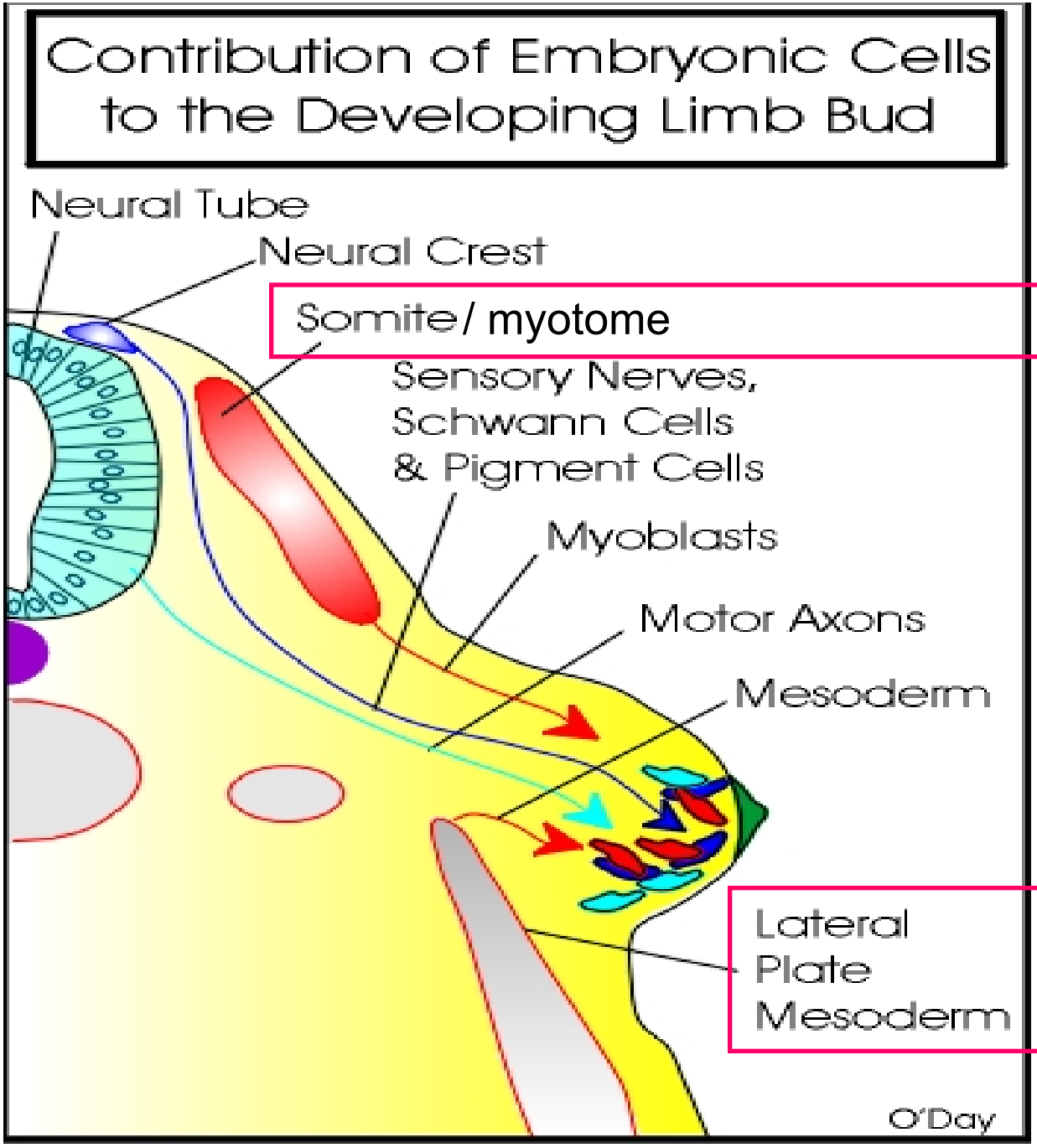
3. DEVELOPMENT OF MUSCLE, CARTILAGE AND BONE



muscle

cartilage/bone

AT THE BEGINNING THERE WAS VITAMIN A……..

LETTERS TO NATURE

Limbs generated at site of tail amputation in marbled balloon frog after vitamin A treatment

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NIAZI and Saxena[1] first observed that vitamin A has an inhibitory and modifying influence on tail regeneration in *Bufo andersonii* tadpoles. A positive relationship was later found between the inhibiting influence of vitamin A and the developmental stage of the regenerating tail in the same species[2]. There have been several subsequent reports[3–7] on the effects of vitamin A and its derivatives on limb development and regeneration. Thus in regenerating amphibian limbs, application of retinoids produces pattern duplication in the proximodistal and anteroposterior axes of the limb[3,8,9], and local application of retinoic acid to the anterior side of developing chick limbs causes duplications in the anteroposterior axis of limb[10,11]. Here we show that vitamin A can cause limb development when applied to amputated tail stumps of the tadpoles of the marbled balloon frog *Uperodon systoma* (Anura Microhylidae). This is the first report of homeotic transformation mediated through vitamin A in vertebrates.

Following amputation through the middle of the tail at the hind-limb bud stage, tadpoles were exposed to a solution of 10 IU per ml vitamin A palmitate (Arovit, Roche; see Table 1 for details) for 24 h (set I), 48 h (set II), 72 h (set III), 96 h (set

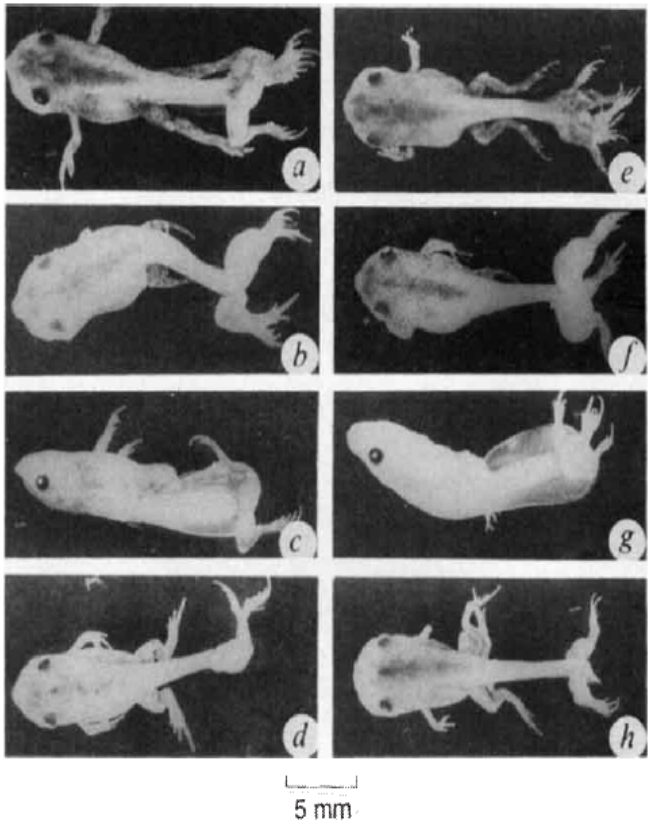
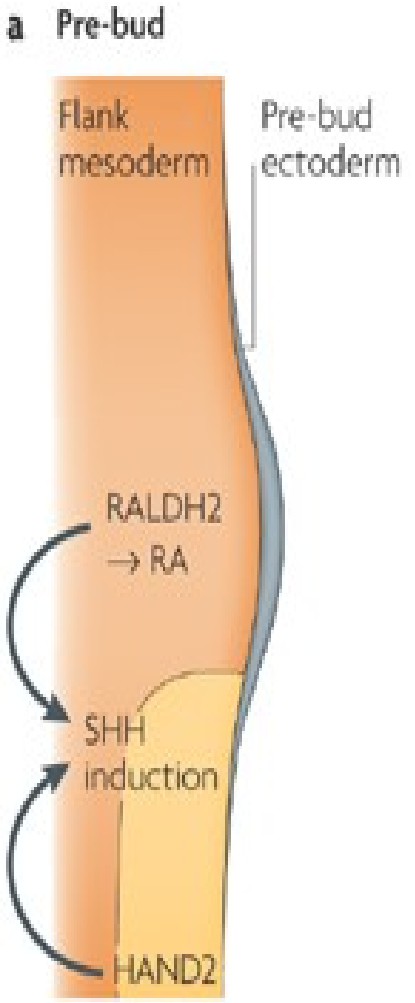


FIG. 1 Effects of vitamin A (10 IU) treatment for various times on limb generation from amputated tail stump. *a*, Treatment for 24 h, 3 limbs are generated; *b*, 72 h, 4 limbs; *c* and *d*, 96 h, 2 limbs; *e*, 120 h, 7 limbs; *f*, 120 h, 3 limbs; *g*, 144 h, 3 limbs; *h*, 144 h, 2 limbs, plus an extra pair of limbs below the original hindlimb.

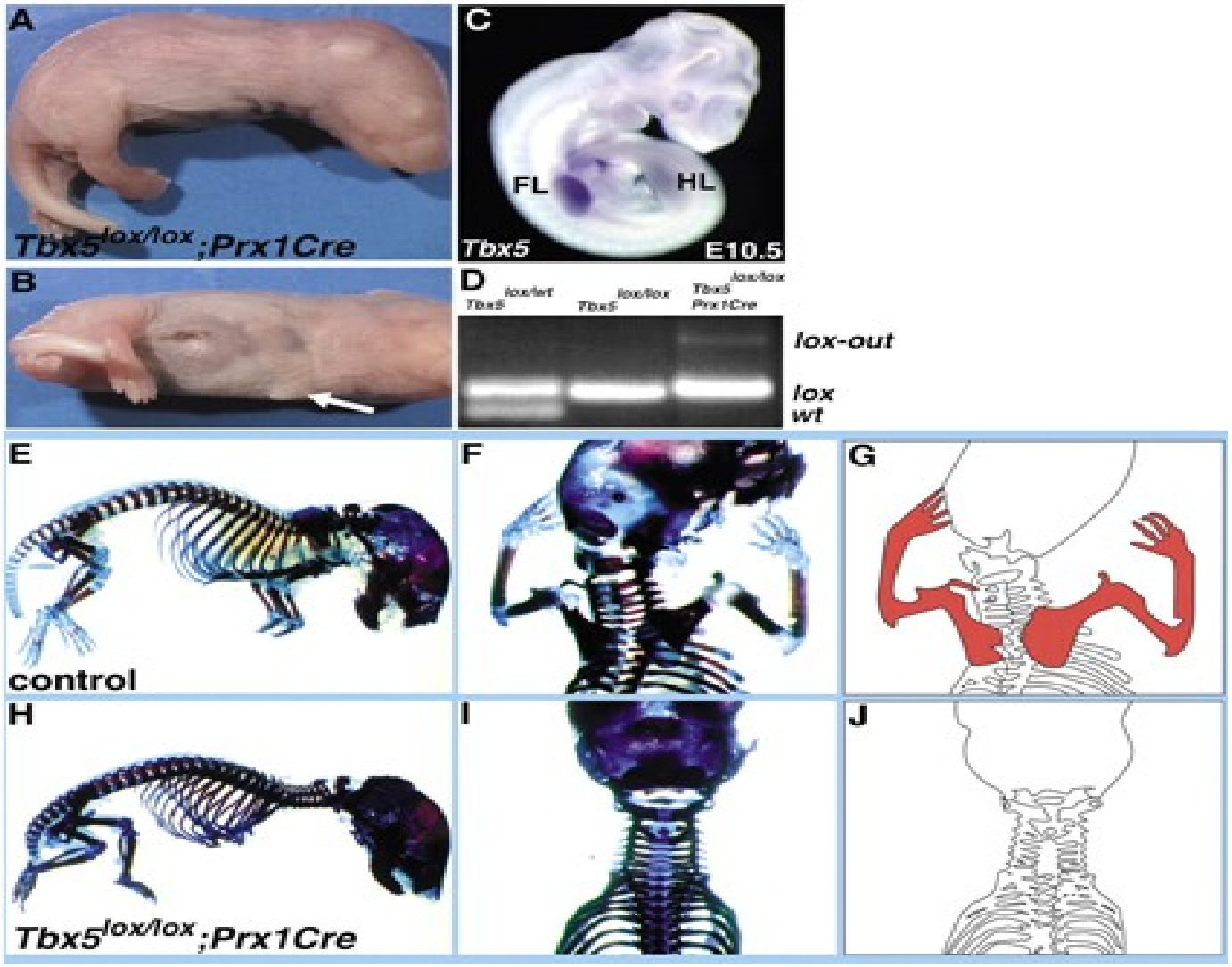


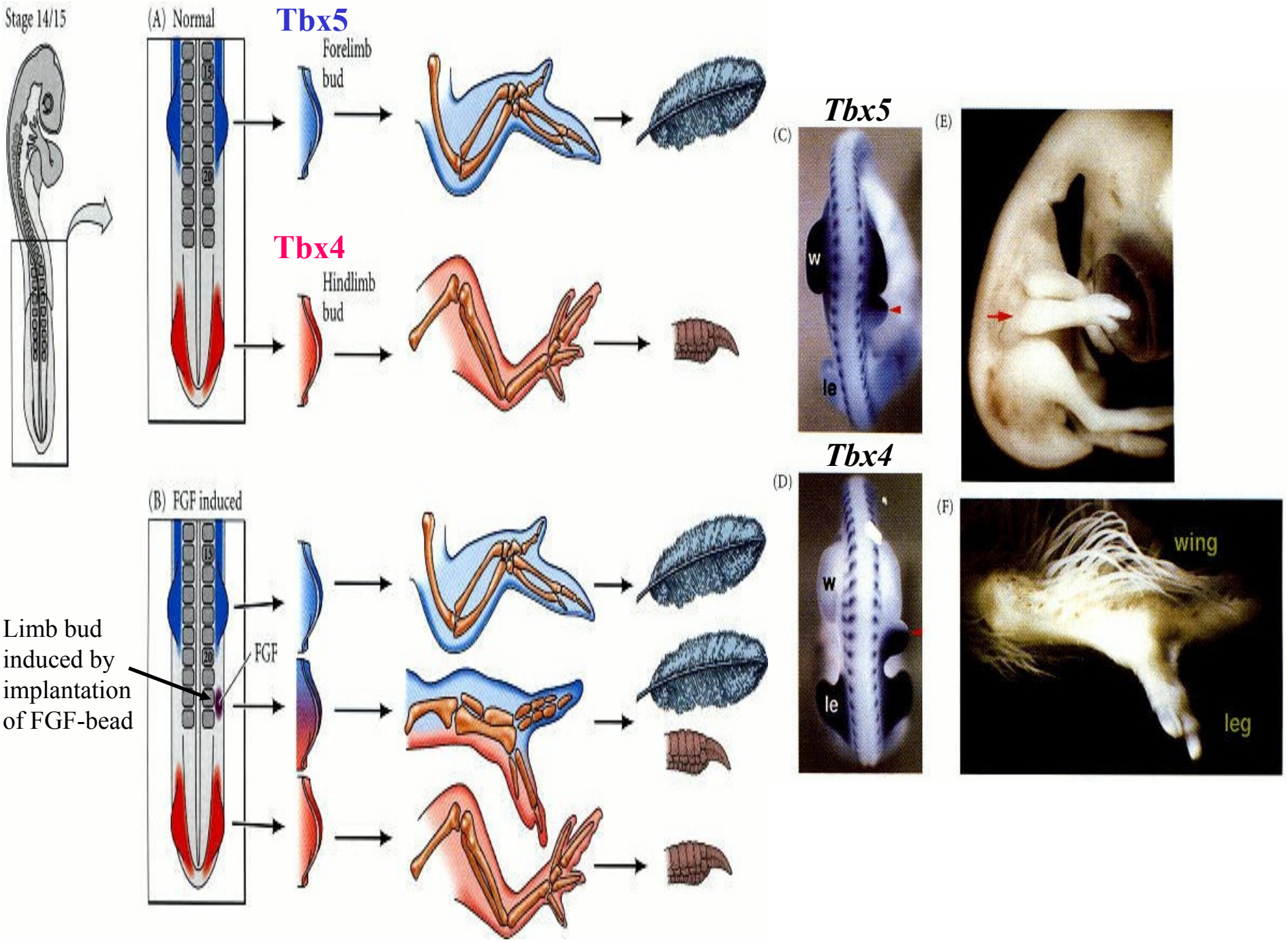
Nature. 1992 Jan 23;355(6358):352-3.

RALDH2- retinaldehyde dehydrogenase 2

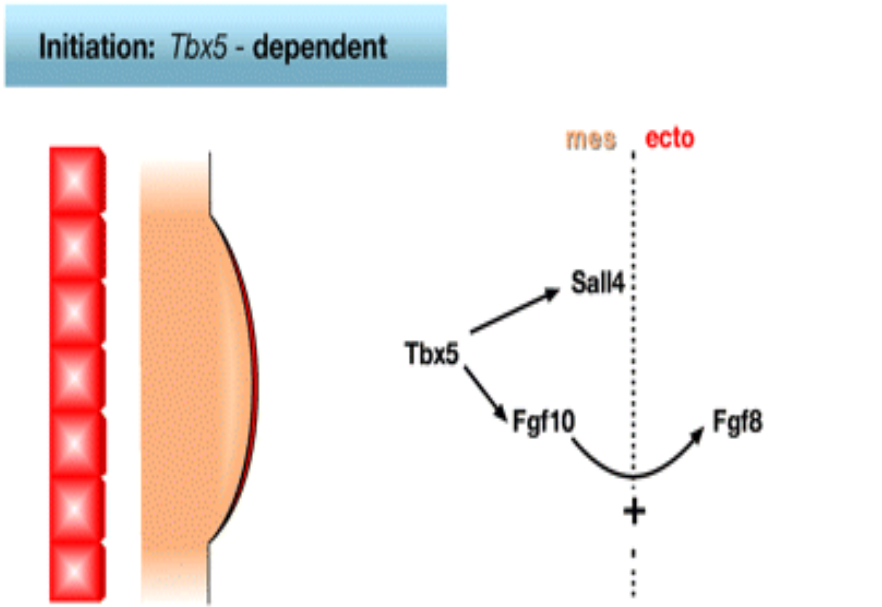
……LATER CAME TBX

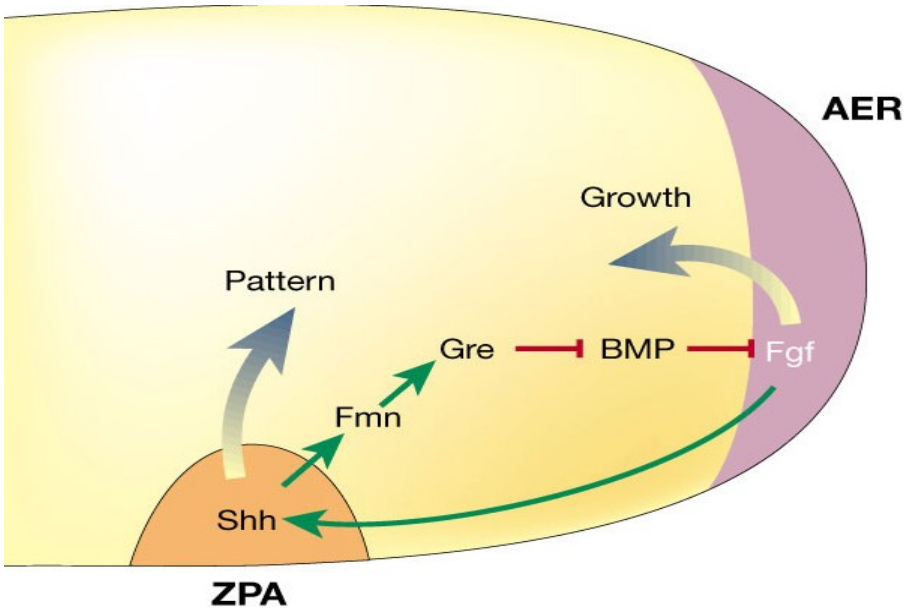
DNA binding domain derived from the prototype gene called transcription factor T. Limb identity factors Tbx4 (hindlimb) and Tbx5 (forelimb)



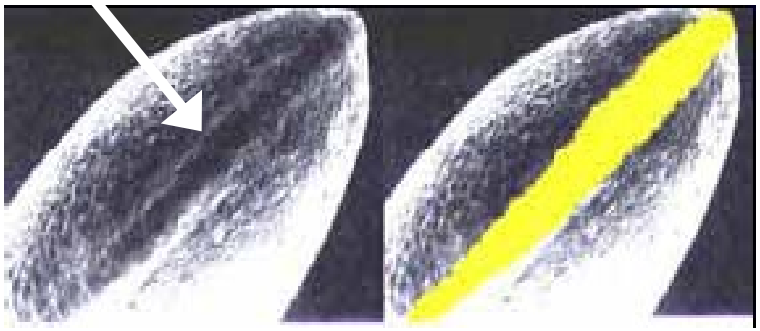

Stage 14/15
(A) Normal
Tbx5
Forelimb bud
Tbx4
Hindlimb bud
(B) FGF induced
FGF
Limb bud induced by implantation of FGF-bead
Tbx5
(C)
w
le
Tbx4
(D)
w
le
(E)
(F)
wing
leg

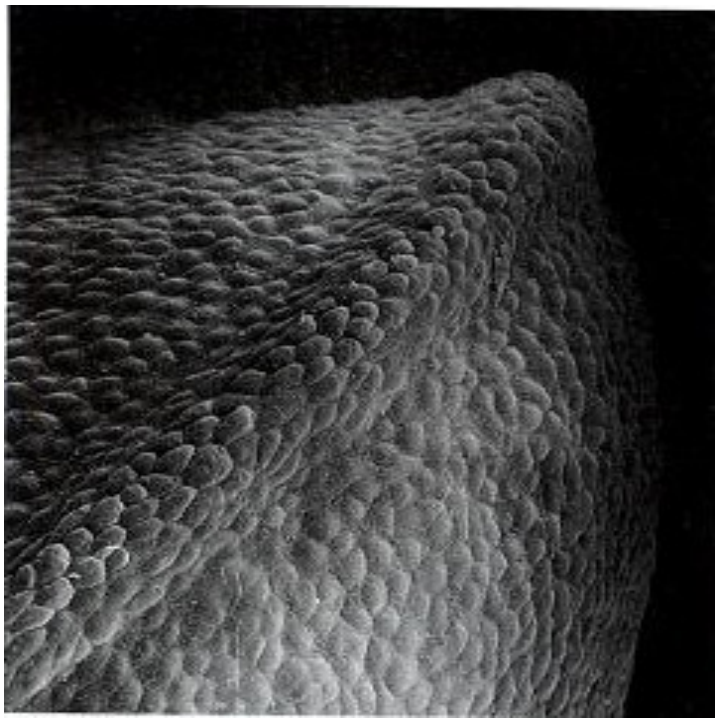
RA → Hox6 (limb field) → Tbx (limb identity) → limb growth/patterning





AER





……..FOLLOWED BY FGF

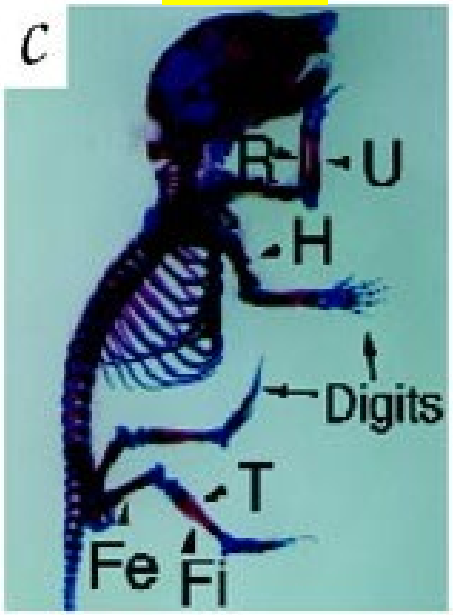
FGF10 → proliferation in the mesoderm → limb bud growth

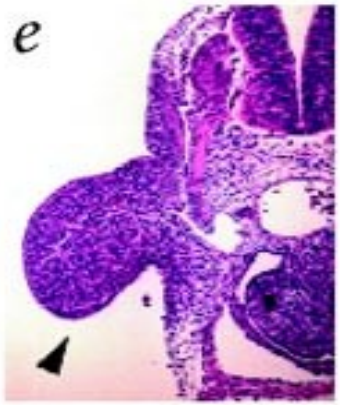
wt

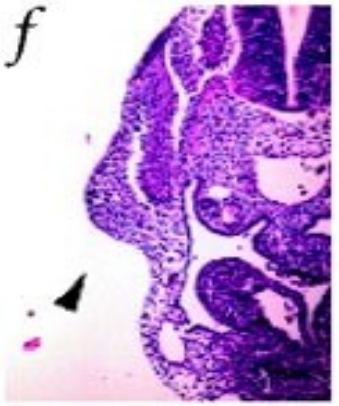
Fgf10$^{-/-}$

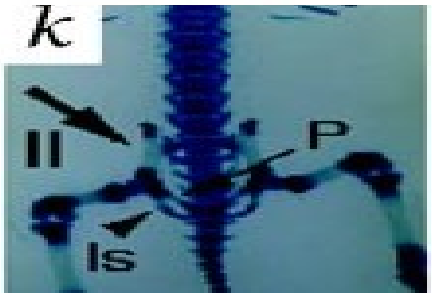
wt

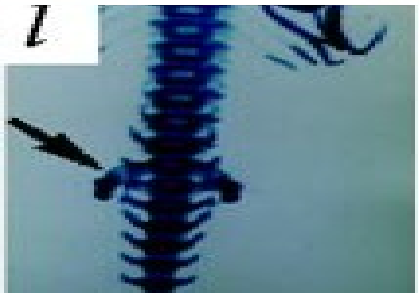
Fgf10$^{-/-}$

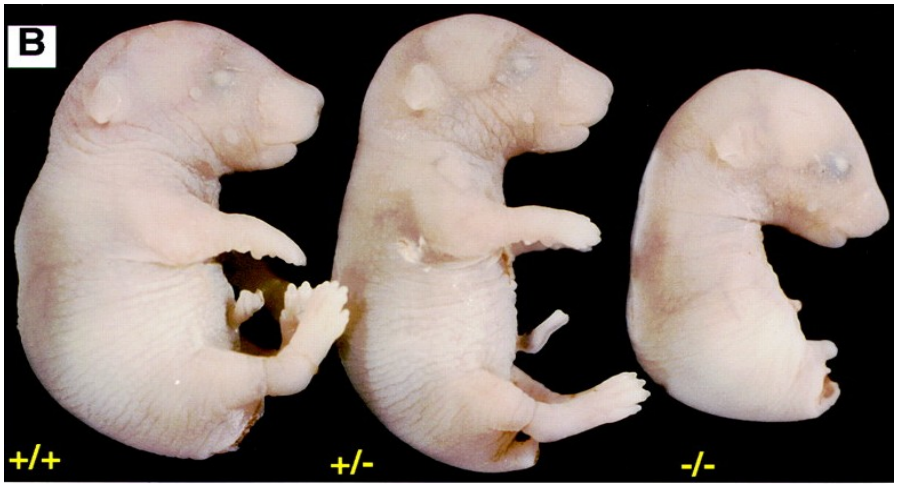










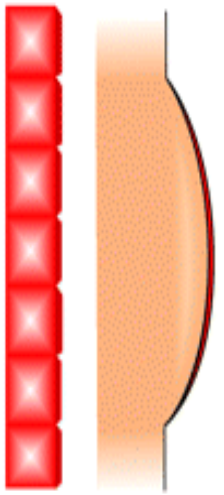


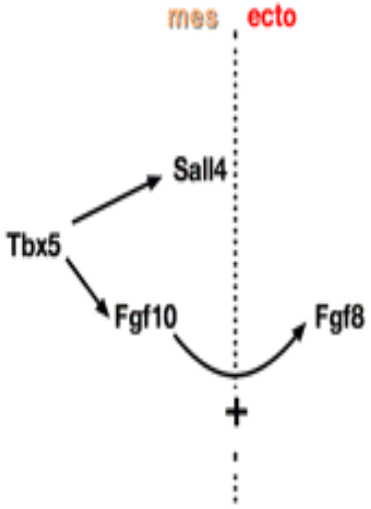
Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless

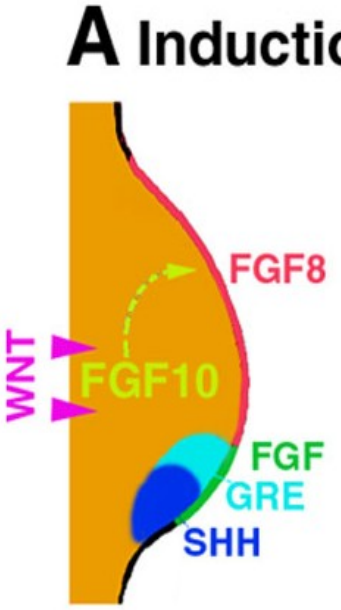
Hosung Min, Dimitry M. Danilenko, Sheila A. Scully, et al.

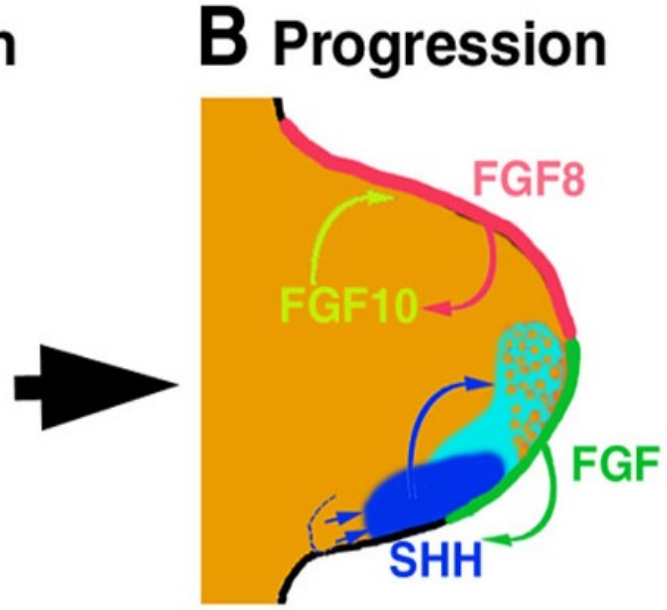
Genes Dev. 1998 12: 3156-3161
Access the most recent version at doi:10.1101/gad.12.20.3156

Initiation: *Tbx5* - dependent



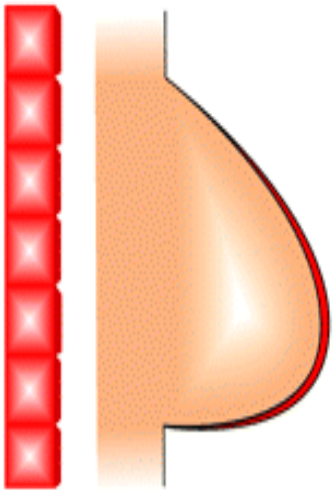


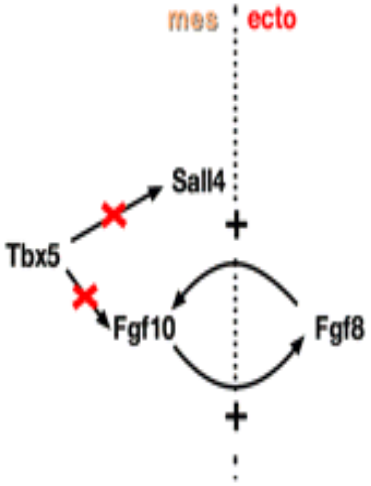


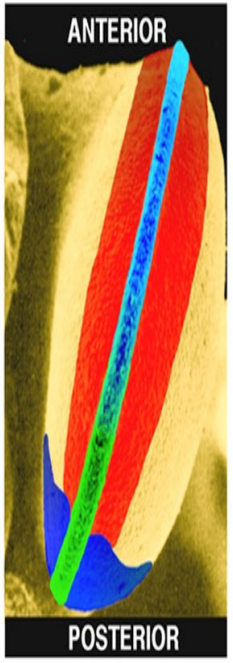


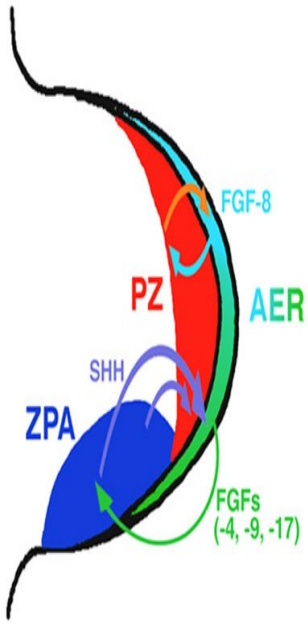
Fgf8

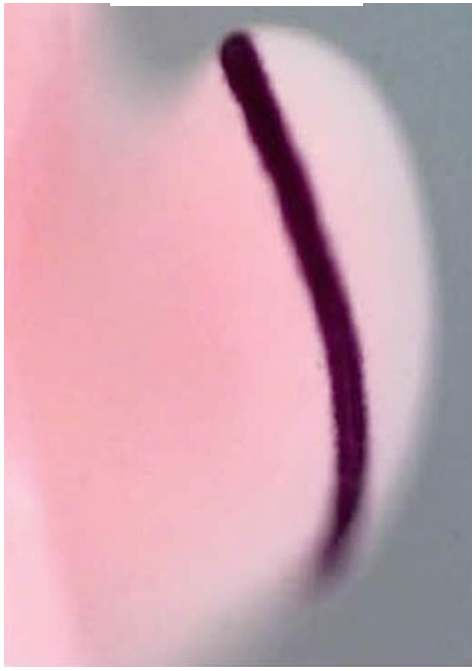
Outgrowth: *Tbx5* - independent









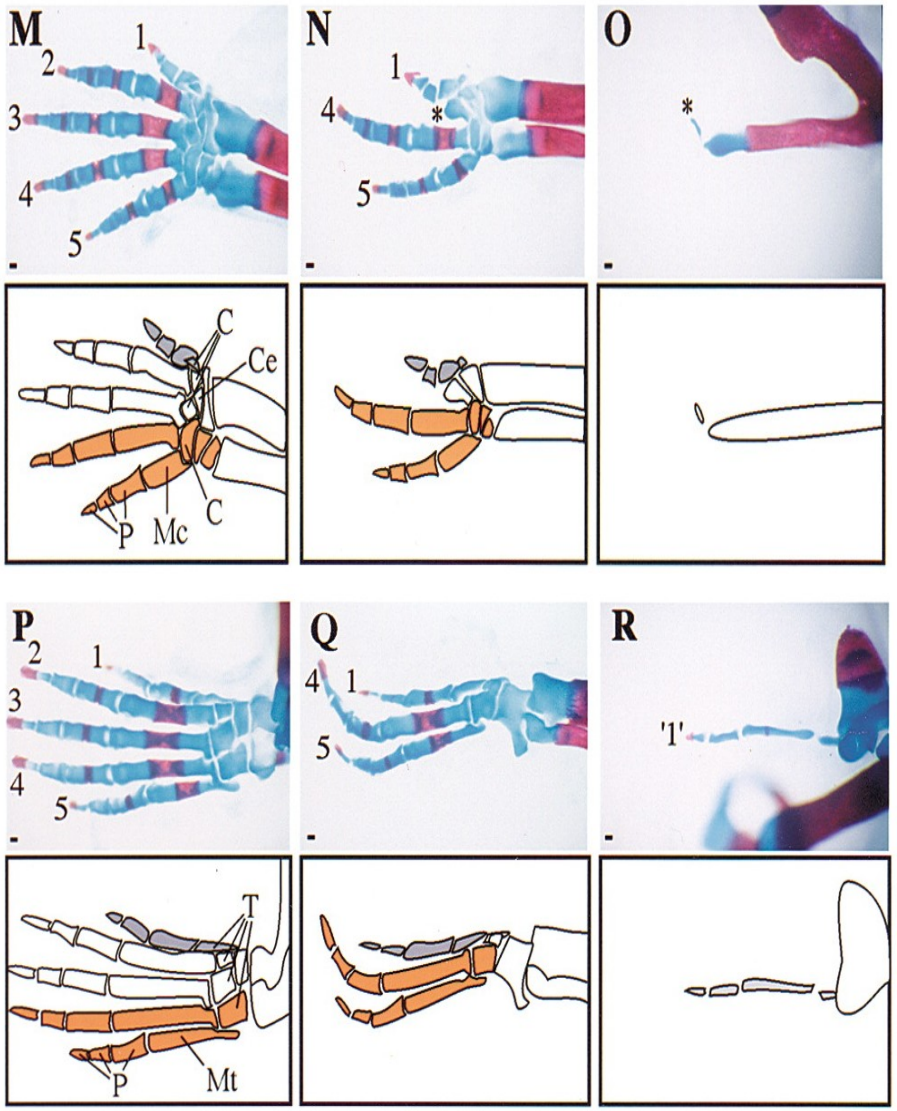


……FOLLOWED BY SHH

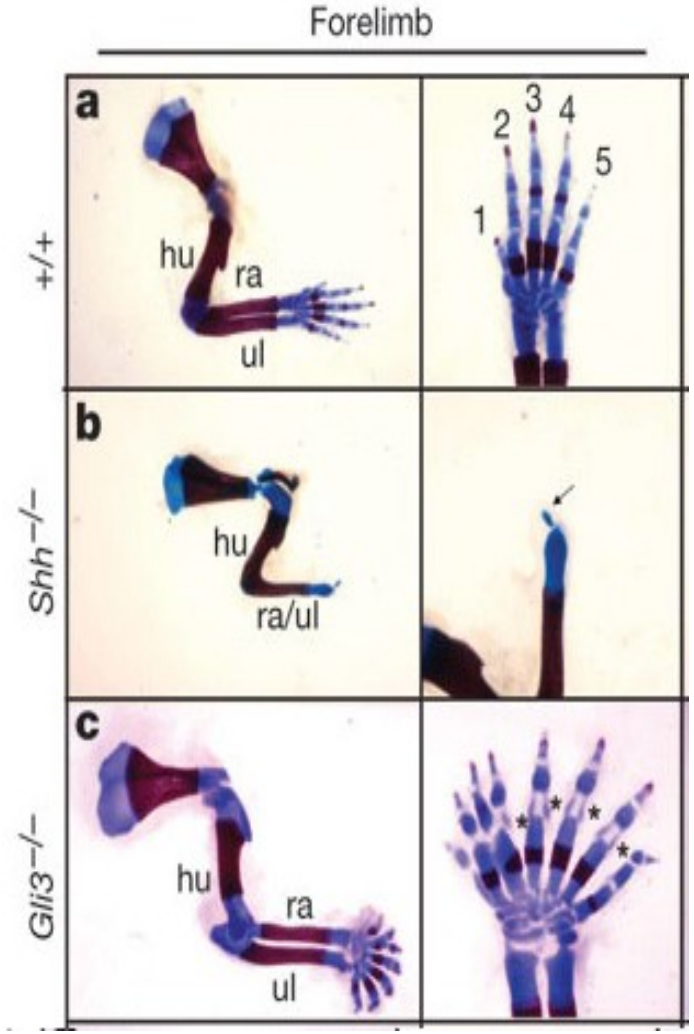
SHH function: normal attenuated lost

Forelimbs

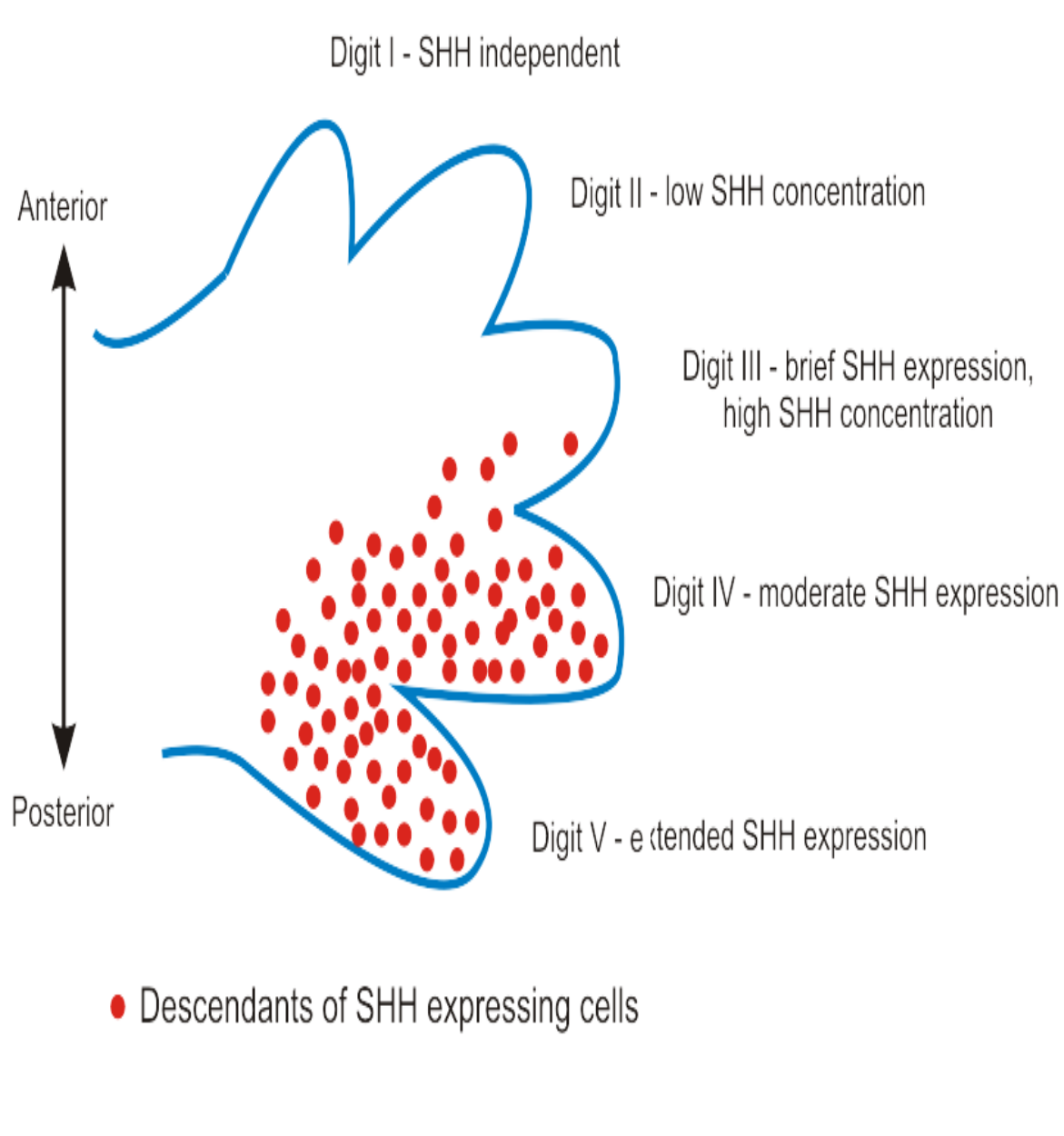
Hindlimbs

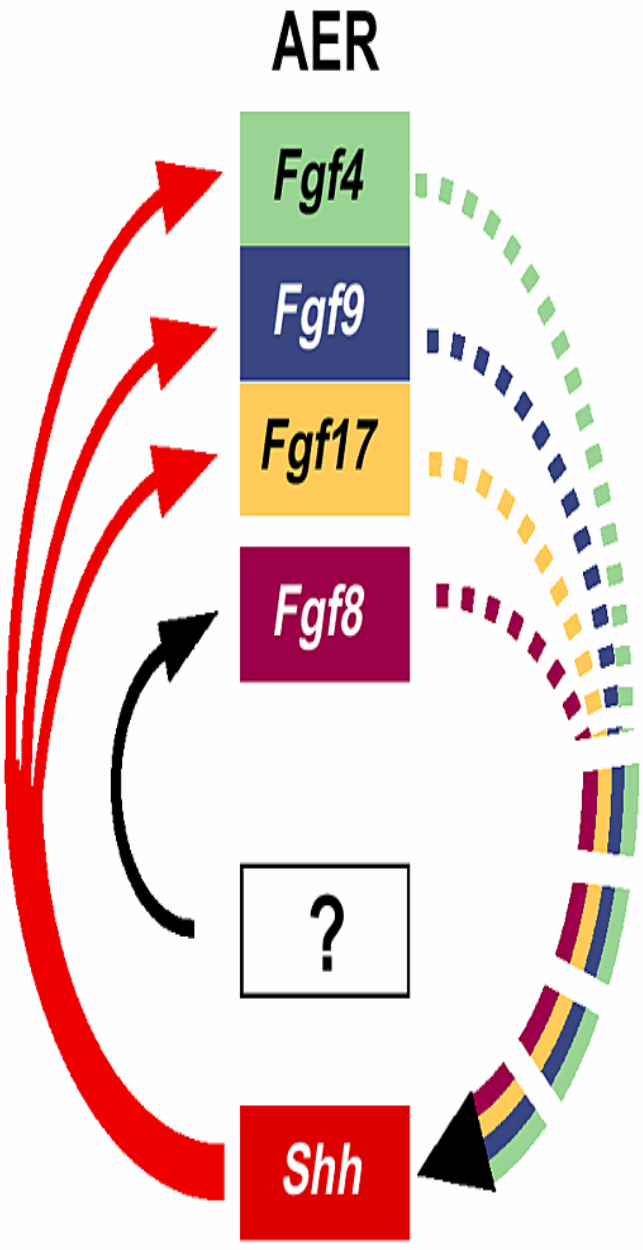


Cell, Vol. 105, 599–612, June 1, 2001

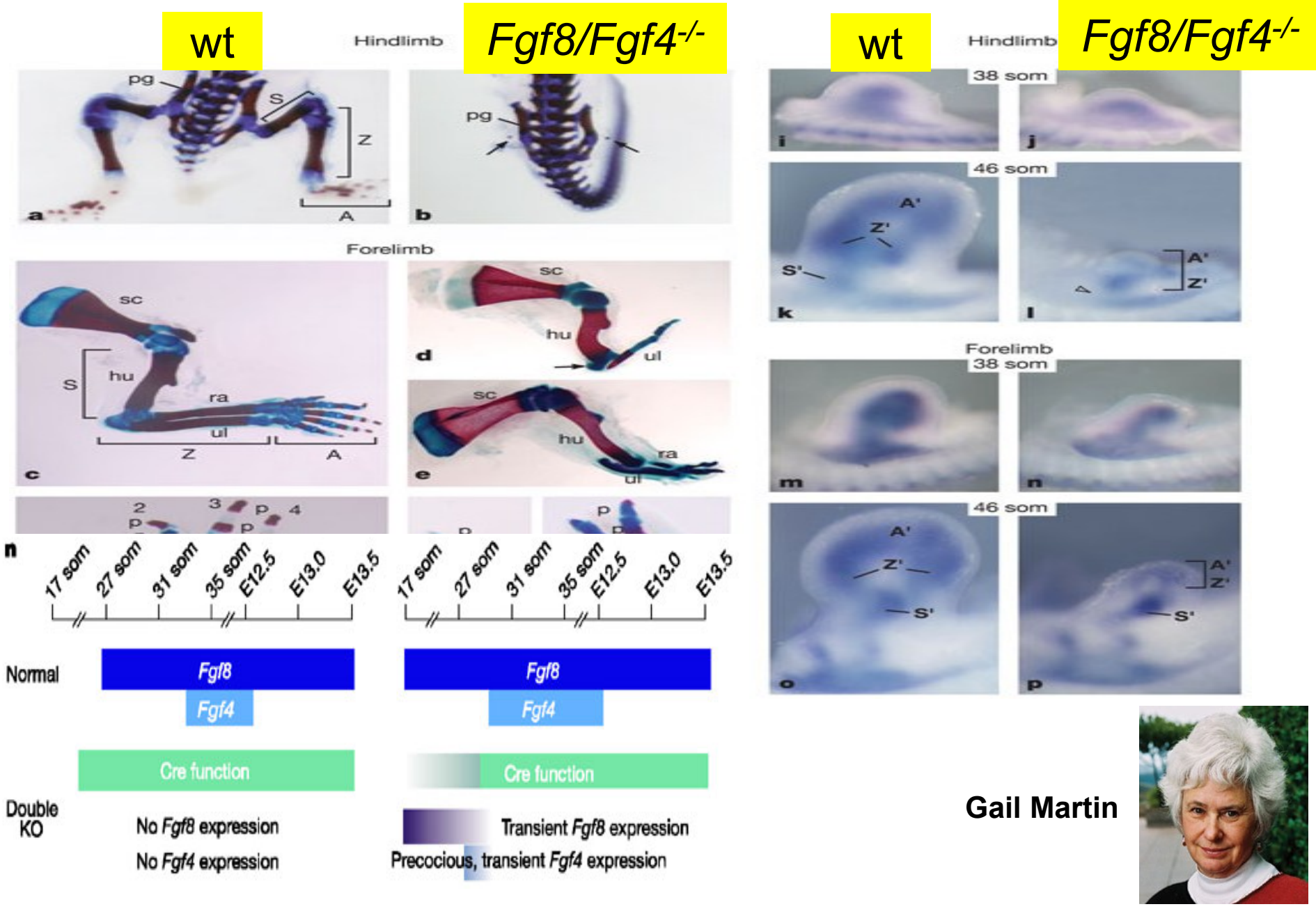


Nature 418, 979-983 (29 August 2002)

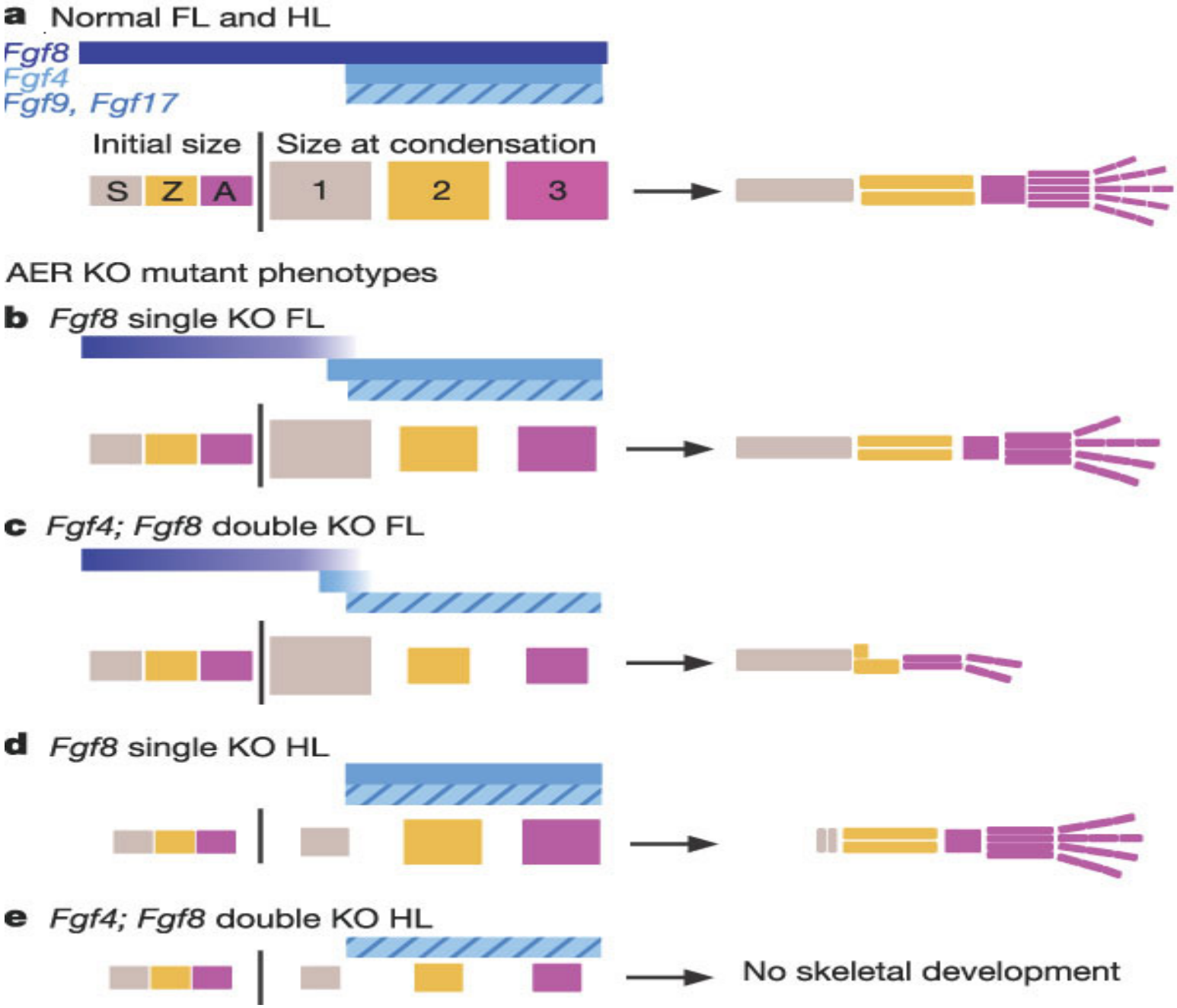

Digit I - SHH independent
Anterior
Digit II - low SHH concentration
Digit III - brief SHH expression, high SHH concentration
Digit IV - moderate SHH expression
Posterior
Digit V - extended SHH expression
Descendants of SHH expressing cells


AER
Fgf4
Fgf9
Fgf17
Fgf8
?
Shh

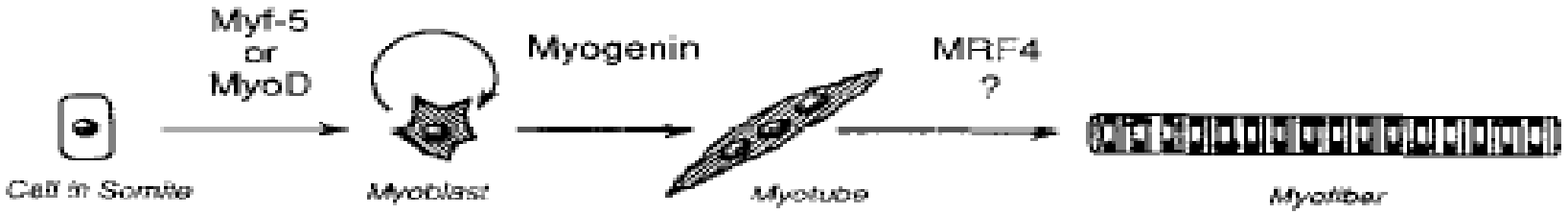
……FOLLOWED BY MORE FGF



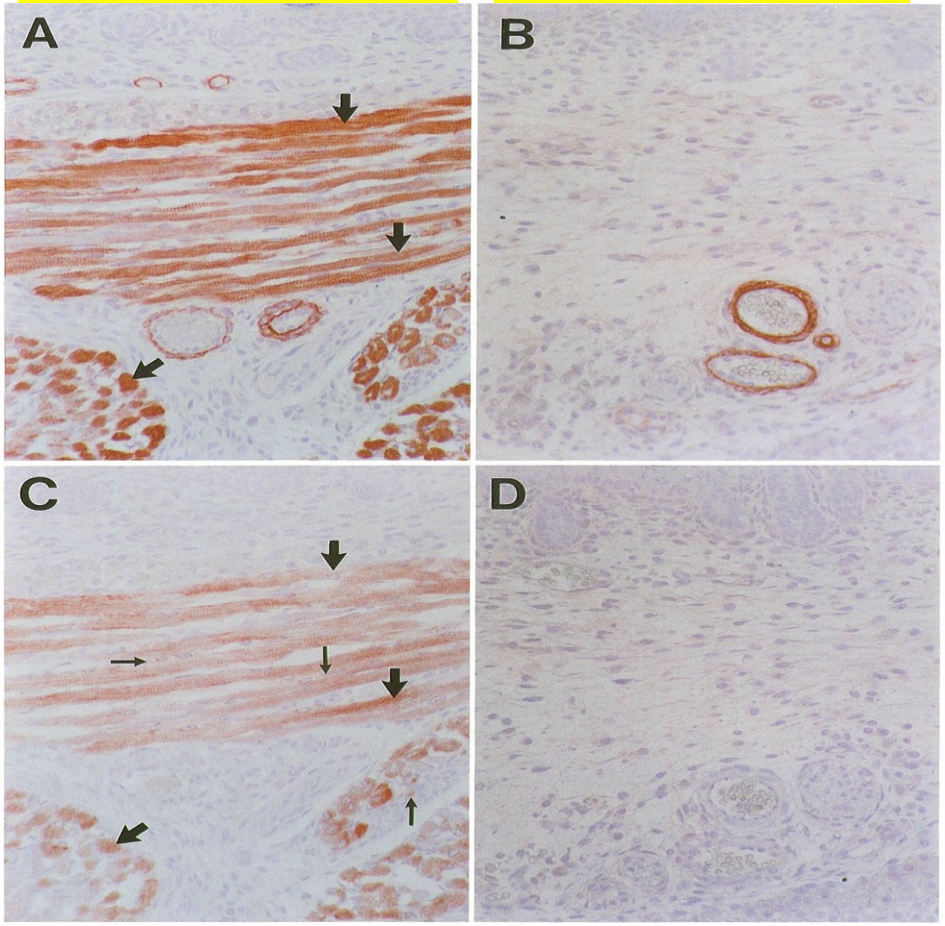
Gail Martin


a Normal FL and HL
Fgf8
Fgf4
Fgf9, Fgf17
Initial size
Size at condensation
S
Z
A
1
2
3
AER KO mutant phenotypes
b Fgf8 single KO FL
c Fgf4; Fgf8 double KO FL
d Fgf8 single KO HL
e Fgf4; Fgf8 double KO HL
No skeletal development

MUSCLE DIFFERENTIATION BY BASIC HELIX-LOOP-HELIX (bHLH) FACTORS



MyoD$^{+/-}$ *Myf-5*$^{+/-}$ | *MyoD*$^{-/-}$ *Myf-5*$^{-/-}$



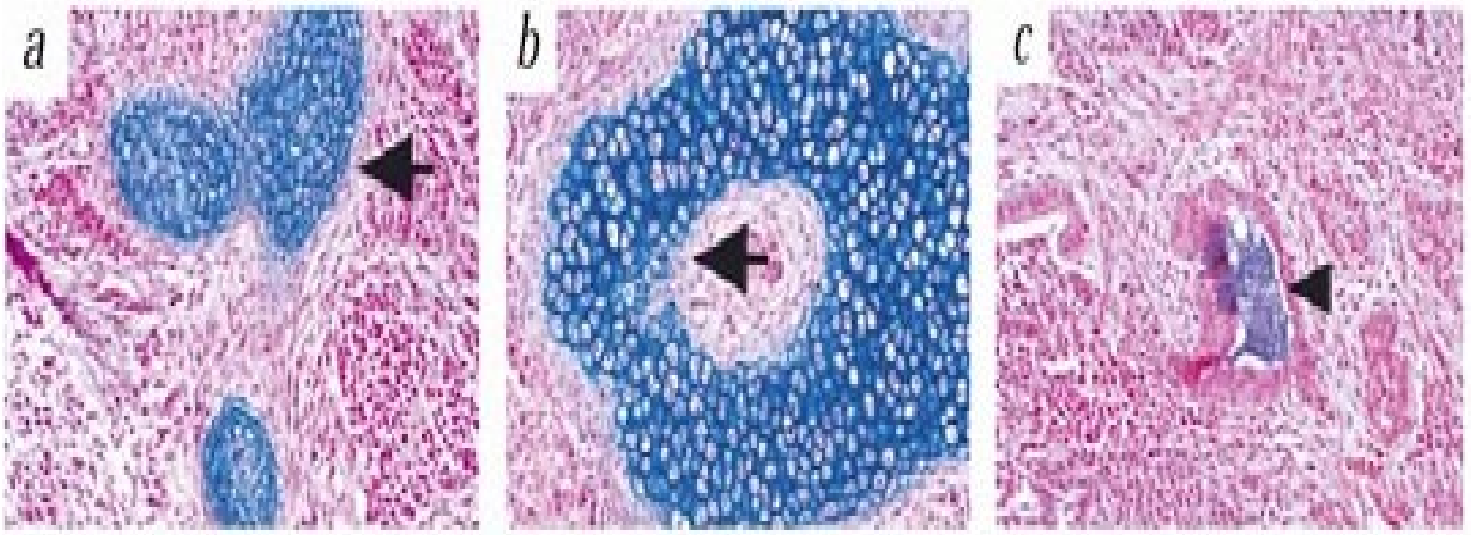
α-actin IHC
(smooth and striated muscle fibers)

desmin IHC
(skeletal muscle fibers, myoblast-like cells)

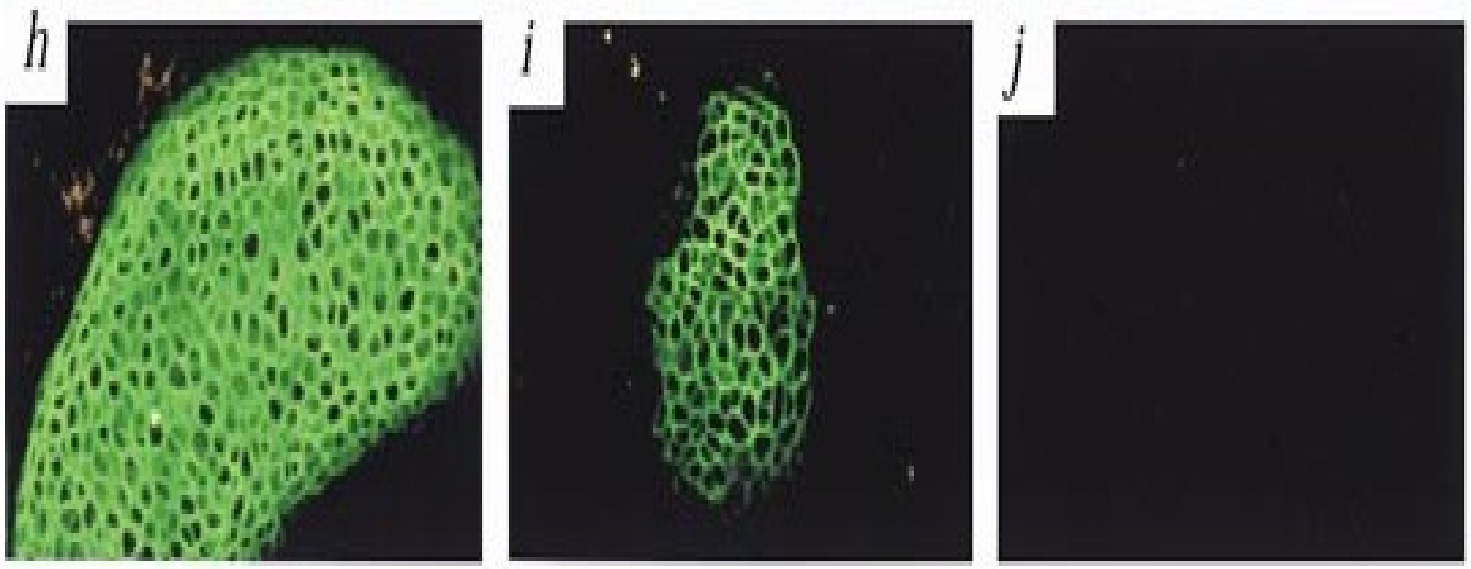
CARTILAGE DIFFERENTIATION BY SOX9 (Sry-Box9)

wt | *Sox9*$^{+/-}$ | *Sox9*$^{-/-}$

alcian blue

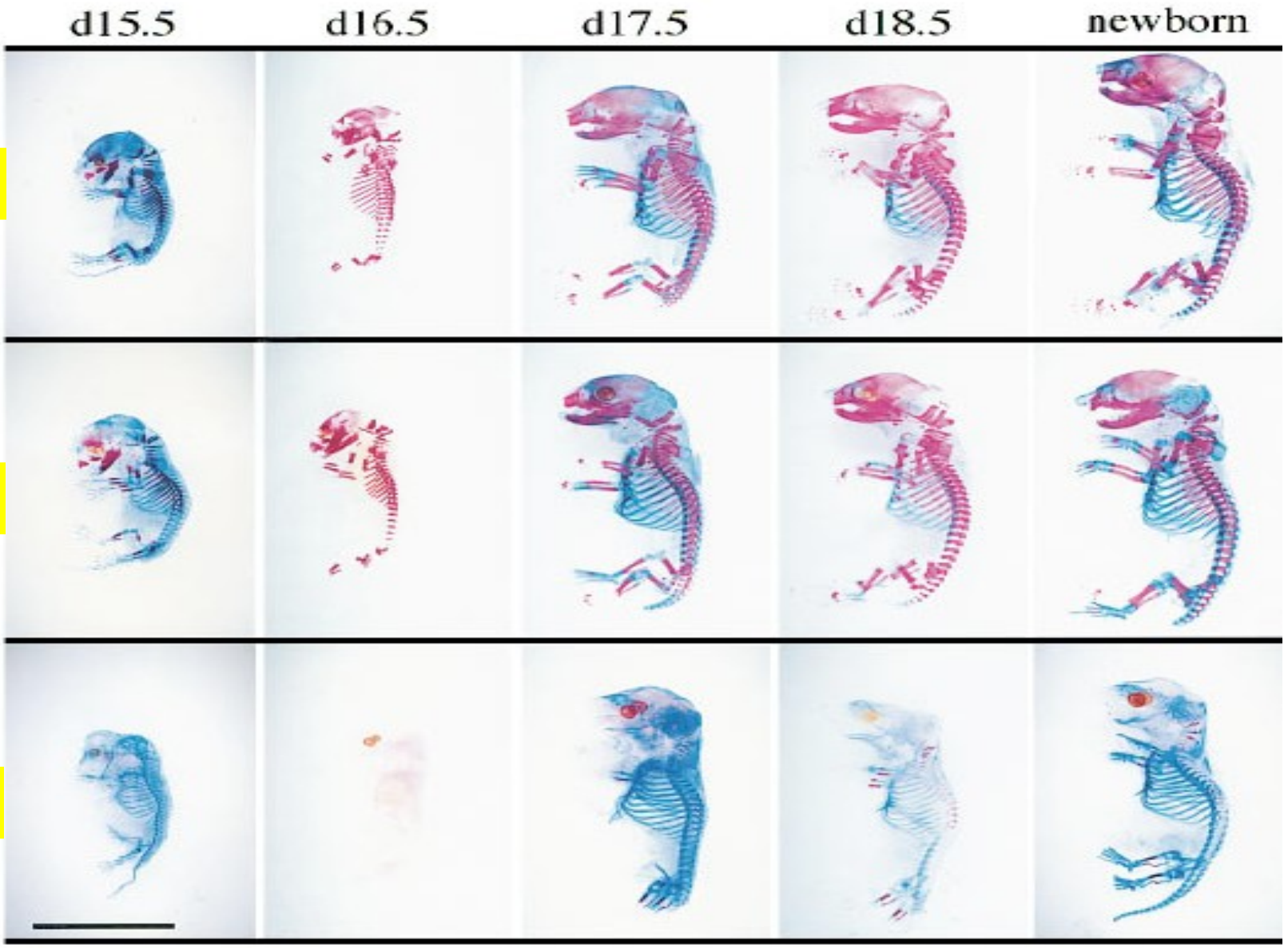


collagen type II

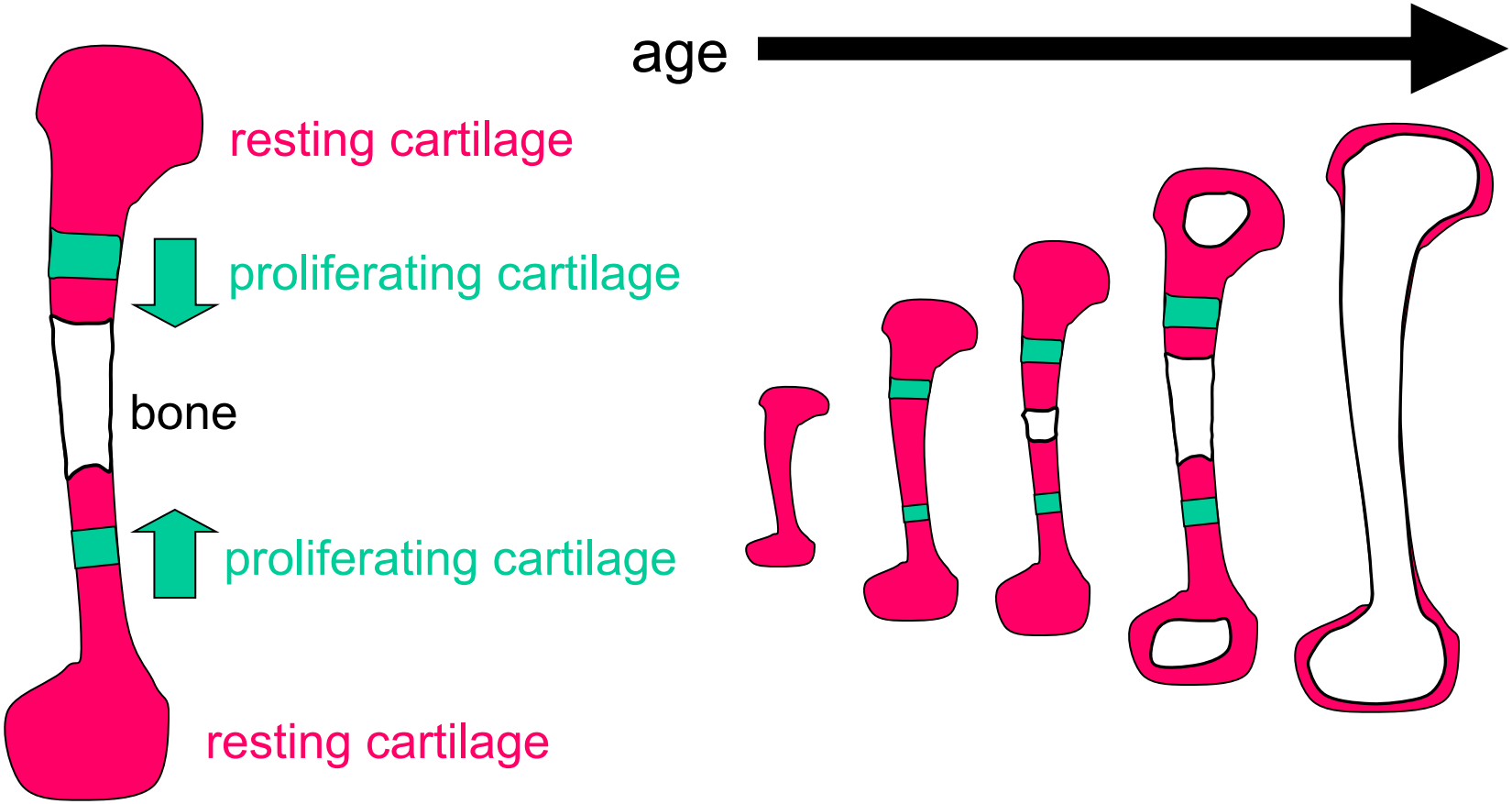


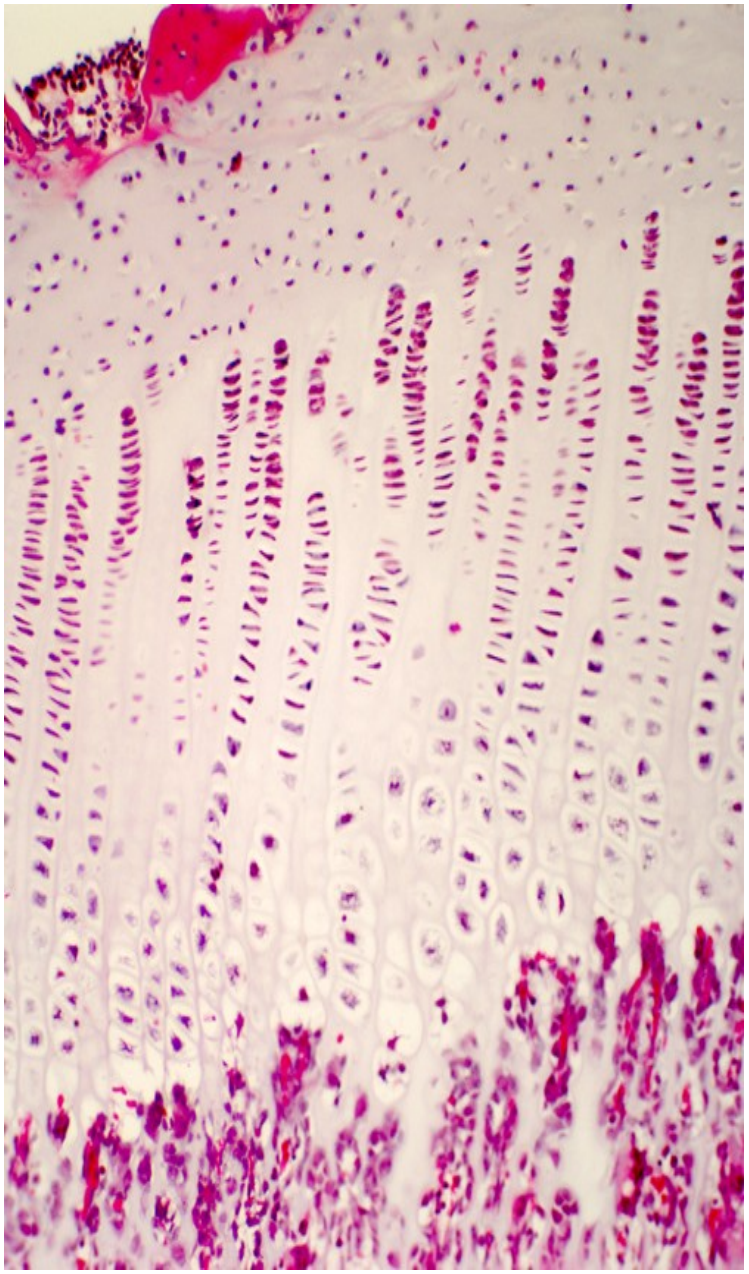
BONE DIFFERENTIATION BY CBFA1 (RUNX2)

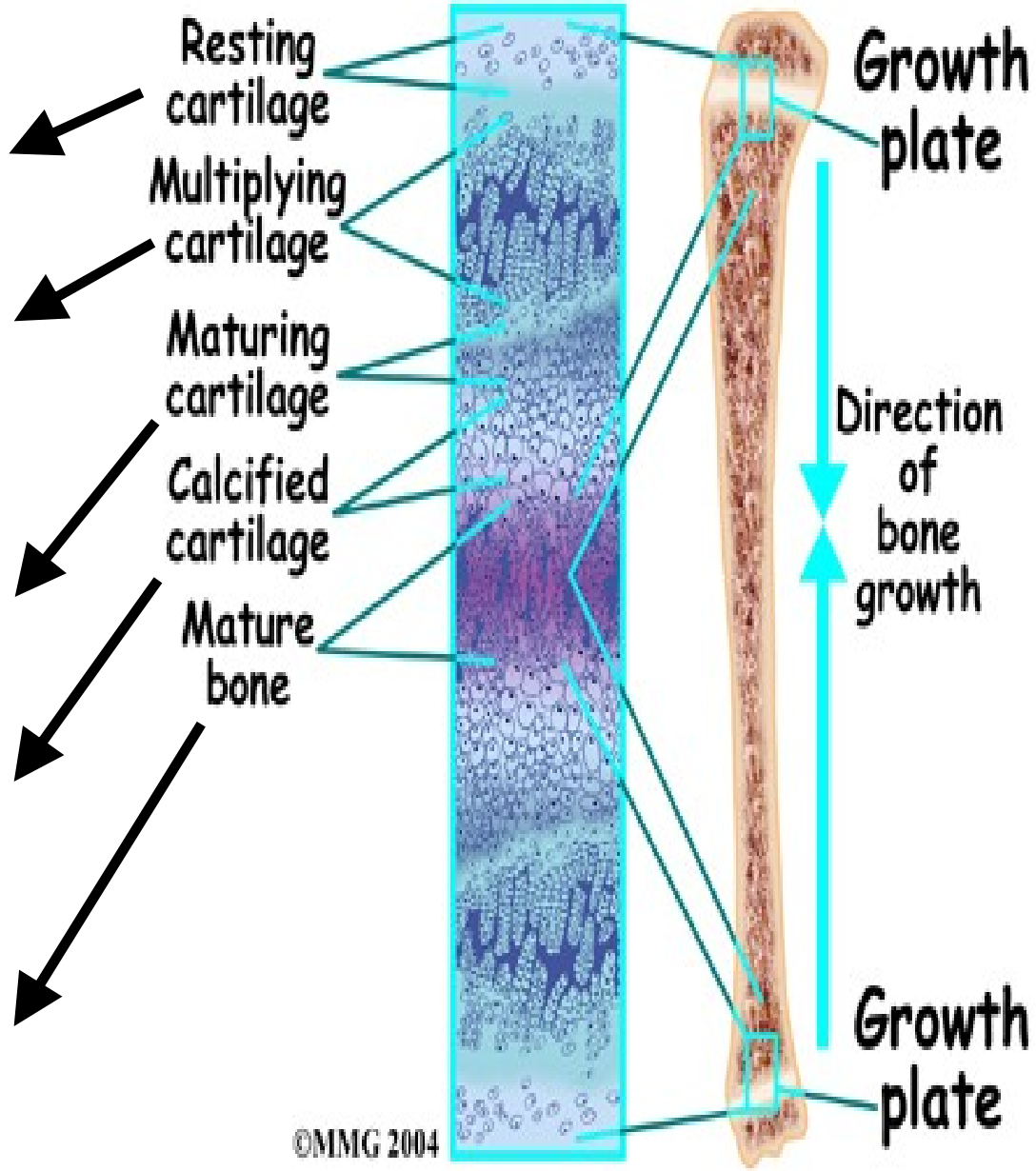
Runx2 (Runt-related transcription factor 2)



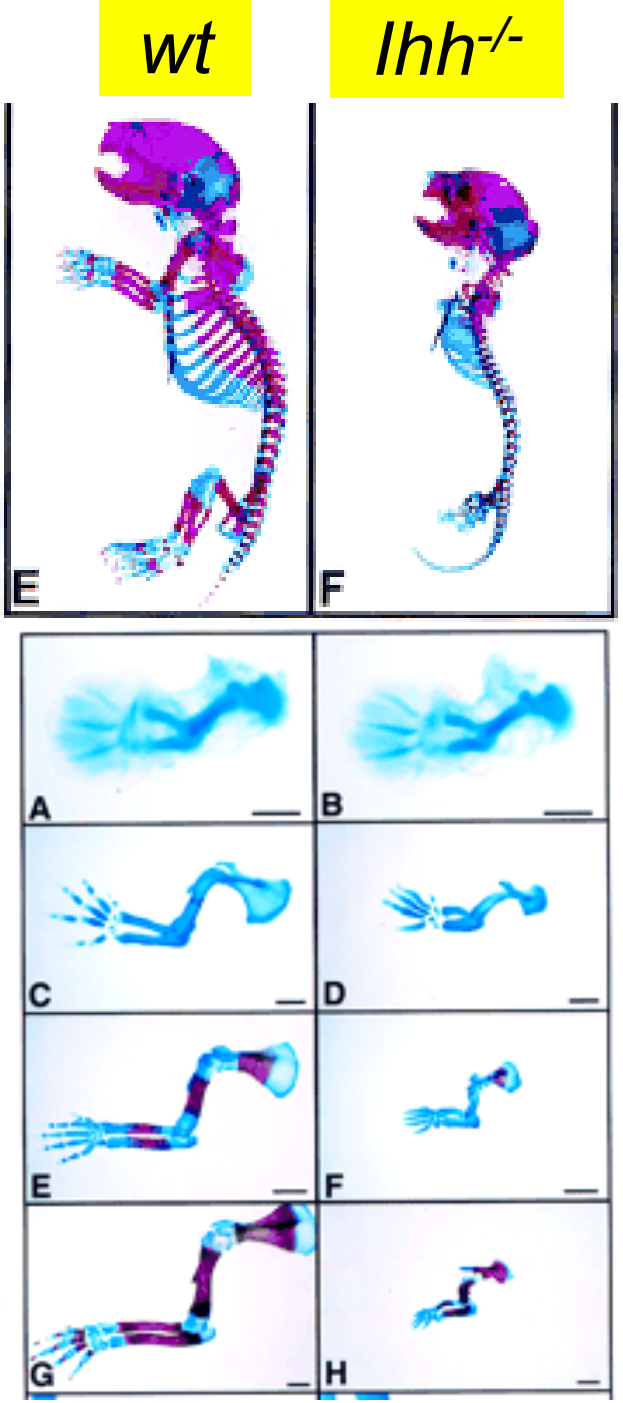
How do the limbs grow?

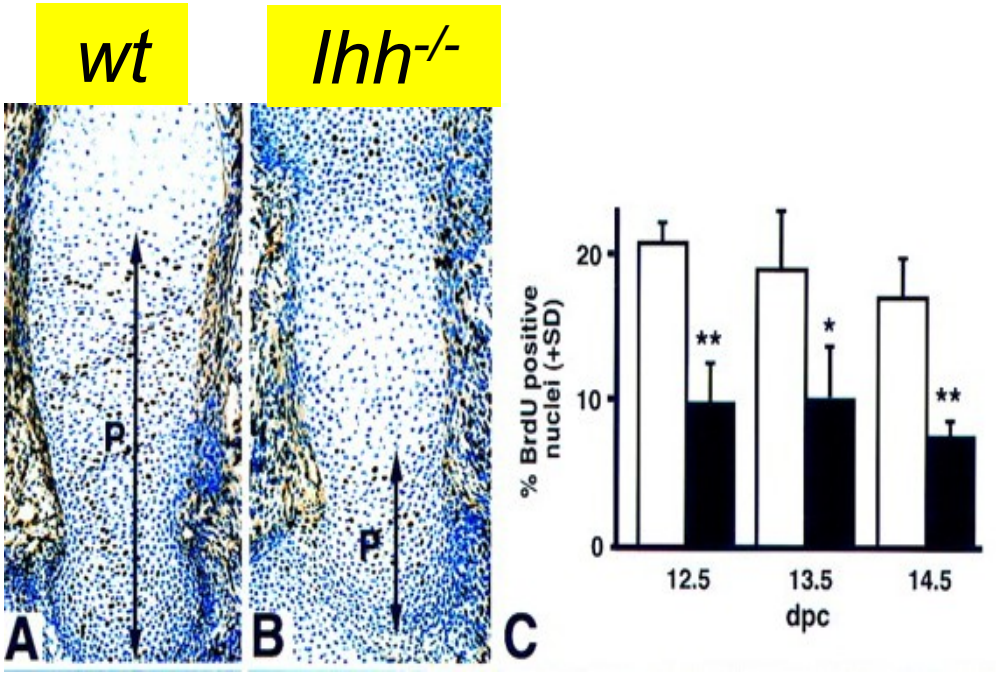




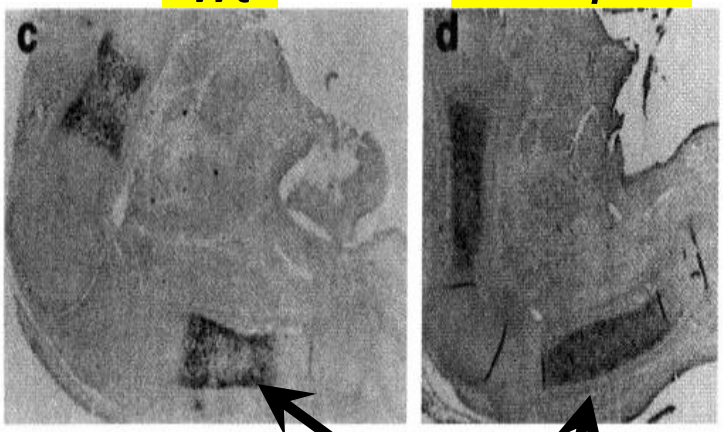

Resting cartilage
Multiplying cartilage
Maturing cartilage
Calcified cartilage
Mature bone
Growth plate
Direction of bone growth
Growth plate
©MMG 2004

Indian hedgehog (Ihh)

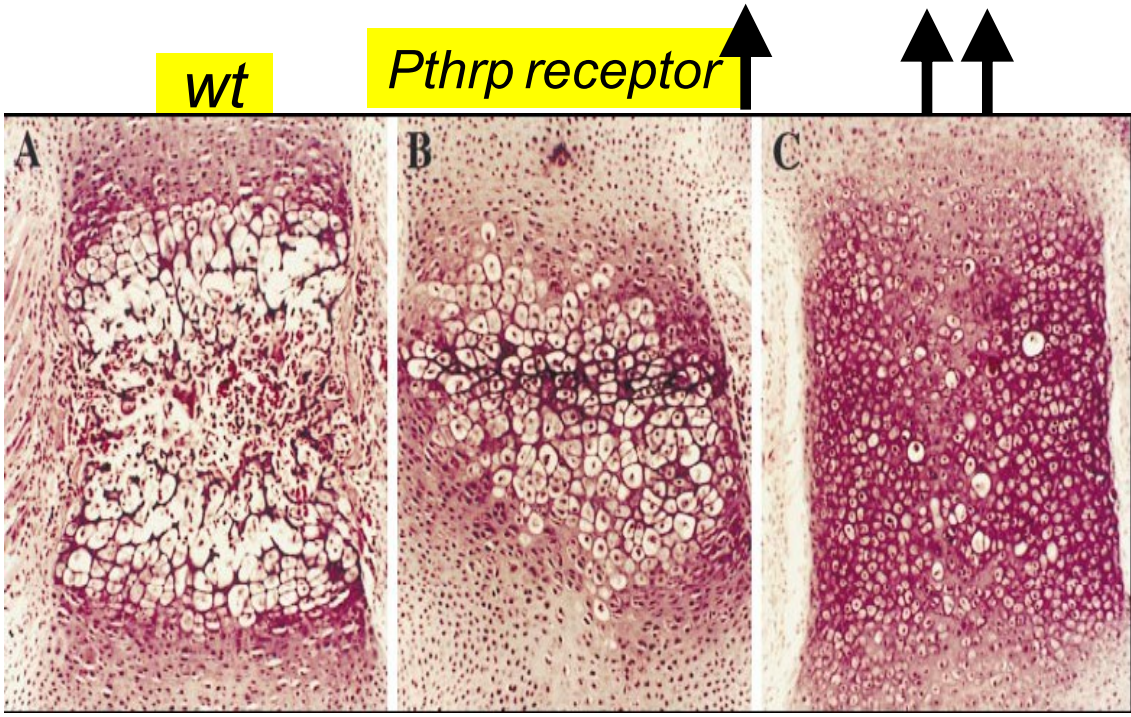




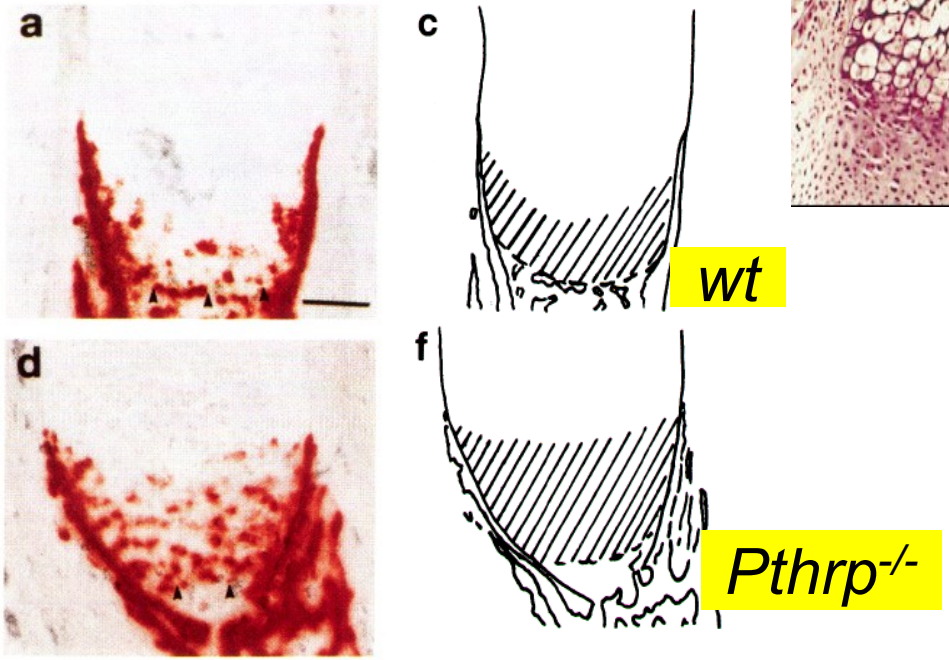
Parathyroid hormone-related peptide (PTHrP)

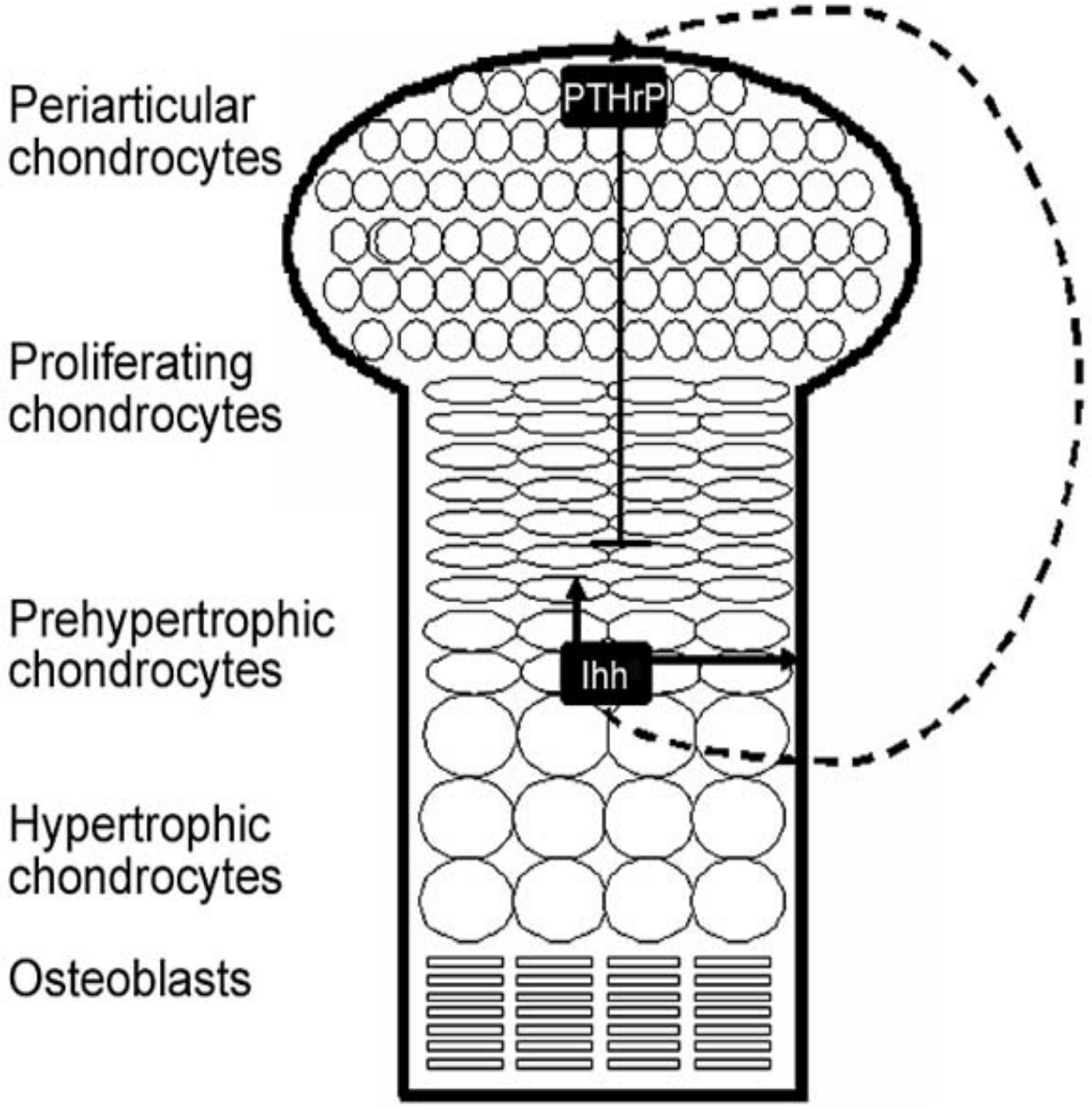


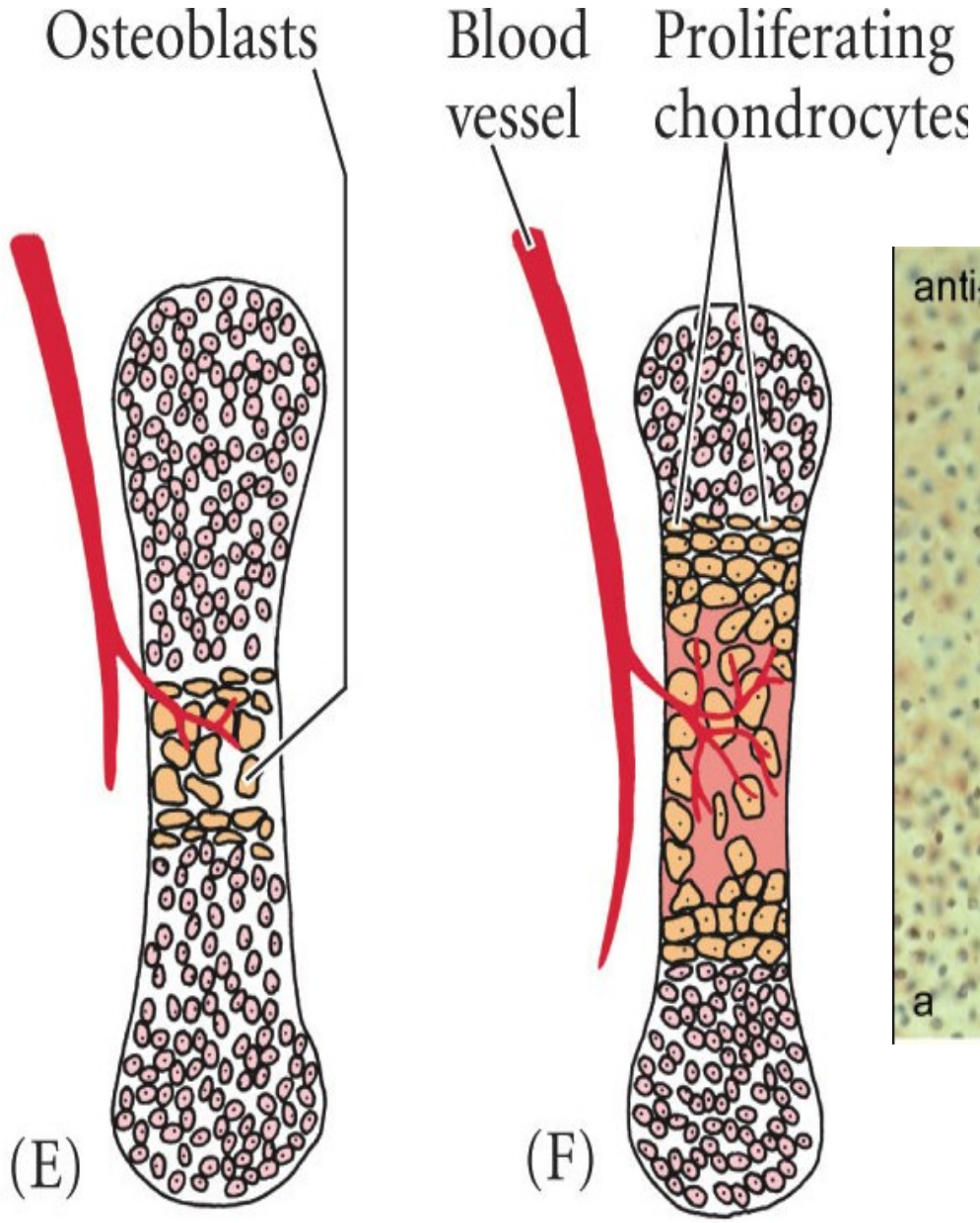
Coll type X in situ

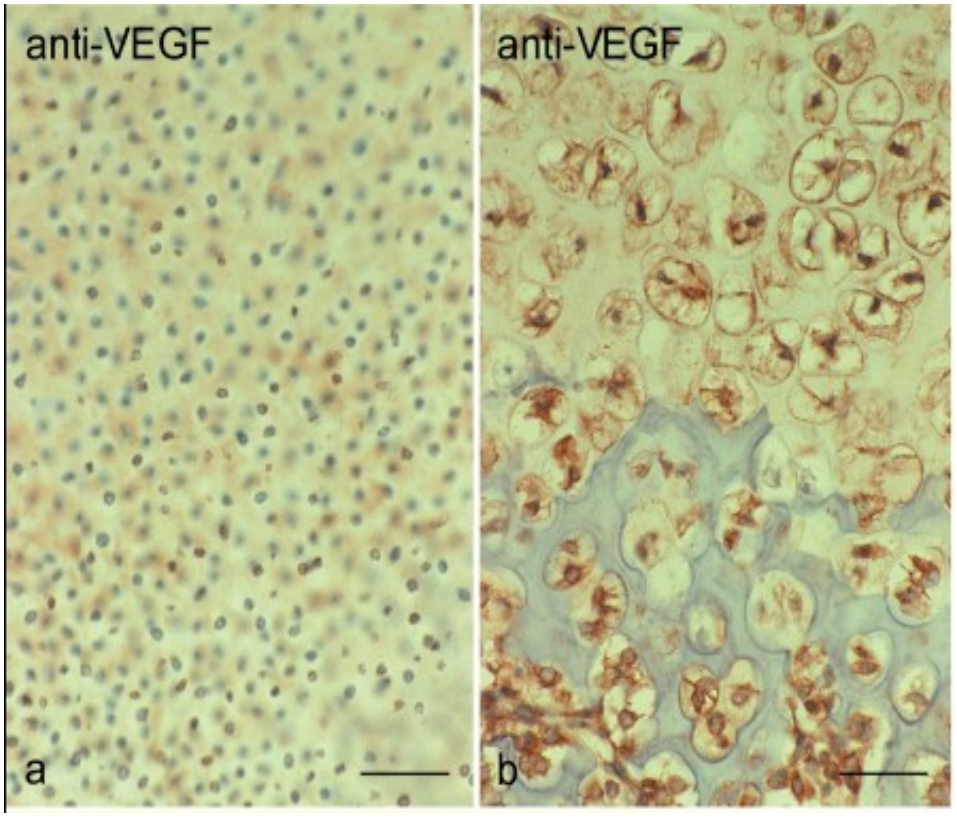


Sternal cartilage




Periarticular chondrocytes
Proliferating chondrocytes
Prehypertrophic chondrocytes
Hypertrophic chondrocytes
Osteoblasts
PTHrP
Ihh


Osteoblasts
Blood vessel
Proliferating chondrocytes
(E)
(F)


anti-VEGF
anti-VEGF
a
b

VEGFA is necessary for chondrocyte survival during bone development

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Bjorn R. Olsen[1,*]

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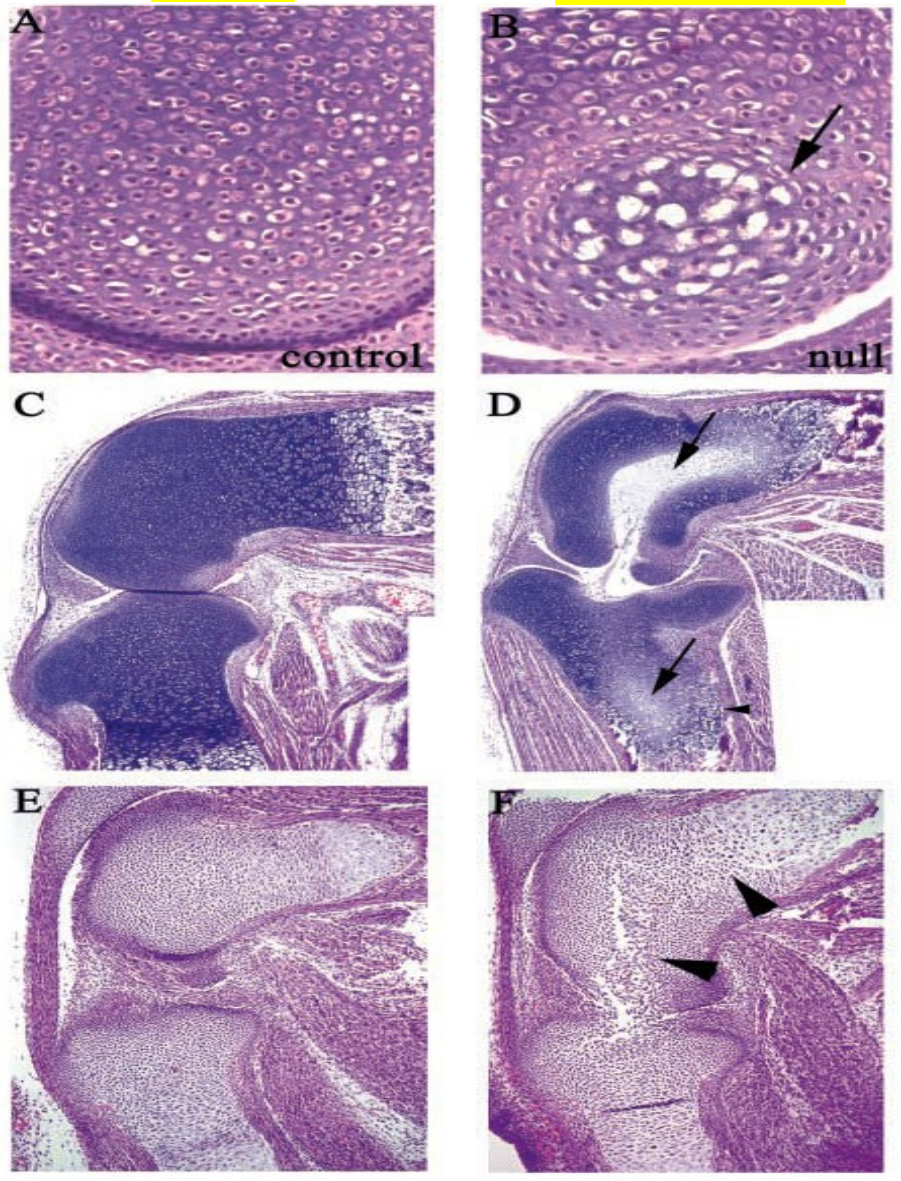
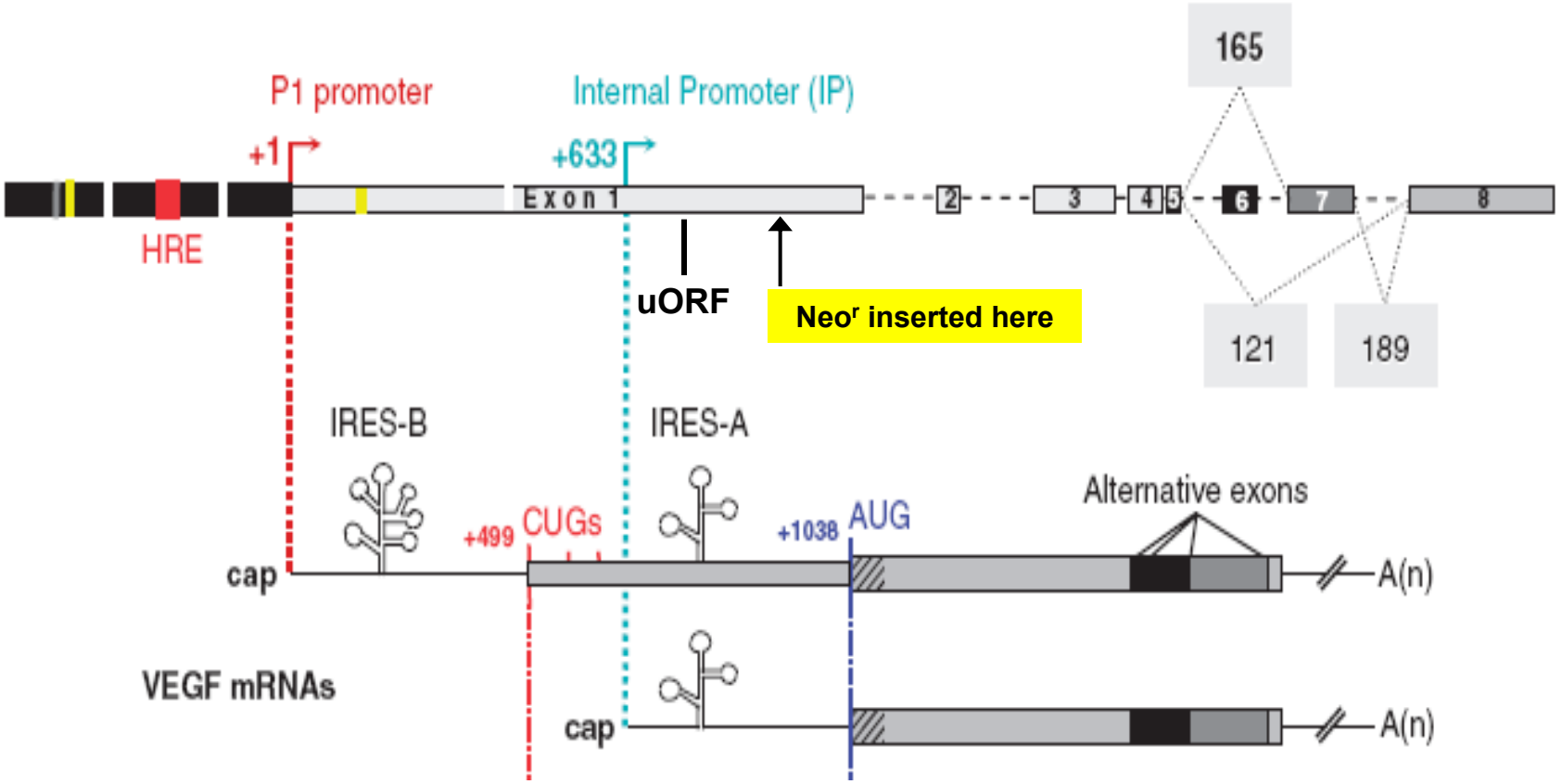
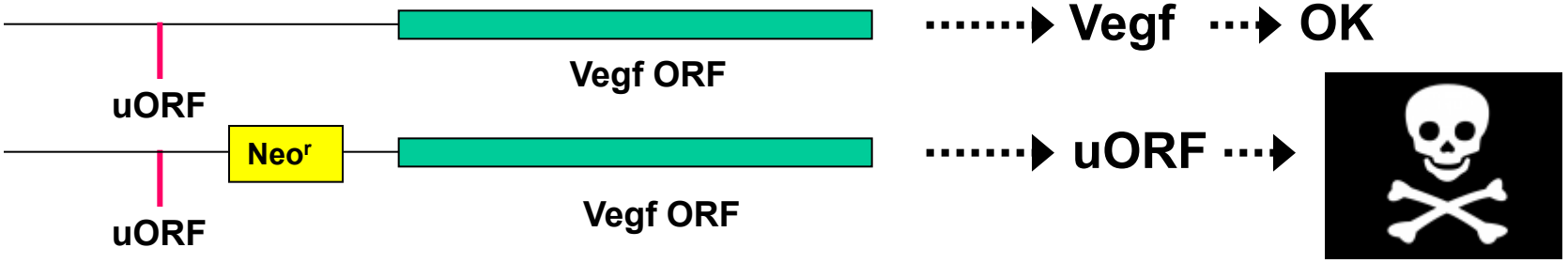
wt

Vegfa$^{-/-}$

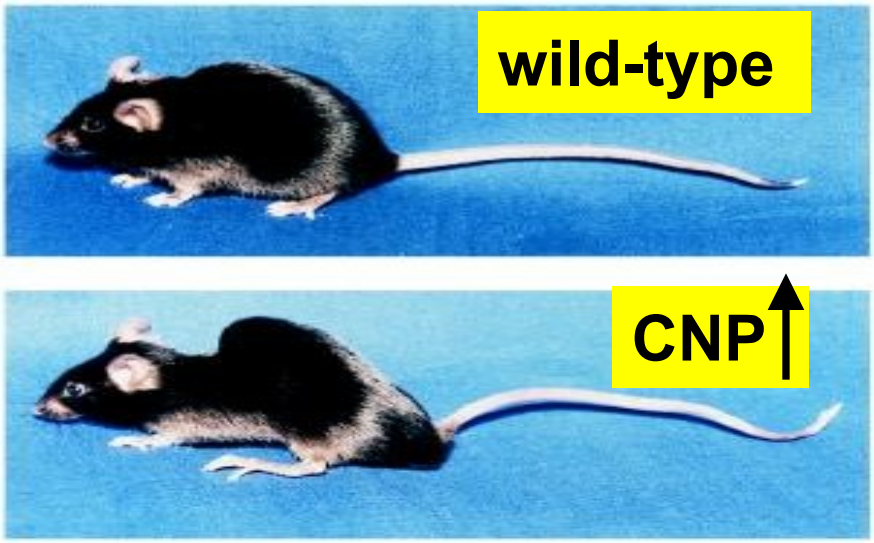


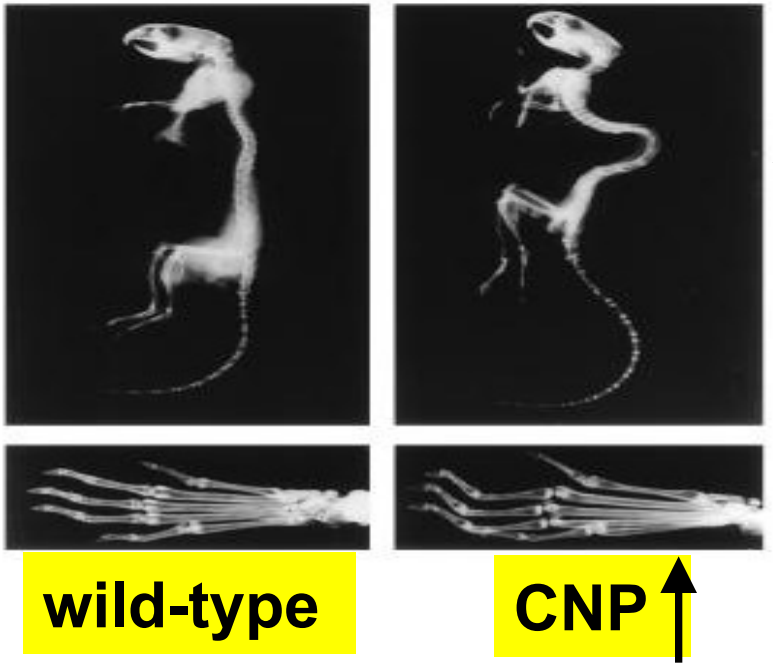


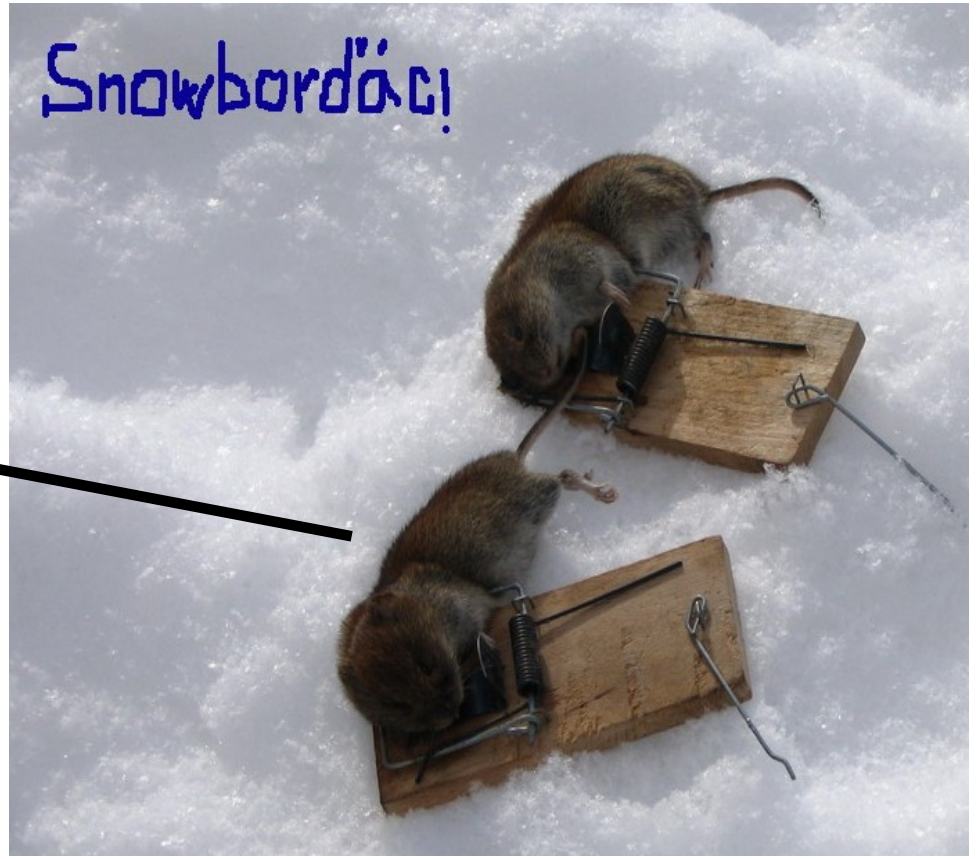
***Vegf* transcription**



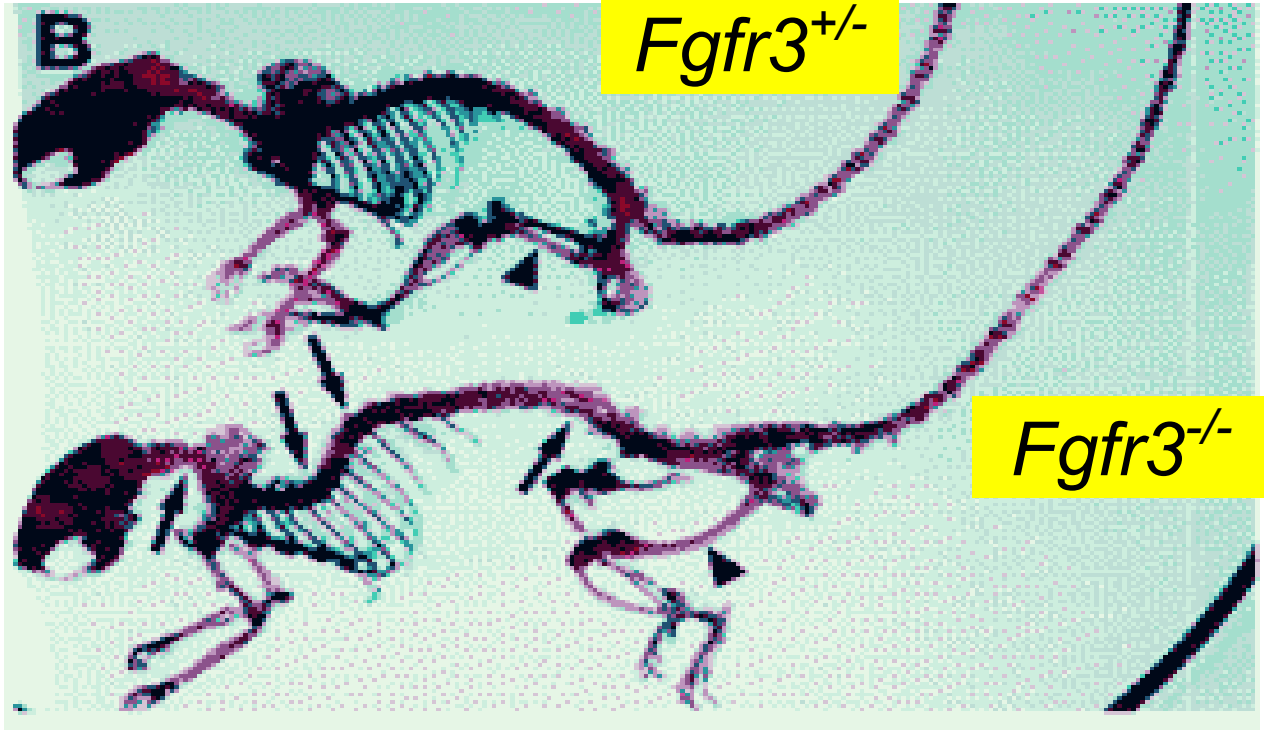
C-type Natriuretic Peptide (CNP)

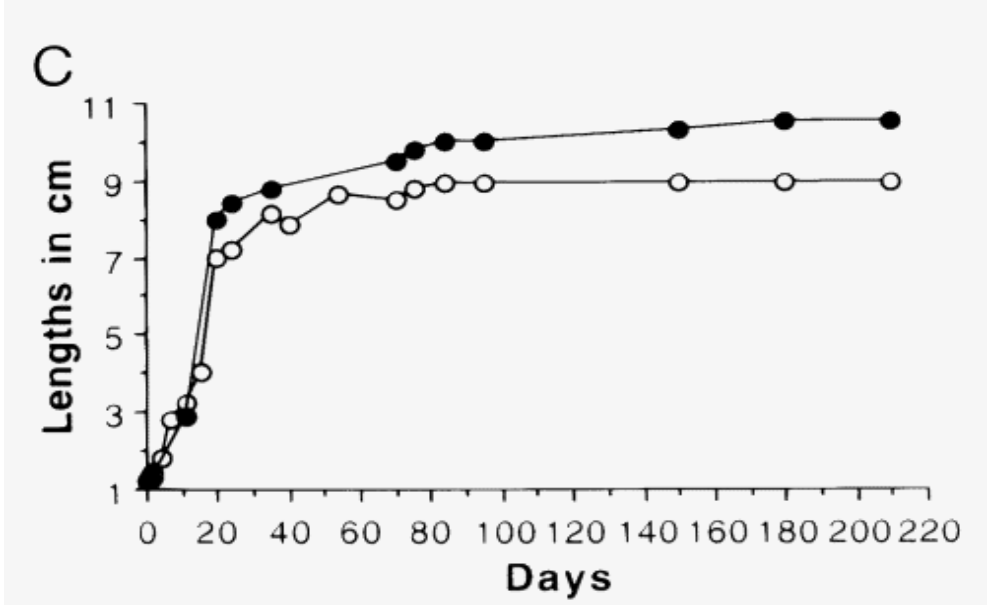




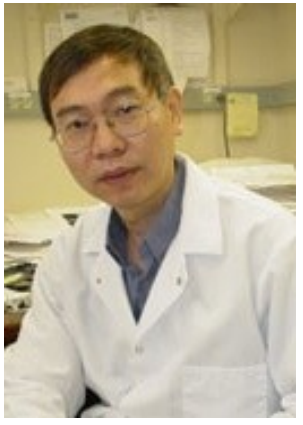


CNP over-expression???


B
Fgfr3+/-
Fgfr3-/-


C
Lengths in cm
11
9
7
5
3
1
0 20 40 60 80 100 120 140 160 180 200 220
Days

Chuxia Deng



WHEN SOMETHING GOES WRONG WITH FGF4

An Expressed *Fgf4* Retrogene Is Associated with Breed-Defining Chondrodysplasia in Domestic Dogs

Heidi G. Parker,[1] Bridgett M. VonHoldt,[2] Pascale Quignon,[1] Elliott H. Margulies,[3] Stephanie Shao,[1] Dana S. Mosher,[1] Tyrone C. Spady,[1] Abdel Elkahloun,[1] Michele Cargill,[4]* Paul G. Jones,[5] Cheryl L. Maslen,[6] Gregory M. Acland,[7,8] Nathan B. Sutter,[8] Keiichi Kuroki,[9] Carlos D. Bustamante,[10] Robert K. Wayne,[2] Elaine A. Ostrander[1]†

Retrotransposition of processed mRNAs is a common source of novel sequence acquired during the evolution of genomes. Although the vast majority of retroposed gene copies, or retrogenes, rapidly accumulate debilitating mutations that disrupt the reading frame, a small percentage become new genes that encode functional proteins. By using a multibreed association analysis in the domestic dog, we demonstrate that expression of a recently acquired retrogene encoding fibroblast

dachshund, Pekingese, and basset hound, where it was found to be dominant and allelic on the basis of arranged crosses (*5*). The phenotype primarily affects the length of the long bones, with growth plates calcifying early in development, thus producing shortened bones with a curved appearance (Fig. 1A) (*6*, *7*).

To identify the genetic foundations of breed-defining phenotypes such as canine chondrodysplasia, we developed a multibreed approach for mapping fixed canine traits. A total of 835 dogs from 76 distinct breeds that provided maximal coverage of phenotypic variation were genotyped by using the Affymetrix version 2.0 single-nucleotide polymorphism (SNP) chip (*8*, *9*). Chondrodysplastic breeds, or cases, were defined on the basis of specific morphologic criteria set forth in each

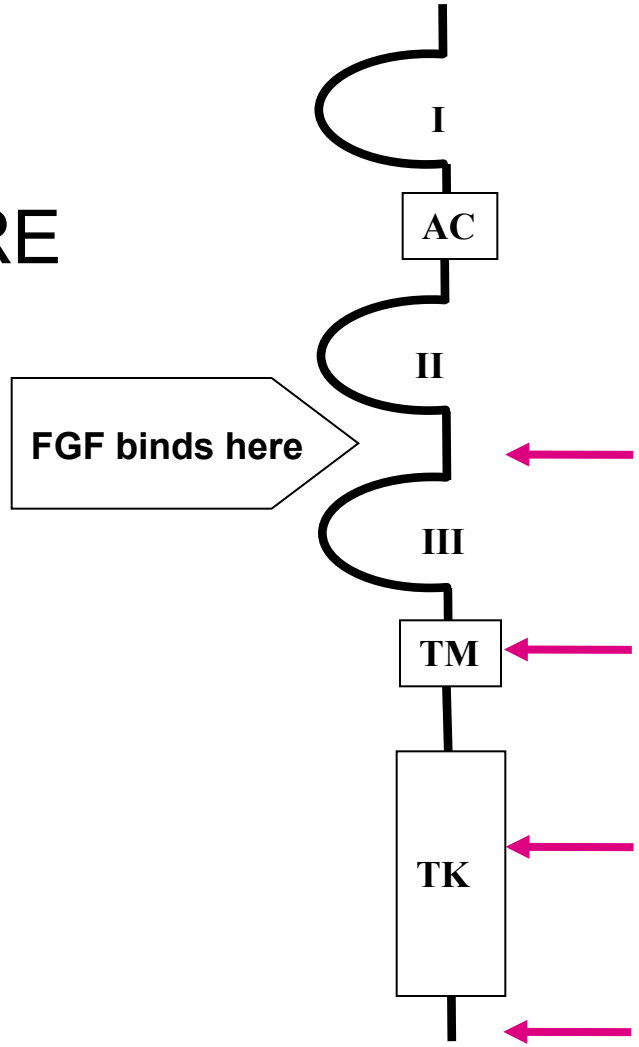
FGF4 ↑↑↑

FGF4 normal

WHEN SOMETHING GOES WRONG WITH FGFR3

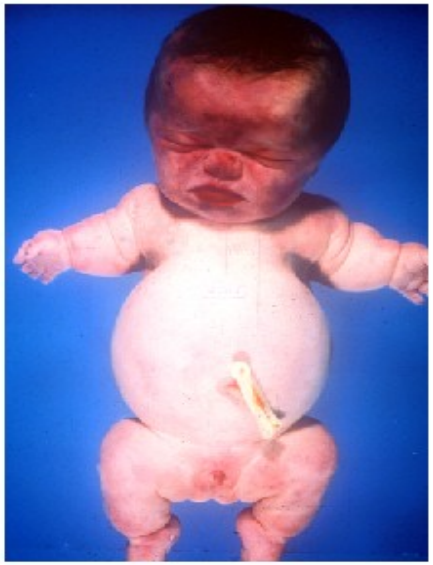
STATURE

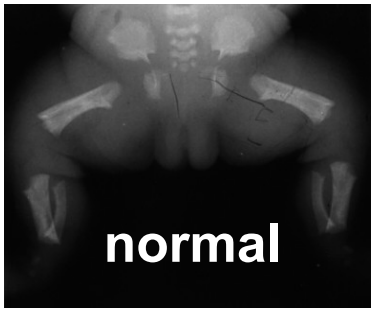
Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia

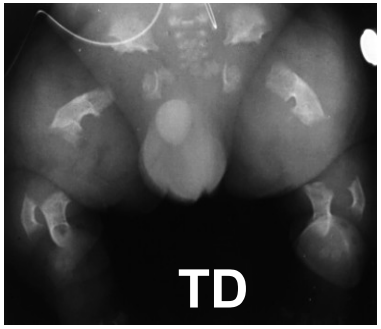


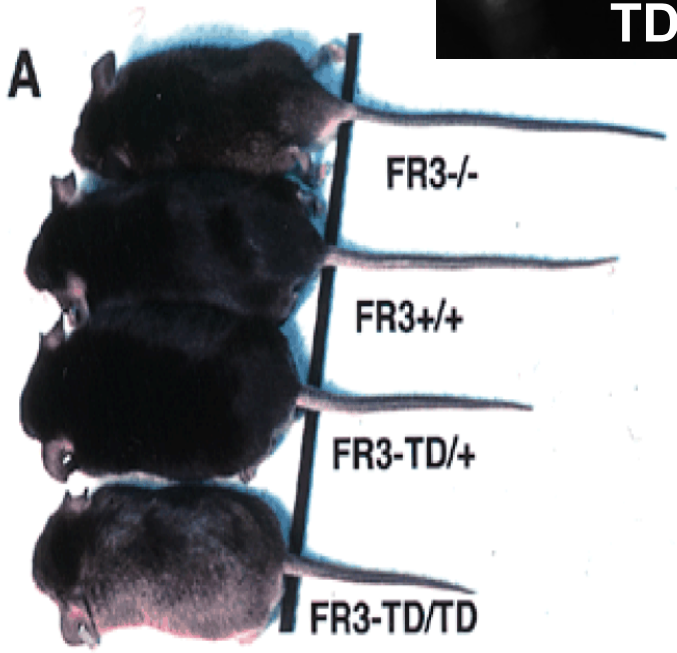
Achondroplasia

Thanatophoric dysplasia



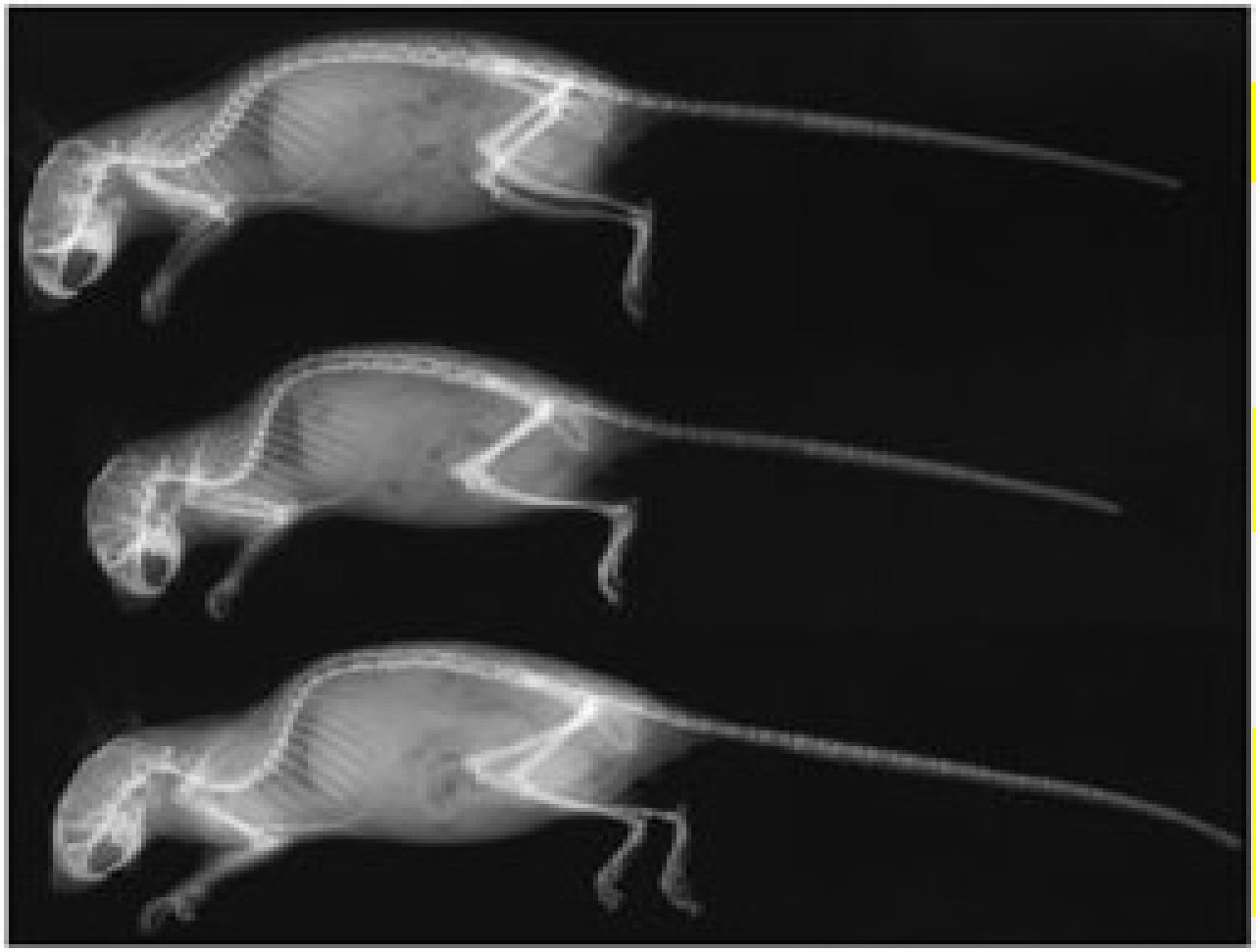






CNP rescues dwarfism caused by ACH

c



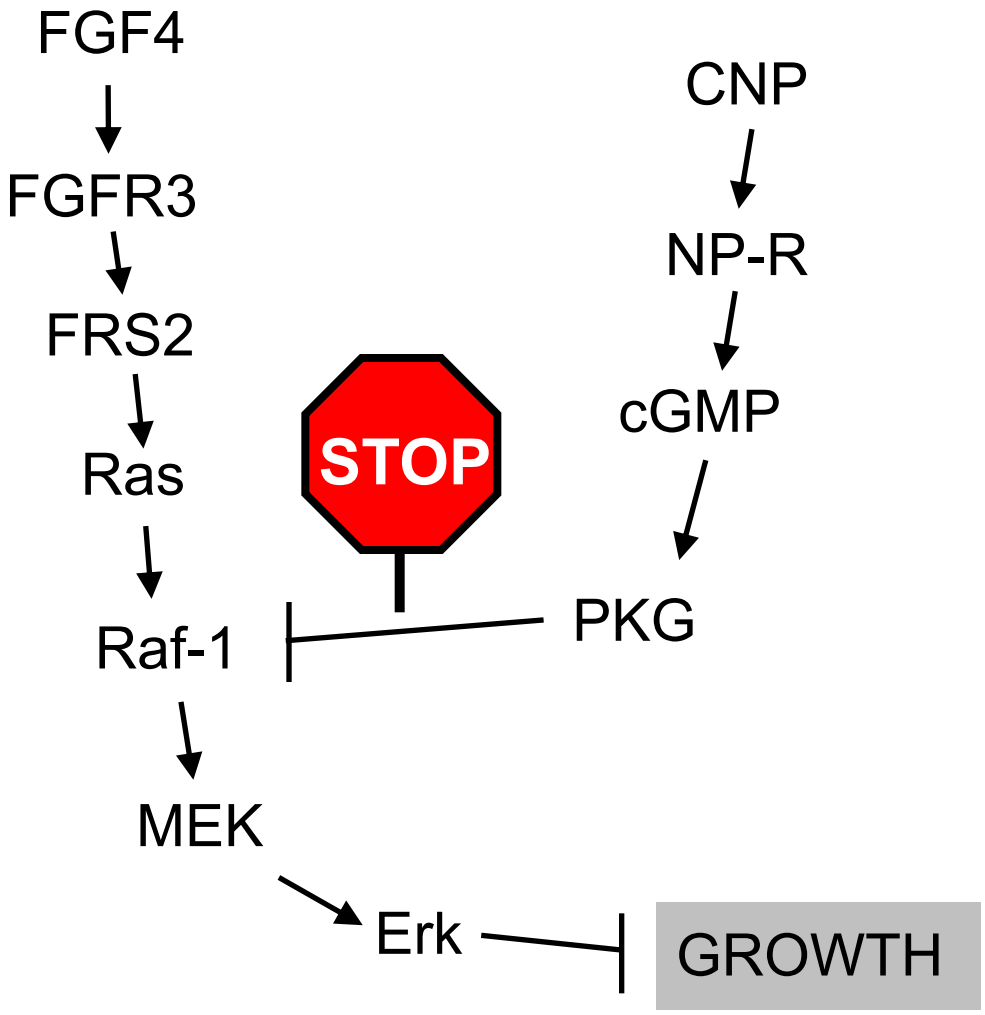
wild-type

Fgfr3^{Ach}

Fgfr3$^{Ach/CNP}$ ↑

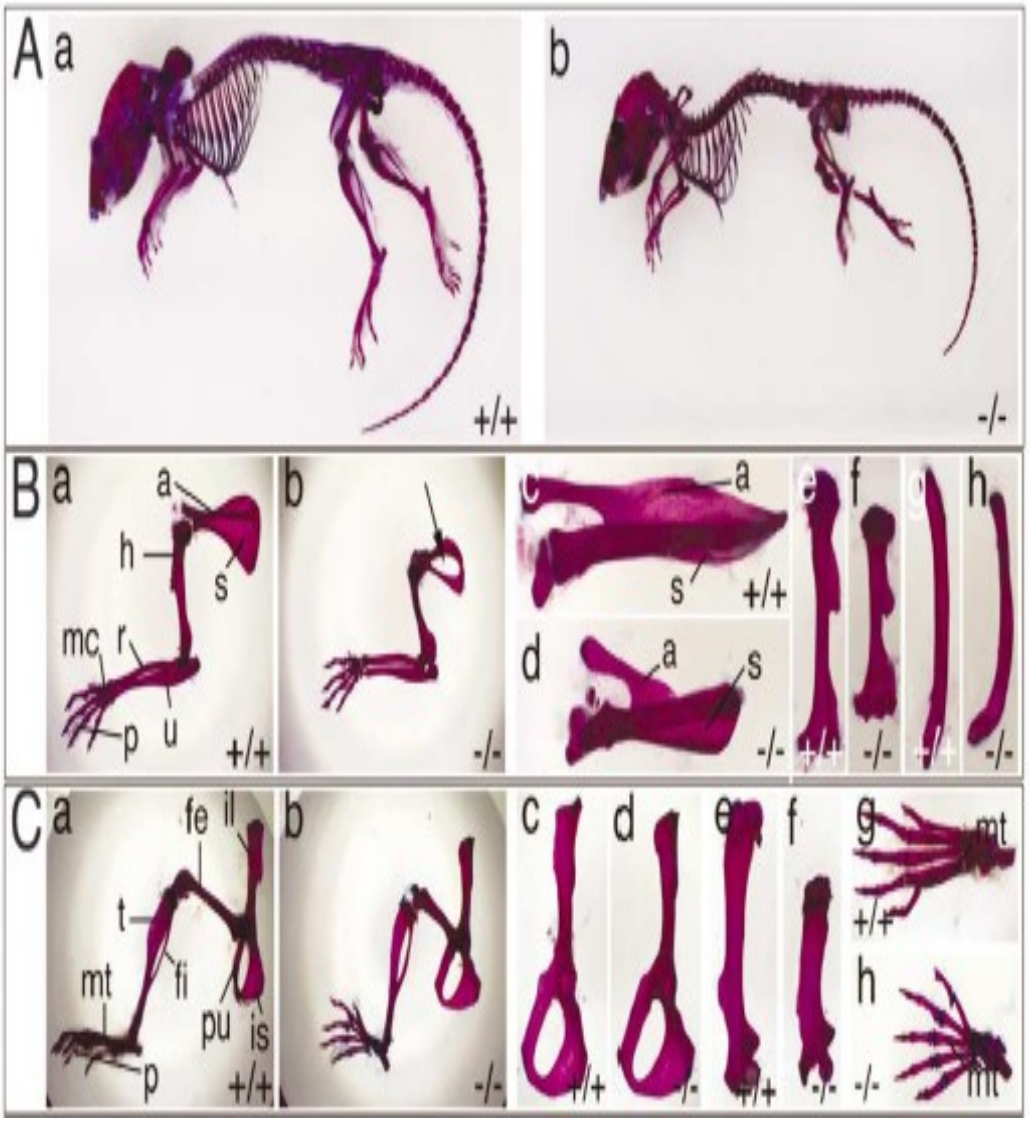
Nature Medicine **10, 80 - 86 (2004)**

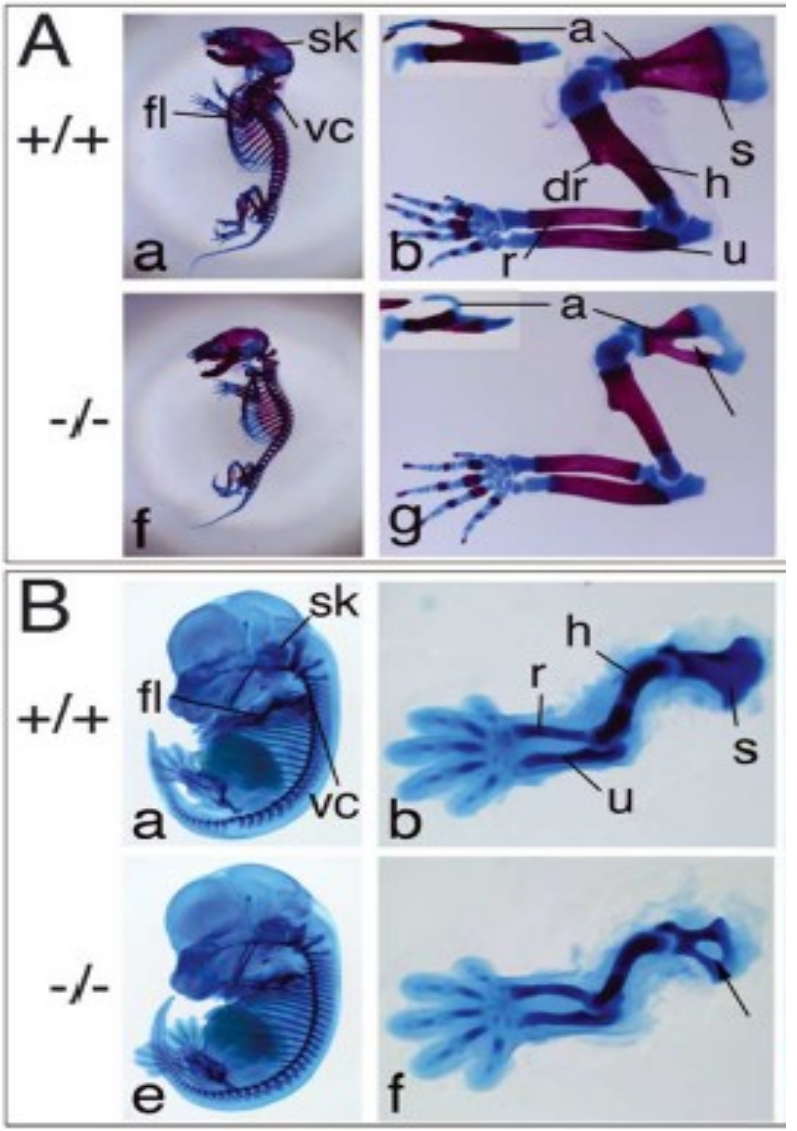
CNP and FGFR3 pathways interact to maintain normal growth



The T-box transcription factor *Tbx15* is required for skeletal development

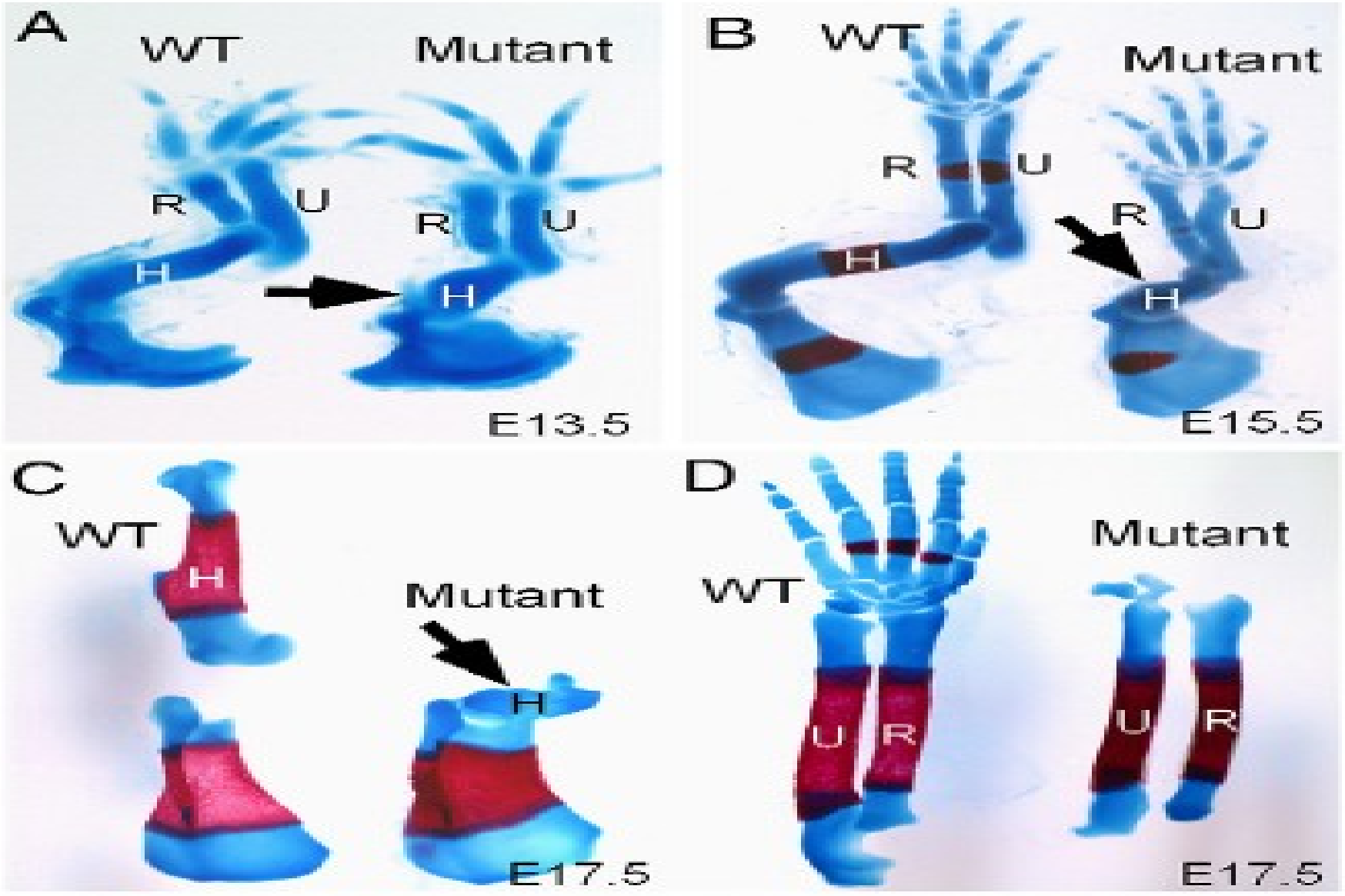
Mechanisms of Development 122 (2005) 131–144





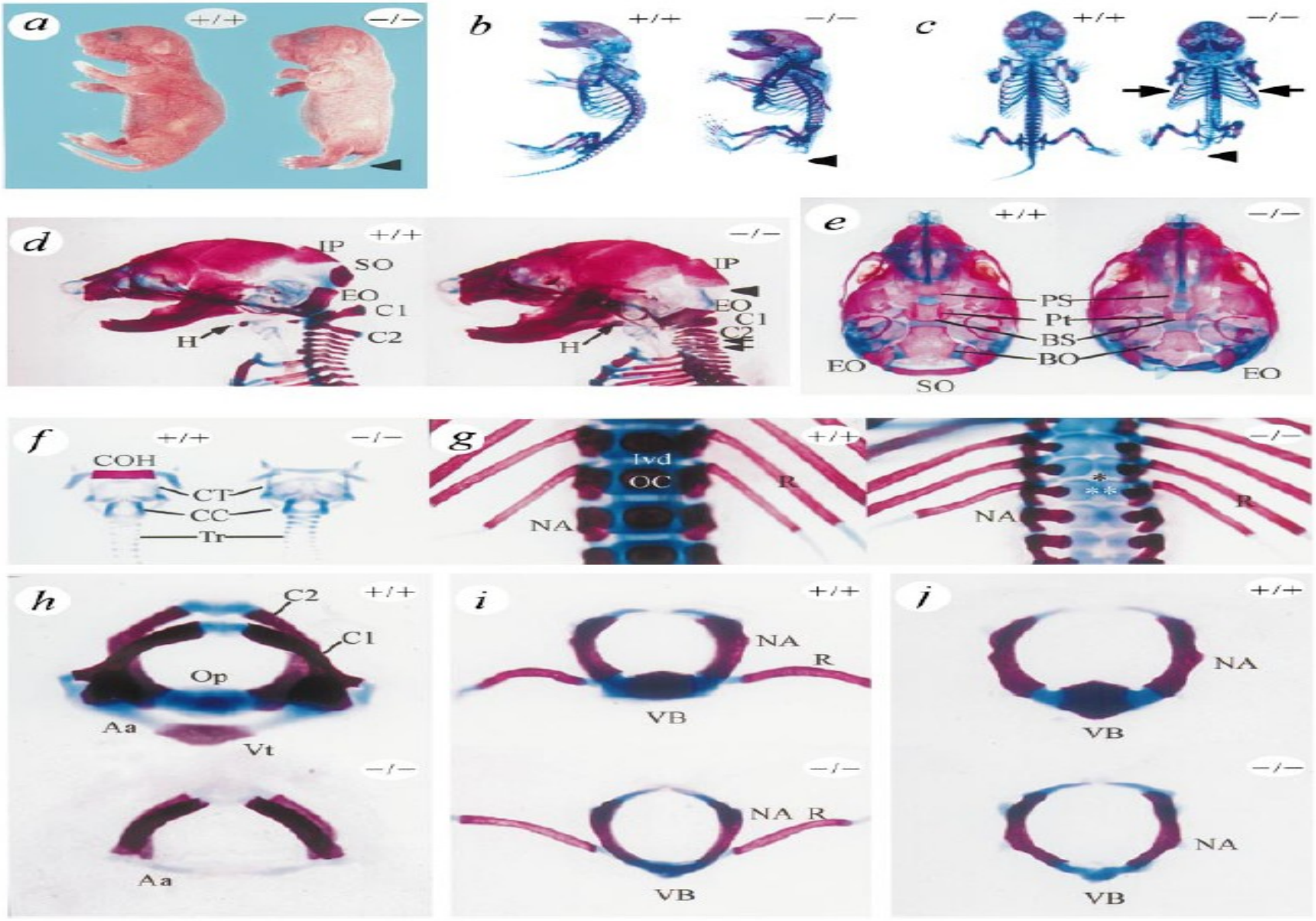
Shox2 is required for chondrocyte proliferation and maturation in proximal limb skeleton

Developmental Biology 306 (2007) 549–559

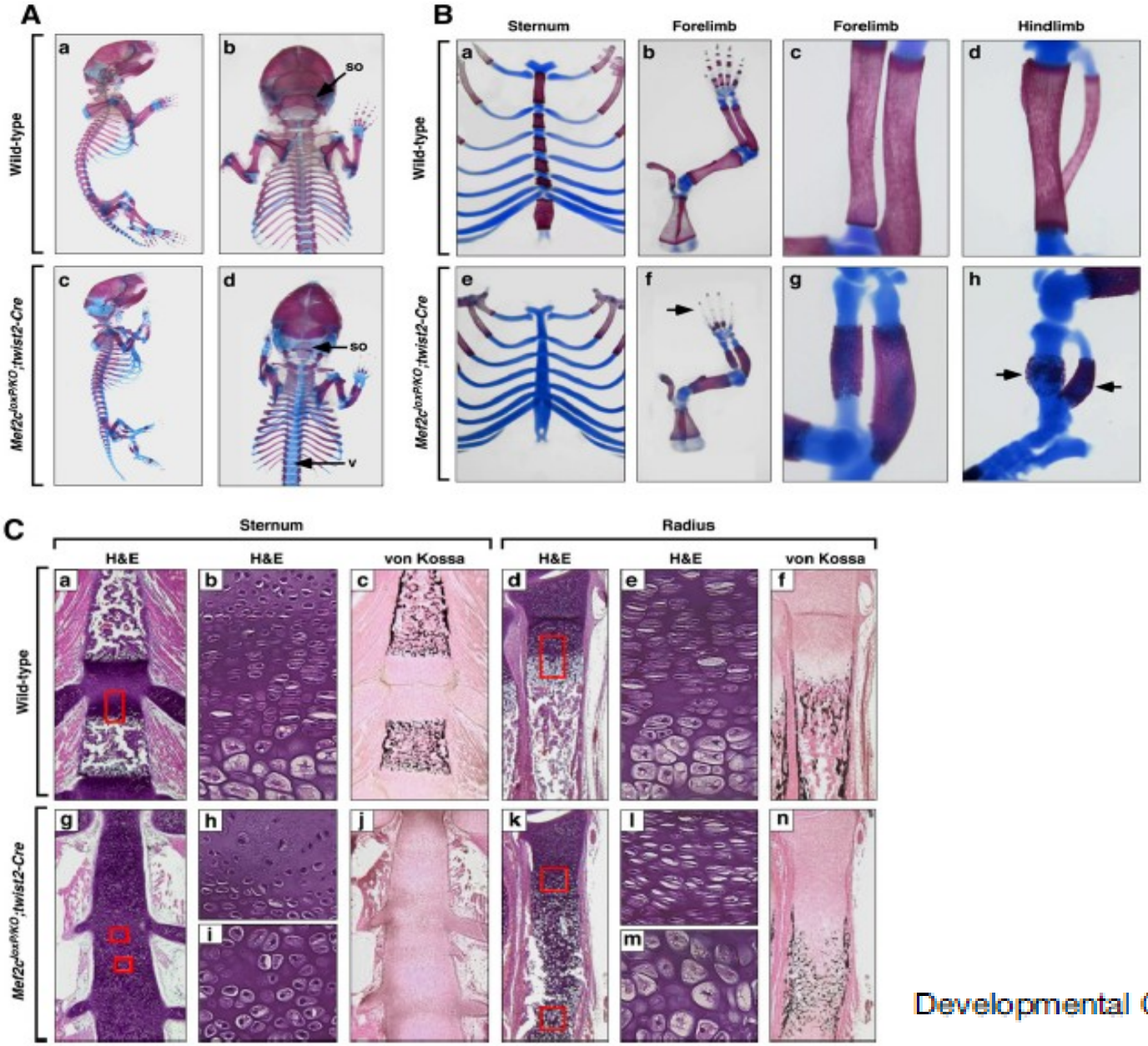


Targeted disruption of the homeobox transcription factor *Bapx1* results in lethal skeletal dysplasia with asplenia and gastroduodenal malformation

Genes to Cells (2000) 5, 499–513



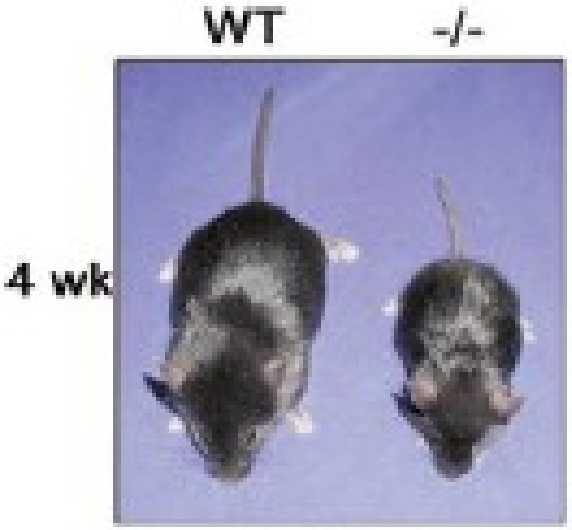
MEF2C Transcription Factor Controls Chondrocyte Hypertrophy and Bone Development

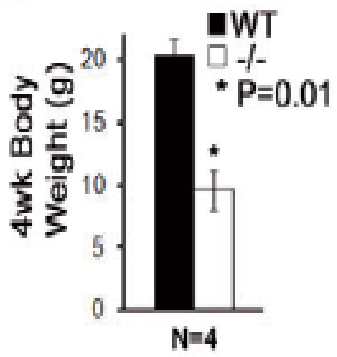


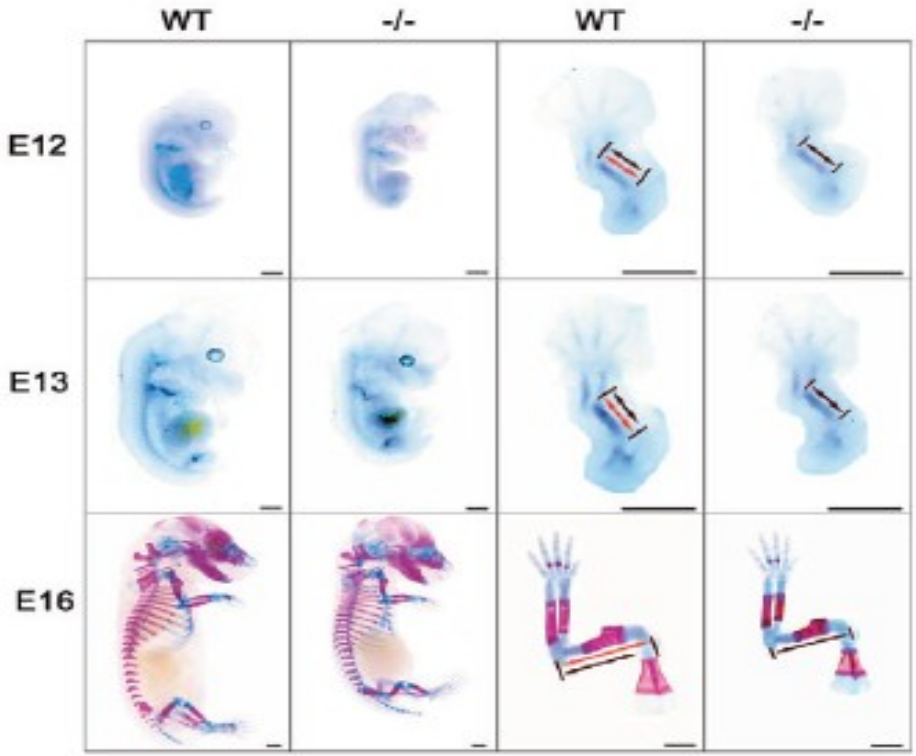
Developmental Cell 12, 377–389, March 2007

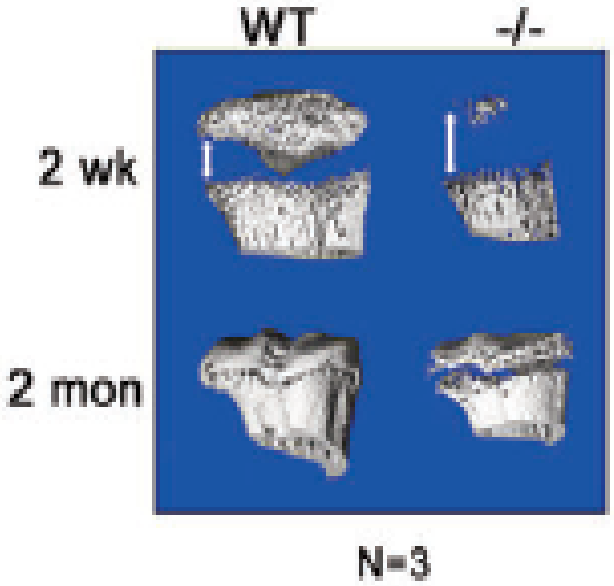
Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating *Ihh* transcription

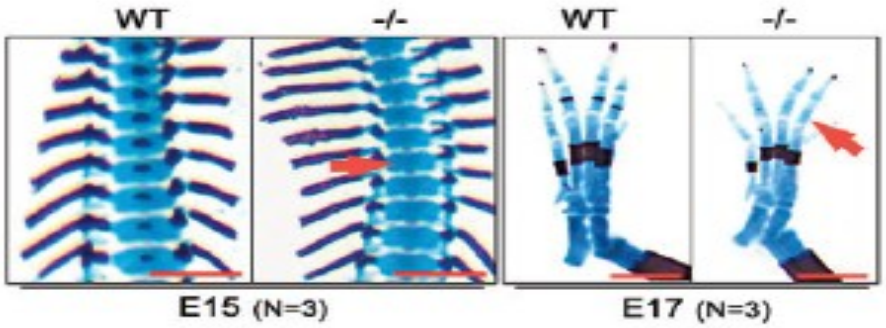
Development 136, 4143-4153 (2009)





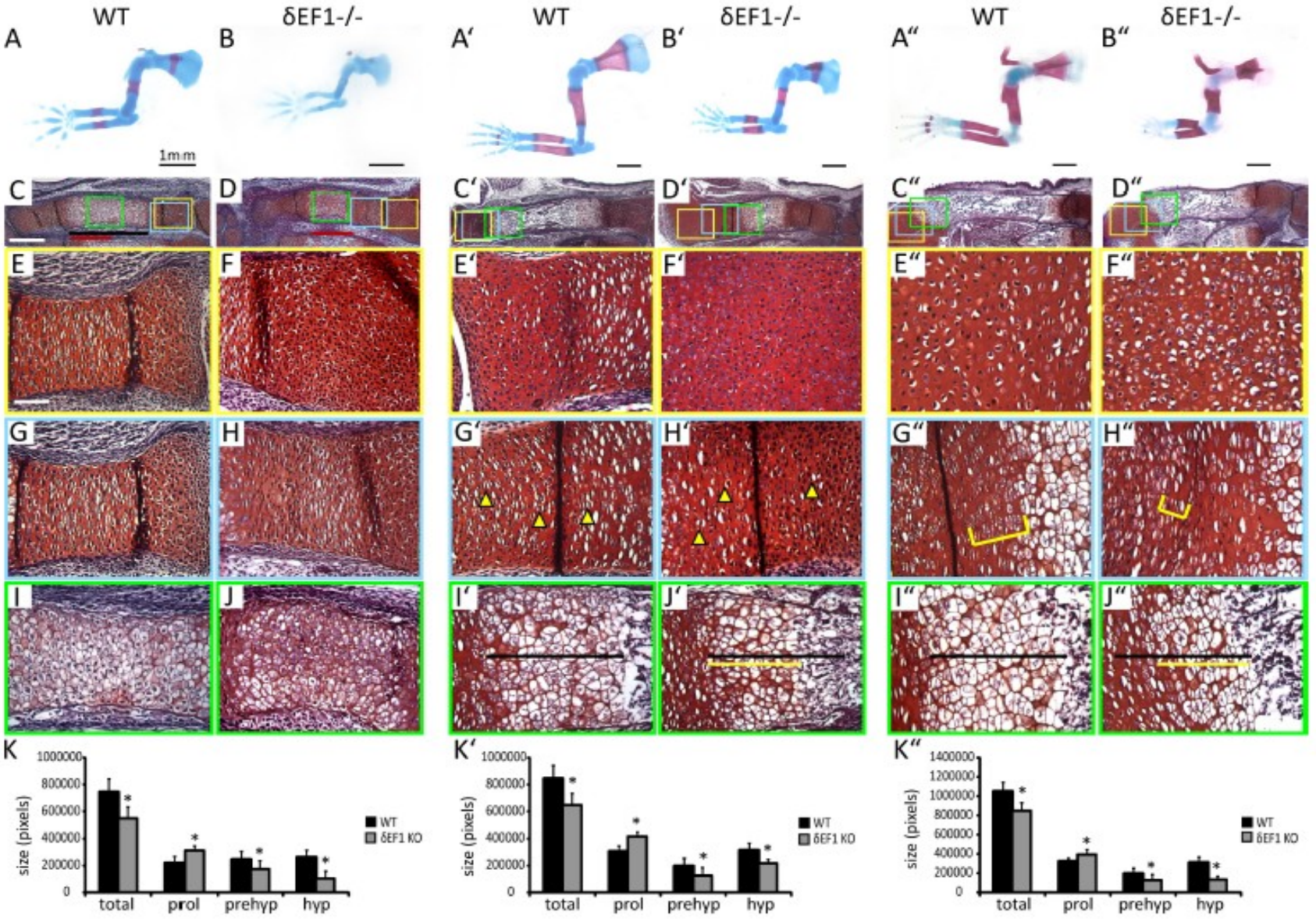




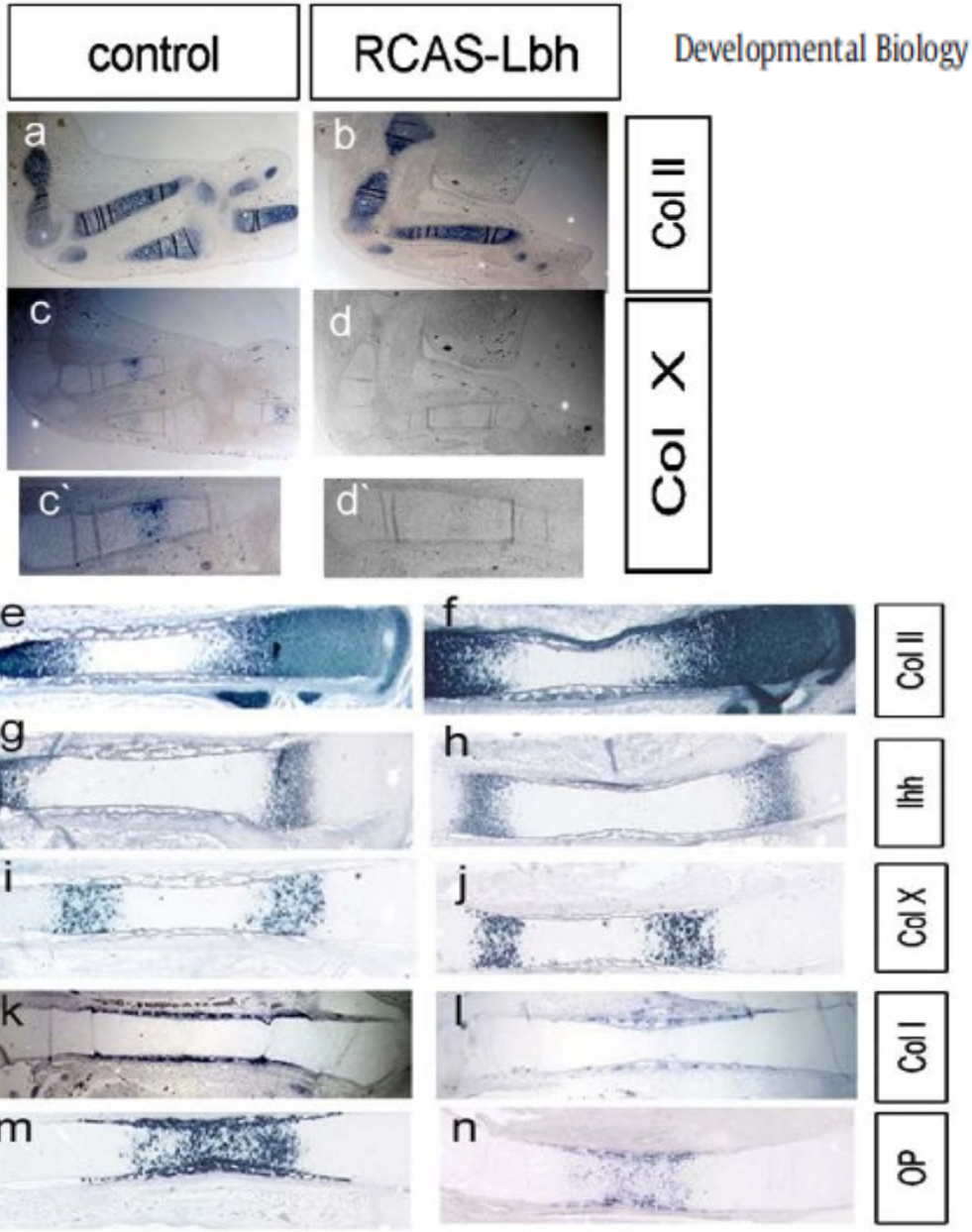


δ-EF1 is a negative regulator of *Ihh* in the developing growth plate

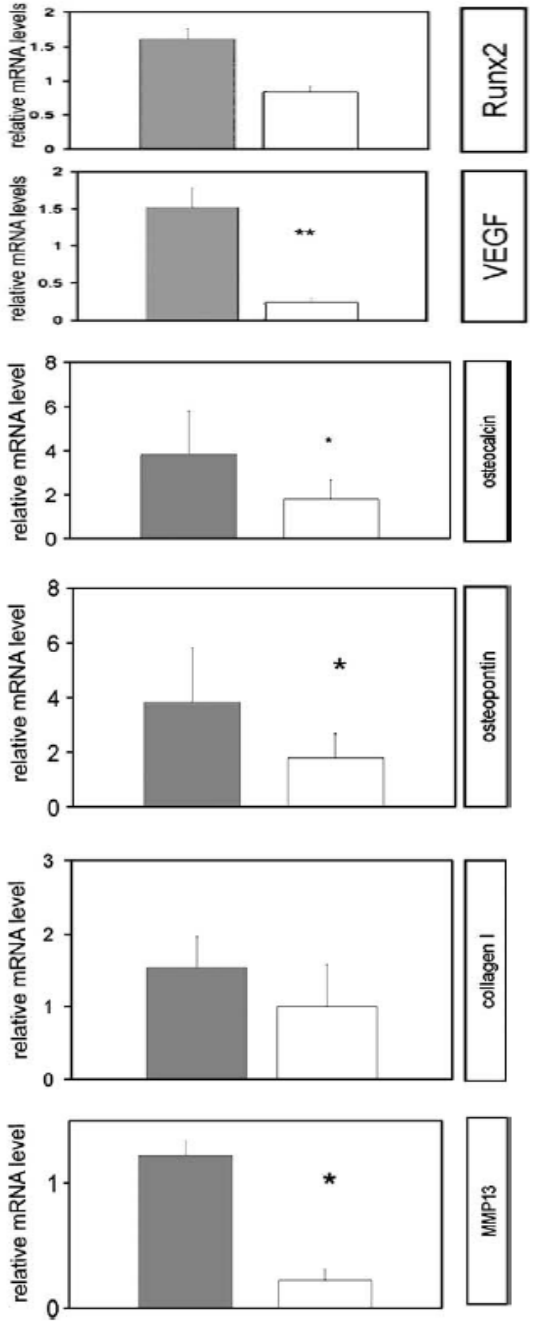
J. Cell Biol. Vol. 187 No. 5 685–699



The transcriptional cofactor Lbh regulates angiogenesis and endochondral bone formation during fetal bone development

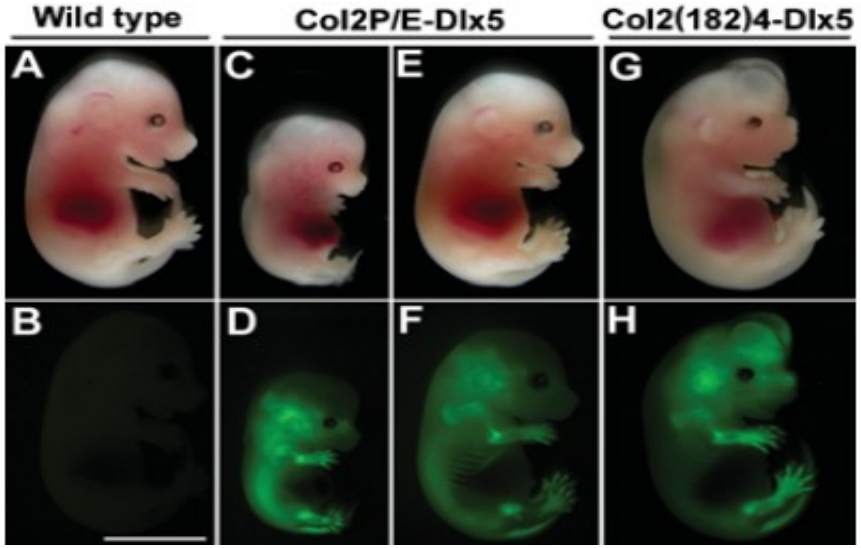


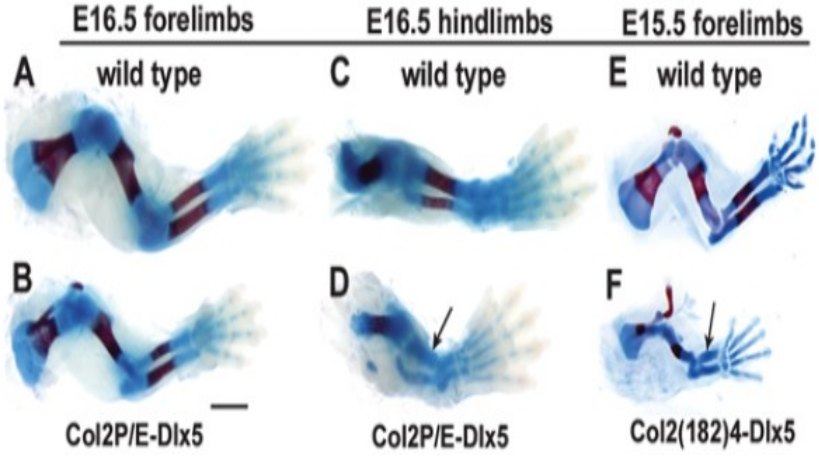
Developmental Biology 333 (2009) 348–358

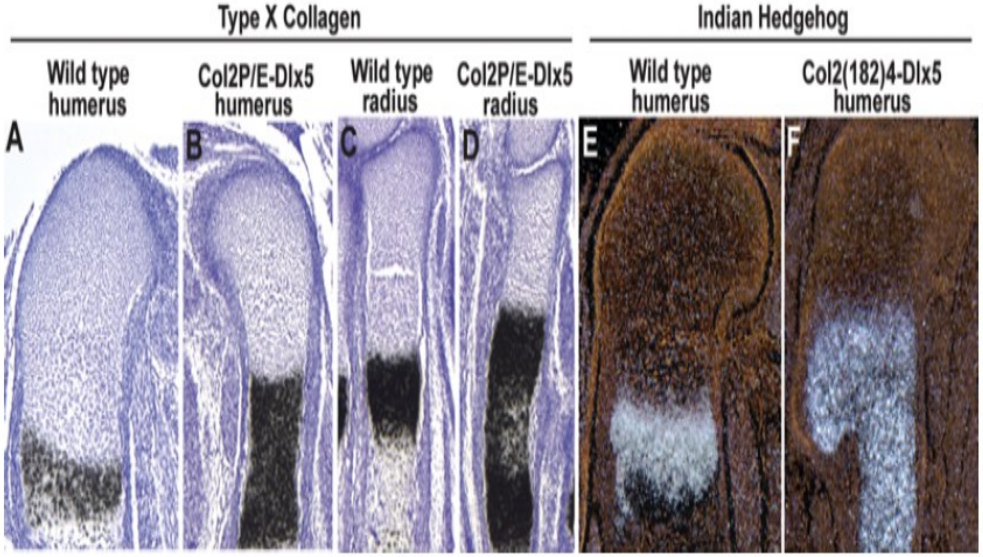


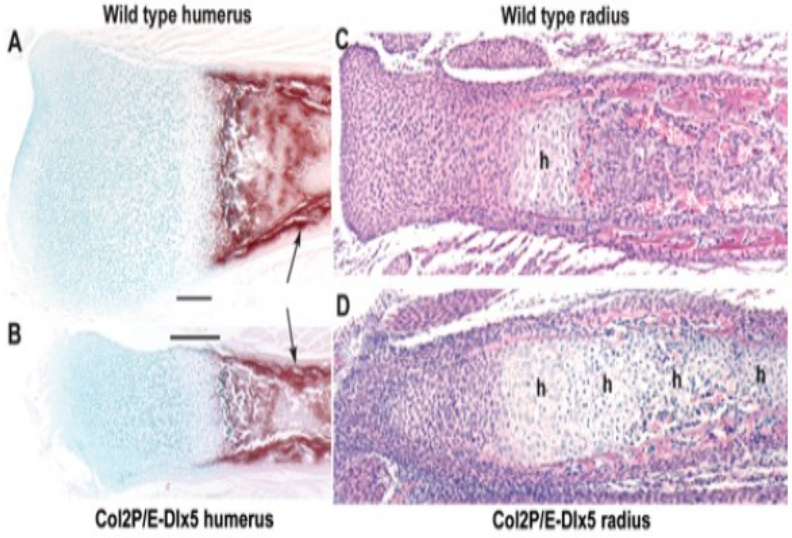
Studies on the role of *Dlx5* in regulation of chondrocyte differentiation during endochondral ossification in the developing mouse limb

Develop. Growth Differ. (2007) **49**, 515–521

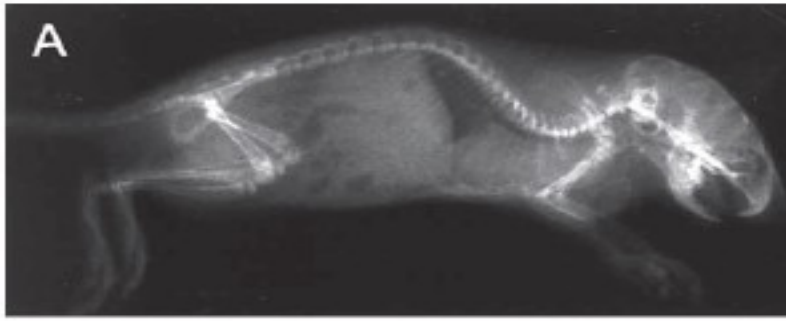


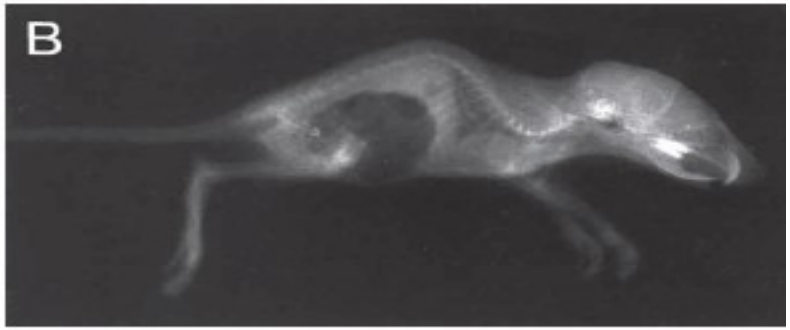


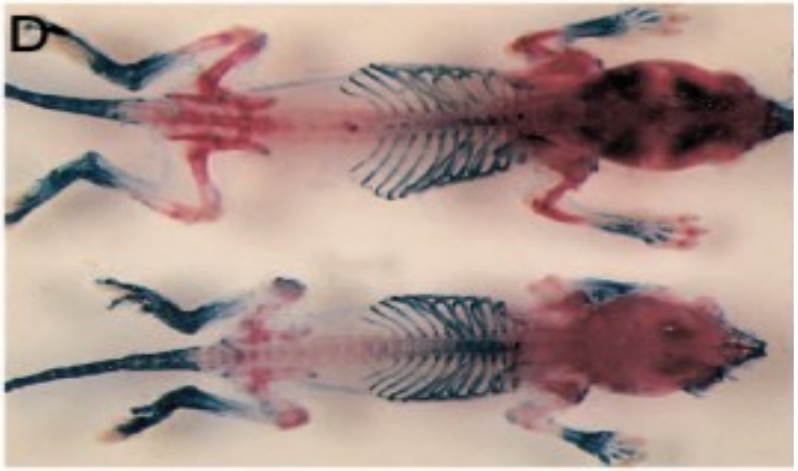




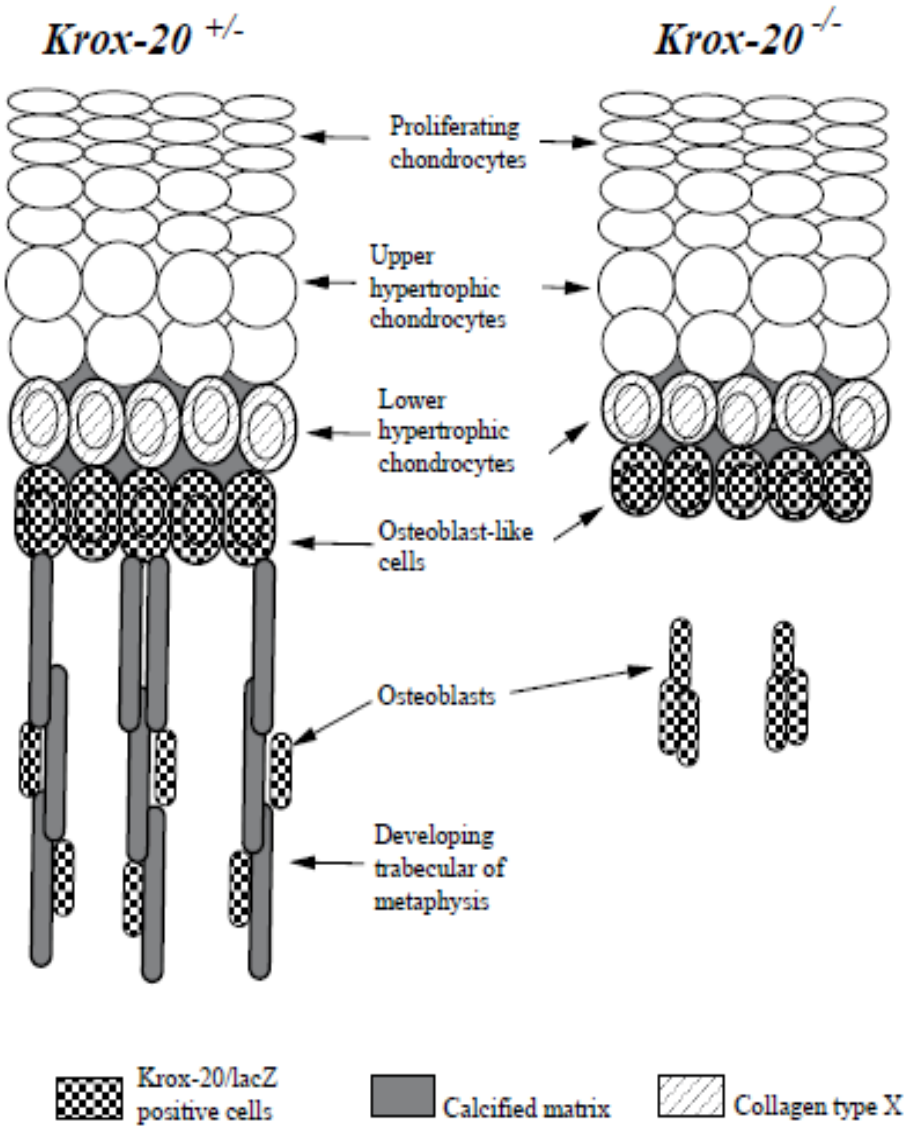
Defective bone formation in *Krox-20* mutant mice



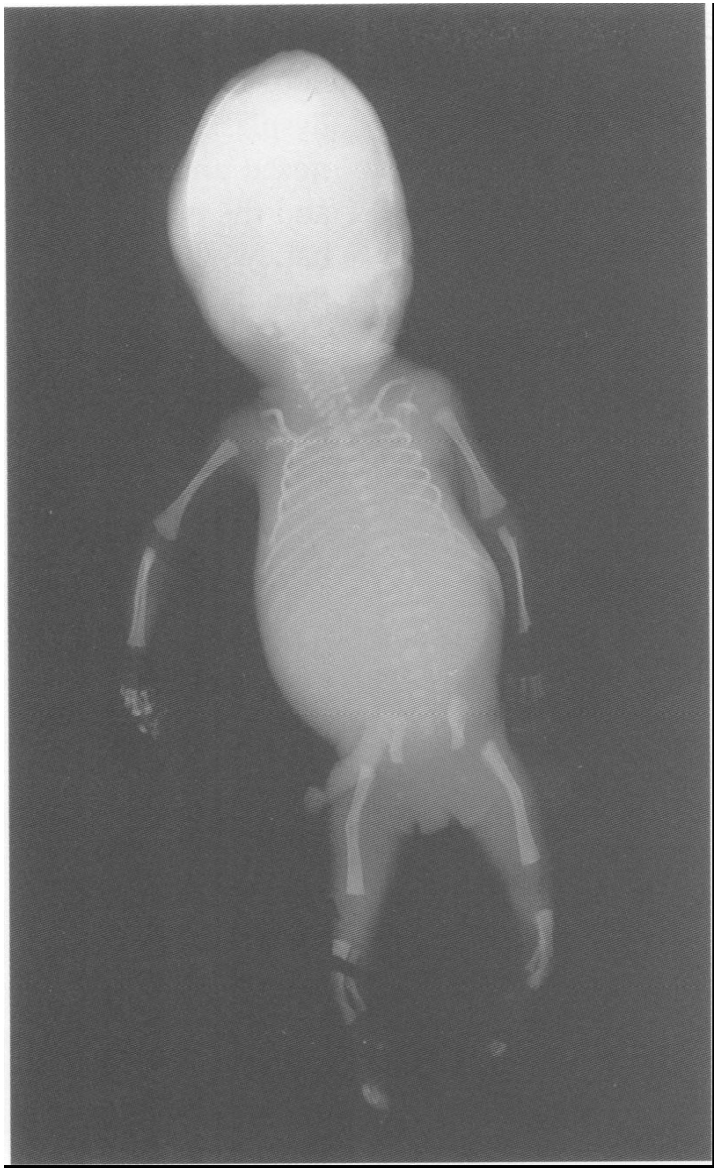


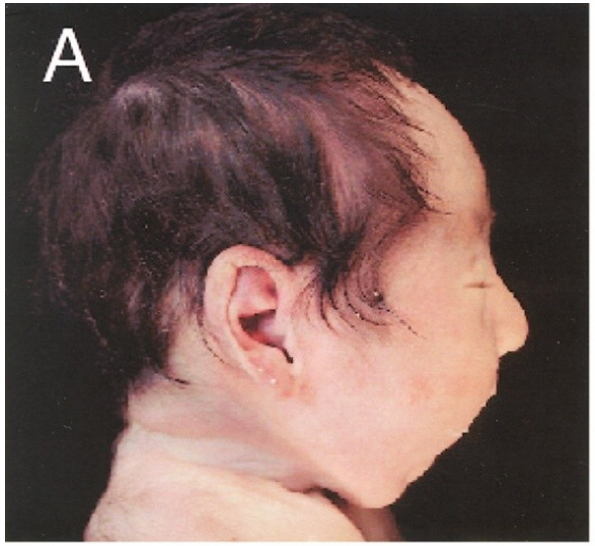


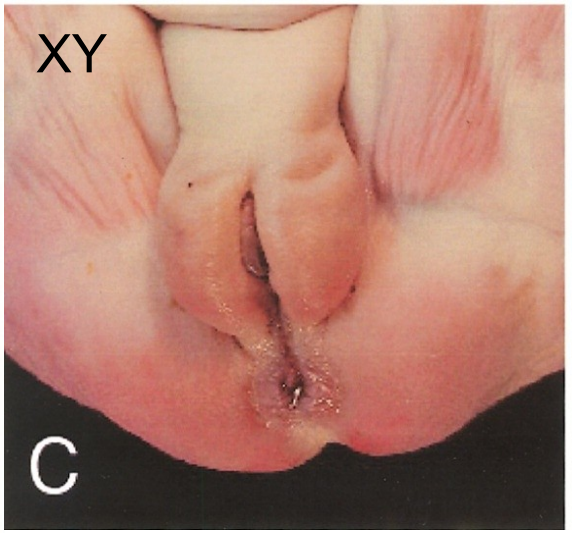
Development 122, 113-120 (1996)



WHEN SOMETHING GOES WRONG WITH SOX9

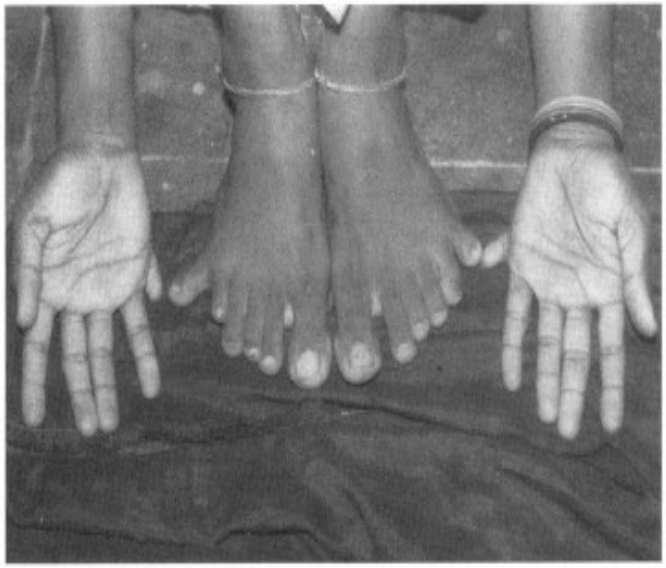




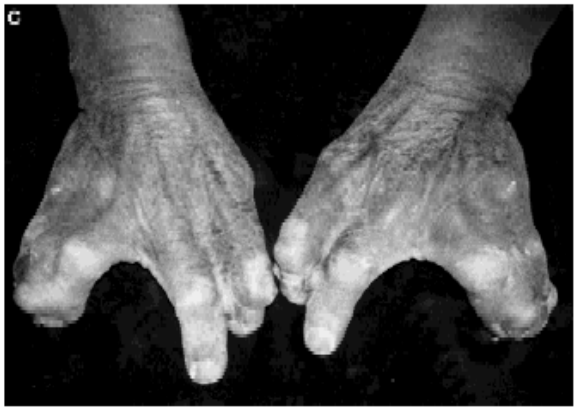


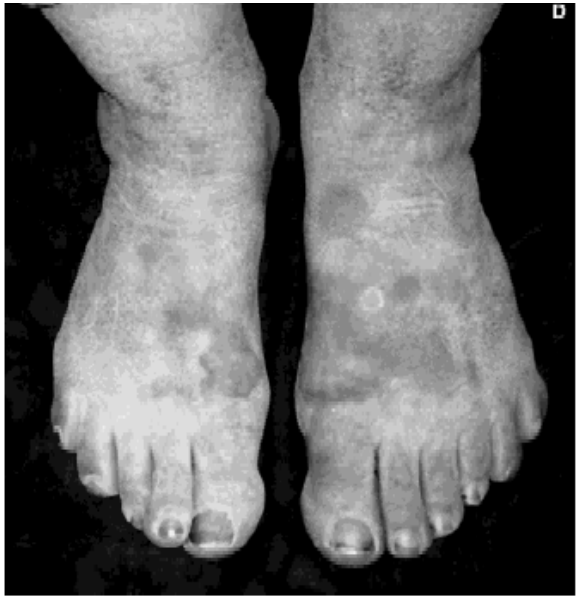
CAMPOMELIC DYSPLASIA
Sox9 haploinsufficiency

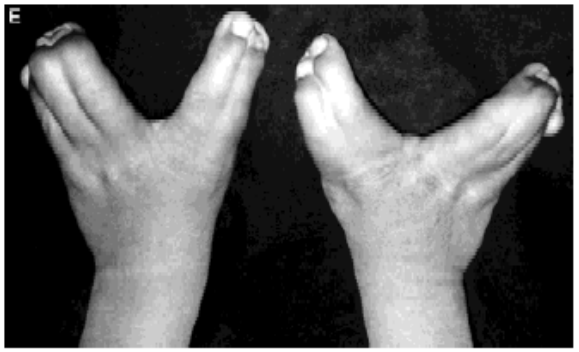
……. OR WITH SHH

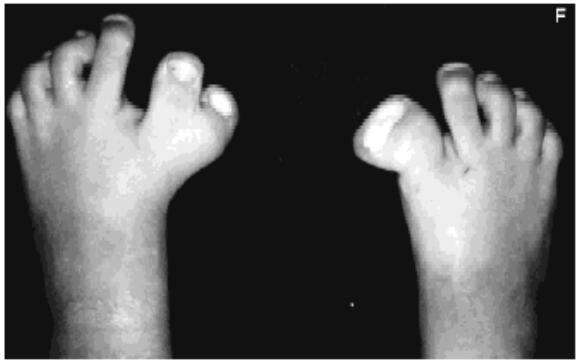


POLYDACTYLY TYPE-A
Loss-of-function mutation in
Gli3 (negative regulator of SHH)



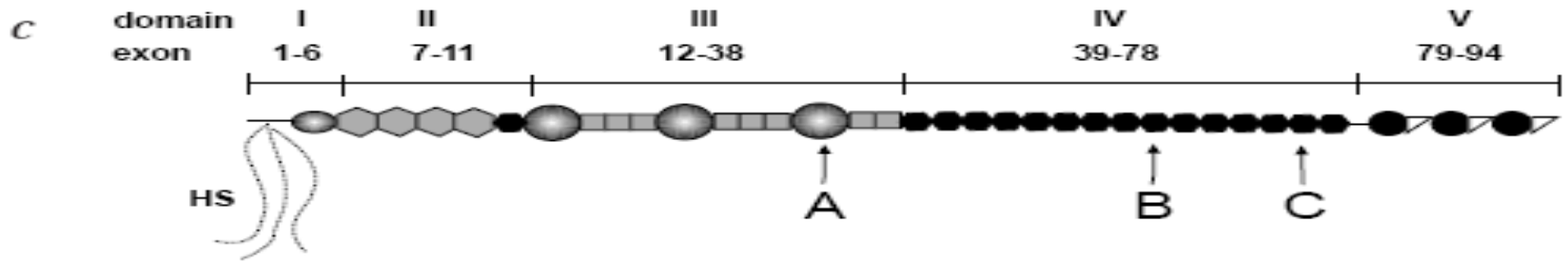




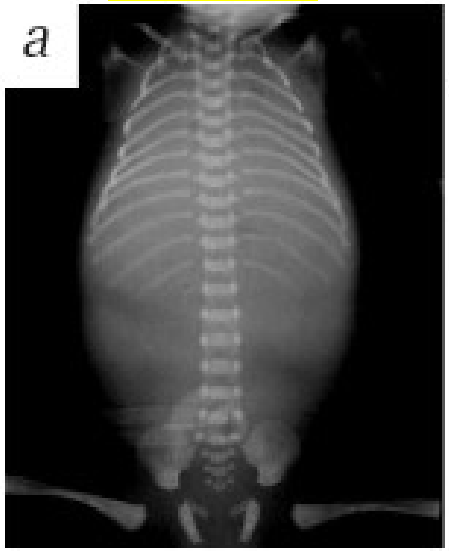


POLYDACTYLY TYPE-II
SHH upregulation via transcriptional enhancer mutation

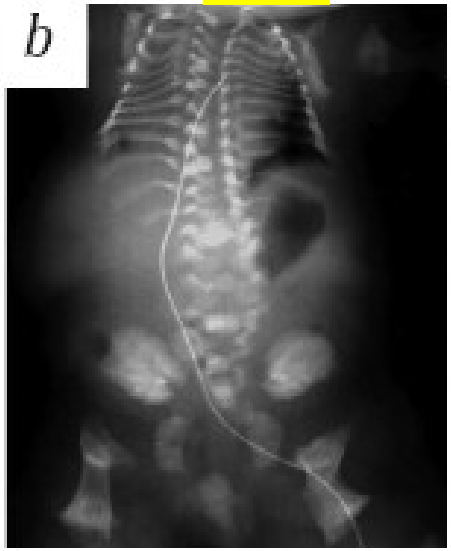
……. OR WITH PERLECAN (Dyssegmental dysplasia - perlecan loss-of-function)



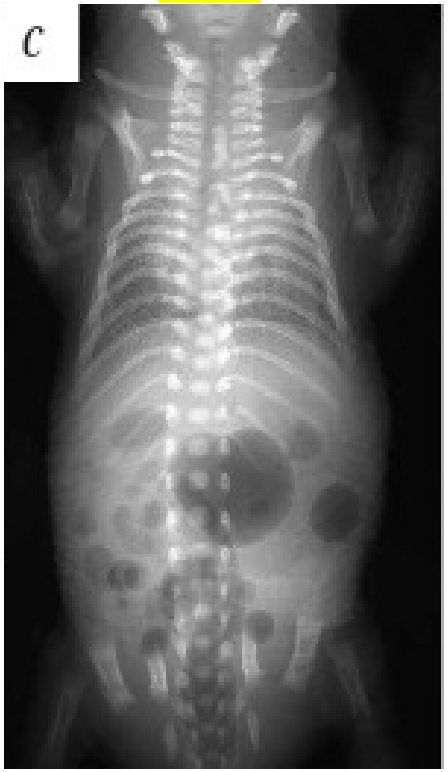
normal



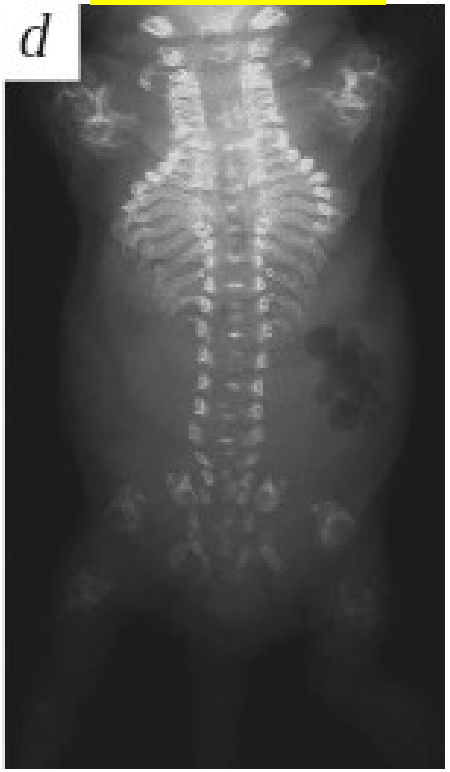
DD



wt



perlecan$^{-/-}$



American Journal of Medical Genetics Part A 143A:1–18 (2007)

Nosology and Classification of Genetic Skeletal Disorders: 2006 Revision

Andrea Superti-Furga,[1]* Sheila Unger,[1,2] and the Nosology Group of the International Skeletal Dysplasia Society

[1]Center for Pediatrics and Adolescent Medicine, Department of Pediatrics, University of Freiburg, Freiburg, Germany
[2]Institute for Human Genetics, University of Freiburg, Freiburg, Germany

Received 23 July 2006; Accepted 7 August 2006

The objective of the paper is to provide the revision of the Nosology of Constitutional Disorders of Bone that incorporates newly recognized disorders and reflects new molecular and pathogenetic concepts. Criteria for inclusion of disorders were (1) significant skeletal involvement corresponding to the definition of skeletal dysplasias, metabolic bone disorders, dysostoses, and skeletal malformation and/or reduction syndromes, (2) publication and/or MIM listing, (3) genetic basis proven or very likely, and (4) nosologic autonomy confirmed by molecular or linkage analysis and/or distinctive diagnostic features and observation in multiple individuals or families. Three hundred seventy-two different conditions were included and placed in 37 groups defined by molecular, biochemical and/or radiographic criteria. Of these conditions, 215 were associated with one or more of 140 different genes. Nosologic status was classified as final (mutations or locus identified), probable (pedigree evidence), or *bona fide* (multiple observations and clear diagnostic criteria, but no pedigree or locus evidence yet). The number of recognized genetic disorders with a significant skeletal component is growing and the distinction between dysplasias, metabolic bone disorders, dysostoses, and malformation syndromes is blurring. For classification purposes, pathogenetic and molecular criteria are integrating with morphological ones but disorders are still identified by clinical features and radiographic appearance. Molecular evidence leads to confirmation of individual entities and to the constitution of new groups, but also allows for delineation of related but distinct entities and indicates a previously unexpected heterogeneity of molecular mechanisms; thus, molecular evidence does not necessarily simplify the Nosology, and a further increase in the number of entities and growing complexity is expected. By providing an updated overview of recognized entities with skeletal involvement and of the underlying gene defects, the new Nosology can provide practical diagnostic help, facilitate the recognition of new entities, and foster and direct research in skeletal biology and genetic disorders. © 2006 Wiley-Liss, Inc.

Key words: nosology; skeletal disorders; osteochondrodysplasias; dysostoses; malformation syndromes; developmental biology; molecular defects

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