

Lecture 10:

Wnt morphogenetic system

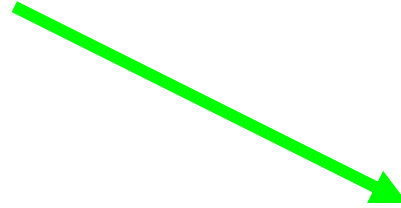
Wnts (Wingless/Int)

- 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

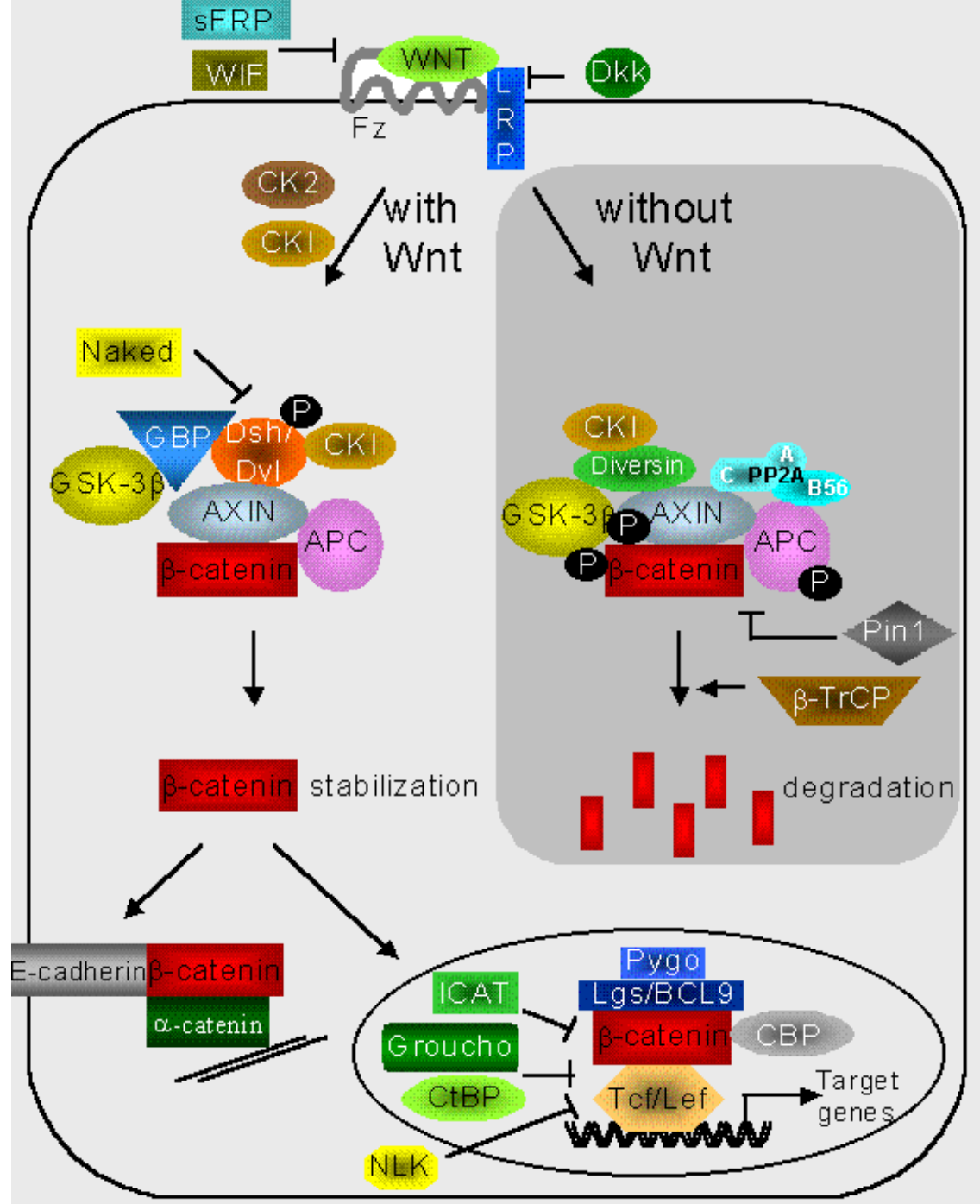


- induce axis duplication in *Xenopus*
- induce transformation of mammary cell line C57mg
- signal via nuclear translocation of β -catenin



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

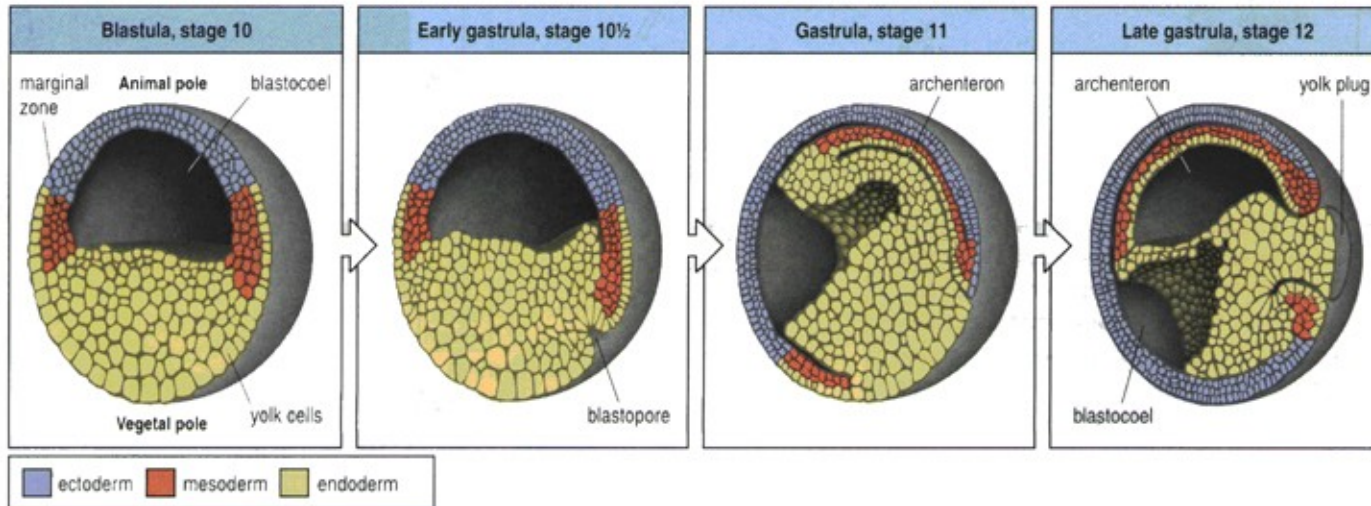
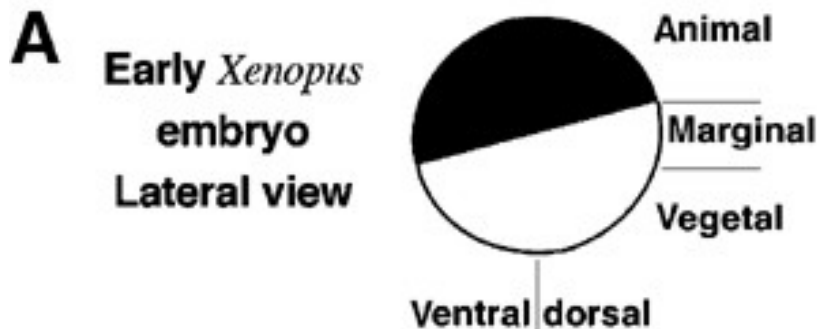
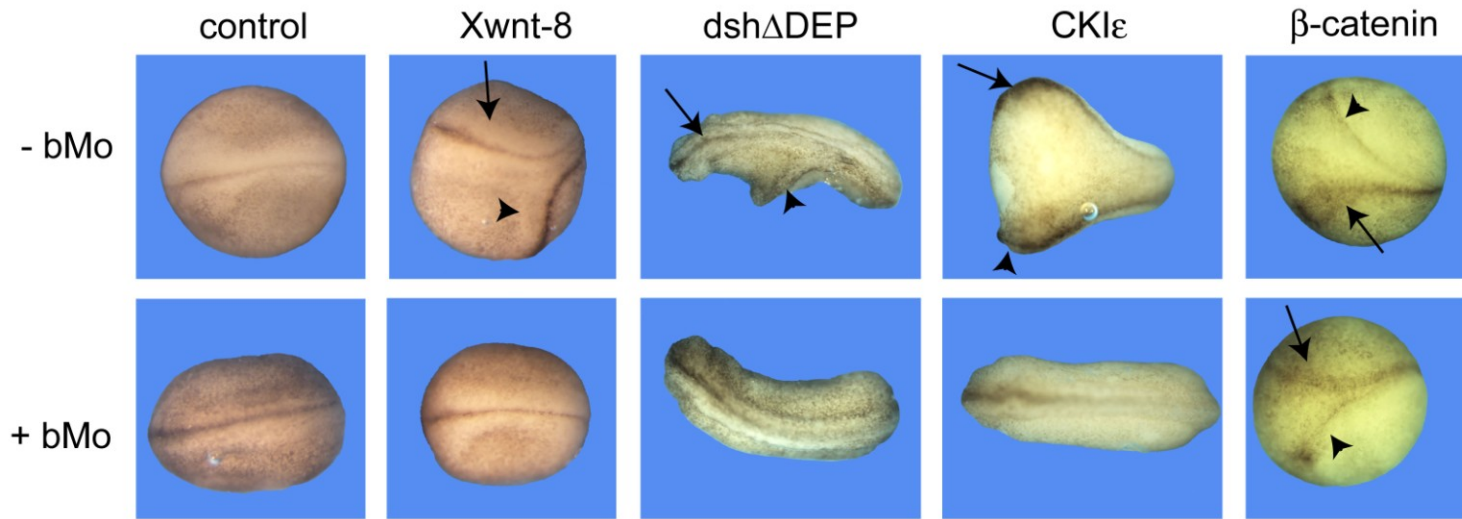


Fig. 2.6 Gastrulation in amphibians. The blastula (first panel) contains several thousand cells and there is a fluid-filled cavity, the blastocoel, beneath the cells at the animal pole. Gastrulation begins (second panel) at the blastopore, which forms on the dorsal side of the embryo. Future mesoderm 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.



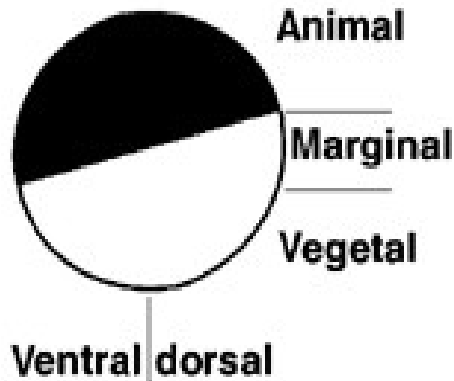
axis duplication assay:



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



Zygotic Wnt pathway
(marginal zone, stage 9)

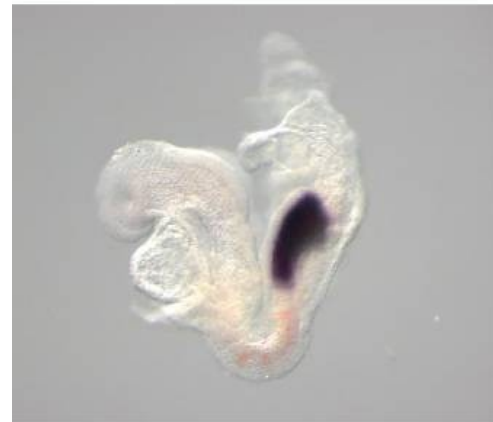
Maternal Wnt pathway
(dorsal side, stage 8)

myší embryo po gastrulaci (E8.5):

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

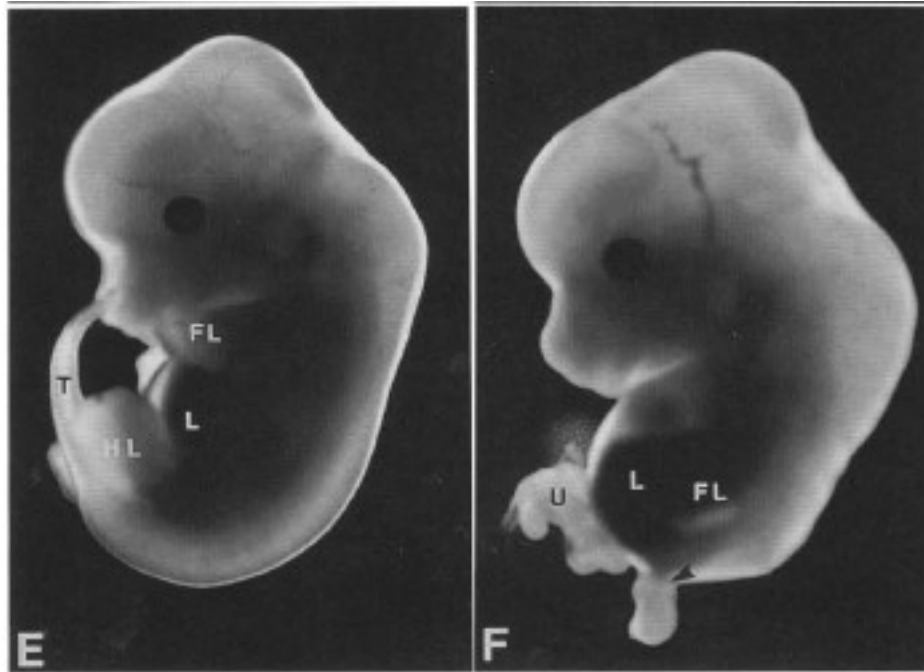
Uncx4.1/Mesogenin

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



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

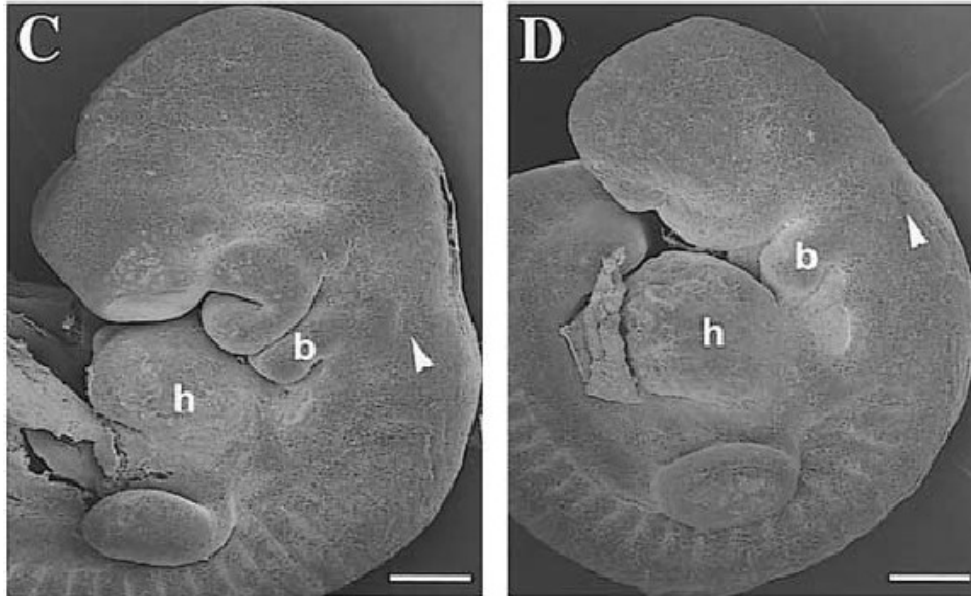
Deplece Wnt/ β -kateninové dráhy při gastrulaci = ztráta zadních částí těla



wild type

Wnt-3a knockout

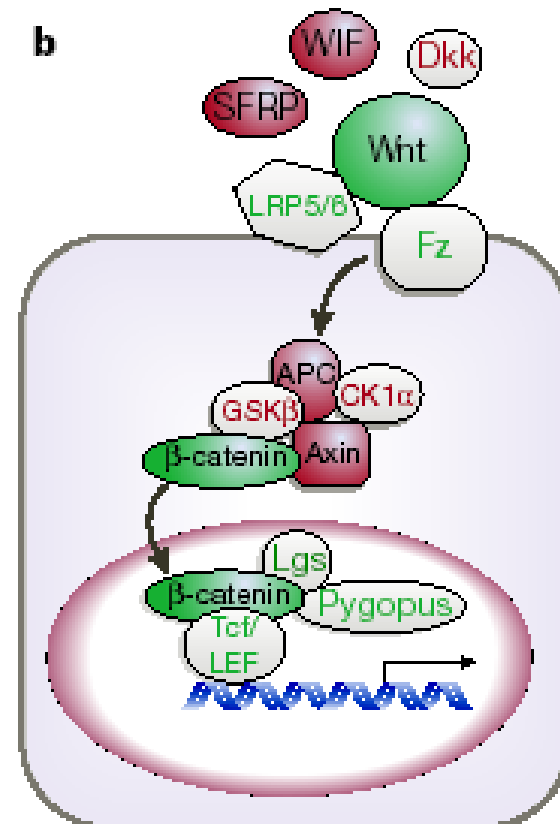
Deplece inhibitorů Wnt/ β - kateninové dráhy při gastrulaci = ztráta předních částí těla



wild type vs. Dkk1 knockout

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

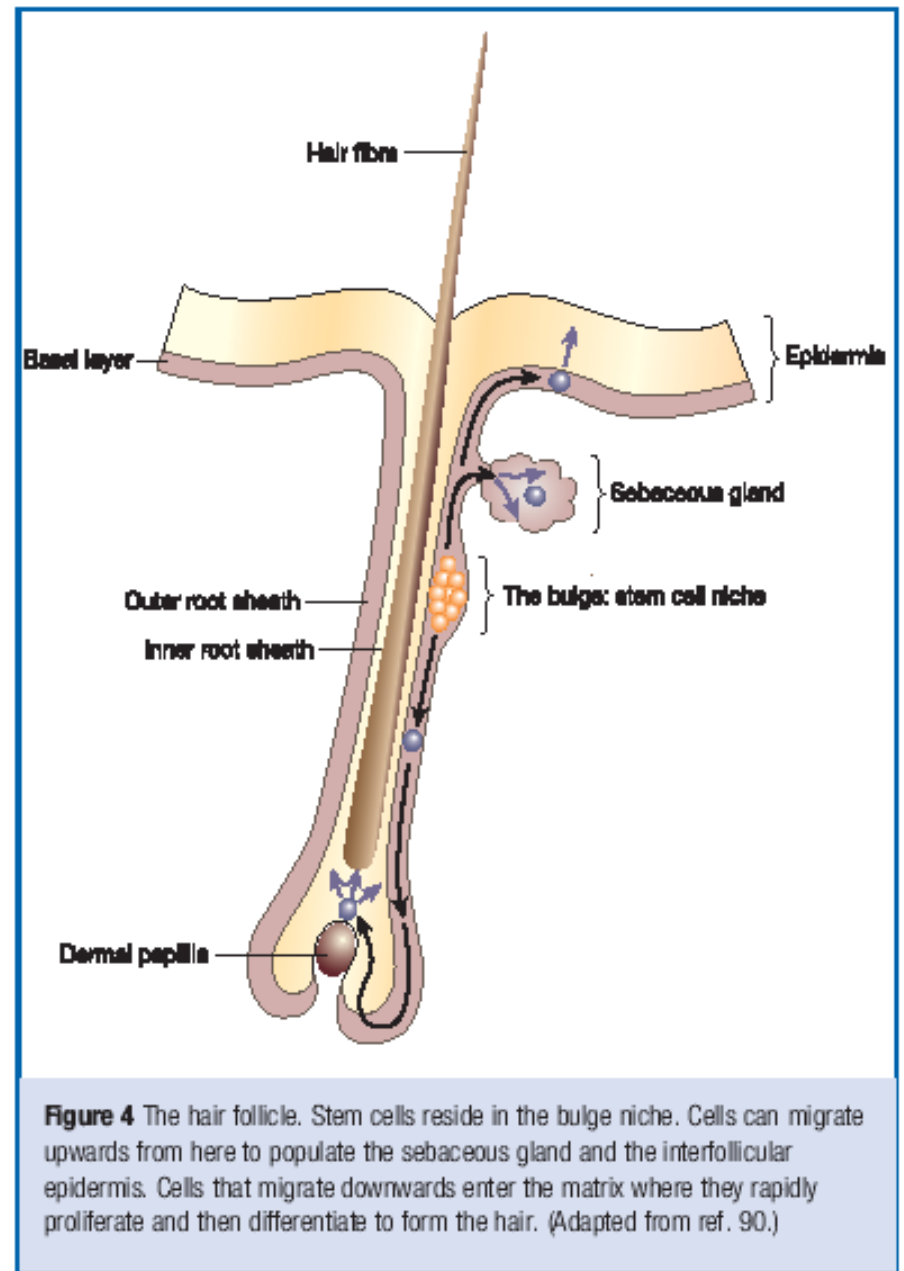
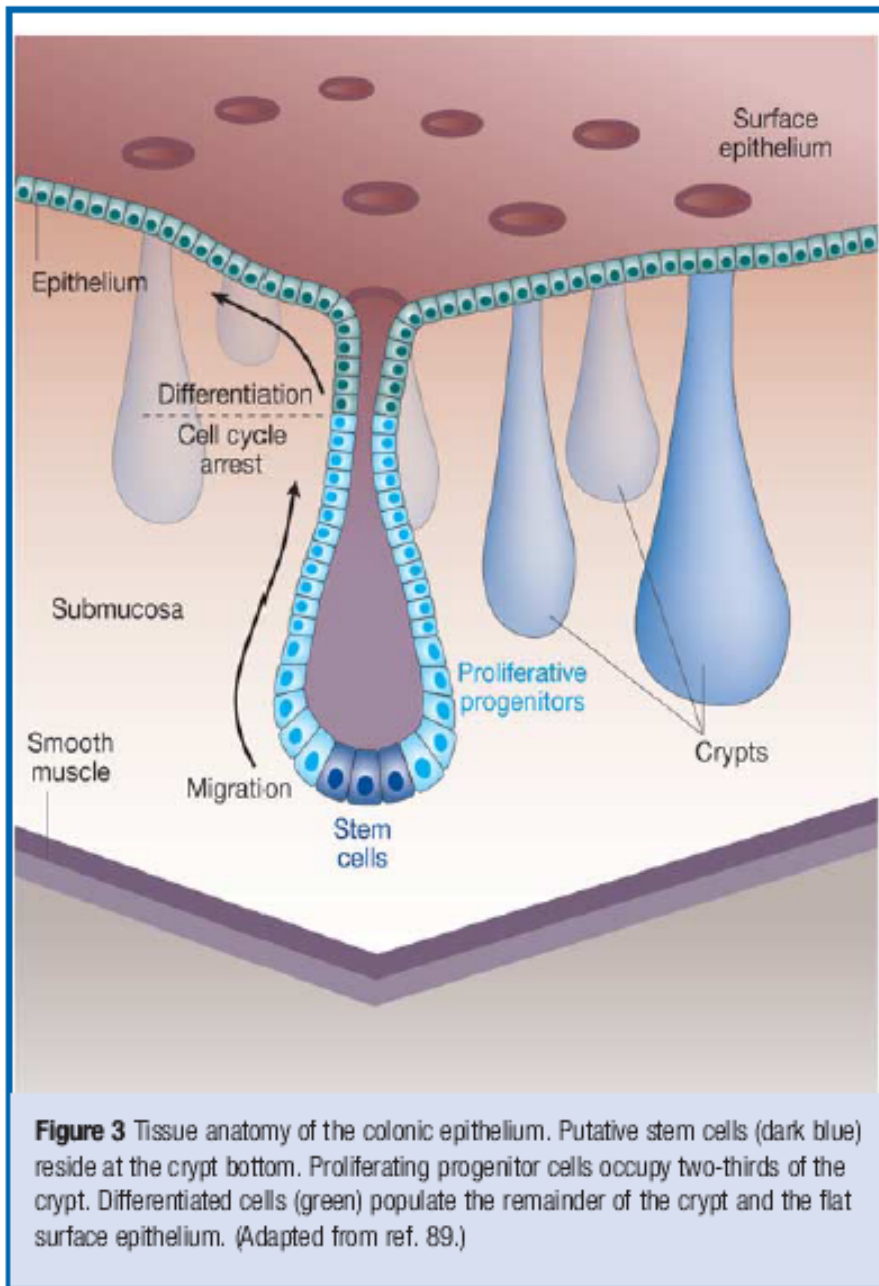
Wnt/ β -catenin dráha je velmi často deregulovaná u nádorů!



according to Beachy et al., Nature 2004

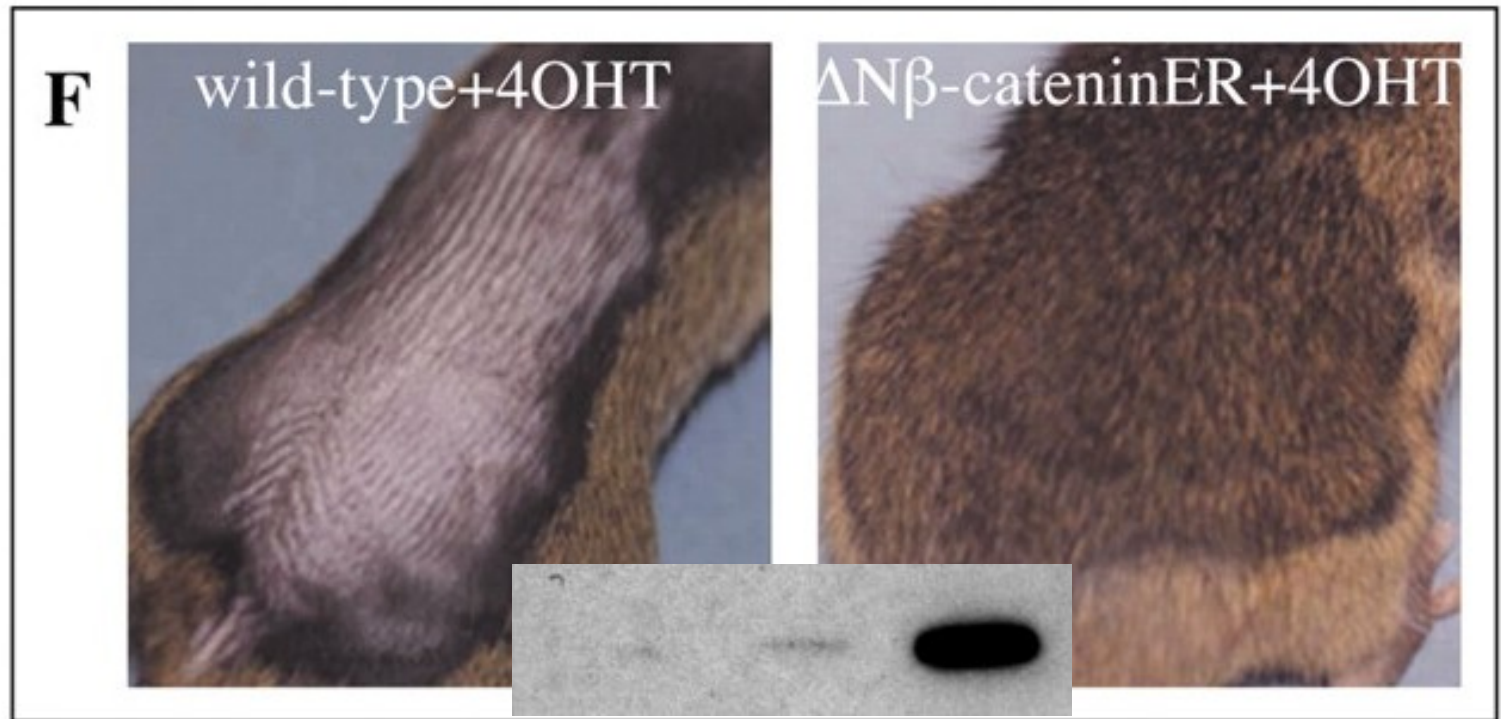
Wnt pathway

| | | |
|---------------|--------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 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 | Pilomatricoma | 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 |



Reya & Clevers 2005, Nature

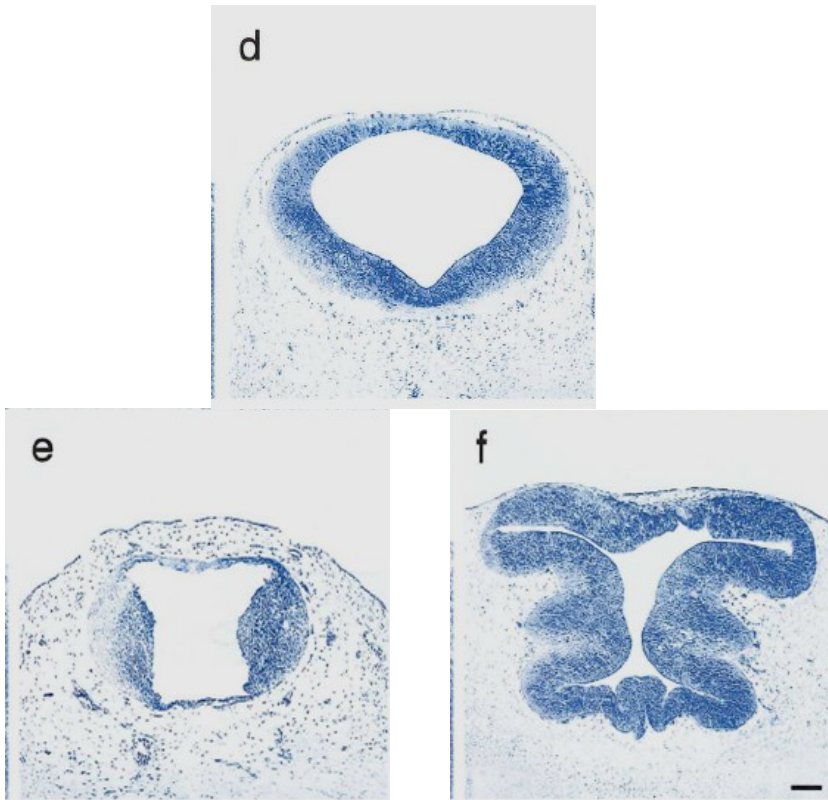
Důsledky aktivace β -catenin v epidermis (po depilaci)



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

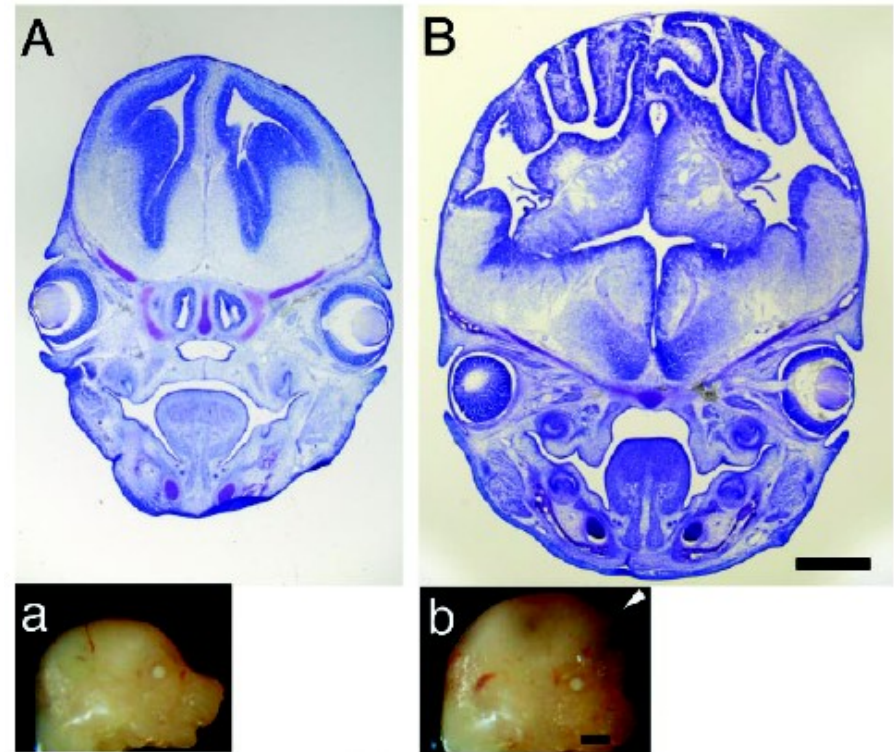
Aktivace β -catenin u vyvíjející se mozkové trubici:

midbrain (Brn4-promotor)



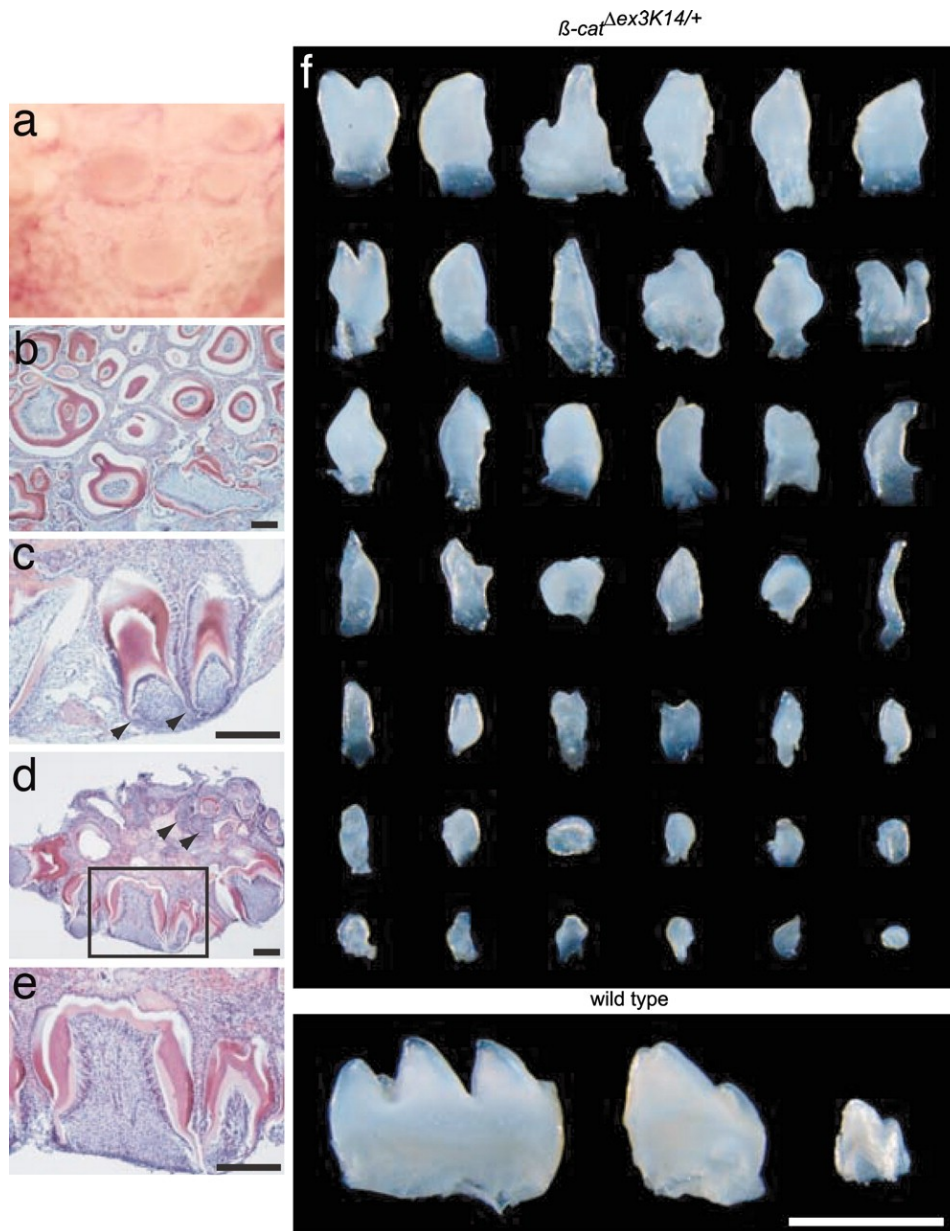
Zechner et al., 2003: Dev. Biol.;258:406-418.

cortex (nestin enhancer)



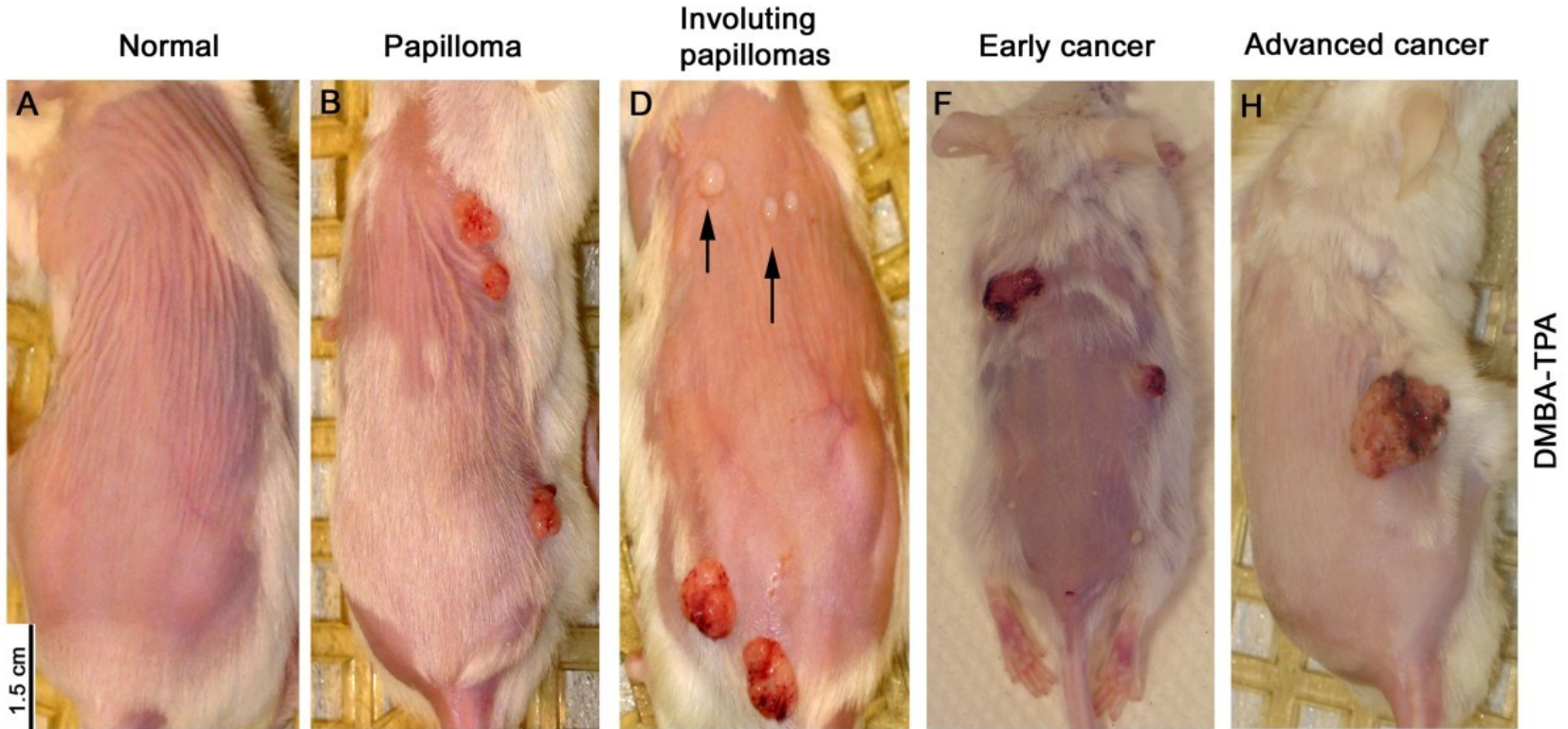
Chenn & Walsh, 2002: Science;297:365-369.

Aktivace beta-cateninu v kmenových buňkách zubu:



DMBA/TPA-induced skin carcinogenesis

- benign papillomas, which in some cases progress into squamous cell carcinoma (SCC)



DMBA/TPA-induced skin carcinogenesis

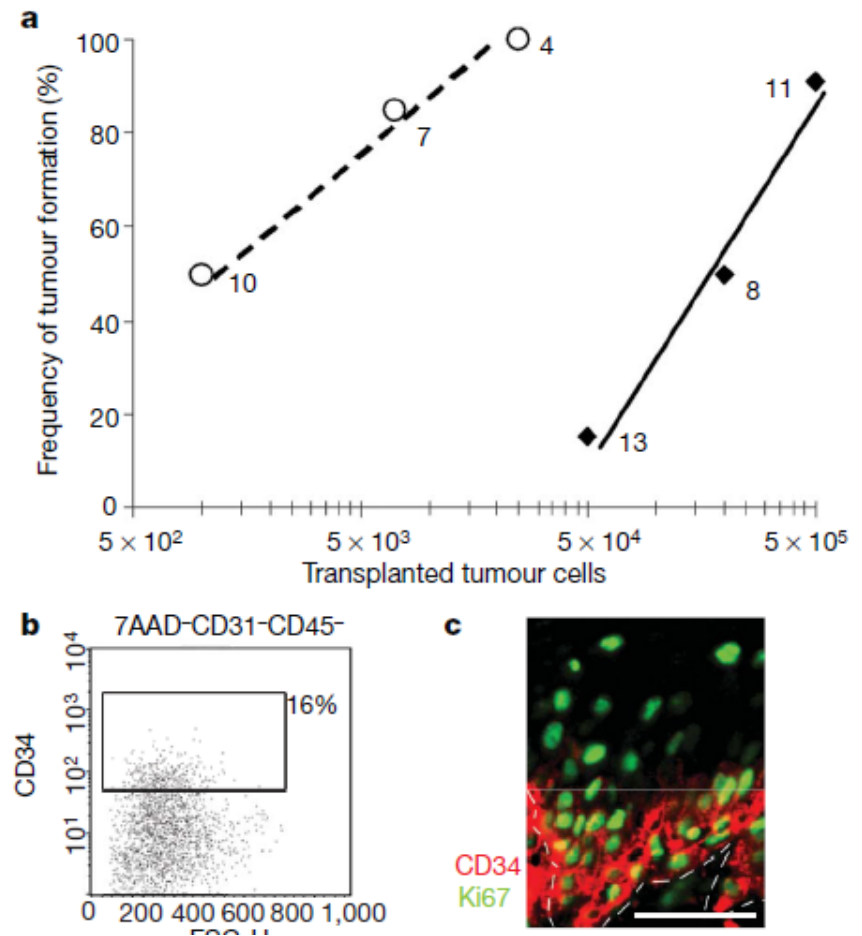
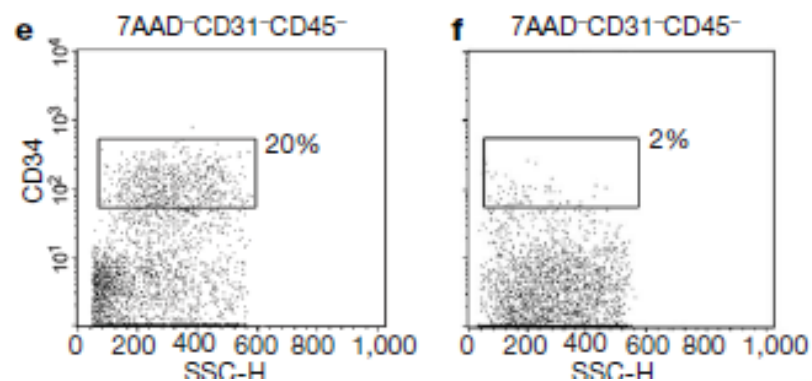
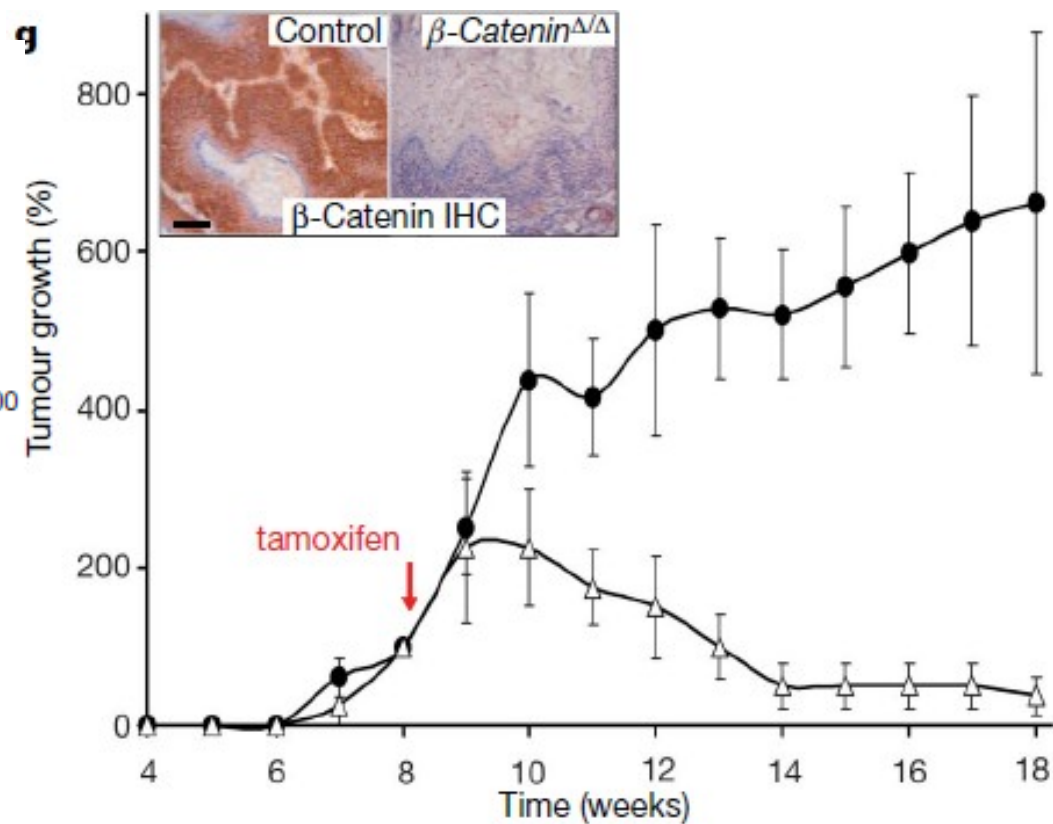


Figure 2 | Cancer stem cells efficiently initiate secondary tumours that recapitulate the organization of the primary tumour. **a**, Diagram summarizing the frequency of tumour formation in orthotopic tumour transplants using unsorted cells (filled diamonds) or CD34⁺ cells (open circles) in varying amounts. The *n* value for each point is shown. **b**, Abundance of CSCs (CD34⁺7AAD⁻CD31⁻CD45⁻) in secondary tumours derived from orthotopic transplantations of CD34⁺ cells.



characterized by abundant keratinization. **e, f**, Abundance of CD34⁺ cells in control tumours (**e**) and β -catenin-deficient tumours (**f**) two weeks after induction of deletion. **g**, Tumour regression after tamoxifen-induced β -catenin deletion in established skin tumours ($n = 6$; control (filled circles), $K14$ -creER^{T2}; β -catenin^{+/-}; mutant (open triangles), $K14$ -creER^{T2}; β -catenin ^{Δ/Δ}). Tumour numbers at 8 weeks were set to 100%. Error bars show



Malanchi et al. 2008, Nature

Wnt/ β -cateninová dráha v regulaci stárnutí

Augmented Wnt Signaling in a Mammalian Model of Accelerated Aging

Hongjun Liu,¹ Maria M Fergusson,^{1*} Rogerio M. Castilho,^{2*} Jie Liu,¹ Liu Cao,¹ Jichun Chen,³ Daniela Malide,⁴ Ilsa I. Rovira,¹ Daniel Schimel,⁵ Calvin J. Kuo,⁶ J. Silvio Gutkind,² Paul M. Hwang,¹ Toren Finkel^{1†}

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Increased Wnt Signaling During Aging Alters Muscle Stem Cell Fate and Increases Fibrosis

Andrew S. Brack,¹ Michael J. Conboy,¹ Sudeep Roy,¹ Mark Lee,² Calvin J. Kuo,² Charles Keller,³ Thomas A. Rando^{1,4*}

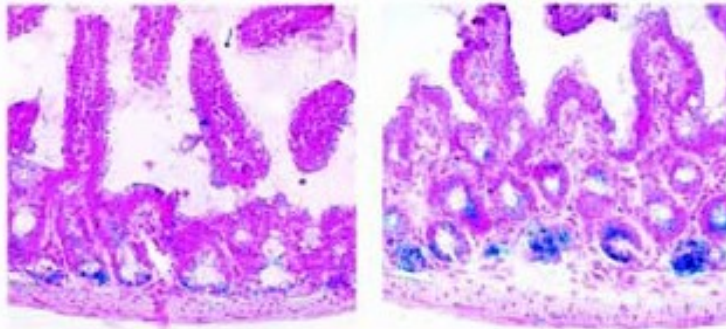
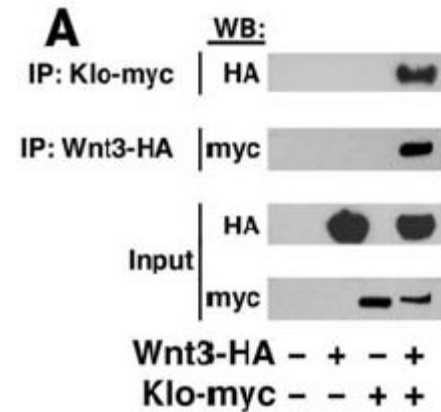
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Klotho myš

-mutantní kmen myši s fenotypem akcelerovaného stárnutí: např. kratší život, arterioskleróza, snížená plodnost nebo kožní atrofie

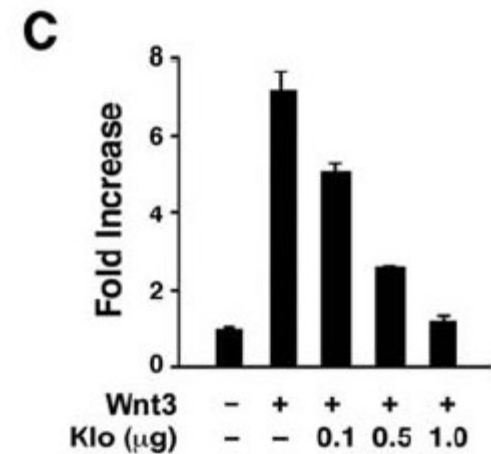
- protein Klotho je transmembránový protein s velkou extracelulární doménou, ta může být odštěpena a volně cirkulovat v krvi



WT

Klo

aktivita Wnt/ β -cateninové dráhy
ve střevním epitelu

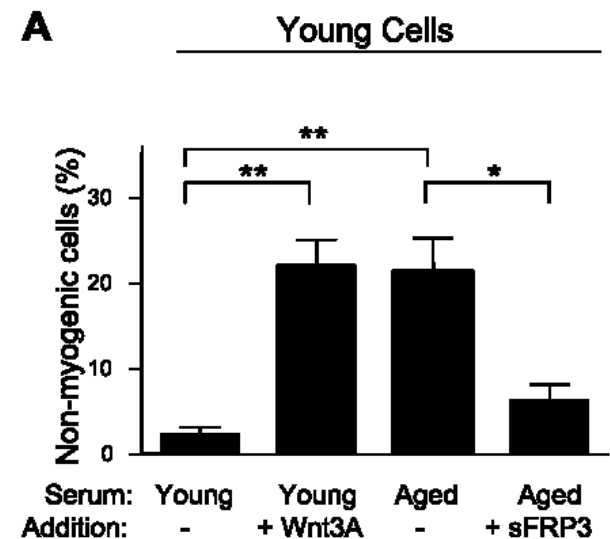
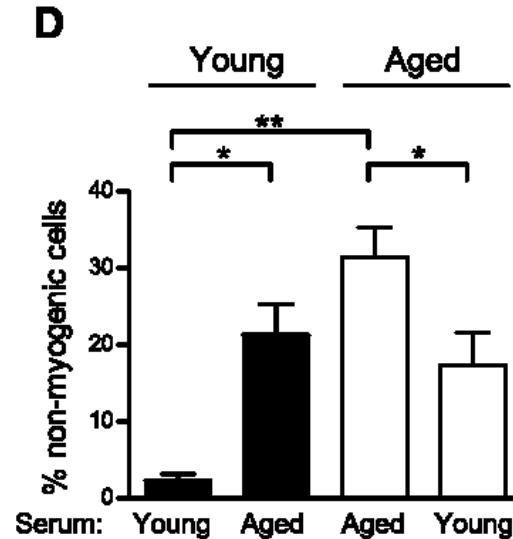
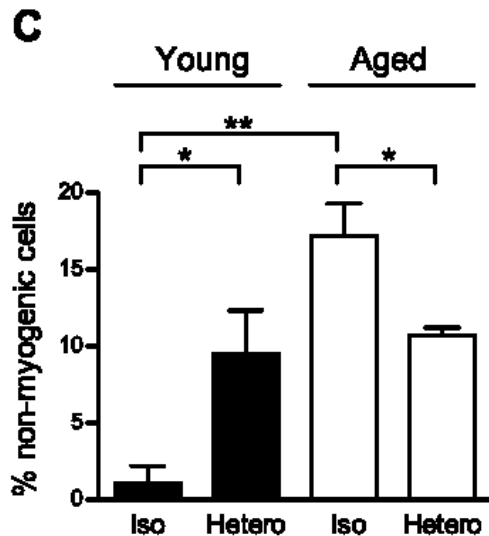
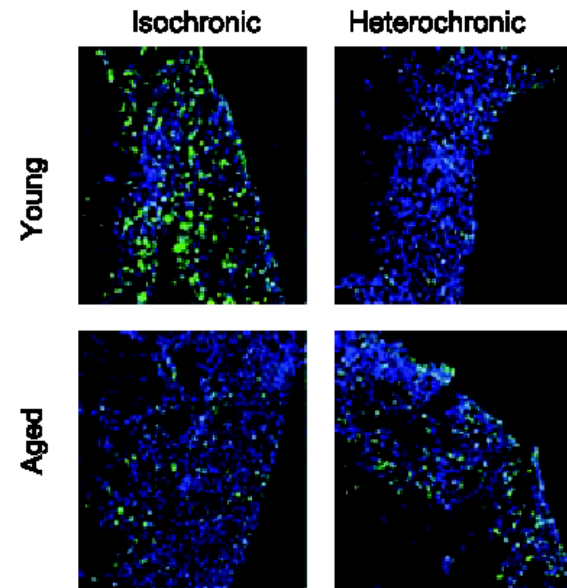


Model 1 – svalová fibróza

- s prodlužujícím se věkem stále častěji při regeneraci svalu vznikají místo svalových buněk buňky fibrózní tkáně – tak přispívají k nižší výkonnosti svalu, která souvisí se stárnutím

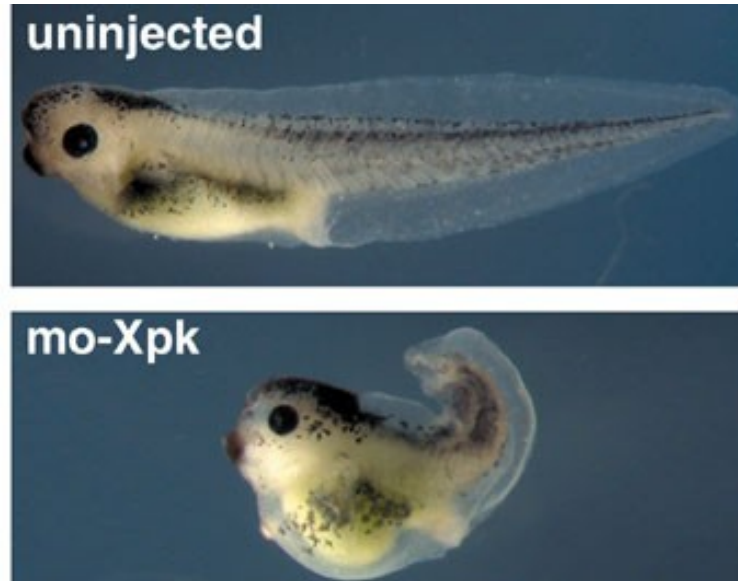
Model 2 – parabiotické párování

Fyzické propojení dvou krevních systémů (a tím i dvou vnitřních prostředí) u myši



Nekanonická Wnt dráha

- e.g. Wnt5a

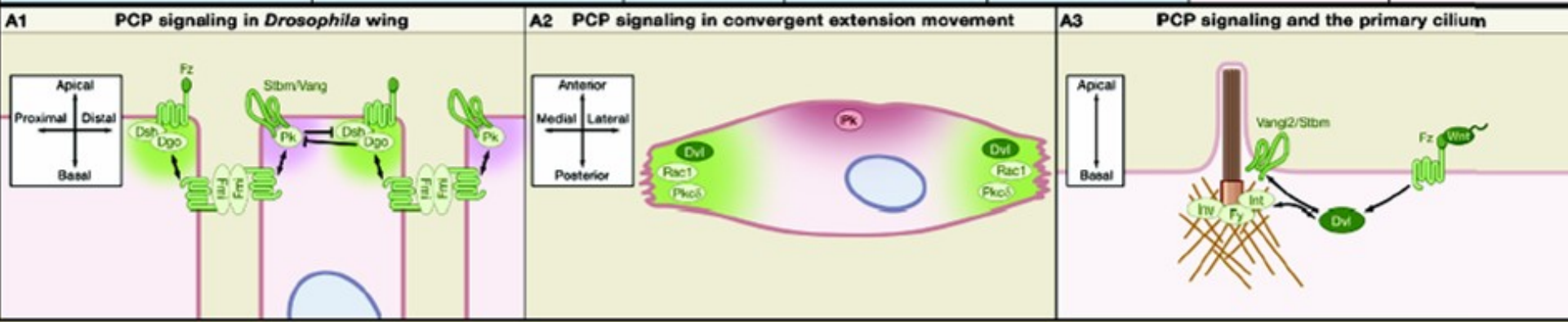
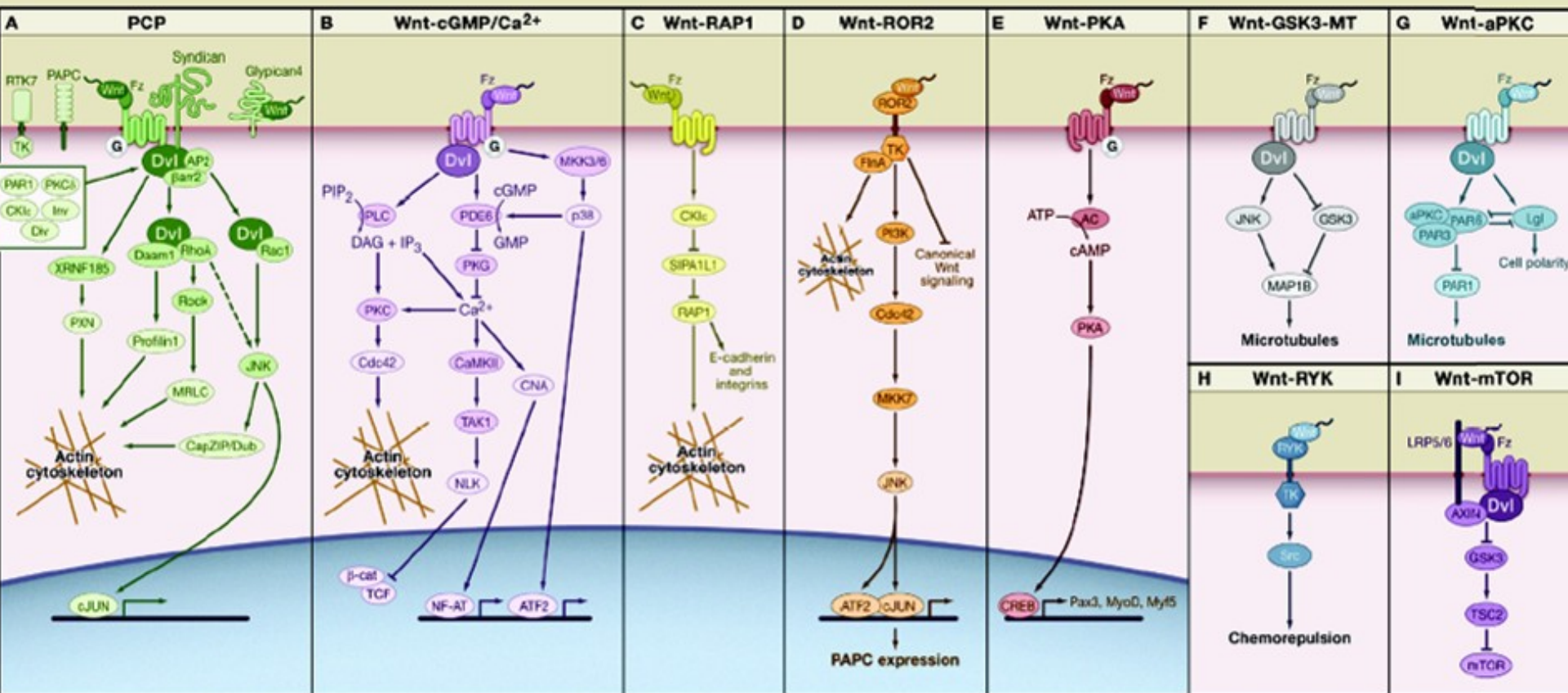


- do not induce axis duplication in *Xenopus*
- do not induce transformation of mammary cell line C57mg
- do not signal via nuclear translocation of β -catenin

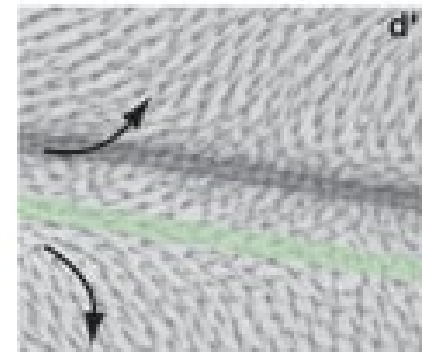
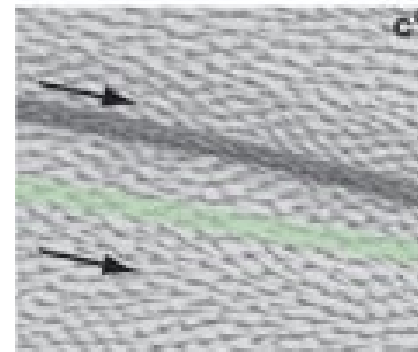
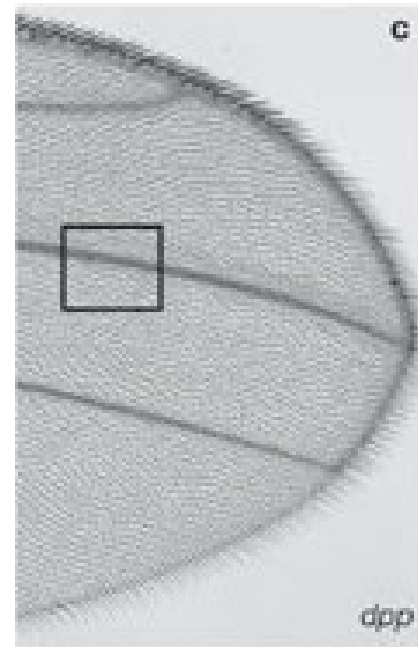
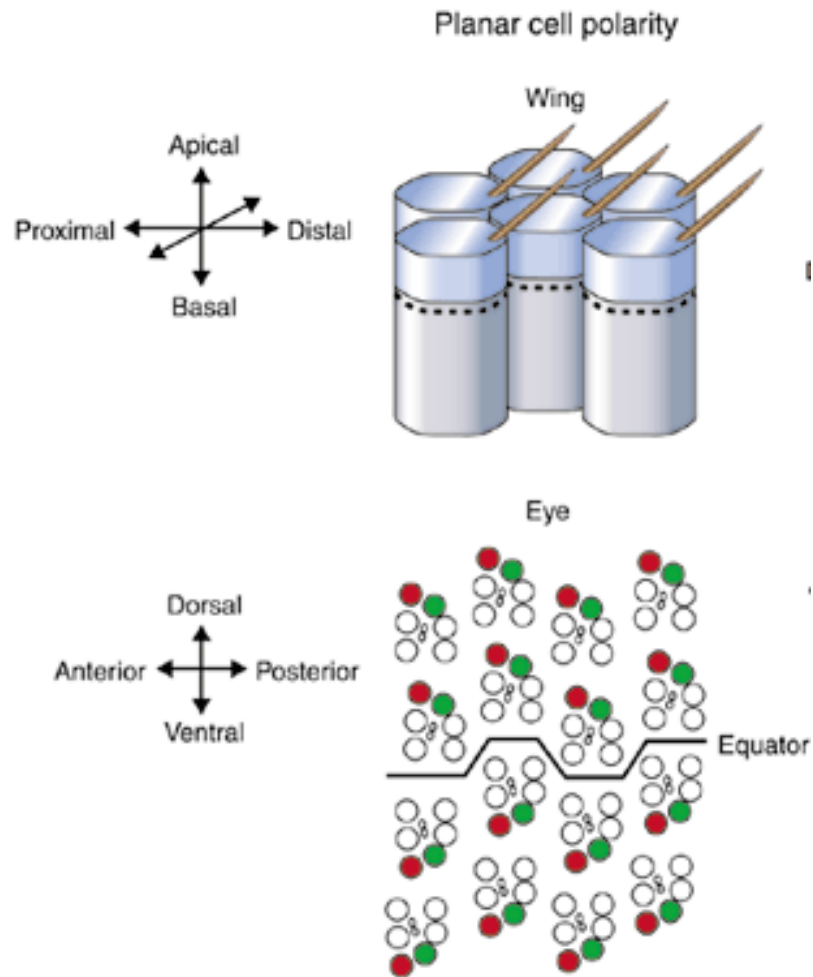
SnapShot: Noncanonical Wnt Signaling Pathways

Mikhail V. Semenov,¹ Raymond Habas,² Bryan T. MacDonald,¹ and Xi He¹

¹Children's Hospital Boston, Harvard Medical School, Boston, MA 02115, USA; ²University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, USA



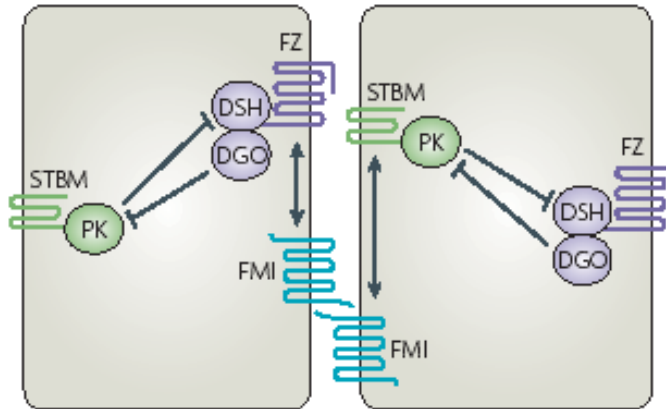
Drosophila – PCP (planar cell polarity)



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



Seifert and Mlodzik, Nature Reviews in Genetics, 2007

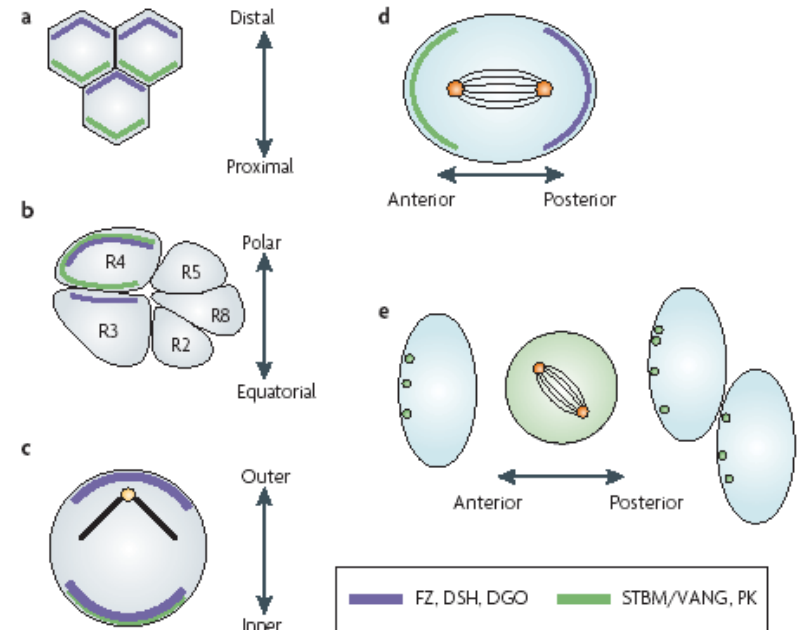
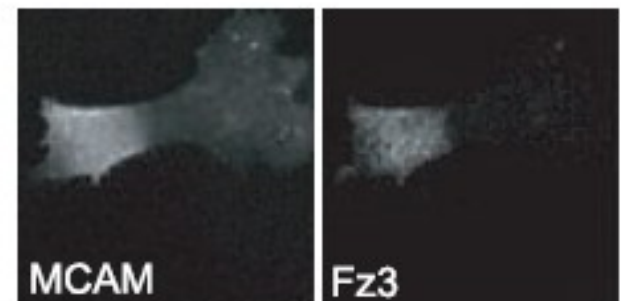
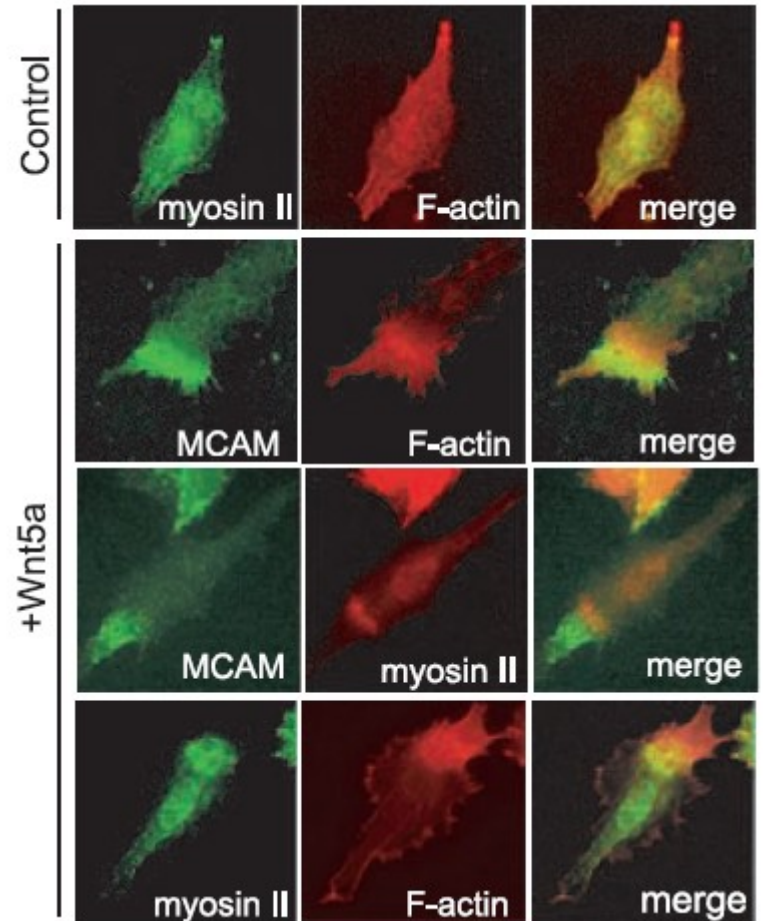


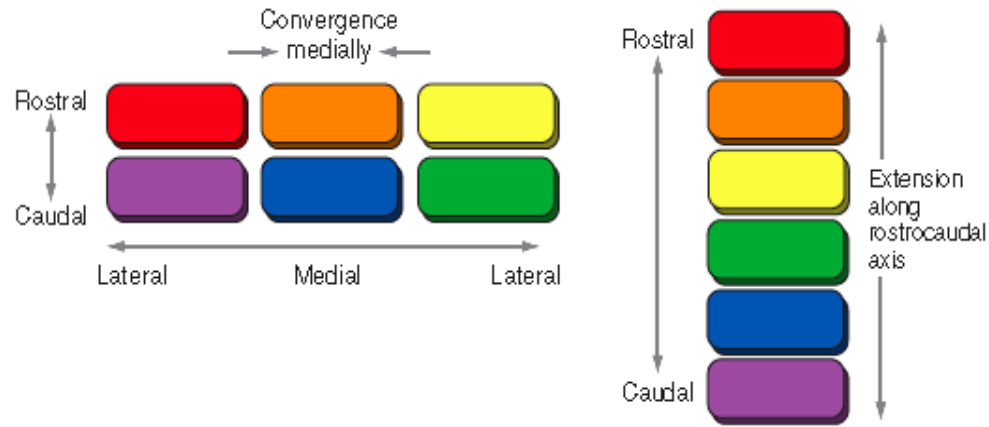
Figure 2 | Subcellular distribution of core Fz/PCP factors in *Drosophila melanogaster* and vertebrates. a–c | Examples of cells with epithelial character (marked by grey shading). *Drosophila melanogaster* wing cells and eye R3 and R4 cells and mouse sensory hair cells in the cochlea (inner ear) are 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 unknown⁹². 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).

Wnt-induced assymetry

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

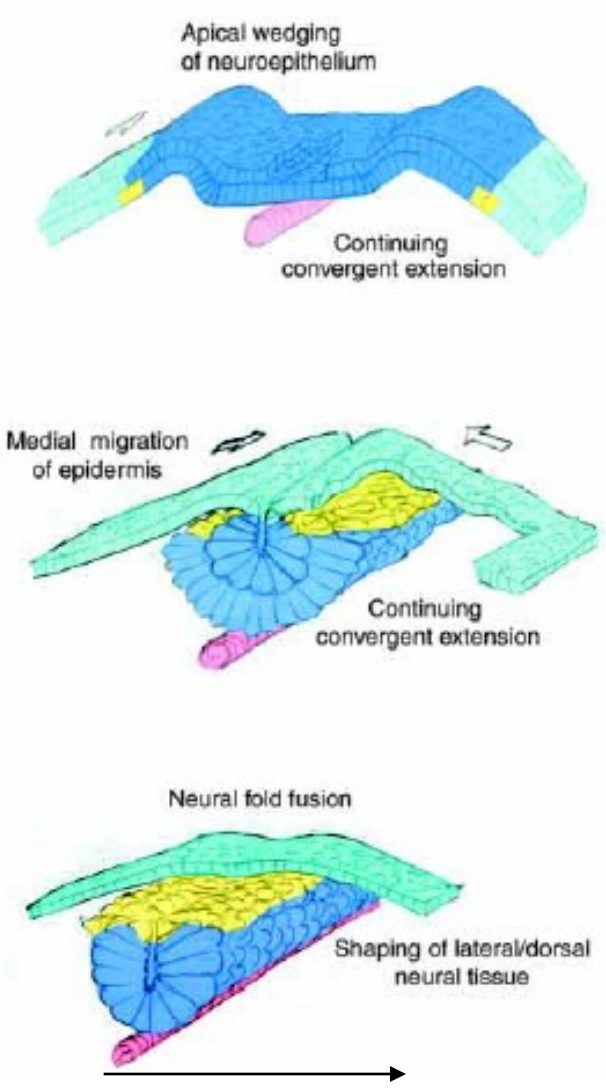


Non-canonical/PCP (Planar cell polarity) in mouse (and human) convergent extension

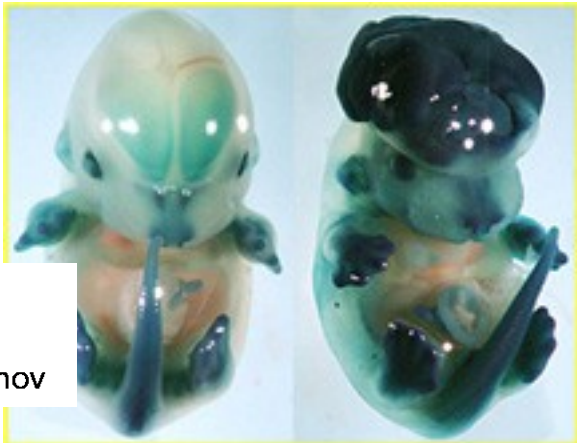


Konvergentní extenze – migrace buněk směrem ke středu těla – vede k prodlužování tělní osy

Důsledky narušené konvergentní extenze (CE)



Exencephaly



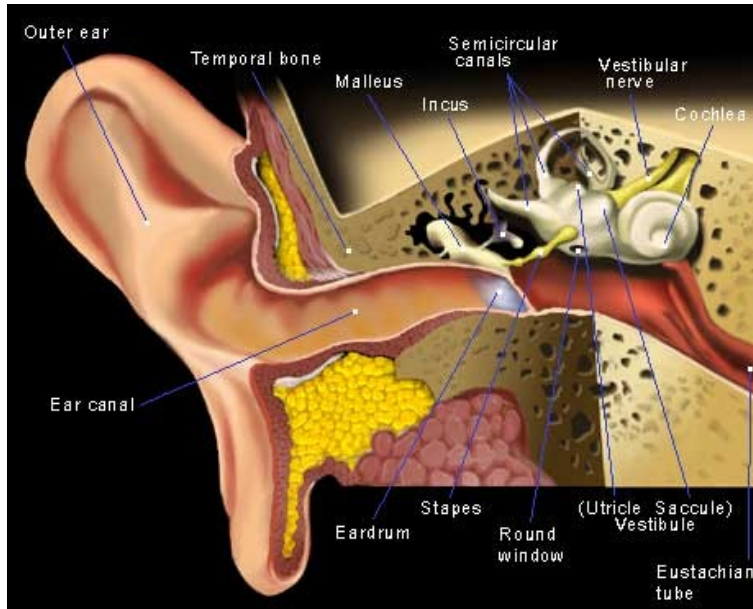
 neurulation.mov

Open neural tube

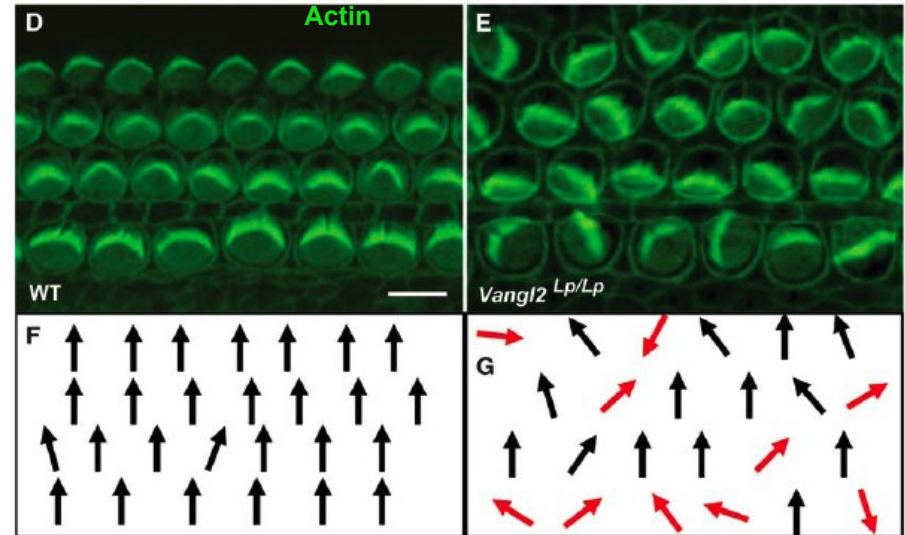


Hamblet et al., 2002, Development

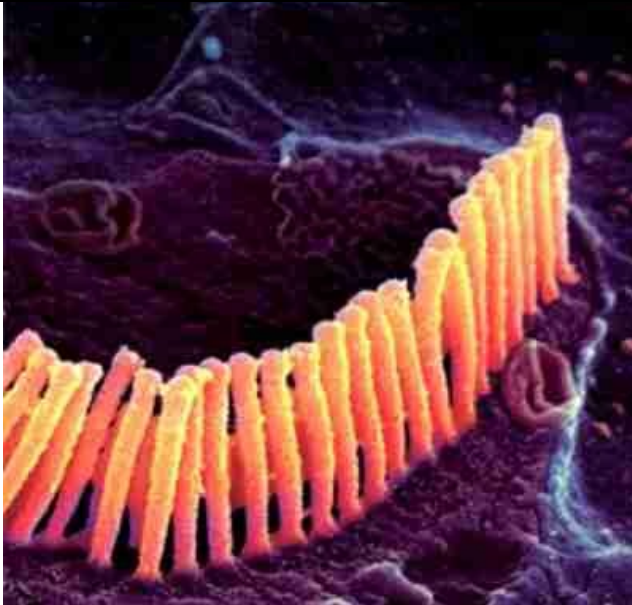
Non-canonical/PCP (Planar cell polarity) pathway: phenotypes in mouse



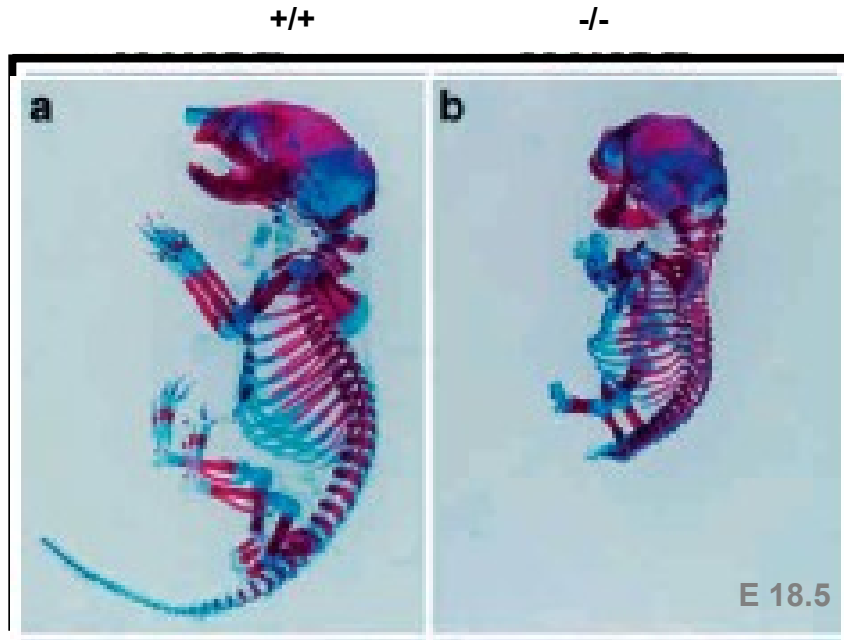
Stereocilia orientation in inner ear hair cells



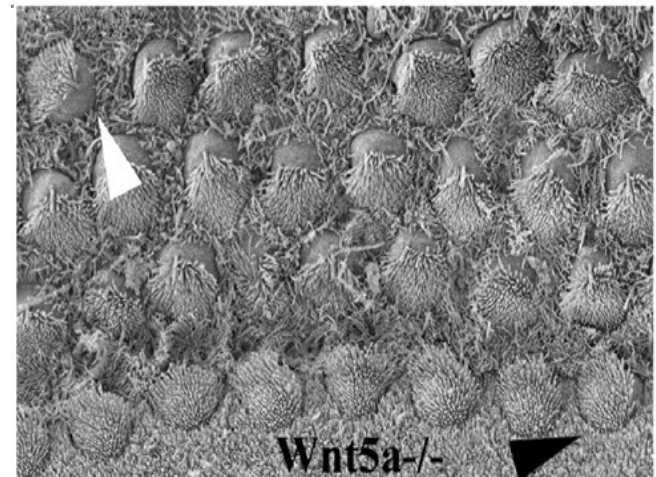
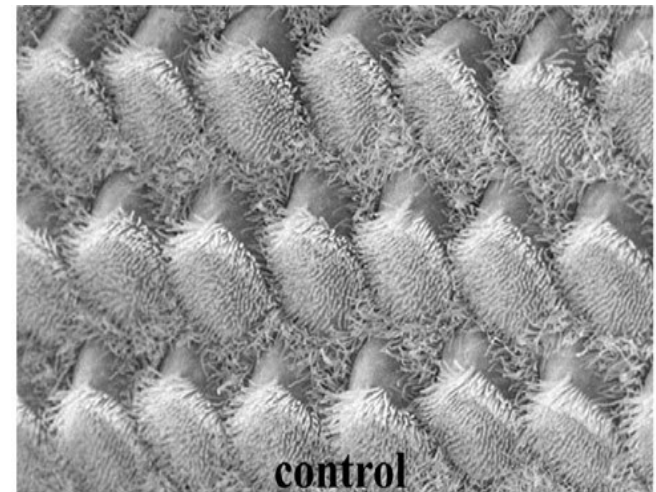
Qian et al., 2007, Dev. Biol.



Known Wnt5a knockout phenotypes

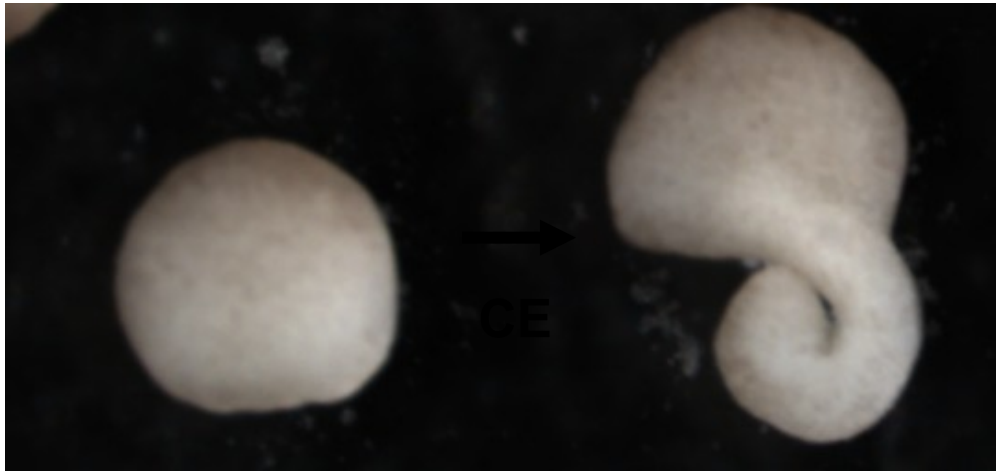


Yamaguchi et al., 1999

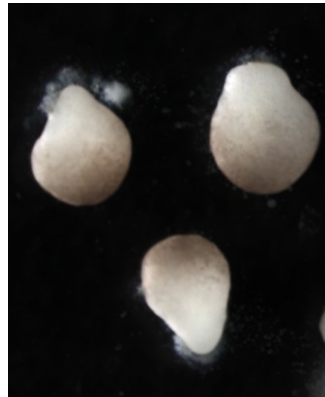


Qian et al, 2007

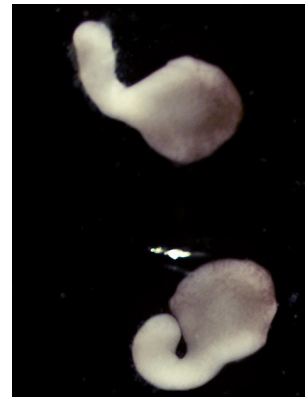
Možnosti studia CE - Kellerovy explantáty (Xenopus)



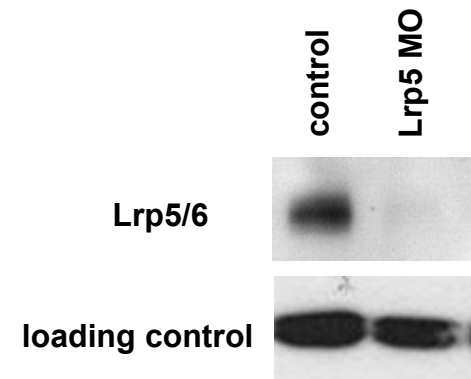
control



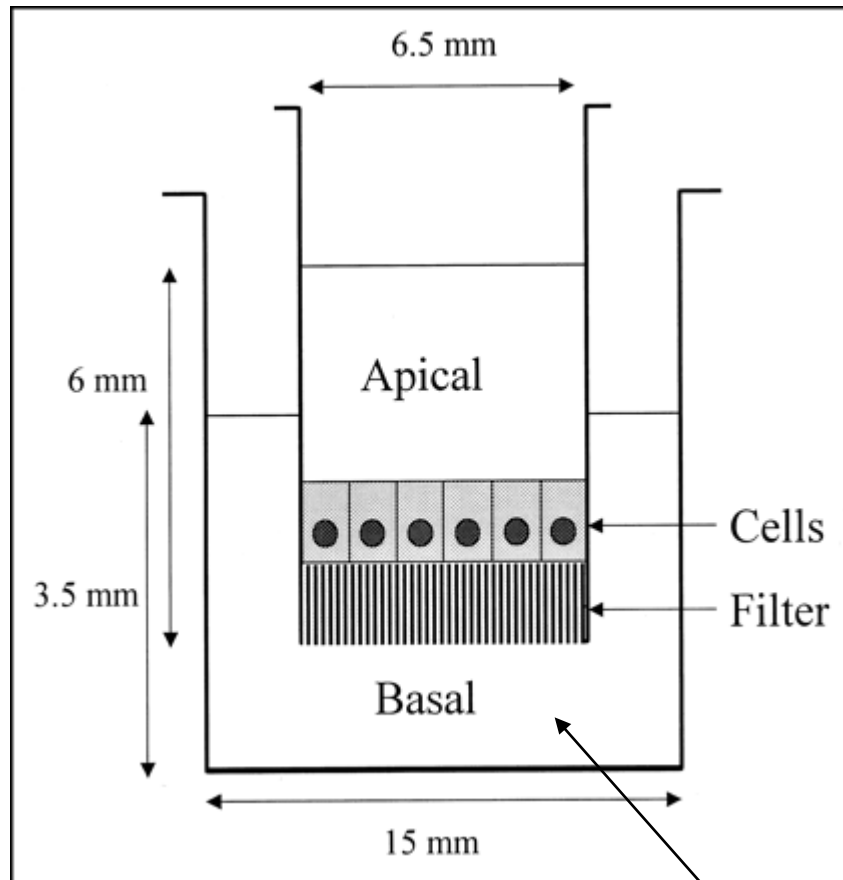
XLRP5 MO



**XLRP5 MO
+ mLRP5**



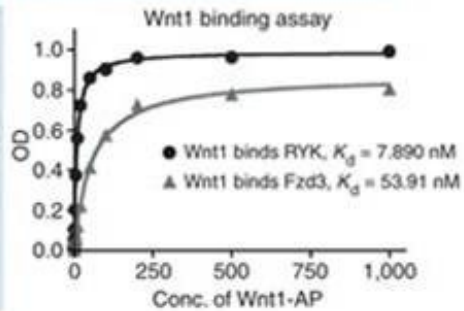
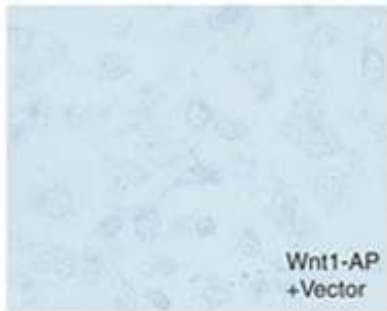
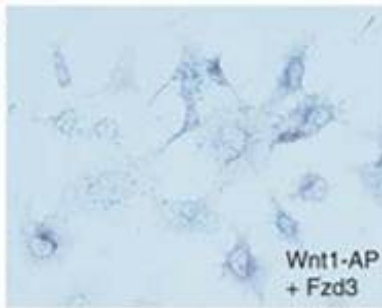
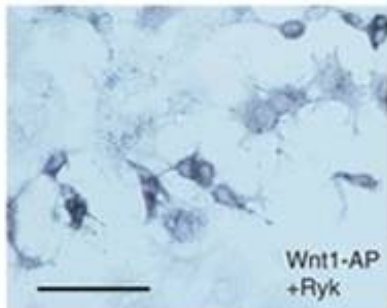
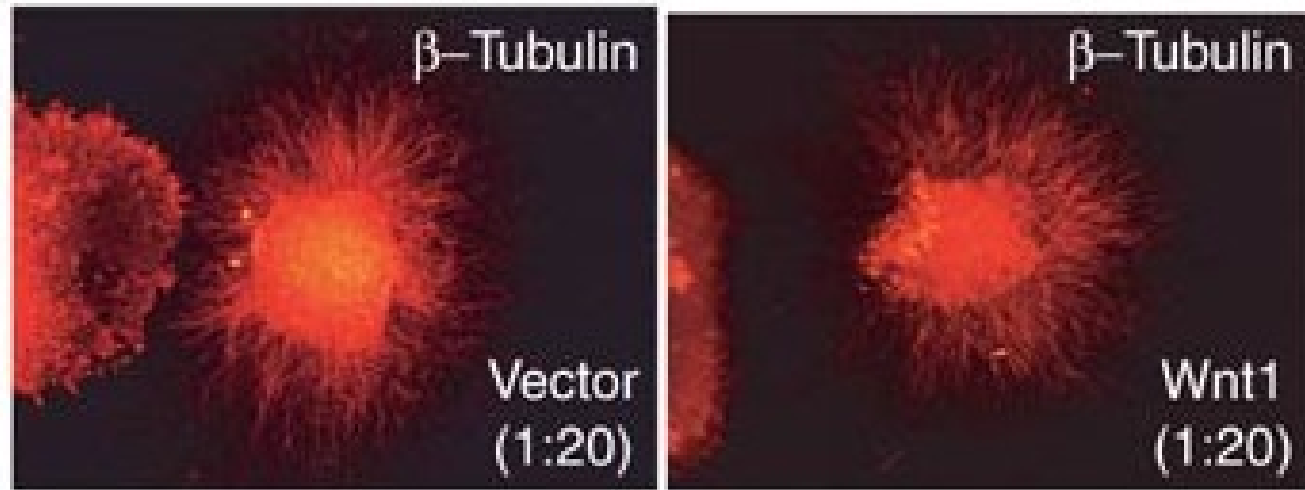
Možnosti studia migrace – Transwell assays (mammalian cells)



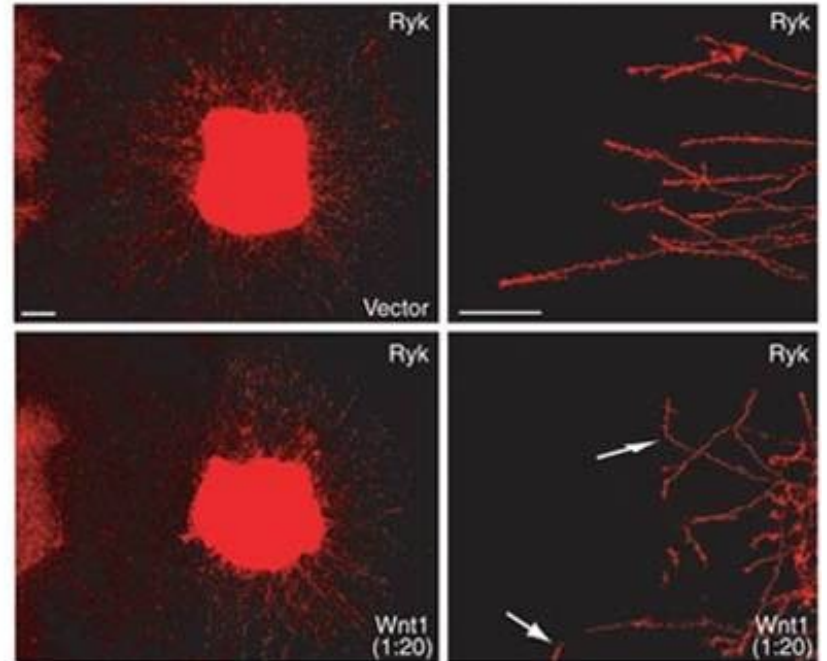
Attractant

Možnosti studia migrace – attractant/repellent assays

Attractant/repellent →

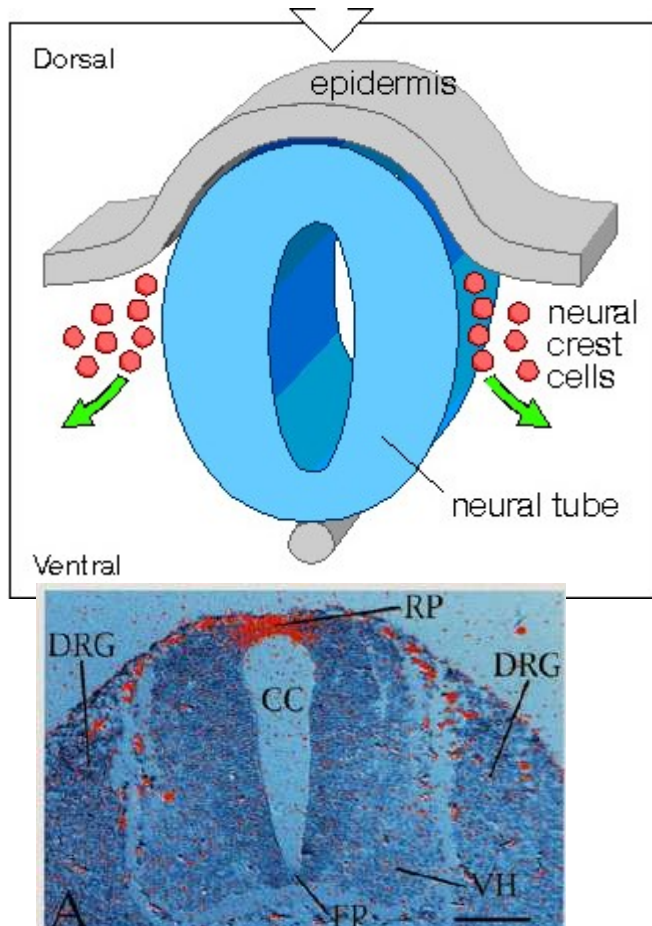


g



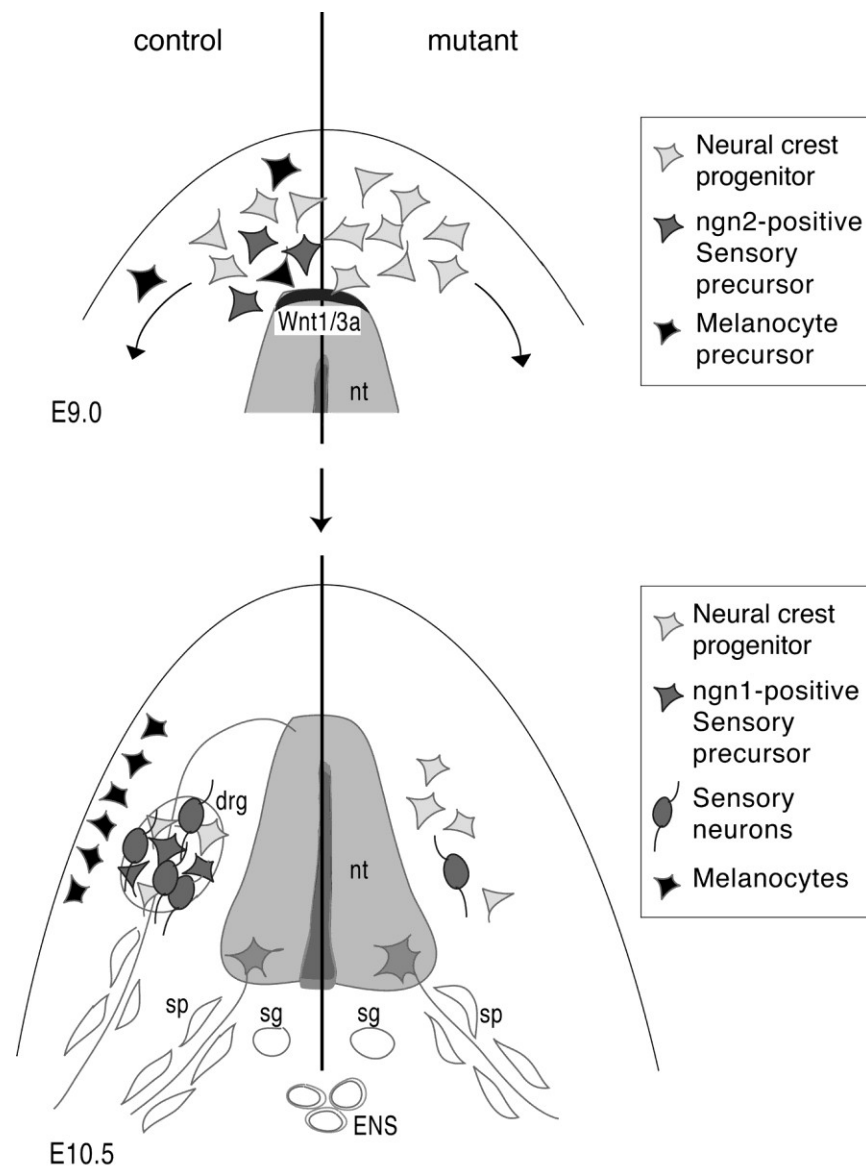
Kanonické a nekanonické Wnt signálování často regulují odlišné části téhož vývojového procesu.

I. Vývoj neurální lišty:

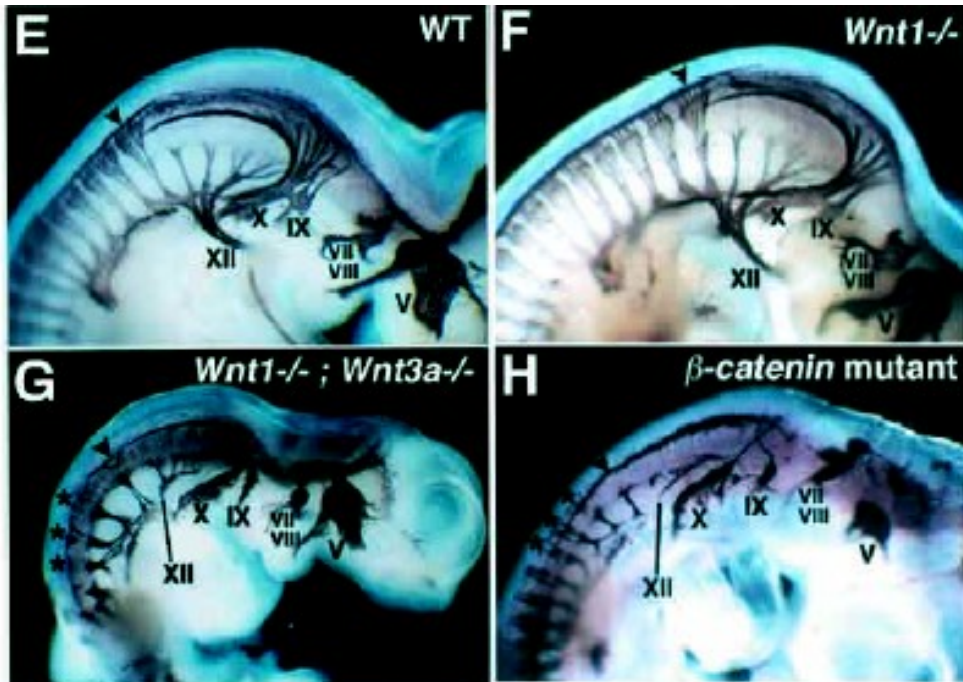
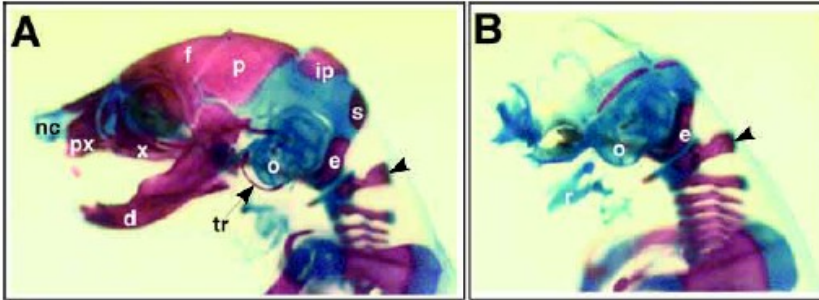


Wnt-3a

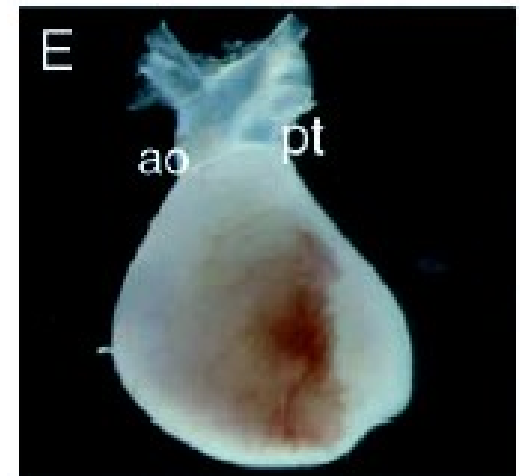
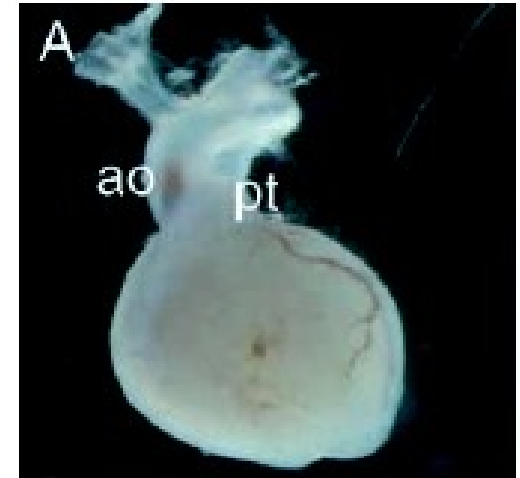
- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, srdce a dalších



Wnt1/3a DKO



Heart outflow tract development

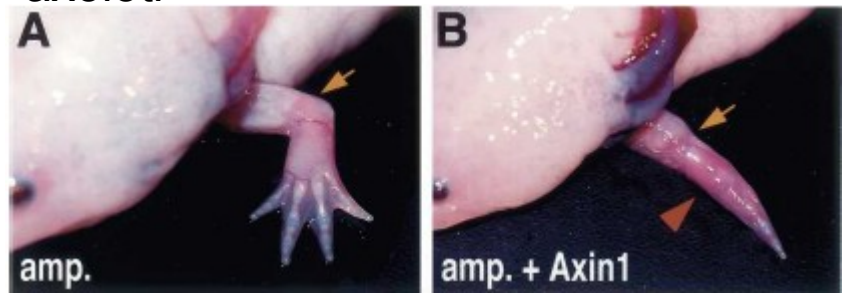


Wnt5a KO

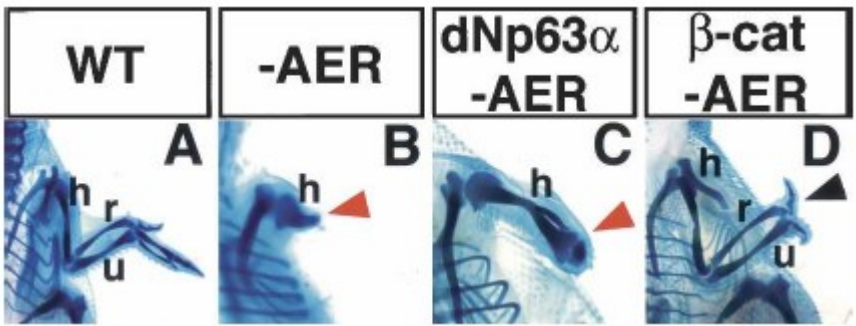
Henderson DJ et al., 2006, TrendsCard. Res.

II. regenerace

axolotl



chick



zebrafish

