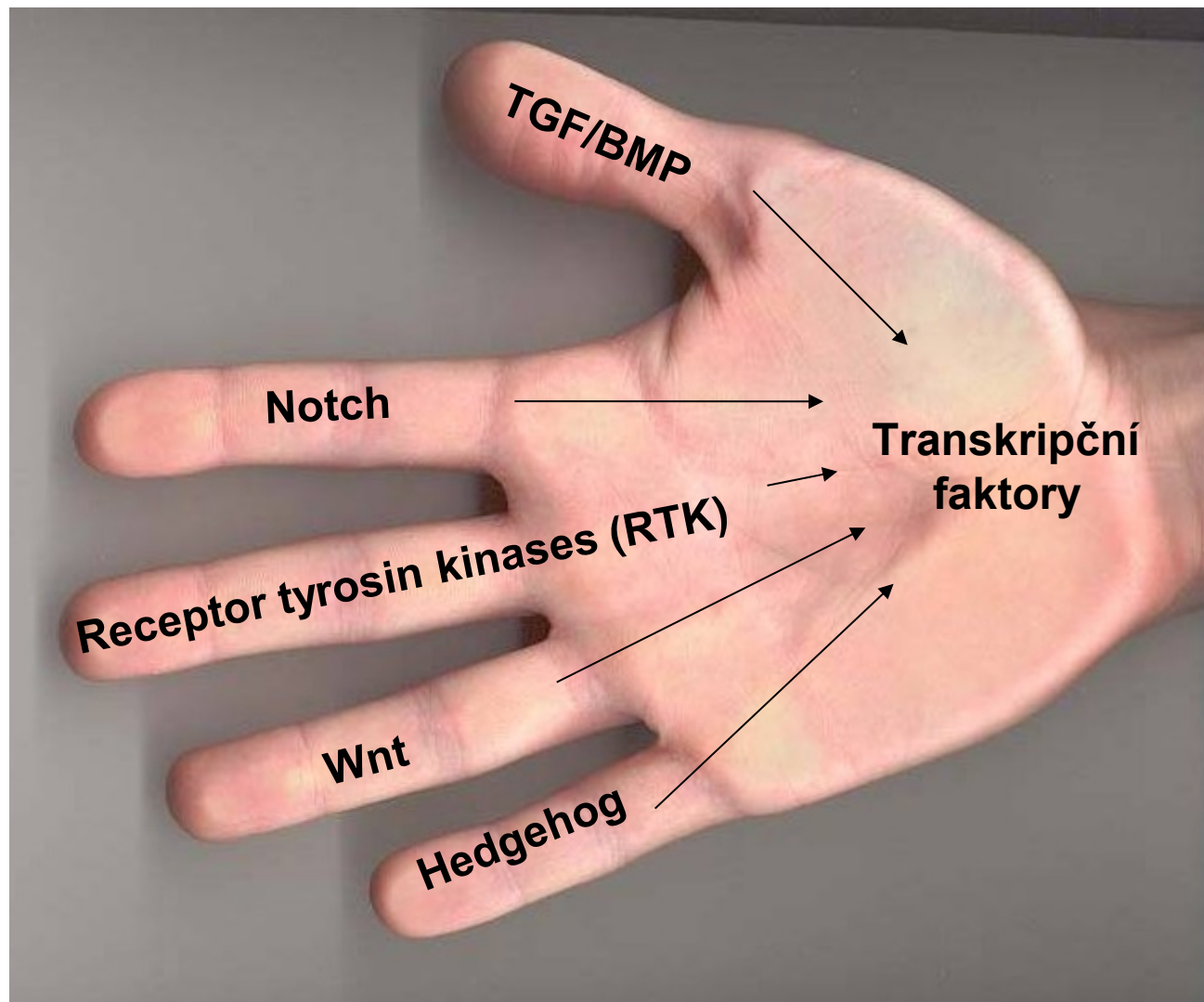


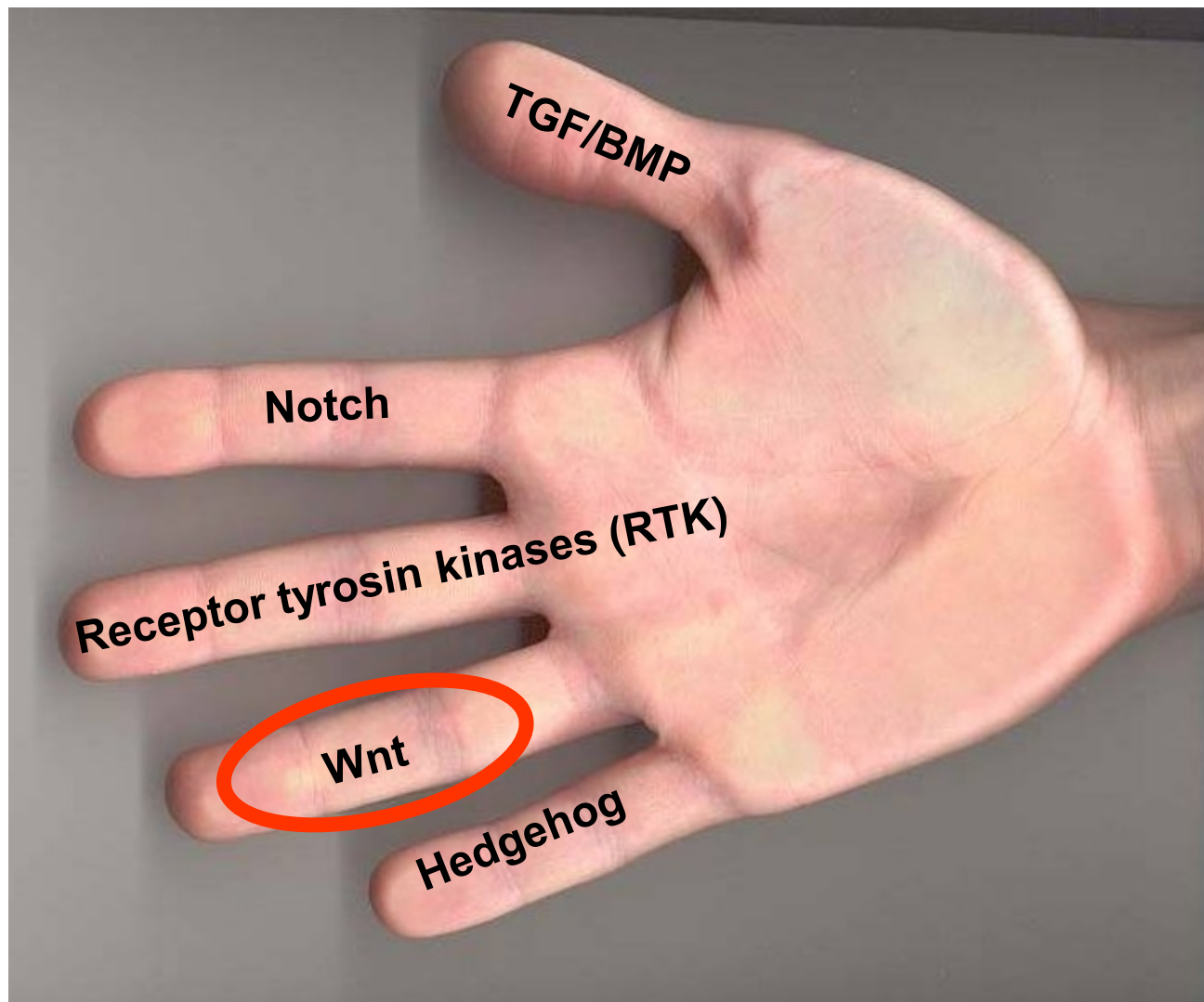
Buněčné systémy ve vývoji: Wnt signální dráha a kmenové buňky

doc. Mgr. Vítězslav Bryja, Ph.D.

Klíčové molekulární komponenty vývoje



Klíčové molekulární komponenty vývoje

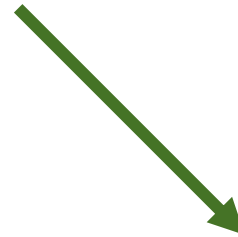


Wnt (Wingless/Int)

- rodina ligandů
- 19 genů pro proteiny Wnt u člověka a u myši
- extracelulární proteiny modifikované glykosylací a palmitoylací
- působí na krátké vzdálenosti, váží se k extracelulární matrix
- pouze u mnohobuněčných živočichů



kanonická dráha
/závislá na β -kateninu/
(např. Wnt-1 or Wnt-3a)



nekanonická dráha
/na β -kateninu nezávislá/
(např. Wnt-5a)

Wnt/ β -kateninová dráha (= kanonická dráha)



- indukuje duplikaci tělní osy u *Xenopus*
- indukuje transformaci buněčné linie odvozené od lidských prsních epiteliálních buněk C57mg
- signál přenášen přes translokaci β -kateninu do jádra

Sekrece Wnt

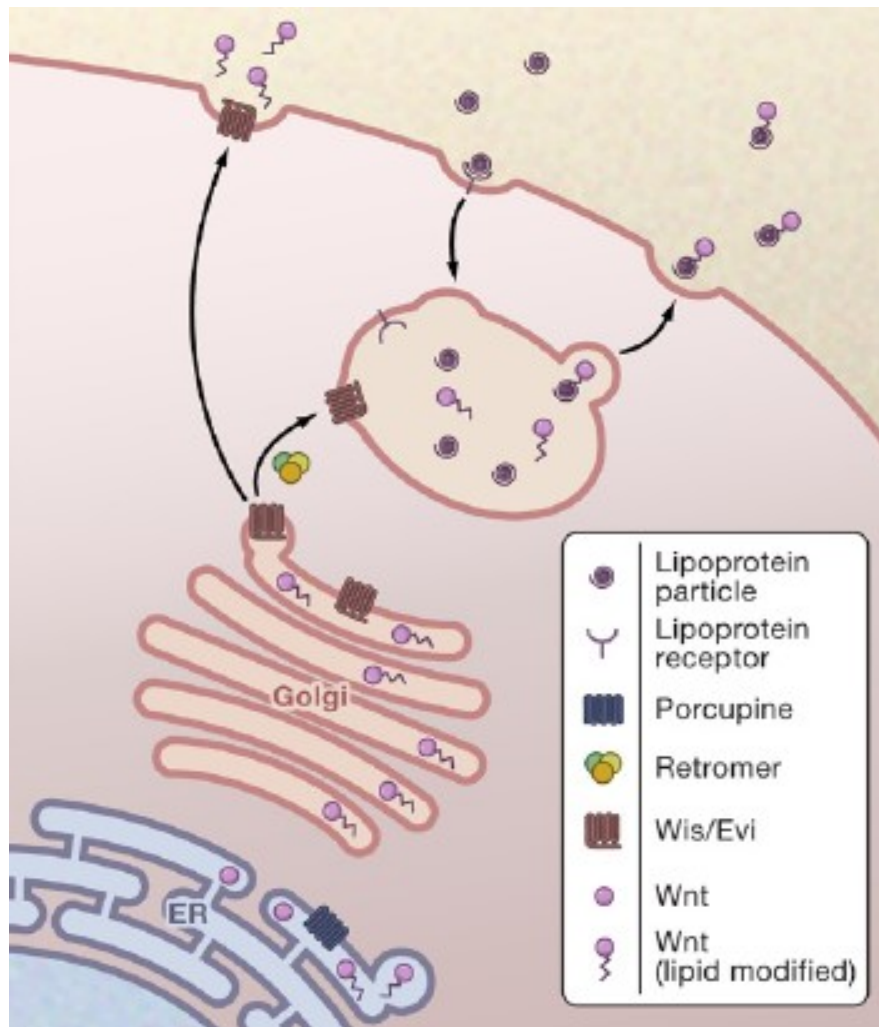
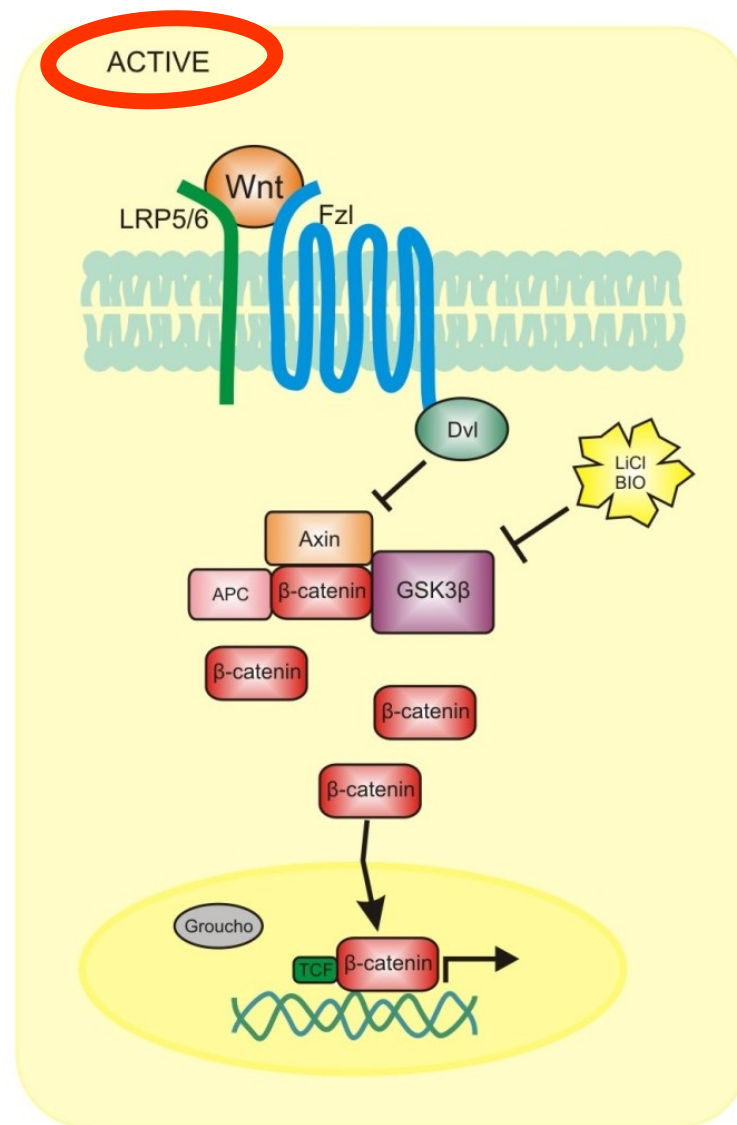
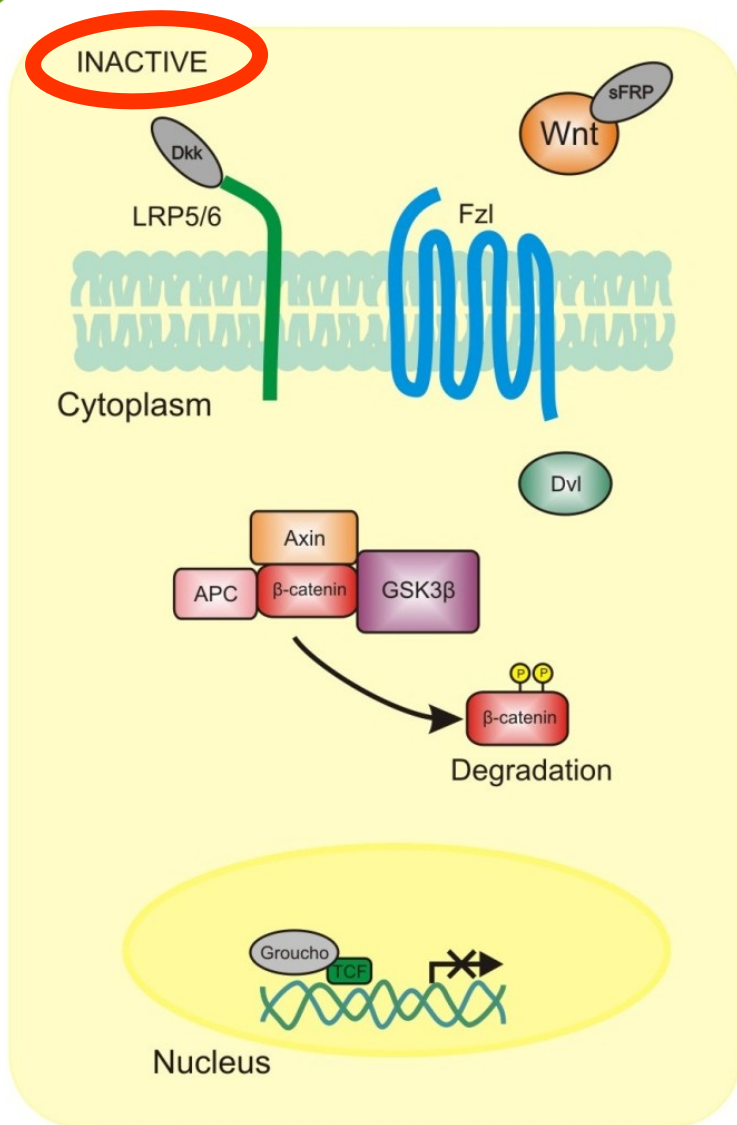


Figure 1. Wnt Secretion

To be secreted, Wnt proteins in the endoplasmic reticulum (ER) need to be palmitoylated by the action of Porcupine. Wnt proteins also require Wntless (Wis/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 Wis between the Golgi and the endo/exocytic compartment.

Kanonická signální dráha Wnt



Kanonická signální dráha Wnt

- legenda k obrázku:

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 (GSK3 β). 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 GSK3 β phosphorylates β -catenin, leading to degradation by ubiquitination. Copyright BTR ©

Frizzled – klíčový receptor většiny (všech?) signálních drah Wnt

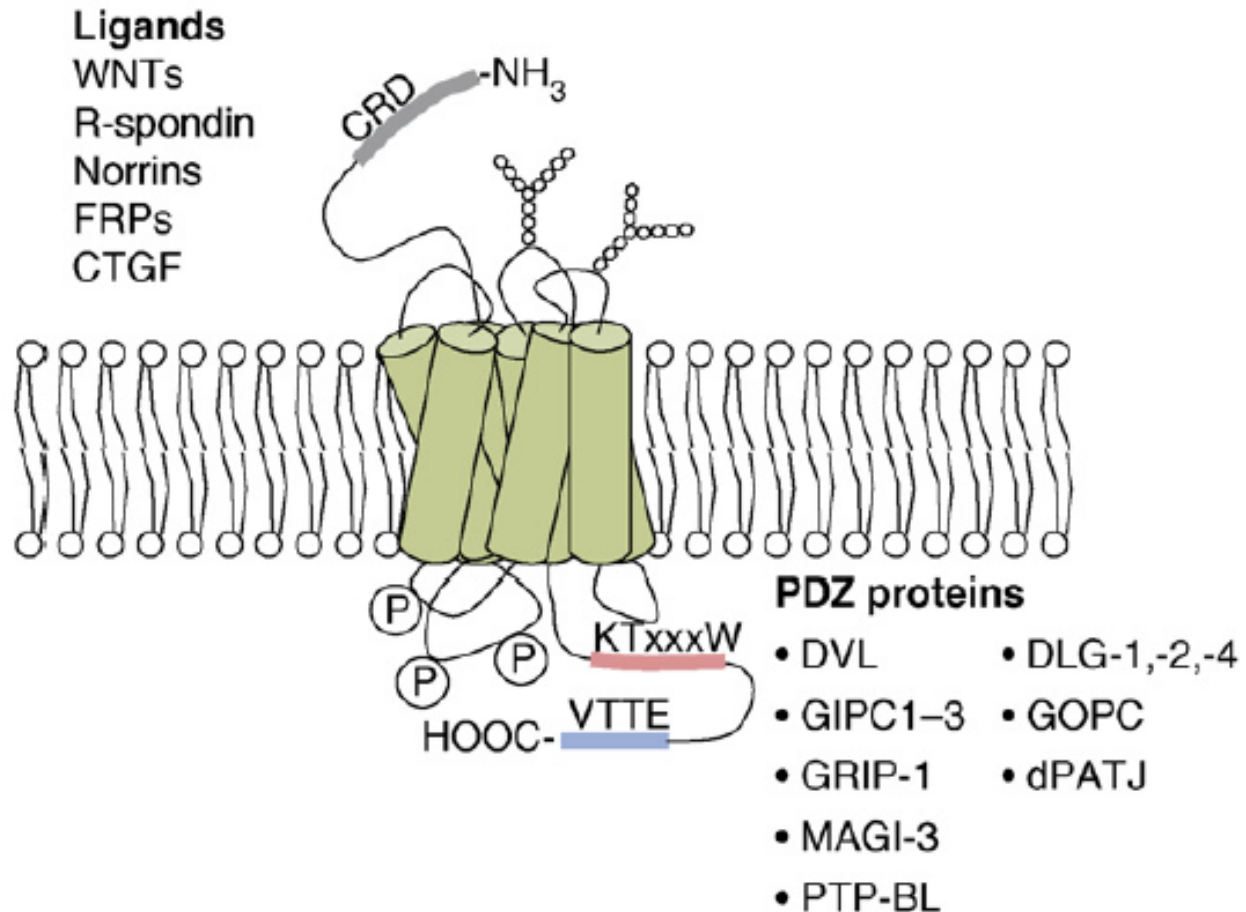
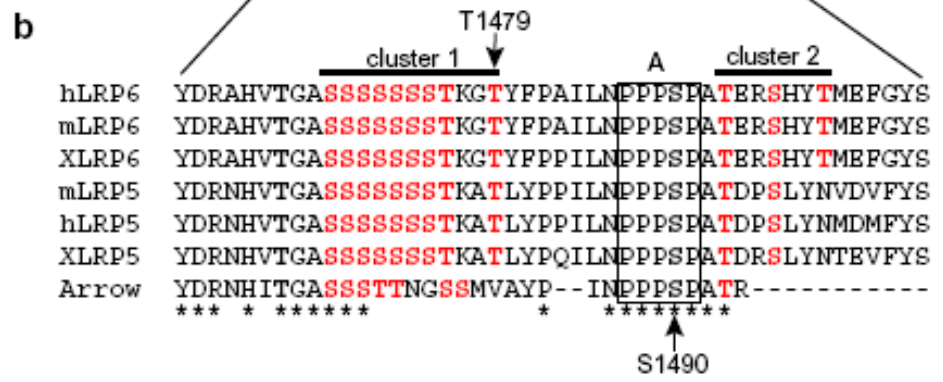
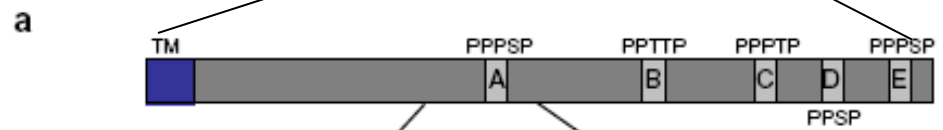
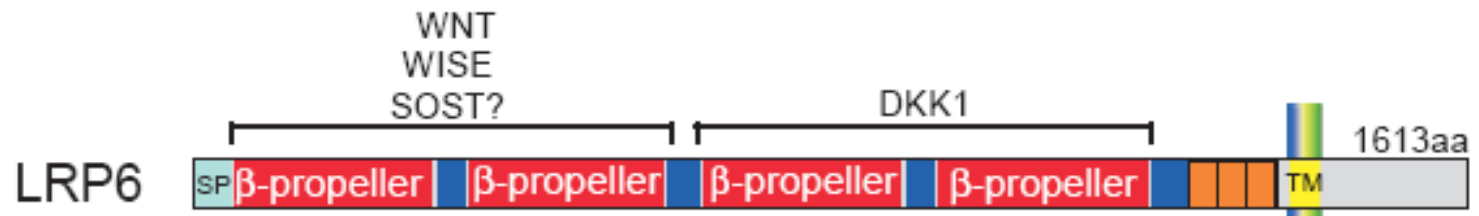
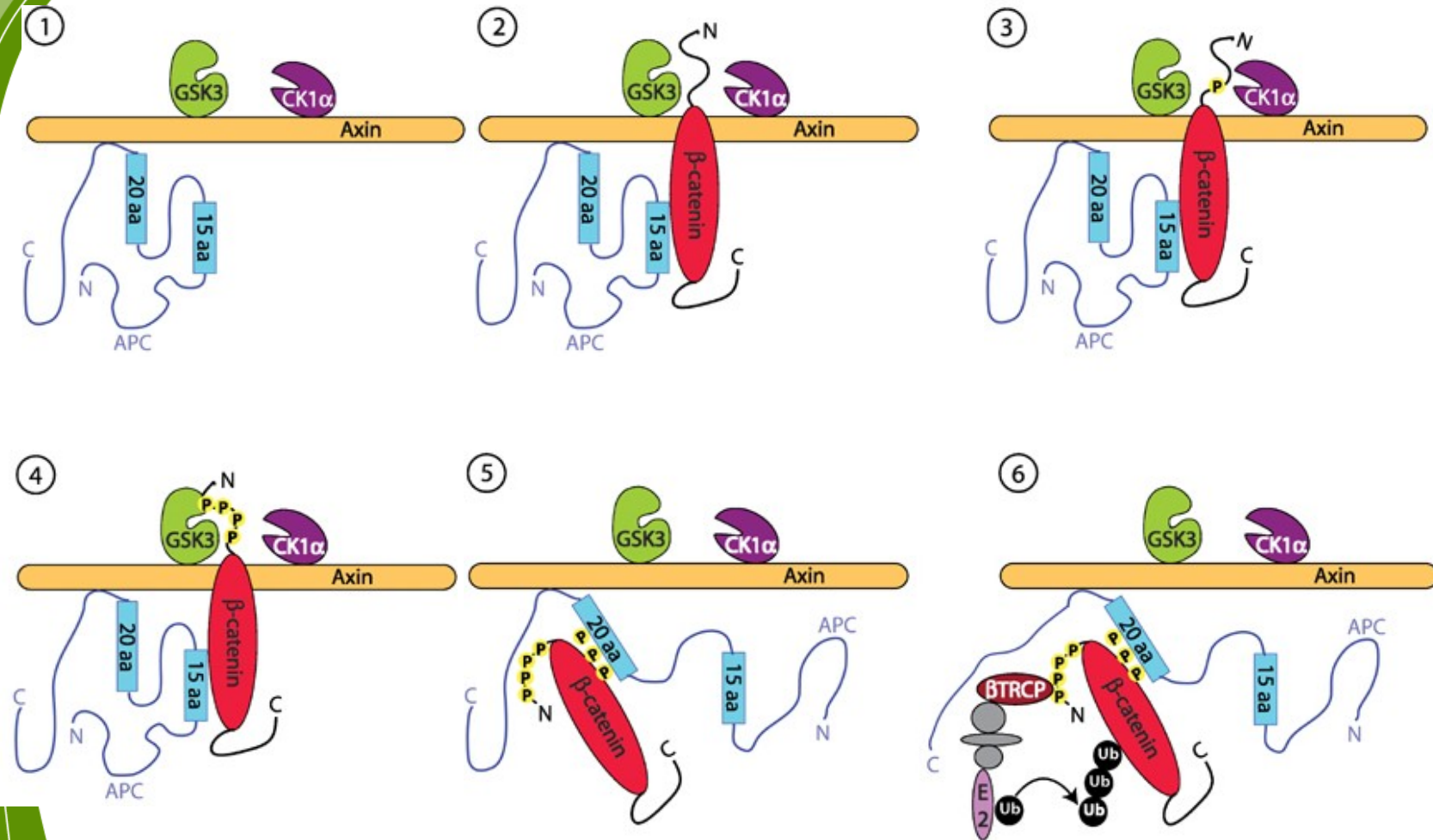


Figure 1. Schematic view of the 7TM model of FZDs. The model indicates extra- and intracellularly interacting proteins, putative glycosylation and phosphorylation sites. The N-terminal CRD is the primary binding site for ligands. The pink stretch in the C terminus indicates the internal PDZ-interacting 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 – stěžejní ko-receptor kanonické signální dráhy Wnt



Destrukční komplex



Destrukční komplex

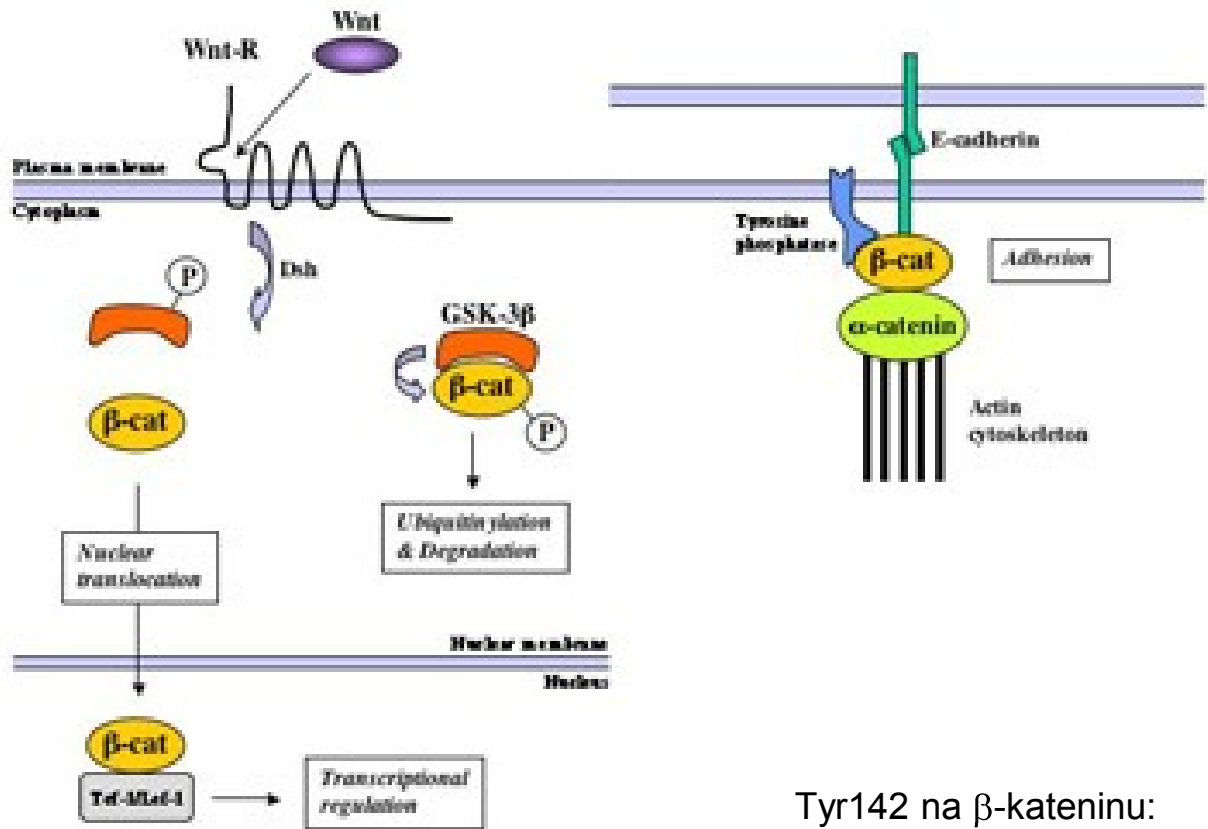
- legenda k obrázku destrukčního komplexu:

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

Kanonická signální Wnt dráha - video

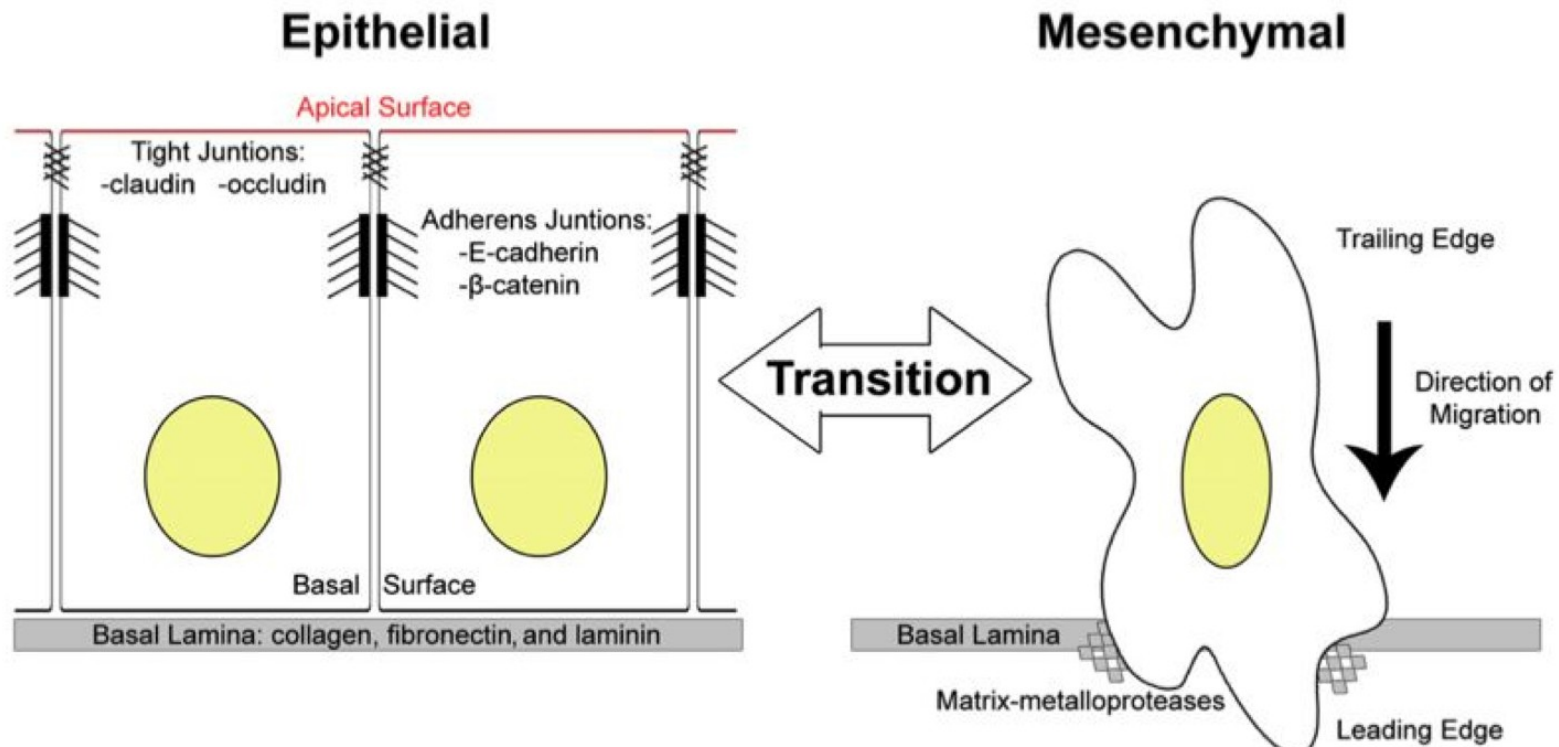
Beta-catenin se v buňkách vyskytuje ve dvou hlavních „poolech“ – biochemických komplexech

Wnt and E-cadherin pathways

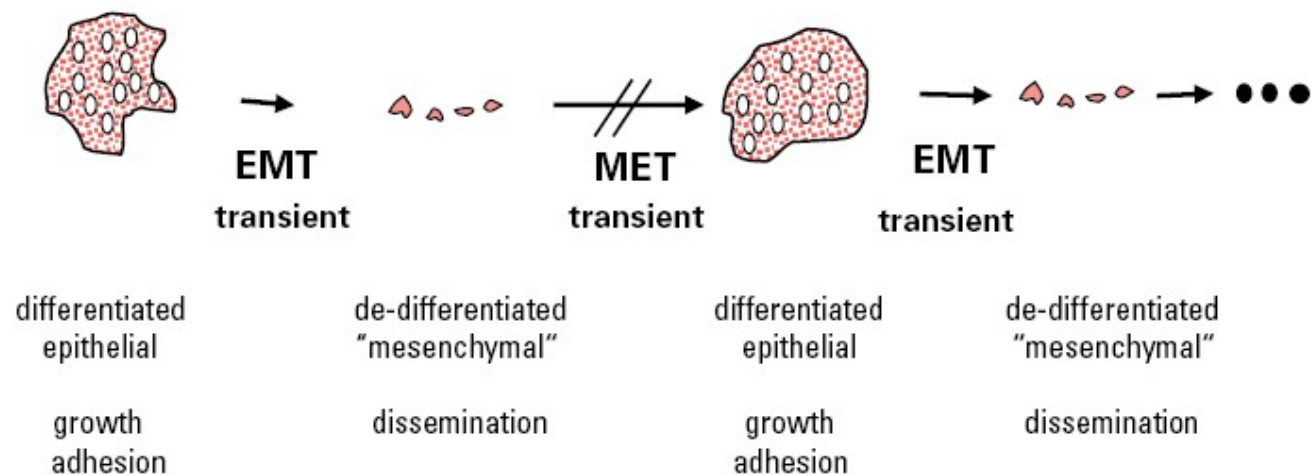
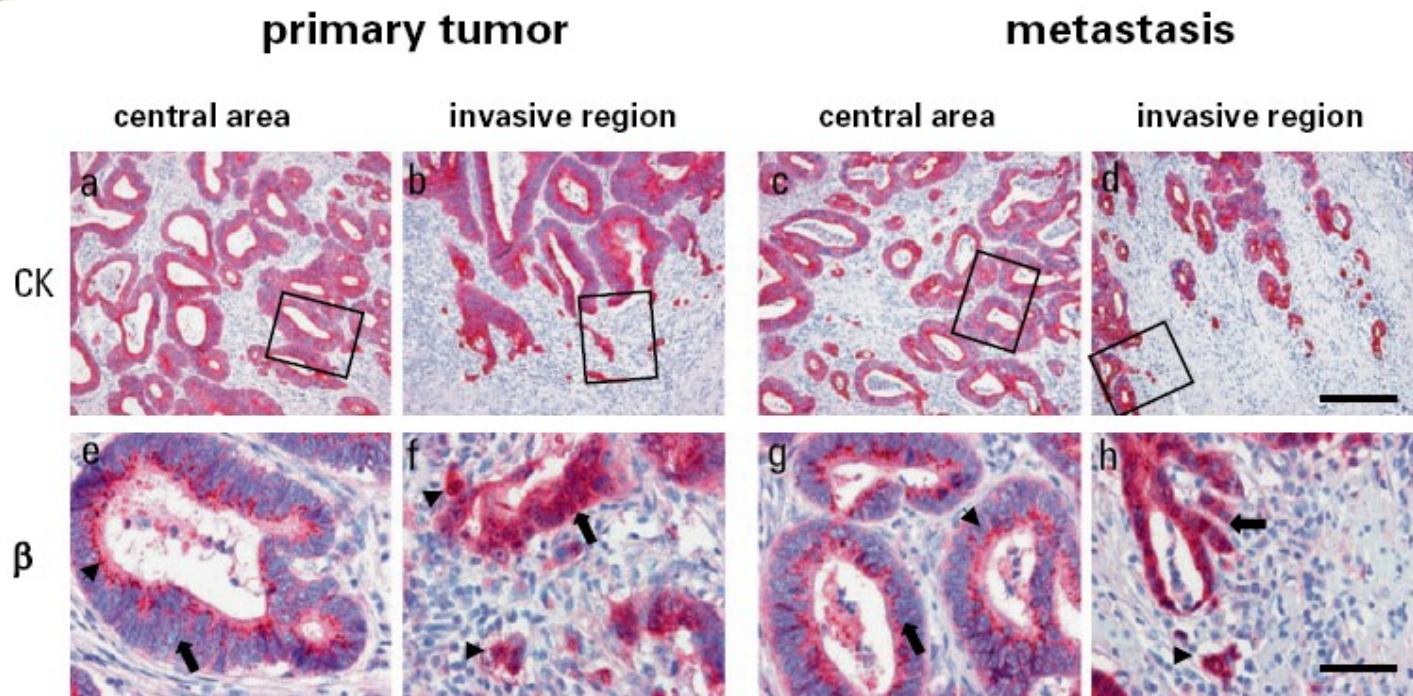


Tyr142 na β-kateninu:
 fosforylován: váže Bcl9
 bez fosforylace: váže α-katenin

Epithelio-mesenchymální transice a role β -kateninu v tomto procesu



Epiteliálně-mezenchymální tranzice (EMT) v nádorech



Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

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

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

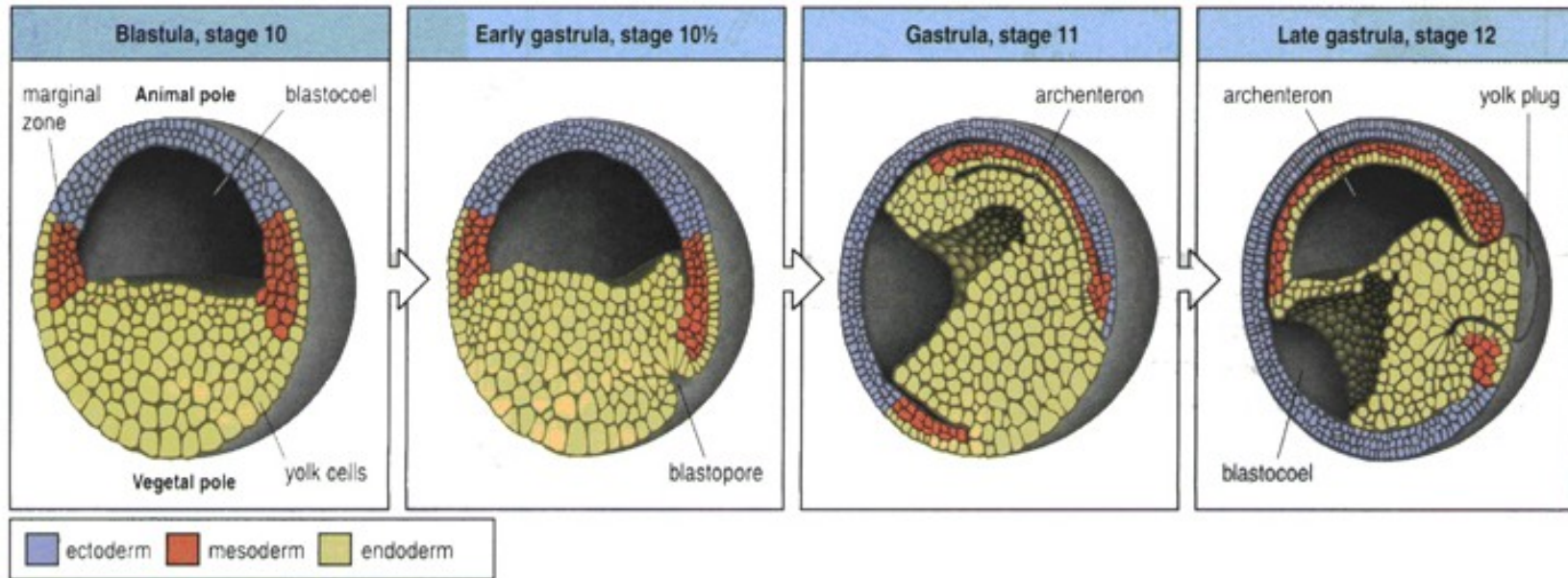
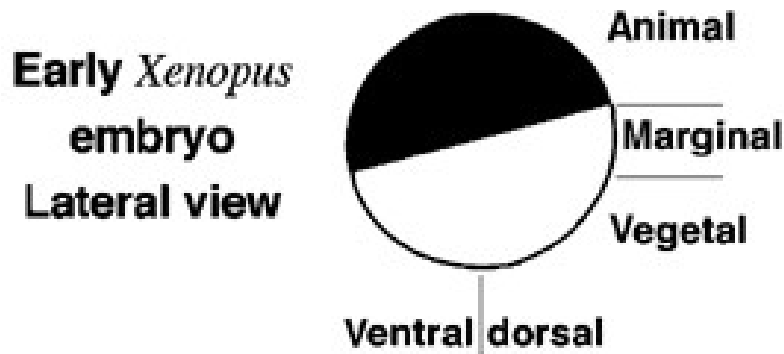


Fig. 2.6 Gastrulation in amphibians. The blastula (first panel) contains several thousand cells and there is a fluid-filled cavity, the blastocoel, beneath the cells at the animal pole. Gastrulation begins (second panel) at the blastopore, which forms on the dorsal side of the embryo. Future mesoderm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

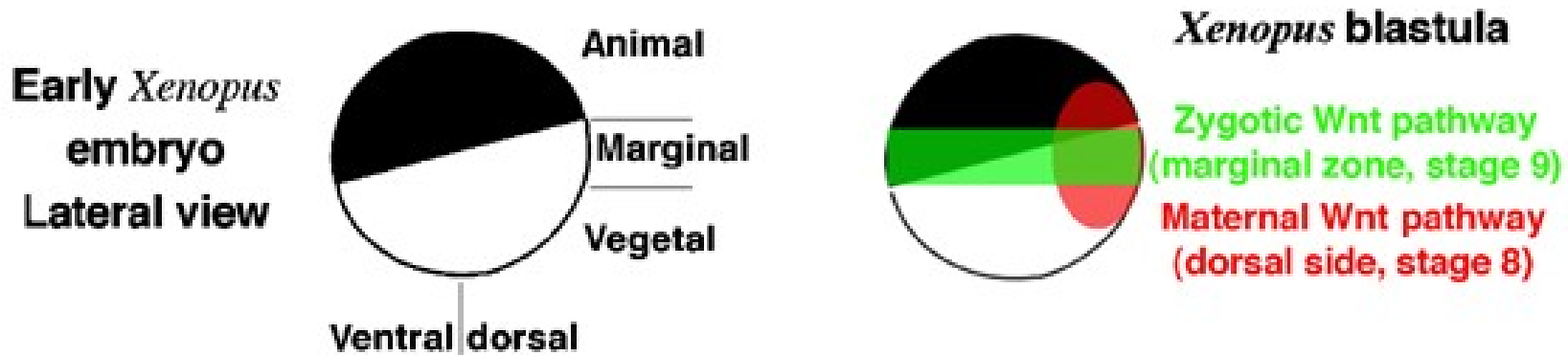
between the endoderm and ectoderm in the animal region (third panel). The tissue movements create a new internal cavity—the archenteron—which will become the gut. Endoderm in the ventral region also moves inside through the ventral lip of the blastopore (fourth panel) and will eventually completely line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

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



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

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



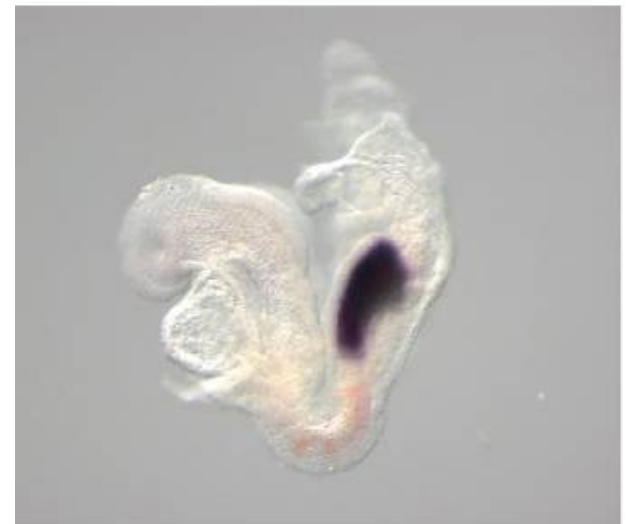
Maternální Wnt/ β -cateninová dráha determinuje dorsální (horní) pól vyvíjející se zygoty a embrya

myší embryo po gastrulaci (E8.5):

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

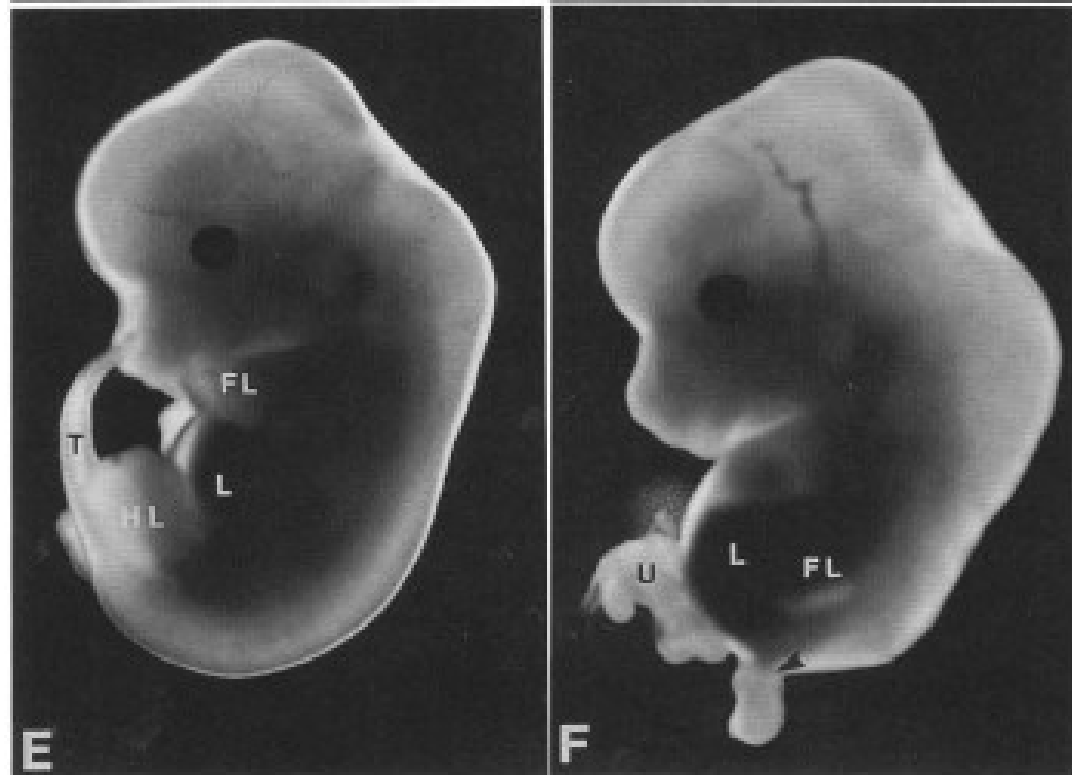
Uncx4.1/Mesogenin

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



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

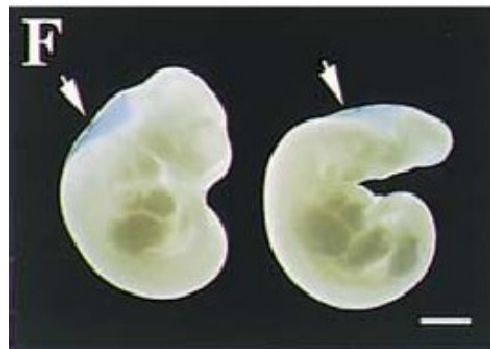
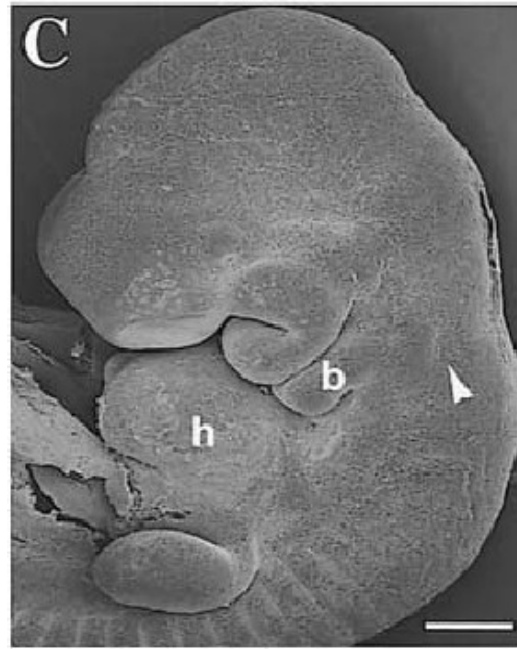
Deplece Wnt/ β -kateninové dráhy při gastrulaci = ztráta zadních částí těla



wild type

Wnt-3a knockout

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



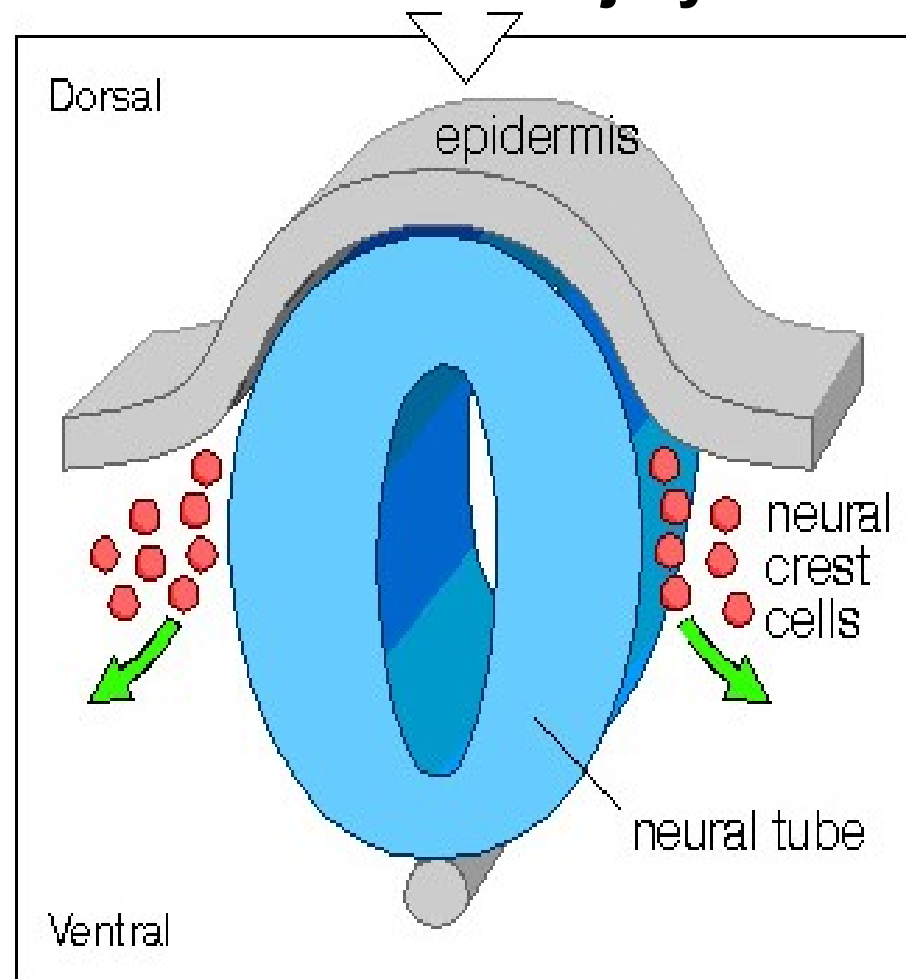
wild type vs. Dkk1 knockout

Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

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

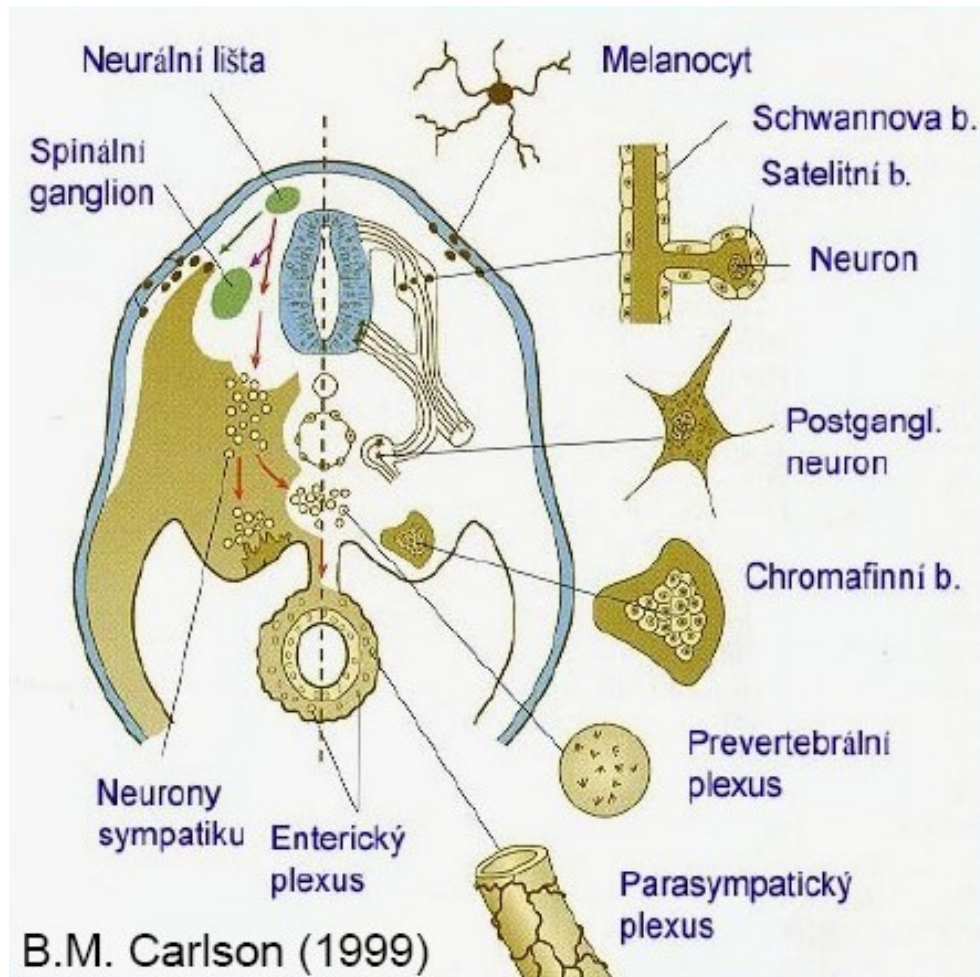


Co všechno z NC vzniká?

- podíl na vzniku cca 40 různých tkání a orgánů

Oblast trupu:

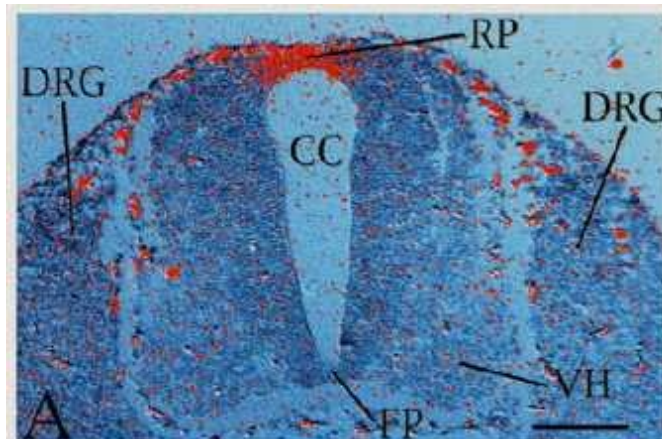
- Neurony a gliální buňky senzorického, sympatického a parasympatického systému
- Buňky dřeně nadledvin
- Pigmentové buňky epidermis
- Svalové buňky některých cév



Co všechno z NC vzniká?

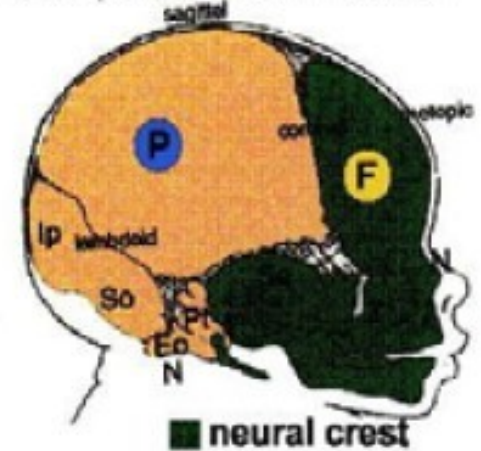
Vývoj neurální lišty:

Wnt-3a



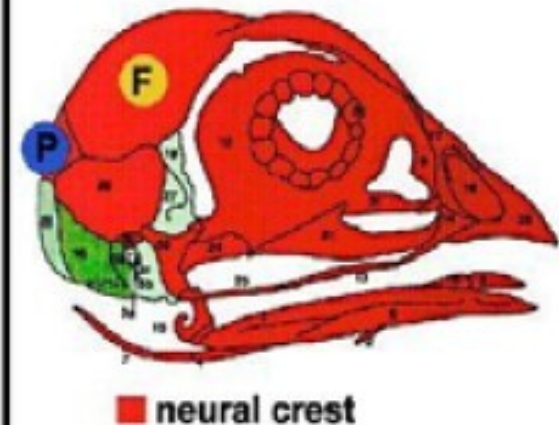
- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, částí srdce a dalších

human skull, extrapolated from mouse:



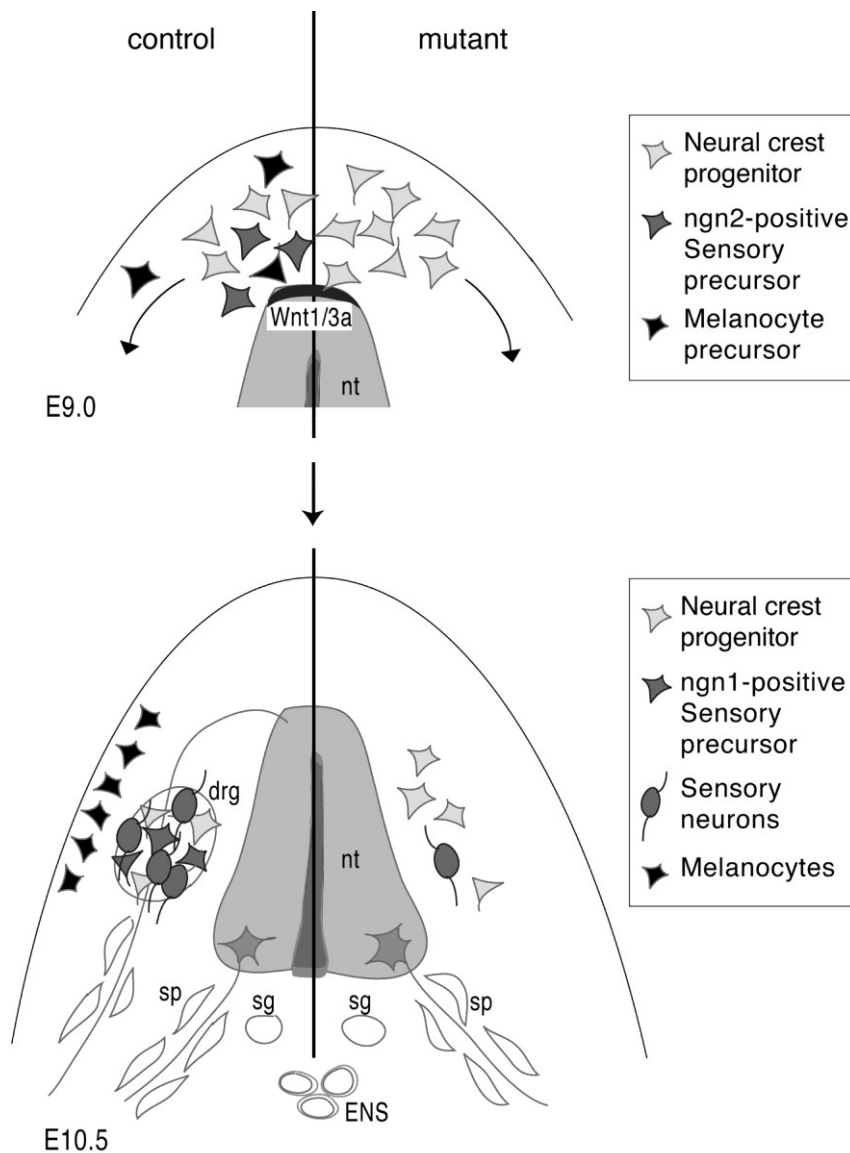
J.Anat. 2001

chick skull:



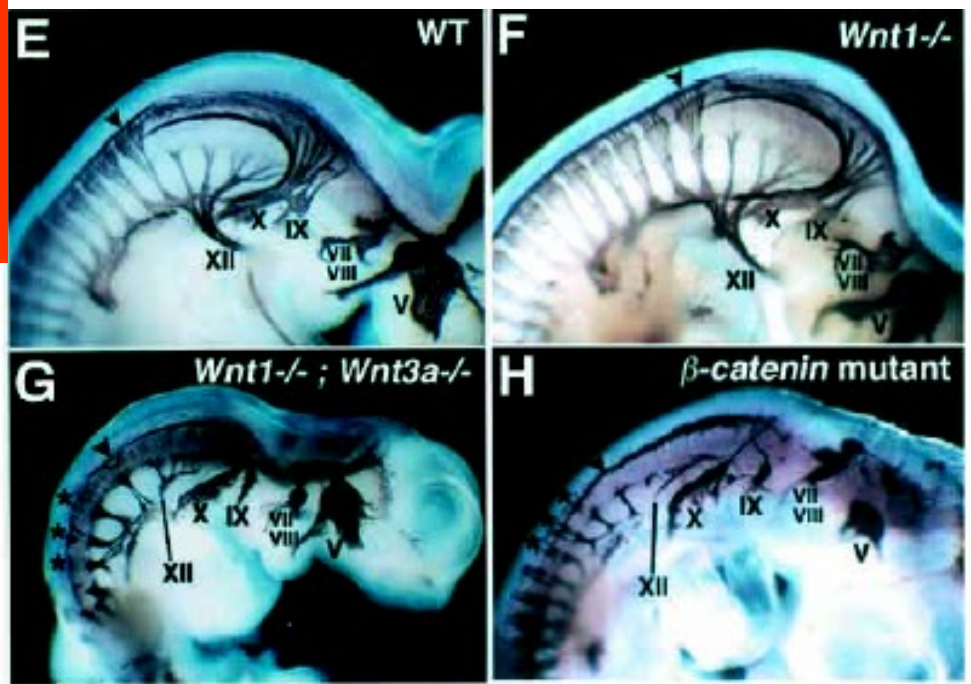
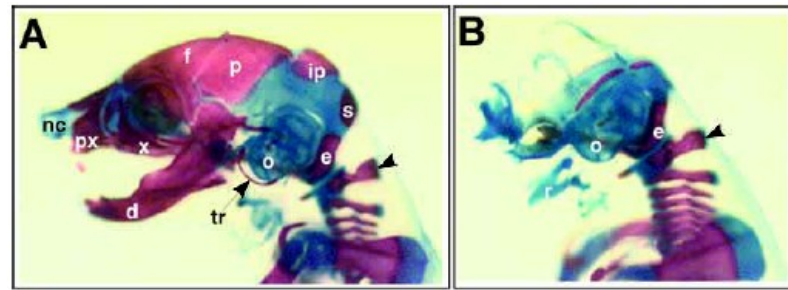
The neural crest (2. nd, 1999)

Wnt signalizace při vývoji neurální lišty



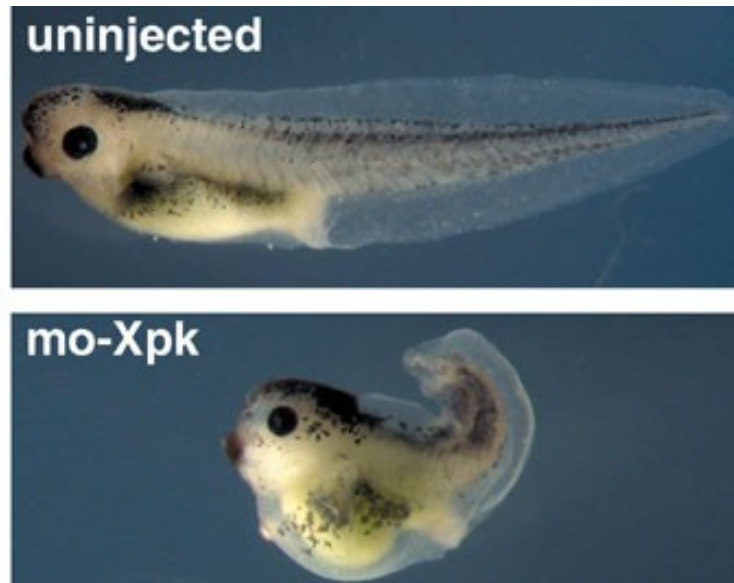
Fyziologie buň. systémů

Wnt1/3a DKO



Nekanonická Wnt dráha

- indukovaná např. ligandem Wnt5a

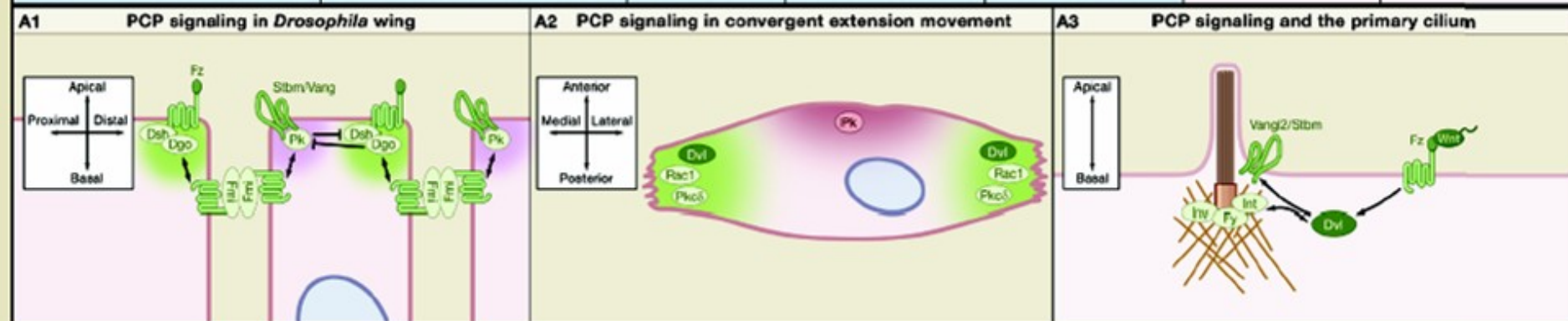
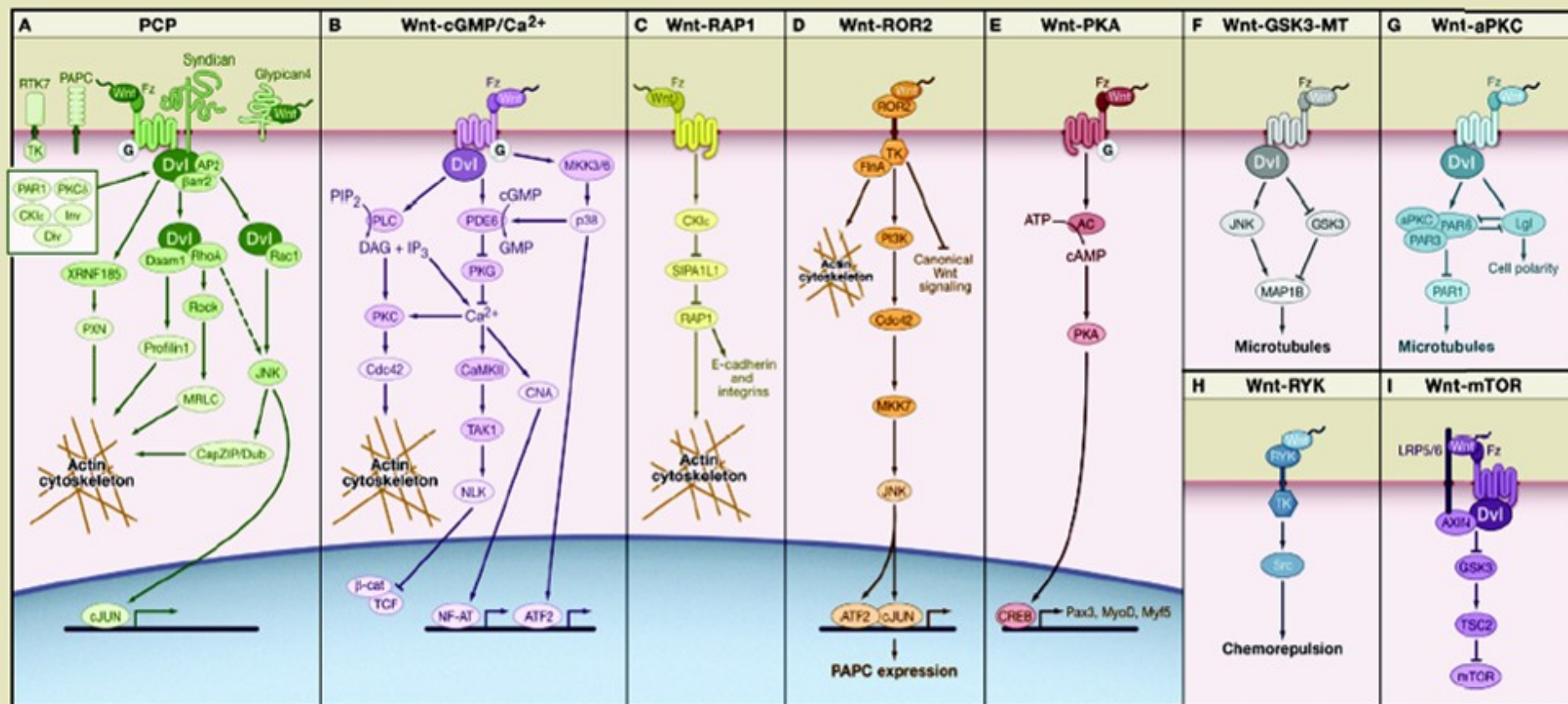


- neindukuje duplikaci tělní osy u *Xenopus*
- neindukuje transformaci buněčné linie odvozené od lidských prsních epiteliálních buněk C57mg
- signál NENÍ přenášen přes translokaci β -kateninu do jádra

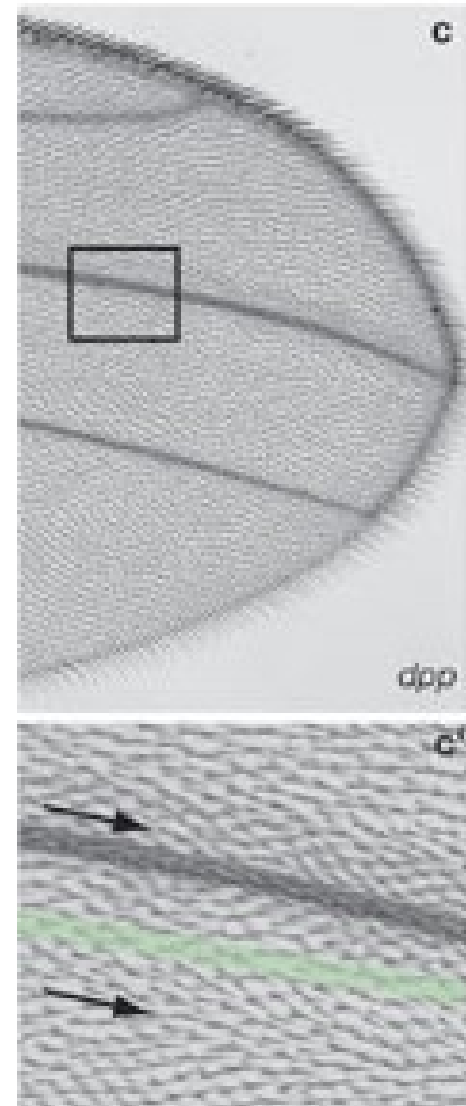
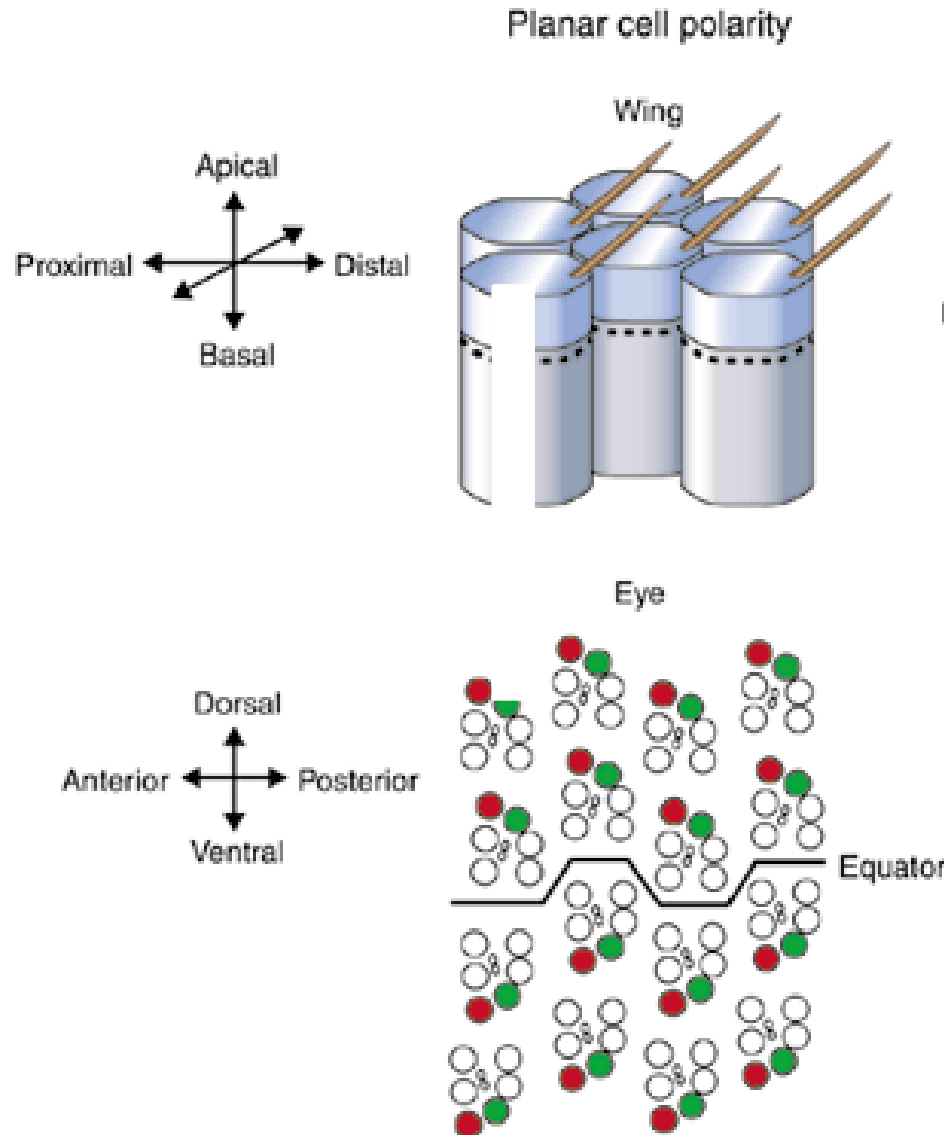
SnapShot: Noncanonical Wnt Signaling Pathways

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



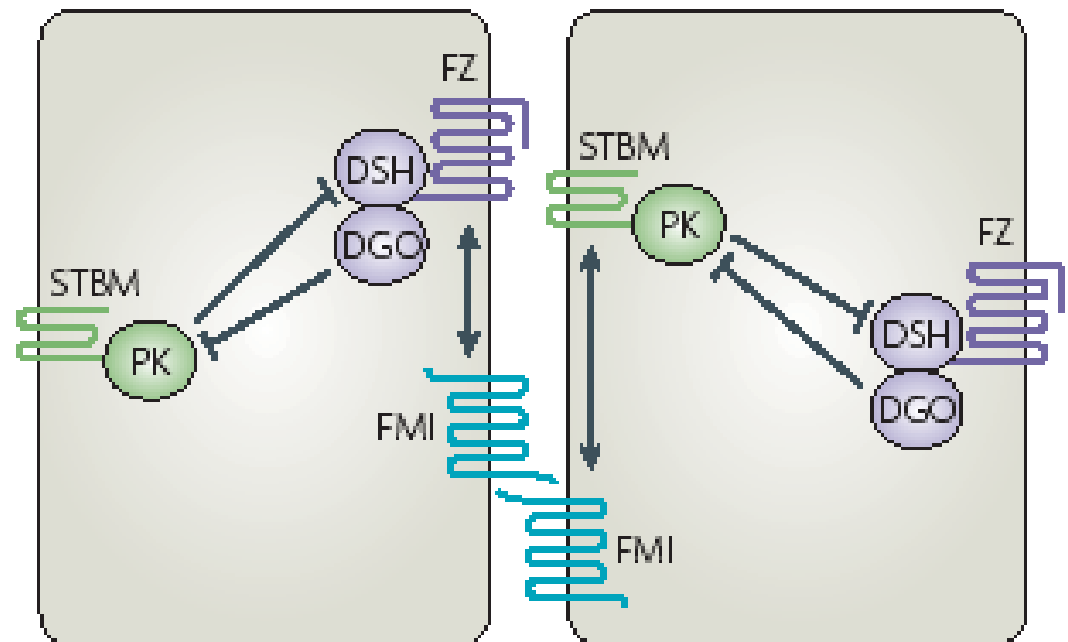
Drosophila – planární buněčná polarita (planar cell polarity, PCP)



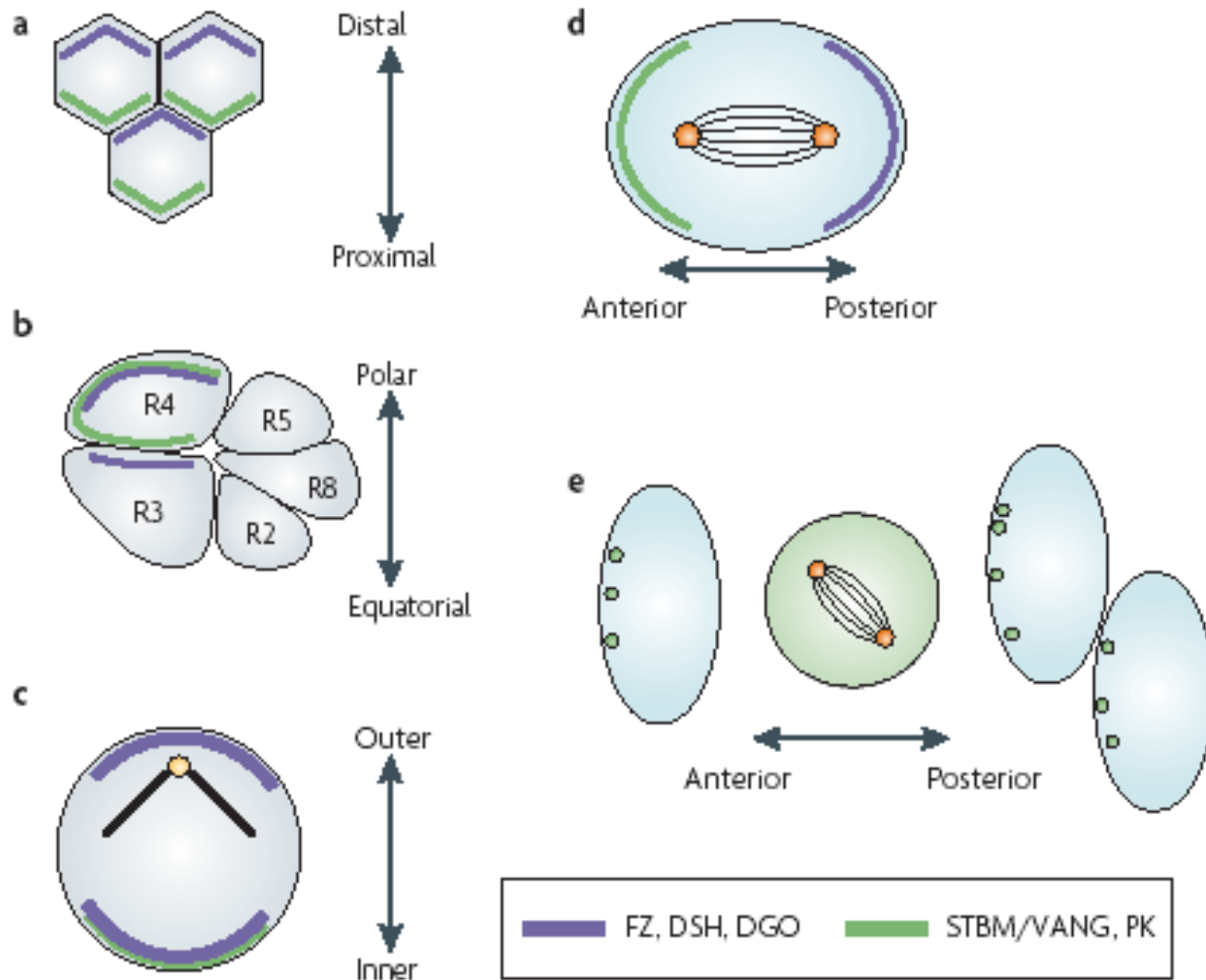
Molekulární mechanismus ustavení PCP

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.



Molekulární mechanismus ustavení PCP

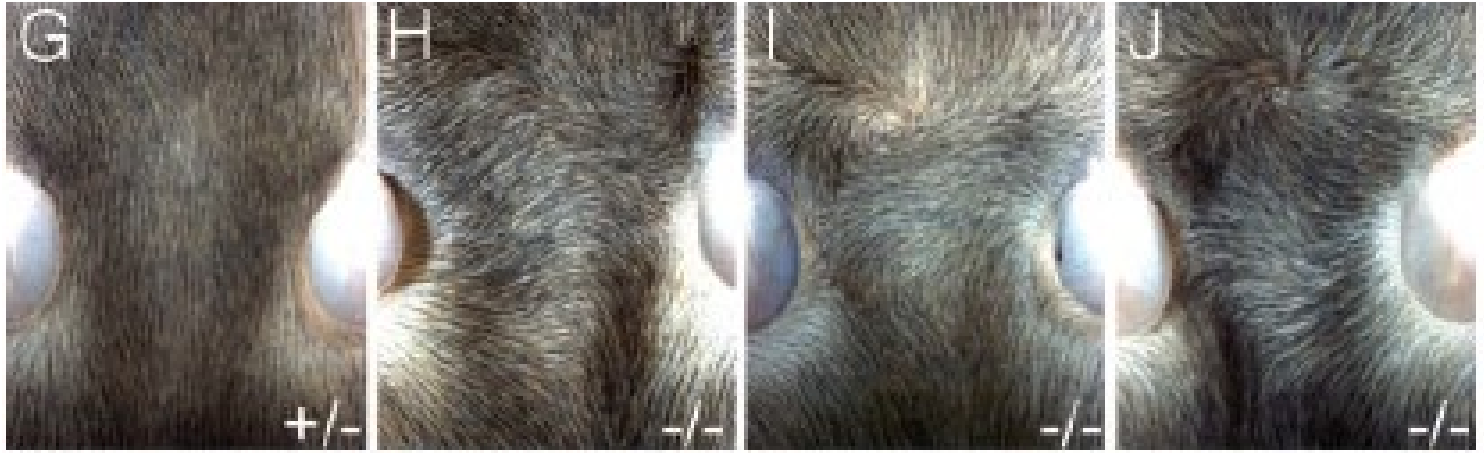


Molekulární mechanismus ustavení PCP

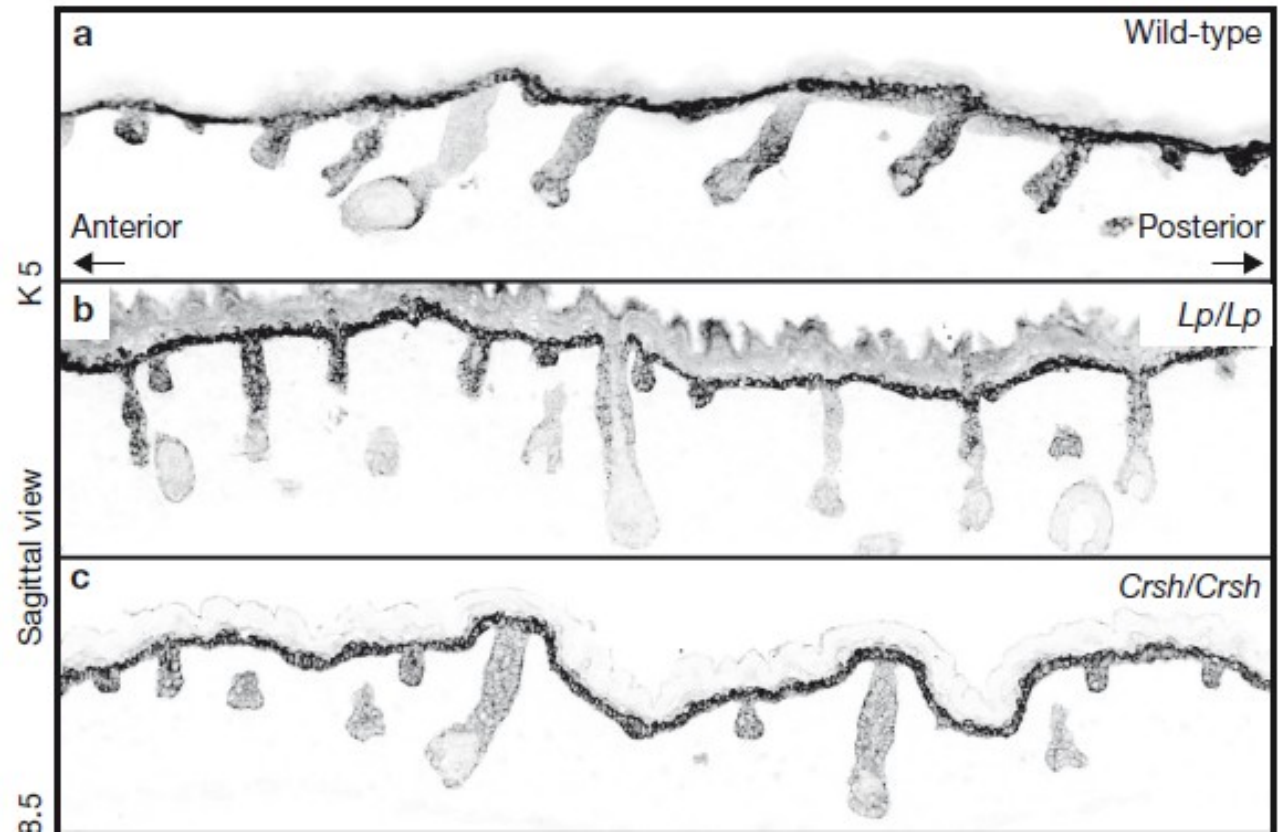
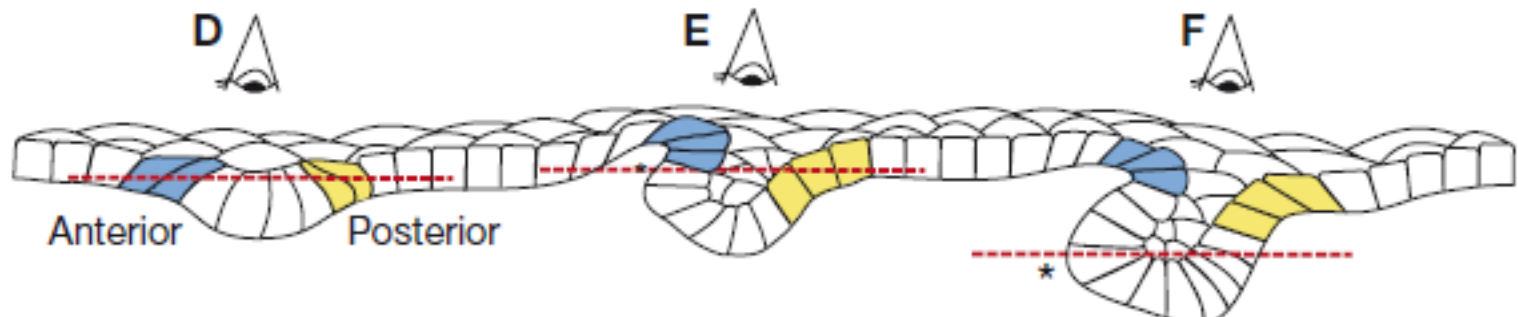
- legenda k obrázku:

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).

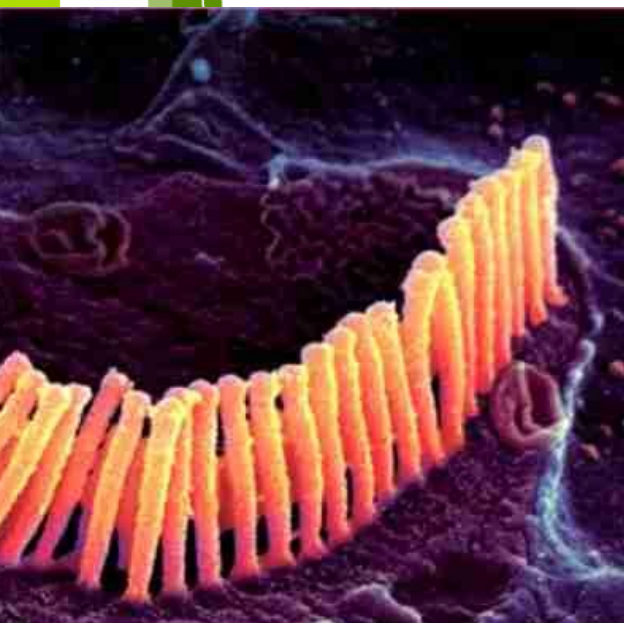
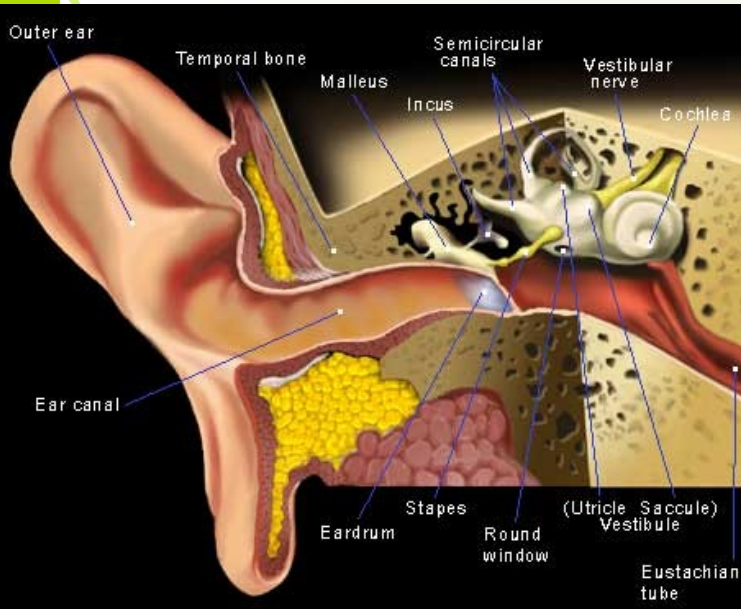
Poruchy v nekanonické signální dráze Wnt u savců



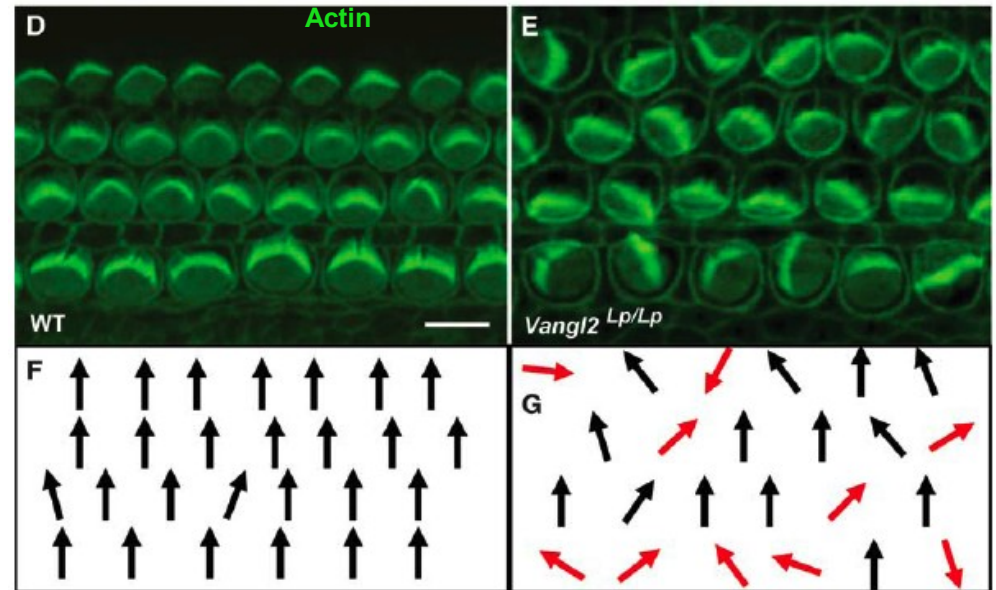
Poruchy v nekanonické signální dráze Wnt u savců



Nekanonická dráha/dráha PCP: fenotypy u myši

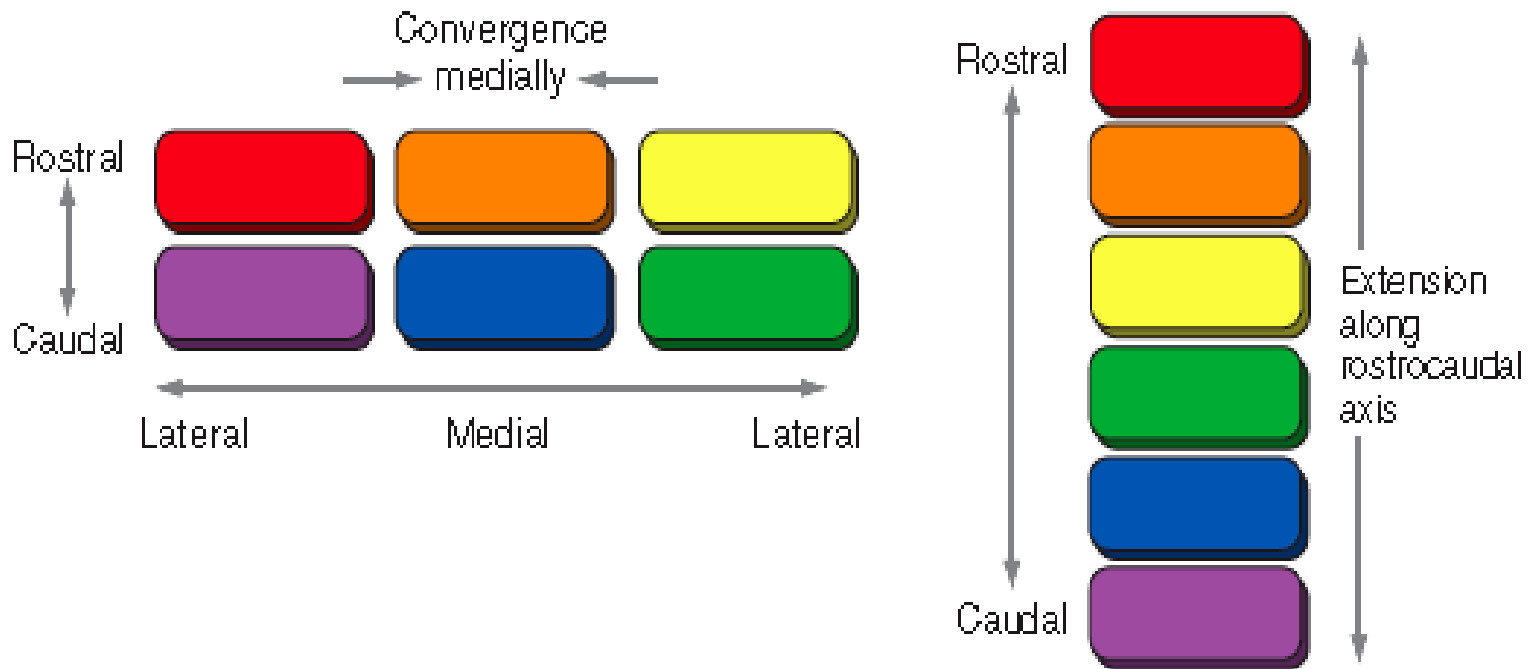


Orientace stereocilií vláskových buněk ve vnitřním uchu



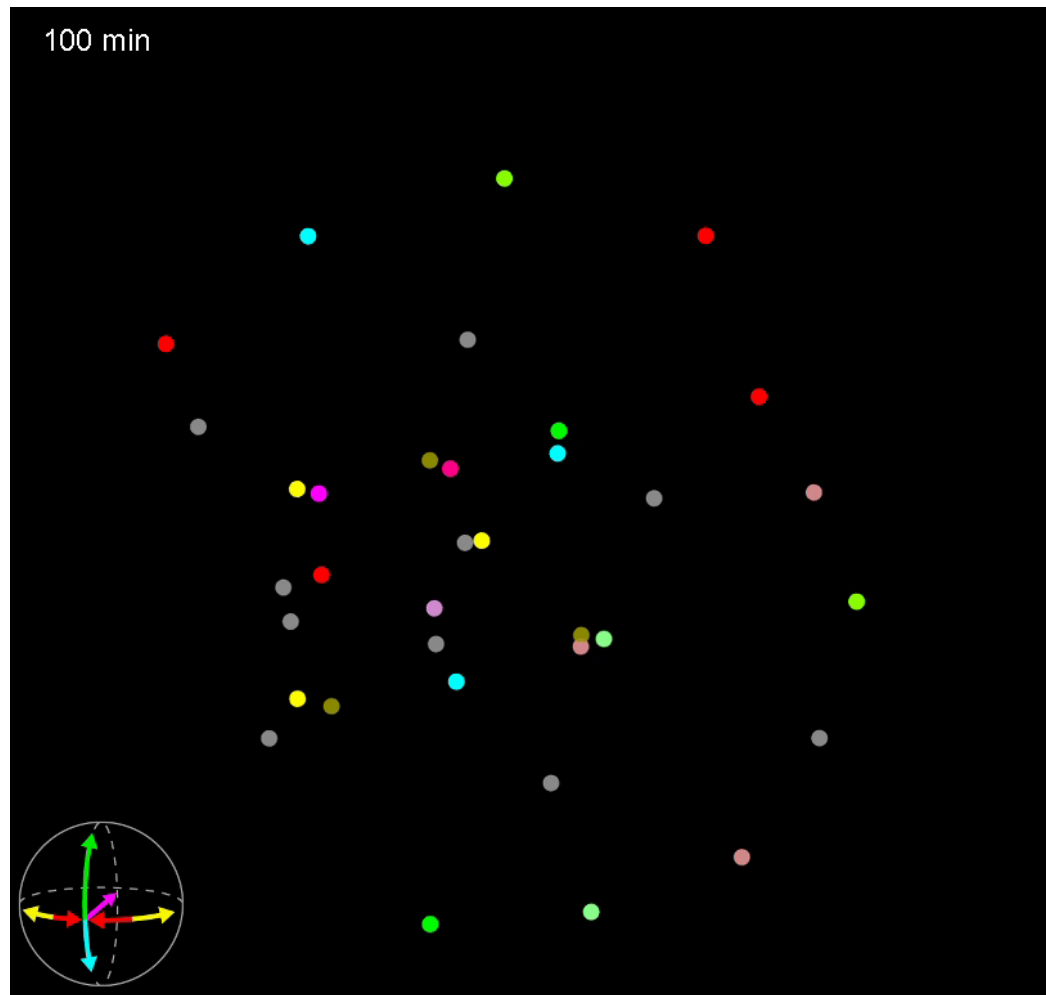
Qian et al., 2007, Dev. Biol.

Nekanonická dráha/dráha PCP při konvergentní extenzi u myši (a člověka)



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

Konvergentní extenze - video



Důsledky narušené konvergentní extenze (CE)



Principles of Development
Fifth Edition

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Axolotl neural tube closure

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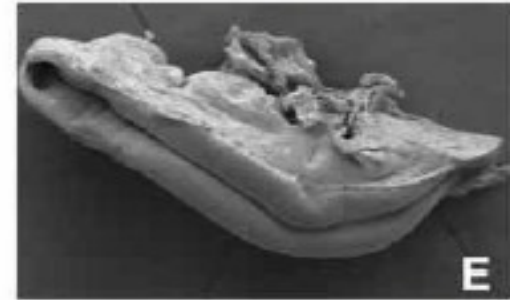


Důsledky narušené konvergentní extenze (CE)

Exencefalie:

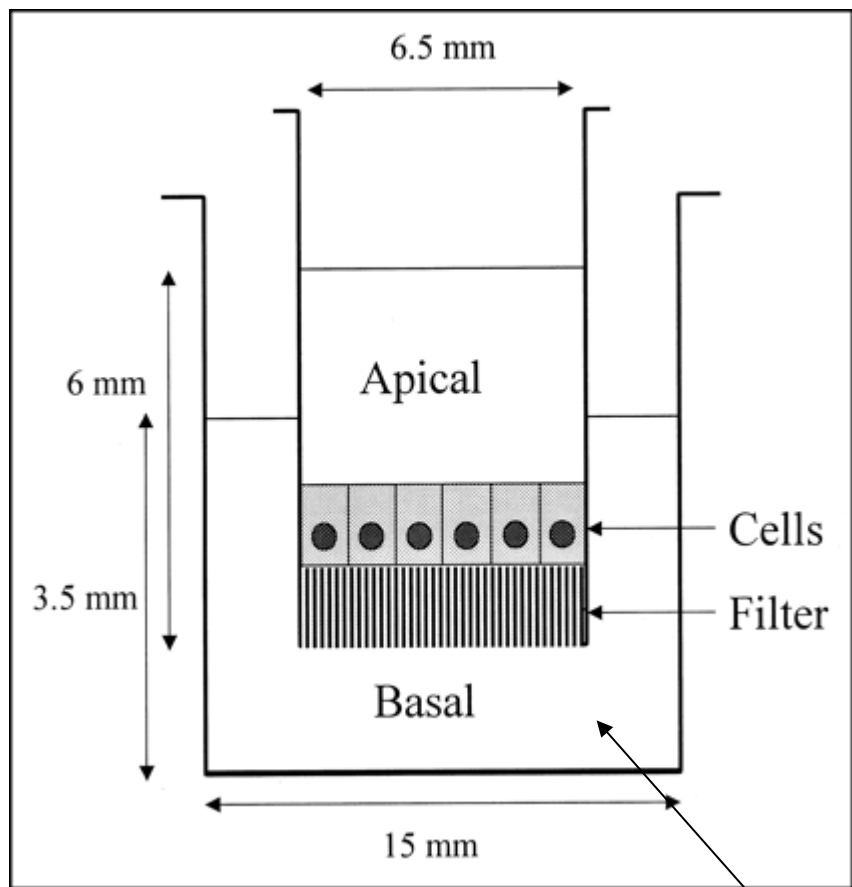


Otevřená nervová trubice:



Hamblet et al., 2002, Development

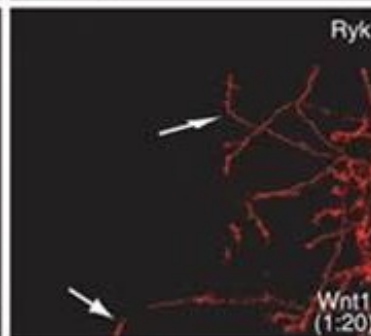
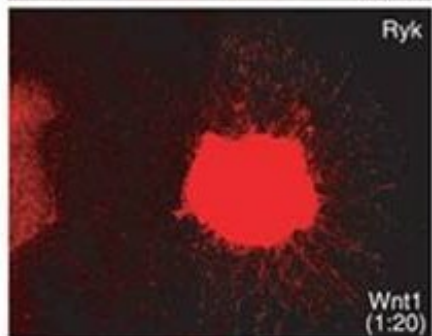
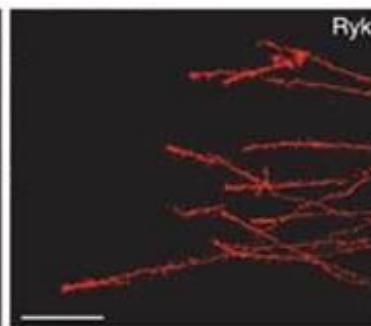
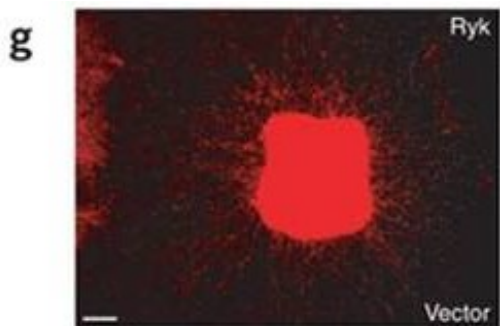
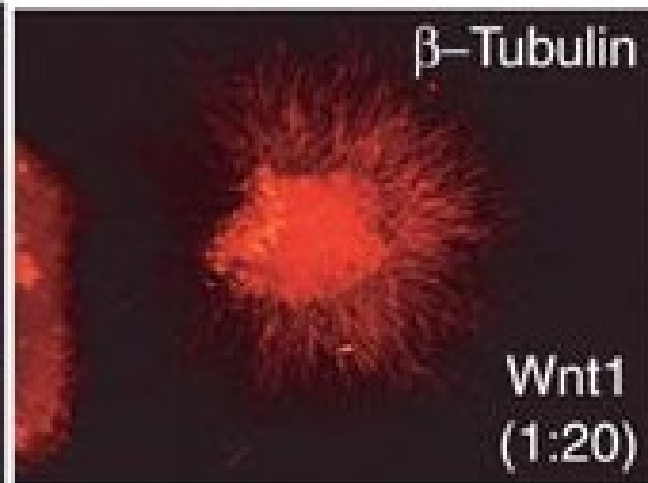
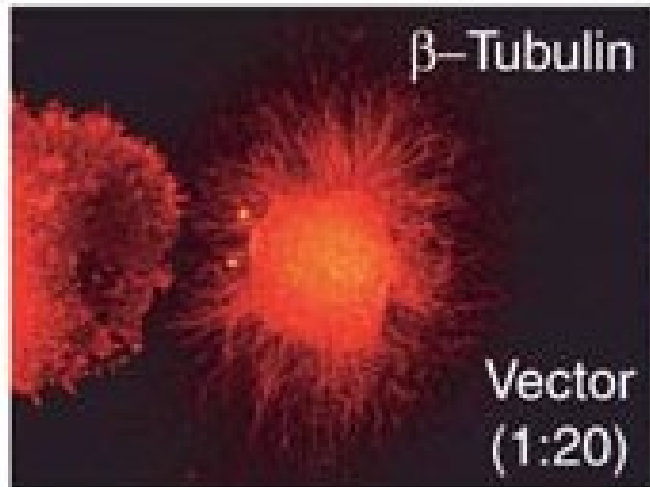
Možnosti studia migrace – polarizace a migrace: Transwell assays (savčí buňky)



Atraktant

Možnosti studia migrace a polarizace – attractant/repeleant assays

Attractant /
repeleant



Kmenové buňky

➤ Pro zájemce o tuto problematiku:

Dr. Jiří Pacherník: Bi7575 Fyziologie kmenových buněk

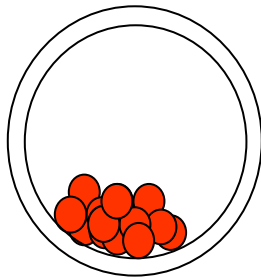
Kmenové buňky

a) sebeobnova (**selfrenewal**)

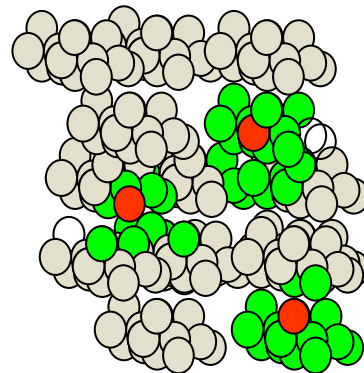
b) multipotence – schopnost dávat vznik dalším buněčným typům

- tkáňově specifické
- embryonální kmenové (ES)

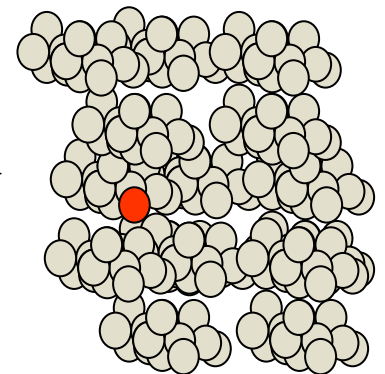
blastocysta



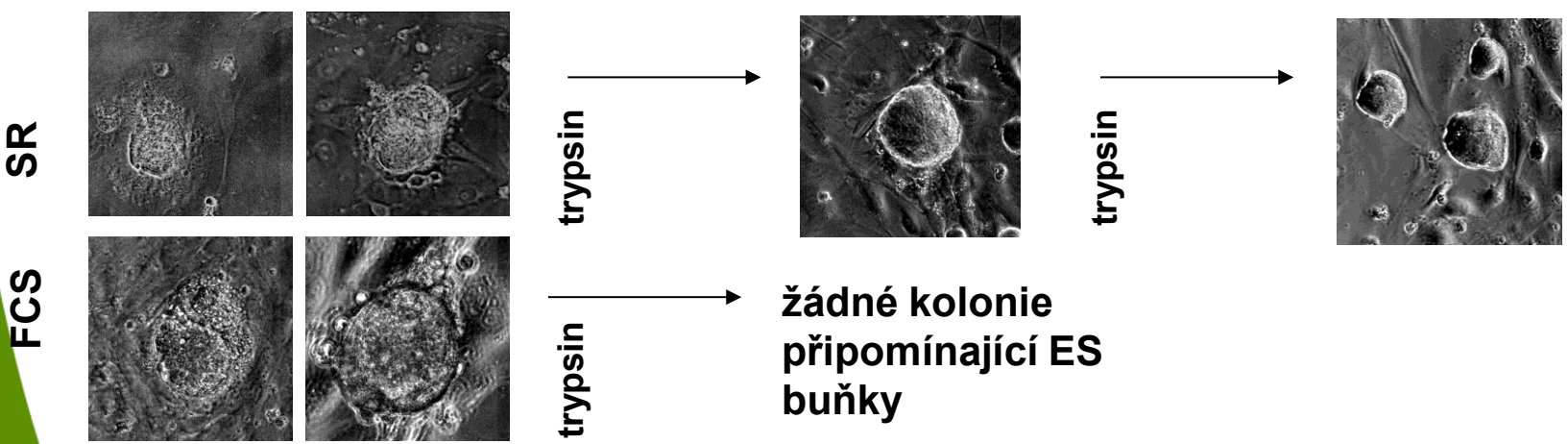
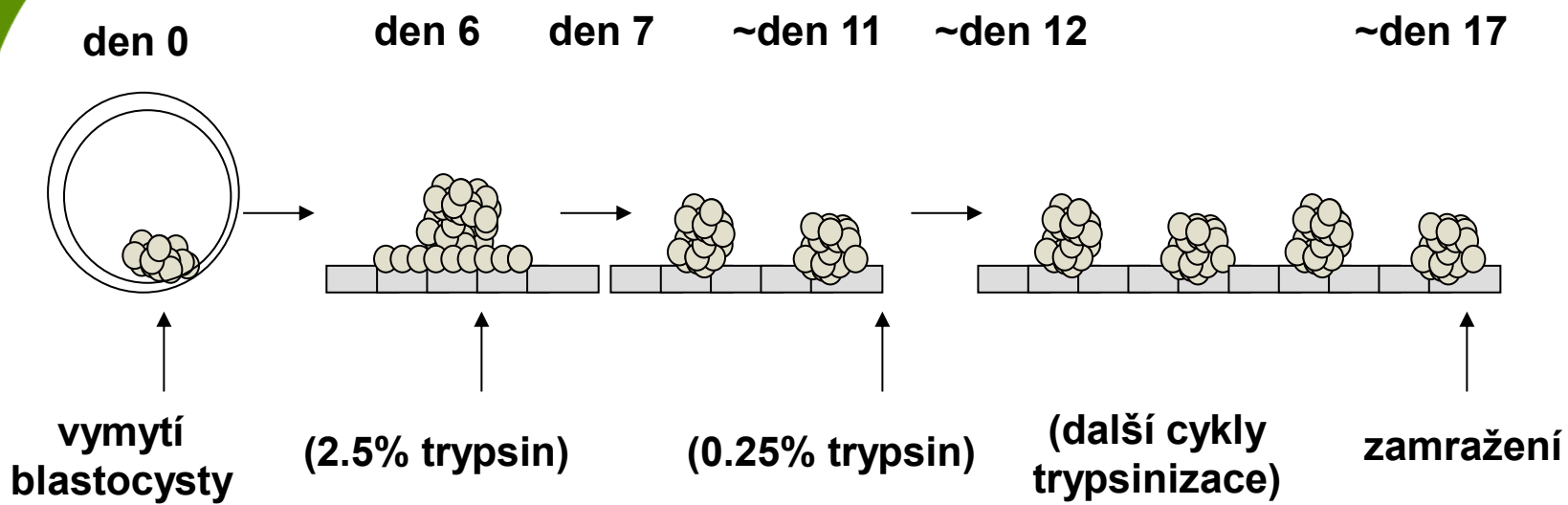
vyvíjející se tkáň (orgán)



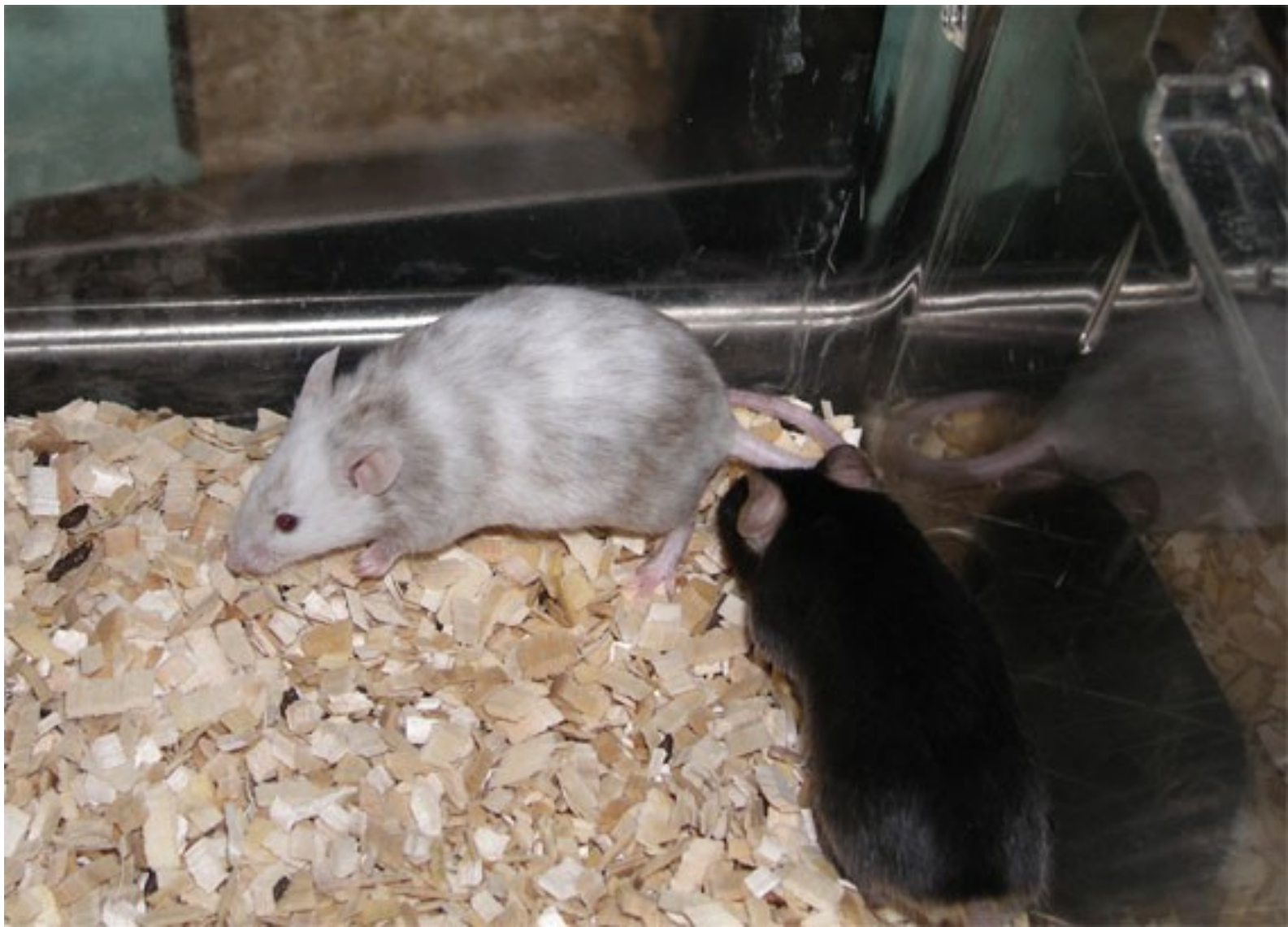
dospělá tkáň



Příprava myších embryonálních kmenových buněk:



Využití myších ES buněk pro transgenezi



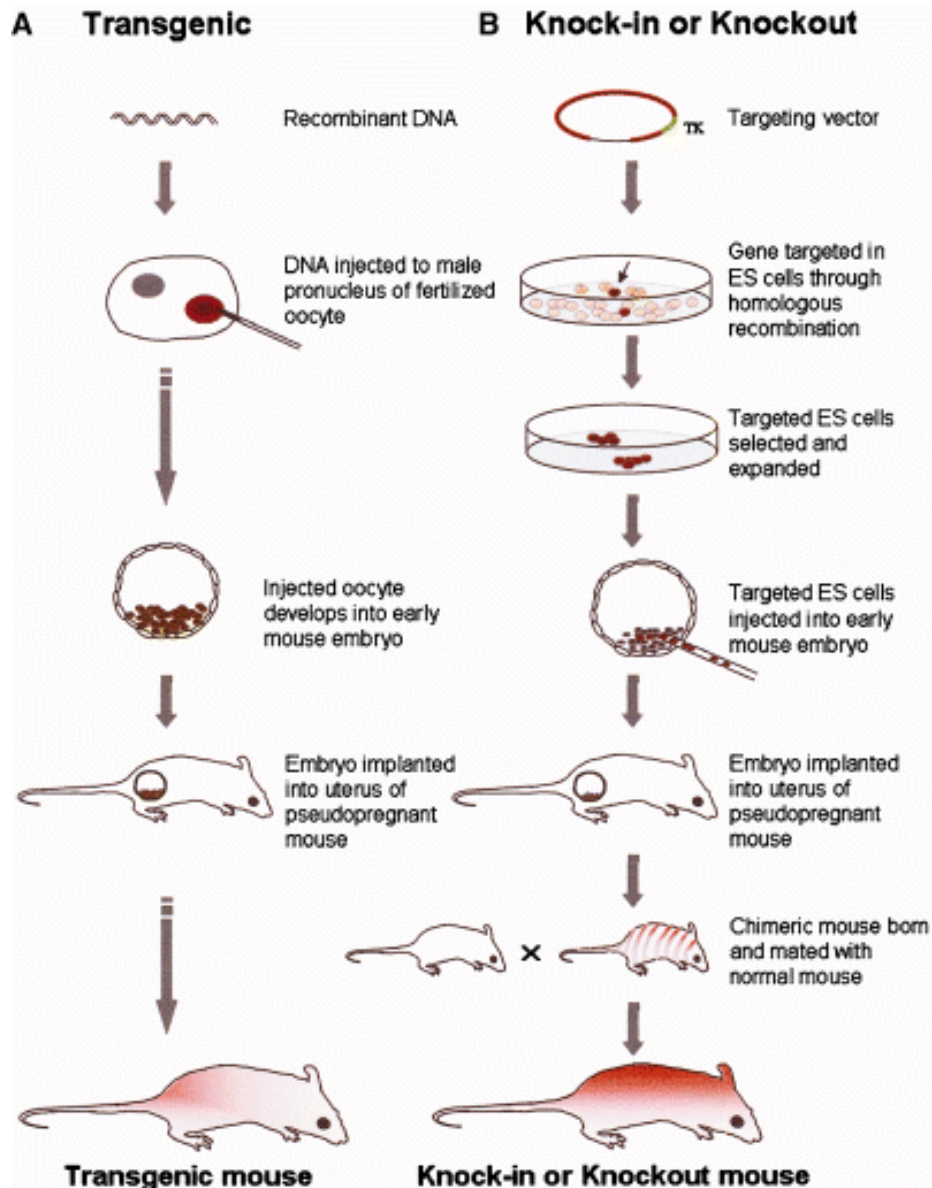
Využití myších ES buněk pro transgenezi

Nobelova cena 2007

**Mario R. Capecchi,
Martin J. Evans and
Oliver Smithies**

za

„principles for
introducing specific
gene modifications in
mice by the use of
embryonic stem cells“



REPORTS

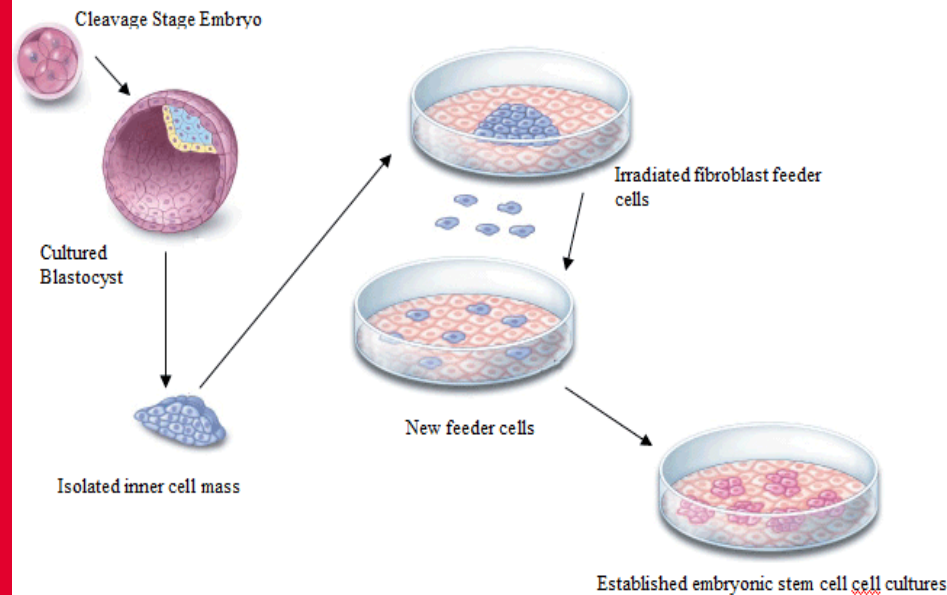
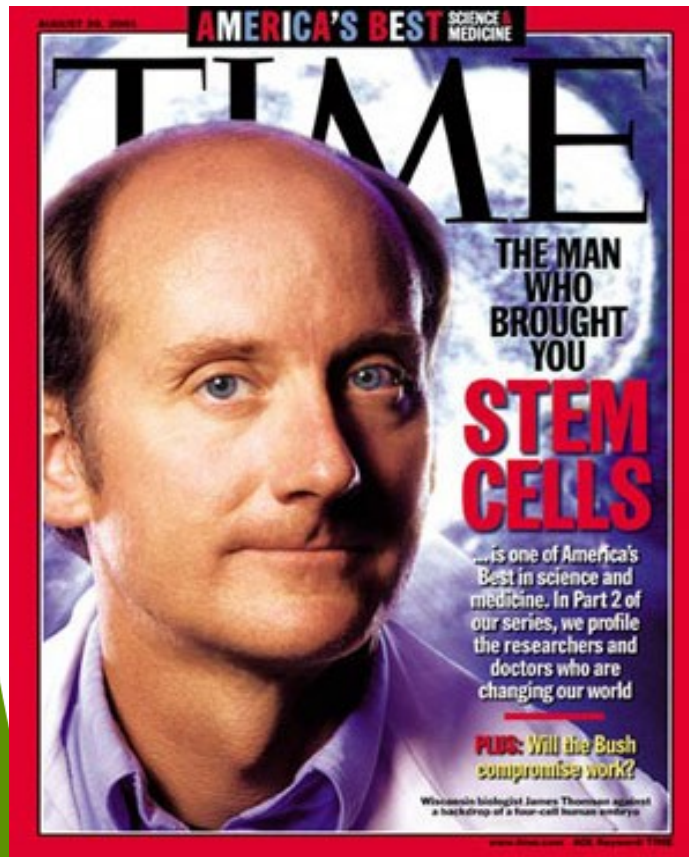
Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro,
Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall,
Jeffrey M. Jones

XX karyotype after 6 months of culture and has now been passaged continuously for more than 8 months (32 passages). A period of replicative crisis was not observed for any of the cell lines.

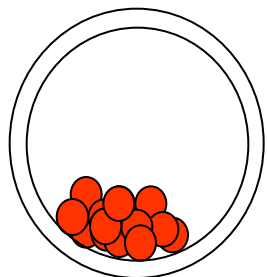
The human ES cell lines expressed high levels of telomerase activity (Fig. 2). Telomerase is a ribonucleoprotein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative lifespan (7, 8). Telomerase expression is

Science. 1998 Nov 6;282(5391):1145-7



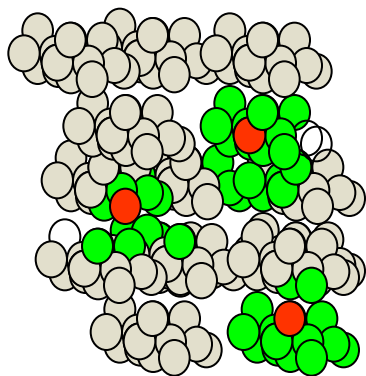
embryonální vývoj

In vivo

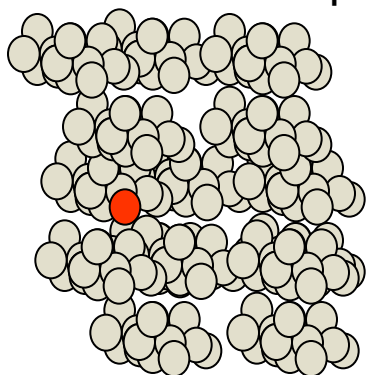


blastocysta

vyvíjející se tkáň (orgán)



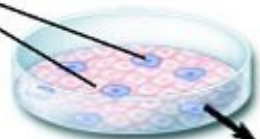
dospělá tkáň



In vitro



Inner cell mass of blastocyst



Undifferentiated embryonic stem cells



EMBRYOID BODIES

lIFSn medium (insulin/transferrin/fibronectin/selenium)

Adherent substrate

SELECTION OF NESTIN-POSITIVE CELLS

N2 medium/bFGF/aminin

N2 medium/bFGF/B27 media supplement

Expansion Phase

NESTIN-POSITIVE NEURONAL PRECURSOR CELLS

NESTIN-POSITIVE PANCREATIC PROGENITOR CELLS

Remove bFGF

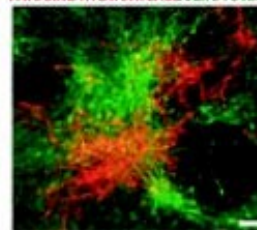
Remove bFGF
Add nicotinamide

Differentiation Phase

DOPAMINE- AND SEROTONIN-SECRETING NEURONS

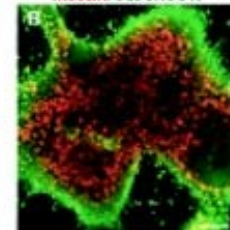
INSULIN-SECRETING PANCREATIC ISLET-LIKE CLUSTERS

TYROSINE HYDROXYLASE/SEROTONIN



Reproduced with permission from Nature Biotechnology

INSULIN/GLUCAGON



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**Kde jsou, jak vypadají a co kontroluje
tkáňové kmenové buňky?**

Uslyšíte příště

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

Kazutoshi Takahashi¹ and Shinya Yamanaka^{1,2,*}

¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

*Contact: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2006.07.024

SUMMARY

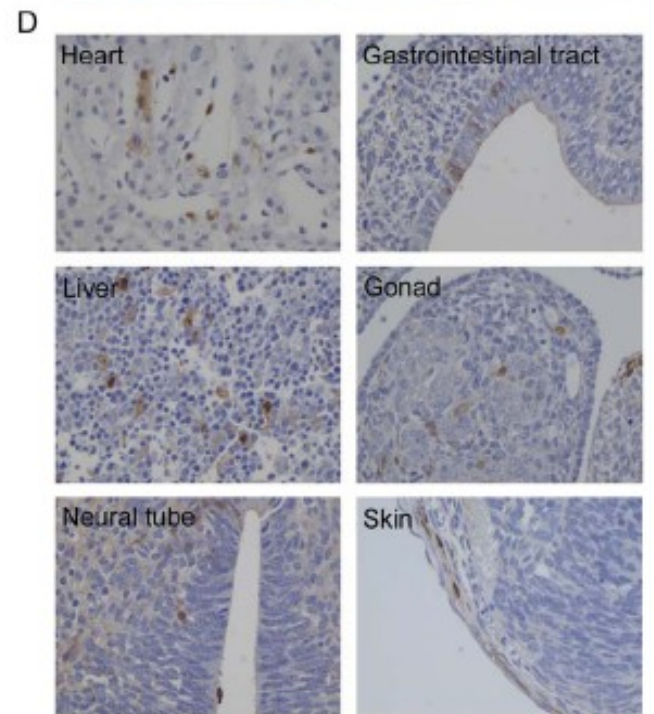
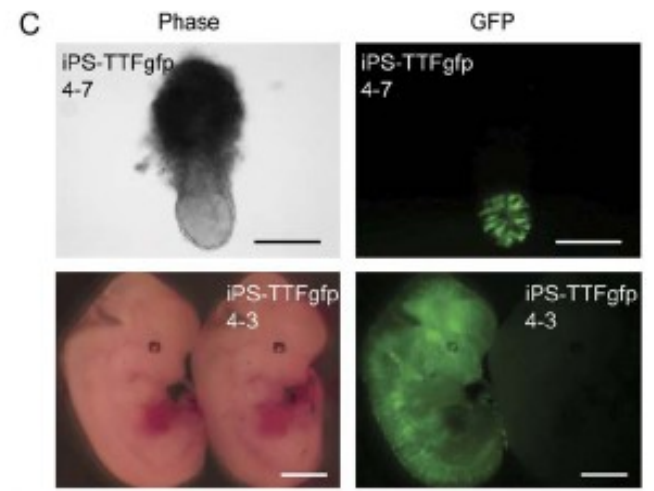
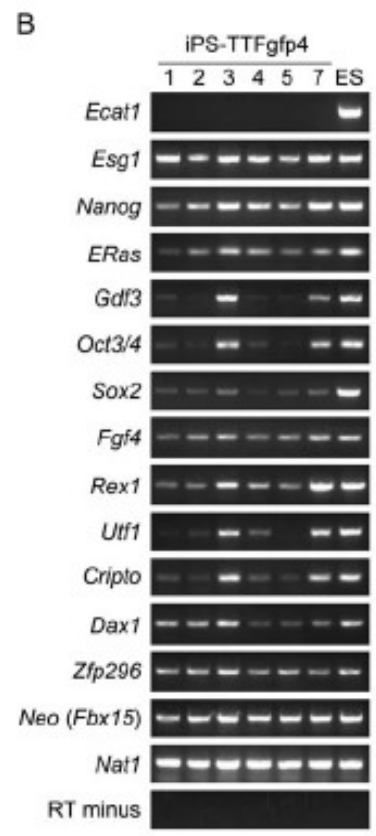
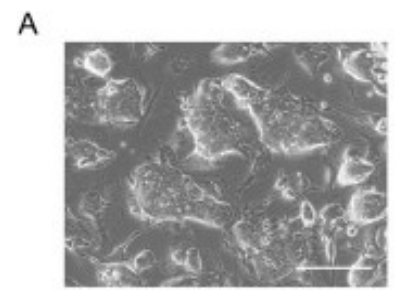
Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we dem-

onstrate that a limited number of transcription factors can induce pluripotency in somatic cells 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.

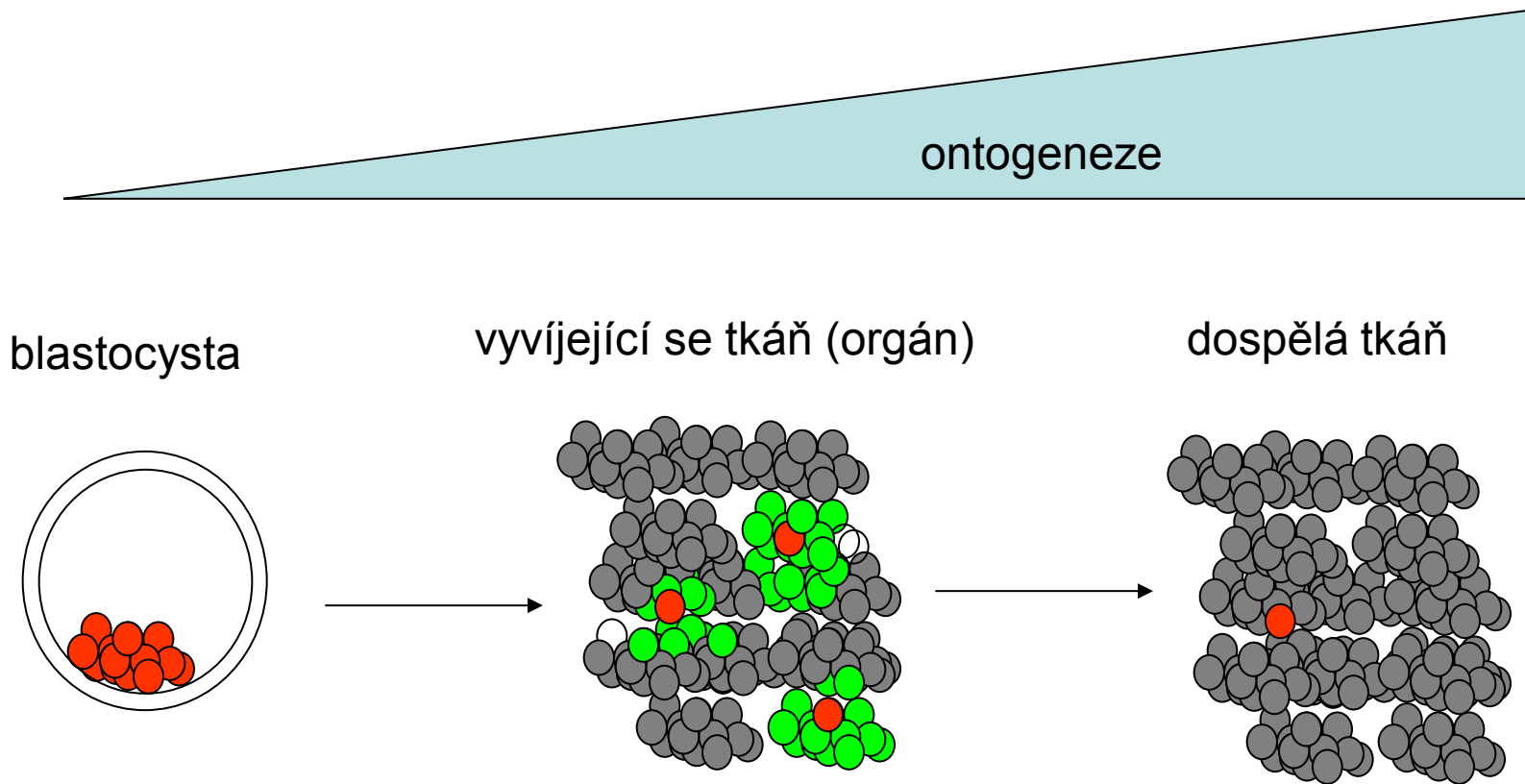
Indukované kmenové buňky - iPSCs



↓
 Oct3/4
 Sox2
 c-Myc
 Klf4
 →



Kmenové buňky



K čemu jsou, jak vypadají a jak jsou regulovány kmenové buňky v dospělém organismu?

K čemu jsou tkáňově specifické kmenové buňky?

- 1) K zajištění homeostázy
 - v lidském organismu běžně regenerují celé tkáně – např. vlasové kořínky (doba „života“ 3-4 roky), epitel střeva, epitel plic, krevní buňky nebo játra

- 2) K zajištění procesu hojení a regenerace

Časy – délka „života“ buněk

cell type	turnover time	BNID
small intestine epithelium	2–4 days	107812, 109231
stomach	2–9 days	101940
blood neutrophils	1–5 days	101940
white blood cells eosinophils	2–5 days	109901, 109902
gastrointestinal colon crypt cells	3–4 days	107812
cervix	6 days	110321
lungs alveoli	8 days	101940
tongue taste buds (rat)	10 days	111427
platelets	10 days	111407, 111408
bone osteoclasts	2 weeks	109906
intestine paneth cells	20 days	107812
skin epidermis cells	10–30 days	109214, 109215
pancreas beta cells (rat)	20–50 days	109228
blood B cells	1 month	111516
trachea	1–2 months	101940
hematopoietic stem cells	2 months	109232
sperm (male gametes)	2 months	110319, 110320
bone osteoblasts	3 months	109907
red blood cells	4 months	101706, 107875
liver hepatocyte cells	0.5–1 year	109233
fat cells	8 years	103455
cardiomyocytes	0.5–10% per year	107076, 107077, 107078
central nervous system	life-time	101940
skeleton	10% per year	109908
lens cells	life-time	109840
oocytes (female gametes)	life-time	111451

Jak zjistit délku „života“ buněk/intenzitu obnovy tkání?

Využití přechodného zvýšení ^{14}C z dob studené války
(Jonas Frisén, Karolinska)

Nervové buňky mozkové kůry

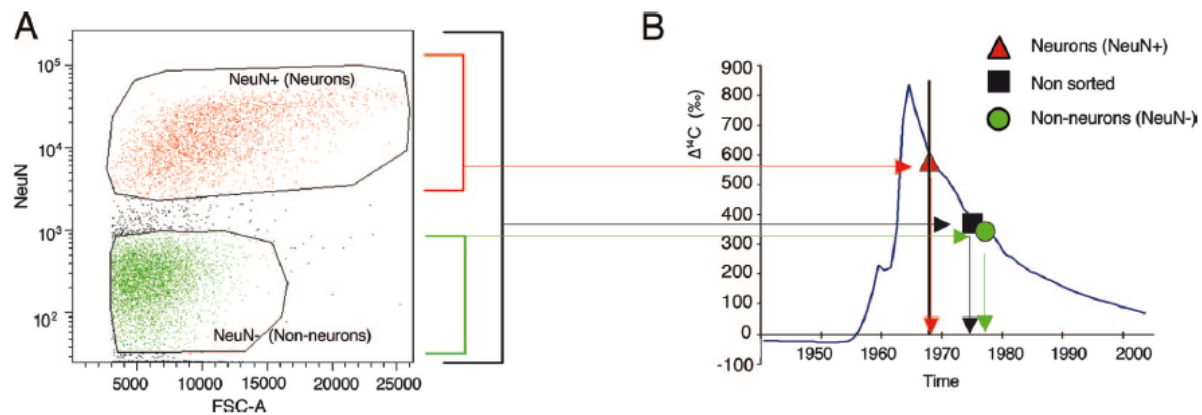


Fig. 1. Determination of the age of neocortical neurons. (A) Neuronal (NeuN-positive) and nonneuronal (NeuN-negative) cell nuclei from the adult human cerebral neocortex were separated and isolated by flow cytometry. (B) The levels of ^{14}C in the atmosphere have been stable over long time periods, with the exception of a large addition of ^{14}C in 1955–1963 as a result of nuclear weapons tests (blue line, data from ref. 26), making it possible to infer the time of birth of cell populations by relating the level of ^{14}C in DNA to that in the atmosphere (horizontal arrows) and reading the age off the x axis (vertical arrows). The average age of all cells in the prefrontal cortex is younger than the individual (black arrows), indicating cell turnover. Dating of nonneuronal cells demonstrates they are younger, whereas neurons are approximately as old as the individual. The vertical bar indicates the year of birth of the individual. ^{14}C levels from modern samples are, by convention, given in relation to a universal standard and corrected for radioactive decay, giving the $\Delta^{14}\text{C}$ value (50).

Regenerace srdce:

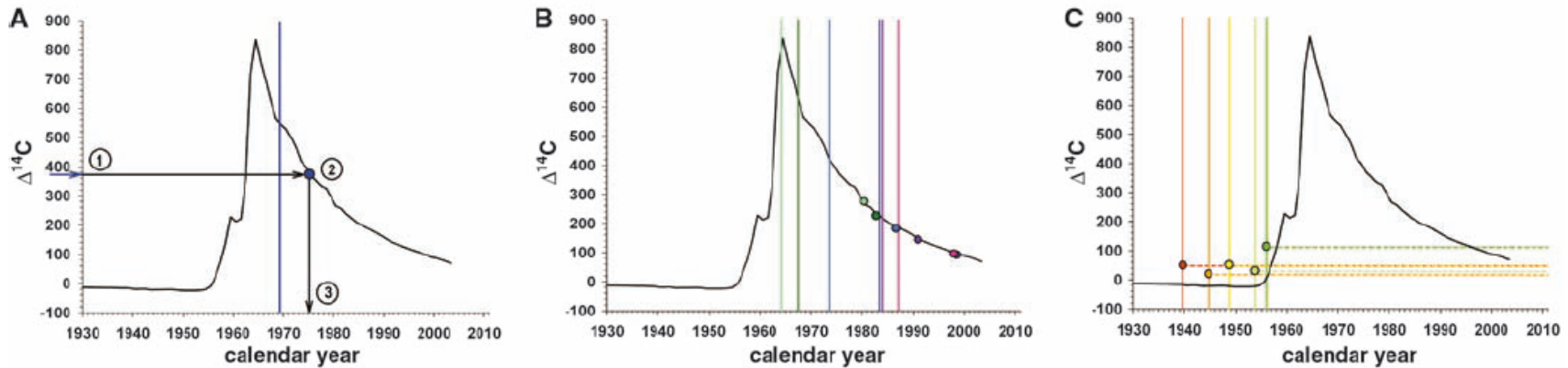
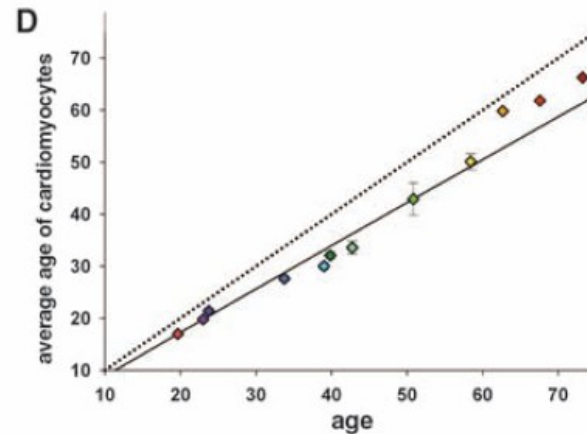
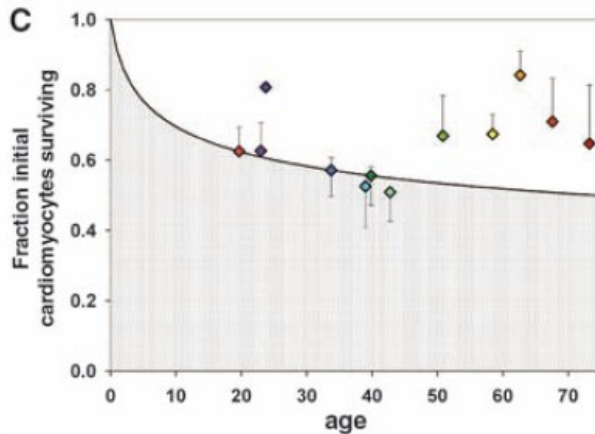
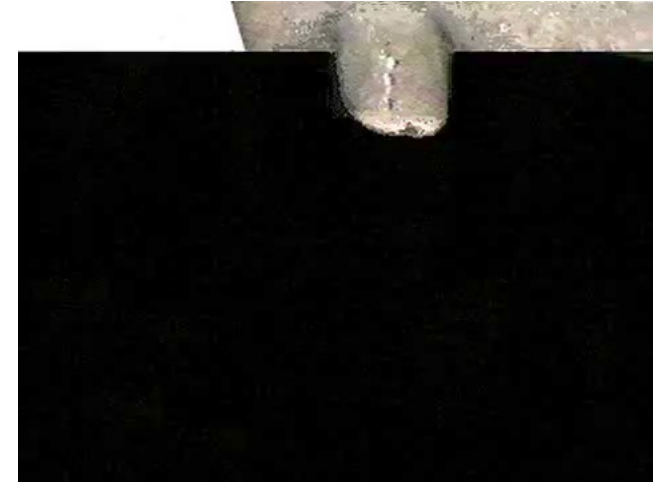
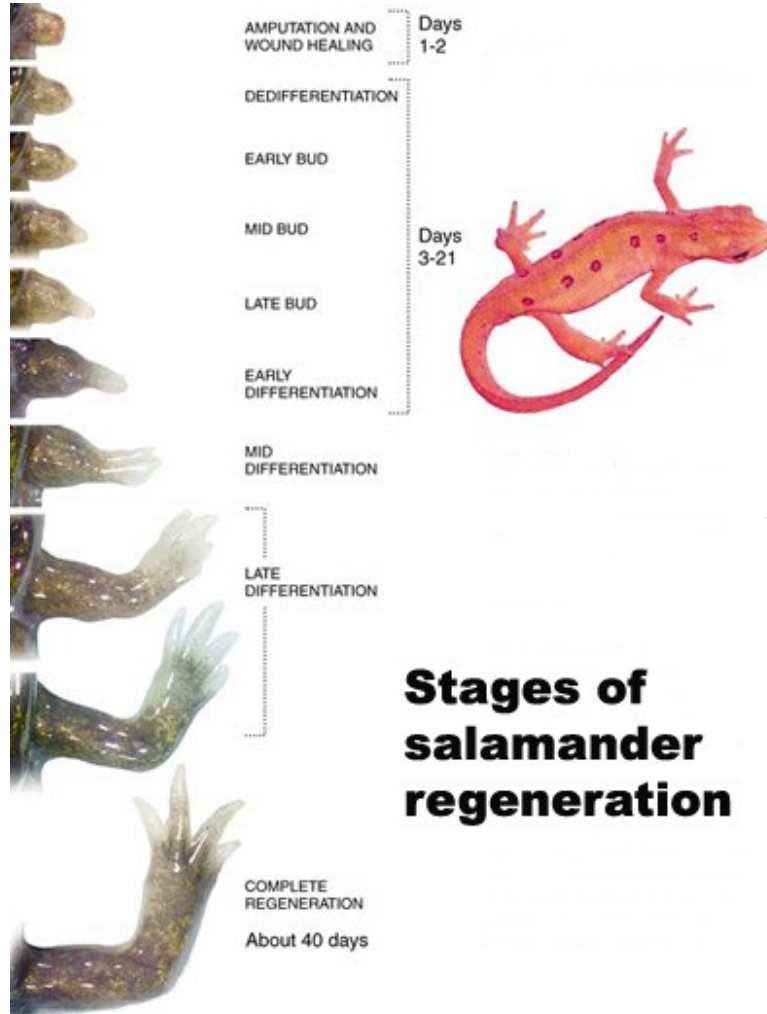


Fig. 1. Cell turnover in the heart. **(A)** Schematic figure demonstrating the strategy to establish cell age by ^{14}C dating. The black curve in all graphs shows the atmospheric concentrations of ^{14}C over the decades since 1930 [data from (14)]. The vertical bar indicates the date of birth of the individual. The measured ^{14}C concentration (1) is related to the atmospheric ^{14}C concentration by use of the established atmospheric ^{14}C bomb curve (2). The average birth date of the population can be inferred by determining where the data point intersects the x axis (3). ^{14}C concentrations in DNA of cells from the left ventricle myocardium in

individuals born after **(B)** or before **(C)** the nuclear bomb tests correspond to time points substantially after the time of birth, indicating postnatal cell turnover. The vertical bar indicates the date of birth of each individual, and the similarly colored dots represent the ^{14}C data for the same individual. For individuals born before the increase in ^{14}C concentrations, it is not possible to directly infer an age because the measured concentration can be a result of ^{14}C incorporation during the rising and/or falling part of the atmospheric curve, and thus the concentration is indicated by a dotted horizontal line.



Schopnosti regenerace se liší mezi jednotlivými organismy

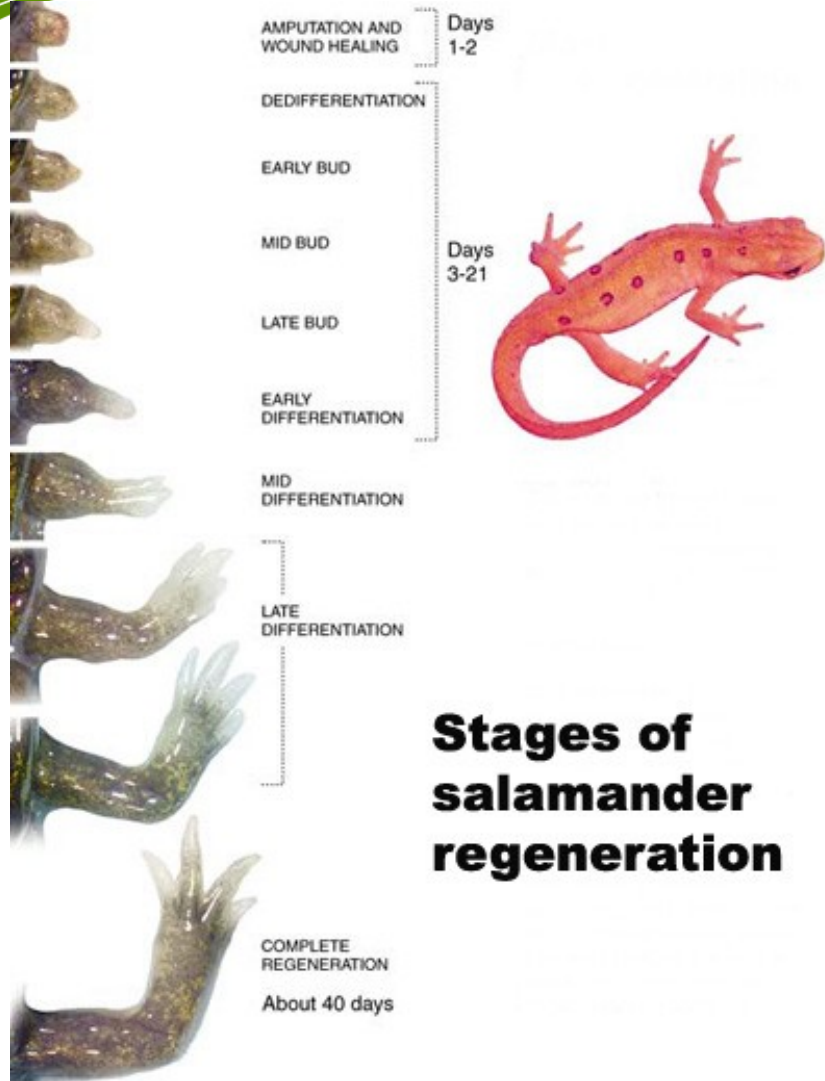


JMG
reptile™

“GECKO TAIL REGENERATION”

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Schopnosti regenerace se liší mezi jednotlivými organismy



po amputaci:



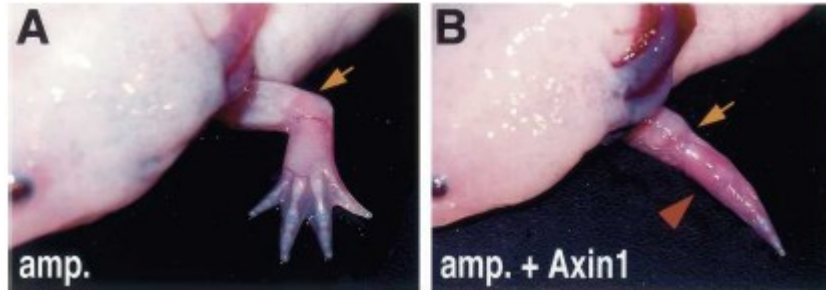
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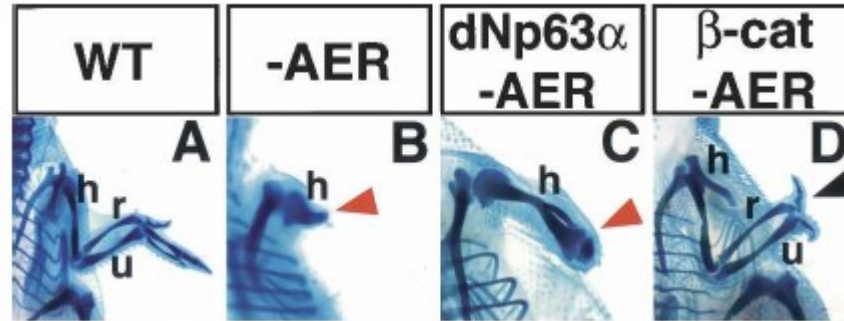
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Morfogenetické sign. dráhy (kanonická Wnt signalizace, Hedgehog, TGF, Notch) jsou nezbytné pro regeneraci u řady mnohobuněčných organismů

axolotl

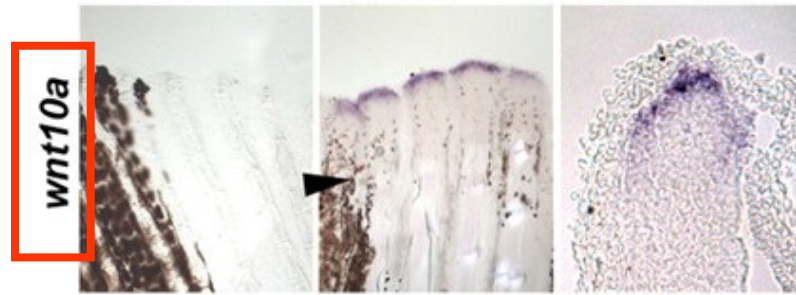
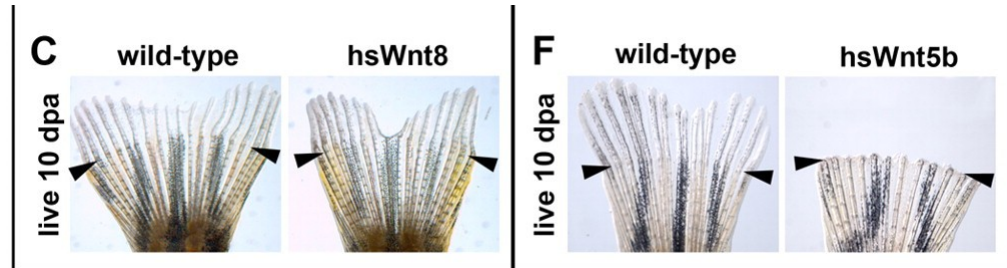


chick

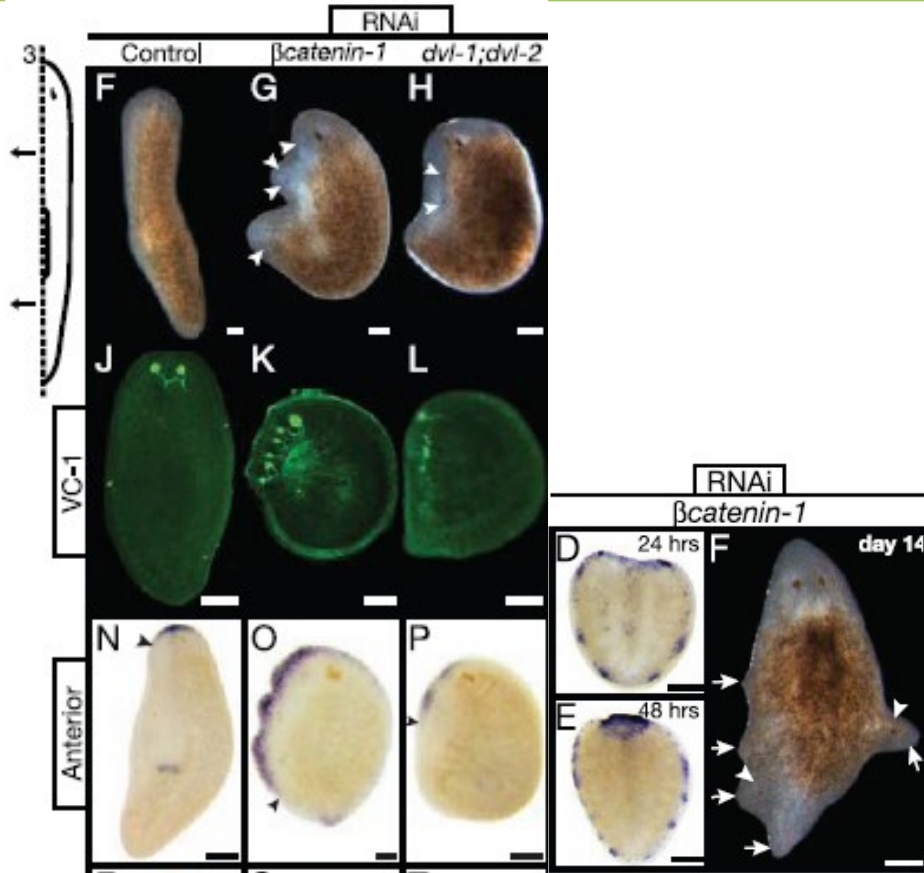
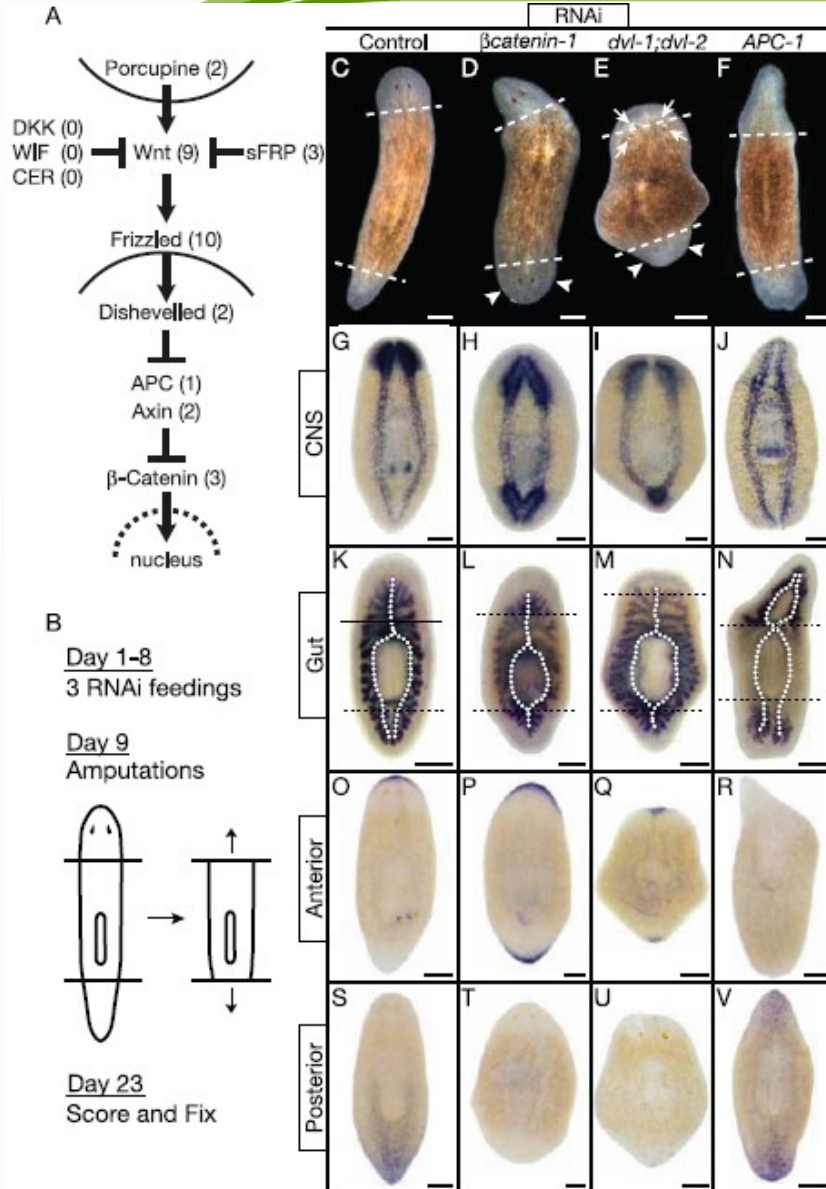


Morfogenetické sign. dráhy (kanonická Wnt signalizace, Hedgehog, TGF, Notch) jsou nezbytné pro regeneraci u řady mnohobuněčných organismů

zebrafish



... Regenerace pod kontrolou Wnt signální dráhy u ploštěnky



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