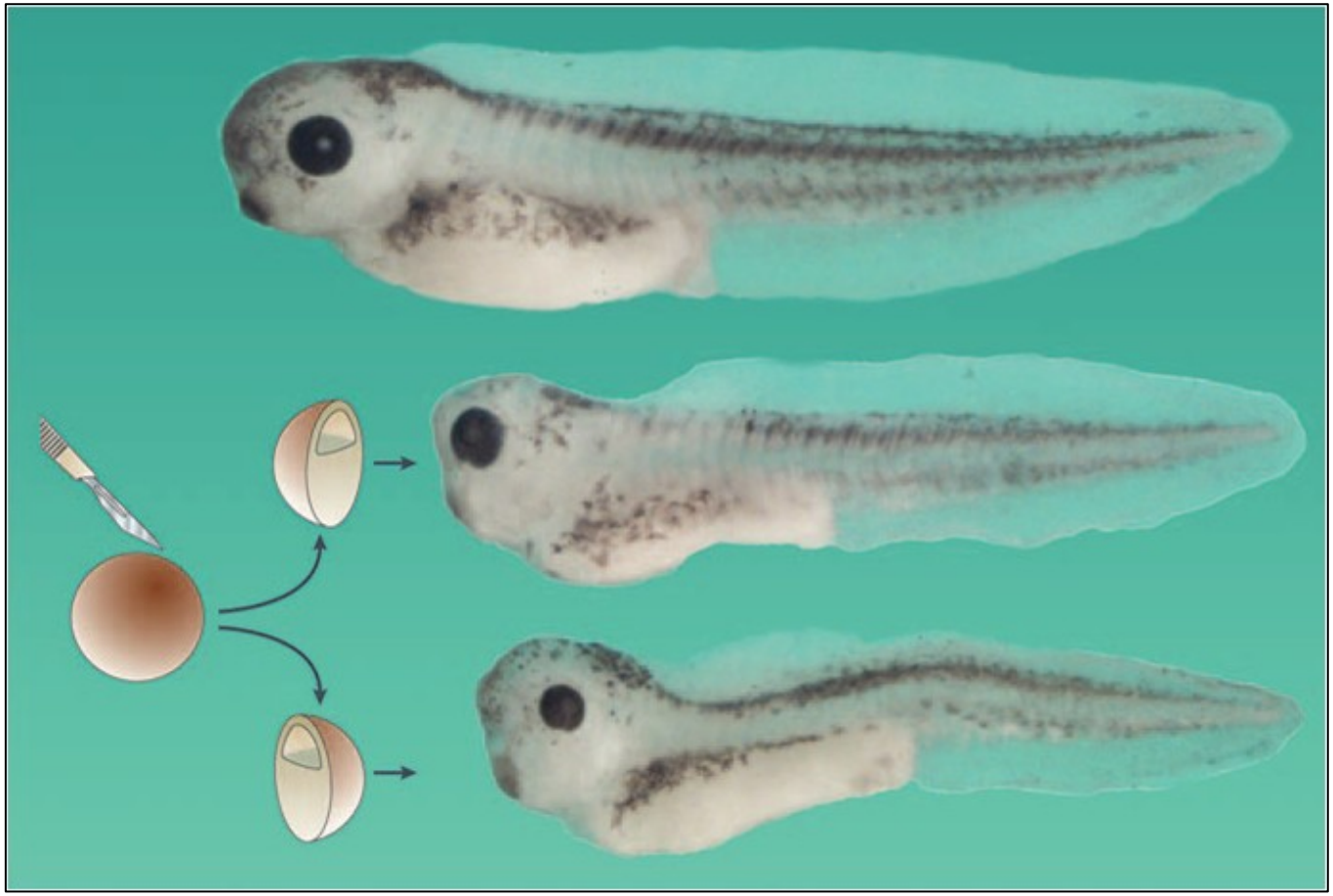
Buněčné systémy ve vývoji

doc. Mgr. Vítězslav Bryja, Ph.D.

Klíčové molekulární komponenty vývoje



Klíčové molekulární komponenty vývoje

Co poskytuje buňce tyto informace?

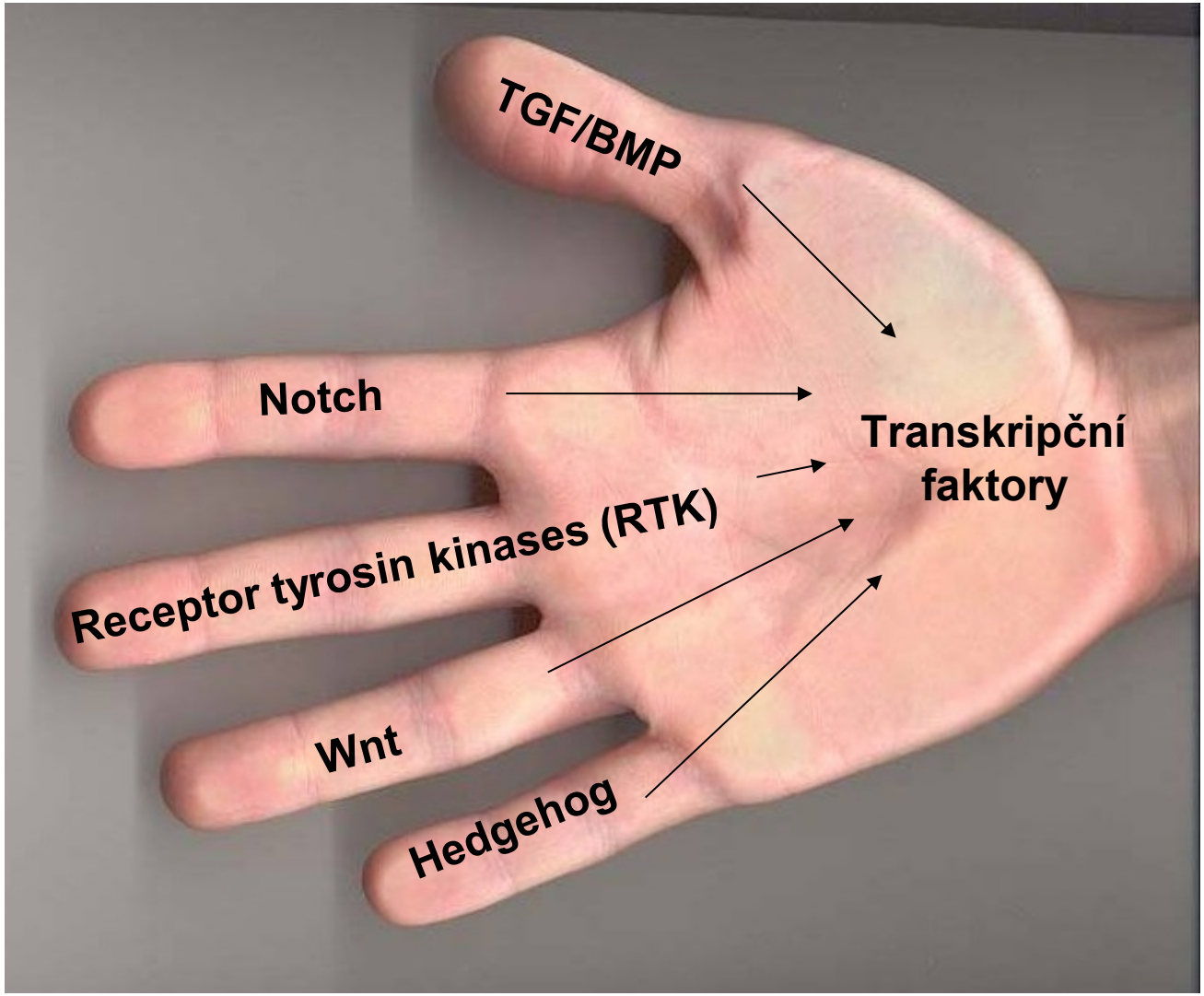
a) signály z okolního prostředí

jednotlivé signální dráhy modulují transkripci a strukturu chromatinu

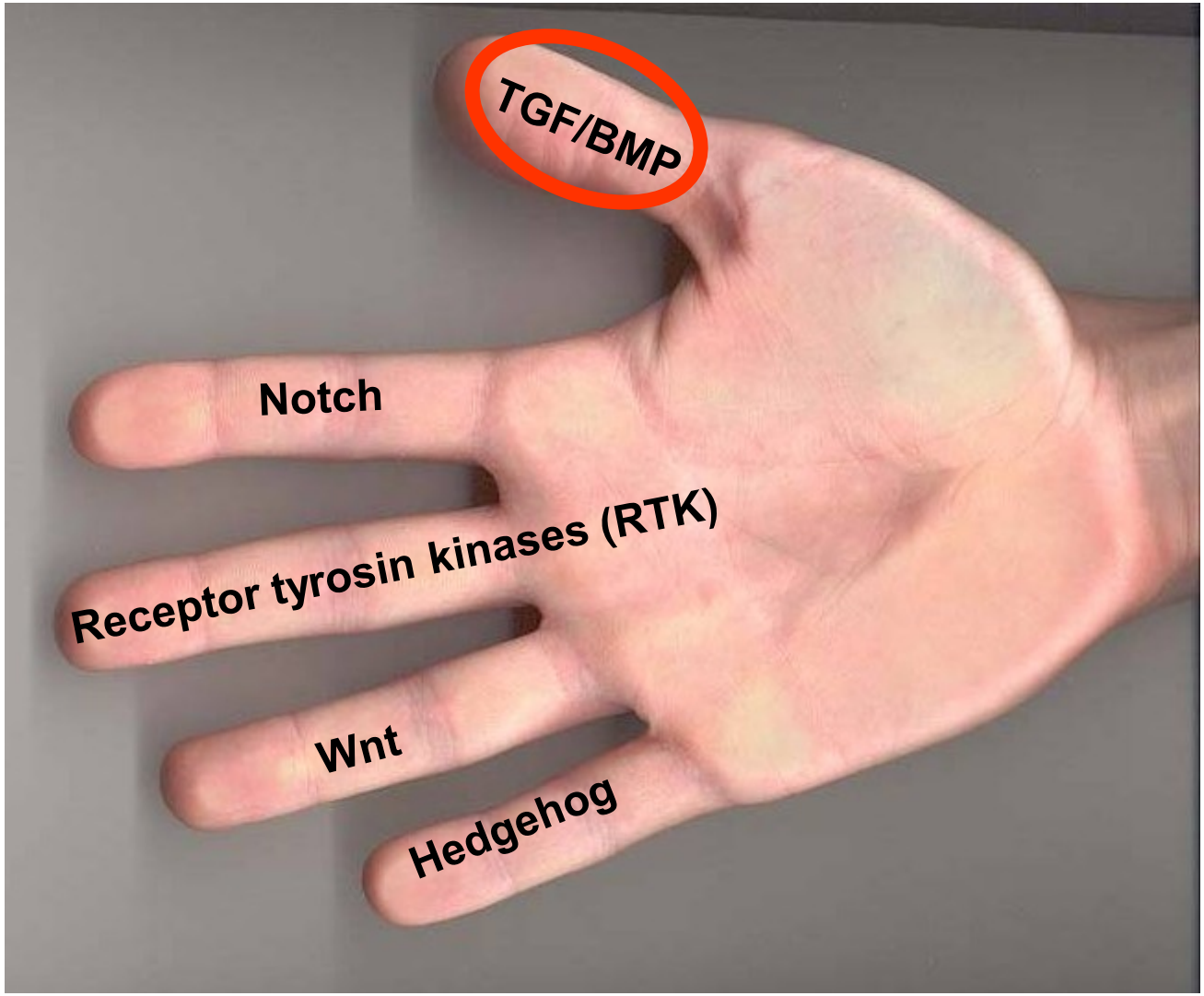
transkripce určuje citlivost buňky k vnějším signálům (např. regulací exprese receptorů či komponent přenosu signálu)

b) transkripční program v jádře

Klíčové molekulární komponenty vývoje



Klíčové molekulární komponenty vývoje



TGF/BMP

- TGF – transforming growth factor
- BMP – bone morphogenetic protein

- patří do TGFβ nadrodiny

TGF/BMP

TGFβ nadrodina má následující podrodiny:

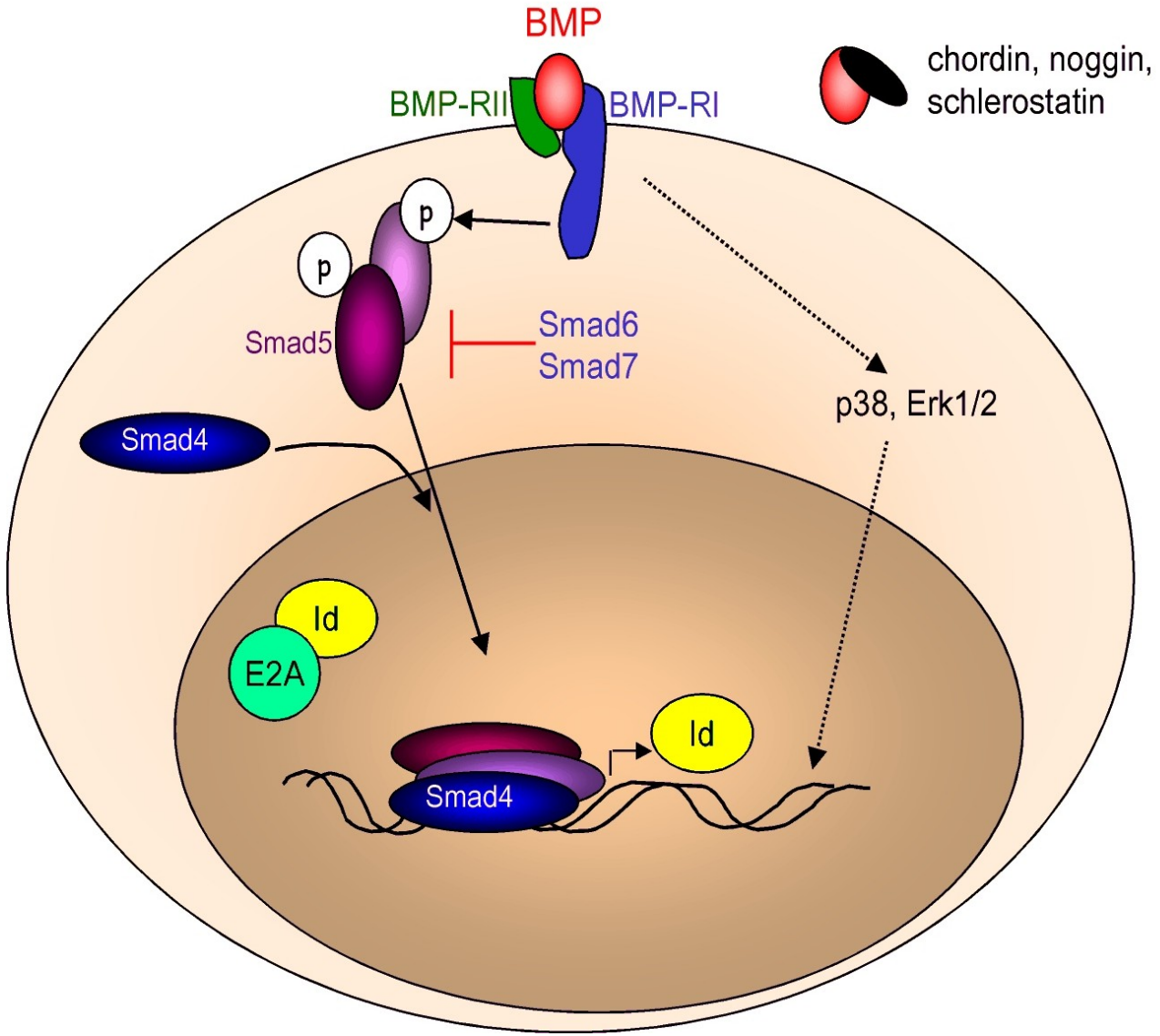
1. TGFβ1-3
2. BMPs – 20 různých ligandů
3. GDF (growth differentiation factor): 9 ligandů
4. activin/inhibin/nodal

Společným znakem je signalizace přes:

- konzervativní rodinu Ser/Thr kinázových receptorů – jsou dvou typů a po vazbě ligandu dimerizují

- cytoplazmatická signalizace přes tzv. SMAD proteiny

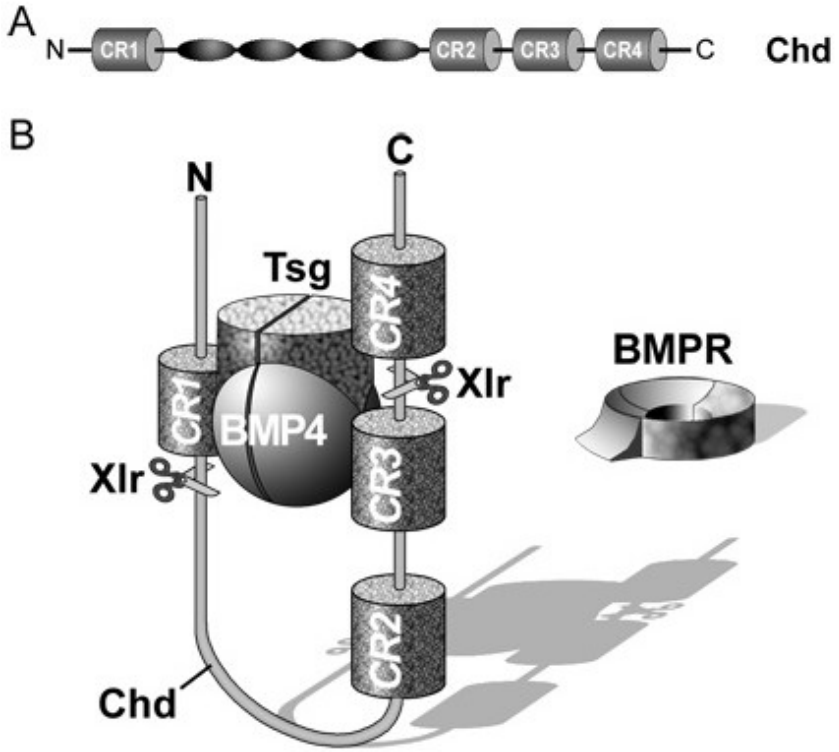
Signální dráha BMP



Inhibitory BMP faktorů

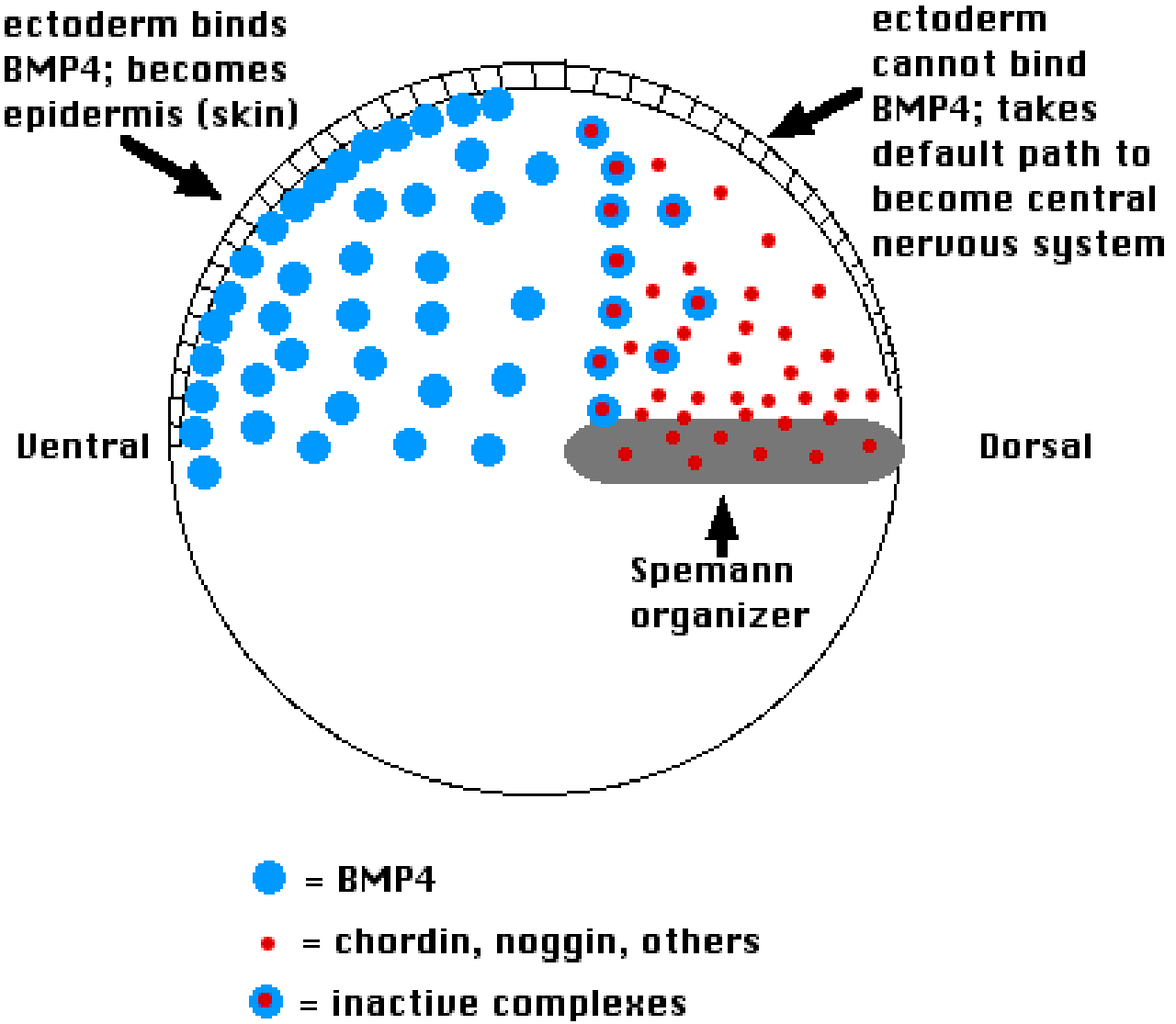
jsou klíčové pro fyziologické funkce BMP

- noggin
- chordin (Chd)
- sklerostin

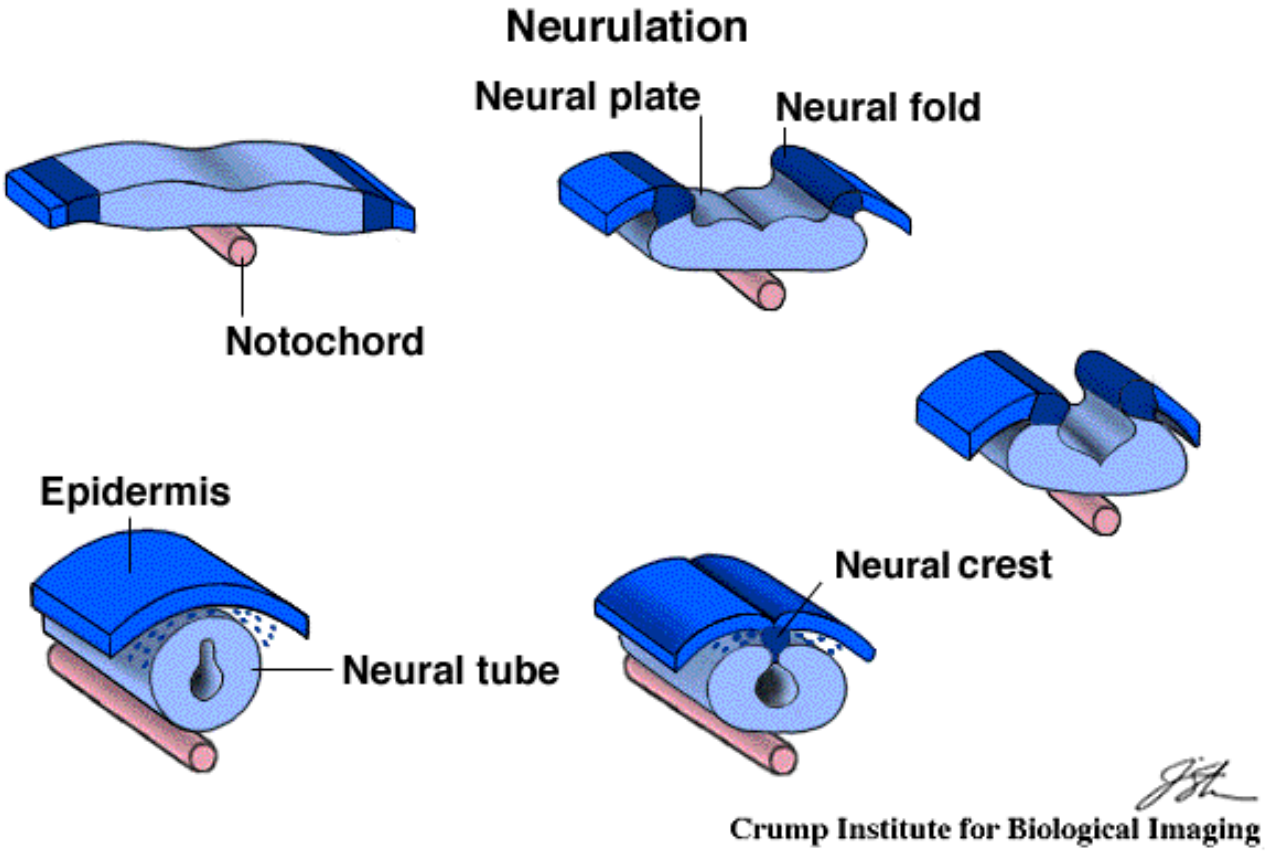


Přímá fyzická interakce mezi chordinem a BMP je podstatou inhibičního působení chordinu

Role BMP inhibitorů v Spemannově organizátoru



Klíčová role BMP inhibitorů produkovaných notochordem při indukci nervové ploténky



notochord (= chorda) produkuje faktory, které specifikují ektoderm a vedou ke tvorbě nervové ploténky (neural plate). Jde zejména o následující faktory: **noggin**, **chordin** a **follistatin** (inhibitory BMP a aktivinu). Samotná produkce těchto BMP inhibitorů specifikuje anteriorní (přední) nervovou trubici, v kombinaci s FGF specifikuje posteriorní (zadní) nervovou trubici.

Klíčové molekulární komponenty vývoje

Nodal

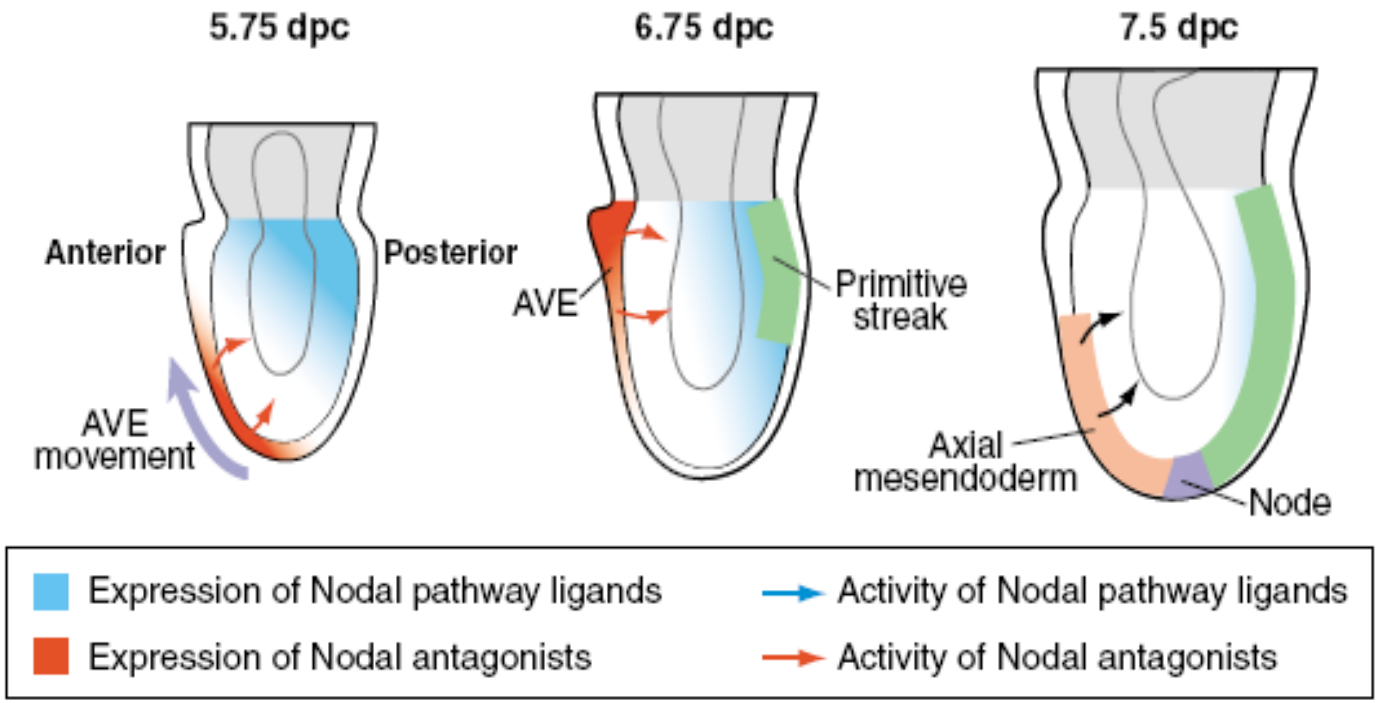
Table 1. Key components of the Nodal signaling pathway

Role	Gene	Function
Pathway ligands	*Nodal* (mouse, chick), *cyclops, squint, southpaw* (fish), *Xnr1, Xnr2, Xnr4, Xnr5, Xnr6* (frog)	Nodal-related TGFβ ligands
	Vg1 (frog, fish, chick)	TGFβ ligand; signals through Nodal pathway
	Gdf1 (mouse)	TGFβ ligand; signals through Nodal pathway
	Gdf3 (mouse)	TGFβ ligand; signals through Nodal pathway
Receptors and co-receptors	*ALK4*	Type I serine-threonine kinase receptor
	ActRII, ActRIIB	Type II serine-threonine kinase receptors
	Cripto, Cryptic (mouse), *one-eyed pinhead* (fish), *FRL-1/XCR1, XCR2, XCR3* (frog)	EGF-CFC co-receptors; interact with ALK4
Inhibitors	*Lefty1, Lefty2*	TGFβ proteins; interact with Nodal ligands and EGF-CFC co-receptors
	Cer1, Cer2	Cerberus/DAN family members; interact with Nodal ligands
Smads	*Smad2, Smad3*	Receptor-Smads
	Smad4	Co-Smad

Nodal a pravo-levá symetrie

gastrulace u myši

AVE – anterior visceral endooderm



Nodal a pravo-levá symetrie

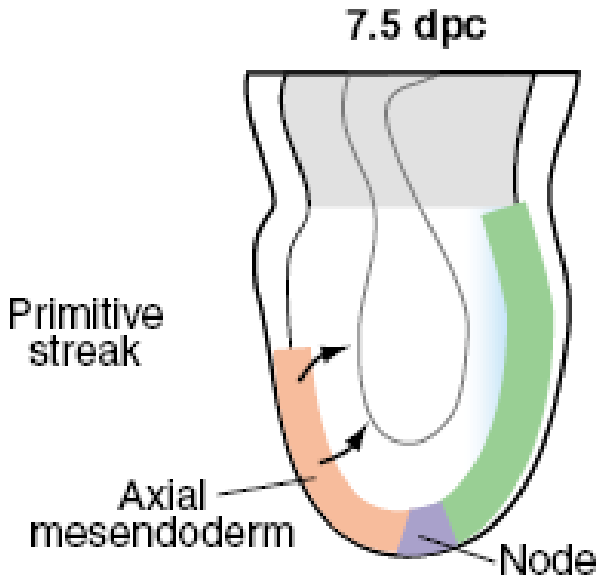
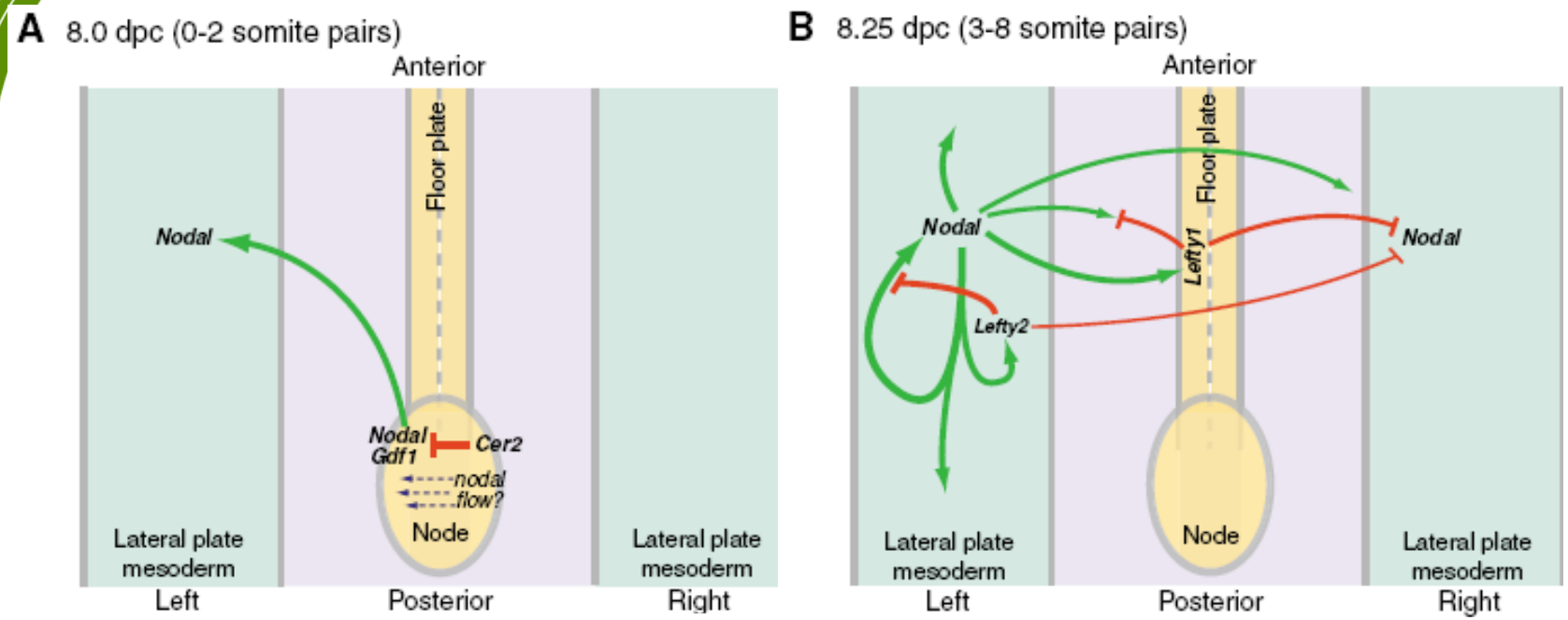


Fig. 5. Sequential function of Nodal signaling in left-right patterning in the mouse embryo. (**A**) Following initial symmetry breaking around the node, possibly as a consequence of ciliary-based nodal flow, Nodal (green arrow) and/or Gdf1 signals become elevated on the left side of the node, and are antagonized by Cer2 (red). Nodal pathway activity then propagates to the left lateral plate mesoderm to activate left-sided *Nodal* expression, most likely through direct long-range action. (**B**) *Nodal* auto-regulates its own expression, which spreads through the left lateral plate mesoderm (green) through a positive-feedback loop. *Lefty2* is induced through a negative-feedback loop, and subsequently downregulates *Nodal* expression (red bar). Axial midline expression of *Lefty1* prevents the spread of left-sided *Nodal* signals, and suppresses ectopic *Nodal* activation on the right side.

Nodal a pravo-levá symetrie



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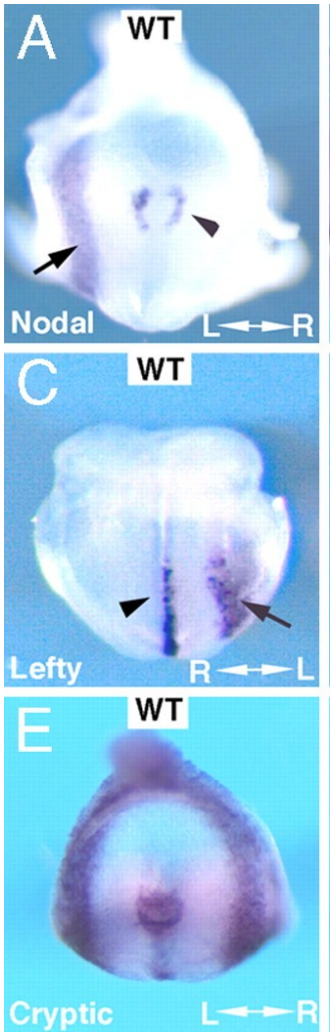
Nodal a pravo-levá symetrie

Analýza pomocí hybridizace in situ ukazuje rozdílnou expresi genů určujících levou stranu

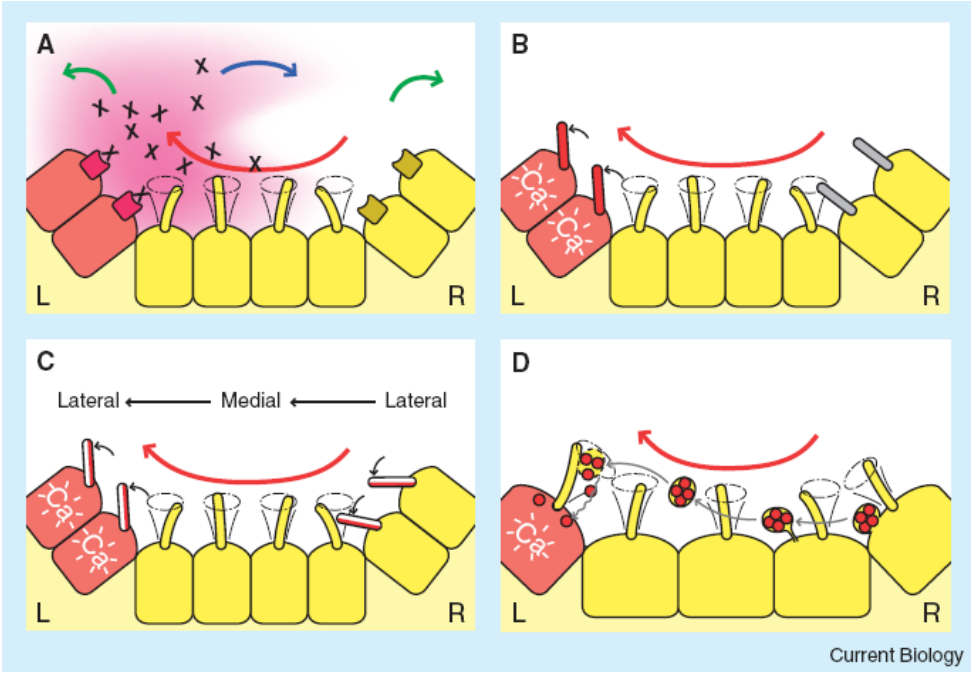


PNAS

Vytváření levopravé asymetrie těla - role cilií

• asymetrická exprese genů jako *lefty1*, *lefty2*, *nodal* a *pitx2*

• nodální cilie (9+0, dynein → pohyblivé) během gastrulace vytváří svým rotačním pohybem tzv. **nodální proud**



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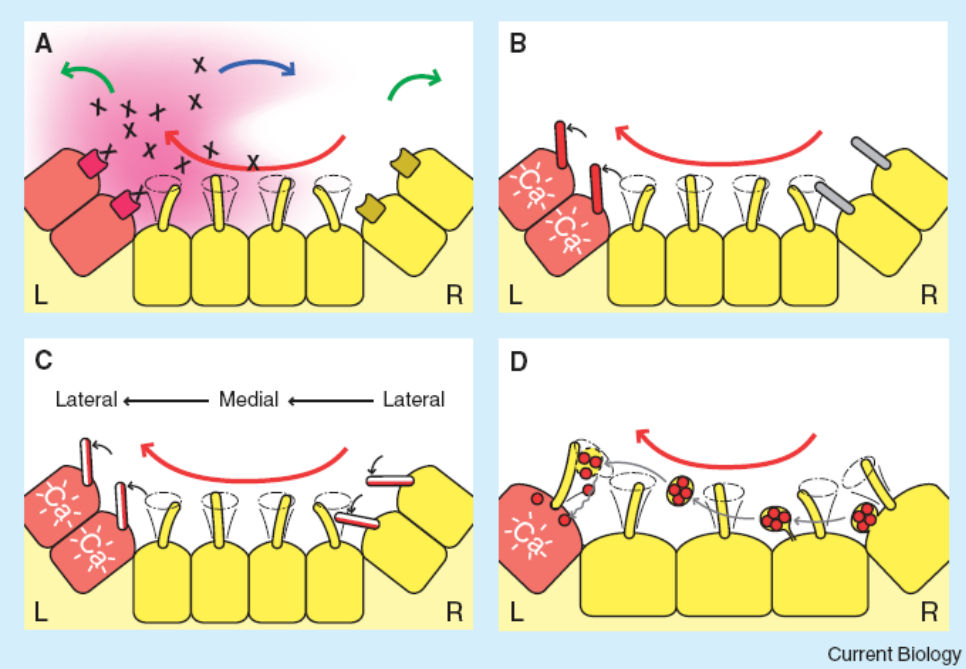
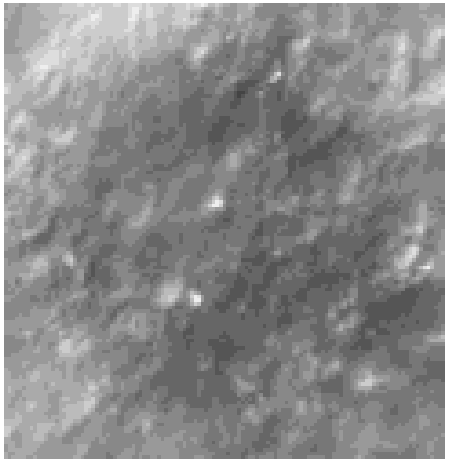


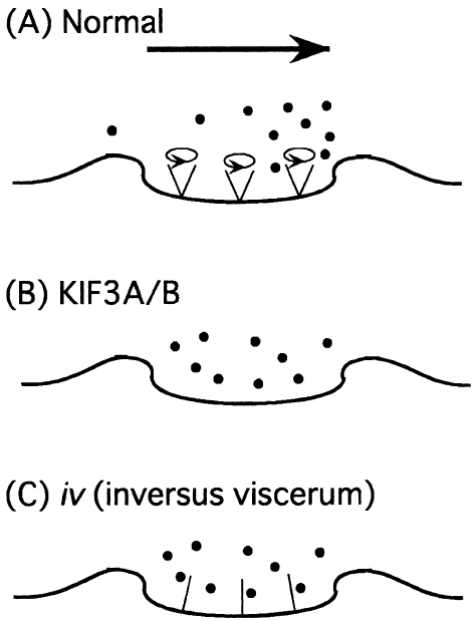


- narušená funkce cilií → vzniká až ***situs inversus*** (vnitřní orgány uspořádány obráceně podle střední osy těla) nebo ***situs ambiguus***

Yokoyama, 2004

Nodal a pravo-levá symetrie

KIF3A/B knockout myši, *iv* mutanti (nodální proud není vytvářen → *lefty* exprimován bilaterálně)



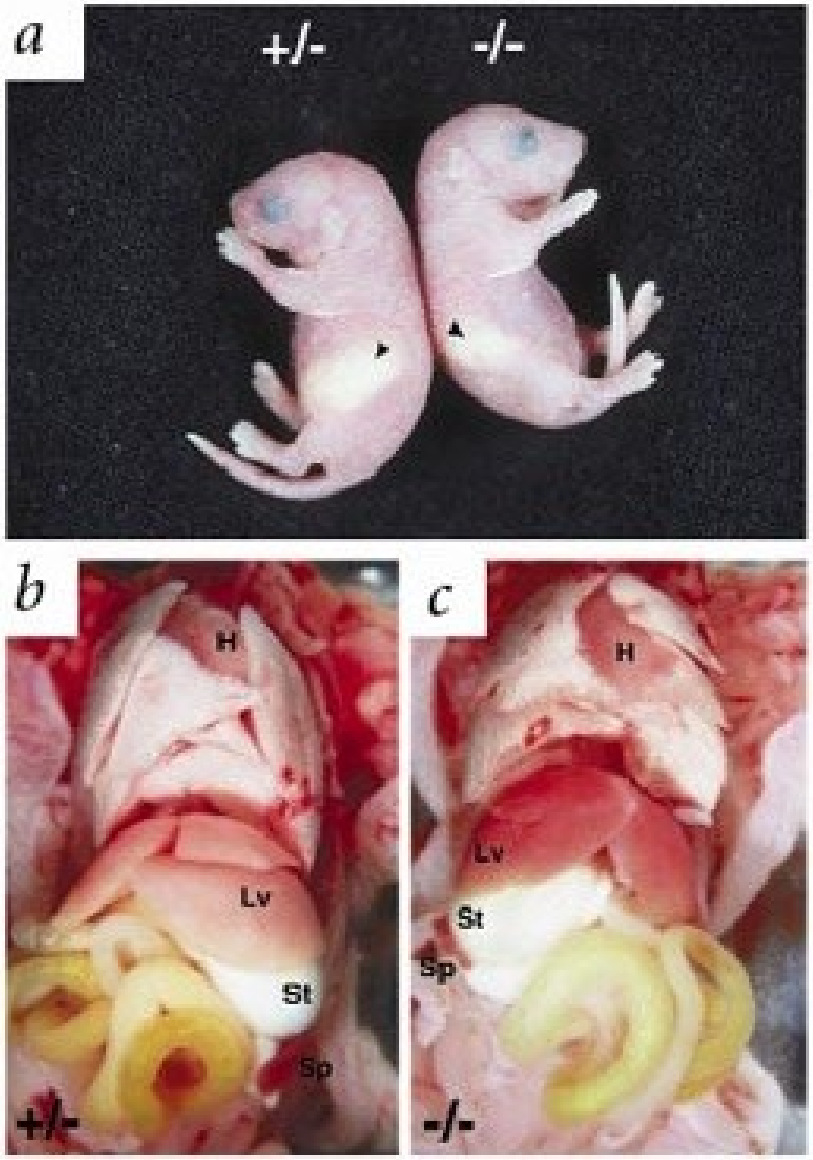
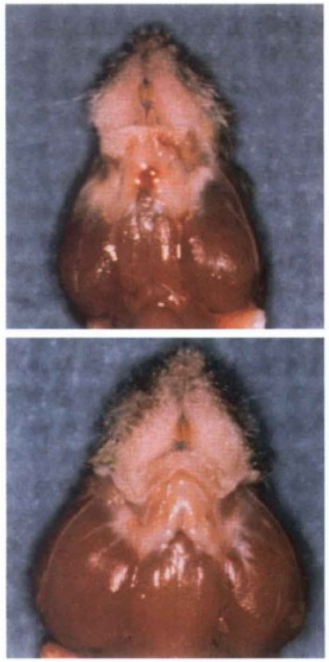
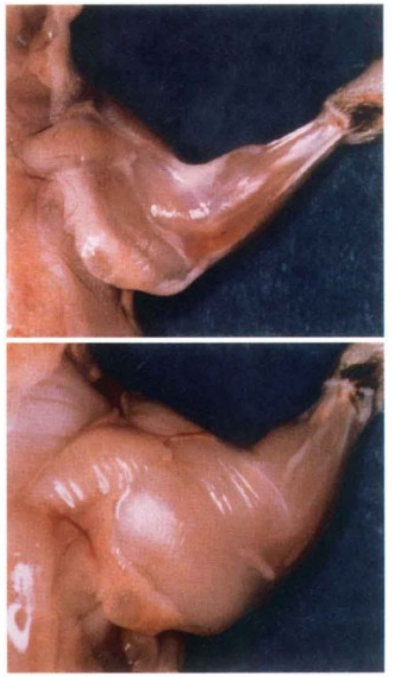


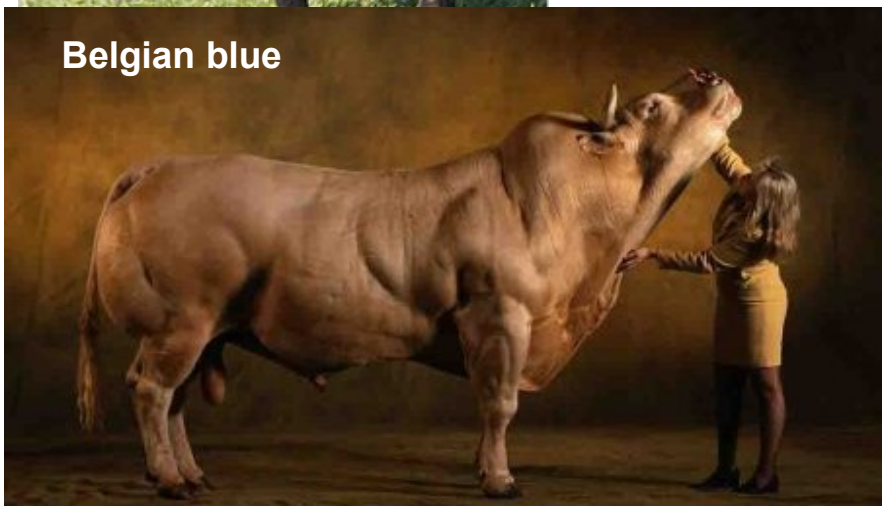
Figure 2. Analysis of *situs* defects in *Gdf1*-/- mice.
a, *Gdf1* +/- and *Gdf1* -/- newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1* +/- (*b*,*d*,*f*,*h*) and *Gdf1* -/- (*c*,*e*,*g*,*i*) mice are shown. ***b***,***c***, Reversal of the orientation of the abdominal organs in *Gdf1* -/- mice. Note also the streak-like appearance of the spleen and the abnormally shaped medial lobe of the liver.

H, heart; Lv, liver; St, stomach; Sp, spleen; AC,

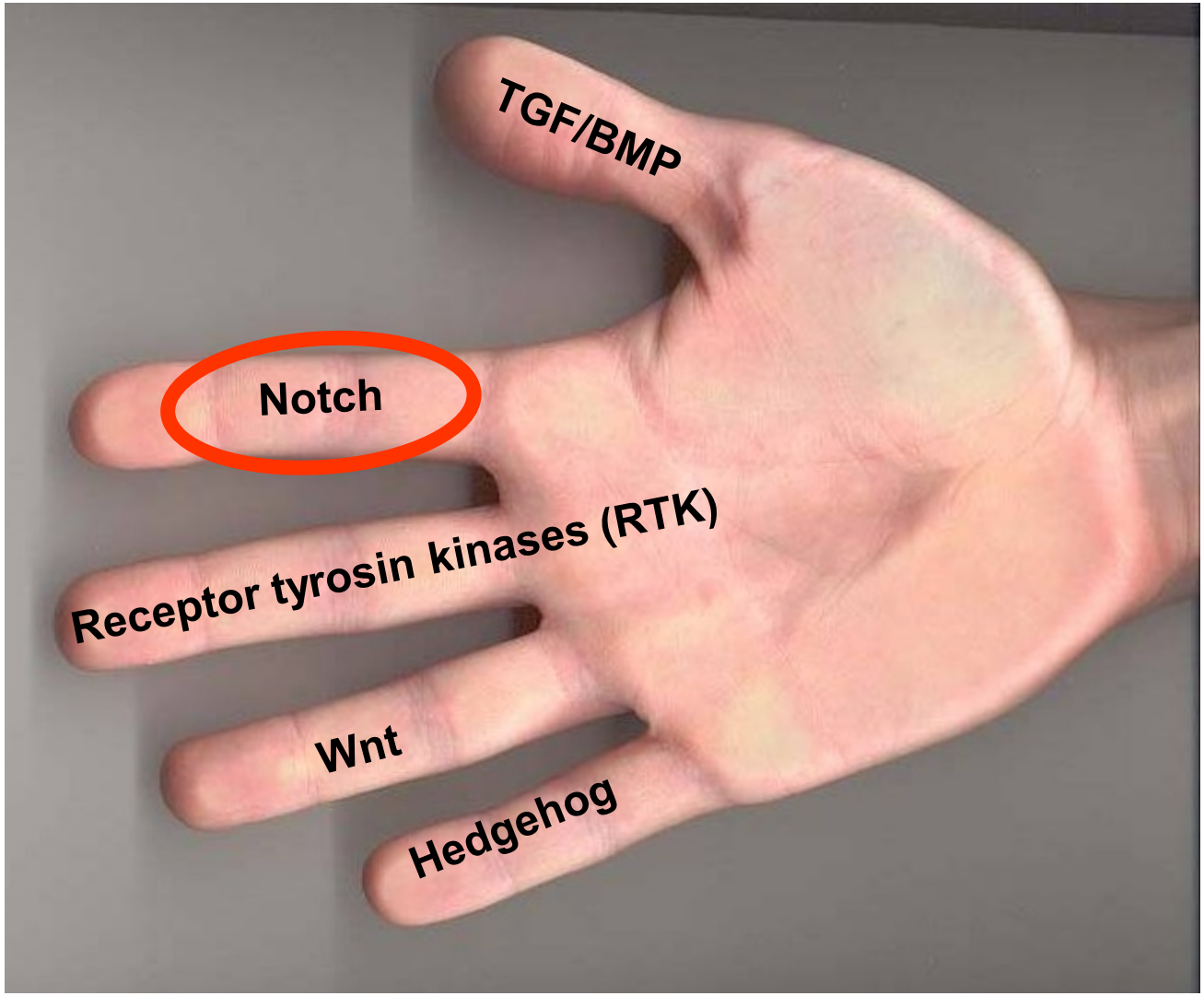
GDF8 (myostatin)





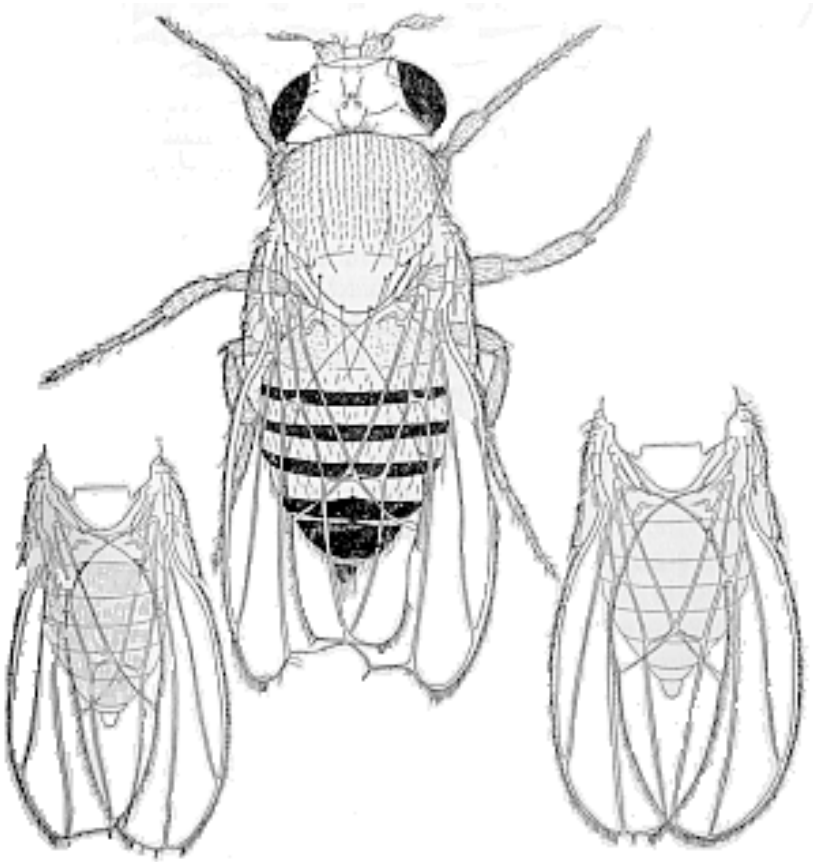


Klíčové molekulární komponenty vývoje

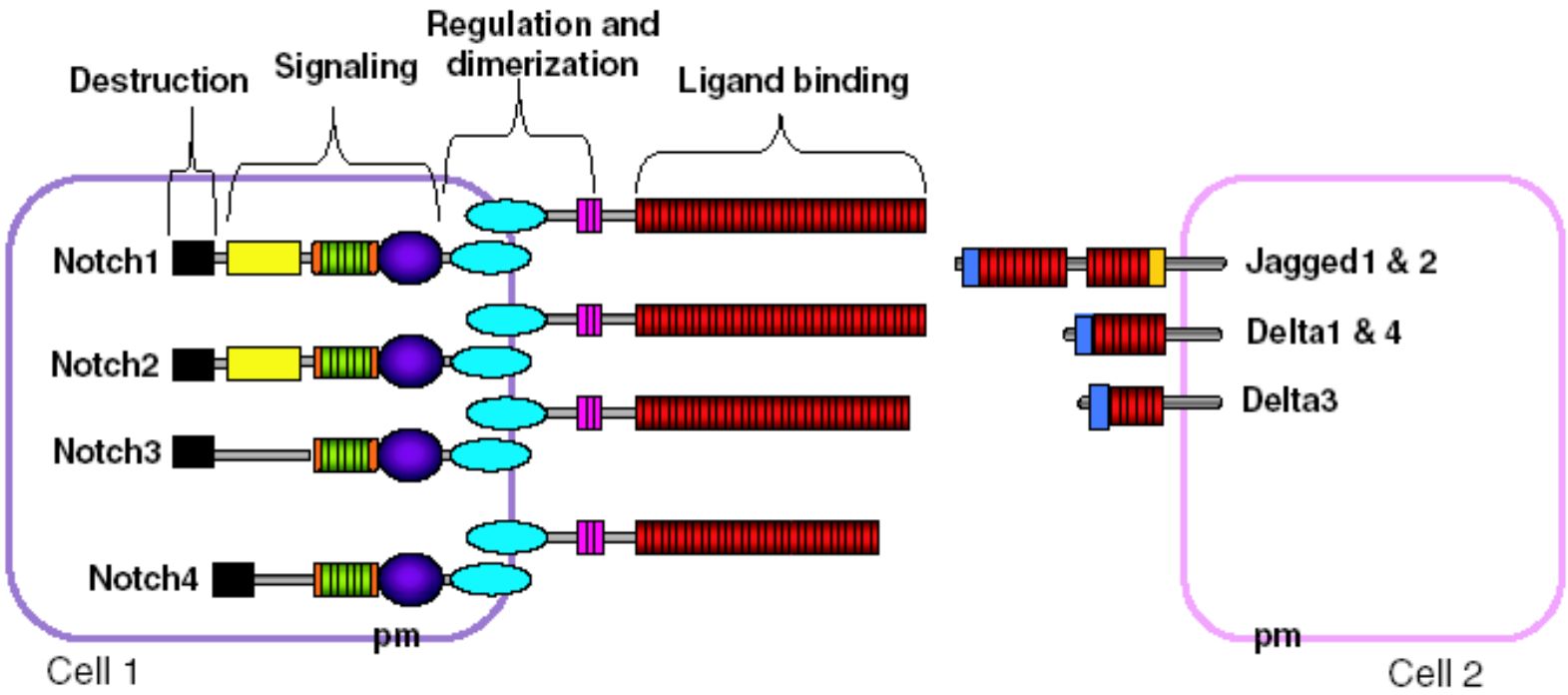


Notch

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



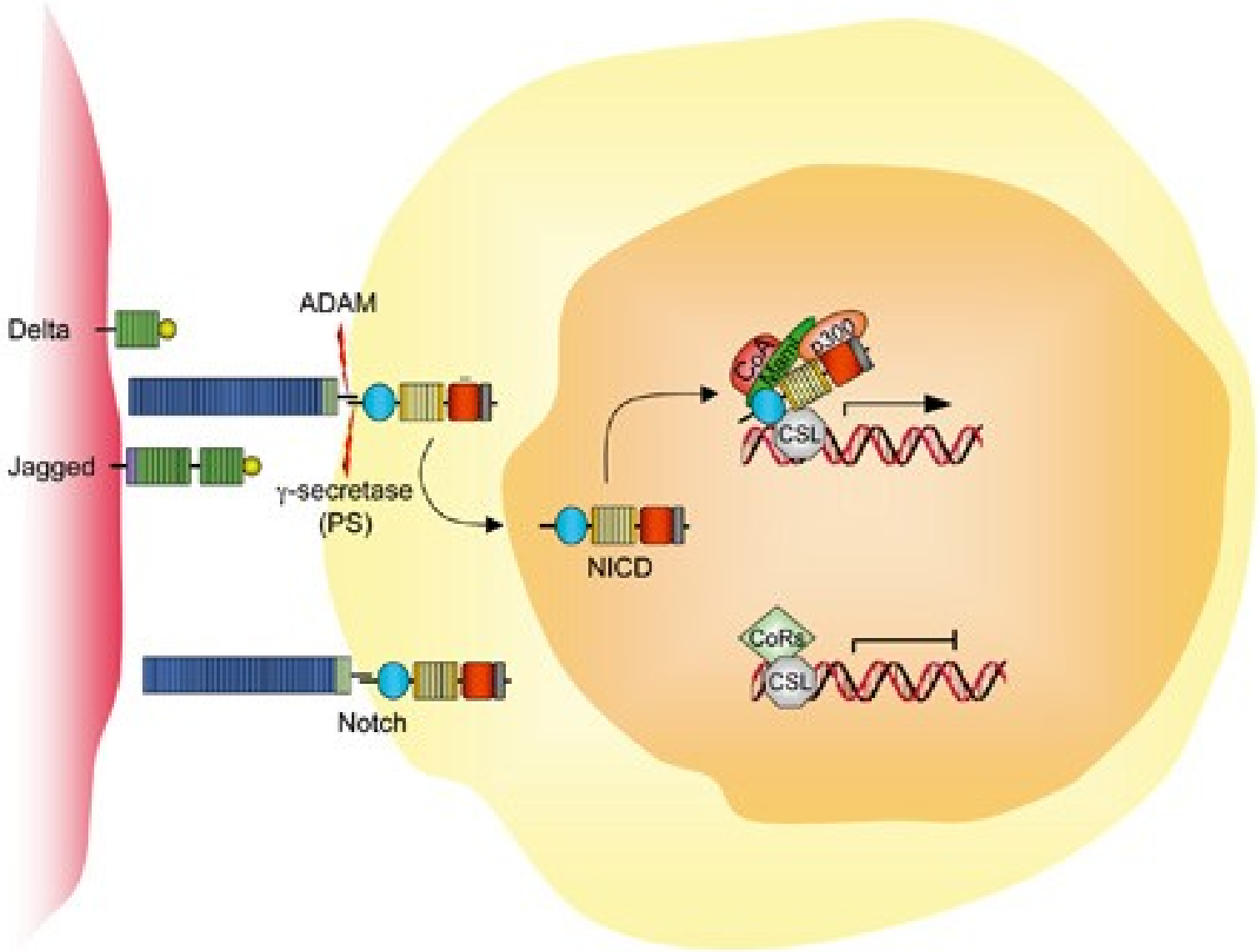
Notch



receptory Notch1-4

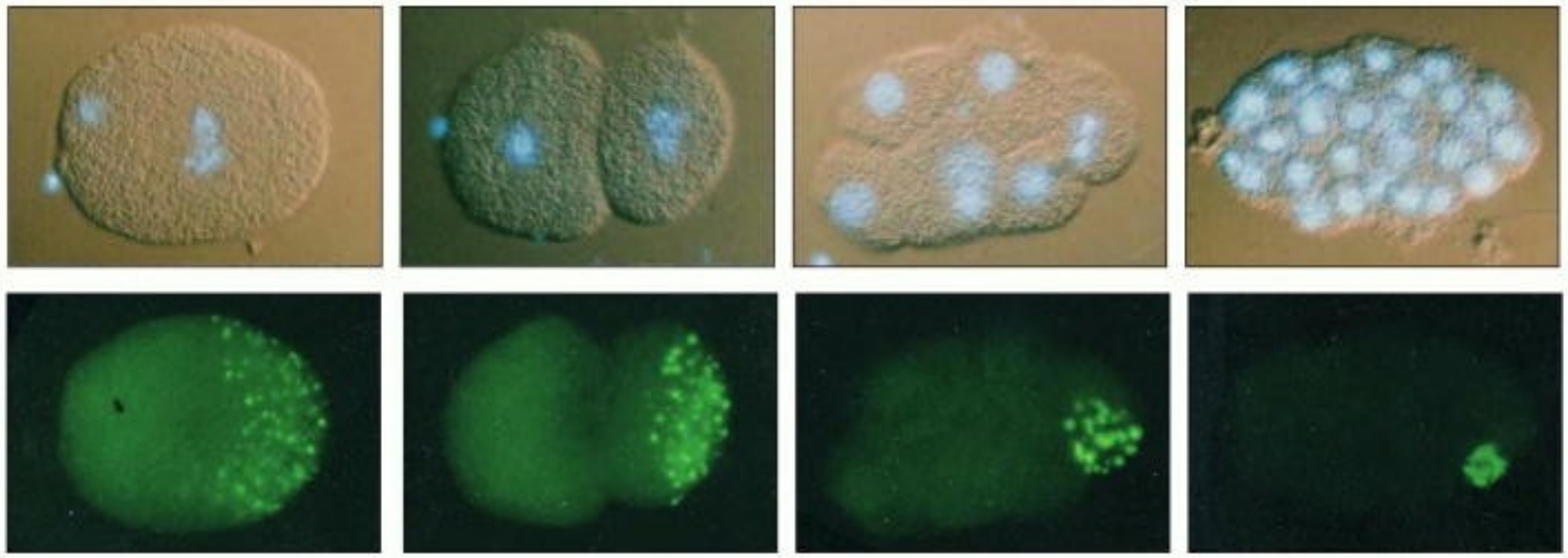
Notch ligandy – jsou vázány na buněčný povrch

Notch dráha - overview



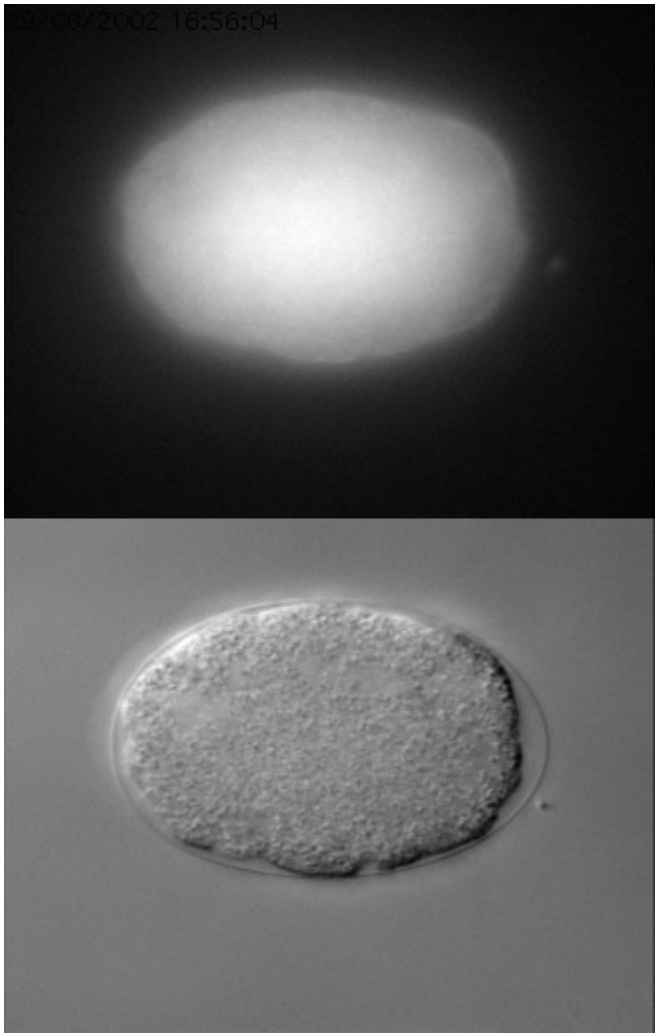
Notch a vznik asymetrie

Model: První buněčná dělení na modelu háďátka (*Caenorhabditis elegans*)



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 μm 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.)

Notch a vznik asymetrie



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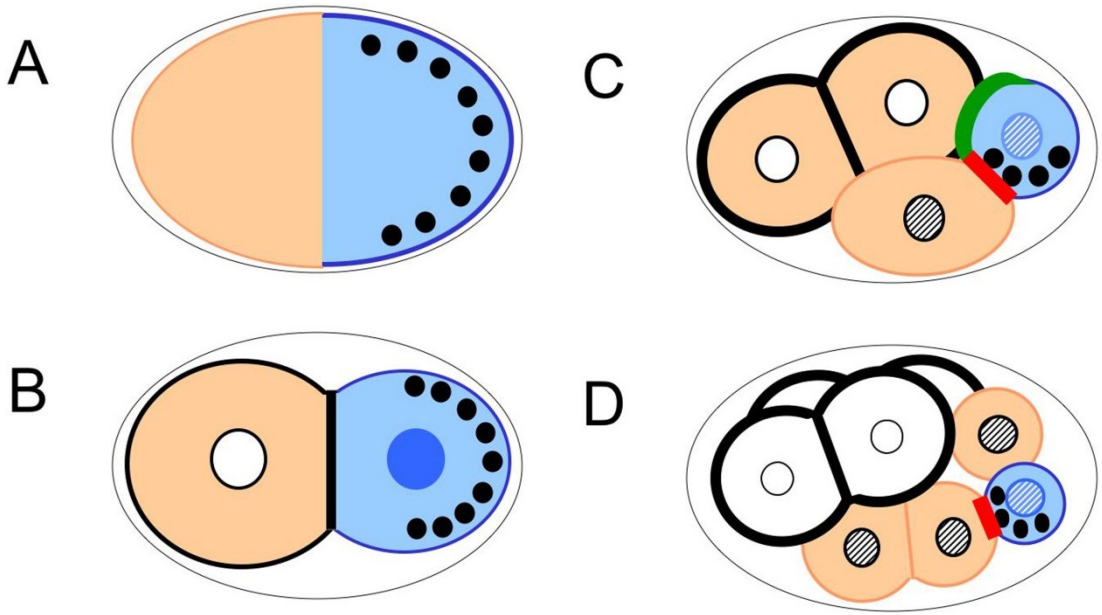
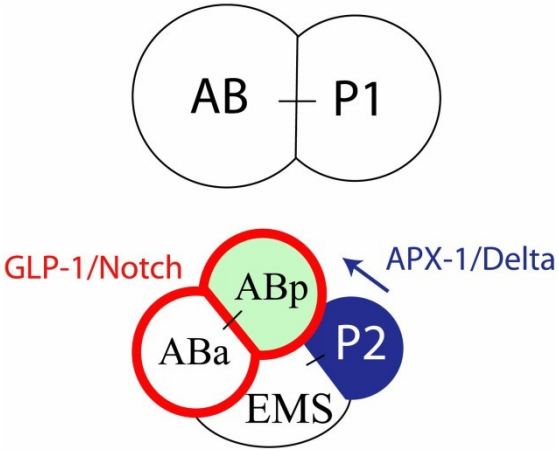




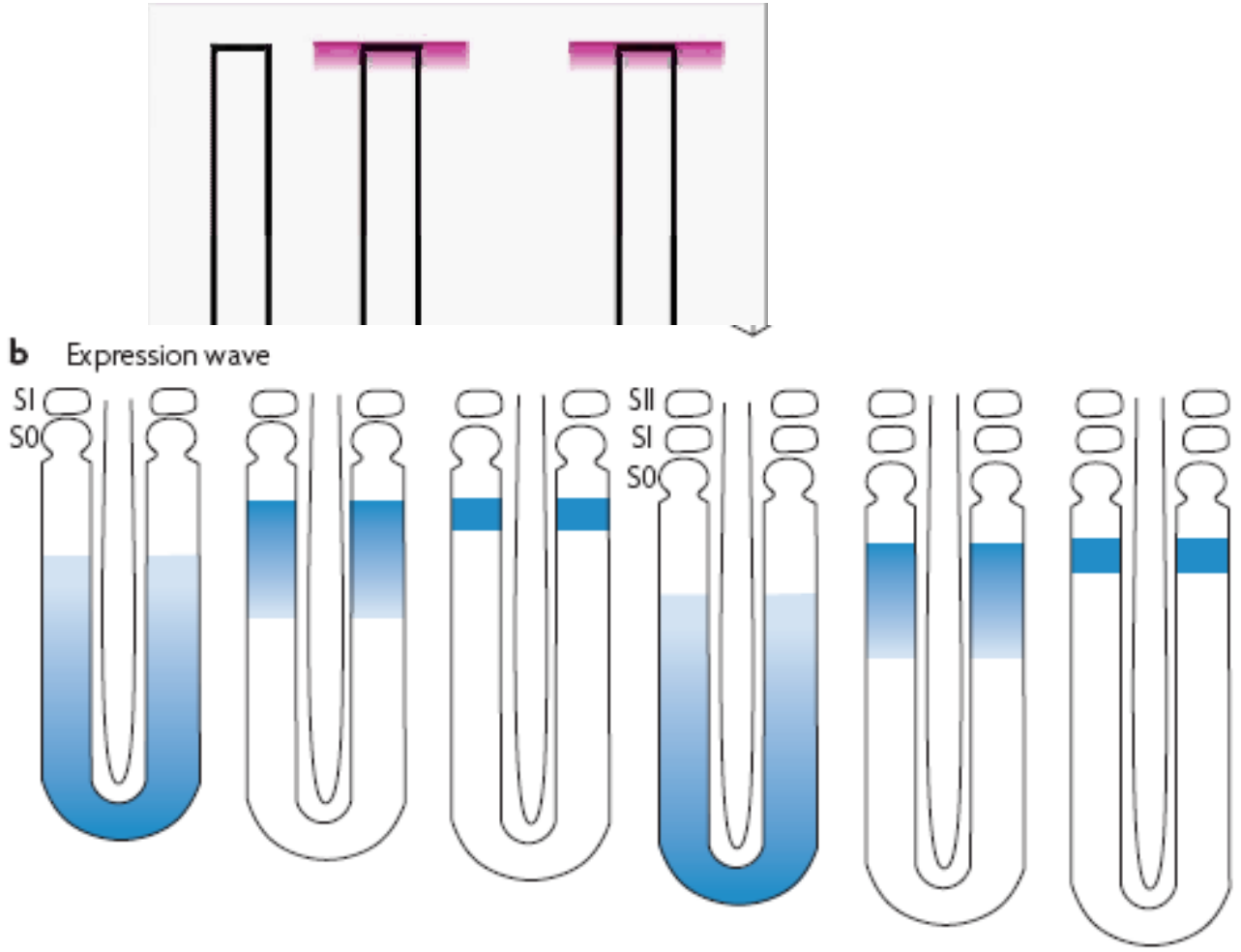
Notch a vznik asymetrie

- legenda k obrázku:

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 discreetly 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.

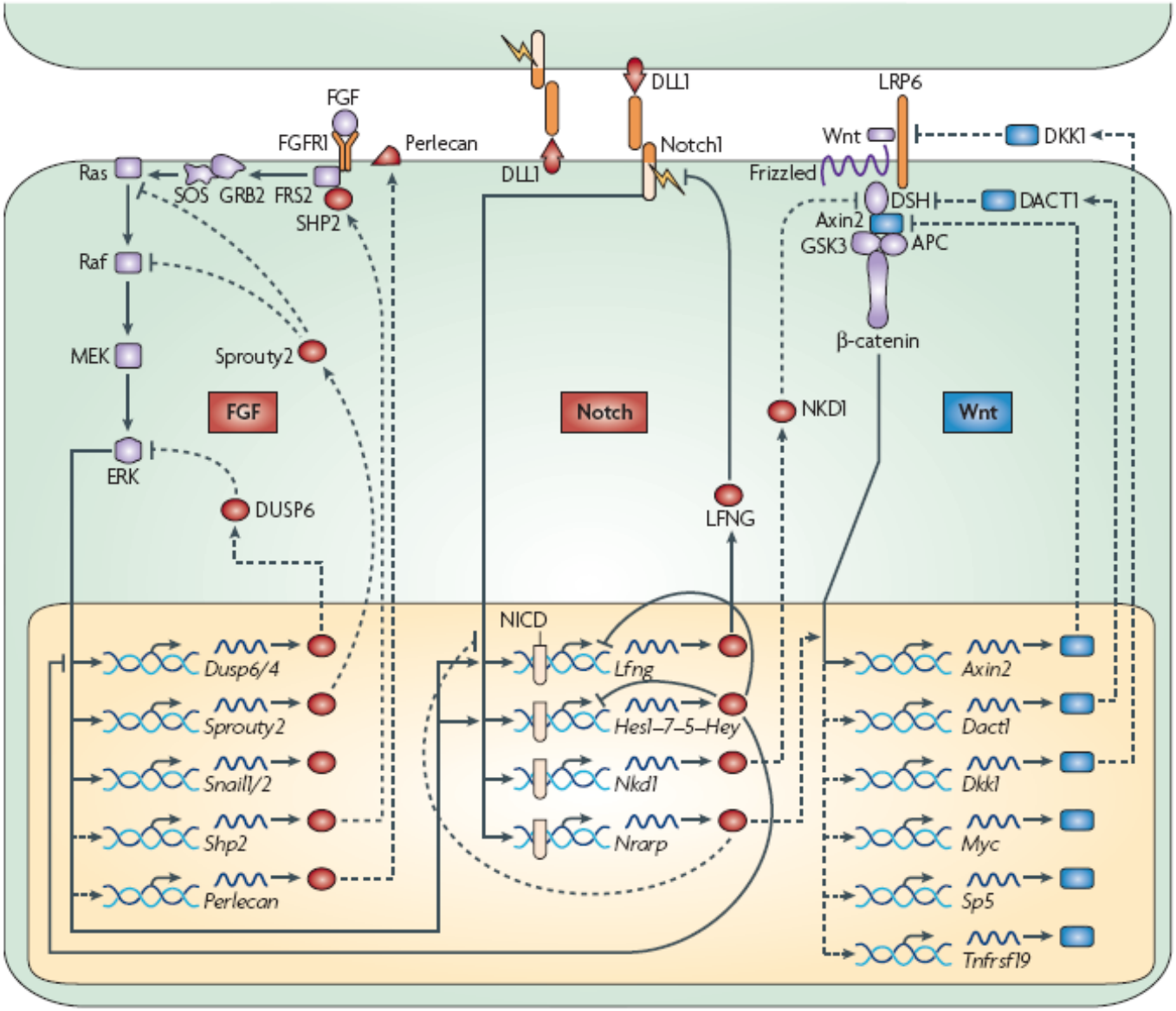
Notch a „segmentační“ hodiny

Notch a „segmentační“ hodiny



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[18]. Part **a** modified with permission from REF. 14 © (1976) Elsevier Ltd.

Notch


FGF
FGFR1
Perlecan
DLL1
DLL1
Notch1
LRP6
Wnt
DKK1
Frizzled
Ras
SOS
GRB2
FRS2
SHP2
DSH
DACT1
Axin2
GSK3
APC
Raf
β-catenin
MEK
Sprouty2
FGF
Notch
NKD1
Wnt
ERK
DUSP6
LFNG
NICD
Dusp6/4
Sprouty2
Snail1/2
Shp2
Perlecan
Lfng
Hes1–7–5–Hey
Nkd1
Nrarp
Axin2
Dact1
Dkk1
Myc
Sp5
Tnfrsf19

Notch

- legenda k obrázku:

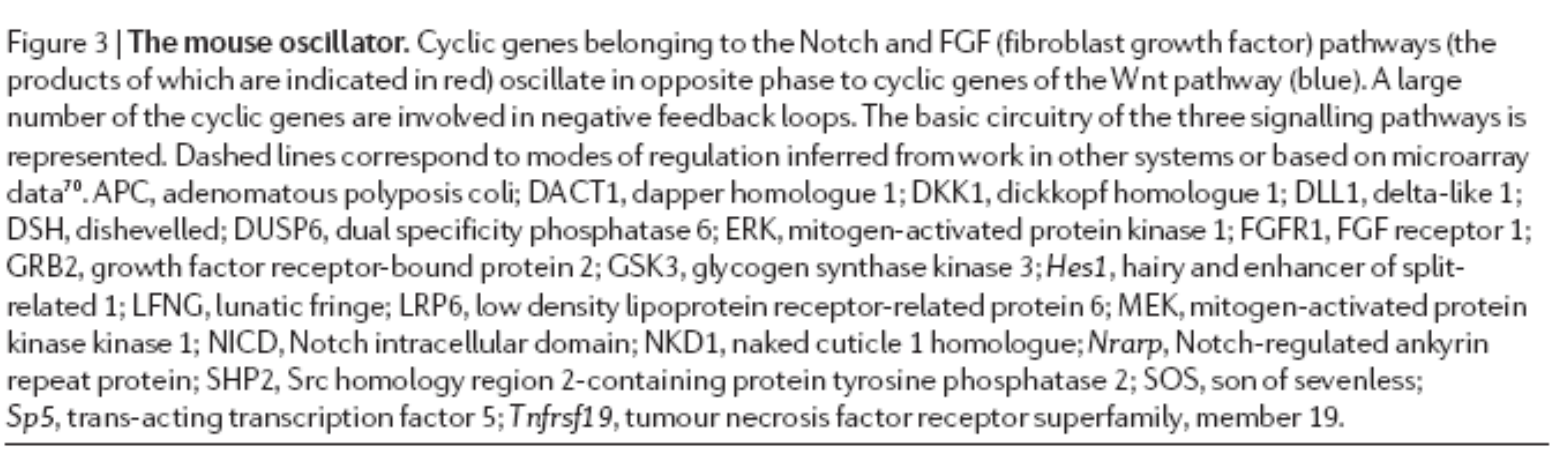
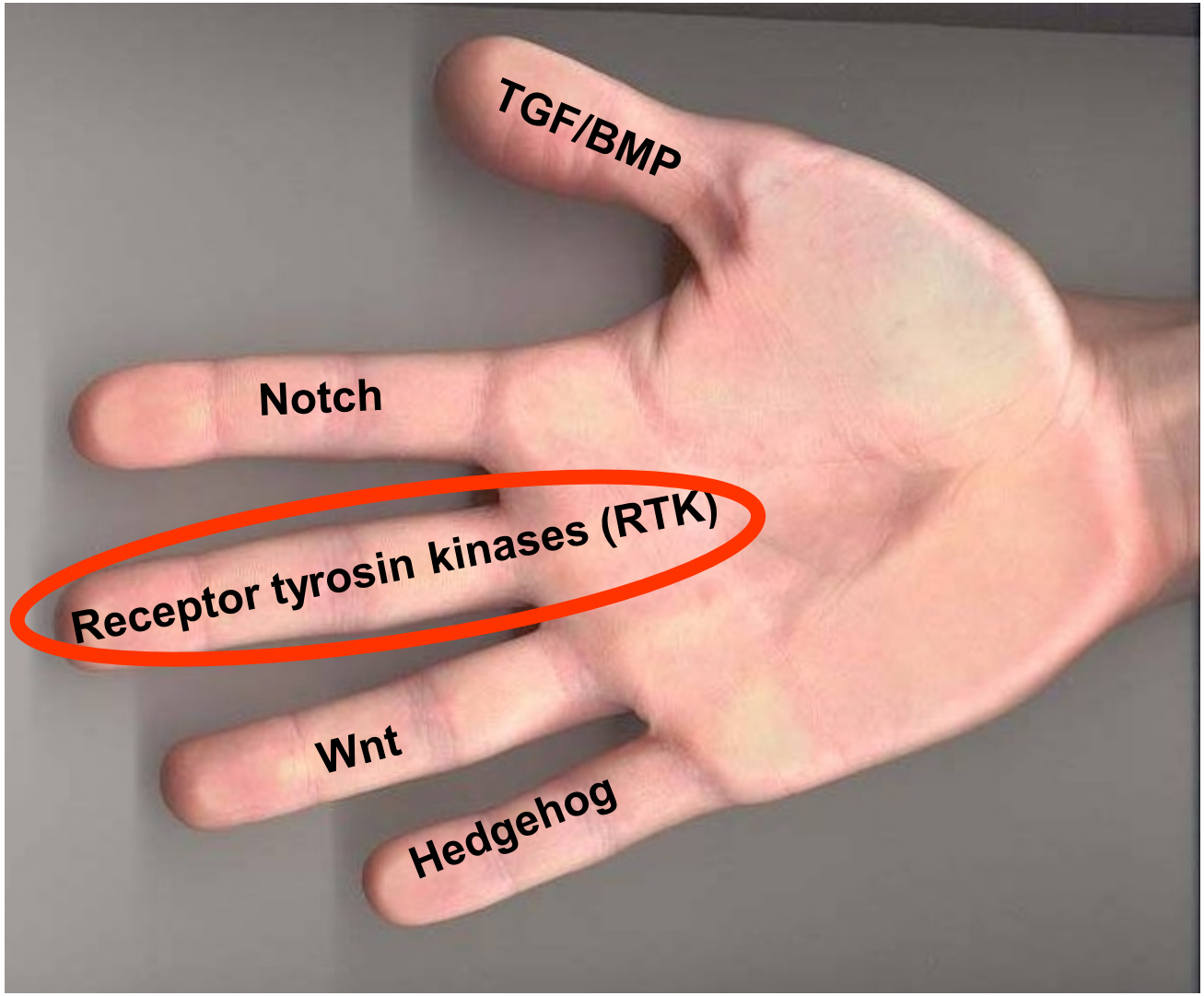
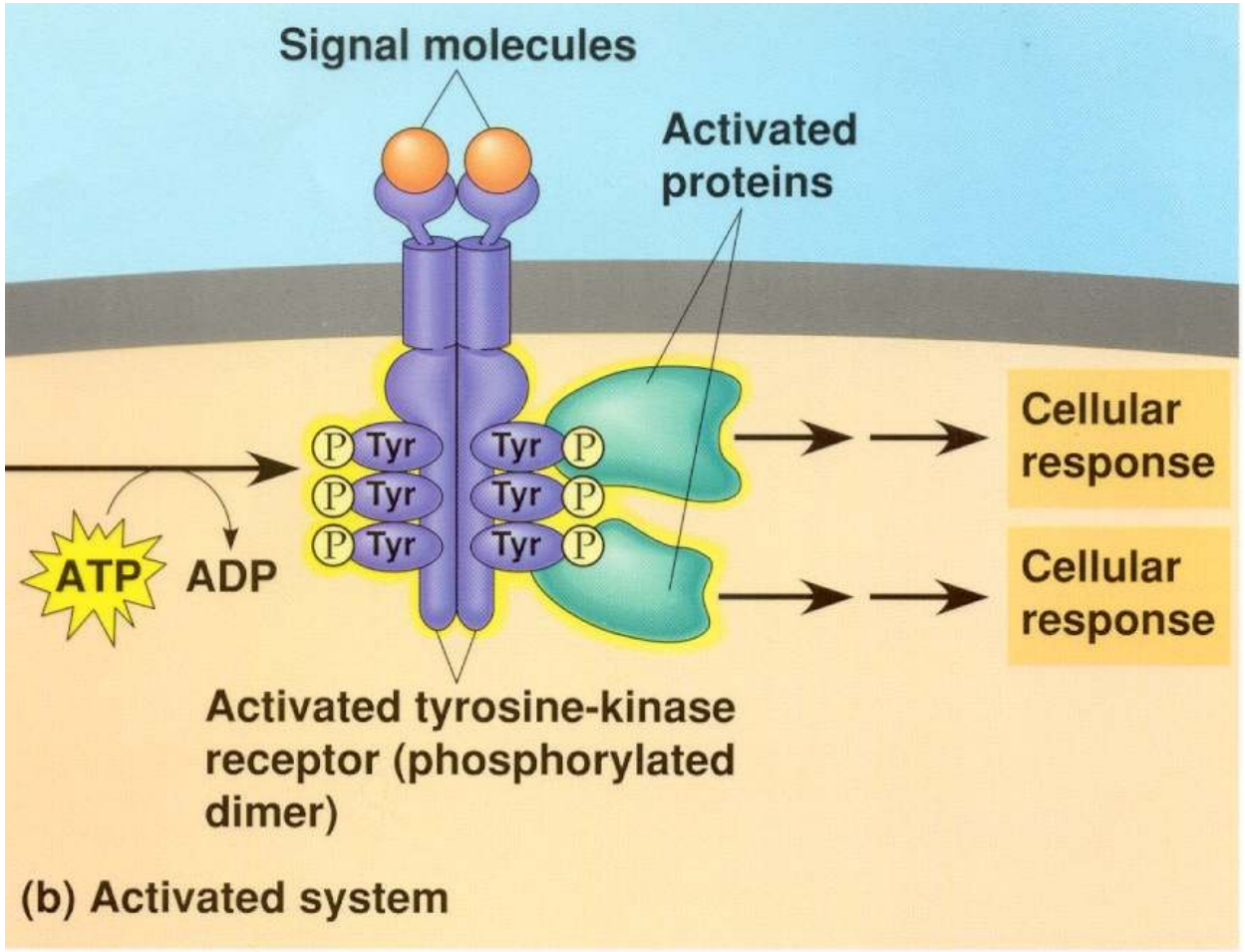


Figure 3 | **The mouse oscillator.** Cyclic genes belonging to the Notch and FGF (fibroblast growth factor) pathways (the products of which are indicated in red) oscillate in opposite phase to cyclic genes of the Wnt pathway (blue). A large number of the cyclic genes are involved in negative feedback 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 data[70]. 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.

Klíčové molekulární komponenty vývoje



Receptorové tyrosin kinázy (RTK)



Hlavní skupiny RTKs:

EGF (epidermal growth factor) receptor family

Insulin receptor family

PDGF (platelet-derived GF) receptor family

FGF (fibroblast GF) receptor family – přednáška č. 3

VEGF (vascular endothelial GF) receptor family

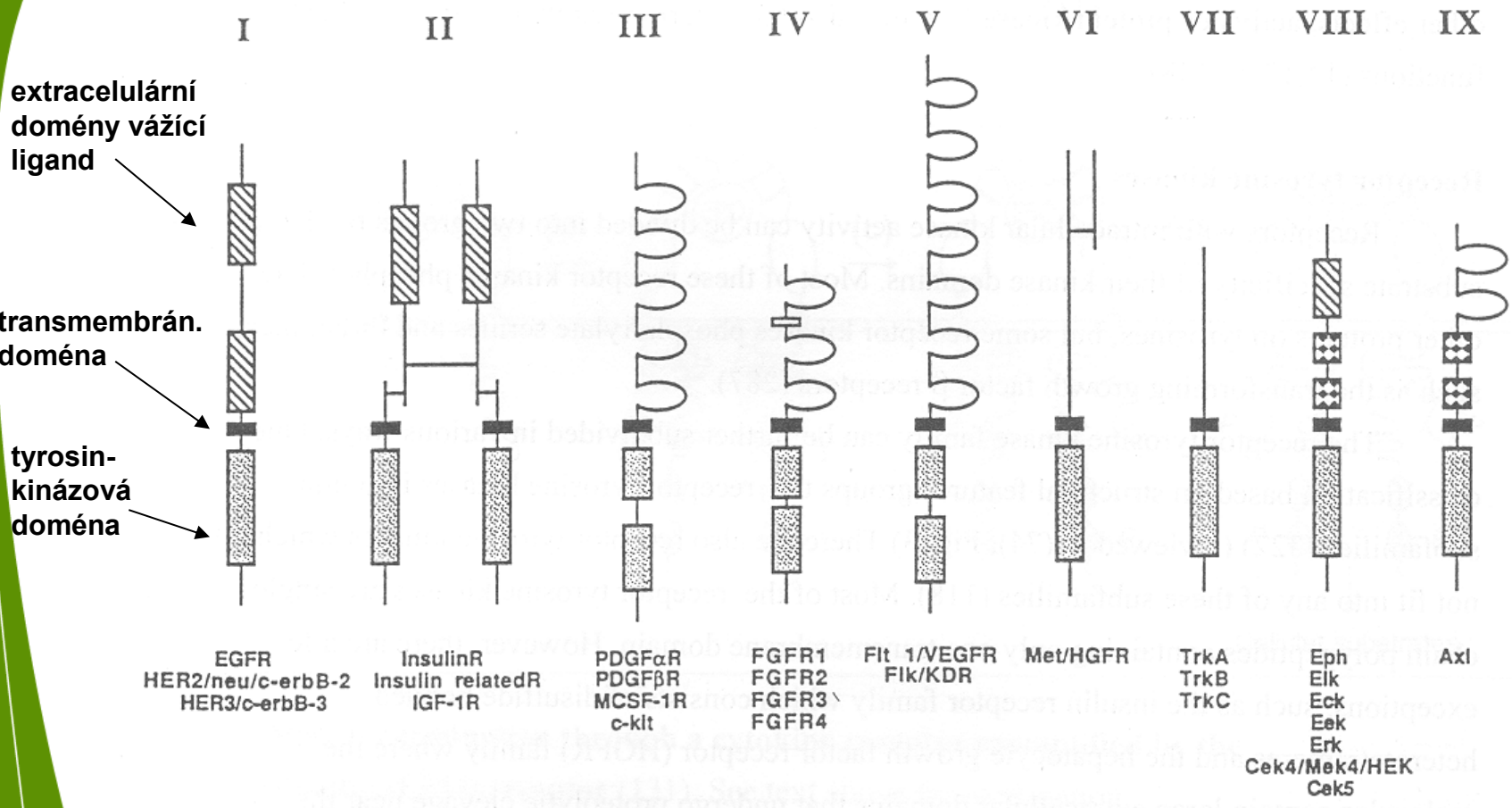
HGF (hepatocyte GF) receptor family

Trk receptor family

Eph receptor family

RET receptor family

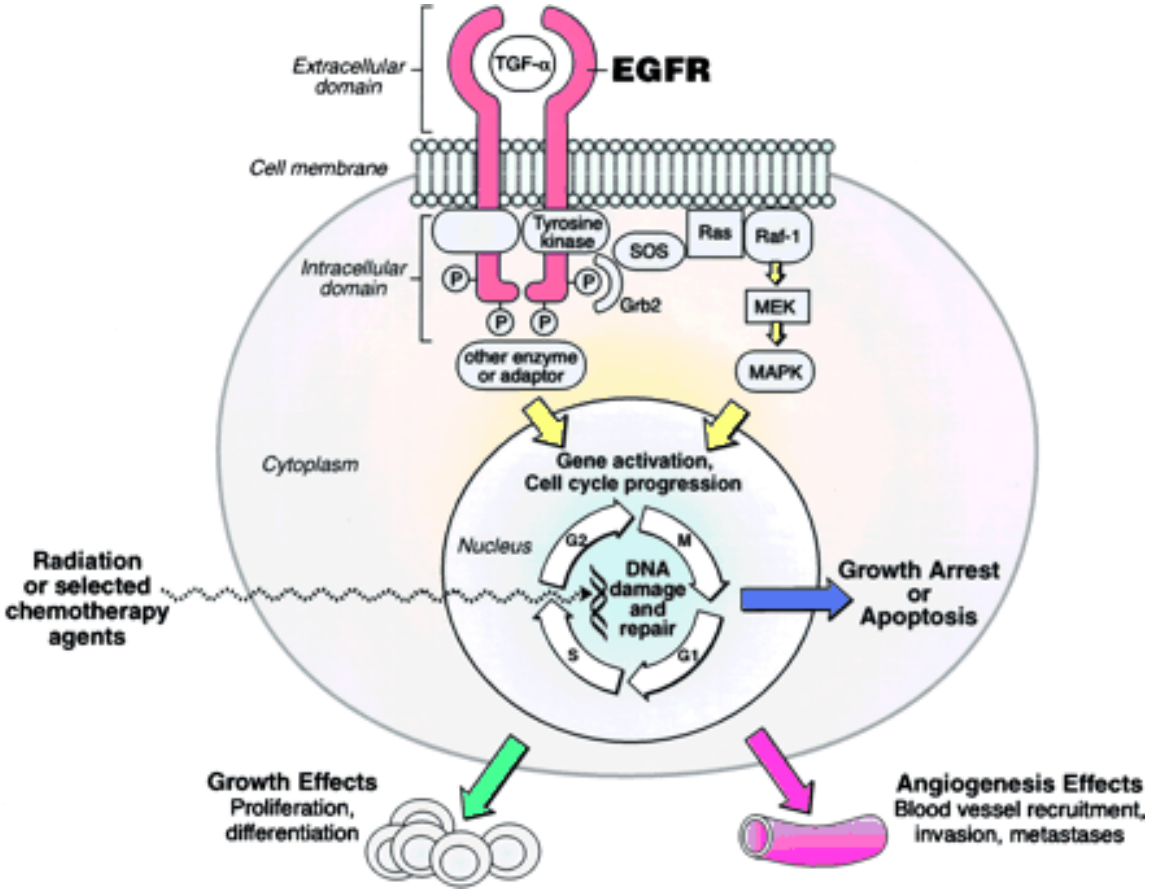
Schematická struktura jednotlivých receptorů



Obecné schéma aktivace RTKs

(zde na příkladu EGFR)

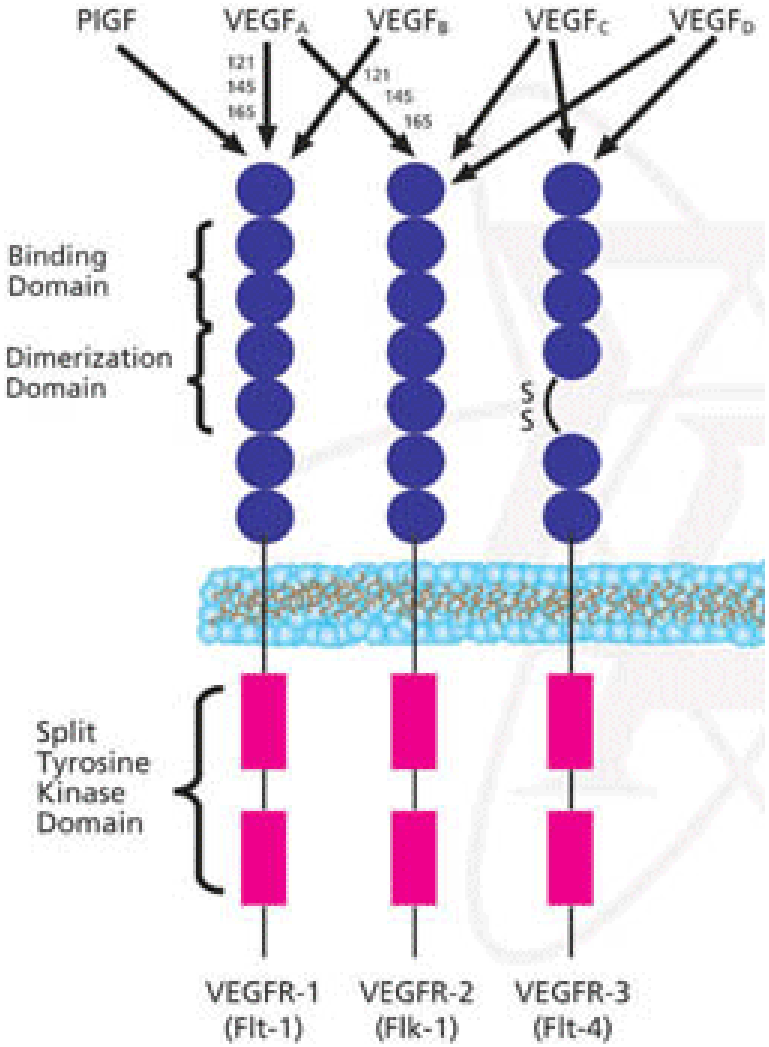
1. ligand se specificky váže na receptor
2. receptor dimerizuje
3. tyrosin-kinázové domény se navzájem fosforylují
4. autofosforylace vede k navázání (recruitment) adaptérových proteinů (zde Grb2)
5. v závislosti na receptoru se aktivují „downstream“ signální dráhy – zde např. Ras/Raf1/MEK/MAPK kinázová dráha,
6. která vede k regulaci transkripce



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

- VEGF/VEGFR
- ephrin/Eph
- FGF/FGFR – viz přednáška č. 3

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



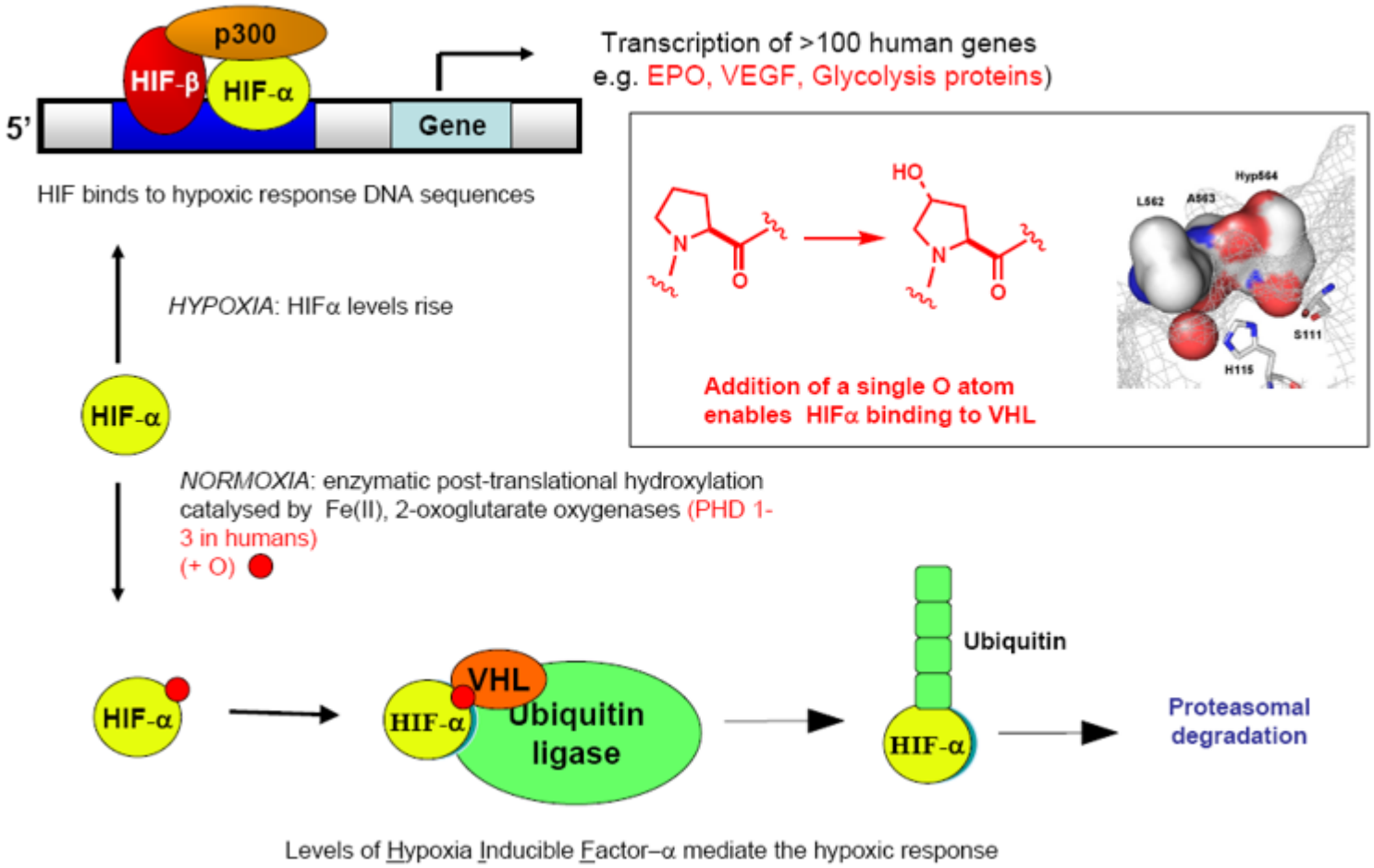
VEGF/VEGFR ve vývoji

- reguluje vznik a vývoj cévní soustavy
- 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 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, erytropoeza, metabolismus Fe)
  - Heterodimer sestává ze tří **α** podjednotek (HIF1α, 2α, 3α) a jedné podjednotky **β** (HIFβ=ARNT)
  - **α podjednotky jsou při normoxii silně labilní**, podjednoteka β je na koncentraci O_2 nazávislá

HIF při normoxii a hypoxii

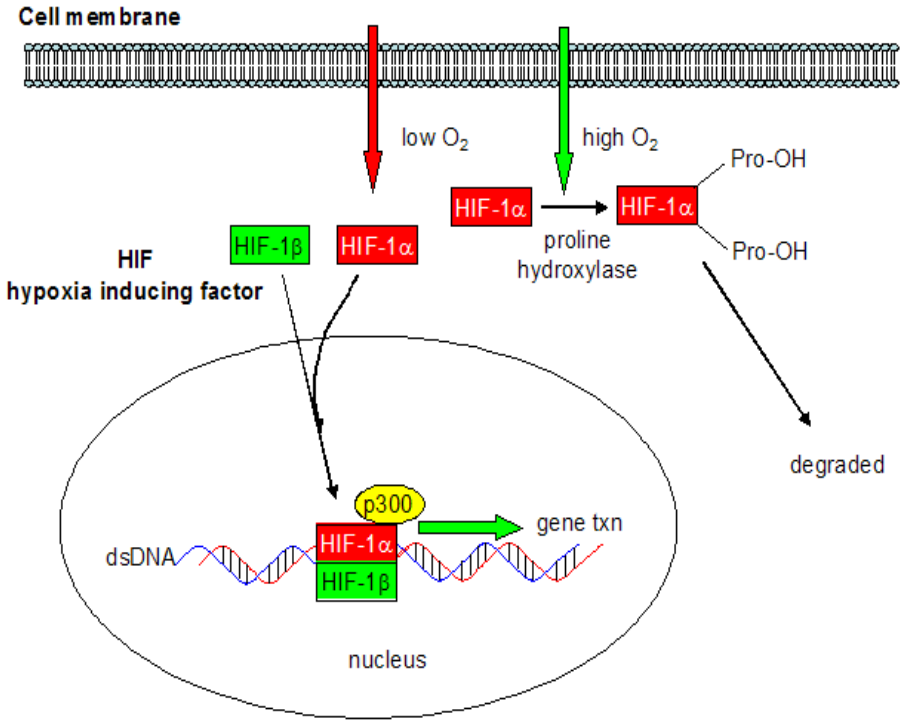


VHL (von Hippel-Lindau) - tumor supresorový gen

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

Modelové vývojové změny spojené s hypoxií/HIF systémem

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



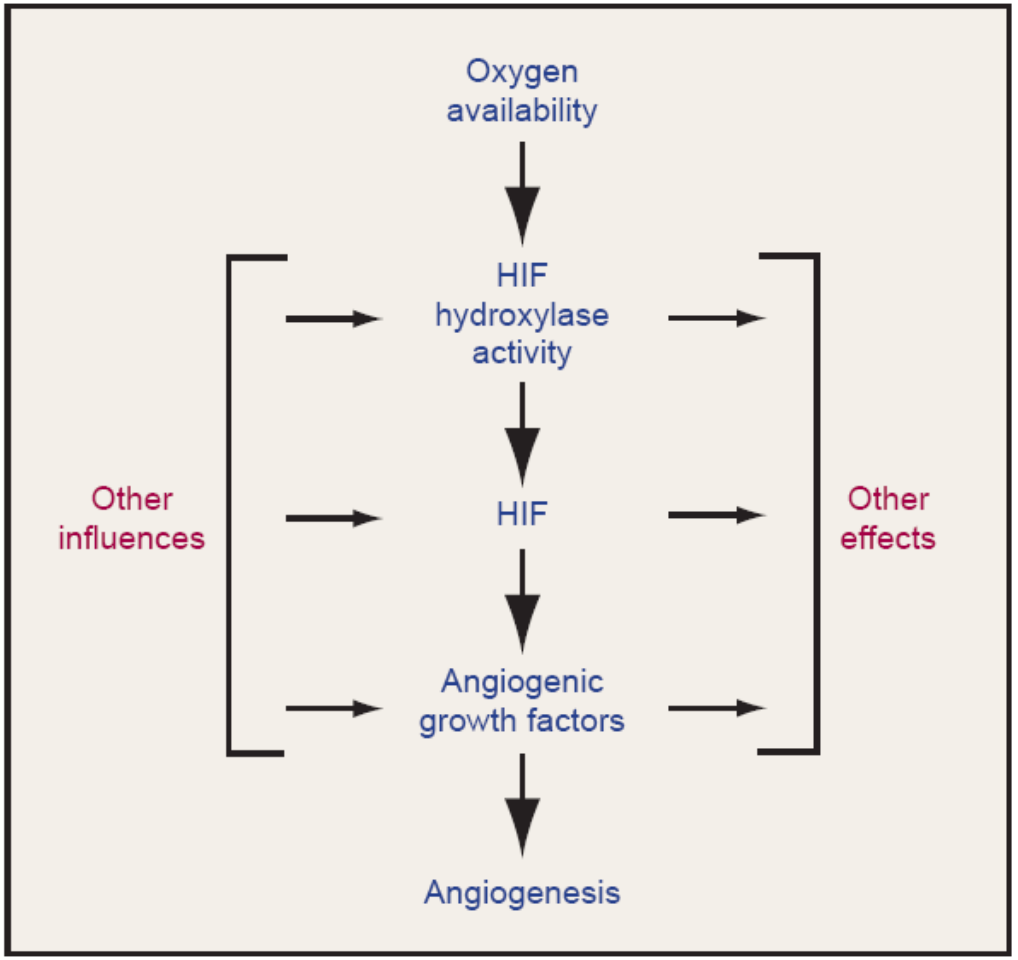
Genes upregulated:

- erythrpoietin (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

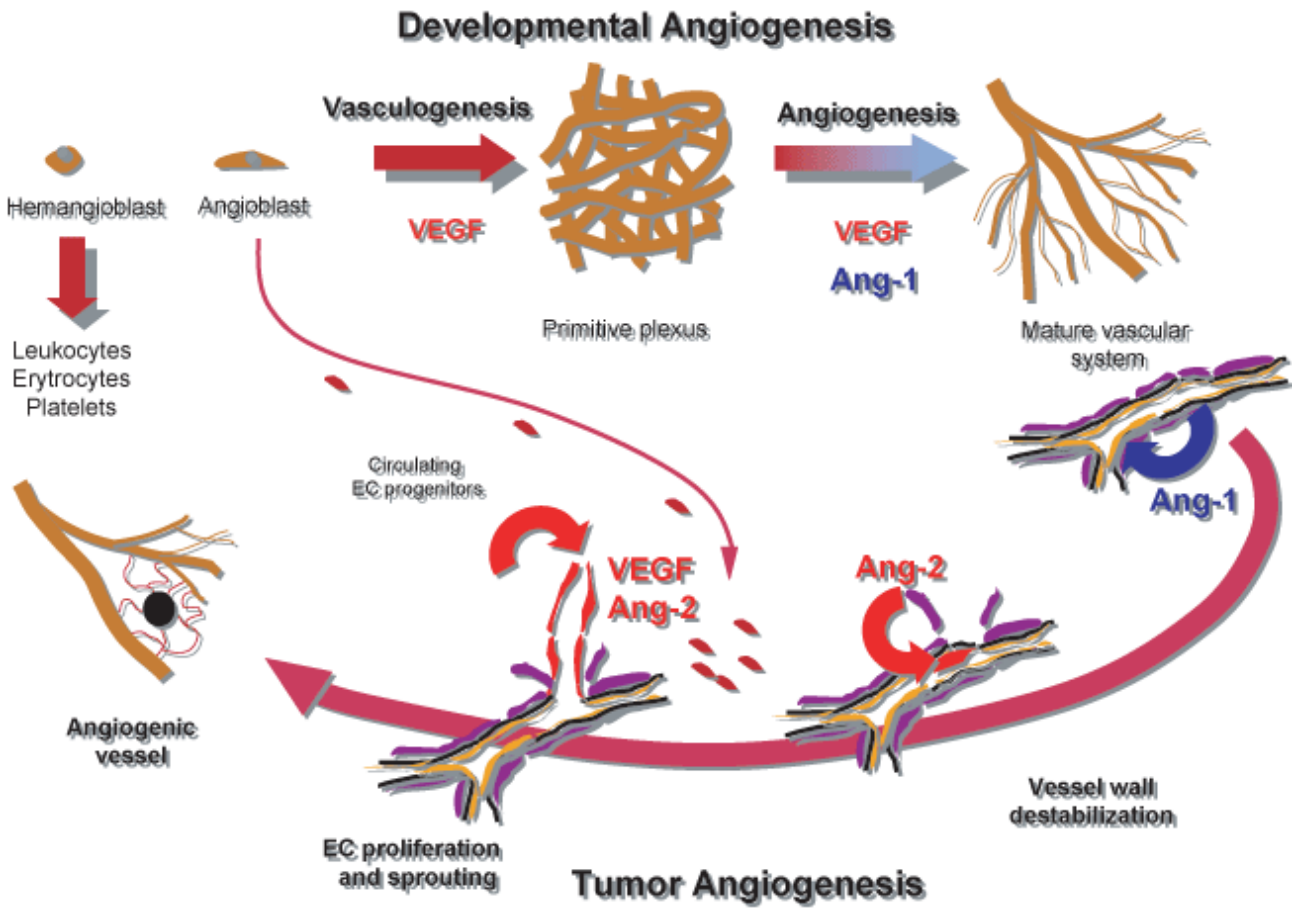
After: Science, 303, pg 1454 (2004)

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



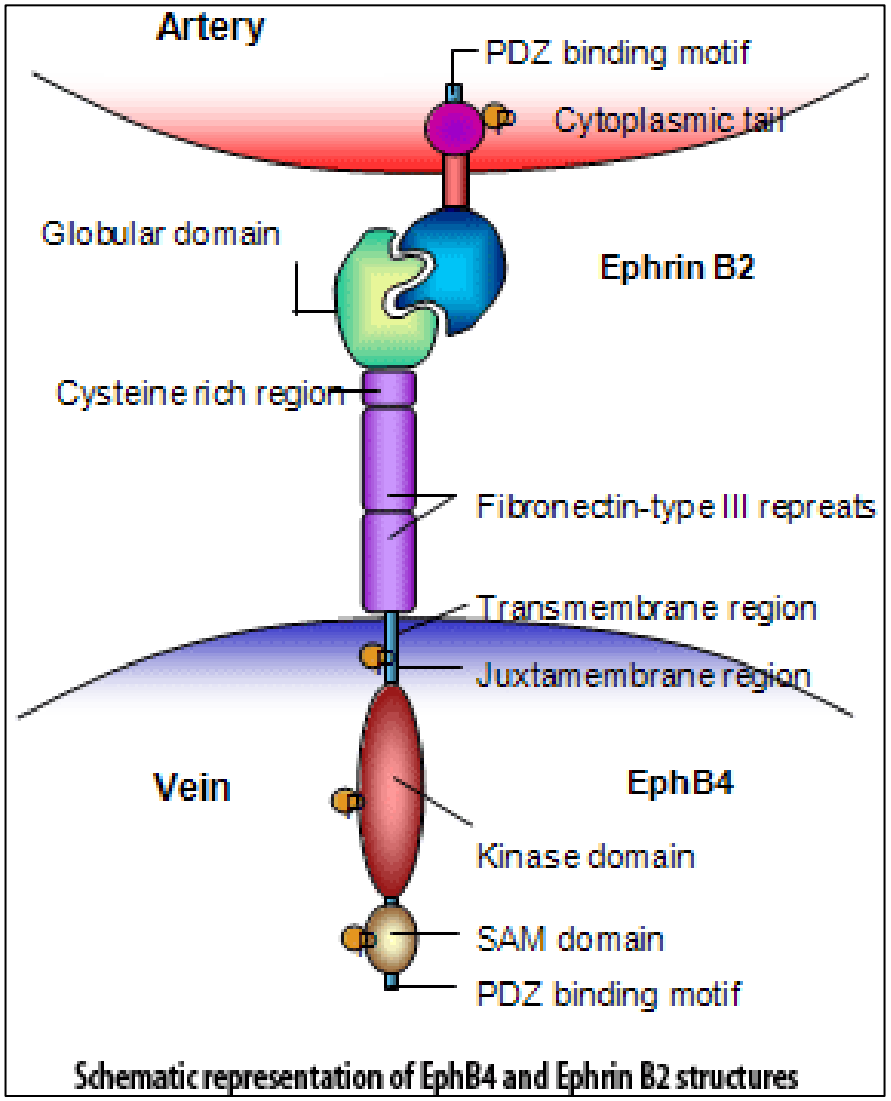
VEGF/VEGFR ve vývoji



Blood vessel formation and tumor angiogenesis. During development, VEGF induces differentiation and proliferation of endothelial cells from its progenitors (the hemangioblast and angioblast) to form a poorly differentiated primitive vascular plexus (vasculogenesis). Angiopoietin-1 (Ang-1) and other morphogens (e.g. Ephrins-Eph) induce remodeling of the vascular plexus into a hierarchically structured mature vascular system through endothelial cell sprouting, trimming differentiation and pericytes recruitment (angiogenesis). During tumor angiogenesis, angiopoietin-2 (Ang-2) destabilizes the vessel wall of mature vessels. Quiescent endothelial cells become sensitive to VEGF (or other angiogenic factors), proliferate and migrate to form new vessels. Bone marrow-derived endothelial cell progenitors are found in the peripheral blood and can recruit at sites of angiogenesis.

Eph/ephrin komplex

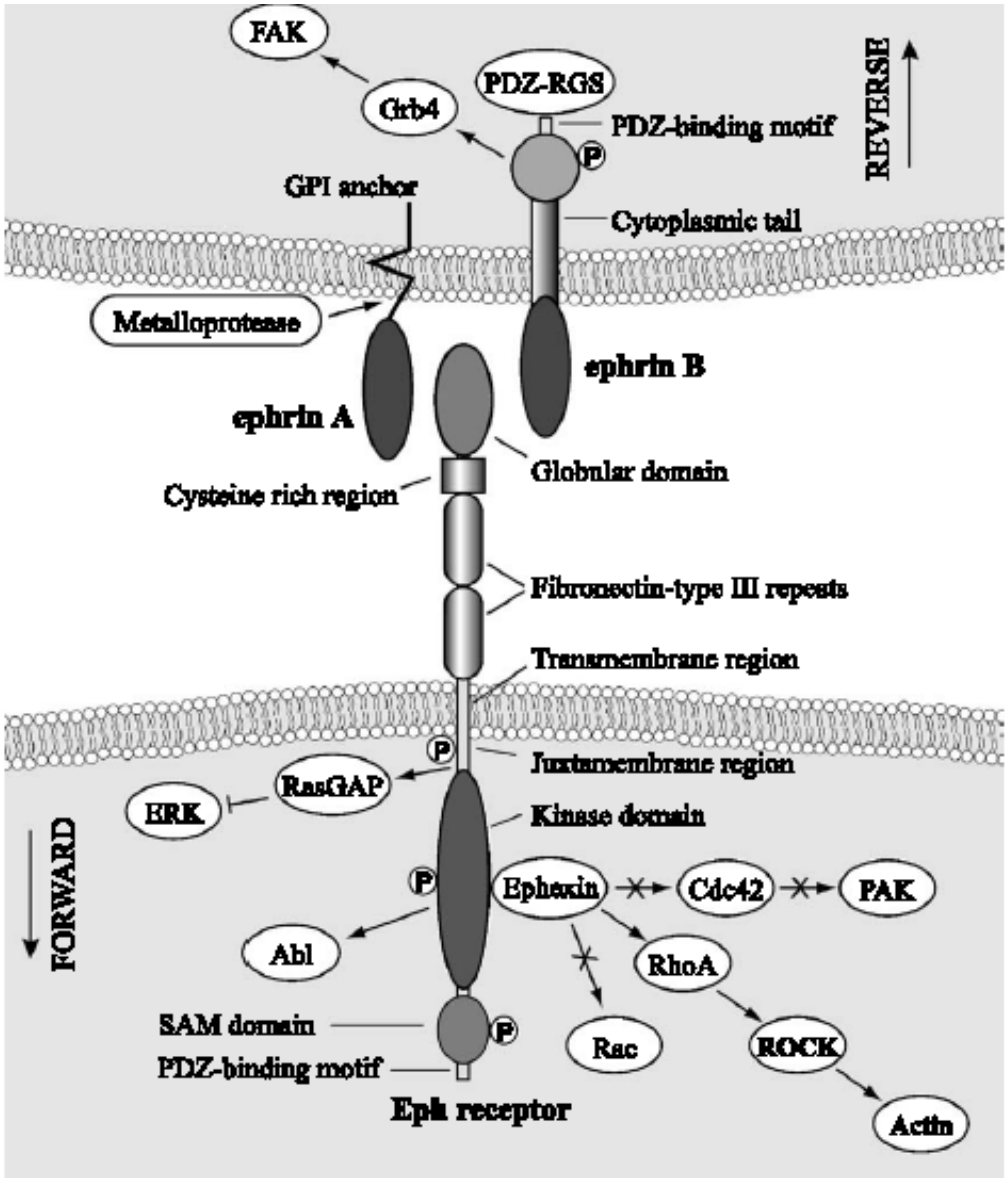
- ephrin – jsou **membránově vázané ligandy** (podobně jako ligandy Notch dráhy)
- ephriny A – na membráně upevněny pomocí tzv. **GPI kotvy**
- ephriny B – transmembránové ligandy, které samy jsou schopny signálovat do buňky
- Eph/ephrin systém je zapojen zejména do „navigace“ buněk (např. buněk cév) 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.



Schematic representation of EphB4 and Ephrin B2 structures

Eph/ephrin komplex

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

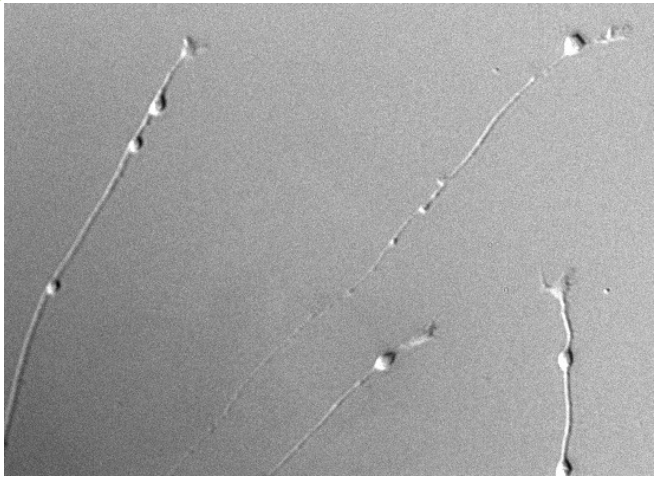


Eph/ephrin komplex

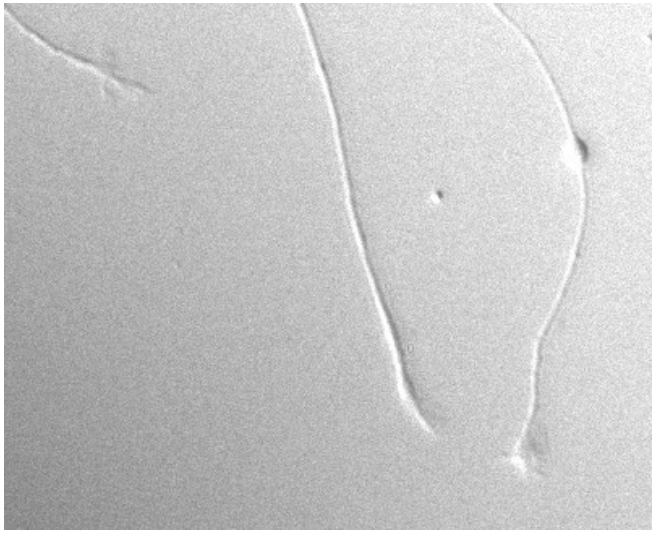
- legenda k obrázku:

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



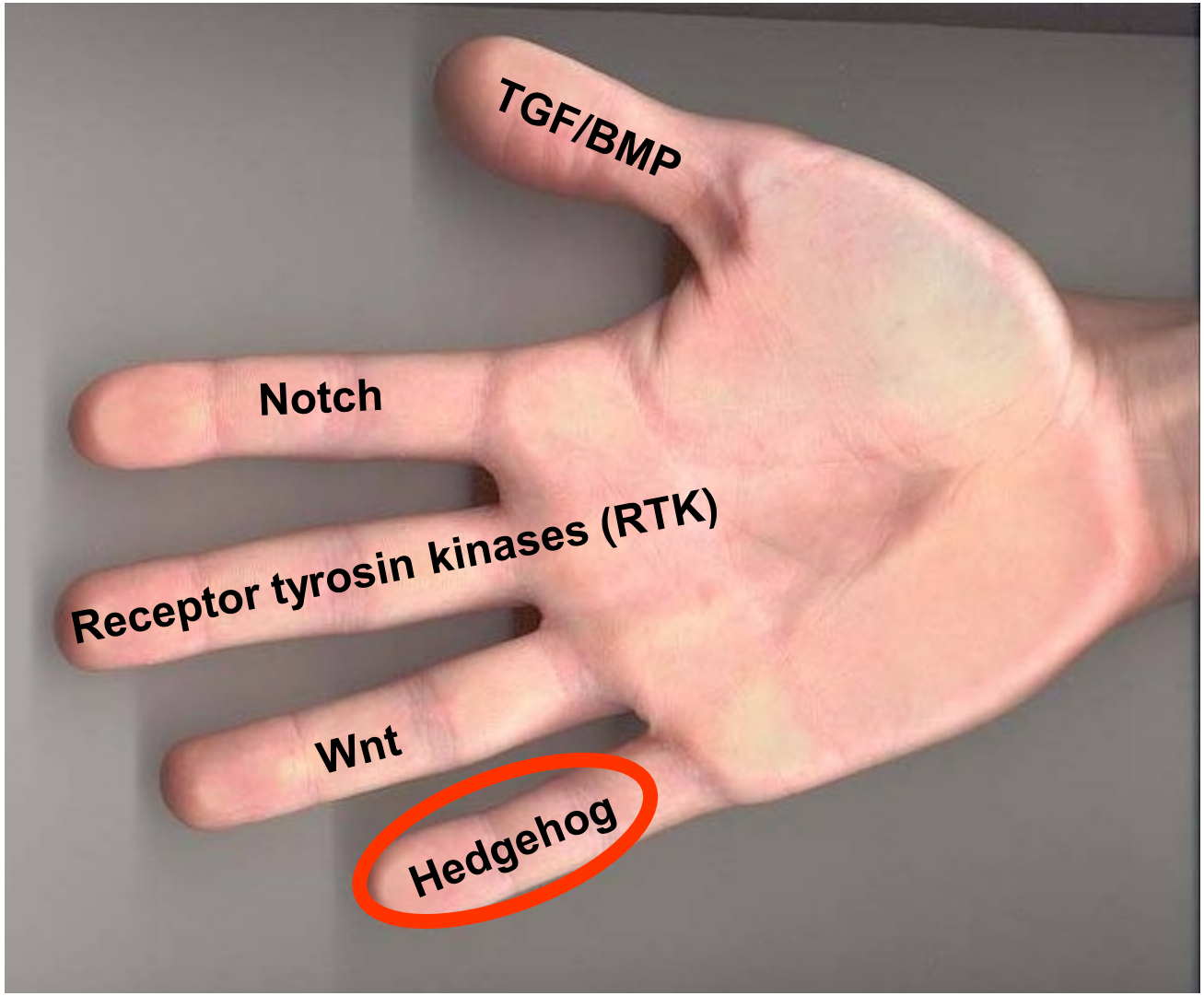
Supporting Information Movie 2. Ephrin-B2 induces extremely rapid growth cone collapse and axon retraction in VT RGCs. Movie depicts VT growth cones treated with ephrin-B2. Frames were captured at 30-second intervals for 45 minutes, replayed at 15 frames per second. 0.5 µg/ml pre-clustered ephrin-B2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

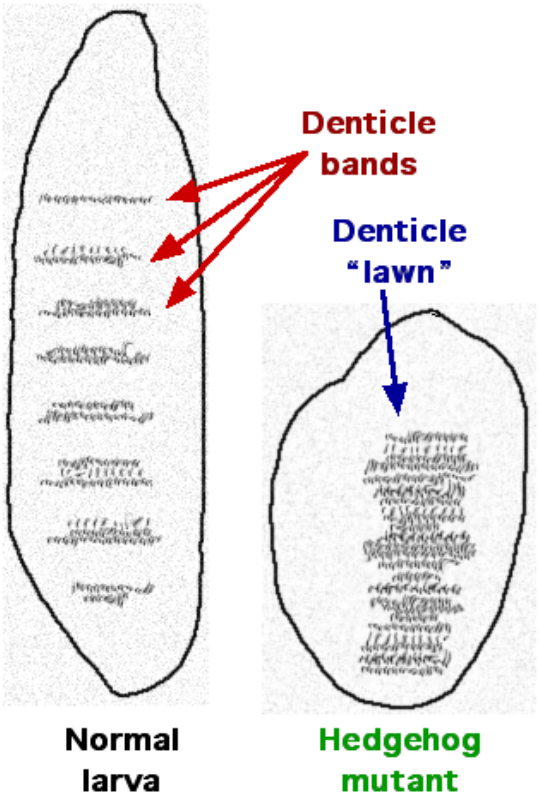
videa z Petros et al., Dev. Neurobiol. 2010

Klíčové molekulární komponenty vývoje



Hedgehog dráha

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



Sonic the Hedgehog

Schéma Shh dráhy

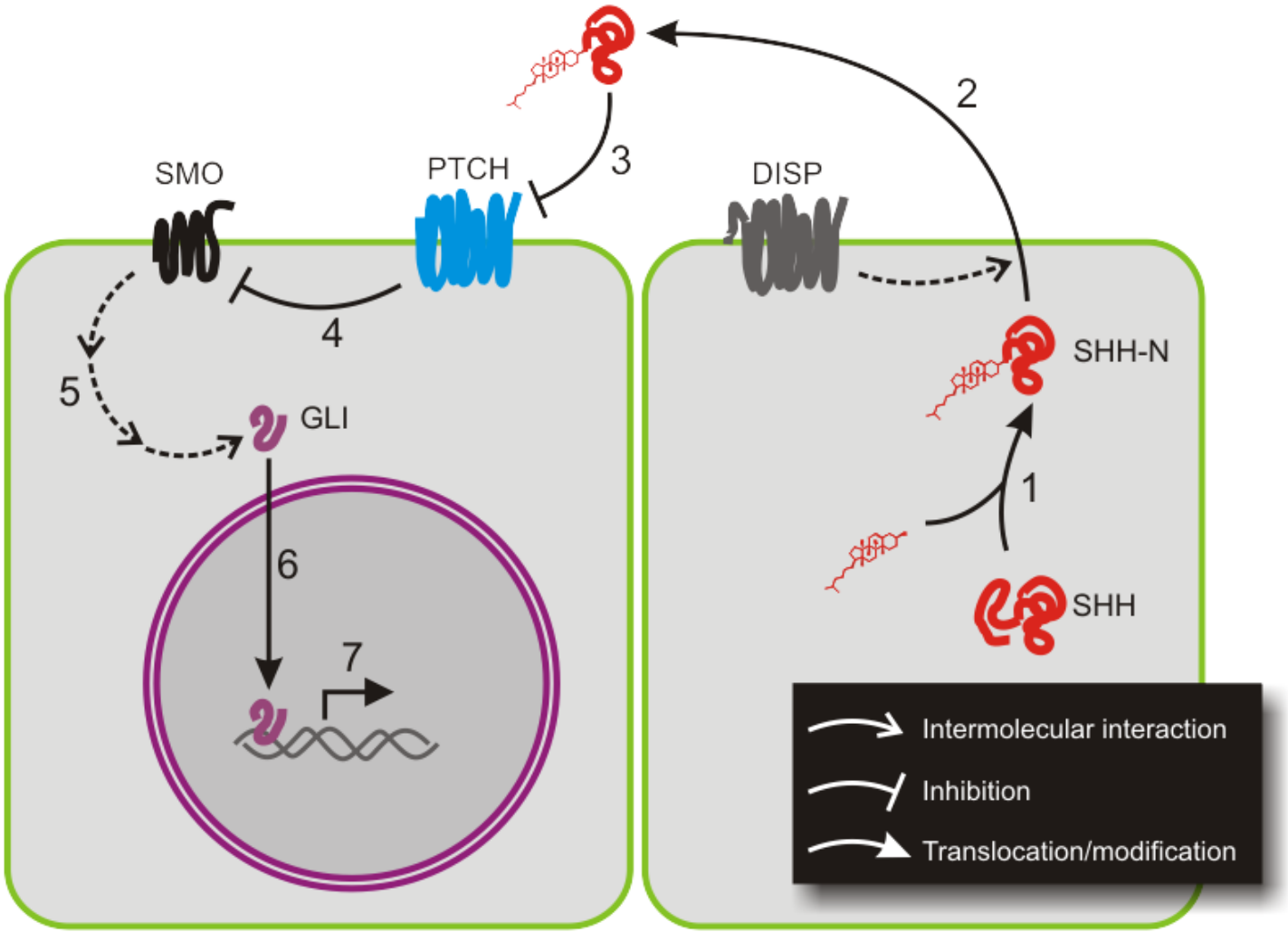


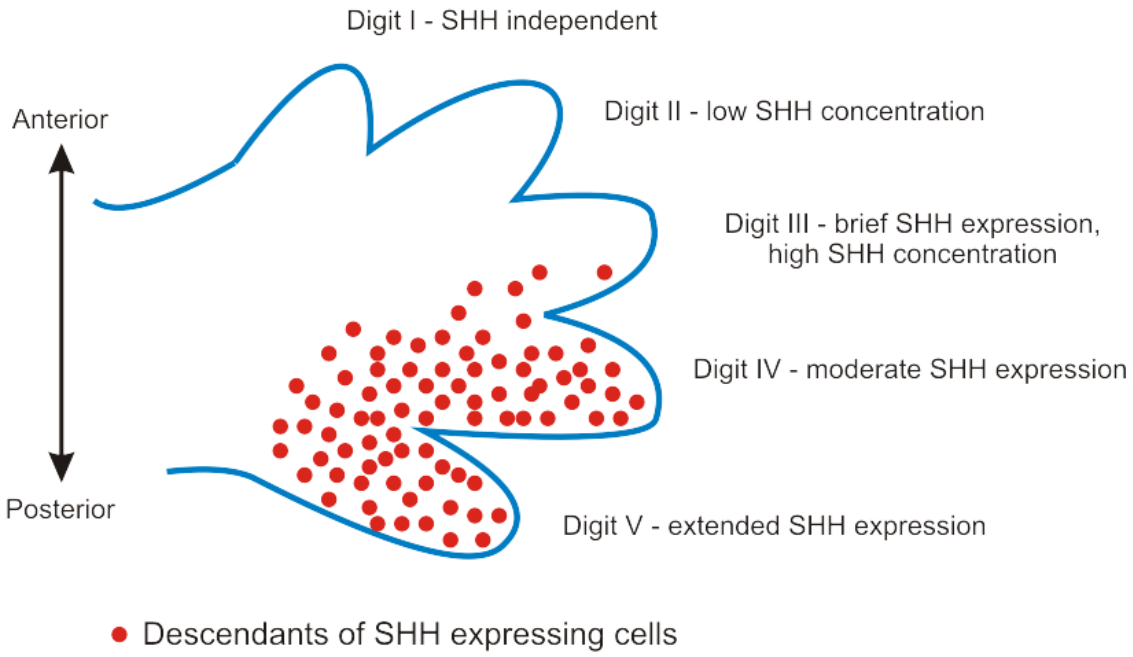
Schéma Shh dráhy

- legenda k obrázku:

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).

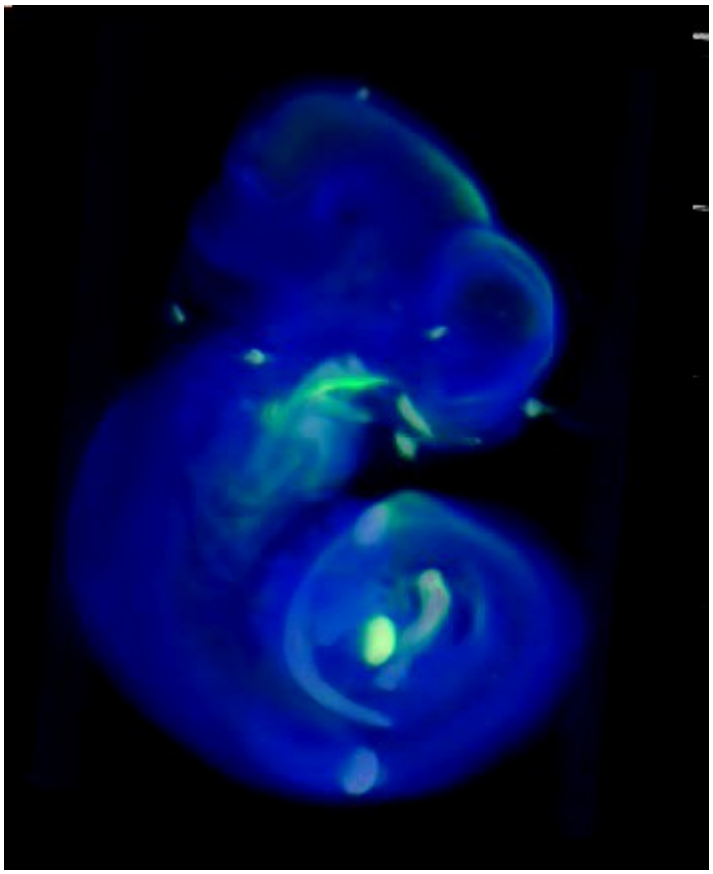
Shh

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



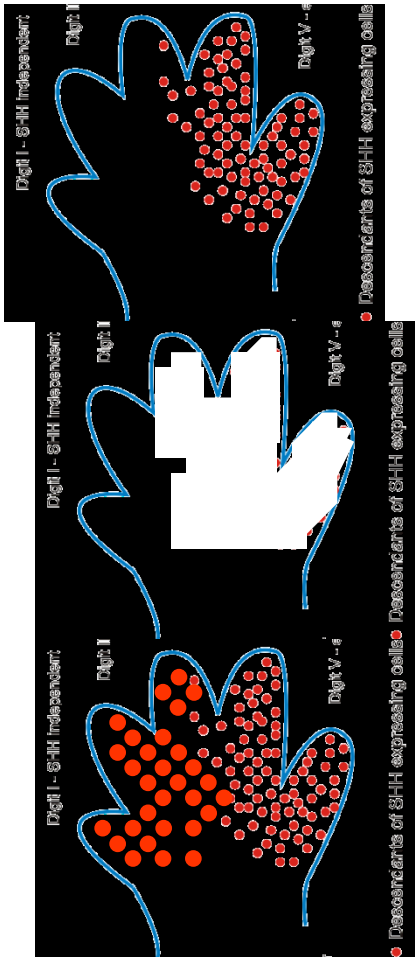
Např. specifikace jednotlivých prstů končetiny

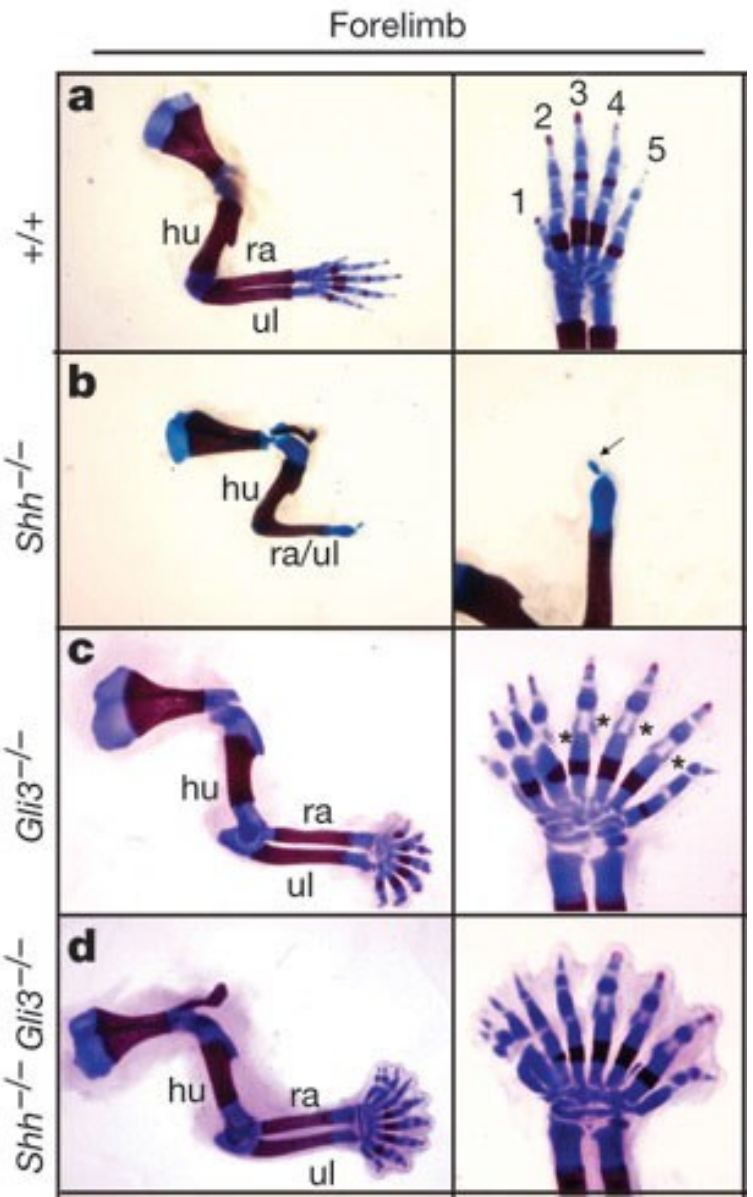
Shh



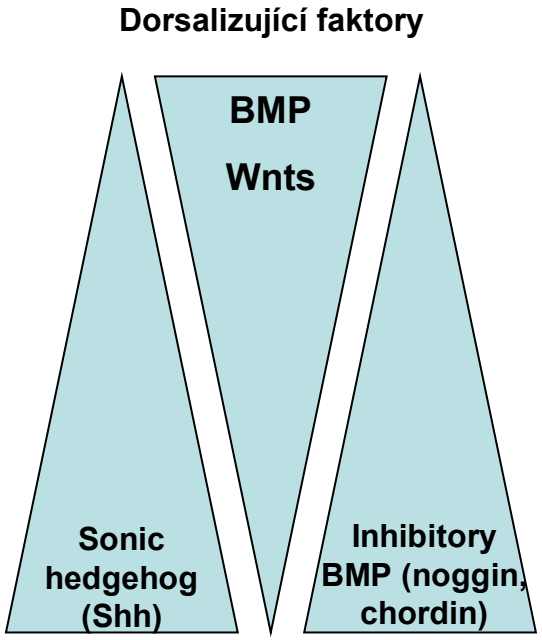
Např. specifikace jednotlivých prstů končetiny

Shh


Digit I - SHH independent
Digit I
Digit V - 4
Descendants of SHH expressing cells


Forelimb
+/+
Shh−/−
Gli3−/−
Shh−/− Gli3−/−
a
b
c
d
hu
ra
ul
ra/ul
1
2
3
4
5

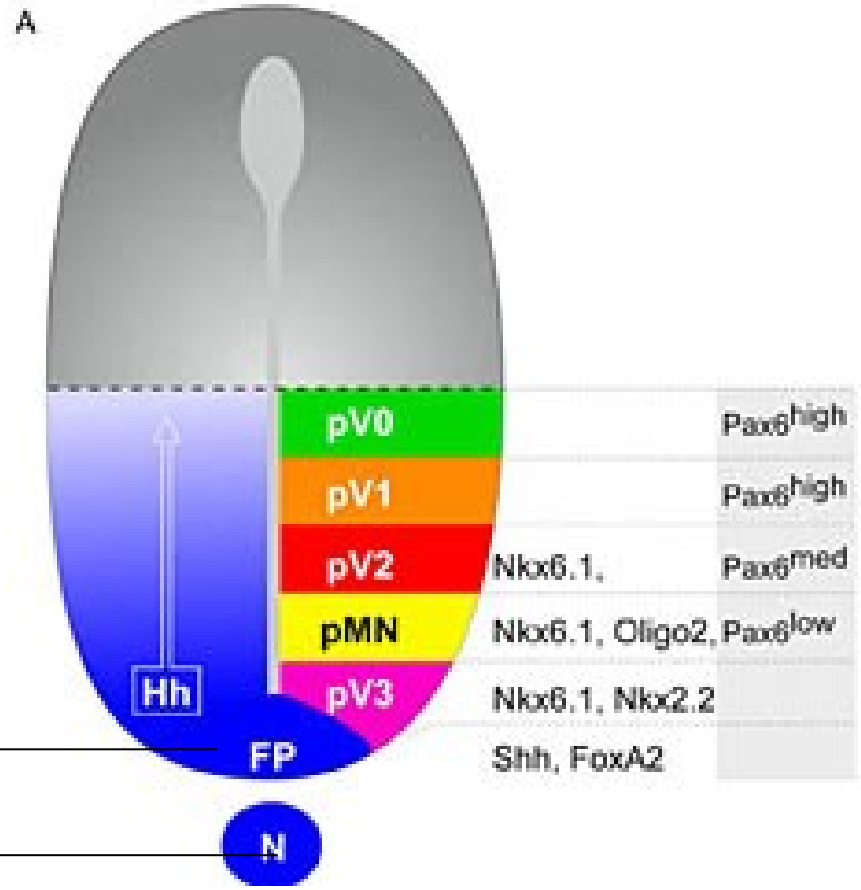
Shh



ventralizující faktory produkované notochordem

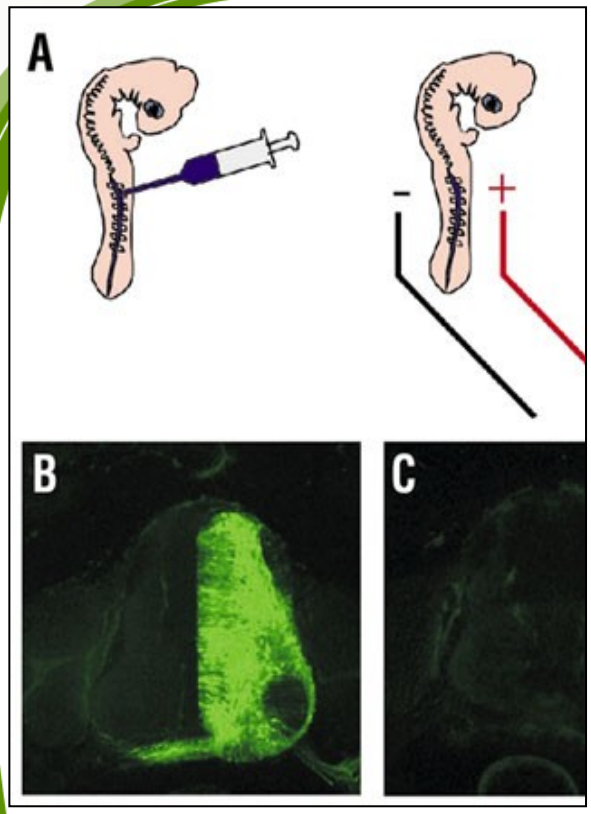
floor plate – nejspodnější část nervové trubice

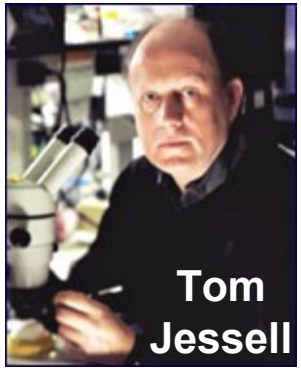
notochord – zdroj Shh

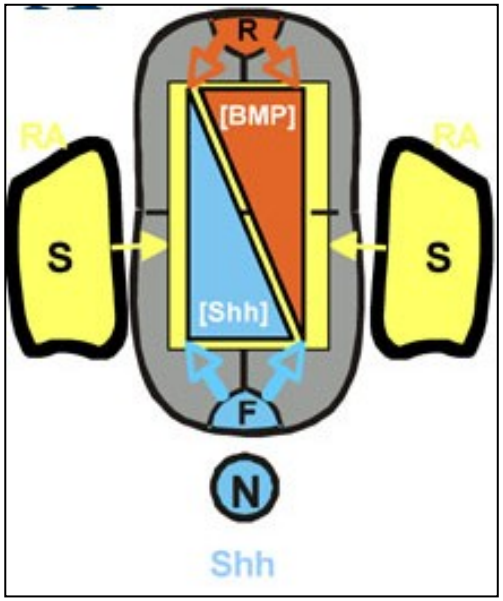


Např. specifikace jednotlivých neuronálních typů ve vyvíjející se nervové trubici

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







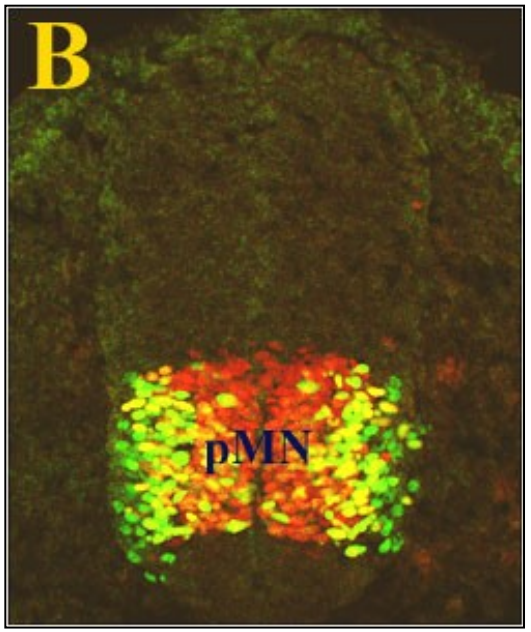
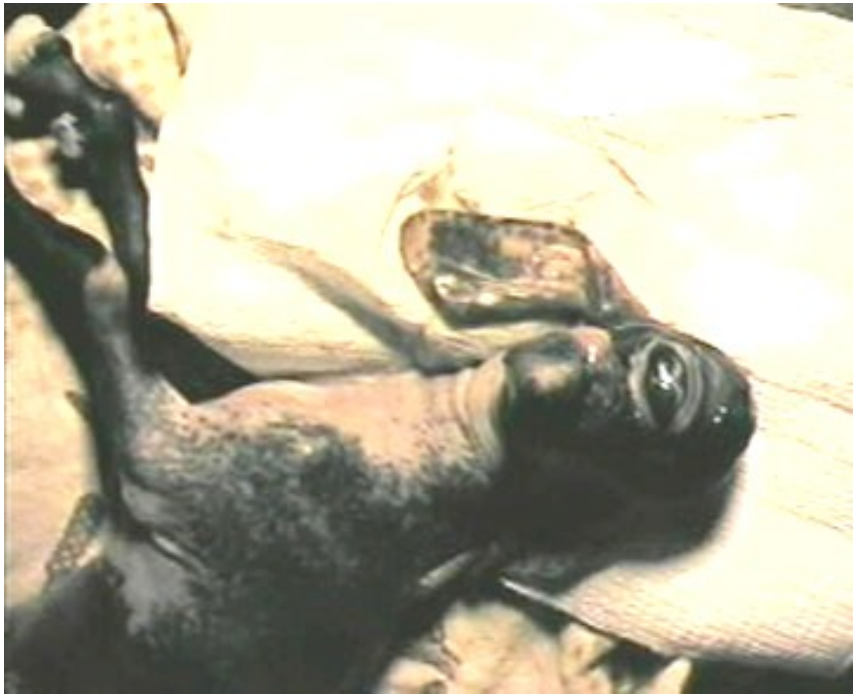


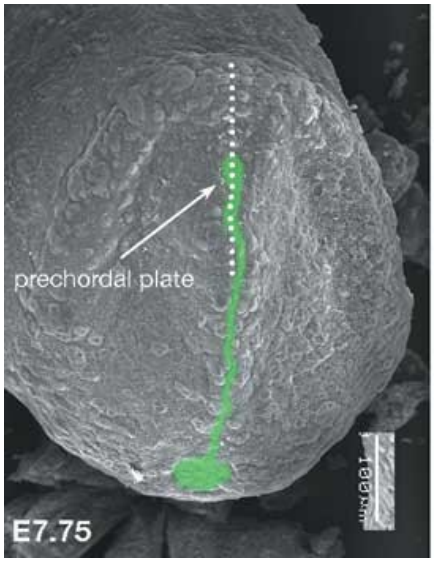
Fig. A - A model for early spinal cord development. The neural tube which will form the spinal cord is patterned into specific domains by multiple external signals which include a ventralizing Sonic Hedgehog (Shh) signal from the notochord (N) and floor plate (F), a dorsalizing BMP signal from the roof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

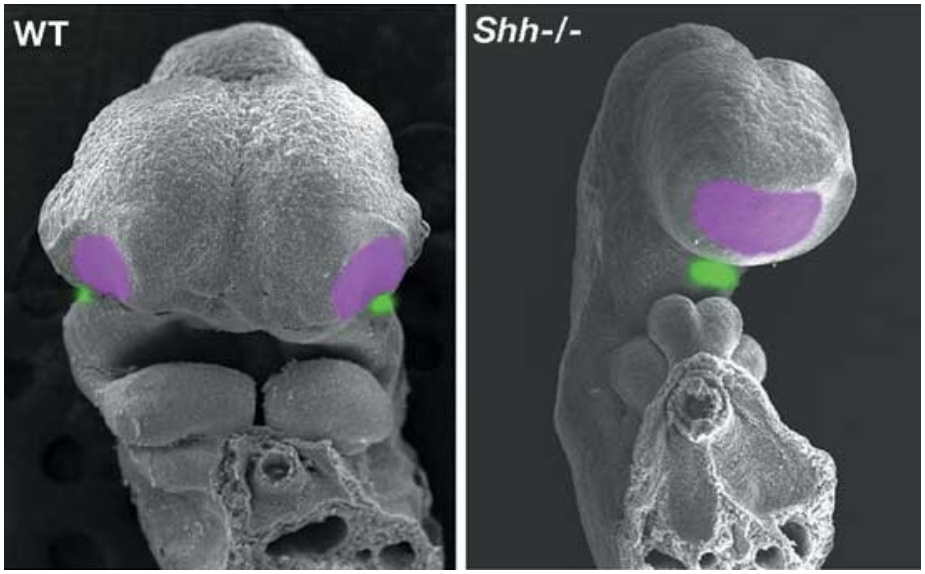
Přirozené inhibitory Shh dráhy



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



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 perchordal plate. Shh (in green) that is expressed in the prechordal plate induces midline structure formation.

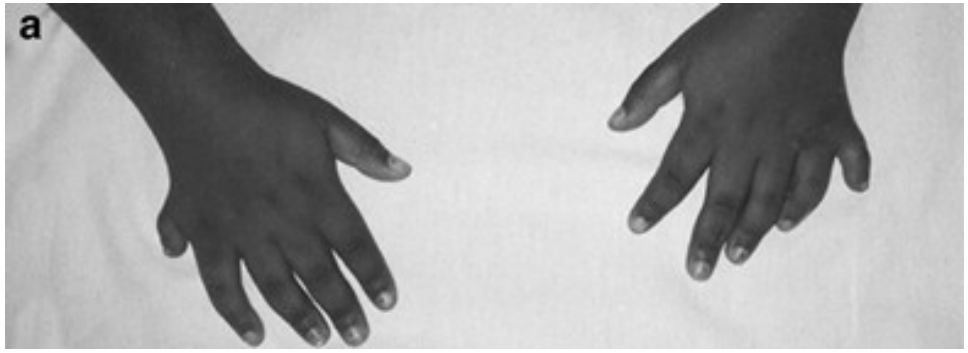


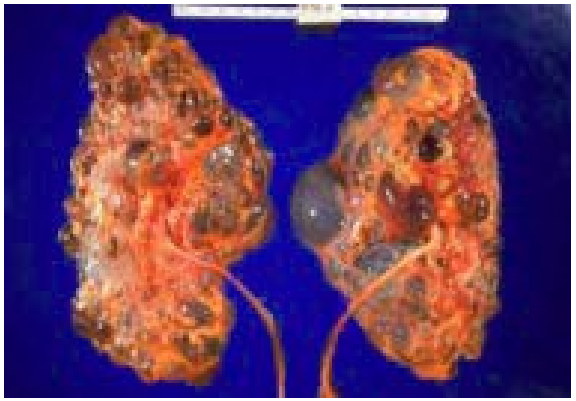
Model mice with Holoprosencephaly due to a Sonic Hedgehog (Shh) deficiency.

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.

Hedgehog (Hh) dráha je vázána na primární cilie

- Abnormální Hh/Wnt a s nimi spojená onemocnění jsou způsobena defekty ve tvorbě primárních cilií (infertilita, polydaktylie, polycystické ledviny, degenerace retiny).
- Hh je přímo vázán na primární cílie.





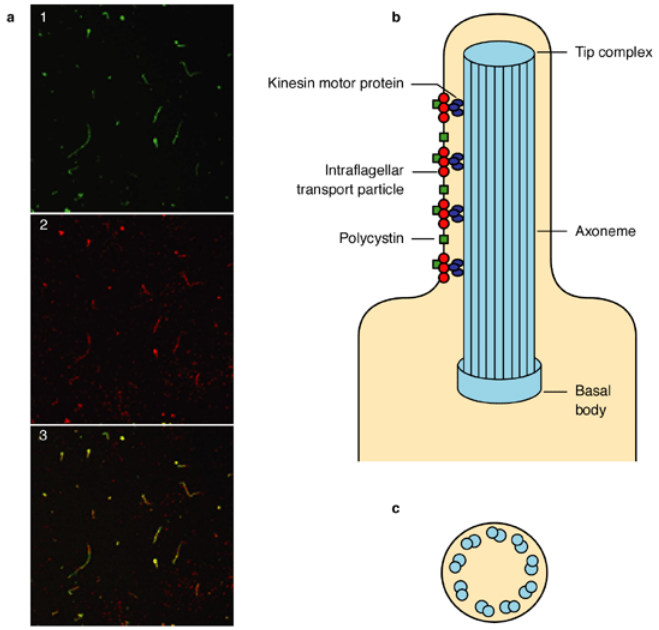
Primary cilia vs. motile (secondary) cilia

PRIMÁRNÍ

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

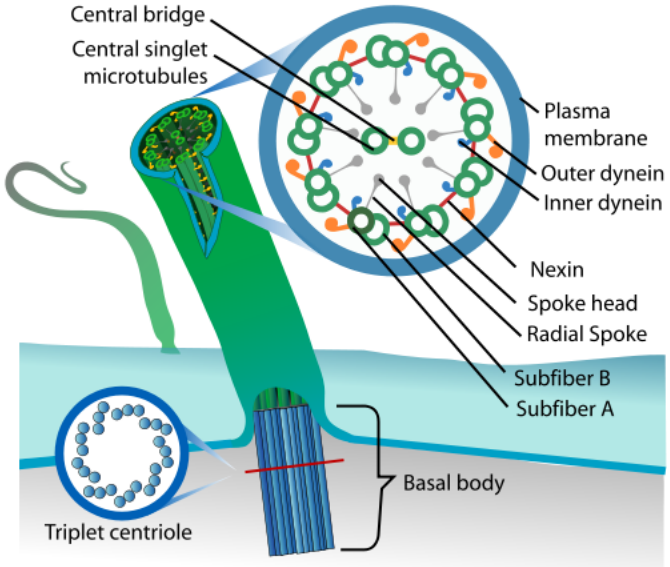
SEKUNDÁRNÍ

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



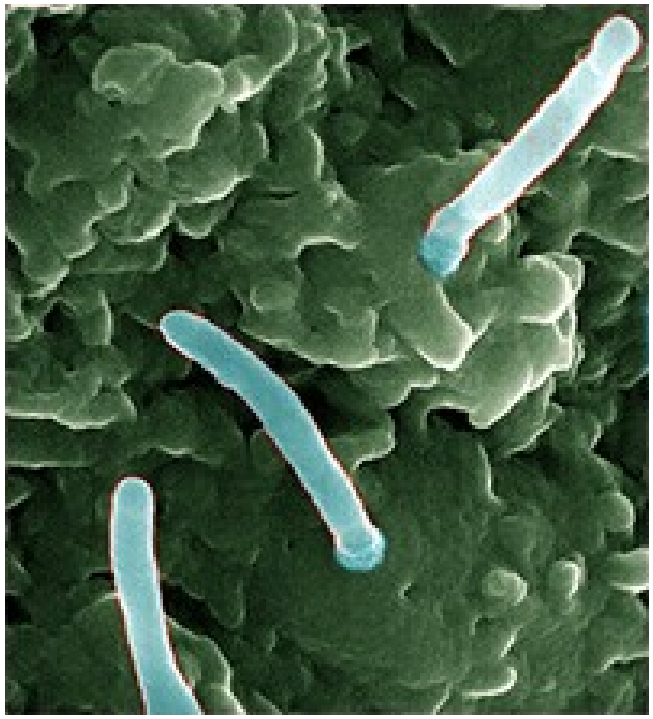
The primary cilium

Expert Reviews in Molecular Medicine©2006 Cambridge University Press



Primární cilie - funkce

- délka 2-10μm, průměr 0.25μm



- chemo- a osmosenzory
- fotoreceptory
- mechanoreceptory

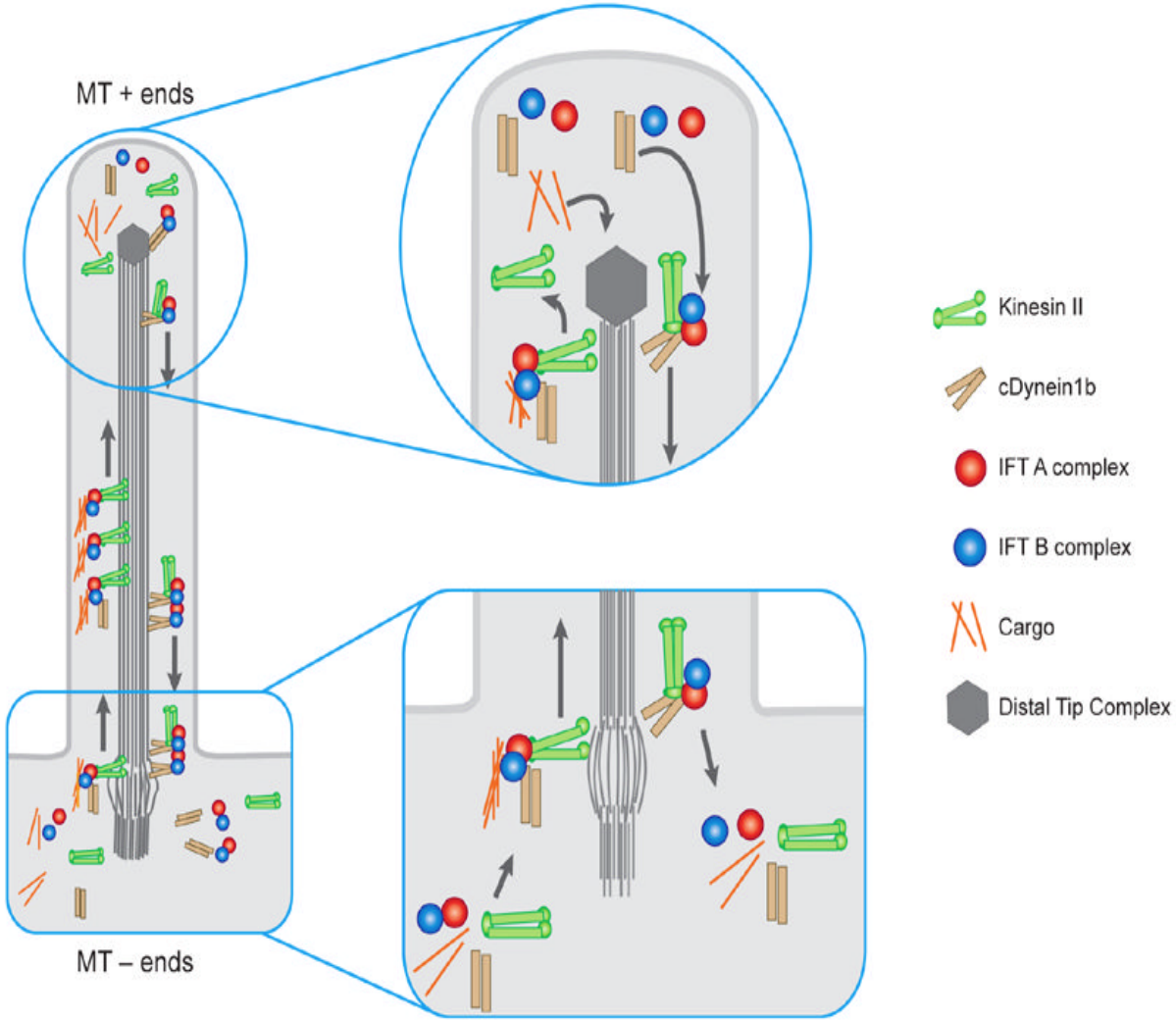
- komunikace v extracelulární matrix

- nodal cilia
  - pohyblivá
  - blastocysta
  - pravolevá souměrnost

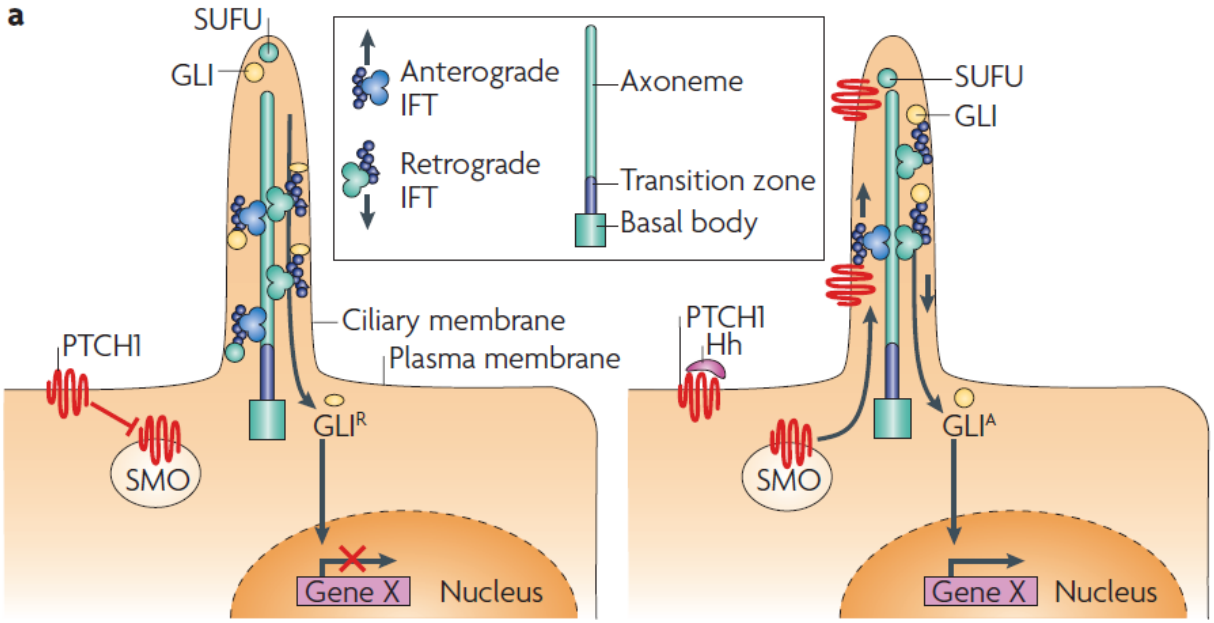
- model trandukce – receptory iontové kanály, efektorové proteiny, transkripční faktory

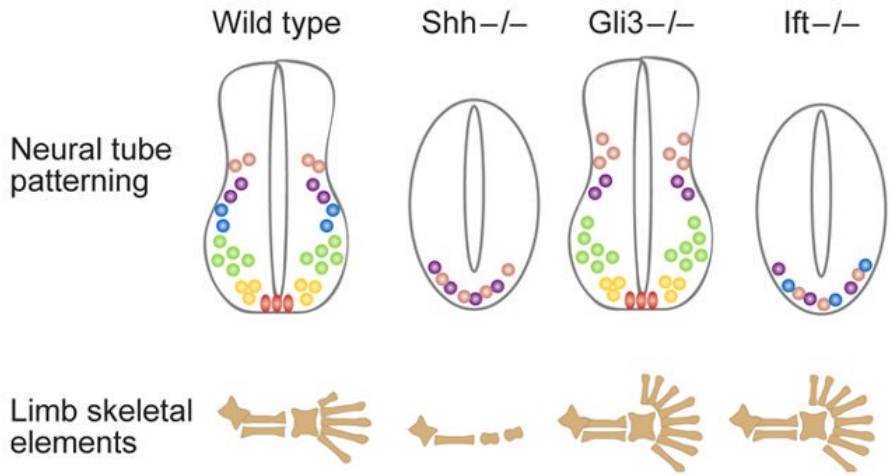
Intraflagelární transport (IFT)

- 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

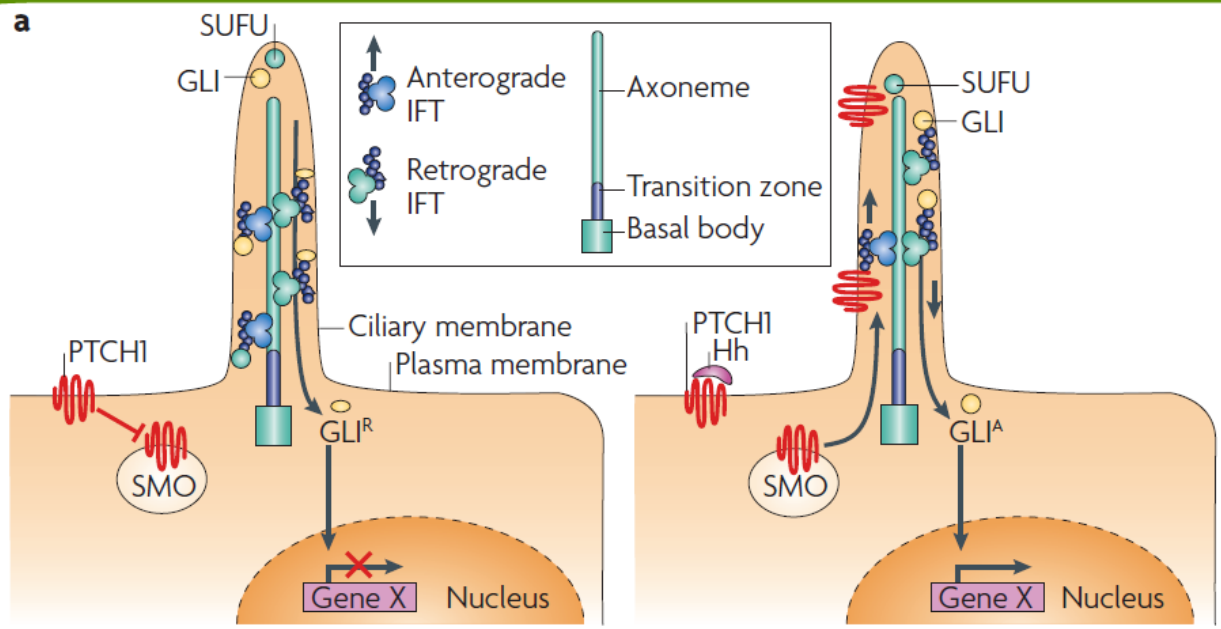


Primární cilie a Hh signalizace





Primární cilie a Hh signalizace

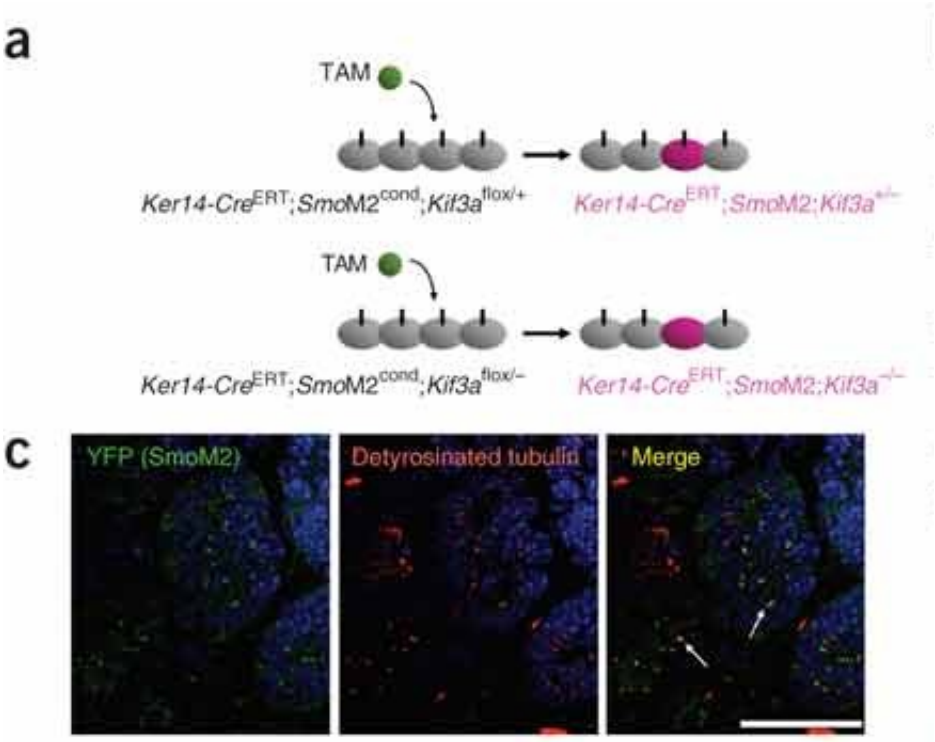


- Je spojen s primárními ciliemi
- Ligand se naváže na patch (Ptc) protein, což způsobí zrušení inhibičního efektu Ptc na protein smoothened (Smo), který transdukuje signál přes glioma transkripční faktory (Gli) do jádra, kde řídí expresi Hh genů. (Gli1, Gli2 a Gli3A jsou aktivátory a Gli3R je represor). Hlavním represorem je SuFu.
- IFT hraje klíčovou úlohu ve funkci regulace Hh signální dráhy (spojuje Smo a Gli)

- Mutace Kif3A a Kif3B mají podobné fenotypy v důsledku ztráty cilie.
- 3 typy Hh – Sonic Hh (Shh), Indian Hh (Ihh) a Desert Hh (Dhh)

Primární cilie a Hh signalizace: důkaz

Basal cell carcinoma – způsobena aktivací Smoothened



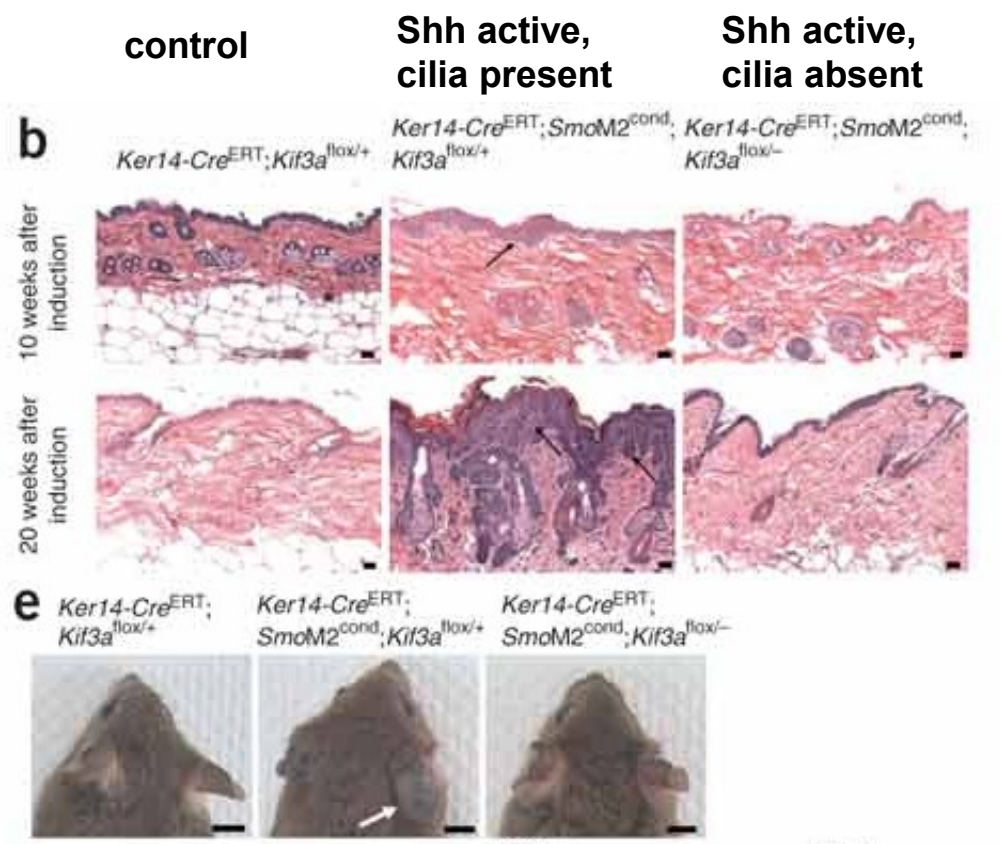
Ker14-Cre: drives expression to the epidermis

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

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

Wong et al., Nat. Med. 2009

Primární cilie a Hh signalizace: důkaz



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Klíčové molekulární komponenty vývoje