

Lecture 10:

Wnt morphogenetic system

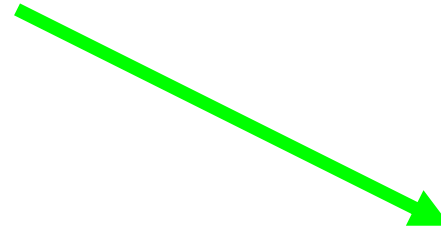
Wnts (Wingless/Int)

- 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 -catenin dráha (= kanonická dráha)

- např. Wnt-1 nebo Wnt-3a



- induce axis duplication in Xenopus

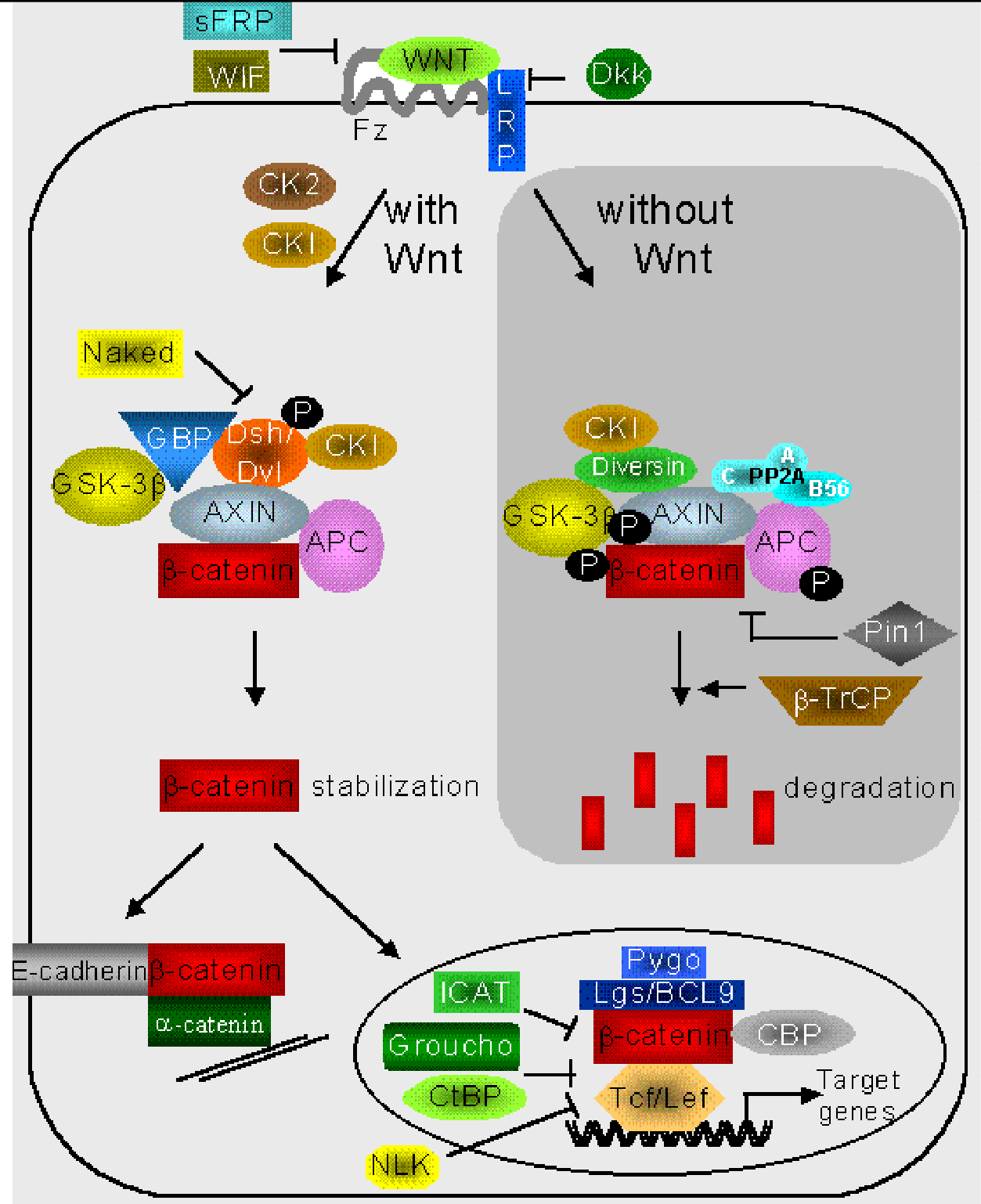
- induce transformation of mammary cell line C57mg

-signal via nuclear translocation of β -catenin



Moon-cel2[1].swf

Wnt/ β - kateninová dráha



Příklady vývojových procesů
regulovaných kanonickou Wnt
dráhou

Maternální Wnt/ β -cateninová dráha
determinuje dorsální (horní) pól
vyvíjející se zygoty a embrya

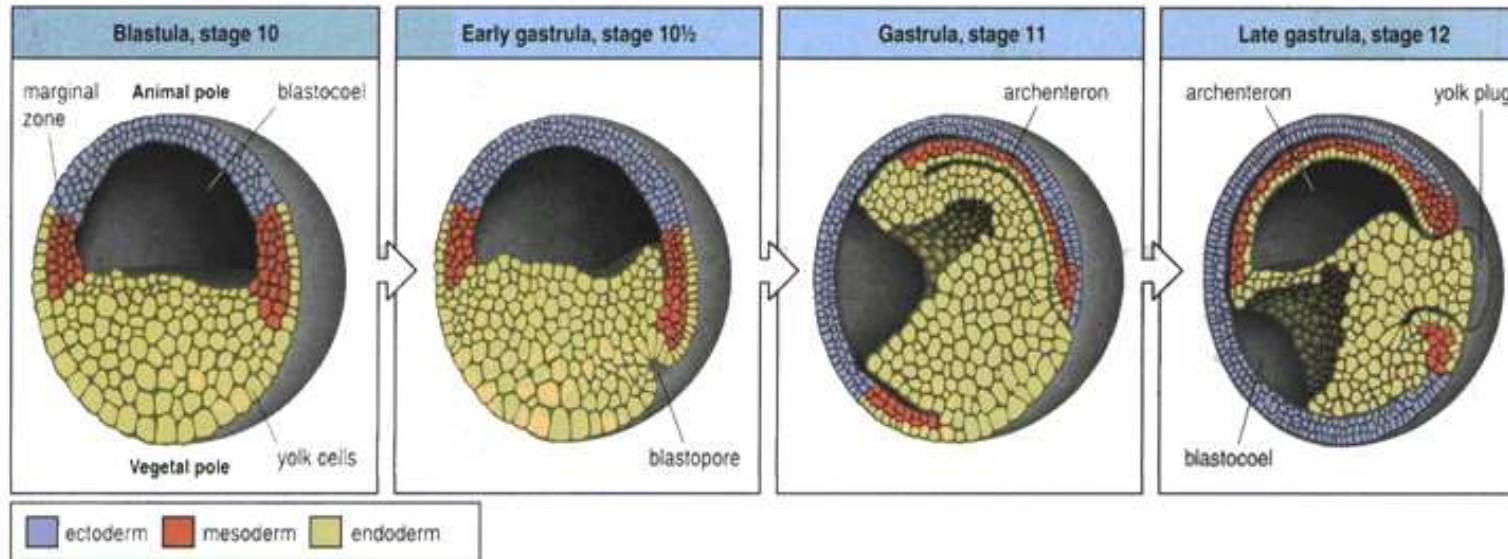
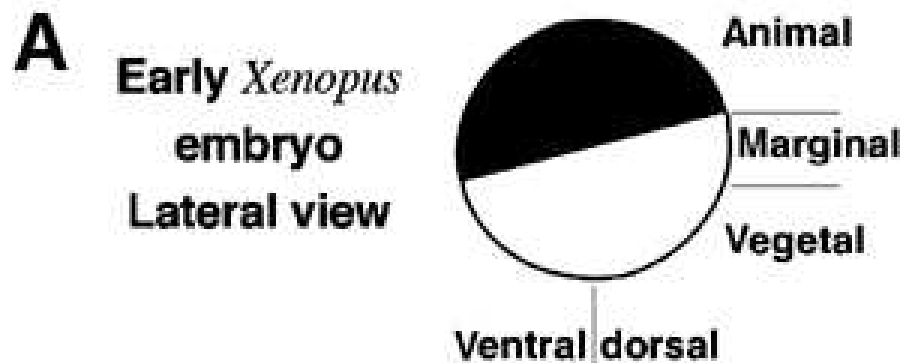
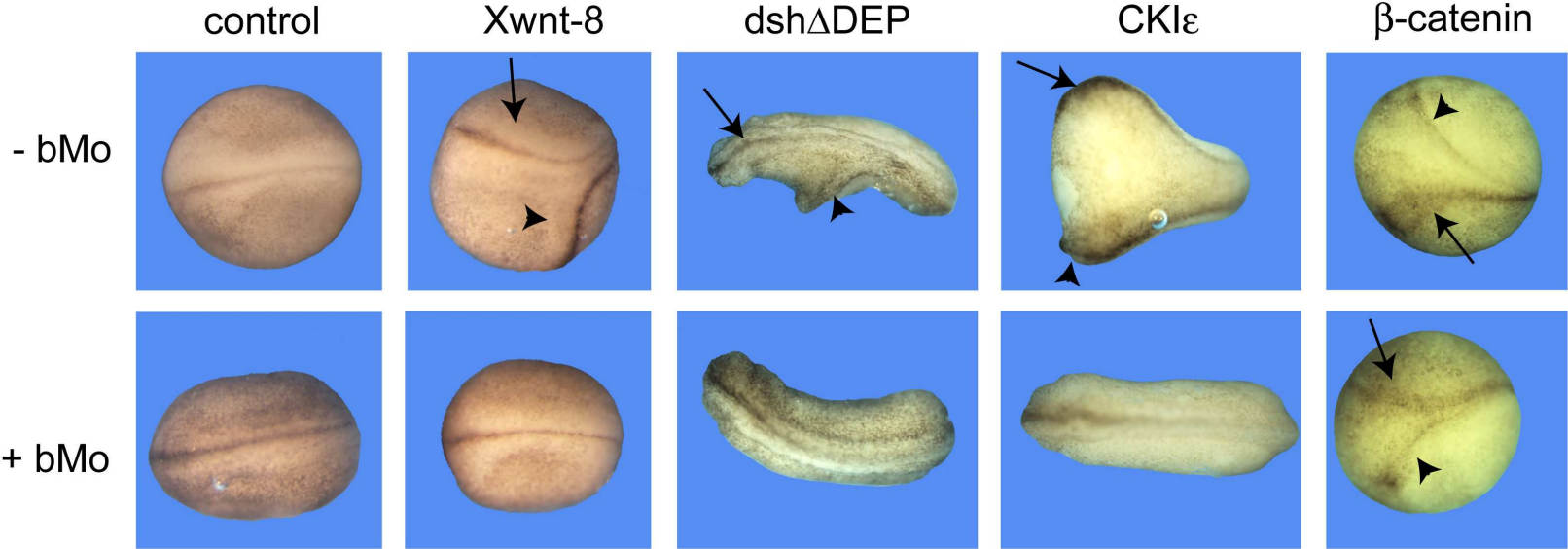


Fig. 2.6 Gastrulation in amphibians. The blastula (first panel) contains several thousand cells and there is a fluid-filled cavity, the blastocoel, beneath the cells at the animal pole. Gastrulation begins (second panel) at the blastopore, which forms on the dorsal side of the embryo. Future mesoderm and endoderm of the marginal zone move inside at this site through the dorsal lip of the blastopore, the mesoderm ending up sandwiched

between the endoderm and ectoderm in the animal region (third panel). The tissue movements create a new internal cavity—the archenteron—which will become the gut. Endoderm in the ventral region also moves inside through the ventral lip of the blastopore (fourth panel) and will eventually completely line the archenteron. At the end of gastrulation the blastocoel has considerably reduced in size. After Balinsky, B.I.: 1975.



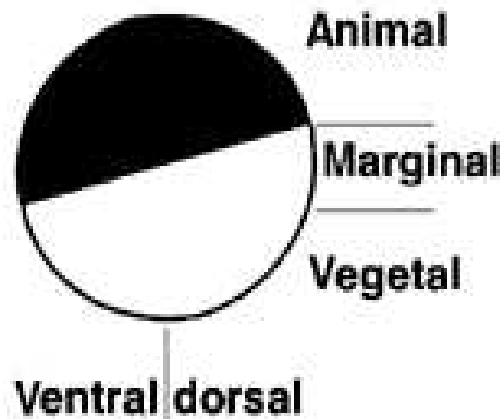
axis duplication assay:



Wnt/ β -cateninová dráha určuje antero-posteriorní (AP, předozadní) osu těla během gastrulace – podporuje vznik zadních a blokuje vznik předních částí těla

A

Early *Xenopus*
embryo
Lateral view



Xenopus blastula

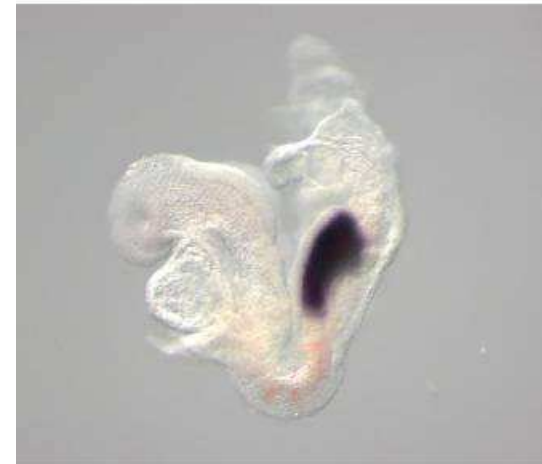
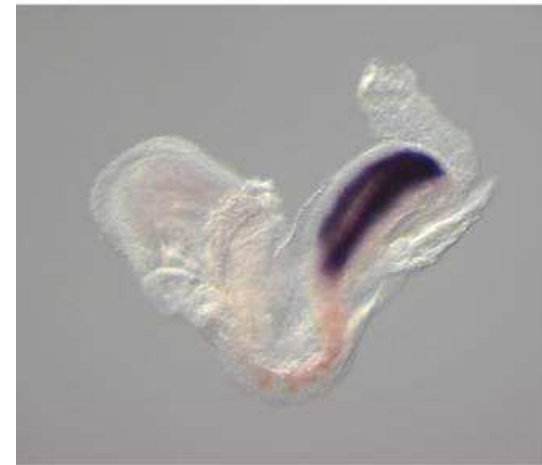


myší embryo po gastrulaci (E8.5):

Cílové geny Wnt/ β -cateninové dráhy jsou exprimovány v zadní části těla.

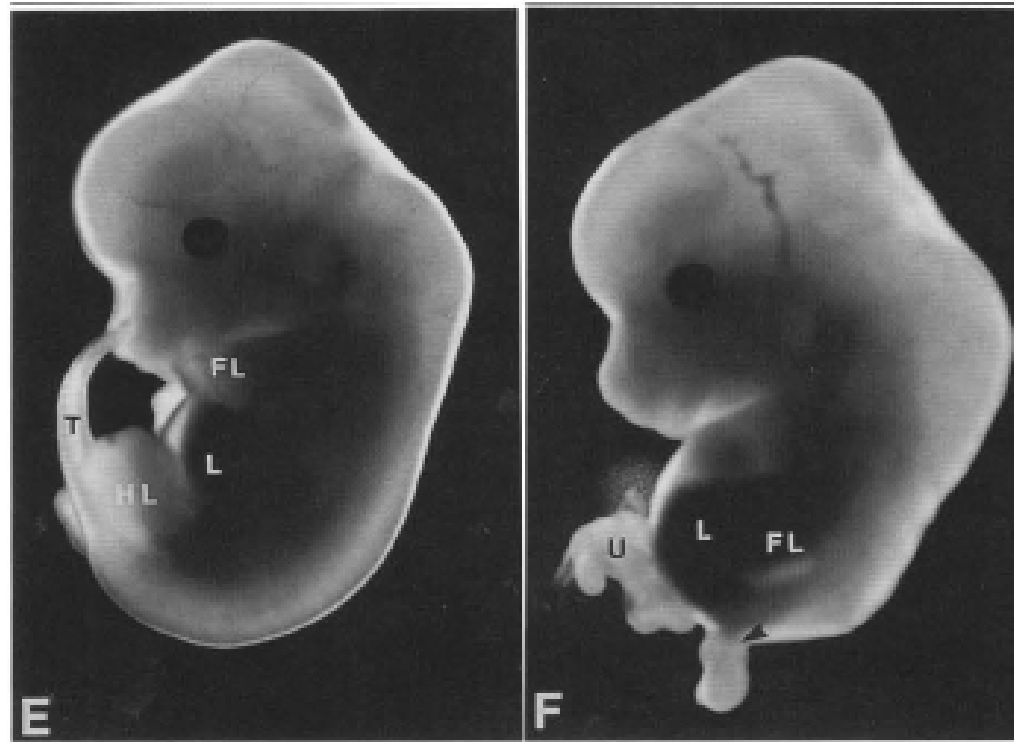
Uncx4.1/Mesogenin

Wnt5a^{+/+};LRP6^{+/+}



Wnt5a^{-/-};LRP6^{+/-}

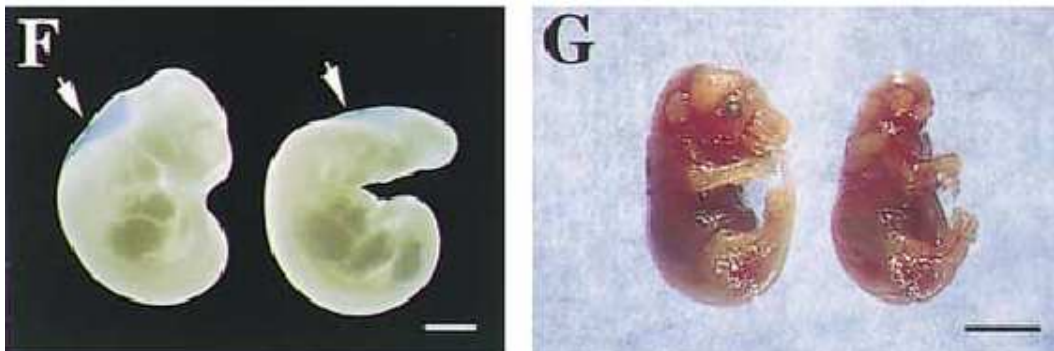
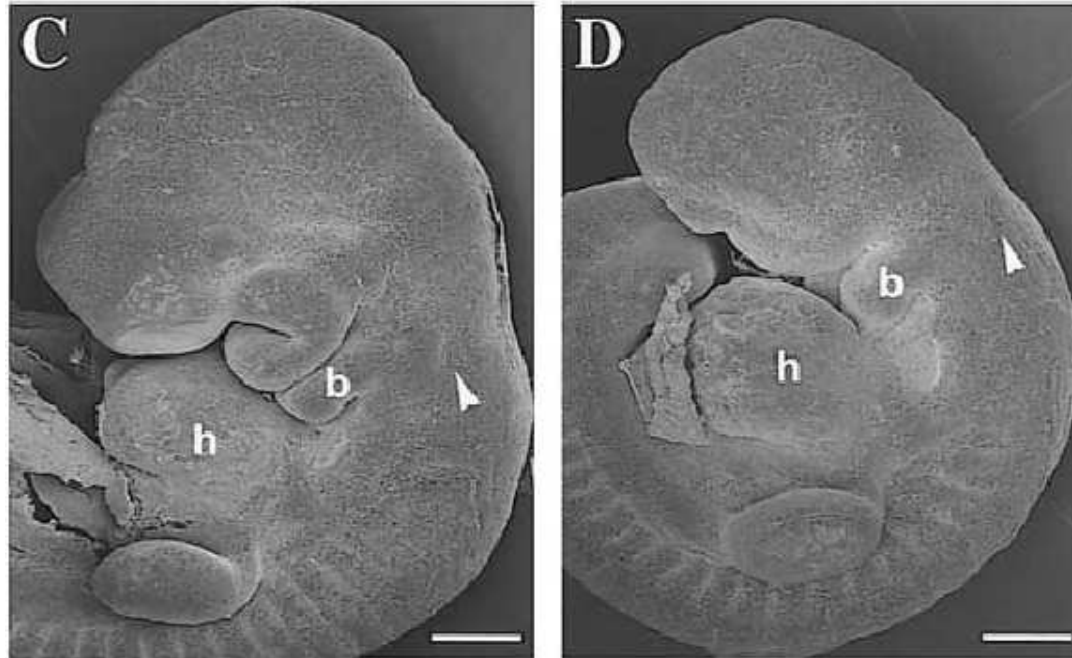
Deplece Wnt/ β -kateninové dráhy při gastrulaci = ztráta zadních částí těla



wild type

Wnt-3a knockout

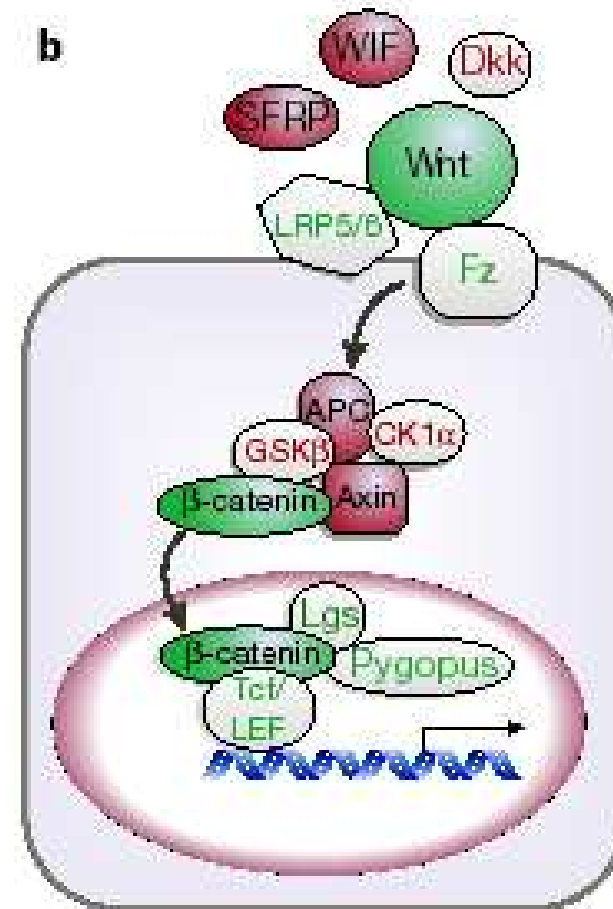
Deplece inhibitorů Wnt/ β - kateninové dráhy při gastrulaci = ztráta předních částí těla



wild type vs. Dkk1 knockout

Wnt/ β -cateninová dráha je
klíčovým regulátorem aktivace
kmenových buněk jak v
embryogenezi, tak v dospělých
tkáních

Wnt/ β -catenin dráha je velmi často deregulovaná u nádorů!



according to Beachy et al., Nature 2004

Wnt pathway		
Colon	Adenocarcinoma	Tumorigenesis by inactivation of APC, Axin; tumorigenesis by stabilization of β -catenin; epigenetic inactivation of SFRPs
Liver	Hepatoblastoma	Tumorigenesis (in mouse) by inactivation of APC and by stabilization of β -catenin
Blood	Multiple myeloma	Cell-growth inhibition by dominant negative TCF4; growth stimulation by Wnt ligand
Hair follicle	Pilomatrixoma	Tumorigenesis (in mouse) by overexpression of β -catenin
Bone	Osteosarcoma	Dkk3 and LRP5 expression inhibits tumour cell growth <i>in vitro</i>
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short interfering RNA and a blocking antibody against Wnt2
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP

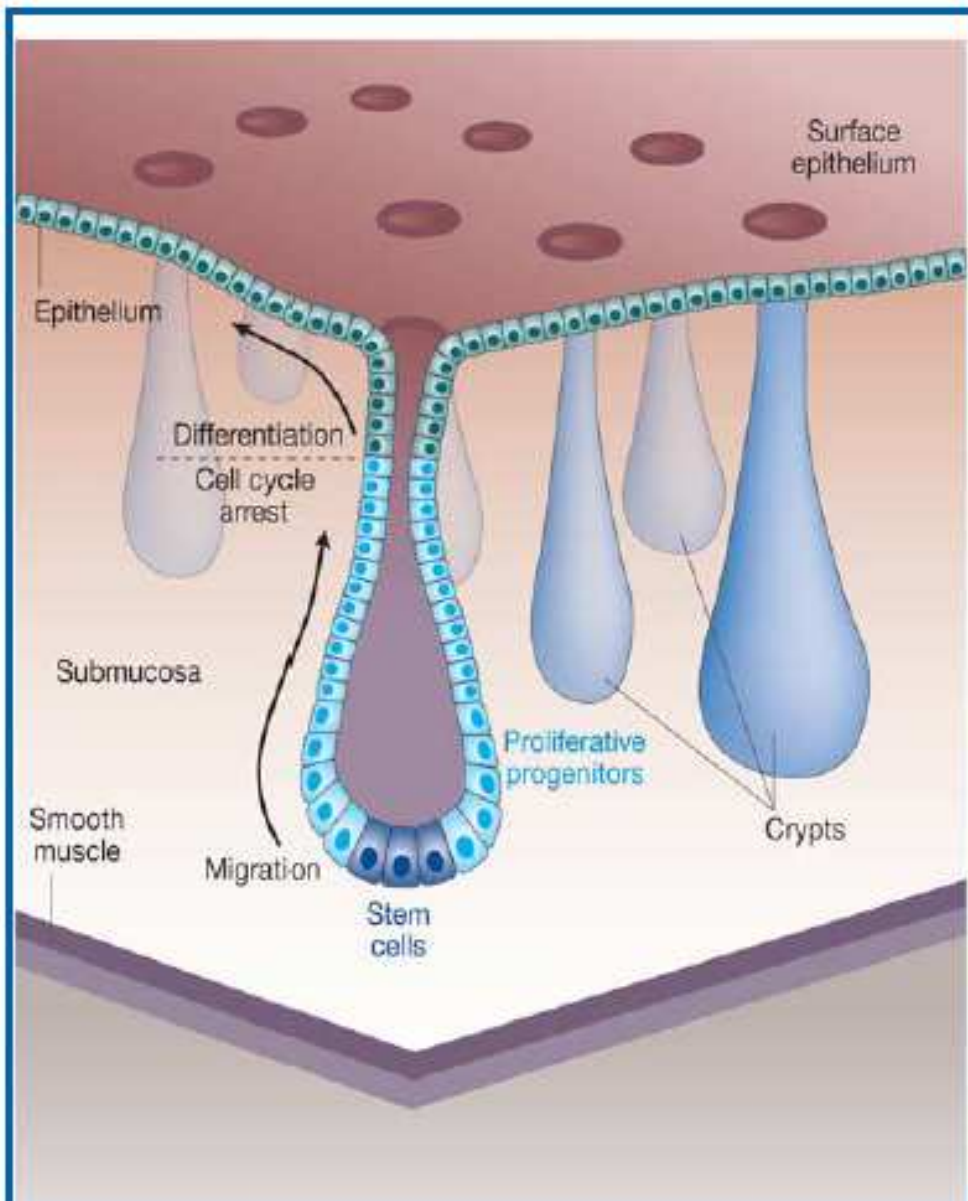


Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)

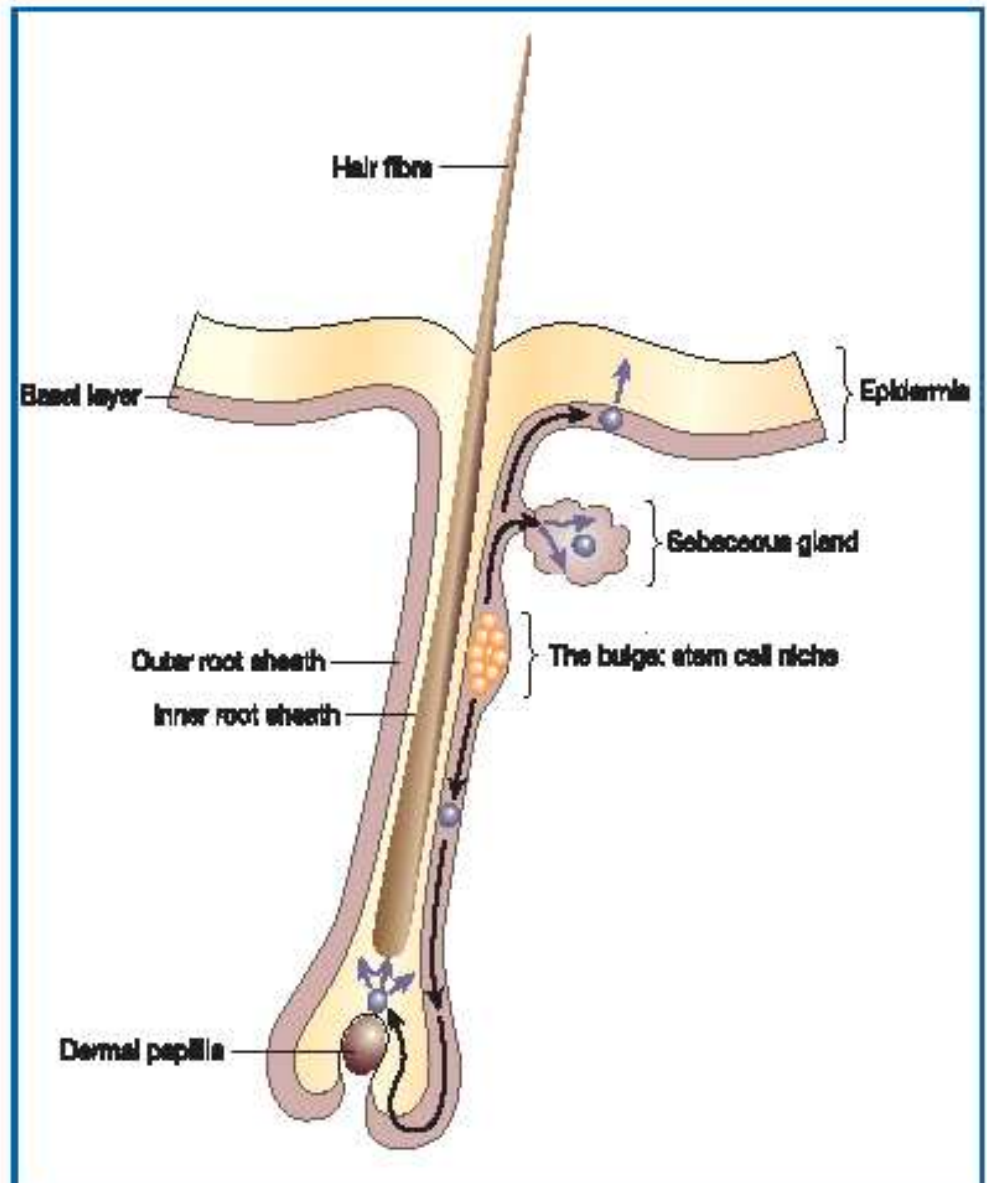
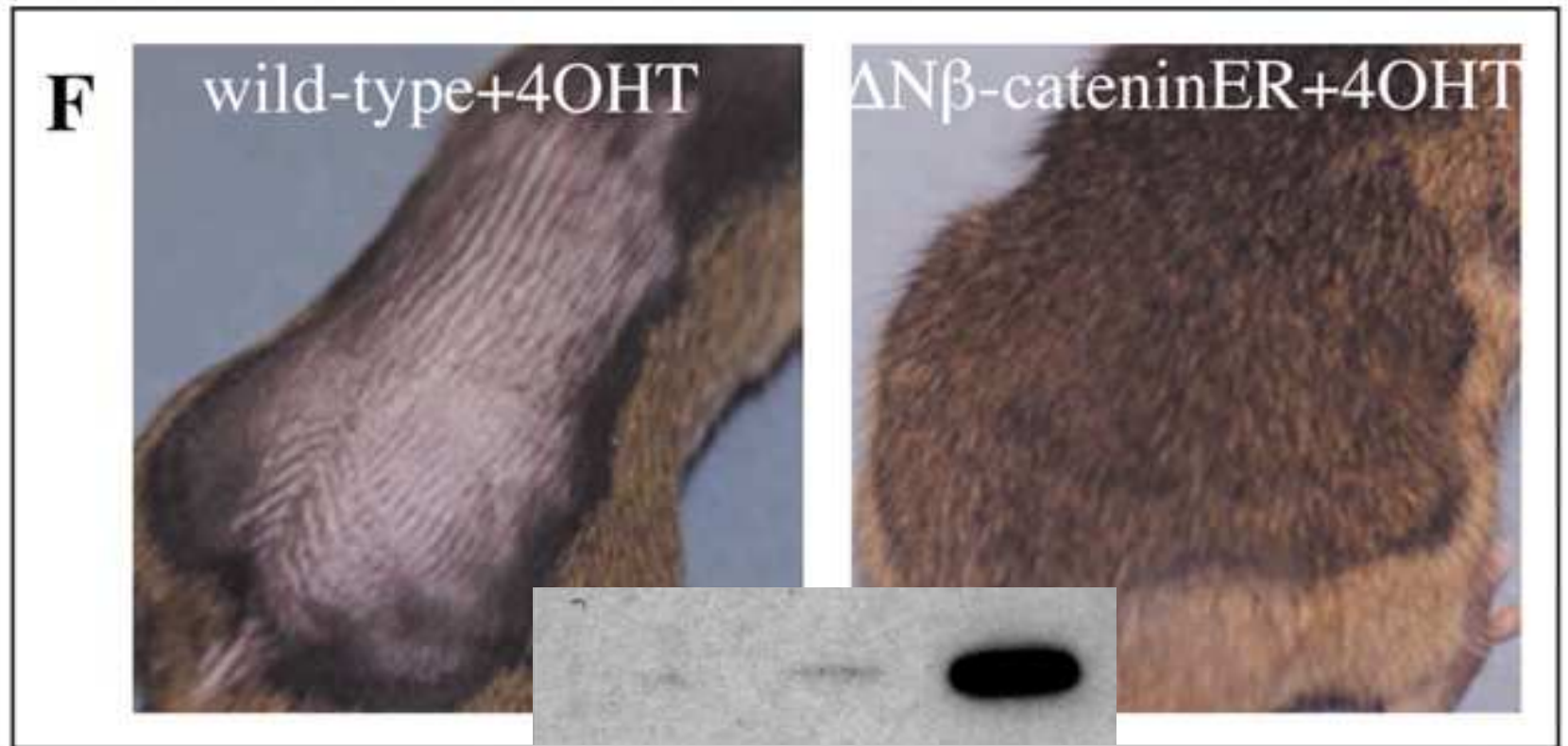


Figure 4 The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from ref. 90.)

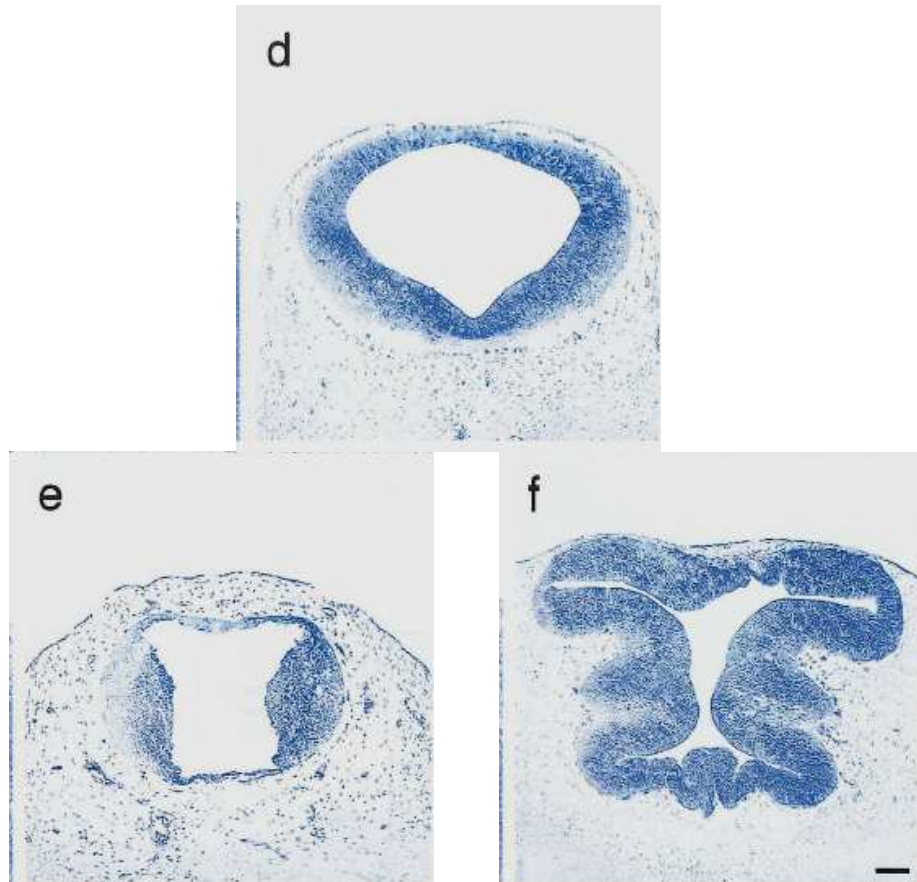
Důsledky aktivace β -catenin v epidermis (po depilaci)



Lo Celso, C. L. et al. Development 2004;131:1787-1799

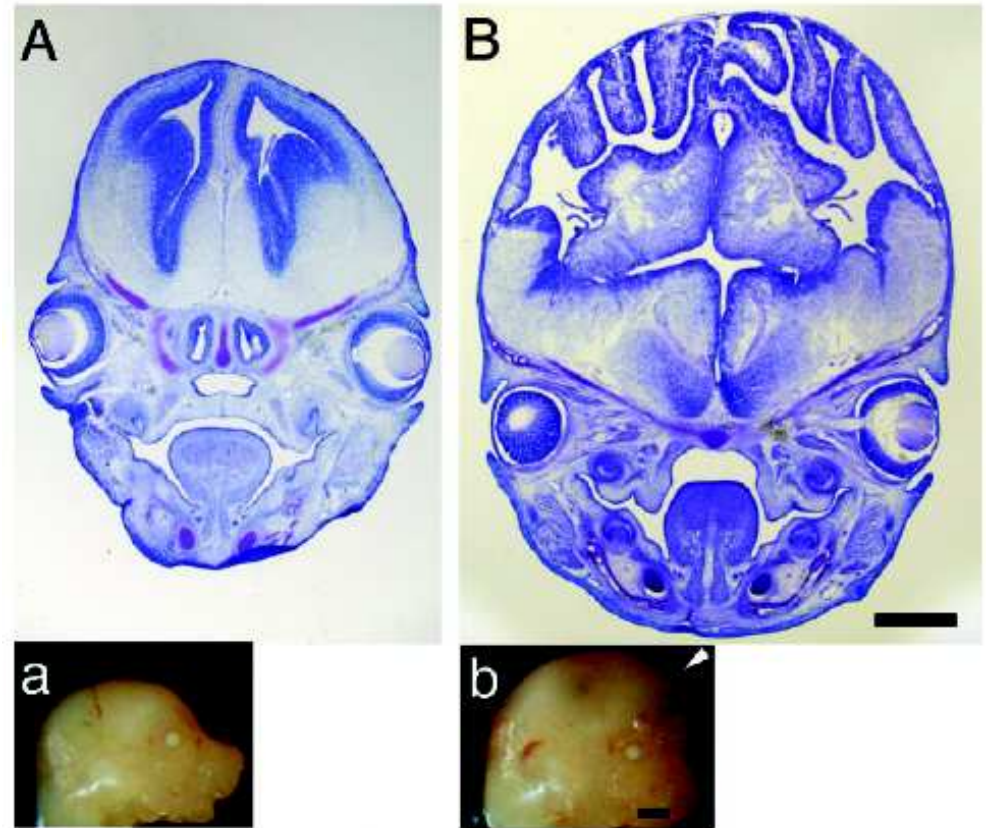
Aktivace β -catenin u ve vyvíjející se mozkové trubici:

midbrain (Brn4-promotor)



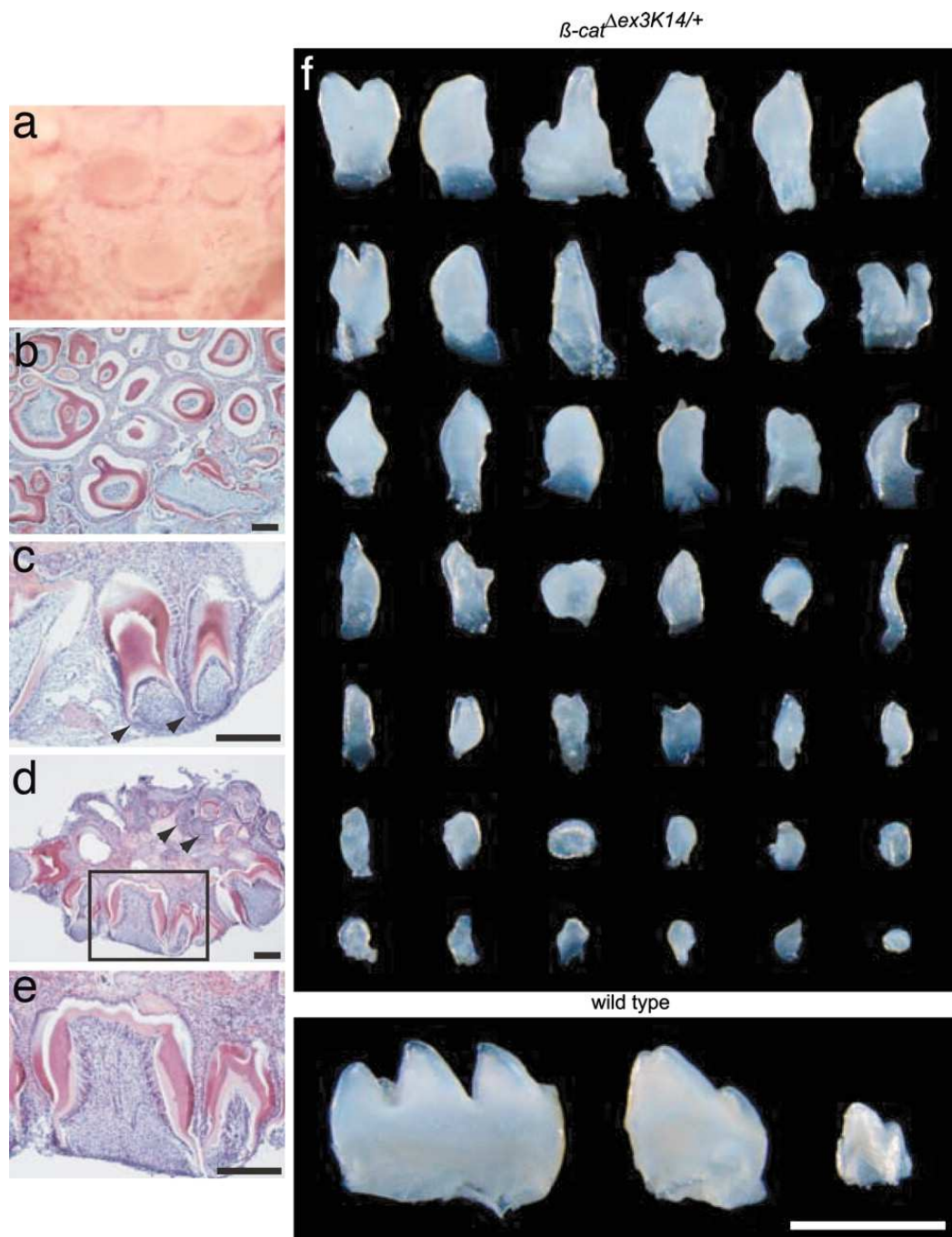
Zechner et al., 2003: Dev. Biol.;258:406-418.

cortex (nestin enhancer)



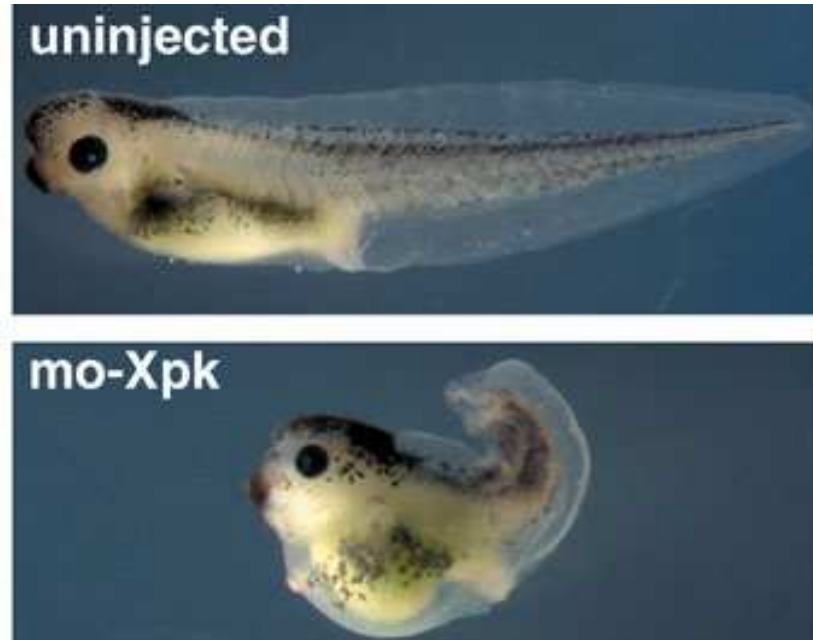
Chenn & Walsh, 2002: Science;297:365-369.

Aktivace beta-cateninu v kmenových buňkách zubu:



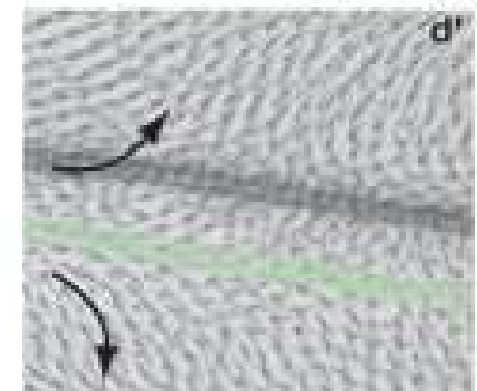
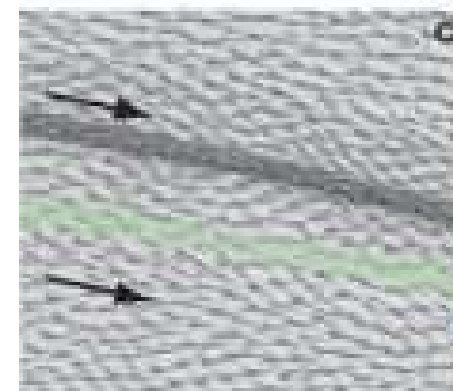
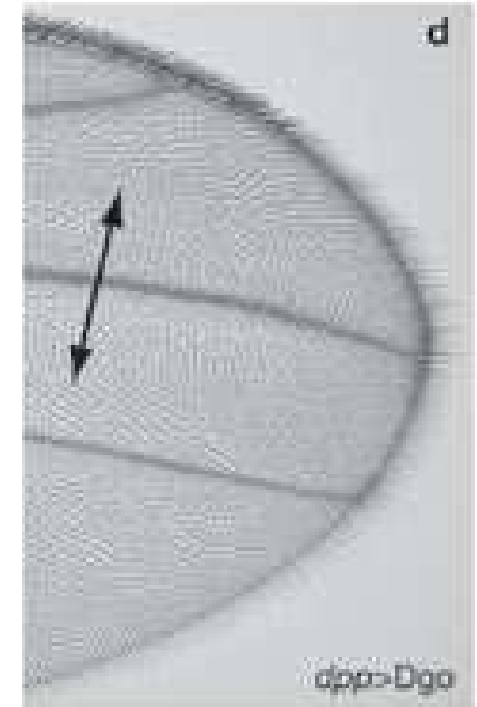
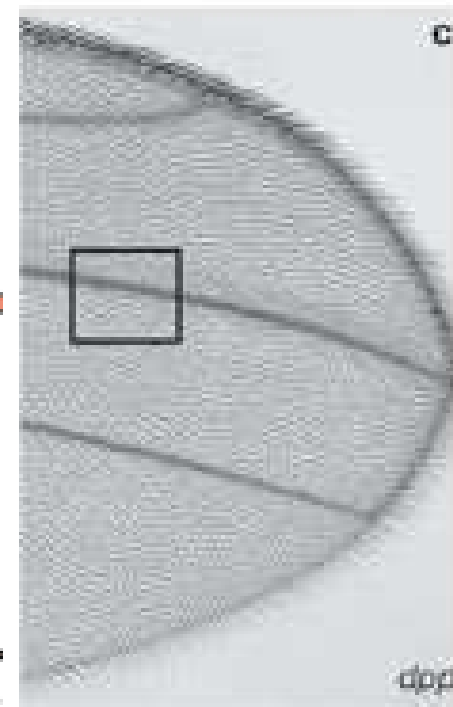
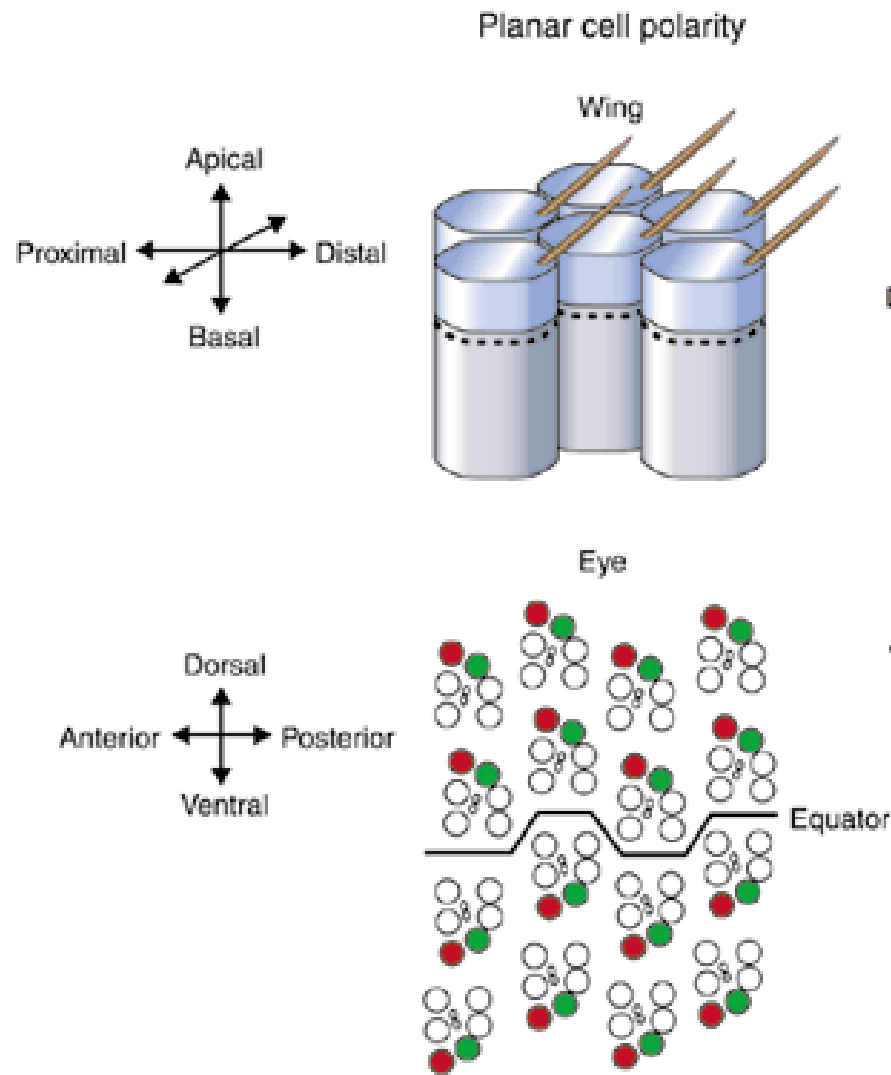
Nekanonická Wnt dráha

- e.g. Wnt5a



- do not induce axis duplication in *Xenopus*
- do not induce transformation of mammary cell line C57mg
- do not signal via nuclear translocation of β -catenin

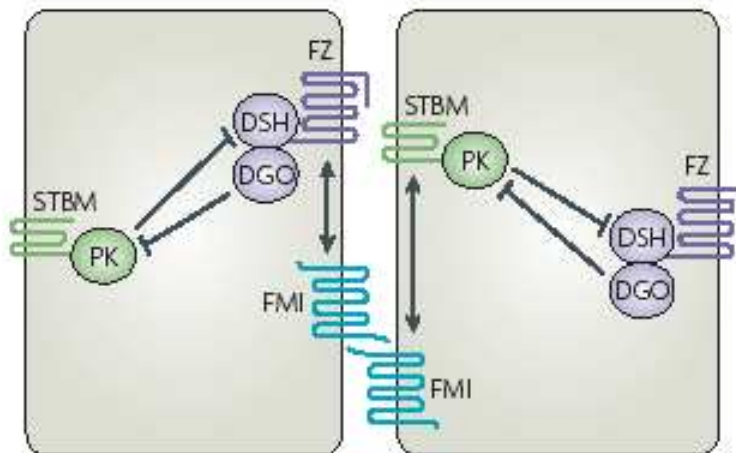
Drosophila – PCP (planar cell polarity)



Molekulární mechanismus ustavení PCP

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

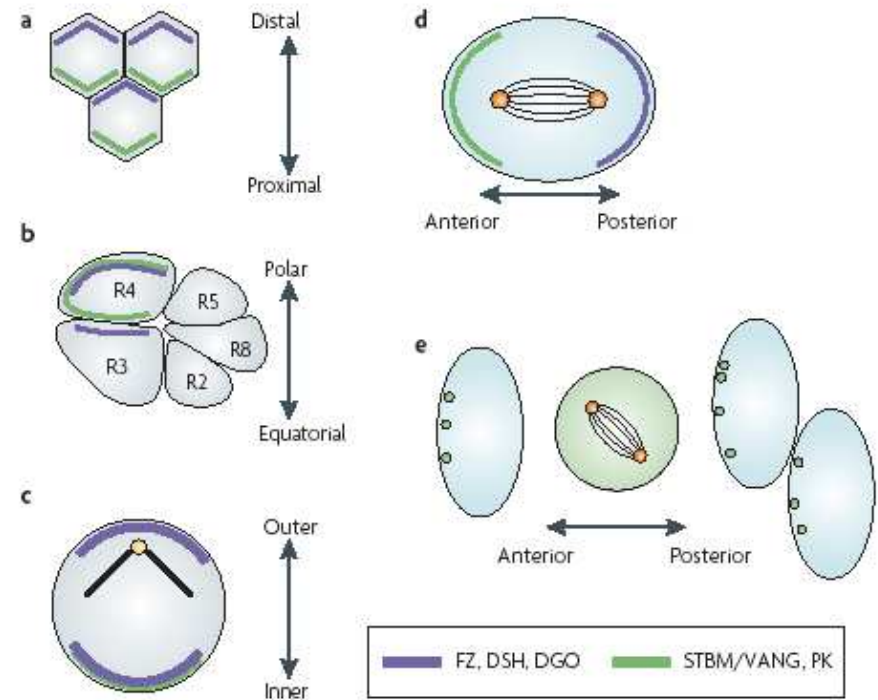
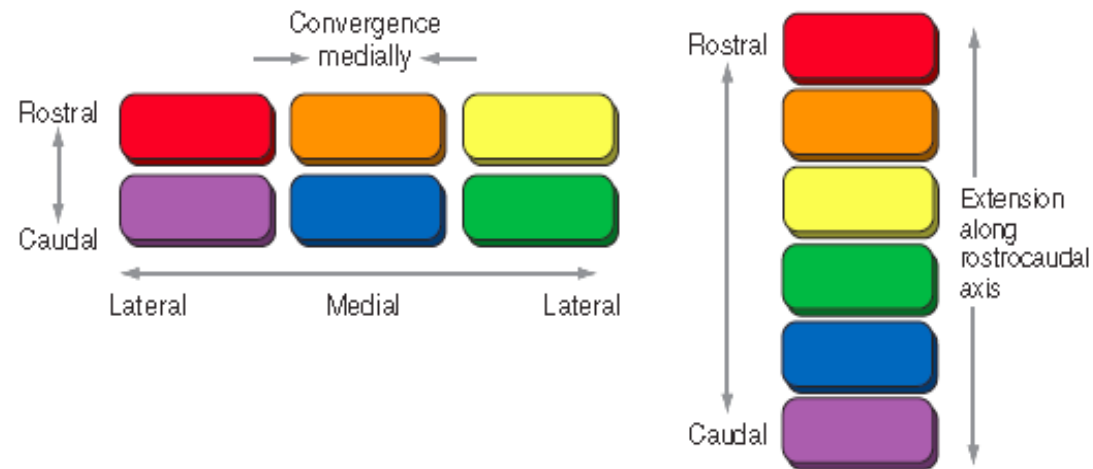


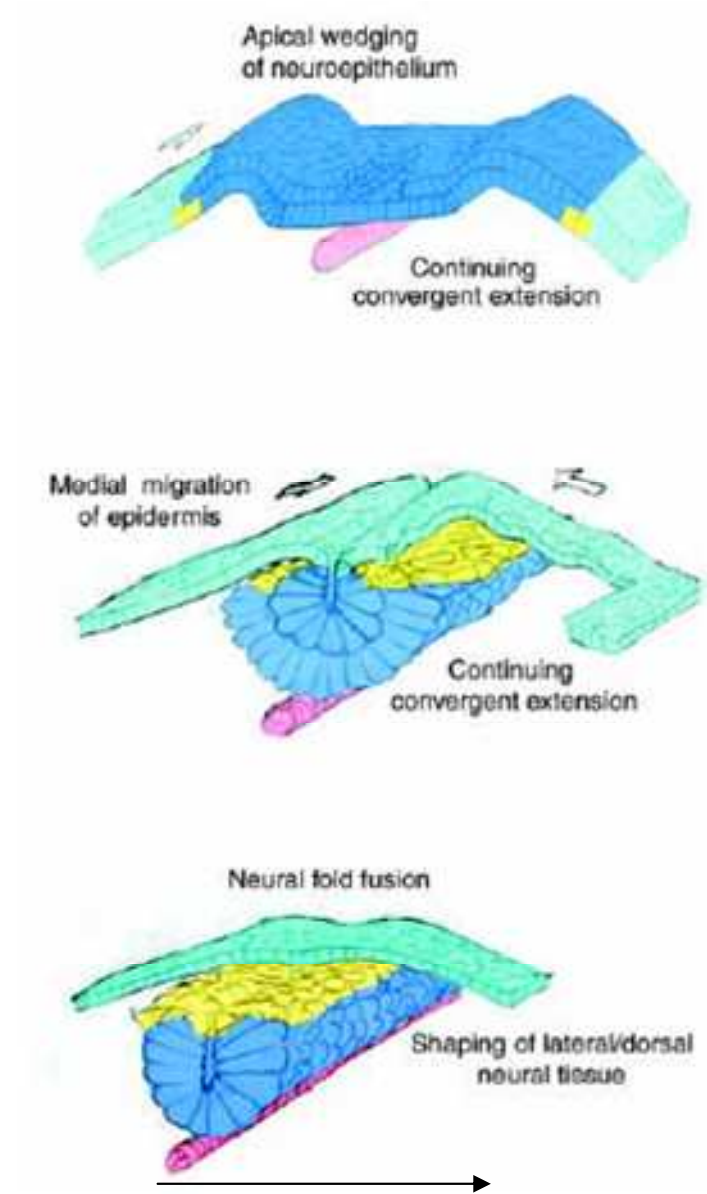
Figure 2 | Subcellular distribution of core Fz/PCP factors in *Drosophila melanogaster* and vertebrates. a–c | Examples of cells with epithelial character (marked by grey shading). *Drosophila melanogaster* wing cells and eye R3 and R4 cells and mouse sensory hair cells in the cochlea (inner ear) are 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 unknown⁸². 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).

Non-canonical/PCP (Planar cell polarity) in mouse (and human) convergent extension



Konvergentní extenze – migrace buněk směrem ke středu těla – vede k prodlužování tělní osy

Důsledky narušené konvergentní extenze (CE)



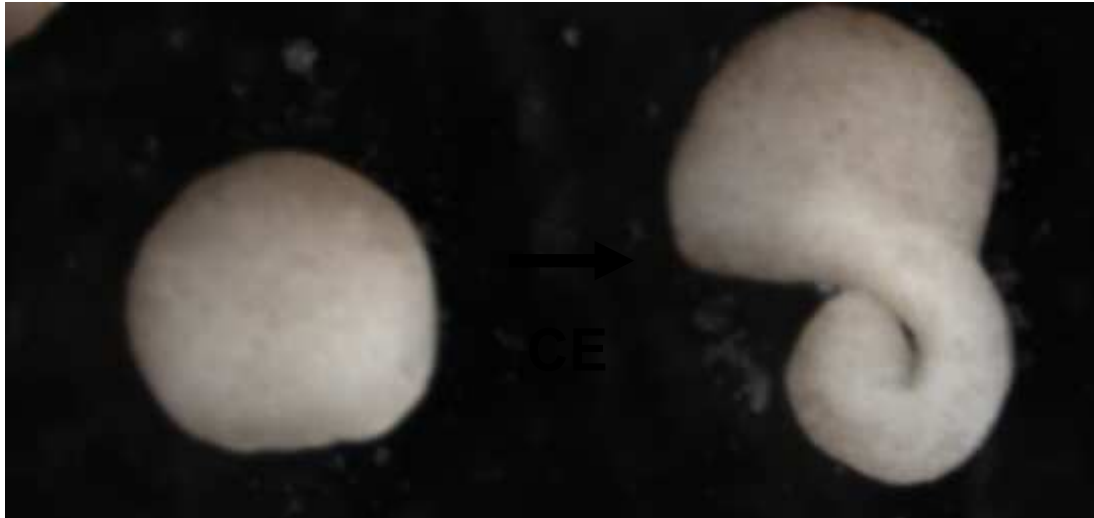
Exencephaly



Open neural tube



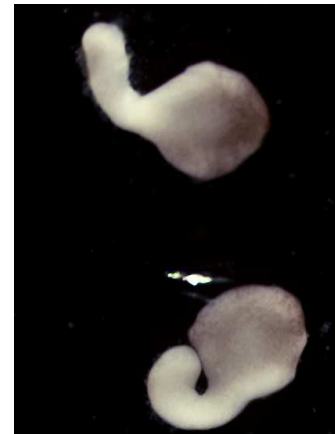
Možnosti studia CE - Kellerovy explantáty (Xenopus)



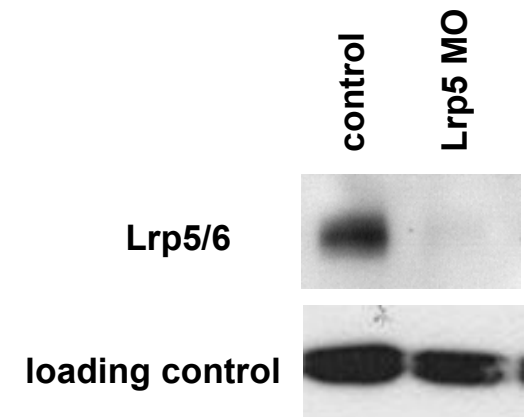
control



XLRP5 MO

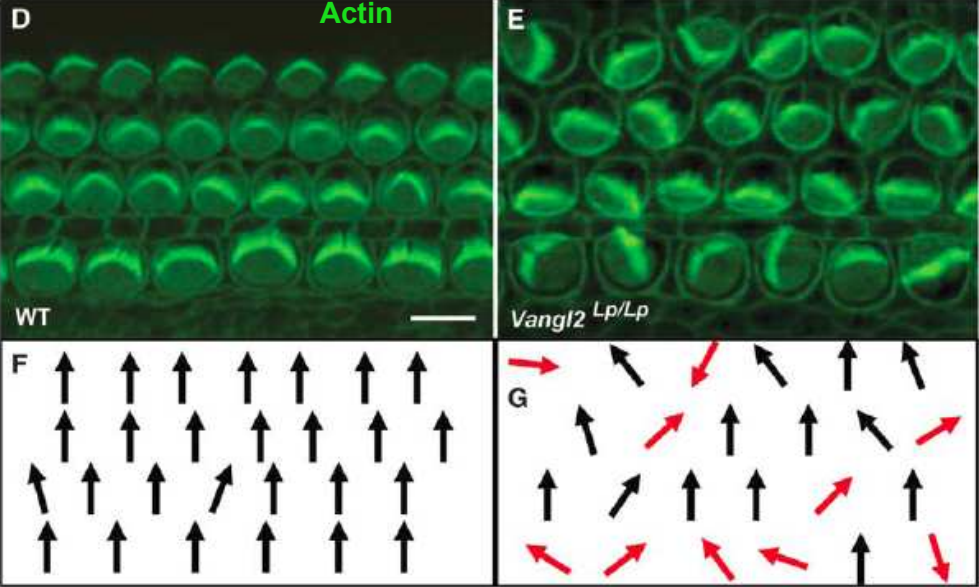


XLRP5 MO
+ mLrp5



Non-canonical/PCP (Planar cell polarity) pathway: phenotypes in mouse

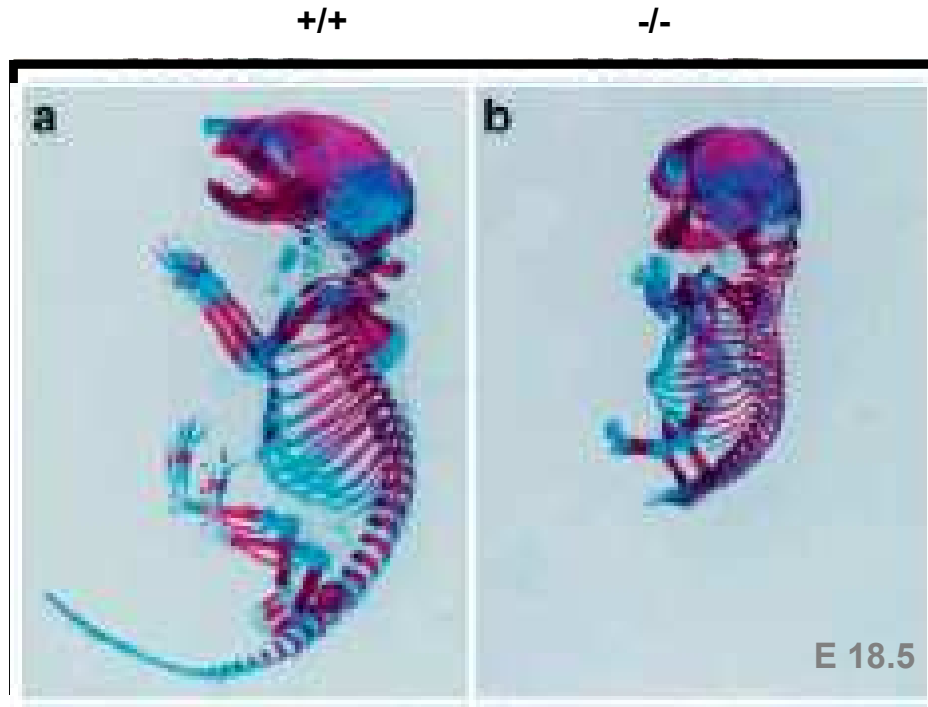
Stereocilia orientation in inner ear hair cells



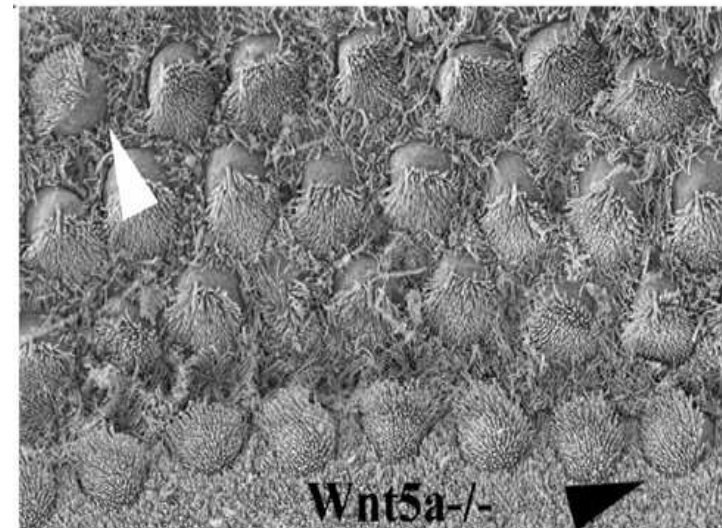
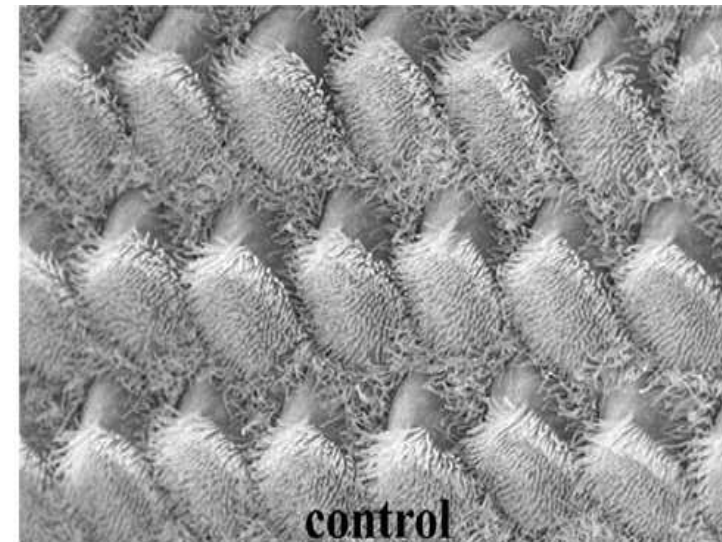
Qian et al., 2007, Dev. Biol.

Increased migration and metastatic potential!

Known Wnt5a knockout phenotypes



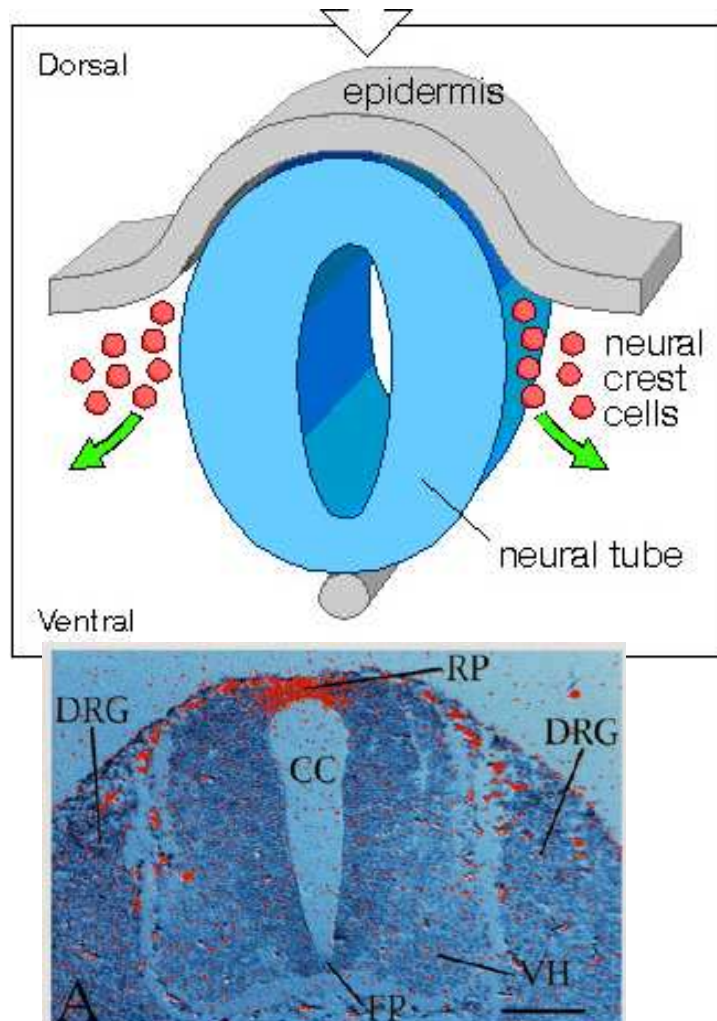
Yamaguchi et al., 1999



Qian et al, 2007

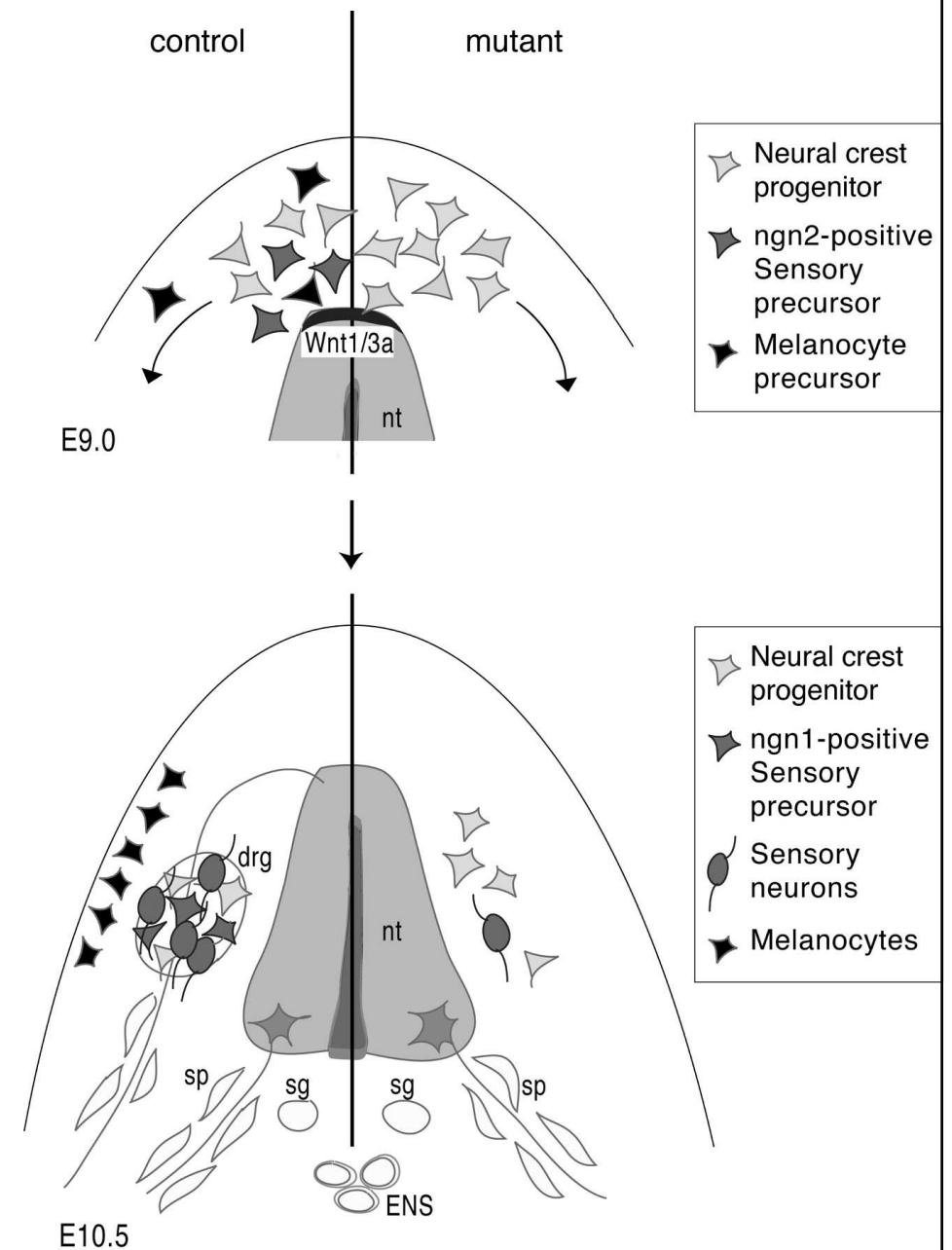
Kanonické a nekanonické Wnt signálování často regulují odlišné části téhož vývojového procesu.

I. Vývoj neurální lišty:

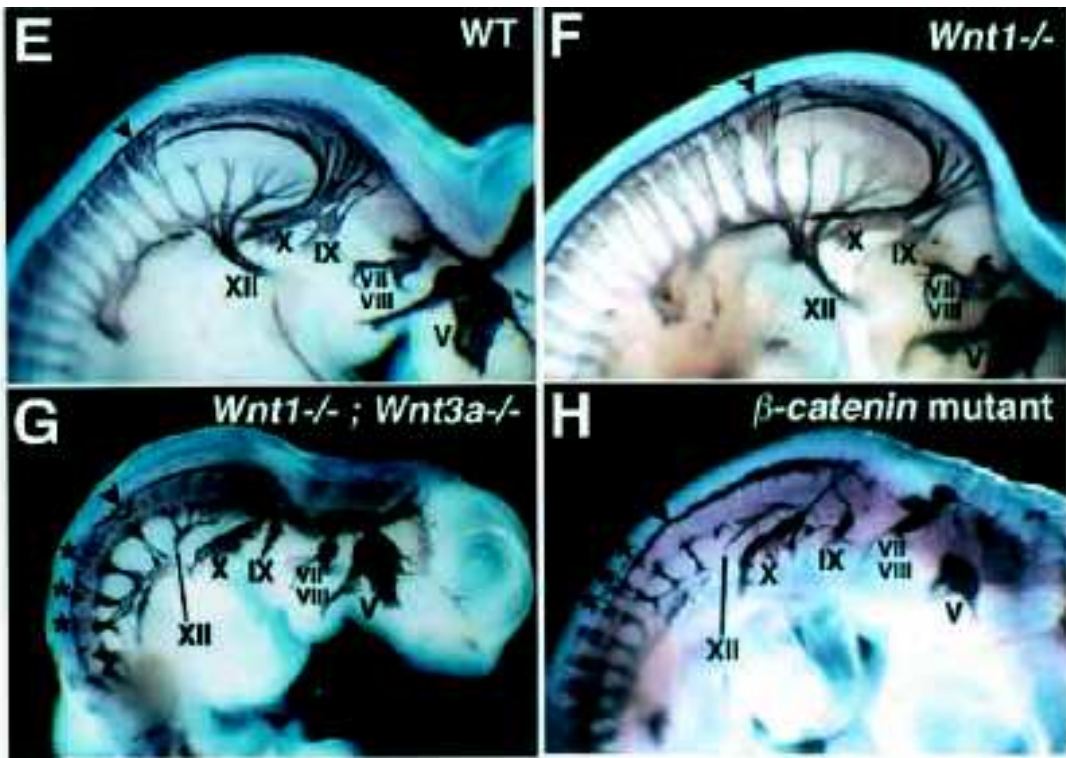
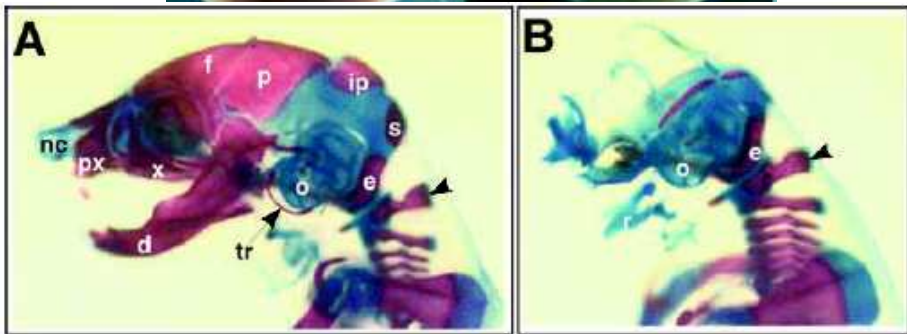


Wnt-3a

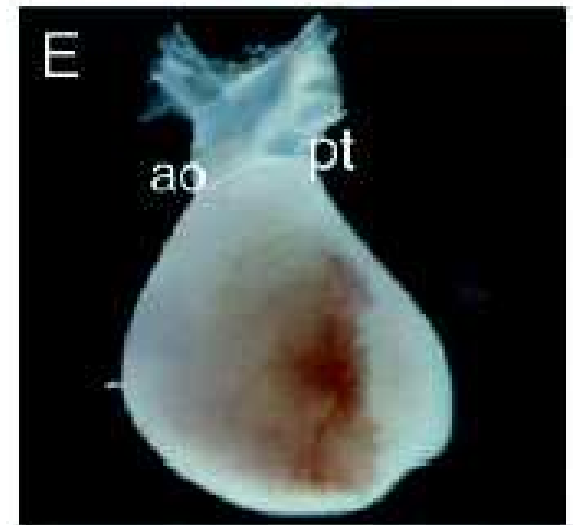
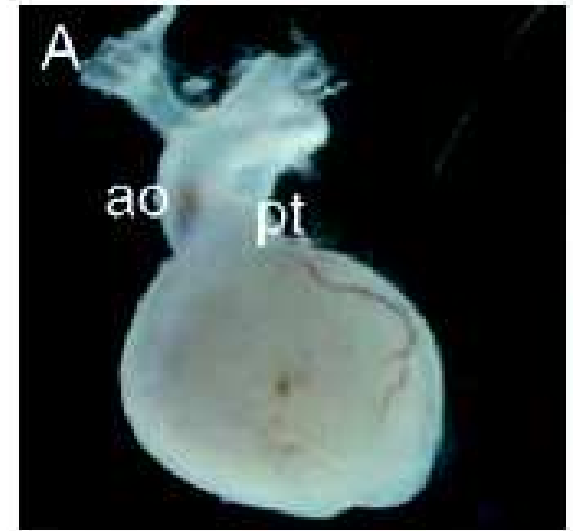
- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, srdce a dalších



Wnt1/3a DKO



Heart outflow tract development



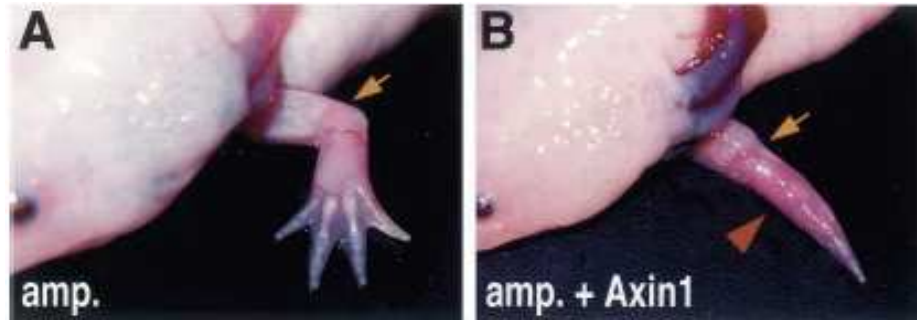
Wnt5a KO

Henderson DJ et al., 2006, TrendsCard. Res.

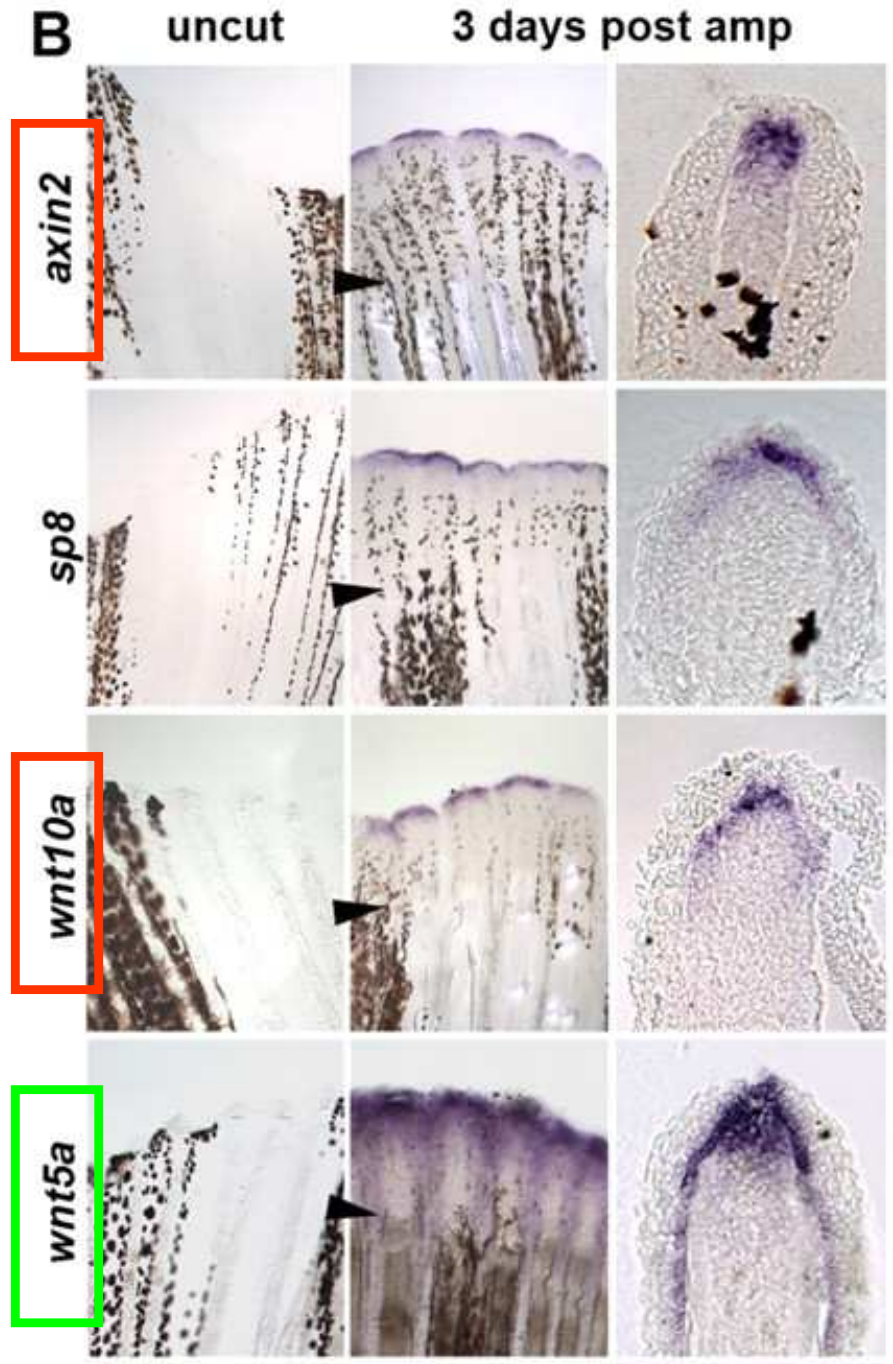
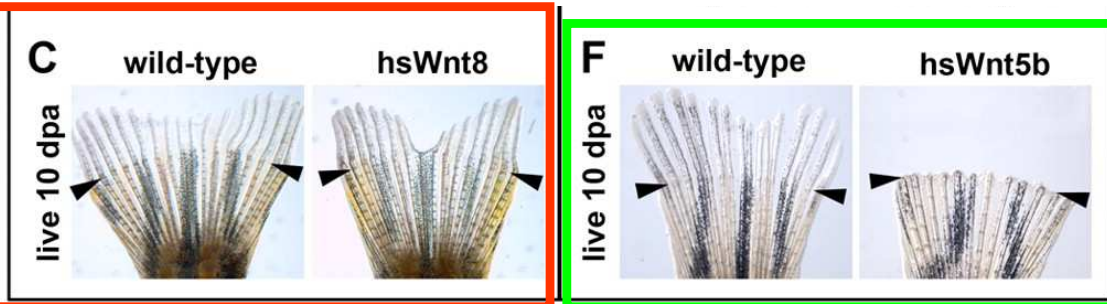
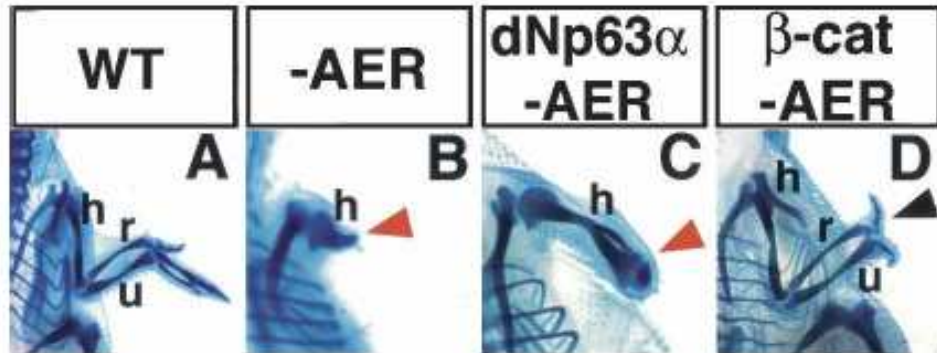
II. regenerace

zebrafish

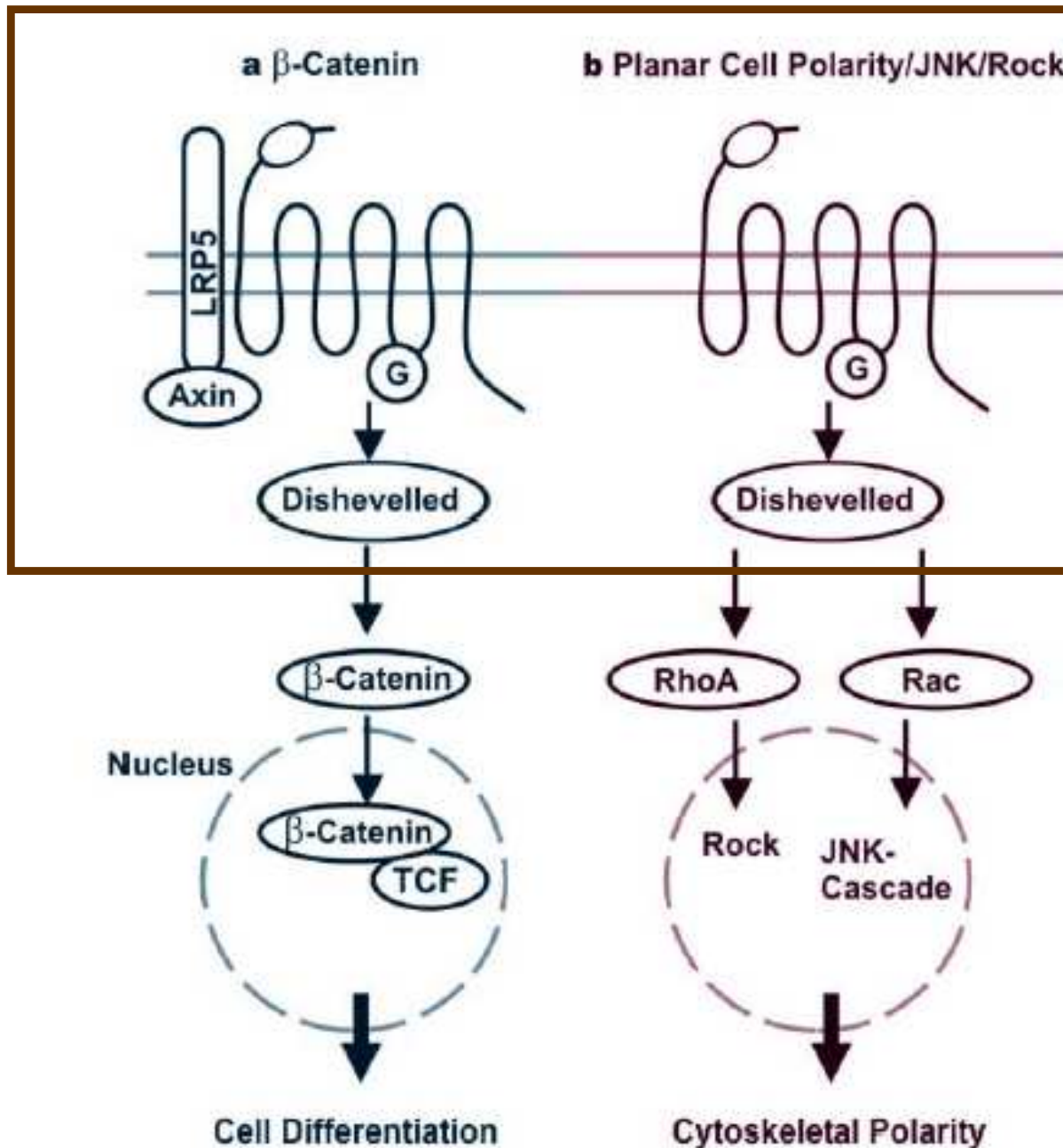
axolotl



chick



Fundamental question of Wnt signalling: How the specificity is achieved?



DISHEVELLED

(Dvl – mouse, XDsh – Xenopus, Dsh – Drosophila)

- phosphoprotein – phosphorylated by numerous kinases, significance often unknown

- acts between Frizzled and downstream signalling components

- required for signal transduction of all Wnt signalling pathways

Purification of Wnt ligands

.....
**Wnt proteins are lipid-modified and
can act as stem cell growth factors**

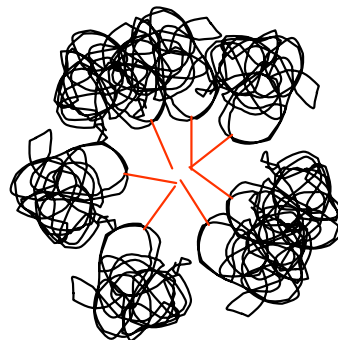
**Karl Willert^{*}, Jeffrey D. Brown^{*}, Esther Danenberg^{*}, Andrew W. Duncan[†],
Irving L. Weissman[‡], Tannishtha Reya[†], John R. Yates III[§] & Roel Nusse^{*}**

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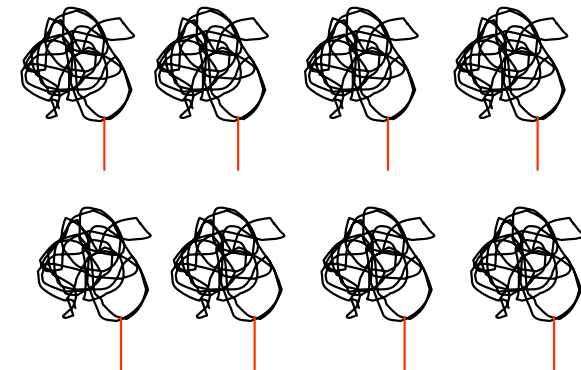
Wnt-3a



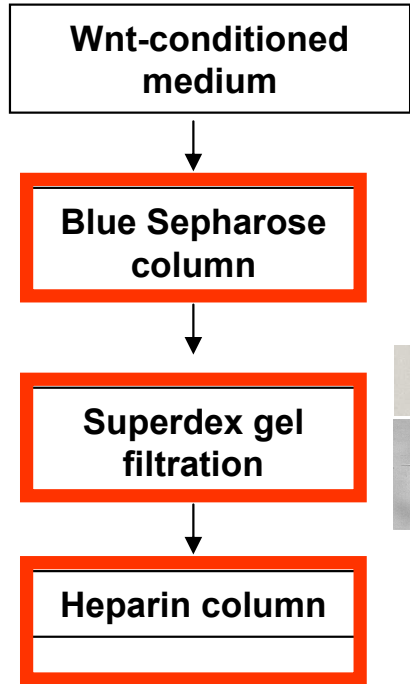
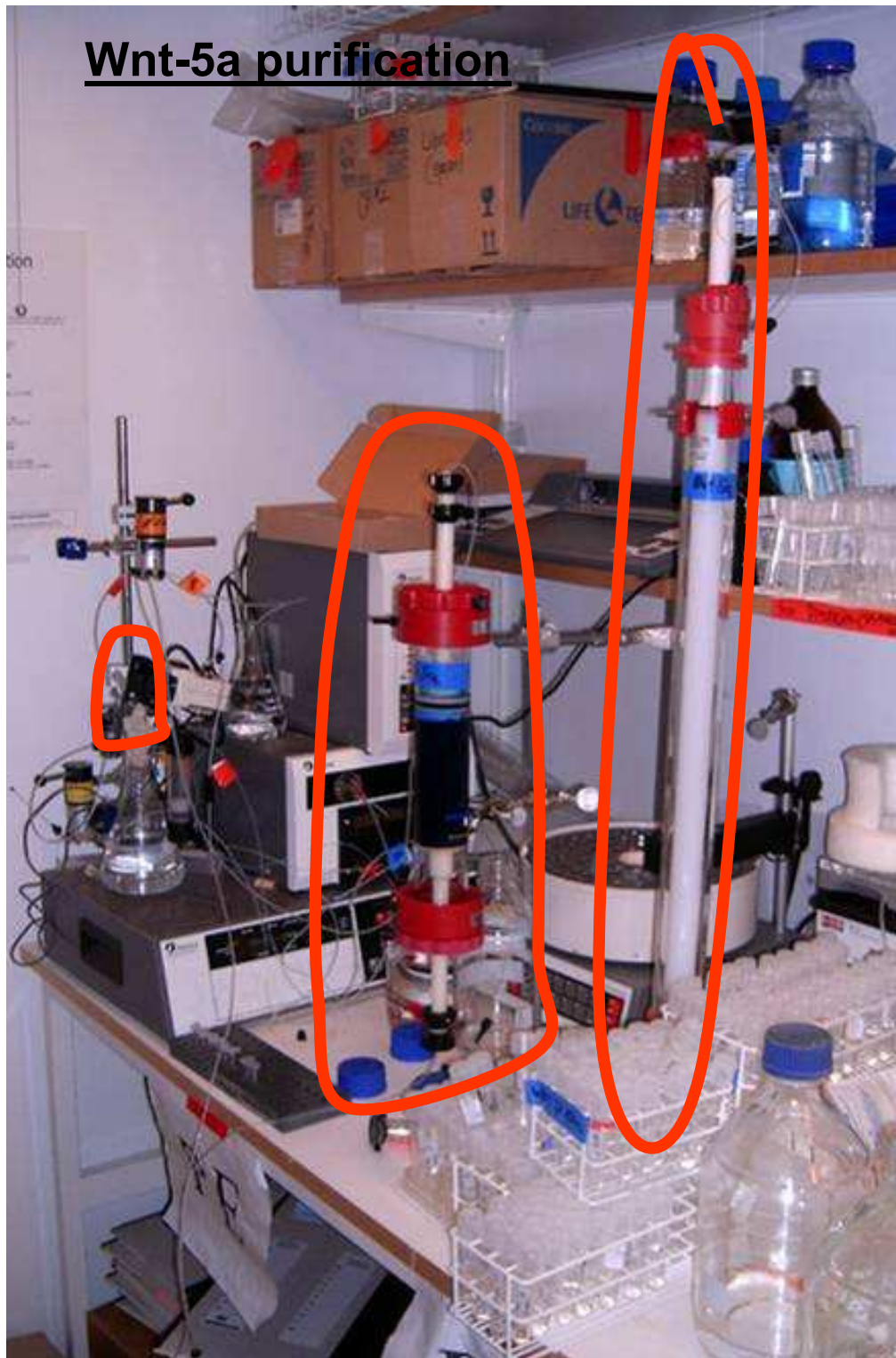
no detergent



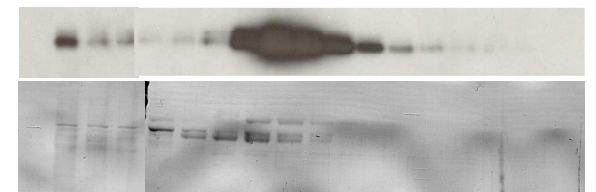
detergent added



Wnt-5a purification



4 liters of media conditioned by fibroblasts expressing HA tagged Wnt-5a



Medium
Blue Sepharose
Superdex
Heparin



WB: Wnt-5a



silver staining