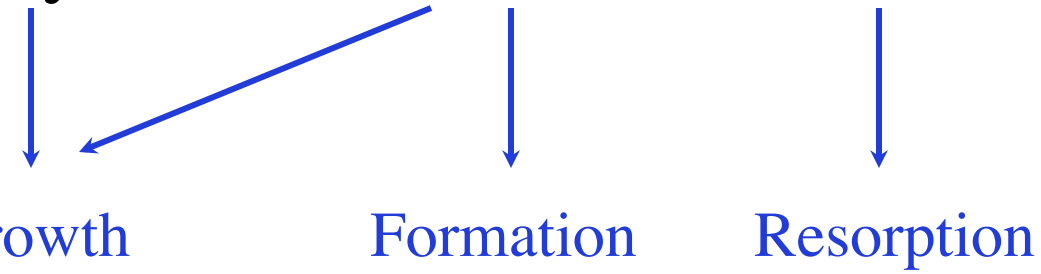
# Skeleton Development

Patricia Ducy
HHSC1616
x5-9299
pd2193@columbia.edu

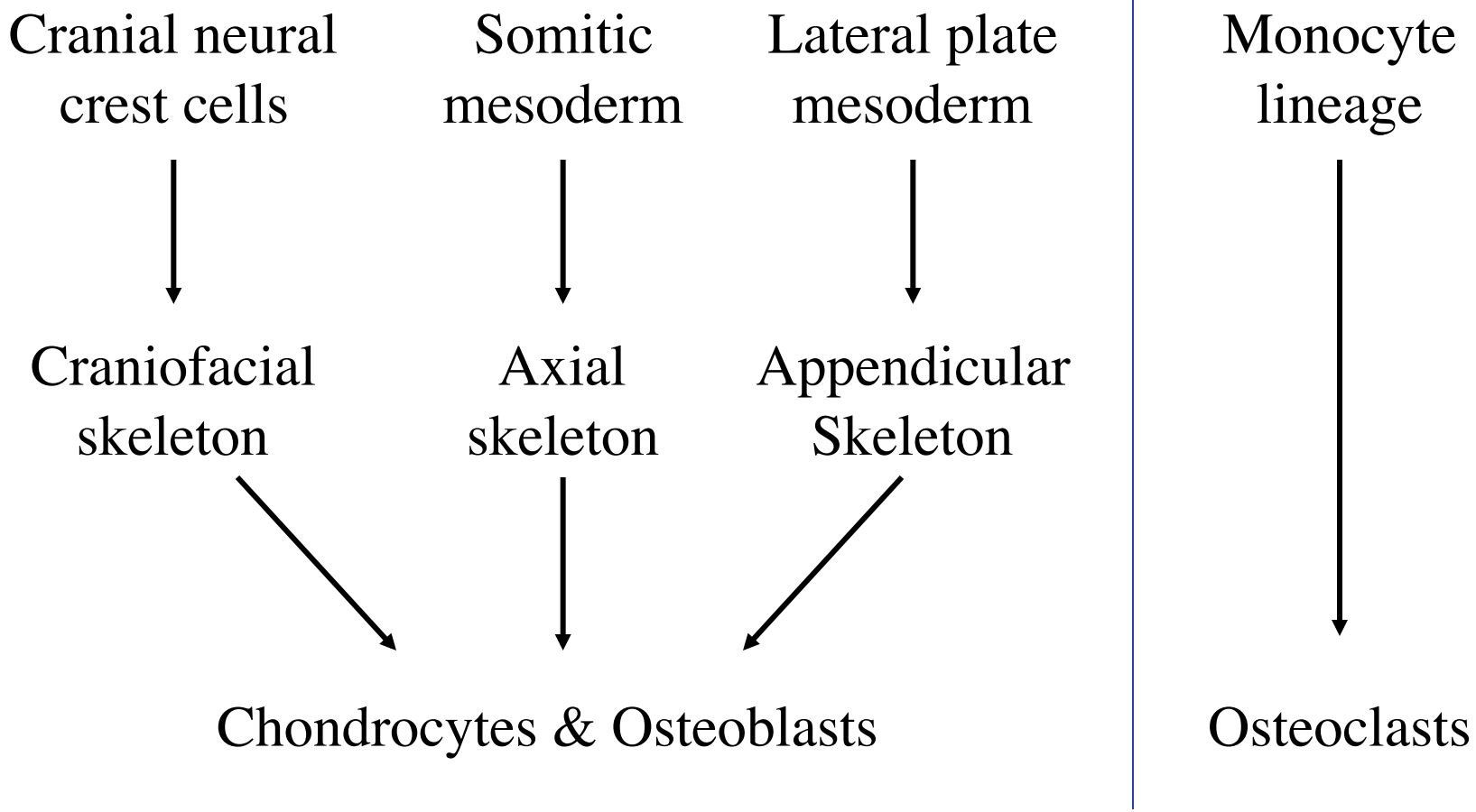
# Skeleton

- ≥ 200 elements
- Two tissues: cartilage, bone
- Three cell types:
  chondrocytes, osteoblasts, osteoclasts

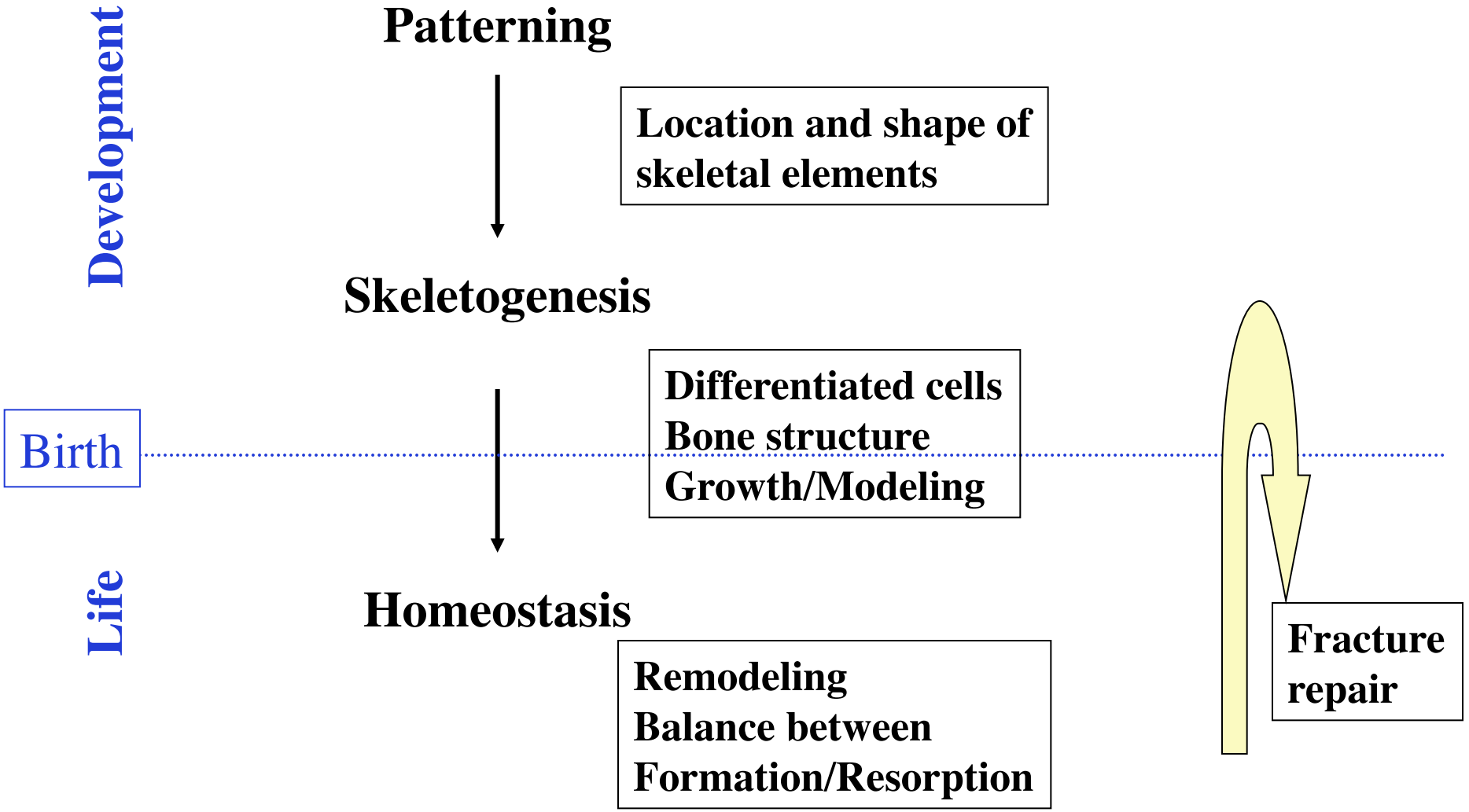



- Three “environments”: marrow, blood, SNS

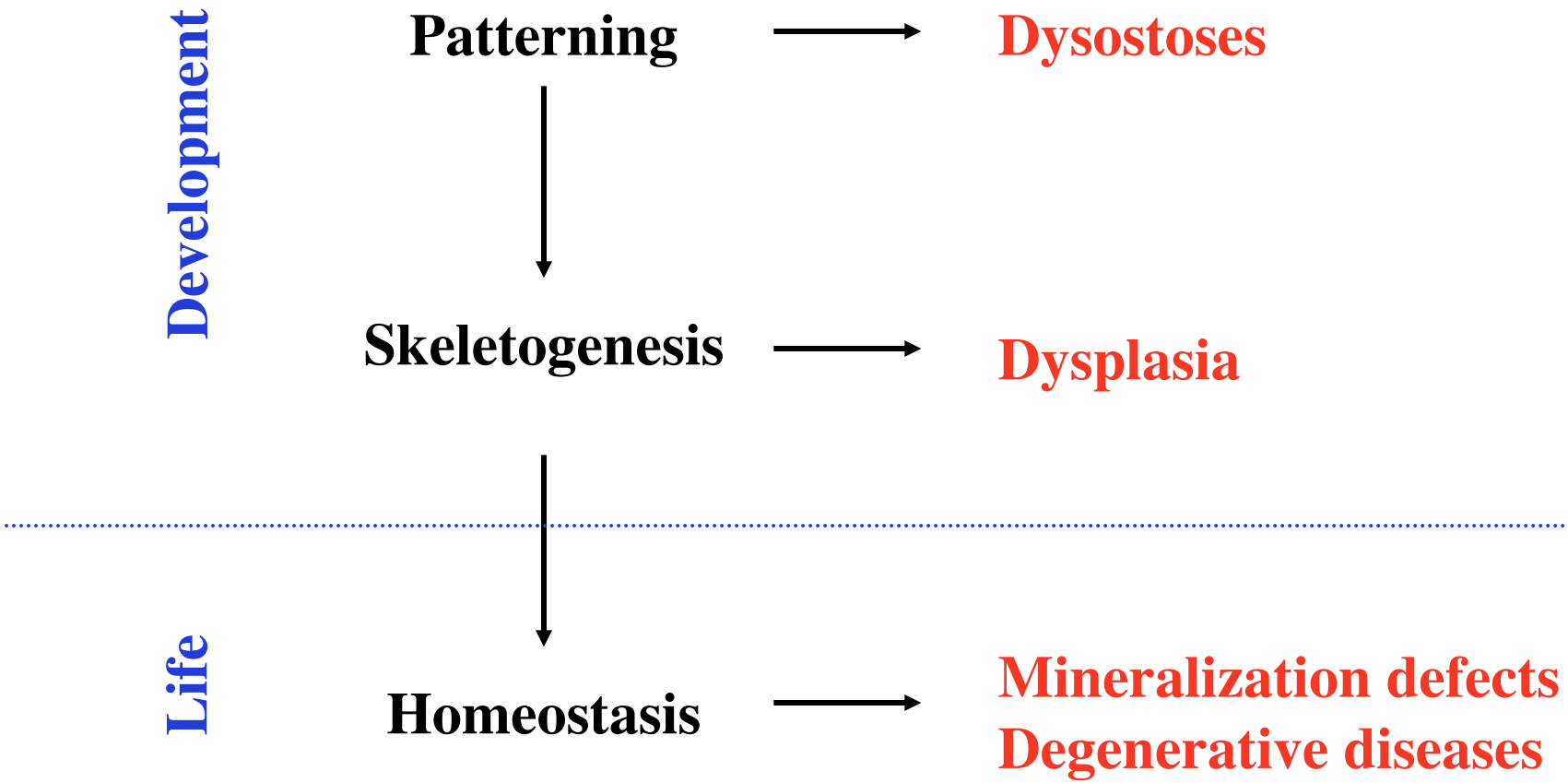
# Embryonic origin of the skeleton



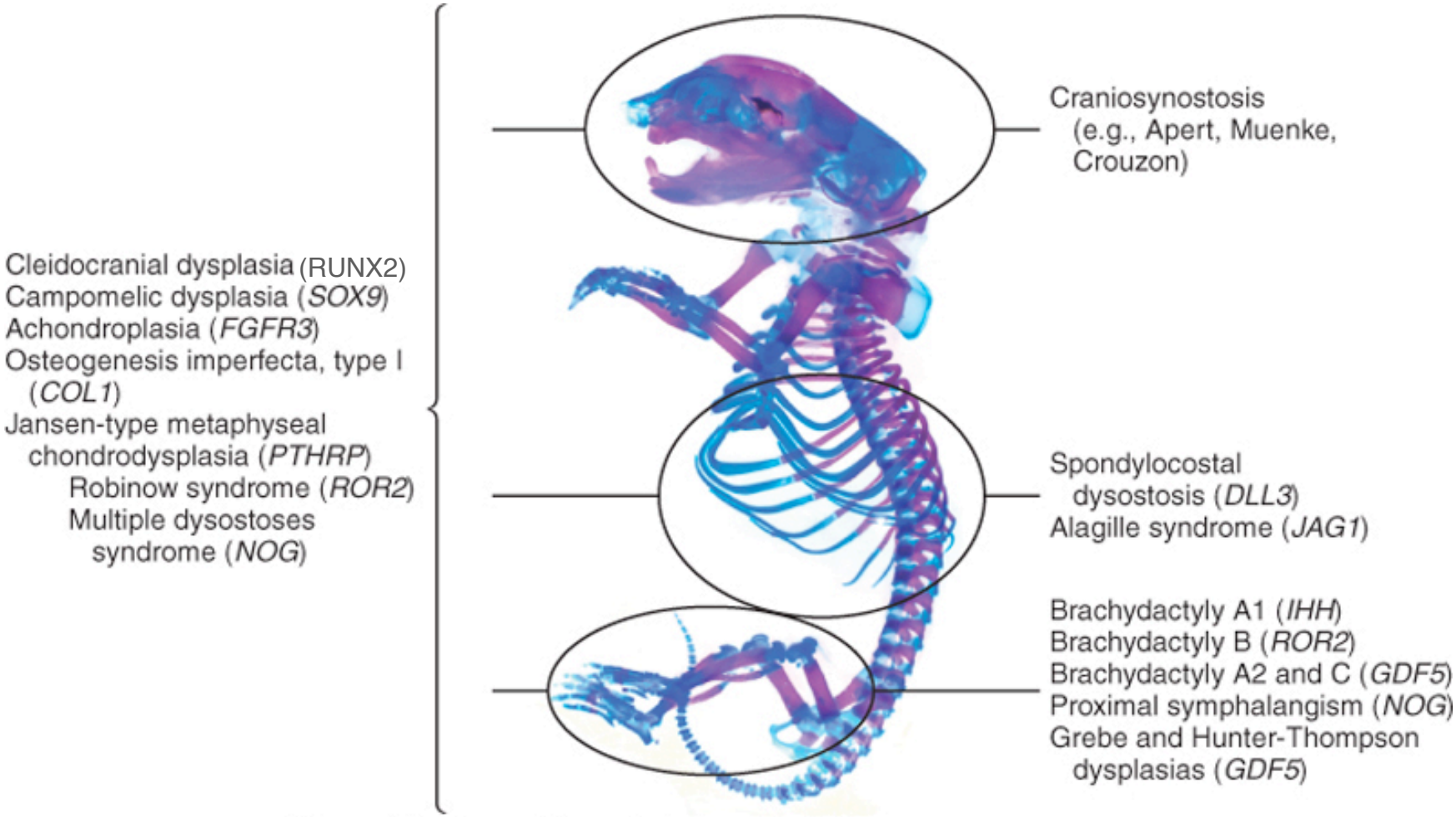
# Skeleton Biology



# Skeleton Pathologies



# Genetic defects associated with skeleton development



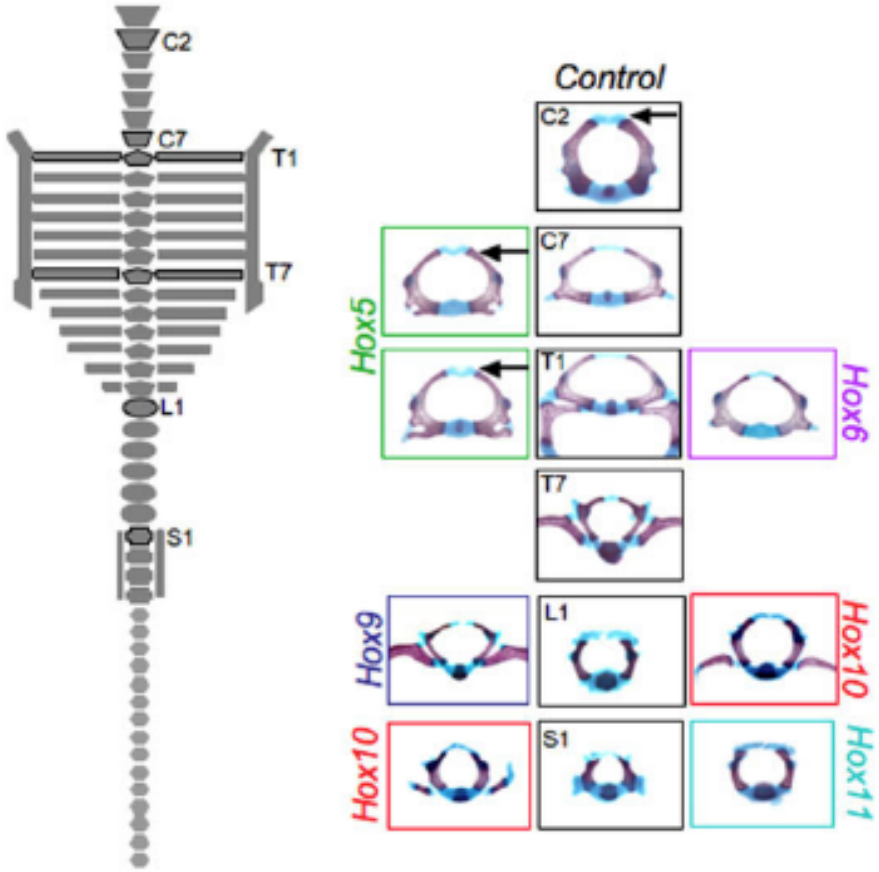
# Skeleton patterning

- Condensation of mesenchymal cells to form the scaffold of each future skeletal element
  - Migration
  - Adhesion
  - Proliferation
- Early steps use signaling molecules and pathways generally involved in patterning other tissues (FGFs, Wnts, BMPs)
- Orchestrated by specific set of genes acting as territories organizers
- When not embryonic lethal disorders often localized

# Hox transcription factors

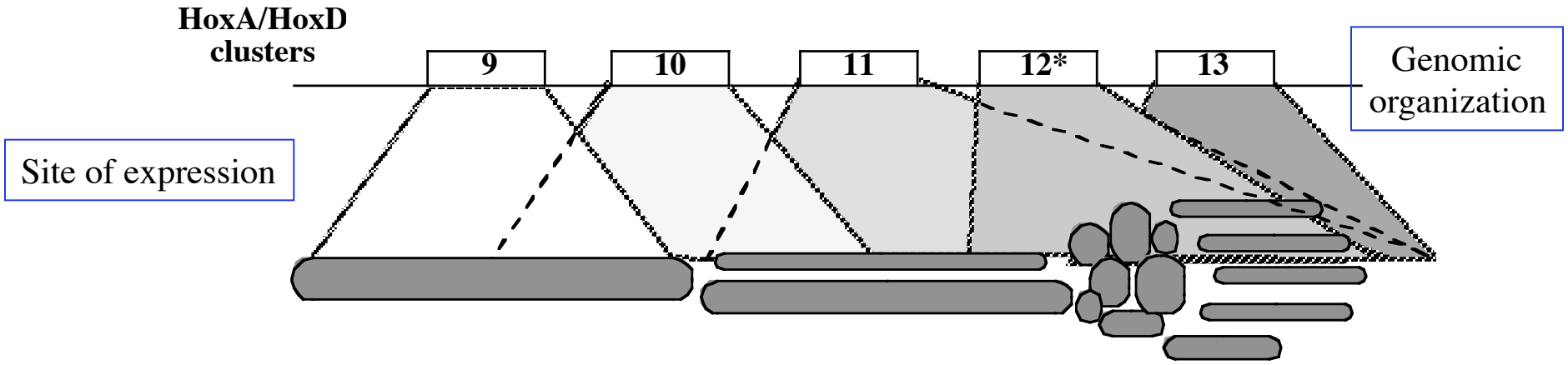
- First described in Drosophila where they control body plan organization
- Arranged in 4 genomic clusters in mammals
- Expression patterns follow the cluster arrangement

# Homeotic transformations in absence of Hox transcription factors




Wellik, *Dev. Dynamics* 236 (2007)

# Hox transcription factors control vertebrate limb patterning

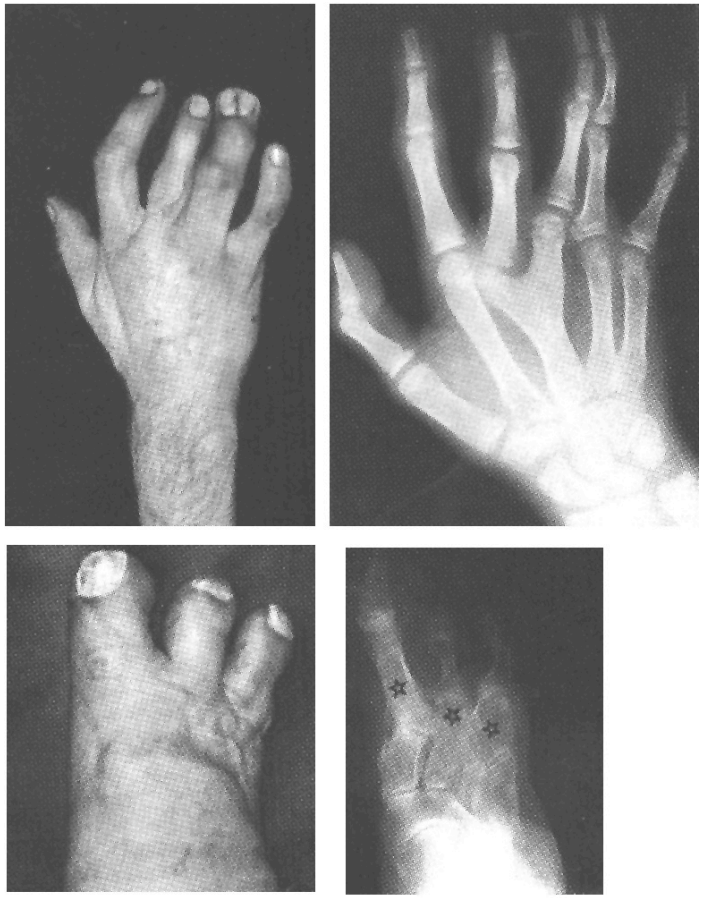



* only in HoxD cluster

# Mutations in HOXD13 cause synpolydactyly* in humans

*OMIM 18600, 186300

Patient with increased number of Ala repeat in HOXD13



**Muragaki et al., *Science* 272 (1996)**

# Skeletogenesis

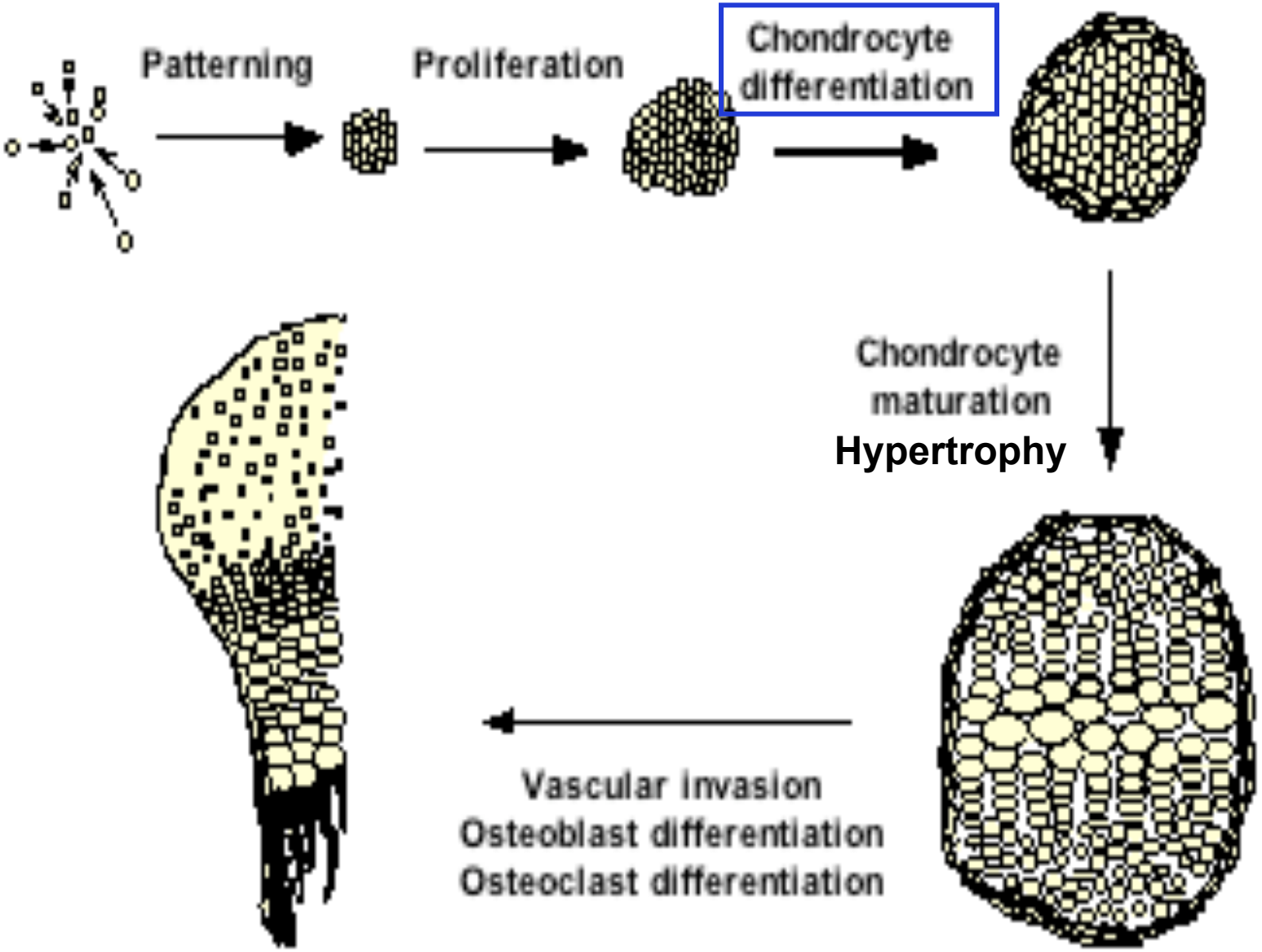
- Cell differentiation
  - Chondrocytes, osteoblasts, osteoclasts
- Bone morphogenesis
  - Formation of growth plate cartilage, bone shaft and marrow cavity
  - Vascular invasion and innervation
- Defects generalized

# Two skeletogenetic mechanisms

- Endochondral ossification
  - Differentiation of a cartilaginous scaffold (chondrocytes) later replaced by bone (osteoblasts)
  - Most of the skeletal elements

- Intramembranous ossification
  - Direct differentiation of the condensed mesenchymal cells into osteoblasts
  - Many bones of the skull, clavicles

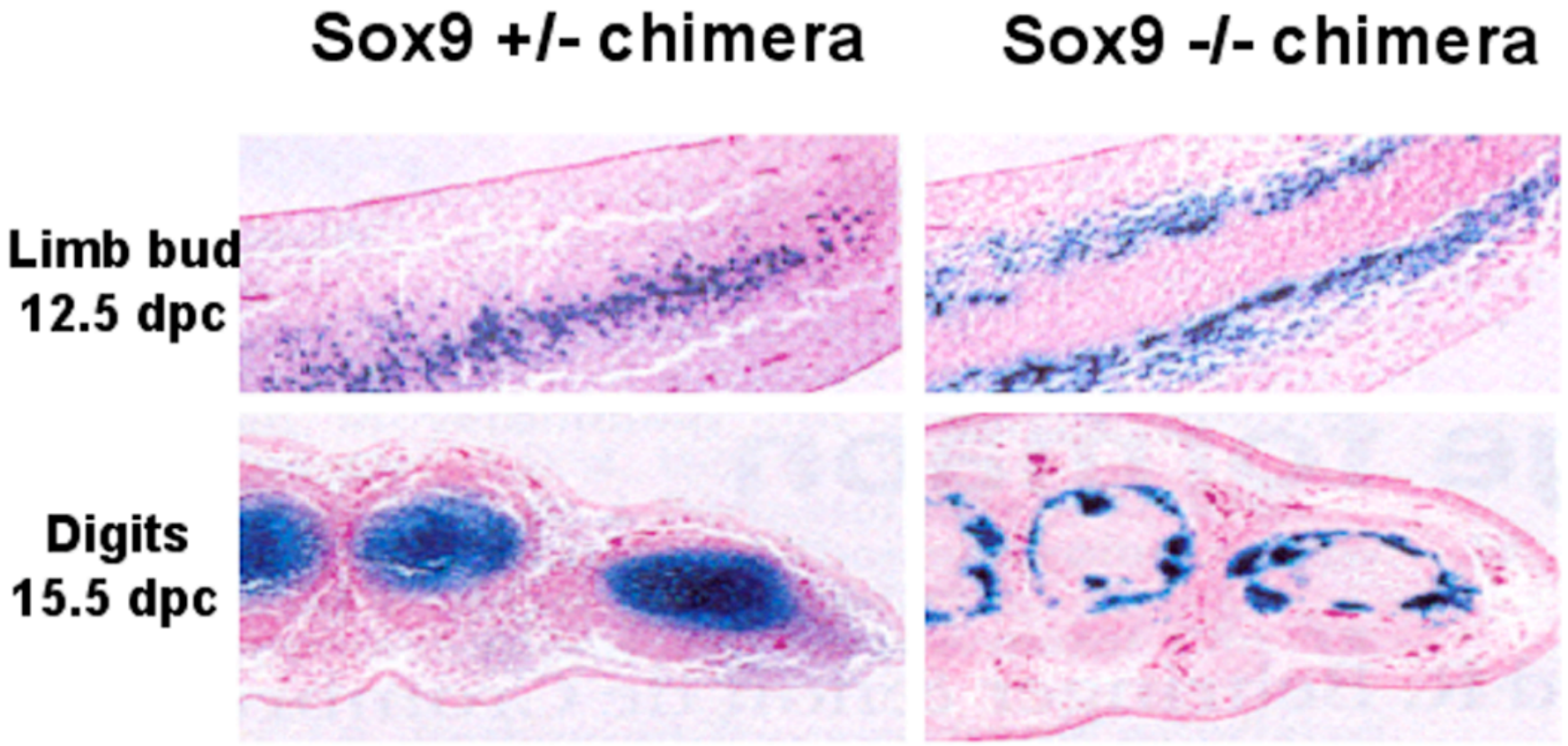
# Endochondral ossification



# Sox9

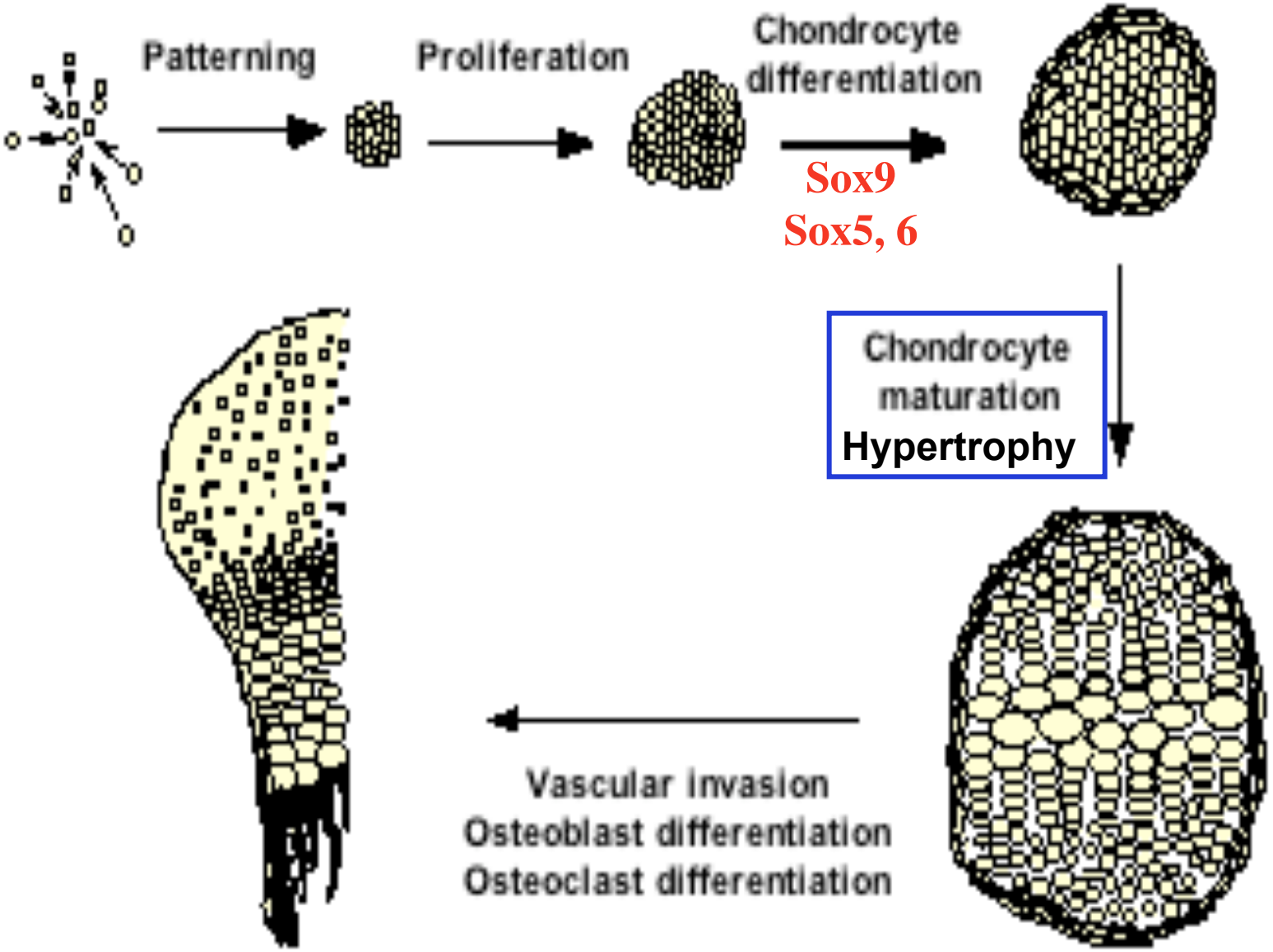
- Transcription factor of the HMG family
- Regulates the expression of chondrocyte-specific genes
- Sox9 haploinsufficiency causes Campomelic dysplasia (OMIM 114290)
- Earliest known regulator of chondrocyte differentiation

# Sox9-deficient cells cannot differentiate into chondrocytes

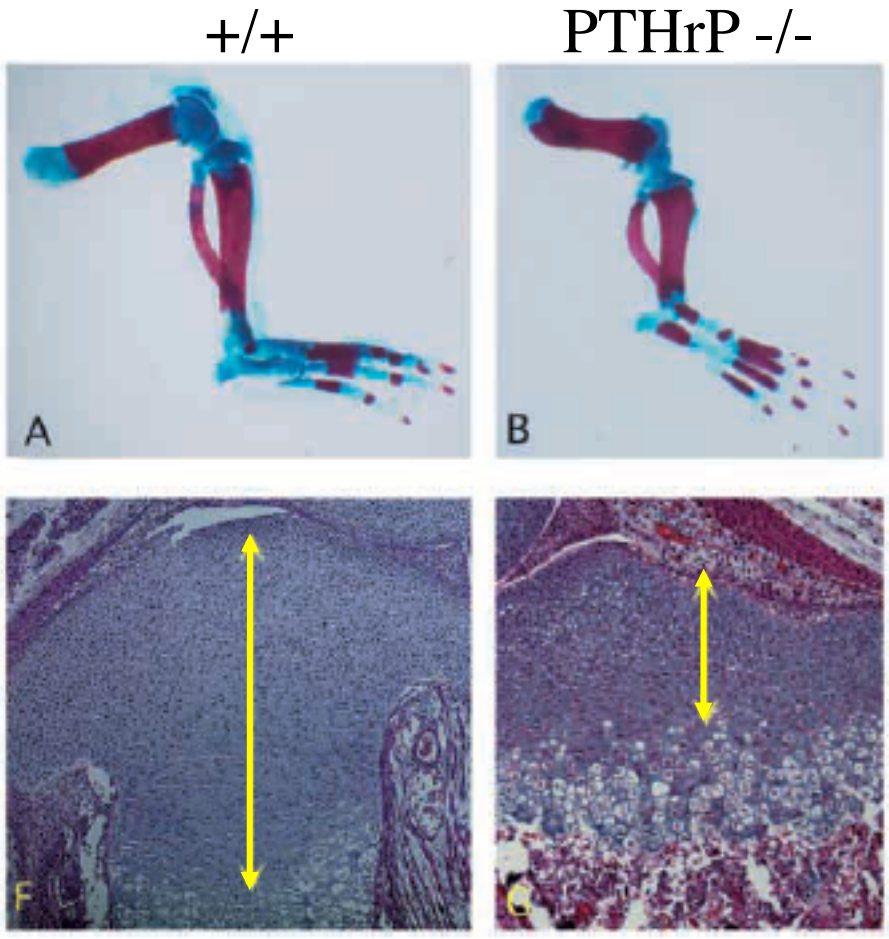



Bi et al., Nat. Genet 22 (1999)

# Endochondral ossification



# Accelerated chondrocyte hypertrophy in PTHrP-deficient mice

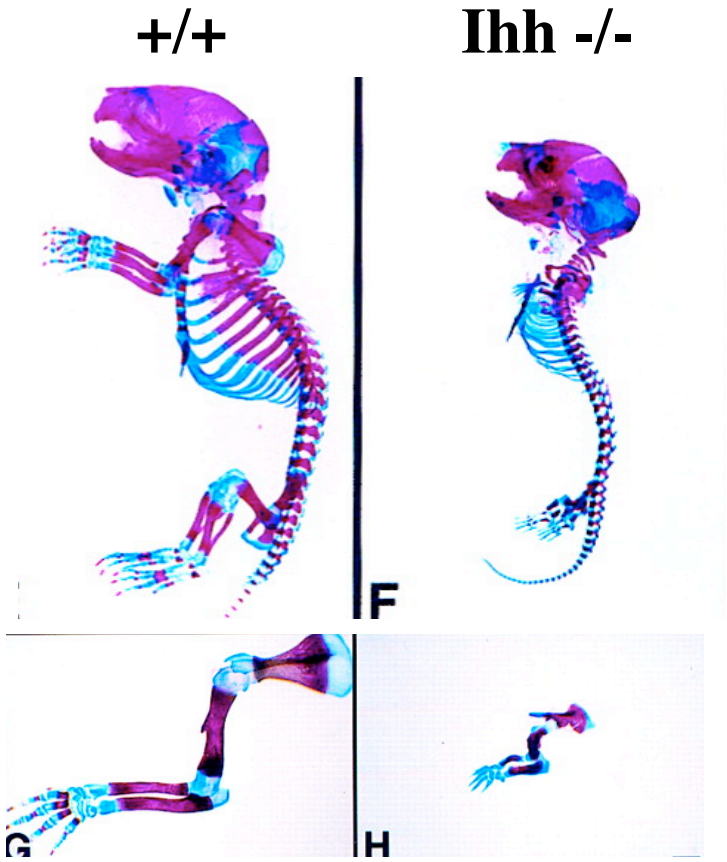



**Karp et al., Development 127 (2000)**

# PTHrP

- Ubiquitously expressed growth factor
- Shares the same receptor with PTH
- Mice “knockout” only phenotype is a generalized growth plate cartilage defect
- PTHrP protein signals to its receptor in the prehypertrophic chondrocytes and blocks their hypertrophic differentiation

# Dwarfism in Ihh-deficient mice

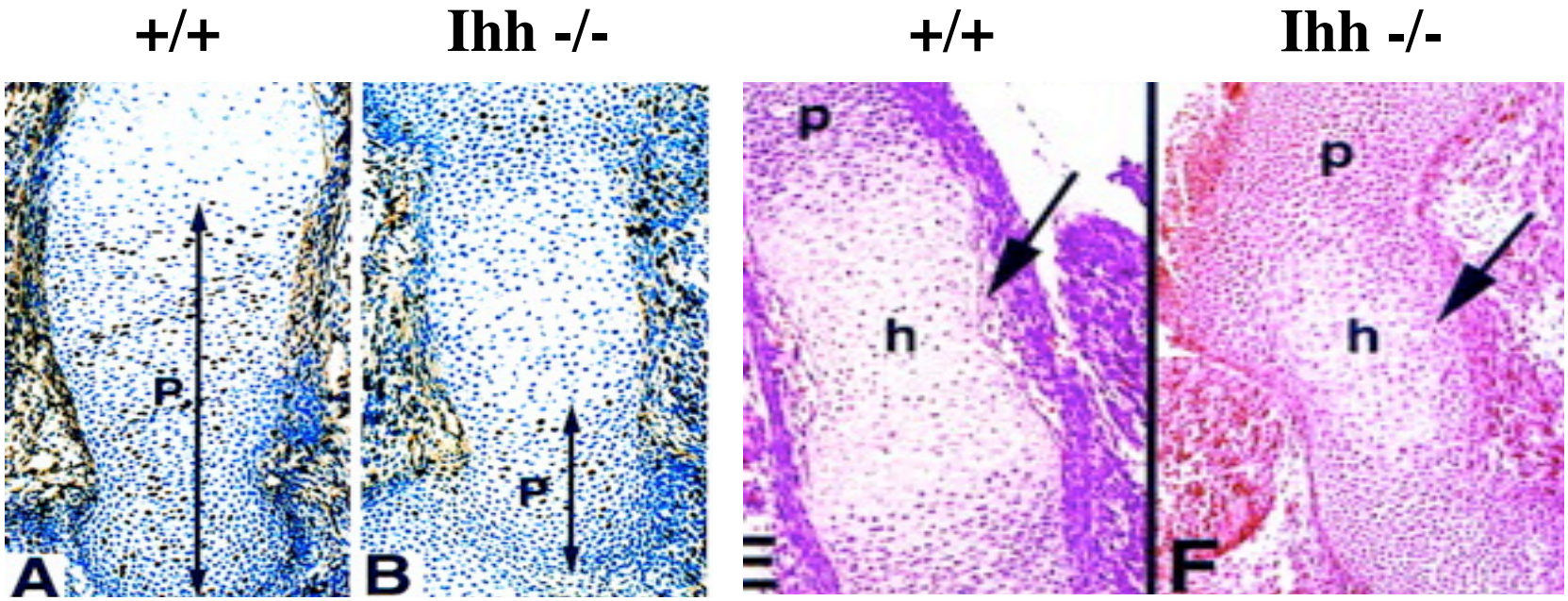



St-Jacques et al. , Genes Dev. 13 (1999)

# Indian hedgehog (Ihh)

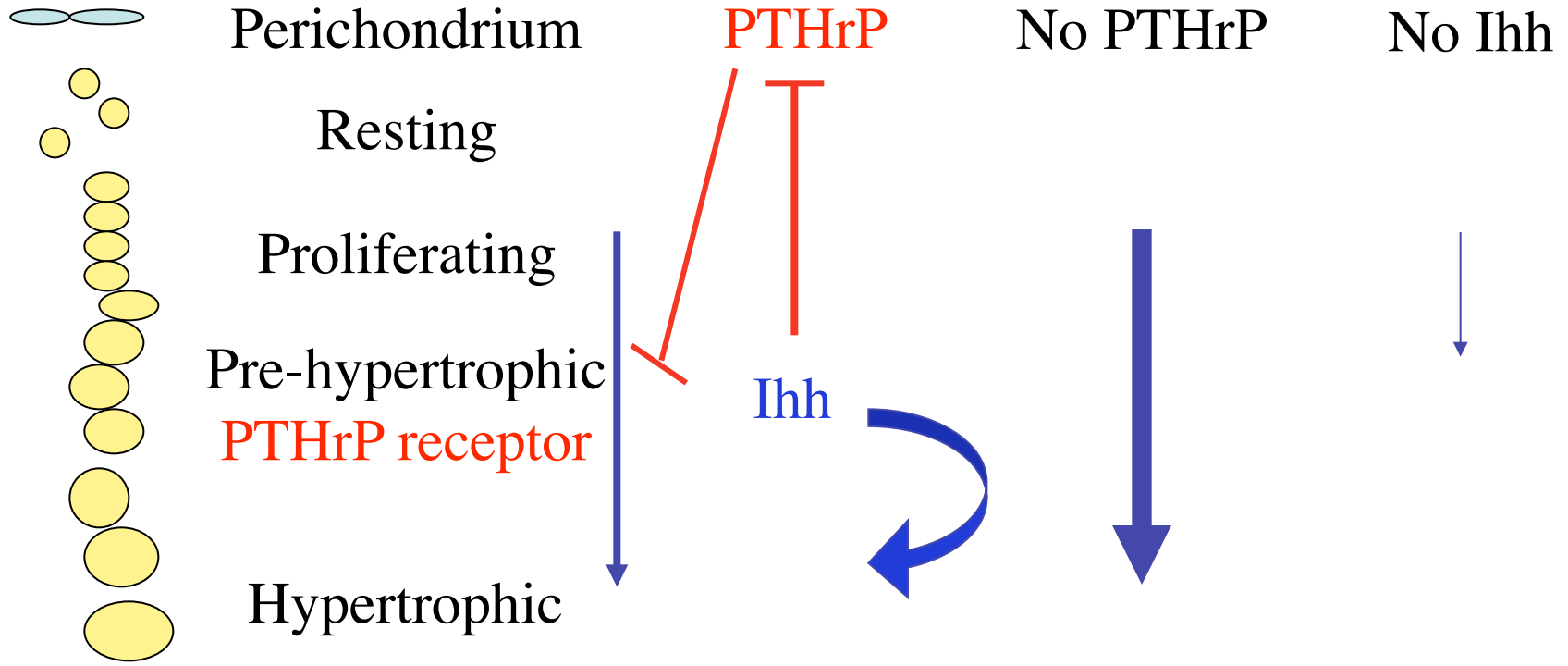
- One of 3 members of the Hedgehog family of growth factors
- Widely expressed during development
- Expression positively regulated by the transcription factor Runx2

# Reduced chondrocyte proliferation and delayed chondrocyte hypertrophy in Ihh-deficient mice



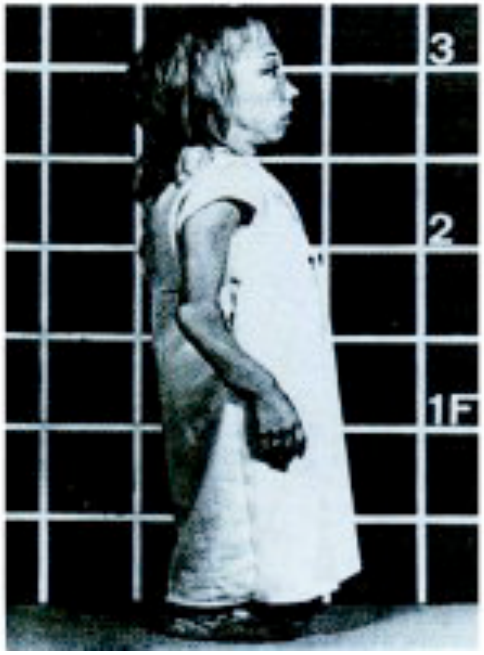

St-Jacques et al. , Genes Dev. 13 (1999)

# Chondrocyte maturation is regulated by a PTHrP/Ihh feedback loop

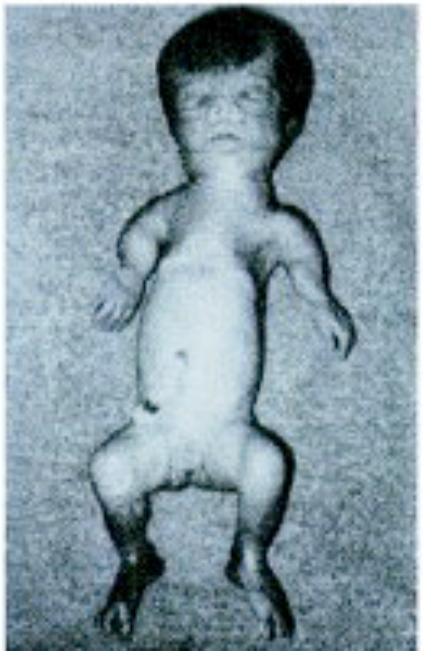


# Mutations in the PTH/PTHrP receptor cause Jansen and Bloomstrand chondrodysplasia

Activating mutations




**Jansen metaphyseal chondrodysplasia OMIM 156400**

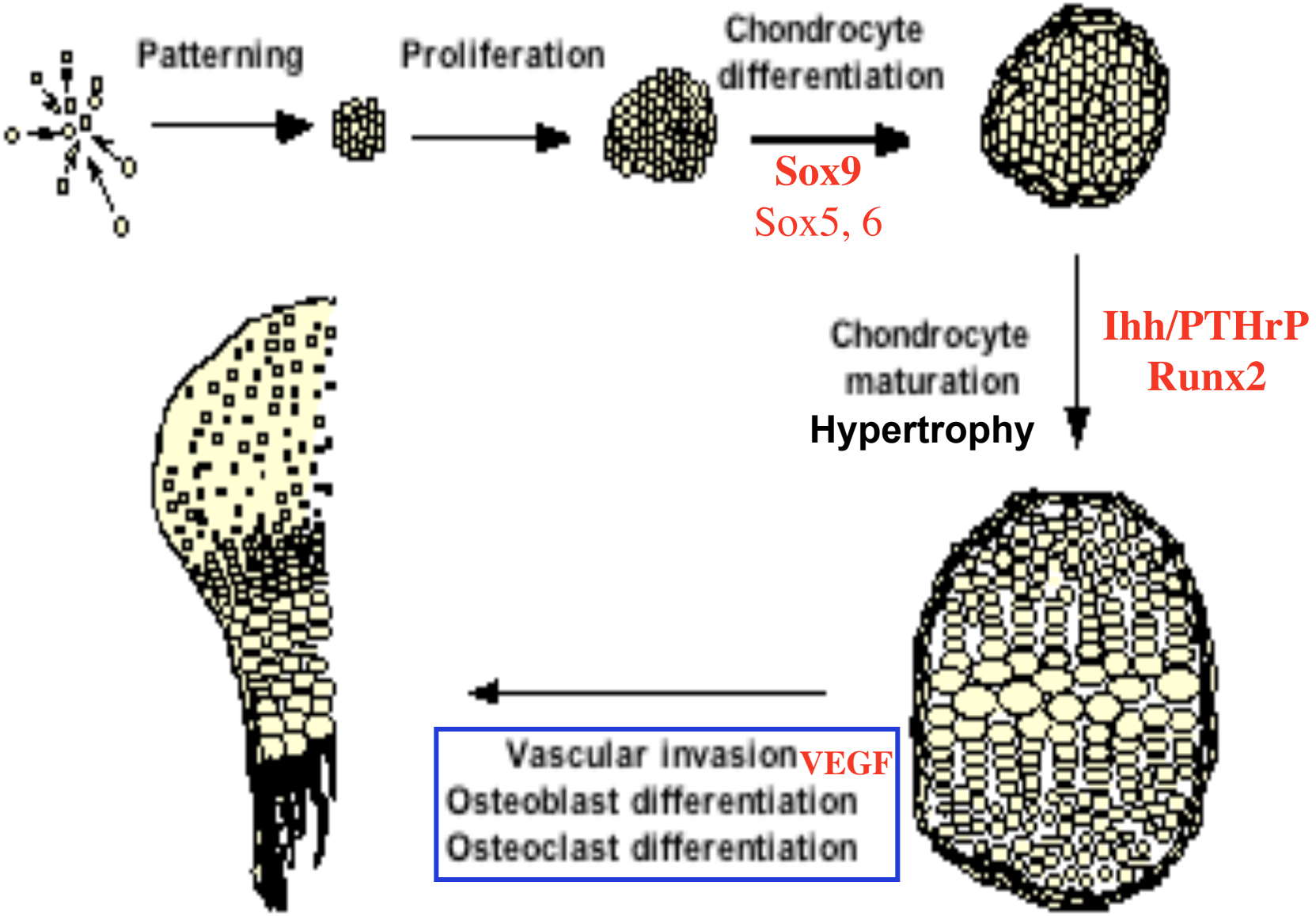


**Blomstrand's lethal chondrodysplasia OMIM 215045**

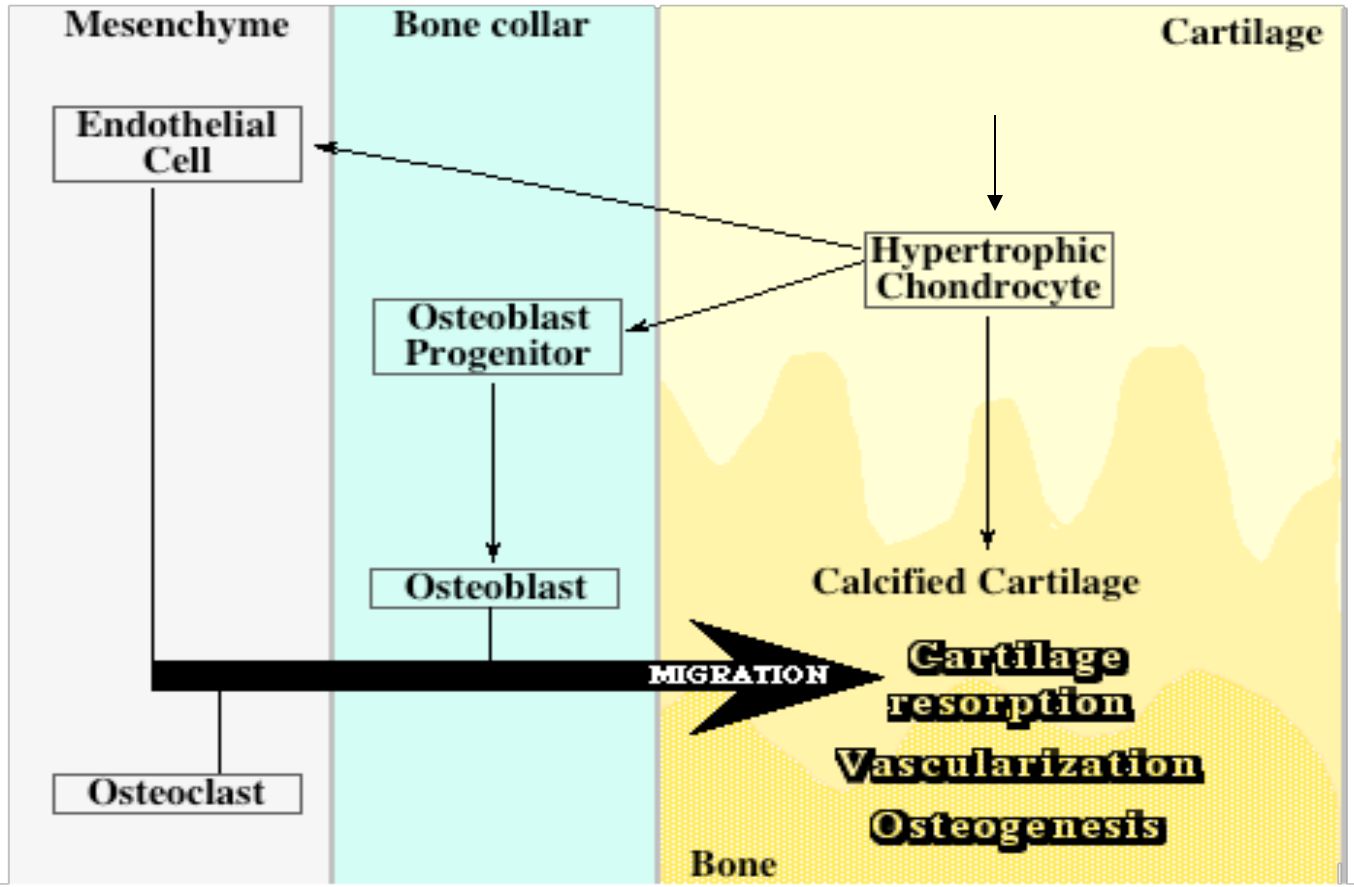
Loss-of-function mutations

**Schipani & Provost. *Brith Defects Res.* 69 (2003)**

# Endochondral ossification

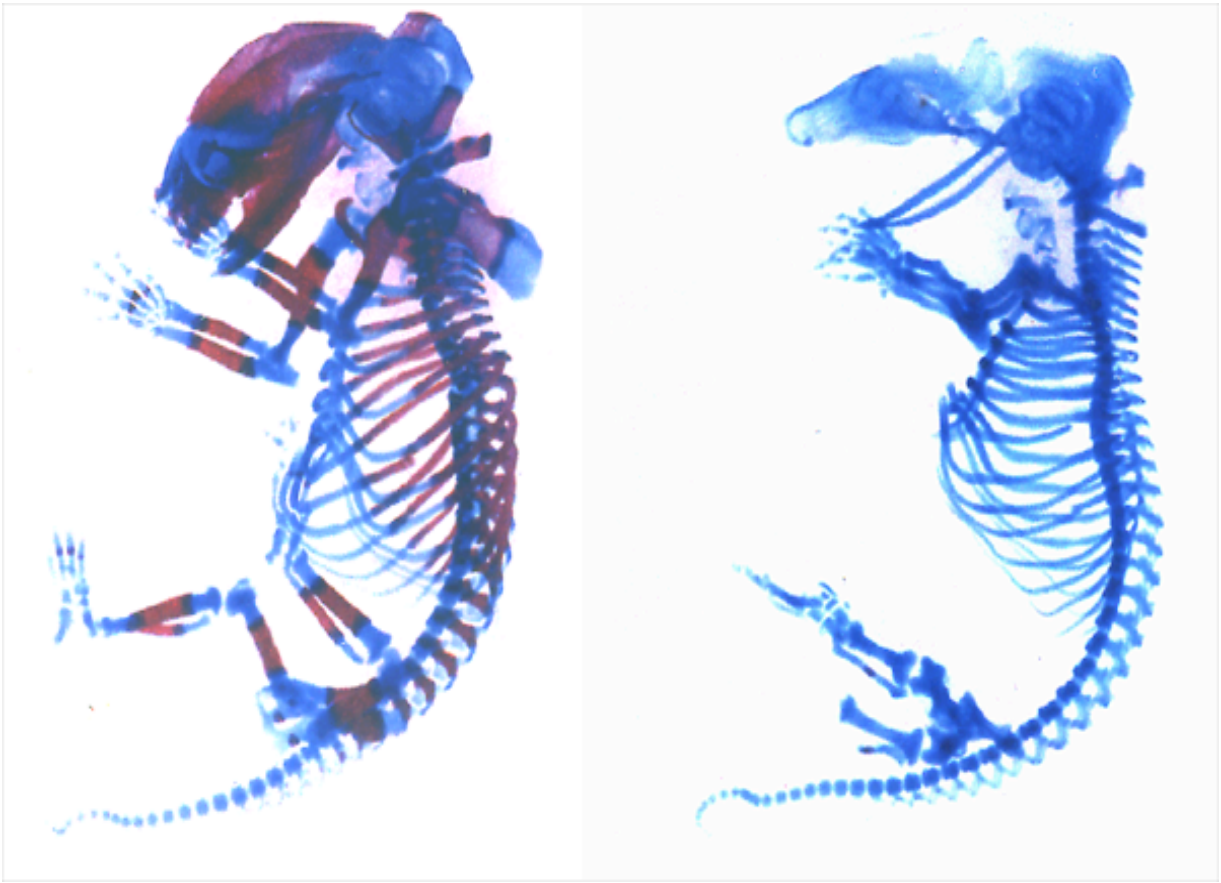


# Endochondral ossification



# Arrest of osteoblast differentiation in Runx2-deficient mice

**+/+** **Runx2 -/-**

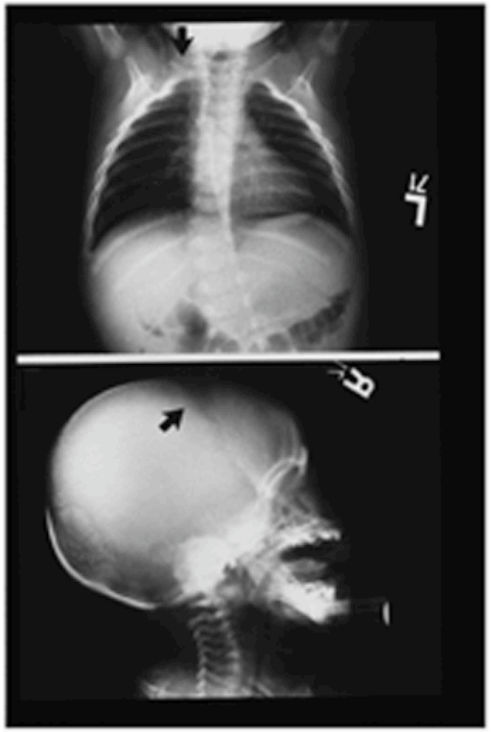


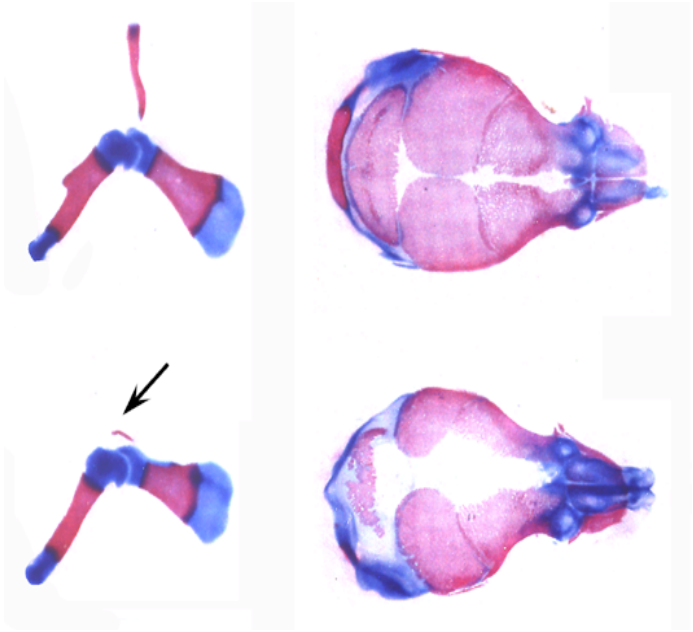
**Otto et al., *Cell* 89 (1997)**

# Runx2

- One of three members of the runt family of transcription factors
- Identified as a regulator of the Osteocalcin promoter
- Necessary and sufficient for osteoblast differentiation

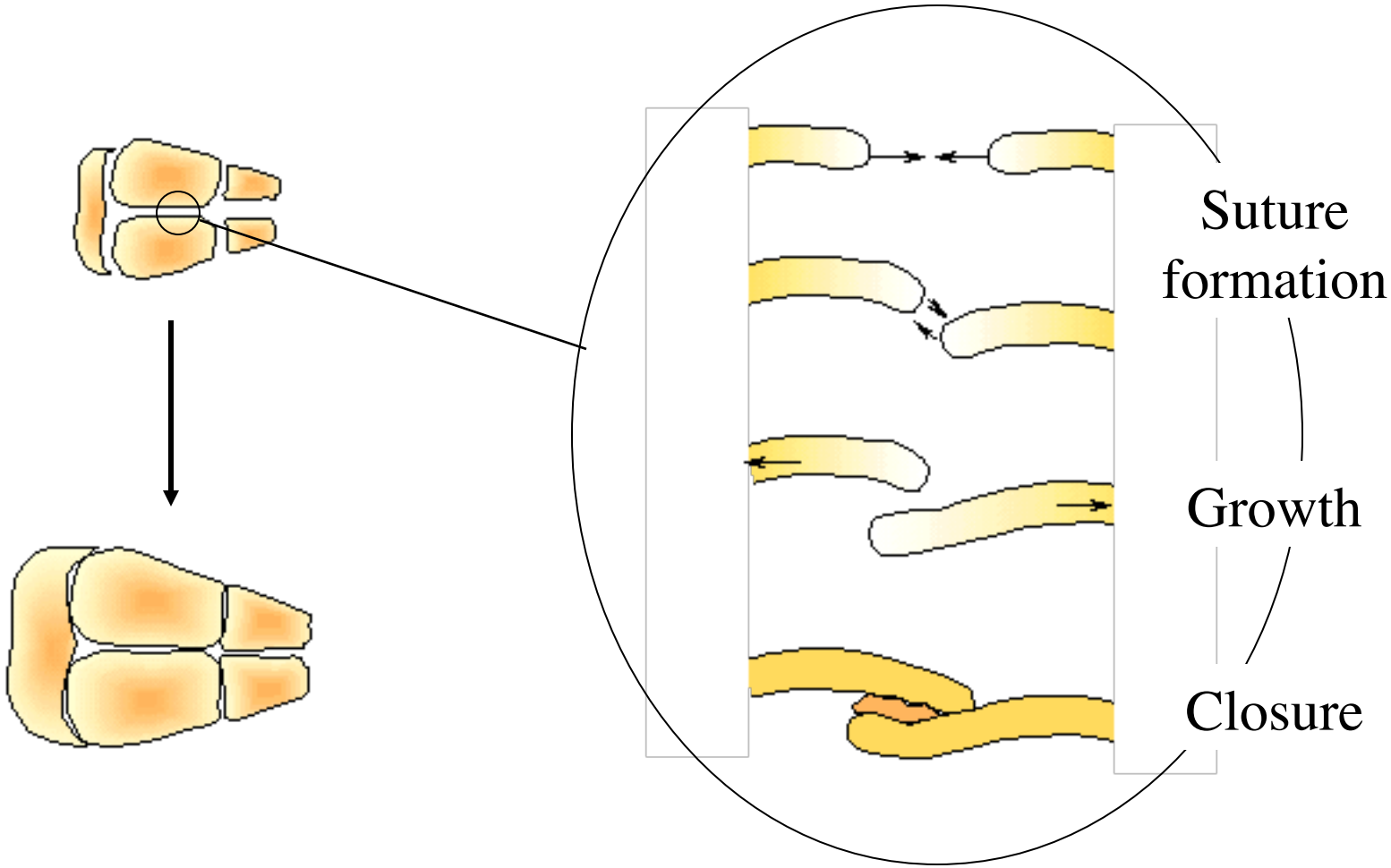
# Cleidocranial dysplasia (CCD, OMIM 119600) is caused by Runx2 haploinsufficiency





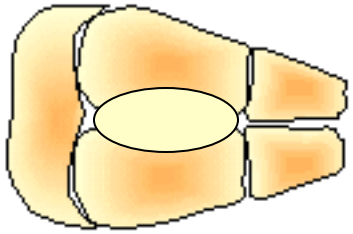

**Mundlos et al., *Cell* 89 (1997)**
**Lee et al. , *Nat Genetics* 16 (1997)**

# Intramembranous ossification



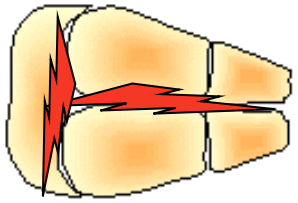
# Disorders of suture fusion

Delay



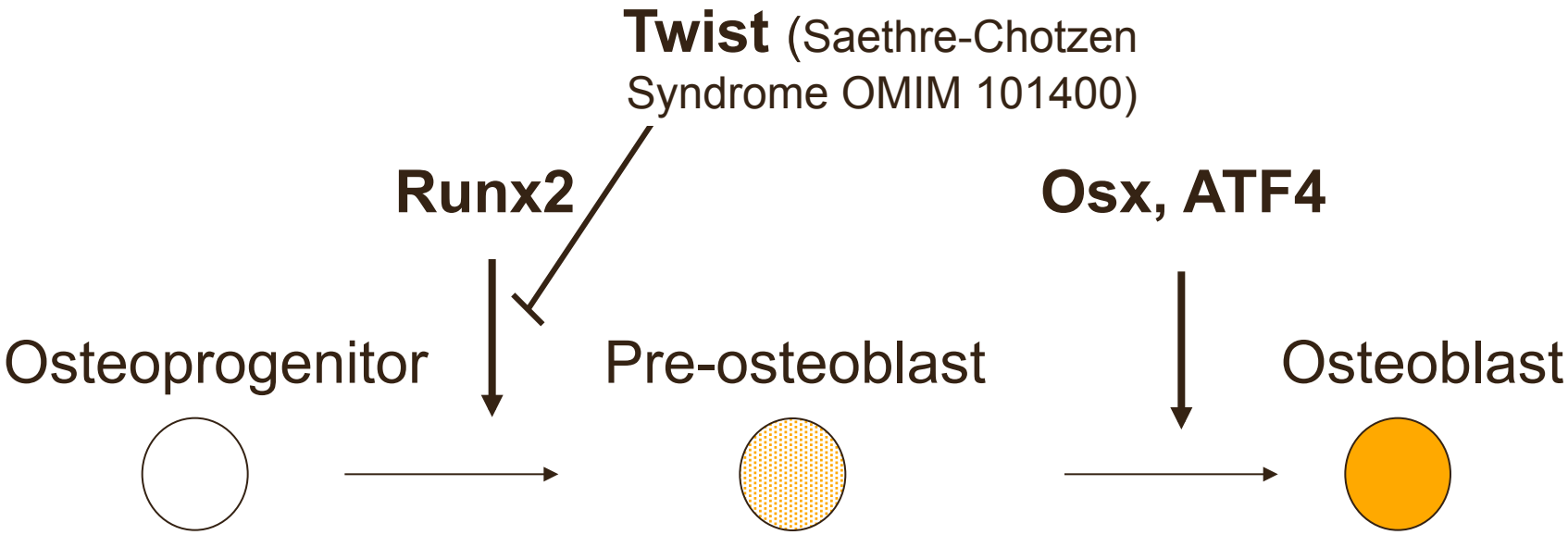
Msx2, Runx2 haploinsufficiency

Acceleration = craniosynostosis



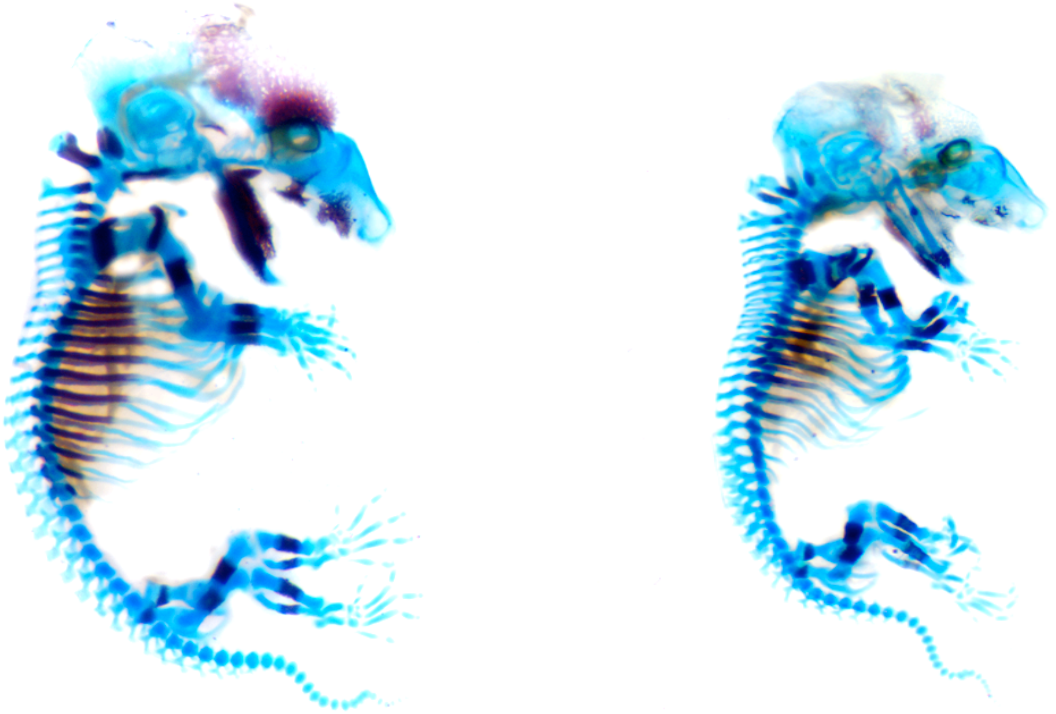
FGFR1, 2, 3 activating mutations
Msx2 activating mutations
Twist haploinsufficiency

# Osteoblast differentiation



# Delayed osteogenesis in absence of *Atf4*

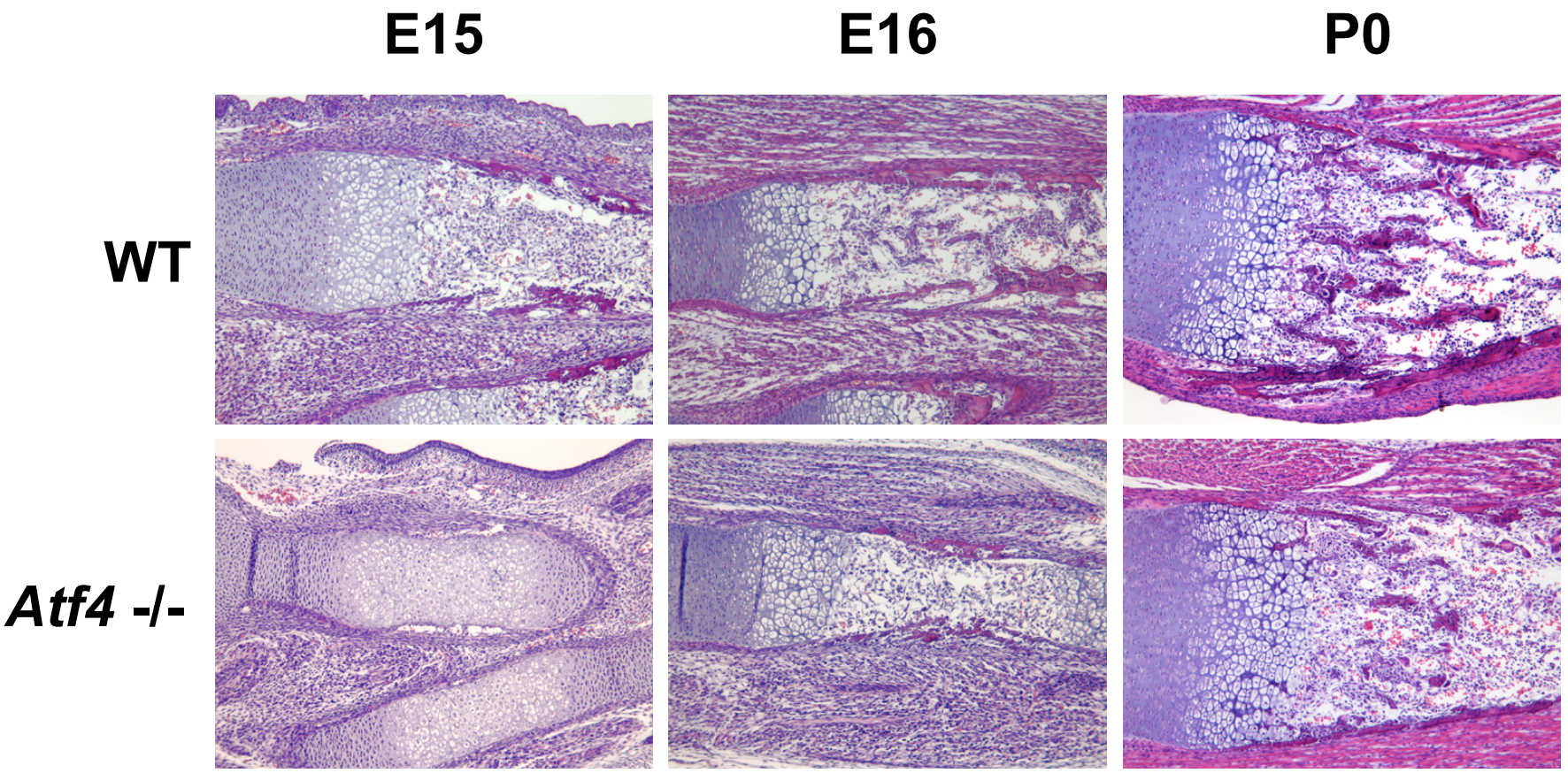
**WT** **Atf4-/-**



E14

**Yang et al., *Cell* 117 (2004)**

# Delayed osteogenesis in *Atf4-deficient mice*




Yang et al., *Cell* 117 (2004)

# ATF4

- Divergent member of the ATF/CREB family of leucine-zipper transcription factors
- Required for amino-acid import
- Identified as a regulator of the Osteocalcin promoter
- Activated by the Rsk2 kinase

# ATF4

- Lack of ATF4 phosphorylation by inactivating mutations in Rsk2 causes the skeletal defects associated with Coffin-Lowry syndrome (OMIM 303600)

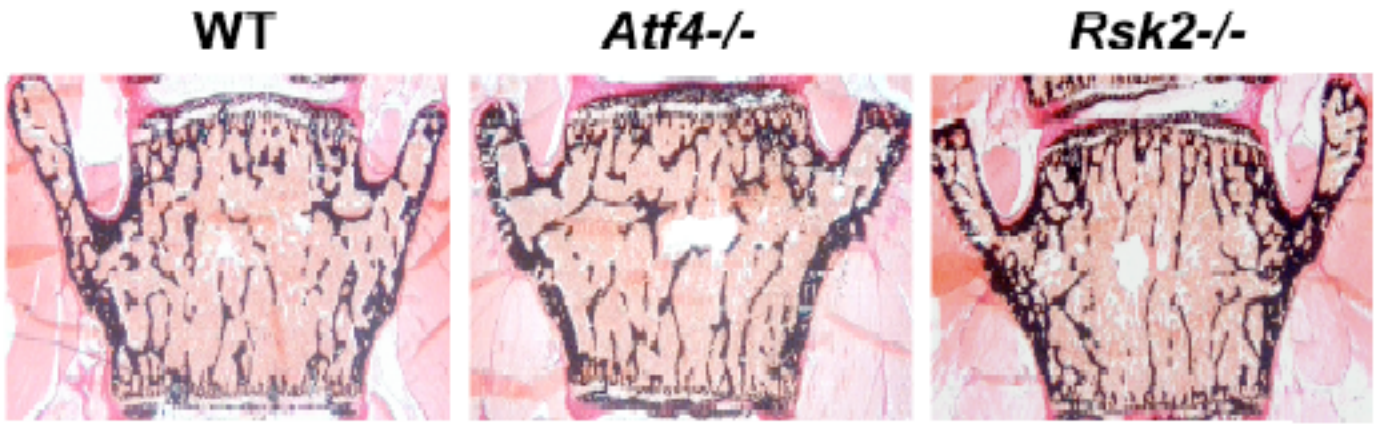
- Increased ATF4 phosphorylation by Rsk2 causes the skeletal defects associated with Neurofibromatosis Type I (OMIM 162200)

# ATF4

- Divergent member of the ATF/CREB family of leucine-zipper transcription factors
- Required for amino-acid import
- Identified as a regulator of the Osteocalcin promoter
- Activated by the Rsk2 kinase

# A high protein diet normalizes bone formation in *Atf4*-/- and *Rsk2*-/- mice

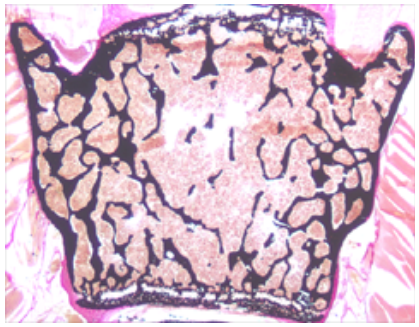
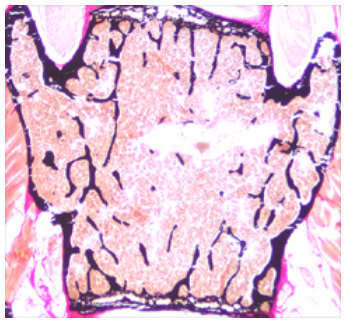
**High protein diet**




| | WT | *Atf4*-/- | *Rsk2*-/- |
|---|---|---|---|
| BV/TV | 14.2±0.4 | 14.5±0.7 | 13.8±0.7 |
| BFR | 199.5±43 | 189.5±49 | 200.5±41 |
| Ob.S/BS | 10.8±0.8 | 11.8±1.3 | 11.3±0.3 |

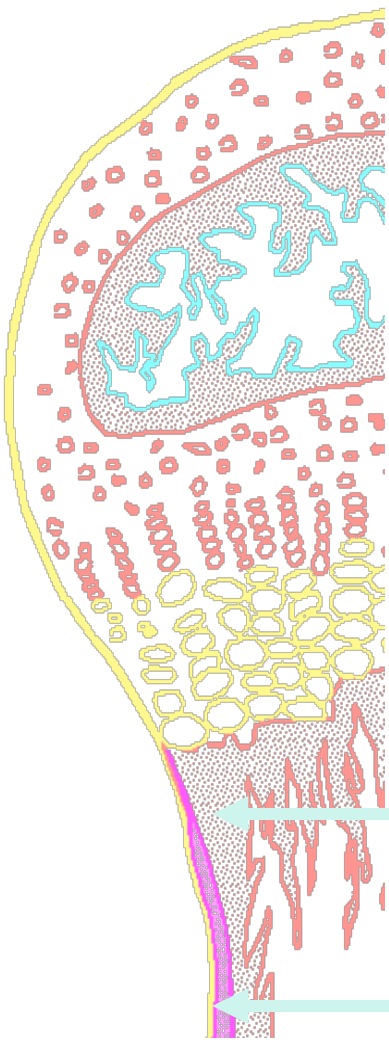
**Elefteriou et al., *Cell Metab*. 4 (2006)**

# A low protein diet normalizes bone formation in a mouse model of Neurofibromatosis type I

| | Normal diet | | Low protein diet | |
|---|---|---|---|---|
| | wt | *Nf1*$_{ob}$-/- | wt | *Nf1*$_{ob}$-/- |
| |  |  |  |  |
| BV/TV | 15.3±1 | 19.6±0.4* | 14.8±0.7 | 15.3±0.6 |
| BFR | 153.3±11 | 313.9±7.0* | 157.0±11 | 186.2±22 |
| ObS/BS | 19.1±0.8 | 31.8±1.6* | 19.0±0.5 | 19.7±1.0 |

Elefteriou et al., *Cell Metab.* 4 (2006)

# Structure of a growing long bone



articular cartilage (chondrocytes)

secondary ossification centre (osteoblasts/osteoclasts)

reserve cartilage

**proliferating cells**

**hypertrophic cells**

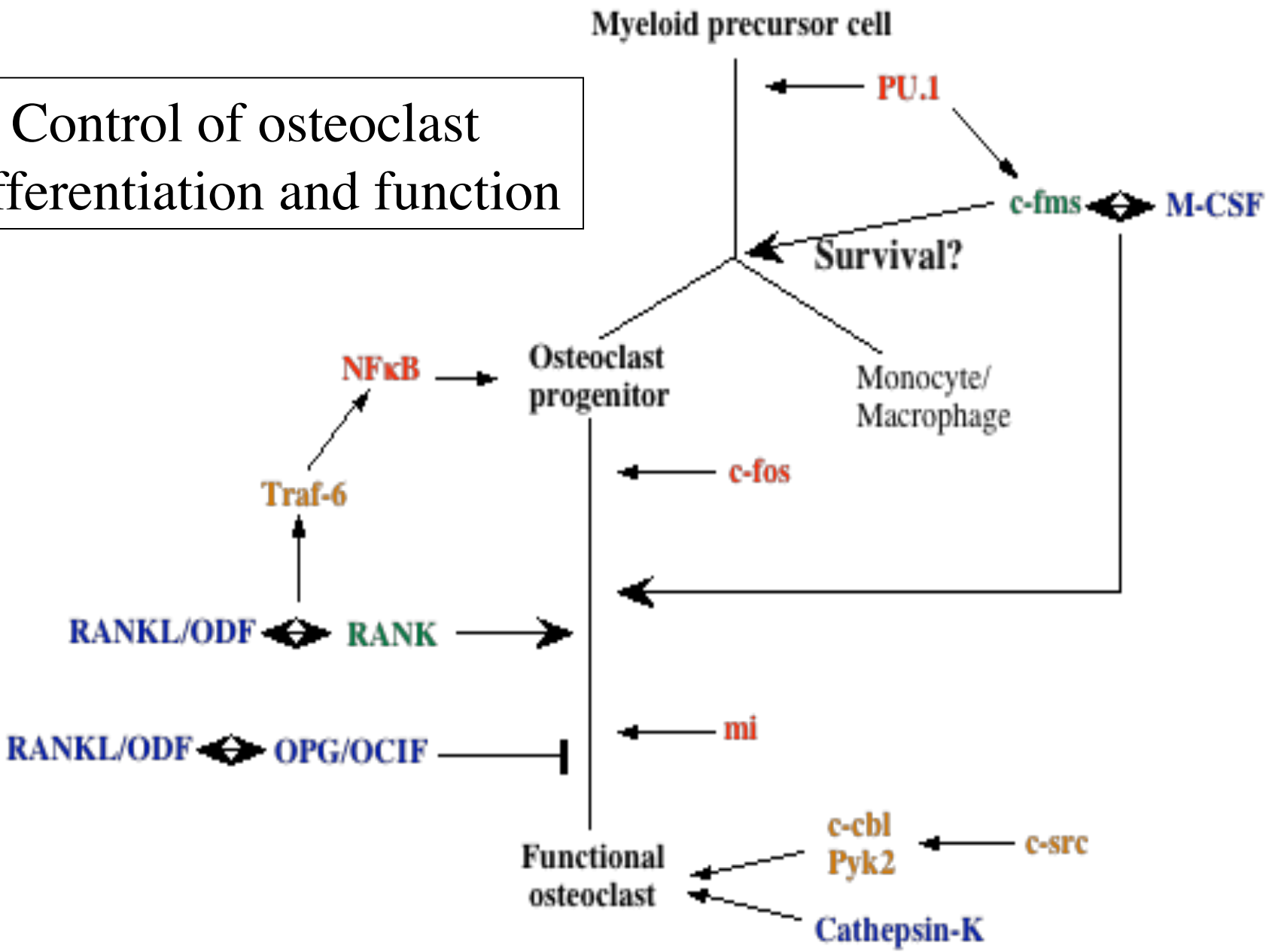
calcified cartilage

Growth plate Cartilage (chondrocytes)

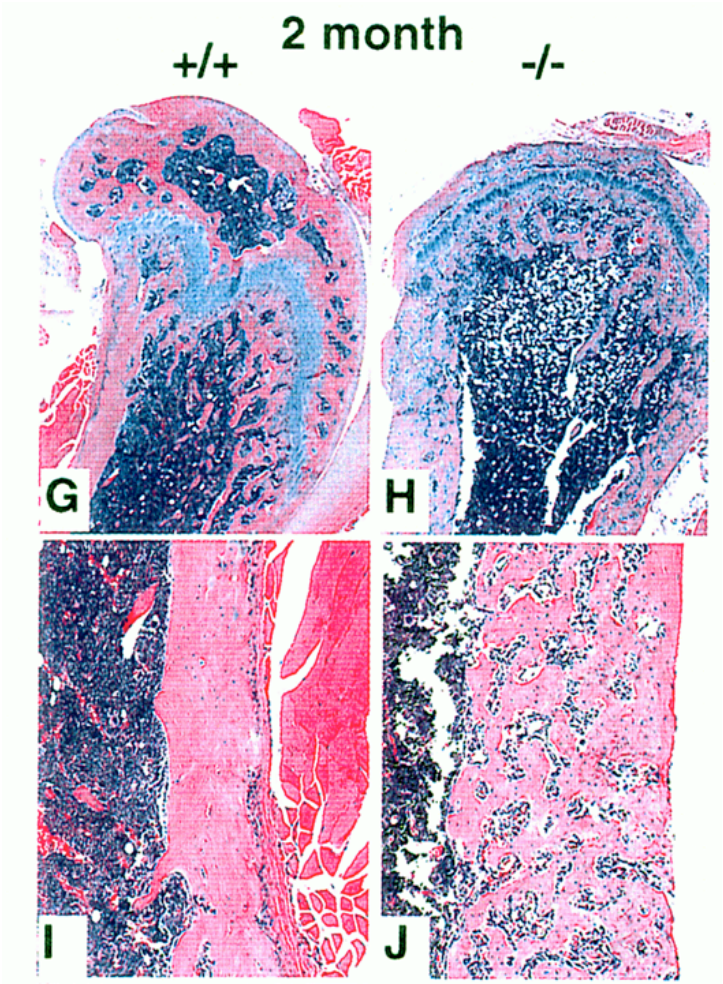
trabecular bone (osteoblasts/osteoclasts)

cortical bone (osteoblasts)

# Control of osteoclast differentiation and function

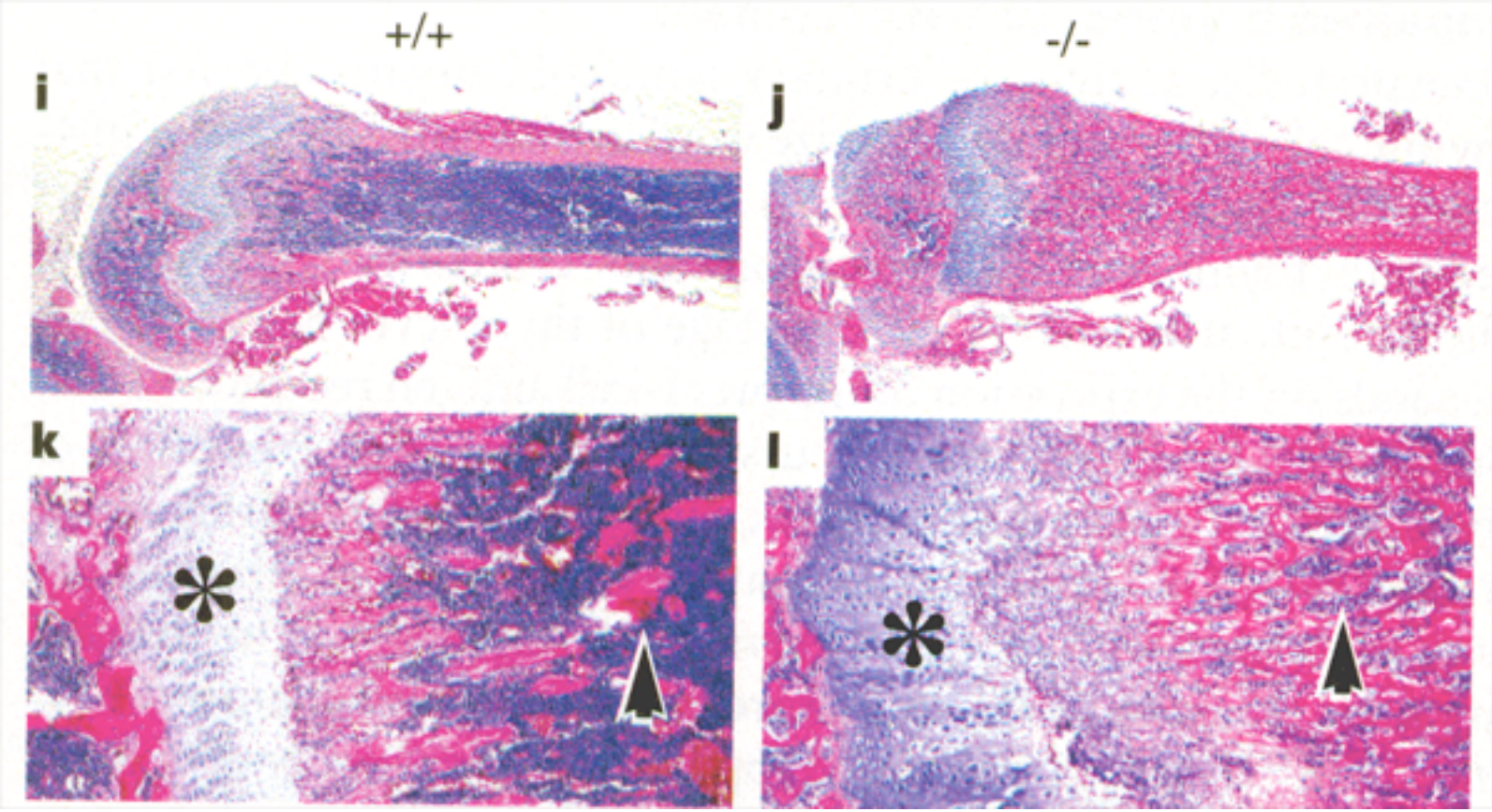


# Osteopenia in OPG-deficient mice

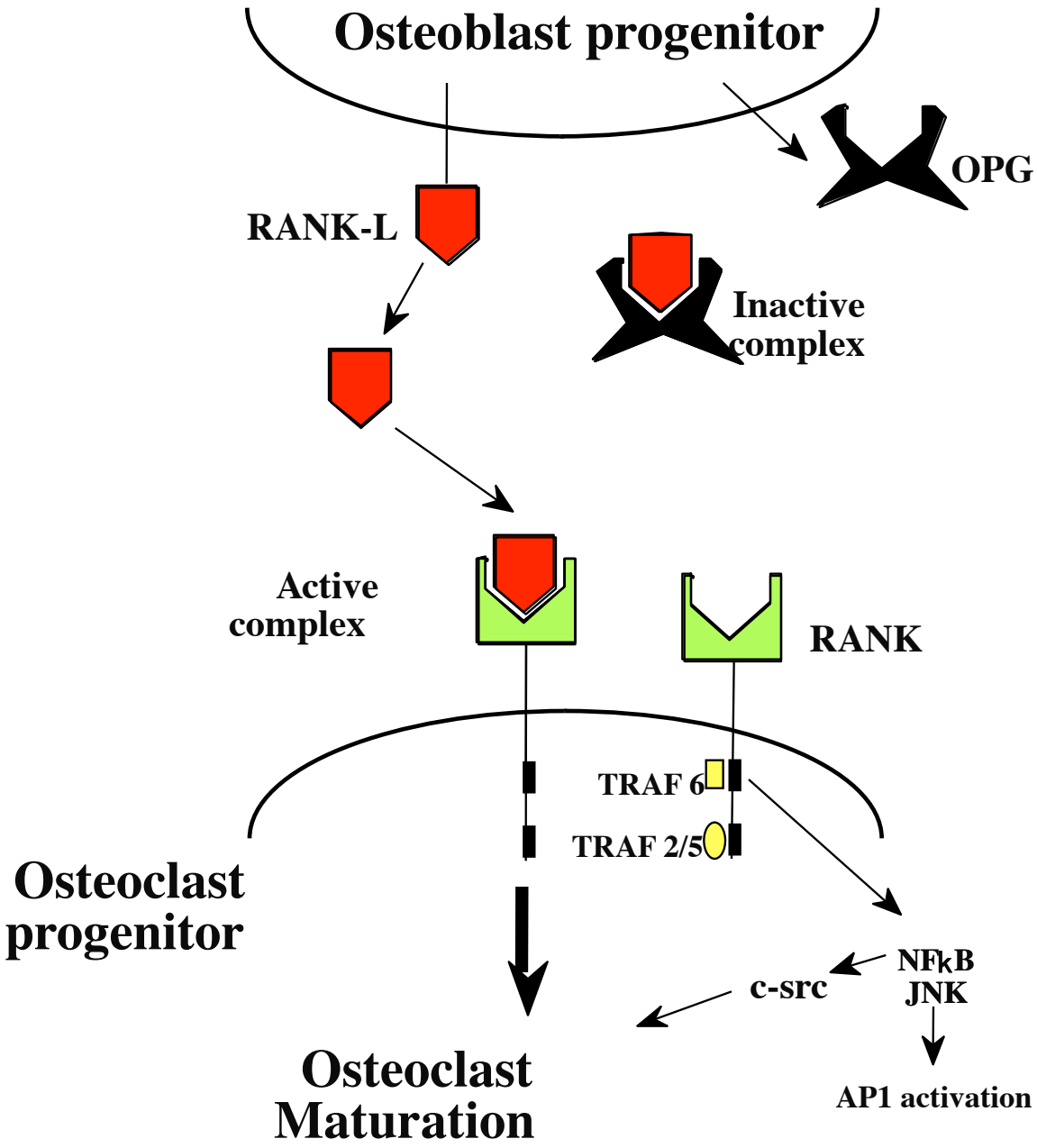



**Bucay et al., *Genes Dev*. 12 (1998)**

# Osteopetrosis in RANK-L deficient mice




**Lacey et al., *Cell* 93 (1998)**


Osteoblast progenitor
OPG
RANK-L
Inactive complex
Active complex
RANK
TRAF 6
TRAF 2/5
Osteoclast progenitor
NFkB
JNK
c-src
Osteoclast Maturation
AP1 activation

# Research directions