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)

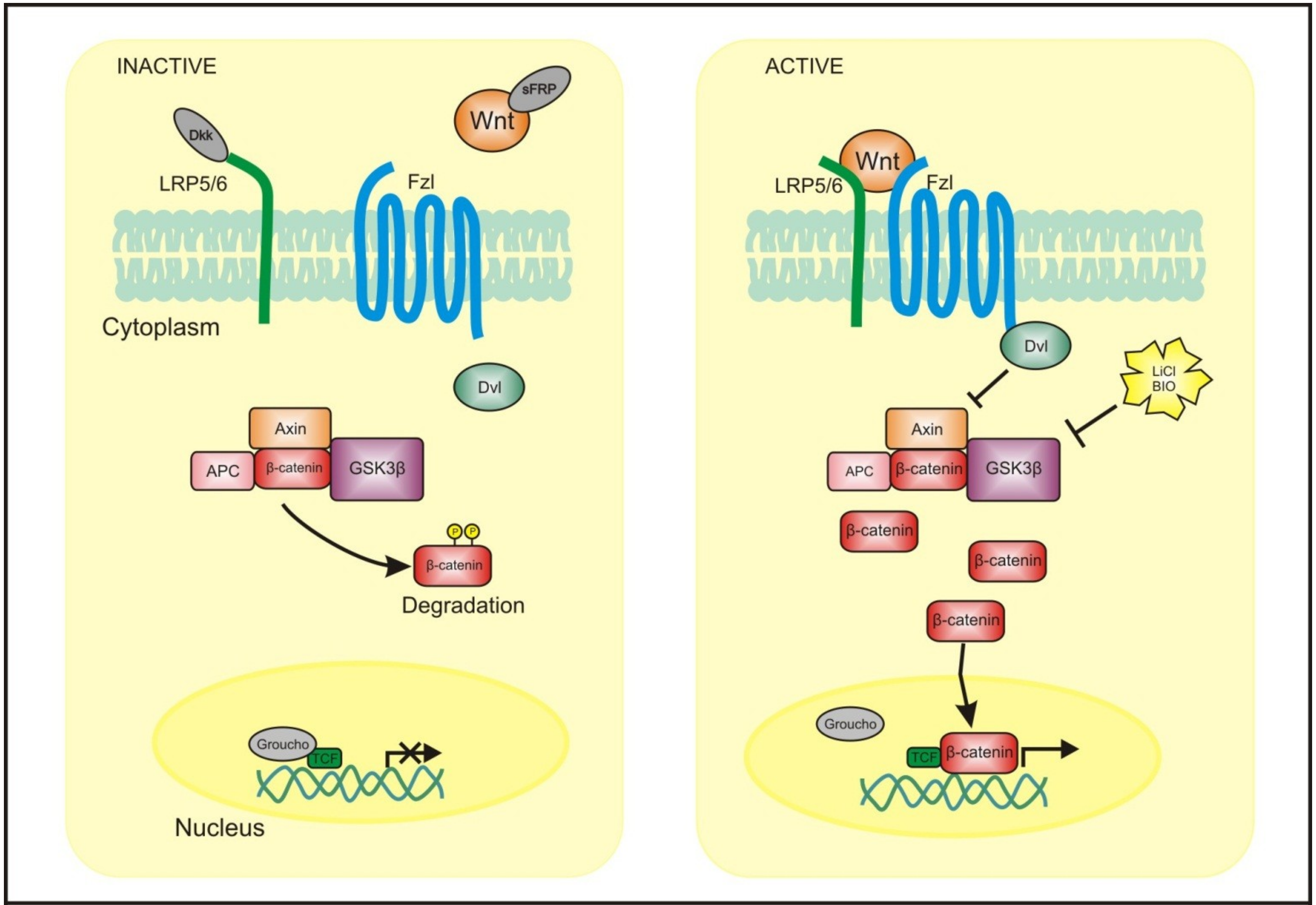
Wnt/β-kateninová dráha (= kanonická dráha)

- induce axis duplication in Xenopus

- induce transformation of mammary cell line C57mg

-signal via nuclear translocation of β-catenin

Wnt/β-catenin pathway



The Canonical Wnt signalling cascade. Canonical Wnt signalling mediates its effect by binding to their receptors frizzled (Fzd) and co-receptors, LRP 5/6. This causes activation of intracellular Dishevelled (Dvl) which, in turn, inhibits glycogen synthase kinase-3 (GSK3β). This results in the stabilisation and nuclear translocation of β-catenin, inducing gene transcription via the LEF/TCF family of transcription factors. In the absence of Wnt signalling, a complex containing GSK3β phophorylates β-catenin, leading to degradation by ubiquitination. Copyright BTR©

Wnt secretion

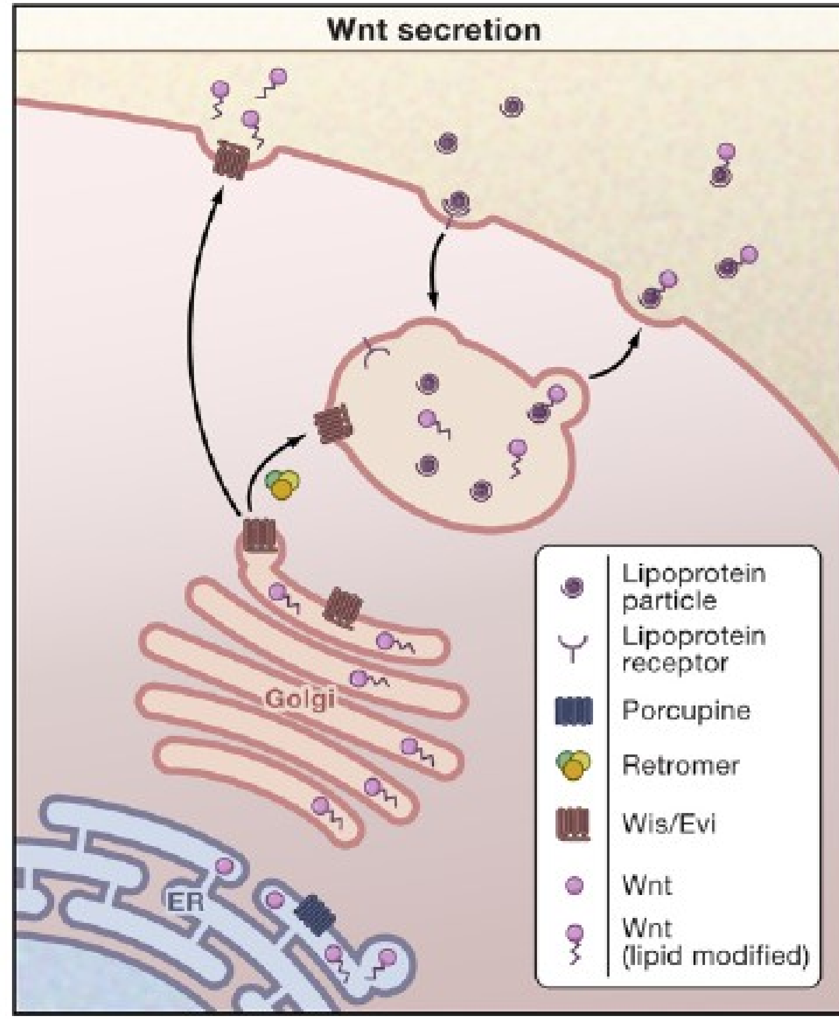


Figure 1. Wnt Secretion

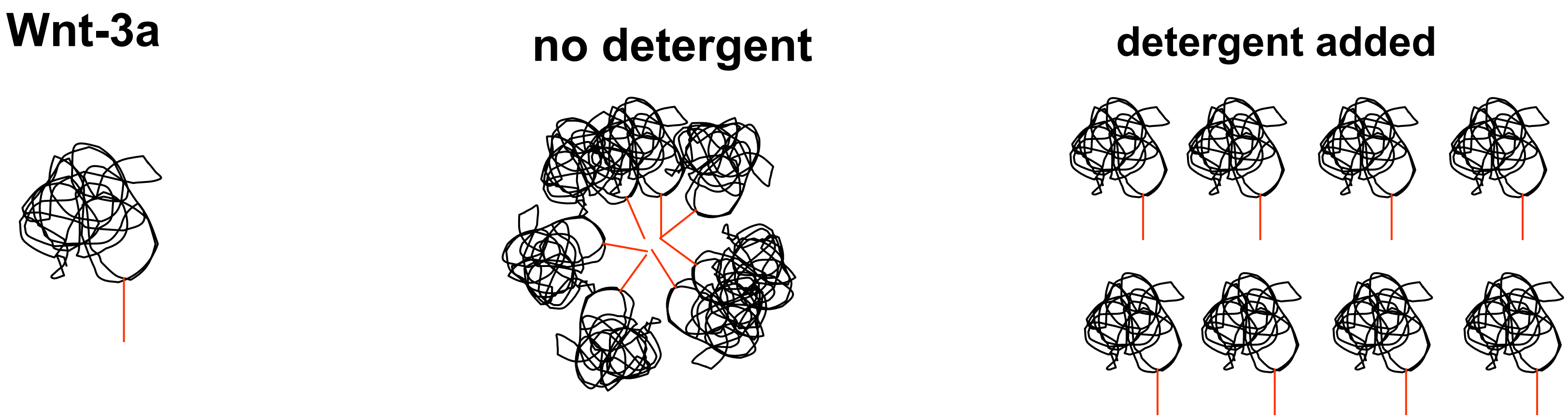
To be secreted, Wnt proteins in the endoplasmic reticulum (ER) need to be palmitoylated by the action of Porcupine. Wnt proteins also require Wntless (Wls/Evi) in order to be routed to the outside of the cell. Loading onto lipoprotein particles may occur in a dedicated endo/exocytic compartment. The retromer complex may shuttle Wls between the Golgi and the endo/exocytic compartment.

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*

NATURE | VOL 423 | 22 MAY 2003 |



Frizzled – crucial receptor of most (all?) Wnt pathways

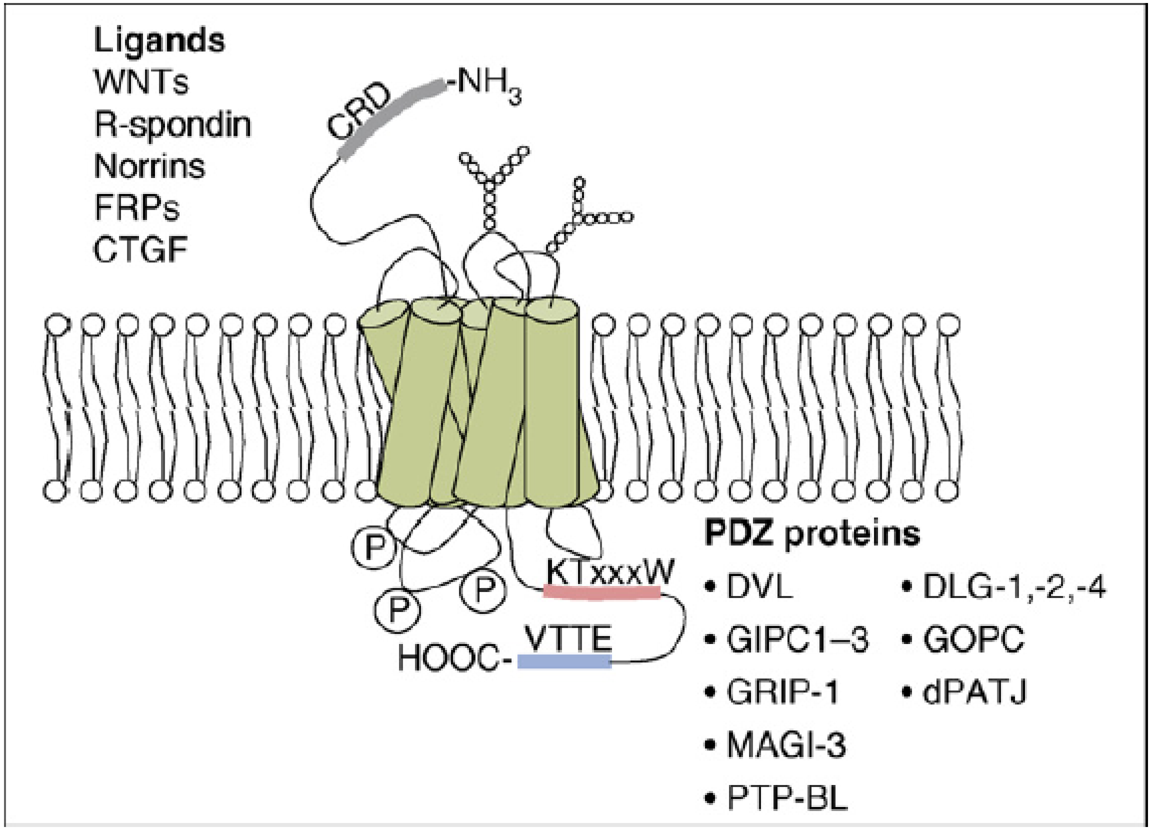
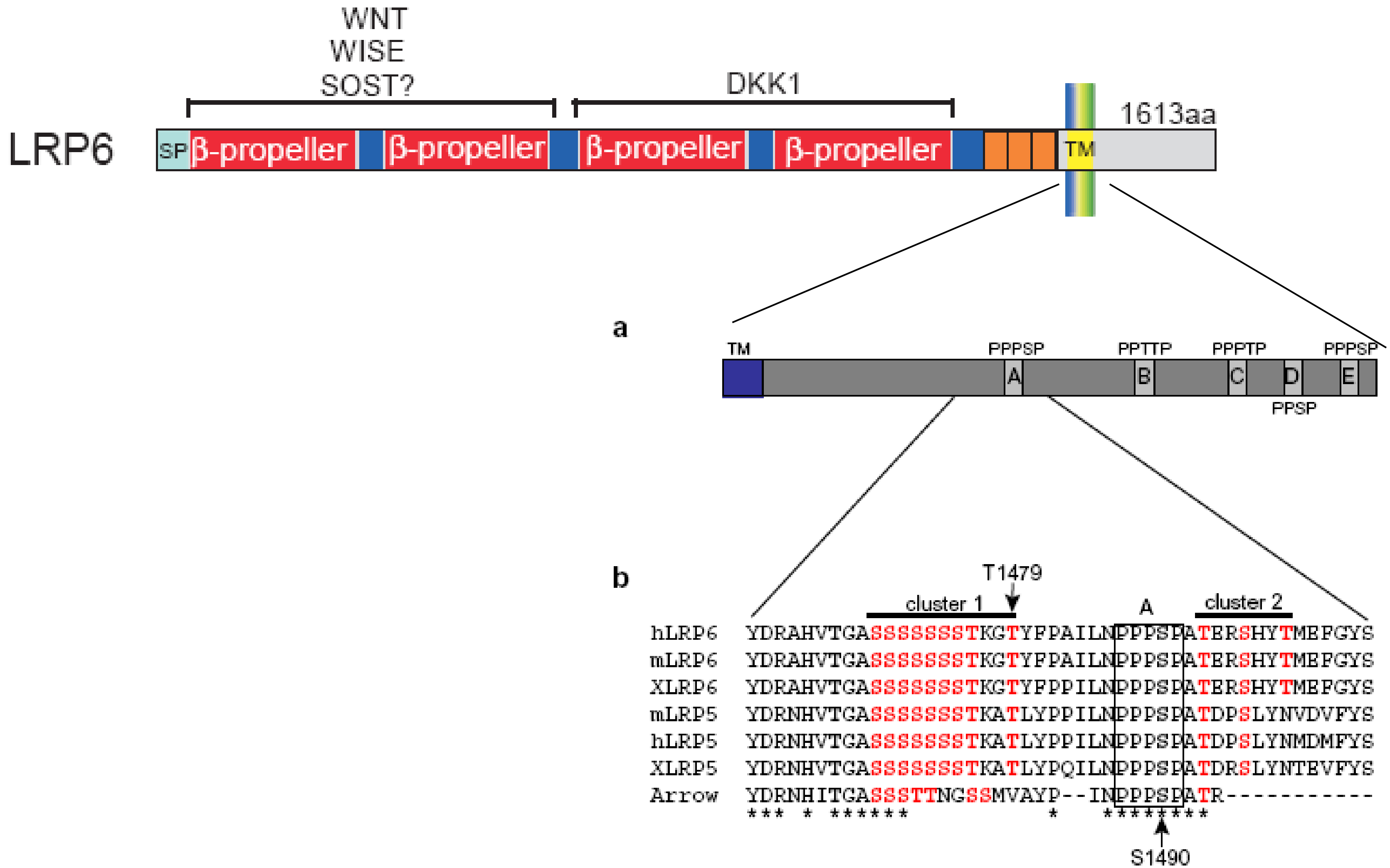


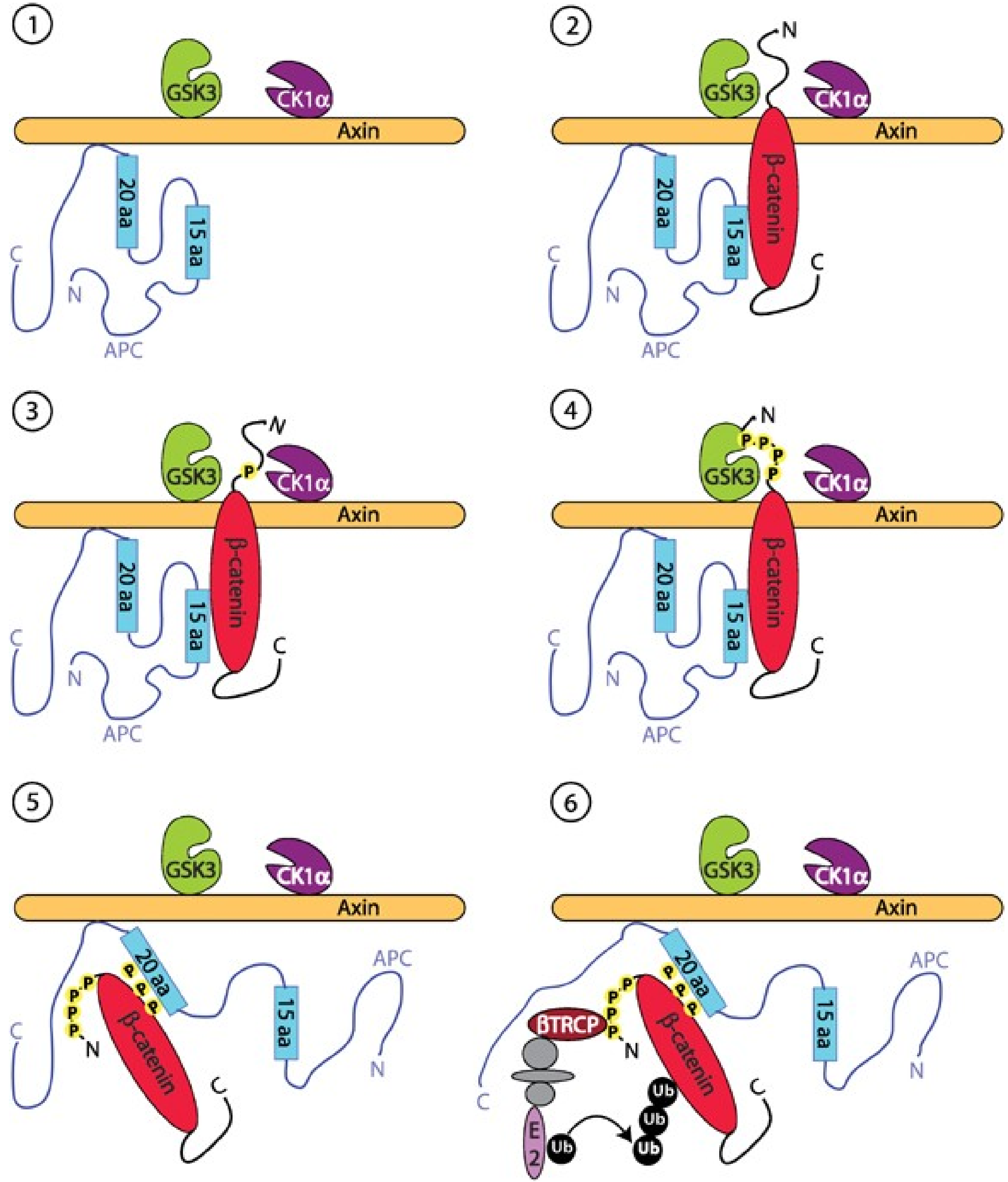
Figure 1. Schematic view of the 7TM model of FZDs. The model indicates extra- and intracellularly interacting proteins, putative glycosylation and phosphorylation sites. The N-terminal CRD is the primary binding site for ligands. The pink stretch in the C terminus indicates the internal PDZ-interacting motif (KTxxxW), which is absolutely conserved in the different FZD isoforms and necessary for DVL binding and signaling. The blue stretch at the far C terminus indicates the presence of a classical, less well-conserved PDZ-ligand sequence present in a subset of FZDs.

Lrp5/6 – crucial co-receptor of the canonical Wnt pathway

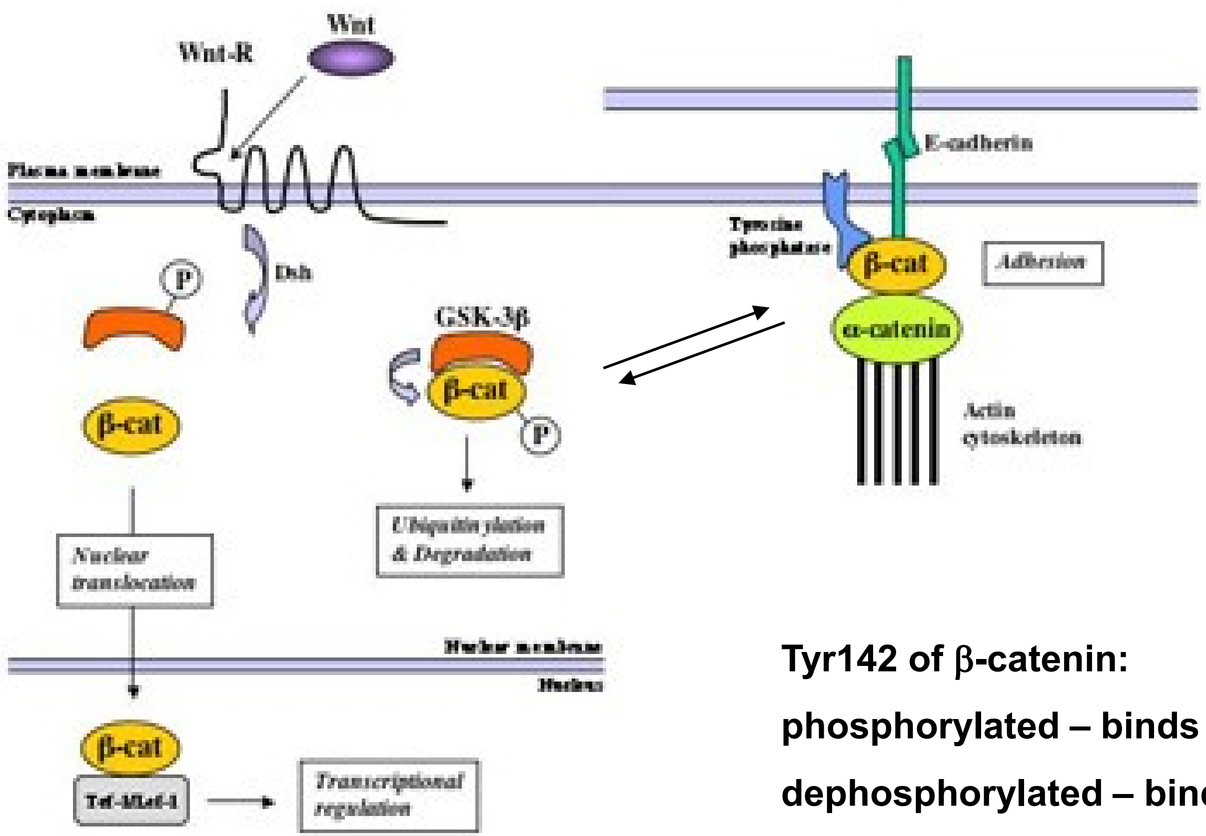


Destruction complex

- A working model for the destruction complex. (1) Initially, the destruction complex contains Axin, GSK3, CK1 and APC (with the 15 aa and 20 aa repeat regions shown). The complex contains other components such as PP2A, which are not shown here. (2) -Catenin enters the complex by binding Axin and potentially the APC 15 aa repeats. This positions the N-terminus of -catenin near CK1 and GSK3. (3) CK1 phosphorylates -catenin at Ser45. (4) GSK3 phosphorylates -catenin at, successively, Thr41, Ser37 and Ser33. (5) The 20 aa repeats, particularly repeat 3, are phosphorylated by a CK1 (and possibly GSK3) which greatly increases their affinity for -catenin. The binding of a phosphorylated 20 aa repeat to -catenin displaces Axin from -catenin. (6) -TRCP1 binds the phosphorylated N-terminus of -catenin, causing the ubiquitination of -catenin by an E2 ligase. APC is then either desphosphorylated within the complex, allowing the ubiquitinated -catenin to leave the complex, or the ubiquitinated -catenin bound to APC leaves the complex and is separated from APC at the proteasome. The complex then returns to Step 1



Wnt and E-cadherin pathways

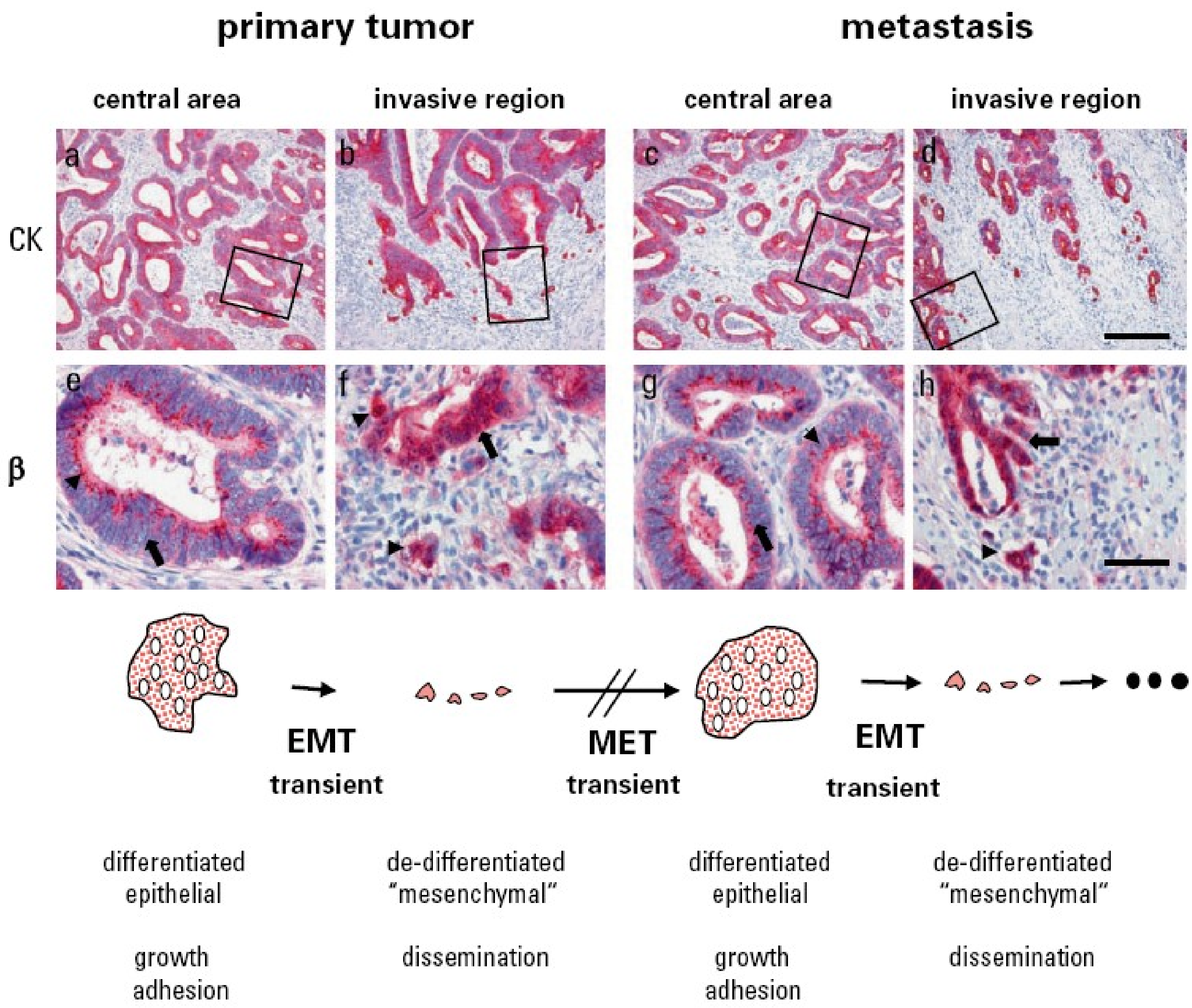


Tyr142 of β-catenin:

phosphorylated – binds Bcl9

dephosphorylated – binds α-catenin

Epithelio-mesenchymal transition (EMT)



Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

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

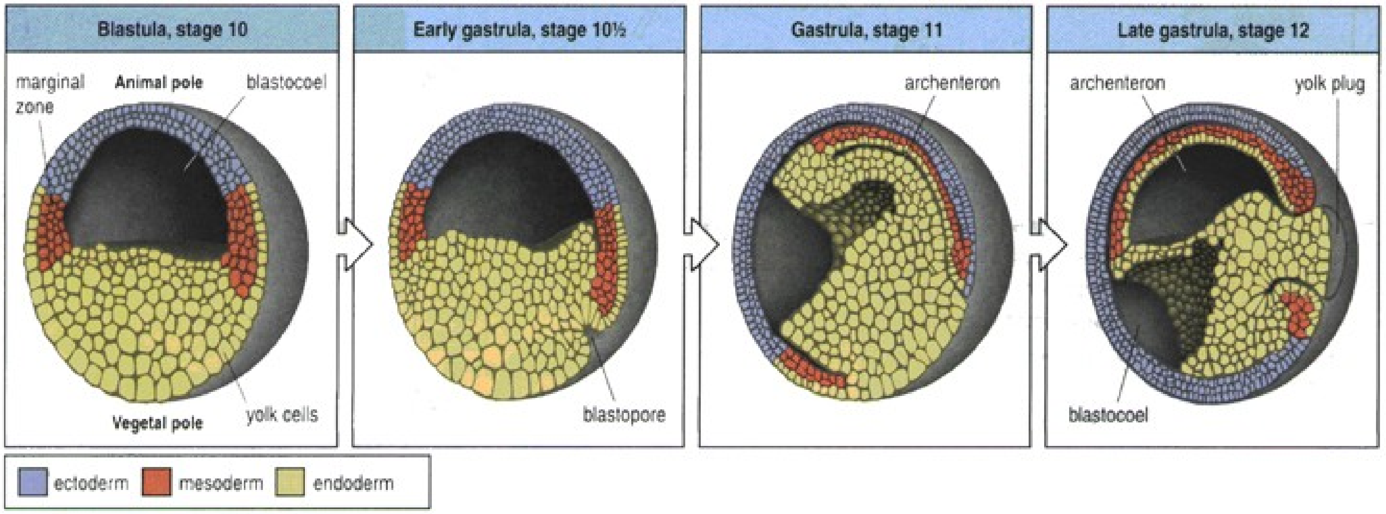


Fig. 2.6 Gastrulation in amphibians. The blastula (first panel) contains several thousand cells and there is a fluid-filled cavity, the blastocoel, beneath the cells at the animal pole. Gastrulation begins (second panel) at the blastopore, which forms on the dorsal side of the embryo. Future mesoderm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

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

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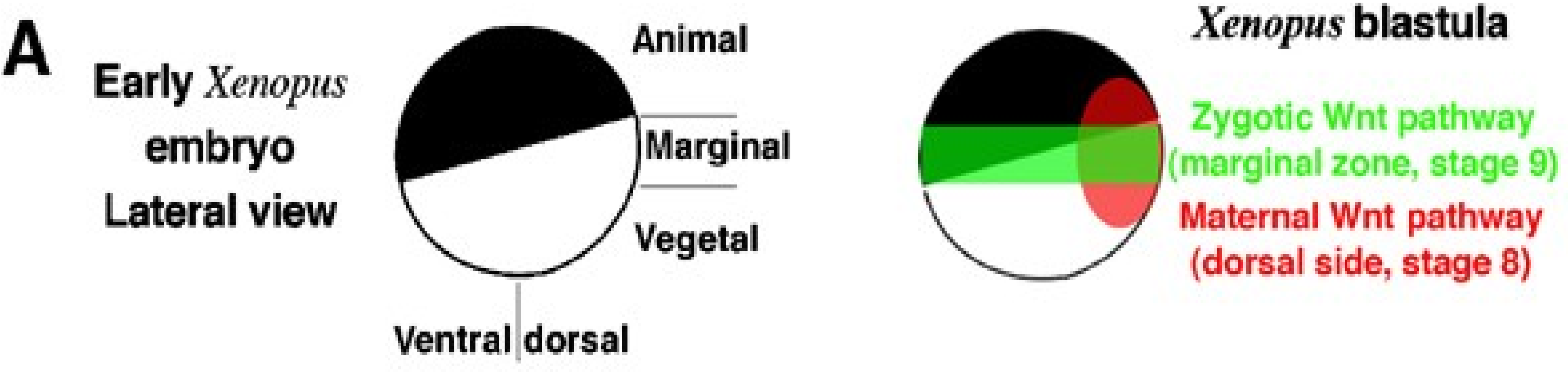
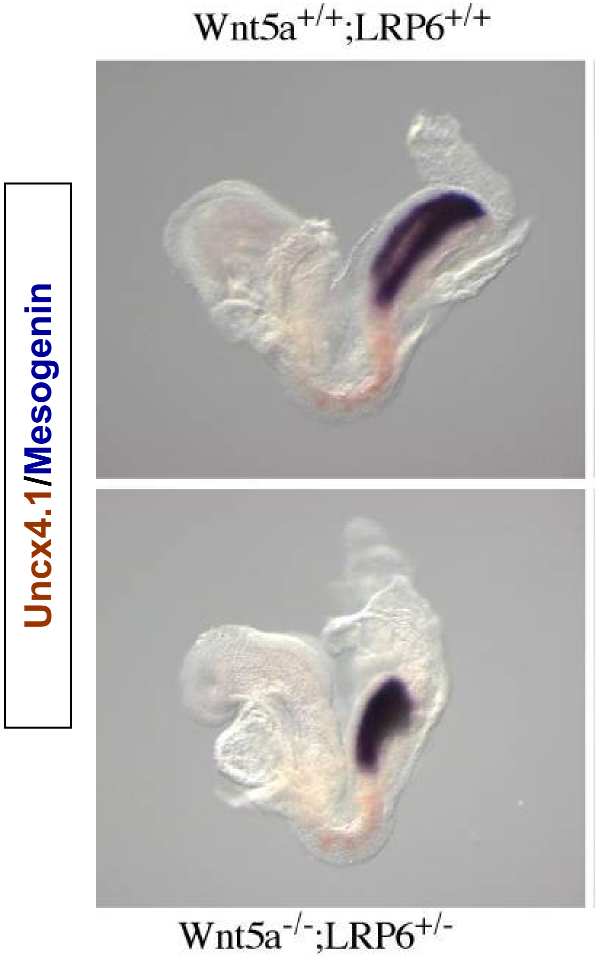
Wnt/β-cateninová dráha určuje anterioro-posteriorní (AP, předo-zadní) osu těla během gastrulace – podporuje vznik zadních a blokuje vznik předních částí těla

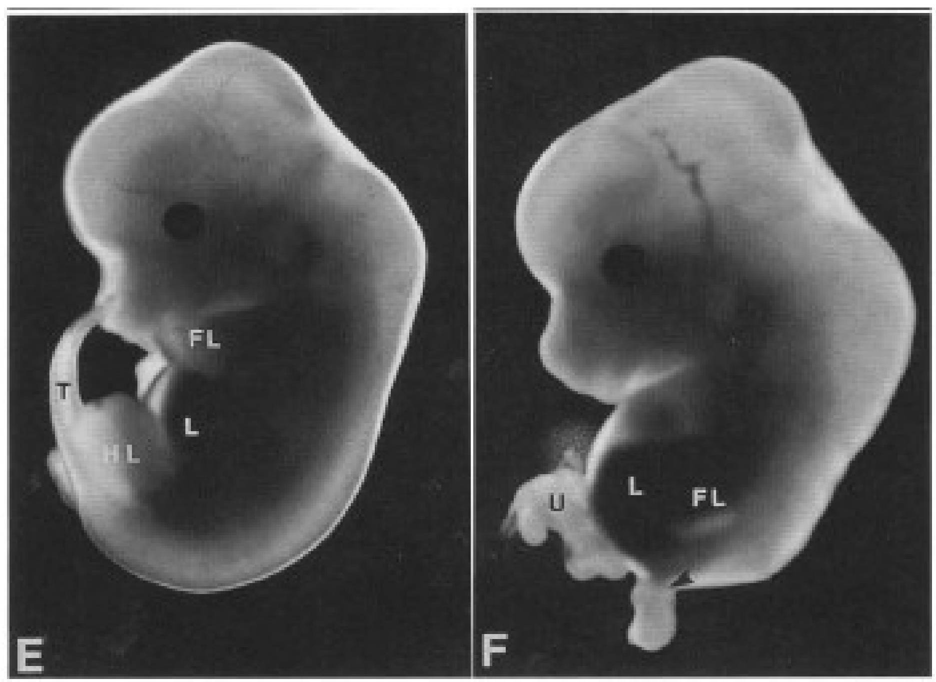


myší embryo po gastrulaci (E8.5):

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

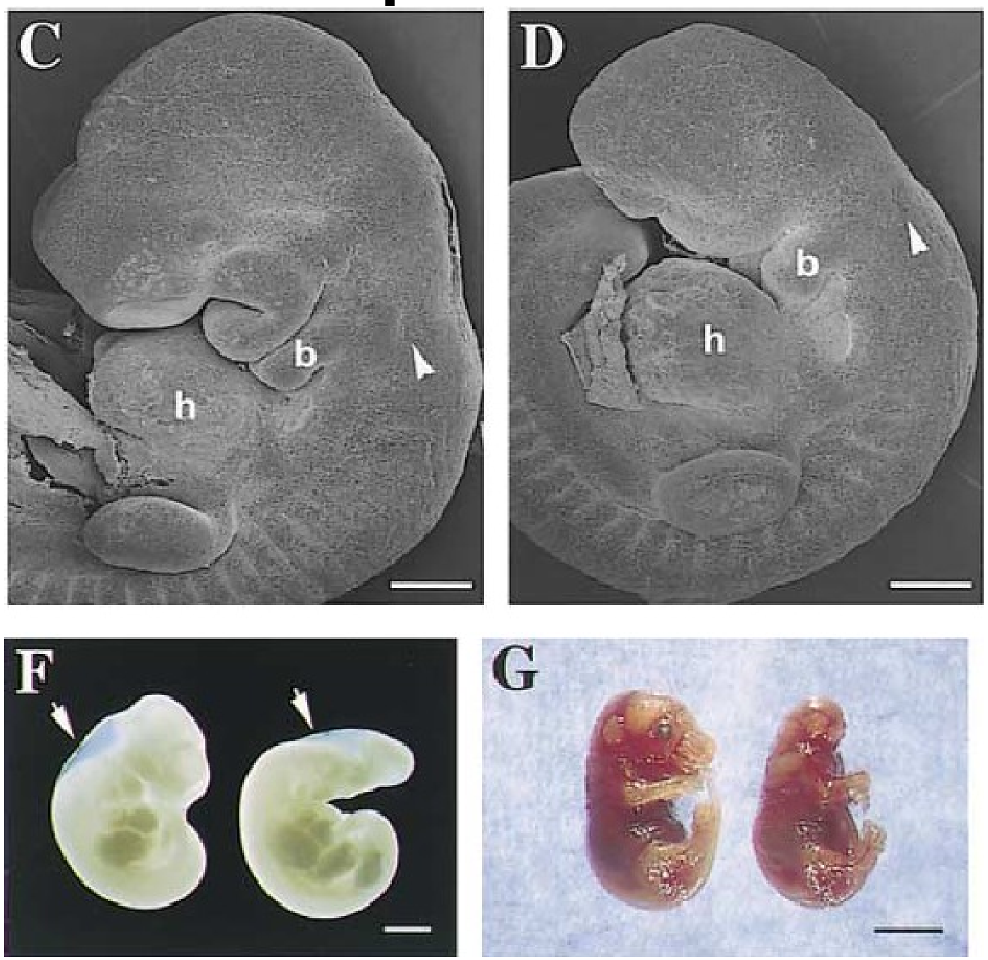


Deplece Wnt/β-kateninové dráhy při gastrulaci = ztráta zadních částí těla

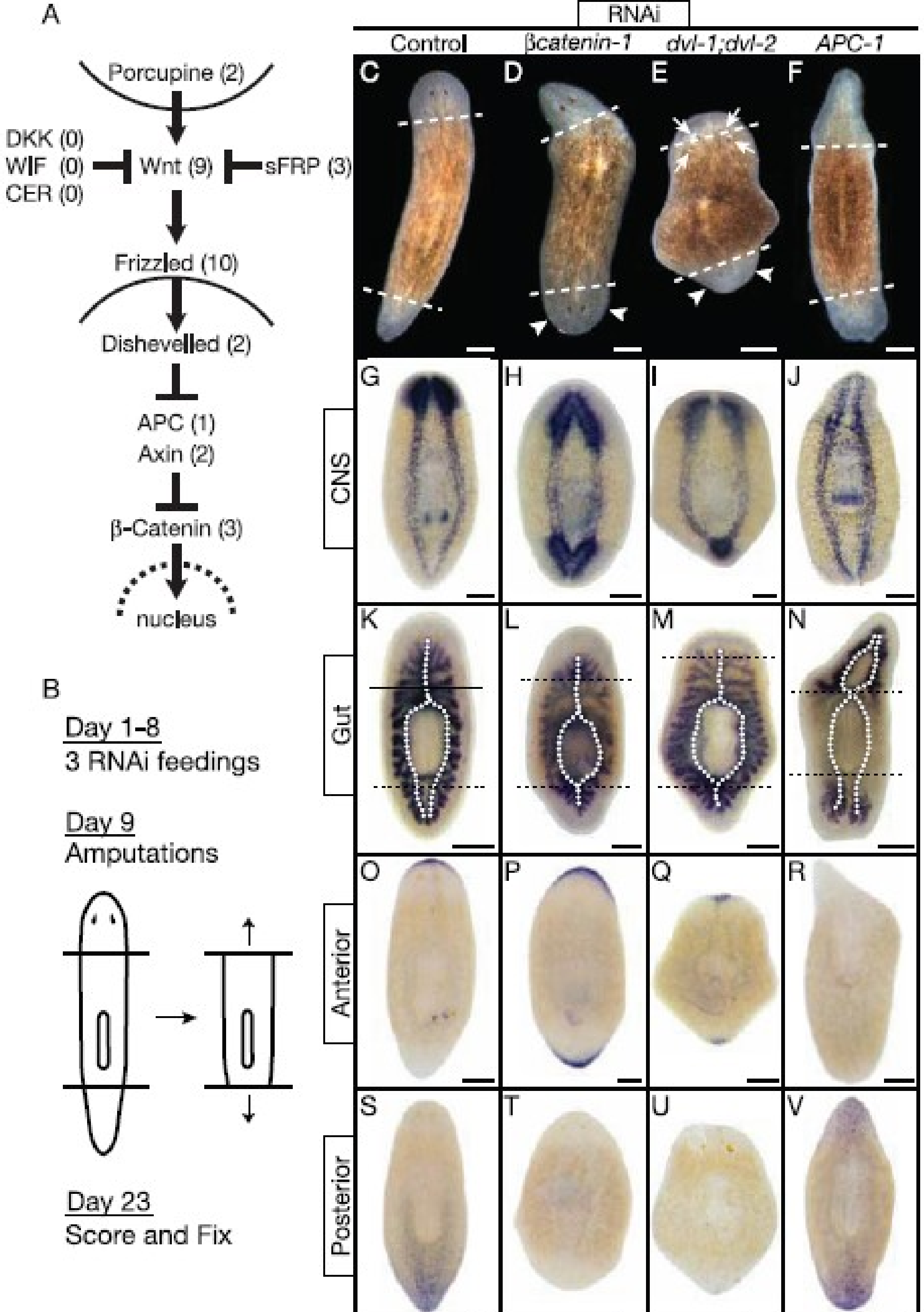


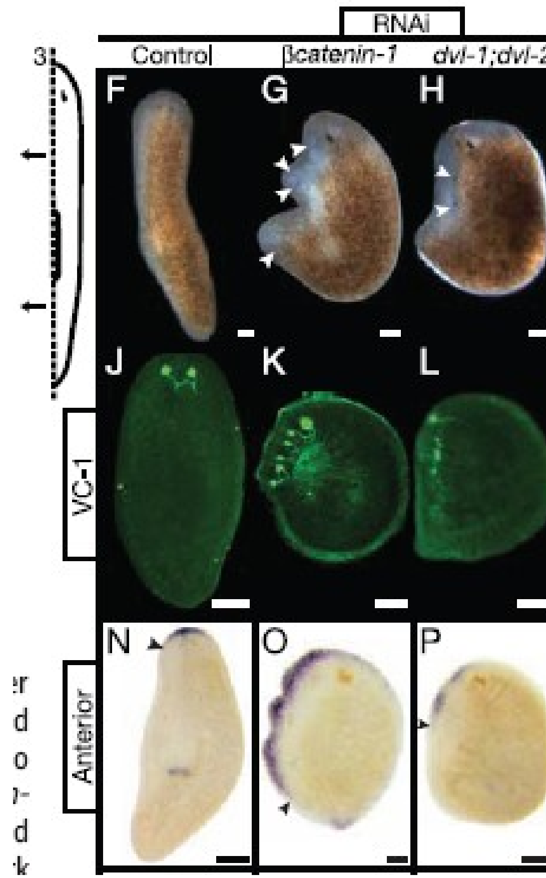
wild type Wnt-3a knockout

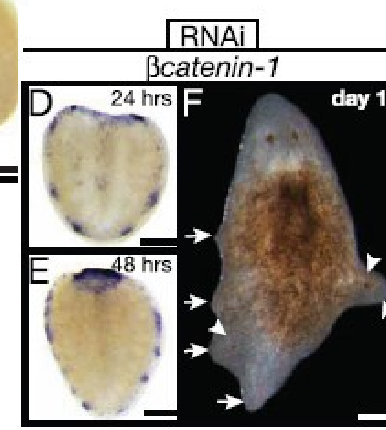
Deplece inhibitorů Wnt/β-kateninové dráhy při gastrulaci = ztráta předních částí těla



wild type vs. Dkk1 knockout







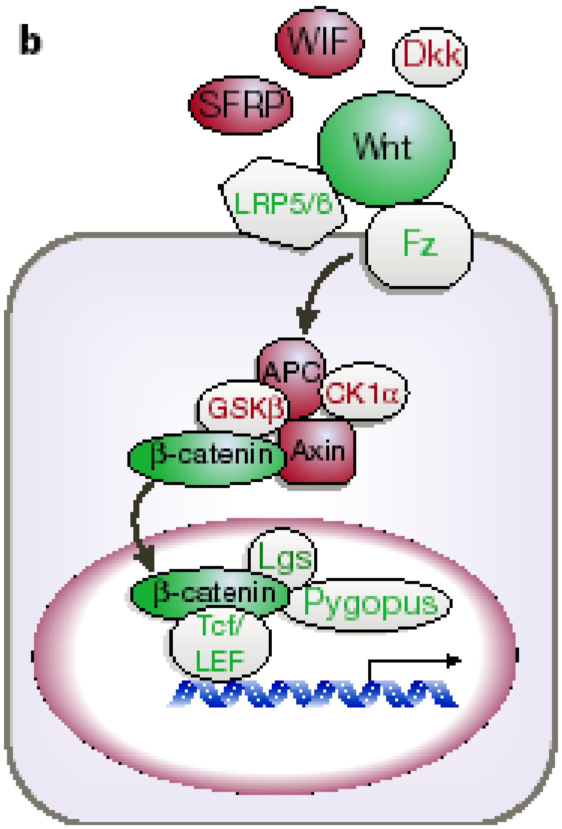
β-Catenin Defines Head Versus Tail Identity During Planarian Regeneration and Homeostasis

Kyle A. Gurley, Jochen C. Rink, Alejandro Sánchez Alvarado*

SCIENCE VOL 319 18 JANUARY 2008

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 *in vitro*
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short intefering RNA and a blocking antibody against Wnt2
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP

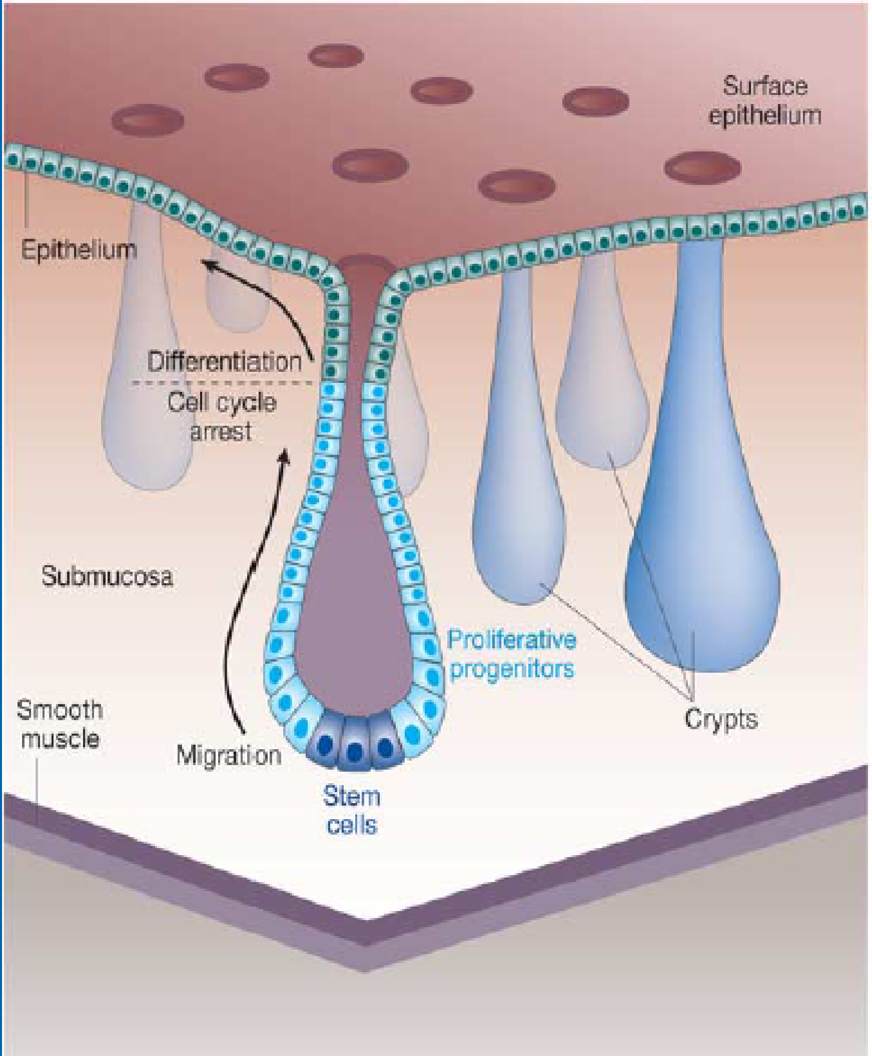


Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)

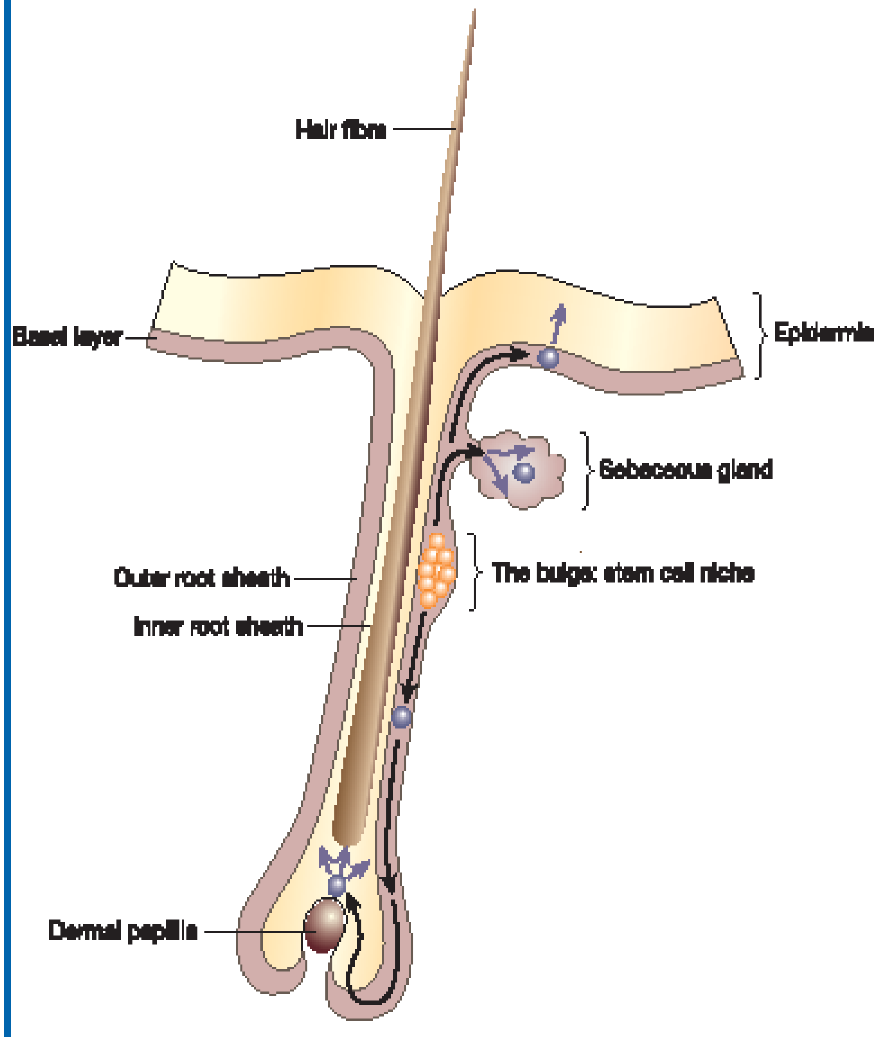
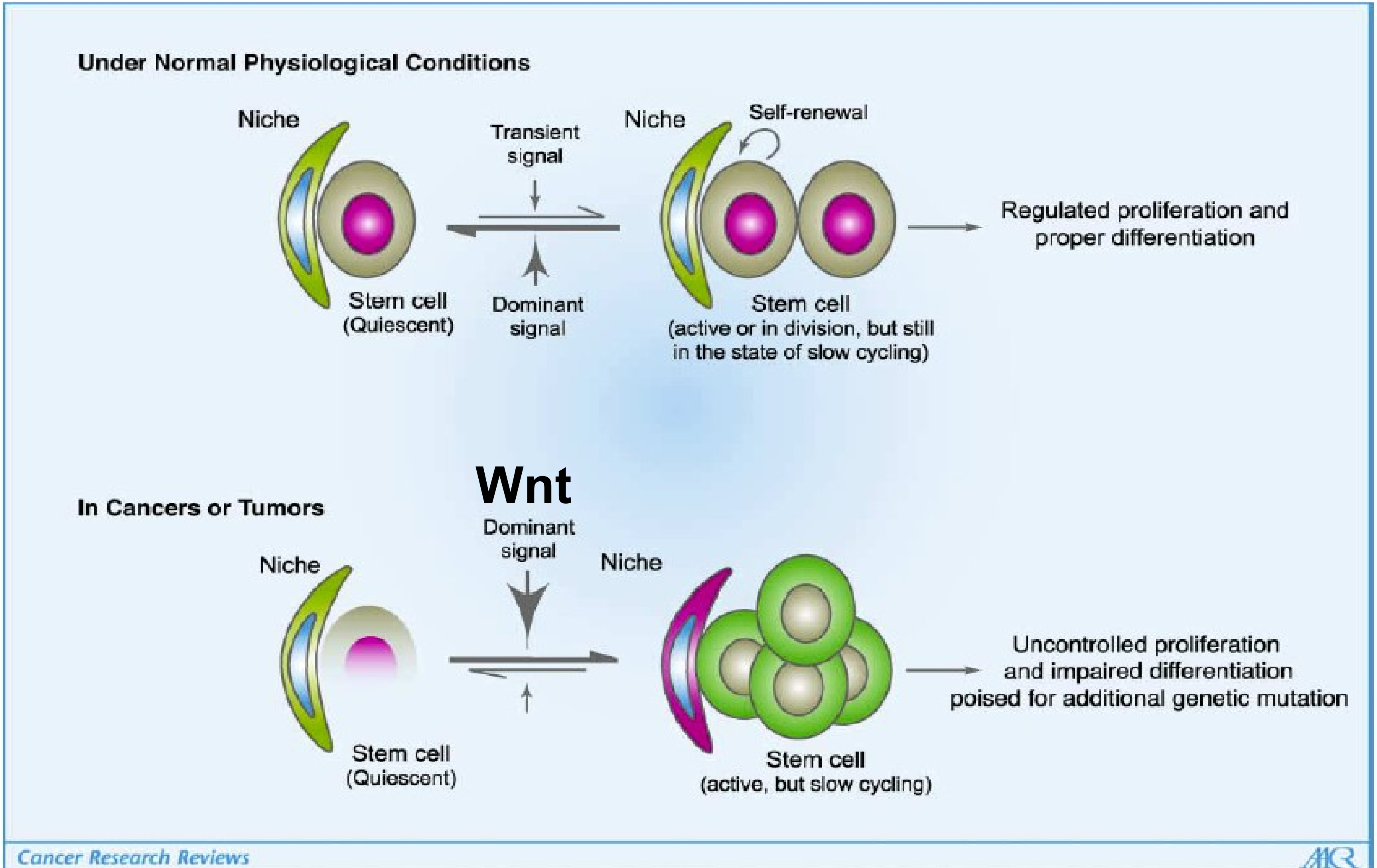


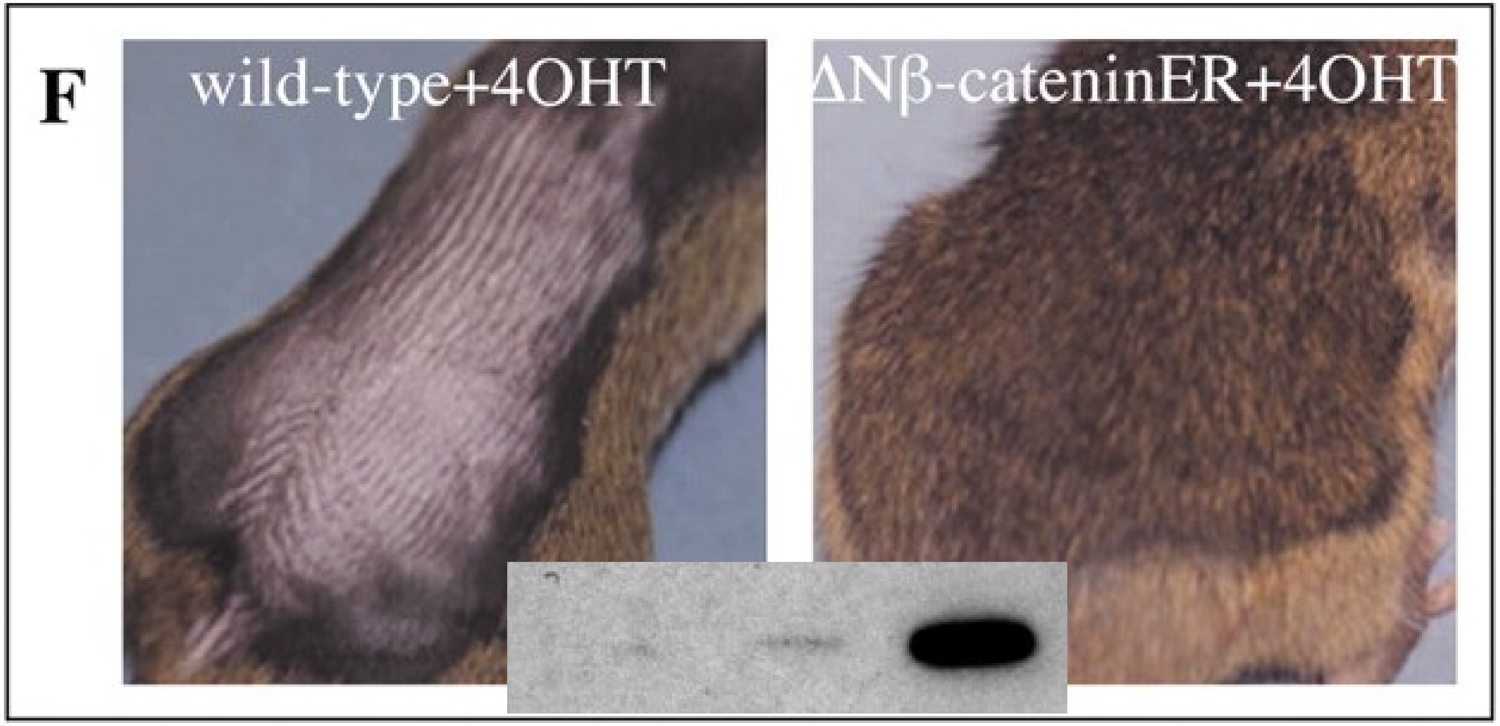
Figure 4 The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from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

Reya & Clevers 2005, Nature

The effects of Wnts on stem cells in their niche

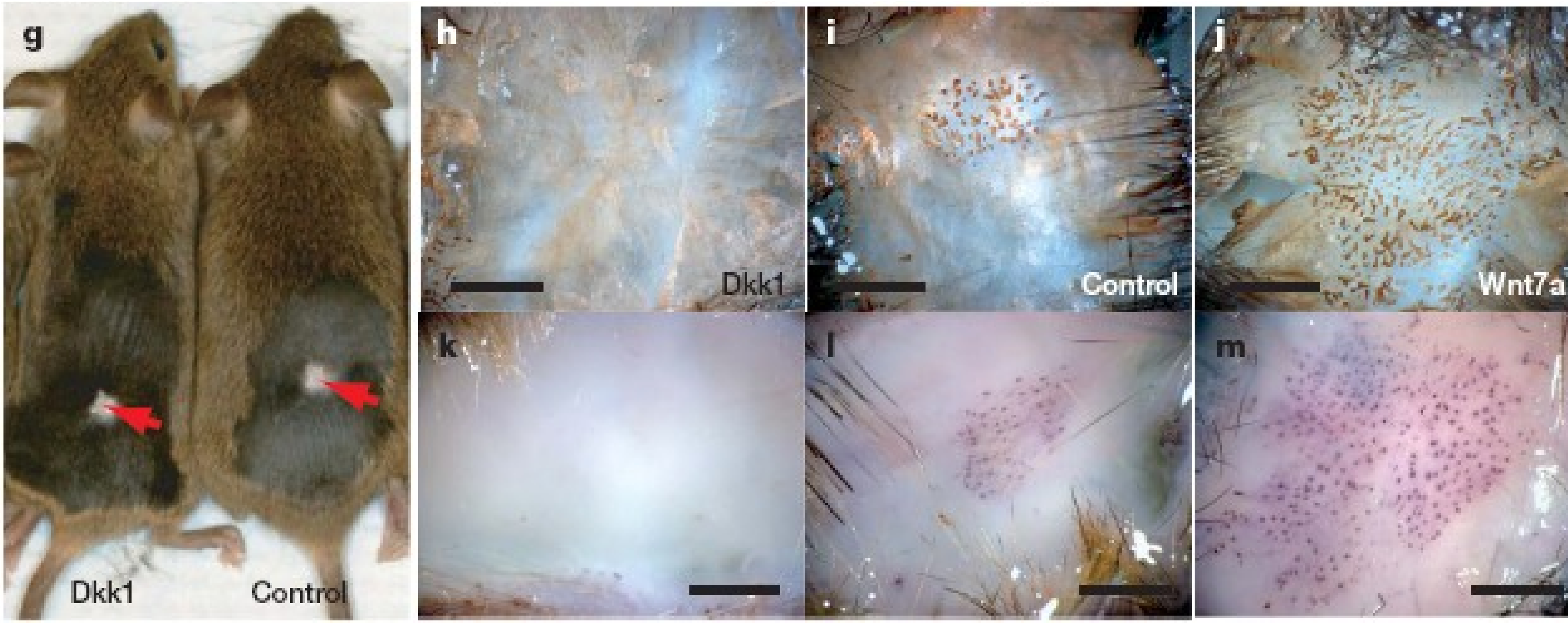


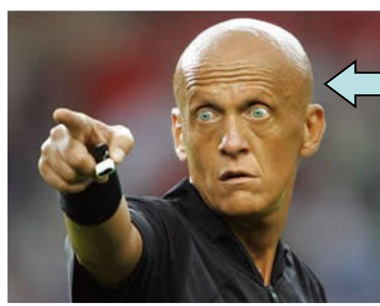
Důsledky aktivace β-cateninu v epidermis (po depilaci)



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

Wnt pathway induces de novo formation of hair follicles





Wnt signaling pathway related polymorphism?

Wnt-dependent *de novo* hair follicle regeneration in adult mouse skin after wounding

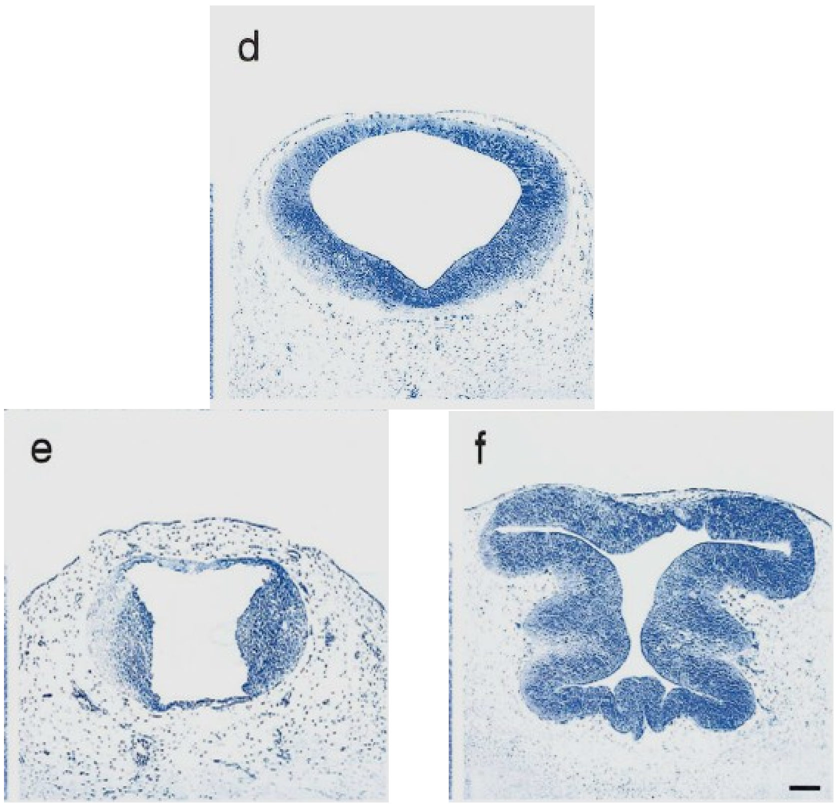
Mayumi Ito[1], Zaixin Yang[1], Thomas Andl[1], Chunhua Cui[1], Noori Kim[1], Sarah E. Millar[1] & George Cotsarelis[1]

nature Vol 447 | 17 May 2007 | doi:10.1038/nature05766

LETTERS

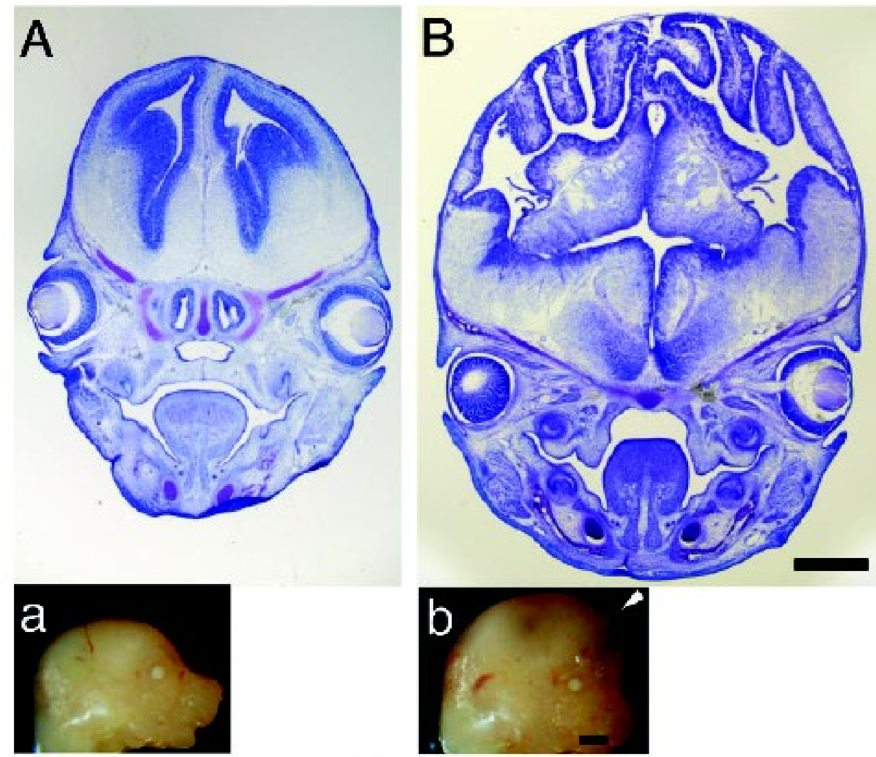
Aktivace β-cateninu ve vyvíjející se mozkové trubici:

<u>midbrain (Brn4-promotor)</u>



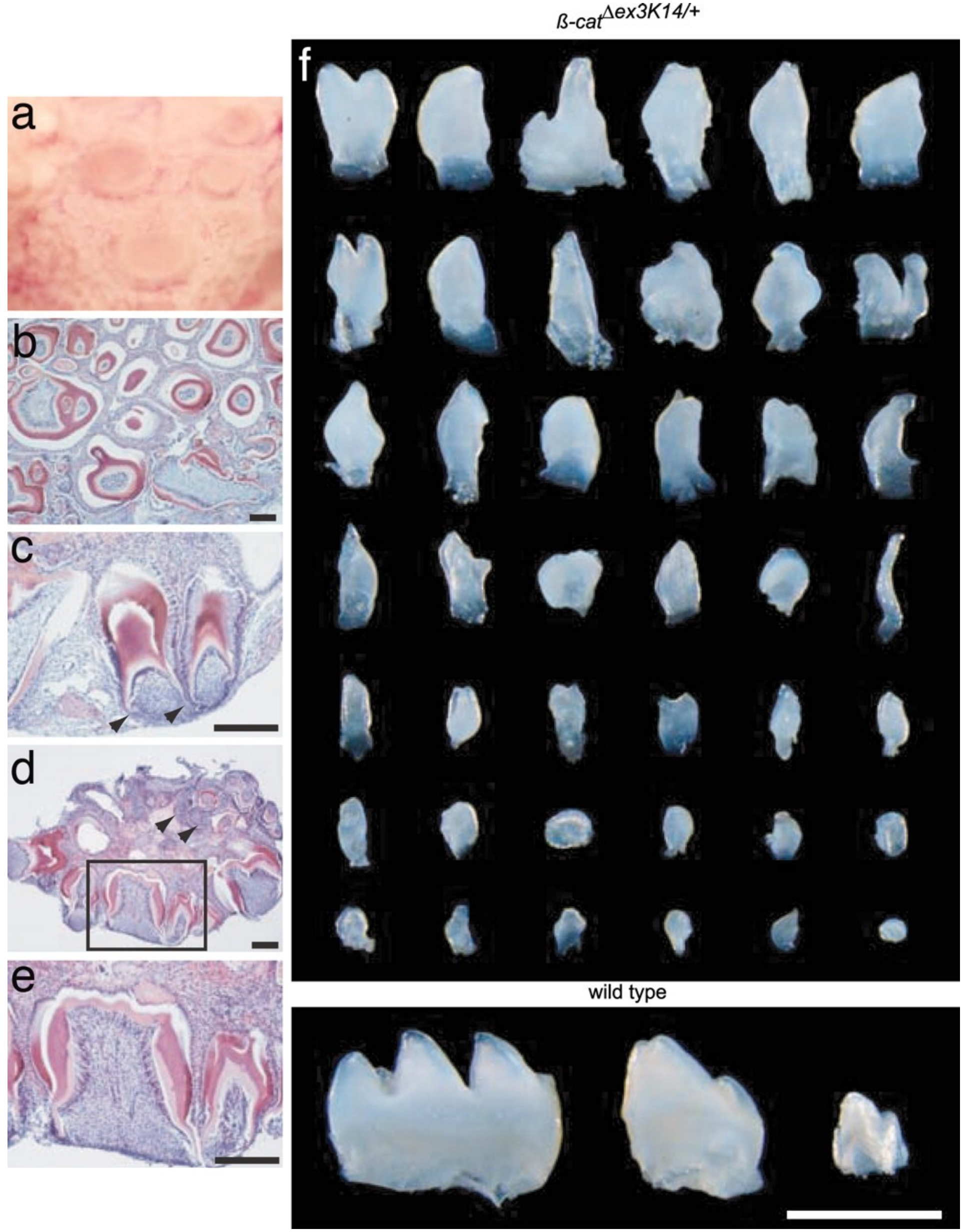
Zechner et al., 2003: Dev. Biol.;258:406-418.

<u>cortex (nestin enhancer)</u>



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

Aktivace beta-cateninu v kmenových buňkách zubu:

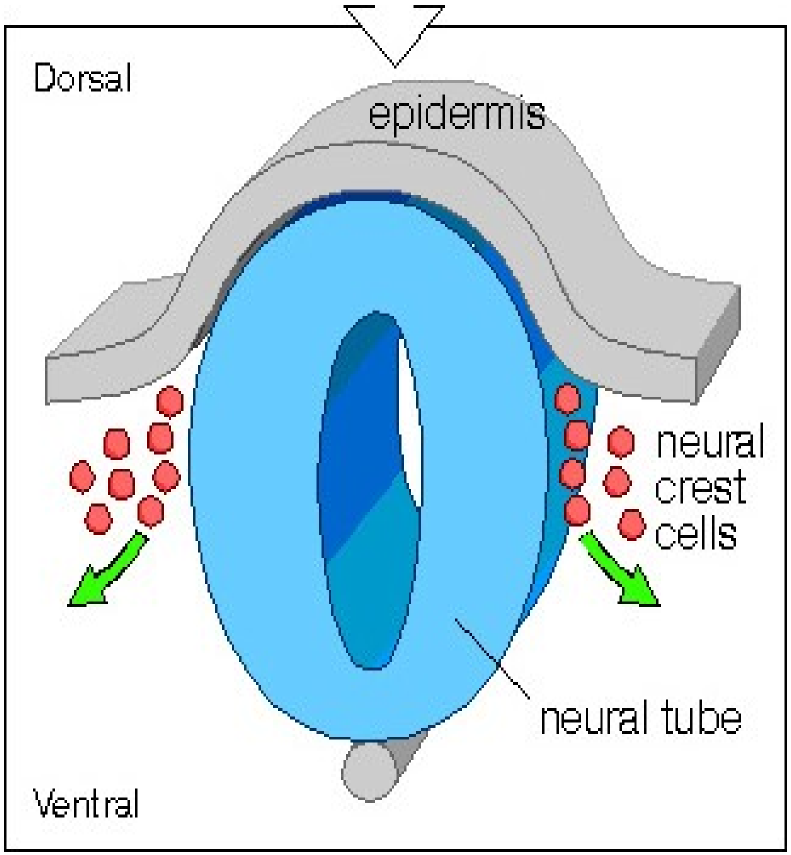


Järvinenet al. (2006) Proc. Natl. Acad. Sci. USA 103, 18627-18632

Wnt/β-cateninová dráha reguluje vývoj neurální lišty (neural crest)

Nervová lišta (neural crest)

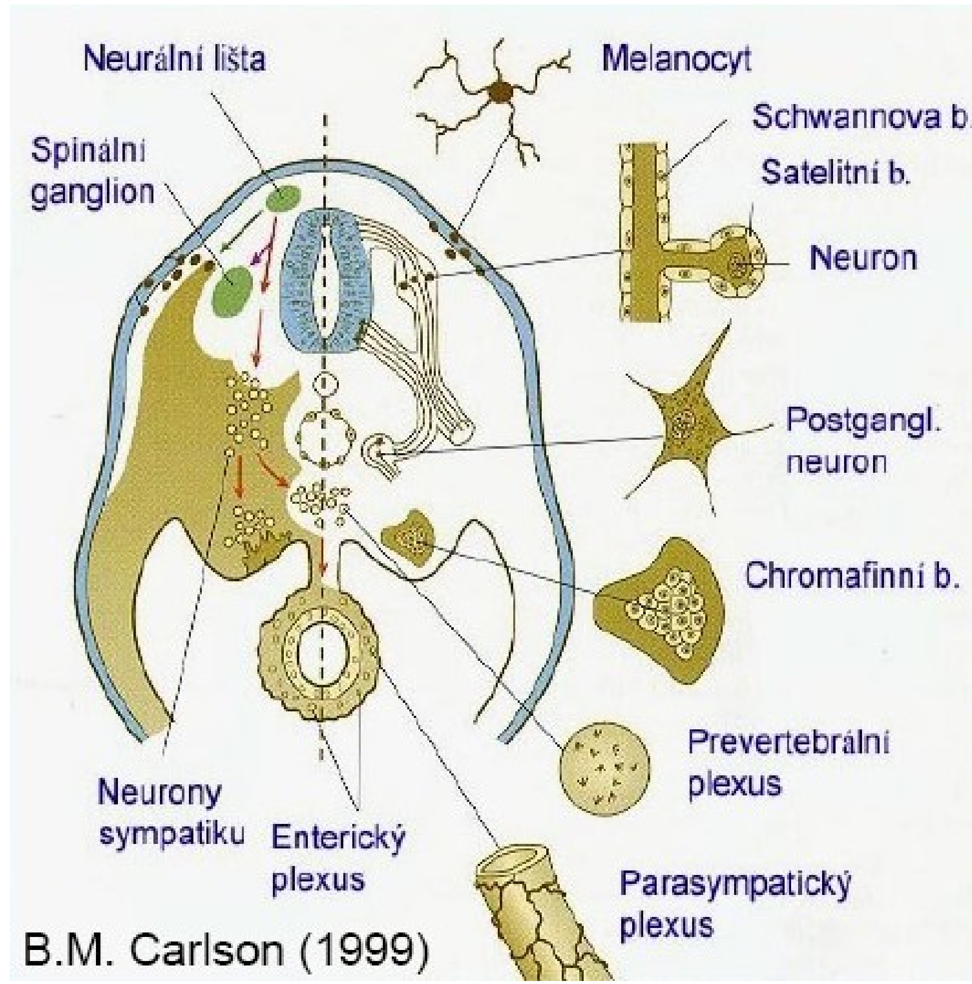
- **populace buněk, vzniká z dorsální (= horní) strany nervové trubice procesem delaminace a migruje několika hlavními cestami do jiných částí embrya**



Co všechno z NC vzniká?

- podíl na vzniku cca 40 různých tkání a orgánů

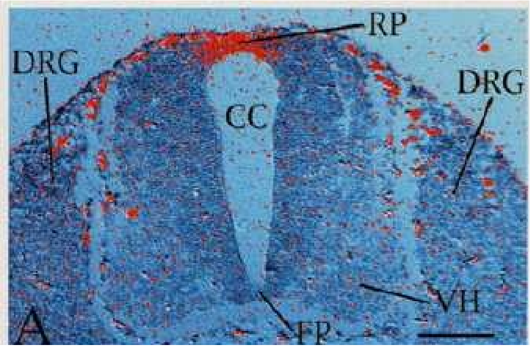
1. Oblast trupu:

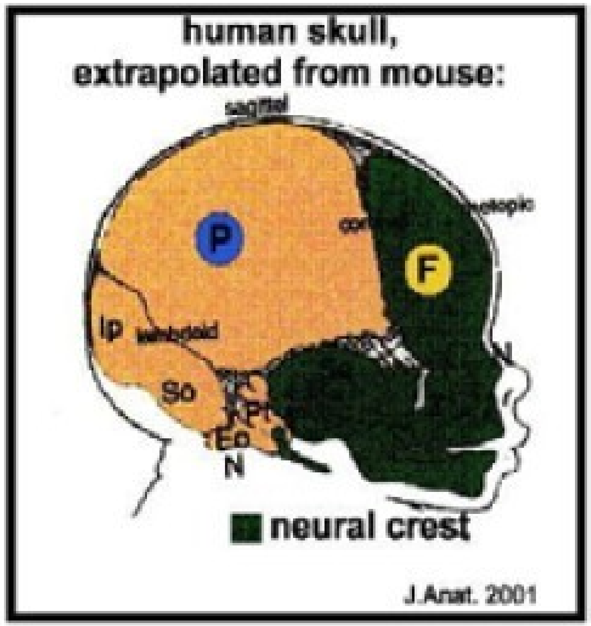


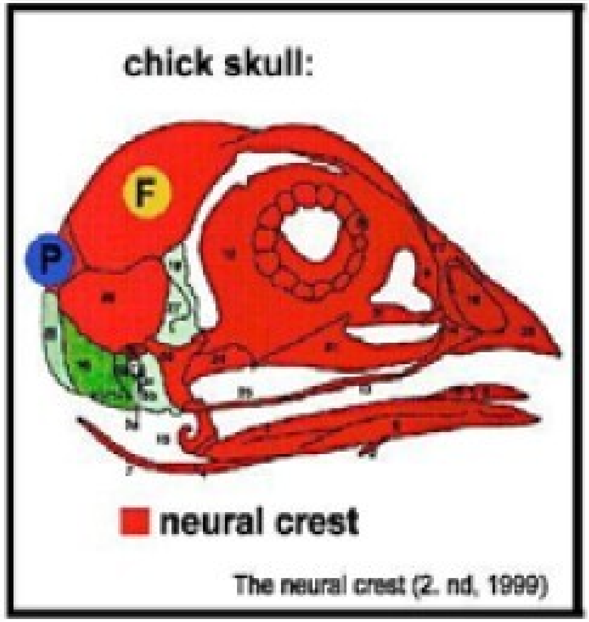
- Neurony a gliální buňky senzorického, sympatického a parasympatického systému
- Buňky dřeně nadledvin
- Pigmentové buňky epidermis
- Svalové buňky některých cév

I. Vývoj neurální lišty:

Wnt-3a

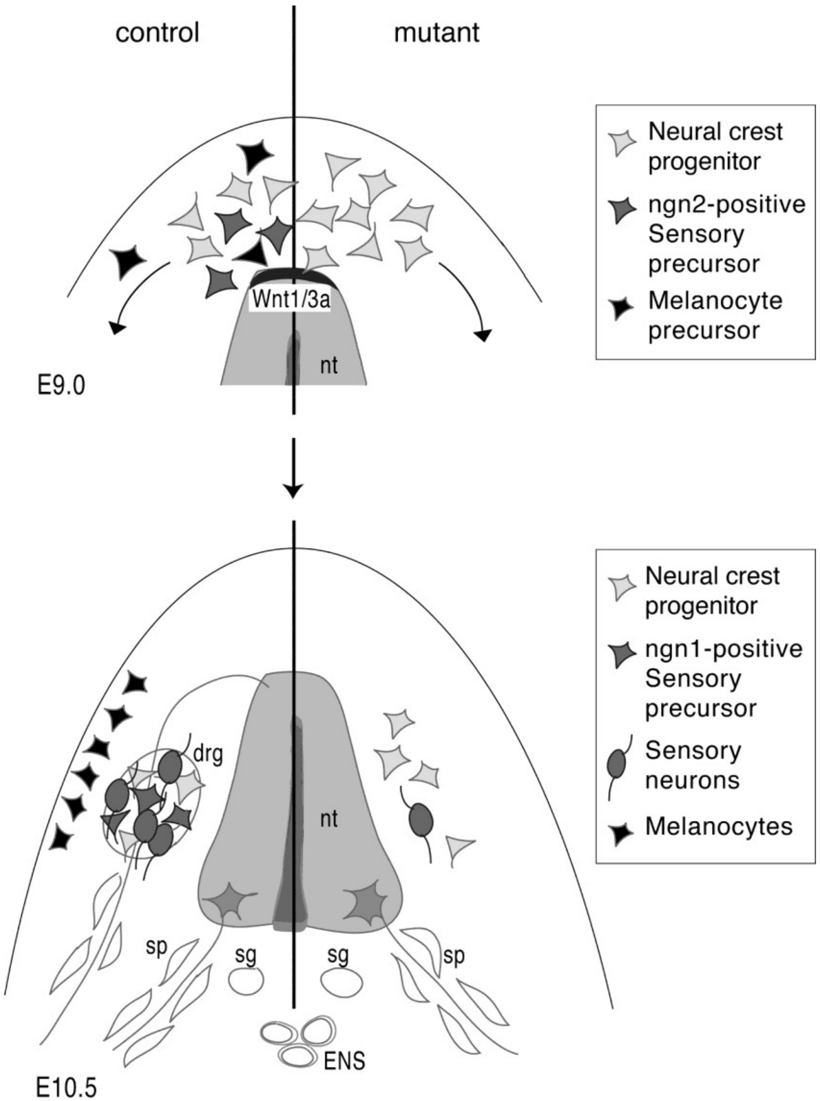


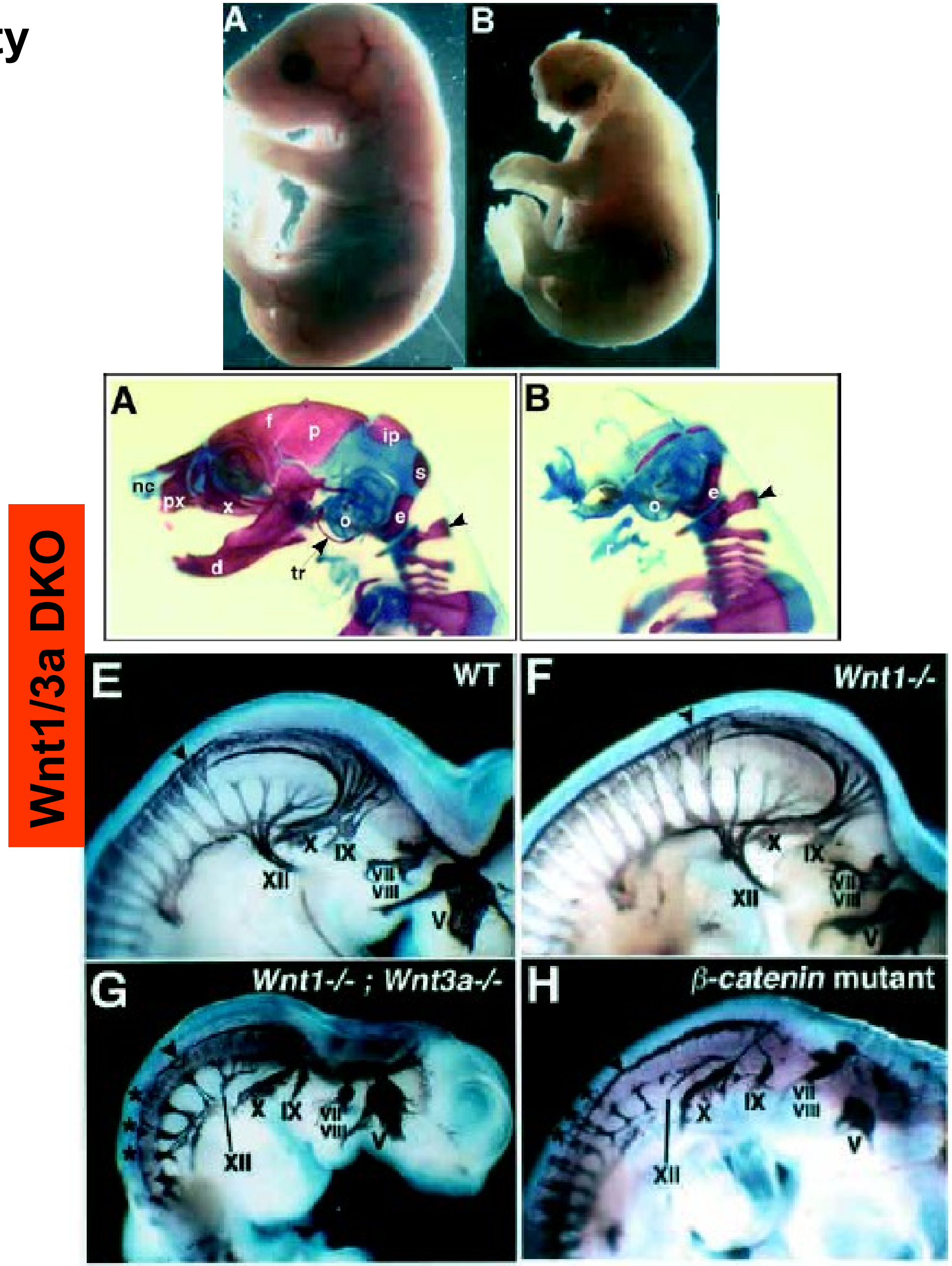




- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, částí srdce a dalších

Wnt signaling při vývoji neurální lišty





Hari, L. et al. J. Cell Biol. 2002;159:867-880

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]†

SCIENCE VOL 317 10 AUGUST 2007 803

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

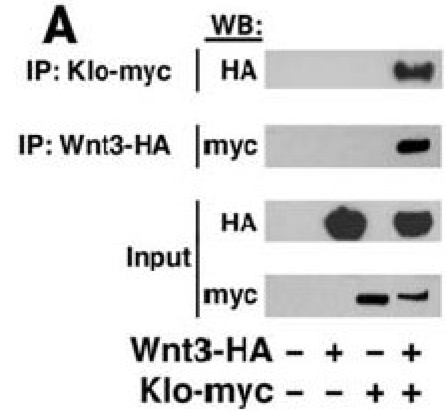
Andrew S. Brack,[1] Michael J. Conboy,[1] Sudeep Roy,[1] Mark Lee,[2] Calvin J. Kuo,[2] Charles Keller,[3] Thomas A. Rando[1,4]*

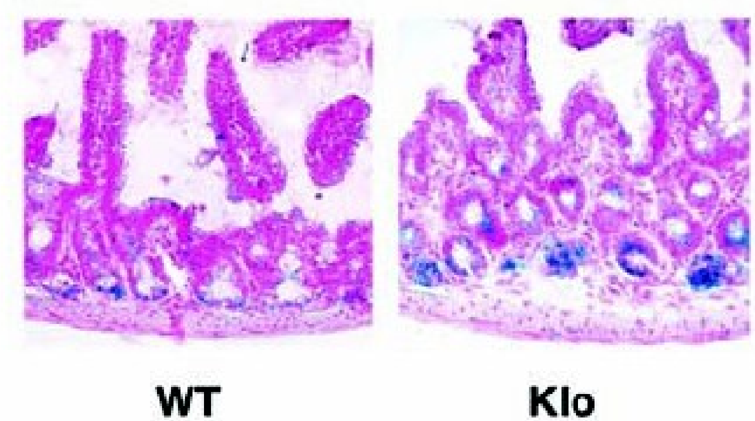
SCIENCE VOL 317 10 AUGUST 2007 807

Klotho myš

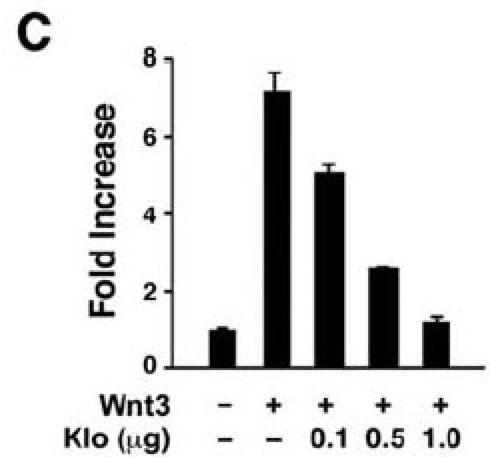
-mutantní kmen myši s fenotypem akcelerovaného stárnutí: např. kratší život, arterioskleróza, snížená plodnost nebo kožní atrofie

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



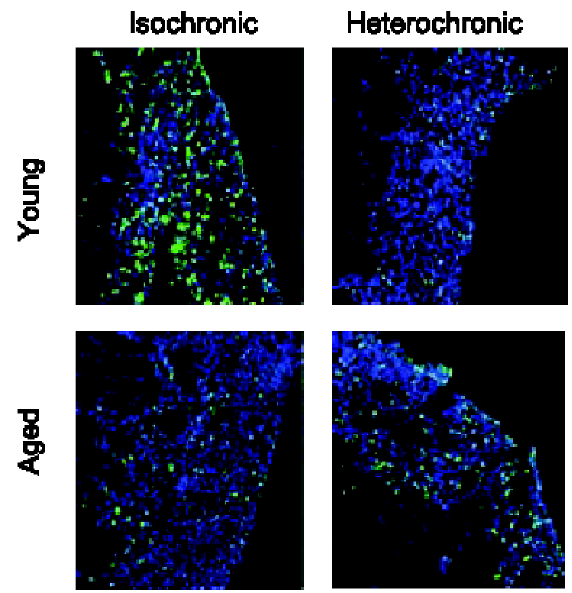


aktivita Wnt/β-cateninové dráhy
ve střevním epitelu



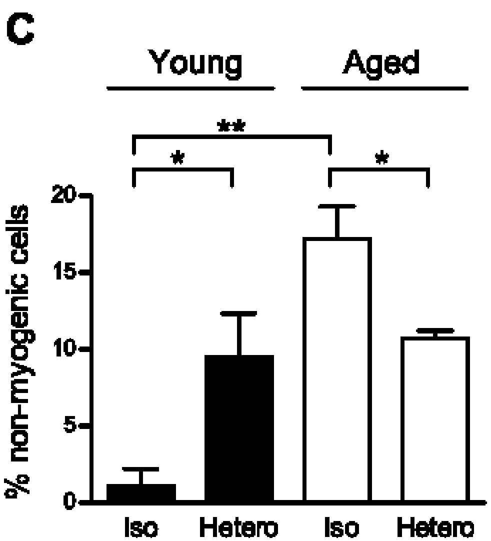
Model 1 – svalová fibróza

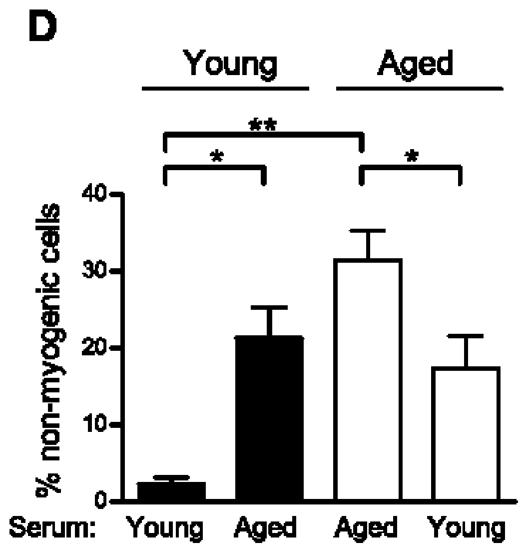
- s prodlužujícím se věkem stále častěji při regeneraci svalu vznikají místo svalových buněk buňky fibrózní tkáně – tak přispívají k nižší výkonnosti svalu, která souvisí se stárnutím

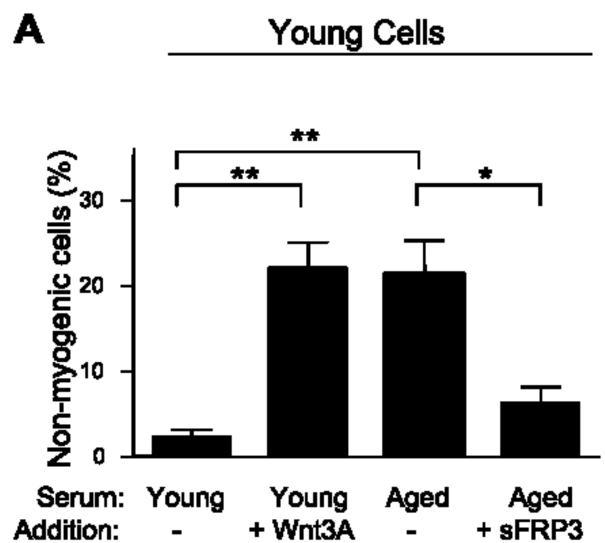


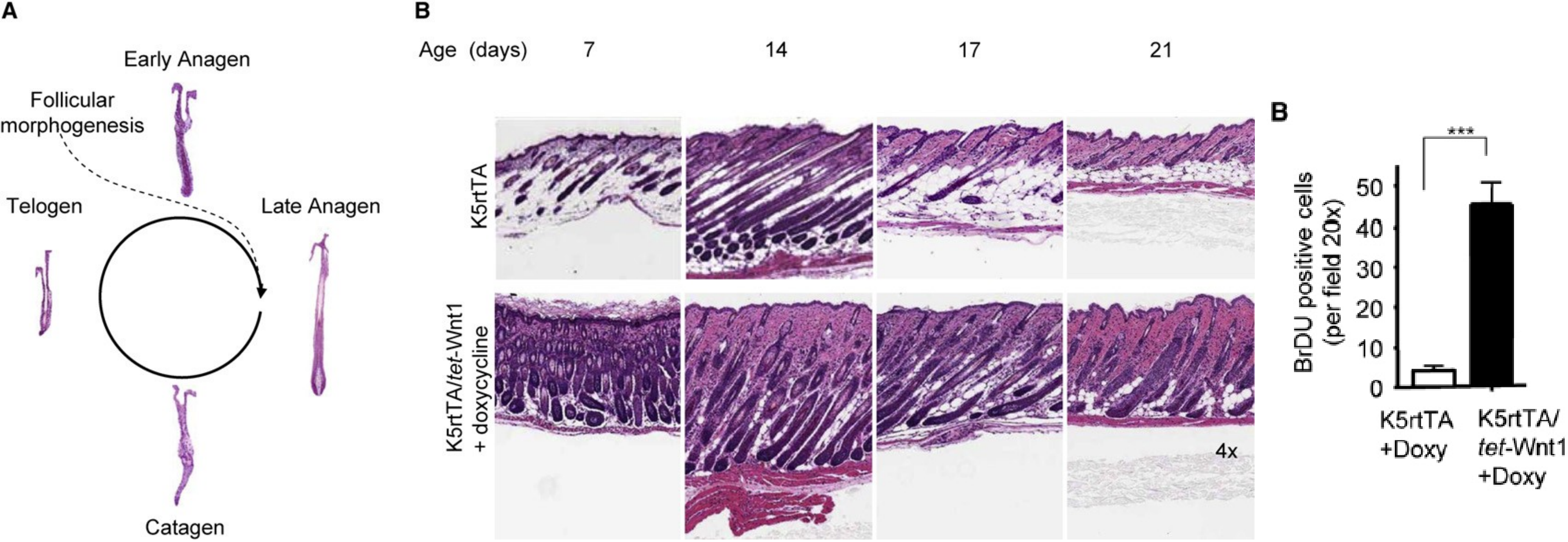
Model 2 – parabiotické párování

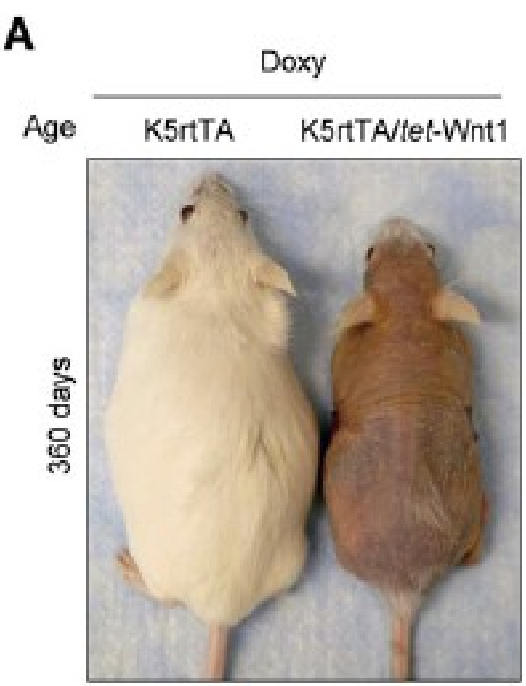
Fyzické propojení dvou krevních systémů (a tím i dvou vnitřních prostředí) u myši

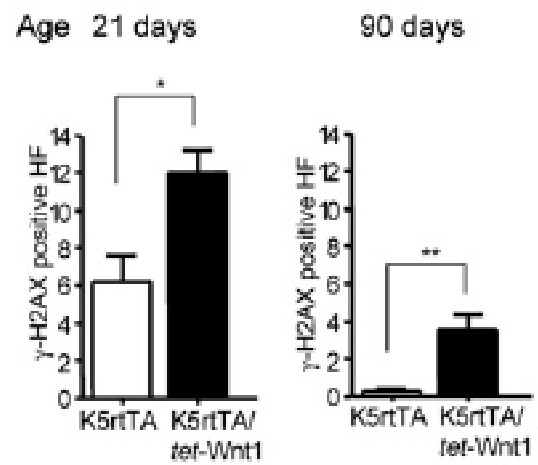












Cell Stem Cell
Article

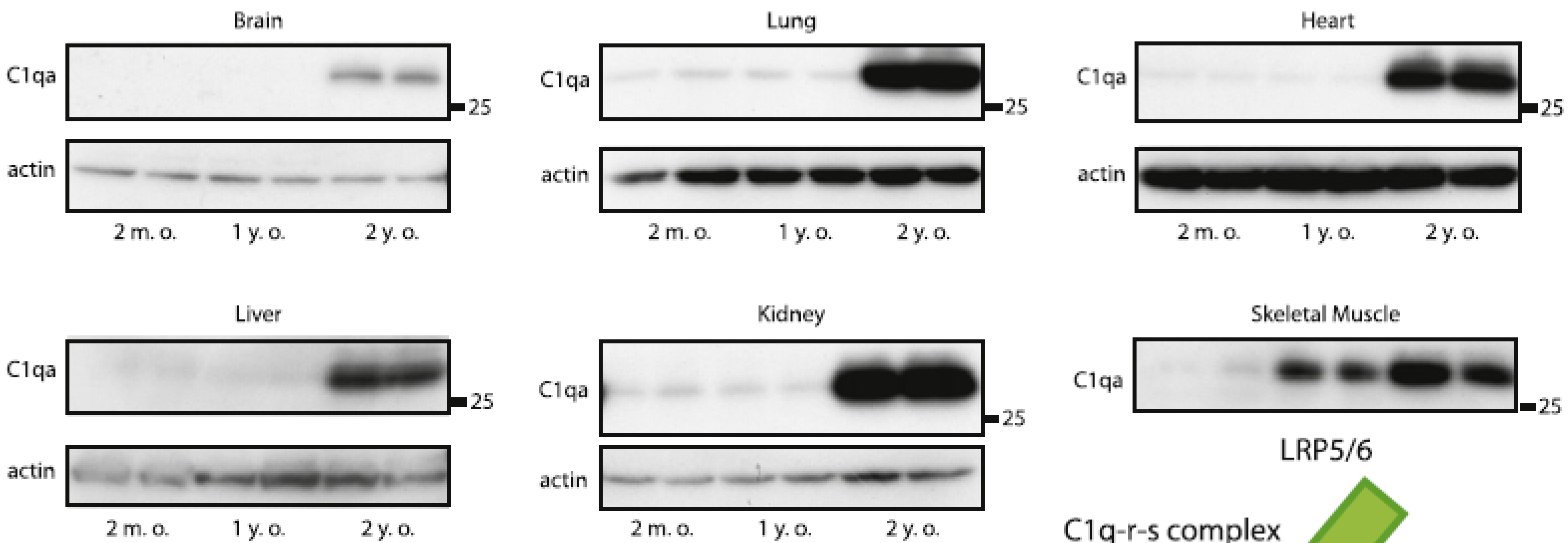
mTOR Mediates Wnt-Induced Epidermal Stem Cell Exhaustion and Aging

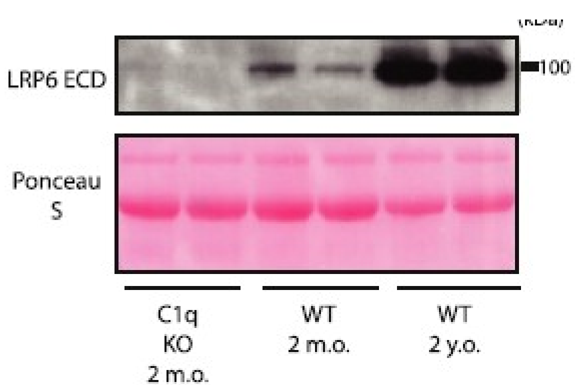
Rogerio M. Castilho,[1] Cristiane H. Squarize,[1] Lewis A. Chodosh,[2] Bart O. Williams,[3] and J. Silvio Gutkind[1,*]

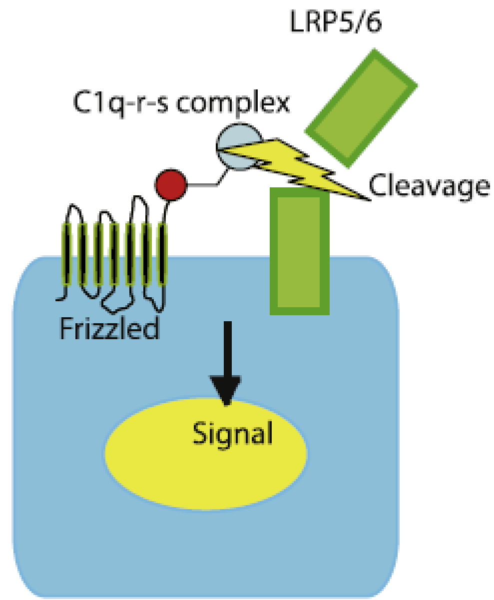
Cell

Complement C1q Activates Canonical Wnt Signaling and Promotes Aging-Related Phenotypes

Atsuhiko T. Naito,[1,3] Tomokazu Sumida,[4] Seitaro Nomura,[4] Mei-Lan Liu,[4] Tomoaki Higo,[1] Akito Nakagawa,[1] Katsuki Okada,[1] Taku Sakai,[1] Akihito Hashimoto,[1] Yurina Hara,[1] Ippei Shimizu,[4] Weidong Zhu,[4] Haruhiro Toko,[4] Akemi Katada,[4] Hiroshi Akazawa,[1,3] Toru Oka,[1,3] Jong-Kook Lee,[1,3] Tohru Minamino,[4] Toshio Nagai,[4] Kenneth Walsh,[5] Akira Kikuchi,[2] Misako Matsumoto,[6] Marina Botto,[7] Ichiro Shiojima,[1,3] and Issei Komuro[1,3,4,*]

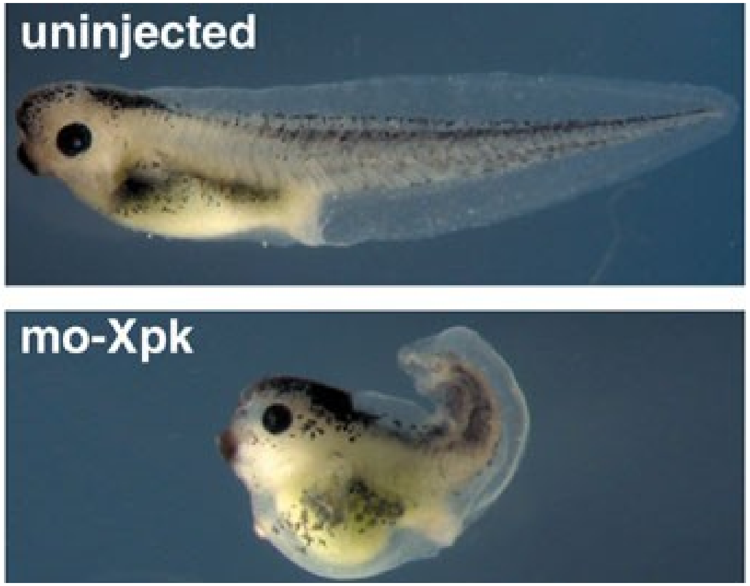






Nekanonická Wnt dráha

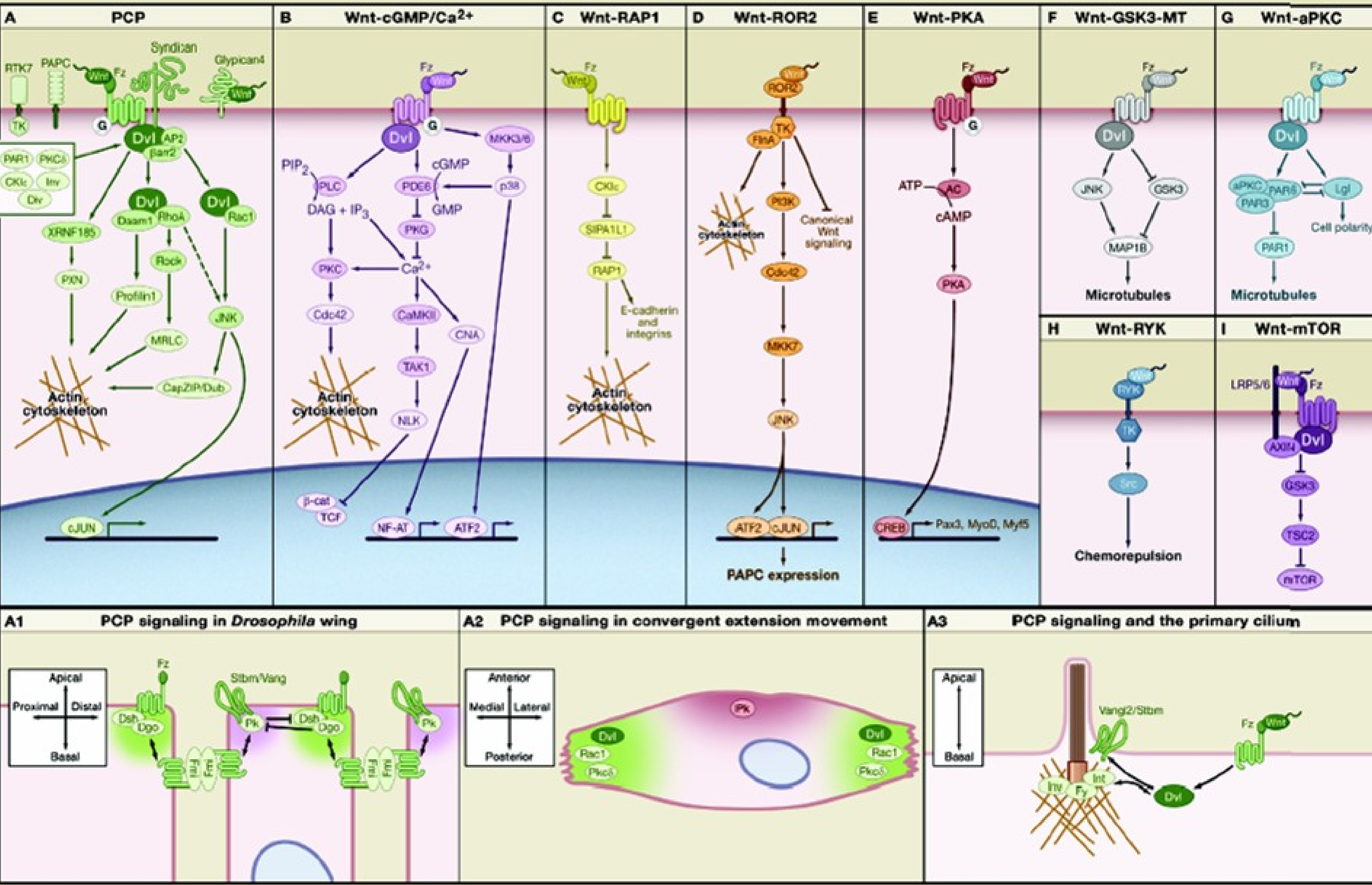
- e.g. Wnt5a



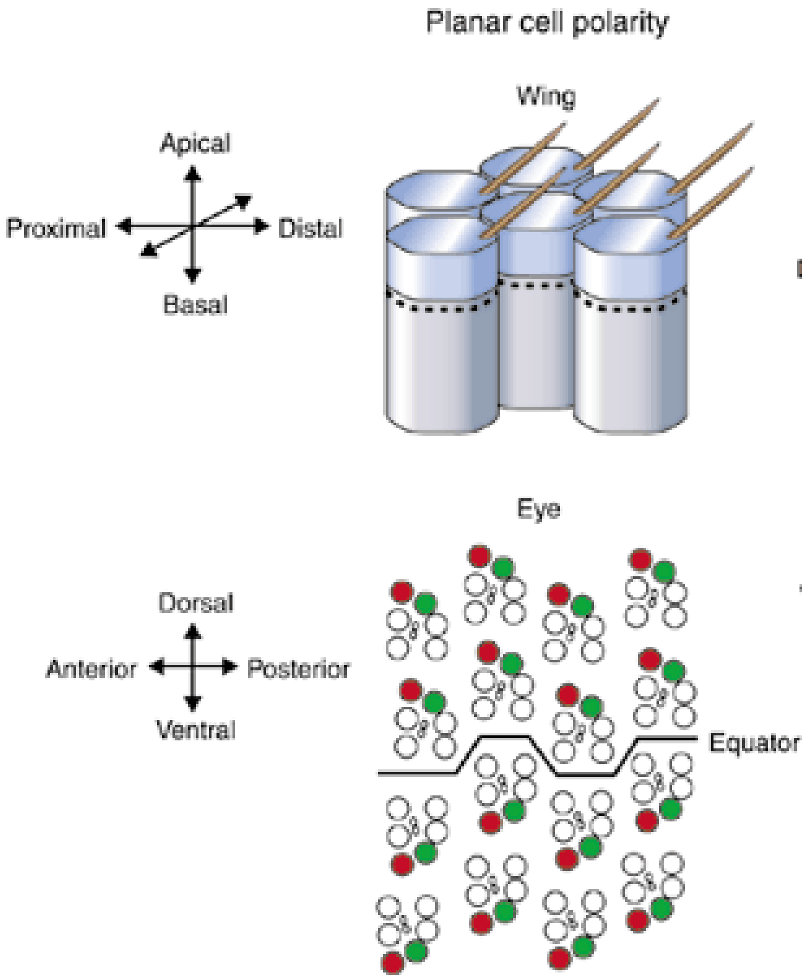
- do not induce axis duplication in Xenopus
- do not induce transformation of mammary cell line C57mg
- do not signal via nuclear translocation of β-catenin

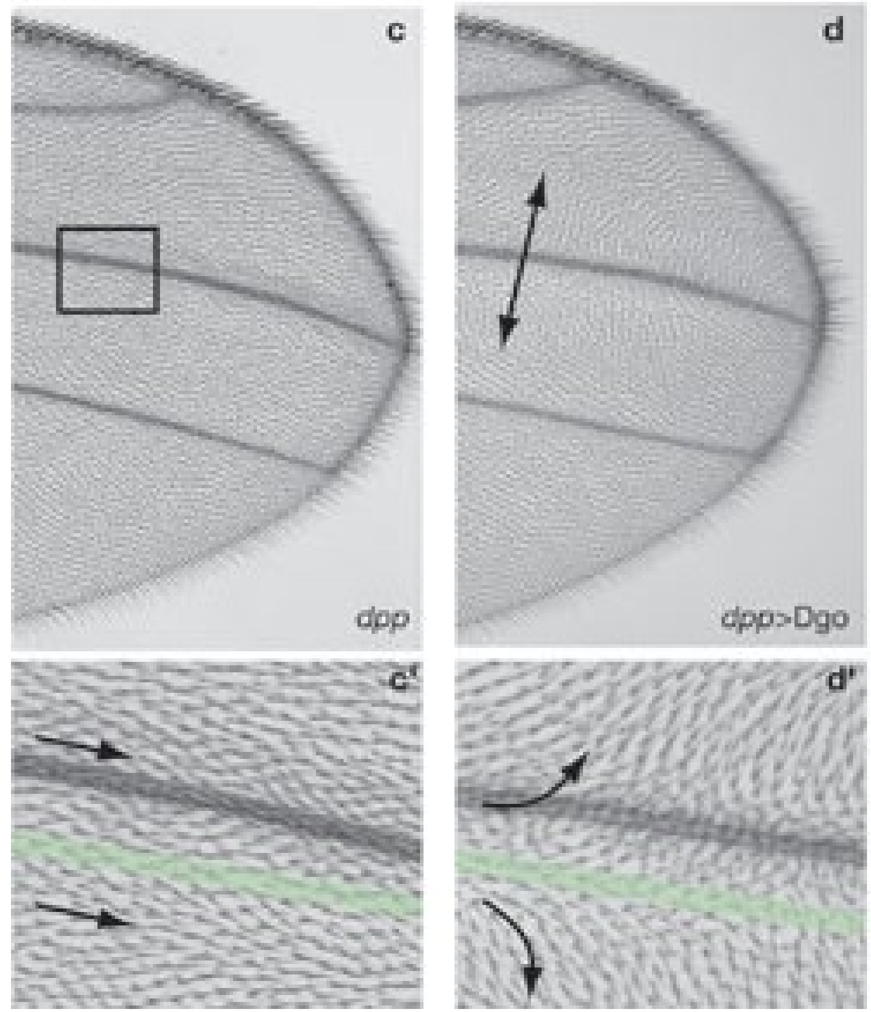
SnapShot: Noncanonical Wnt Signaling Pathways

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



Drosophila – PCP (planar cell polarity)





Molekulární mechanismus ustavení PCP

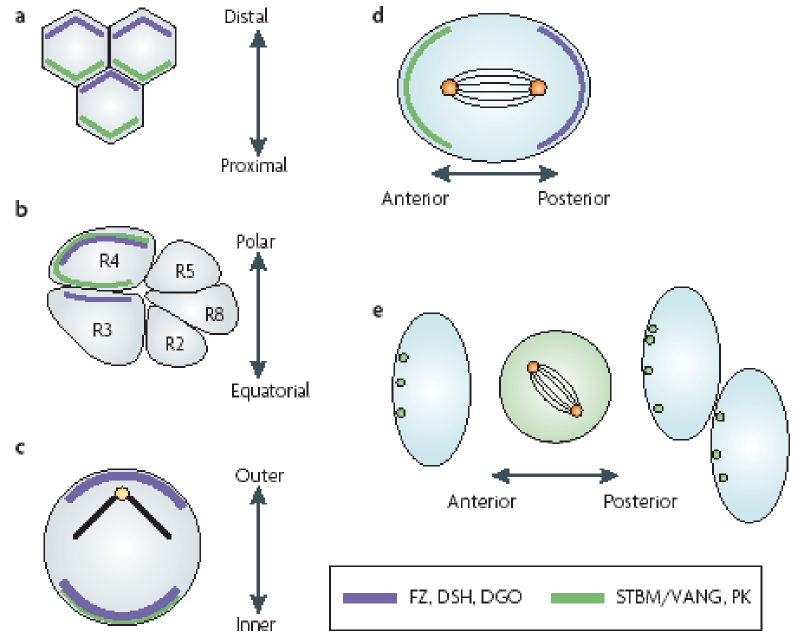
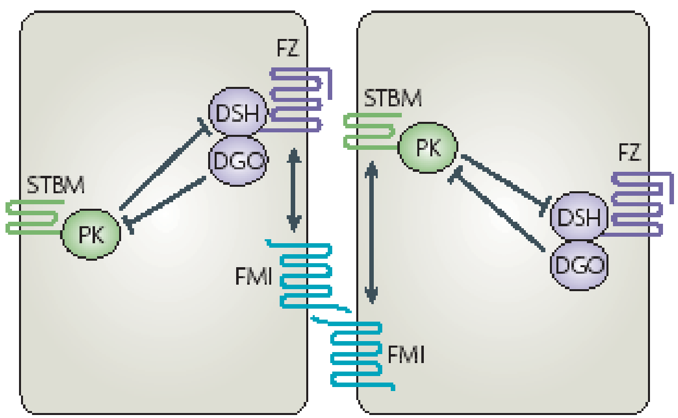


Figure 2 | **Subcellular distribution of core Fz/PCP factors in *Drosophila melanogaster* and vertebrates. a–c** | Examples of cells with epithelial character (marked by grey shading). *Drosophila melanogaster* wing cells and eye R3 and R4 cells and mouse sensory hair cells in the cochlea (inner ear) 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[52]. 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**

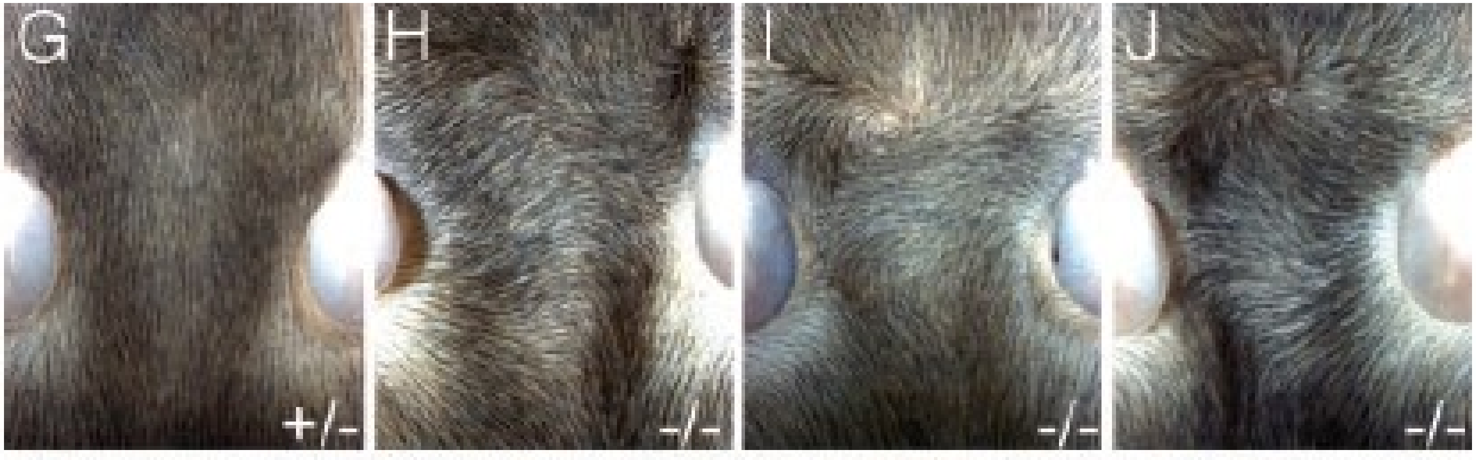
The molecular logic of the formation and separation of the Frizzled–Dishevelled–Diego (FZ–DSH–DGO) and Prickle–Strabismus (PK–STBM) complexes has started to be unravelled. In FIG. 2 are reported examples of the localization of each complex in various tissues. The figure is an apical view of two cells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

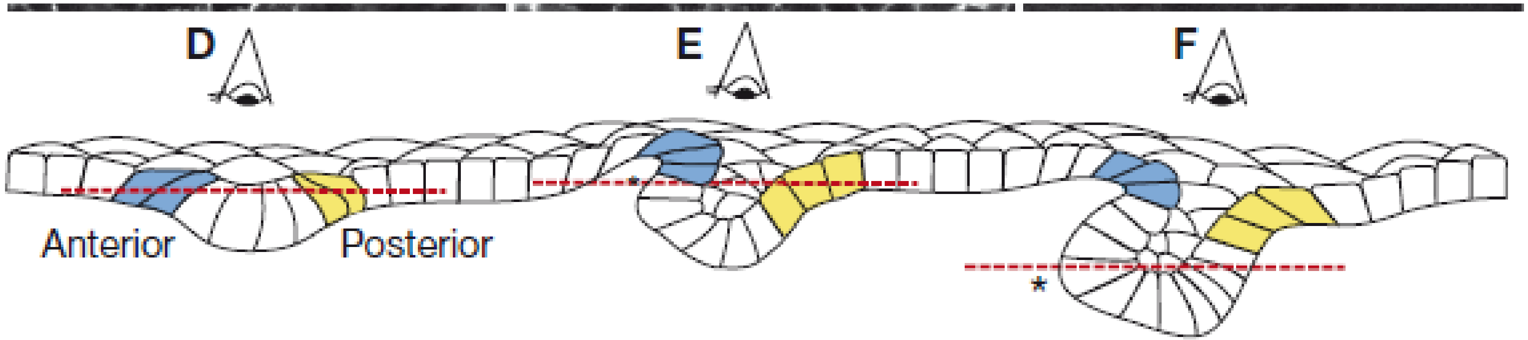


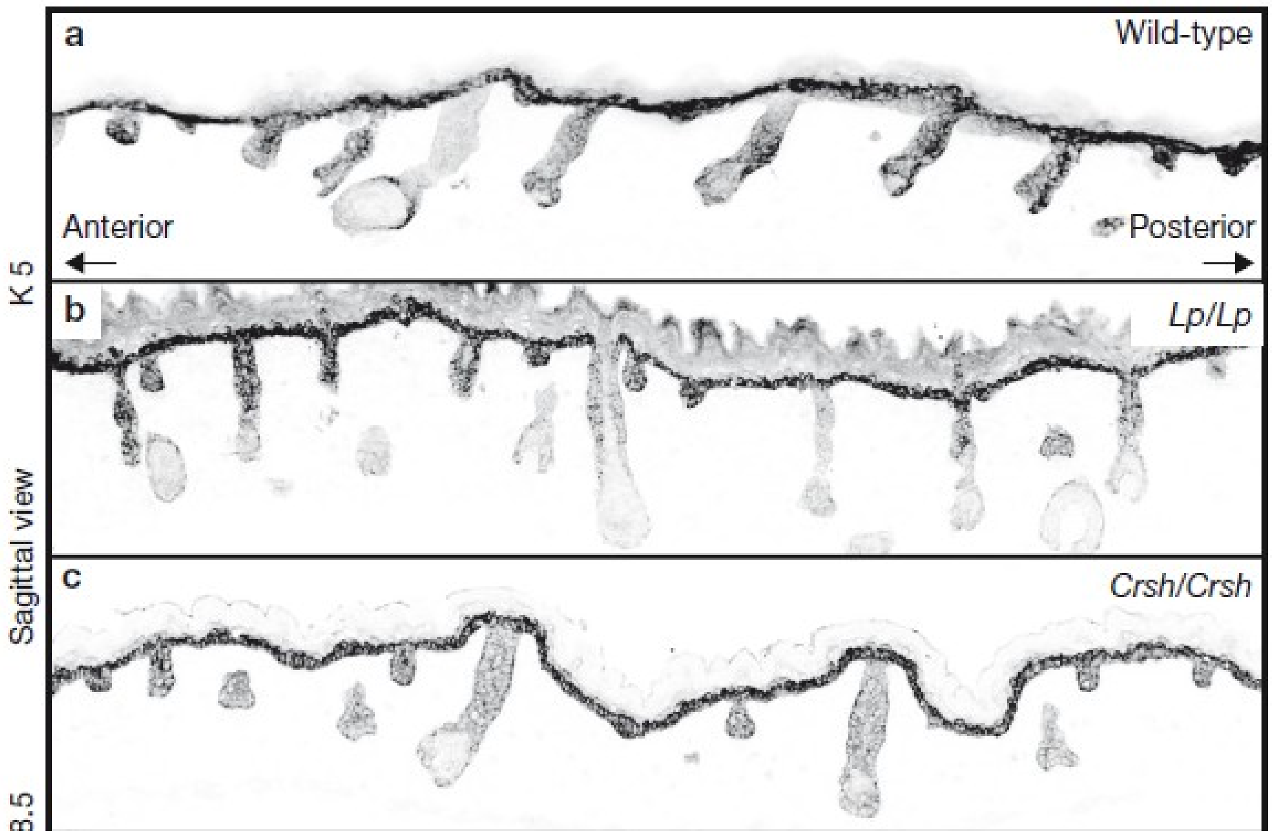
Seifert and Mlodzik, Nature Reviews in Genetics, 2007

Defect in the non-canonical Wnt pathway in mammals

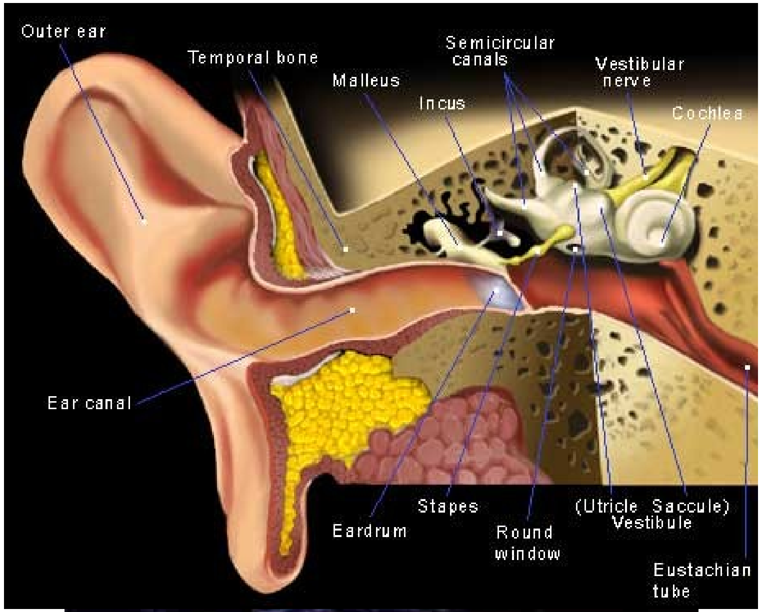
Changes in the "haircut"

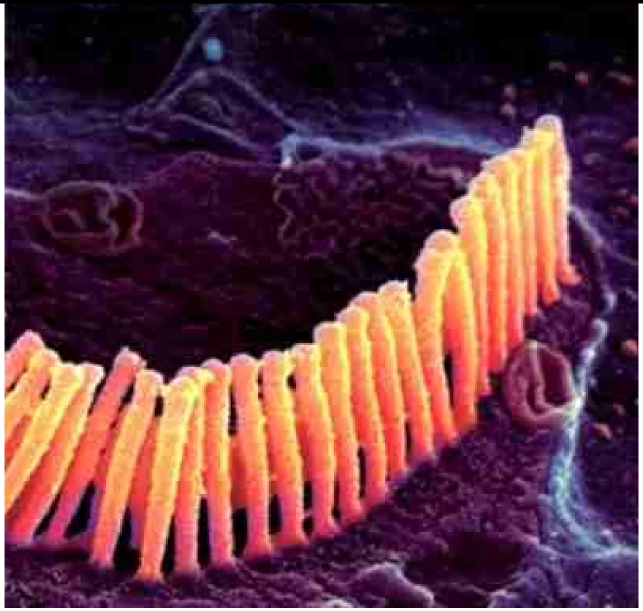



D
E
F
Anterior
Posterior

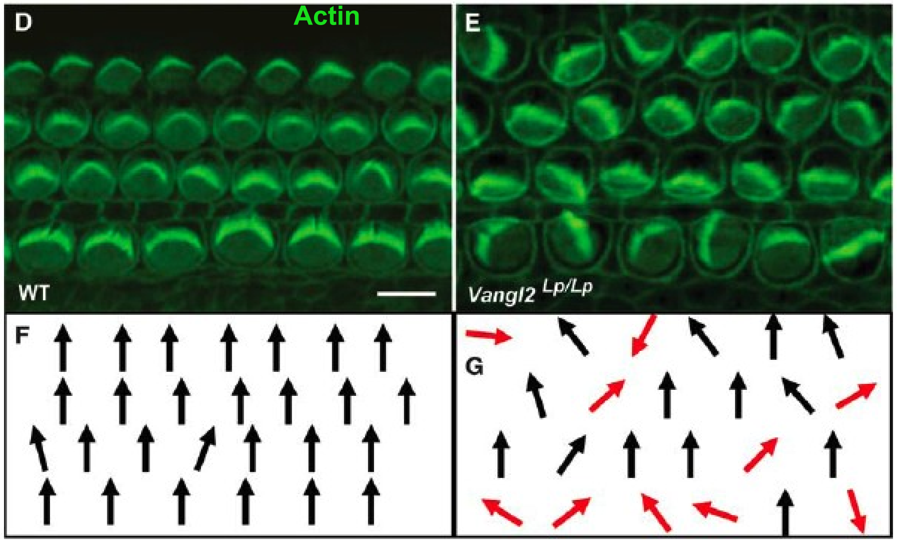

a
Wild-type
Anterior
Posterior
b
Lp/Lp
c
Crsh/Crsh
K 5
Sagittal view
8.5

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





Stereocilia orientation in inner ear hair cells

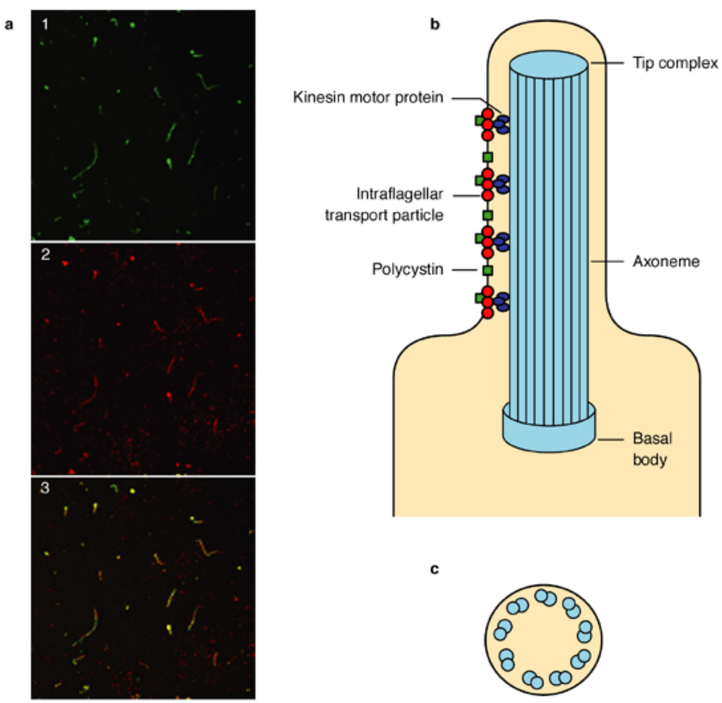


Qian et al., 2007, Dev. Biol.

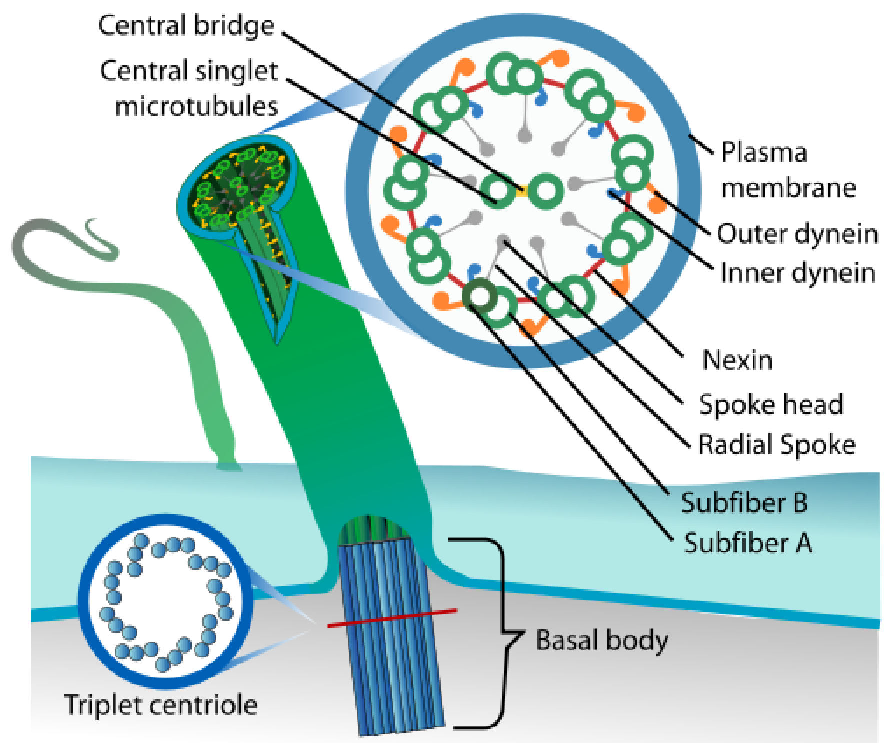
Primary cilia vs. motile (secondary) cilia

- struktura 9+0
- nepohyblivé
- téměř všechny buňky (www.primary–cilium.co.uk)
- solitérní

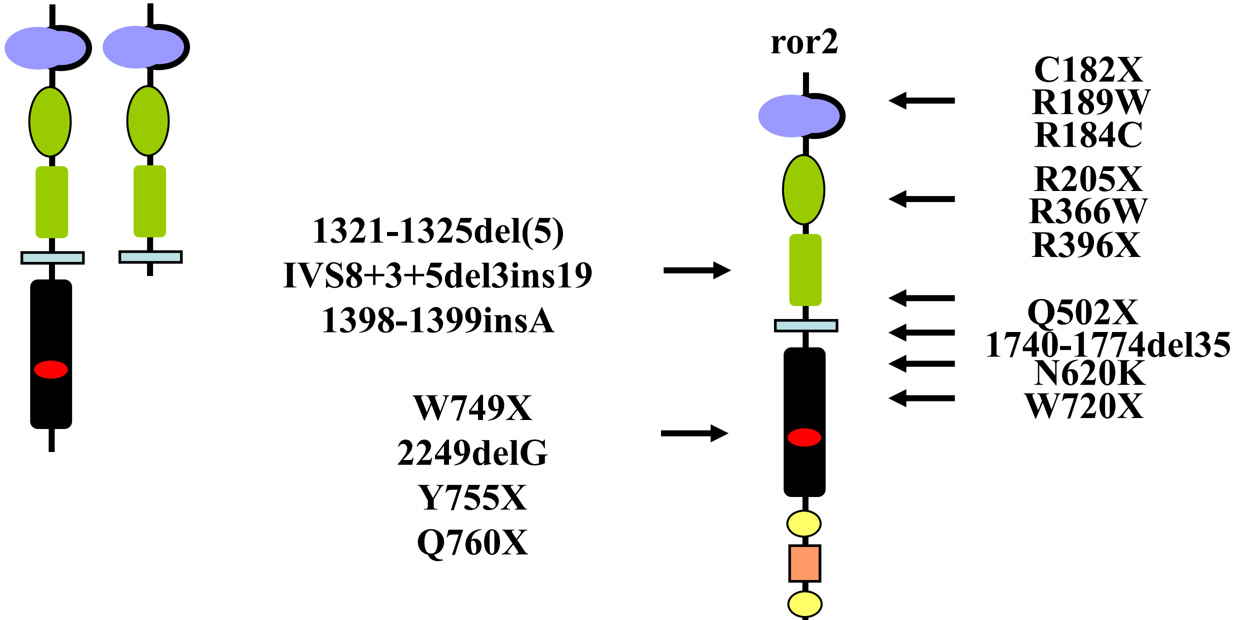
- struktura 9+2
- pohyblivé
- epitely tracheje, vejcovodů, ependym…



The primary cilium



Ror2

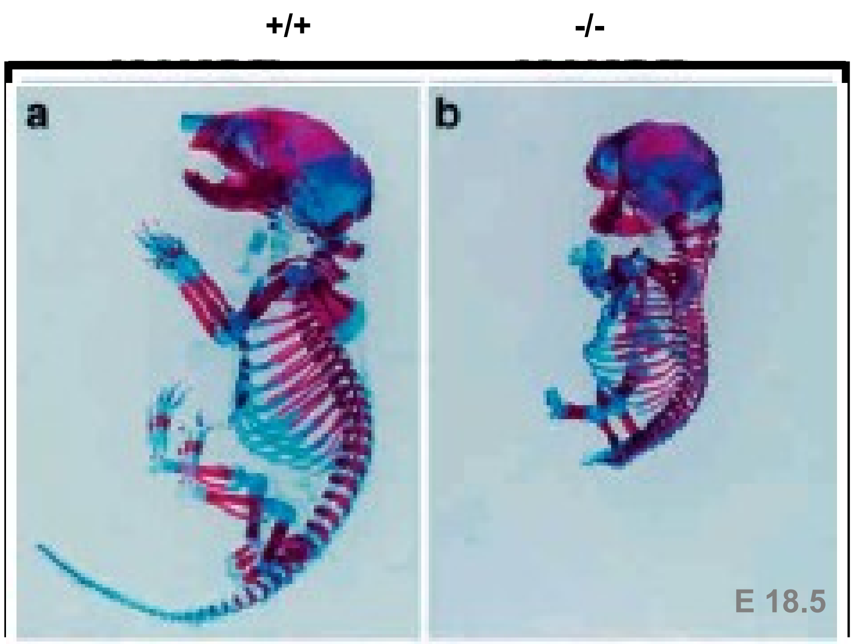


Mutations in *Ror2* cause dominant brachydactyly type B (BDB) and recessive robinow syndrome (RRS)

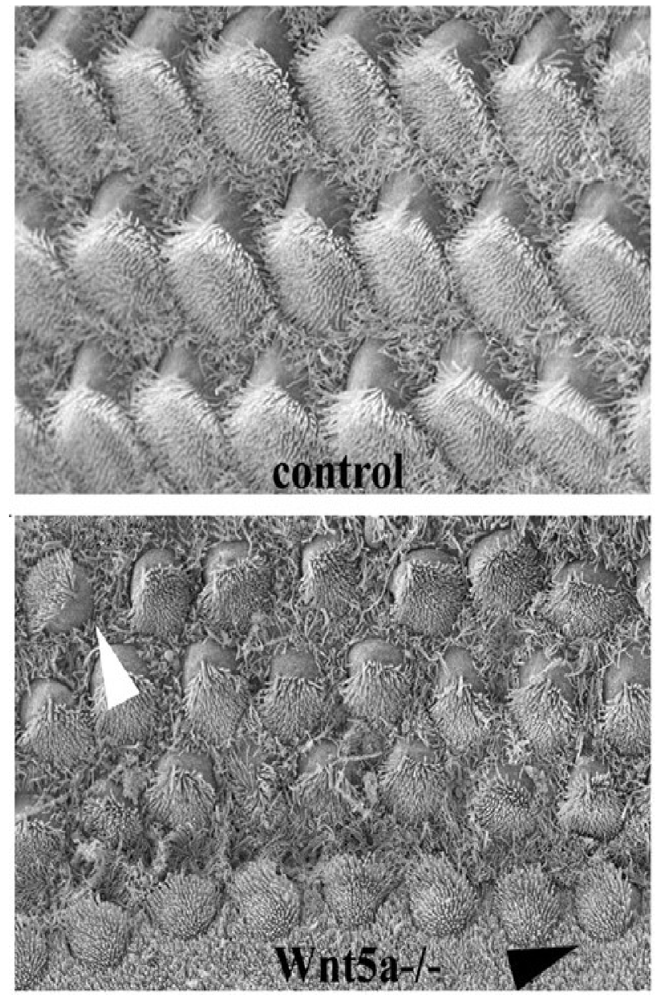
BDB

Provided by S. Stricker

Known Wnt5a knockout phenotypes

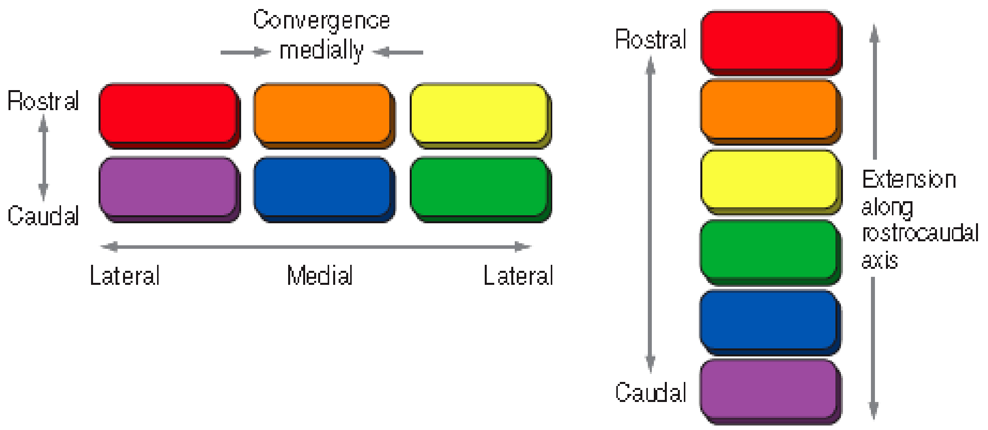


Yamaguchi et al., 1999



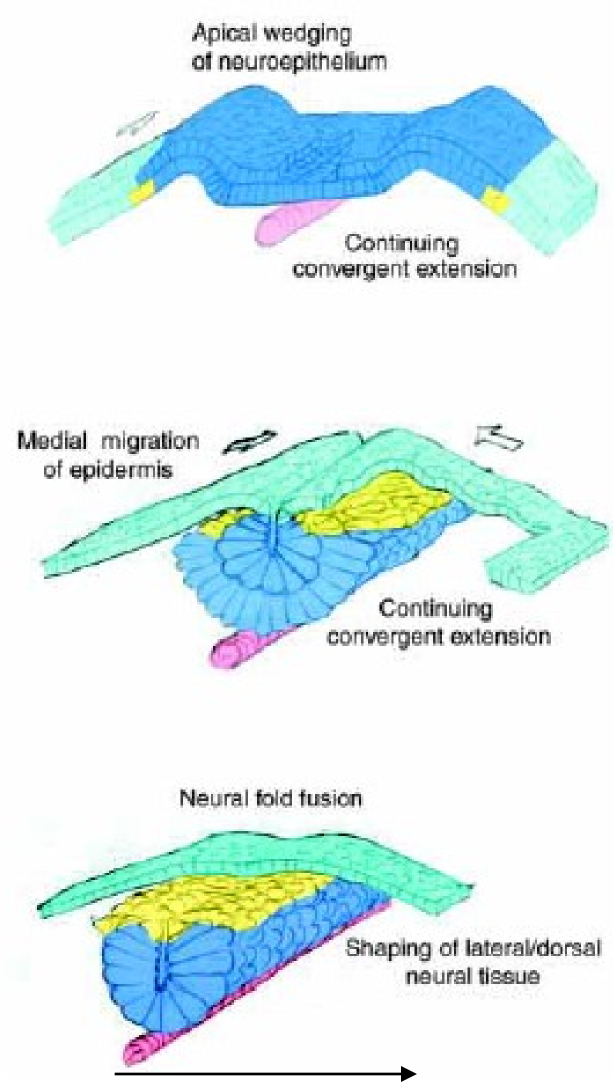
Qian et al, 2007

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

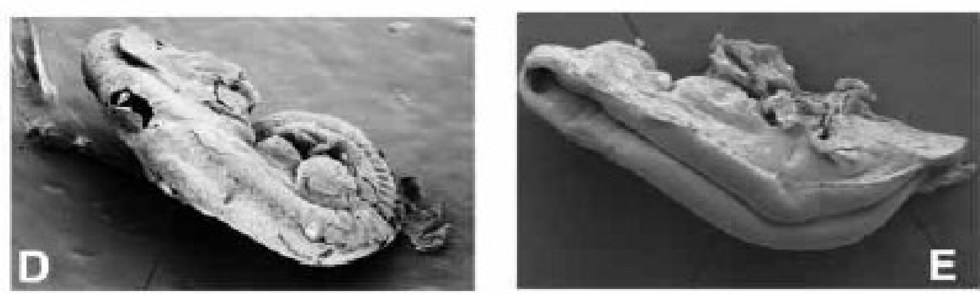
Důsledky narušené konvergentní extenze (CE)



neurulation.mov

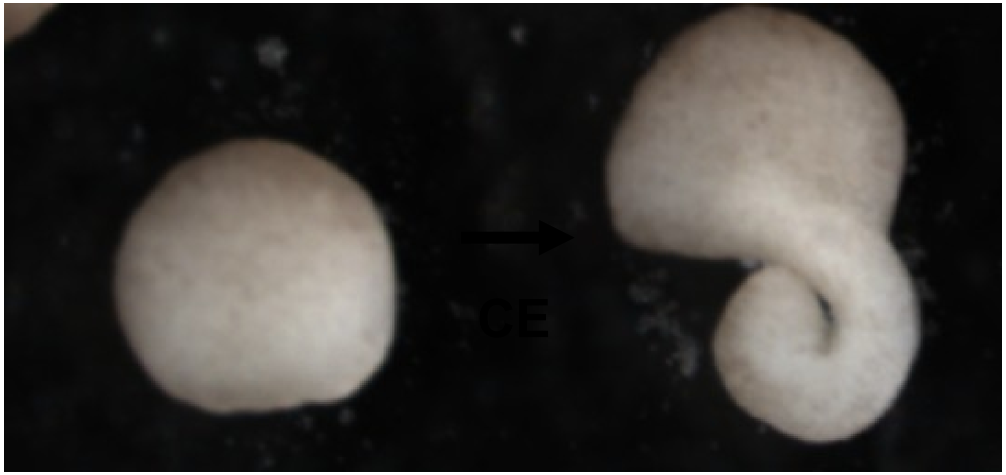
Exencephaly

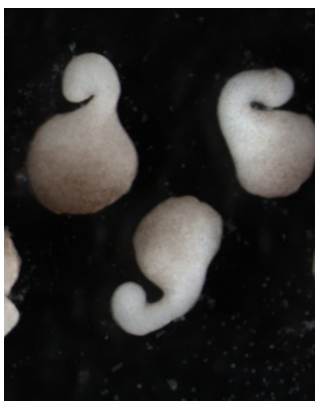
Open neural tube



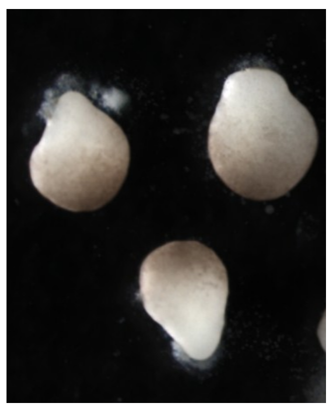
Hamblet et al., 2002, Development

Možnosti studia CE - Kellerovy explantáty (Xenopus)

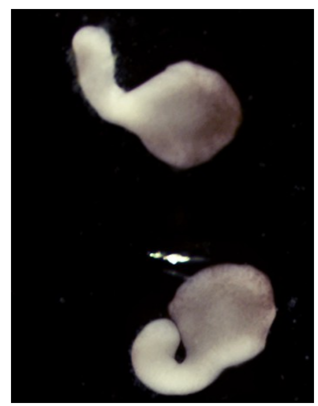




control

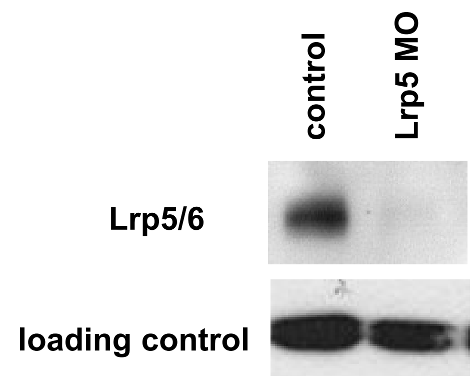


XLRP5 MO



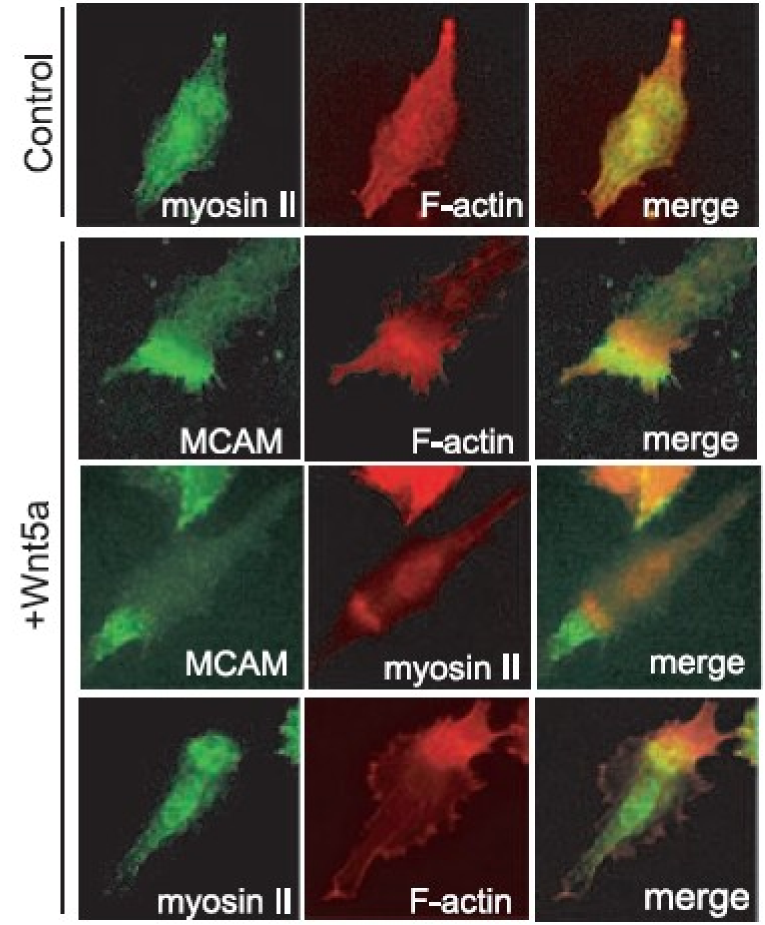
XLRP5 MO

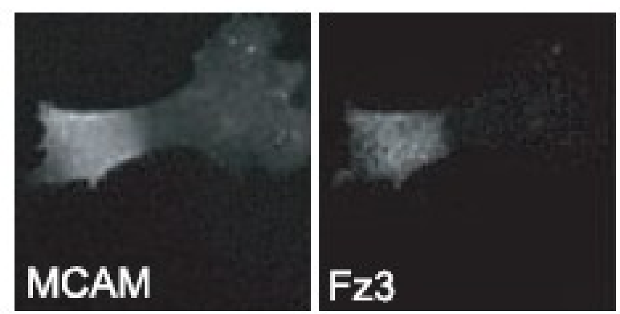
+ mLrp5



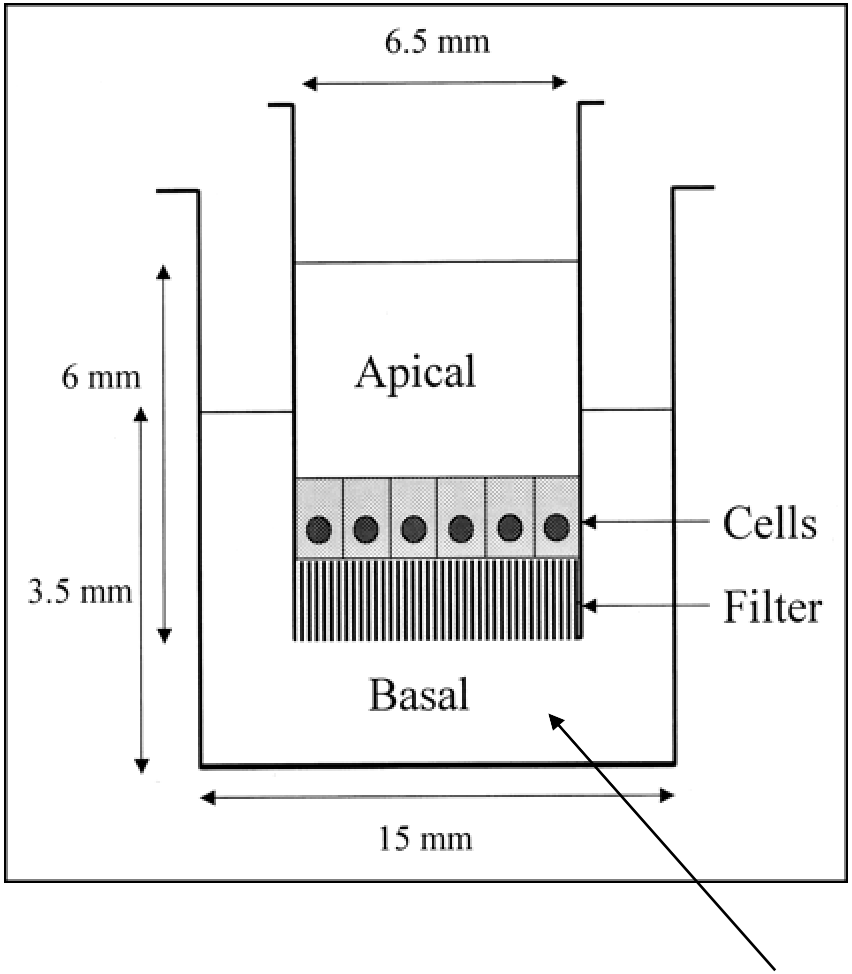
Wnt-induced assymetry

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



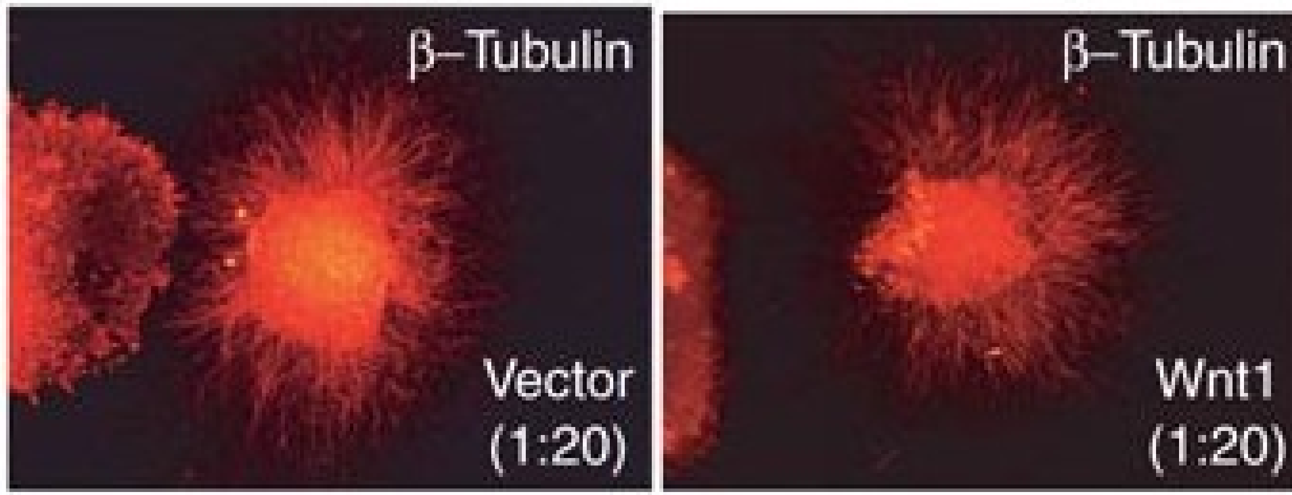


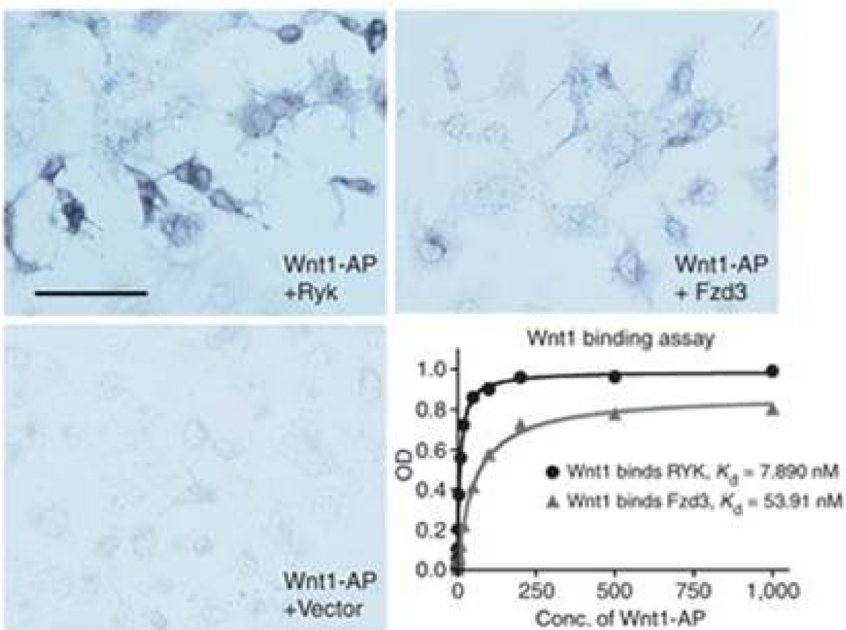
Možnosti studia migrace – Transwell assays (mammalian cells)



Možnosti studia migrace – attractant/repelent assays

Attractant/repelent →





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