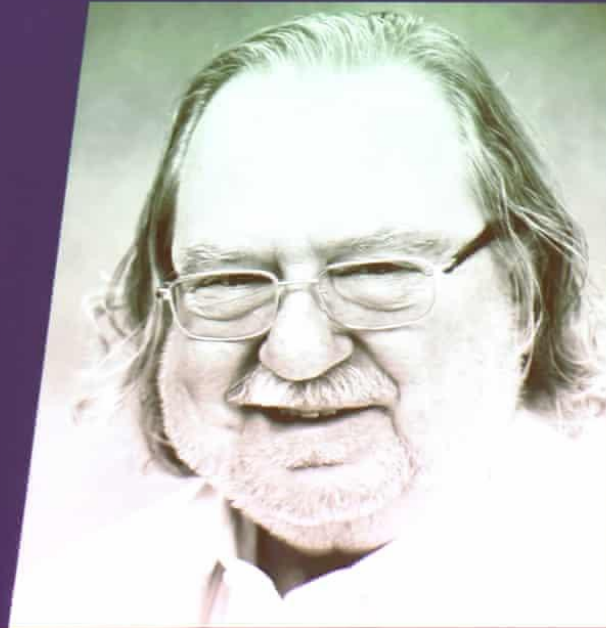


Buněčné systémy ve vývoji

doc. Mgr. Vítězslav Bryja, Ph.D.

Nobelova cena za fyziologii a medicínu 2018



James P Allison
MD Anderson Cancer Center



Tasuku Honjo
Kyoto University



Nobelova cena za fyziologii a medicínu 2018

- Objev inhibičních mechanismů imunitní odpovědi a jejich využití pro protinádorovou terapii

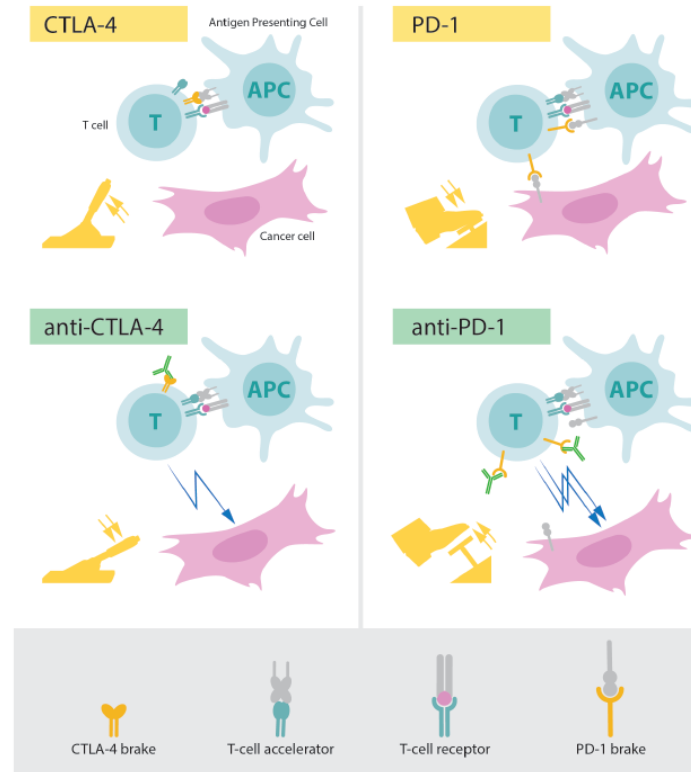


Figure: Upper left: Activation of T cells requires that the T-cell receptor binds to structures on other immune cells recognized as "non-self". A protein functioning as a T-cell accelerator is also required for T cell activation. CTLA-4 functions as a brake on T cells that inhibits the function of the accelerator. **Lower left:** Antibodies (green) against CTLA-4 block the function of the brake leading to activation of T cells and attack on cancer cells.

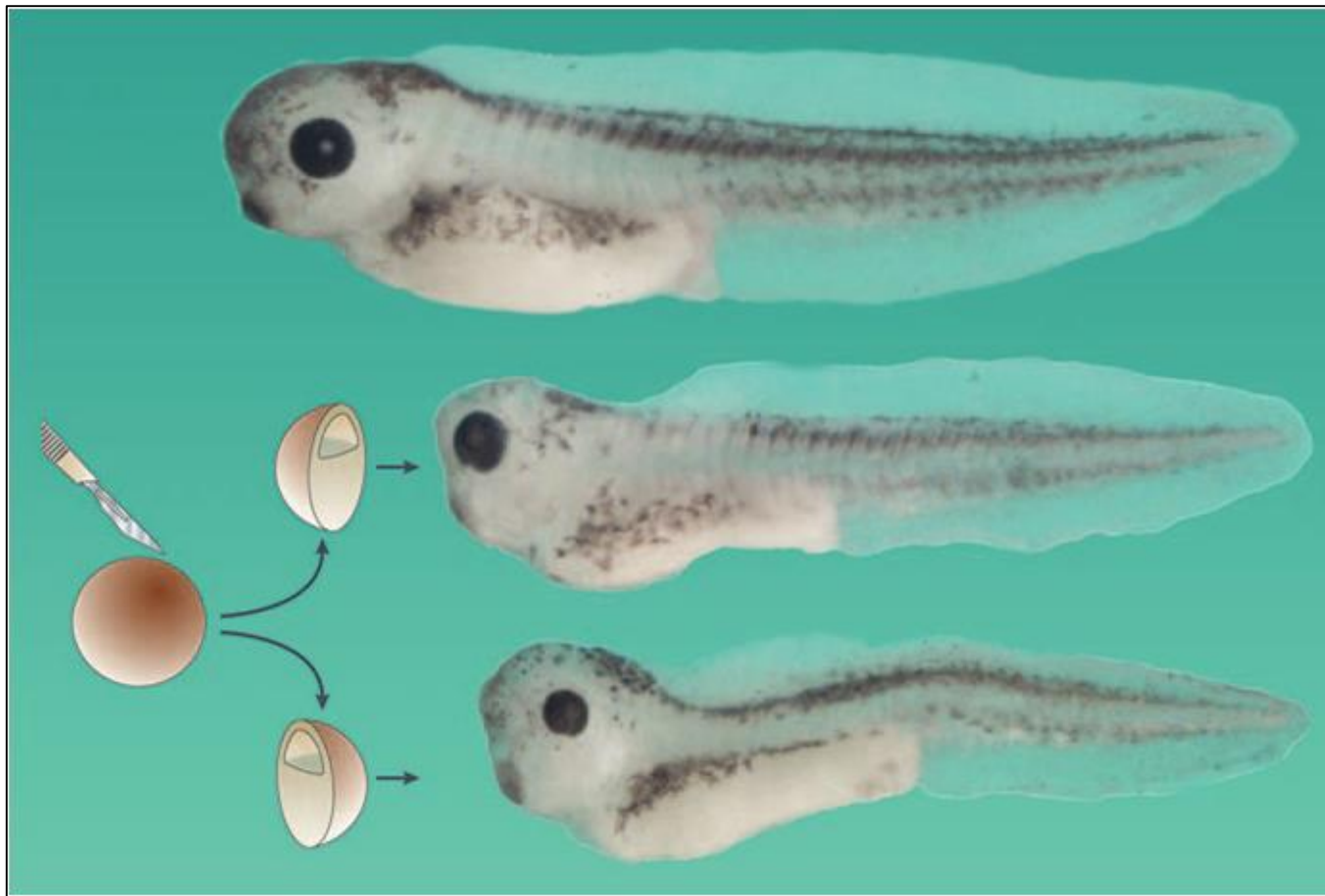
Upper right: PD-1 is another T-cell brake that inhibits T-cell activation. **Lower right:** Antibodies against PD-1 inhibit the function of the brake leading to activation of T cells and highly efficient attack on cancer cells.

Vlastnosti buněk v průběhu embryonálního vývoje

Danio rerio



Vlastnosti buněk v průběhu embryonálního vývoje



Vlastnosti buněk v průběhu embryonálního vývoje

- Rychlé buněčné dělení
- Dynamické změny diferenciačního stavu (cell fate)
- Intenzivní migrace
- Změny tvaru a velikosti buňky
- Schopnost odpovědi na poškození celého embrya

Klíčové molekulární komponenty vývoje

Co poskytuje buňce tyto informace?

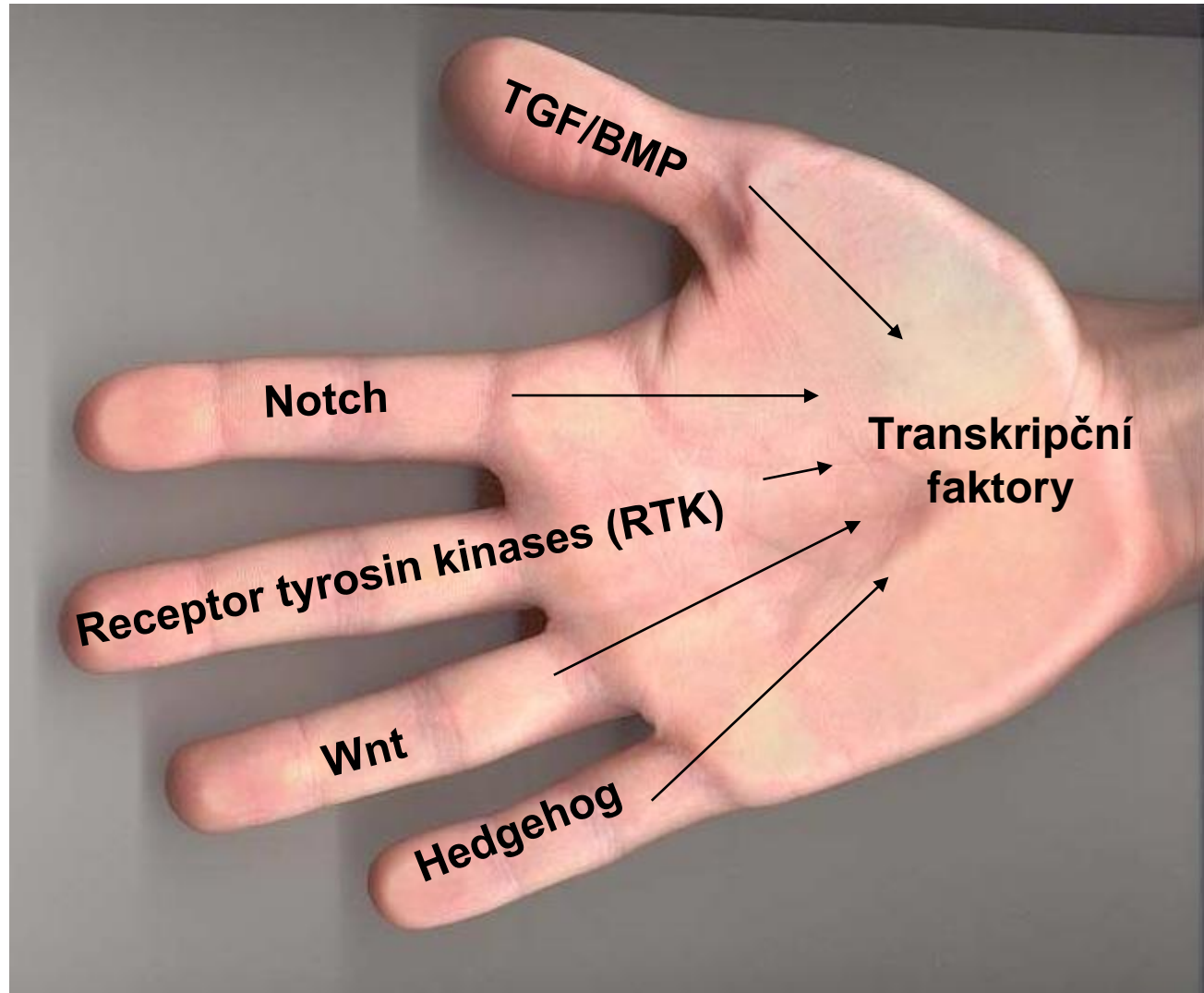
a) signály z okolního prostředí

jednotlivé
signální
dráhy
modulují
transkripci a
strukturu
chromatinu

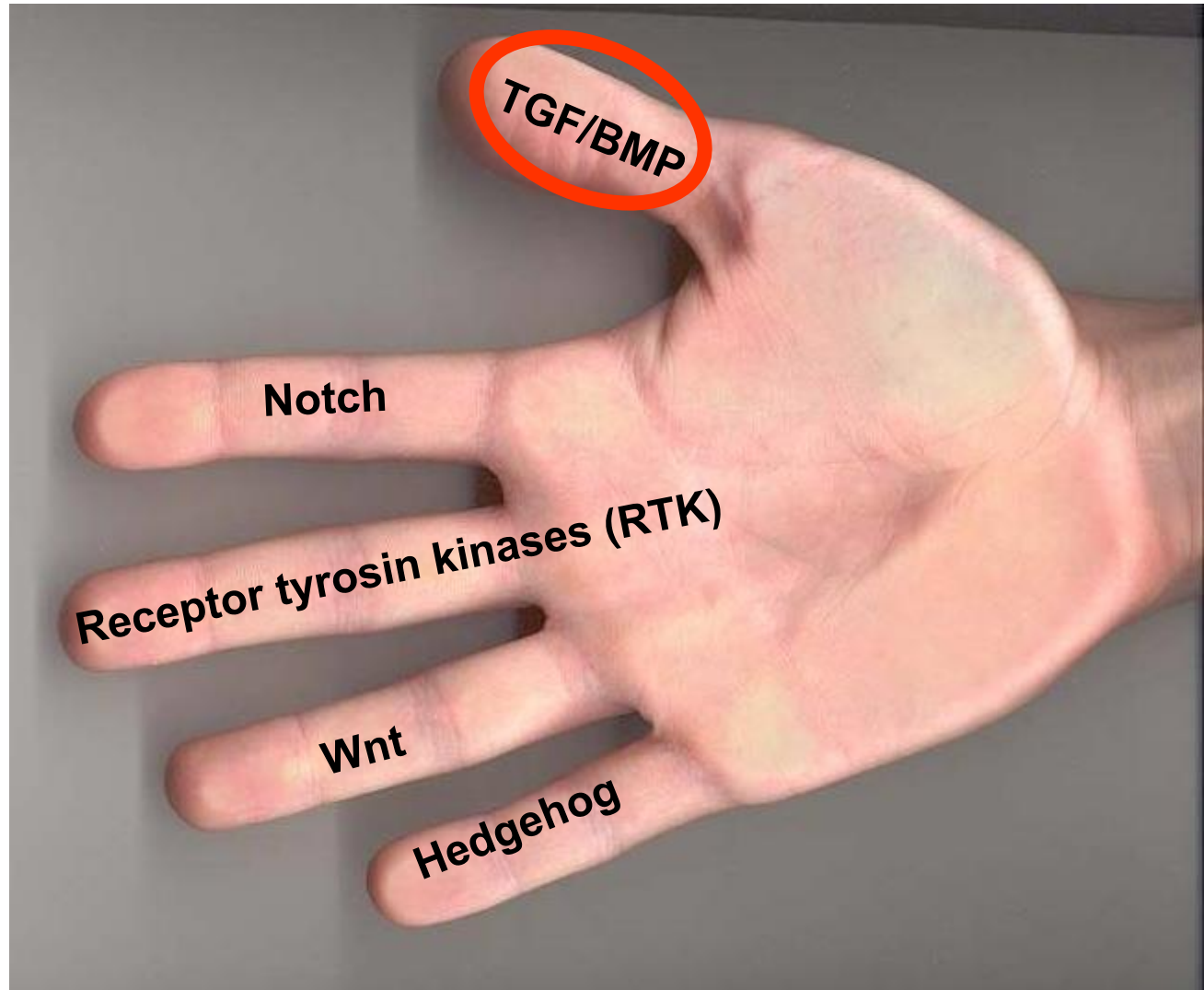
transkripce určuje
citlivost buňky k
vnějším signálům
(např. regulací
exprese receptorů
či komponent
přenosu signálu)

b) transkripční program v jádře

Hlavní signální dráhy důležité v embryonálním vývoji



Klíčové molekulární komponenty vývoje



TGF/BMP

- TGF – transforming growth factor (transformující růstový faktor)
- BMP – bone morphogenetic protein (kostní morfogenetický protein)
- patří do TGF β nadrodiny

TGF beta nadrodina:

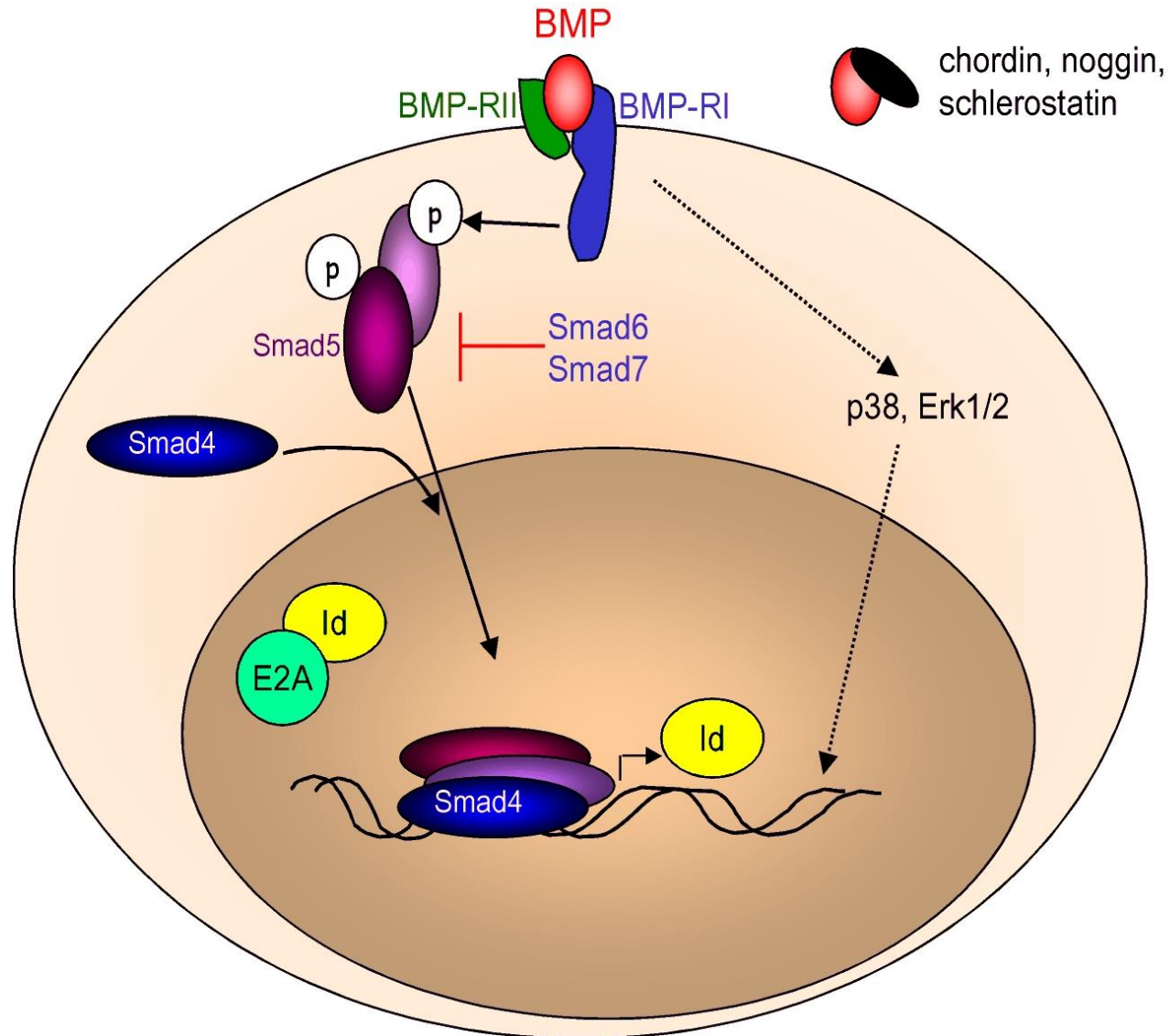
TGF β nadrodina má následující podrodiny:

1. TGF β 1-3
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

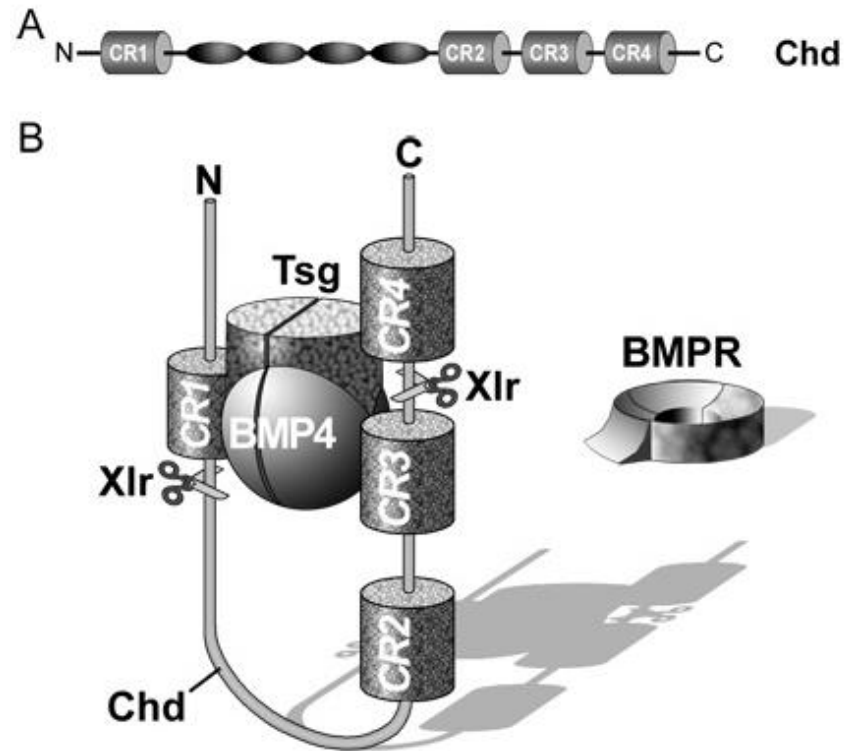
Signální dráha BMP



Inhibitory BMP faktorů

jsou klíčové pro fyziologické funkce BMP

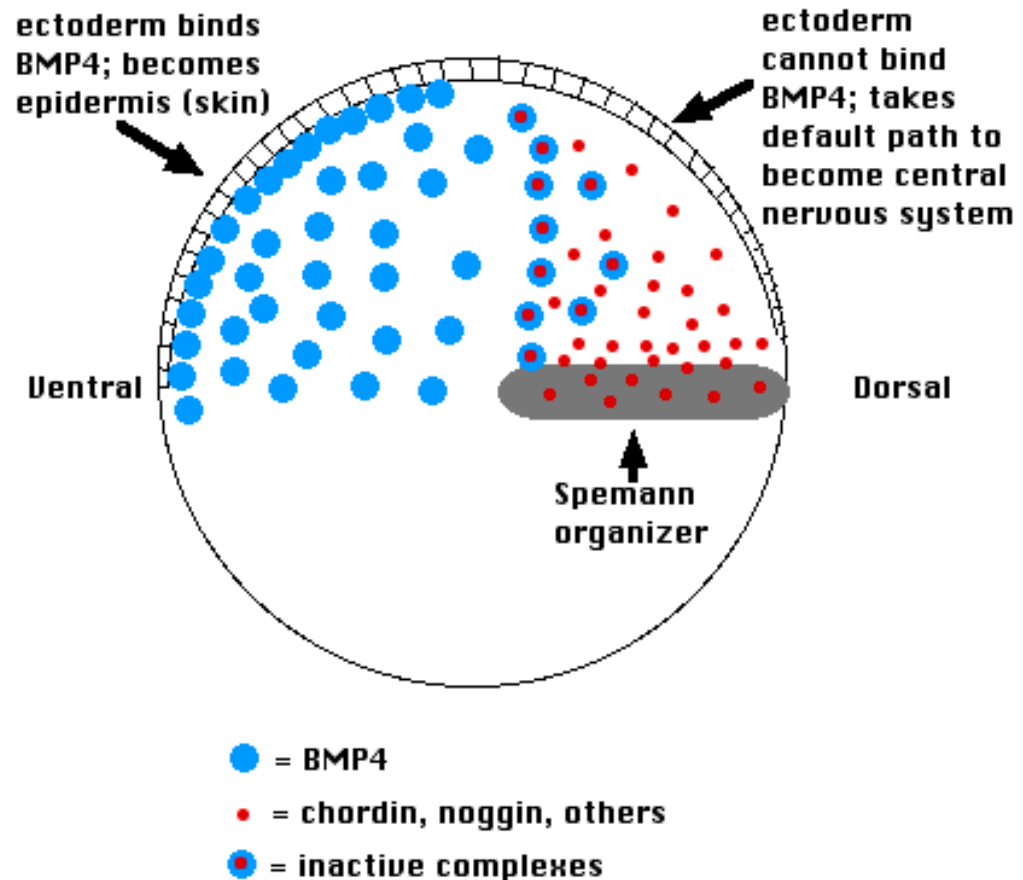
- noggin
- chordin (Chd)
- sklerostin



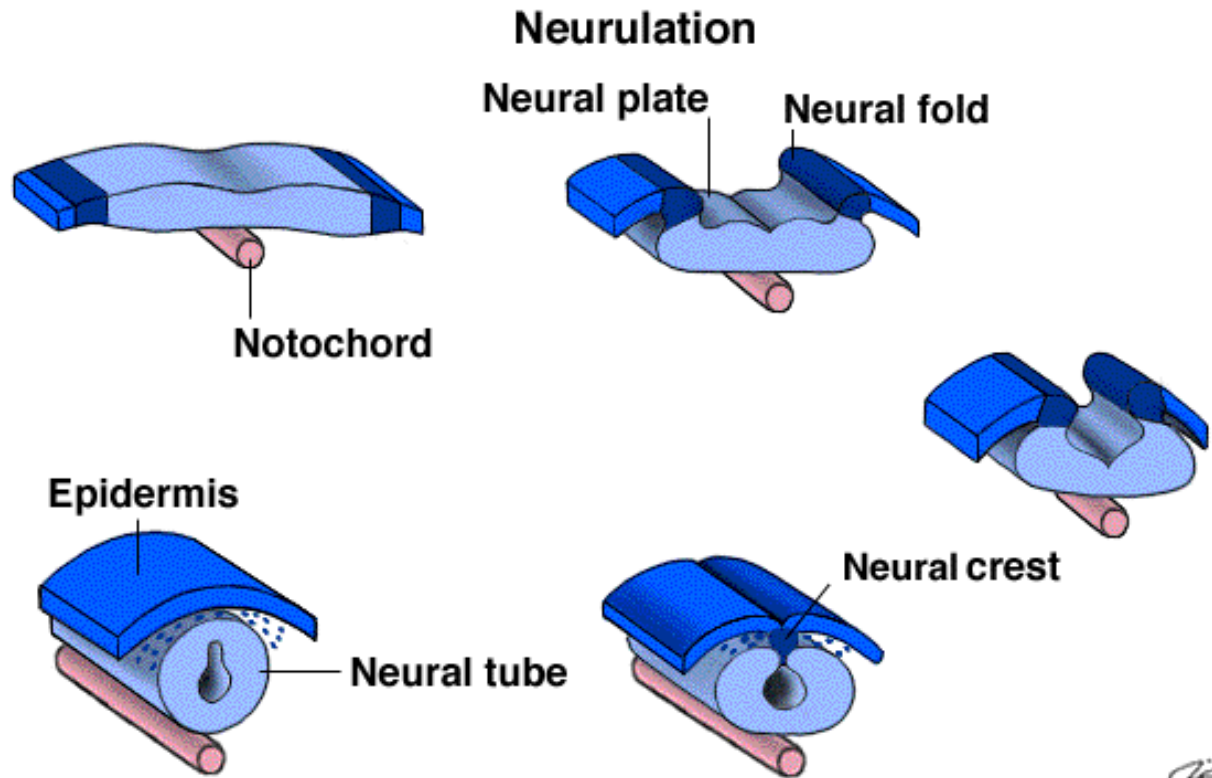
Přímá fyzická interakce mezi chordinem a BMP je podstatou inhibičního působení chordinu

Role BMP inhibitorů v Spemannově organizátoru (Xenopus)

- Spemannův organizátor u obojživelníků (= Hensenův nodus u ptáků) – organizační centrum, které umožňuje správný průběh gastrulace



Klíčová role BMP inhibitorů produkovaných notochordem při indukci nervové ploténky



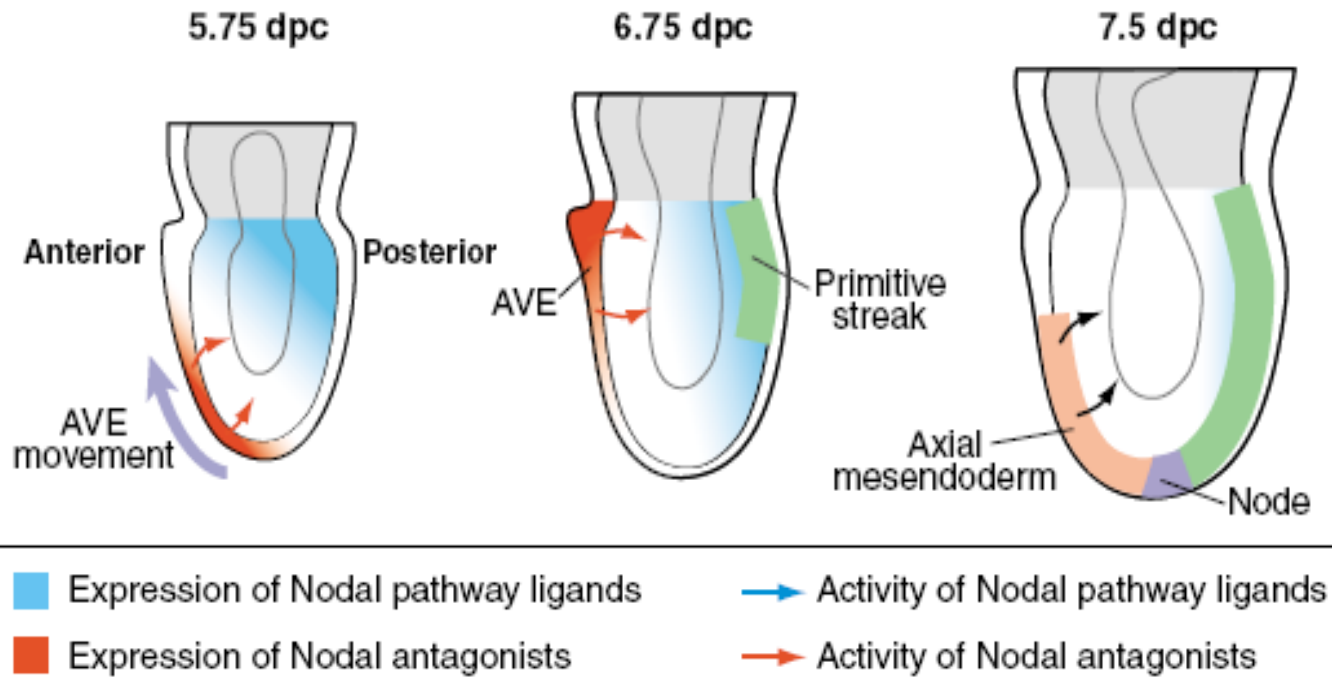
Crump Institute for Biological Imaging

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.

Nodal a pravo-levá symetrie

gastrulace u myši

AVE – anterior visceral endoderm



Nodal a pravo-levá symetrie

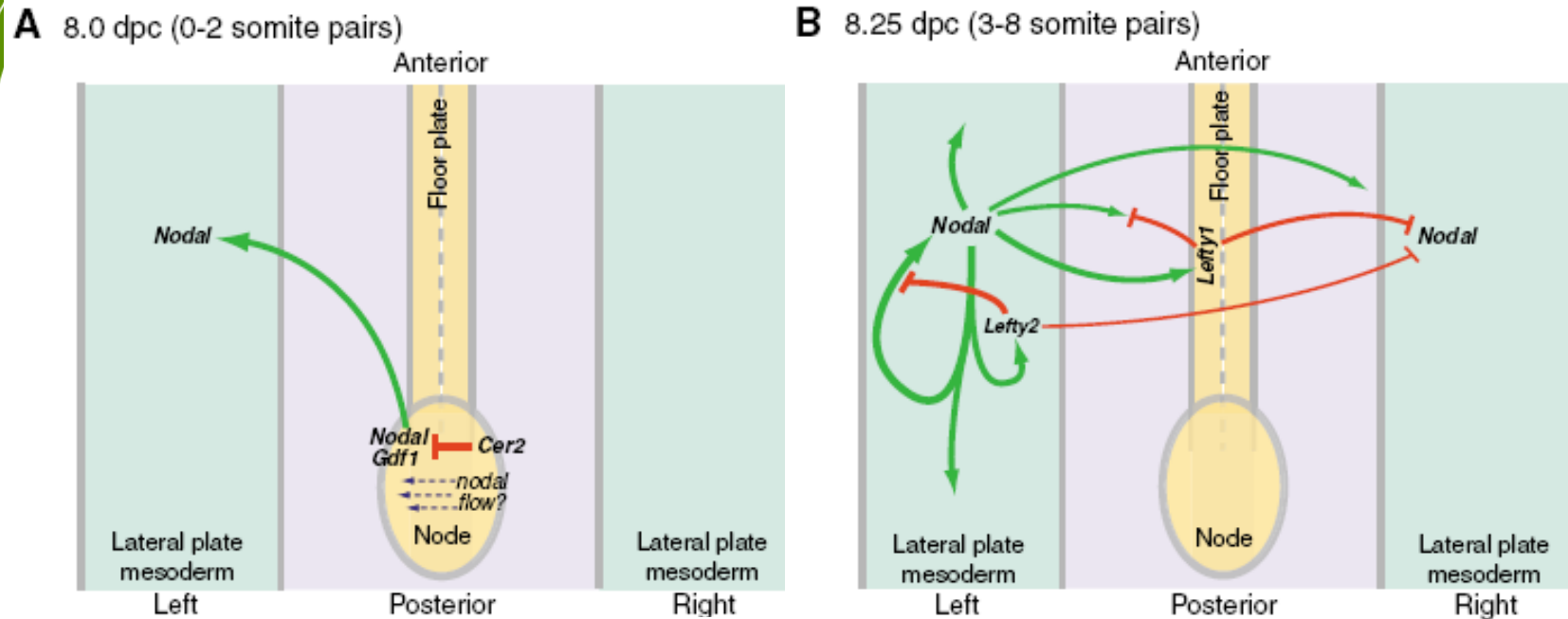
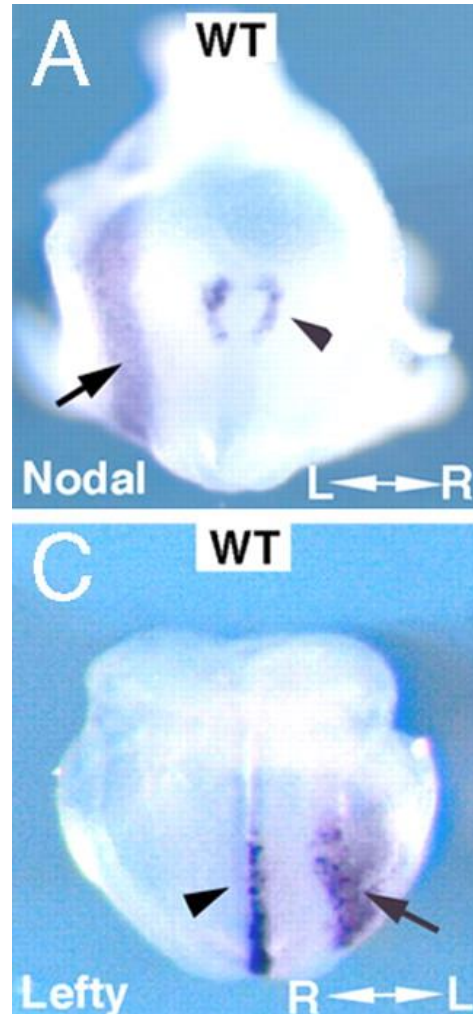


Fig. 5. Sequential function of Nodal signaling in left-right patterning in the mouse embryo. (A) Following initial symmetry breaking around the node, possibly as a consequence of ciliary-based nodal flow, Nodal (green arrow) and/or Gdf1 signals become elevated on the left side of the node, and are antagonized 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.

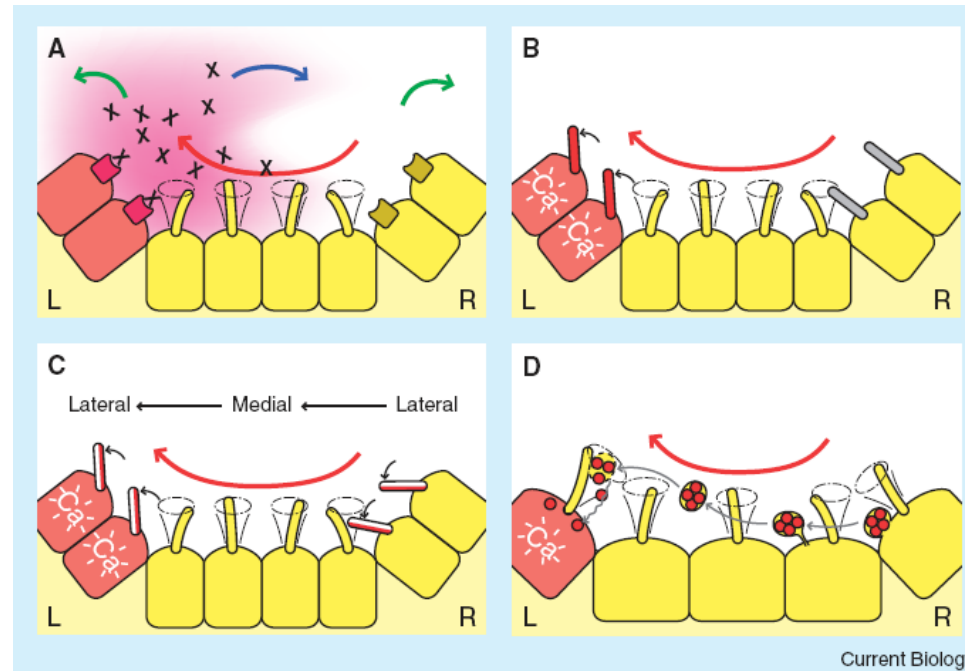
Nodal a pravo-levá symetrie

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

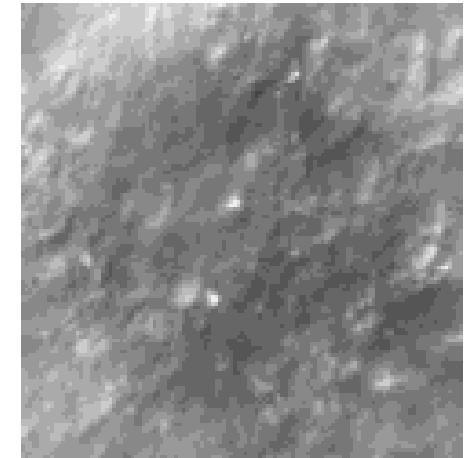
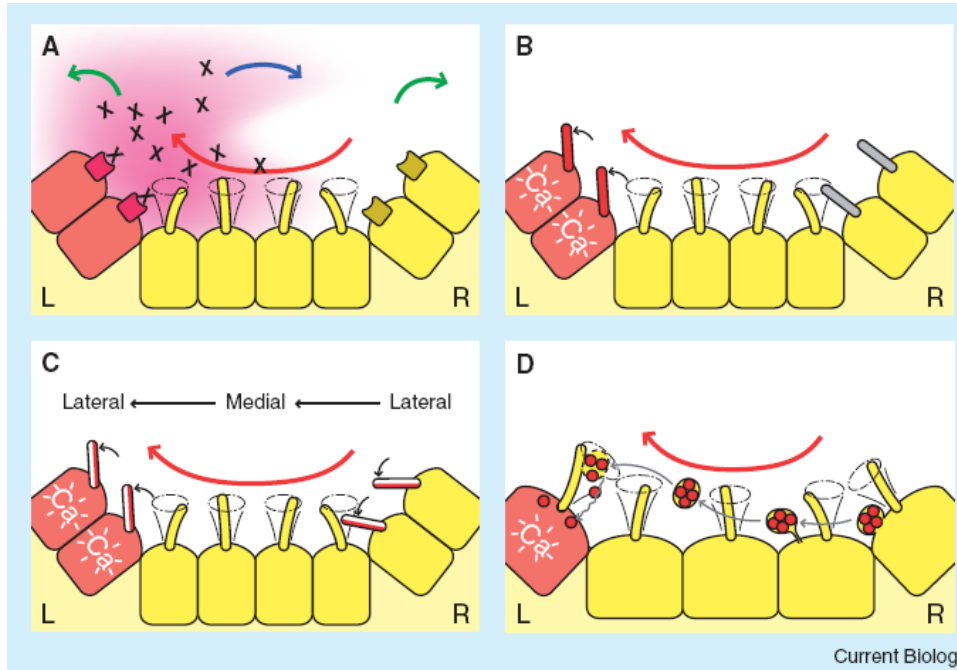


Vytváření levopravé asymetrie těla - role cilií

- asymetrická exprese genů jako *lefty1*, *lefty2*, *nodal* a *pitx2*
- nodální cilie (9+0, dynein → pohyblivé) během gastrulace vytváří svým rotačním pohybem tzv. **nodální proud**



Vytváření levopravé asymetrie těla - role cilií



- narušená funkce cilií → vzniká až **situs inversus** (vnitřní orgány uspořádané 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)

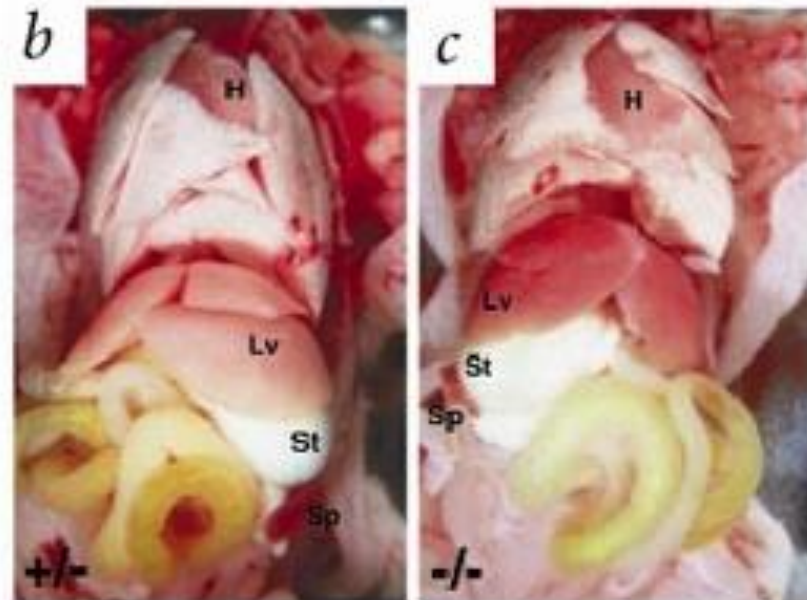
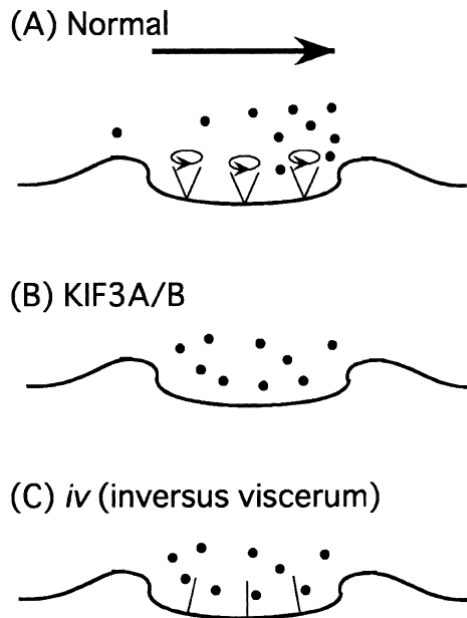
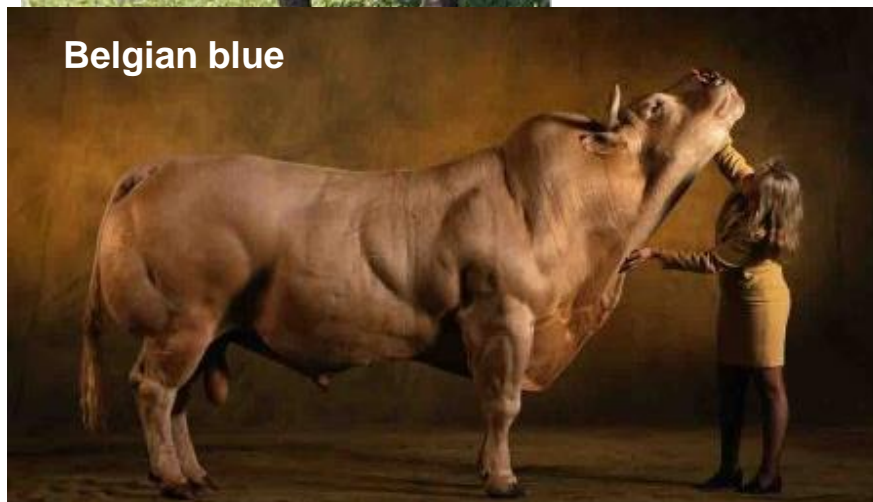


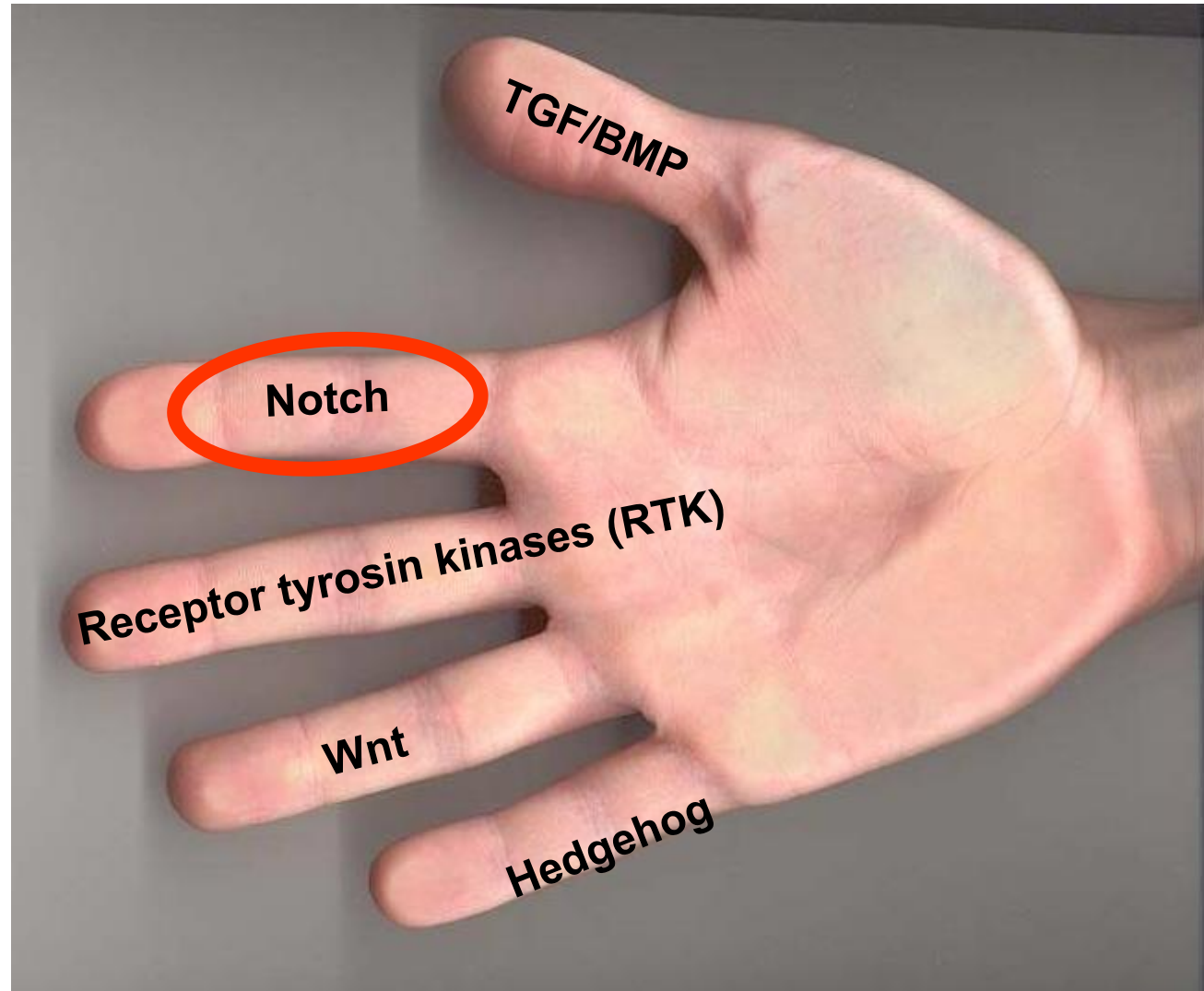
Figure 2. Analysis of situs defects in *Gdf1*^{-/-} mice.
a, *Gdf1*^{+/-} and *Gdf1*^{-/-} newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1*^{+/-} (**b,d,f,h**) and *Gdf1*^{-/-} (**c,e,g,i**) mice are shown. **b,c**, Reversal 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) – příklad tzv. master regulátoru konkrétní tkáně

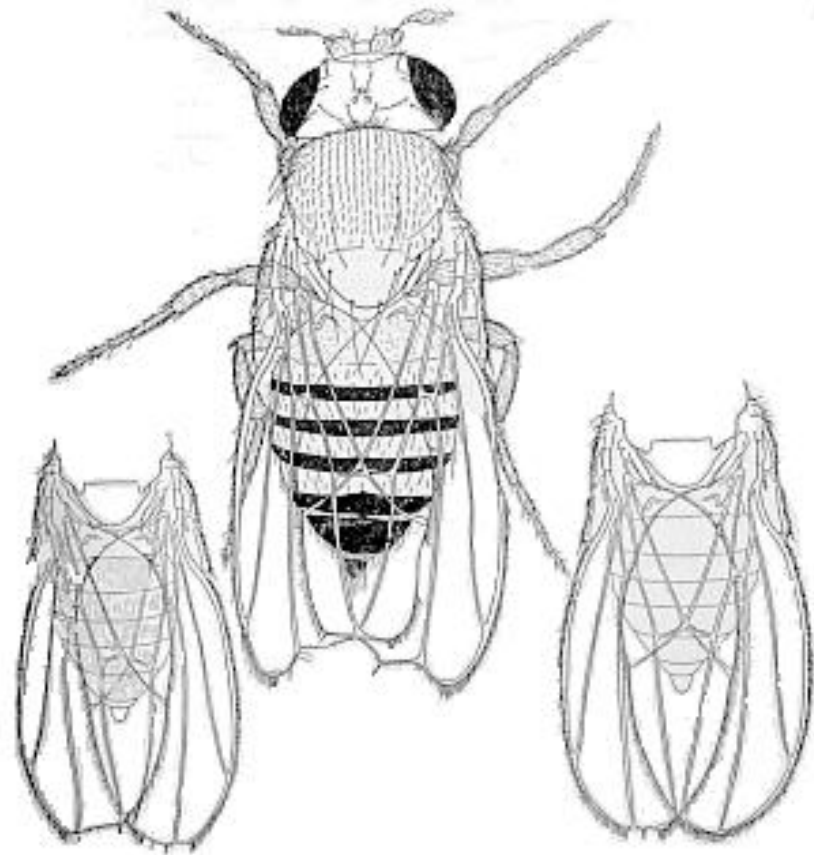


Klíčové molekulární komponenty vývoje

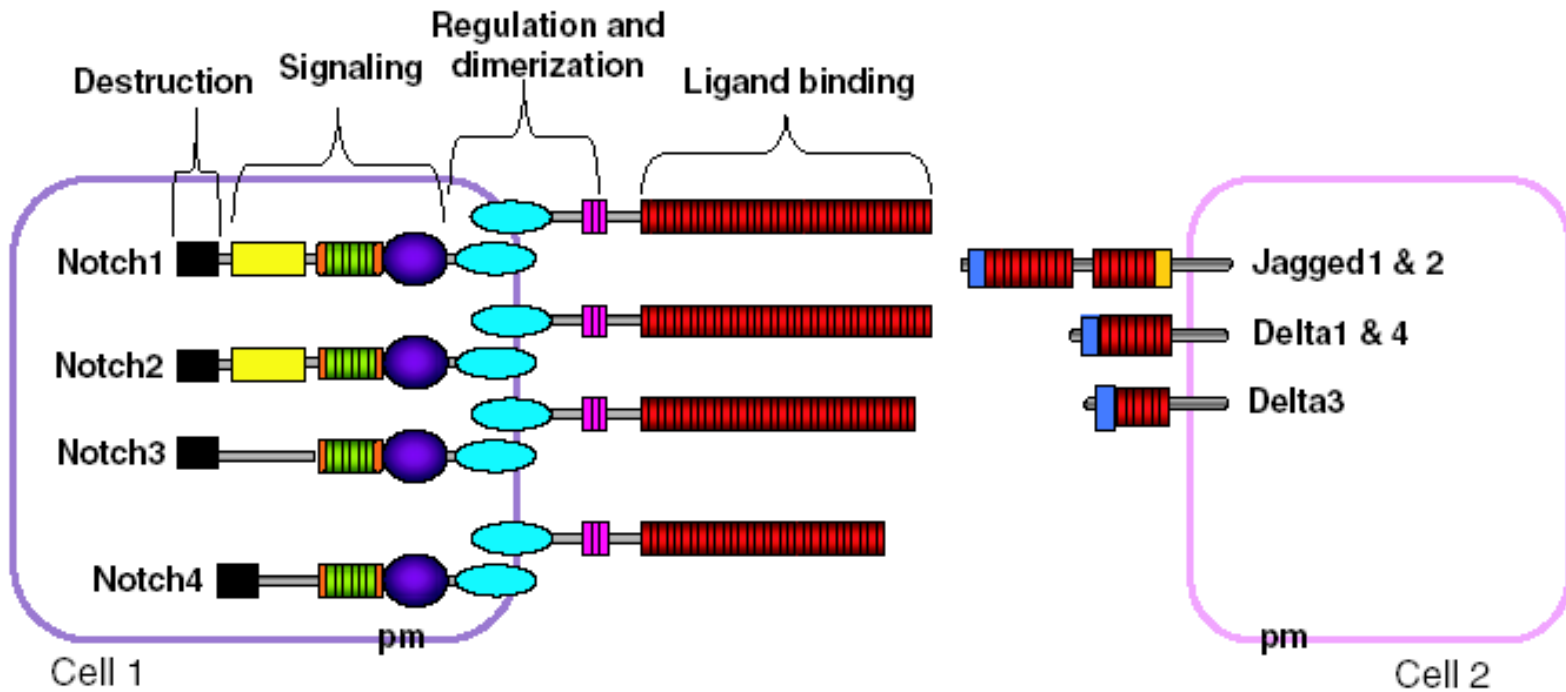


Notch

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



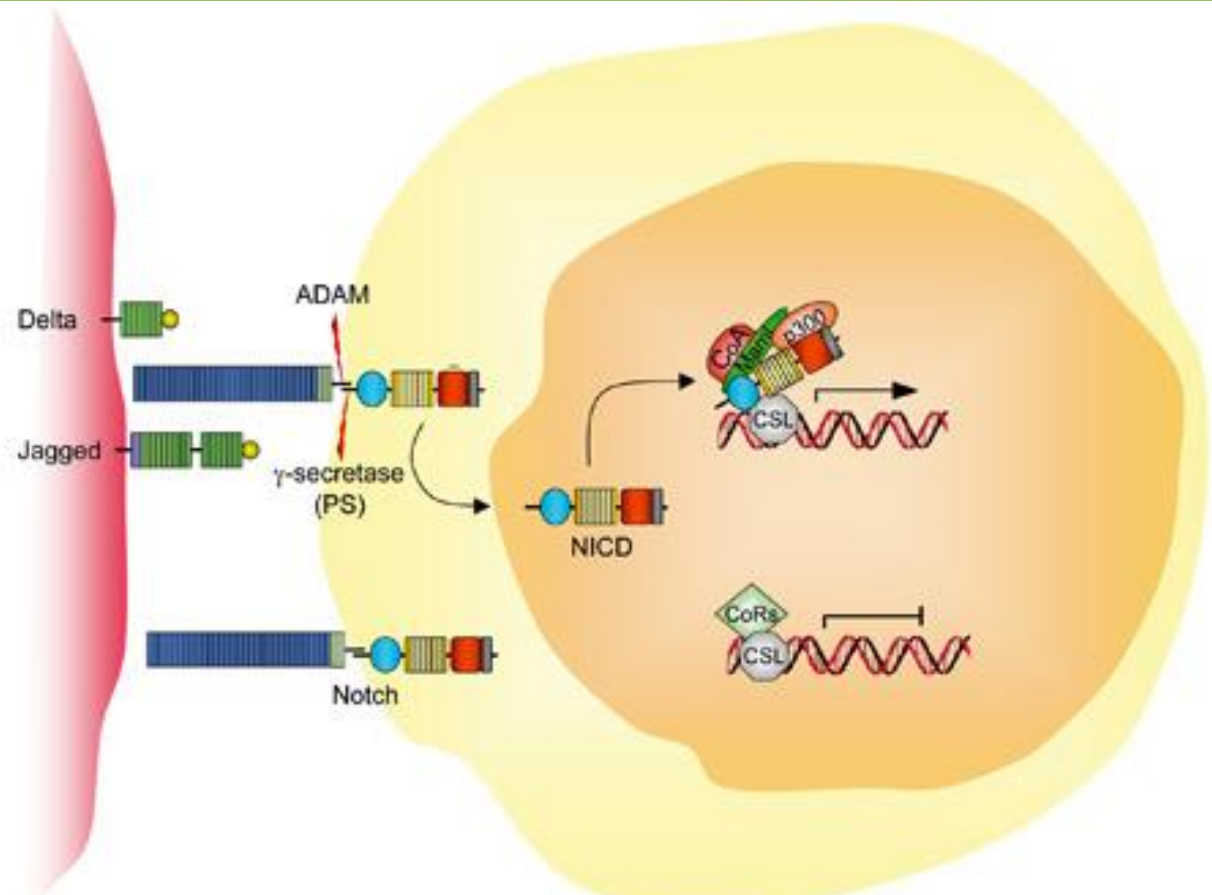
Notch



Transmembránové heterodimerické receptory Notch 1-4

Notch ligandy Jagged a Delta – jsou vázány na buněčný povrch

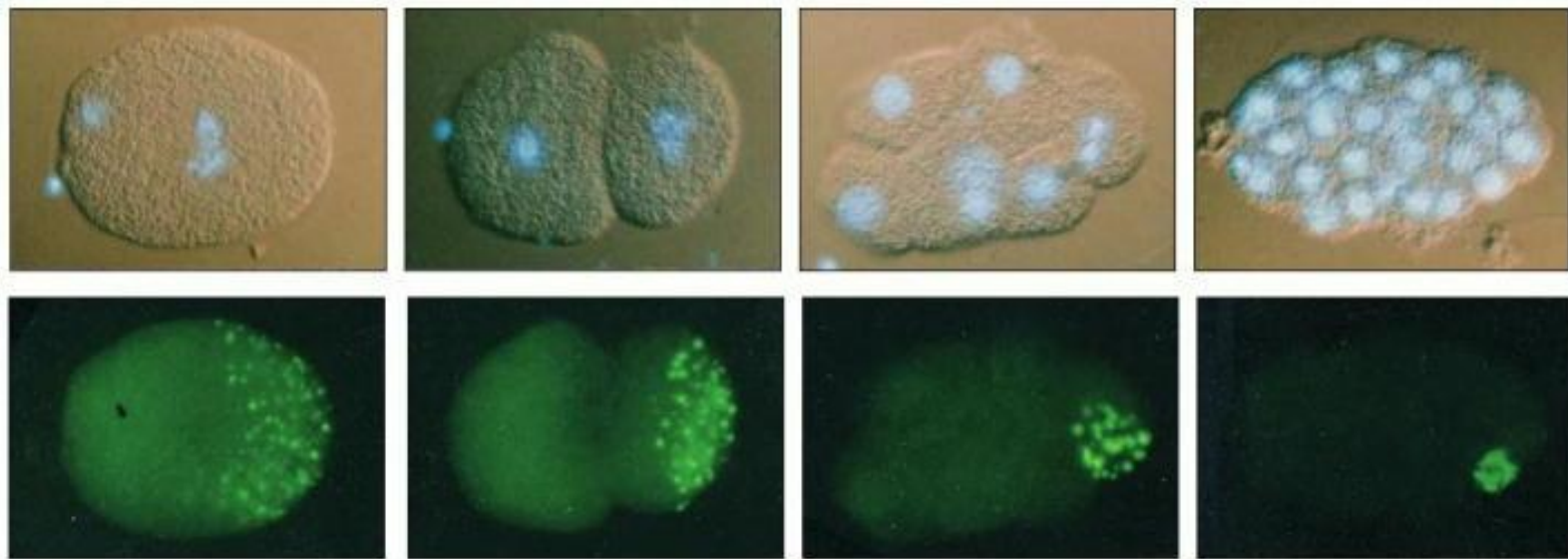
Notch dráha - overview



- Vazba ligandu Notch receptor aktivuje dvě proteázy (enzymy specificky štěpící protein)
- Proteázy jsou z rodiny ADAM a gamma-sekretáz
- Štěpení Notch těmito proteázami uvolňuje tzv. NICD (Notch intracellular domain), který se uvolňuje a do cytoplazmy a následně přesouvá do jádra
- NICD přímo interaguje s transkripčními faktory CBF1/Mastermind a spouští transkripci

Notch a vznik asymetrie

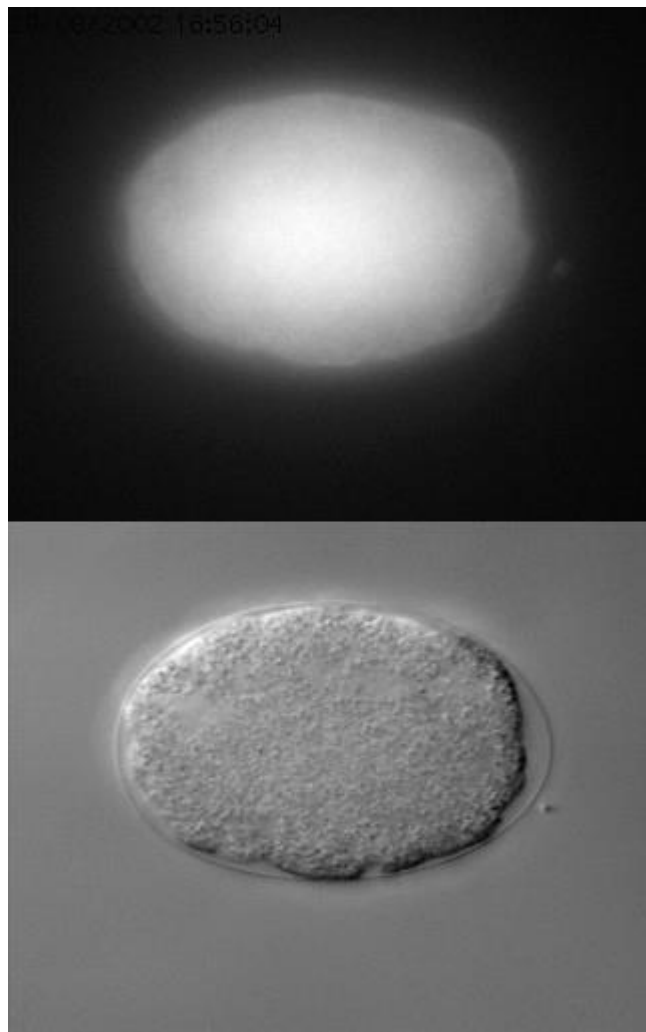
Model: První buněčná dělení na modelu háďátka (*Caenorhabditis elegans*)



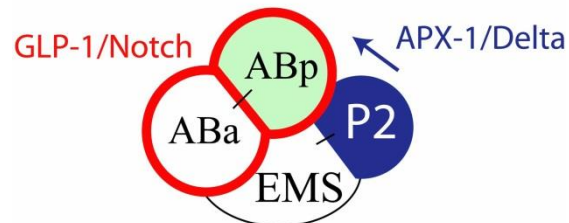
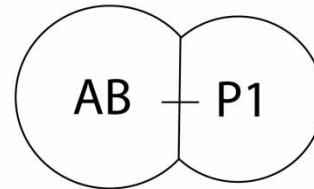
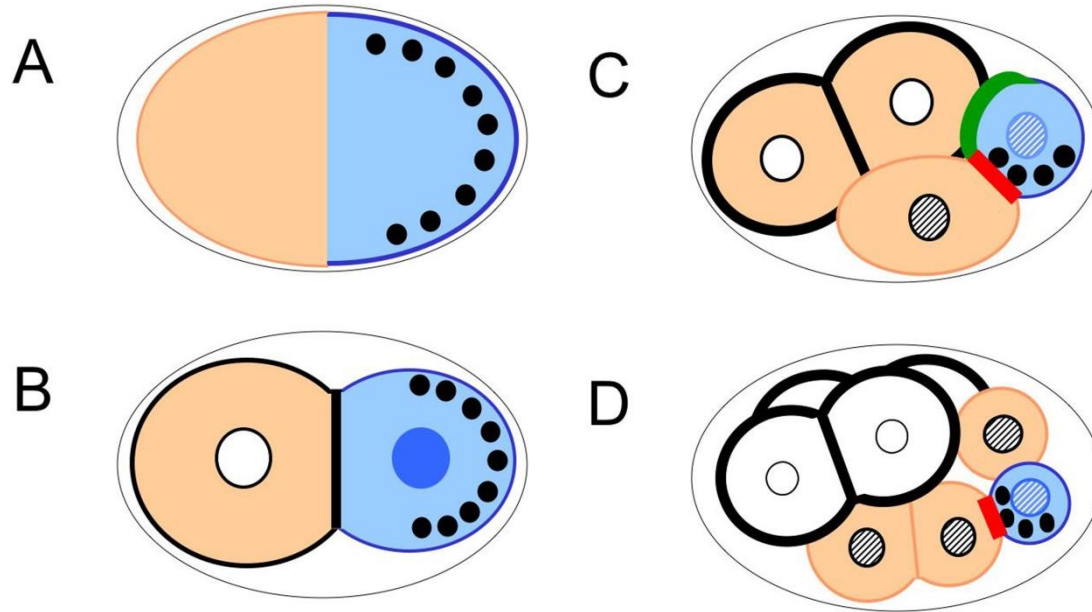
Asymmetric divisions segregating P granules into the founder cell of the *C. elegans* germ line.

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



Notch a vznik asymetrie



Notch a vznik asymetrie

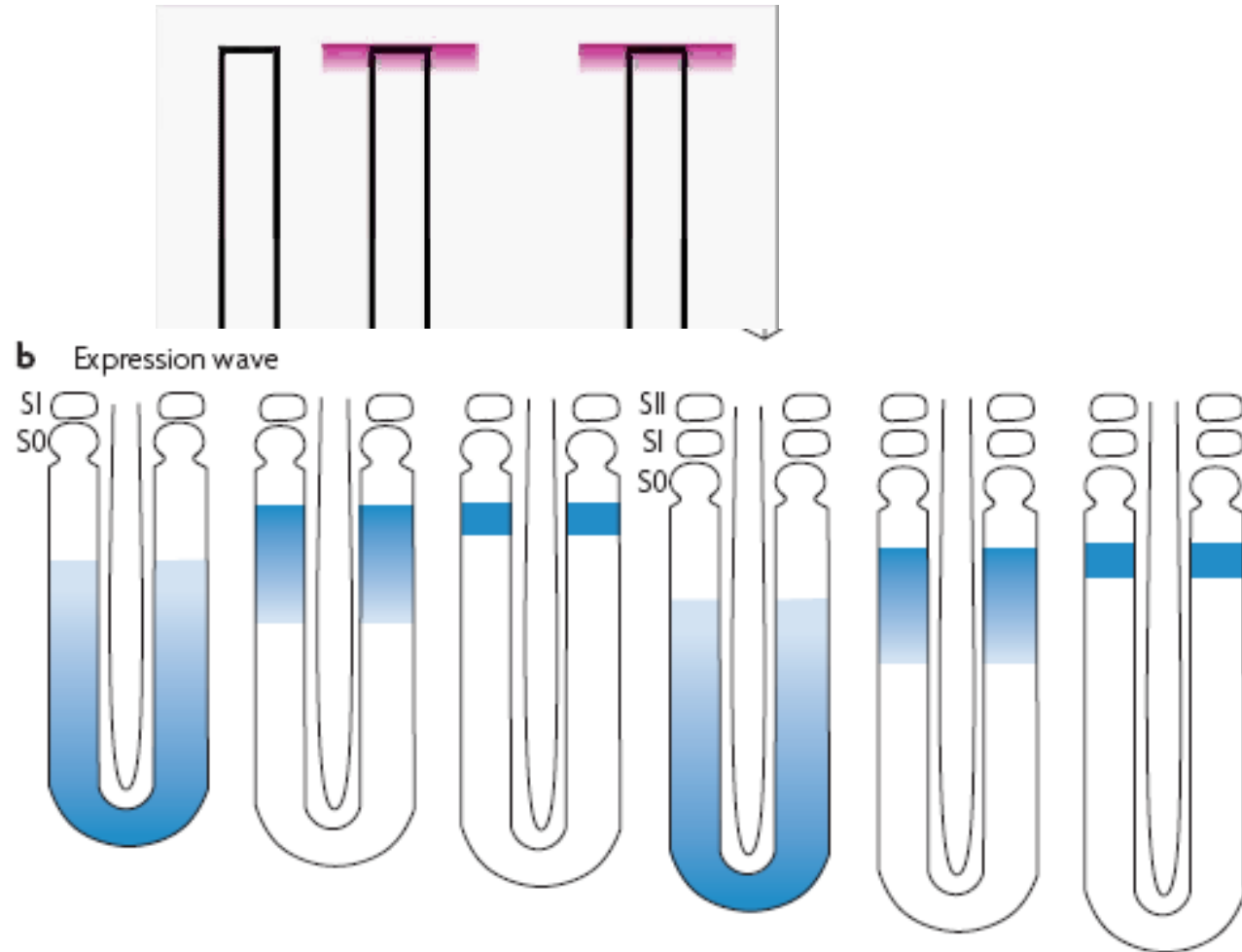
- legenda k obrázku:

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

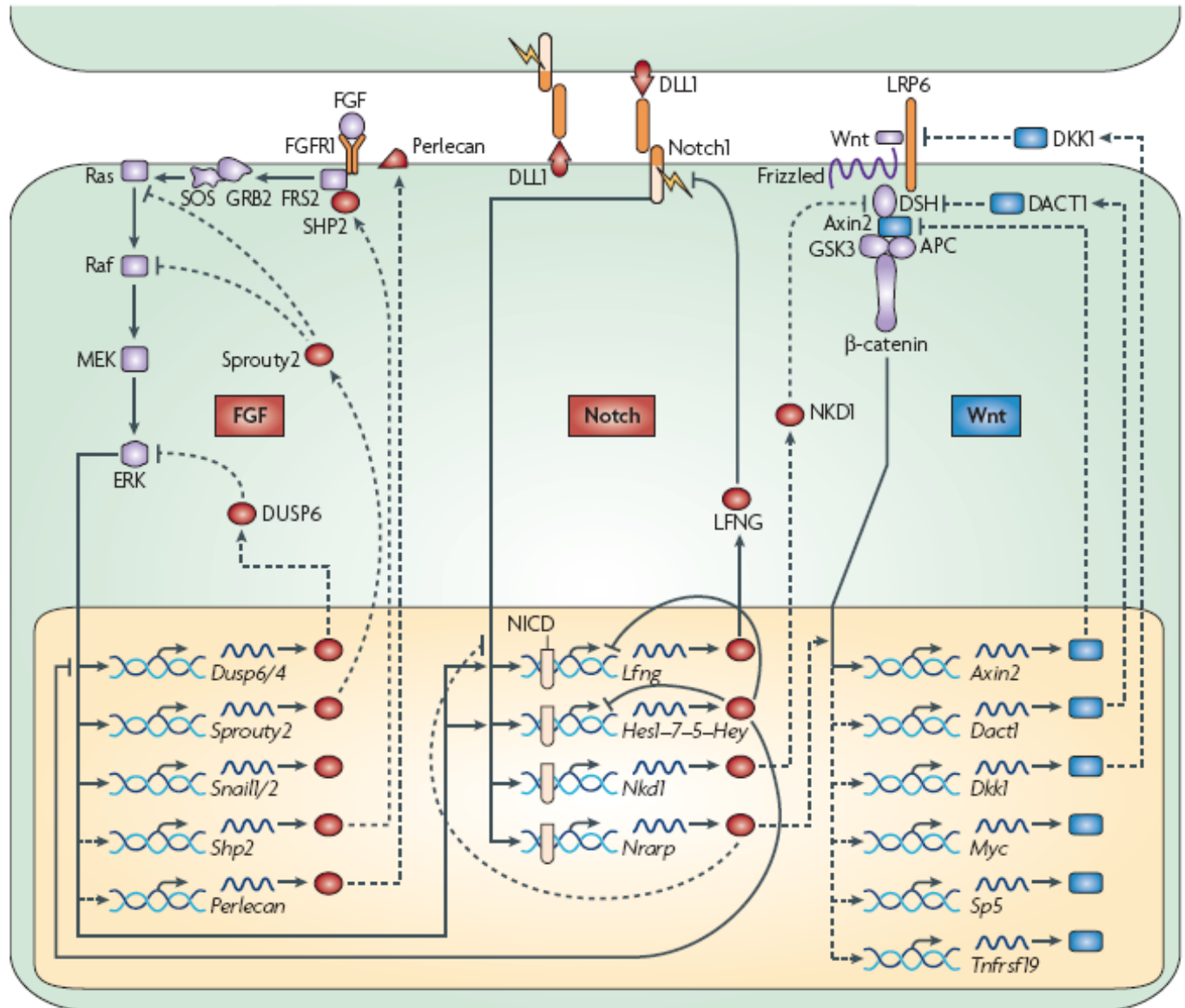


Notch a „segmentační“ hodiny



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

Notch

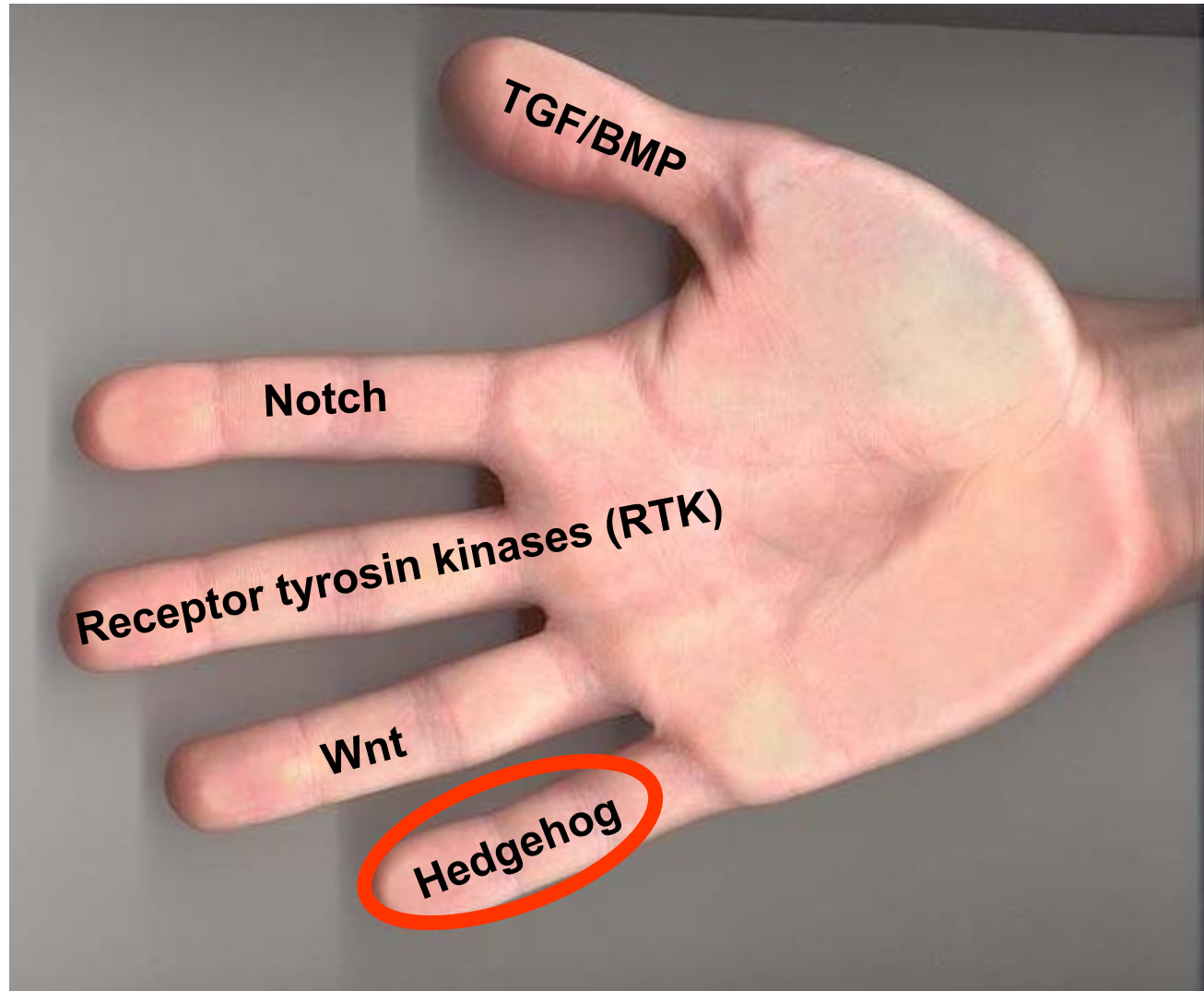


Notch

- legenda k obrázku:

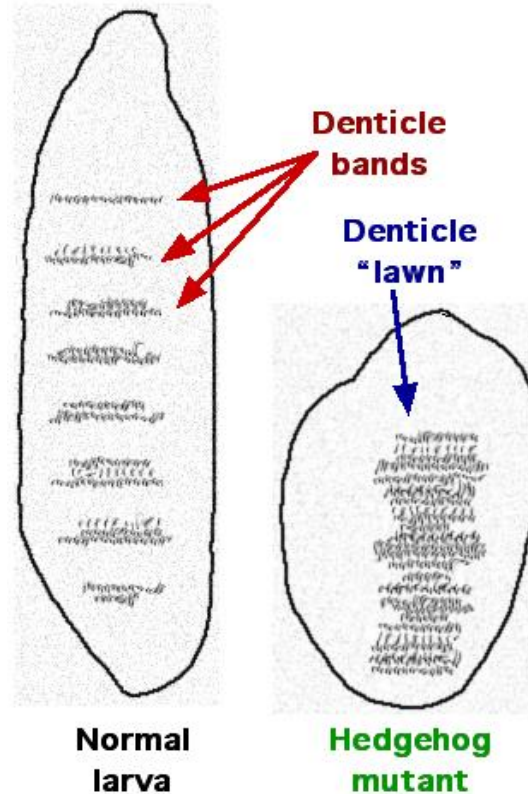
Figure 3 | **The mouse oscillator.** Cyclic genes belonging to the Notch and FGF (fibroblast growth factor) pathways (the products of which are indicated in red) oscillate in opposite phase to cyclic genes of the Wnt pathway (blue). A large number of the cyclic genes are involved in negative feedback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

Klíčové molekulární komponenty vývoje



Hedgehog dráha

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

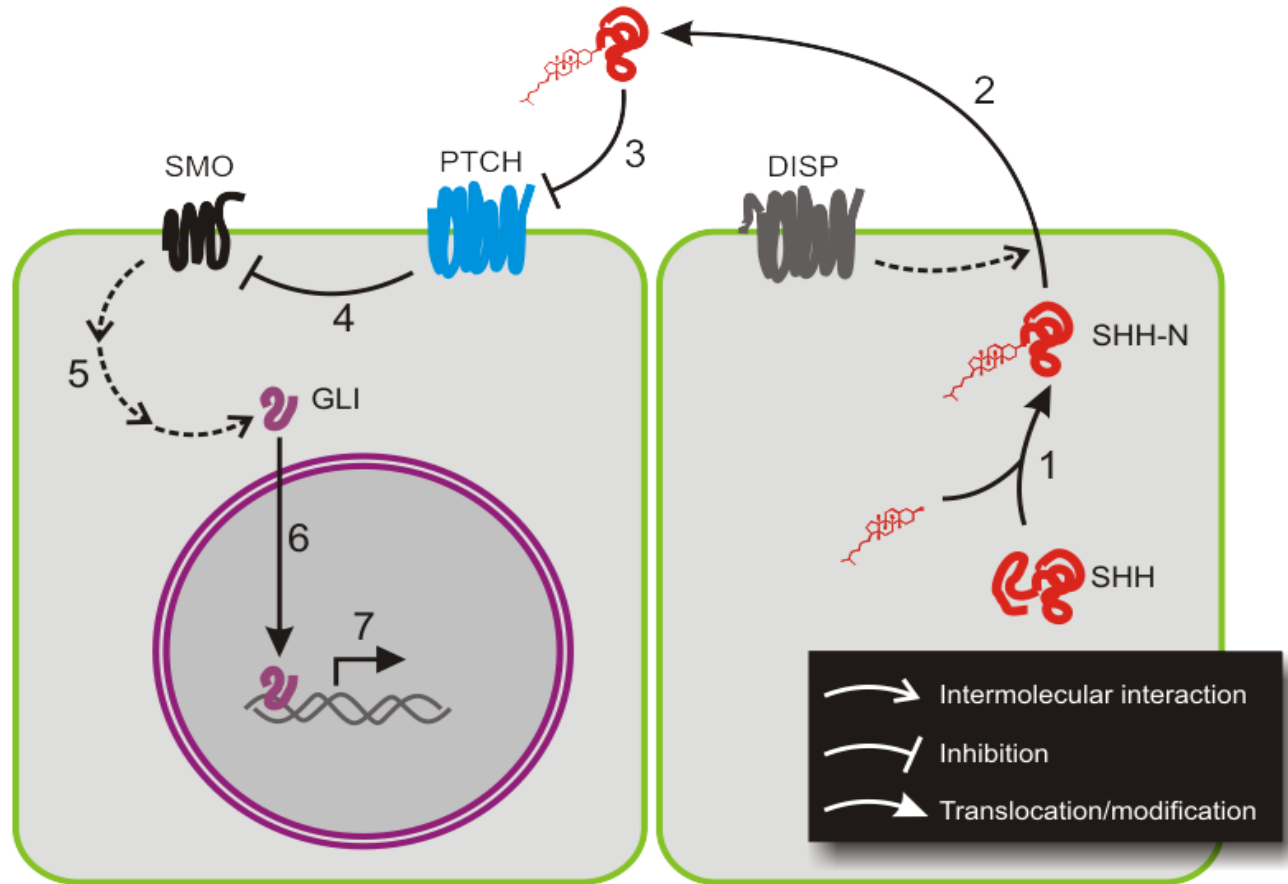


Sonic the Hedgehog

Historie přichází do Brna už tento týden!

The hedgehog gene (hh) was first identified in the fruit-fly *Drosophila melanogaster* in the classic Heidelberg screens of Christiane Nüsslein-Volhard and **Eric Wieschaus**, as published in 1980. These screens, which led to them winning the Nobel Prize in 1995 along with developmental geneticist Edward B. Lewis, identified genes that control the segmentation pattern of the *Drosophila* embryos.

Schéma Shh dráhy



- Sonic hedgehog (SHH) je modifikován oxysterolem a pro sekreci vyžaduje protein Dispatched (Disp)
- Shh váže Patched (PTCH), který je za normálních okolností inhibítozem Smoothened (SMO), po vazbě Shh je tato inhibice přerušena
- Uvolnění SMO umožňuje aktivaci transkripčních faktorů z rodiny GLI, které se přesouvají do jádra a spouští transkripci

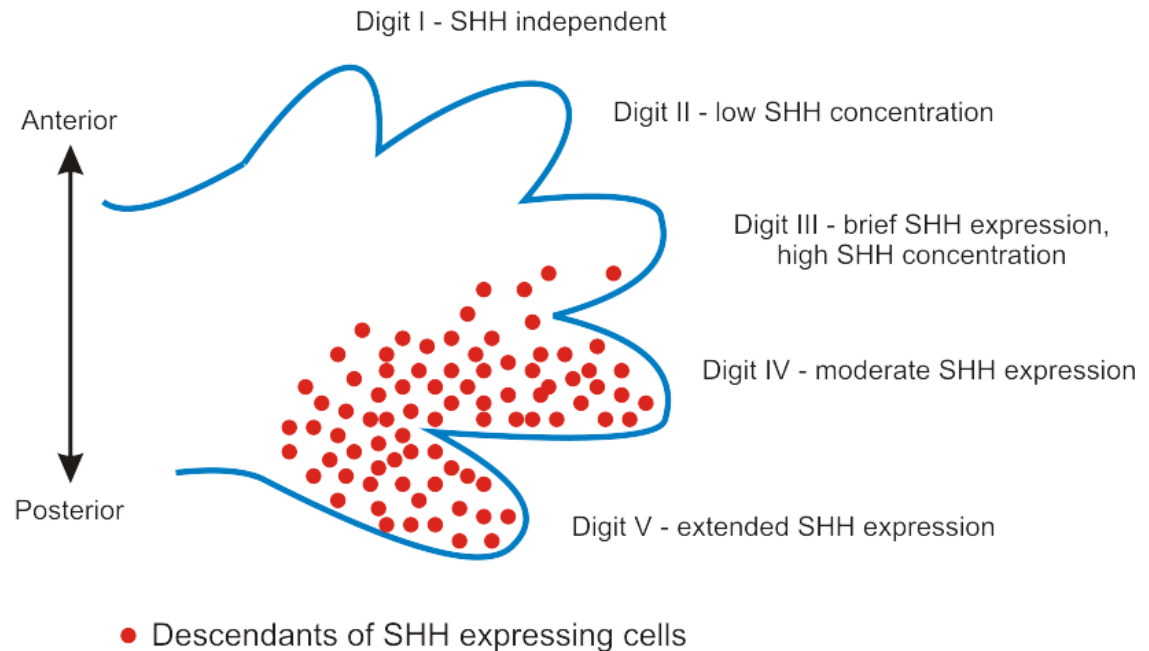
Schéma Shh dráhy

- 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 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).

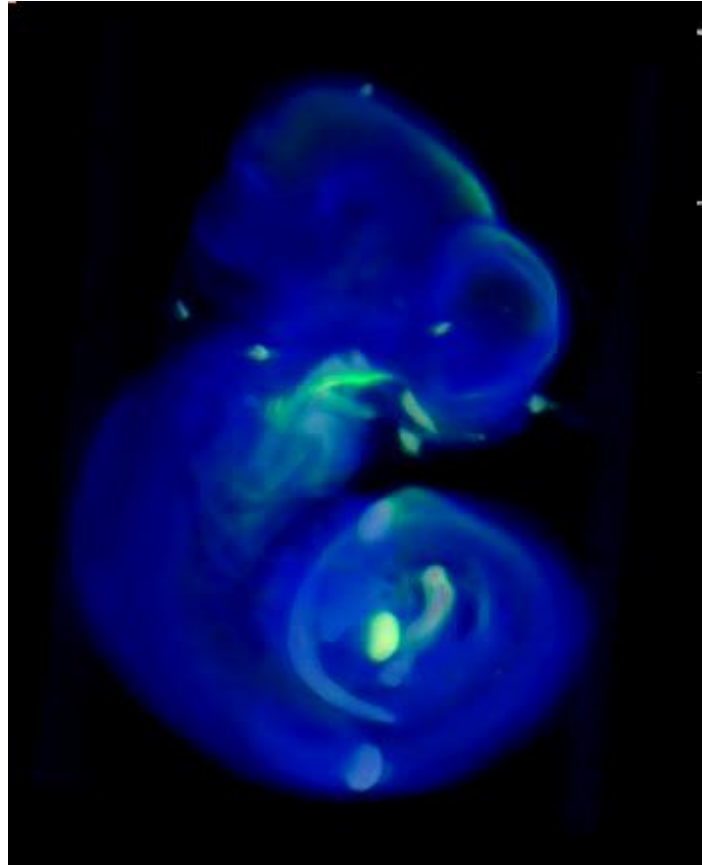
Shh

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



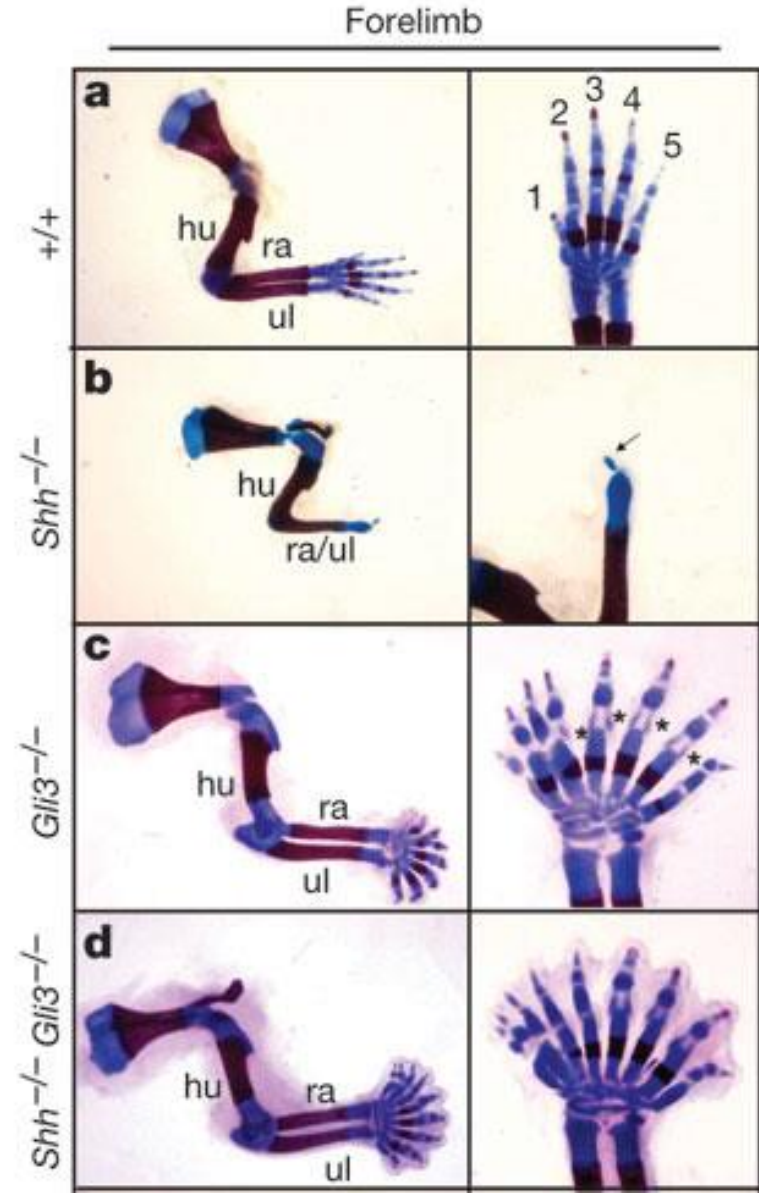
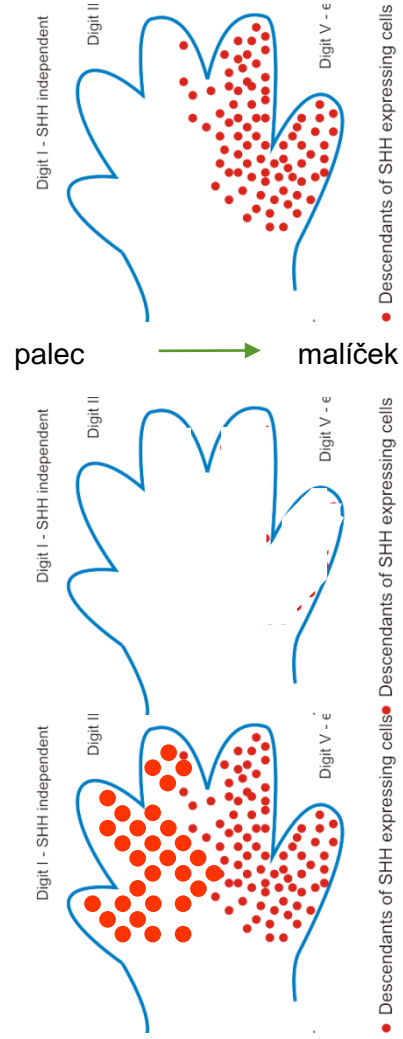
Např. specifikace jednotlivých prstů končetiny

Shh

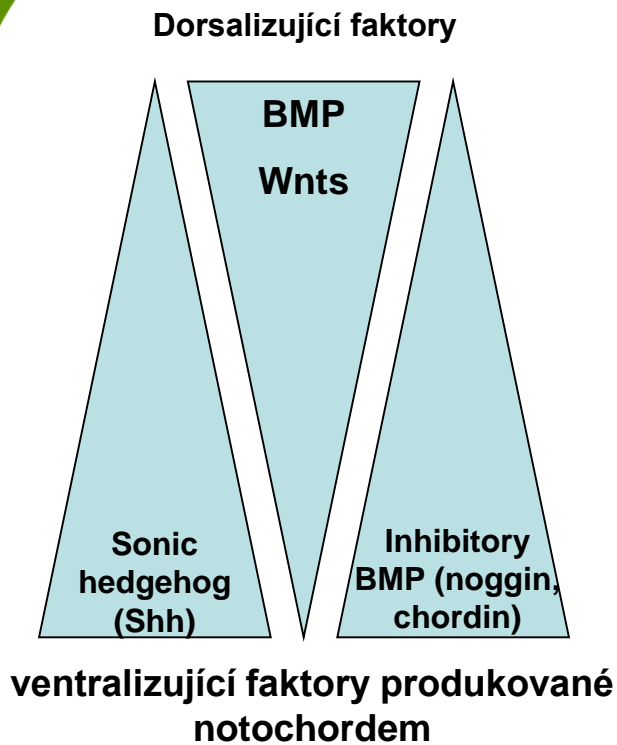


Např. specifikace jednotlivých prstů končetiny

Shh

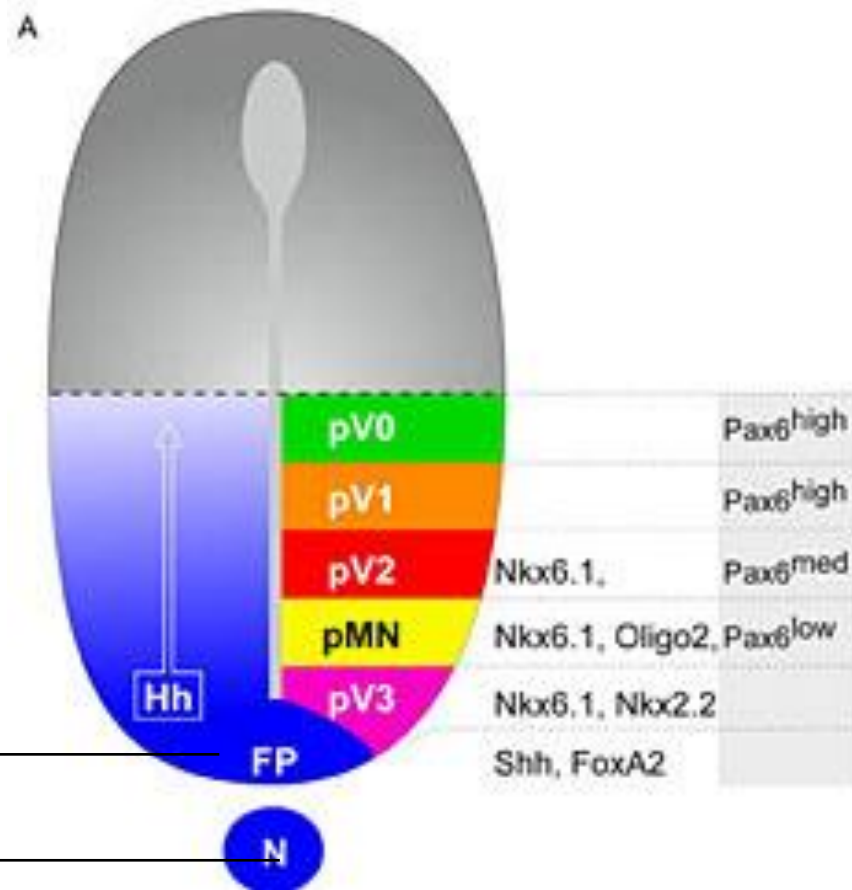


Shh



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

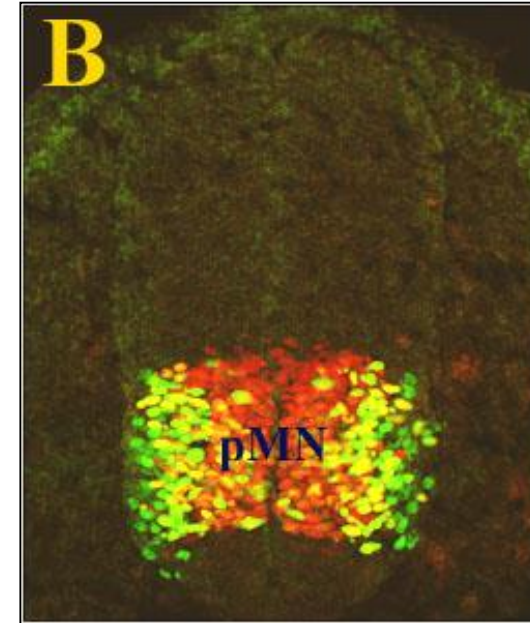
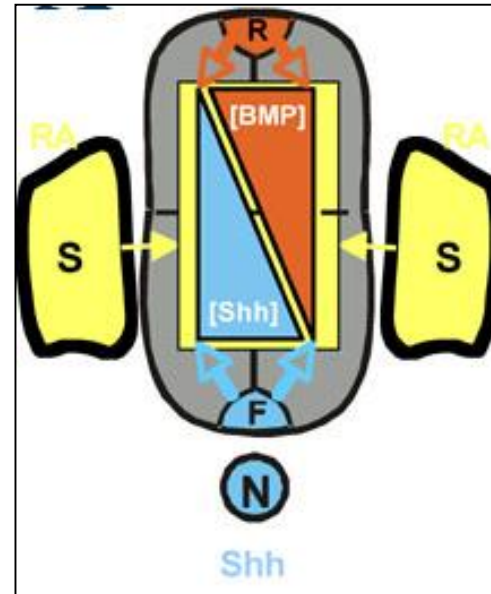
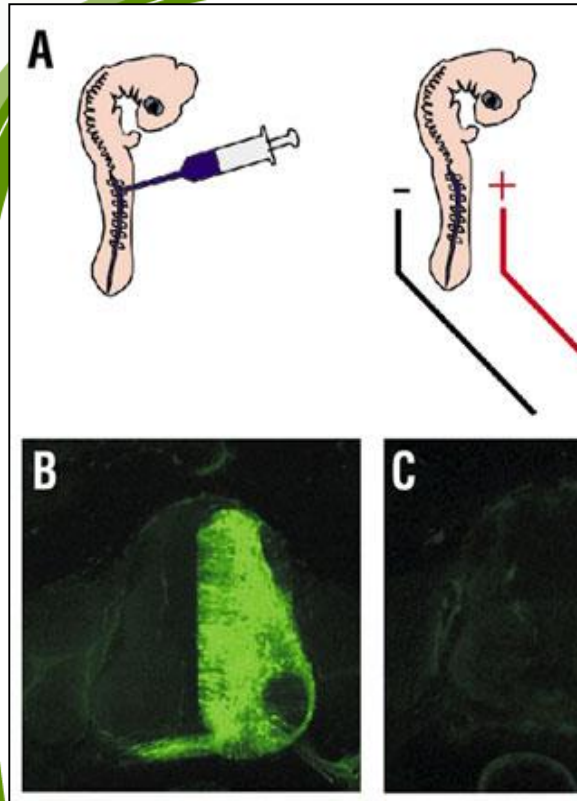
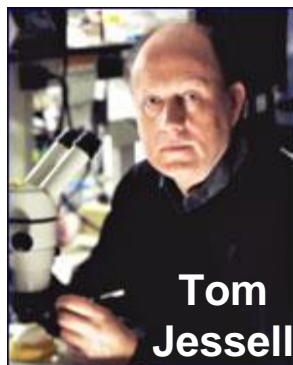


Fig. A - A model for early spinal cord development. The neural tube which will form the spinal cord is patterned into specific domains by multiple external signals which include a ventralizing Sonic Hedgehog (Shh) signal from the notochord (N) and floor plate (F), a dorsalizing BMP signal from the roof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

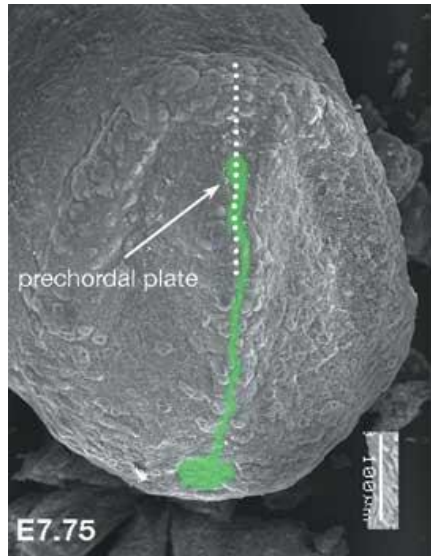


Tom Jessell

Přirozené inhibitory Shh dráhy

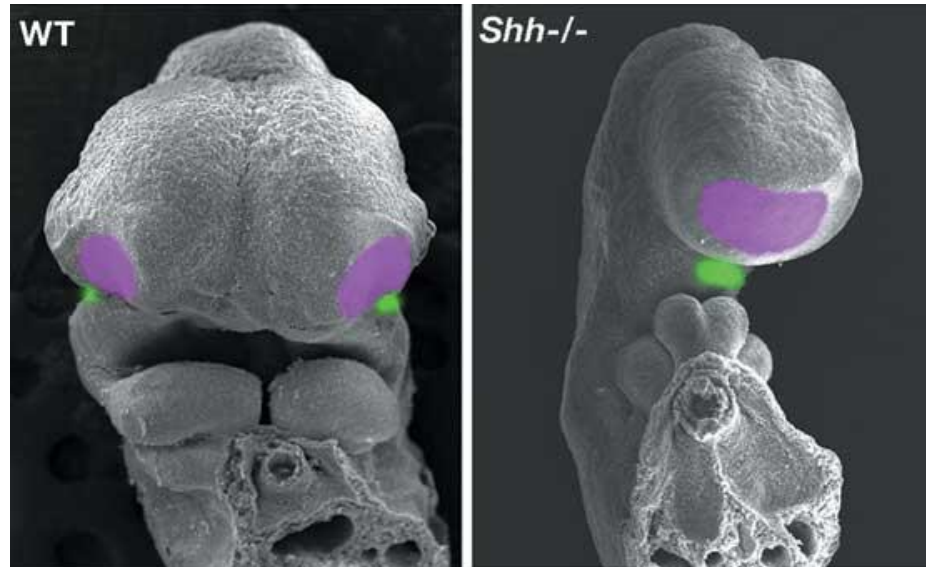


cyclopamin – teratogenní alkaloid z kýchavice (*Veratrum californicum*), poprvé identifikován jako látku způsobující cyklopii (= 1 oko) a holoprosencephalii u ovcí



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.

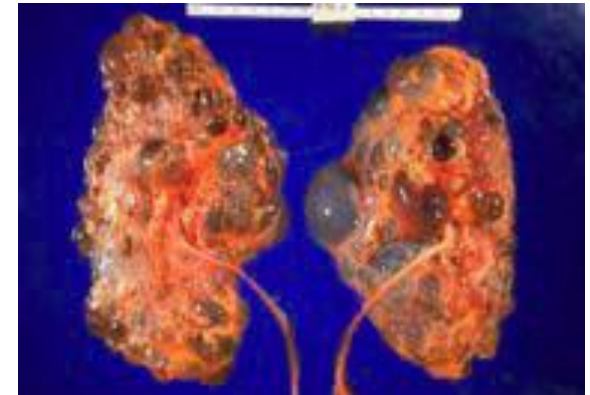


Model mice with Holoprosencephaly due to a Sonic Hedgehog (Shh) deficiency.

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.

Hedgehog (Hh) dráha je vázána na primární cilie

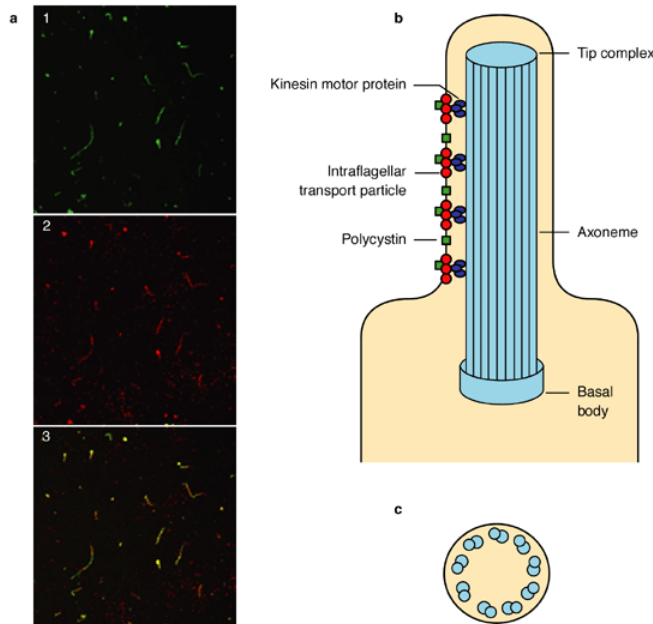
- Abnormální Hh/Wnt a s nimi spojená onemocnění jsou způsobena defekty ve tvorbě primárních cilií (infertilita, polydaktylie, polycystické ledviny, degenerace retiny).
- Hh je přímo vázán na primární cílie.



Primary cilia vs. motile (secondary) cilia

PRIMÁRNÍ

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

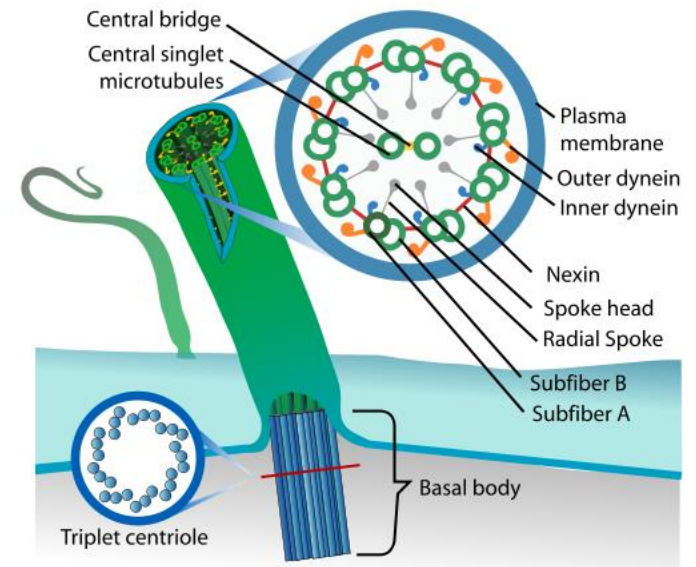


The primary cilium

Expert Reviews in Molecular Medicine © 2006 Cambridge University Press

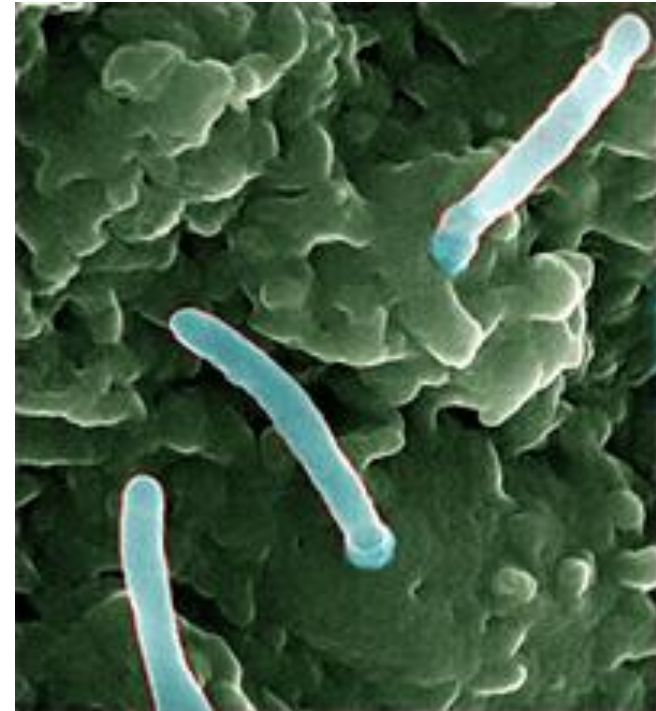
SEKUNDÁRNÍ

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



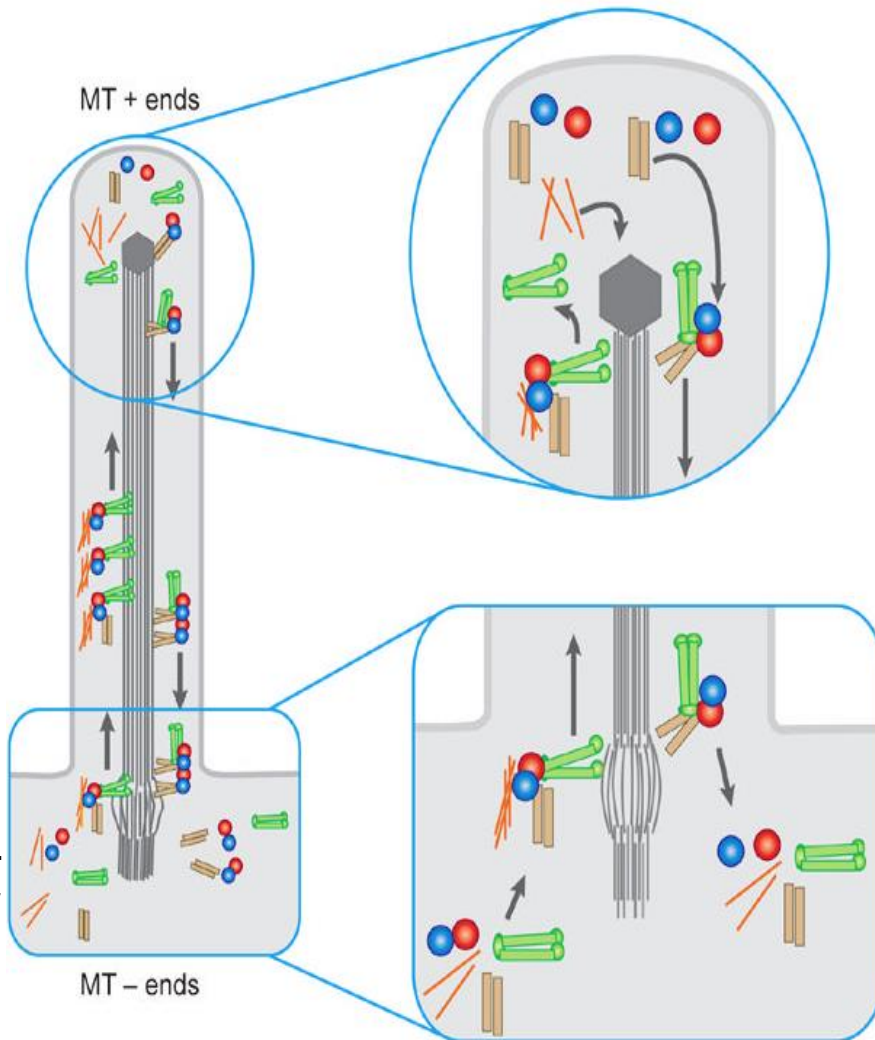
Primární cilie - funkce

- délka 2-10 μm , průměr 0.25 μm
- chemo- a osmosenzory
- fotoreceptory
- mechanoreceptory
- komunikace v extracelulární matrix
- nodální cilie
 - pohyblivá
 - blastocysta
 - pravolevá souměrnost
- model transdukce – receptory iontové kanály, efektorové proteiny, transkripční faktory



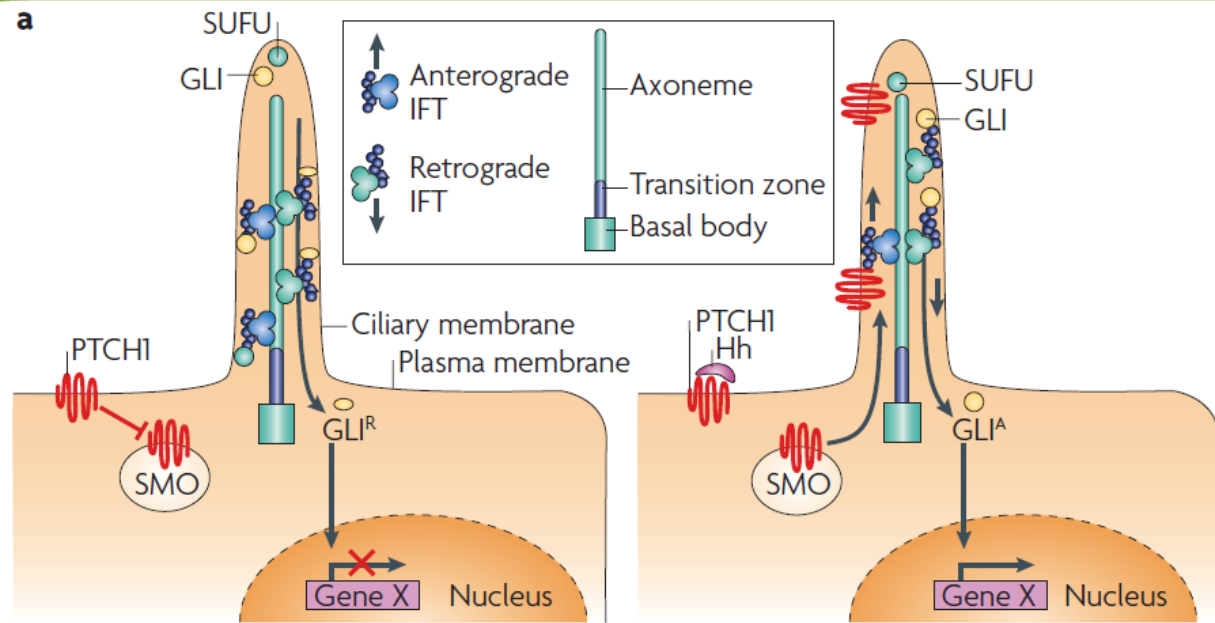
Intraflagelární transport (IFT)

- 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 cile.
- IFT je zodpovědný za regulaci signálních drah vázaných na primární cile



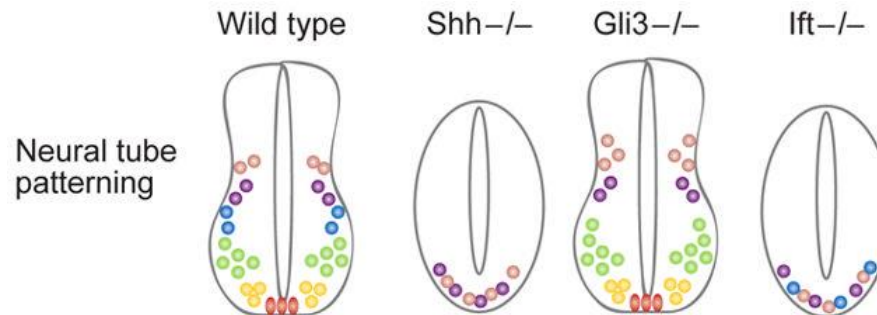
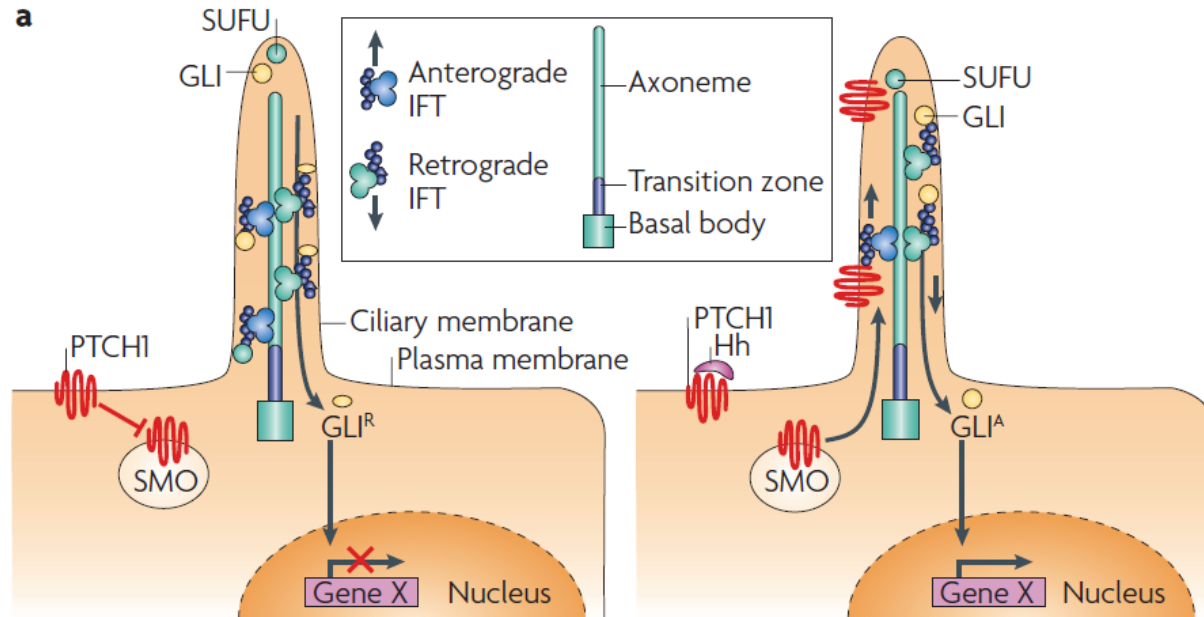
- Kinesin II
- cDynein1b
- IFT A complex
- IFT B complex
- Cargo
- Distal Tip Complex

Primární cilie a Hh signalizace



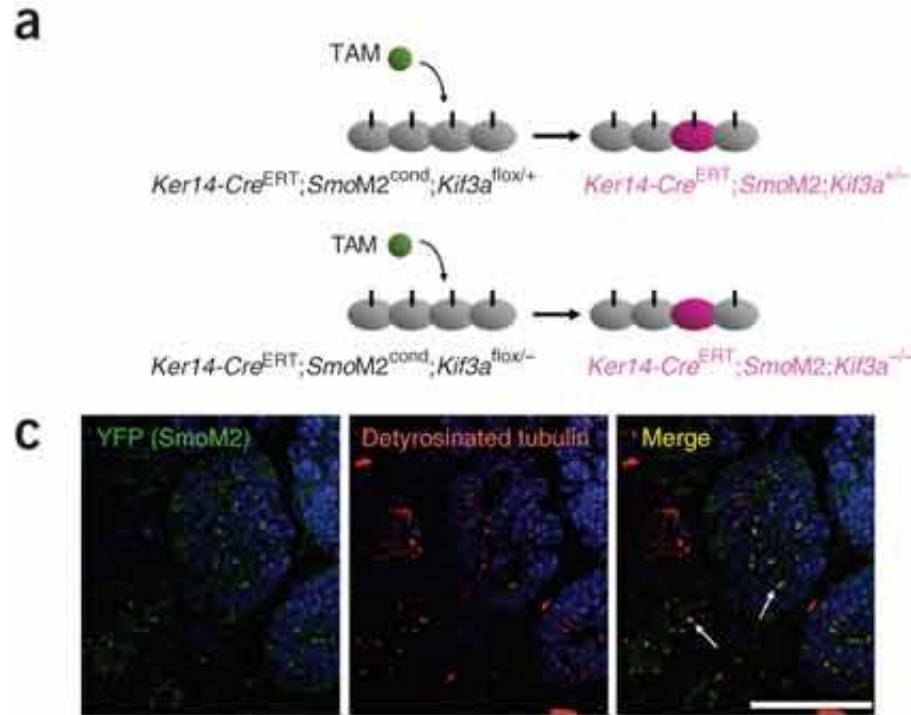
- Je spojen s primárními ciliemi
- Ligand se naváže na patch (Ptc) protein, což způsobí zrušení inhibičního efektu Ptc na protein smoothened (Smo), který transdukuje signál přes glioma transkripční faktory (Gli) do jádra, kde řídí expresi Hh genů. (Gli1, Gli2 a Gli3A jsou aktivátory a Gli3R je represor). 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.

Primární cilie a Hh signalizace



Primární cilie a Hh signalizace: důkaz

Basal cell carcinoma – způsobena aktivací Smoothened

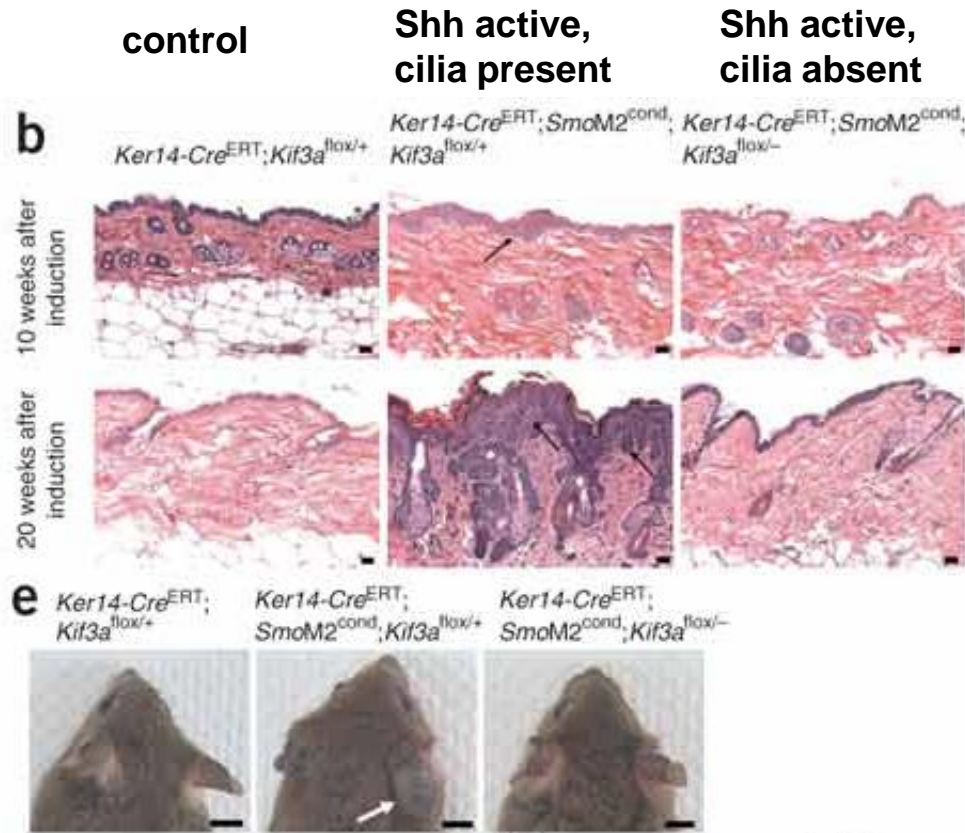


Ker14-Cre: drives expression to the epidermis

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

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

Primární cilie a Hh signalizace: důkaz



Ker14-Cre: drives expression to the epidermis

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Klíčové molekulární komponenty vývoje

