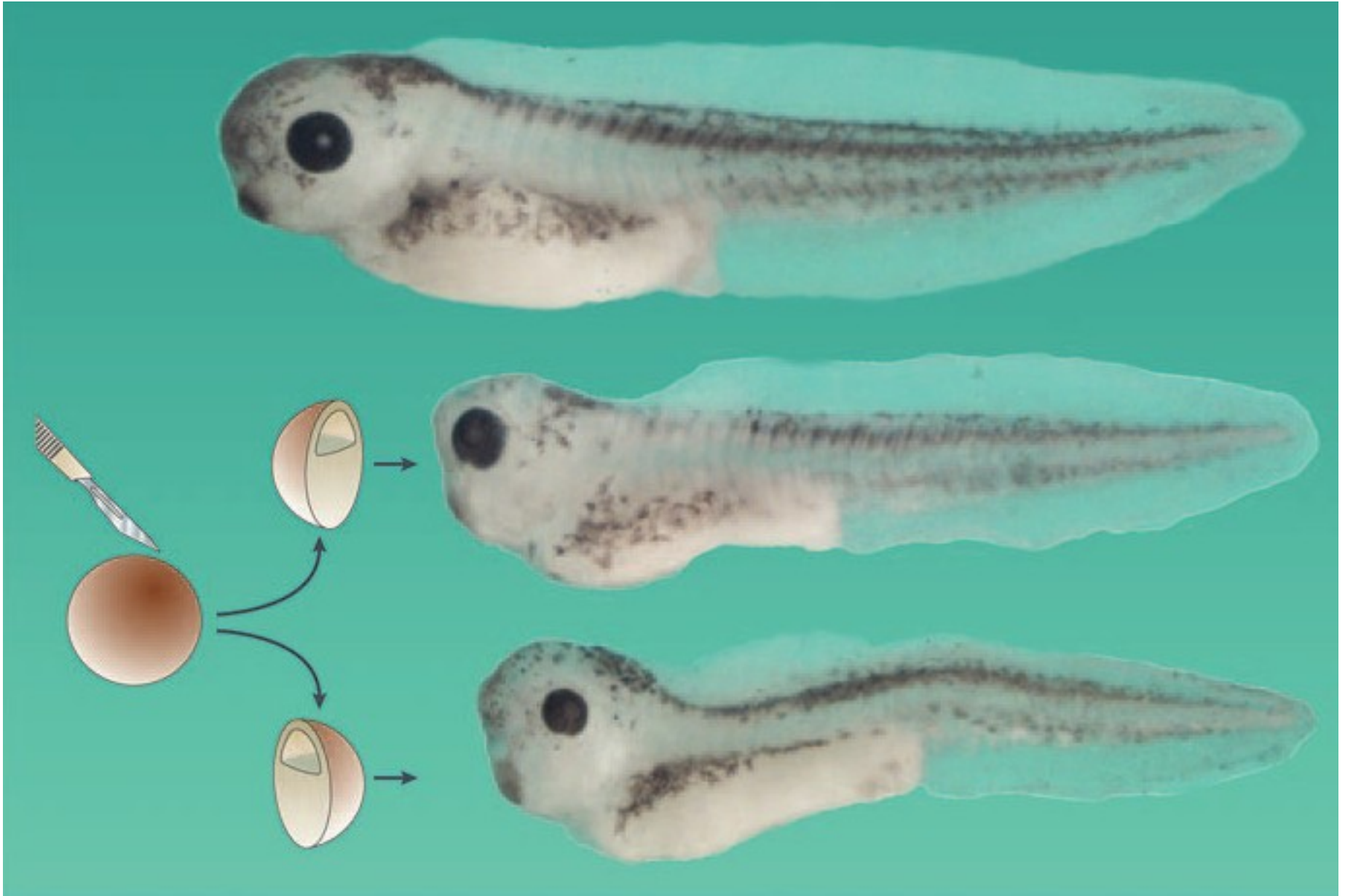


#6

Klíčové molekulární komponenty  
vývoje



# Co poskytuje buňce tyto informace?

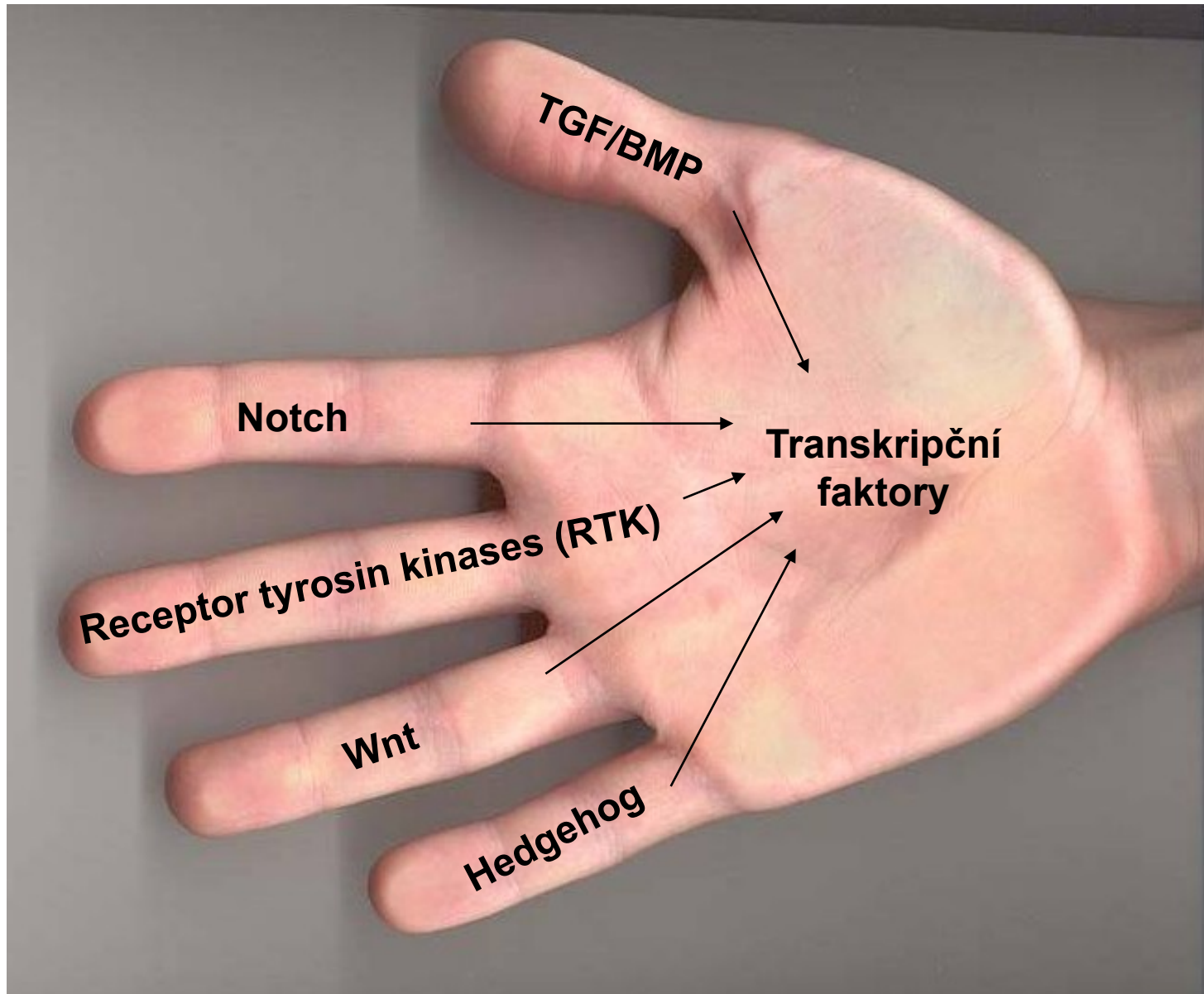
a) signály z okolního prostředí

jednotlivé  
signální dráhy  
modulují  
transkripci a  
strukturu  
chromatinu

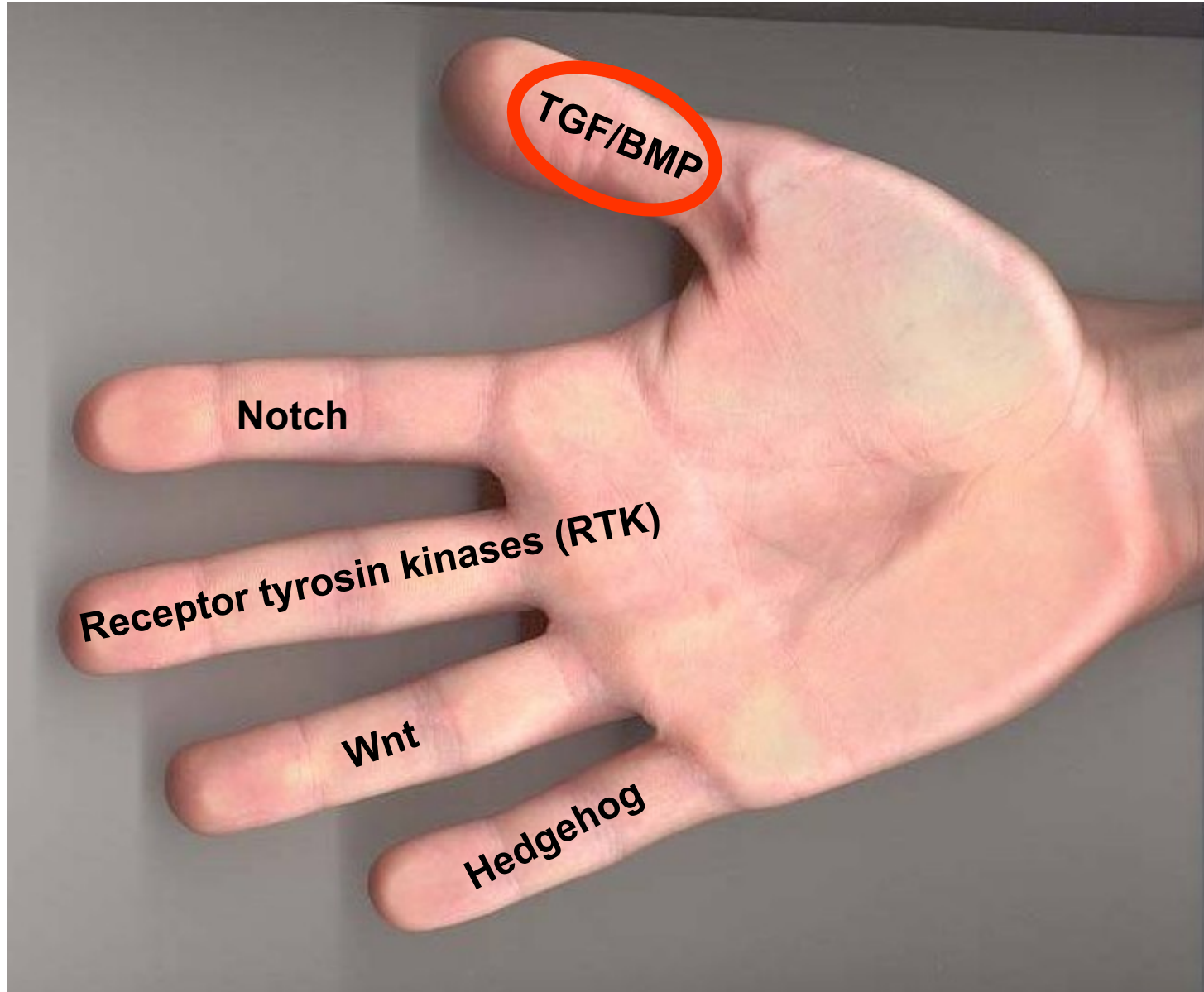
transkripce určuje  
citlivost buňky k  
vnějším signálům  
(např. regulací  
exprese receptorů či  
komponent přenosu  
signálu)

b) transkripční program v jádře

# Signály z vnějšího prostředí



# Signály z vnějšího prostředí



# TGF/BMP

- TGF – transforming growth factor
- BMP – bone morphogenetic protein
  
- patří do TGF $\beta$  nadrodiny

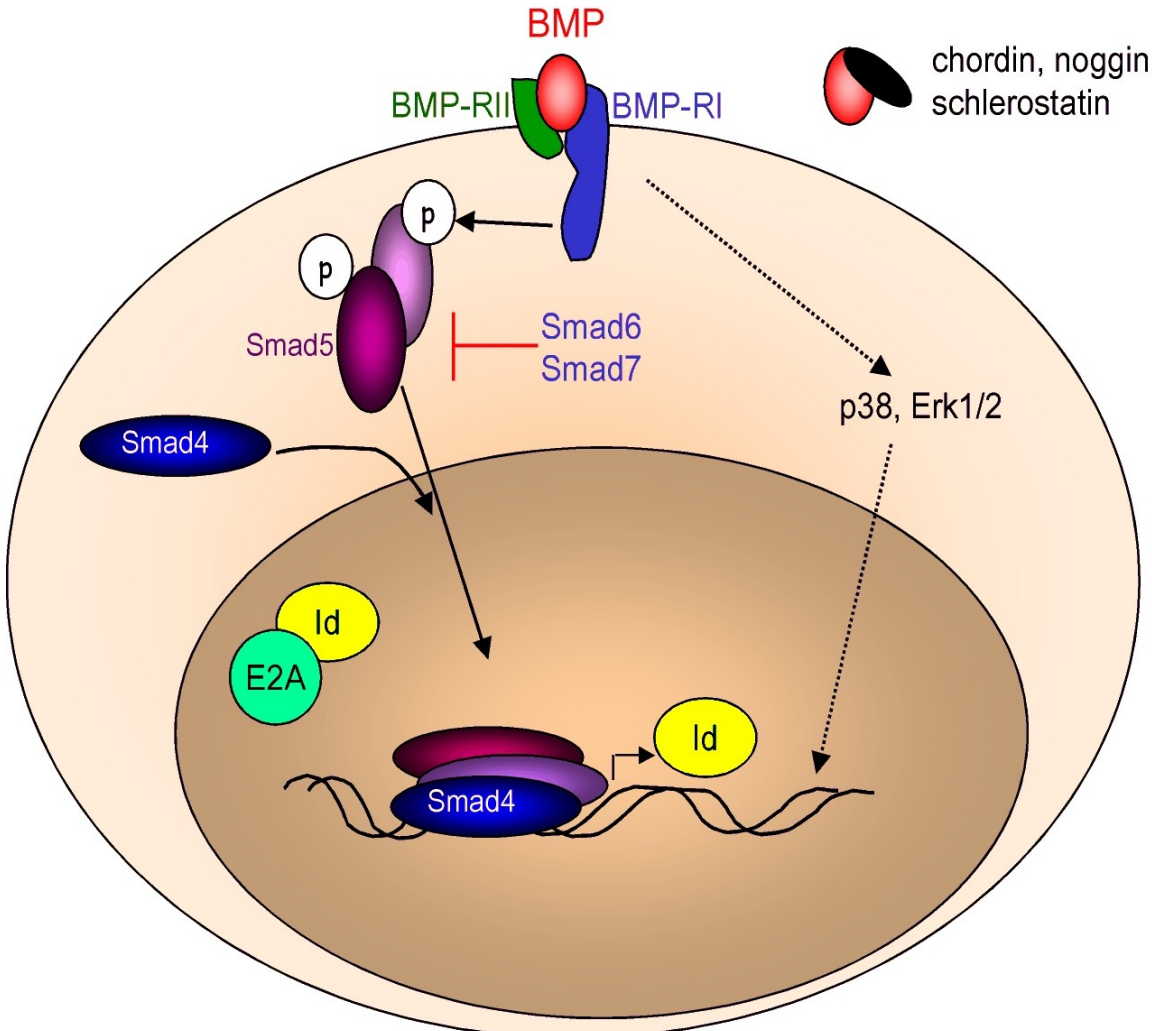
# TGF $\beta$ nadrodina má následující podrodiny:

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

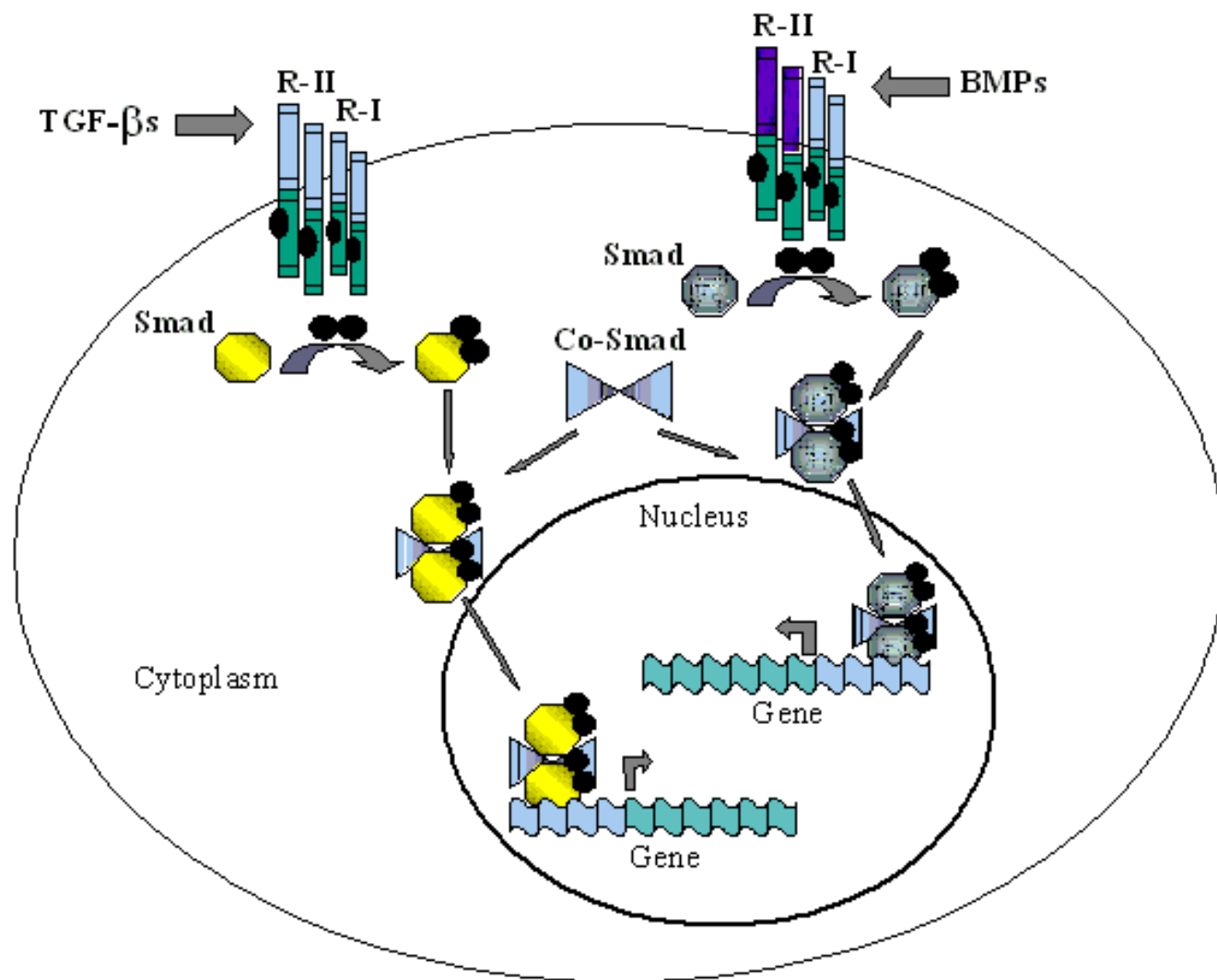
Společným znakem je signalizace přes:

- konzervativní rodinu Ser/Thr kinázových receptorů – jsou dvou typů a po vazbě ligandu dimerizují
- cytoplazmatická signalizace přes tzv. SMAD proteiny

# BMP signaling pathway



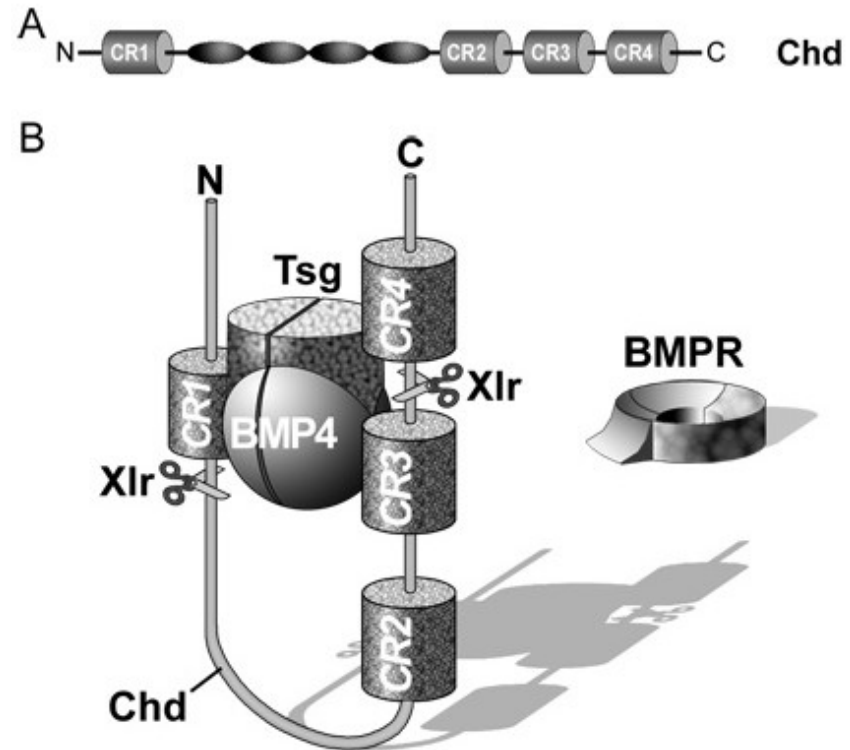




# Inhibitory BMP faktorů,

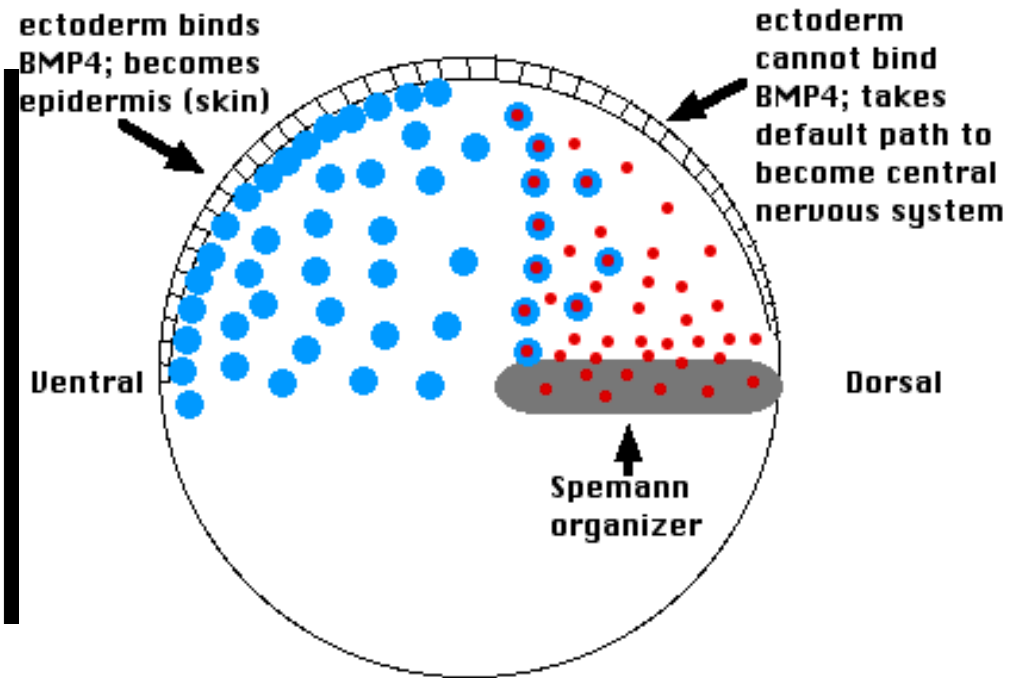
jsou klíčové pro fyziologické funkce BMP.

- noggin
- chordin (Chd)
- sklerostin



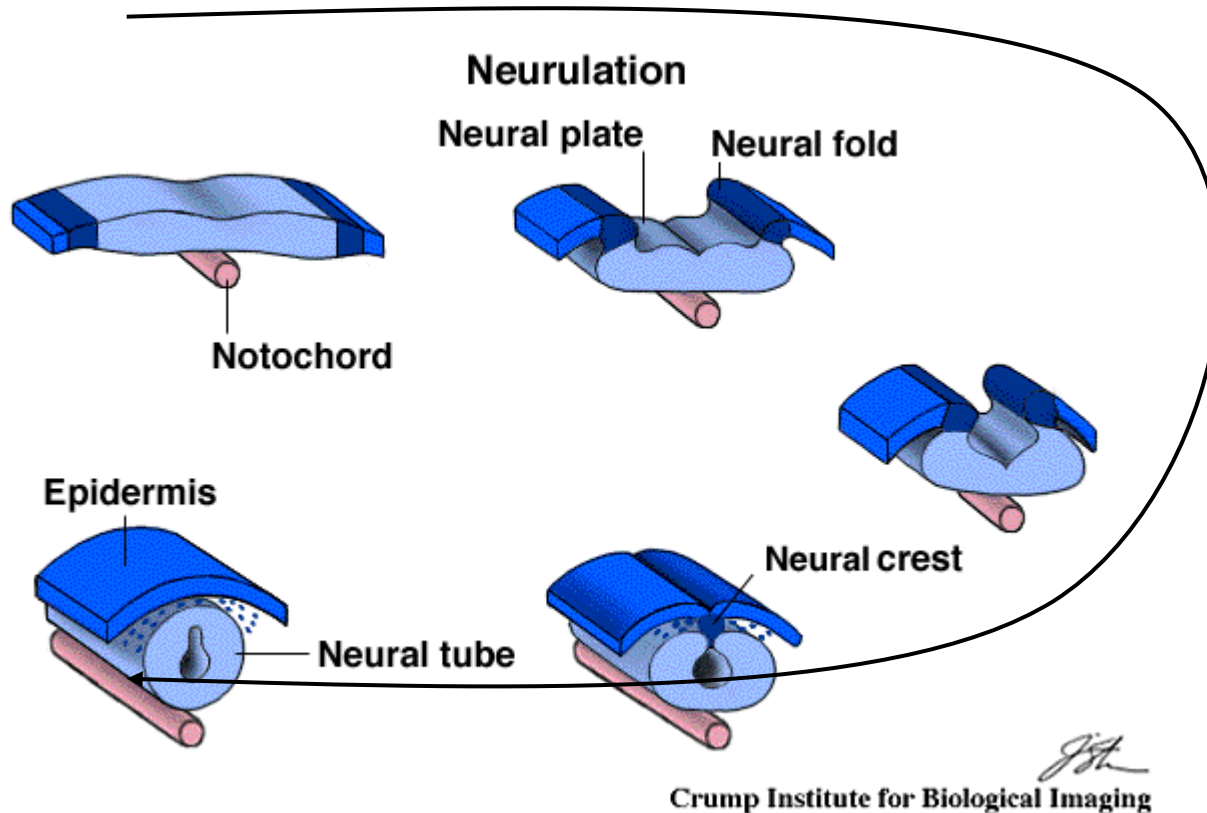
Přímá fyzická interakce mezi chordinem a BMP je podstatou inhibičního působení chordinu

# Role BMP inhibitorů v Spemannově organizátoru



- = BMP4
- = chordin, noggin, others
- = inactive complexes

# Klíčová role BMP inhibitorů produkovaných notochordem při indukci nervové ploténky



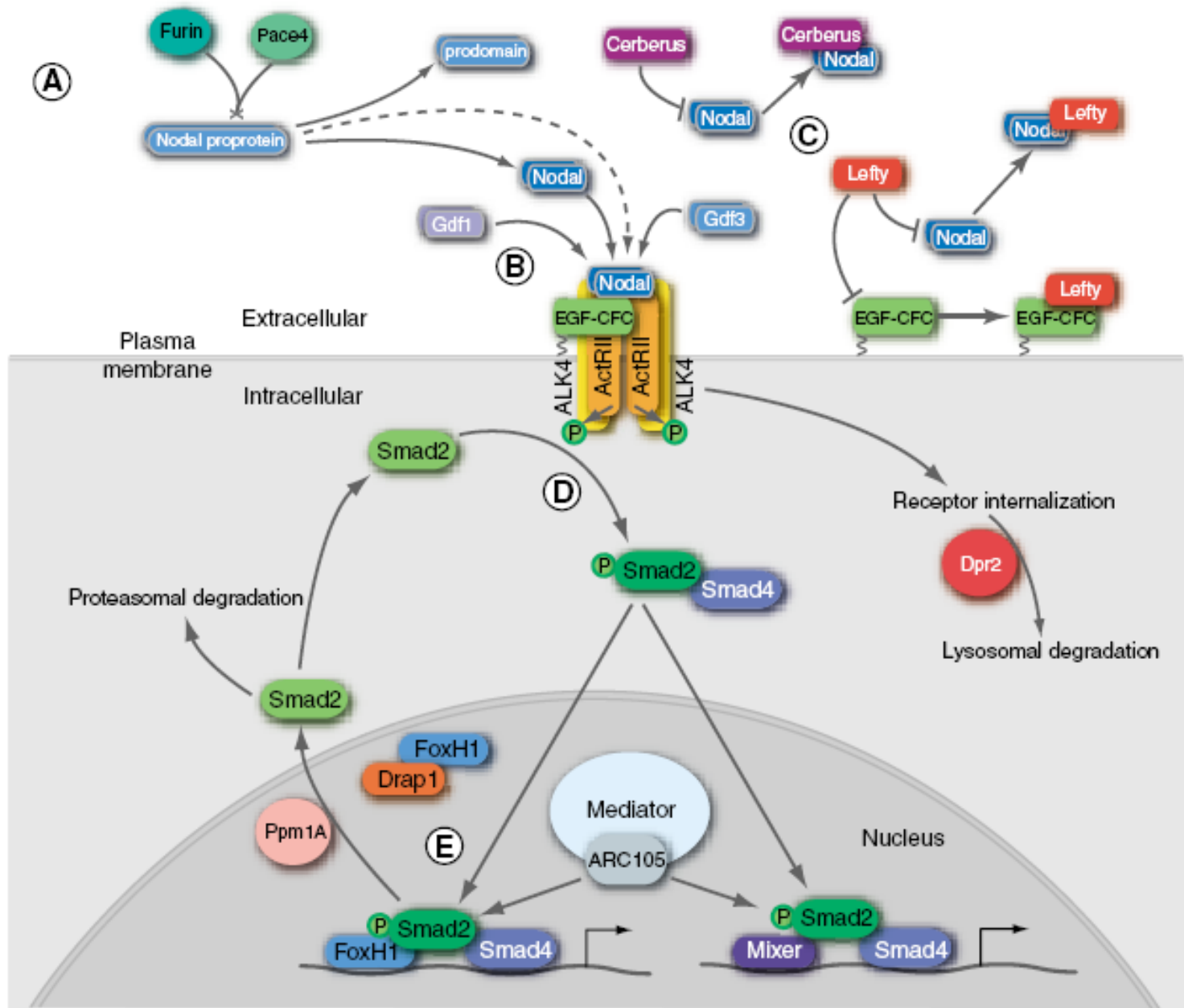
**notochord** (= chorda) produkuje faktory, které specifikují ektoderm a vedou ke tvorbě nervové ploténky (neural plate). Jde zejména o následující faktory: **noggin**, **chordin** a **follistatin** (inhibitory BMP a aktivinu). Samotná produkce těchto BMP inhibitorů specifikuje anteriorní (přední) nervovou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

# Nodal

**Table 1. Key components of the Nodal signaling pathway**

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

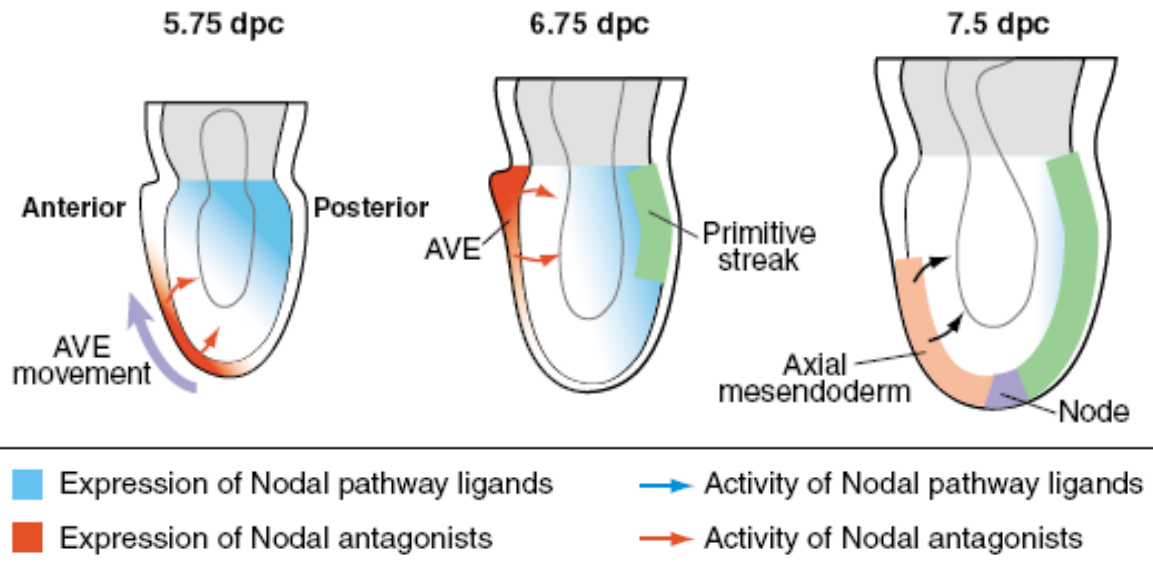
pool  
inaktivního  
proproteinu



# Nodal and left-right asymmetry

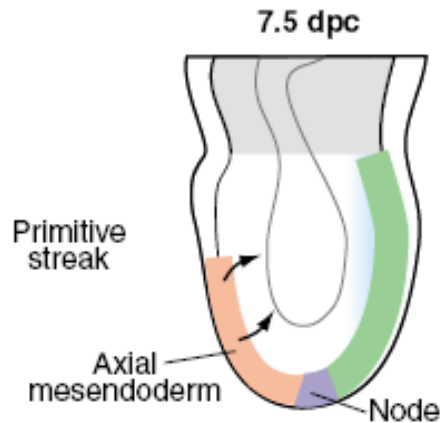
mouse gastrulation

AVE – anterior visceral endoderm



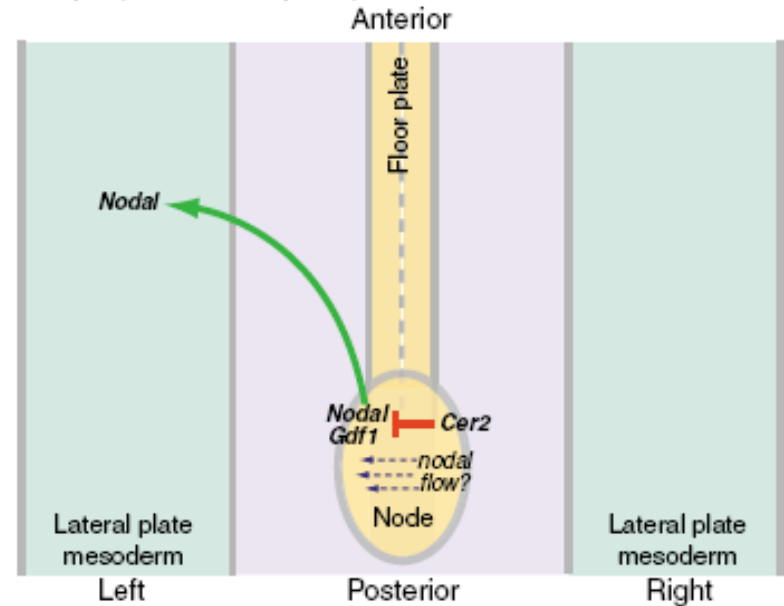
Movie16\_1.mov

# Nodal and left-right asymmetry

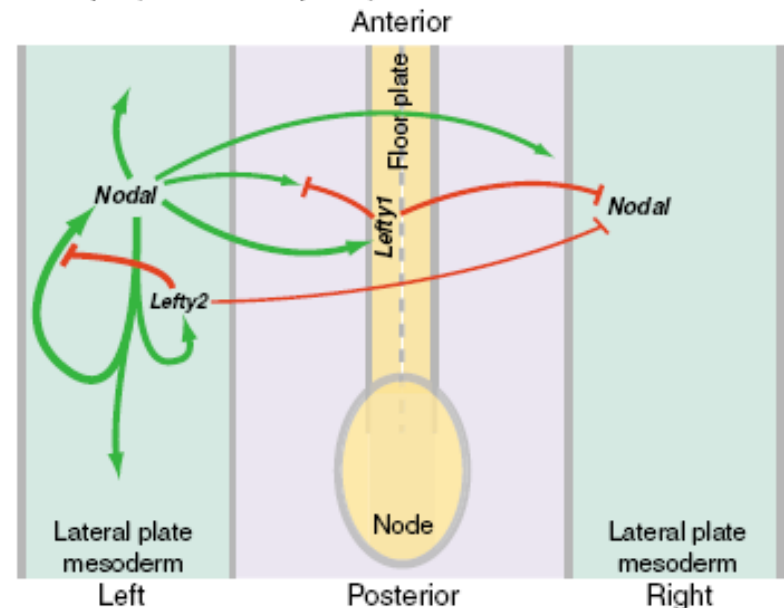


**Fig. 5. Sequential function of Nodal signaling in left-right patterning in the mouse embryo.** (A) Following initial symmetry breaking around the node, possibly as a consequence of ciliary-based nodal flow, Nodal (green arrow) and/or Gdf1 signals become elevated on the left side of the node, and are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

## A 8.0 dpc (0-2 somite pairs)

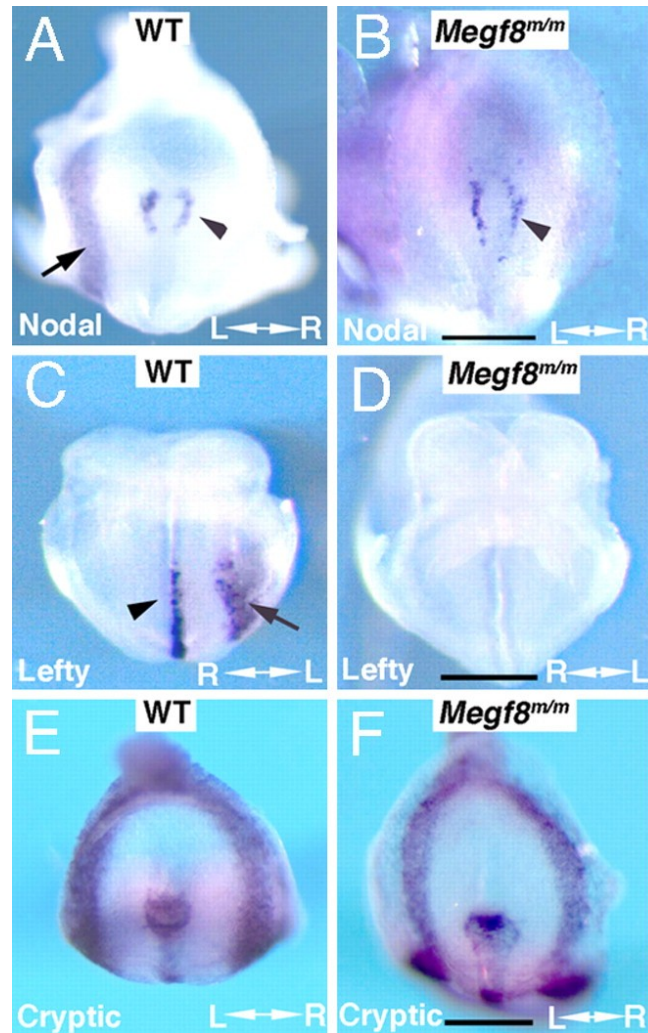


## B 8.25 dpc (3-8 somite pairs)





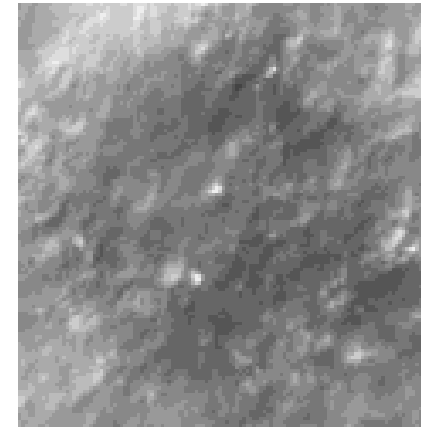
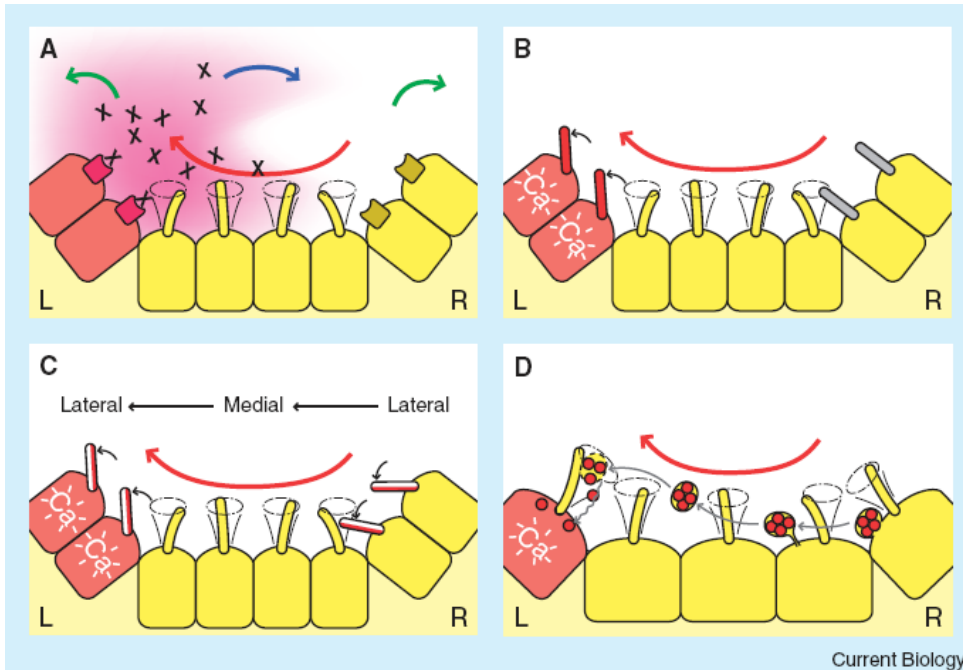
In situ hybridization analysis show altered expression of left determinant genes in *Megf8<sup>m/m</sup>* embryos.



Zhang Z et al. PNAS 2009;106:3219-3224

# Vytváření levopravé asymetrie těla - role cilií

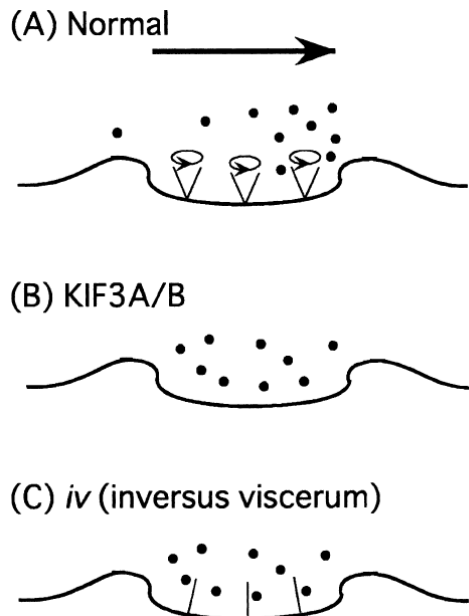
- asymetrická exprese genů jako *lefty1*, *lefty2*, *nodal* a *pitx2*
- nodální cilie (9+0, dynein → pohyblivé) během gastrulace vytváří svým rotačním pohybem tzv. nodální proud



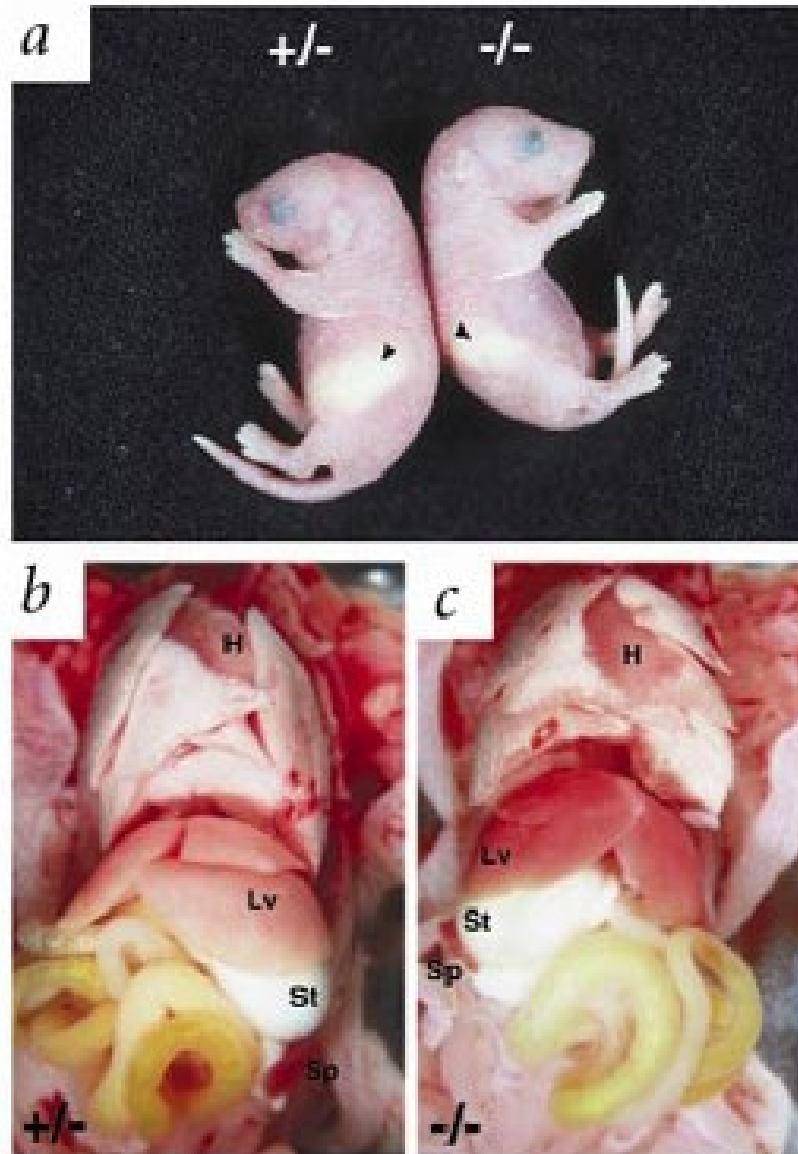
cshperspect-SYM-a000802001.mov

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

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



## GDF1 mutanti



**Figure 2. Analysis of situs defects in *Gdf1*<sup>-/-</sup> mice.**

**a**, *Gdf1*<sup>+/-</sup> and *Gdf1*<sup>-/-</sup> newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1*<sup>+/-</sup> (**b,d,f,h**) and *Gdf1*<sup>-/-</sup> (**c,e,g,i**) mice are shown. **b,c**, Reversal of the orientation of the abdominal organs in *Gdf1*<sup>-/-</sup> 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,

# Emerging functions of Nodal

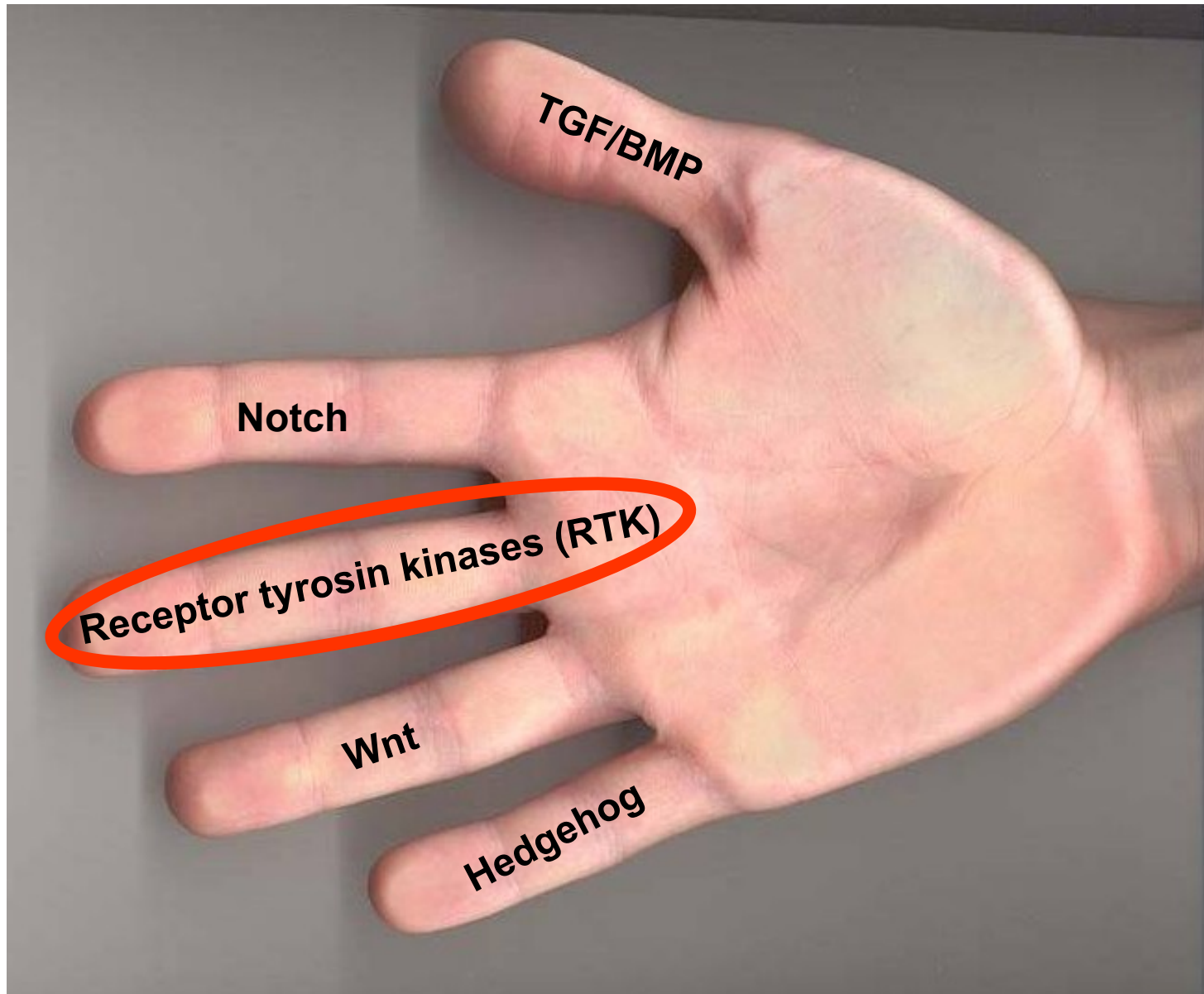
- promotes pluripotency of the epiblast in vivo and of ES cells in vitro
- controls fate of cancer stem cells – eg. in pancreatic cancer, very promising treatment

# GDF8 (myostatin)

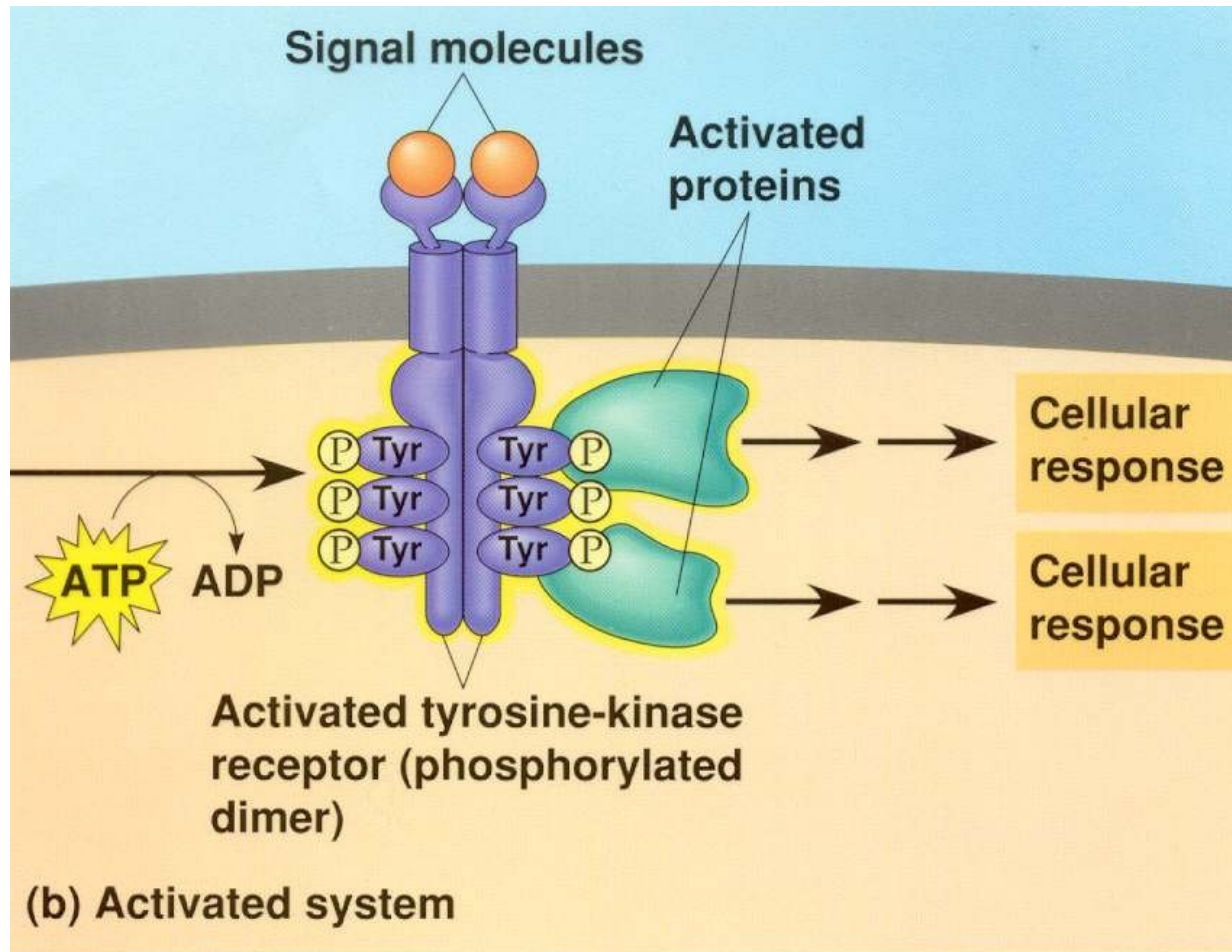




# Signály z vnějšího prostředí



# 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. 9

VEGF (vascular endothelial GF) receptor family

HGF (hepatocyte GF) receptor family

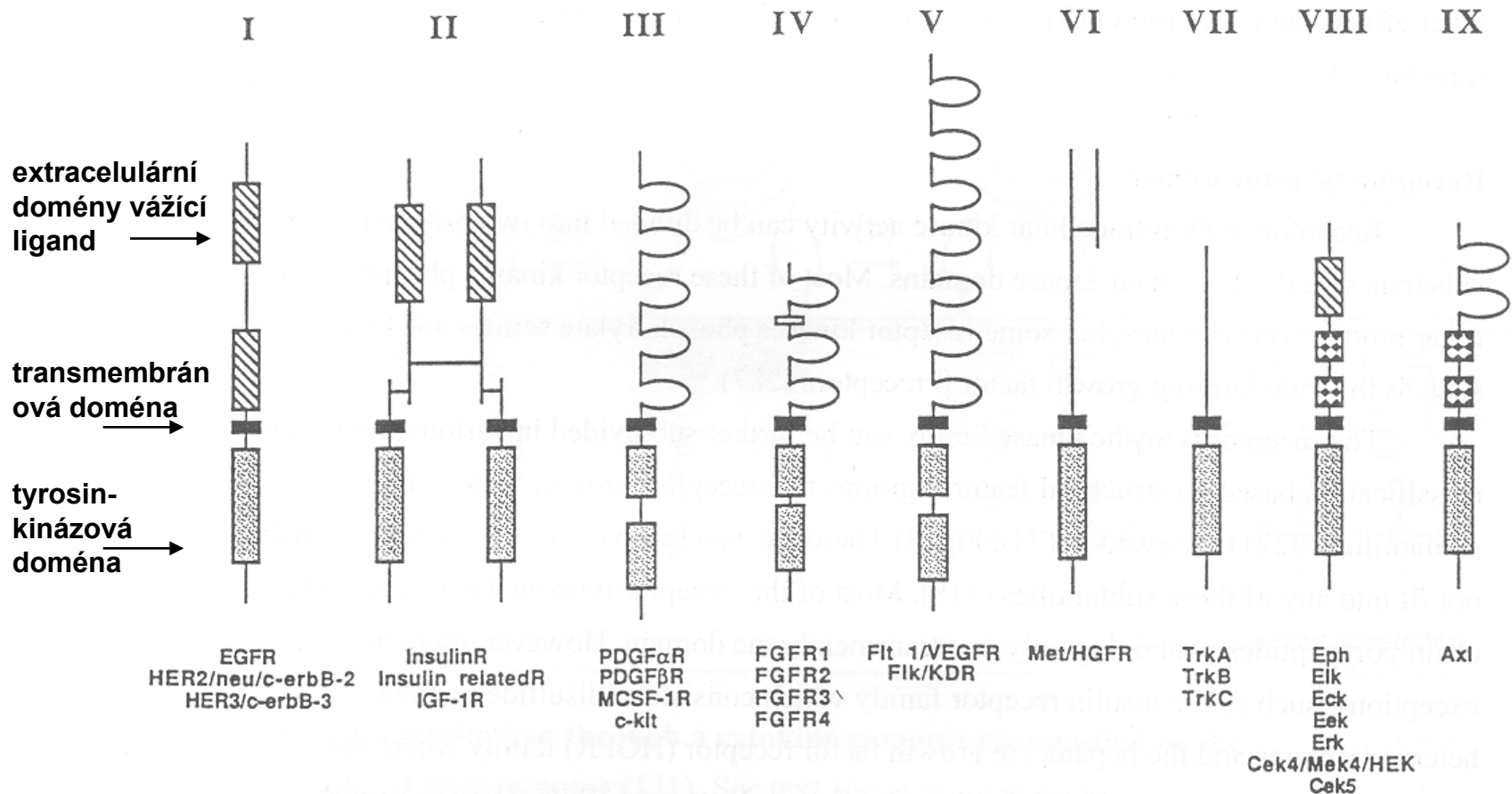
Trk receptor family

Eph receptor family

RET receptor family



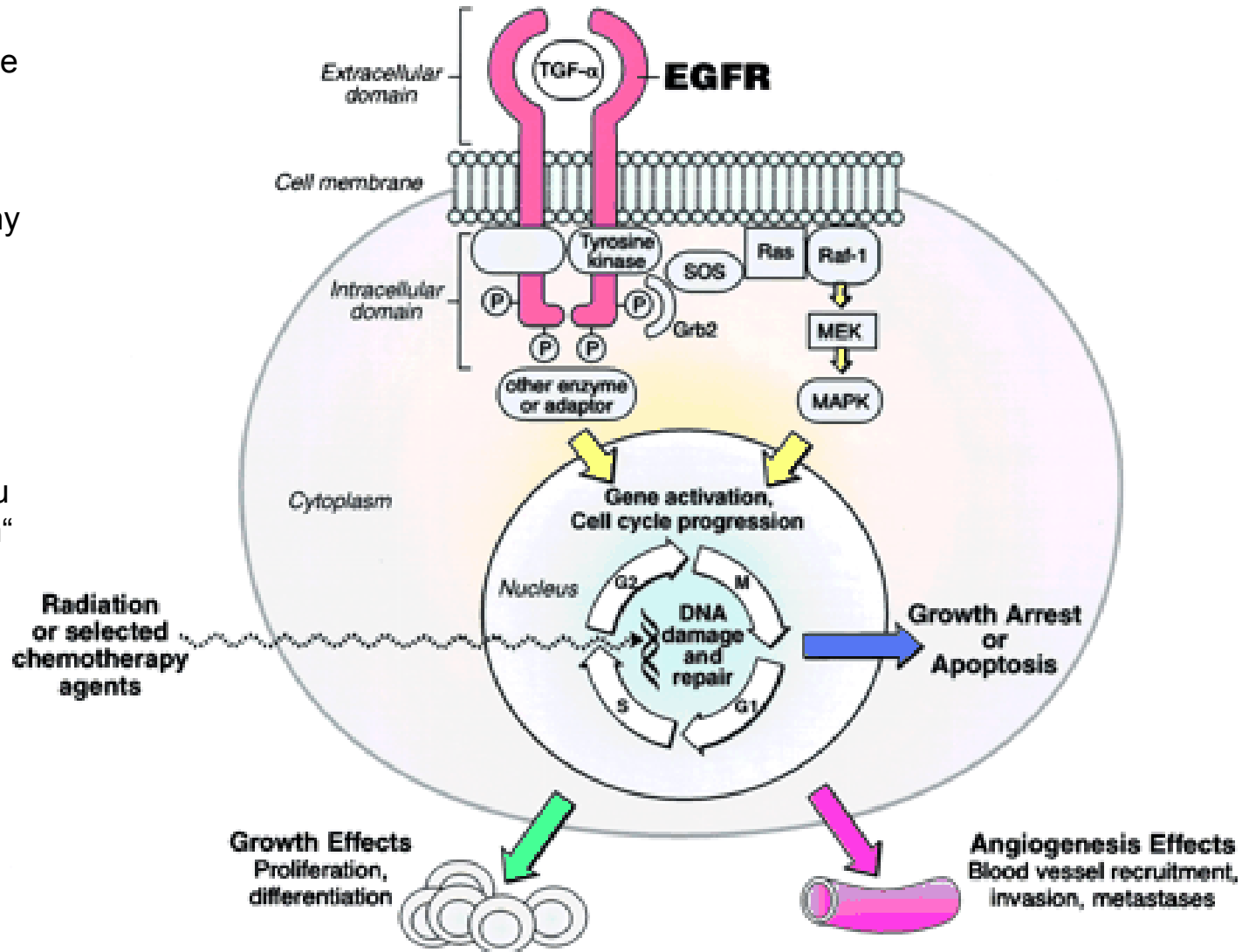
# Schematická struktura jednotlivých receptorů



# Obečné schéma aktivace RTKs

(zde na příkladu EGFR)

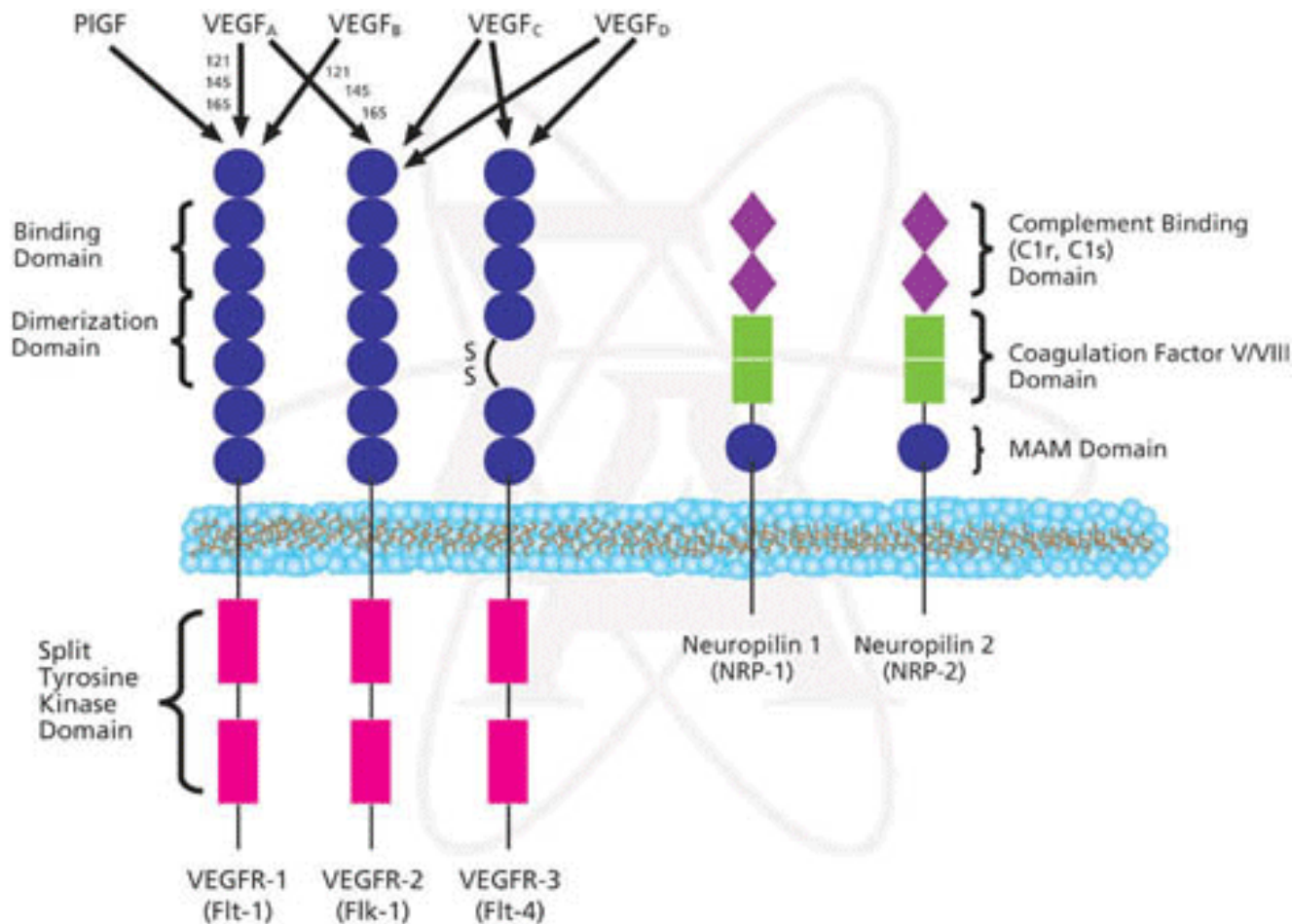
1. ligand se specificky váže na receptor
2. receptor dimerizuje
3. tyrosin-kinázové domény se navzájem fosforylují
4. autofosforylace vede k navázání (recruitment) adaptérových proteinů (zde Grb2)
5. v závislosti na receptoru se aktivují „downstream“ signální dráhy – zde např. Ras/Raf1/MEK/MAPK kinázová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



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

- VEGF/VEGFR
  - ephrin/Eph
    - PDGF
    - Trk family
- FGF/FGFR – viz přednáška č. 9

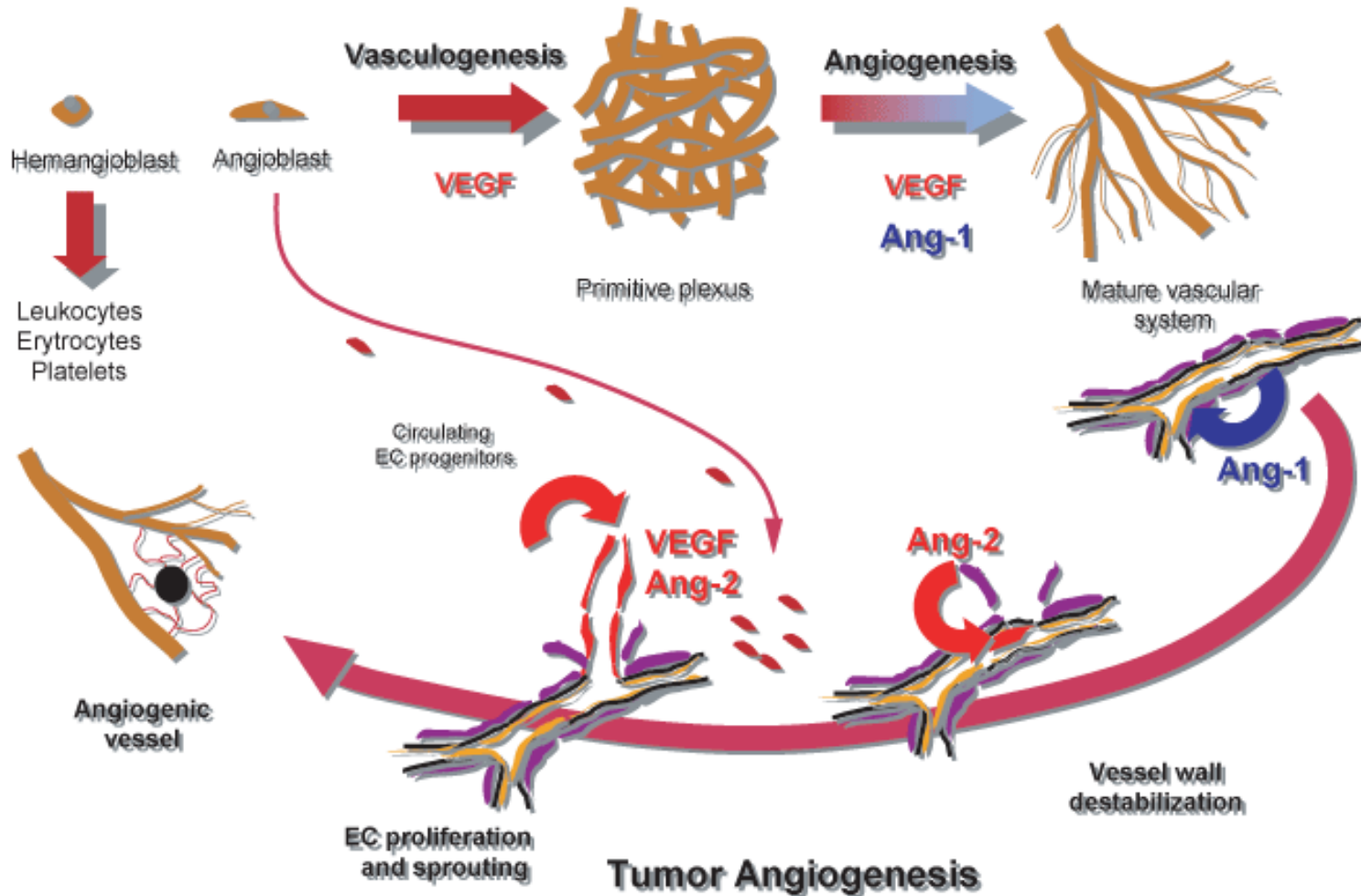
# Vascular endothelial growth factors (VEGF) a jejich receptory (VEGFR)



# VEGF/VEGFR ve vývoji

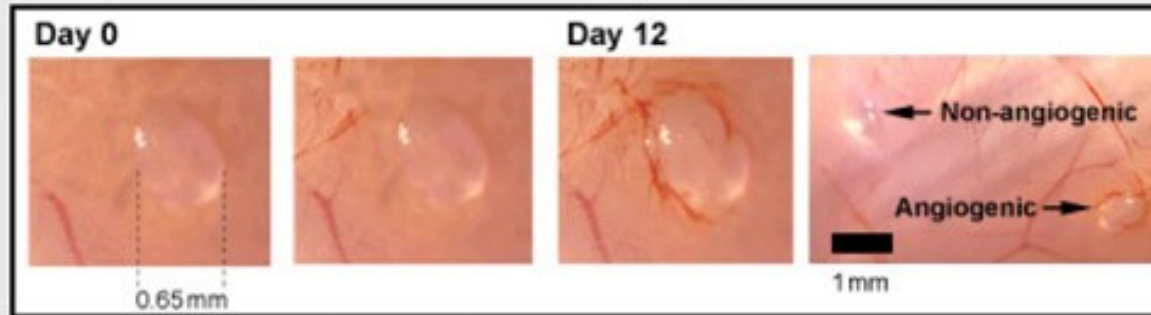
- reguluje vznik a vývoj cévní soustavy
- hypoxie (=nedostatek kyslíku) indukuje HIF (hypoxia-induced factor), který reguluje produkci VEGF; viz přednáška č. 7.
- VEGF je schopen regulovat vznik de novo cév v hypoxické části embrya
- - podobný mechanismus se uplatňuje i při onkogenezi, kde VEGF podporuje prokrvení nádorů a tím podporuje jejich růst

## Developmental Angiogenesis



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.

## Physiologic appearance of the “angiogenic switch”



Images reproduced with permission from Judah Folkman.

- The “angiogenic switch” leads to neovascularization, as shown in a rat tumor model<sup>1</sup>

Reference: 1. Folkman J. *N Engl J Med.* 1971;285:1182-1186.

## Functions of the VEGF family of receptors

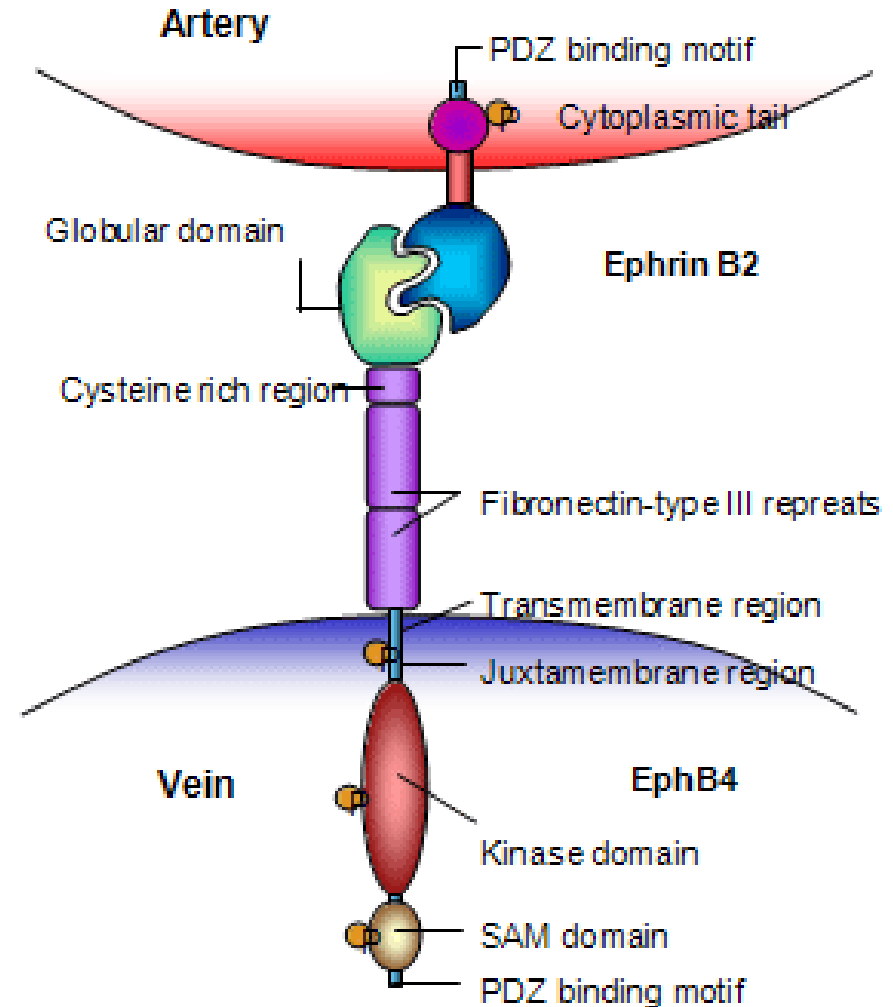
VEGFR-1 <sup>1,2</sup>	Crucial to embryonic angiogenesis  Does not appear to be critical in pathogenic angiogenesis
VEGFR-2 <sup>1,3</sup>	Most important VEGF receptor in tumor angiogenesis  Mediates the majority of VEGF angiogenic effects
VEGFR-3 <sup>1,4</sup>	Found only in lymphatic endothelial cells  Associated with lymph node metastasis

**References:** 1. Hicklin DJ, Ellis LM. *J Clin Oncol.* 2005;23:1011-1027. 2. Olofsson B, Korpelainen E, Pepper MS, et al. *Proc Natl Acad Sci USA.* 1998;95:11709-11714. 3. Ogawa S, Oku A, Sawano A, et al. *J Biol Chem.* 1998;273:31273-31282. 4. Dumont DJ, Jussila L, Taipale J, et al. *Science.* 1998;282:946-989.

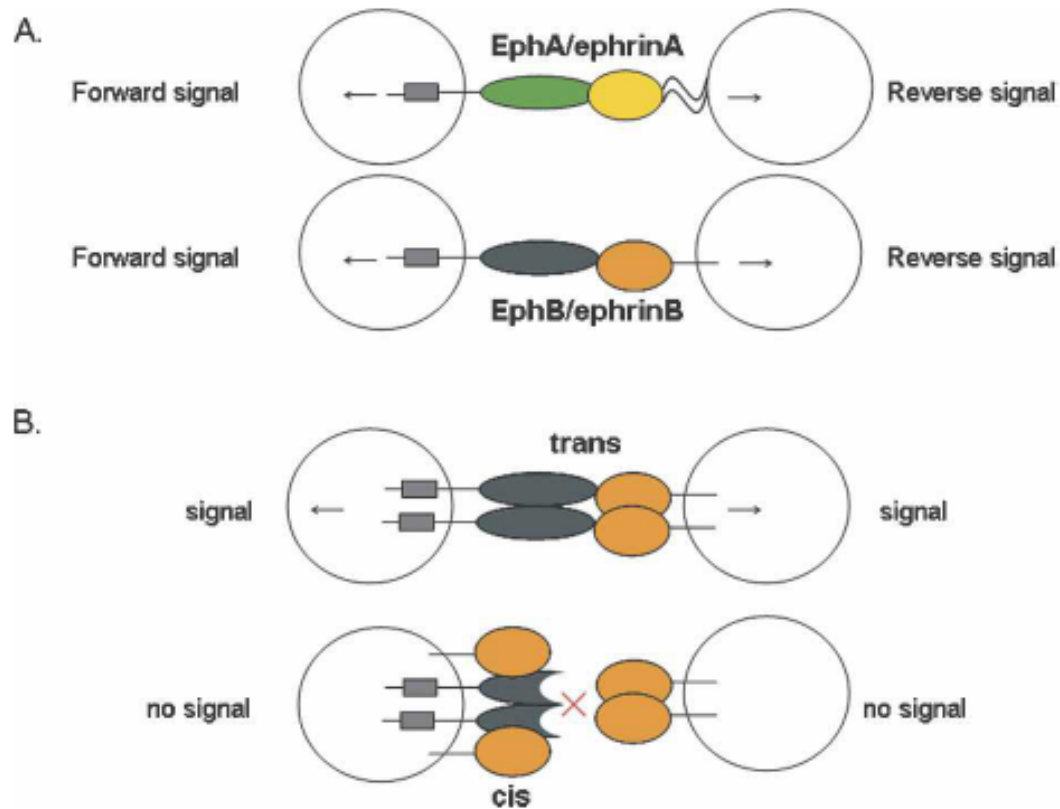


# Eph/ephrin komplex

- 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

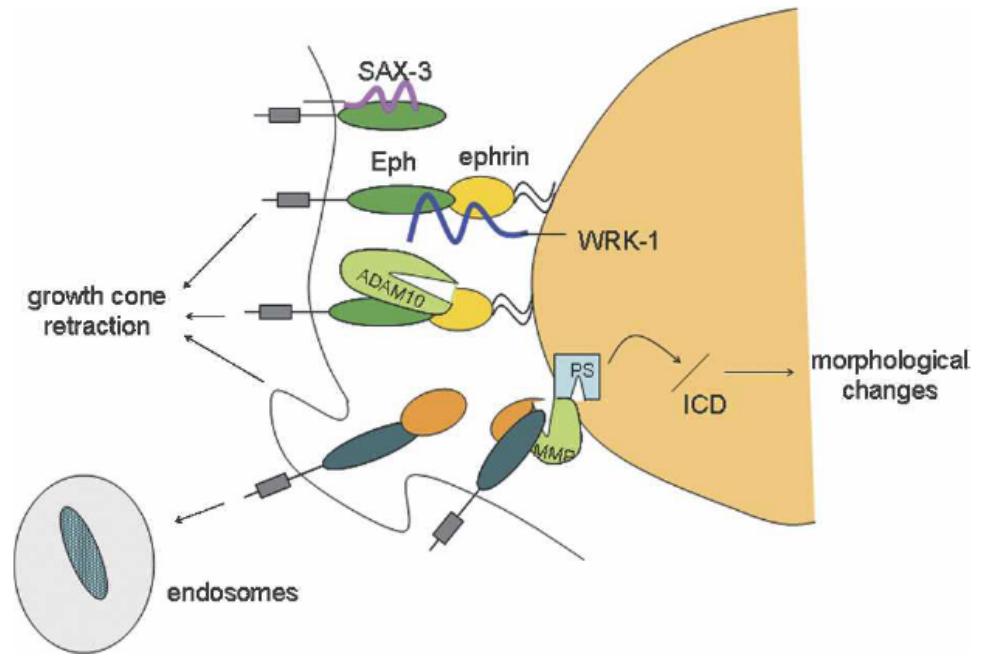
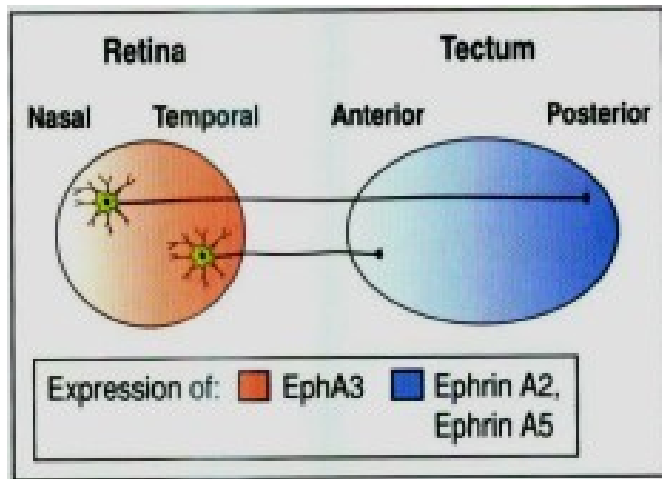


**Figure 1.** Main features of Eph/ephrin signaling. (A) Both classes of Eph receptors and ephrins activate bidirectional signaling. Interaction between Eph receptors and ephrins leads to activation of forward and reverse signaling in neighboring cells. (B) Eph receptors and ephrins expressed in opposing cells interact in *trans* and activate bidirectional signaling. Eph receptors and ephrins coexpressed in the same cell interact in *cis*. *Cis* interaction has been shown to inhibit *trans* interaction and/or signaling.

## Eph/ephrin

- při „axon guidance“ tj. navádění jednotlivých axonů v nervovém systému

- growth cone retraction



**Figure 5.** Interactions at growth cones. Binding of WRK-1 and Eph/VAB-1 in *trans* prevents midline crossing. VAB-1 also interacts with SAX-3 in *cis*. Growth cone retraction requires termination of contact between Eph- and ephrin-expressing cells. This is achieved by cleavage of ephrin ectodomain by ADAM10 in *trans* and/or endocytosis of Eph/ephrin complexes, followed by processing of the ectodomain in the endosomal/lysosomal pathway. Subsequent to ectodomain shedding, processed ephrins are targets for PS cleavage that releases the ICD in the cytosol.

## Eph/ephrin

Unique feature: reverse signalling – tj. nesignáluje jen receptor, ale i ligand

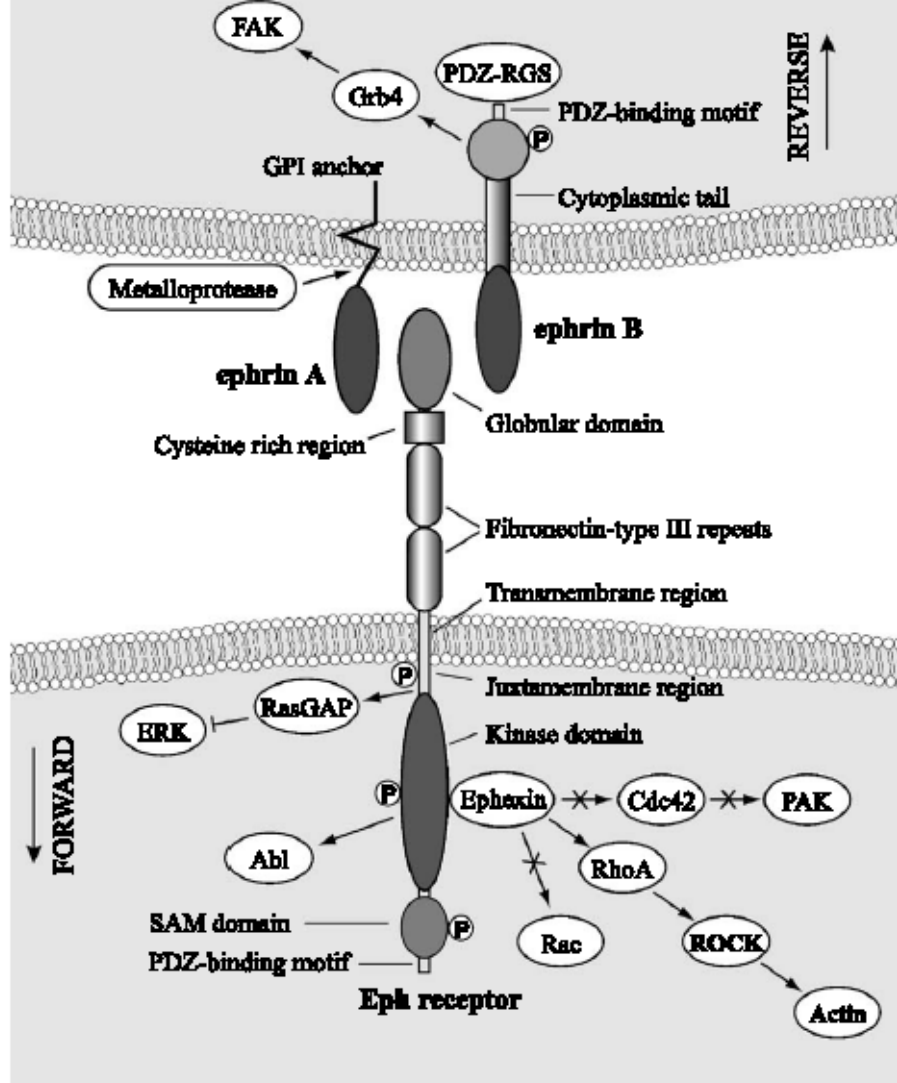


Fig. 1. Forward and reverse signaling by the ephrin-Eph complex. Glycosylphosphatidylinositol (GPI)-anchored ephrin-As bind to EphA receptors whereas the transmembrane ephrin-Bs bind to EphB receptors. The ephrin-Eph receptor binding initiate forward signaling in the Eph receptor bearing cells and reverse 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 Ctrb4 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  $\alpha$  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)).



ephrinB induced growth cone collapse.mov

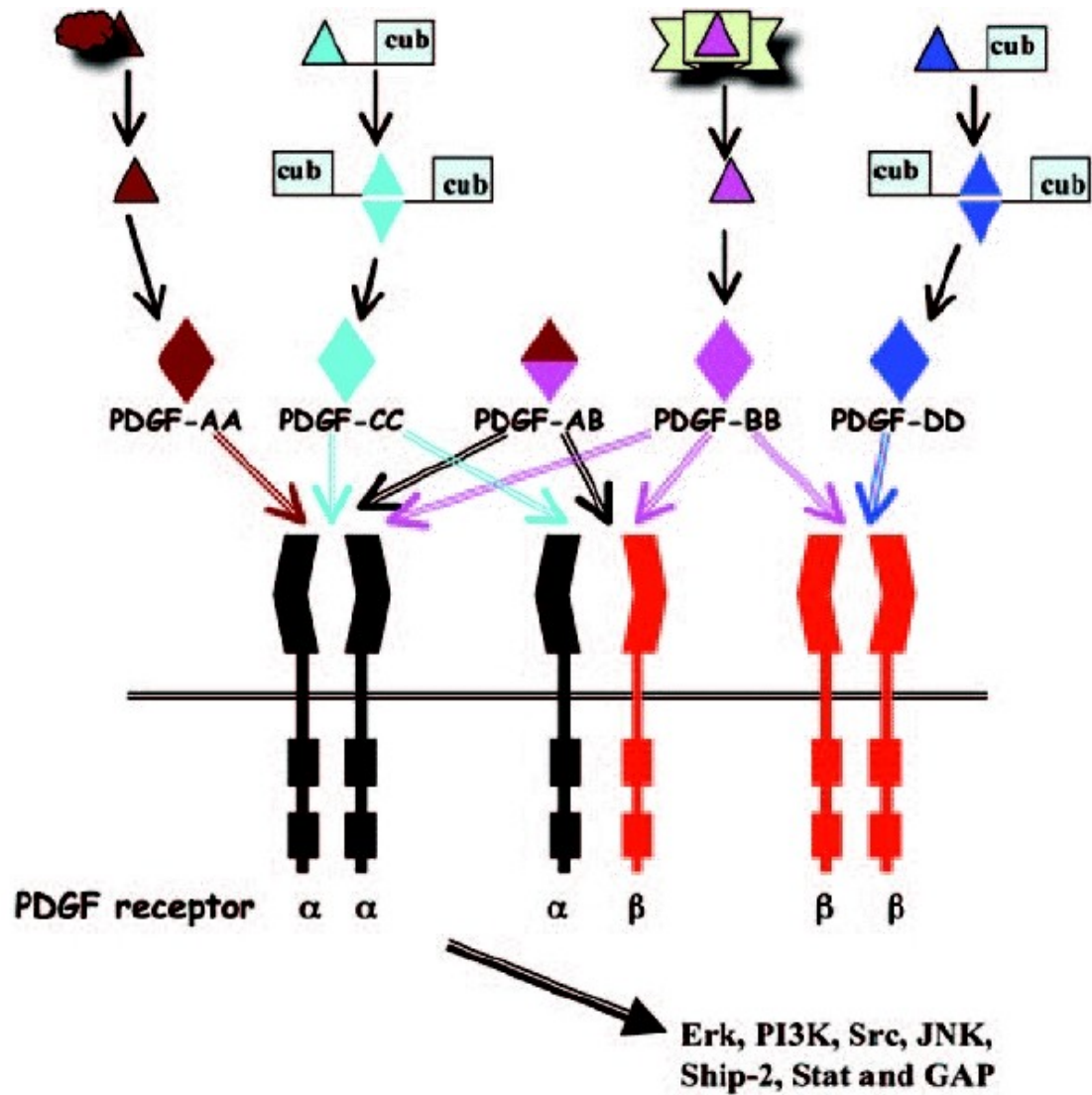
Supporting Information Movie 2. Ephrin-B2 induces extremely rapid growth cone collapse and axon retraction in VT RGCs. Movie depicts VT growth cones treated with ephrin-B2. Frames were captured at 30-second intervals for 45 minutes, replayed at 15 frames per second. 0.5  $\mu\text{g/ml}$  pre-clustered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

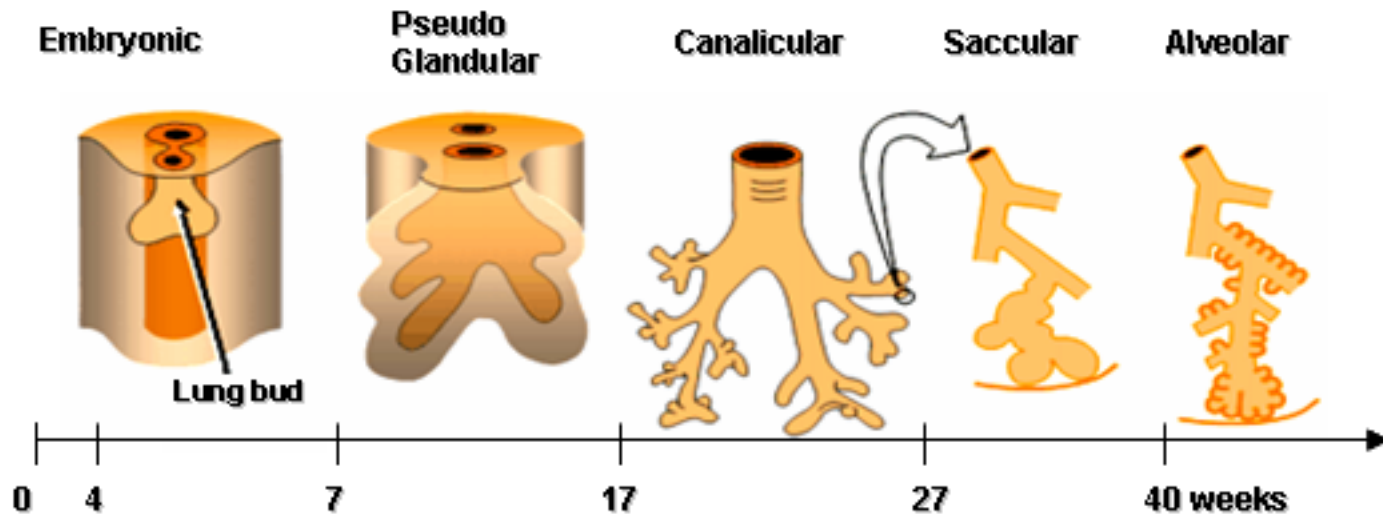


EphrinB2 with Rho kinase inhibitor.mov

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

# PDGF

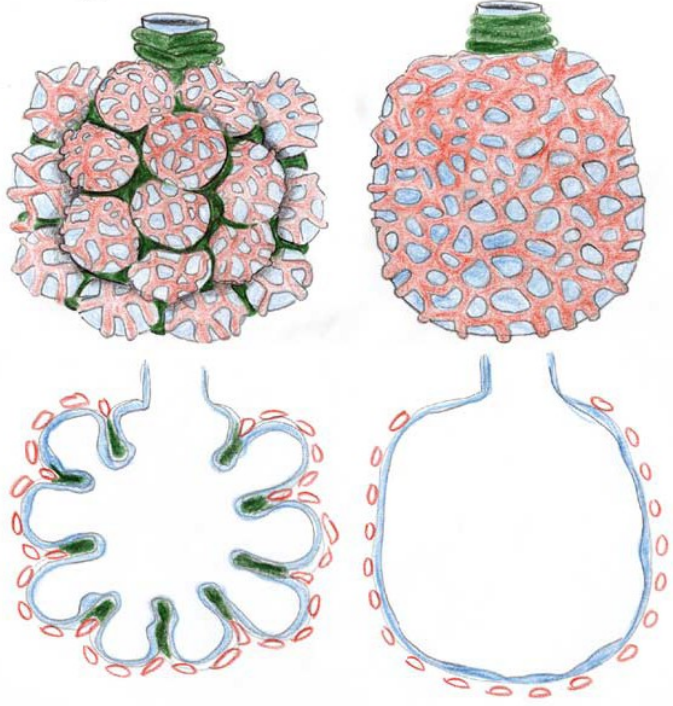




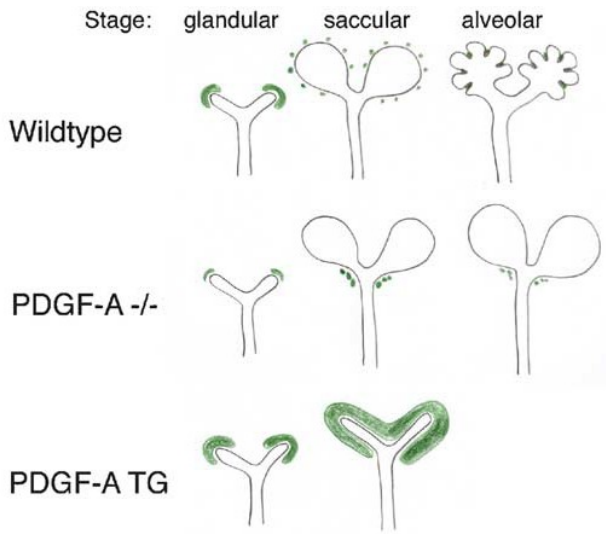
**The stages of pulmonary development**



Wildtype alveolar sac      PDGF-A <sup>-/-</sup> alveolar sac



(A)



(B)

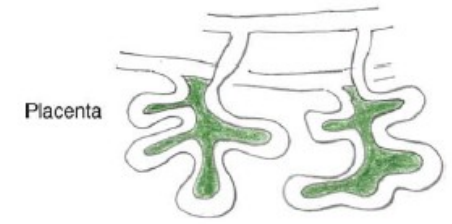
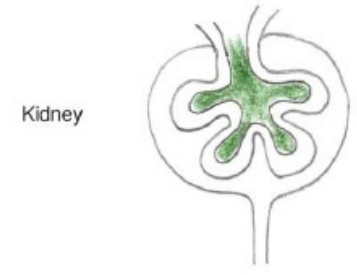
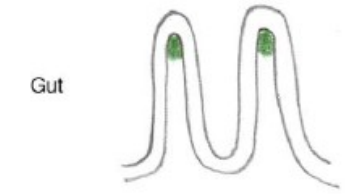
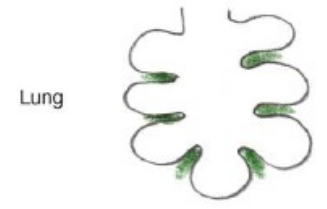
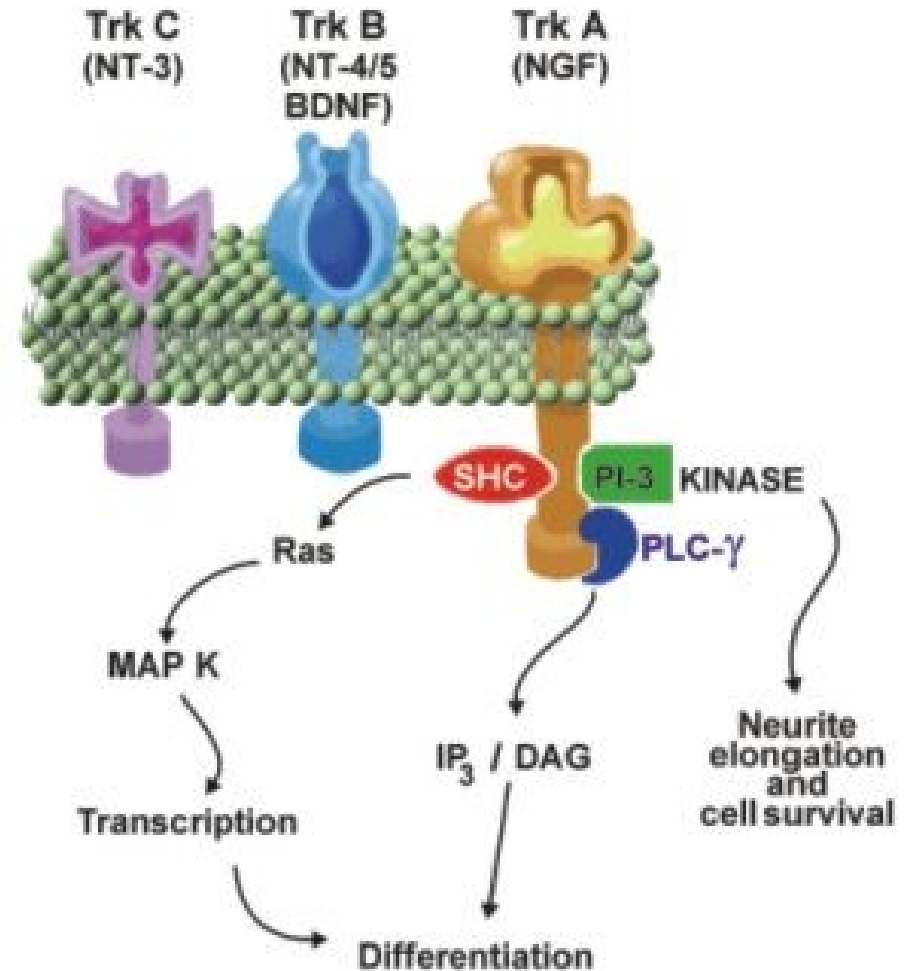


Fig. 5. Illustration of analogous epithelial or endothelial folding processes controlled by PDGFR $\alpha$  or PDGFR $\beta$  positive cells (green). The individual examples are discussed in the text.

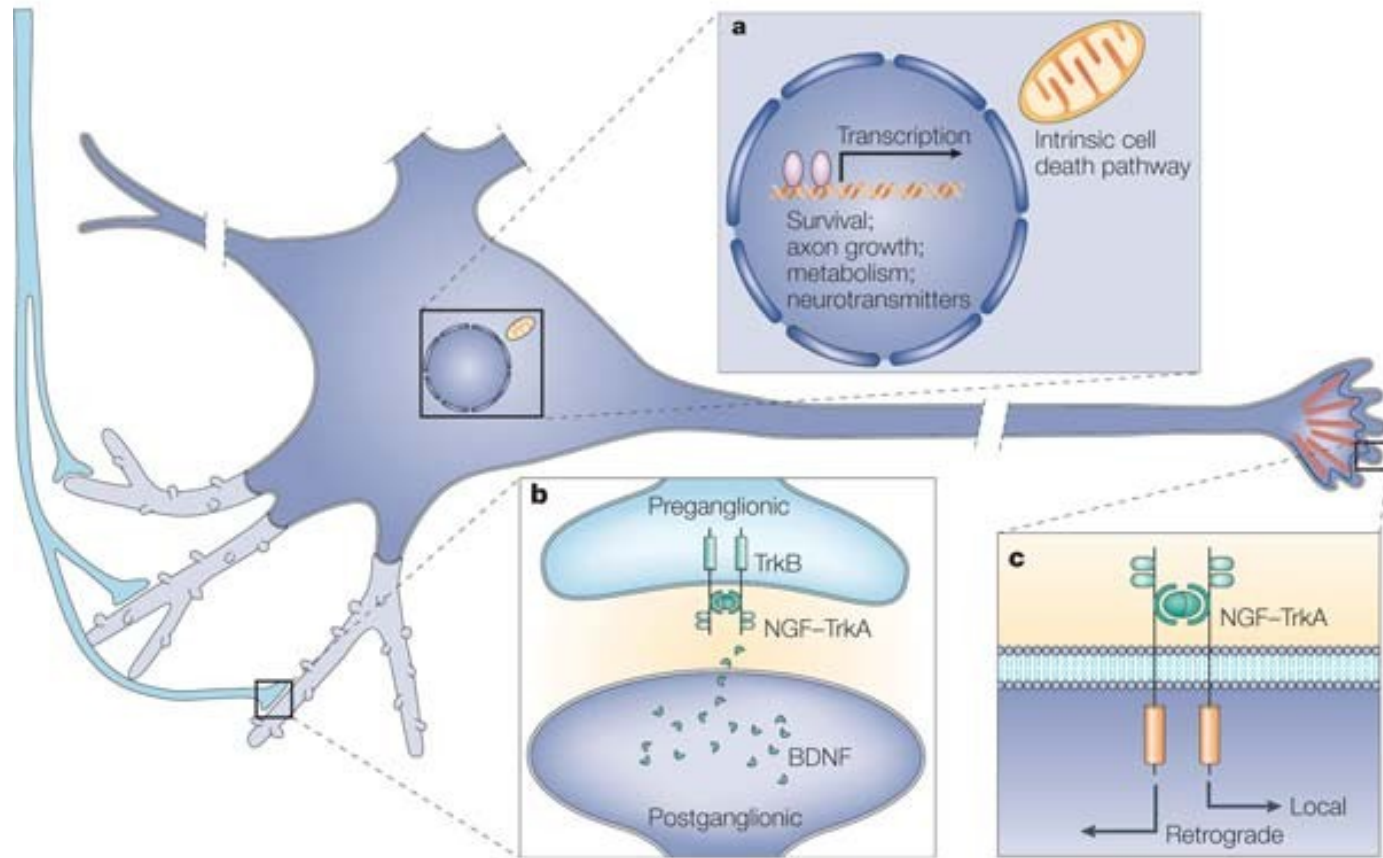


# Trk (tropomyosine-related kinase)

- tři receptory exprimované v nervovém systému – TrkA, TrkB a TrkC
- specificky váží neurotrofické peptidy – NT3-5, BDNF=brain derived neurotrophic factor, NGF=neural growth factor



Neurotrofní látky – podporují přežívání neuronů – a tak regulují vzájemné propojení nervových buněk (po lopatě – neuron, který není synapticky spojen s jiným zahyne)

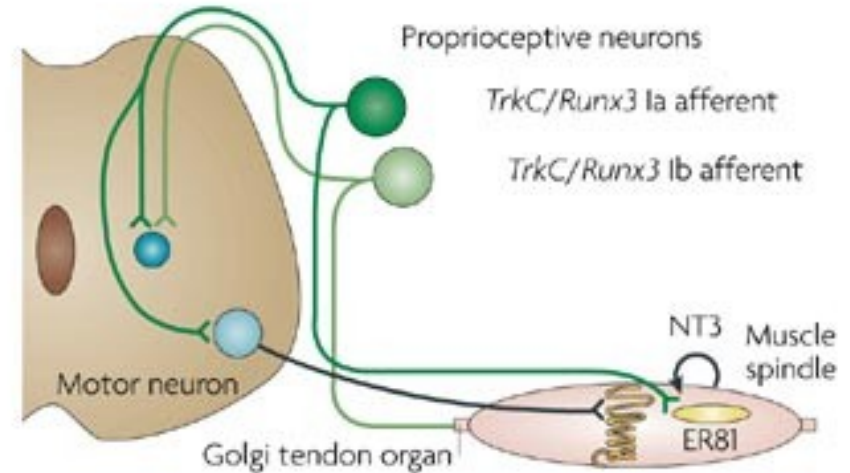
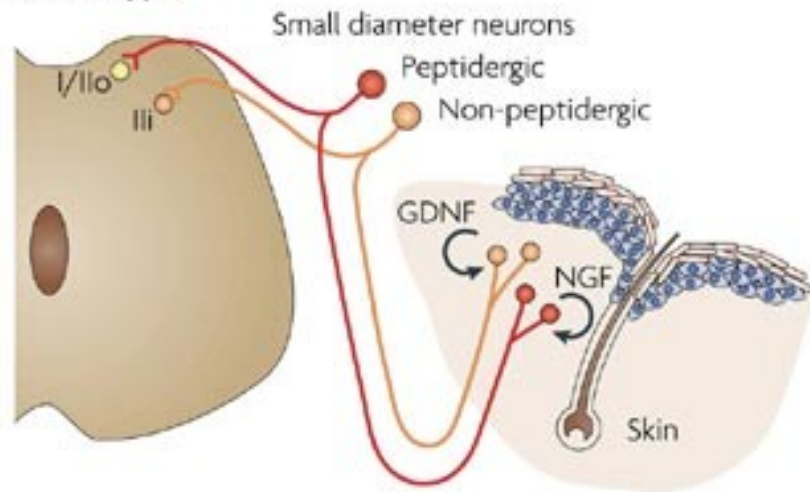


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a | Neurotrophin signalling events initiated in developing axons signal retrogradely to neuronal cell bodies to inhibit intrinsic cell death pathways. These signalling events also influence transcriptional programs that are involved in cell survival, axon growth, synaptogenesis, metabolism and the establishment of neurotransmitter and neuropeptide phenotypes. b | The establishment of pre- and postganglionic synaptic contacts in sympathetic neurons is influenced by retrograde nerve growth factor (NGF)-TrkA signalling. A potential synaptogenic signal downstream of retrograde NGF-TrkA signalling is the neurotrophin brain-derived neurotrophic factor (BDNF). BDNF regulates the formation and maintenance of presynaptic contacts by signalling trans-synaptically to TrkB receptors on preganglionic sympathetic neurons. c | Neurotrophin-dependent axon growth is supported by both local and retrograde signalling through the activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) signalling pathways. Retrograde neurotrophin signalling also regulates axon growth and target innervation through the activation of transcriptional programs.

# TrkA/C systém determinuje synaptická spojení v periferním nervstvu

a Wild type



nociceptory (receptory bolesti) – jsou determinovány expresí TrkA a přežívají díky NGF v kůži

proprioceptory (receptory polohy/napětí svalu) – jsou determinovány expresí TrkC a přežívají díky NT3 ve svalu

# HGF (hepatocyte growth factor)/SF (scatter factor)

One ligand – one receptor system

Diversity determined by cytoplasmic components and adaptor proteins

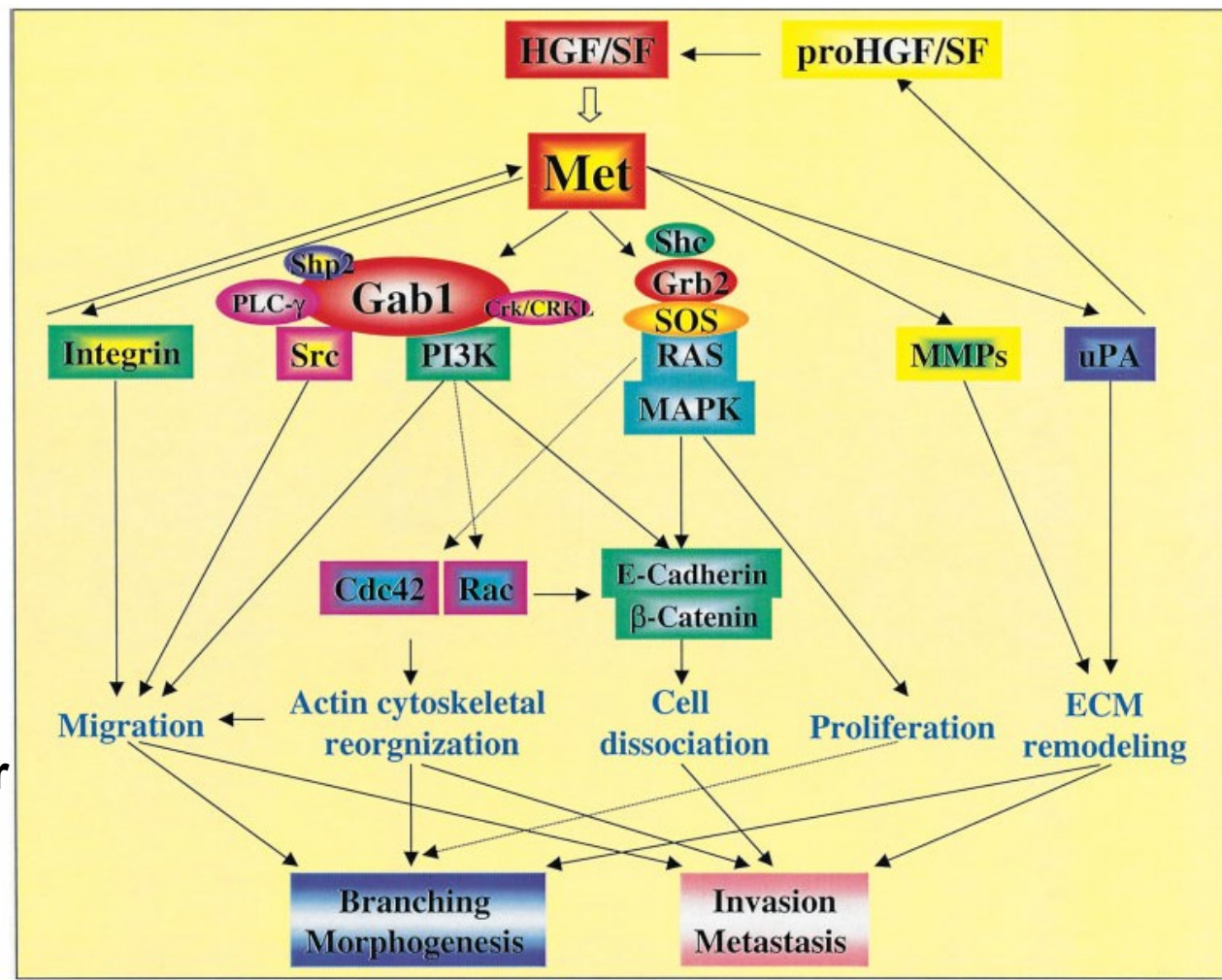


Fig. 1. Schematic representation of HGF/SF-Met signaling cascades in the control of branching morphogenesis and invasion. Upon HGF/SF binding, Met can activate a series of intracellular signaling pathways such as Ras/MAPK, PI3K, and Src through the two major adaptor molecules Grb2 and Gab1, which induces the biological events including proliferation, cell dissociation, and migration. Met can activate Cdc42 and Rac through a manner without direct interaction, which lead to the

actin cytoskeletal reorganization, an event crucial for branching morphogenesis and invasion. Interaction of Met with integrin in a ligand-dependent or independent manner is also critical for Met-mediated invasive growth. Except the enzymatic changes of downstream signaling molecules, HGF/SF-Met signaling also increases the activities of MMPs and uPA by up-regulating these gene expressions, which contributes to the ECM remodeling as well as activation of HGF/SF.