formation

(A)





# Apical Ectodermal Ridge and Mesoderm

- Mesenchyme induces thickening of ectoderm = AER
- Interaction AER with underlying mesoderm generates A-P polarity
- Both are important





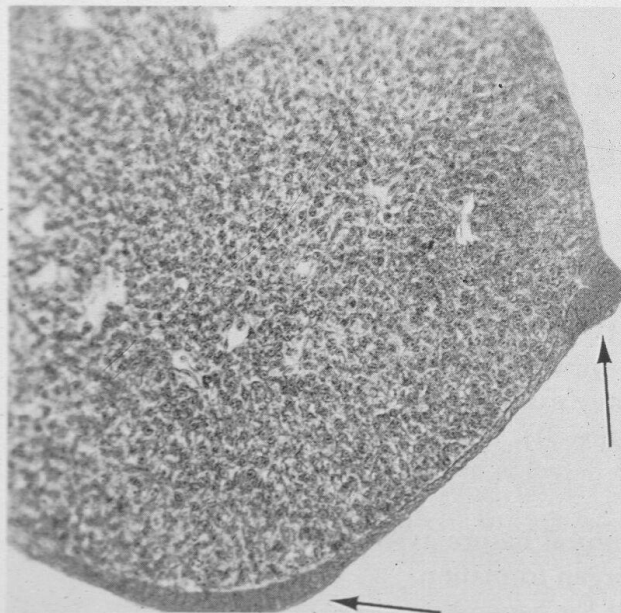
# Tissue Grafts using Mutants

- *Polydactyly* (extra digits)
  - meso (*pd*) + ecto (wt) → polydactyly
  - meso (wt) + ecto (*pd*) → normal phenotype
- *Eudiplopodia* (extra digits; secondary AER forms)
  - meso (*eu*) + ecto (wt) → normal phenotype
  - meso (wt) + ecto (*eu*) → 2 AERs, extra digits
- *Limbless* (no limb; no AER)
  - same as *eu* so acts in ecto

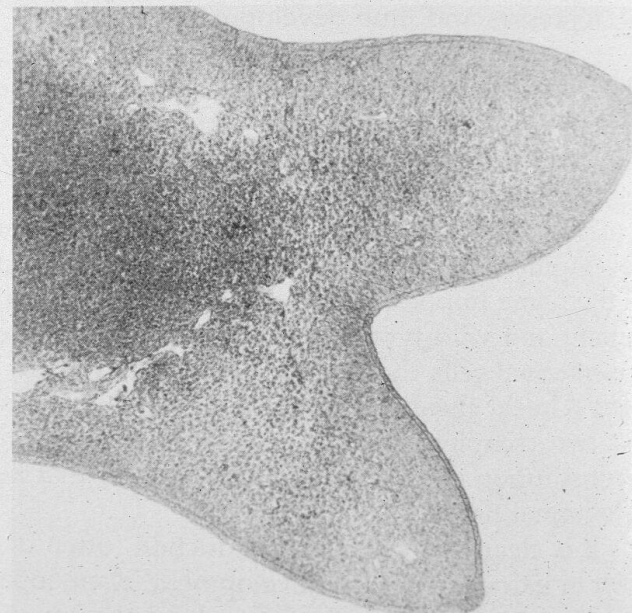
# Eudiplopodia

FIGURE 31

Cross sections of hindlimb buds from *eudiplopodia* chick embryos. (A) Two AERs (arrows) on hindlimb bud; extra outgrowth on the dorsal side will form an extra set of toes. (B) Both outgrowth regions are covered by an AER. (From Goetinck, 1964; photographs courtesy of P. Goetinck.)

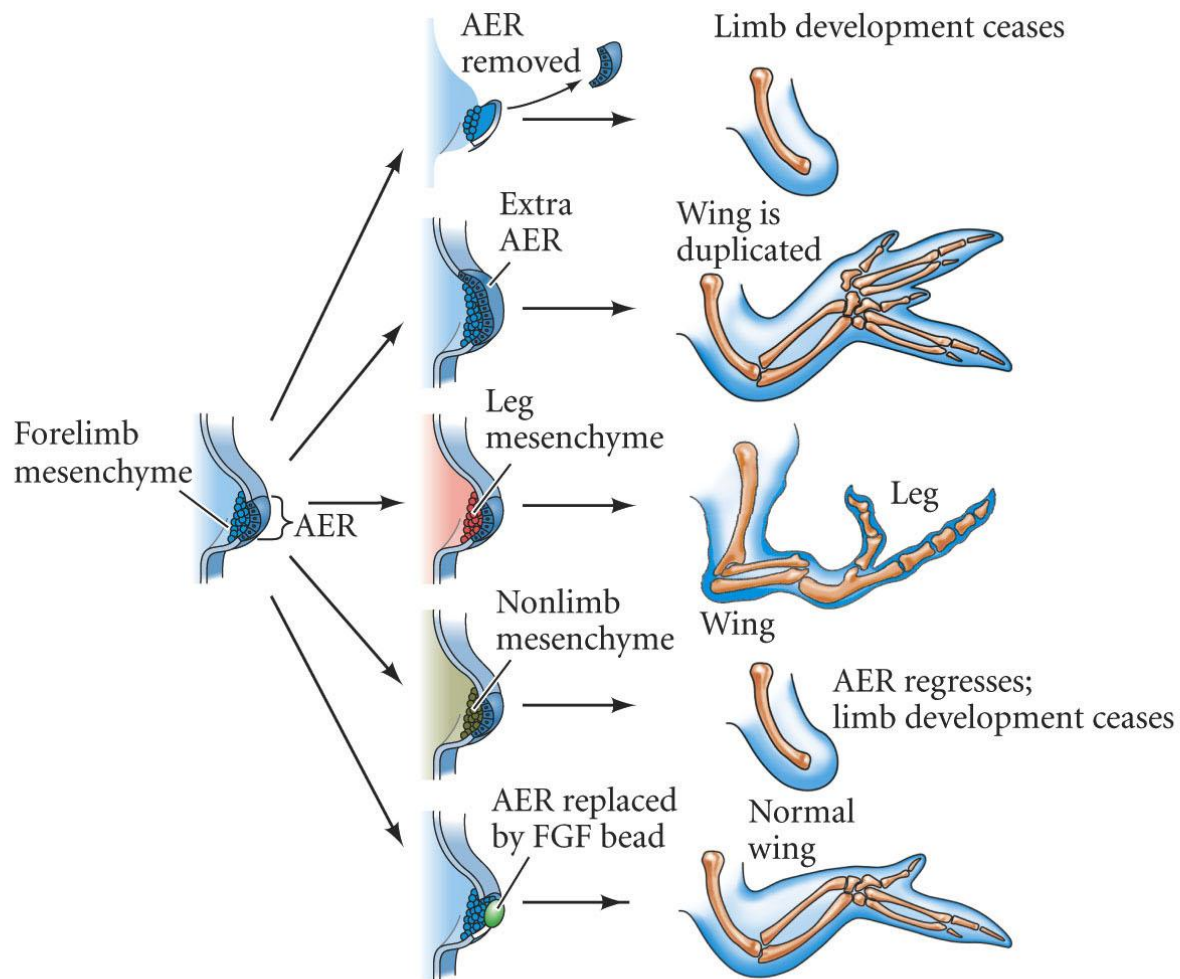


(A)



(B)

# Surgical Experiments



# Summary of Surgical Experiments

- Ectoderm
  - Sequential removal of AER
    - more distal structures do not develop
  - Extra AER gives duplications
  - FGF beads can replace AER
- Mesoderm
  - Leg mesoderm will signal leg structures
  - Non-limb mesoderm get no limb formation

# What Does FGF Do?

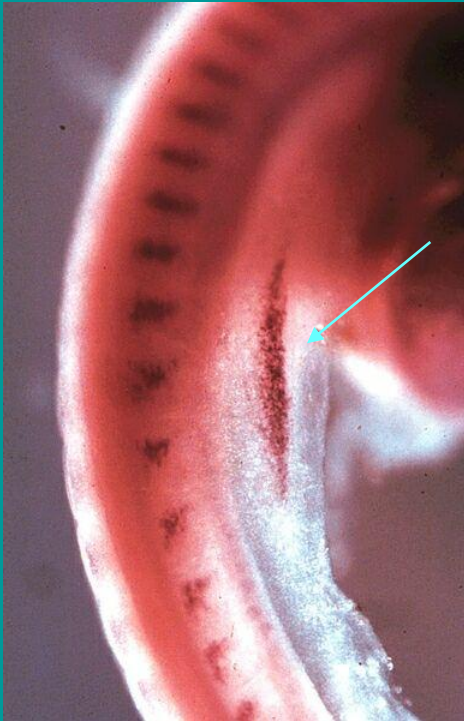
- FGF-10 made by lateral plate mesoderm
- Restricted to limb forming region by Wnt proteins
- Bead will induce extra limbs
- FGF-10 localizes FGF-8 production in AER



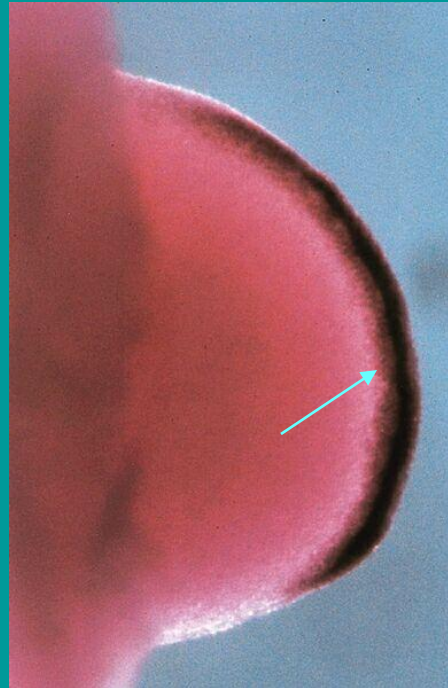


# Where is Ectodermal FGF-8 mRNA Localized?

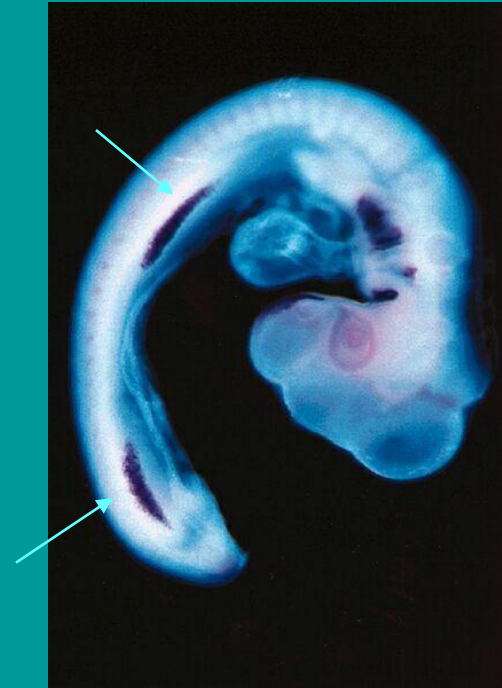
Limb Bud  
Ectoderm



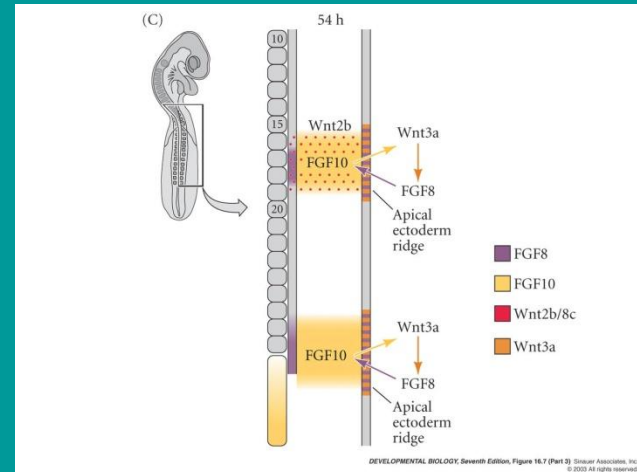
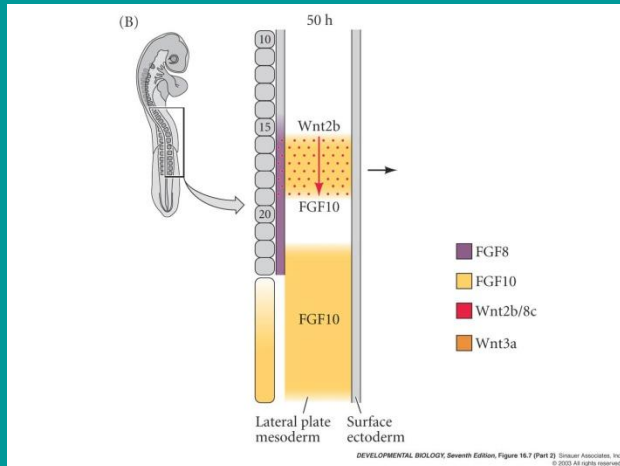
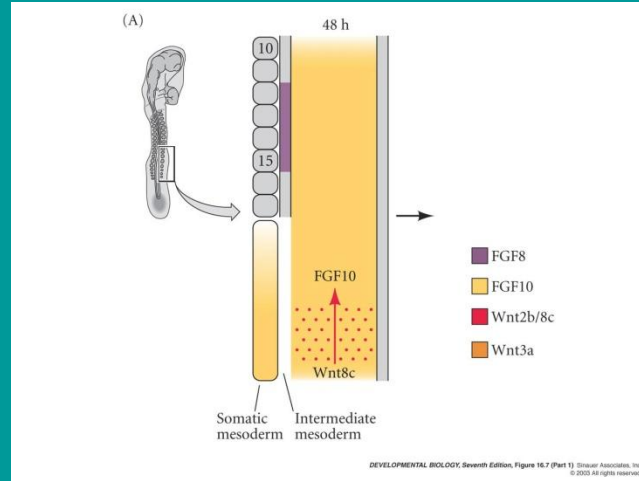
Apical Ectodermal  
Ridge



AER of Hindlimb  
and Forelimb



# Model



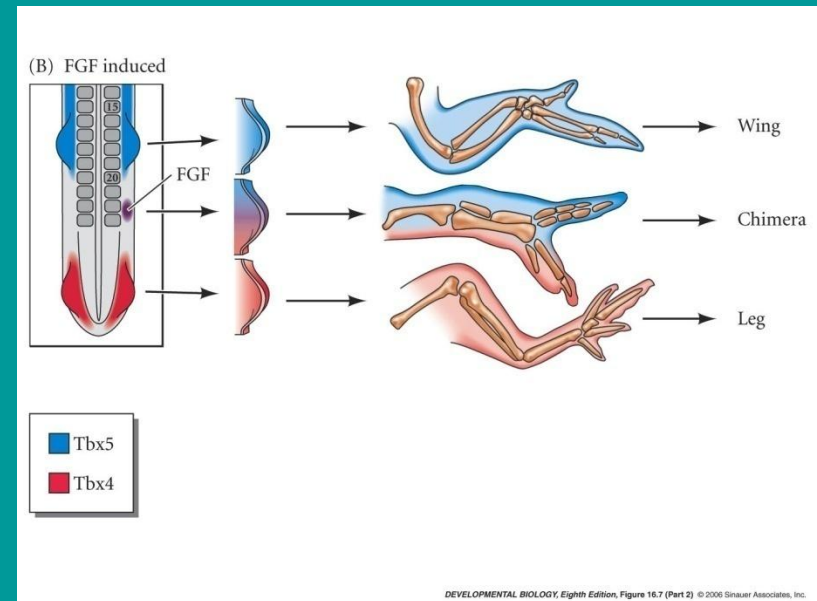
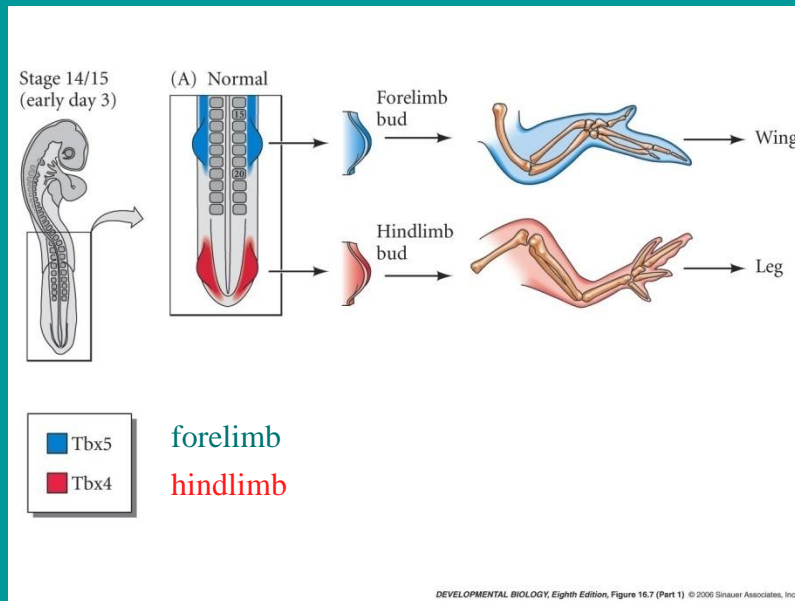


# Feedback Loops

- AER makes FGF-8
- Induces SHH in mesenchyme
- SHH maintains FGF-8
- FGF-8 and FGF-10 maintain SHH

# Induces TBX Transcription Factors: Forelimb and Hindlimb

Hox genes specify Tbx transcription factors that control FGF production



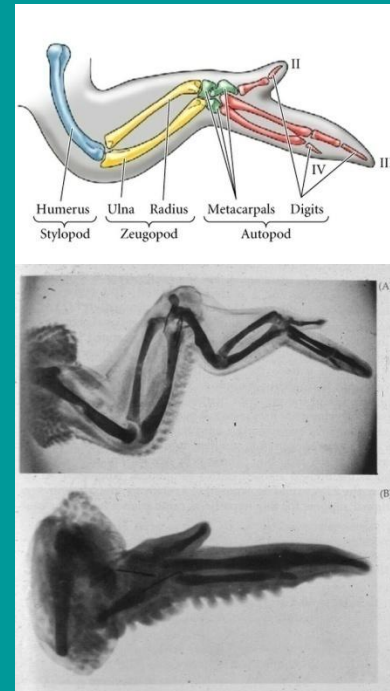
# Proximal-Distal Polarity

# What is the Progress Zone ?

- Region of mesoderm under the AER
  - undergoes cell division
  - permits elongation of limb
- Ca. 0.2-0.35 mm thick
- Kept dividing under FGF-8 stimulation by AER
- Continues to make FGF-10
- Source of positional information

# Positional Information from the PZ

- First cells leaving form proximal (humerus)
- Last cells form digits
- Experiment
  - A. control H - R/U - D
  - B. graft young zone to older limb get duplication
    - H - R/U - H - R/U - D
  - C. graft old zone on younger limb get deletion
    - H - D



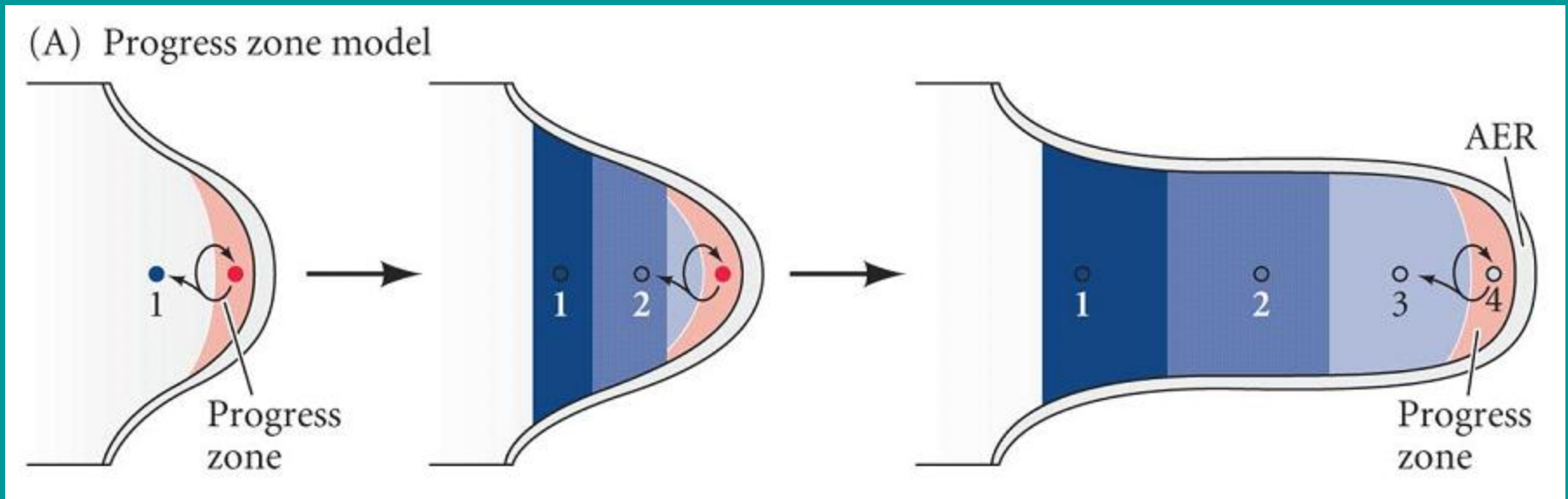
A.

B.

C.

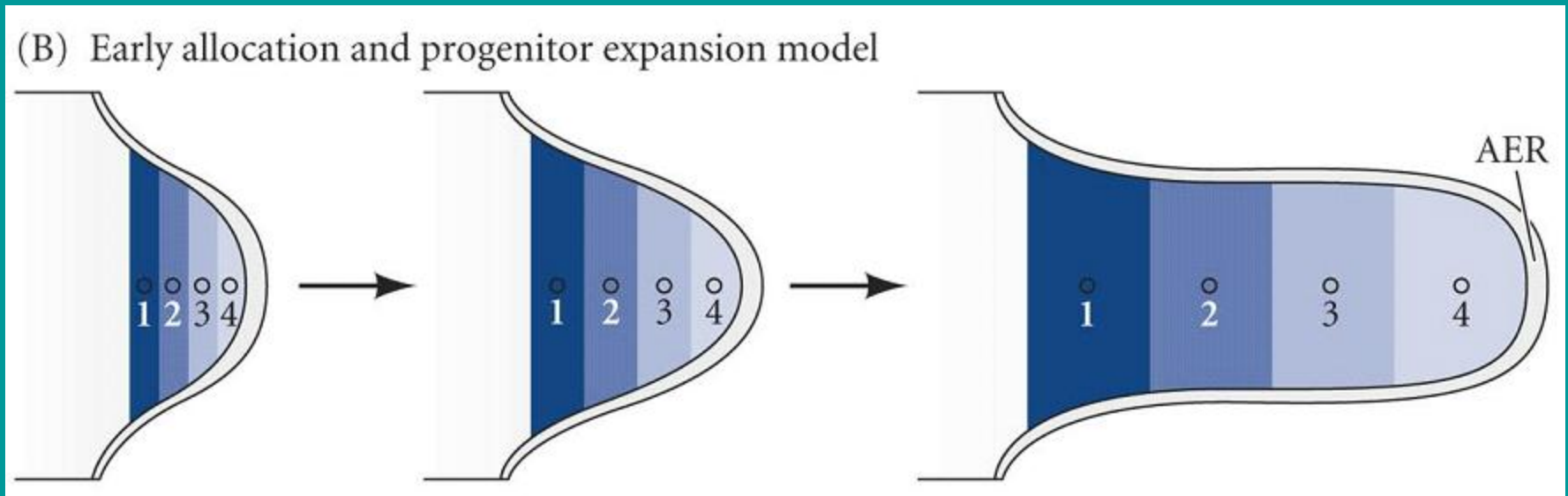
# Progress Zone Model

- Time cells spend in progress zone specifies position
- Constantly removed -> cartilage
- Longer in zone = more distal



# Early Allocation Model

- All precursors already present
- When AER is removed nearest cells die
  - so only early precursors continue to form more proximal structures





# Reaction-Diffusion Model

- Near AER, FGFs from AER prevent synthesis of fibronectin matrix and condensation → no cartilage
- Further away, TGF- $\beta$  (positive feedback) responsive cells make Fn and a self-inhibitor
- Lead to periodic patterns TGF- $\beta$  and Fn

# Hox Genes

# What is the Role of the Hox Genes?

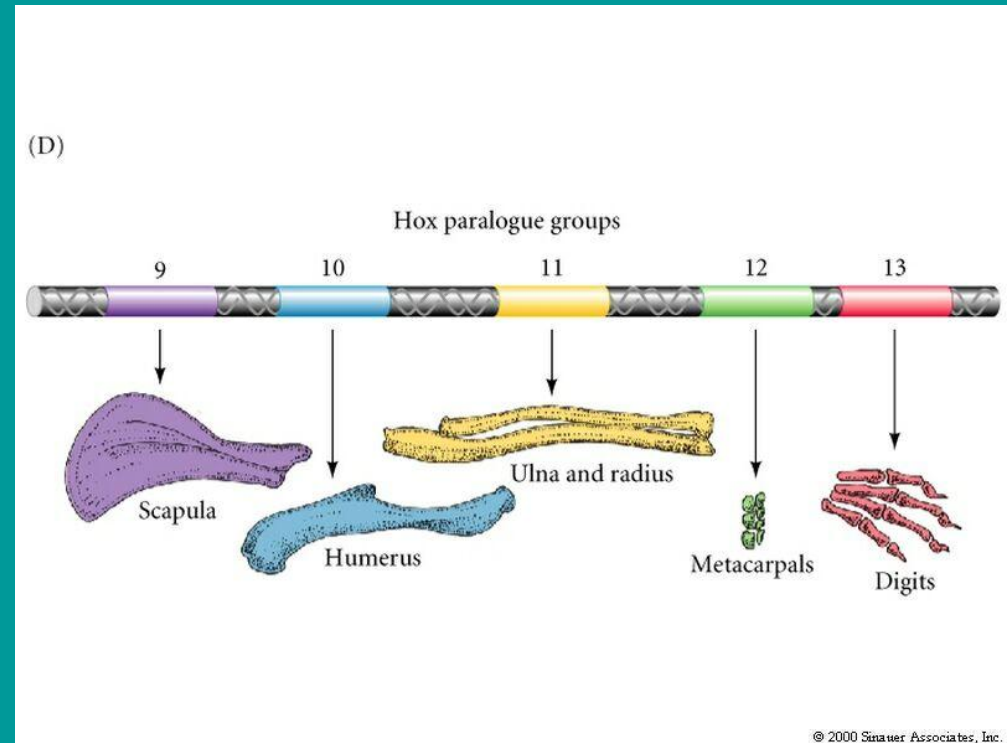
- Hox 9-13 genes in mesenchyme specify limb in mouse

- KO Hoxa-11, b-11, c-11, d-11
  - no ulna and radius in forelimb
- KO Hoxa-13, b-13, c-13, d-13
  - no toes



# Hox Genes

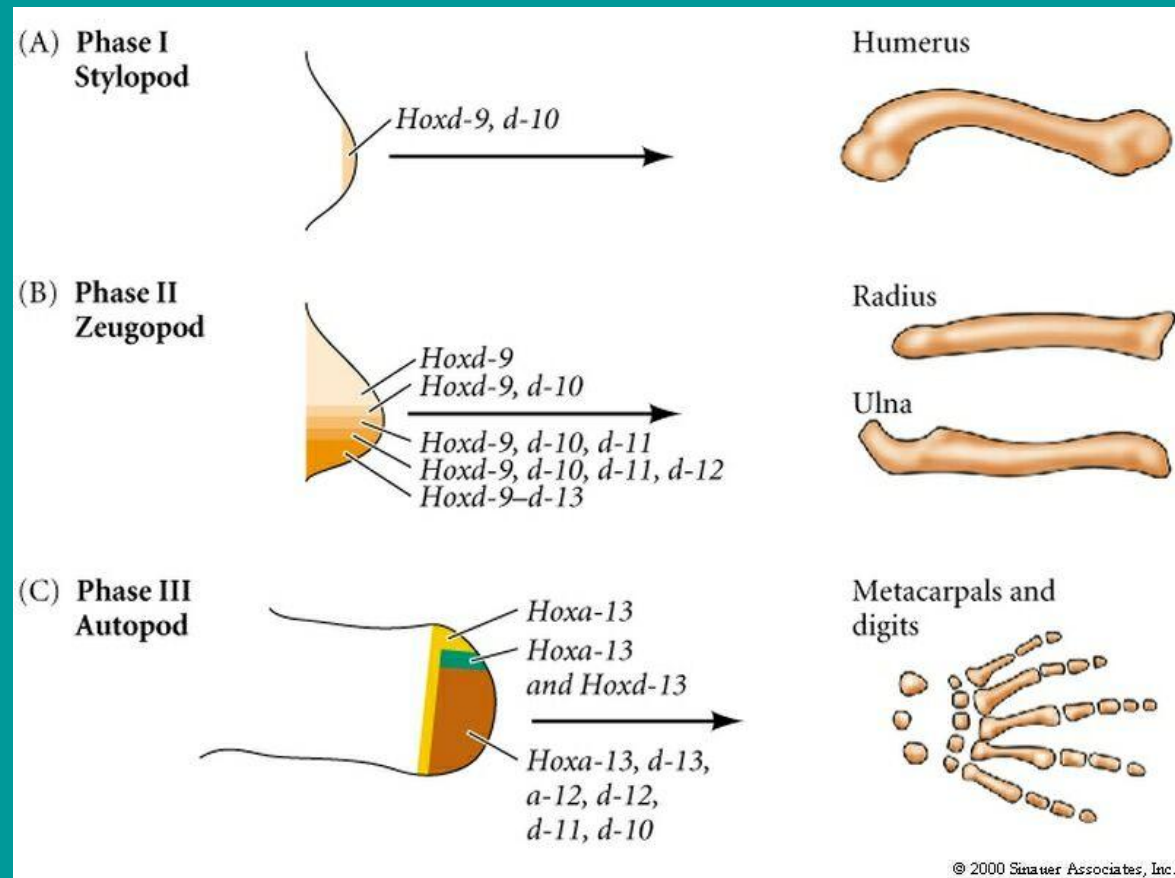
- The most 5' genes control (among other things) regions of the forelimb



# Shifting Hox Expression during Limb Formation Specifies Position

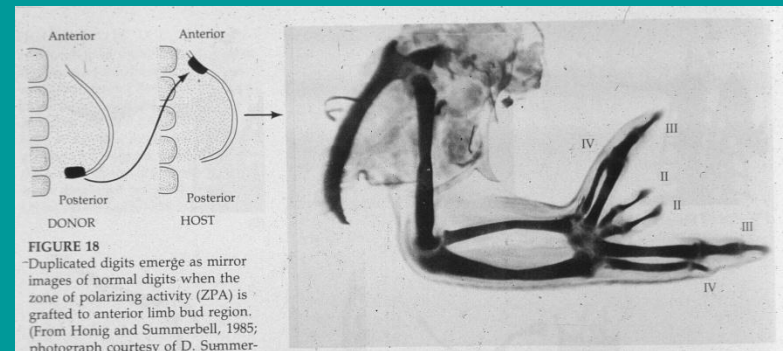
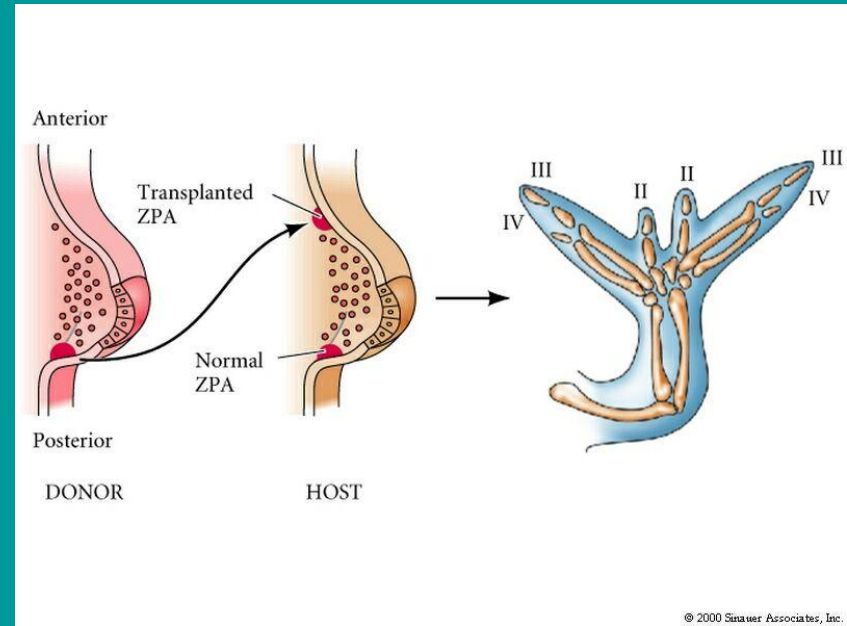
Nested

Combinatorial?



# Anterior-Posterior Axes

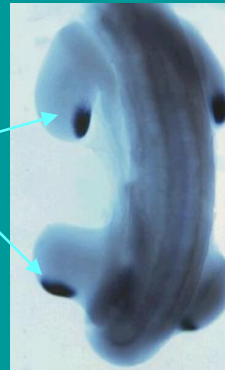
- Zone polarizing activity = ZPA
- Transplant
  - Mirror image digit duplication
  - Maintains polarity
- Source of positional signals



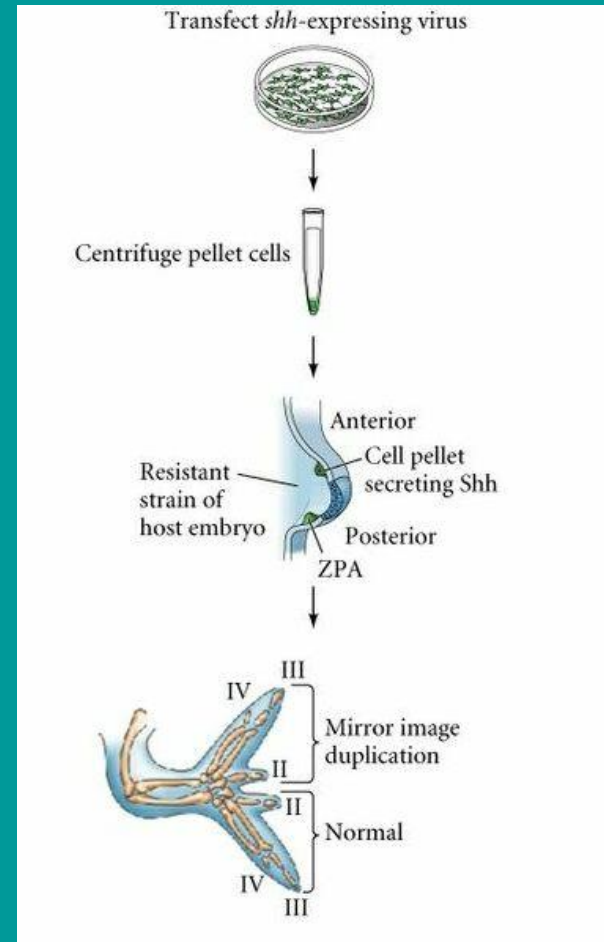
# What Signals Come From ZPA?

- Shh is made in ZPA

Shh mRNA



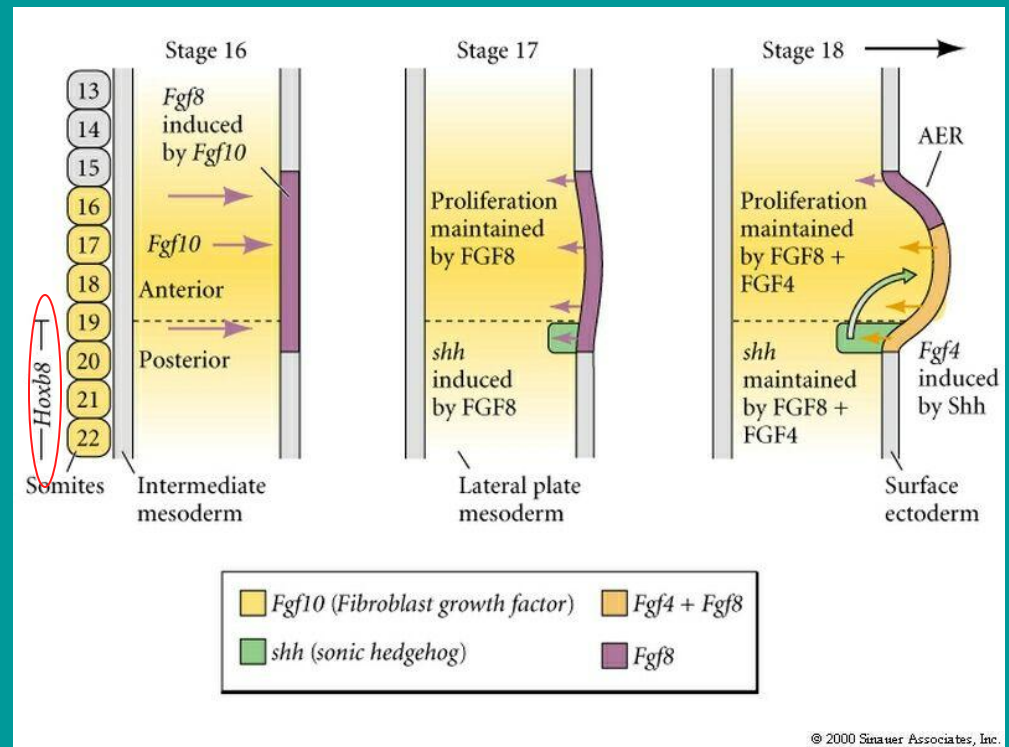
- Cells that make Shh can substitute
  - Beads soaked in Shh can substitute
- Turns on gradient of BMPs
- Controls Hoxd expression





# What Localizes Shh?

- FGF-8 from AER
- Cell in posterior of bud have different transcription factors (*hoxb-8* and dHAND) that allow them to respond and make Shh
- Shh then feeds back to AER



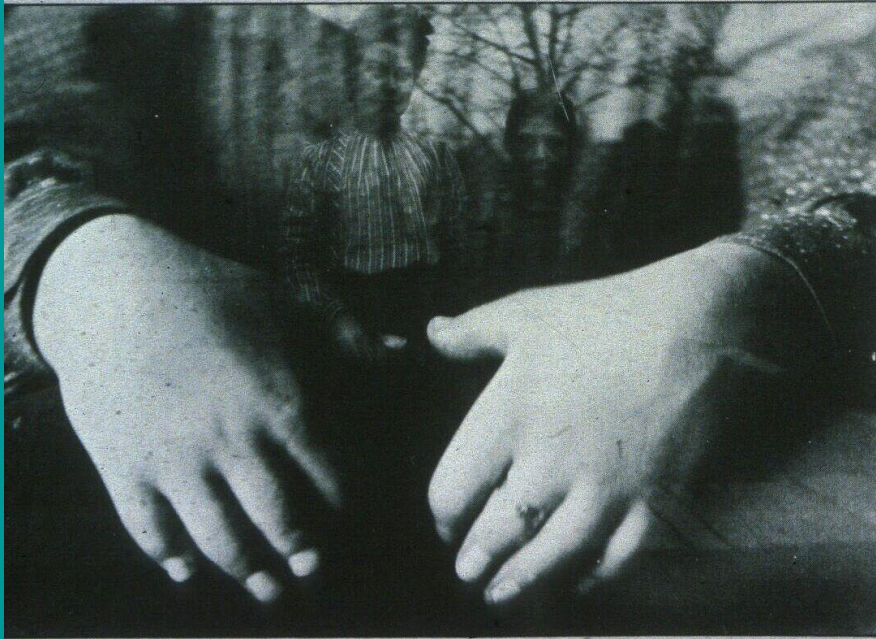
# What Controls Dorsal-Ventral?

- Ectodermally controlled
  - rotate ectoderm, reverse DV
- Wnt7a expressed dorsally
  - deletion of *wnt7a*
  - mouse has pads on both sides

# Digits

Changeling fingers:

genetics...



or surrealism?

# How Are the Digits Sculpted?

- Carved out by programmed cell-death
- Also inter-radius/ulna
- BMPs drive, noggin opposes

