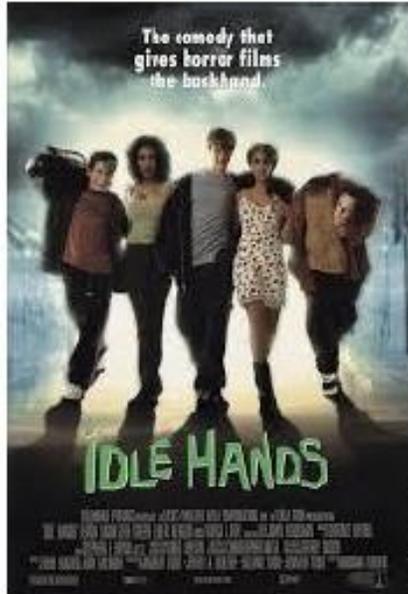


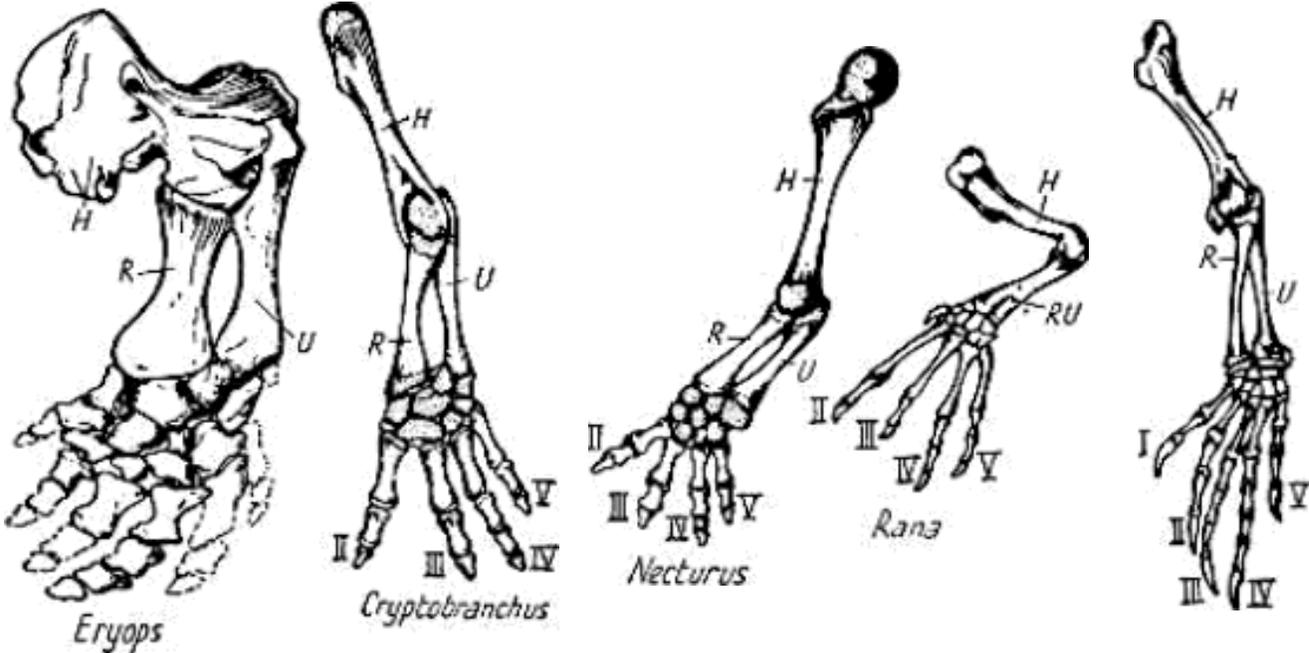
Axis determination in limb
patterning

Ramray Bhat

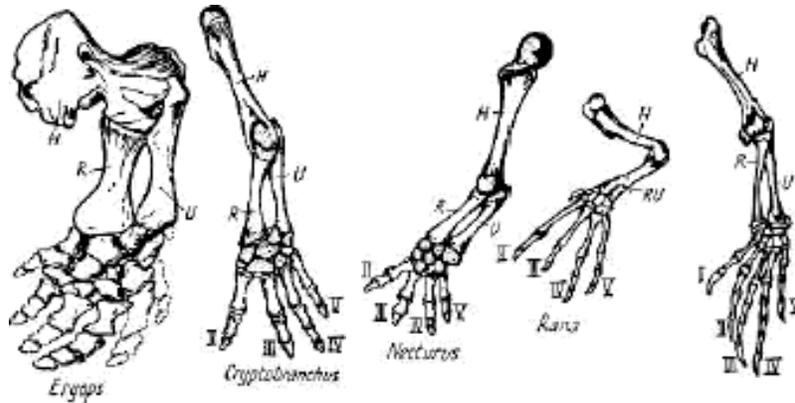
Ramray@iisc.ac.in



Homology in limb skeletal pattern

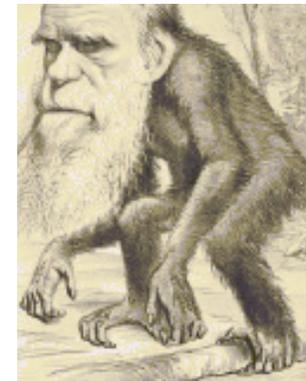


Young, *The life of vertebrates*, 1981



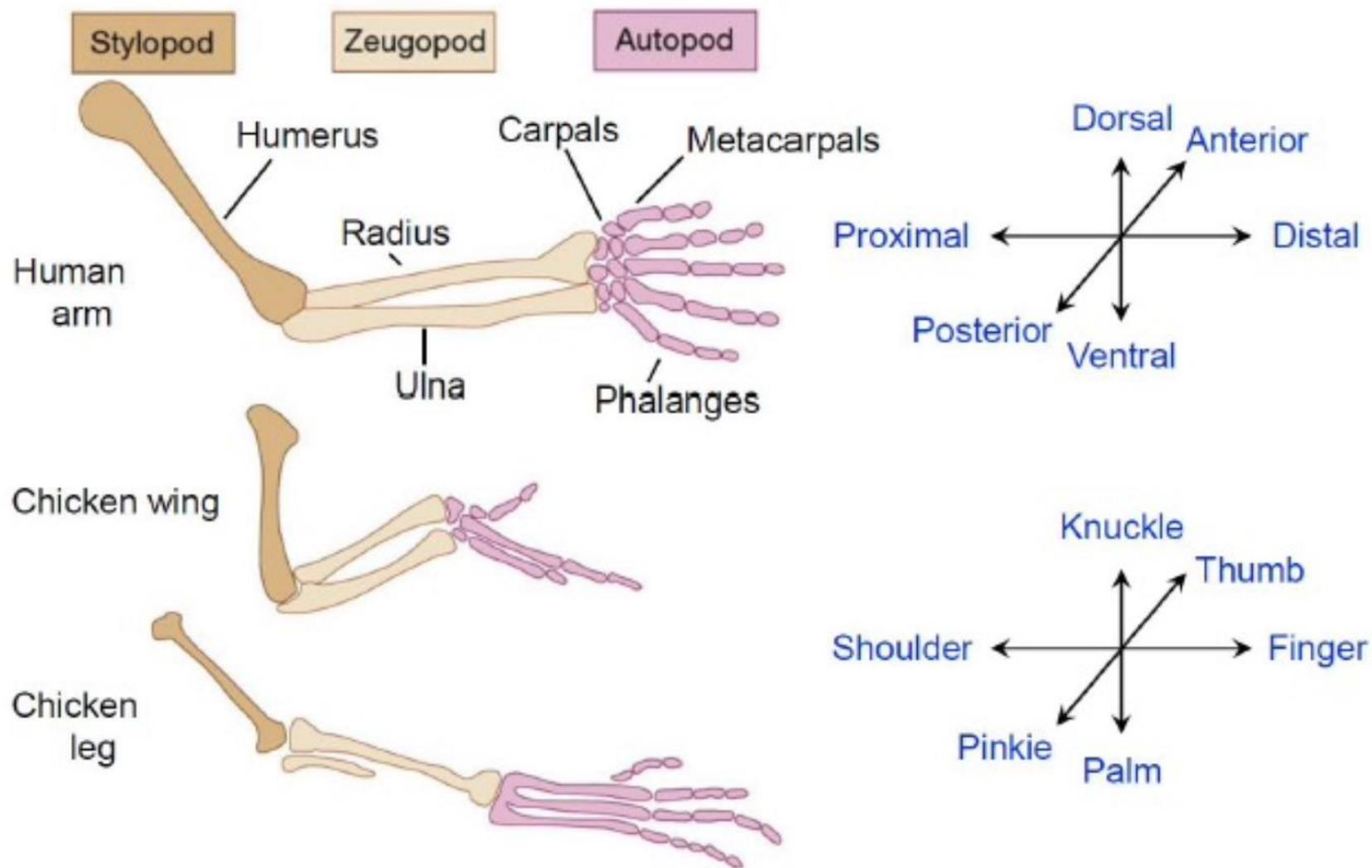
What can be more curious than that the hand of a man, formed for grasping that of a mole for digging, the leg of the horse, the paddle of the porpoise and the wing of the bat should all be constructed on the same pattern and should include the same bones, in the same relative positions? Geoffroy St. Hilaire has insisted strongly on the high importance of relative connexion in homologous organs: the parts may change to almost any extent in form and size, and yet they always remain connected together in the same order.

Darwin. On The The Origin of Species. 1859

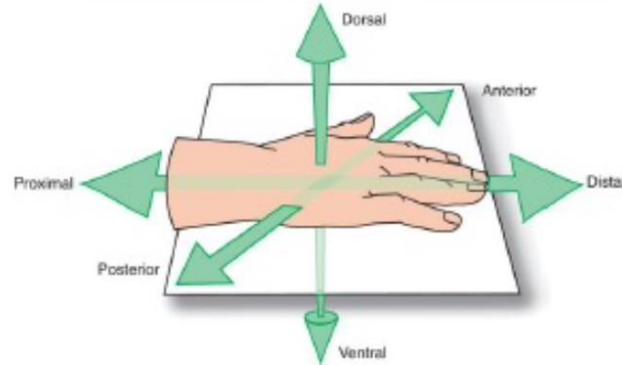
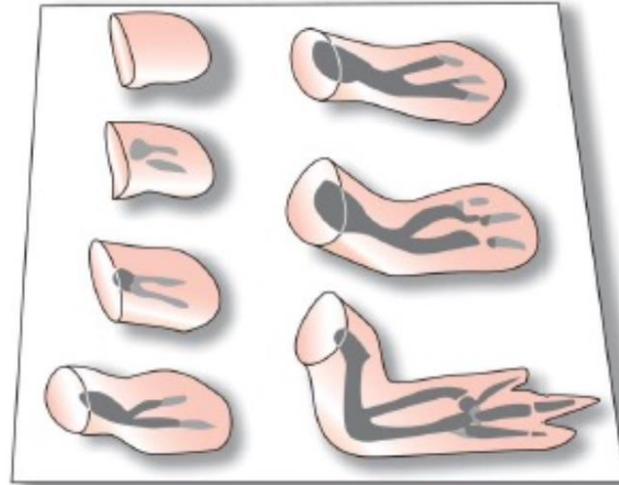


Source. wikipedia

Limb Patterning

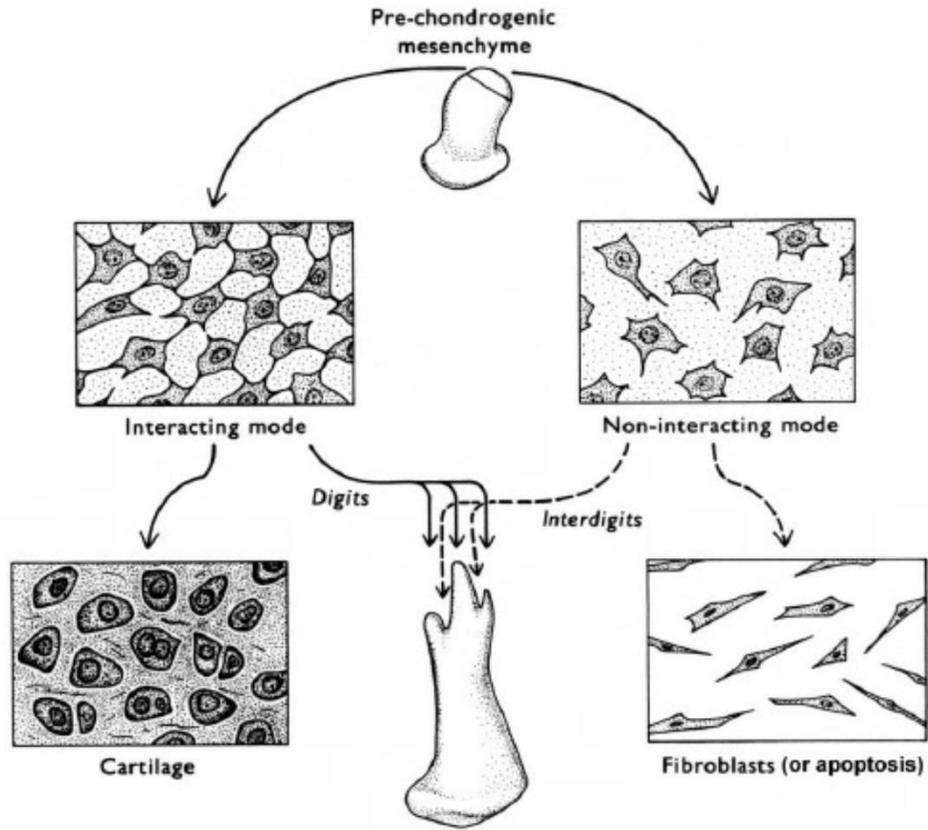


Skeletal pattern formation in the avian limb

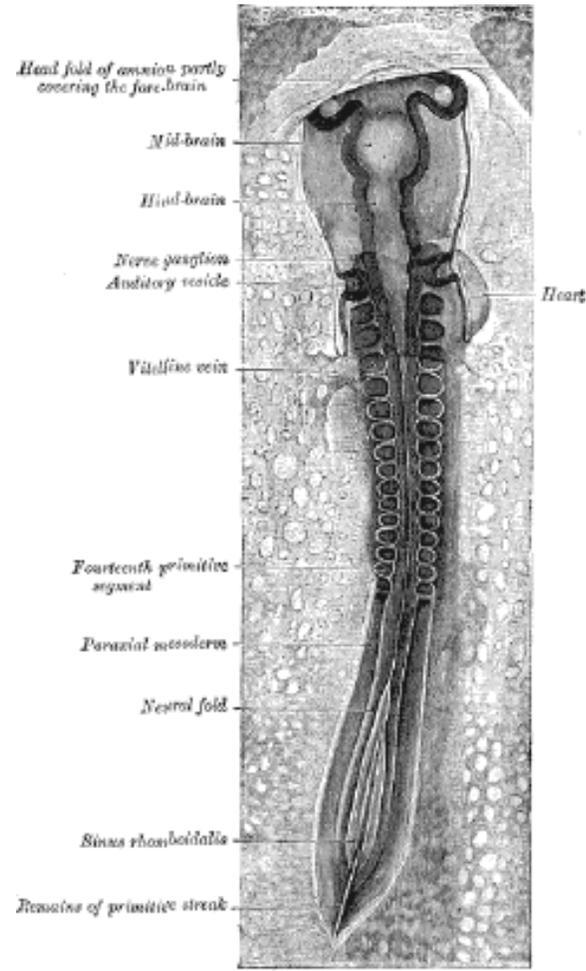


Forgacs & Newman, *Biological Physics of the Developing Embryo*; 2005

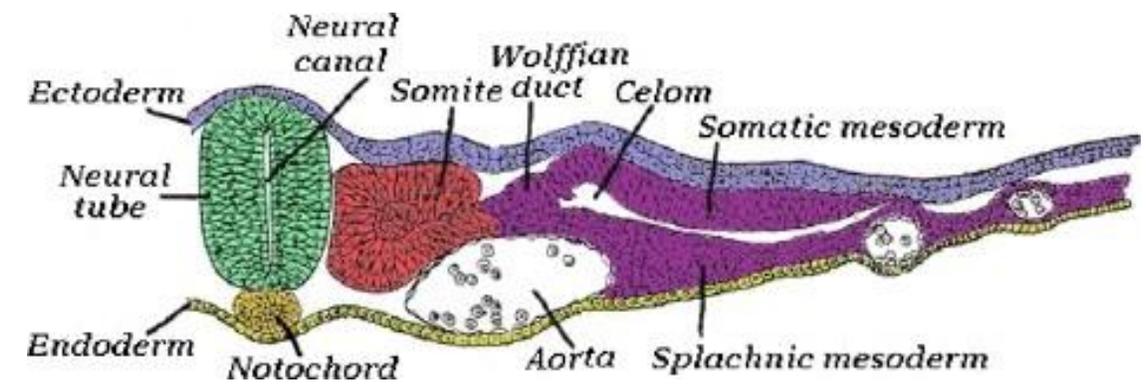
Limb chondrogenesis *in vitro* and *in vivo*



The source of limb mesenchyme

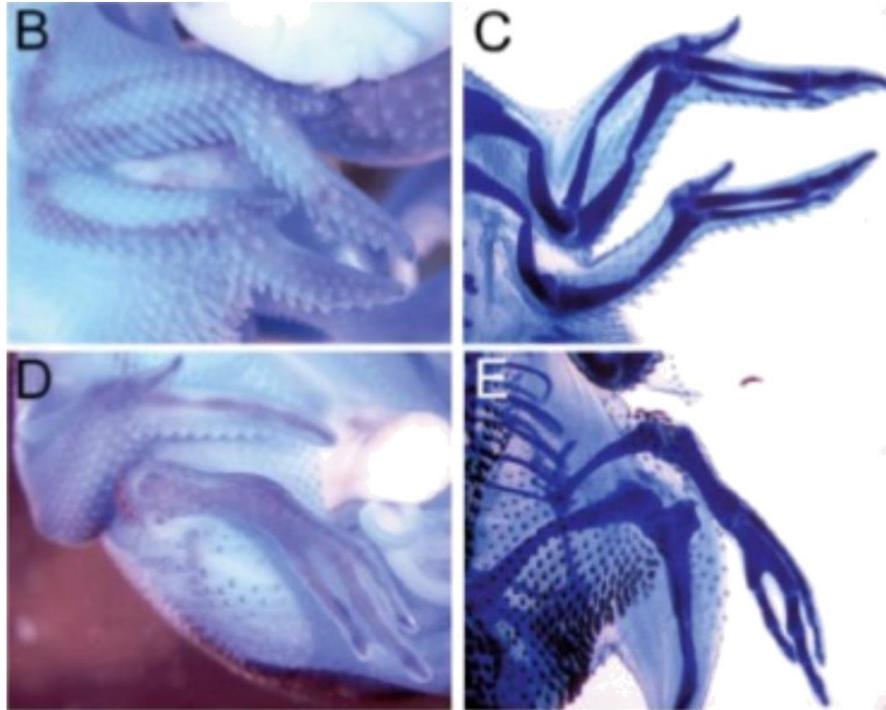


Duvall's embryological atlas



Gray's anatomy, 1918

Splanchnic mesoderm can form limbs



B: Wing level of the splanchnic mesoderm formed a wing-type limb.

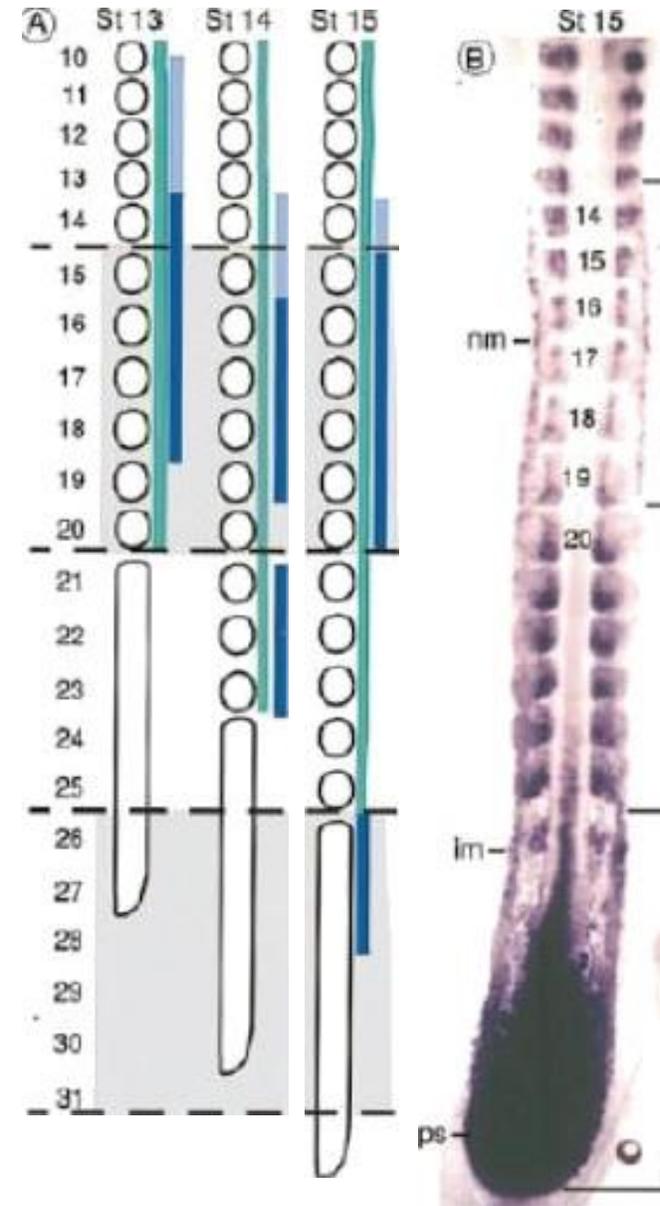
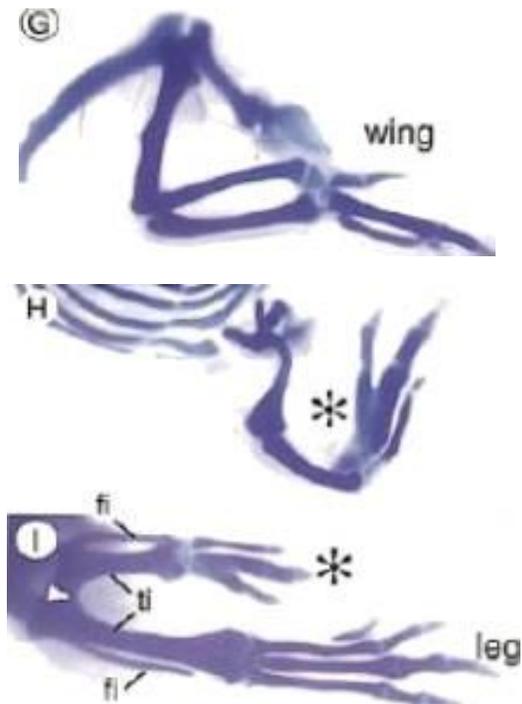
C: Skeletal pattern of B.

D: Leg level of the splanchnic mesoderm formed a leg-type limb.

E: Skeletal pattern of D.

Endogenous signals for limb specification

1. Nephrogenic mesoderm is associated with FGF8 expression
2. FGF8 expression is then associated with limb mesenchyme

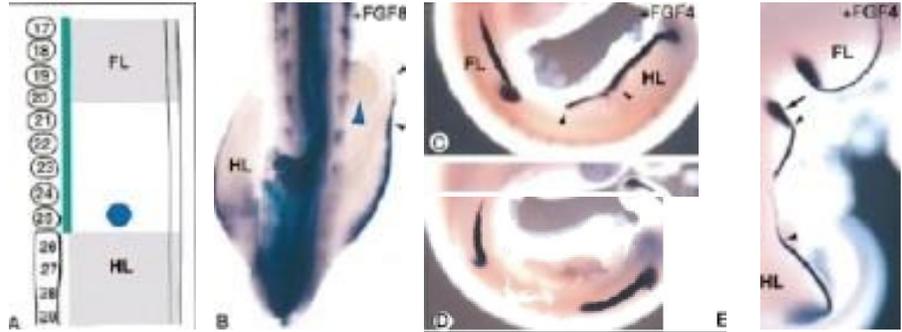


Role of FGF8

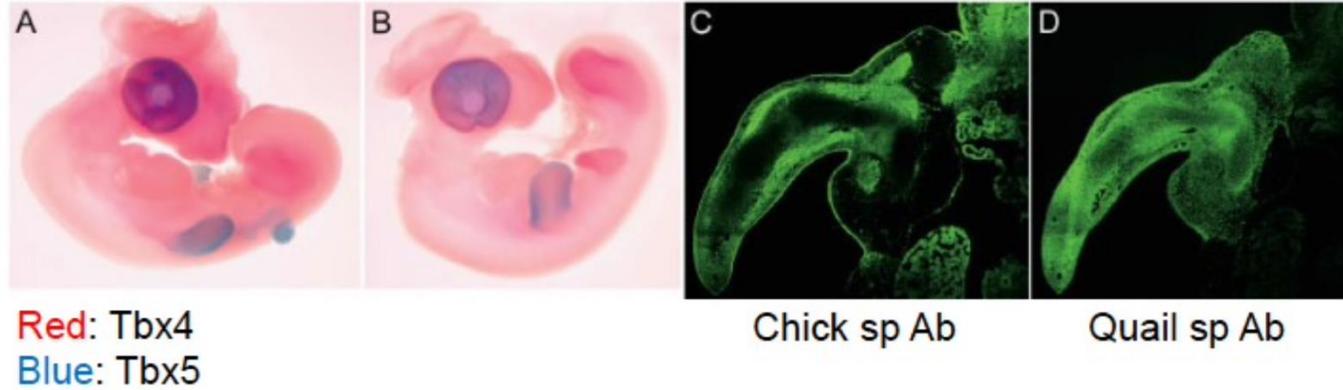


FGF expression in the ectoderm
Shh in the posterior mesoderm

Implantation of FGF4/8 bead in interlimb region leads to expression of ectopic ectodermal FGF and mesodermal Shh



Graft position specificity
splanchnic mesoderm forms limb mesenchyme

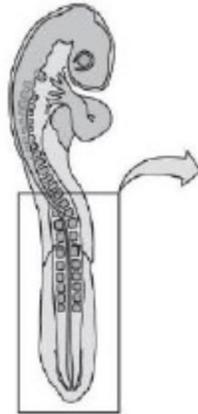


Limb from splanchnic mesoderm of
wing level: Tbx5
Limb from splanchnic mesoderm of
leg level: Tbx4

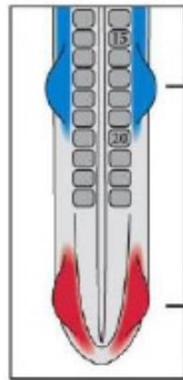
Splanchnic mesoderm from quail put
into chick forms limb mesenchyme

Tbx Genes Specify Limb Type

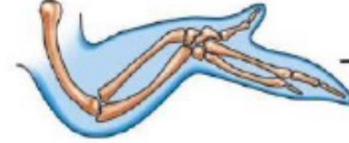
Stage 14/15
(early day 3)



(A) Normal



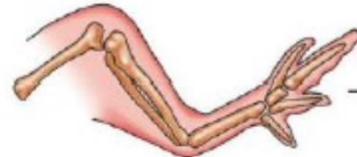
Forelimb
bud



Wing



Hindlimb
bud



Leg

■ Tbx5

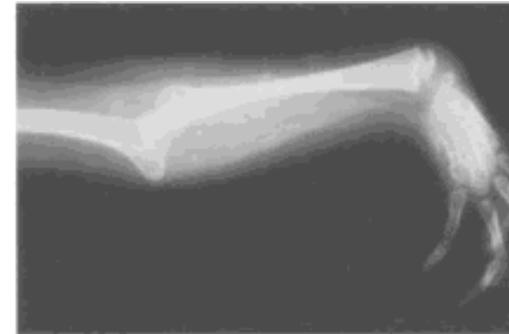
■ Tbx4

Tbx5 mutation: Holt-Oram syndrome

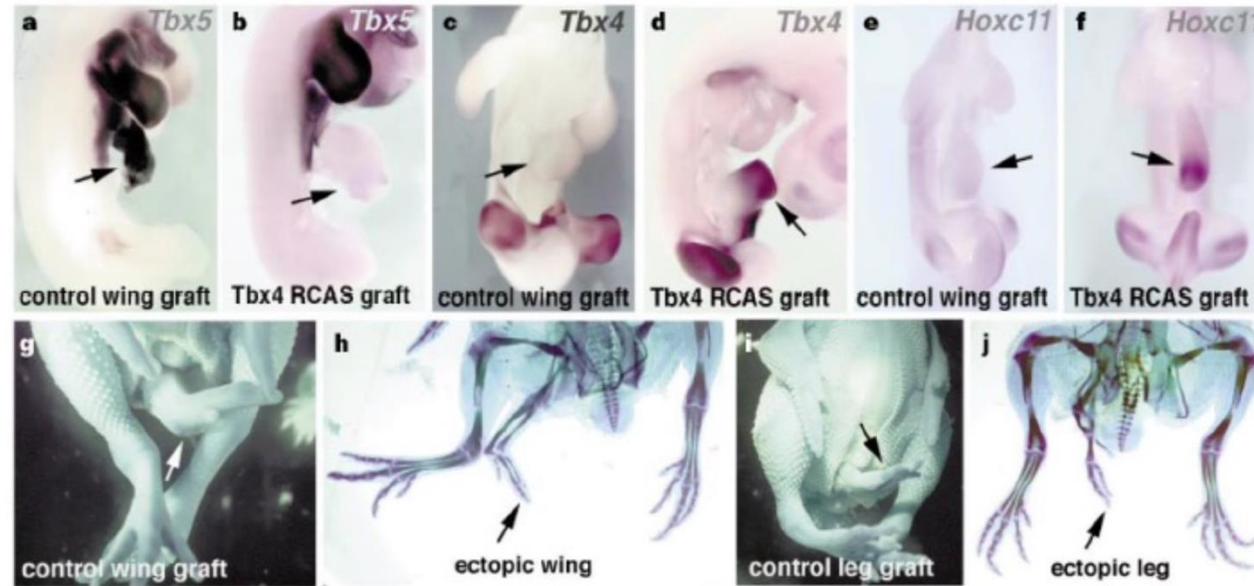
T-box domain containing protein

Domain binds to DNA

Tbx4 mutation leads to clubfoot



Tbx expression associates with limb identity



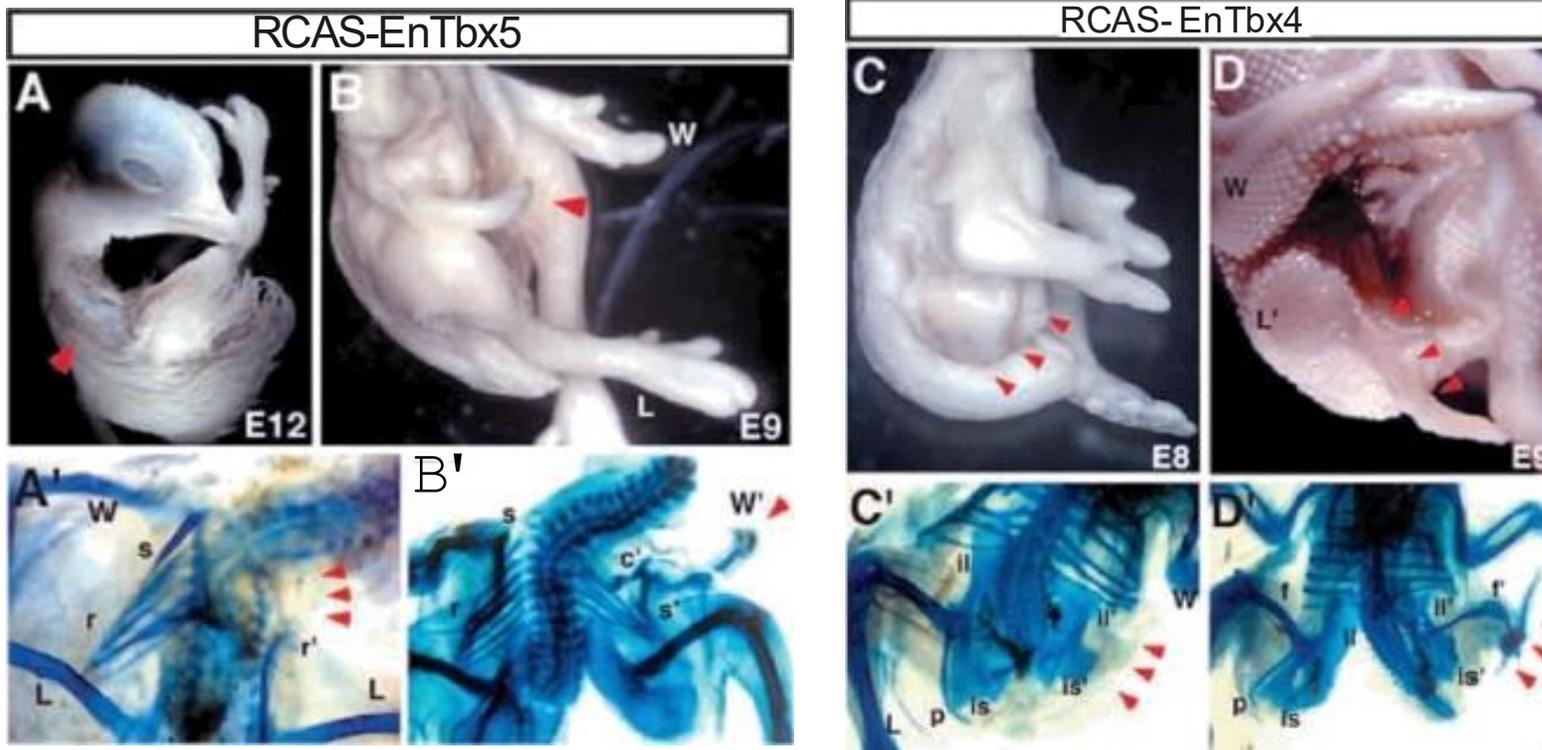
a,c,e: grafting control wing cells: assumes wing identity (Tbx5, not Tbx4)

g,h: becomes wing

b,d,f: grafting Tbx4 expressing wing cells: assumes leg identity (Tbx4, not Tbx5)

i,j: becomes leg

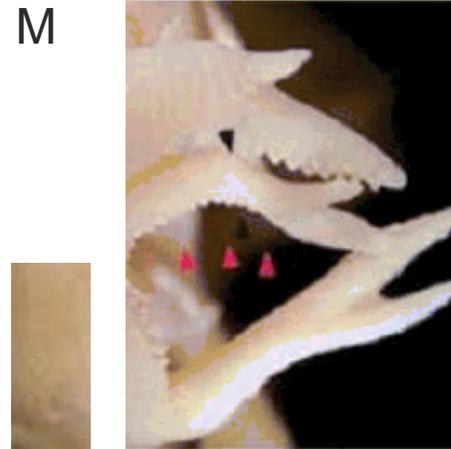
Tbx dn misexpression: limbless phenotype



dnTbx5: No wing

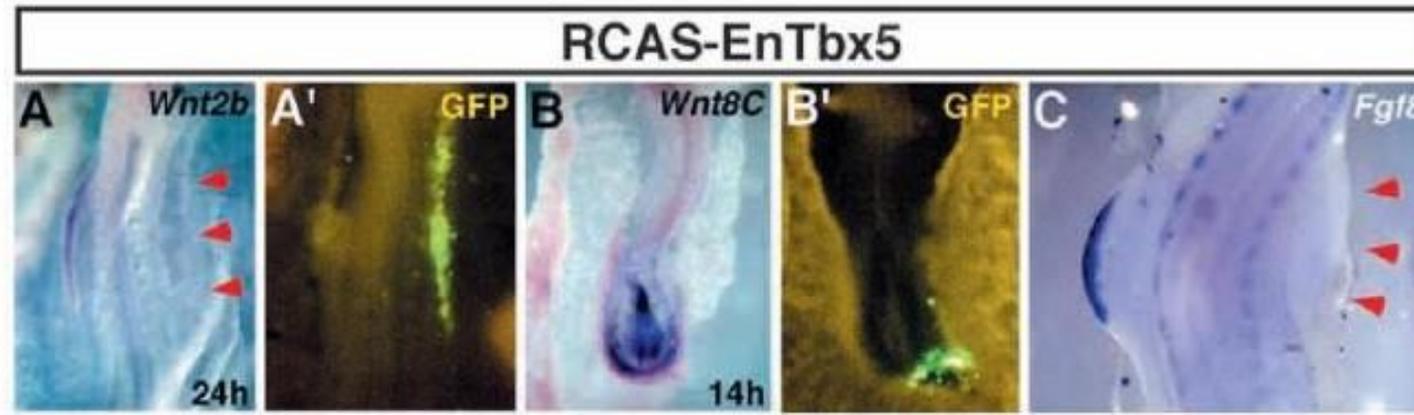
dnTbx4: No leg

Tbx misexpression in flank: ectopic limbs



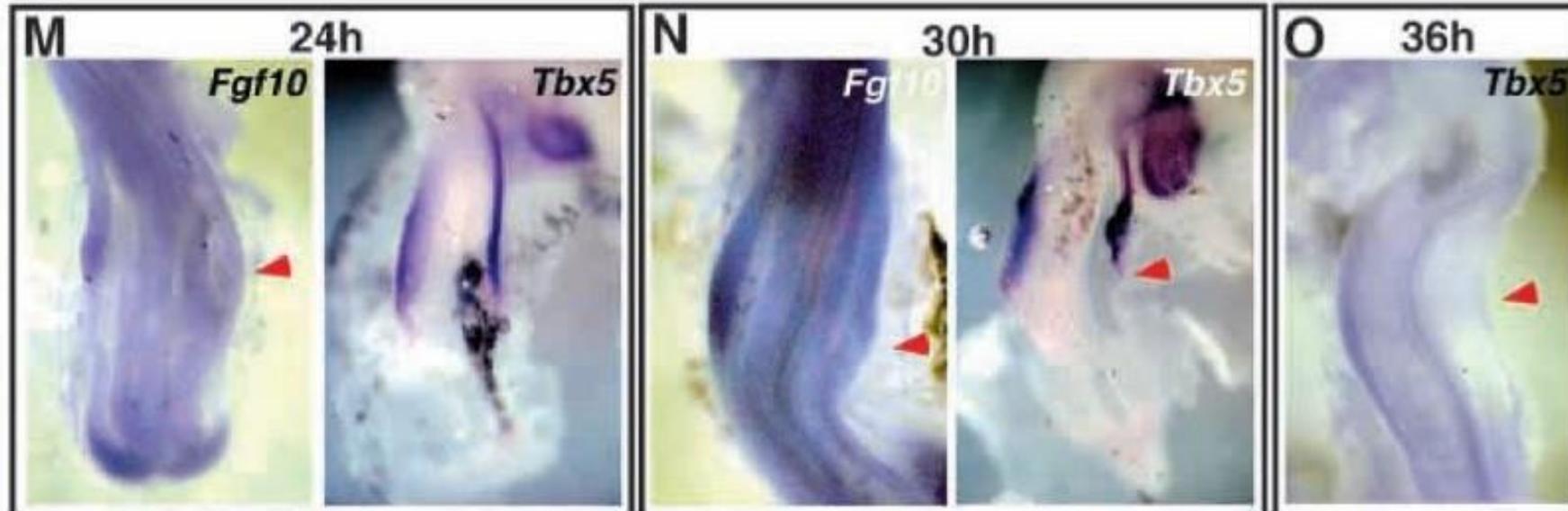
Takeuchi et al, *Development*, 2003

Tbx dn misexpression: repression of Wnt2b and Fgf8 expression



Inhibition of Tbx5 expression: inhibition of Wnt2b and Fgf8 expression

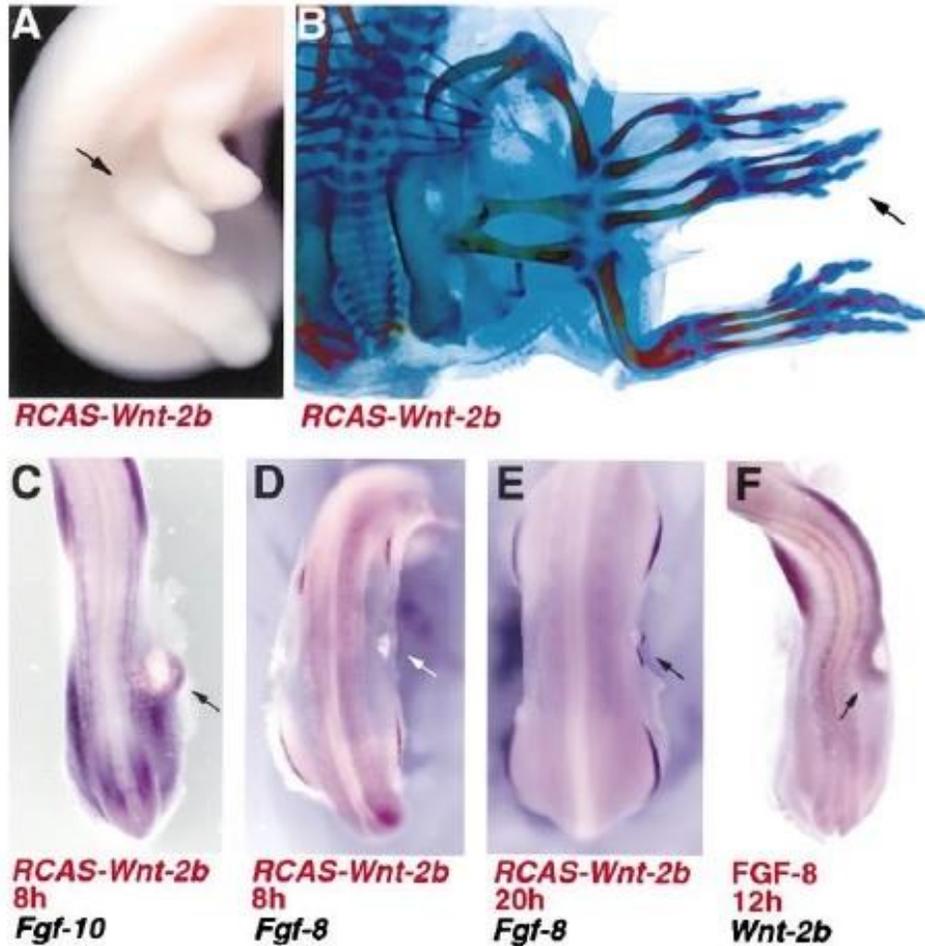
Hierarchical or reciprocal? dnLef1 expression



inhibition of Wnt signaling: inhibition of mesodermal Fgf10 and Tbx5 expression

Cross talk between fgf, wnt and tbx

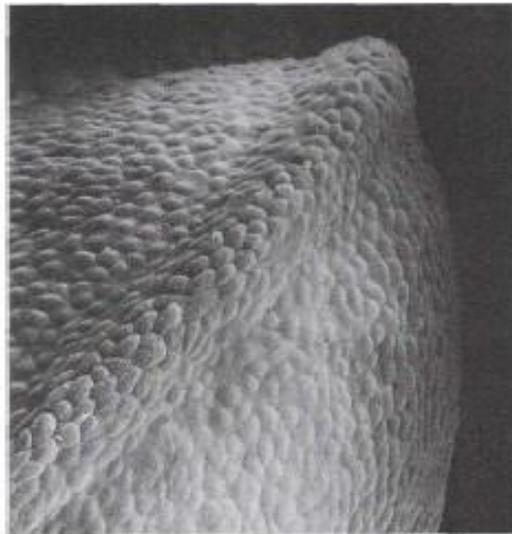
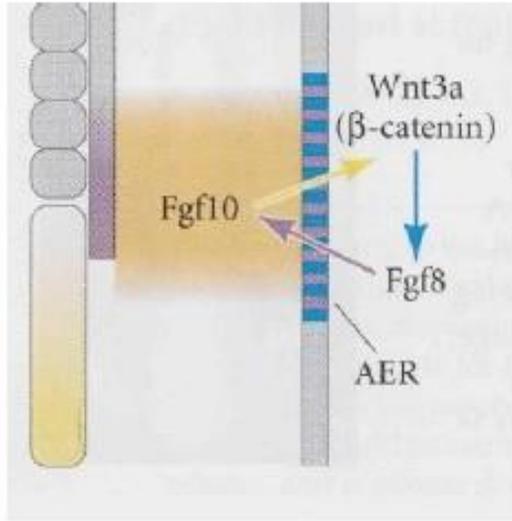
Wnt2b effect on FGF10: induction and limb initiation



crosstalk between fgf and wnt signaling. Wnt can initiate limb bud formation

FGF8: ectodermal FGF
FGF10: mesodermal FGF

Development of the Apical ectodermal ridge



Induced by the flank FGF, Wnt, Tbx axis

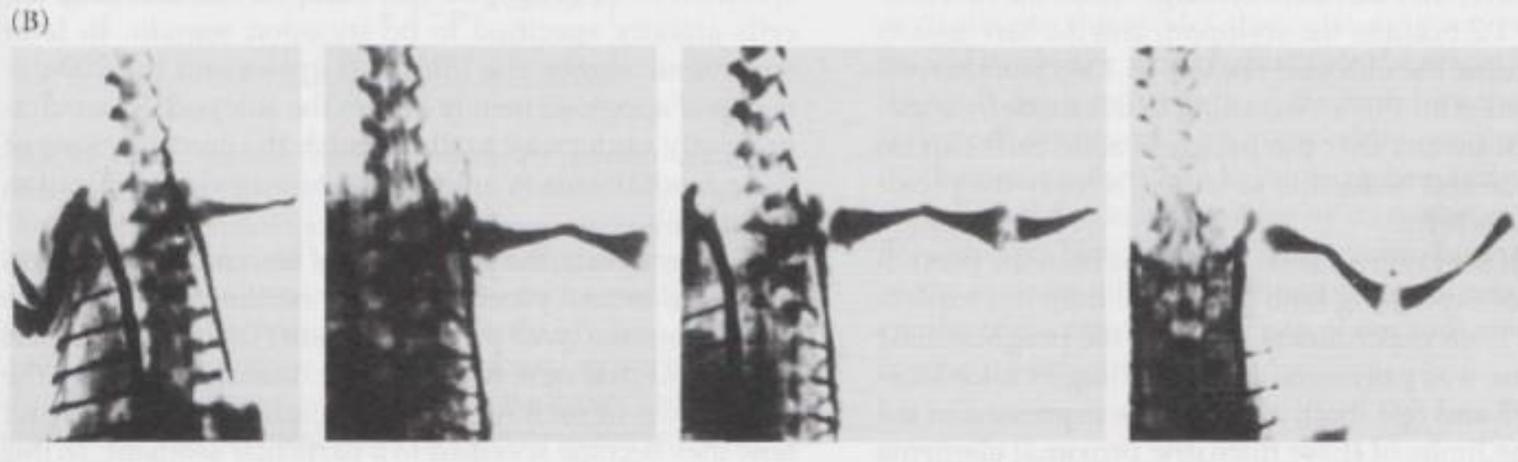
Functions of the AER

1. Secretion of the FGF cocktail consisting primarily of FGF8, which keeps the underlying mesoderm in an undifferentiated state.
2. FGFs also induce the underlying mesoderm to proliferate.
3. Interact with other signals that specify the AP/DV axis

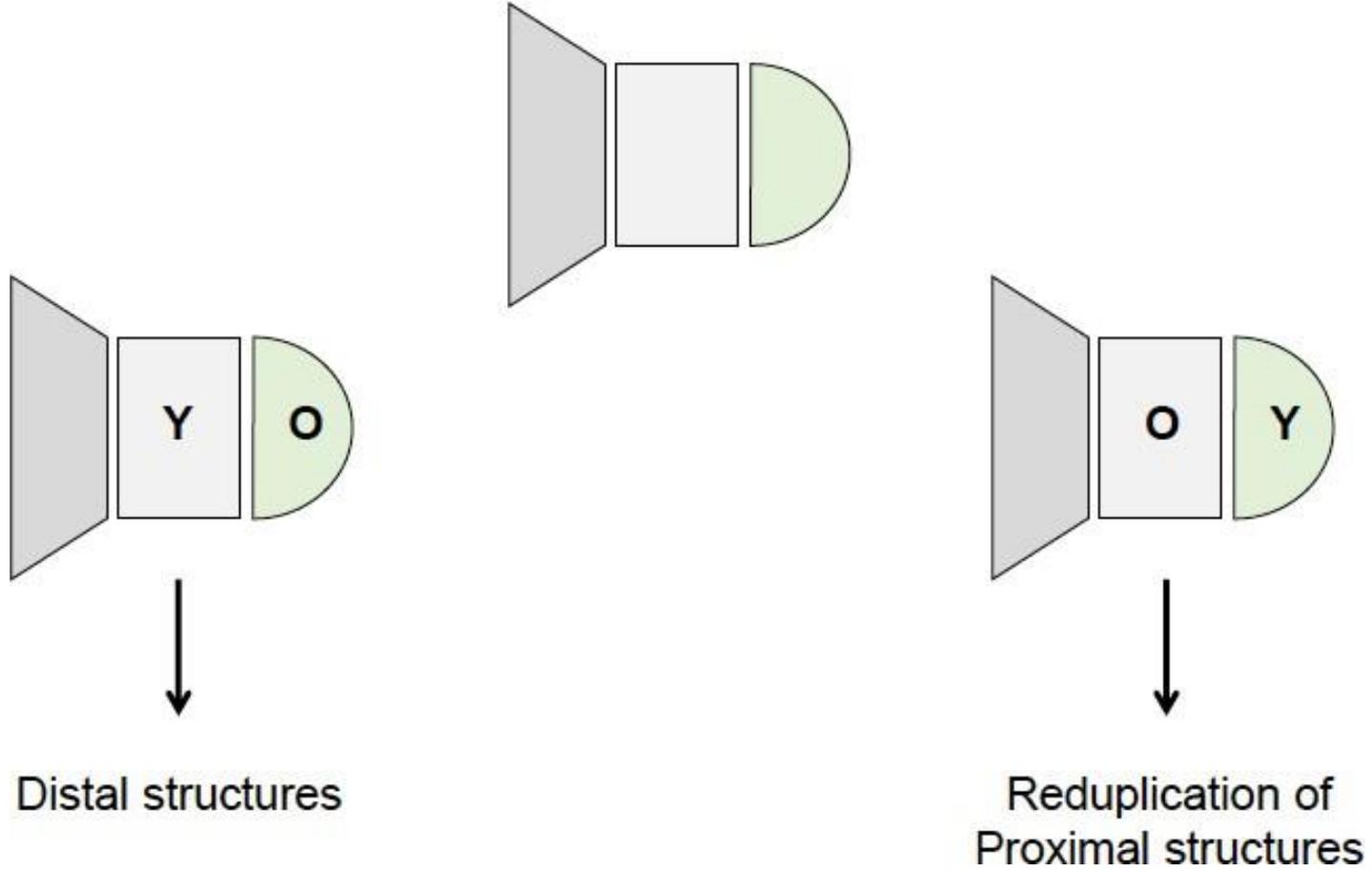
DEVELOPMENT OF THE TETRAPOD LIMB



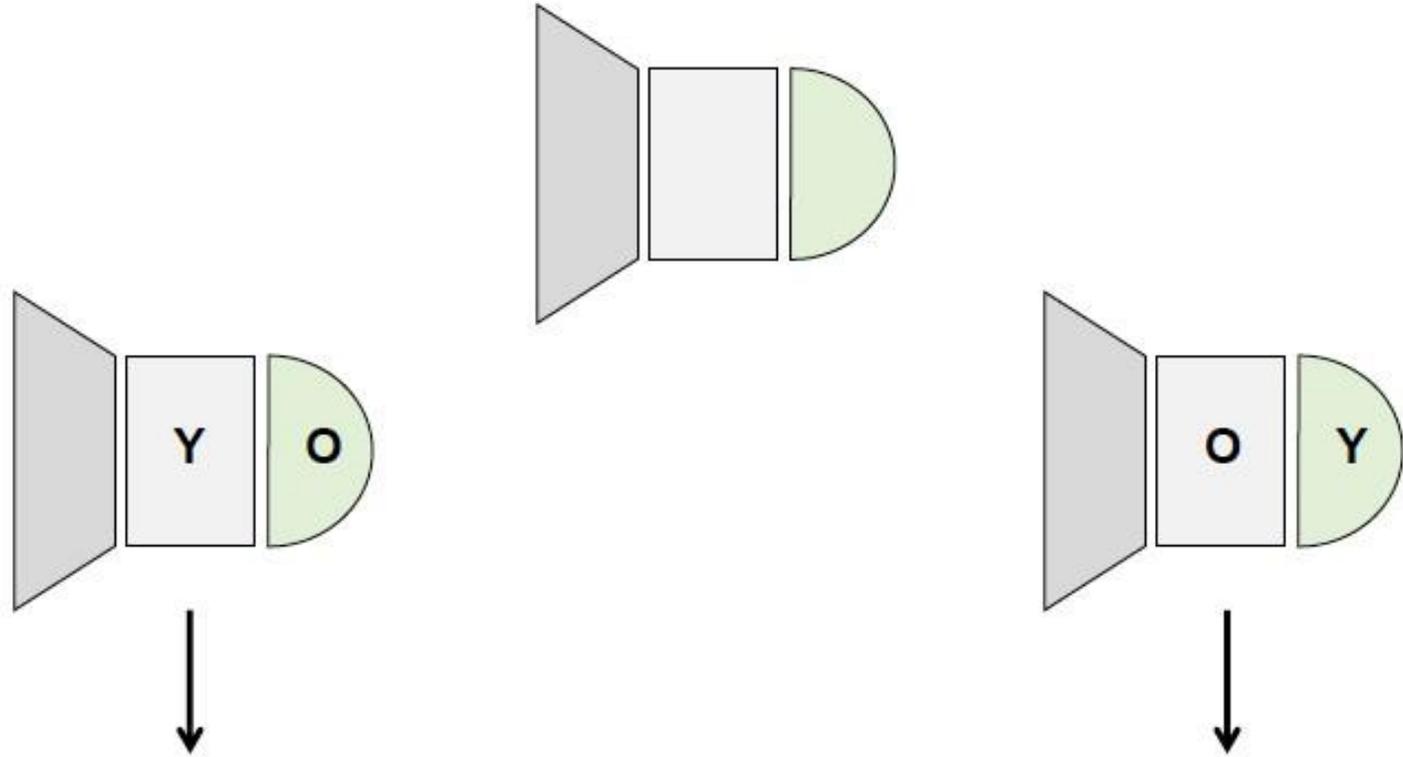
FIGURE 13.11 The AER is necessary for wing development. (A) Dorsal view of a normal chick wing (dorsal view). (B) Dorsal views of skeletal patterns after removal of the entire AER from the right wing bud embryos at various stages. (From Iten 1982, courtesy of L. Iten.)



Where does the instructional signal for P-D signals lie? **AER?**



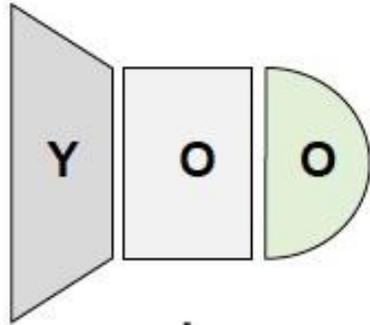
Where does the instructional signal for P-D signals lie? **AER? NO**



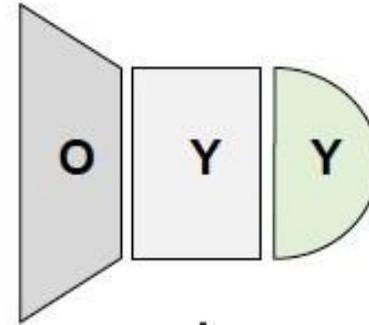
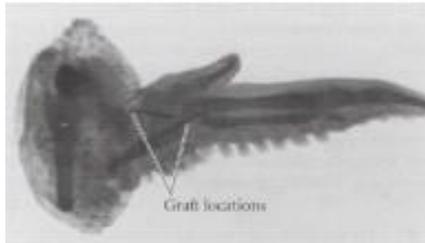
Normal structures!

Normal structures!

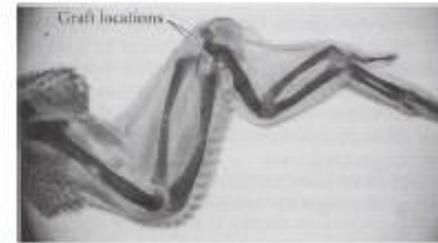
Where does the instructional signal for P-D signals lie? **PROGRESS ZONE?**



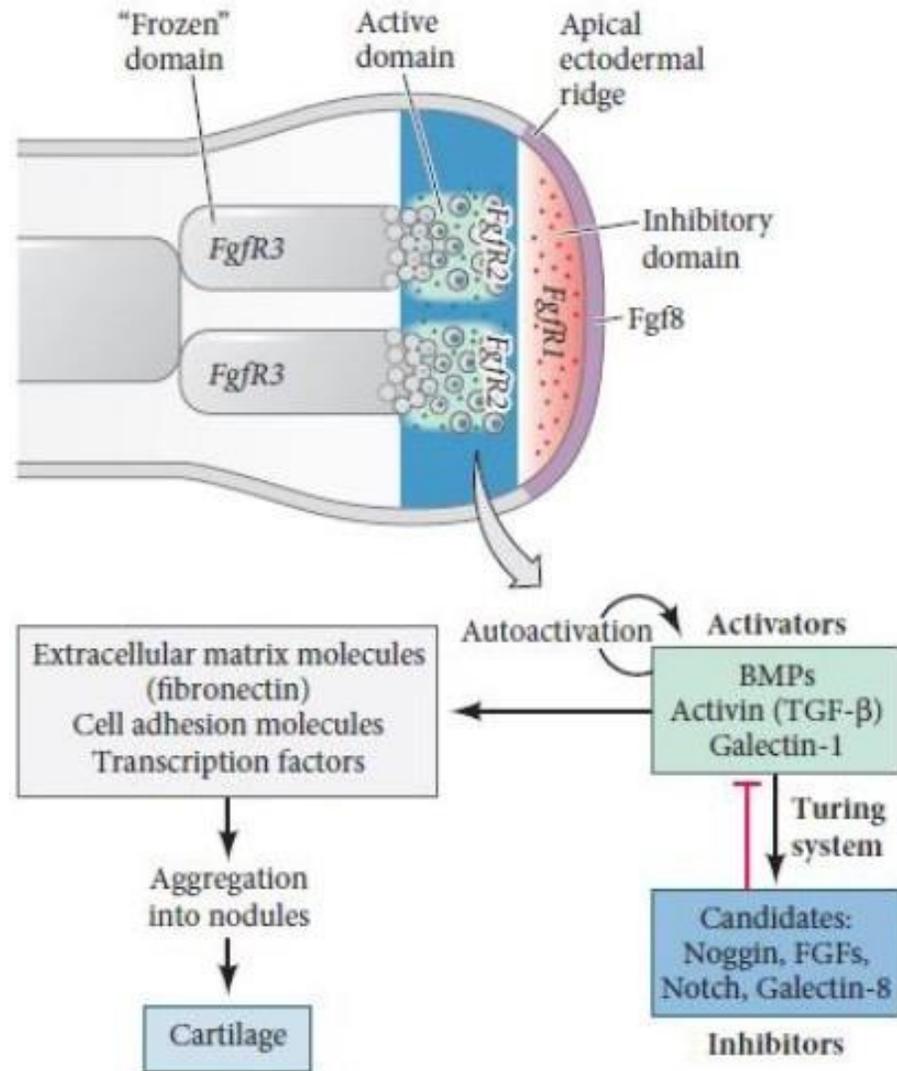
Digits next to humerus



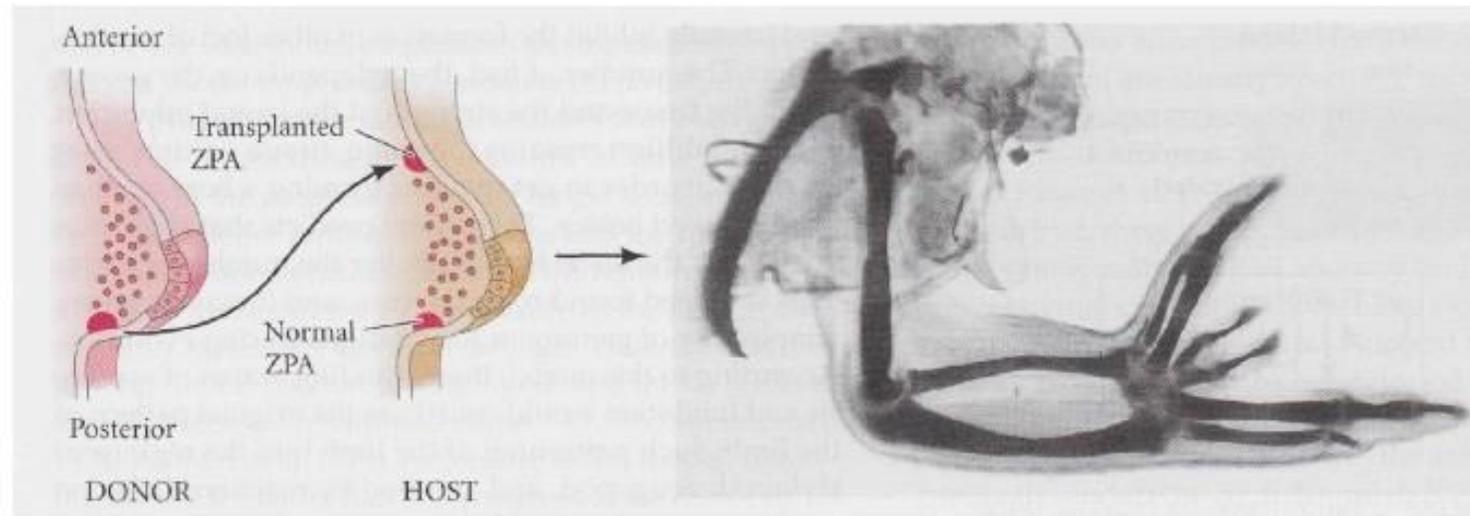
Duplication of proximal structures



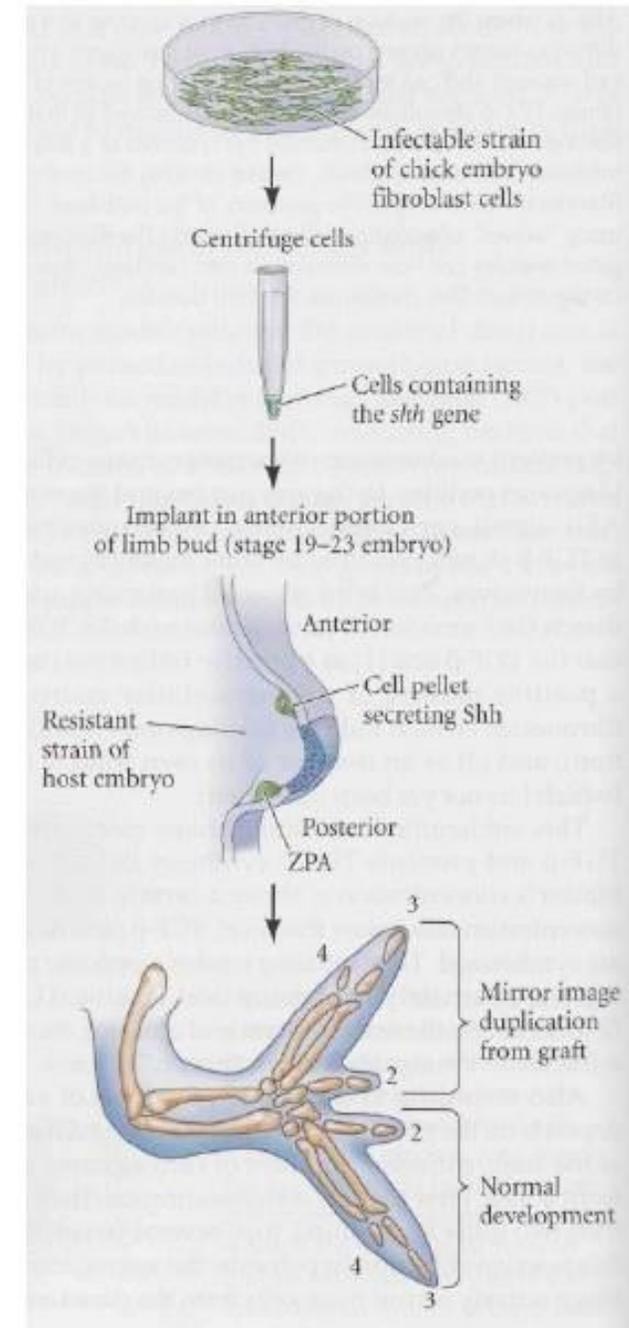
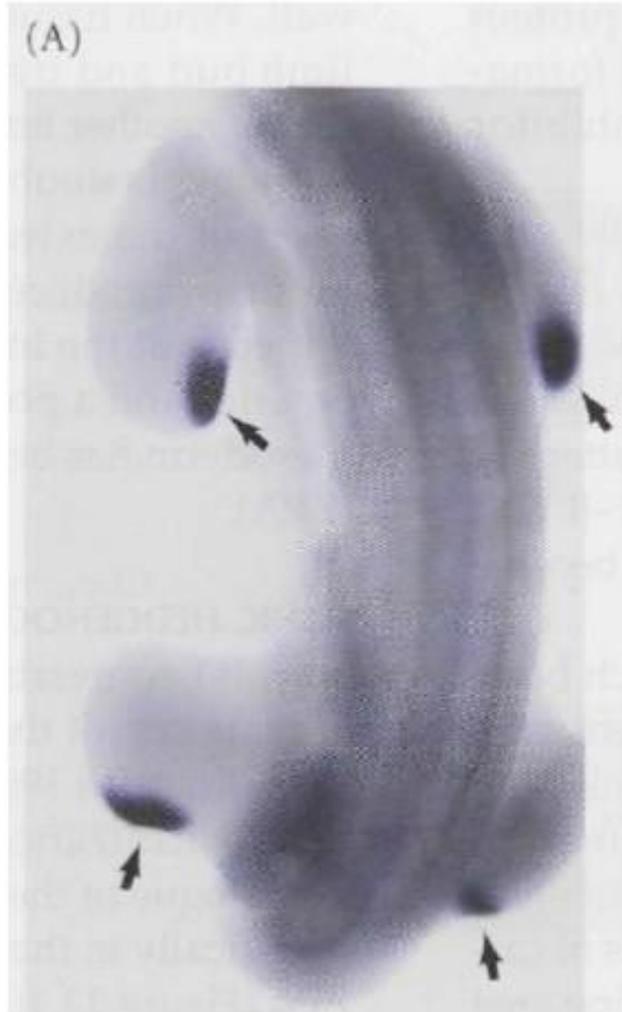
How do condensations form?



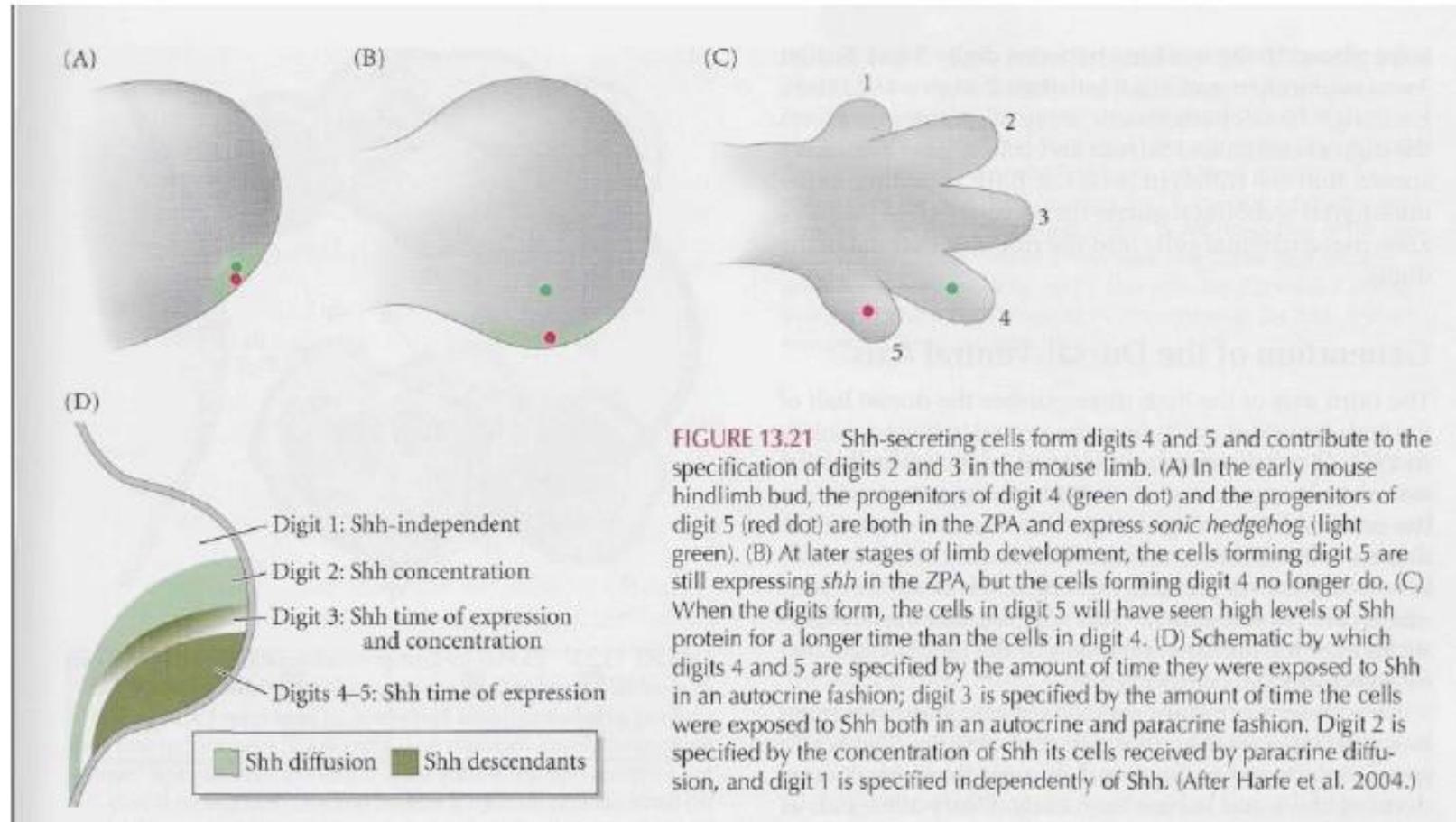
Antero-posterior patterning: discovery of ZPA

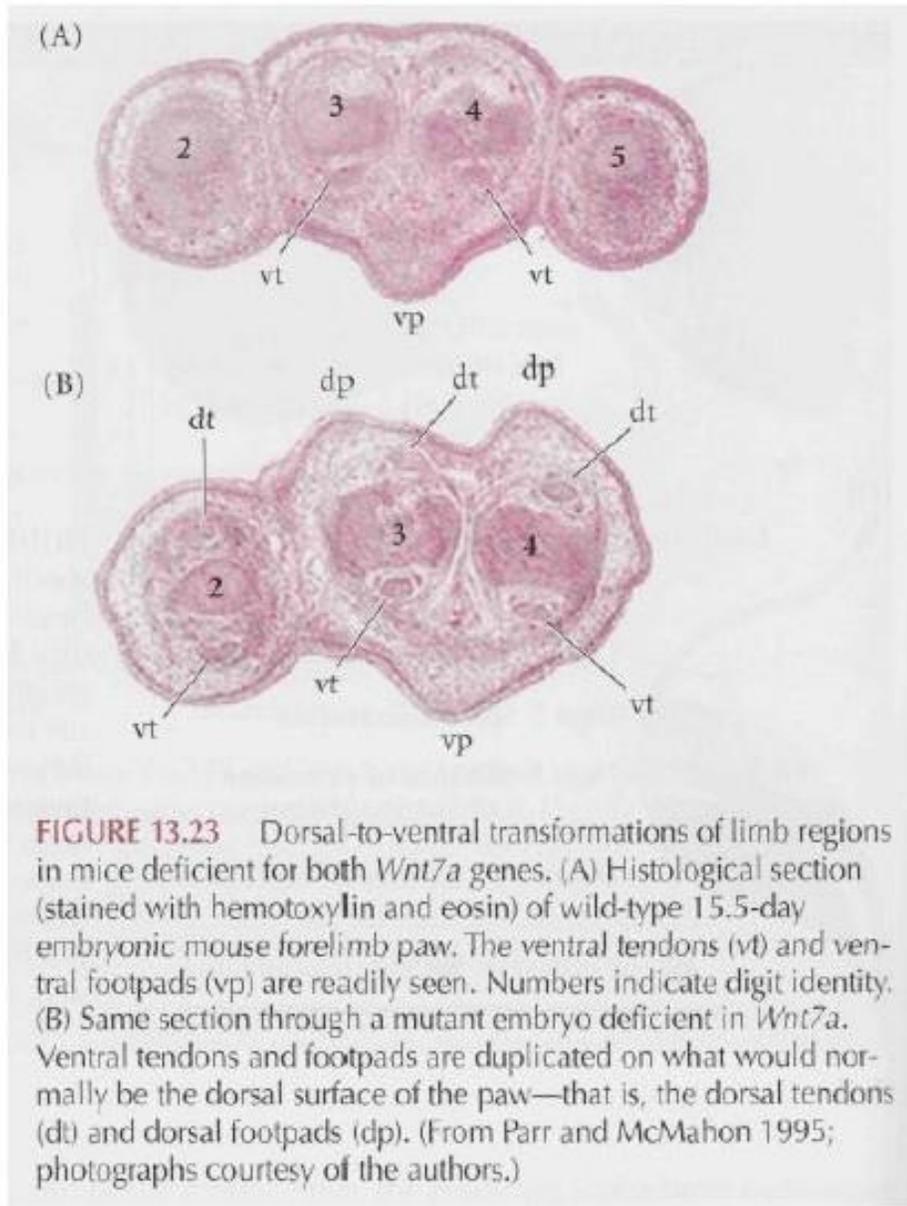


ZPA signal is Sonic Hedgehog



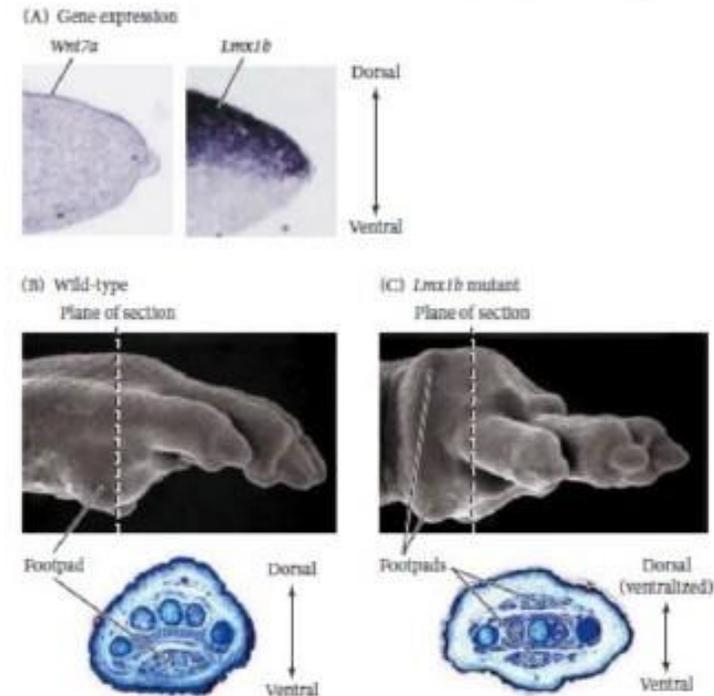
Sonic hedgehog patterns digits differently





The dorsalizing center

Wnt7a



Integrating the axes

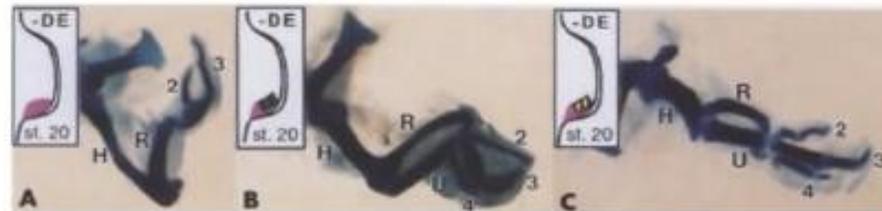
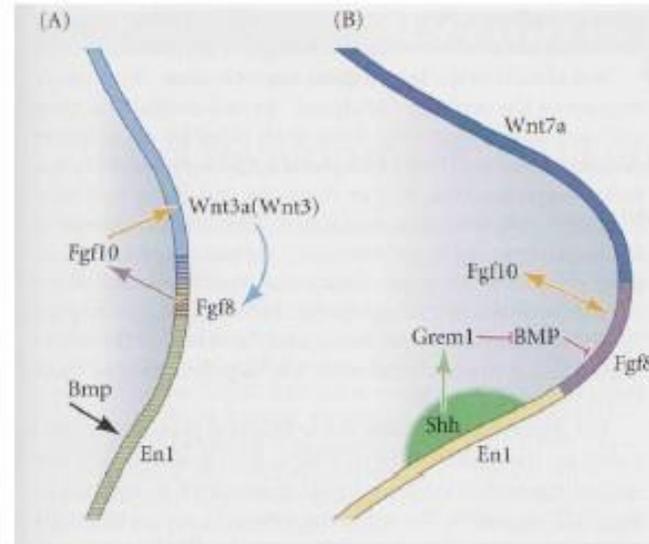


Figure 4. Dorsal Ectoderm Removal Results in a Mutant Limb That Lacks Posterior Structures

Shh- or *Wnt7a*-expressing cells can rescue posterior skeletal formation. Skeletal pattern 7 days after removal of the dorsal ectoderm and grafting of a pellet of *Shh*- or *Wnt7a*-expressing cells to the distal tip of a stage 20 wing bud:

no graft (A); *Shh* cells (B); *Wnt7a* cells (C). In the schematic drawings, the green and yellow hatched boxes represent a graft of *Shh*- or *Wnt7a*-expressing cells, respectively. Abbreviations are as listed in the legends to Figures 2 and 3.

