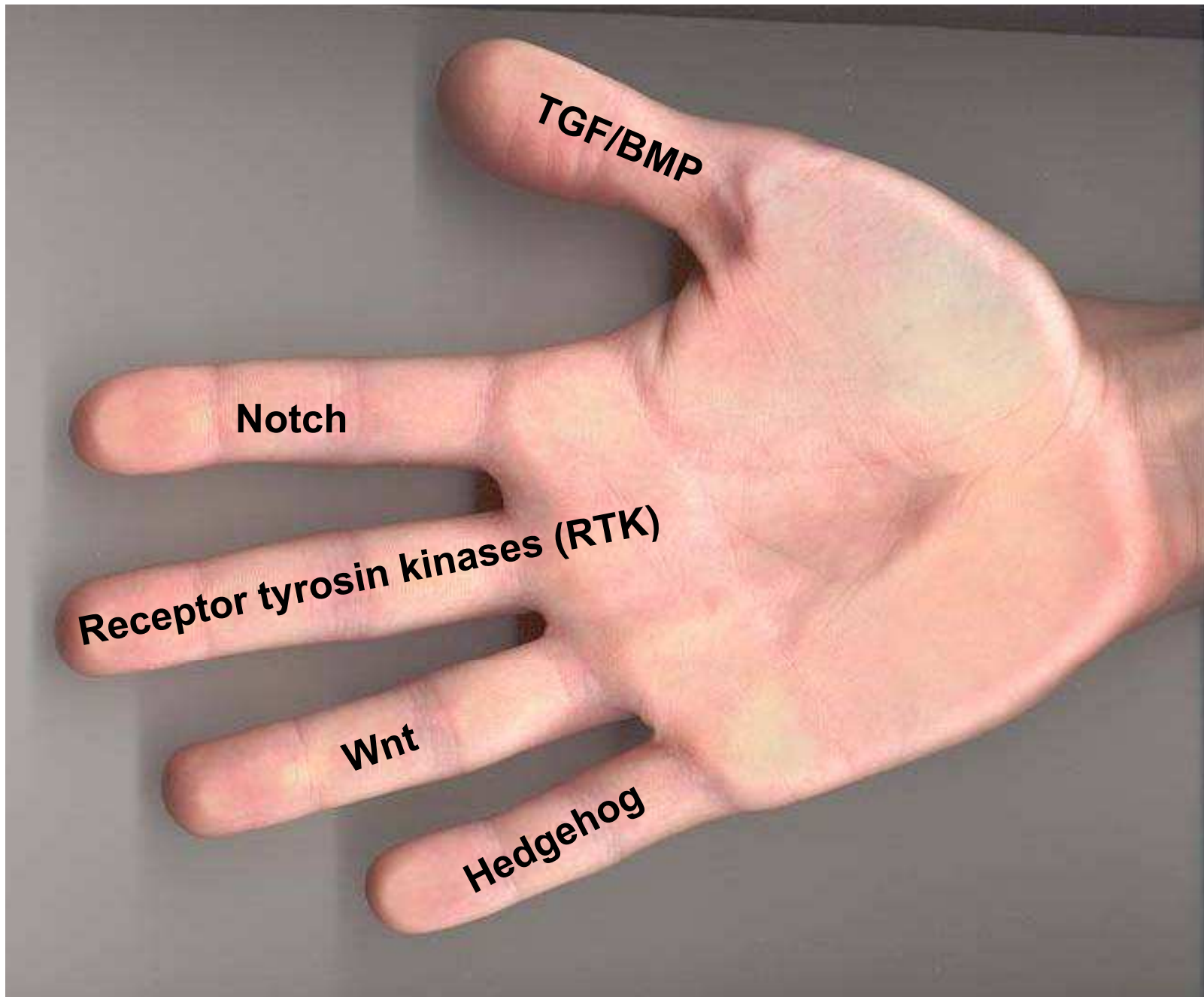


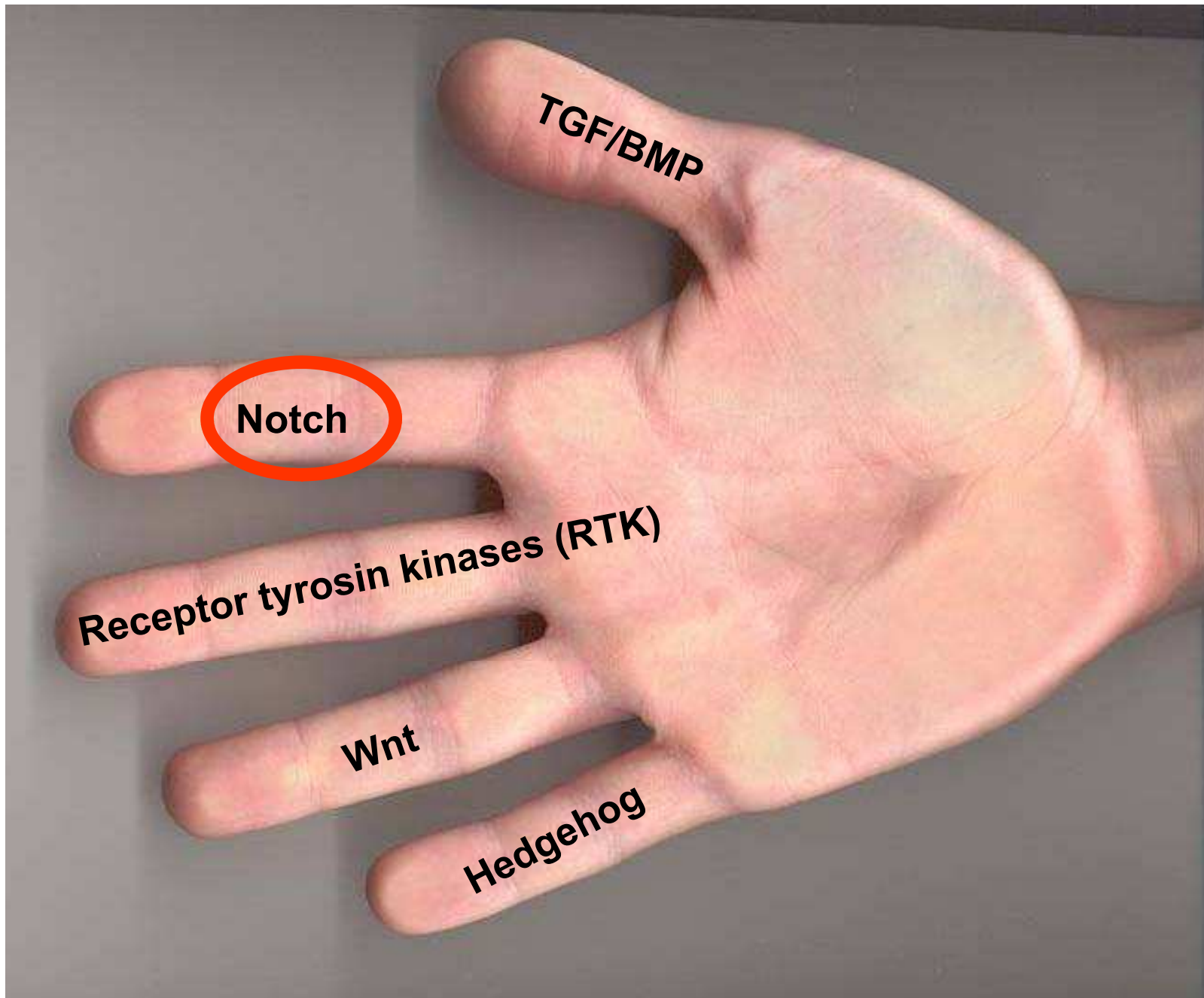
#7

**Klíčové molekulární komponenty
vývoje II**

Signály z vnějšího prostředí

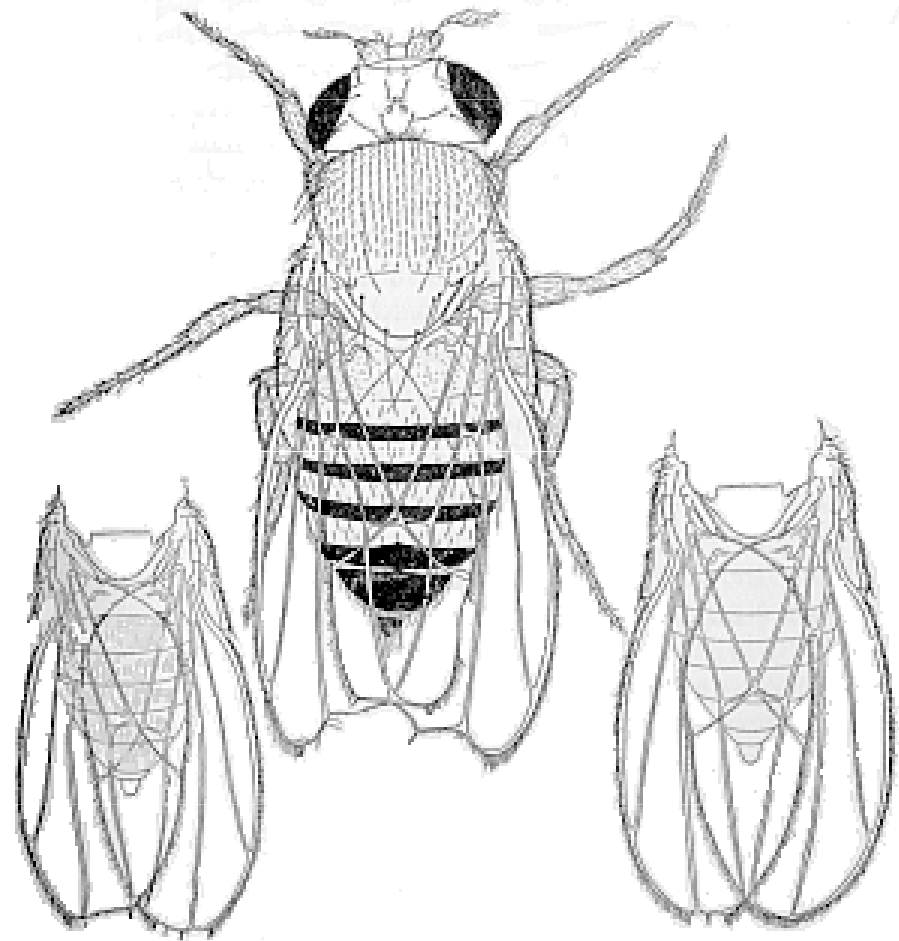


Signály z vnějšího prostředí

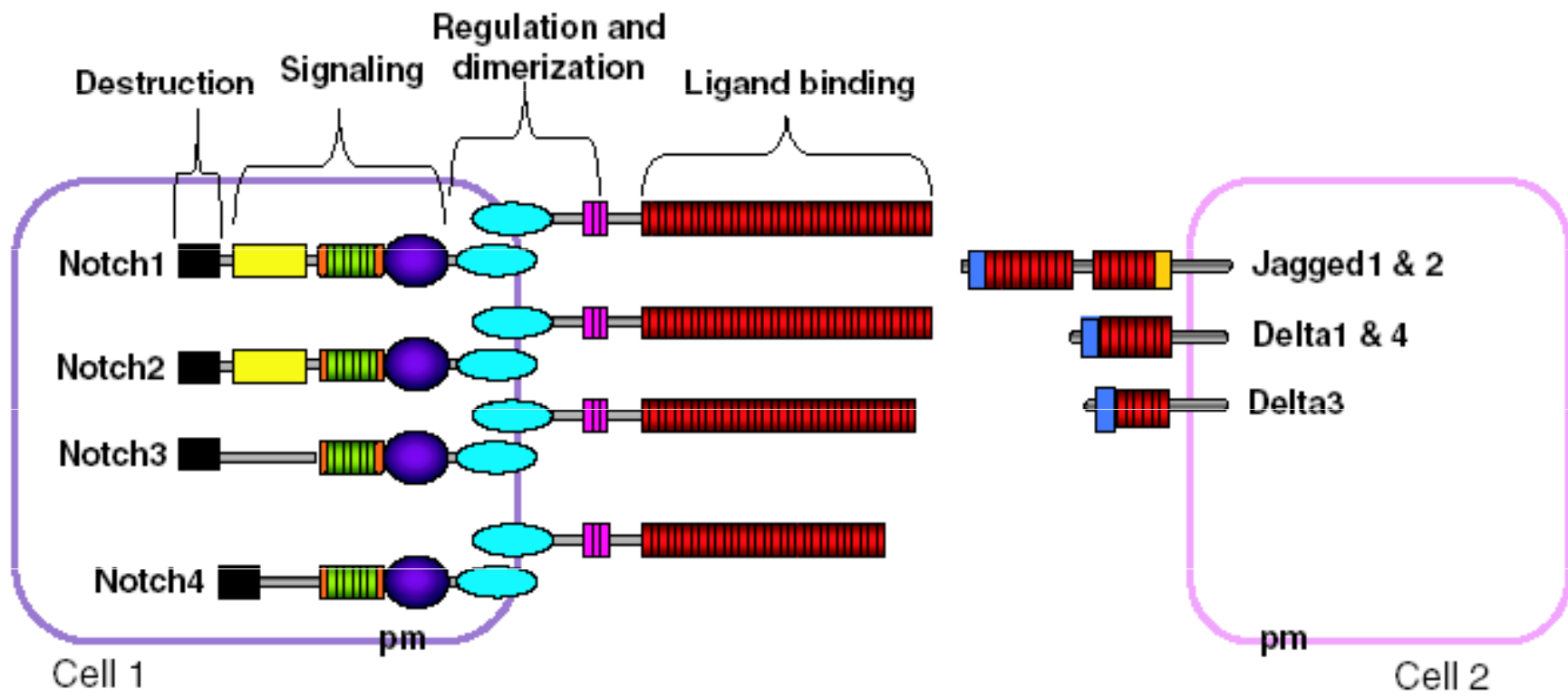


Notch

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



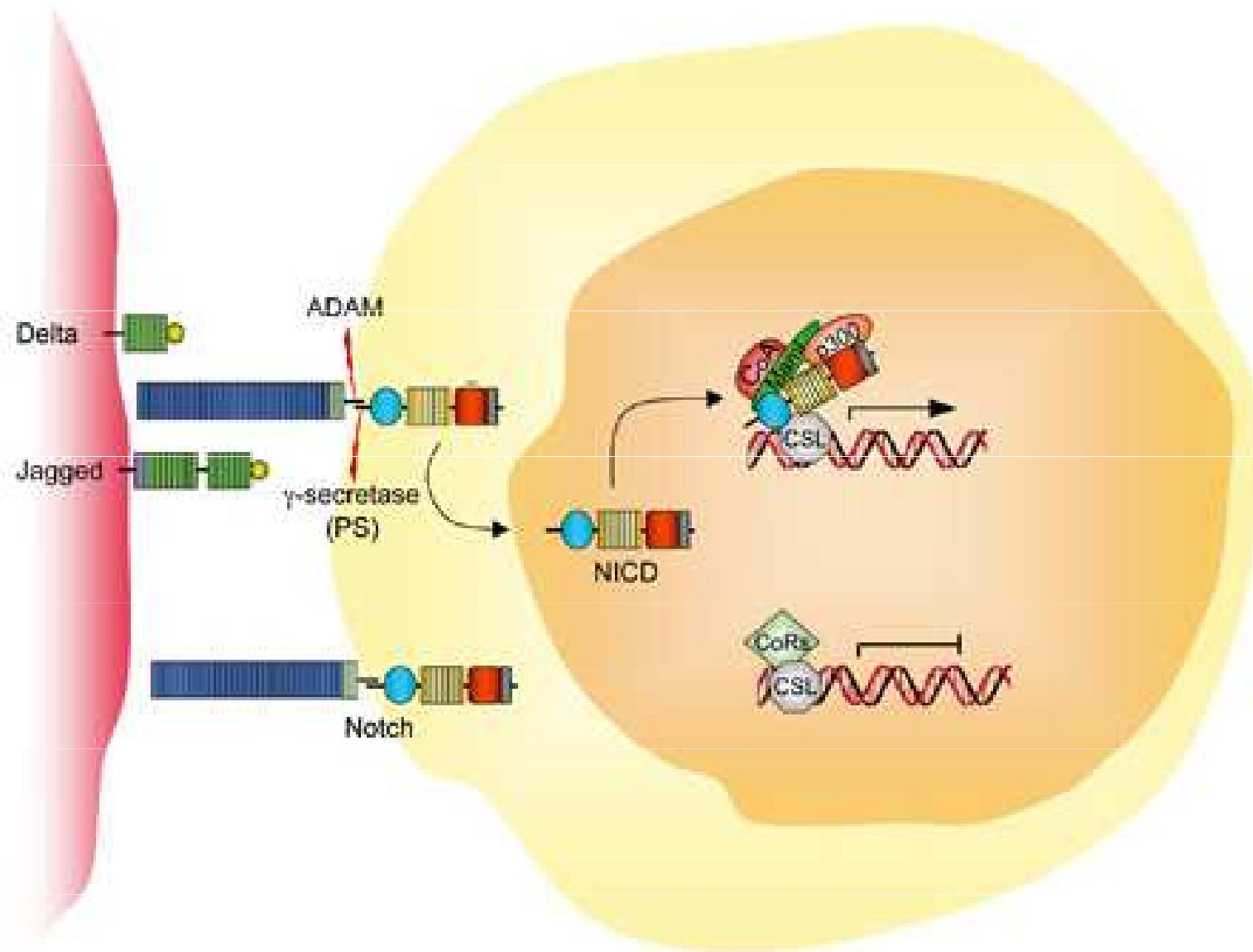
A



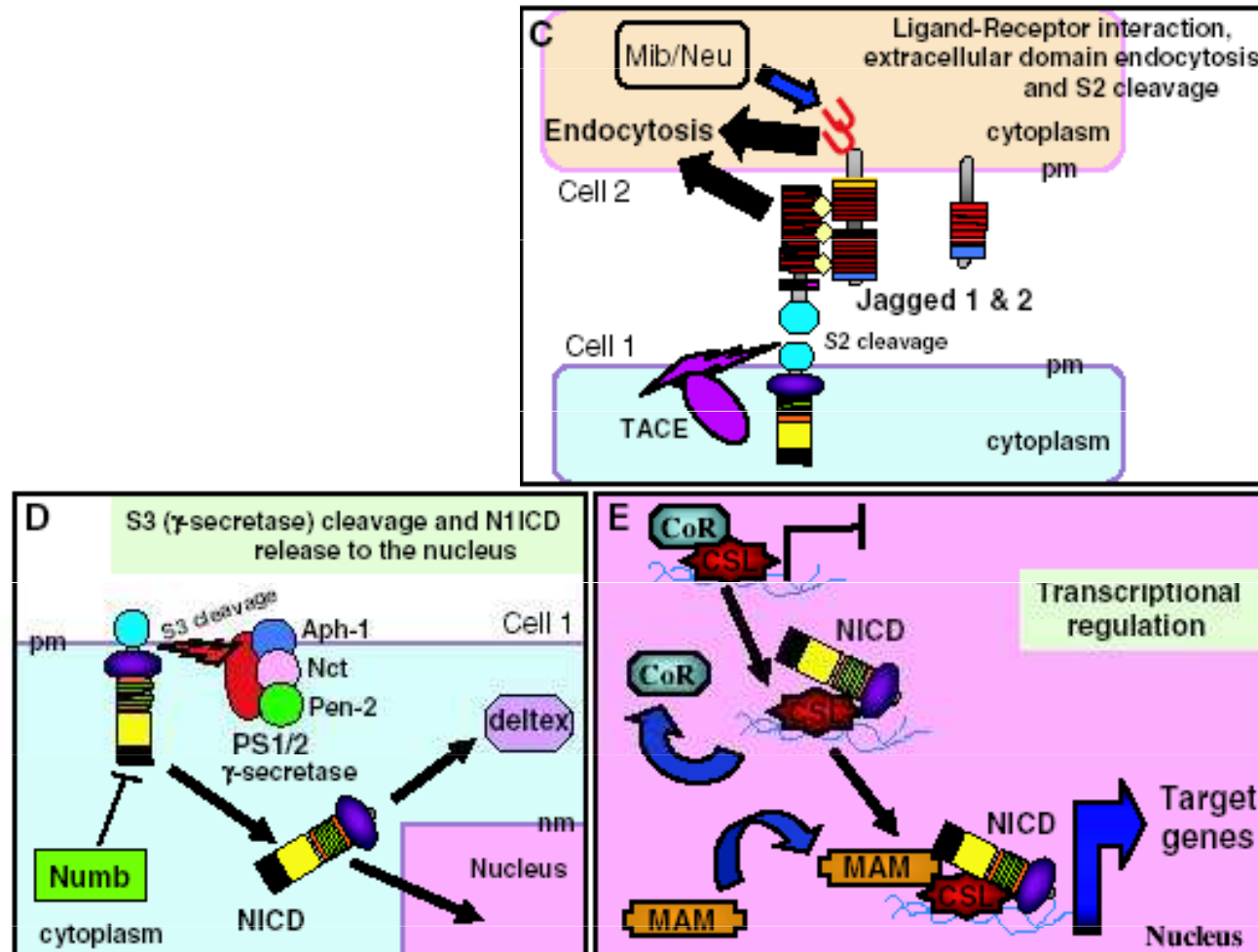
receptory Notch1-4

Notch ligandy – jsou vázány na buněčný povrch

Notch dráha - overview



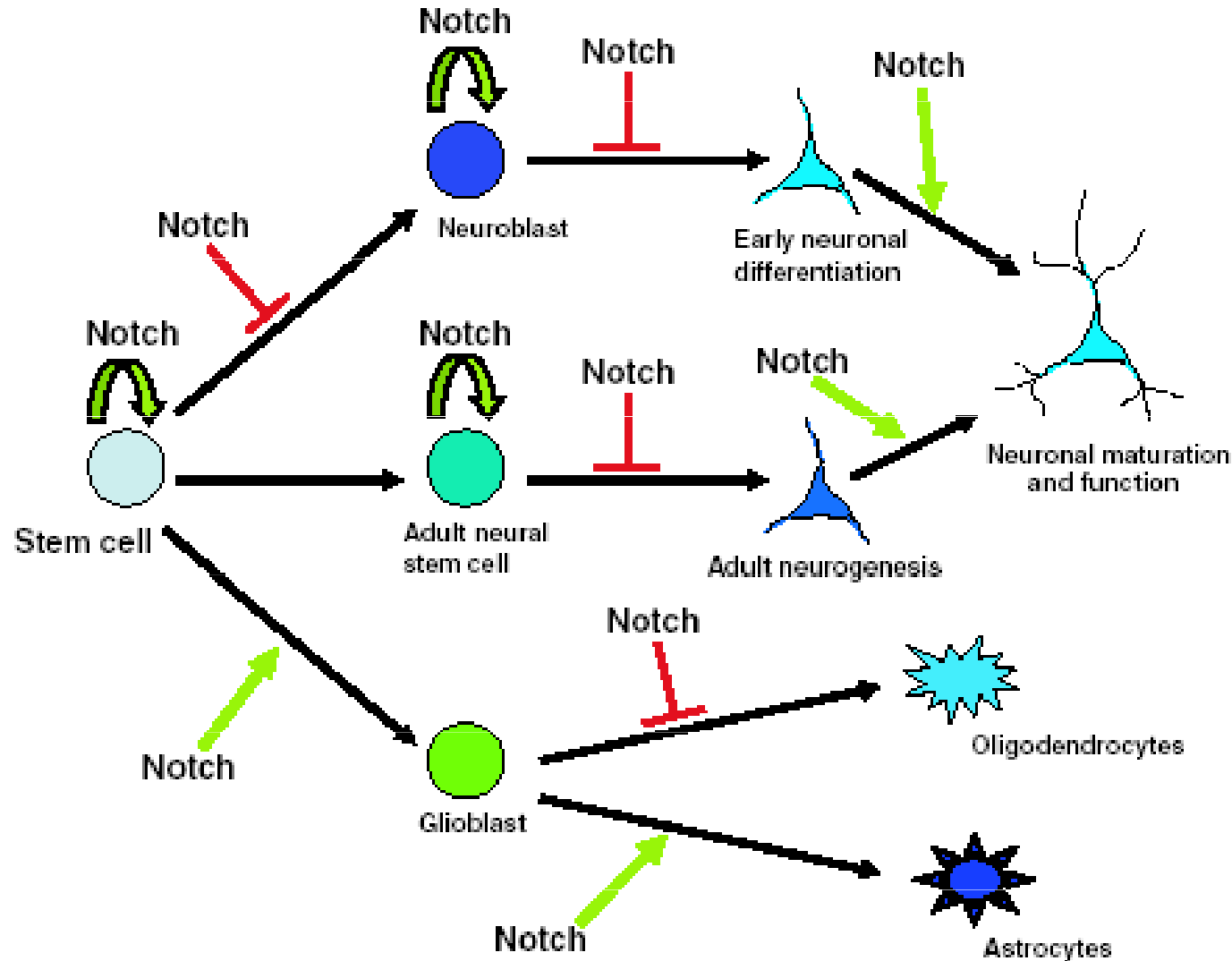
Notch signalling step-by-step



Notch is expressed in the cell membrane as a heterodimer. C, Binding to Delta or Jagged ligands initiates two consecutive proteolytic cleavage events; the first is mediated by the ADAM protease TACE and occurs on the extracellular side of Notch, near the transmembrane domain (site 2 cleavage). D, The second cleavage (S3) occurs within the transmembrane domain and is mediated by γ -secretase activity, a complex composed of four different integral membrane proteins: presenilin, nicastrin (Nct), Aph-1, and Pen-2 (286). NICD is released and translocates to the nucleus. In the cytoplasm, the Numb protein negatively regulates Notch signaling, possibly by promoting receptor turnover. Deltex proteins may transduce Notch signals independently of CSL. E, In the nucleus NICD binds to the CSL transcription factor, converting it from a transcriptional repressor into a transcriptional activator by displacing a CoR complex and recruiting coactivators such as MAML1 (MAM). This leads to transcriptional activation of downstream target genes. pm, Plasma membrane; nm, nuclear membrane.

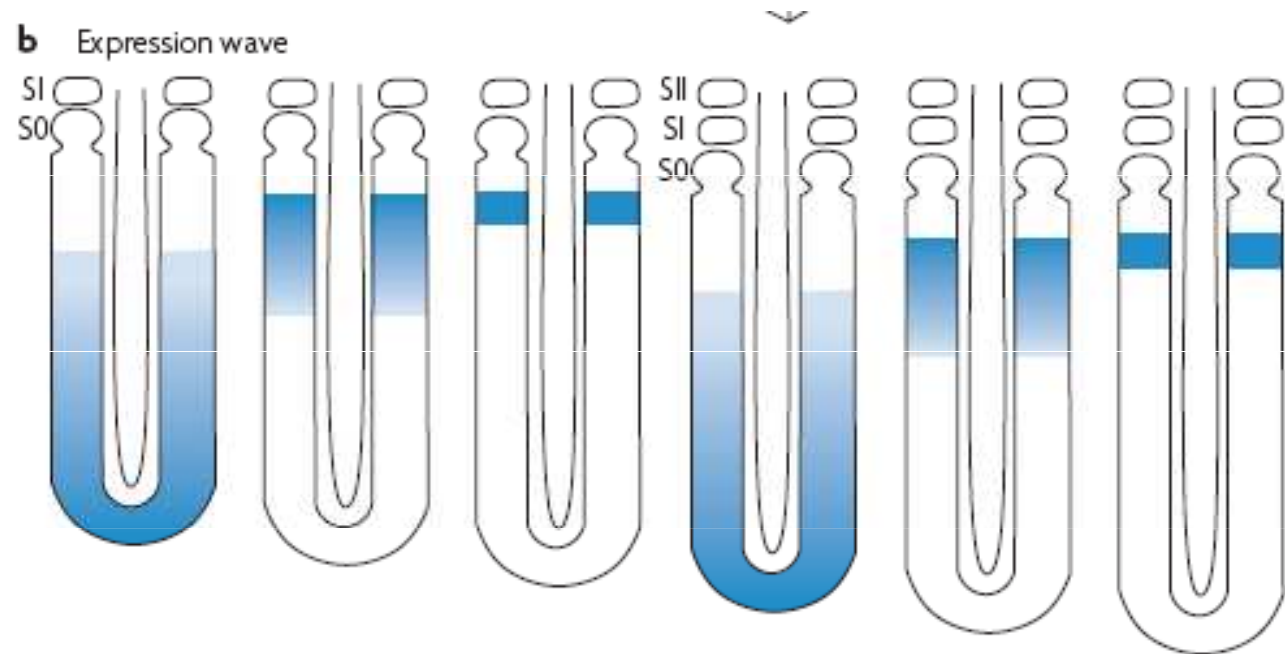
Notch dráha, stejně jako ostatní morfogenetické systémy, reguluje (ať už negativně nebo pozitivně) jednotlivé vývojové procesy na několika stupních.

Příklad: vývoj jednotlivých buněčných typů v nervovém systému



Notch and the segmentation clock

segmentation clock movie.mov



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 sequentially¹⁸. Part a modified with permission from REF. 14 © (1976) Elsevier Ltd.

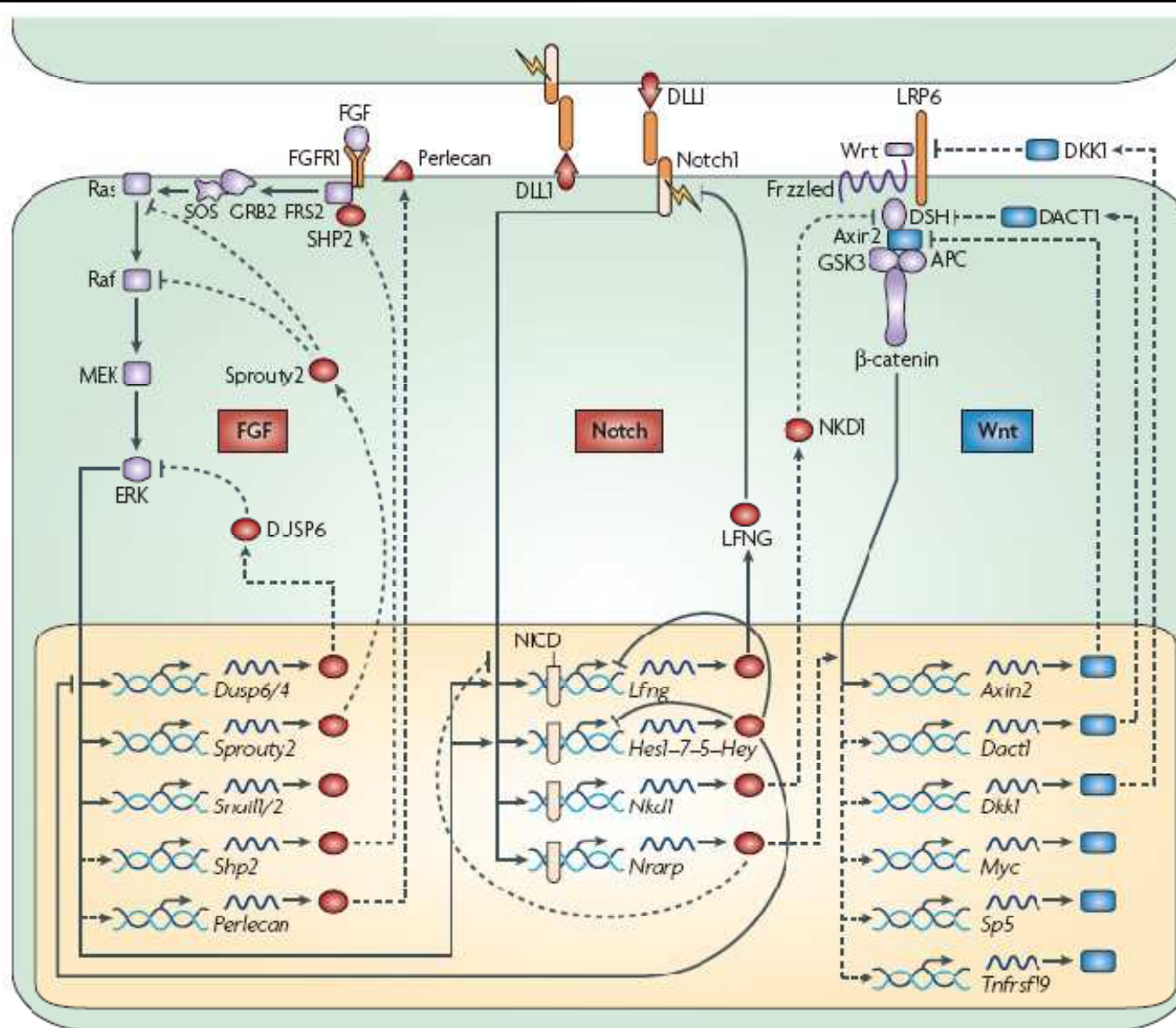


Figure 3 | The mouse oscillator. Cyclic genes belonging to the Notch and FGF (fibroblast growth factor) pathways (the products of which are indicated in red) oscillate in opposite phase to cyclic genes of the Wnt pathway (blue). A large number of the cyclic genes are involved in negative feedback loops. The basic circuitry of the three signalling pathways is represented. Dashed lines correspond to modes of regulation inferred from work in other systems or based on microarray data⁷⁰. APC, adenomatous polyposis coli; DACT1, dapper homologue 1; DKK1, dickkopf homologue 1; DLL1, delta-like 1; DSH, dishevelled; DUSP6, dual specificity phosphatase 6; ERK, mitogen-activated protein kinase 1; FGFR1, FGF receptor 1; GRB2, growth factor receptor-bound protein 2; GSK3, glycogen synthase kinase 3; *Hes1*, hairy and enhancer of split-related 1; LFNG, lunatic fringe; LRP6, low density lipoprotein receptor-related protein 6; MEK, mitogen-activated protein kinase kinase 1; NICD, Notch intracellular domain; NKD1, naked cuticle 1 homologue; *Nrarp*, Notch-regulated ankyrin repeat protein; SHP2, Src homology region 2-containing protein tyrosine phosphatase 2; SOS, son of sevenless; *Sp5*, trans-acting transcription factor 5; *Tnfrsf19*, tumour necrosis factor receptor superfamily, member 19.

Jak studovat somitogenezi u myši?

T/Mesp2

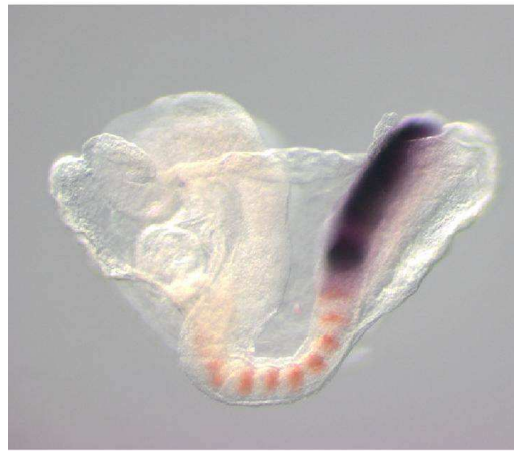
Wnt5a^{+/+}; *LRP6*^{+/+}



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



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



Uncx4.1/Hes7

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



Mesp2/Ripply2

Wnt5a^{+/-}; *Lrp6*^{+/-}



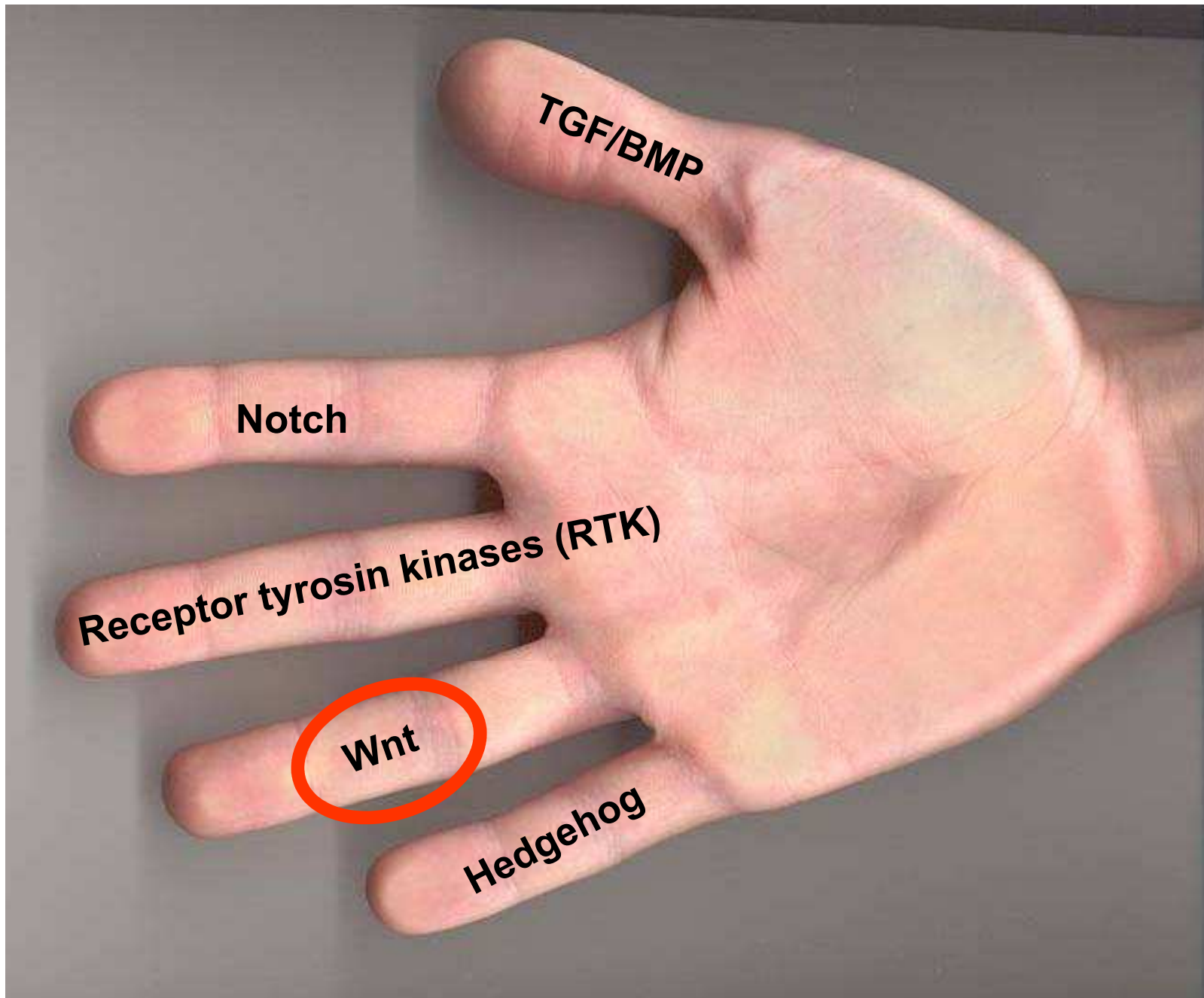
Wnt5a^{+/-}; *Lrp6*^{-/-}



Wnt5a^{+/+}; *Lrp6*^{-/-}



Signály z vnějšího prostředí

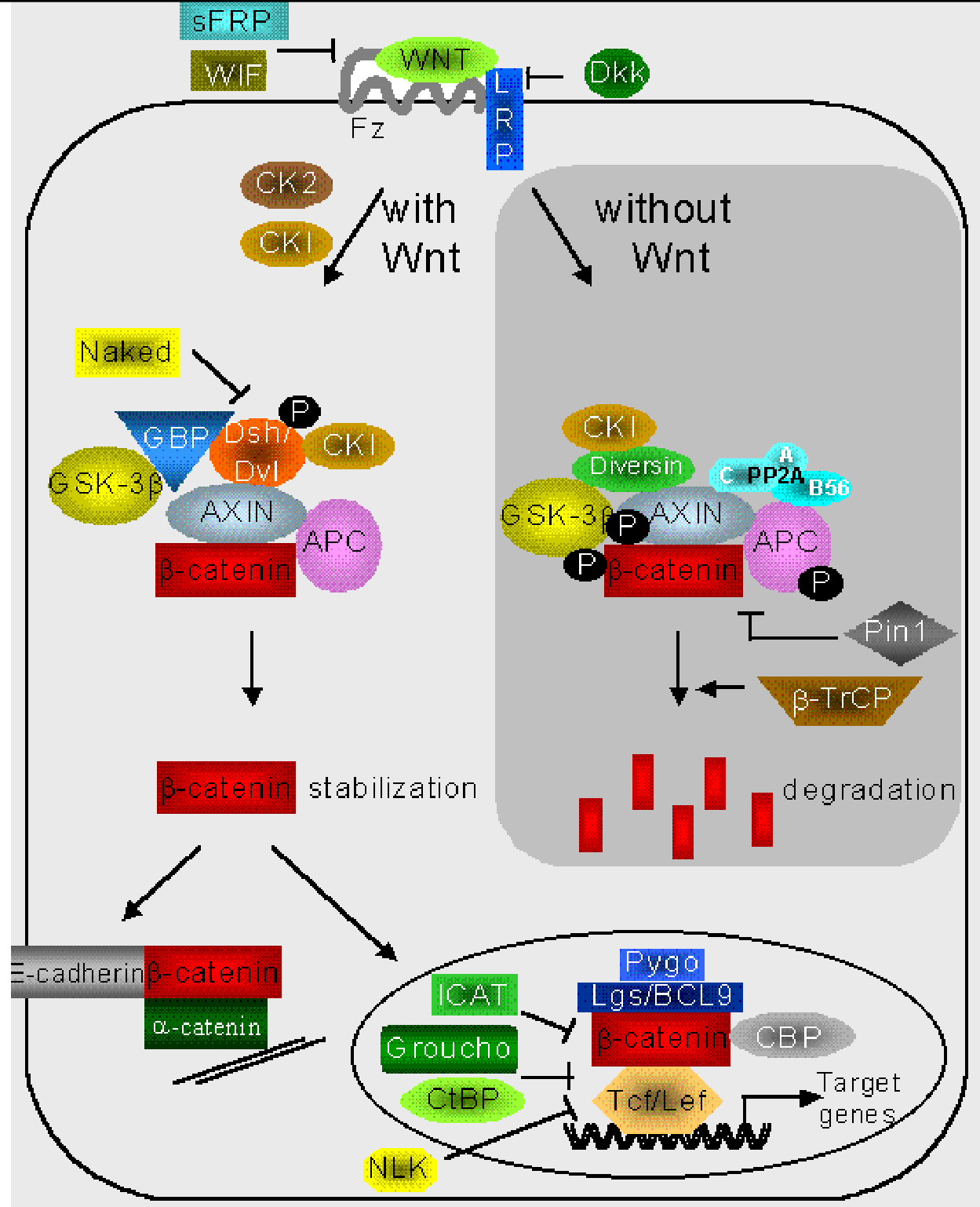


Wnts – viz přednáška č. 10

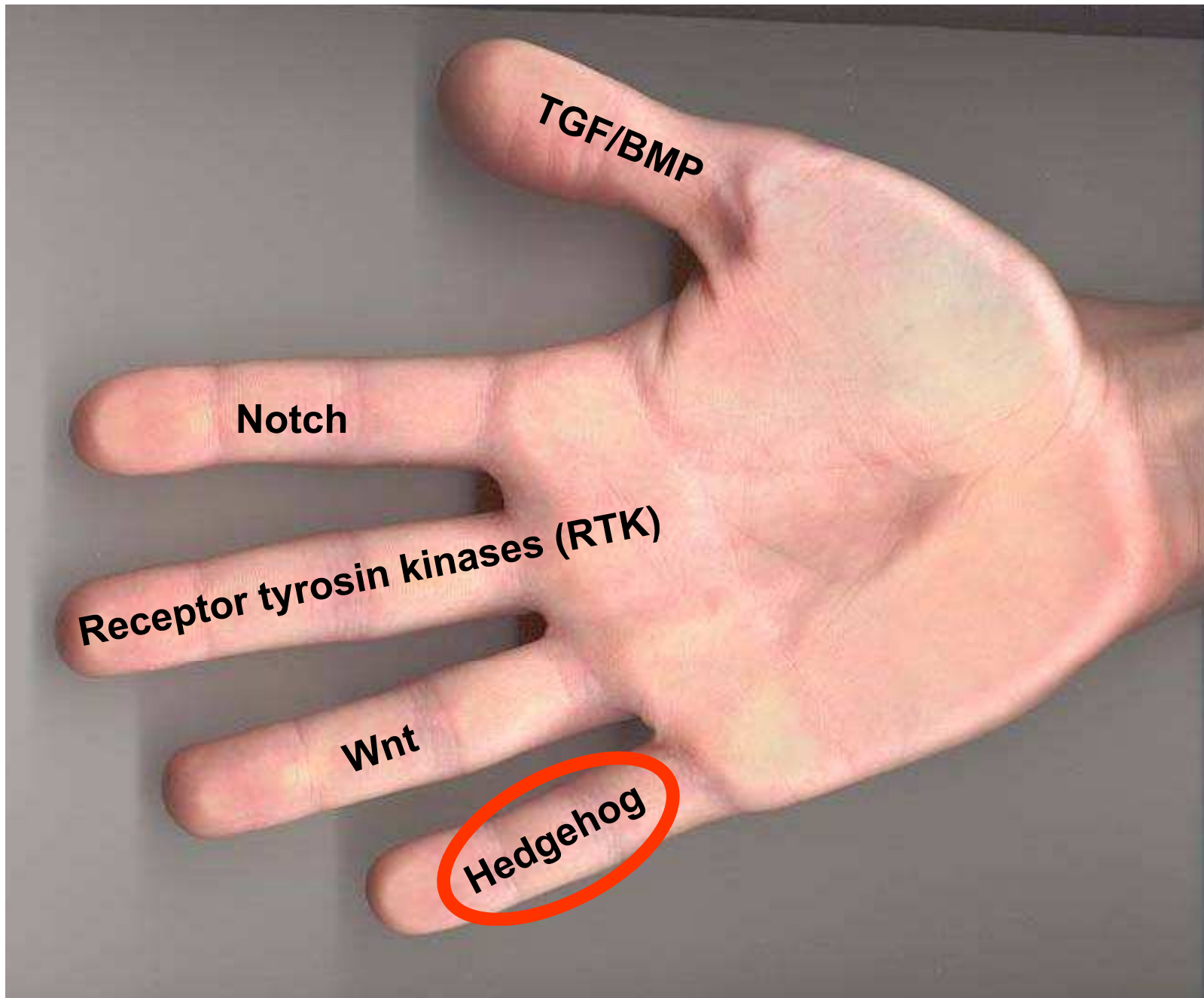
- rodina ligandů
- 19 členů u člověka a myši
- glykosylované a palmitoylované extracelulární proteiny
- Název: z Wingless – první mutace u Drosophily a Int – onkogen přenášený MMTV-virem a způsobující prsní karcinomy

Wnt/ β - kateninová dráha

- nejlépe poznaná
- Wnt=ligand
- Frizzled=receptor
- beta-katenin je hlavní součástí této dráhy, po aktivaci aktivuje ve spolupráci s dalšími faktory transkripci

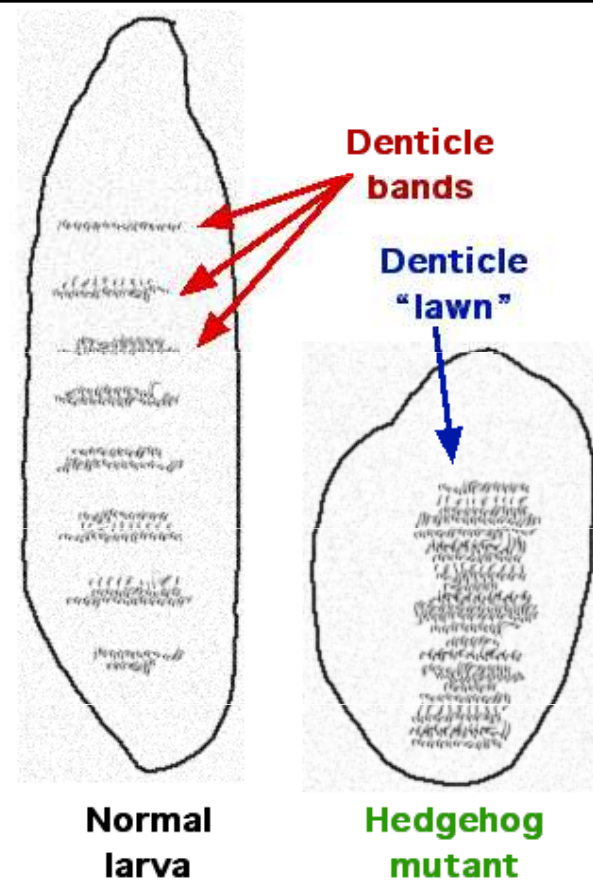


Signály z vnějšího prostředí



Hedgehog dráha

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



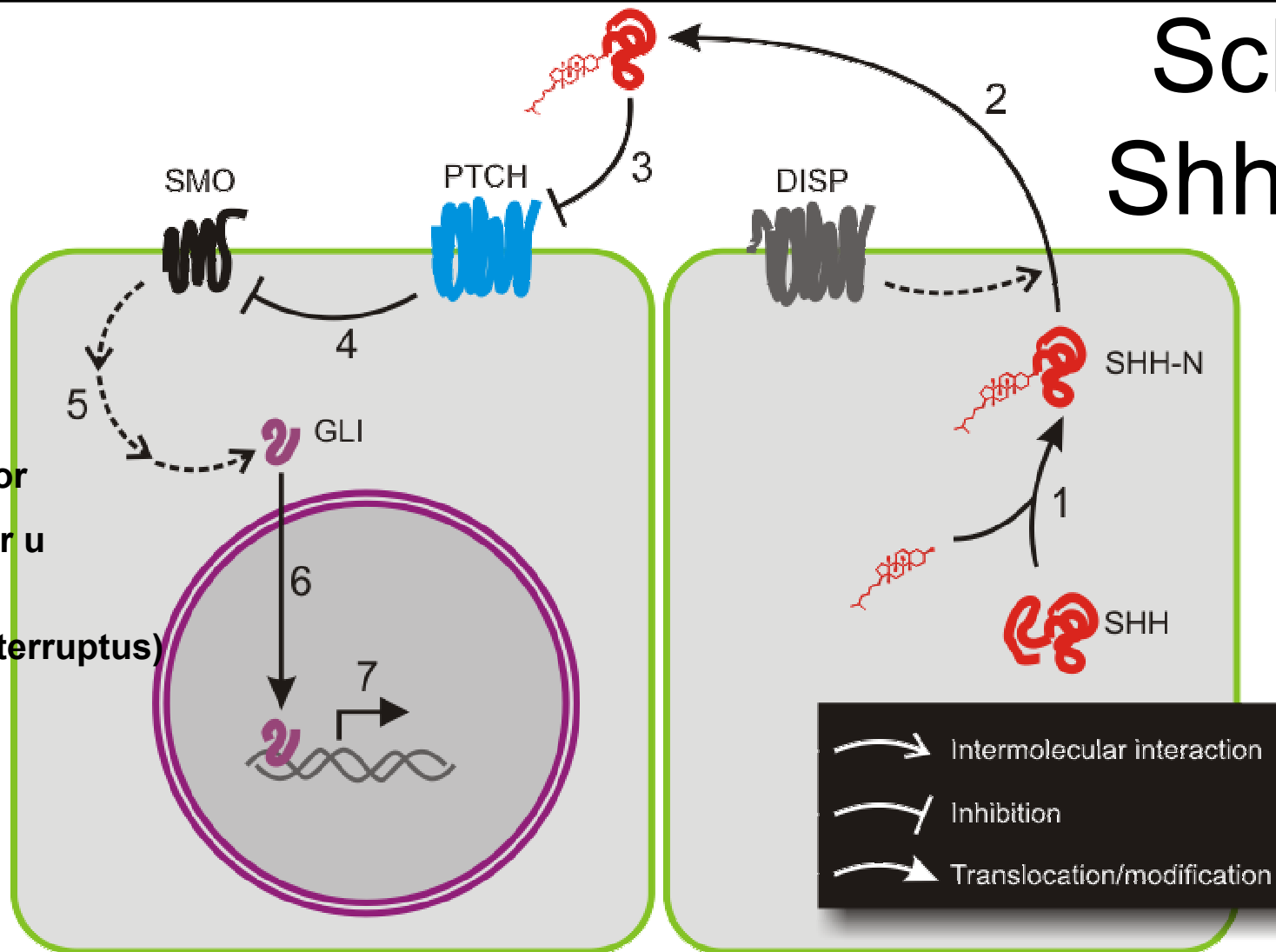
Sonic the Hedgehog

Schéma Shh dráhy

Gli1,2-aktivátor

Gli3 - represor u obratlovců

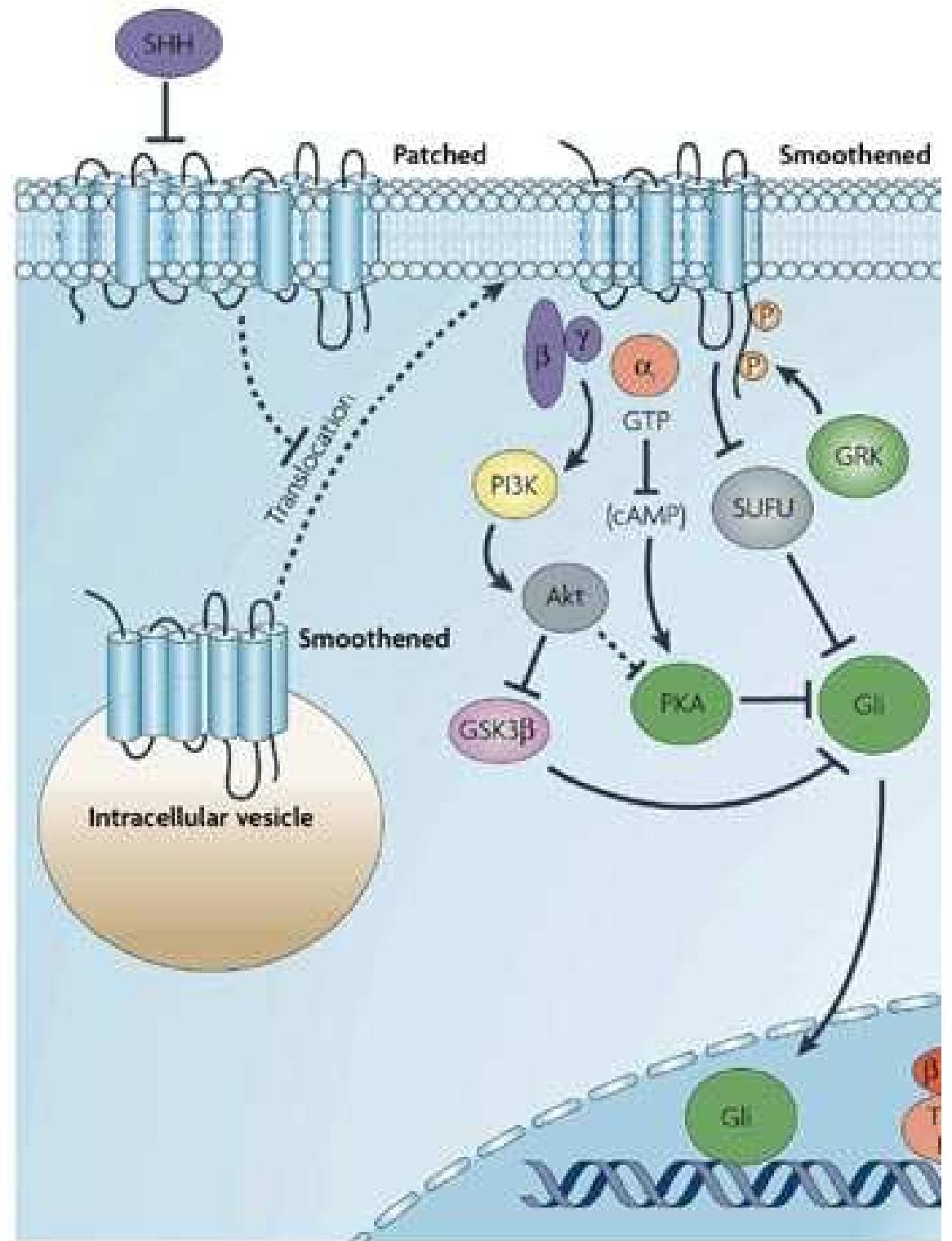
Ci (cubitus interruptus) u octomilky



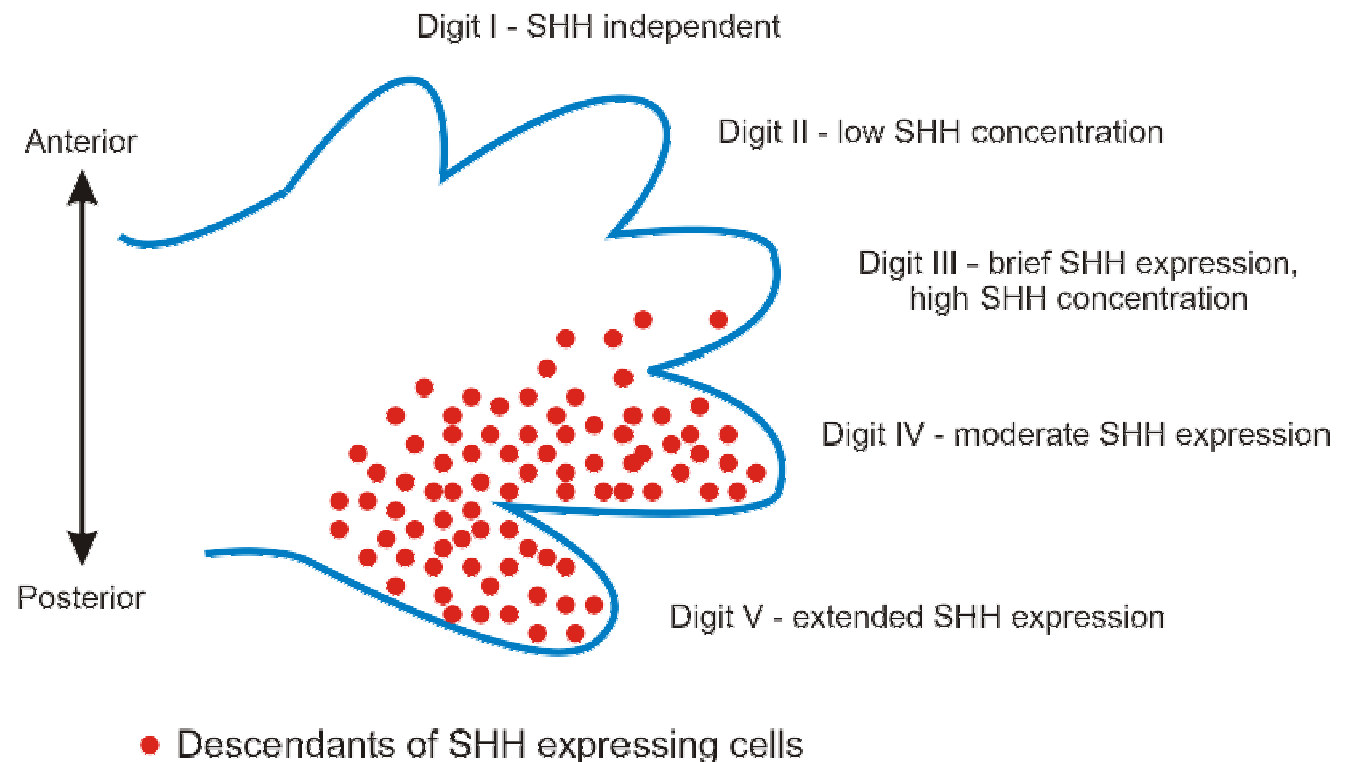
Sonic hedgehog (SHH) is [translated](#) as a ~45kDa precursor and undergoes [autocatalytic](#) processing to produce an ~20kDa N-terminal signaling domain (referred to as SHH-N) and a ~25kDa C-terminal domain with no known signaling role (1 on figure 5). During the cleavage, a [cholesterol](#) molecule 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 Smoothed (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)

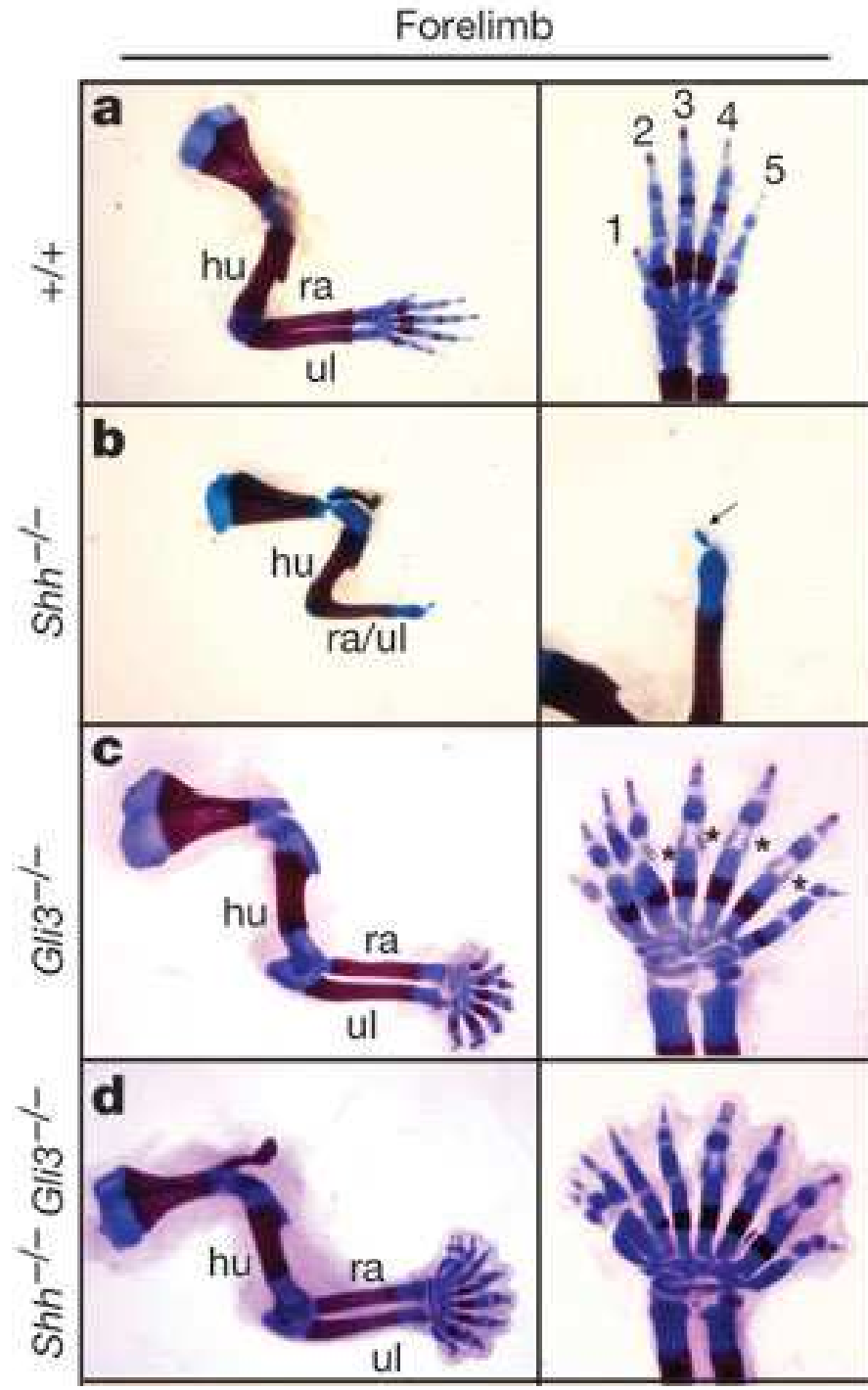
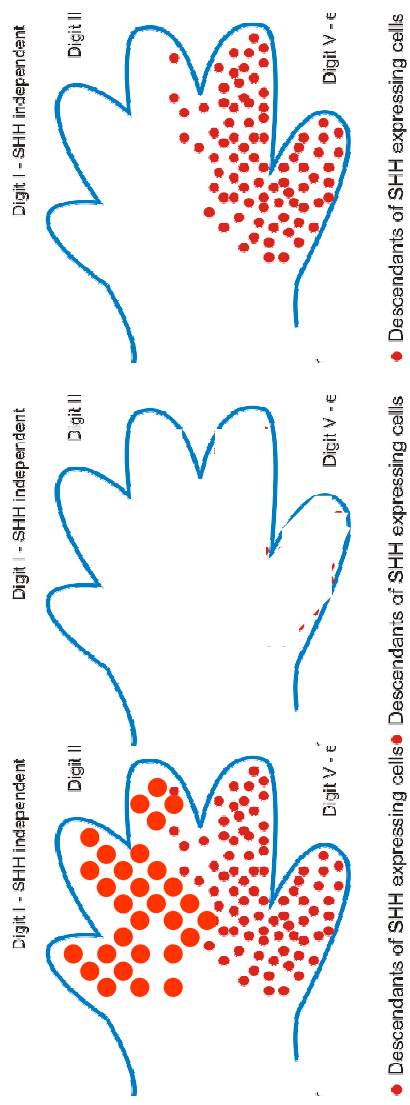
The 12-transmembrane domain protein patched (PTCH), the receptor for hedgehog proteins, negatively regulates the seven-transmembrane receptor smoothed (SMO) in the resting state. The binding of sonic hedgehog (SHH) to PTCH relieves this inhibition and SMO changes its localization to the membrane, thereby 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).



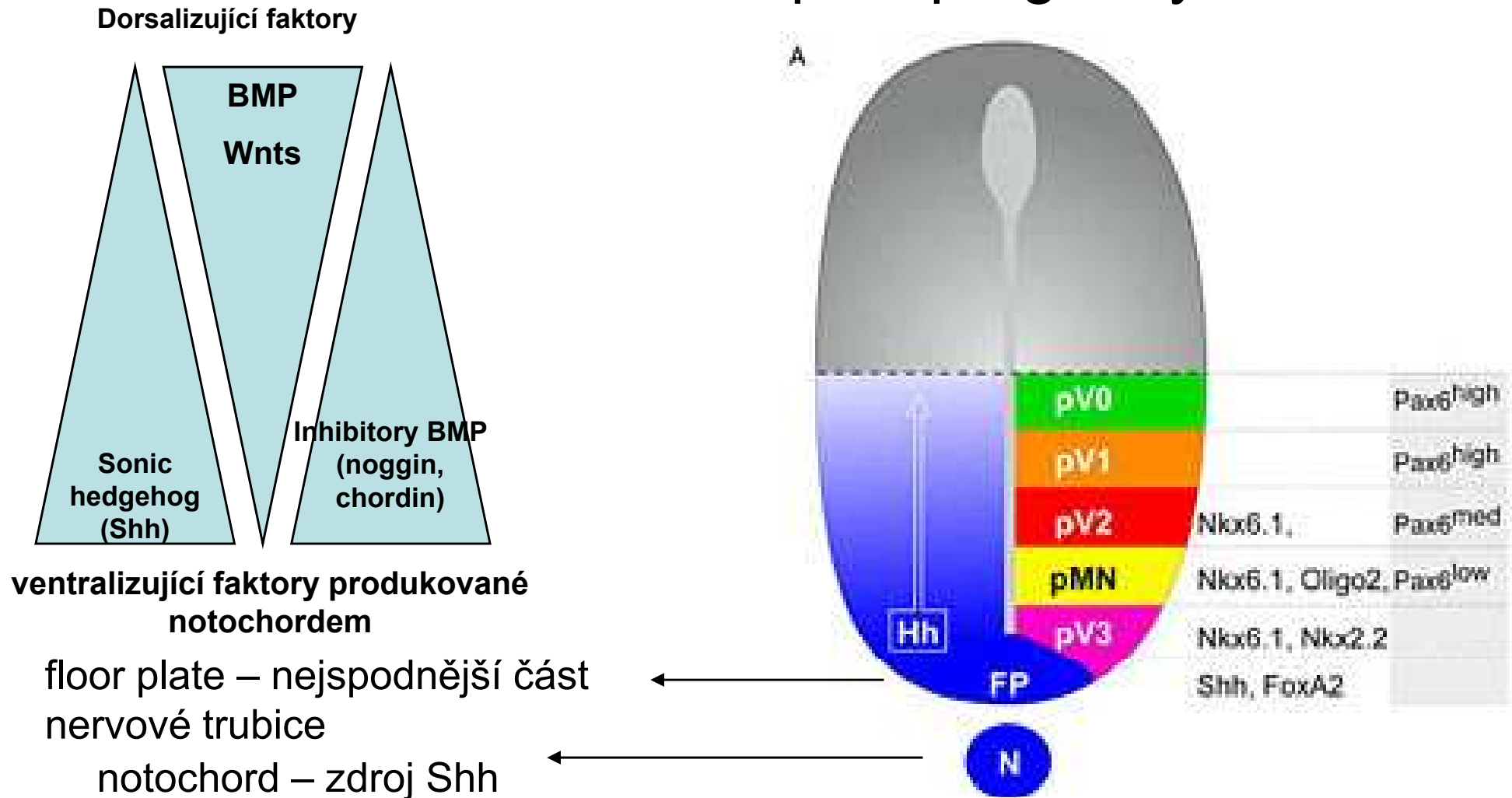
Shh – jeden z nejlépe popsaných klasických morfogenů (tzv. model francouzské vlajky) – v závislosti na koncentraci morfogenu se spouští odlišné transkripční programy



Např. specifikace jednotlivých prstů končetiny



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



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

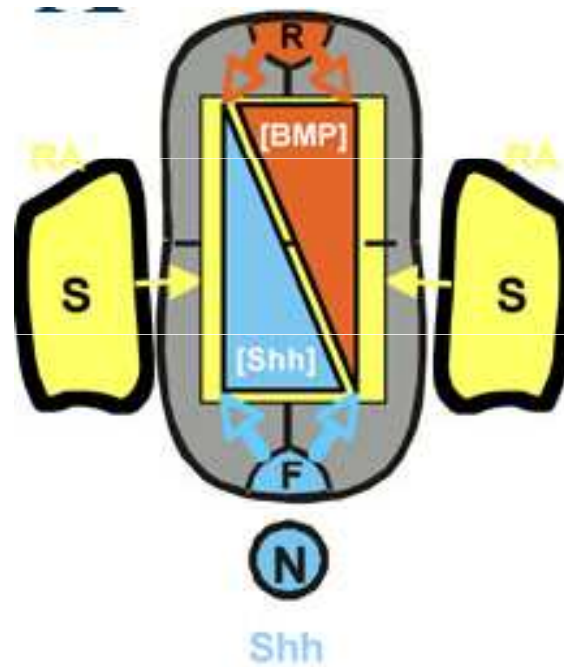
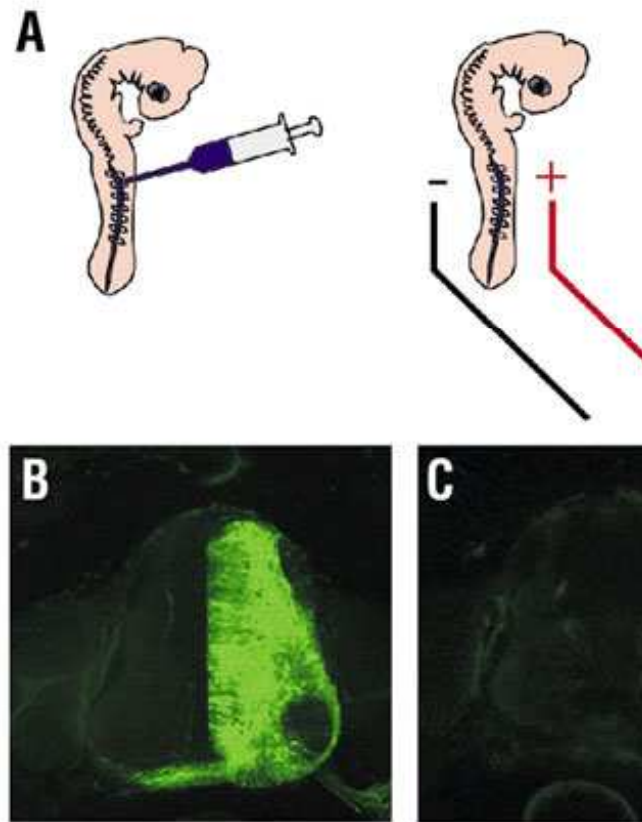
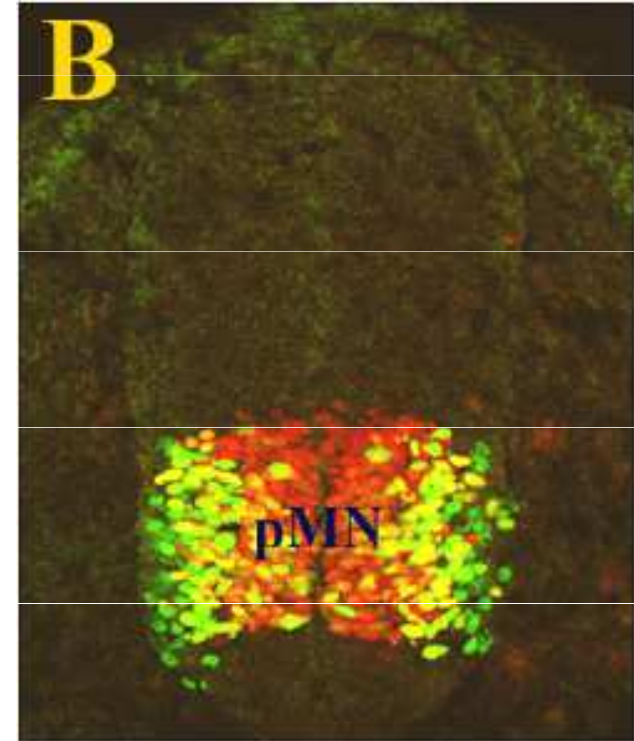


Fig. A - A model for early spinal cord development. The neural tube which will form the spinal cord is patterned into specific domains by multiple external signals which include a ventralizing Sonic Hedgehog (Shh) signal from the notochord (N) and floor plate (F), a dorsalizing BMP signal from the roof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

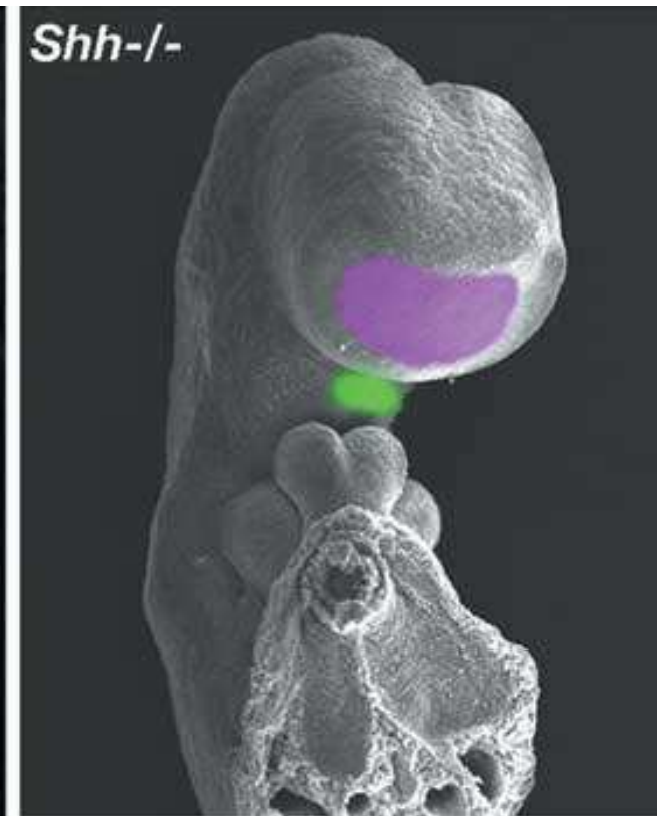
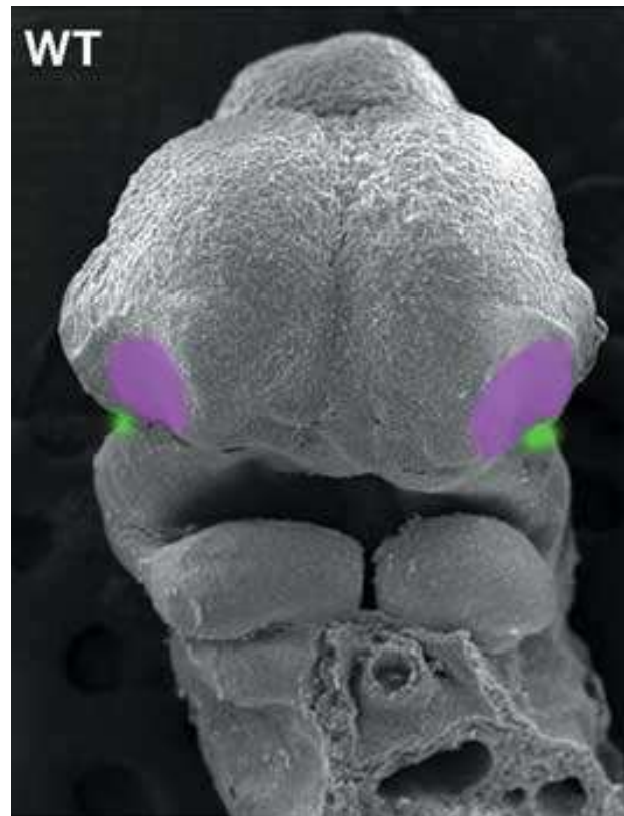
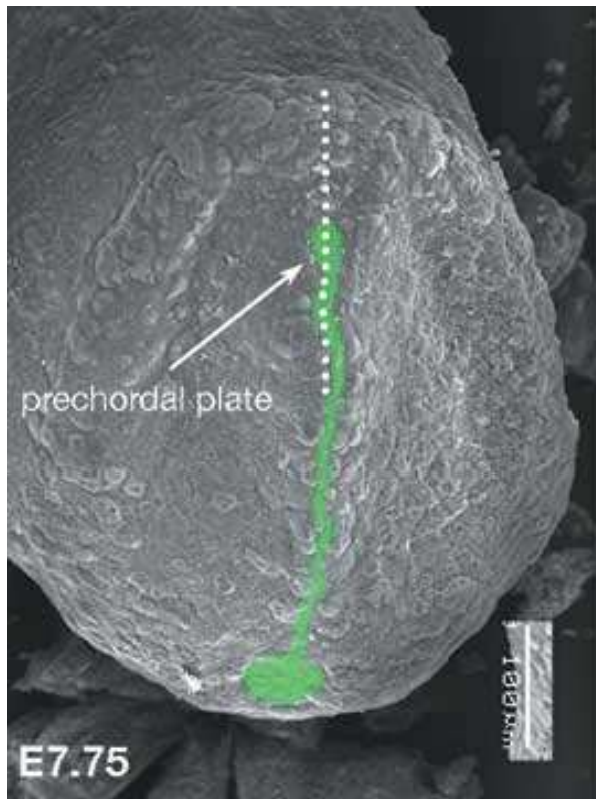


Cross section of the spinal cord of an embryonic day three chicken embryo stained with fluorescent antibodies. Shown here in red is the motor neuron progenitor domain (pMN), one of many precise domains established by earlier signaling events. The pMN domain is here labelled through the use of antibodies 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



Expression of Sonic hedgehog (Shh) protein and the determination of the midline structure in mouse embryo.

An SEM micrograph of the frontal view of a mouse embryo (fetal age 7.75 days). Shh protein is green. The dotted line in the micrograph shows the region: Shh antibody reveals Shh. The part that will become the brain (head fold) is followed by the prechordal plate. Shh (in green) that is expressed in the prechordal plate induces midline structure formation.

Model mice with Holoprosencephaly due to a Sonic Hedgehog (Shh) deficiency.

An SEM micrograph of ten-day old mouse embryos (front view of face). The mouse deficient in Shh gene (right) has no midline structure and only one region (eye position shown in green). Note, too, the lack of nostril separation due to no midline structure. The normal embryo (left), by contrast, has both the eyes and nostrils separated to between the two hemispheres.

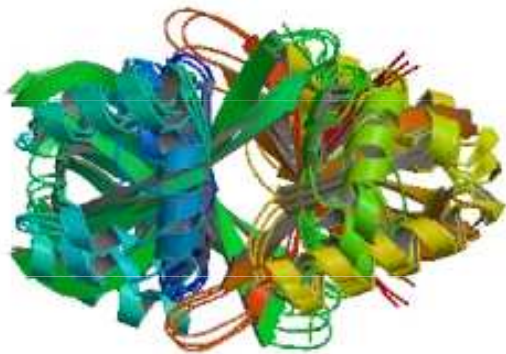
Hypoxie a HIF

Část podkladů dodali:

Eva Lincová

Jana Kamarýtová

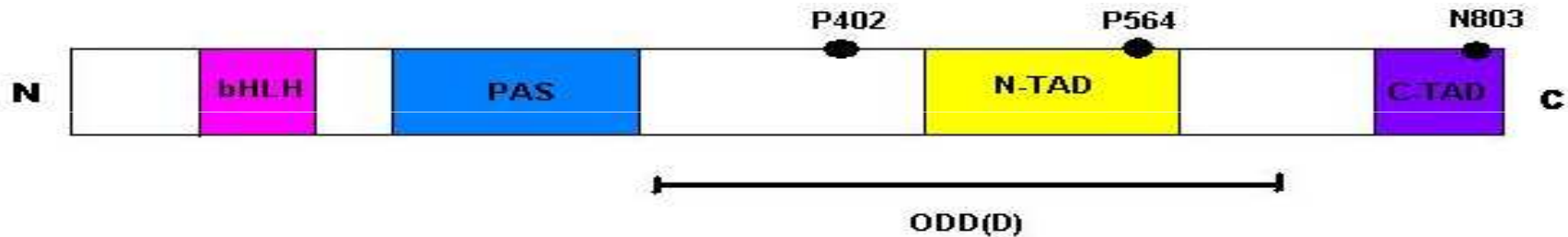
Filip Trčka



Hypoxie a HIF

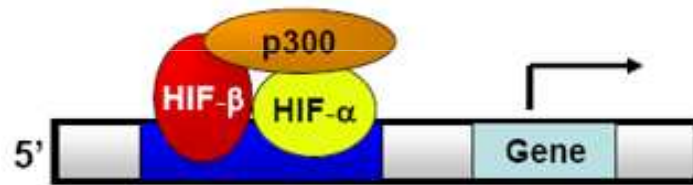
- **Hypoxie:** snížený parciální tlak O_2 ve tkáni X normoxie
- **HIF** – Hypoxia-Inducible Factor:
 - Heterodimerický TF aktivující geny obsahující v promotorové sekvenci HRE (Hypoxia response element), vlastní transkripce je iniciována pomocí koaktivátorů **p300** a **CBP** (CREB-binding protein)
 - 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 **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

Struktura HIF α podjednotek



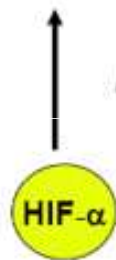
- bHLH (basic Helix-Loop-Helix) motiv: vazba na DNA, dimerizace
- PAS (Per/ARNT/Sim) doména: usnadňuje heterodimerizaci
- N-TAD (N-terminal transactivation domain)
- C-TAD (C-terminal transactivation domain)
- ODD(D) (Oxygen-dependent degradation domain)
- P402, P564: místa specifické hydroxylace v ODD při normoxii
- N803: místo specifické hydroxylace asparaginu mimo ODD

HIF při normoxii a hypoxii

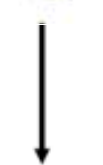


Transcription of >100 human genes
e.g. **EPO, VEGF, Glycolysis proteins**)

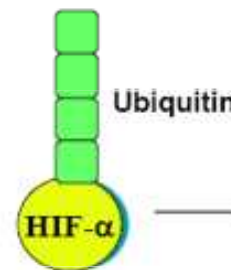
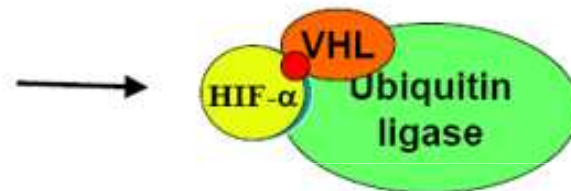
HIF binds to hypoxic response DNA sequences



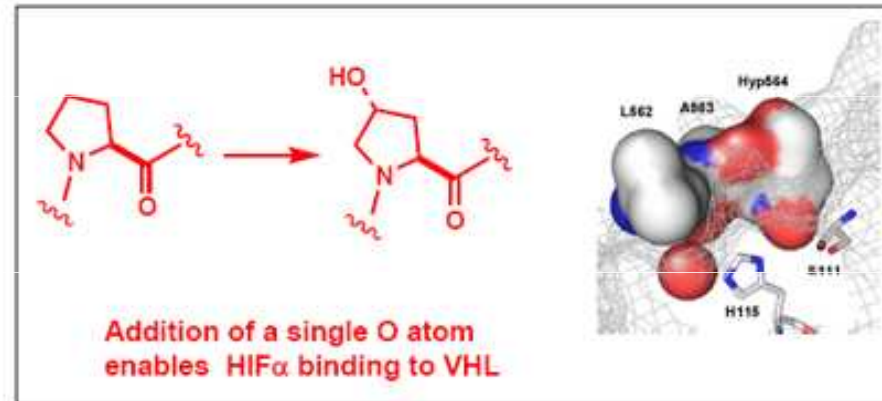
HYPOXIA: HIF α levels rise



NORMOXIA: enzymatic post-translational hydroxylation
catalysed by Fe(II), 2-oxoglutarate oxygenases (**PHD 1-3** in humans)
(+ O) ●



Proteasomal degradation



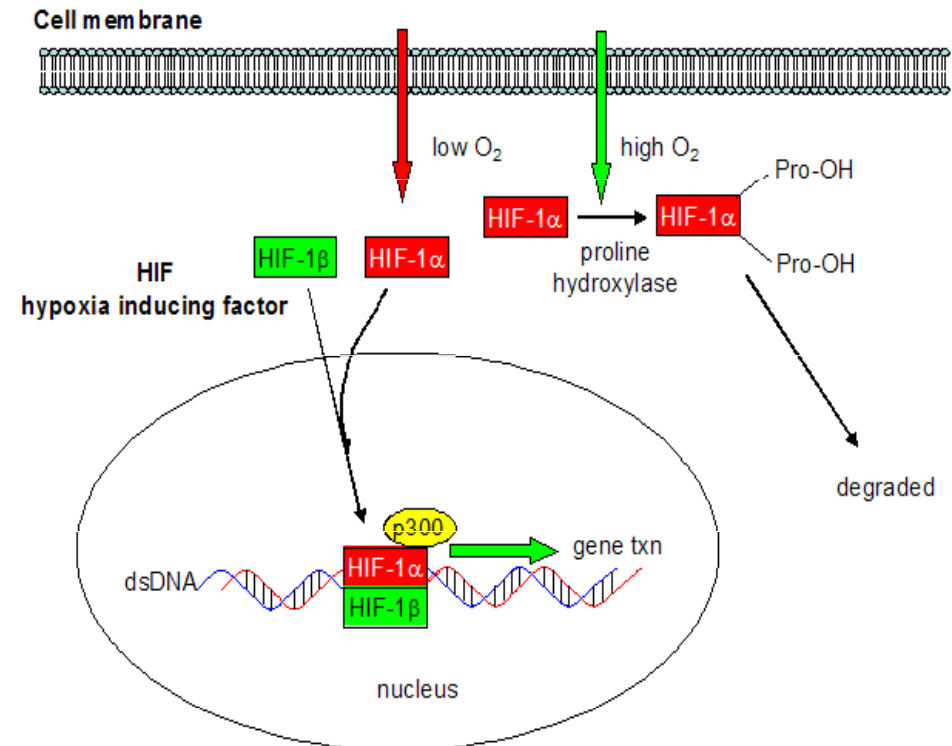
<http://www.chem.ox.ac.uk/oc/cjschofield/images/new-2.png>

Levels of Hypoxia Inducible Factor- α mediate the hypoxic response

VHL (von Hippel-Lindau) - tumor supresorový gen

Modelové vývojové změny spojené s hypoxií/HIF systémem

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

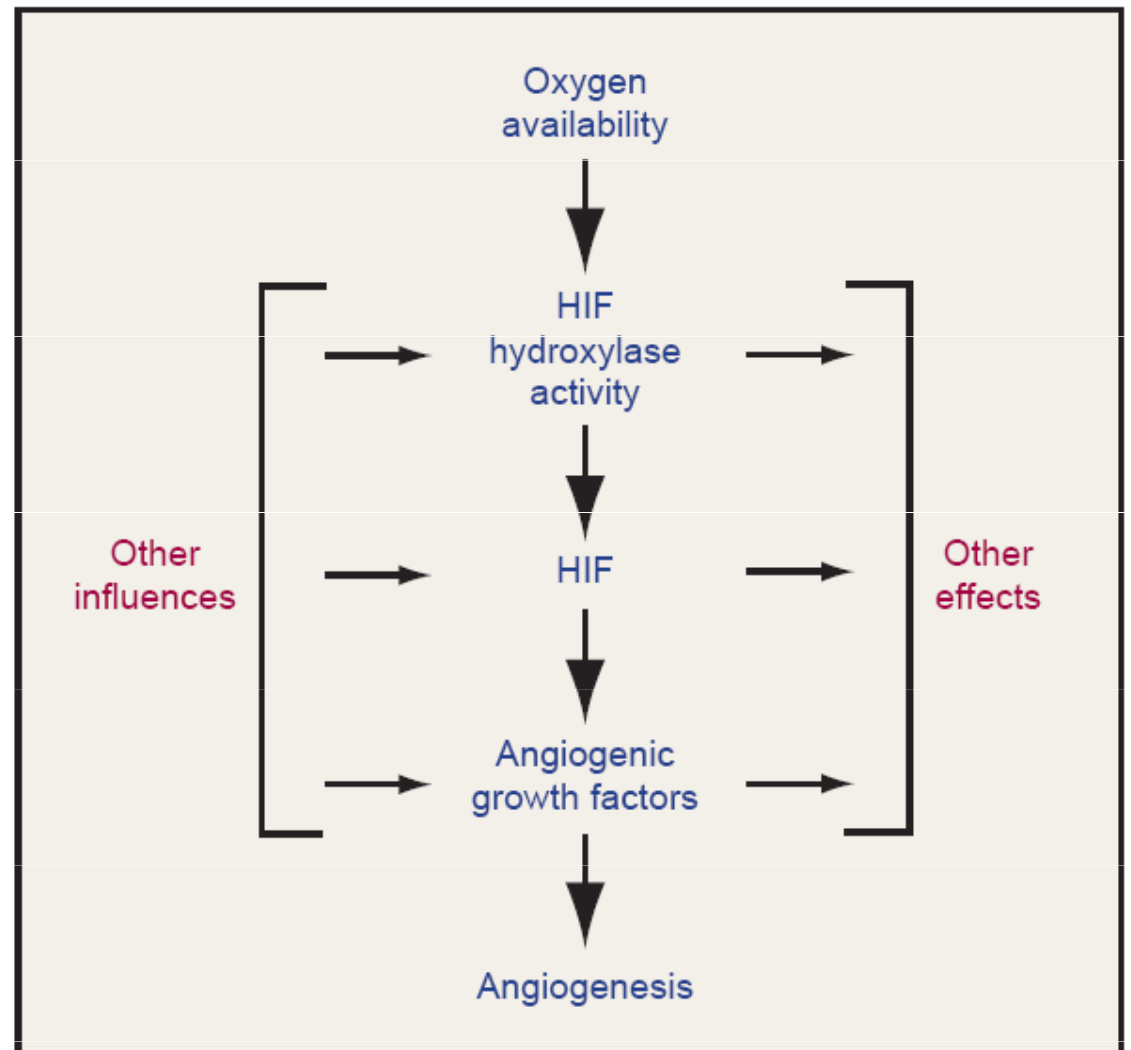


Genes upregulated:

- 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)

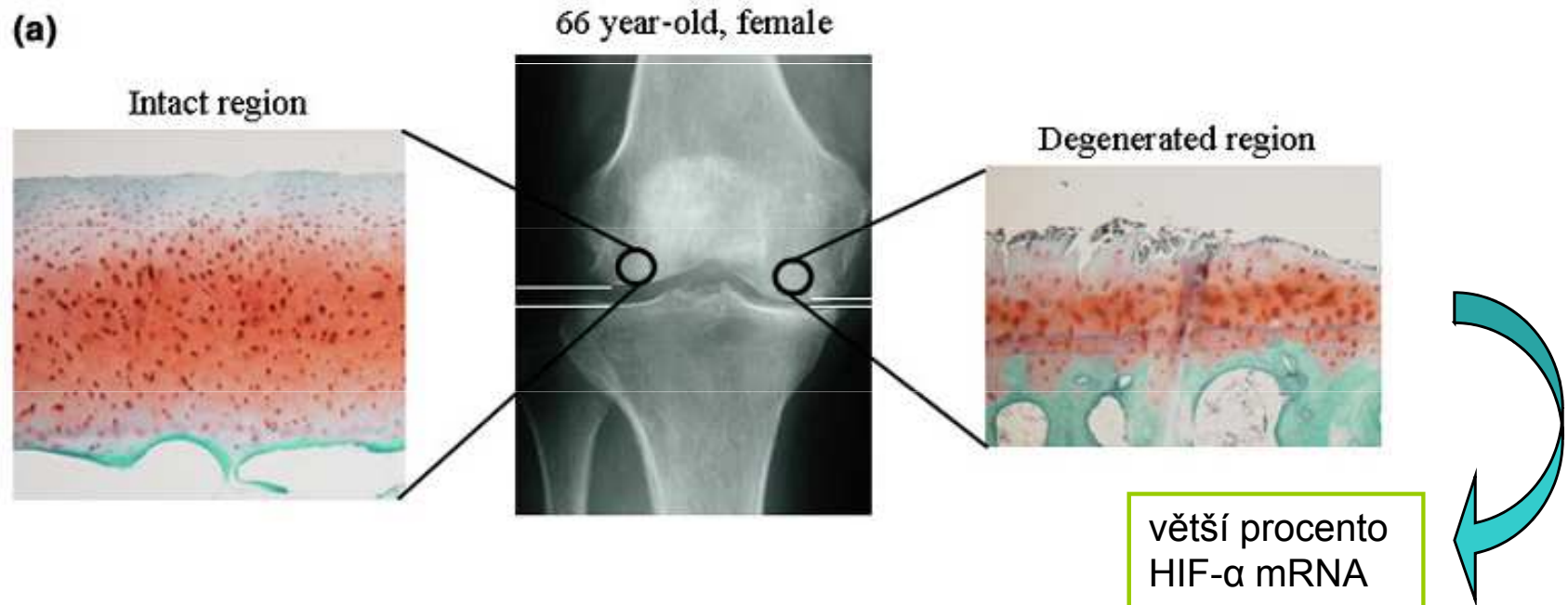
Angiogenesis

- 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

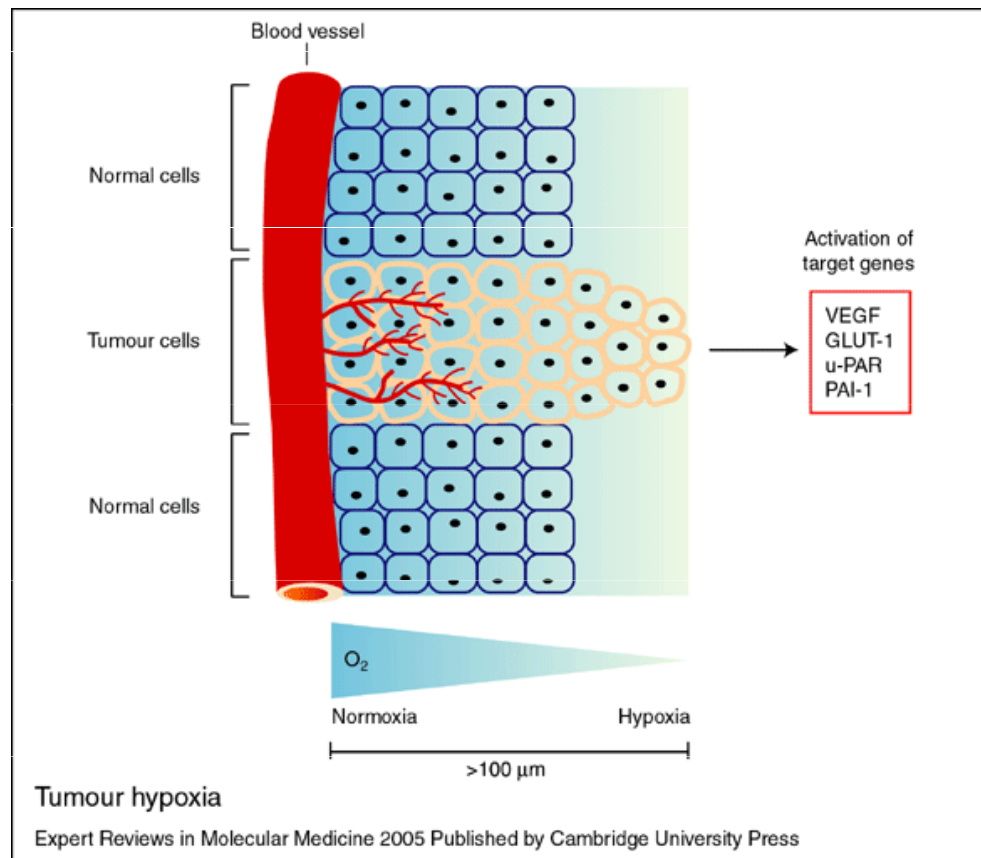


Růst chrupavek

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



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)

