Skeleton Development

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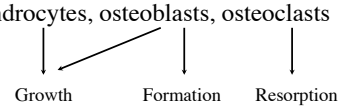
Skeleton

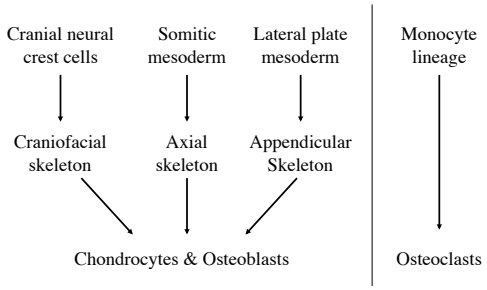
- ≥ 200 elements
- Two tissues: cartilage, bone
- Three cell types:
  chondrocytes, osteoblasts, osteoclasts



- Three "environments": marrow, blood, SNS

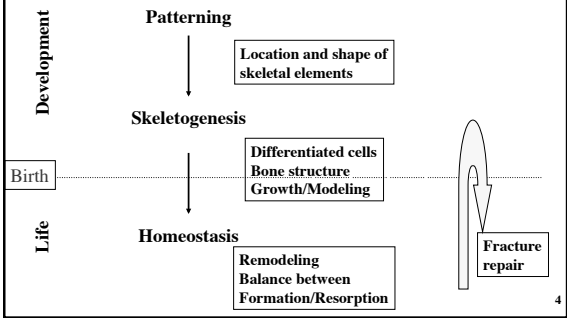
2

Embryonic origin of the skeleton



3

Skeleton Biology



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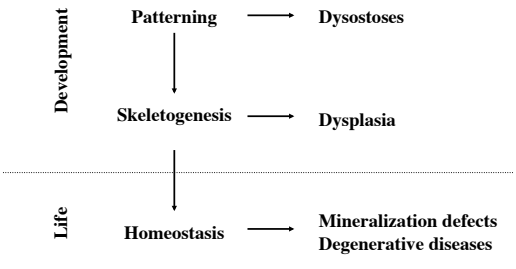
Skeleton Pathologies



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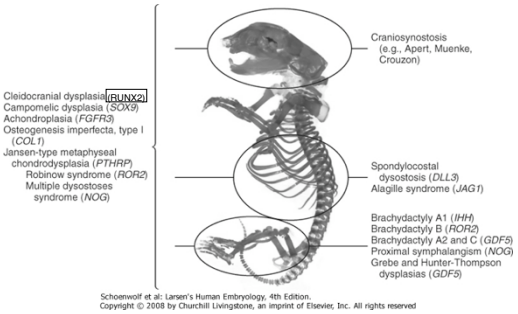
Genetic defects associated with skeleton development



Schoenwolf et al: Larsen's Human Embryology, 4th Edition.

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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

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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

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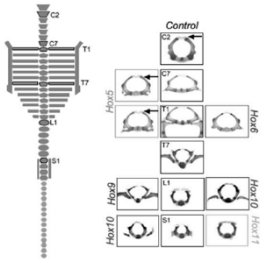
Homeotic transformations in absence of Hox transcription factors



Wellik, *Dev. Dynamics* 236 (2007)

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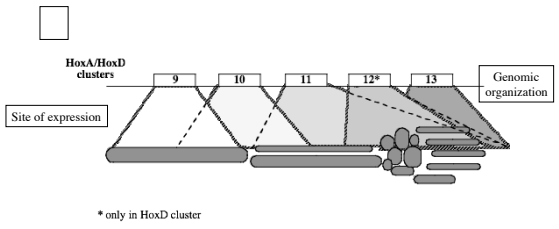
Hox transcription factors control vertebrate limb patterning



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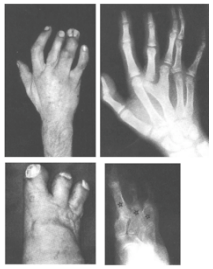
Mutations in HOXD13 cause synpolydactyly* in humans

*OMIM 18600, 186300



Patient with increased number of Ala repeat in HOXD13

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

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Skeletogenesis

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

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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

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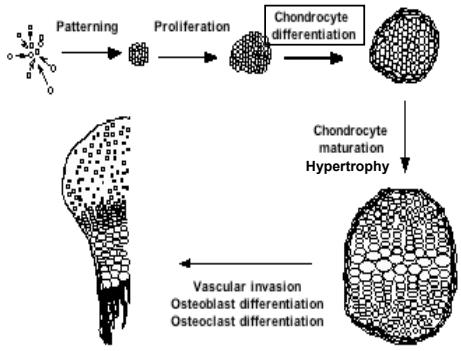
Endochondral ossification



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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

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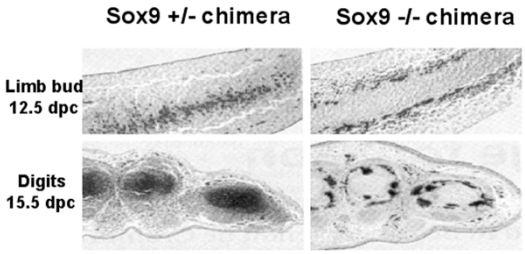
Sox9-deficient cells cannot differentiate into chondrocytes



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

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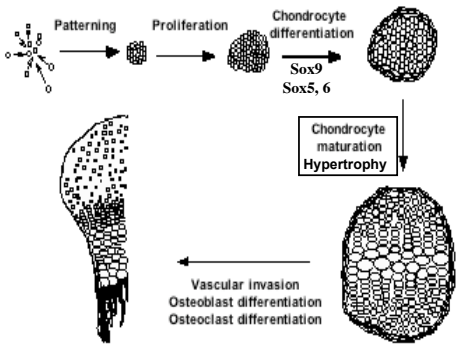
Endochondral ossification



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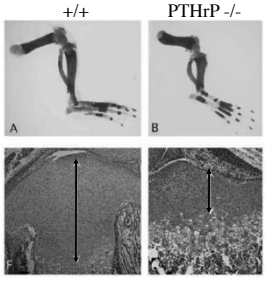
Accelerated chondrocyte hypertrophy in PTHrP-deficient mice



Karp et al., Development 127 (2000)

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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

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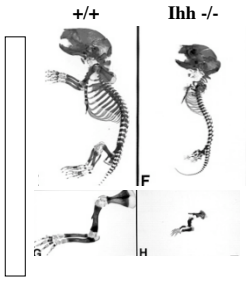
Dwarfism in Ihh-deficient mice



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

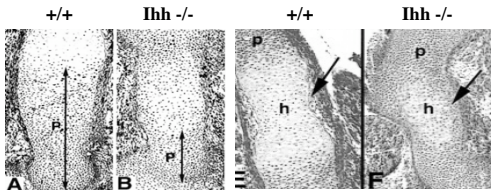
20

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

21

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



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

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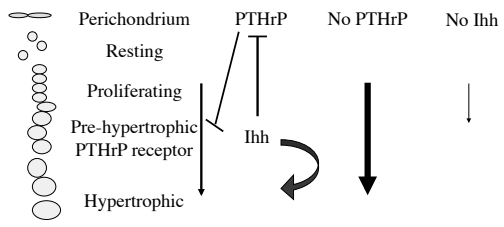
Chondrocyte maturation is regulated by a PTHrP/Ihh feedback loop



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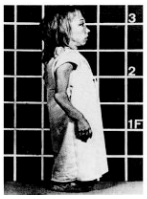
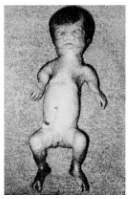
Mutations in the PTH/PTHrP receptor cause Jansen and Bloomstrand chondrodysplasia

Activating mutations





Loss-of-function mutations

Jansen metaphyseal chondrodysplasia OMIM 156400

Blomstrand's lethal chondrodysplasia OMIM 215045

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

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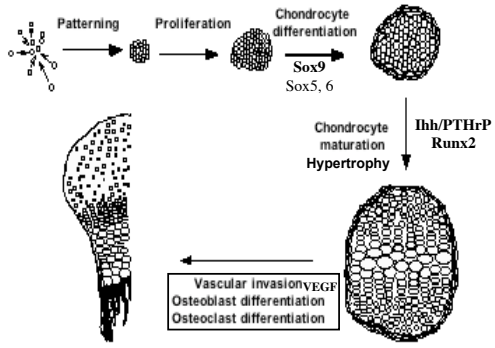
Endochondral ossification



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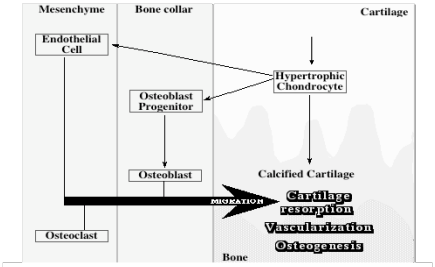
Endochondral ossification



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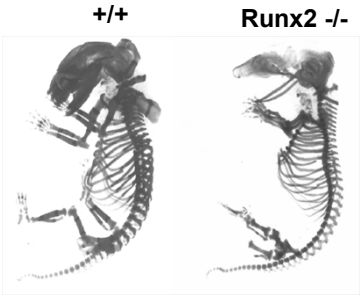
Arrest of osteoblast differentiation in Runx2-deficient mice



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

27

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

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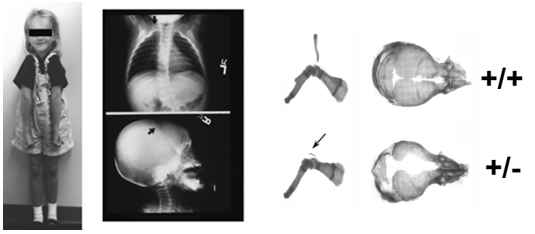
Cleidocranial dysplasia (CCD, OMIM 119600) is caused by Runx2 haploinsufficiency



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

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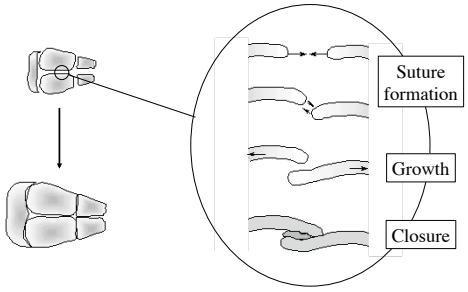
Intramembranous ossification



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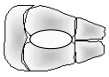
Disorders of suture fusion

Delay  Msx2, Runx2 haploinsufficiency

Acceleration = craniosynostosis

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

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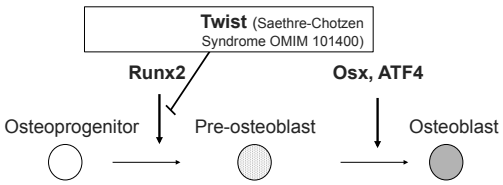
Osteoblast differentiation



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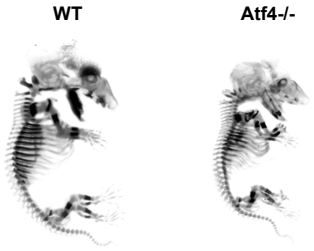
Delayed osteogenesis in absence of *Atf4*

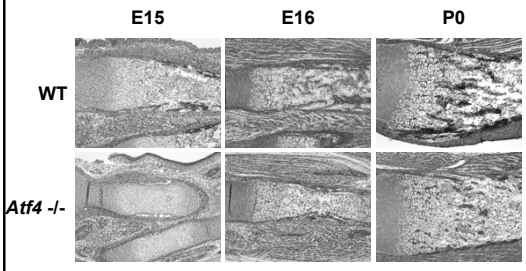


E14

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

33

Delayed osteogenesis in *Atf4-deficient mice*



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

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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

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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)

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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

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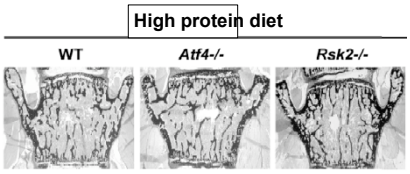
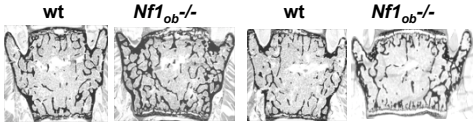
A high protein diet normalizes bone formation in *Atf4*-/- and *Rsk2*-/- mice



	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) 38

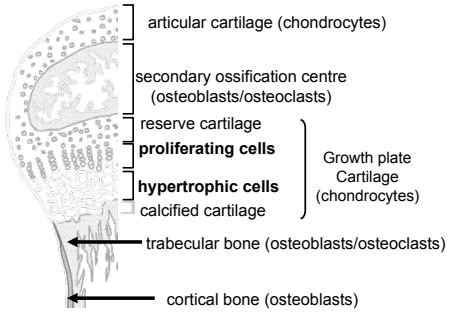
A low protein diet normalizes bone formation in a mouse model of Neurofibromatosis type I



	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) 39

Structure of a growing long bone



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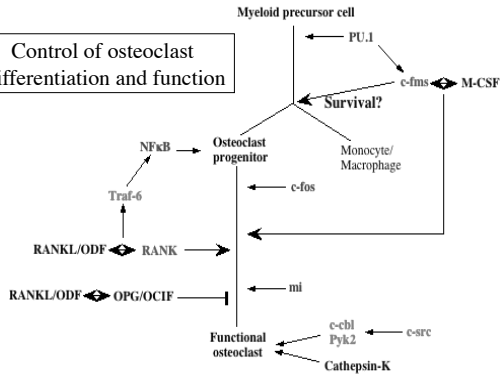
Control of osteoclast differentiation and function



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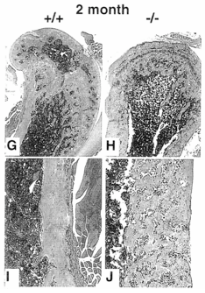
Osteopenia in OPG-deficient mice



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

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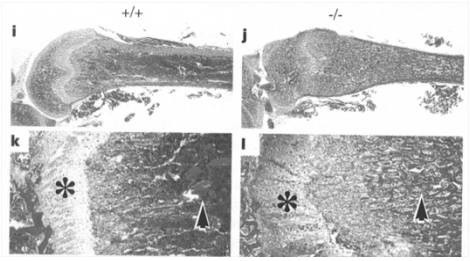
Osteopetrosis in RANK-L deficient mice



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

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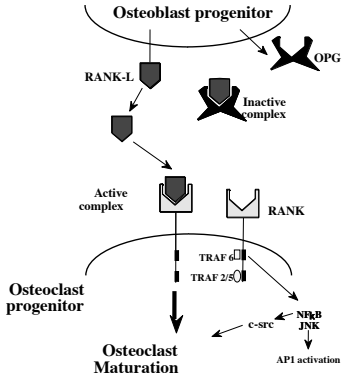

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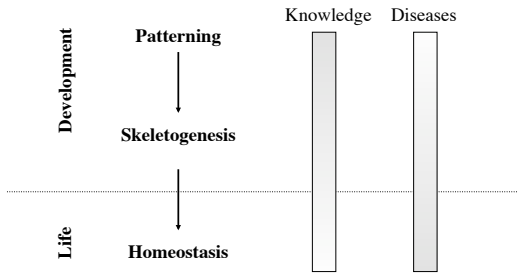
Research directions



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