Krátké shrnutí základů z Vývojové fyziologie živočichů I

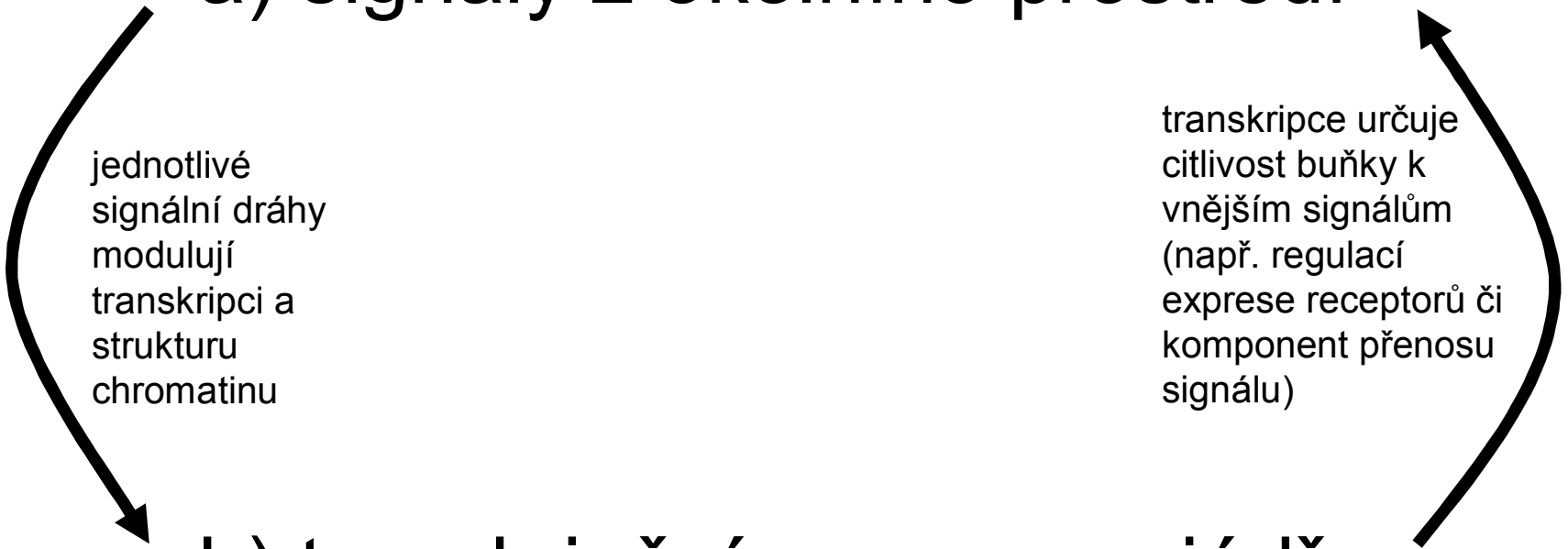
Buňka ve vyvíjejícím se embryu musí umět a vědět nejrůznější věci:

- vědět kdo je, kde je, kým bude a co bude dalším krokem v jejím buněčném životě
- buňka činí rozhodnutí na otázky typu jako: Dělit se? Diferencovat? A v co diferencovat? Migrovat? A kam? Zemřít?

Pavlovy movies

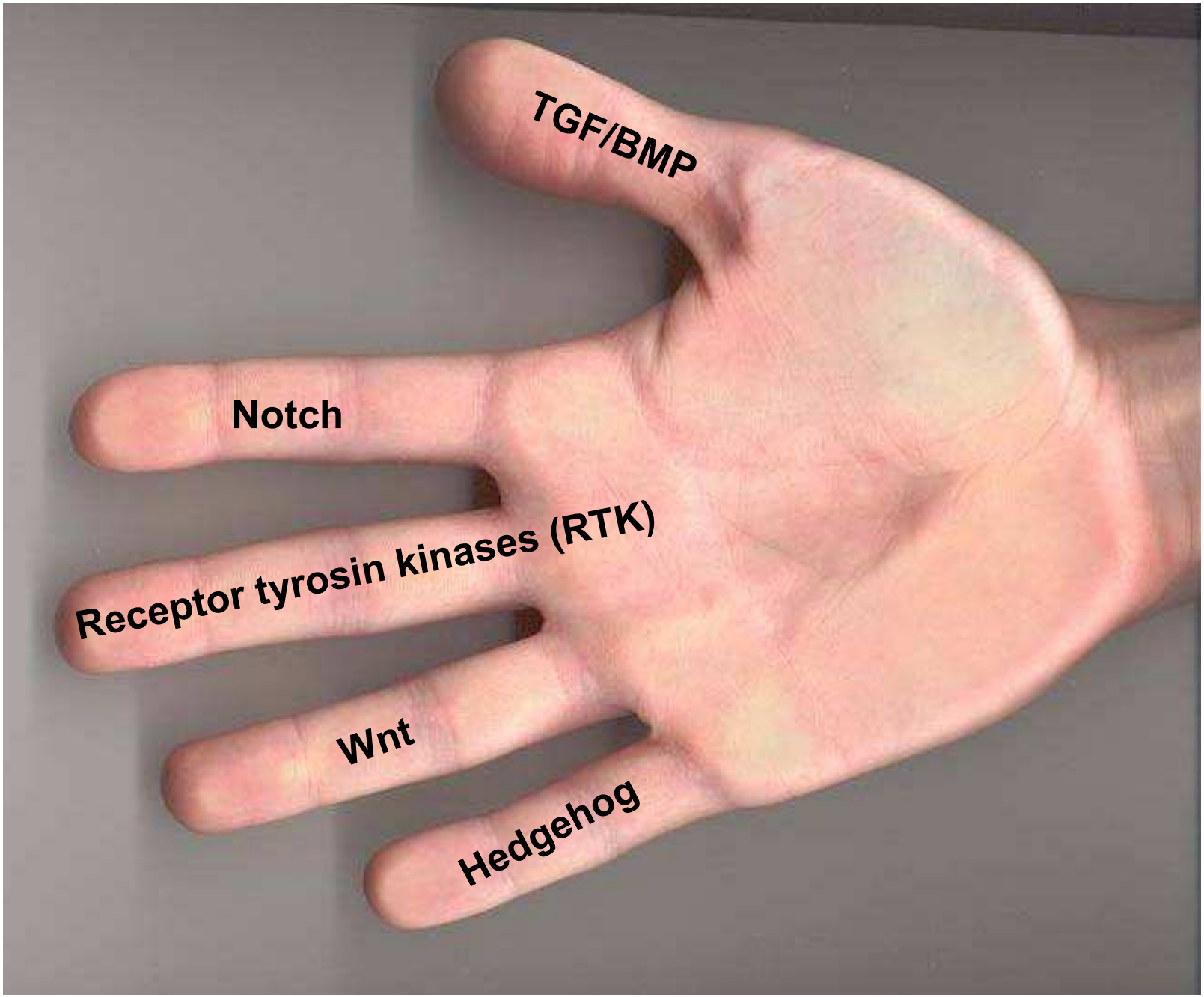
Co poskytuje buňce tyto informace?

a) signály z okolního prostředí

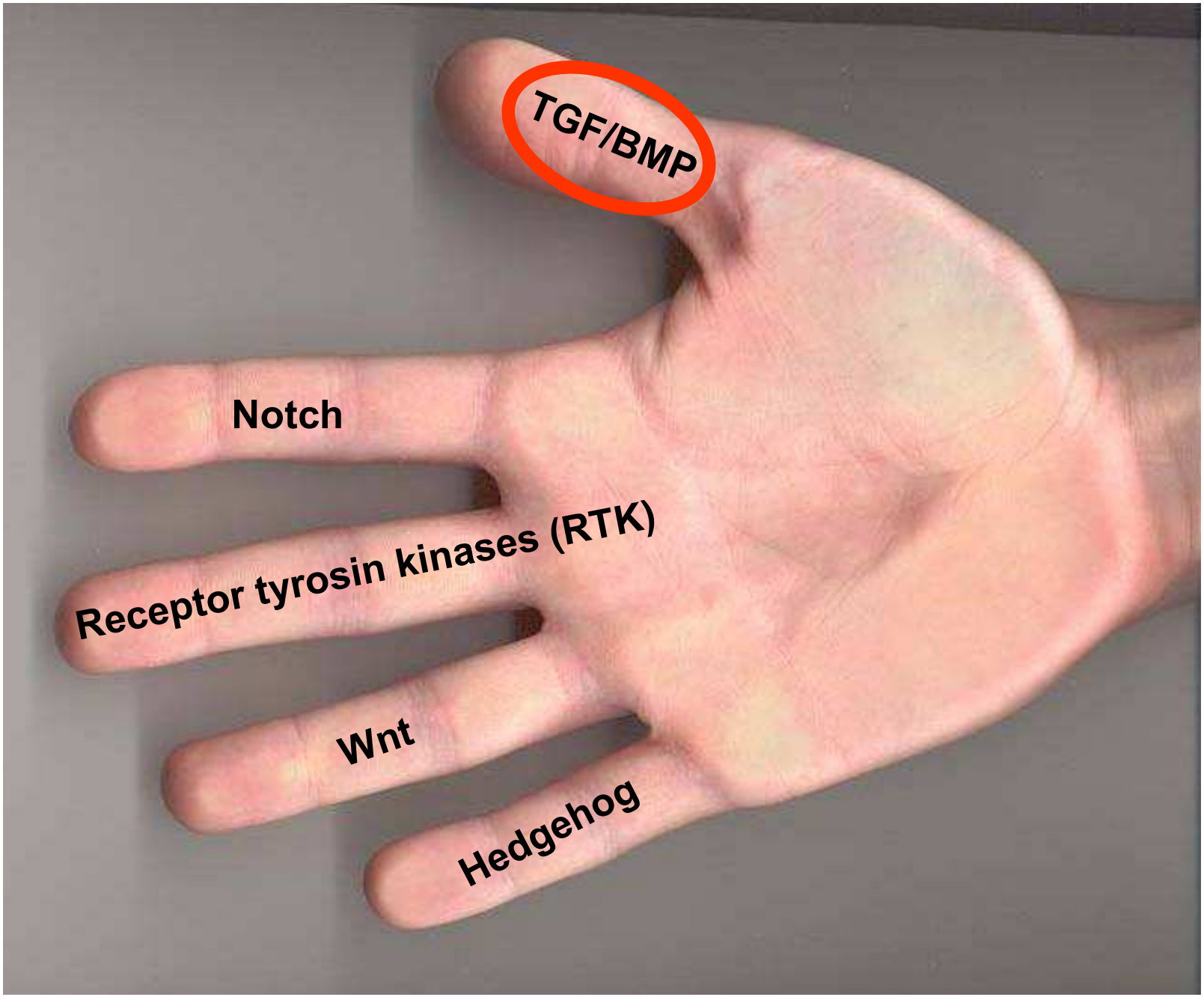


b) transkripční program v jádře

Signály z vnějšího prostředí



Signály z vnějšího prostředí



TGFβ nadrodina má následující podrodiny:

1. TGFβ1-3
2. BMPs – 20 různých ligandů
3. GDF (growth differentiation factor): 9 ligandů
4. activin/inhibin/nodal

Společným znakem je signalizace přes:

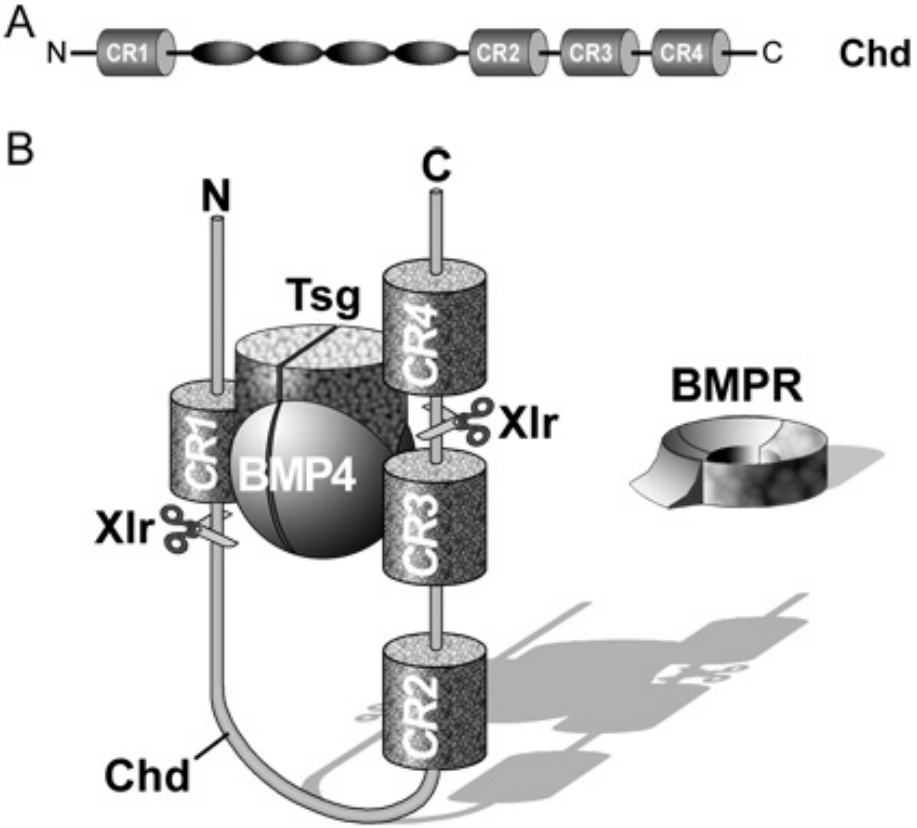
- konzervativní rodinu Ser/Thr kinázových receptorů – jsou dvou typů a po vazbě ligandu dimerizují

- cytoplazmatická signalizace přes tzv. SMAD proteiny

Inhibitory BMP faktorů,

jsou klíčové pro fyziologické funkce BMP:

- noggin
- chordin (Chd)
- sklerostin



Přímá fyzická interakce mezi chordinem a BMP je podstatou inhibičního působení chordinu

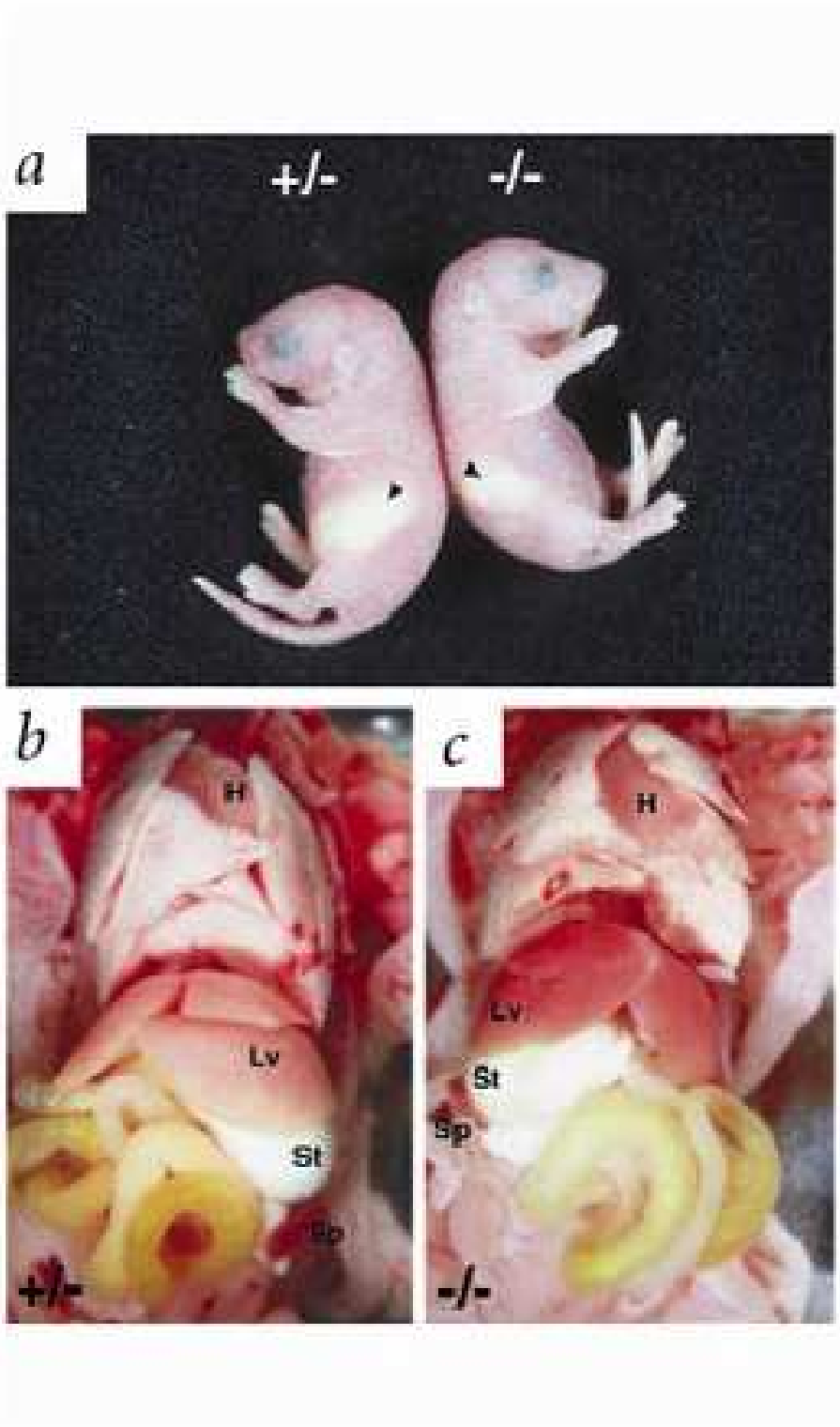
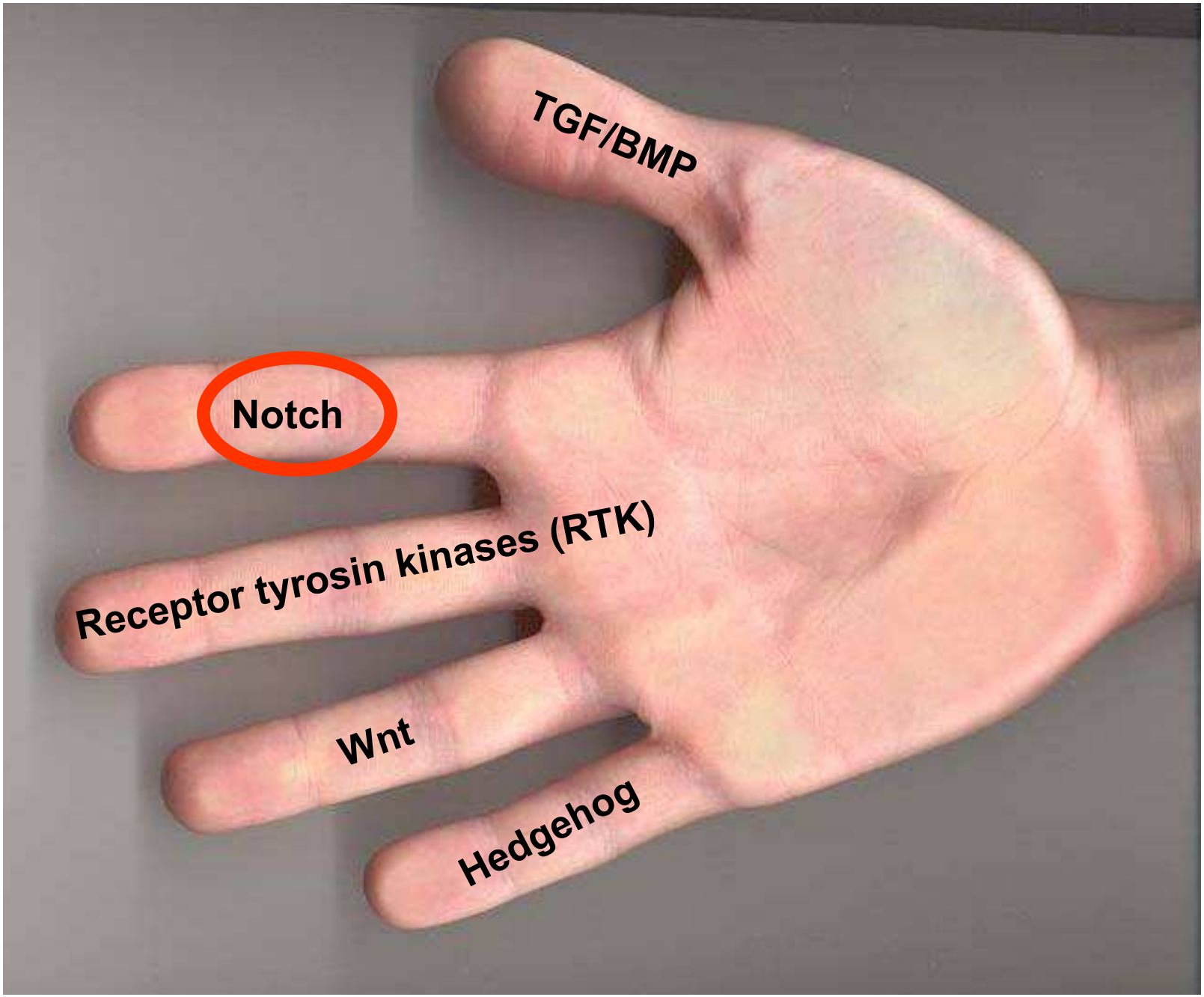


Figure 2. Analysis of *situs* defects in *Gdf1*-/- mice.
a, *Gdf1* +/- and *Gdf1* -/- newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1* +/- (*b*,*d*,*f*,*h*) and *Gdf1* -/- (*c*,*e*,*g*,*i*) mice are shown. ***b***,***c***, Reversal of the orientation of the abdominal organs in *Gdf1* -/- mice. Note also the streak-like appearance of the spleen and the abnormally shaped medial lobe of the liver.

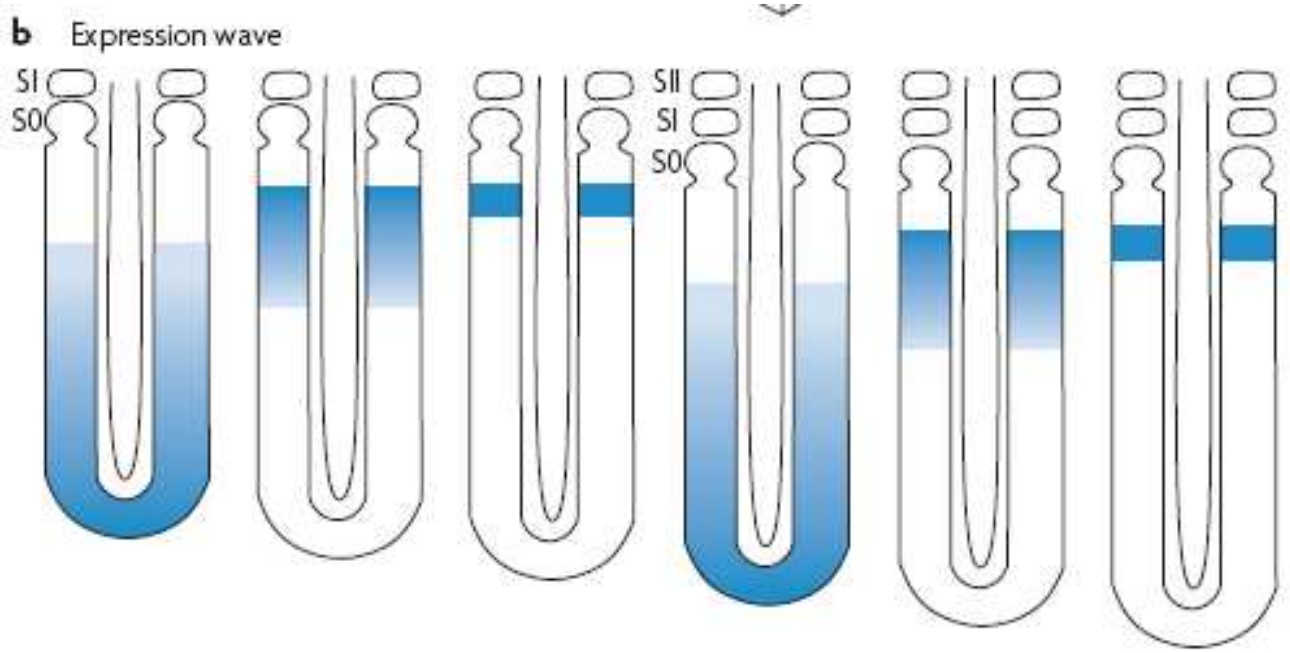
H, heart; Lv, liver; St, stomach; Sp, spleen; AC,

Signály z vnějšího prostředí



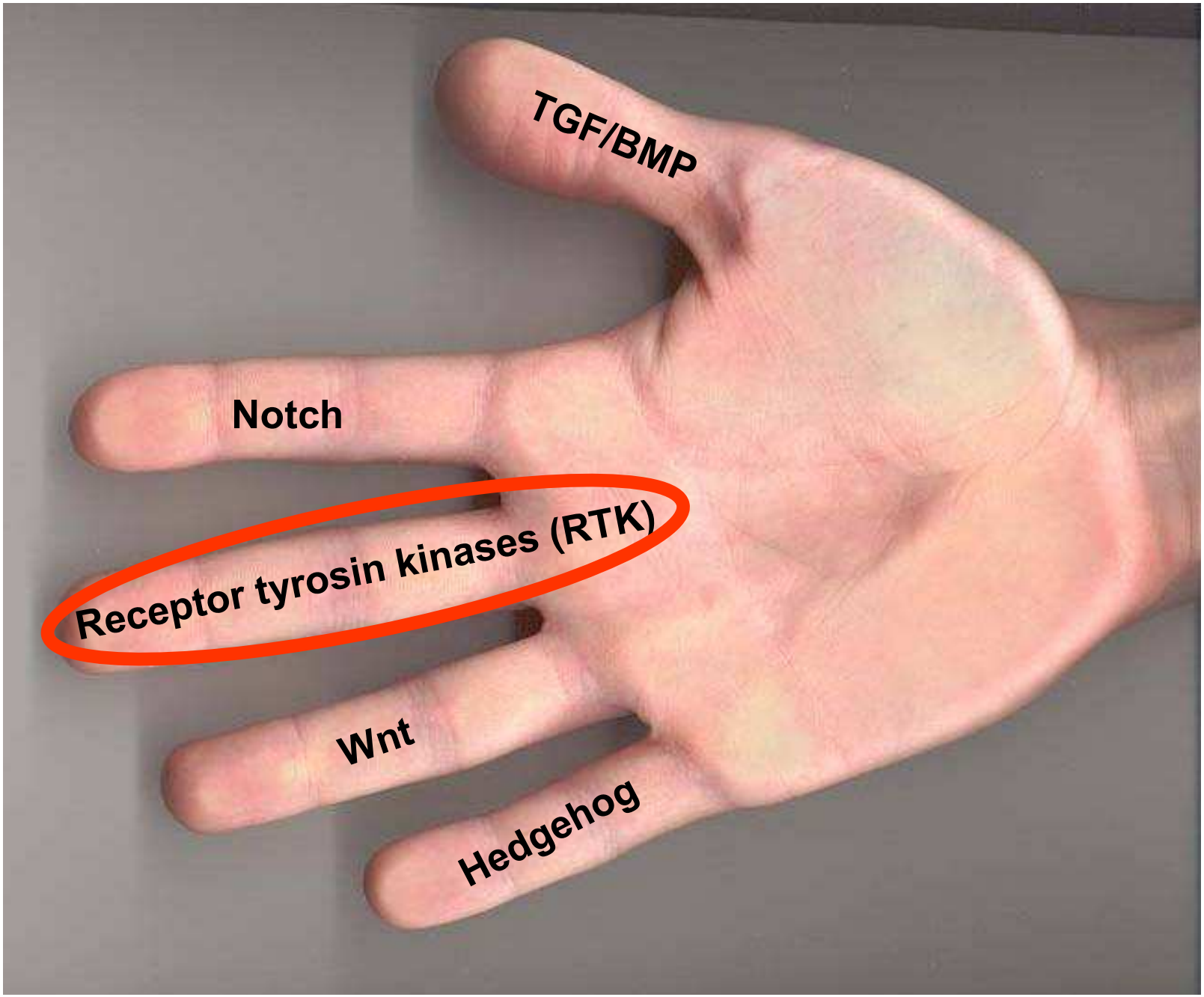
Notch and the segmentation clock

segmentation clock movie.mov

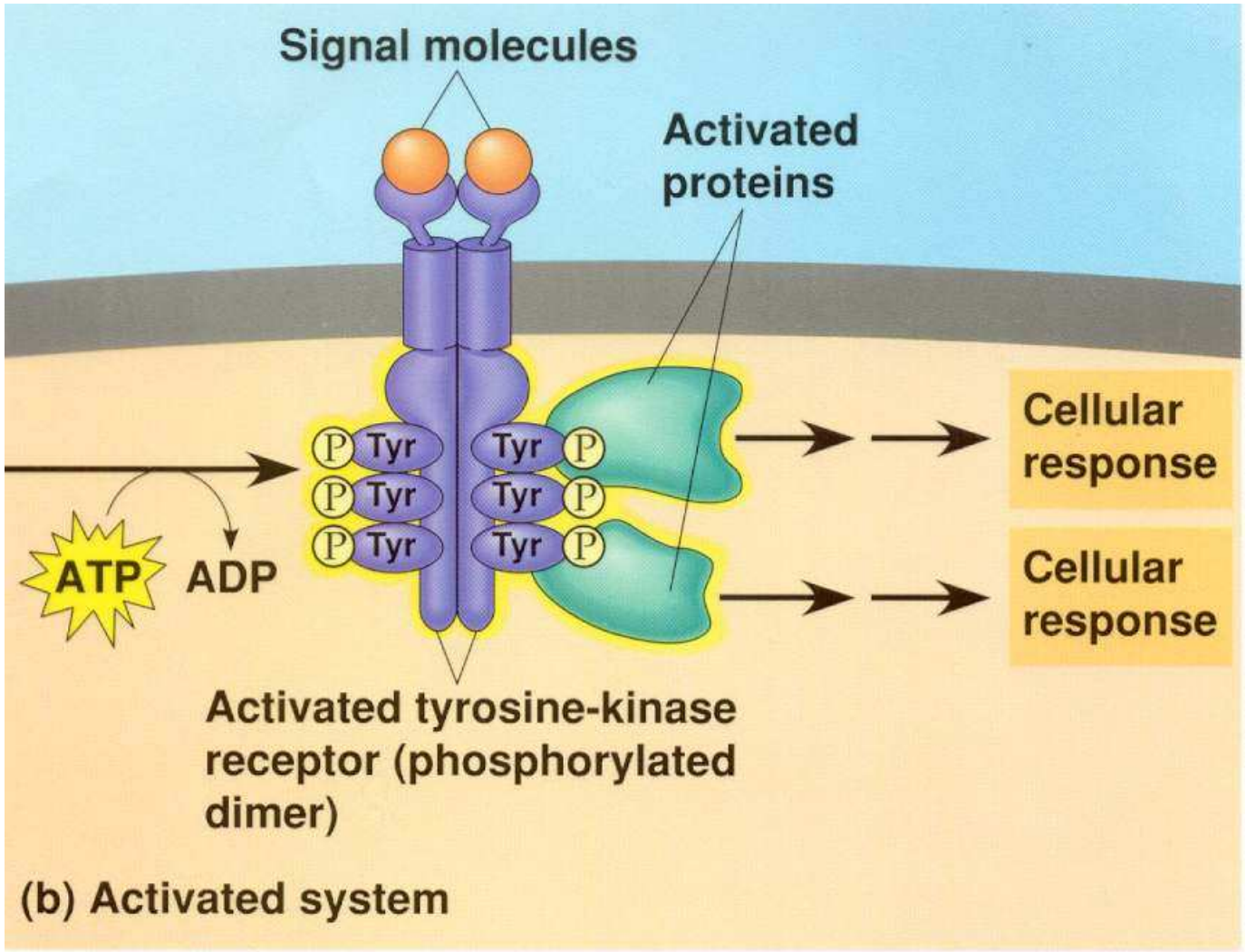


cellular states. **b** | Evidence for an oscillator underlying vertebrate segmentation. Periodic waves of transcriptional expression of the *hairy1* gene (blue) in PSM cells are associated with the formation of each pair of somites added sequentially[18]. Part **a** modified with permission from REF. 14 © (1976) Elsevier Ltd.

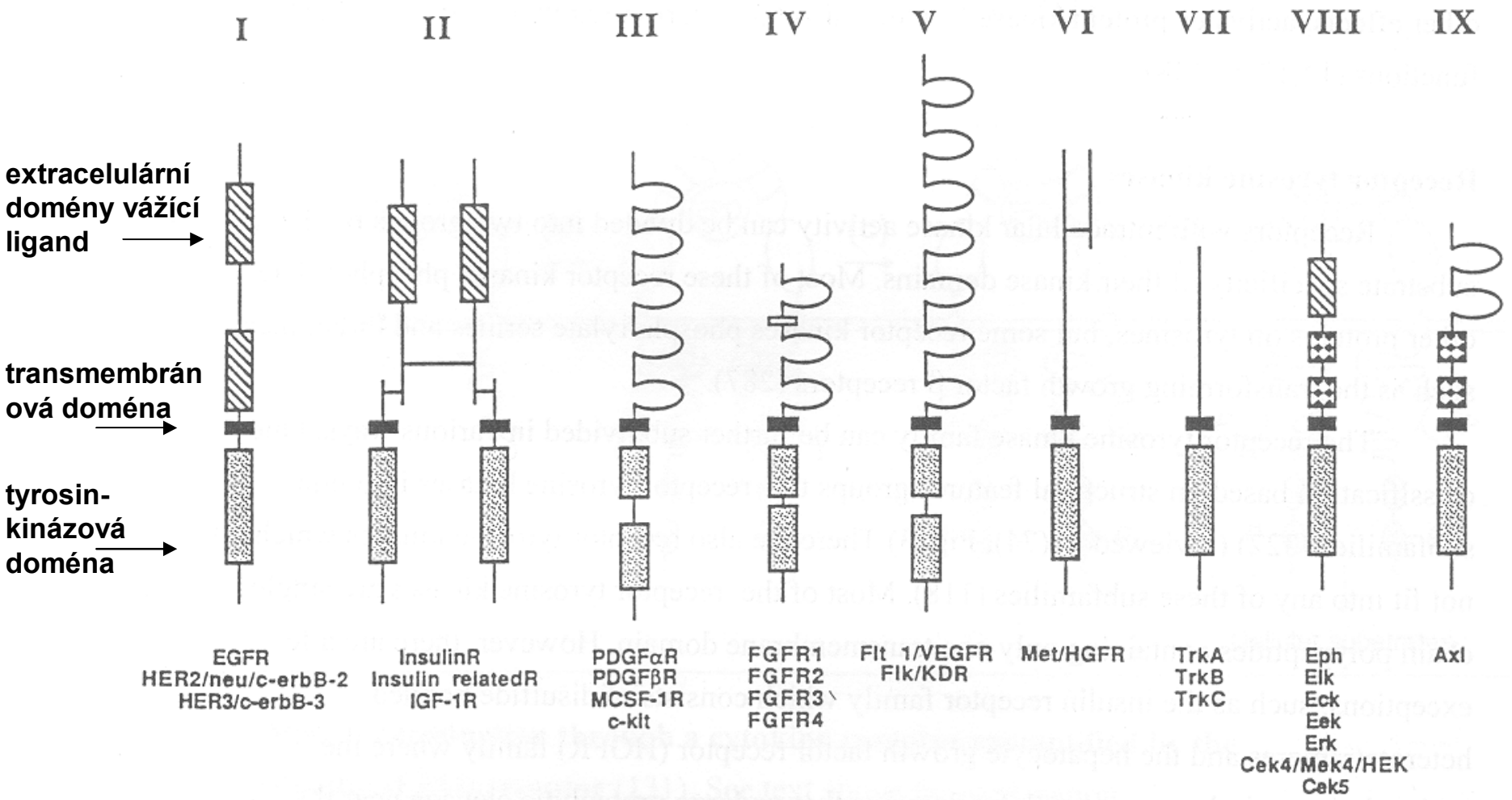
Signály z vnějšího prostředí



Receptorové tyrosin kinázy (RTK)



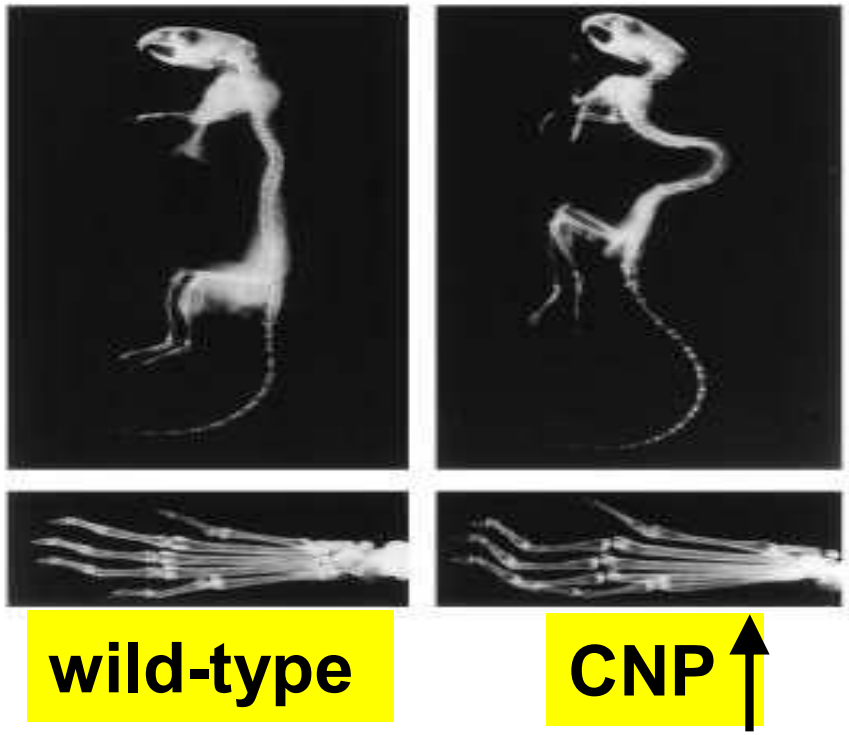
Schematická struktura jednotlivých receptorů

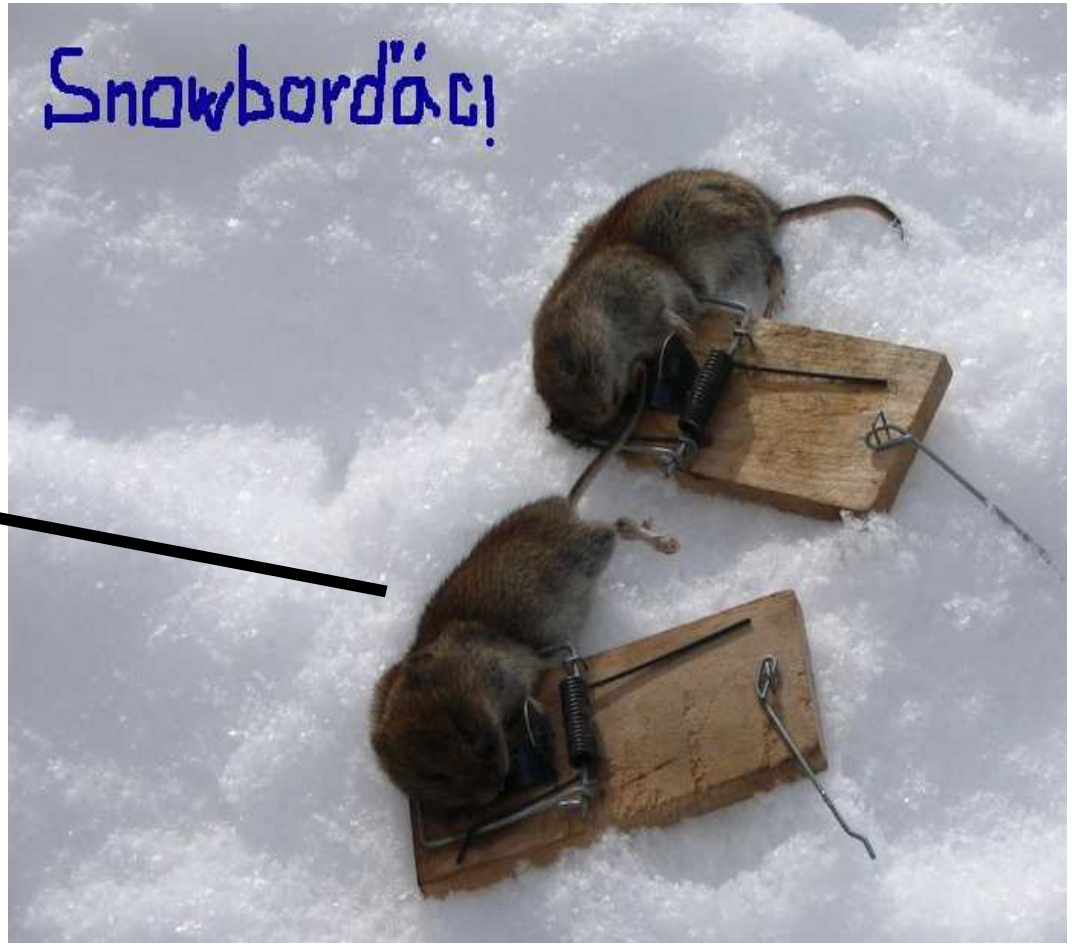


Erk MAP kinase activity is necessary for FGFR3 phenotype in cartilage

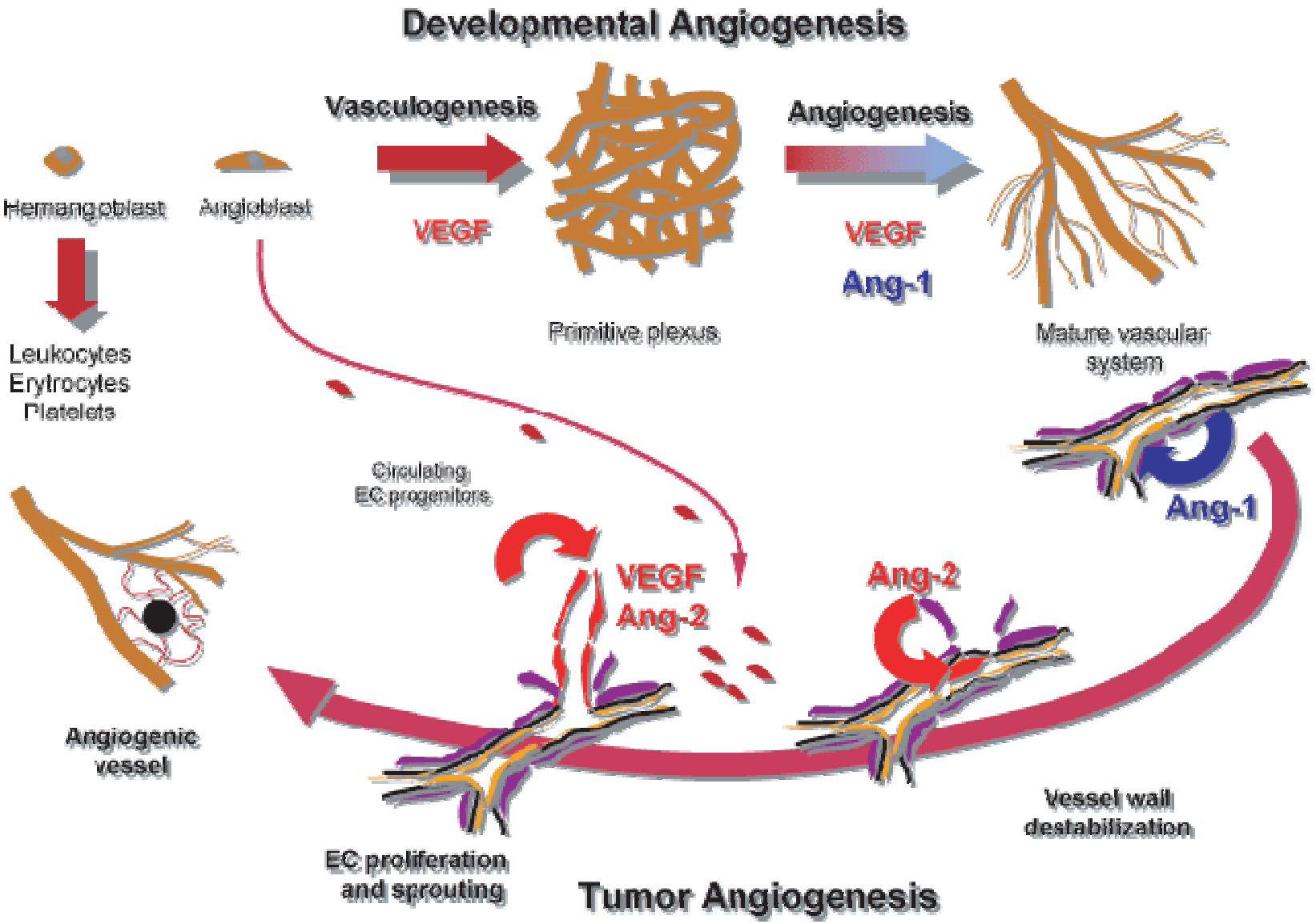
FGF2
↓
FGFR3
↓
FRS2
↓
Ras
↓
Raf-1
↓
MEK
↓
Erk →

C-type Natriuretic Peptide (CNP) over-expression results in skeleton overgrowth in mice





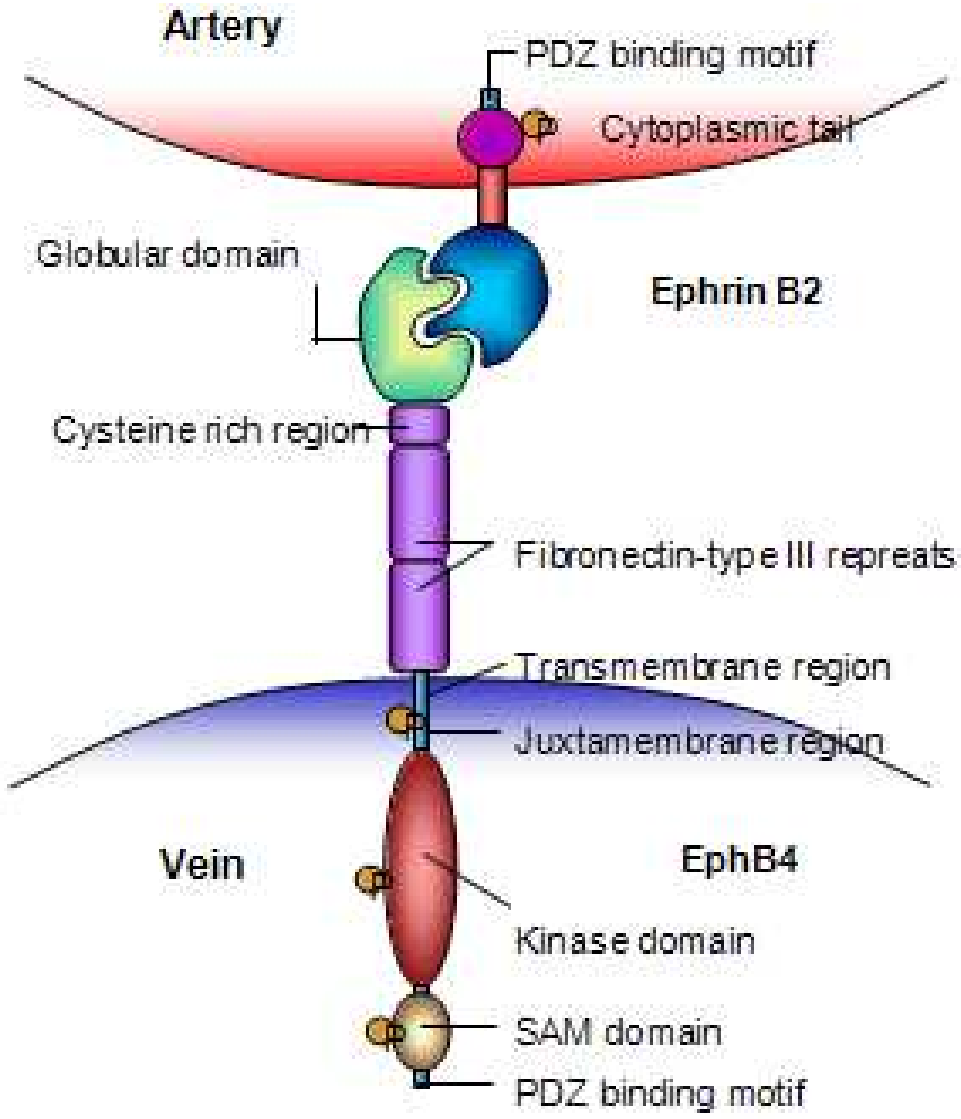
CNP over-expression???



Blood vessel formation and tumor angiogenesis. During development, VEGF induces differentiation and proliferation of endothelial cells from its progenitors (the hemangioblast and angioblast) to form a poorly differentiated primitive vascular plexus (vasculogenesis). Angiopoietin-1 (Ang-1) and other morphogens (e.g. Ephrins-Eph) induce remodeling of the vascular plexus into a hierarchically structured mature vascular system through endothelial cell sprouting, trimming differentiation and pericytes recruitment (angiogenesis). During tumor angiogenesis, angiopoietin-2 (Ang-2) destabilizes the vessel wall of mature vessels. Quiescent endothelial cells become sensitive to VEGF (or other angiogenic factors), proliferate and migrate to form new vessels. Bone marrow-derived endothelial cell progenitors are found in the peripheral blood and can recruit at sites of angiogenesis.

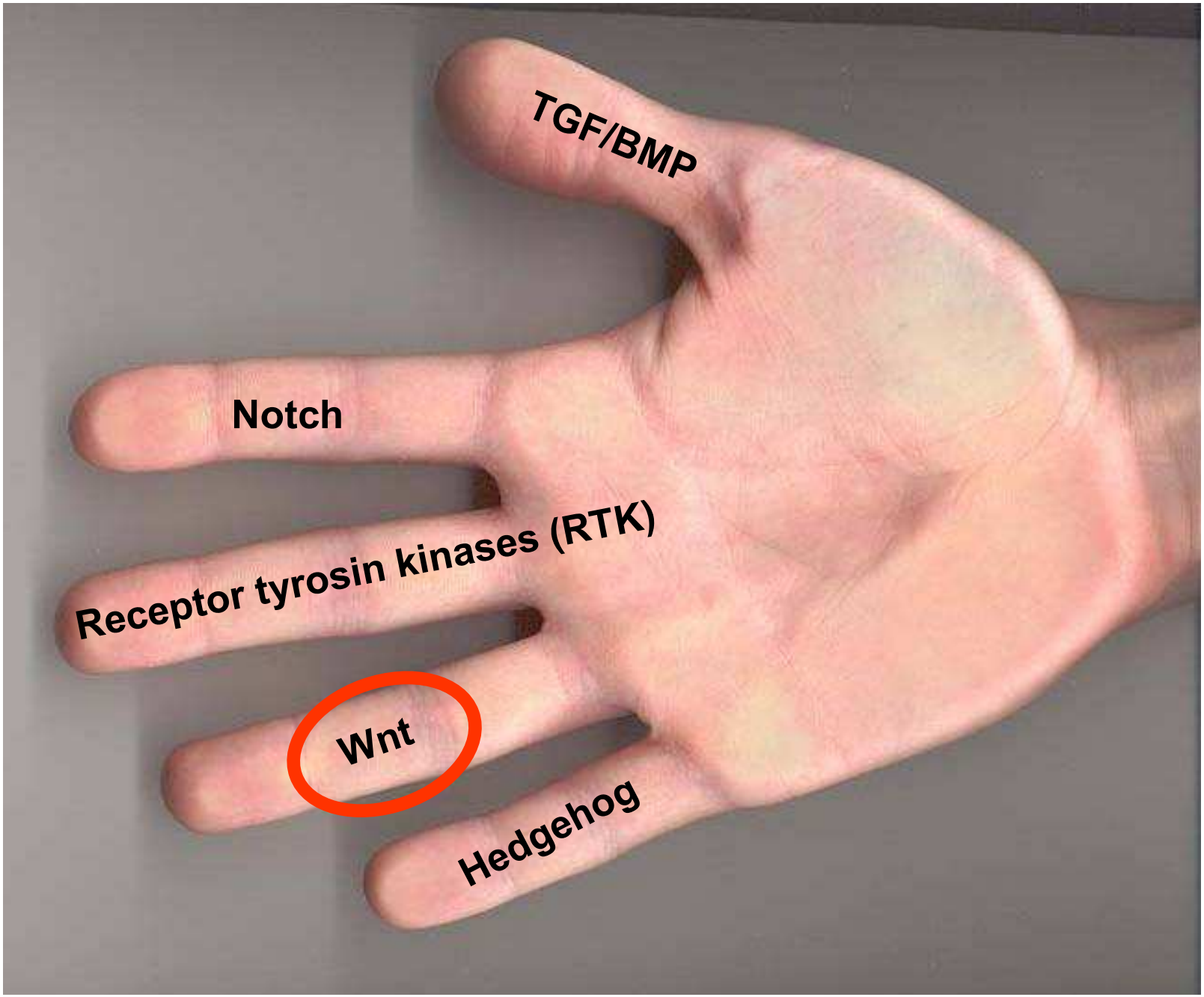
Eph/ephrin komplex

- ephrin – jsou membránově vázané ligandy (podobně jako ligandy Notch dráhy)
- ephriny A – na membráně upevněny pomocí tzv. GPI kotvy
- ephriny B – transmembránové ligandy, které samy jsou schopny signálovat do buňky
- Eph/ephrin systém je zapojen zejména do „navigace“ buněk (např. buněk cév) či jejich částí (např. navádění axonů v nervové soustavě), a do „contact-mediated cell sorting“ ve vyvíjejícím se embryu. Jde o obecný mechanismus regulující migraci buněk.



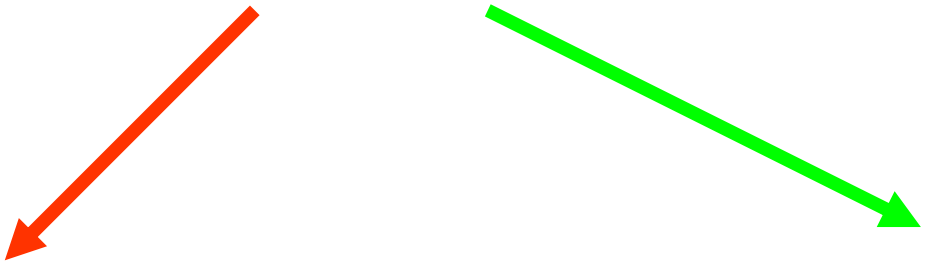
Schematic representation of EphB4 and Ephrin B2 structures

Signály z vnějšího prostředí



Wnts (Wingless/Int)

- family of ligands
- 19 members in human and mouse
- glycosylated and palmitoylated extracellular proteins
- short range of action, bind to extracellular matrix
- only in multicellular animals



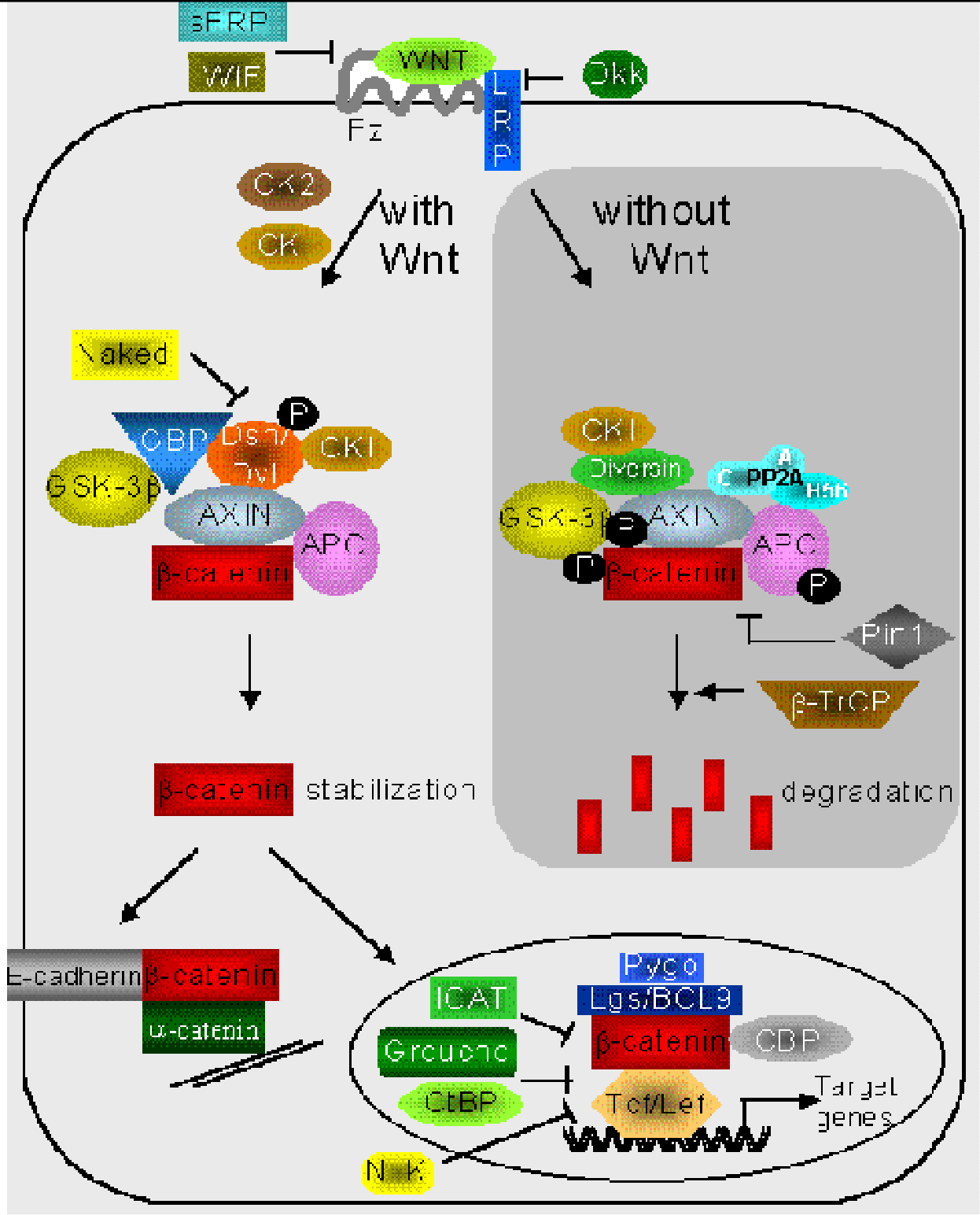
canonical

(eg. Wnt-1 or Wnt-3a)

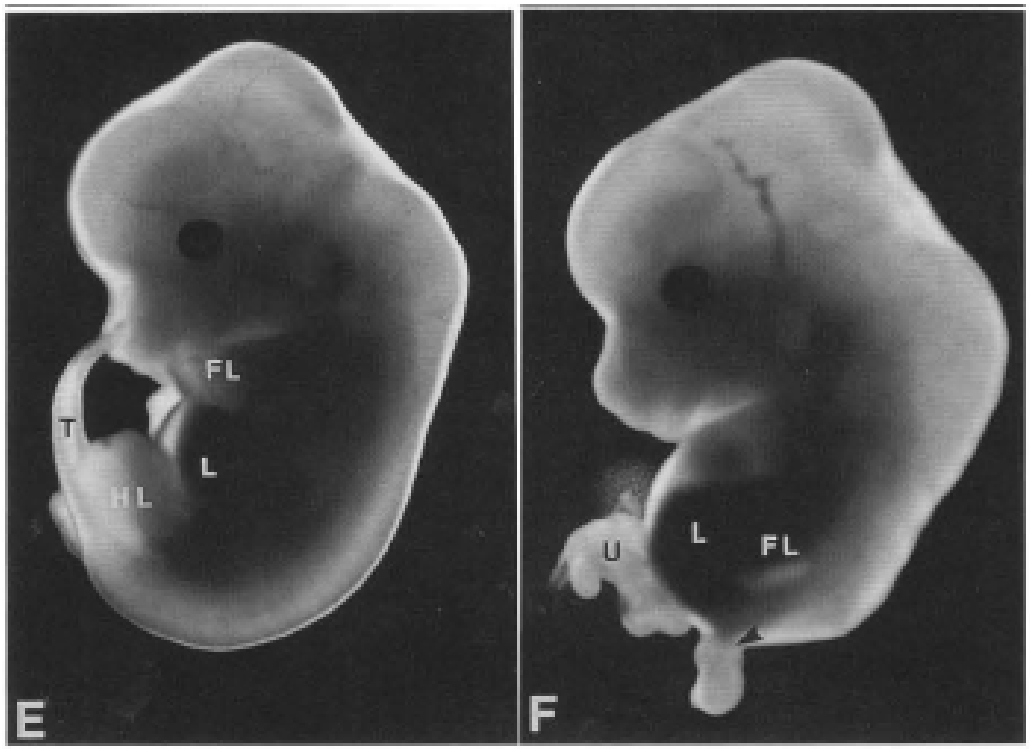
non-canonical

(eg. Wnt-5a)

Wnt/β-kateninová dráha



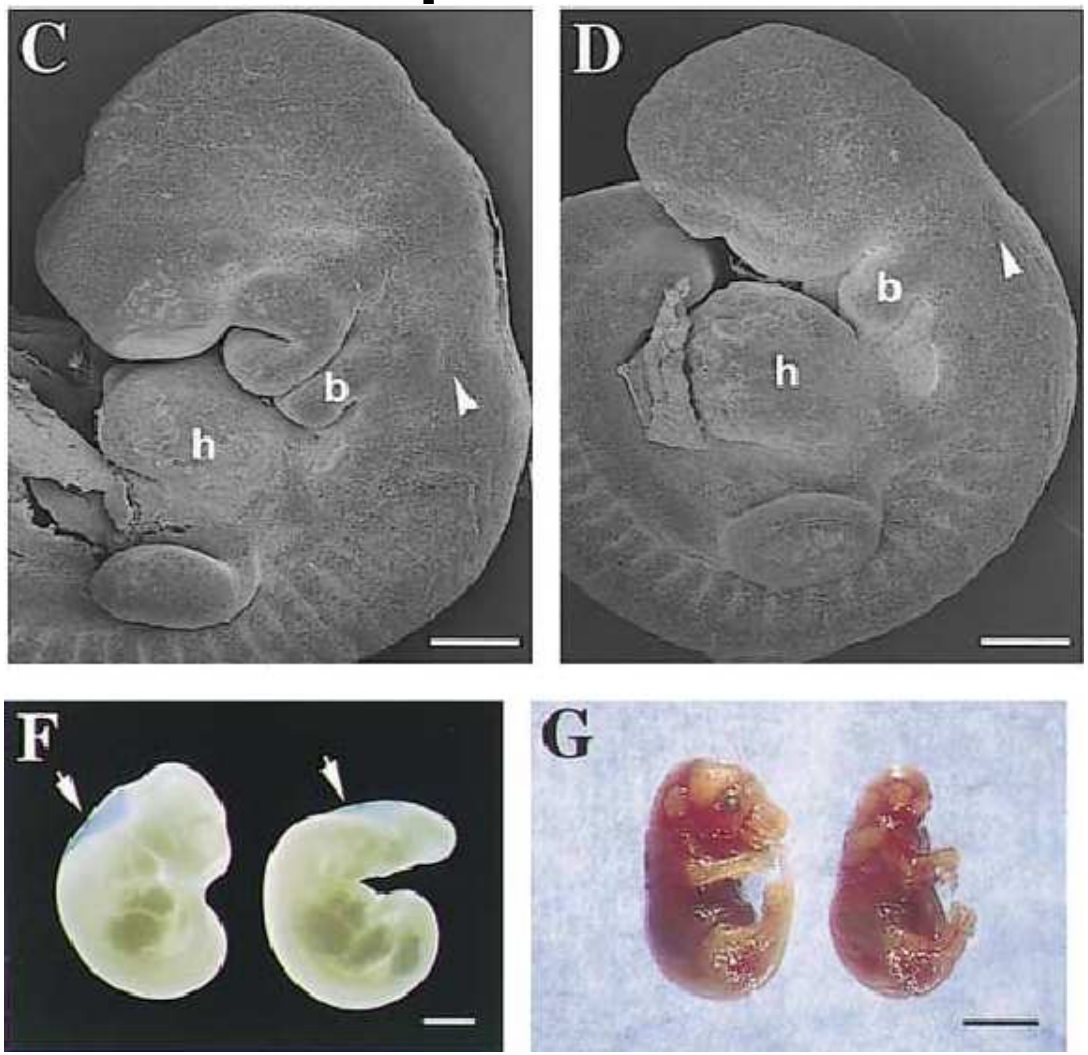
Deplece Wnt/β-kateninové dráhy při gastrulaci = ztráta zadních částí těla



wild type

Wnt-3a knockout

Deplece inhibitorů Wnt/β-kateninové dráhy při gastrulaci = ztráta předních částí těla

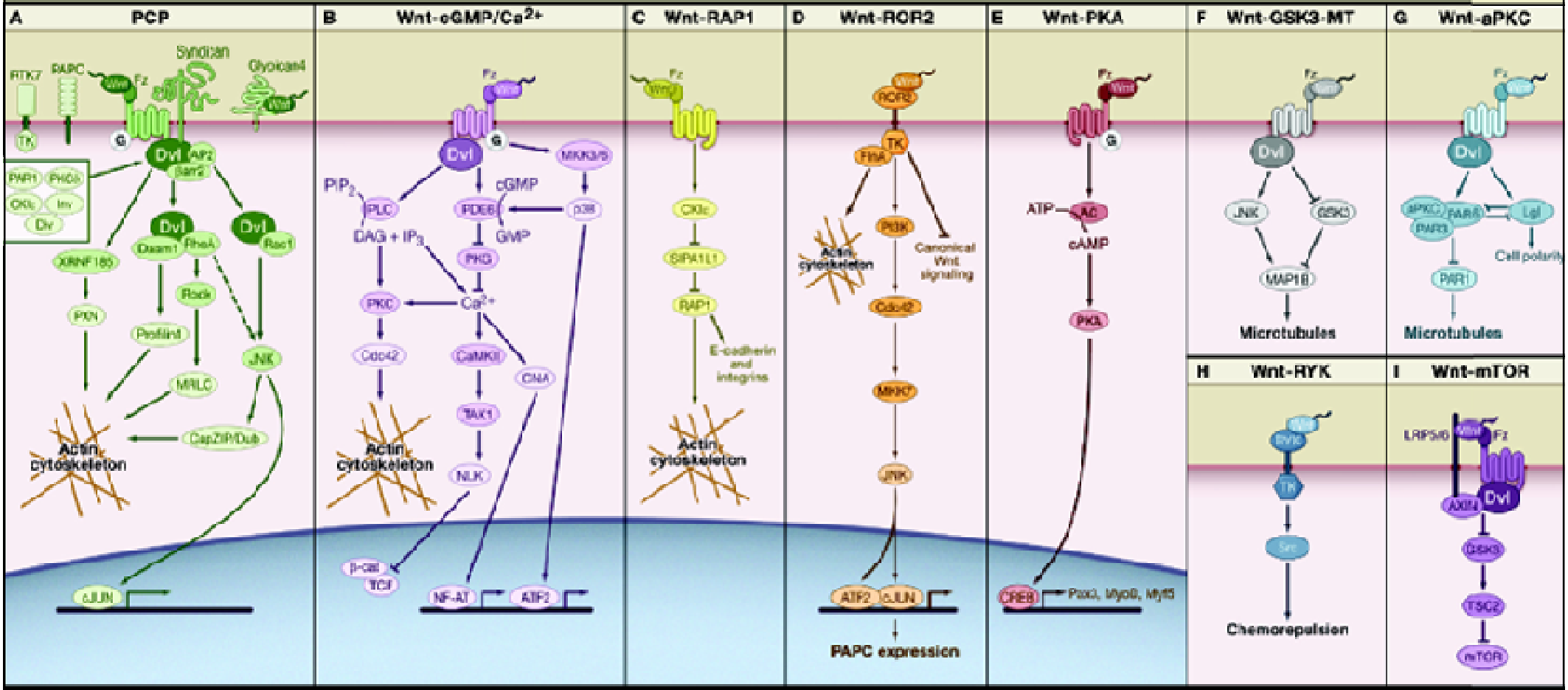


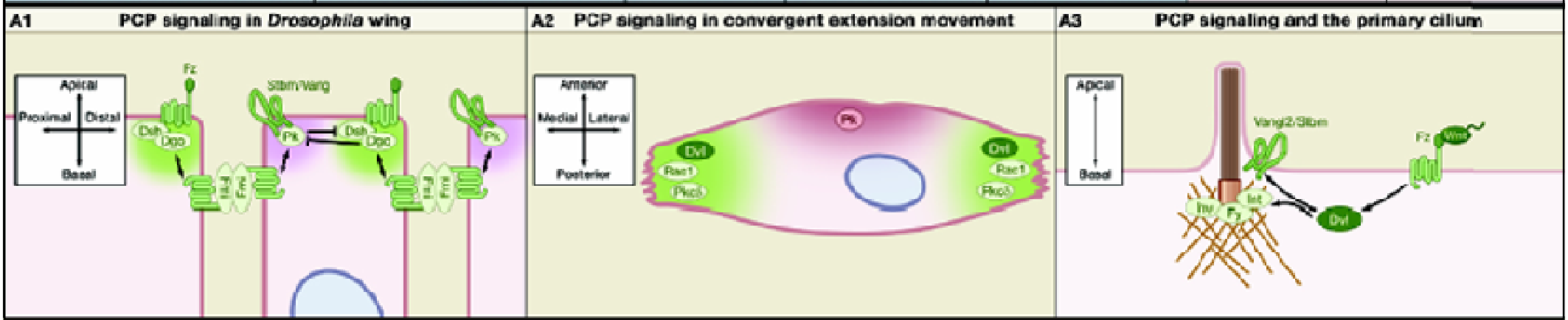
wild type vs. Dkk1 knockout

SnapShot: Noncanonical Wnt Signaling Pathways

Mikhail V. Semenov,[1] Raymond Habas,[2] Bryan T. MacDonald,[1] and Xi He[1]
[1]Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA; [2]University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, USA

Cel





Molekulární mechanismus ustavení PCP

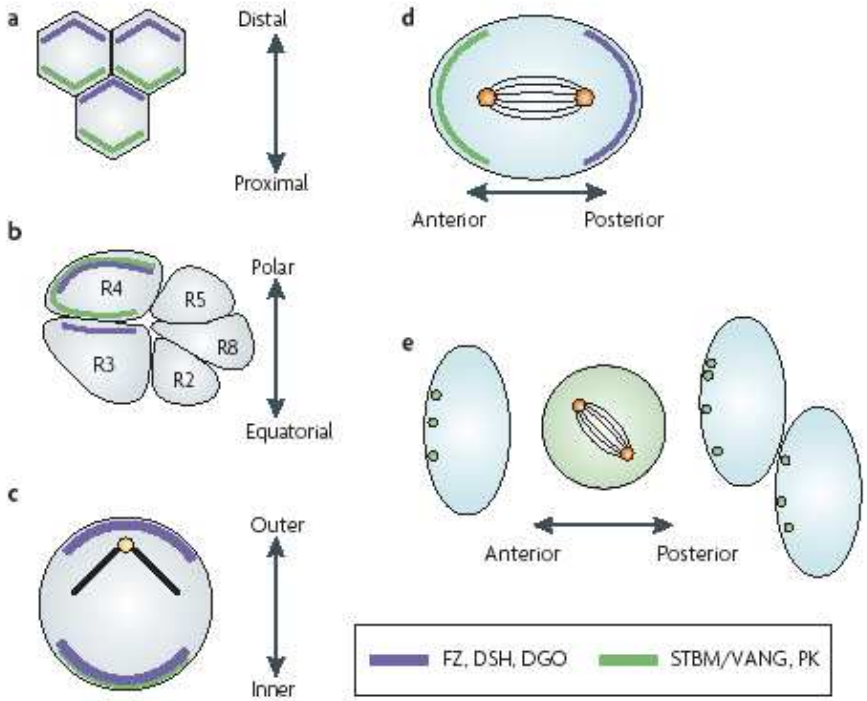
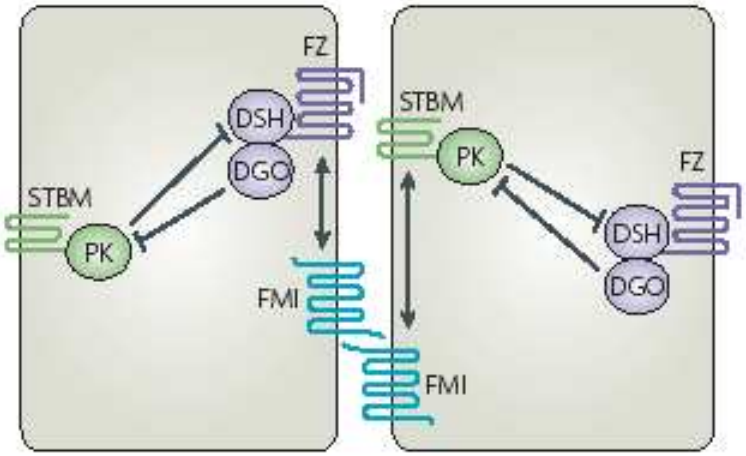


Figure 2 | **Subcellular distribution of core Fz/PCP factors in *Drosophila melanogaster* and vertebrates. a–c** | Examples of cells with epithelial character (marked by grey shading). *Drosophila melanogaster* wing cells and eye R3 and R4 cells and mouse sensory hair cells in the cochlea (inner ear) are shown in **a**, **b** and **c**, respectively. **d**,**e** | Examples of dividing cells. The spindle orientation in the *D. melanogaster* sensory organ precursor (SOP) cells depends on the asymmetric distribution of the Frizzled (Fz)/planar cell polarity (PCP) factors (as shown in **d**), as does the orientation of neuroectodermal cells in zebrafish (as shown in **e**; note that during mitosis the asymmetric distribution of PK is lost and then re-established). Depending on the tissue, only a subset of the respective proteins has been analysed (the *D. melanogaster* wing is the only tissue in which all proteins were analysed; all but DSH have been analysed in the eye). These illustrations represent the localizations patterns of PCP proteins at the proposed time of signalling. In the wing, asymmetry of Flamingo (FMI) has been reported earlier, but the relevance of this is unknown[82]. Note that in the mouse inner ear (as shown in **c**) vang-like 2 (VANGL2) and FZ3/FZ6 localize to the same side of the cells; it is not known whether other Fz family members localize with the DSH homologues DVL1 and DVL2 to the opposite side. During zebrafish gastrulation (as shown in **e**) Prickle (Pk), which is represented by green circles, is cytoplasmic during cell division but regains polarity after separation of the daughter cell. Only PK has been analysed in this context, but its localization depends on the presence of Strabismus (STBM).

Box 1 | **Molecular interactions between the Fz/PCP core factors**

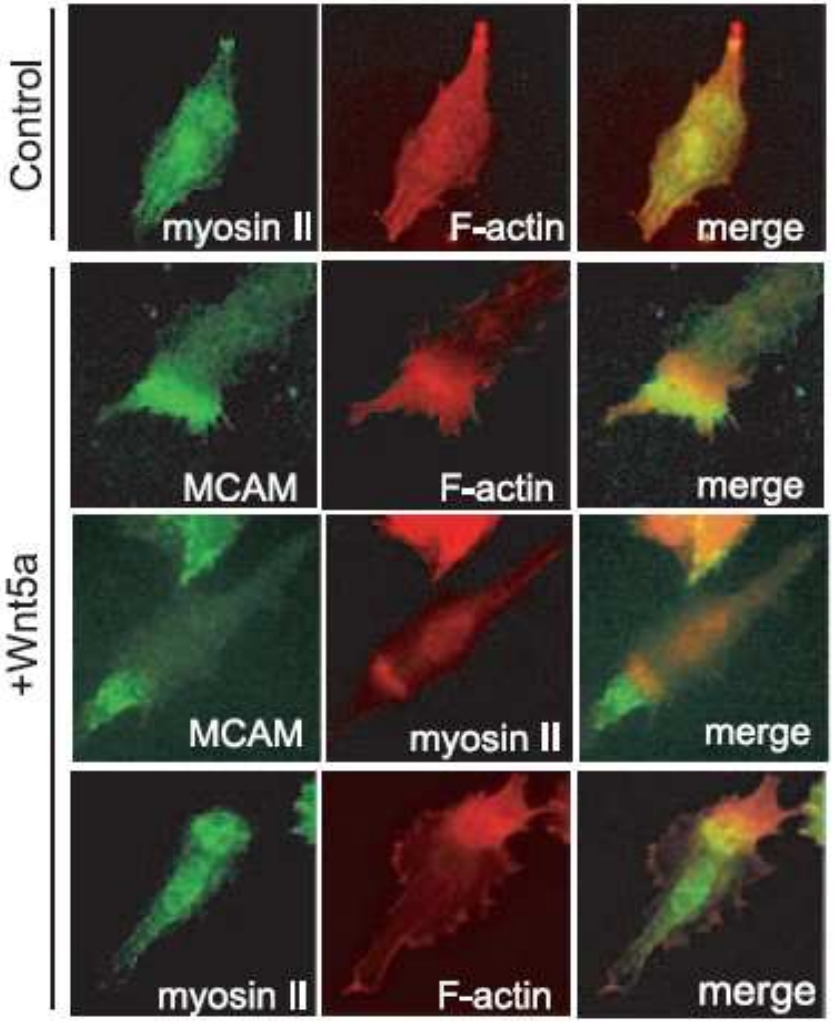
The molecular logic of the formation and separation of the Frizzled–Dishevelled–Diego (FZ–DSH–DGO) and Prickle–Strabismus (PK–STBM) complexes has started to be unravelled. In FIG. 2 are reported examples of the localization of each complex in various tissues. The figure is an apical view of two cells that have attained asymmetric localization of the two complexes. Several lines of

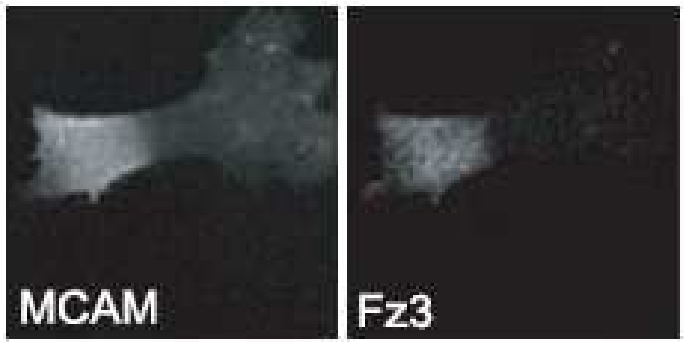


Seifert and Mlodzik, Nature Reviews in Genetics, 2007

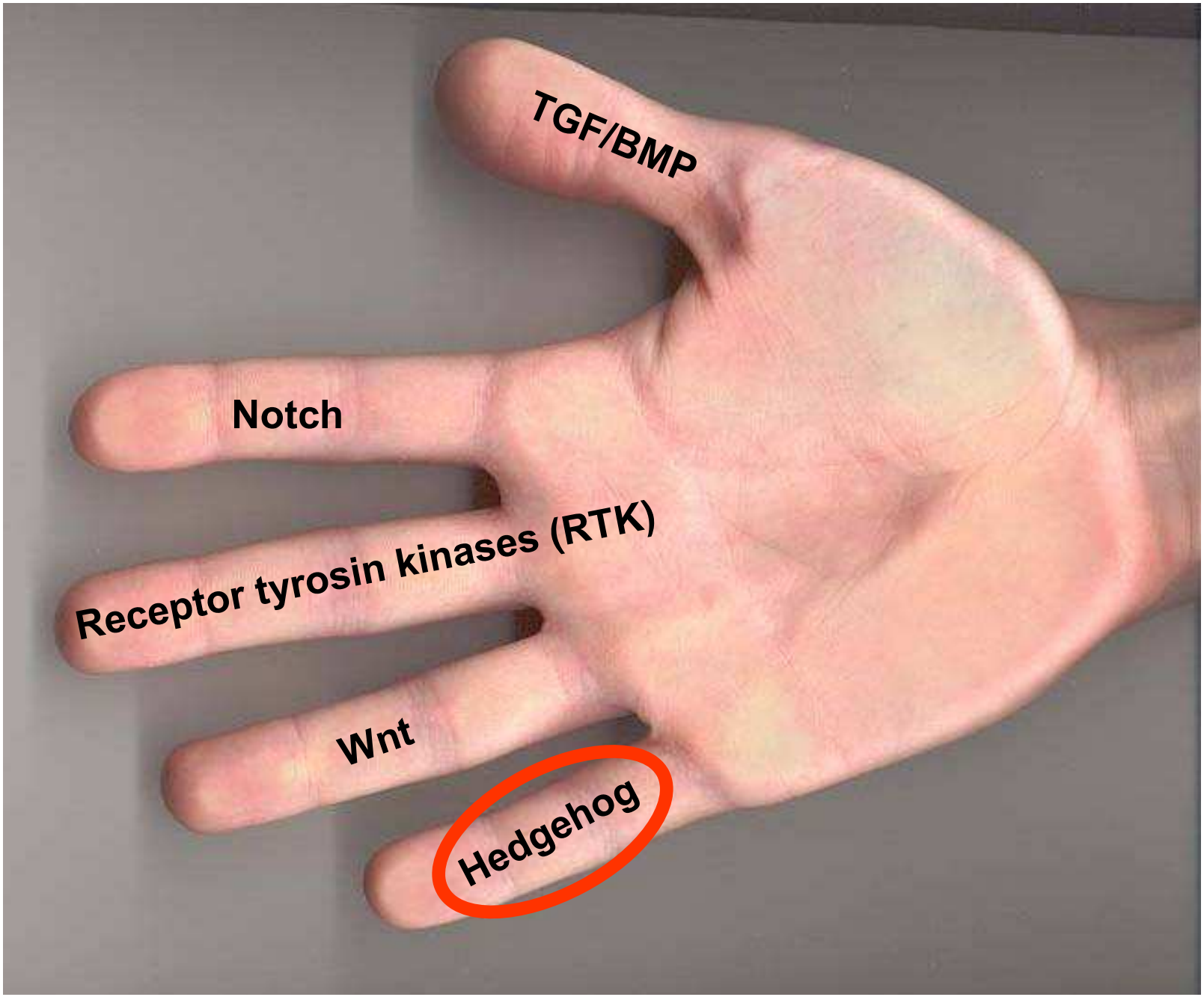
Wnt-induced assymetry

W-RAMP = Wnt-mediated receptor-actin-myosin polarity



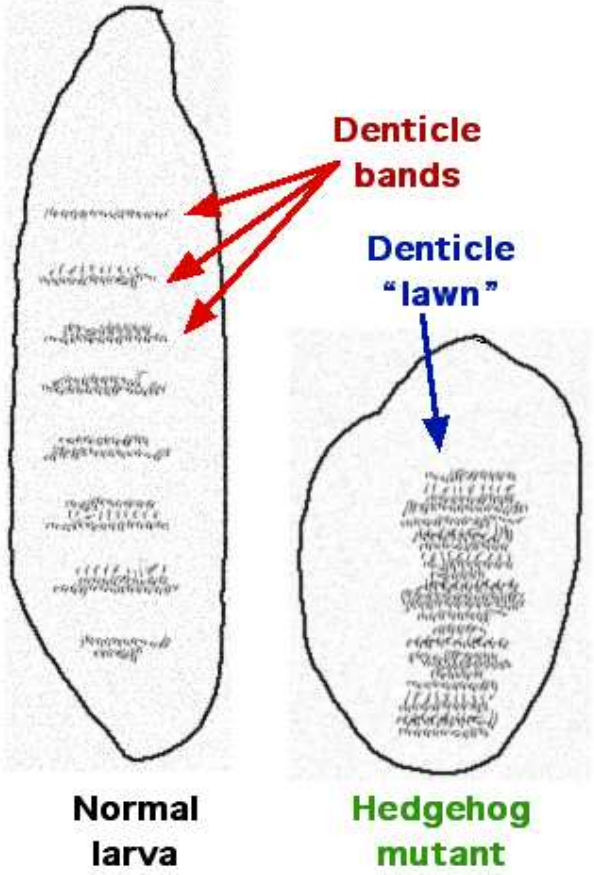


Signály z vnějšího prostředí



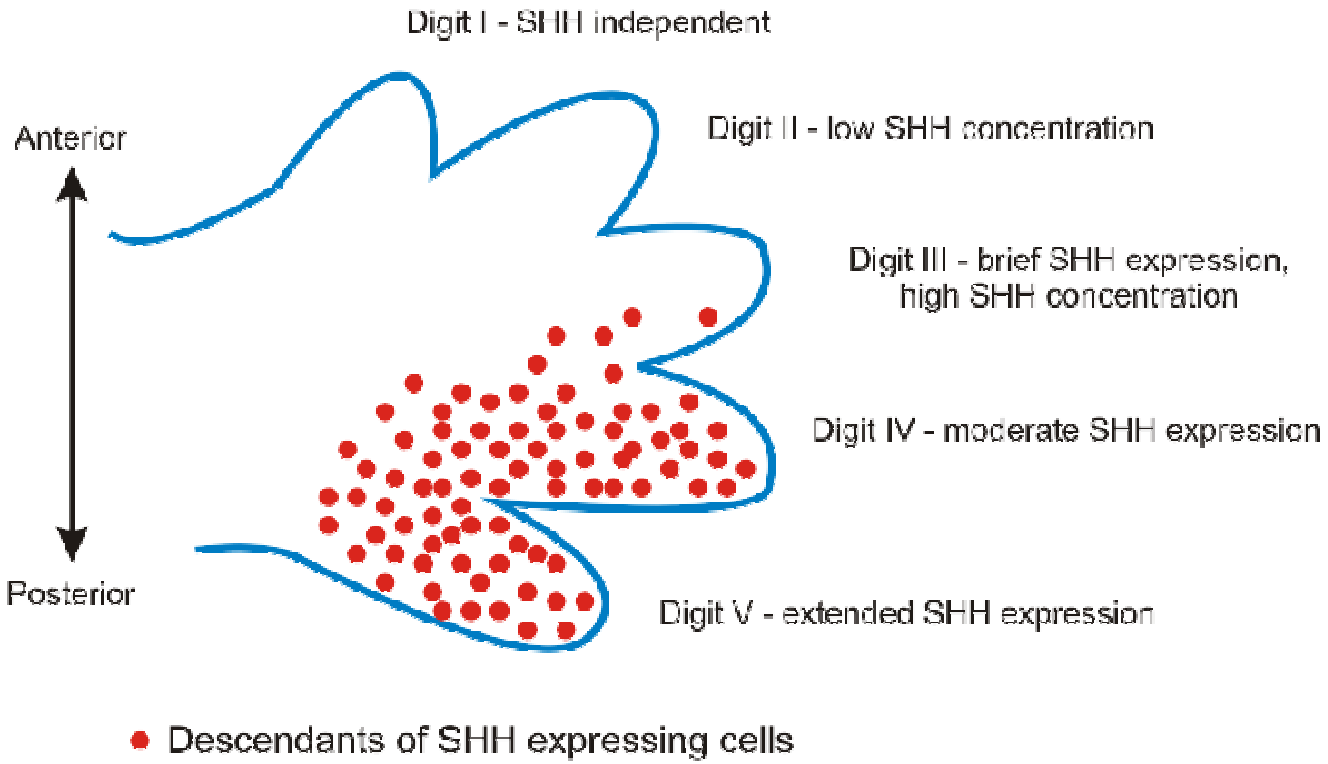
Hedgehog dráha

- hedgehog (Hh) u octomilky – název „ježek“ podle fenotypu larvy
- u savců jsou tři homology – sonic hedgehod (Shh), indian hedgehog (Ihh) a desert hedgehog (Dhh)

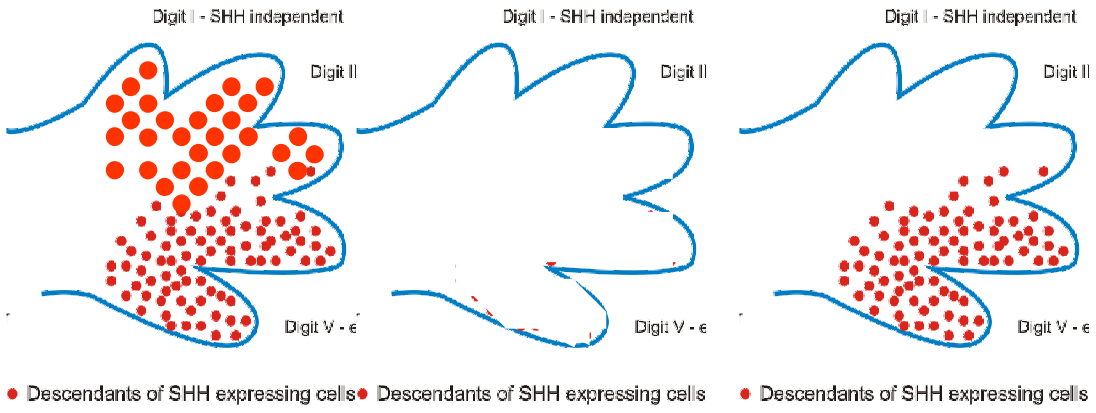


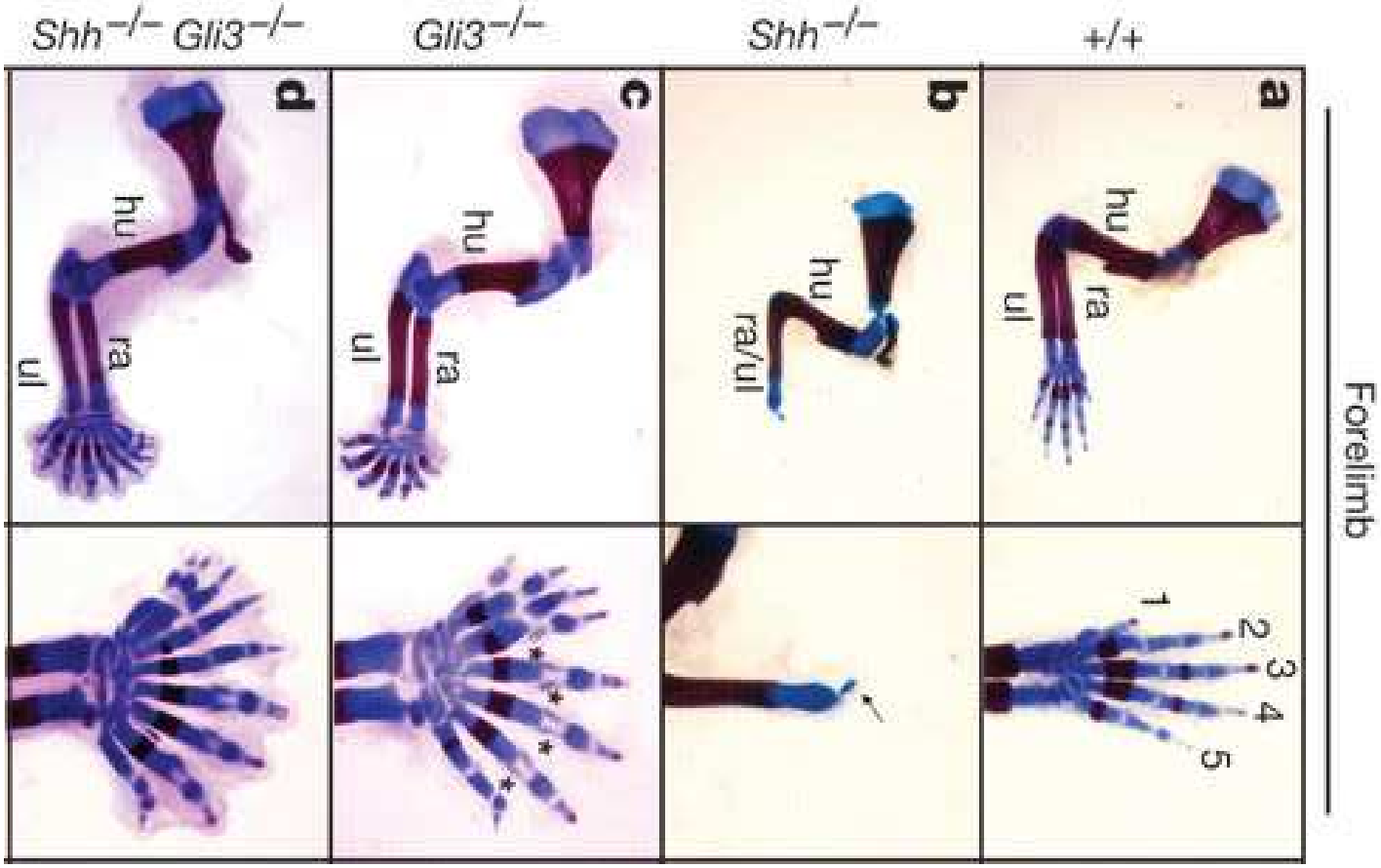
Sonic the Hedgehog

Shh – jeden z nejlépe popsaných klasických morfogenů (tzv. model francouzské vlajky) – v závislosti na koncentraci morfogenu se spouští odlišné transkripční programy



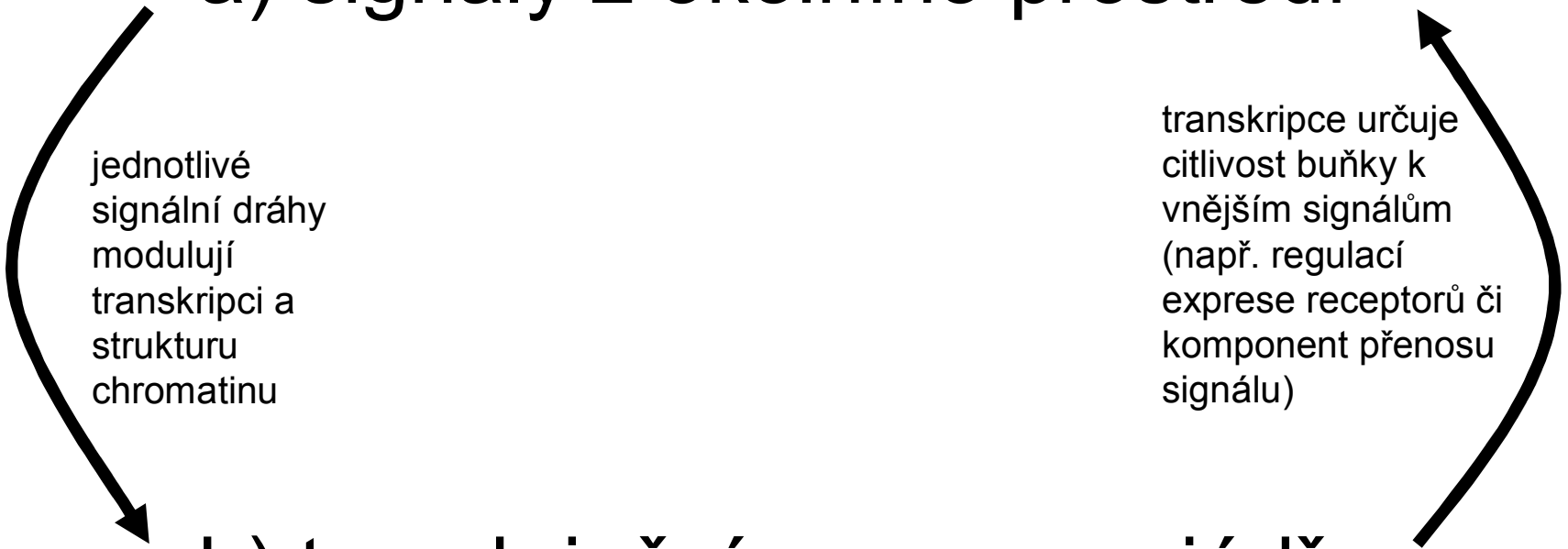
Např. specifikace jednotlivých prstů končetiny


Digit I - SHH independent
Digit II
Digit V - e
Descendants of SHH expressing cells
Digit I - SHH independent
Digit II
Digit V - e
Descendants of SHH expressing cells
Digit I - SHH independent
Digit II
Digit V - e
Descendants of SHH expressing cells


Shh−/− Gli3−/−
Gli3−/−
Shh−/−
+/+
d
c
b
a
hu
ra
ul
hu
ra
ul
hu
ra/ul
hu
ra
ul
1
2
3
4
5
Forelimb

Co poskytuje buňce tyto informace?

a) signály z okolního prostředí



b) transkripční program v jádře

TRANSCRIPTION FACTORS

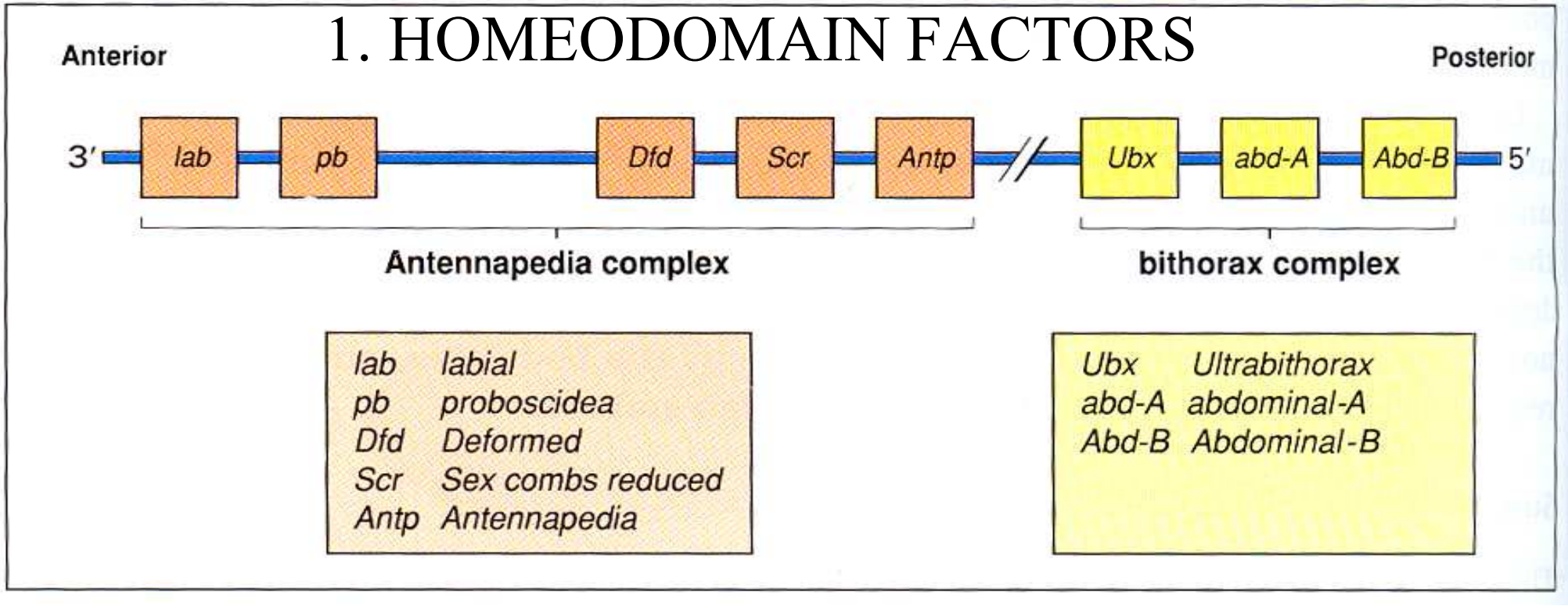
-ultimate effectors of the outside-in intercellular signaling
-one of the most important molecules in shaping of the embryo
-classified in families by DNA-binding domain

1. HOMEODOMAIN FACTORS

Homeodomain – 60aa sequence that interact with DNA, encoded by homeobox in the particular gene. Many homeobox genes regulate segment identity.
Homeotic genes cause **homeosis** – a transformation of one whole segment into another related one, such as antenna into leg.

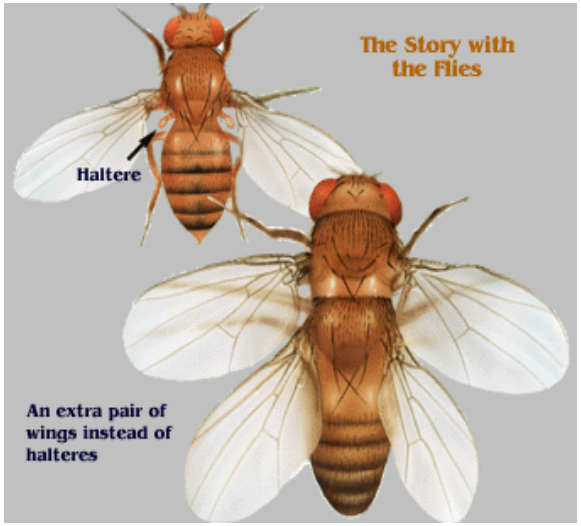
Segment polarity genes – basic shape of segment, same for all segments.
Homeotic selector genes – control the differences in segment development via initiation of future developemntal pathways in each segment.

1. HOMEODOMAIN FACTORS



Antennapedia

Bithorax

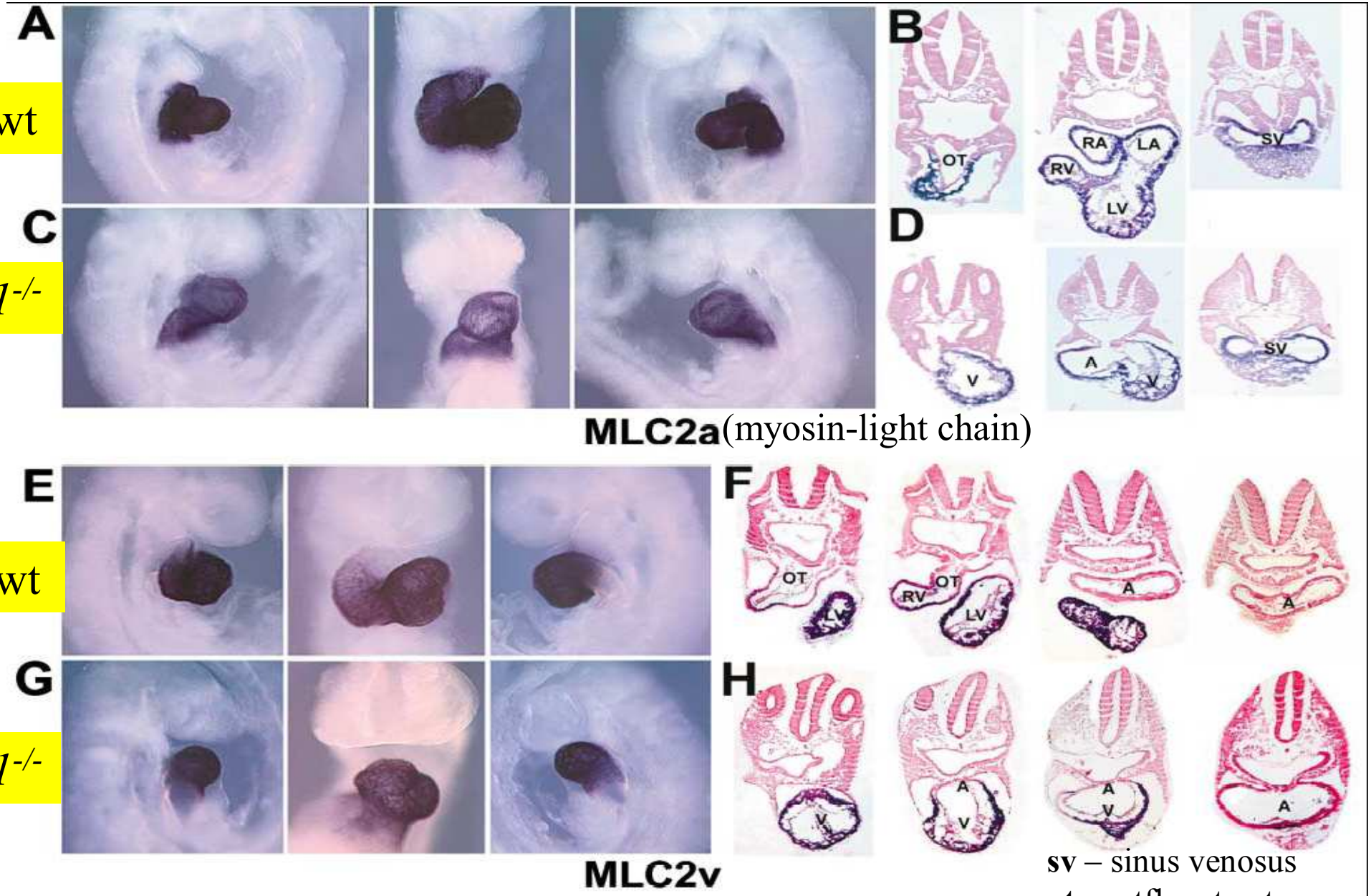


Chinook

2. LIM-HOMEODOMAIN PROTEINS

Two LIM domains fused to DNA-binding homeodomain

Islet-1 E9.5

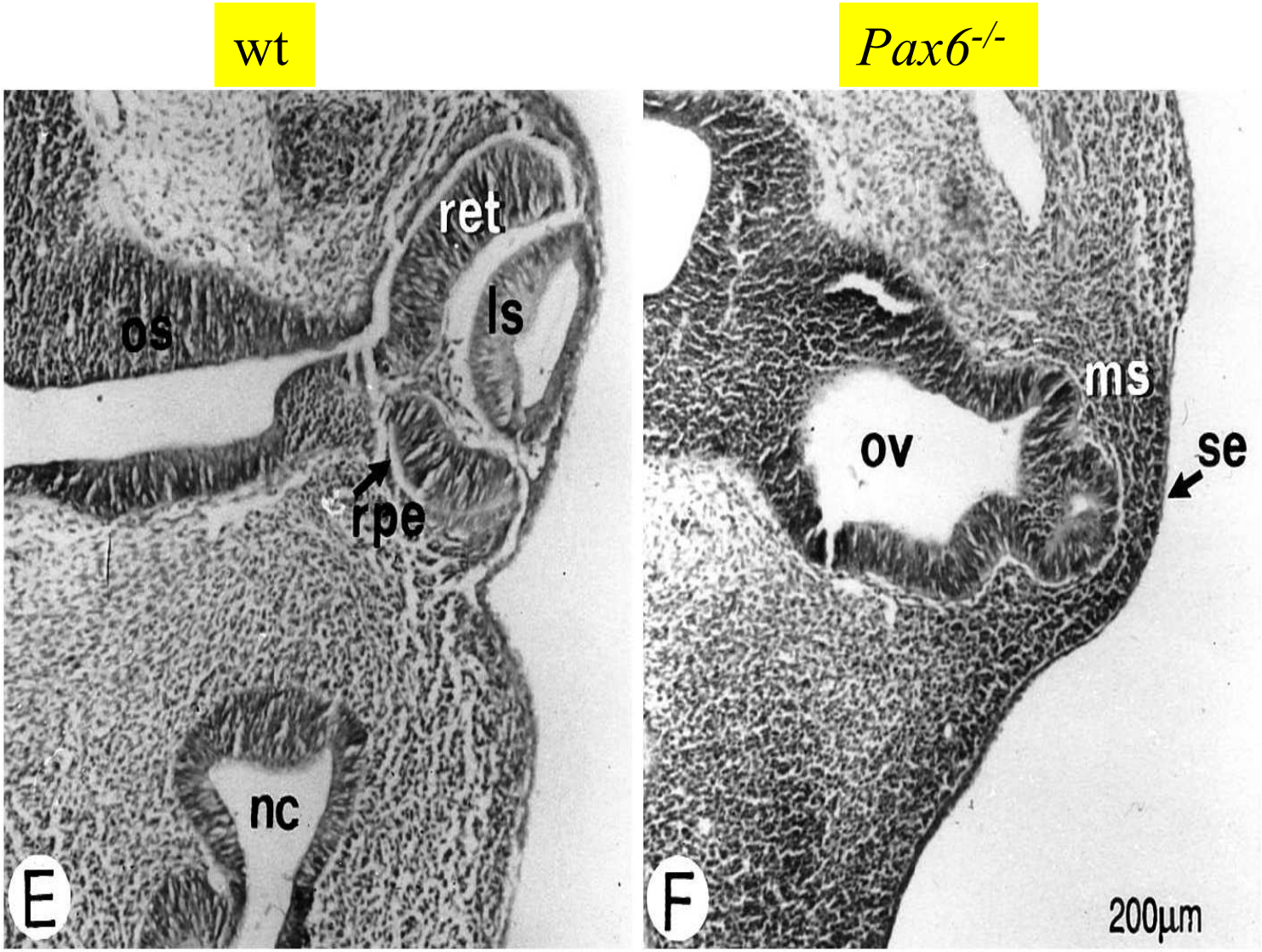


3. PAX PROTEINS

DNA binding region: paired domain with 6 α-helical segments

Pax6 (paired box gene 6)

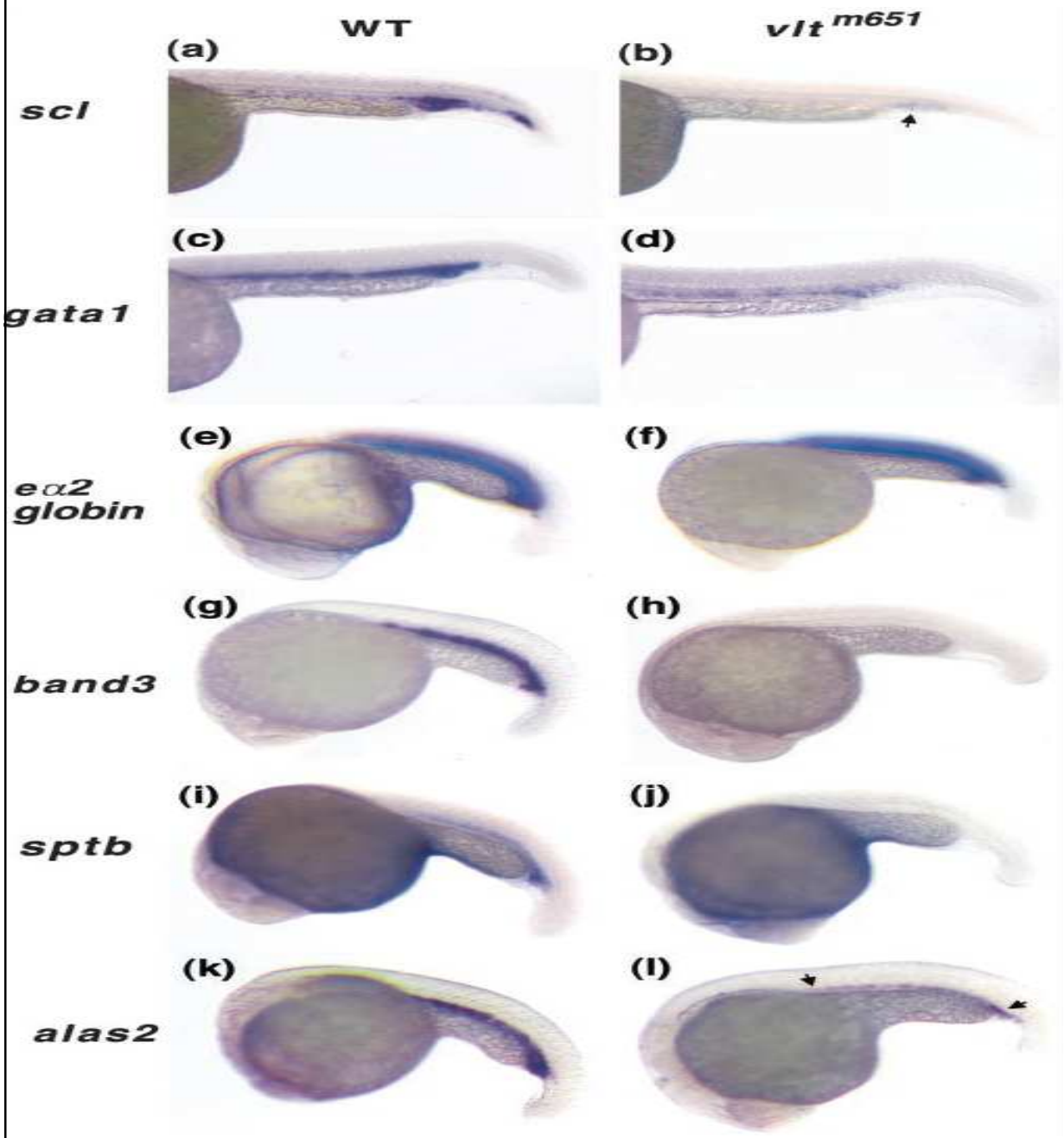
se - surface ectoderm
ms –mesenchymal-like cells
rpe – pigmented retinal epithelium
ret – retina
os – optic stalk
ov- optic vesicle
nc – nasal cavity
ls -lens



E11.5

4. ZINC-FINGER PROTEINS

Bind DNA via zinc-finger motif

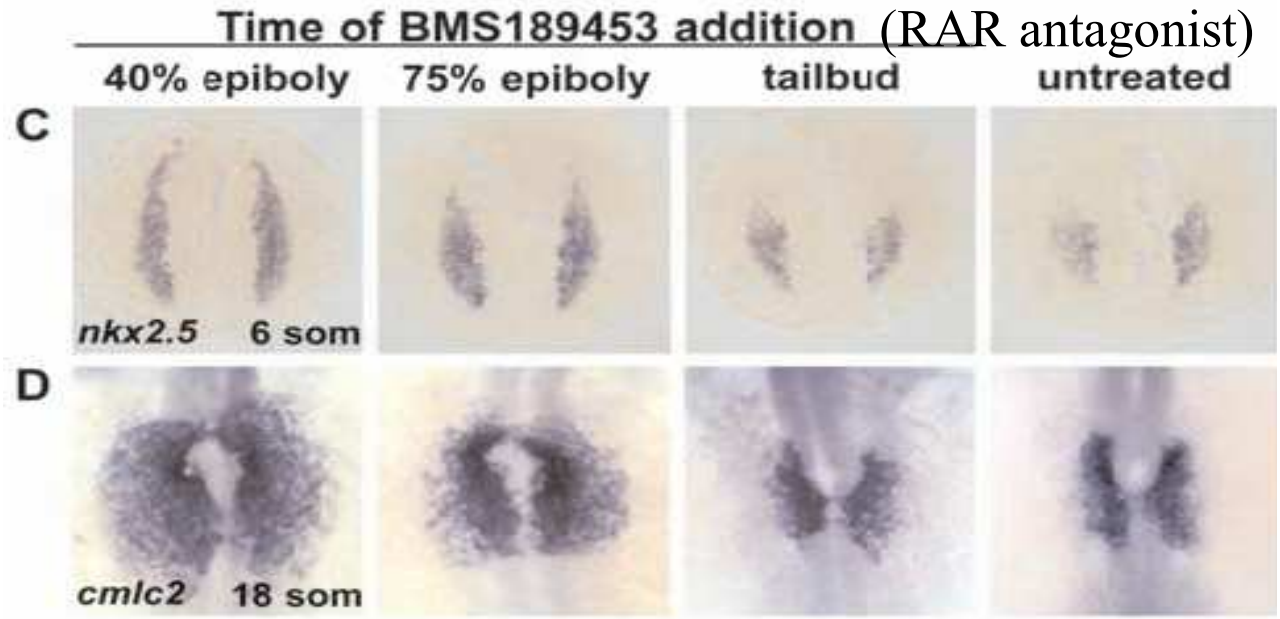


Dracula fish – loss-of-function mutation in GATA1 – impaired erythroid differentiation

5. NUCLEAR RECEPTOR SUPERFAMILY

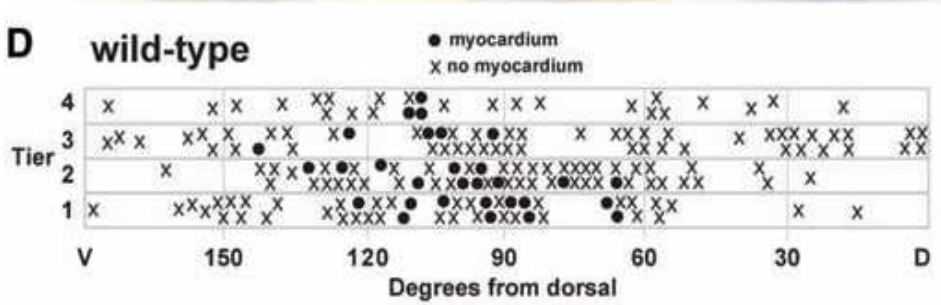
Intracellular receptor that function as transcription factors. Lipophilic ligands: steroids, thyroid hormone, retinoid acid. Inactive – Hsp90-bound in cytoplasm, complexed with ligand, - nuclear.

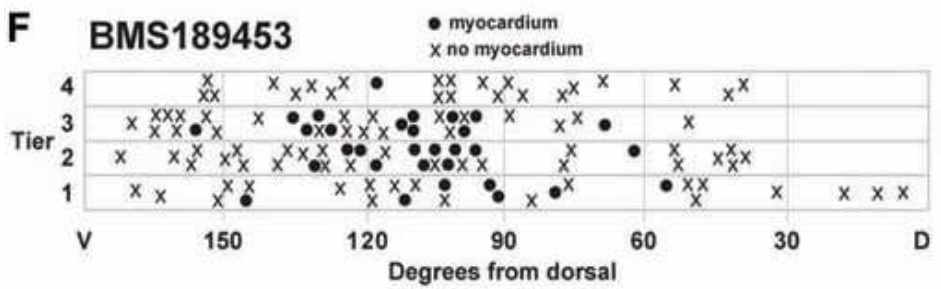
Retinoid acid signaling in limiting the cardiac progenitor pool

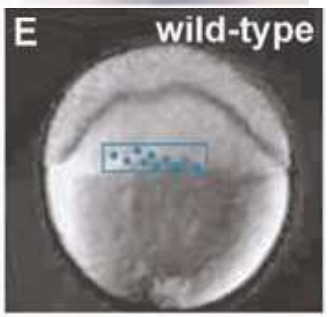


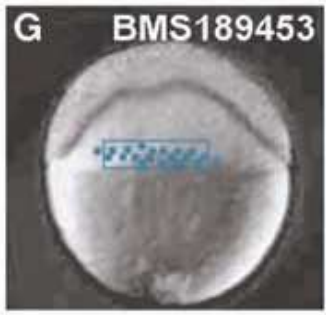
precardiac mesoderm

cardiomyocytes

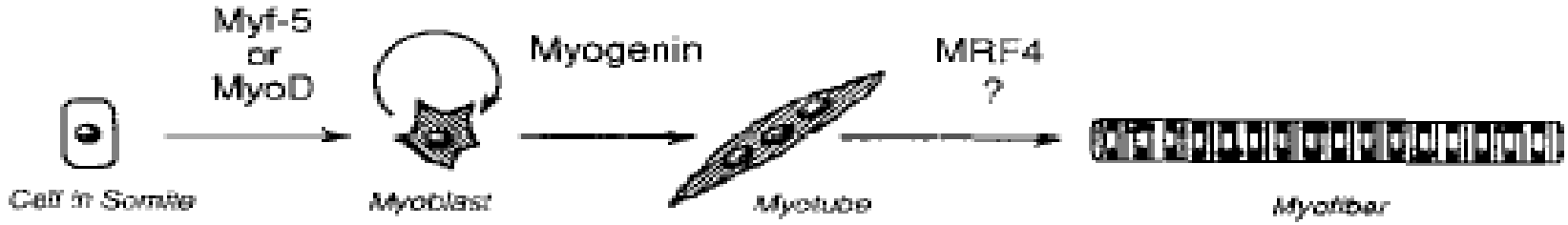




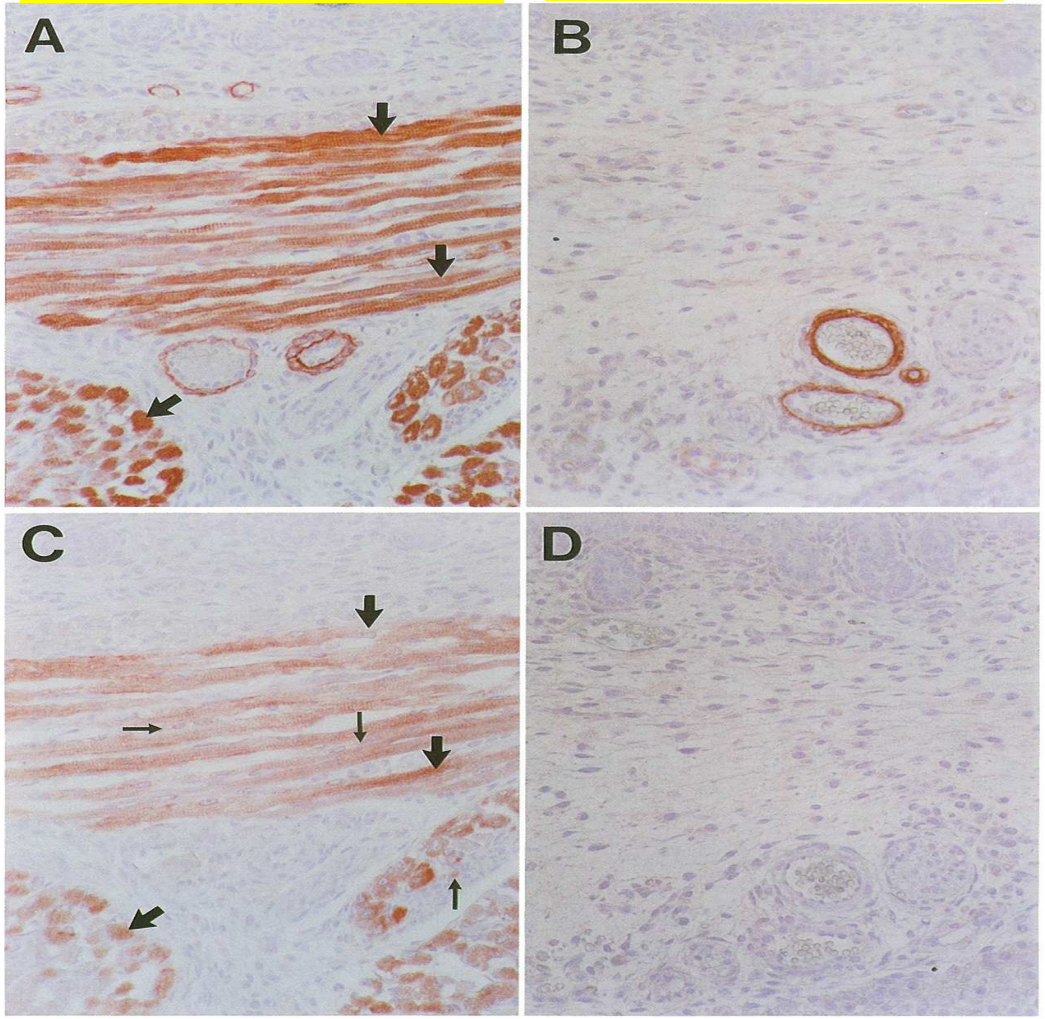




6. BASIC HELIX-LOOP-HELIX (bHLH) FACTORS



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



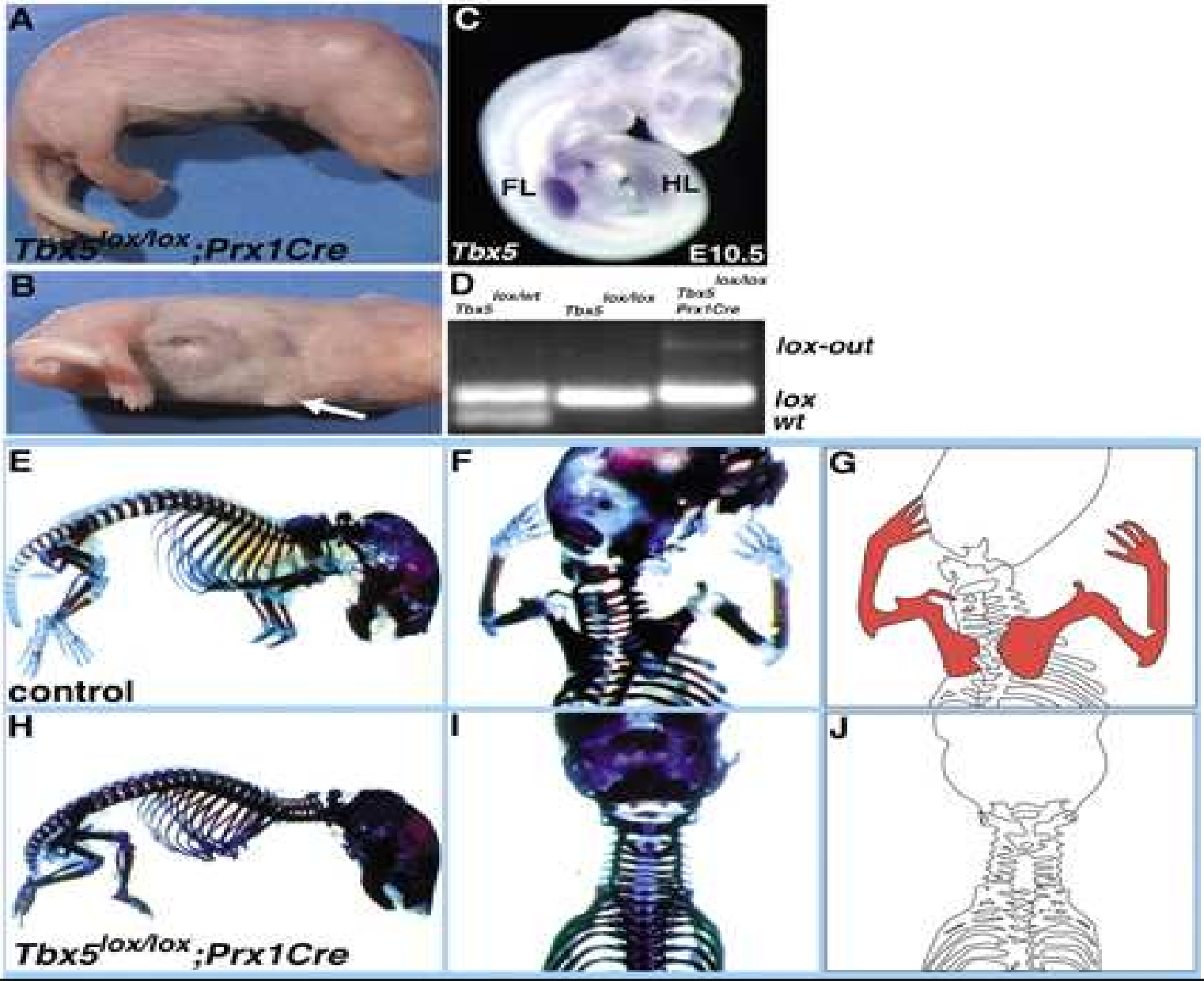
Contain basic DNA binding region and hydrophobic helix-loop-helix region responsible for dimerisation

α-actin IHC
(smooth and striated muscle fibers)

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

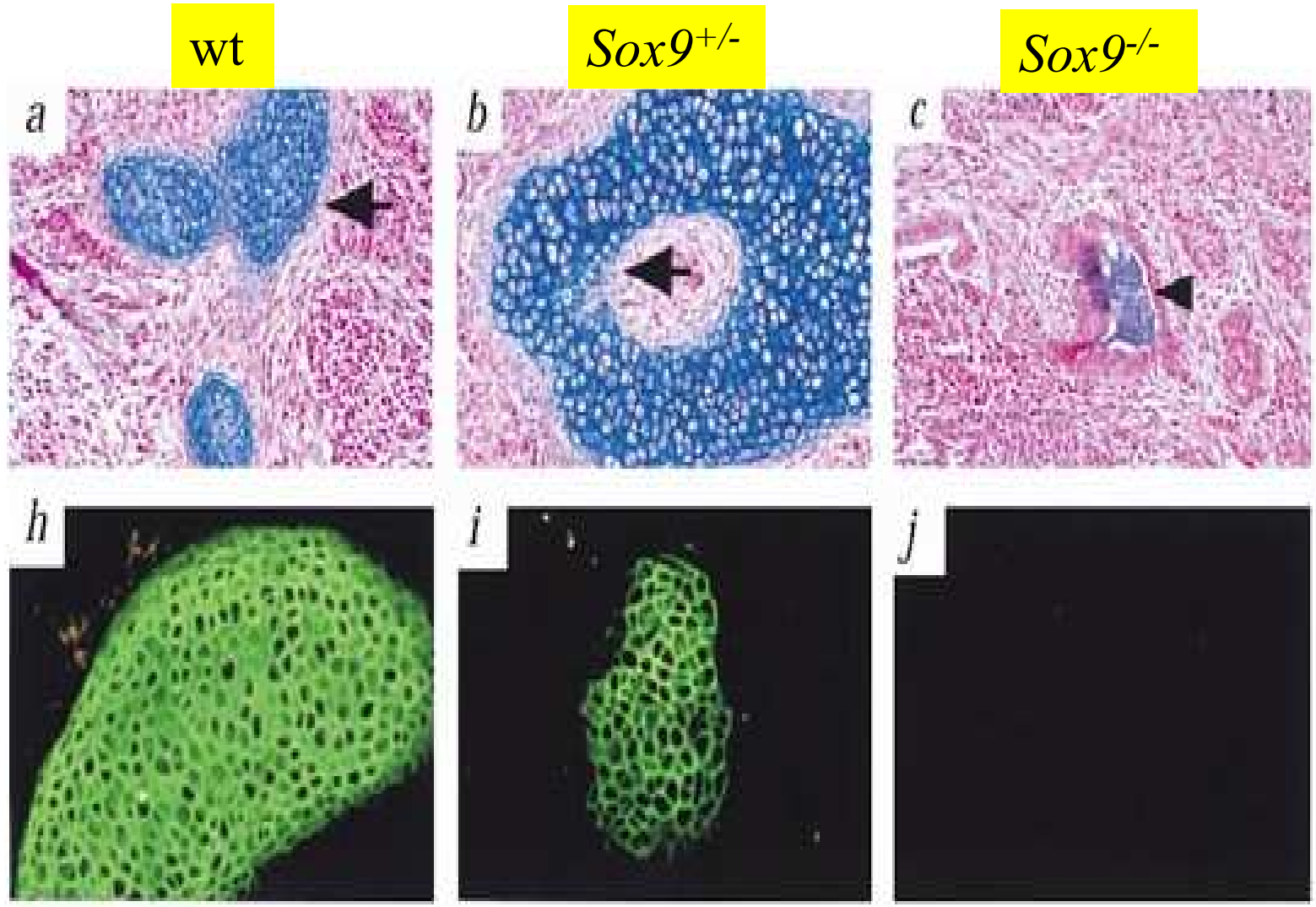
7. T-BOX FACTORS

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



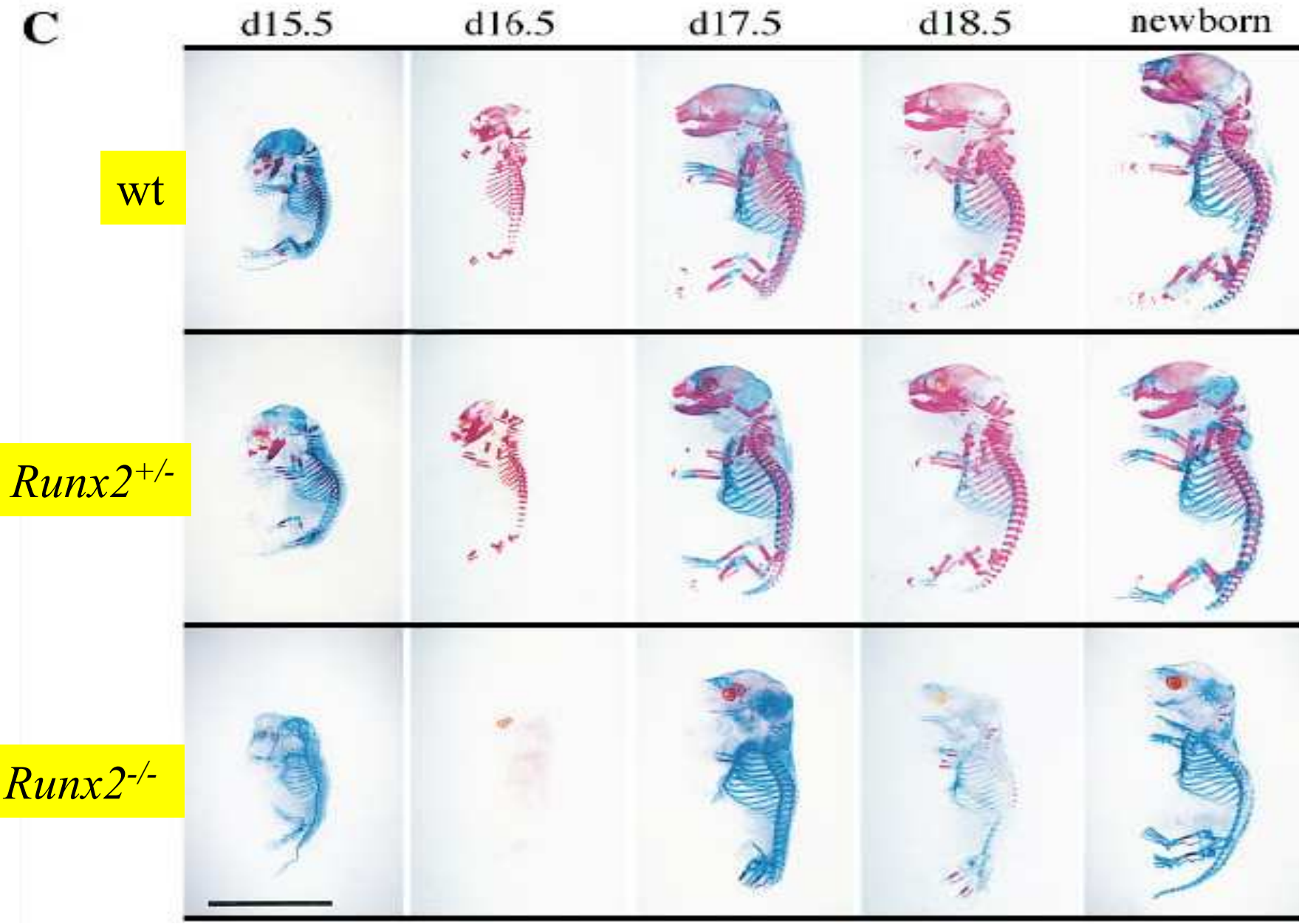
8. HIGH MOBILITY GROUP (HMG)-box FACTORS

Operate via bending DNA to bring regulatory sites with transcriptional complex
Sox9 (Sry-Box9) – master inducer of cartilage



10. RUNT DOMAIN-CONTAINING FACTORS

Runx2 (Runt-related transcription factor 2)



2006- INDUCIBLE PLURIPOTENT CELLS (iPS)

Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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DOI 10.1016/j.cell.2006.07.024

SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors

or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.