Instituts thématiques

Inserm

Institut national de la santé et de la recherche médicale

université PARIS DIDEROT PARIS 7

Osteoblasts

(my favorite cell…)

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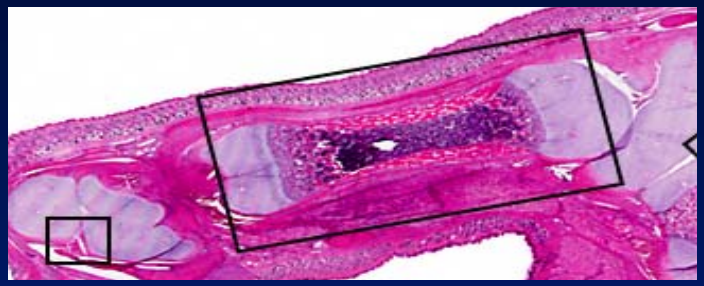
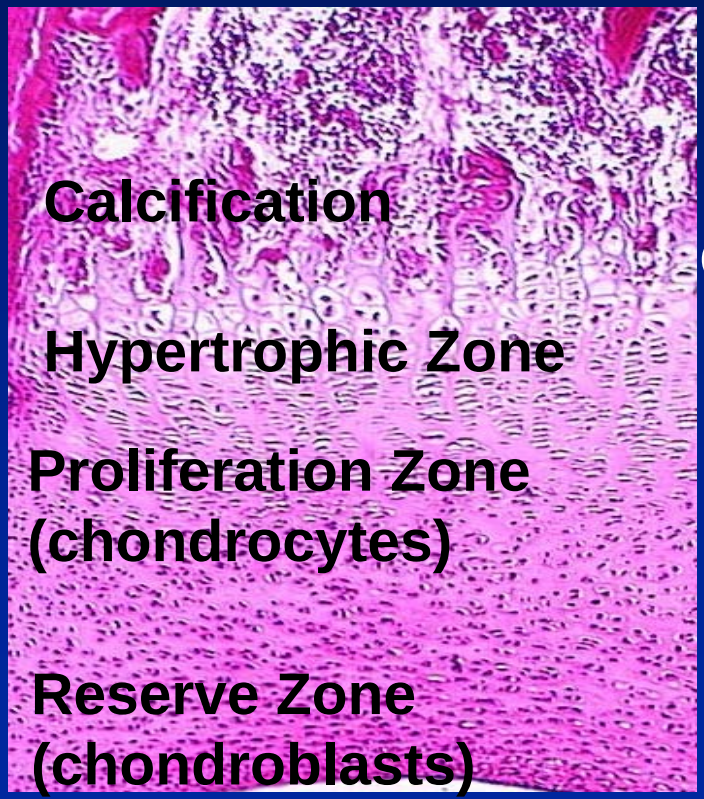
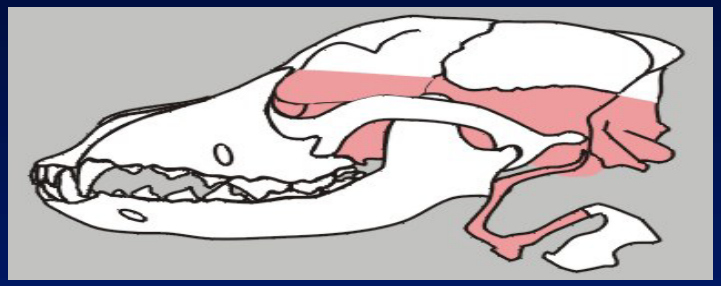
Bone Formation During Development: Two Types

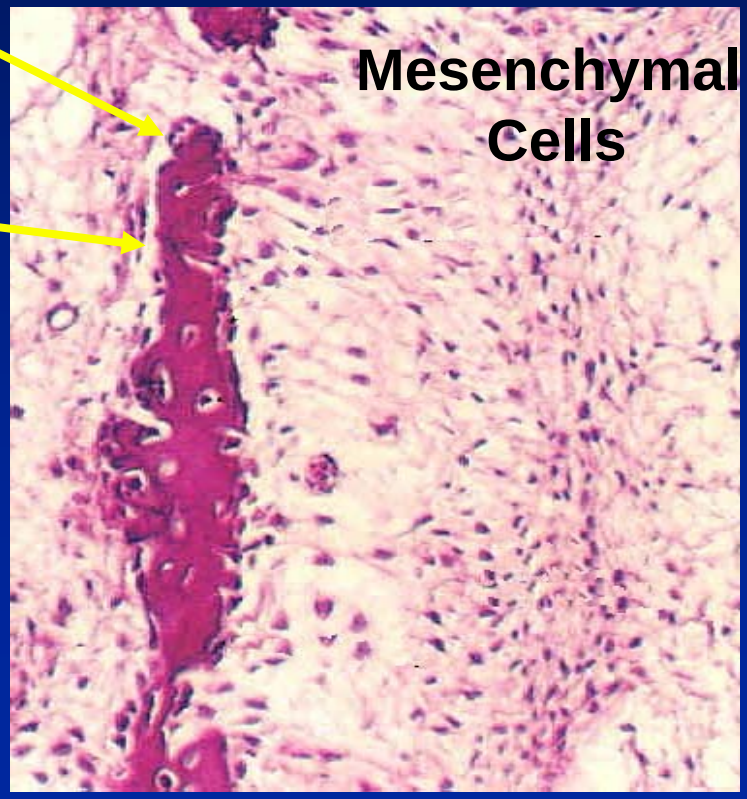
Long Bones (Humerus)



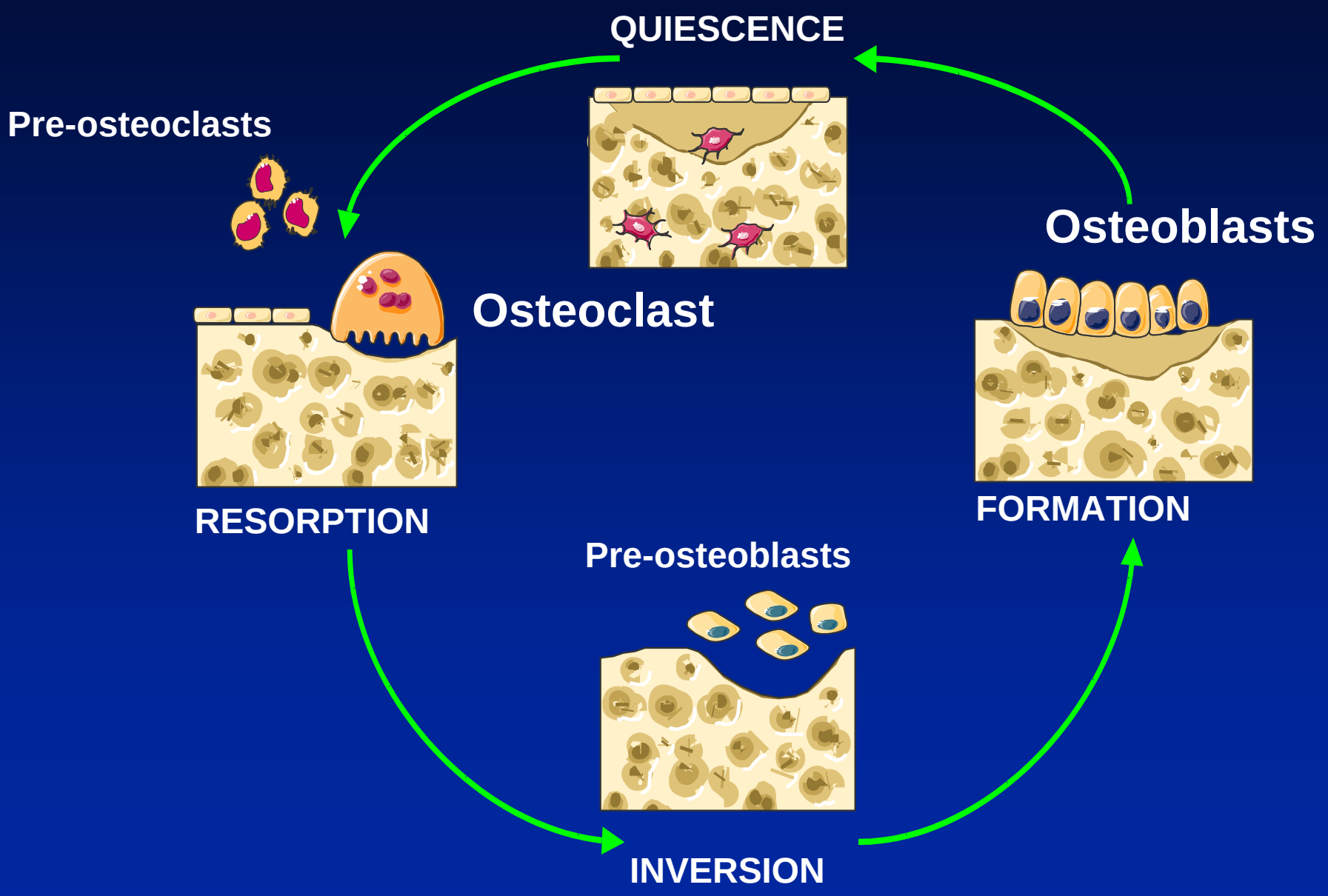


Membranous Bones (Cranial Vault)





Bone formation during post-natal remodeling



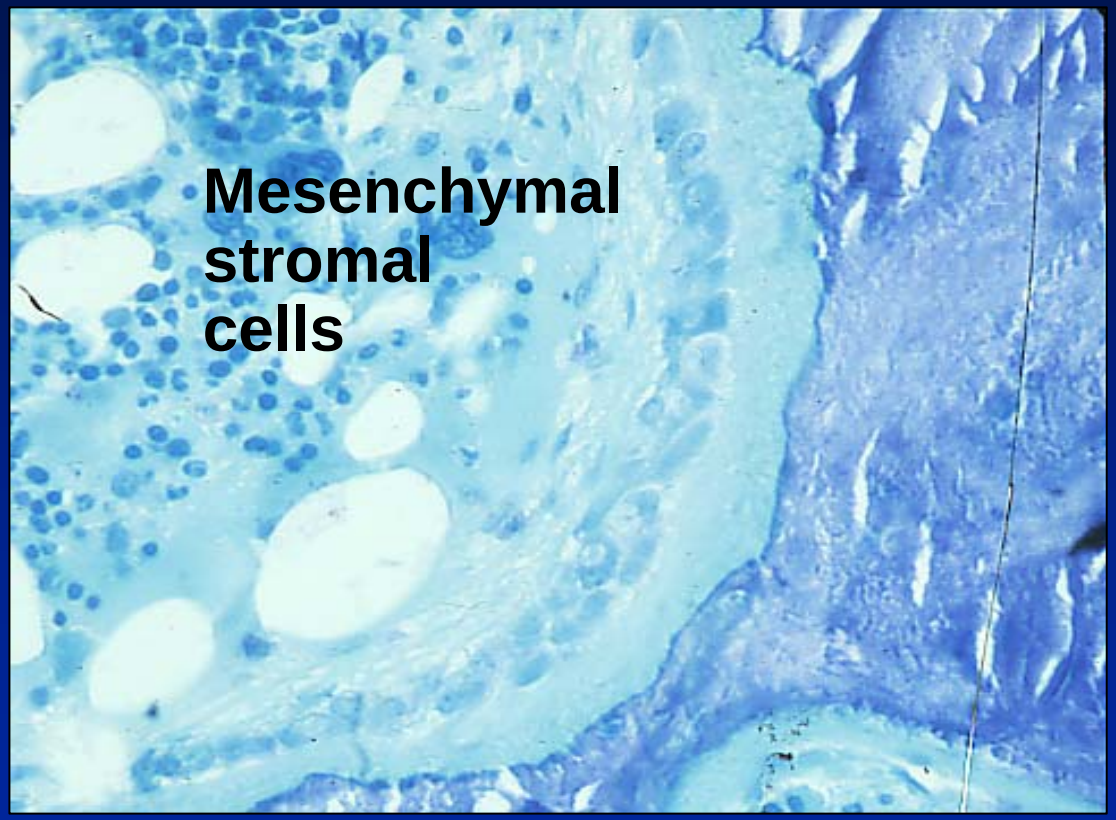
Sources of osteoblasts in the post natal life

Several sources

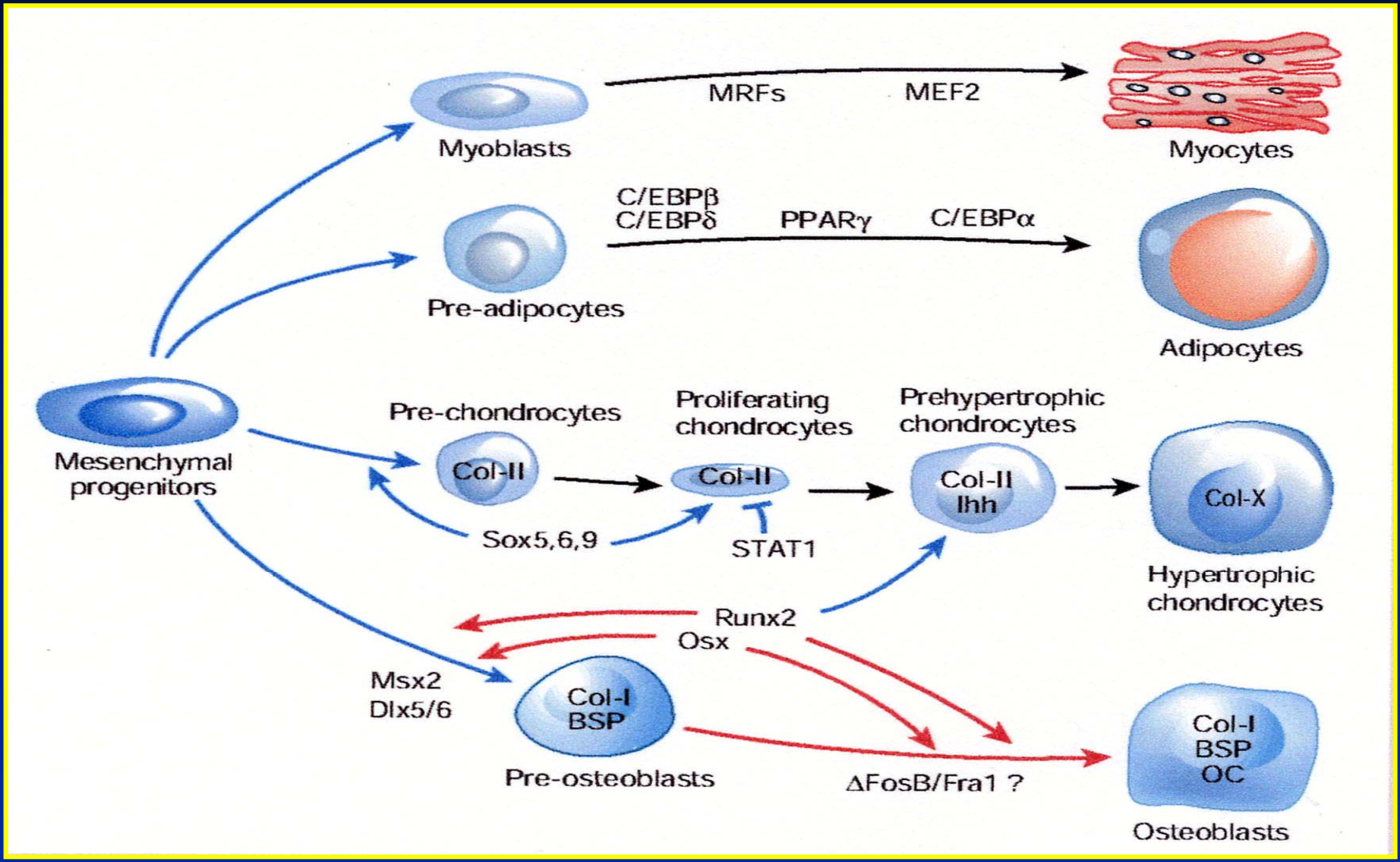
- Bone marrow stroma

-Pericytes

- adipose tissue
- muscle
- circulation (?)

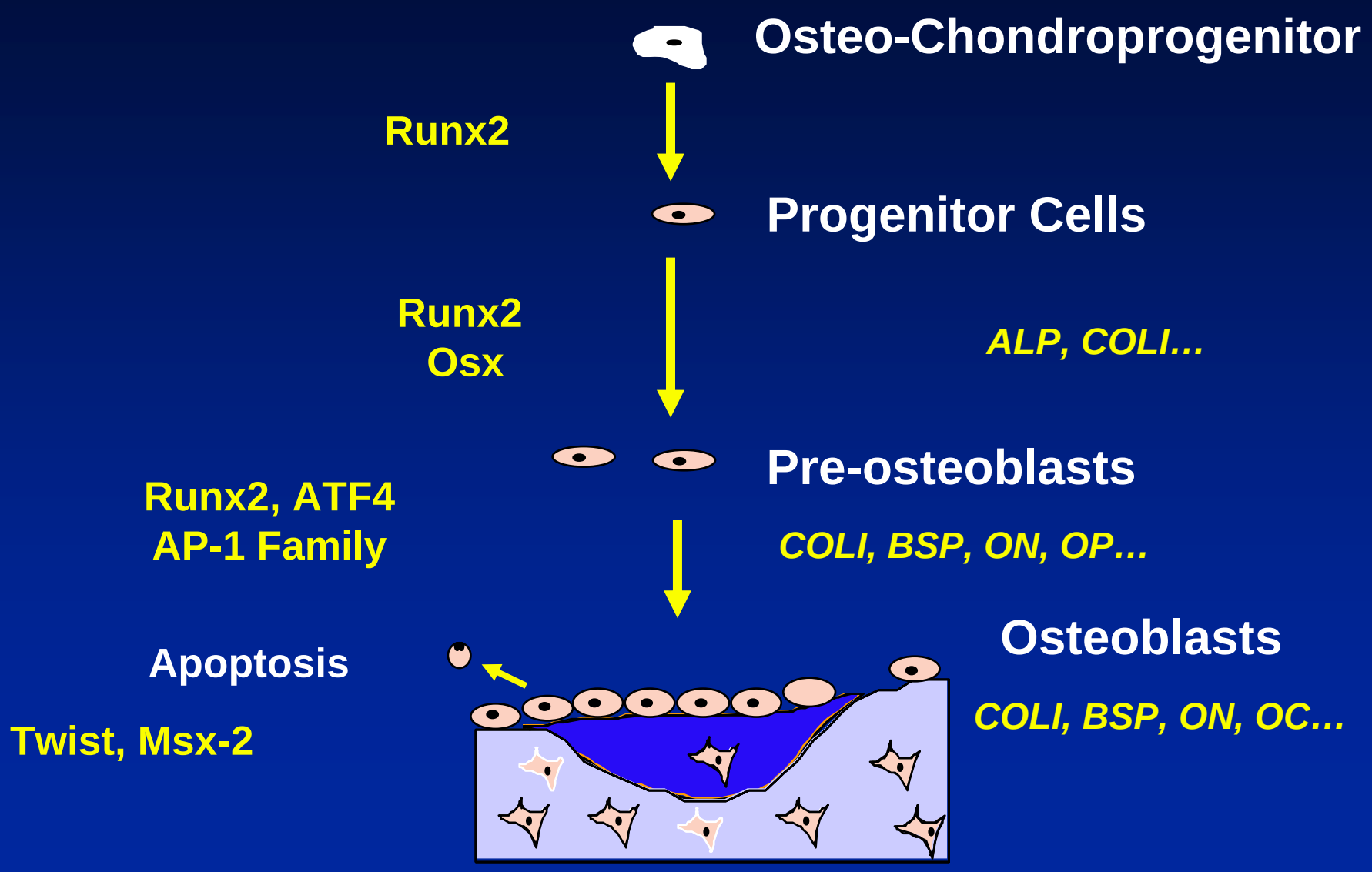


Osteoblast Differentiation

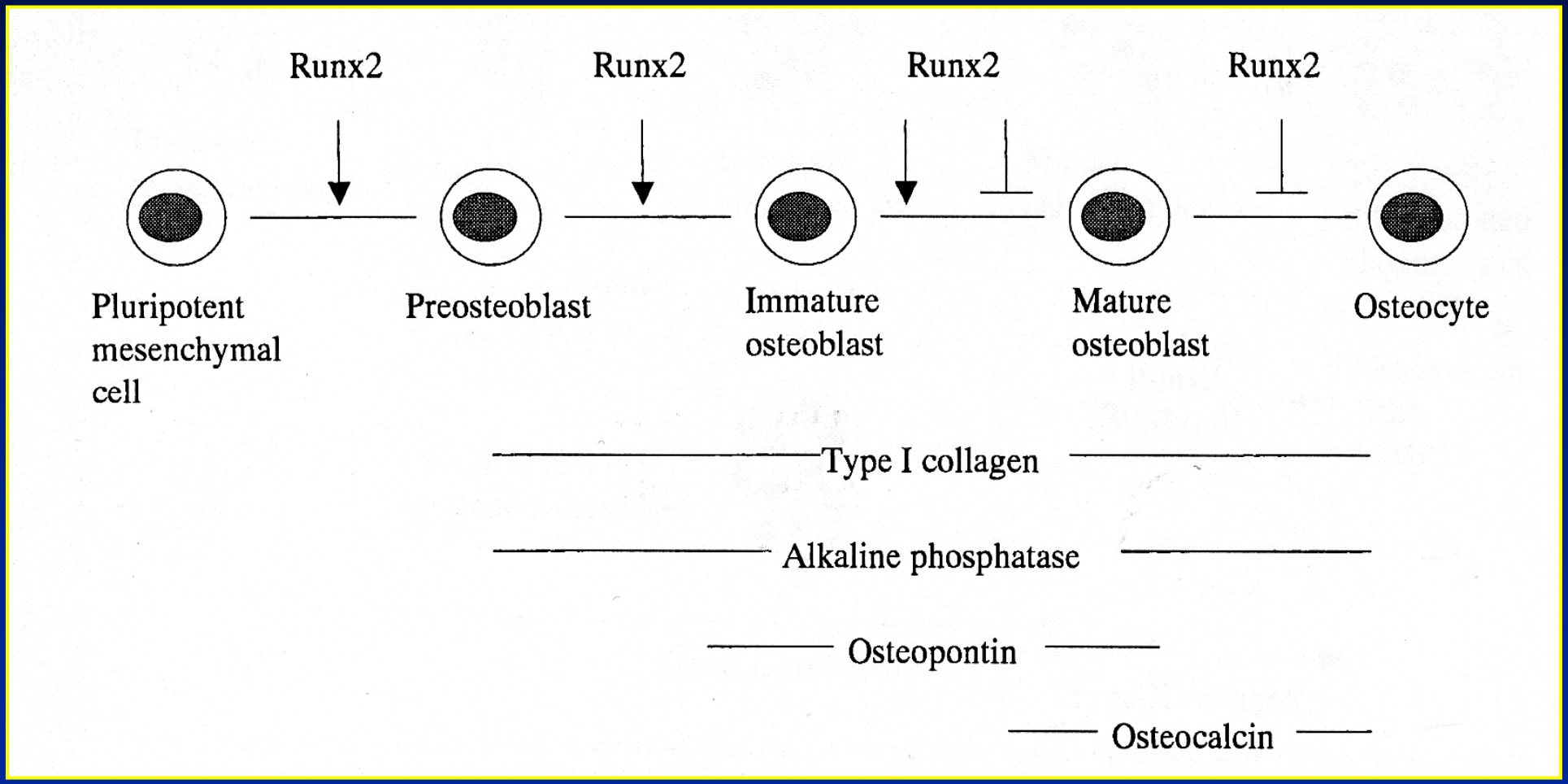


Harada & Rodan, Nature, 2003

Main Transcription Factors Involved in Postnatal Osteogenesis *in vivo*

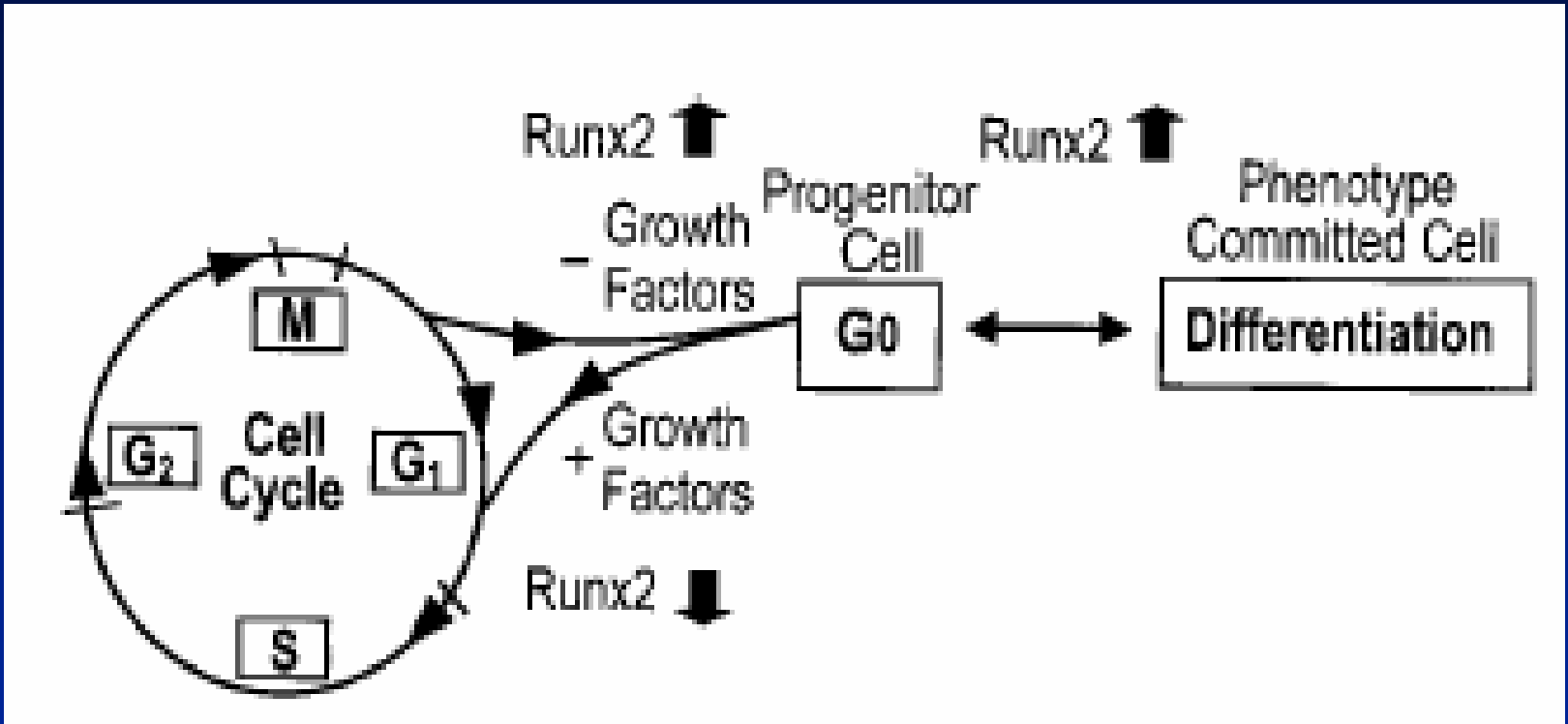


Runx2 Controls Osteoblast Differentiation



Ducy and Karsenty, 1997; Komori, 1997

Runx2 Controls Osteoblast Proliferation



Pratap et al., Cancer Res. 2003

Galindo et al., JBC 2005

Runx2 is Highly Regulated

Hoxa-2 (homeobox)
Dlx3 (homeobox)
Aj18 (ZNF protein, competes for DNA binding))
Myeloid Elf-1 factor (MEF)
Stat1
Sox8, Sox9
Nrf2

-

Runx2
Expression or function

+

Msx2 (homeobox)
Bapx1(homeobox)
Dlx5 (homeobox)
Rb
SATB2 (nuclear matrix protein)
Hoxa-10 (homeobox)
Taz (transcriptional activator)
C-Fos, JunD, JunB, Fra2

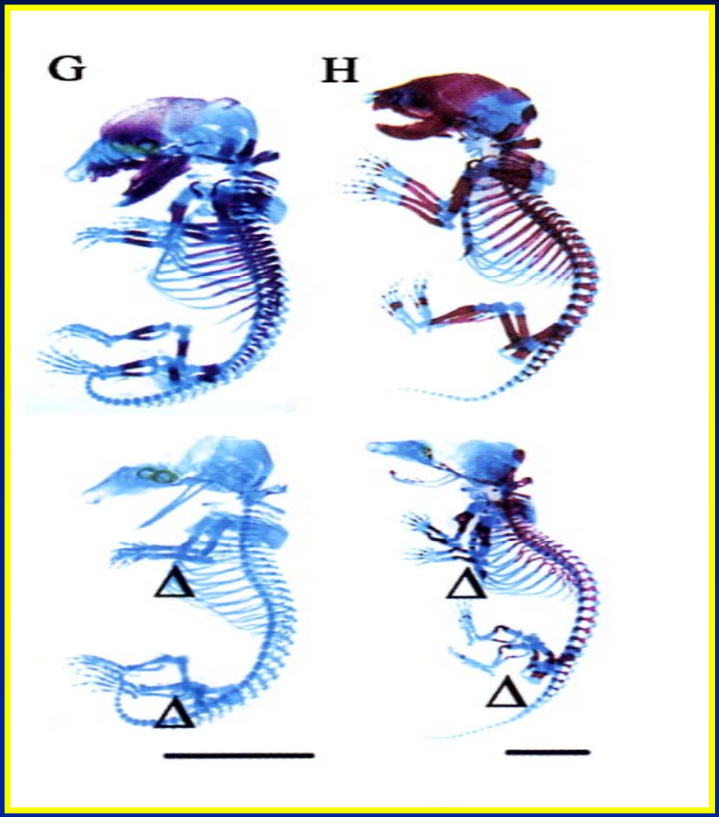
Smurf1
Schnurri-3
CyclinD1

(promotes proteasome degradation)

Marie PJ, ABB, 2008

Osterix (Osx) Controls Bone Formation

E15.5 Newborn

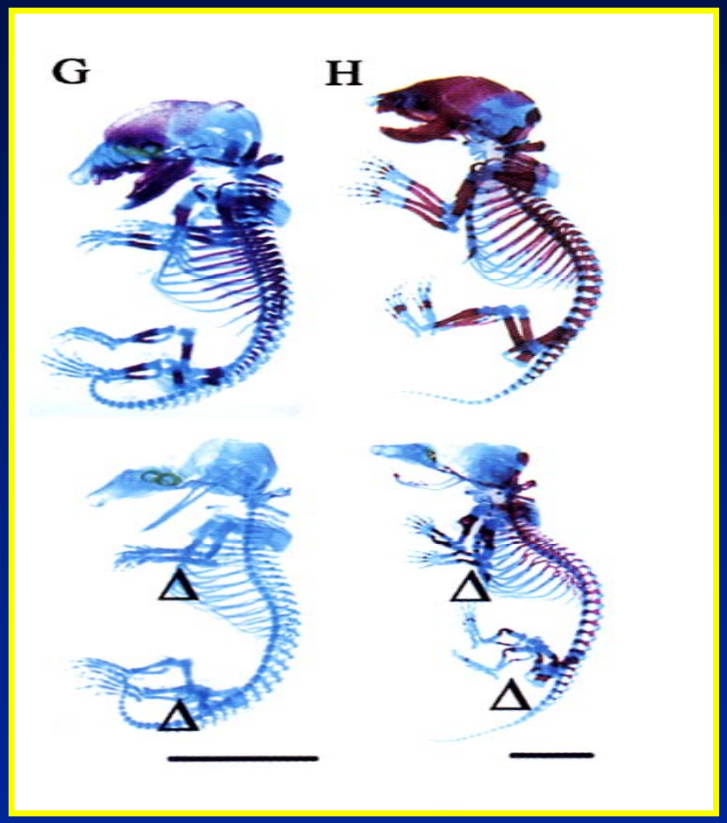


Defective mineralization

Nakashima et al., Cell, 2002

Osterix (Osx) Controls Bone Formation

E15.5 Newborn



Defective mineralization

Nakashima et al., Cell, 2002

Osx Regulation

NFAT
Nuclear factor of activated T cells:
Interacts with Osx

↓

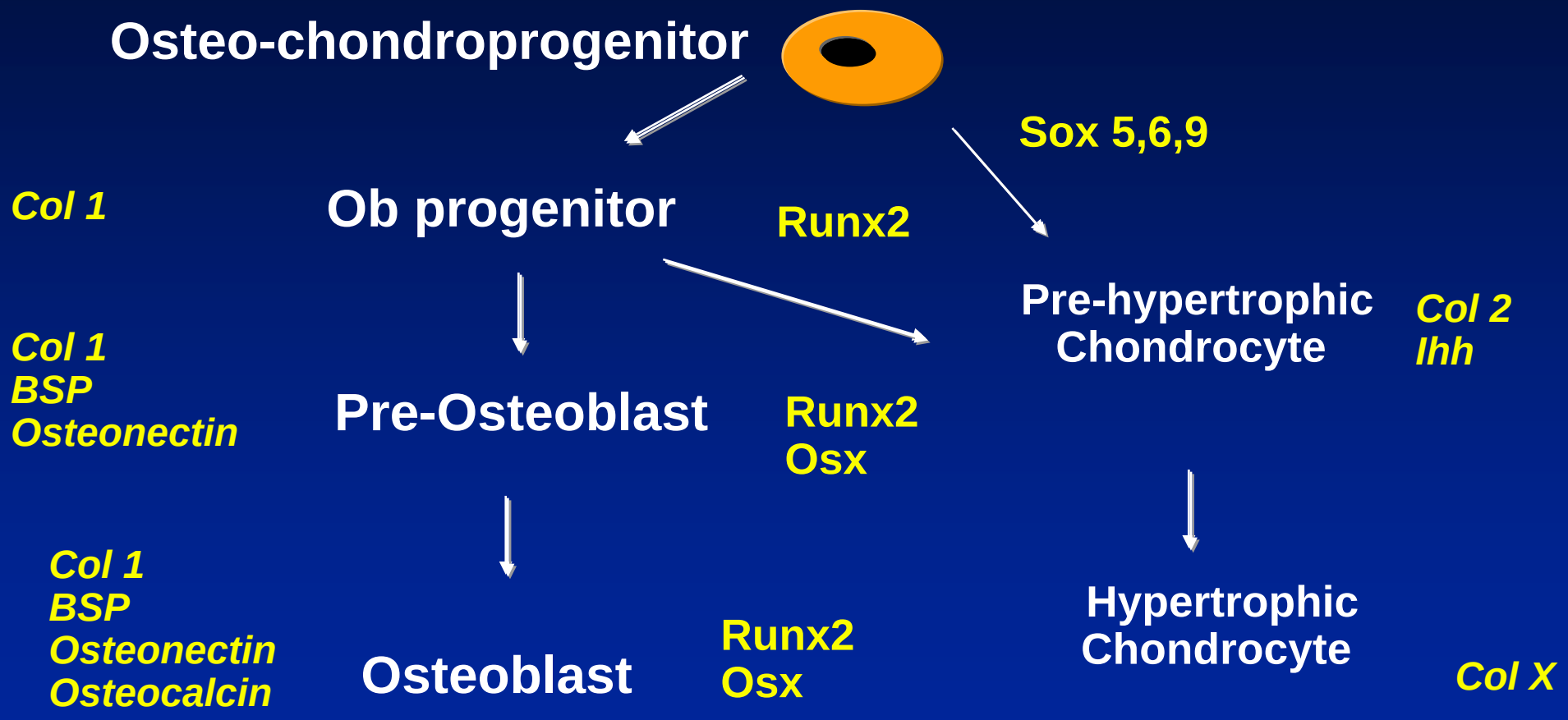
Osx

⊤

p53
(tumor suppressor)

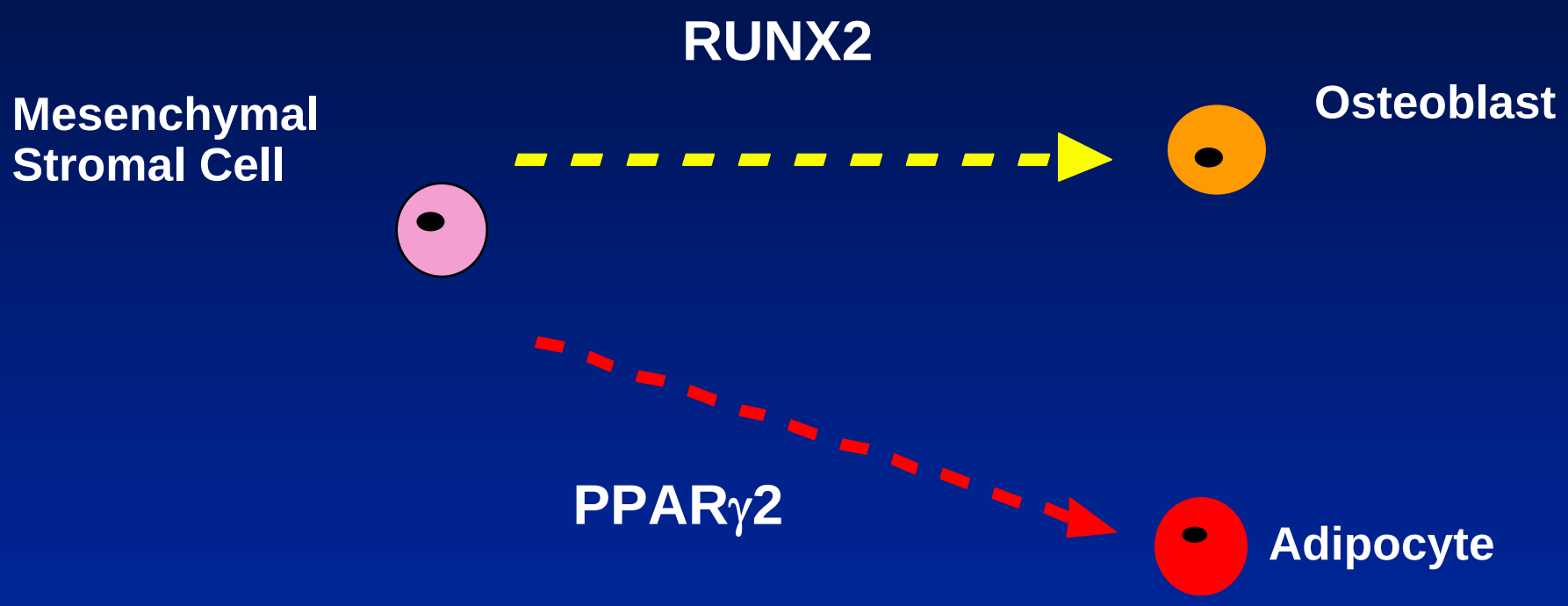
Koga et al., Nat Med, 2005
Wang et al., J Cell Biol, 2006

Osx Acts Downstream of Runx2



Nakashima et al., Cell, 2002

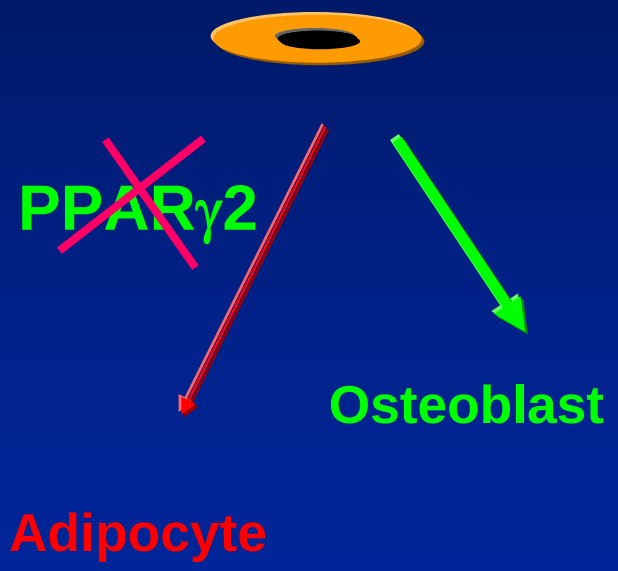
PPARγ Controls Mesenchymal Cell Differentiation

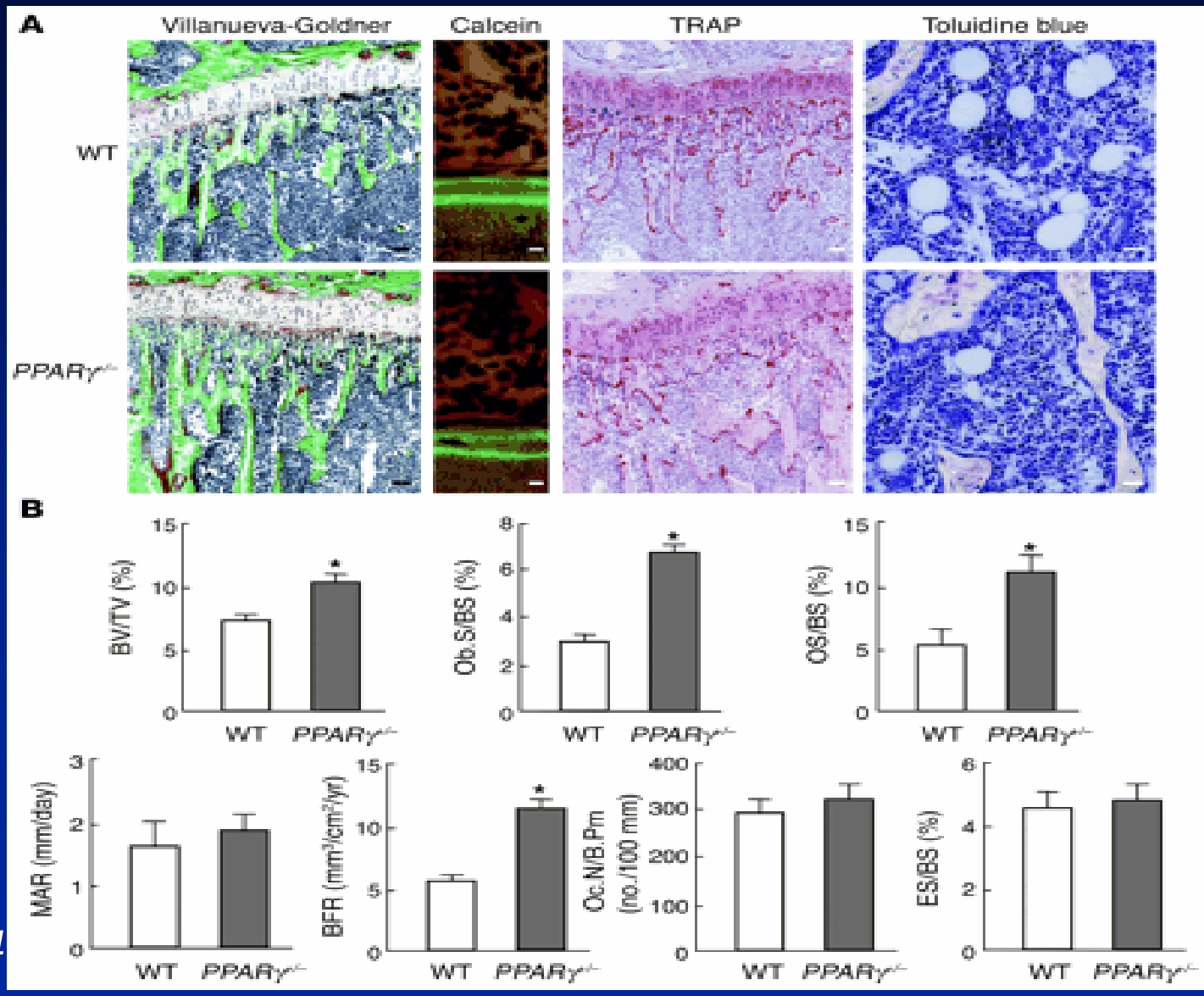


Invalidation of PPARγ2 :

Increased bone formation

Mesenchymal cell





Akune et al, J Clin Invest, 2004

ATF4 : an atypical transcription factor that regulates osteoblasts

•**Coffin-Lowry syndrome:** mutation in **RSK2** (growth factor related kinase): inactivation of RSK2 and bone loss

RSK2

↓ phosphorylation

ATF4 (cAMP-response element-binding protein)

↓

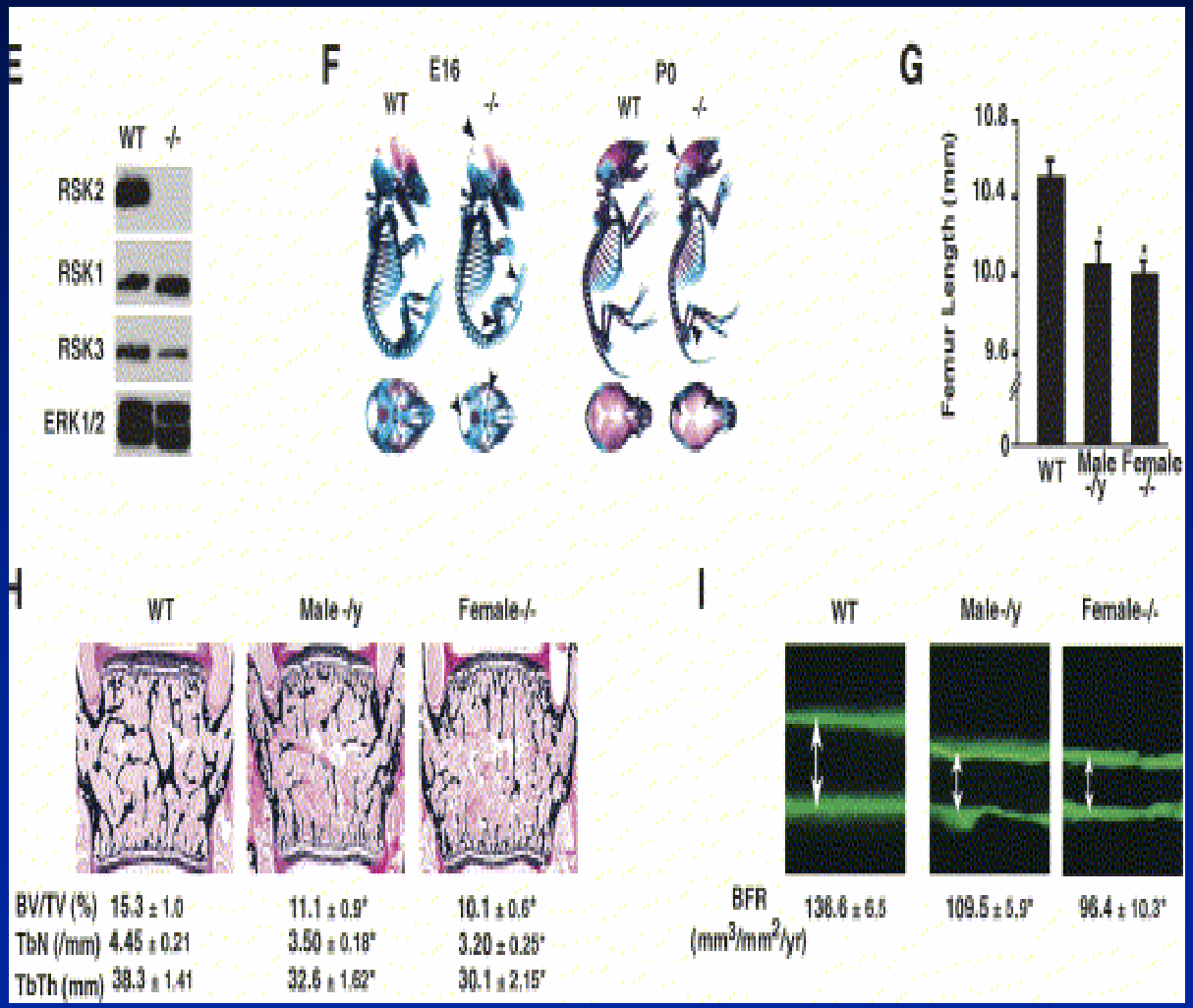
- ATF4 accumulation (low degradation) in Ob: binds OSE1 in the OC promoter induces OC expression

- Enhances collagen synthesis postranscriptionally

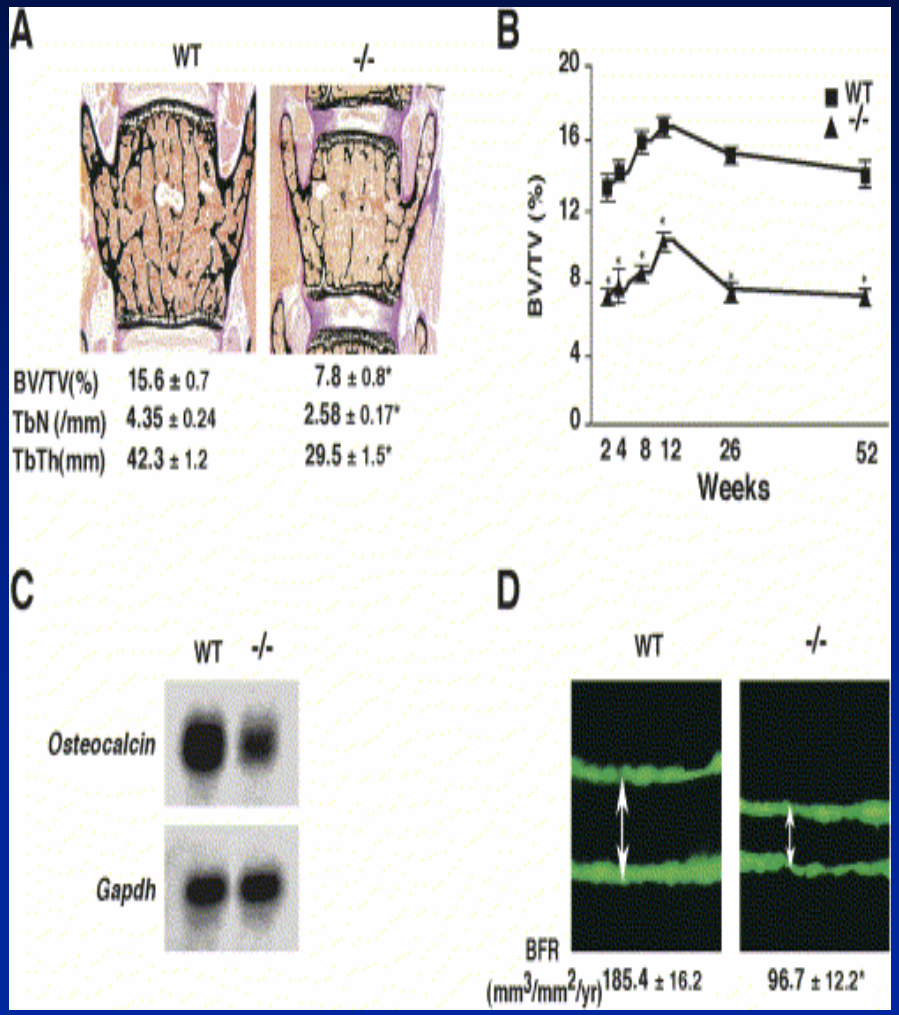
Yang et al., Cell, 2004; JBC, 2004

ATF4 is a Substrate for RSK2 and Controls Osteoblast Differentiation

Invalidation of RSK2 : Osteopenia

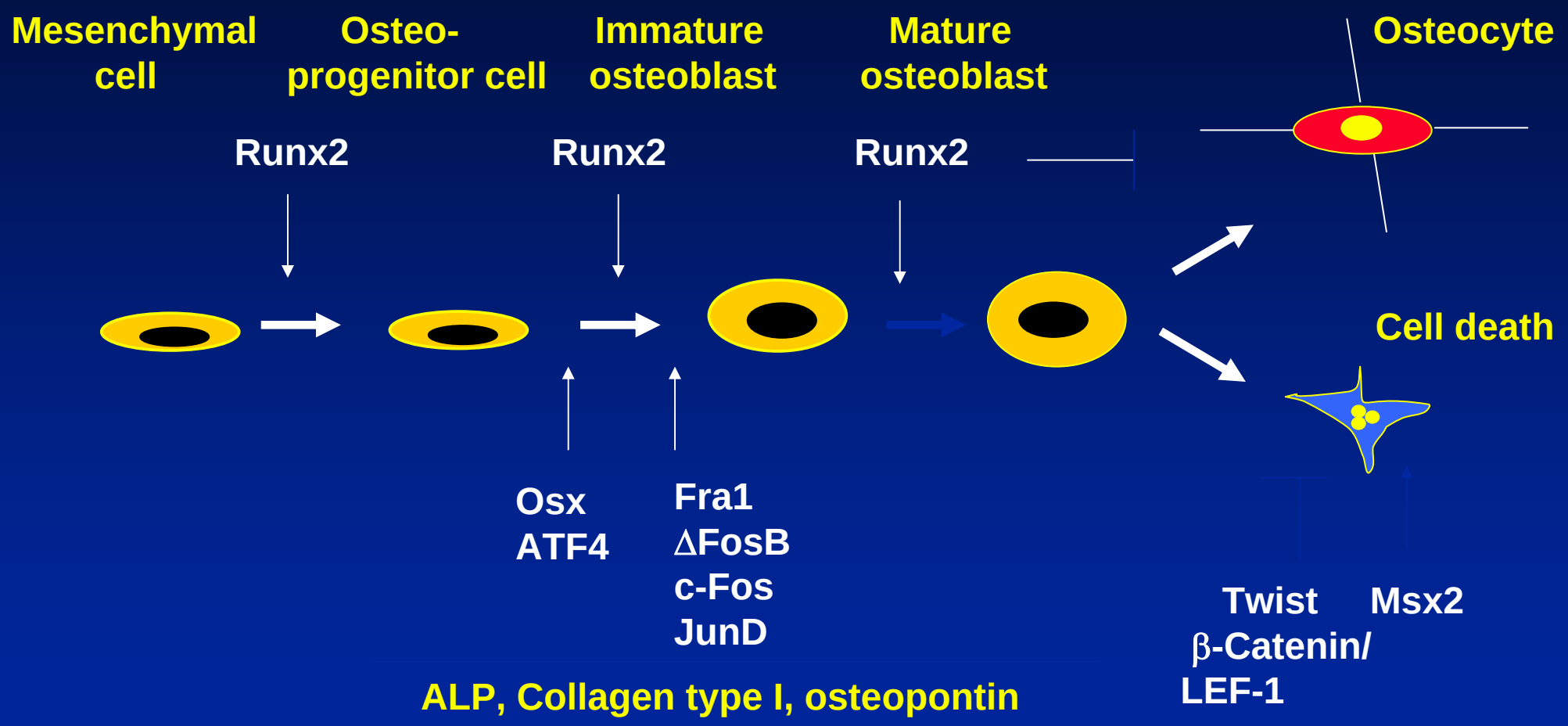


Invalidation of ATF4 : Osteopenia



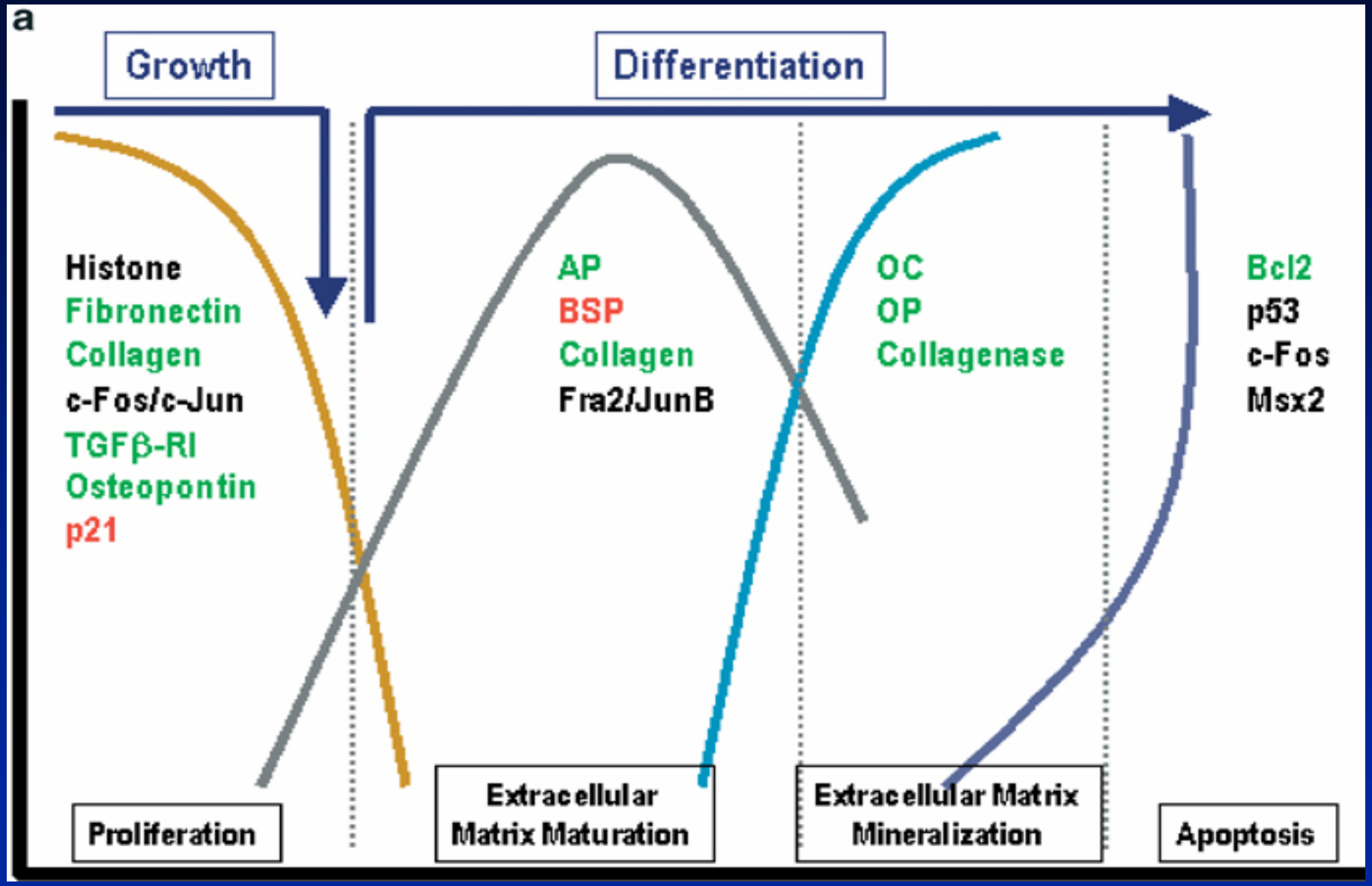
Yang et al., Cell, 2004; JBC, 2004

Summary: Multiple transcription factors control osteoblastogenesis



Marie PJ. Arch Biochem Biophys, 2008

Sequence of Osteoblast Differentiation *in vitro*



Stein et al., Oncogene 2004

The Main Function of Osteoblasts: Production of Bone Matrix

Main components:

Collagen (type 1: 90 %)

Non collagenous proteins

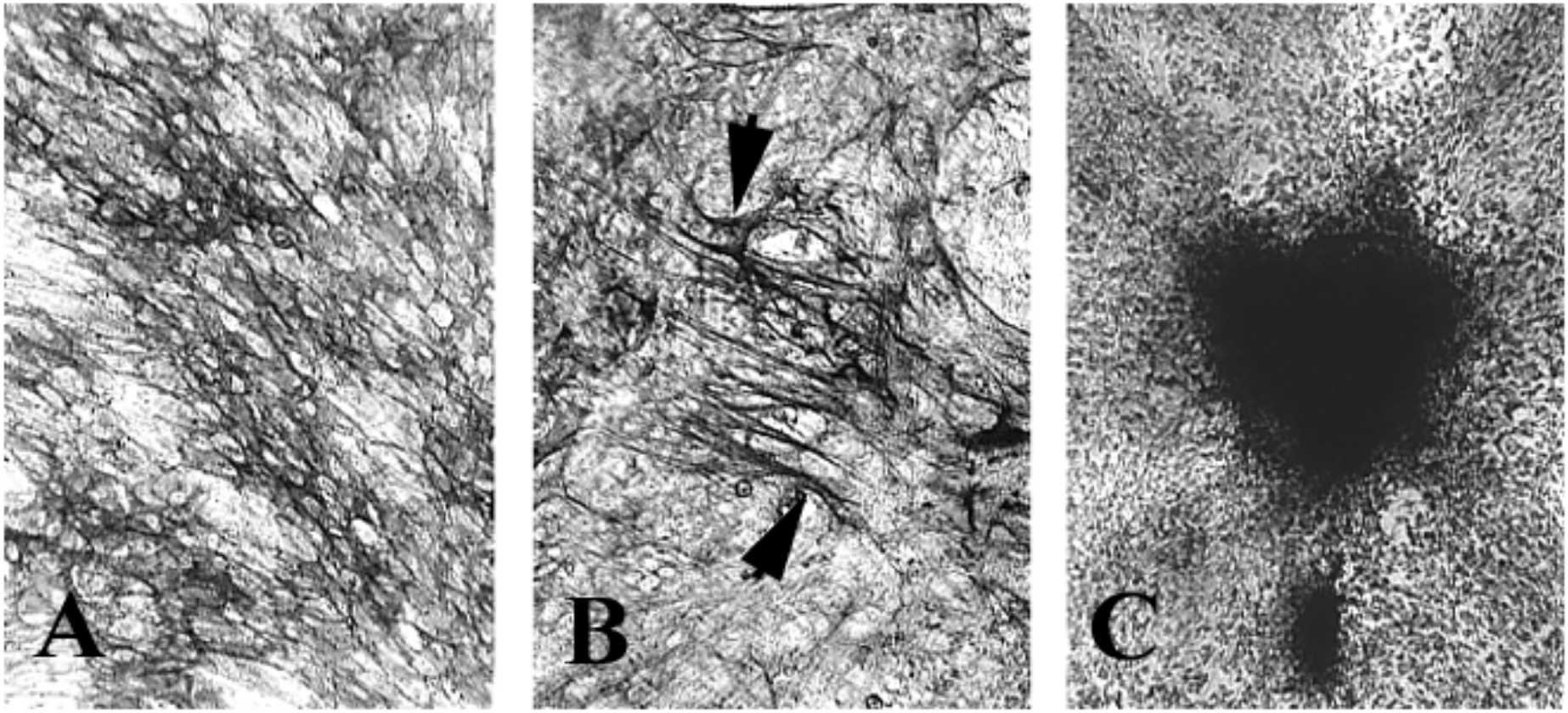
Glycosaminoglycans

Growth Factors

Type 1 collagen

Sequence of Mineralization *in vitro*

Alkaline phosphatase **Type I collagen** **Bone nodules**

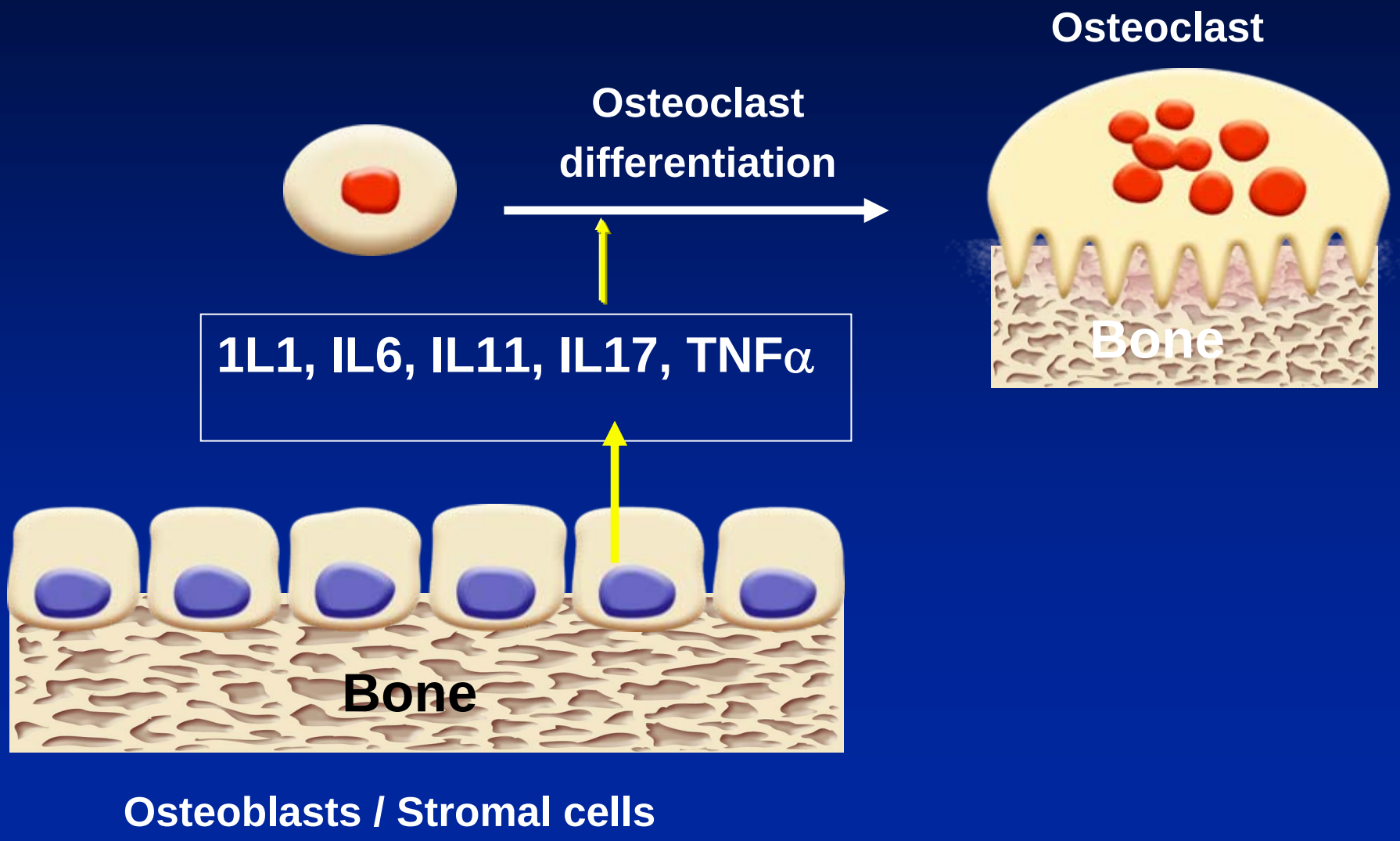


Marie et al., 1989

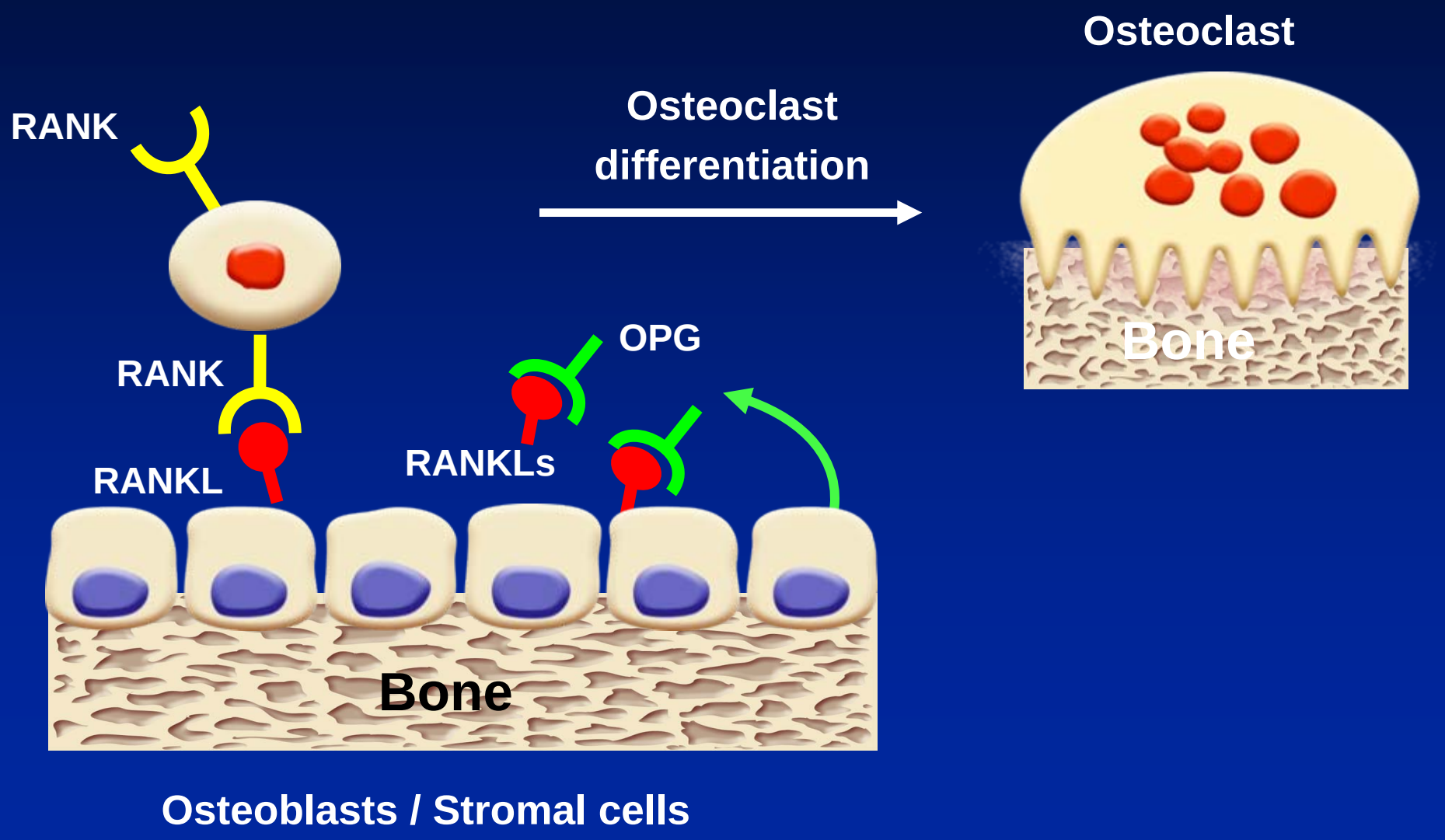
Role of Proteins Produced by Osteoblasts

Gene	Role in bone	KO / Mutation
ALP	Mineralization	Osteomalacia
Collagen	Structure	Abnormal bone matrix (OI)
Osteocalcin		Increased bone mass
Osteopontin	Osteoclast adhesion	Increased bone mass
Sialoprotein	Mineralization	Decreased bone formation & bone repair
Ostonectin	?	Decreased bone formation
Matrix Gla Protein	Inhibits mineralization	Blood vessel calcification
Biglycan	Binds TGFß	Decreased bone formation

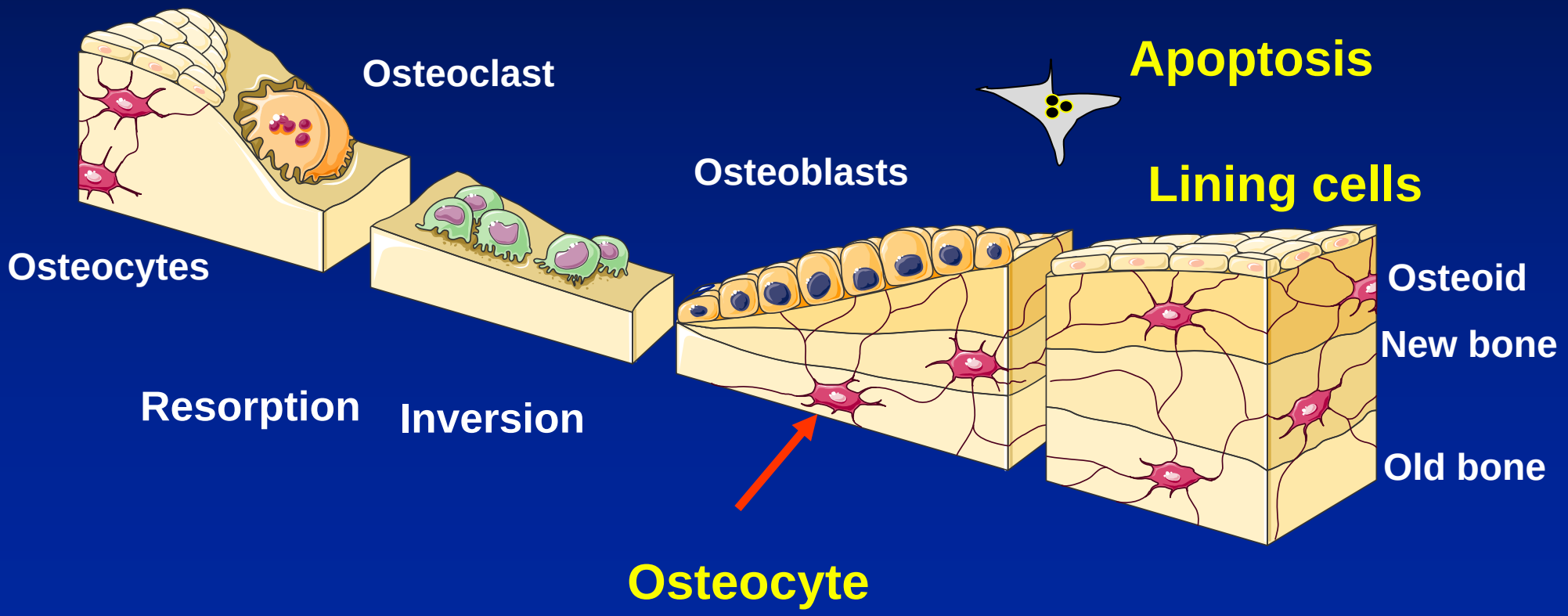
Other function of osteoblasts: Osteoblasts control osteoclastogenesis



Osteoblasts control osteoclastogenesis through RANKL / OPG

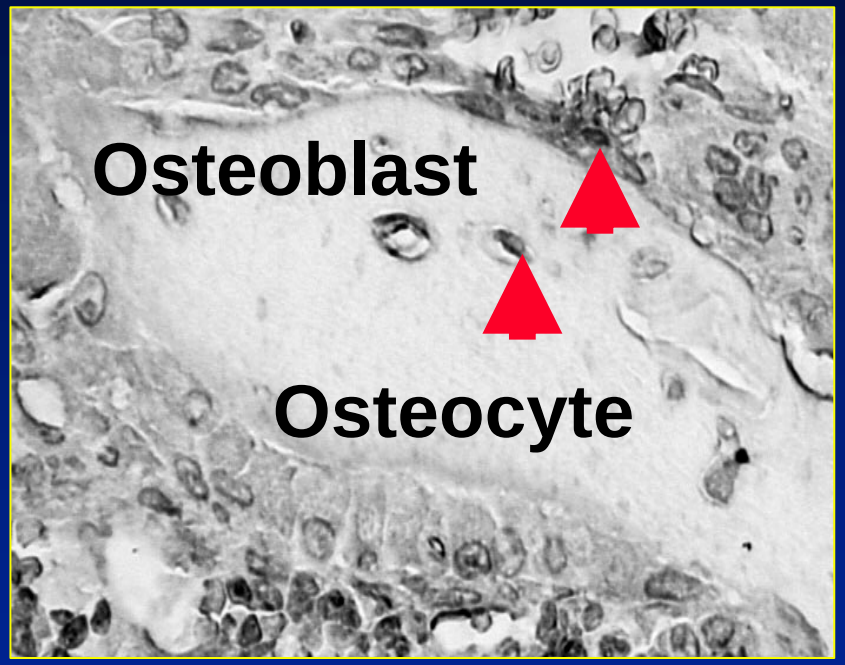


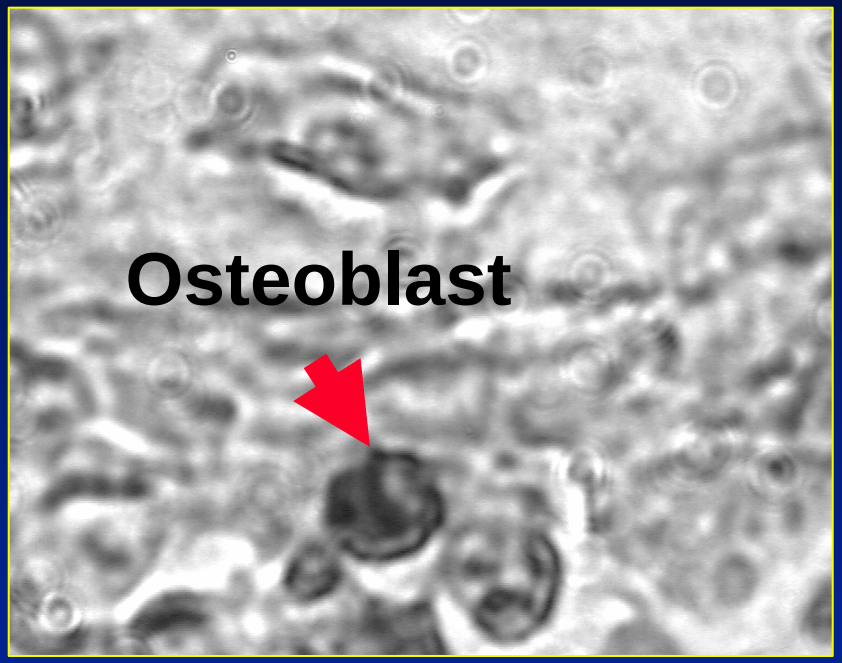
Final Fate of Osteoblasts: Lining cells / Osteocytes / Cell Death



Osteoblast apoptosis

5-10 % osteoblasts and osteocytes die by apoptosis

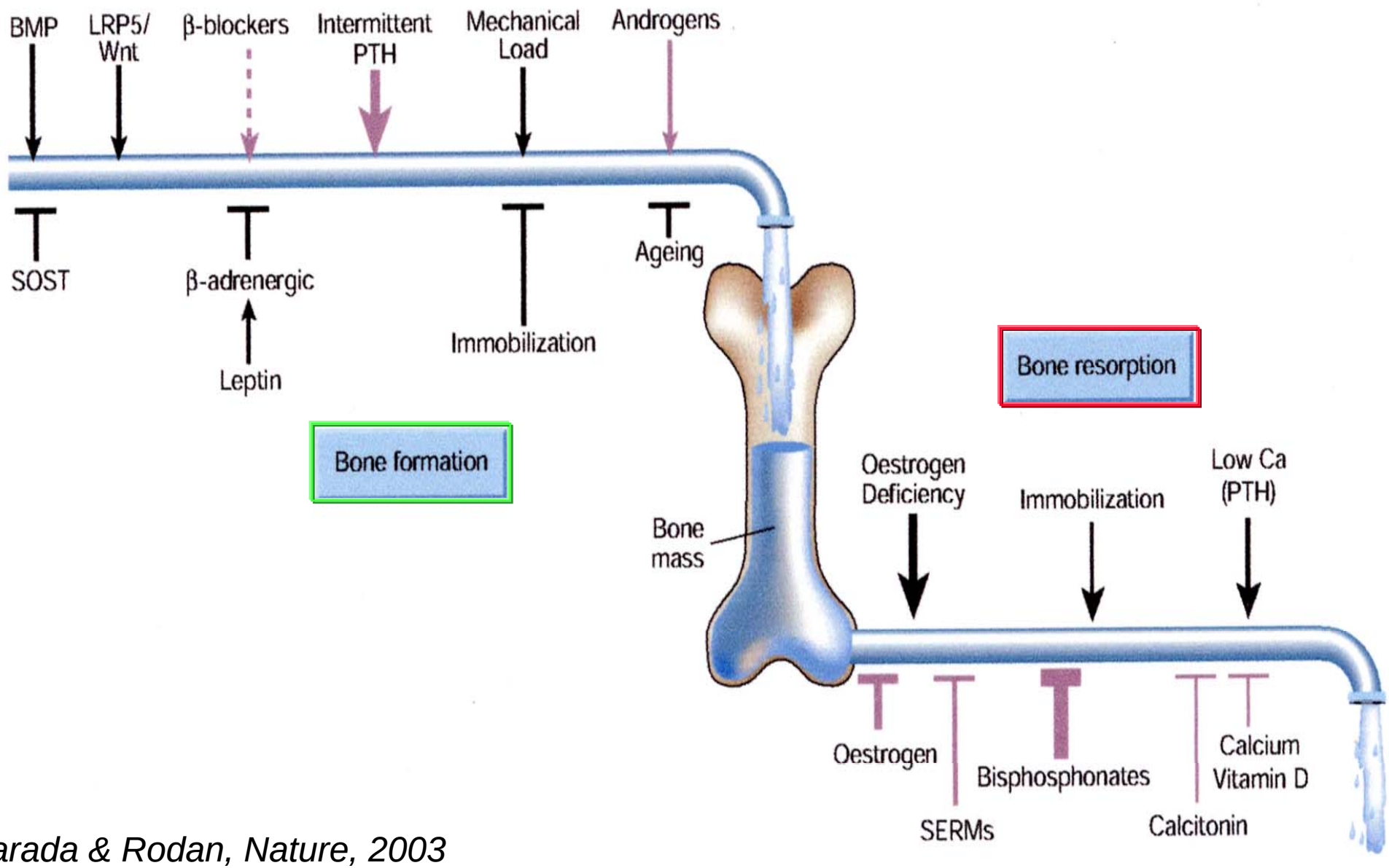




Importance: Increased osteoblast apoptosis in bone loss induced by glucocorticoids and skeletal unloading

O Brien et al., 2004; Dufour et al., Bone 2007

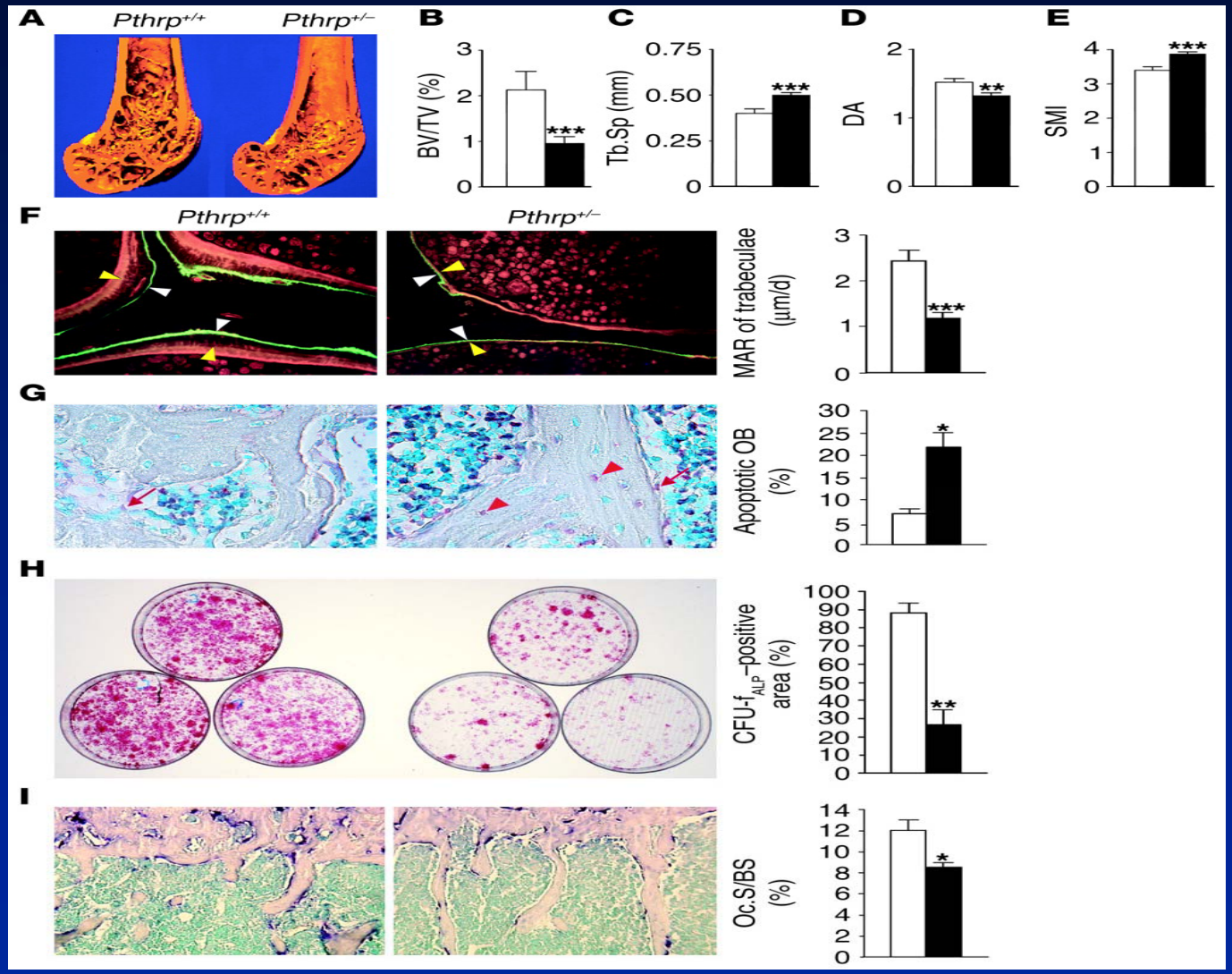
Regulation of osteoblastogenesis



Harada & Rodan, Nature, 2003

PRH/PTHrP Signaling is Important for the Control of Bone Mass

PTHrP haploinsufficiency causes osteopenia by decreasing bone formation



Miao, D. et al. J. Clin. Invest. 2005

PTH: A Bipotent Molecule Active on Bone Cells

Continuous PTH

↑ RANKL
↓ OPG

↓

↑ Osteoclastogenesis

↓

↑ Bone Resorption

↓

Bone Loss

Intermittent PTH

↑ Pre-osteoblast number

↓ BMC adipogenesis

↓Osteoblast/osteocyte apoptosis

↓

↑Number of osteoblasts

↓

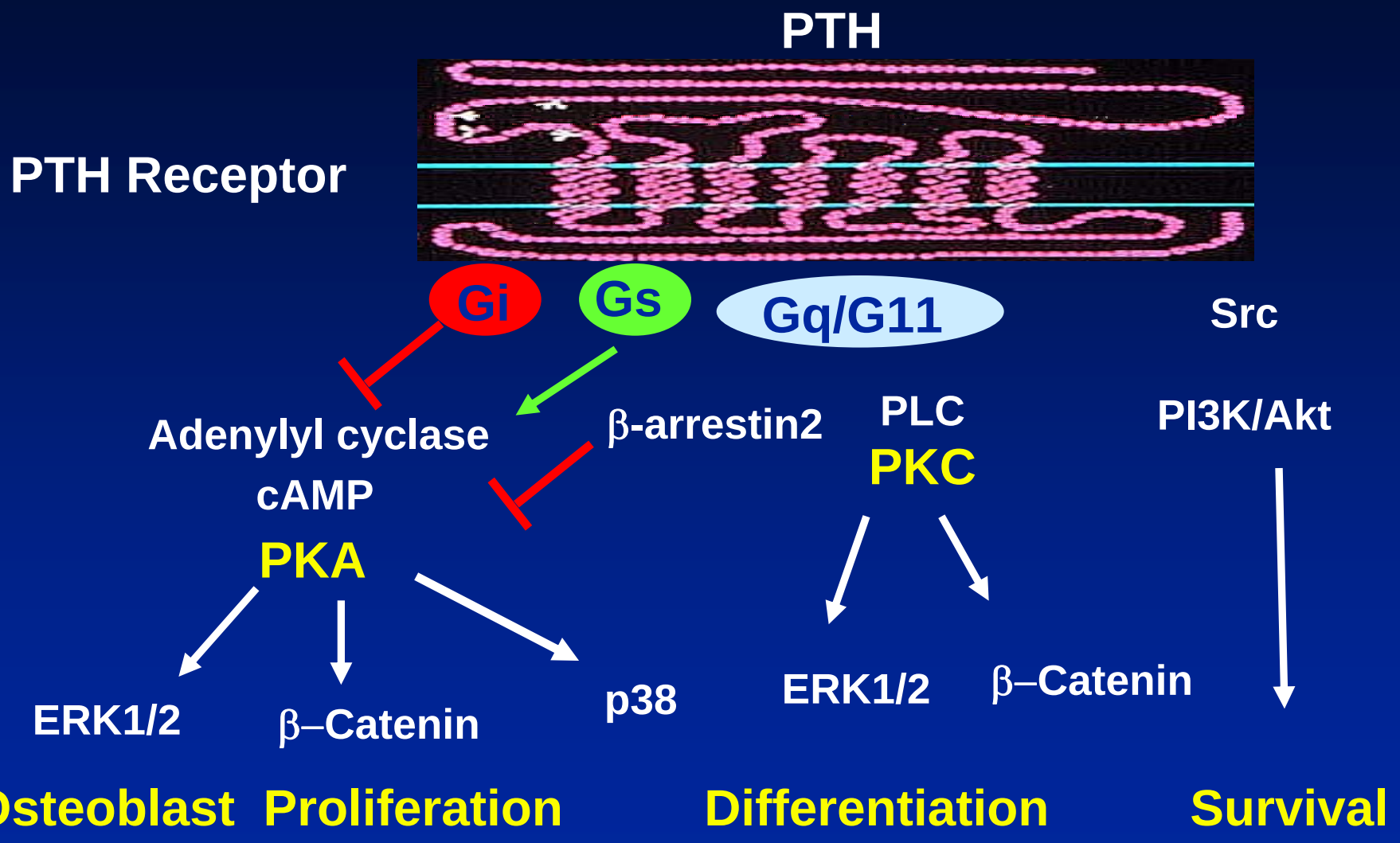
↑ Trabecular Bone Formation

↓

↑ Bone Gain

Lanske et coll. JCI 1999

PTH Signaling in Osteoblasts



Partidge, Gene 2002; Rey et al., Bone 2007; JBC 2006; Yamamoto et al., Bone 2007; Yang et al., Bone 2006; Ferrari et al., Endo. 2005; Chen et al., JBC 2004

Target Genes induced by PTH Signaling in Osteoblasts

PTH Signaling

+

Runx2
Runx2-P
Runx2 degradation
(explains intermittent
vs continuous effects)
Osterix

Decreases PPARγ

Osteoblast commitment

+

c-fos
cyclin D1

Osteoblast proliferation

+

IGF-I
FGF2
TGFβ
Sclerostin

Osteoblast function

+

Bad-P
β-catenin

Osteoblast survival

Jilka, Bone 2007

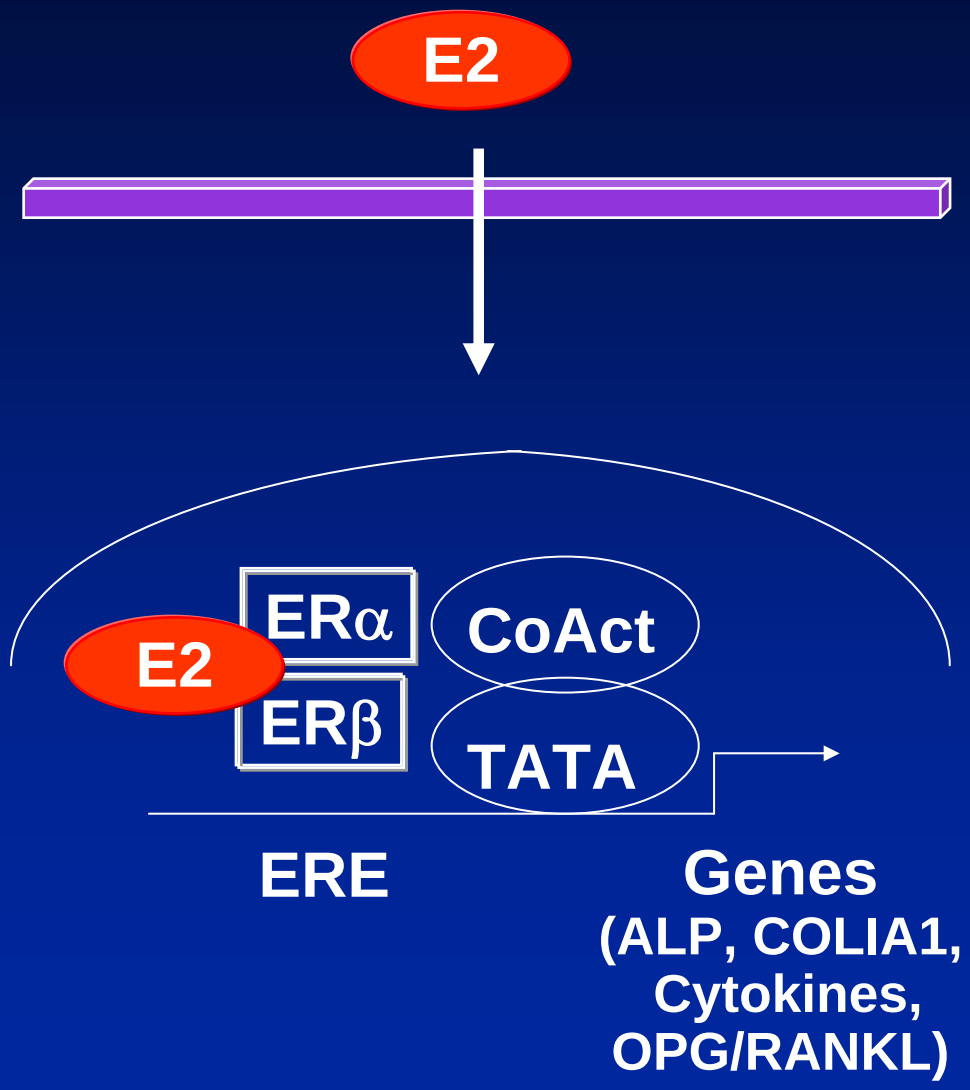
Estrogens and Bone Formation

- Important role in the acquisition of peak bone mass during skeletal growth

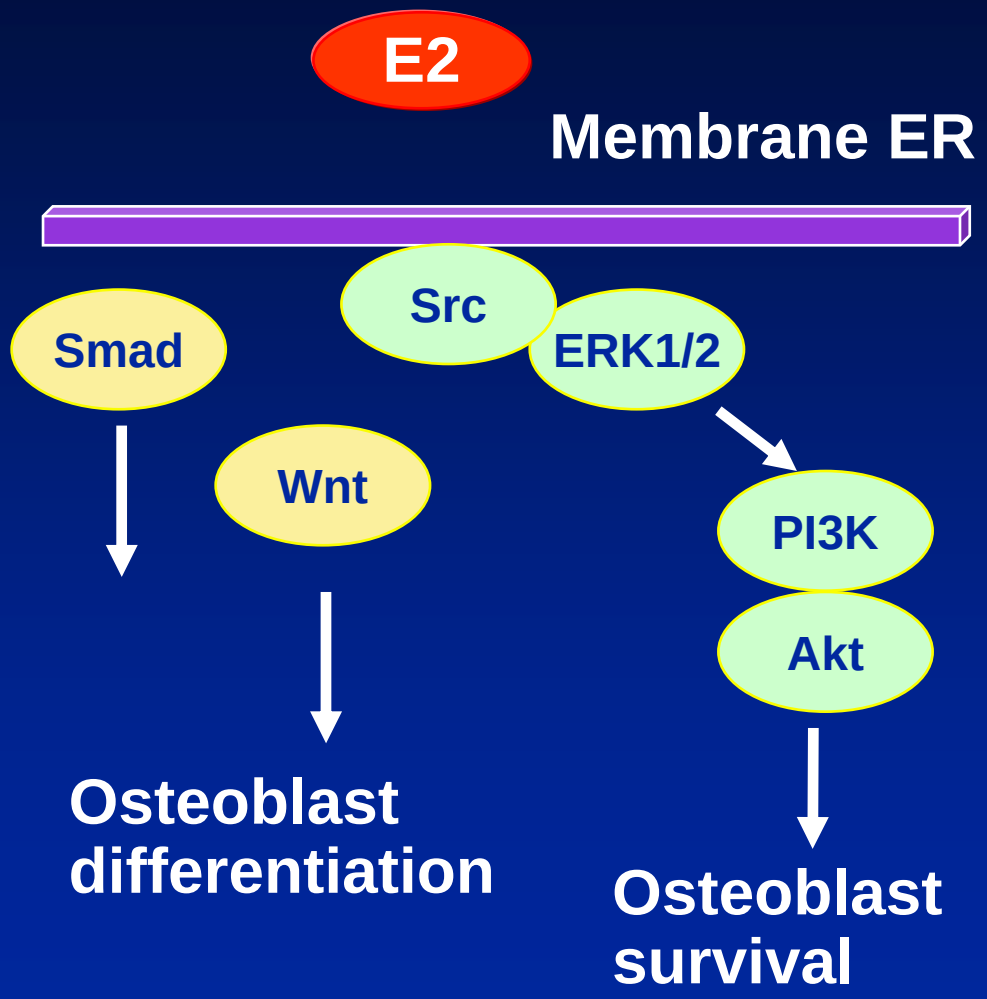
- Act on osteoblasts through genomic and nongenomic effects

Estrogen Receptor Pathways

Genomic (simplified)

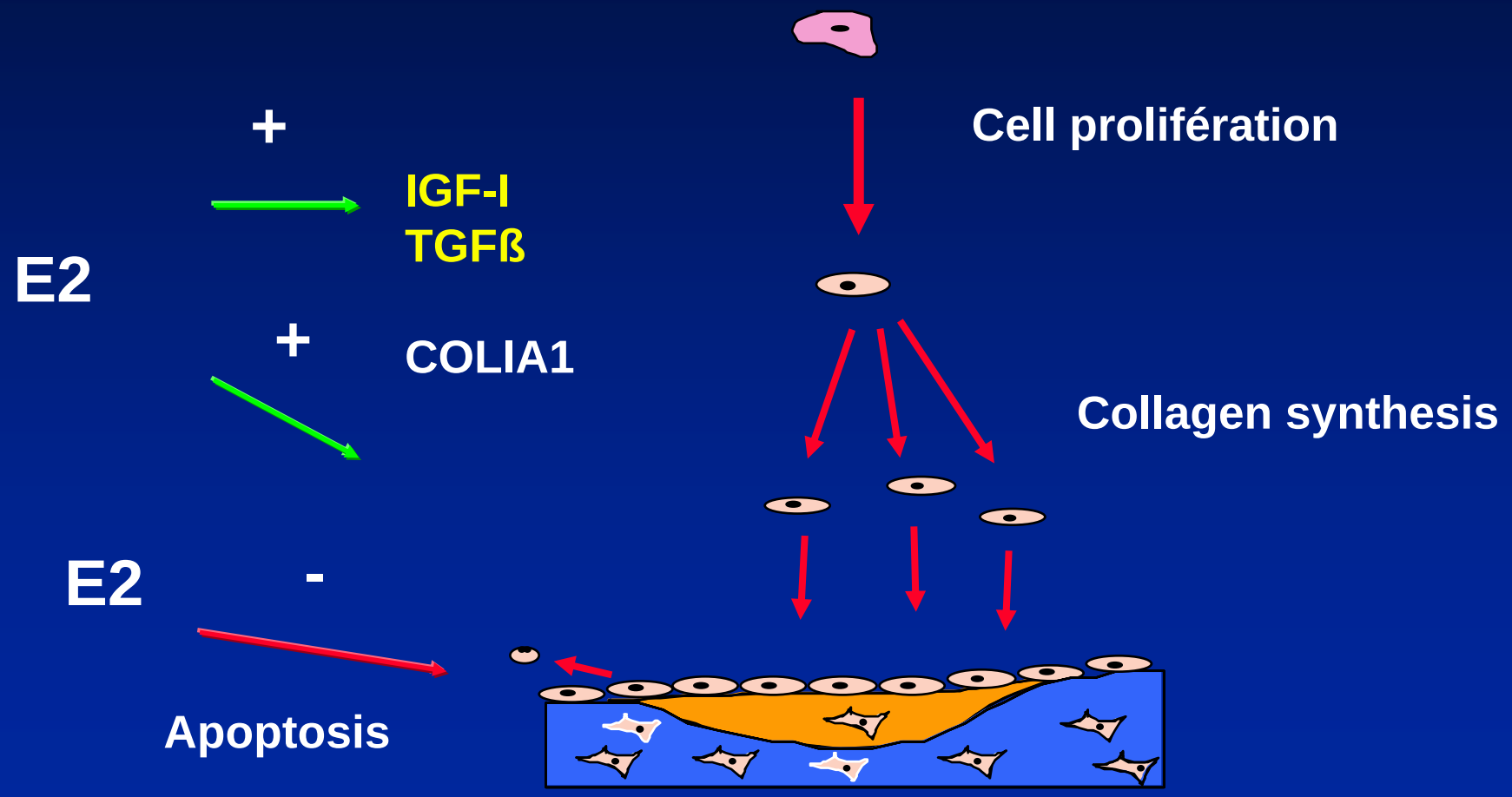


Non-Genomic

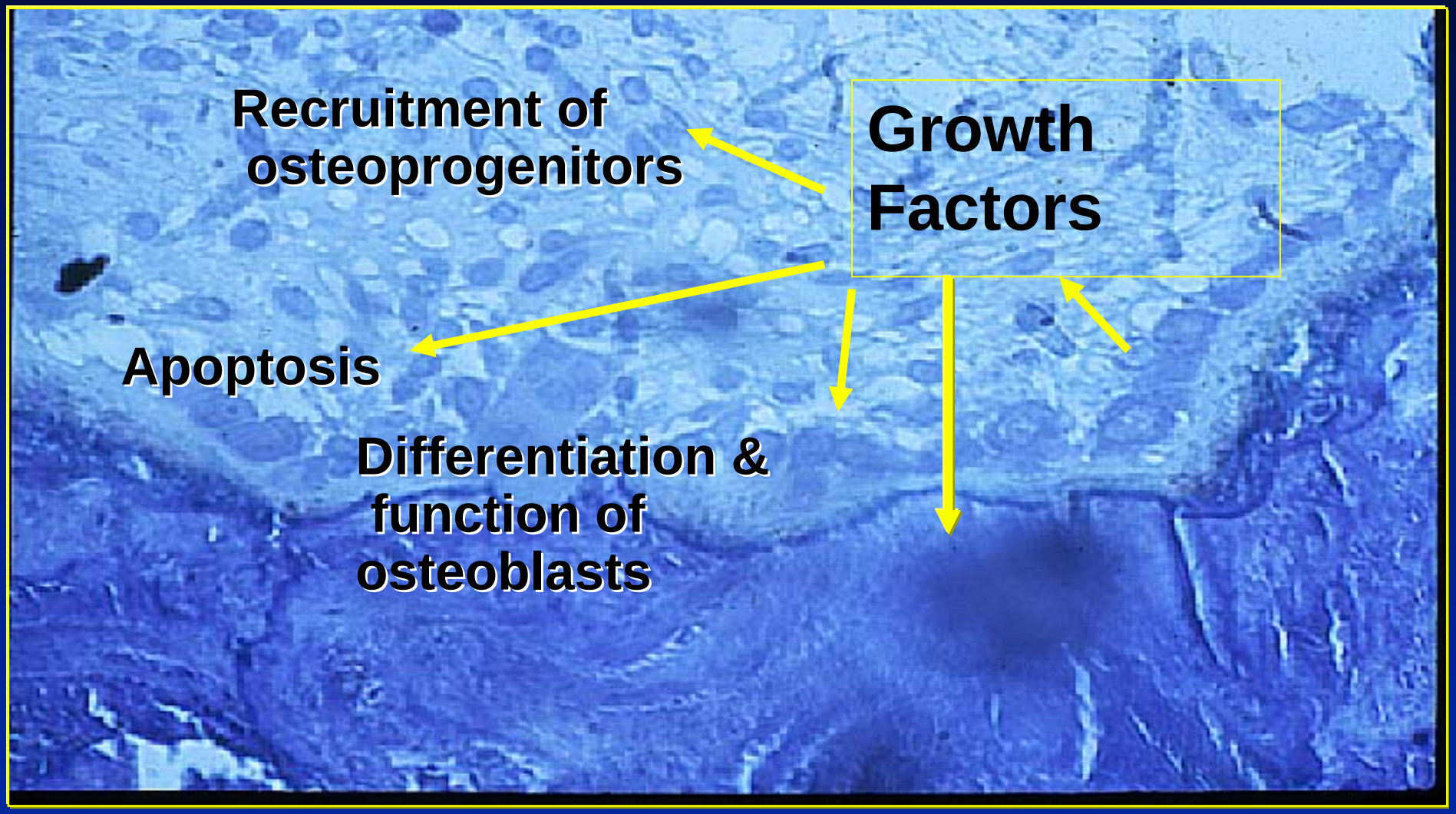


Aquirre JBC 2007; Kousteni MCB 2007

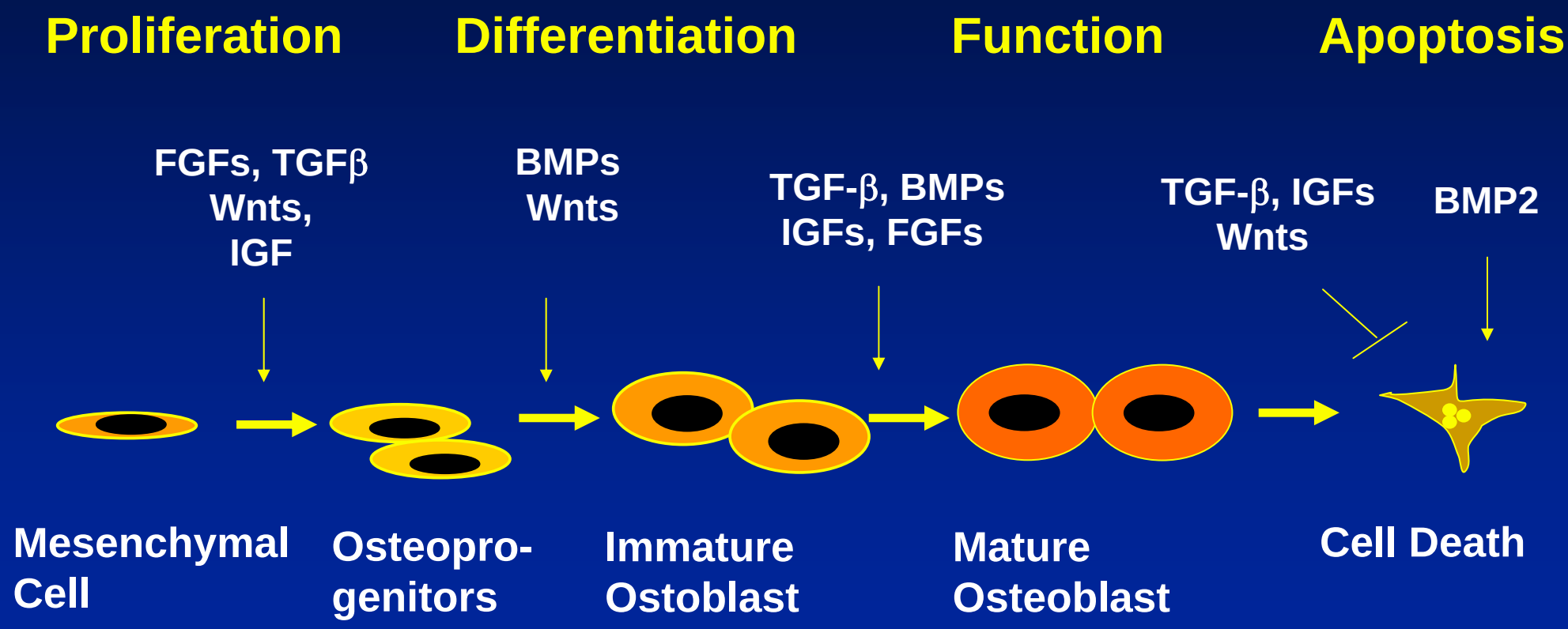
Estrogens Promote Bone Formation Through Growth Factor Expression



Regulation of Osteoblasts by Growth Factors



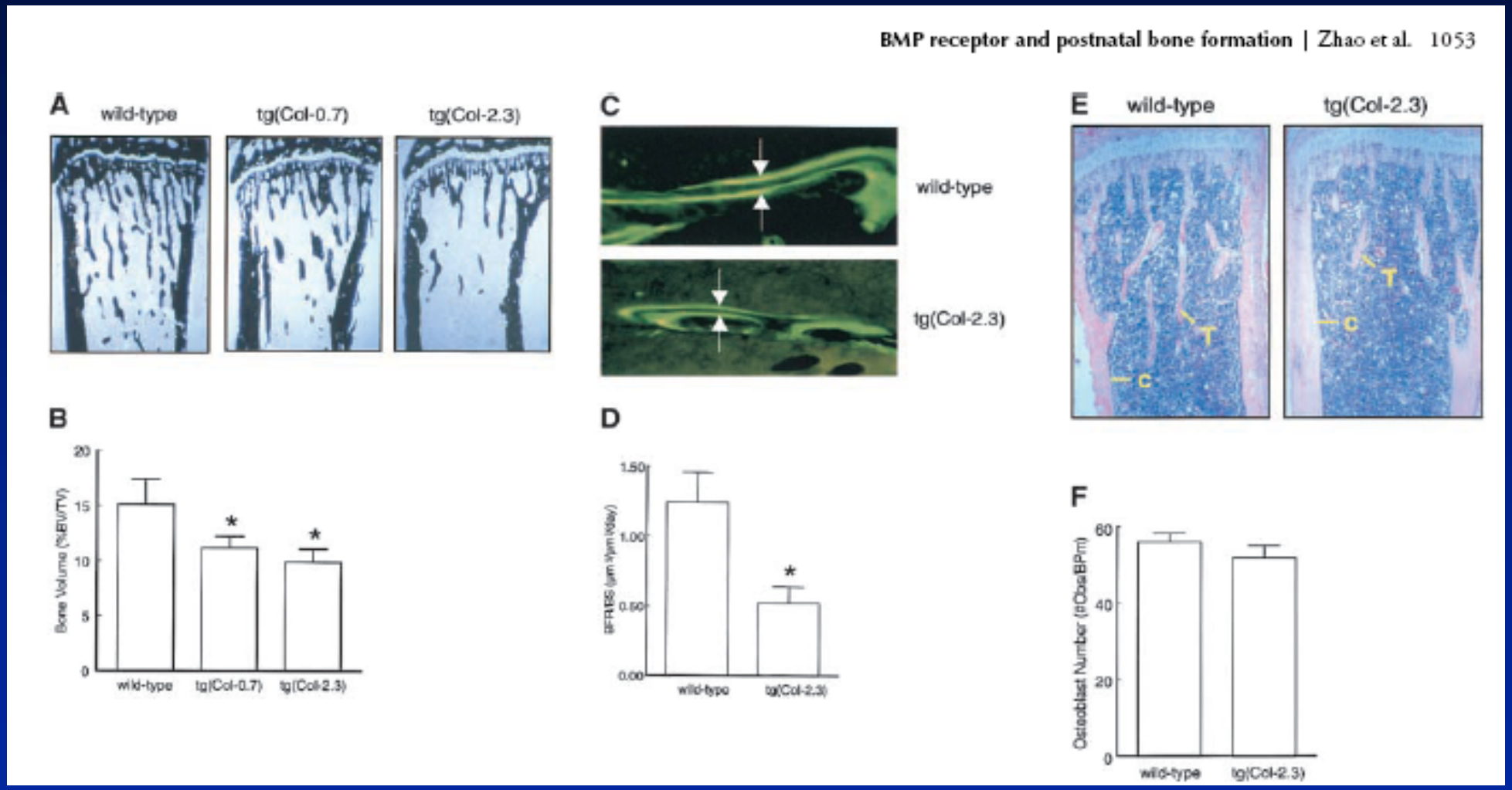
Control of osteoblastogenesis by growth factors



Marie, ABB 2008

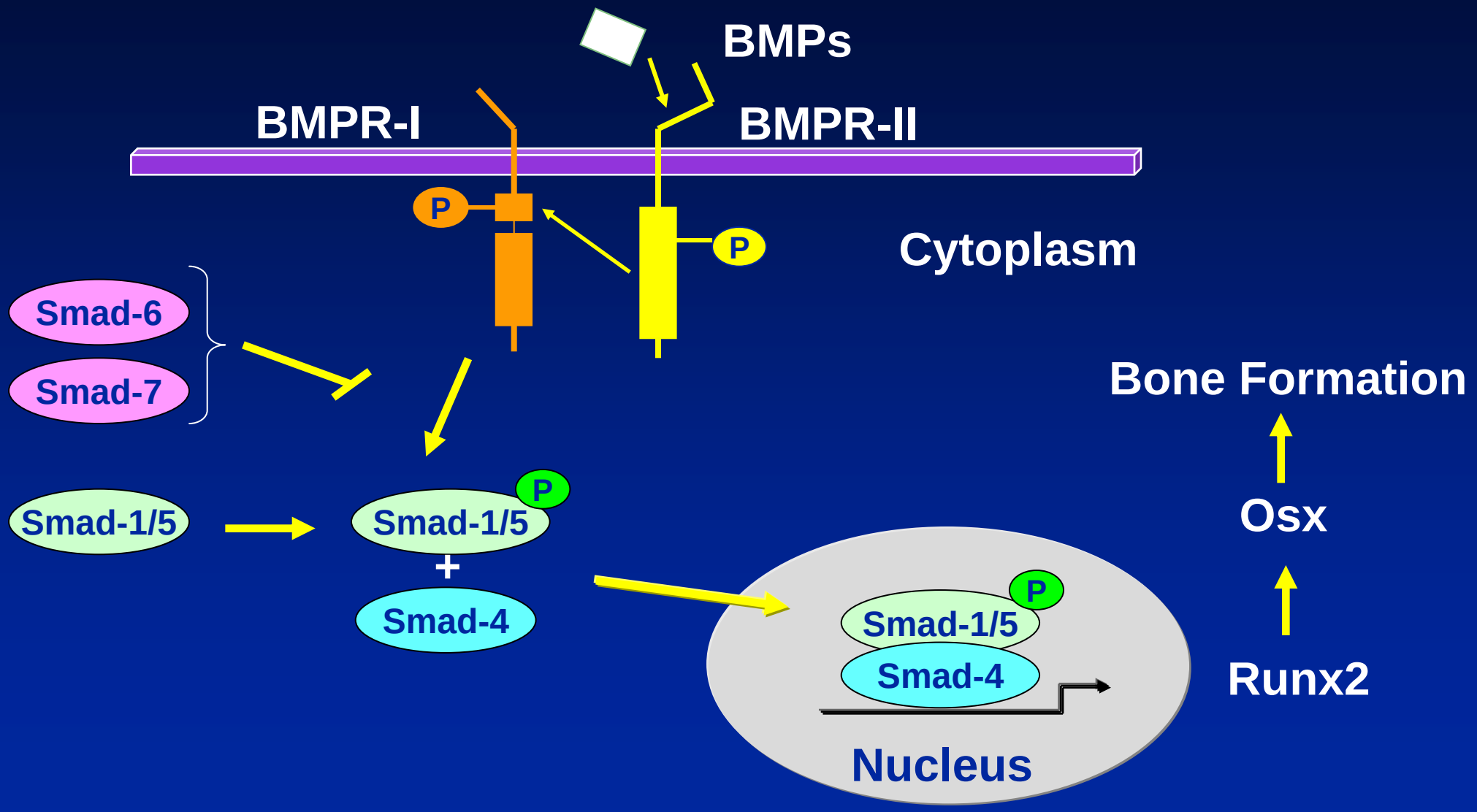
BMP Signaling is Important in Post-Natal Bone Formation

Invalidation of BMP-RIB: Decreased bone formation and bone loss



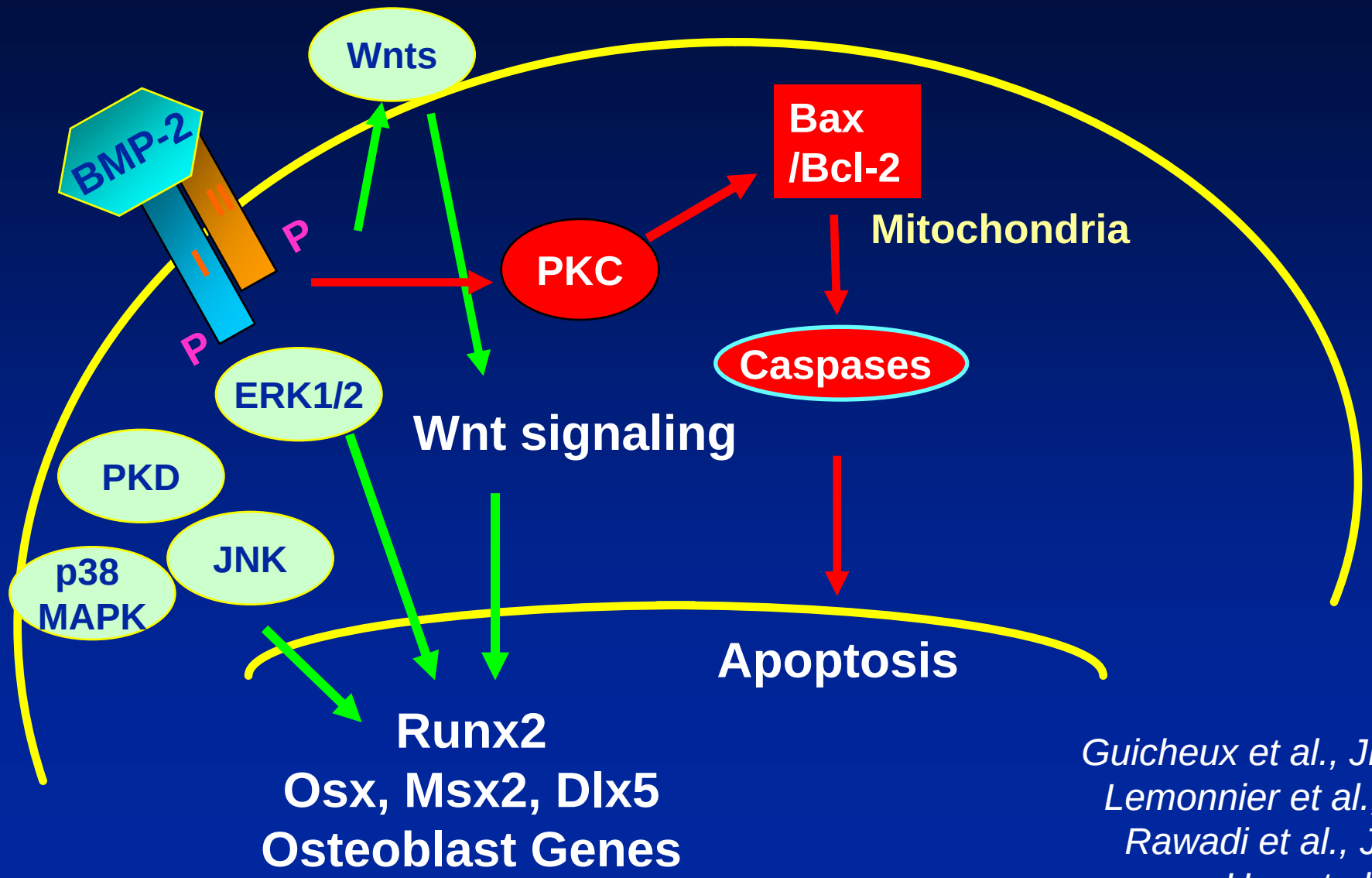
Zhao et al., JCB 2002

Canonical Pathway of Bone Morphogenetic Proteins



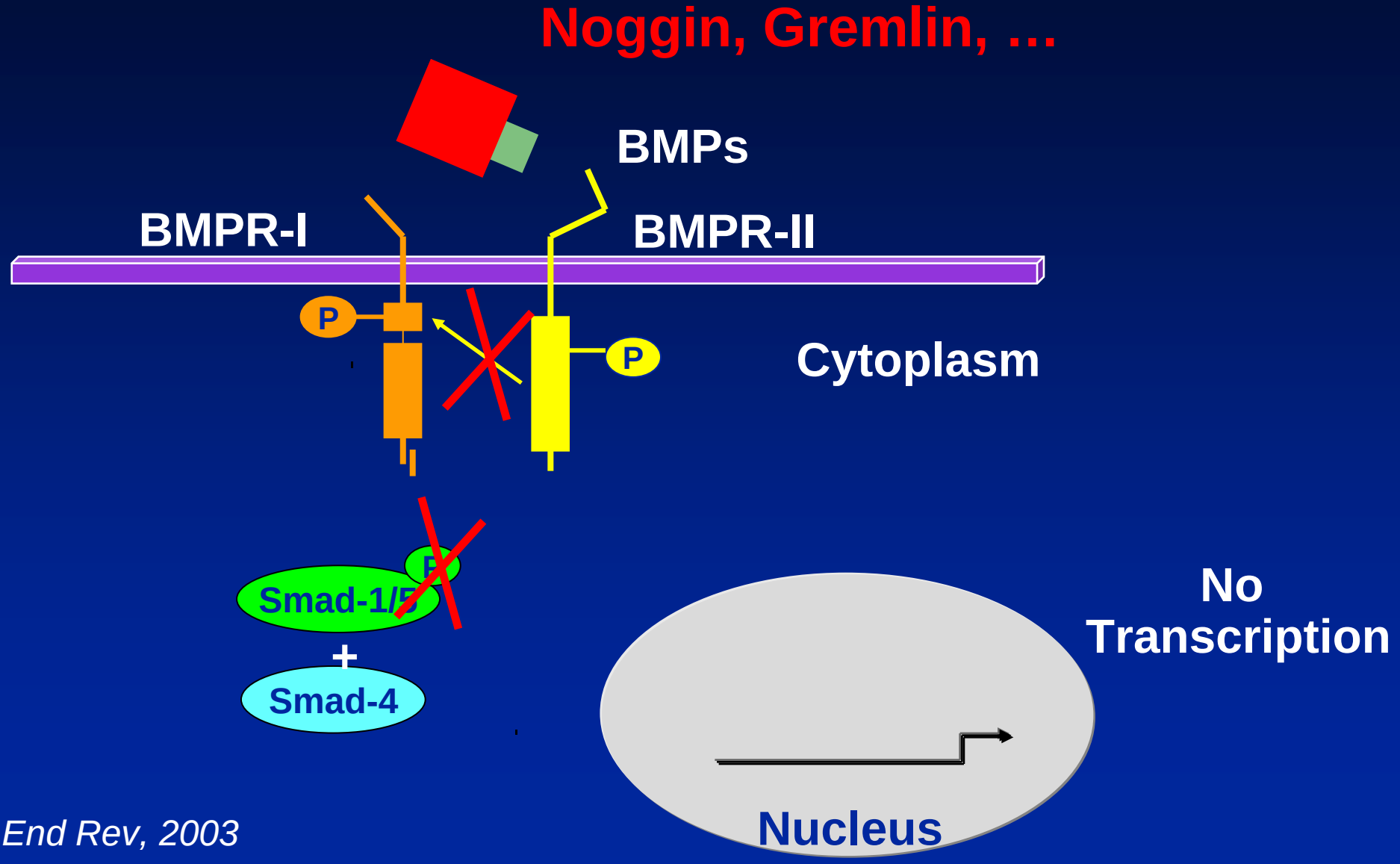
Ten Dijke, et al., 2003

BMP Non-Canonical Pathways



Guicheux et al., JBMR 2003
Lemonnier et al., JBC 2004
Rawadi et al., JBMR 2003
Hay et al. JBC 2001

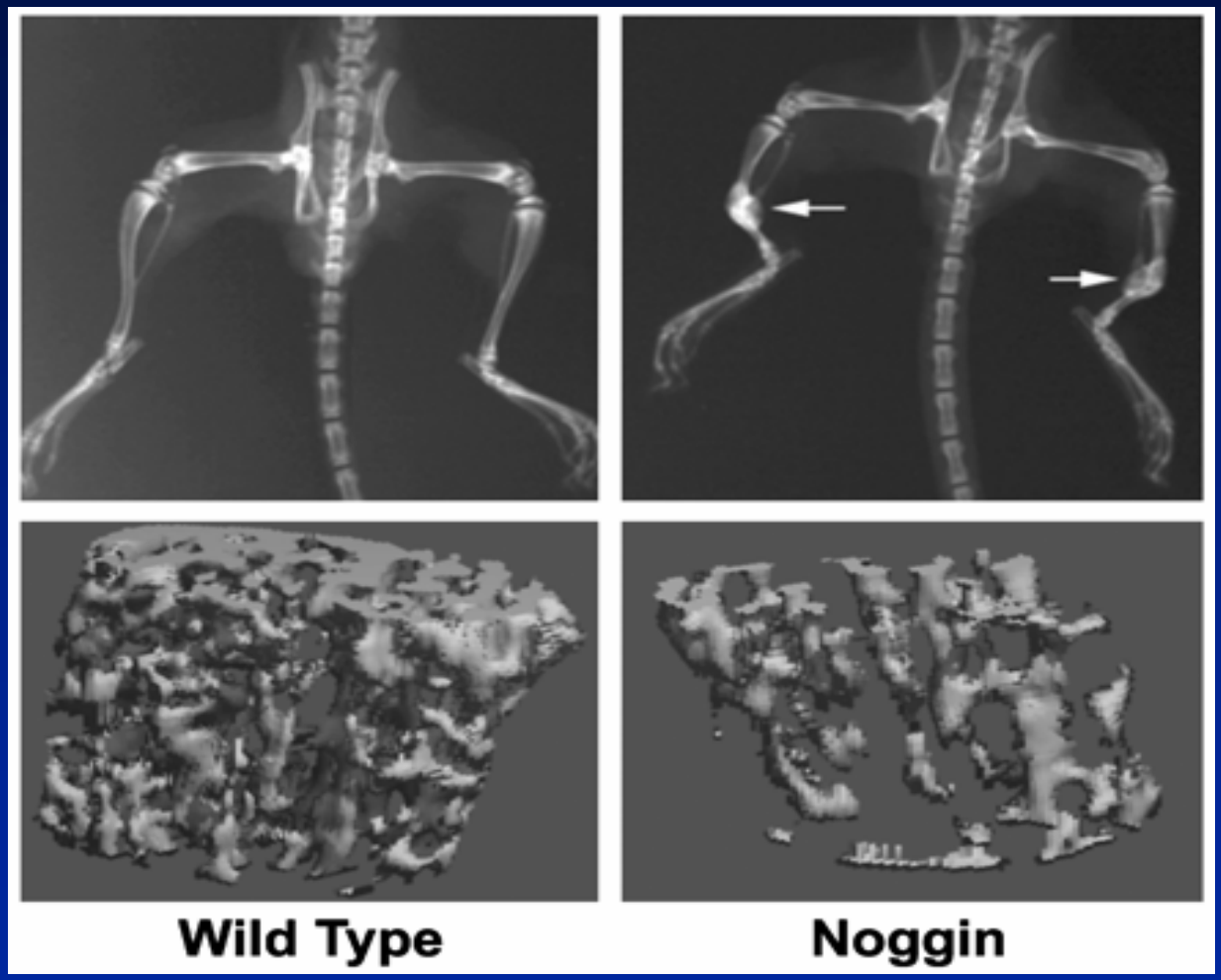
Negative Regulation of BMP Signaling by Antagonists



Canalis, End Rev, 2003

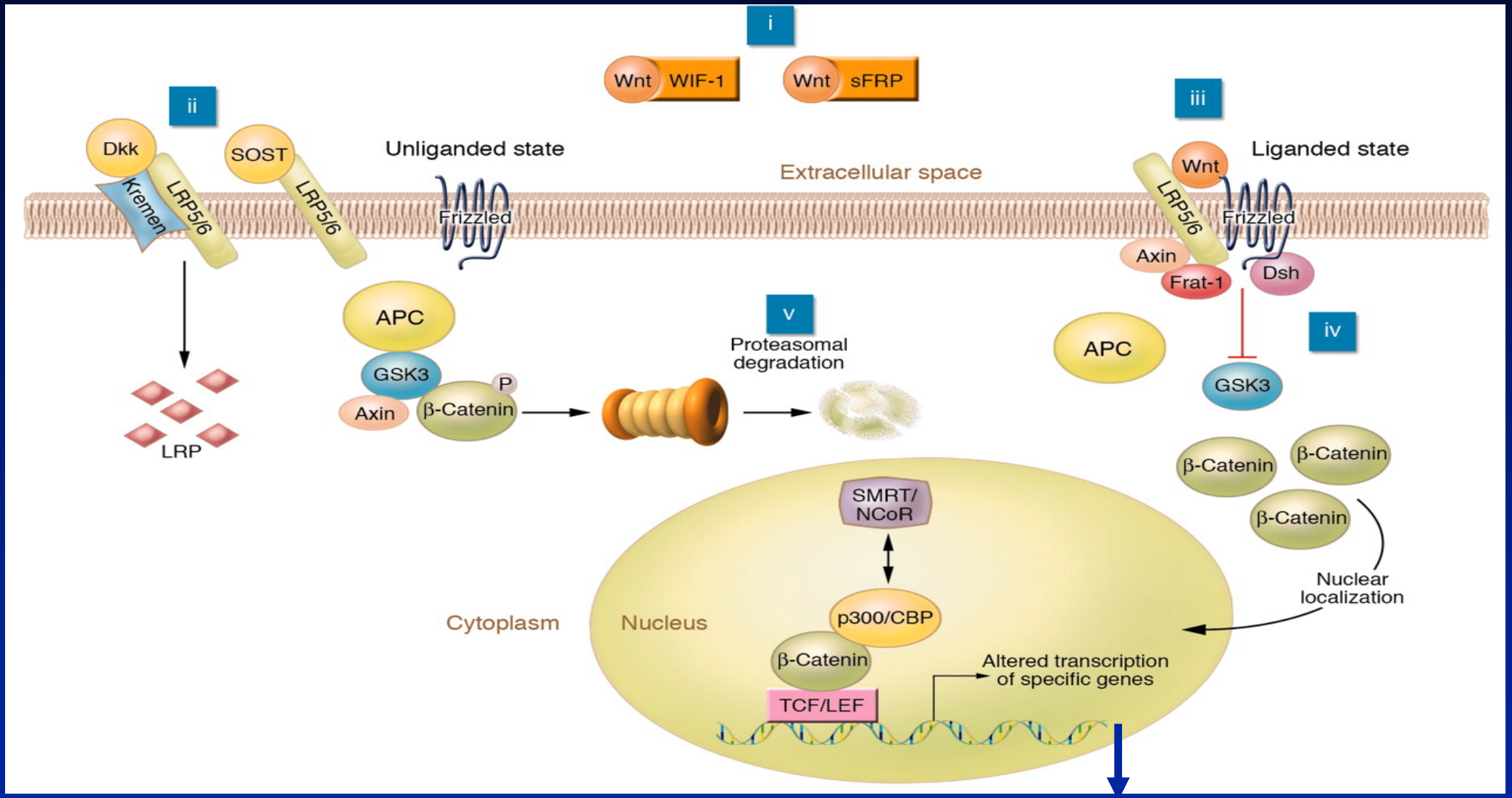
Chen et al., Growth factors, 2004

Overexpression of Noggin (or Gremlin) Inhibits BMP Activity and Induces Bone Loss



Devlin et al, J Clin Invest, 2004

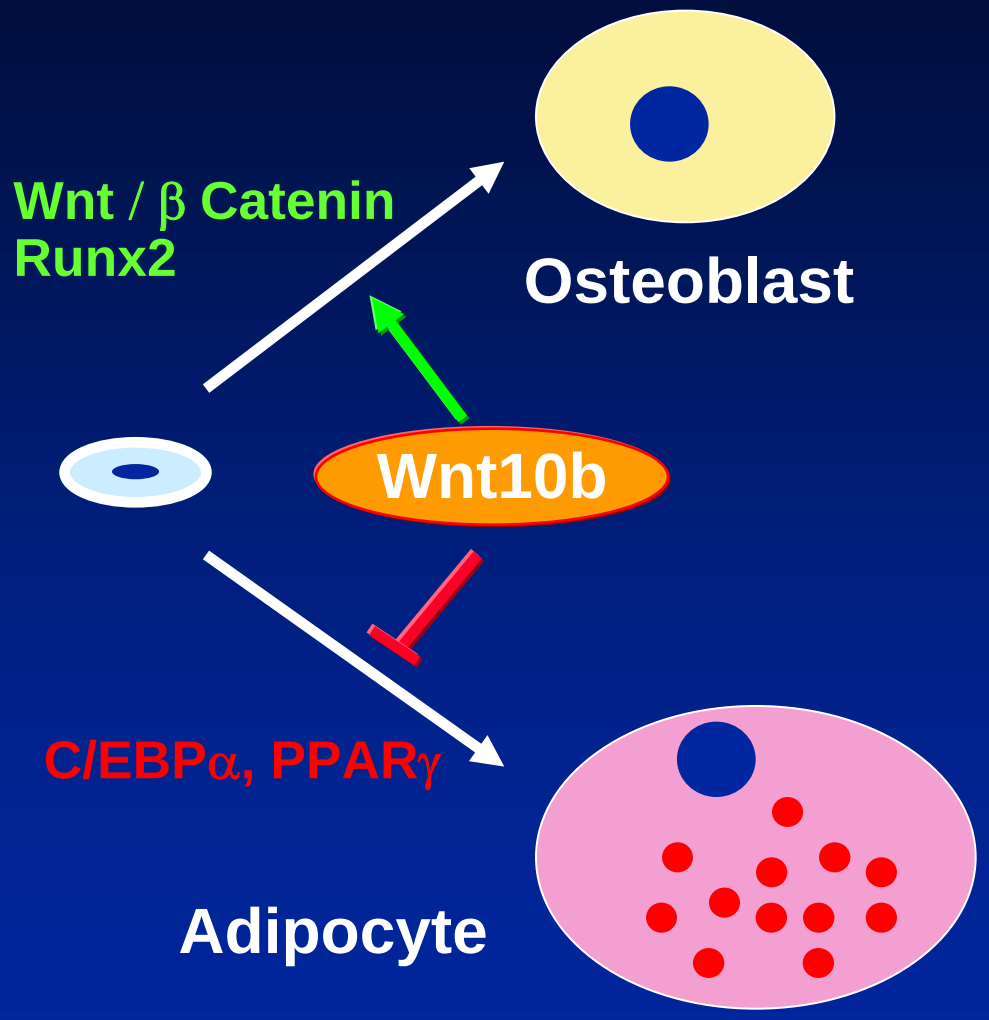
The Wnt Canonical Pathway



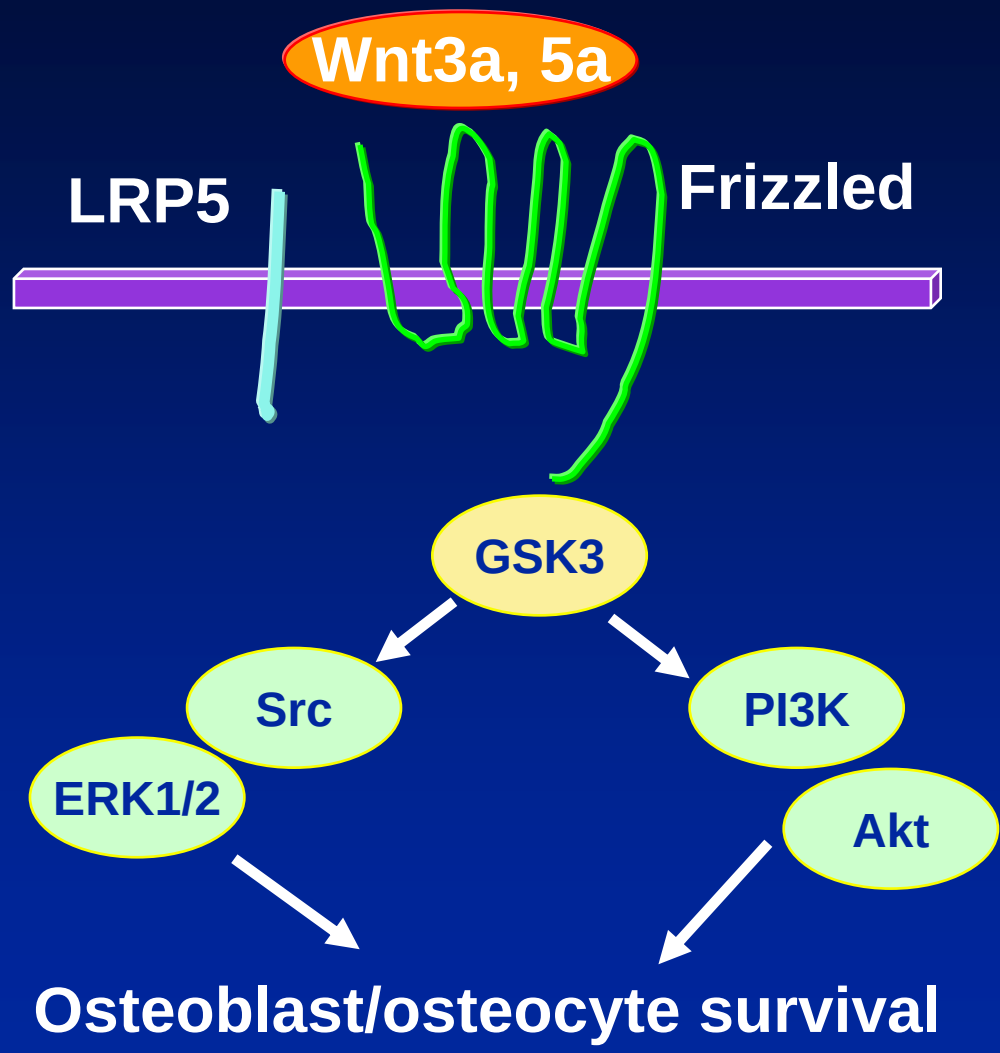
Krishnan J. Clin. Invest. 2006

ALP, COLIAI, Runx2
Osteoblast differentiation
Bone formation, bone gain

Wnt Non-Canonical Pathways Control Osteoblasts

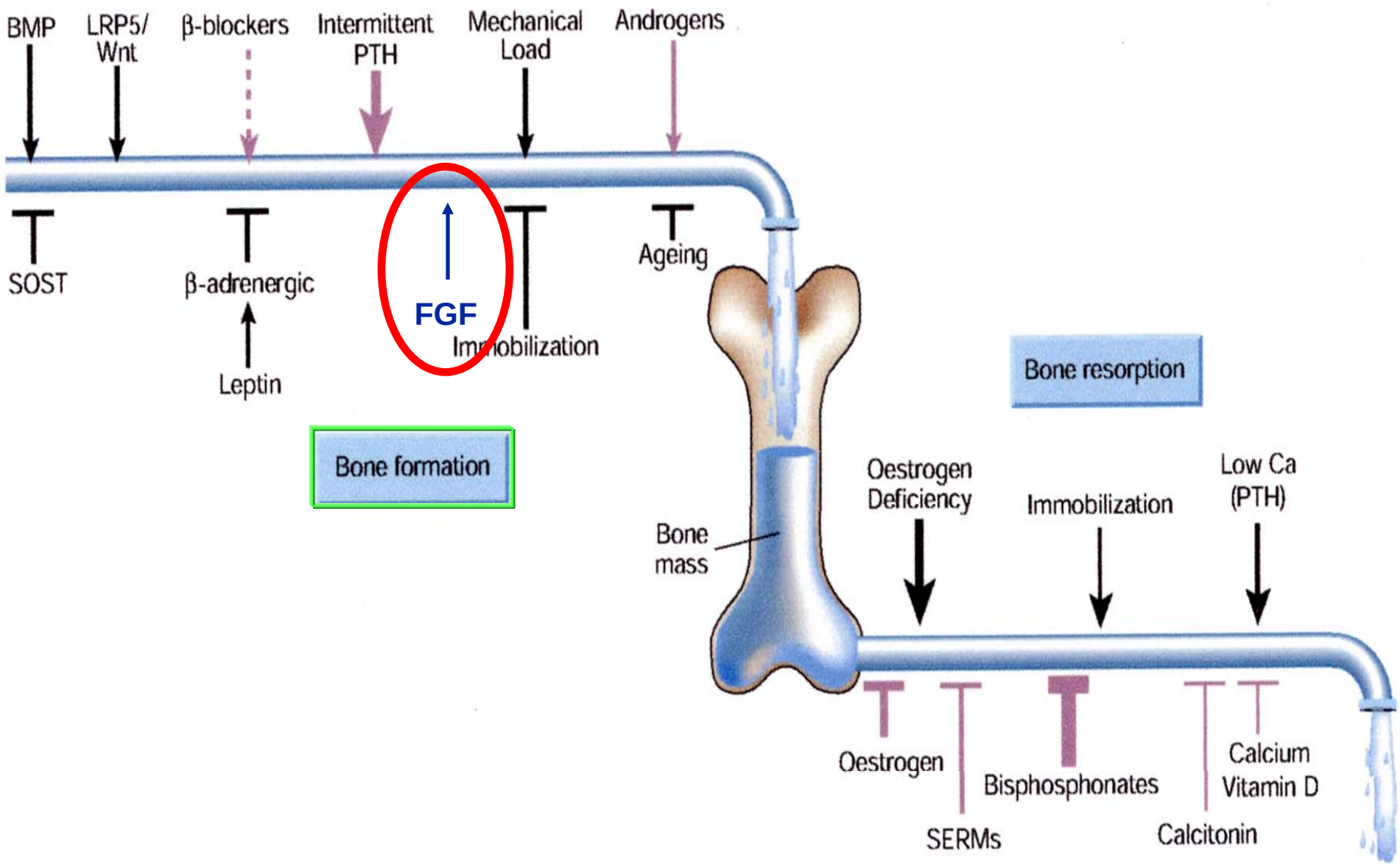


Kang et al. JBC 2007



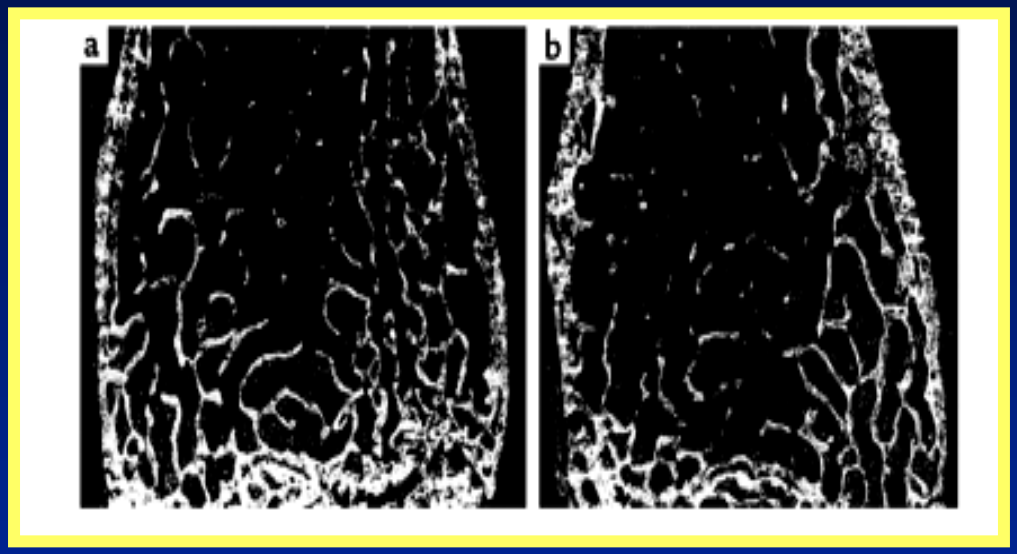
Almeida et al. JBC 2005

FGF Signaling



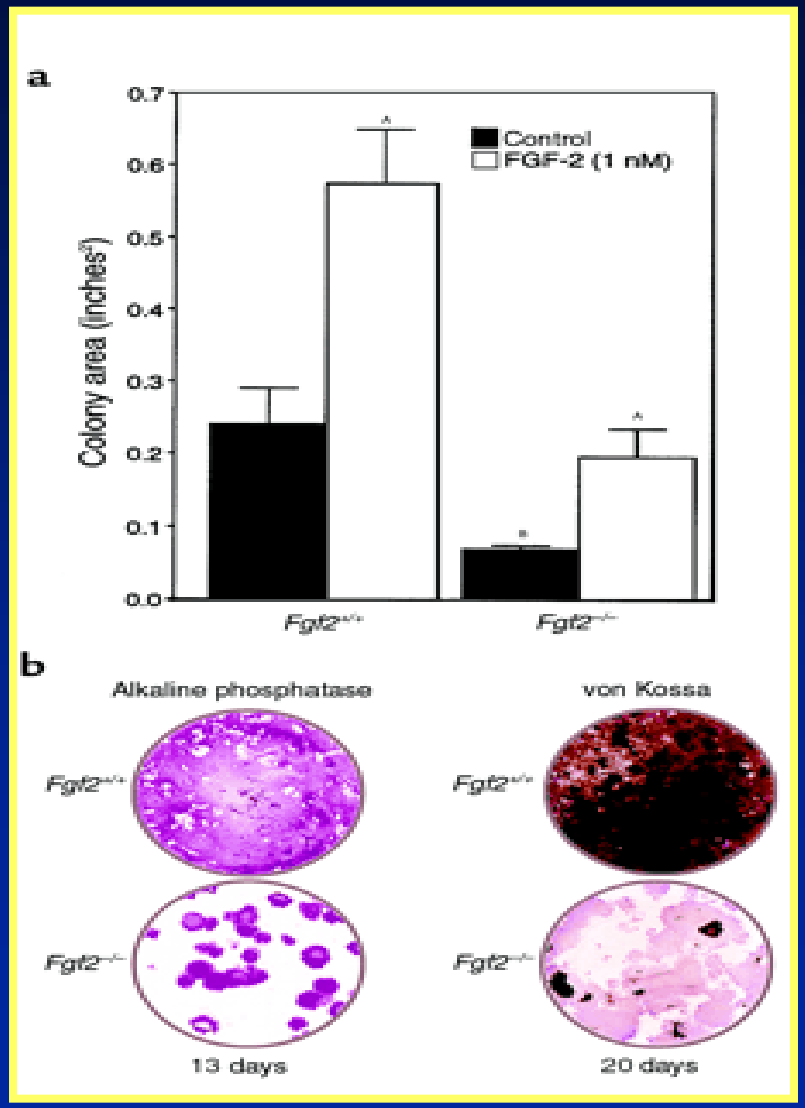
FGF2 Invalidation Reduces Bone Formation

FGF2 +/+ FGF2 -/-



8 months mice: bone loss

Defective bone formation



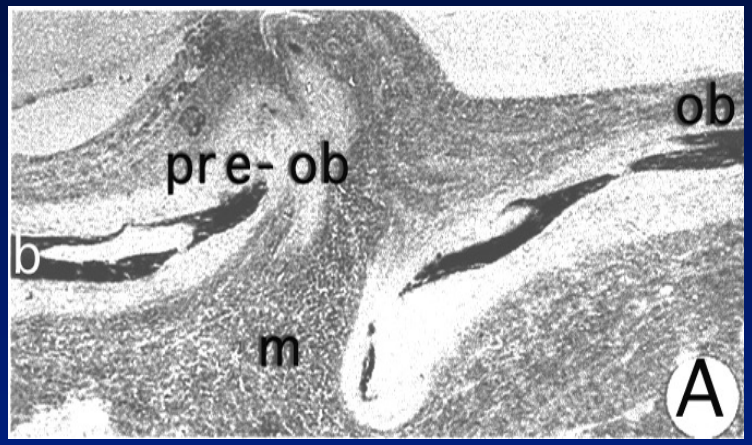
FGF2+/+

FGF2-/-

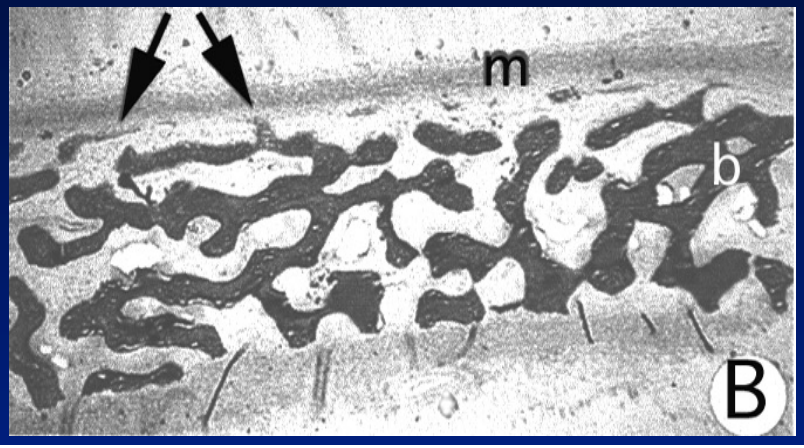
Montero et al., J Clin Invest, 2000

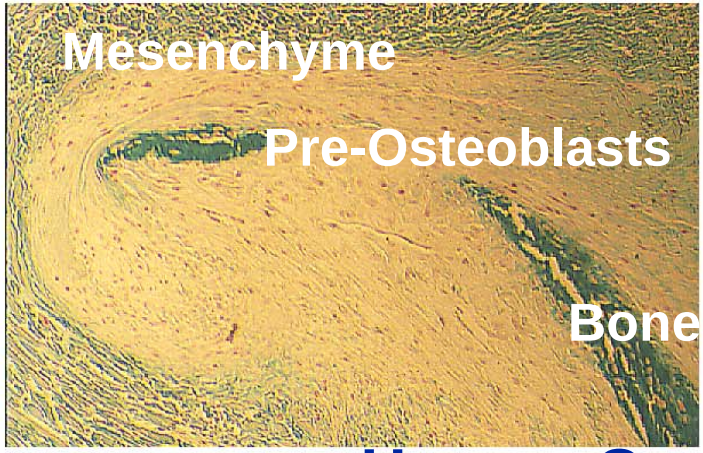
Activation of FGF Receptor-2 Increases Bone Formation (Apert Syndrome)

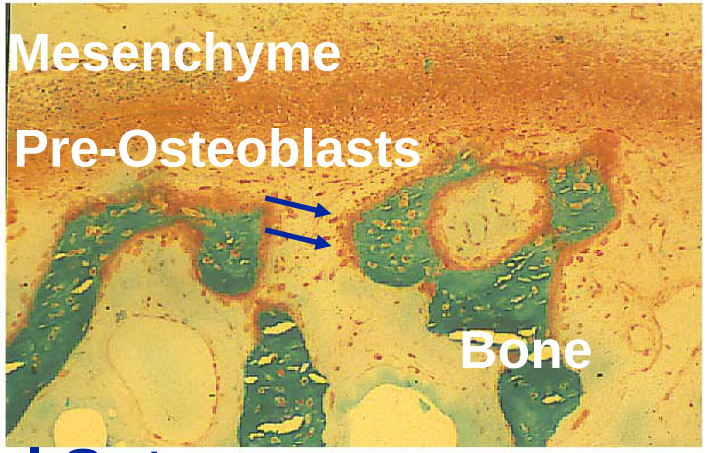
Normal Suture



Apert Suture (Activating FGFR2 Mutation)



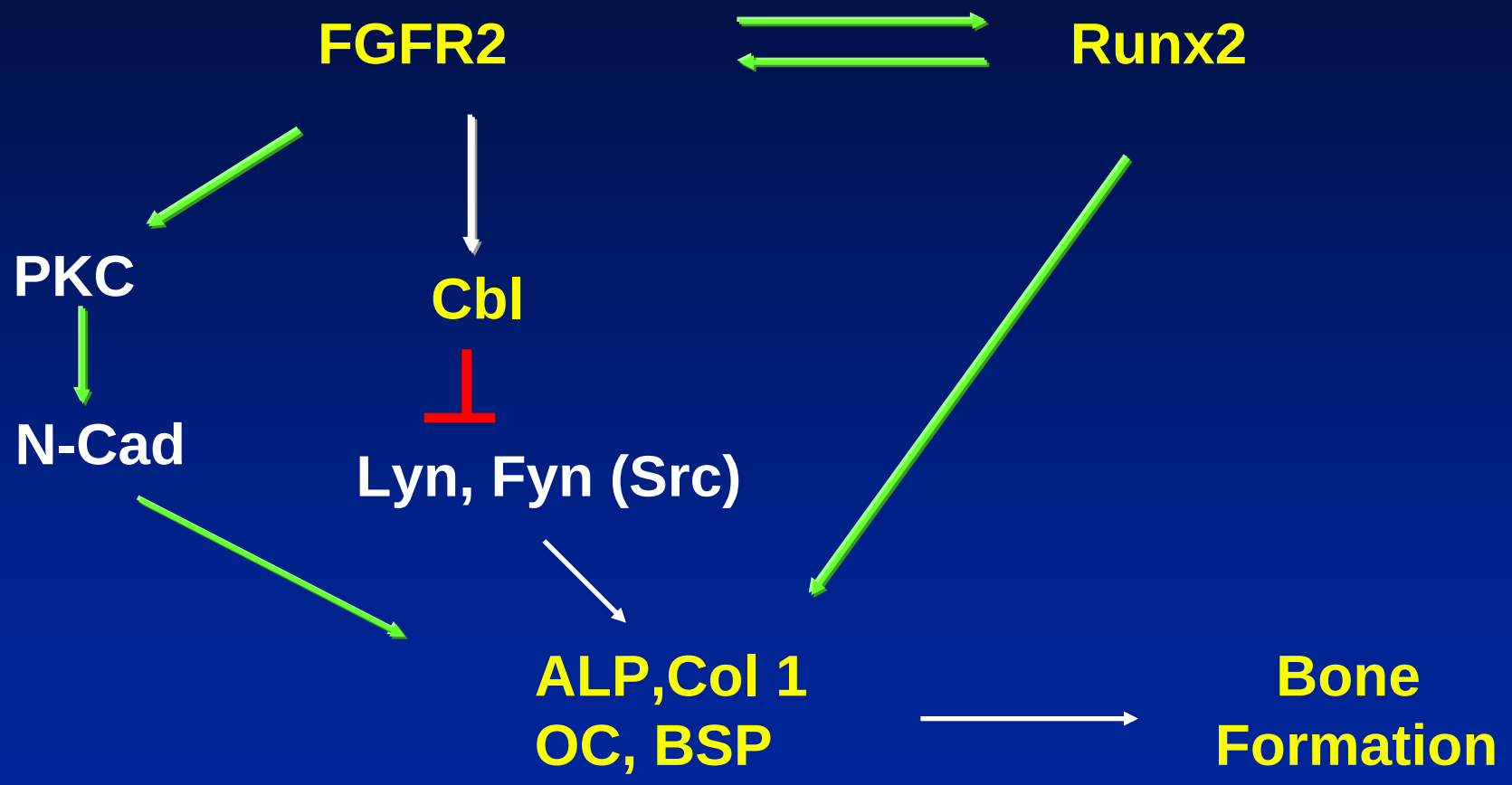




Human Coronal Suture

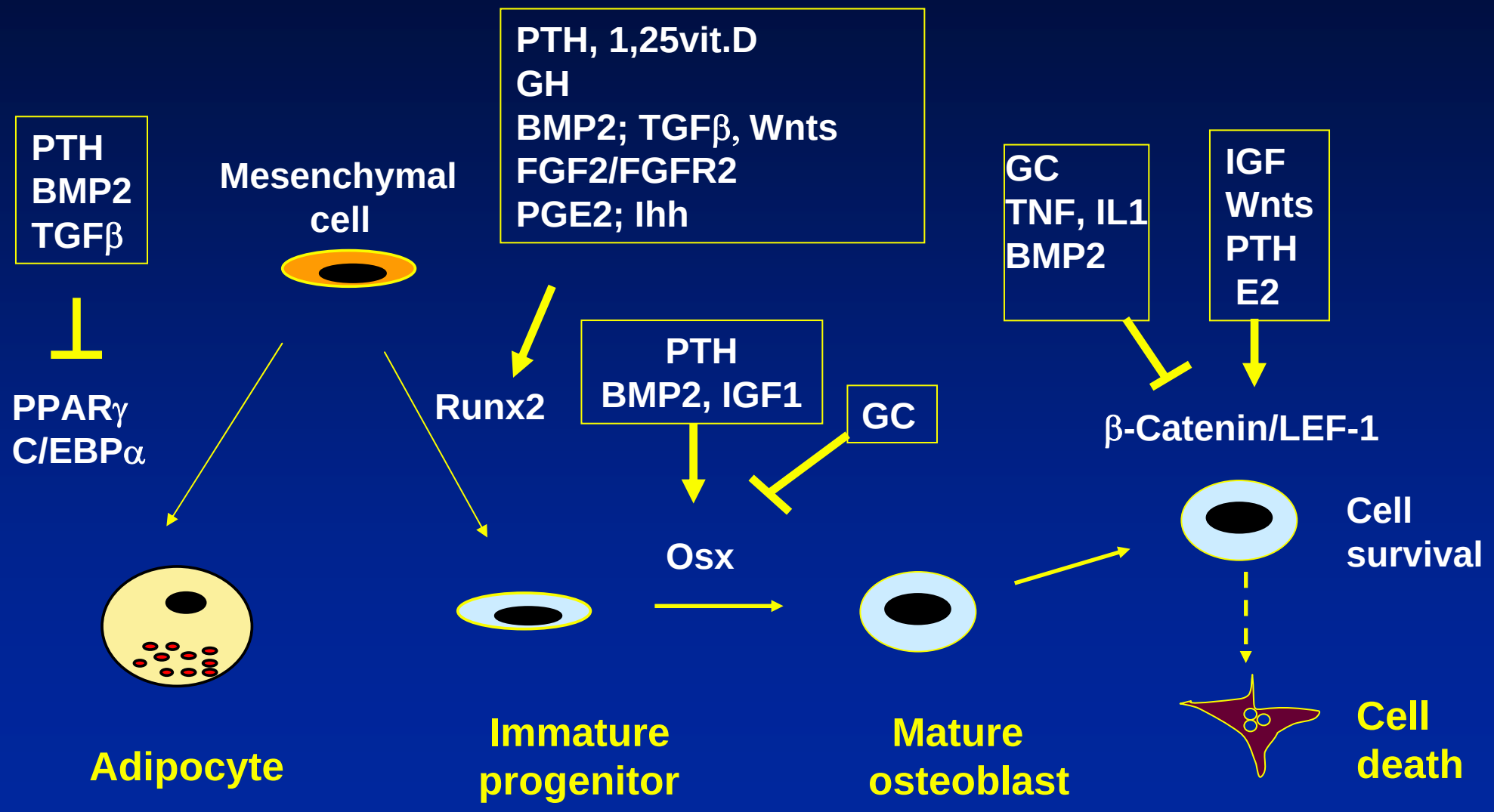
Lomri & Marie, J Clin Invest, 1998; Ornitz & Marie; Genes and Dev, 2002

FGFR2 Signaling Promotes Human Osteoblast Differentiation



Lomri & Marie, JCI, 1998; Ornitz & Marie; Genes & Dev, 2002; Kaabeche et al., JBC 2004; Marie, Gene 2003; Marie, JCB 2005

Overall Regulation of Osteoblastogenesis



Marie P, ABB 2008

Questions ?