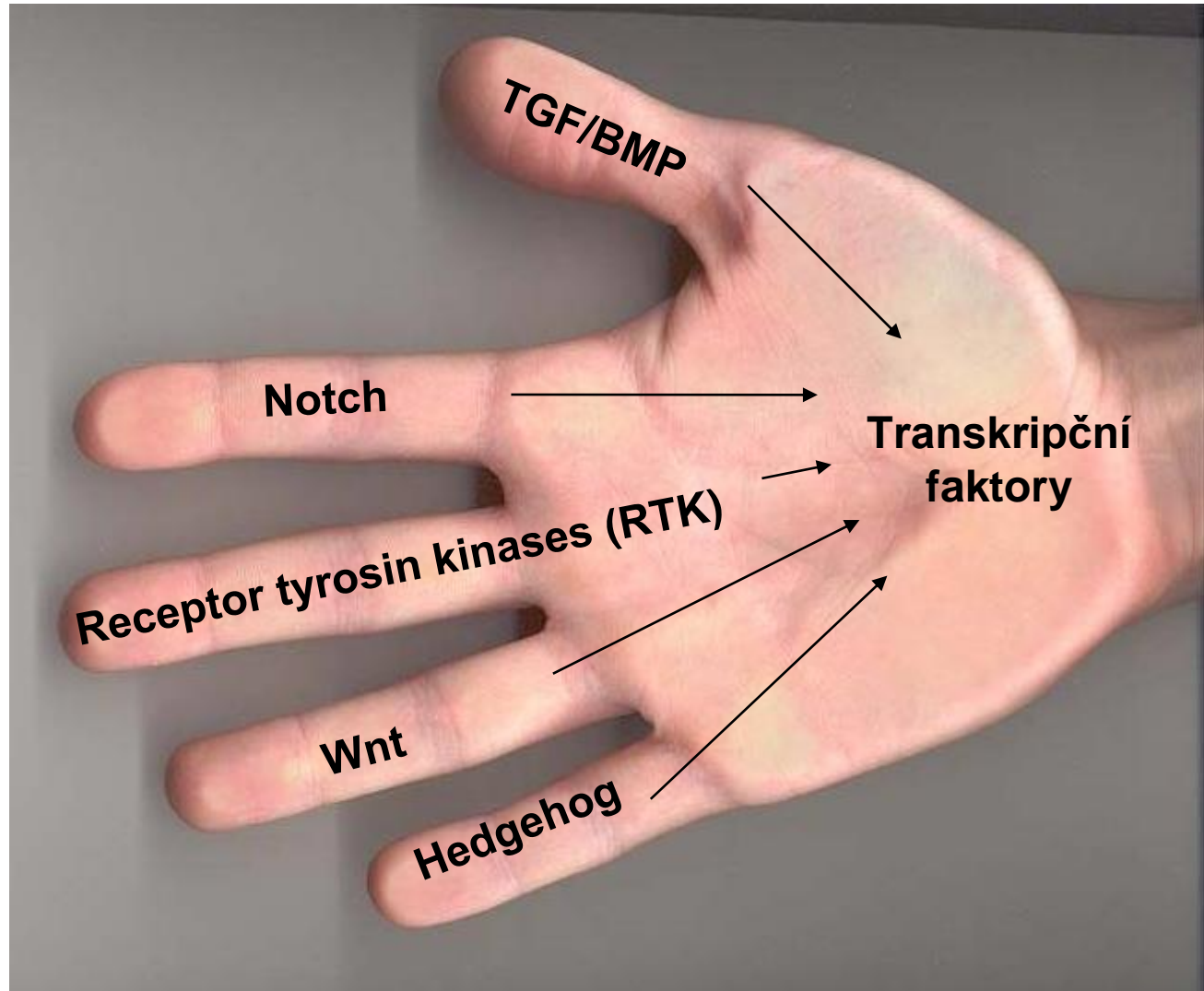


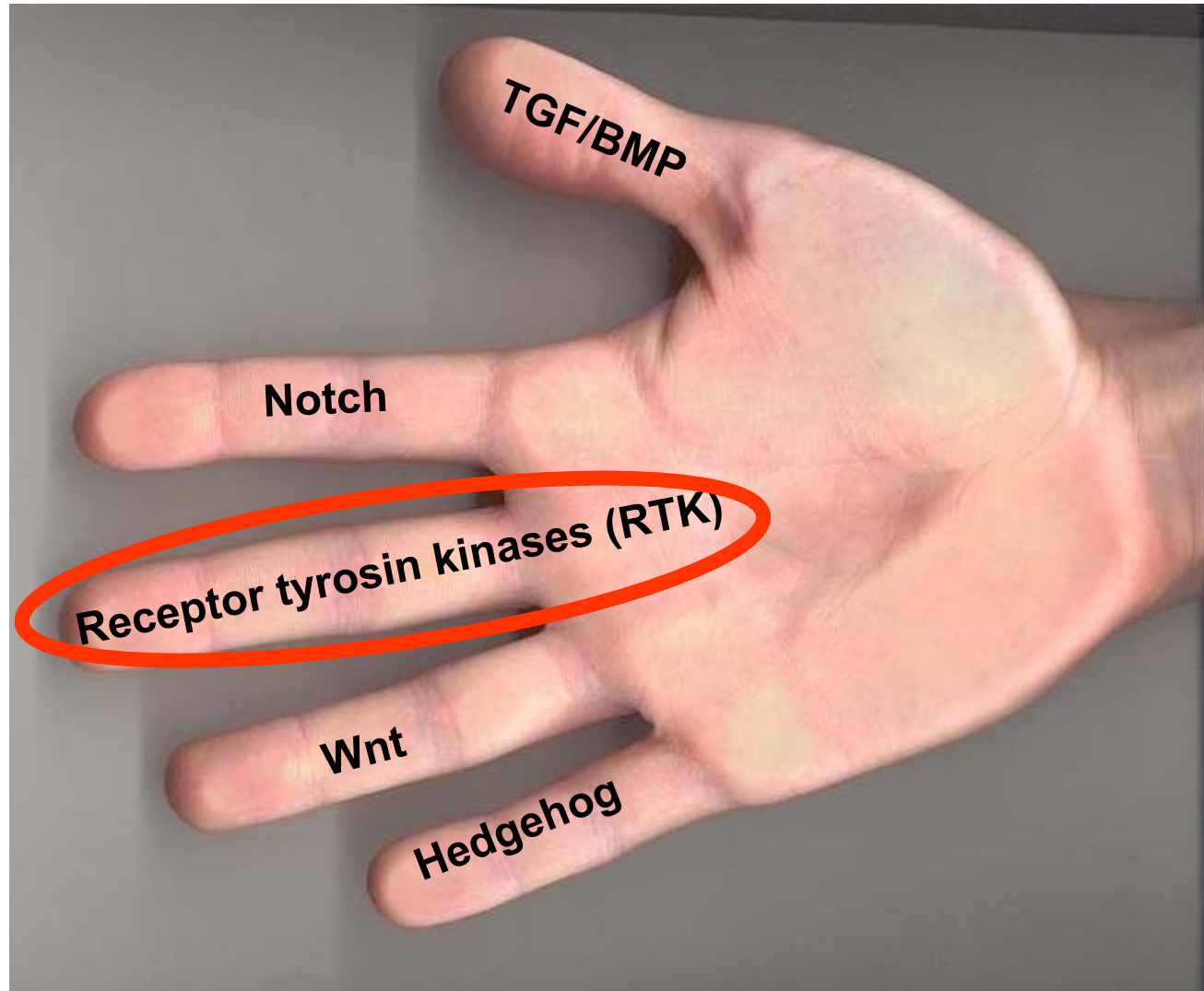
**Buněčné systémy ve vývoji:
Receptorové tyrosin kinázy, hypoxie a
Wnt signální dráha**

doc. Mgr. Vítězslav Bryja, Ph.D.

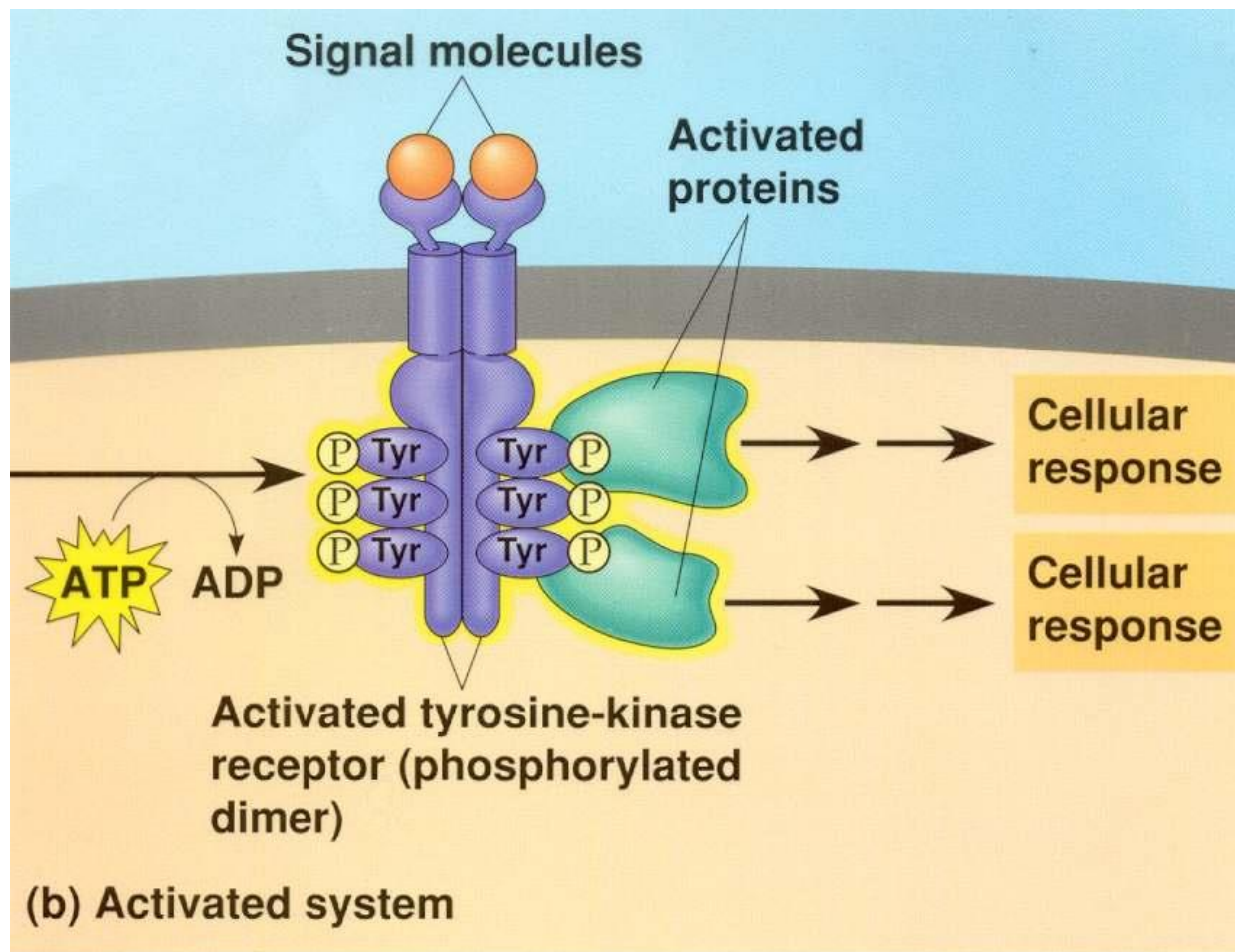
Klíčové molekulární komponenty vývoje



Klíčové molekulární komponenty vývoje



Receptorové tyrosin kinázy (RTK)



Receptorové tyrosin kinázy

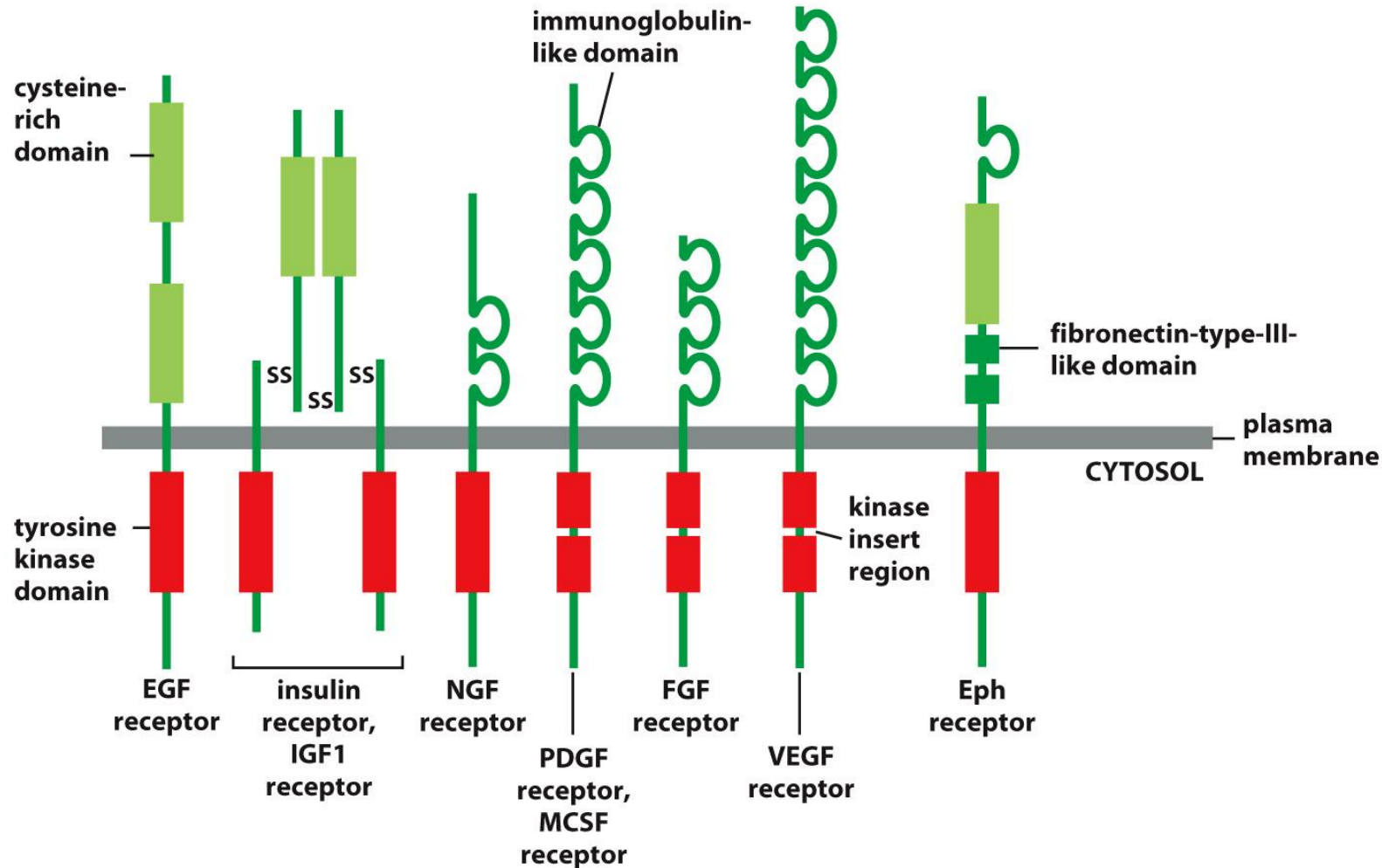


Figure 15-43 Molecular Biology of the Cell 6e (© Garland Science 2015)

Hlavní skupiny receptorových tyrosin kináz

TABLE 15-4 Some Signal Proteins That Act Via RTKs

Signal protein family	Receptor family	Some representative responses
Epidermal growth factor (EGF)	EGF receptors	Stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	Insulin receptor	Stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factor (IGF1)	IGF receptor-1	Stimulates cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk receptors	Stimulates survival and growth of some neurons
Platelet-derived growth factor (PDGF)	PDGF receptors	Stimulates survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	Stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factor (FGF)	FGF receptors	Stimulates proliferation of various cell types; inhibits differentiation of some precursor cells; acts as inductive signal in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	Stimulates angiogenesis
Ephrin	Eph receptors	Stimulates angiogenesis; guides cell and axon migration

Table 15-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

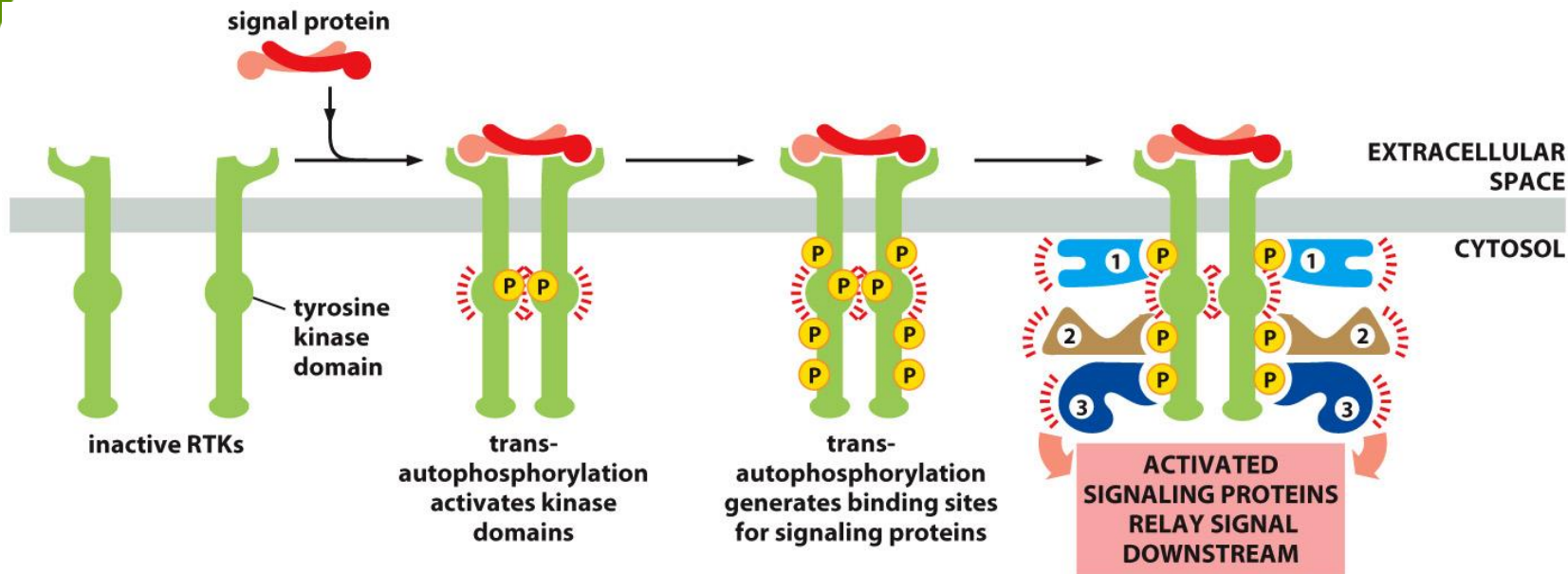


Figure 15-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

1. ligand se specificky váže na receptor
2. receptor dimerizuje
3. tyrosin-kinázové domény se navzájem fosforylují
4. autofosforylace vede k navázání (recruitment) adaptérových proteinů
5. V závislosti na receptoru se aktivují „downstream“ signální dráhy –např. Ras/Raf1/MEK/MAPK kinázová dráha,
6. která vede k buněčné odpovědi

Adaptorové proteiny s SH2 doménou

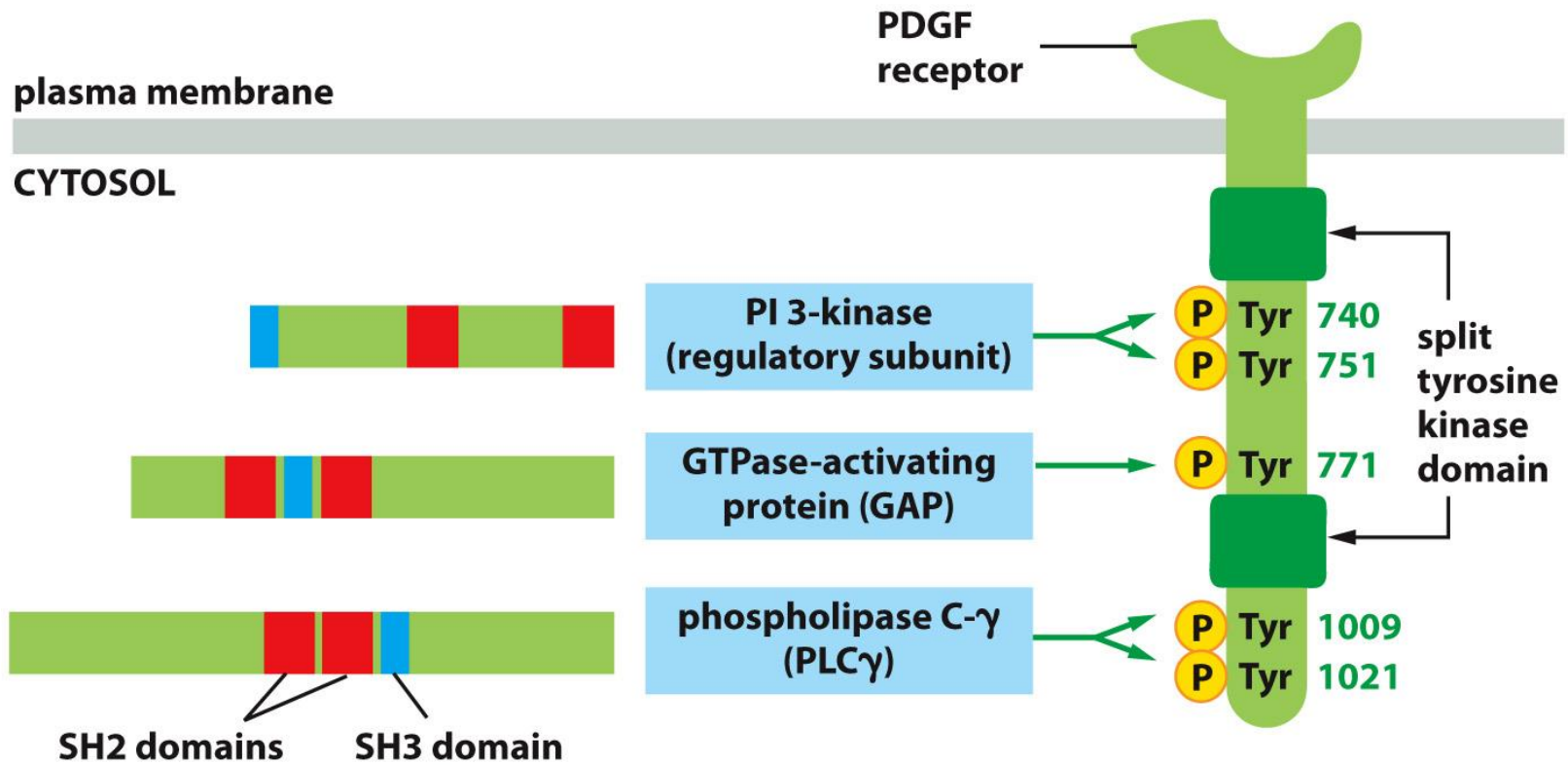


Figure 15-46a Molecular Biology of the Cell 6e (© Garland Science 2015)

Doména SH2 rozpoznává fosfo-tyrosin

binding site for amino acid side chain

binding site for phosphotyrosine

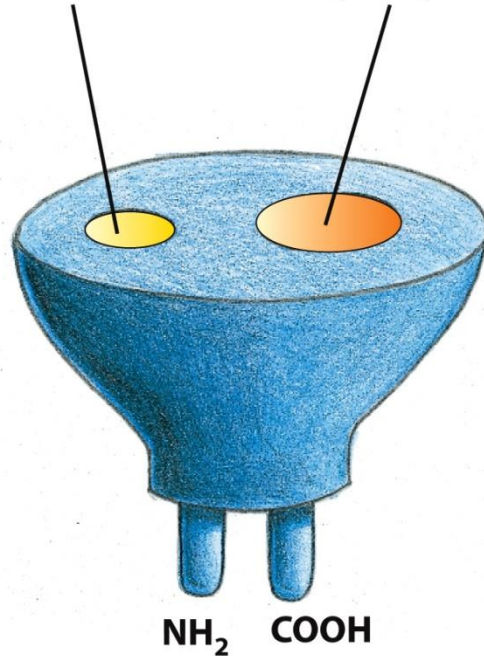


Figure 15-46c Molecular Biology of the Cell 6e (© Garland Science 2015)

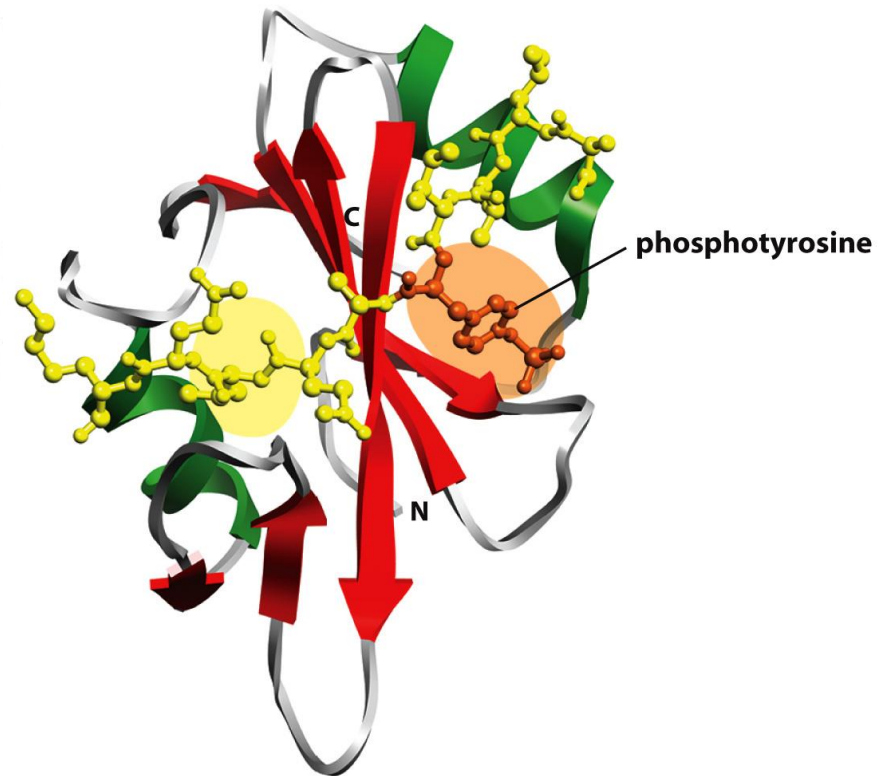
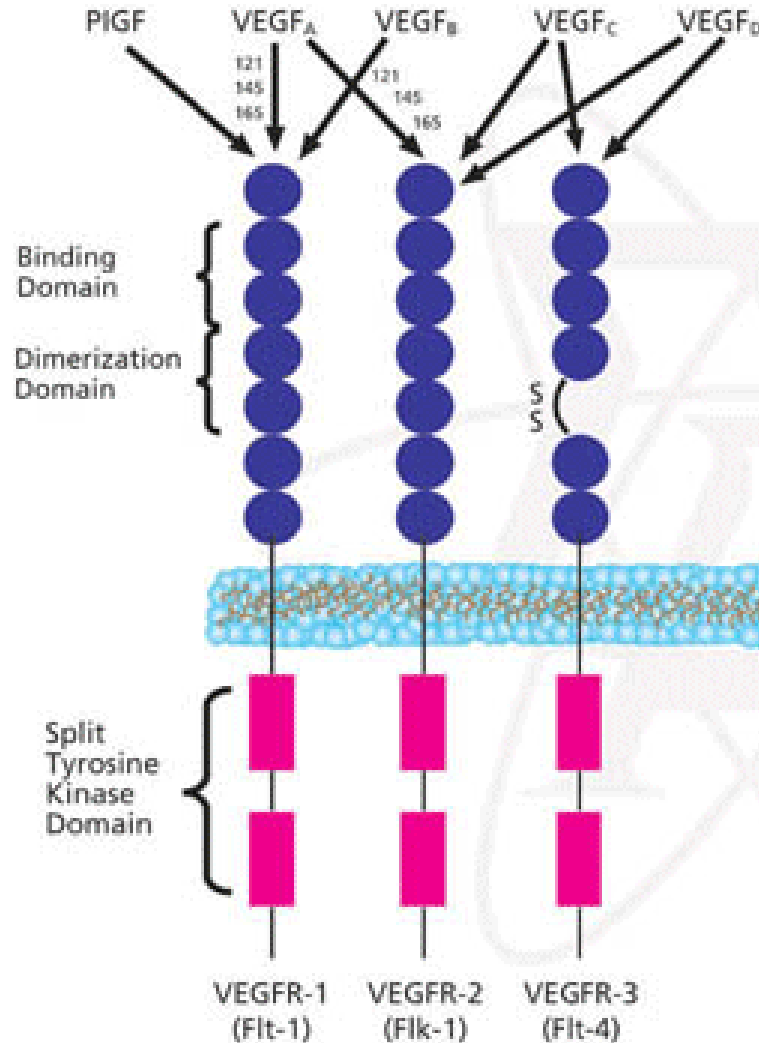


Figure 15-46b Molecular Biology of the Cell 6e (© Garland Science 2015)

Vybrané ligand:RTK receptorové systémy a jejich modelové funkce ve vývoji

- VEGF/VEGFR
- FGF
- ephrin/Eph

Vascular endothelial growth factors (VEGF) a jejich receptory (VEGFR)



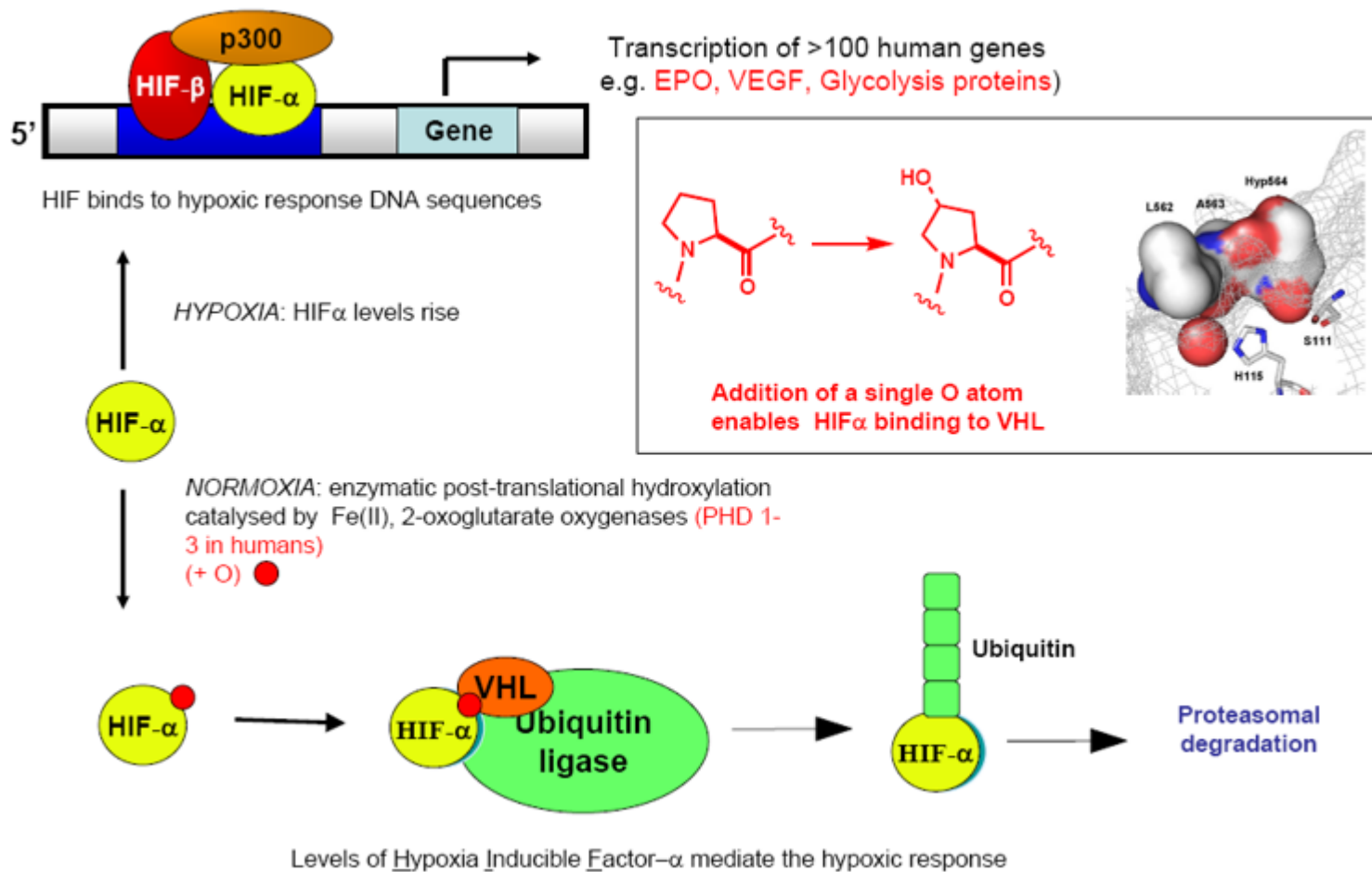
VEGF/VEGFR ve vývoji

- reguluje vznik a vývoj cévní soustavy
- master regulátor angiogeneze (vývoje cév)
- hypoxie (=nedostatek kyslíku) indukuje HIF (hypoxia-induced factor), který reguluje produkci VEGF.
- VEGF je schopen regulovat vznik de novo cév v hypoxické části embrya
- - podobný mechanismus se uplatňuje i při onkogenezi, kde VEGF podporuje prokrvení nádorů a tím podporuje jejich růst

Hypoxie a HIF

- **Hypoxie:** snížený parciální tlak O_2 ve tkáni X normoxie
- **HIF** – Hypoxia-Inducible Factor:
 - Heterodimerický transkripční faktor aktivující geny obsahující v promotorové sekvenci HRE (Hypoxia response element), vlastní transkripce je iniciována pomocí koaktivátorů **p300** a **CBP** (CREB-binding protein)
 - Prozatím je známo kolem 60 (100) genů regulovaných HIF, řada z nich reguluje odpověď na hypoxii (angiogeneze, proliferace, metabolismus glukózy, migrace, apoptóza, erythropoeza, metabolismus Fe)
 - Heterodimer sestává ze tří α podjednotek (HIF1 α , 2 α , 3 α) a jedné podjednotky β (HIF β =ARNT)
 - **α podjednotky jsou při normoxii silně labilní**, podjednotka β je na koncentraci O_2 nezávislá

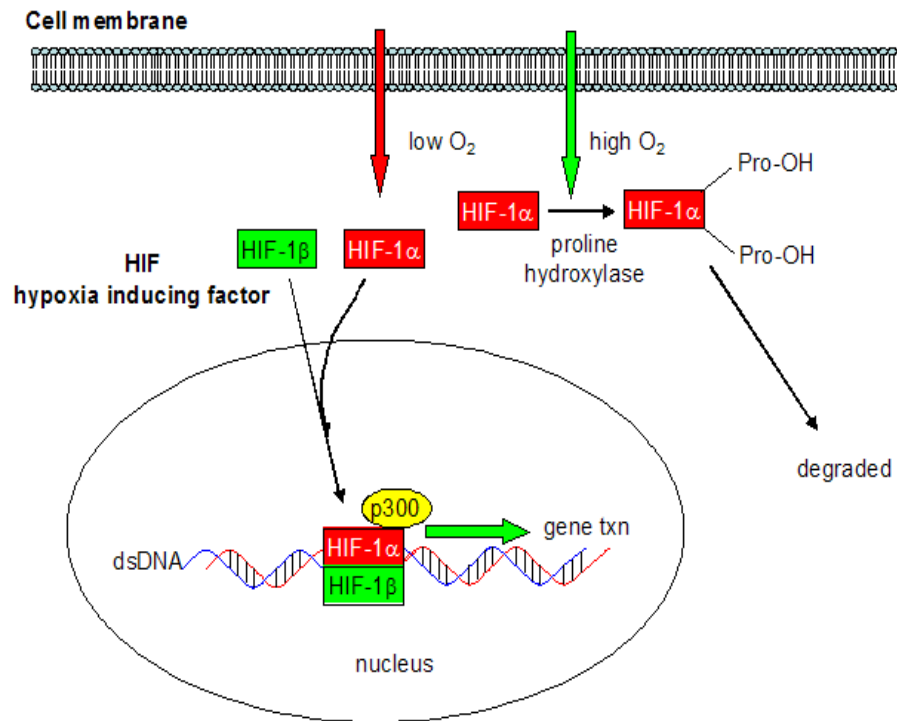
HIF při normoxii a hypoxii – význam hydroxylace prolinu



VHL (von Hippel-Lindau) - tumor supresorový gen

Modelové vývojové změny spojené s hypoxií/HIF systémem

- embryonální vývoj
- angiogenese
- růst chrupavek
- krvetvorba – aktivace EPO genu

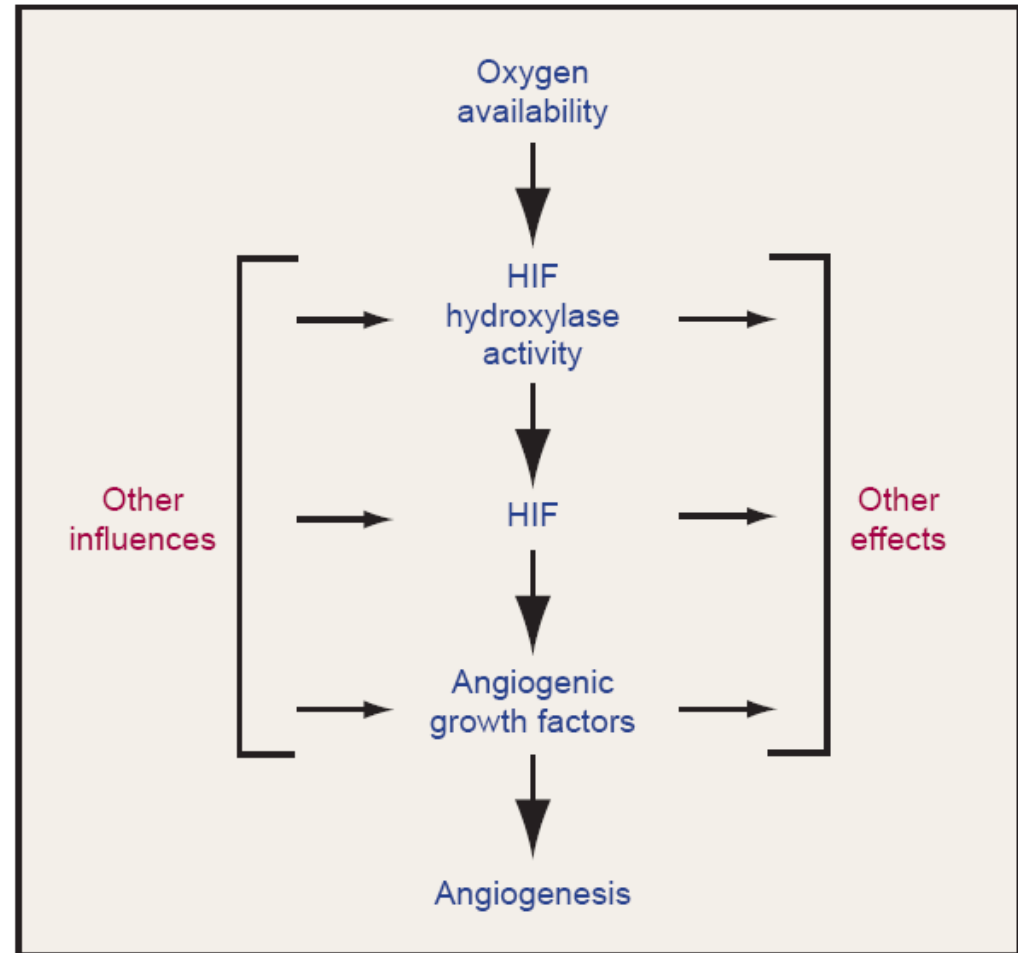


Genes upregulated:

- erythropoietin (induce RBC formation)
- glycolytic enzymes (needed if O₂ low)
- angiogenesis (new blood vessel growth)
- embryonic development
- placenta (for vascularization)
- macrophage and neutrophils (work in hypoxic wound conditions)

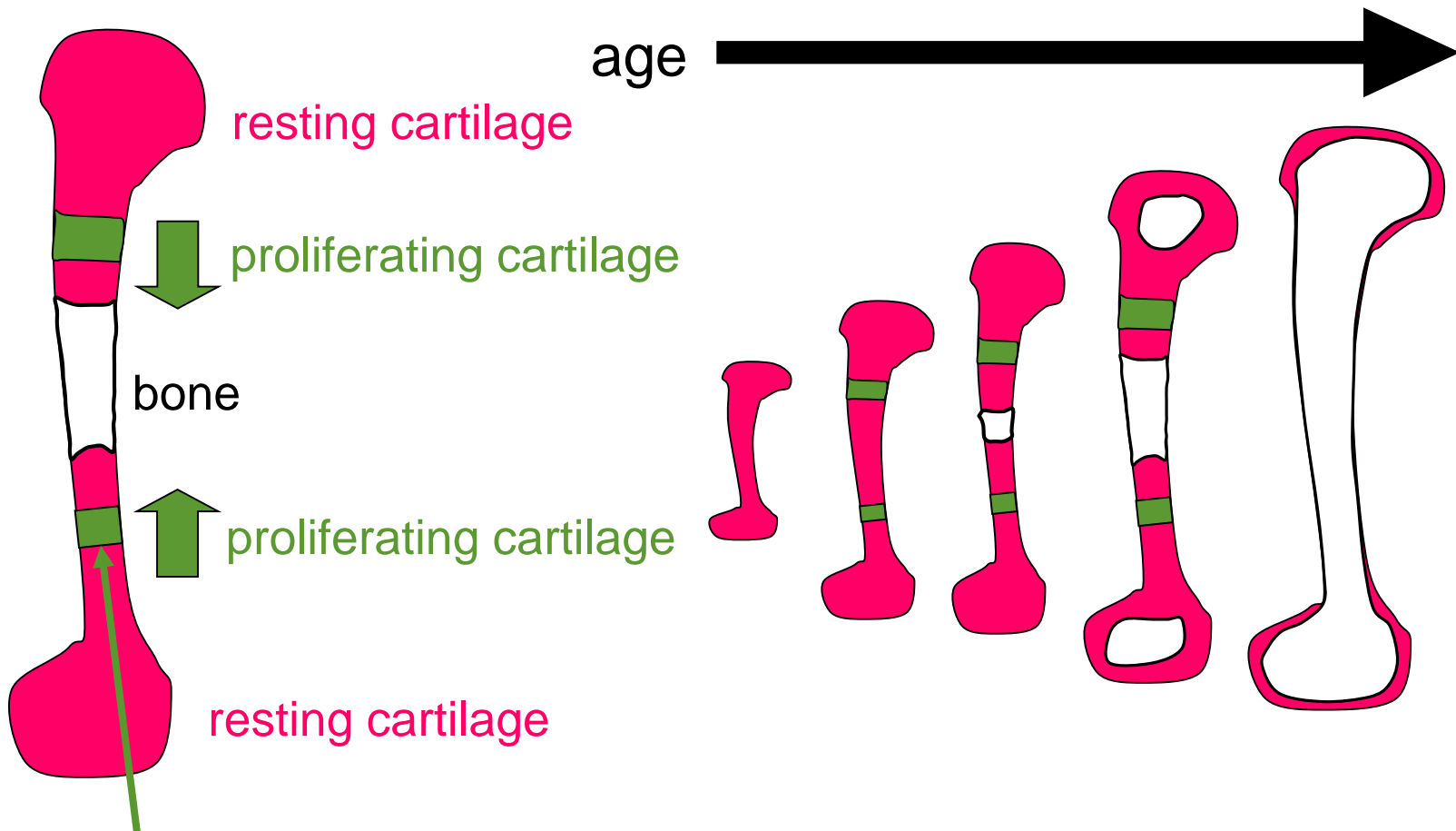
Angiogenese

- Angiogenese
 - tvorba nových krevních cév
- **HIF** se váže do oblasti promotoru a iniciuje transkripci receptoru **VEGFR 2** i expresi **VEGF** (Vascular Endothelial Growth Factor)
 - hlavní faktor angiogenese
- v normálním vývoji ale i během nádorového růstu



FGF dráha na příkladu regulace růstu kostí

Jak rostou dlouhé kosti?
- klíčová role chrupavky a růstové ploténky

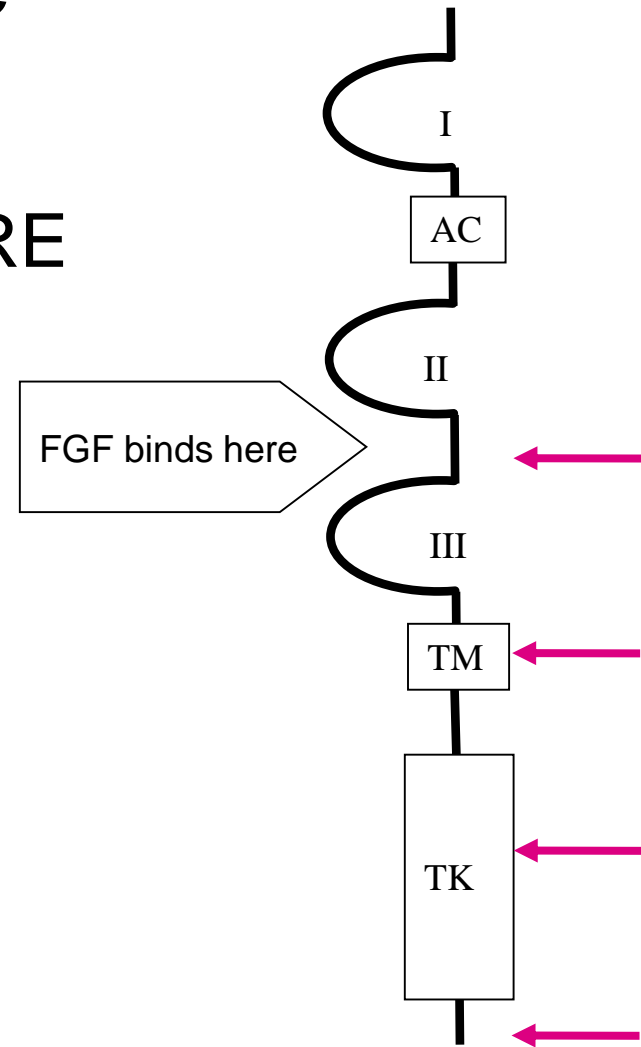


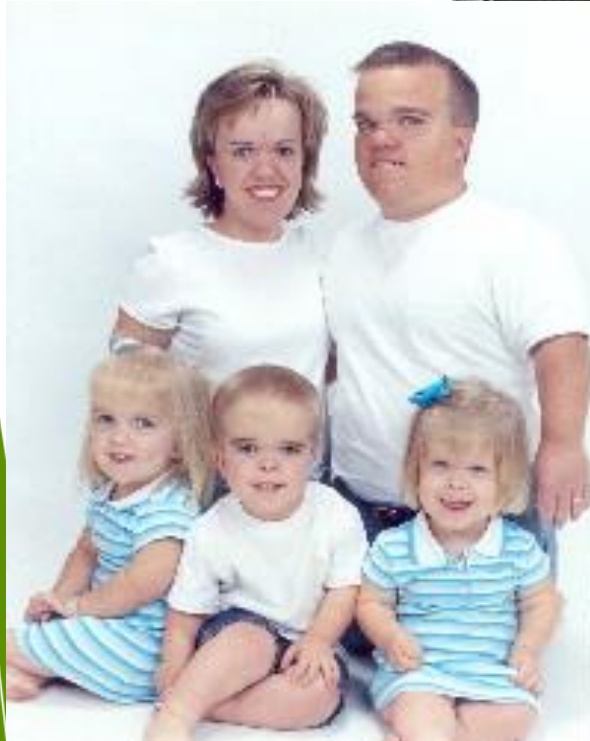
růstová ploténka (growth plate) – zaniká v dospělosti

Mutace v FGFR3 způsobují skeletální dysplázie

Hypochondroplasia
Achondroplasia
SADDAN
Thanatophoric Dysplasia

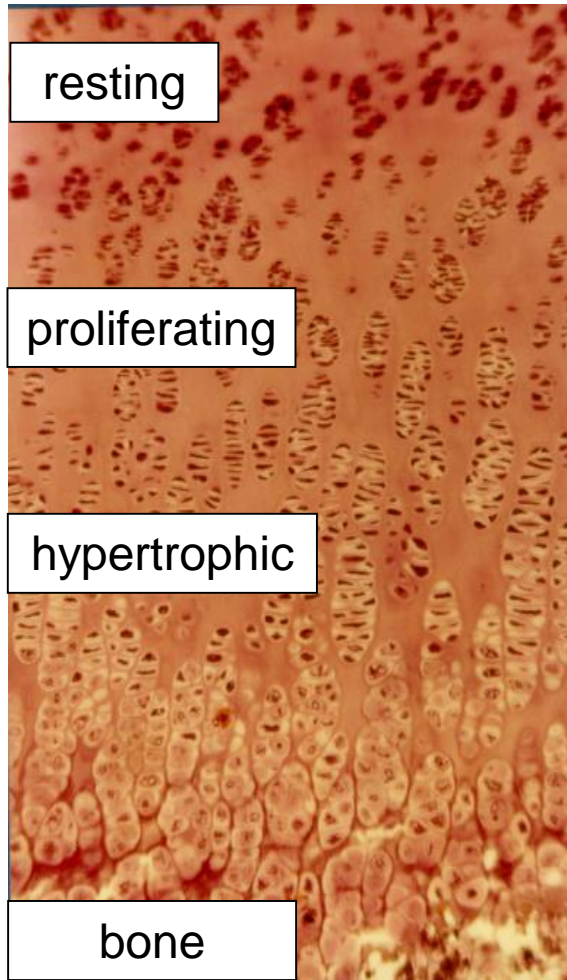
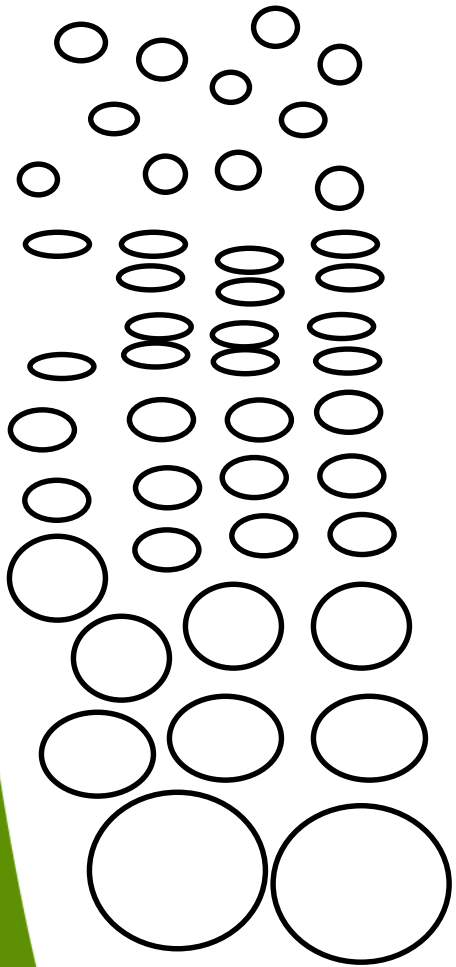
STATURE



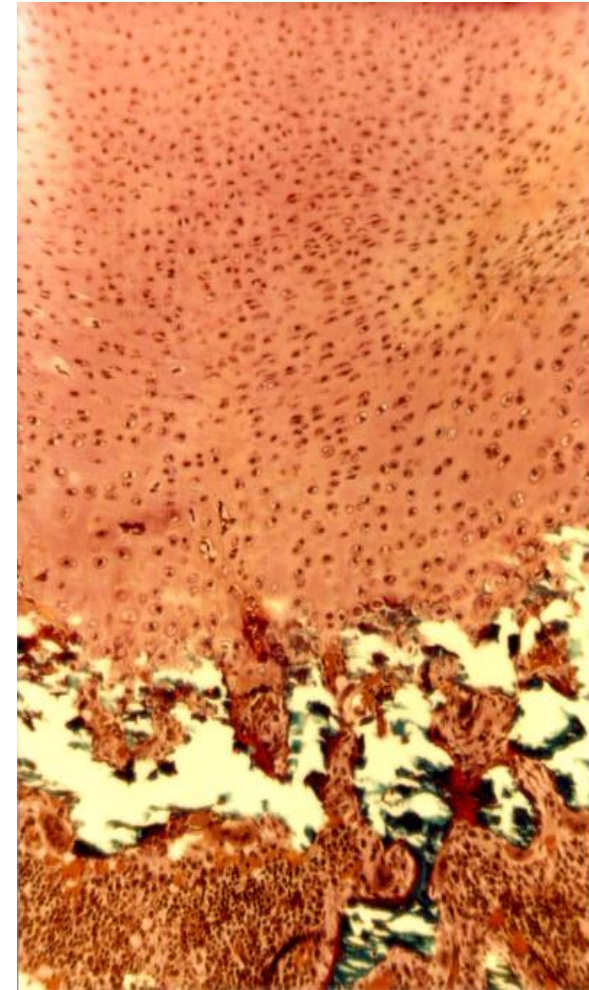


Achondroplasia

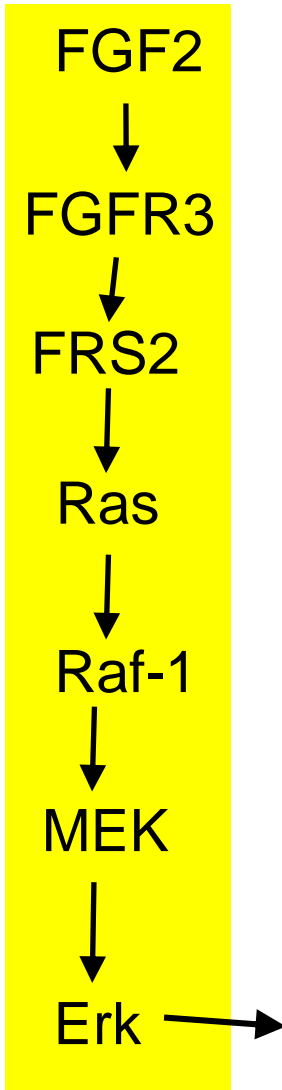
Růstová ploténka v detailu



healthy



TD

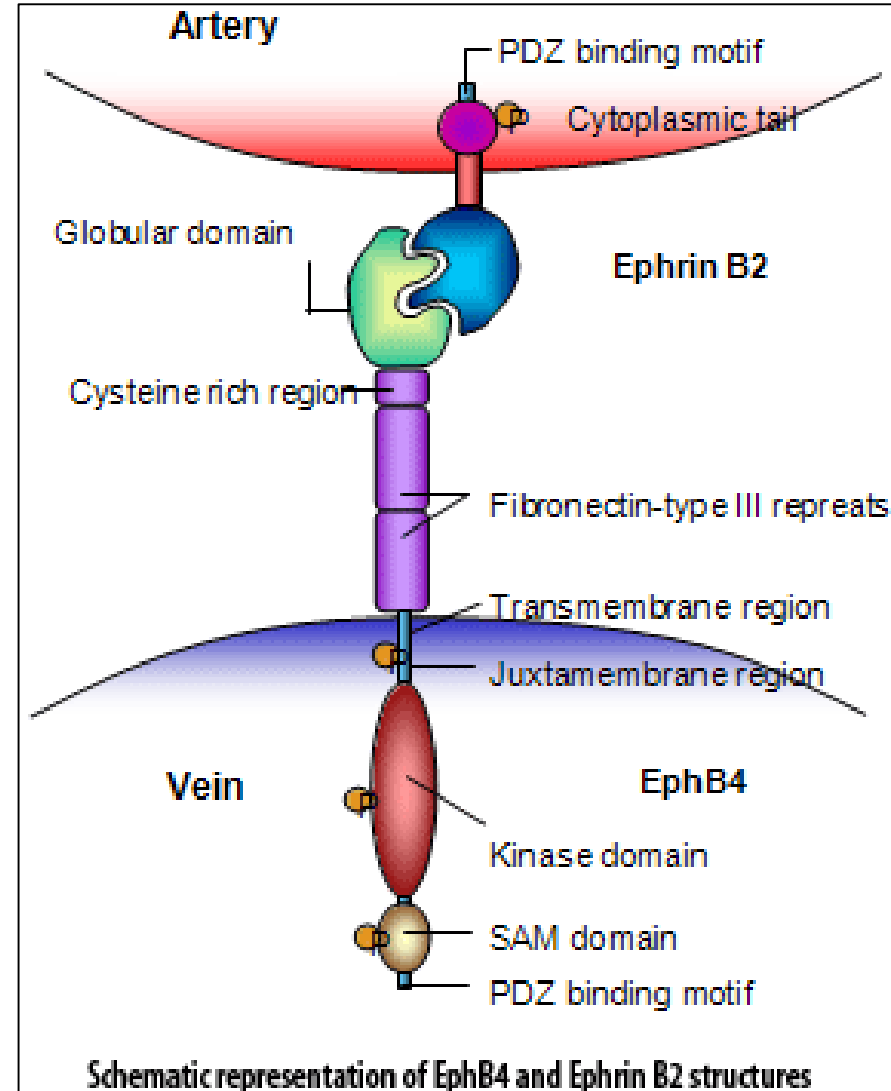


Erk MAP kinase activity is necessary for FGFR3 phenotype in cartilage



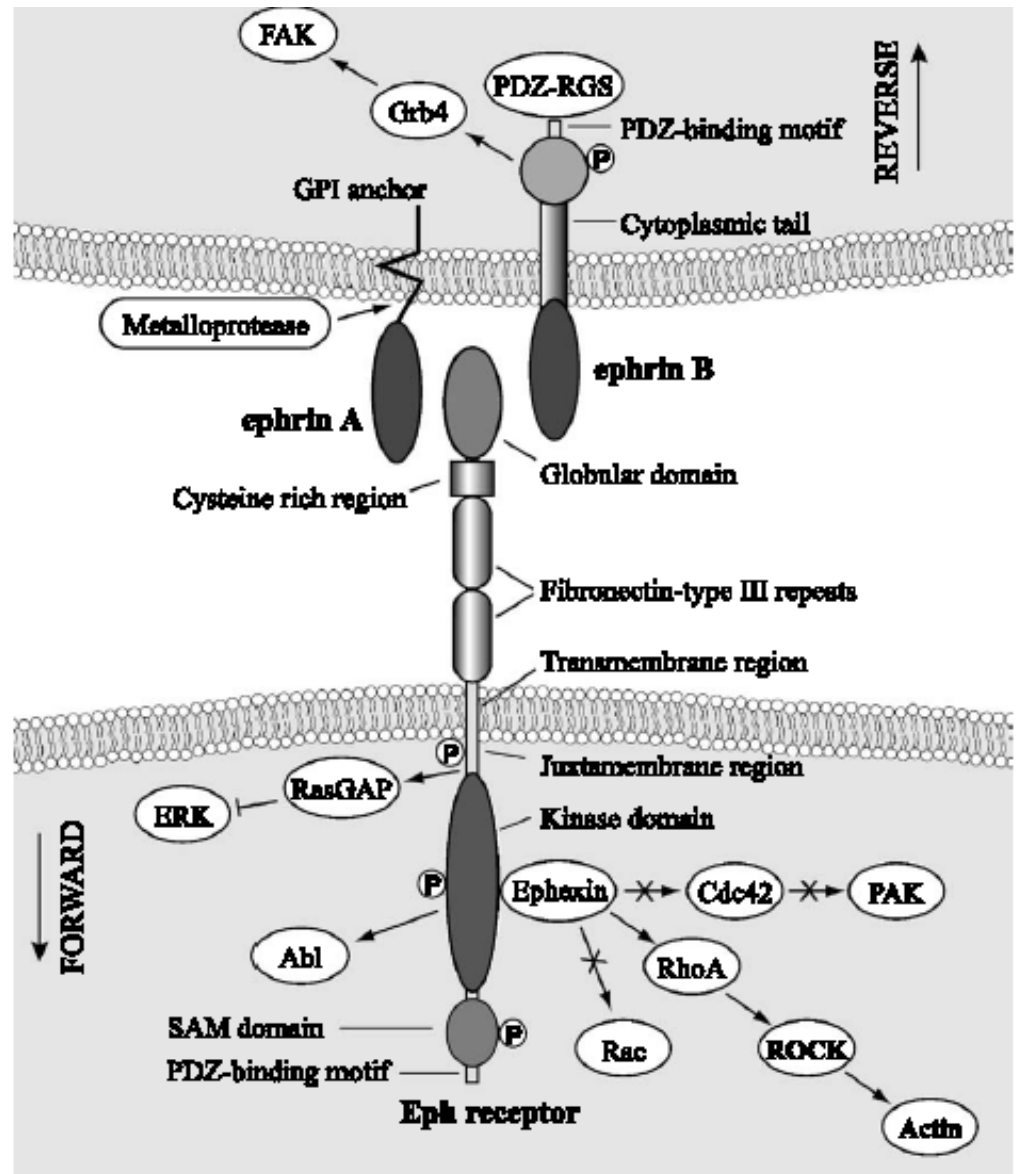
Eph/ephrin komplex

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



Eph/ephrin komplex

Jedinečná vlastnost ephrinů: reverse signalling – tj. nesignáluje jen receptor, ale i ligand

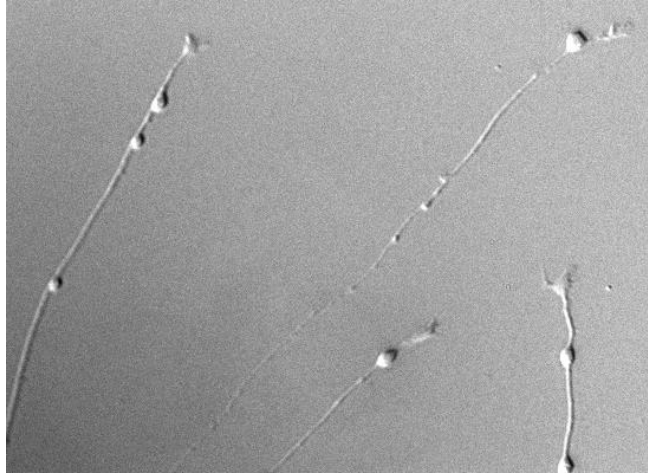


Eph/ephrin komplex

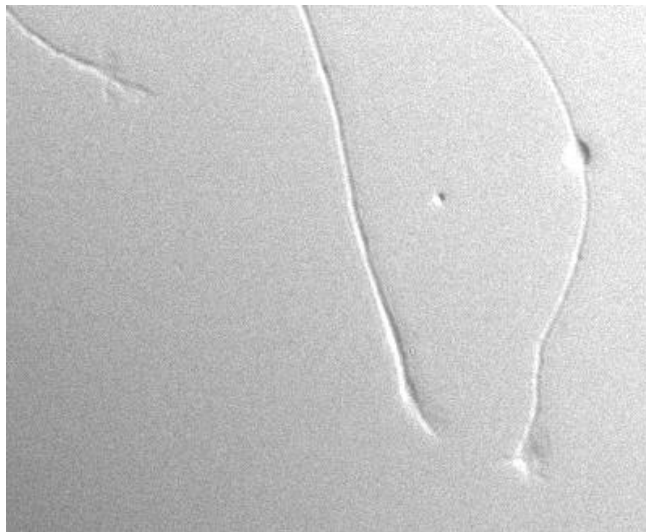
- legenda k obrázku:

Fig. 1. Forward and reverse signaling by the ephrin–Eph complex. Glycosylphosphatidylinositol (GPI)-anchored ephrin-As bind to EphA receptors whereas the transmembrane ephrin-Bs bind to EphB receptors. The ephrin–Eph receptor binding initiate forward signaling in the Eph receptor bearing cells and reverse signaling in the cells that express ephrins. Major events associated with forward signaling involve the exchange factor ephexin, which links the Eph receptor with the Rho GTPases and then to regulation of actin remodeling. Other important events implicate the inactivation of focal adhesion kinase (FAK) and decreased integrin-mediated adhesion through activation of the phosphatases Shp2 by EphA. In contrast, the recruitment of the adaptor protein Nck to EphB and the activation of Src are associated with increased integrin-mediated adhesion. Reverse signaling by ephrin-Bs is characterized by the recruitment of SH2 domain containing protein such as Grb4 to phosphotyrosine residues on ephrin-Bs. PDZ-RGS3 are PDZ-binding proteins that bind to ephrin-Bs to modulate signaling through G-protein-coupled receptors. In the case of ephrin-As, the reverse signaling implies their aggregation with signaling molecules in membrane raft microdomains. Interestingly, their activity can be modulated by enzymatic cleavage by metalloproteases. SAM, sterile α motif; PDZ, PSD-95 disc large zonula occludens-1. Reproduced with permission from Nature Reviews Molecular Cell Biology, Kullander and Klein. Copyright 2002 Macmillian Magazines Ltd. (Kullander and Klein (2002)).

Eph/ephrin komplex

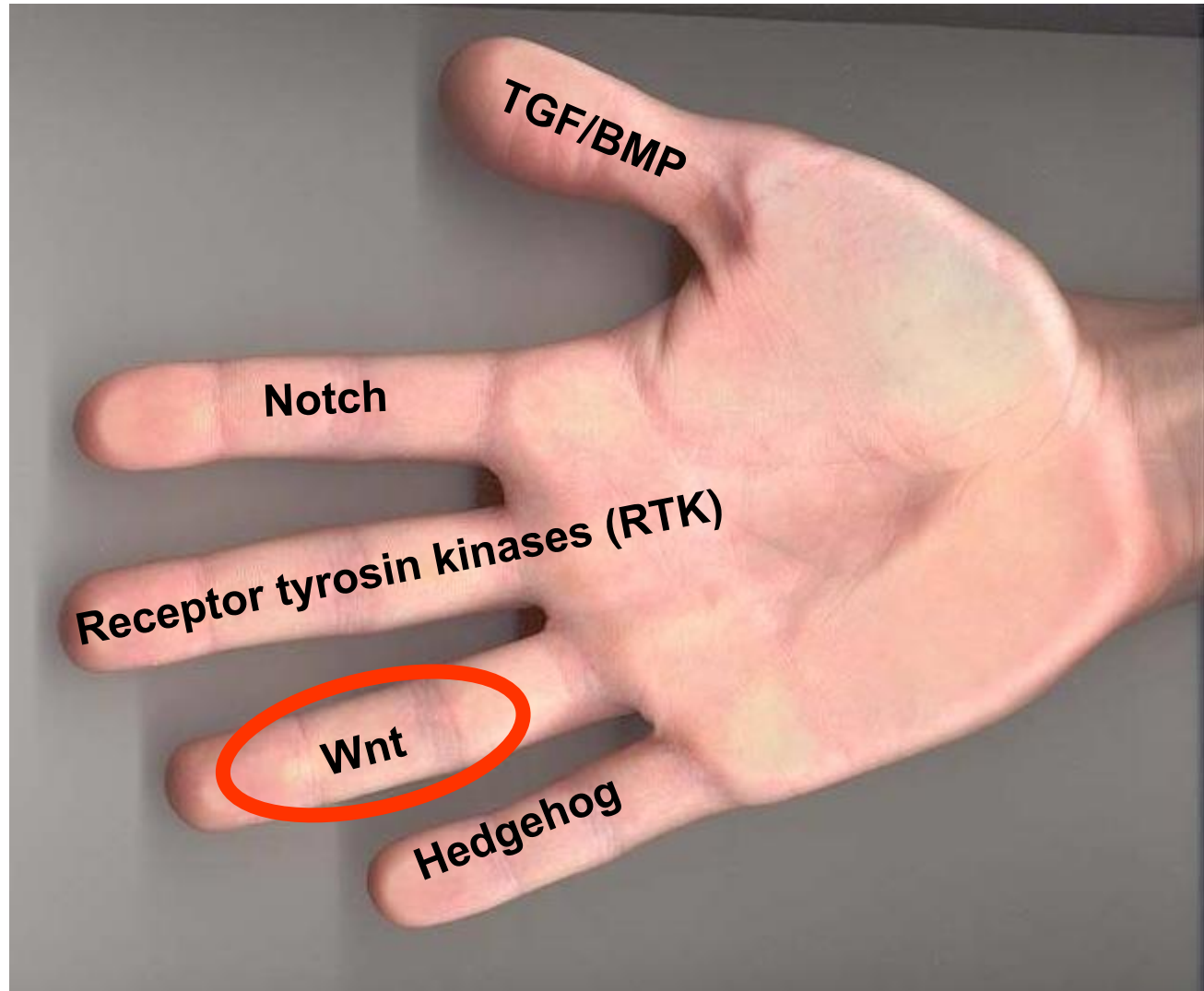


Supporting Information Movie 2. Ephrin-B2 induces extremely rapid growth cone collapse and axon retraction in VT RGCs. Movie depicts VT growth cones treated with ephrin-B2. Frames were captured at 30-second intervals for 45 minutes, replayed at 15 frames per second. 0.5 $\mu\text{g/ml}$ pre-clustered ephrin-B2 was added after 15 minutes (2 second interval in movie).



Supporting Information Movie 4. Inhibiting Rho kinase strongly diminishes axon retraction but does not affect growth cone collapse. Movie depicts VT growth cones pre-treated the Rho kinase inhibitor Y-27632 for 1 hour, followed by treatment with ephrin-B2. Frames were captured at 30-second intervals for 45 minutes, replayed at 15 frames per second. 0.5 $\mu\text{g/ml}$ pre-clustered ephrin-B2 was added after 15 minutes (2 second interval in movie).

Klíčové molekulární komponenty vývoje

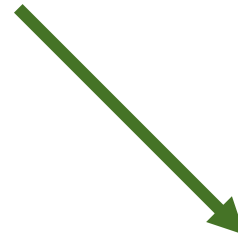


Wnt (Wingless/Int)

- 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

Speciální buněčný aparát pro sekrece Wnt proteinů

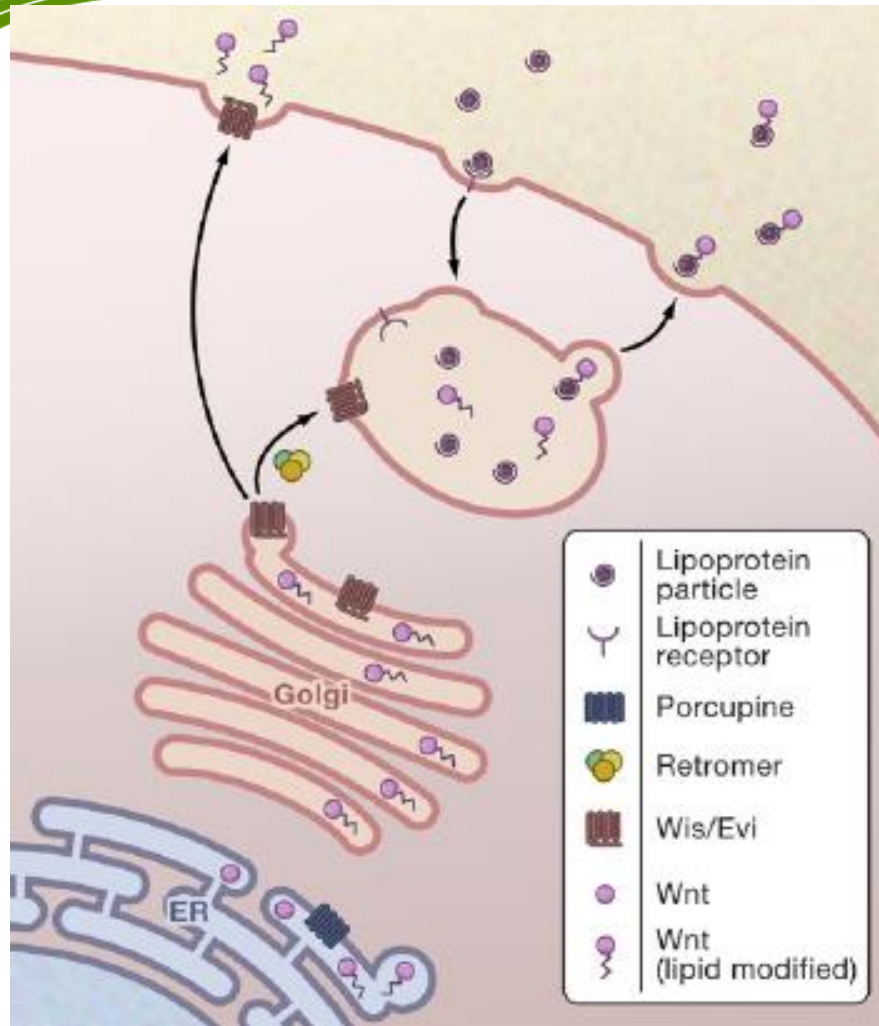
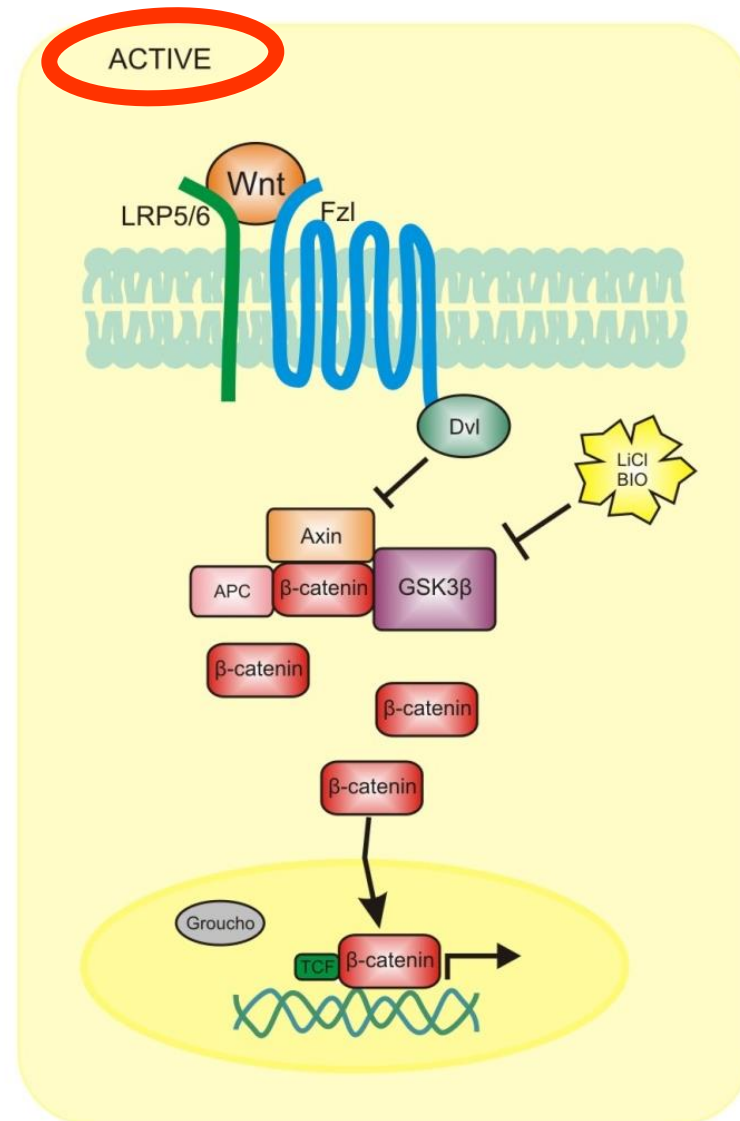
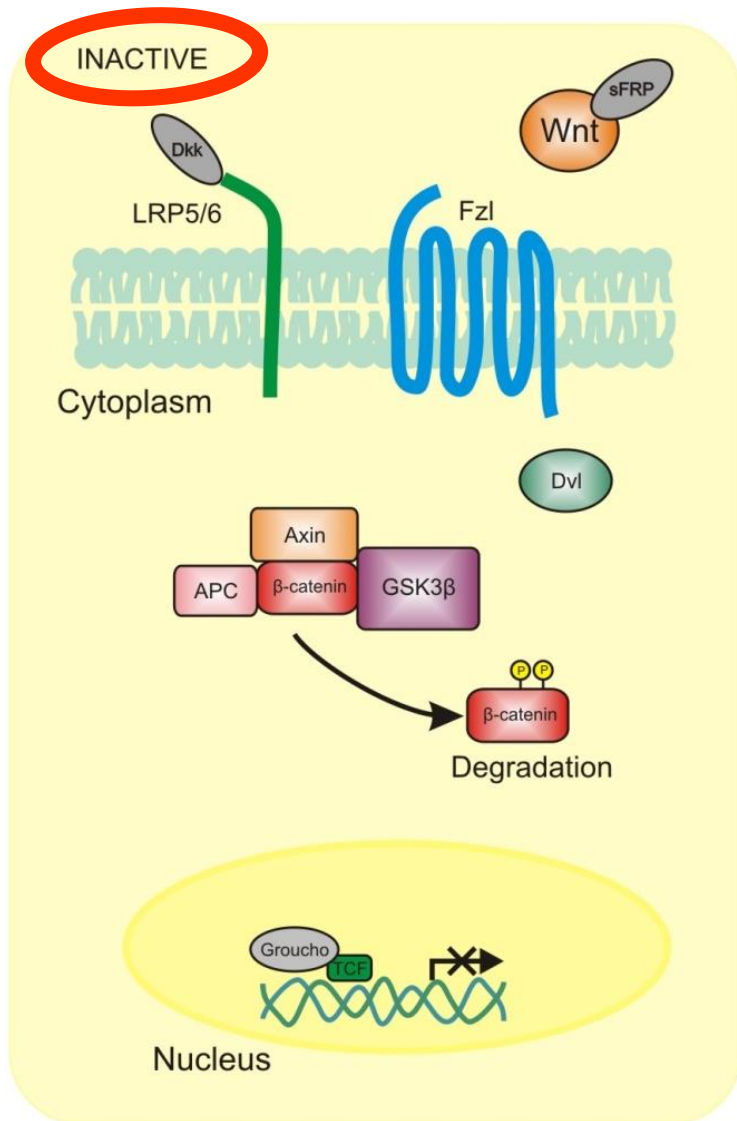


Figure 1. Wnt Secretion

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

Kanonická signální dráha Wnt



LiCl
BIO

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 signálních drah Wnt

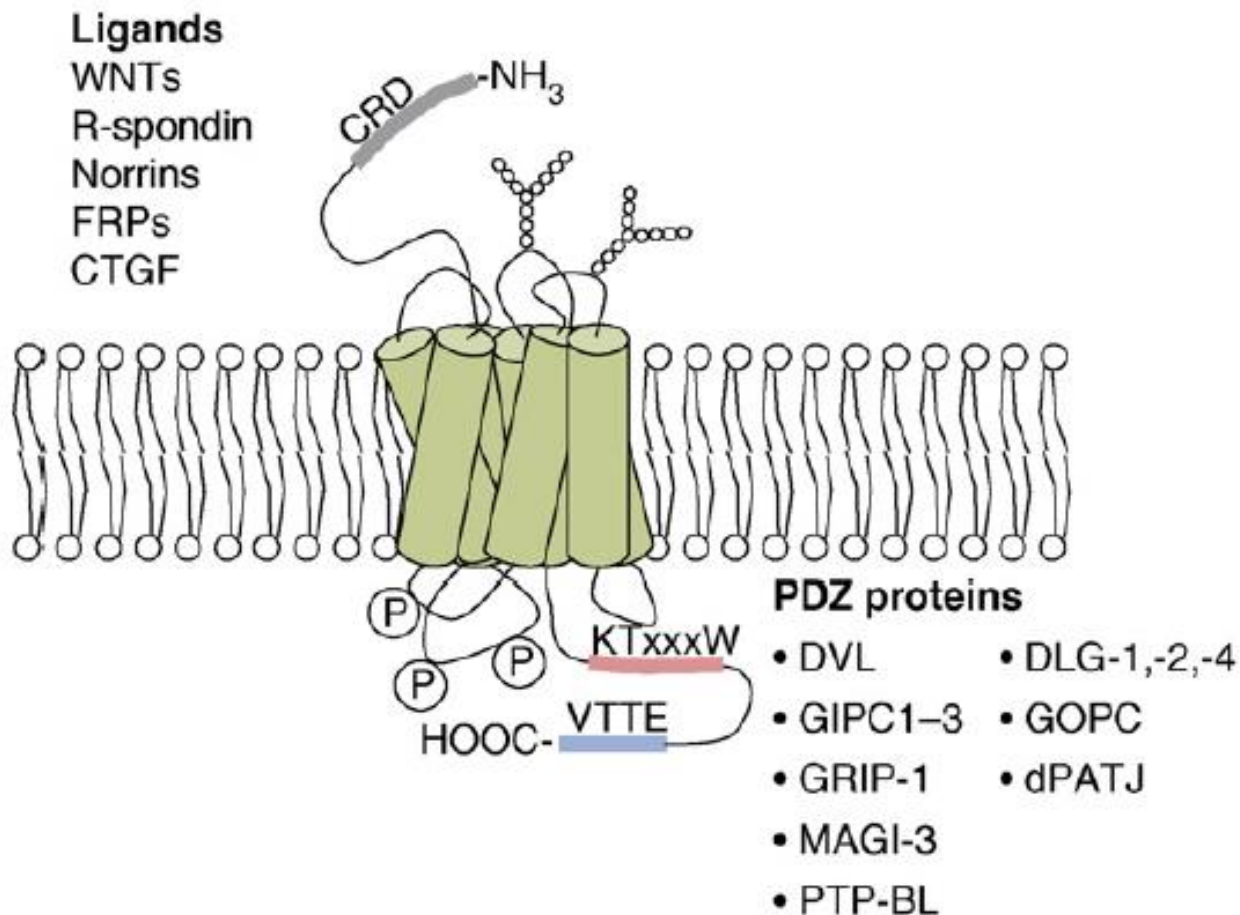
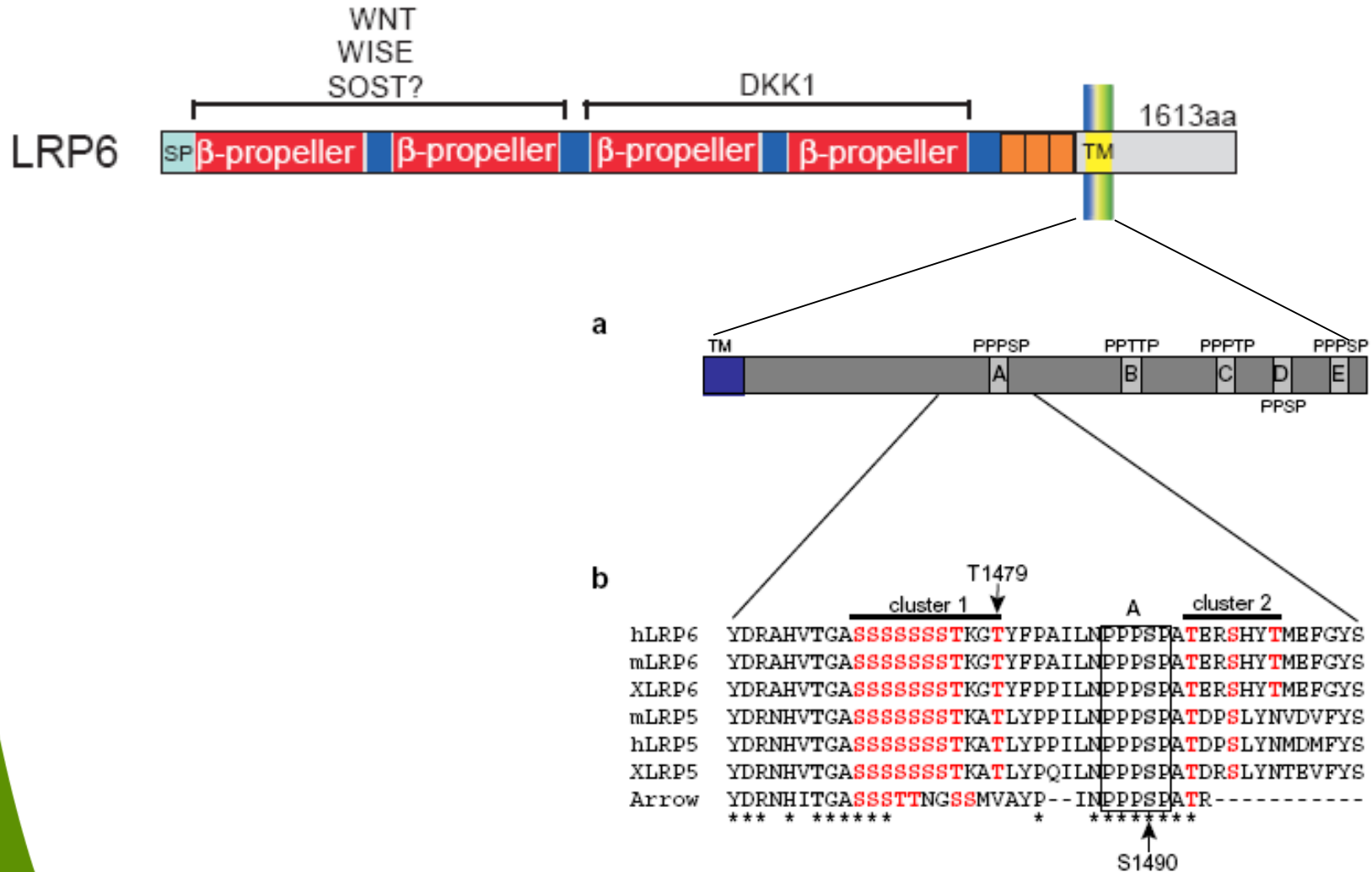
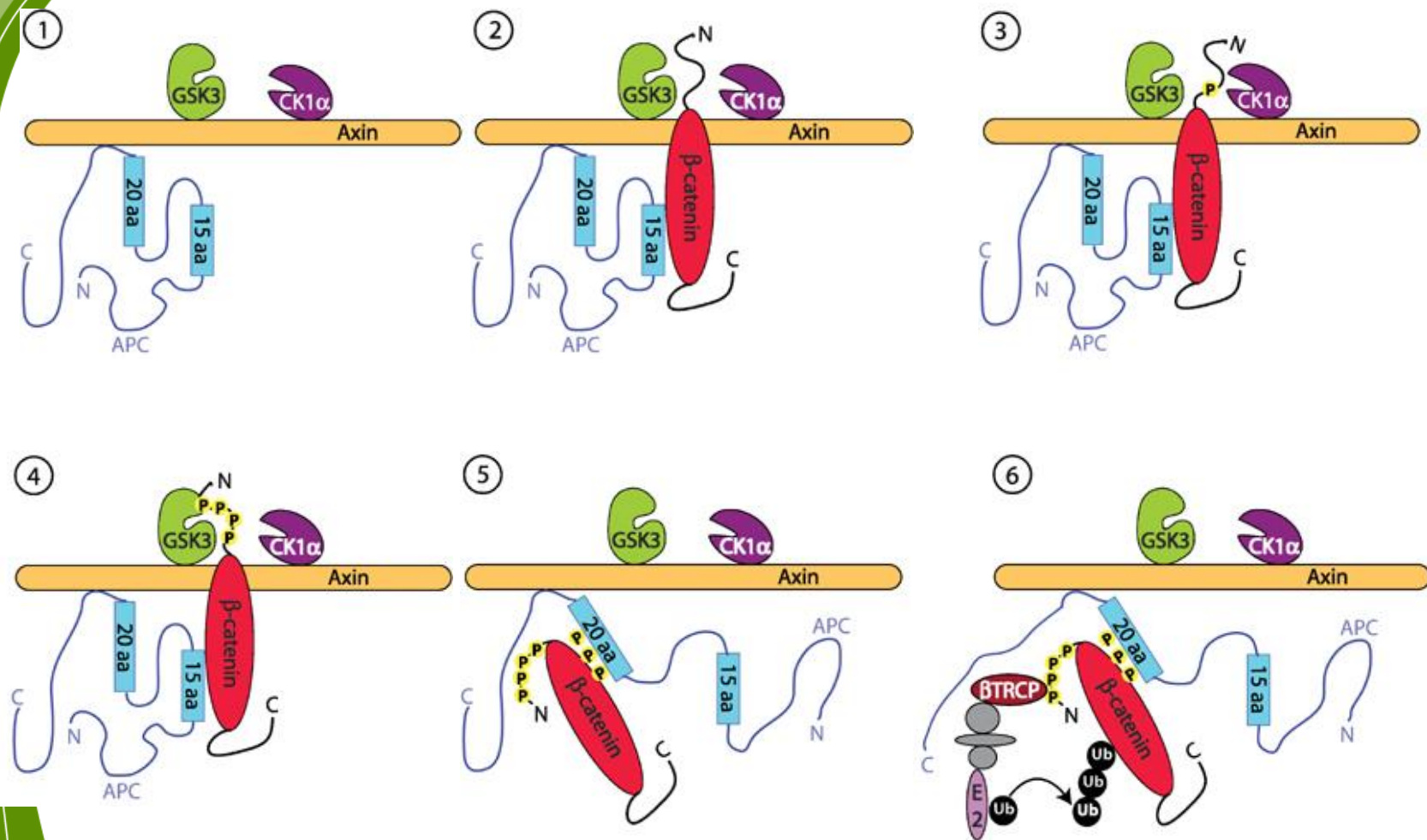


Figure 1. Schematic view of the 7TM model of FZDs. The model indicates extra- and intracellularly interacting proteins, putative glycosylation and phosphorylation sites. The N-terminal CRD is the primary binding site for ligands. The pink stretch in the C terminus indicates the internal PDZ-interacting 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 pro beta-kenatin



Destrukční komplex

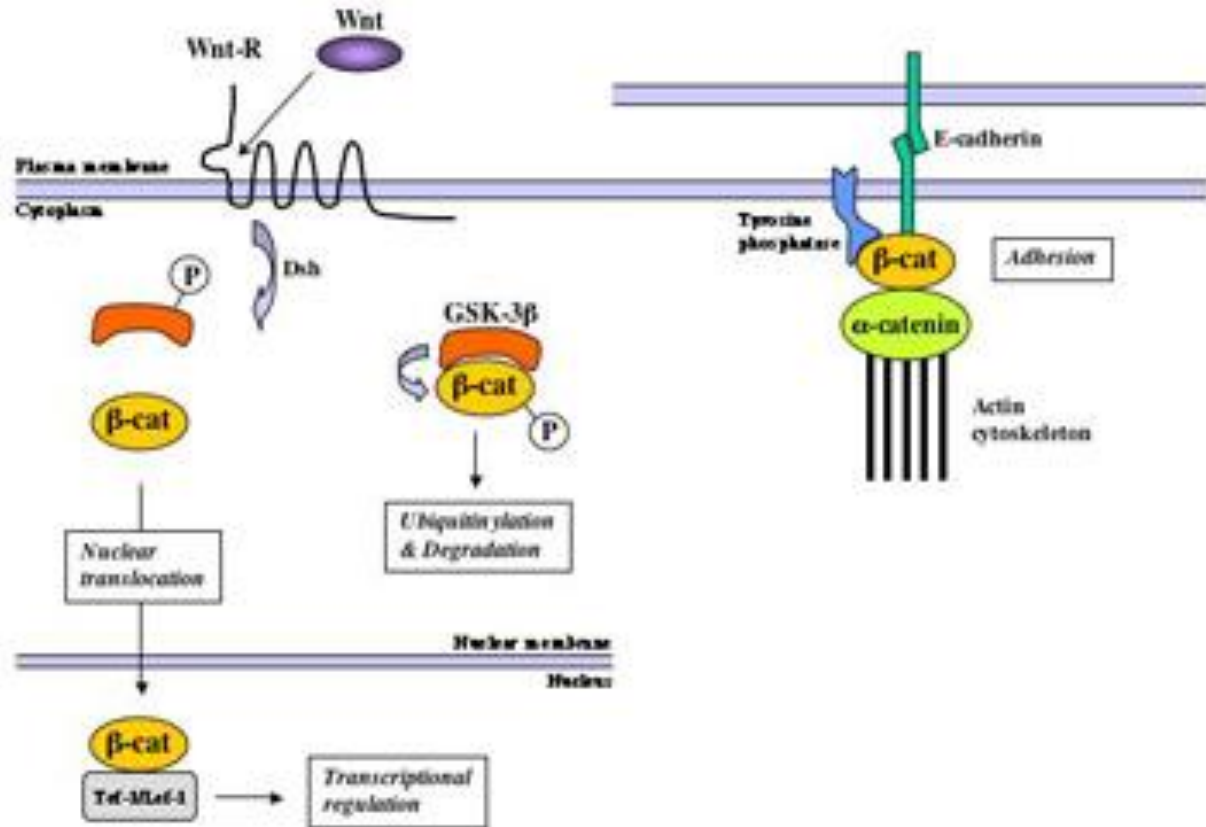
- legenda k obrázku destrukčního komplexu:

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) β -Catenin enters the complex by binding Axin and potentially the APC 15 aa repeats. This positions the N-terminus of β -catenin near CK1 and GSK3. (3) CK1 phosphorylates β -catenin at Ser45. (4) GSK3 phosphorylates β -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 β -catenin. The binding of a phosphorylated 20 aa repeat to β -catenin displaces Axin from β -catenin. (6) TRCP1 binds the phosphorylated N-terminus of β -catenin, causing the ubiquitination of β -catenin by an E2 ligase. APC is then either desphosphorylated within the complex, allowing the ubiquitinated β -catenin to leave the complex, or the ubiquitinated β -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 „polech“ – biochemických komplexech

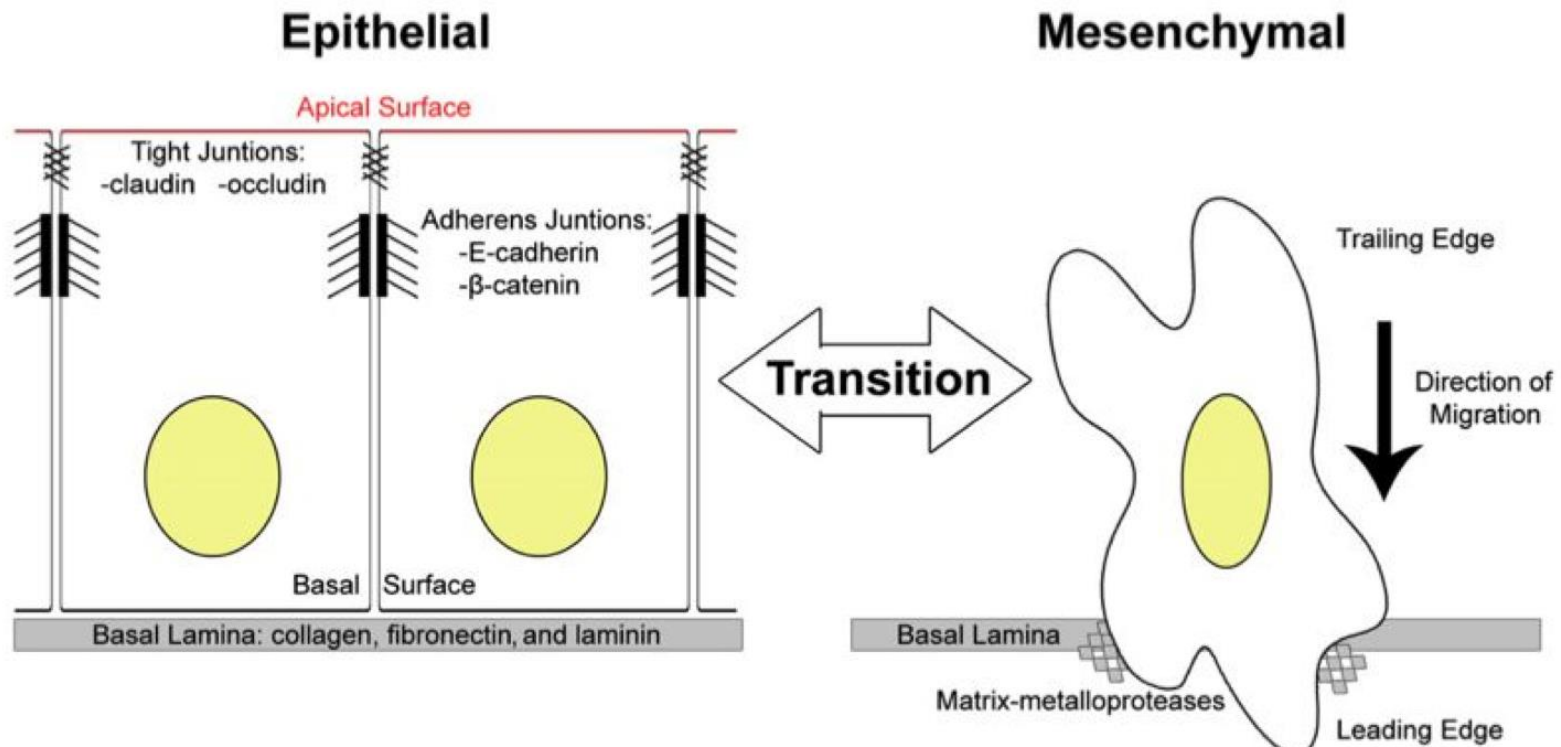
Wnt and E-cadherin pathways



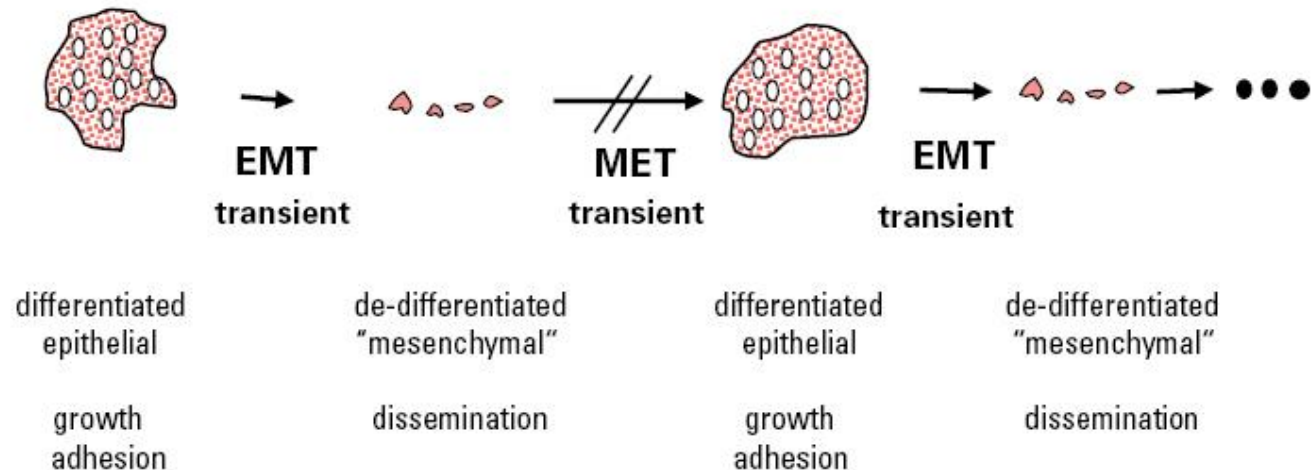
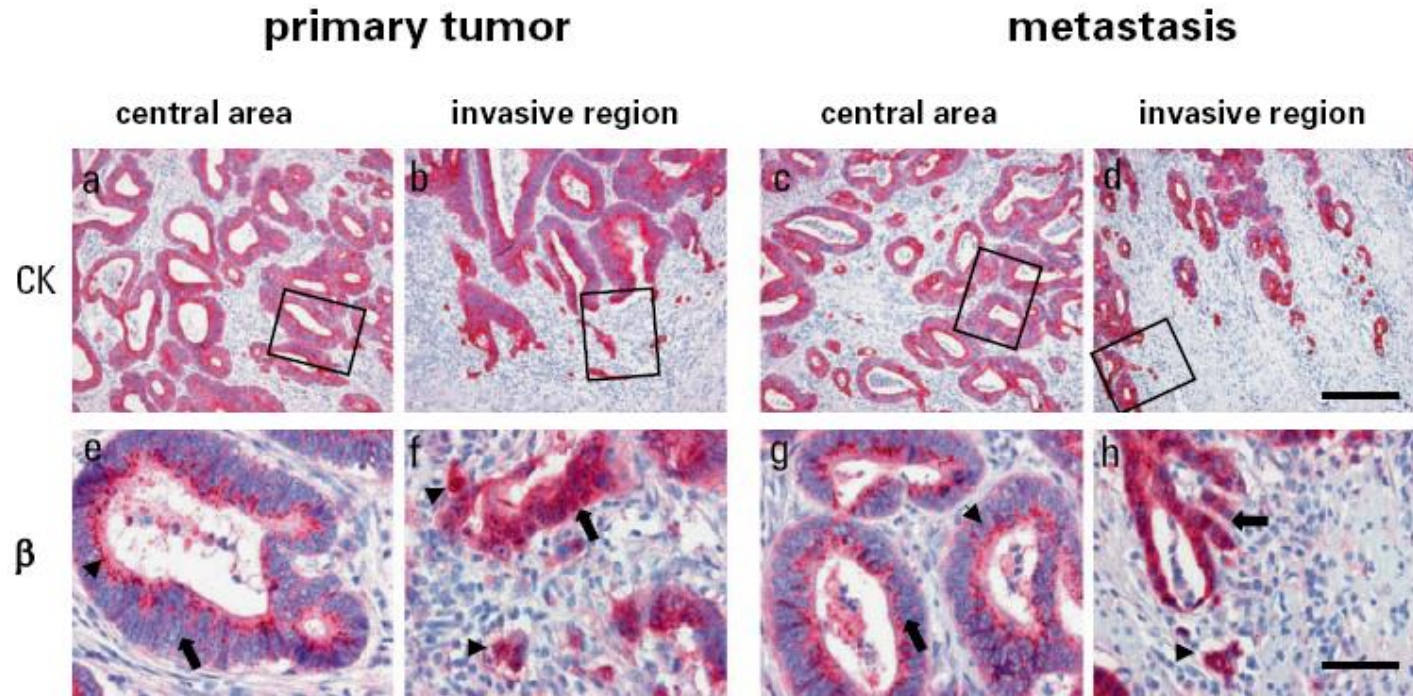
1. Jako součást Wnt signalizace

2. V komplexu s E-cadherin jako součást „adherens junctions“

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



Epiteliálně-mezenchymální tranzice (EMT) v nádorech

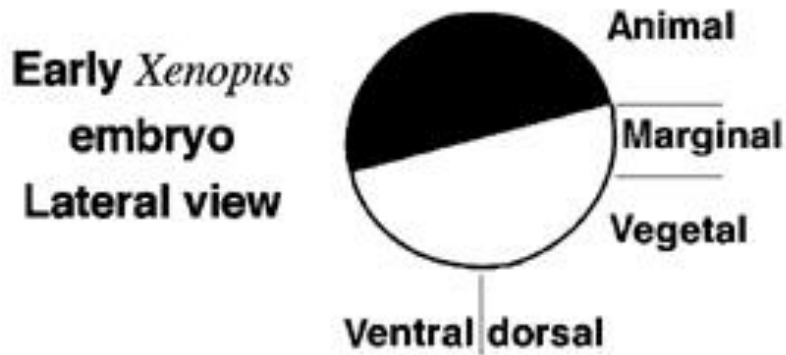


Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

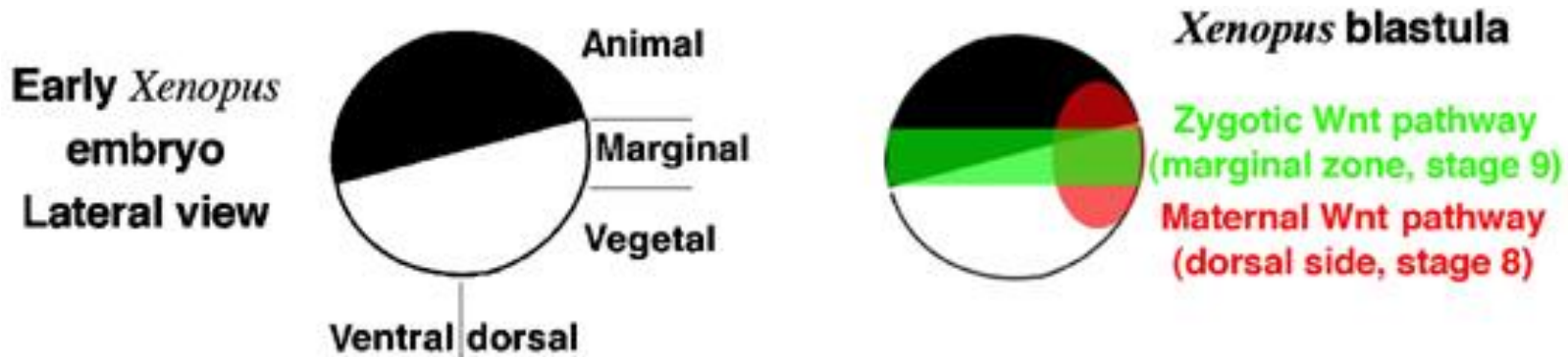
Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

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

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



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



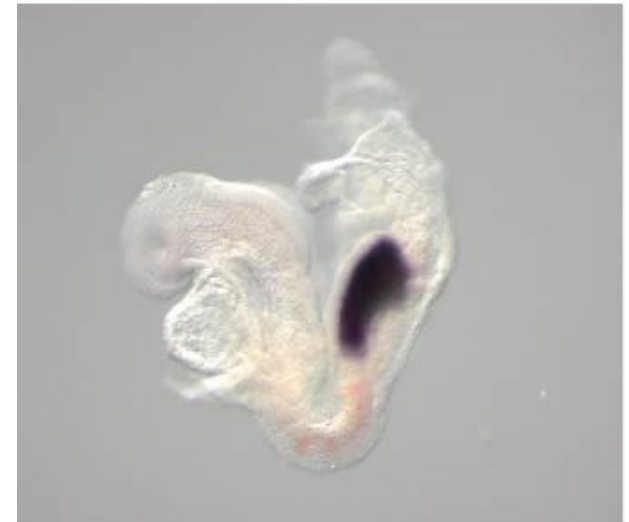
Wnt/ β -cateninová dráha určuje zadní část embrya

myší embryo po gastrulaci (E8.5):

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

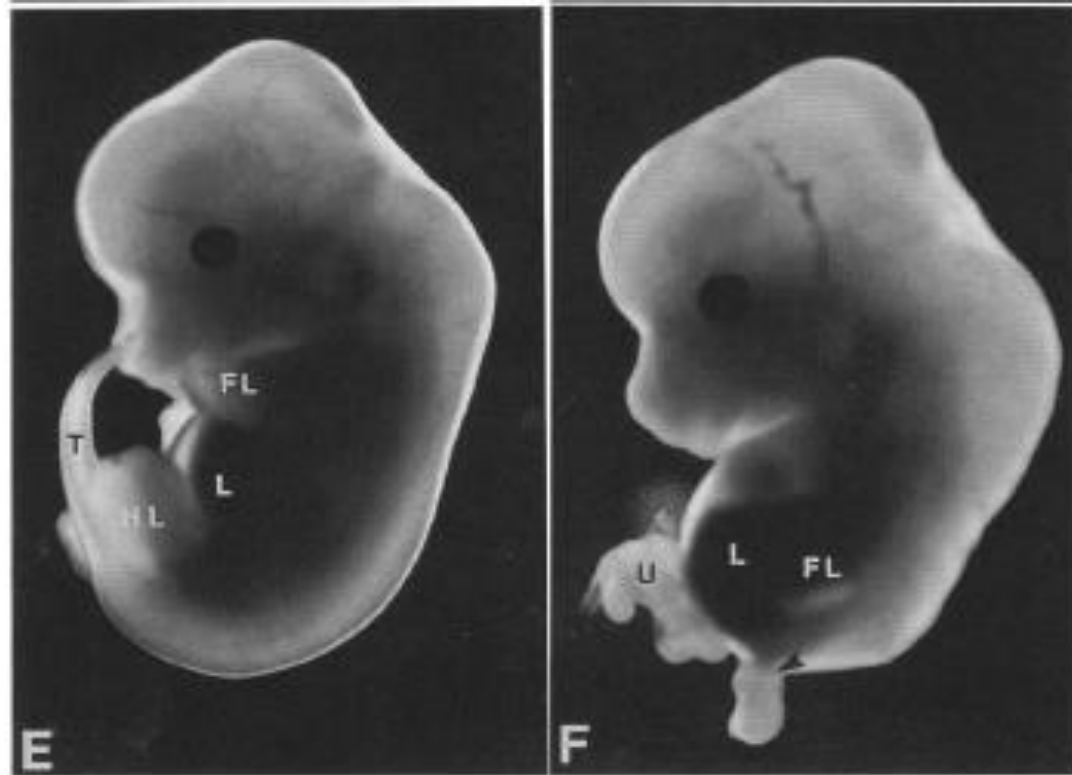
Uncx4.1/Mesogenin

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



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

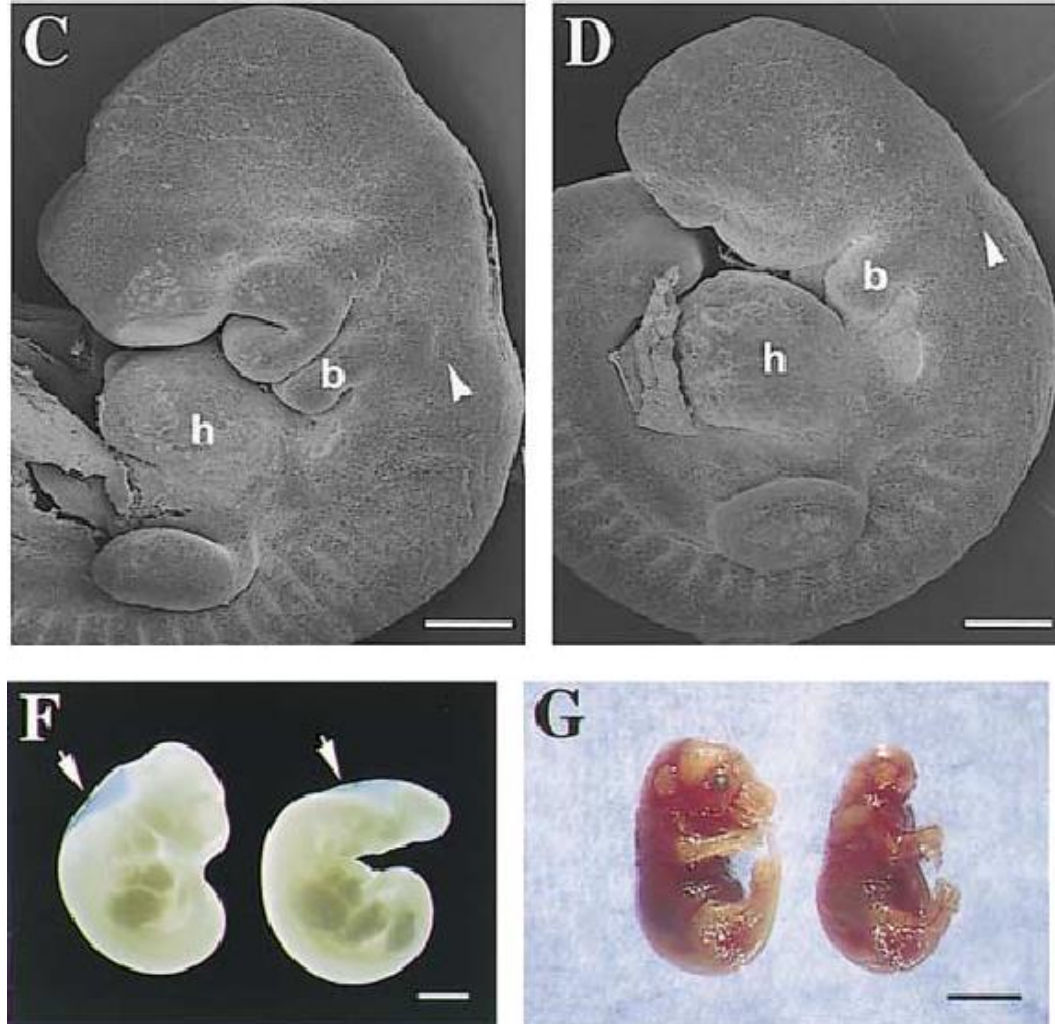
Deplece Wnt/ β -kateninové dráhy při gastrulaci = ztráta zadních částí těla



wild type

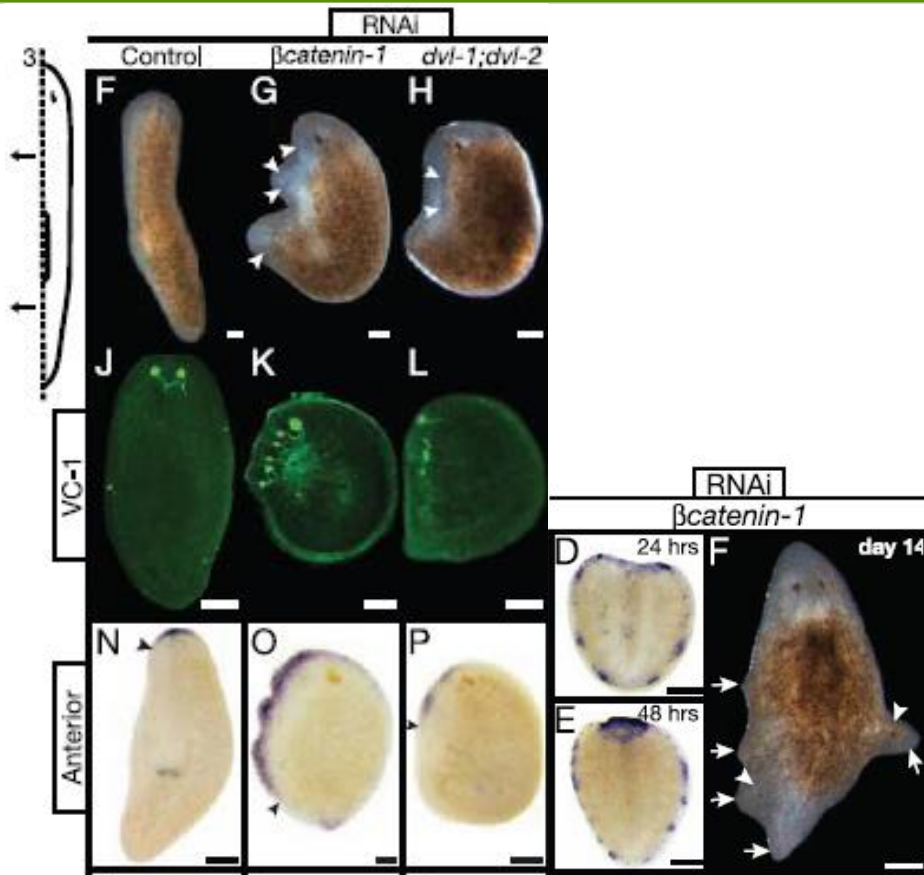
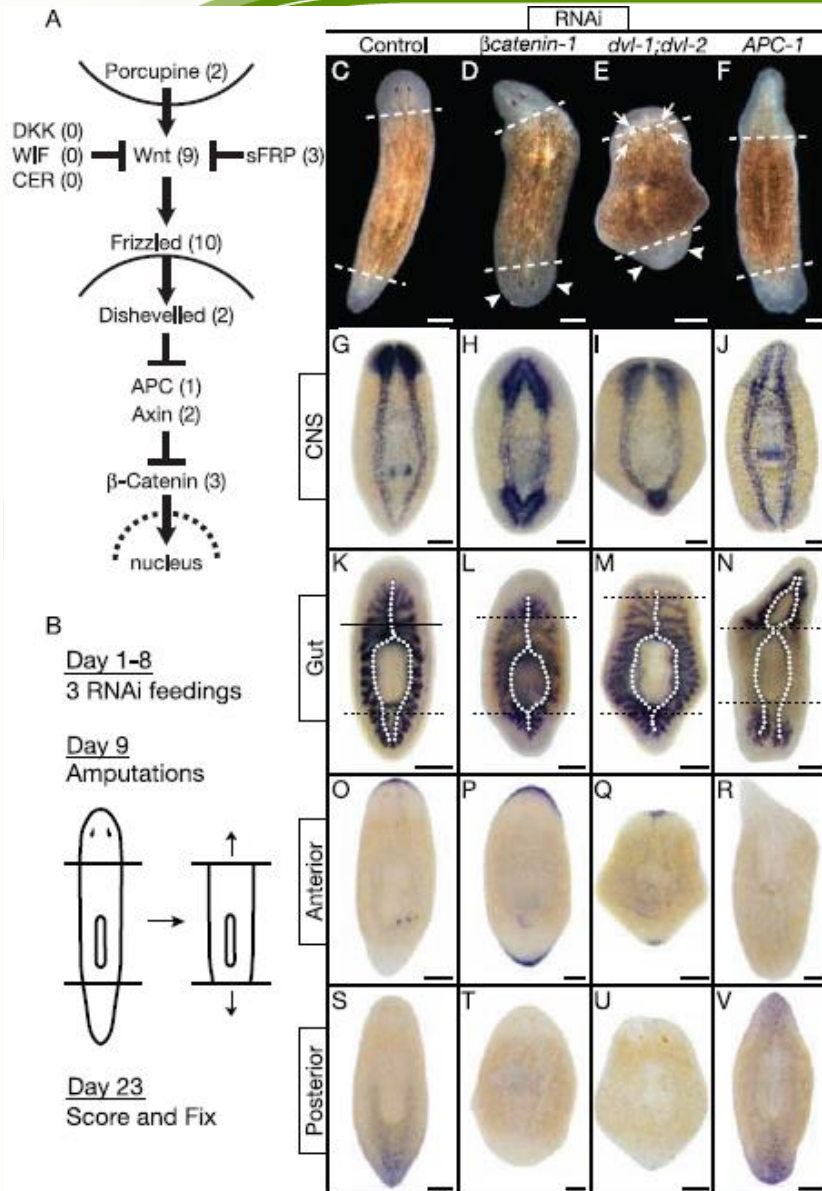
Wnt-3a knockout

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



wild type vs. Dkk1 knockout

... Wnt signální dráha určuje předozadní osu i při regeneraci u ploštěnky



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

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

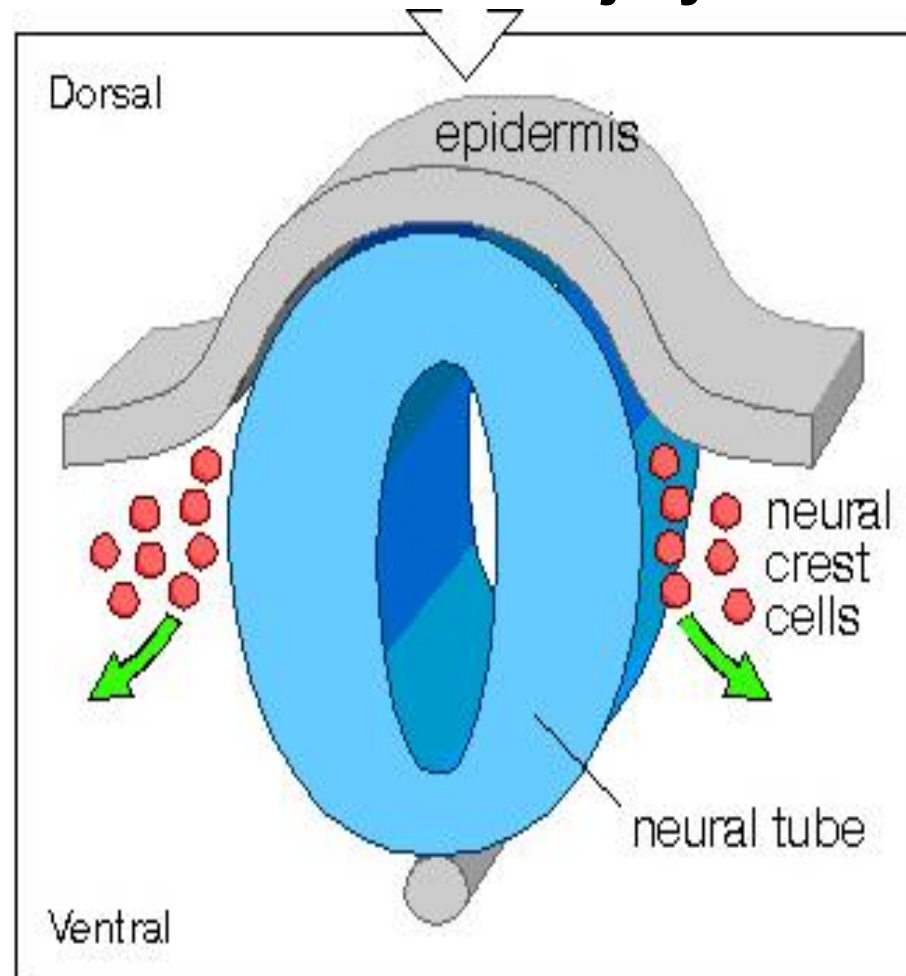
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Příklady vývojových procesů regulovaných kanonickou Wnt dráhou

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

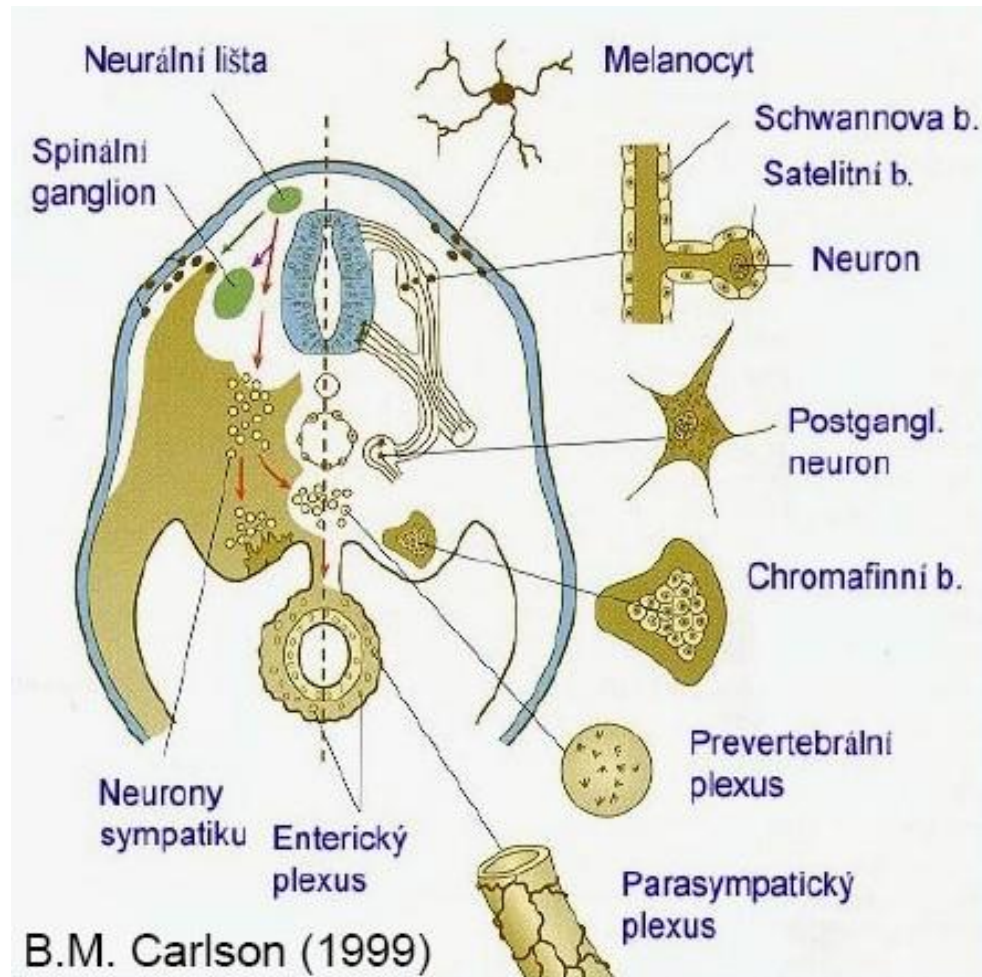


Co všechno z NC vzniká?

- podíl na vzniku cca 40 různých tkání a orgánů

Oblast trupu:

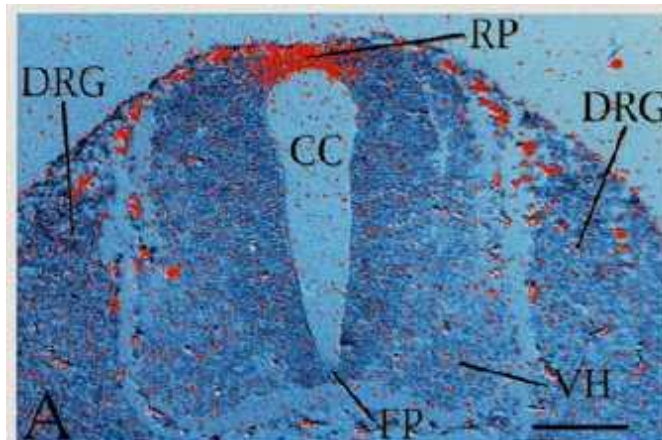
- Neurony a gliální buňky senzorického, sympatického a parasympatického systému
- Buňky dřeně nadledvin
- Pigmentové buňky epidermis
- Svalové buňky některých cév



Co všechno z NC vzniká?

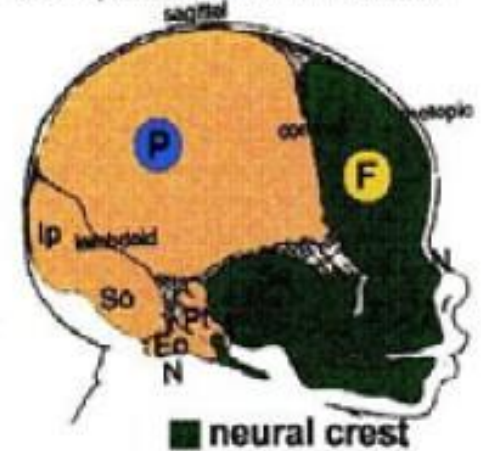
Vývoj neurální lišty:

Wnt-3a



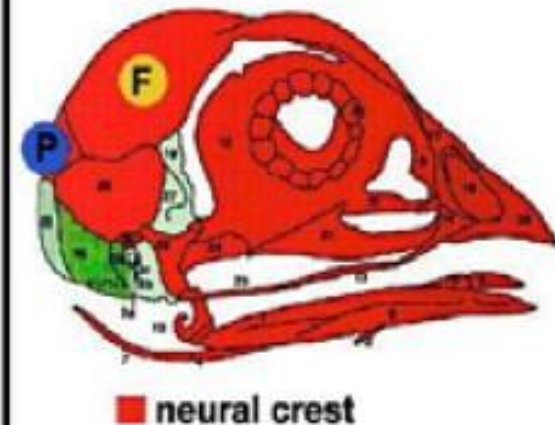
- neurální lišta je zdrojem periferního nervového systému, melanocytů, obličejových kostí a svalů, částí srdce a dalších

human skull, extrapolated from mouse:



J.Anat. 2001

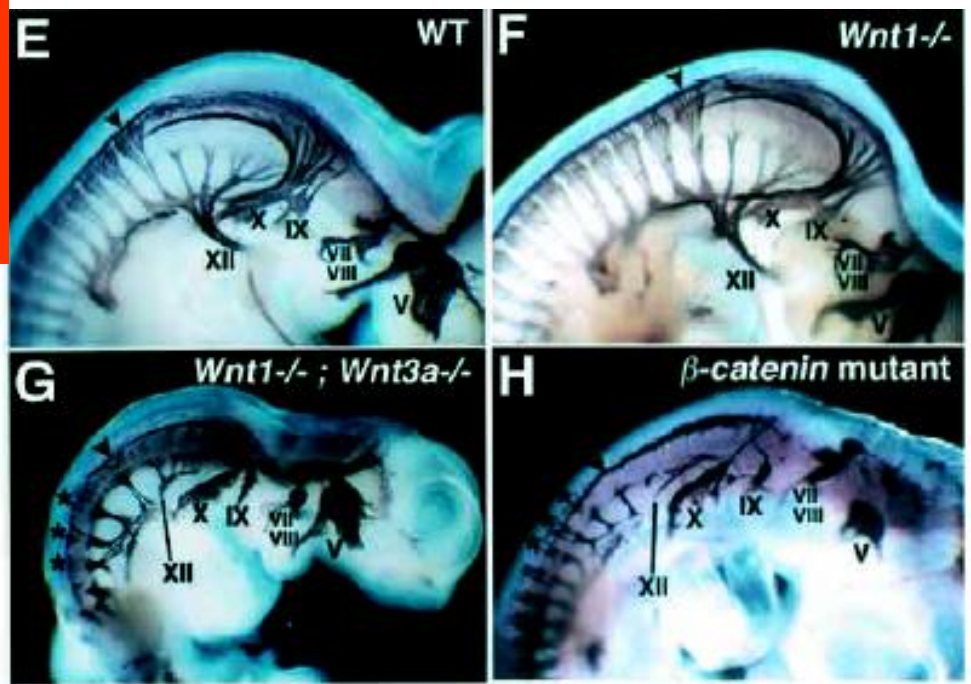
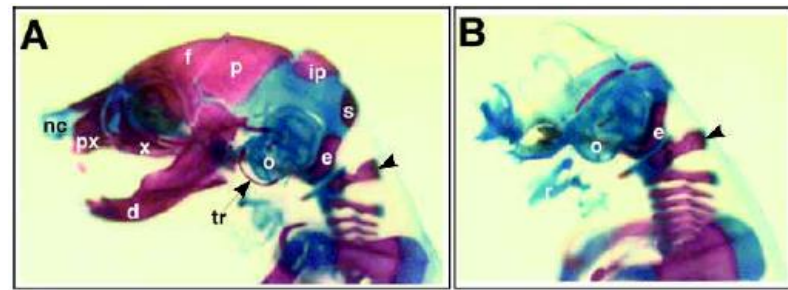
chick skull:



The neural crest (2. nd, 1999)

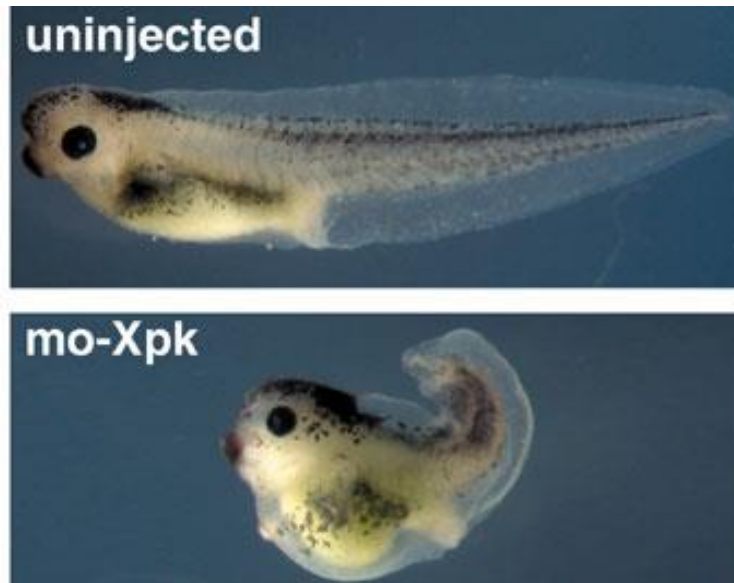
Fyziologie buň. systémů

Wnt1/3a DKO



Nekanonická Wnt dráha

- indukovaná např. ligandem Wnt5a

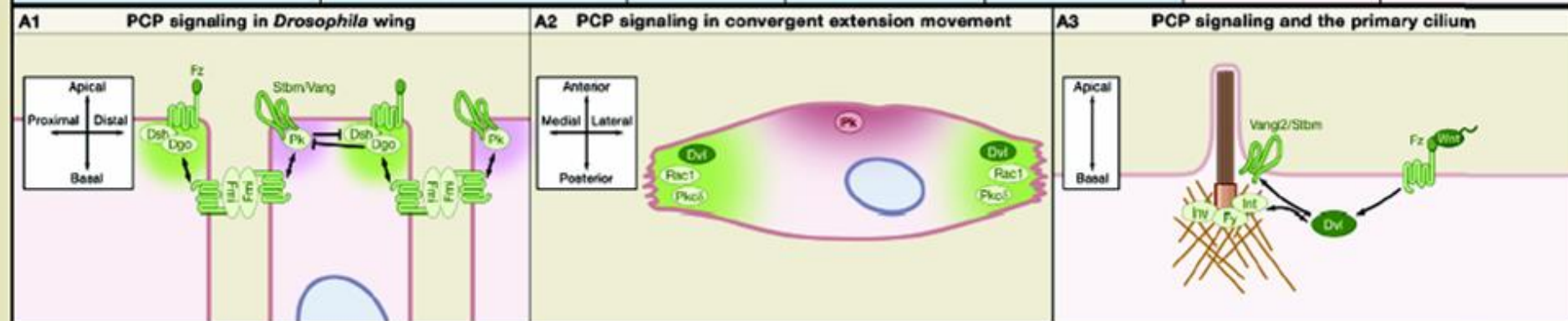
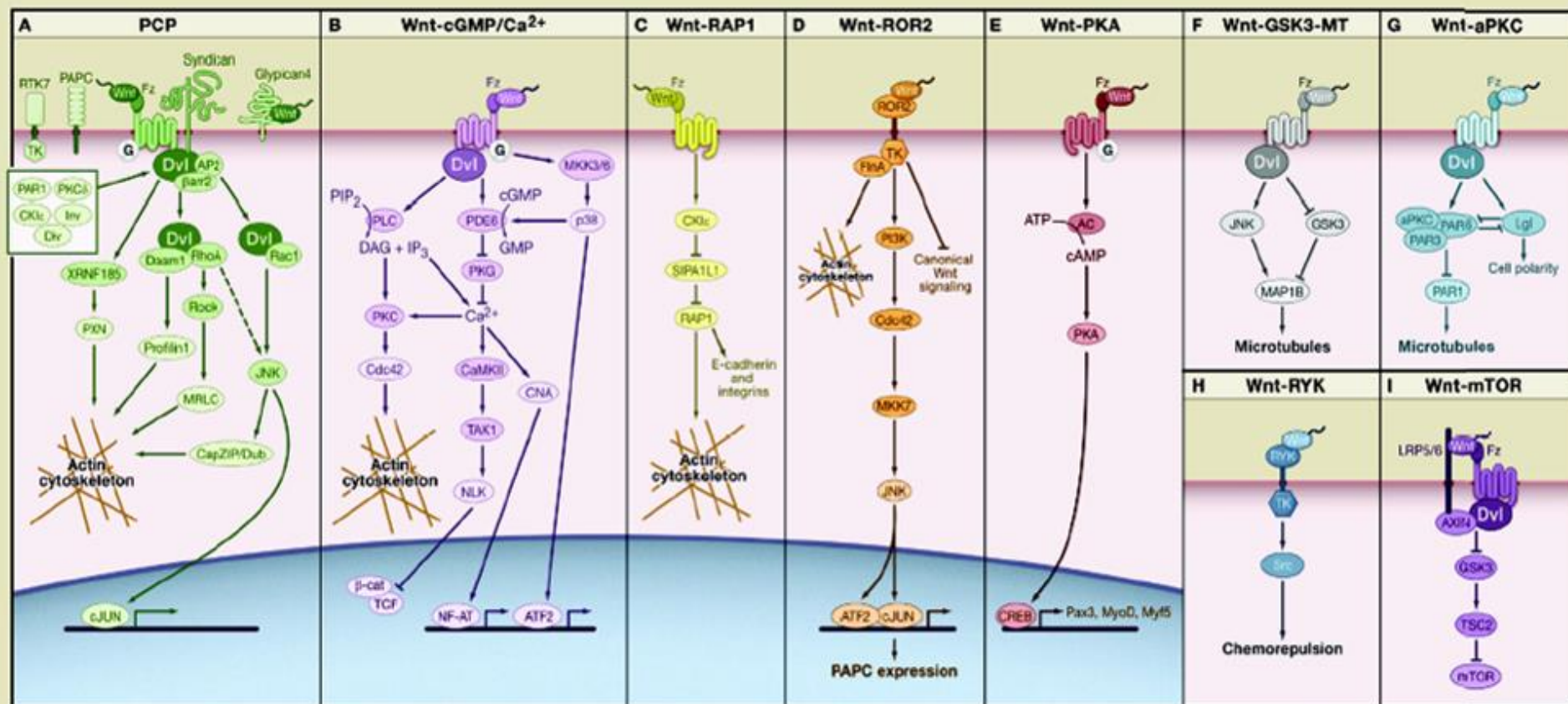


- neindukuje duplikaci tělní osy u *Xenopus*
- neindukuje transformaci buněčné linie odvozené od lidských prsních epiteliálních buněk C57mg
- signál NENÍ přenášen přes translokaci β -kateninu do jádra

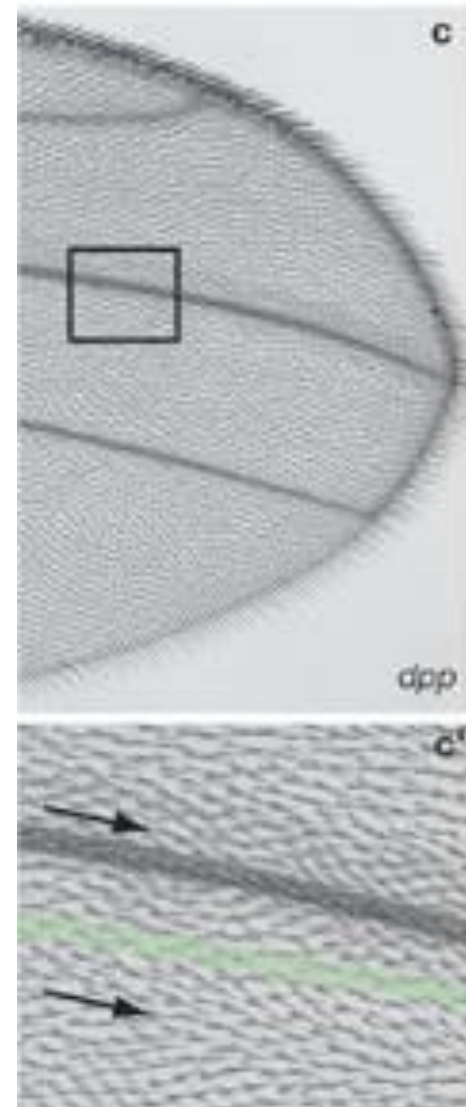
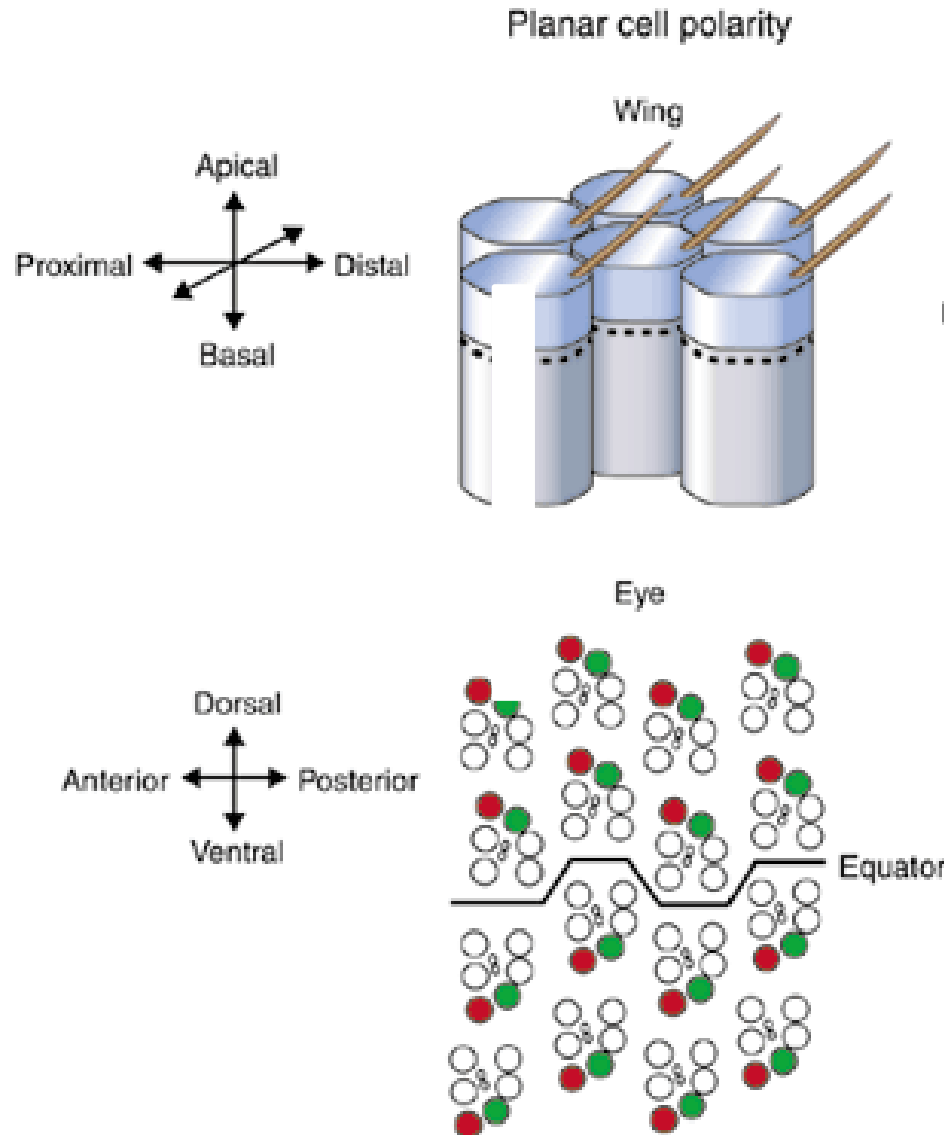
SnapShot: Noncanonical Wnt Signaling Pathways

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



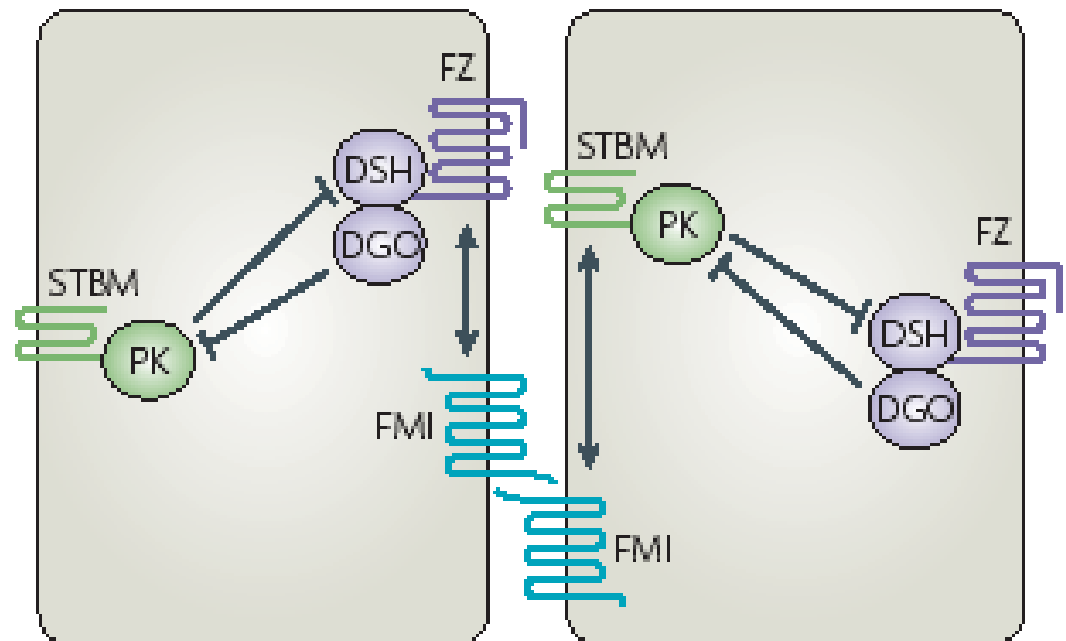
Drosophila – planární buněčná polarita (planar cell polarity, PCP)



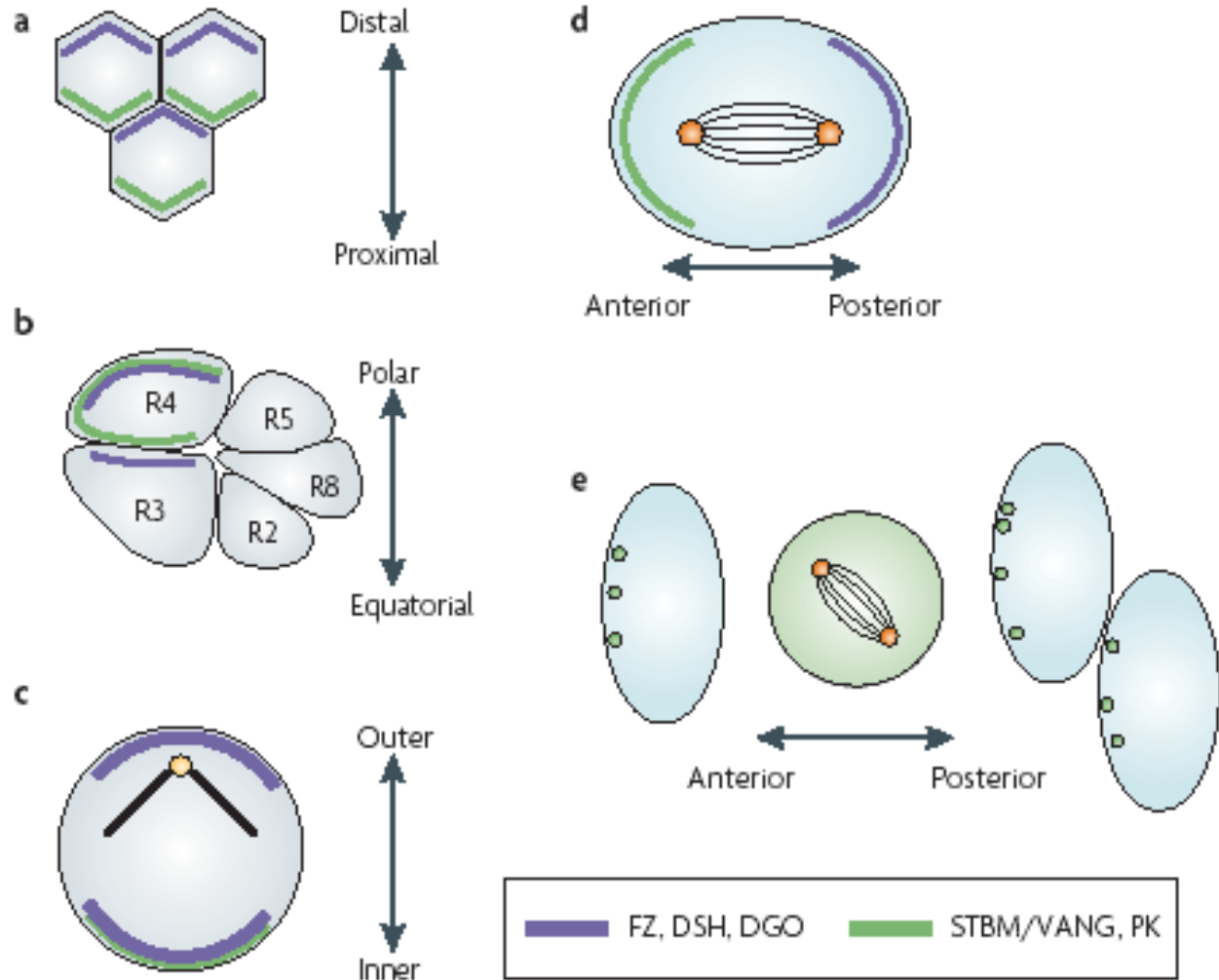
Molekulární mechanismus ustavení PCP

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

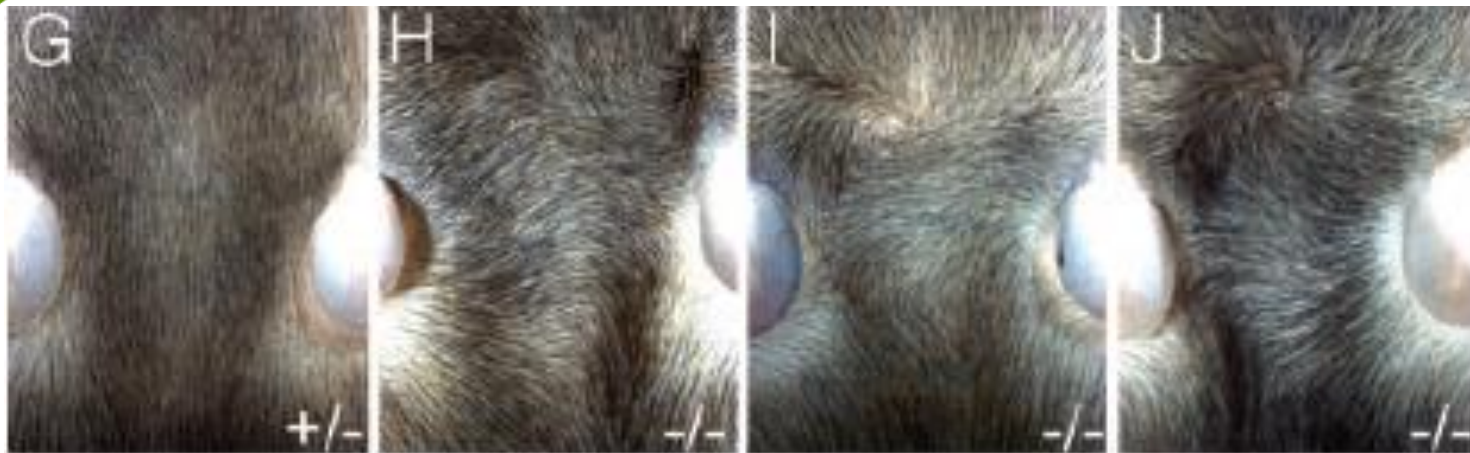


Molekulární mechanismus ustavení PCP

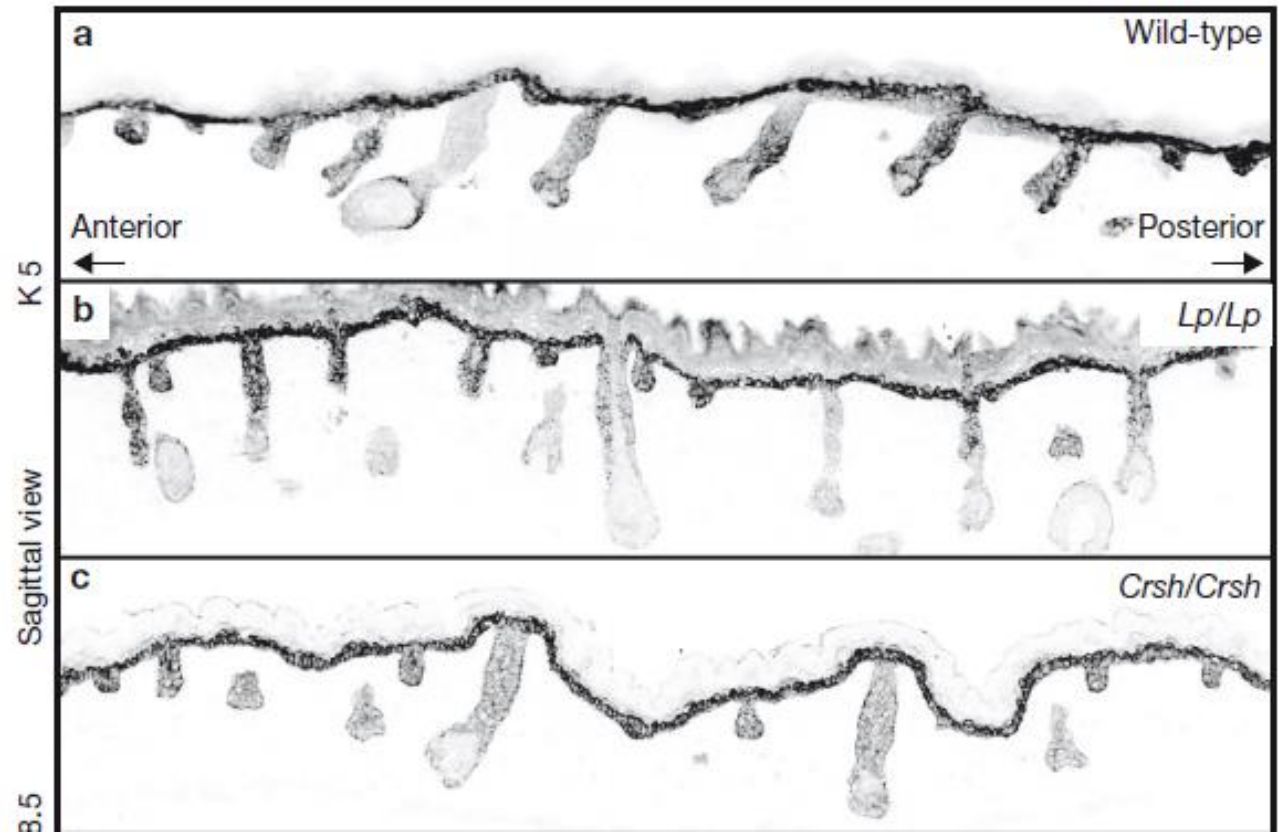
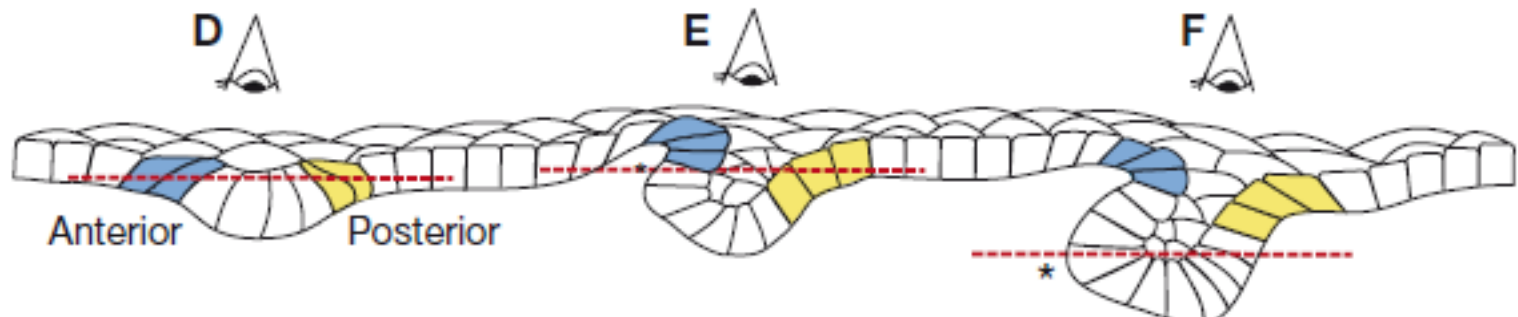
- legenda k obrázku:

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).

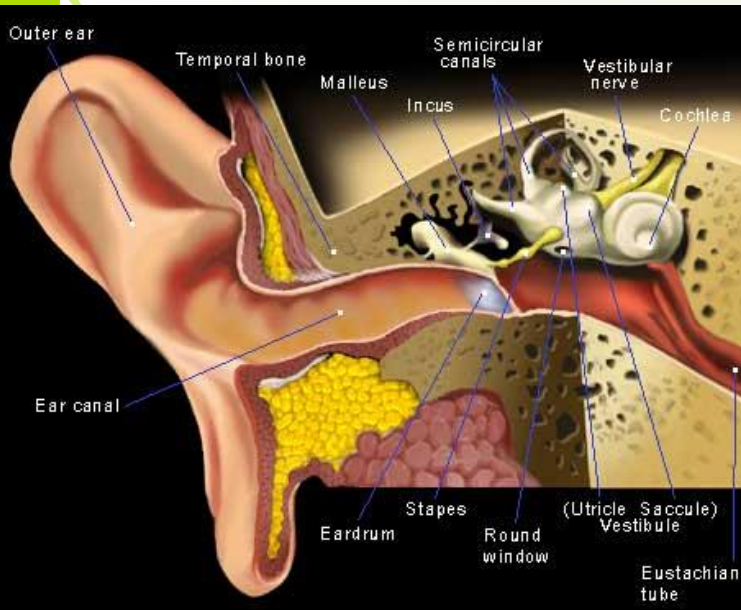
Poruchy v nekanonické signální dráze Wnt u savců



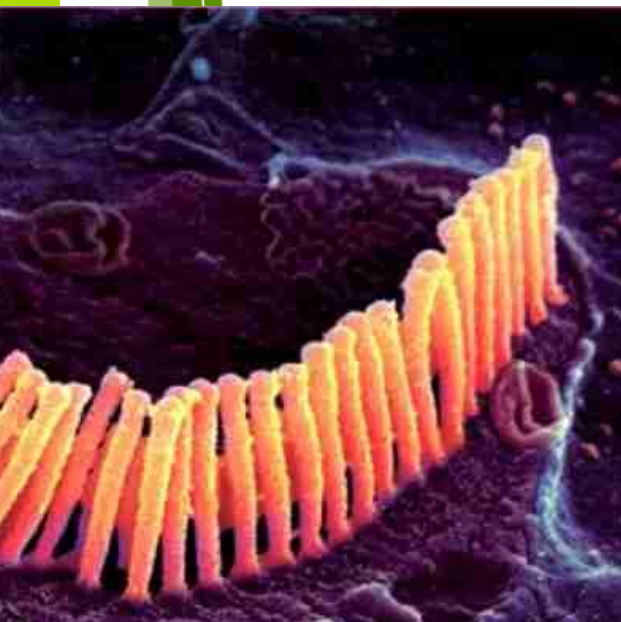
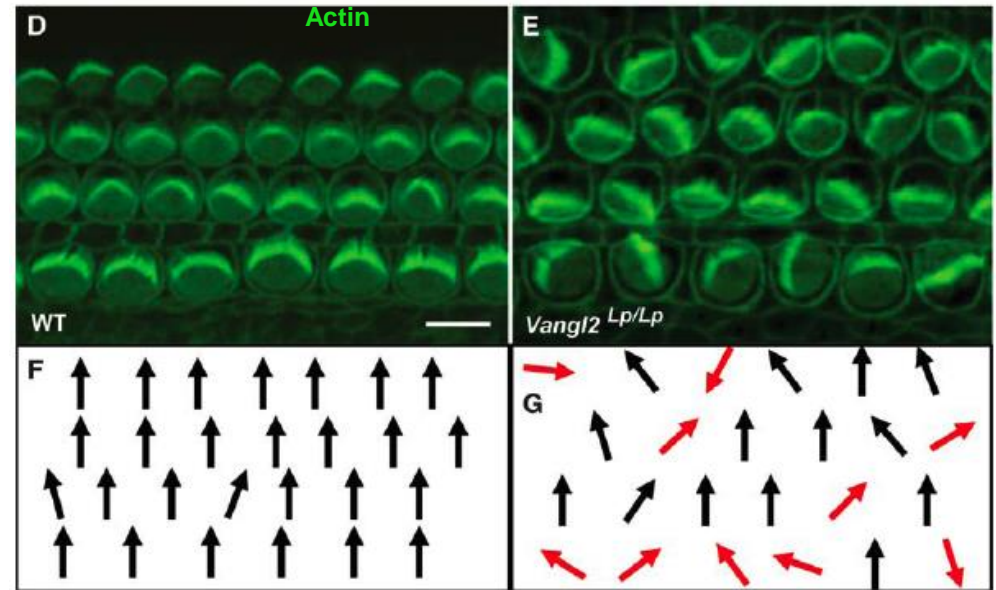
Poruchy v nekanonické signální dráze Wnt u savců



Nekanonická dráha/dráha PCP: fenotypy u myši

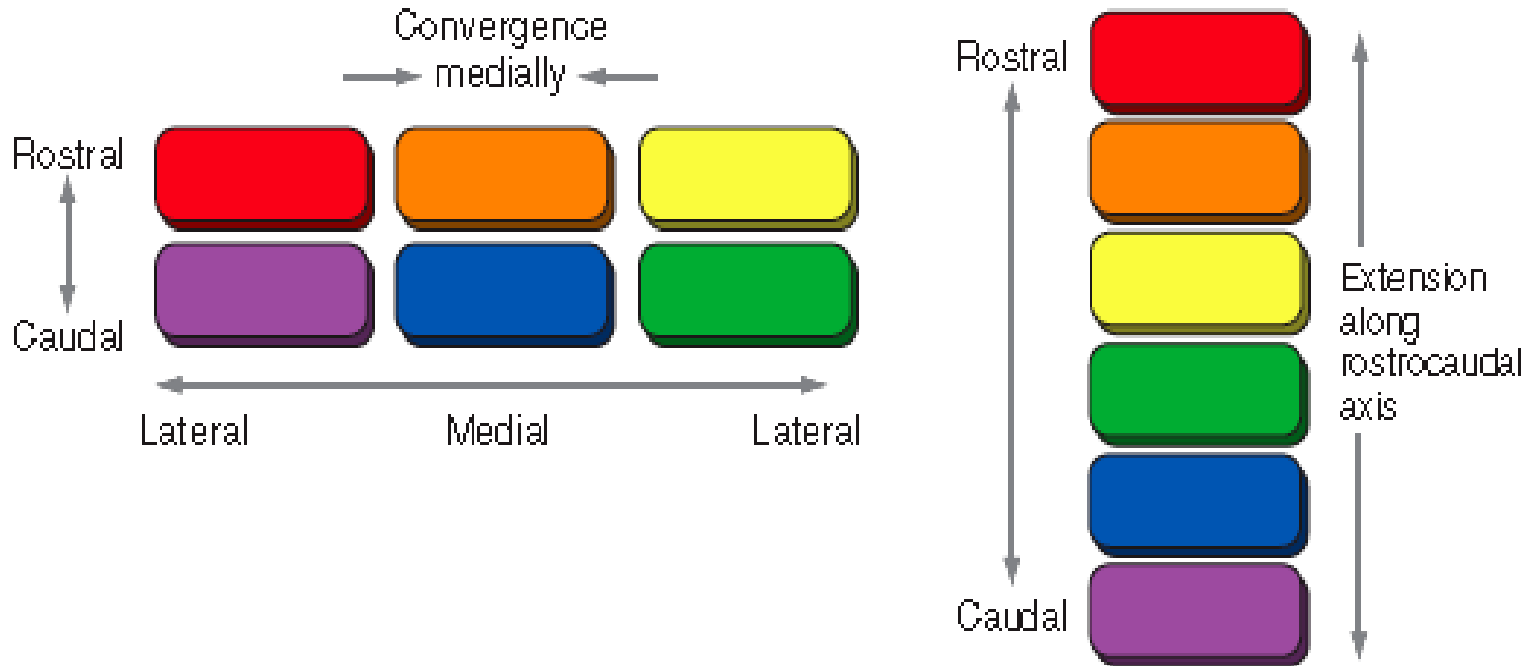


Orientace stereocilií vláskových buněk ve vnitřním uchu



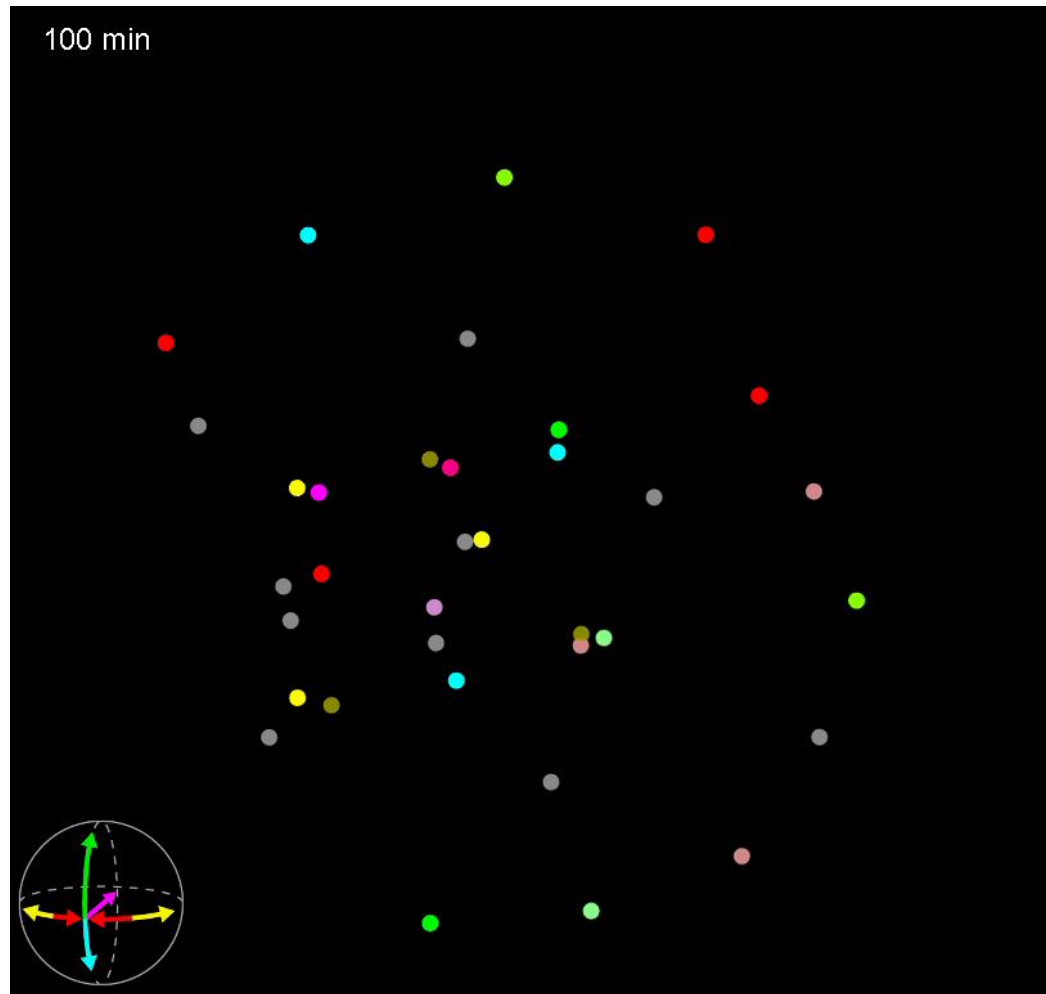
Qian et al., 2007, Dev. Biol.

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

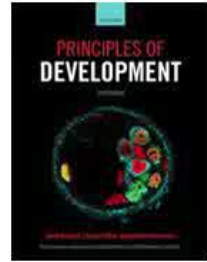


Konvergentní extenze – migrace buněk směrem ke středu těla – vede k prodlužování tělní osy

Konvergentní extenze - video



Důsledky narušené konvergentní extenze (CE)



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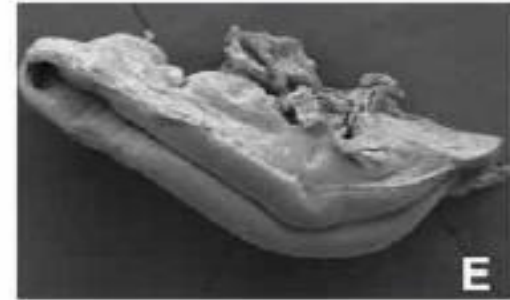


Důsledky narušené konvergentní extenze (CE)

Exencefalie:



Otevřená nervová trubice:



Hamblet et al., 2002, Development