Fyziologie živočichů a člověka

Fyziologie buňky

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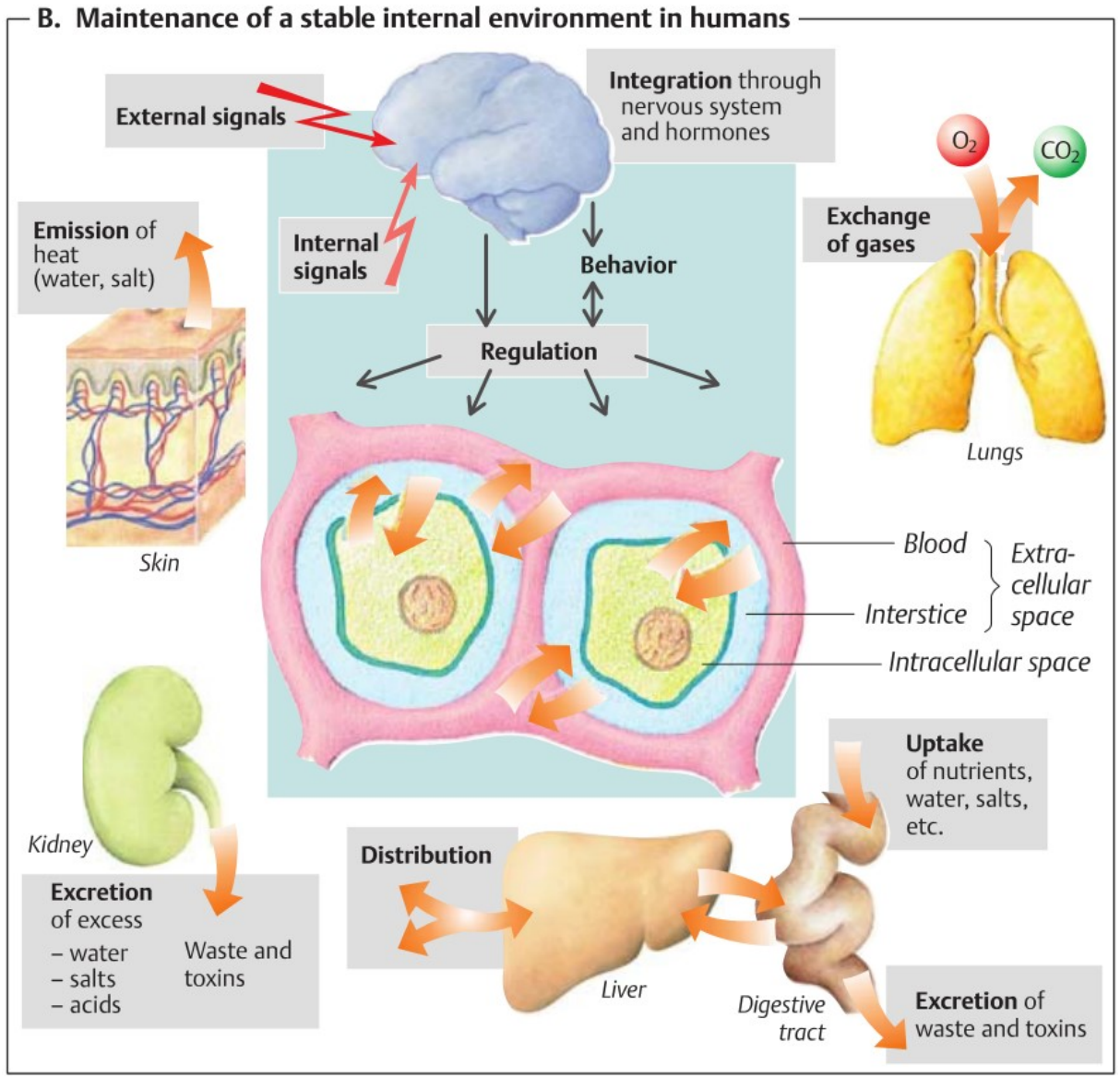
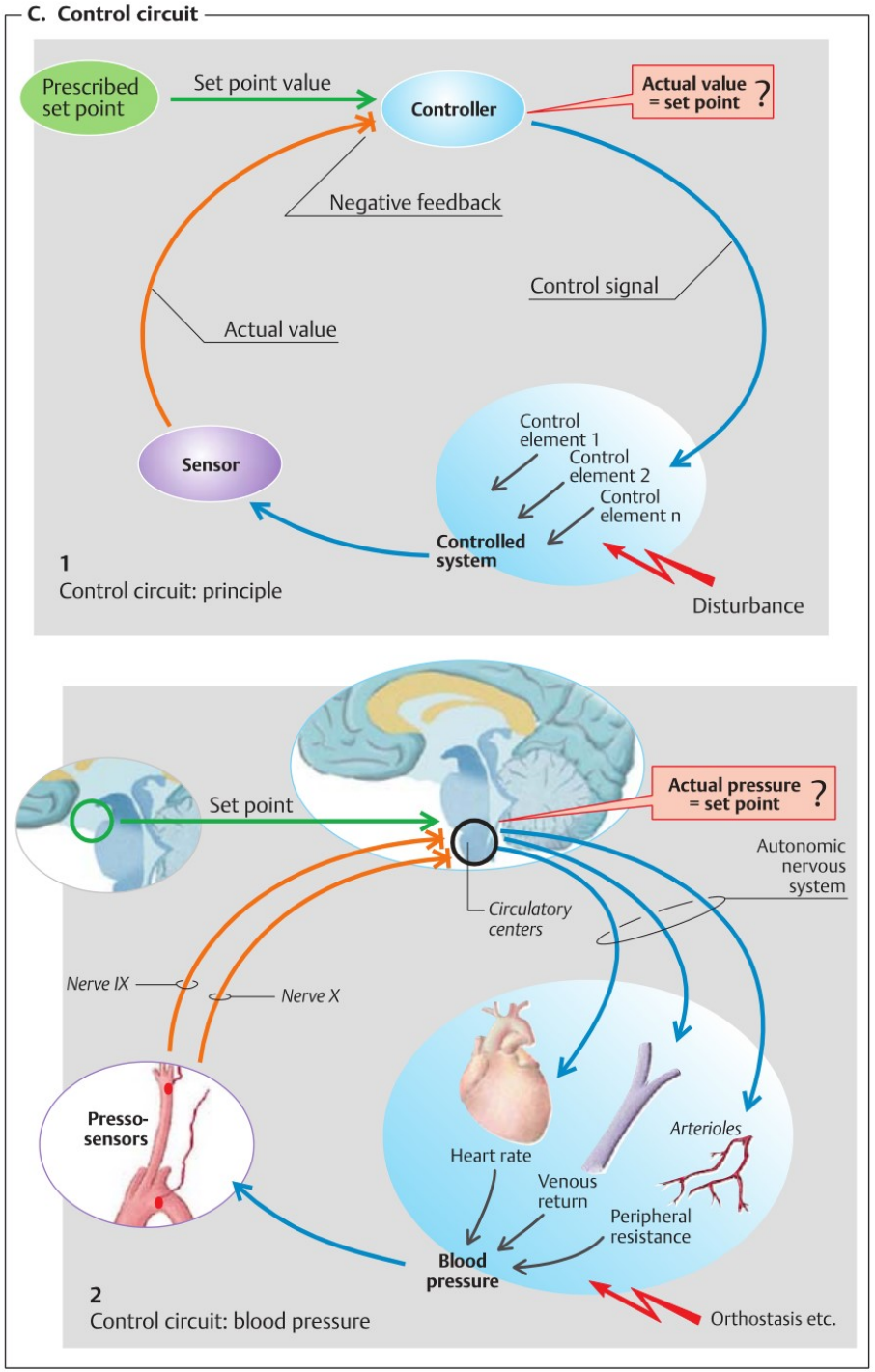
Intro

- Fyziologie – spojení struktury, funkce a regulace
- Není problém vyvinout si orgán/protein/buněčnou strukturu

Základní fyziologické pochody u člověka

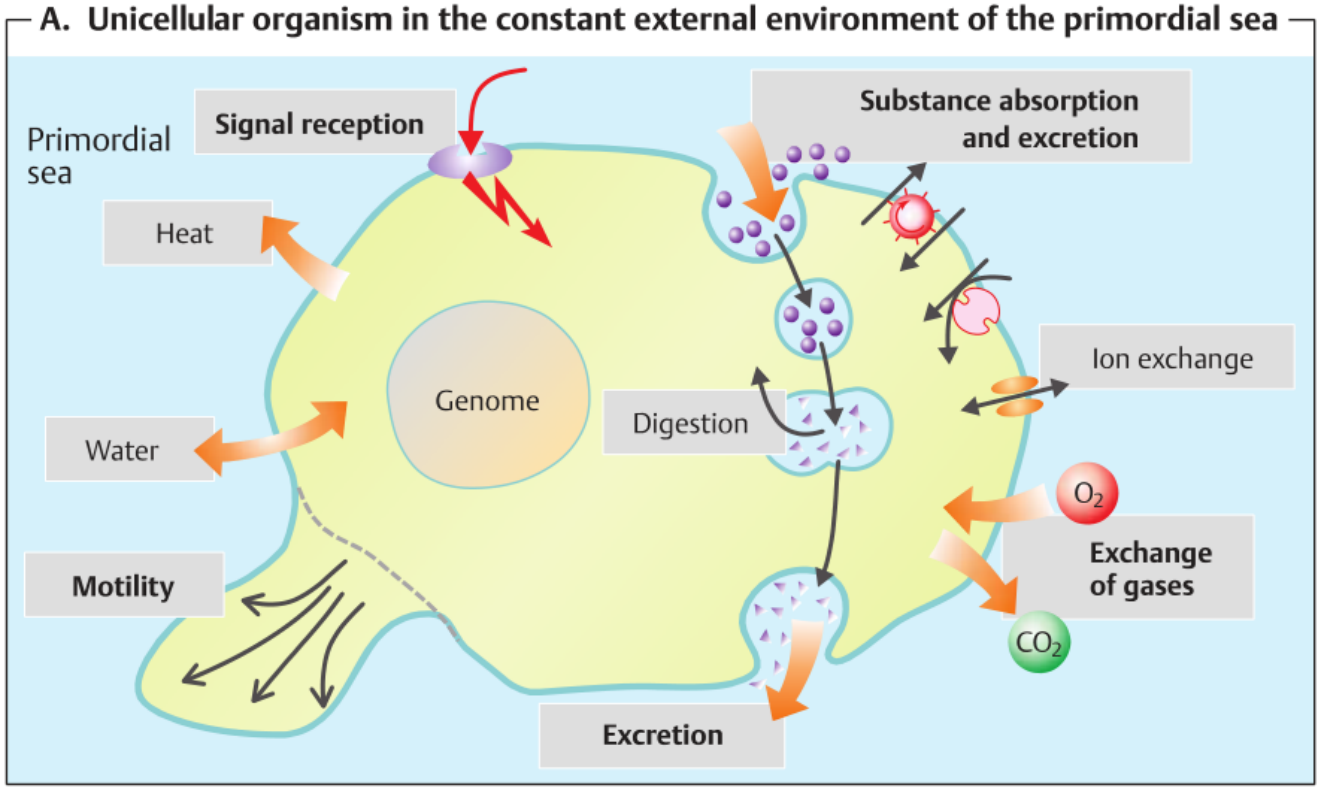




Regulační okruhy

- Controller hlídá a nastavuje nějaký systém, a sám je jeho činností regulován.
- **<u>Pozitivní zpětná vazba</u>**: systém↑ - controller↑
- **<u>Negativní zpětná vazba</u>**: systém↑ - controller↓

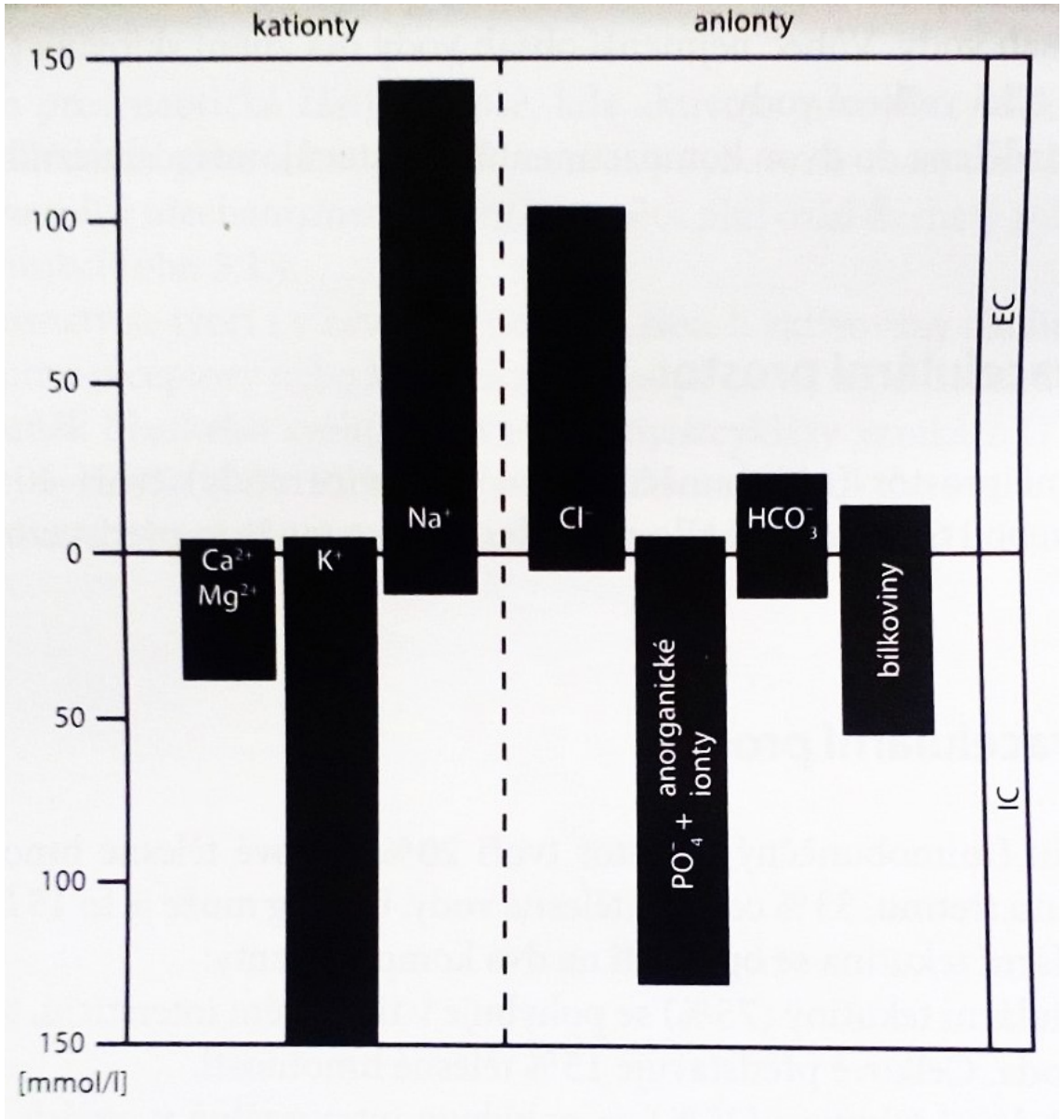
Buňka – základní životní pochody



- Izolace od okolí
- ALE(!) nutnost komunikace („otevřený systém“)

- Extracelulární prostor (měnící se)
- Intracelulární prostor (stabilní)
- Homeostáze

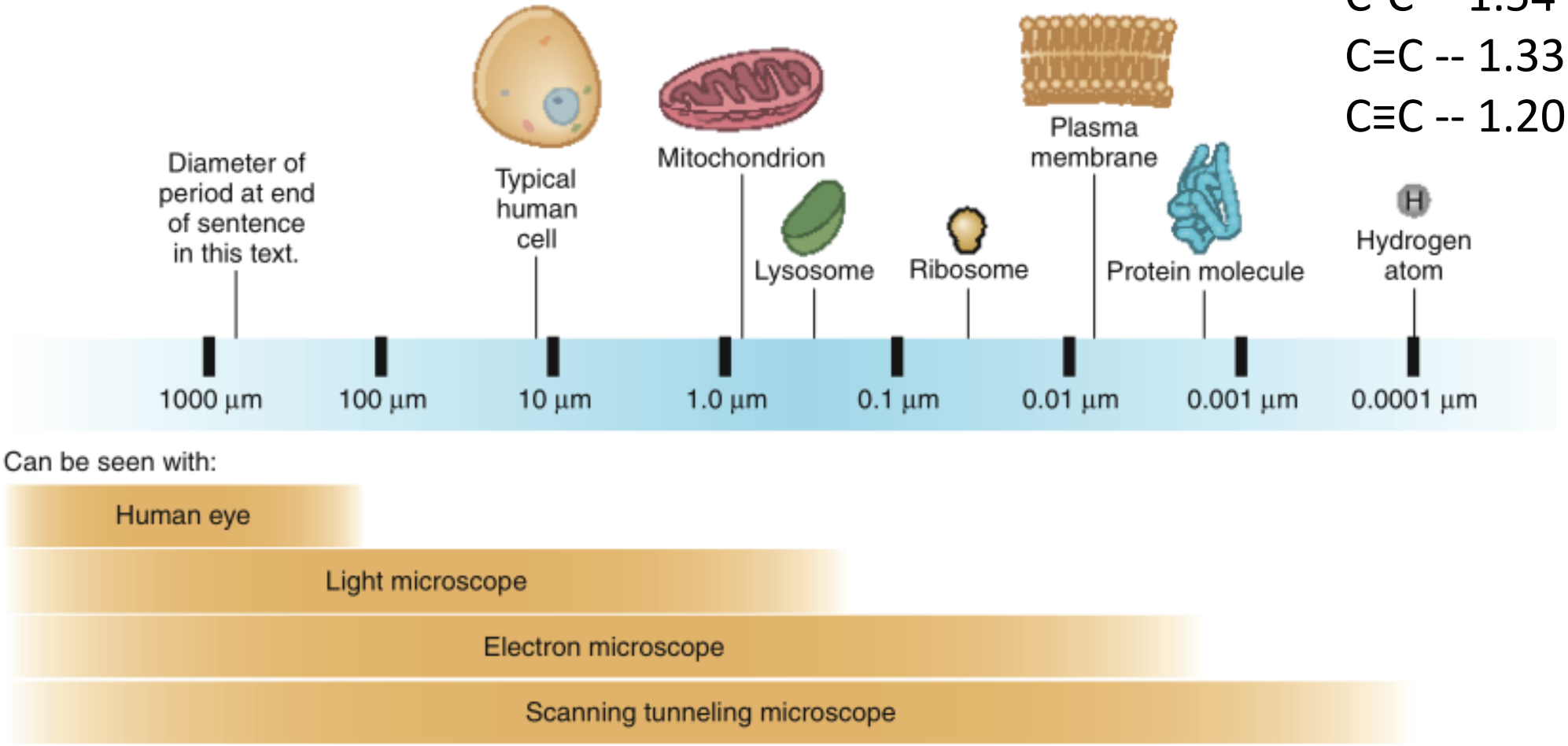
Ionty v buňce a EC prostoru



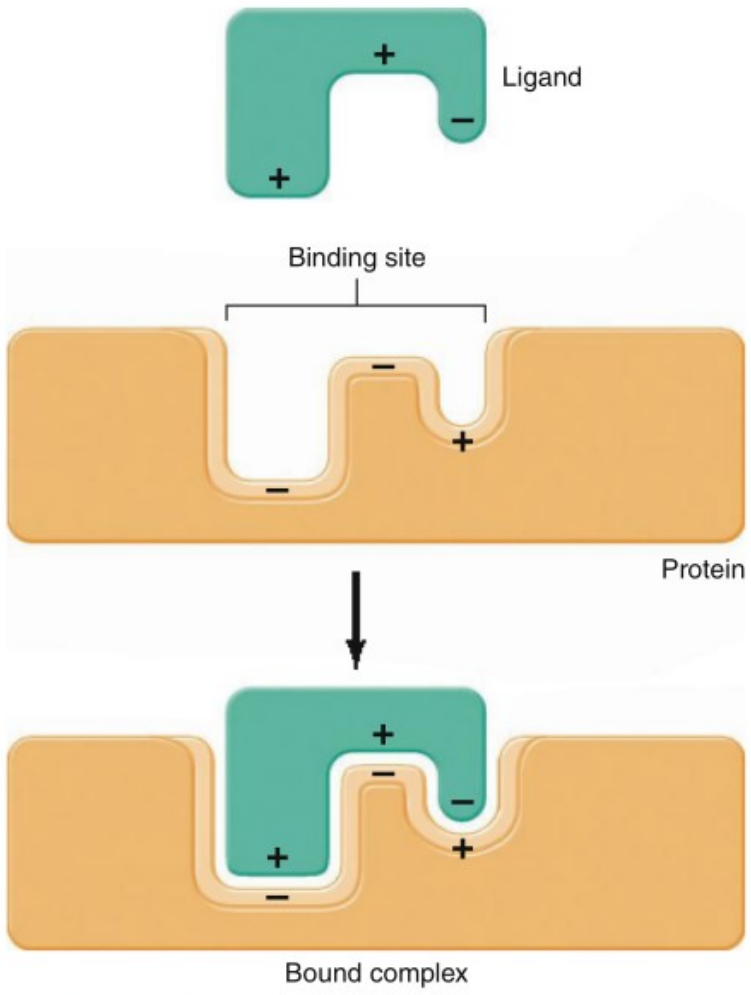
- Na^+ hlavní extracelulární kationt
- K^+ hlavní intracelulární kationt
- Intracelulárně více proteinů + fosforečnanů – výsledný IC náboj ***negativní***

Buňka – srovnání velikosti struktur

1 Å = 10^{-10} m = 0.1 nm
C-C -- 1.54 Å
C=C -- 1.33 Å
C≡C -- 1.20 Å



Interakce ligand – protein



AP|R **Figure 3.26** The complementary shapes of ligand and the protein-binding site determine the chemical specificity of binding.

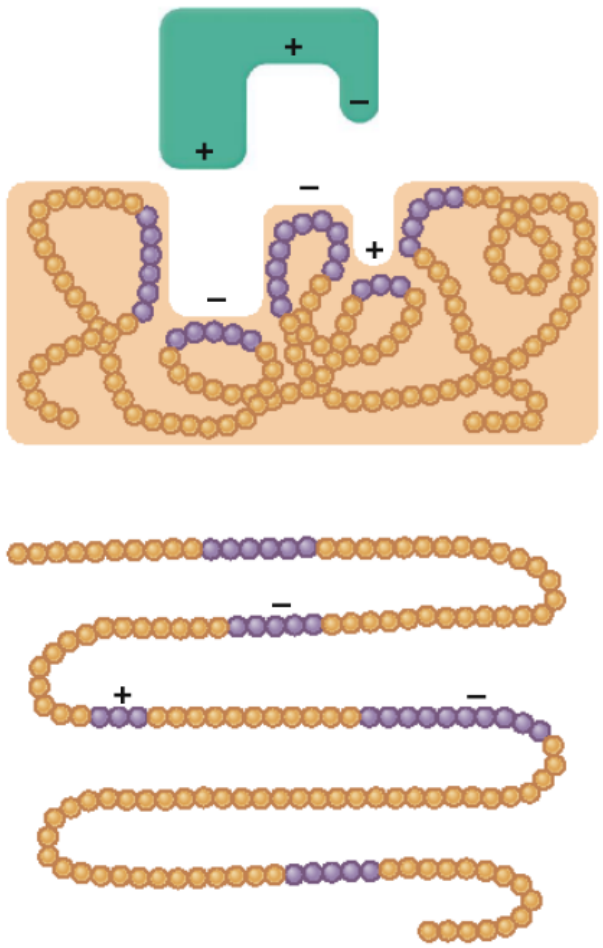


Figure 3.27 Amino acids that interact with the ligand at a binding site need not be at adjacent sites along the polypeptide chain, as indicated in this model showing the three-dimensional folding of a protein. The unfolded polypeptide chain appears at the bottom.

Interakce ligand – protein

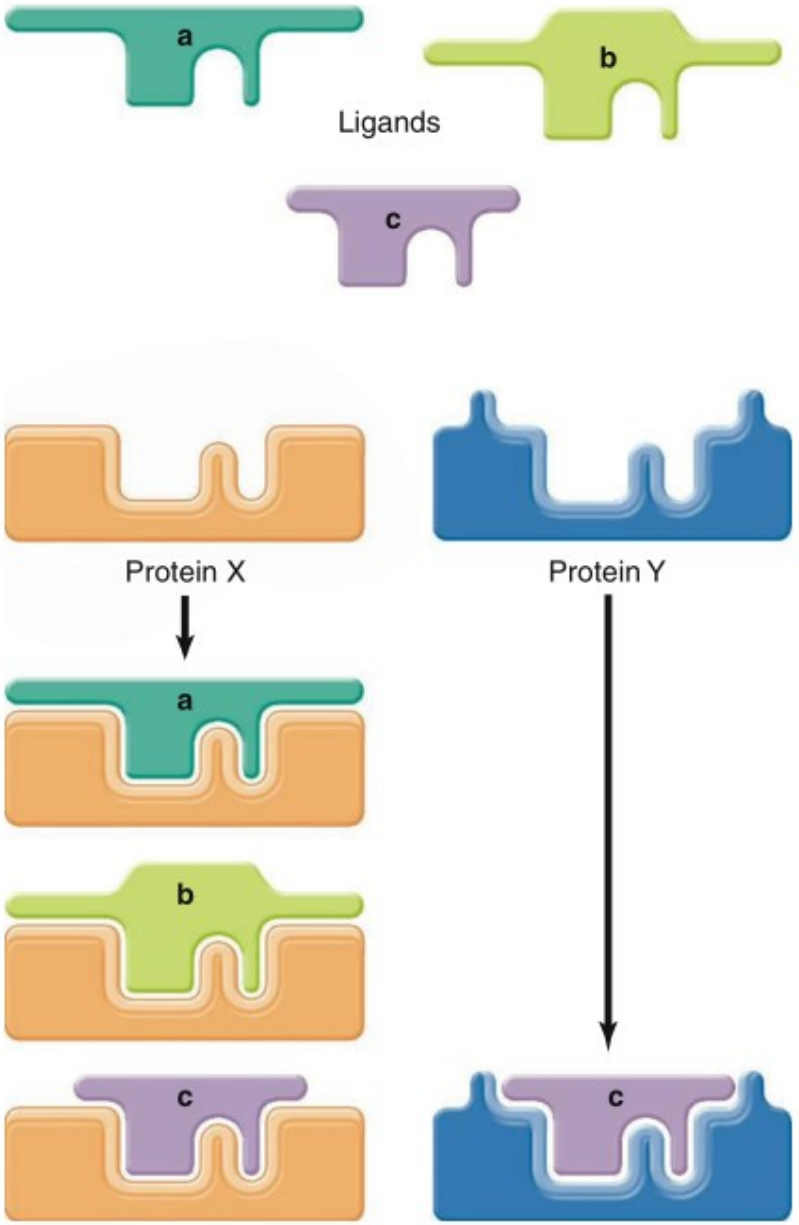


Figure 3.28 Protein X can bind all three ligands, which have similar chemical structures. Protein Y, because of the shape of its binding site, can bind only ligand c. Protein Y, therefore, has a greater chemical specificity than protein X.

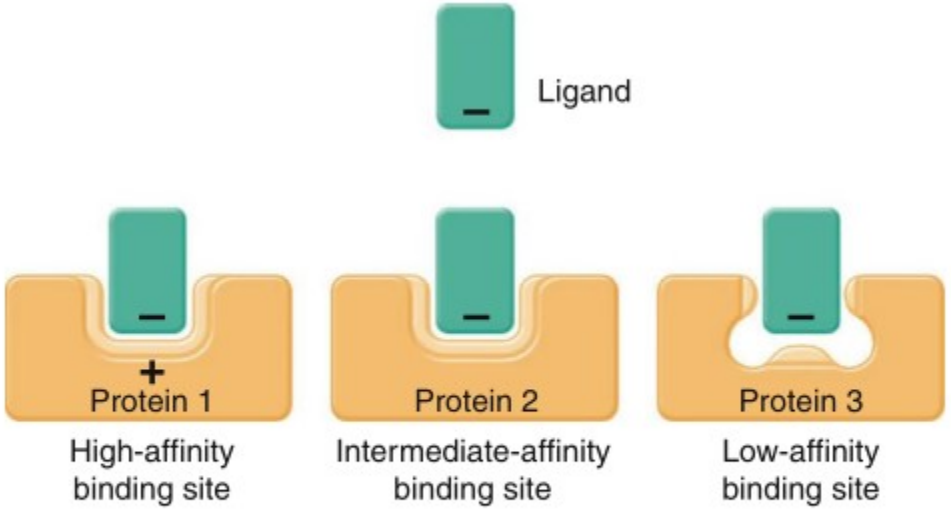


Figure 3.29 Three binding sites with the same chemical specificity but different affinities for a ligand.

Modulace vazby ligand – protein

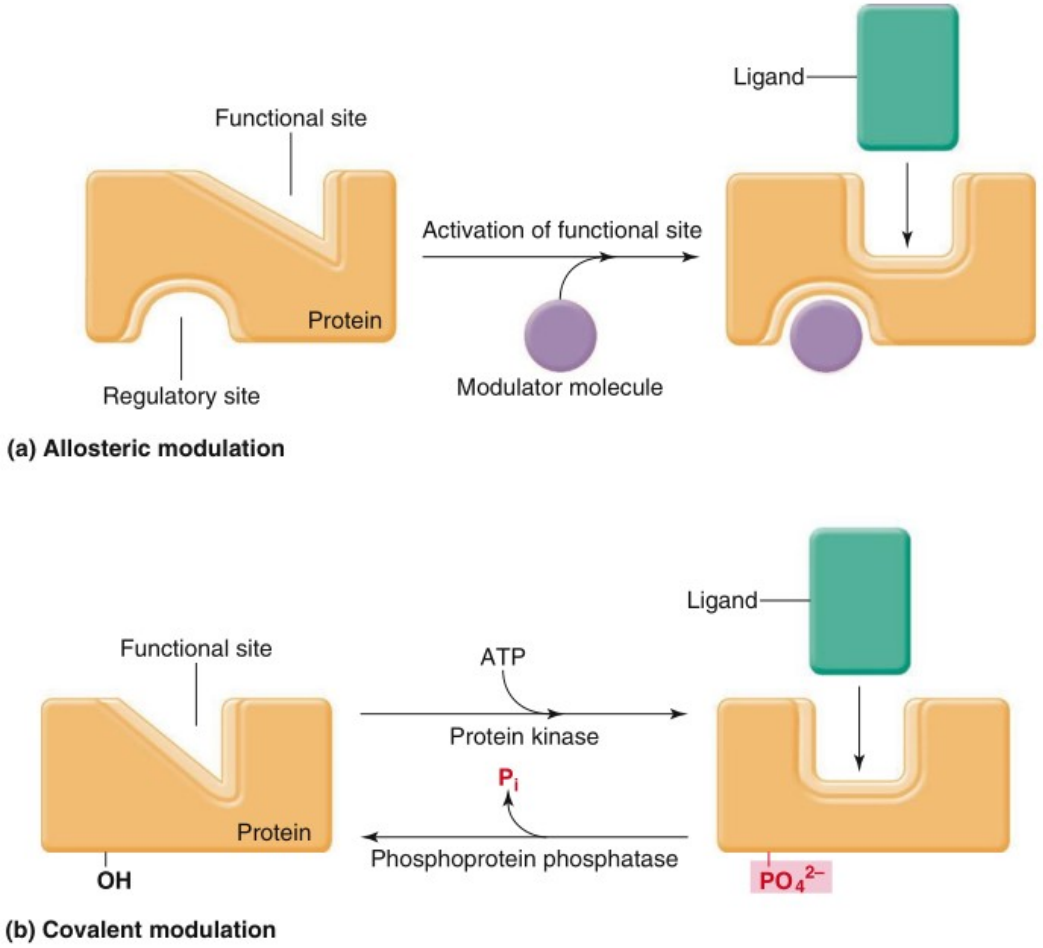
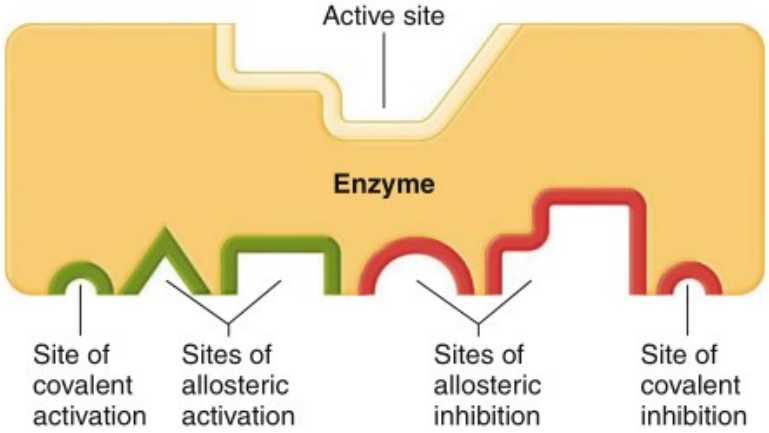


Figure 3.32 (a) Allosteric modulation and (b) covalent modulation of a protein's functional binding site.

- Alosterická – slabá (nekovalentní) vazby modulátoru změní afinitu l-p vazby
- Kovalentní – kovalentní navázání nějaké skupiny (fosforylace, metylace…) změní sílu vazby ligandu



Saturace vazby ligand - protein

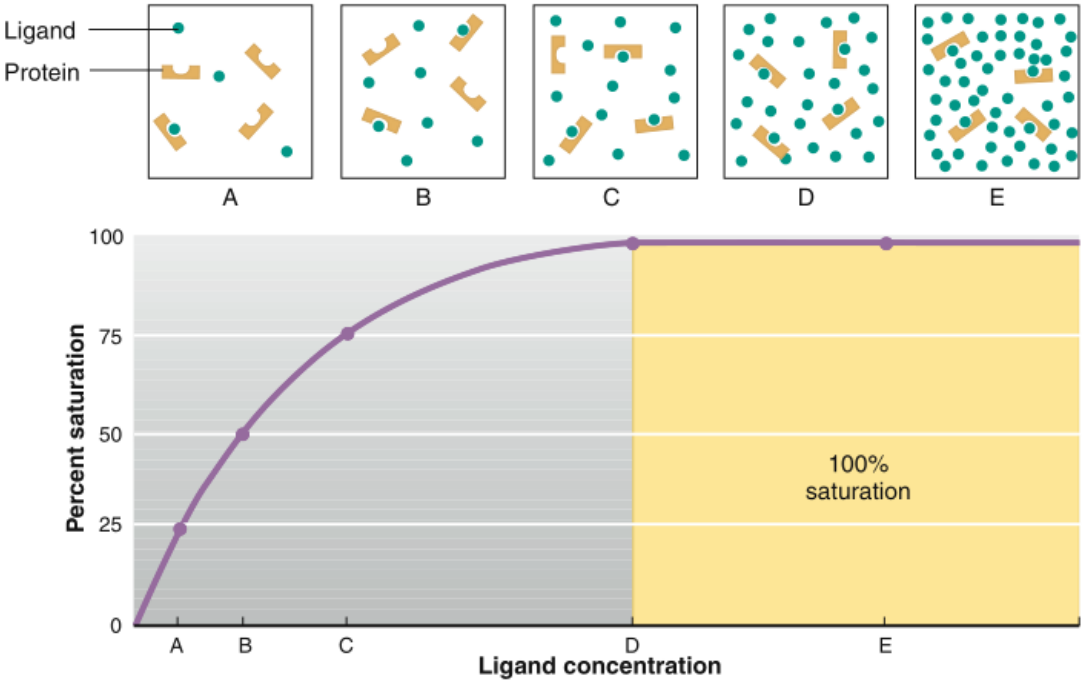


Figure 3.30 Increasing ligand concentration increases the number of binding sites occupied—that is, it increases the percent saturation. At 100% saturation, all the binding sites are occupied, and further increases in ligand concentration do not increase the number bound.

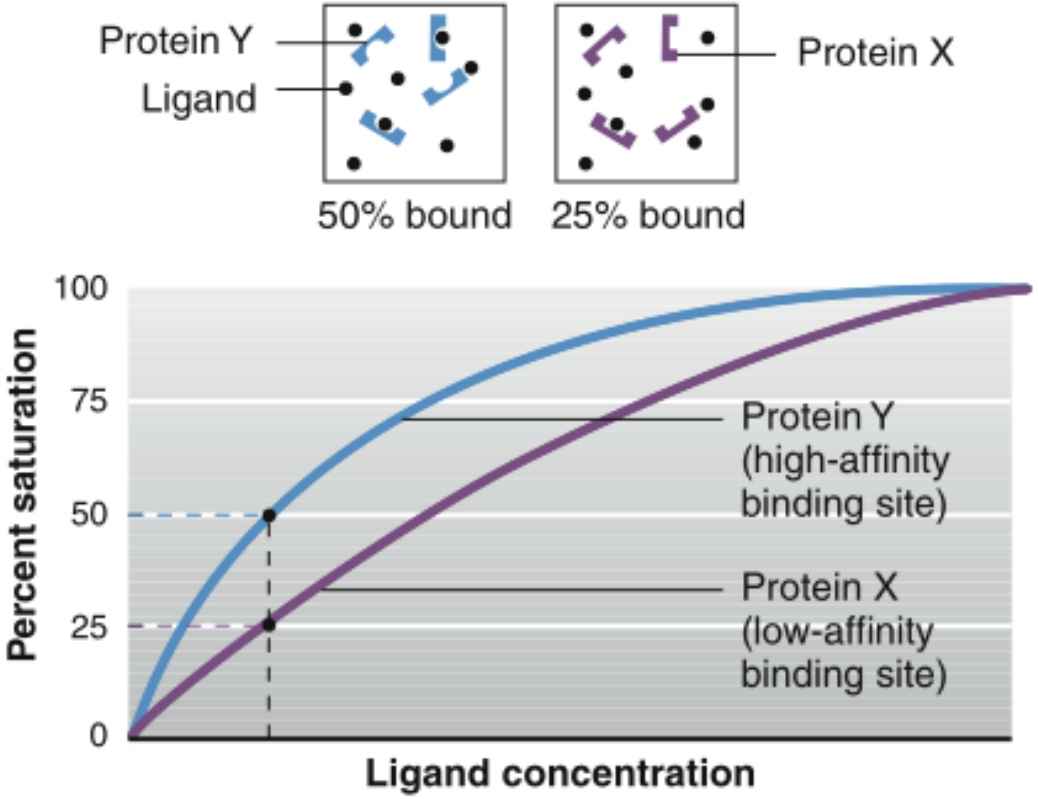
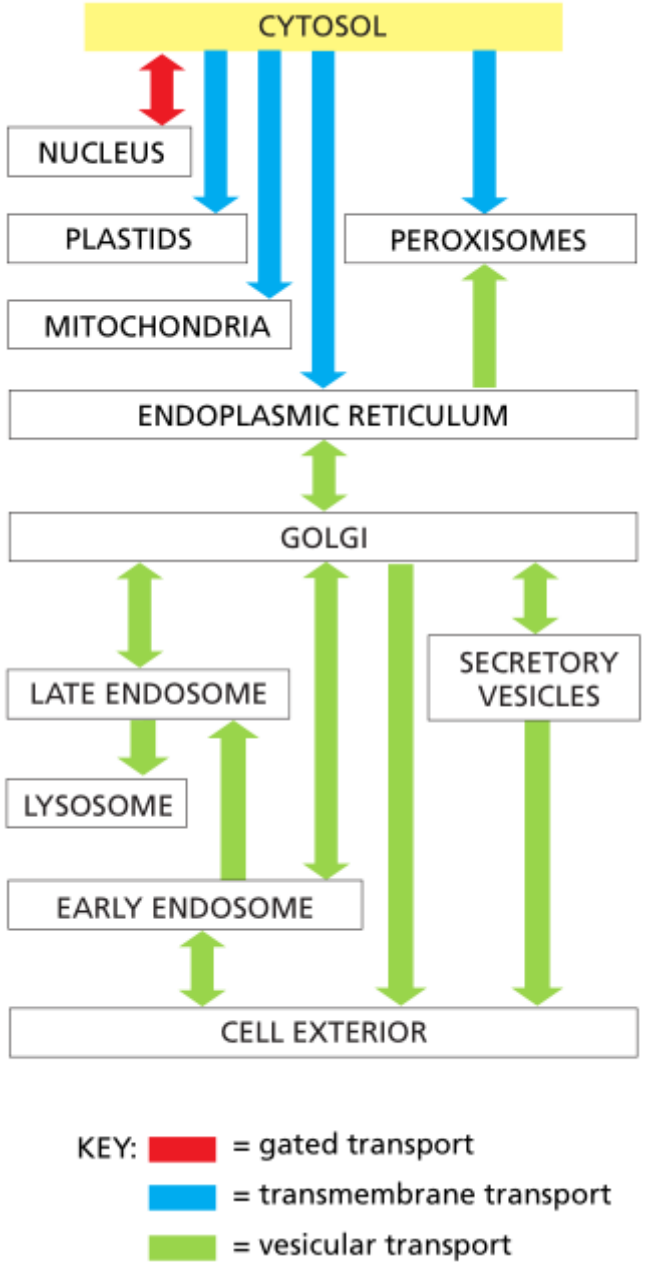
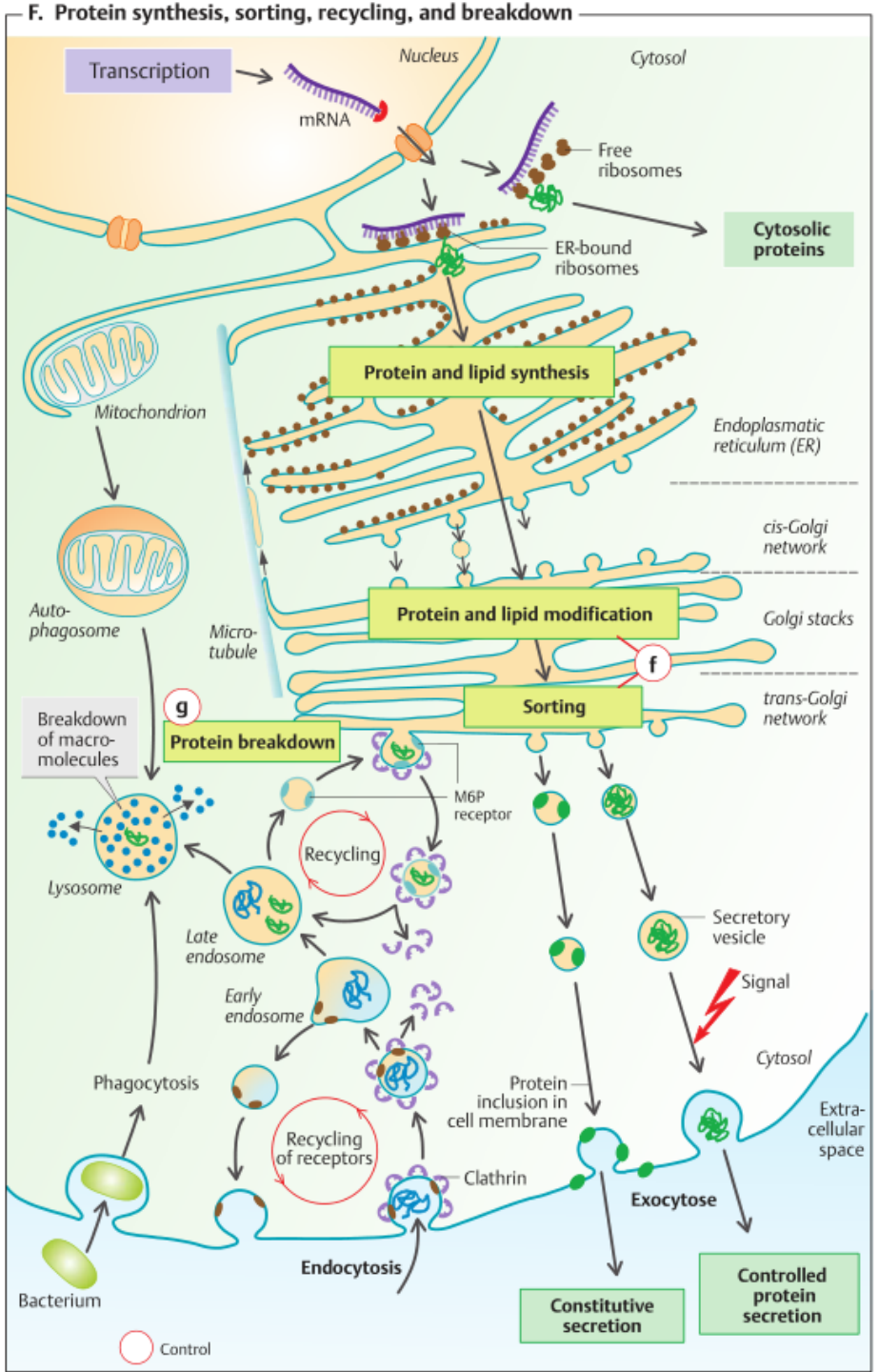


Figure 3.31 When two different proteins, X and Y, are able to bind the same ligand, the protein with the higher-affinity binding site (protein Y) has more bound sites at any given ligand concentration up to 100% saturation.

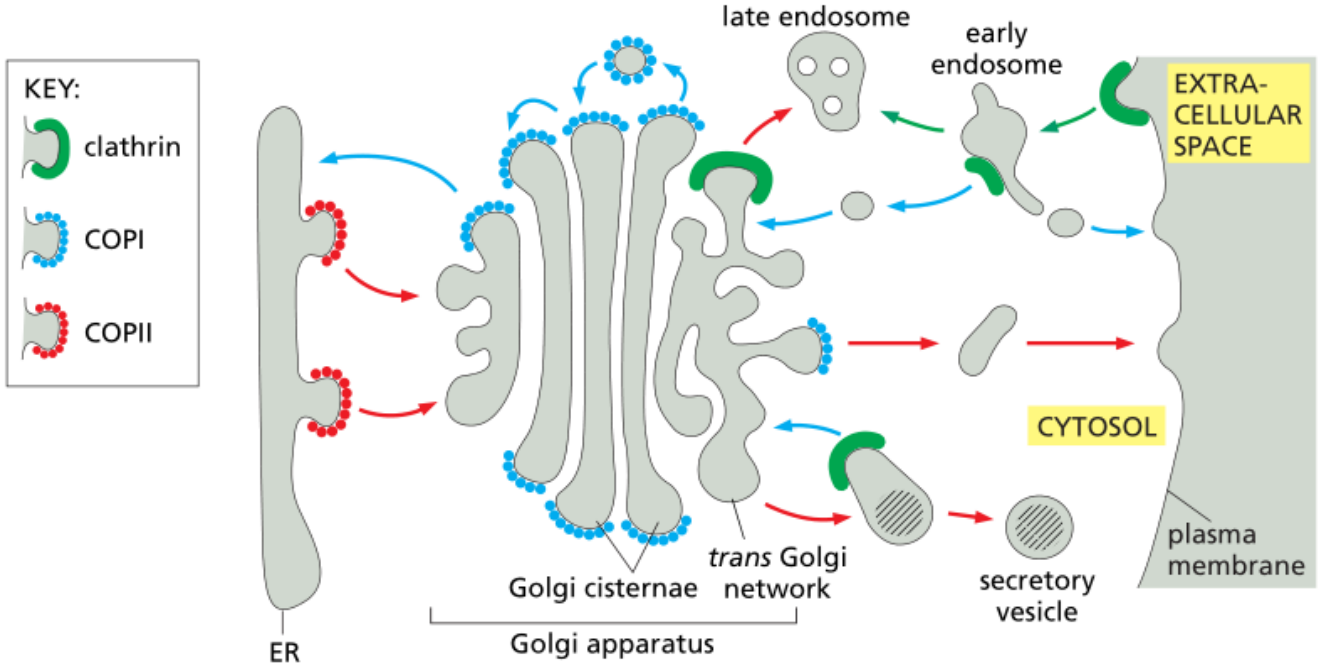
Buňka – transport

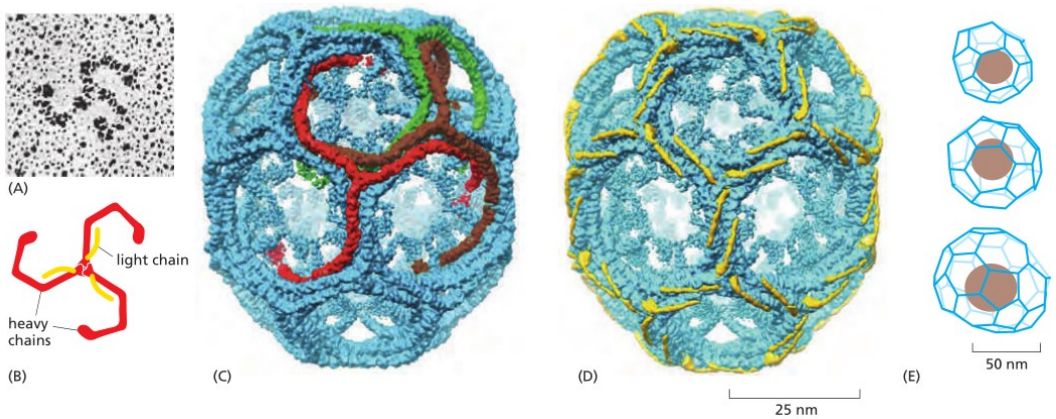
- Transport z jádra
- ER
- Cis a trans-Golghiho systém
- Exocytosa
- Endocytosa
- Lysosom

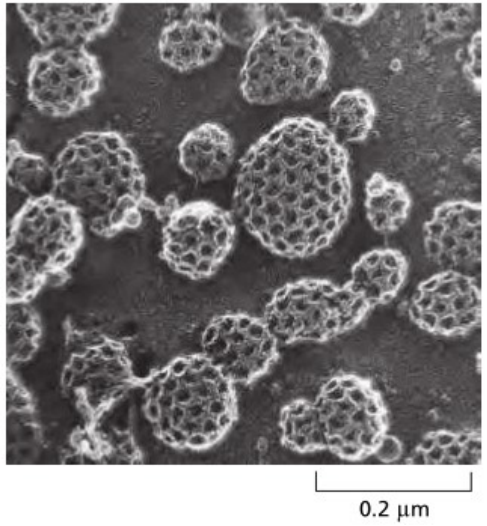




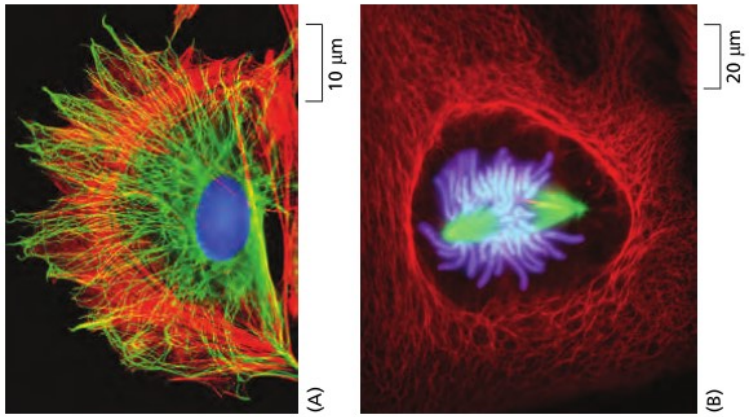
Clathrin, COPI a COPII v buněčné transdukci

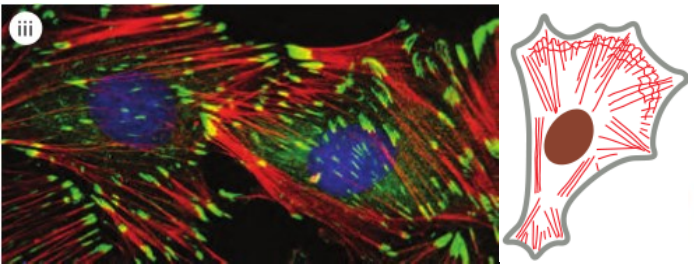


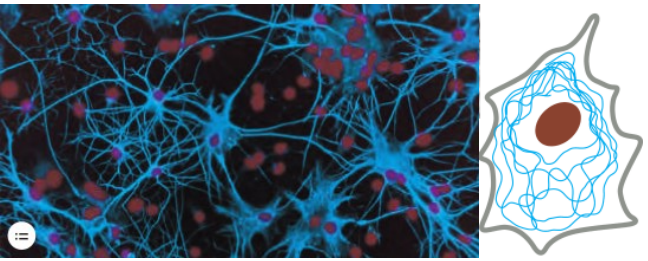


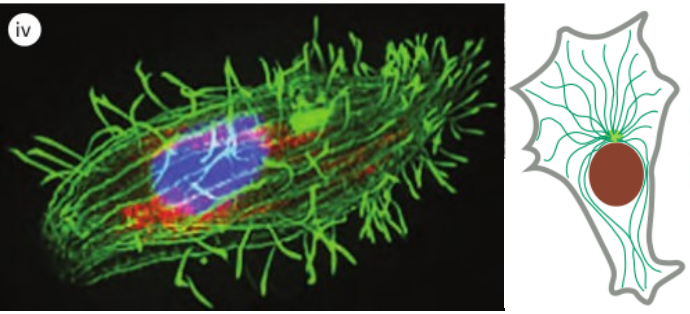


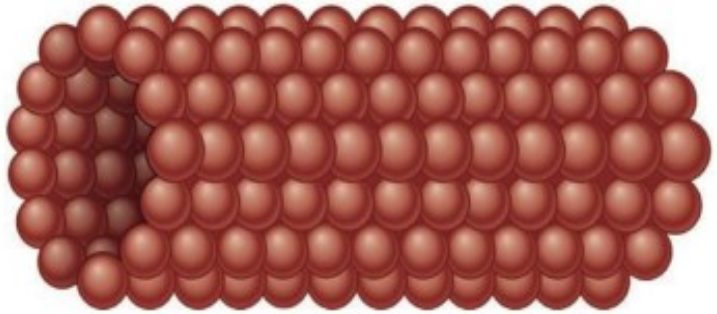
Buněčná kostra - cytoskelet





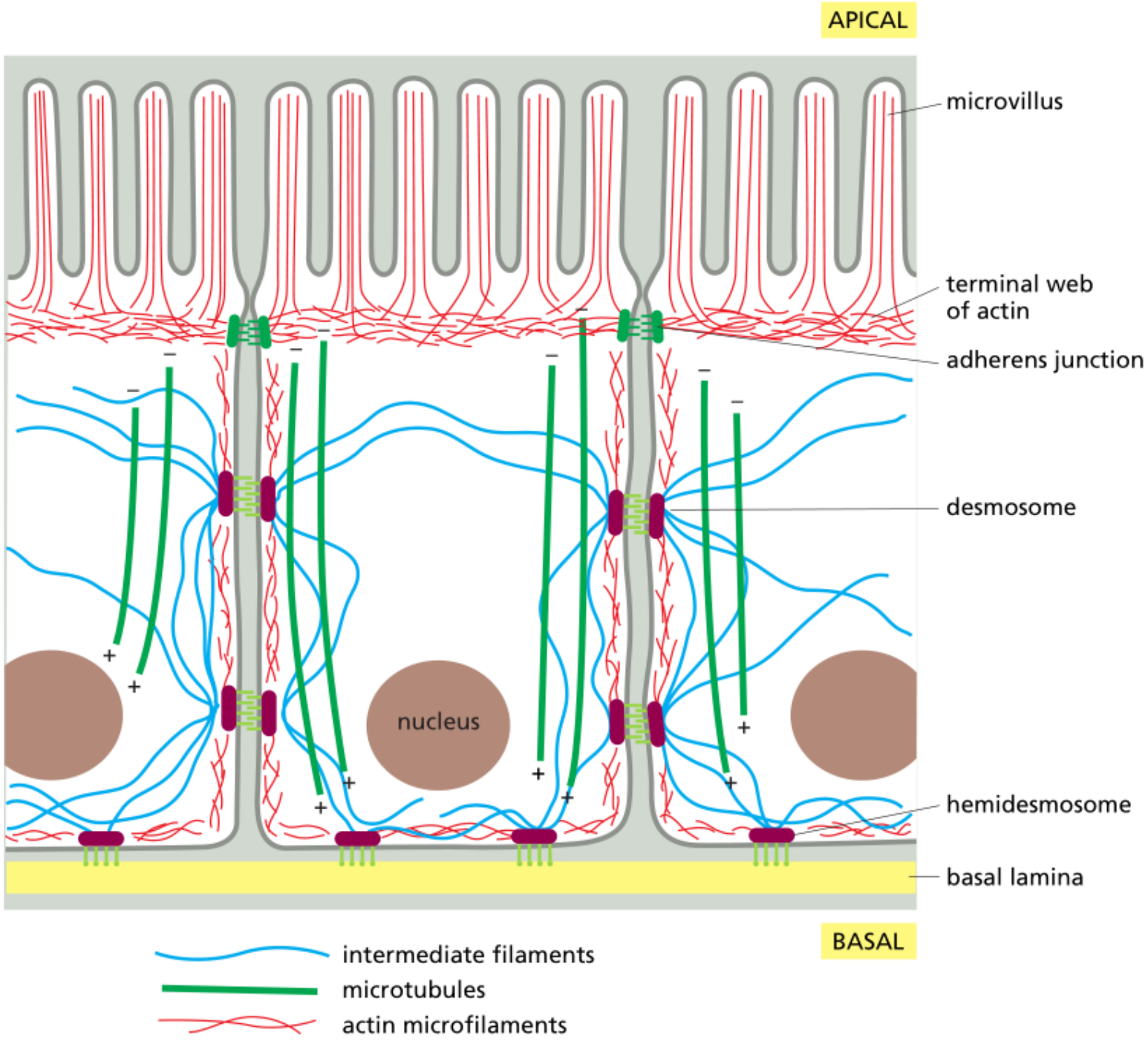






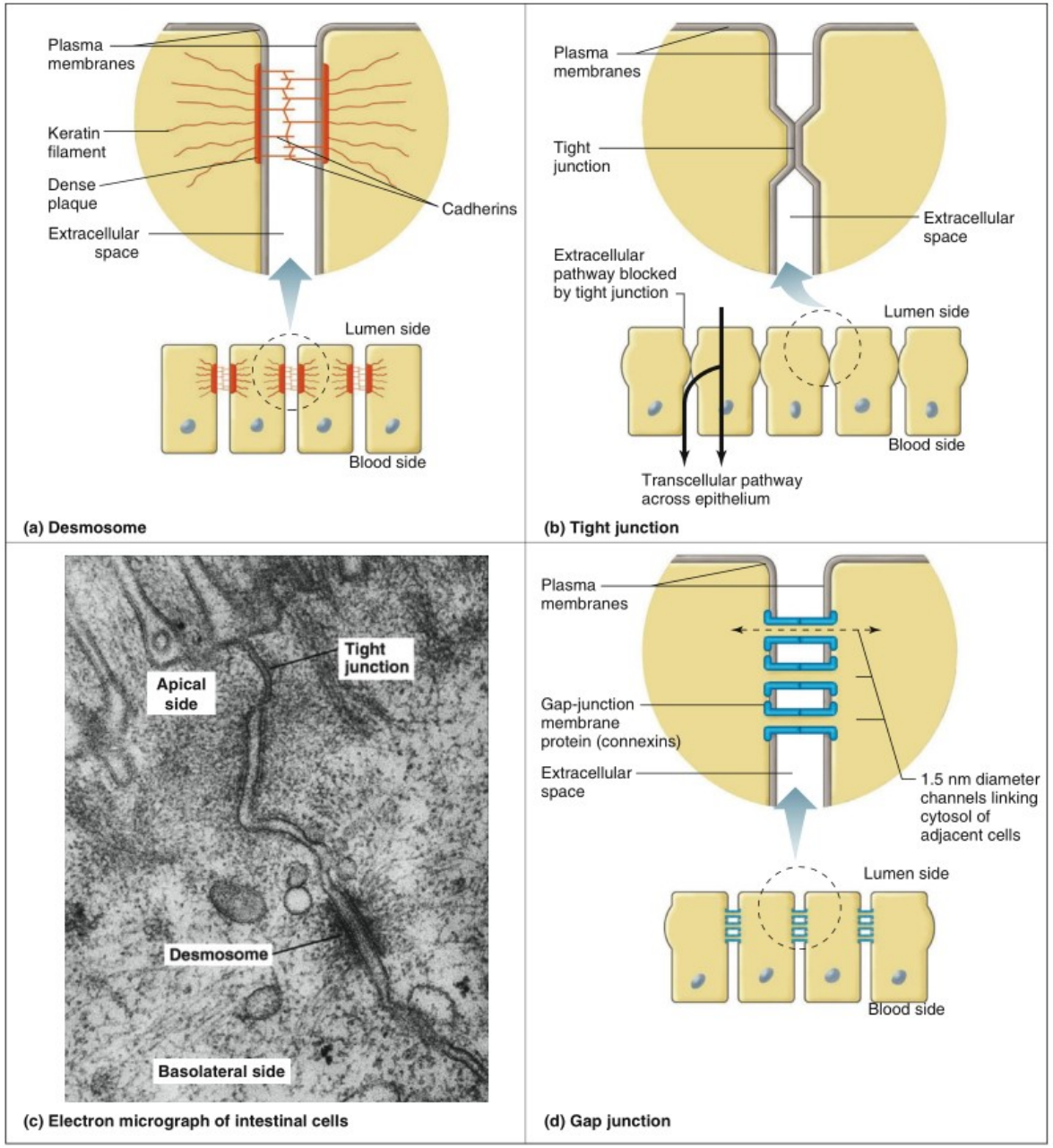
Cytoskeletal filaments	Diameter (nm)	Protein subunit
Actin filament	7	G-actin
Intermediate filament	10	Several proteins
Microtubule	25	Tubulin

Cytoskelet a polarizace buňky



Kontakty mezi buňkami

- Integrace buněk do tkání – kontaktní proteiny *integriny*
- Desmosomy – pevné v tahu, elastické spojení, kůže
- Tight junctions – překážka volného průchodu látek, střevní sliznice
- Gap junctions – výměna nízkomolekulárních látek, srdeční svalovina



AP|R **Figure 3.9** Three types of specialized membrane junctions: (a) desmosome; (b) tight junction; (c) electron micrograph of two intestinal epithelial cells joined by a tight junction near the apical (luminal) surface and a desmosome below the tight junction; and (d) gap junction. Electron micrograph from M. Farquhar and G. E. Palade, *J. Cell. Biol.*, 17:375–412.

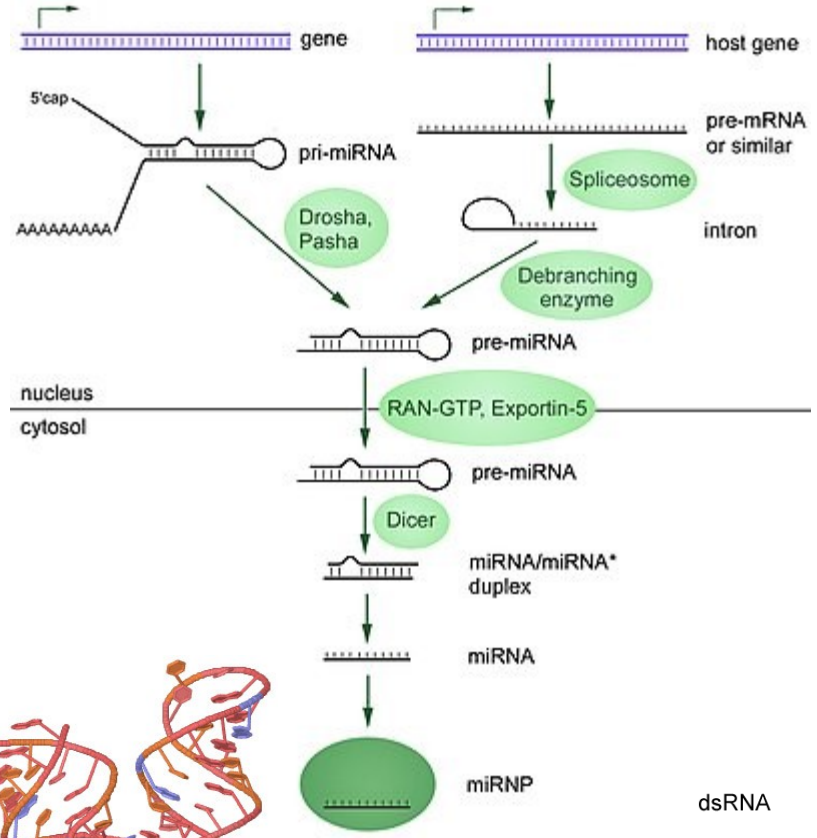
RNA – struktura a funkce v buňce

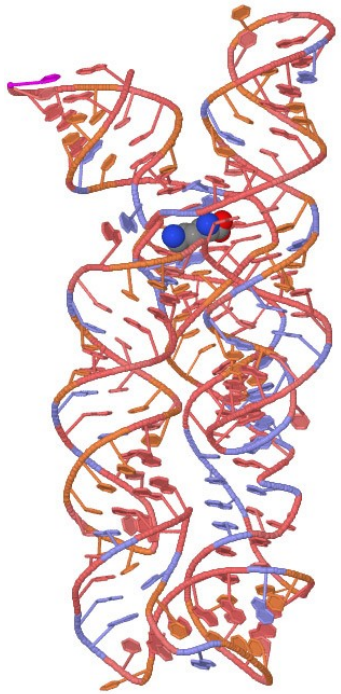
- Total RNA per cell: 1-10 pg
- **rRNA** - ribosomal RNA (translation) 85%
- **tRNA** - transfer RNA (translation) 15%
- **mRNA** - messenger RNA (codes for protein) 1-5%,
  - 1-20k molecules per gene per cell

- SRP RNA - signal recognition particles
- tmRNA - transfer-messenger RNA (only in bacteria)
- Other RNA's:
  - miRNA - micro, siRNA - small interfering (gene regulation)
  - piRNA (transposon defense)
  - lncRNA – long non-coding (regulation, epigenetics)

RNA funkce

- mRNA (riboswitch)
- rRNA
- tRNA
- In nuclear export
- Part of spliceosome (snRNA)
- Regulatory molecules (RNAi)
- Enzymes (Ribozymes)
- Viral genomes
- Retrotransposons





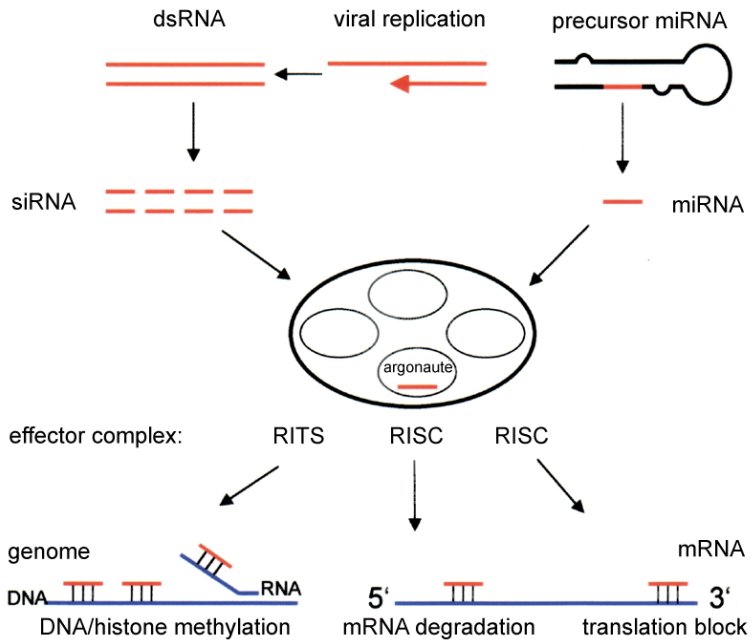
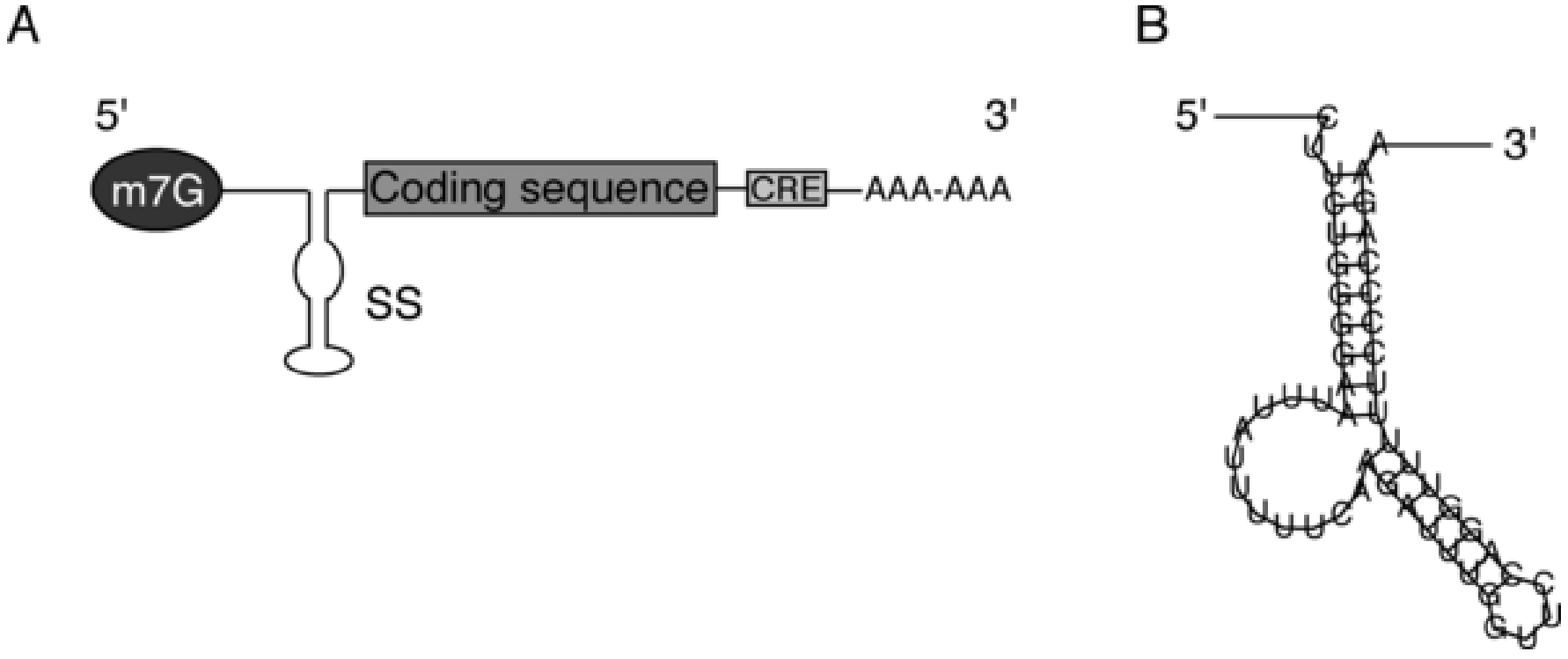


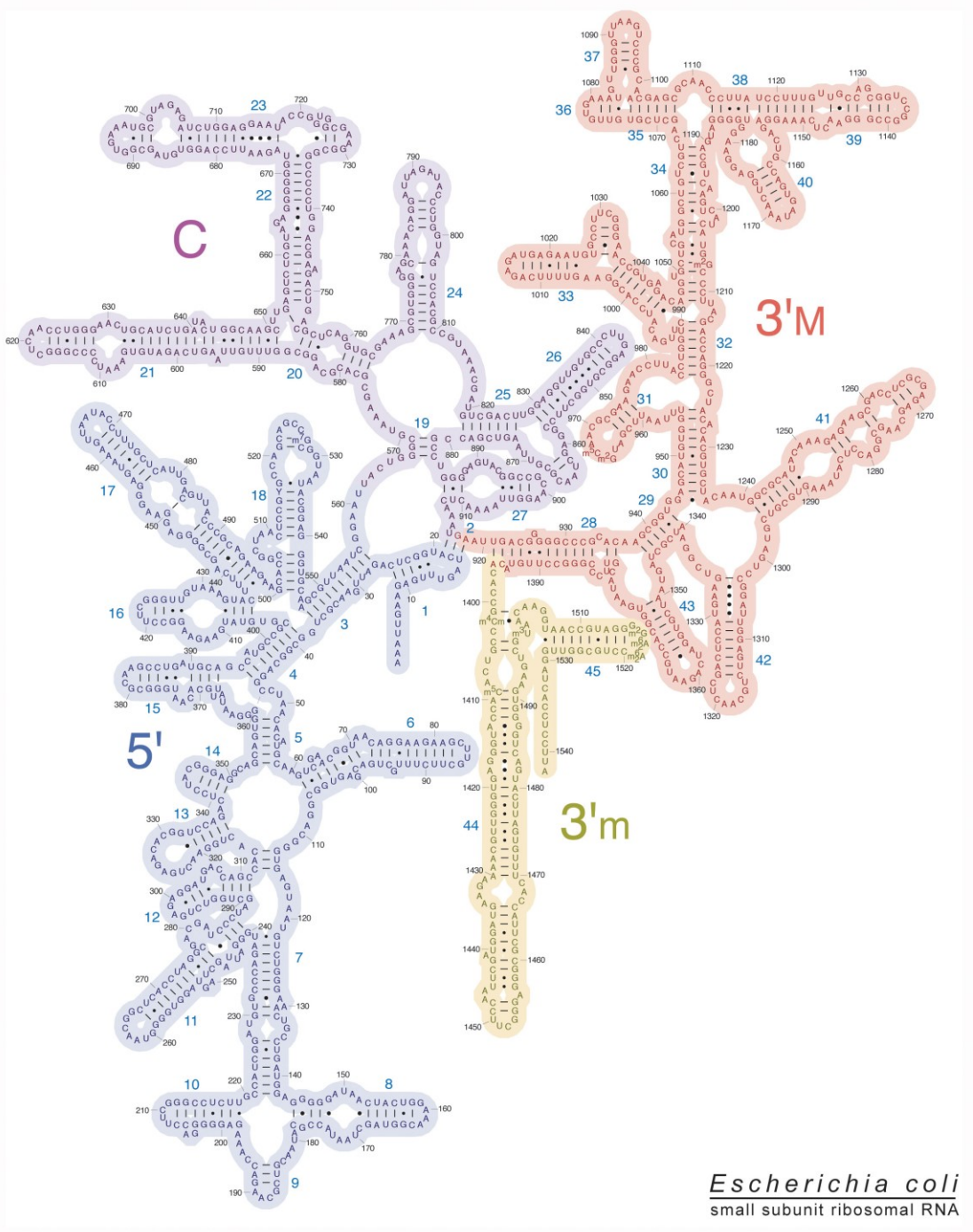
Figure 1. Structure of Yeast mRNA



Ringnér M, Krogh M (2005) Folding Free Energies of 5'-UTRs Impact Post-Transcriptional Regulation on a Genomic Scale in Yeast. PLoS Comput Biol 1(7): e72. doi:10.1371/journal.pcbi.0010072
http://journals.plos.org/ploscompbiol/article?id=info:doi/10.1371/journal.pcbi.0010072

PLOS | COMPUTATIONAL BIOLOGY

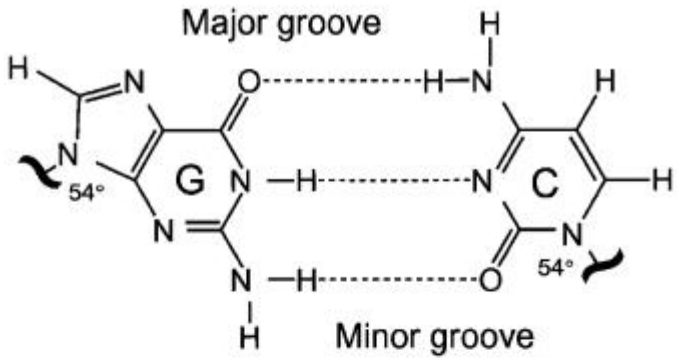
RNA struktura

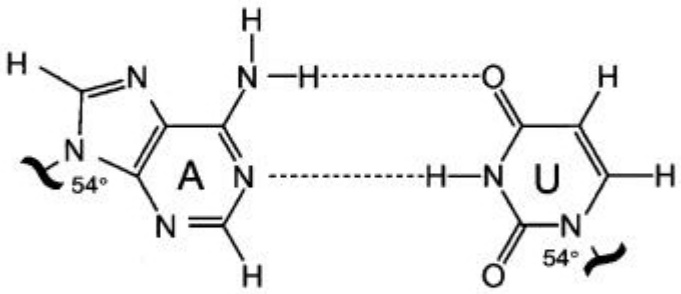


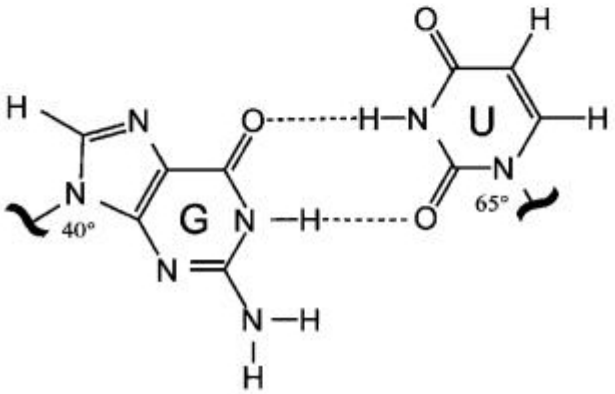
http://rna.ucsc.edu/rnacenter/ribosome_images.html

Párování bází v RNA

- Canonical Watson-Crick pairing
  - A-U
  - C-G
- Non-canonical pairing
  - G-U (closing pair)







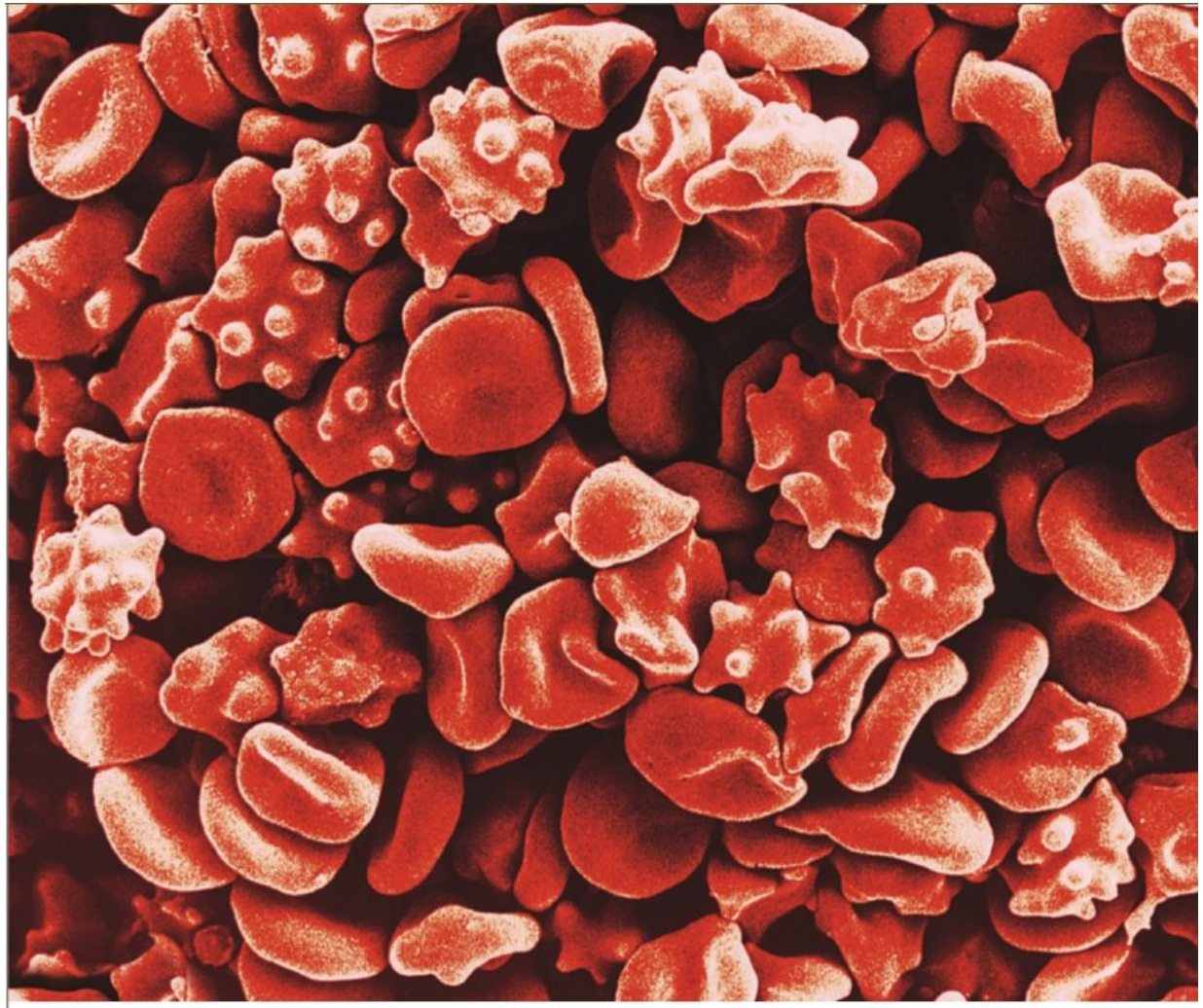
Párování bází v RNA

bpC	C:G	U:A	U:G	G:A	C:A	U:C	A:A	C:C	G:G	U:U	Total
WC	49.8%	14.4%	0.01%	1.2%	0.1%	0.5%	-	-	-	-	66.1%
Wb	0.06%	0.06%	7.1%	-	0.2%	-	0.3%	0.5%	0.2%	0.9%	9.6%
Other	0.8%	5.8%	1.5%	9.4%	2.3%	0.6%	2.6%	0.5%	0.7%	0.3%	24.3%
Total	50.7%	20.3%	8.7%	10.6%	2.6%	1.0%	2.9%	1.0%	0.9%	1.3%	100.0%

Just 71.3% of rRNA contacts are canonical or G:U wobble!

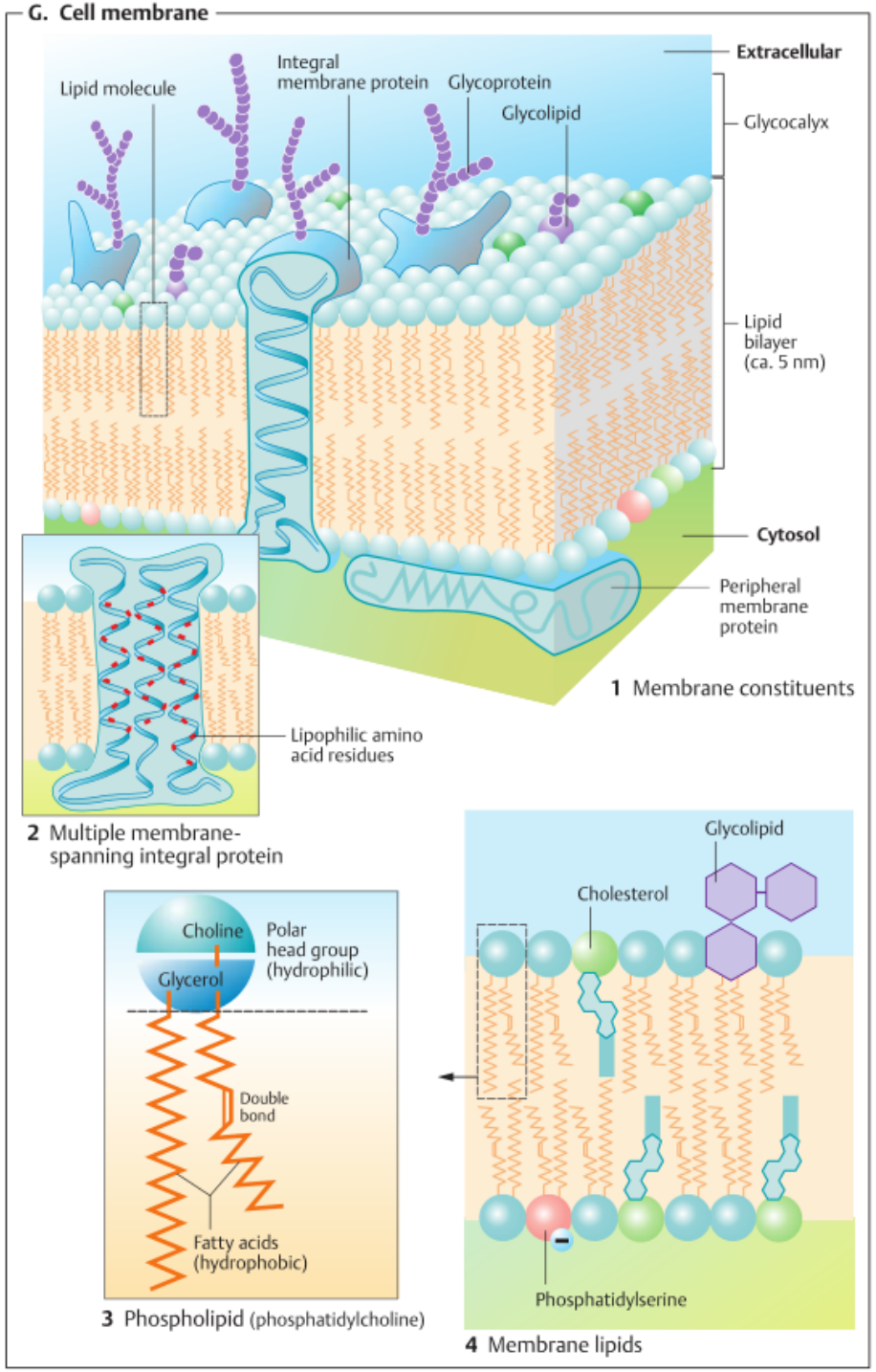
Lee & Gutell (2004) Diversity of base-pair conformations and their occurrence in rRNA structure and RNA structural motifs *J Mol Biol.*

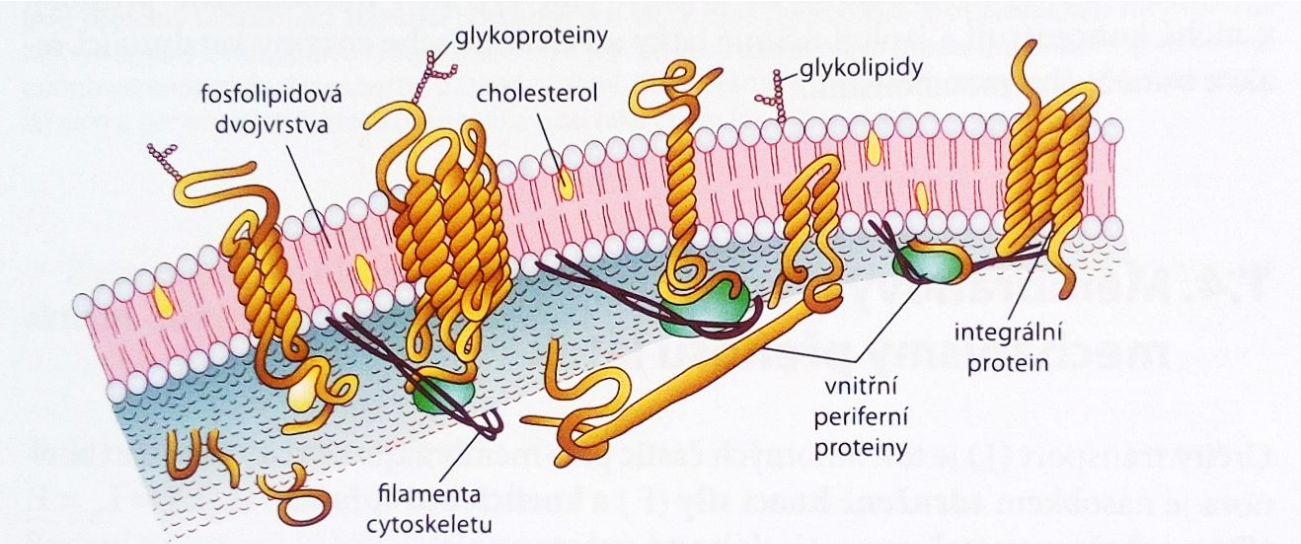
Buněčná membrána – přenos látek



Changes in red blood cell shape due to osmosis; the knobby appearance of some cells is due to water leaving the cell.

Buněčná membrána



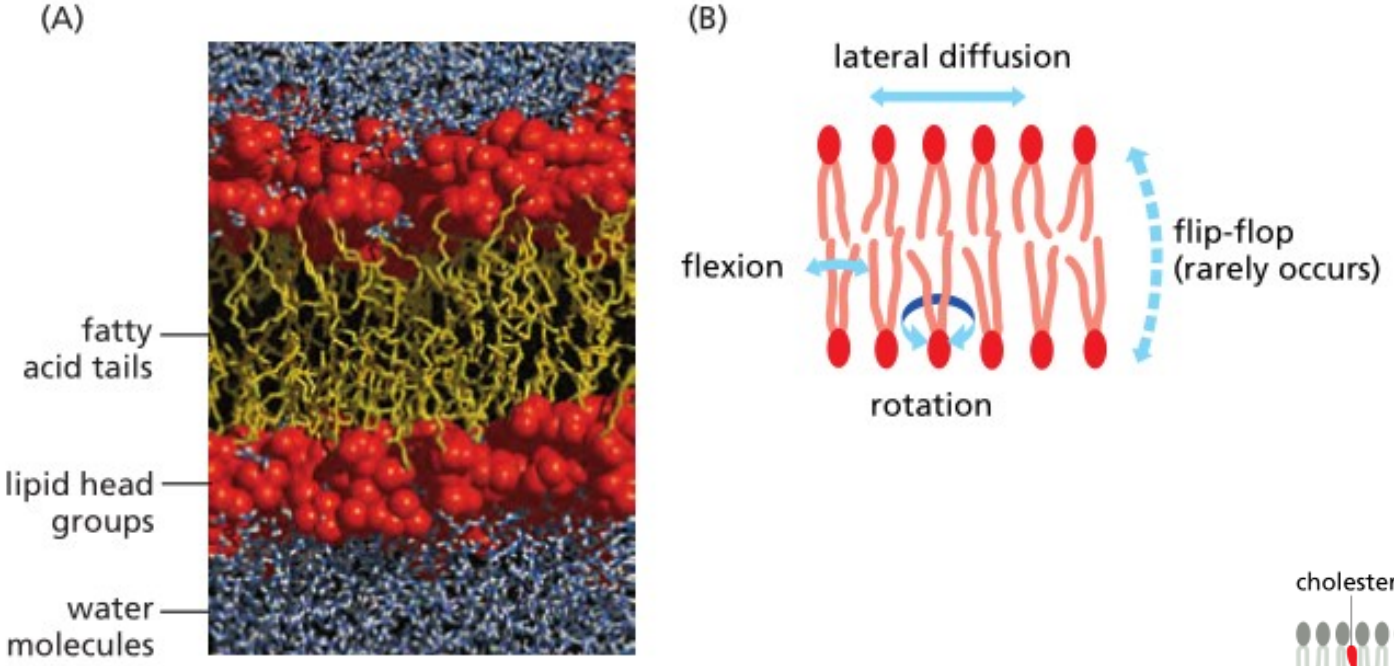


proteiny membrány { receptory kanály, kanály, přenašeče, enzymy } princip funkce: změna prostorové konformace

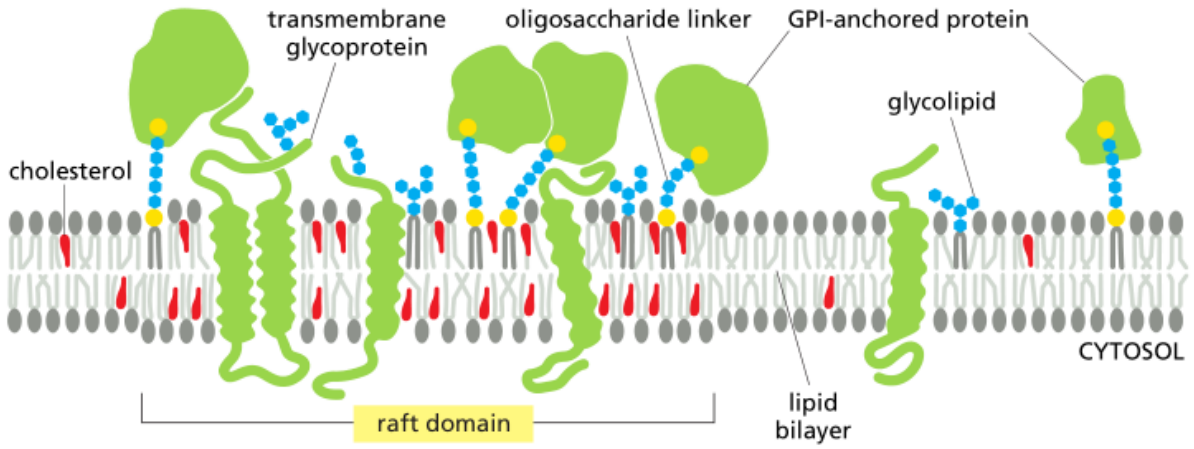
Obr. 1.2. Schéma struktury plazmatické membrány živočišné buňky. Pohled z vnitřku buňky na dvojvrstvu lipidů, do níž jsou vsazeny integrálně nebo jednostranně komplexní proteiny. Vzhledem k tekutosti lipidů se mohou proteiny v membráně pohybovat a měnit svoji konformaci, což je základem přenosu energie a informace. Podle: http://vschool.nides.bc.ca

Buněčné membrány

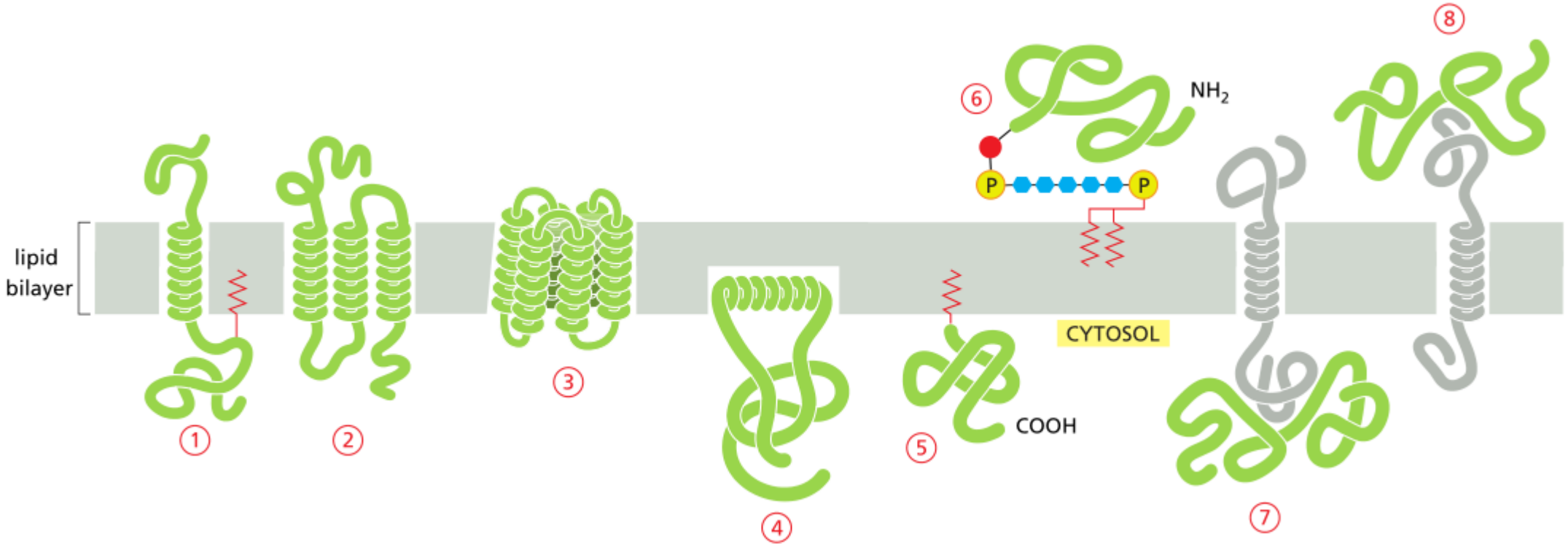
Model fosfolipidové dvouvrstvy



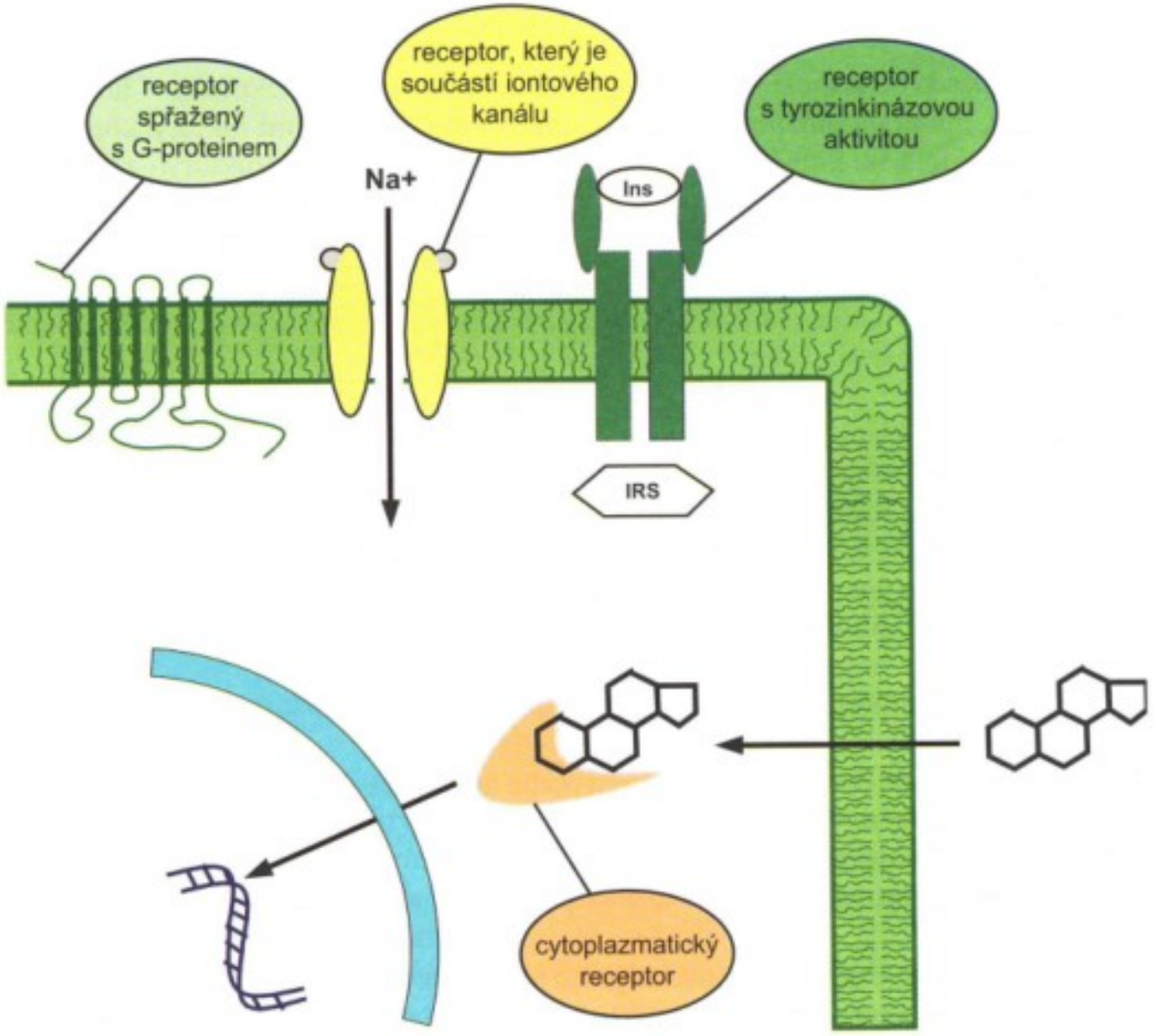
Rafty – pohyblivé oblasti



Membránové proteiny



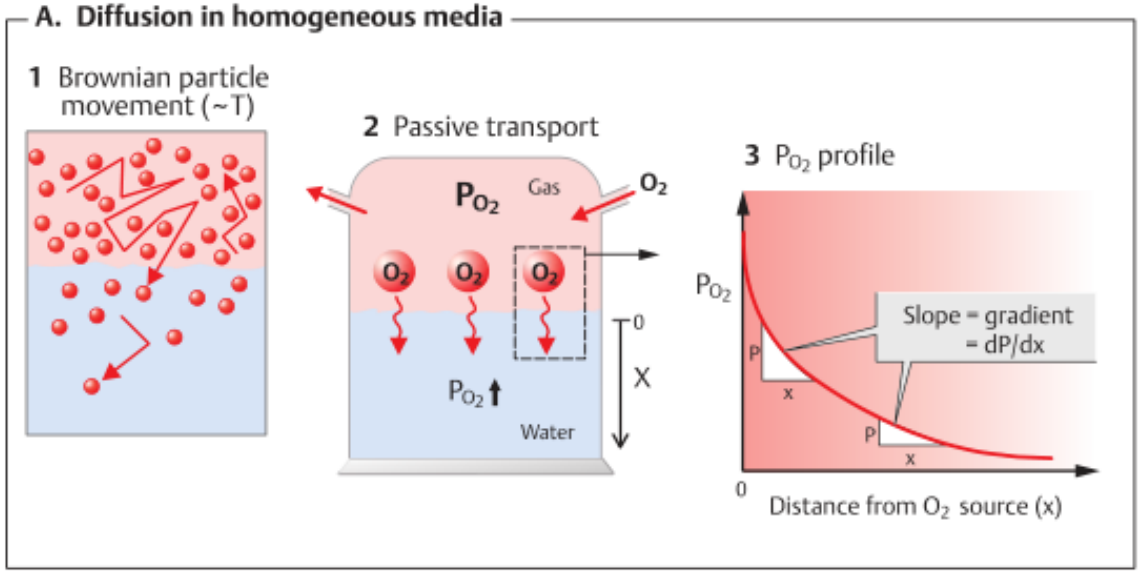
Most membrane proteins extend across the bilayer as (1, with covalently attached fatty acid chain) a single α helix, (2) as multiple α helices, or (3) as a rolled-up β sheet (a β barrel).
Some of these are anchored to the cytosolic surface by an amphiphilic α helix (4). (5) Others are attached to the bilayer solely by a covalently bound lipid chain (6) via an oligosaccharide linker—called a GPI anchor. (7, 8) Some proteins are attached to the membrane only by noncovalent interactions with other membrane proteins.

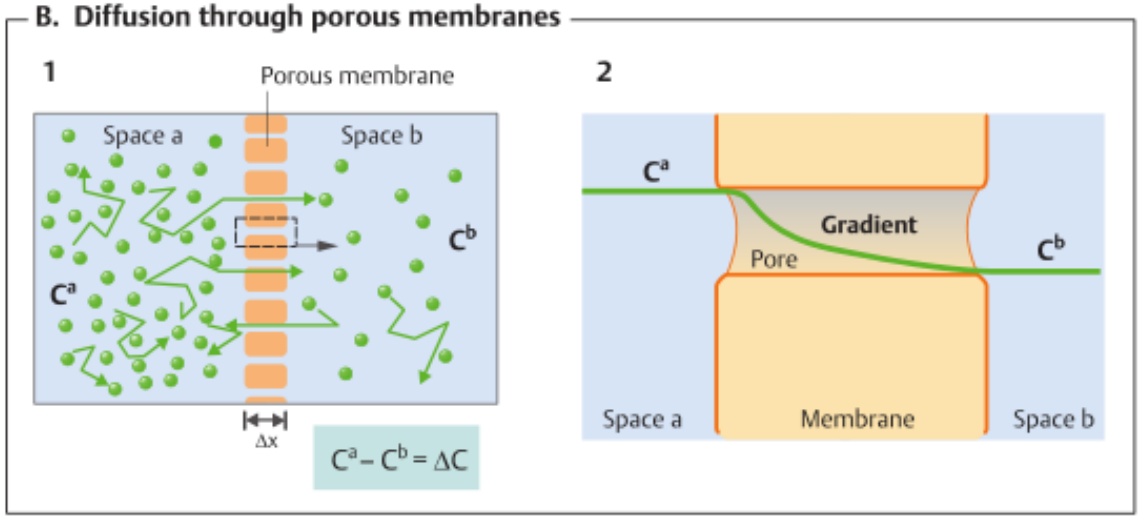


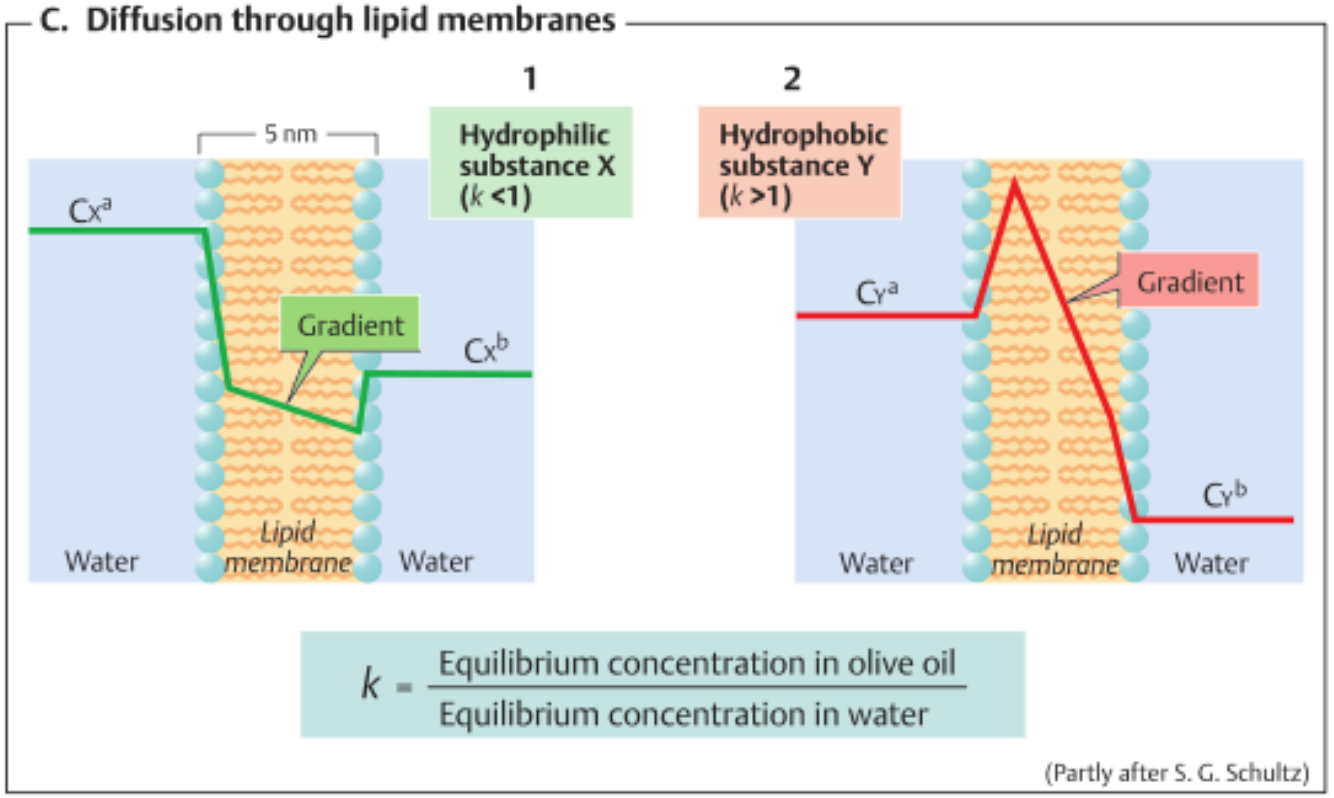
Obr. 1.28 *Schéma jednotlivých typů receptorů – shora zleva: receptor spřažený s G-proteiny; receptor, který je součástí iontového kanálu; receptor s vlastní tyrozinkinázovou aktivitou; cytoplazmatický (steroidní) receptor*

Receptory membránové a cytoplasmatické

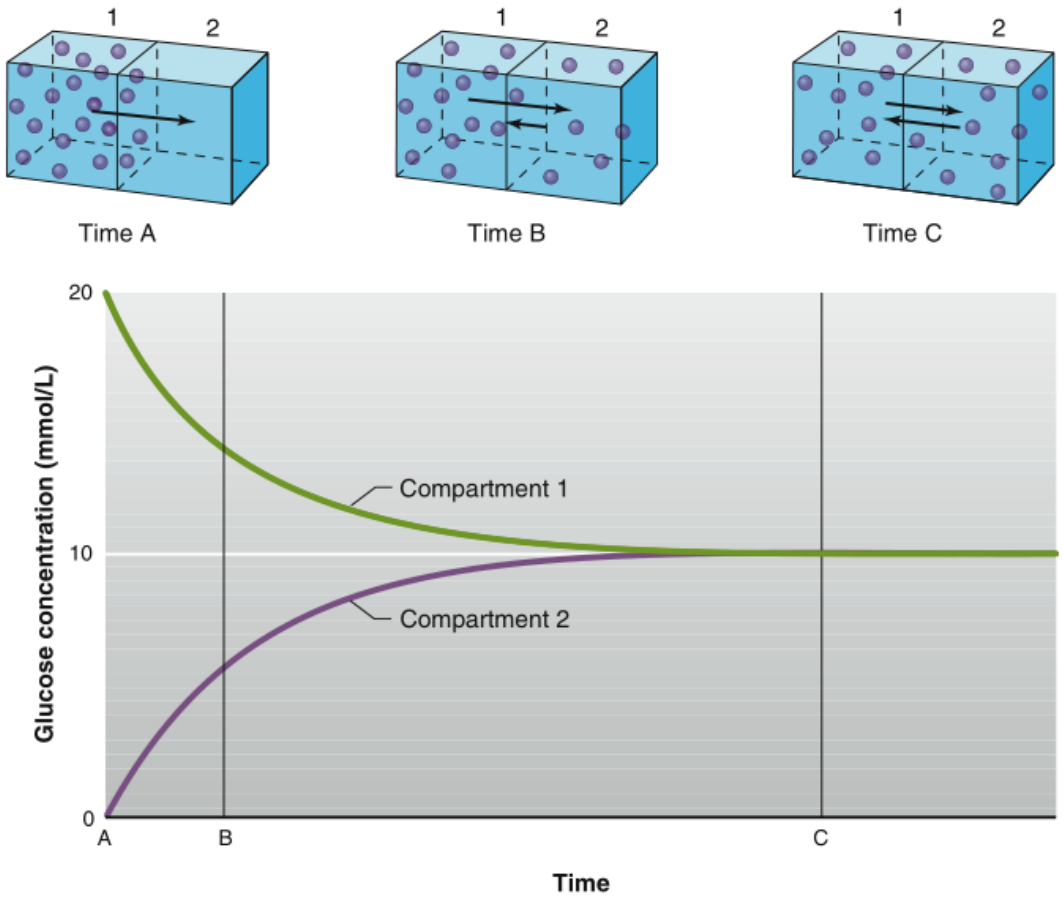
Difuze – hydrofilní a lipofilní látky



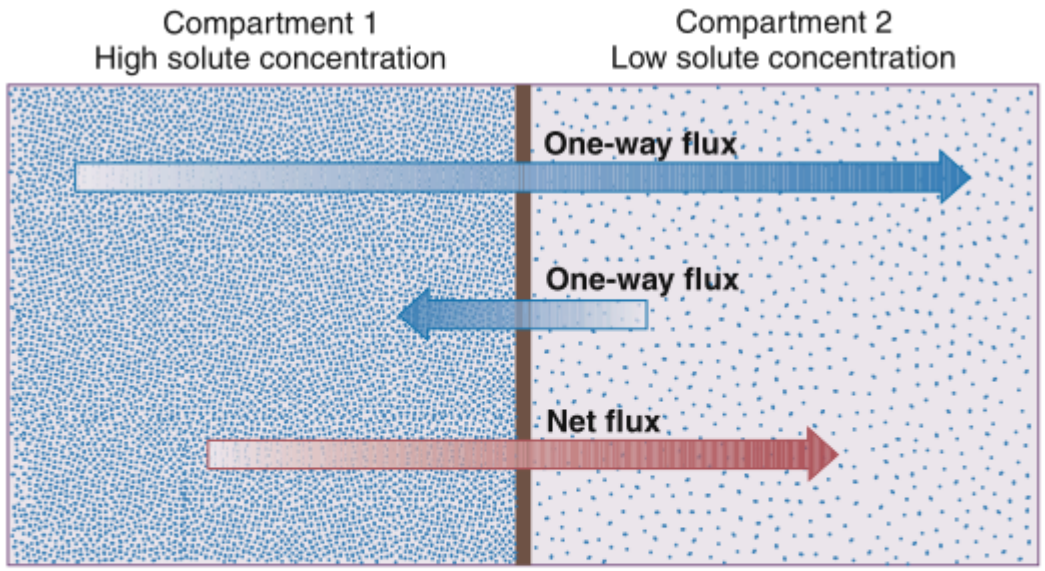




Difuze glukosy přes membránu - model



Difuze glukosy přes membránu - model



AP|R **Figure 4.3** The two one-way fluxes occurring during simple diffusion of solute across a boundary and the net flux (the difference between the two one-way fluxes). The net flux always occurs in the direction from higher to lower concentration. Lengths of arrows indicate magnitude of the flux.

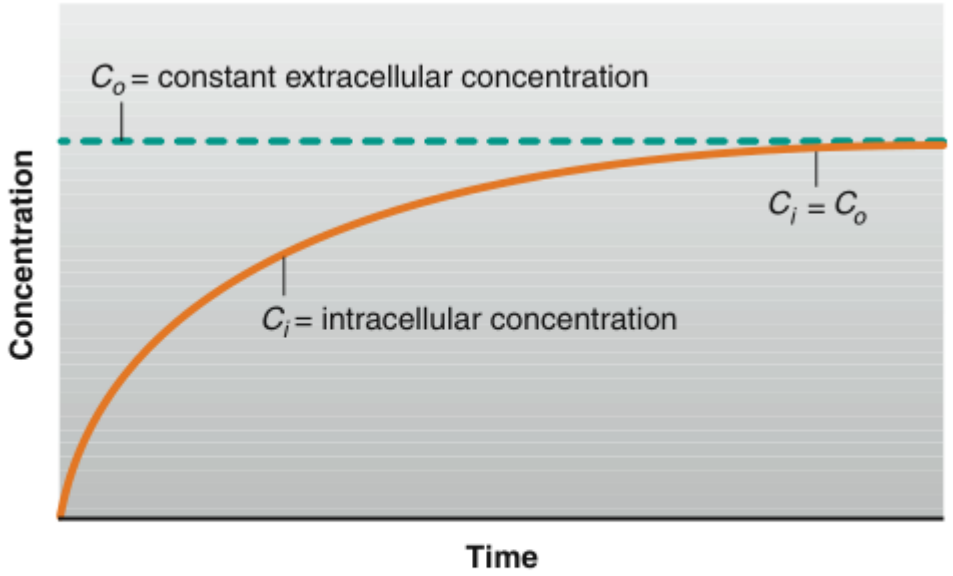


Figure 4.4 The increase in intracellular concentration as a solute diffuses from a constant extracellular concentration until diffusion equilibrium ($C_i = C_o$) is reached across the plasma membrane of a cell.

Rozdíly mezi pasivním a aktivním transportem

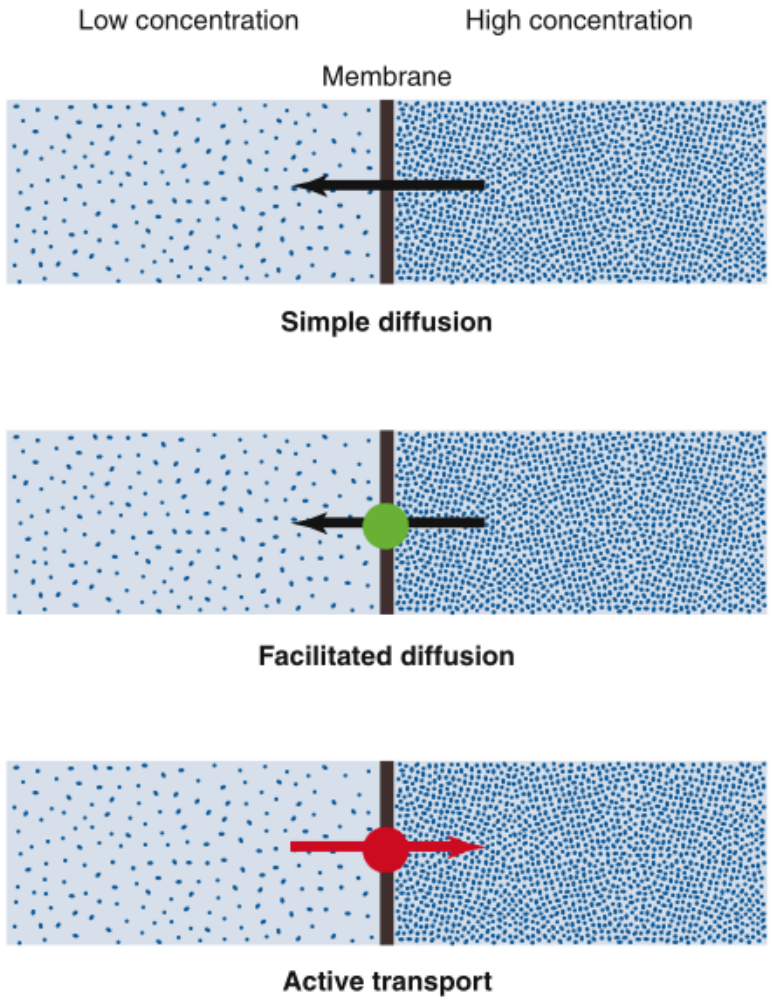
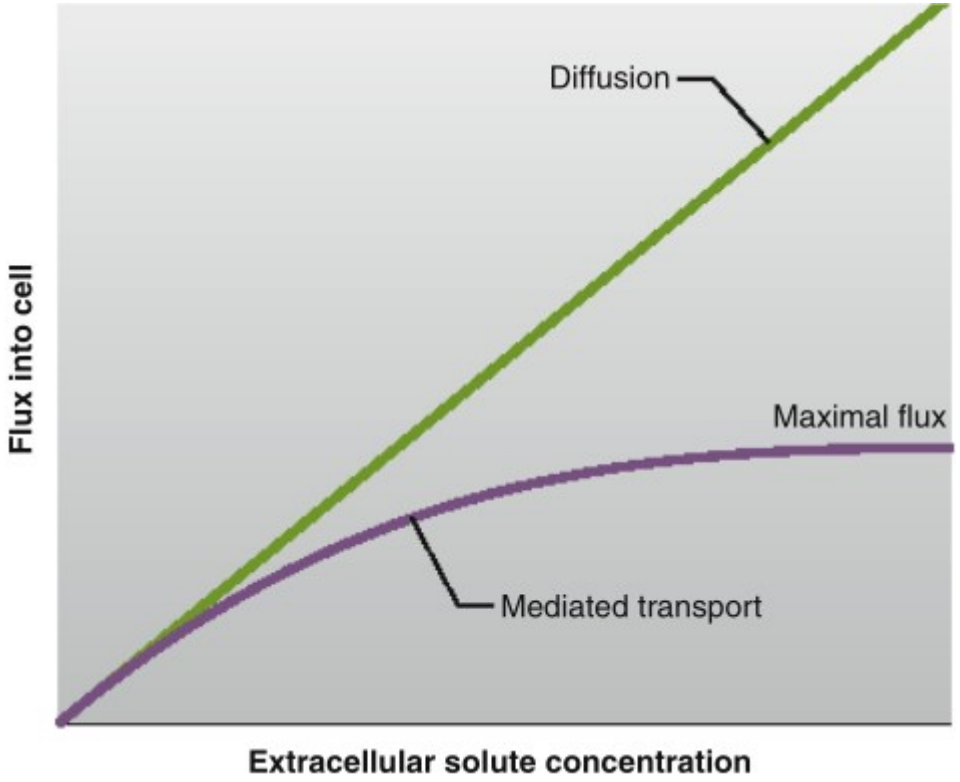


Figure 4.10 Direction of net solute flux crossing a membrane by simple diffusion (high to low concentration), facilitated diffusion (high to low concentration), and active transport (low to high concentration). The colored circles represent transporter molecules.



Difuze (pasivní) vs. aktivní transport do buňky v závislosti na koncentraci transportované látky

Aktivní transport (AT)

- Proti koncentračnímu spádu, nutno dodat energii (různými způsoby), nutné pro udržování intracelulárního prostředí odlišného od extracelulárního, omezená kapacita

- **Primární AT** – ATPasy, iontové kanály (Na^+-K^+ pumpa)
- **Sekundární AT** – kotransport (symport/antiport), energie dodána transportem druhé molekuly (elektroneutrální nebo rheogenní, dle výsledného náboje obou molekul)
- **Terciární AT** – endo/exo-cytosa, váčky s transportovanou látkou (ACh), typicky řízený vazebně

Iontové kanály – model

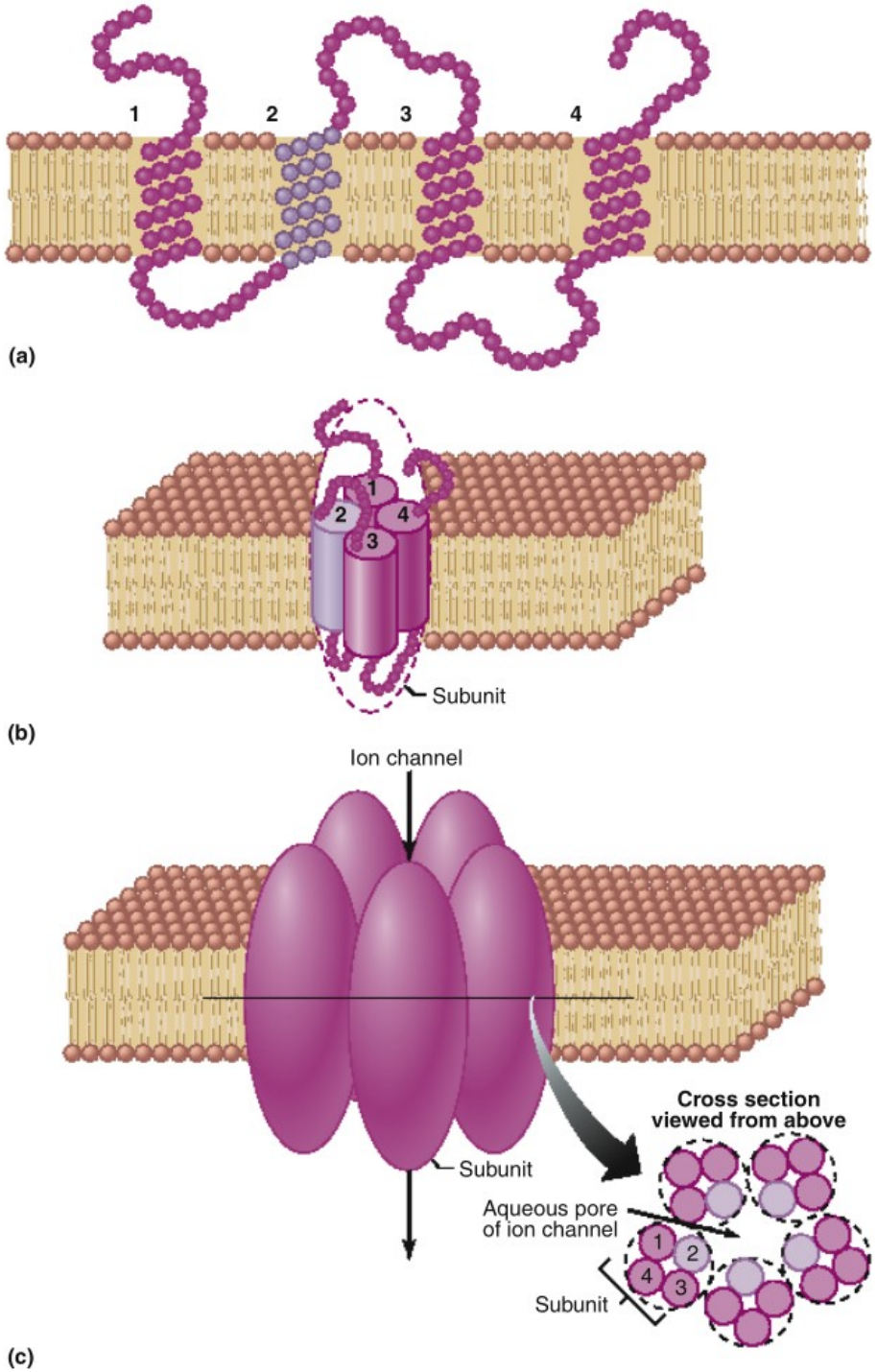


Figure 4.5 Model of an ion channel composed of five polypeptide subunits. Individual amino acids are represented as beads. (a) A channel subunit consisting of an integral membrane protein containing four transmembrane segments (1, 2, 3, and 4), each of which has an alpha-helical configuration within the membrane. Although this model has only four transmembrane segments, some channel proteins have as many as 12. (b) The same subunit as in (a) shown in three dimensions within the membrane, with the four transmembrane helices aggregated together and shown as cylinders. (c) The ion channel consists of five of the subunits illustrated in (b), which form the sides of the channel. As shown in cross section, the helical transmembrane segment 2 (light purple) of each subunit forms each side of the channel opening. The presence of ionized amino acid side chains along this region determines the selectivity of the channel to ions. Although this model shows the five subunits as identical, many ion channels are formed from the aggregation of several different types of subunit polypeptides.

Iontové kanály

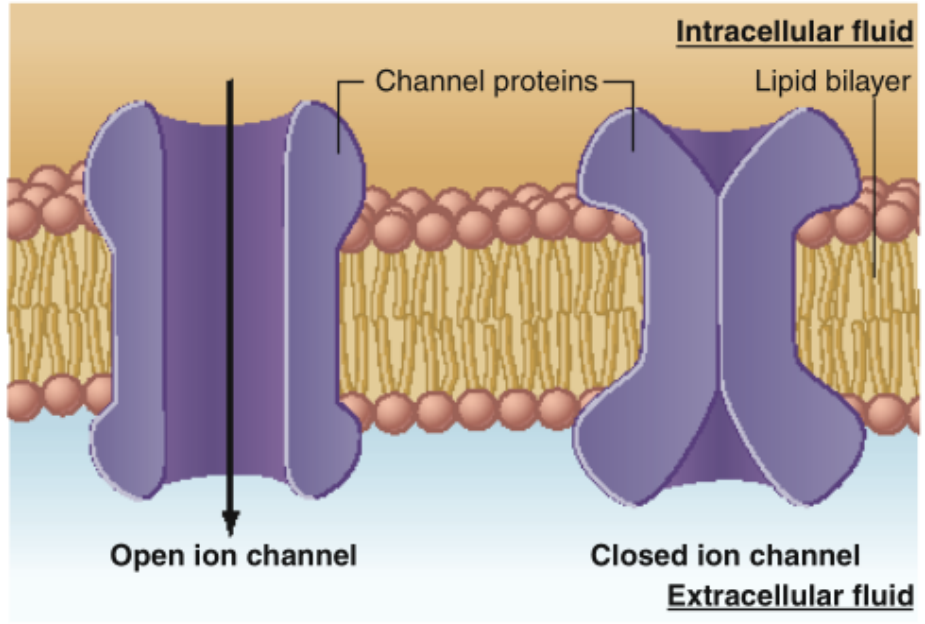
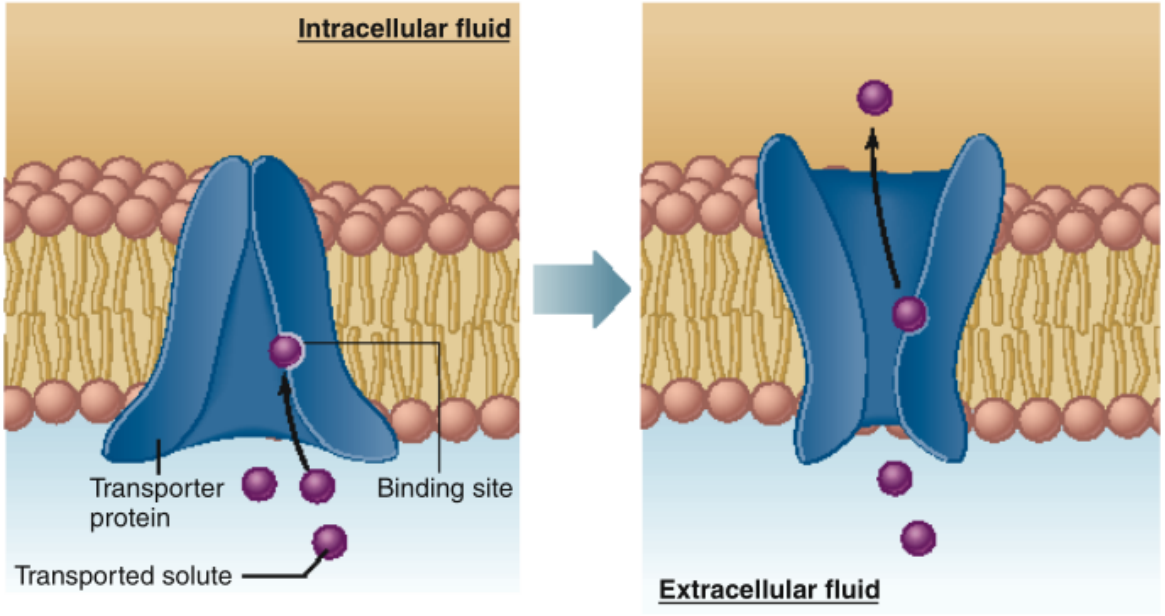
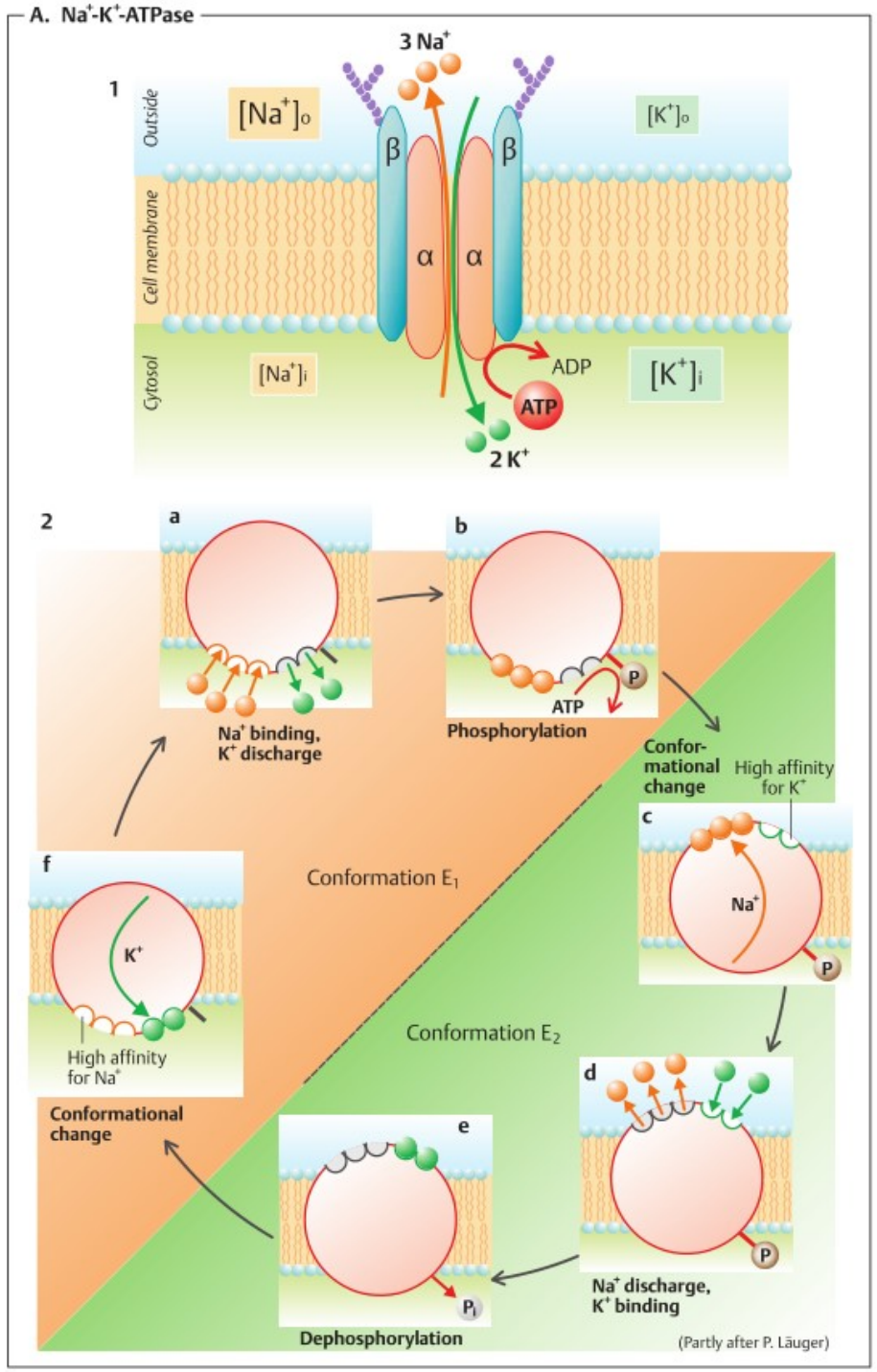


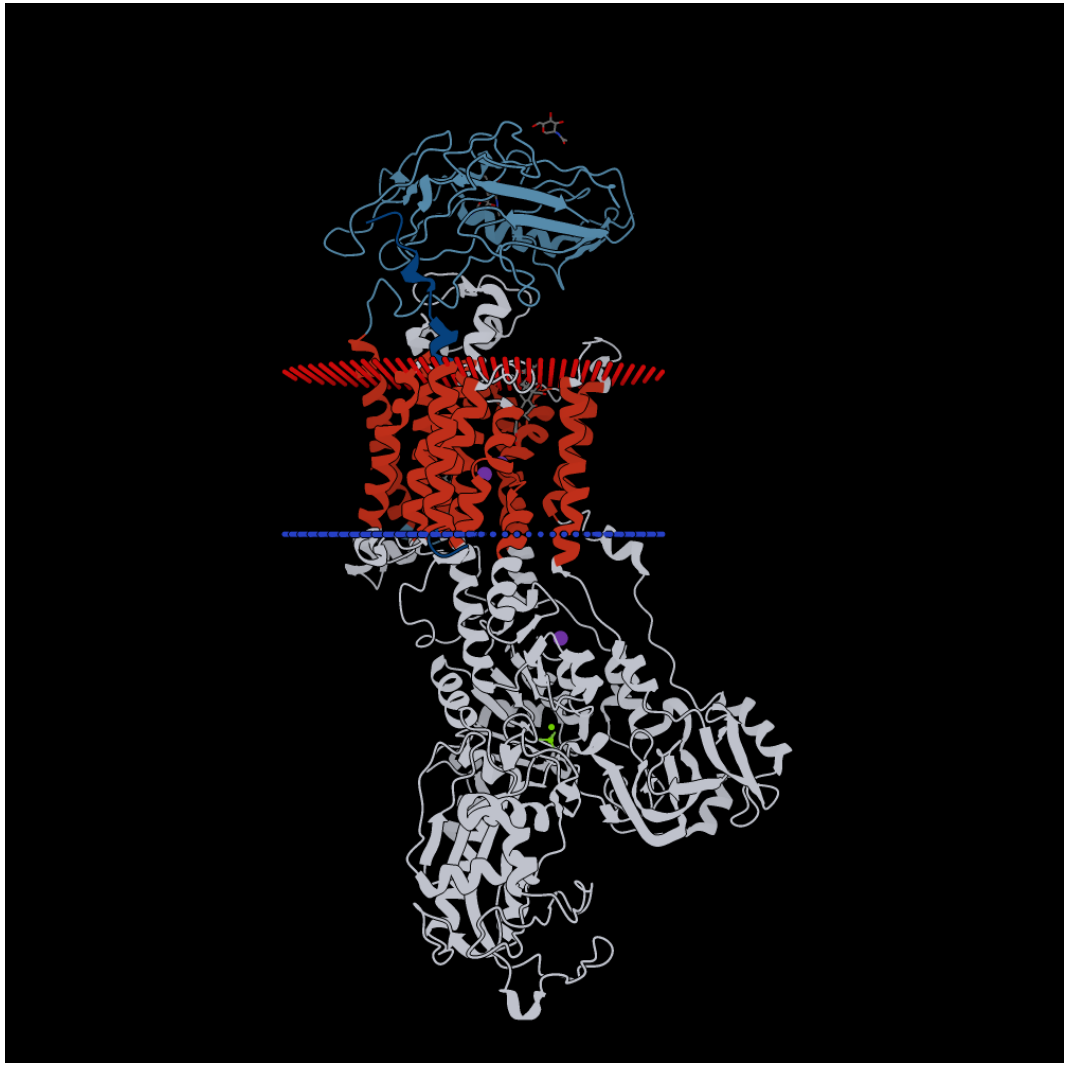
Figure 4.7 As a result of conformational changes in the proteins forming an ion channel, the channel may be open, allowing ions to diffuse across the membrane, or may be closed. The conformational change is grossly exaggerated for illustrative purposes. The actual conformational change is more likely to be just sufficient to allow or prevent an ion to fit through.



AP|R **Figure 4.8** Model of mediated transport. A change in the conformation of the transporter exposes the transporter binding site first to one surface of the membrane then to the other, thereby transferring the bound solute from one side of the membrane to the other. This model shows net mediated transport from the extracellular fluid to the inside of the cell. In many cases, the net transport is in the opposite direction. The size of the conformational change is exaggerated for illustrative purposes in this and subsequent figures.

Na^+-K^+ pumpa



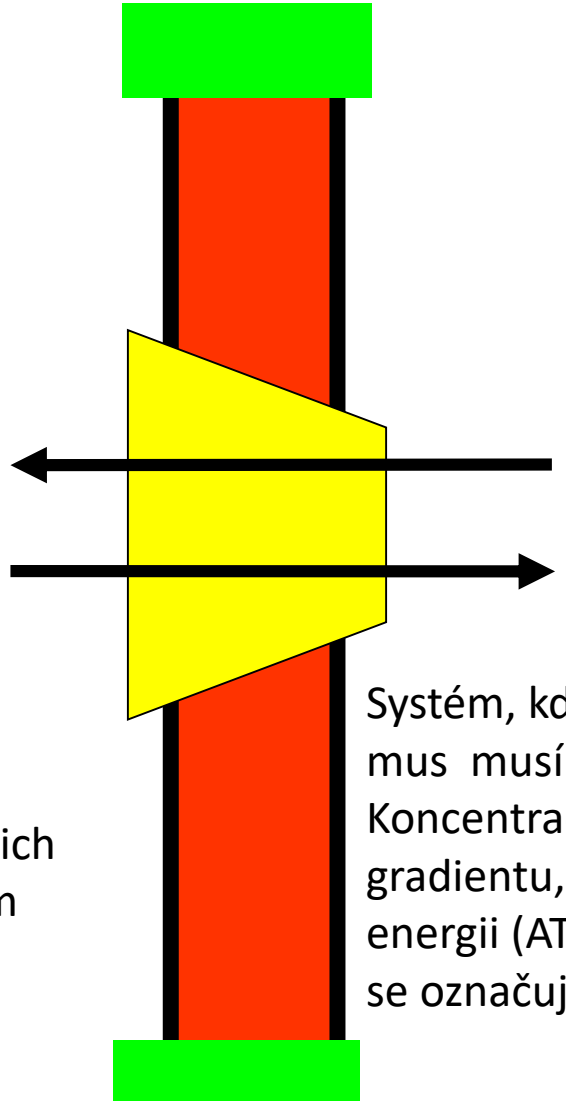


3A3Y

Iontové poměry Na-K-ATPasy

EXTRACELULÁRNÍ TEKUTINA

INTRACELULÁRNÍ TEKUTINA



Na^+ = 145 mMol/l

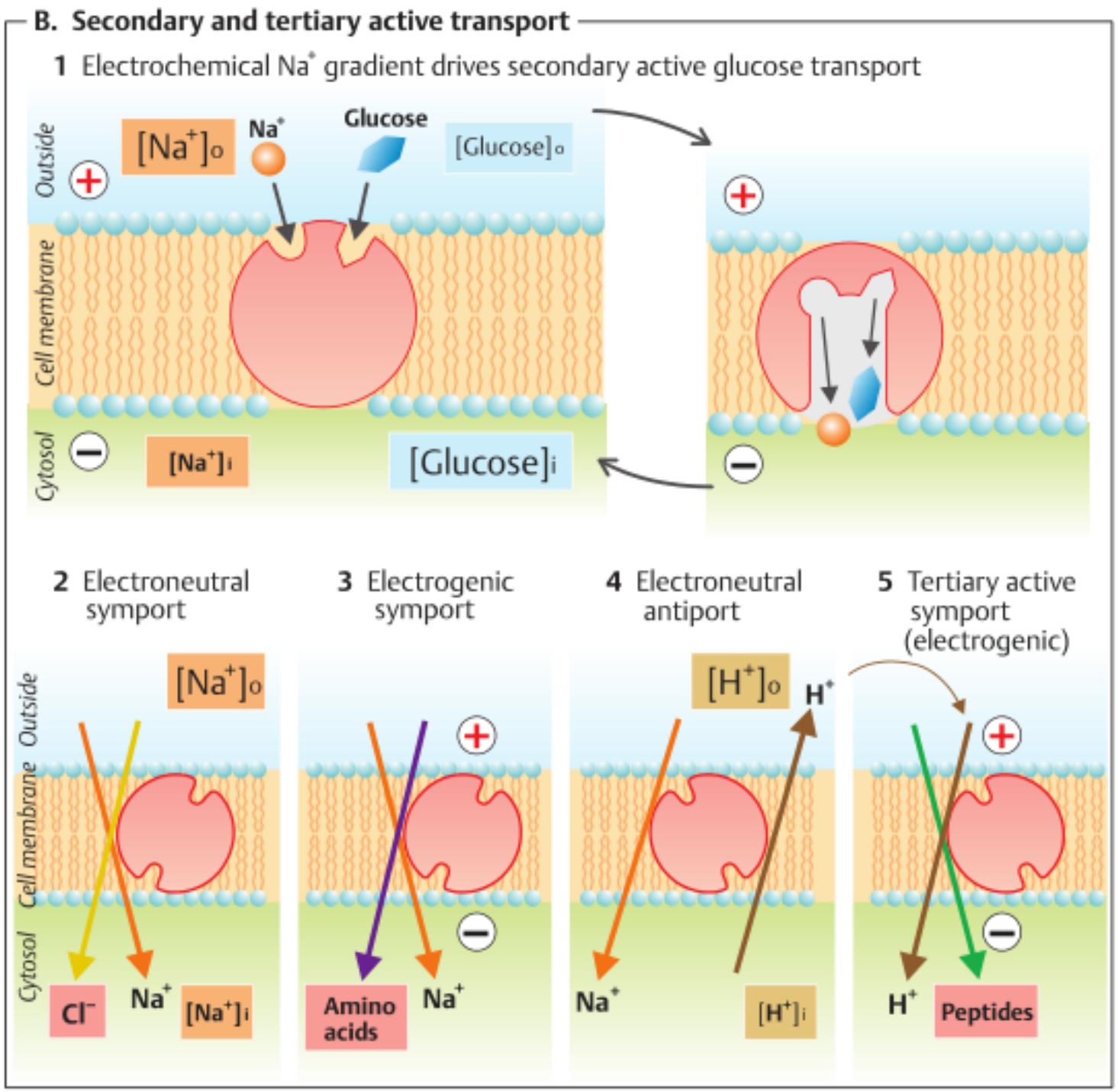
K^+ = 5 mMol/l

Na^+ = 10(15) mMol/l

K^+ = 150 mMol/l

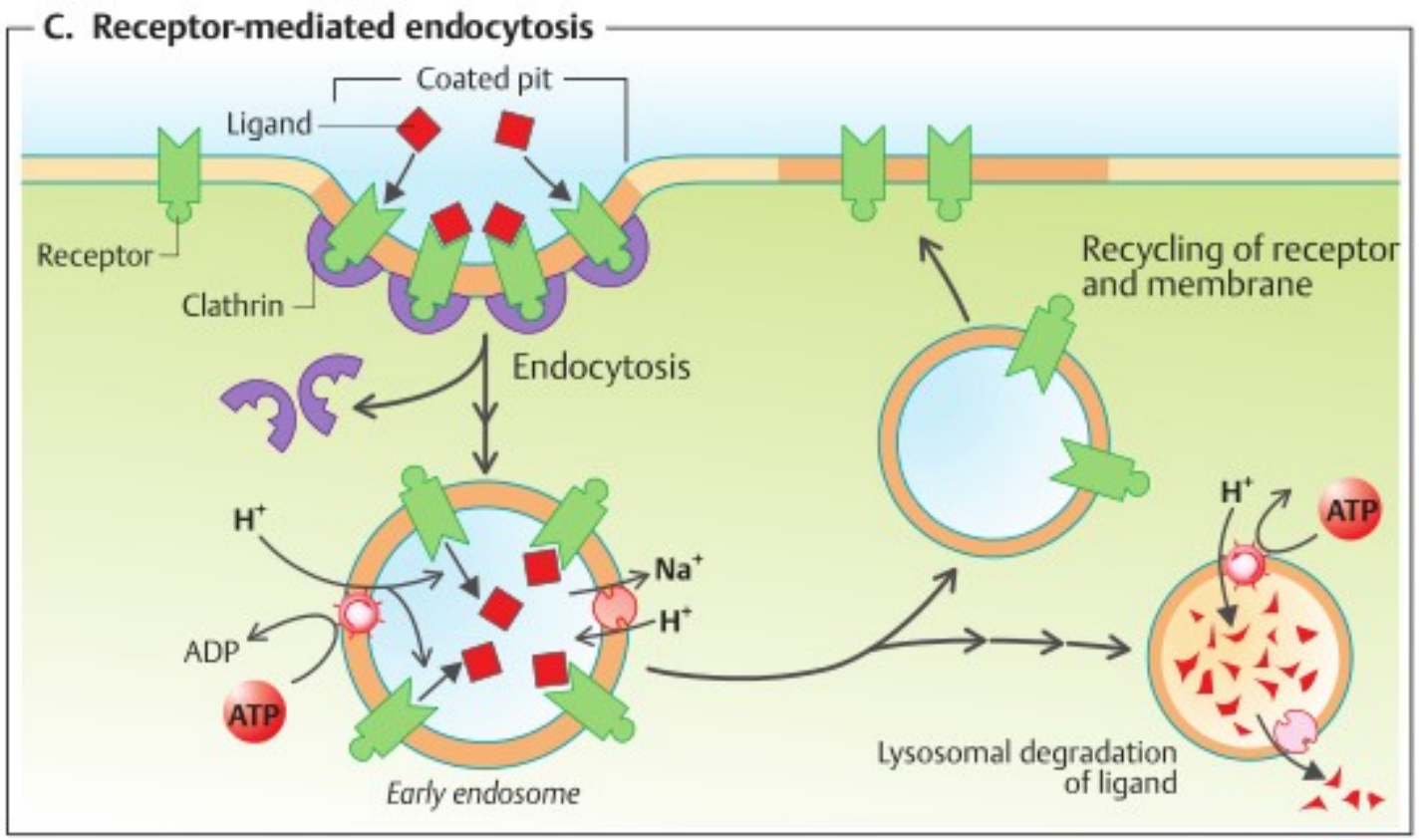
Je zřejmé, že Na-K-ATPasa přesouvá Na^+ a K^+ proti jejich koncentračním gradientům

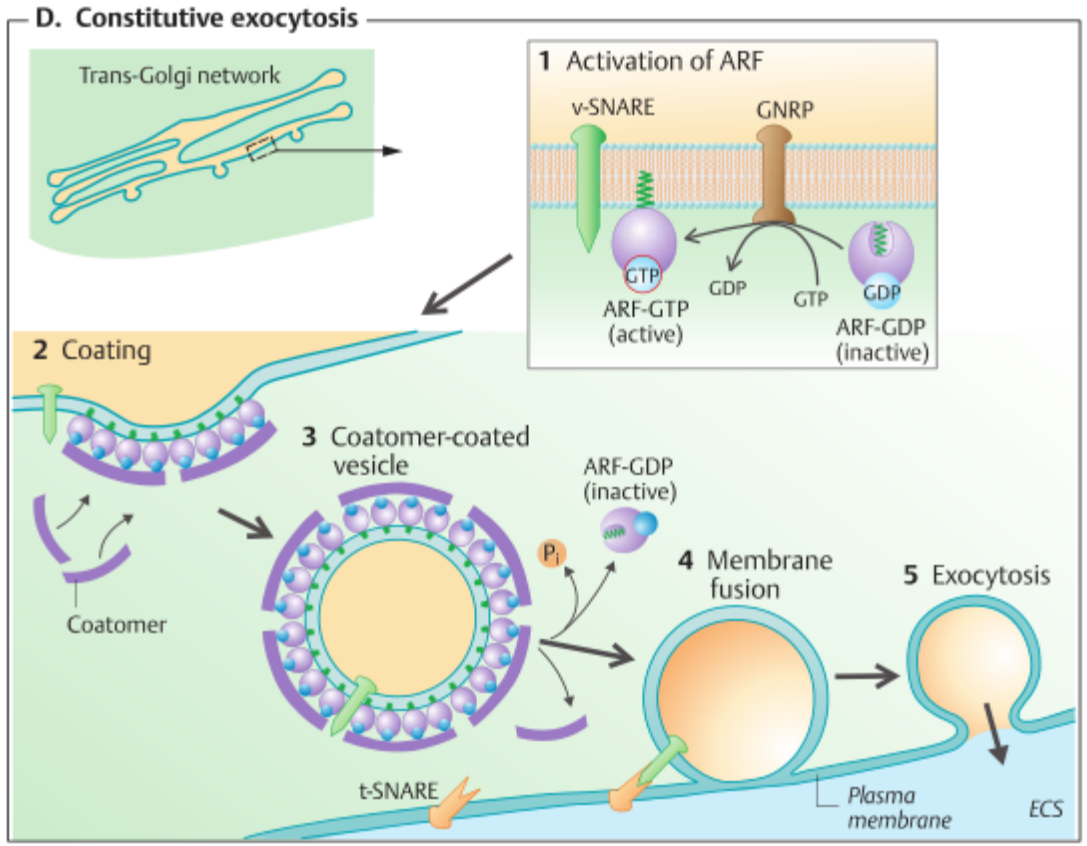
Systém, kdy transportní mechanismus musí působit silou proti síle Koncentračního (elektrochemického) gradientu, spotřebovává chemickou energii (ATP). Tento mechanismu se označuje AKTIVNÍ TRANSPORT



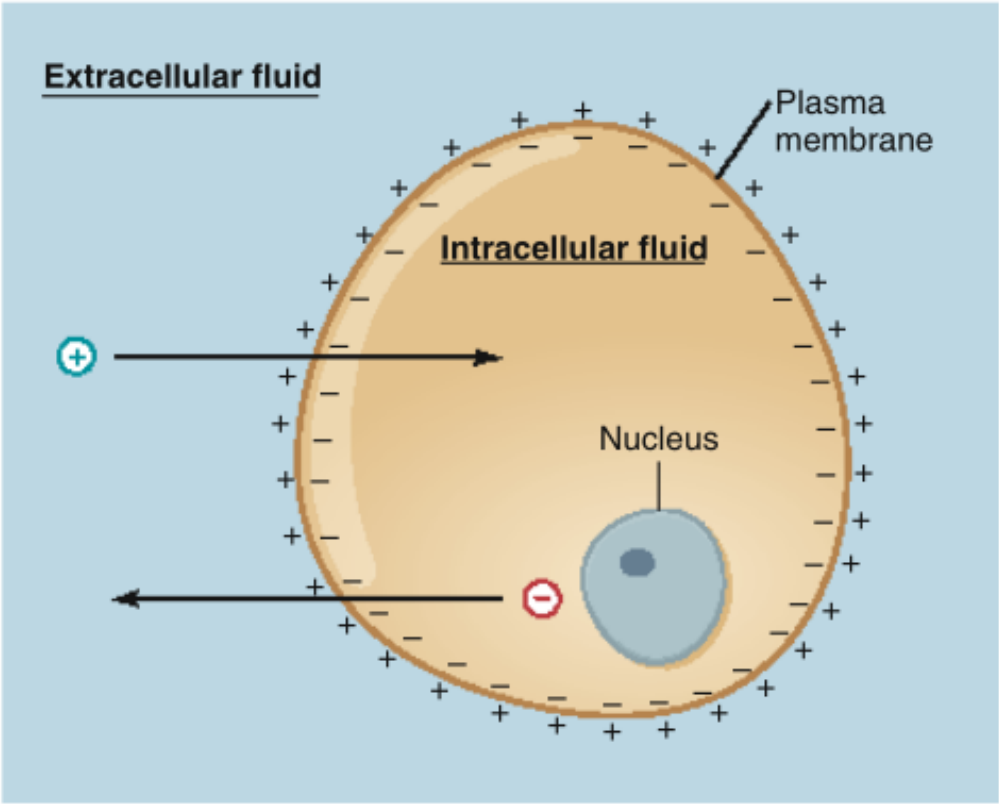
Sekundární aktivní transport - kotransport

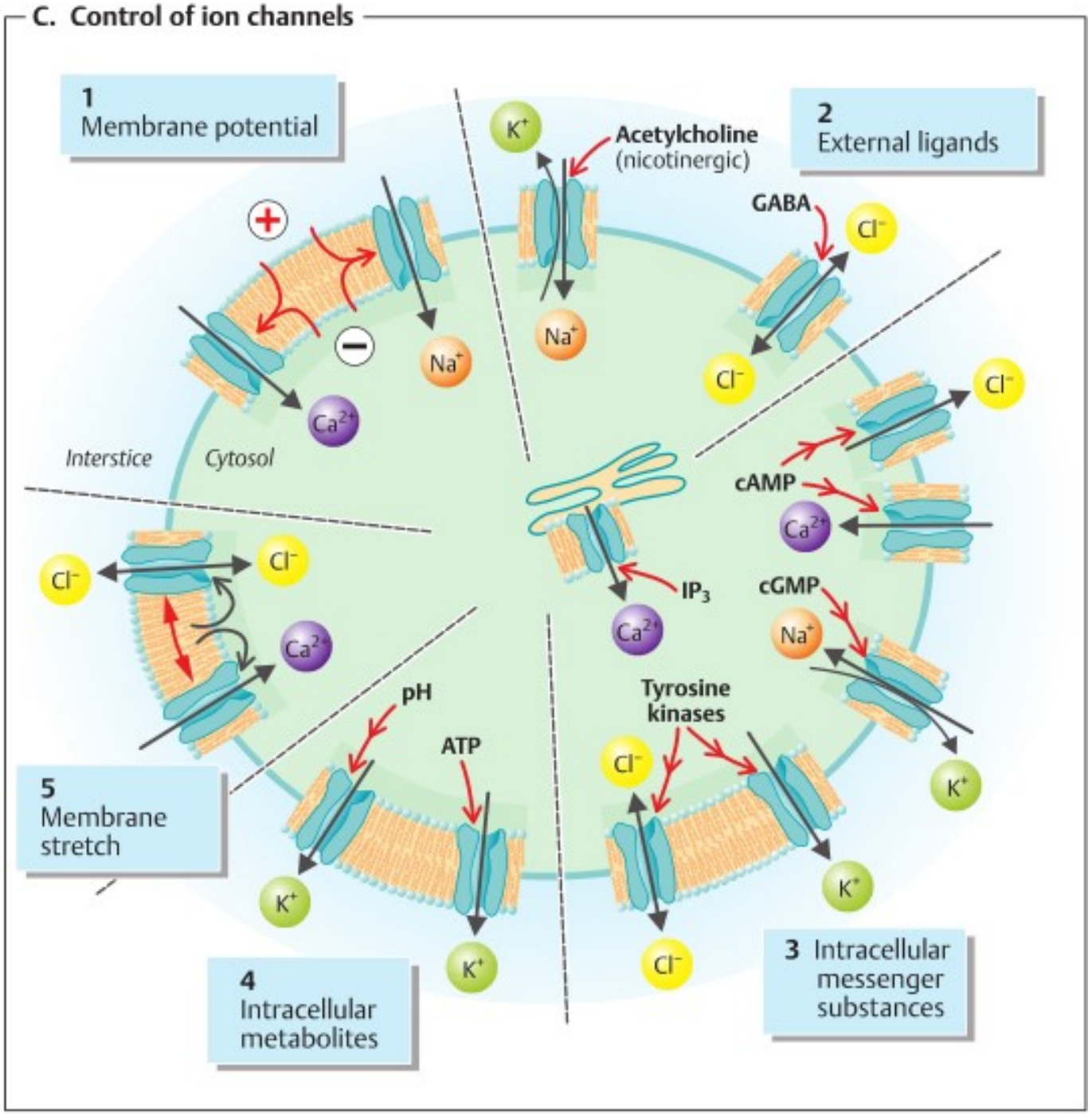
Terciární aktivní transport – endo/exo-cytosa





Iontové kanály – rozložení el. náboje





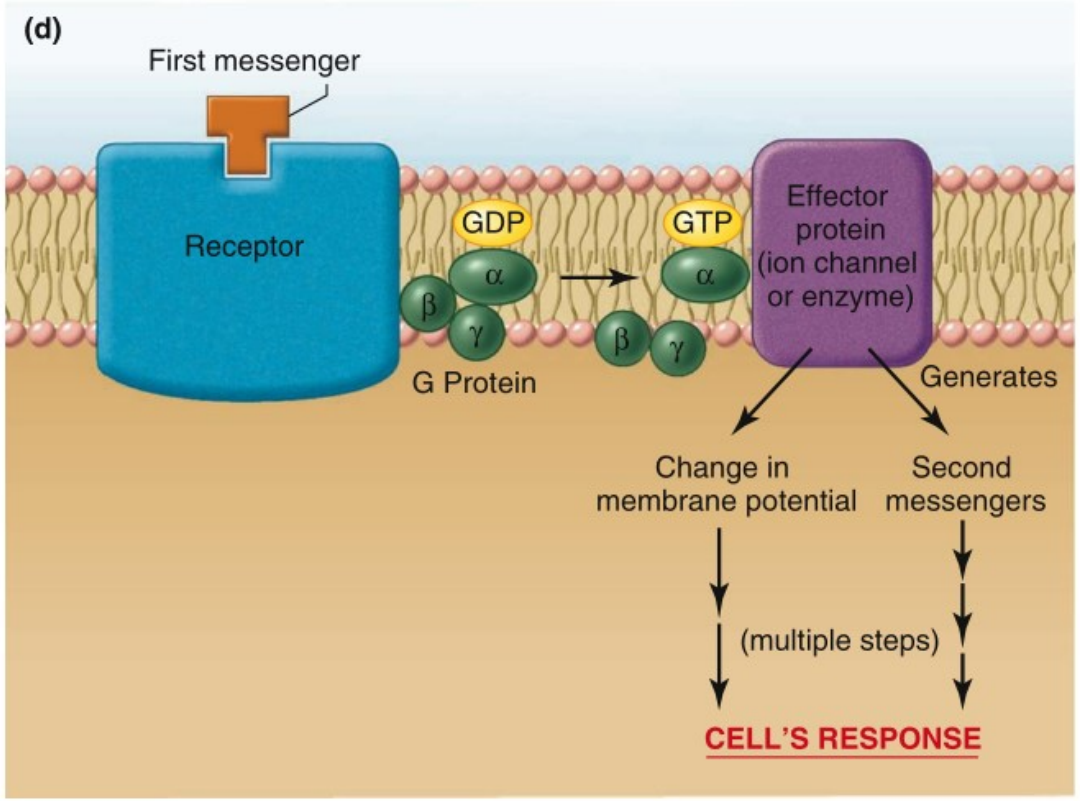
Přenos signálů přes membránu

Tab. 1.1. Zjednodušený přehled přenosu informace extracelulárními chemickými signály. AA – kyselina arachidonová, cAMP – cyklický adenosinmonofosfát, DAG – diacylglycerol, proteiny G – po vazbě GTP uvolňuje své podjednotky α, β a γ spouštějící reakce enzymatické nebo ovlivňující přímo kanály, HETE – hydroxyeikosatetraenoáty, IP_3 – inositoltrifosfát, MLCK – myozinkináza, PKA – proteinkináza A, PKC – proteinkináza C, PLC – fosfolipáza C, ER – endoplazmatické retikulum. [1,2] viz kap. 2

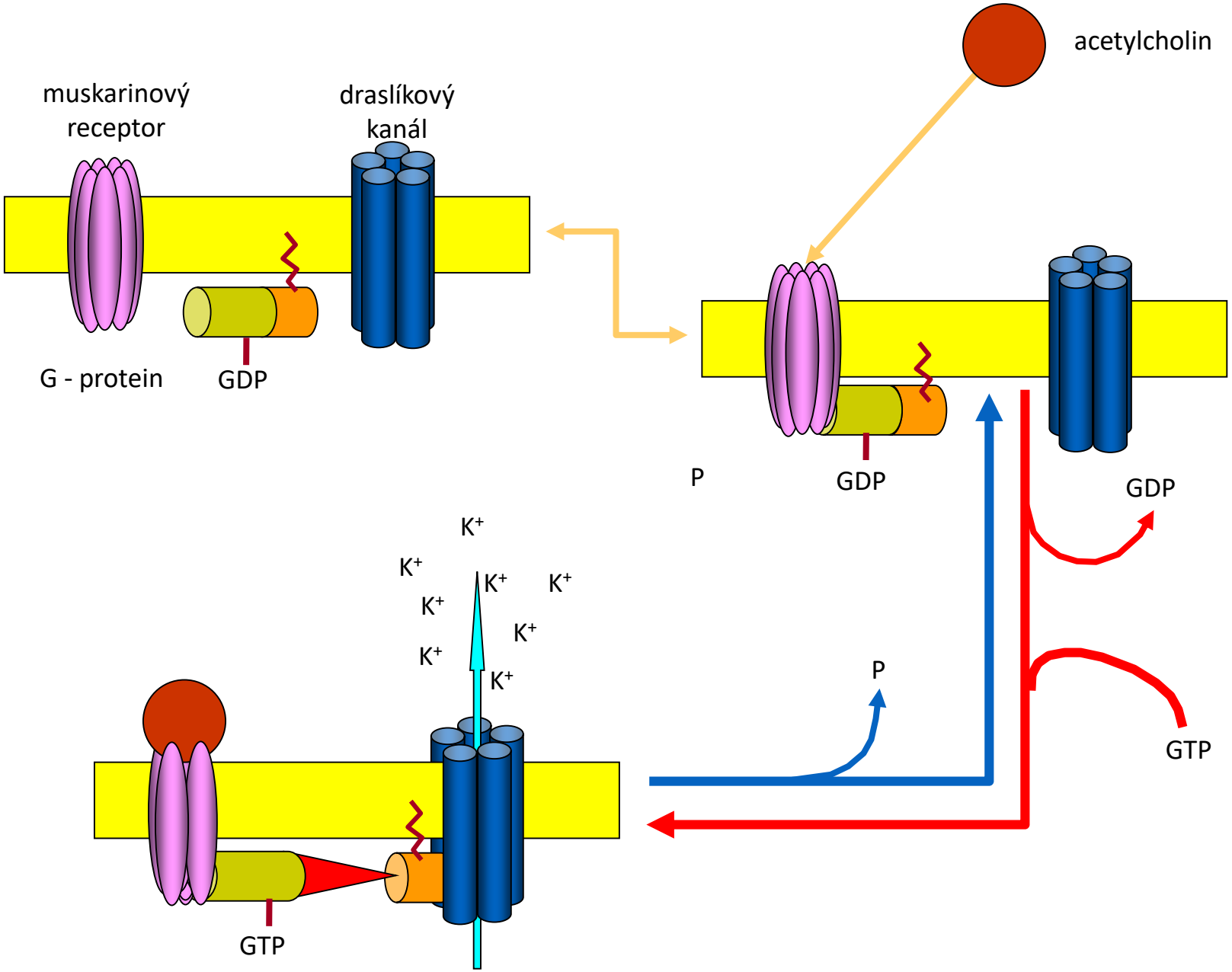
První posel (signální molekula)	Receptor	Převaděč (transducer)	Primární efektor	Druhý posel	Sekundární efektor	Konečný mechanismus	Příklady řízených funkcí
Neurotransmiter Hormon Protilátka Peptid Lék Toxin	**membránový**						
	ionotropní[1]		iontový kanál			transport iontů, fosforylace kanálů a proteinů	depolarizace/hyperpolarizace membrány
	metabotropní[2]	proteiny Gαβγ	adenylátcykláza	cAMP	PKA		
			fosfolipáza C	DAG	PKC		
					Ca kalmodulin	MLCK	kontrakce svalové buňky
				IP_3	Ca^{2+} kanál ER	↑ Ca_i^{2++}	
			fosfolipáza A_2	AA	cyklooxygenáza	prostaglandiny, prostacykliny, tromboxan	motorika cév a bronchů, alergie, zánět, tromboagregace, buněčná proliferace
					lipooxygenáza	leukotrieny	
					epoxygenáza	HETE	
	katalytický (7 typů)	autofosforylace (tyrosin, serin)	aktivace kináz nebo fosfatáz			transkripční faktory	kontrola transkripce genů, aktivace lymfocytů
	jaderný	cytoplazmatický protein	difúze do jádra buňky				

G-proteiny – přenos signálu přes membránu

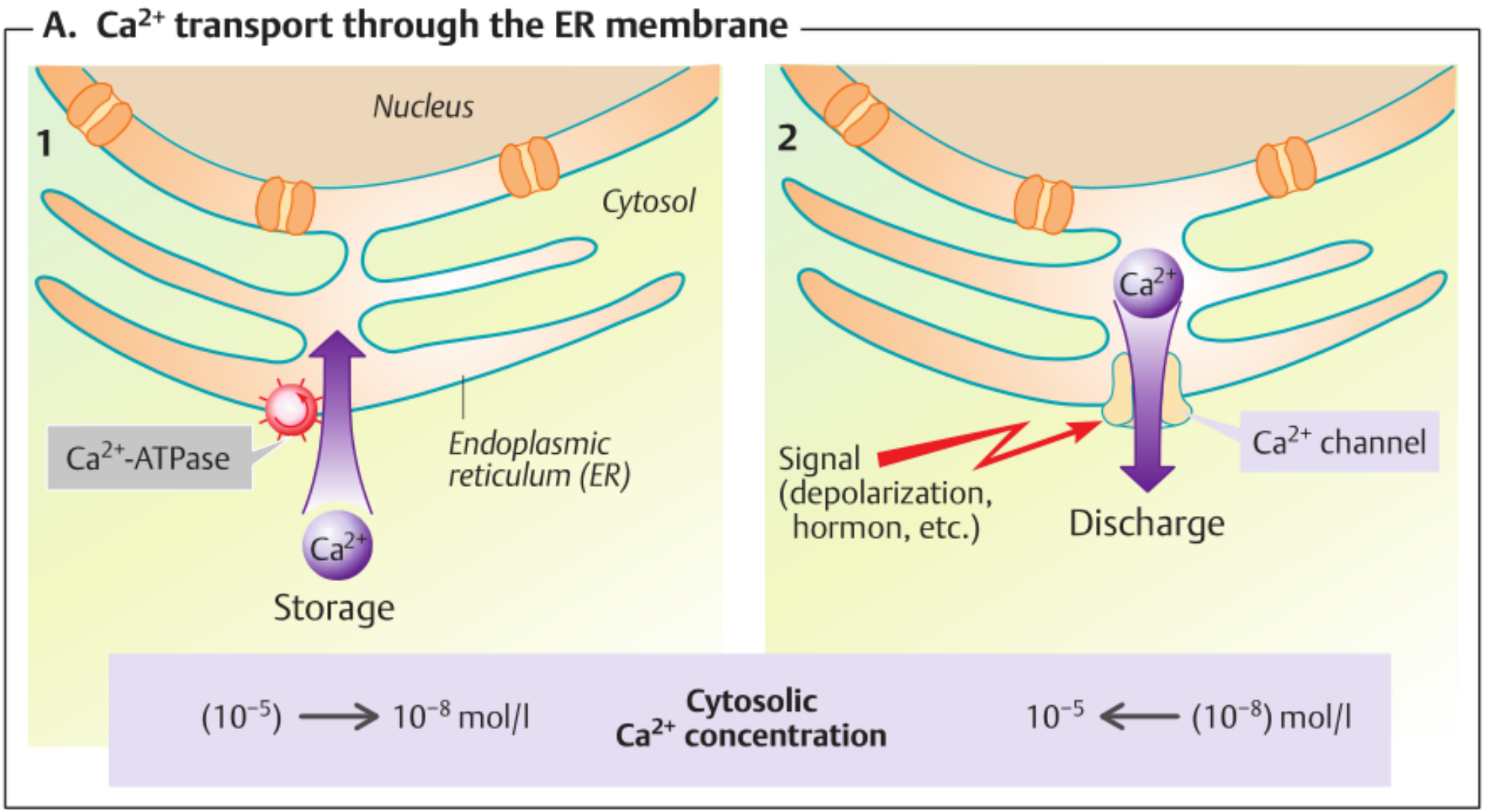
- 3 podjednotky (α, β, γ)
- Při vazbě **<u>prvního posla</u>** -> disociace α
- α aktivuje vazebně řízený iontový kanál (jiný membránový protein)
- **<u>Druhý posel</u>**, nejčastěji cAMP, inositol trifosfát (IP_3), diacyl glycerol (DAG), fosfolipasa C
- Zesílení signálu



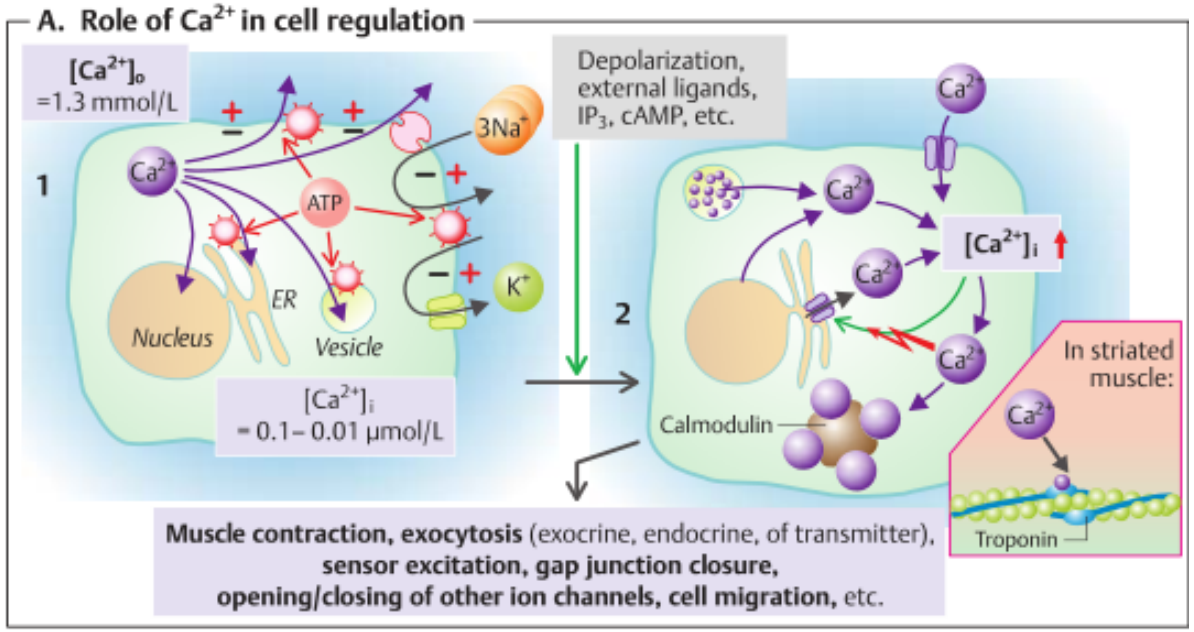
Vazebně řízený kanál spojený s G-proteinem

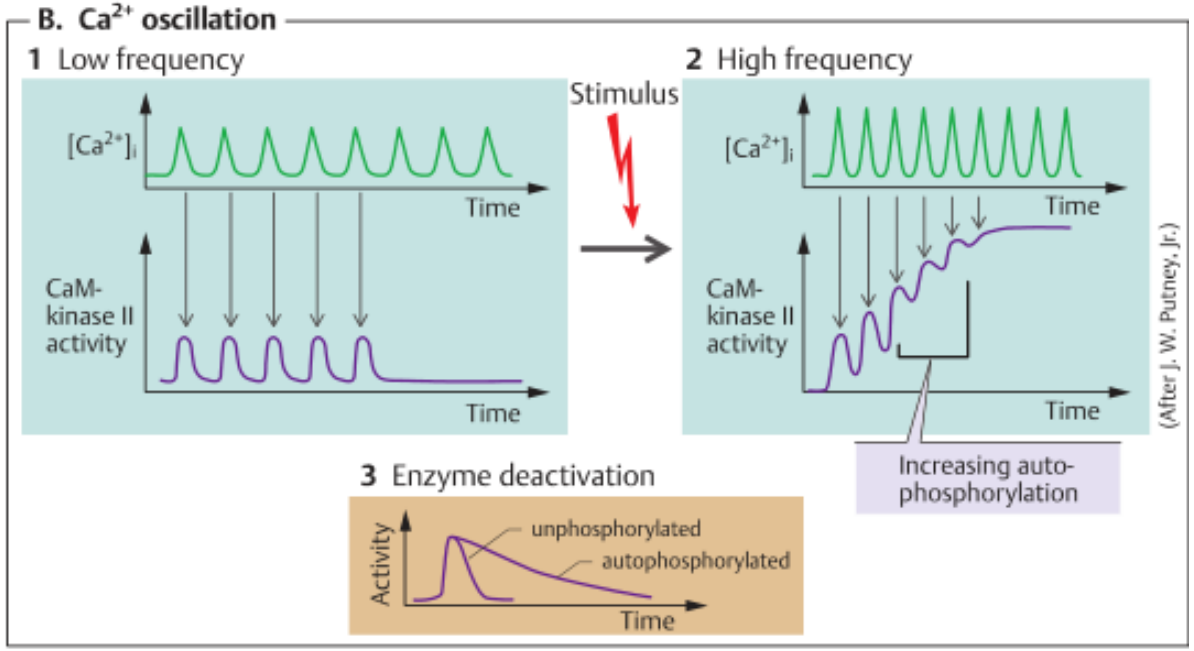


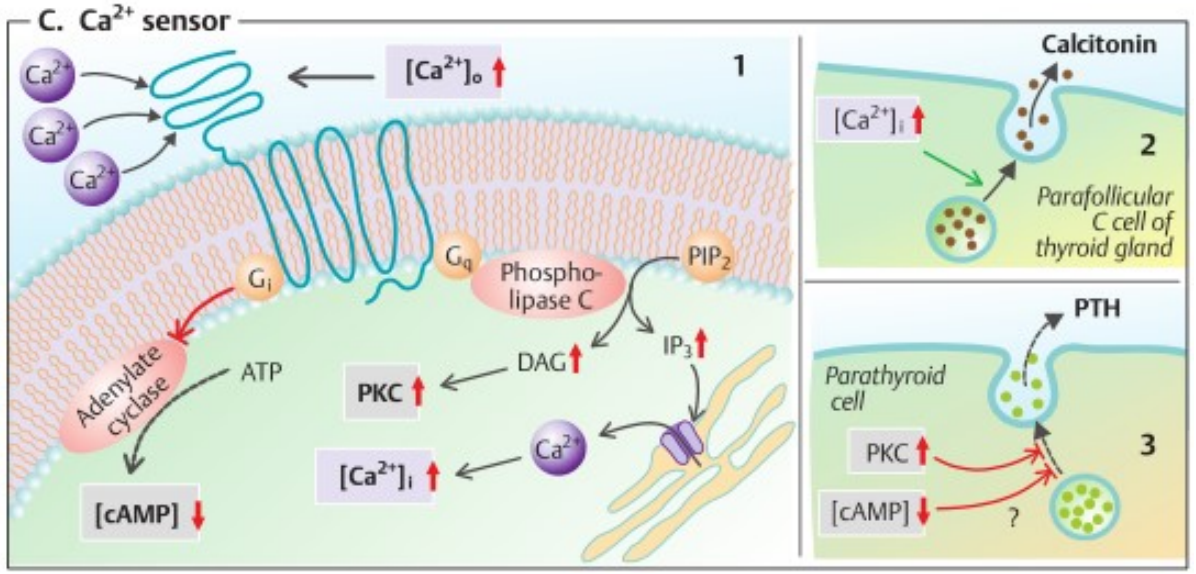
Transport vápníku z ER



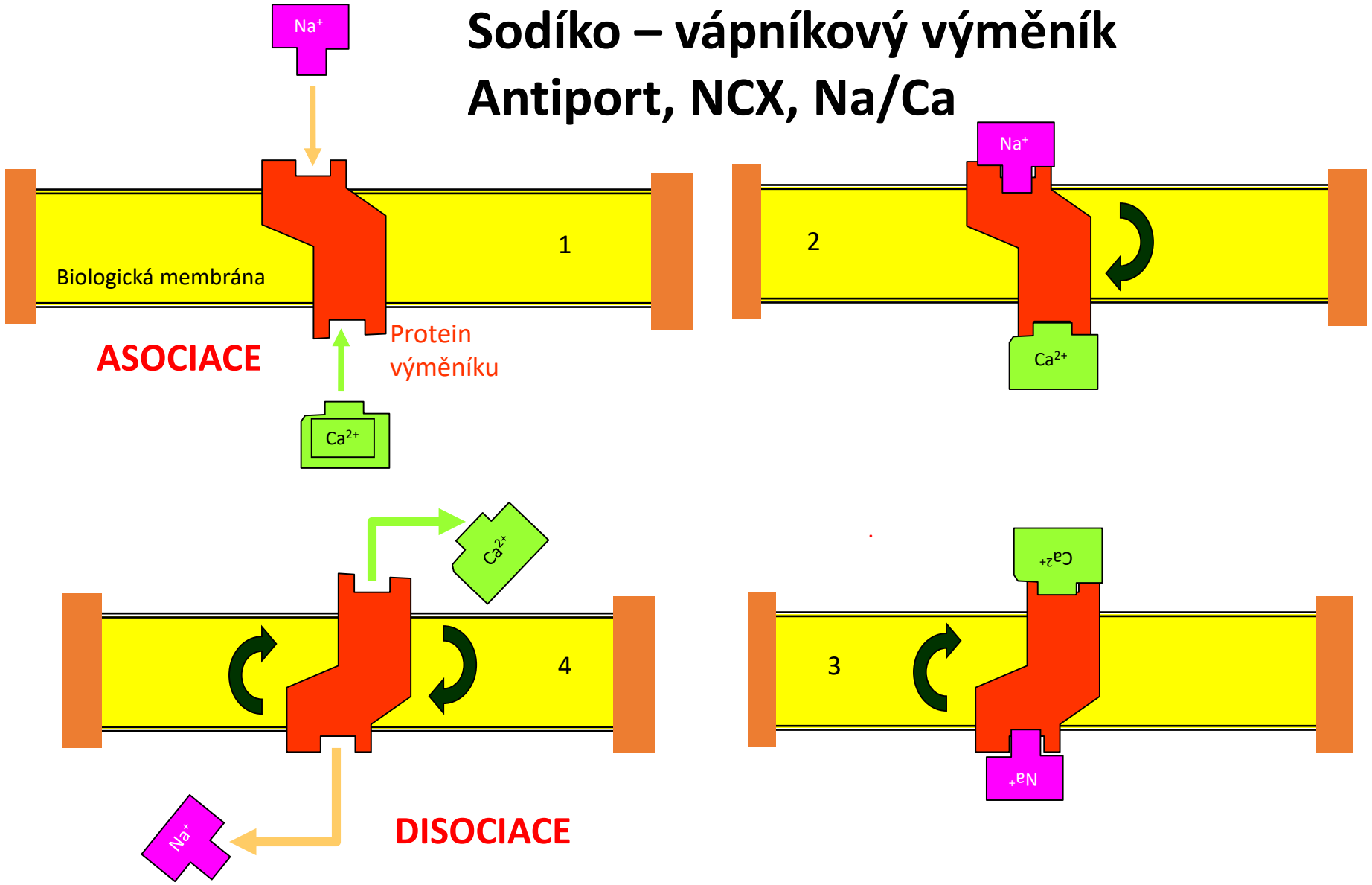
Role Ca^{2+} v buněčné regulaci

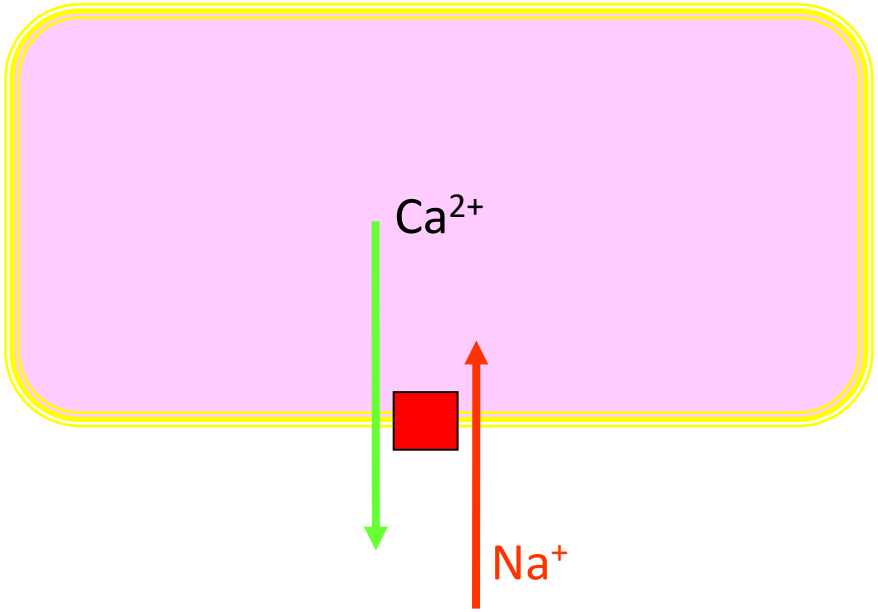






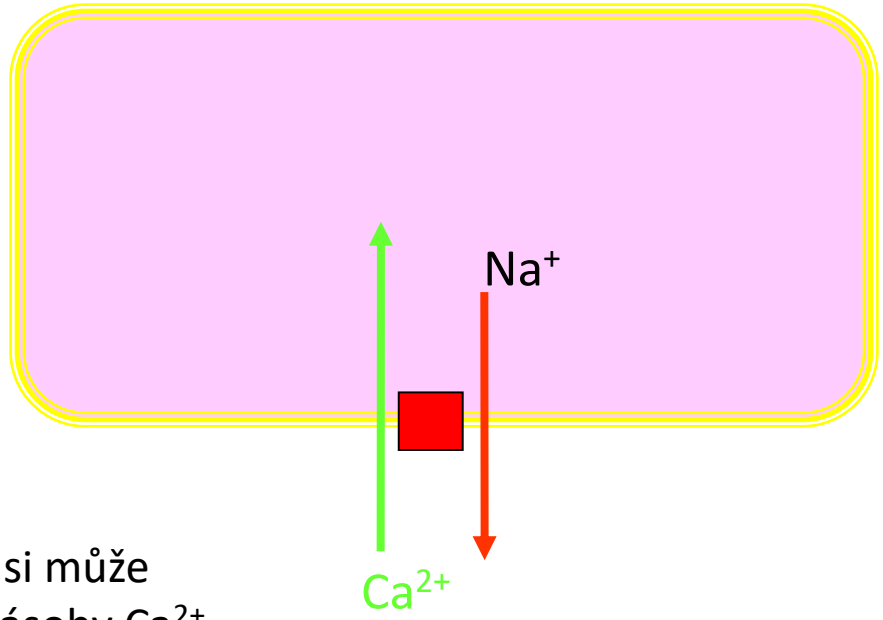
Sodíko – vápníkový výměník
Antiport, NCX, Na/Ca

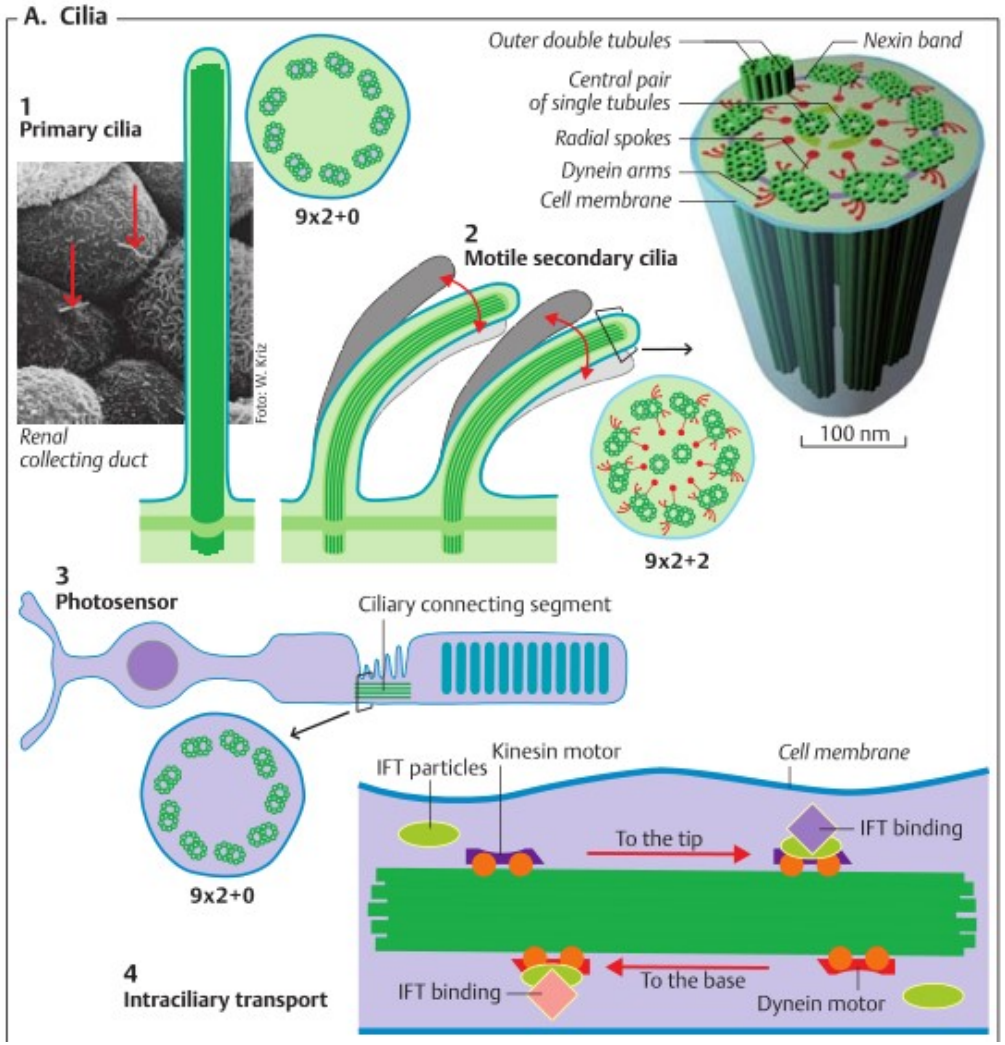
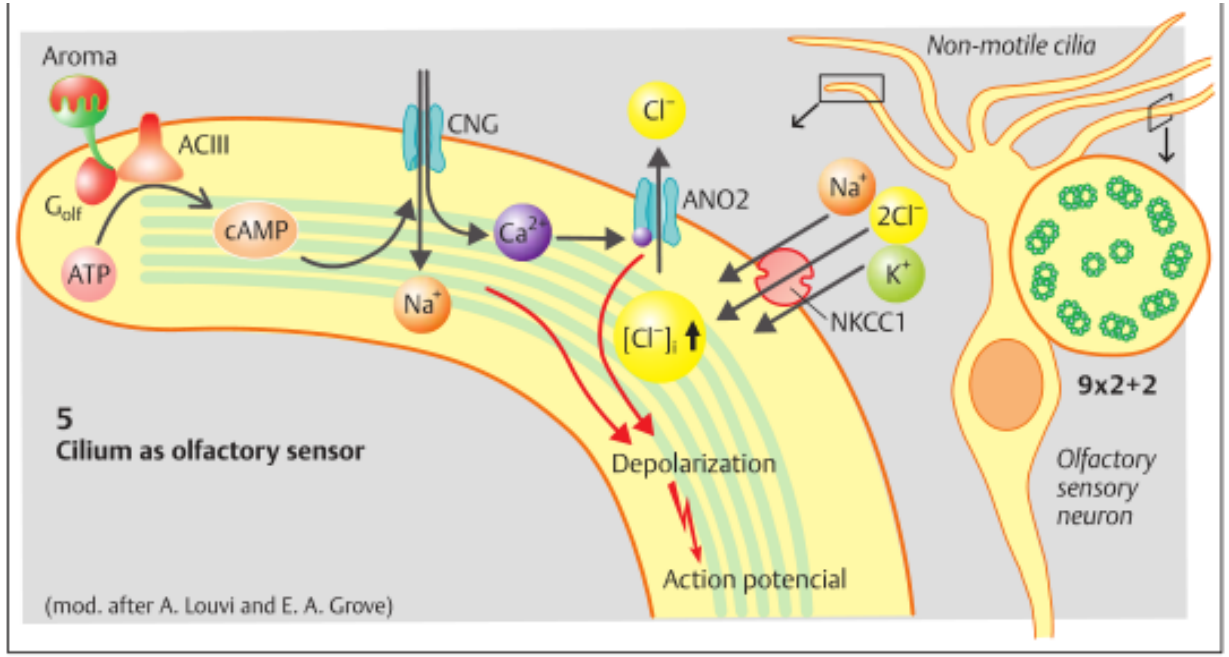




Každá buňka se brání tzv. <u>vápníkovému přetížení</u>.
Normální intracelulární koncentrace Ca^{2+} je menší než 10^{-7}mol.l^{-1}.
K vápníkovému přetížení jsou mimořádně citlivé buňky myokardu a nervové buňky.

NCX se stává významným nástrojem buněčné vápníkové homeostázy.



Za zcela mimořádných okolností si může Buňka doplňovat intracelulární zásoby Ca^{2+}

Cilie – primární v každé buňce, sekundární ve specializovaných