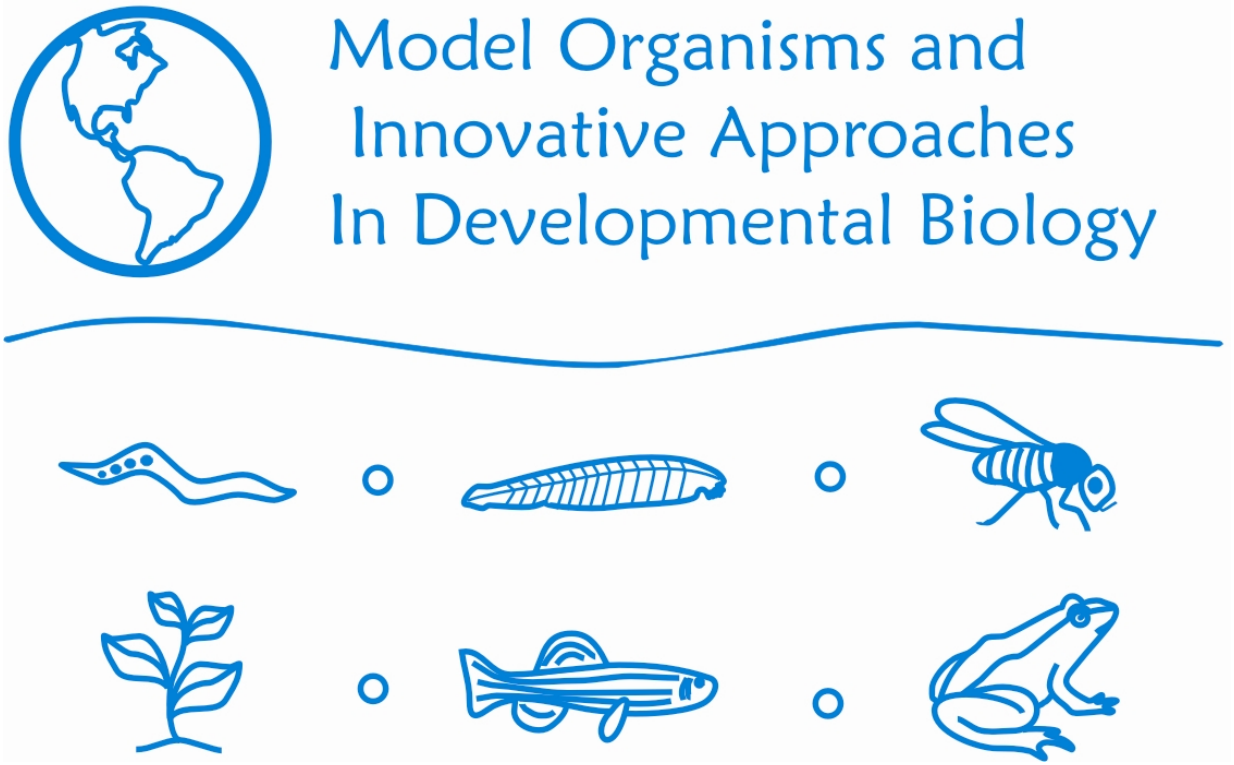
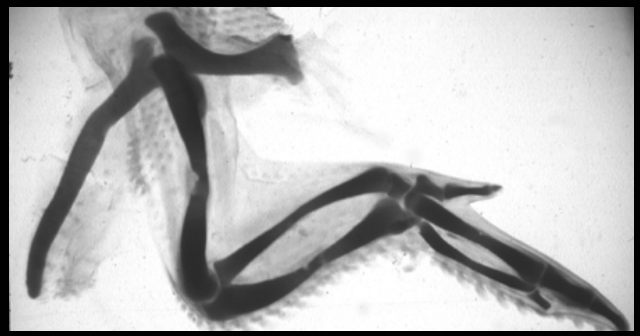


Genetic and Molecular Control of Vertebrate Limb Development and Morphological Diversity

Lee Niswander
HHMI/ U Colorado Health Sciences Center

lee.niswander@uchsc.edu

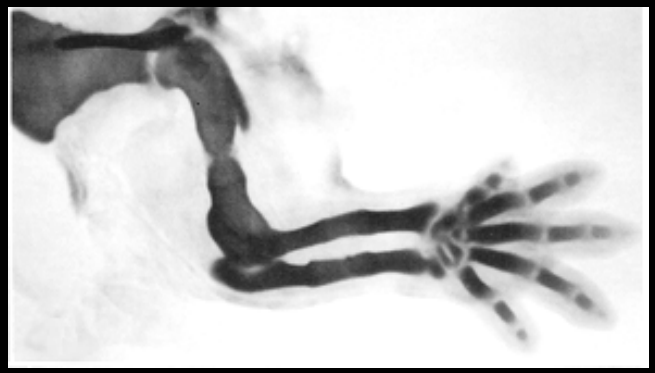
Limb Development



Chick

Pro: experimental embryology,
rapid functional analysis

Con: relative lack of genetics



Mouse

Pro: genetics,
genetic manipulation

Con: in utero development

Bat

Pro: evo-devo studies,
experimental embryology

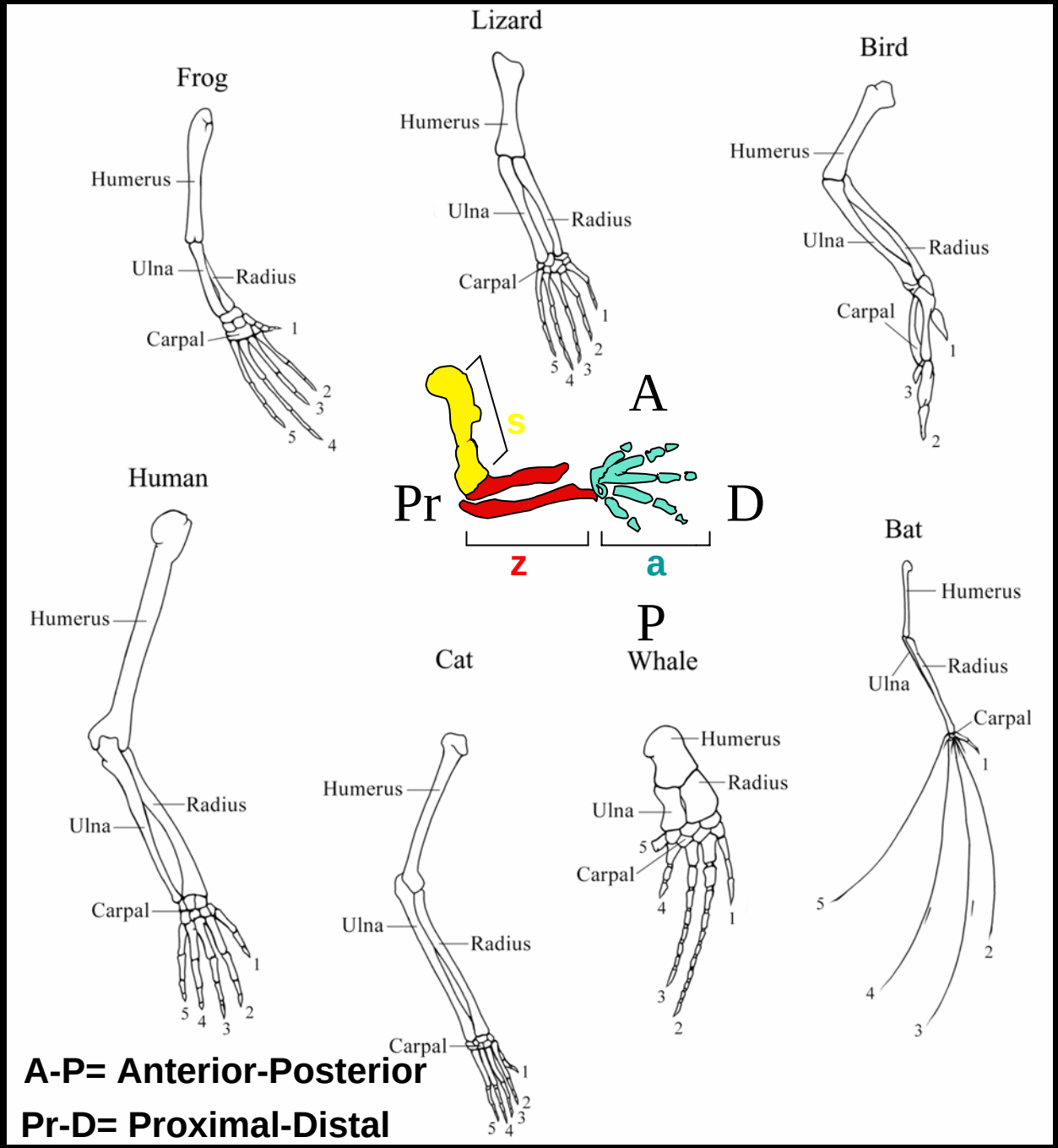
Con: relative lack of genetics
relatively difficult to obtain

Diversity of Vertebrate Limbs

s= stylopod

z= zeugopod

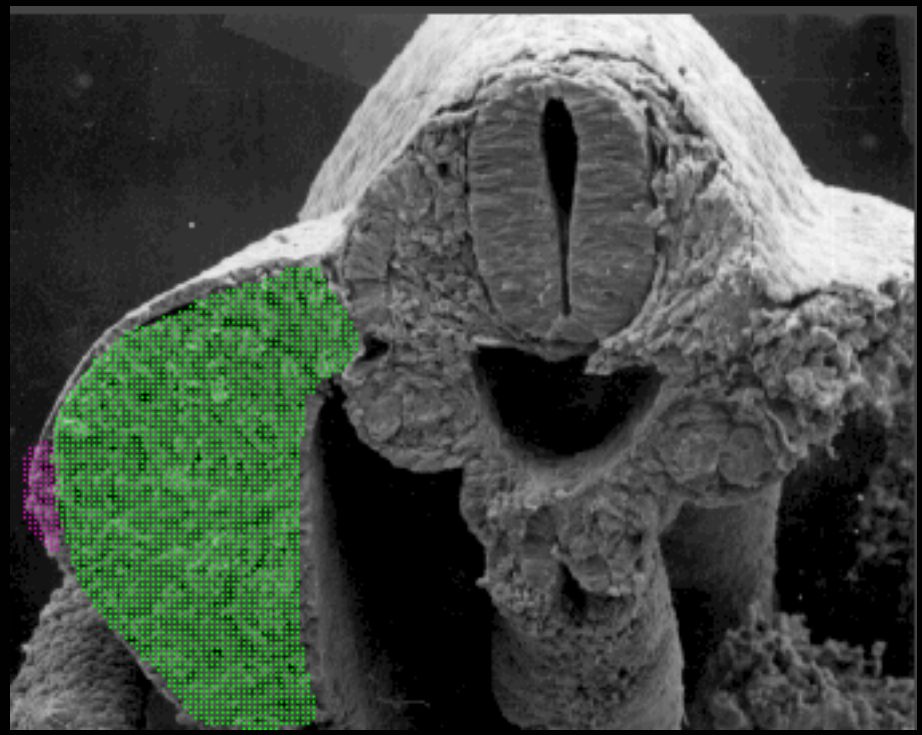
a= autopod



A-P= Anterior-Posterior

Pr-D= Proximal-Distal

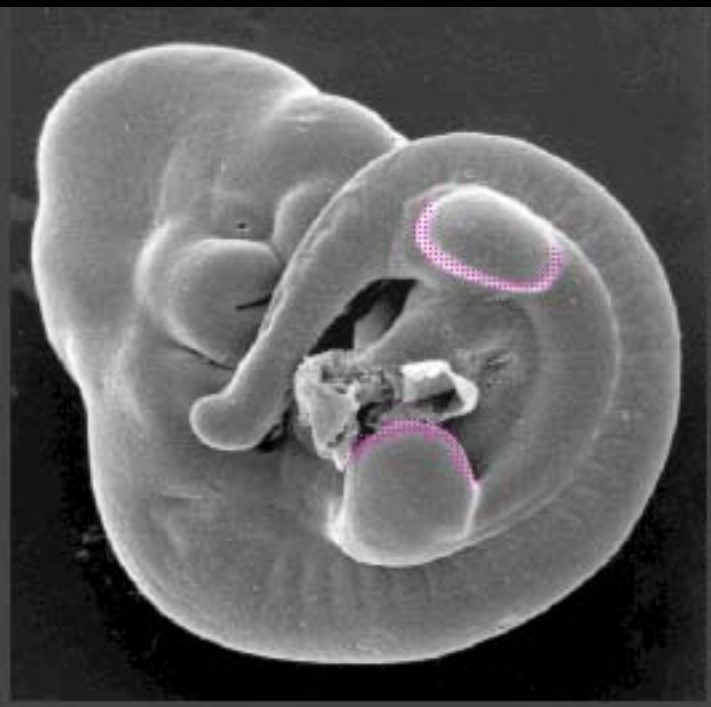
Modified from:
Ridley M. Evolution, 2nd ed. Blackwell Science 1996

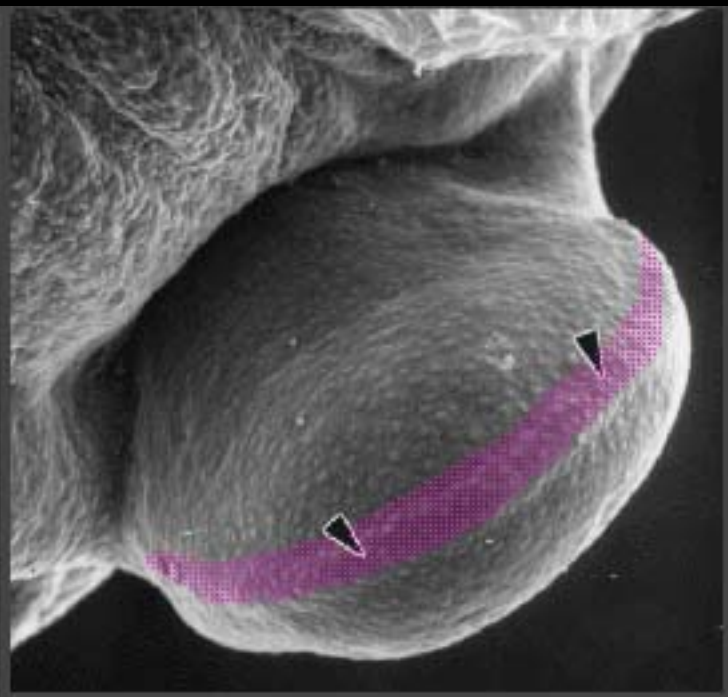


P. Martin
Int. J. Dev. Biol 1990

Species: Mouse
Day Gestation: 9
Approx. Human Age: 28 days
View: Lateral

http://www.med.unc.edu/embryo_images/unit-mslimb





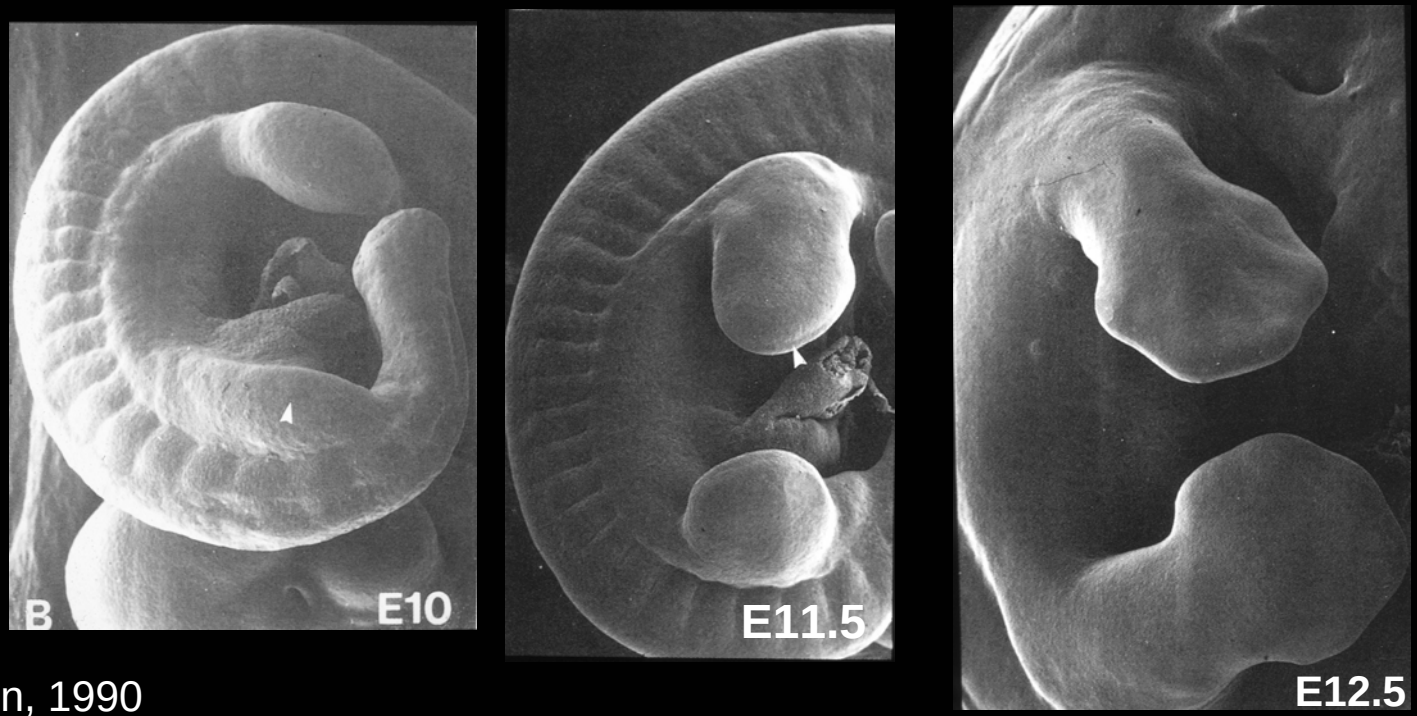
The thickened ectoderm at the distal rim of the limb bud is termed the **apical ectodermal ridge.**

Species: Mouse
Day Gestation: 11
Approx. Human Age: 33 days
View: Lateral

Limb Development
17 of 26

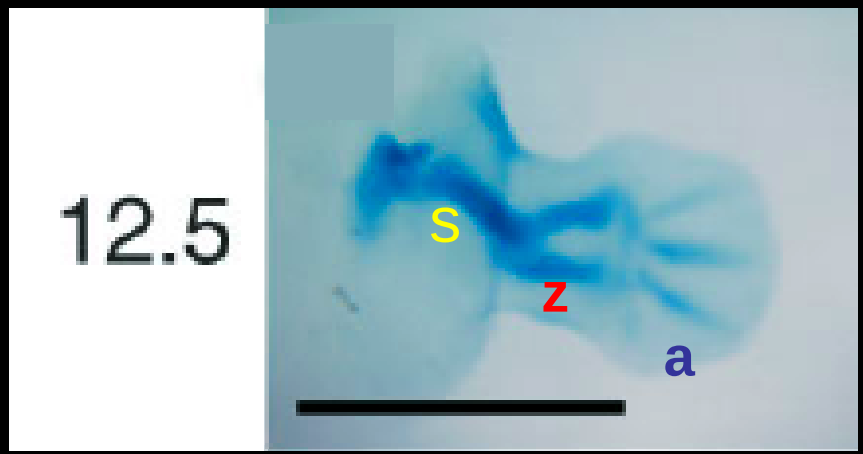
http://www.med.unc.edu/embryo_images/unit-mslimb/mslimb_htms/mslimb017.htm

Laying down of the cartilage condensations

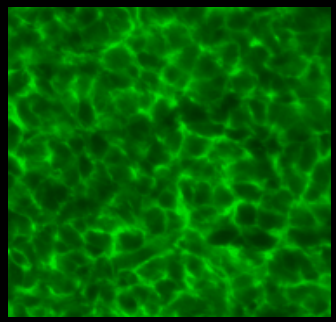


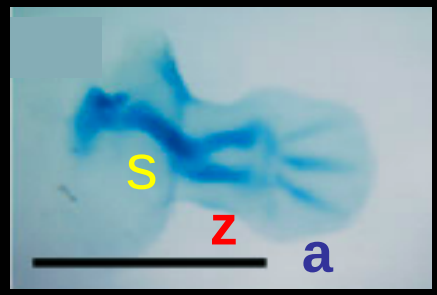
Martin, 1990

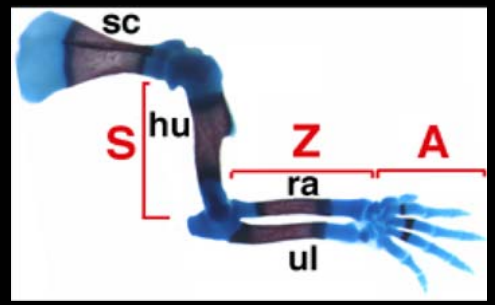
Khokha et al. 2003

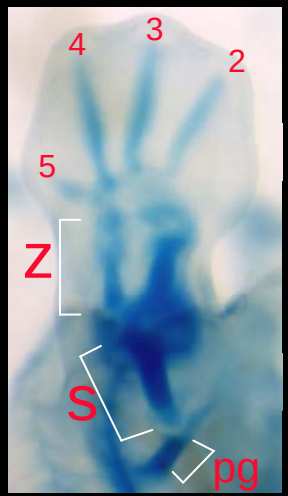


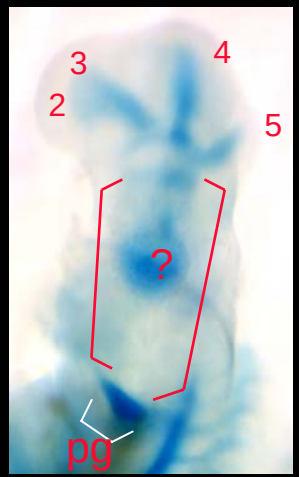
Specification → Elaboration of Patterning



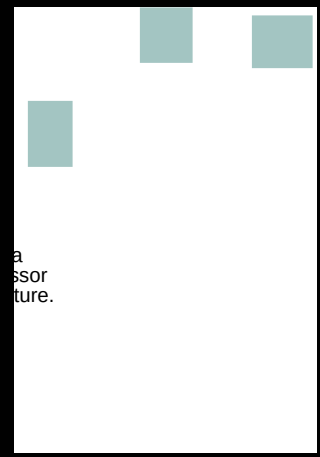






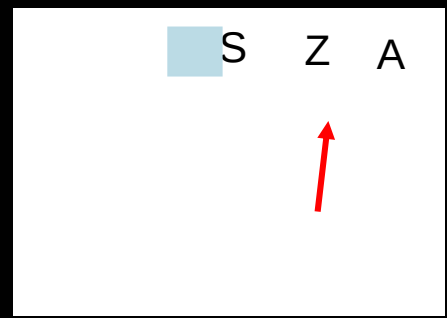


QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



Plzf/Gli3 dKO
Barna et al.

Fgf4/Fgf8 dKO
Sun et al. Nature (2002)



Hoxa11/d11 dKO
Davis et al. Nature (1995)

Maria Barna

PLZF (also known as ZFP 145)

PLZF translocated to the Retinoic Acid Receptor Alpha (RARα)gene is associated with acute promyelocytic leukemia (APL)

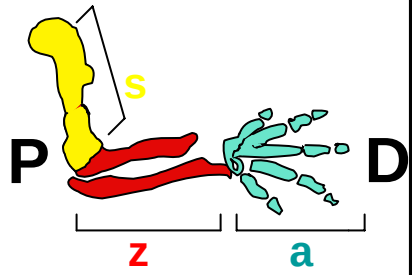
PLZF is a sequence specific DNA binding transcriptional repressor belonging to the POK (POZ and Kruppel) family of proteins that can remodel chromatin to a heterochromatic state.

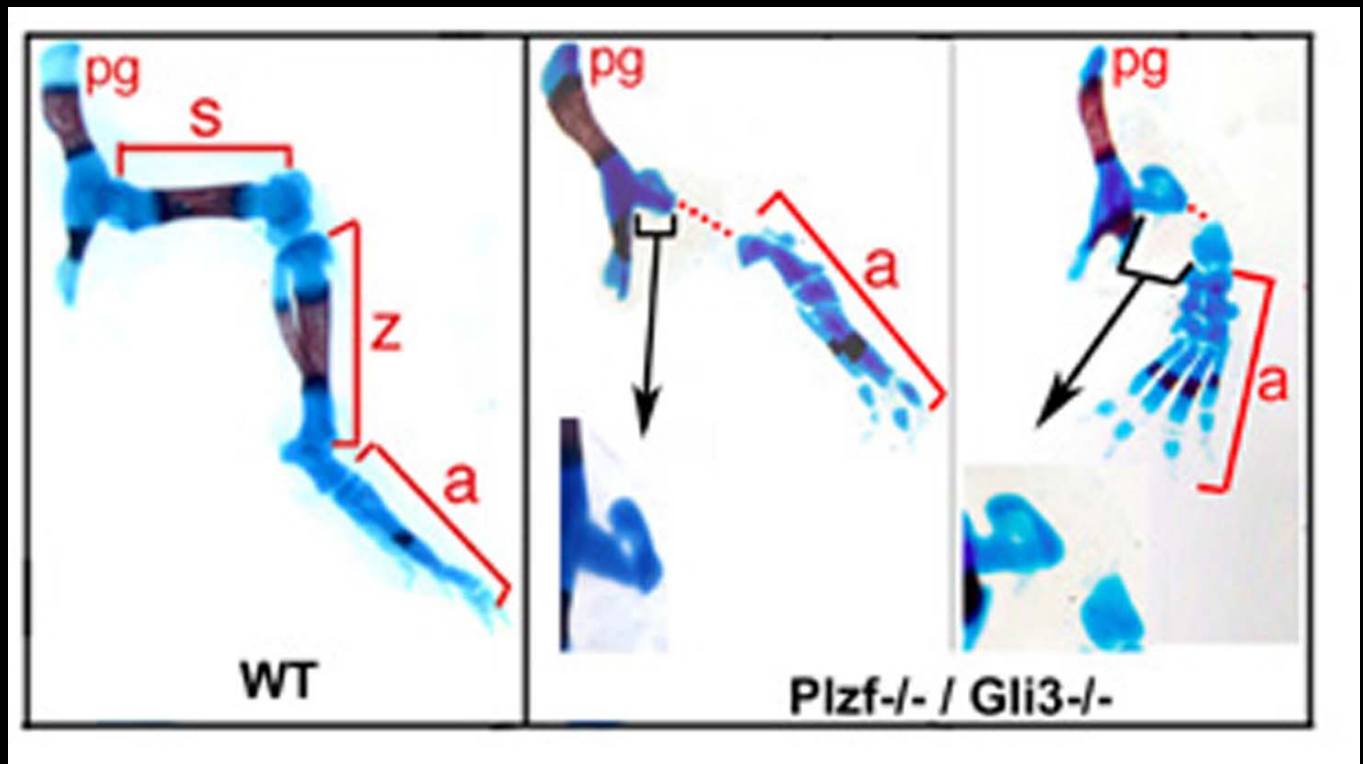
Genetic interaction between the transcription factors Plzf and Gli3

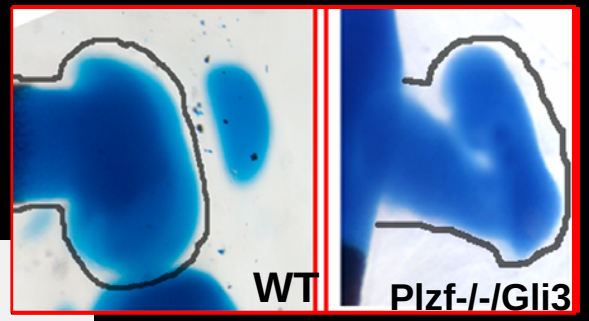
s= stylopod

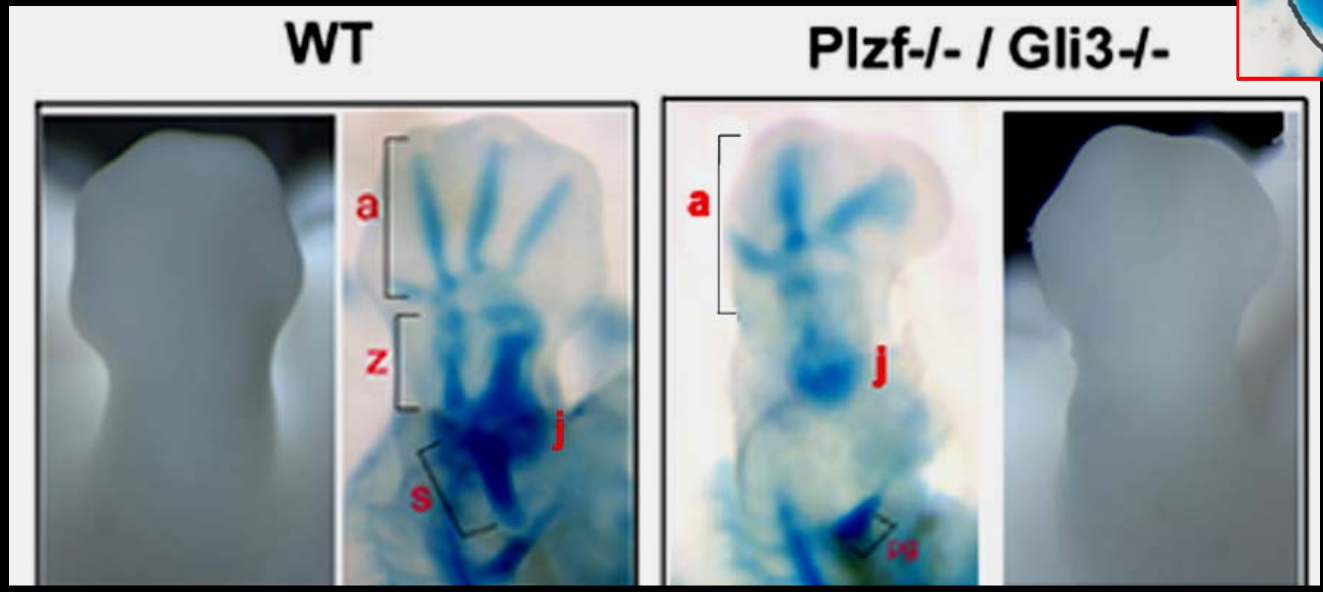
z= zeugopod

a= autopod






WT
Plzf-/-/Gli3

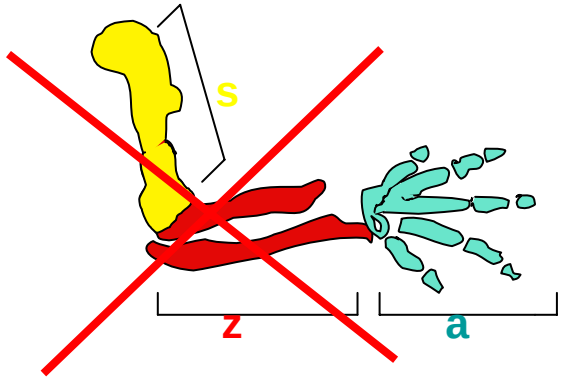

WT
Plzf-/- / Gli3-/-
a
z
j
s
a
j

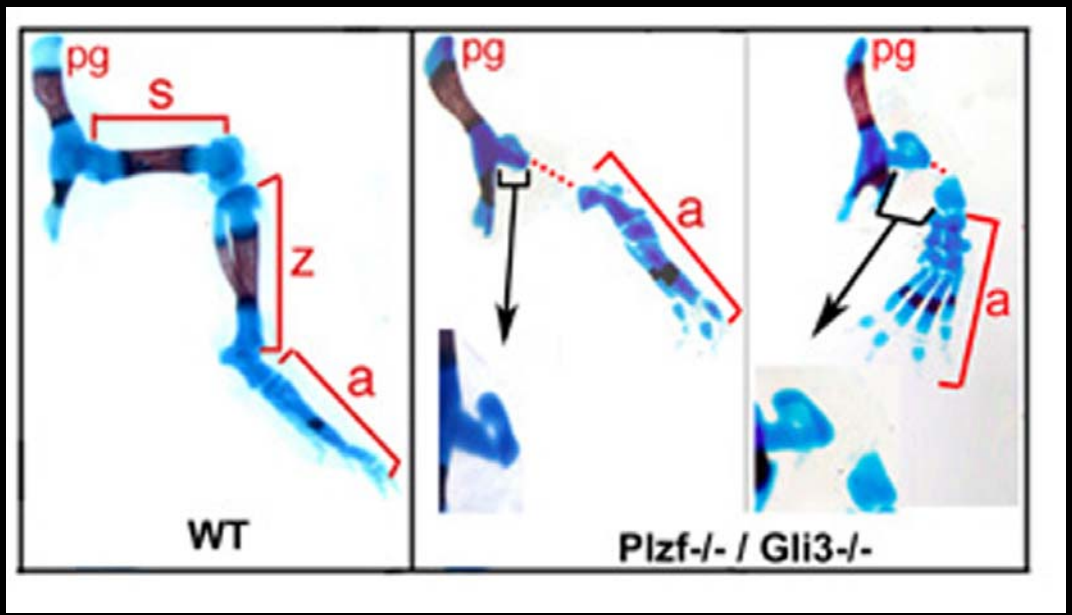
Skeletal elements of the limb are independently regulated

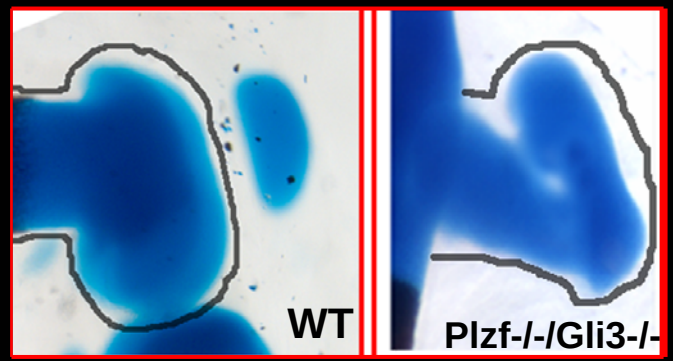
s= stylopod

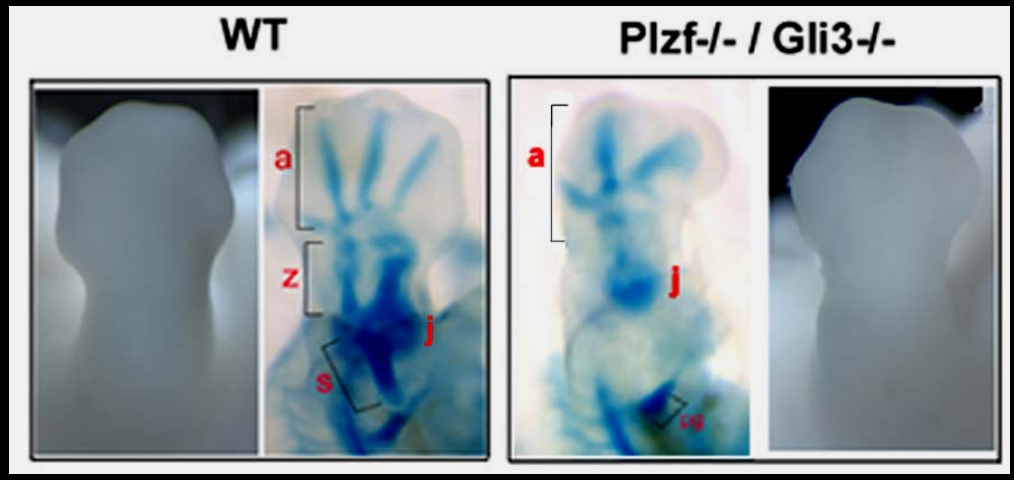
z= zeugopod

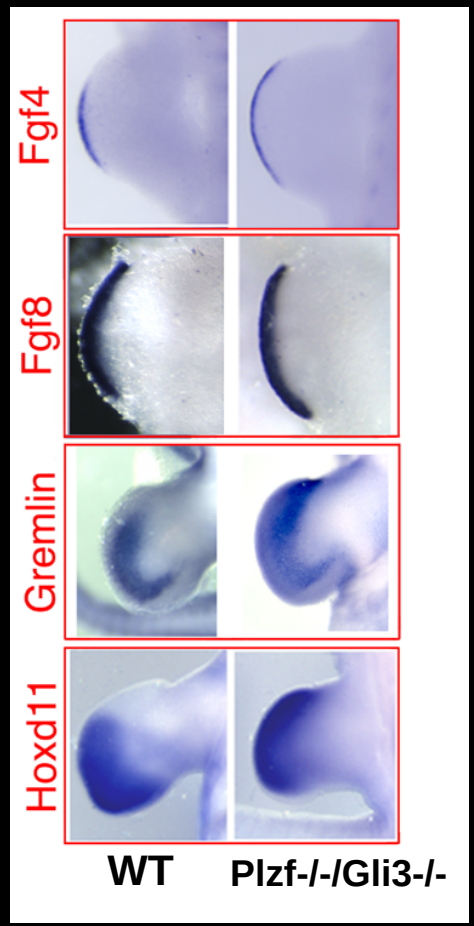
a= autopod

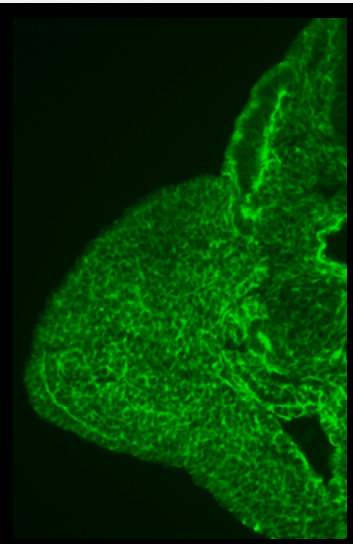


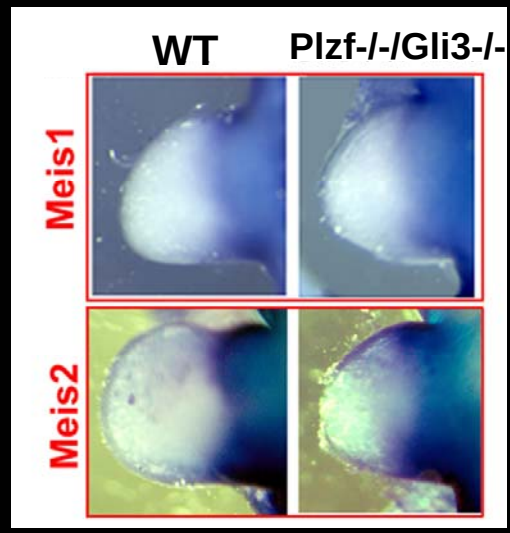


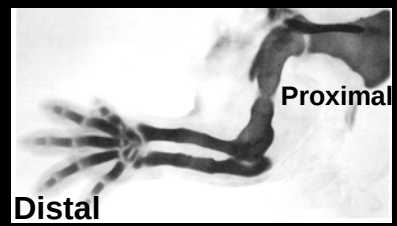



WT
Plzf-/- / Gli3-/-
a
z
j
s
a
j

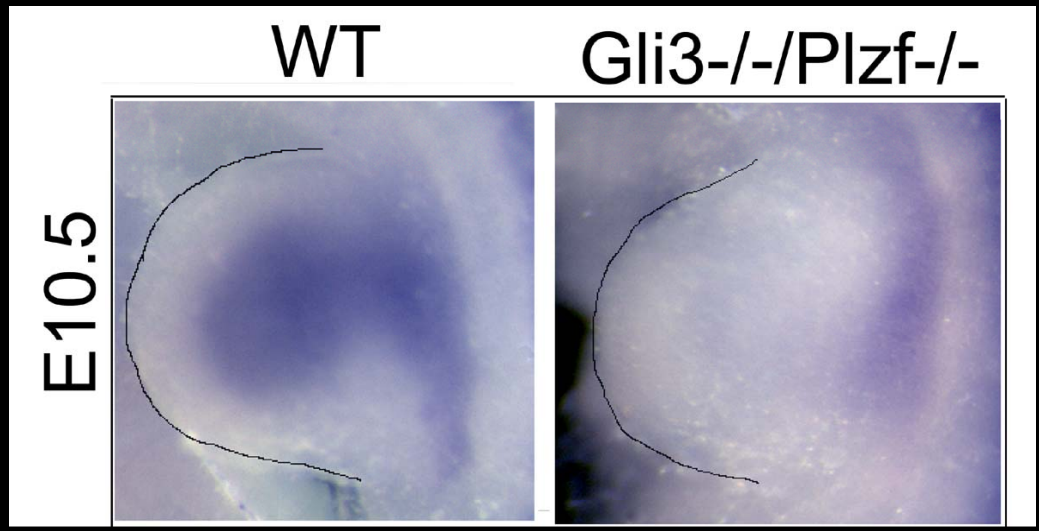

Fgf4
Fgf8
Gremlin
Hoxd11
WT
Plzf-/-/Gli3-/-



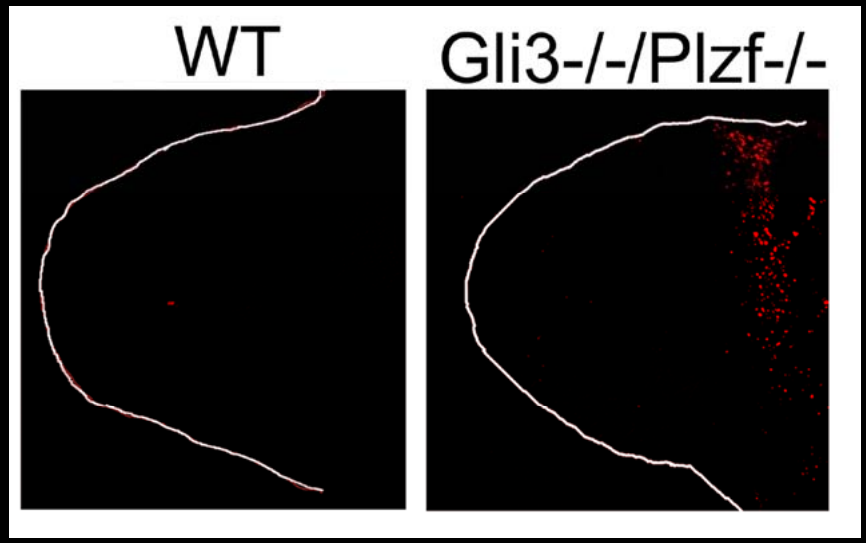

WT
Plzf-/-/Gli3-/-
Meis1
Meis2


Proximal
Distal

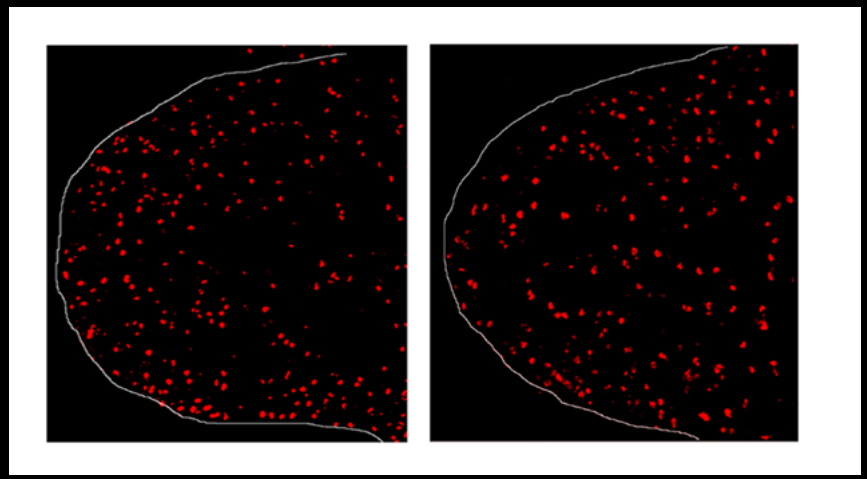
Sox9:the earliest known marker of cartilage differentiation



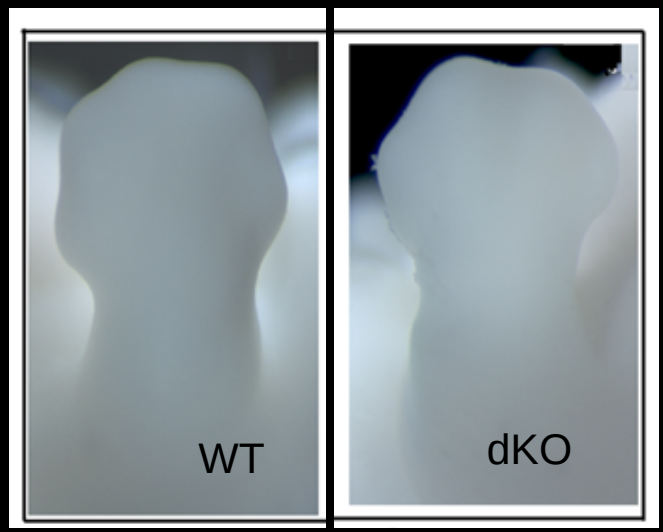
Plzf and Gli3 are required for the survival of a small population of proximal mesenchyme



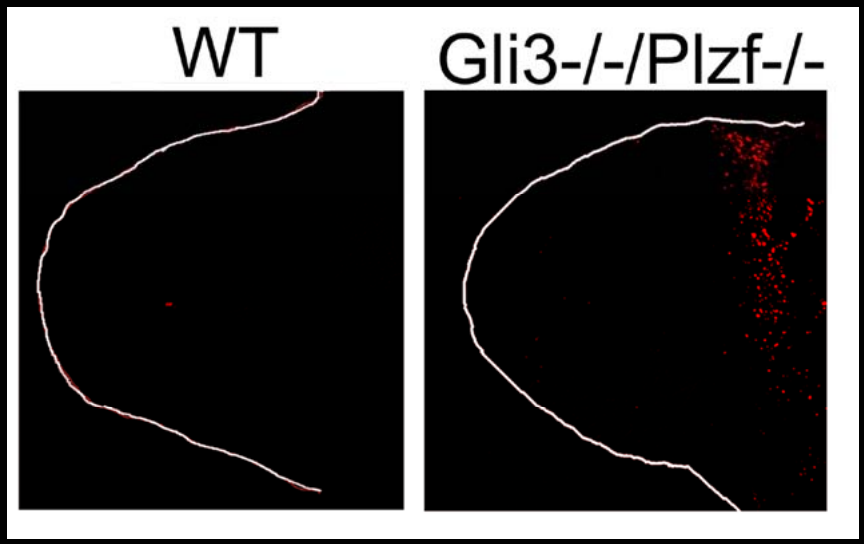
Tunel E10.5



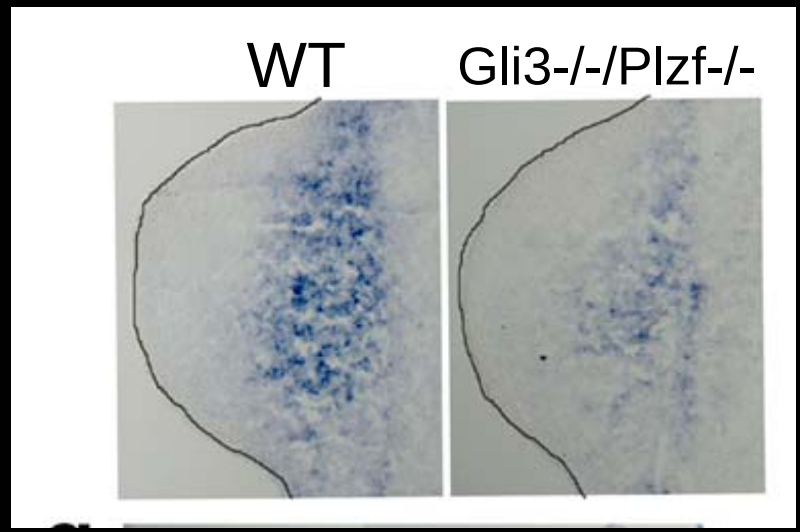
H3-Mitotic Cells E10.5



The dying cells in the Plzf-/-/Gli3-/- limb are marked by BmprIB expression



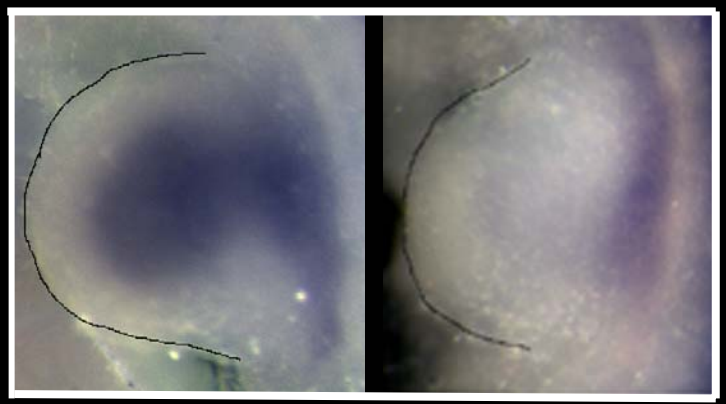
Tunel E10.5



BmprIB E10.5

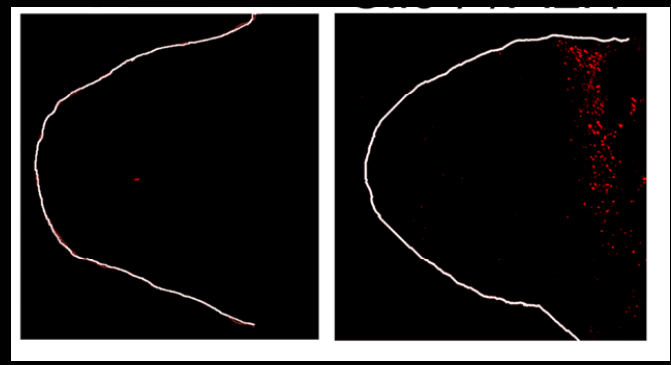
The molecular basis for Plzf-/-/Gli3-/- proximal limb defects

Sox9



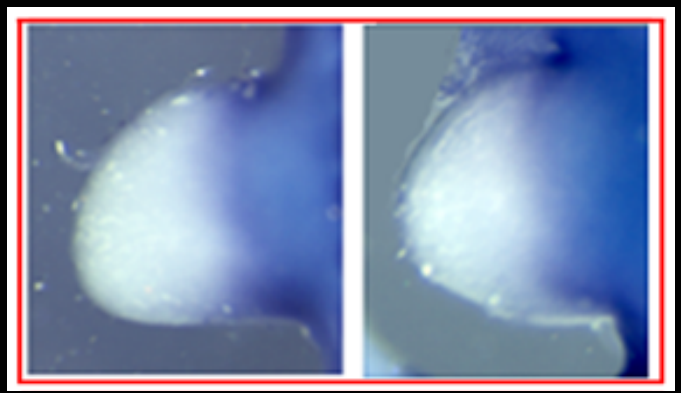
WT Plzf-/-/Gli3-/-

Tunel



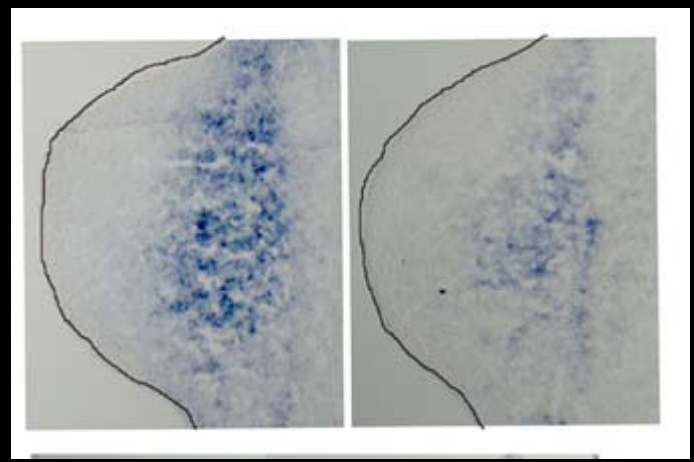
WT Plzf-/-/Gli3-/-

Meis1

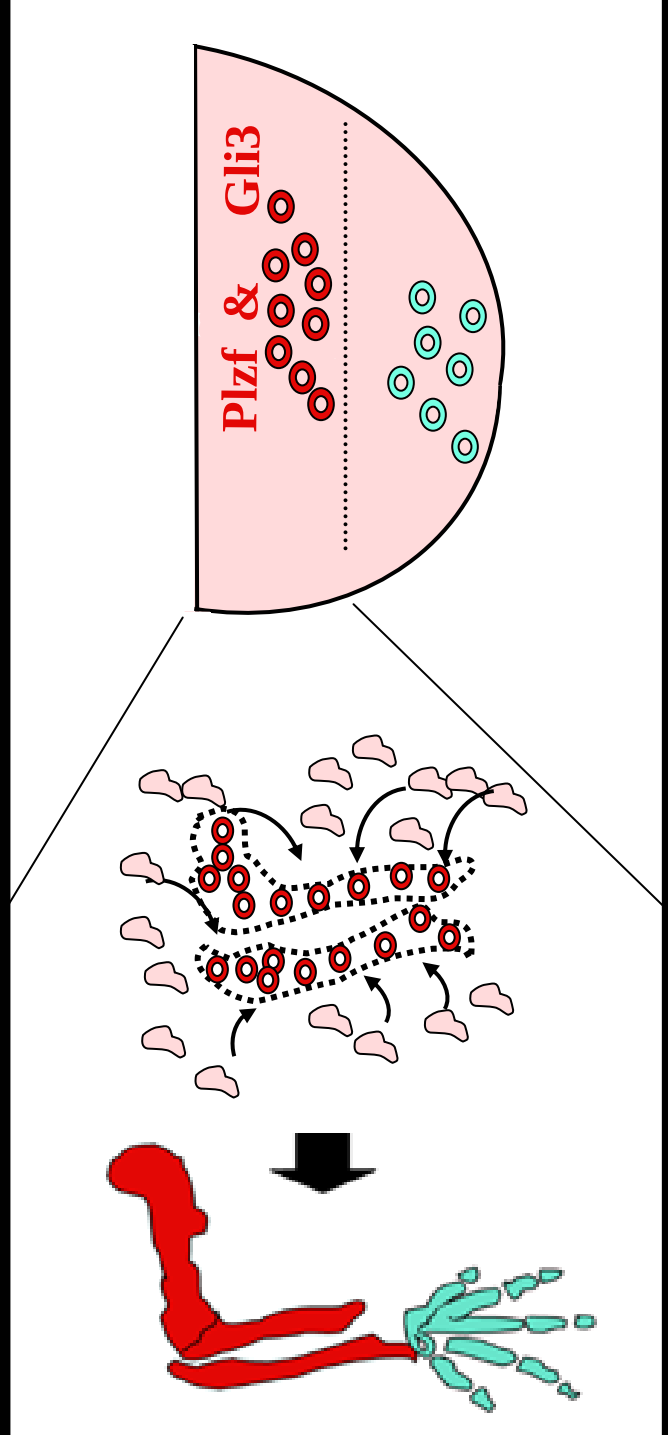


WT Plzf-/-/Gli3-/-

BMPR1B



WT Plzf-/-/Gli3-/-



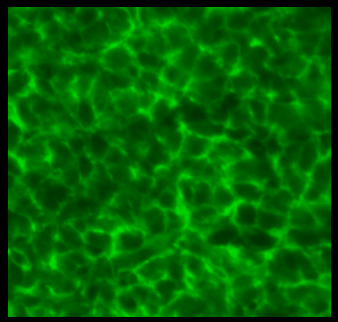
= Mesenchyme

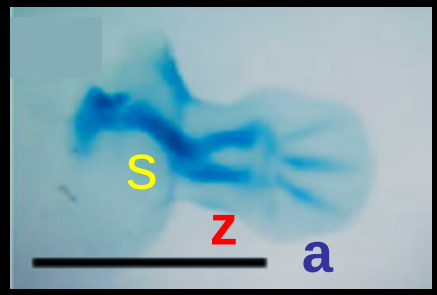
= Proximal Progenitors

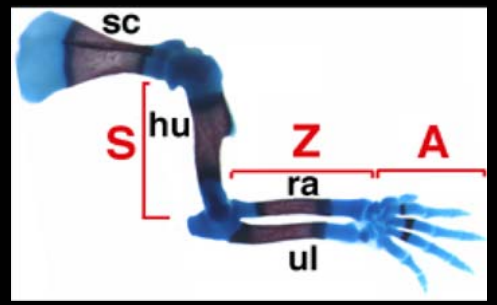
= Distal Progenitors

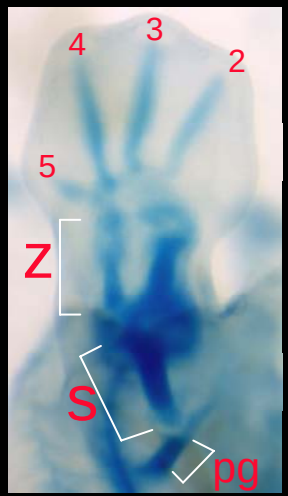
Specification

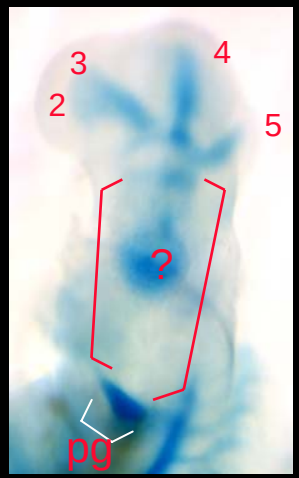
Elaboration of Patterning

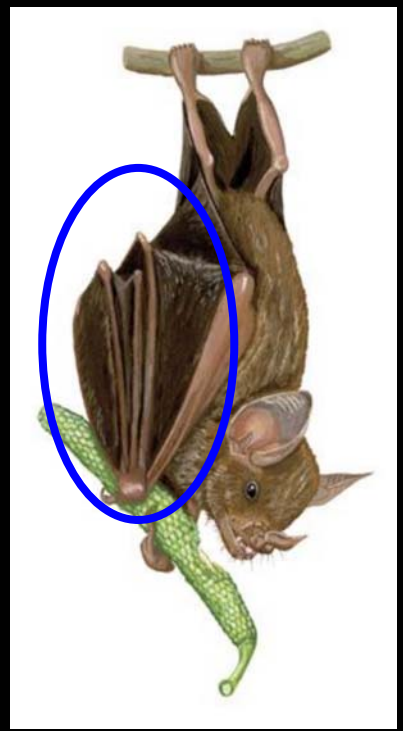






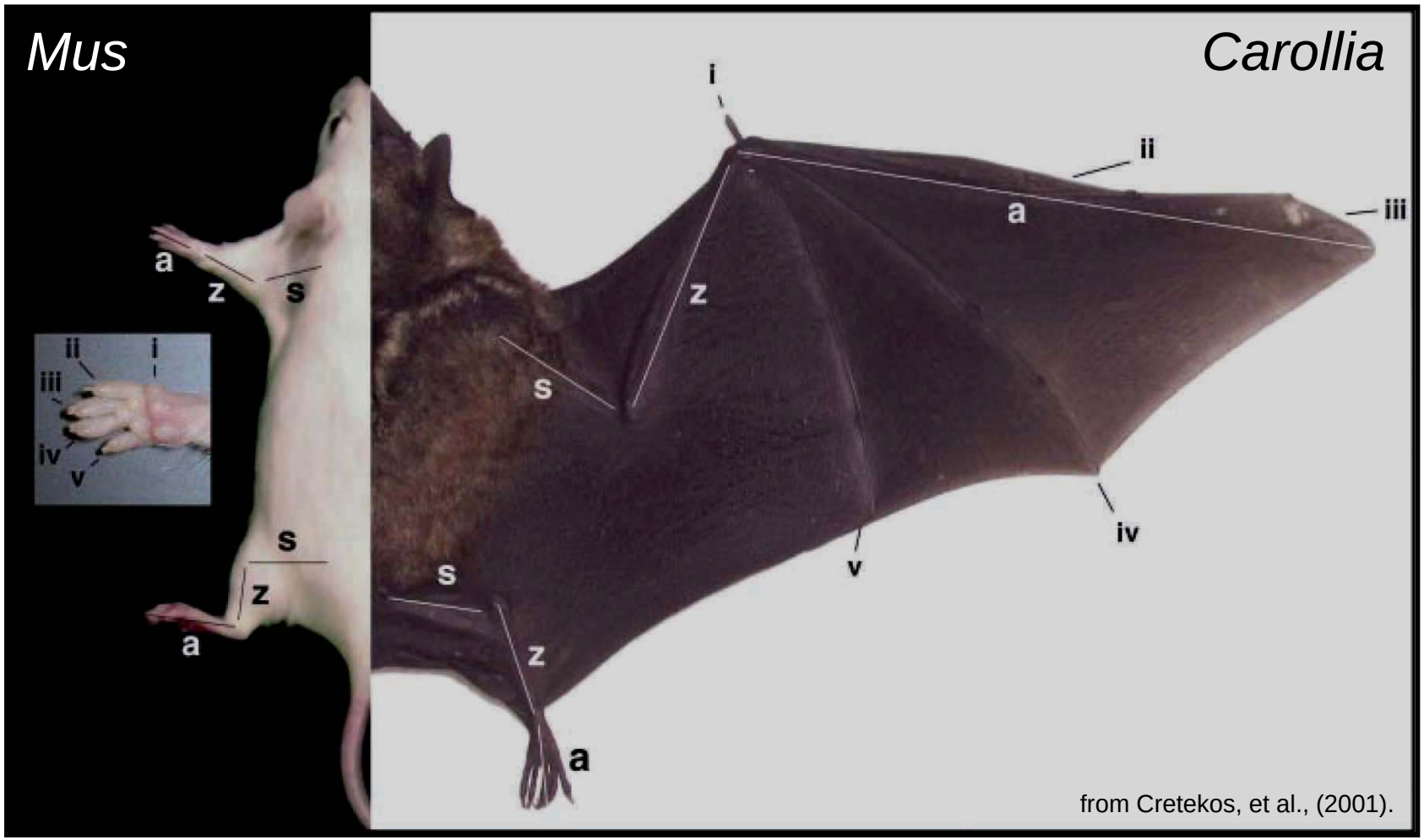






Plzf/Gli3 dKO
Barna et al.

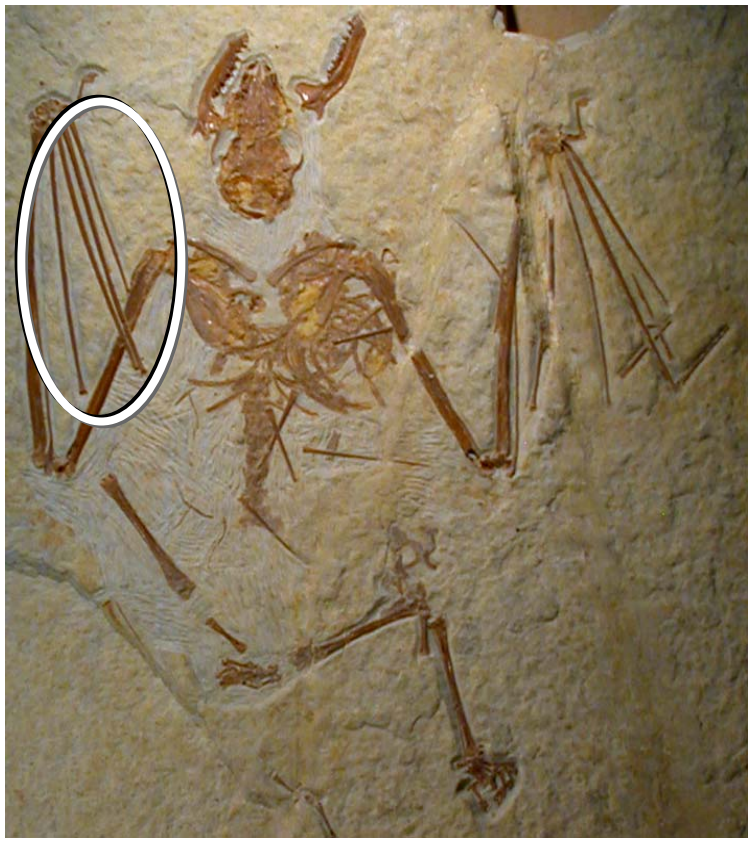
Comparative evo-devo



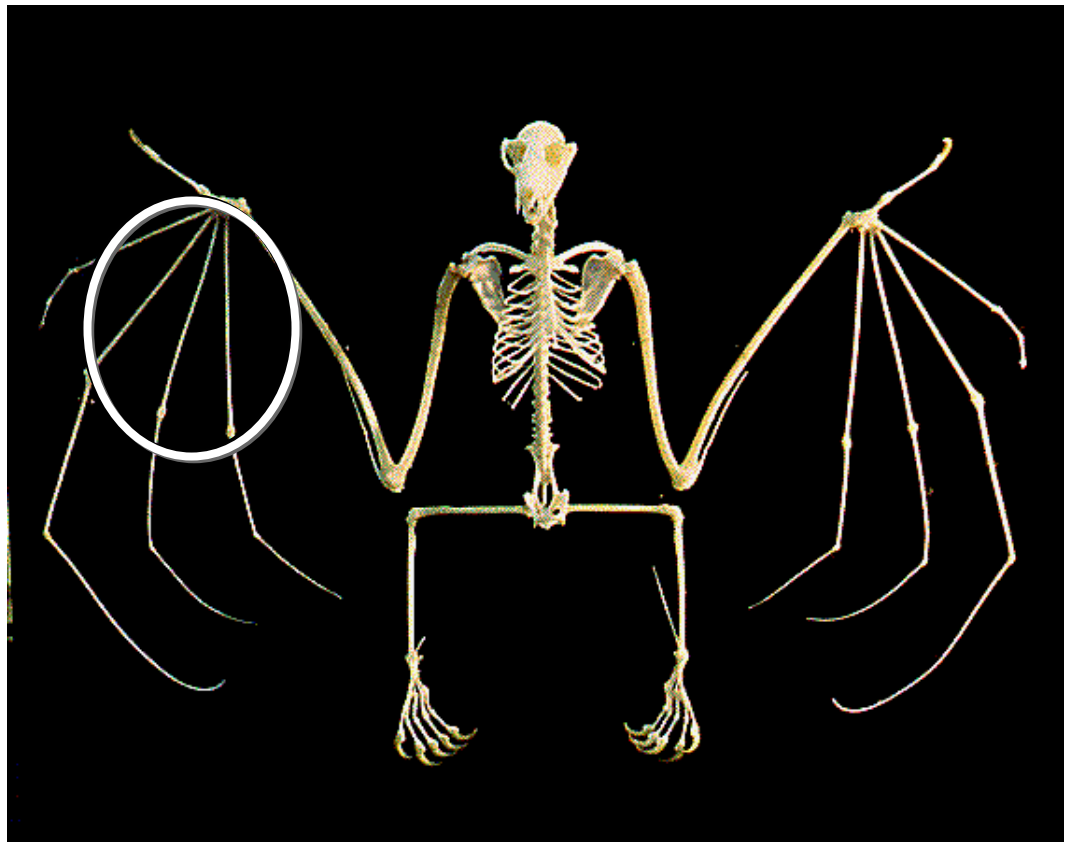
from Cretekos, et al., (2001).

The evolution of bat morphology

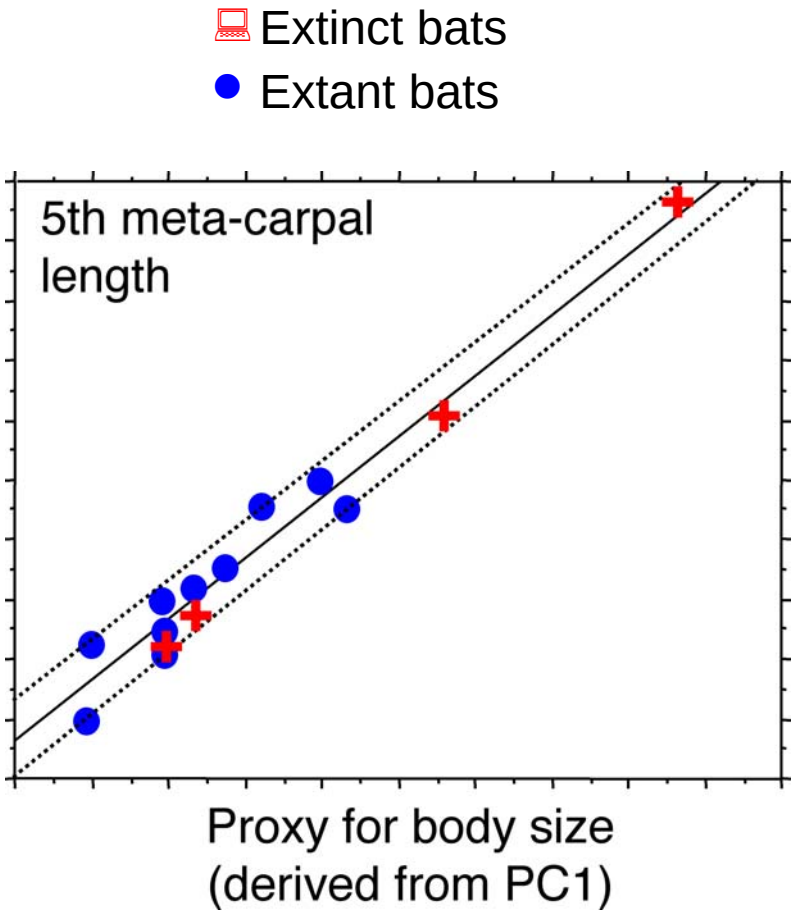
Early fossil bat
(50 million years old)



Modern bat



Bat metacarpals have remained constant in length for the past fifty million years



- **Extinct bats**: *Icaronycteris*, *Hipposideros*, *Archaeonycteris*, *Palaeochiropteryx*
- **Extant bats**: representatives of every modern family

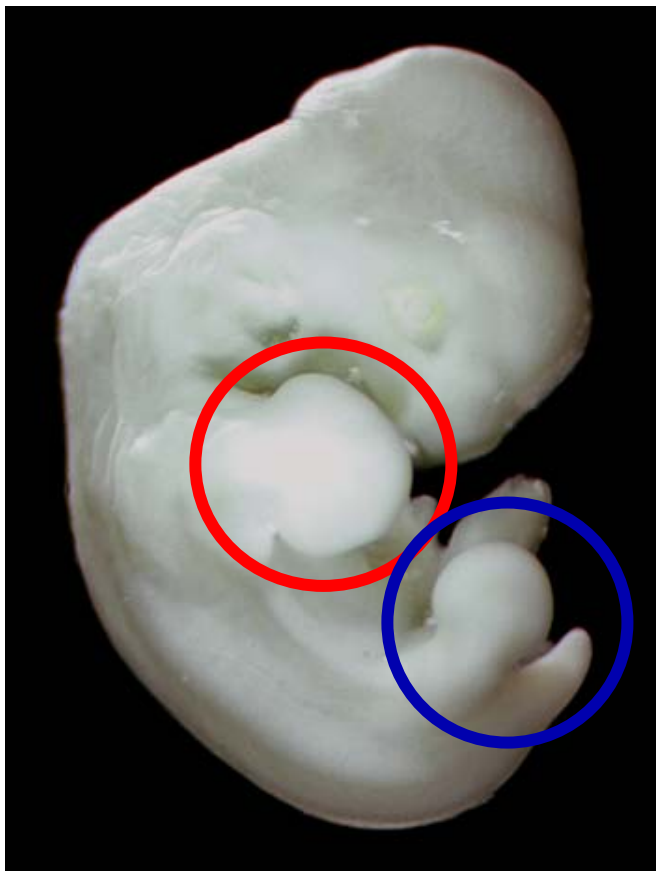
Carollia perspicillata

- **Common name:** Seba's short-tailed bat
- **Abundance:** Very Common
- **Gestation:** 115-120 days
- **Reproduction:** Bi-annually (June to Aug. and Feb. to May) give birth to 1 offspring
- **Diet:** Fruit, pollen, insects
- **Location:** Central and South America

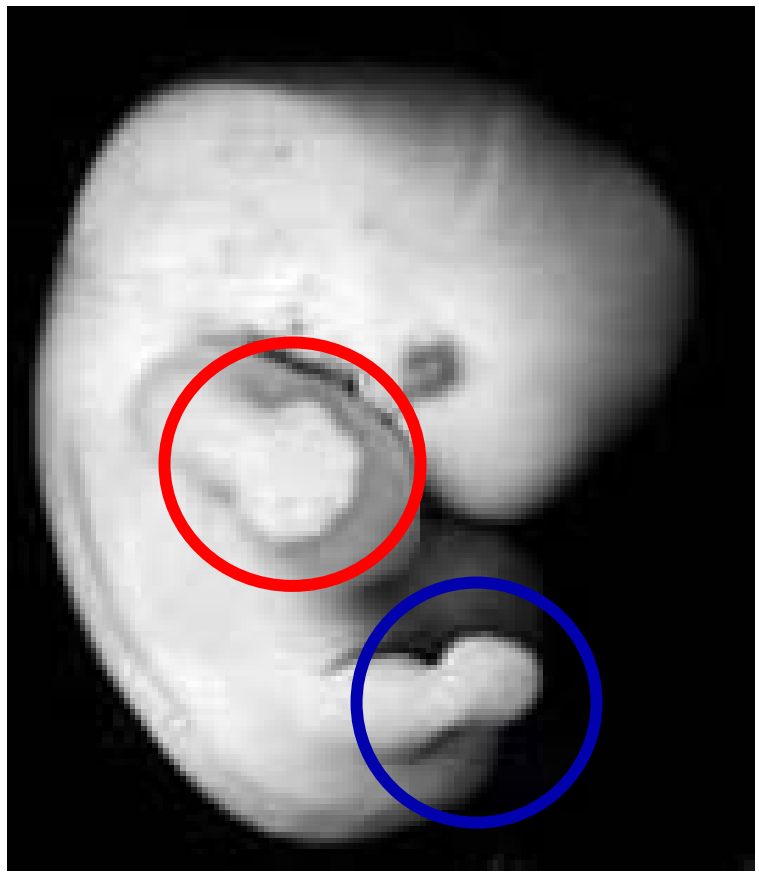
Hypothesis:

The elongation of bat digits resulted from changes in the temporal and/or spatial expression of a few key regulators of limb development.

Early bat digits are similar in size to those of other mammals



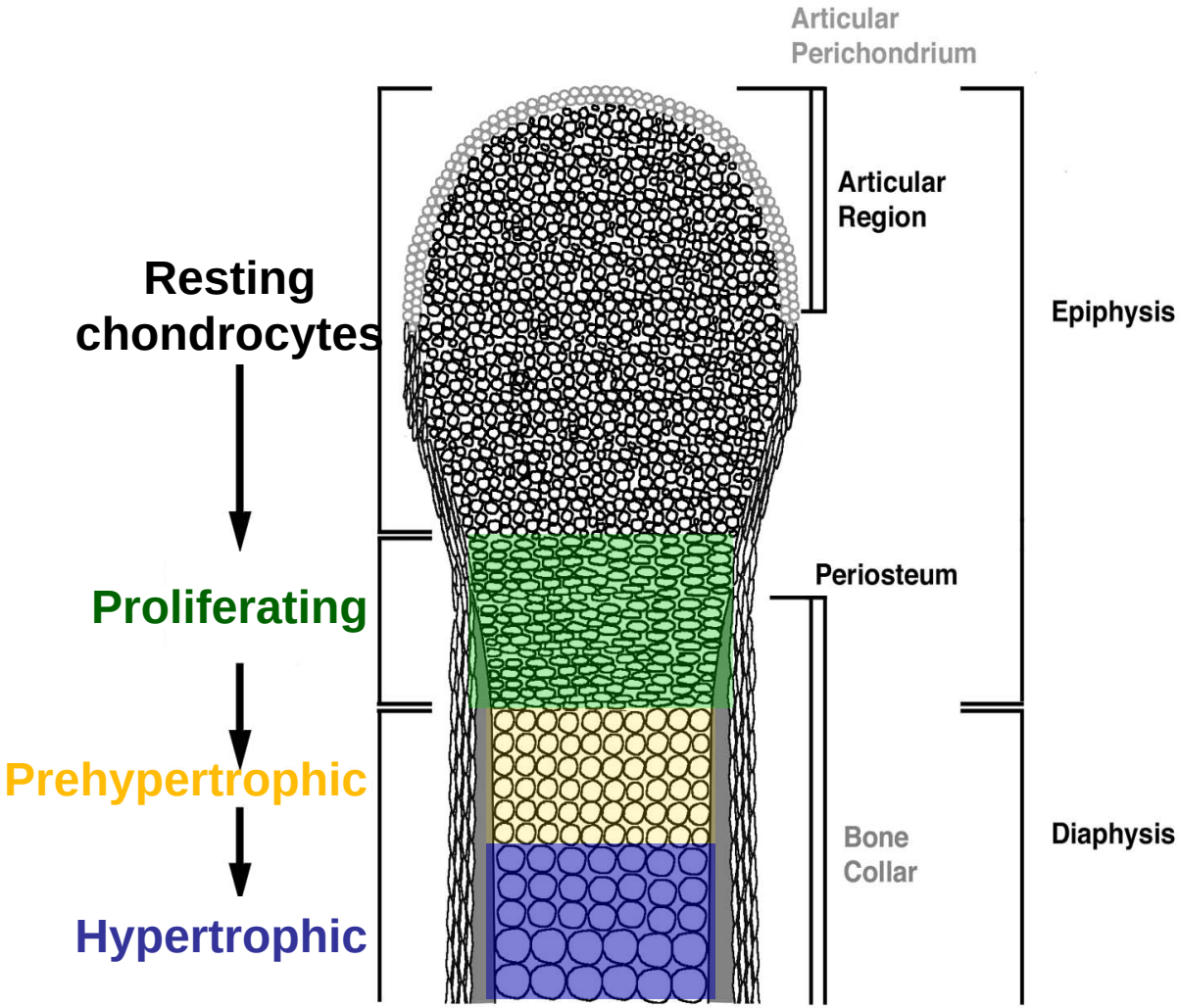
Day 48 Bat Embryo
(comparable to Day 12 mouse)



Day 48 Human Embryo
(comparable to Day 13.5 mouse)

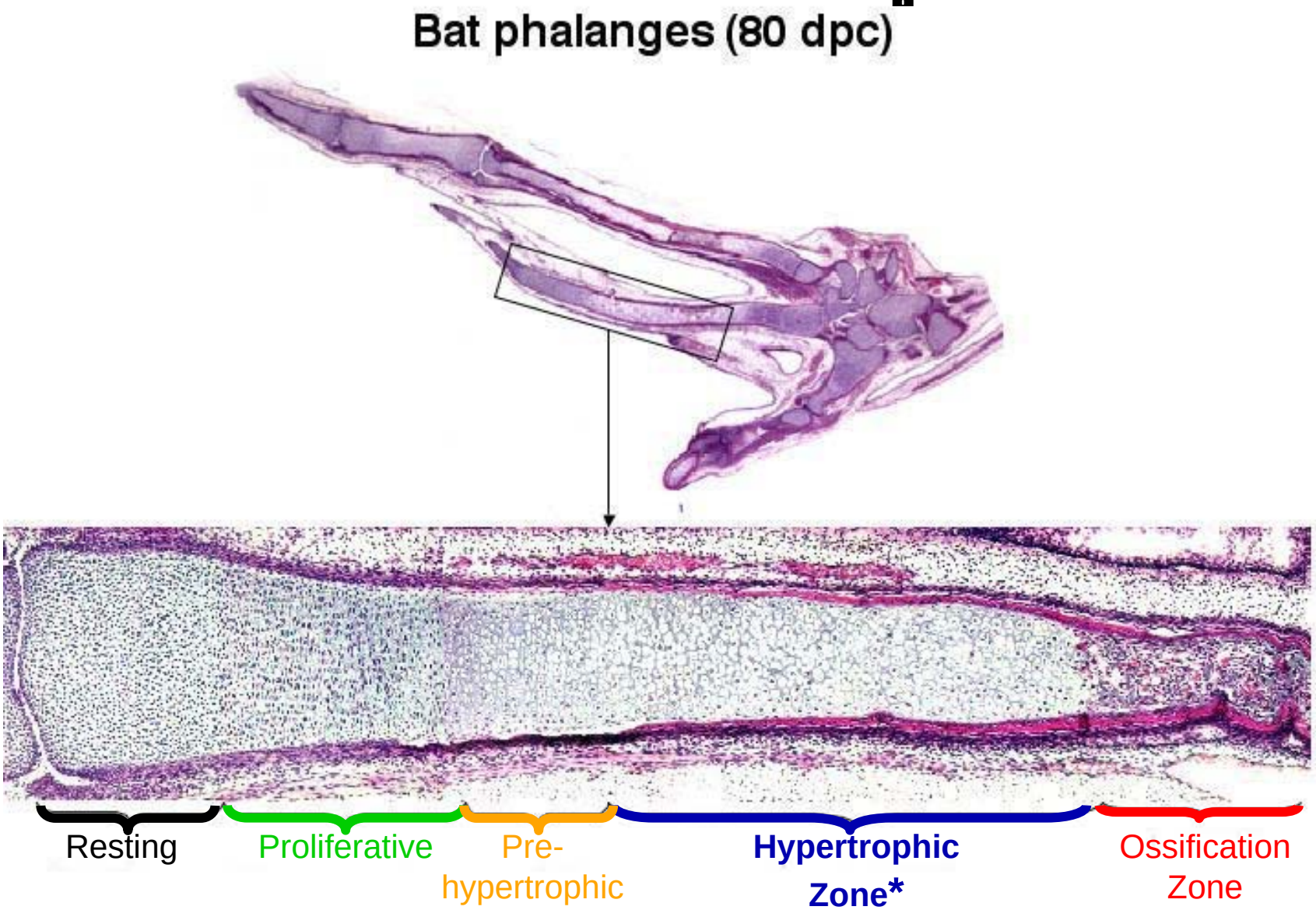
Growth plate morphology:

The relative rate of cartilage proliferation and differentiation controls digit length



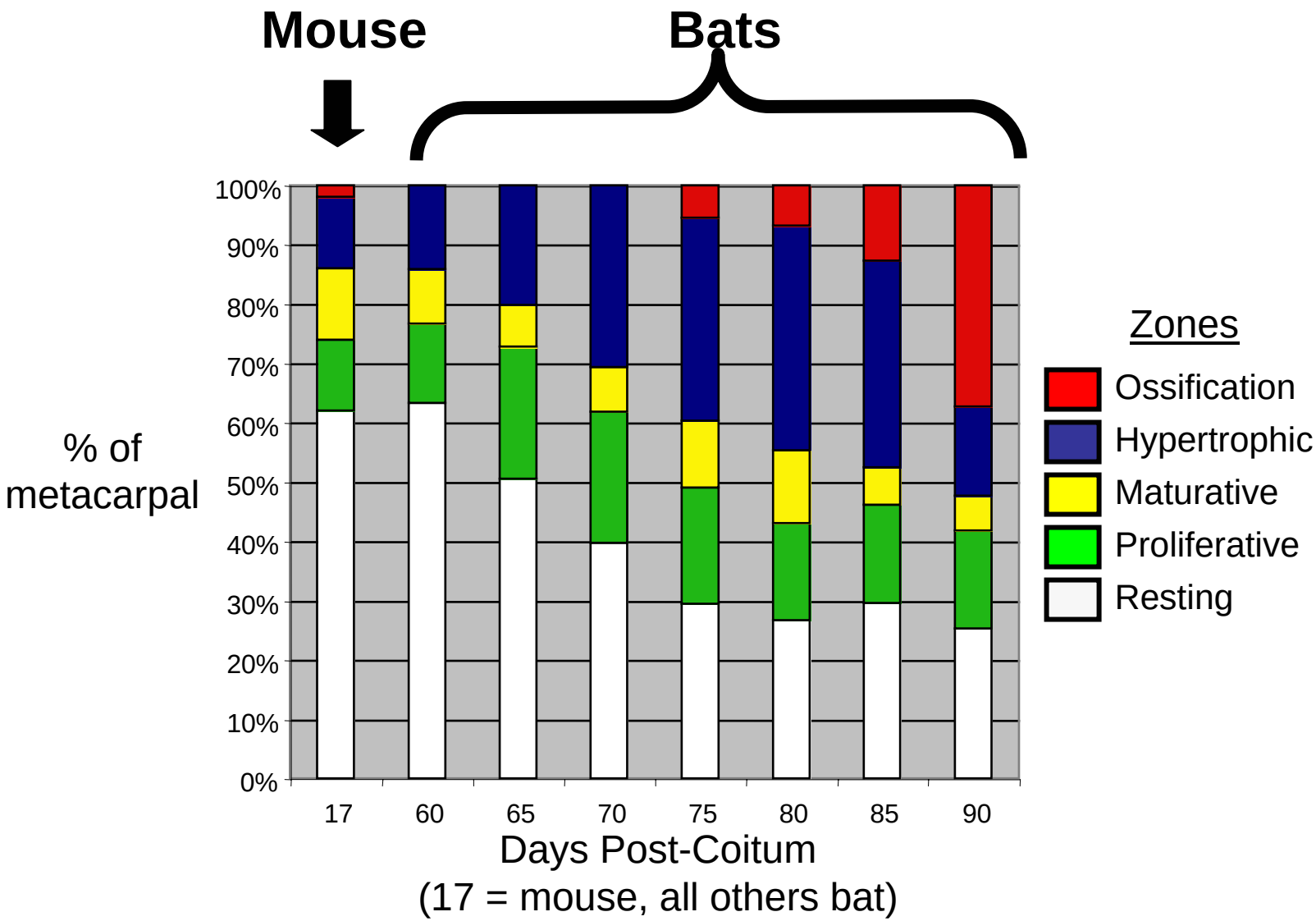
*adapted from Weatherbee and Niswander (in press)

Hypertrophic zone greatly enlarged in bat

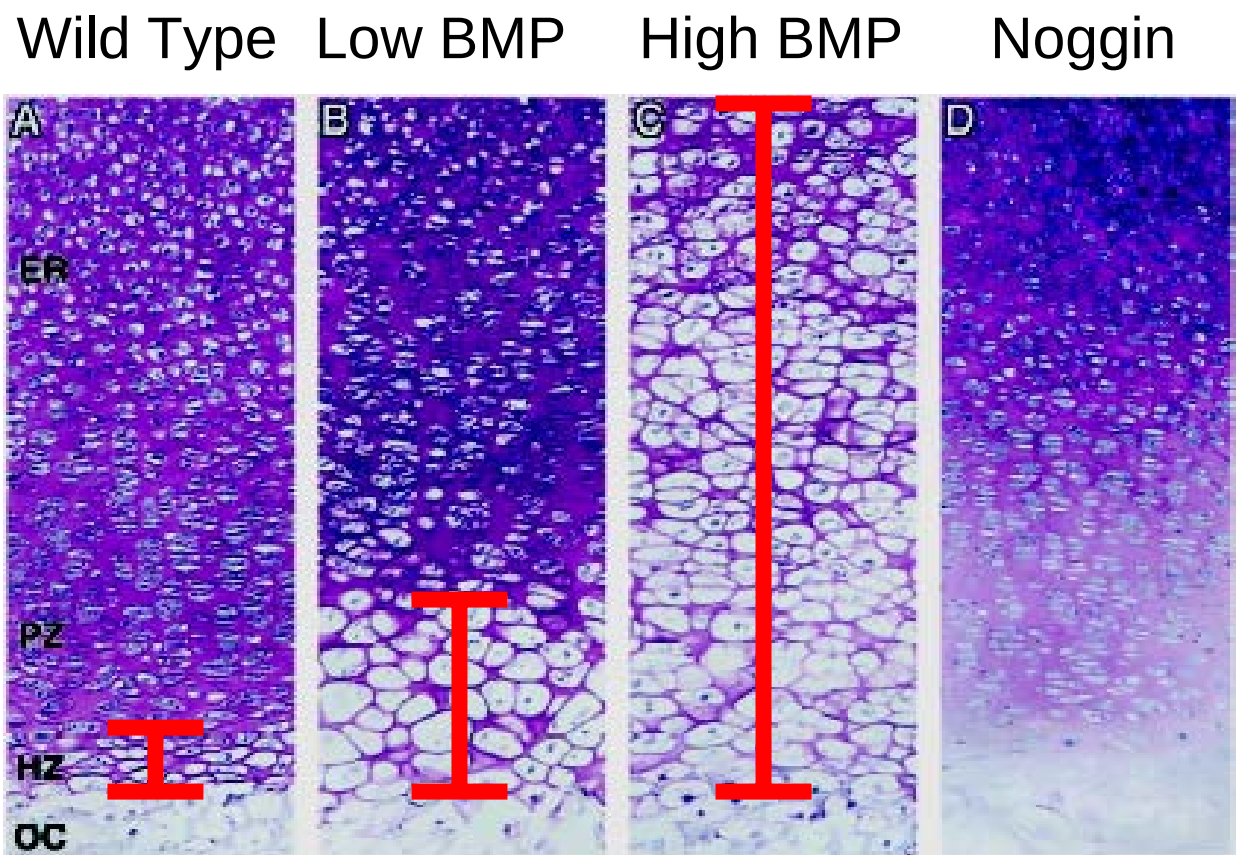


Paraffin embedded, sectioned and stained with hematoxylin and eosin

Hypertrophic zone greatly enlarged in bat



Application of BMP protein to mouse and rat limbs results in a bat-like morphology (De Luca, et. al., 2001)



Can BMP stimulate digit elongation in bats?

Bat metacarpals were cultured in either *Bmp* or *Noggin* (a BMP antagonist) proteins, or control media

Left Limb

Right Limb

BMP
or
Noggin

Let grow for four days

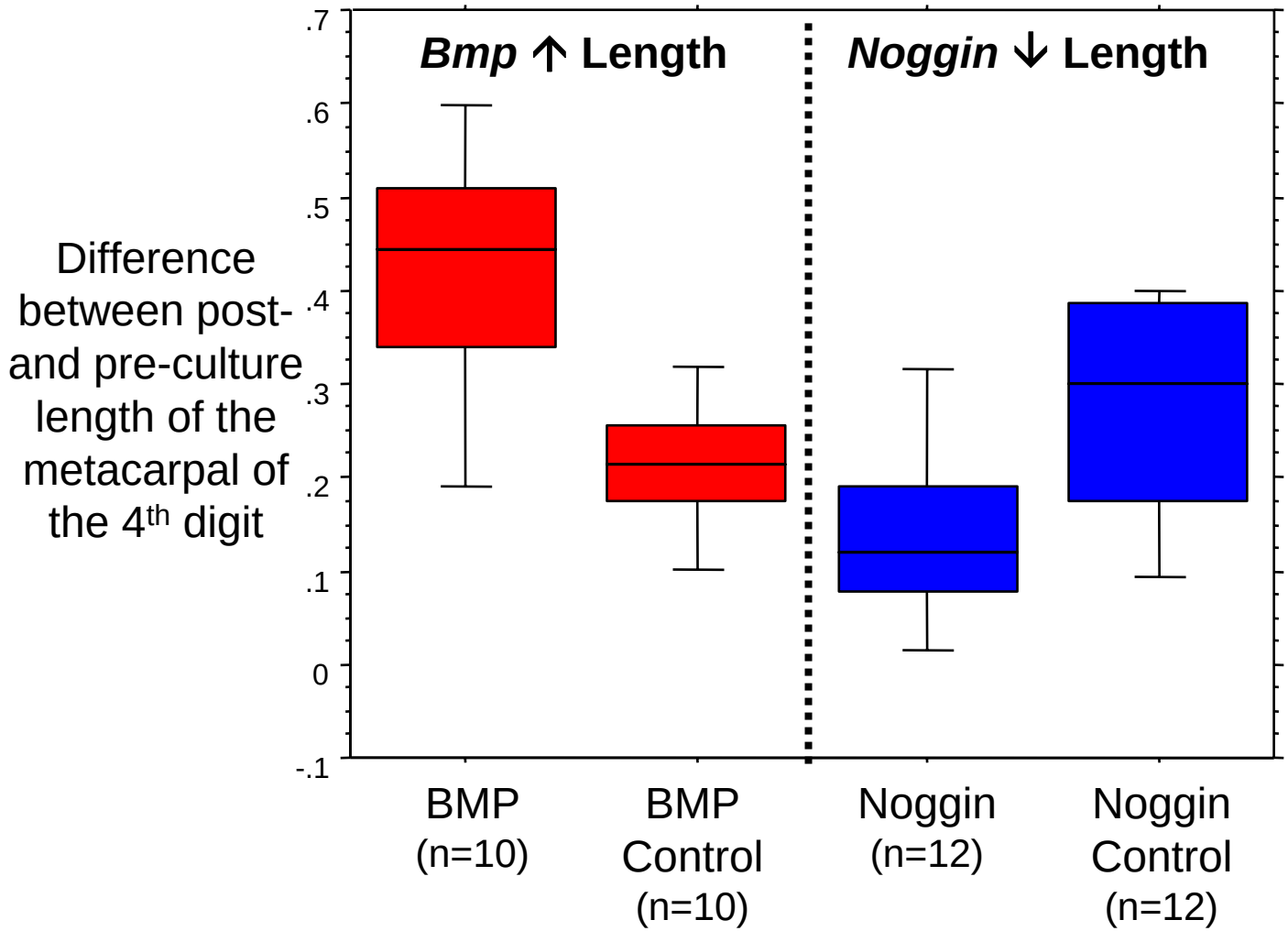
Digit length can be changed by application of either *Bmp* or *Noggin* protein

Pre-*Bmp* culture*

Post-*Bmp* culture*

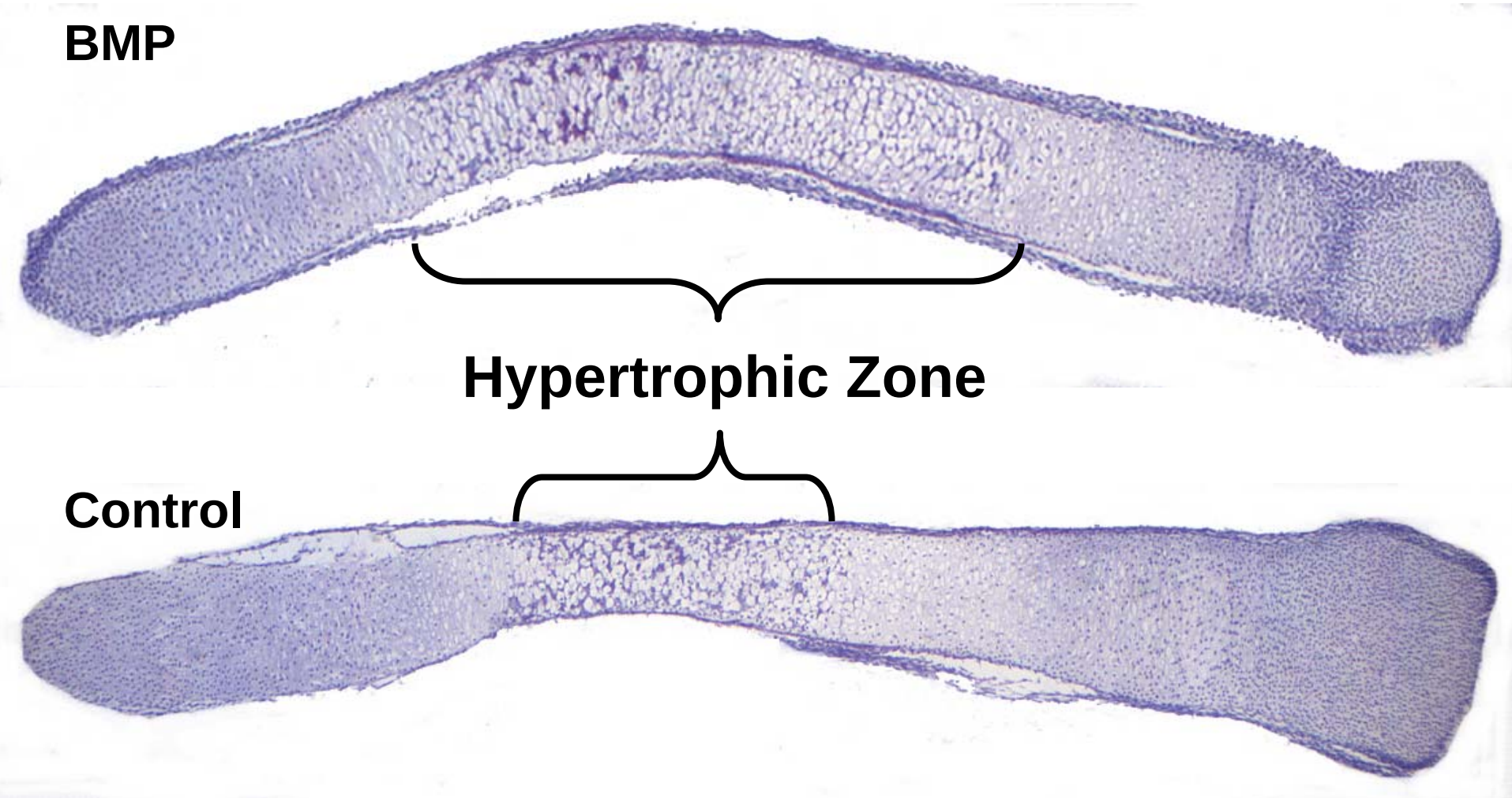
*images shown at same scale

Digit length can be changed by application of either *Bmp* or *Noggin* protein

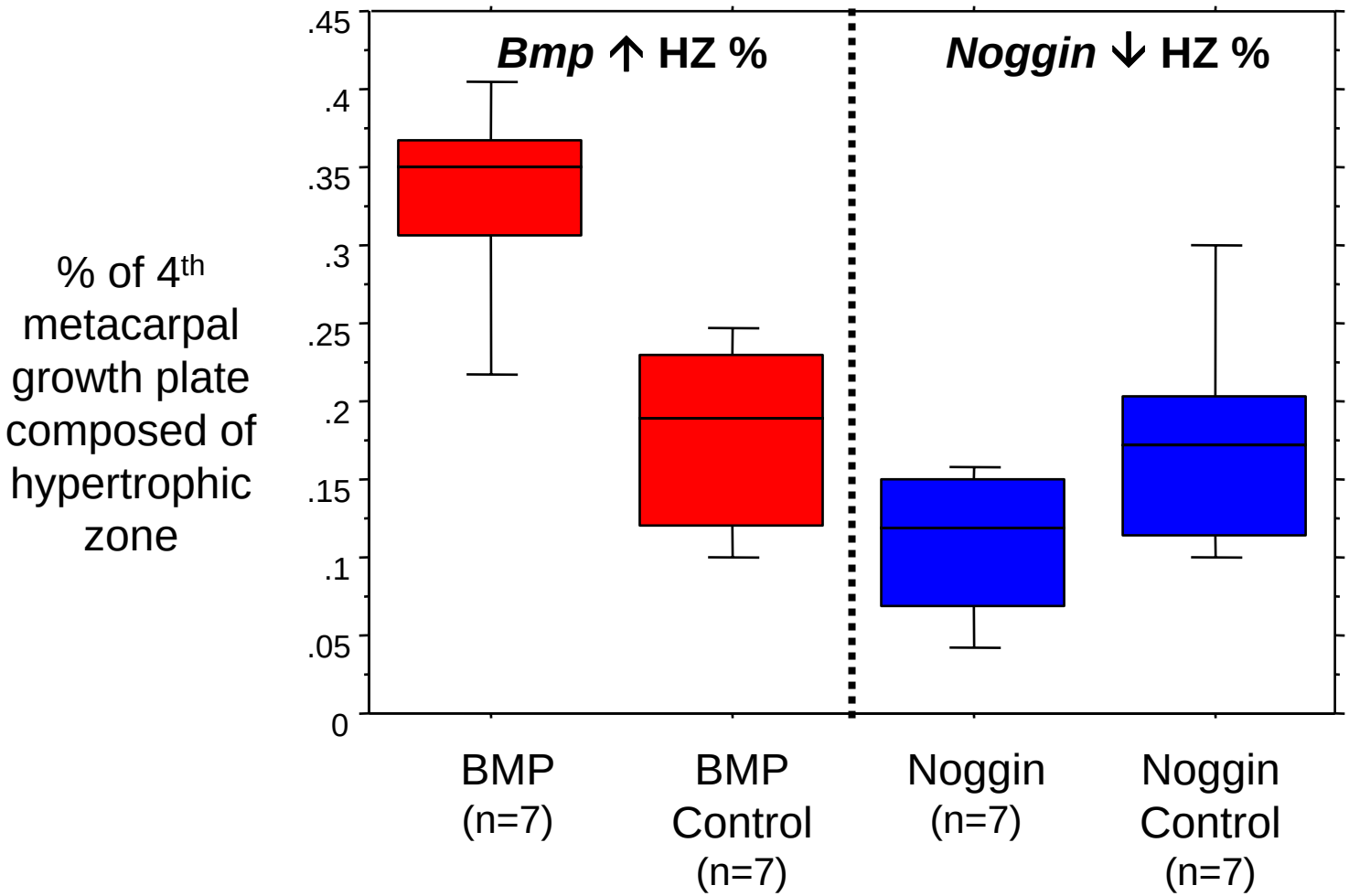


• Differences between *Bmp* & *Bmp* Control and *Noggin* & *Noggin* Control are statistically significant (p-values = 0.003 and 0.015, respectively)

Relative size of the hypertrophic zone can be changed by application of either *Bmp* or *Noggin*

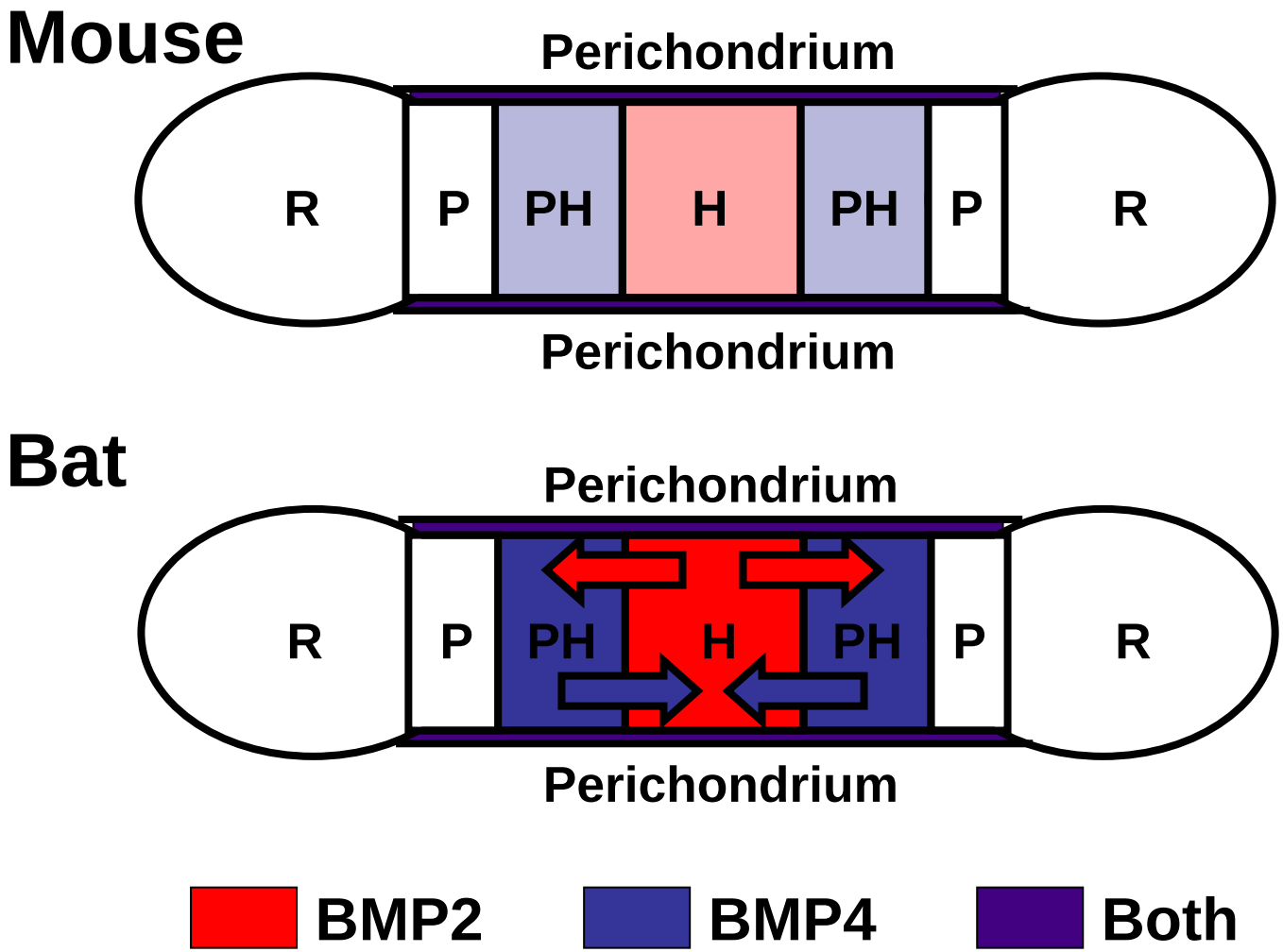


Relative size of the hypertrophic zone can be changed by application of either *Bmp* or *Noggin* protein

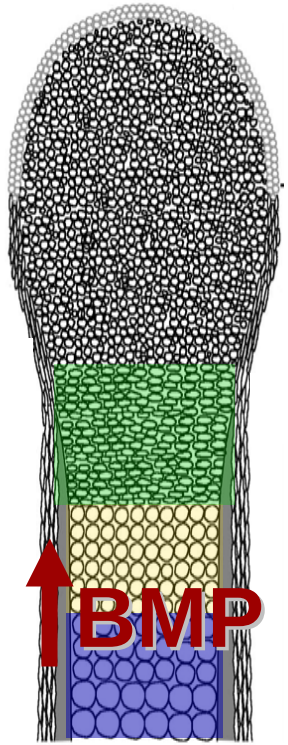


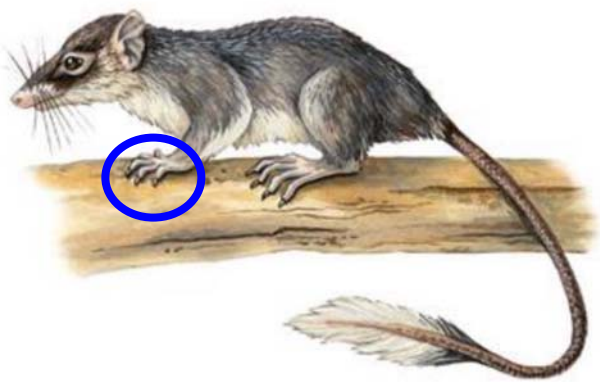
• Differences between *Bmp* & *Bmp* Control and *Noggin* & *Noggin* Control are statistically significant (p-values = 0.028 and 0.046, respectively)

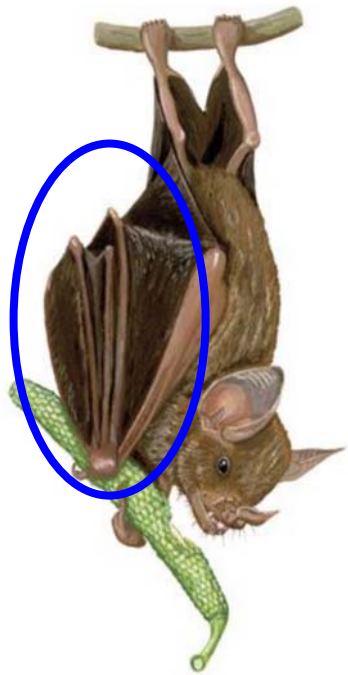
Did a change in *Bmp* expression stimulate digit elongation?



Conclusion

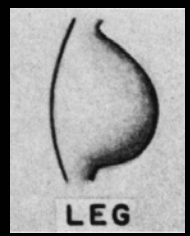






Chicken Embryo

In ovo surgical and molecular experiments



stage 20 chick embryo

BMP signaling is required for interdigital cell death

Molecular manipulation in the chick embryo

* Block BMP signaling - inhibit cell death

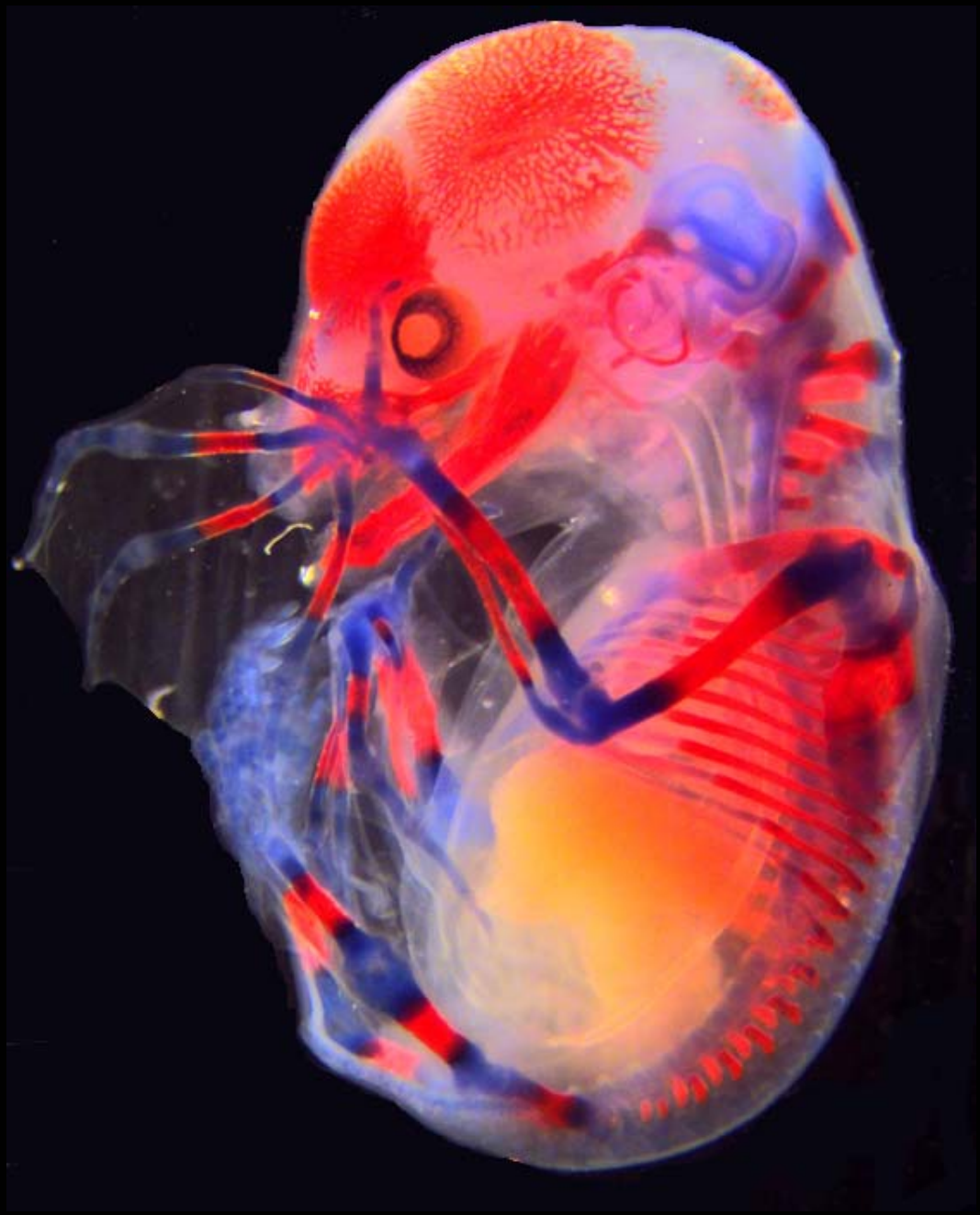
* Activate BMP signaling - premature cell death

Zou and Niswander 1996, Science 272, 738-741
Pizette et al. 1999, Development 126, 883-894

Bat Wing Development

How is the webbing between the digits maintained in bat wings?

Scott Weatherbee



Bat

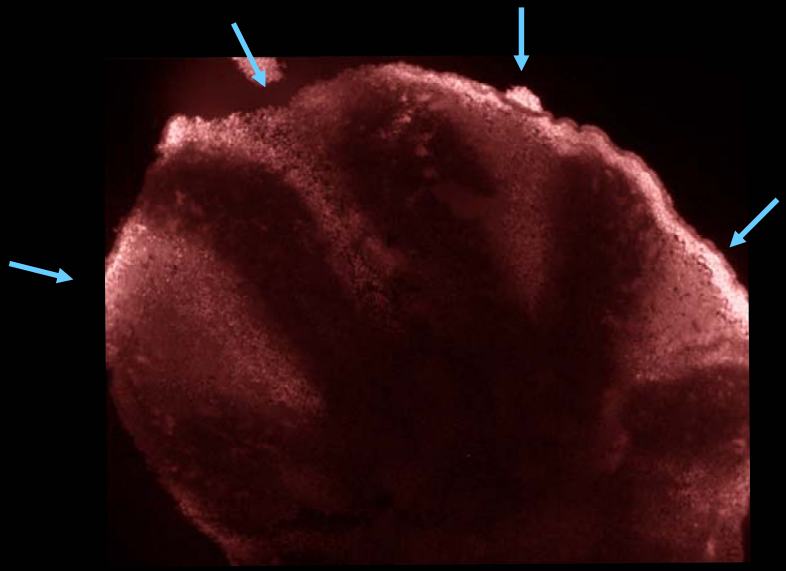
Molecular Evolution

Scott Weatherbee
Karen Sears

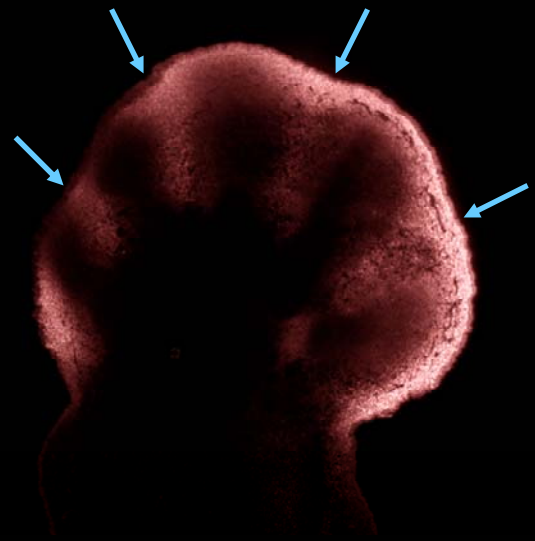
MD Anderson Cancer Center
Richard Behringer
Chris Cretekos

SUNY Downstate
John Rasweiler

BMP signaling occurs in the bat interdigits



Forelimb
webbing retained



Hindlimb
webbing removed

Similar levels of BMP target (anti-Msx1/2 antibody) in forelimb and hindlimb

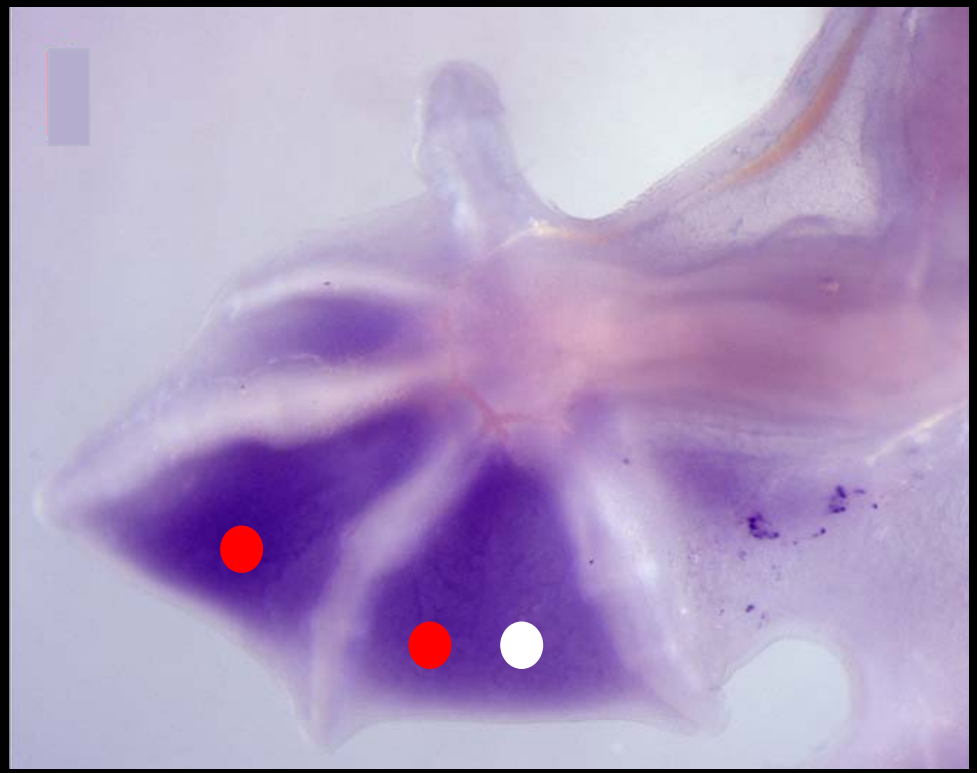
Fgf8 is expressed in bat interdigital tissue

scale bars = 0.5mm

Webbing is retained by attenuation of BMP signaling and acquisition of a novel domain of FGF signaling

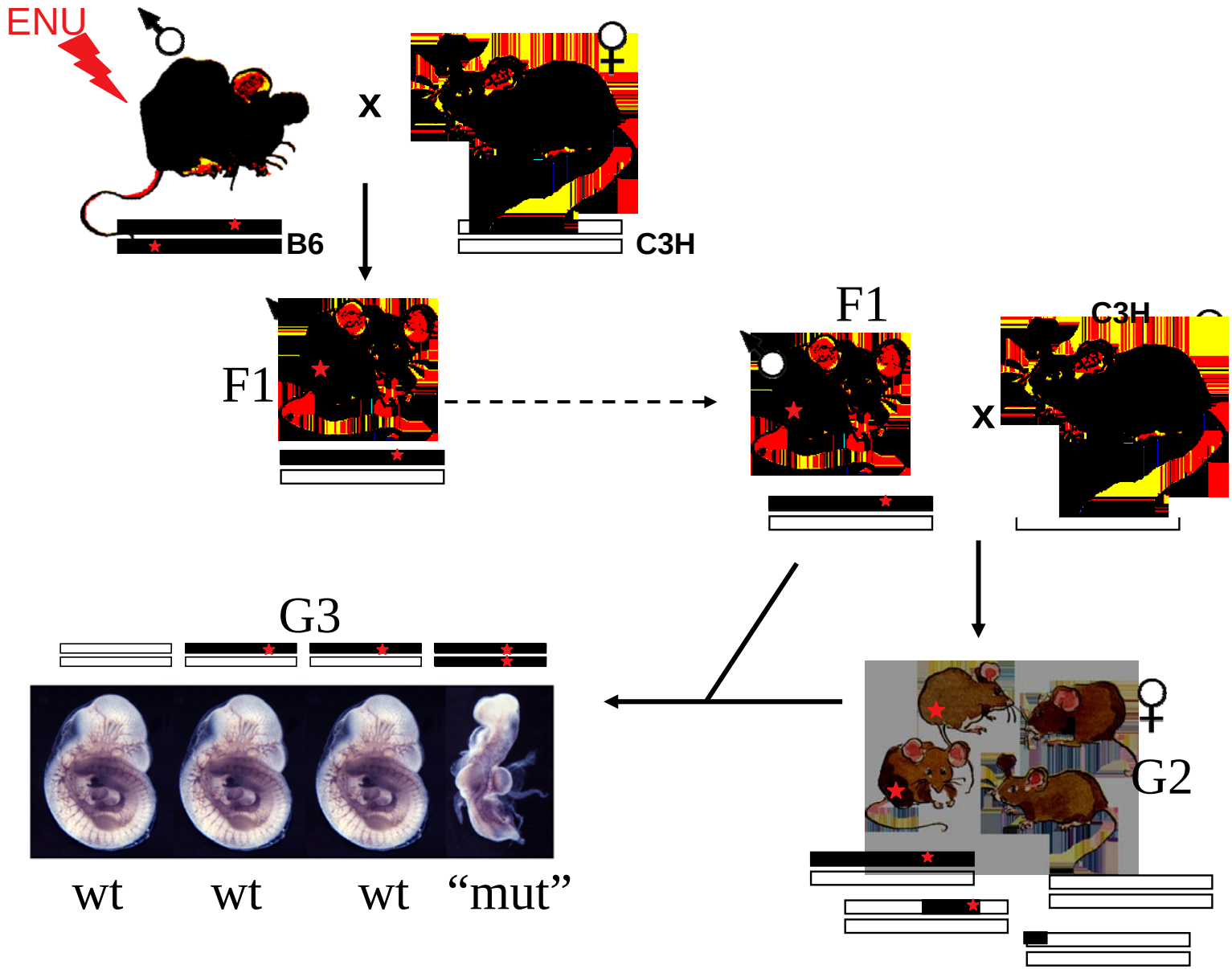
= FGF inhibitor (SU5402)

= BMP protein



Insert beads
Culture for 19-48 hours
Analyze for cell death

Genome-wide forward genetic screen to directly identify genes required for mouse embryonic development

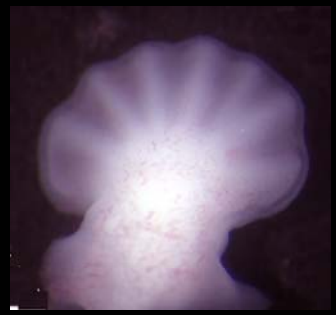


Limb mutations

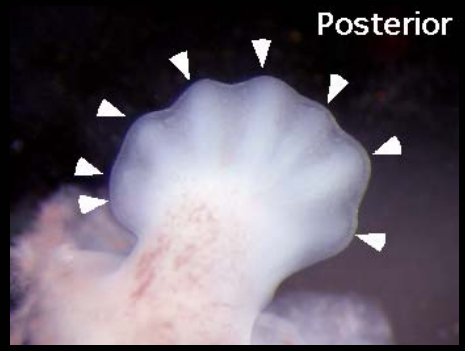
Maria Barna
Tae-Hee Kim
Aimin Liu
Scott Weatherbee

control

Line 37
Line 33D



Line 11A
Cardiac
Eye
Neural



Line V8 (*flexo*)
L-R patterning
Midbrain defect

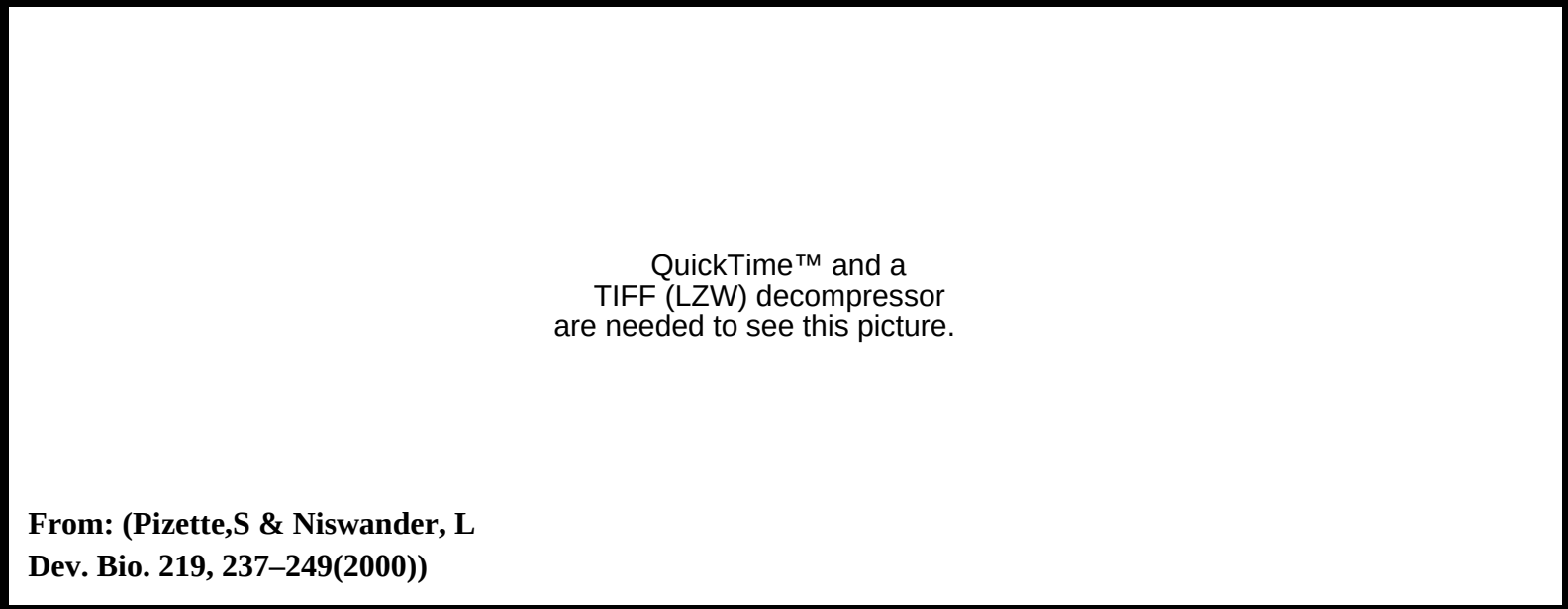
Line 20
Syndactyly
Exencephaly
Lung fusion

control

Line 10
Exencephaly
Lack of eyes

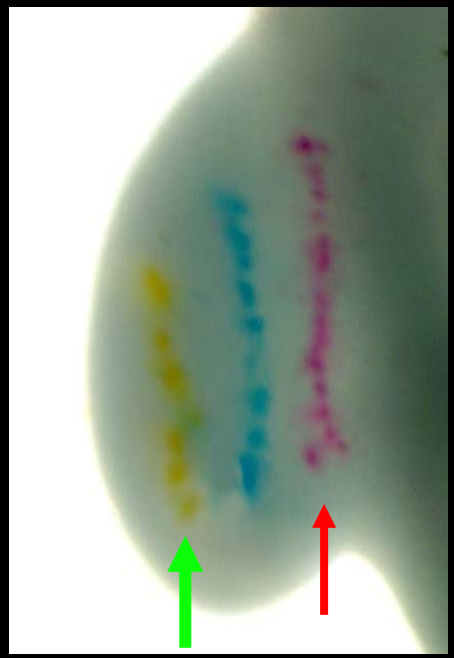
Lines 99K1/43B
Digit loss and fusion
D-V digit duplications
Kidney small/missing
Neonatal lethality

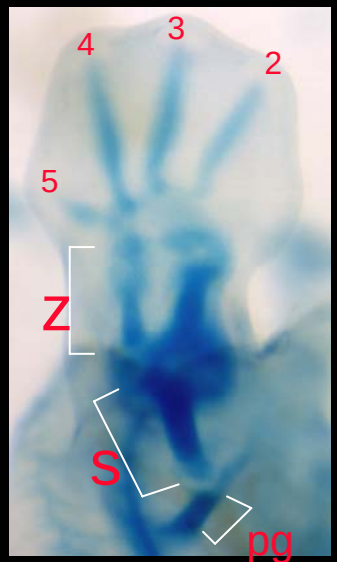
“ There is no thought without an image”
-Aristotle



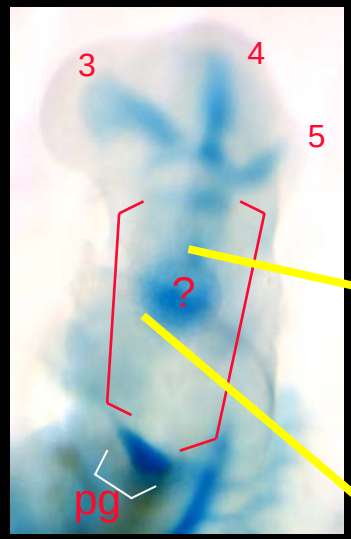
From: (Pizette,S & Niswander, L
Dev. Bio. 219, 237–249(2000))

Functional Evidence for Early Specification





WT



Plzf -/-Gli3-/-

Gdf5 Expression