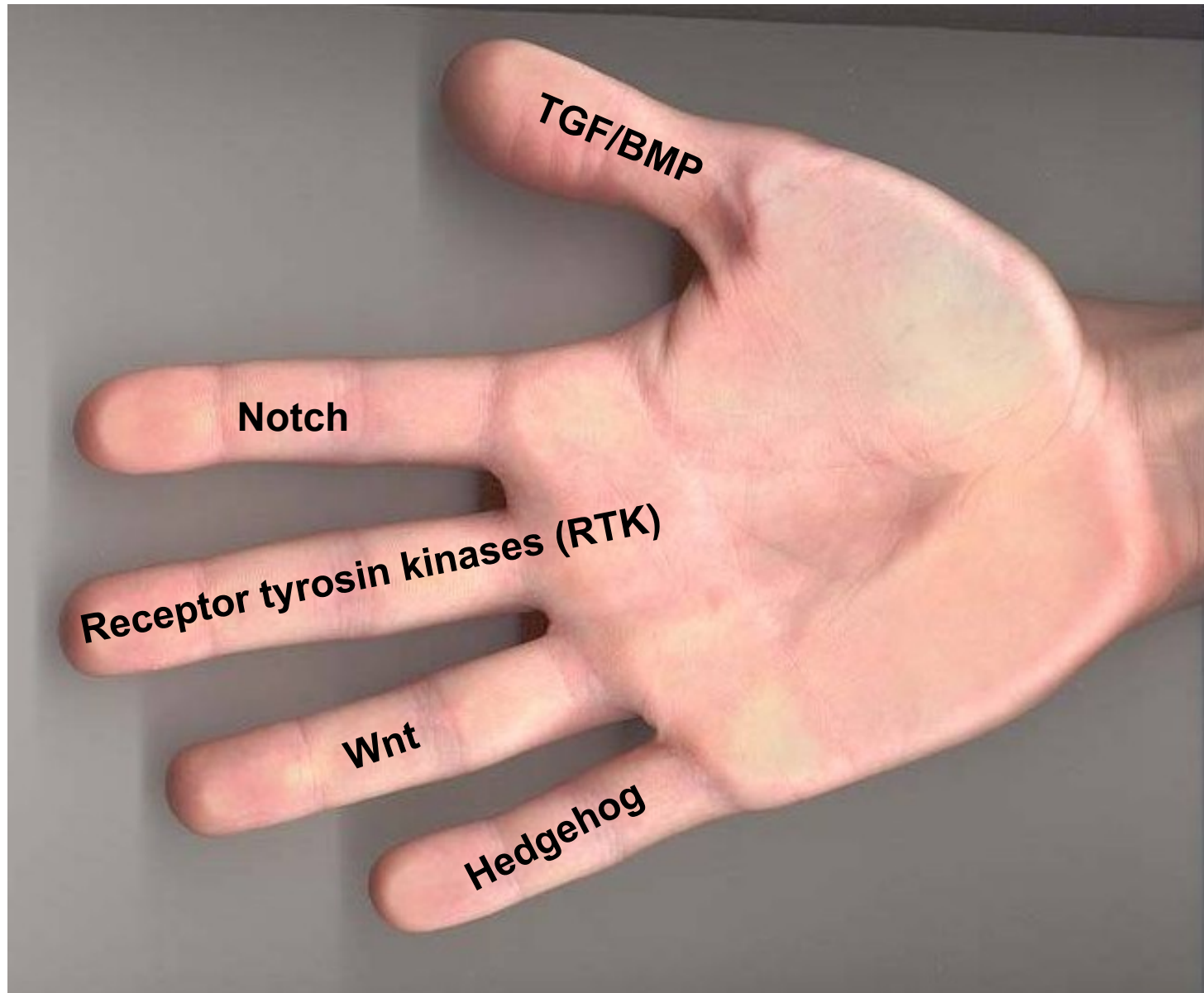


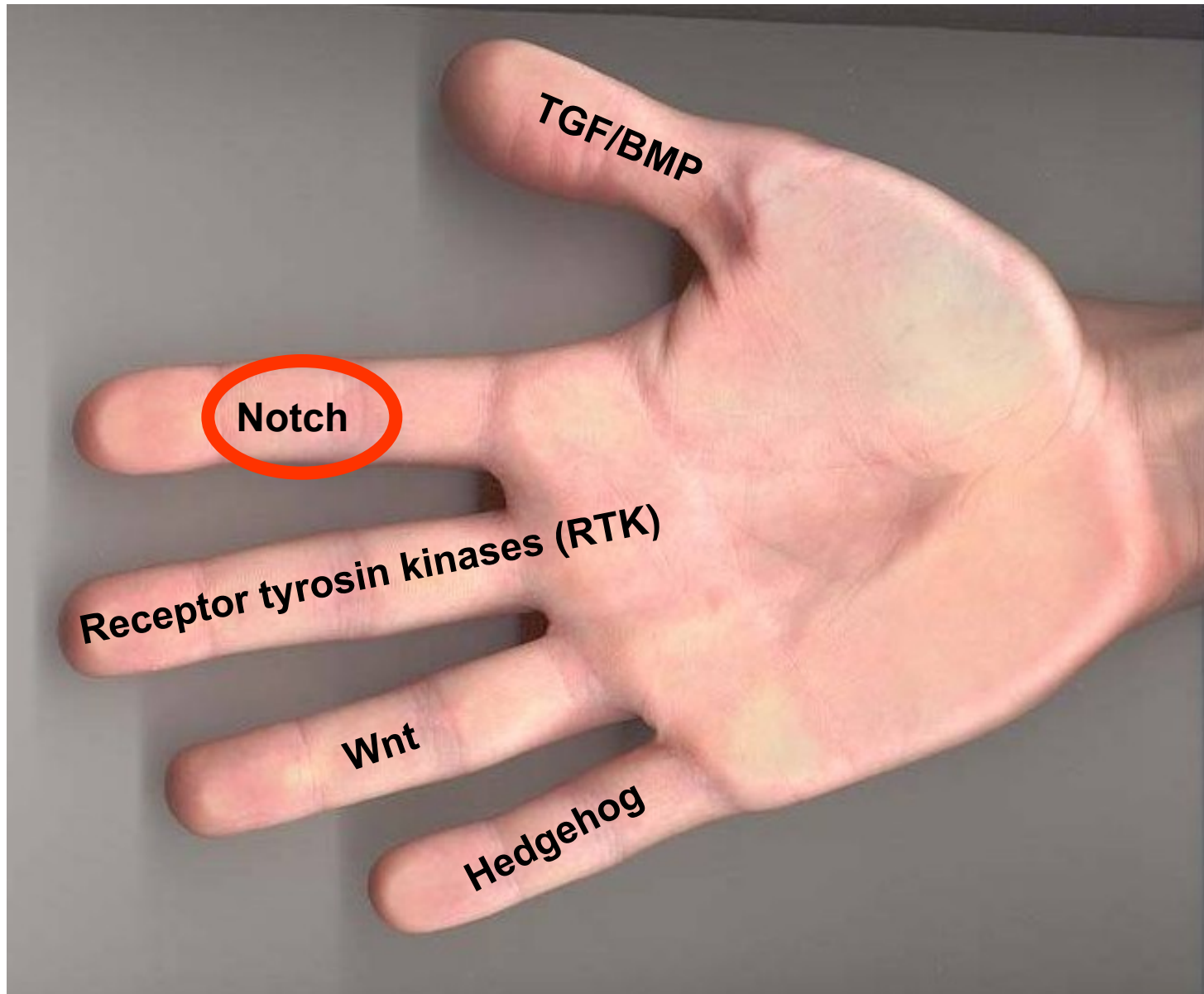
**#7**

**Klíčové molekulární komponenty  
vývoje II**

# Signály z vnějšího prostředí

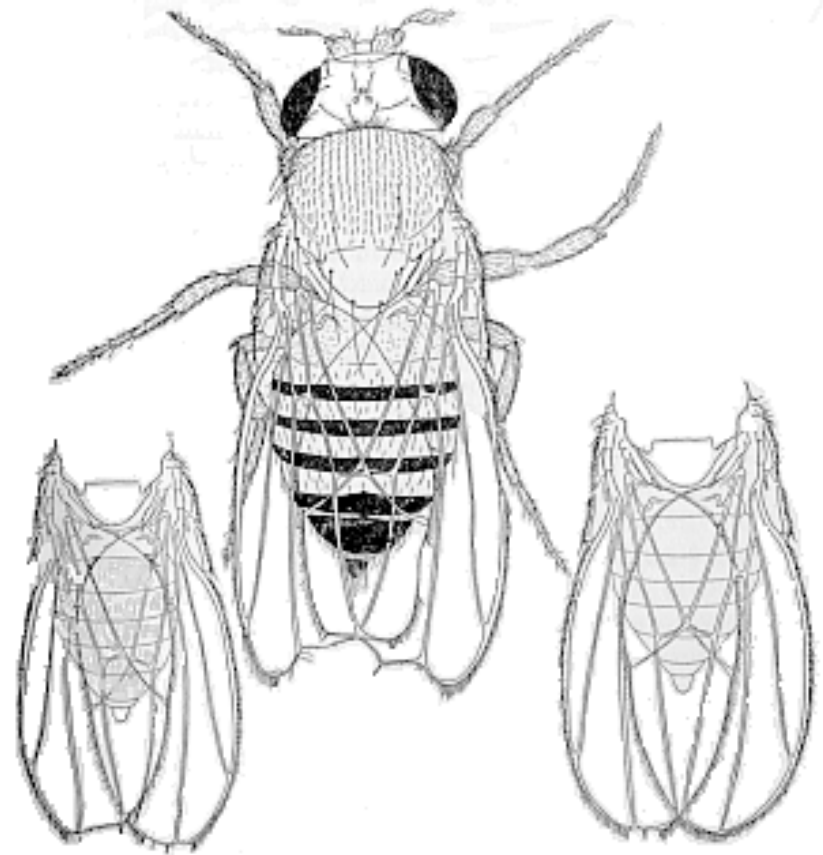


# Signály z vnějšího prostředí

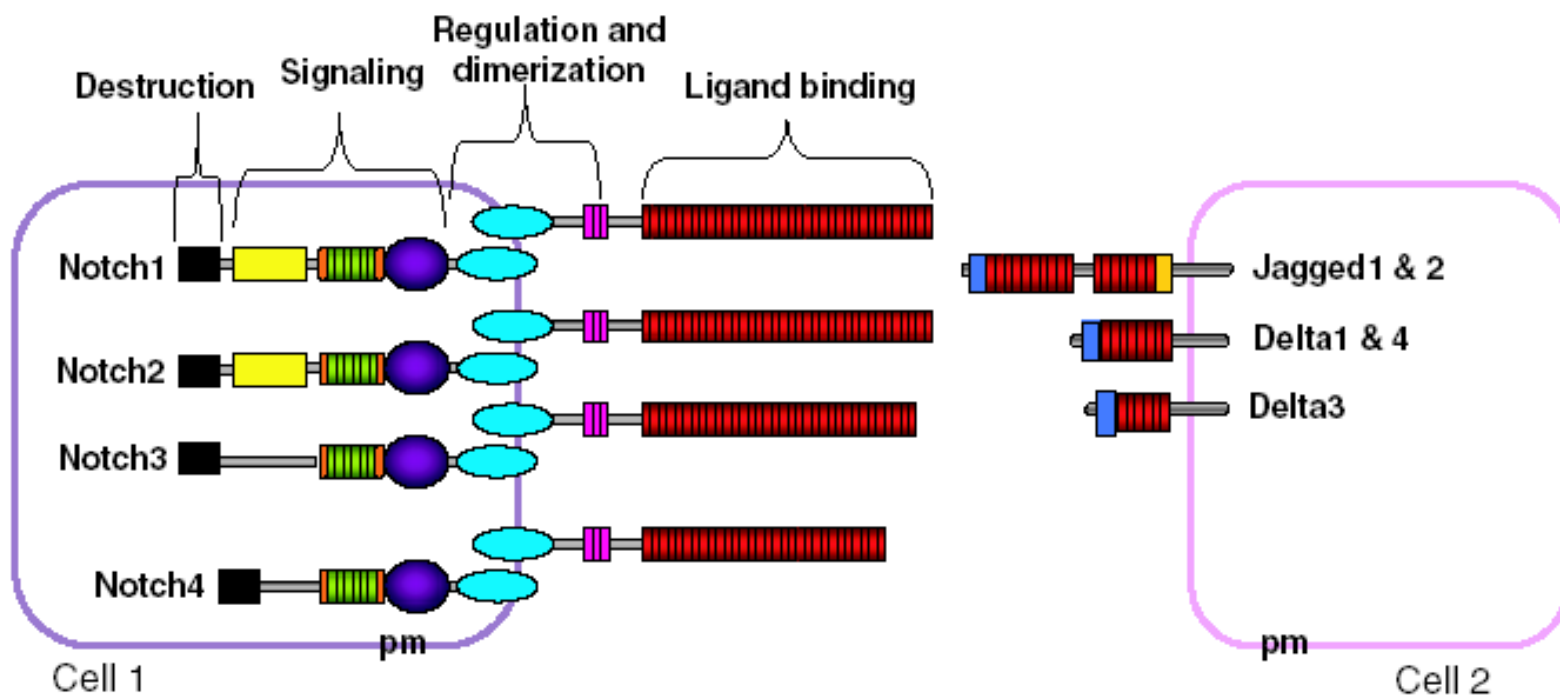


# Notch

- Notch=zářez – podle prvního fenotypu octomilky se zářezy na křídlech (T.H. Morgan, 1919)



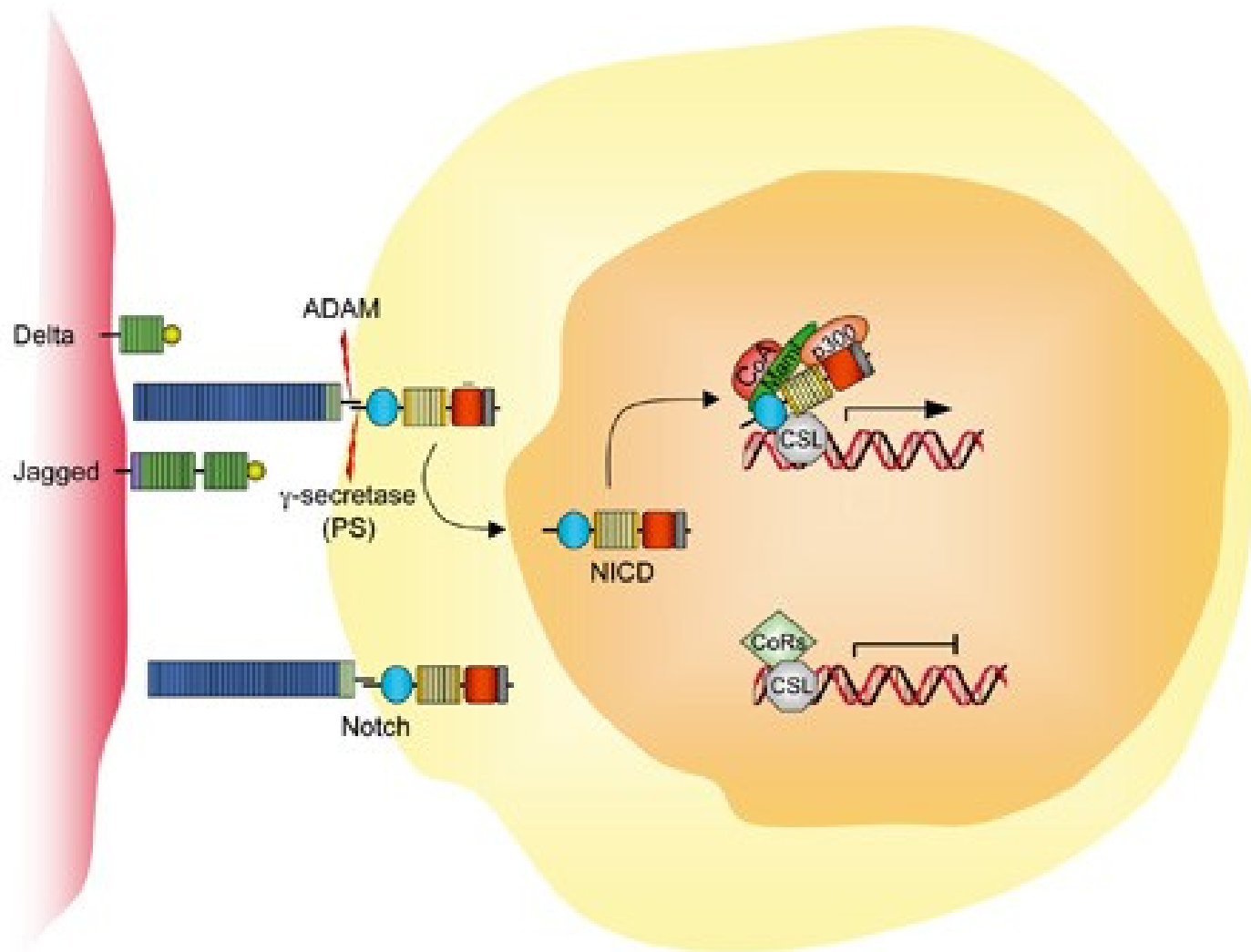
A



receptory Notch1-4

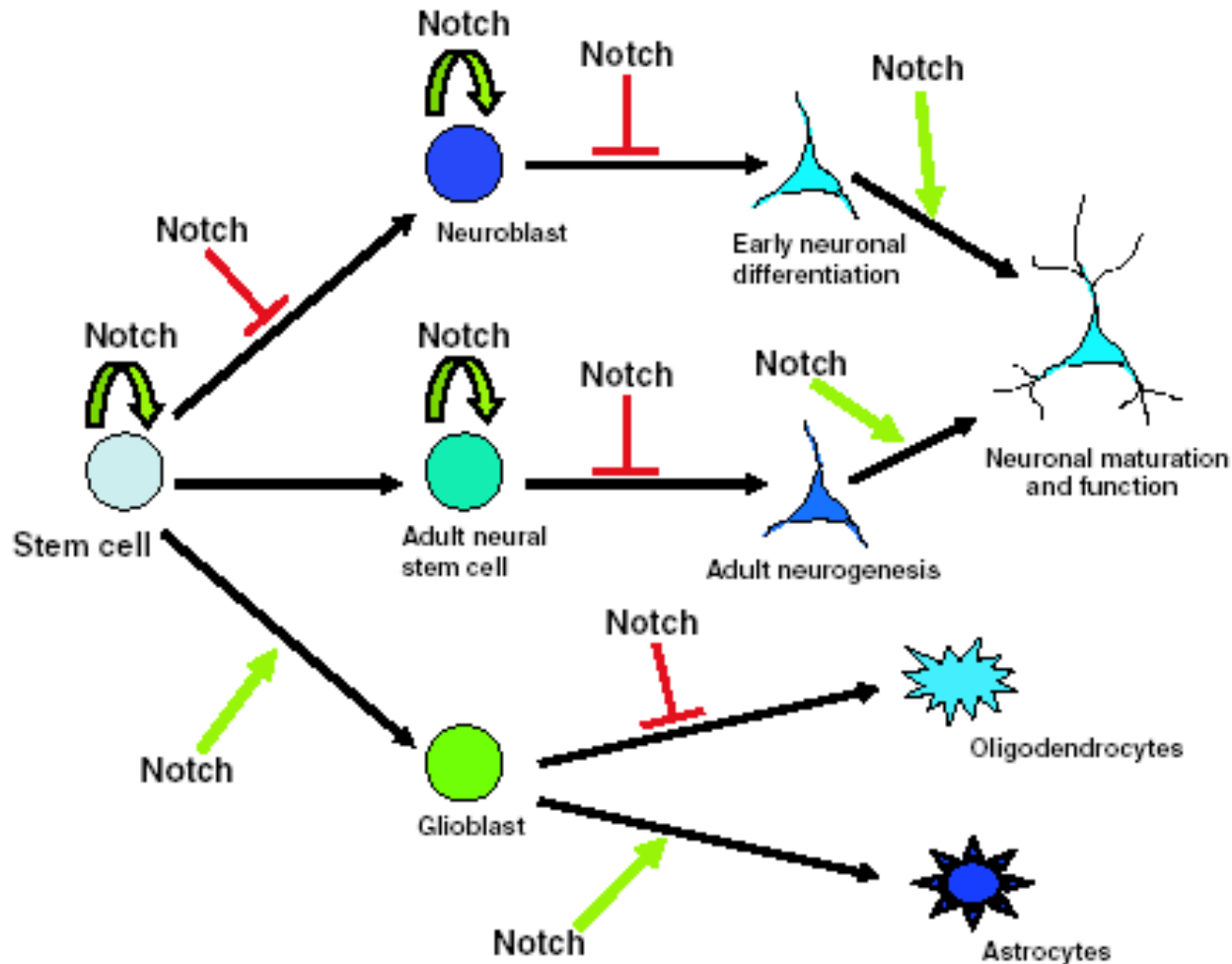
Notch ligandy – jsou vázány na buněčný povrch

# Notch dráha - overview

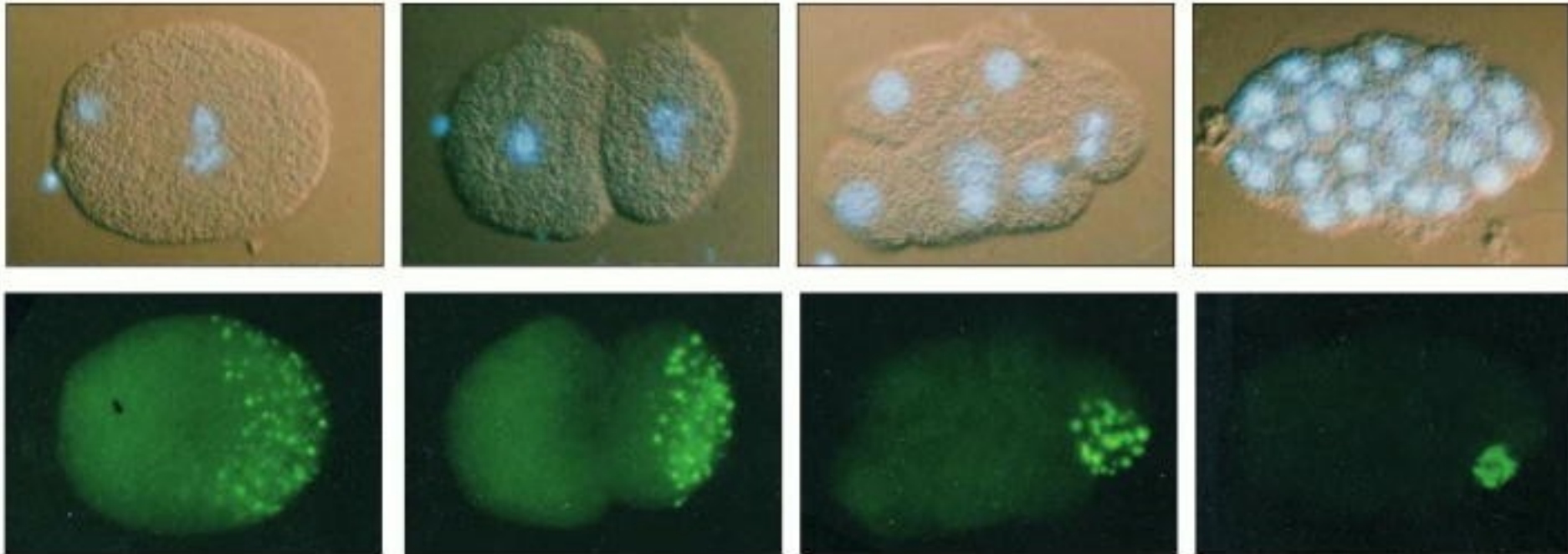


Notch dráha, stejně jako ostatní morfogenetické systémy, reguluje (ať už negativně nebo pozitivně) jednotlivé vývojové procesy na několika stupních.

**Příklad: vývoj jednotlivých buněčných typů v nervovém systému**



# Notch a asymetrické buněčné dělení



## **Asymmetric divisions segregating P granules into the founder cell of the *C. elegans* [germ line](#).**

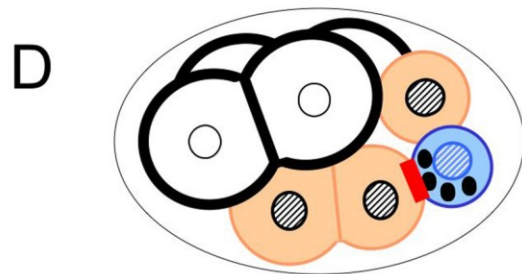
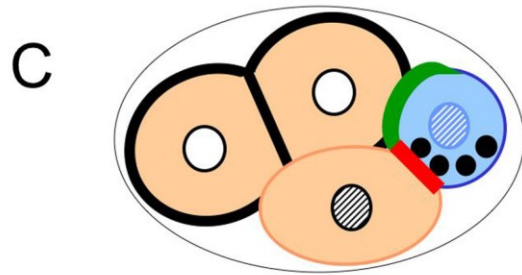
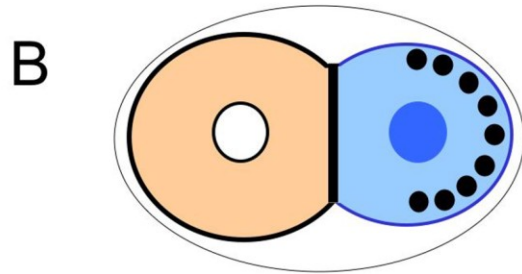
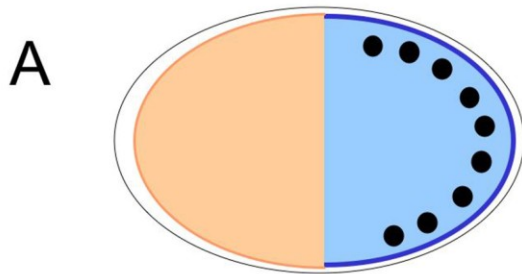
The micrographs in the upper row show the pattern of cell divisions, with cell nuclei stained blue with a DNA-specific fluorescent dye; below are the same cells stained with an antibody against P granules. These small granules (0.5–1  $\mu\text{m}$  in diameter) are distributed randomly throughout the cytoplasm in the unfertilized egg (not shown). After fertilization, at each cell division up to the 16-cell stage, both they and the intracellular machinery that localizes them asymmetrically are segregated into a single daughter cell. (Courtesy of Susan Strome.)



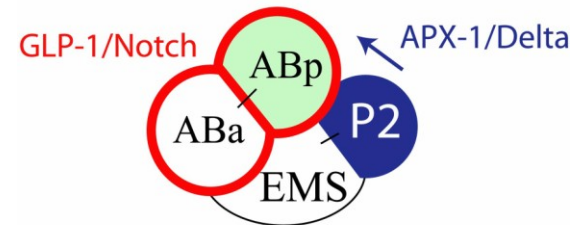
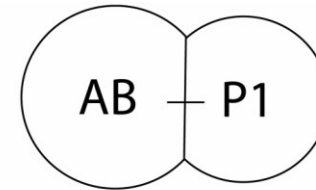
MOVIE06 PIE-1 .m



# Notch a asymetrické buněčné dělení



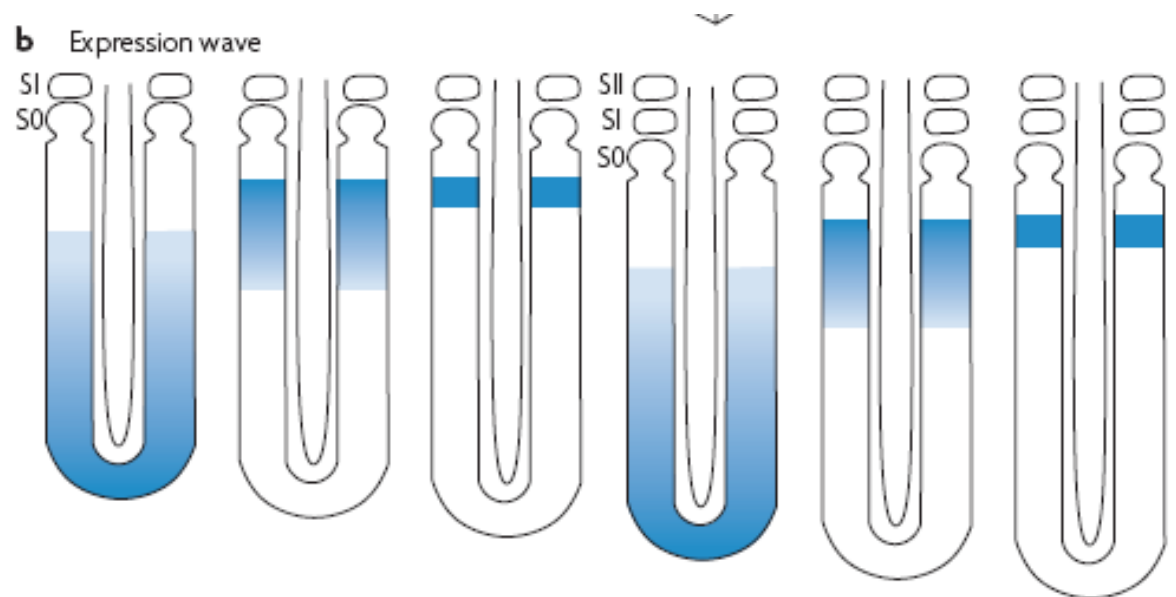
**Asymmetric localization of polarity mediators and cell fate determinants in the early embryo.** P granules: black discs; cytoplasmic POS-1, MEX-1, and cytoplasmic and nuclear PIE-1: blue; nuclear PAL-1: hatched; MEX-5 and MEX-3: peach; plasma membrane localized GLP-1: black; membrane localized APX-1: green; membrane localized MES-1: red. Although shown discretely localized for simplicity, the cytoplasmic proteins are present at low levels in the opposite domain before division, and in the sister cell after division. In addition, MEX-5, MEX-3, MEX-1, POS-1 and PIE-1 are also present on P granules. (A) MEX-5, MEX-3, MEX-1, PIE-1, POS-1 and P granules are uniformly present in the cytoplasm just after fertilization, but become asymmetrically localized during the one-cell stage. (B) The anterior and posterior determinants are differentially segregated to AB and P1 as a result of the first asymmetric division. GLP-1 protein first appears in AB at the two-cell stage, and PIE-1 protein enters the nucleus in addition to being cytoplasmic. As the cell cycle proceeds (not shown), posterior determinants become restricted to the posterior half of P1, while MEX-5 appears in the anterior half of P1. (C) In the four-cell embryo, GLP-1 is expressed on membranes of both AB cells, but only ABp is in contact with the P2 cell expressing APX-1. MES-1 is enriched at the cell contact between P2 and EMS; MES-1/SRC-1 signaling in conjunction with Wnt signaling polarizes the EMS cell, such that it will divide asymmetrically. As the cell cycle proceeds, posterior determinants within P2 become asymmetrically localized as in previous P cells. MEX-5 disappears from the AB cells, but is still present in the anterior daughters of each P division.



# Notch and the segmentation clock



segmentation clock movie.r



cellular states. **b** | Evidence for an oscillator underlying vertebrate segmentation. Periodic waves of transcriptional expression of the *hairy1* gene (blue) in PSM cells are associated with the formation of each pair of somites added sequentially<sup>18</sup>. Part **a** modified with permission from REF. 14 © (1976) Elsevier Ltd.



# Jak studovat somitogenezi u myši?

## T/Mesp2

## Uncx4.1/Hes7

*Wnt5a*<sup>+/+</sup>; *LRP6*<sup>+/+</sup>

*Wnt5a*<sup>-/-</sup>; *LRP6*<sup>+/+</sup>

*Wnt5a*<sup>+/+</sup>; *LRP6*<sup>+/+</sup>

*Wnt5a*<sup>-/-</sup>; *LRP6*<sup>+/+</sup>

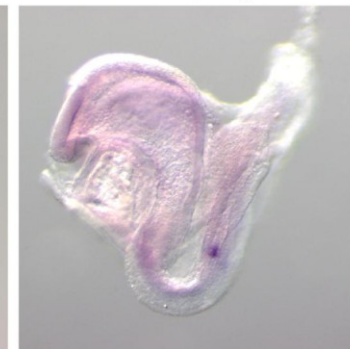
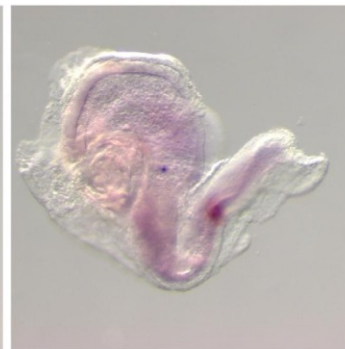


## Mesp2/Ripply2

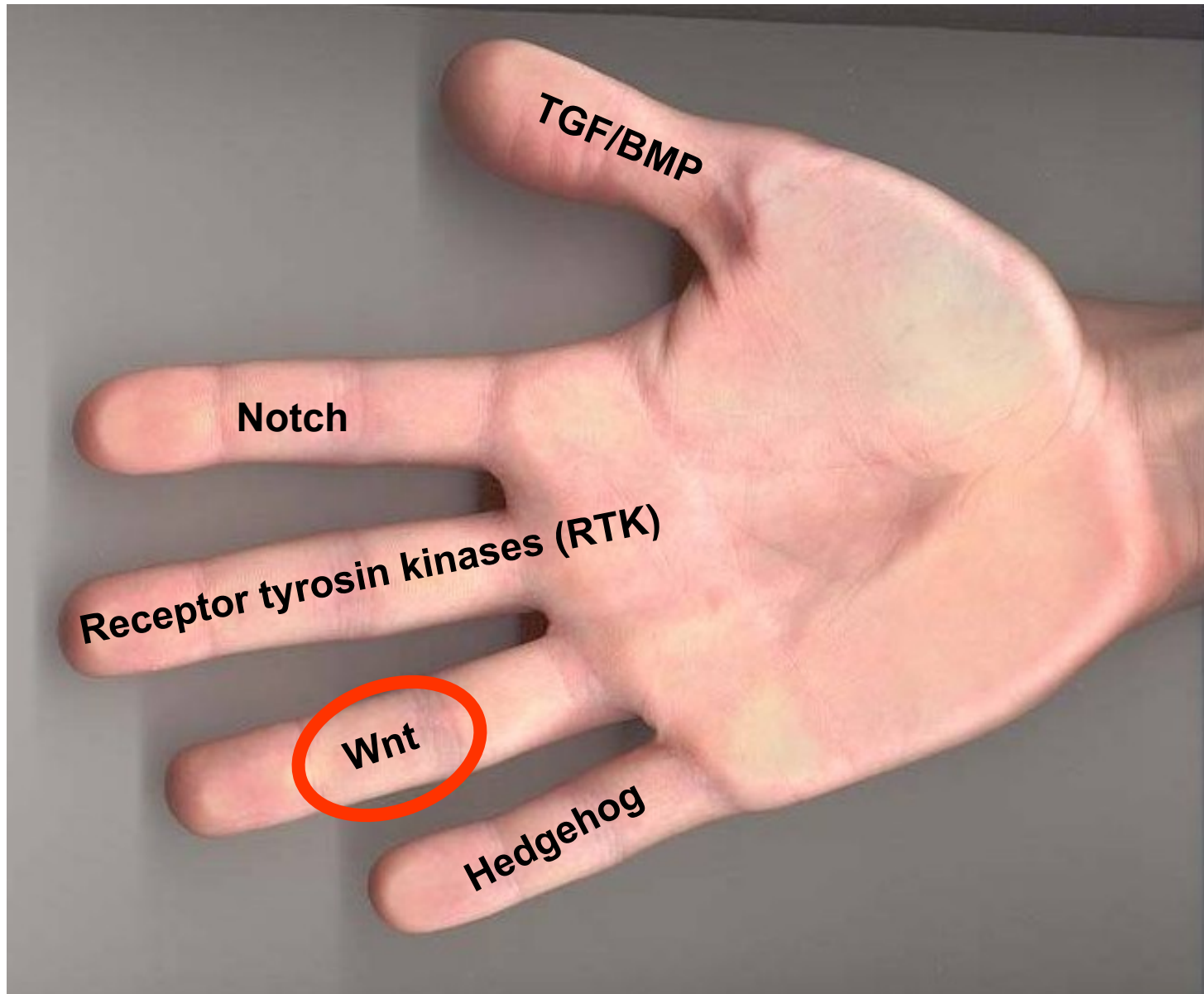
*Wnt5a*<sup>+/-</sup>; *Lrp6*<sup>+/-</sup>

*Wnt5a*<sup>+/-</sup>; *Lrp6*<sup>-/-</sup>

*Wnt5a*<sup>+/+</sup>; *Lrp6*<sup>-/-</sup>



# Signály z vnějšího prostředí

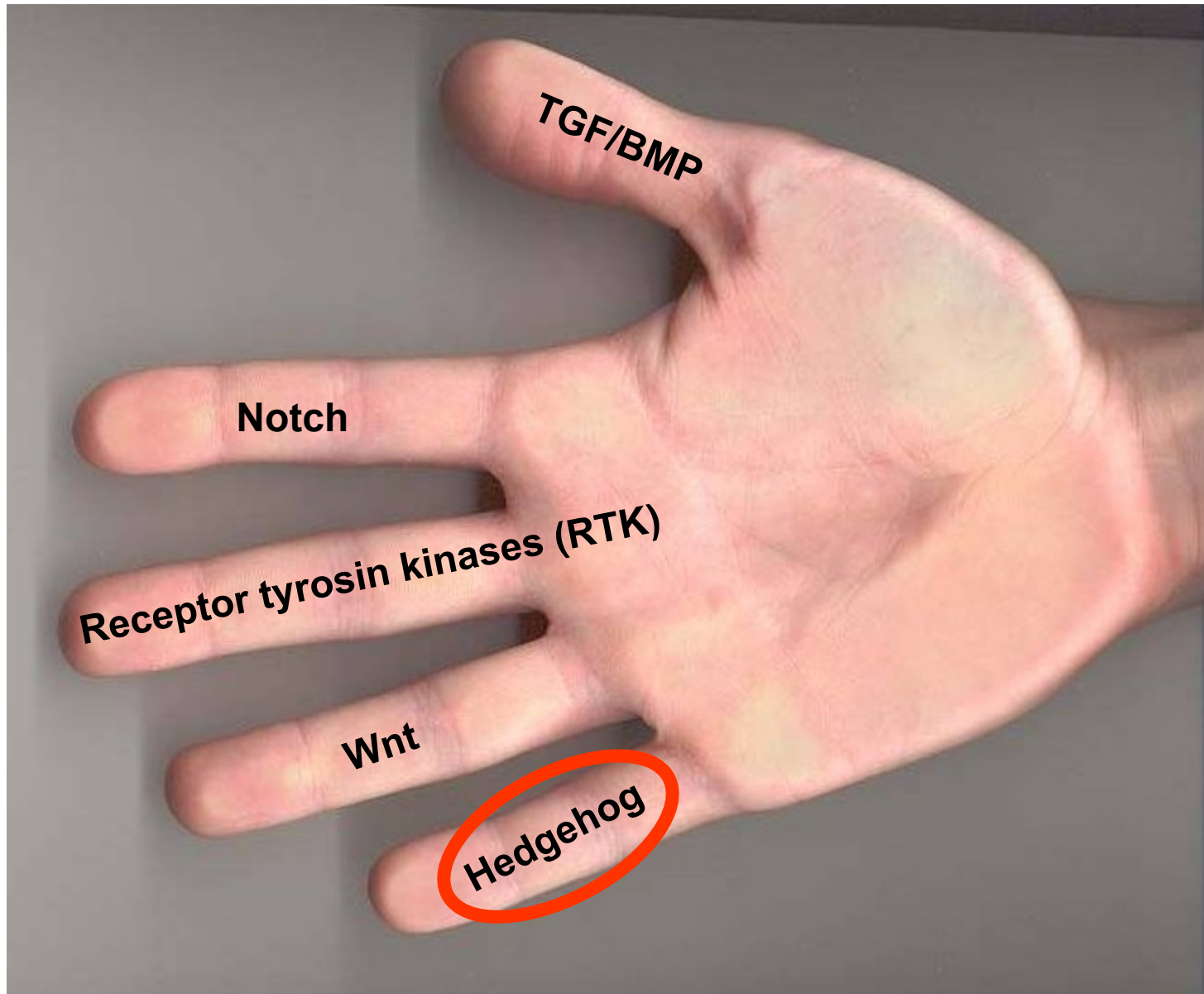


# Wnts – viz přednáška č. 10

- rodina ligandů
- 19 členů u člověka a myši
- glykosylované a palmitoylované extracelulární proteiny
- Název: z Wingless – první mutace u *Drosophily* a Int – onkogen přenášený MMTV-virem a způsobující prsní karcinomy

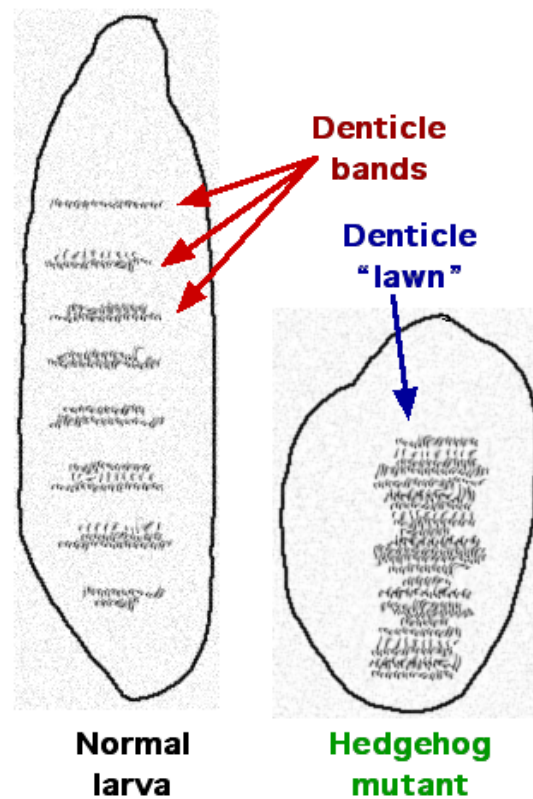


# Signály z vnějšího prostředí



# Hedgehog dráha

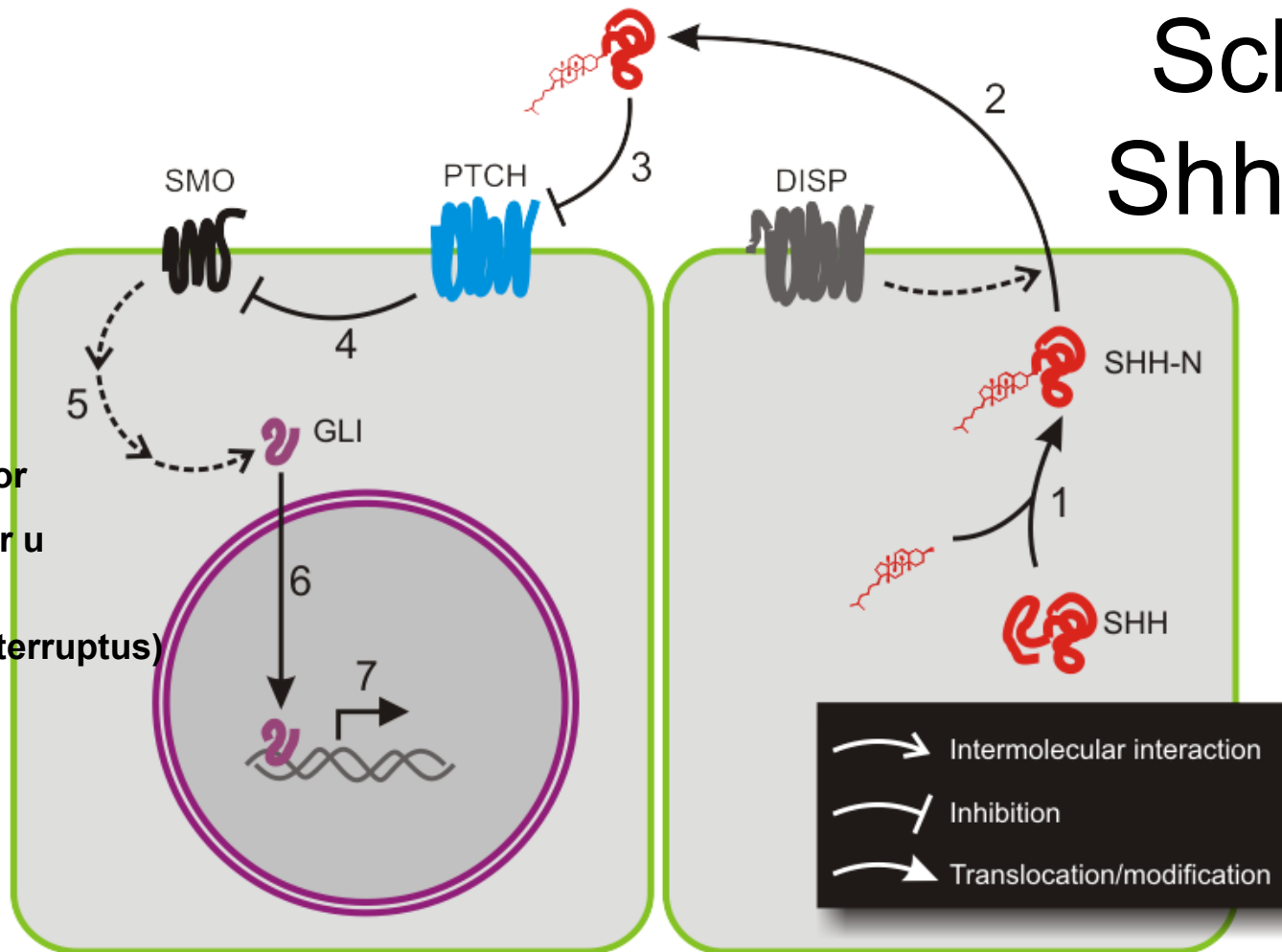
- hedgehog (Hh) u octomilky – název „ježek“ podle fenotypu larvy
- u savců jsou tři homology – sonic hedgehod (Shh), indian hedgehog (Ihh) a desert hedgehog (Dhh)



Sonic the Hedgehog



# Schéma Shh dráhy



**Gli1,2-aktivátor**

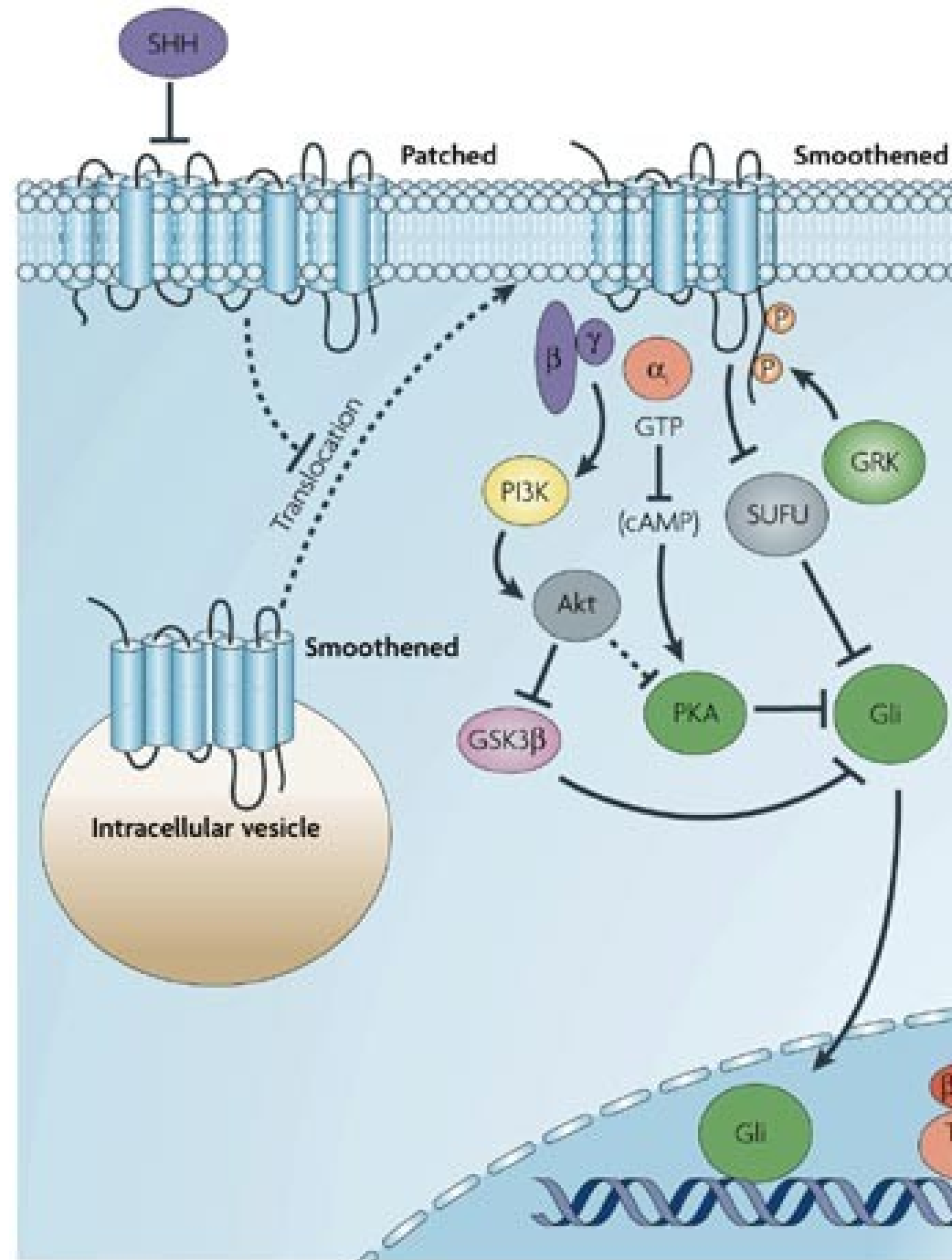
**Gli3 - represor u obratlovců**

**Ci (cubitus interruptus) u octomilky**

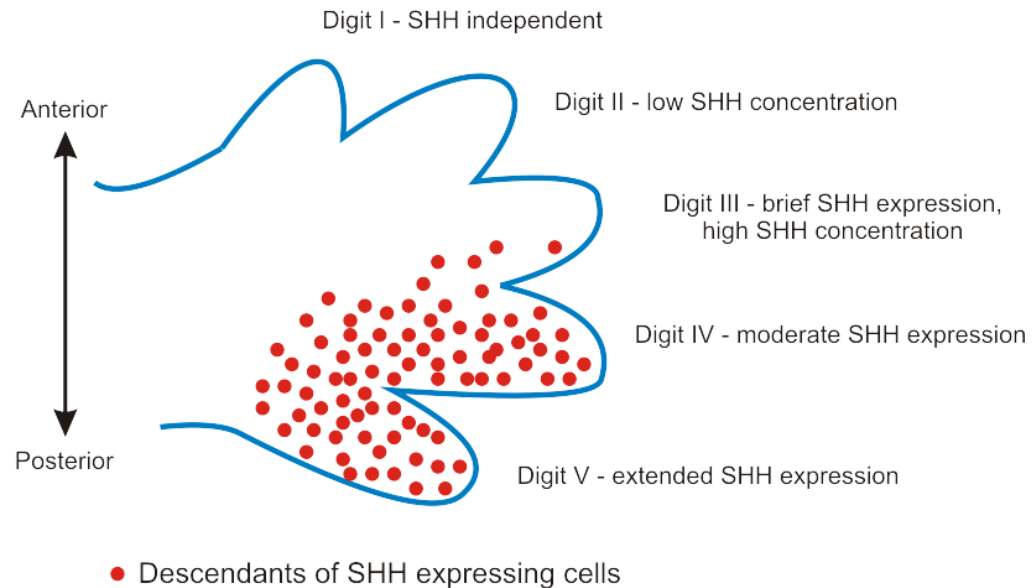
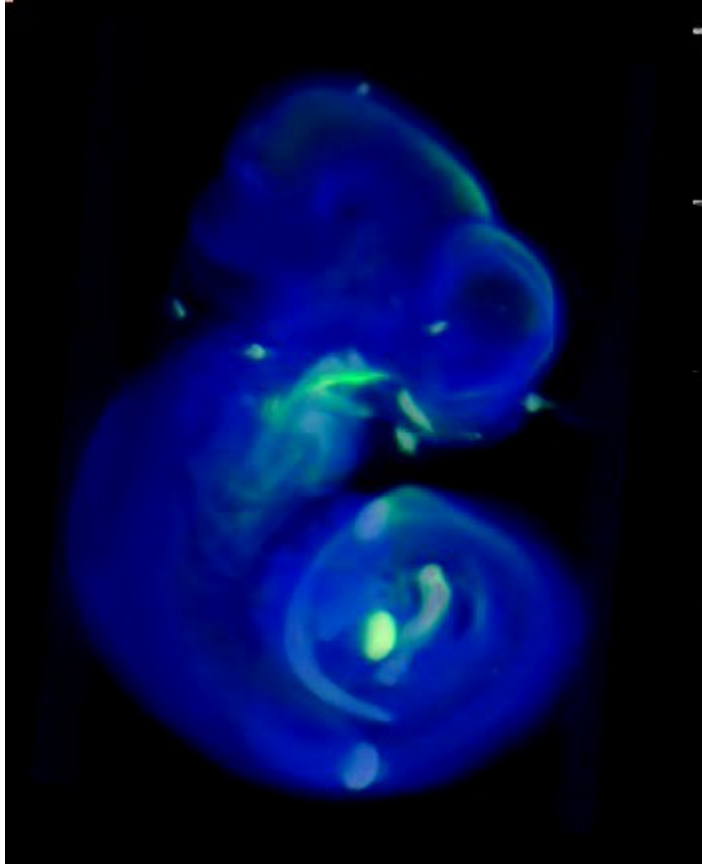
**Sonic hedgehog (SHH)** is translated as a ~45kDa precursor and undergoes **autocatalytic** processing to produce an ~20kDa N-terminal signaling domain (referred to as SHH-N) and a ~25kDa C-terminal domain with no known signaling role (1 on figure 5). During the cleavage, a **cholesterol** molecule is added to the carboxyl end of the N-terminal domain, which is involved in trafficking, secretion and receptor interaction of the ligand. When SHH reaches its target cell, it binds to the **Patched-1 (PTCH1)** receptor(3). In the absence of ligand, PTCH1 inhibits **Smoothened (SMO)**, a downstream protein in the pathway(4). It has been suggested that SMO is regulated by a small molecule, the cellular localisation of which is controlled by PTCH. PTCH1 has a sterol sensing domain (SSD), which has been shown to be essential for suppression of Smo activity. A current theory of how PTCH regulates SMO is by removing oxysterols from SMO. PTCH acts like a sterol pump and remove oxysterols that have been created by 7-dehydrocholesterol reductase. Upon binding of a Hh protein or a mutation in the SSD of PTCH the pump is turned off allowing oxysterols to accumulate around SMO. This accumulation of sterols allows SMO to become active or stay on the membrane for a longer period of time. The binding of SHH relieves SMO inhibition, leading to activation of the GLI transcription factors(5): the **activators Gli1** and **Gli2** and the **repressor Gli3**. The sequence of molecular events that connect SMO to GLIs is poorly understood. Activated GLI accumulates in the nucleus(6) and controls the transcription of hedgehog target genes(7).

# Schéma aktivace transkripčního faktoru Gli – současný pohled (2007)

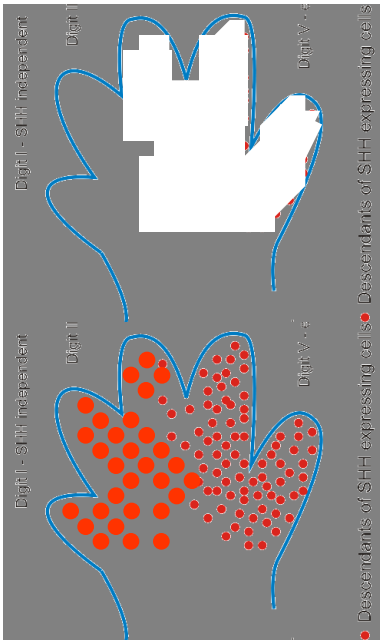
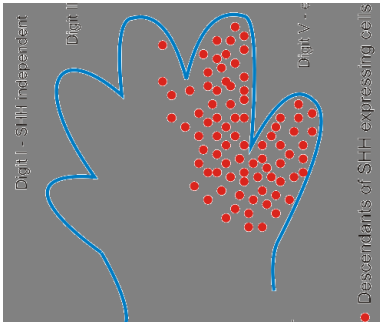
The 12-transmembrane domain protein patched (PTCH), the receptor for hedgehog proteins, negatively regulates the seven-transmembrane receptor smoothed (SMO) in the resting state. The binding of sonic hedgehog (SHH) to PTCH relieves this inhibition and SMO changes its localization to the membrane, thereby stimulating the Gli family of transcription factors, which are responsible for most of the effects of SMO. SMO activates Gli through G proteins that inhibit adenylyl cyclases, and G activates phosphatidylinositol 3-kinase (PI3K) and Akt. Both of these mechanisms seem to prevent the protein kinase A (PKA)-regulated inhibitory phosphorylation of Gli. Gli is also constitutively suppressed by suppressor of fused (SUFU), and this inhibition is relieved by SMO activation through a still unclear mechanism that might involve G protein-coupled receptor kinase 2 (GRK2).



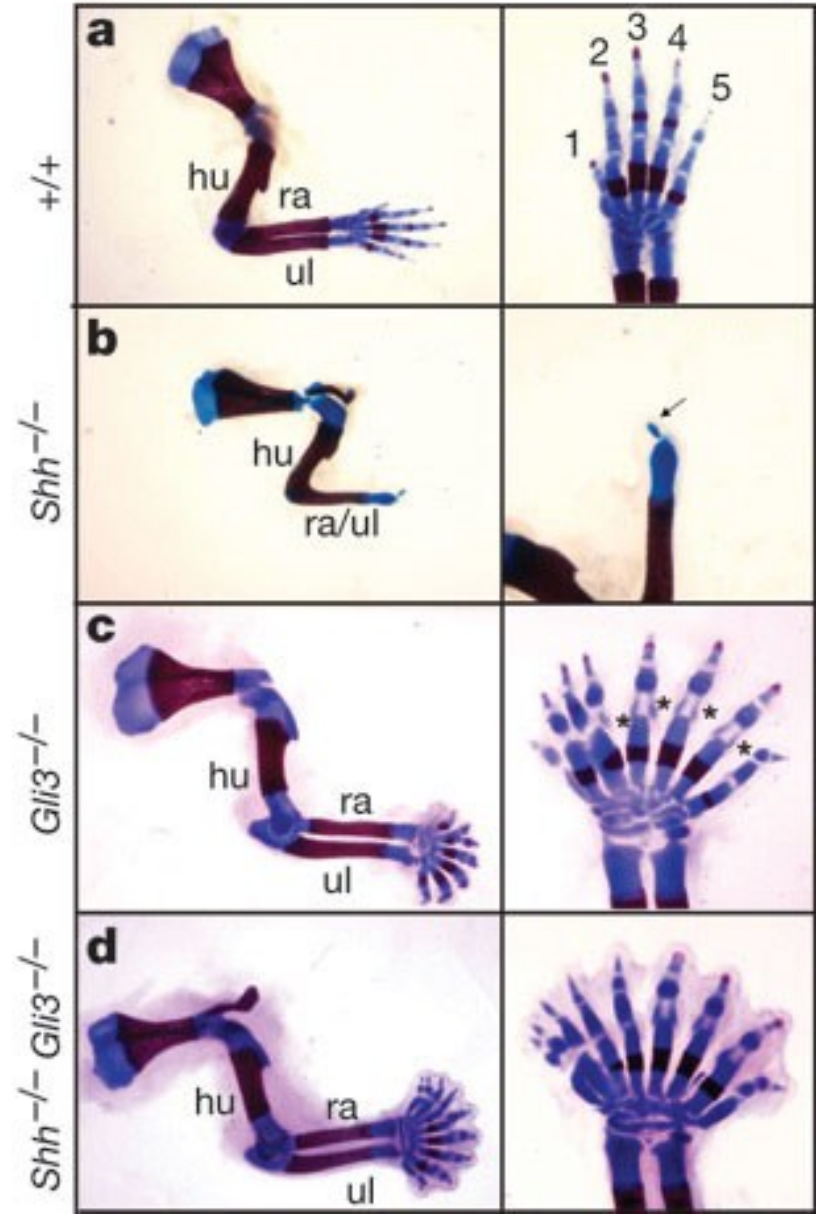
Shh – jeden z nejlépe popsaných klasických morfogenů (tzv. model francouzské vlajky) – v závislosti na koncentraci morfogenu se spouští odlišné transkripční programy



Např. specifikace jednotlivých prstů končetiny

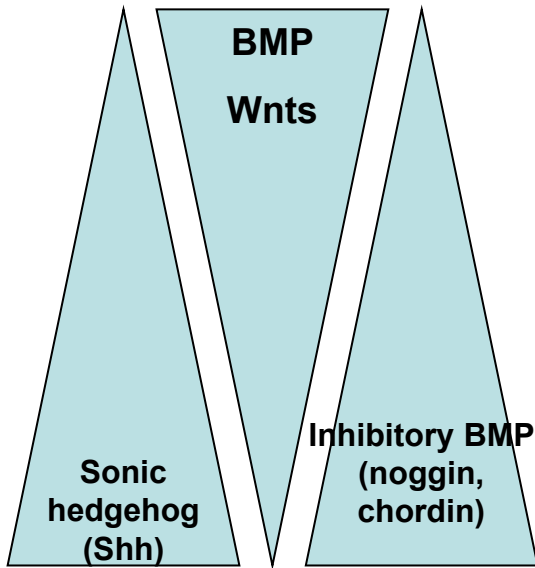


Forelimb



Shh – jeden z nejlépe popsáných klasických morfogenů (tzv. model francouzské vlajky) – v závislosti na koncentraci morfogenu se spouští odlišné transkripční programy

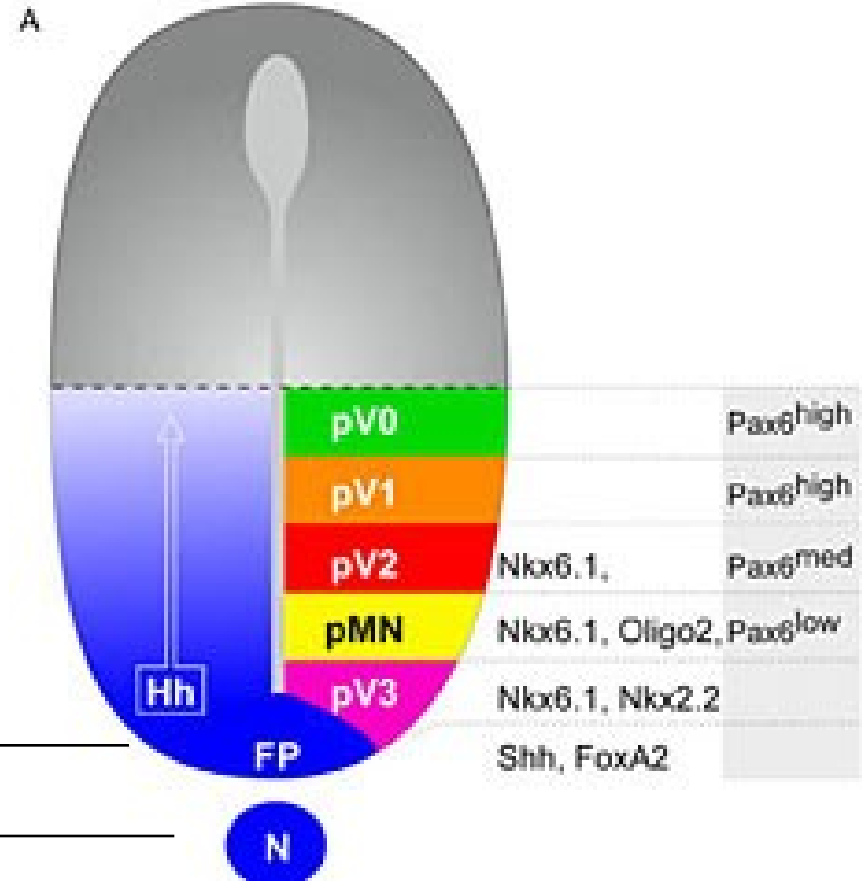
Dorsalizující faktory



ventralizující faktory produkované notochordem

floor plate – nejspodnější část nervové trubice

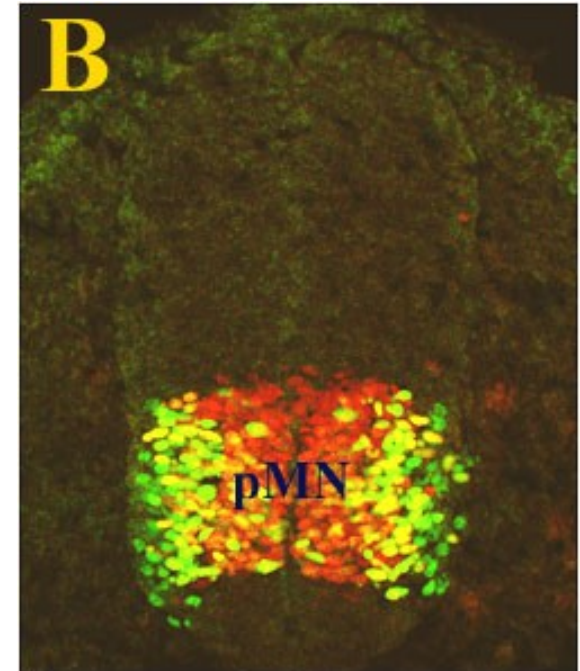
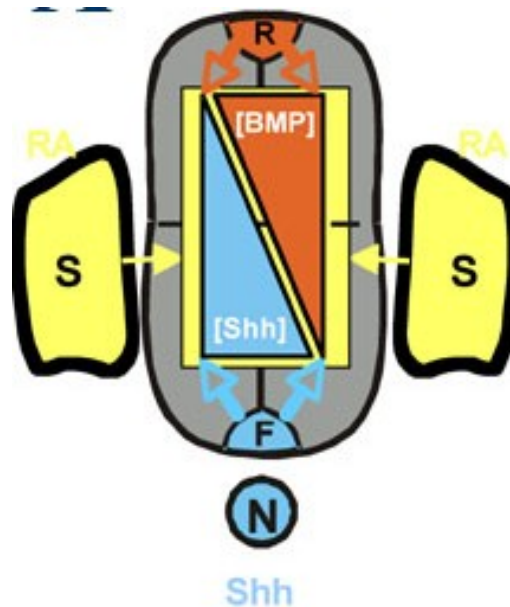
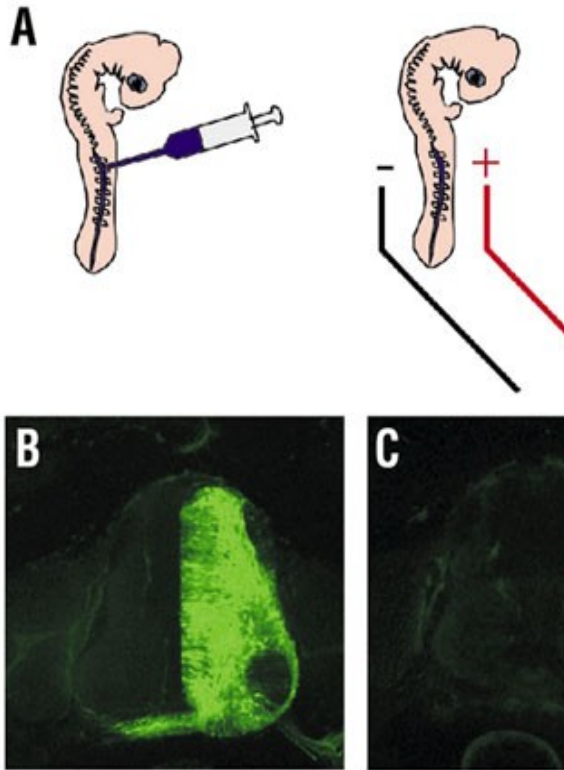
notochord – zdroj Shh



Např. specifikace jednotlivých neuronálních typů ve vyvíjející se nervové trubici

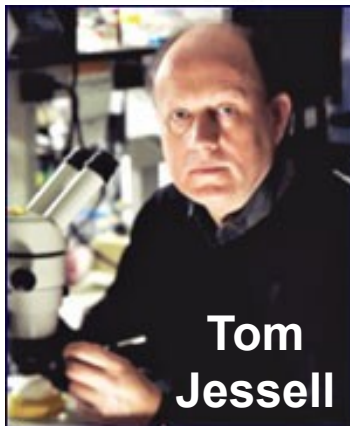


# Elektroporace kuřecí nervové trubice umožnila poznat jakým způsobem buňky během vývoje získávají a udržují svou identitu



*Fig. A - A model for early spinal cord development. The neural tube which will form the spinal cord is patterned into specific domains by multiple external signals which include a ventralizing Sonic Hedgehog (Shh) signal from the notochord (N) and floor plate (F), a dorsalizing BMP signal from the roof plate (R), and retinoic acid (RA) signaling from the adjacent somites (S).*

Cross section of the spinal cord of an embryonic day three chicken embryo stained with fluorescent antibodies. Shown here in red is the motor neuron progenitor domain (pMN), one of many precise domains established by earlier signaling events. The pMN domain is here labelled through the use of antibodies specific for Olig2, a critical regulator of motor neuron formation. Developing motor neurons emerging from the pMN are shown labelled in green.



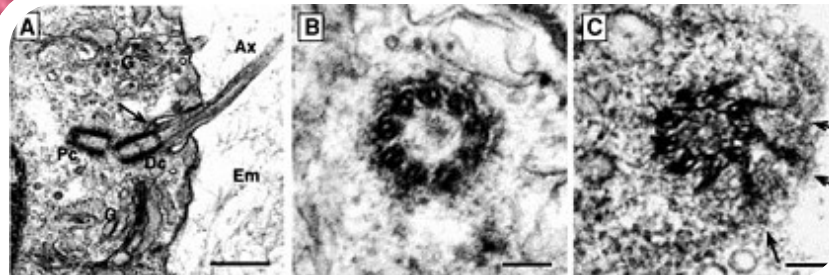
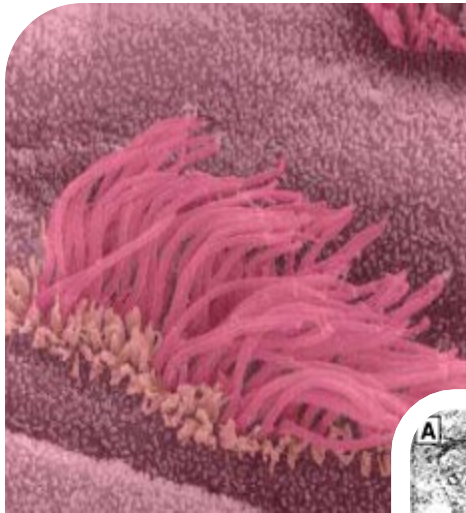
# Přirozené inhibitory Shh dráhy



**cyclopamin** – teratogenní alkaloid z kýchavice (*Veratrum californicum*), poprvé identifikován jako látku způsobující cyklopii (= 1 oko) a holoprosencephalii u ovcí

# Primary cilia in development and as signaling centers

Podklady dodali  
Karolina Babíčková  
Pavel Dobeš  
Tomáš Bárta

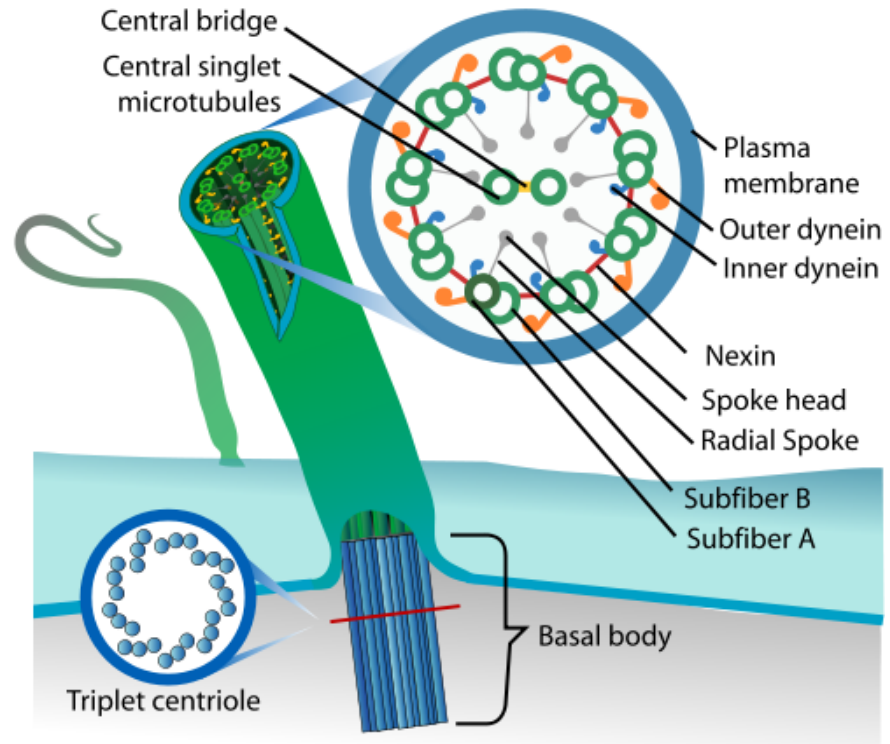
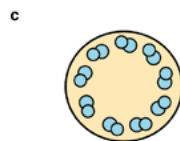
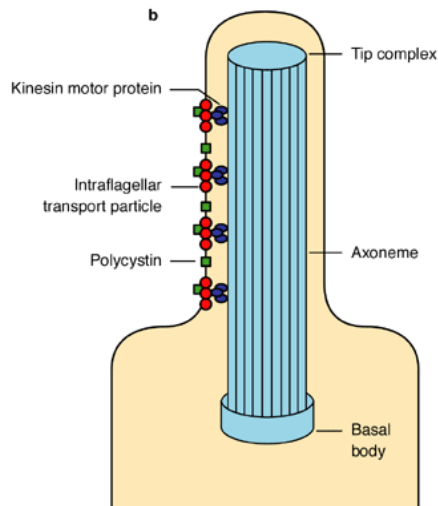
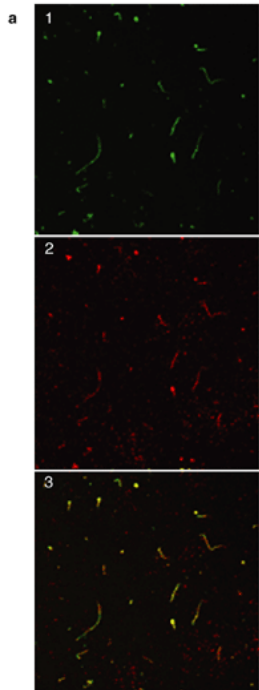




# Primary cilia vs. motile (secondary) cilia

- struktura 9+0
- nepohyblivé
- téměř všechny buňky ([www.primary-cilium.co.uk](http://www.primary-cilium.co.uk))
- solitérní

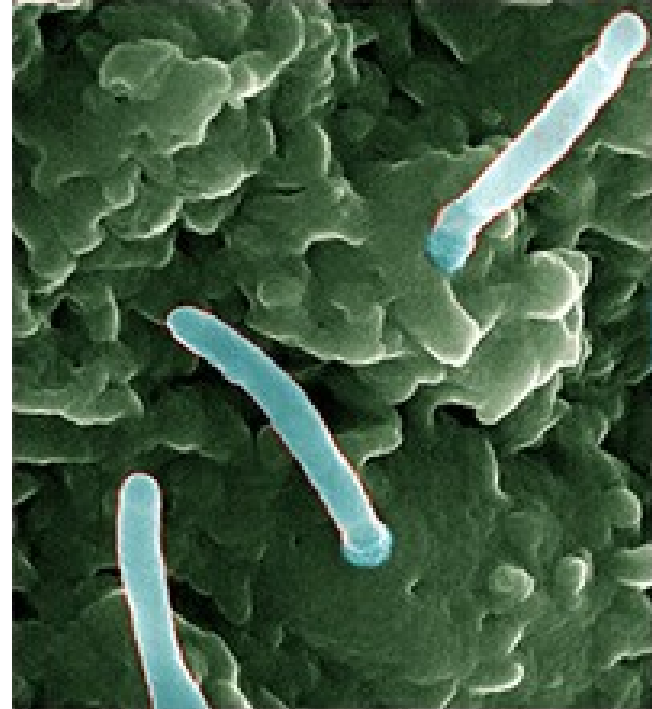
- struktura 9+2
- pohyblivé
- epitely tracheje, vejcovodů, ependym...



The primary cilium

# Primary cilia - function

- délka 2-10 $\mu\text{m}$ , průměr 0.25 $\mu\text{m}$
- chemo- a osmosenzory
- fotoreceptory
- mechanoreceptory
- komunikace v extracelulární matrix
- nodal cilia
  - pohyblivá
  - blastocysta
  - pravolevá souměrnost
- model transdukce – receptory iontové kanály, efektorové proteiny, transkripční faktory



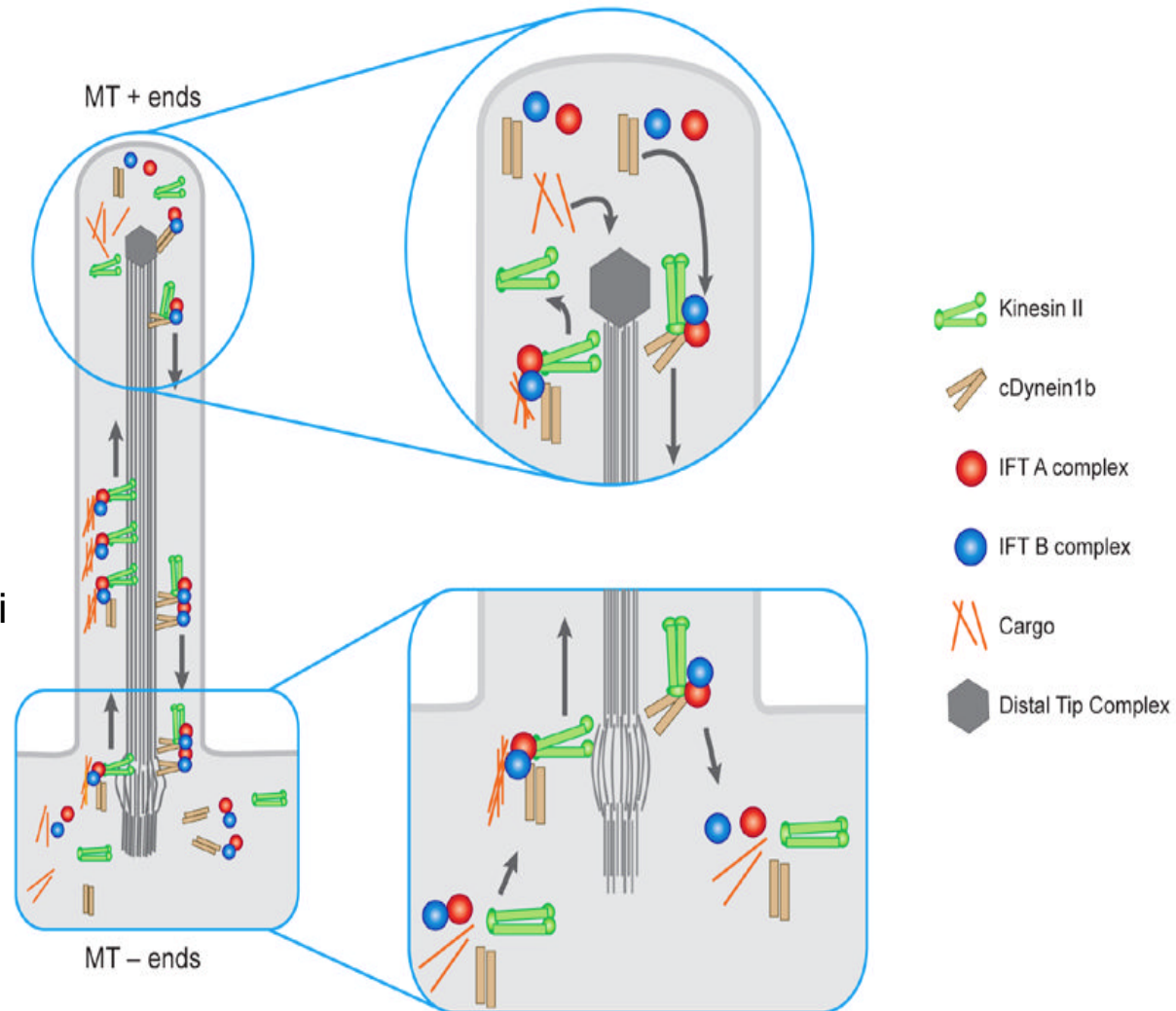
# Hedgehog (Hh) a Wnt signální dráhy jsou vázané na primární cilie

- Wnt a Hh signální dráhy regulují embryonální vývoj a formování mnoha tkání.
- Abnormální Hh/Wnt a s nimi spojená onemocnění jsou způsobena defekty ve tvorbě primárních cilií (infertilita, polydaktylie, polycystické ledviny, degenerace retiny).
- Hh, Wnt jsou přímo vázány na primární cílie.



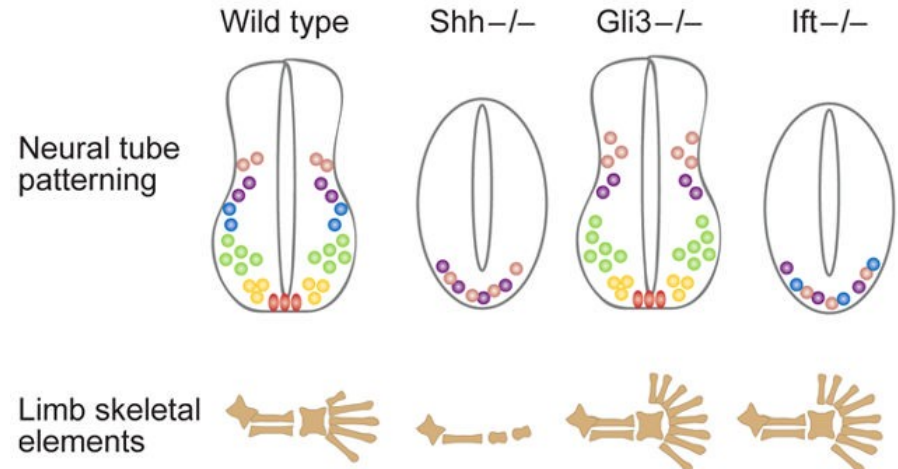
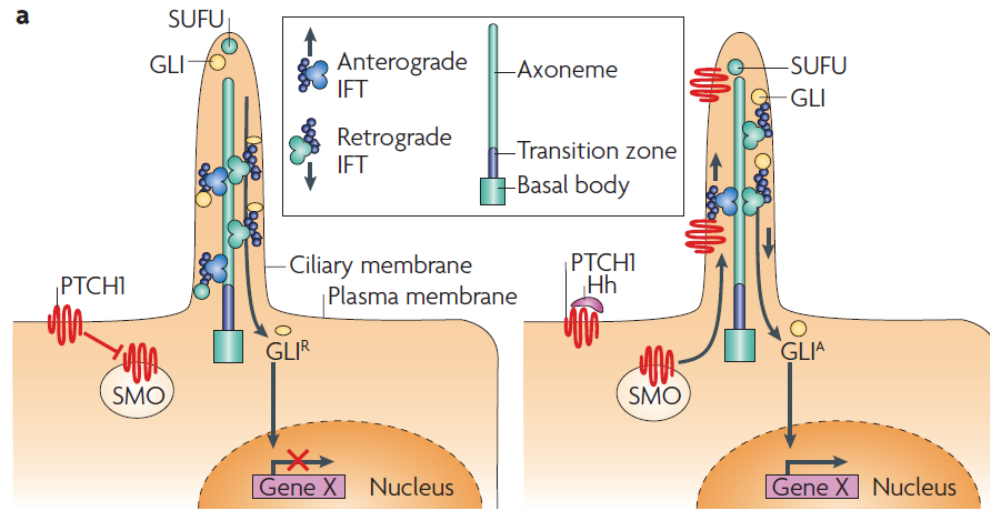
# Intraflagelární transport (IFT)

- Poprvé popsali Kozminski et al. 1993 pomocí DIC mikroskopie
- Za transport zodpovědný kinesin-II – transport k distálnímu „+“ konci a dynein zodpovědný za transport k „-“ konci.
- Kif3A, Kif3B (podjednotky kinesinu) KO buňky netvoří cilie.
- IFT je zodpovědný za regulaci signálních drah vázaných na primární cilie

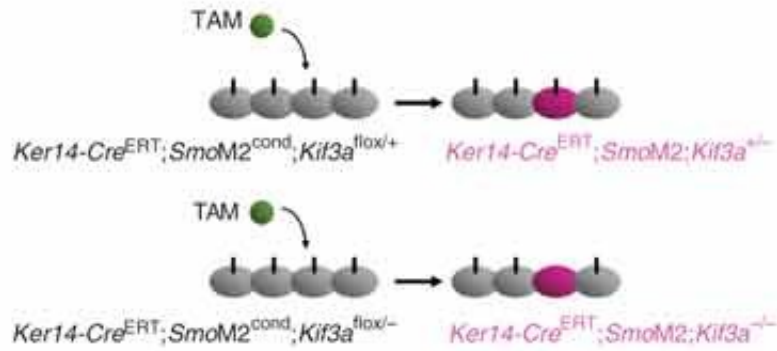


# Primary cilia and Hh signaling

- Je spojen s primárními ciliemi
- Ligand se naváže na patch (Ptc) protein, což způsobí zrušení inhibičního efektu Ptc na protein smoothened (Smo), který transdukuje signál přes glioma transkripční faktory (Gli) do jádra, kde řídí expresi Hh genů. (Gli1, Gli2 a Gli3A jsou aktivátory a Gli3R je represor). Hlavním represorem je SuFu.
- IFT hraje klíčovou úlohu ve funkci regulace Hh signální dráhy (spojuje Smo a Gli)
- Partial loss-of-function v genu *polaris* vede u myši k preaxiální polydaktýlii a k poruchám při formování končetin. Při úplné ztrátě dojde v embryu k formování náhodné pravo-levé symetrie -> důležitost primárních cílů a Hh ve vývoji.
- Mutace Kif3A a Kif3B mají podobné fenotypy v důsledku ztráty cíle.
- 3 typy Hh – Sonic Hh (Shh), Indian Hh (Ihh) a Desert Hh (Dhh)



a



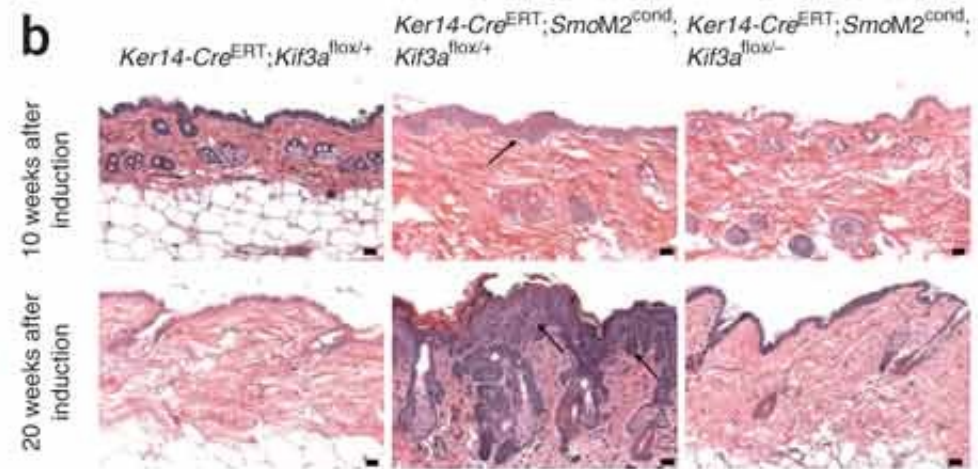
c



control

Shh active,  
cilia presentShh active,  
cilia absent

b



e



Ker14-Cre: drives expression to the epidermis

SmoM2 (cond): constitutively active Smoothed (activated by Cre)

Kif3a Flox: following Cre leads to Kif3a deletion and primary cilia loss



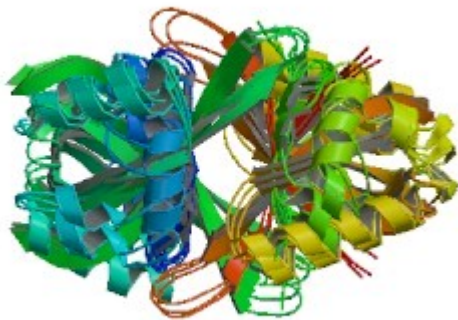
# Hypoxie a HIF

Část podkladů dodali:

Eva Lincová

Jana Kamarýtová

Filip Trčka

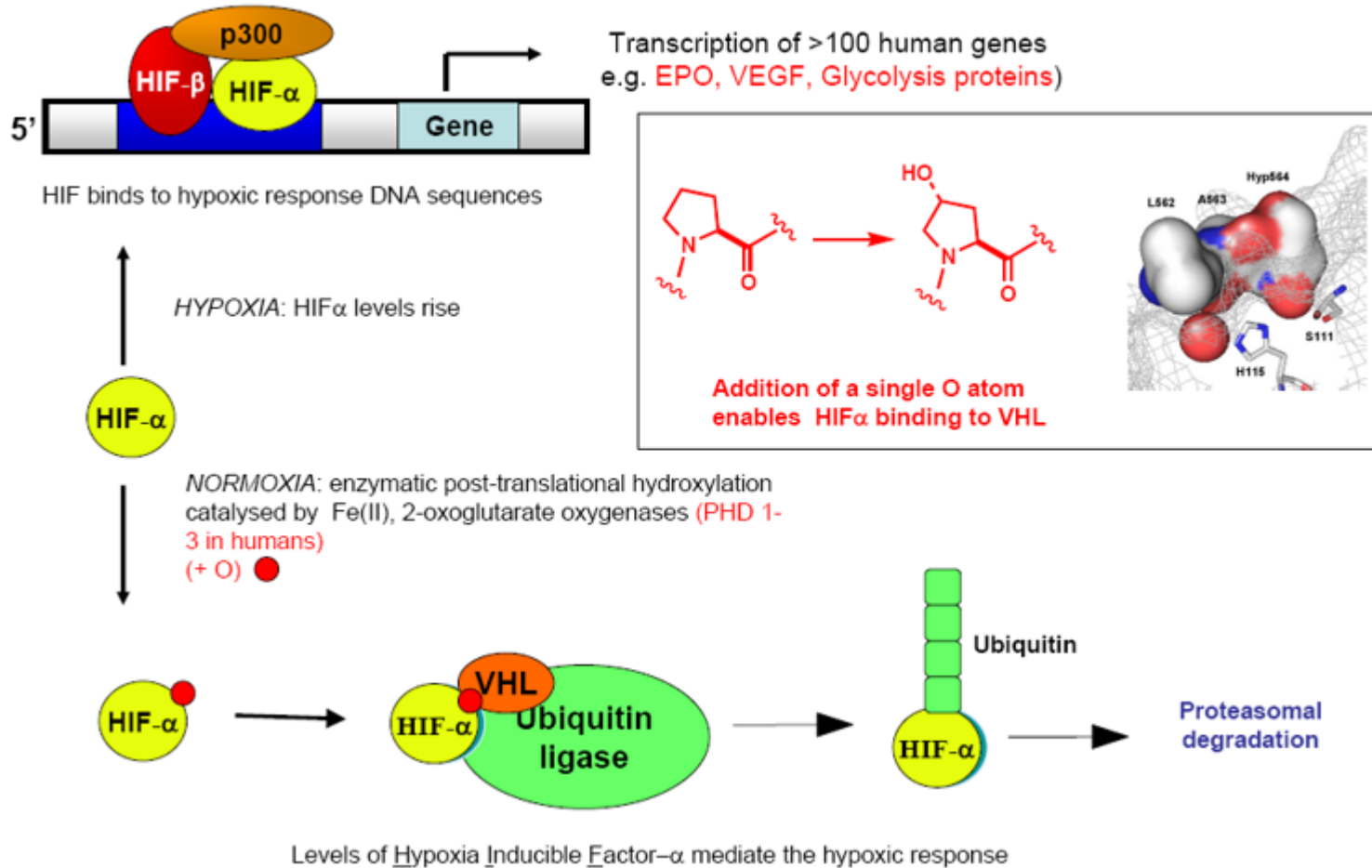


# Hypoxie a HIF

- **Hypoxie:** snížený parciální tlak  $O_2$  ve tkáni X normoxie
- **HIF** – Hypoxia-Inducible Factor:
  - Heterodimerický TF 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  $\alpha$  podjednotek (HIF1 $\alpha$ , 2 $\alpha$ , 3 $\alpha$ ) a jedné podjednotky  $\beta$  (HIF $\beta$ =ARNT)
  - **$\alpha$  podjednotky jsou při normoxii silně labilní**, podjednotka  $\beta$  je na koncentraci  $O_2$  nezávislá



# HIF při normoxii a hypoxii

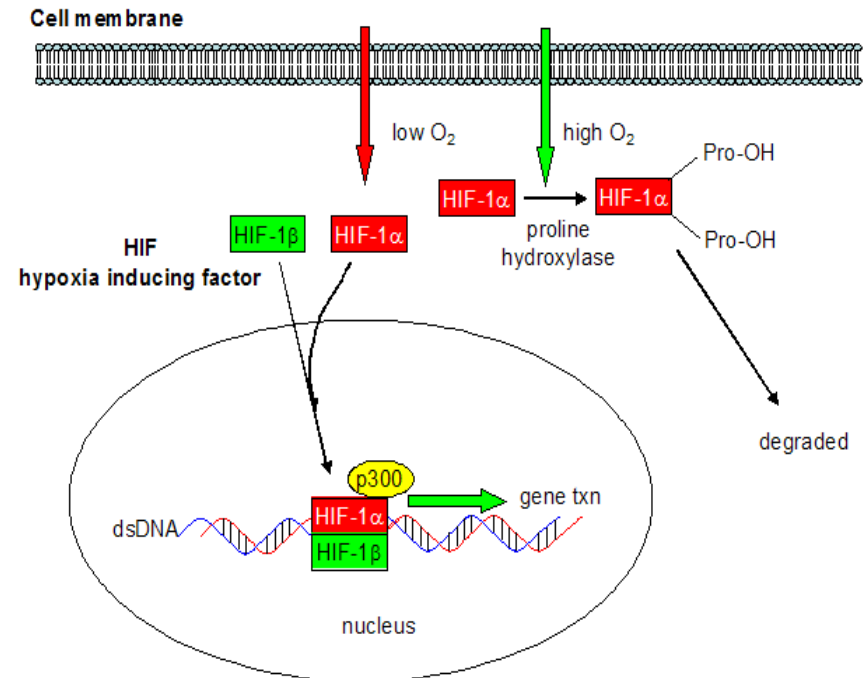


<http://www.chem.ox.ac.uk/oc/cjschofield/images/new-2.png>

**VHL (von Hippel-Lindau) - tumor supresorový gen**

# Modelové vývojové změny spojené s hypoxií/HIF systémem

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

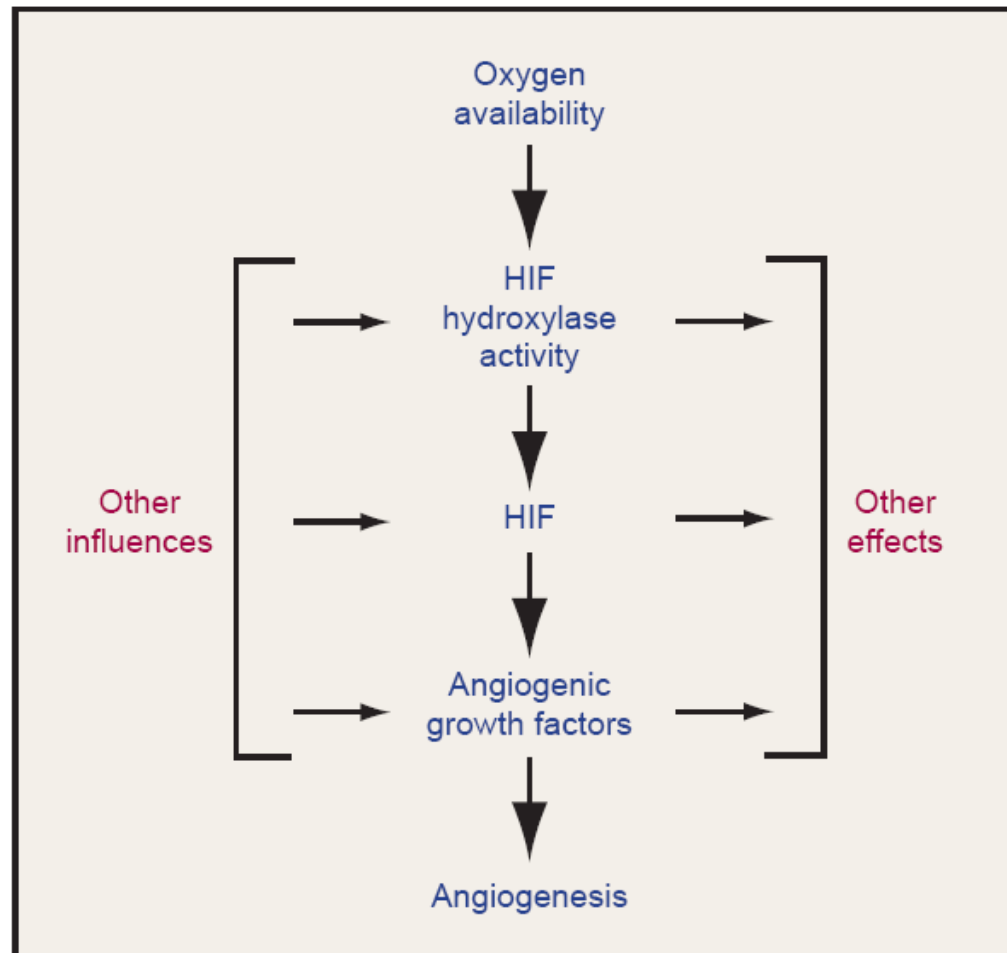


## Genes upregulated:

- erythropoietin (induce RBC formation)
- glycolytic enzymes (needed if  $O_2$  low)
- angiogenesis (new blood vessel growth)
- embryonic development
- placenta (for vascularization)
- macrophage and neutrophils (work in hypoxic wound conditions)

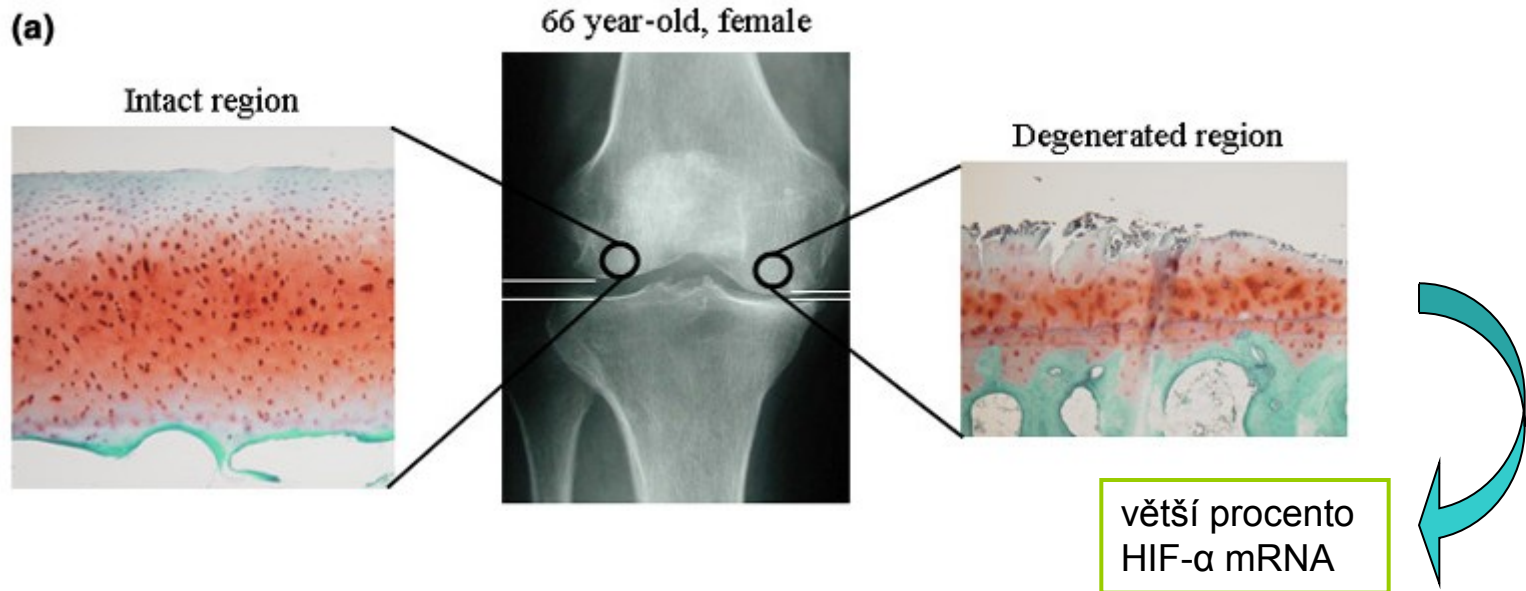
# Angiogenesis

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

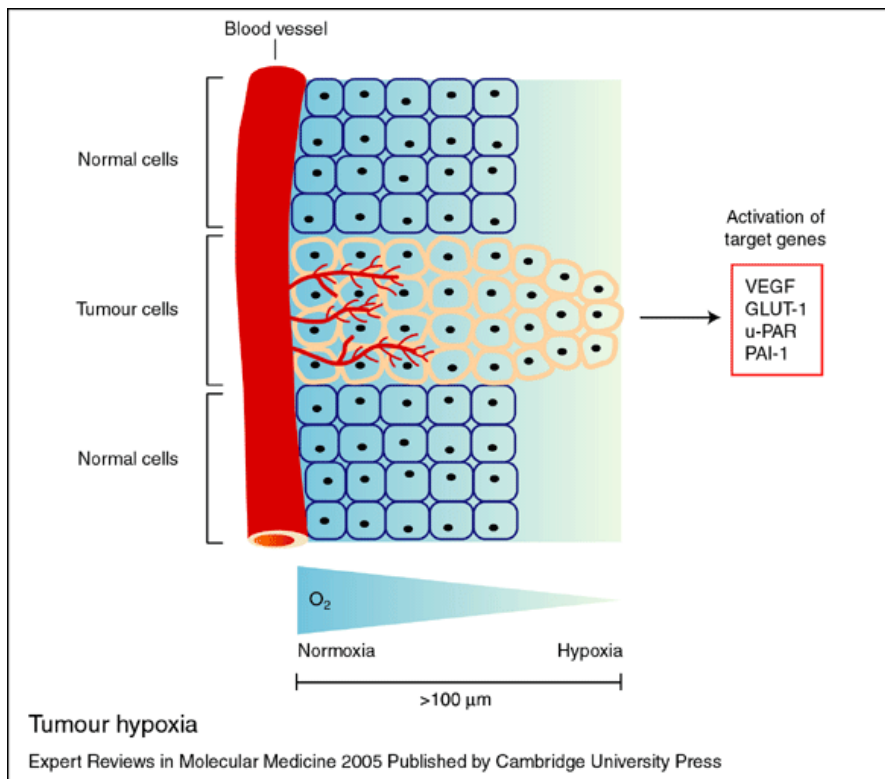


# Růst chrupavek

- HIF přítomen při růstu chrupavek
- HIF-1 může hrát roli v adaptaci chondrocytů na nízký tlak kyslíku
- osteoarthritis



# Hypoxie v karcinogenezi



- Hypoxie je již ve vzdálenosti 100 μM od cévy

- Aktivované geny podporují angiogenezi a metastázování

# Příčiny a důsledky aktivace HIF

- PŘÍČINY
- Hypoxie
- Působení růstových faktorů a onkogenních signálních drah (EGF, Ras, Src, ...)
- Mutace nádorových supresorů (p53, PTEN, **pVHL**)
- Nedostatečná aktivita hydroxyláz

- DŮSLEDKY
- Podpora angiogeneze a růst nádoru
- Metabolická adaptace na hypoxii (podpora glykolýzy)

