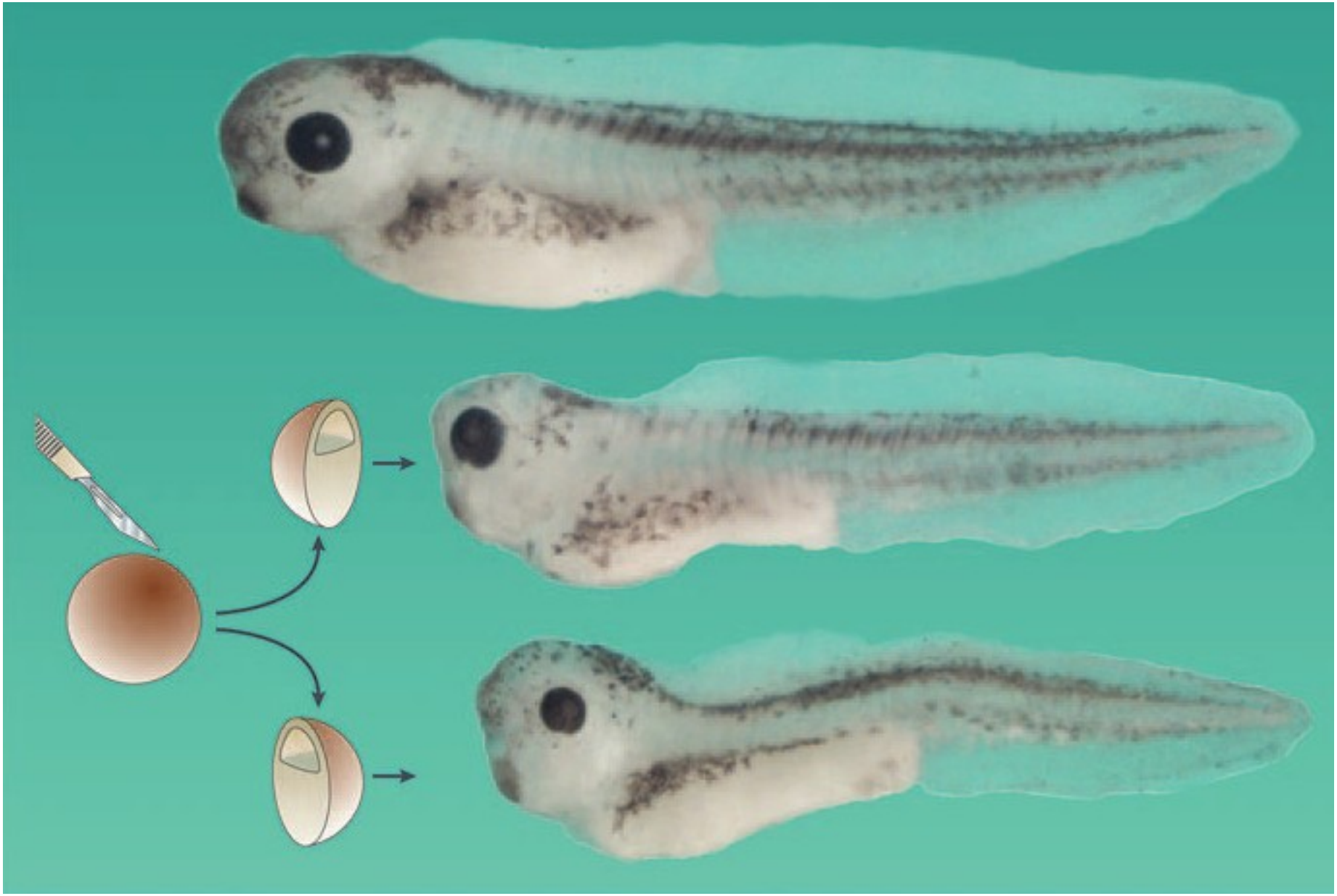
#6

Klíčové molekulární komponenty vývoje

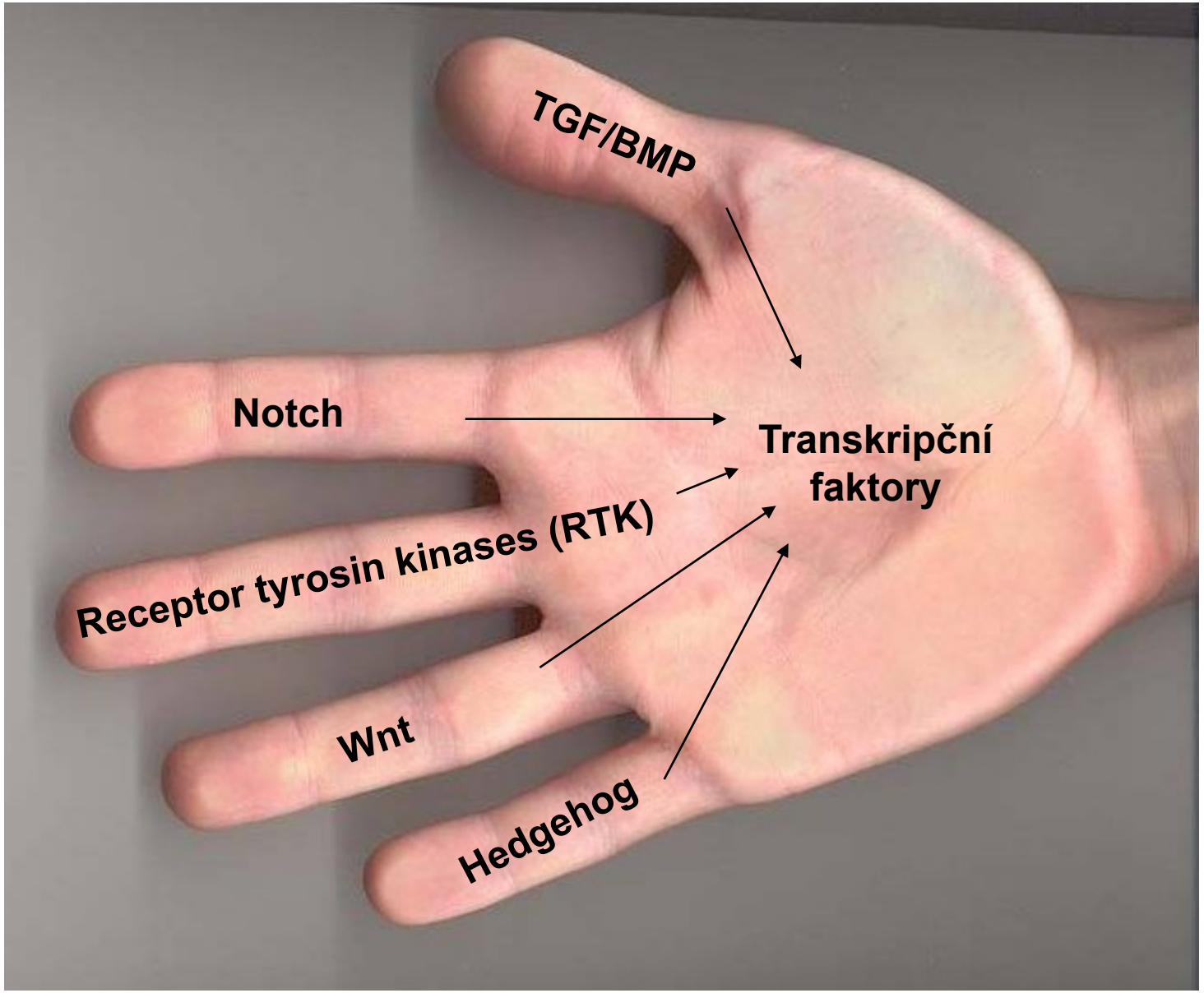


Co poskytuje buňce tyto informace?

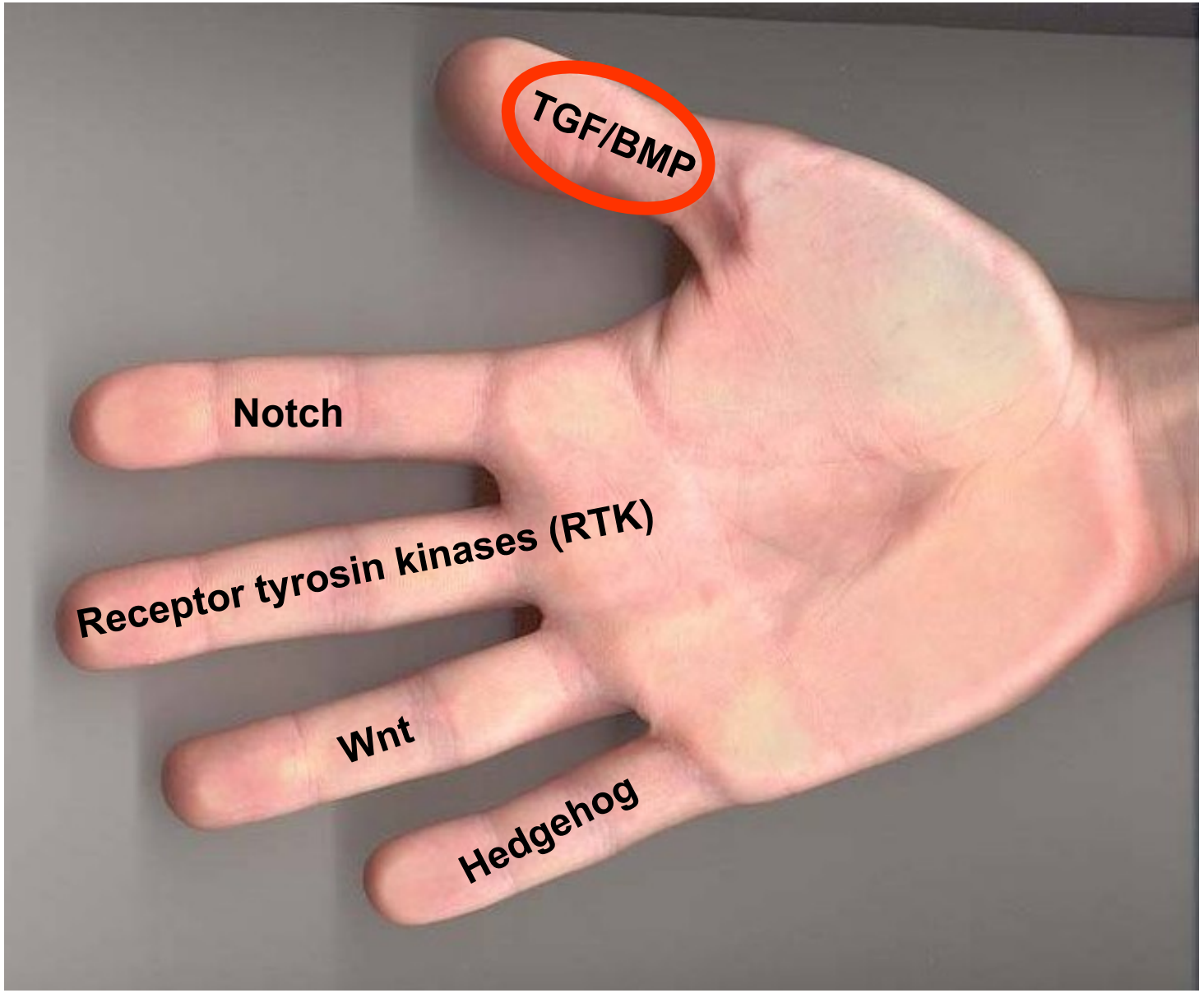
a) signály z okolního prostředí

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 TGFβ nadrodiny

TGFβ nadrodina má následující podrodiny:

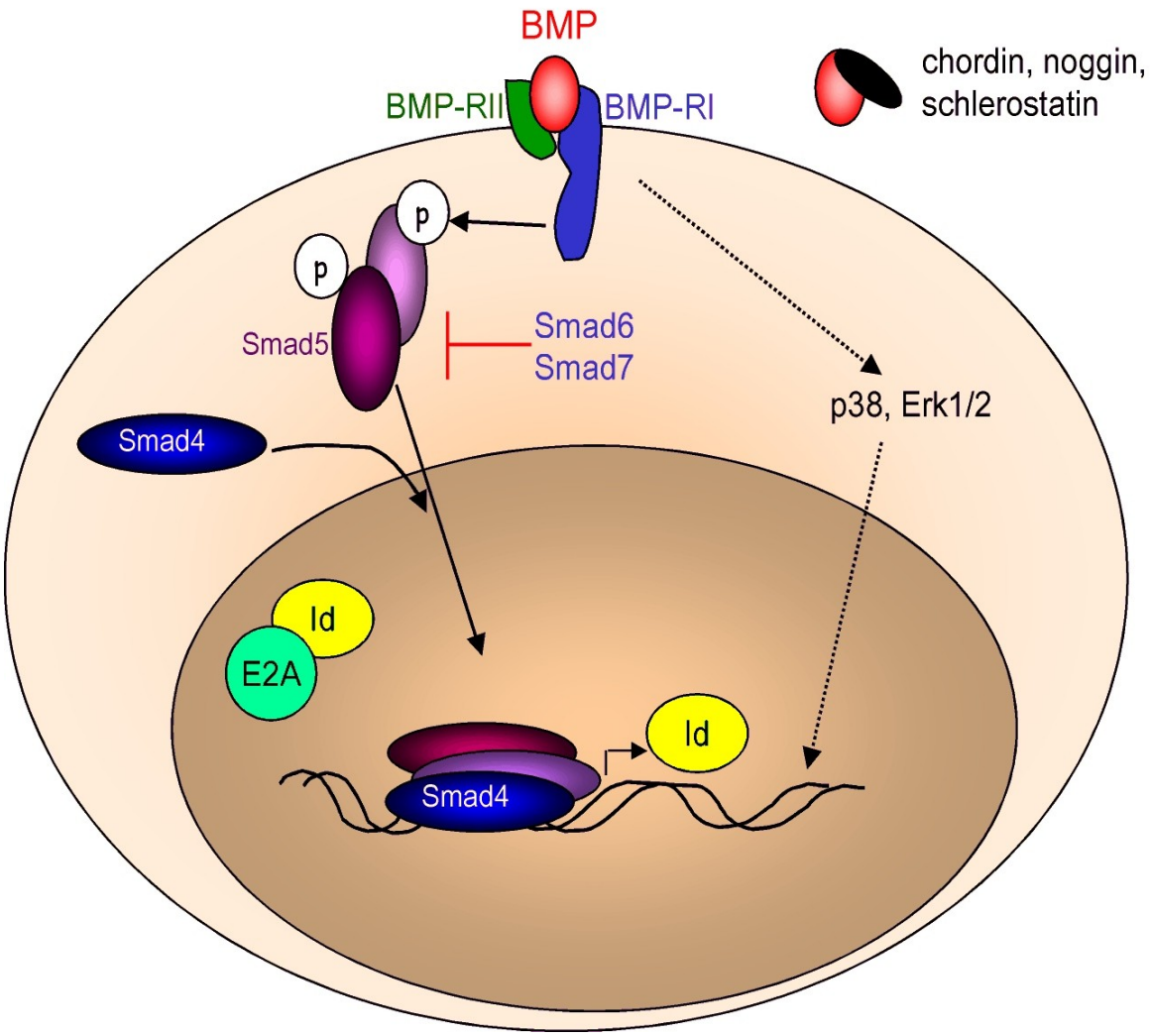
1. TGFβ1-3
2. BMPs – 20 různých ligandů
3. GDF (growth differentiation factor): 9 ligandů
4. activin/inhibin/nodal

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

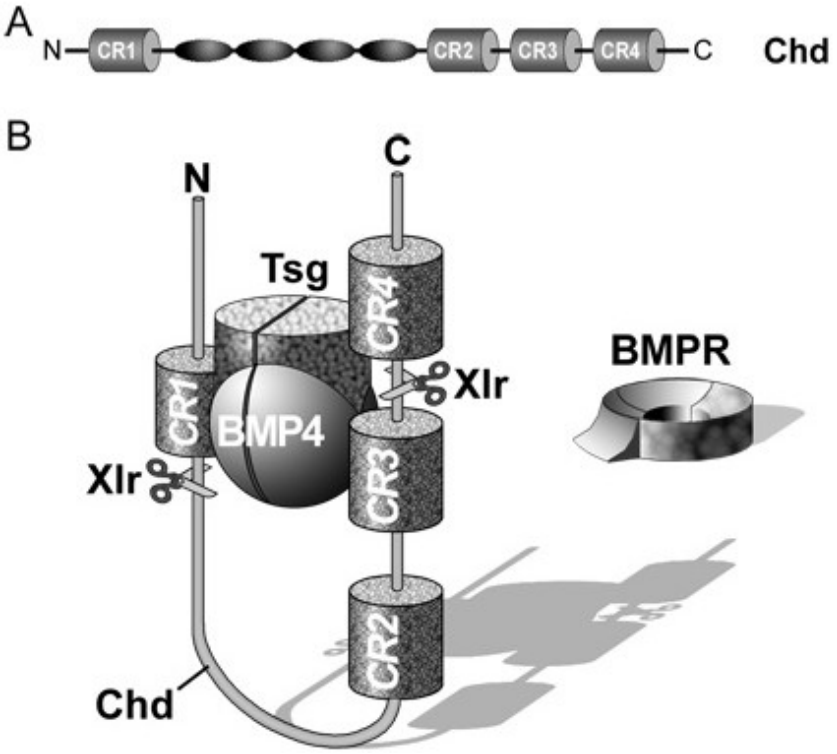
BMP signaling pathway



Inhibitory BMP faktorů,

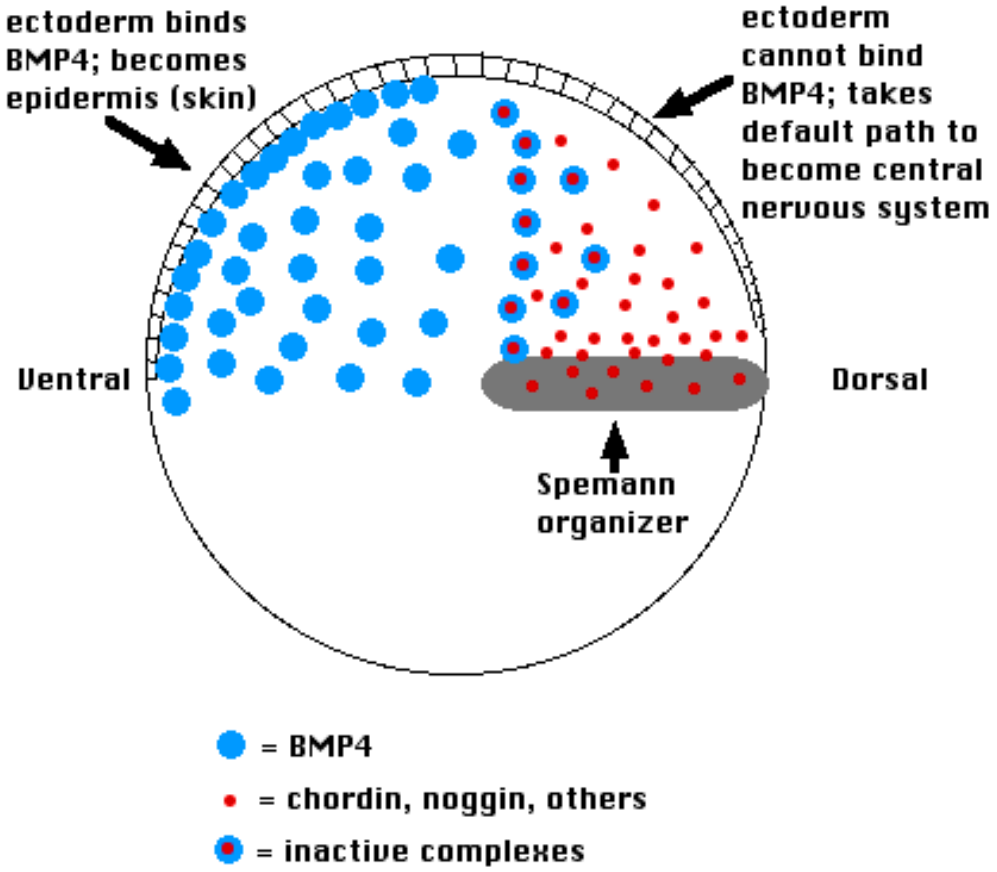
jsou klíčové pro fyziologické funkce BMP

- noggin
- chordin (Chd)
- sklerostin

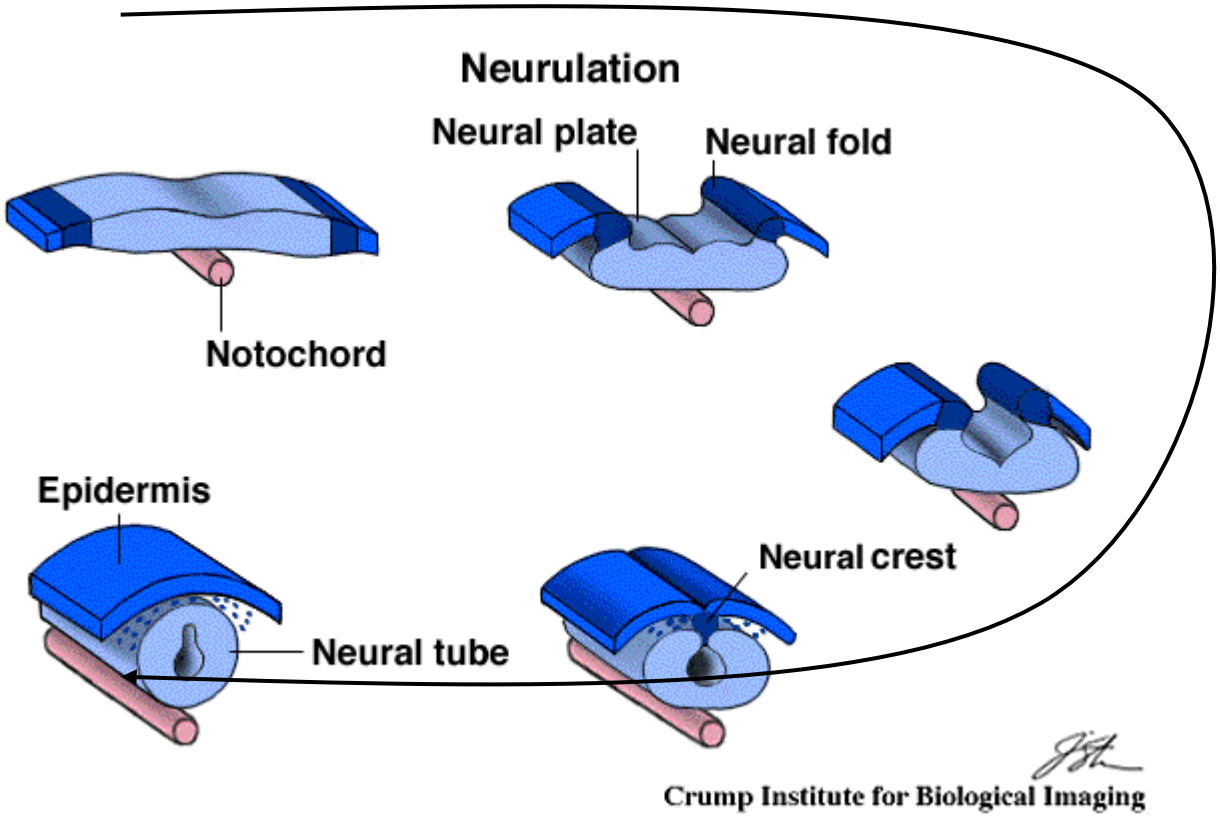


Přímá fyzická interakce mezi chordinem a BMP je podstatou inhibičního působení chordinu

Role BMP inhibitorů v Spemannově organizátoru



Klíčová role BMP inhibitorů produkovaných notochordem při indukci nervové ploténky



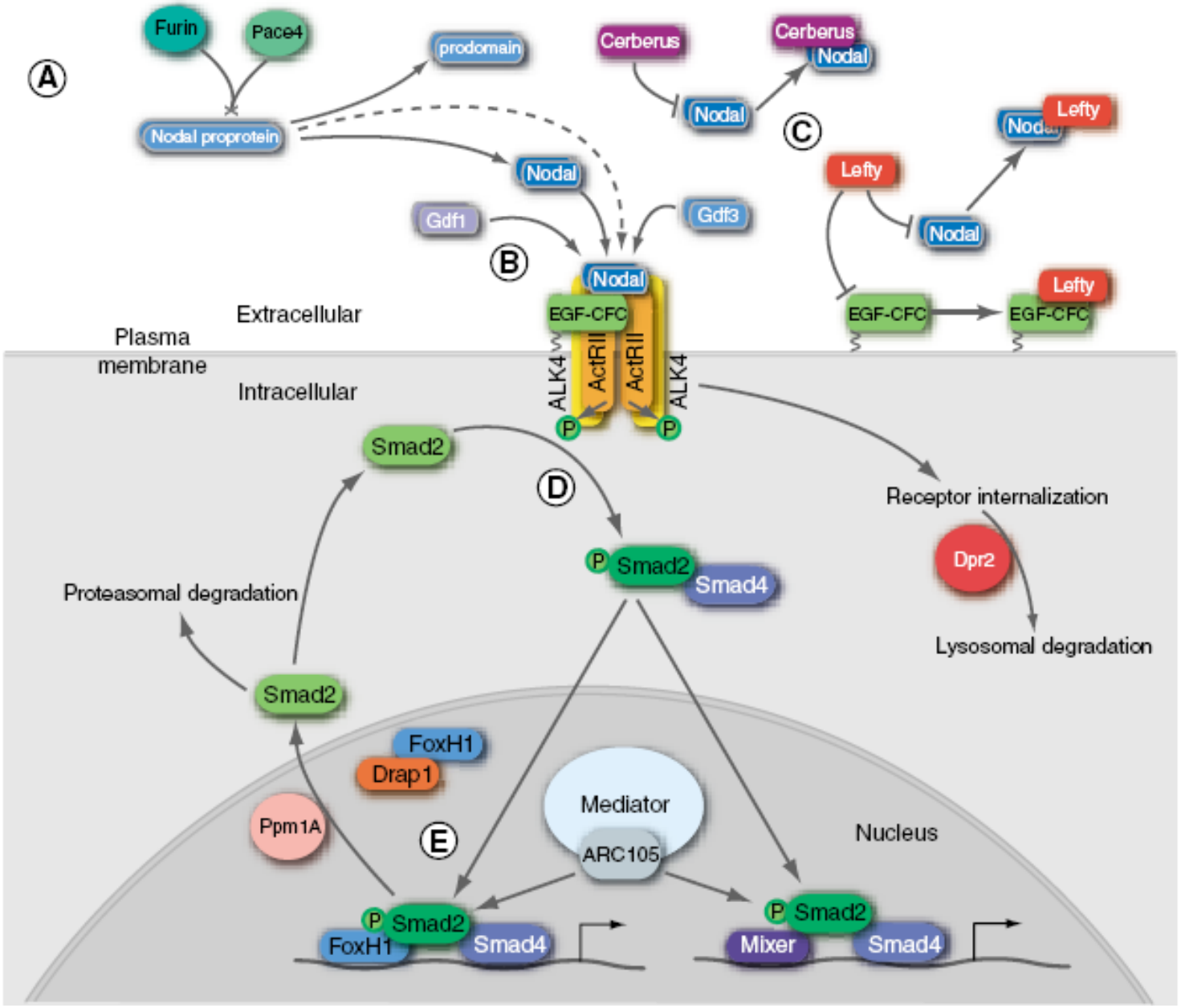
notochord (= chorda) produkuje faktory, které specifikují ektoderm a vedou ke tvorbě nervové ploténky (neural plate). Jde zejména o následující faktory: **noggin**, **chordin** a **follistatin** (inhibitory BMP a aktivinu). Samotná produkce těchto BMP inhibitorů specifikuje anteriorní (přední) nervovou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

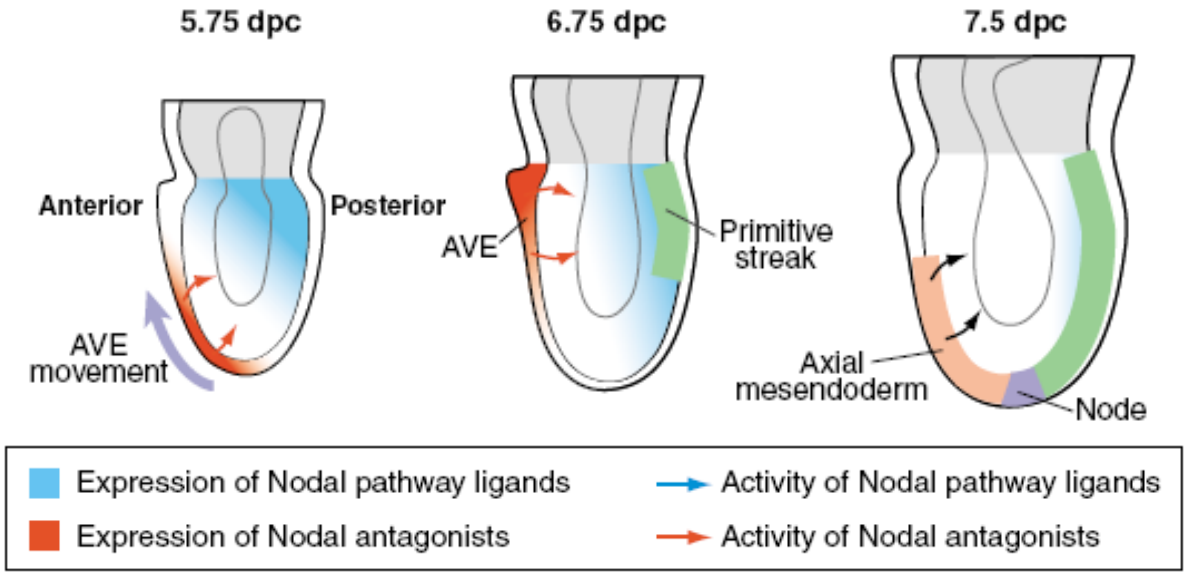
pool inaktivního proproteinu



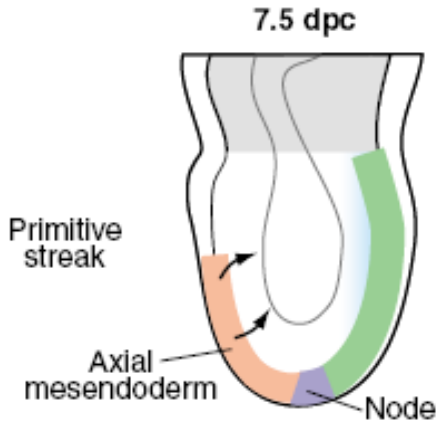
Nodal and left-right assymetry

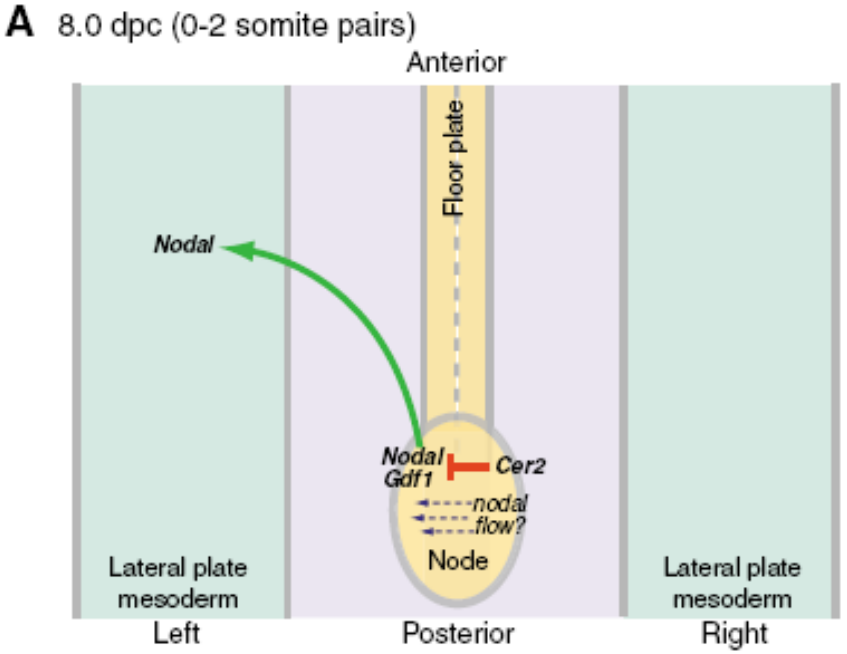
mouse gastrulation

AVE – anterior visceral endooderm



Nodal and left-right assymetry





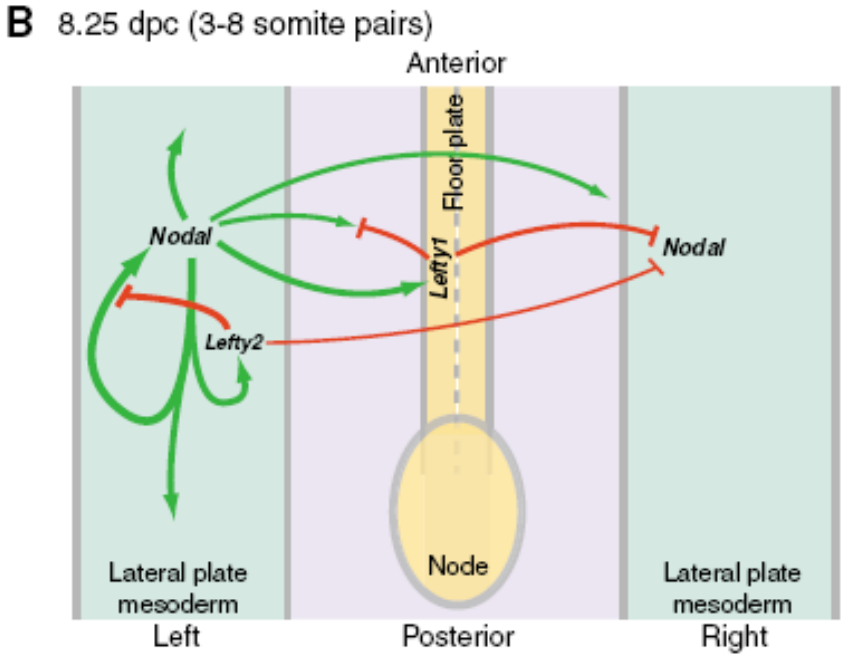
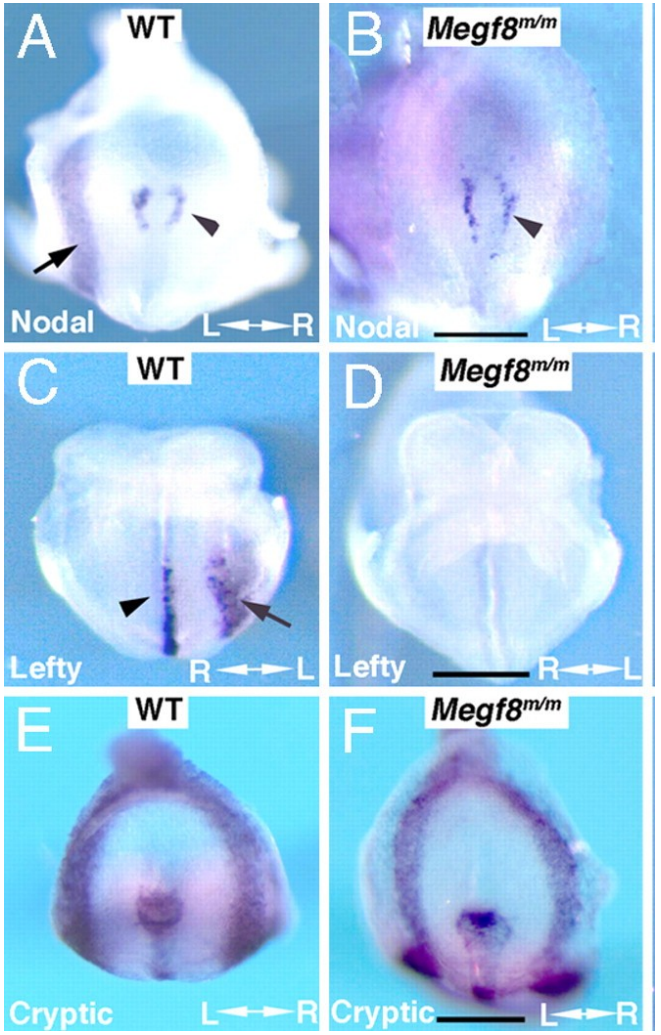


Fig. 5. Sequential function of Nodal signaling in left-right patterning in the mouse embryo. (**A**) Following initial symmetry breaking around the node, possibly as a consequence of ciliary-based nodal flow, Nodal (green arrow) and/or Gdf1 signals become elevated on the left side of the node, and are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

In situ hybridization analysis show altered expression of left determinant genes in Megf8m/m embryos.

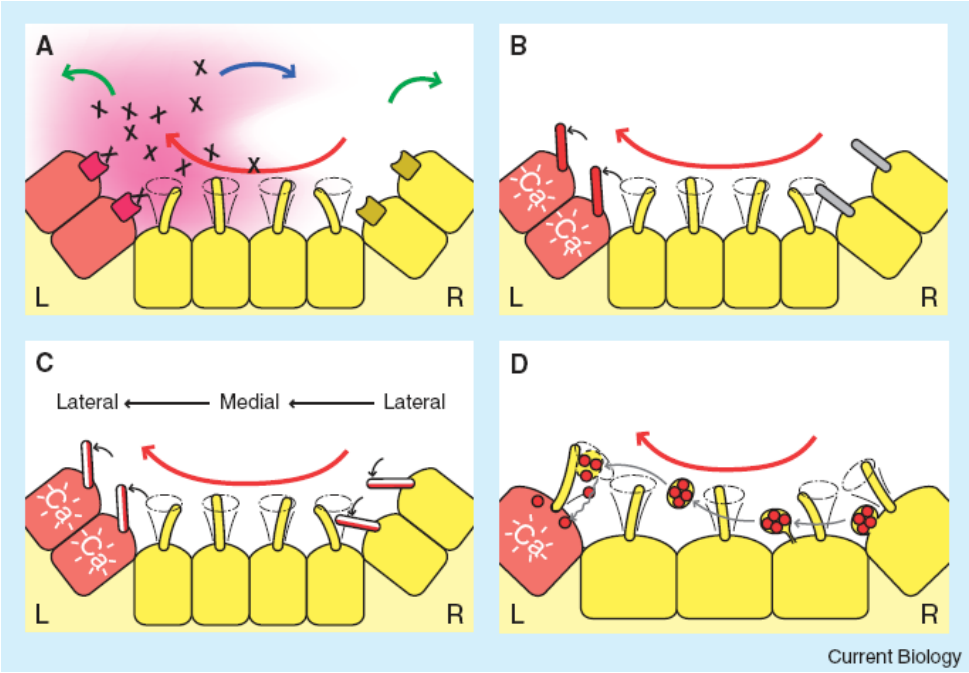


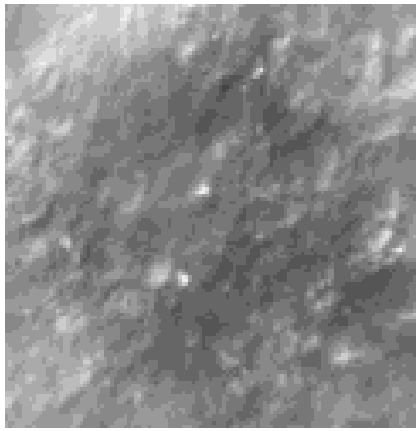
Zhang Z et al. PNAS 2009;106:3219-3224

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

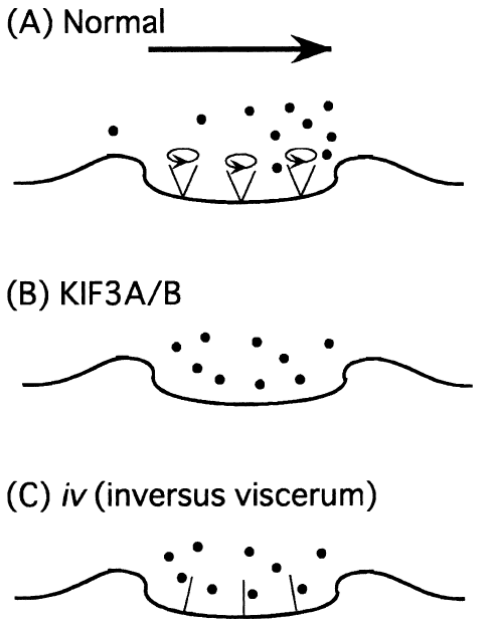




• narušená funkce cilií → vzniká až *situs inversus* (vnitřní orgány uspořádány obráceně podle střední osy těla) nebo *situs ambiguus*

Yokoyama, 2004

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



GDF1 mutanti

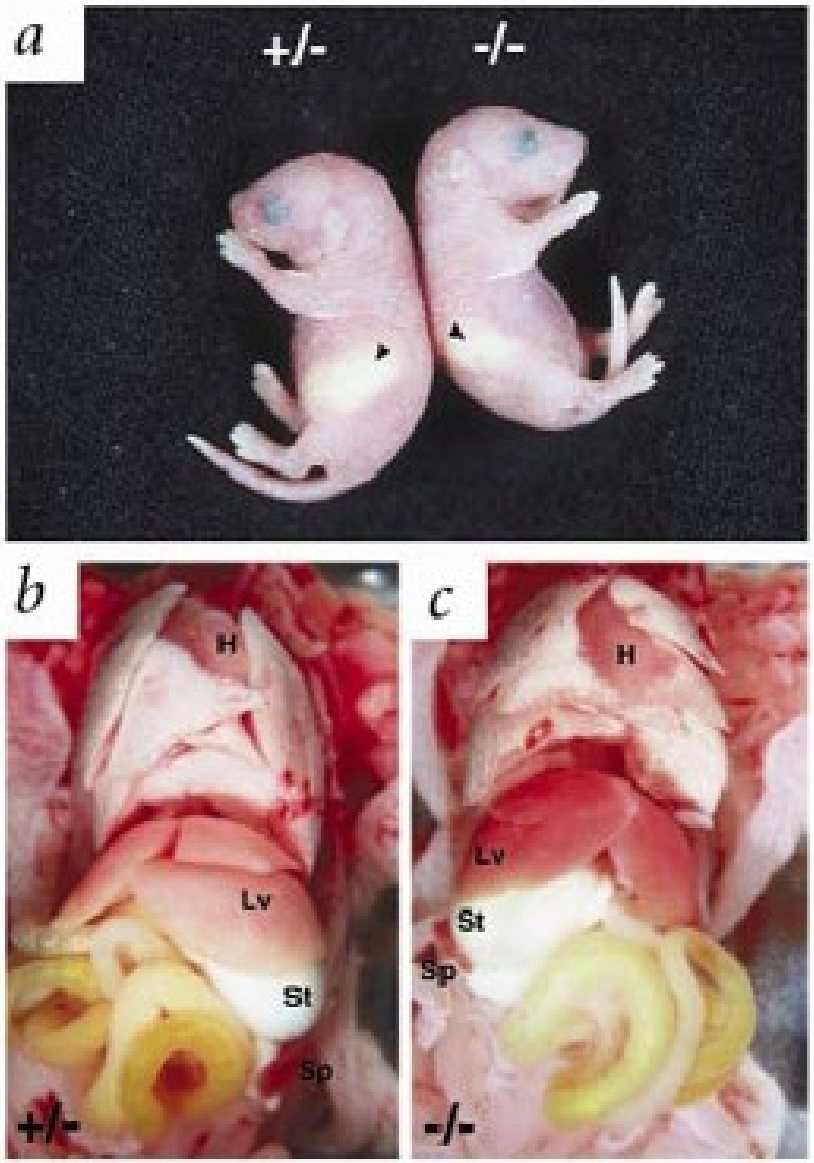
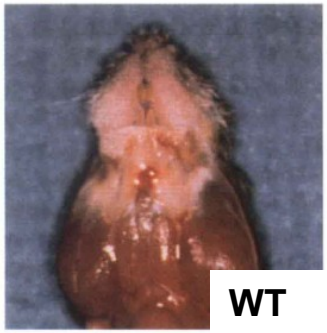
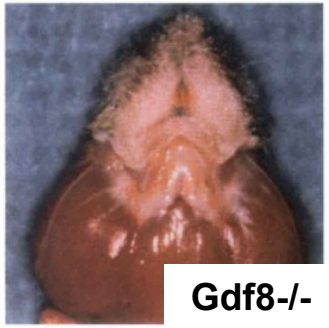


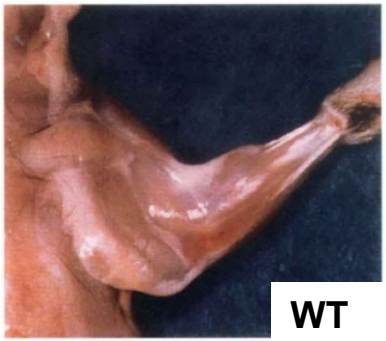
Figure 2. Analysis of *situs* defects in *Gdf1*-/- mice.
a, *Gdf1* +/- and *Gdf1* -/- newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1* +/- (*b*,*d*,*f*,*h*) and *Gdf1* -/- (*c*,*e*,*g*,*i*) mice are shown. ***b***,***c***, 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.

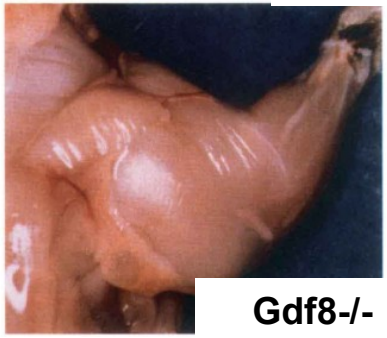
H, heart; Lv, liver; St, stomach; Sp, spleen; AC,

GDF8 (myostatin)

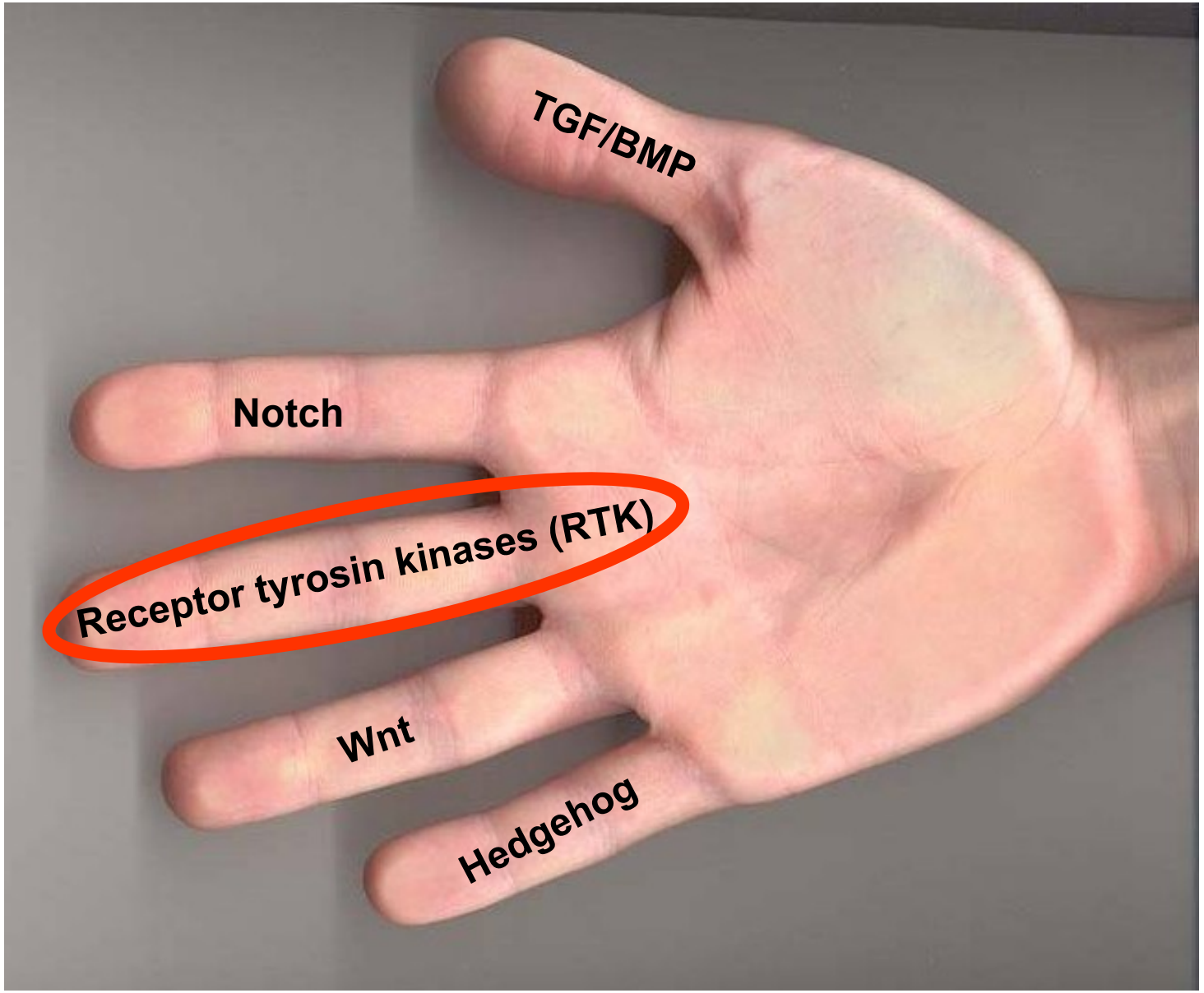




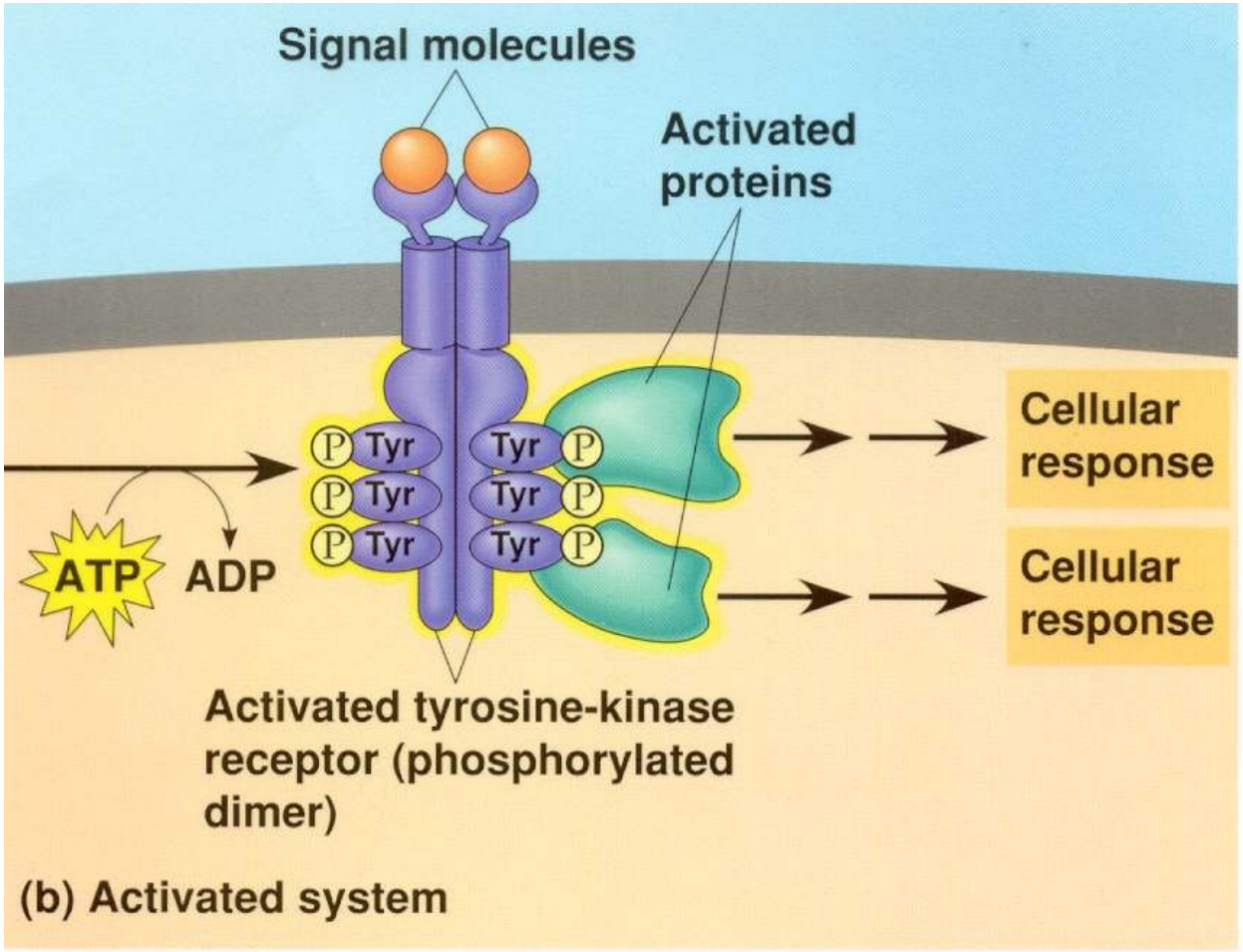




Signály z vnějšího prostředí



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

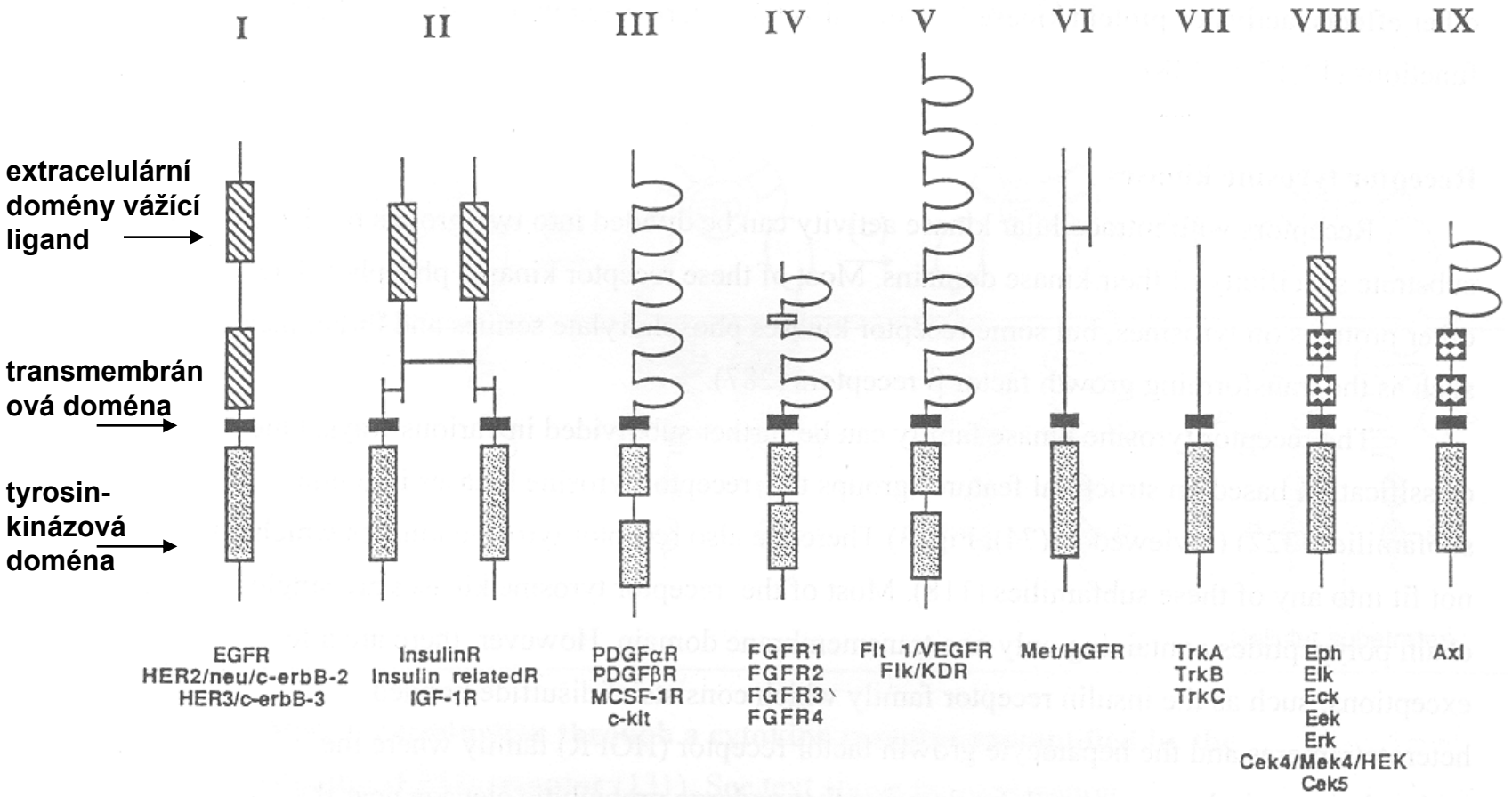
HGF (hepatocyte GF) receptor family

Trk receptor family

Eph receptor family

RET receptor family

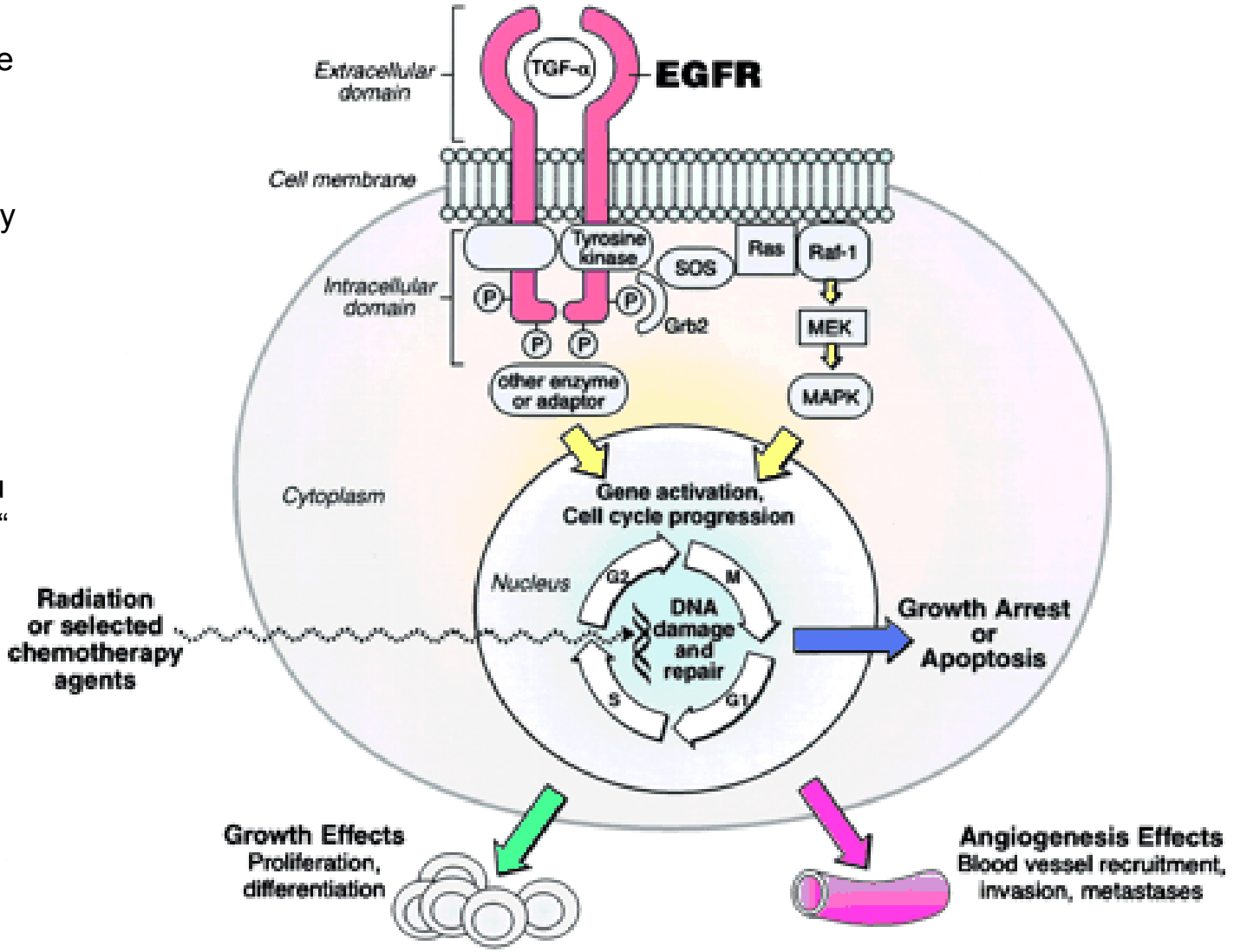
Schematická struktura jednotlivých receptorů



Obecné schéma aktivace RTKs

(zde na příkladu EGFR)

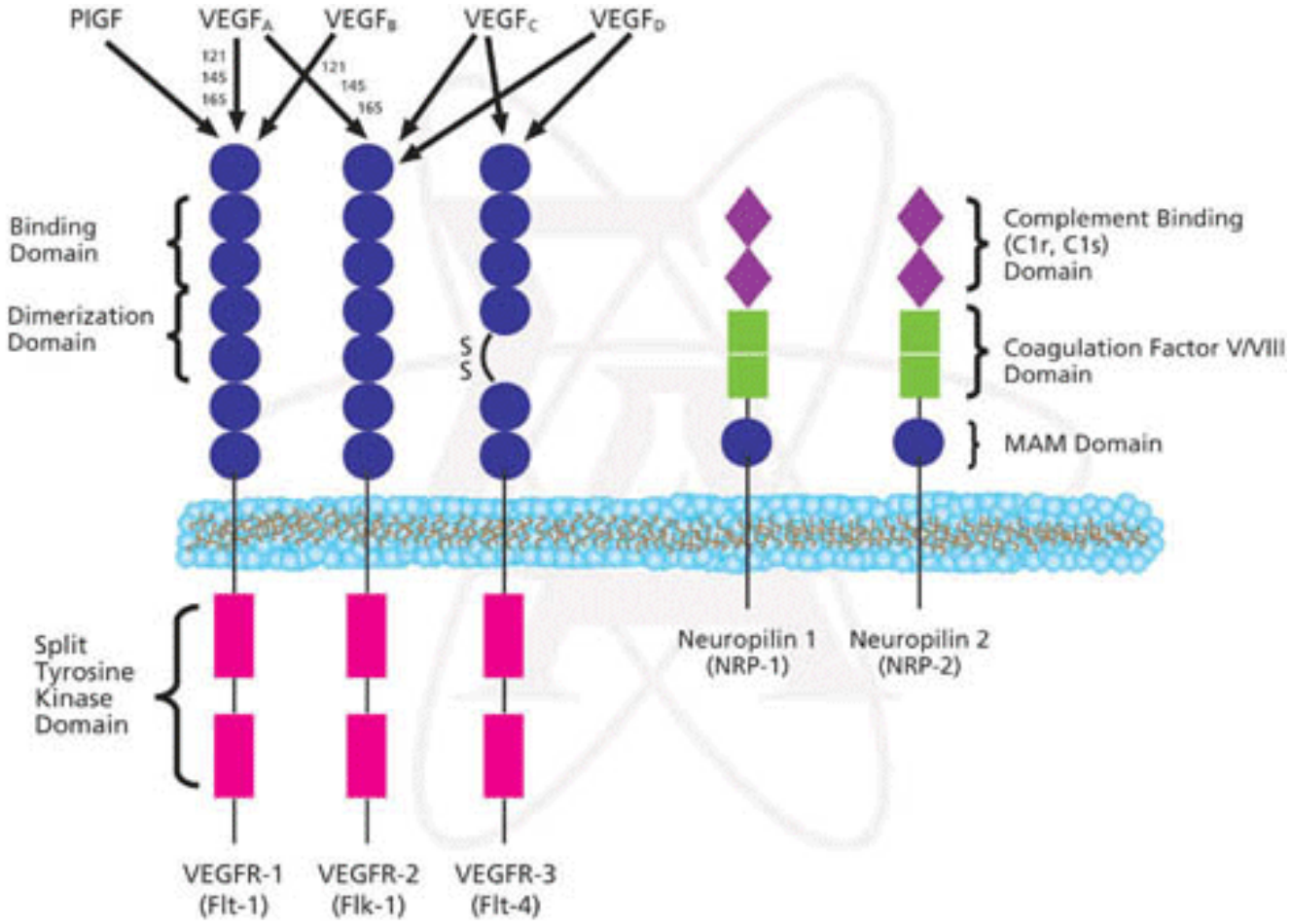
1. ligand se specificky váže na receptor
2. receptor dimerizuje
3. tyrosin-kinázové domény se navzájem fosforylují
4. autofosforylace vede k navázání (recruitment) adaptérových proteinů (zde Grb2)
5. v závislosti na receptoru se aktivují „downstream“ signální dráhy – zde např. Ras/Raf1/MEK/MAPK kinázová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



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

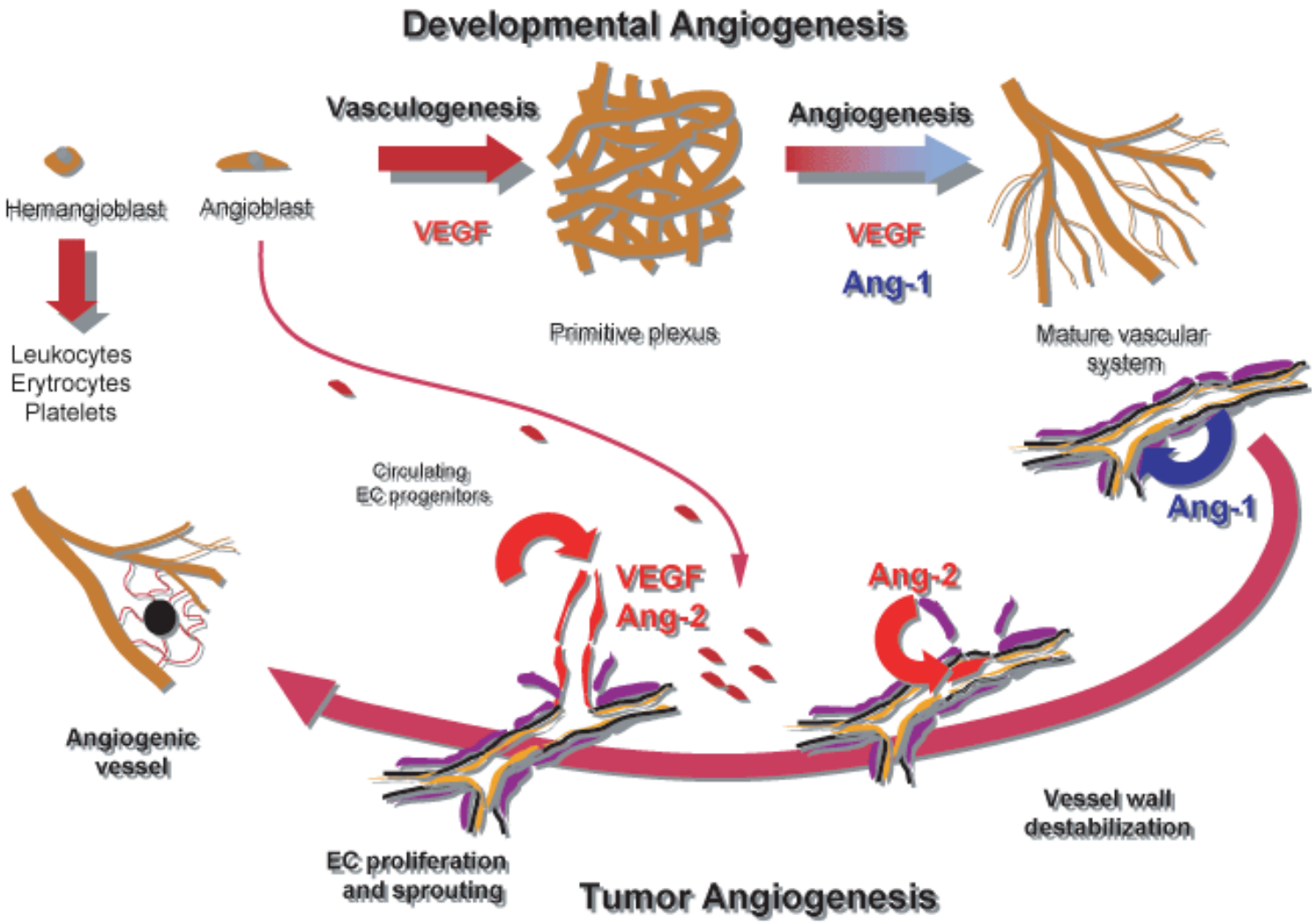
- VEGF/VEGFR
- ephrin/Eph
- PDGF
- Trk family
- FGF/FGFR – viz přednáška č. 9

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



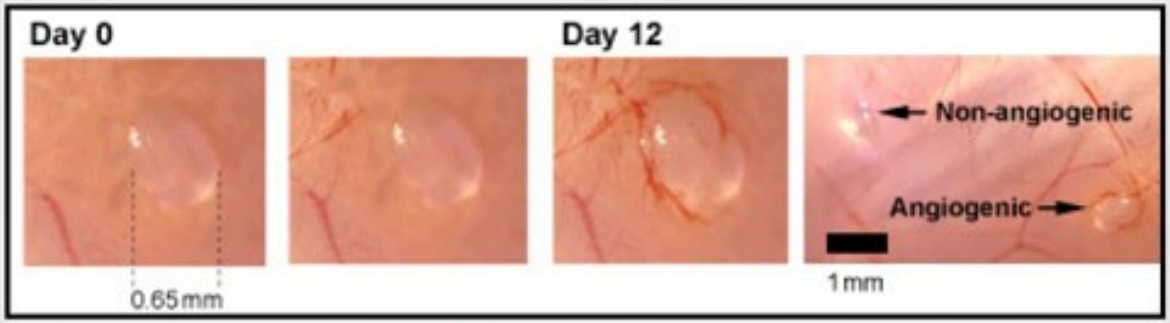
VEGF/VEGFR ve vývoji

- 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



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[1]

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

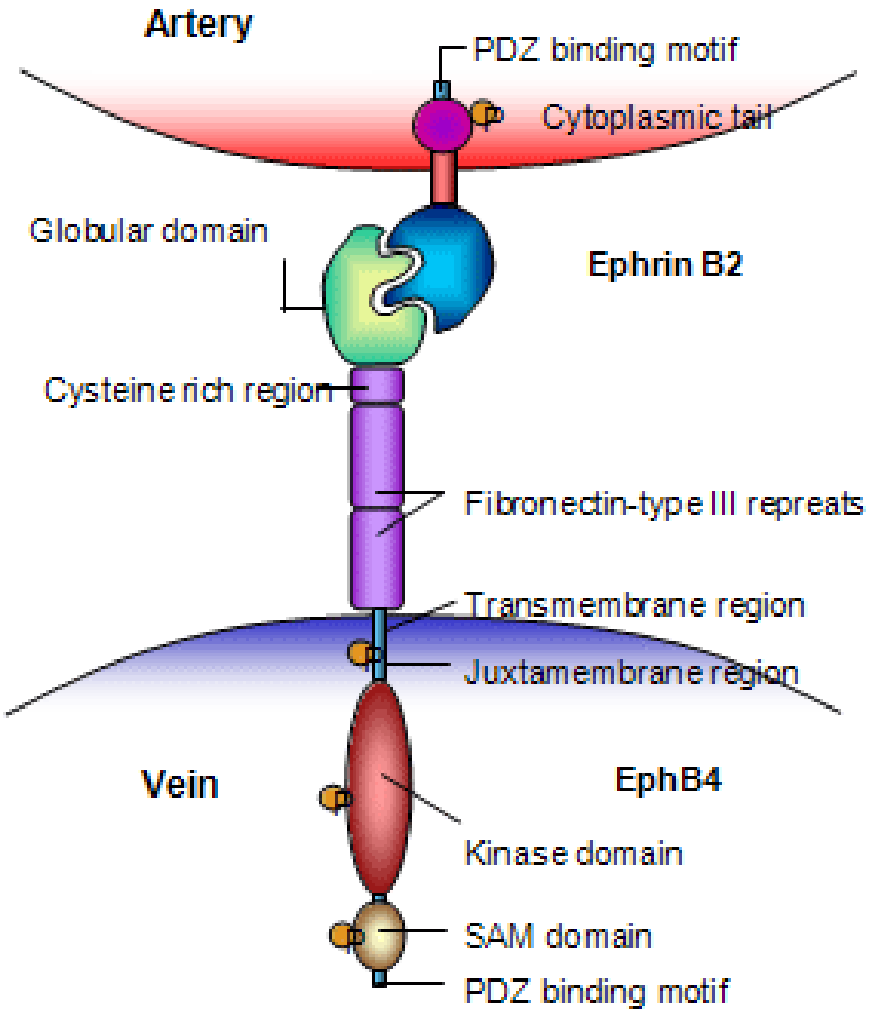
Functions of the VEGF family of receptors

VEGFR-1[1,2]	Crucial to embryonic angiogenesis Does not appear to be critical in pathogenic angiogenesis
VEGFR-2[1,3]	Most important VEGF receptor in tumor angiogenesis Mediates the majority of VEGF angiogenic effects
VEGFR-3[1,4]	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

Eph/ephrin

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

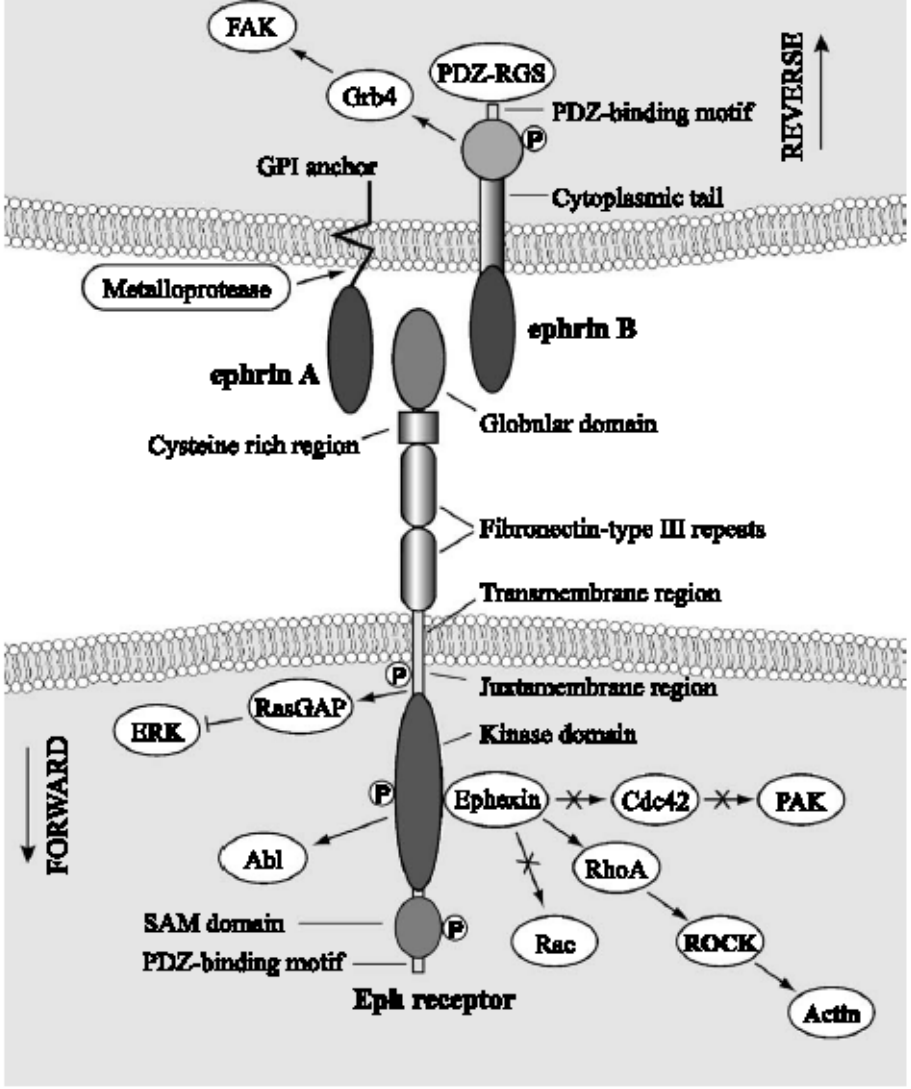


Fig. 1. Forward and reverse signaling by the ephrin–Eph complex. Glycosylphosphatidylinositol (GPI)-anchored ephrin-As bind to EphA receptors whereas the transmembrane ephrin-Bs bind to EphB receptors. The ephrin–Eph receptor binding initiate forward signaling in the Eph receptor bearing cells and reverse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

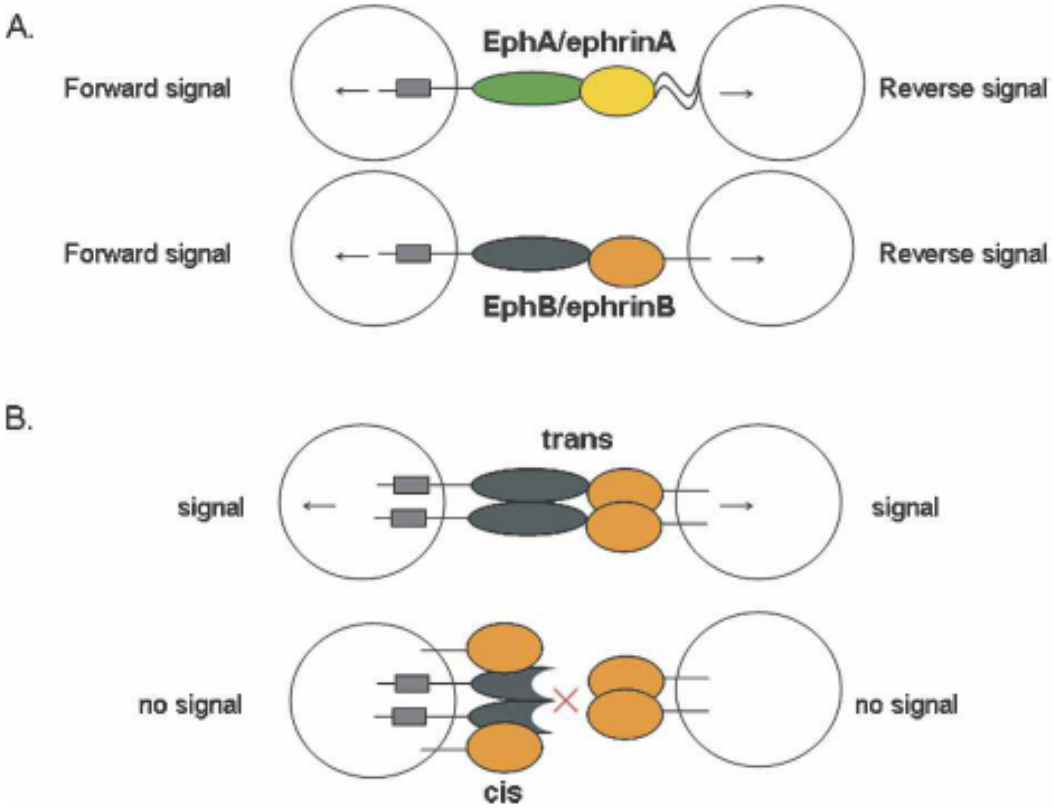


Figure 1. Main features of Eph/ephrin signaling. (*A*) Both classes of Eph receptors and ephrins activate bidirectional signaling. Interaction between Eph receptors and ephrins leads to activation of forward and reverse signaling in neighboring cells. (*B*) Eph receptors and ephrins expressed in opposing 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.

nB induced growth cone collaps

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).

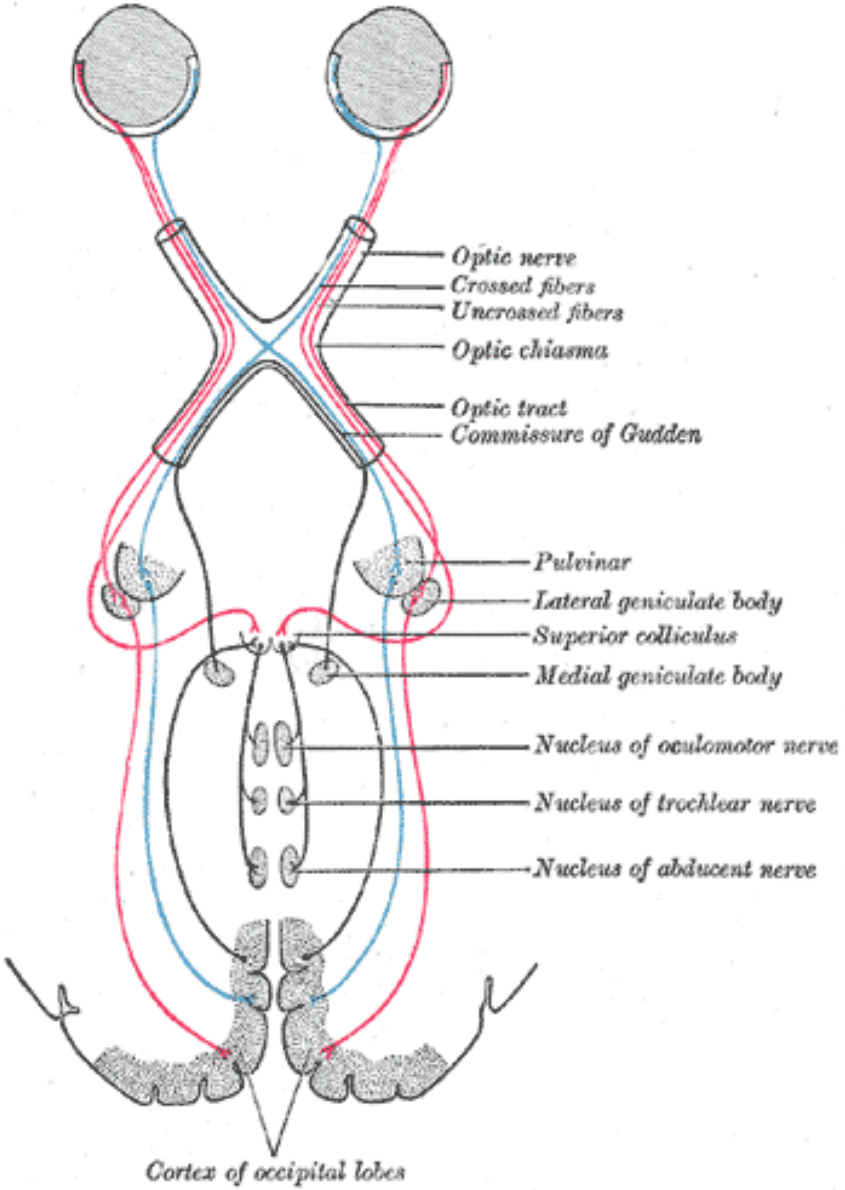
nB2 with Rho kinase inhibitor

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

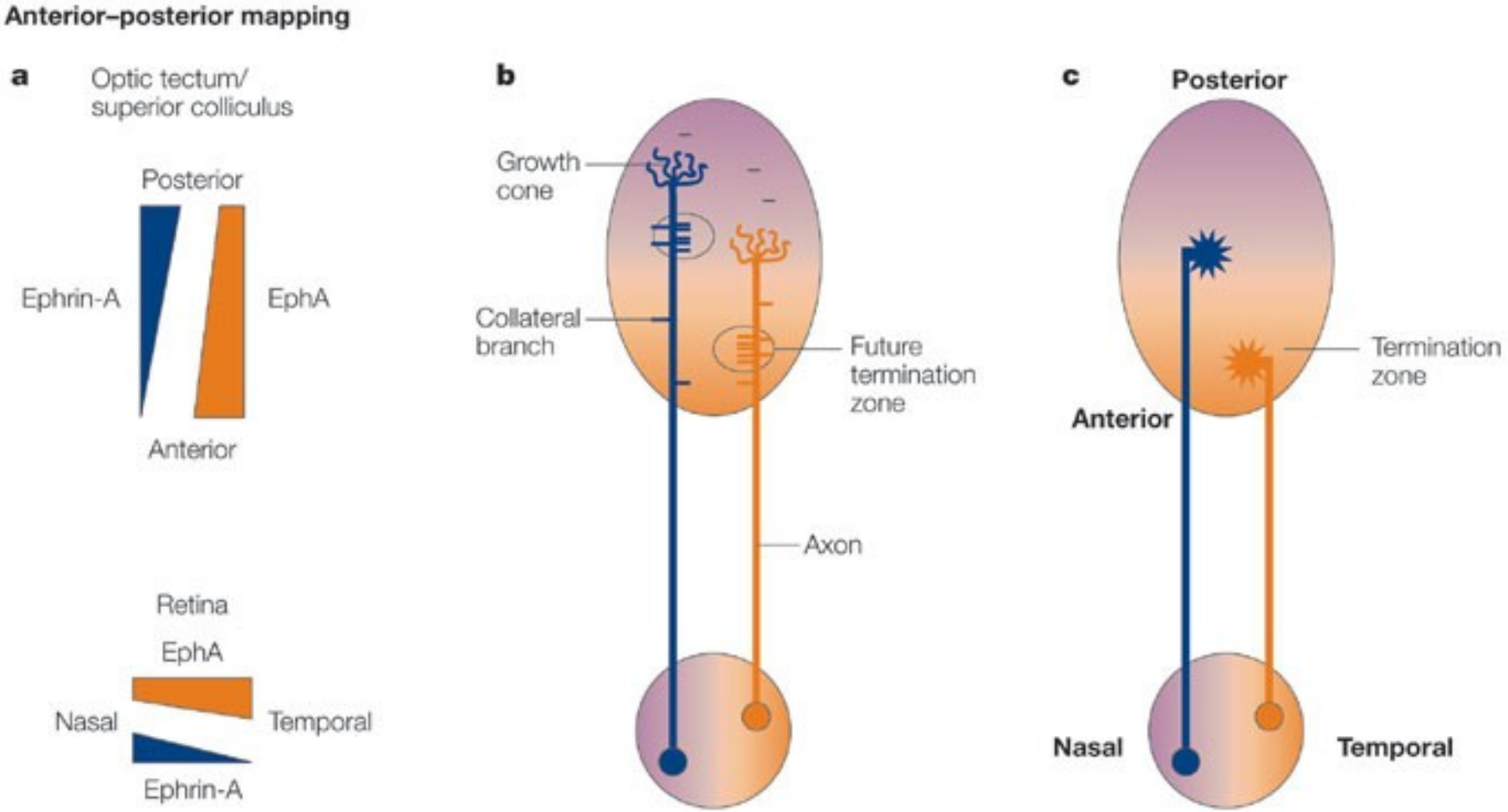
Eph/ephrin

- při „axon guidance“ tj. navádění jednotlivých axonů v nervovém systému (growth cone retraction)

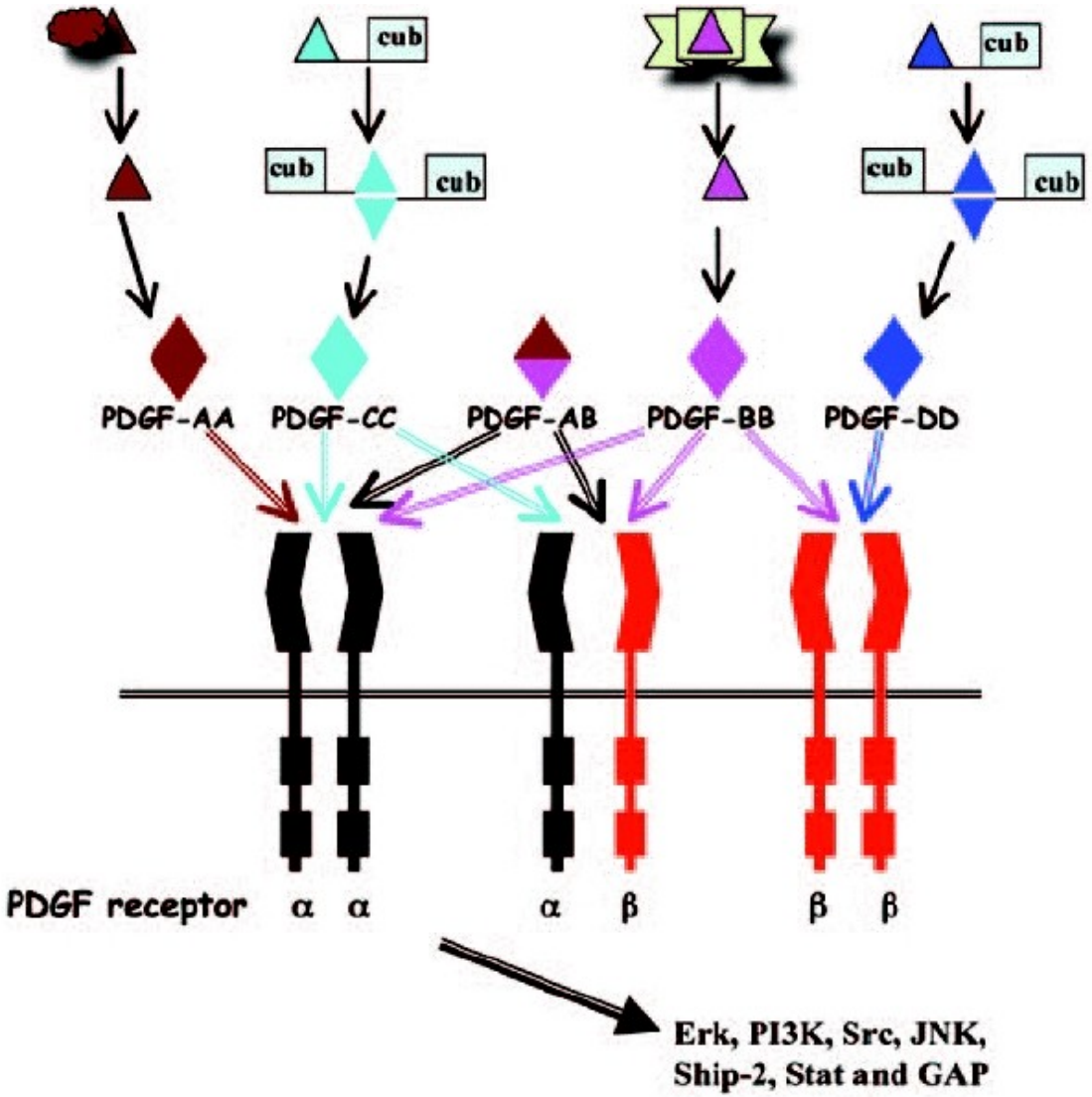


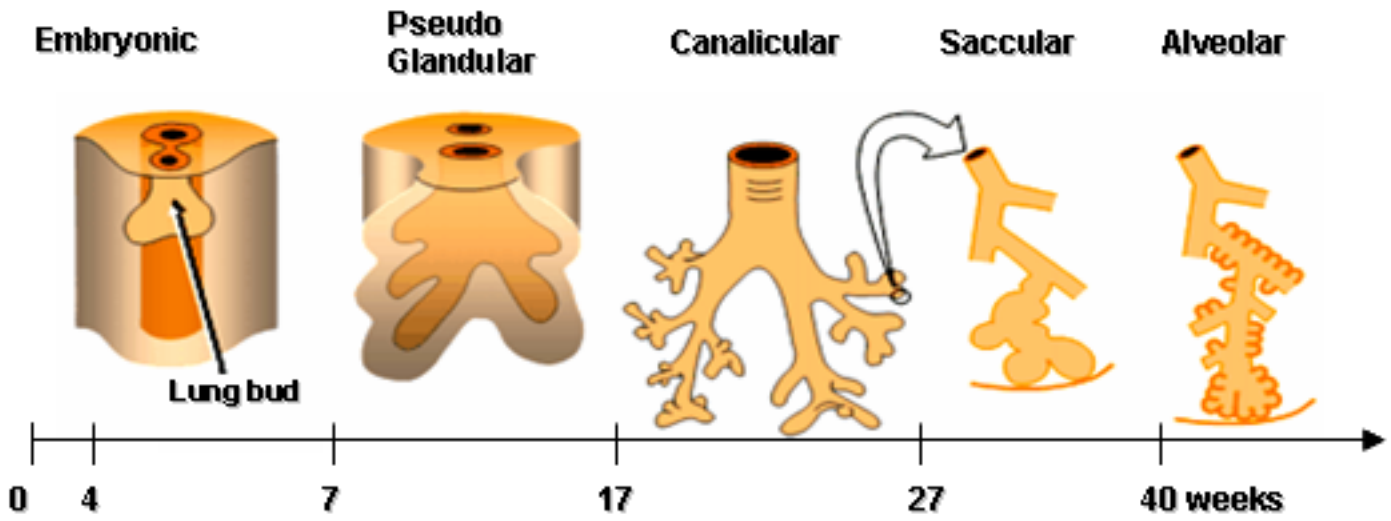
Eph/ephrin

- při „axon guidance“ tj. navádění jednotlivých axonů v nervovém systému (growth cone retraction)

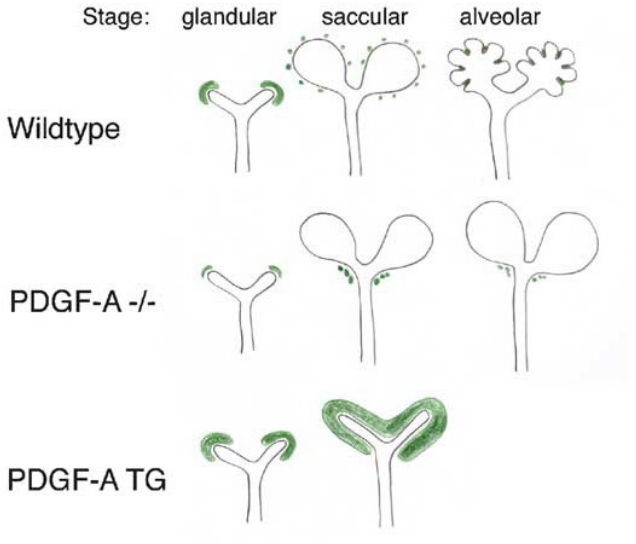


PDGF


cub
cub
cub
cub
cub
cub
PDGF-AA
PDGF-CC
PDGF-AB
PDGF-BB
PDGF-DD
PDGF receptor
α α
α β
β β
Erk, PI3K, Src, JNK,
Ship-2, Stat and GAP



The stages of pulmonary development



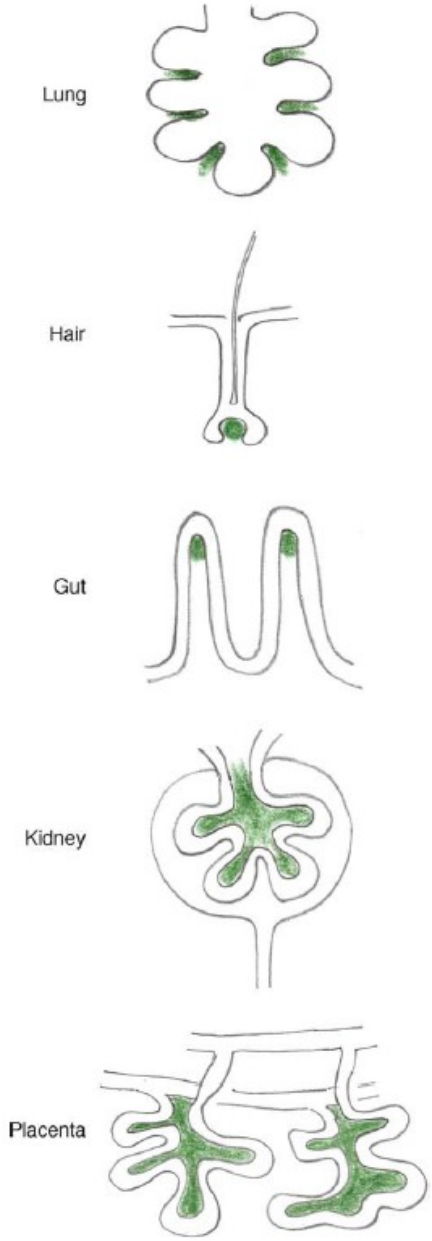
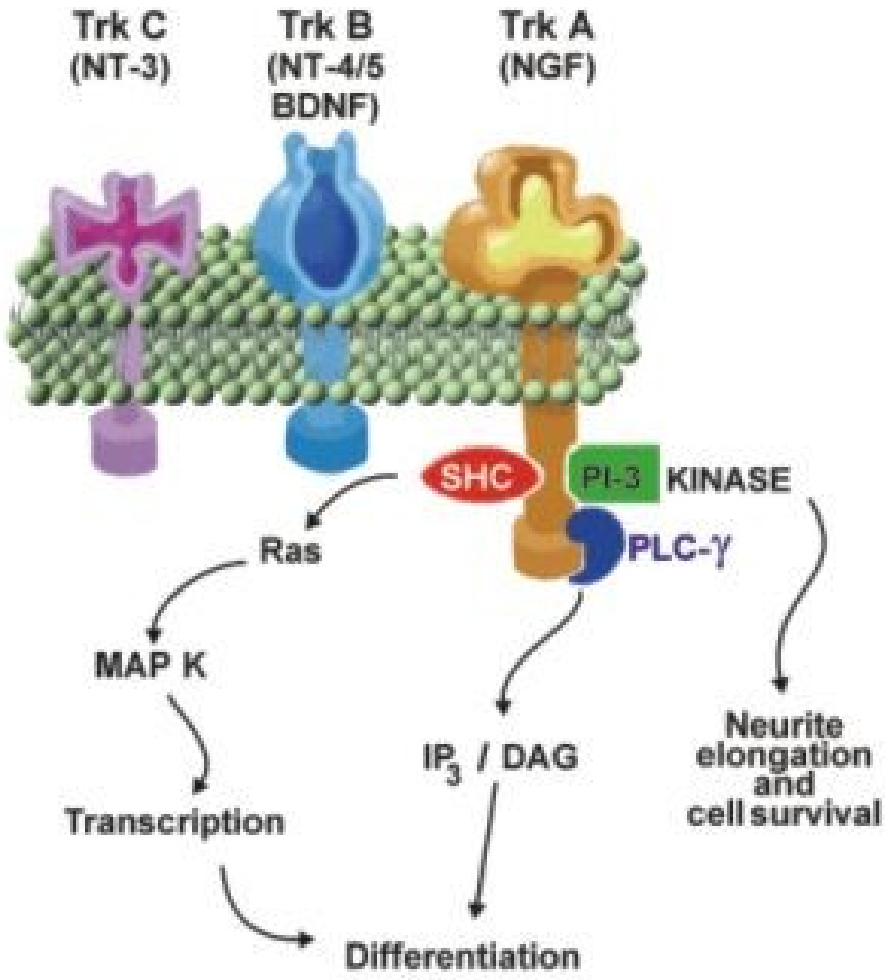


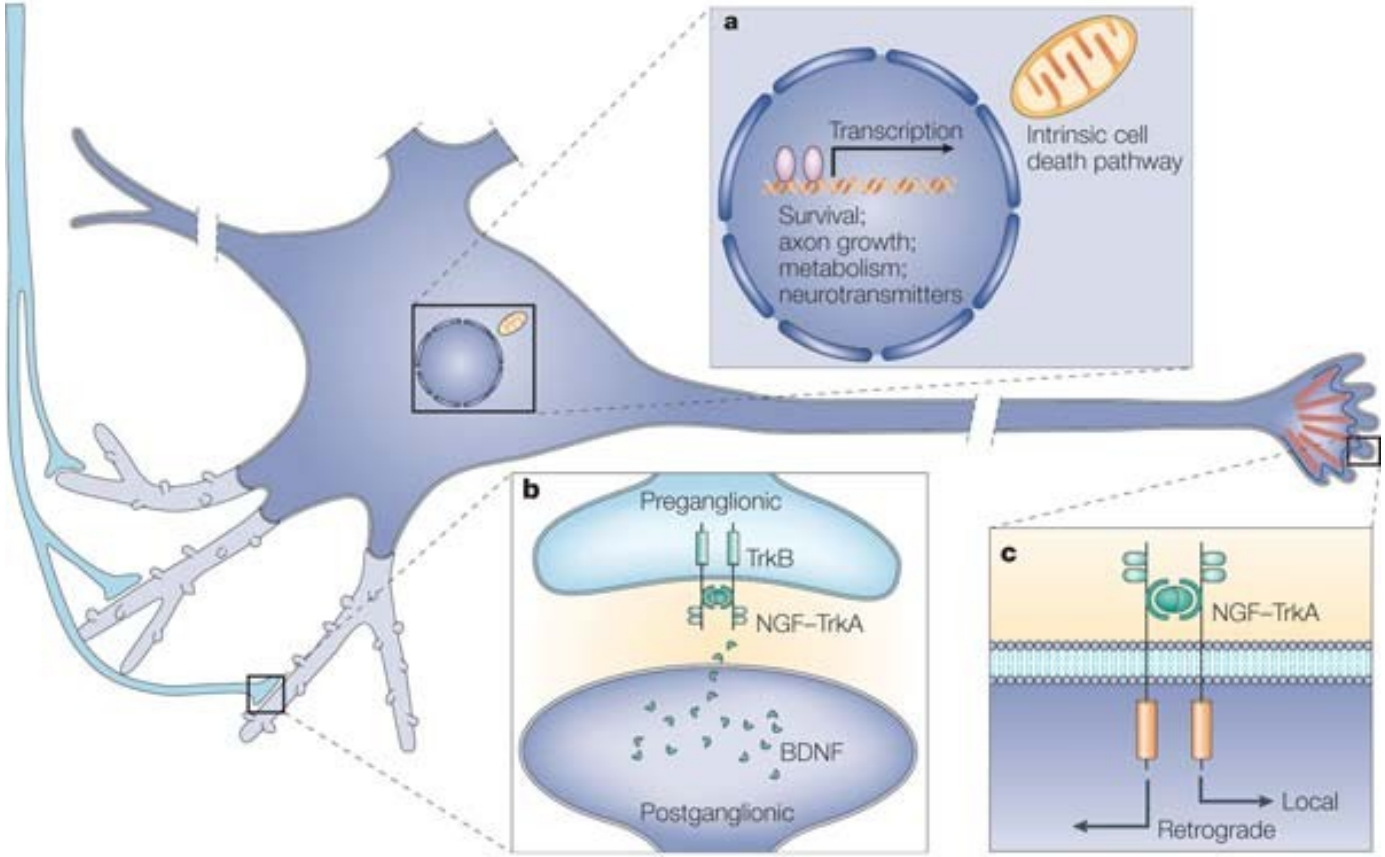
Fig. 5. Illustration of analogous epithelial or endothelial folding processes controlled by PDGFRα or PDGFRβ positive cells (green). The individual examples are discussed in the text.

Trk (tropomyosine-related kinase)

- tři receptory exprimované v nervovém systému – TrkA, TrkB a TrkC
- specificky váží neurotrofické peptidy – NT3-5, BDNF=brain derived neurotrofic 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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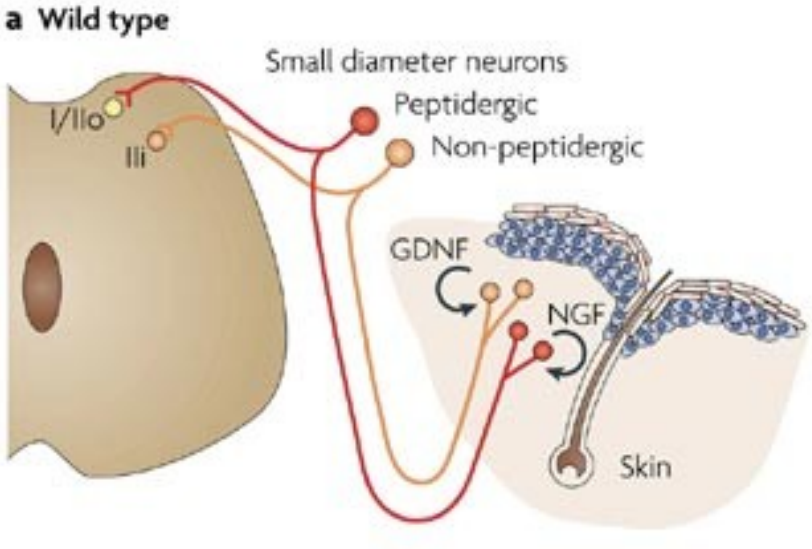
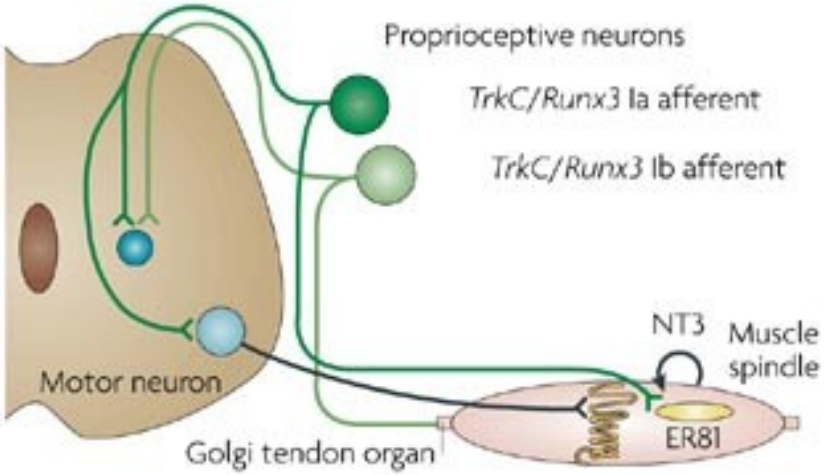
a | Neurotrophin signalling events initiated in developing axons signal retrogradely to neuronal cell bodies to inhibit intrinsic cell death pathways. These signalling events also influence transcriptional programs that are involved in cell survival, axon growth, synaptogenesis, metabolism and the establishment 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





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

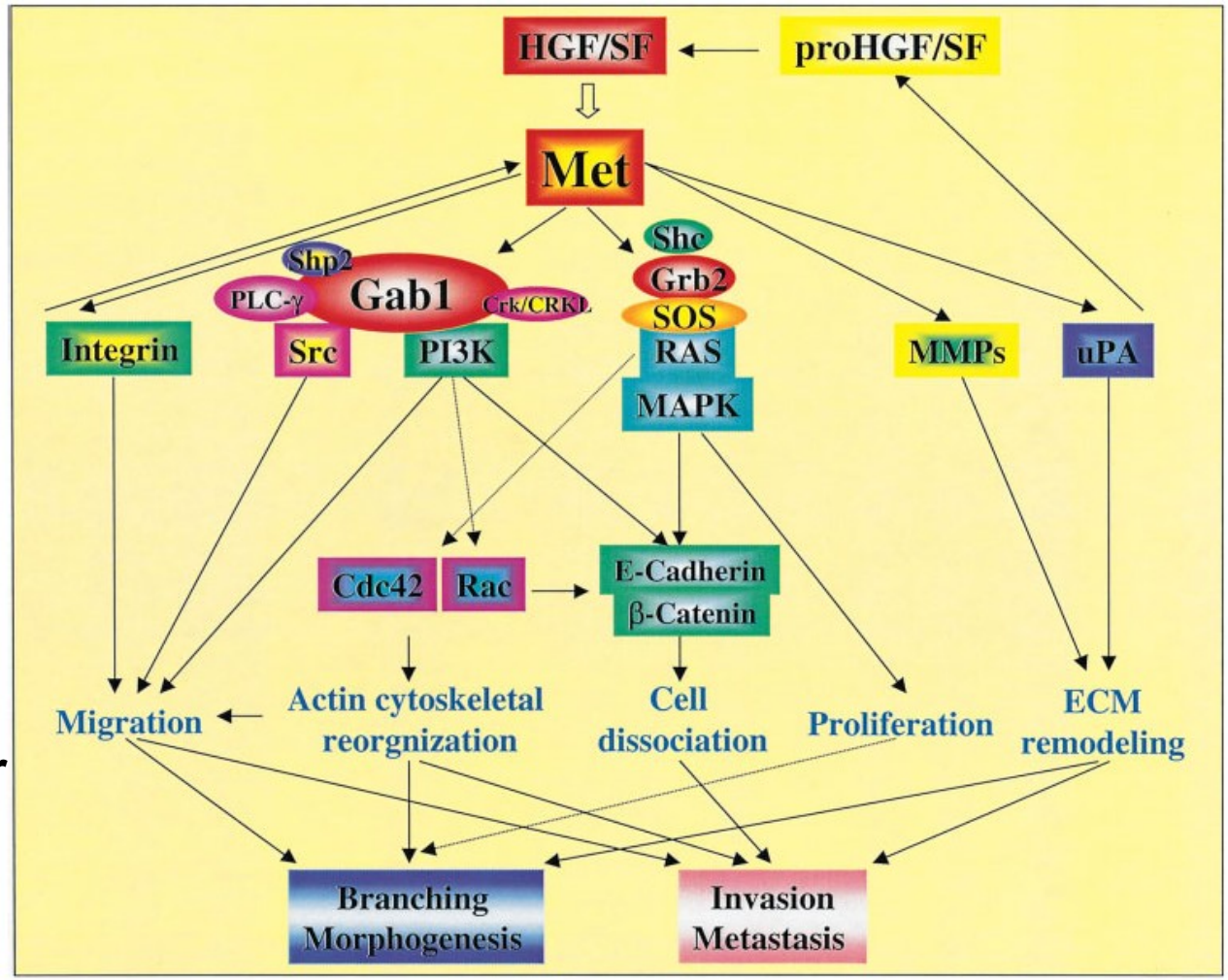


Fig. 1. Schematic representation of HGF/SF-Met signaling cascades in the control of branching morphogenesis and invasion. Upon HGF/SF binding, Met can activate a series of intracellular signaling pathways such as Ras/MAPK, PI3K, and Src through the two major adaptor molecules Grb2 and Gab1, which induces 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 reorgnization, 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.

- In the map that connects the retina to the optic tectum/superior colliculus in the midbrain, retinal ganglion cells in the nasal retina project their axons to the posterior part of the superior colliculus, whereas retinal ganglion cells in the temporal retina project to targets in the anterior colliculus (see figure **a**–**c**). Along the dorsoventral axis, ventral retinal axons project to the medial (dorsal) colliculus and dorsal retinal axons project to the lateral (ventral) colliculus (**d–f**)[5, 6, 33]. Eph receptors and ephrins that are distributed in gradients on both the projecting retinal axons and their targets (**a**,**d**) contribute to the specificity of the connections in several ways. First, the high levels of ephrin-A ligands in the posterior colliculus repel (-) retinal ganglion cell growth cones — particularly those from temporal retinal axons, which have higher EphA levels (**b**,**c**; axons are indicated in darker colours for visibility). Second, the lower ephrin-A levels in the anterior colliculus might stimulate axon extension[45]. Third, ephrin-As on nasal axons silence the co-expressed EphA receptors (regions of co-expression are indicated in blue and orange, resulting in purple), thereby sharpening the gradient of responsive EphA receptors[28, 33]. Fourth, ephrin-As in the colliculus suppress collateral branches in the part of the axons between the growth cone and the future termination zone[43]. Ephrin-B1, which is more concentrated in the medial colliculus, does not seem to affect the growth cones of retinal axons[37] (**e**,**f**). However, it attracts (+) medially the collateral branches from EphB-positive ventral retinal axons that are lateral to the future termination zone[38]. Ephrin-B1 might also repel (-) collateral branches from ventral axons that are medial to the future termination zone, causing them to grow laterally. So, ephrin-B1 might mediate attraction at concentrations that are lower than in the termination zone and repulsion at higher concentrations.