Limb Development

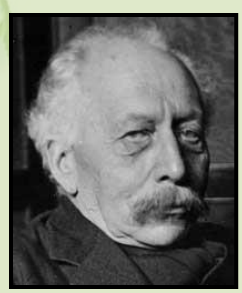
Fejlődés- és Molekuláris Genetika, 2021

I. Insect Leg Development

Homeotic mutants and Hox genes

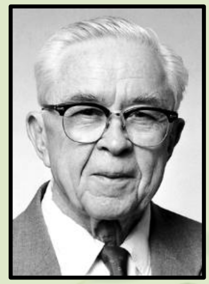
- The term *homeotic variation* refers to mutant alleles in which one body part is replaced by another (homeosis) (William H. Bateson)
- Fisrt homeotic mutant : bithorax

- The term Hox genes was introduced by Edward B. lewis



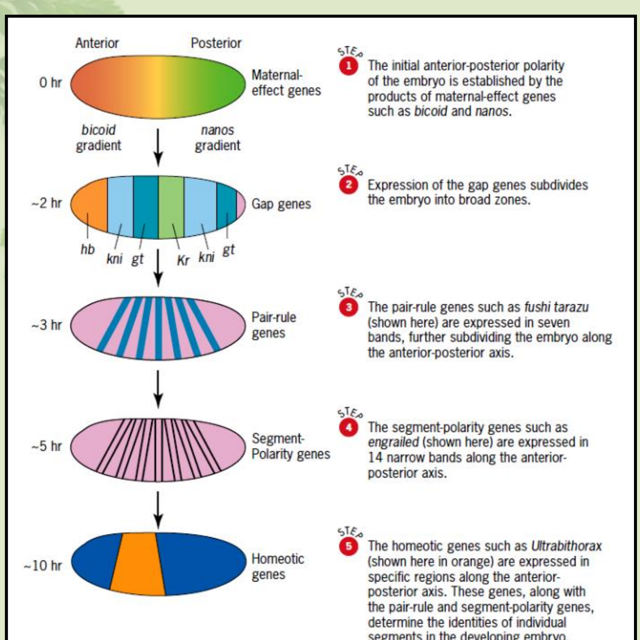
(1861-1926)

1889 - 1938

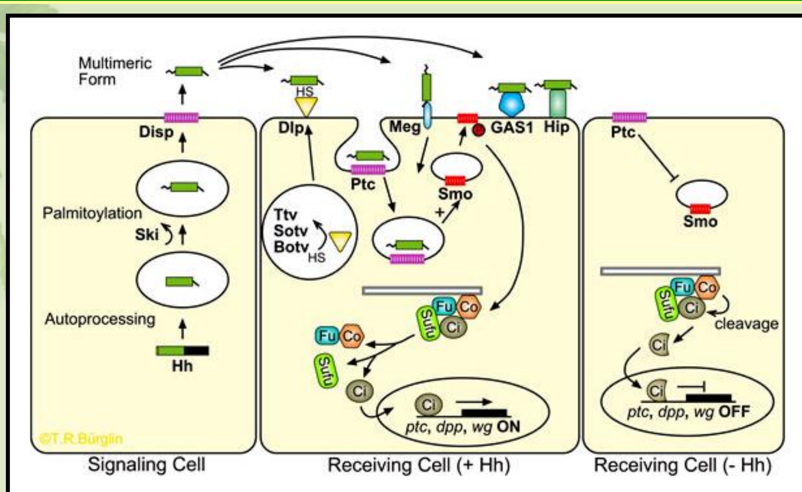


(1918-2004)

Cascade of gene expression in *Drosophila* embryos



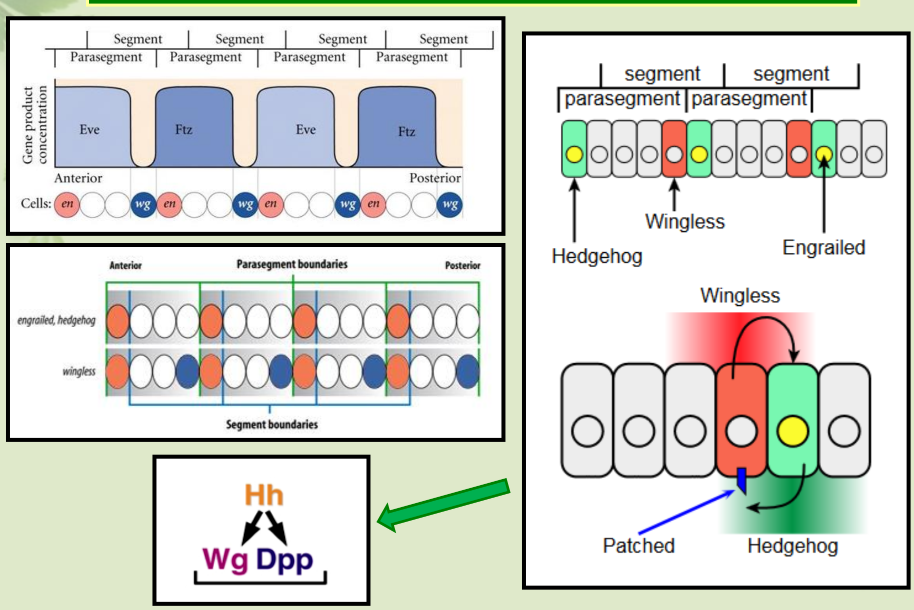
Hedgehog (Hh) signaling pathway



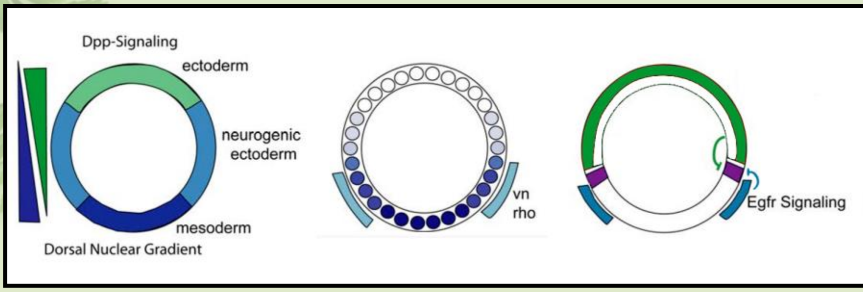
Disp: Dispatched
Ptc: Patched
Smo: Smoothened
Co: Costal 2

Fu: Fused
Sufu: Supressor of fused
Ci: Cubitus interruptus (**CiA** and **CiR**)
(Transcriptional activator (**A**) or repressor (**R**))

Segment polarity genes

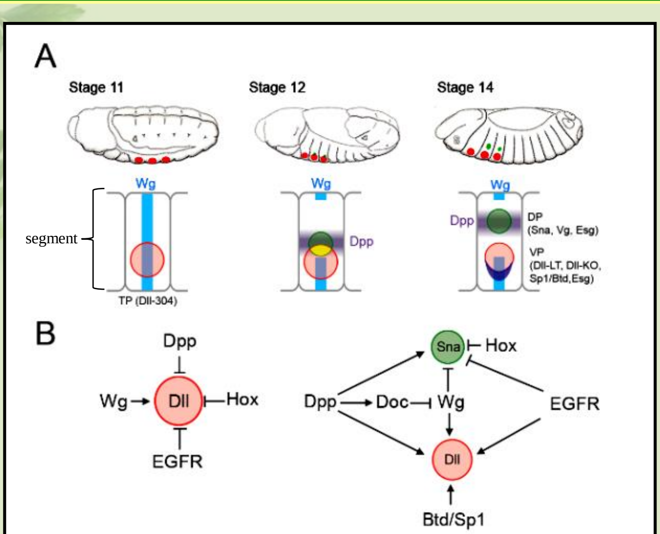


Background: dorso-ventral polarity



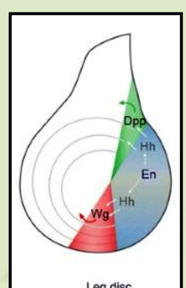
decapentaplegic (dpp) encodes a ligand of the transforming growth factor-β signaling pathway
dorsal (dl) encodes a transcription factor
vein (vn) encodes a secreted neuregulin-like EGFR ligand.
Epidermal growth factor receptor (Egfr) encodes the transmembrane tyrosine kinase receptor for signaling ligands in the TGFα family,

Specification of the thoracic appendages.



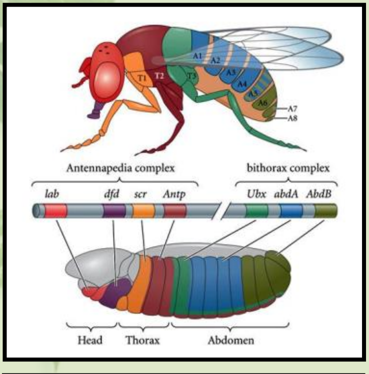
Ruiz-Losada et al., Dev. Biol., 2018

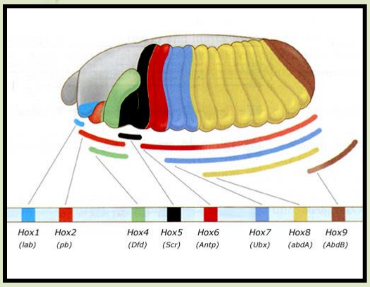
- Thoracic/ventral primordia
- Dorsal primordia

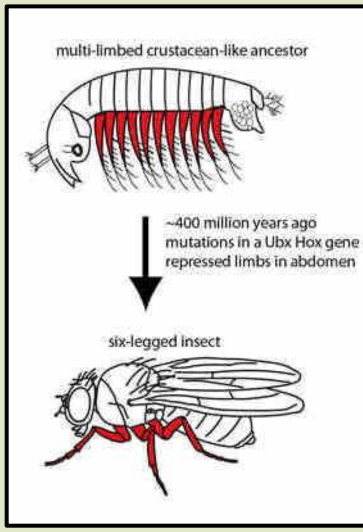


Beira and Paro, Chromosome, 2016

Leg discs' progenitor domains and the homeobox genes





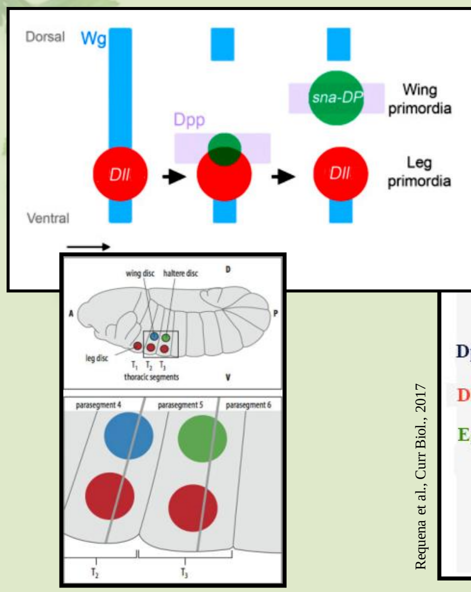


Ronshaugen et al., *Nature*, 2002

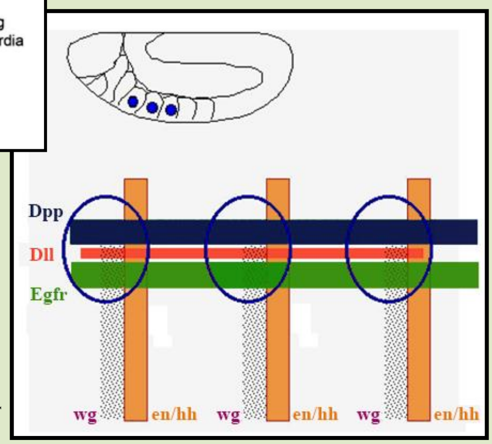
Homeobox genes *vs* Hox genes

Homeobox genes	Hox genes
Homeobox genes are a group of genes that encode for transcription factors that regulate the anatomical development of organisms	Hox genes are a subset of homeobox genes that specifically regulate the development of body axes and structures during embryonic development
Regulate morphogenesis	Regulate body segmentation and development of appendages
Around 200	Around 39

First step to proximo-distal patterning

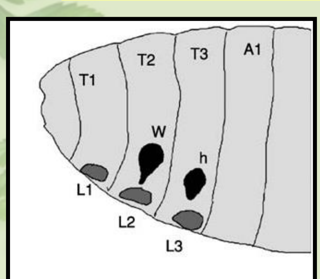


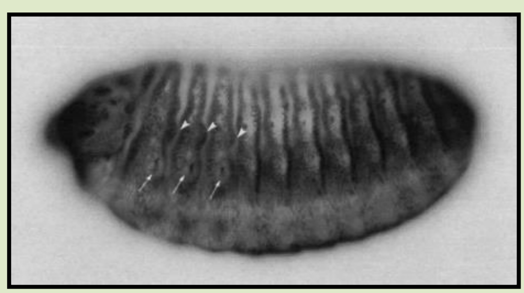
Dpp: Decapentaplegic – a BMP (secreted ligand)
Dll: Distalless – a homeodomain transcription factor
Egfr: EGF receptor

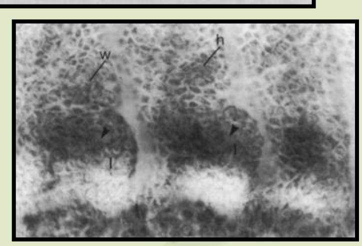


Requena et al., Curr Biol., 2017

Positions of leg discs' precursor cells

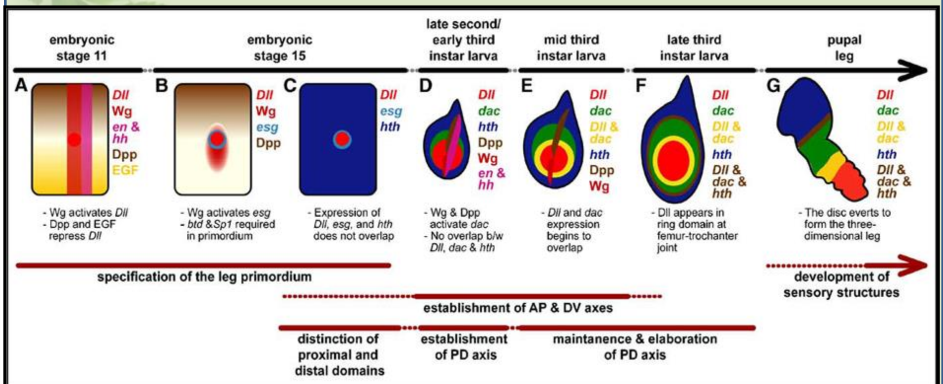






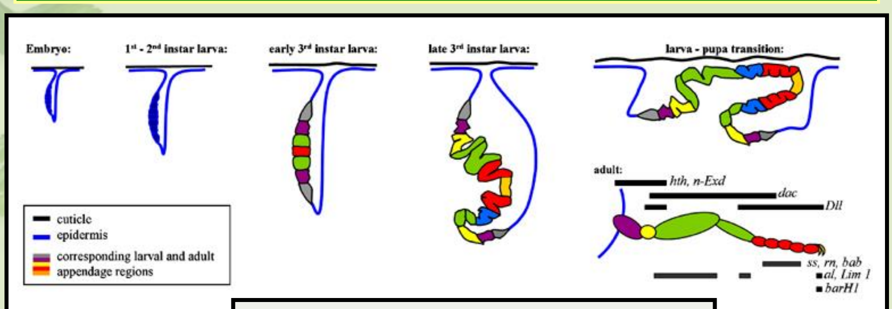
Bate and Arias, *Development*, 1991

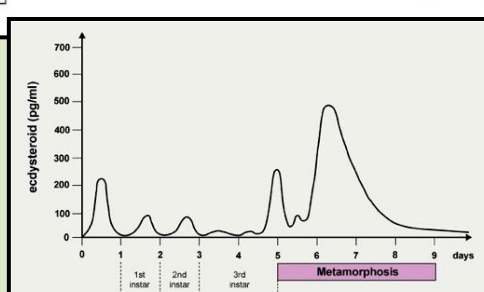
An overwiev of leg development



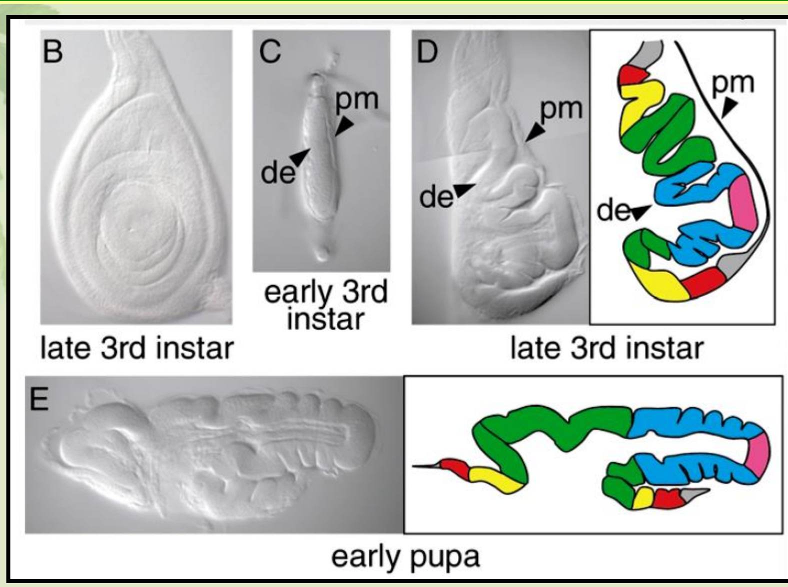
Esg maintains the imaginal state through the repression of endoreplication

Leg disc's morphogenesis

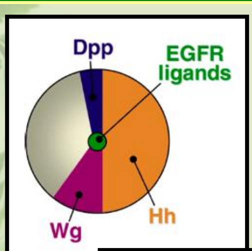


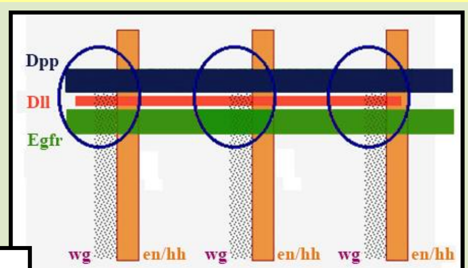


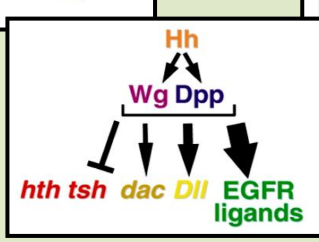
Steps of leg morphogenesis



Leg discs' progenitor domains

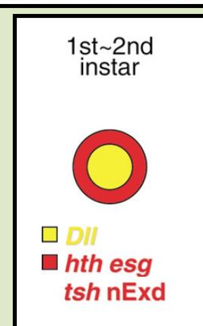




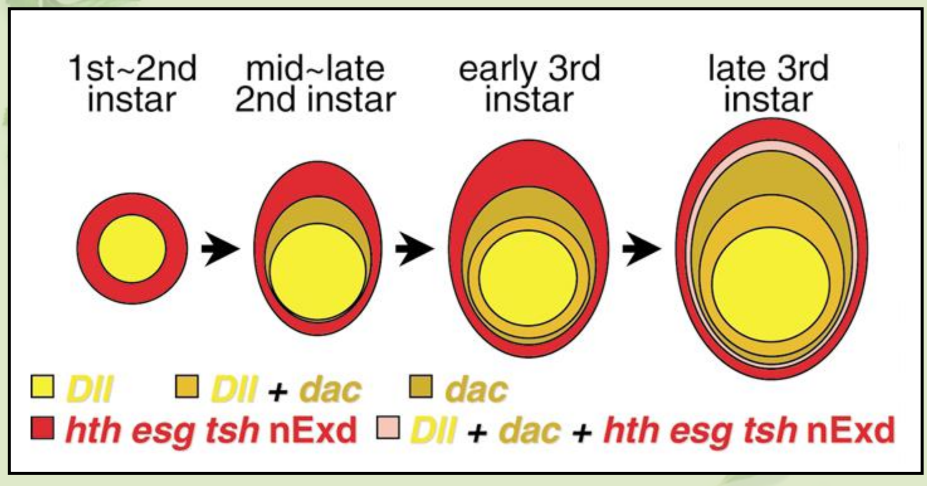


Formation of most distal area of developing leg

hth: homothorax (homeobox)
tsh: teashirt (transcription f.)
dac: dachshund (transcription f.)
Dll: distalles (homeobox)
Esg: Escargot (transcription f.)
Exd: extradenticle (transcription f.)

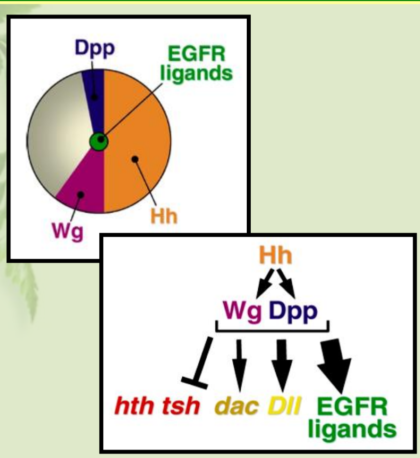


Gene expression domains in leg discs



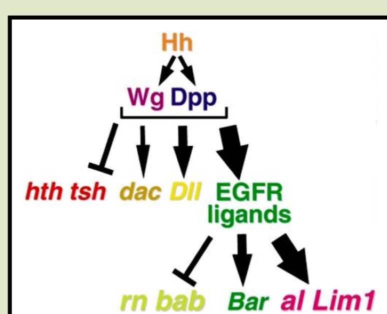
Kojima, *Dev. Growth Differ.*, 2004

Leg discs' progenitor domains

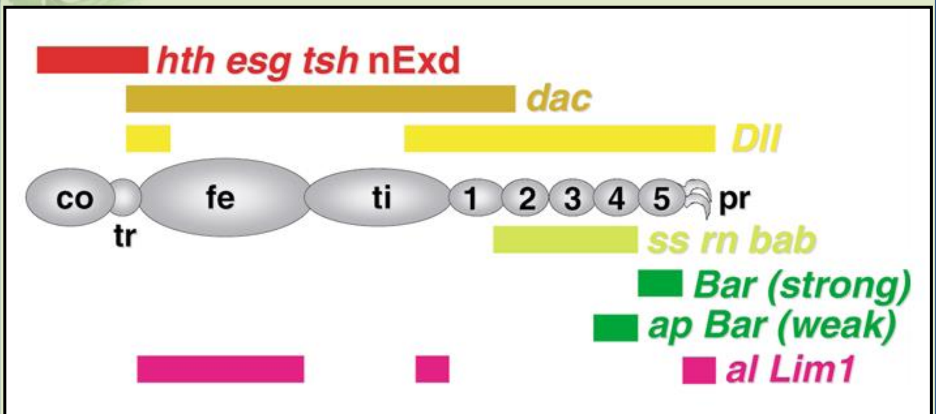


Proximal → distal

rn: round
(transcription f.)
bab: bric-a brac
(transcription f.)
Bar: BARH1/2
(homeobox)
al: aristaless
(homeobox)
LIM:LIM1
(homeobox)

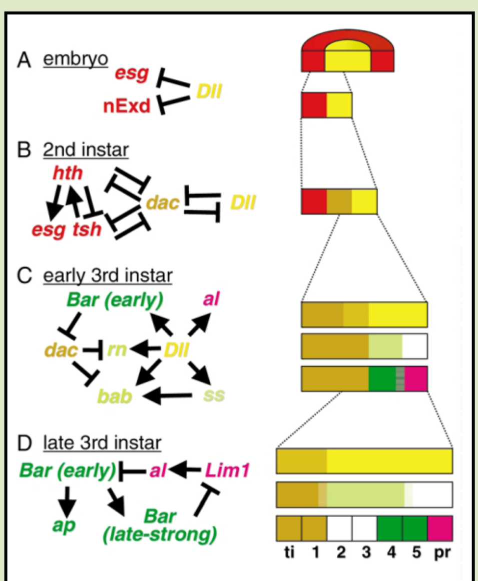


Proximodistal pattern of gene expression



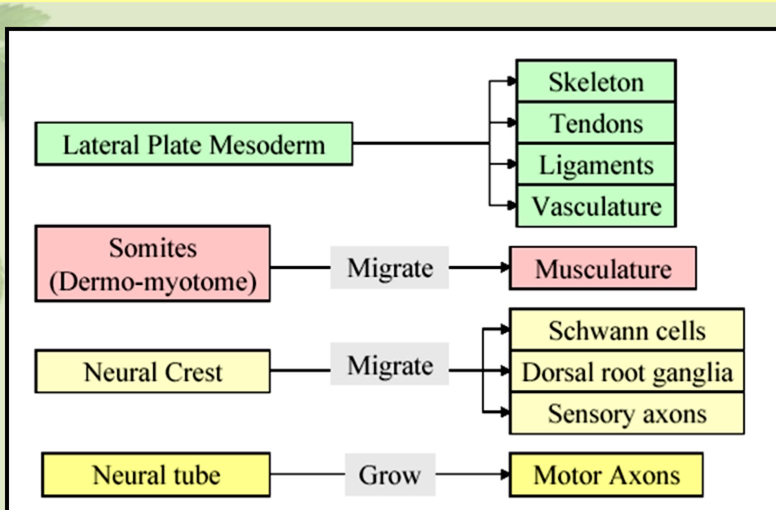
rn: rotund (transcription f.)
bab: bric-a brac (transcription f.)
al: aristaless (homeobox)
SS: spineless (HLH protein)
Bar: BARH1/2 (homeobox)
LIM:LIM1 (homeobox)

PD: crosstalk between the genes

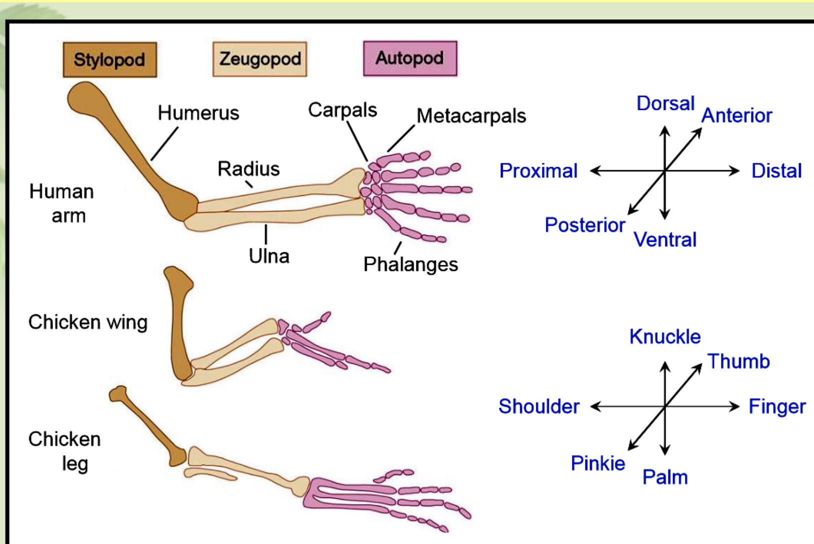


II. Vertebrate Limb Development

From four sources

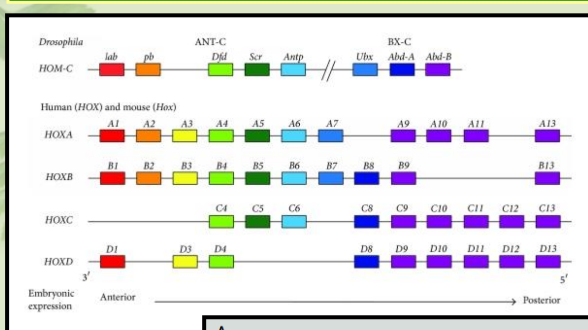


Parts and dimensions of a limb

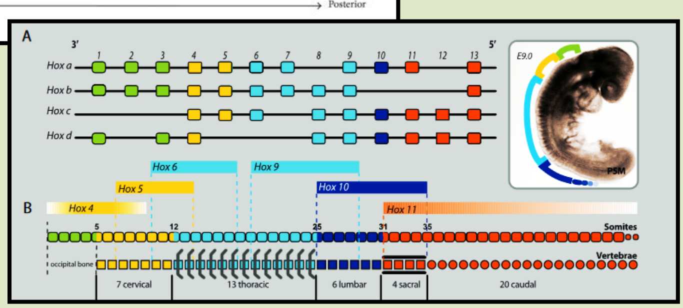


Manini and Martin, Nature 2003

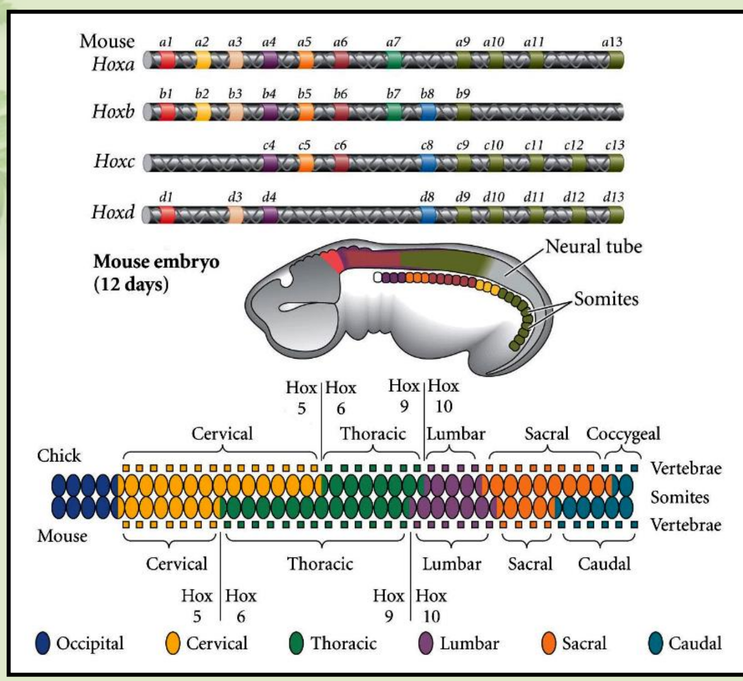
Hox genes in Drosophila and mammals



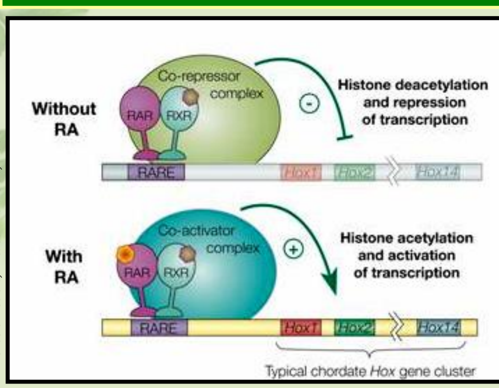
HoxA: 7th chromosome
HoxB: 17th chromosome
HoxC: 12th chromosome
HoxD: 2th chromosome



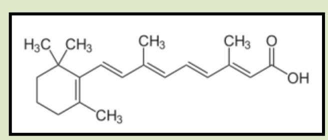
Chal and Pourquié, 2009, The Skeletal System

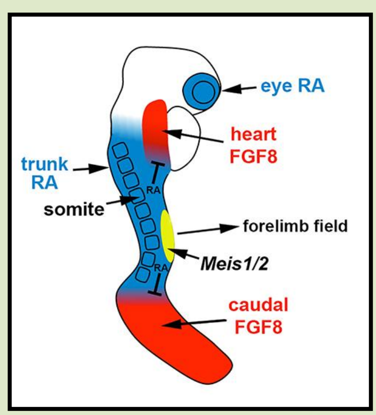


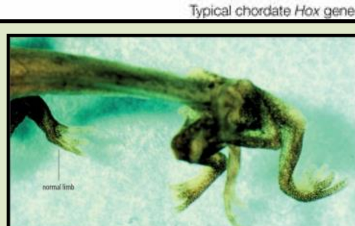
Retinoic acid signaling



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

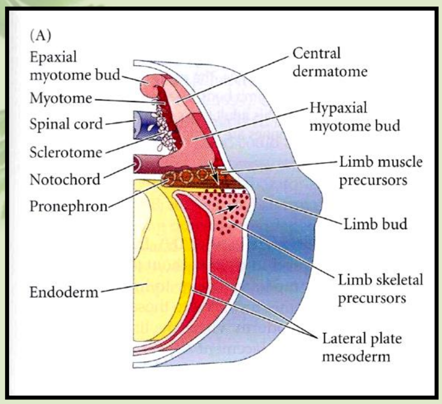




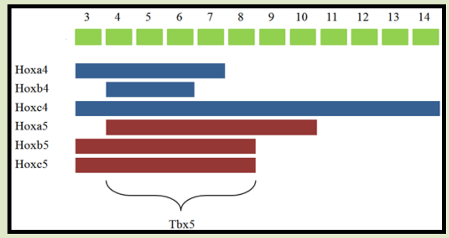


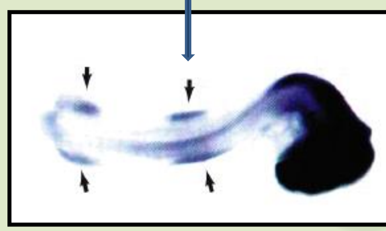
Meis1/2: homeobox genes

First morphological sign



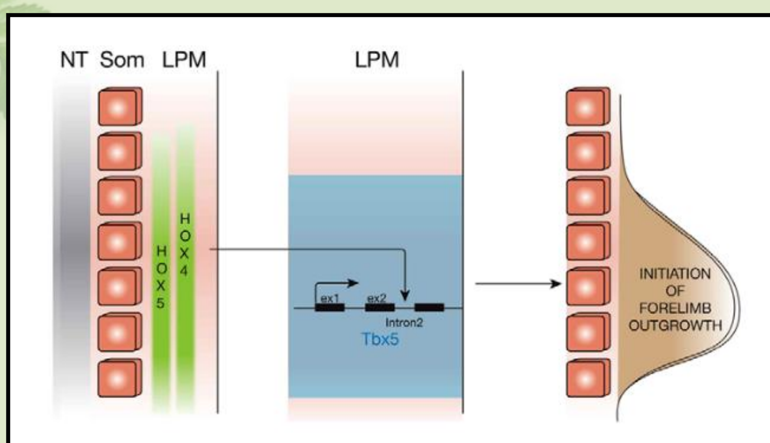
Posterior lateral plate of mesoderm (PLPM) + ectoderm





T-box genes encode transcription factors

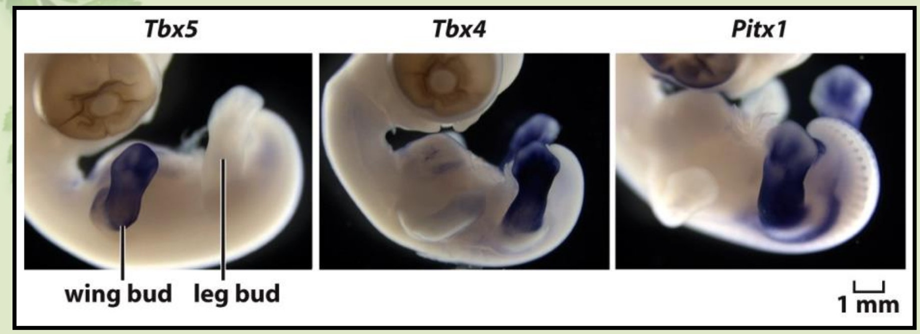
Hox4/5 genes control the expression of Tbx5



Minguillon et al., Development, 2012

361 bp sequence in the intron 2 ➡ 6 Hox binding site (Hbs): 1, 3, 4, 5, and 6: activators (Hox4, Hox5)
(CNE – conserved non-coding element) Hbs2: inhibitor (Hox8, Hox9 and 10)

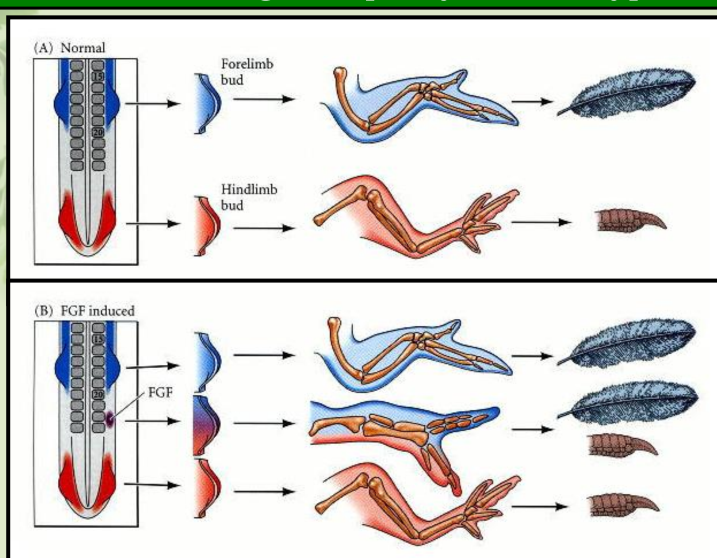
Tbx expression



- Tbx4/Tbx5: T-box transcription factors
- Pitx-1: Pituitary Homeobox1
- Tbx4 and Pitx1: Expressed in the leg (hindleg) bud.
- Misexpression of Pitx1in the wing bud causes the limb to develop with leg-like characteristics.
- Tbx2 and Tbx3 expressed in both buds

Molecular Biology of the Cell

Tbx genes specify the limb type



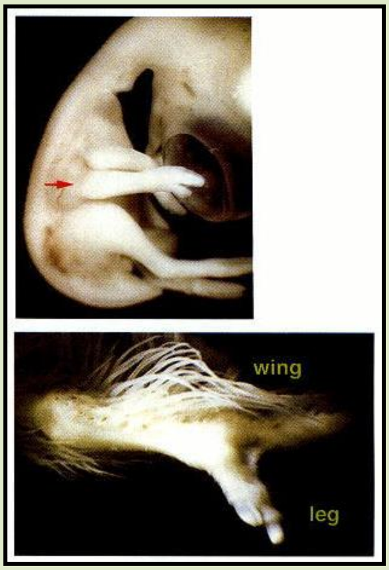
Retinoic acid
↓
Hox genes
↓
Tbx genes
↓
Wnt/FGF

Tbx4/5 and Pitx genes are expressed in the mesoderm.

Gradients of Tbx5 and Tbx4

Tbx5 – forelimb

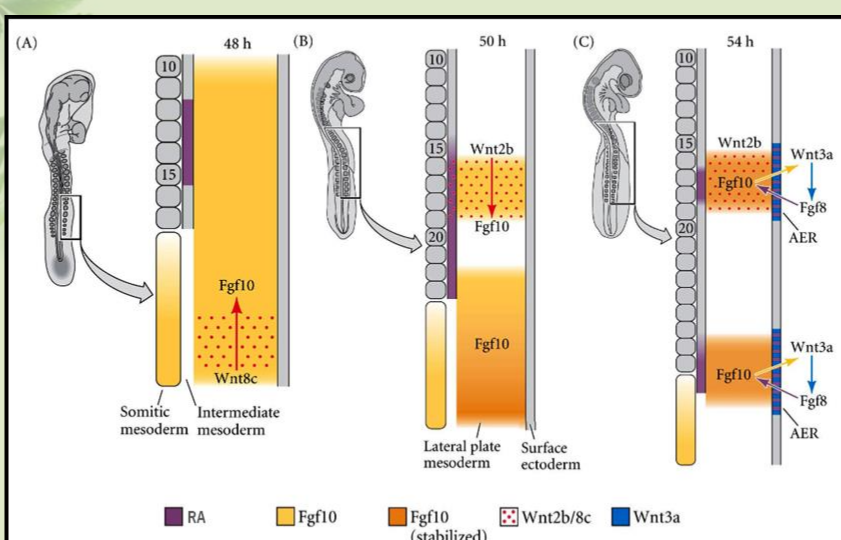
Tbx4 - hindlimb



Tbxs initiate the expression of Fgfs (and Wnt ?)

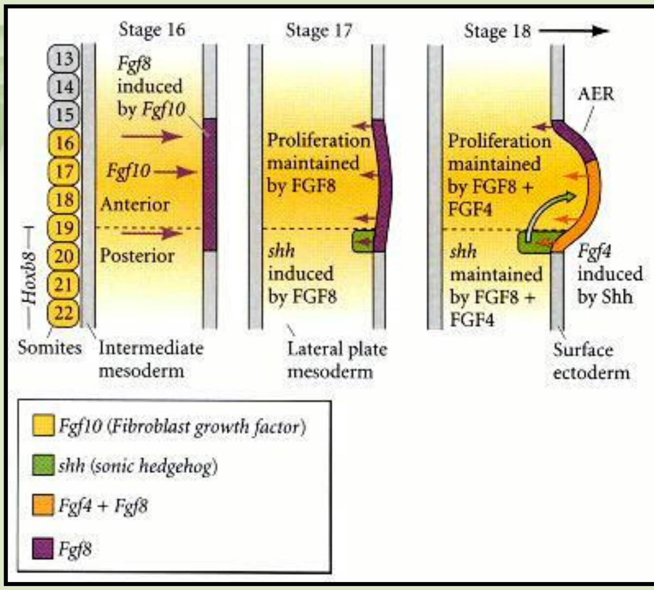
Ohuchi and Noji, *Cell Tissue Res.*, 1999

Fgf/Wnt – limb bud initiation



Gilbert: Developmental Biology, 6th ed.

Appearance of AER

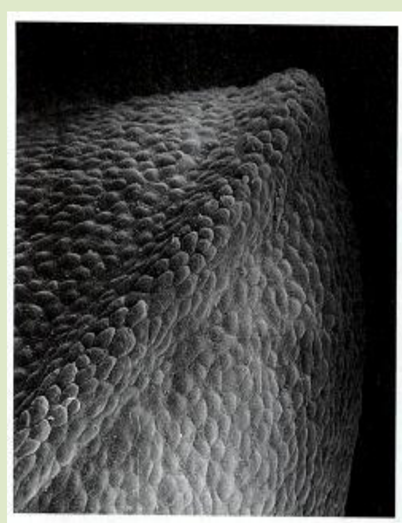


Gilbert: Developmental Biology, 6th ed.

Apical ectodermal ridge (AER)

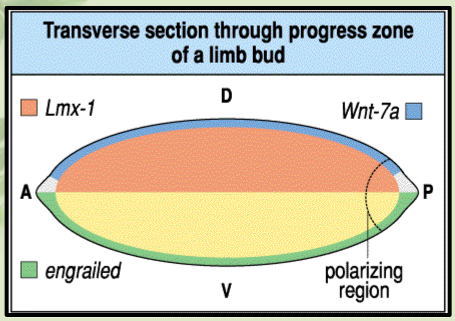
Three functions of AER

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.

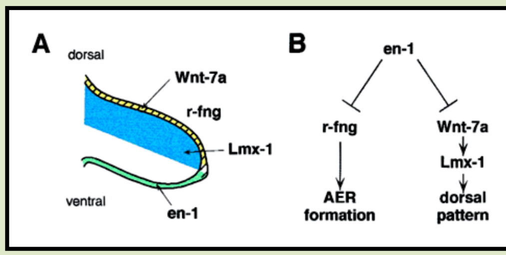


The major signaling center for the developing limb.

Dorsoventral polarity



Wnt: wingless-nt
Lmx-1: LIM homeobox 1
r-fng: radical fringe

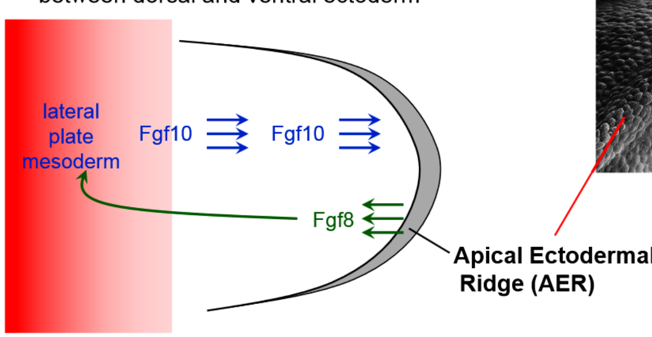


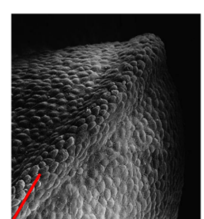
AER: apical ectodermal ridge

Formation of proximodistal axis

Proximal-Distal Axis

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



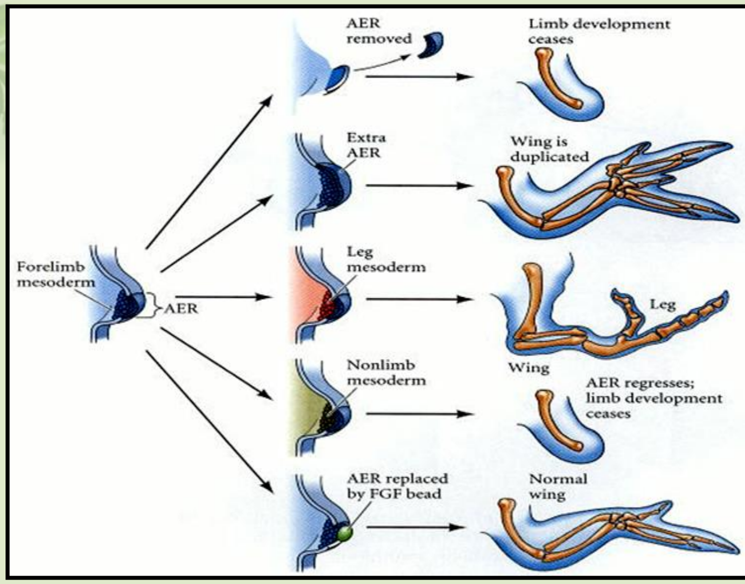


Lateral plate mesoderm expresses Fgf10

Fgf10 initiates AER via Wnt3a, β-catenin

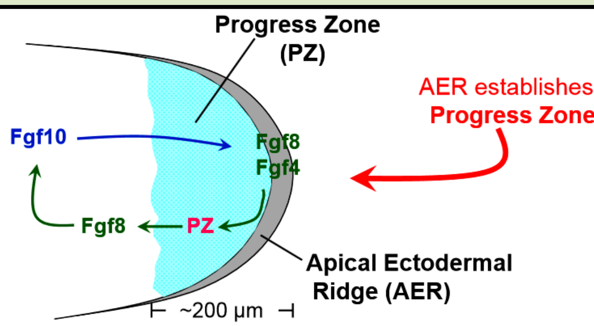
AER expresses Fgf8, Fgf4; maintains Fgf10 expression

AER manipulations



Gilbert: Developmental Biology, 6th ed.

The progress zone

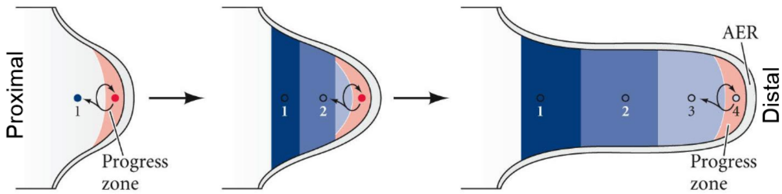


Progress Zone – mesodermal mesenchyme; receives AER signals:
- promotes proliferation (mitosis)
- prevents differentiation into cartilage
- maintains expression of A/P and D/V-related signals

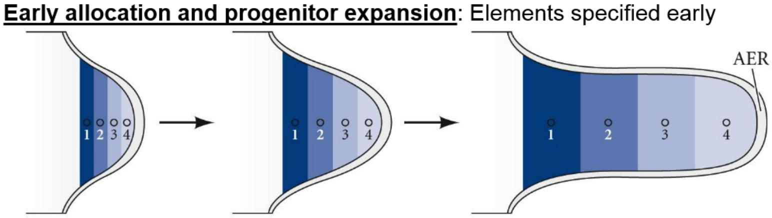
PZ mesenchyme specifies proximal-distal axis
- transplantation experiments demonstrated that positional information was carried by PZ cells
- PZs conveyed age-appropriate specification instructions

Proximal-distal specification models 1.

Progress zone model: Identity established by residence time in PZ

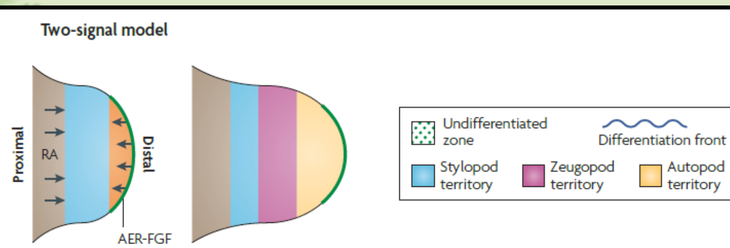


Early allocation and progenitor expansion: Elements specified early

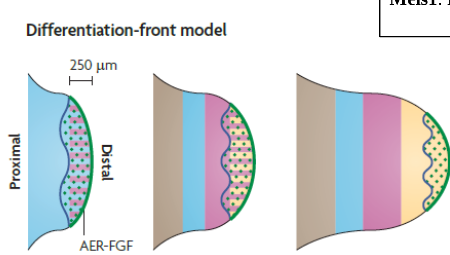


Specifying mechanism - ??

Proximal-distal specification models 2.



Meis1: Myeloid ecotropic viral integration site 1 (homeobox gene)

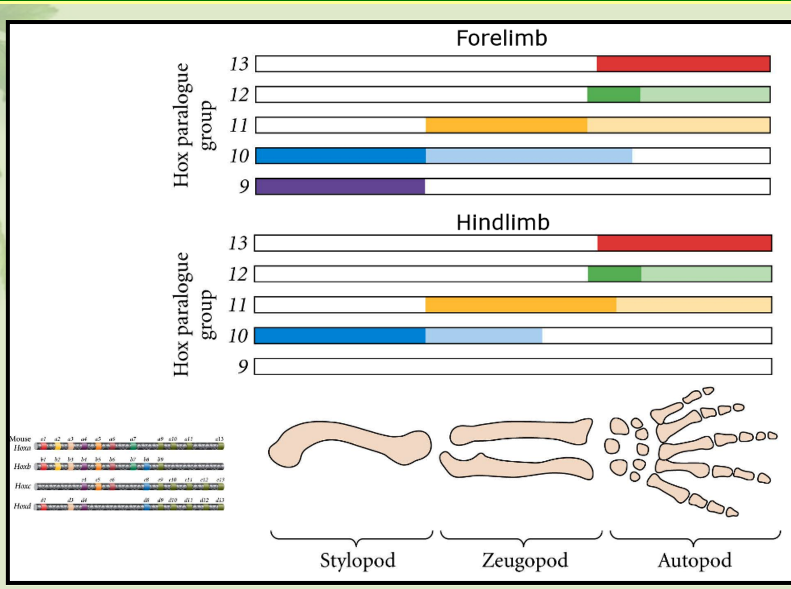


Marker genes:

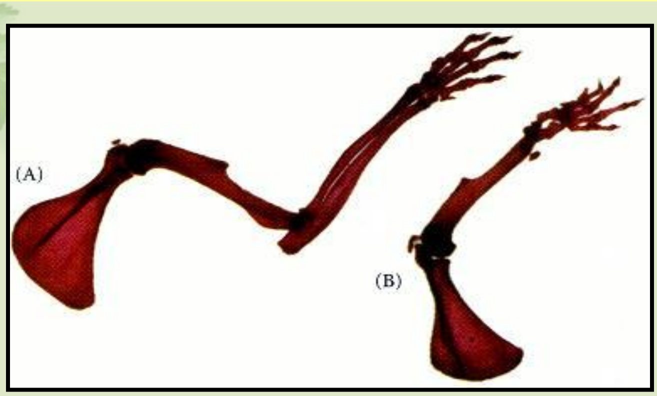
Meis1 – Hoxa11 – Hoxa13
S Z A

Zeller et al., Nature Rev. Gen, 2009

5’ Hox genes pattern



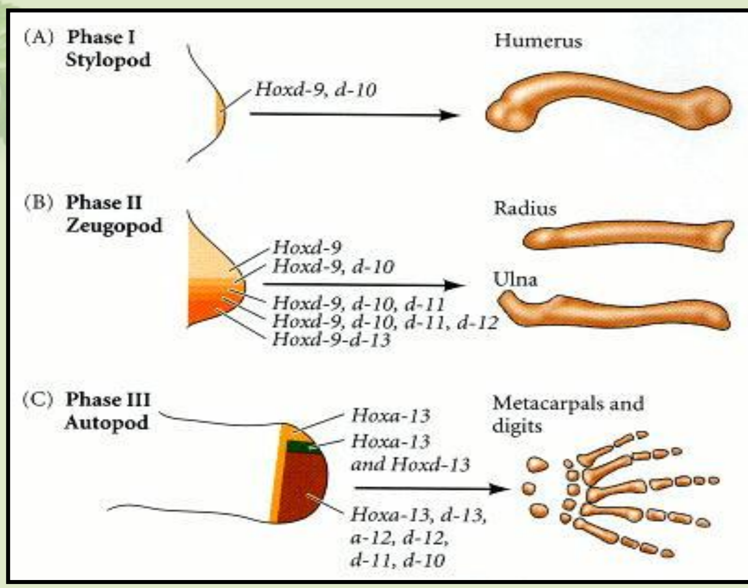
Deletion of limb bone elements by the deletion of paralogous Hox genes



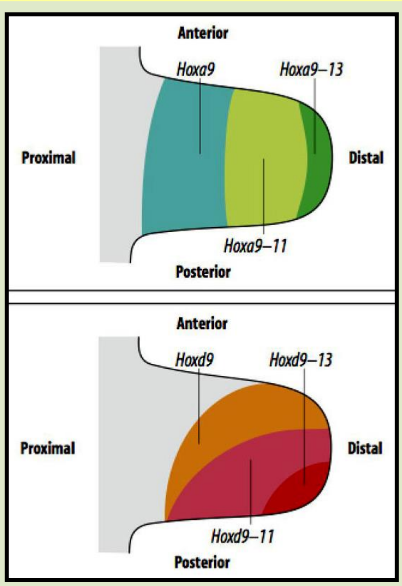
(A) Wild-type mouse forelimb.

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

Determination of S-Z-A by paralogous Hox genes



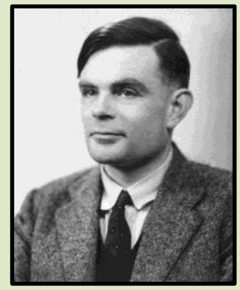
Expression pattern of HoxA and HoxD genes



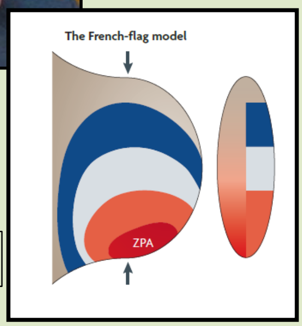
Anterior – posterior specification

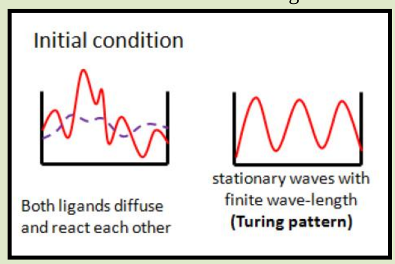
Peter Wolpert

Theories of pattern making



Alan Turing

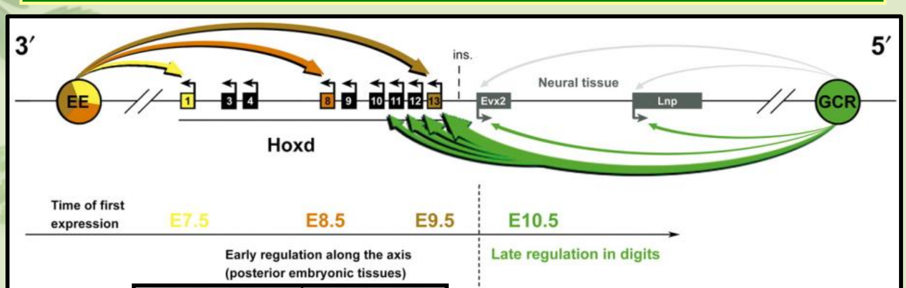


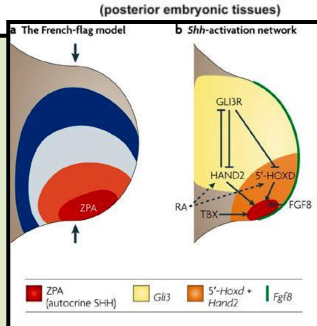


ZPA:
Zone of Polarizing Activity

Wolpert, J. Theor. Biol., 1969

Creation of ZPA



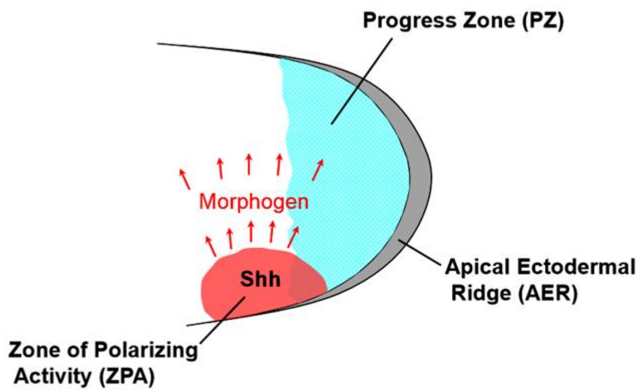


EE: Early enhancer (Early Limb Control Region)
GCR: Global Control Region

Wnt AER (Fgf8)

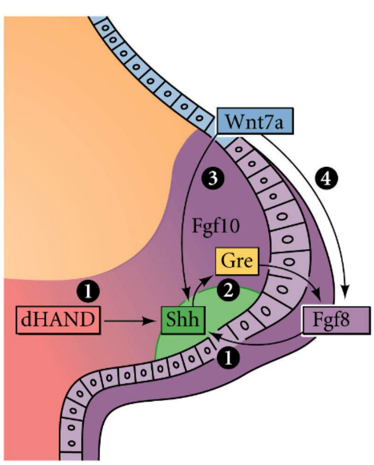
Hoxb8 ➡ **ZPA**

Anterior – posterior specification



Shh *necessary and sufficient* for establishing ZPA
(NOTE – Shh not necessary for polarity of styolpod)
Shh induced by **dHAND** and **Hox genes**
ZPA maintained by feedback loop with AER

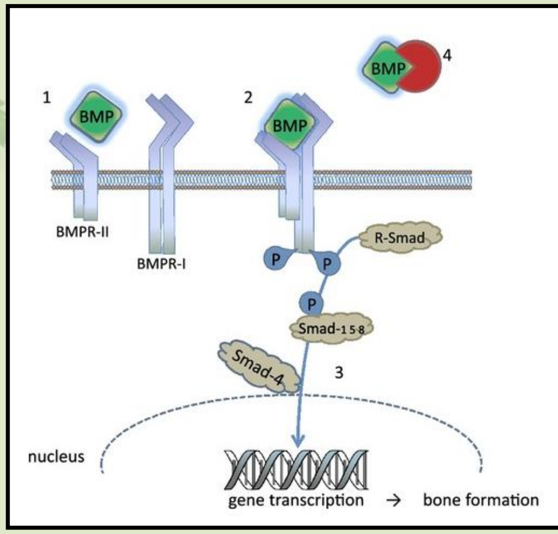
ZPA/AER feedback loop model in details



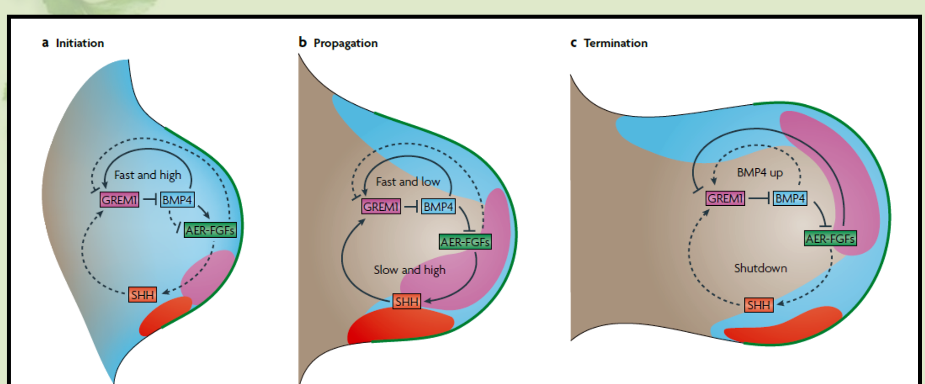
1. dHAND - bHLH transcription factor and Fgf8 from AER stimulate *Shh*
   - Fgf8 (and Fgf4) maintains *Shh* expression
2. Shh up-regulates *Gremlin1* in posterior mesenchyme
   - Grem1 antagonizes BMP ligands (BMPs repress Fgf expression in AER)
3. Wnt7a maintains *Shh*
   Wnt7a determines the size of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

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

Gremlin1 antagonizes BMP ligands

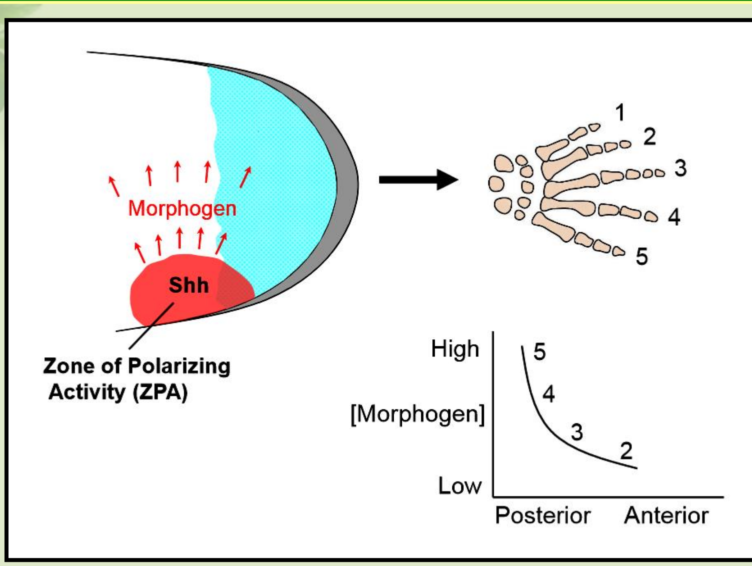


Self-regulation of limb signaling system

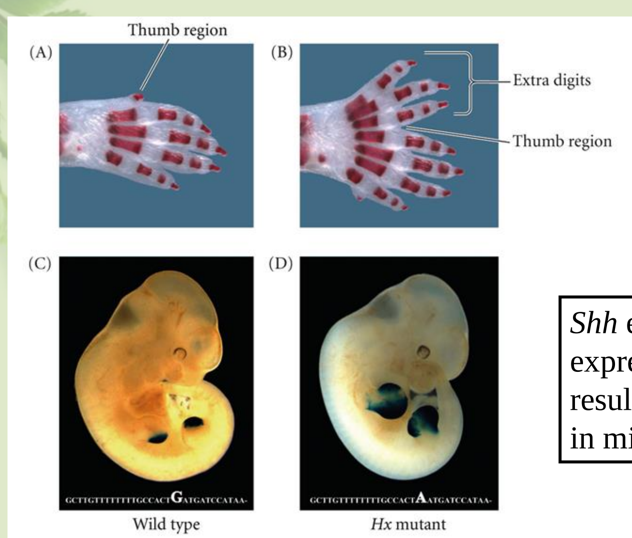


Zeller et al., Nature Rev. Gen, 2009

ZPA morphogen gradient



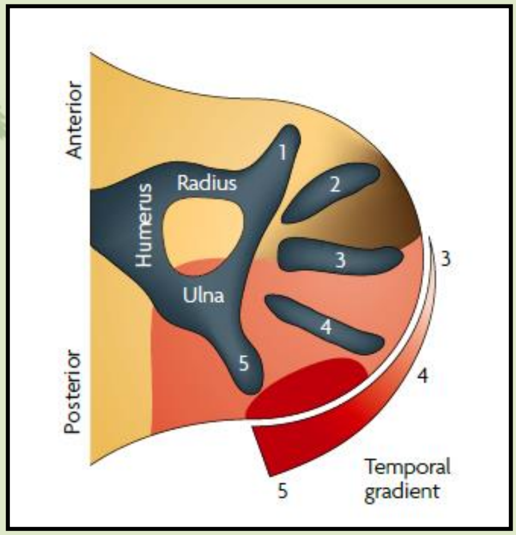
Ectopic expression of shh



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

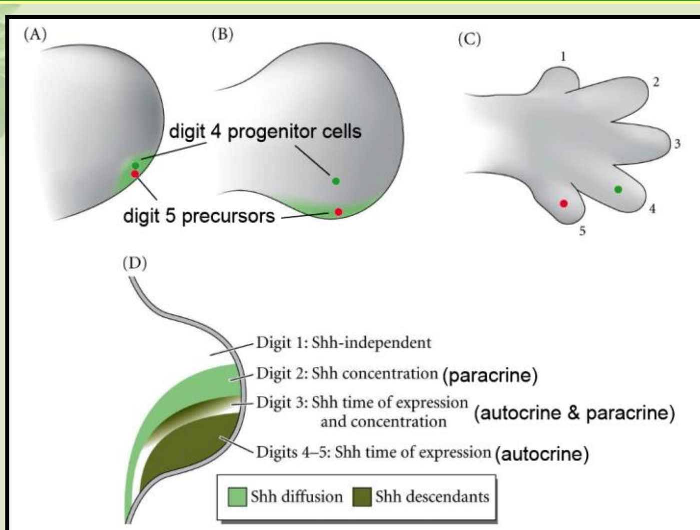
Shh ectopic expression can result in polydactyly in mice.

The temporal gradient model for A-P

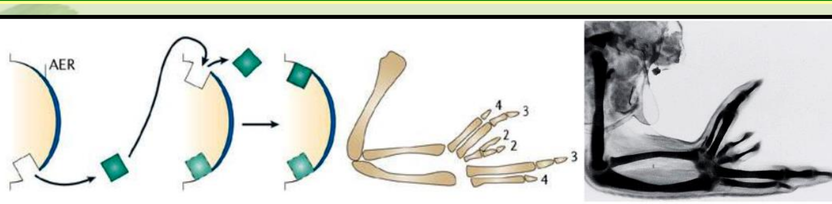


Zeller et al., Nature Rev. Gen, 2009

Shh specify digit identity



ZPA transplantation

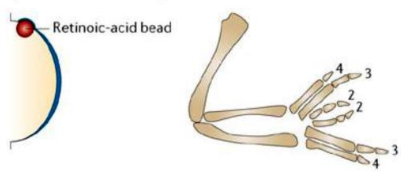


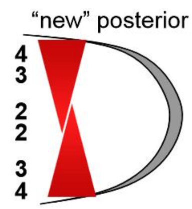
Posterior tissue transplant to anterior = duplicated autopod

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

Retinoic acid operates upstream of Shh

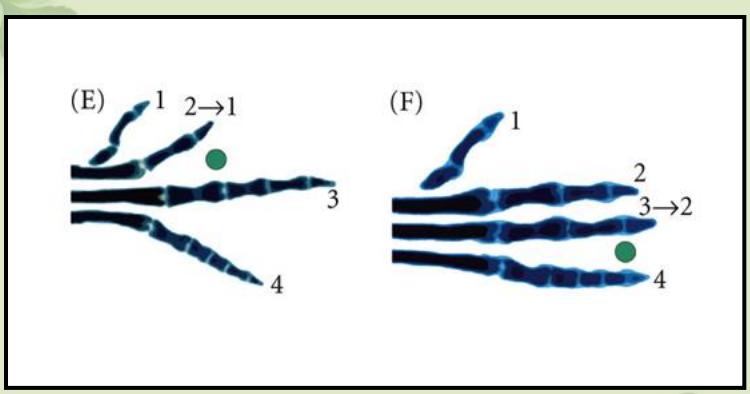
- implant RA-soaked bead =mirror-image duplication
- possible Hox gene involvement





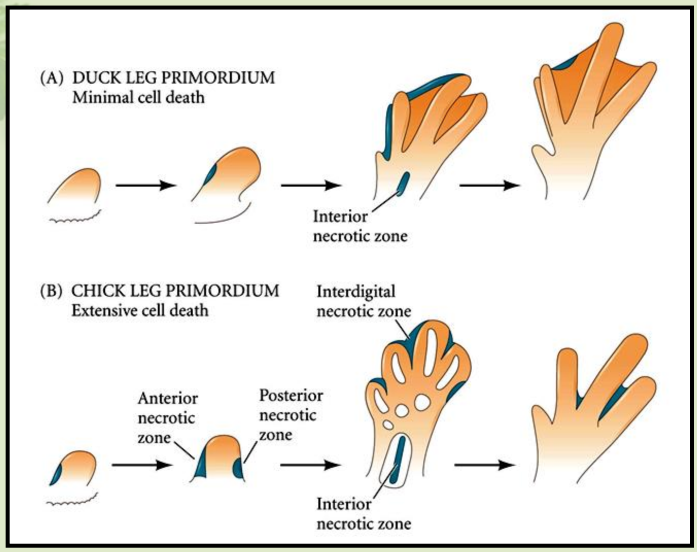
Tickle, Nature Mol. Biol., 2006

BMPs also regulate identity of digits



Noggin / BMP antagonist

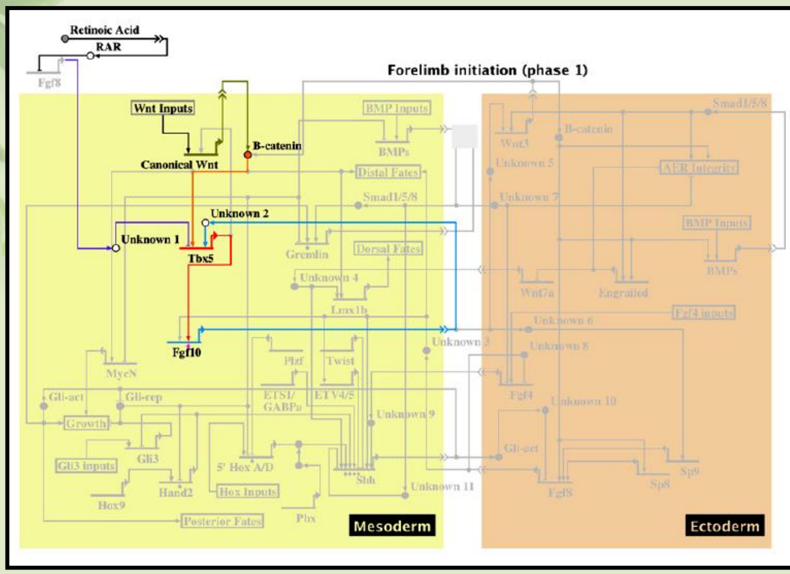
Patterns of cell death in leg primordia



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

Control of forelimb initiation phase



Rabinovitz et al., Dev. Biol., 2012

The End