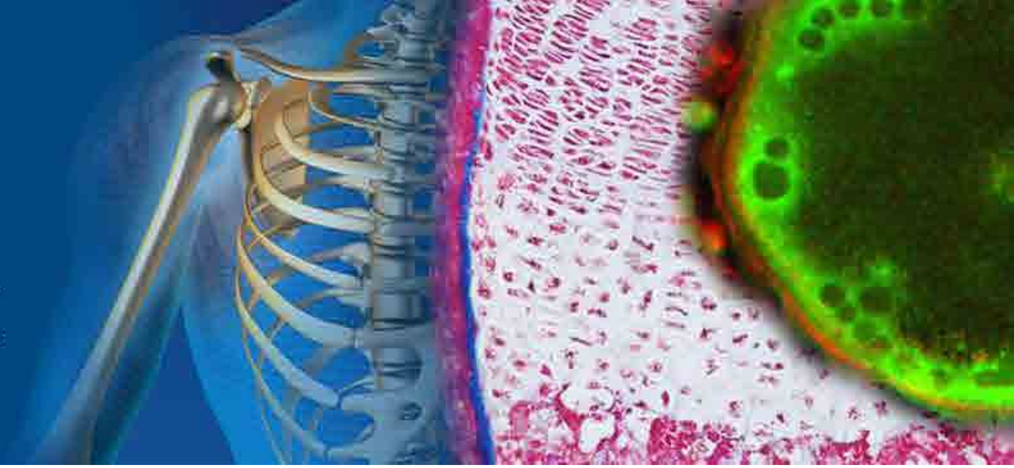
CENTER for MUSCULOSKELETAL RESEARCH



Musculoskeletal Development

Jennifer H. Jonason, Ph.D.

Department of Orthopaedics

Center for Musculoskeletal Research

IND464: Musculoskeletal Basic Science

Fall 2019

Musculoskeletal System

- **Bone**
  - 206 bones in the adult human skeleton
  - Major functions:
    - Mechanical support
    - Protects organs
    - Provides environment for hematopoiesis
    - Stores minerals, growth factors, and hormones
- **Cartilage**
  - 3 types: hyaline, elastic, and fibrocartilage
  - Provides low friction surface for motion and withstands tensile, shear, and compressive forces
- **Skeletal Muscle**
  - Provides contractile forces to move the bones of the skeleton
  - Maintains body temperature
- **Tendon**
  - Attach muscle to bone and transmit contractile forces from muscle to bone
- **Ligament**
  - Attach bone to bone

https://cpd.cqu.edu.au/course/info.php?id=456

Musculoskeletal System

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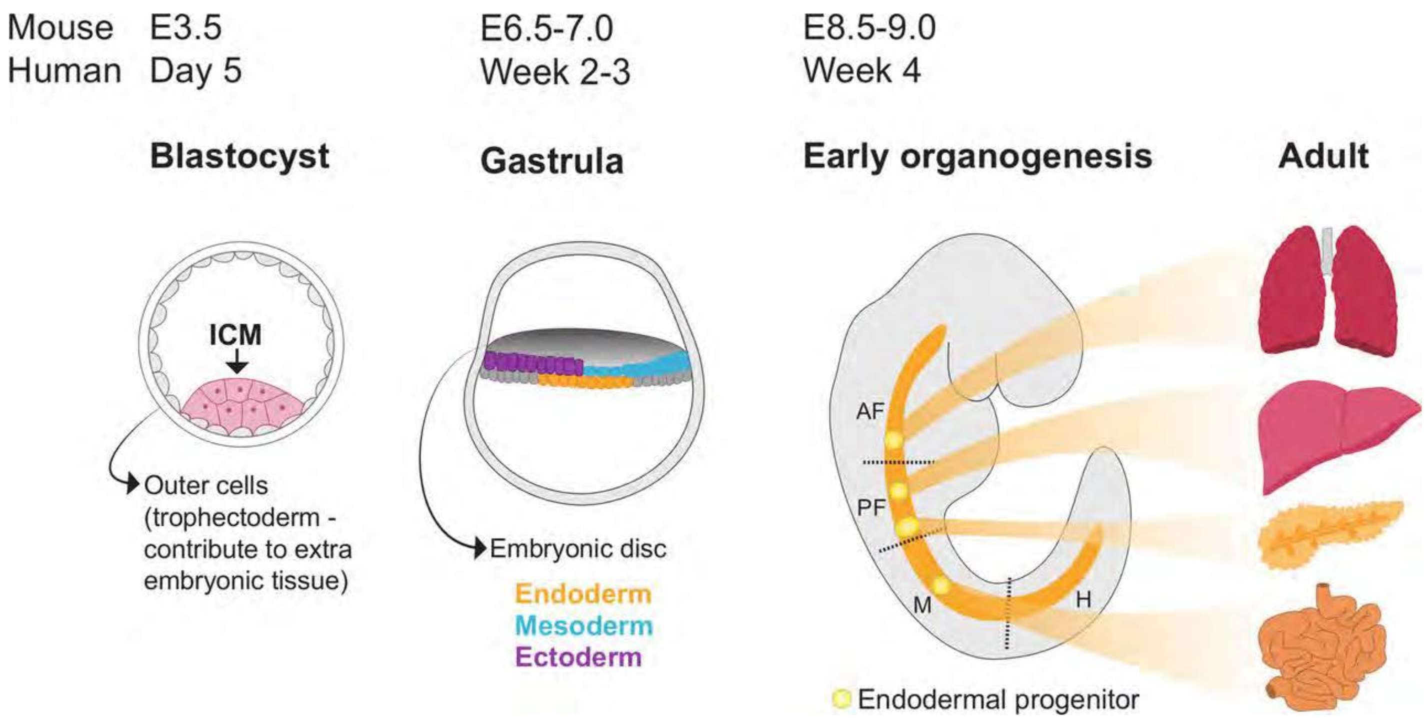
https://cpd.cqu.edu.au/course/info.php?id=456

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

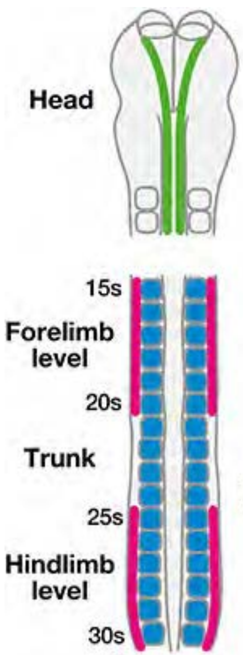
Embryogenesis/Organogenesis

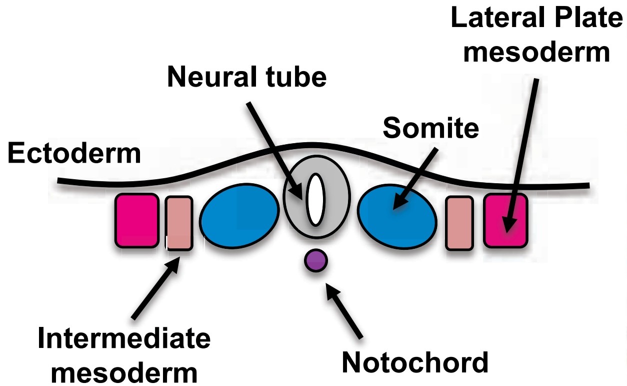


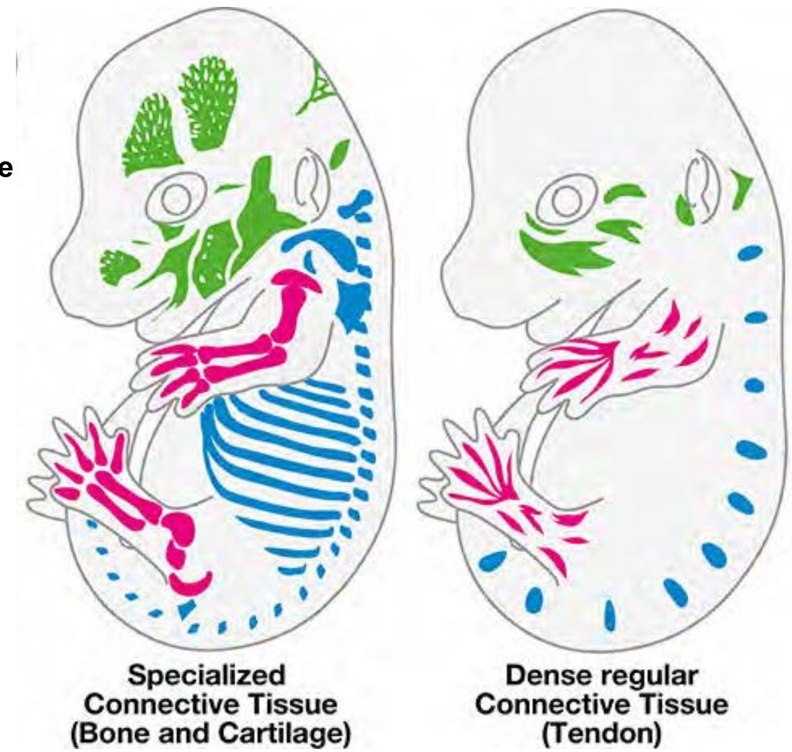
Endoderm: respiratory system, digestive system
Mesoderm: musculoskeletal system, circulatory system
Ectoderm: skin, nervous system

Prior, *et. al.* (2019) *Gut* doi:10.1136/gutjnl-2019-319256.

Embryonic Origins of Musculoskeletal Tissues

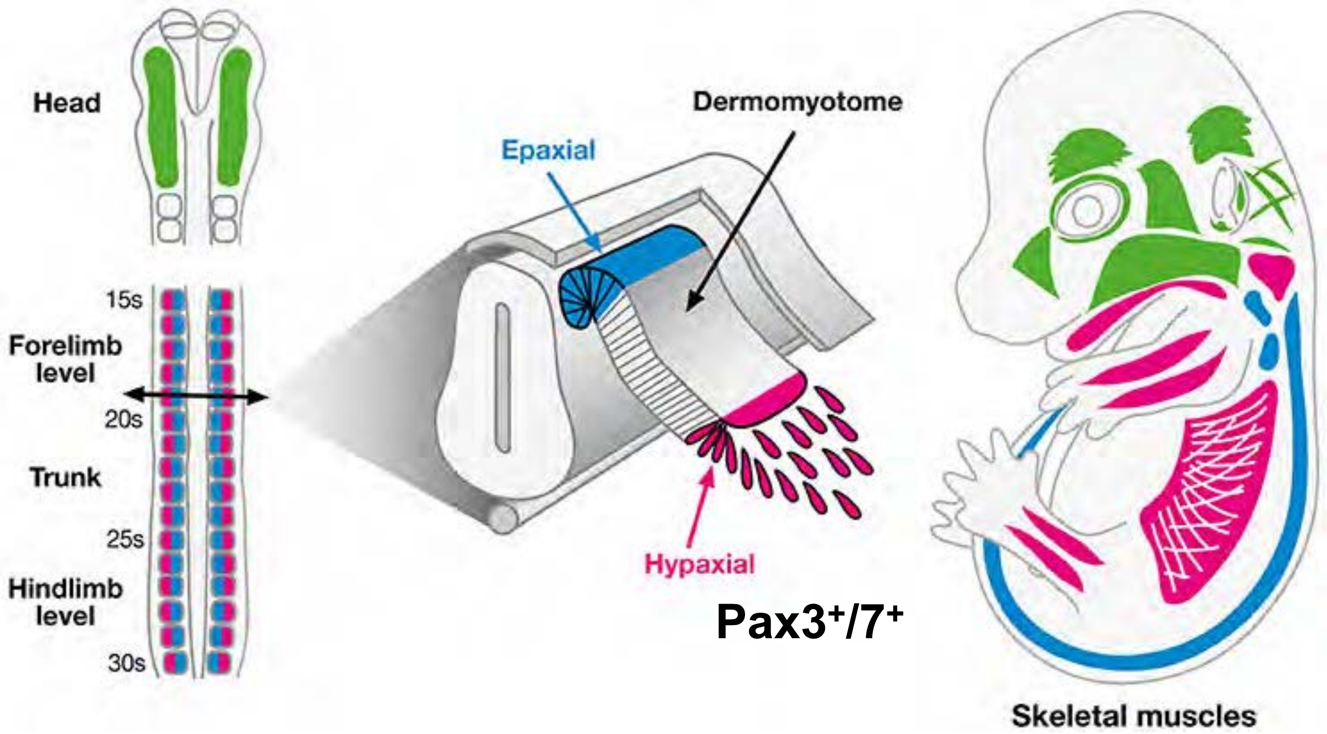






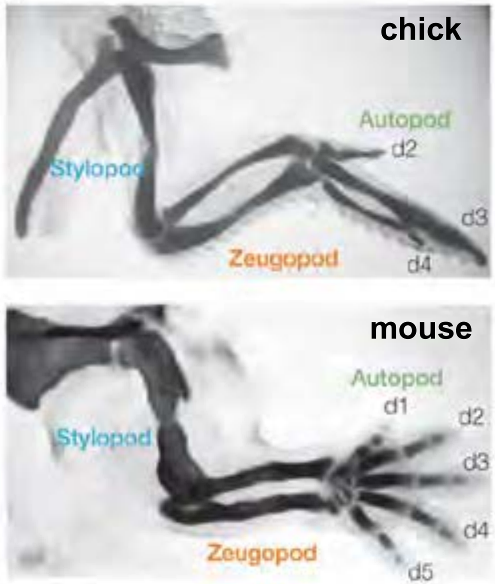
Adapted from Nassari *et. al.* (2017) *Front Cell Dev Biol.* 5:22.doi: 10.3389/fcell.2017.00022.

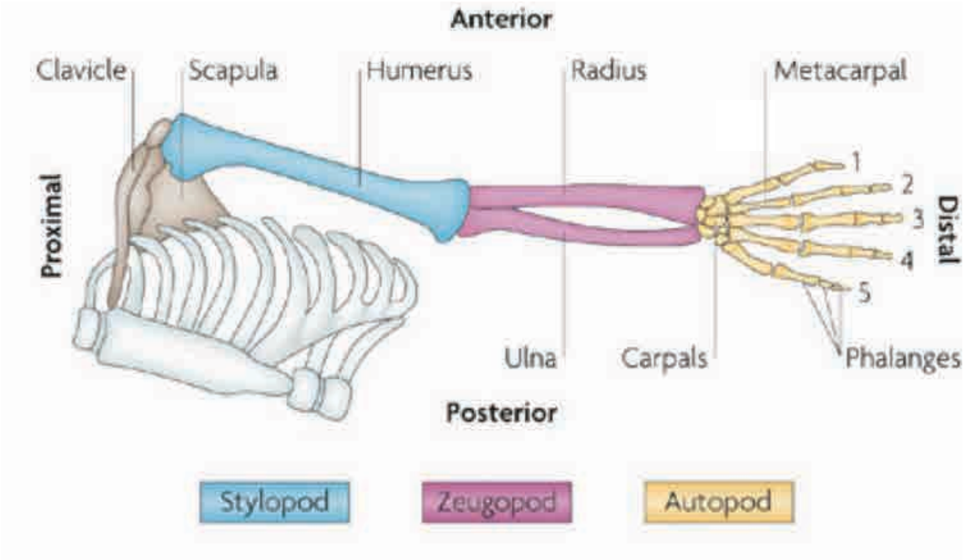
Embryonic Origins of Musculoskeletal Tissues



Adapted from Nassari *et. al.* (2017) *Front Cell Dev Biol.* 5:22.doi: 10.3389/fcell.2017.00022.

Patterning of the Limb Skeleton





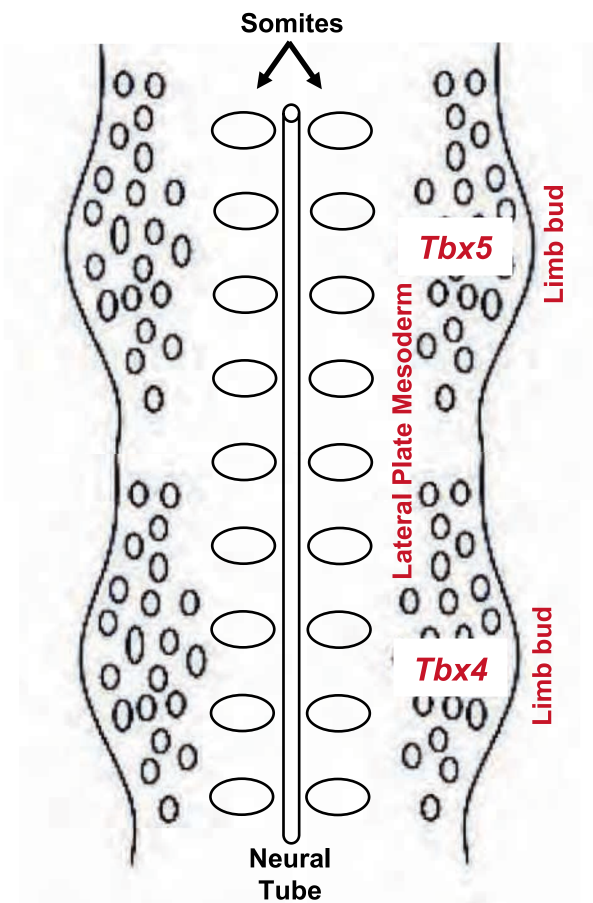
Patterning: the process in which the positions and identities of cells with different fates are laid down.

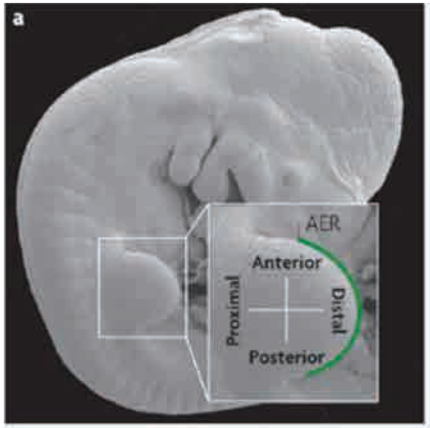
Specification: the process preceding determination during which a cell acquires its fate. The exposure of specified cells to different signals might alter their fates; the fate of specified cells is not fixed.

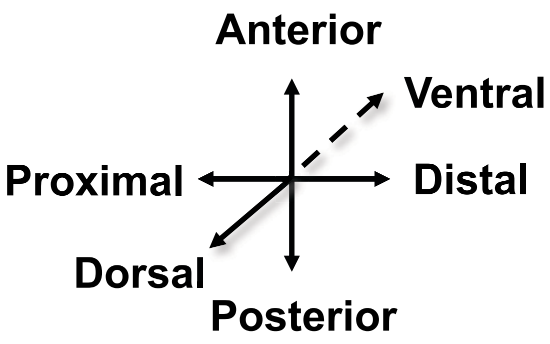
Determination: When cell fate is fixed so that the cell will initiate differentiation into the specified cell type even if the cell is isolated or transplanted into a different environment or tissue; occurs before the appearance of cell-type-specific morphological characteristics, but is often followed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

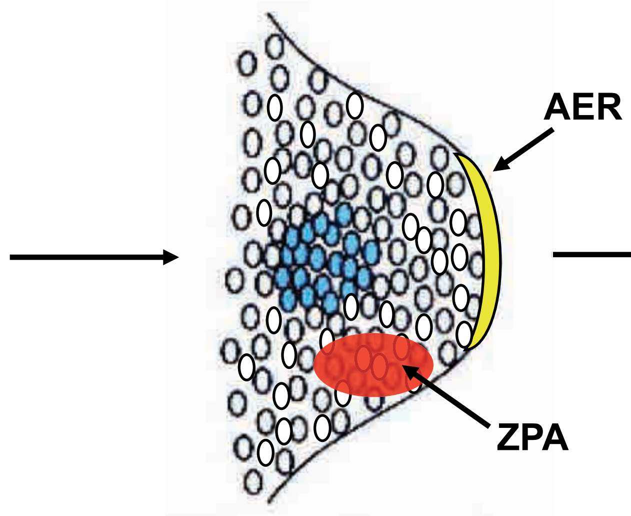
Zeller, *et. al.* (2009) *Nat Rev Gen.* 10: 845-858.
Niswander (2003) *Nat Rev Gen.* 4: 131-142.

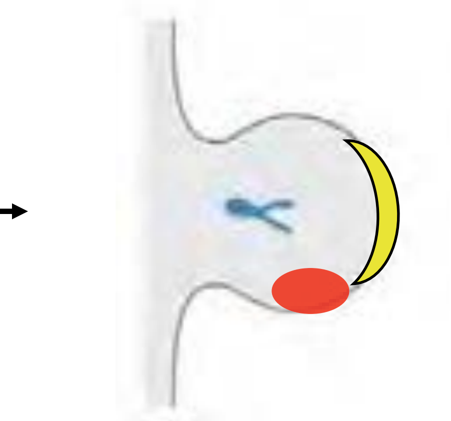
Limb bud Development, Patterning, and Chondrogenesis









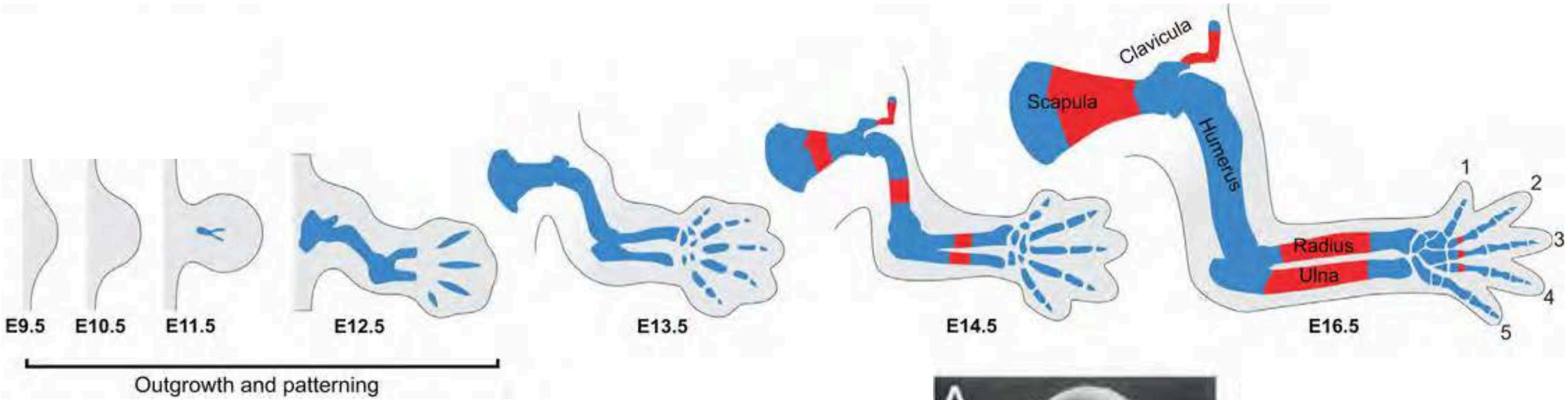


Mouse: E9.5-E10.0 — E10.5-E11.5 — E11.5-E12.5

Human: 4-5 weeks

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

Mouse Limb Patterning and Development

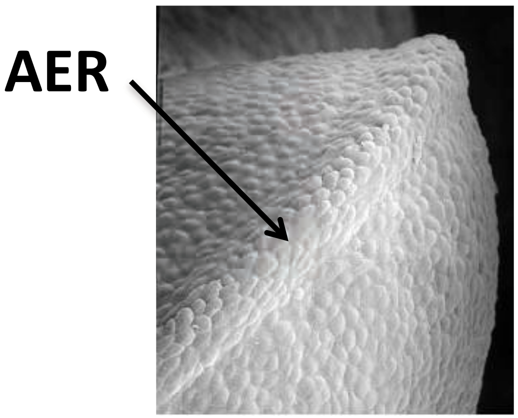


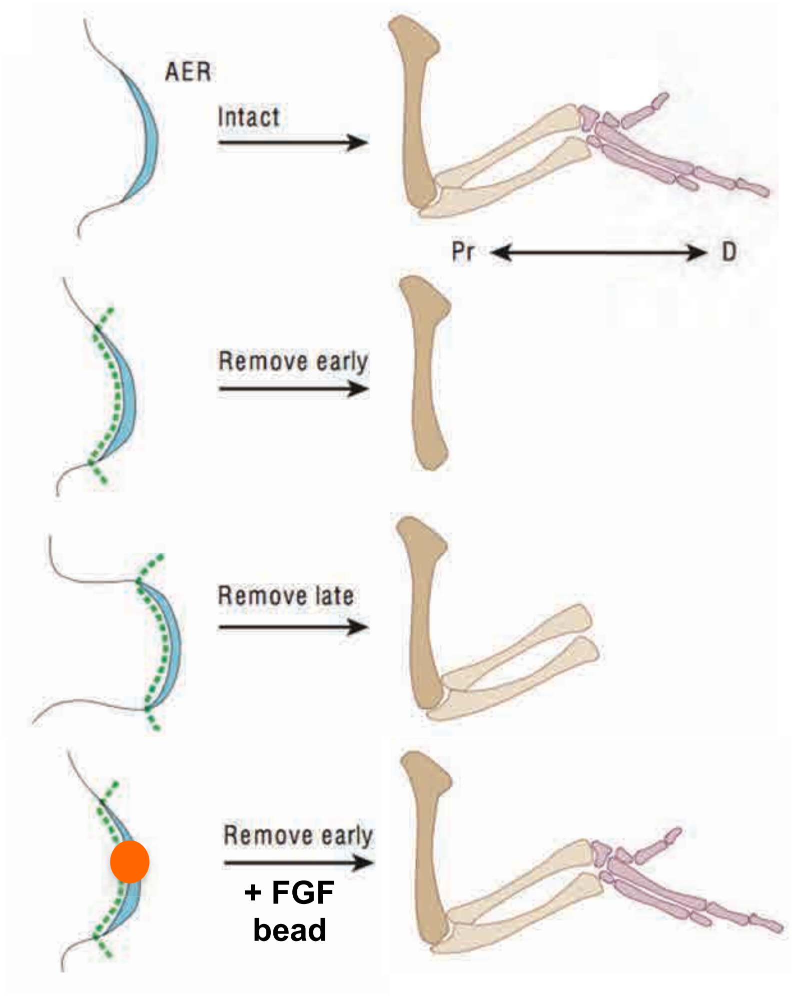
- ***Hindlimb development is delayed by 0.5 days***

Embryo E11.0

Adapted from Zuniga (2015) *Development.* 142: 3810-20.

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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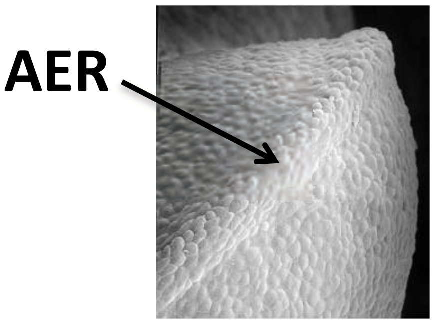
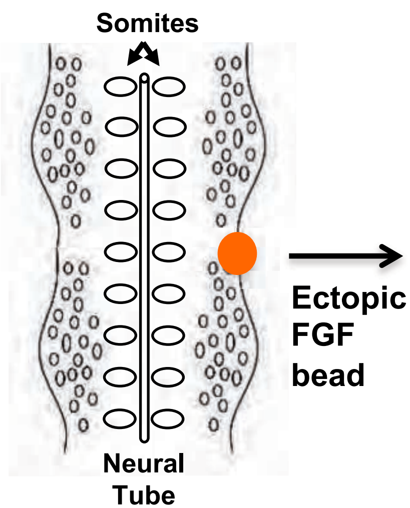
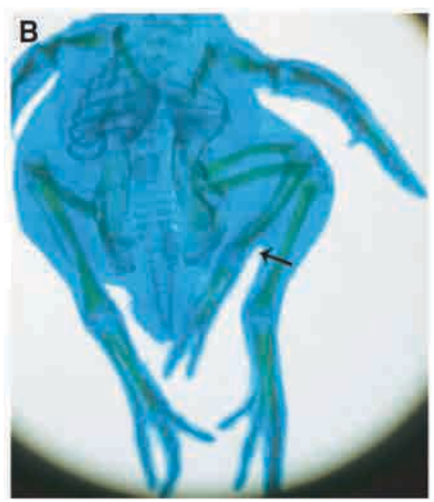
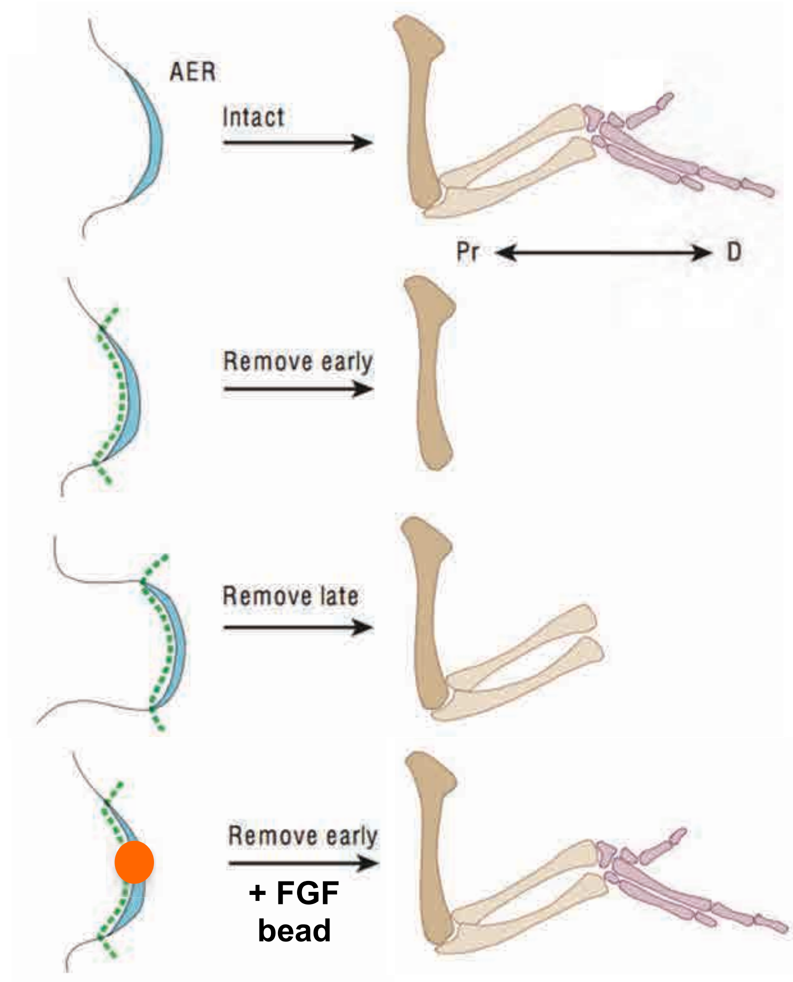




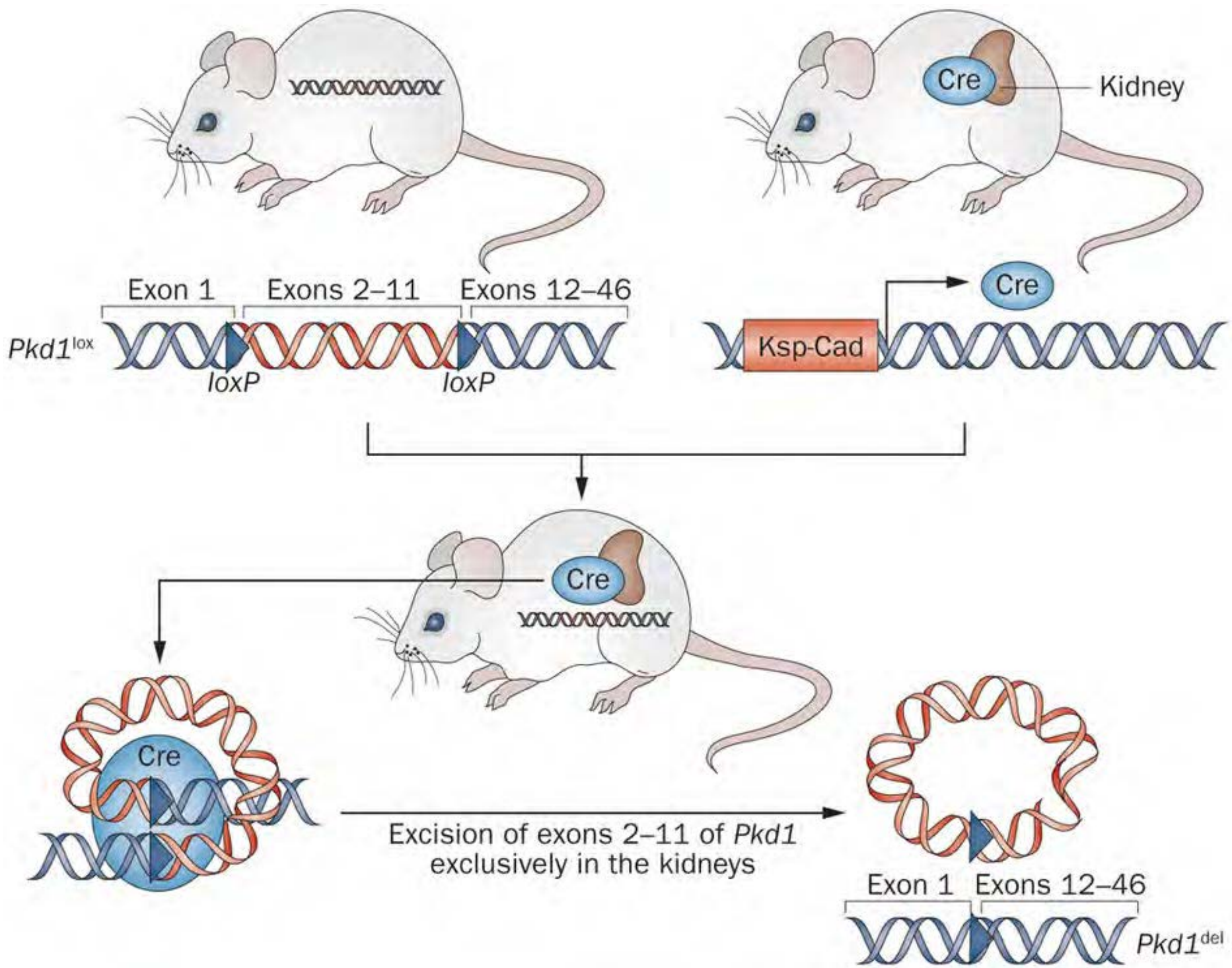


Adapted from Tickle (2015) *J. Anat.* 227: 418-430.



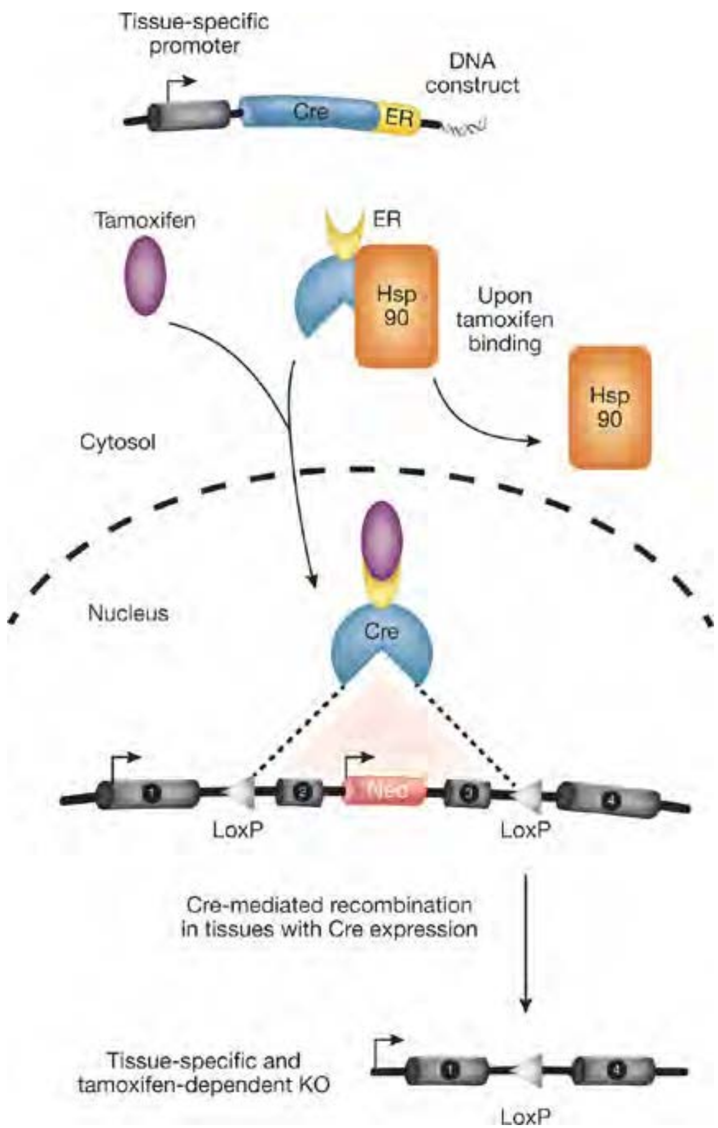
Adapted from Mariani and Martin (2003) *Nature* 423: 319-325.

Overview of the Cre-*loxP* Recombination System



Happé, H. and Peters, D. J. M. (2014) *Nat. Rev. Nephrol.* doi:10.1038/nrneph.2014.137

The Tamoxifen-inducible Cre-*loxP* System



Gunschmann, C. (2014) *J Invest Dermatol*. doi:10.1038/jid.2014.213

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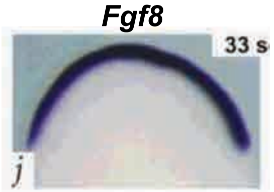
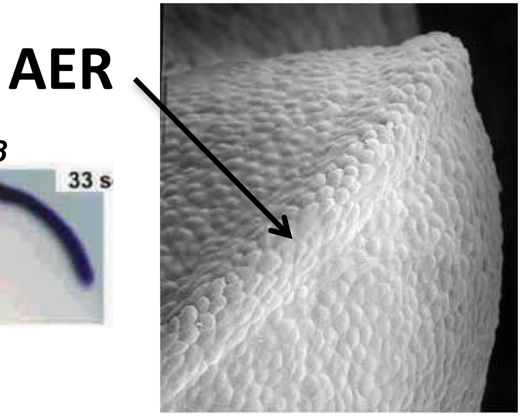
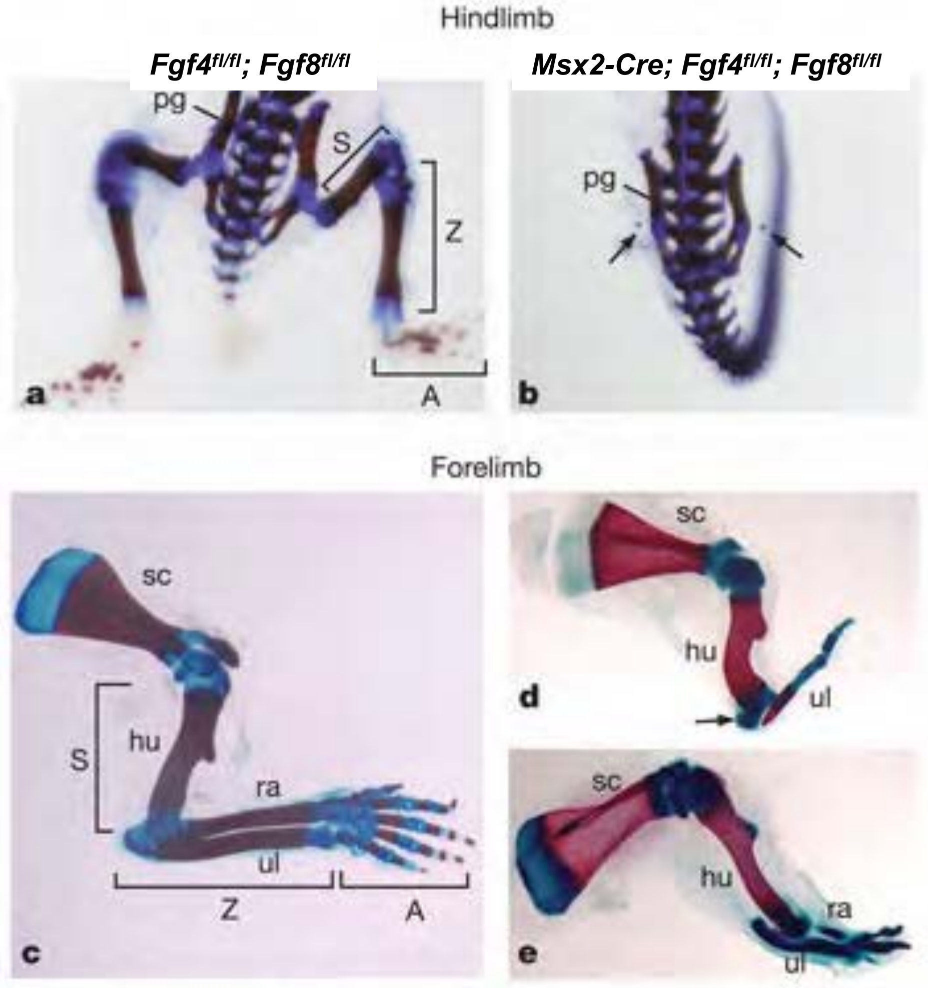


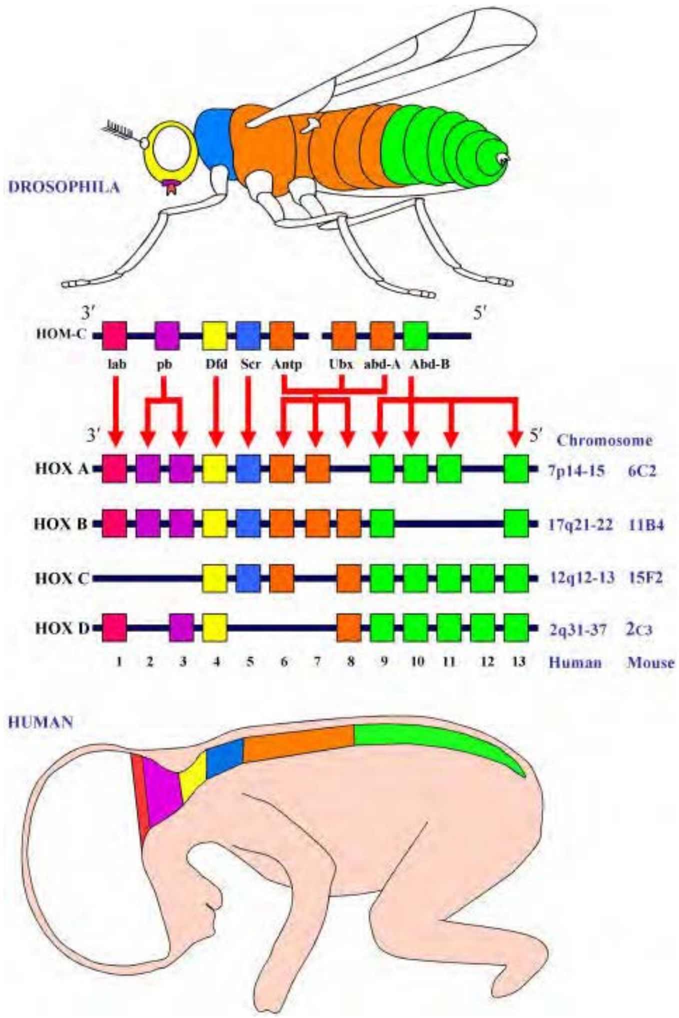


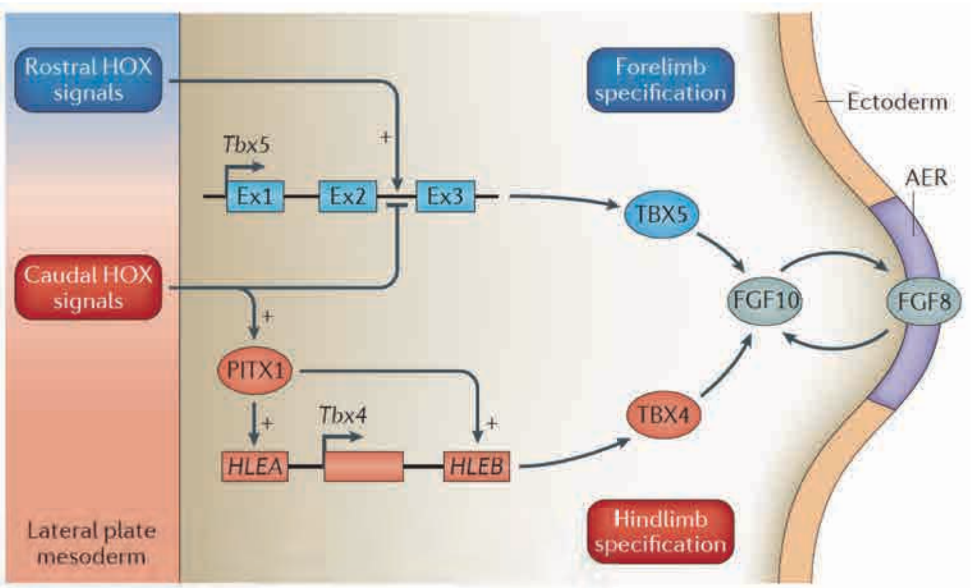


- FGF4, 8, 9, and 17 are all expressed in the AER.
- Only *Fgf8* is essential for normal limb bud development.
- Deletion of both *Fgf4* and *Fgf8* from the AER completely disrupts limb bud development.

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.

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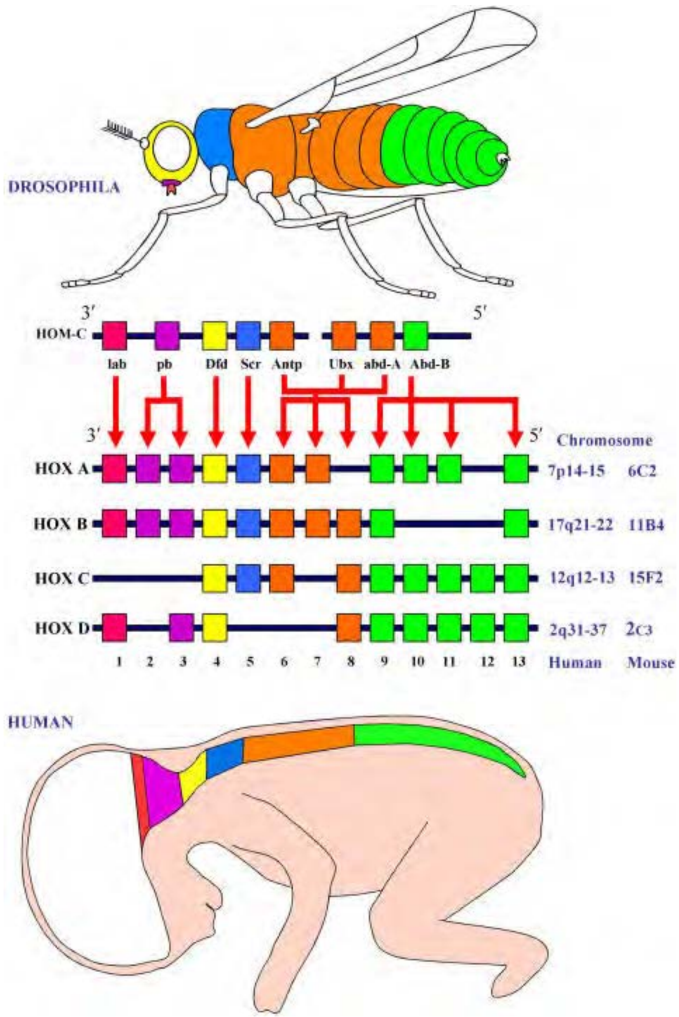
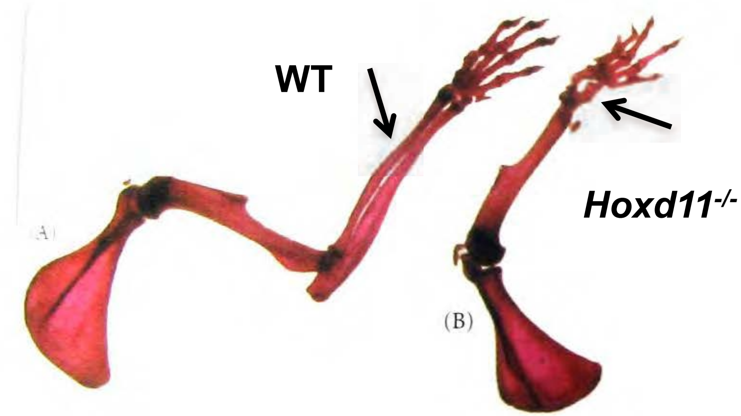
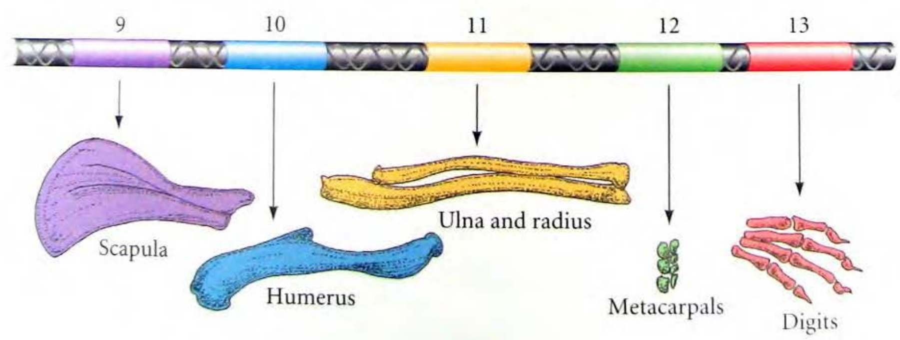


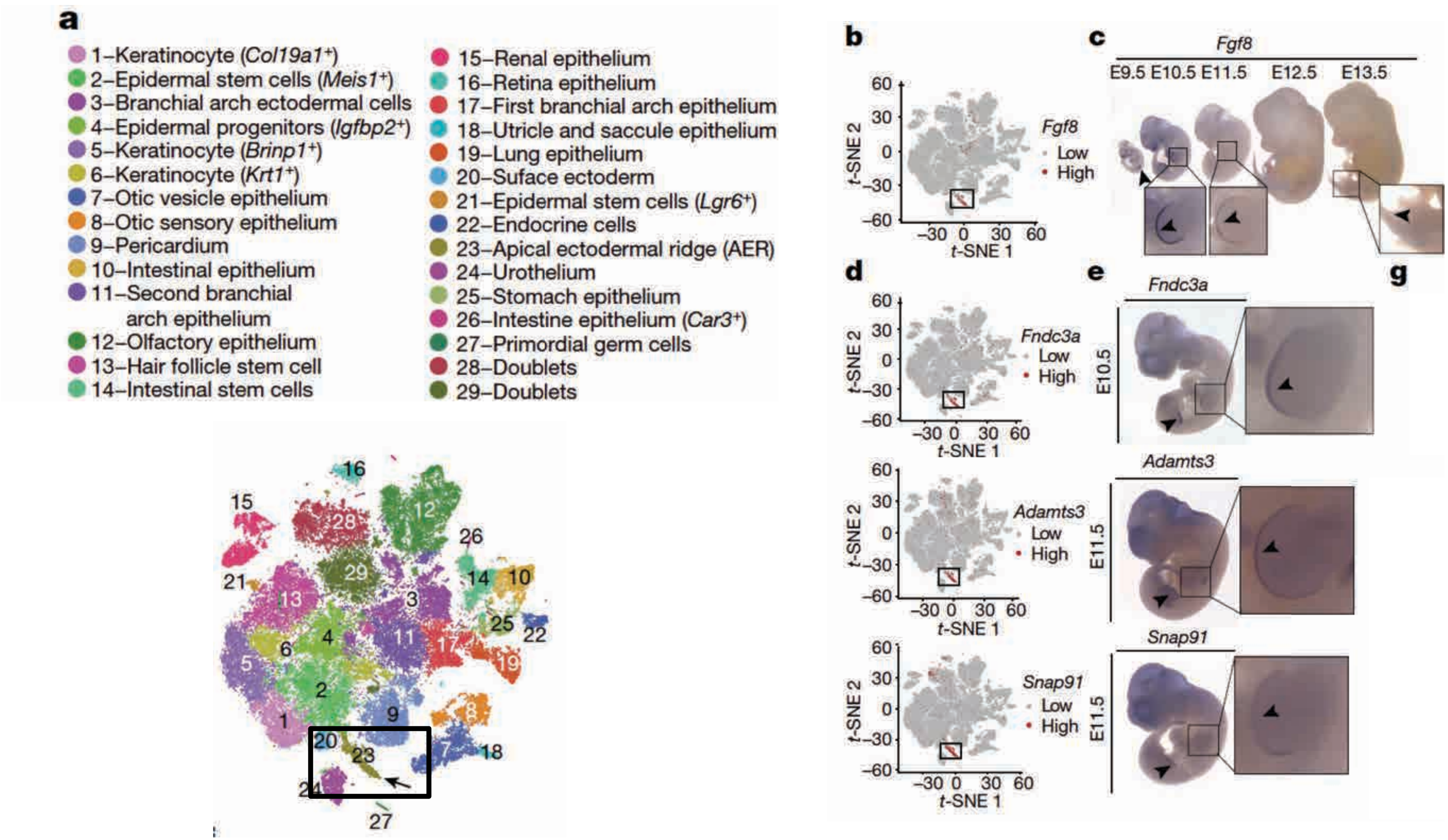




Lappin, *et. al.* (2006) *Ulster Med* 75: 23-31.
Davis, *et. al.* (1995) *Nature* 375: 791-795.

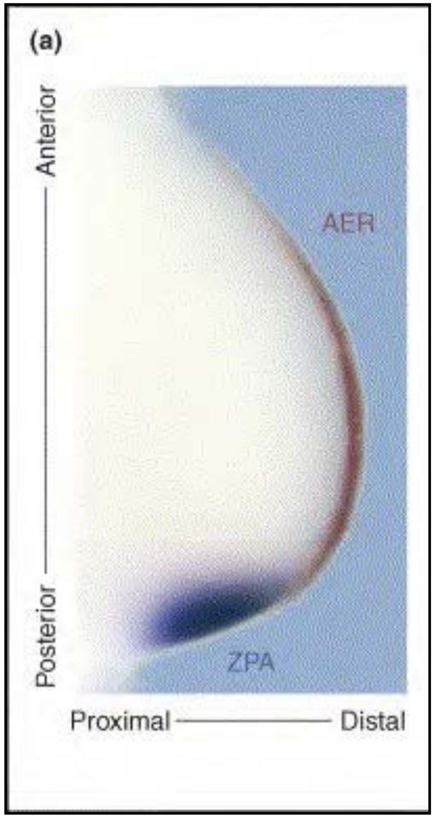
Identification of Novel AER-associated Genes using scRNA-seq

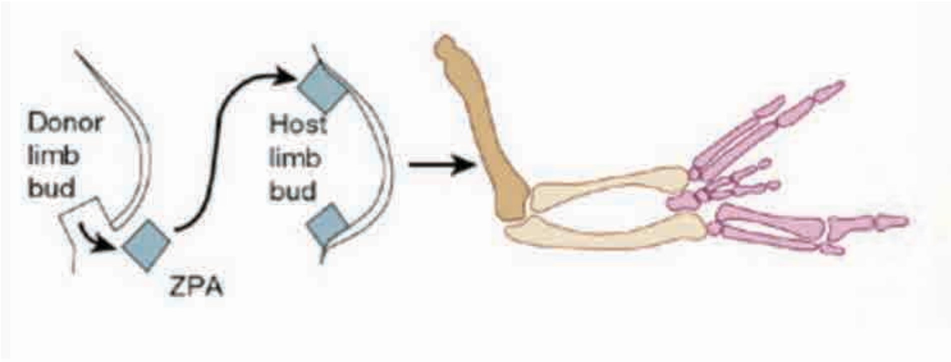
"The single-cell transcriptional landscape of mammalian organogenesis"



Cao, *et. al.* (2019) *Nature* 566: 496-502.

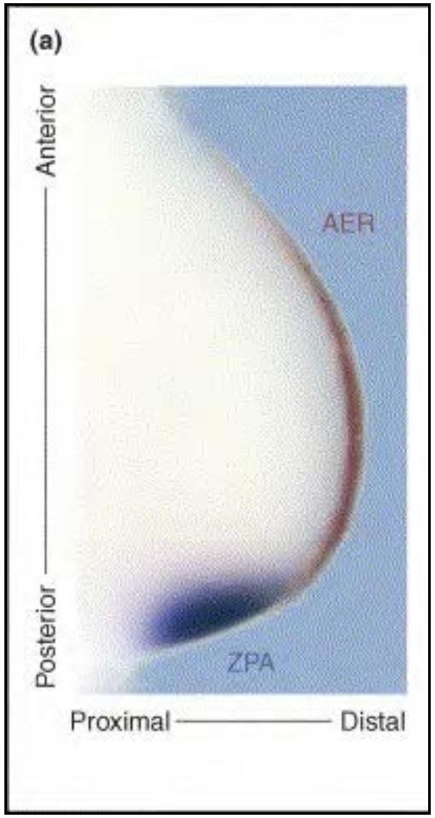
ZPA Regulation of Anterior to Posterior Patterning

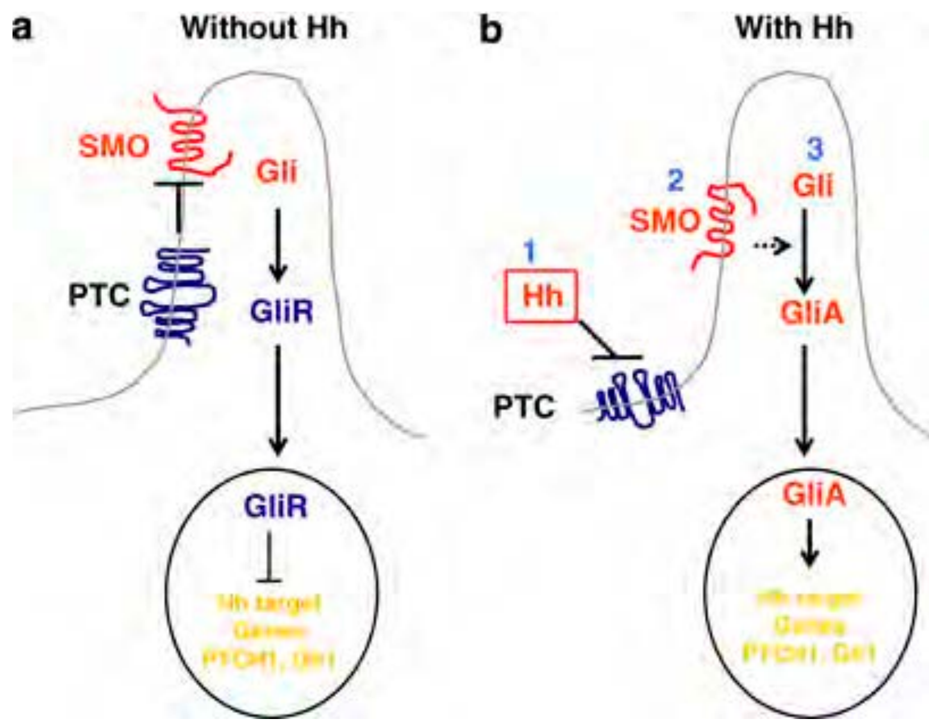




Adapted from Mariani and Martin (2003) *Nature* 423: 319-325.

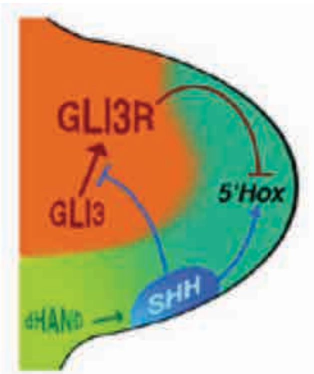
ZPA/SHH Regulation of Anterior to Posterior Patterning

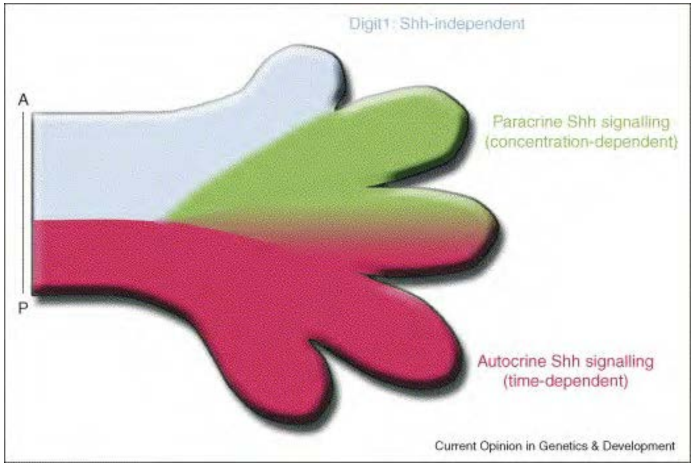


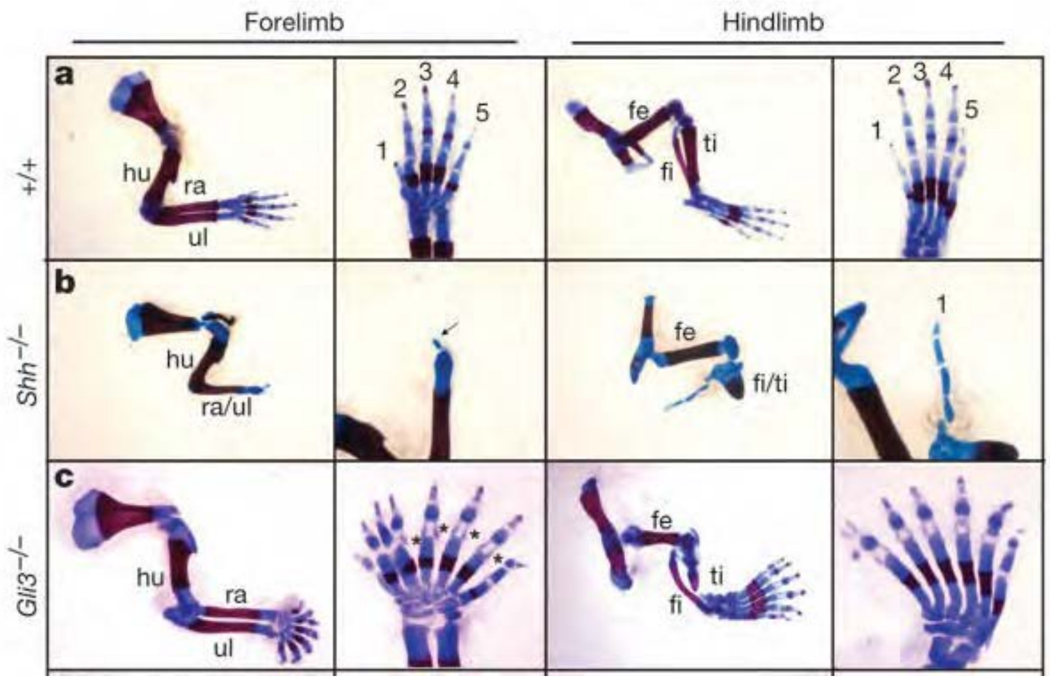


Yang, *et. al.* (2010) *Oncogene* 29: 469-481.

ZPA (Shh) Regulation of Anterior to Posterior Patterning

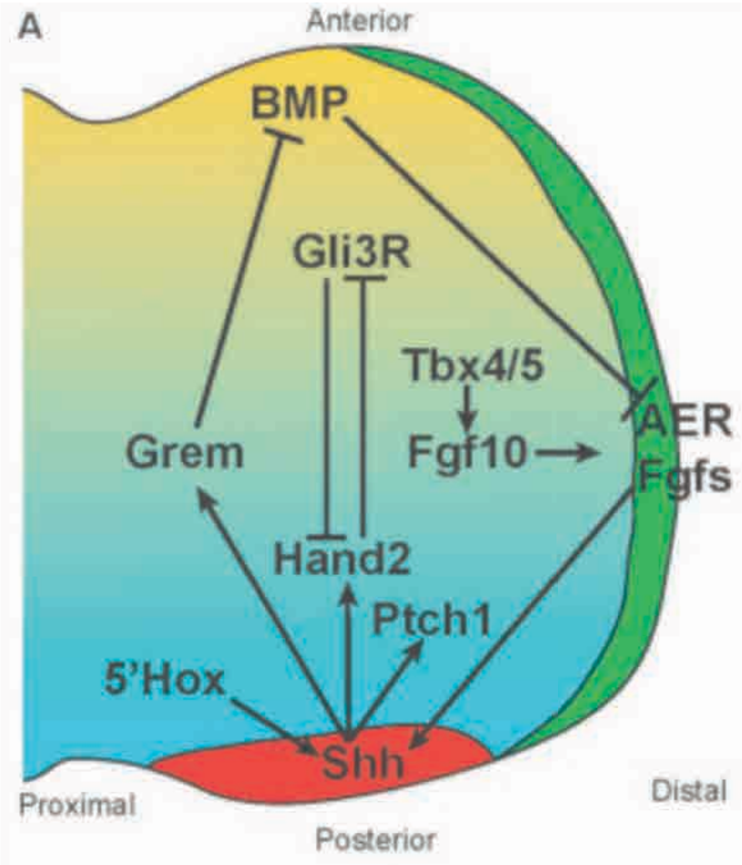


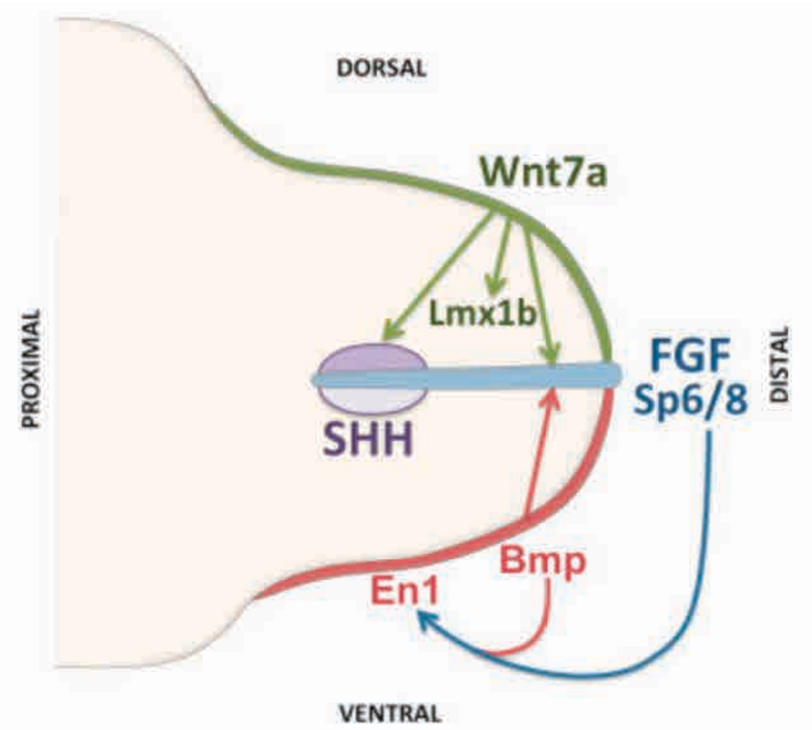




Litingtung *et. al.* (2002) *Nature* 418: 979-983.

Coordinated Development of the Vertebrate Limb Across Axes





Young & Tabin (2017) *Dev Biol.* 429: 401-408.
Delgado & Torres (2017) *Dev Biol.* 429: 382-386.

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)

- **Dwarfing Chondrodysplasias**
  - **Hypochondroplasia**
  - **Achondroplasia**
  - **Thanatophoric Dysplasia**

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

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

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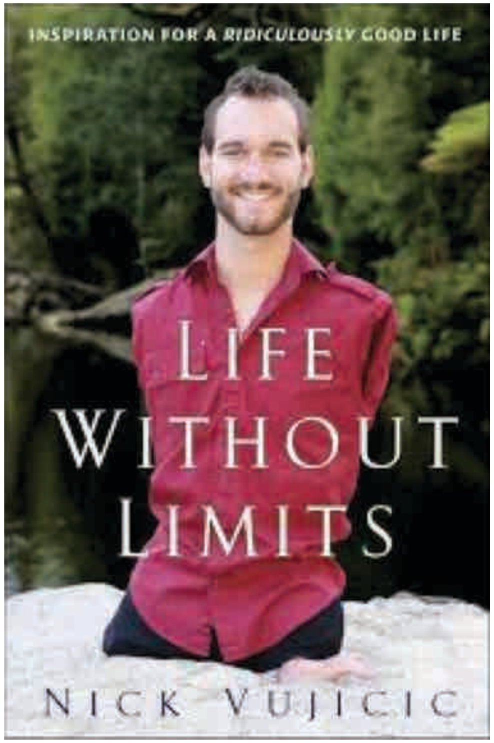
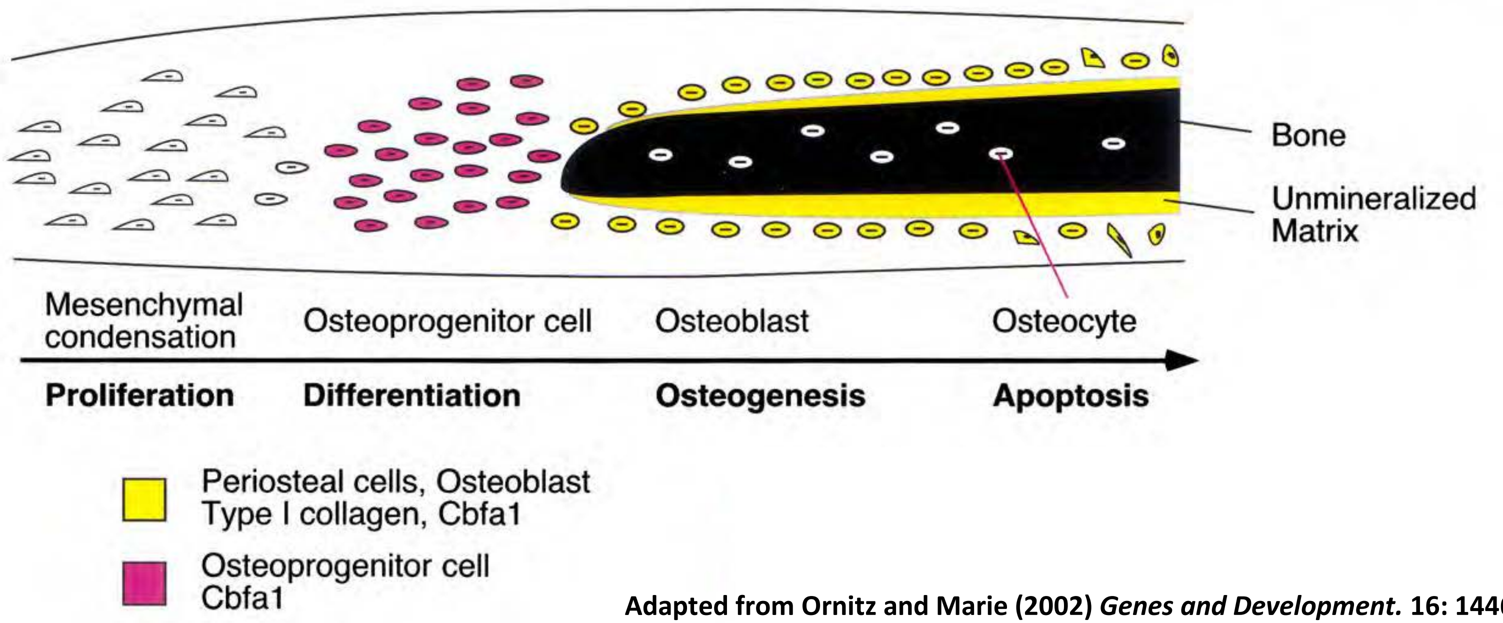
Tetra-amelia (mutations in WNT3)



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

Intramembranous *versus* Endochondral Ossification

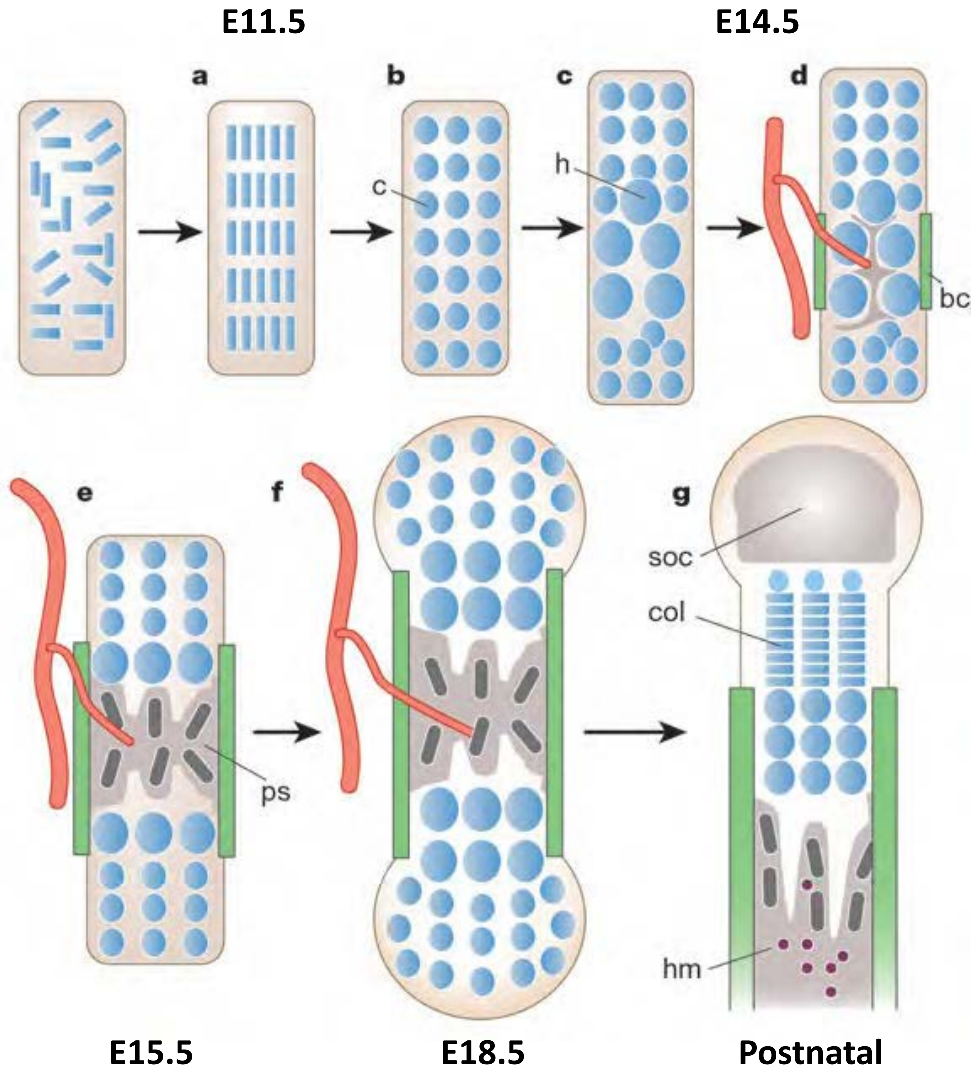
- **Intramembranous:** bone formation directly from mesenchymal condensations; flat bones (skull, mandible, clavicles)



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.)

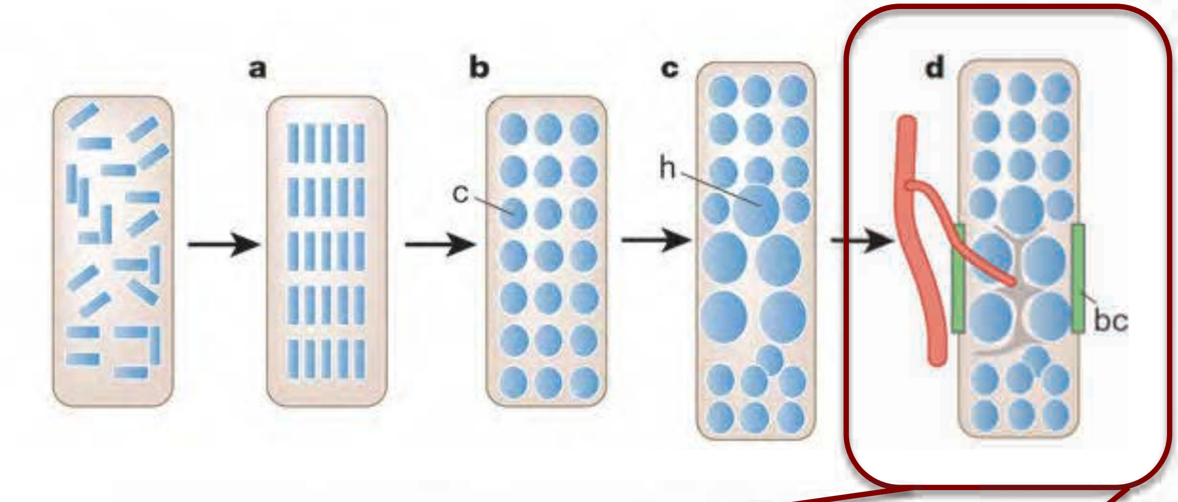
Endochondral Bone Formation

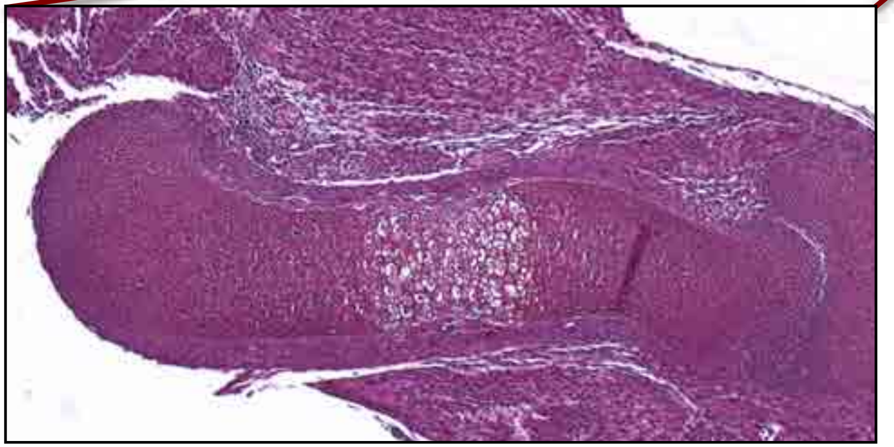


a. Mesenchymal condensations
b. Chondrogenesis and chondrocyte proliferation
c. Onset of hypertrophy
d. Terminal chondrocyte differentiation
e. Primary ossification center formation
f-g. Establishment of epiphyseal growth plates and secondary ossification centers

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

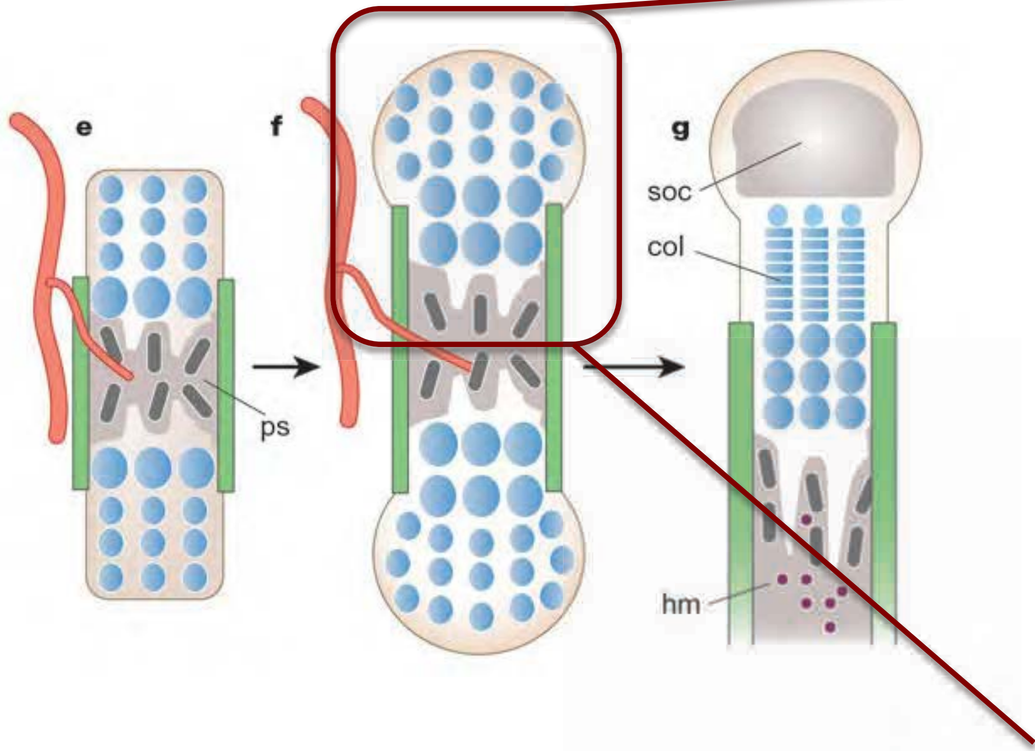
Chondrocyte Maturation – Onset of Hypertrophy



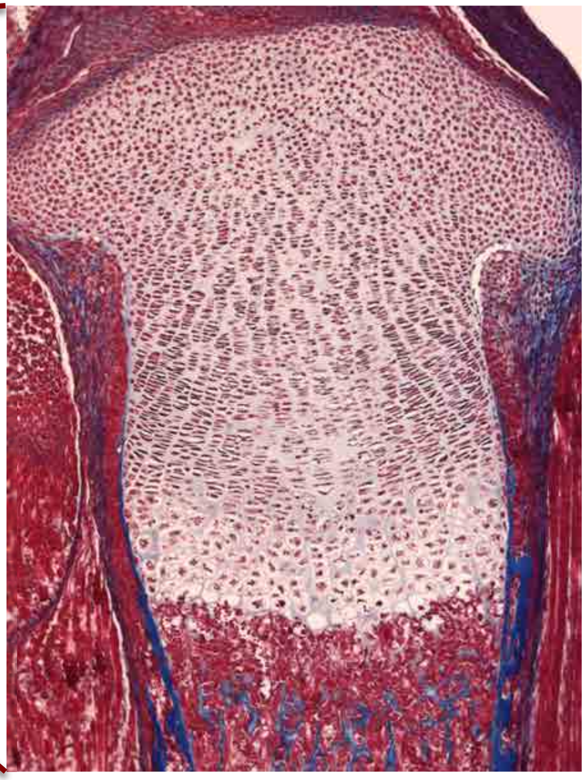


Mouse E14.5 Humerus

Chondrocyte Maturation – Terminal Hypertrophy



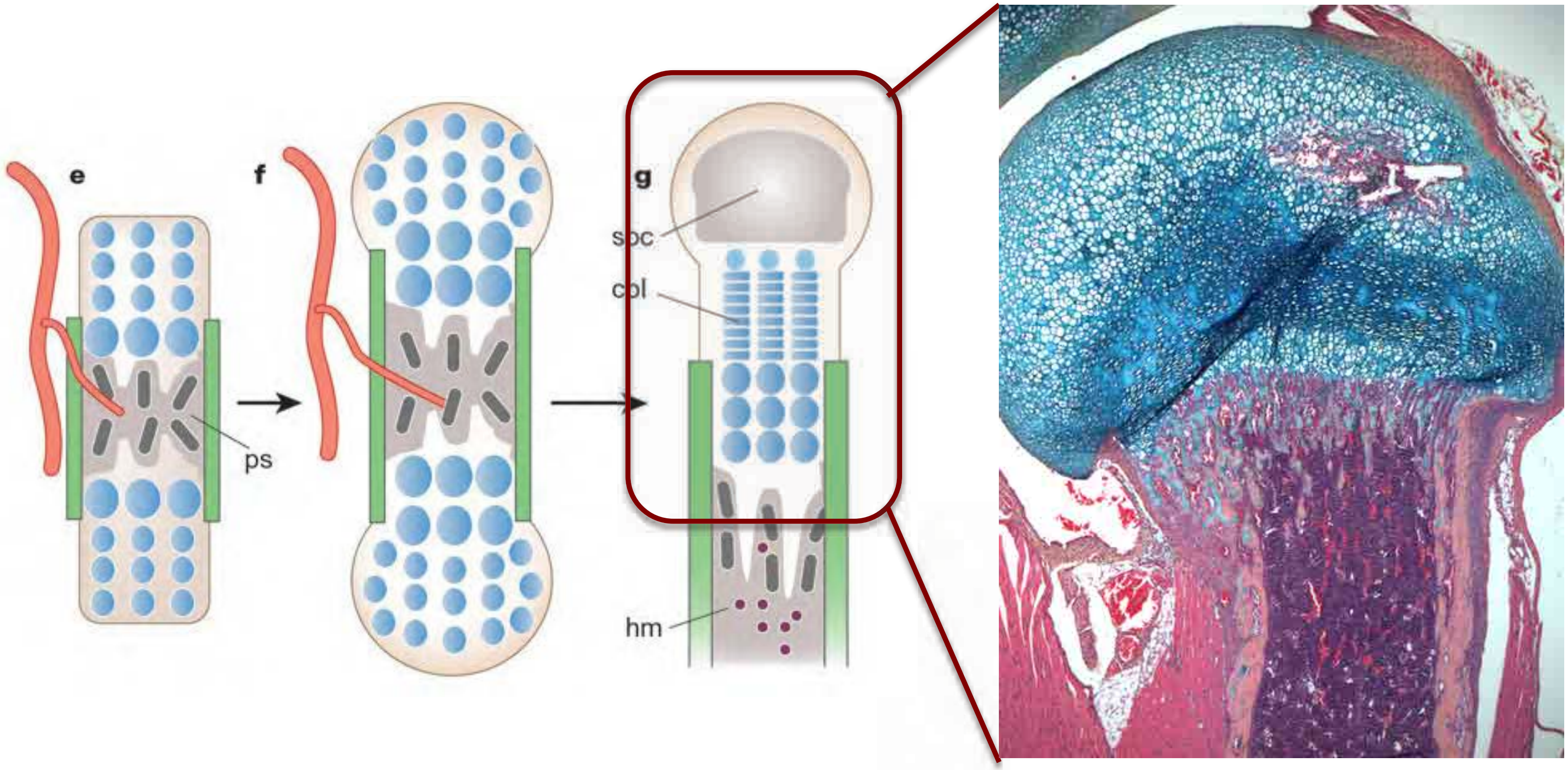
Mouse E18.5 Proximal Tibia



Endochondral Bone Formation

Establishment of Growth Plate and Articular Cartilages

Mouse P7 Proximal Humerus



Endochondral Bone Formation

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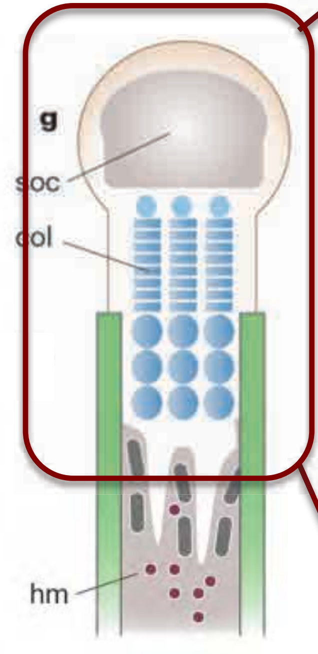
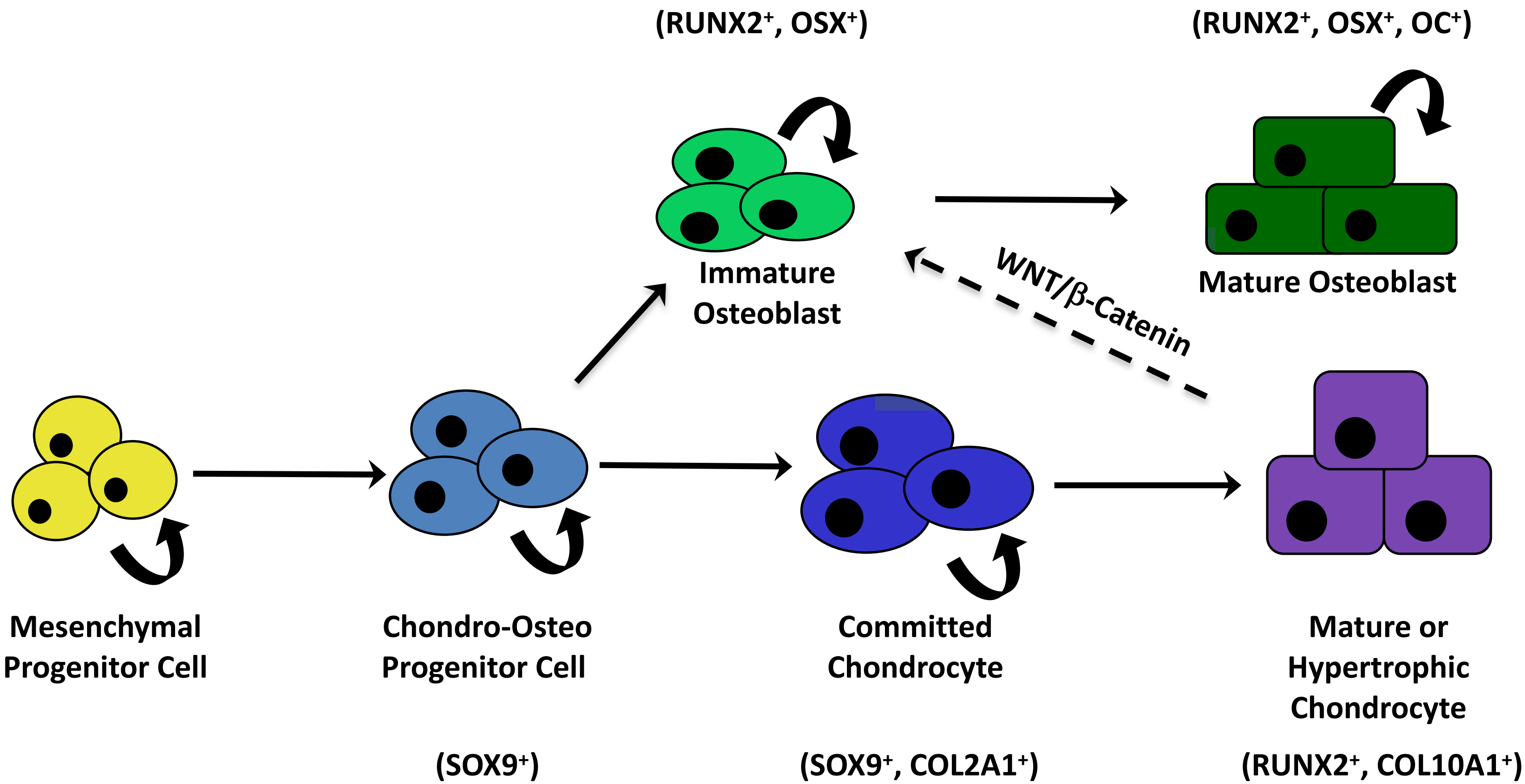


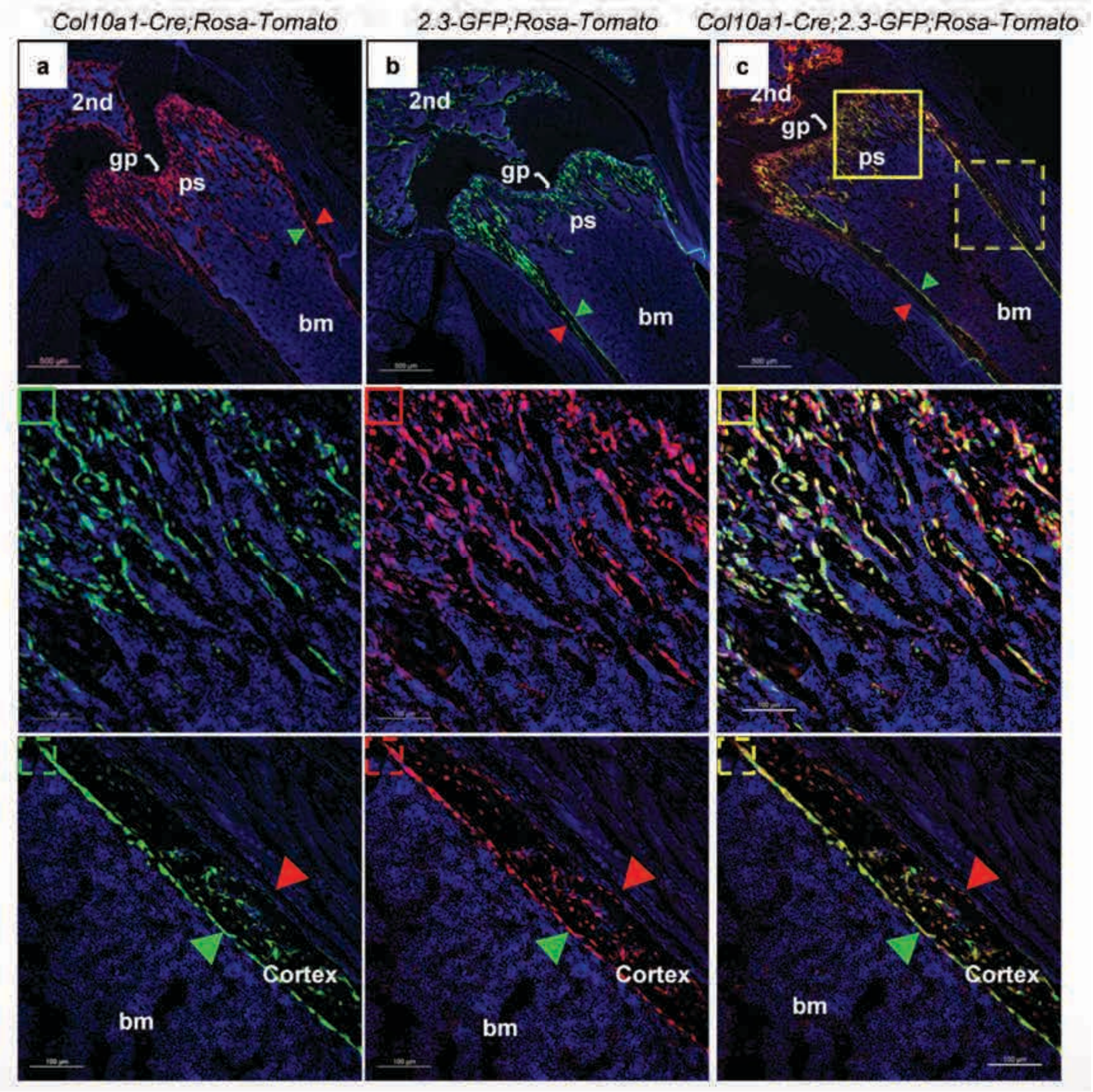
Mouse P7 Proximal Humerus

Mouse P28 Proximal Humerus

Chondrogenesis and Osteoblastogenesis

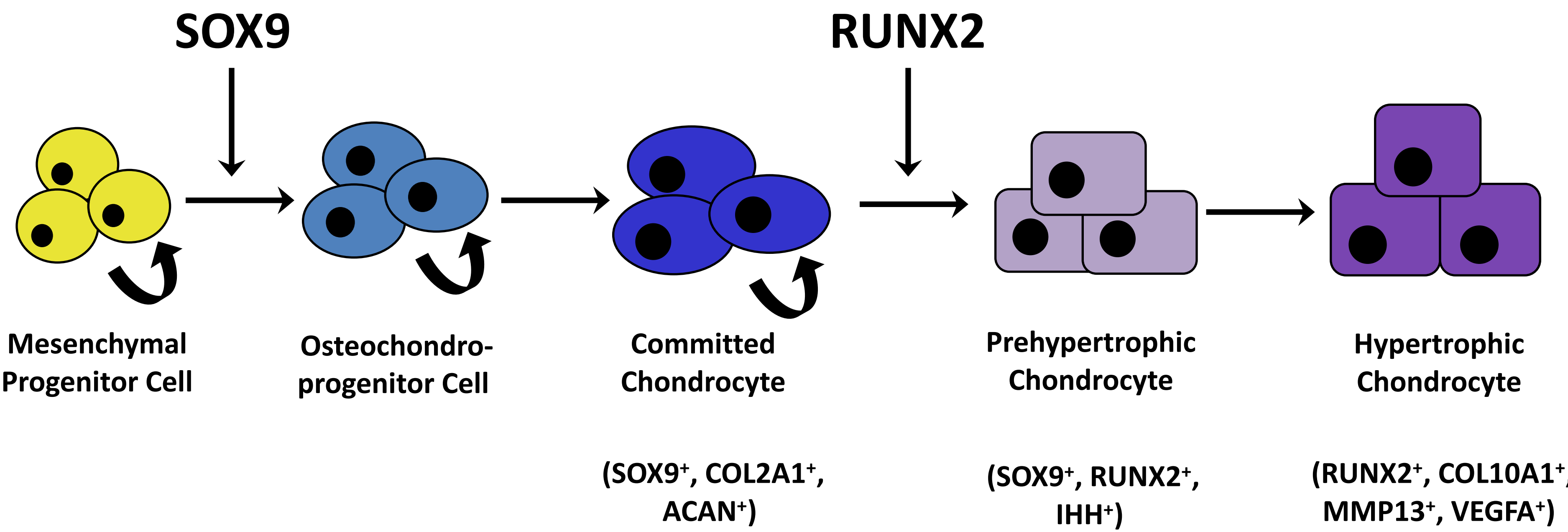


Transdifferentiation of Hypertrophic Chondrocytes to Osteoblasts During Endochondral Bone Formation



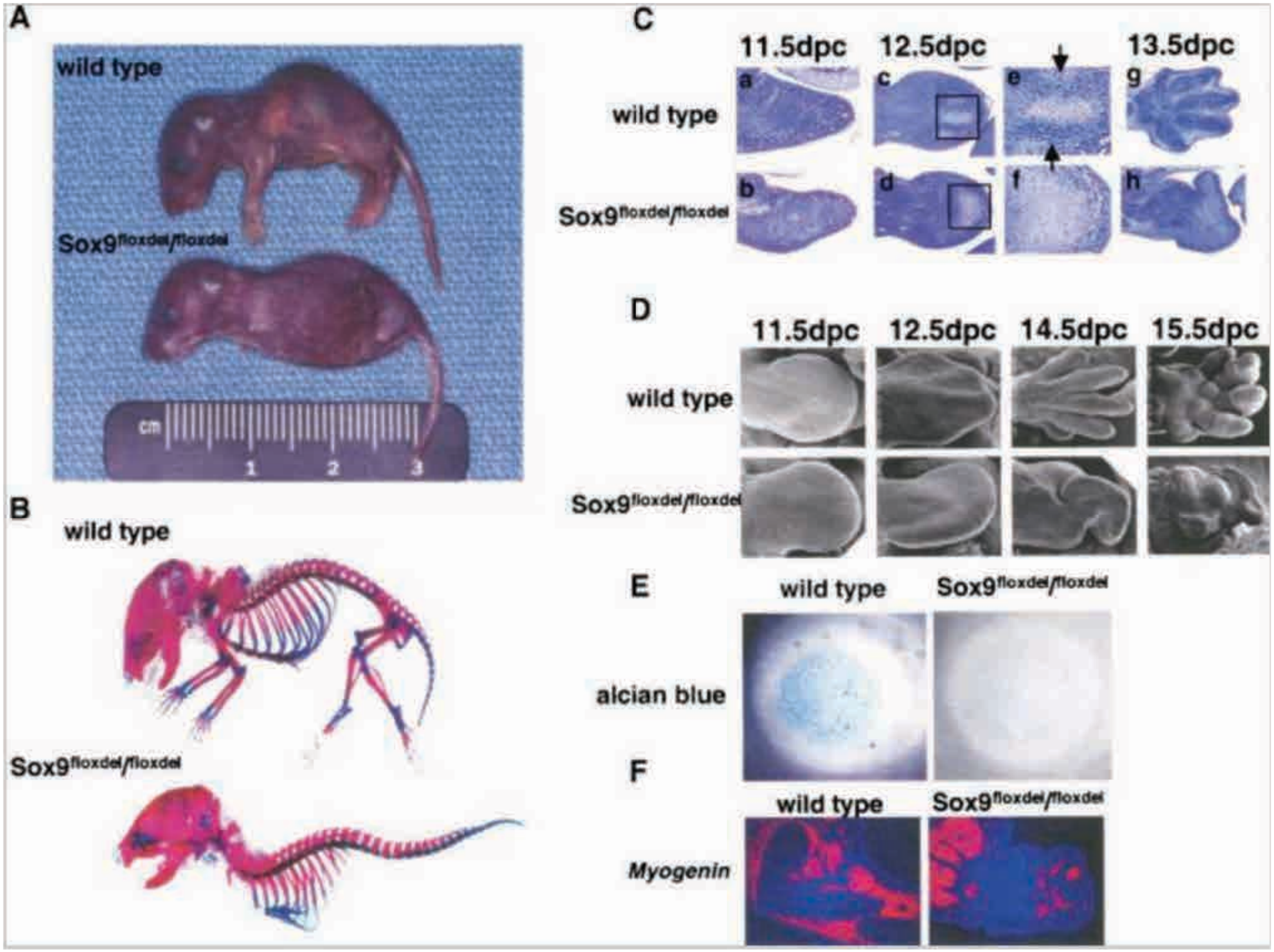
Zhou, *et. al.* (2014) *Plos Gen.* 12: e1004820.

Molecular Regulation of Chondrocyte Maturation



SOX9 is Required for Chondrogenesis

Prx1-Cre (expressed in all MSCs of limb bud approx. E9.5)



Akiyama, *et. al.* (2002) *Genes Dev. 16*: 2813-2828.

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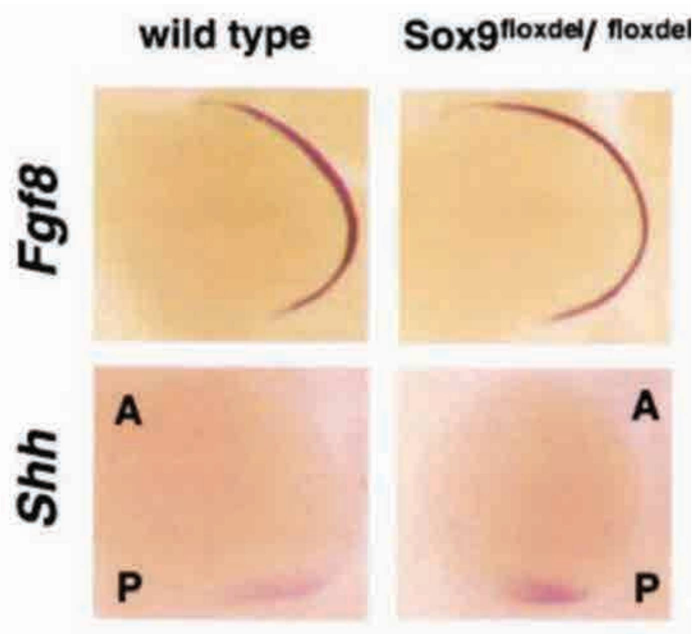
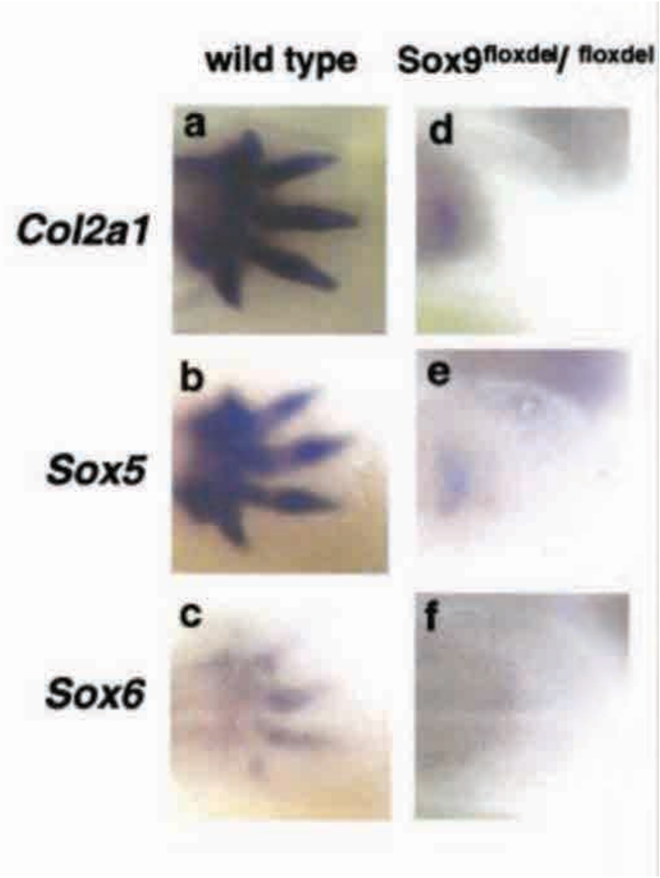
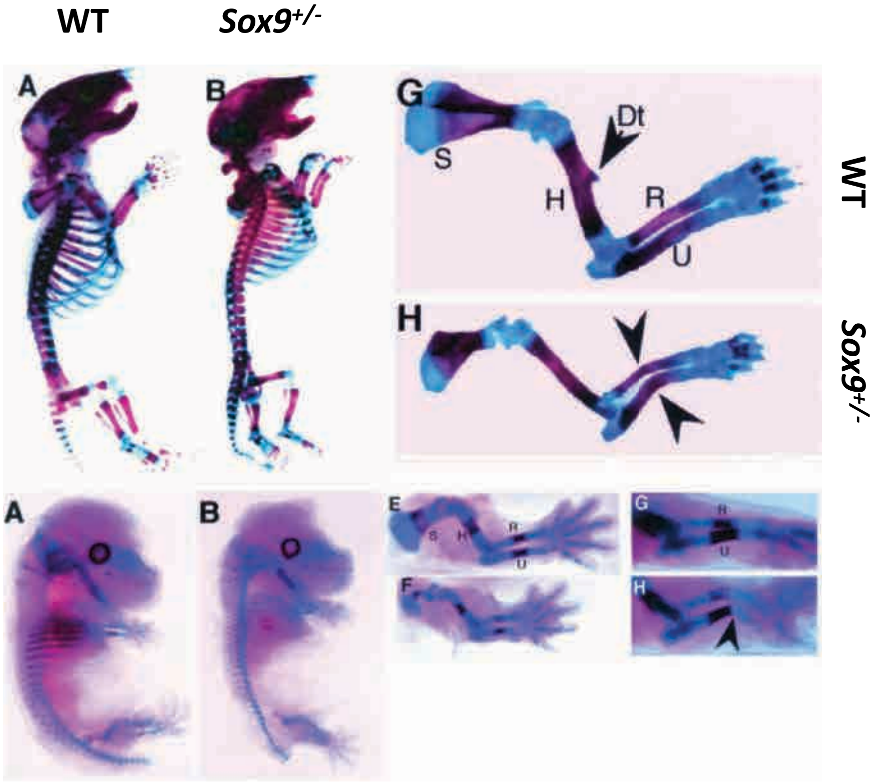




Akiyama, *et. al.* (2002) *Genes Dev. 16*: 2813-2828.

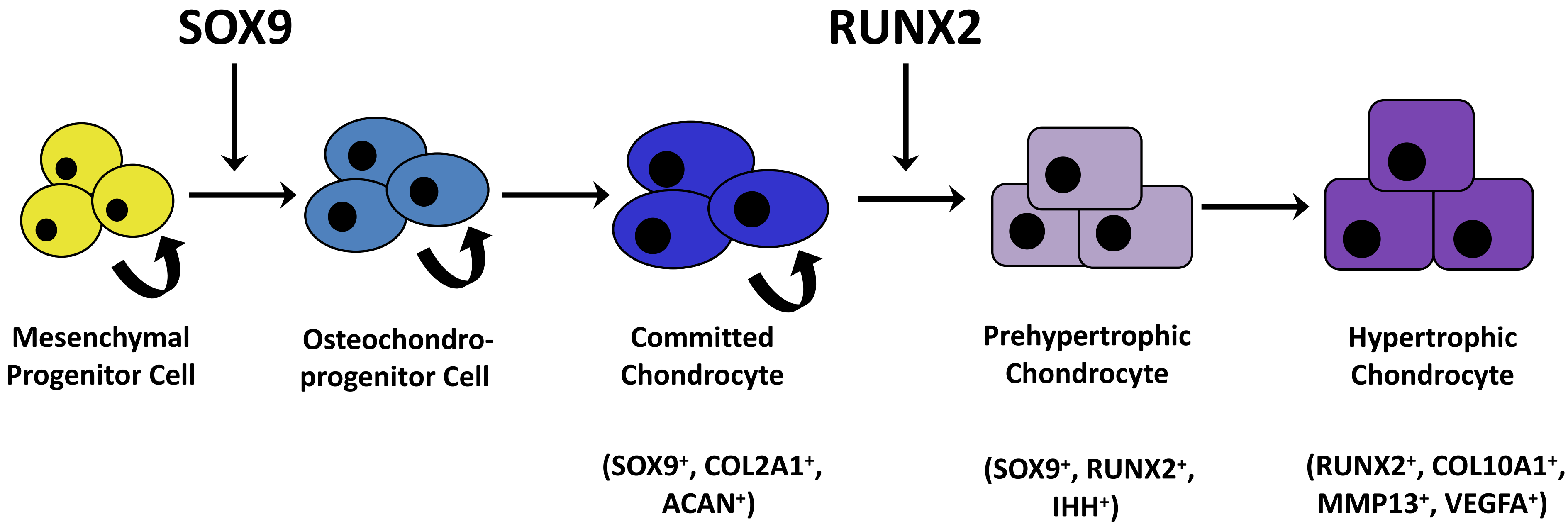
Campomelic Dysplasia

- Autosomal dominant disorder due to haploinsufficiency of *SOX9*
- Clinical features include; bowing of long bones, hypoplasia of the scapula and pelvis, decreased number of ribs, craniofacial abnormalities
- Most affected infants die due to respiratory distress



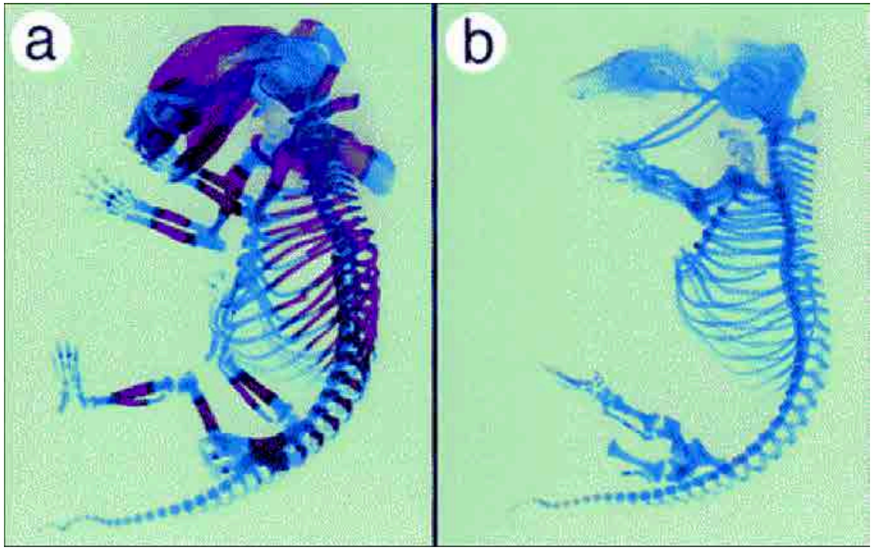
Bi, *et. al.* (2001) *PNAS.* 98: 6698-6703.

Molecular Regulation of Chondrocyte Maturation

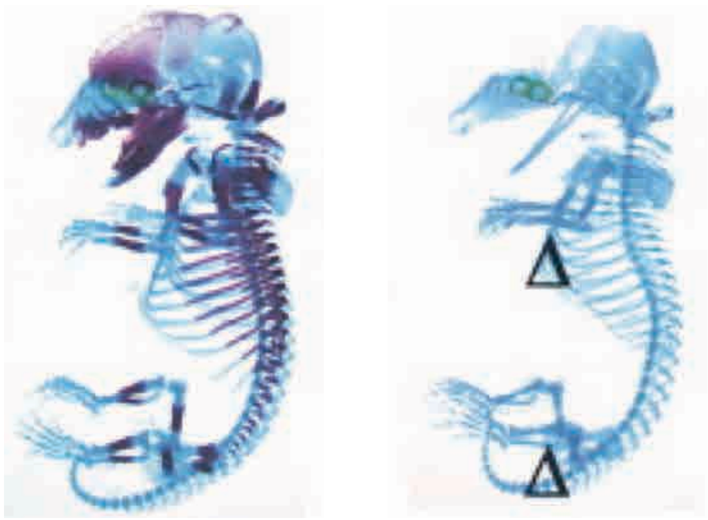


Critical Transcriptional Regulators of Chondrocyte Maturation and Bone Development (Runx2 and Osterix)

Runx2 mutant mice (null)	*Osterix* mutant mice (null)



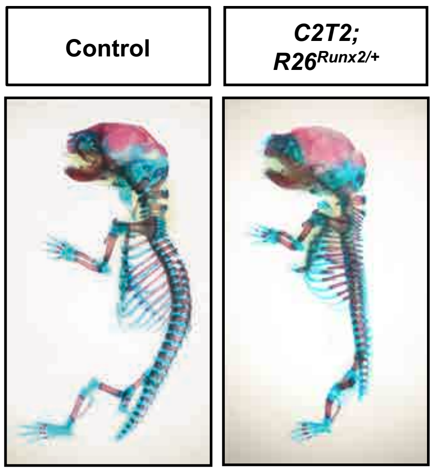
Otto F et al., (1997) Cell (89)5:765-771.

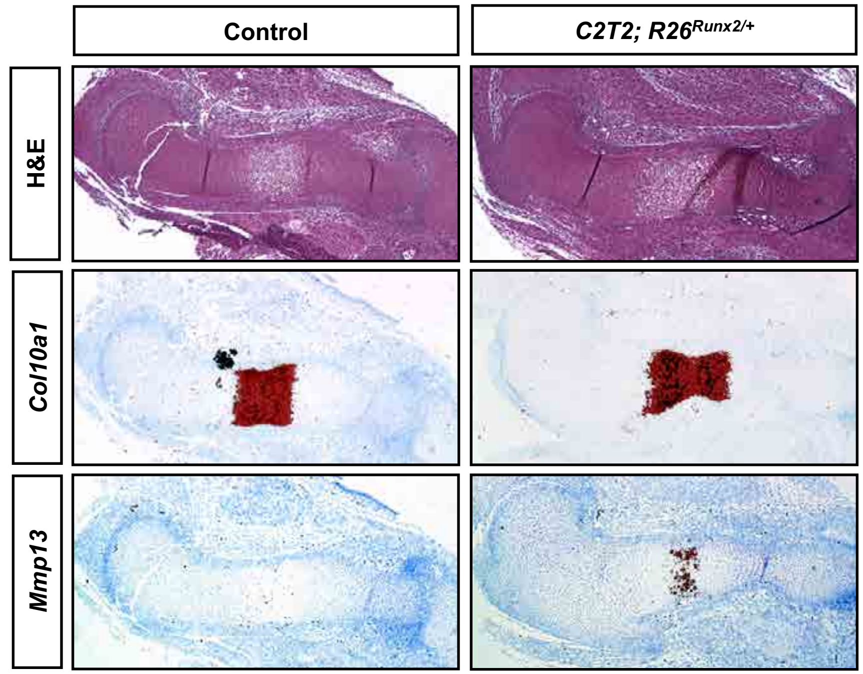


Nakashima K et al., (2002) Cell (108)1:17-29.

RUNX2 Overexpression Promotes Chondrocyte Maturation

Col2a1-CreER^{T2} (induced at E13.5 for target chondrocytes)



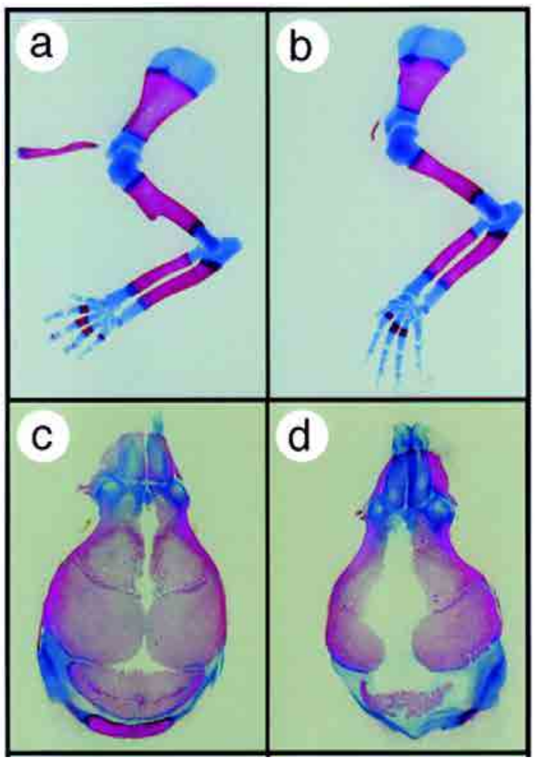


Catheline, *et. al.* (2019) *JBMR.* doi: 10.1002/jbmr.3737

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

WT **$Runx2^{+/-}$**



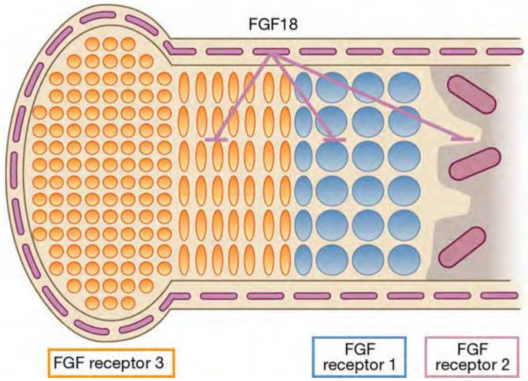
Otto, *et. al.* (1997) *Cell.* 89: 765-771.

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

Supernumerary teeth

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

Human Disorders of Skeletal Patterning, Differentiation, and Growth



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

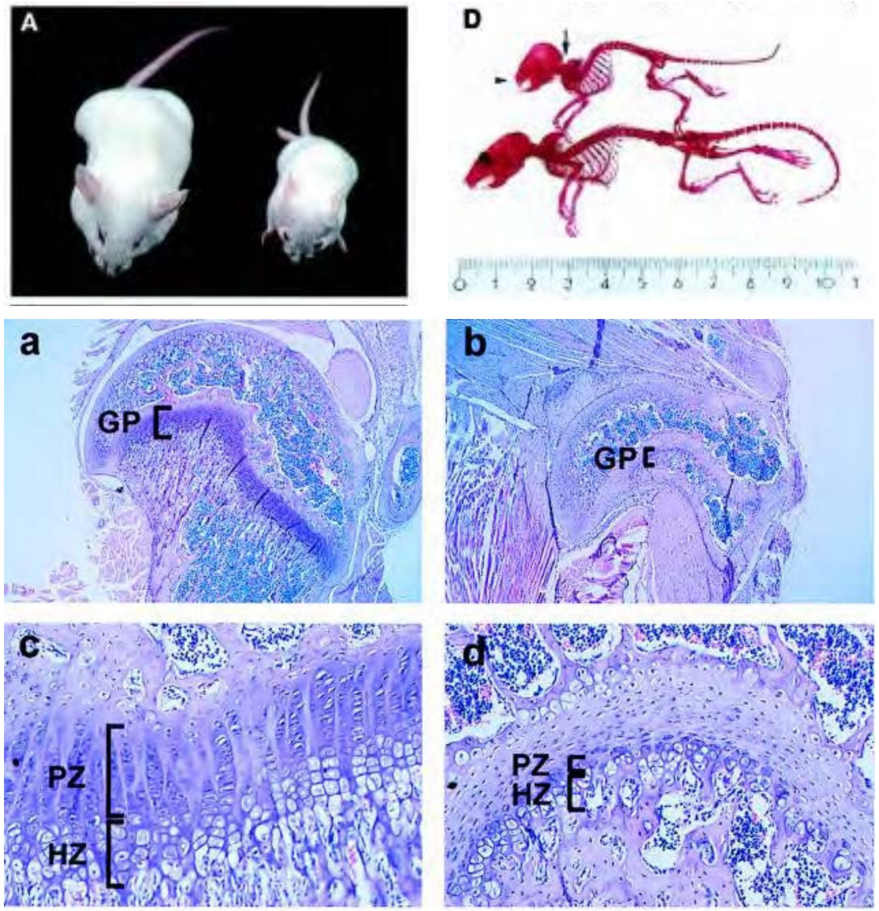
- **Brachydactyly** (shortened digits)
- **Campomelic Dysplasia** (*SOX9* haploinsufficiency)
- **Ellis-van Creveld Syndrome** (*EVC* mutations)
- **Cleidocranial Dysplasia** (*RUNX2* haploinsufficiency)

- **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)

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

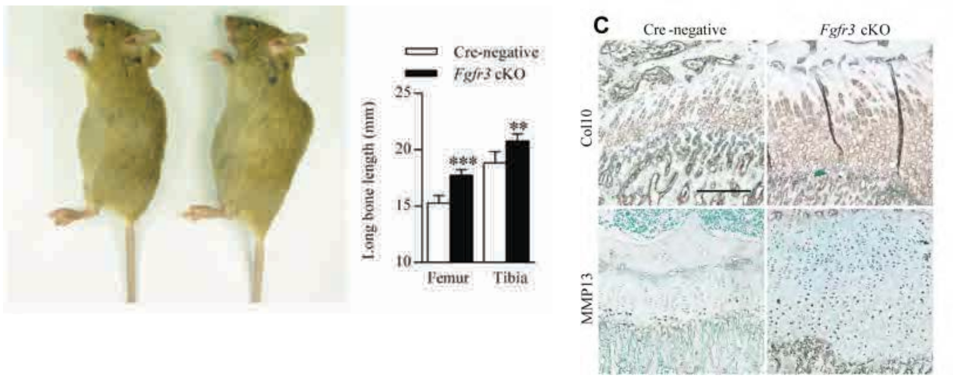
FGFR3 Mouse Models

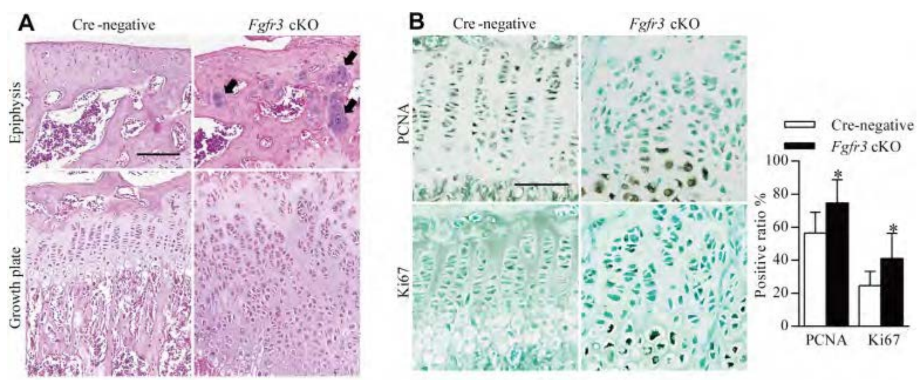
FGFR3 Activation (FGFR3$^{G374Rneo-/+}$)
Achondroplasia model



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

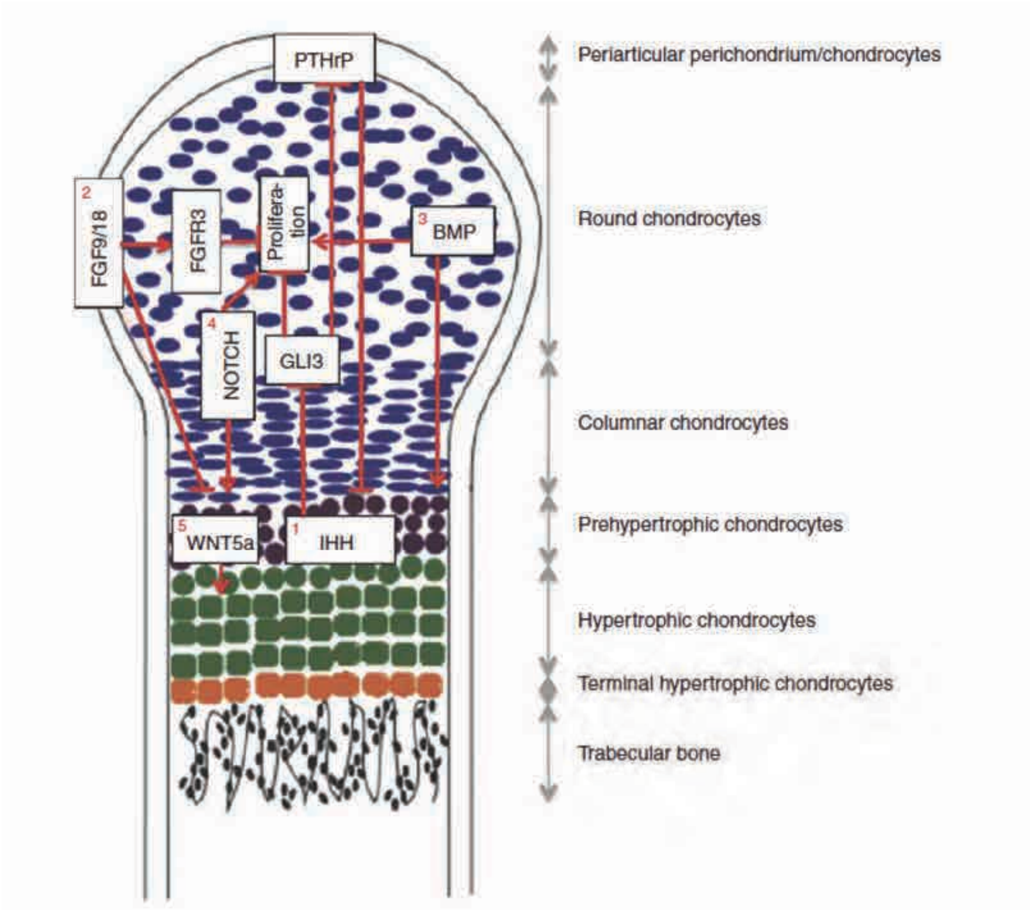
Chondrocyte-specific loss of *Fgfr3* leads to enhanced proliferation and maturation





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

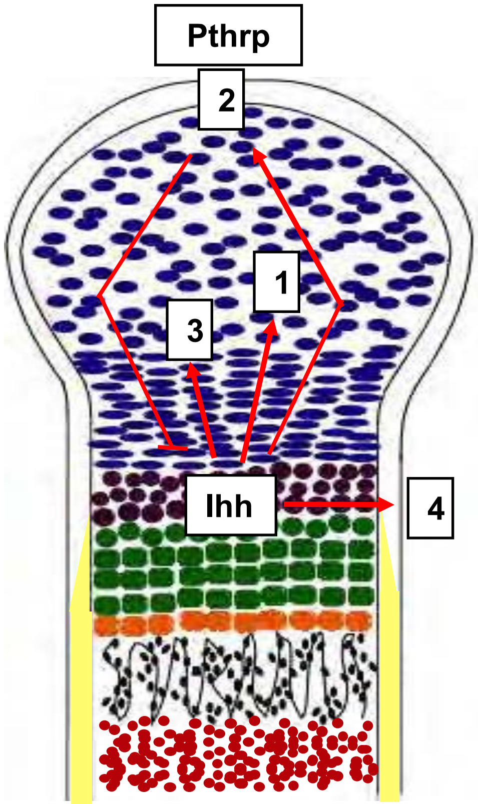
Signaling Pathways that Regulate Growth Plate Development

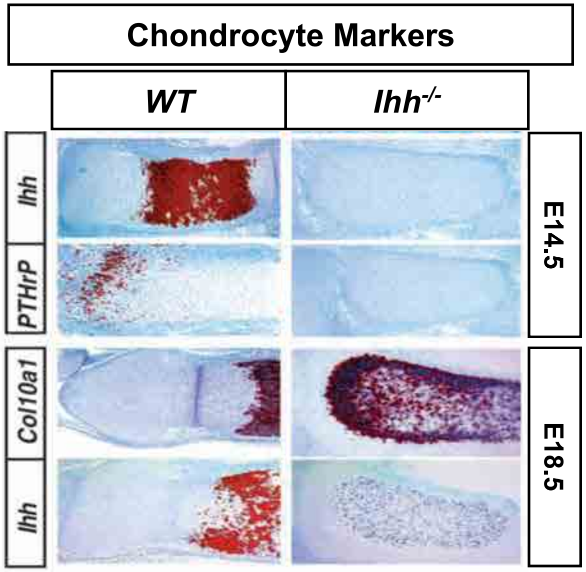


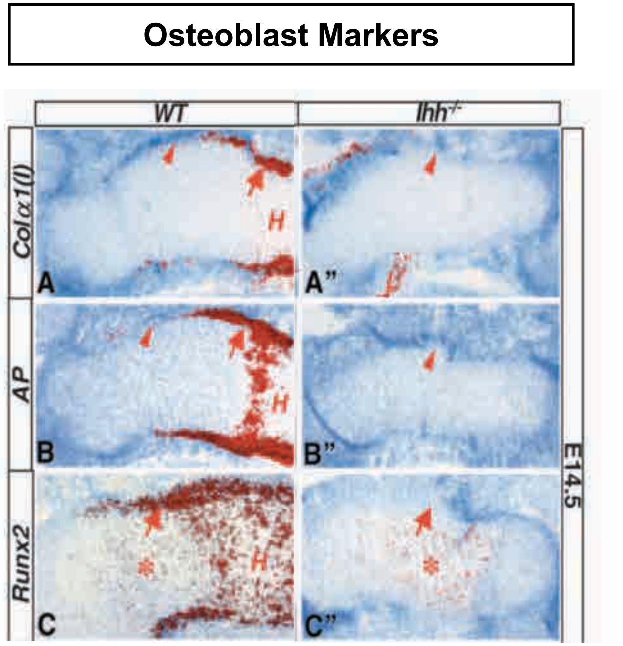
Long & Ornitz (2013) *CSH Perspect Biol.* 5:a008334.

Ihh/Pthrp Signaling During Cartilage and Bone Development

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





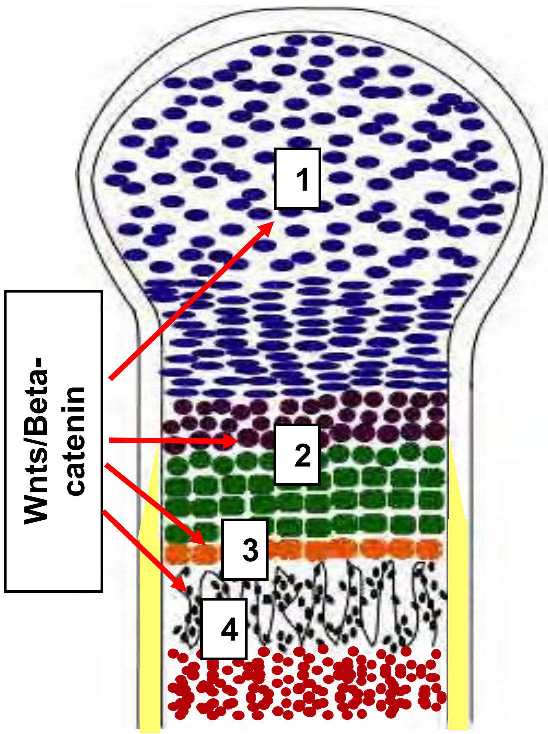


Long, *et al.* (2001) *Development.* 128:5099-5108.

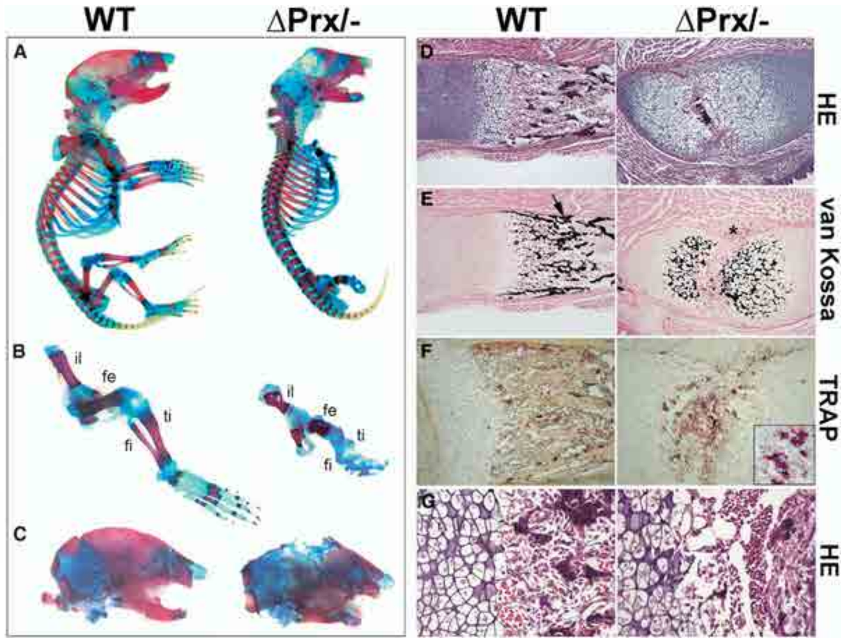
Hu, *et. al.* (2005) *Development.* 132:49-60.

Wnt/β-Catenin Signaling During Cartilage and Bone Development

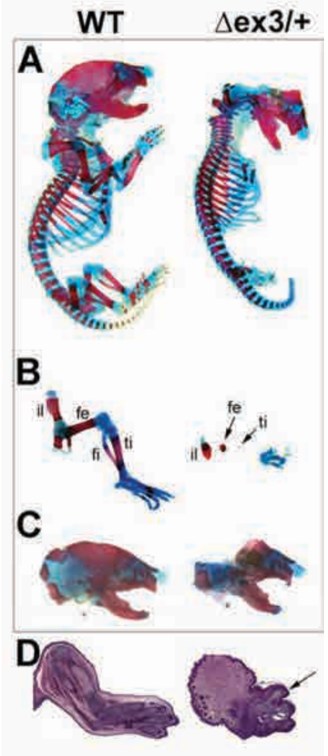
- **Wnt/β-catenin signaling induces chondrocyte proliferation, differentiation, and osteoblast maturation in committed cells.**
- **Wnt/β-catenin signaling suppresses chondrogenic and osteogenic differentiation in mesenchymal progenitor cells.**



β-catenin Loss-of-function (Prx1-Cre)

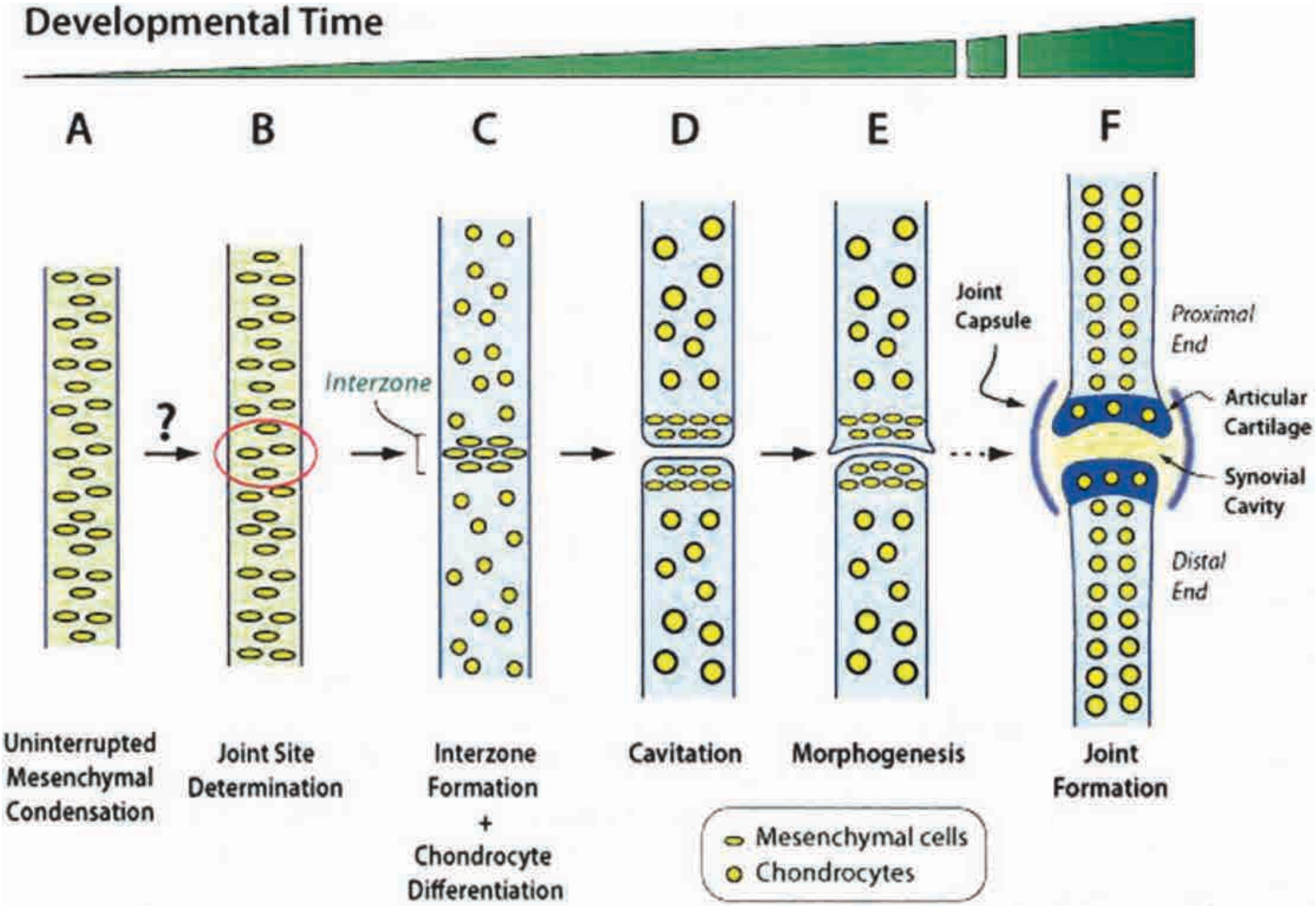


Gain-of-function



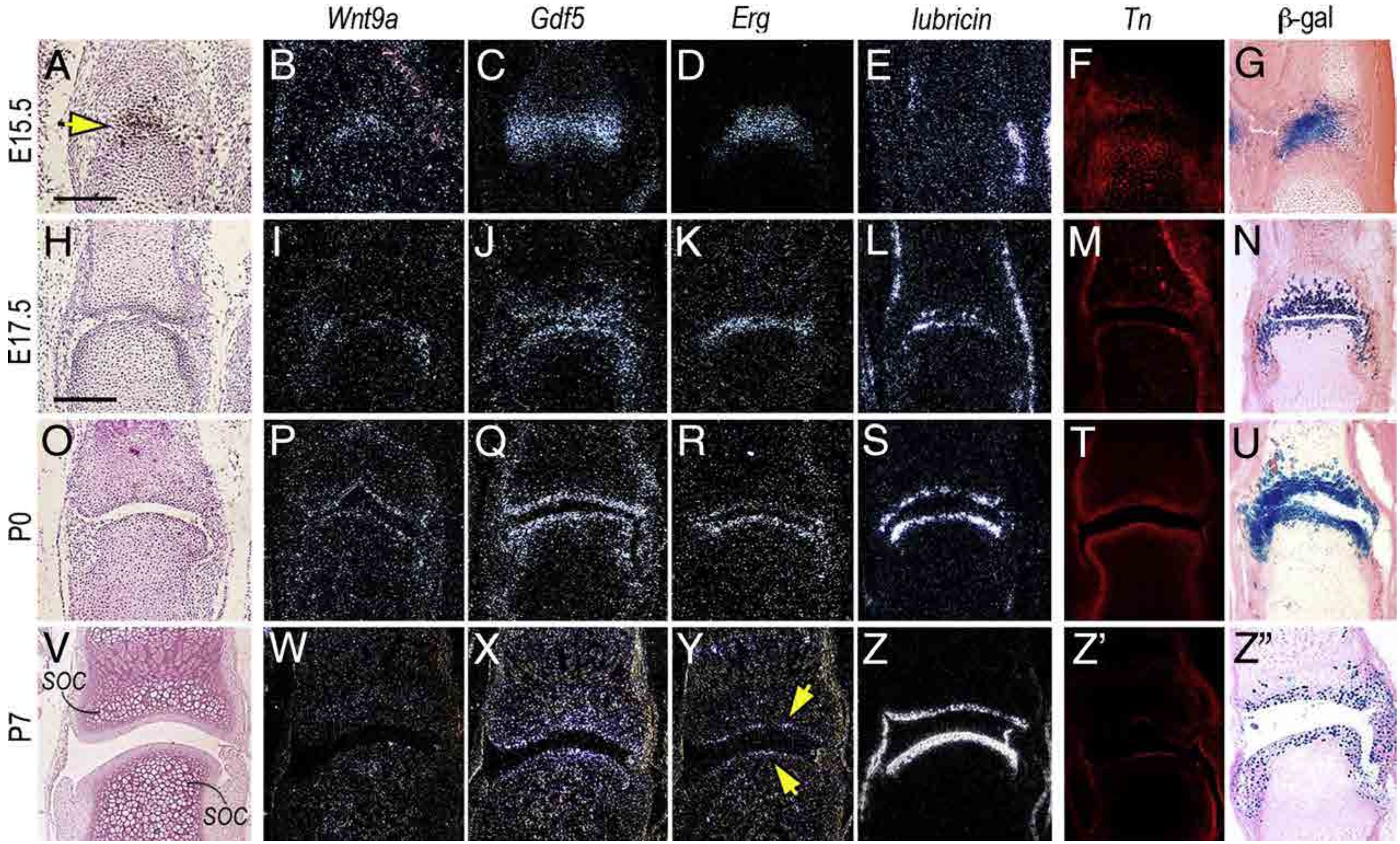
Hill, *et al.* (2005) *Developmental Cell.* 8:727-738.

Synovial Joint Development During Embryogenesis



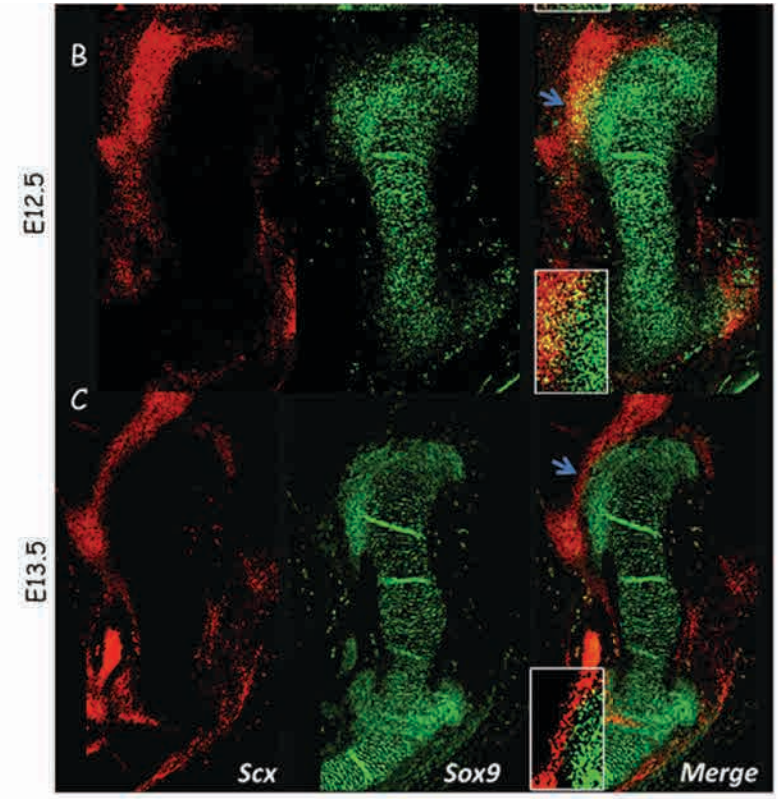
Pacifici, et al. (2005) Birth Defects Res. 75:237-248.

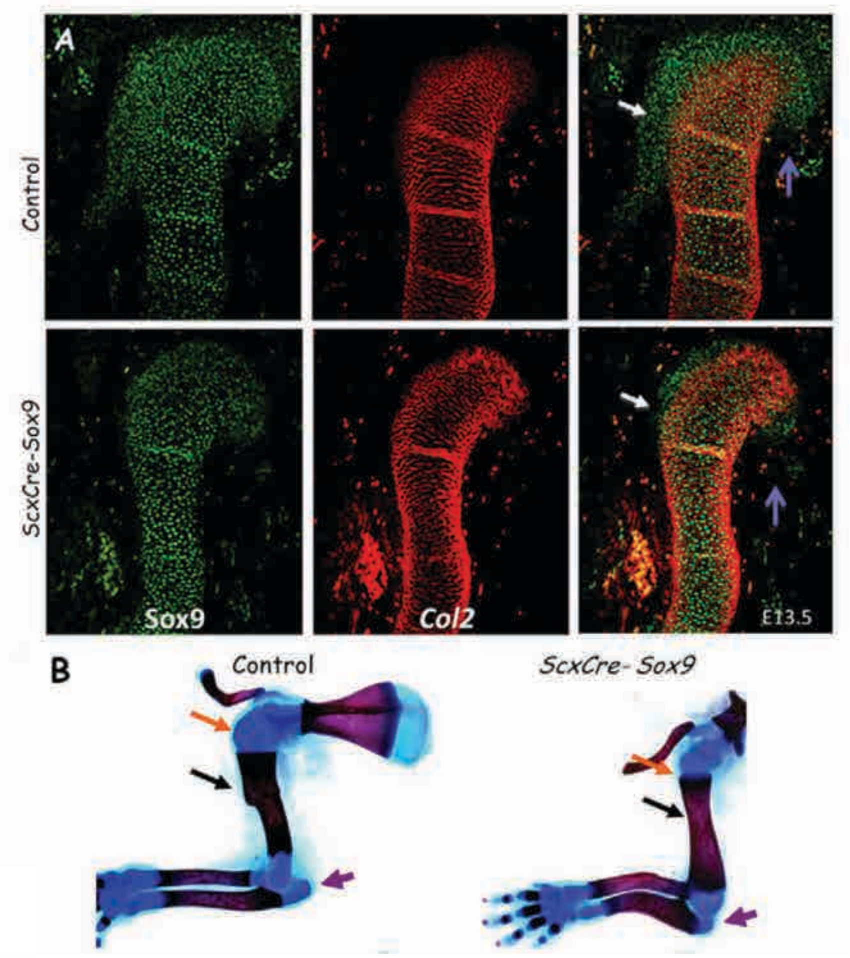
Molecular Regulation of Joint Development

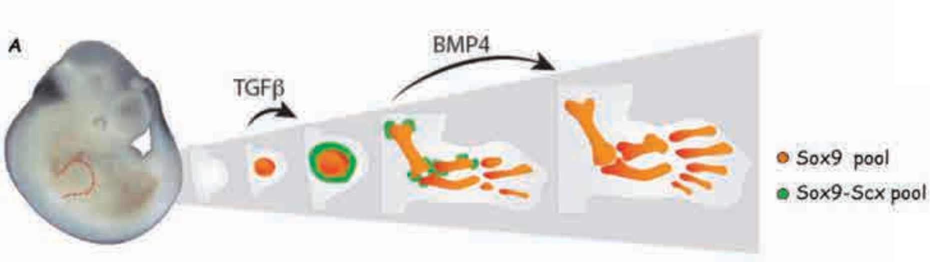


Koyama, et al. (2008) Developmental Biology. 1:62-73.

Bone Eminence Development Requires *Sox9* Expression

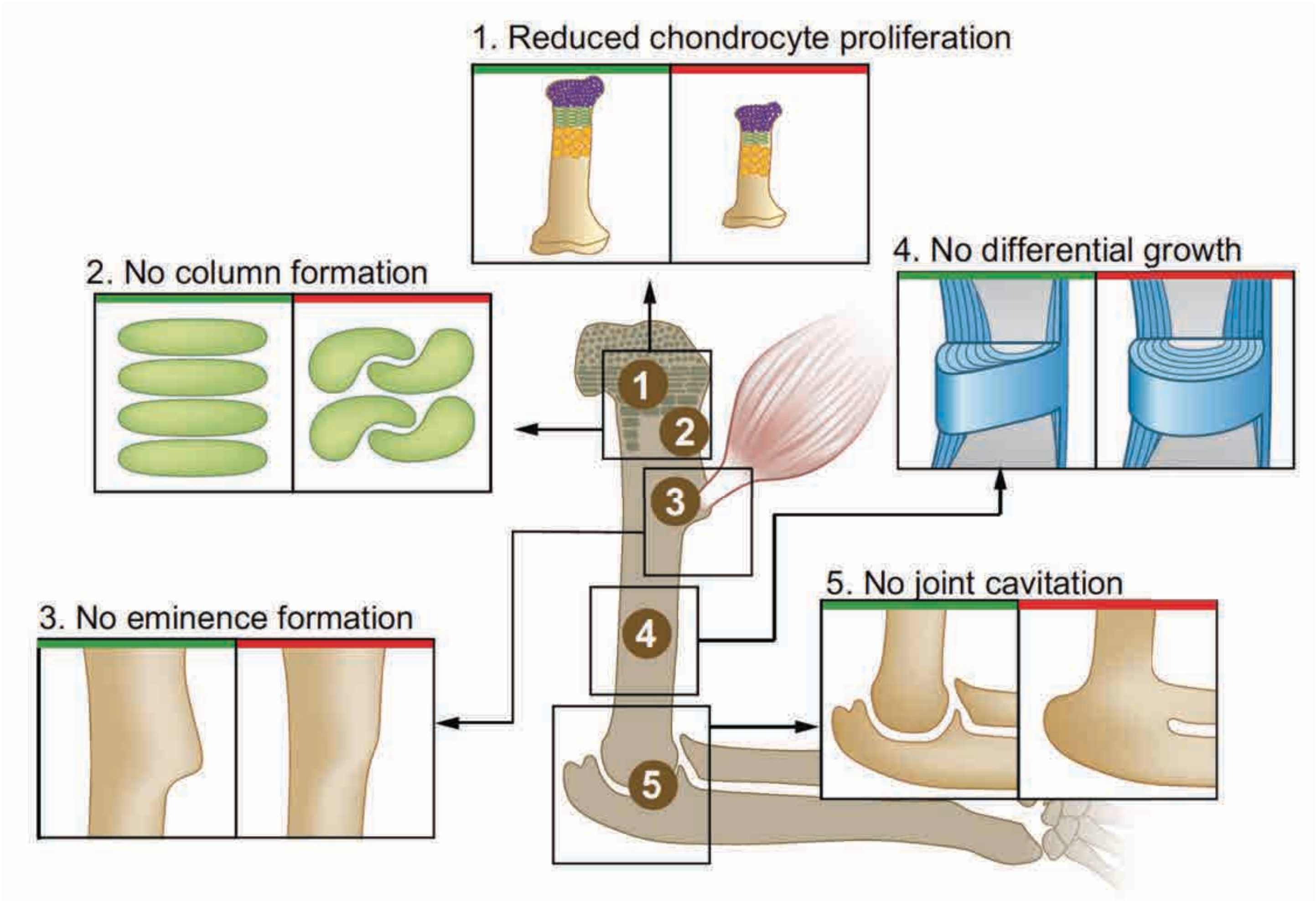






Blitz, *et al.* (2013) *Development*. 140:2680-90.

Reduced Mechanical Forces Affect Musculoskeletal Development



Felsenthal, et al. (2017) *Development*. 144: 4271-83.