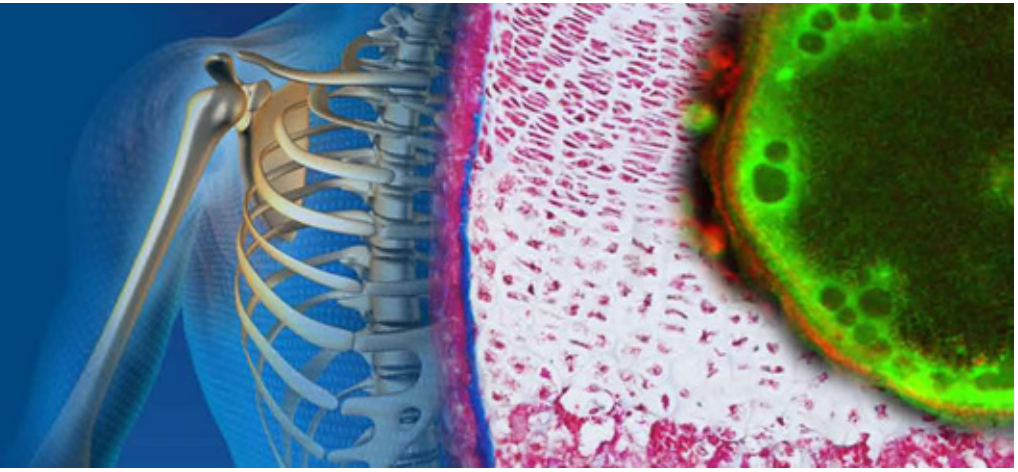
CENTER *for* MUSCULOSKELETAL RESEARCH



Skeletal Development

Jennifer H. Jonason, Ph.D.

Assistant Professor of Orthopaedics and Rehabilitation

Center for Musculoskeletal Research

Orthopaedic Basic Science Course

Spring 2017

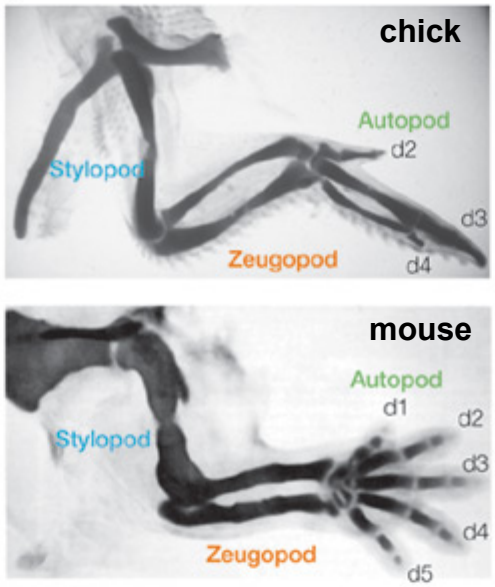
Lecture Objectives

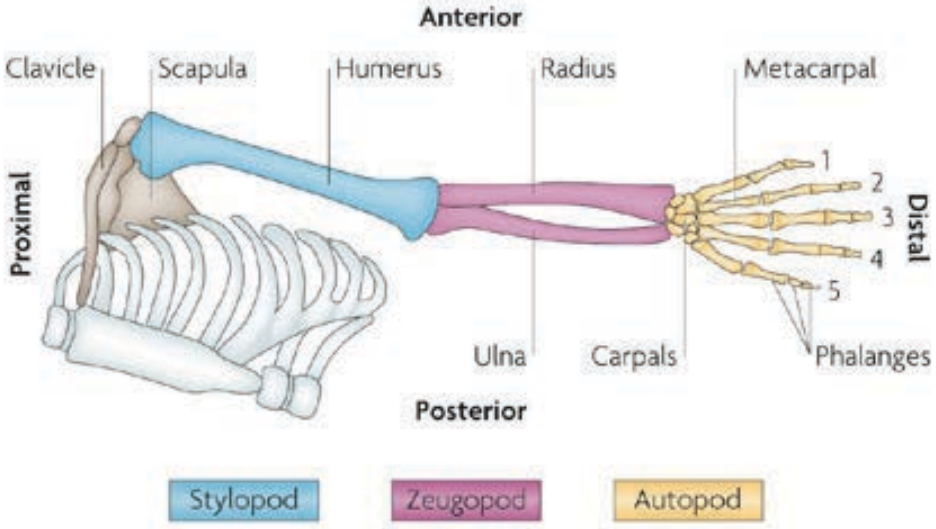
- Introduction to limb patterning and the signaling centers/pathways responsible for proximal-to-distal and anterior-to-posterior patterning

- Introduction/review of intramembranous and endochondral bone formation and mesenchymal progenitor cell differentiation

- Introduction to joint formation and gene expression patterns characteristic of a developing joint

Patterning of the Limb Skeleton





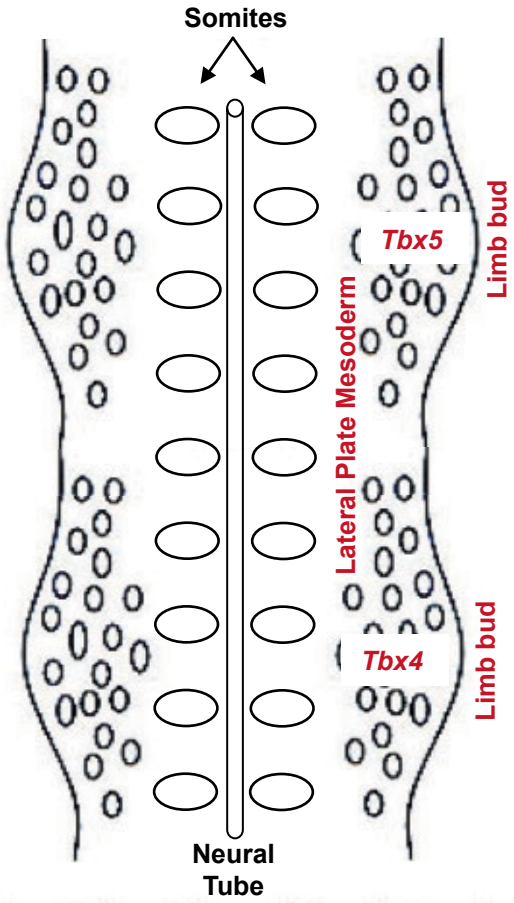
Patterning: the process in which the positions and identities of cells with different fates are laid down.

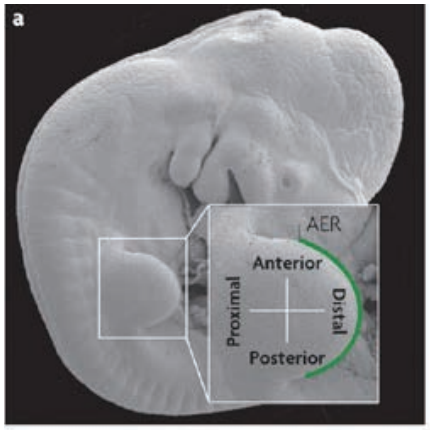
Specification: the process preceding determination during which a cell acquires its fate. The exposure of specified cells to different signals might alter their fates; the fate of specified cells is not fixed.

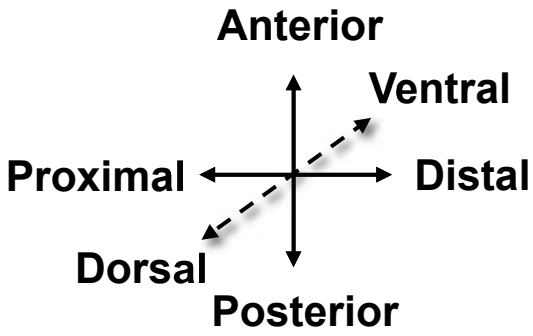
Determination: When cell fate is fixed so that the cell will initiate differentiation into the specified cell type even if the cell is isolated or transplanted into a different environment or tissue; occurs before the appearance of sell-type-specific morphological characteristics, but is often followed by differentiation.

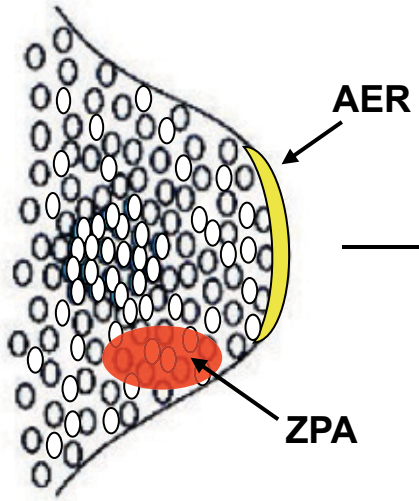
Zeller, *et. al.* (2009) *Nat Rev Gen.* 10: 845-858.
Niswander (2003) *Nat Rev Gen.* 4: 131-142.

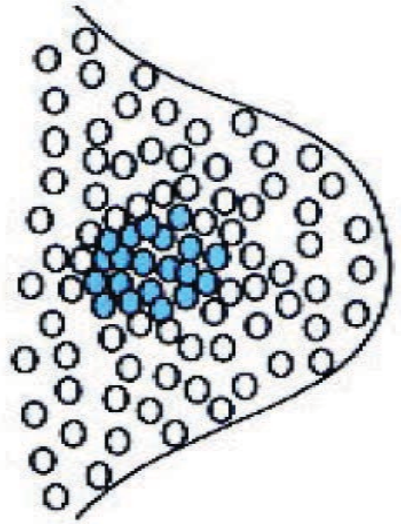
Limb bud Development, Patterning, and Chondrogenesis









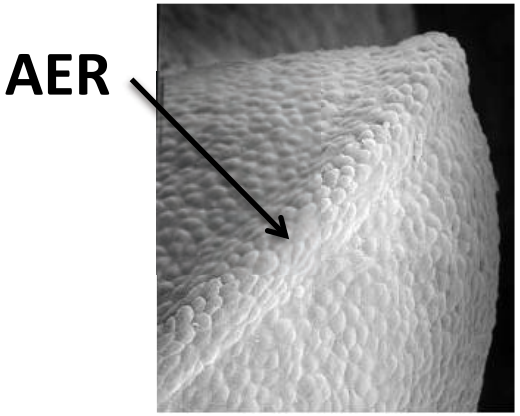


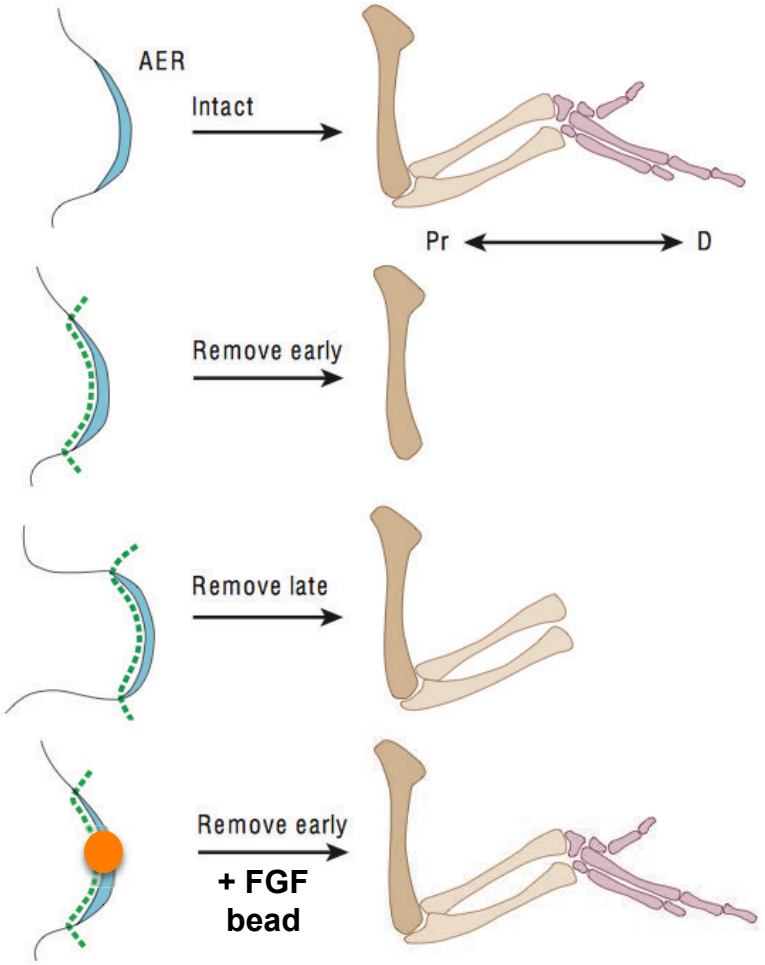
Mouse: E9.5-E10.5 E10.5-E11.5 E11.5-E12.5

Human: 4 weeks

Adapted from Ornitz and Marie (2002) *Genes and Development.* 16: 1446-65.
Zeller, *et. al.* (2009) *Nat Rev Gen.* 10: 845-858.

Influence of the Apical Ectodermal Ridge (AER) on Proximodistal Limb Patterning





Adapted from Mariani and Martin (2003) *Nature* 423: 319-325.

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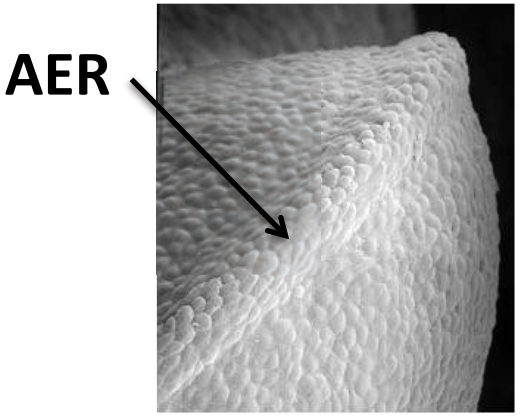
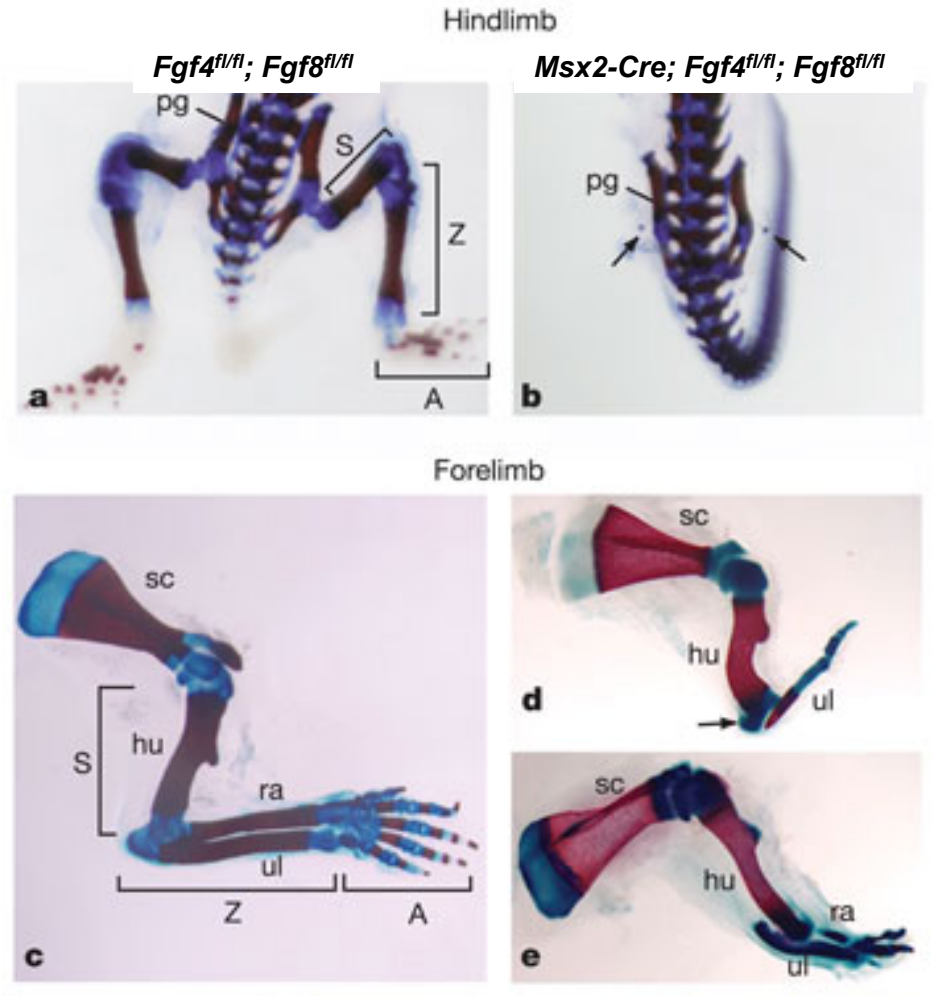
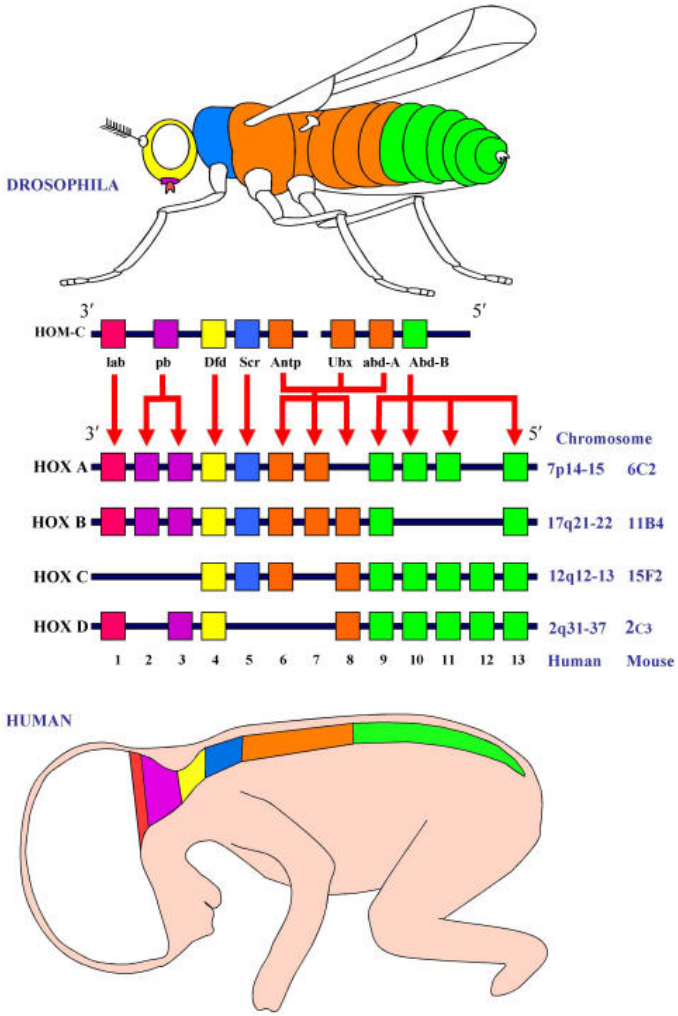


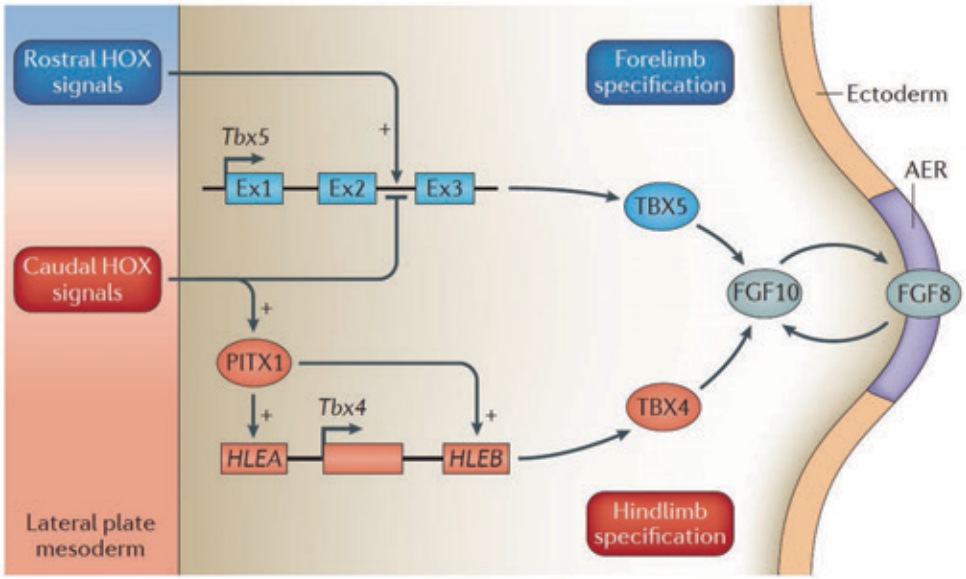
- FGF4, 8, 9, and 17 are all expressed in the AER.
- Only *Fgf8* is essential for normal limb bud development.
- Deletion of both *Fgf4* and *Fgf8* from the AER completely disrupts limb bud development.



Lewandoski, *et. al.* (2000) *Nat Gen* 26: 460-463.
Sun, *et. al.* (2002) *Nature* 418: 501-508.

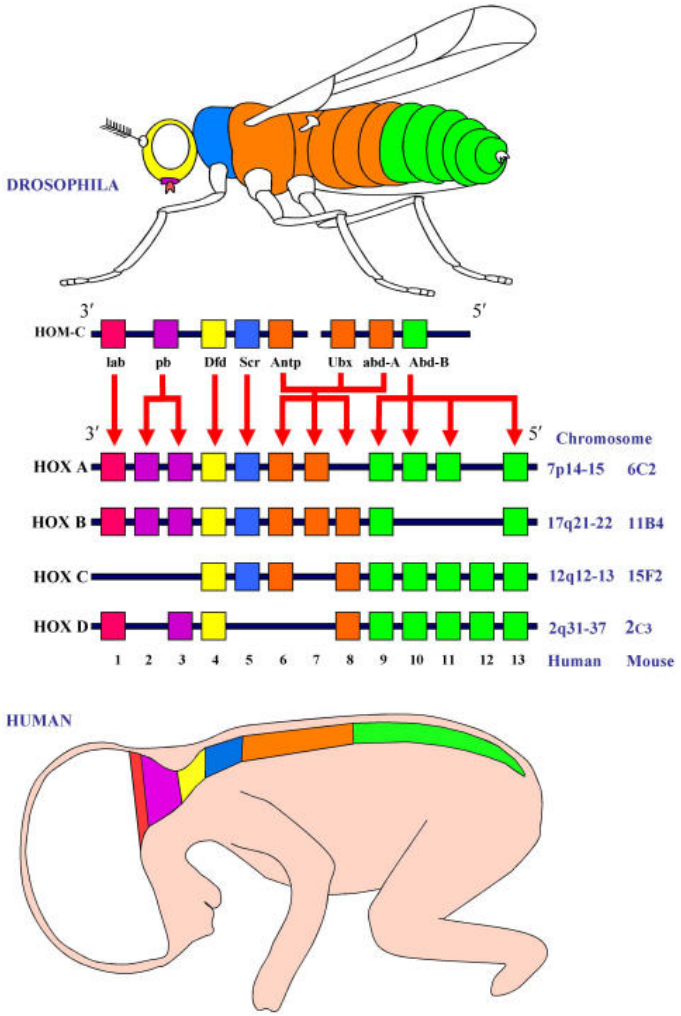
Hox Gene Regulation of Proximodistal Patterning

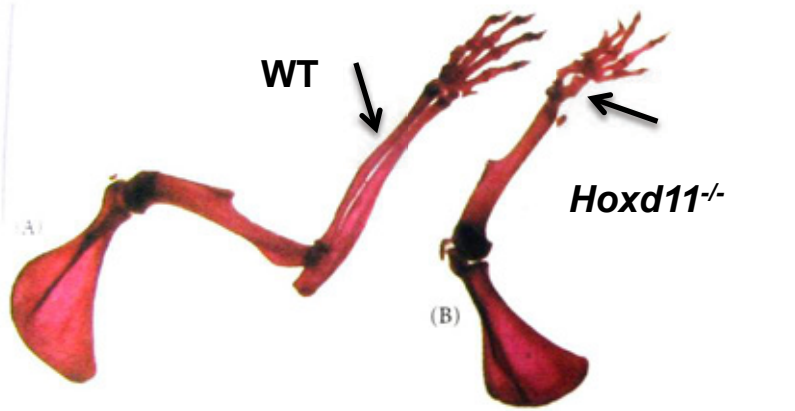


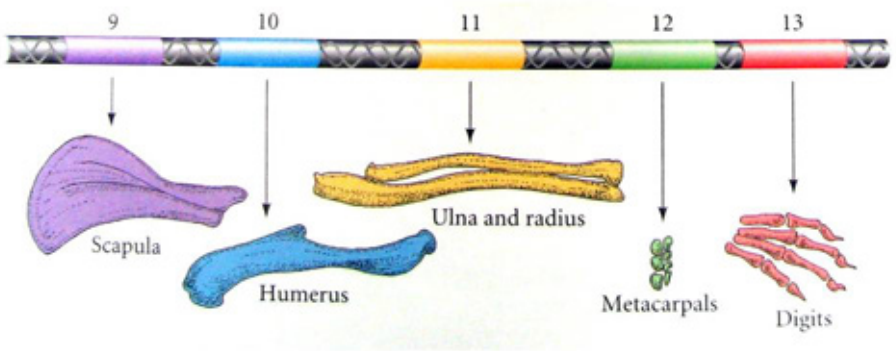


Lappin, *et. al.* (2006) *Ulster Med* 75: 23-31.
Petit, *et. al.* (2017) *Nat Rev Gen,* ePub.

Hox Gene Regulation of Proximodistal Patterning

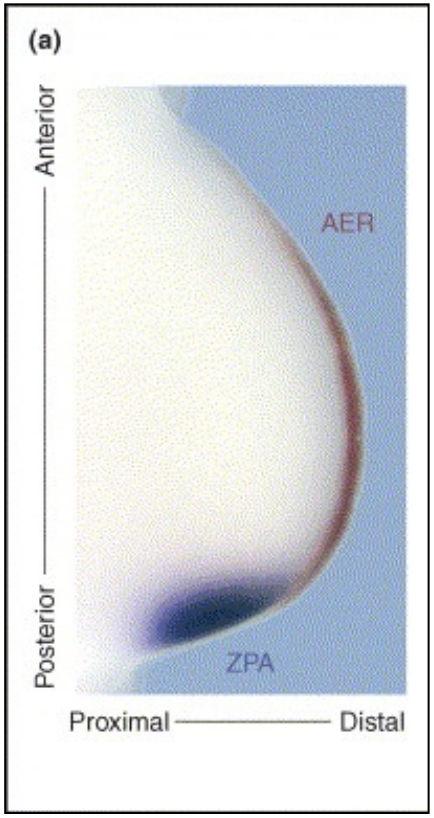


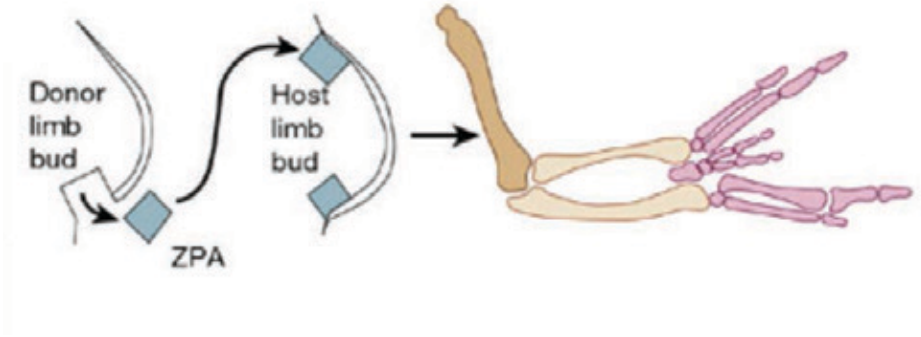




Lappin, *et. al.* (2006) *Ulster Med* 75: 23-31.
Davis, *et. al.* (1995) *Nature* 375: 791-795.

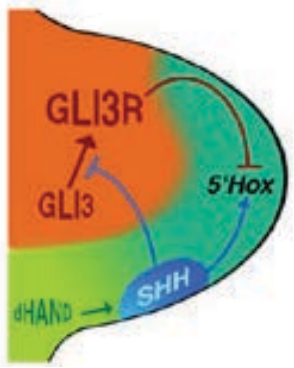
ZPA Regulation of Anterior to Posterior Patterning

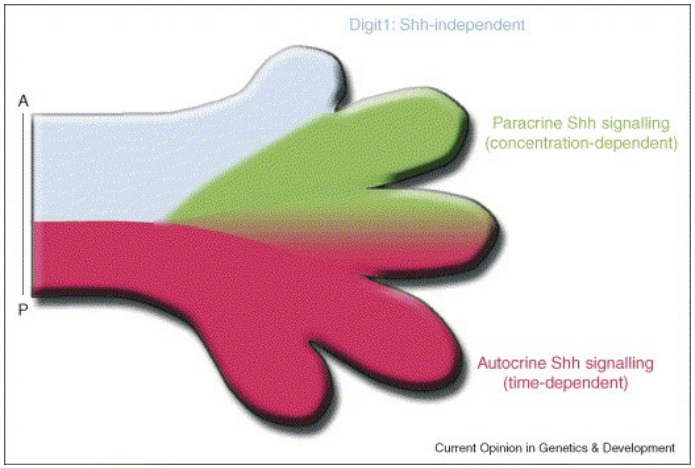


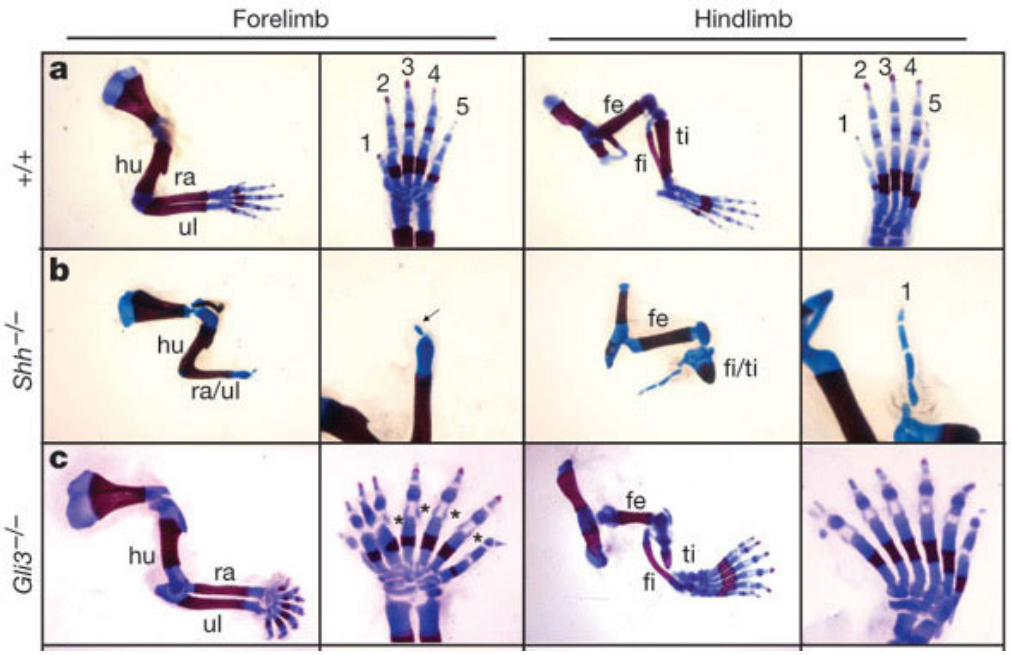


Adapted from Mariani and Martin (2003) *Nature* 423: 319-325.

ZPA (Shh) Regulation of Anterior to Posterior Patterning







Litingtung *et. al.* (2002) *Nature* 418: 979-983.

Human Disorders of Skeletal Patterning, Differentiation, and Growth

- **Polydactyly** (extra digits)
- **Oligodactyly** (missing digits)
- **Amelia** (absence of limbs)
- **Meroamelia** (partial absence of a limb)

- **Brachydactyly** (shortened digits)
- **Campomelic Dysplasia** (*SOX9* haploinsufficiency)
- **Ellis-van Creveld Syndrome** (*EVC* mutations)
- **Cleidocranial Dysplasia** (*RUNX2* haploinsufficiency)

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  - **Hypochondroplasia**
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  - **Thanatophoric Dysplasia**

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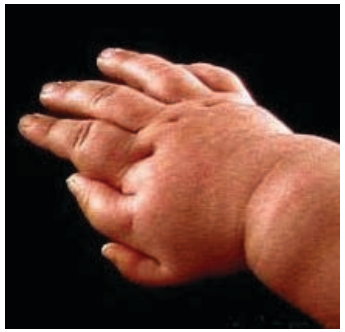

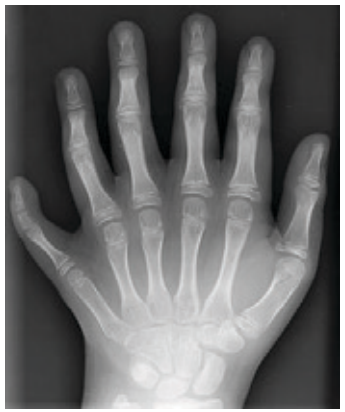

https://runkle-science.wikispaces.com/Polydactyly

For review, Kornak and Mundlos (2003) *Am. J. Hum. Genet.* 73: 447-474.

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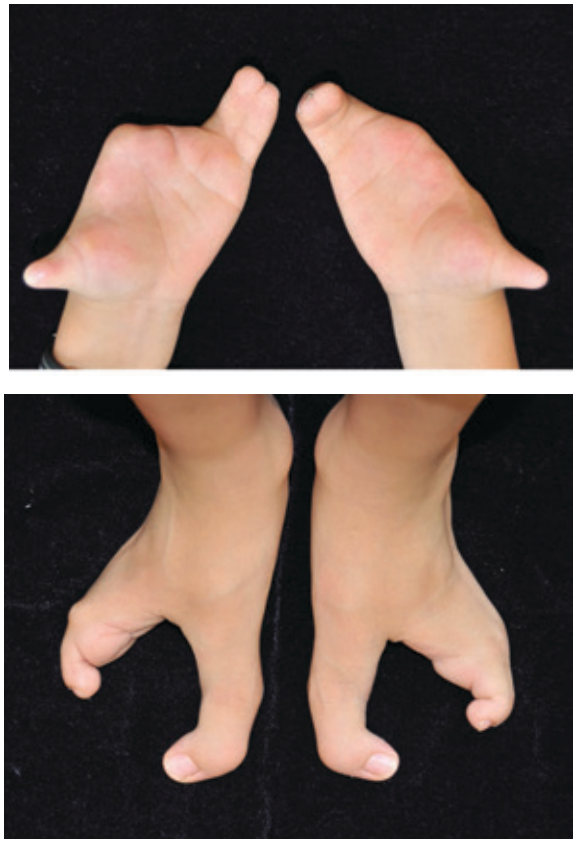

http://drpanossian.com/surgical-solutions/hand-deformities/#ectrodactyly

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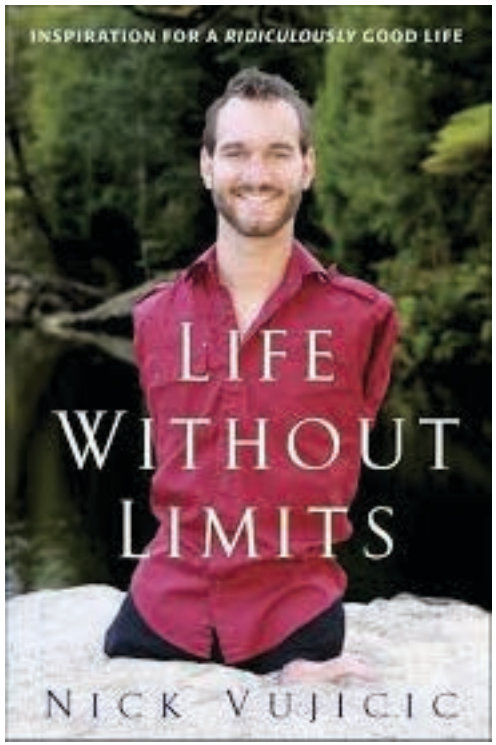
Tetra-amelia (mutations in WNT3)



For review, Kornak and Mundlos (2003) *Am. J. Hum. Genet.* 73: 447-474.

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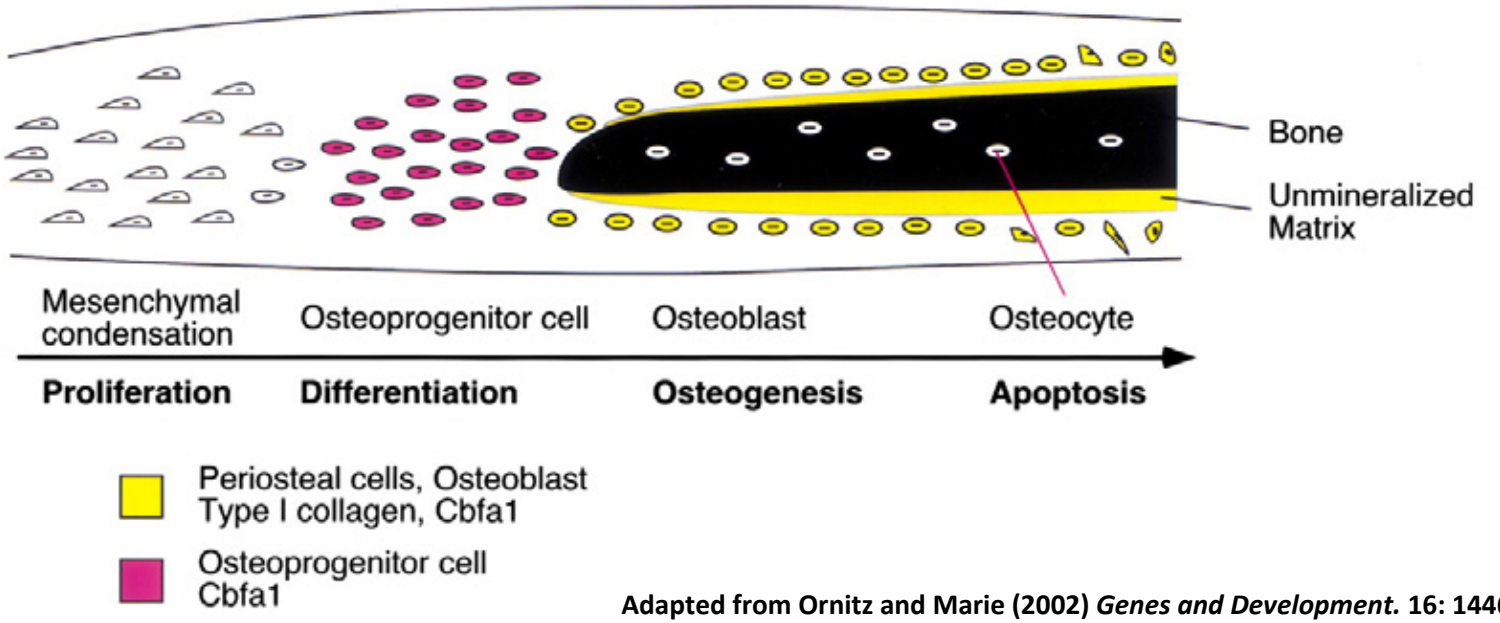
For review, Kornak and Mundlos (2003) *Am. J. Hum. Genet.* 73: 447-474.

Intramembranous *versus* Endochondral Ossification

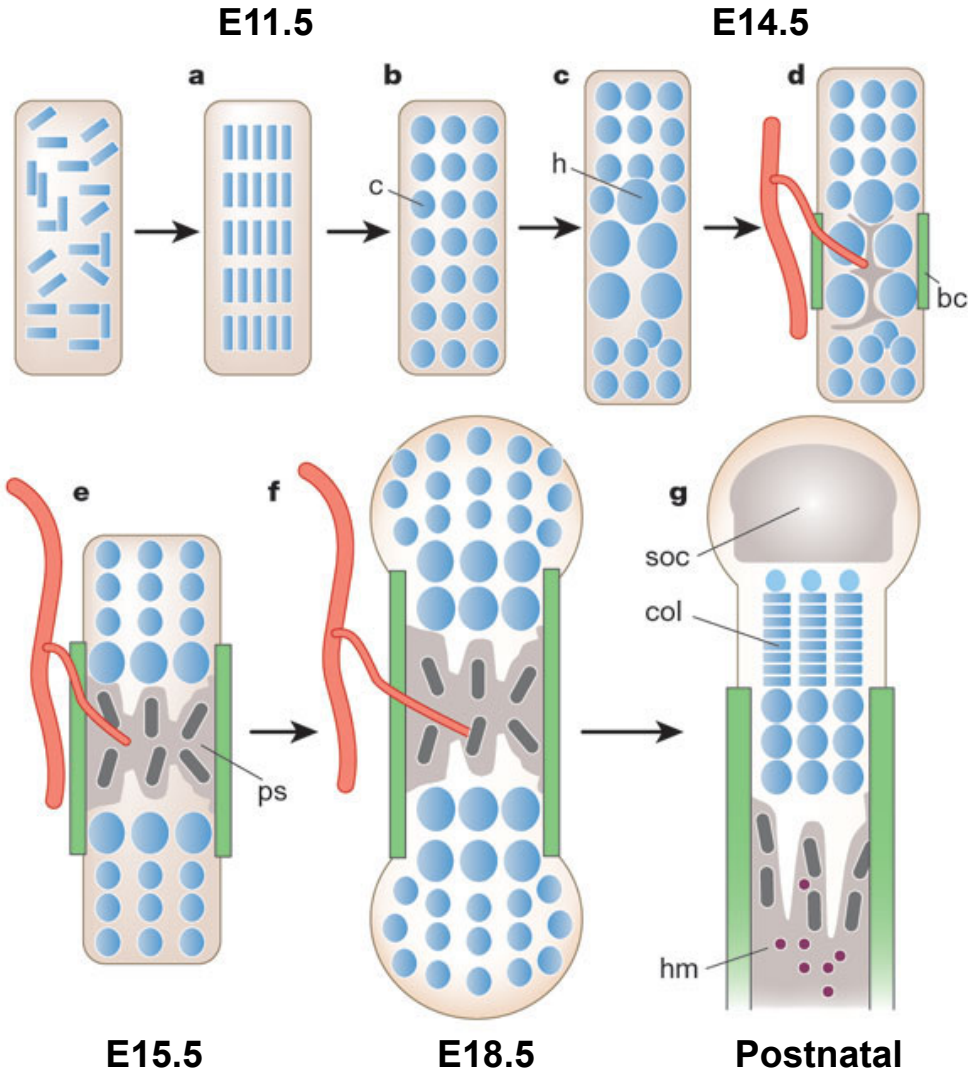
- **Intramembranous:** bone formation directly from mesenchymal condensations; flat bones (skull, mandible, clavicles)



Adapted from Ornitz and Marie (2002) *Genes and Development.* 16: 1446-65.

- **Endochondral:** bone formation following the formation of a hyaline cartilage template; all other bones (long bones, etc.)

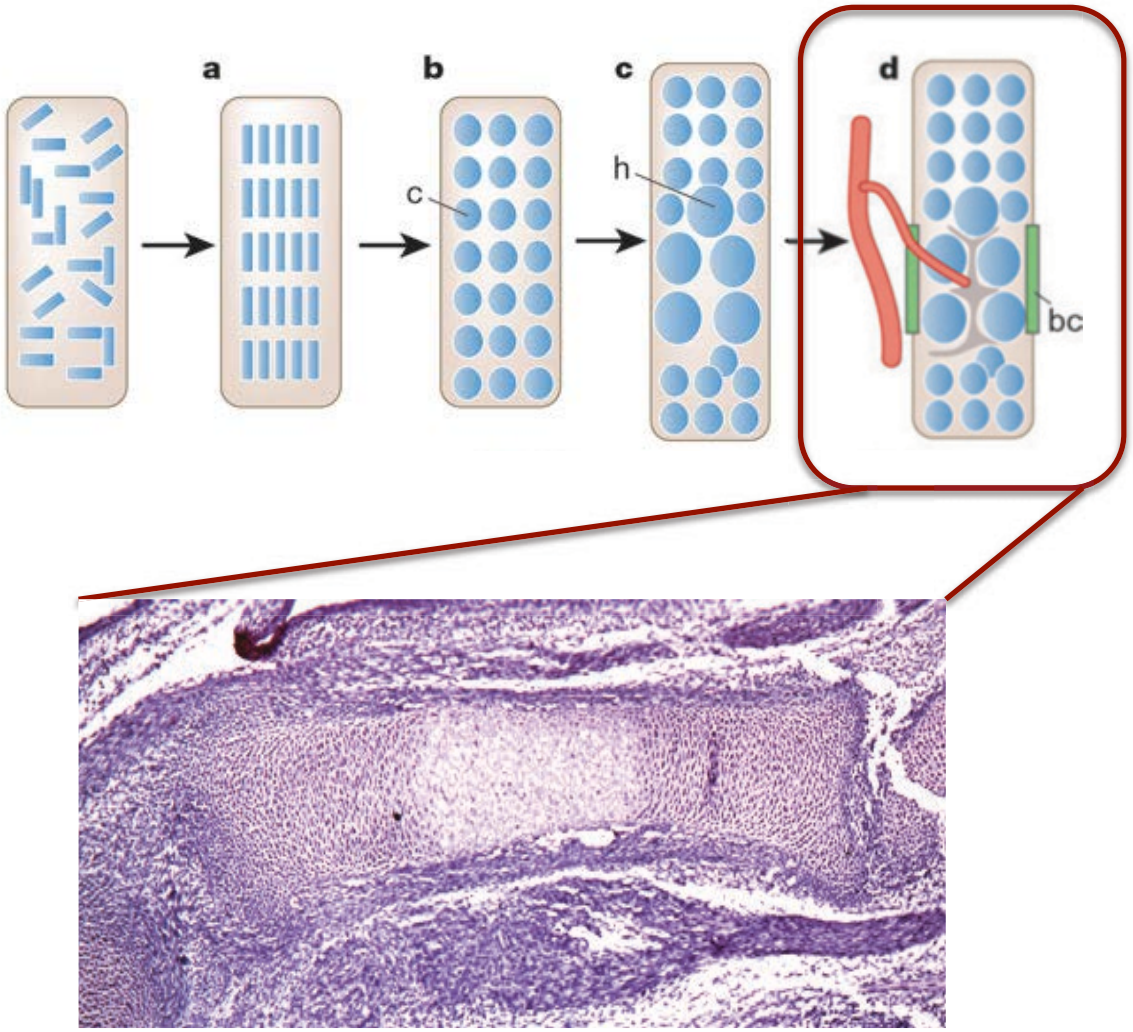
Endochondral Bone Formation



a. Mesenchymal condensations
b. Chondrogenesis and chondrocyte proliferation
c. Onset of hypertrophy
d. Terminal chondrocyte differentiation
e. Primary ossification center formation
f-g. Establishment of epiphyseal growth plates and secondary ossification centers

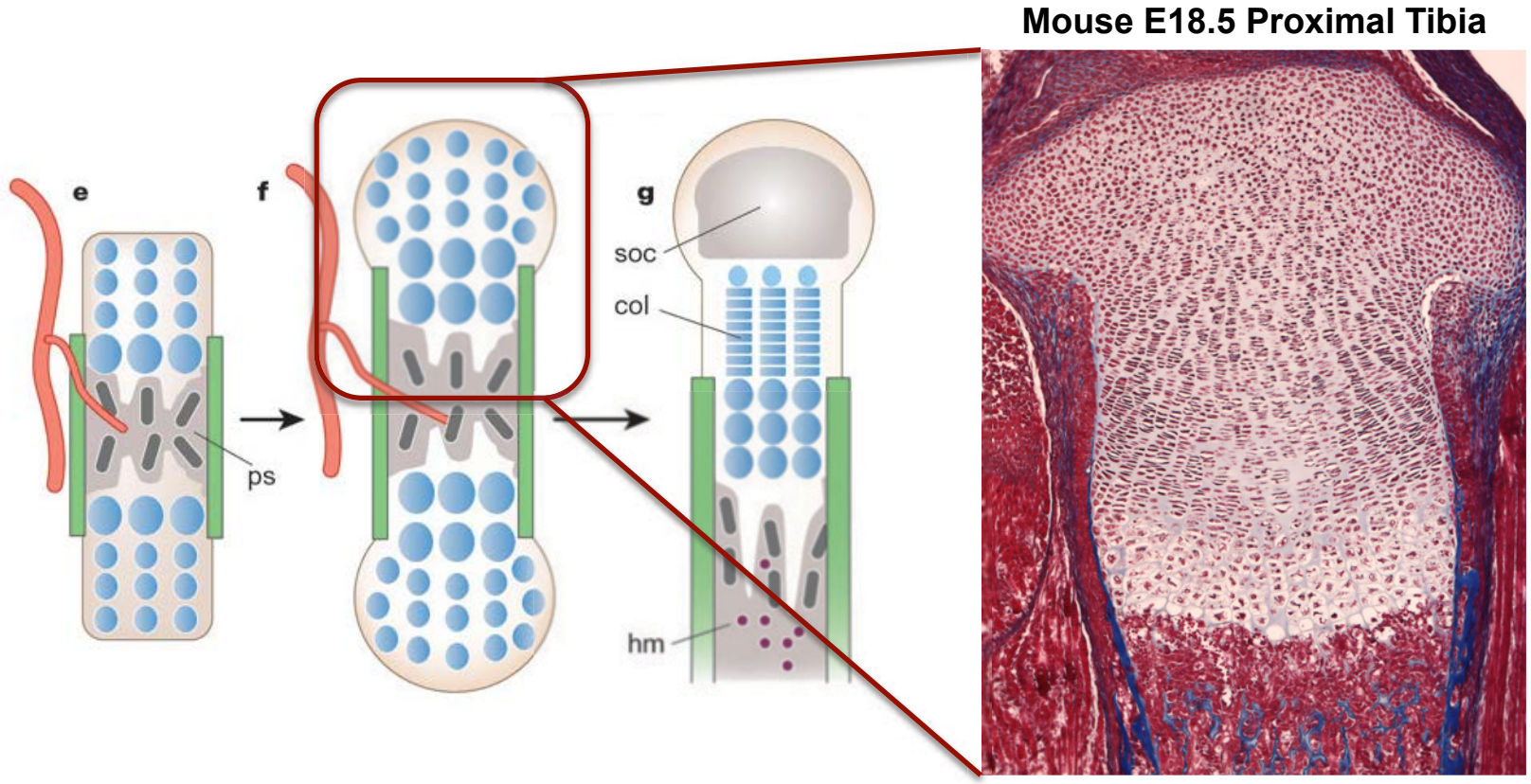
Kronenberg, H. (2003) *Nature*. 423: 332-336.

Chondrocyte Maturation – Onset of Hypertrophy

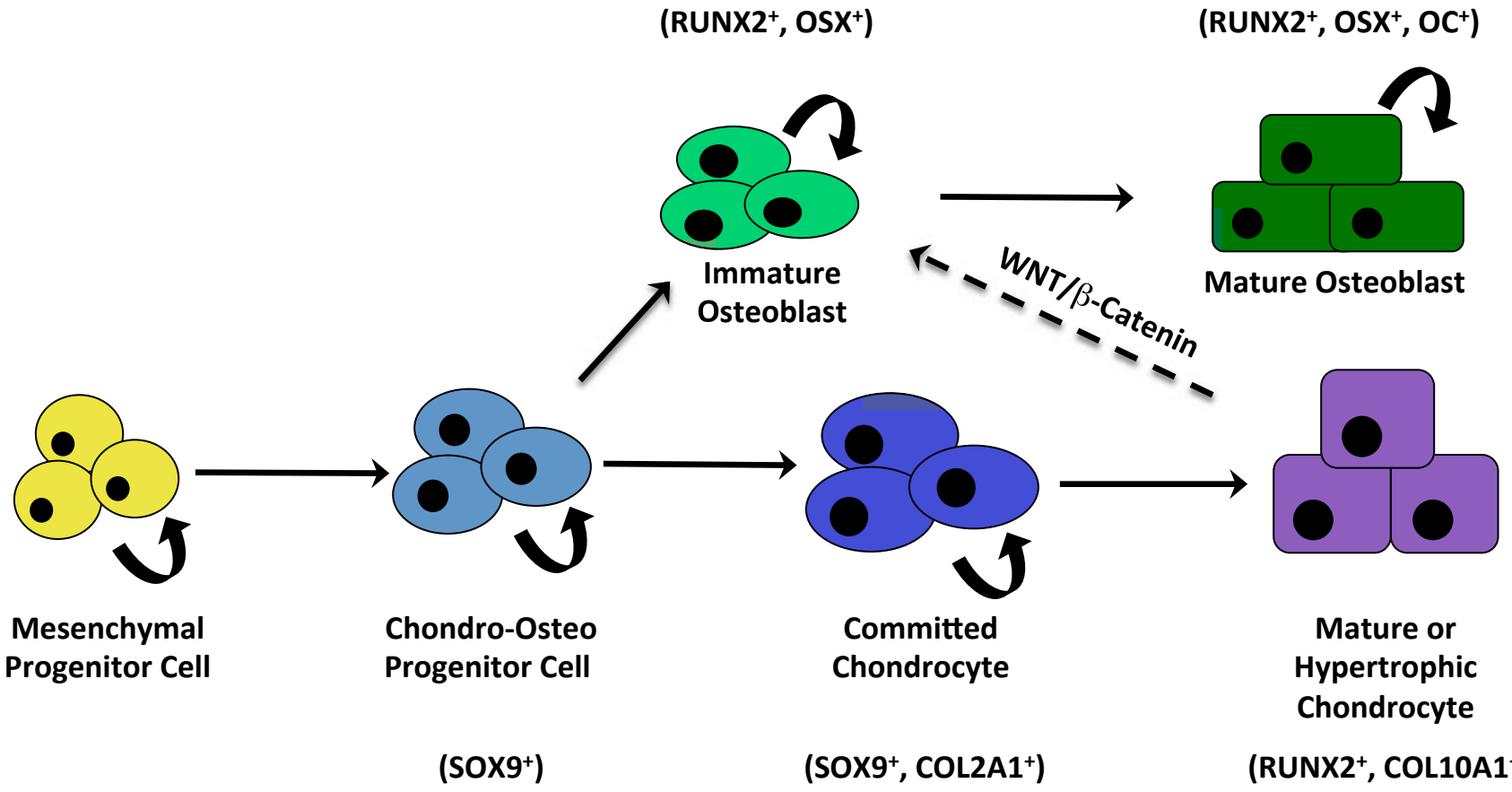


Mouse E14.5 Tibia

Chondrocyte Maturation – Terminal Hypertrophy

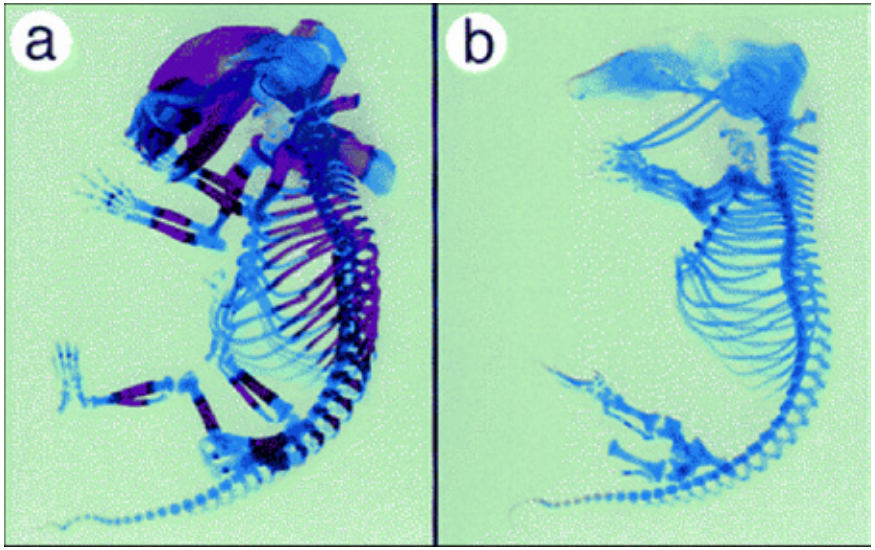


Chondrogenesis and Osteoblastogenesis



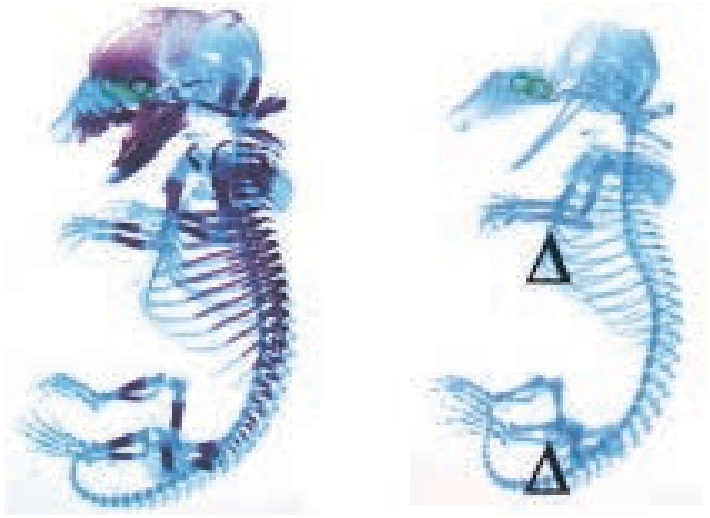
Critical Transcriptional Regulators of Chondrocyte Maturation and Bone Development (Runx2 and Osterix)

Runx2 **mutant mice (null)**



Otto F et al., (1997) Cell (89)5:765-771.

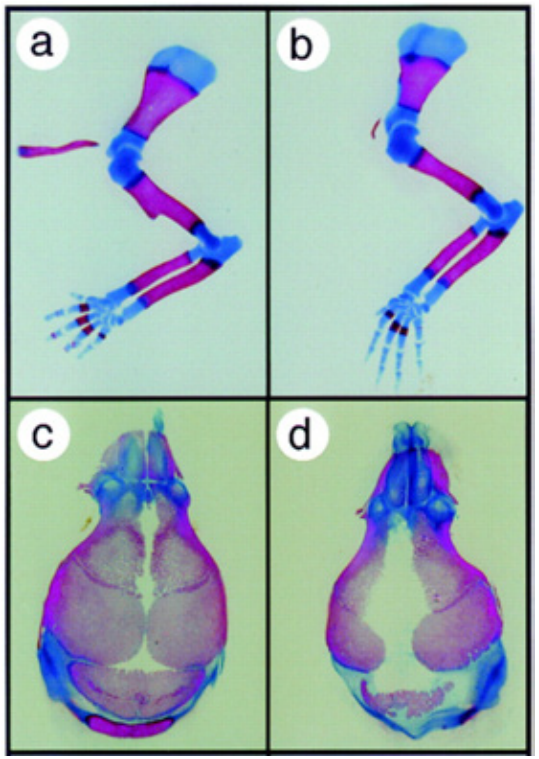
Osterix **mutant mice (null)**



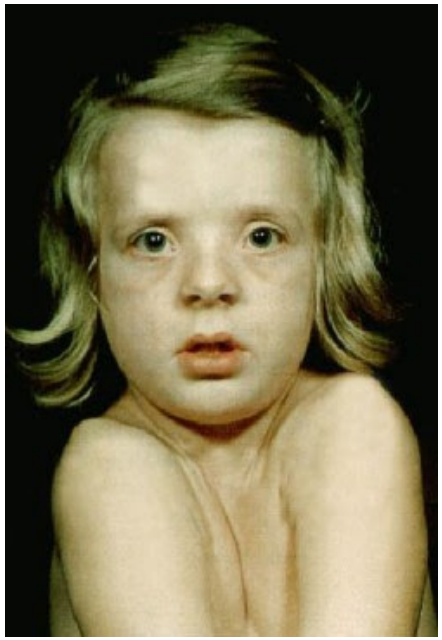
Nakashima K et al., (2002) Cell (108)1:17-29.

Cleidocranial Dysplasia (CCD)

- Autosomal dominant disorder due to haploinsufficiency of *RUNX2*
- Clinical features include; hypoplastic clavicles, delayed ossification of the cranial sutures and fontanelles, dental anomalies, and short stature
- At risk for development of osteoporosis and fracture

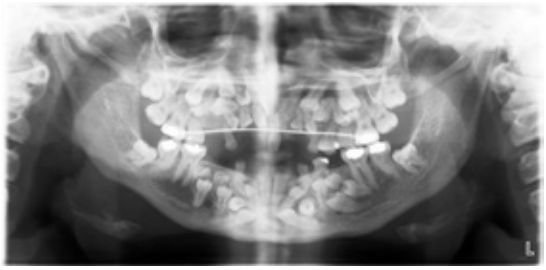


Otto, *et. al.* (1997) *Cell.* 89: 765-771.



http://
dentistryandmedicine.blogspot.
com/2011/05/cleidocranial-
dysostosis-cleidocranial.html

Supernumerary teeth



http://drgstoothpix.com/
2014/05/09/case-of-the-week-
cleidocranial-dysplasia/

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For review, Kornak and Mundlos (2003) *Am. J. Hum. Genet.* 73: 447-474.

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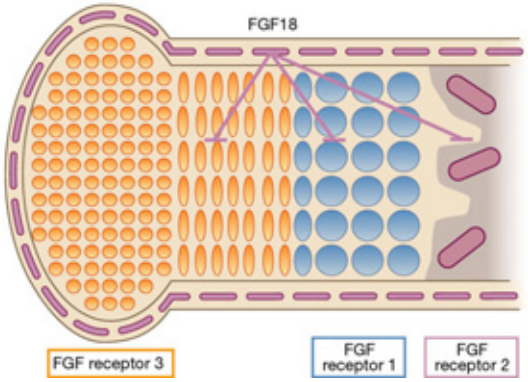


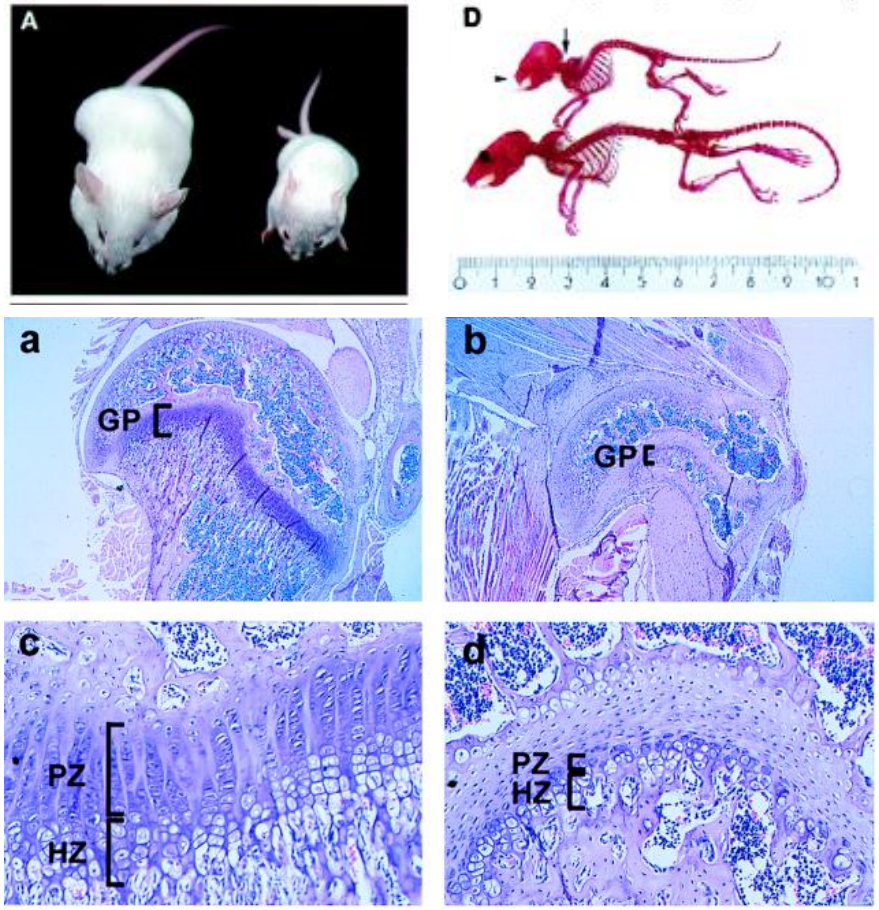
- **Dwarfing Chondrodysplasias** (caused by activating FGFR3 mutations)
  - **Hypochondroplasia** (least severe; short stature)
  - **Achondroplasia** (most common; short stature**,** frontal bossing)
  - **Thanatophoric Dysplasia** (most severe; most patients die of respiratory distress following birth)

- **Craniosynostosis syndromes** (premature closure of sutures)

For review, Kornak and Mundlos (2003) *Am. J. Hum. Genet.* 73: 447-474.

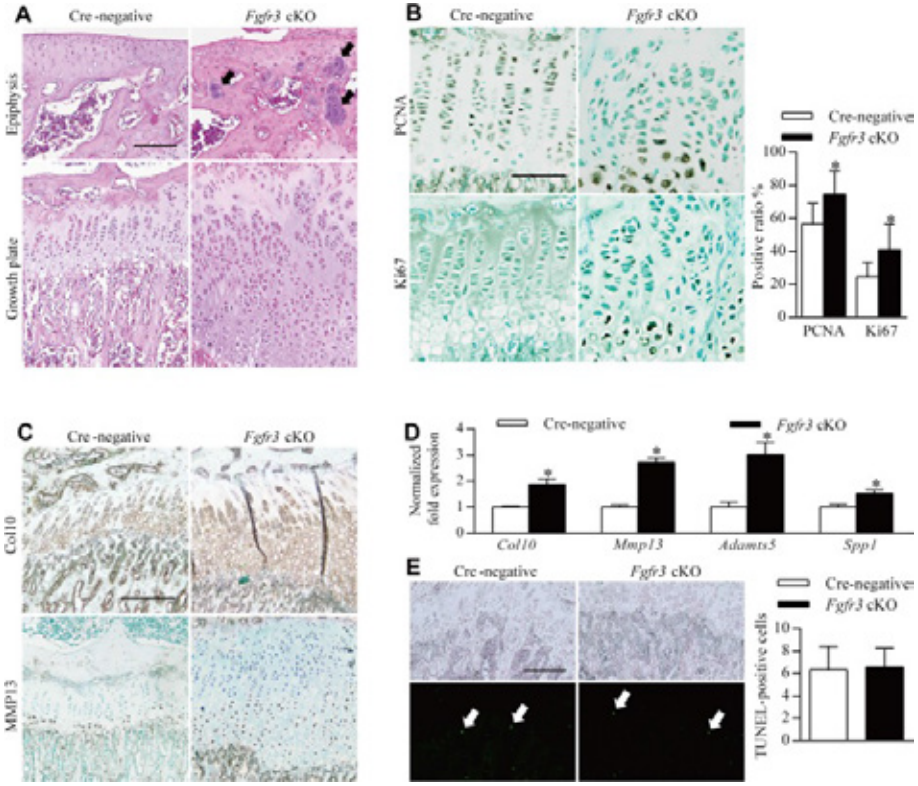
FGFR3 Mouse Models

FGFR3 Activation (FGFR3$^{G374Rneo-/+}$)
Achondroplasia model



Wang, *et. al.* (1999) *PNAS.* 96: 4455-4460.

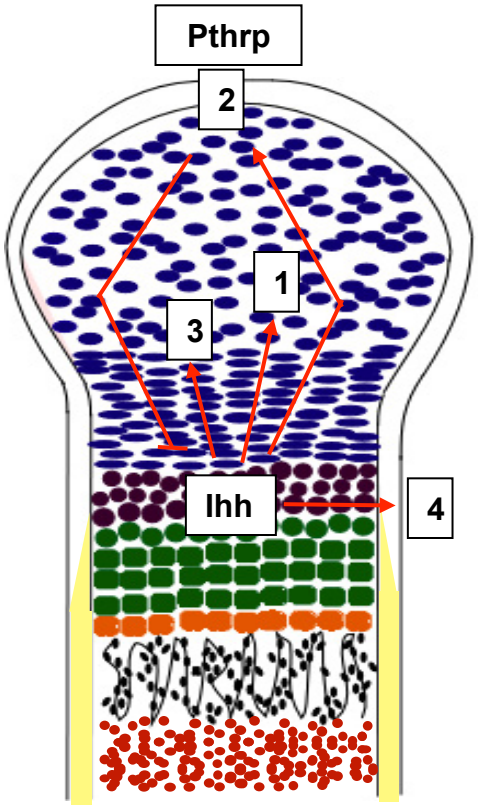
Chondrocyte-specific loss of *Fgfr3* leads to enhanced proliferation and maturation

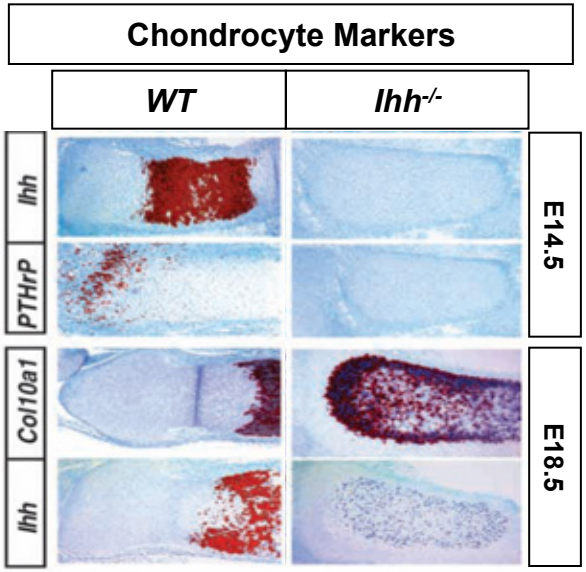


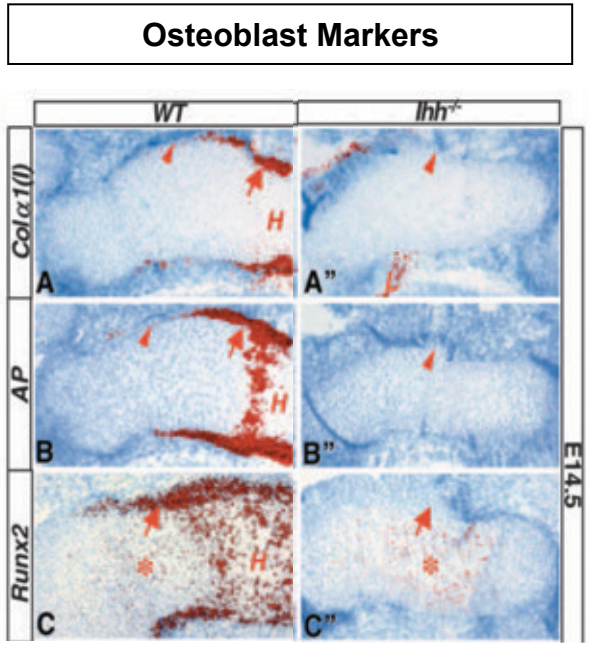
Zhou, *et. al.* (2015) *Plos Genet.* 11: ePub.

Ihh/Pthrp Signaling During Cartilage and Bone Development

- **Ihh signaling induces chondrocyte proliferation, delays chondrocyte differentiation, and is required for early osteoblast maturation.**





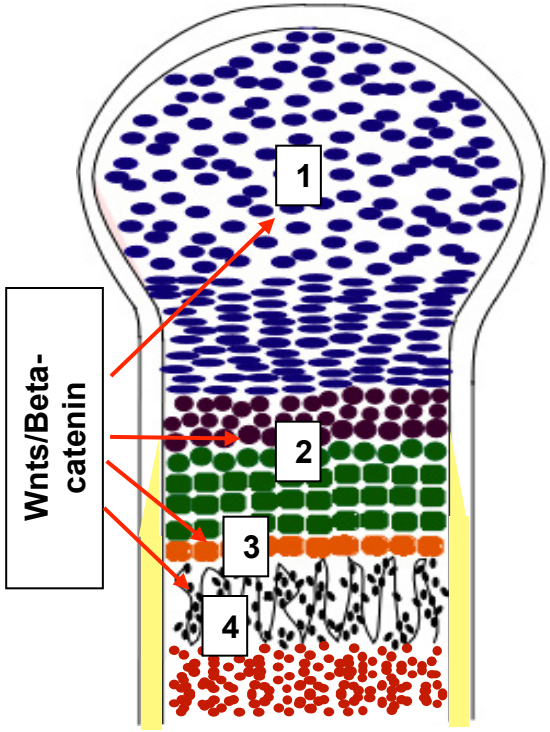


Long, *et al.* (2001) *Development.* 128:5099-5108.

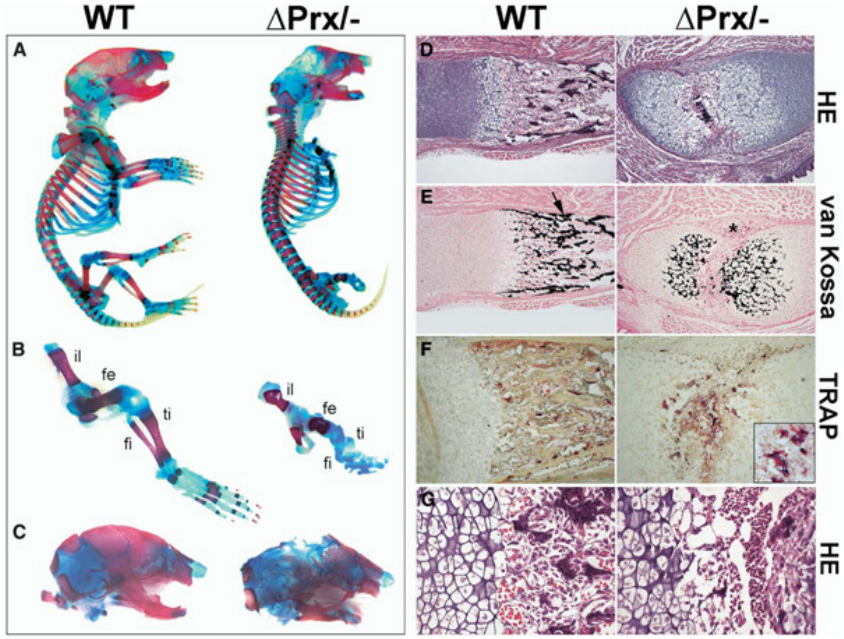
Hu, *et. al.* (2005) *Development.* 132:49-60.

Wnt/β-Catenin Signaling During Cartilage and Bone Development

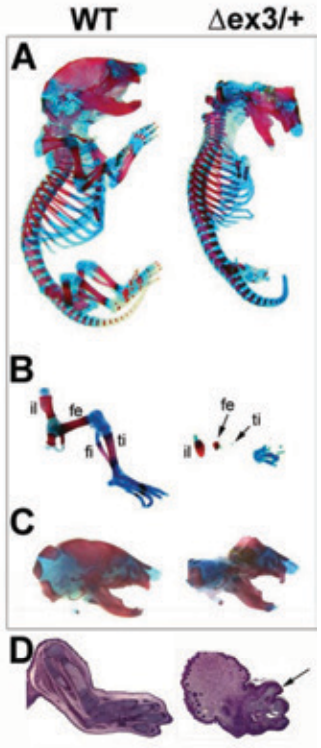
- Wnt/β-catenin signaling induces chondrocyte proliferation, differentiation, and osteoblast maturation in committed cells.
- Wnt/β-catenin signaling suppresses chondrogenic and osteogenic differentiation in mesenchymal progenitor cells.



β-catenin Loss-of-function (Prx1-Cre)



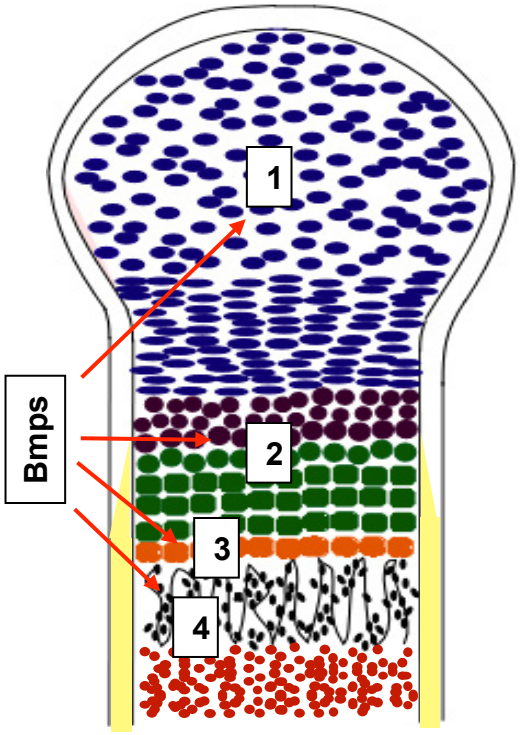
Gain-of-function

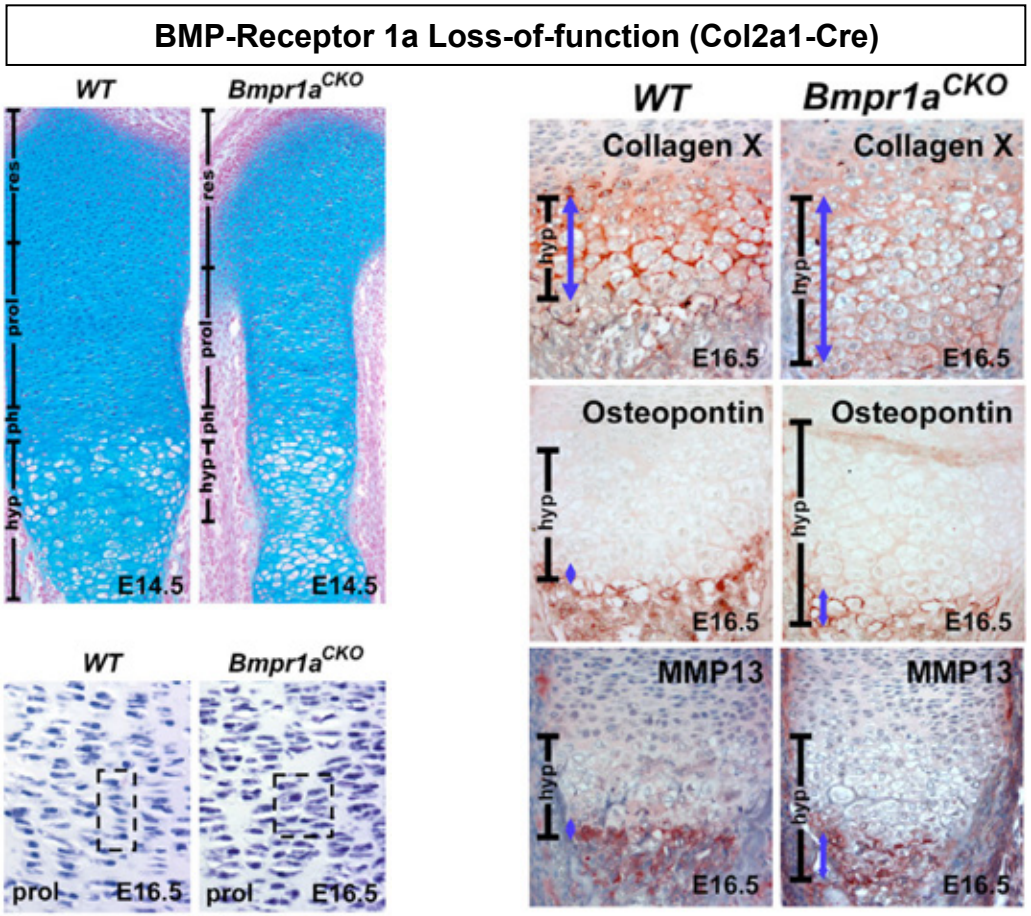


Hill, *et al.* (2005) *Developmental Cell.* 8:727-738.

BMP Signaling During Cartilage and Bone Development

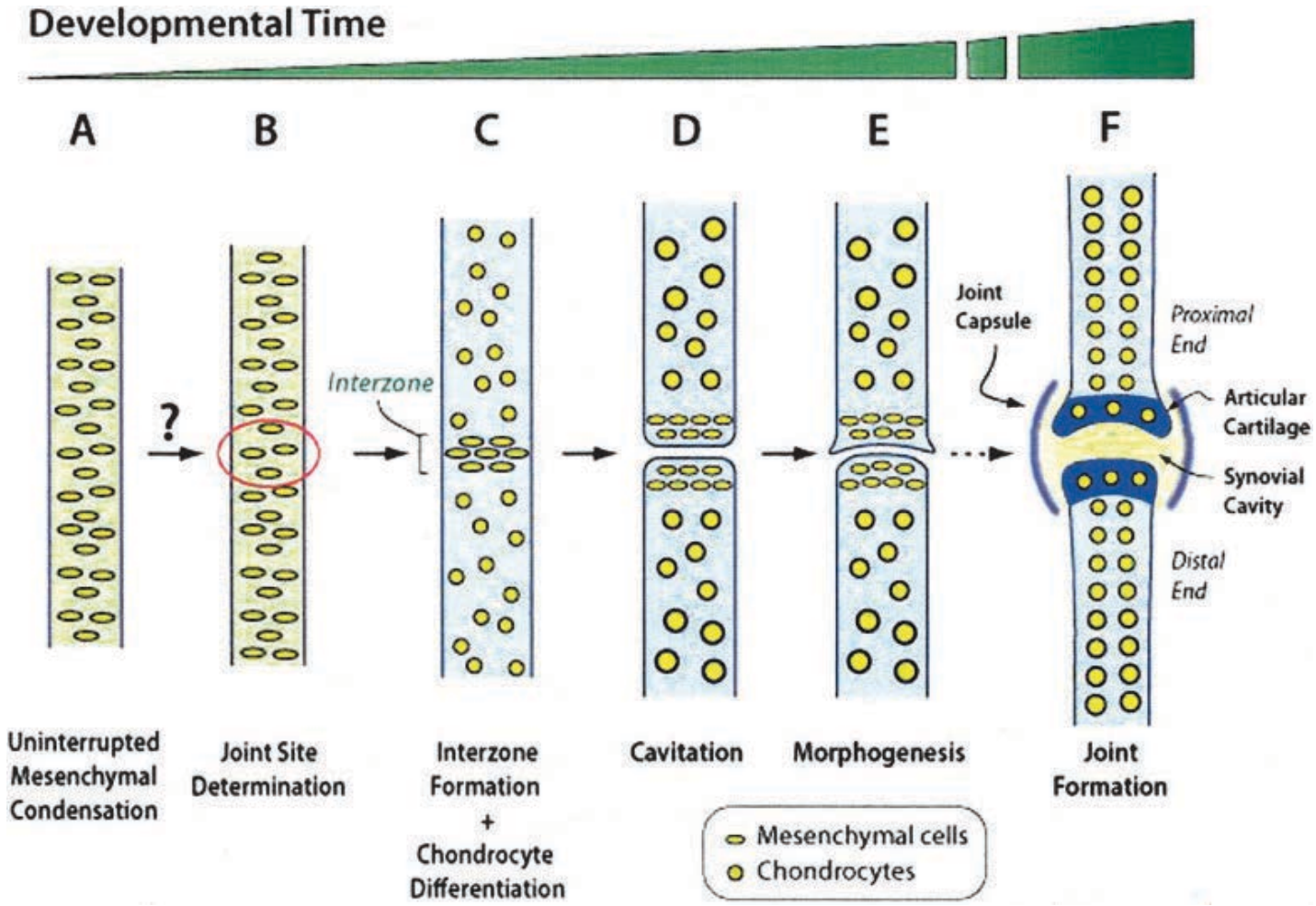
- BMP signaling induces chondrocyte proliferation and differentiation.





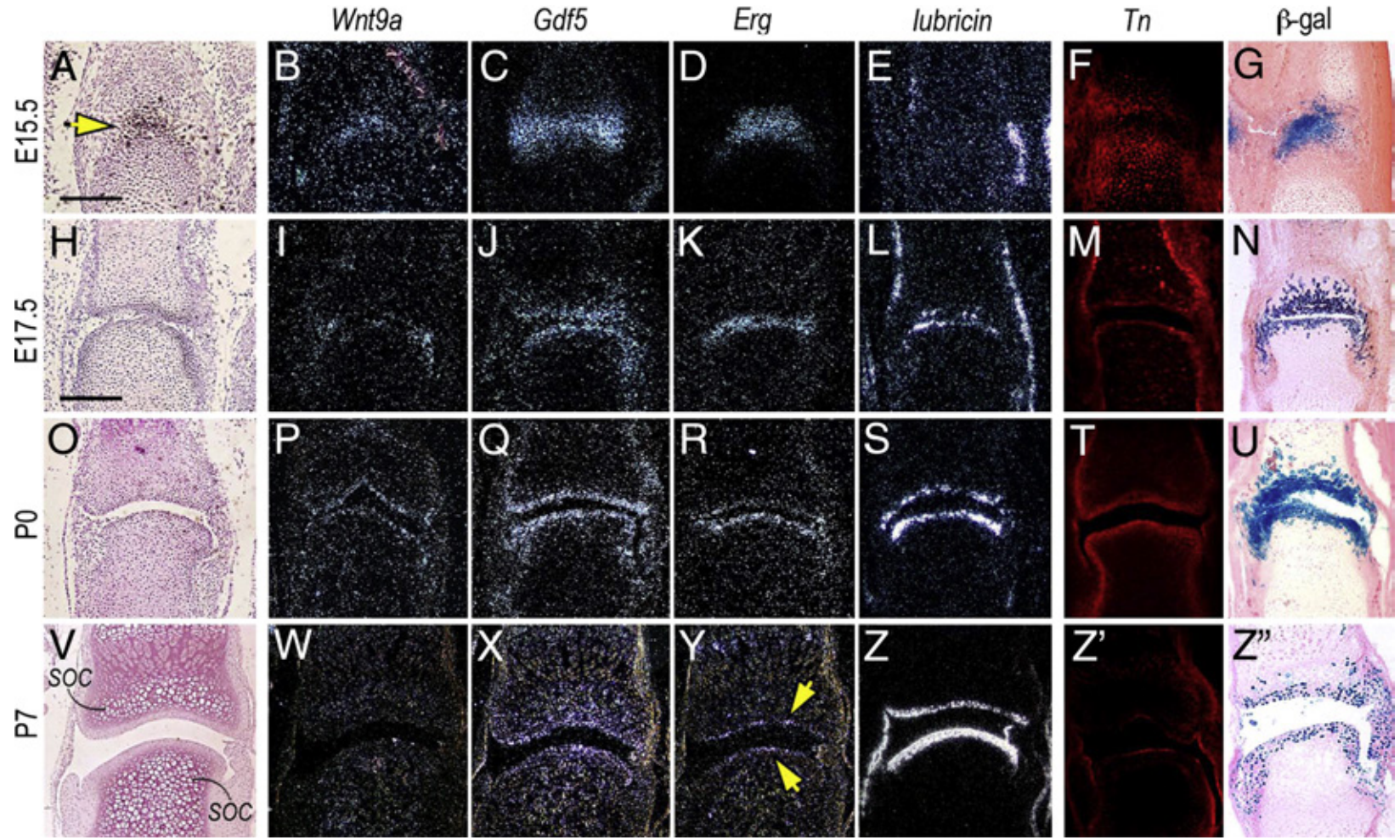
Yoon, *et al.* (2006) *Development.* 133:4667-4678.

Synovial Joint Development During Embryogenesis



Pacifici, et al. (2005) Birth Defects Res. 75:237-248.

Molecular Regulation of Joint Development



Koyama, et al. (2008) Developmental Biology. 1:62-73.