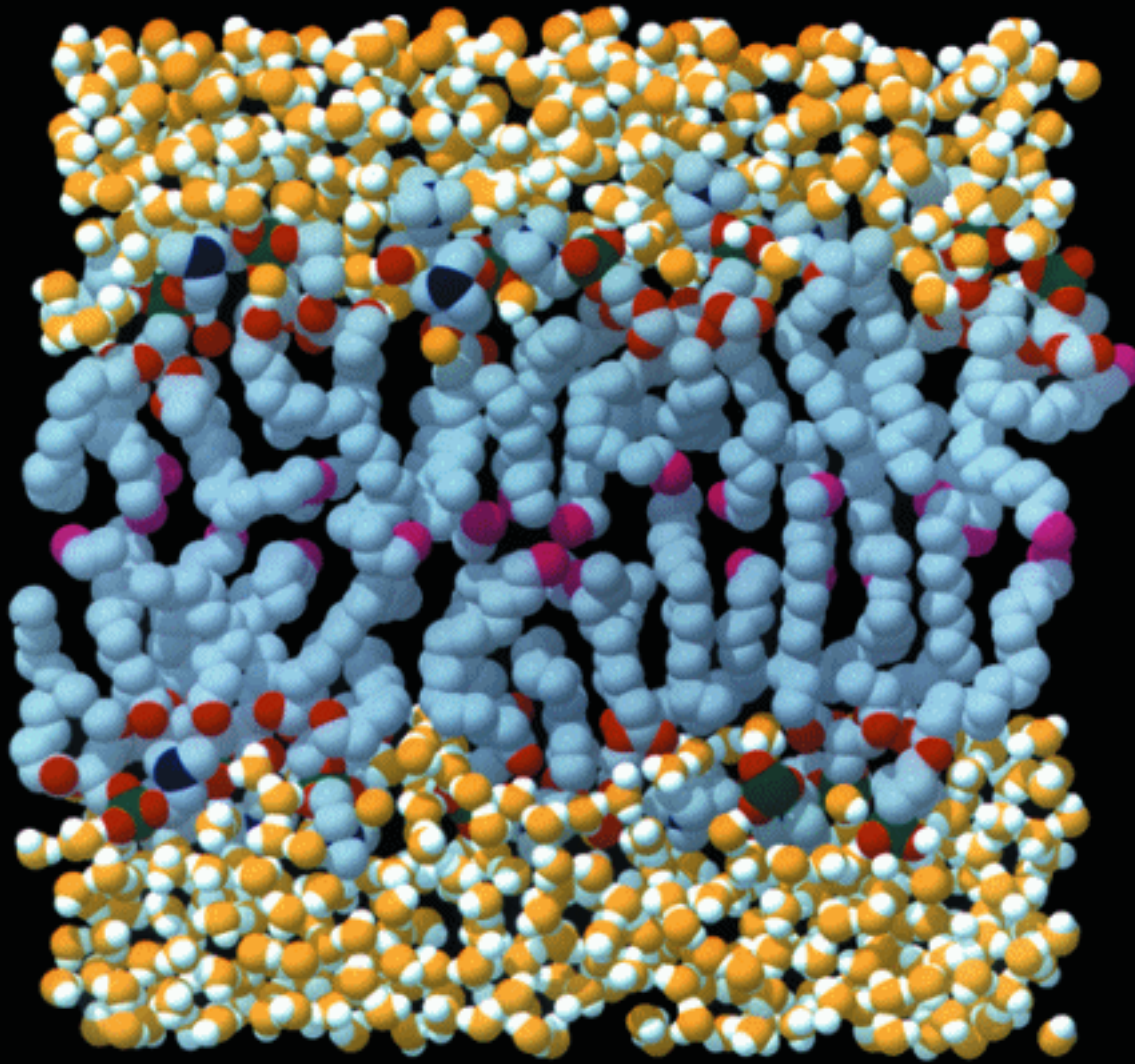


Molekulární aparát membránového transportu



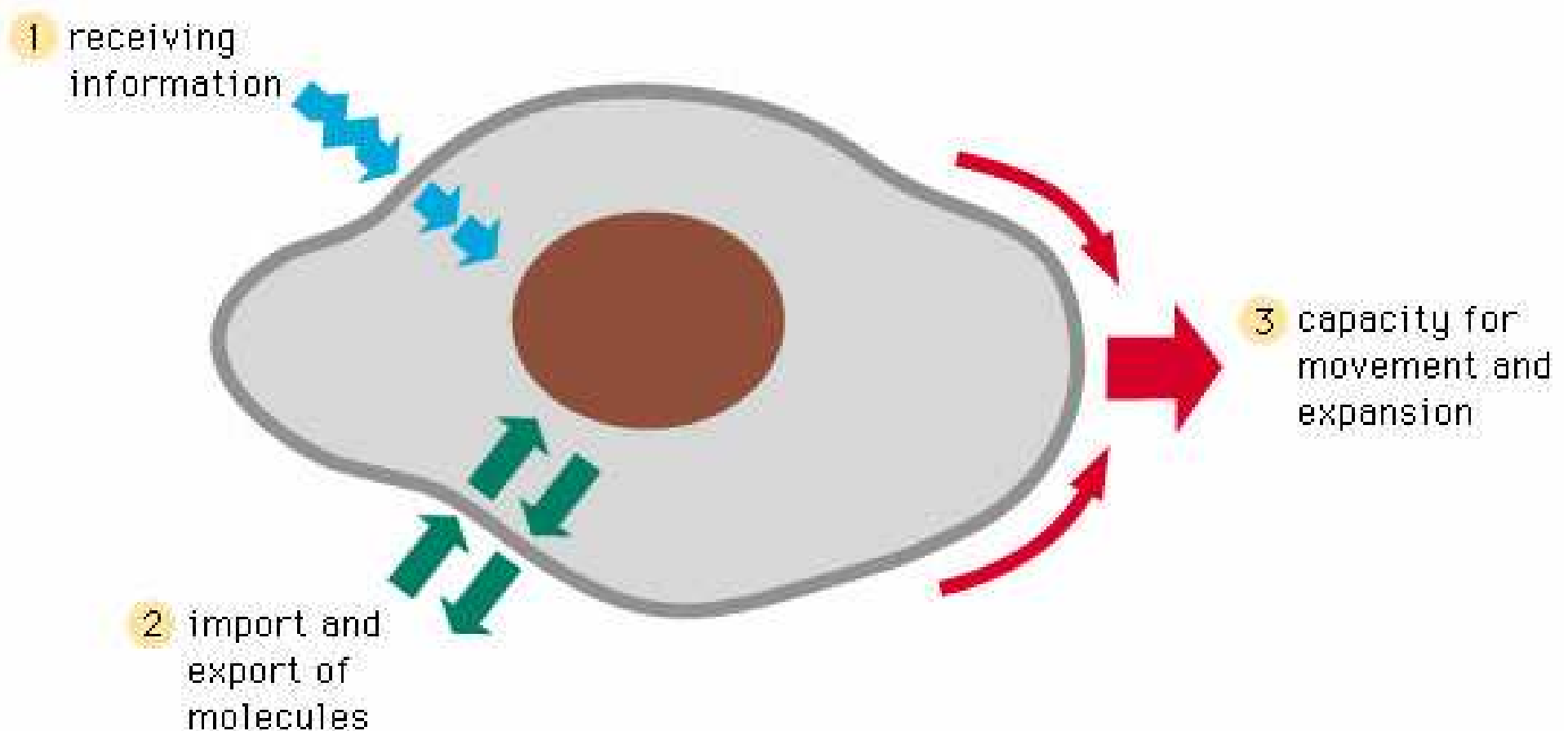
Osnova

- **Základní vlastnosti biologických membrán**
- Transport látek přes membránu
 - úvod
 - pumpy
 - přenašeče
 - kanály
 - transport vody
- Membránový transport v praxi – regulace otevírání a zavírání svěřacích buněk

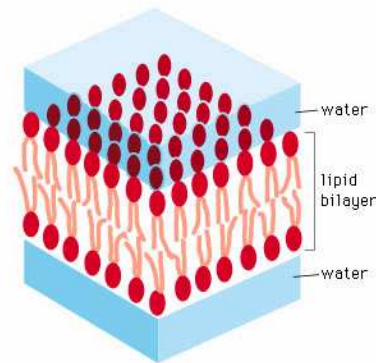
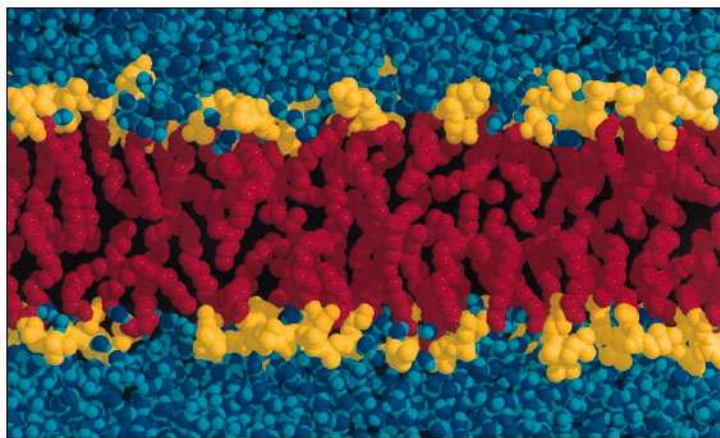
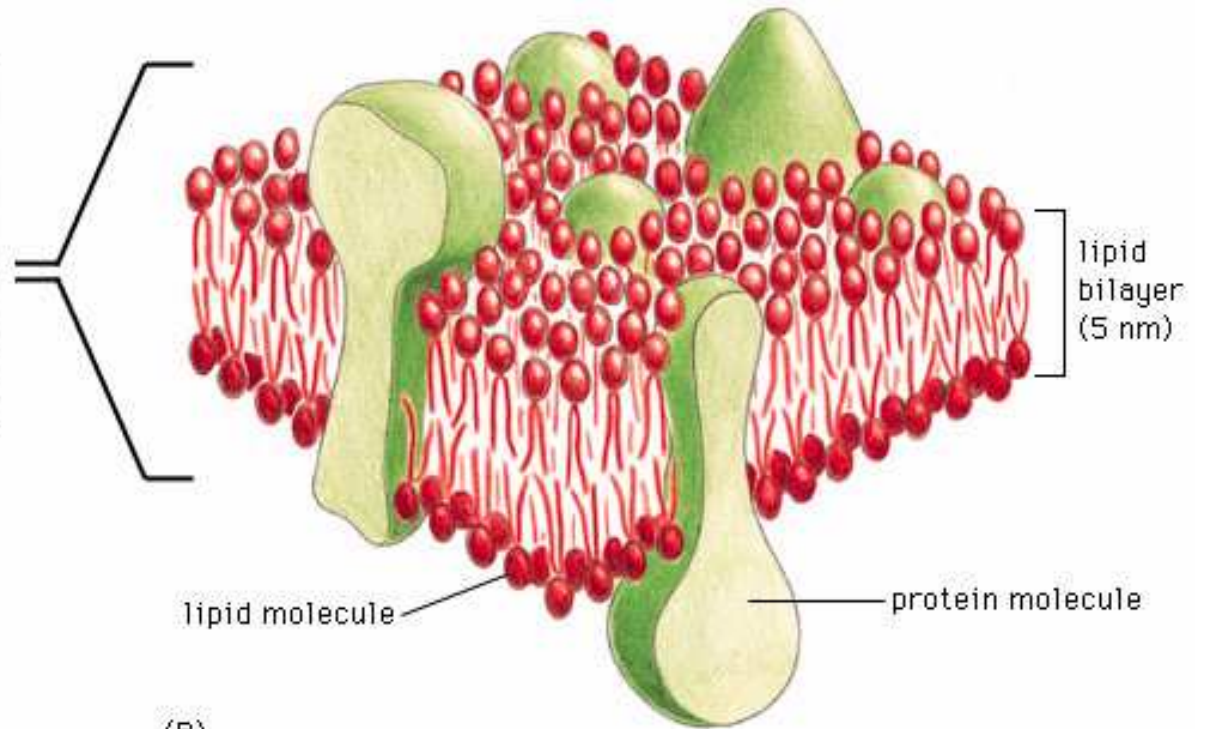
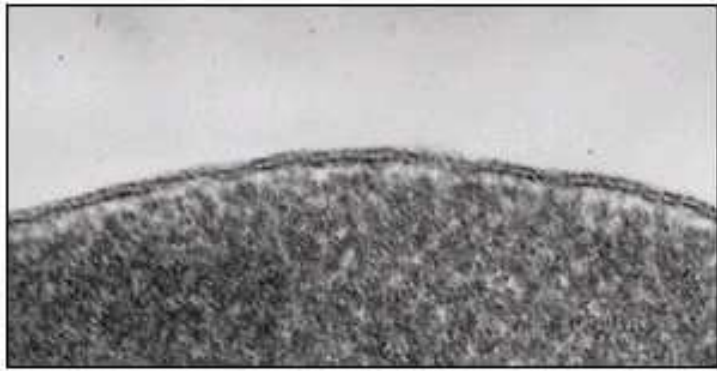
Membrány obsahují

1. sensory které umožňují reakci buňky na podněty z okolí
2. pumpy, přenašeče a kanály pro transport látek skrz.

Mechanické vlastnosti membrán jsou obdivuhodné (např. Schopnost růstu a změny tvaru bez ztráty integrity)



Membrány = lipidová dvojvrstva + proteiny



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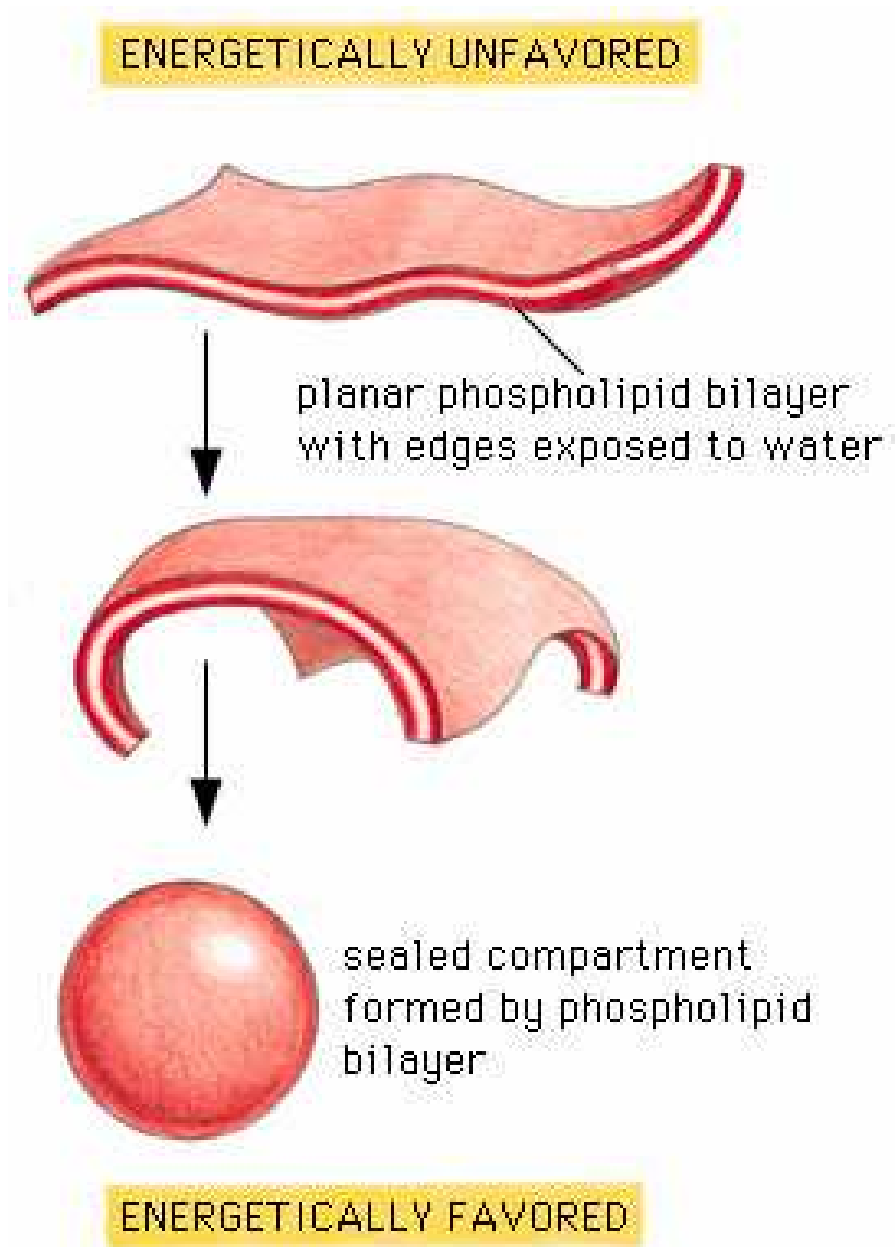
©1998 GARLAND PUBLISHING

Self-sealing property

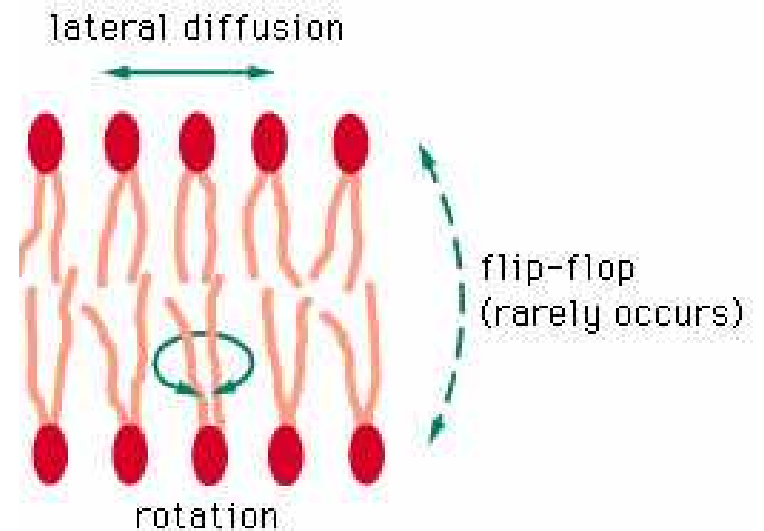
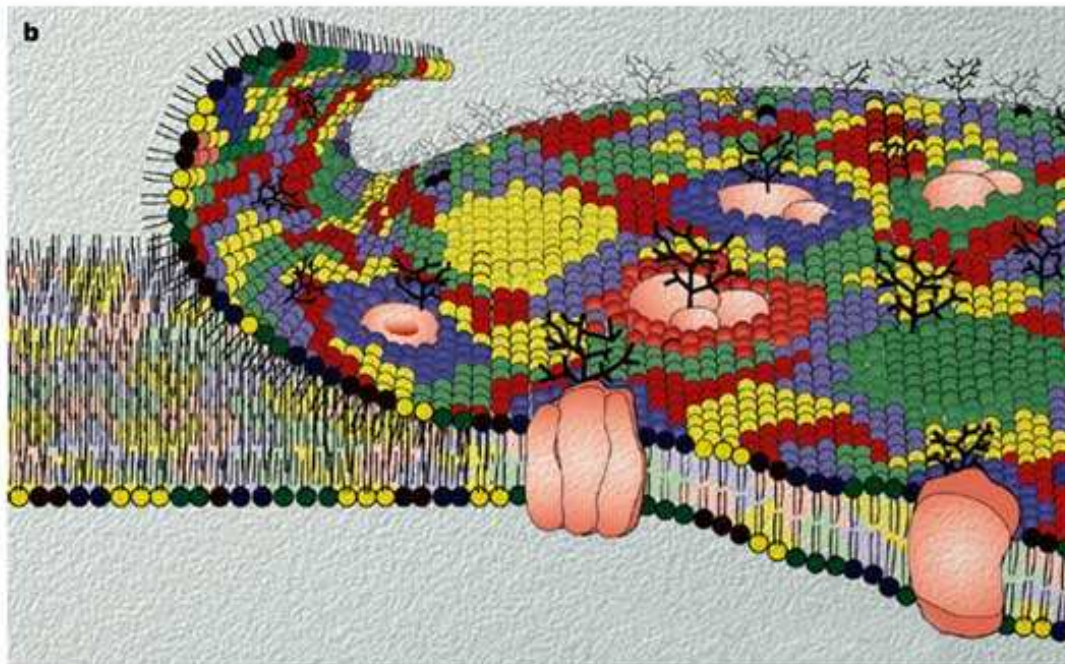
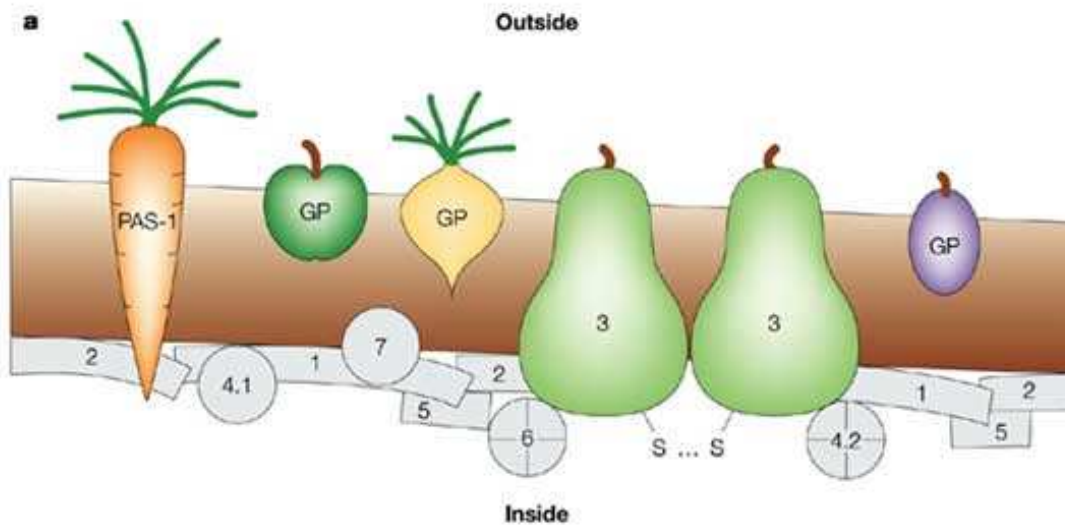
“volné konce” jsou rychle odstraněny – energeticky nevýhodné (kontakt hydrofobních částí s vodou)

“The lipids will spontaneously seal and will always form a closed compartment.

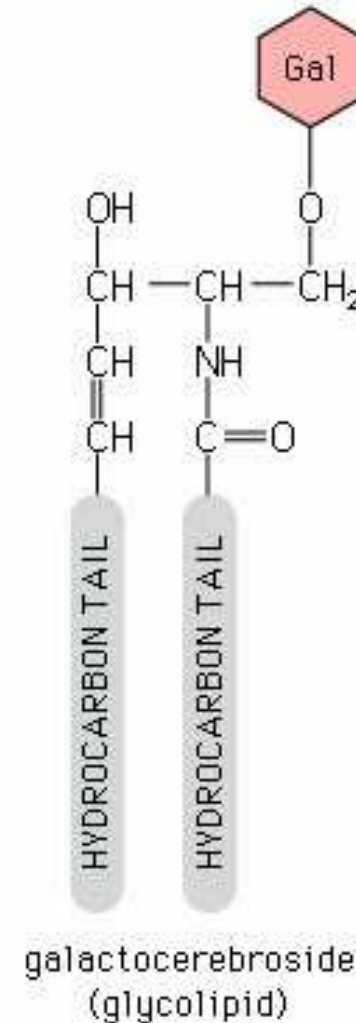
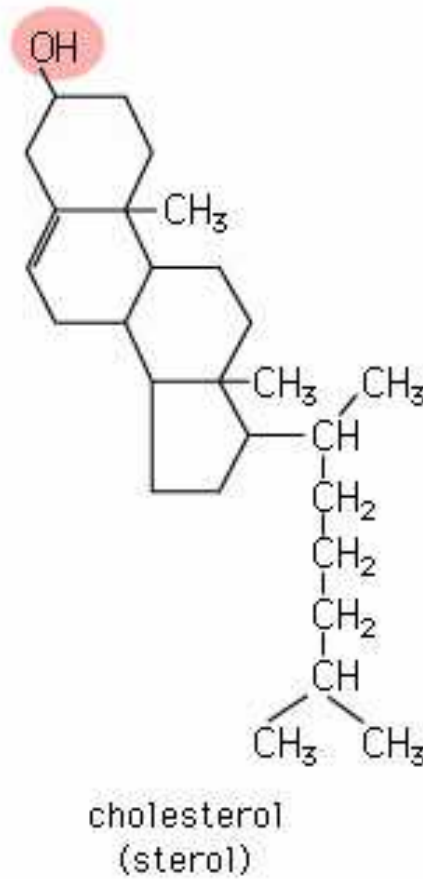
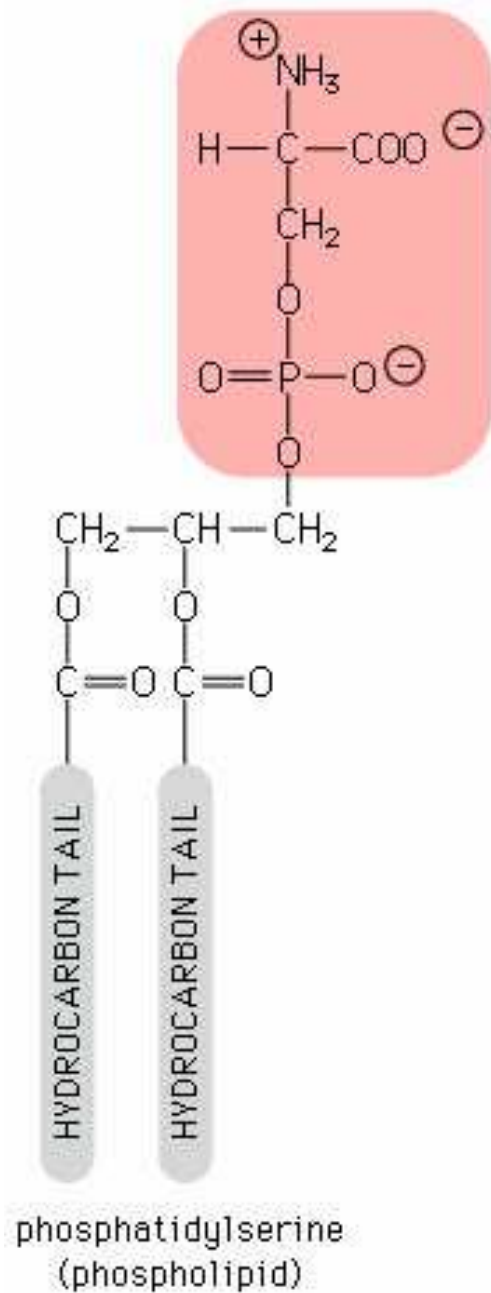
A small tear will be repaired. A larger tear may lead to the break up of the membrane into separate vesicles.”



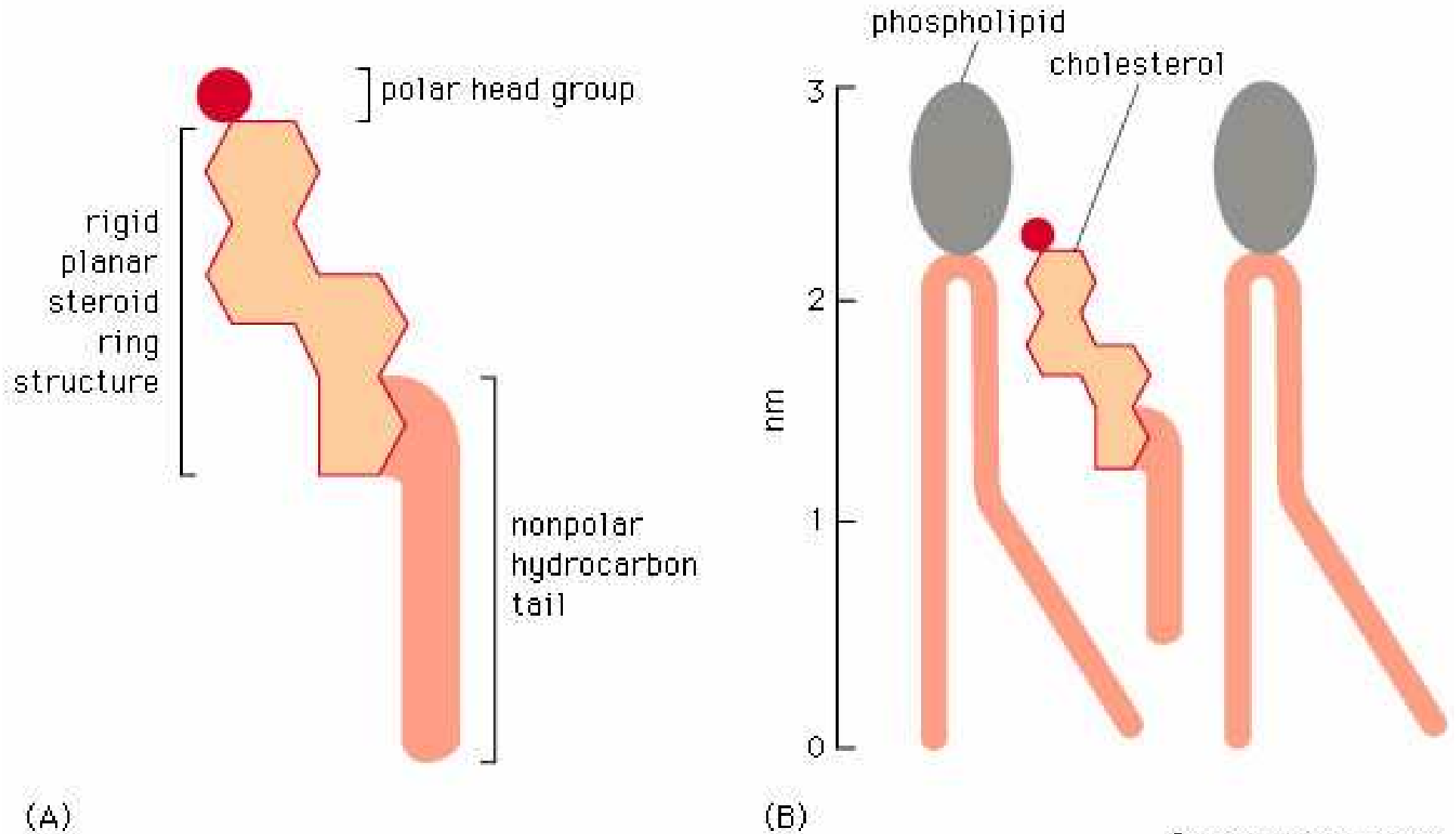
Model membrány jako fludní mozaiky



3 základní stavební kameny lipidových dvouvrstev



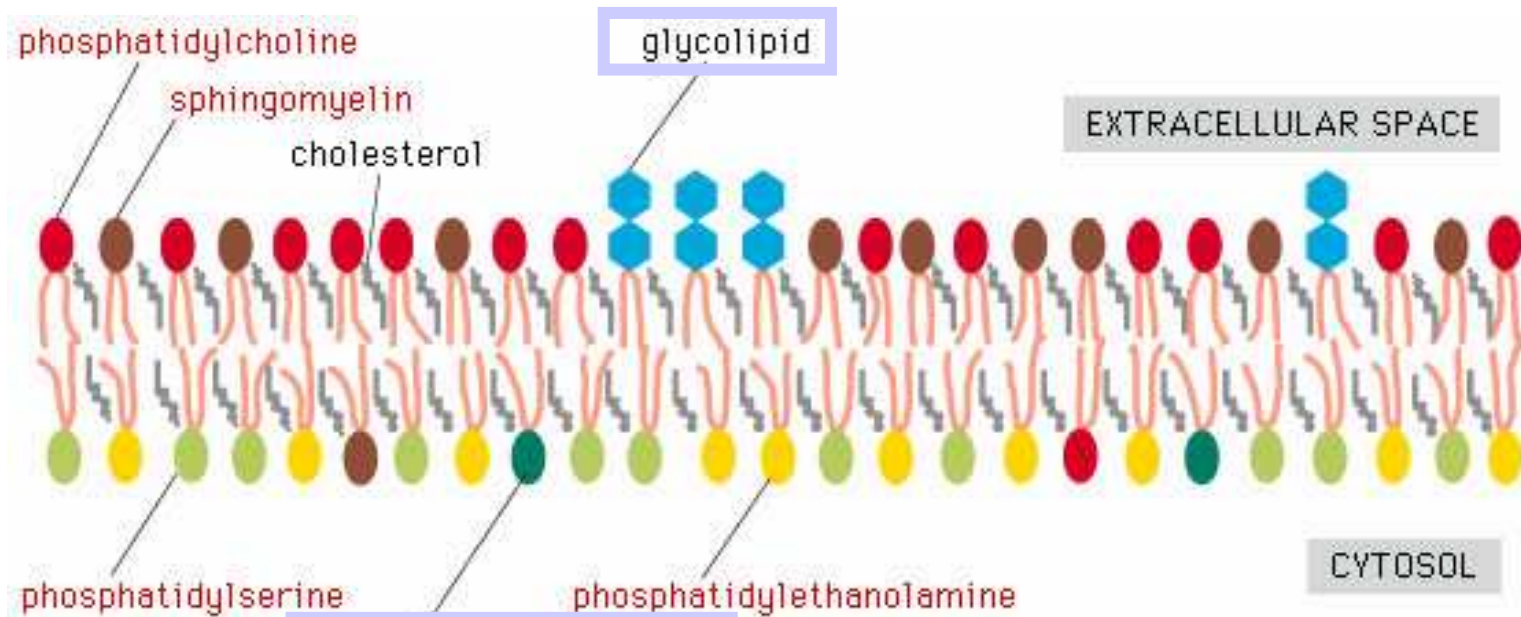
Steroly se vmezeřují do prostor mezi fosfolipidy – zvyšují tuhost dvouvrstvy, snižují fluiditu a permeabilitu.



Lipidová dvouvrstva je složením ASYMETRICKÁ!

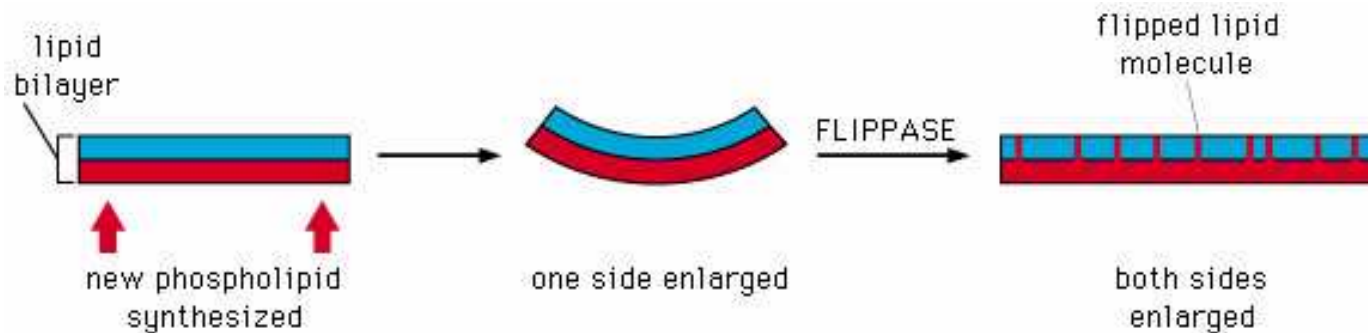
Nové membr. lipidy vznikají v ER, např PC a spol na cytosolické straně - flipasy

Glykolipidy dosyntetizovány v Golgi, pouze na vnější straně (žádné flipasy)



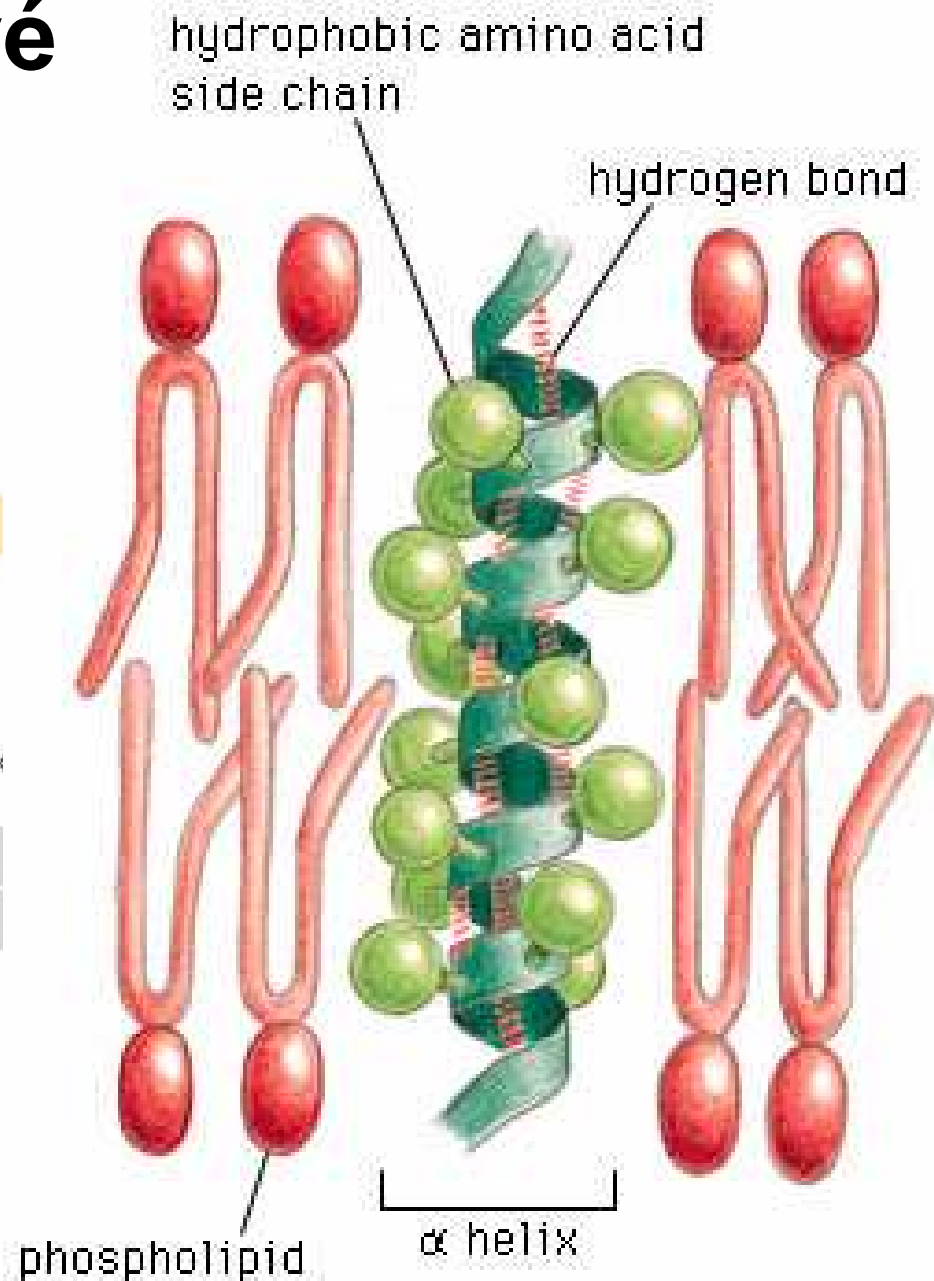
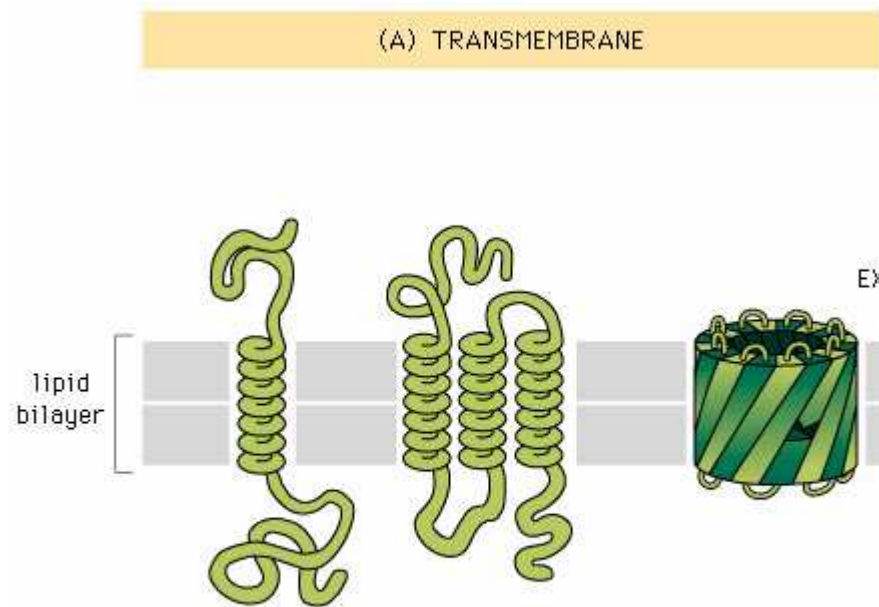
Intracellular signal transduction

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Transmembránové proteiny



Osnova

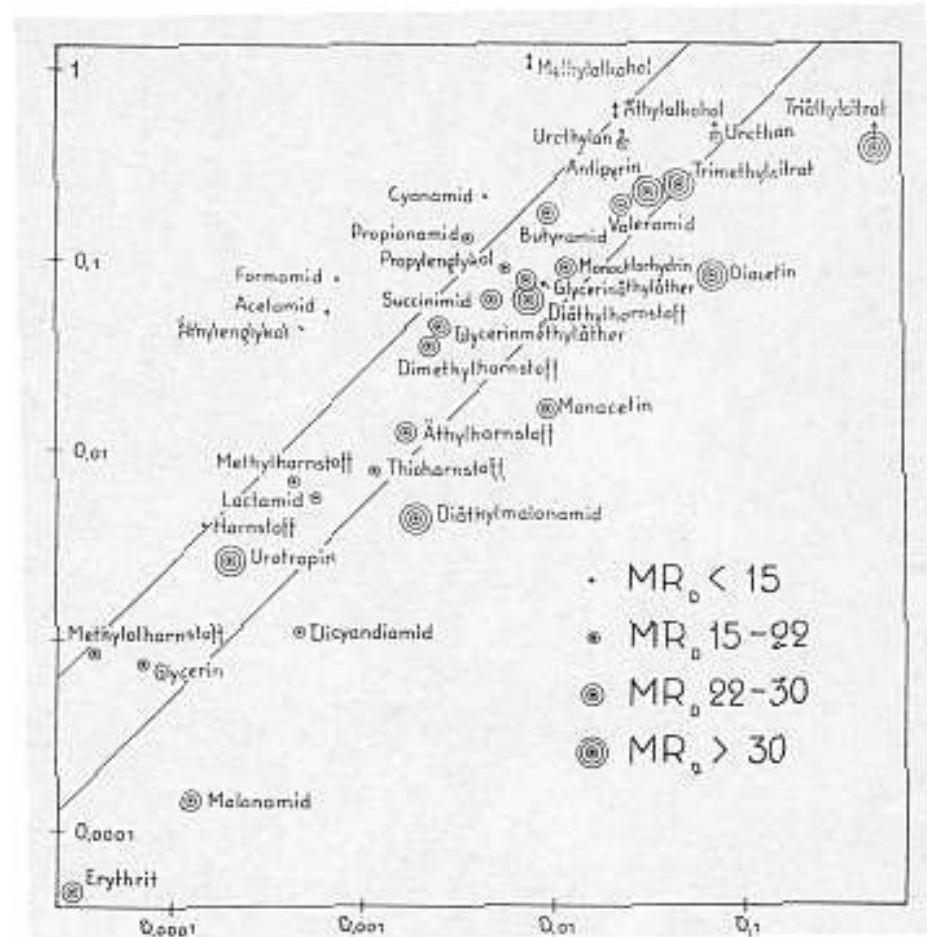
- Základní vlastnosti biologických membrán
- Transport látek přes membránu
 - **úvod**
 - pumpy
 - přenašeče
 - kanály
 - transport vody
- Membránový transport v praxi – regulace otevírání a zavírání svěracích buněk

Co prochází membránou?

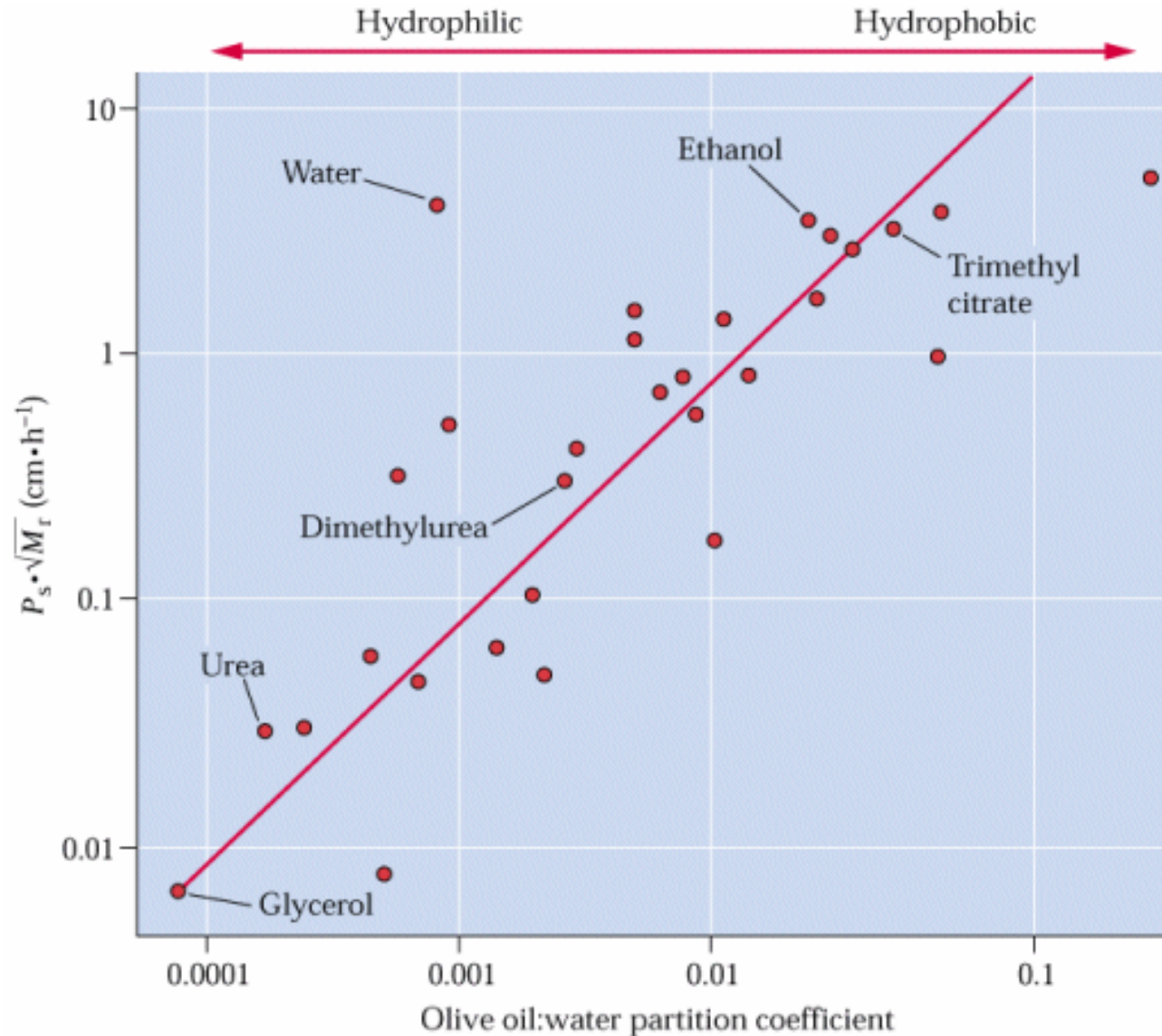


Runar P. Collander
(1894-1973)

membránová permeabilita
buněk Chara sp. (30. léta)



Co prochází membránou „samo od sebe“?



koef. membr.
permeability

$$P_s = k \cdot V / t_{0.5} A$$

objem
poločas dosažení rovnováhy
povrch

- nenabité molekuly!

Can I get through please?

Depends...

Who are you?

How big are you?

Are you charged?

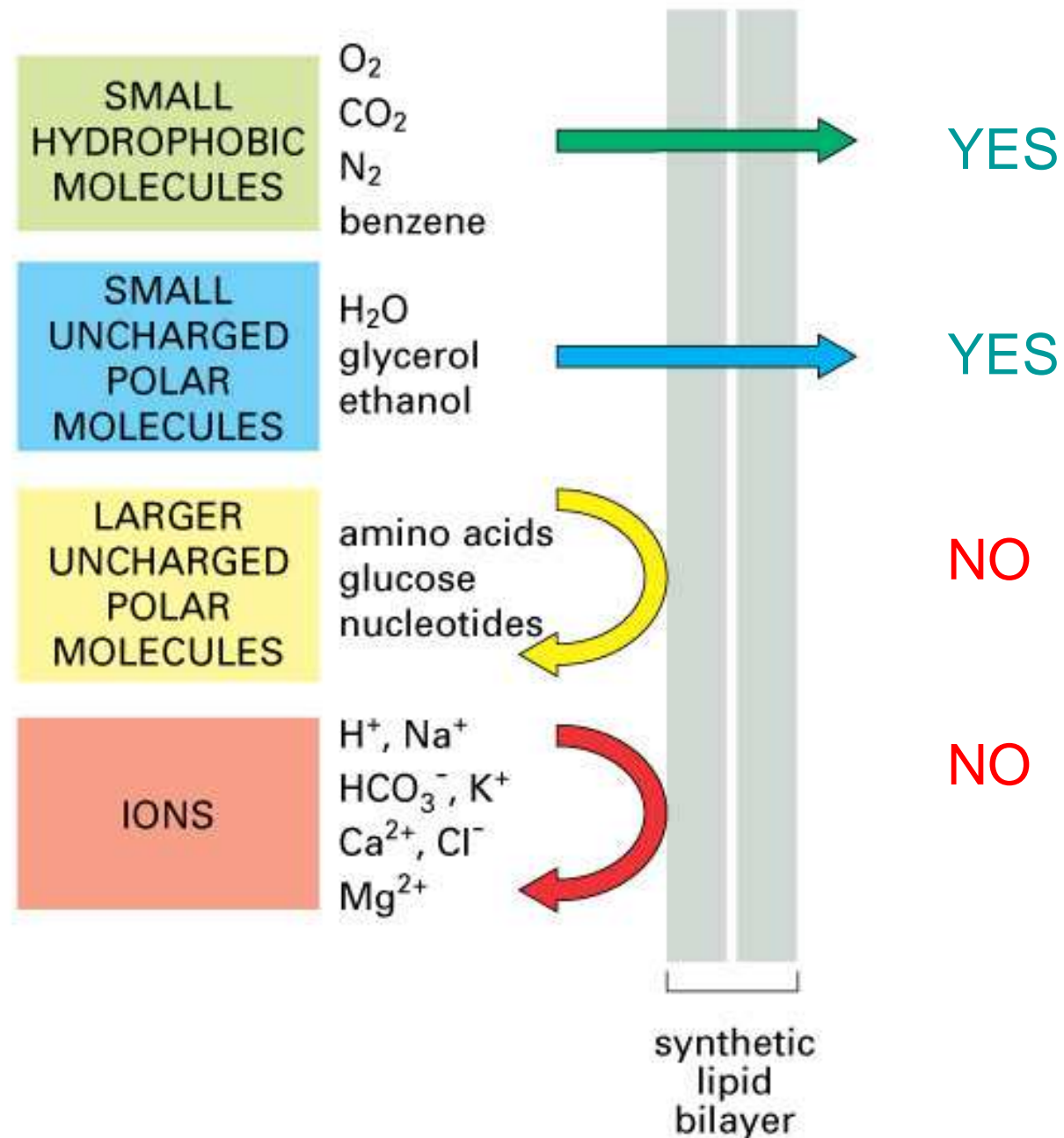


Figure 12-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Membránový transport

- Tvorba turgoru (většinou K^+)
- Příjem minerálů a živin
- Vyloučení odpadních produktů
- Vyloučení toxických látek
- Distribuce metabolitů
- Kompartmentalizace metabolitů
- Přenos energie
- Přenos signálů

Membránové transportery obecně:

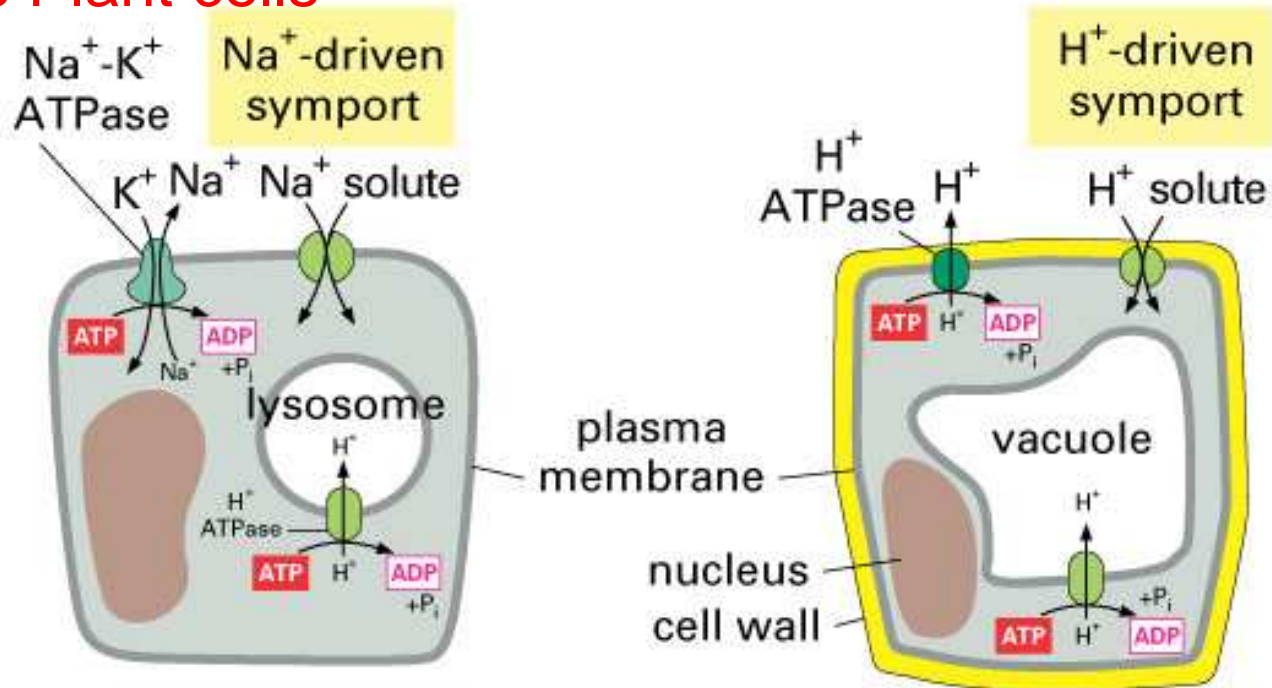
- Pumpy
 - pohon ATP, PP
 - *Primární aktivní transport*
- Přenašeče (Carriers)
 - pohon gradienty na membráně (pmf)
 - *Sekundární aktivní transport*
 - *Pasivní transport (usnadněná difuze)*
- Kanály
 - pohon gradienty na membráně
 - *Pasivní transport (usnadněná difuze)*

Kapacita membránových transportů

- **Pumpy:** kovalentní reakce – 10^2 s^{-1}
- **Přenašeče:** změny konformace – 10^3 s^{-1}
- **Kanály:** ani jedno z toho – $10^6 - 10^8 \text{ s}^{-1}$

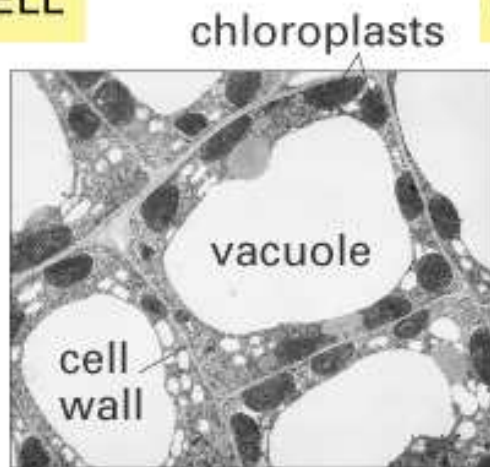
... srv. zastoupení v buňkách

Animal vs Plant cells



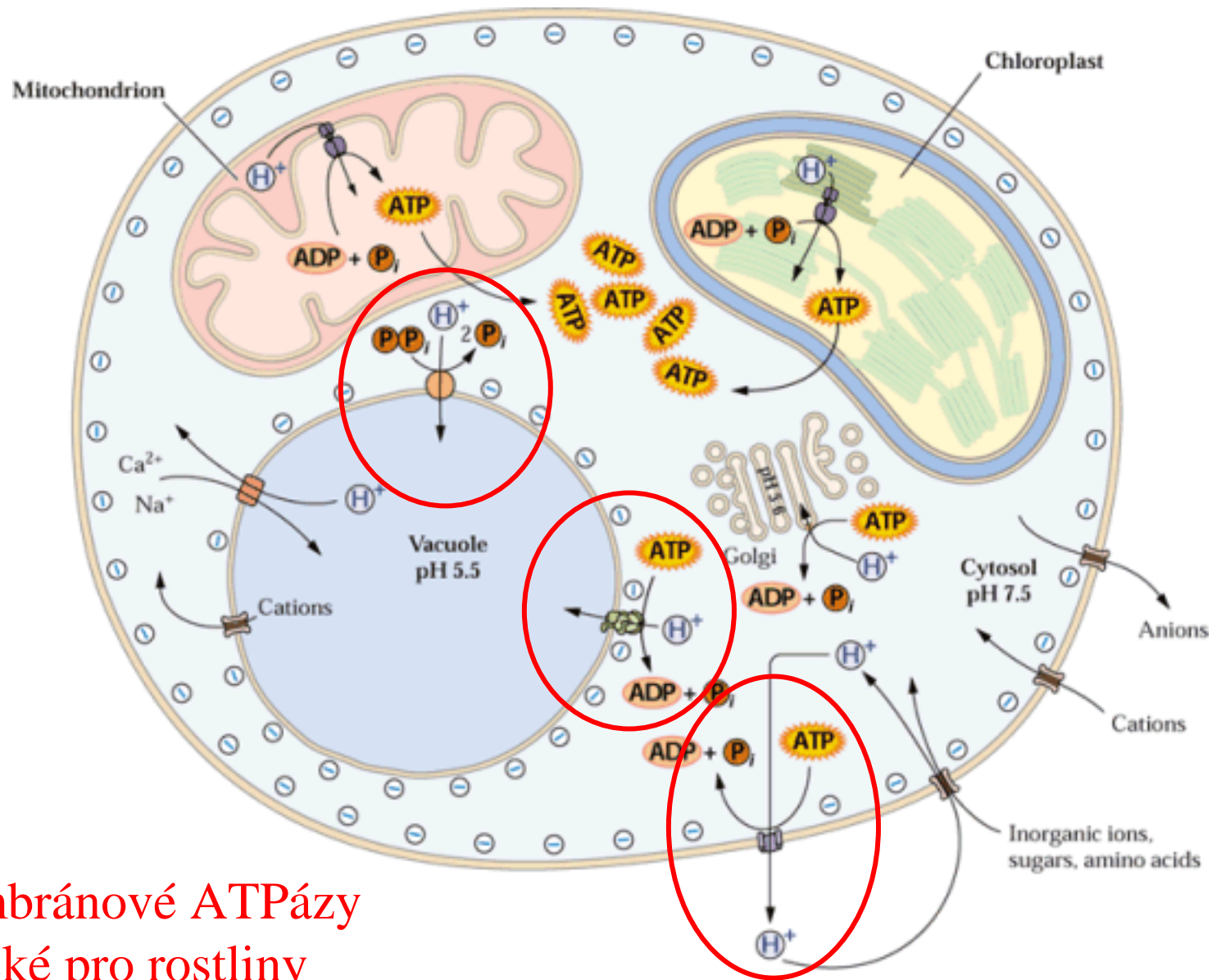
(A) ANIMAL CELL

(B) PLANT CELL



(C)

10 μm



Membránové ATPázy
typické pro rostliny

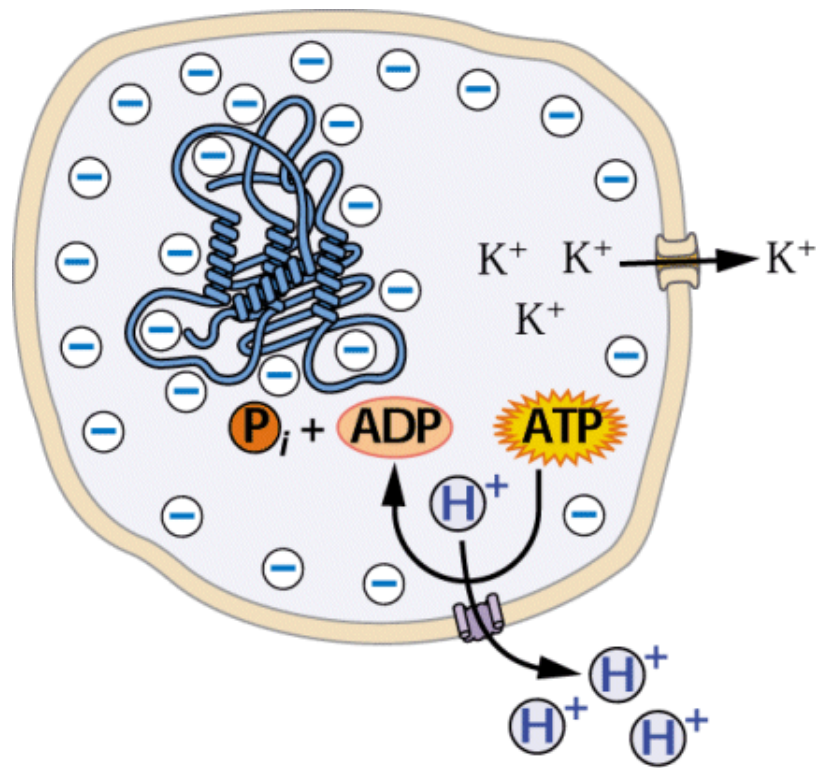
Membránový potenciál V_m

je rozdíl elektrických potenciálů dvou vodných roztoků iontů oddělených membránou.

plasmalema = -150 mV

tonoplast = -20 mV

Dominantním zdrojem
membránového potenciálu V_m
rostlin jsou protonové pumpy.



Proto jsou protonové pumpy
elektrogenní.

Membránový potenciál vytvářený protonovou pumpou je součástí tzv. **proton-motivní síly (pmf)**, jejímž zdrojem je také koncentrační rozdíl protonů.

V_m a **pmf** jsou klíčovou složkou všech transportních dějů na membráně.

Protože platí, že 1 stupňový rozdíl
pH odpovídá asi 59mV,
lze vyjádřit netermodynamicky

$$pmf=59.1x(pH_o-pH_c)+Vm$$

při průměrném V_m -120 až -150 mV a
rozdílu pH ve stěně (pH5) a v cytoplasmě
(pH7) vychází pak pmf rost. b. až -268mV.

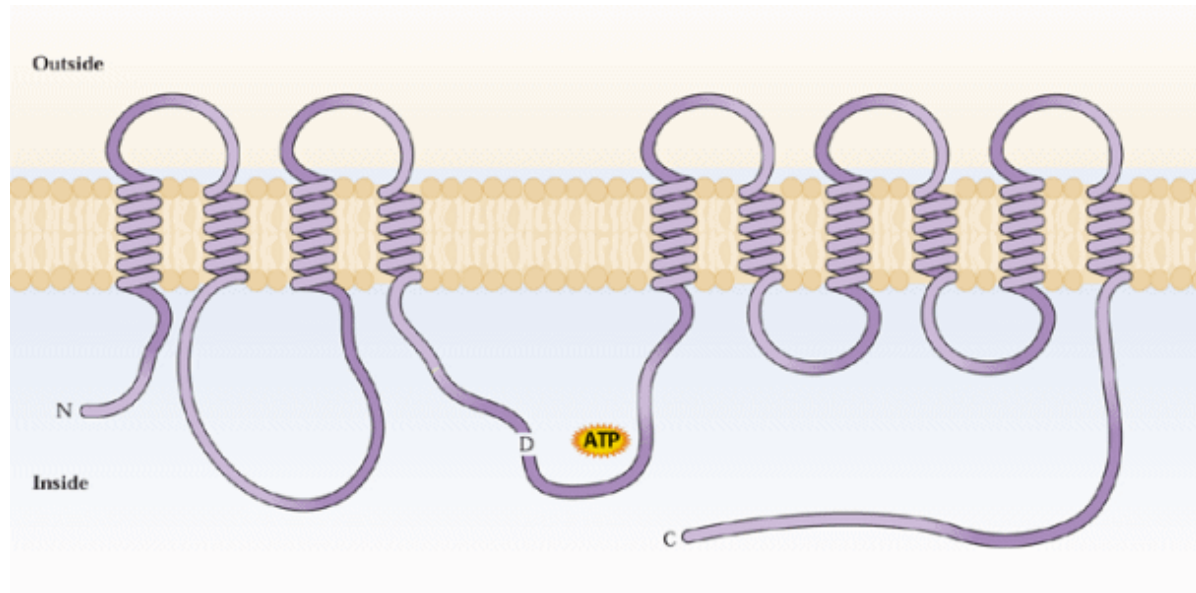
Osnova

- Základní vlastnosti biologických membrán
- Transport látek přes membránu
 - úvod
 - **pumpy**
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 - kanály
 - transport vody
- Membránový transport v praxi – regulace otevírání a zavírání svěřacích buněk

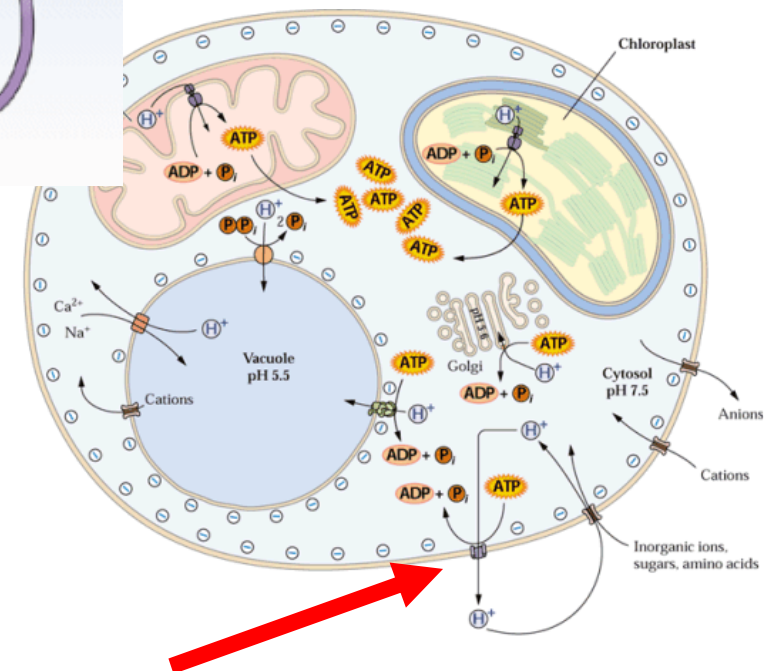
Membránové transportery konkrétně:

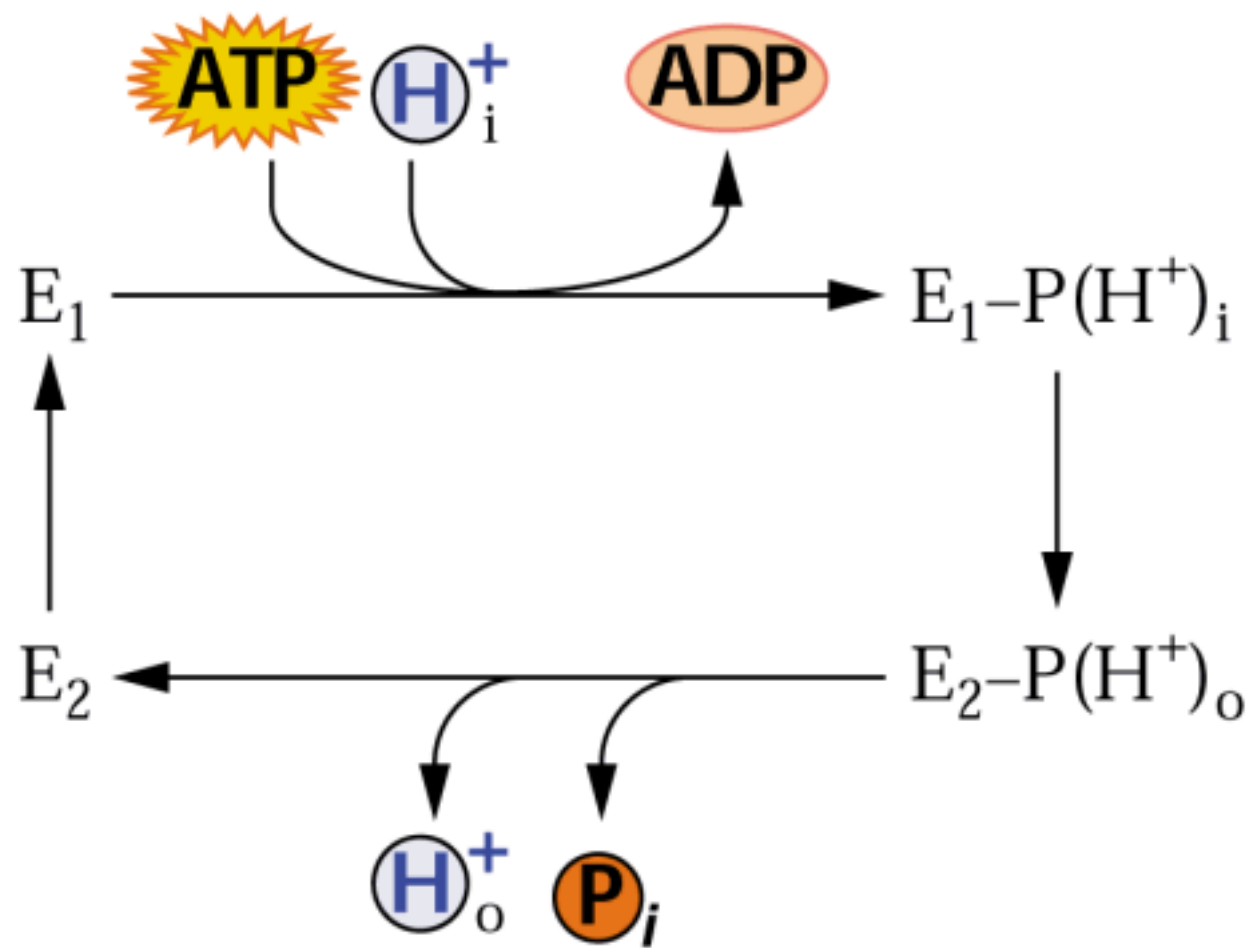
- Pumpy
 - pohon ATP, PP
 - pro rostliny typické H⁺ pumpy plasmalemy, tonoplastu
- Přenašeče
- Kanály

Plasmalemma: P – H⁺-ATPáza



Arabidopsis: genová rodina AHA
(11 genů!)





Proč má Arabidopsis tolik AHA genů?

| | |
|--|--|
| „housekeeping“ | AHA1 (shoots), AHA2 (roots) |
| všude, ale málo (regulace podmínkami?) | AHA5 |
| část. orgán. specif. | AHA3 (floem), AHA11 (list), AHA4 (kořen) |
| prašníky a pyl | AHA6 , AHA7 , AHA8 , AHA9 |
| obal semene | AHA10 |

esenciální

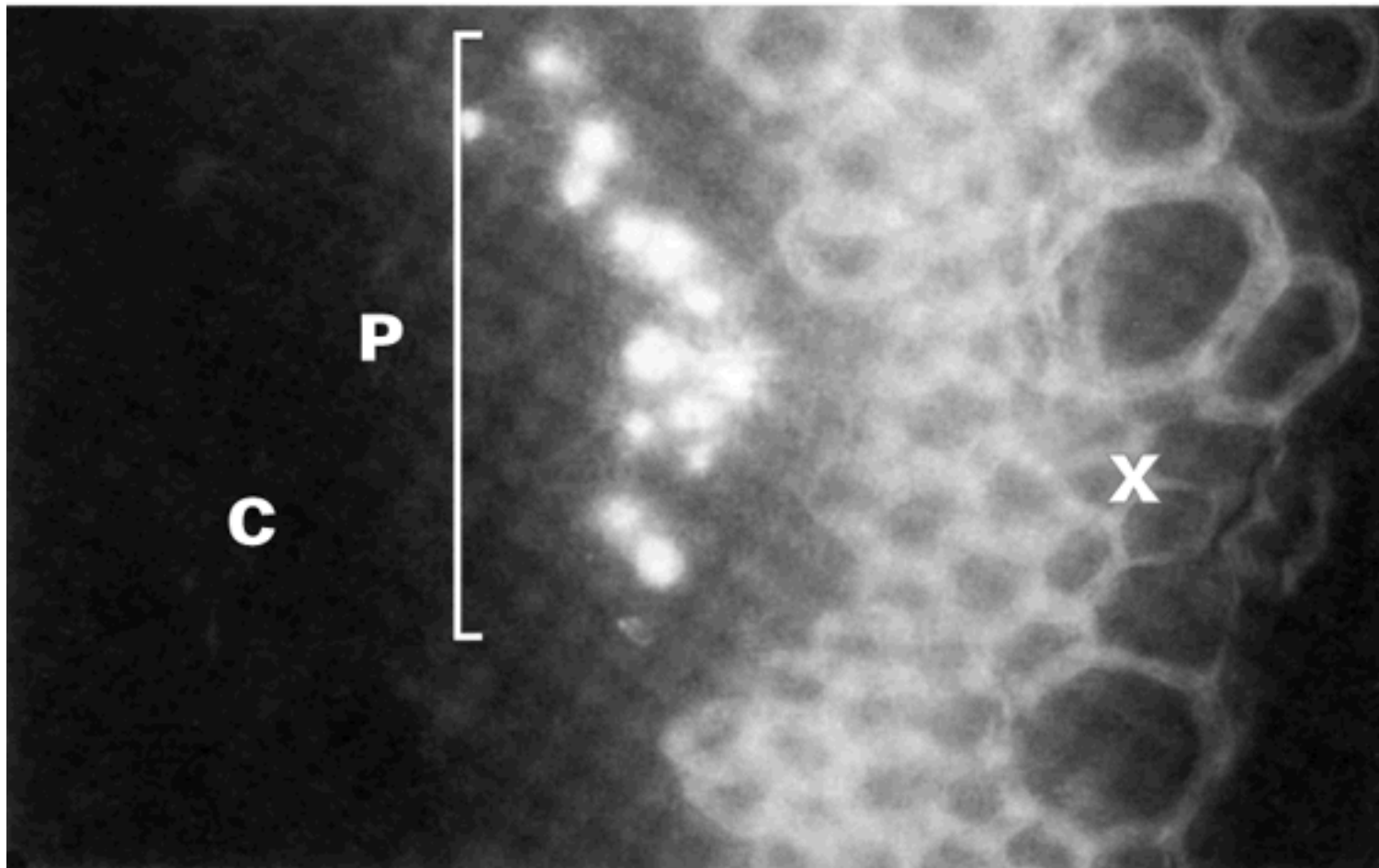
mutantní fenotyp (AHA4 dominant. alela: salt sens., aha10: transparent testa)

v DRMs („raftech“)

(Gaxiola et al., FEBS Lett. 2007)

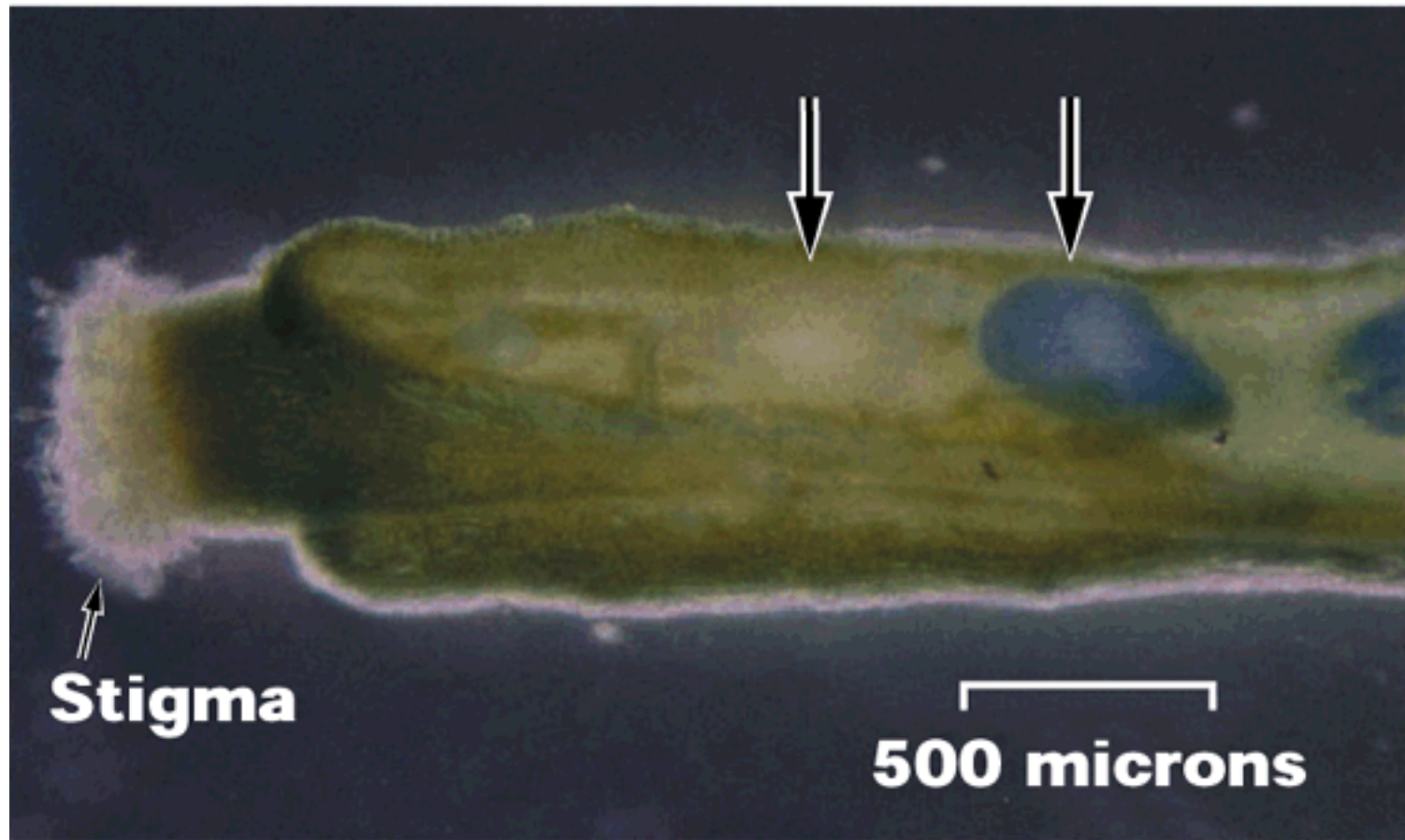
AHA3 exprese ve floému (Myc-tag)

(A)



AHA10 v semeni – GUS

(B)



Posttranslační regulace P-ATPázy: fosforylace a 14-3-3 protein

(transkripční regulace je,
ale nevýrazná!)

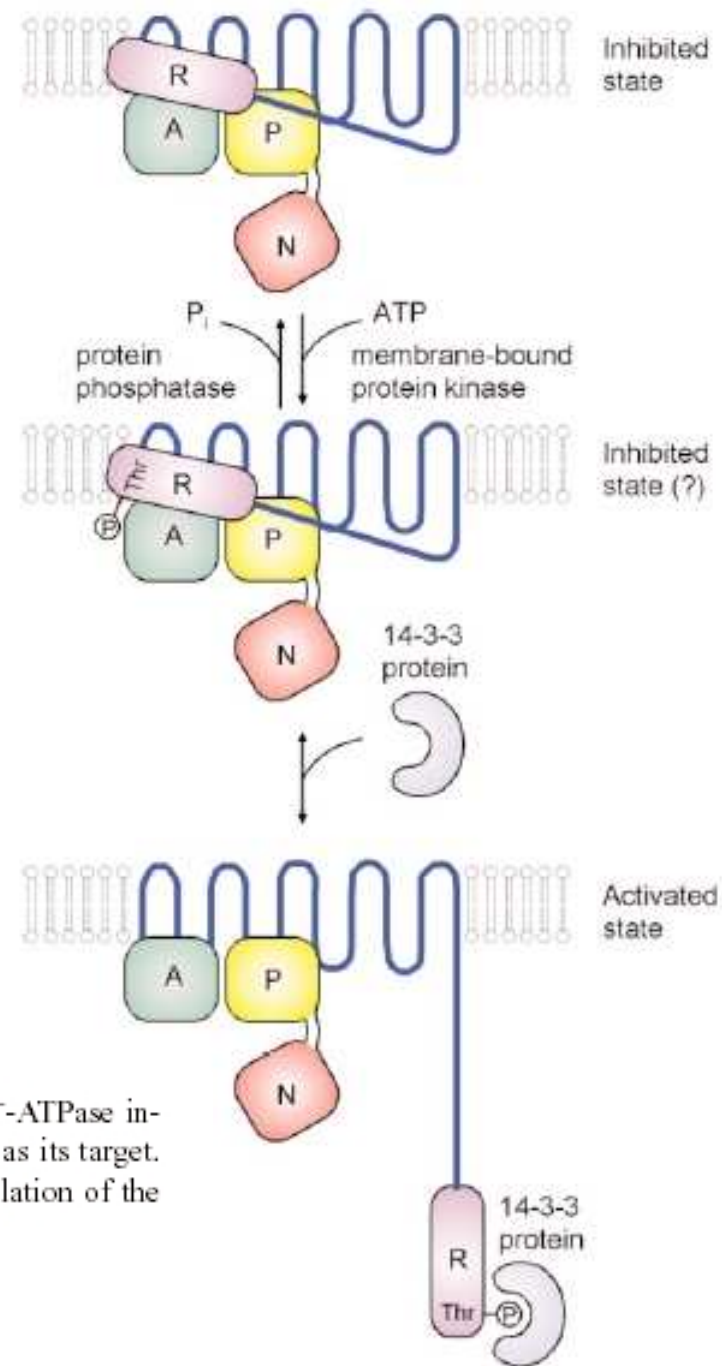
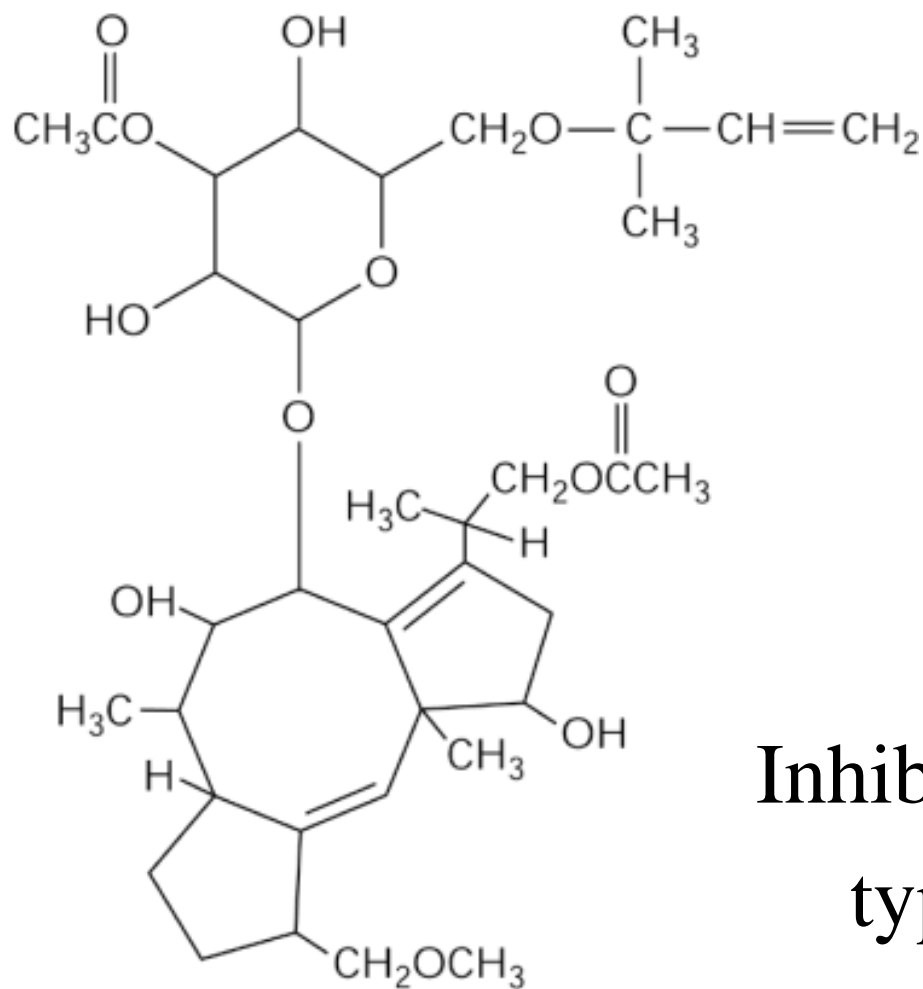


Figure 6 A model for posttranslational regulation of plasma membrane H⁺-ATPase involving a protein kinase and protein phosphatase pair that has the R-domain as its target. Regulatory 14-3-3 protein recognizes a binding site generated by phosphorylation of the R-domain.

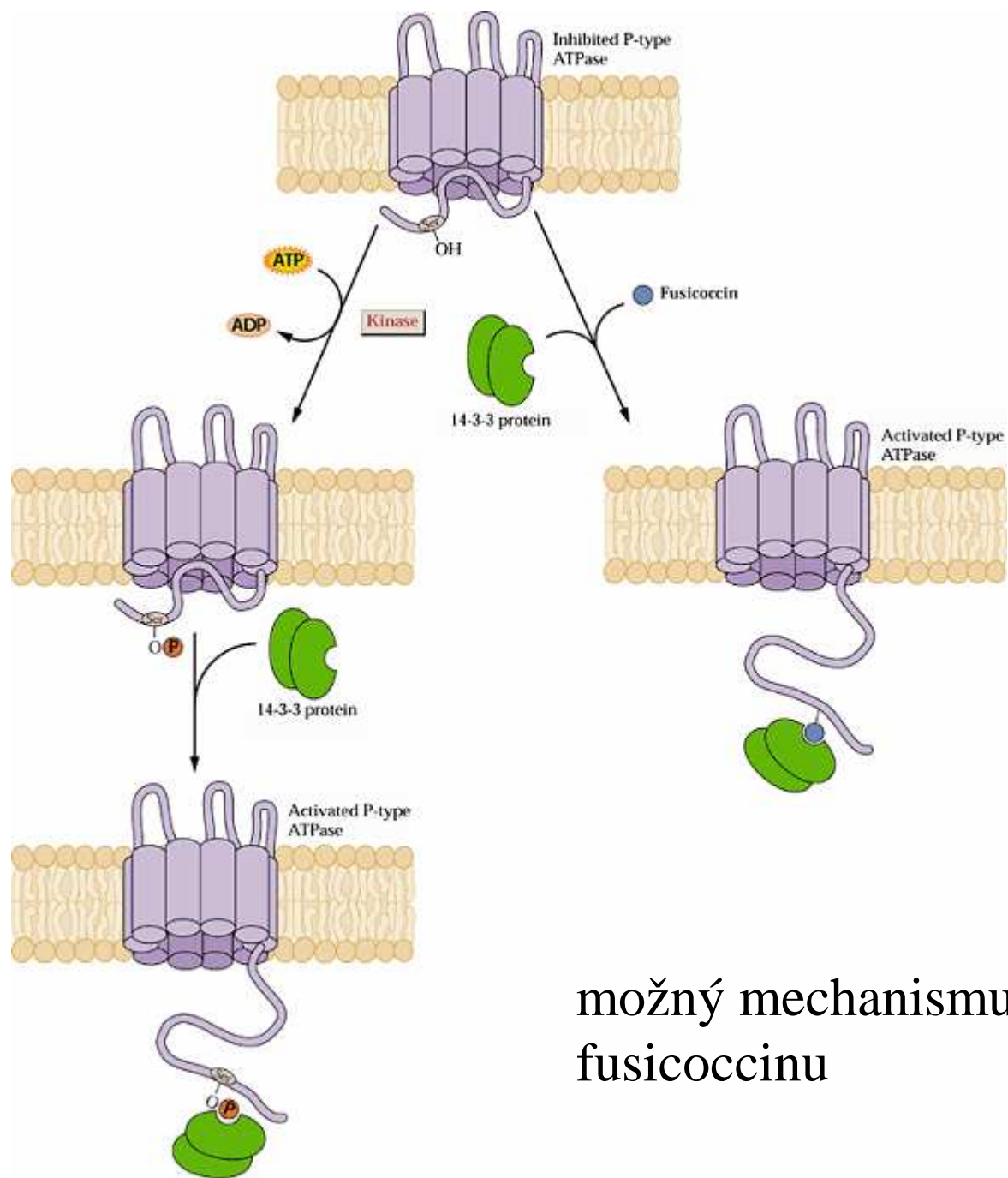
Agonista P – H⁺-ATPázy – **fusicoccin** (ireversibilní)



Fusicoccin

z houby *Fusicoccum amygdali*
působí trvalé otevření
průduchů a uschnutí.

Inhibitor všech! ATPáz P-
typu je **ortovanadát**



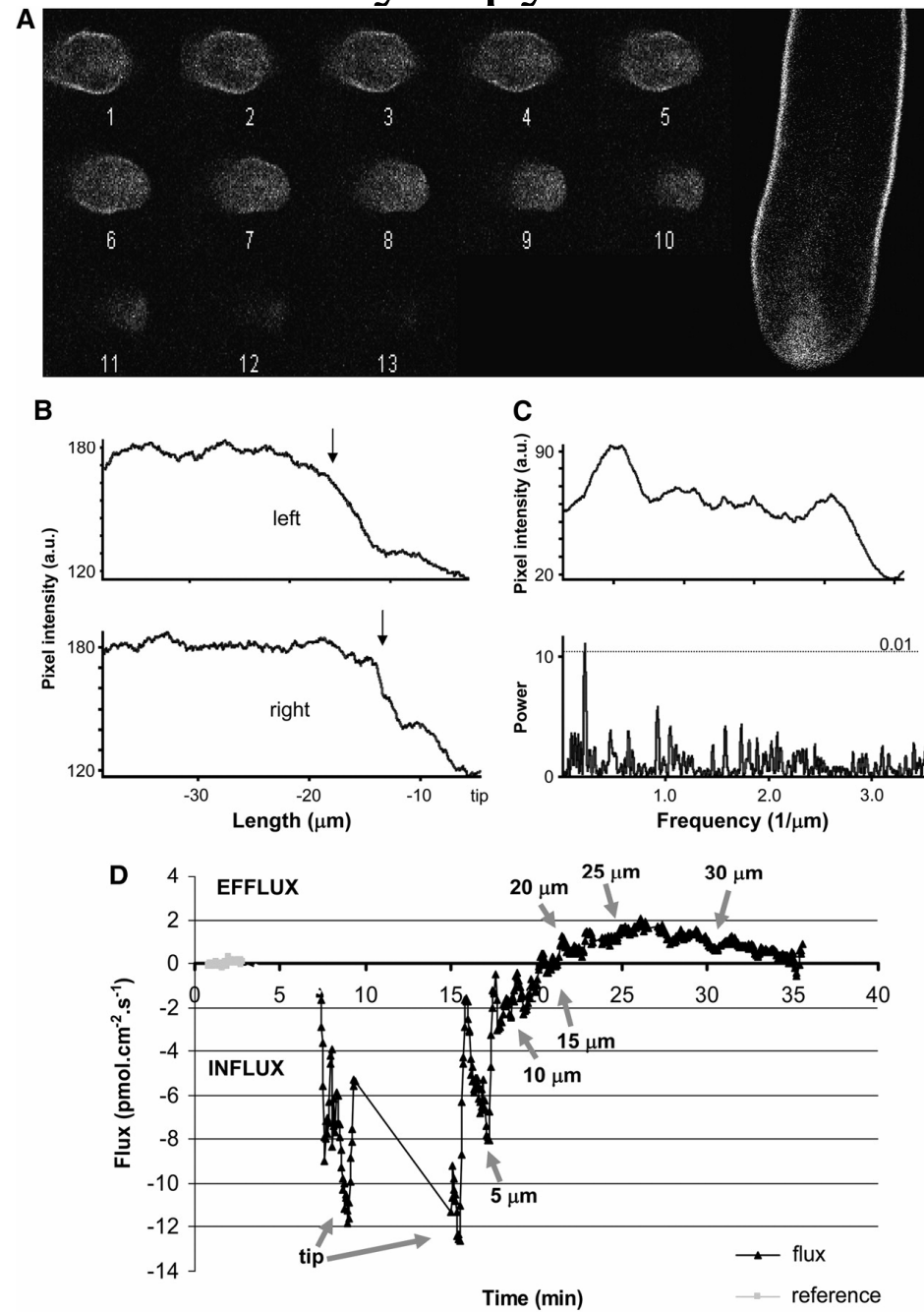
možný mechanismus působení fusicoccinu

Fyziologické signály regulující fosforylaci?

- Blue light - phytochrom -
 - aktivace ATPázy- (otevření průduchů špenátu)
 - inaktivace ATPázy - (snížení turgoru - pohyb listů fazolu)
- Sucho - ABA - defosforylace - inaktivace ATPázy- (zavření průduchů)
- též odpověď na cukry ... v různ. systémech

- kontext ovlivňuje výsledek!

Lokalizace H-ATPasy v pylové láčce tabáku



Gradients pH v rostoucích buňkách: příklad pyl. láček a koř. vlásků

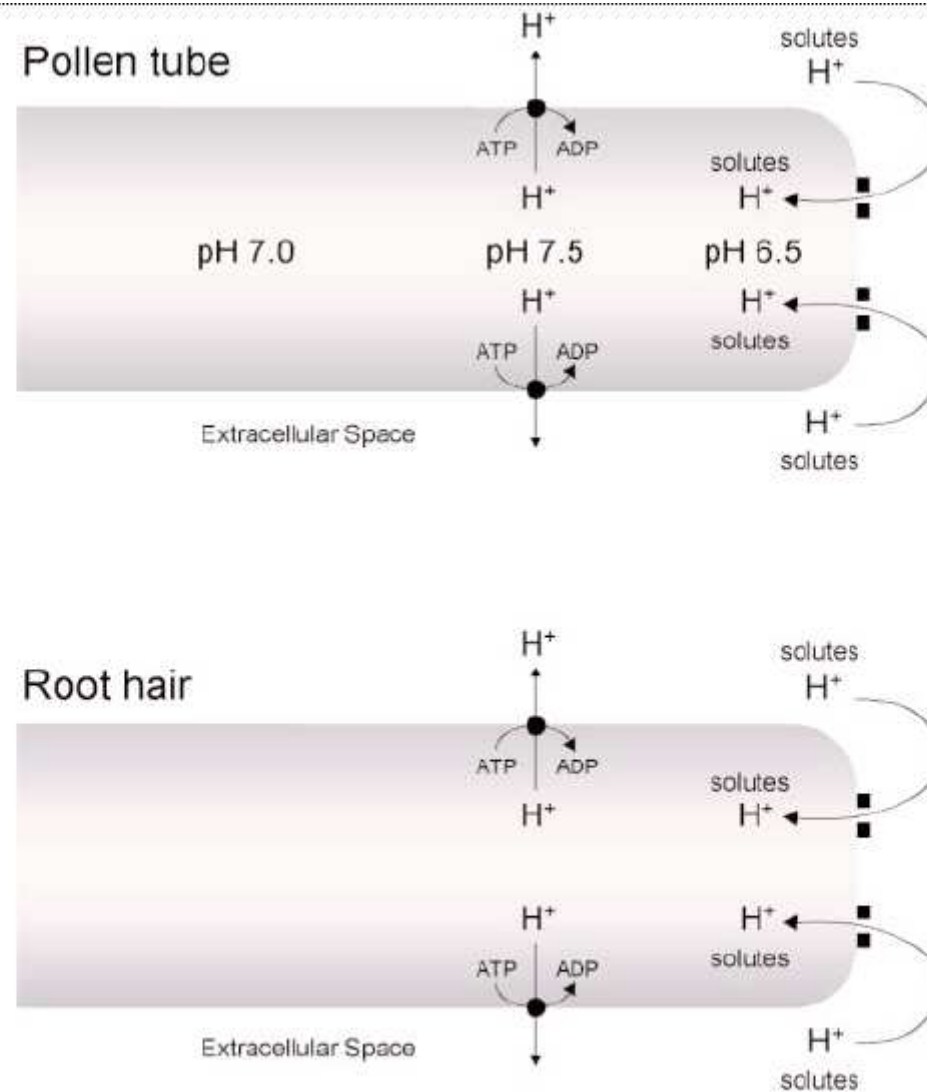
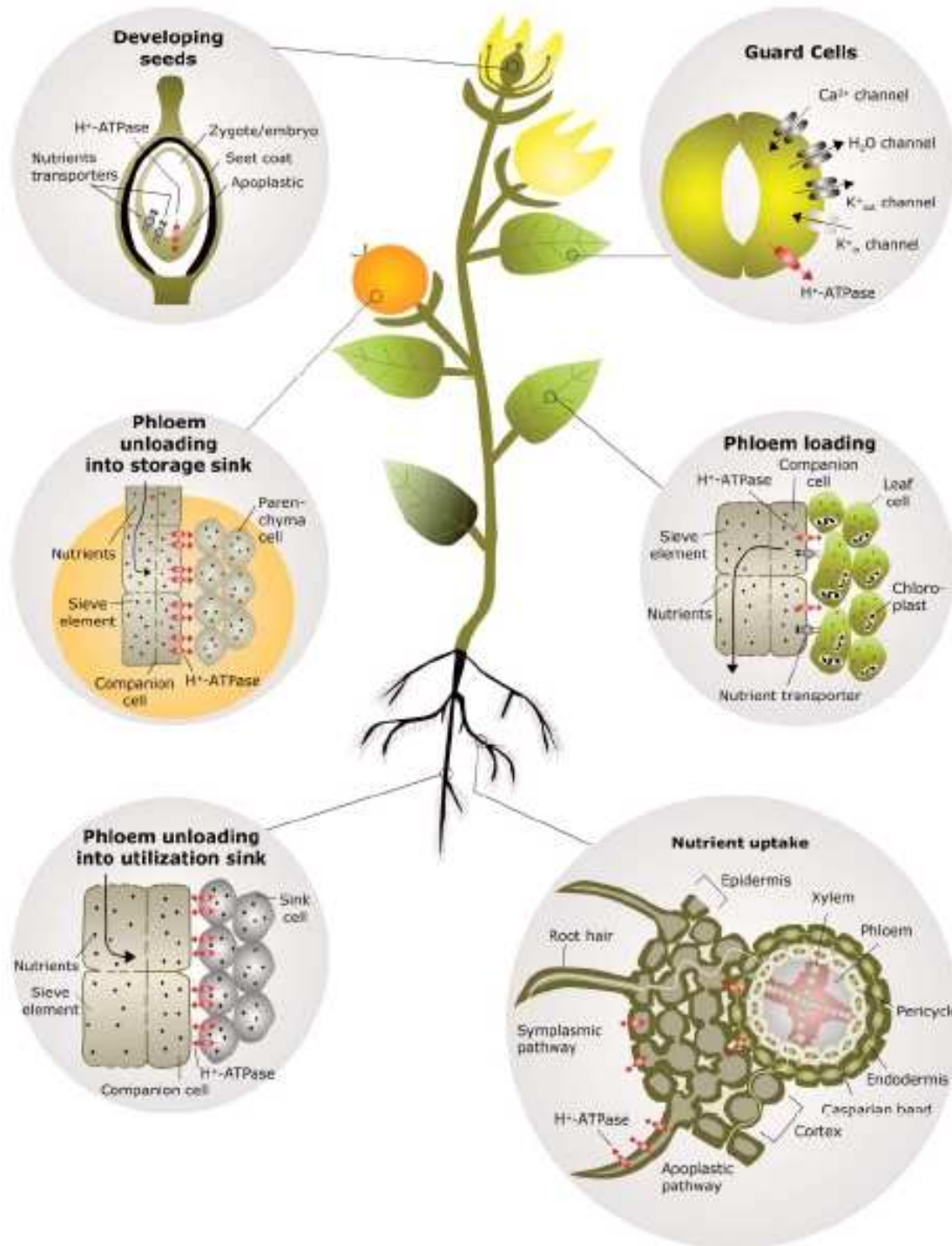


Figure 5 A model for proton gradients in pollen tubes and root hairs. Data related to pollen tubes are from Feijo et al (35).



Ca-ATPase

(další pumpa P-
typu)

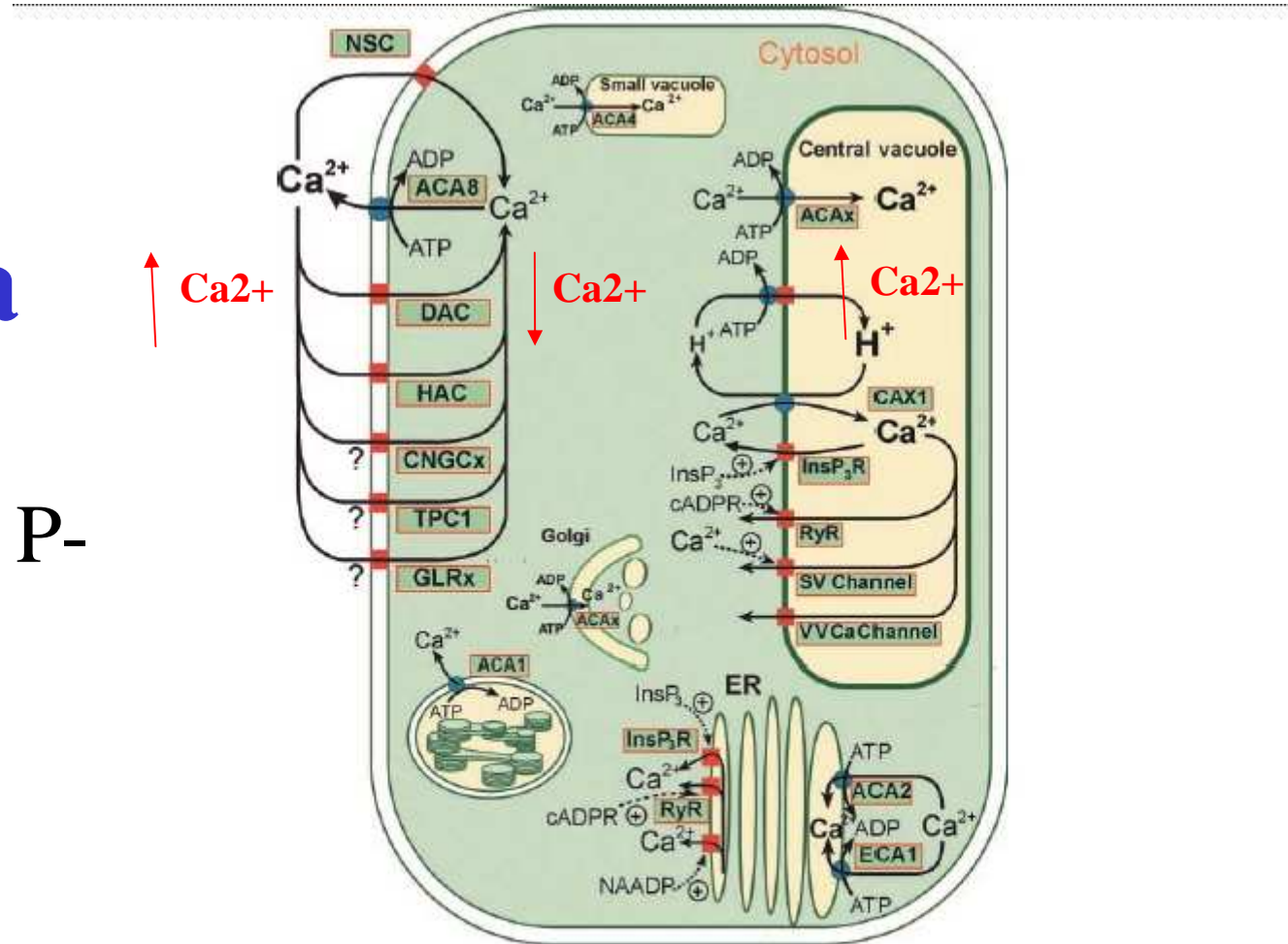


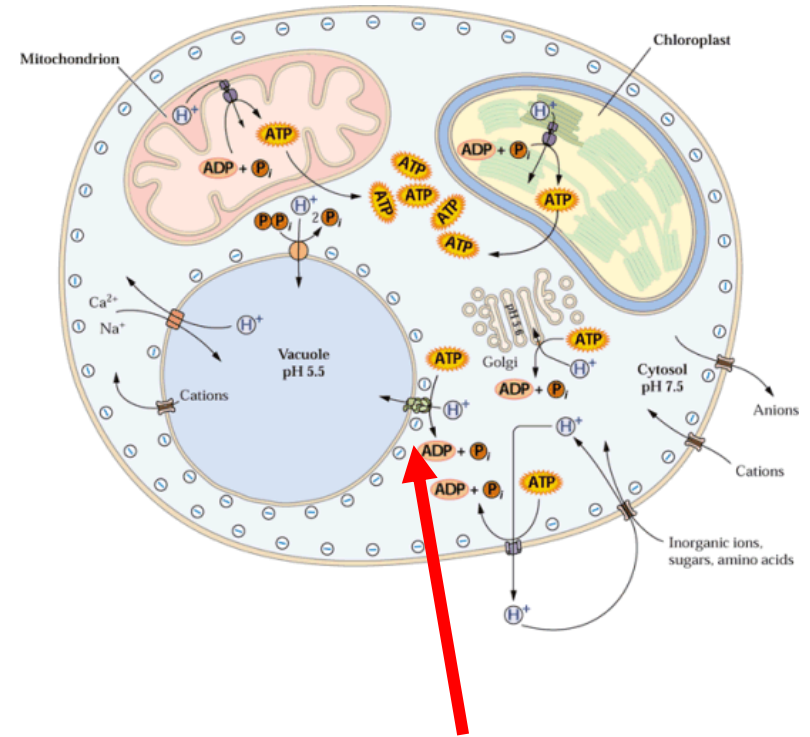
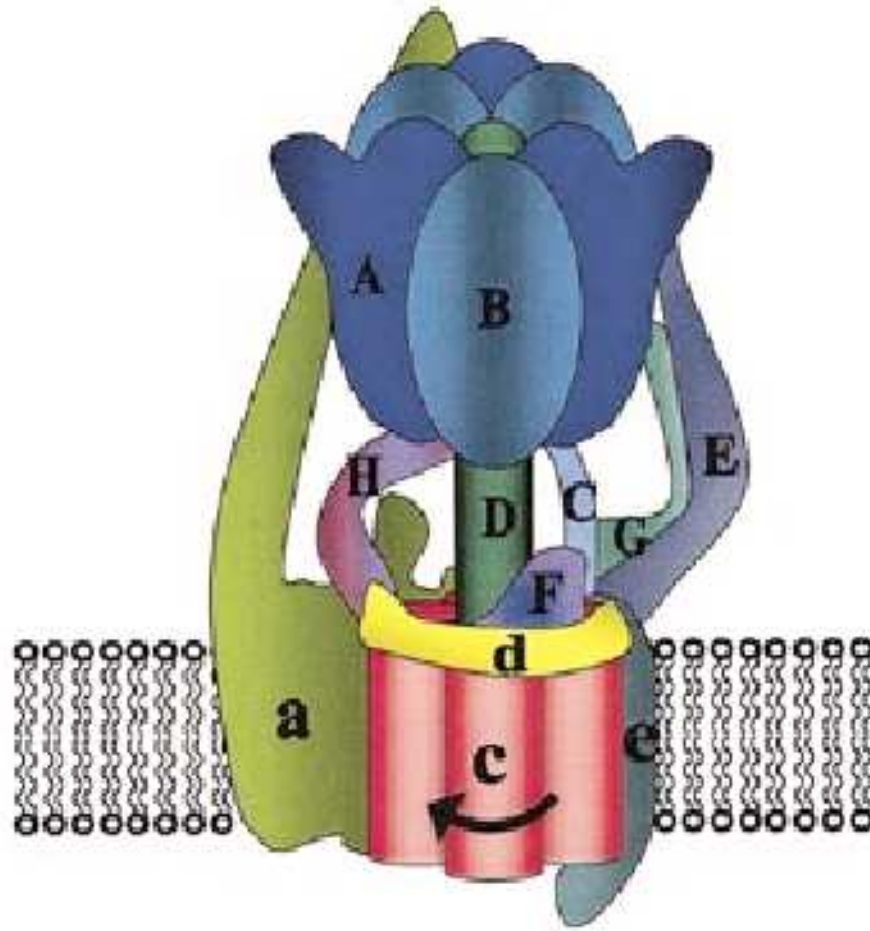
Figure 2. Schematic Representation of Major Identified Ca^{2+} Transport Pathways in Arabidopsis Cell Membranes.

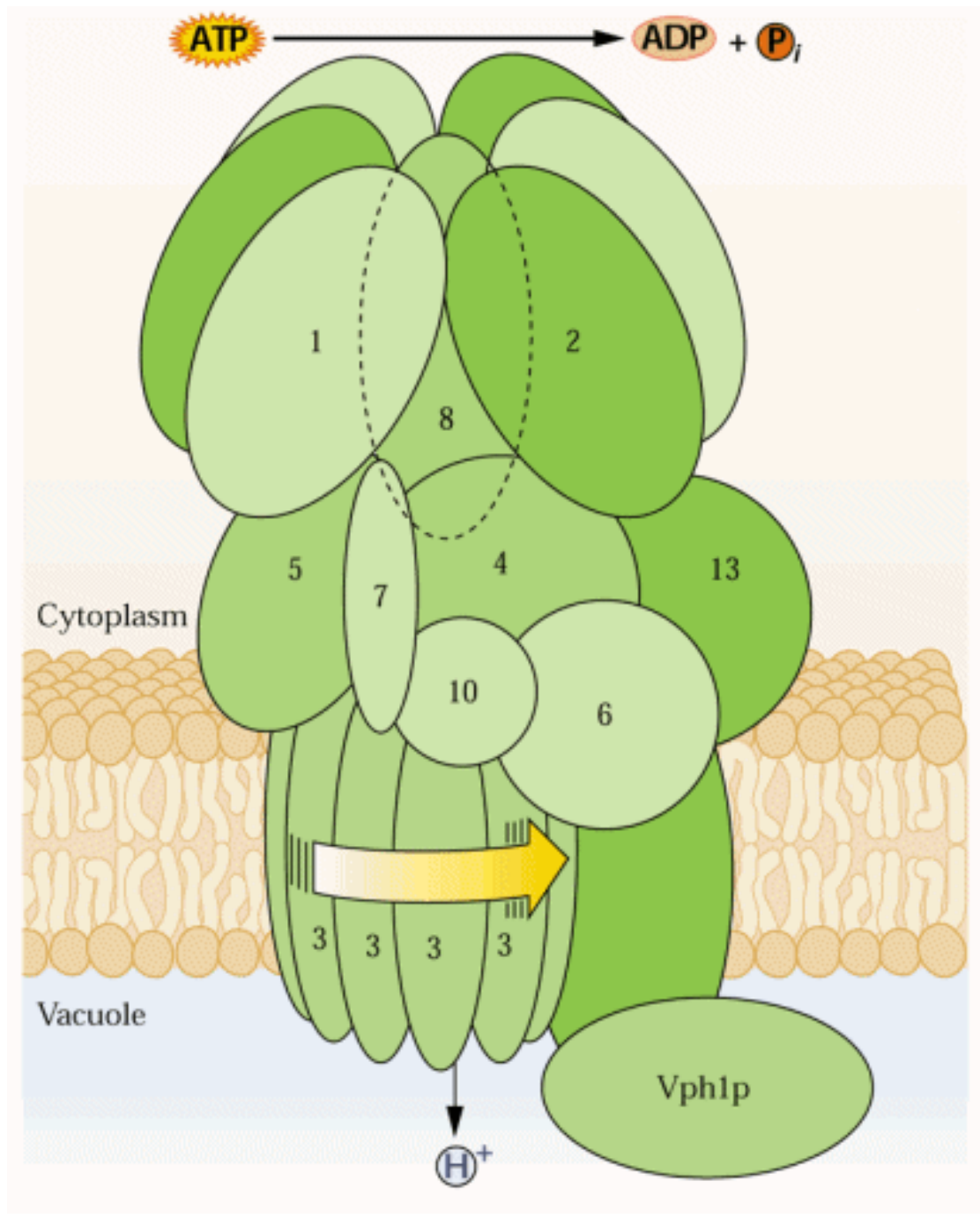
Blue circles represent energized transport systems. ACA1, ACA4, ACA8 are autoinhibited calcium ATPases identified at a molecular level. The direction of Ca^{2+} pumping for ACA1 is hypothetical. ECA is an ER-type calcium ATPase. ACAx in the central vacuole and in the Golgi has not been identified at a molecular level. CAX1 is a $\text{Ca}^{2+}/\text{H}^{+}$ antiporter expected to be localized at the vacuolar membrane. Red squares represent Ca^{2+} -permeable channels. At the plasma membrane, nonselective cation (NSC) channels, depolarization activated channels (DACs) and hyperpolarization activated channels (HACs) have been characterized at an electrophysiological but not at a molecular level. A two-pore channel (TPC1) has been shown to complement a yeast mutant deficient in Ca^{2+} uptake, but channel location is hypothetical. Using electrophysiological techniques, cyclic nucleotide gated channels (CNGC1 and CNGC2) were shown to be permeable to calcium. Plasma membrane location is

Dva typy rostlinných Ca^{2+} pump

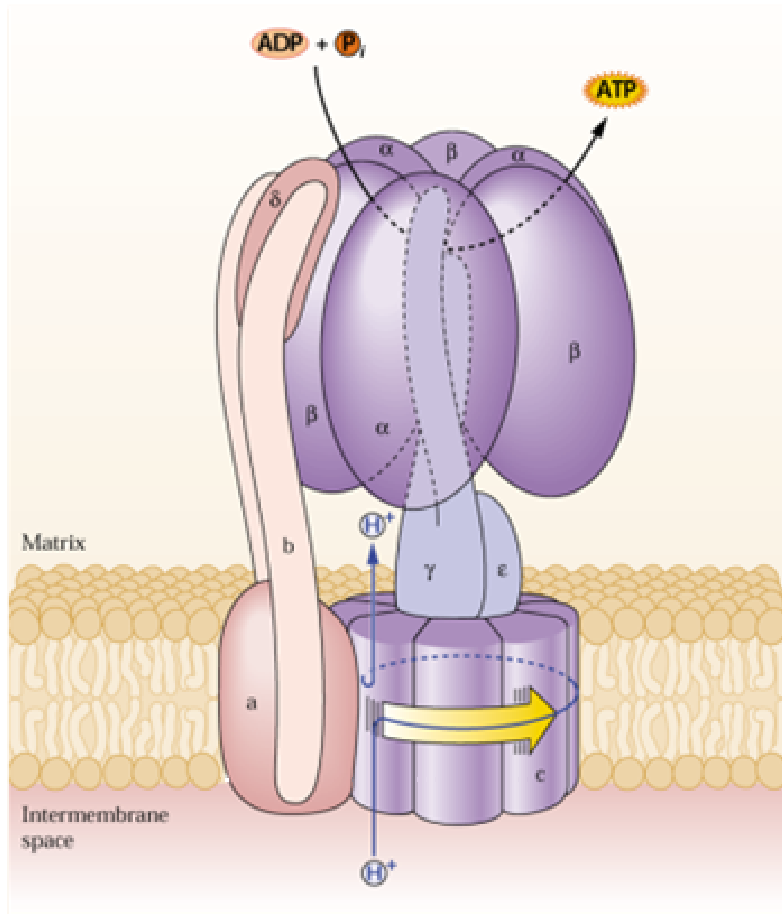
1. IIB (ACA) - v PM, ale i ER, plastidu a tonoplast. Mají **autoinhibiční regulační doménu** (fosforylace, vazba CaM) .
Obě jsou P-typu, tedy inhibovány ortovanadátem.
2. IIA (ECA) - v ER, není aktivována CaM.

V-H+ATPase

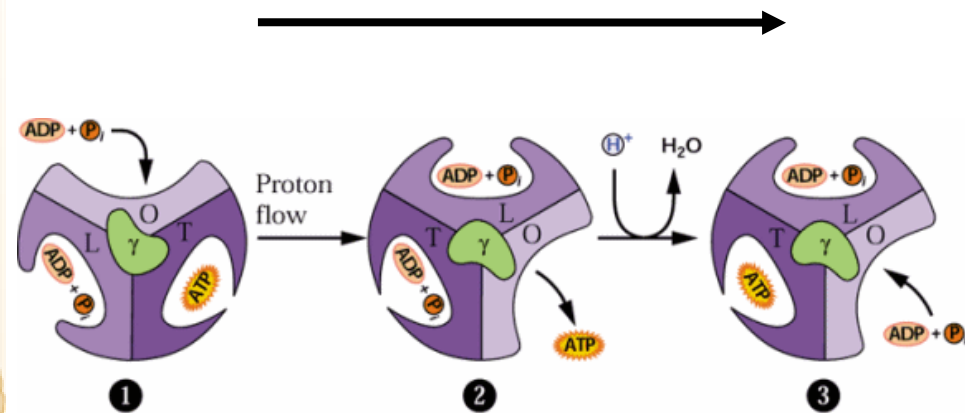




Připomeňme si: obrátíme-li elektromotor, dostaneme dynamo.

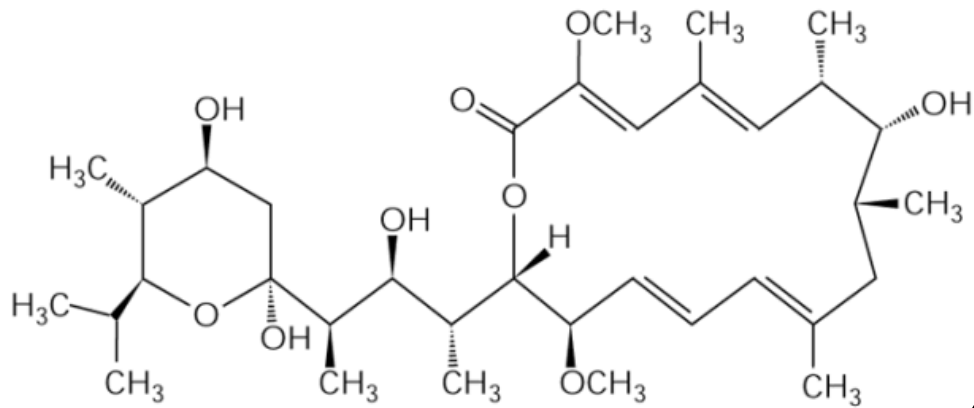


F₀F₁ – ATP synthasa



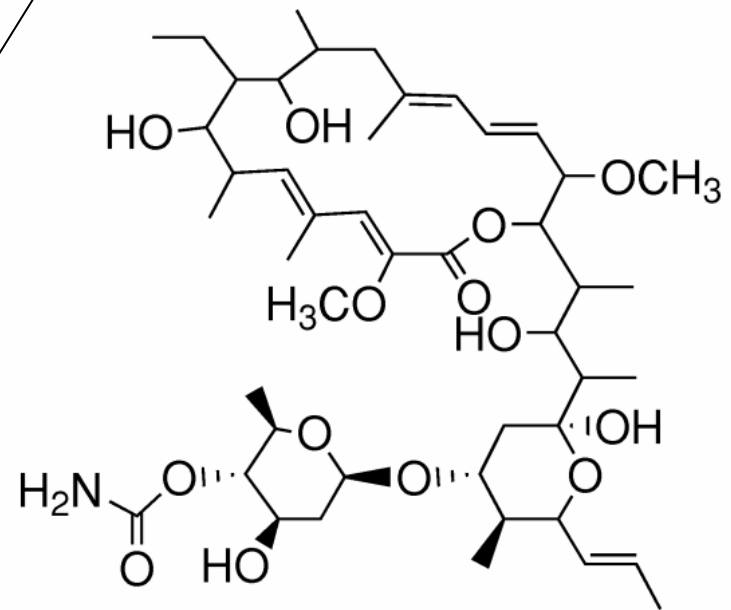
V - ATPasa

Specifické inhibitory V – H⁺ATPázy



Bafilomycin A₁

Streptomyces



Concanamycin A

(váže se na **V_o** podjednotku **c**)

det/cop mutanti



Dark

Light

Dark

Light

Wild Type

***det* Mutant**

det3 je mutant C podjednotky
V-H⁺-ATPázy

V-H+ATPasa

Table I. Number of Isogenes Encoding VHA Subunits in Plant Genomes

| VHA-subunit | Isogene numbers | | |
|-------------|--------------------|------------------------|------------------|
| | <i>A. thaliana</i> | <i>M. crystallinum</i> | <i>O. sativa</i> |
| A | 1 | 2 | 2 |
| B | 3 | 3 | 2 |
| C | 1 | 4 | 1 |
| D | 1 | 1 | 1 |
| E | 3 | 3 | 2 |
| F | 1 | 2 | 1 |
| G | 3 | 1 | 3 |
| H | 1 | 1 | 2 |
| a | 3 | 3 | 3 |
| c/c' | 5 | Unknown | 4 |
| d | 2 | 2 | 1 |
| e | 2 | 1 | 1 |

mutace
letální
pro pyl

Note. The number of isogenes was determined for *A. thaliana* through a FASTA search in the MATDB database (www.mips.gsf.de), and estimated for *M. crystallinum* through a BLAST search in the database dbEST (www.ncbi.nlm.nih.gov/dbEST) and for *O. sativa* through a BLAST search in the TIGR database (www.tigr.org).

13 podjednotek, 27 genů ...
kolik kombinací??

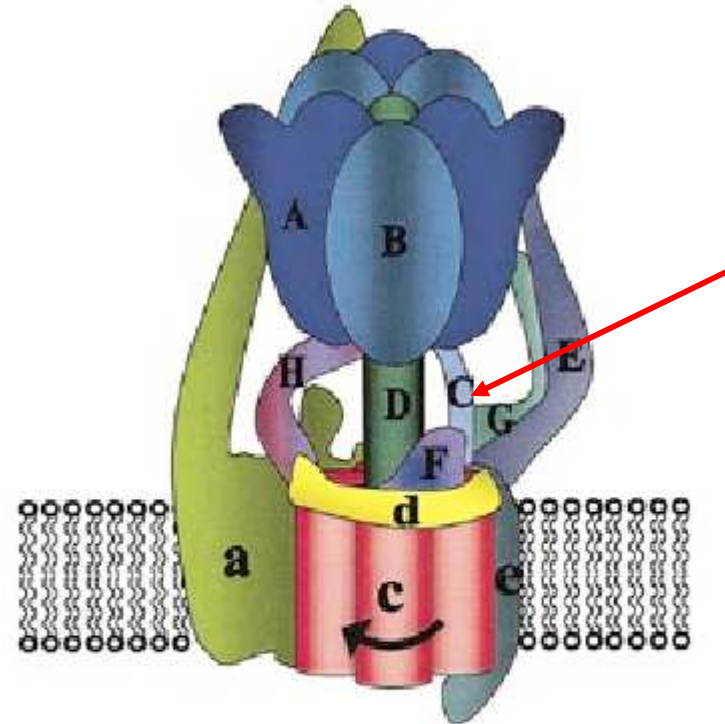
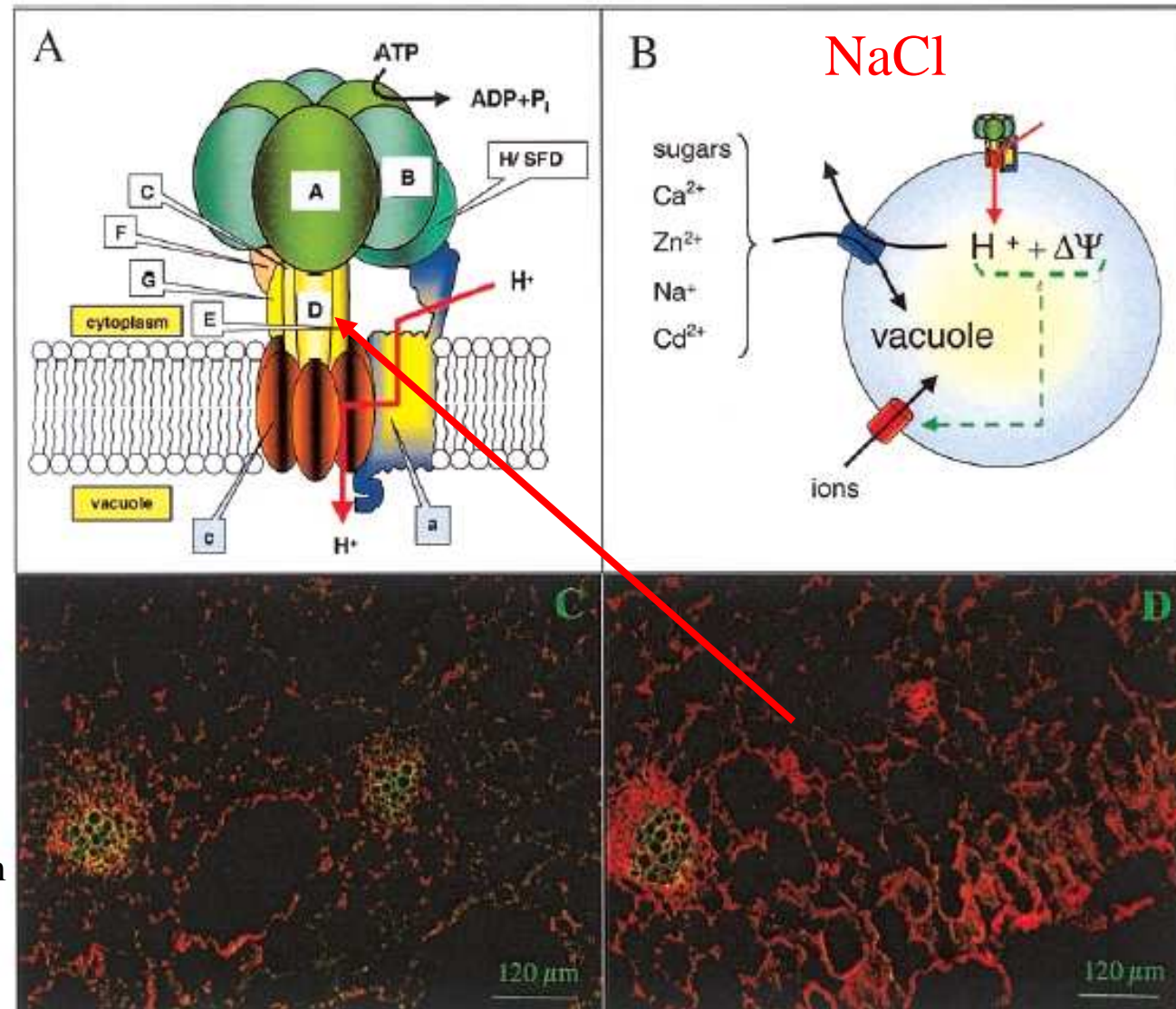


Fig. 2. Model of the plant V-ATPase. The arrangement and interaction of VHA-subunits is based on results from cross-linking studies, the 3D map of V-ATPase of *Kalanchoe daigremontiana* (Domgall *et al.*, 2002) and model suggestions for V-ATPase of *Manduca sexta* (Radermacher *et al.*, 2001) and *Bos bovis* (Wilkins *et al.*, 1999; Wilkins and Forgacs, 2001). Subunits of the V₁-part are labelled with capital letters, subunits of the V₀ are labelled with lower case letters.

Rozdíly isoformem:
např. regulace
podmínkami

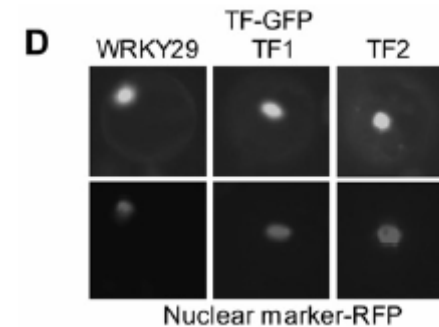


E-subunit induk. stresem

Fig. 1. Structure, basic functions and cell-specific expression of V-ATPase. (A) Hypothetical arrangement of atpv A-H, a and c in the holocomplex of the functional V-ATPase. Subunits coloured in brown or yellow constitute the proposed rotor structure, subunits in green and blue the stator. (B) Function of the V-ATPase in energizing the tonoplast or other membranes of the secretory pathway. The proton motive force built up by primary electrogenic pumps consists of the ΔpH and a small membrane potential $\Delta\Psi$ and drives H^+ -antiporters which carry ions such as Na and Zn (blue transporter). Uniporters and channels (red) transport other substrates which may accumulate following protonation (acid trap) or are distributed according to $\Delta\Psi$. (C, D) Immunolocalization of V-ATPase subunit E in leaf cross-sections of 10-week-old *Mesembryanthemum crystallinum*. (C) Control plant. (D) Plant stressed with 400 mM NaCl for 72 h. Subunit E localization is shown with red fluorescence signals.

Další možné role?

- kvasinky -
homotypic
vacuole fusion
- Drosophila -
synaptic vesicle
fusion
- savci - A4 jako
pH sensor
- **Arabidopsis -
VHA-B1 v Glu
signalling!!**



(D) TF1-GFP and TF2-GFP are localized in the nucleus. The nuclear red-fluorescent protein (RFP) serves as a nuclear marker in cotransfected protoplasts. WRKY29-GFP is a positive control.

(Cho, Yoo, Sheen 2006)

V-H⁺-PPase

(membránová pyrofosfatáza) rostlinná specialita v tonoplastu užívá jako zdroj energie pyrofosfát P_{Pi} ("odpad" při polymeraci DNA, RNA a biosyntetických proc. např. ADPG či UDPG)

příbuzné pumpy ale i v membránách bakterií, hmyzu a parazitů

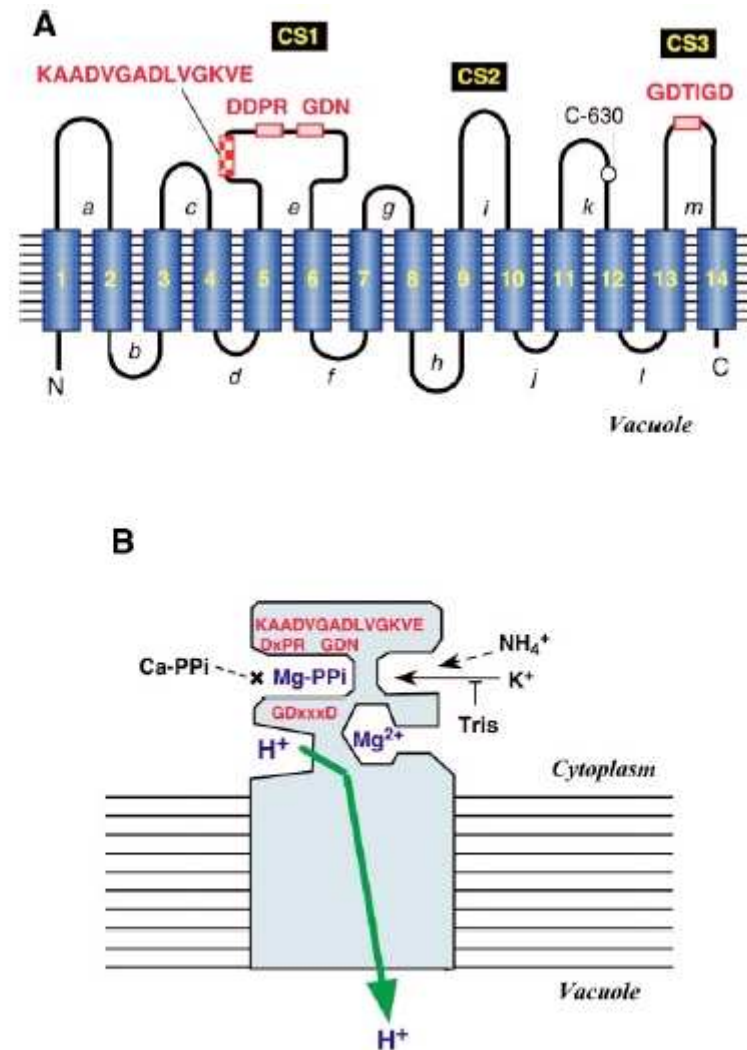


Figure 2 A structural model of V-PPase. (A) The 14 putative transmembrane helices are depicted as cylinders and conserved motifs are shown in boxes. The NEM-binding cysteine residue (Cys630 of *Vigna* V-PPase, Cys634 of Arabidopsis enzyme) (161) is shown as a circle. The hydrophilic loops are numbered from a to m. The conserved segments in the cytosolic loops are indicated as CS1, CS2, and CS3. (B) Schematic presentation of the

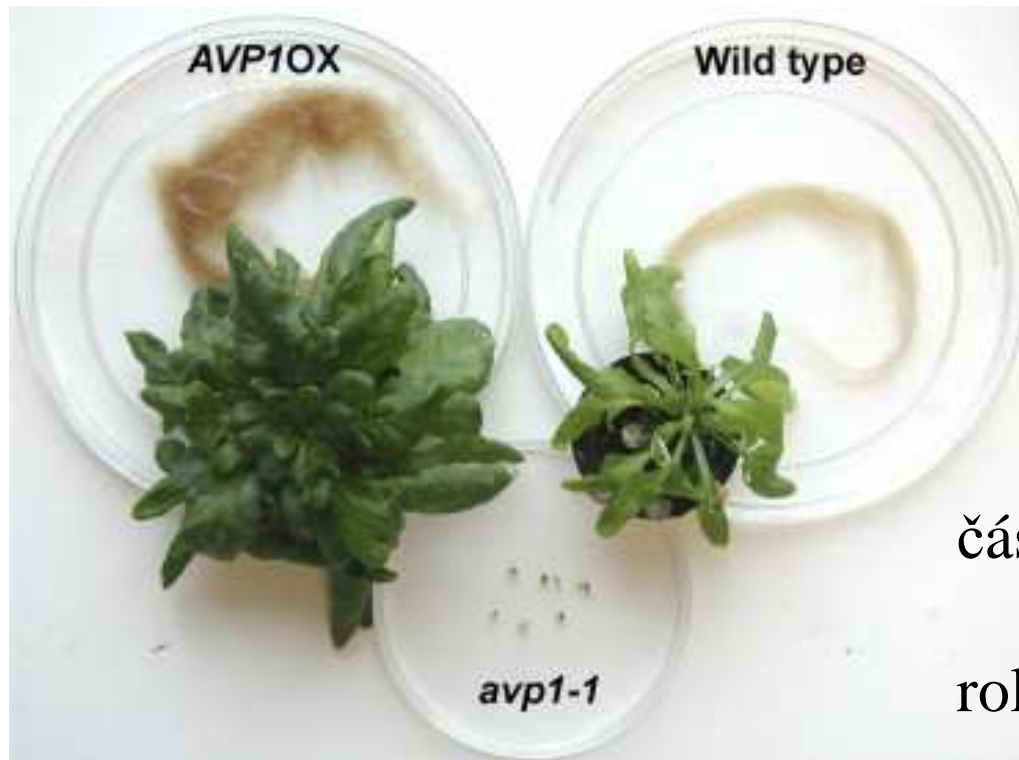
2 typy PPasy

Typ I – závislý na cytosolickém K^+
středně inhibovány Ca^{2+}

Typ II – K^+ insenzitivní
extrémně Ca^{2+} senzitivní

Evolve rostlinné vakuoly je těsně
spojena s evolucí V H⁺-PPasy,
která je nutnou součástí funkčního tonoplastu
(minoritně je ovšem lokalizována i do PM) za
normálních podmínek i ve stresu.

AVP1 - vak. Ppasa typ I



část proteinu v plasmalemě

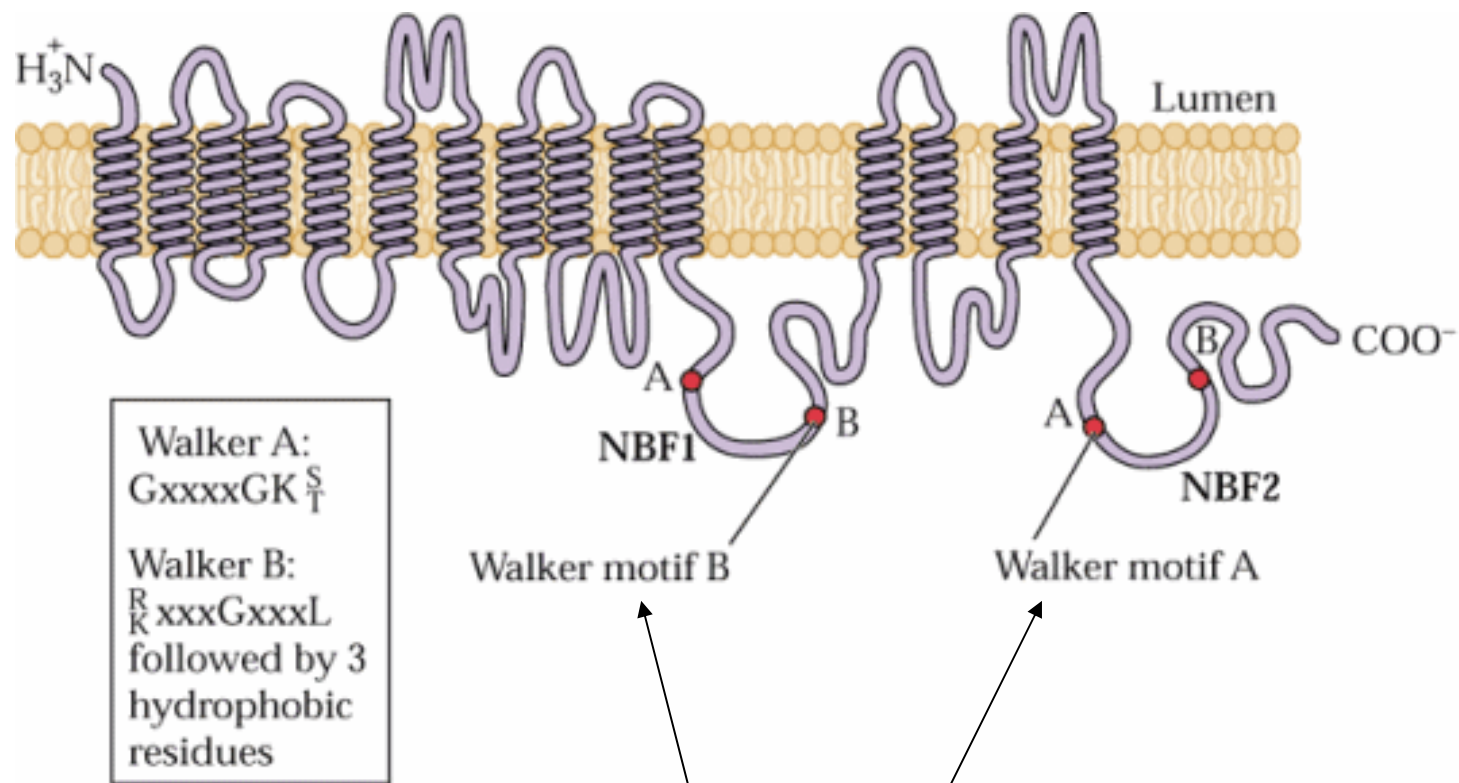
role v transportu auxinu?

overexpresse zvyšuje rezistenci k sol. stresu...

ABC transportery

(**A**TP **B**inding **C**assette)

fungují nejen jako na ATP závislé pumpy, ale také jako iontové kanály, či jejich regulátory.



ATP vazebná místa.

Fungují jako **pumpy** (tj prochází substrát hydrofilní doménou uvnitř ABC) či "**flipázy**" (substrát je během přenosu „překlopen“)?

jejich specifita kolísá a jsou klíčové pro řadu detoxifikačních transportů zvl. do vakuoly.

ABC a kutikula: CER5 kóduje ABC transp.

wt

cer5

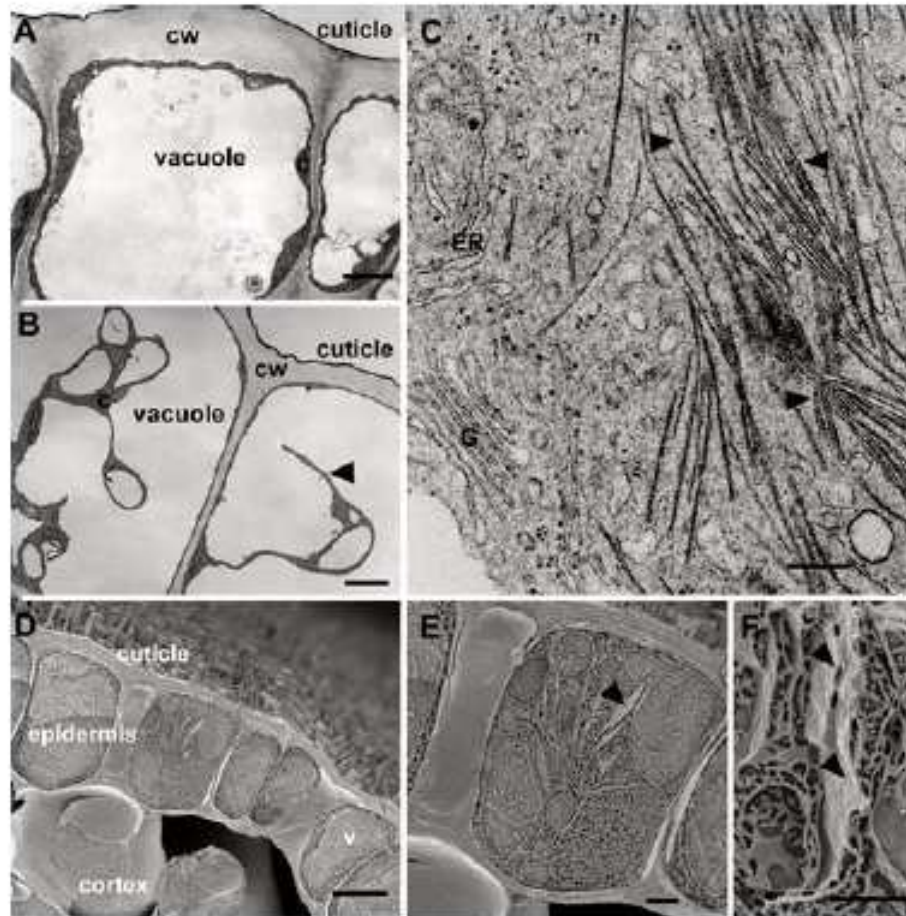


Fig. 1. Epidermal wax-secreting cells of *Arabidopsis* stems in transverse section. (A) Wild-type cells. c indicates cytoplasm; cw, cell wall. Scale bar, 2 μ m. (B) *cer5* cells with intrusions of cytoplasm in vacuoles (arrowhead). Scale bar, 2 μ m. (C) *cer5* cytoplasm contains unusual linear inclusions (arrowheads). ER, endoplasmic reticulum; G, Golgi. Scale bar, 200 nm. (D) Cryo-SEM of *cer5* epidermis, covered with cuticle. Scale bar, 5 μ m. (E) *cer5* epidermal cell with inclusions (arrow). Scale bar, 2 μ m. (F) High-magnification view showing sheetlike nature of inclusions. Scale bar, 2 μ m.

ABC a transport IAA

The Plant Journal (2005) **44**, 179–194

doi: 10.1111/j.1365-313X.2005.02519.x

Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1

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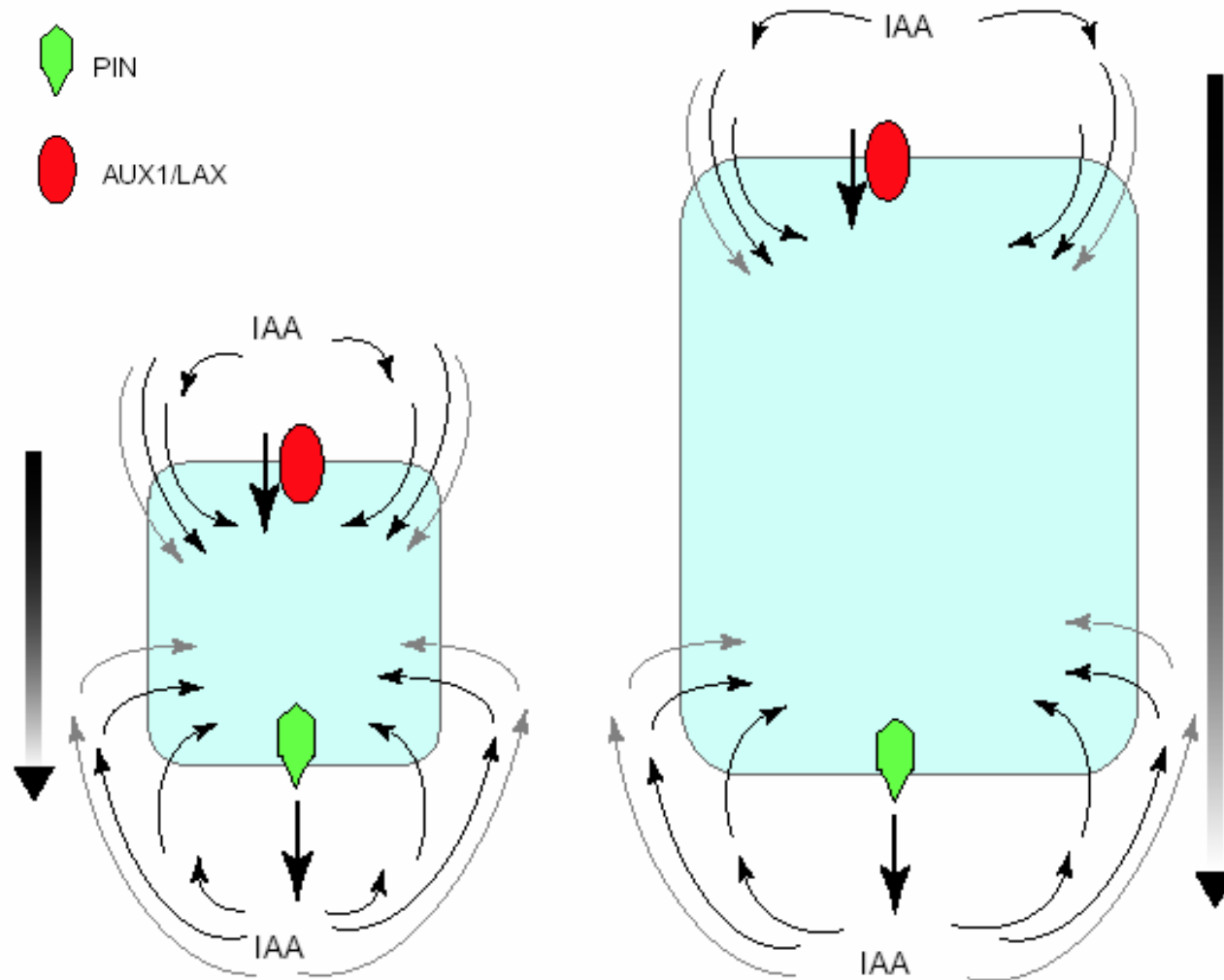
*For correspondence (fax +1 765 494 0391; e-mail murphy@purdue.edu).

†These authors contributed equally to this work.

‡Present address: Department of Genetics, University of Georgia, Athens, GA, USA.

§Present address: Carbogen AG, C11-5001 Aarau, Switzerland.

Modely transportu auxinu



Klasicky: přenašeče PIN a AUX/LAX

Co dělají PGP (ABC) transportery?



PIN



AUX1/LAX

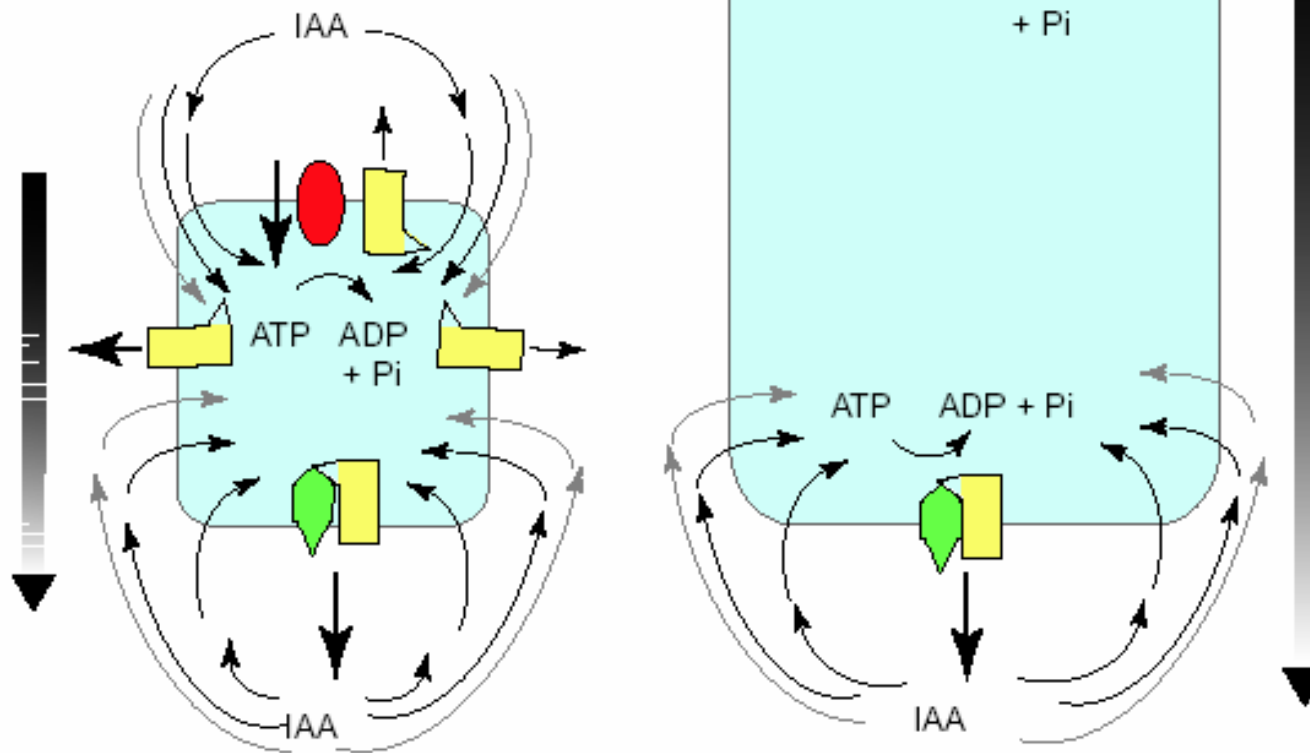


PGP



Direction and
gradient of
IAA movement

Current Opinion in Plant Biology



ABC a IAA "fenotypy" jen malé

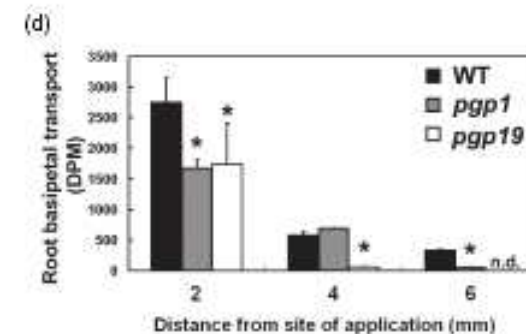
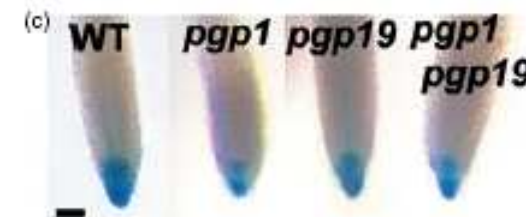
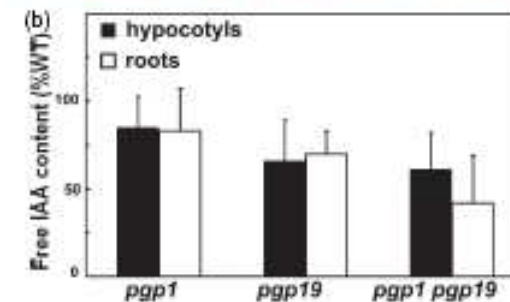
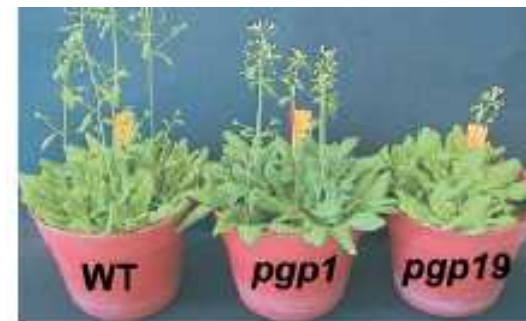


Figure 1. *pgg1* mutants have a subtle phenotype.

(a) *pgg1* and *pgg19* mutants have a dwarf phenotype under short-day conditions.

(b) Free IAA content in 5-day seedlings. Values are mean \pm SD from 500 seedlings per replicate, $n = 3$. WT values = $100\% \pm 0.074$ for hypocotyls, and $100\% \pm 11.114$ for roots.

(c) Pro_{DR5} :GUS expression in 5-day seedling root tips. Bar, 0.1 mm.

(d) Basipetal ^3H -IAA transport from the root tip in 5-day seedlings. Values are mean \pm SD from 10 seedlings per replicate, $n = 3$.

Lokalizace
„klasických“
PIN IAA
výtokových
přenašečů není
narušena

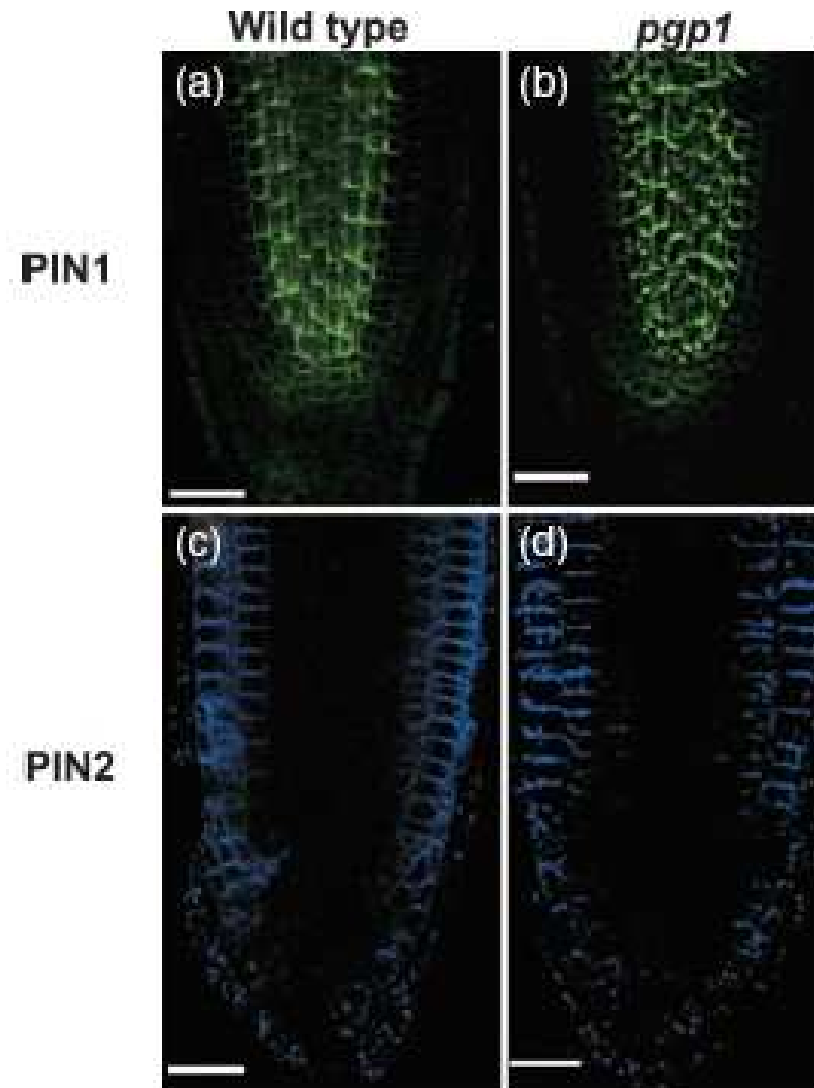
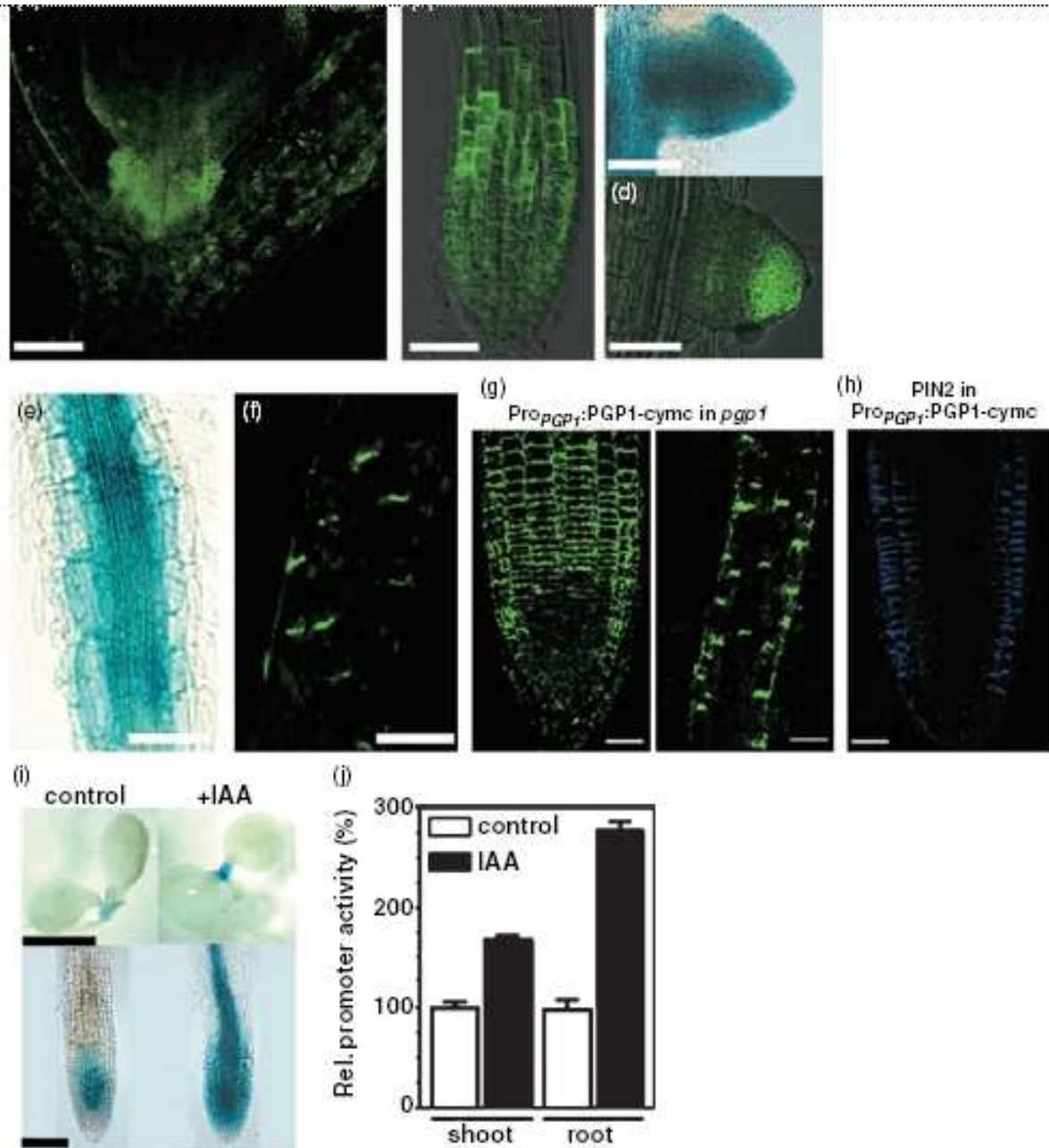


Figure 2. PIN1 and PIN2 localization is unaltered in *pgp1* root tips. (a) PIN1 immunolocalization in 5-day wild type root tips. (b) PIN1 immunolocalization in 5-day *pgp1* root tips. (c) PIN2 immunolocalization in 5-day wild type root tips. (d) PIN2 immunolocalization in 5-day *pgp1* root tips. Bar, 125 μ m.

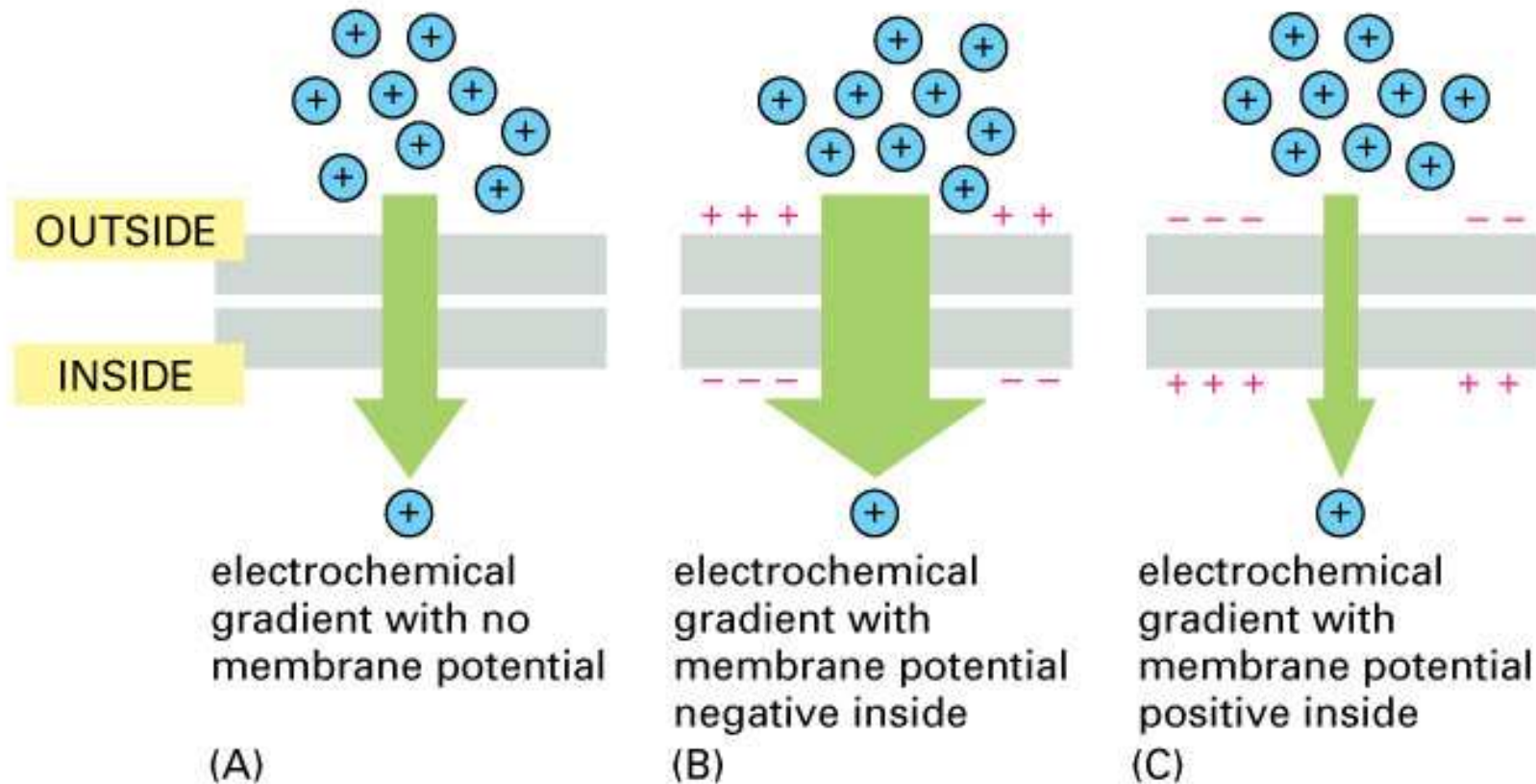
PGP1 je
 nepolárně
 lokalizován v
 mladých
 pletivech, ale
 polárně v
 diferencovan-
 ých b.kořene
 (f). Jeho
 exprese je
 aktivována
 IAA



Membránové transportery konkrétně :

- Pumpy
- **Přenašeče**
 - **saturovatelné, poháněny gradienty (pmf)**
- Kanály

Two forces may be at work in passive transport - if we are dealing with a charged solute across the membrane.



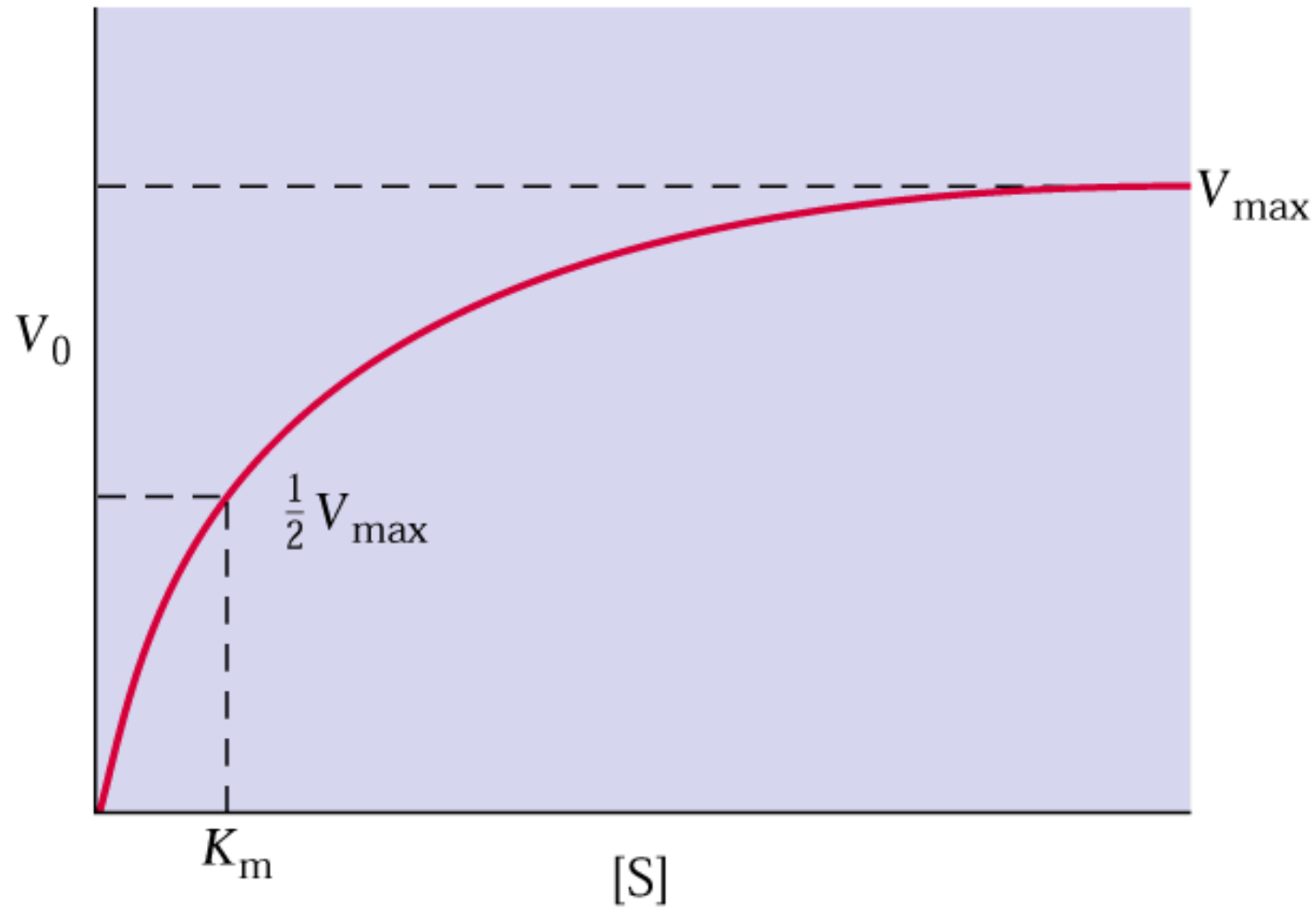
1) CONCENTRATION

2) MEMBRANE POTENTIAL

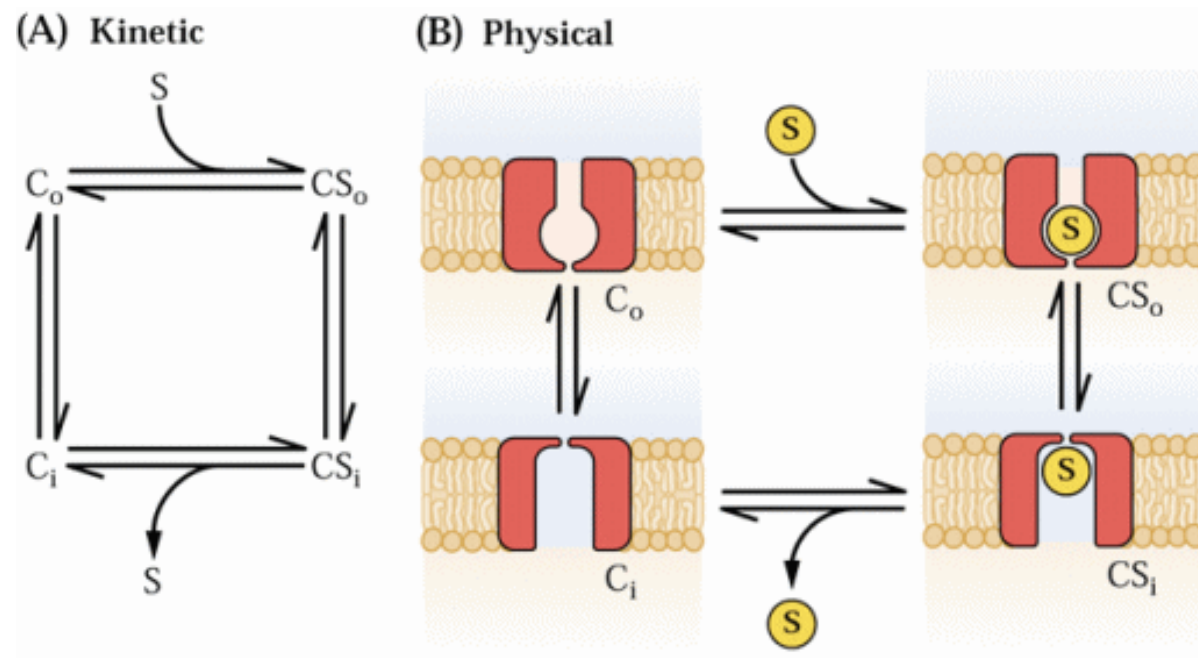
Přenašeče

- Hlavní anorganické živiny (NH_4^+ , NO_3^- , P_i , K^+ , SO_4^{2-})
- Uptake dalších iontů (Cl^-)
- Organické látky (cukry (mono- a di-), aa, baze)

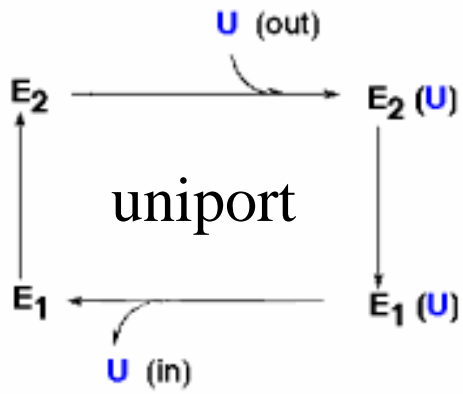
Saturační kinetika přenašeče



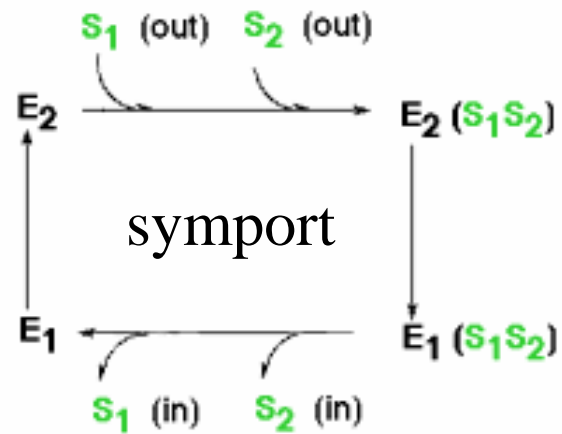
Model obecného přenašeče



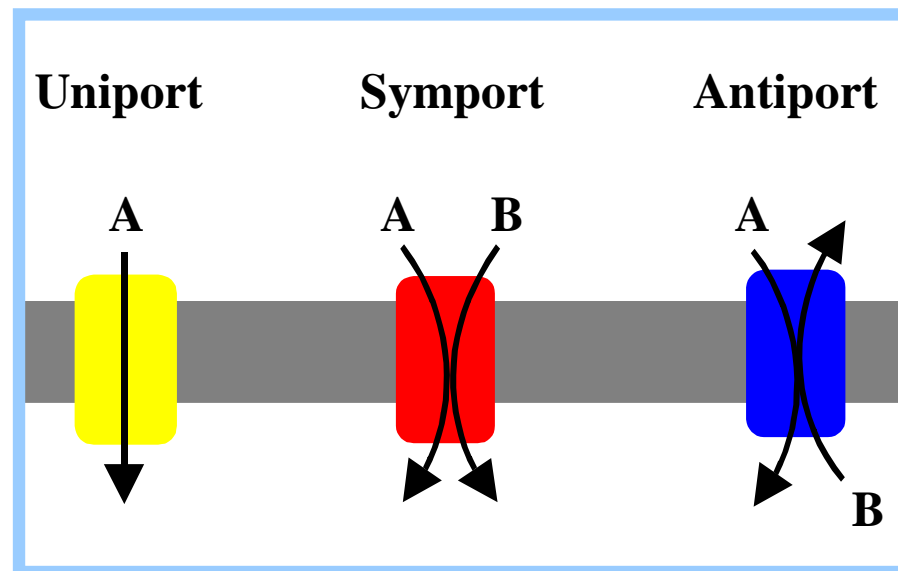
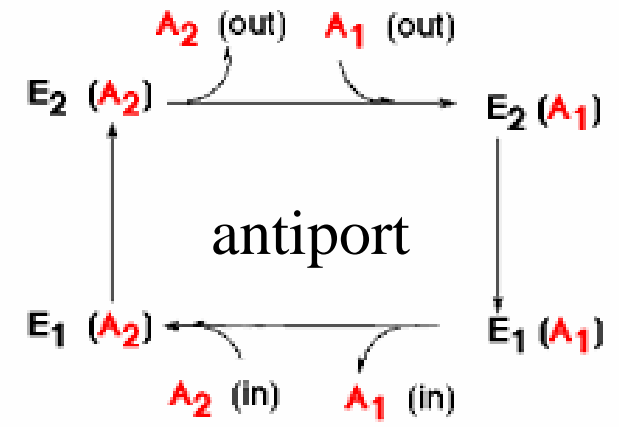
Typy přenašečů



“Pasivní” transport

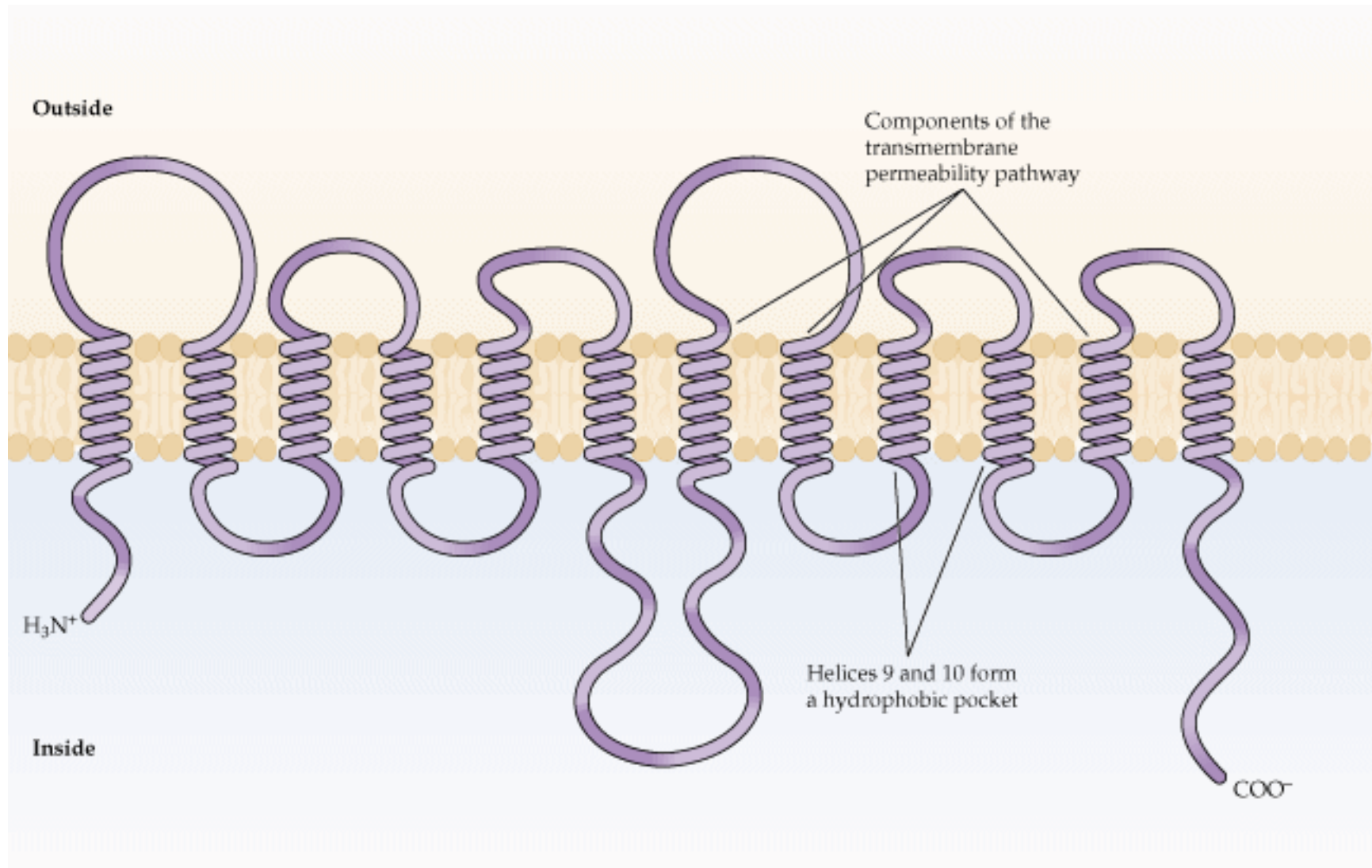


Sekundární aktivní transport



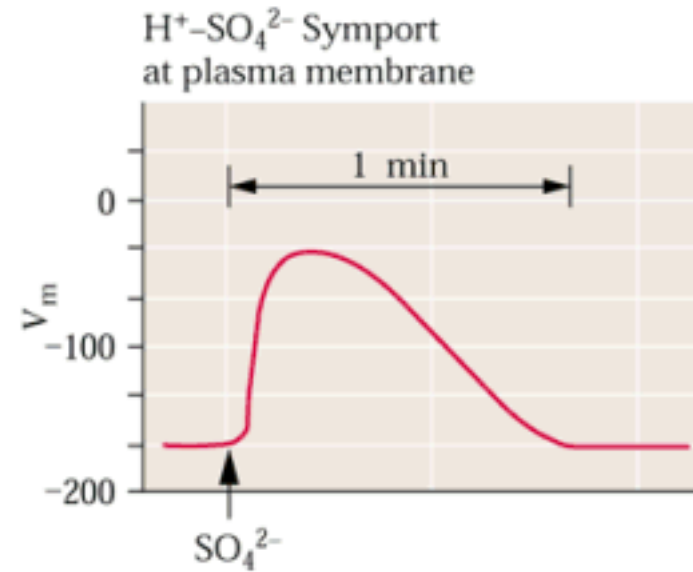
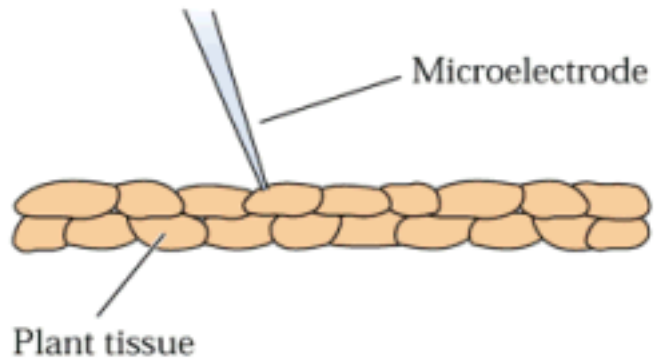
Struktura obecného přenašeče

(Major facilitator superfamily)

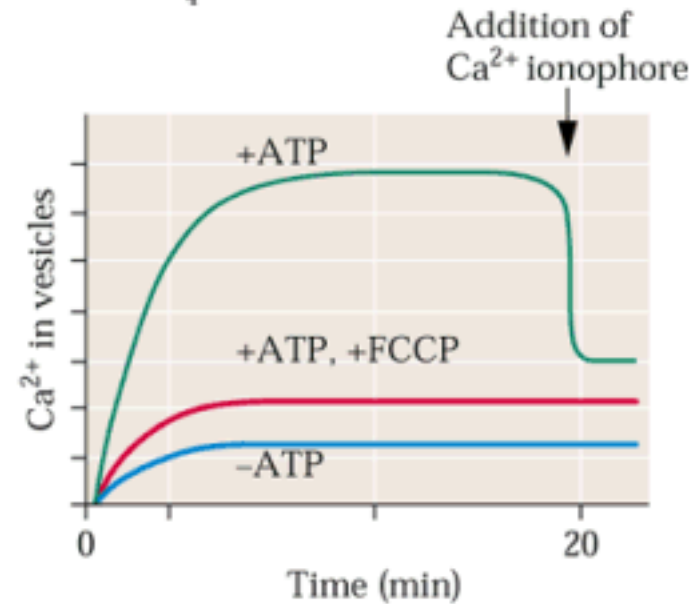
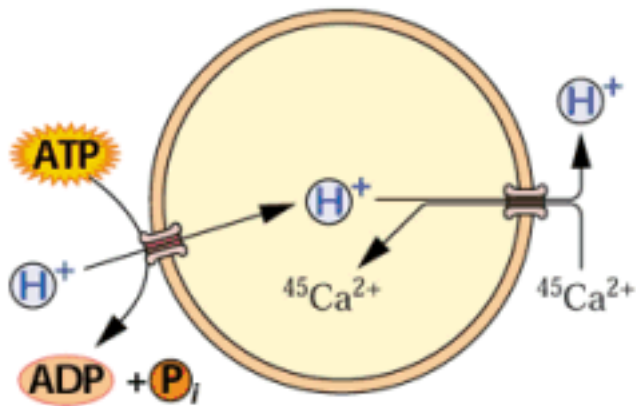


Jak studovat transportní procesy?

(A) Electrophysiological

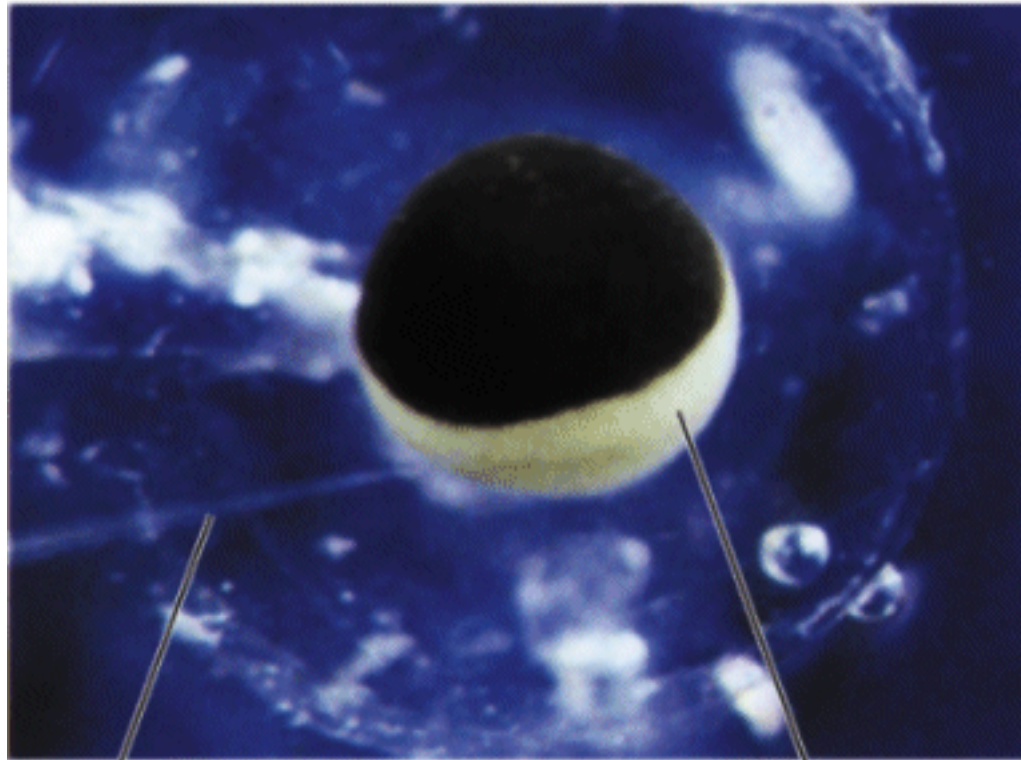


(B) Membrane vesicles



Heterologní exprese: oocyty, kvasinky

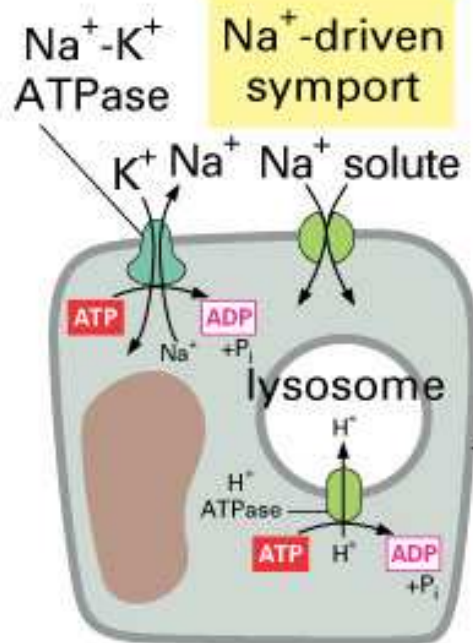
2 mm



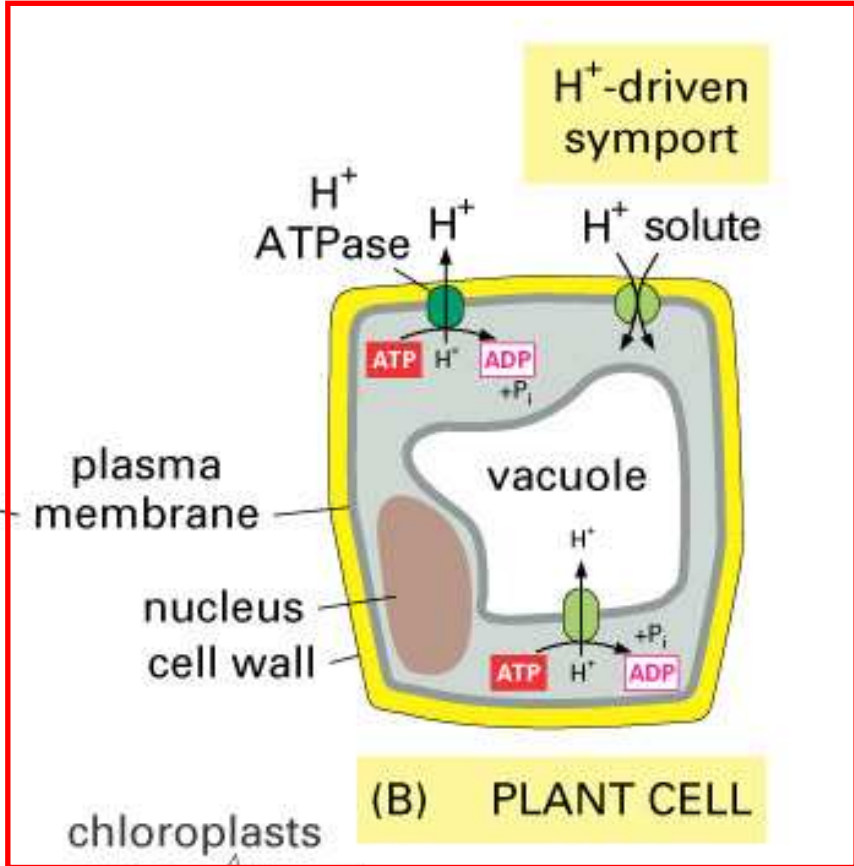
Injection
pipette

Xenopus
oocyte

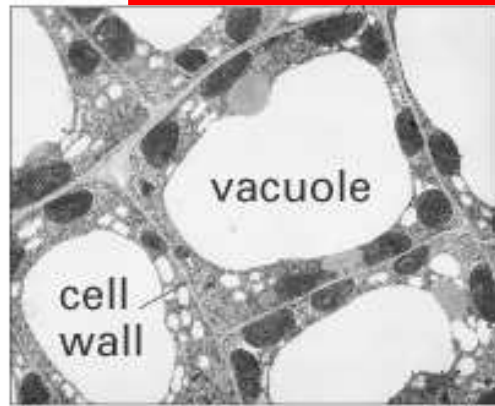
Animal vs Plant cells



(A) ANIMAL CELL



(B) PLANT CELL



(C) 10 μm

Symport H^+ /cukry při plnění floemu

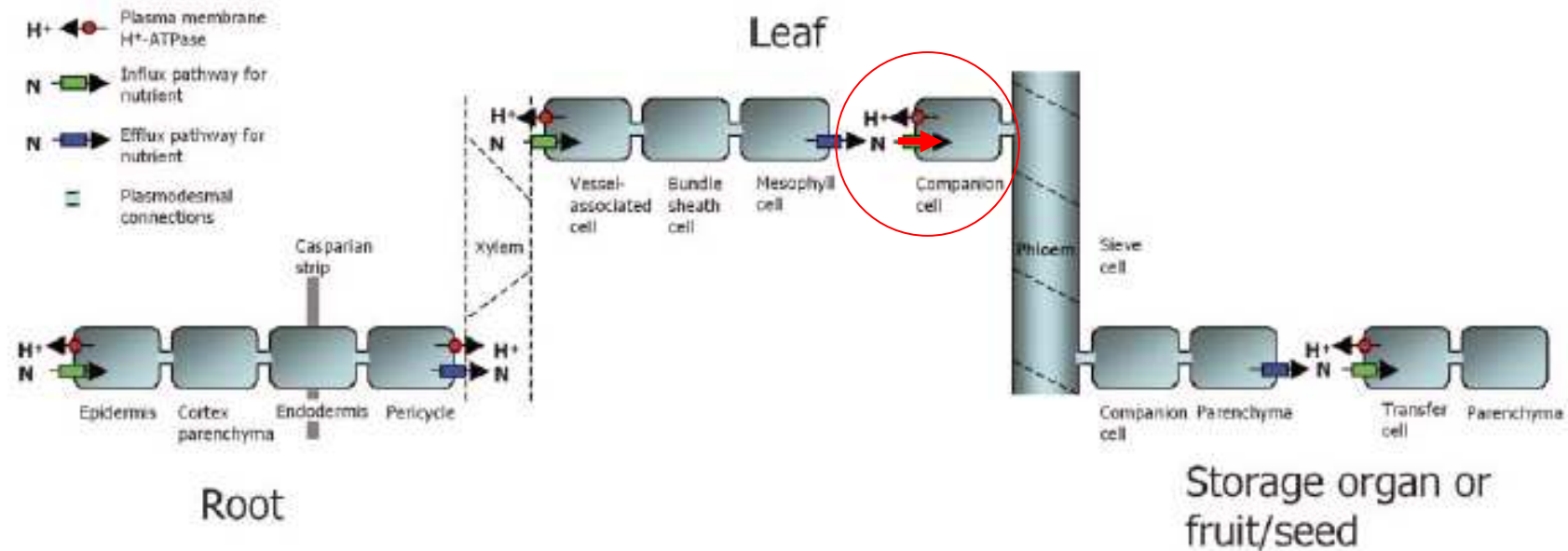
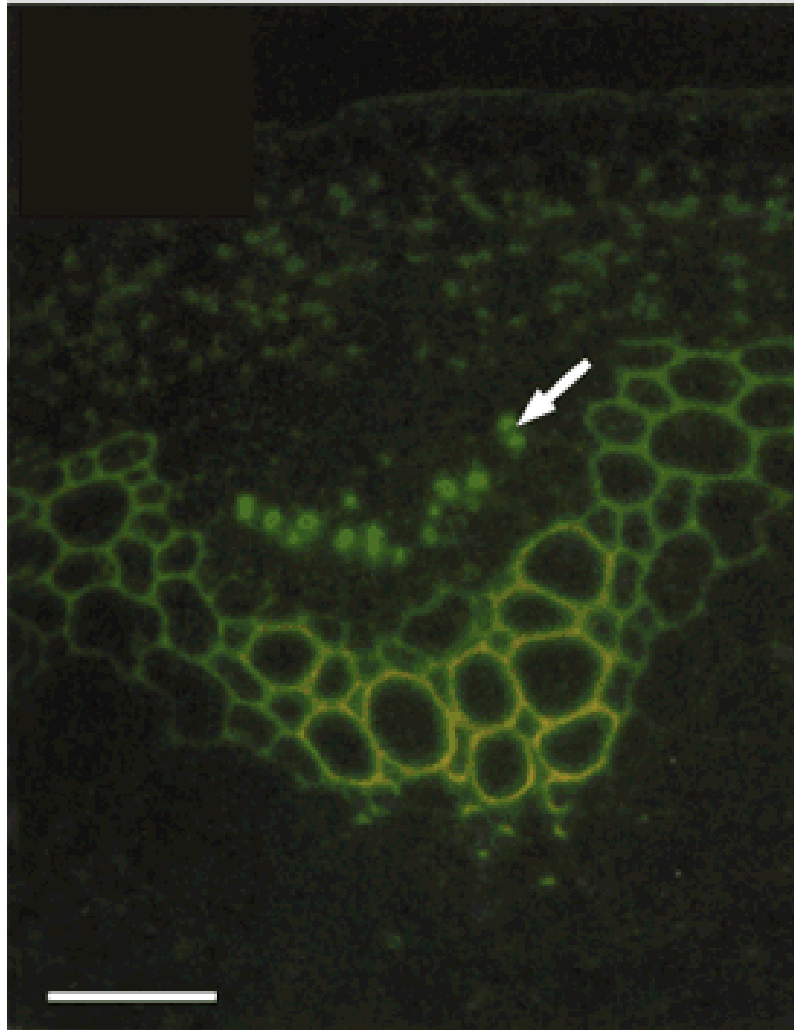


Figure 1. Overview of main transport barriers in the plant body and the energization of cellular nutrient uptake by plasma membrane H^+ -ATPase.

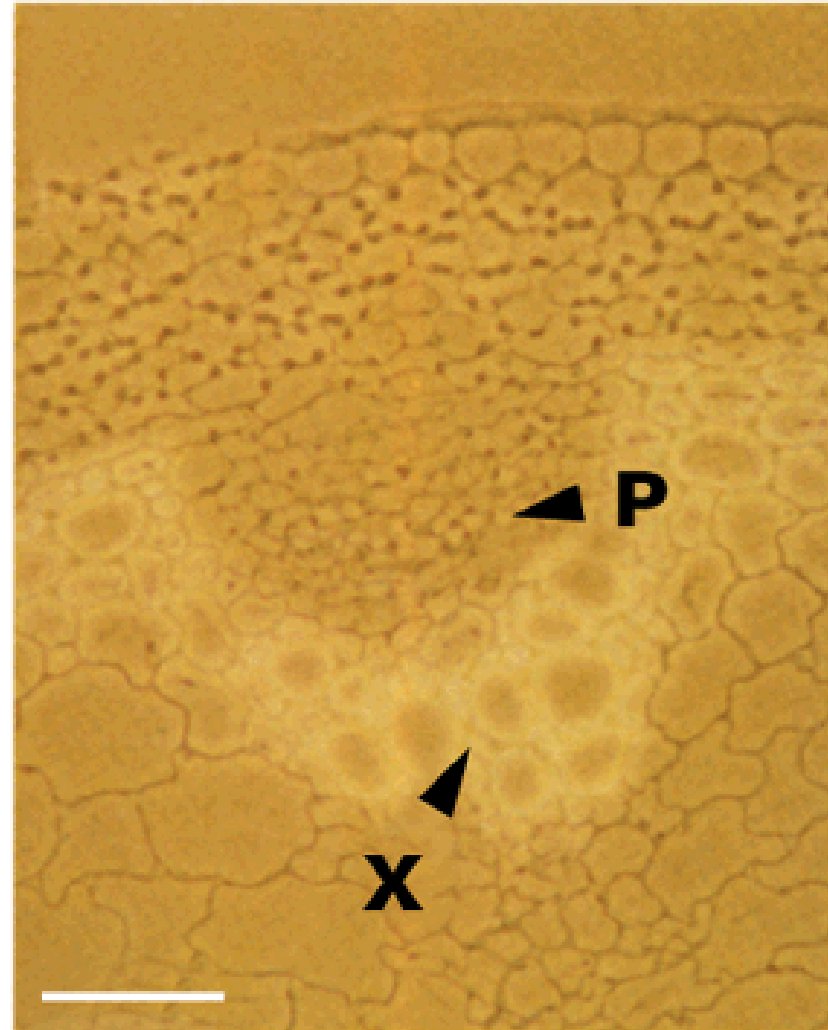
sucrose- H^+ symporters

Arabidopsis SUC2 v průvodních buňkách

(A)

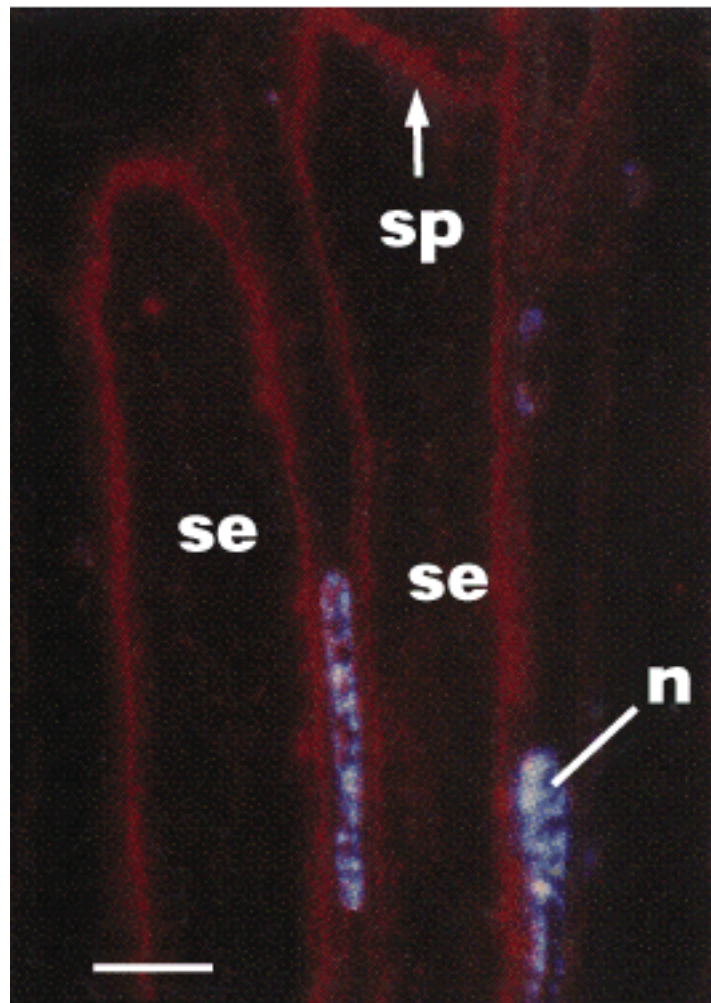


(B)

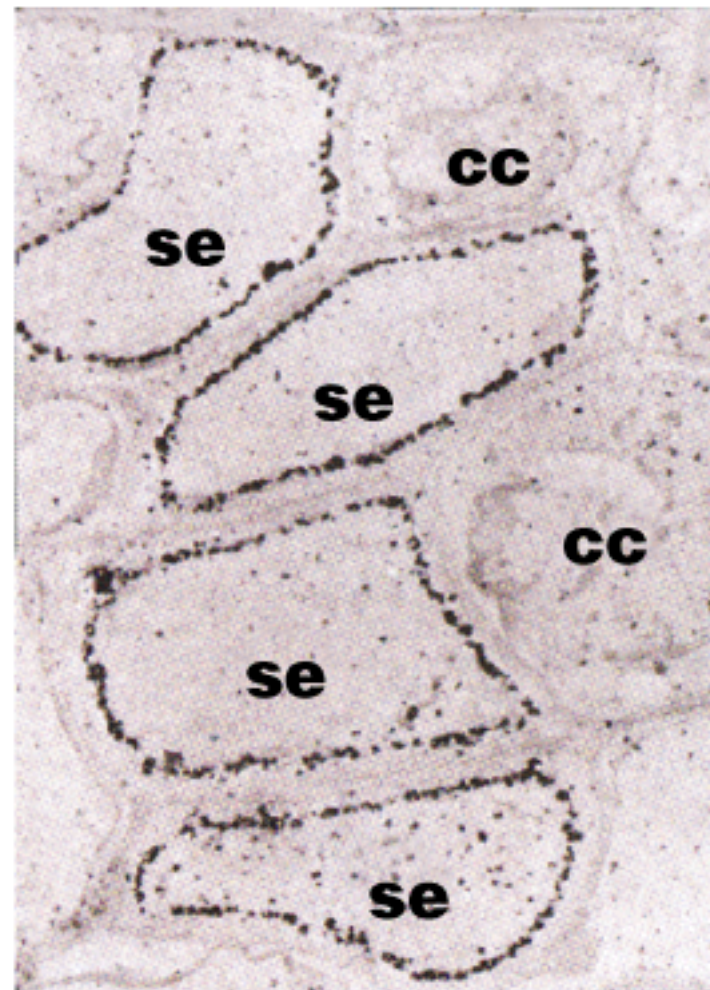


Bramborový SUT1 v sítkovicích

(A)

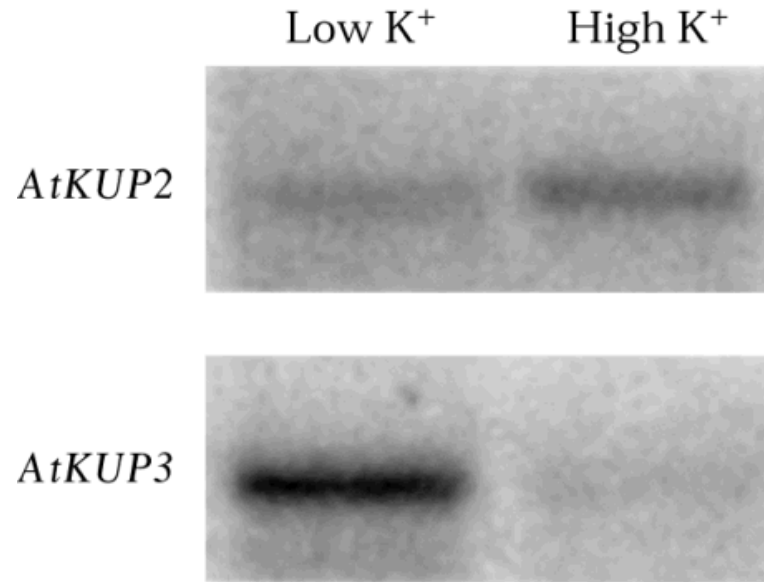


(B)



Proč různé přenašeče téhož iontu?

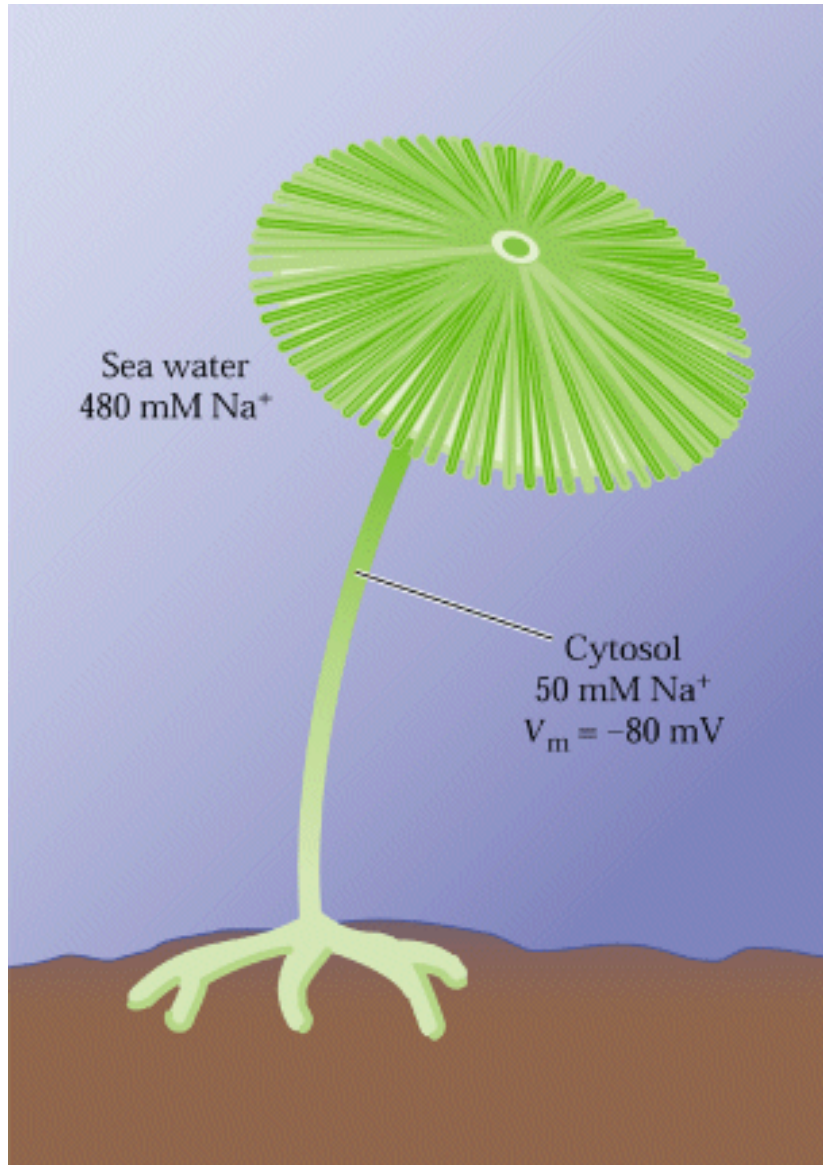
Jemné ladění!



Regulace aktivity přenašečů:

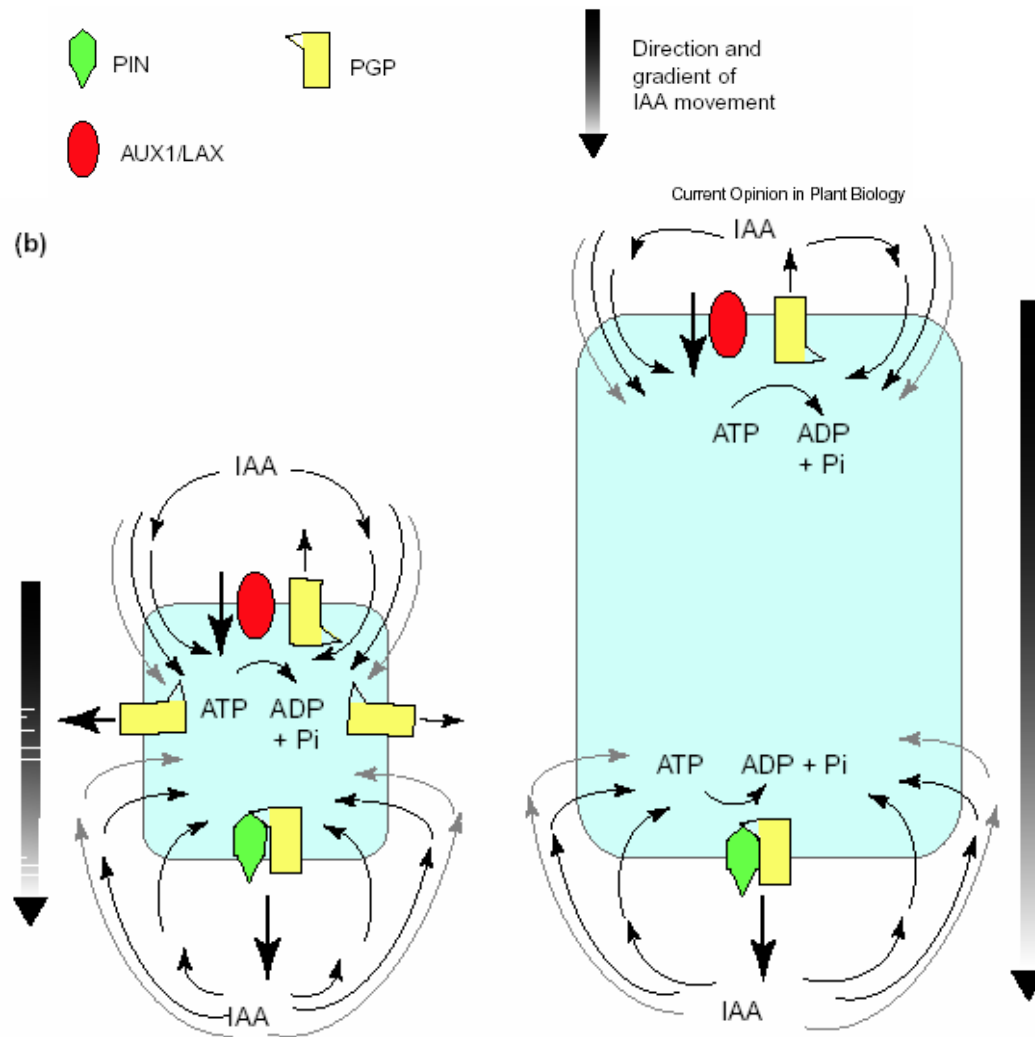
- Transkripční (pravděpodobně převládající)
- Posttranlační - (transinhibice)

Jsou i výjimky z pohonu H^+



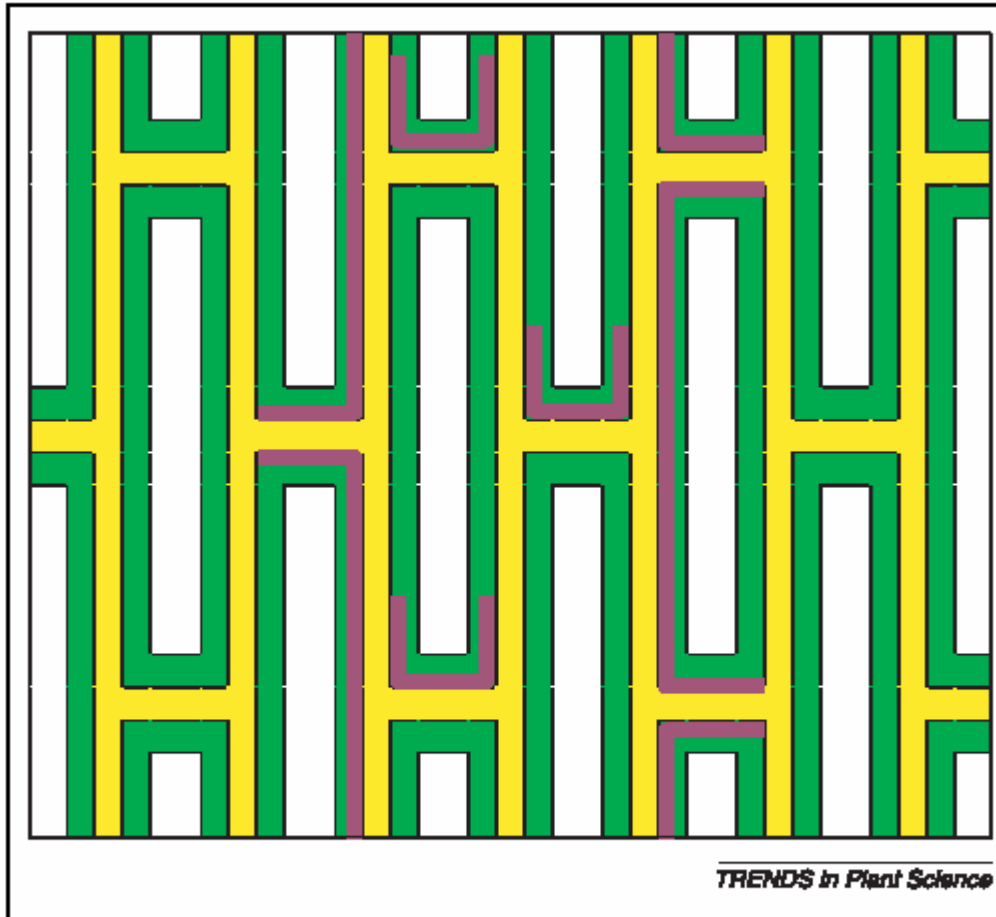
$Na^+ NO_3^-$ coupling

Přenašeče auxinu (IAA)



- ABC transporters (MDR, PGP)
- PIN
- AUX/LAX

Kudy auxin teče?



Přenašeče
(efflux carriers):
AUX1/LAX1
PINs

inhibice – fytotropiny
(NPA)

Figure 1. Schematic representation of auxin transporting tissue. Cell walls are depicted in yellow, cytoplasm in green and vacuoles in white. The localization of PIN carriers is indicated in plum. The figure shows two files of canal cells, bordered on each side by one file of border cells. The remaining files are parenchyma. Fine details of the PIN distribution are not shown.

IAA influx: AUX1

Swarup et al. 2001

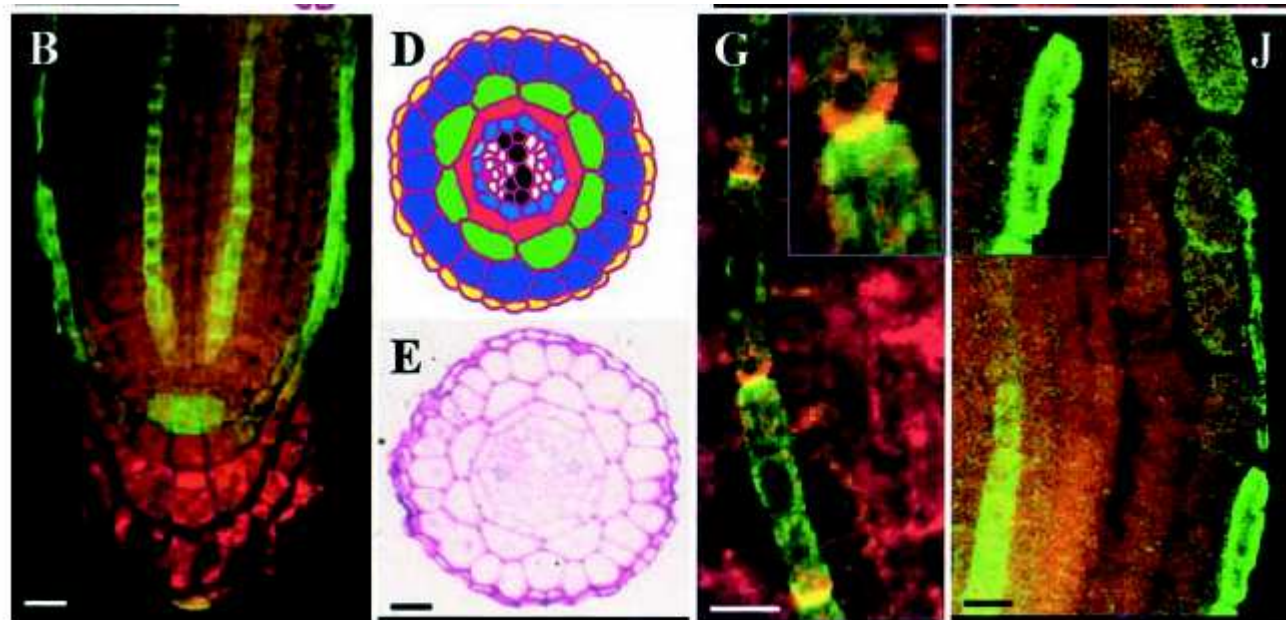


Figure 1. AUX1 is expressed in a subset of columella, lateral root cap, and stele tissues.

Swarup et al.

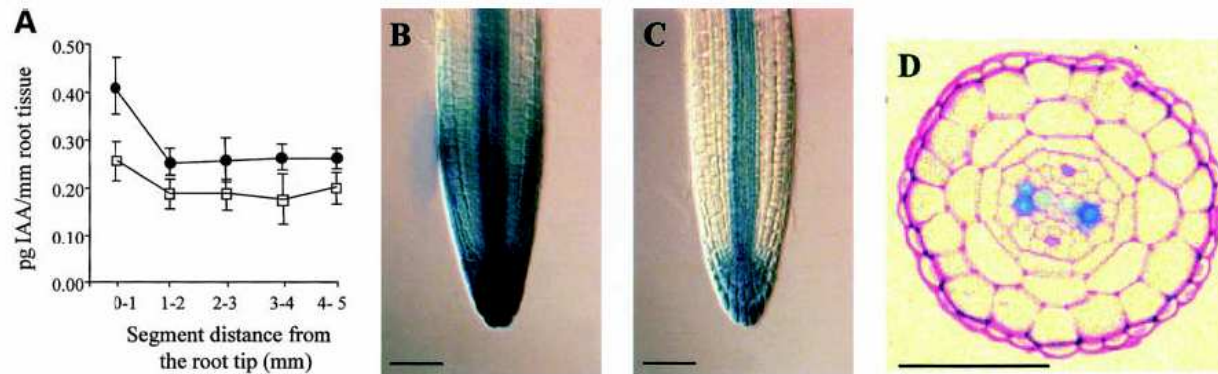
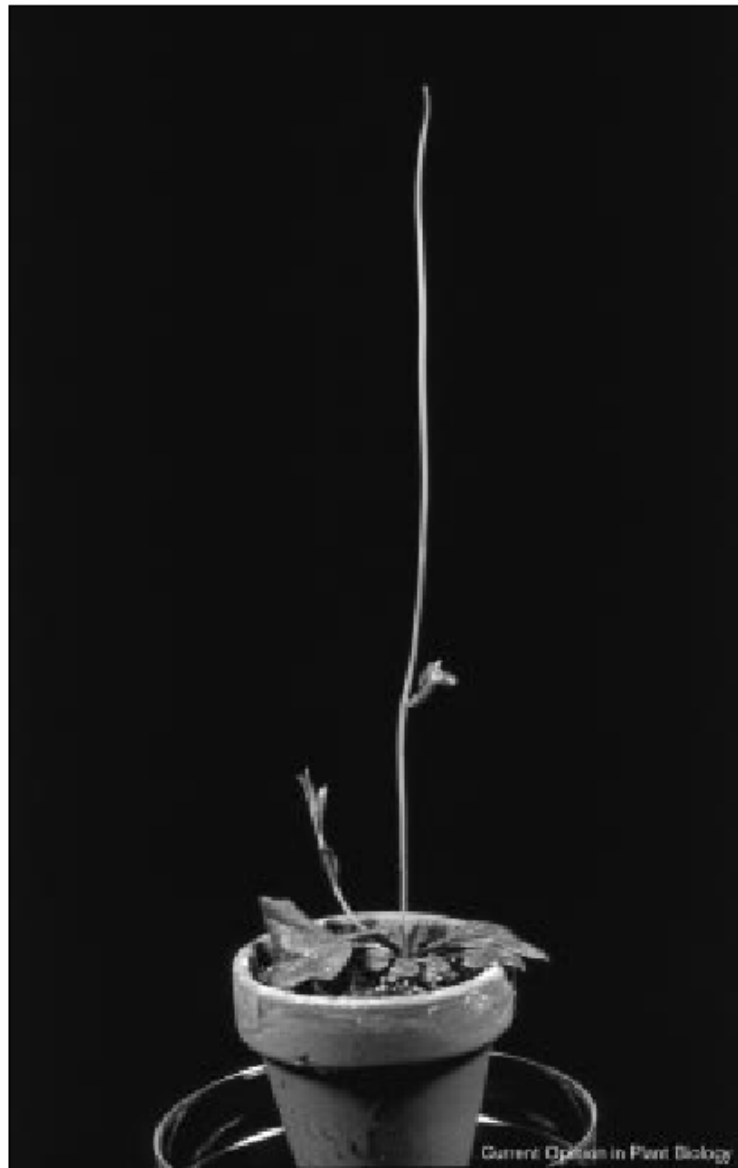


Figure 2. Analysis of IAA accumulation in wild-type and *aux1* *Arabidopsis* root apices. (A) High resolution IAA quantitation in *Arabidopsis* wild-type (●) and *aux1* (□) root segments. (B) DIC image of whole-mount GUS-stained wild-type *IAA2::uidA* root apex. (C) DIC image of whole-mount GUS-stained *aux1 IAA2::uidA* root apex. (D) Radial section of GUS-stained wild-type *IAA2::uidA* root apex. Scale bars, 50 μ m.

PIN přenašeče

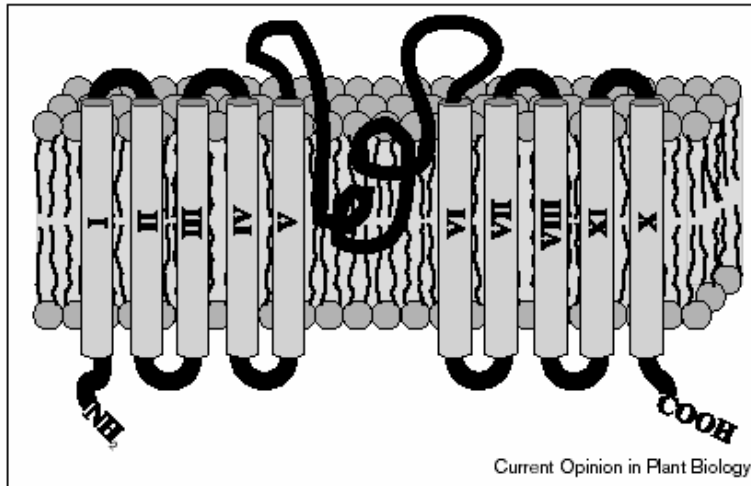
Fenotyp: jako inhibice
transportu (efflux)
auxinu působením
NPA!

pin1 mutant

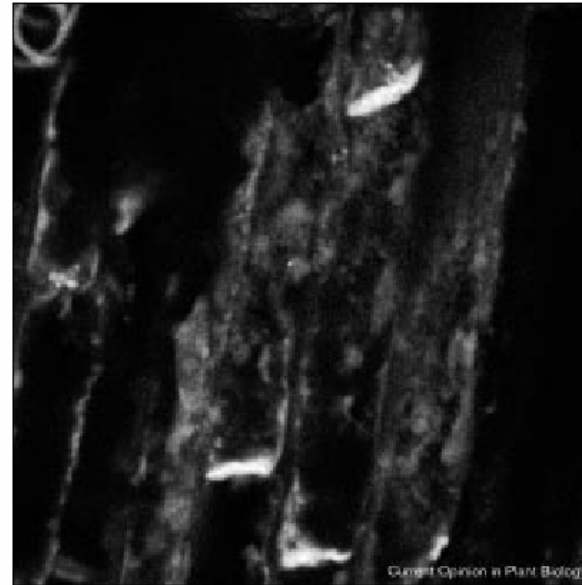


The mutation of the *Arabidopsis thaliana* *AtPIN1* gene changes the phenotypes of *Arabidopsis* plants drastically. The pin-formed, naked inflorescence with no or defective flowers is the main characteristic of these mutants.

PIN proteiny

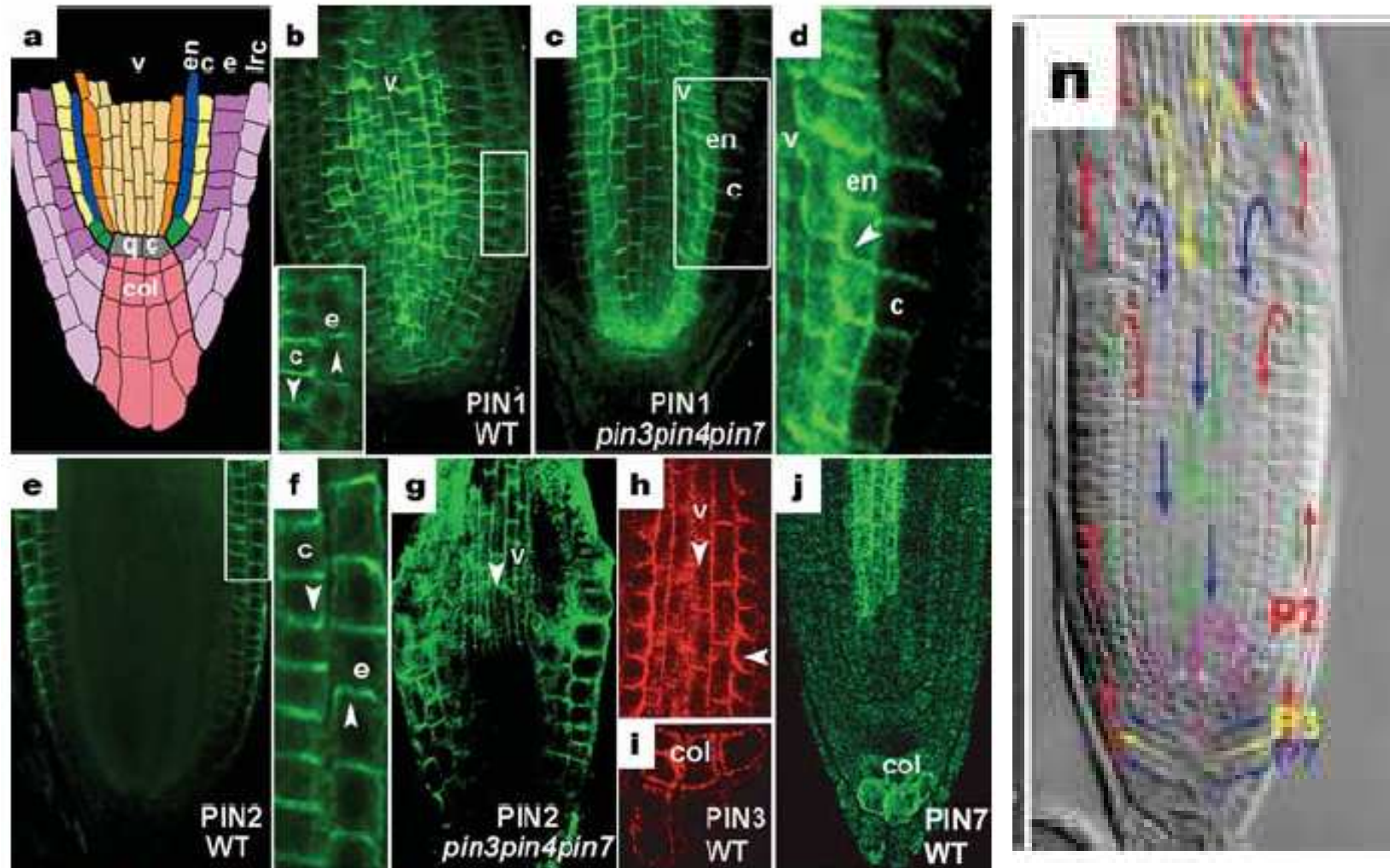


The AtPIN1 protein is a plasma membrane protein with transmembrane segments and a large hydrophilic loop between transmembrane segments five and six.



The AtPIN1 protein was localized at the basal end of xylem parenchyma cells. The bright signals are visualized by confocal laser-scanning microscopy of longitudinal tissue sections incubated with anti-AtPIN1 antibodies and secondary fluorescently labelled antibodies.

Lokalizace členů PIN rodiny



(I. Billou, K. Palme, J. Friml et al.)

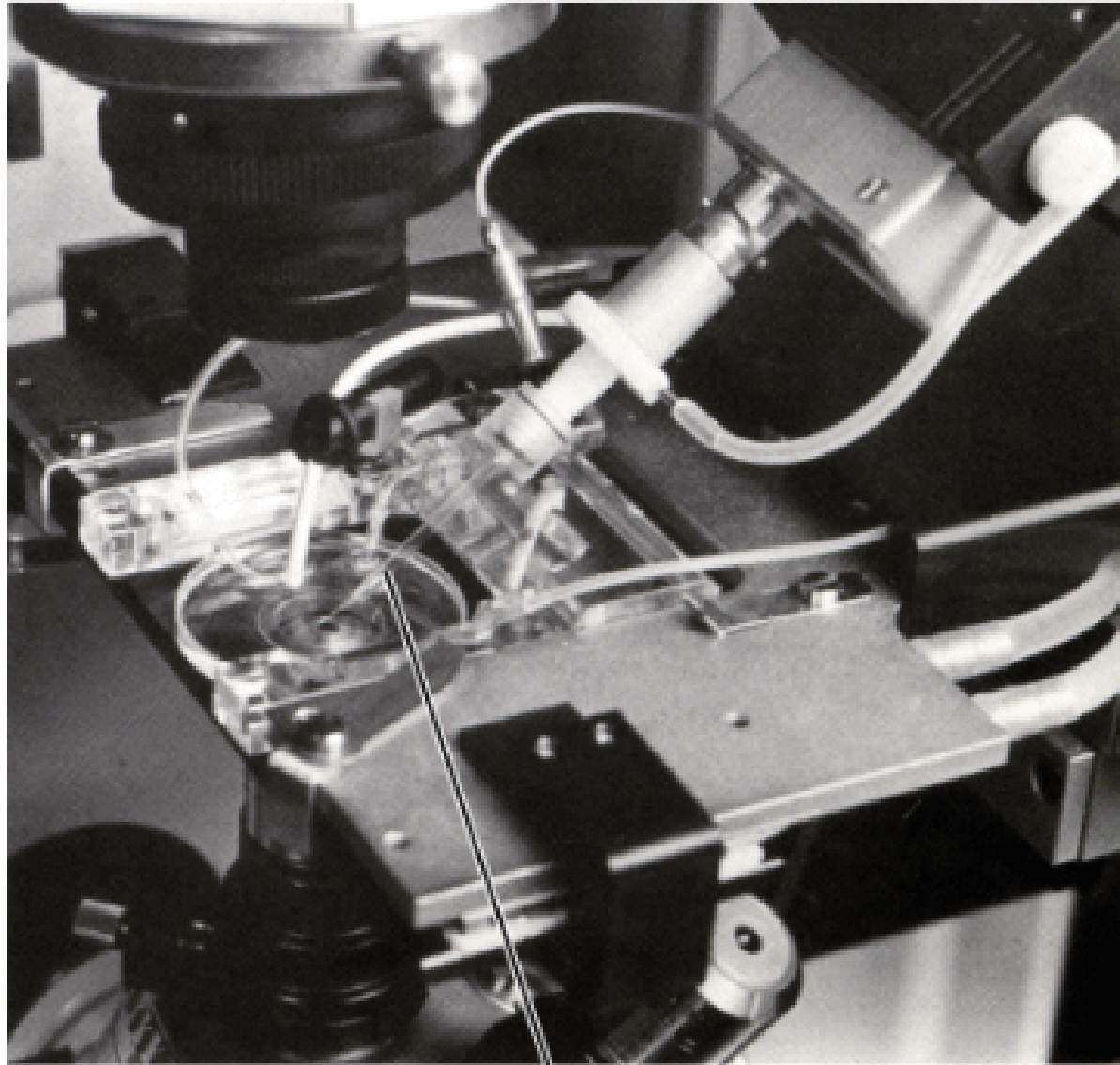
Co pohání transport auxinu?

- ???
- (Samozřejmě) žádá funkční membránu
- Interakce s řadou proteinů – možná role
 - ABC transportérů (PGP)
 - K⁺ kanálů (TRH1)
 - Ppasa (AtVP1)
 - a dalších...

Membránové transportery konkrétně :

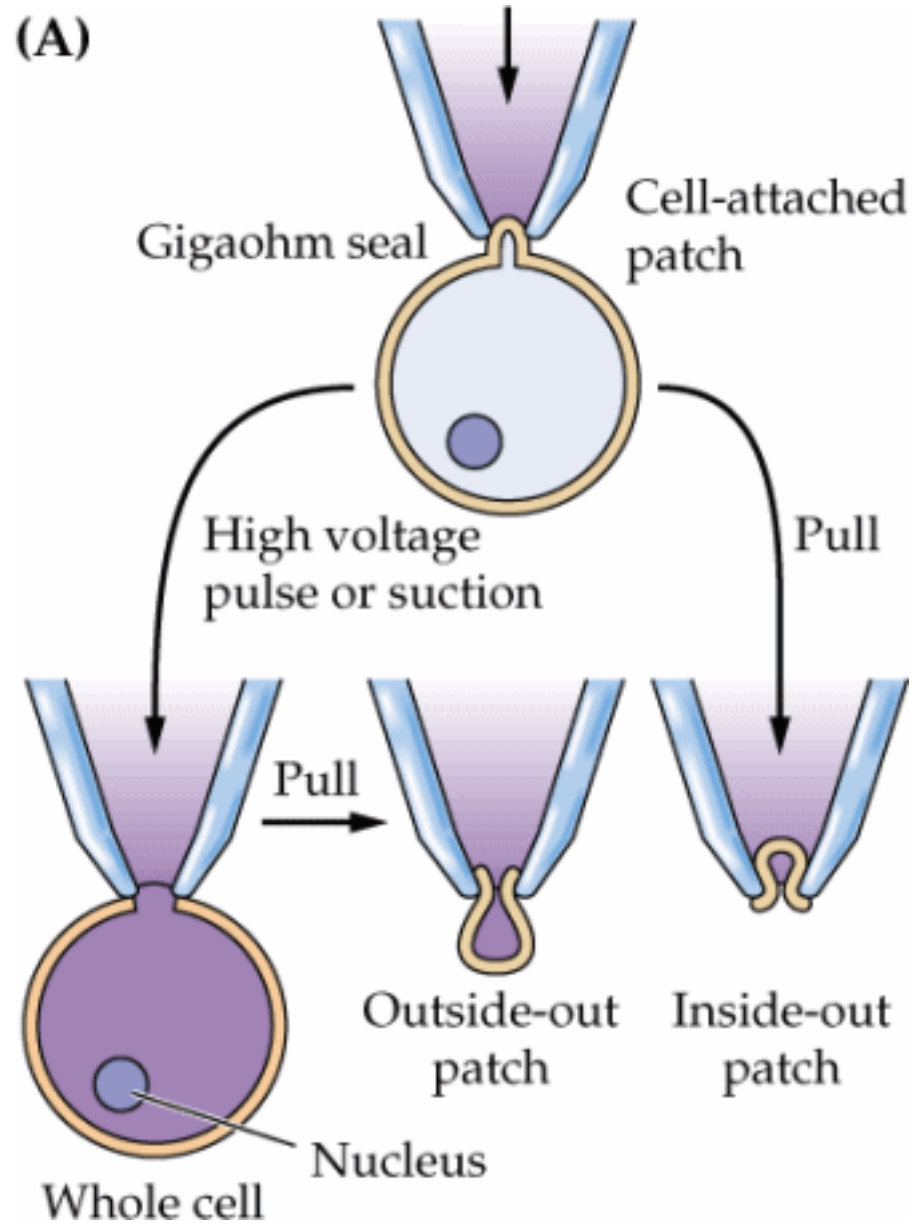
- Pumpy
- Přenašeče
- Kanály
 - řádově vyšší propustnost!
 - tedy je jich málo, biochemie nemusí být dost citlivá ... elektrofyzologie!

(C)

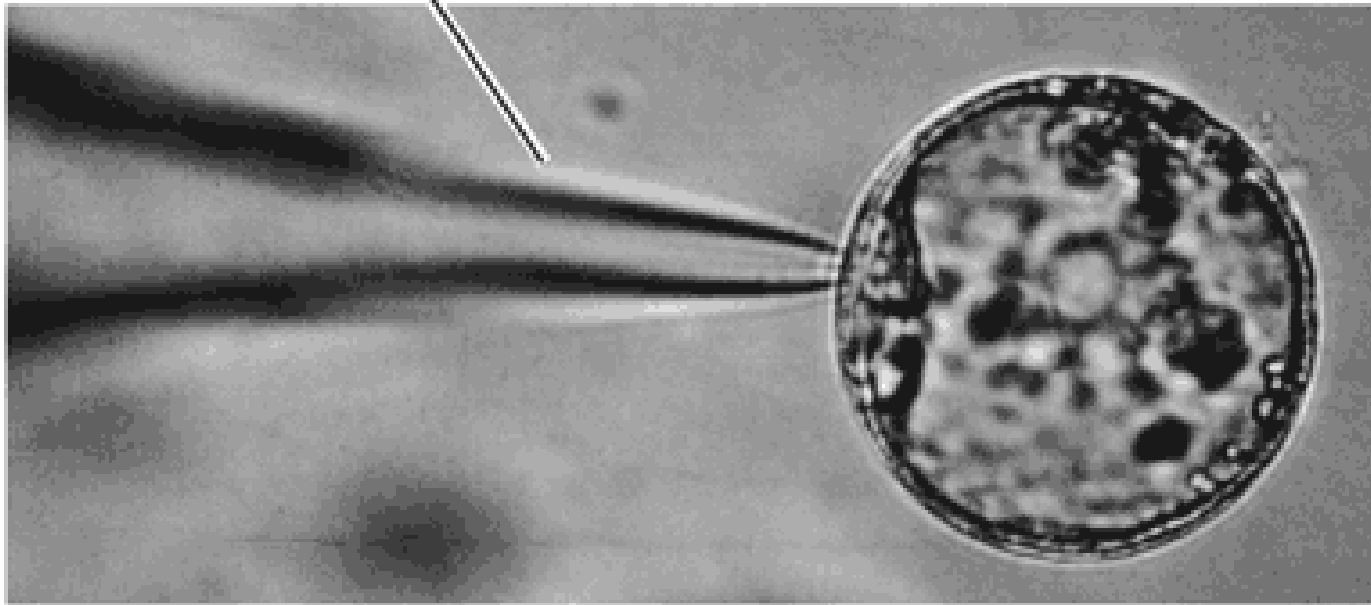


Pipette

Patch-clamp



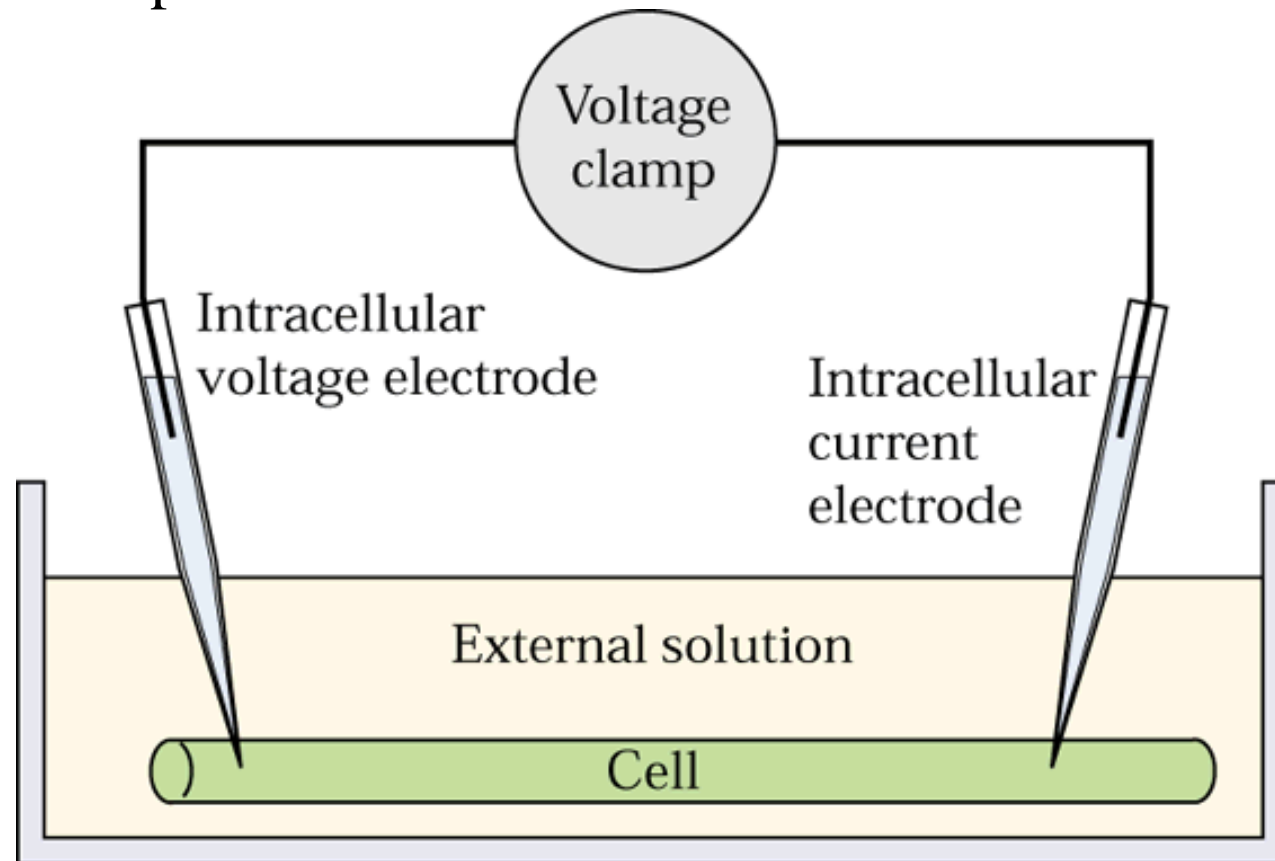
Patch pipette



Micrograph of a patch pipette attached to the surface of a barley aleurone cell protoplast. (Photo courtesy of J. Schroeder and D. Bush.)

Další možnosti:

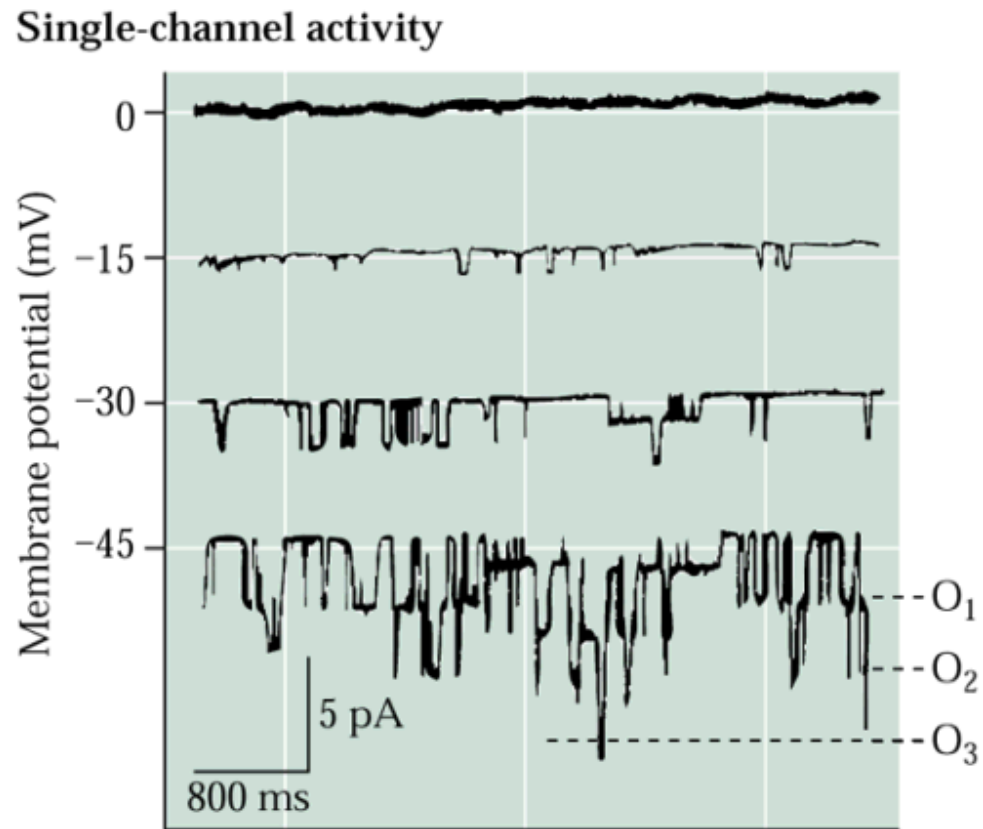
- Voltage clamp



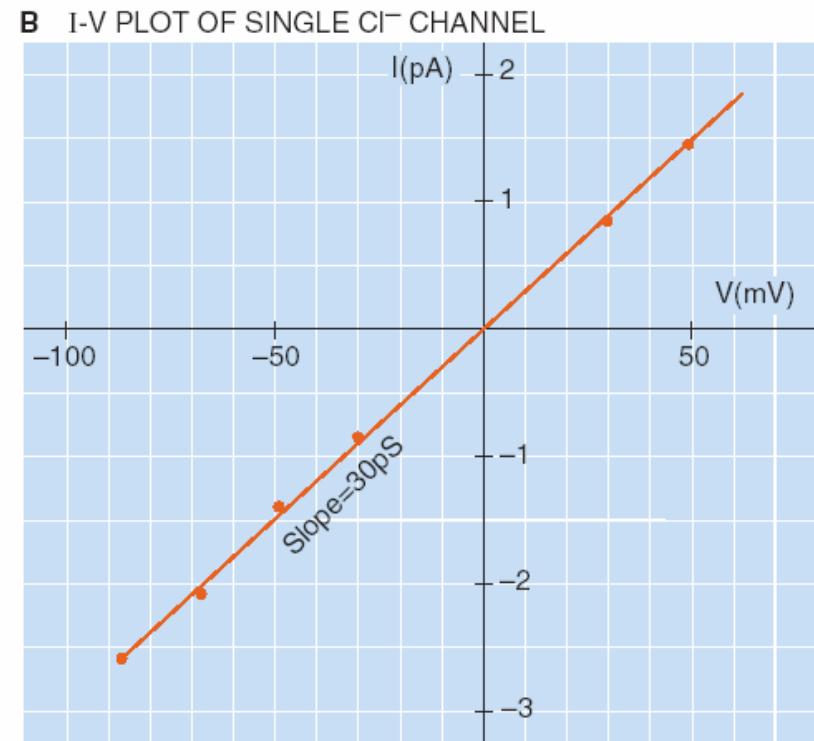
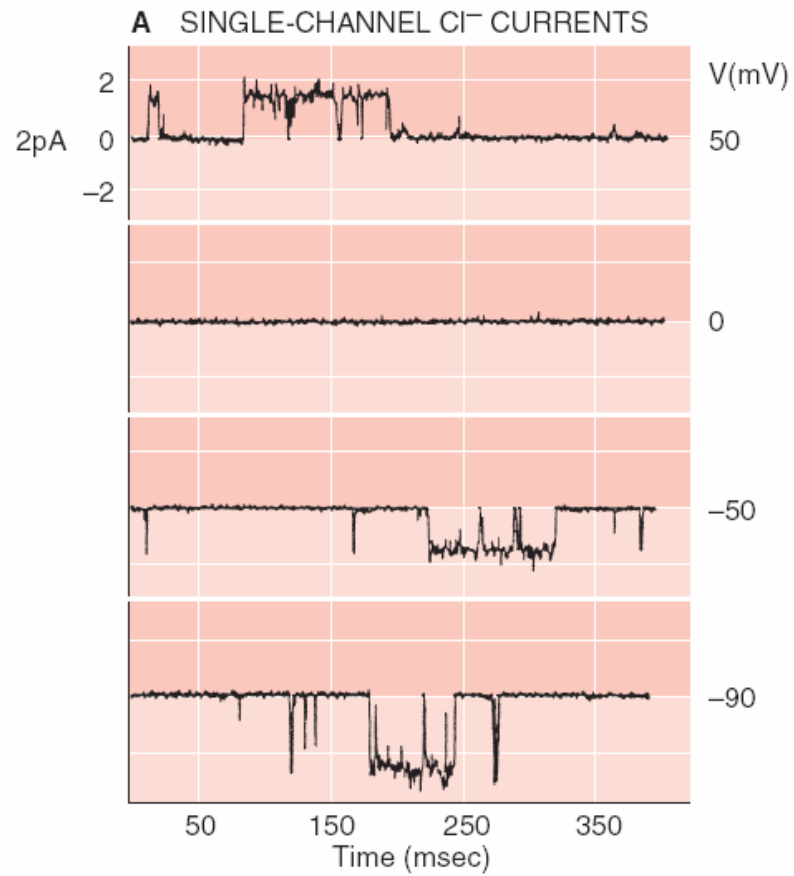
- Planární lipidové dvojvrstvy

Patch-clamp techniky mohou měřit i 1 kanál

- Závislost membránového potenciálu na čase
- I-V křivky



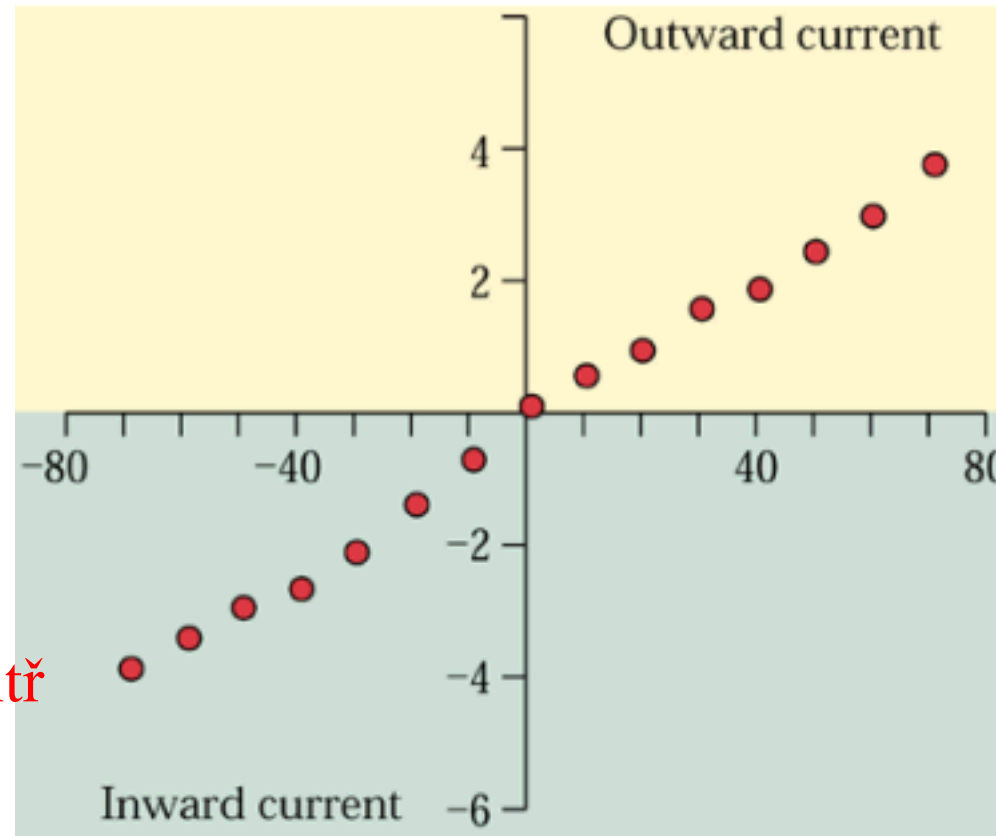
I-V křivky



I-V křivky

Single-channel current

Single-channel current (pA) (měřený)



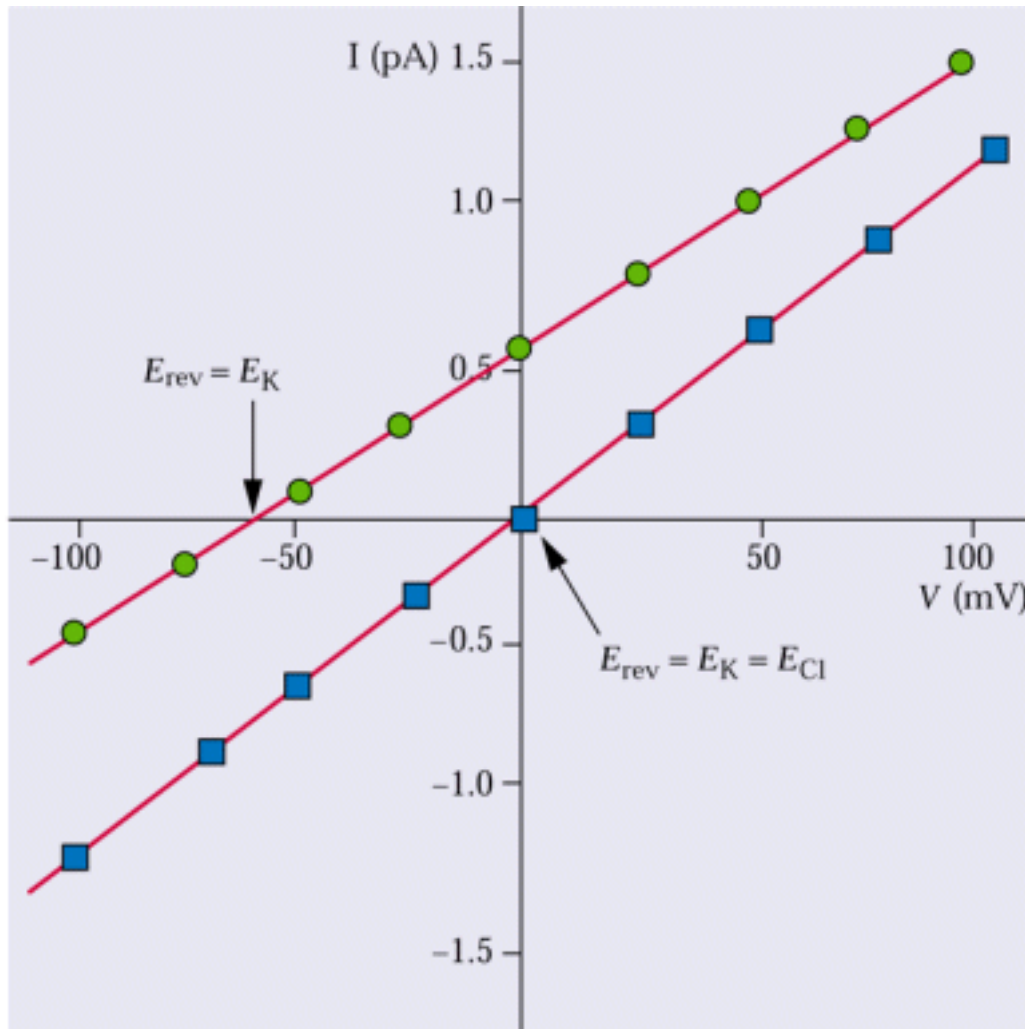
kationty ven
anionty dovnitř

Membrane
potential (mV)

(vložený na
membránu)

anionty ven
kationty dovnitř

Měření selektivity kanálů - závislost I/V na složení média

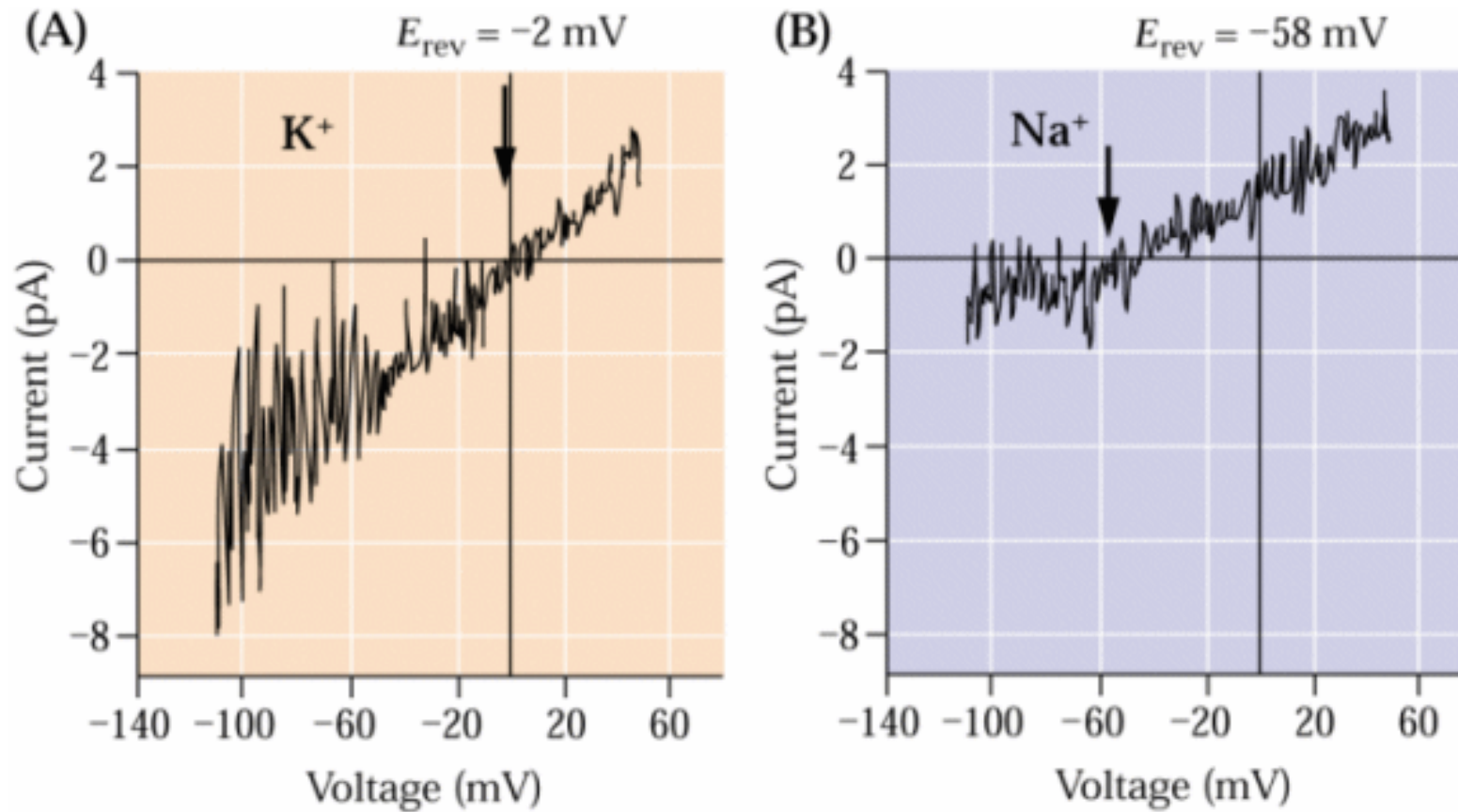


outside-out patch

100 mM KCl
(in and out)

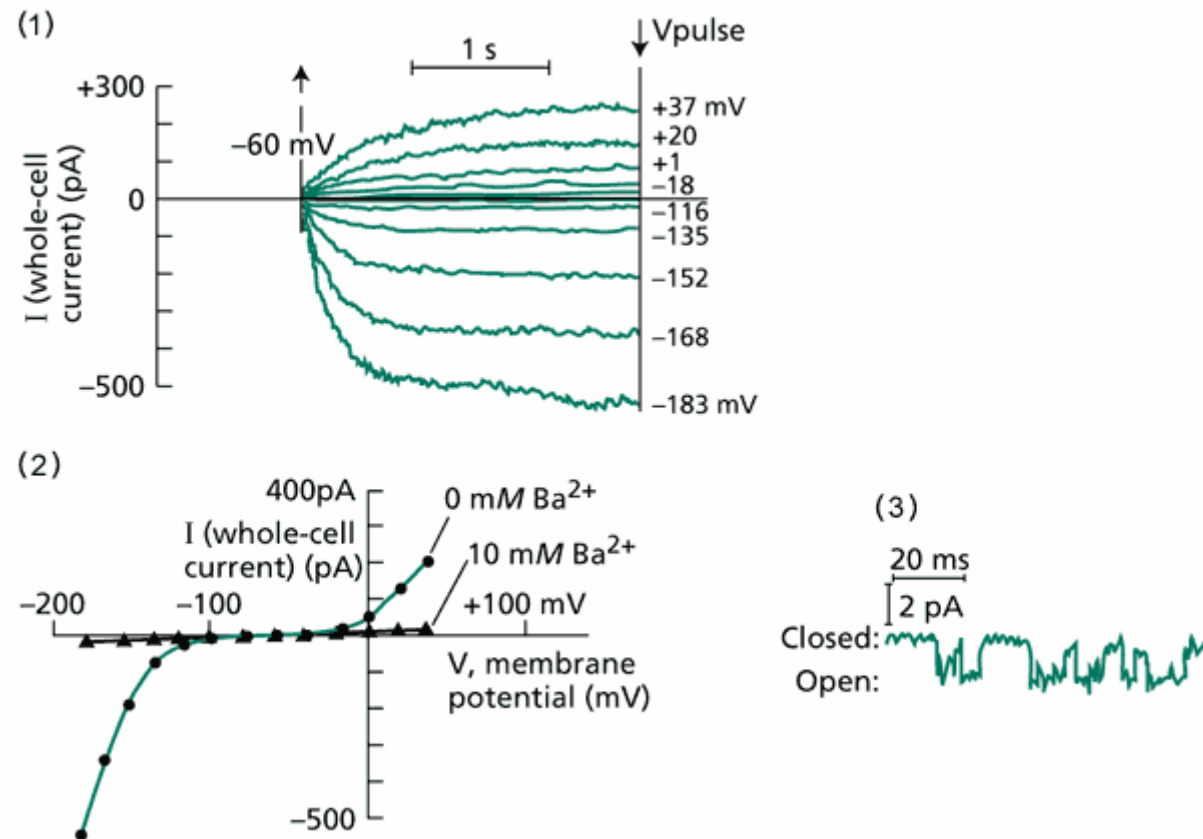
100 mM KCl in
10 mM KCl out

Měření selektivity kanálů - závislost I/V na složení média



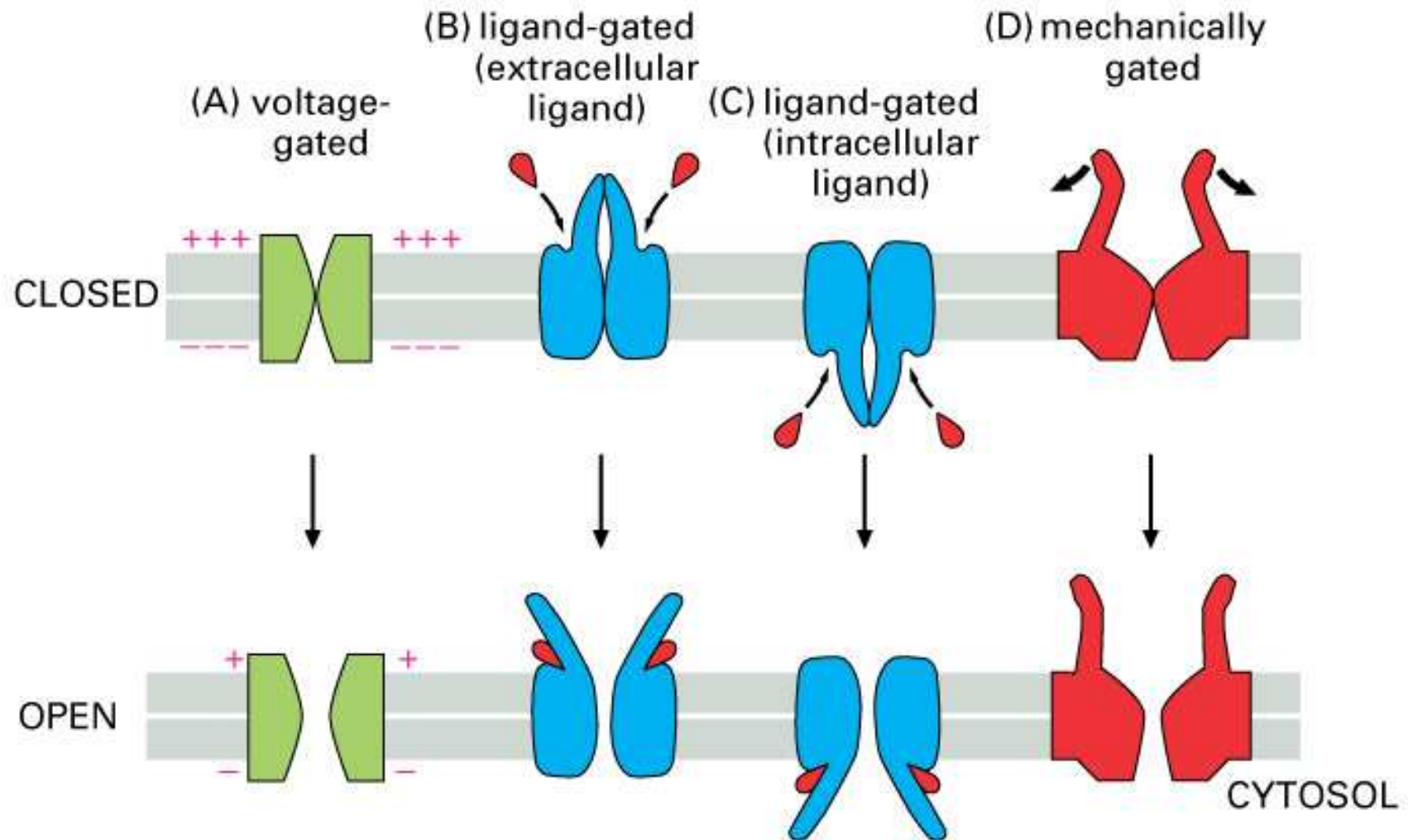
(outside-out patch)

Co můžeme měřit - shrnutí

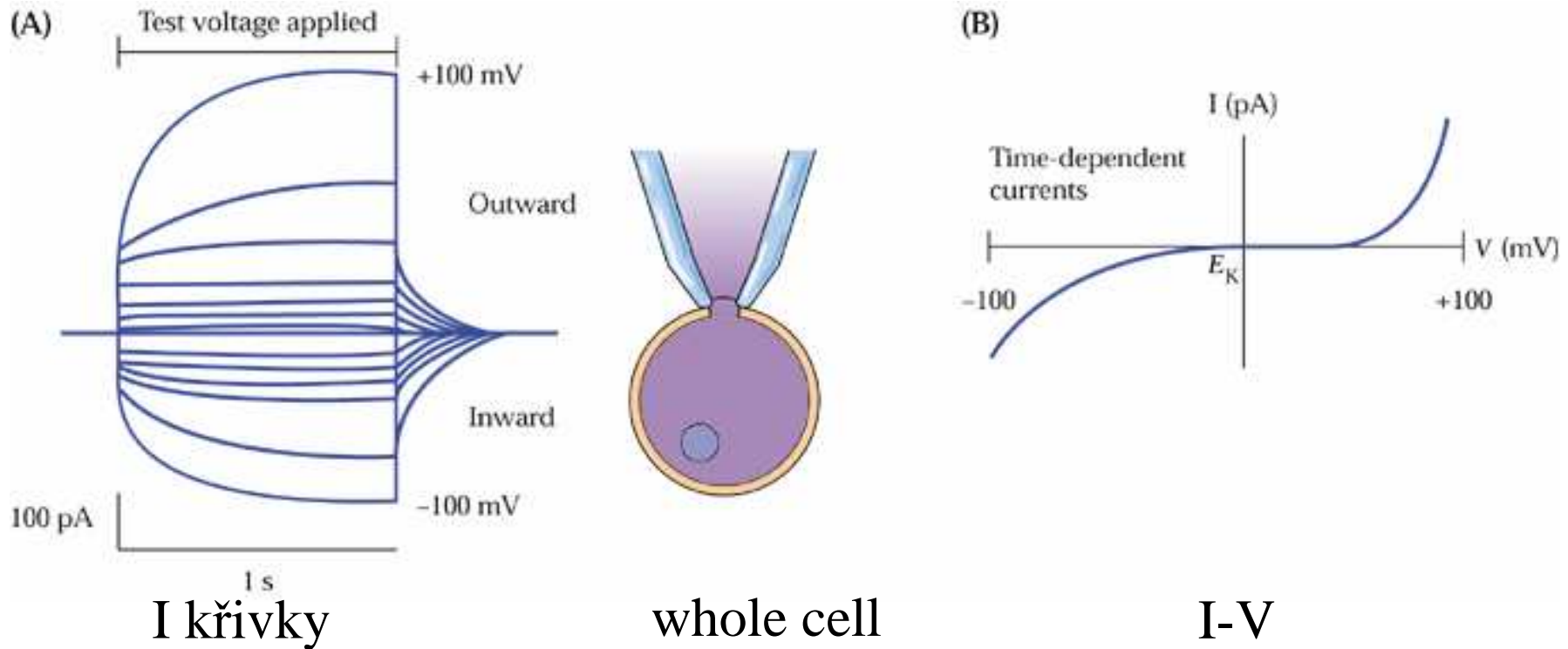


Recordings of K⁺ currents in whole cells and in single K⁺-selective channels of guard cell protoplasts. (1) K⁺ currents recorded in the whole-cell configuration (see Web Figure 6.2.B) when the membrane potential is clamped at different values. Upward deflections show outward currents; downward deflections show inward currents. K⁺ concentrations were 105 mM in the pipette and cytoplasm, and 11 mM in the bathing solution outside the cell. (2) Data from the same experiment plotted as an I/V (current/voltage) curve, before and after the addition of Ba²⁺, an ion that blocks K⁺ channels. (3) Inward K⁺ current through a single channel in a membrane patch. (From Schroeder et al. 1987.)

Různé způsoby regulace otvírání a zavírání kanálů

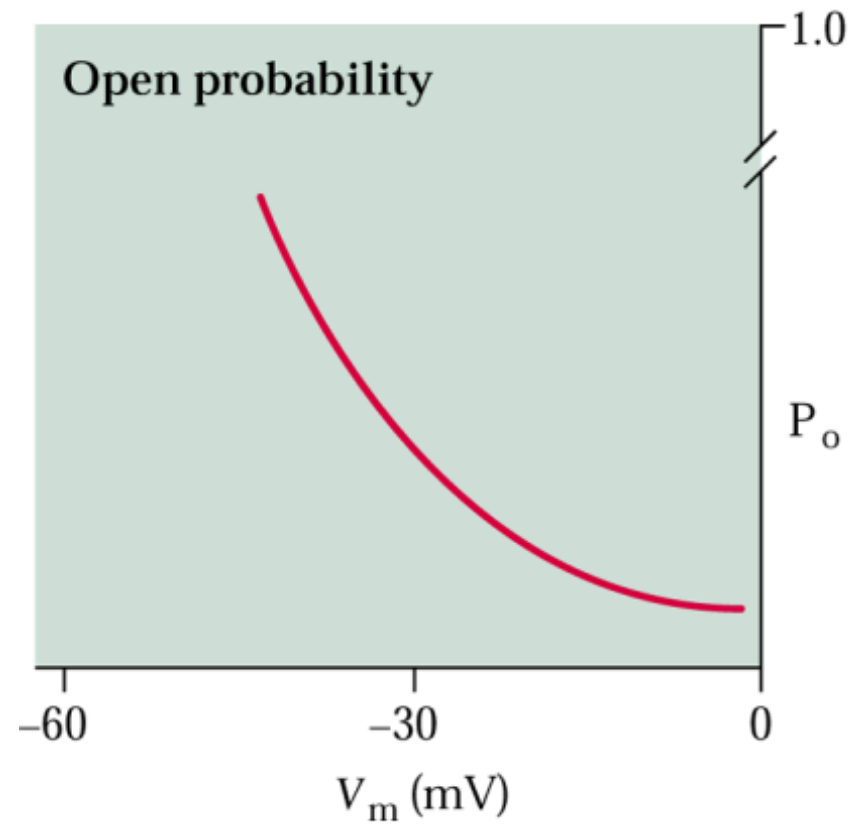
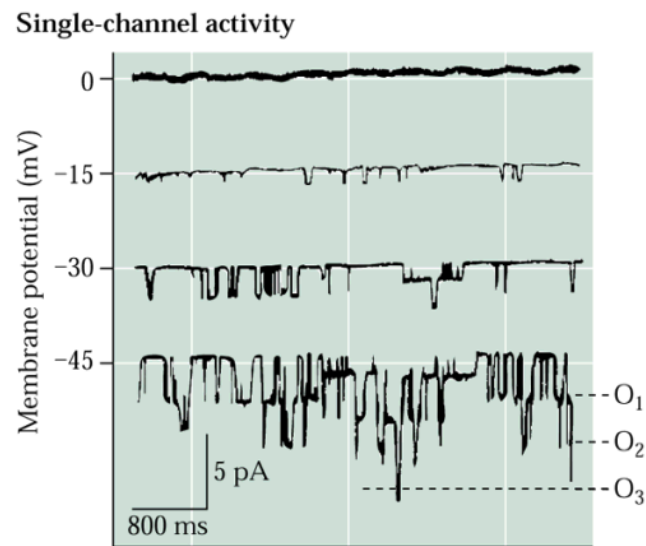


Iontové kanály plasmalemmy: regulace napětím (voltage gated)



Může být i regulace ligandem (ligand gated) nebo obojím

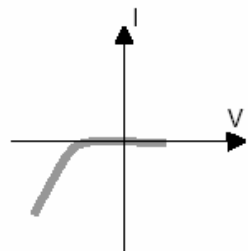
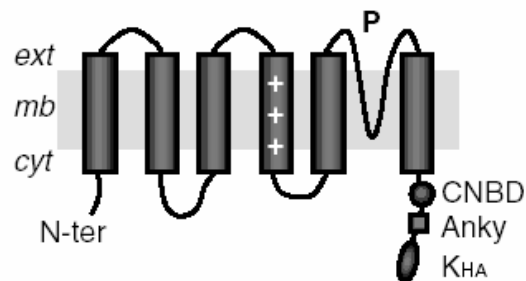
Závislost otvírání obec. kanálu na napětí



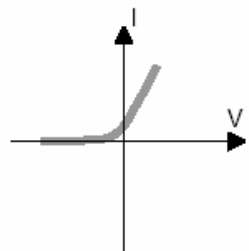
K⁺ kanály v Arabidopsis

- K – až 10 % sušiny rostlin
- 35 genů pro K⁺ transportní systém
 - 15 kanálů
- Plasmalema (shaker, TPK4)
- Tonoplast (ostatní TPK, Kir)

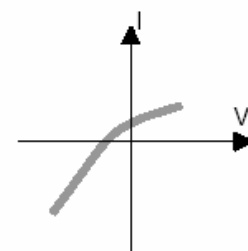
Shaker



Inwardly rectifying

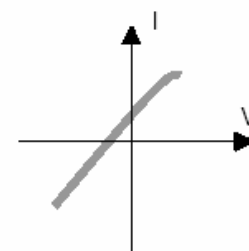
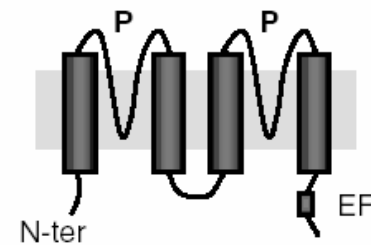


Outwardly rectifying



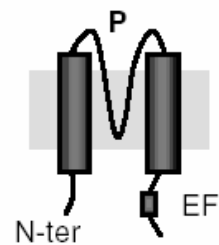
Weakly rectifying

TPK



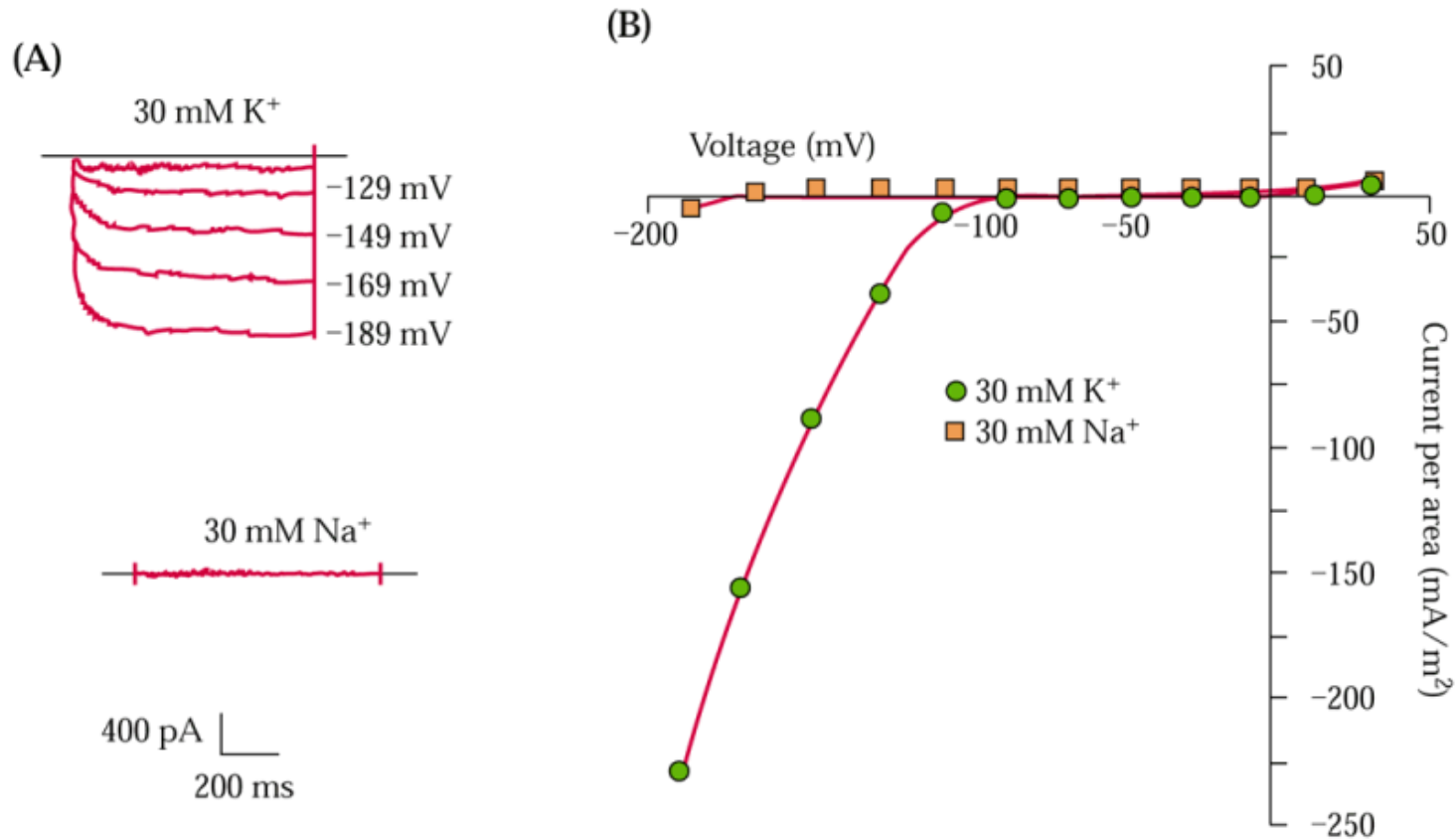
Non voltage-regulated

Kir-like



?

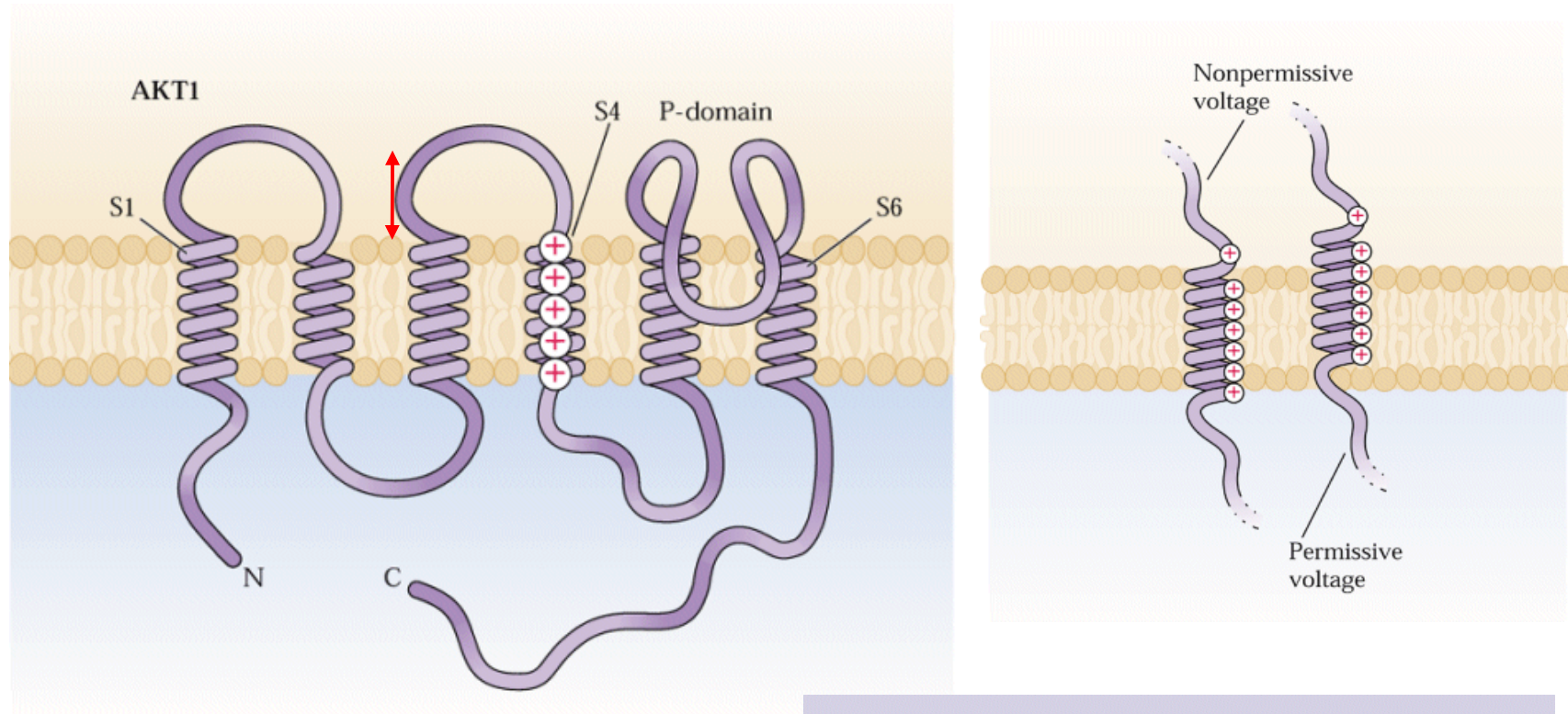
inward rectifying K⁺ kanál



aktivován při hyperpolarizaci membrány ($V_m < -120\text{mV}$)
ve svěracích buňkách regulován G proteiny a fosforylací
inhibován zvýšenými konc cytosolického Ca²⁺

inhibitor - tetraethylammonium

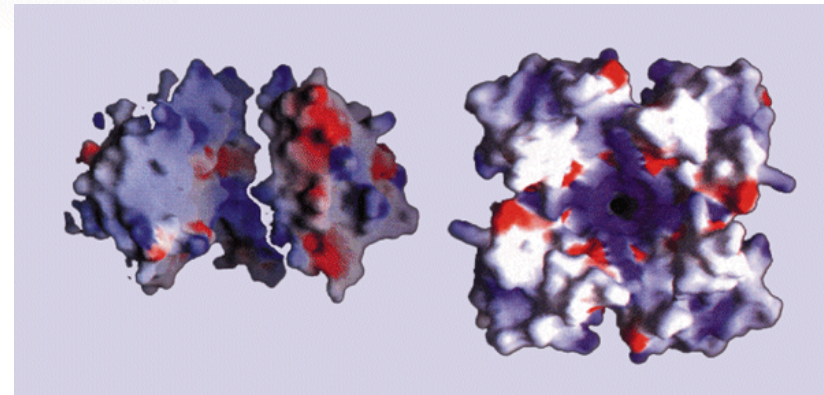
K⁺ kanály typu *shaker* (inward rectifying)



Tetramer!

+ regulační podjednotky!

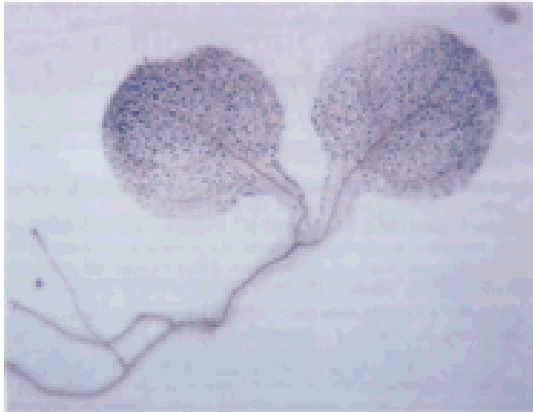
+ fosforylace!! (ABA sig.?)



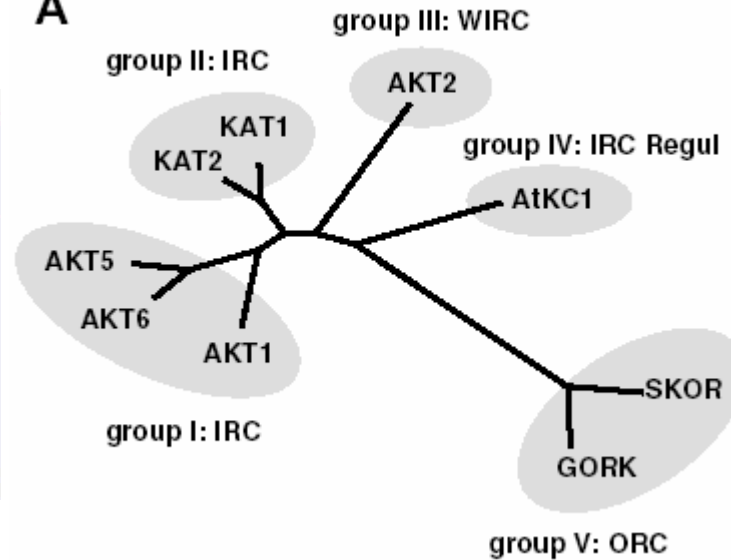
K⁺ kanály typu *shaker* (inward rectifying)

KAT1

(A)



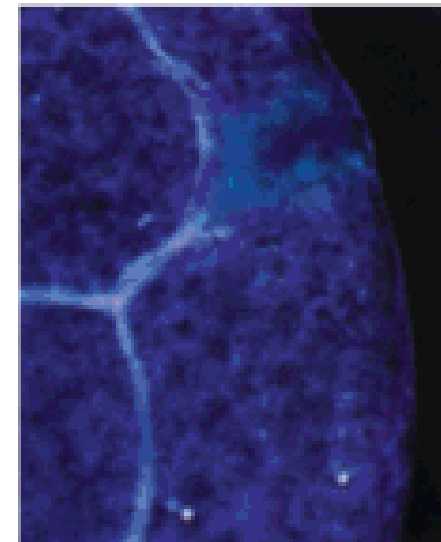
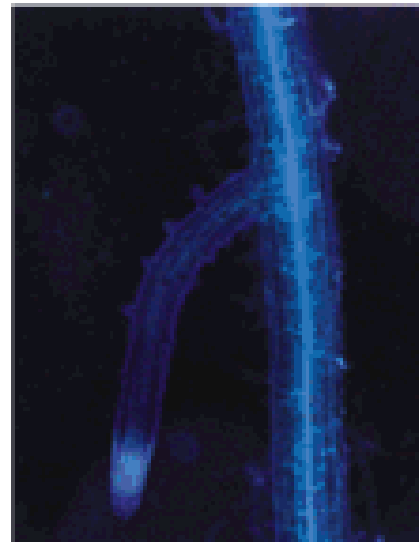
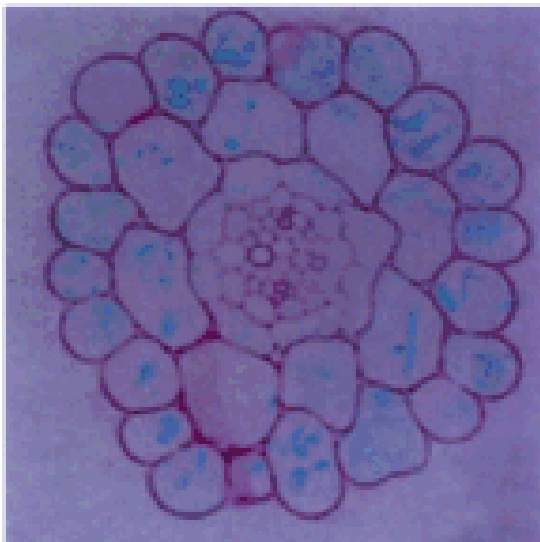
A



Channel type

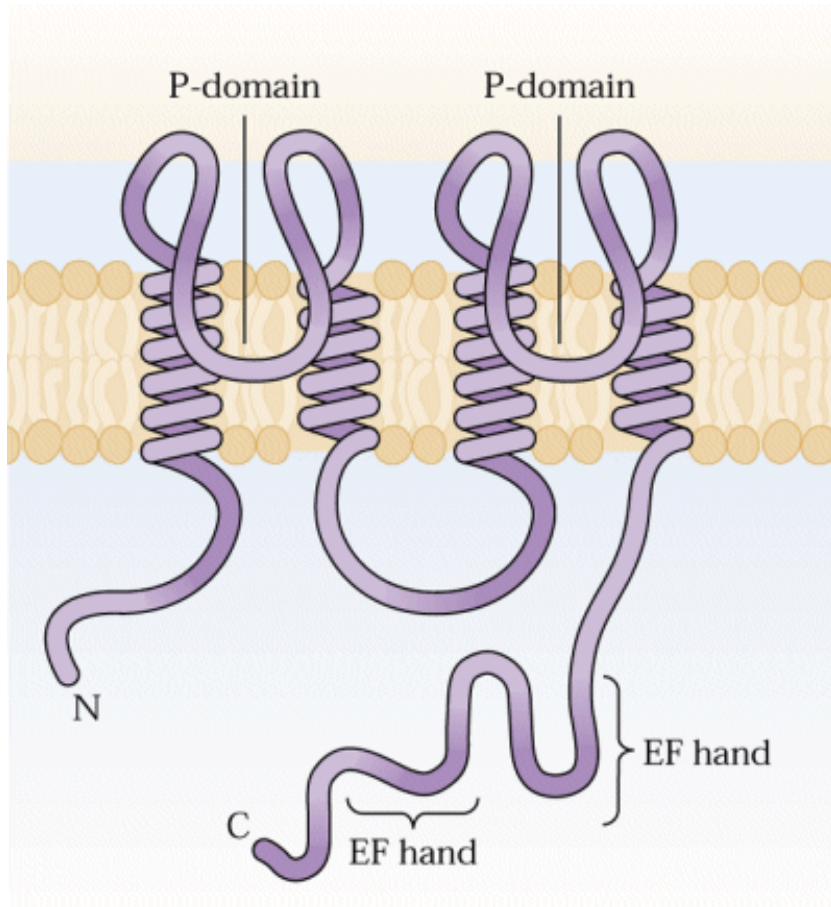
- IRC: Inwardly rectifying conductance
- WIRC: Weakly inwardly rectifying conductance
- ORC: Outwardly rectifying conductance
- IRC Regul: Regulatory subunit involved in

(B)

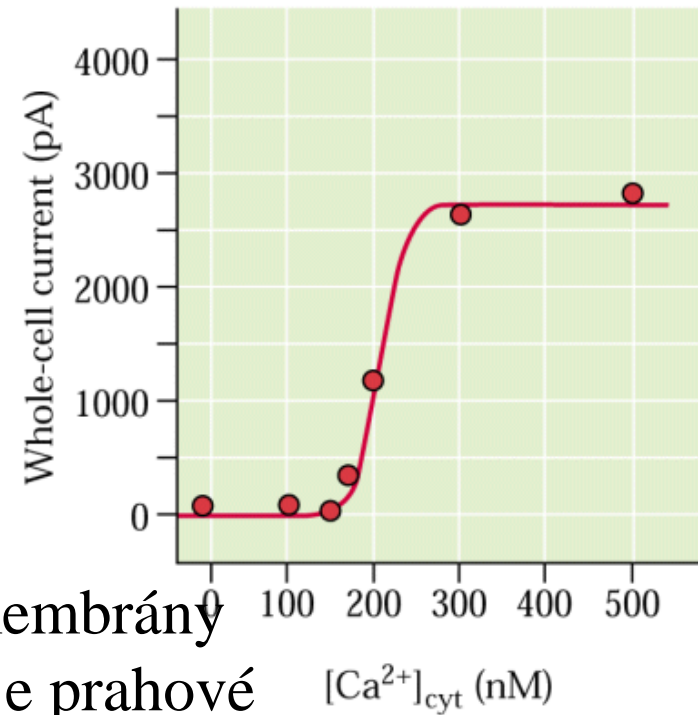


AKT1

KCO1 (outward rectifying K^+)

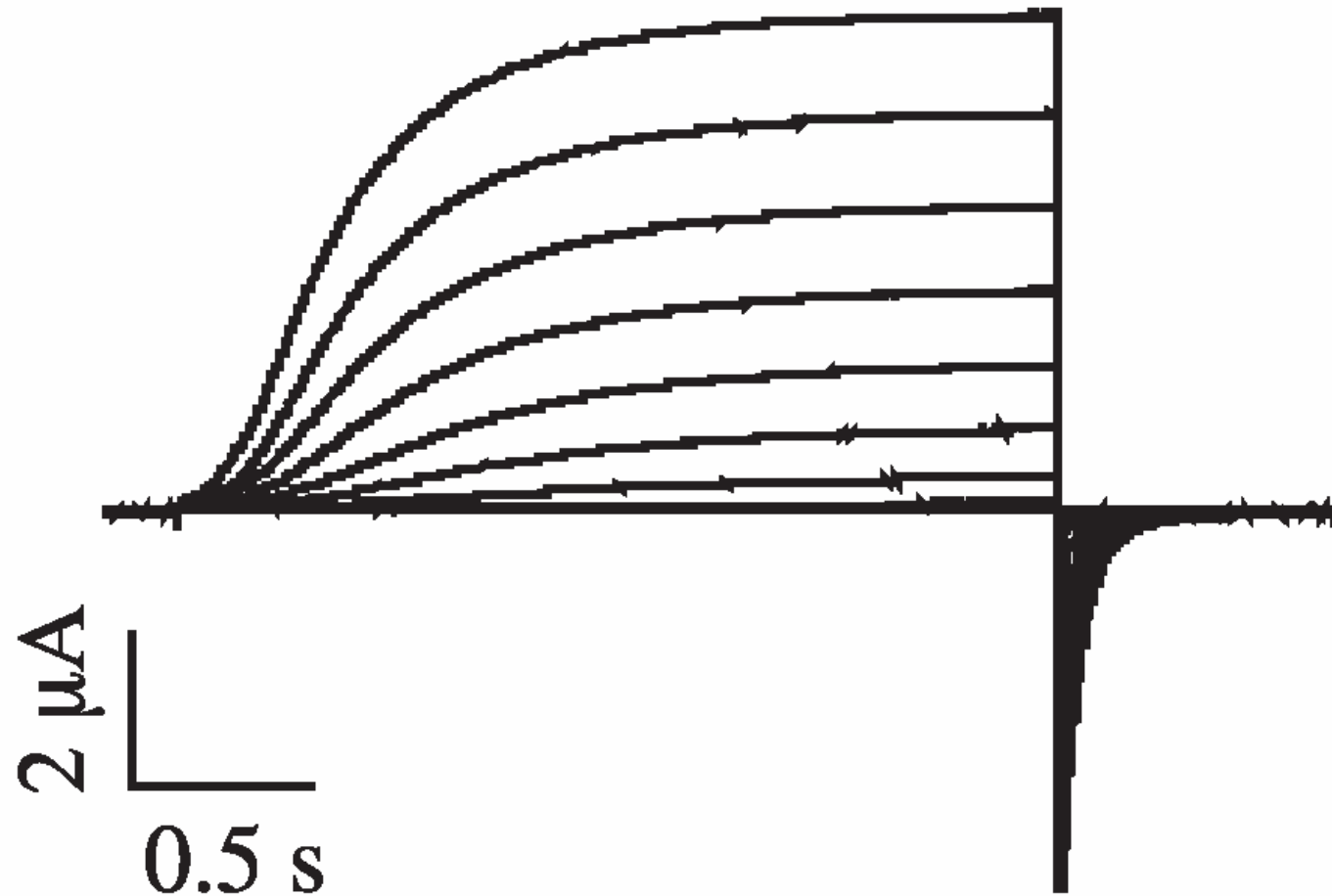


regulace
 Ca^{2+} !



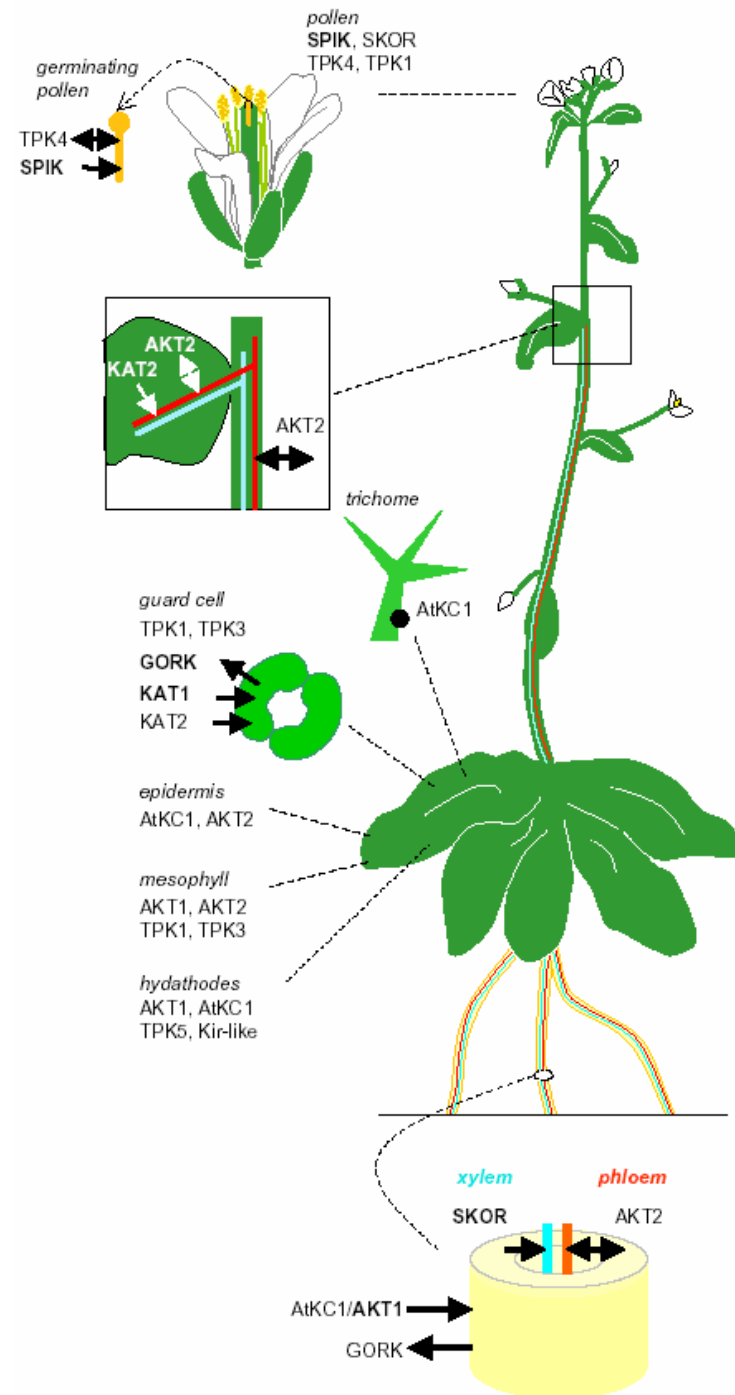
aktivován při depolarizaci membrány
inhibice Ba^{2+} , vápník snižuje prahové
napětí pro aktivaci

GORK (guard cell outward rectifying K^+ channel, shaker)



(expressed in oocytes)

Funkce K⁺ kanálu *in* *planta*



Flowers

| | |
|------|-------|
| TPK1 | [14] |
| TPK4 | [33] |
| AKT2 | [32] |
| SKOR | [86] |
| SPIK | [26] |
| AKT6 | [32] |
| GORK | [101] |

Leaf

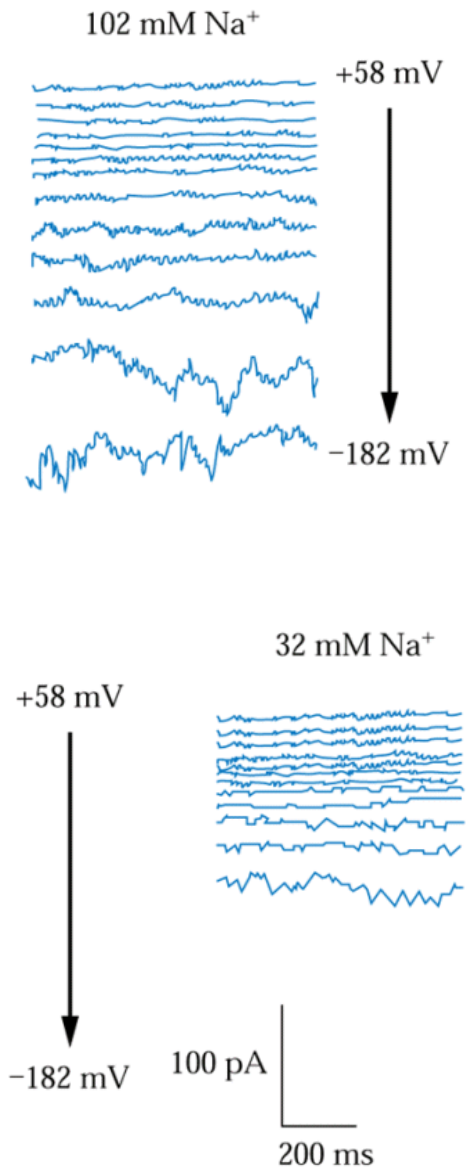
| | |
|----------|------------|
| TPK1 | [102] [14] |
| TPK3 | [102] |
| TPK5 | [11] |
| Kir-like | [11] |
| GORK | [103] |
| AKT1 | [79] |
| AKT2 | [32] |
| AtKC1 | [56] |
| KAT1 | [104] |
| KAT2 | [53] |

Root

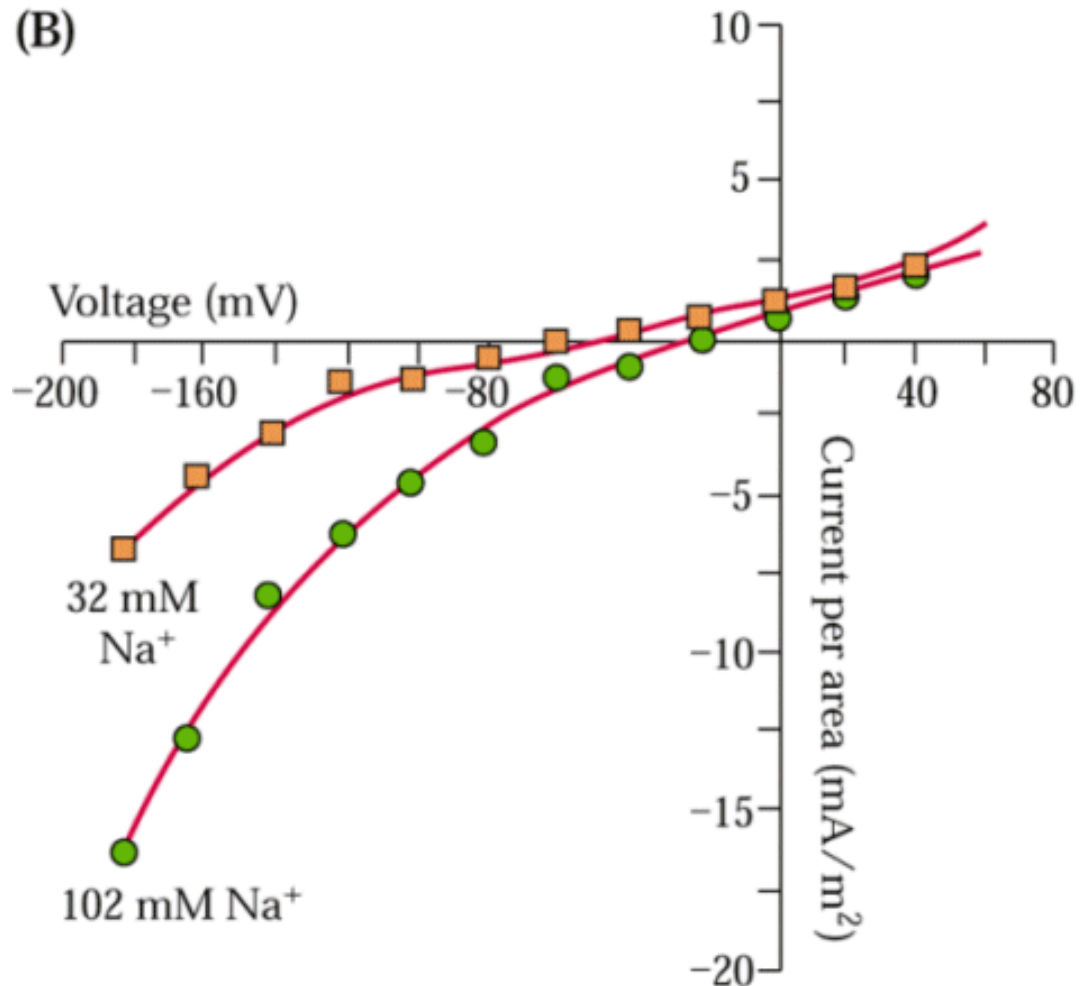
| | |
|----------|-------|
| TPK1 | [14] |
| TPK5 | [11] |
| Kir-like | [11] |
| SKOR | [86] |
| AKT2 | [32] |
| GORK | [103] |
| AKT1 | [79] |
| AtKC1 | [55] |

Na napětí nezávislý (a méně selektivní) Na⁺ kanál

(A)



(B)

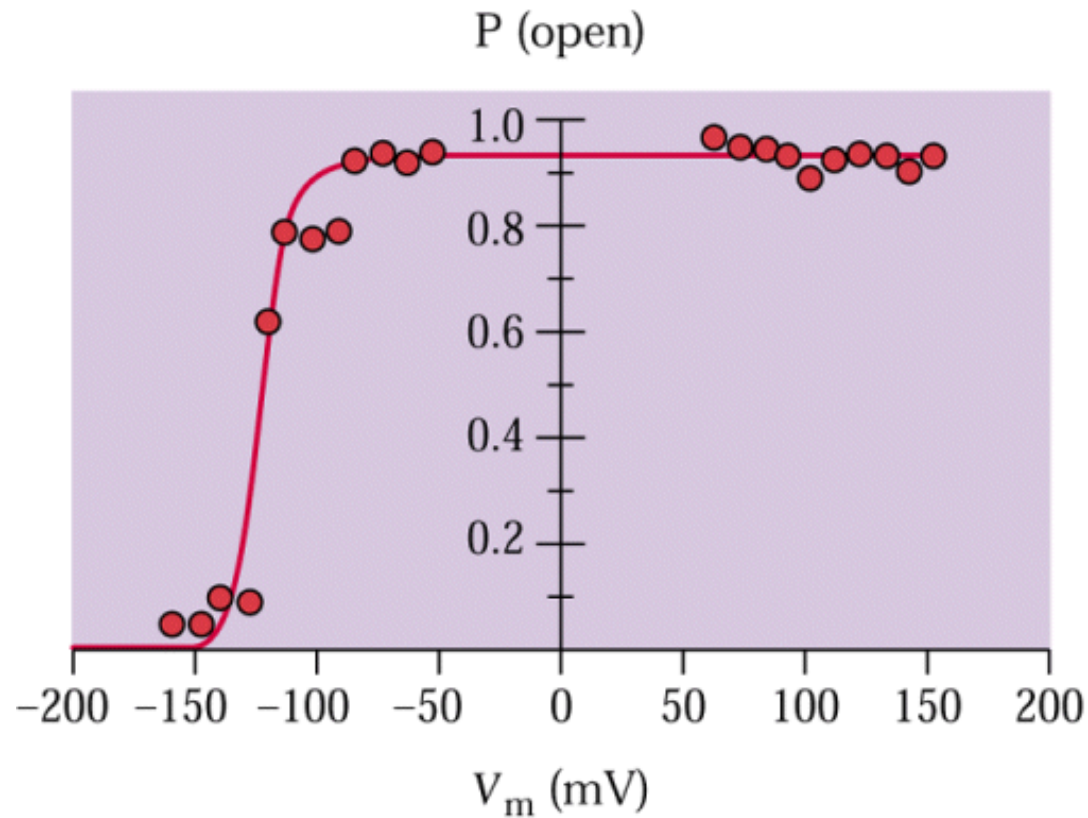


příjem sodíku??

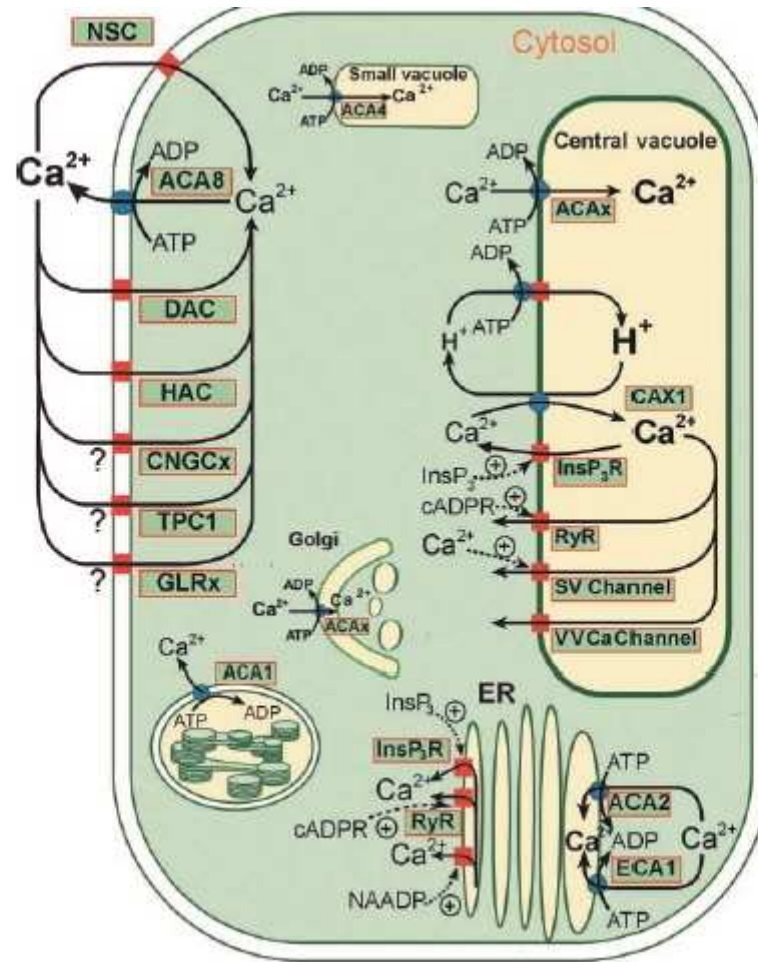
Ca²⁺ kanály plasmalemy

- **aktivované hyperpolarizací**
 - mechanosenzitivní nebo induk. elicitory (**ROS!**)
 - selektivnější (Ca; Ca, K; Ca, Ba)
- **aktivované depolarizací**
(např. DAC, rca)
 - různě selektivní (Ca; Ca, K; Ca, Ba, Sr, Mg; „maxi-cation“ Rb až Mn)
- **nezávislé na napětí**

Ca²⁺ kanály plasmalemy

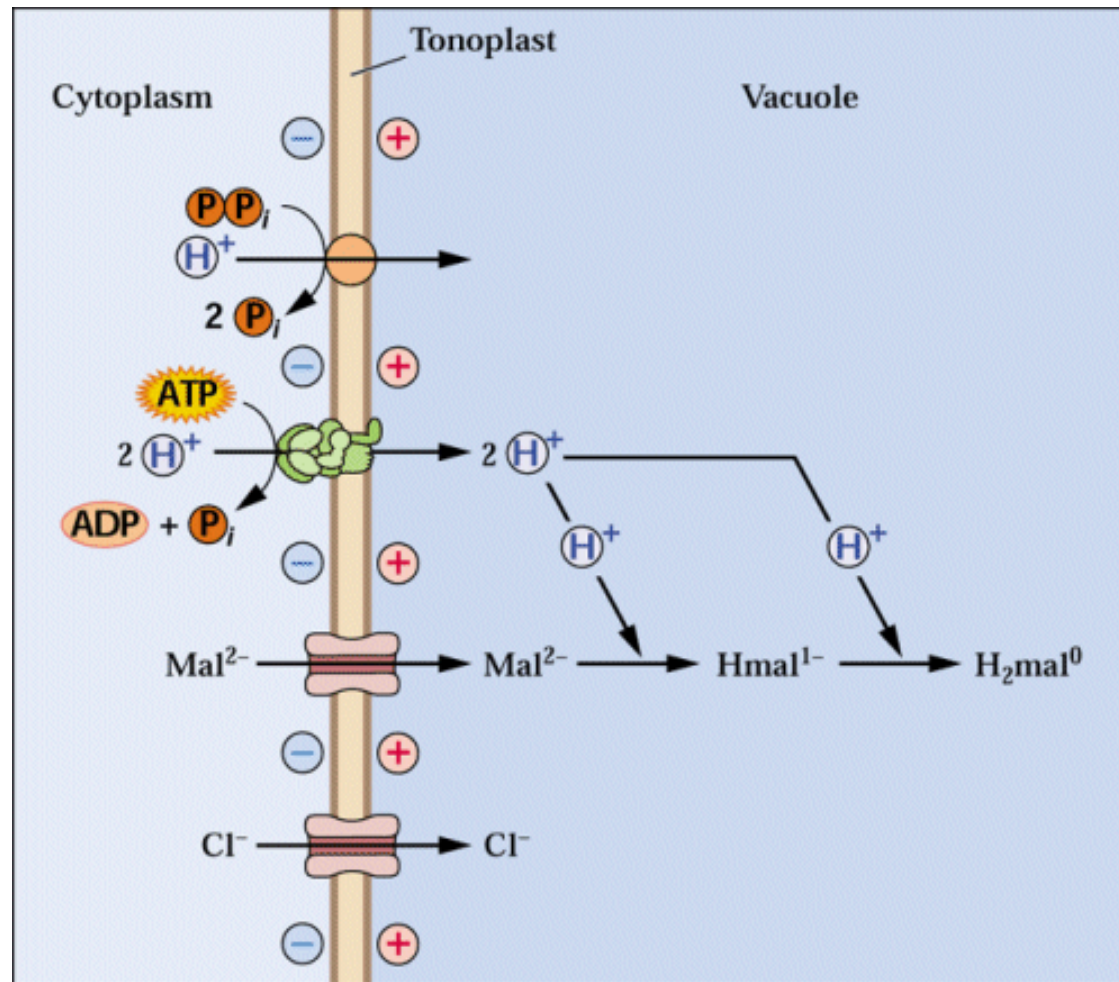


... ale buňka má vápníkových kanálů mnohem víc,
a k tomu ještě pumpy ...

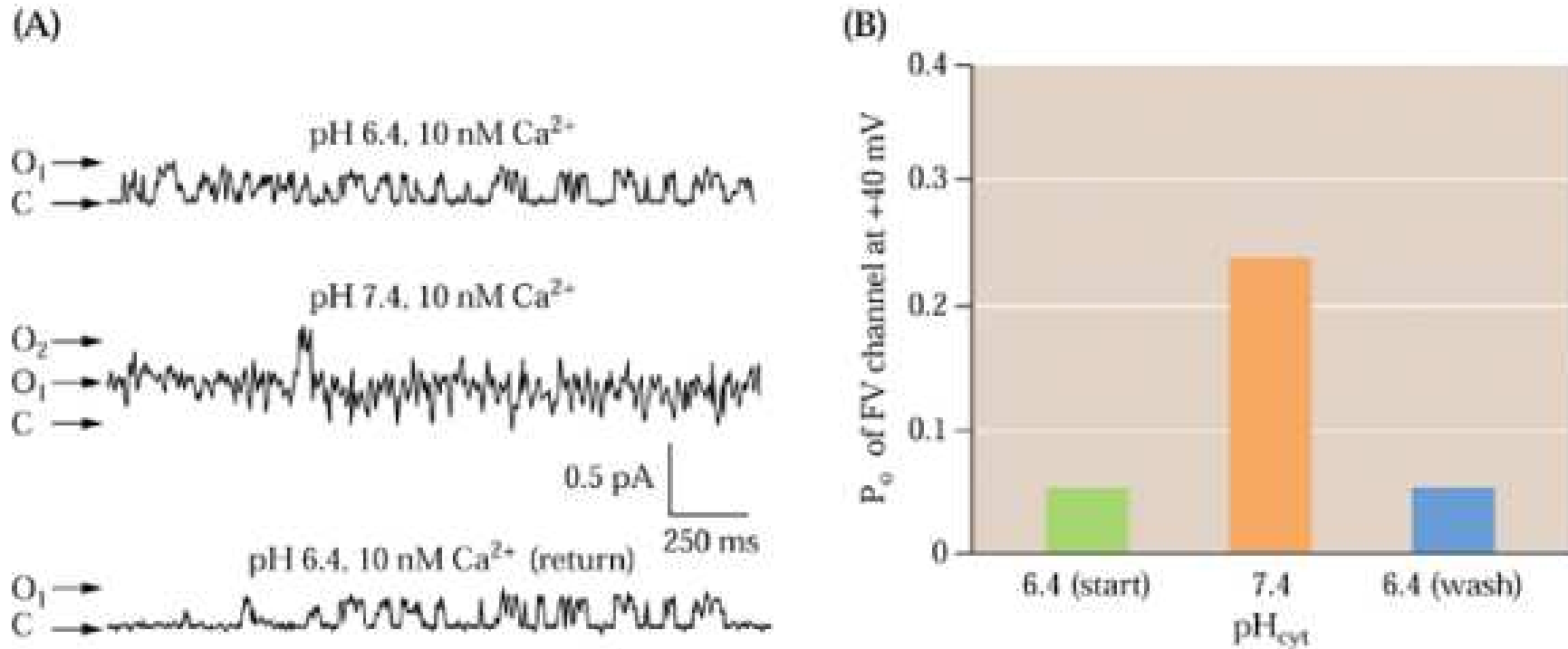


(i na vakuole)]

Iontové kanály tonoplastu a dalších endomembrán



Tonoplastový kanál pro jednomocné kationty (K^+): regulace pH a Ca^{2+}

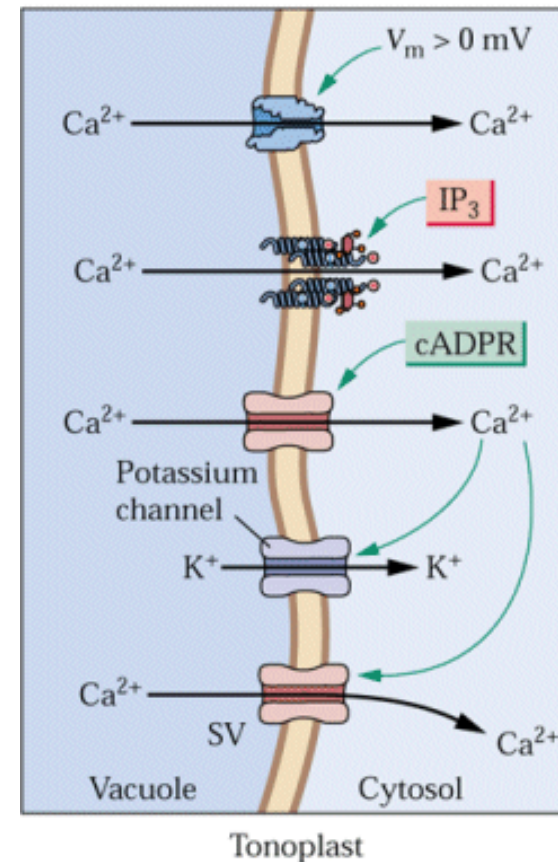


FV – fast vacuolar channel: aktivace $pH \uparrow$, inhibice Ca^{2+}
VK – vacuolar K^+ : inhibice $pH \uparrow$, aktivace Ca^{2+} (zřejmě TPK1)

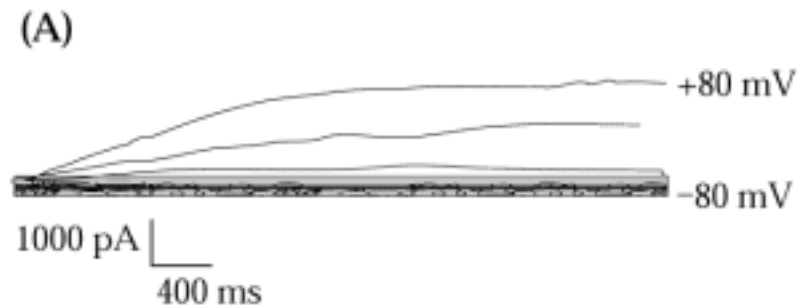
Vápníkové kanály tonoplastu a dalších endomembrán

- tonoplastový, aktivace IP₃
- tonoplastový, aktivace cADPR („ryanodin. receptor“)
- voltage-activated, Ca²⁺ insensitive
- **voltage-activated Ca²⁺ sensitive (SV, slow vacuolar, TPC1)**

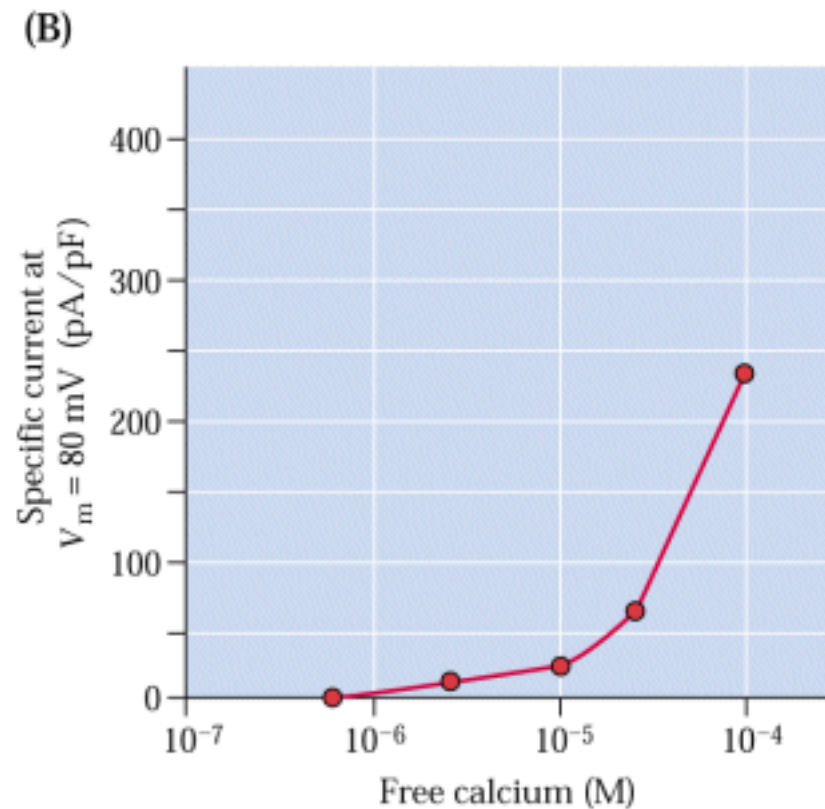
(A) The guard cell vacuolar membrane during ABA-induced stomatal closure



CICR – calcium induced calcium release

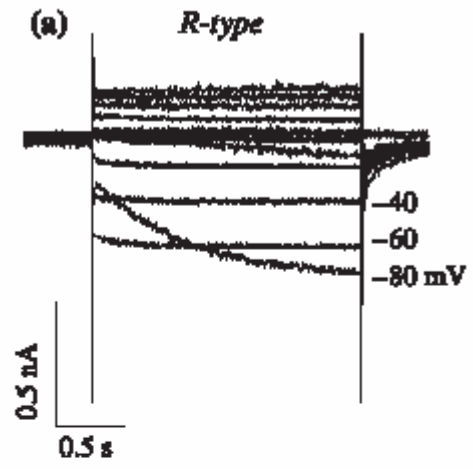


Ca²⁺ stimuluje SV
calcium channel

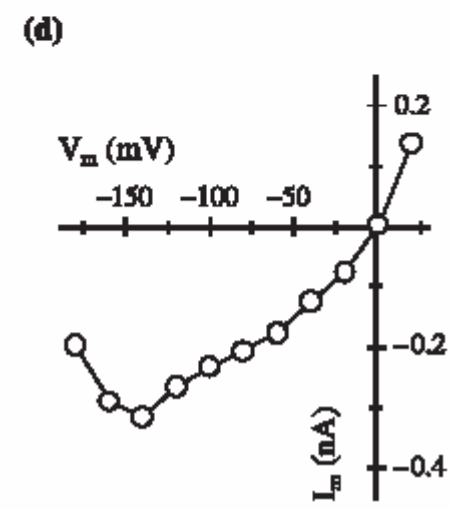
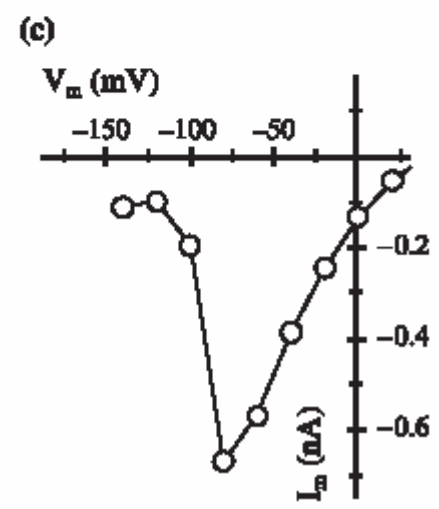
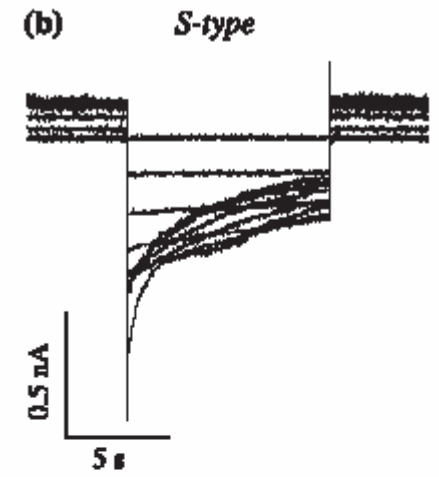


Aniontové kanály (Cl⁻) plasmalemmy (svěracích buněk)

rychlý (R)



pomalý (S)



aktivace Ca²⁺!

Tonoplastové kanály pro malát (VMAL)

Rostlinná specialita!

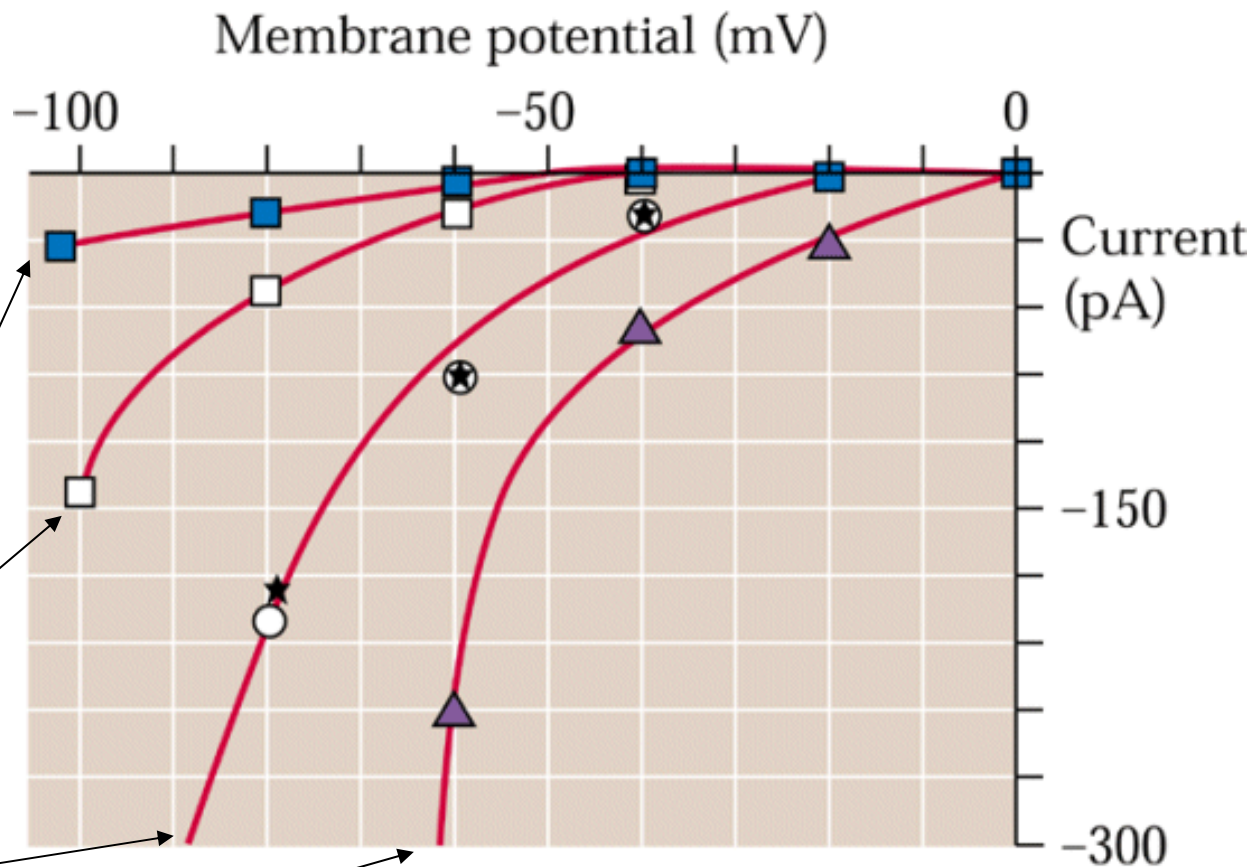
CAM rostliny! (Kalanchoe)

malát 10 mM

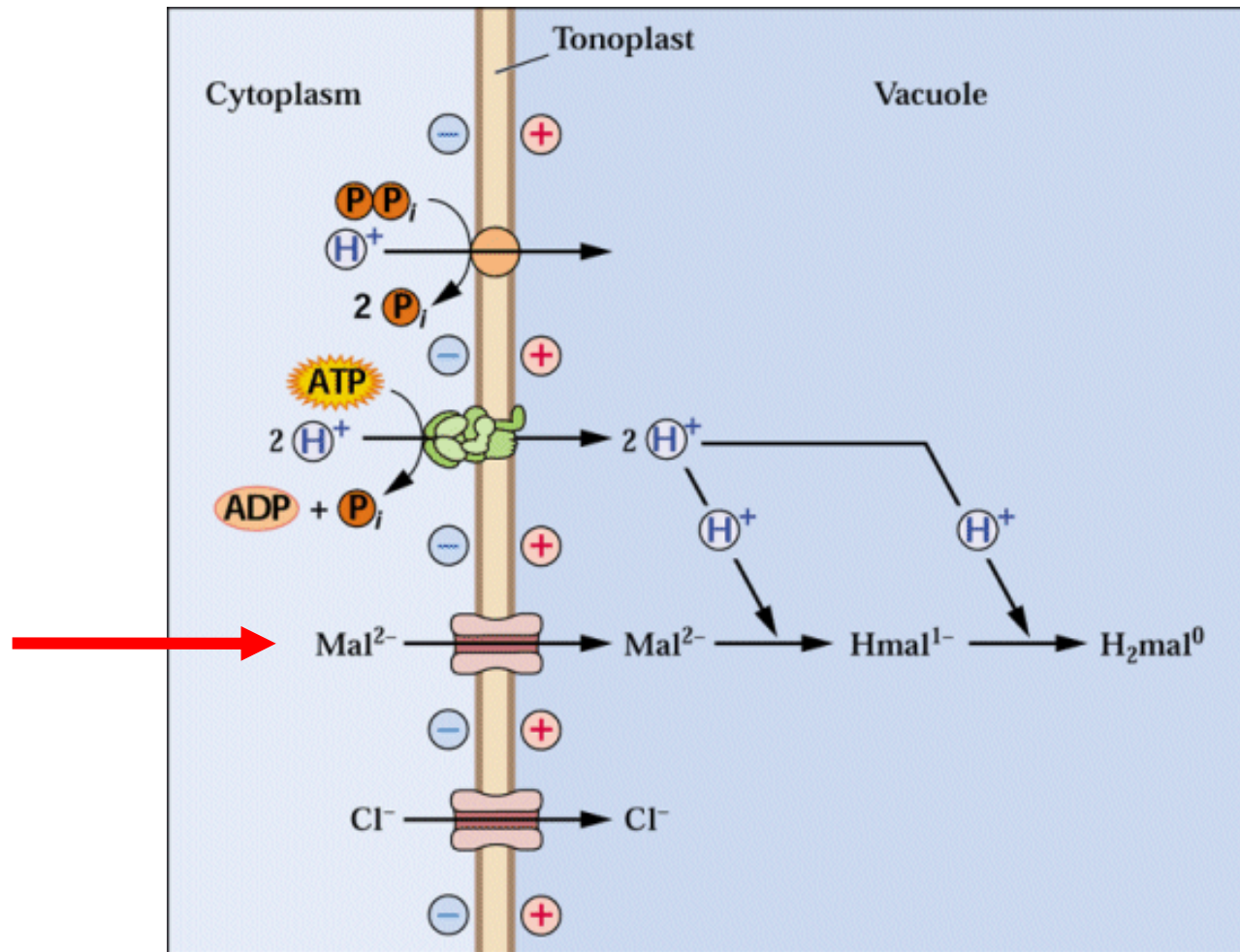
20 mM

50 mM

100 mM



Tonoplastové kanály pro malát (VMAL)

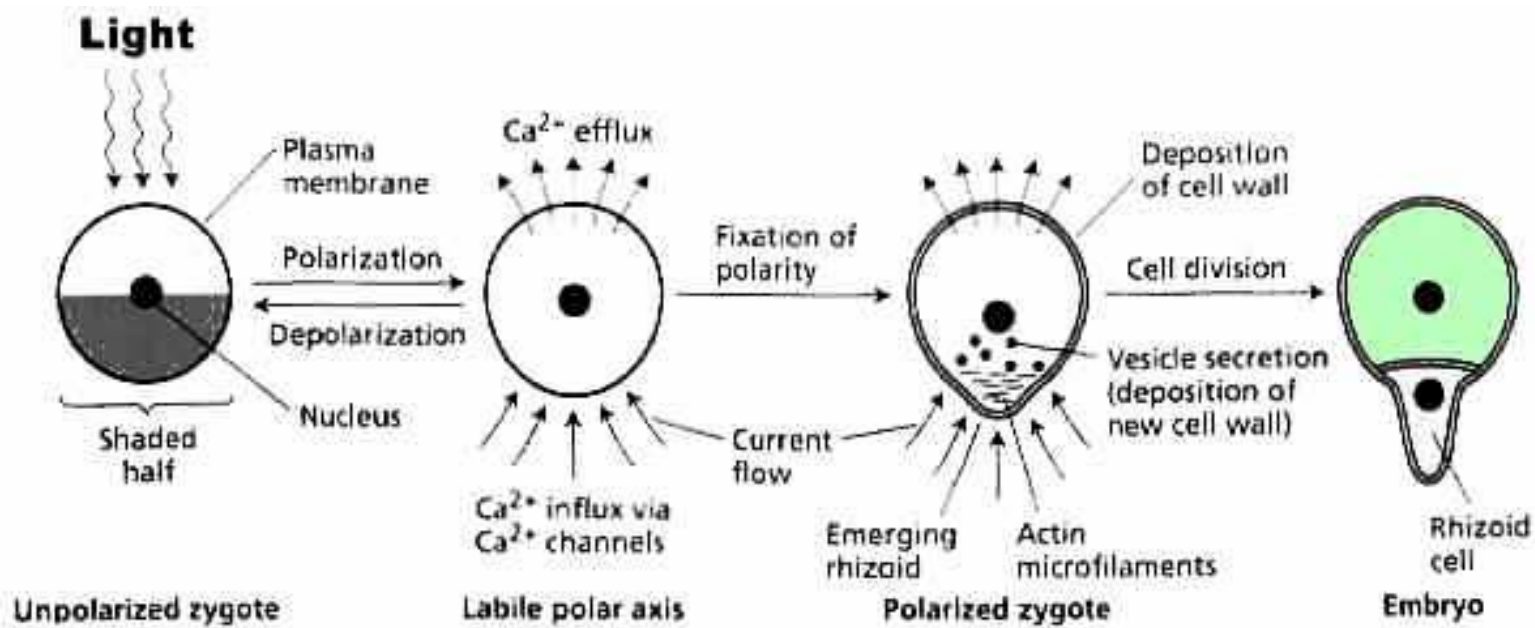


(pohon H⁺ gradientem)

Kanály v regulaci vývojových dějů

Příklad: vrcholový růst

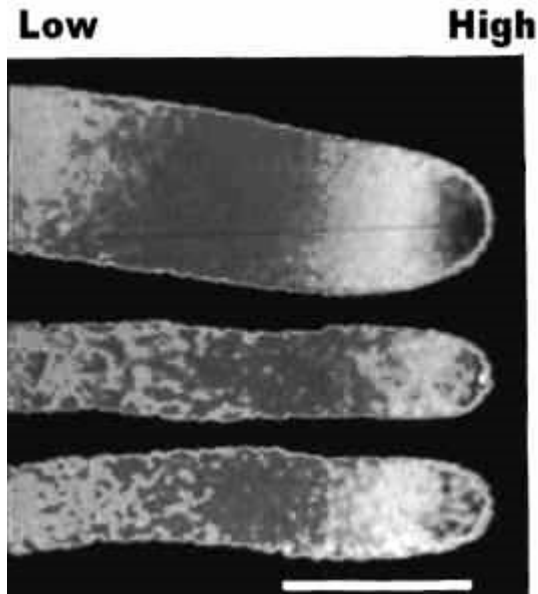
Zygota **Fucus**



- relokalizace Ca^{2+} kanálů jako 1. krok

Gradients Ca^{2+} a pH při vrcholovém růstu

Ca Concentration

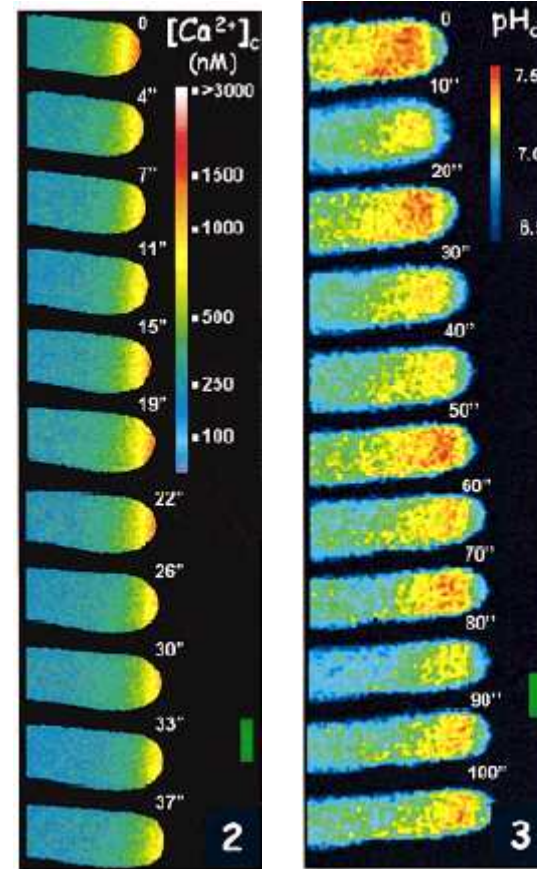


100 nM Ca

450 nM Ca

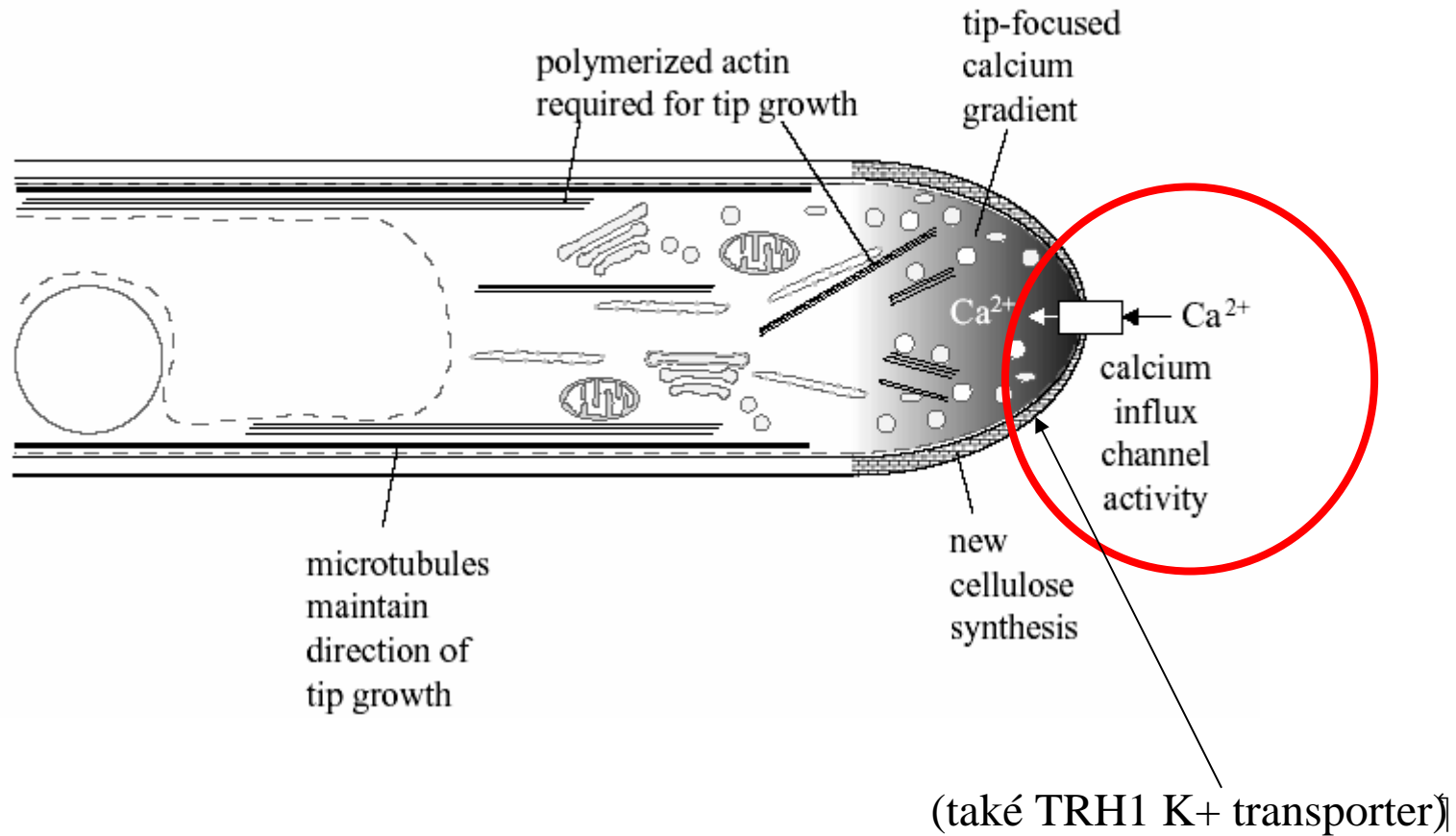
Fucus rhizoid

... dtto kořenné vlásky ...



pylová láčka (Feijo et al.)

Obecný rys vrcholového růstu?

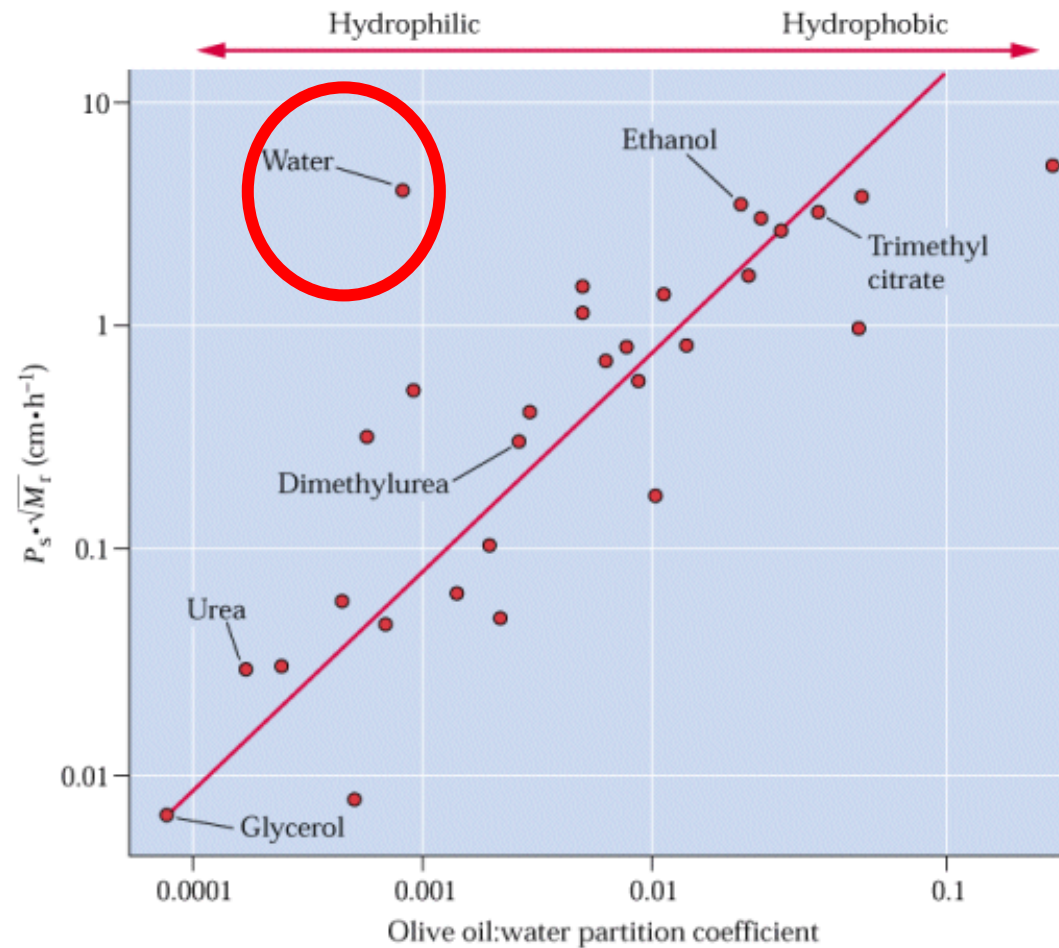


... dále sekreční dráha a cytoskelet ...

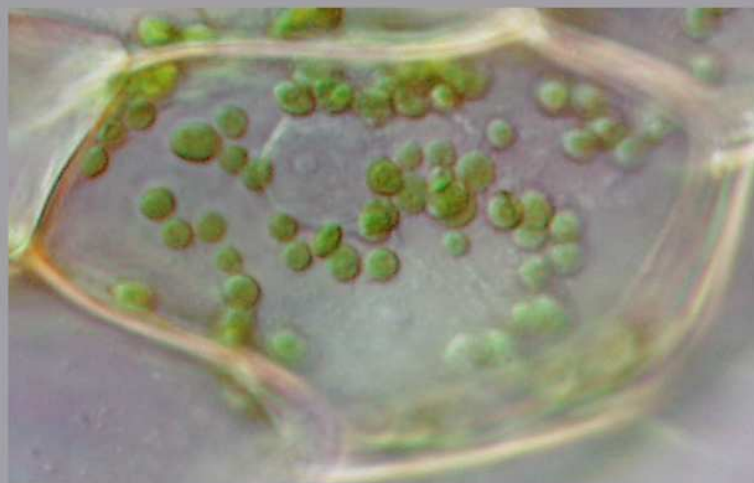
Osnova

- Základní vlastnosti biologických membrán
- Transport látek přes membránu
 - úvod
 - pumpy
 - přenašeče
 - kanály
 - **transport vody**
- Membránový transport v praxi – regulace otevírání a zavírání svěracích buněk

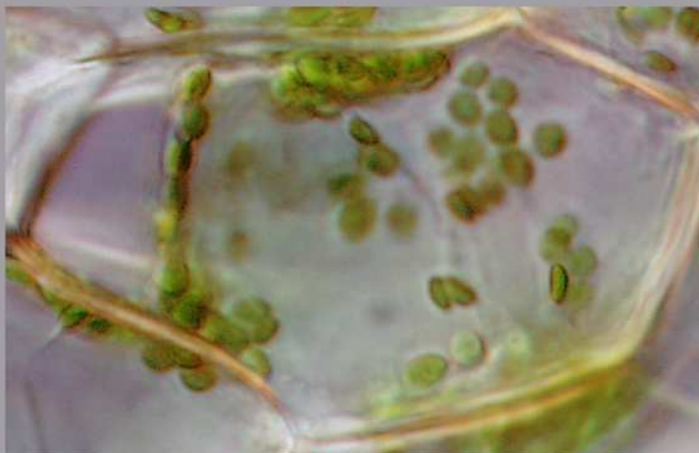
Transport vody přes membrány: aquaporiny



... ale musí to jít rychle!



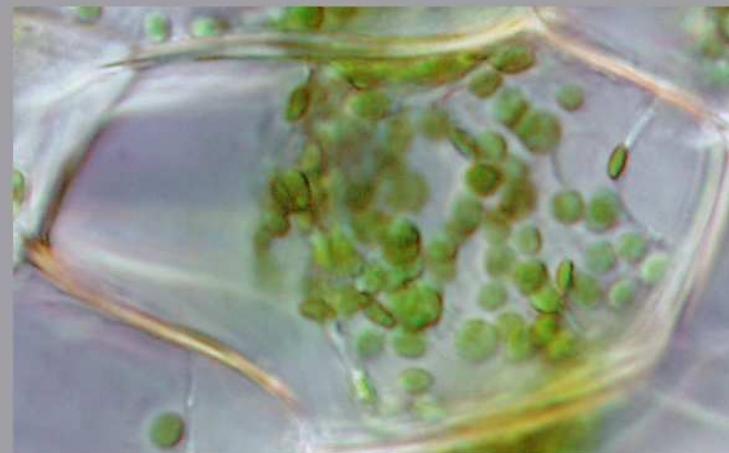
20% Sucrose Added



2 minutes

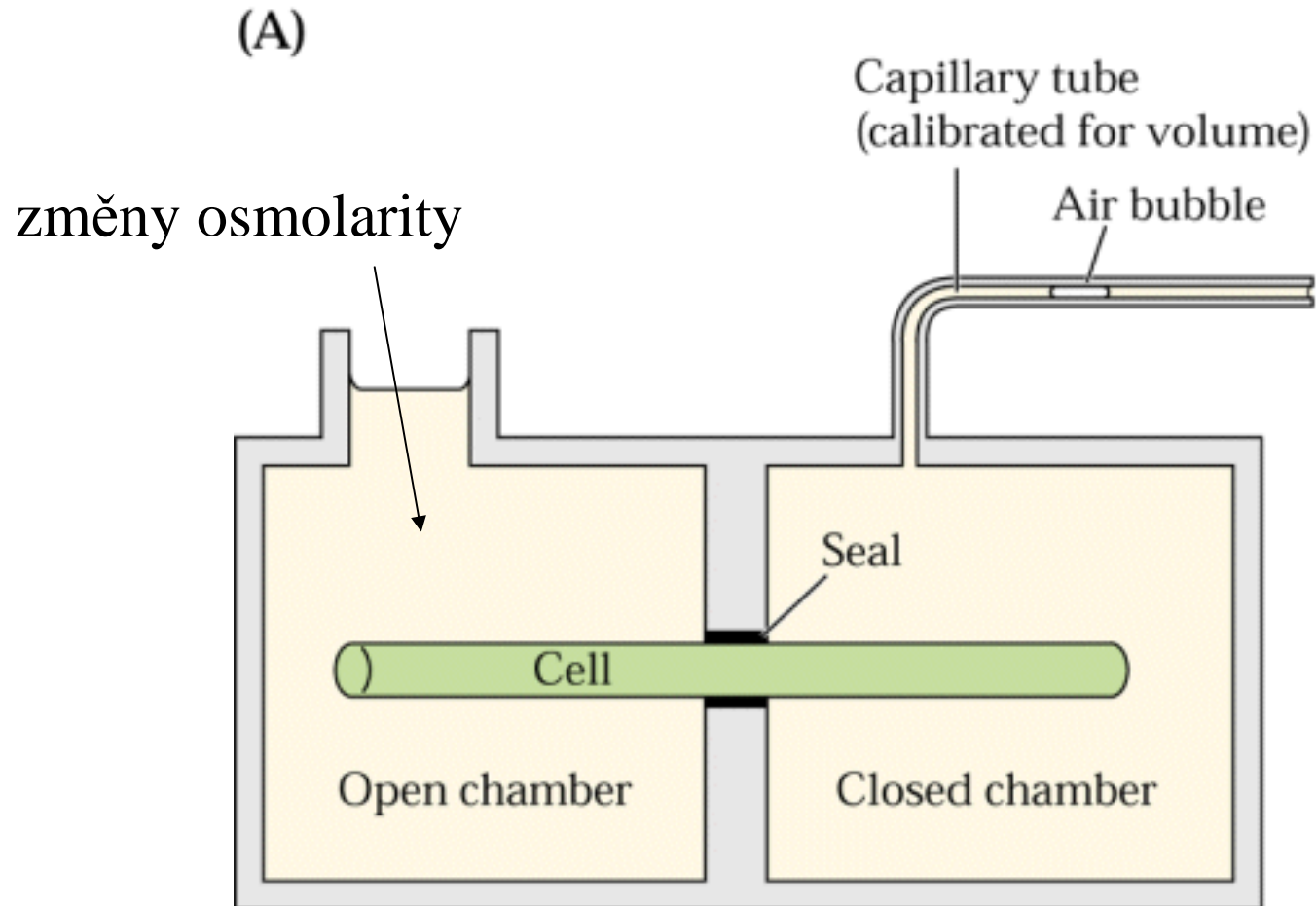


Distilled Water Added

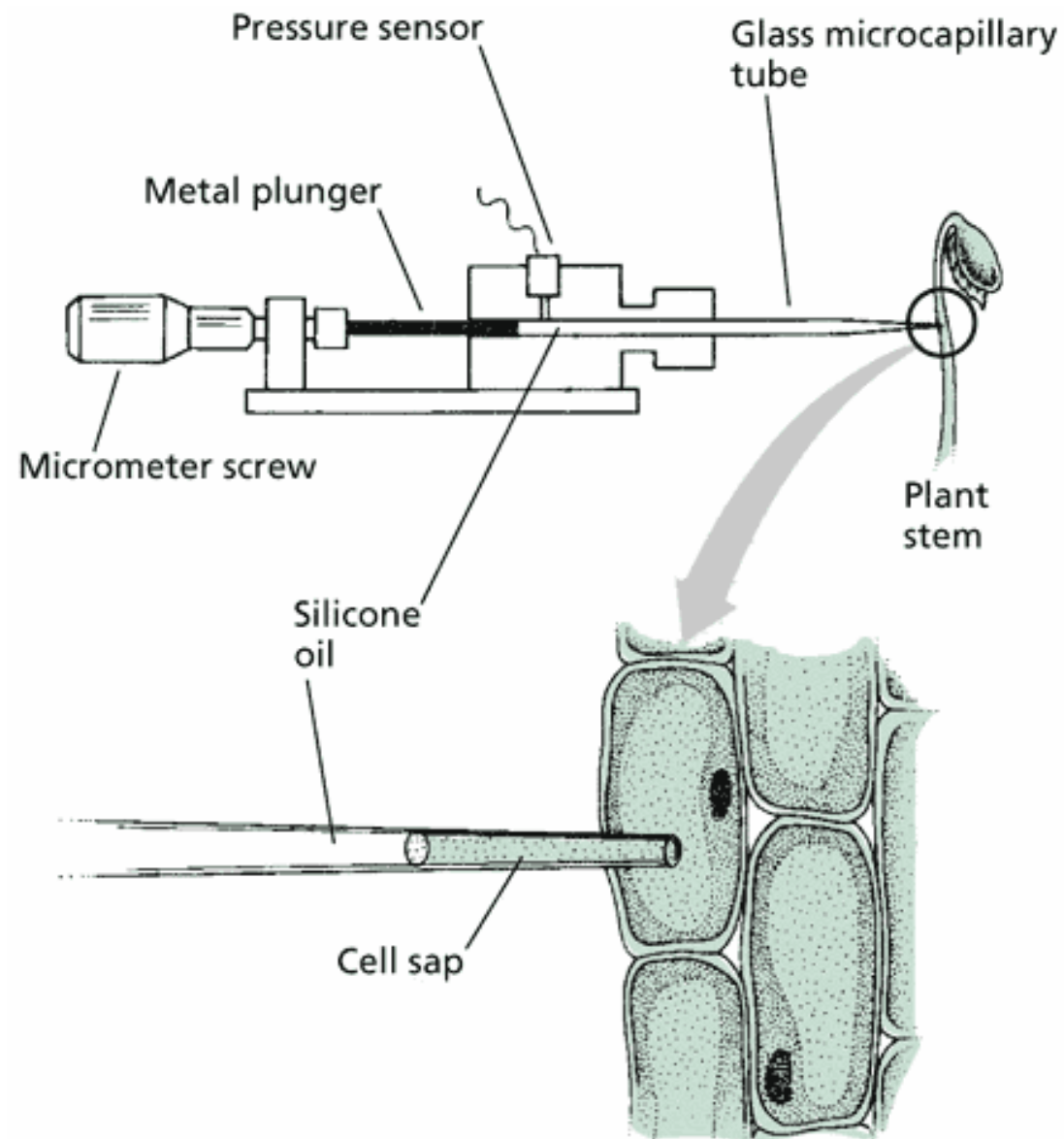


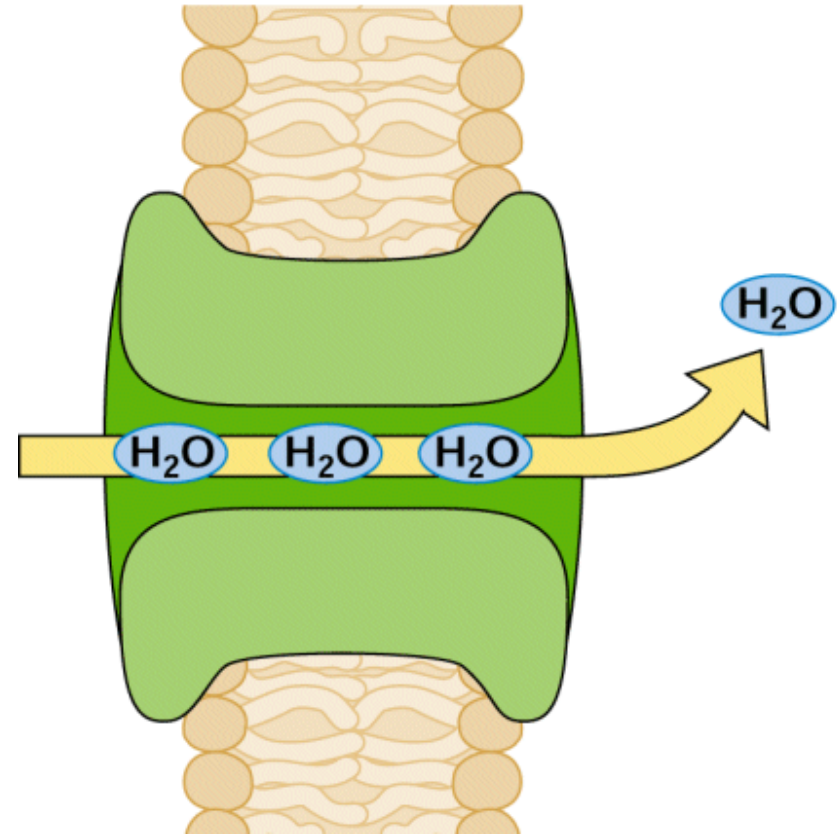
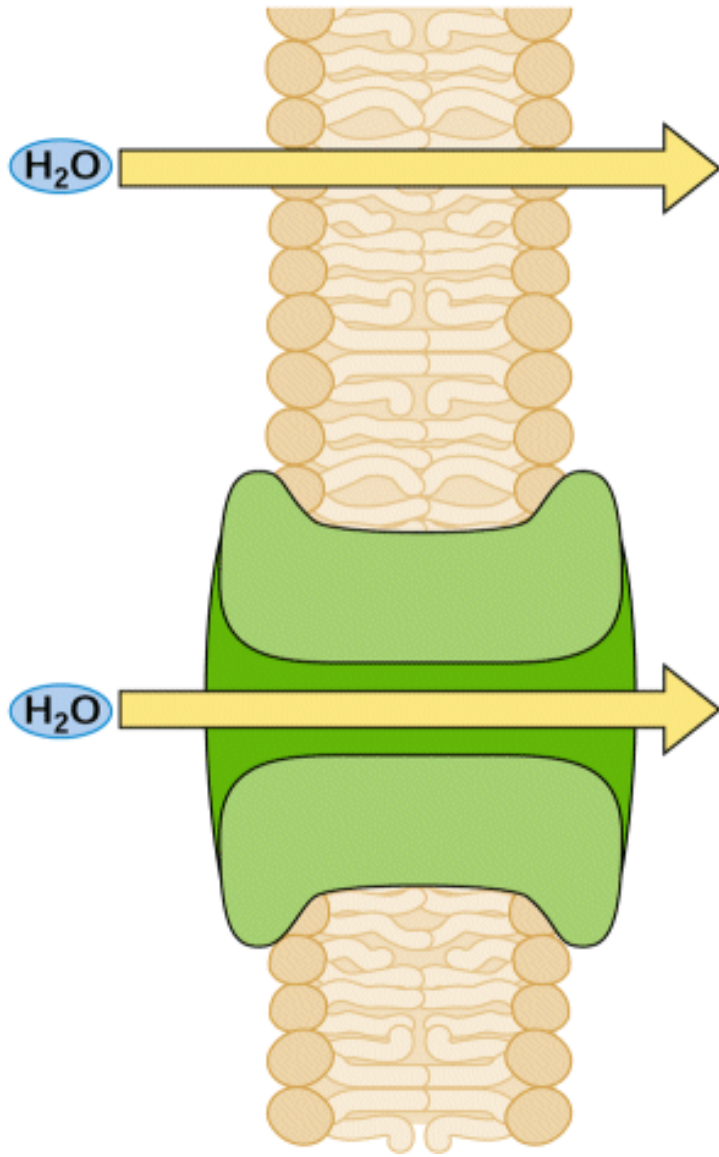
5 minutes

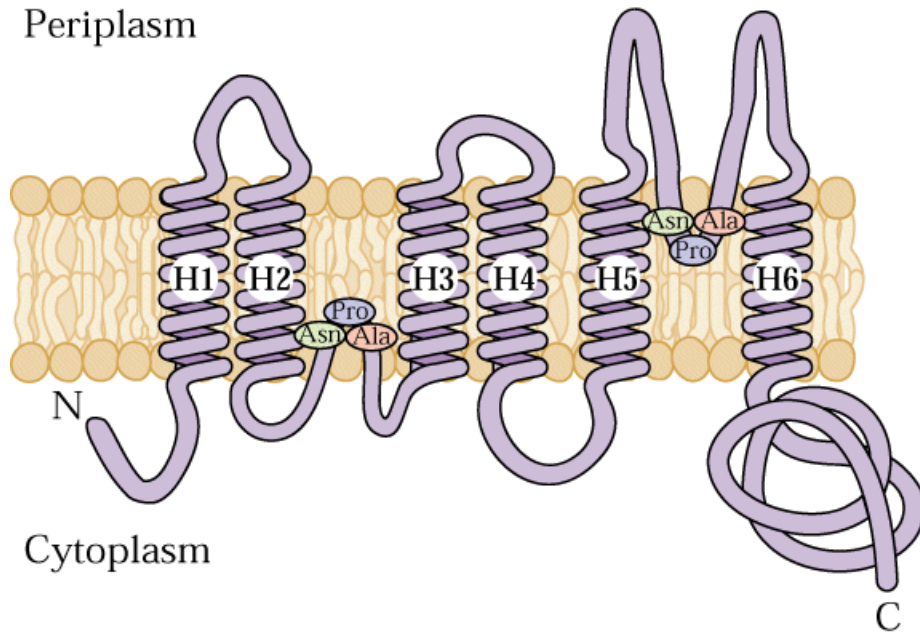
Měření transportu vody



Jak měřit turgor?



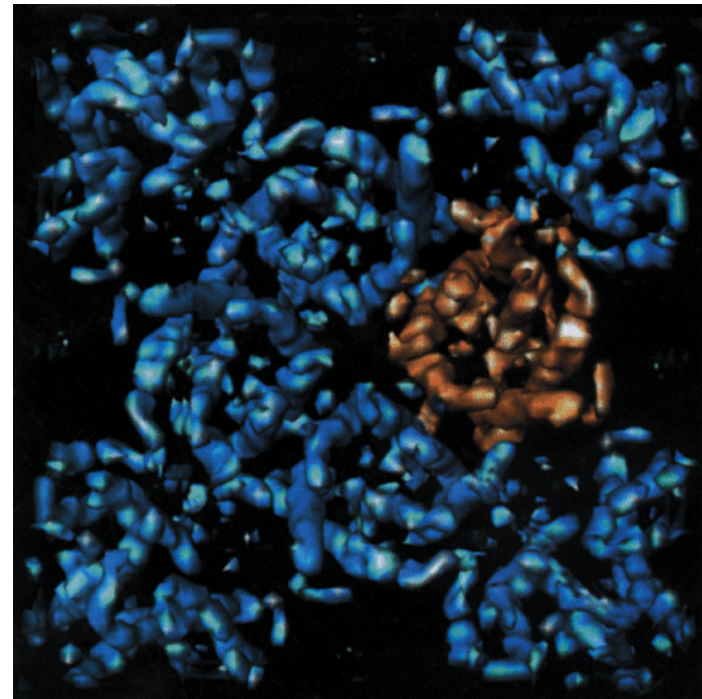




Tonoplast **I**ntrinsic **P**roteins

různé TIPs v různých typech vakuol!

Arabidopsis: 35 genů, 10 prot. ve vakuole, kolik kombinací?



K čemu jsou akvaporiny?

- SPH – „simple permeability hypothesis“
 - regulace buněčného objemu (jsou nutné?)
 - homeostáze objemu cytoplasmy (model??)
 - zprostředkování pohybu vody ... radiálně kořenem (ALE antisense PIP mutanti?)
- Vnímání a regulace osmotického (turgorového) tlaku
- TIPs přenášejí i NH₃!

(Hill et al. 2004, Martinoia et al. 2007)

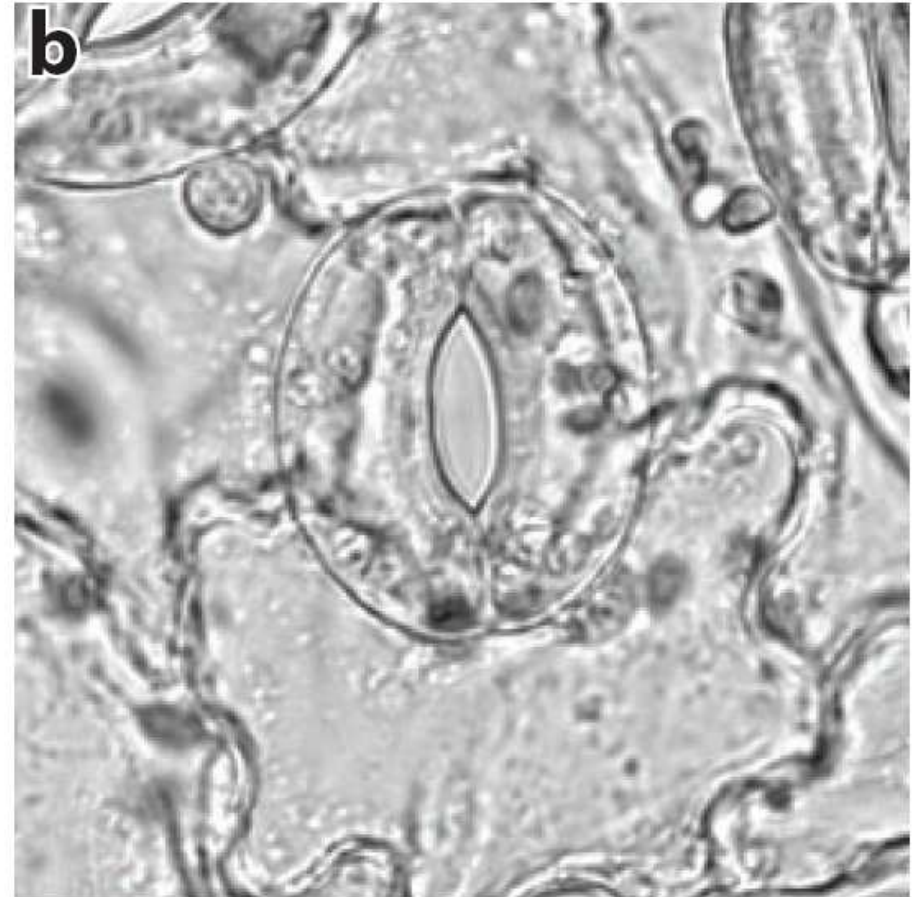
Osnova

- Základní vlastnosti biologických membrán
- Transport látek přes membránu
 - úvod
 - pumpy
 - přenašeče
 - kanály
 - transport vody
- **Membránový transport v praxi – regulace otevírání a zavírání svěřacích buněk**

Stomatologie



Průduchy: integrace transportních systémů



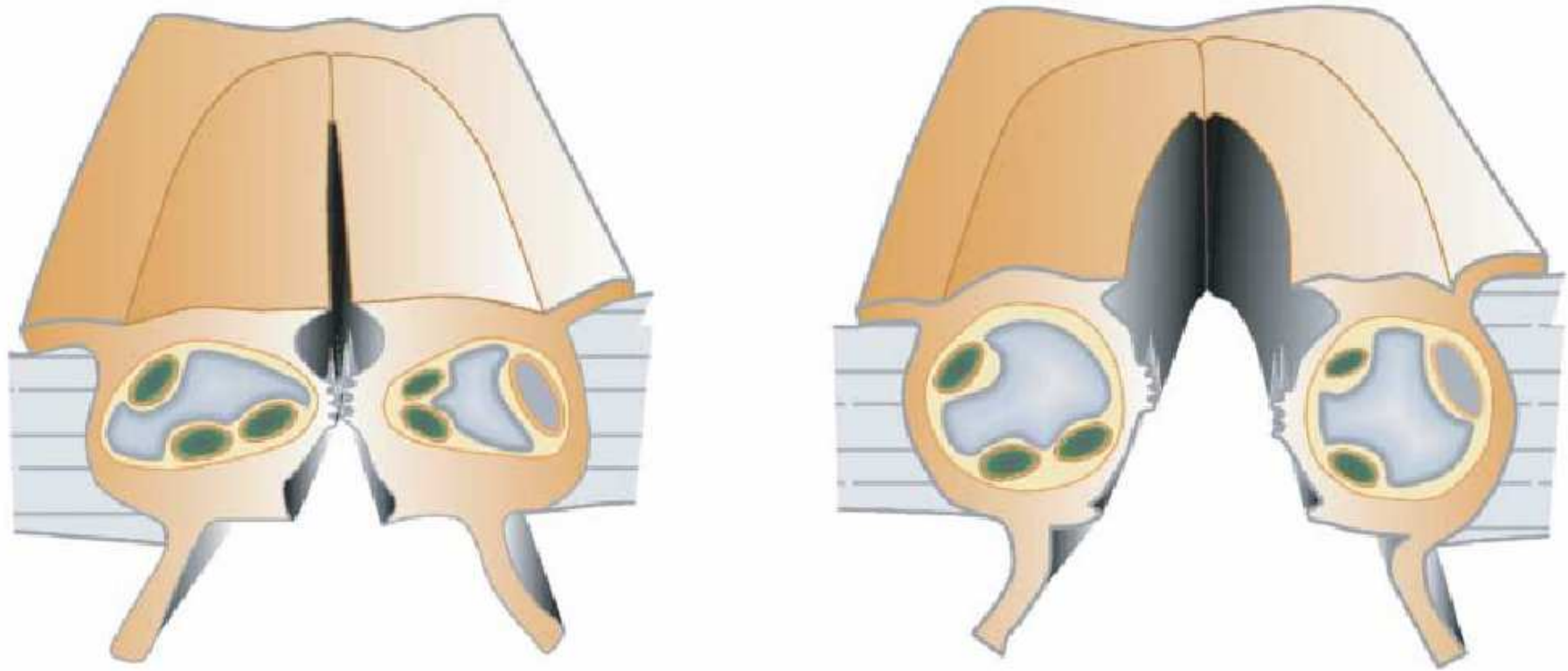
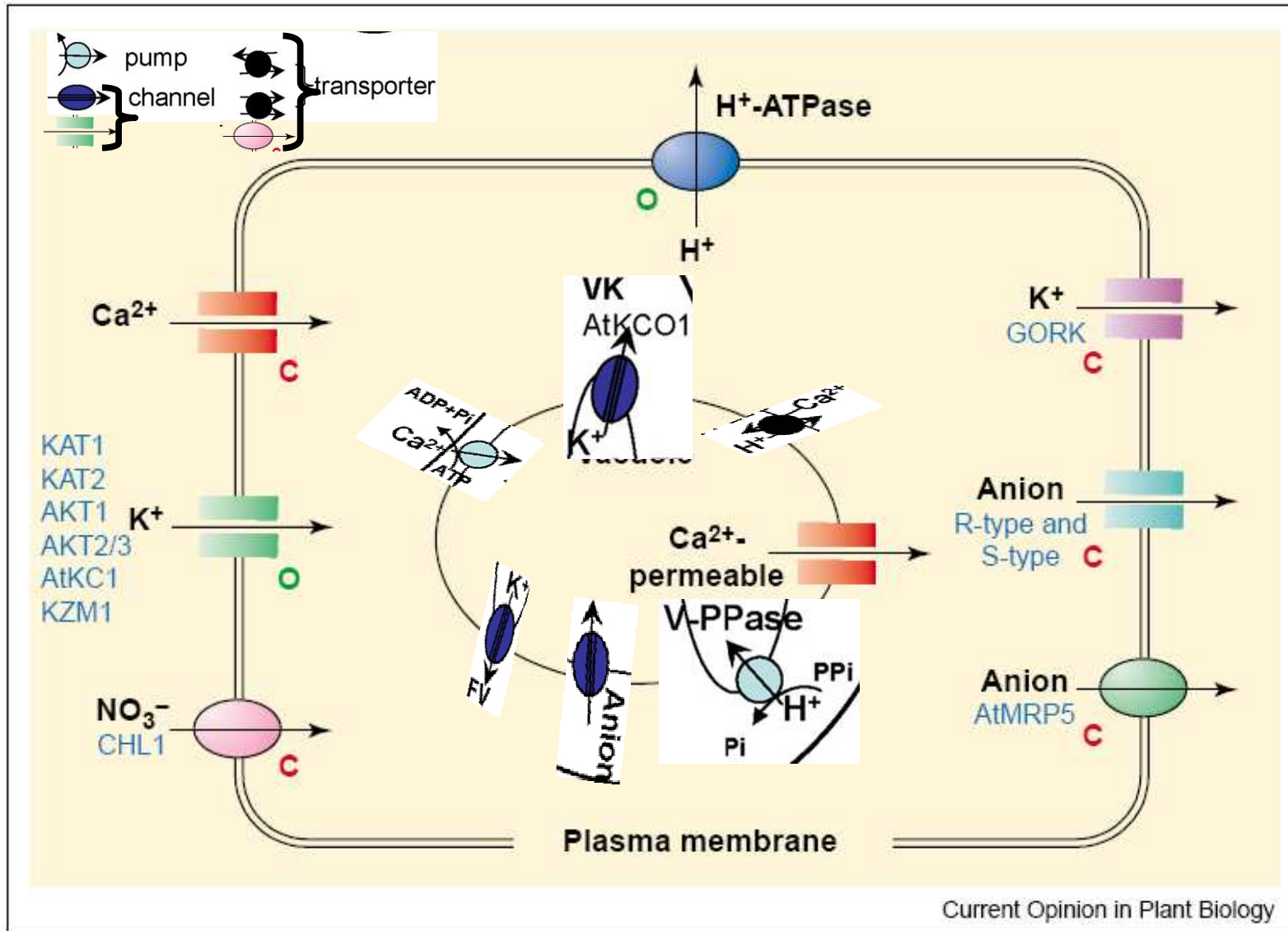
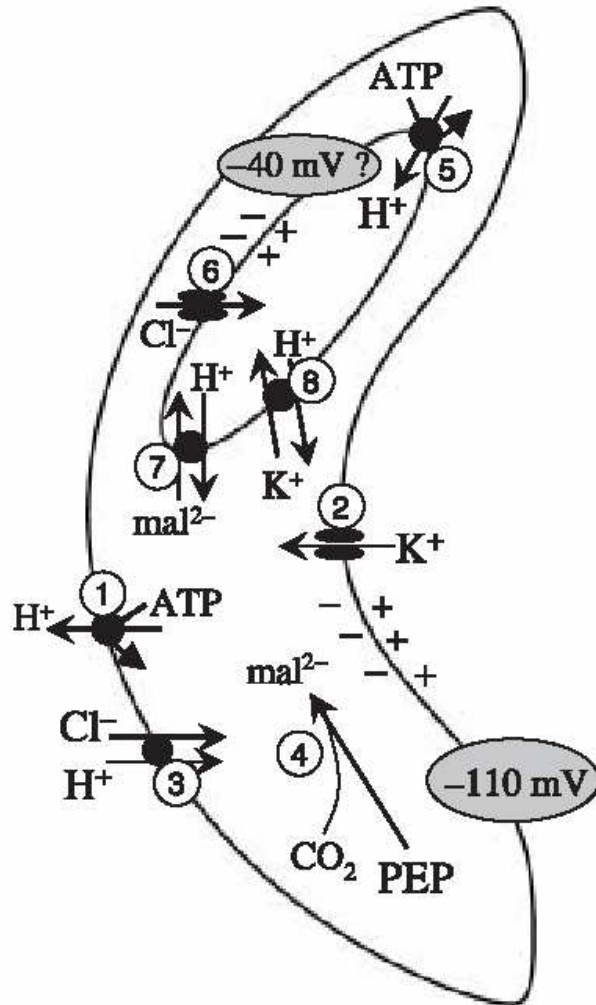


Fig. 1 Changes in the shape of guard cells during stomatal movements. During stomatal opening the guard cell volume increases, which causes them to bend and changes their cross section from an flat oval (right drawing), to a circle (left drawing). Redrawn after Raschke & Dickerson (1972) and Wanner (2004).

Přenašeče, pumpy a kanály - Integrace



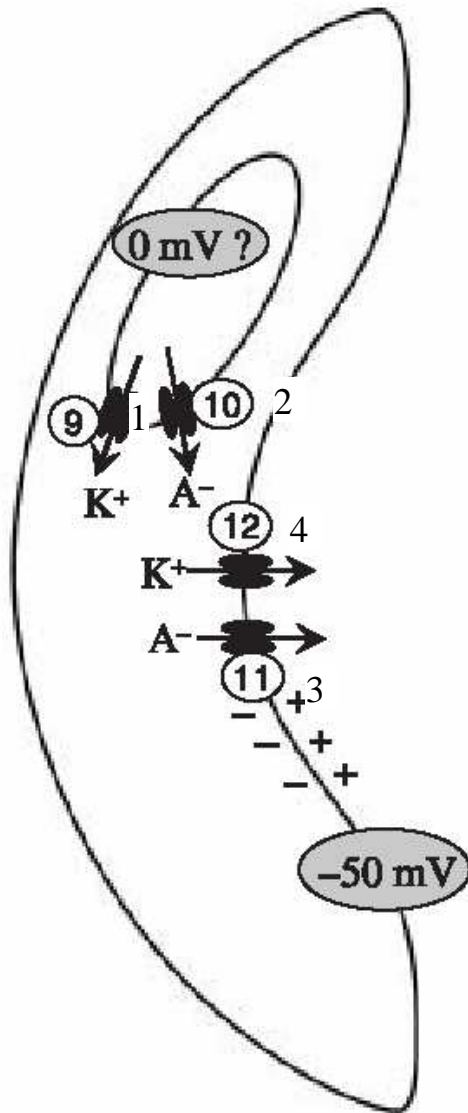
Otvírání



- H⁺ ATPasy (**p**) – pmf
- hyperpolarizace – K⁺ uptake přes inward rectifier (**k**)
- Cl⁻ uptake (asi symportem s H⁺) (**t**) a NO₃⁻ uptake (symport s H⁺) (**t**)
- produkce malátu (fotosyntesa)
- pmf na tonoplastu (H⁺ ATPasa, Ppasa **p**)
- Cl⁻ do vakuoly po spádu (**k**)
- malát do vakuoly (**k**, **t**)
- H⁺/K⁺ antiport (**t**)
- voda jde za solemi do vakuoly → zvýšení objemu (vakuola) → OTEVŘENÍ

pumpa, **tranporter**, **kanál**

Zavírání

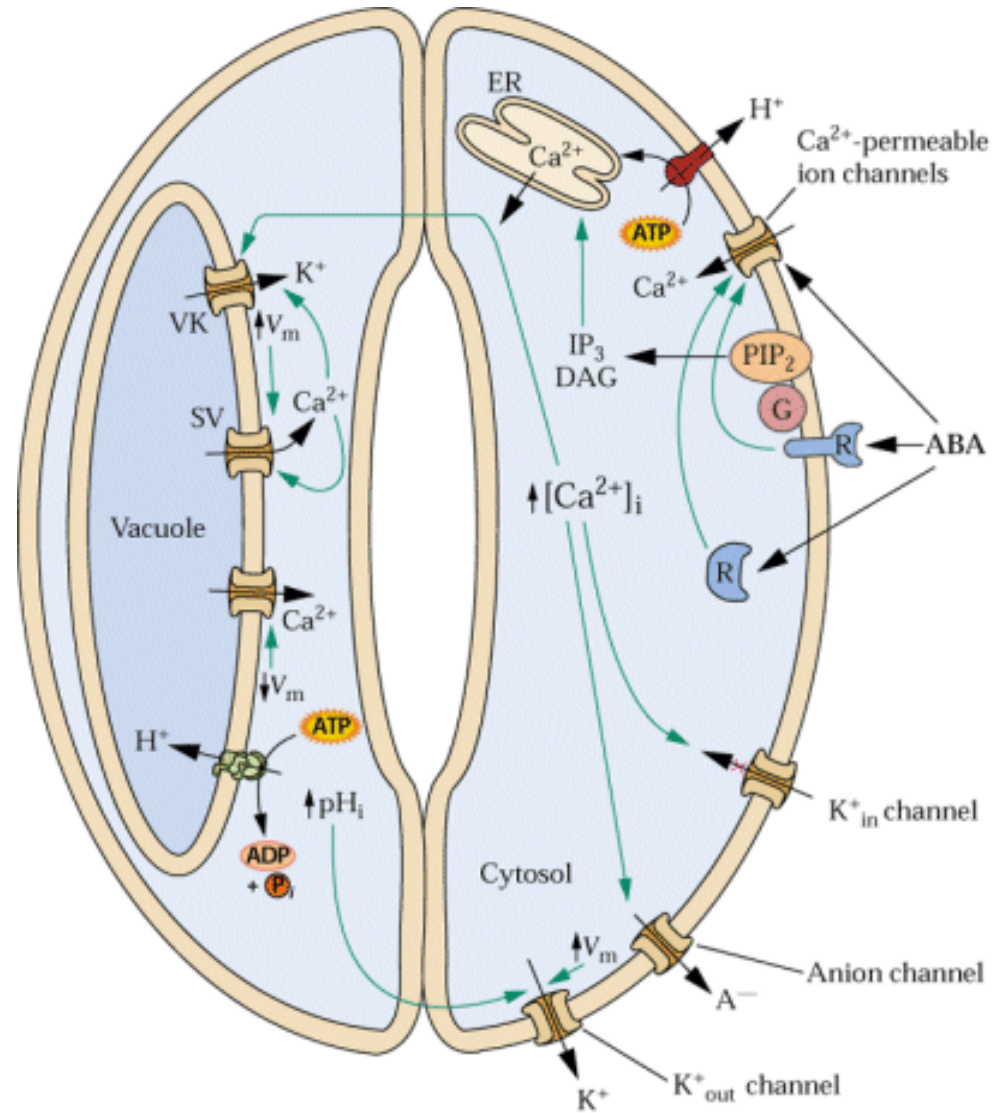


- výtok K^+ z vakuoly po spádu a depolarizace (**k**)
- výtok Cl^- po spádu (**k, t**)
- otevření aniont. kanálů depolarizuje plasmalemu (**k**)
- aktivace outward rectifying K^+ kanálu (**k**)
- voda následuje (vytéká ven) → snížení objemu → ZAVŘENÍ

pumpa, transporter, kanál

Signalizace a regulace

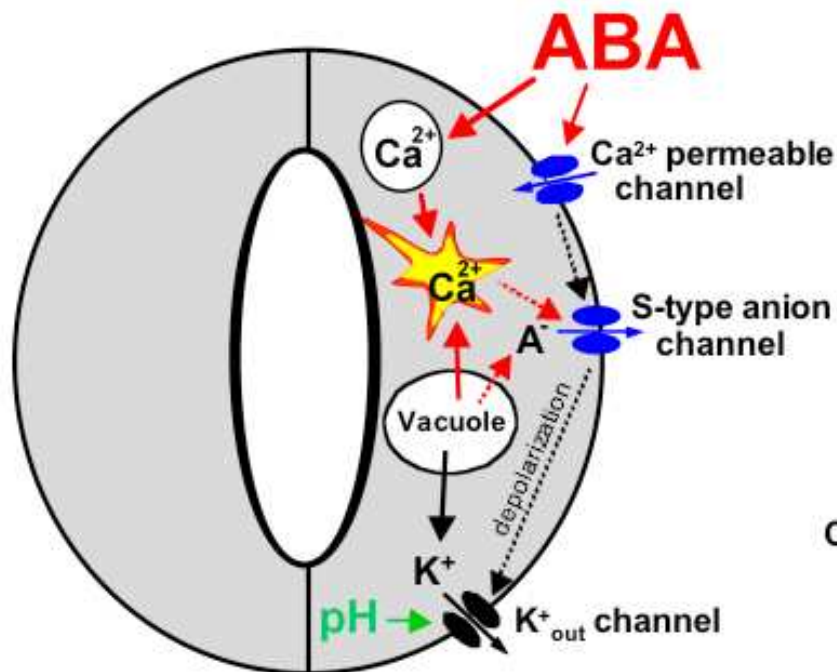
- ABA, modré světlo, CO_2
- zprostředkování změnami v pH a Ca^{2+}
- **Signální molekuly** - ROS, NO, sfingosin-1-fosfát a další fosfolipidy



The Clickable Guard Cell: Electronically linked Model of Guard Cell Signal Transduction Pathways

<http://www-biology.ucsd.edu/labs/schroeder/clickablegc.html>

ABA mediates stomatal closing



ABA inhibits stomatal opening

