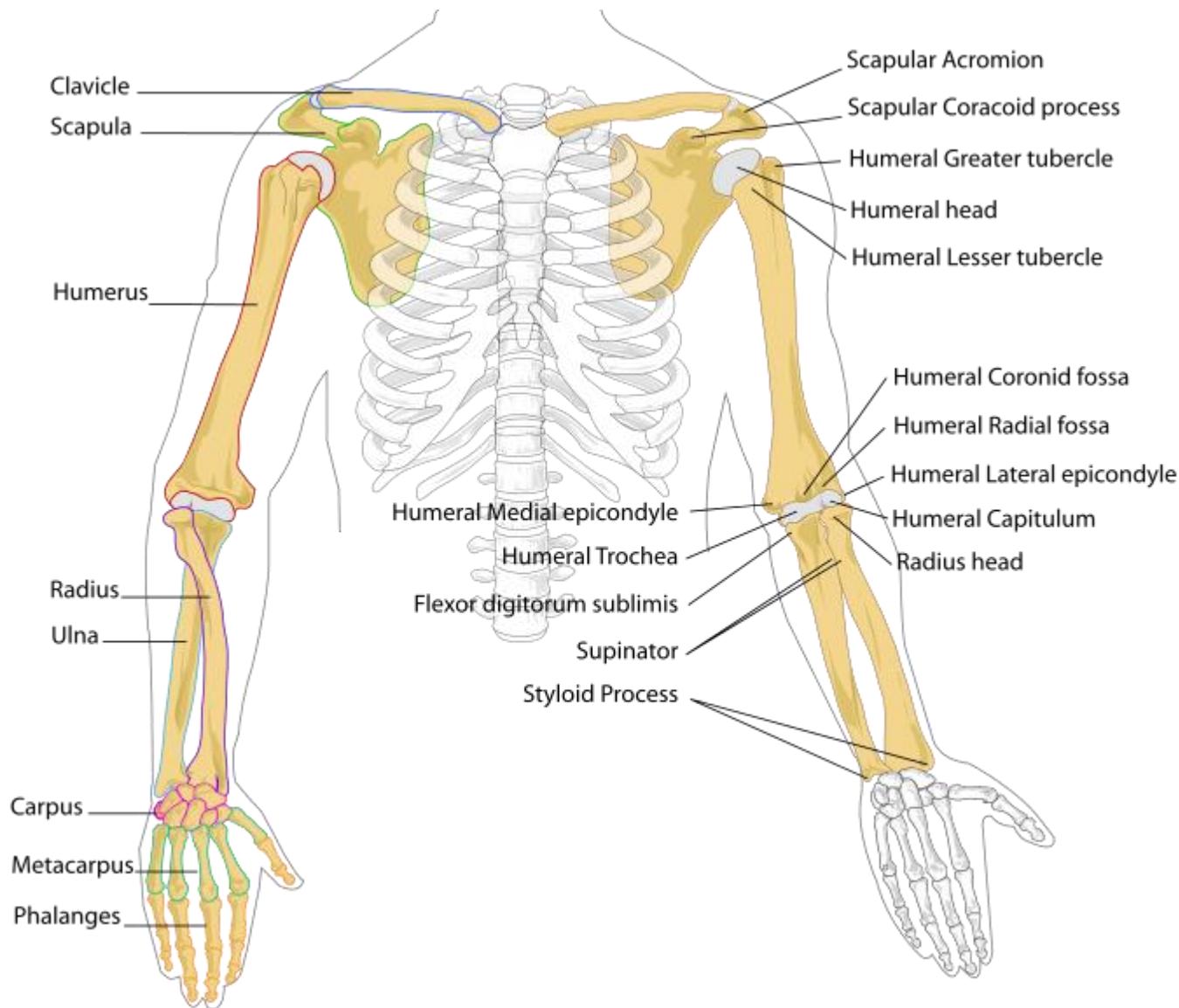


Model organisms and developmental biology

仲寒冰

zhong.hb@sustc.edu.cn

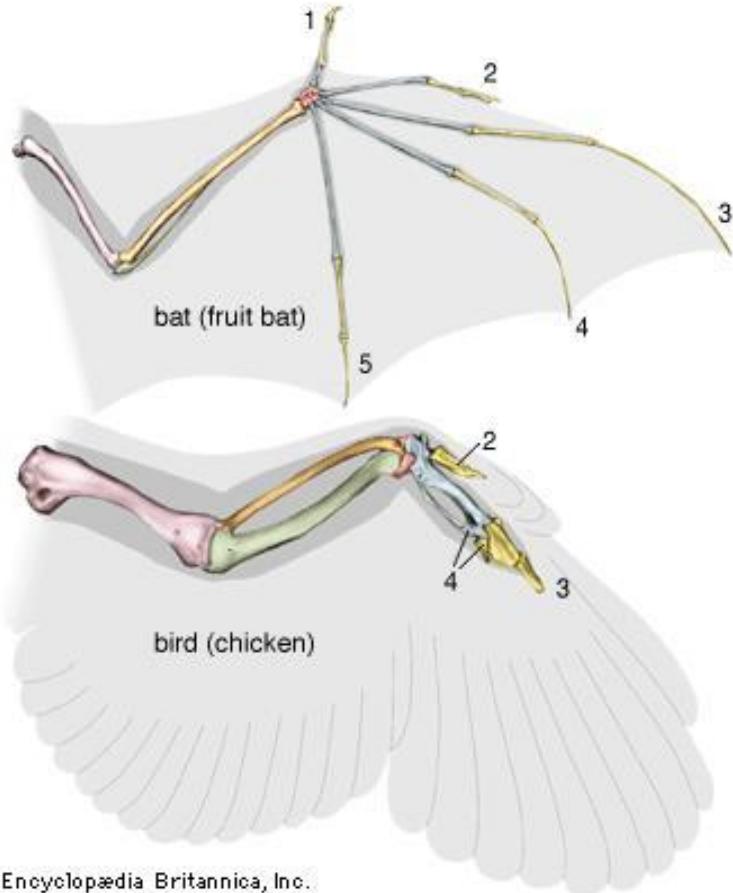
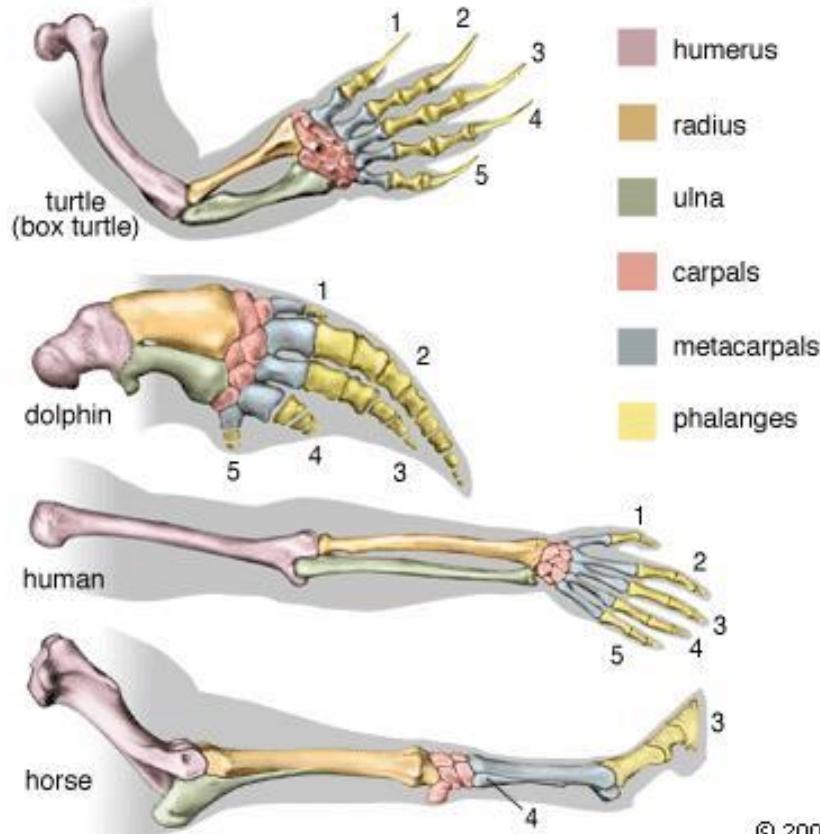
Human forelimb skeleton



Homologies of the forelimb among vertebrates

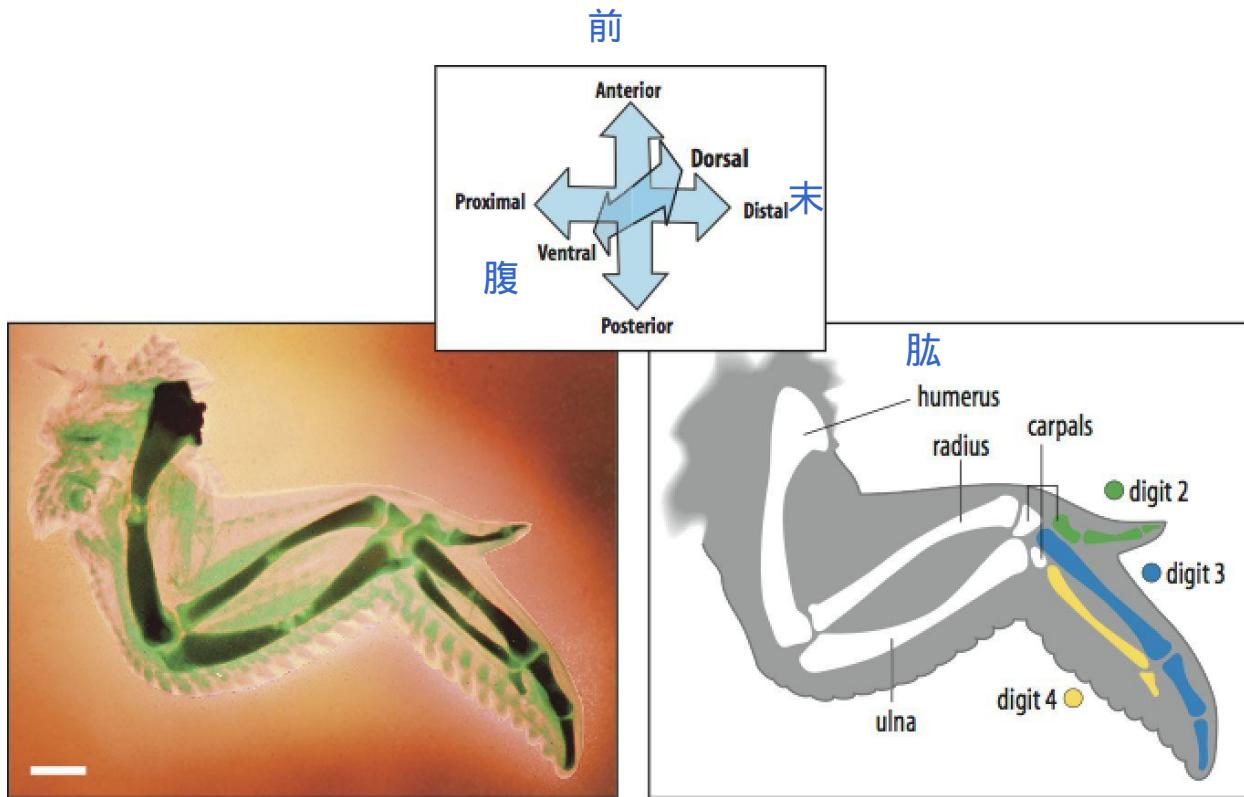
脊椎

Homologies of the forelimb in six vertebrates

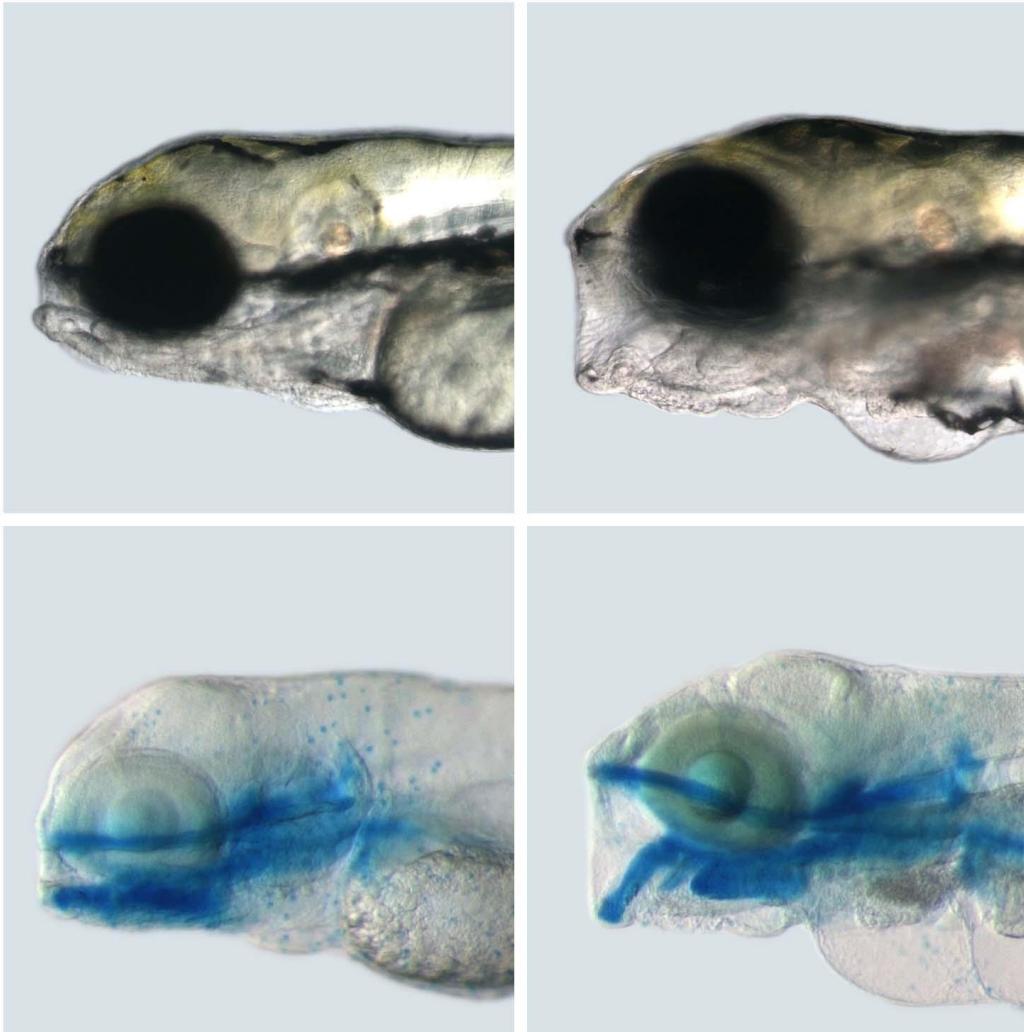


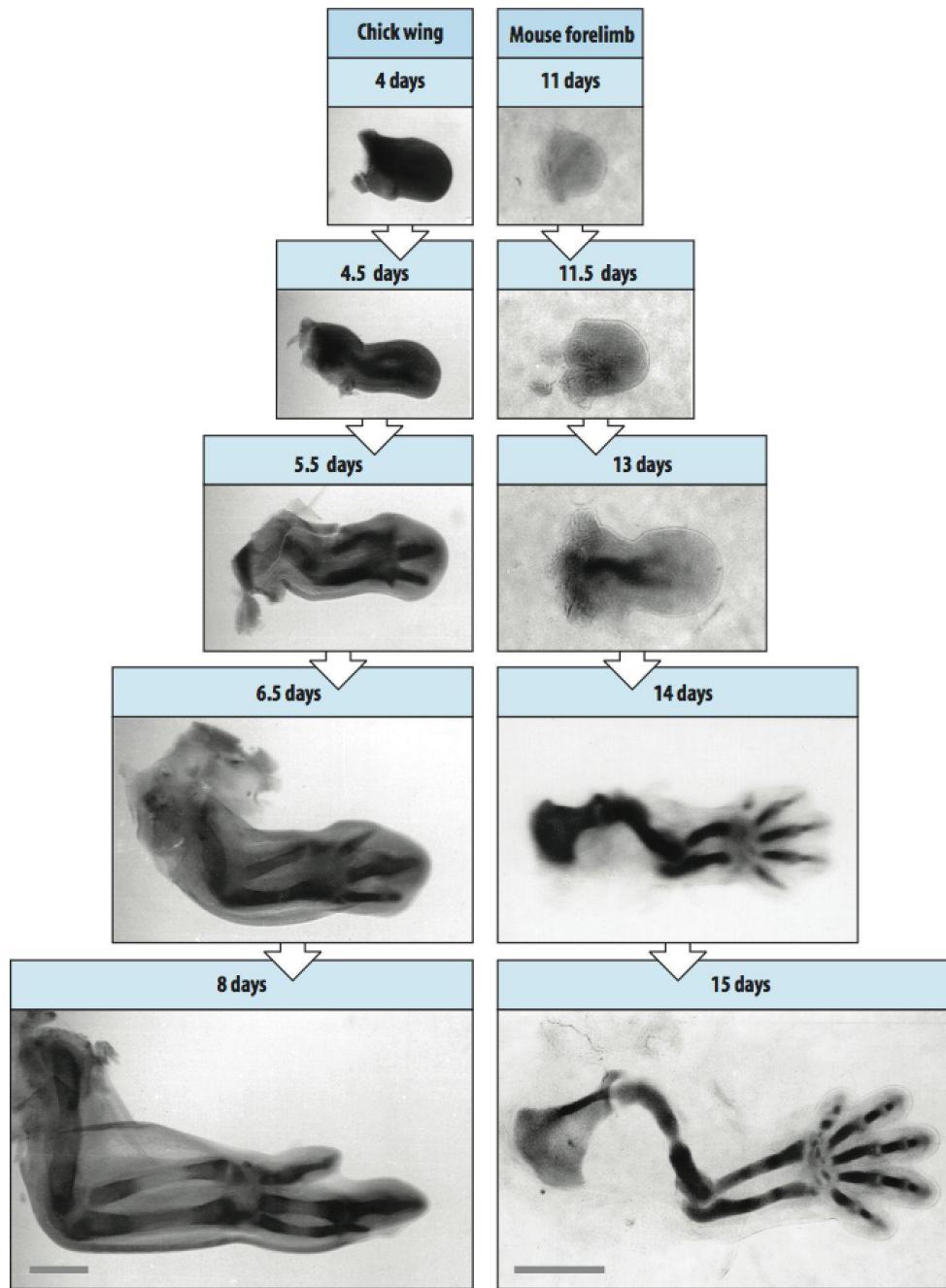
© 2005 Encyclopædia Britannica, Inc.

The chick wing

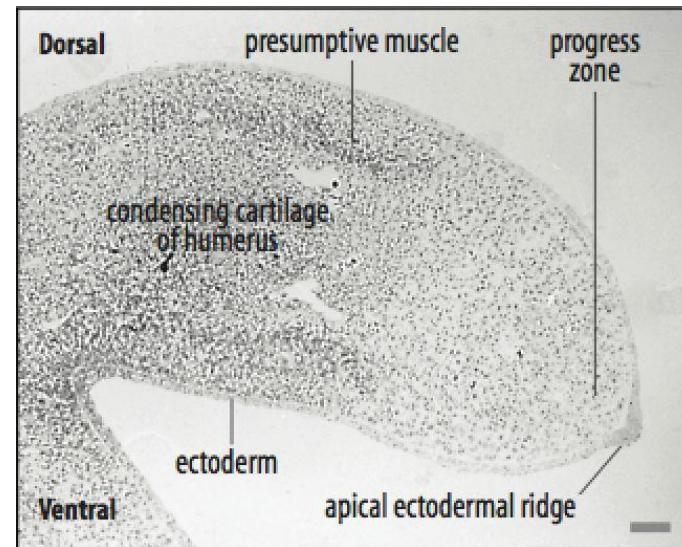
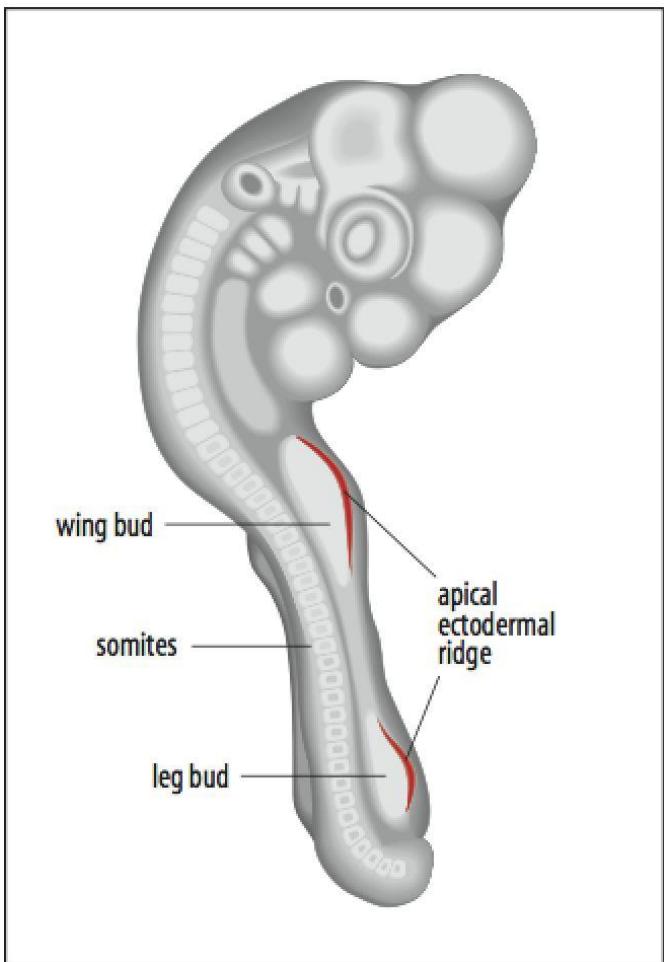


Alcian blue staining of cartilage





The limb buds of the chick embryo of day 3

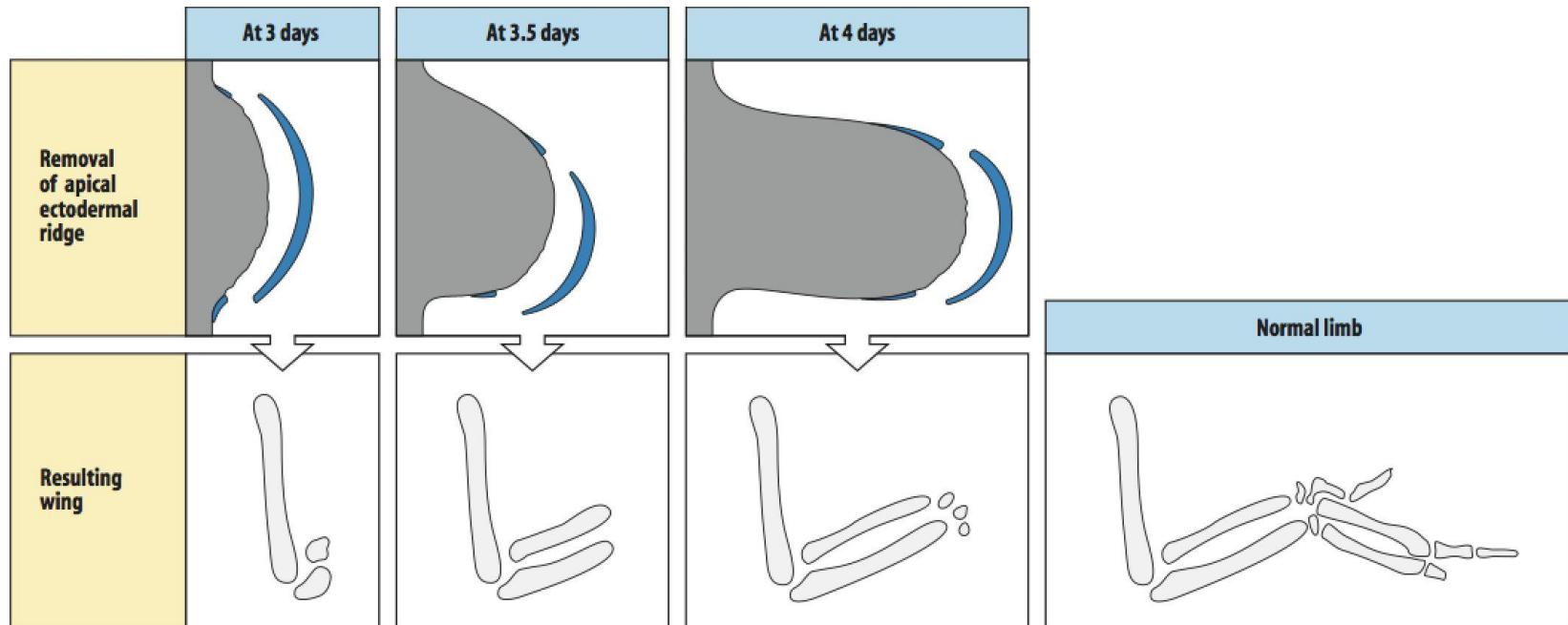


A chick limb bud at 4.5 days, showing the apical ectodermal ridge

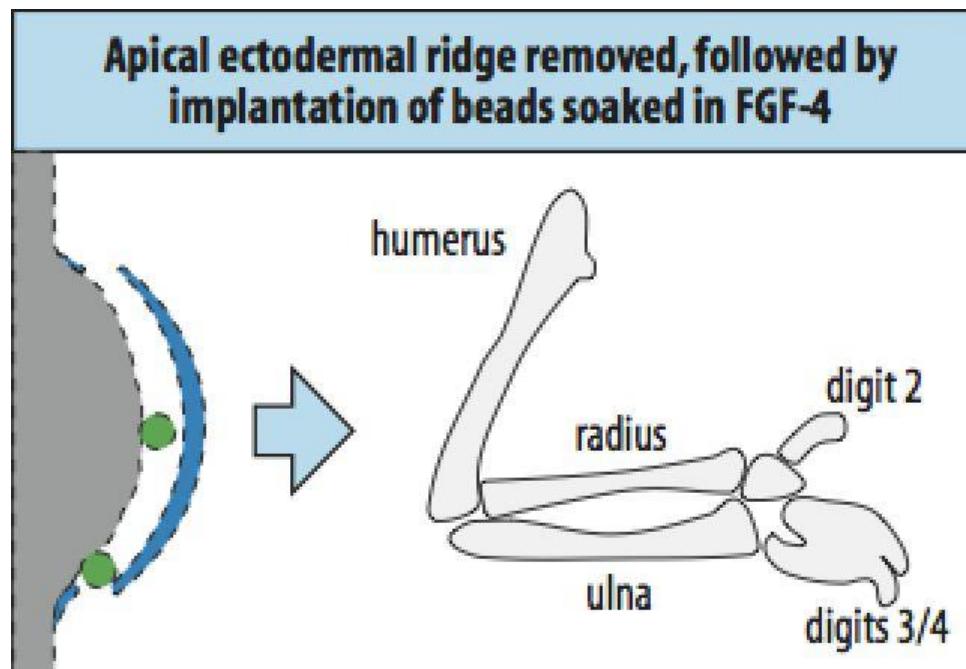


Scale bar = 0.1mm

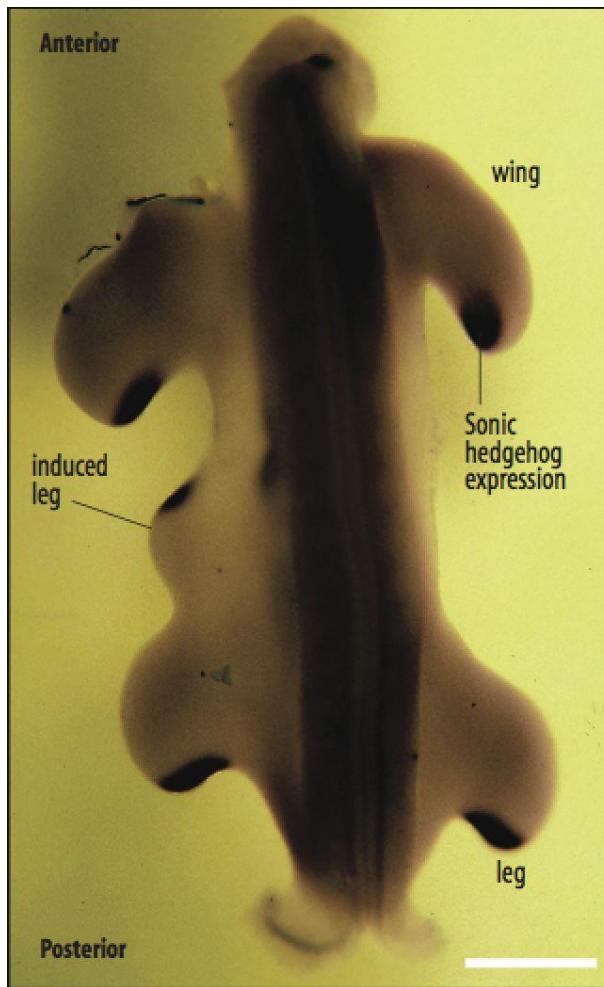
The apical ectodermal ridge is required for proximo-distal development



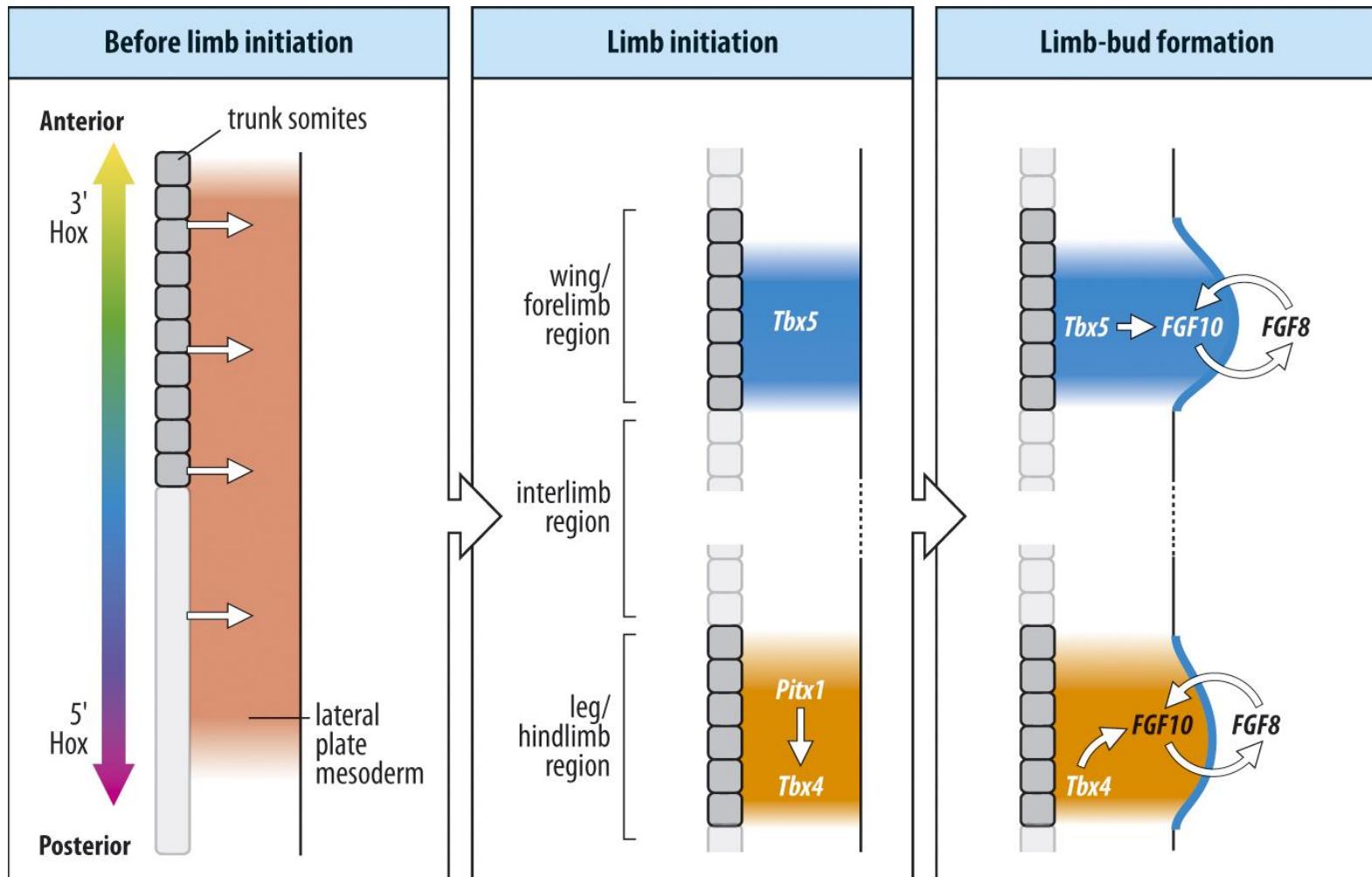
FGF-4 or FGF-8 can substitute for the apical ectodermal ridge



Result of local application of FGF-4 to the flank of a chick embryo



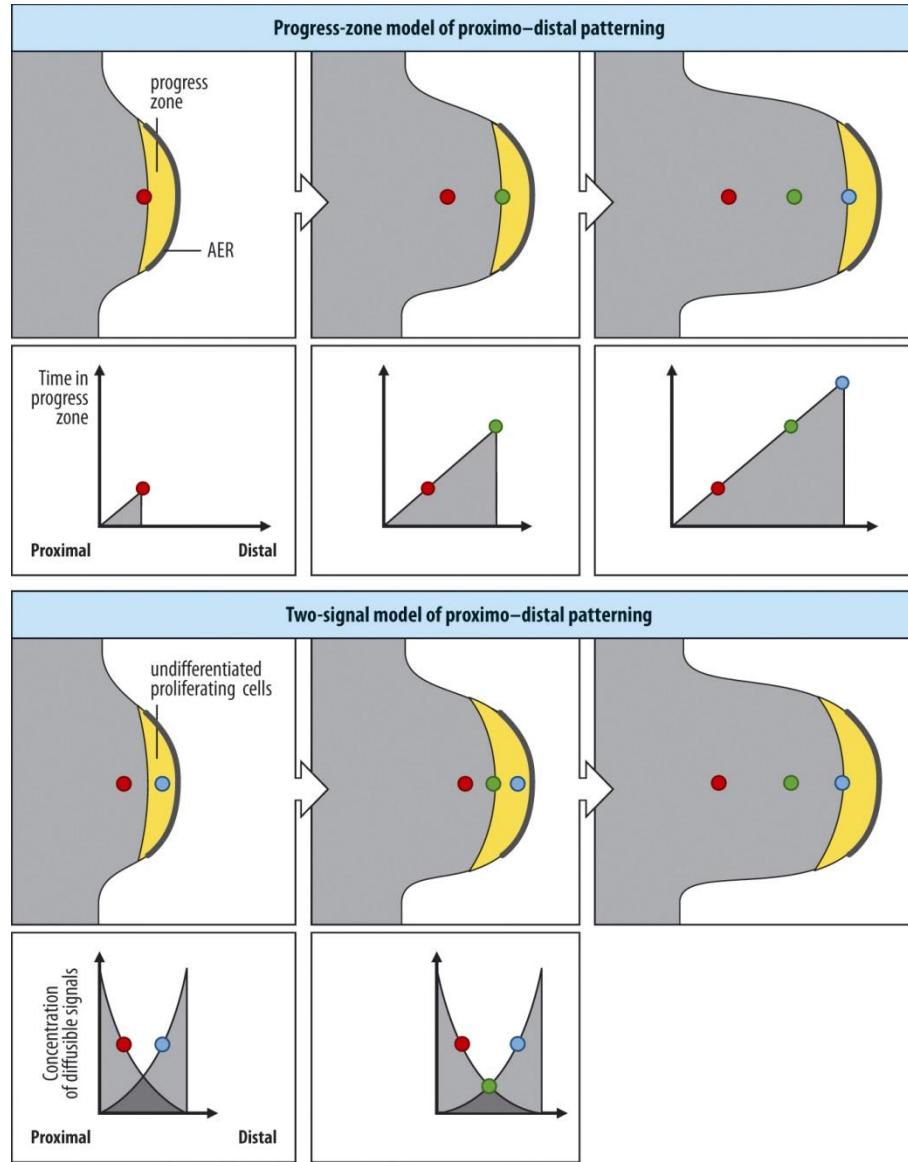
Schematic diagram of limb initiation in chick and mouse



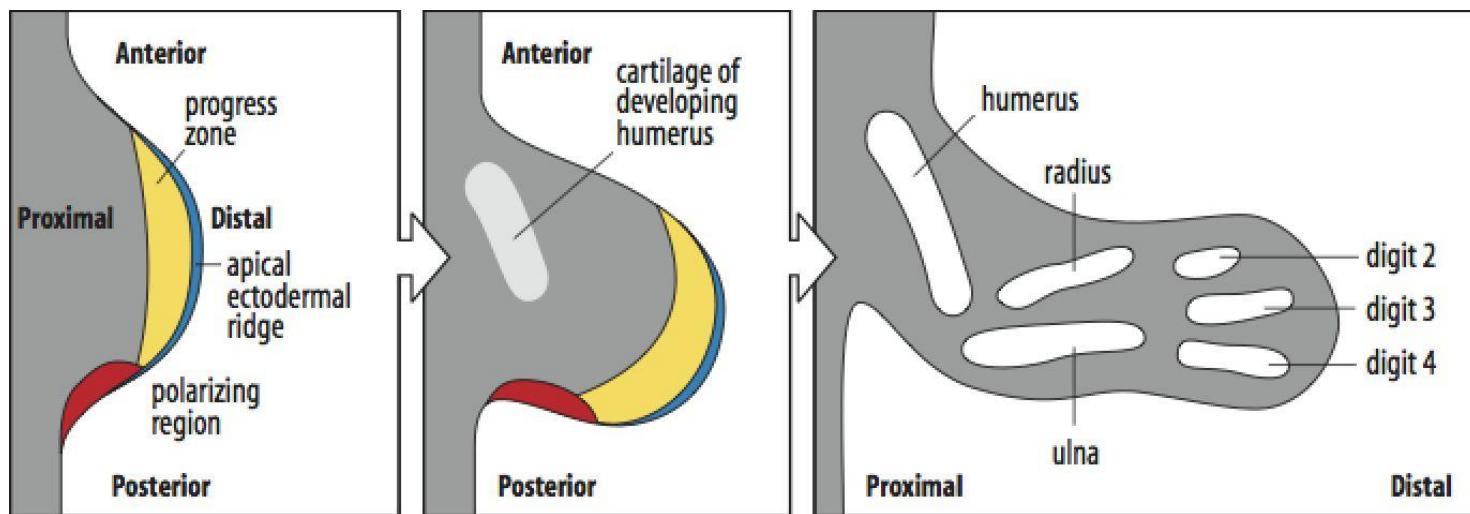
Two popular, but not perfect models

- The timing model, which proposes that proximo-distal patterning is specified by the length of time cells spend in the zone of undifferentiated cells at the tip of the limb.
- The two-signal model, which involves interaction of retinoic acid and FGF.

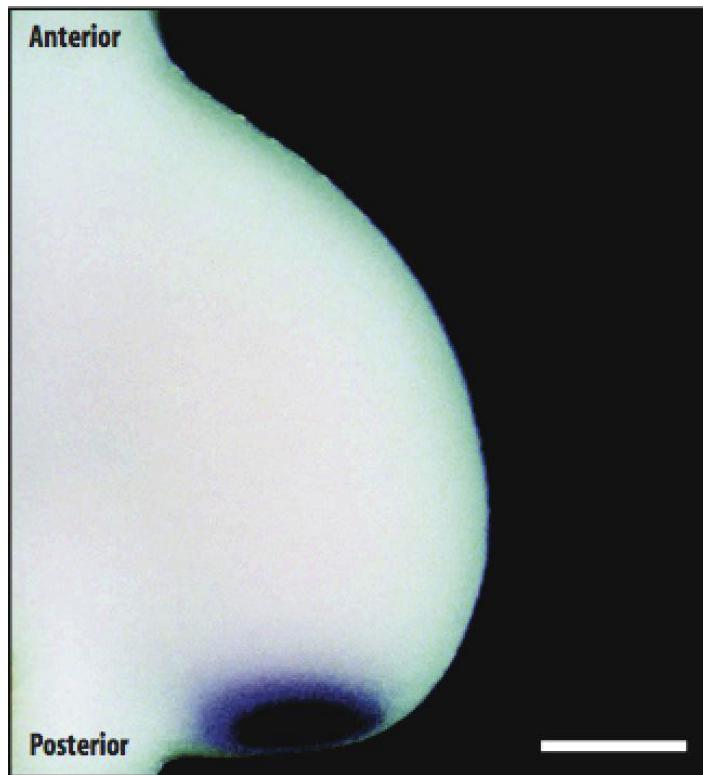
The timing model and the two-signal model



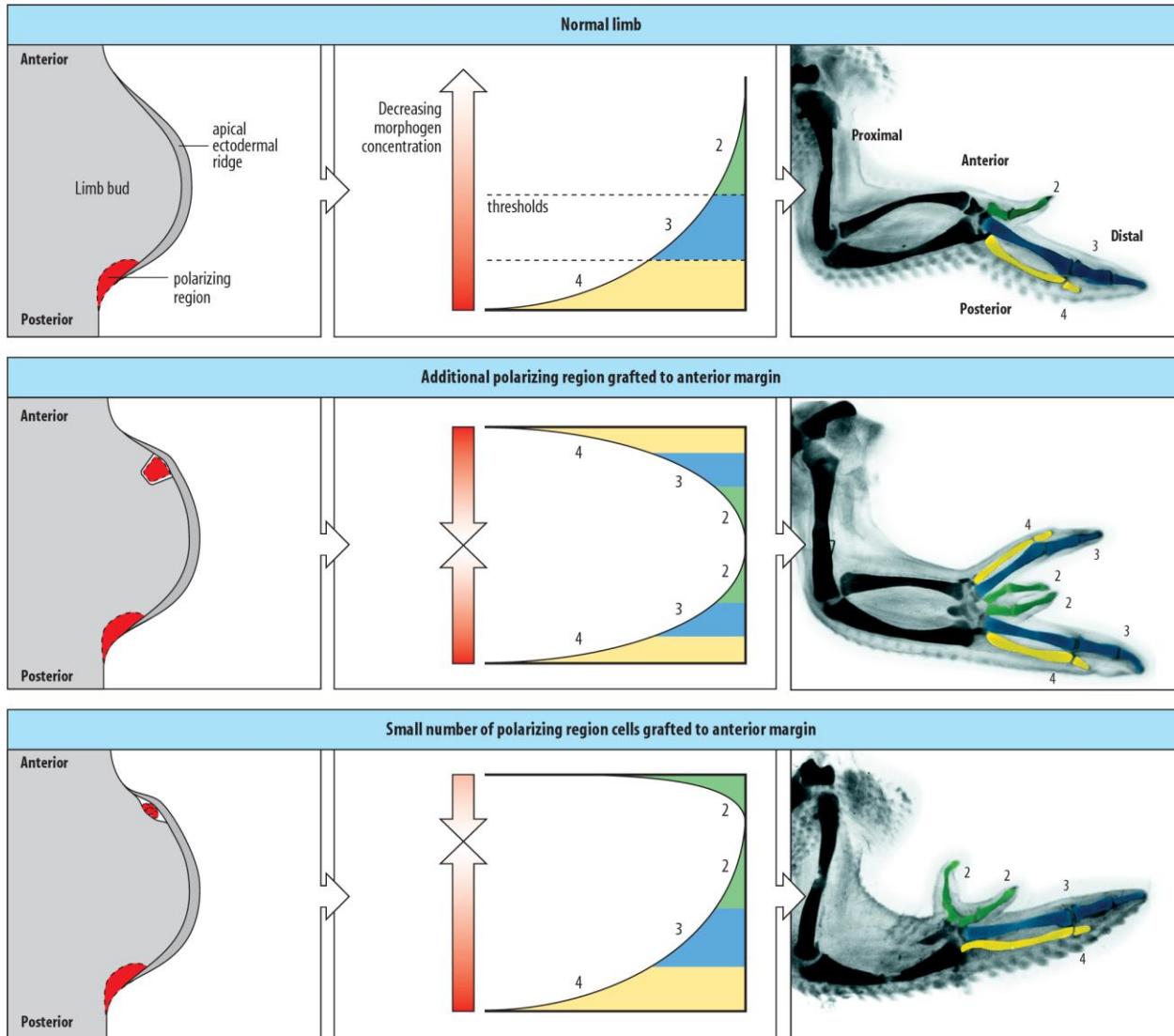
Cell needs to acquire positional values along both PD and AP axes



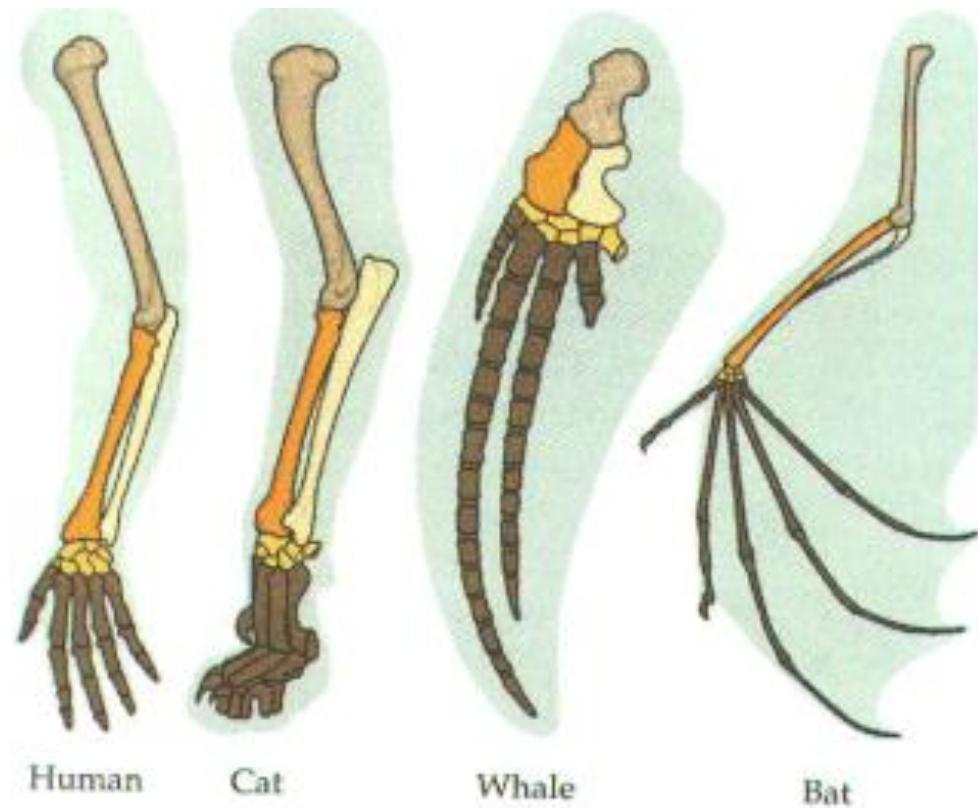
AP axes of limb



The polarizing region can specify pattern along the AP axis



Cat forelimb



The most famous polydactyly cat tribe inhabits Ernest Hemingway's old home in Key west



The polydactylous mouse mutant *Sasquatch*

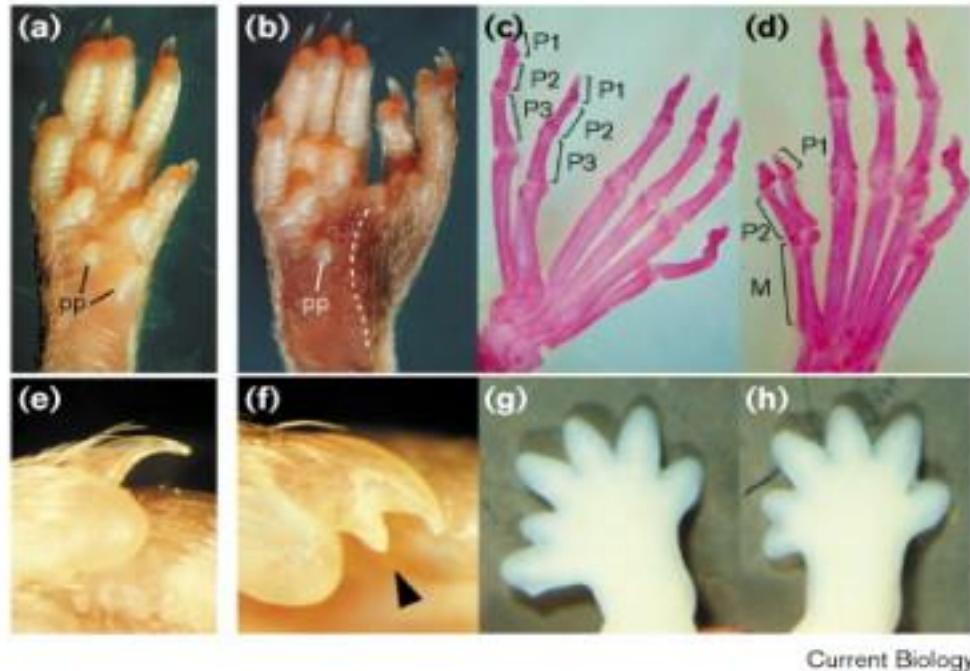


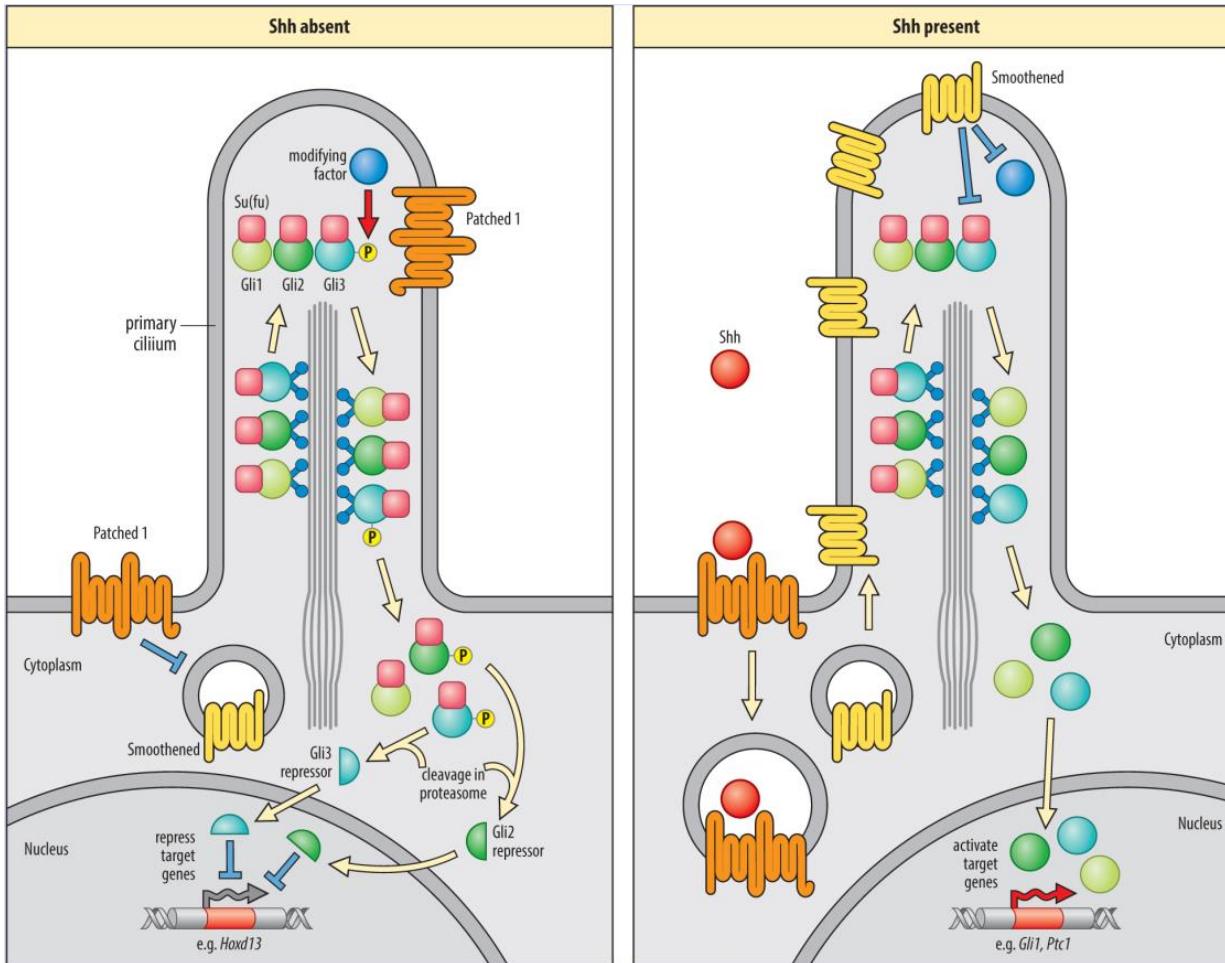
Figure 1 Morphological analysis of limb phenotypes in *Ssq* mutants. (a,b) Comparison of the ventral surface of the adult hindfoot between (a) wild-type and (b) *Ssq*/+ mice revealed preaxial polydactyly. The ventral surface underlying the supernumerary ...*Identification of Sonic hedgehog as a candidate gene responsible for the polydactylous mouse mutant Sasquatch*

James Sharpe , Laura Lettice , Jacob Hecksher-Sørensen , Margaret Fox , Robert Hill , Robb Krumlauf

Zone of polarizing activity regulatory sequence (ZRS)

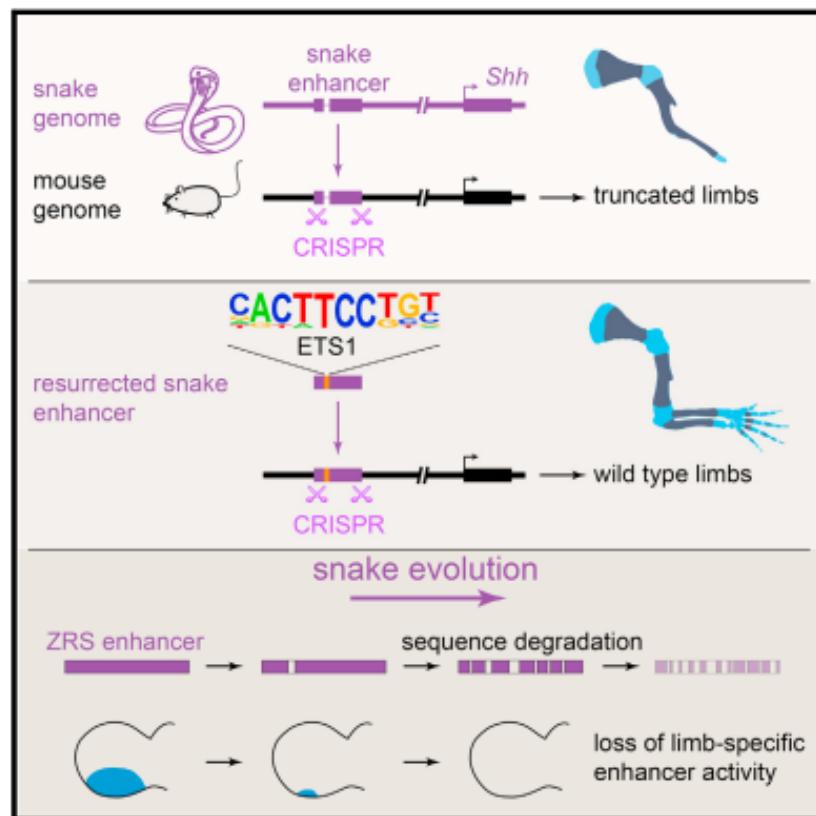
- ZRS is a long-range cis-regulatory region governing limb specific Shh expression. It was first identified in mouse.
- ZRS is 1 Mb away from the Shh gene. It is within intron 5 of the *Lmbr1* gene
- The Hemingway's cat tribe carries a ZRS mutation, a single-nucleotide substitution.

Hedgehog pathway



Progressive Loss of Function in a Limb Enhancer during Snake Evolution

Graphical Abstract



Authors

Evgeny Z. Kvon, Olga K. Kamneva,
Uirá S. Melo, ..., Diane E. Dickel,
Len A. Pennacchio, Axel Visel

Correspondence

lapennacchio@lbl.gov (L.A.P.),
avisel@lbl.gov (A.V.)

In Brief

Morphological disappearance of limbs in snakes is associated with sequence changes disrupting the function of a critical limb enhancer.

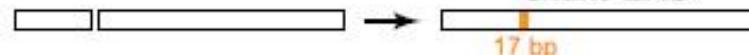
A

17 bp snake-specific deletion

8

Snake ZRS

Resurrected snake ZRS



c

6/6

4/
python

D

A small, white mouse is shown from a side profile, facing left. It has pink ears and a pink tail. The background is dark.

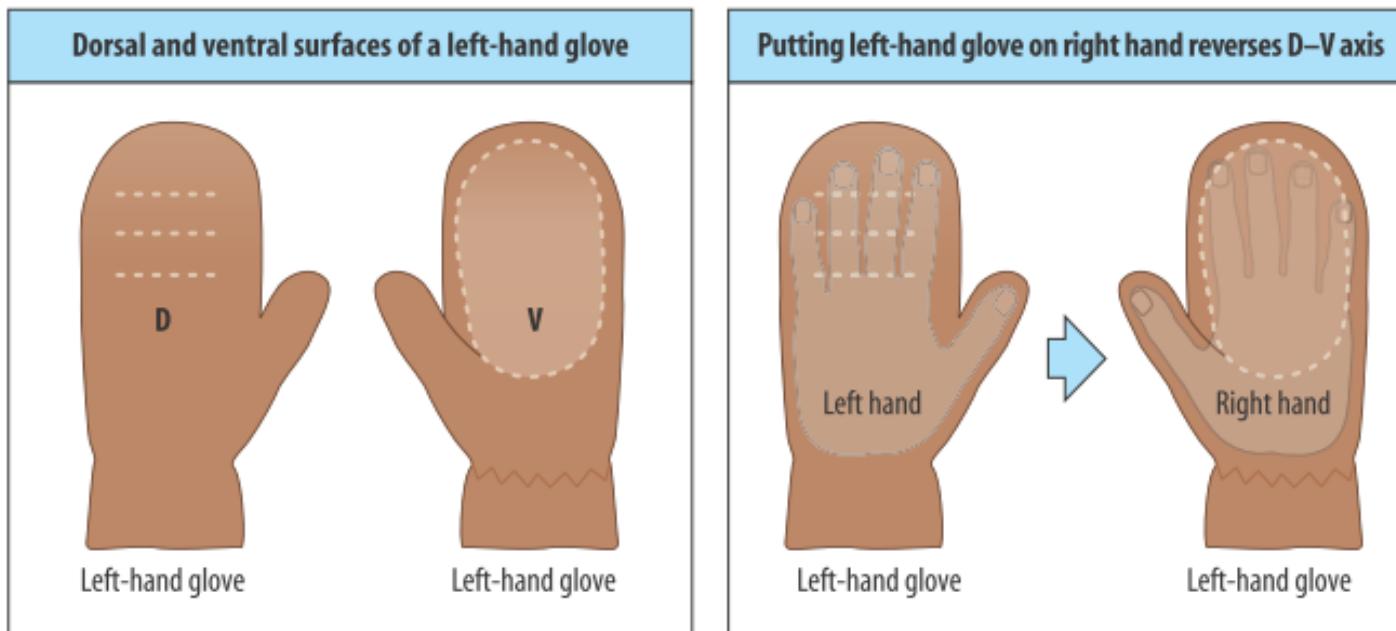
A white mouse is shown from a side profile, walking towards the left on a dark, textured surface. The word "python" is written in white text at the bottom right of the image.

E

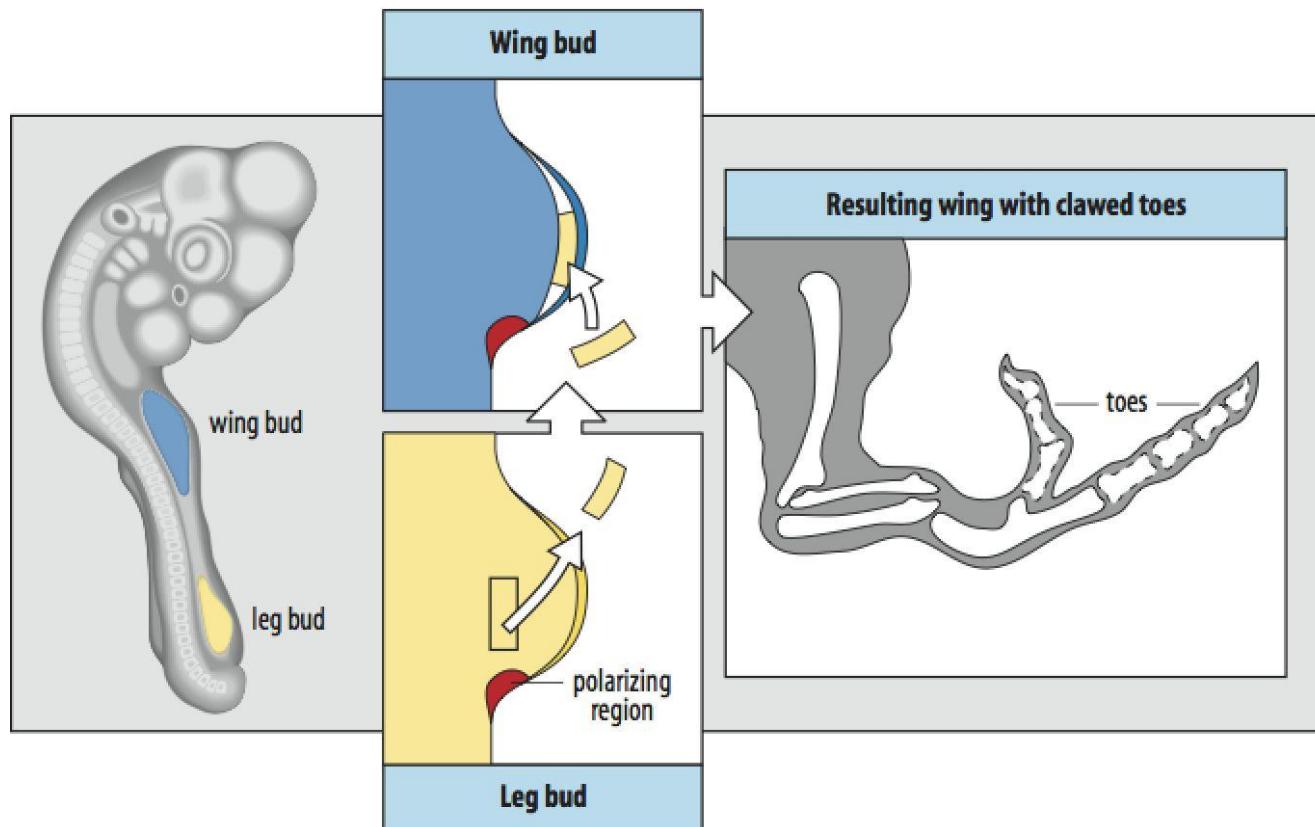
A micrograph of a Python embryo at stage E18.5, showing the development of the nervous system. The neural folds are closing to form the neural tube, with blue staining indicating the dorsal side.

3/3

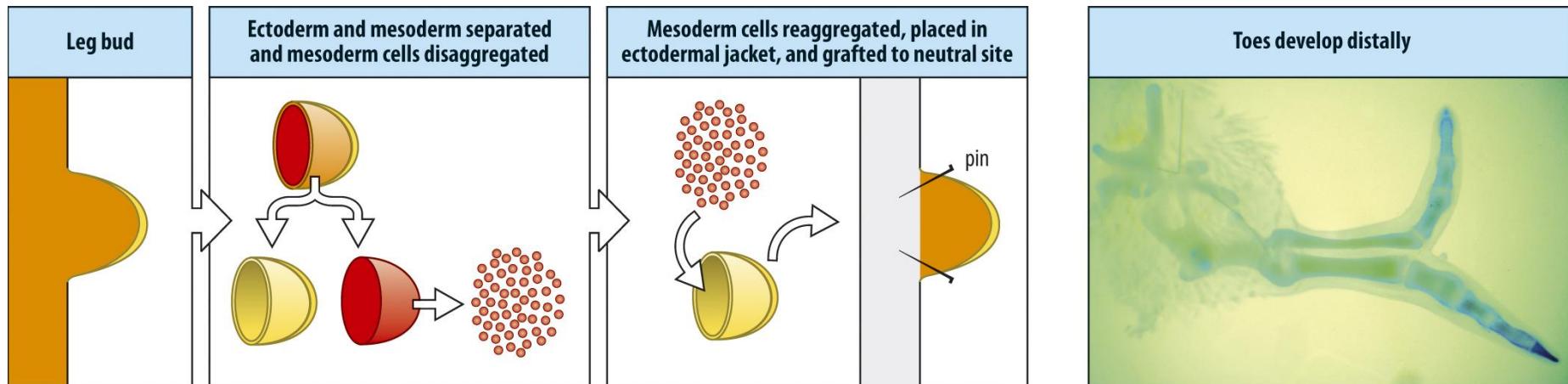
The DV axis of the limb is controlled by the ectoderm



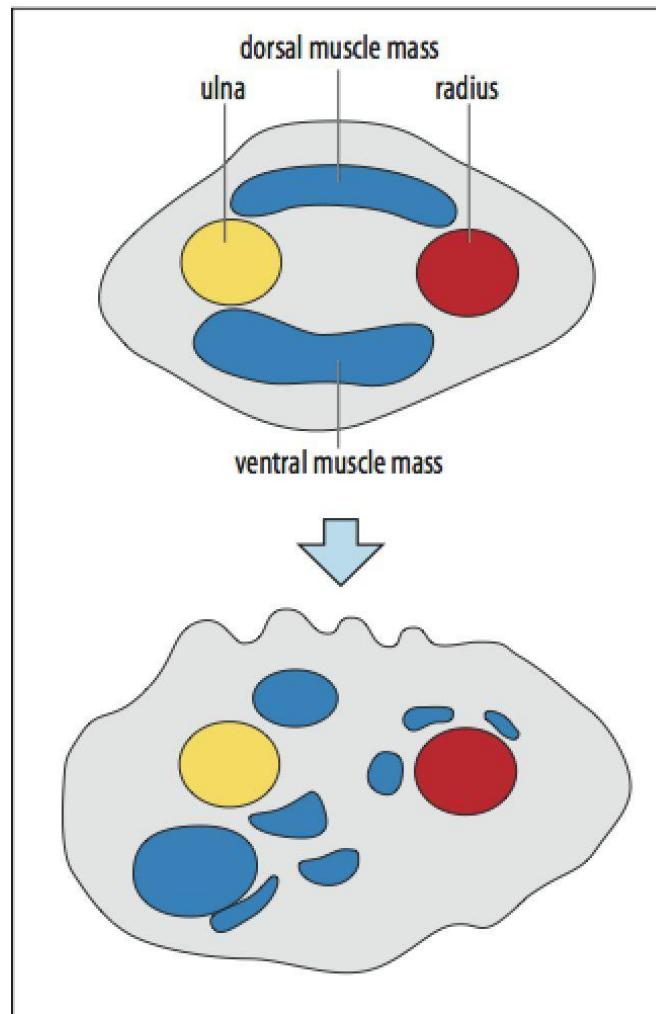
Different interpretations of the same positional signals give different limbs



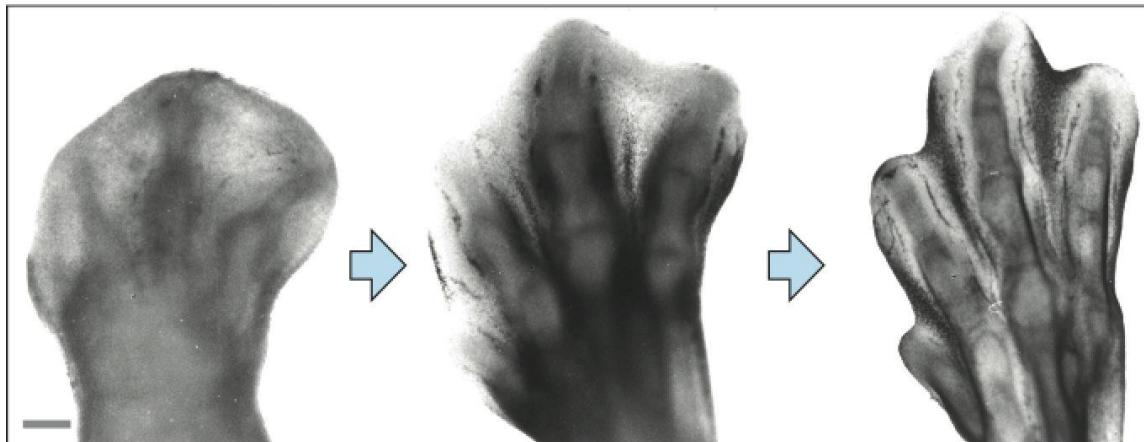
Self-organization may be involved in the development of the limb bud



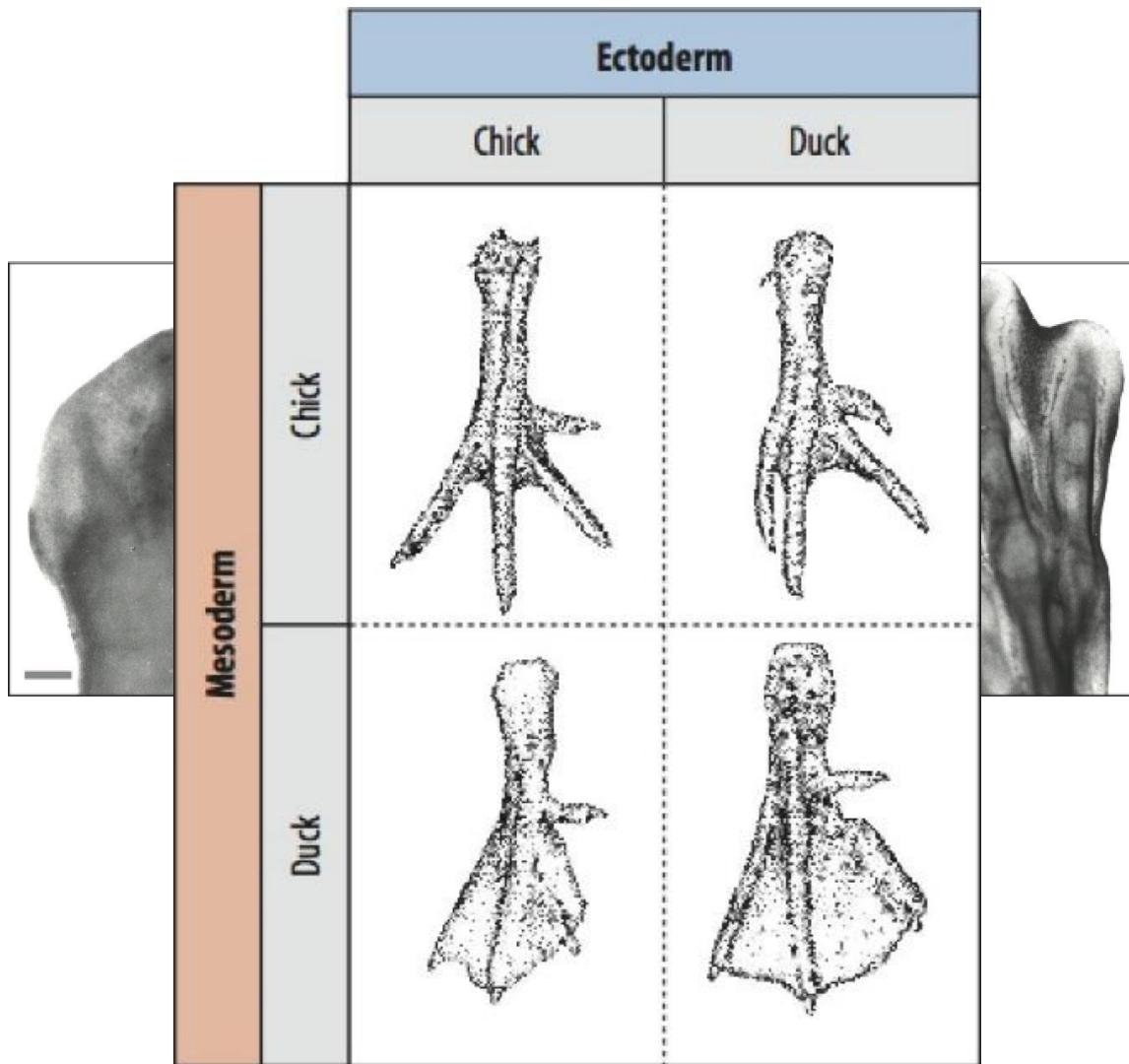
Limb muscle is patterned by the connective tissue



Separation of the digits is the result of programmed cell death



Separation of the digits is the result of programmed cell death



Digits and fin rays share common developmental histories

Tetsuya Nakamura^{1*}, Andrew R. Gehrke^{1*}, Justin Lemberg¹, Julie Szymaszek¹ & Neil H. Shubin¹

Understanding the evolutionary transformation of fish fins into tetrapod limbs is a fundamental problem in biology¹. The search for antecedents of tetrapod digits in fish has remained controversial because the distal skeletons of limbs and fins differ structurally, developmentally, and histologically^{2,3}. Moreover, comparisons of fins with limbs have been limited by a relative paucity of data on the cellular and molecular processes underlying the development of the fin skeleton. Here, we provide a functional analysis, using CRISPR/Cas9 and fate mapping, of 5' *hox* genes and enhancers in zebrafish that are indispensable for the development of the wrists and digits of tetrapods^{4,5}. We show that cells marked by the activity of an autopodial *hoxa13* enhancer exclusively form elements of the fin fold, including the osteoblasts of the dermal rays. In *hoxa13* knockout fish, we find that a marked reduction and loss of fin rays is associated with an increased number of endochondral distal radials. These discoveries reveal a cellular and genetic connection between the fin rays of fish and the digits of tetrapods and suggest that digits originated via the transition of distal cellular fates.

The origin of tetrapod limbs involved profound changes to the distal skeleton of fins. Fin skeletons are composed mostly of fin rays⁶, whereas

hox paralogues—individually and in combination—to the adult fin phenotype and the origin of cells that give rise to the distal fin skeleton. While previous studies have shown that osteoblasts of the fin rays in the caudal fin of zebrafish are derived from either neural crest or paraxial mesoderm, the source of osteoblasts in pectoral fin rays is currently unknown^{22–24}. Consequently, it remains unclear where the cellular and genetic markers of the autopod of the tetrapod limb reside in fish fins.

In order to bridge these gaps in knowledge, we followed the fates of cells marked by early and late phase *hox* enhancers to adult stages in pectoral fins. In addition, we engineered zebrafish that completely lacked each individual *hoxa13* gene, and bred stable lines with multiple gene knockout combinations of *hox* paralogues. The power of these experiments is twofold: 1) to our knowledge, they represent the first functional analyses of *hox* activity in fins, and 2) they enable a direct developmental comparison to experiments performed in tetrapod limbs.

We performed *in situ* hybridization of *hoxa13a*, *hoxa13b*, and *hoxd13a* genes from 48–120 h post fertilization (hpf) in zebrafish to determine whether active *hox* expression has a role in the development of the pectoral fin fold. *Hoxa13* genes in zebrafish are expressed in the

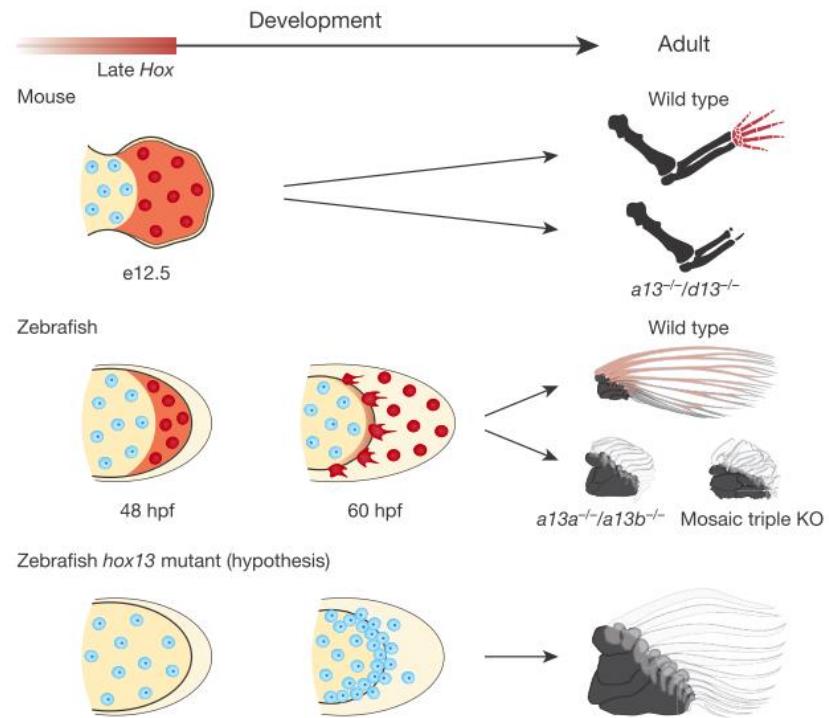
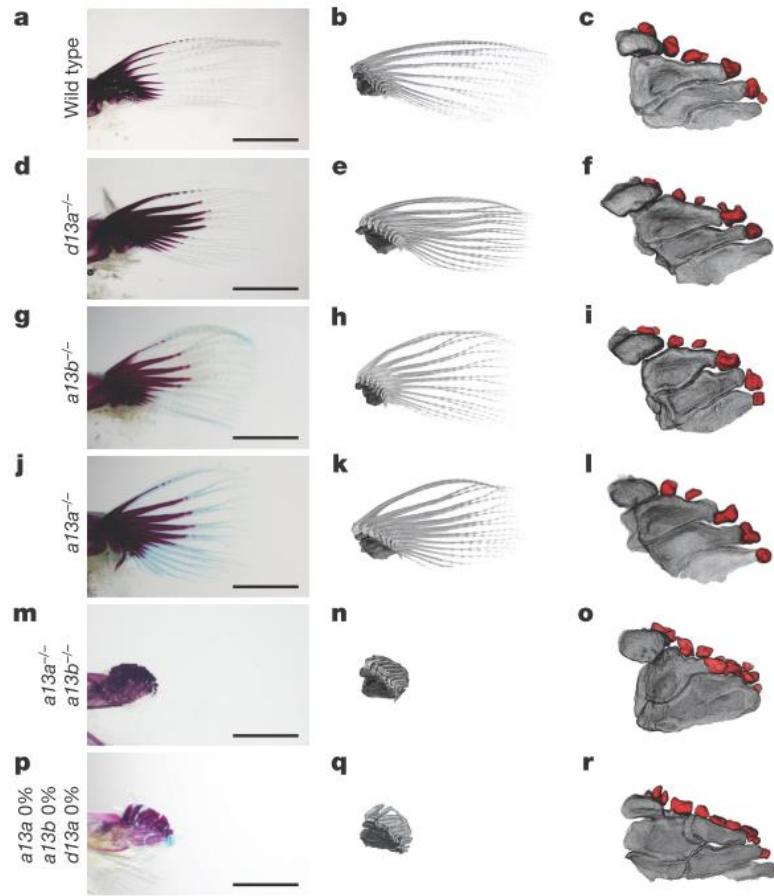
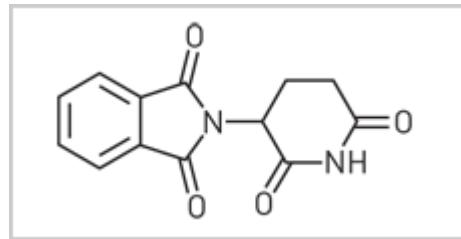


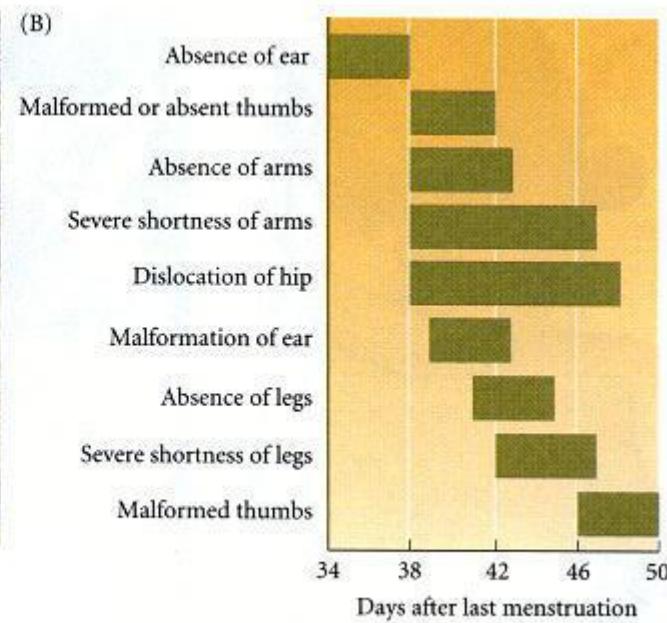
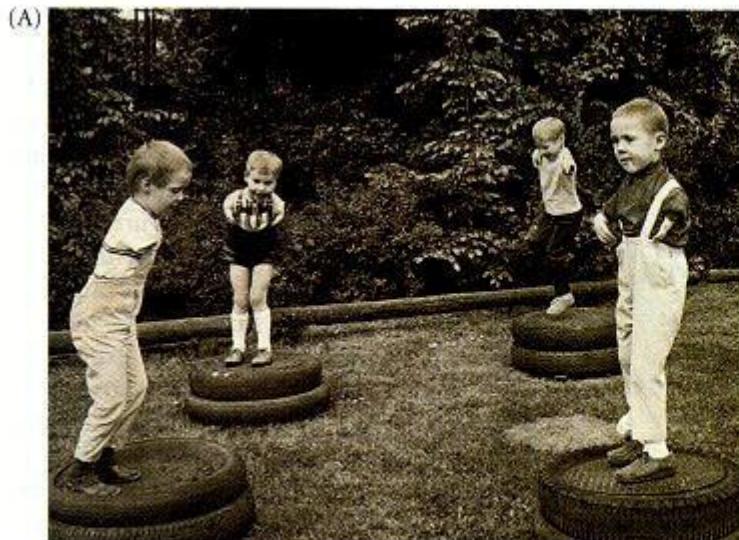
Figure 4 | Shared developmental histories in fin rays and digits. In mice (top row), late phase *Hox* expression (red) marks the distal cells of the limb bud that result in bones of the autopod (wrists and digits). Double knockout of *Hoxa13* and *Hoxd13* results in the loss of the autopod. In zebrafish wild-type fins (middle row), cells marked by late phase *hox* expression (red) end up in the fin fold and within osteoblasts of the

Thalidomide (反应停)



- Developed by German pharmaceutical company Grünenthal.
- Launched on 1st October 1957 as a treatment of morning sickness.
- Withdrawn in 1961 due to severe teratogenicity.
- More than 10,000 children in 46 countries were born with deformities, mainly in Germany, Holland, and Japan.

Deformities



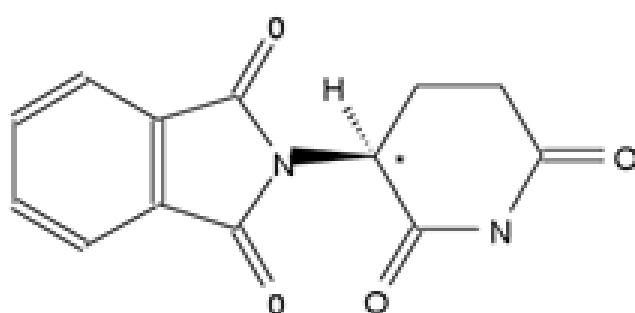
Gilbert et al, Developmental Biology (5th)

FDA blocked sale of Thalidomide in the United States

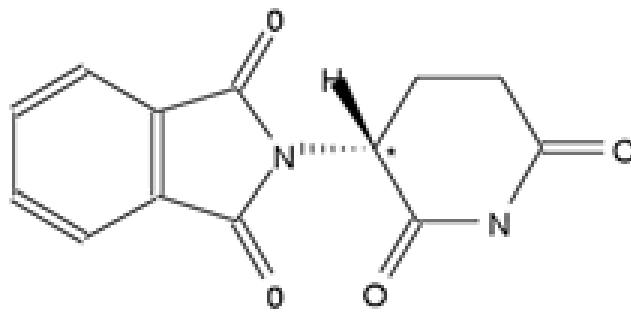


1962: FDA inspector Frances Oldham Kelsey receives an award from President John F. Kennedy for blocking sale of Thalidomide in the United States

One particular optical isomer of thalidomide caused the teratogenicity



Enantiomère (S) : tératogène



Enantiomère (R) : Non-toxique

The non-toxic isomer can be converted to the teratogenic isomer once in the human body.

What's the mechanism?

- The drug is a potent teratogen in zebrafish, chickens, rabbits, and primates including humans, BUT NOT in rats and mice.
- Possible mechanisms: Thalidomide intercalates into DNA in G-C rich regions and inhibits angiogenesis.
- Present primary target: CRBN(cereblon).

RESEARCH ARTICLE

Identification of a Primary Target of Thalidomide Teratogenicity

Takumi Ito,^{1*} Hideki Ando,^{2*} Takayuki Suzuki,^{3,4} Toshihiko Ogura,³ Kentaro Hotta,² Yoshimasa Imamura,⁵ Yuki Yamaguchi,² Hiroshi Handa^{1,2†}

Binding of thalidomide to CRBN and DDB1.

To purify thalidomide-binding proteins, we performed affinity purification using ferrite-glycidyl methacrylate (FG) beads (9). The carboxylic thalidomide derivative FR259625 was covalently conjugated to the beads (fig. S1) and incubated with human HeLa cell extracts (10). After extensive washing, bound proteins were eluted with free thalidomide, and the eluate fractions were subjected to SDS gel electrophoresis and silver staining. Two polv-



► *Hiroshi Handa is a professor in the Integrated Research Institute, Tokyo Institute of Technology and a leader of Medical and Biotechnology Project. He received his M.D. in 1972 and his D.M.S. in 1976 at the Keio University, School of Medicine. In 1976, he was an assistant professor at the University of Tokyo. He worked as a postdoctoral fellow at MIT under the supervision of Prof. Phillip A. Sharp from 1978 to 1980. He was an associate professor in 1984 at the University of Tokyo. He has been a professor from 1991 at Tokyo Institute of Technology. He has conducted research in a wide variety fields including (1) affinity latex particles, (2) regulation of transcription elongation, (3) chemical biology through the identification of chemical targets, (4) highly functionalized magnetic particles used for medical and biotechnology fields, (5) nanocapsules on the basis of self-assembled reconstruction of viral capsid protein and its derivatives for novel DDS. ■*

Magnetic FG beads

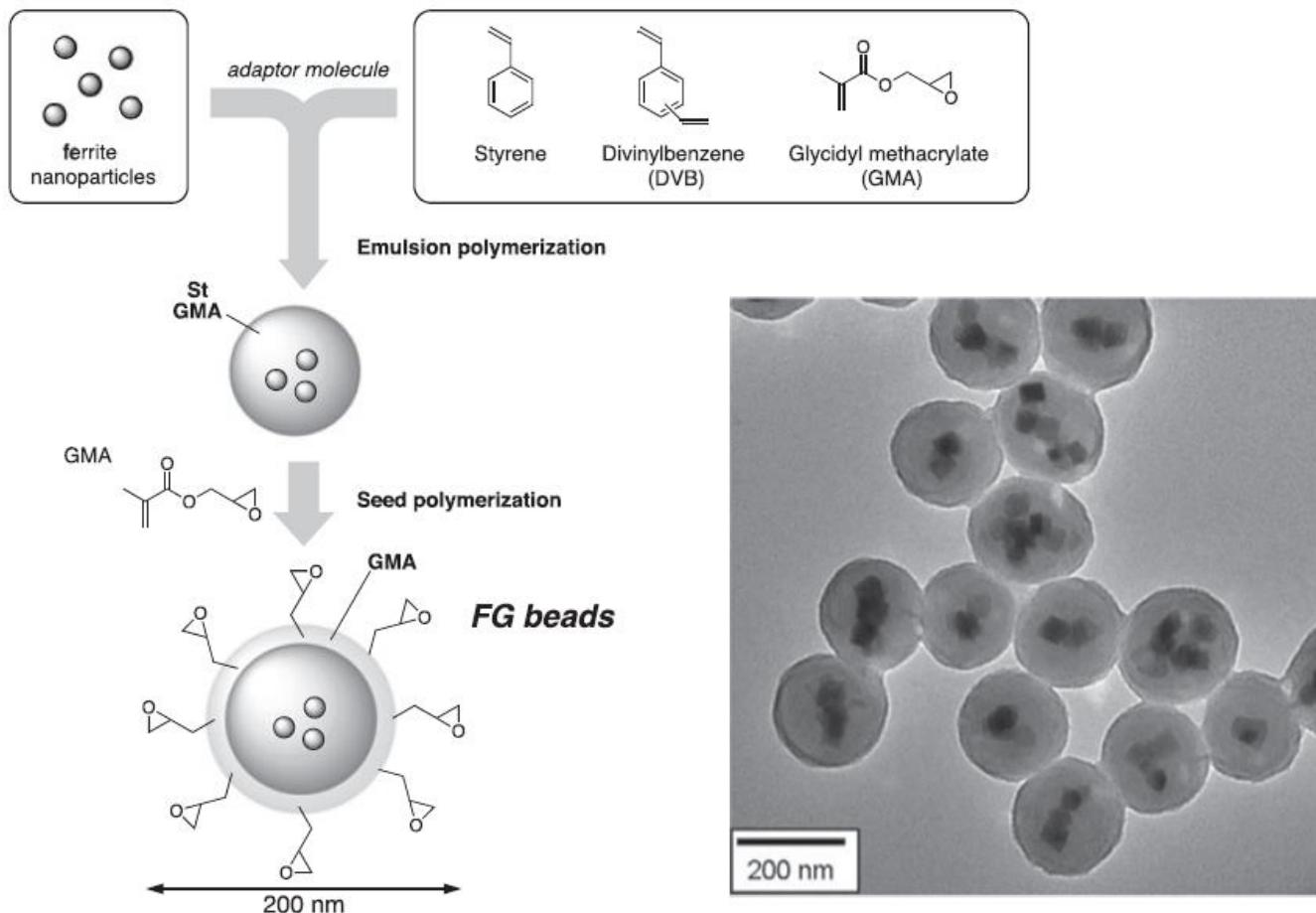
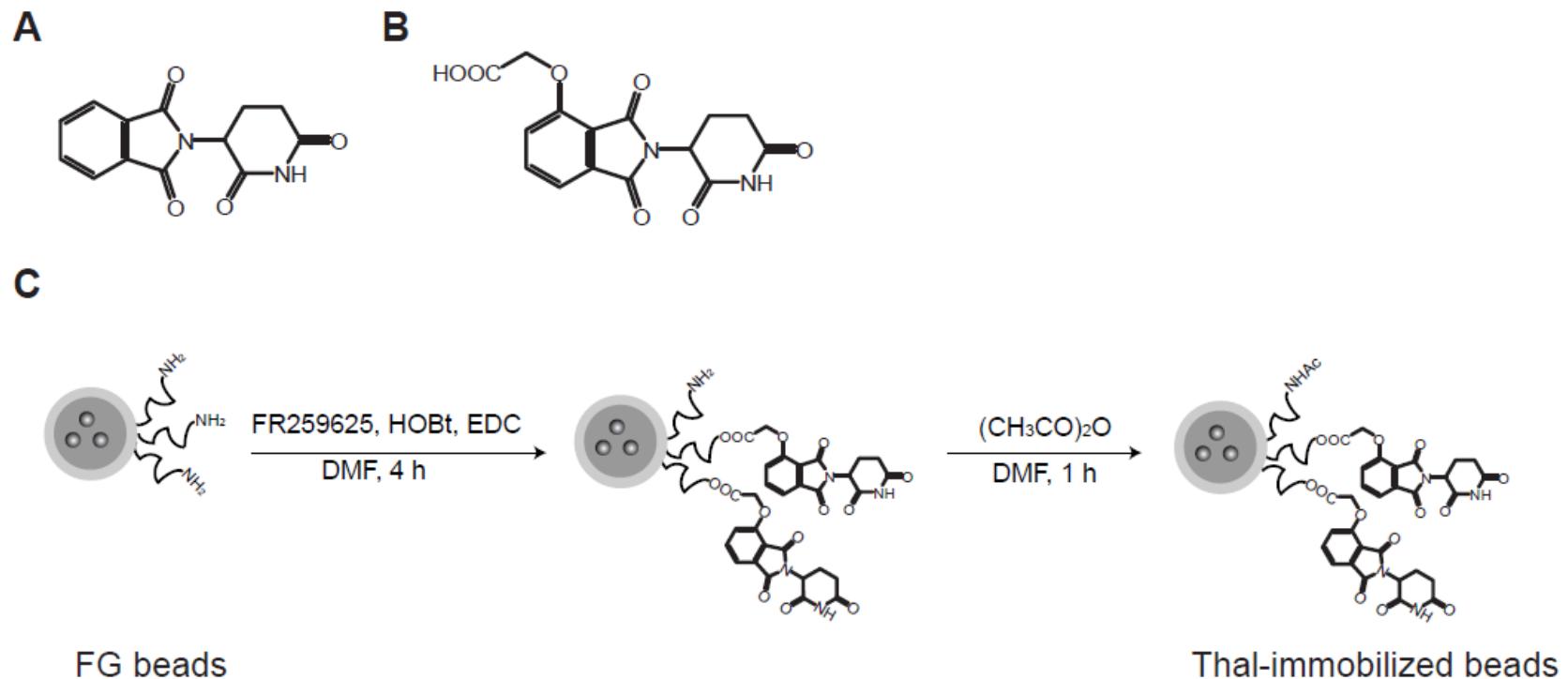
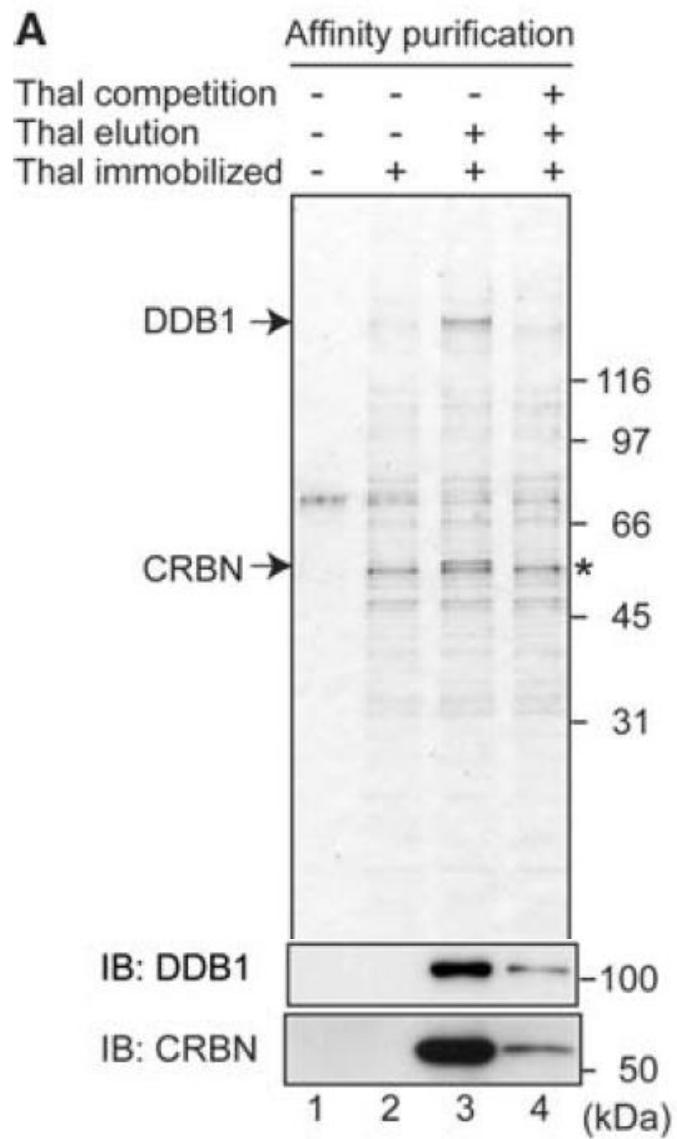


Fig. 4. Transmission electron microscopy image of FG beads.

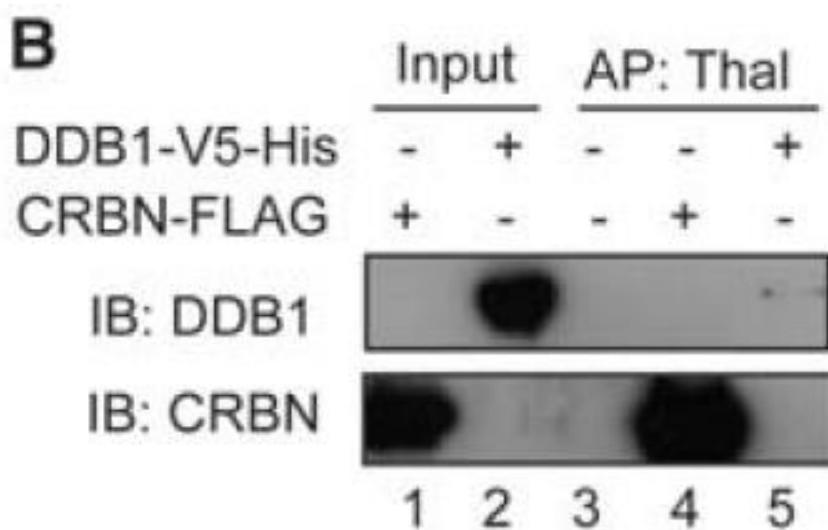
Scheme of thalidomide immobilization to FG beads



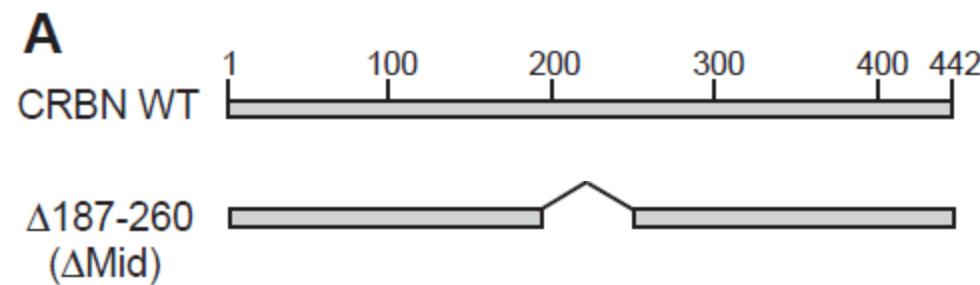
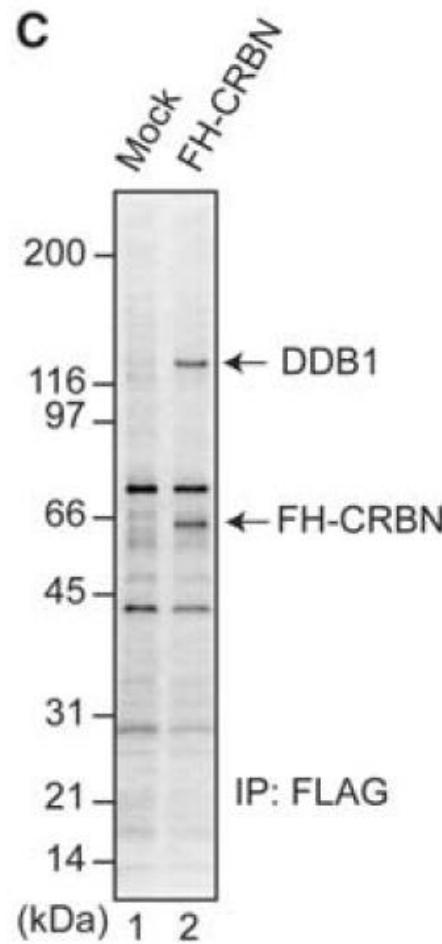
DDB1 and CRBN: candidate thalidomide binding proteins



CRBN but not DDB1 directly binds to thalidomide



DDB1 binds to thalidomide through its interaction with CRBN



Ubiquitination system

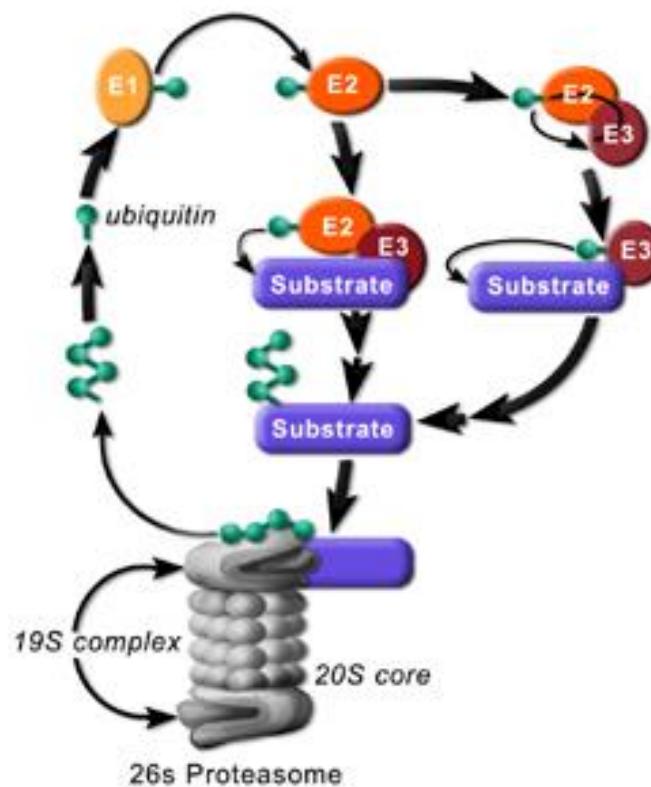
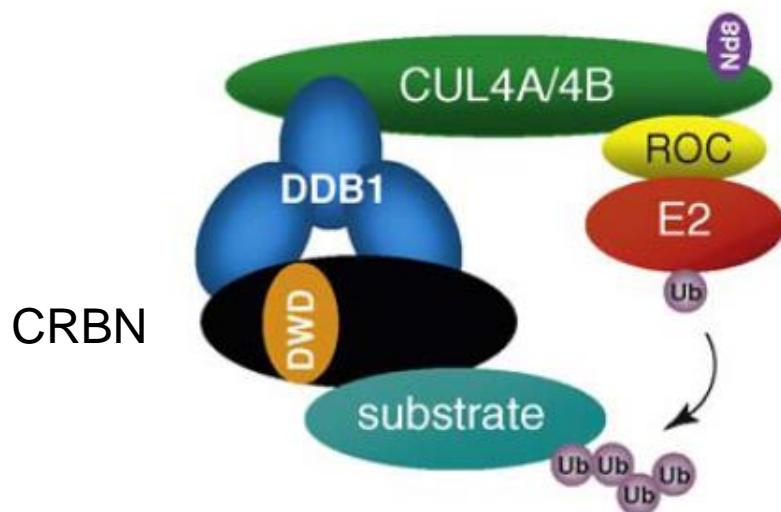
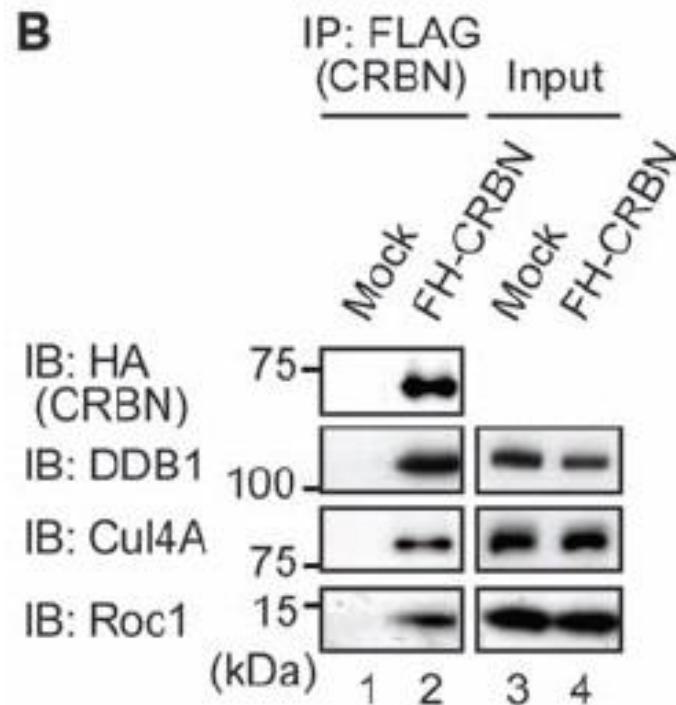


Image by MIT OCW. After W. Hilt, Universitat Stuttgart

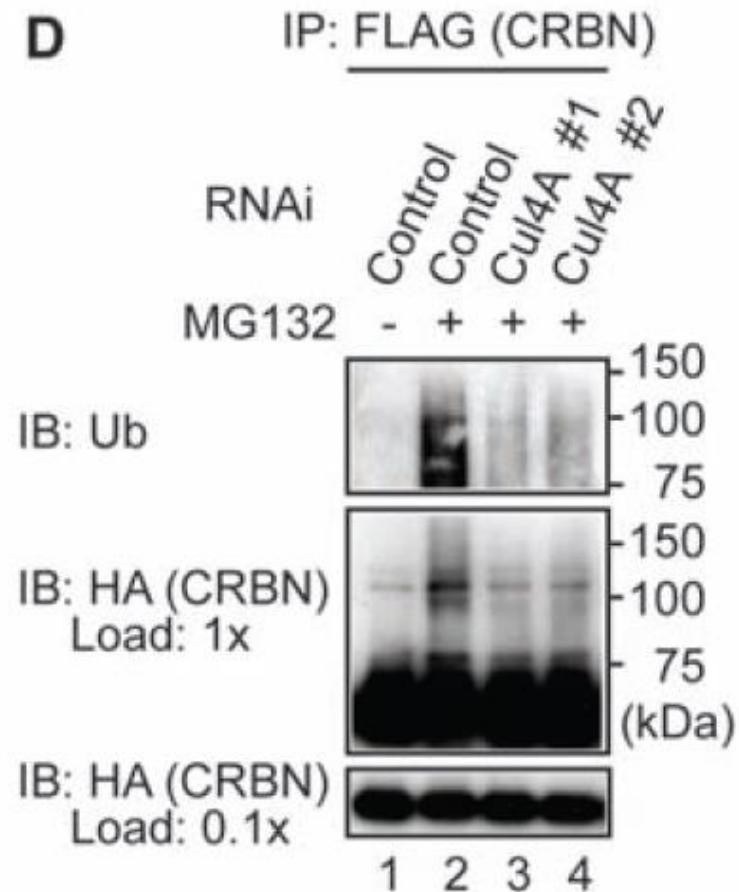
CRBN forms an E3 complex with DDB1 and Cul4A



Jackson, S. et al., 2009

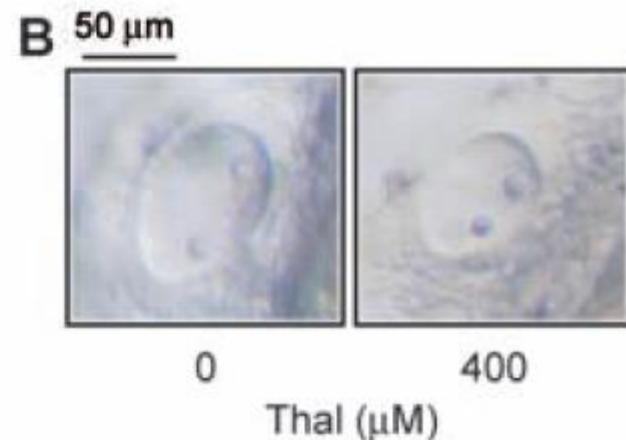
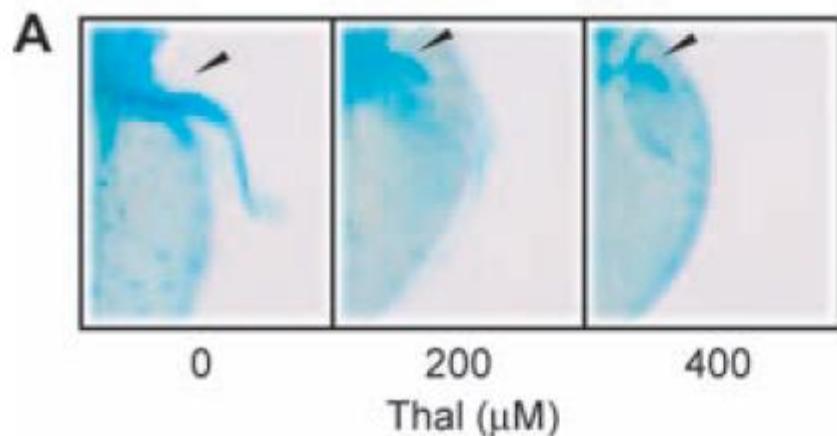


Autoubiquitination of CRBN depends on Cul4a

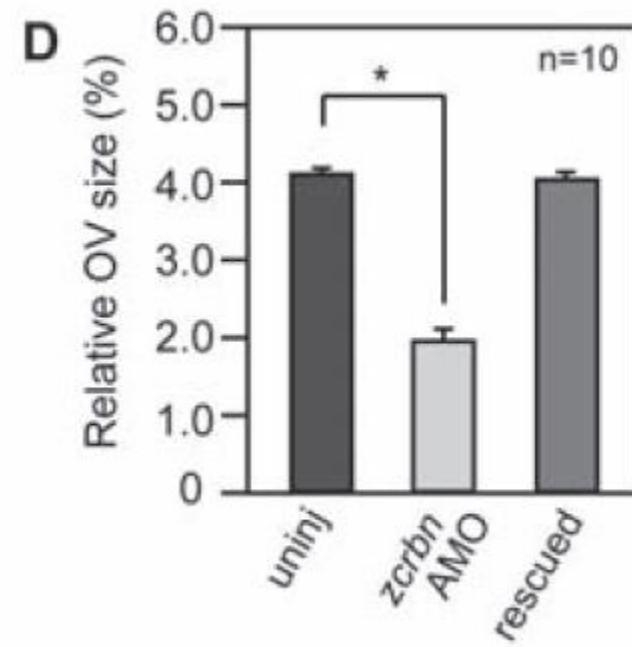
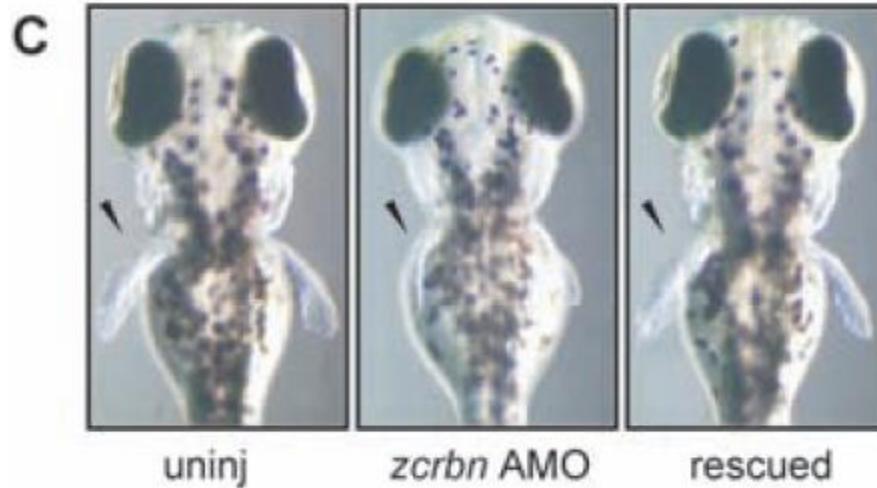


Whether the inhibition of CRBN is responsible for thalidomide teratogenicity?

Treatment of thalidomide in zebrafish induced loss-of-pectoral fins and reduced size of otic vesicles

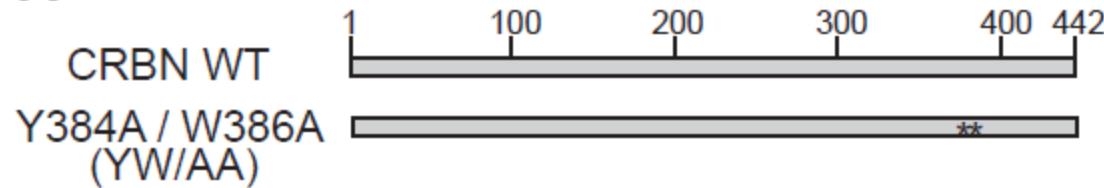


Thalidomide-induced phenotype can be phenocopied by knockdown of *zcrbn*

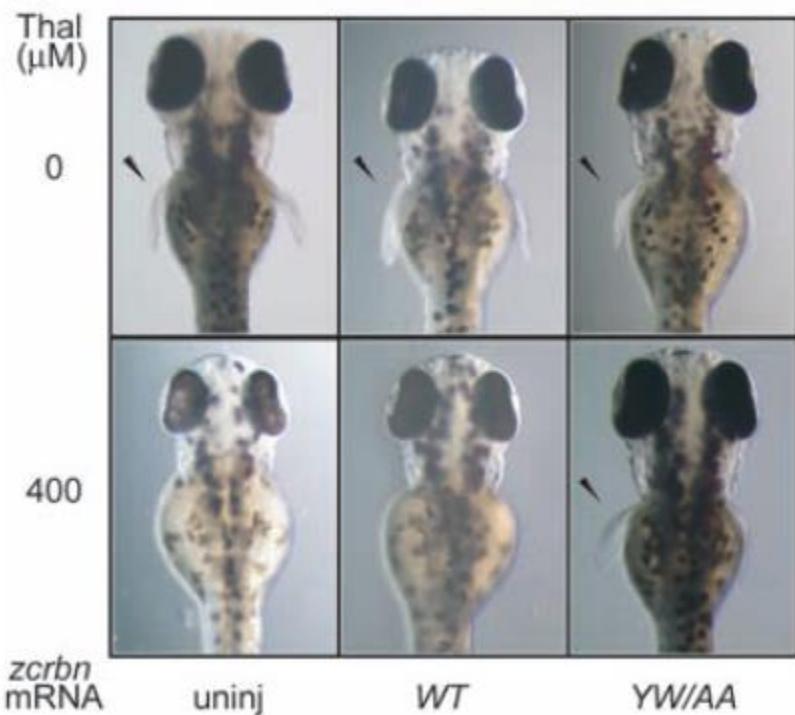


The thalidomide-induced phenotype can be rescued by zcrbn(YW/AA) but not WT zcrbn

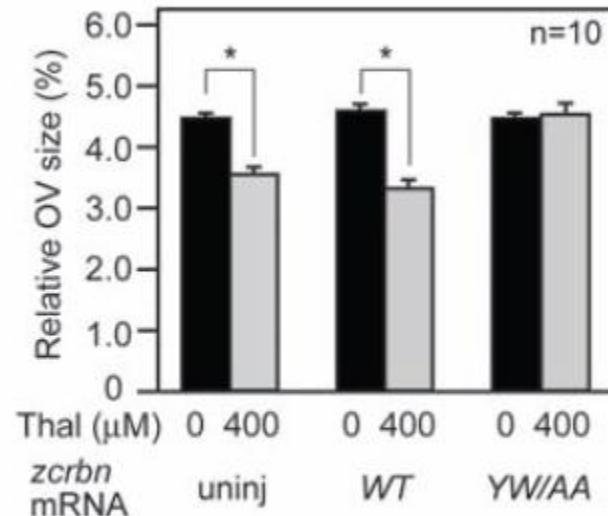
A



A

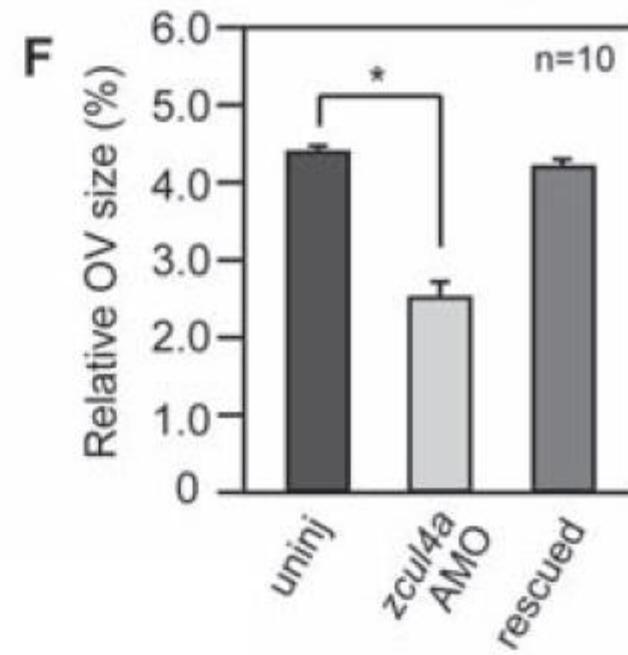
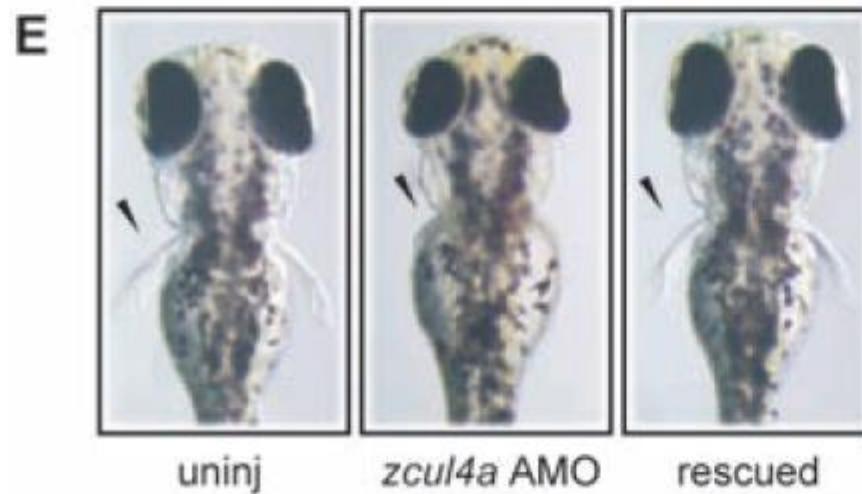


B



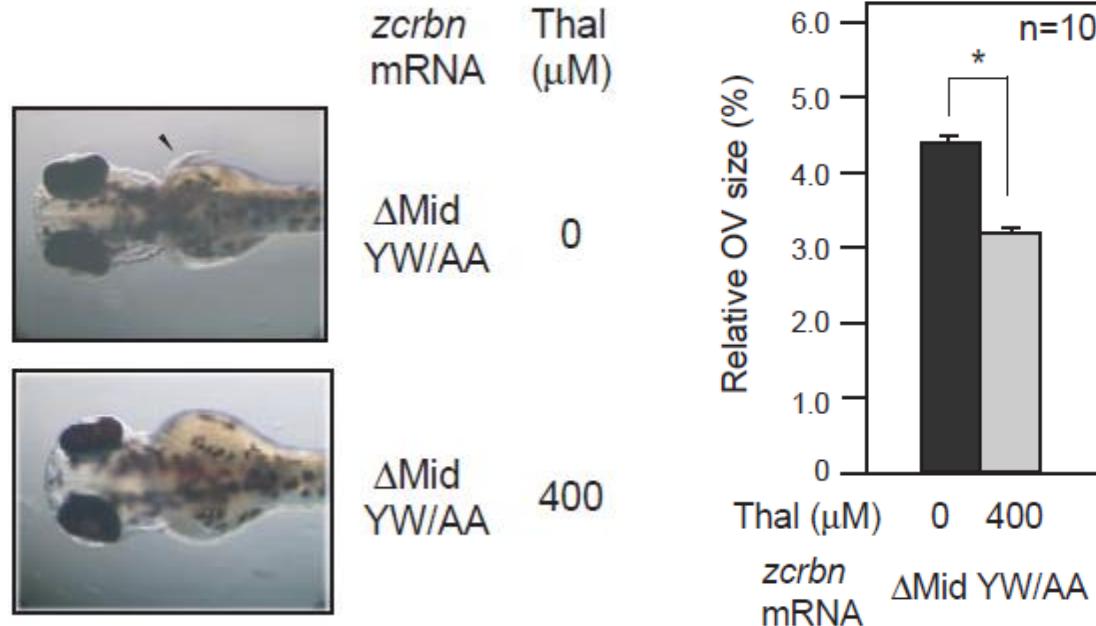
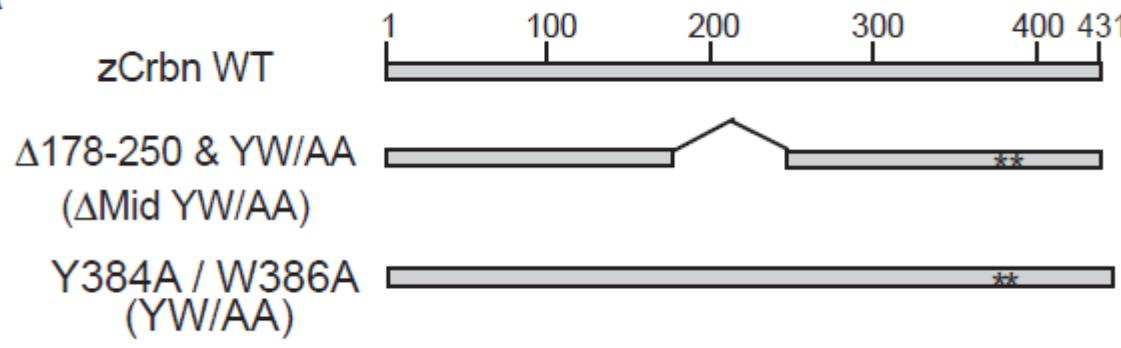
Whether the inhibition of Cul4A-DBB1-CRBN E3 complex is responsible for thalidomide teratogenicity?

Thalidomide-induced phenotype can be phenocopied by knockdown of *zcul4a*



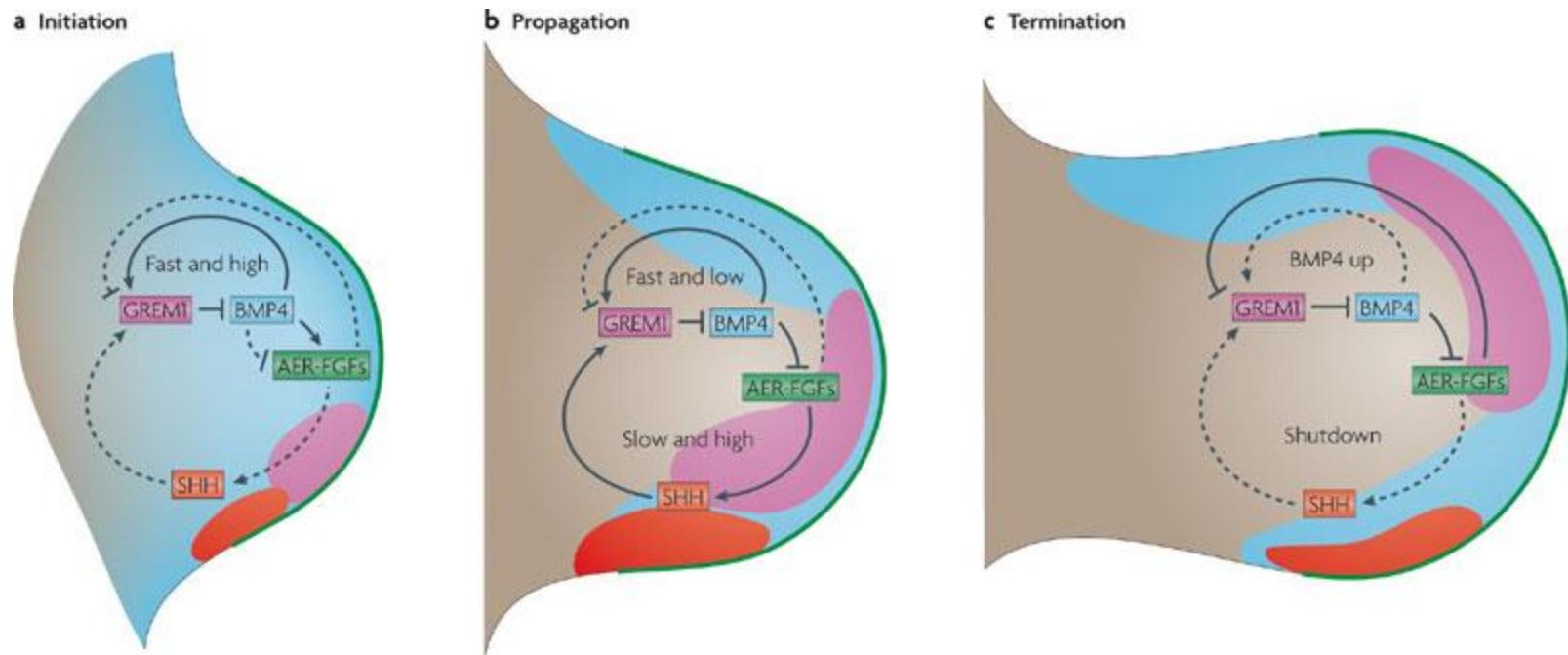
The binding to DBB1 is essential to the rescue of thalidomide phenotype by zcrbn (YW/AA)

A



What is the downstream pathway of
Cul4A-DBB1-CRBN E3 complex?

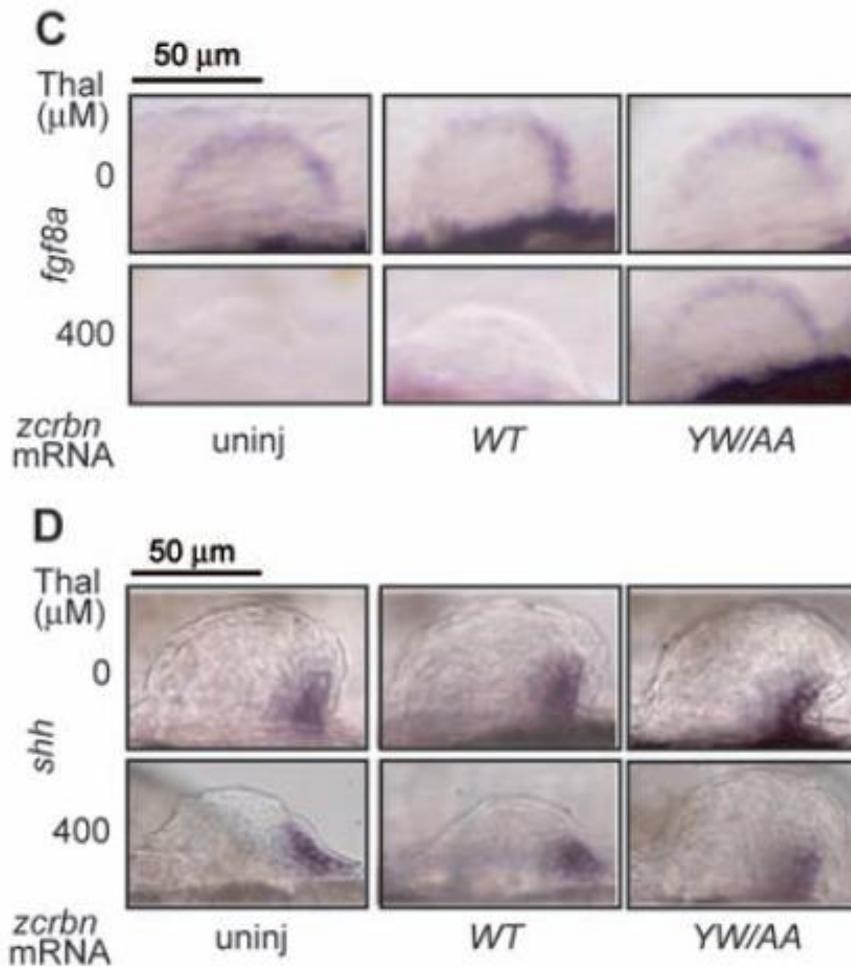
Fgf and Shh signaling during limb development



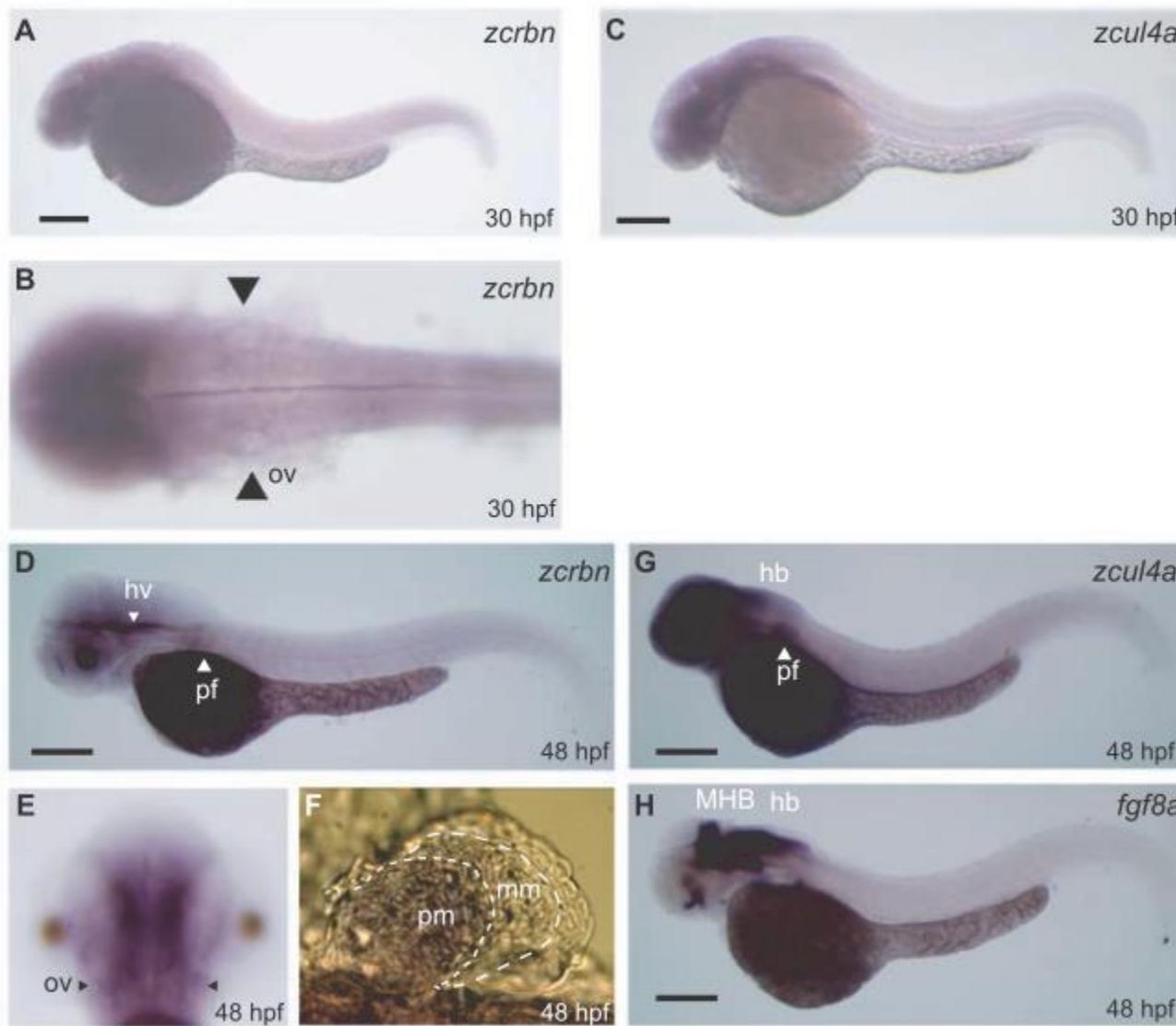
Nature Reviews | Genetics

Zeller, R. et al., 2009

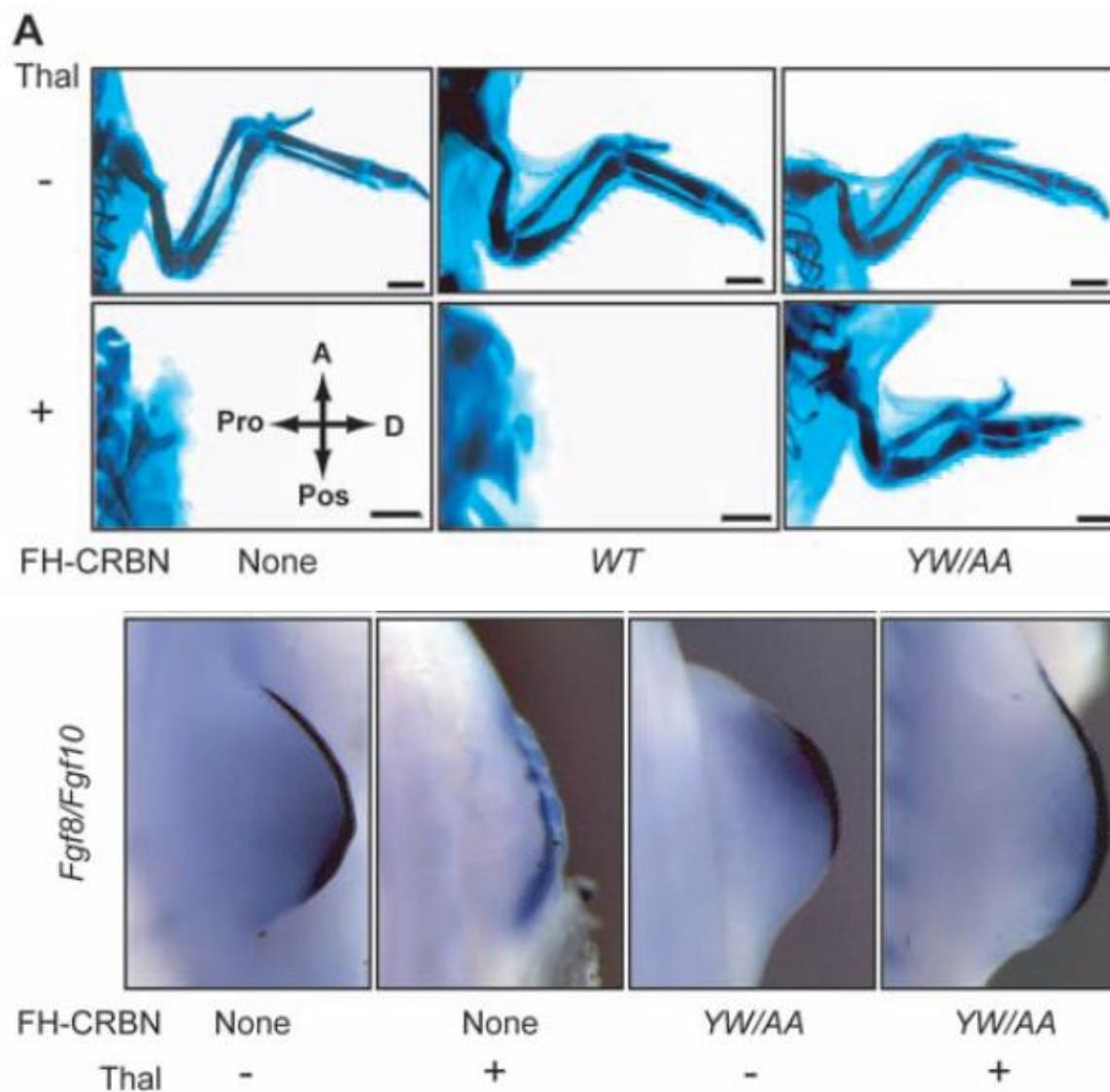
Expression of *fgf8a* but not *shh* is abolished by thalidomide treatment



zcrbn and *zcul4a* expression in 30- and 48-hpf zebrafish embryos



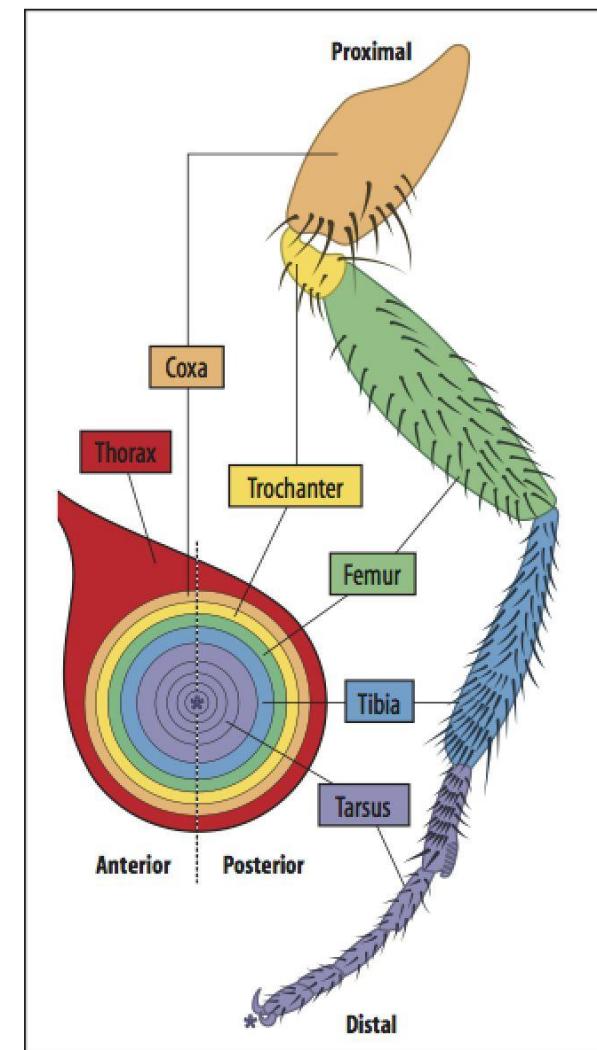
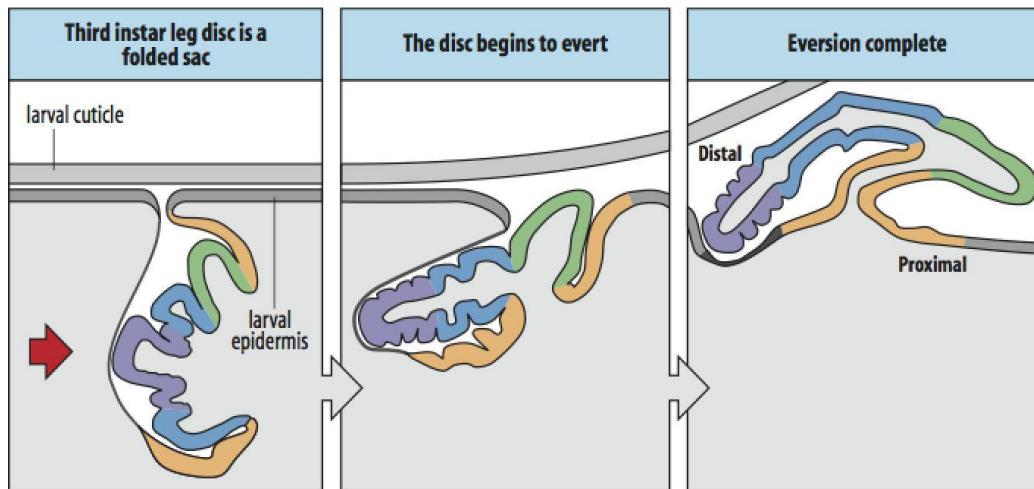
The phenomena observed in zebrafish is well conserved in chick



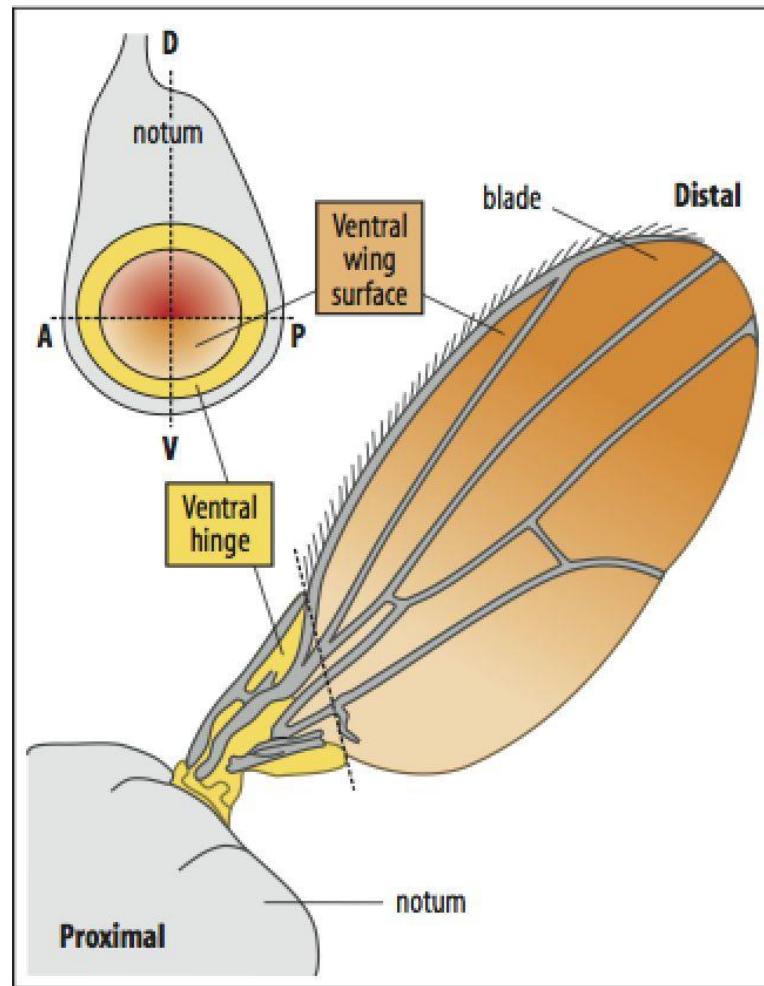
Thalidomine revives

- erythema nodosum leprosum (麻风结节性红斑)
- Multiple myeloma (多发性骨髓瘤)

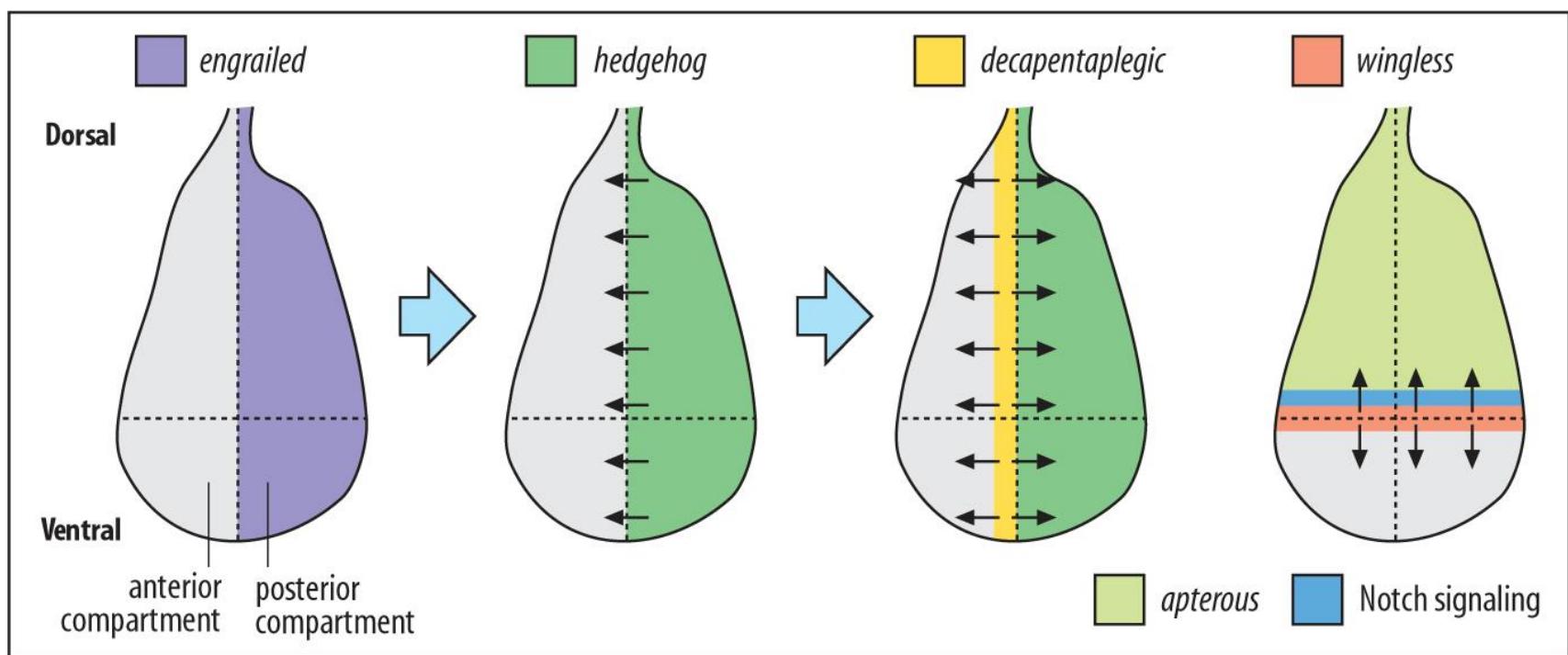
Drosophila leg disc



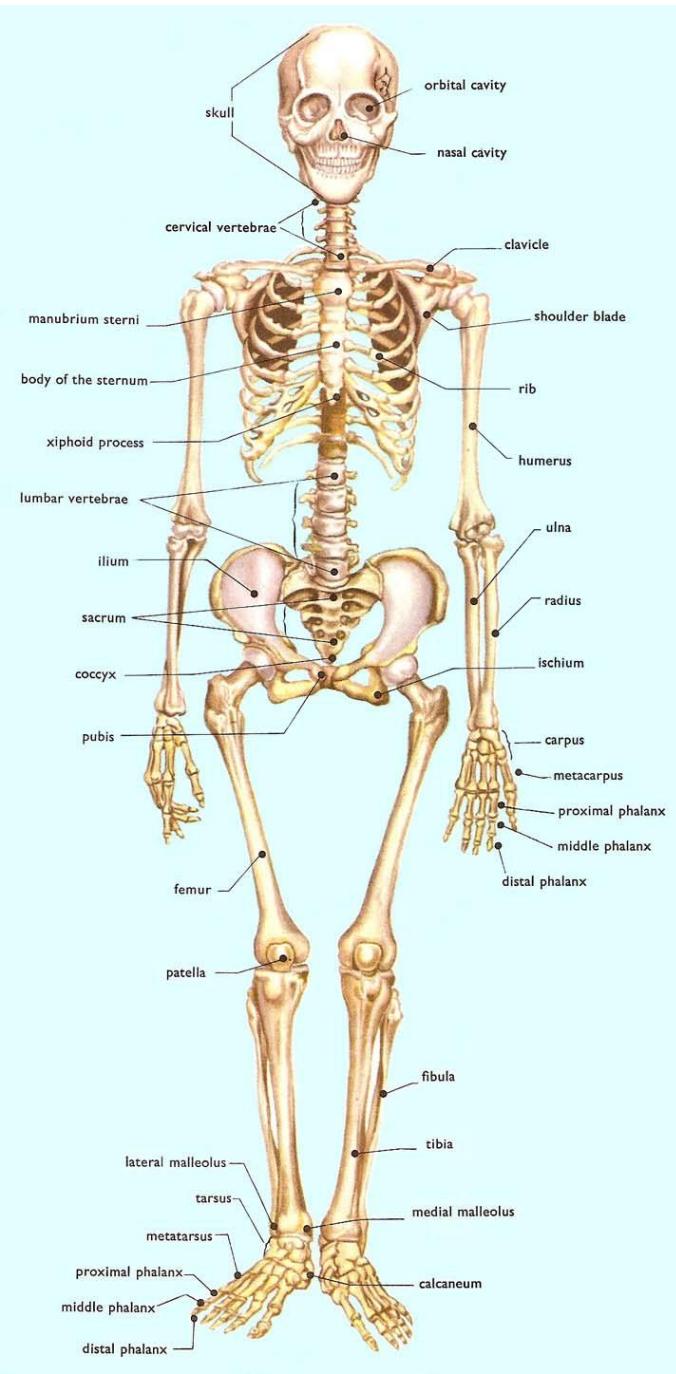
Fate map of the wing imaginal disc of *Drosophila*



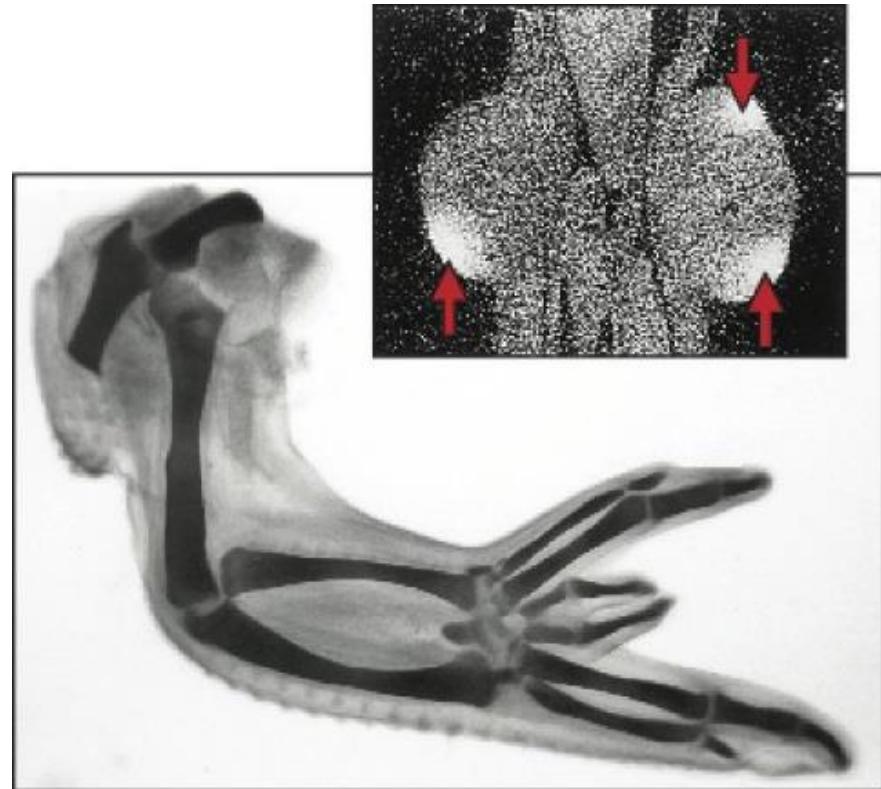
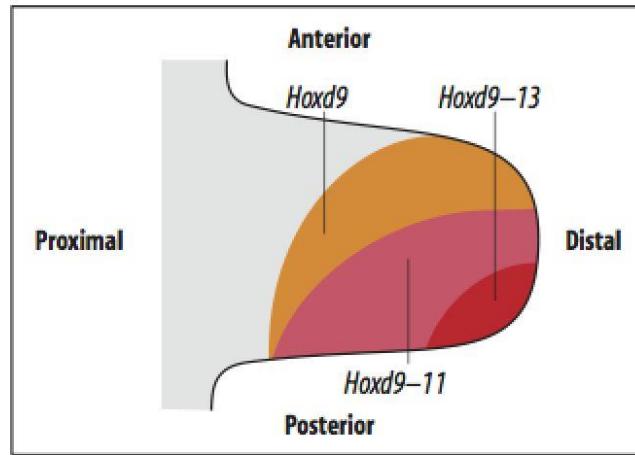
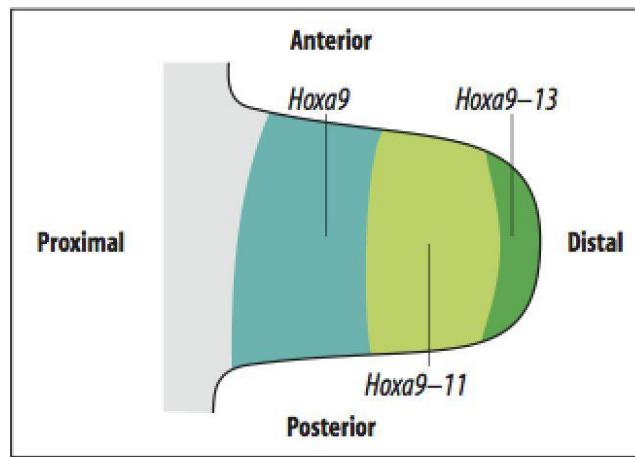
Establishment of signaling regions in the wing disc at the compartment boundaries



Thanks!



Hox genes establish the polarizing region and also provide a code for limb patterning



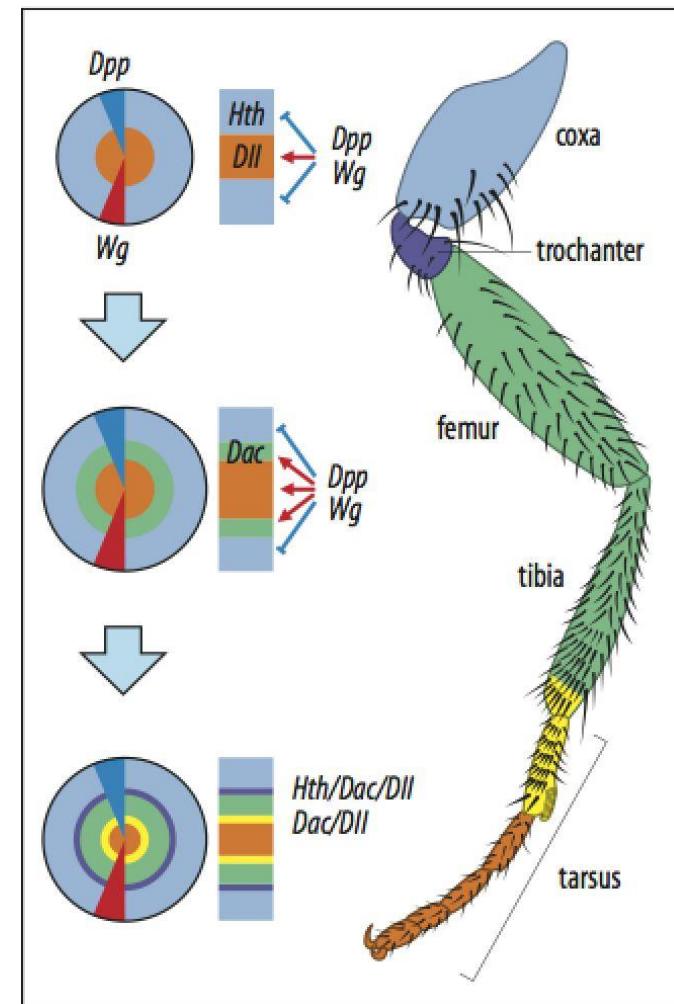
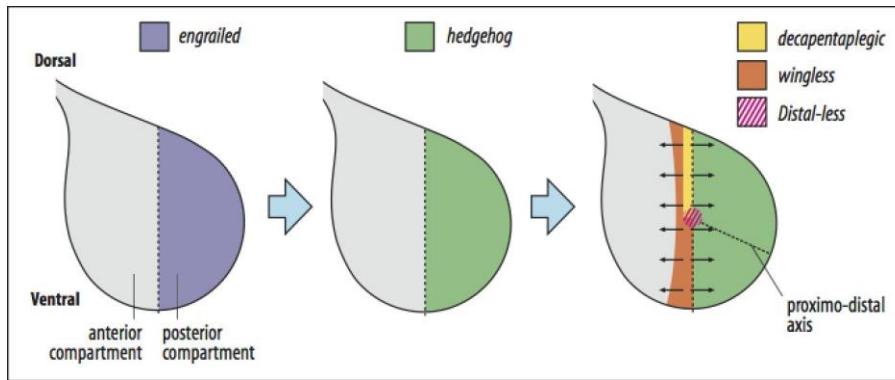
Sox9 expression in the developing mouse hand-plate

Normal

Hoxd11–13^{–/–}, *Gli3*^{–/–}



Establishment of AP compartments and regional subdivision along PD axis



Butterfly wing pattern



Cells interpret their position according to their developmental history and genetic make-up

