Fyziologie buň. systémů

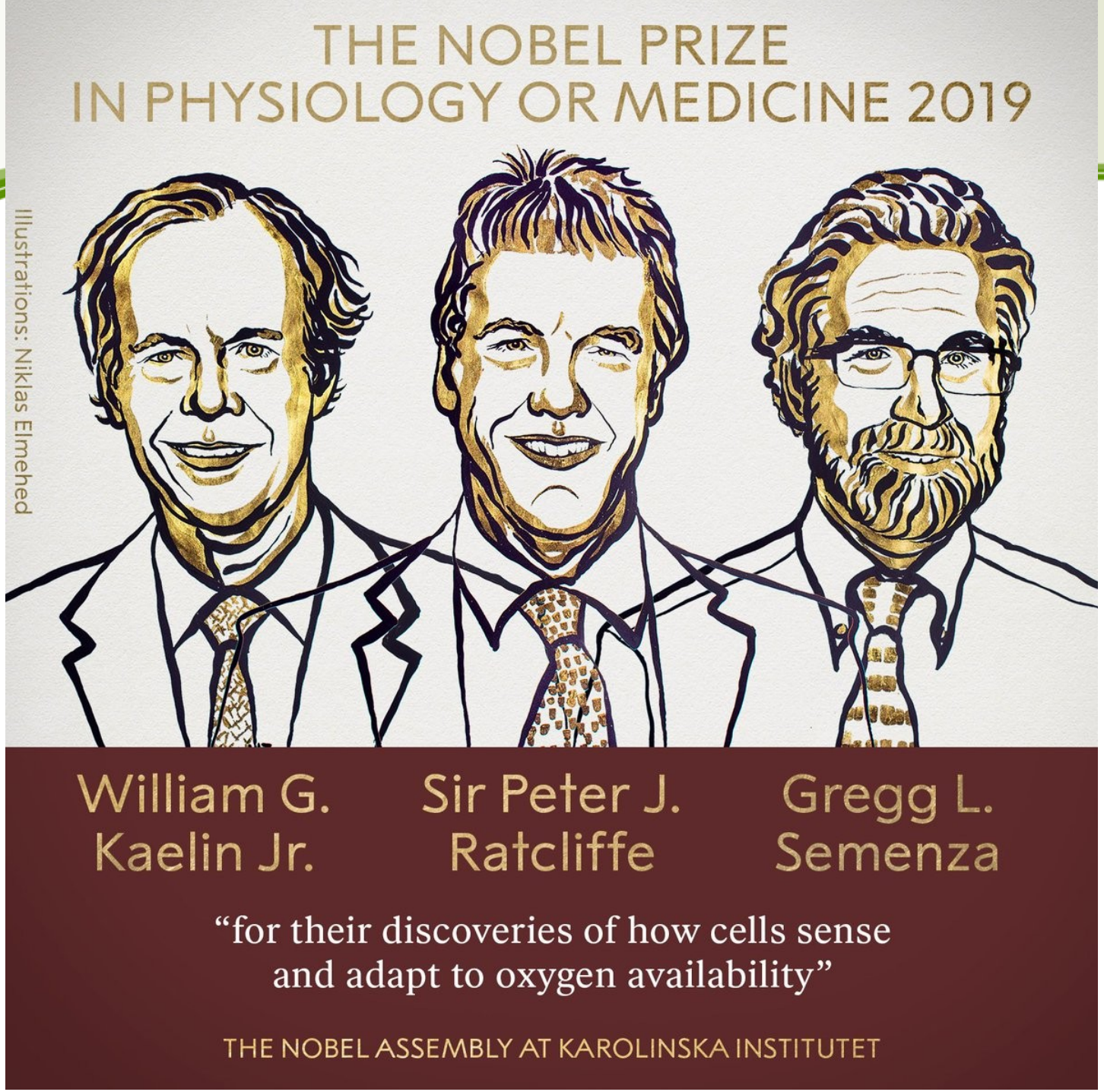
Buněčné regulace III:

Reakce na změny chemických a fyzikálních parametrů prostředí

Vítězslav Bryja

Reakce tkání na změny v dostupnosti kyslíku a regulace angiogeneze

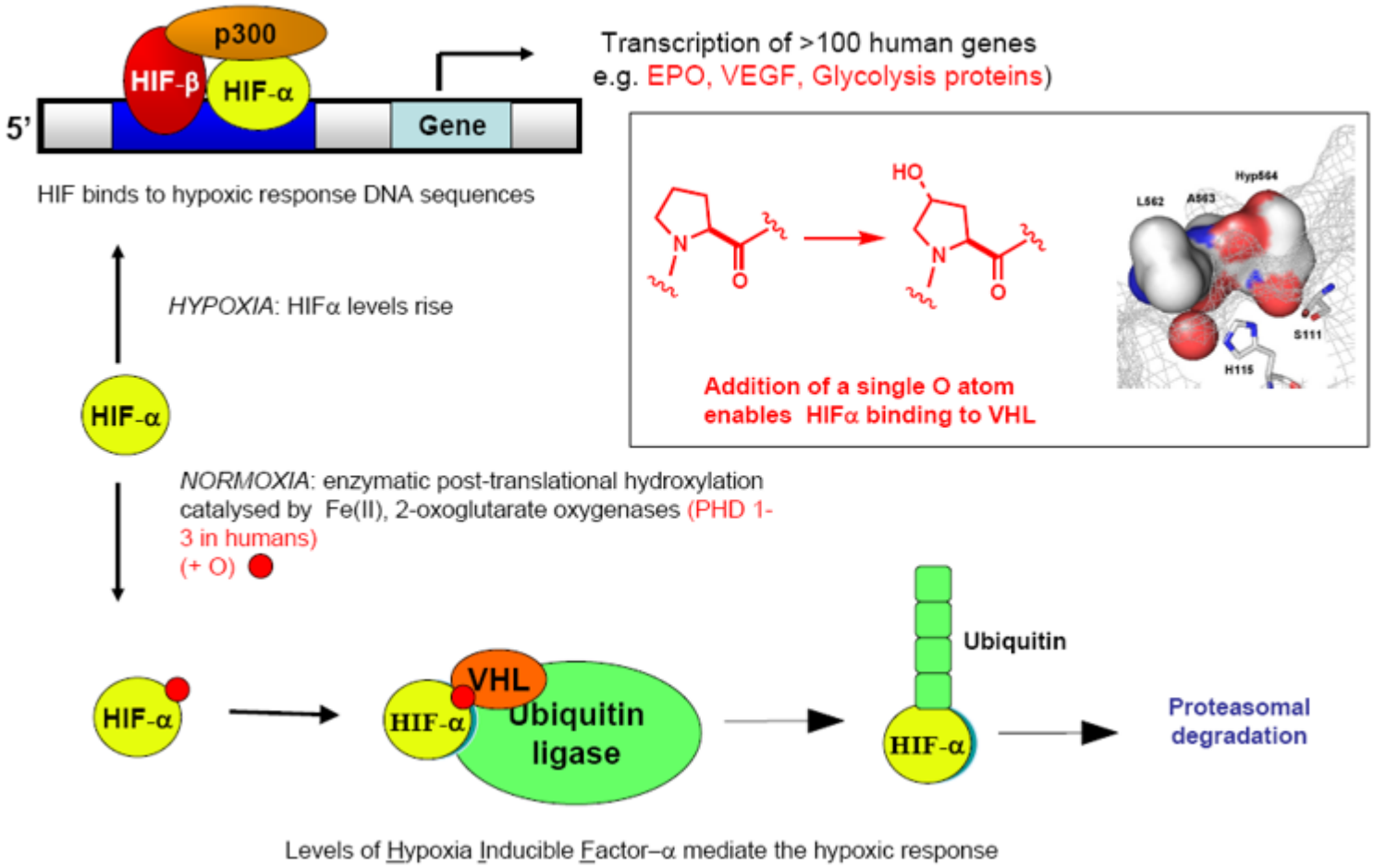
- A) Detekce nedostatku kyslíku - Hypoxia inducible factor (HIF)
- B) iniciace angiogeneze - vaskulární endotheliální růstový faktor VEGF/VEGFR
- C) buněčné mechanismy angiogeneze (role Notch a angiopoetinové signalizace)


THE NOBEL PRIZE
IN PHYSIOLOGY OR MEDICINE 2019
Illustrations: Niklas Elmehed
William G. Kaelin Jr.
Sir Peter J. Ratcliffe
Gregg L. Semenza
“for their discoveries of how cells sense and adapt to oxygen availability”
THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET

Hypoxie a HIF

- O_2 se difuzí šíří asi na 150 μm
- **Hypoxie**: snížený parciální tlak O_2 ve tkáni **X** normoxie
- **HIF** – Hypoxia-Inducible Factor:
  - Heterodimerický transkripční faktor 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 – význam hydroxylace prolinu

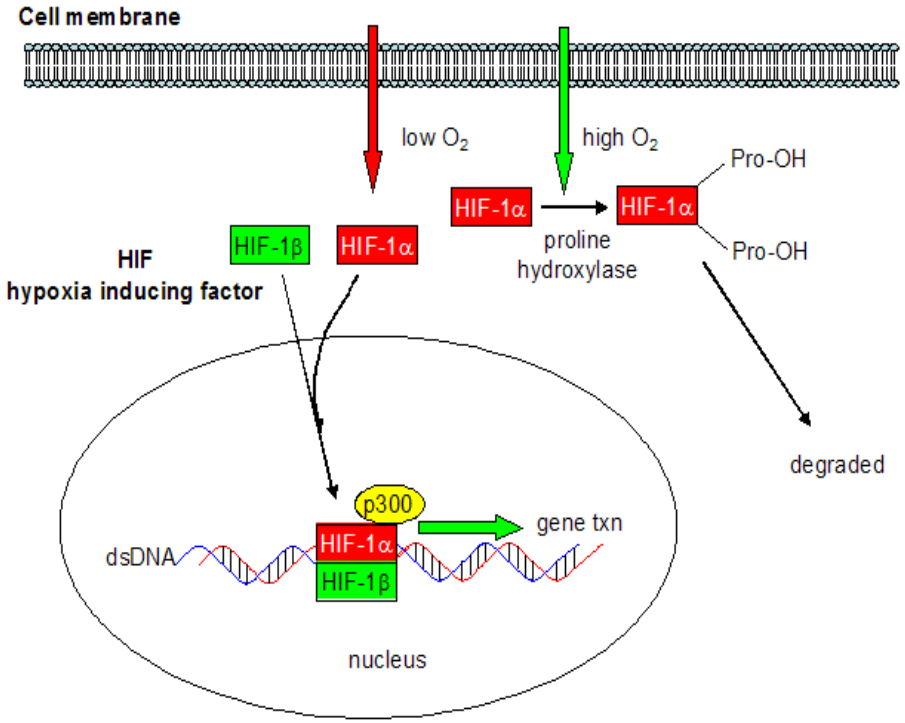


VHL (von Hippel-Lindau) - tumor supresorový gen

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

Modelové 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

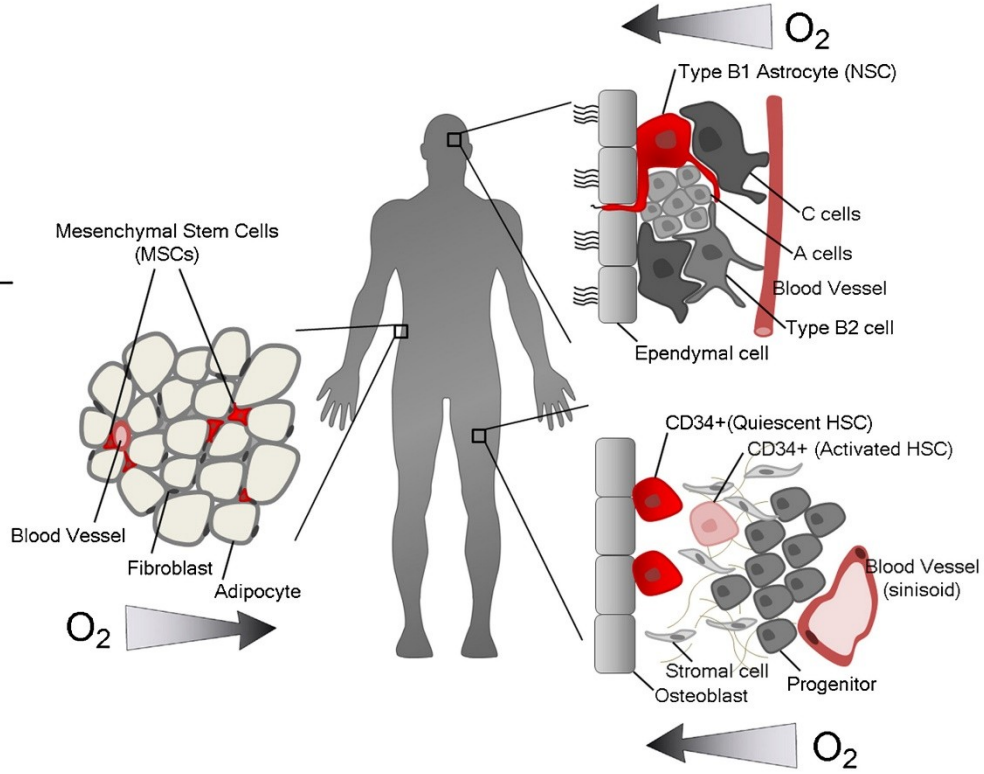
After: Science, 303, pg 1454 (2004)

Hypoxie je přítomna/reguluje niku kmenových buněk

The Mesenchymal Stem Cell Niche

2-8% O_2

Kofoed et. al., 1985
Harrison et. al., 2002
Matsumoto et. al., 2005
Pasarics et. al., 2009



The Neural Stem Cell Niche

<1-8% O_2

Dings et. al., 1998
Erecinska and Silver, 2001
Panshision, 2009

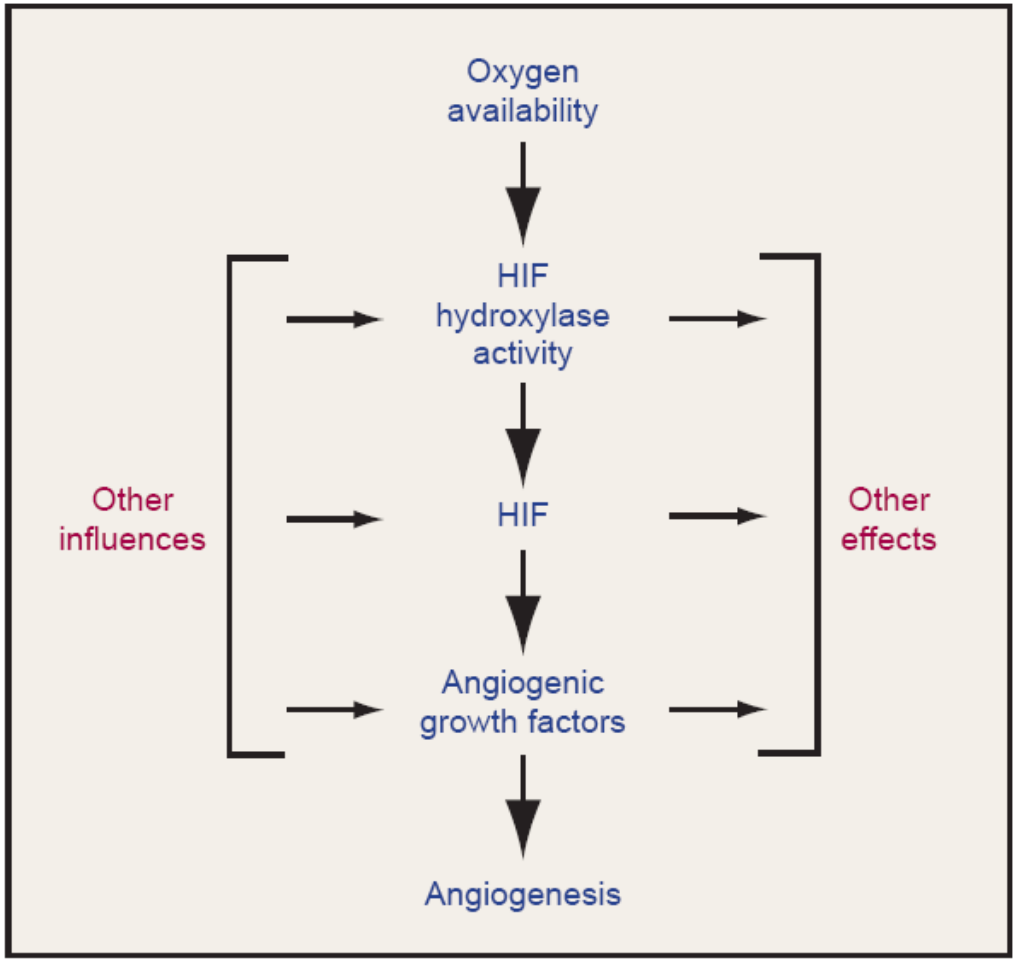
The Hematopoietic Stem Cell Niche

1-6% O_2

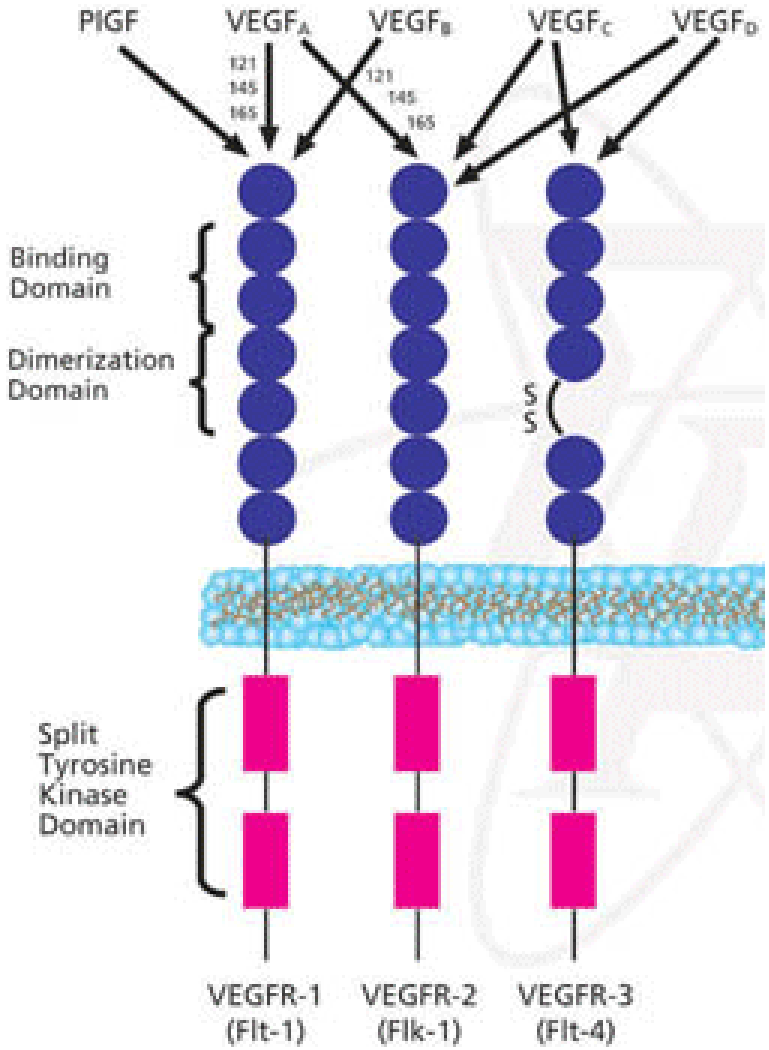
Grant and Root, 1947
Cipolleschi et. al., 1993
Chow et al., 2010
Eliasson and Jonsson, 2010

Angiogenese

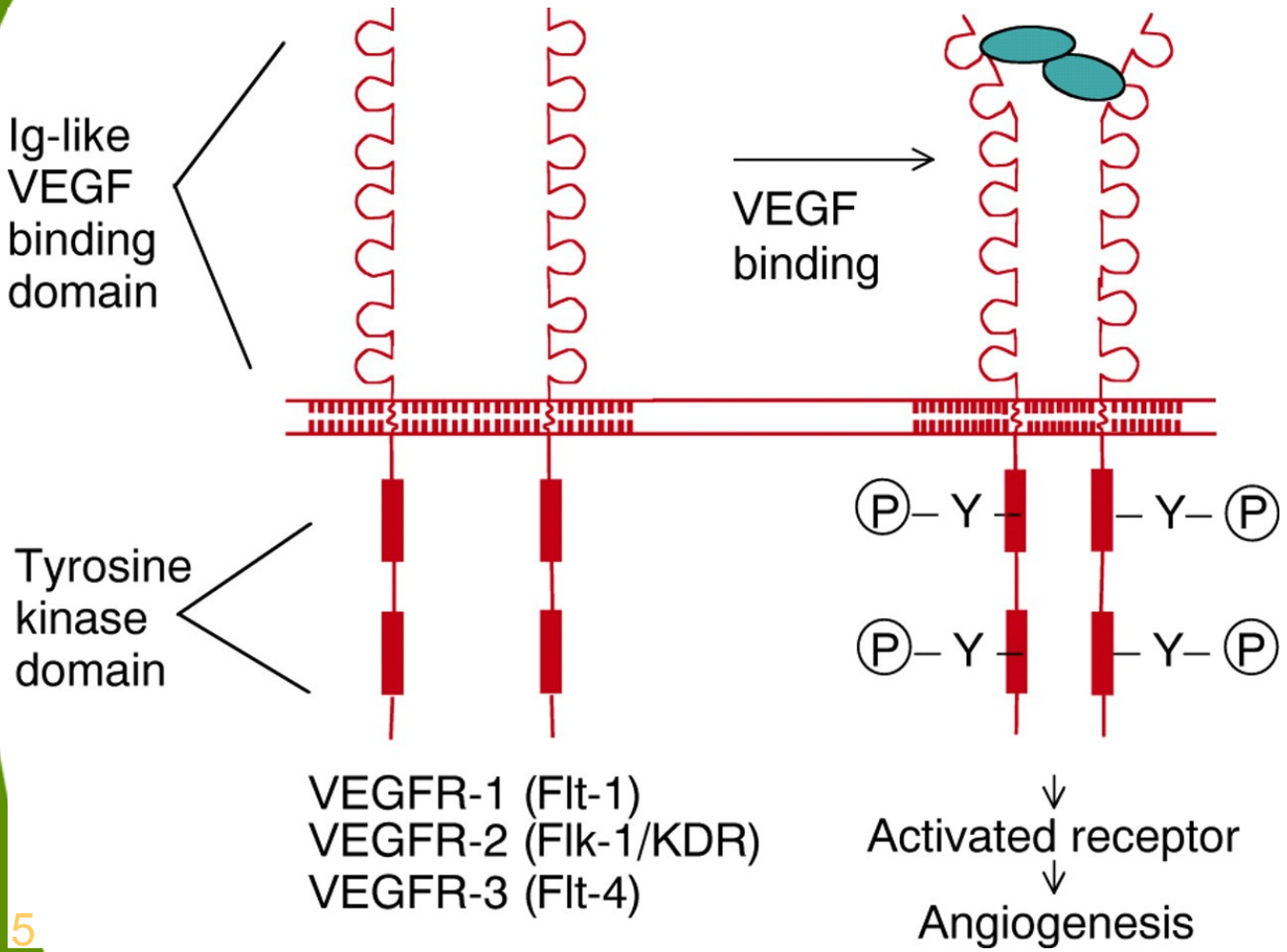
- Angiogenese
  - tvorba nových krevních cév
- **HIF** se váže do oblasti promotoru a iniciuje transkripci receptoru **VEGFR 2** i expresi **VEGF**(Vascular Endothelial Growth Factor)
  - hlavní faktor angiogenese
- v normálním vývoji ale i během nádorového růstu



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



VEGFR2


Ig-like
VEGF
binding
domain
Tyrosine
kinase
domain
VEGF
binding
P– Y
– Y– P
P– Y
– Y– P
VEGFR-1 (Flt-1)
VEGFR-2 (Flk-1/KDR)
VEGFR-3 (Flt-4)
Activated receptor
Angiogenesis

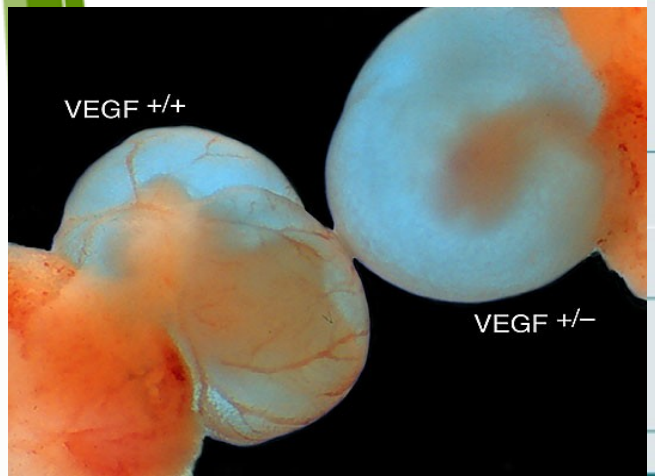
Obr. 5

VEGF/VEGFR ve vývoji

- reguluje vznik a vývoj cévní soustavy
- master regulátor angiogeneze (vývoje cév)
- 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

Shrnutí

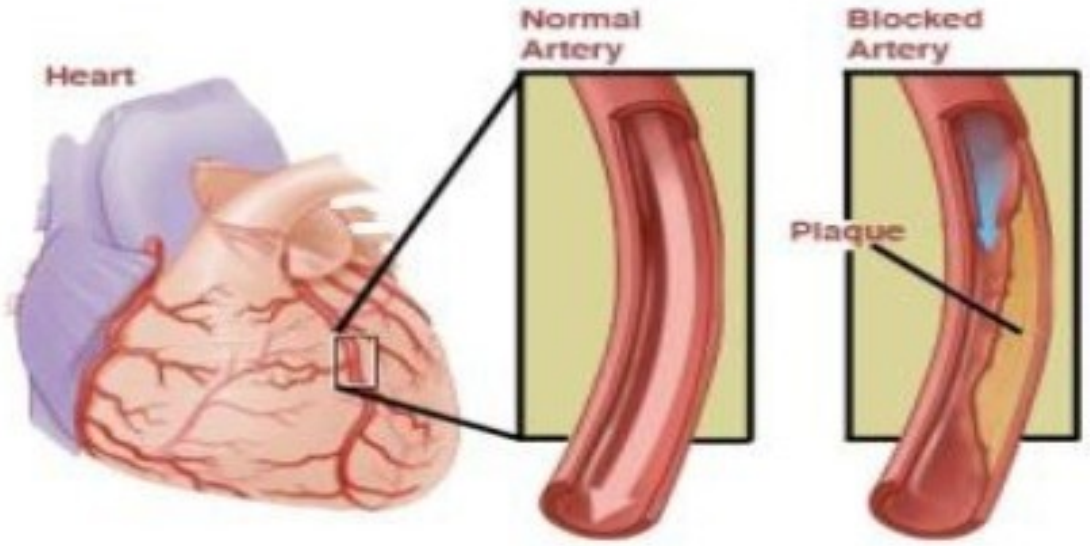
- VEGF je **signální protein (ligand)** schopný indukovat genovou expresi
- Primárním cílem VEGF jsou **vaskulární ECs**
- VEGF přispívá k **zachování stávajících cév** a indukuje **vznik a růst cév nových**
- Významná role VEGF v **embryonálním vývoji** i v **nádorové transformaci**



Proces	Úloha VEGF
Embryogeneze a časný postnatální vývoj	Nezbytný pro vznik krevních cév
	Delece jednoho genu VEGF je letální
	Nezbytný pro časný postnatální vývoj, zejména pro funkci ledvin
Růst kostí	Stimuluje invazi krevních cév, která je nutná pro trabekulární růst kostí
	Účinky inhibice VEGF jsou reversibilní pokud je hladina VEGF obnovena
Vyzrávání žlutého tělíska a angiogeneze v děloze	Stimuluje vyzrávání žlutého tělíska, které pak produkuje progesteron. Společné působení progesteronu a VEGF je nezbytné pro angiogenezi v děloze.
Hojení ran	Podílí se na vzniku nových cév v místě poranění

Angioterapie

- využití léků k regulaci angiogeneze
- proangiogeneze u ischemických chorob srdečních



Obr. 6

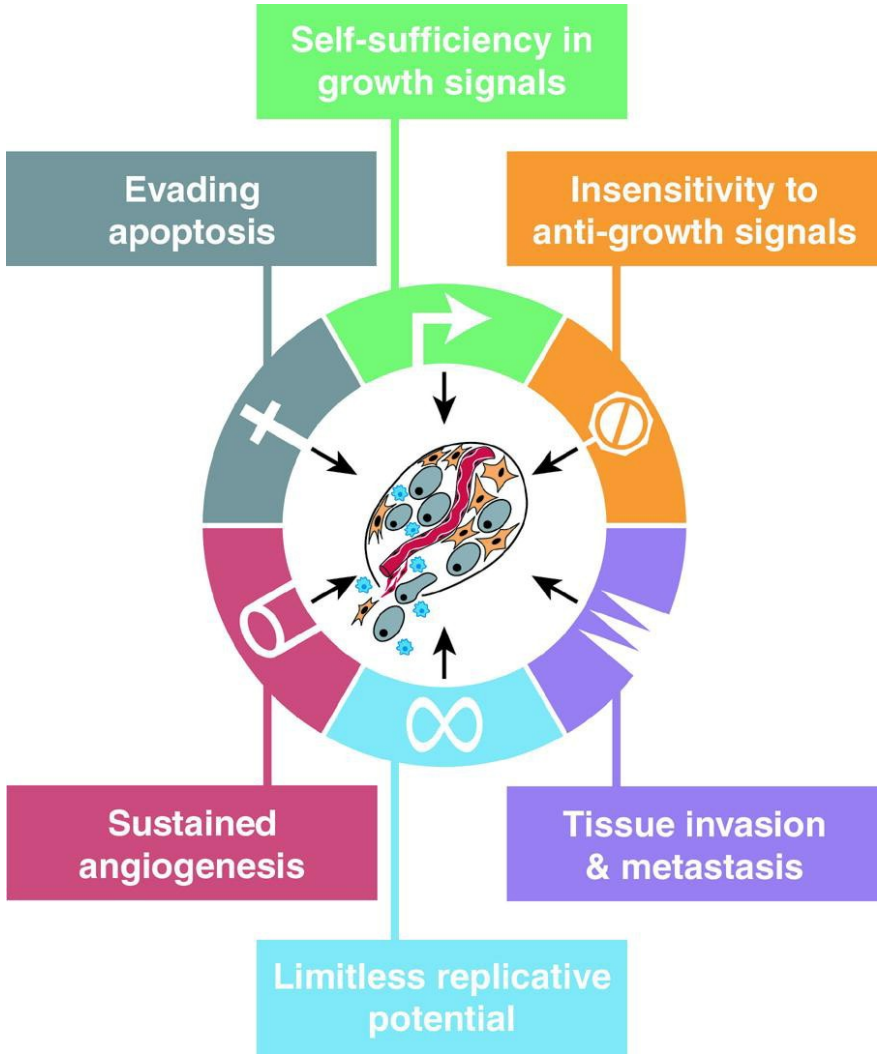
Angioterapie

- patologická angiogeneze:
  - rakovina
  - diabetická retinopatie
- Bevacizumab (Avastin)

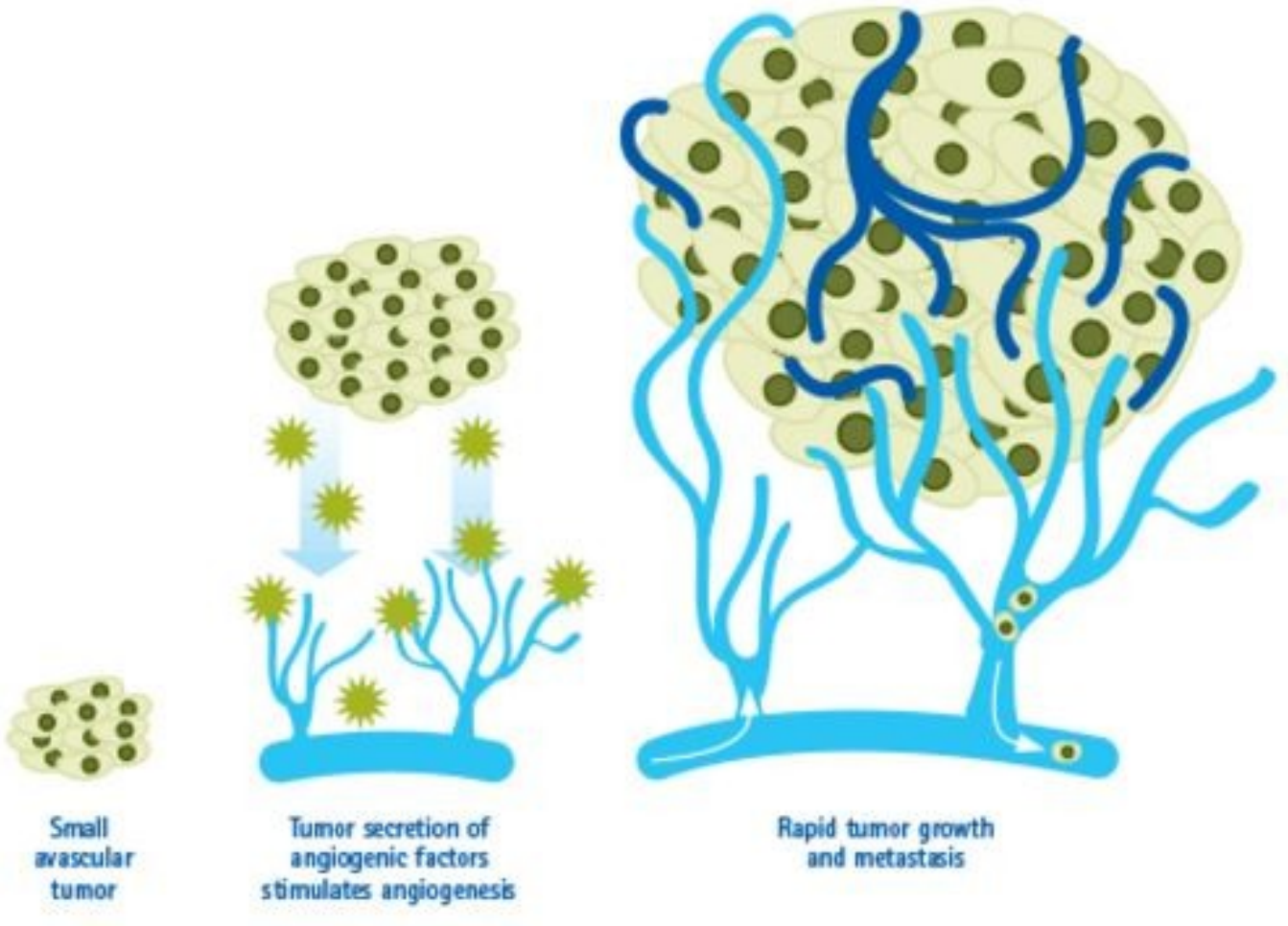
The second option is direct VEGF blocking. Nowadays, this line already has a grounded position in medicine. Drugs acting in this way are:

- Bevacizumab (Avastin, Genentech, San Francisco, CA, USA), a full-length humanised recombinant monoclonal IgG anti-VEGF-A antibody. It binds and inhibits all VEGF-A isoforms [11, 23, 24]. Its molecular weight is 148 kDa, so it is a large molecule with twice the half-life of ranibizumab [12, 13]. It has been approved for the treatment of several solid tumours (colorectal, non-epithelial lung, breast, ovarian, and renal cancers) and glioblastomas [3, 24–26]. In ophthalmology it is used as an off-label procedure [11, 12, 27, 28]. Furthermore, it is probably still the most widely used anti-VEGF drug in ophthalmology due to much lower costs of therapy, compared with other medicines [12, 24, 29].
- Ranibizumab (Lucentis, Genentech, San Francisco, CA, USA/Novartis Ophthalmics, Basel, Switzerland) is a (Fab) fragment of a humanised monoclonal anti VEGF-A antibody, also against all VEGF-A isoforms [10, 13, 23]. Its molecular weight is 48 kDa [24]. This drug was designed for eye diseases, and it was approved for intra-ocular use in neovascular AMD, macular oedema (ME) after retinal vein occlusions (RVO), diabetic macular oedema (DME), and diabetic retinopathy (DR) with DME [30]. In any other ocular diseases it is also used off label.
- Pegaptanib (Macugen, Phizer, New York), a 28-base ribonucleic acid aptamer, covalently linked to two branched 20-kd polyethylene glycol moieties [10, 23]. It specifically binds and blocks activity of extracellular VEGF-A165 isoform [11, 23]. It was used in wet AMD treatment, but it was found to be weaker than the drugs listed above. This is probably due to its specificity for binding only one isoform of VEGF [16].
- Aflibercept (Eylea, Regeneron, Tarrytown, NY, USA), a VEGF-trap: a 115-kDa recombinant fusion decoy protein consisting of VEGF binding domains of human VEGFR-1 and VEGFR-2 fused to the Fc domain of human immunoglobulin G1 [23]. It binds all forms of VEGF-A but also PlGF-1 and PlGF-2 with a very high affinity, greater than bevacizumab or ranibizumab [10, 11, 16]. It was approved for colorectal metastasising carcinoma treatment (Zaltrap). In ophthalmology it has already been approved as a therapy for neovascular AMD, macular oedema after RVO, and diabetic macular oedema [31].

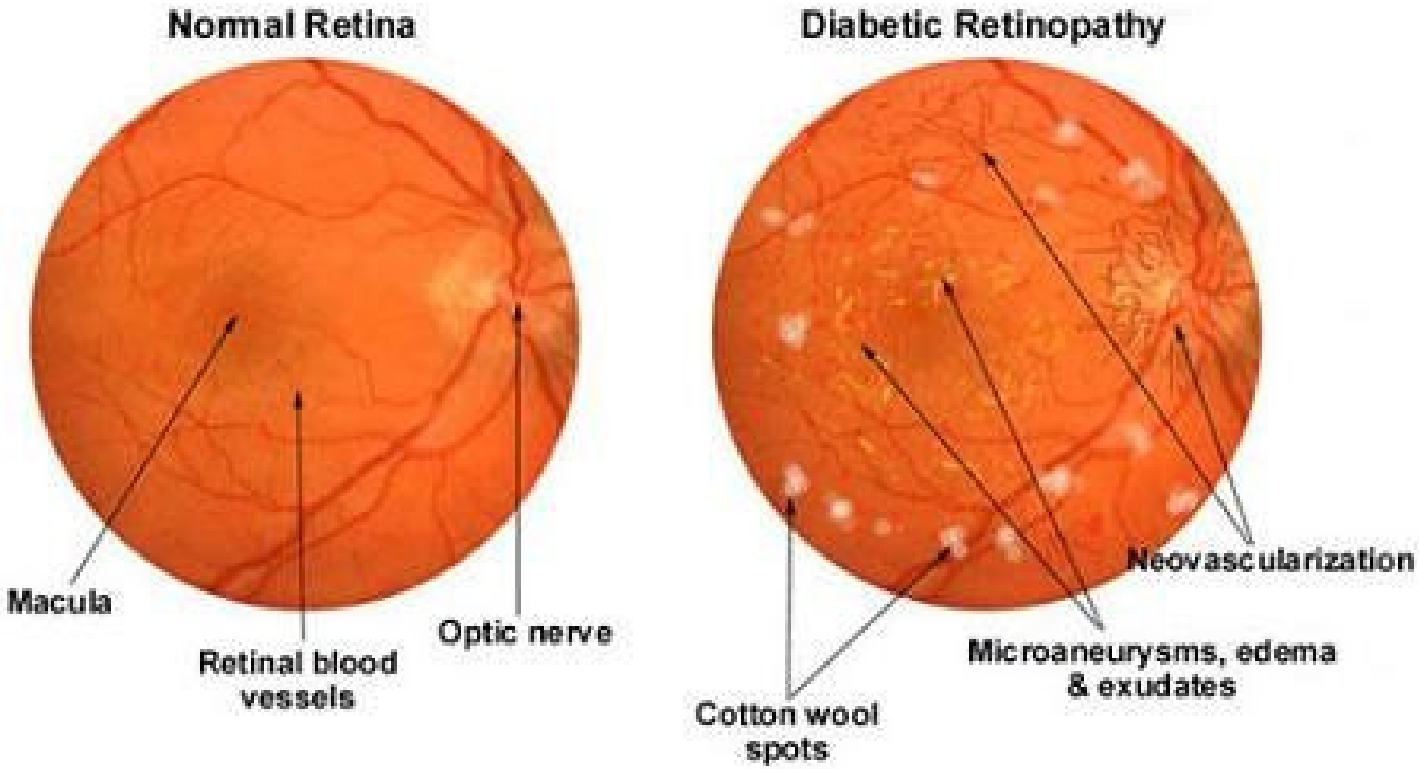
Rakovina a angioterapie



Rakovina a angioterapie



Diabetická retinopatie



- Diabetic retinopathy, also known as diabetic eye disease, is a medical condition in which damage occurs to the retina due to diabetes mellitus. It is a leading cause of blindness.
- Diabetic retinopathy affects up to 80 percent of those who have had diabetes for 20 years or more.
- Depends on VEGF signaling

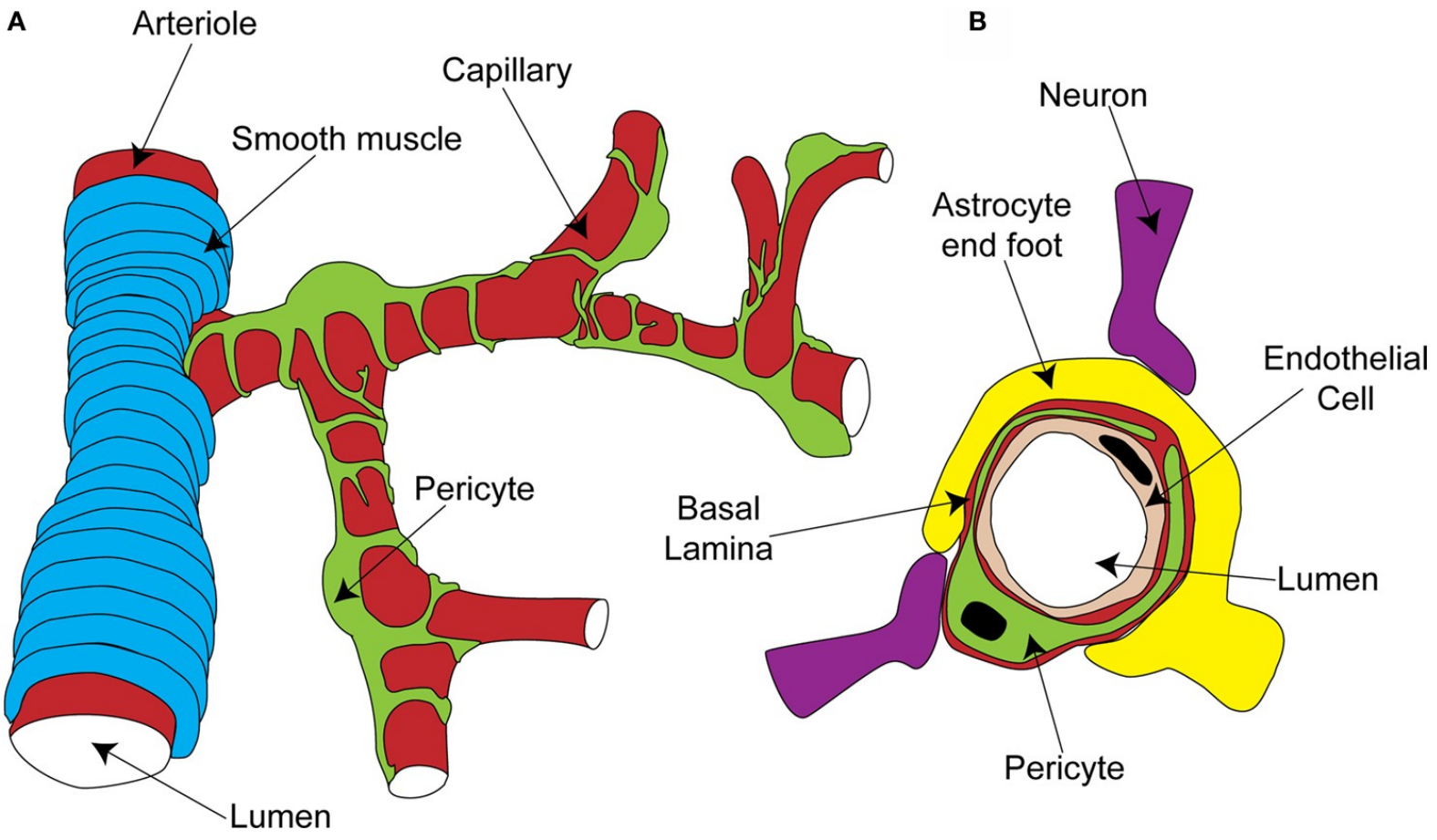
Angiogeneze vs. vaskulogeneze

- vaskulogeneze = vznik a vývoj cév při embryonálním vývoji (de novo)
- angiogeneze (neokapilarizace) = z cév již existujících

Angiogeneze

- v embryogenezi
- iniciovaná:
  - poranění tkáně
  - menstruační cyklus
  - hypoxická tkáň
- sprouting x intususceptive (spliting)

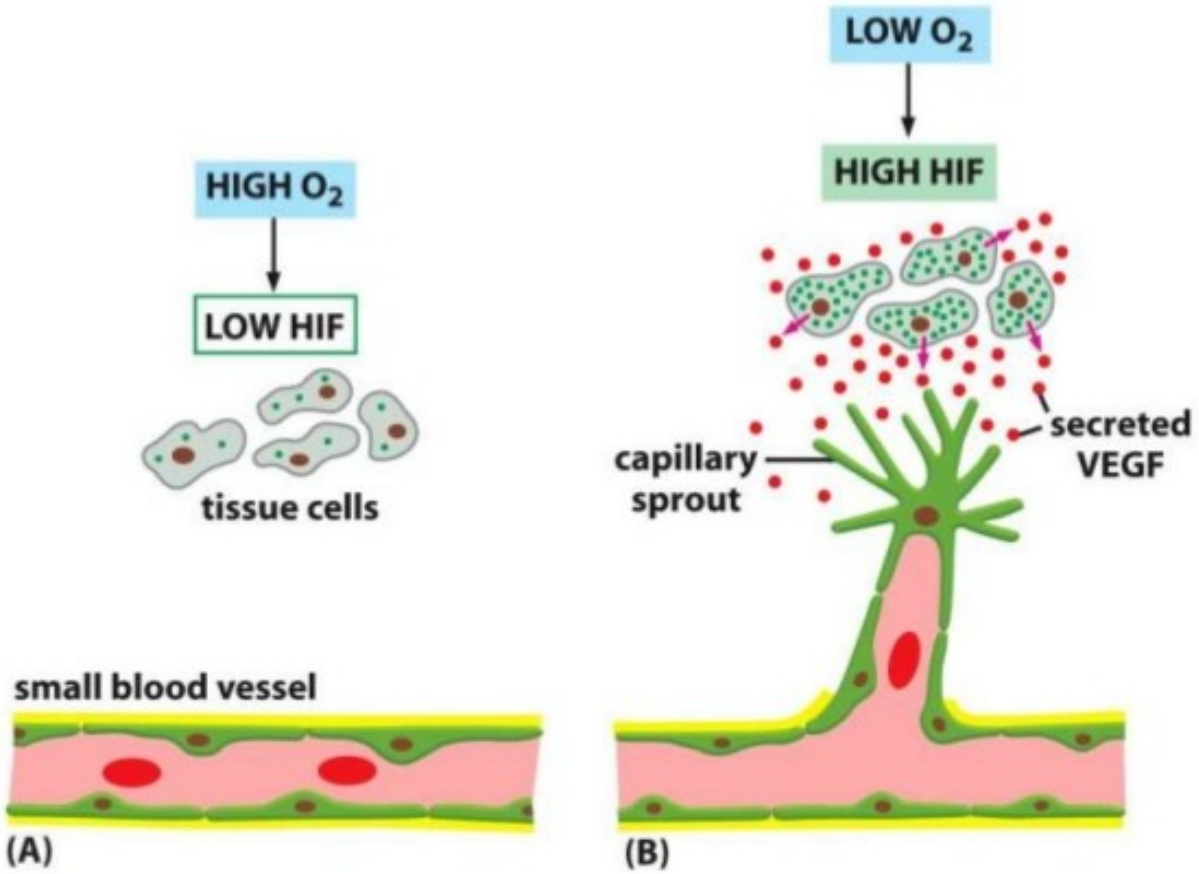
Anatomie cévy



Základní kroky angiogeneze po poranění (sprouting angiogeneze)

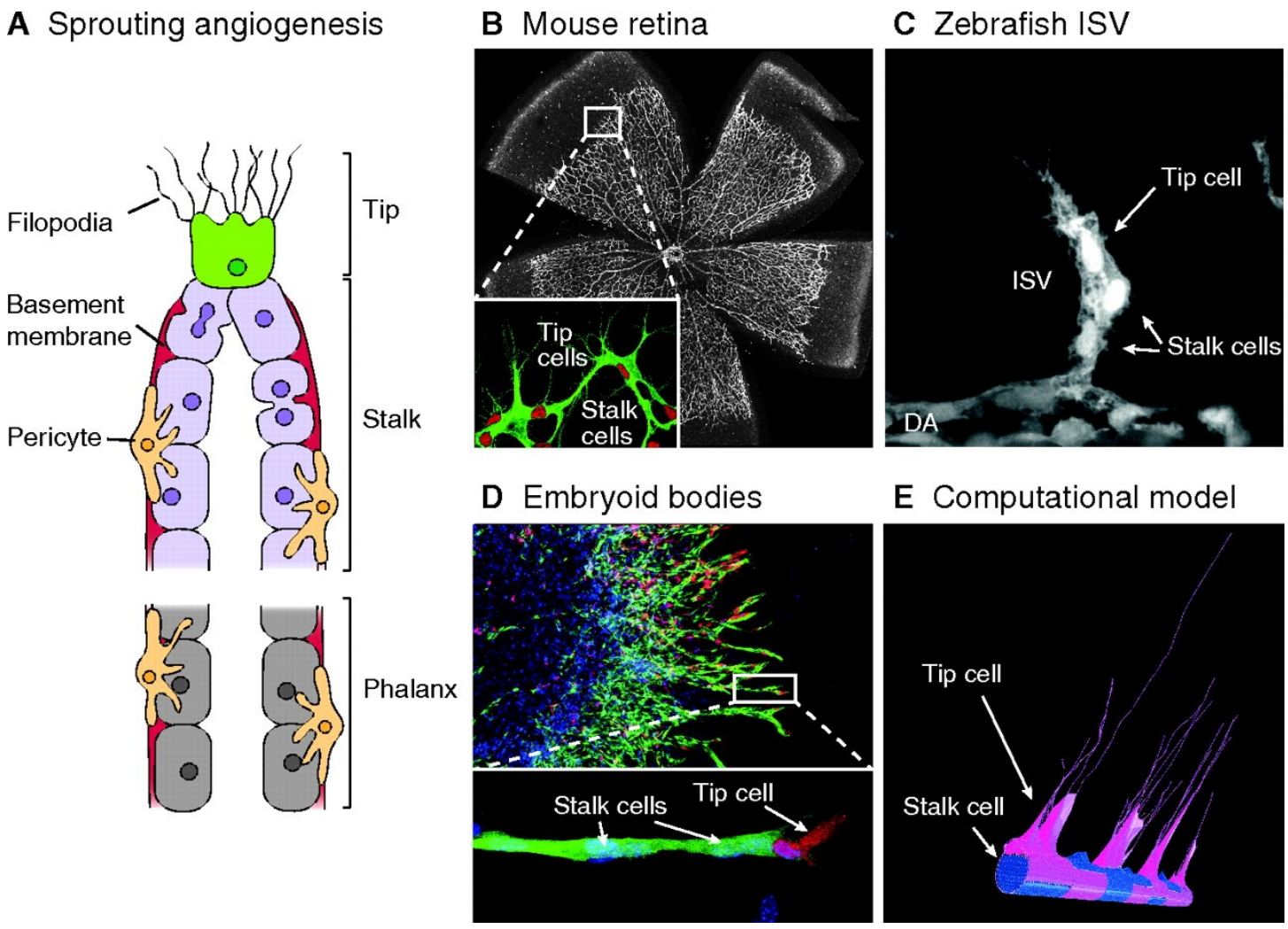
1. dilatace cév (eNOS)
2. EC kontrakce
3. „Tip-cell“ selekce (Notch signalizace)
4. Ustavení „stalk cell“ a jejich proliferace
5. Vakuolizace (vytvoření lumenu)
6. Spojení „výhonků“ (anastomóza)
7. Pericytární stabilizace

Sprouting (klíčení) cév

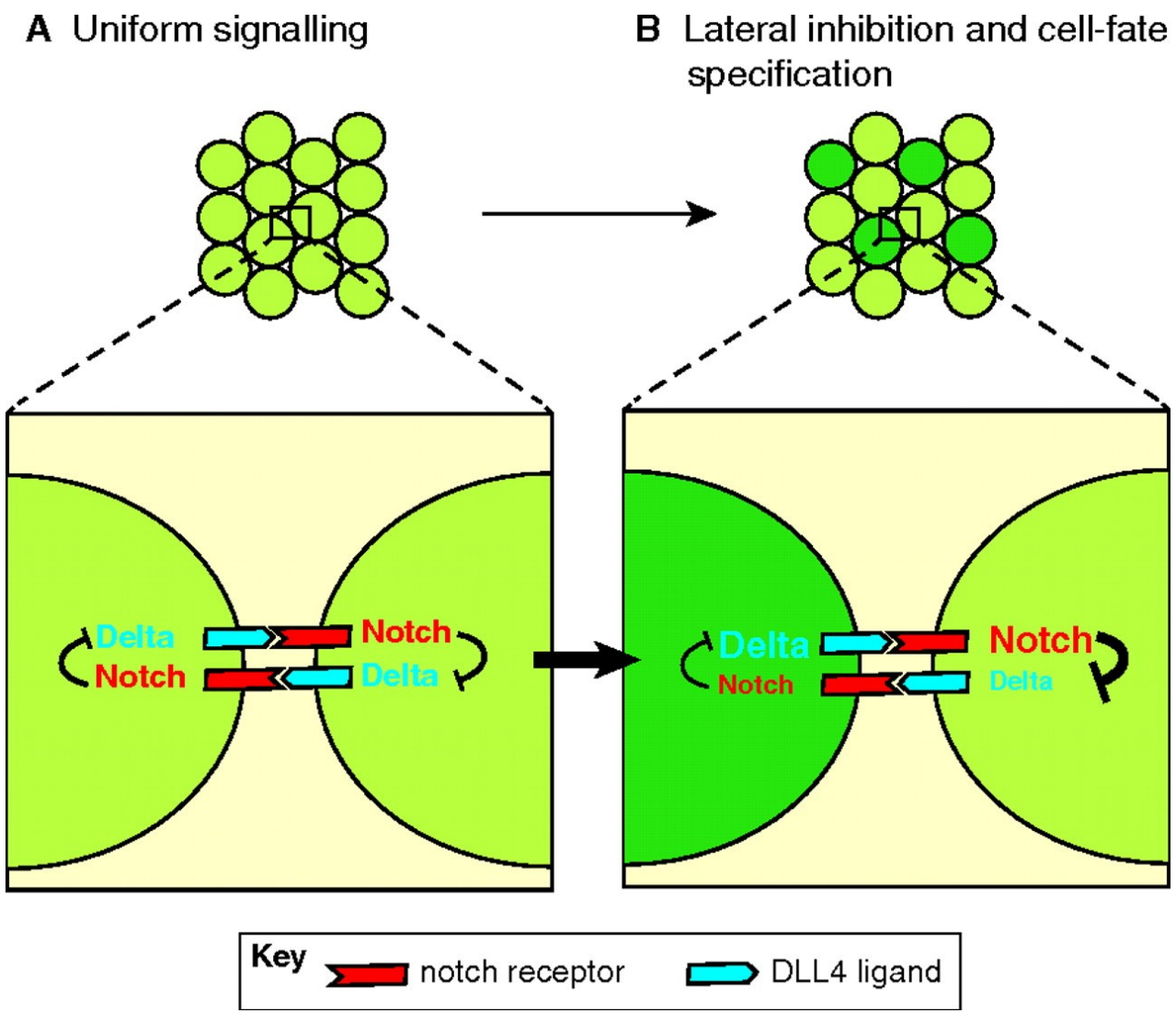


Obr. 2

Cell analysis in sprouting angiogenesis models.

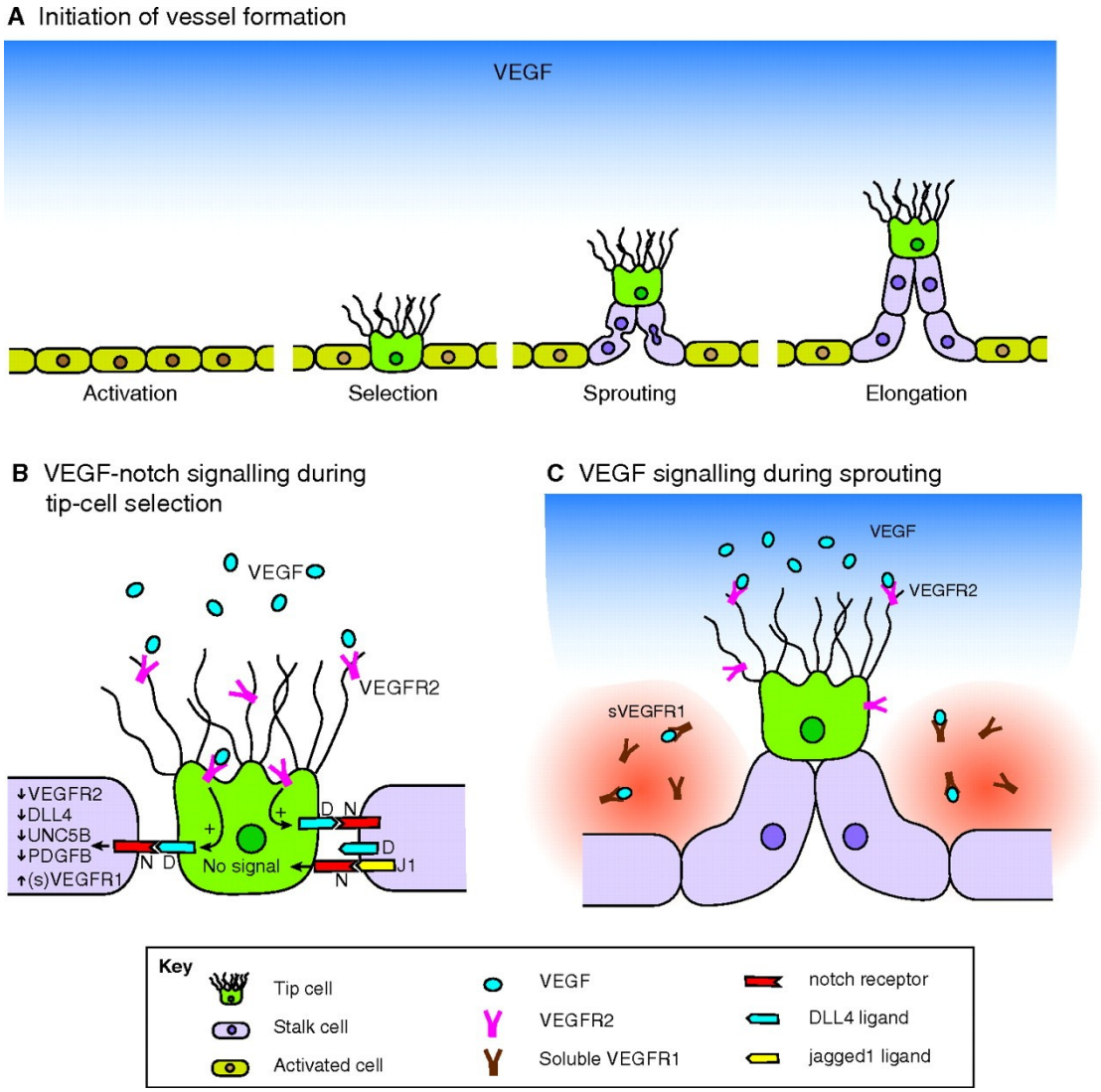


Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583



Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583

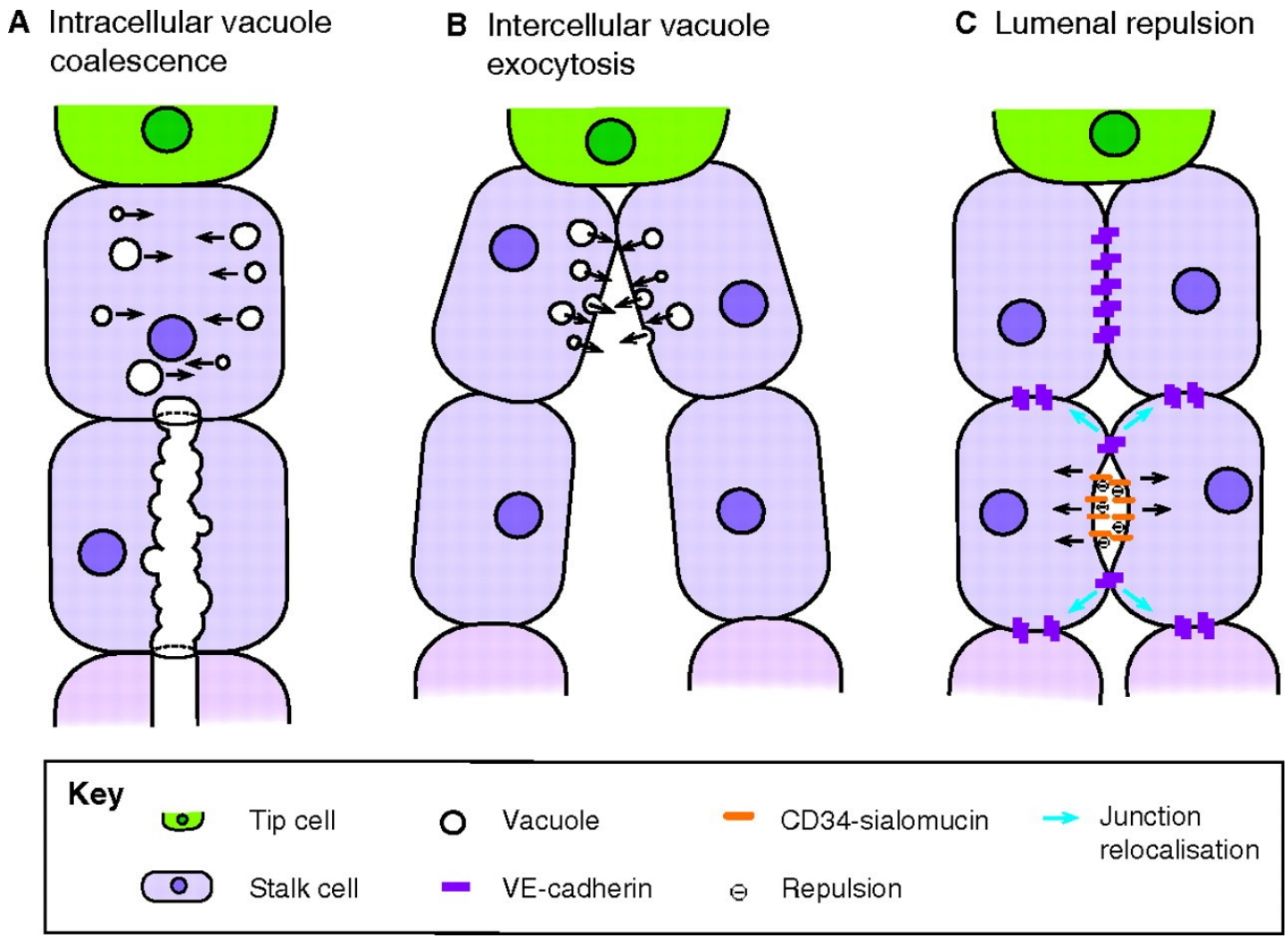
Sprout induction.



Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583

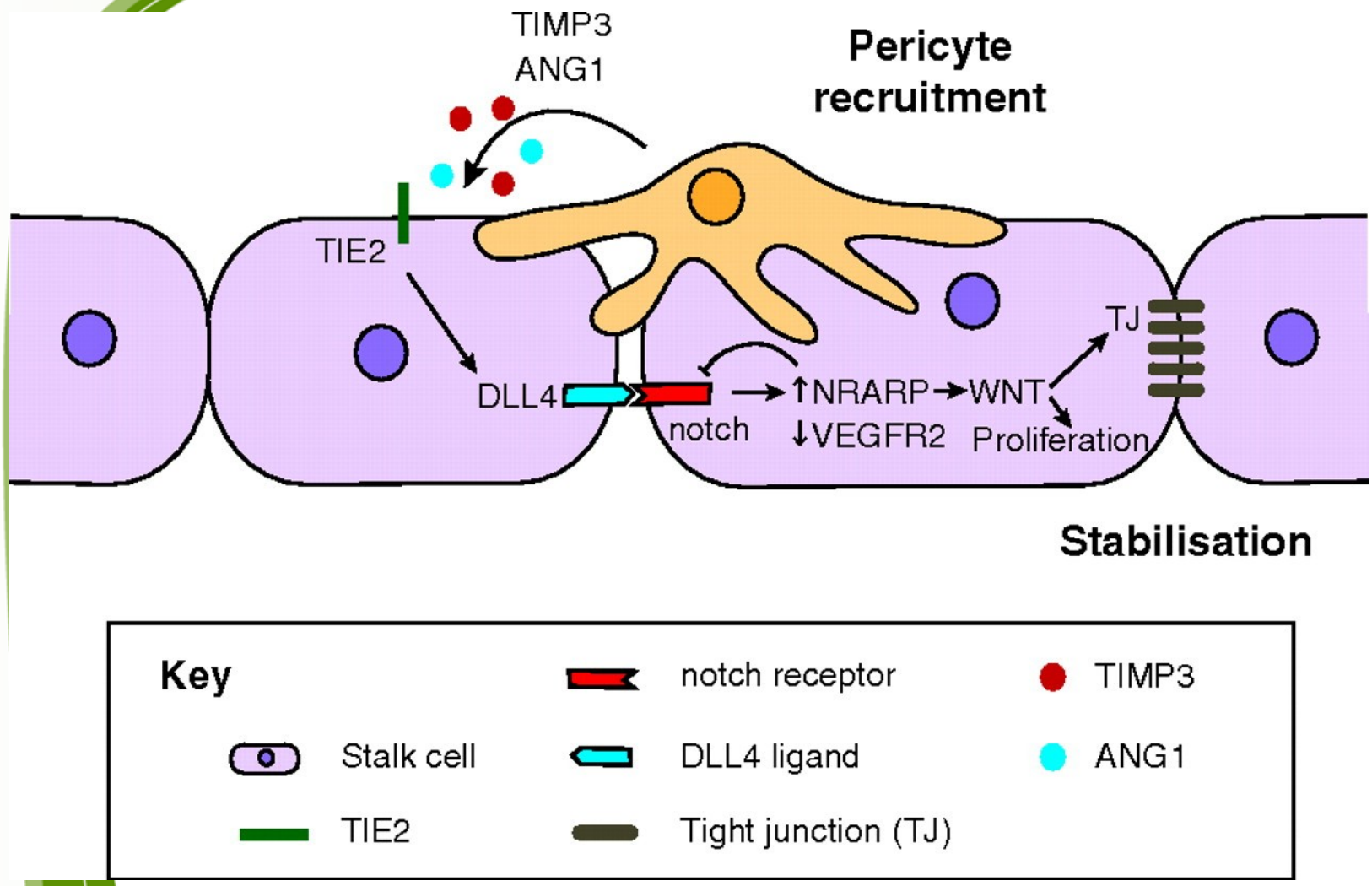
Development

Models of lumen formation during sprout outgrowth.



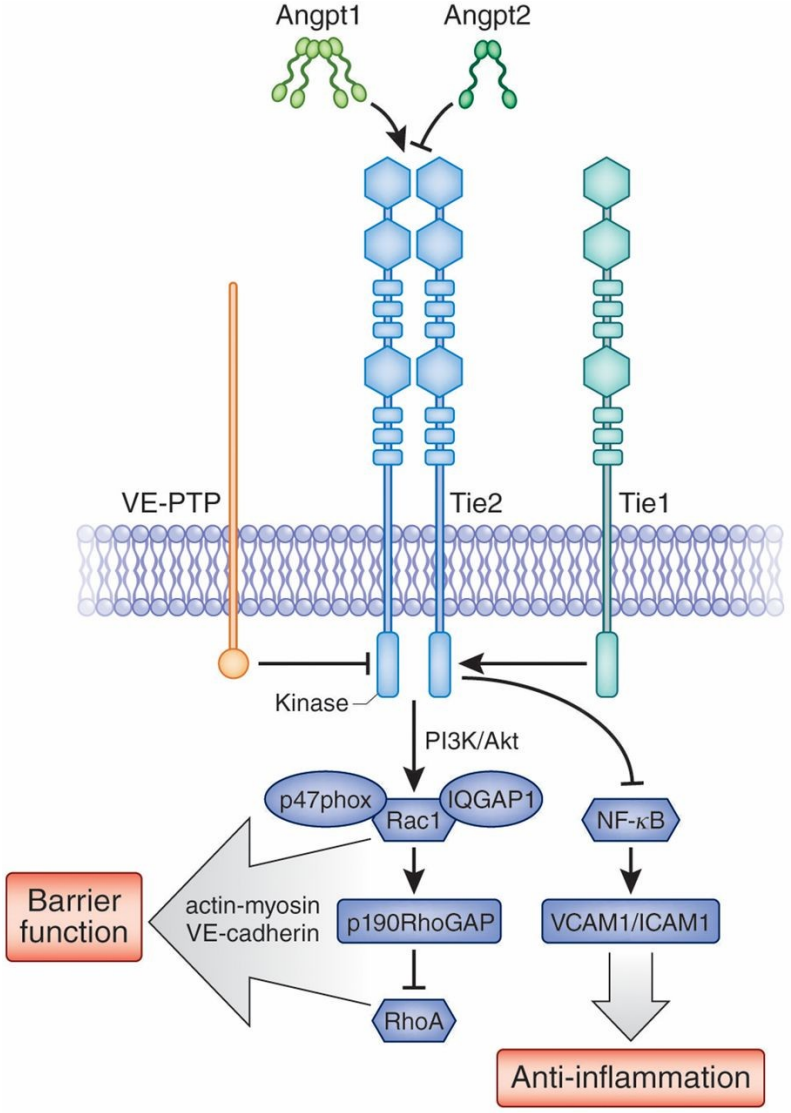
Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583

Vessel stabilisation.

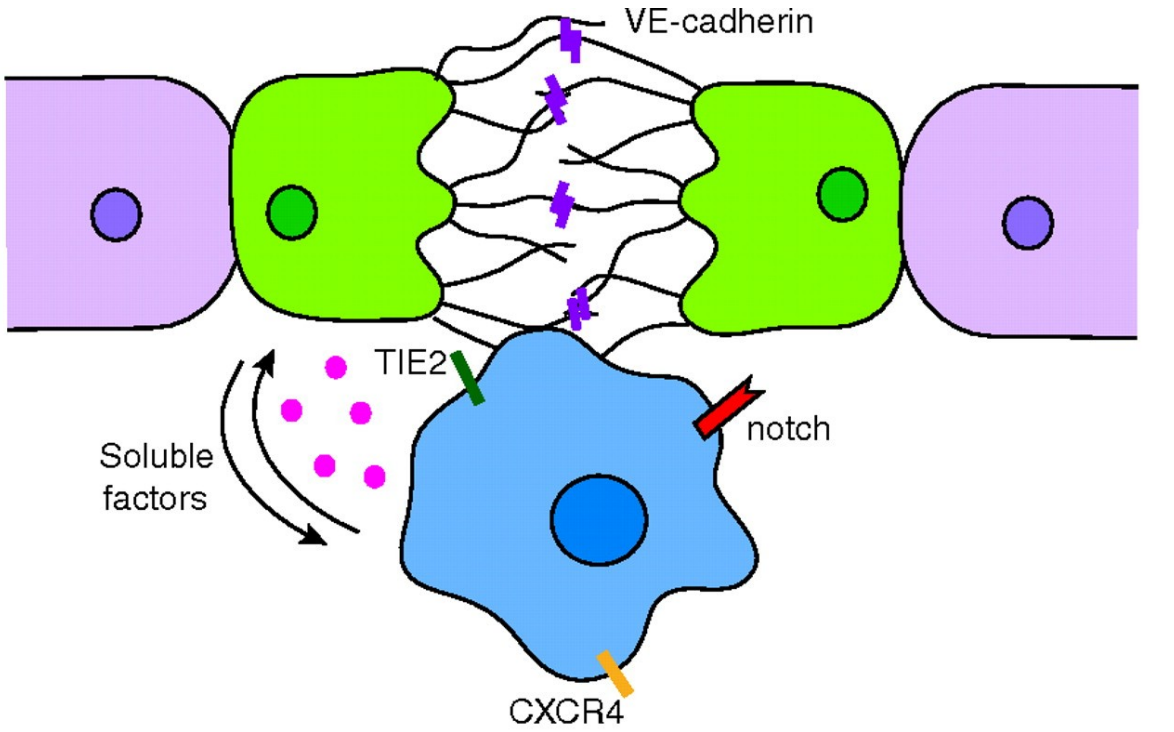


Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583

Angiopoetin-Tie RTK systém



Current concepts of anastomosis.

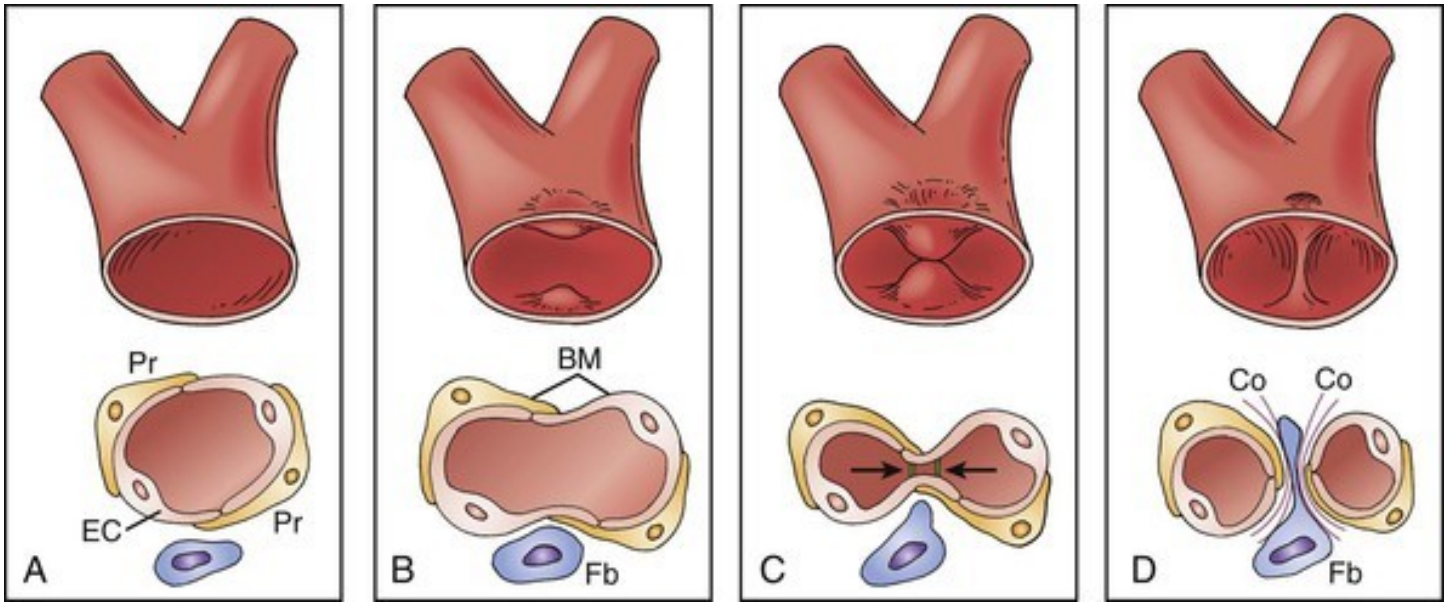


Ilse Geudens, and Holger Gerhardt Development 2011;138:4569-4583

Development

Spliting

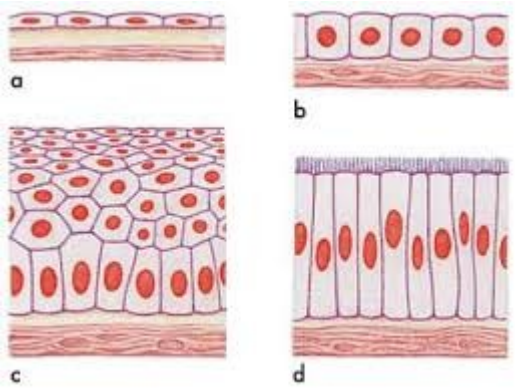
1. protruze dovnitř lumenu
2. rozdělení kapilár
3. „vpáčení“ fibroblastu



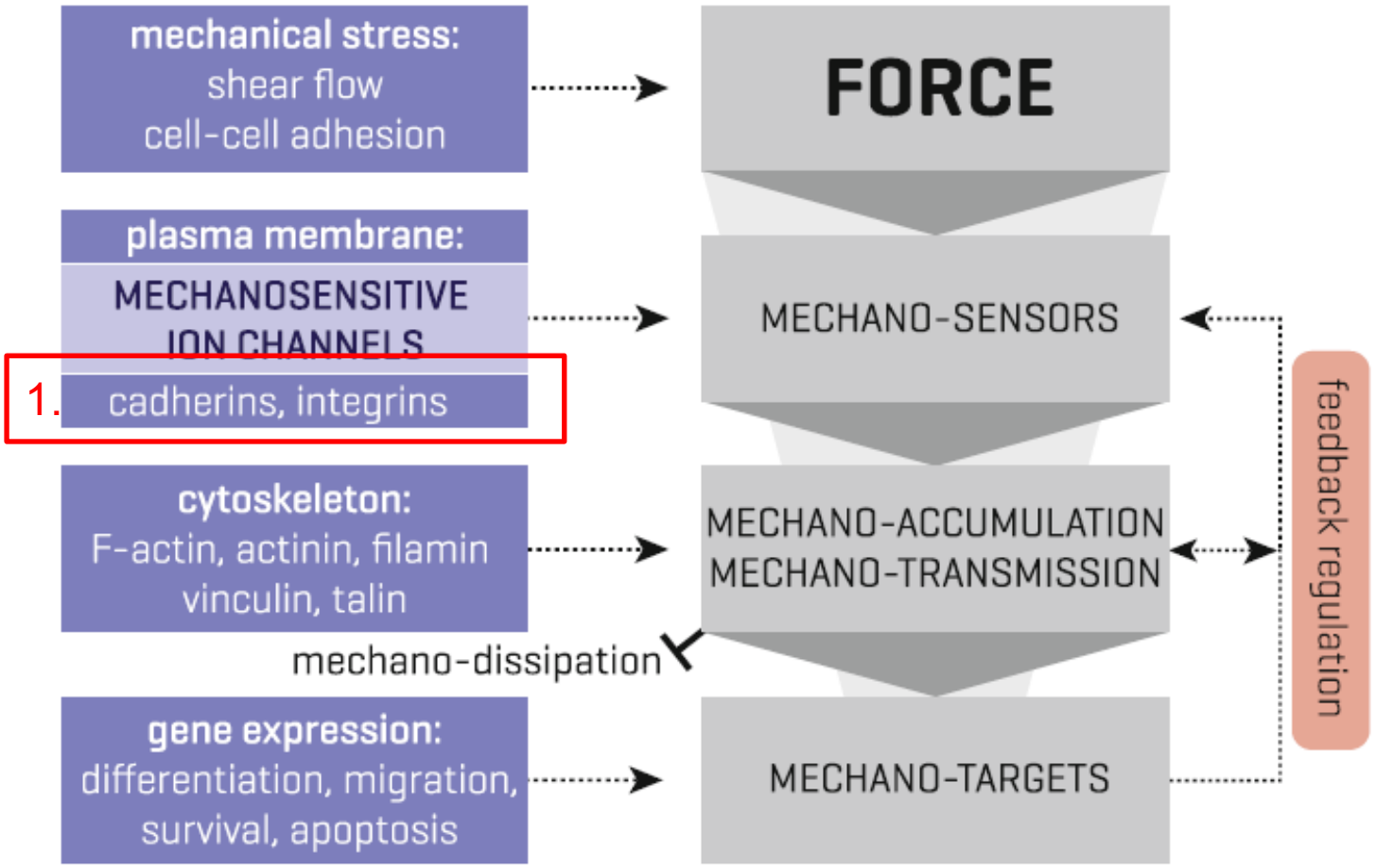
Obr. 3

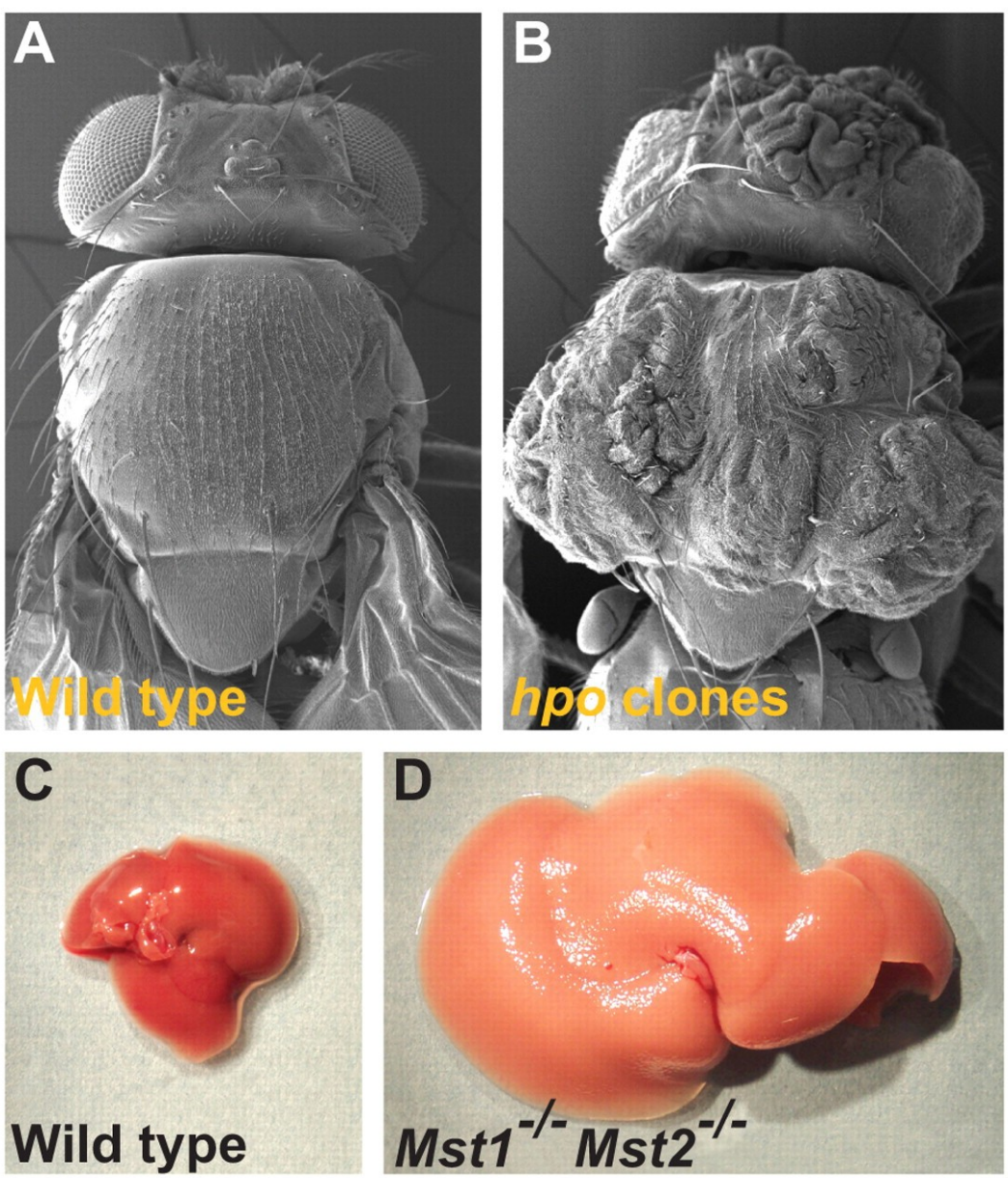
Mechanické vlivy a jejich detekce

Buňky fungují v tkáních

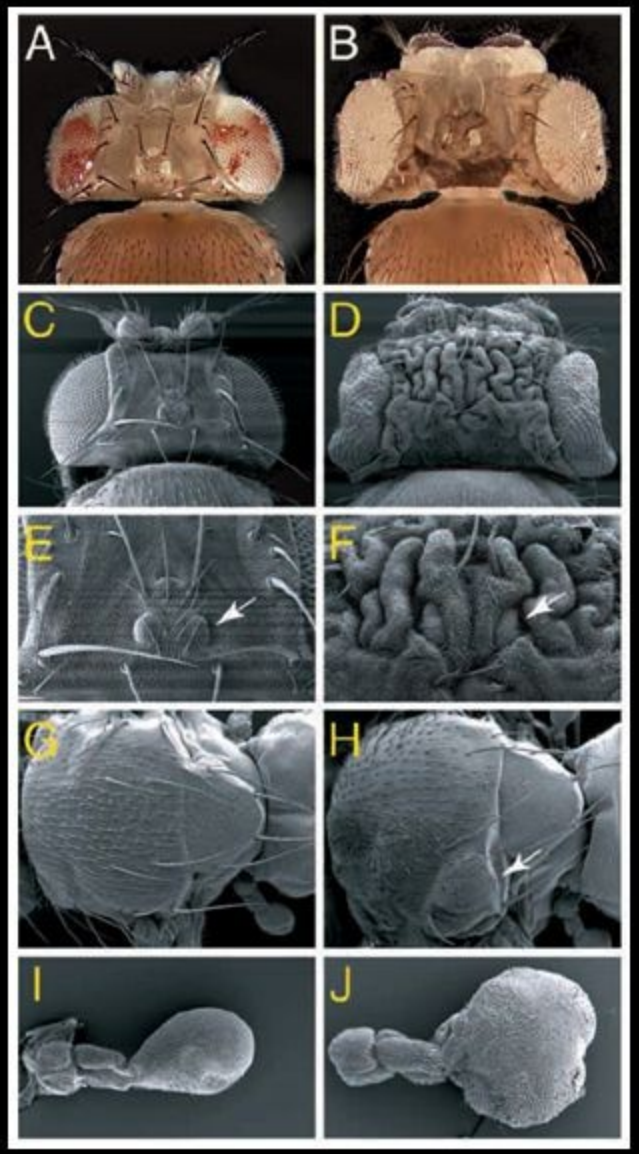


- Koncept: kontaktní inhibice proliferace (contact inhibition of proliferation – CIP):
- Hustota buněk vysoká – dělení je silně inhibováno
- Hustota buněk nízká (ale i při natažení tkáně) – buněčné dělení je umožněno
- Prerekvizita pro CIP: existence mechanismu, který umožní vnímat tenzi v tkáni a přenášet ji do „rozhodnutí buněk“ zda-se dělit nebo ne


mechanical stress:
shear flow
cell-cell adhesion
FORCE
plasma membrane:
MECHANOSENSITIVE ION CHANNELS
cadherins, integrins
MECHANO-SENSORS
1.
cytoskeleton:
F-actin, actinin, filamin
vinculin, talin
MECHANO-ACCUMULATION
MECHANO-TRANSMISSION
mechano-dissipation
gene expression:
differentiation, migration,
survival, apoptosis
MECHANO-TARGETS
feedback regulation


A
Wild type
B
hpo clones
C
Wild type
D
Mst1-/- Mst2-/-

Phenotype of a Hippo Pathway Mutant (shar-pei)

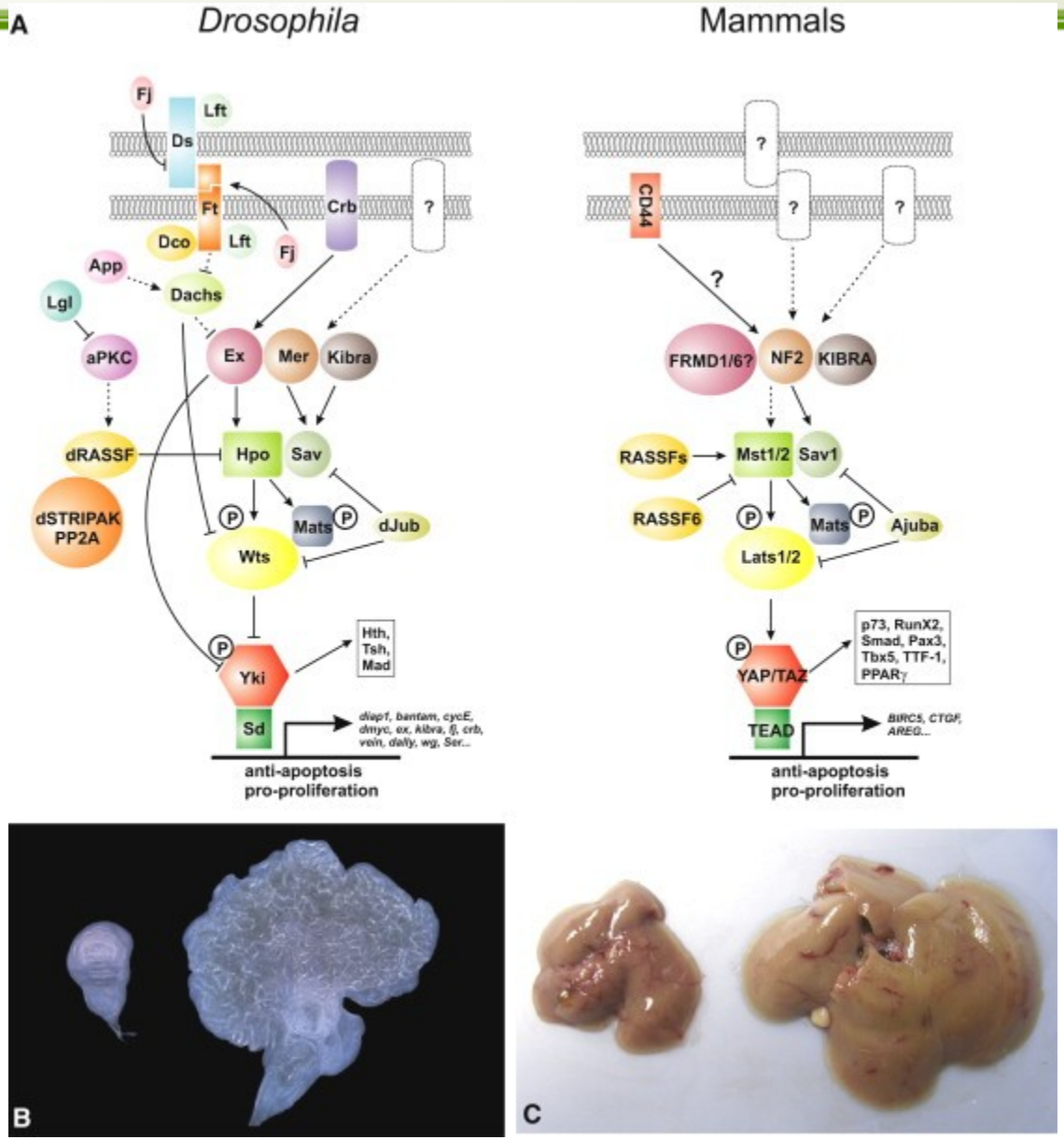


- Georg Halder's research group designed the screen that is depicted on the previous slide and identified the founding member of the Hippo tumor suppressor pathway. Panels C-F (to the left) document the effects of removing this gene from the entire head and retina. In contrast to the wild type control animals which have a flat head cuticle surface (panels C,E) the mutant tissue over-proliferates leaving undulating folds of head capsule tissue. This phenotype resembles the undulating folds of skin on a shar-pei dog – based on this similarity the gene was called shar-pei.

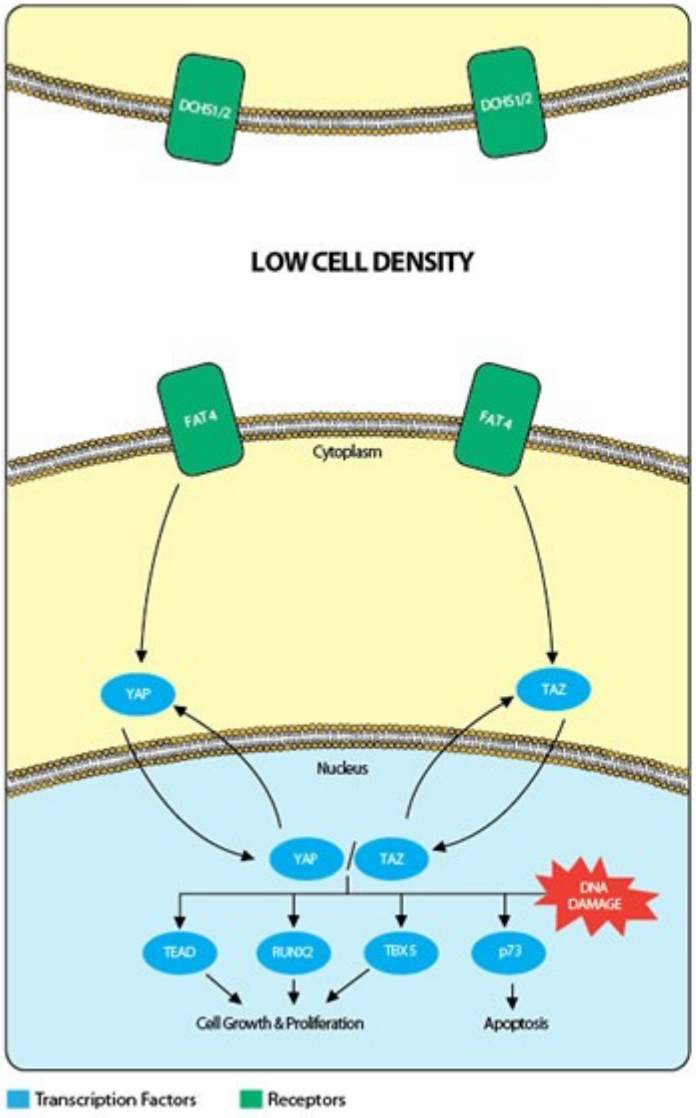
- The ability to suppress cell proliferation is not limited to the head and retina. Removal of shar-pei within a clone of cells within the thorax leads to tumor formation (panel H, arrow). Loss of shar-pei throughout the entire haltere leads to a significant increase in size (panel I,J). In every tissue examined shar-pei (and by extension the entire Hippo pathway) controls organ size throughout all developing Drosophila tissues. The same has been shown for the mammalian Hippo pathway.

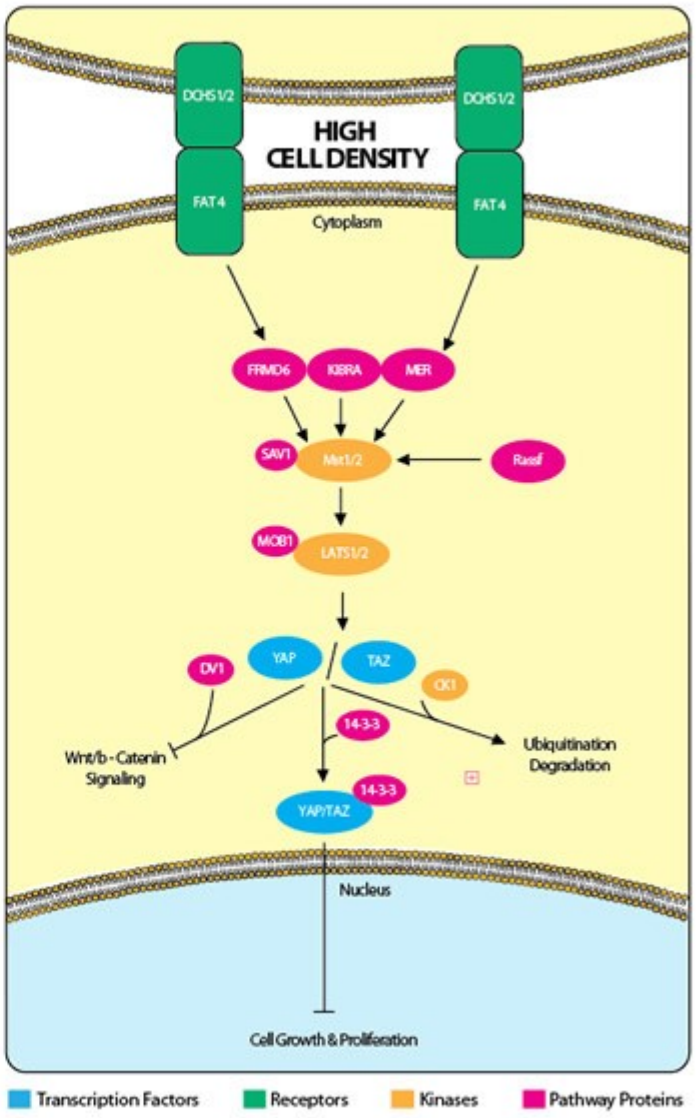
- Tissues can appear larger for two reasons. First, the number of cells in wild type and mutant tissue can be the same but the cells can be bigger in the mutant. Second. The size of wild type and mutant cells can be the same but the number of these cells can be significantly higher in the mutant tissue. In the fly retina each ommatidium is separated from its neighbors by a single cell (panel c below). In the shar-pei mutant there are more cells between the ommatidia (panel b below). These results indicate that the Hippo pathway is a true tumor suppressor pathway and that its role in development is to suppress cellular proliferation.

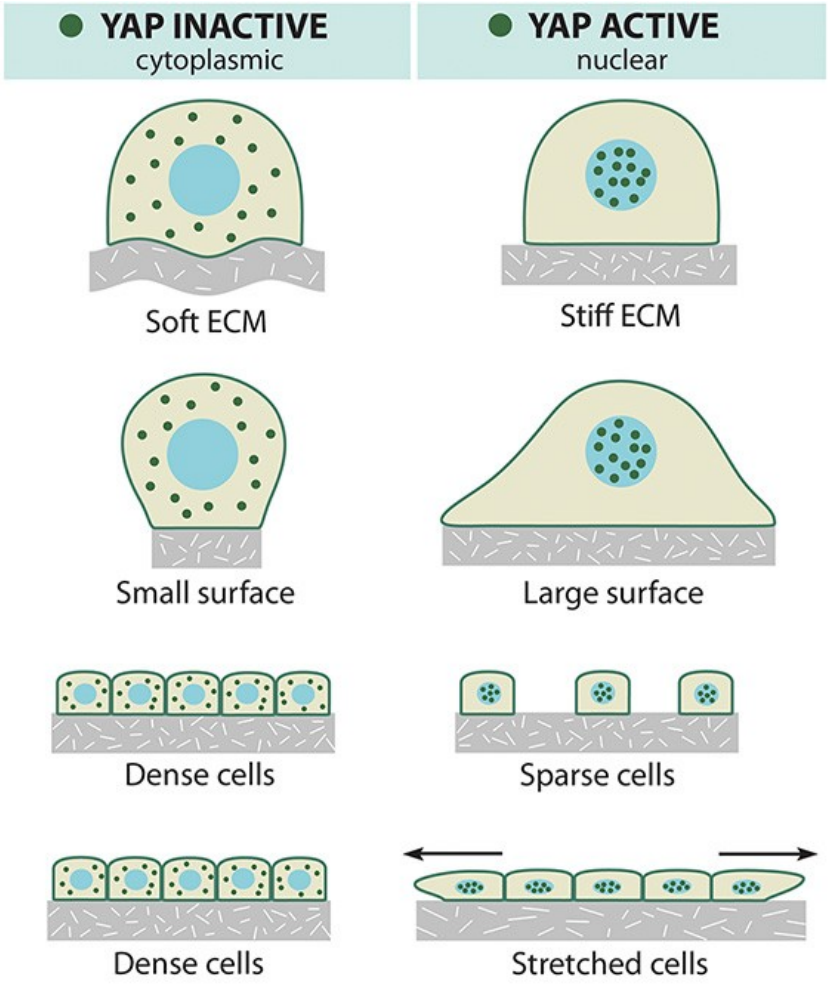
Hippo (Drosophila) = Yap/Taz (obratlovci)

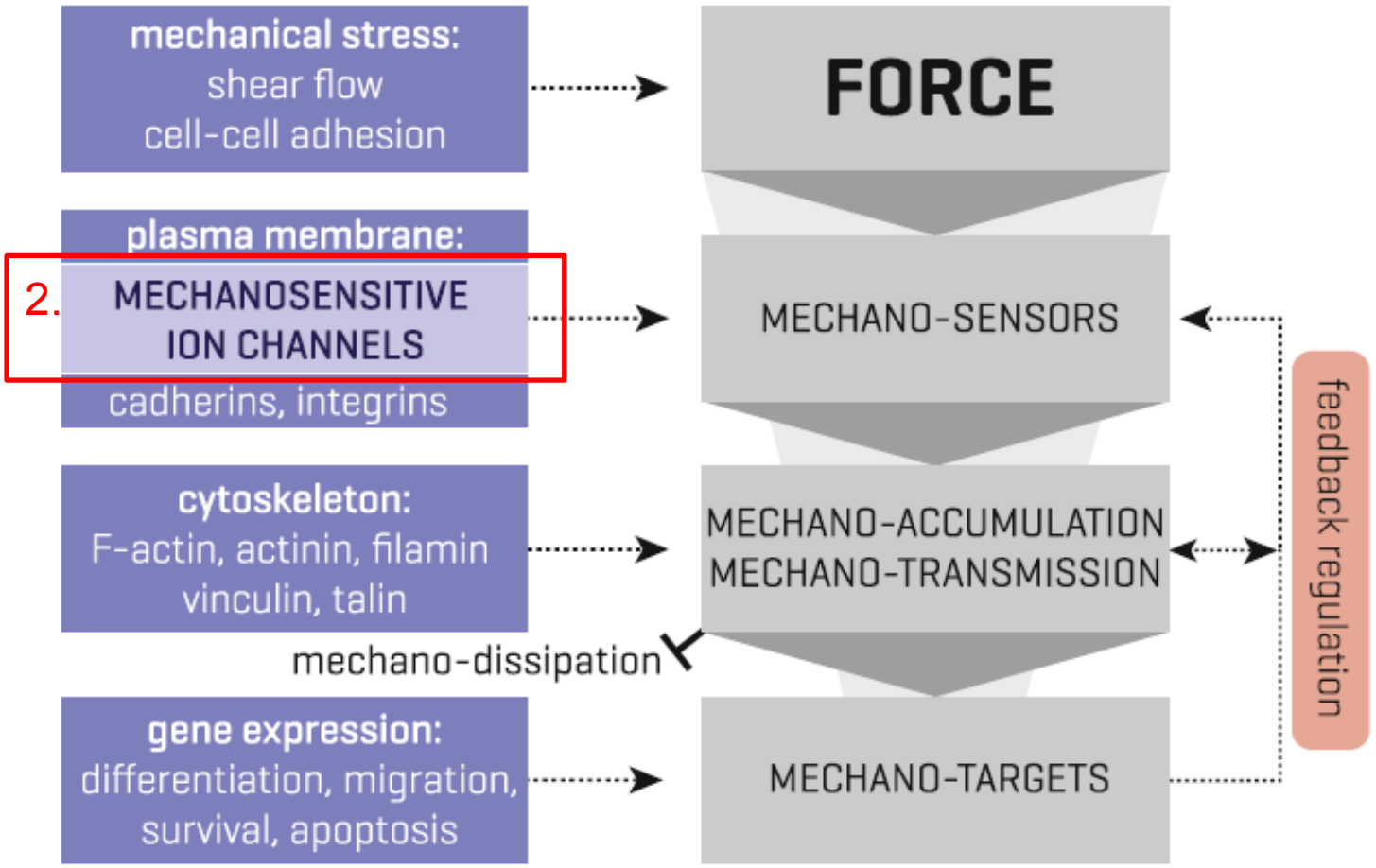


Hippo nebo též Yap/Taz signální dráha jako senzor






YAP INACTIVE
cytoplasmic
YAP ACTIVE
nuclear
Soft ECM
Stiff ECM
Small surface
Large surface
Dense cells
Sparse cells
Dense cells
Stretched cells


mechanical stress:
shear flow
cell-cell adhesion
FORCE
plasma membrane:
2.
MECHANOSENSITIVE
ION CHANNELS
cadherins, integrins
MECHANO-SENSORS
cytoskeleton:
F-actin, actinin, filamin
vinculin, talin
MECHANO-ACCUMULATION
MECHANO-TRANSMISSION
mechano-dissipation
gene expression:
differentiation, migration,
survival, apoptosis
MECHANO-TARGETS
feedback regulation

Citlivost iontových kanálů k mechanickým vlivům

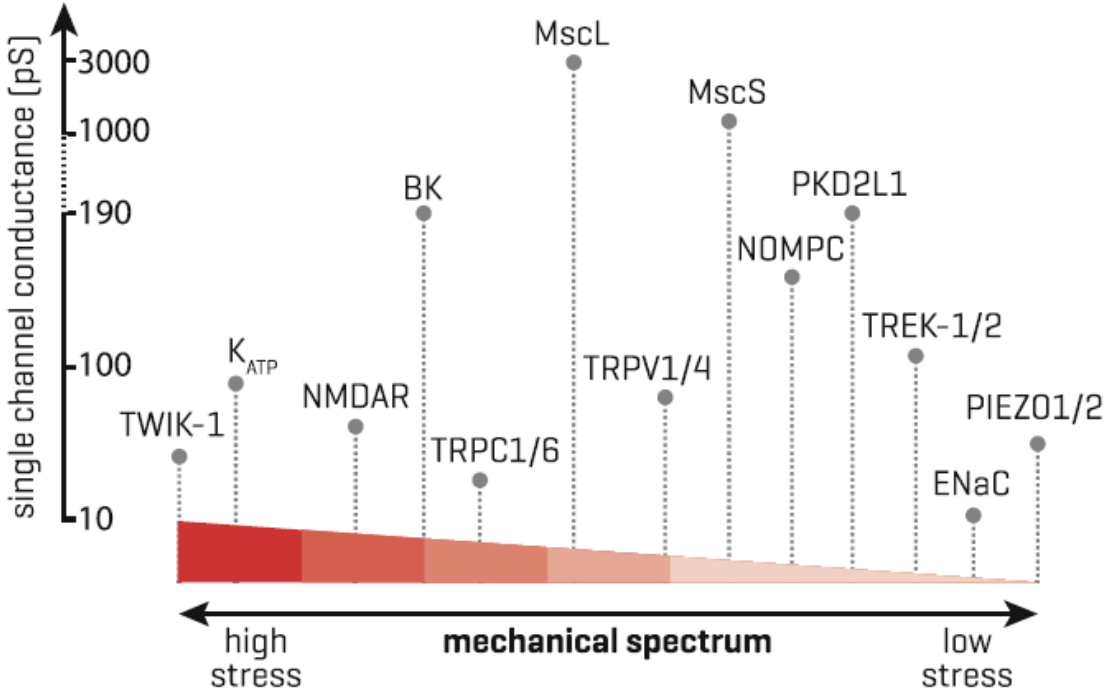


Fig. 4.3 Ion channels sensitivity to mechanical force probed by the patch-clamp technique [32]. All ion channels can be placed on a mechanical continuum from highly sensitive (e.g., Piezo1) to those that are almost insensitive to mechanical force (e.g., TWIK-1). They cover a broad conductance spectrum ranging from tens of pS (e.g., ENaC) to 3 nS (e.g., MscL). Note that several factors such as presence or absence of extracellular or intracellular network and experimental paradigm (i.e., stimulus type) may shift channels along the spectrum (see [32–34]; modified from Cox et al. 2016 [32])

Iontový kanál Piezo1

- Otvírá se při zvýšeném namáhání membrány

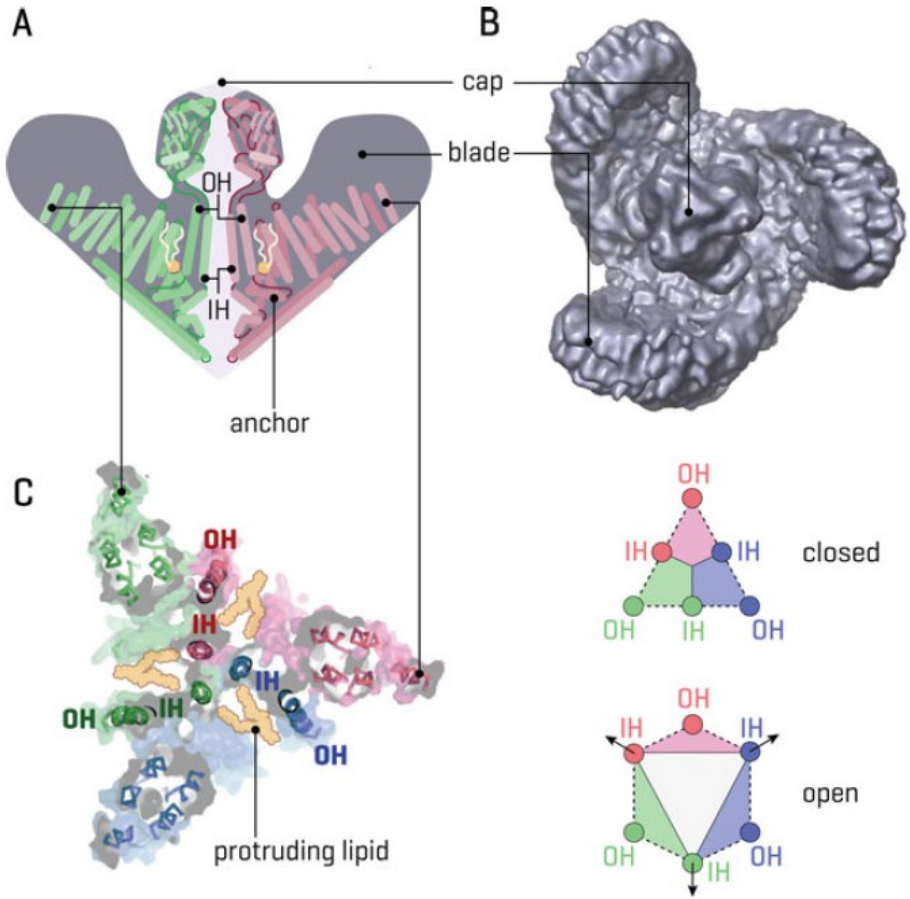
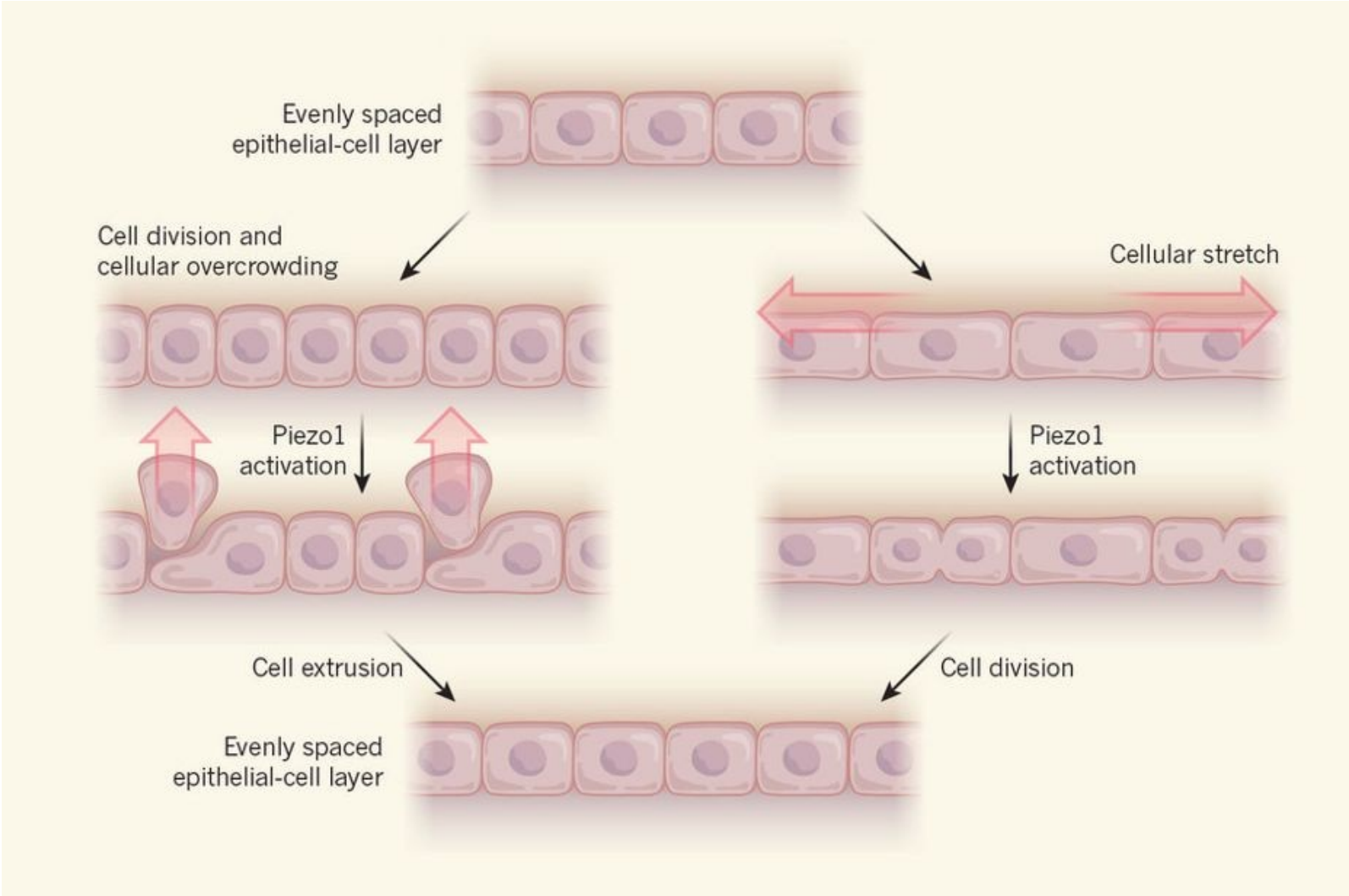
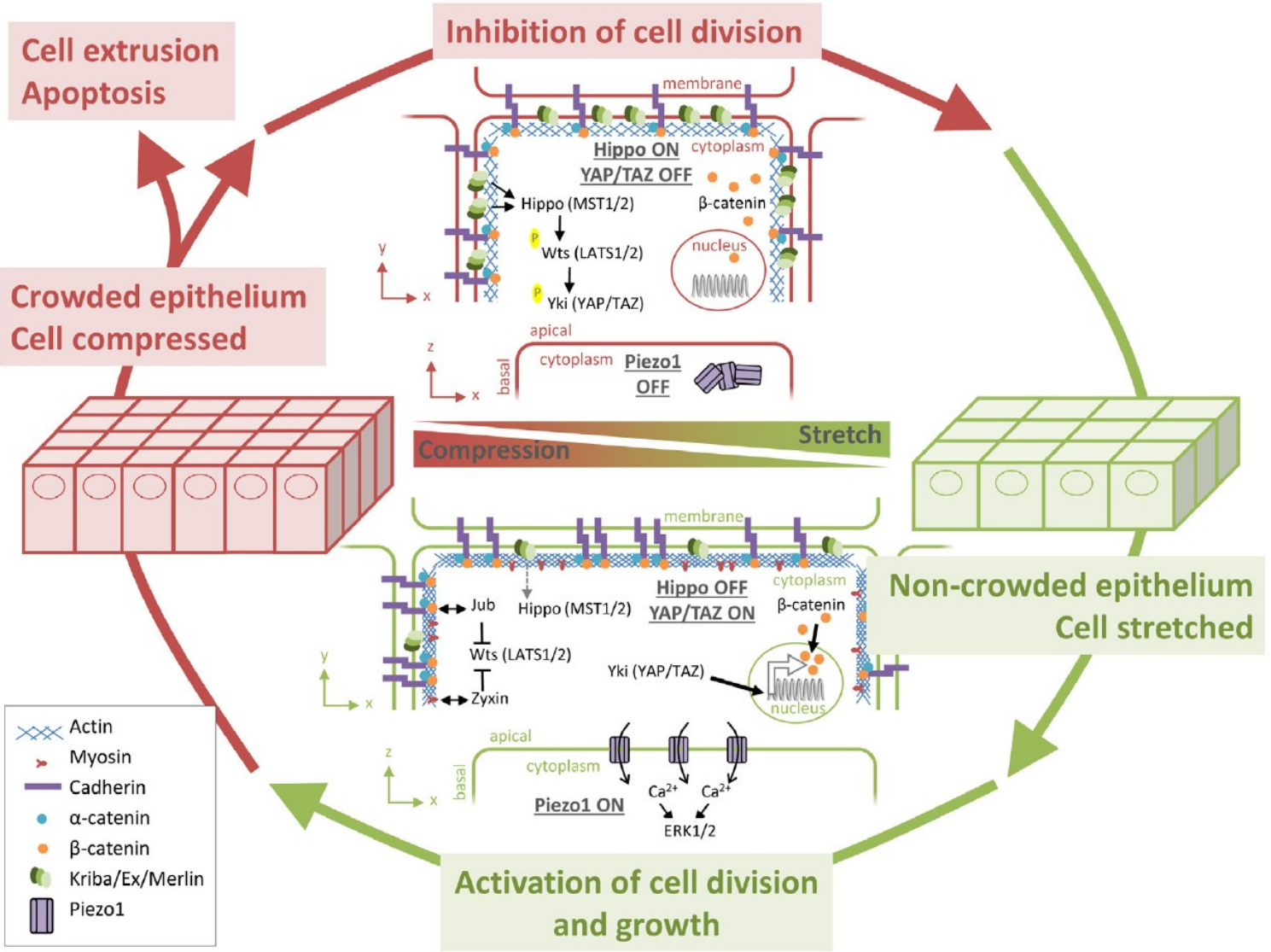


Fig. 4.5 Mammalian mechanosensitive Piezo1 architecture and a putative membrane-mediated gating mechanism. (**a**) Schematic of the side view of Piezo1 structure. (**b**) *Top view* of Cryo-EM structure of mouse Piezo1 as shown in *shaded grey* surface (PDB: 3JAC) [72]. (**c**) View from the *top* of the human Piezo1 (homology model based on mouse Piezo1) shows the interlocked arrangement of its 3 subunits at the level of the hydrophobic core of the lipid bilayer. An increase in lateral bilayer tension is thought to result in a clockwise or counter-clockwise deflection of the 'Blade' domains around the 'Anchor' and outer helix (OH) domains. This movement ultimately results in the displacement of the inner helices (IH) away from the center of the pore to allow ion conduction,

Role Piezo1 v regulaci „density“ epitelu





Mechanical feedback between cell proliferation and tissue stress. Cell proliferation leads to increased cell density within tissues, where cells sense mechanical compression forces that in turn trigger cell extrusion, apoptosis and inhibit cell division. Mechanical inhibition of cell division at high cell density is mediated by activation of Hippo signaling leading to the cytoplasmic retention and thus inactivation of the co-transcriptional activators YAP/TAZ [15,16,17•,18,23] and cytoplasmic localization and thus inactivation of the mechanosensitive Ca^{2+} channel Piezo [21••]. Mechanical induction of cell division at low cell density is mediated by inhibition of Hippo-signaling leading to YAP/TAZ nuclear localization and thus activation [15,16,17•,18,23], and nuclear translocation of β-catenin [18,19] and stretch-mediated activation of plasma membrane localized mechanosensitive Piezo channels [21••].

Article

PIEZO2 in sensory neurons and urothelial cells coordinates urination

https://doi.org/10.1038/s41586-020-2830-7

Received: 18 March 2020

Accepted: 22 July 2020

Published online: 14 October 2020

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Henry Miller stated that "to relieve a full bladder is one of the great human joys".