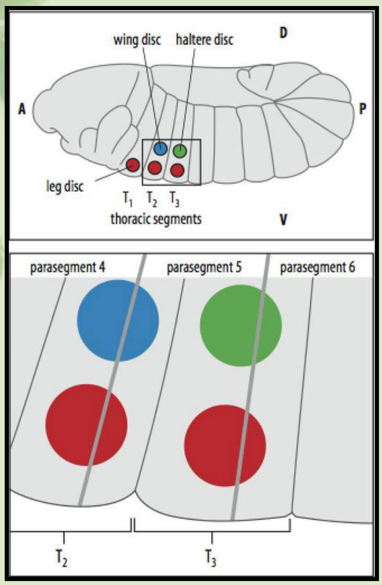
Limb/Leg Development

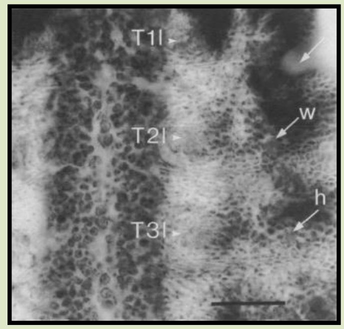
Fejlődés- és Molekuláris Genetika, 2018

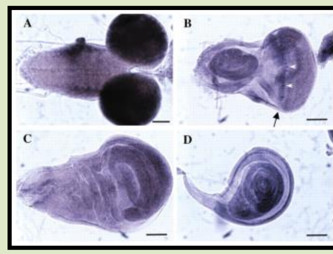
Insect Limb Development

Fejlődés- és Molekuláris Genetika, 2014

Leg discs





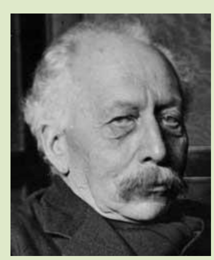


A-brain
B- ant.-eye

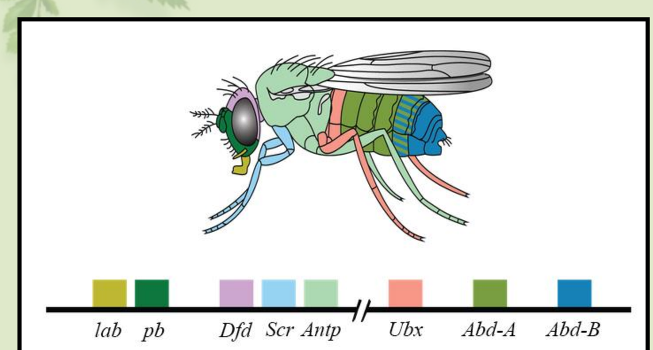
C-wing,
D-leg

Homeotic genes

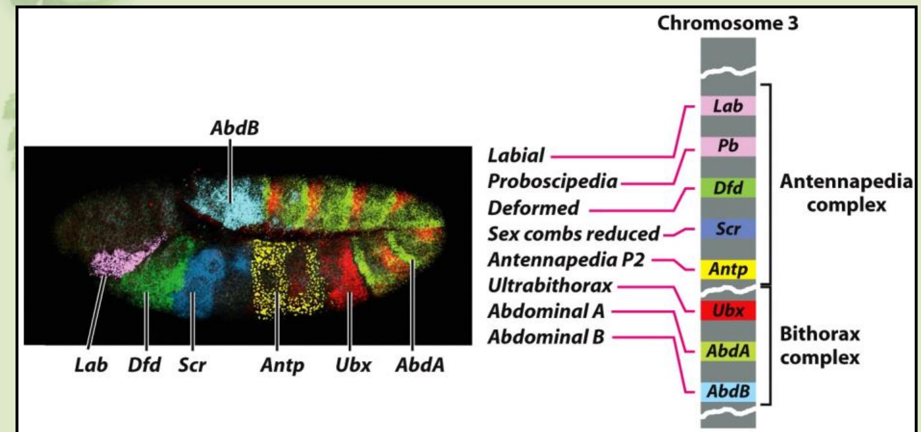
- The term *homeotic* refers to mutant alleles in which one body part is replaced by another.
  - It was coined by the English zoologist William Bateson, for describing of perturbation in the order of segments



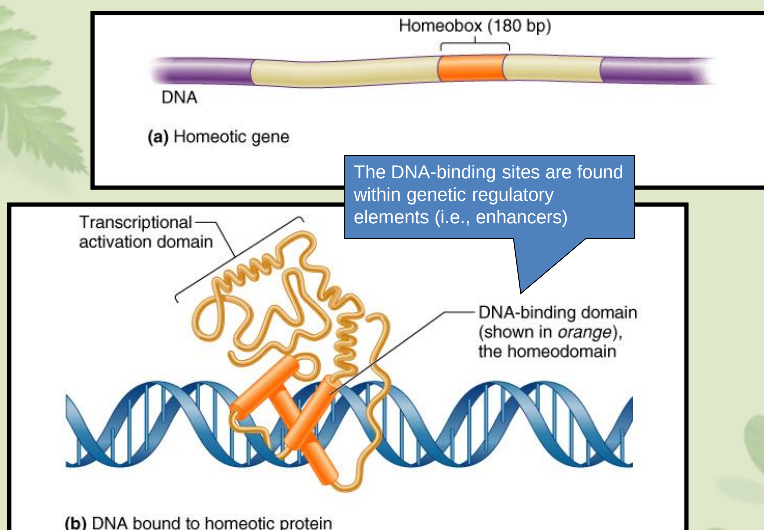
(1861-1926)



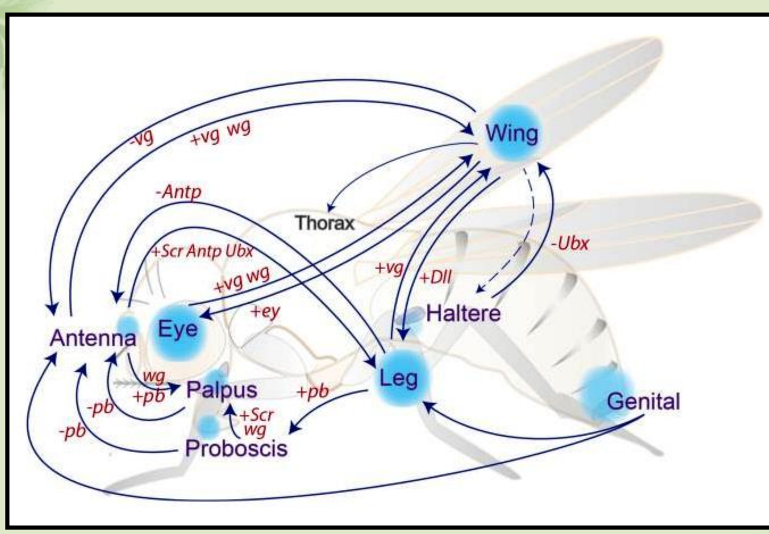
Expression pattern of Hox-genes in *Drosophila* embryo



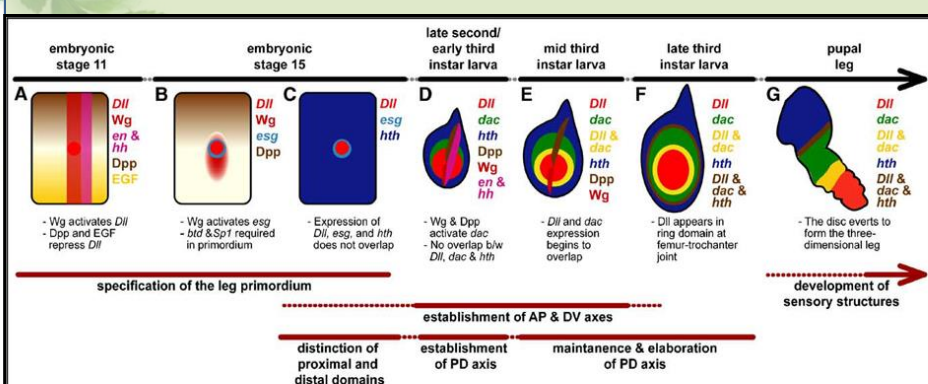
The homeobox



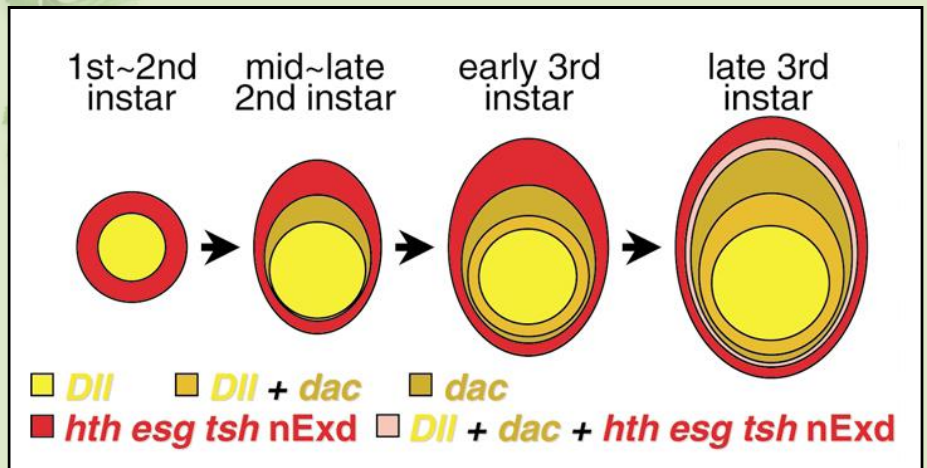
Transdetermination



An overwiev of leg development

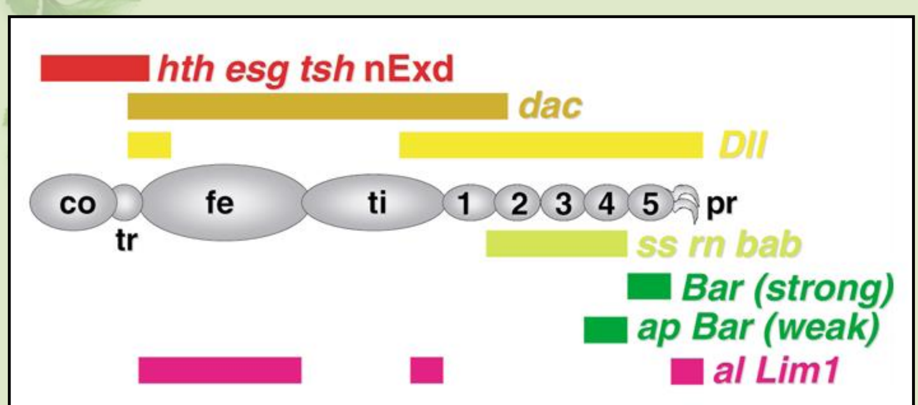


PD: Génexpressziós domének a lábdiszkuszban



Kojima, Dev. Growth Differ. 2004

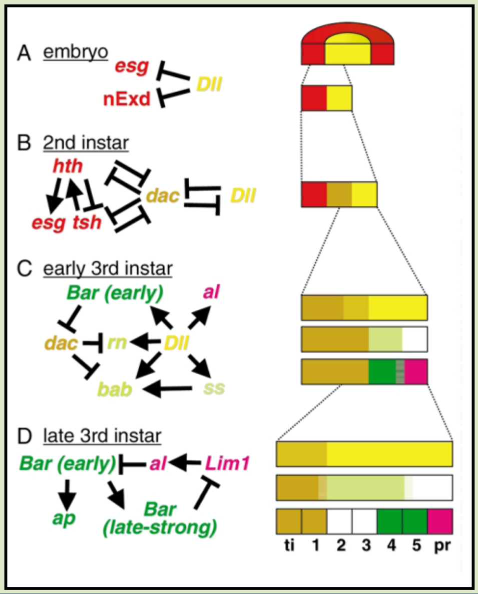
Proximodistal gene expression pattern



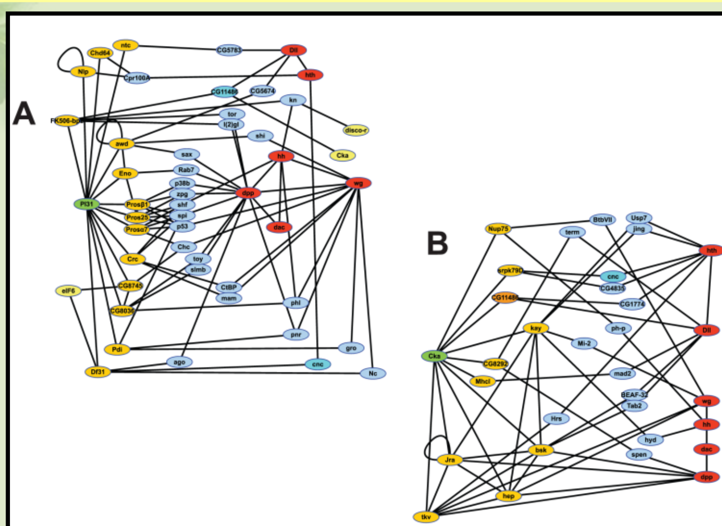
Kojima, Dev. Growth Differ. 2004

Bar: tarsal-segment-specific homeobox *gene*
Exd: a ubiquitously transcribed homeo-box gene
Bab: transcription factor
Ap, LIM1: LIM-homeodomain protein
Rn: zinc finger

PD: crosstalk between the genes



Connections between candidates and canonical leg development genes



Grubbs et al, Plos, 2013

Vertebrate Limb Development

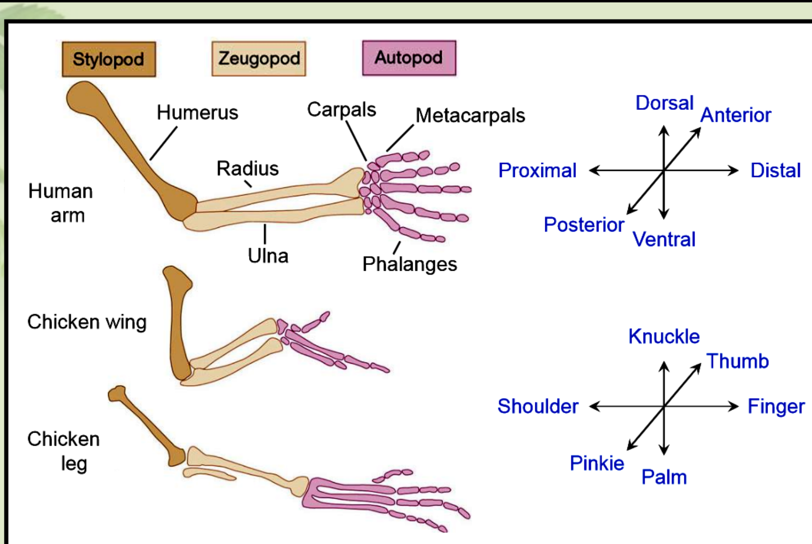
Fejlődés- és Molekuláris Genetika, 2014

"What can be more curious than that the hand of a man, formed for grasping, that of a mole for digging, the leg of a horse, the paddle of the porpoise, and the wing of the bat should all be constructed on the same pattern and should include similar bones in the same relative positions?"

Charles Darwin

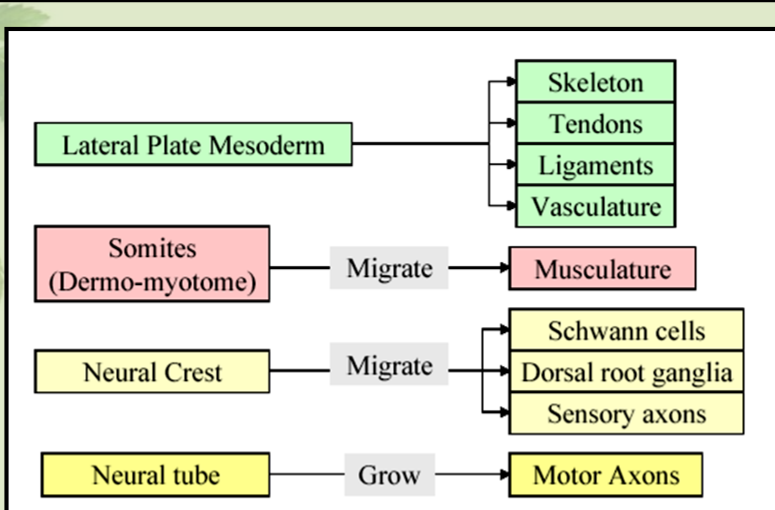
On the Origin of Species

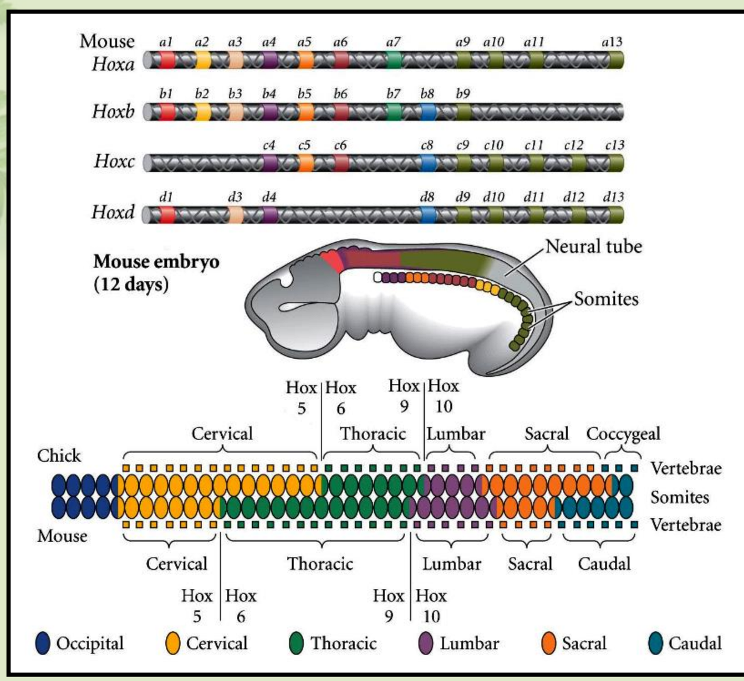
Parts and dimensions of a limb



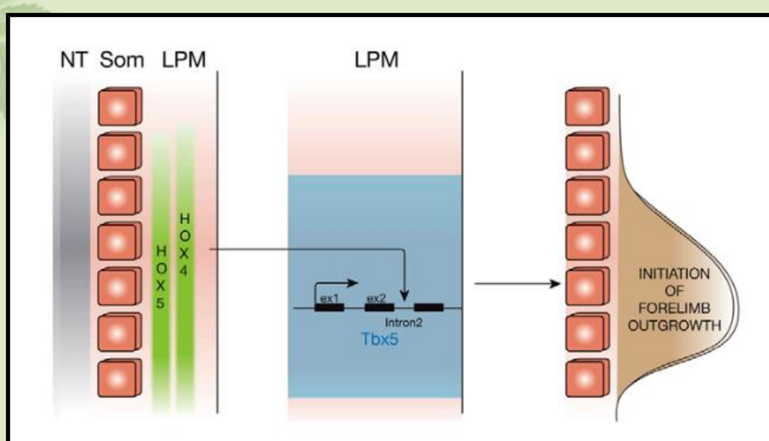
Manini and Martin, Nature 2003

From four sources





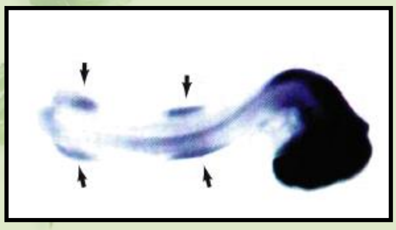
Hox genes control Tbx5/4



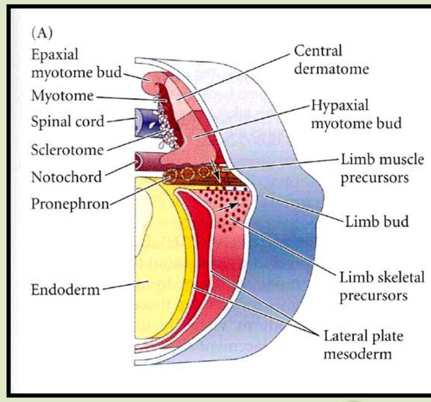
Minguillon et al., Development, 2012

Target for transcription factors coded by Hox4/5 is a 361 bp sequence in the intron 2 (conserved non-coding element)

First morphological sign

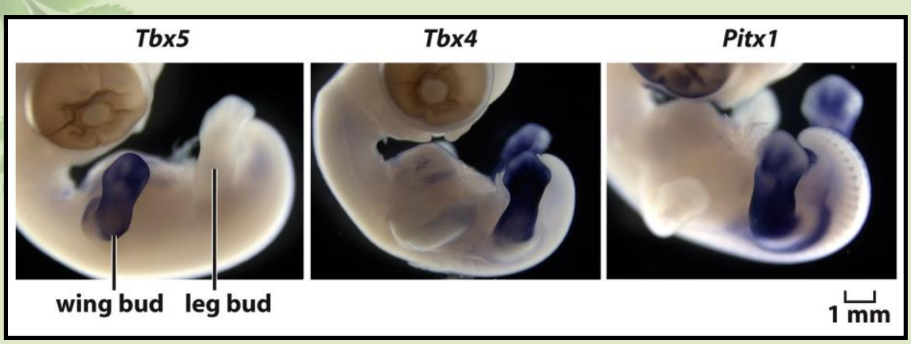


Local expression of T-box transcription factors



Lateral plate of mesoderm + ectoderm

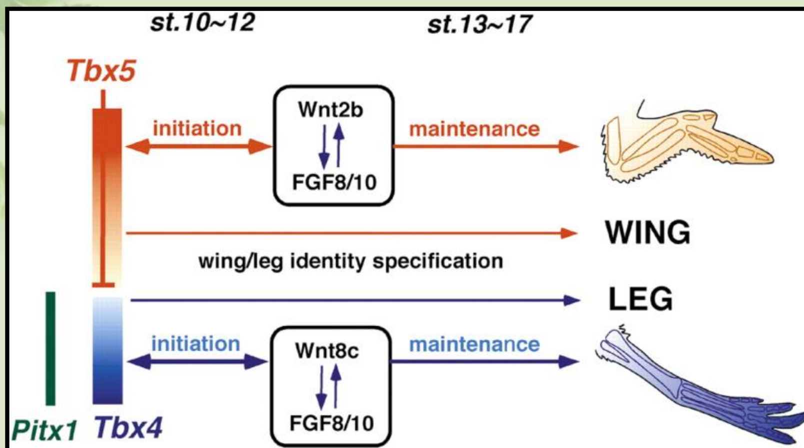
Tbx expression



- Tbx5: Expressed in wing bud
- Tbx4 and Pitx1: Expressed in the leg bud.
- Misexpression of Pitx1in the wing bud causes the limb to develop with leg-like characteristics. Pitx1: paired-like homeodomain 1.

Molecular Biology of the Cell

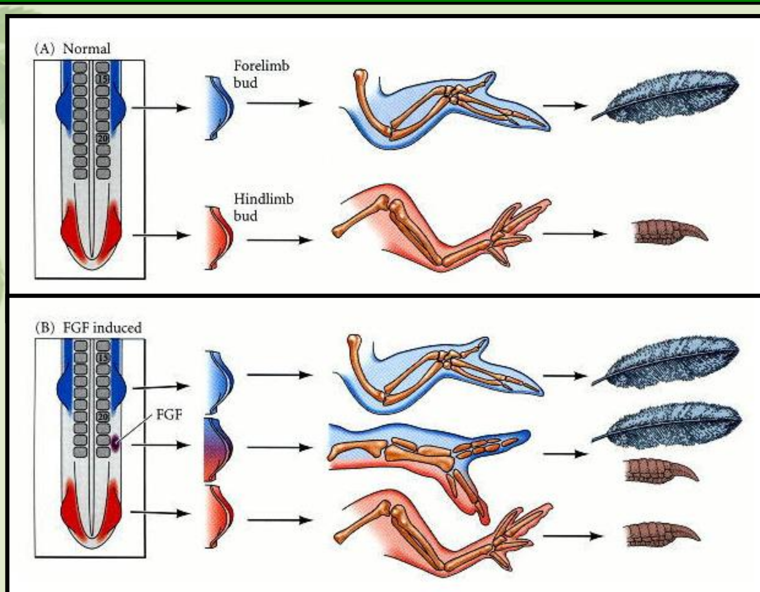
Signal mcascade in limb development



Takeuchi et al, Development, 2003

Once activated, the Wnt/Fgf cascades feedback on to *Tbx5* and *Tbx4* genes to establish a tight positive regulatory loop.

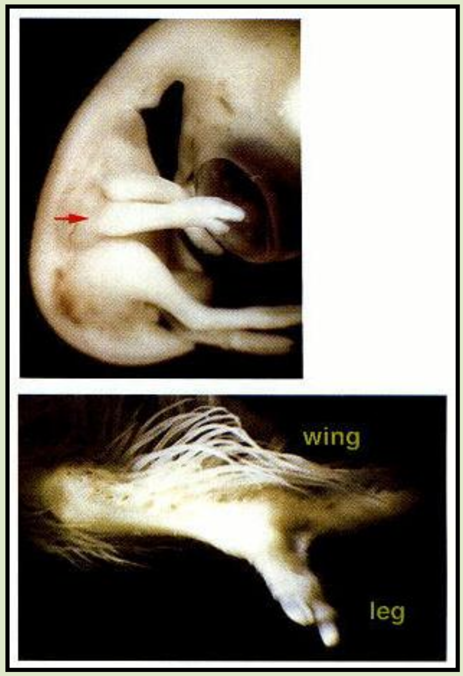
Tbx genes specify the limb type



Tbx5 and Tbx4

Tbx5 – forelimb

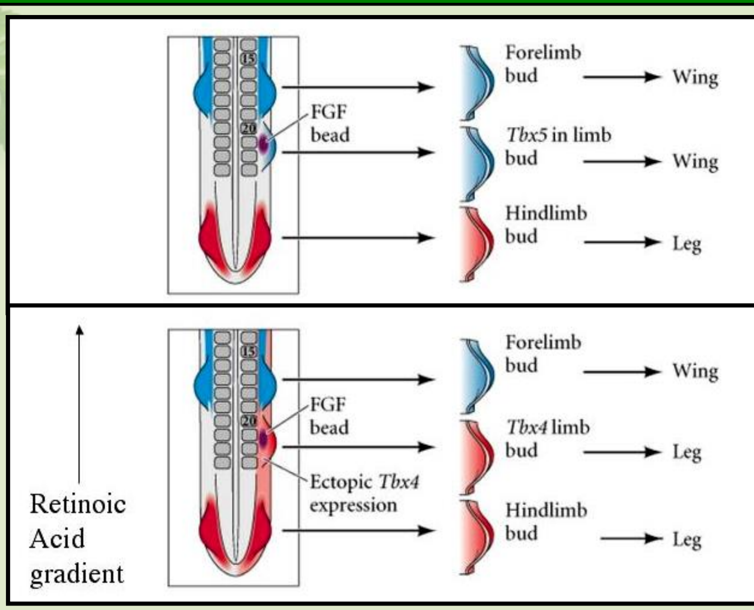
Tbx4 - hindlimb



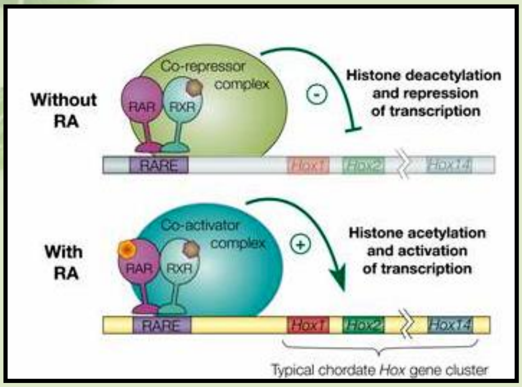
Tbxs expression initiated by Wnts and FGFs

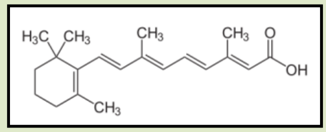
Ohuchi and Noji, Cell Tissue Res., 1999

Not only Tbx genes specify the limb type



Retinoic acid signaling

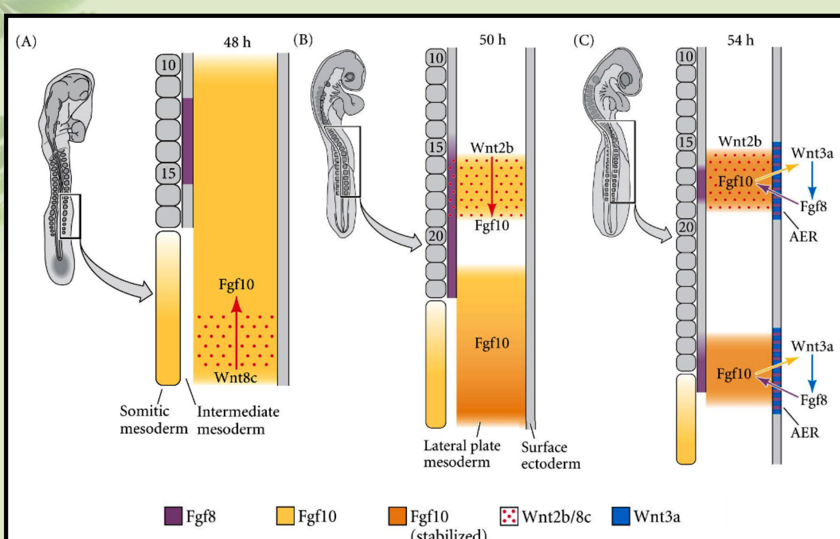




Martelaz et al., Int. J. Biol. Sci., 2006

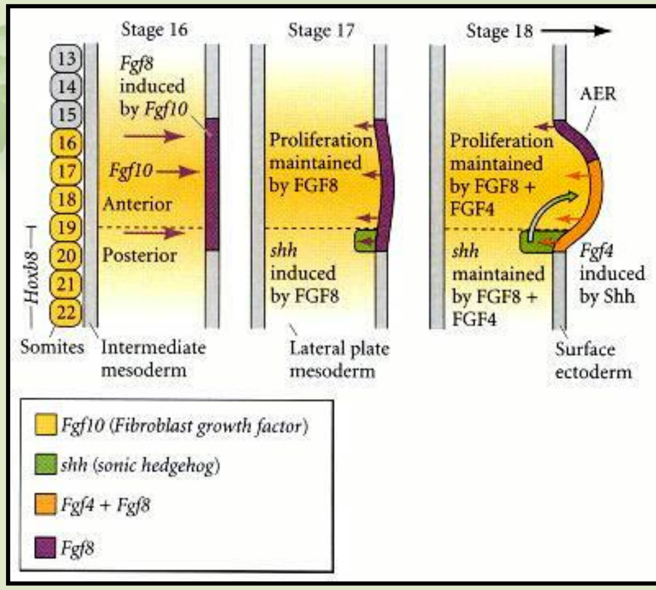
RA: retinoic acid
RAR: RA receptor
RXR: retinoic X receptor
RARE: Ra responsible element

Wnt/FGF – limb bud initiation



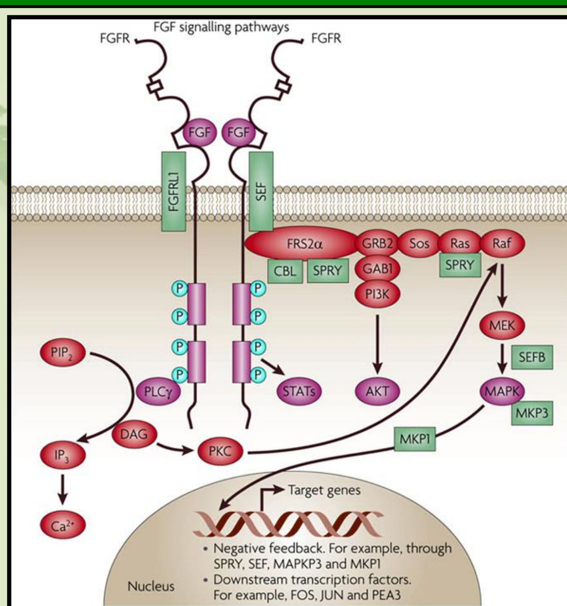
Gilbert: Developmental Biology, 6th ed.

Appearance of AER



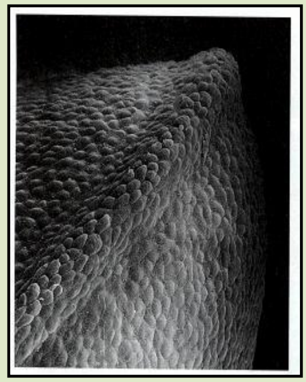
Gilbert: Developmental Biology, 6th ed.

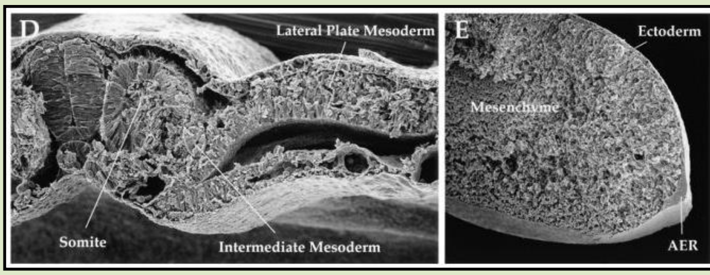
FGF signaling pathways



Apical ectodermal ridge (AER)

– The major signaling center for the developing limb.





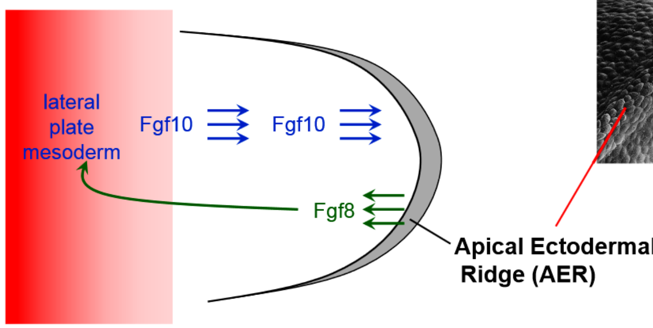
Three functions of AER

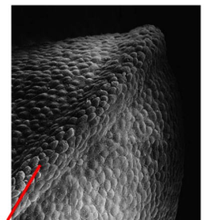
1. Maintain the mesenchyme beneath it in a proliferating phase
2. Maintain the expression of the molecules that generate the anterior-posterior axis
3. Interact with the proteins specifying the anterior-posterior and dorsal-ventral axes so that each cell is given instructions how to differentiate.

Formation of proximodistal axis

Proximal-Distal Axis

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



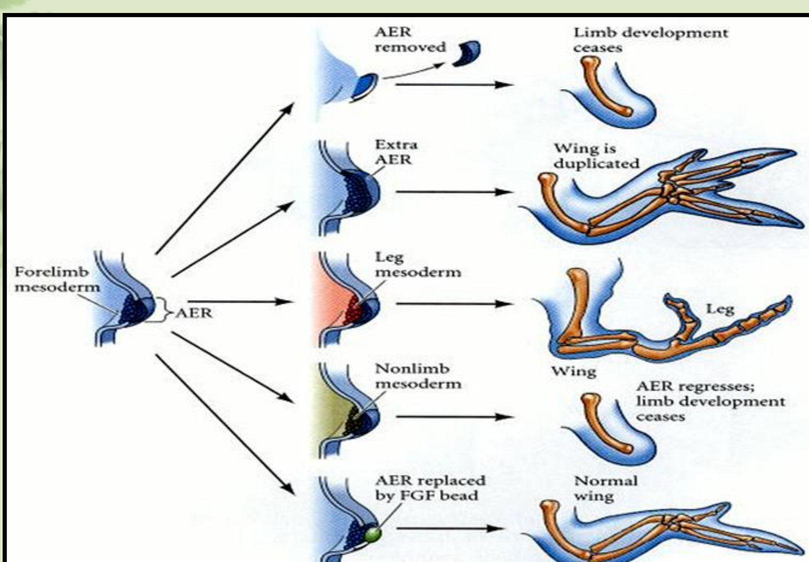


Lateral plate mesoderm expresses Fgf10

Fgf10 initiates AER via Wnt3a, β-catenin

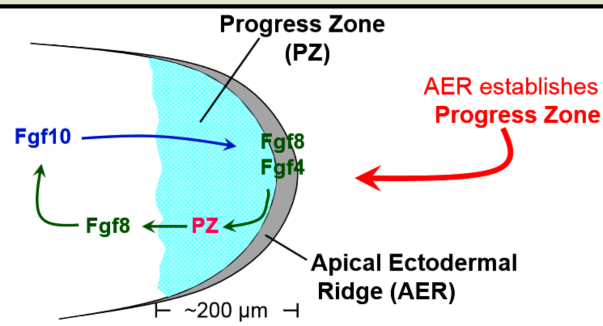
AER expresses Fgf8, Fgf4; maintains Fgf10 expression

AER manipulations



Gilbert: Developmental Biology, 6th ed.

The progress zone

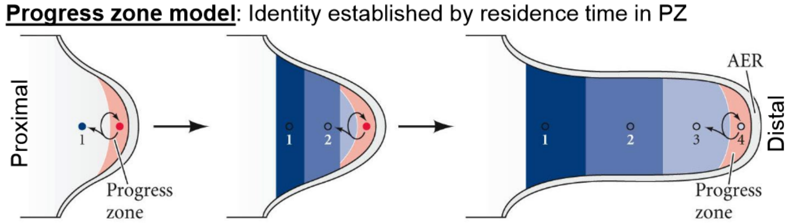


Progress Zone – mesodermal mesenchyme; receives AER signals:
- promotes proliferation (mitosis)
- prevents differentiation into cartilage
- maintains expression of A/P and D/V-related signals

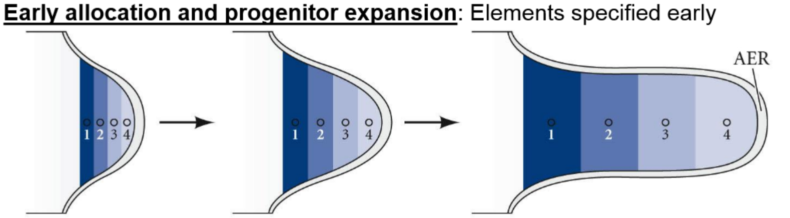
PZ mesenchyme specifies proximal-distal axis
- transplantation experiments demonstrated that positional information was carried by PZ cells
- PZs conveyed age-appropriate specification instructions

Proximal-distal specification models 1.

Progress zone model: Identity established by residence time in PZ

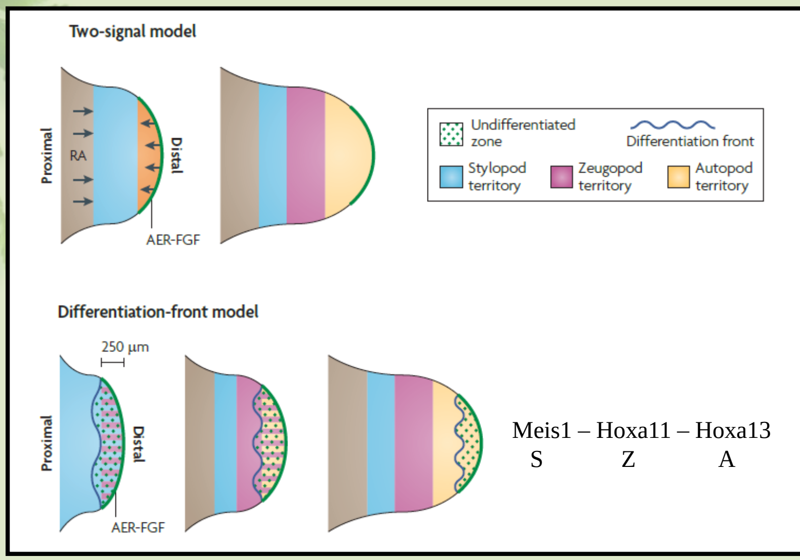


Early allocation and progenitor expansion: Elements specified early



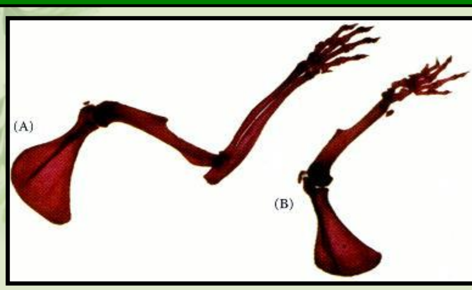
Specifying mechanism - ??

Proximal-distal specification models 2.



Zeller et al., Nature Rev. Gen, 2009

Deletion of limb bone elements by the deletion of paralogous Hox genes

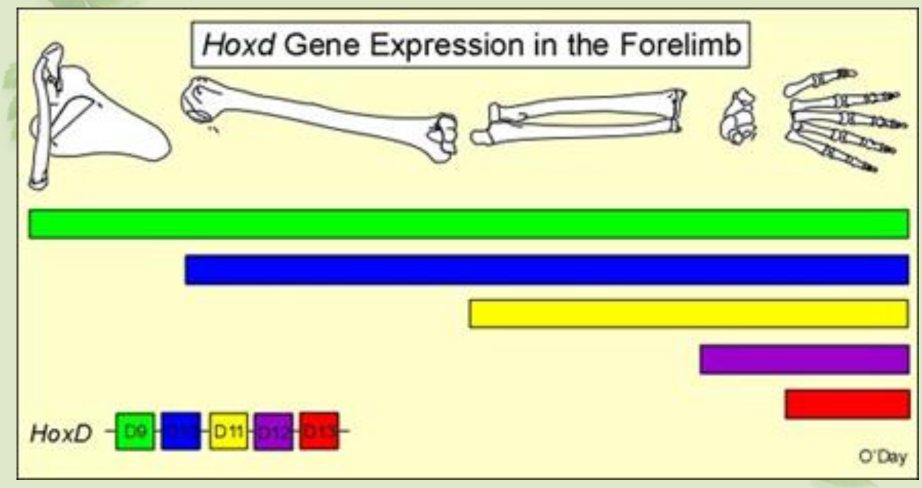


(A) Wild-type mouse forelimb.

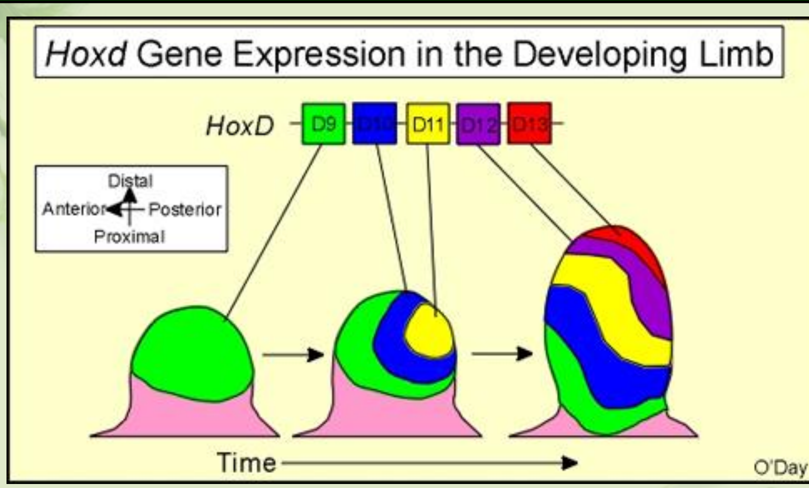
(B) Forelimb of mouse made doubly mutant such that it lacked functional *Hoxa-11* and *Hoxd-11* genes. The ulna and radius are absent.

- The HoxD gene complex is expressed in a specific pattern in the developing mouse forelimb. The pattern of gene expression correlates with the linear arrangement of the genes in the genome.

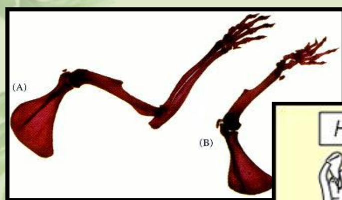
Expression of the Hox genes relative to the final developed limb

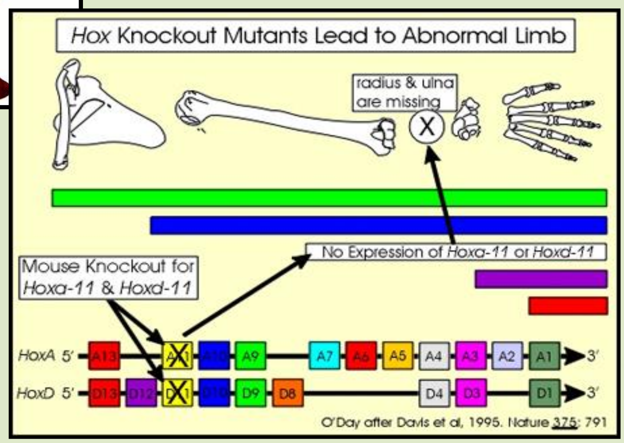


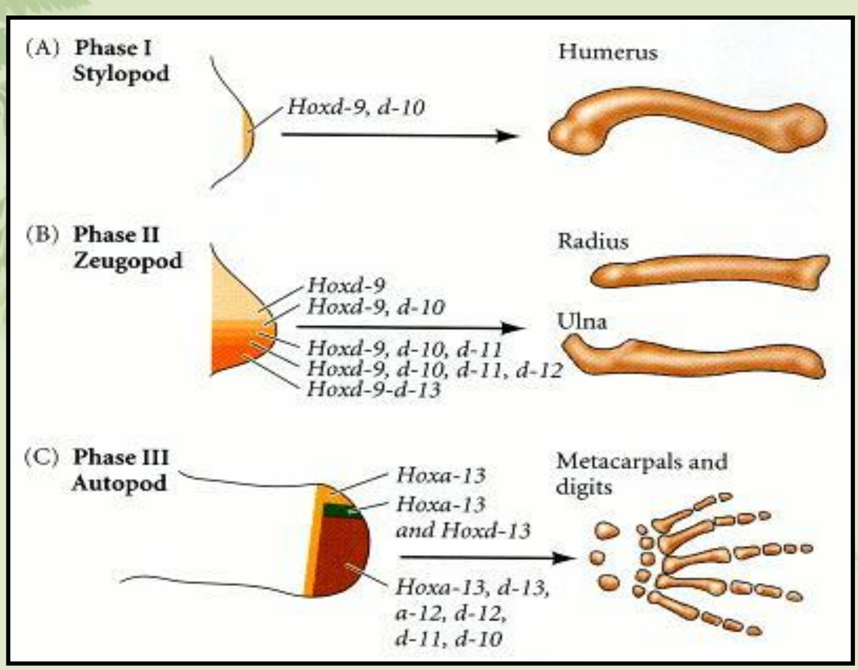
HoxD-9 expressed earliest; HoxD-13 expressed only in final stages of digit formation



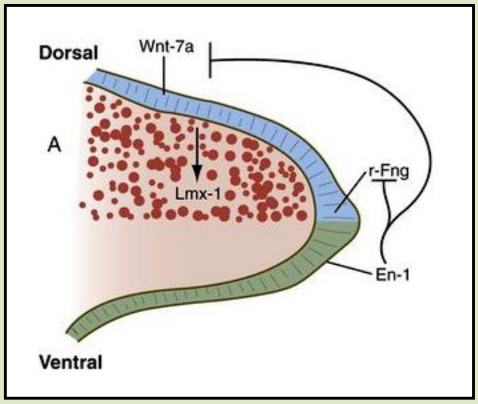
Deletion of limb bone elements by the deletion of paralogous Hox genes





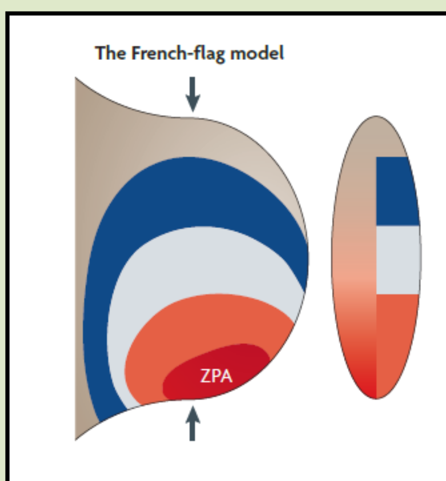


Dorso-ventral polarity



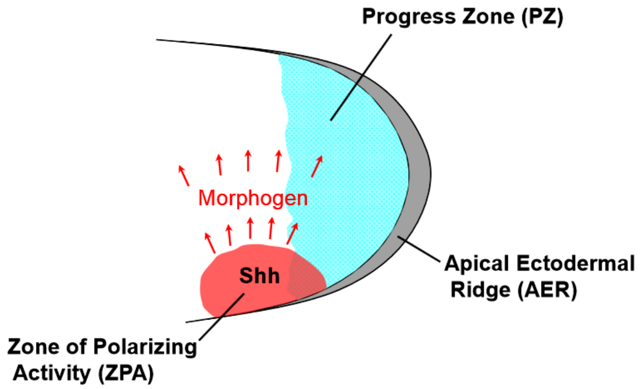
Lmx1: LIM homeobox transcription factor 1
R-Fng: radical fringe
En-1: engrailed 1

Anterior – posterior specification



Wolpert, J. Theor. Biol., 1969

Anterior – posterior specification

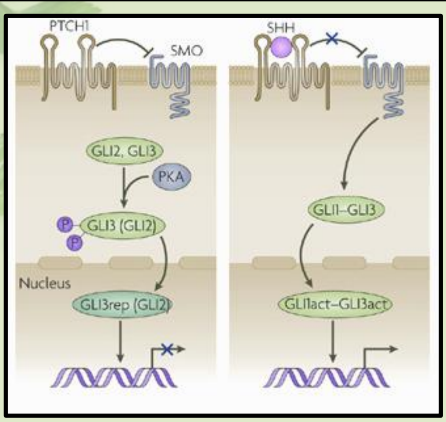


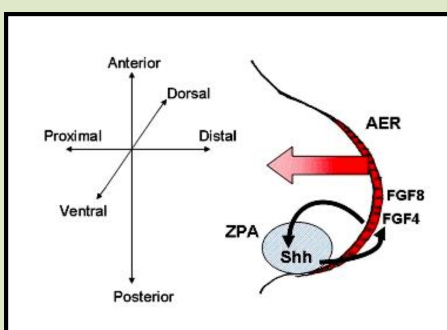
Shh *necessary and sufficient* for establishing ZPA
(NOTE – Shh not necessary for polarity of styolpod)

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

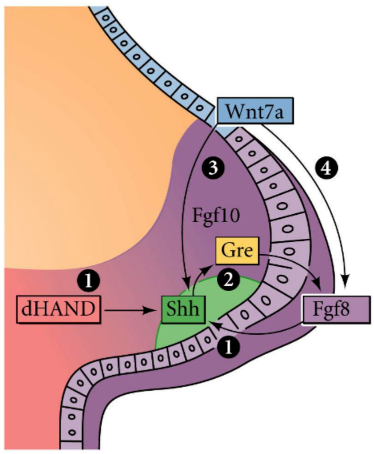
ZPA maintained by feedback loop with AER

Shh signaling and ZPA/AER feedback loop model





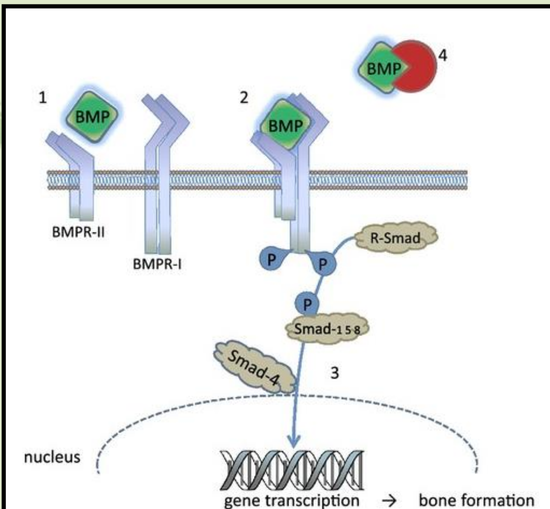
ZPA/AER feedback loop model in details



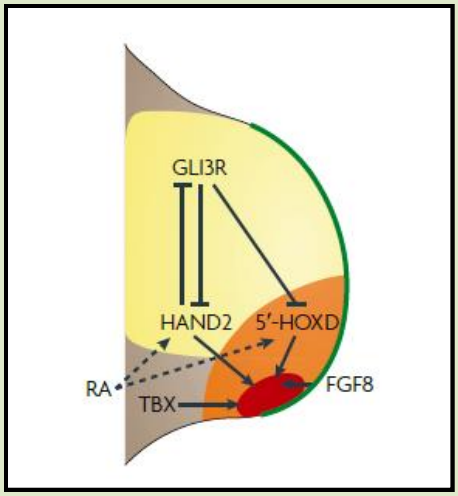
1. dHAND - bHLH transcription factor and Fgf8 from AER stimulate *Shh*
   - Fgf8 (and Fgf4) maintains *Shh* expression
2. Shh up-regulates *Gremlin1* in posterior mesenchyme
   - Grem1 antagonizes BMP ligands (BMPs repress Fgf expression in AER)
3. Wnt7a maintains *Shh*
   Wnt7a determines the size of AER

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

Gremlin1 antagonizes BMP ligands

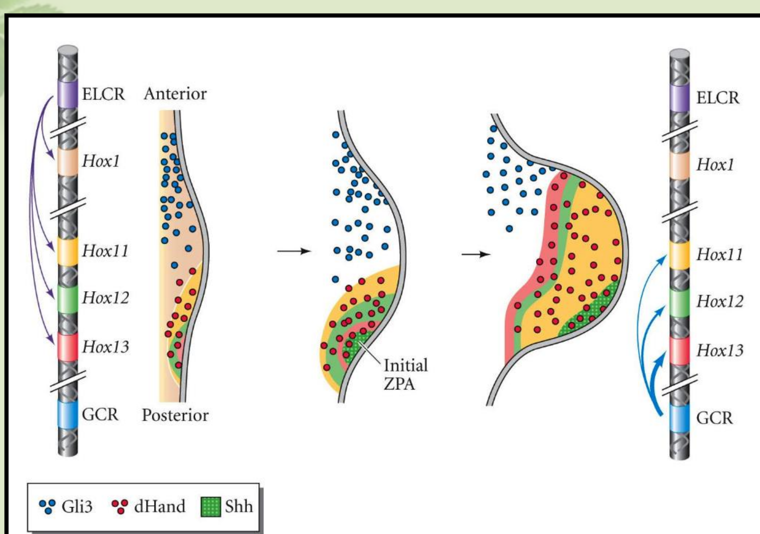


Shh activation network

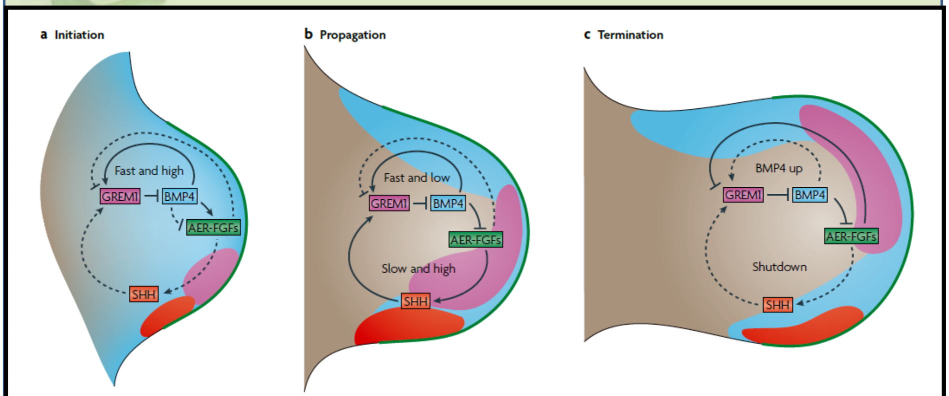


Zeller et al., Nature Rev. Gen, 2009

Hox genes in early limb bud



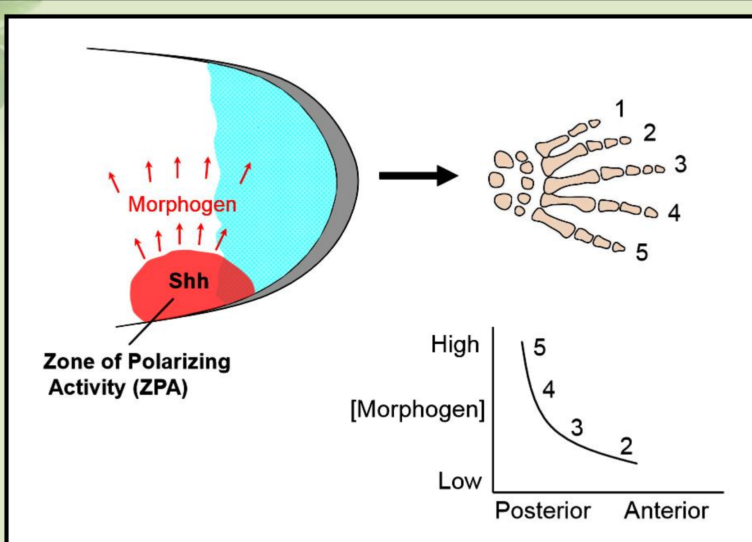
Self-regulation of limb signaling system



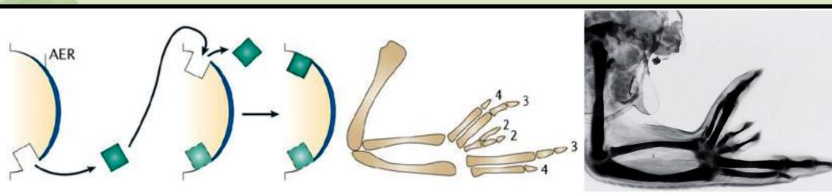
The interlinked signalling feedback loops that operate at each stage are shown as solid lines.
Broken lines indicate inactive loops.

Zeller et al., Nature Rev. Gen, 2009

ZPA morphogen gradient



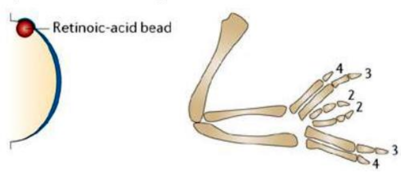
ZPA transplantation



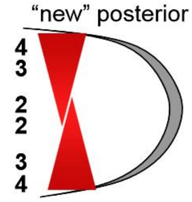
Posterior tissue transplant to anterior = duplicated autopod

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

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

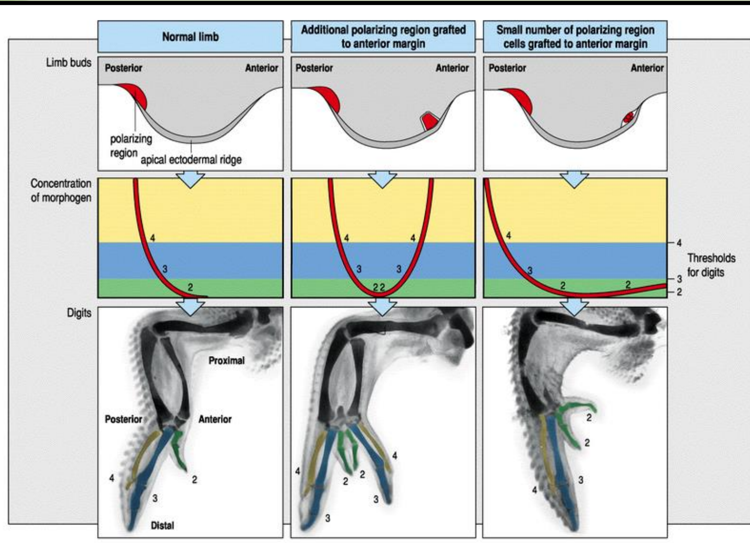


"new" posterior

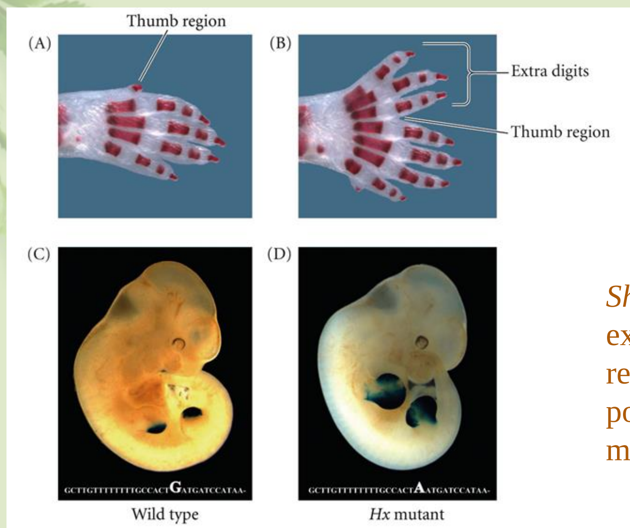


Tickle, Nature Mol. Biol., 2006

ZPA morphogen gradient



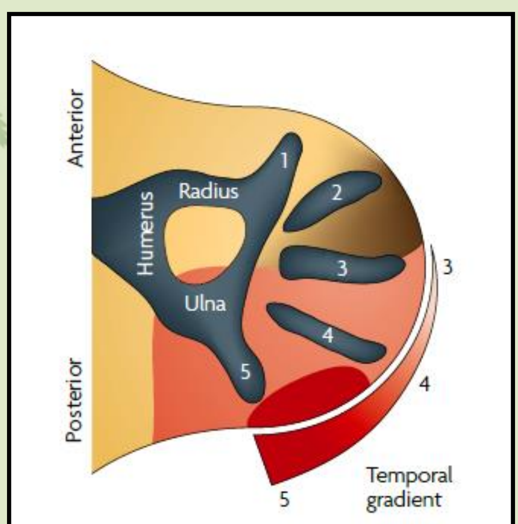
Ectopic expression of shh



The mutant form in (B) is called the *Hx* mutation (hemimelic extratoes).

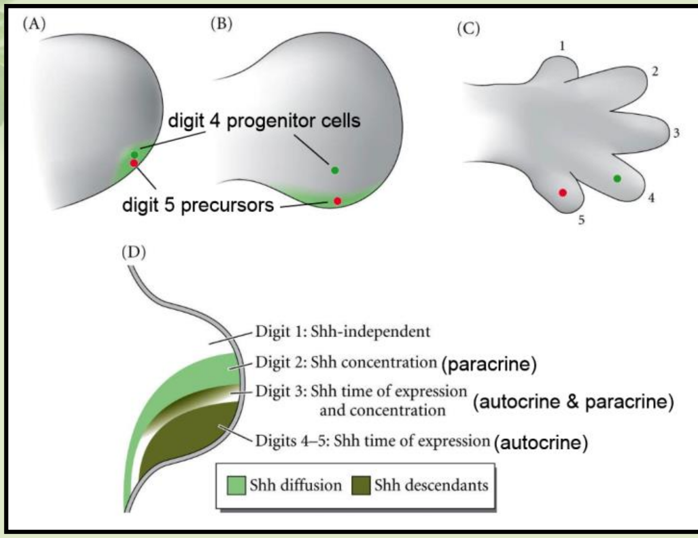
Shh ectopic expression can result in polydactyly in mice.

The temporal gradient model for A-P

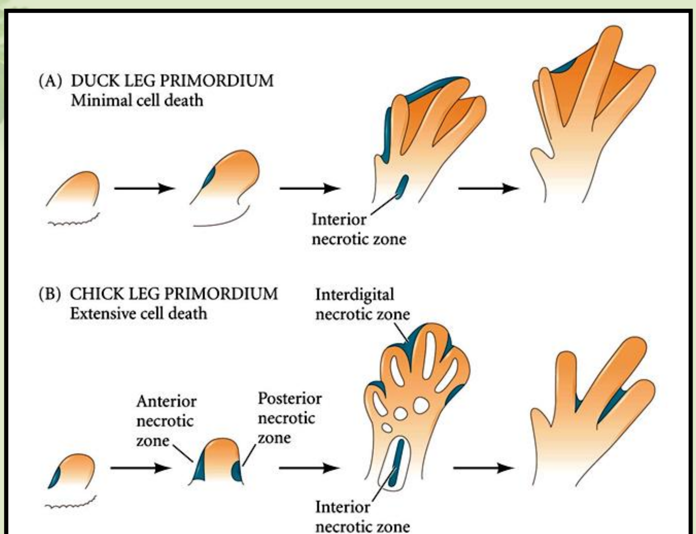


Zeller et al., Nature Rev. Gen, 2009

Shh specify digit identity



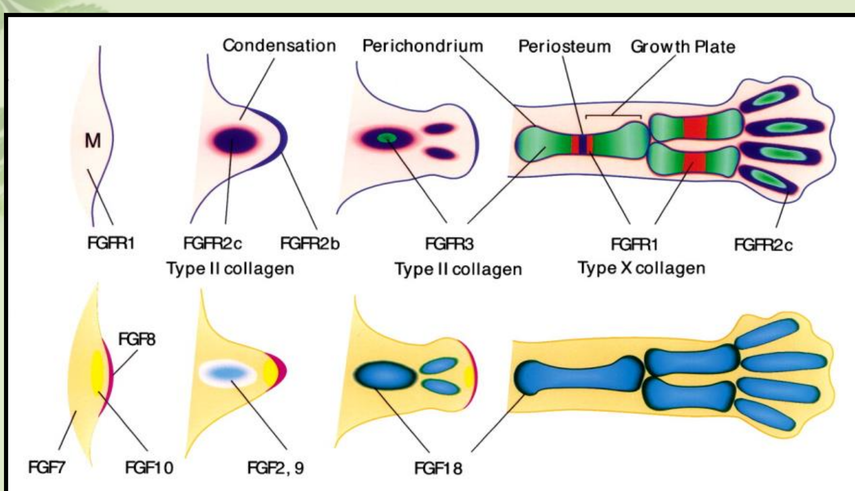
Patterns of cell death in leg primordia



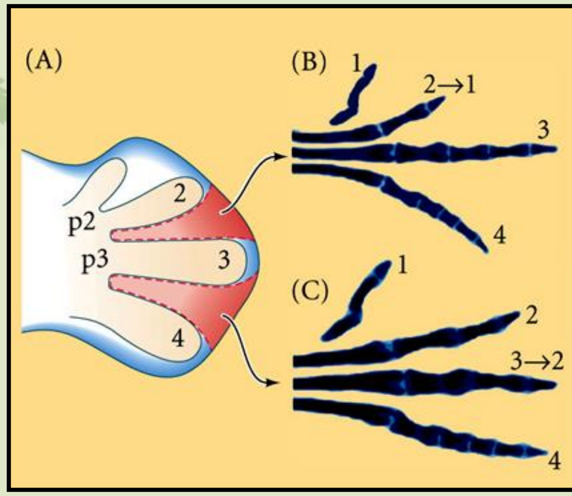
BMPs

- Signals for apoptosis in the autopod are the BMP2, BMP4 and BMP7
- They are expressed in the interdigital mesenchyme
- Blocking BMP signaling prevents interdigital apoptosis – NOGGIN

FGF signaling in bone development

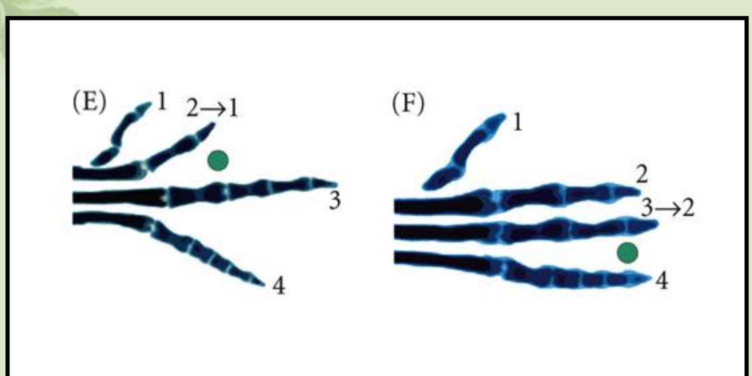


BMPs regulate identity of digits



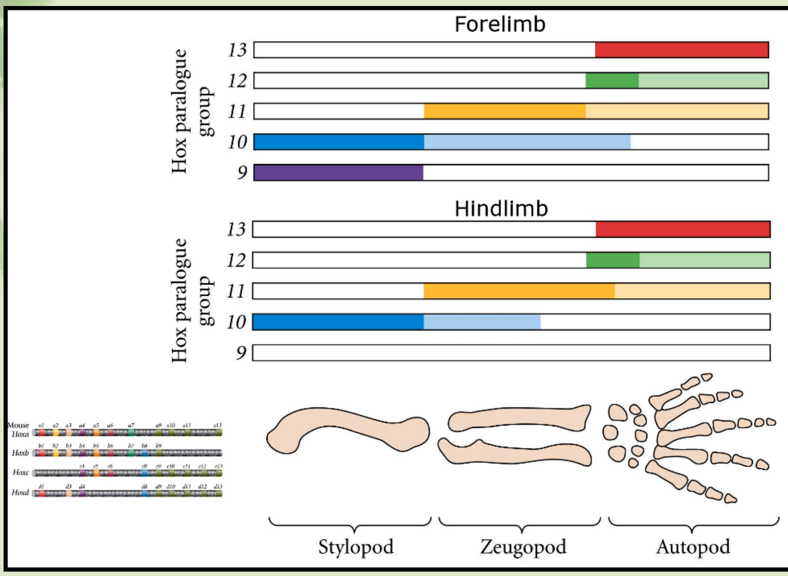
Remove interdigital mesoderm (red tisues).

BMPs regulate identity of digits

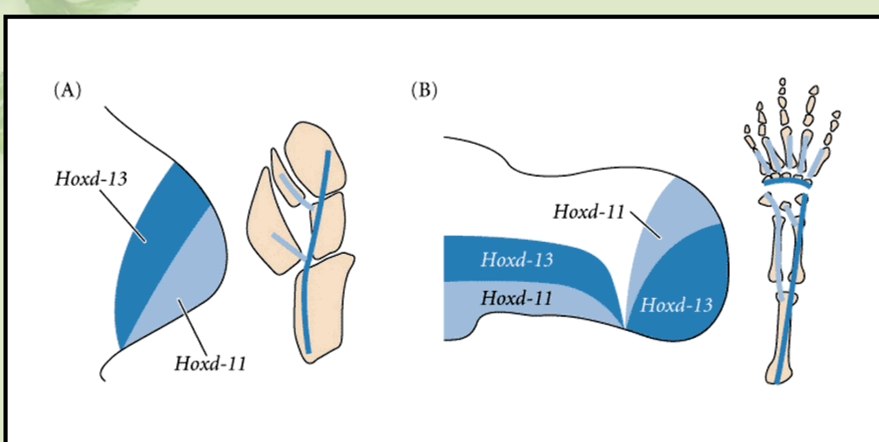


Noggin / BMP antagonist

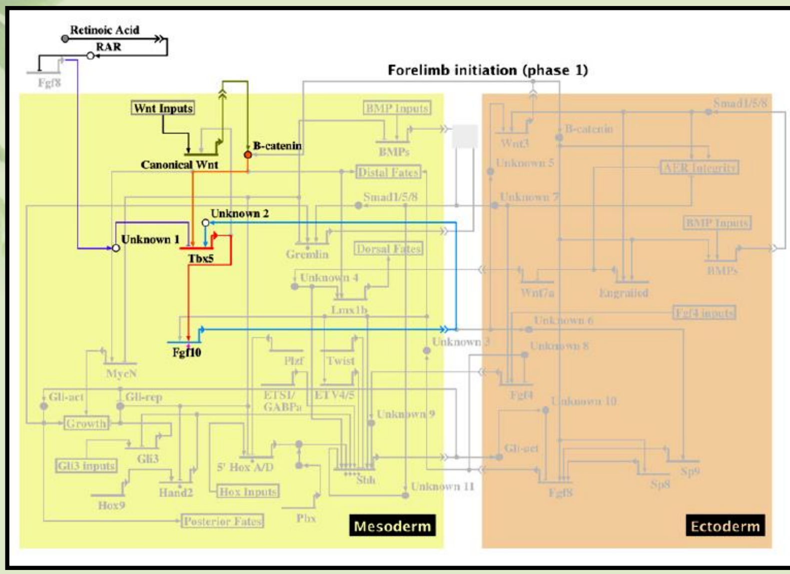
5’ Hox genes pattern



Hox genes in fins and legs



Control of forelimb initiation phase



Rabinovitz et al., Dev. Biol., 2012

The End