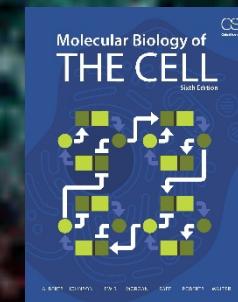


Transport of ions and small molecules across membranes

- Lecture 5 -

- I. Overview
- II. Channels
- III. Transporters
- IV. ATP-pumps
- V. Membrane potential

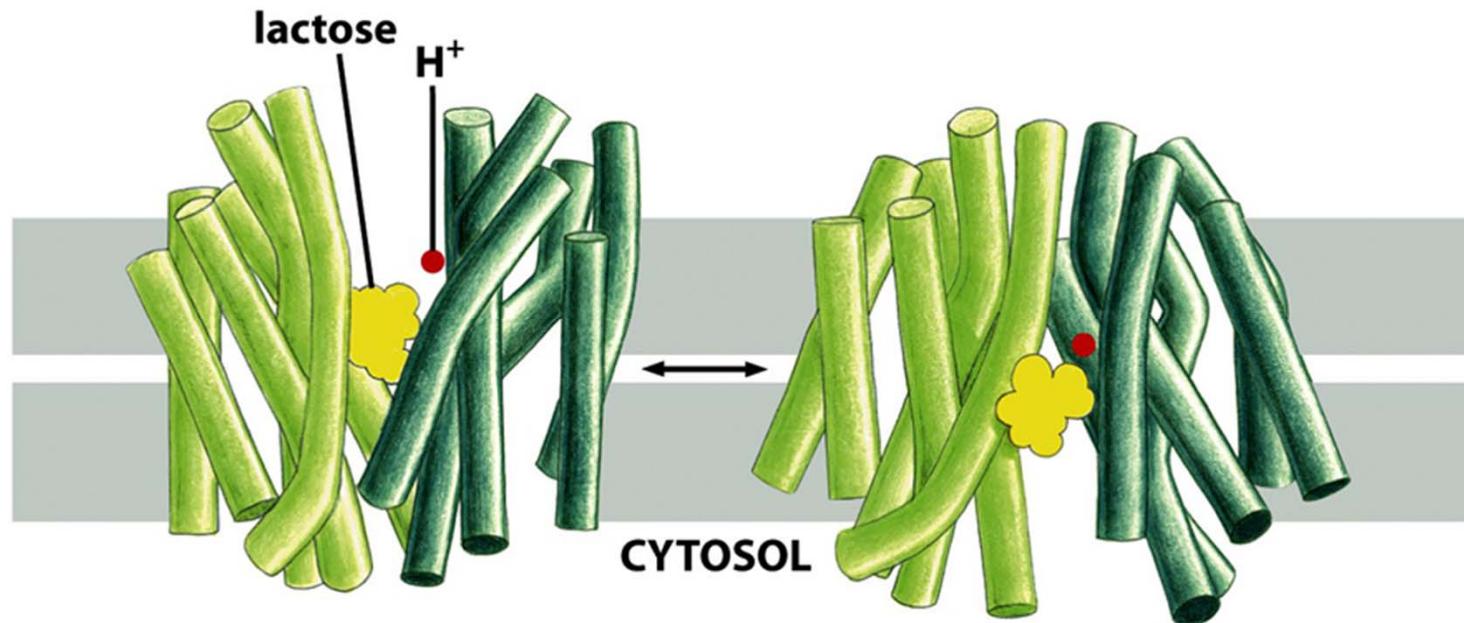


Chapter 11

Lactose permease: symport of lactose and H⁺ in *E.coli*

Lactose transport is driven by H⁺ symport

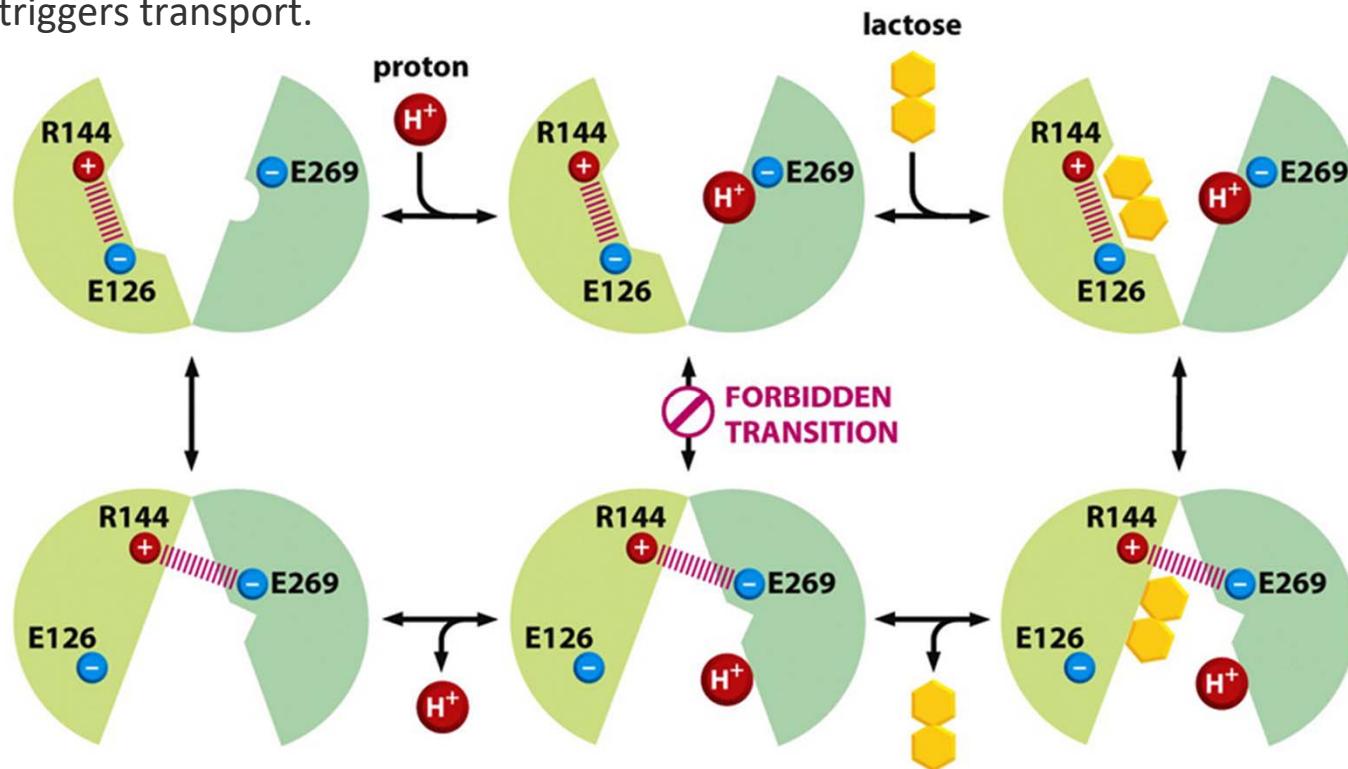
- Lactose permease: 12 TMDs



But how is the molecular mechanism?

Lactose permease transport- at molecular level

- Arginine (R) 144 forms bond with glutamic acid (E) 126, leaving glutamic acid (E)269 free to accept a proton (H^+) and together with lactose binding a conformational exchange occurs that triggers transport.



- After the change, R144 forms now a bond with E269 which destroys the binding site for lactose, triggering release, and after the proton is also released, the molecule flips back to the starting position, exposing the bindin sites (R144 forms the bond with E126).....

Antiporter: a way for cells regulate cellular pH

Facts:

- **pH of cells and intracellular compartments is strictly controlled but pH greatly differs between compartments:** Cellular pH (cytosol): ~7.2
lysosomal pH: ~5.0
endoplasmic reticulum: ~7.2
- **Two options to alkalinize (up) regulate cytosolic pH:**
 - Option 1: Export of H⁺ out of the cell
 - Option 2: Neutralization of H⁺ in the cytosol by HCO₃⁻ import
$$\text{HCO}_3^- + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{CO}_2$$

Antiporter: a way for cells regulate cellular pH

Regulation of cytosolic pH option 1:

Export of H⁺ out of the cell to alkalinize pH (pH goes up!)

- **Na⁺-H⁺ exchanger:**
coupled influx of Na⁺ to the efflux of H⁺
 - the driving force for this antiport is the **Na⁺ gradient** across the PM.
 - Regulation activity of the **Na⁺-H⁺ exchanger** is increased by cytosolic low pH.

Antiporter: a way for cells regulate cellular pH

Regulation of cytosolic pH option 2:

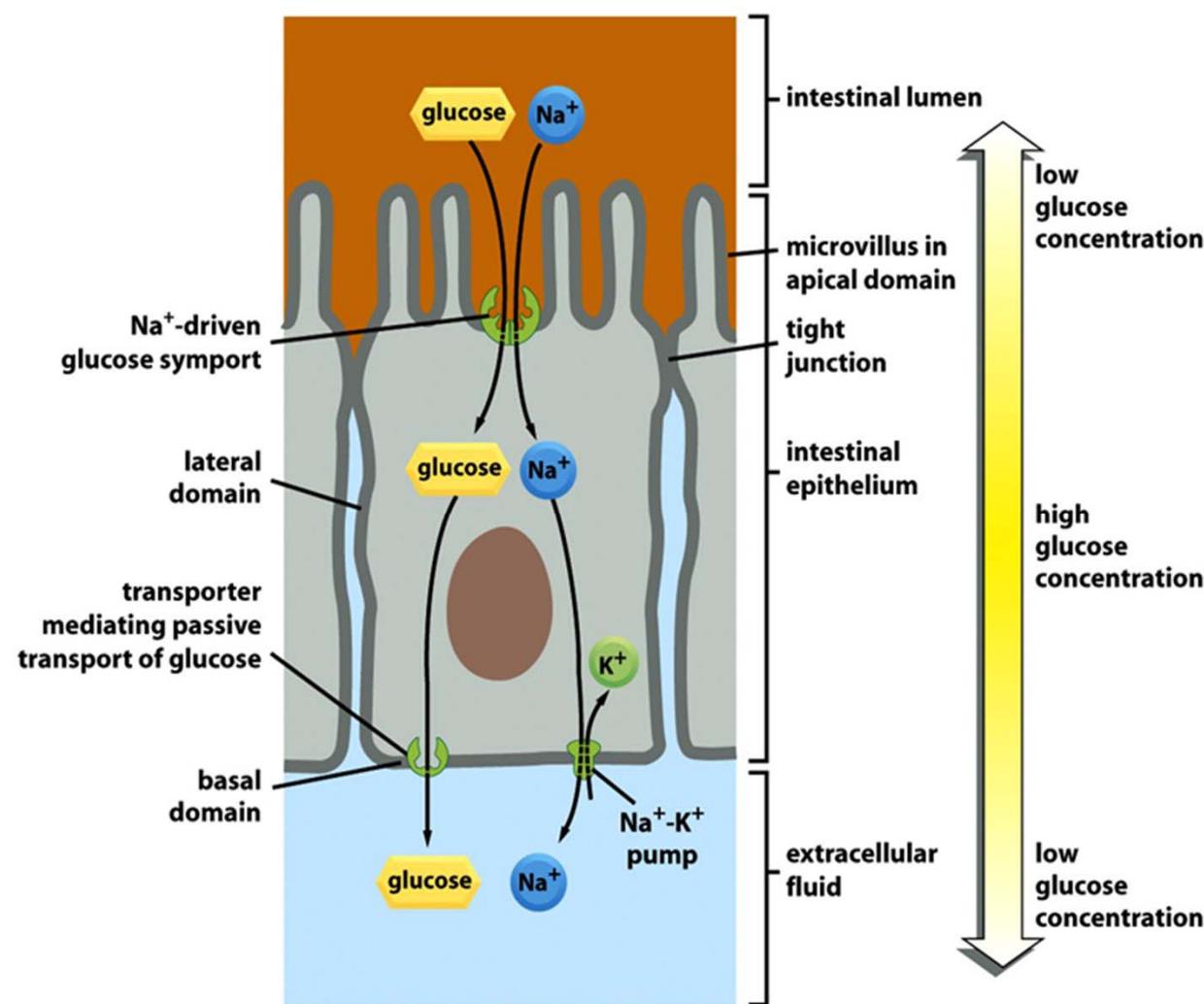
Neutralization of H^+ in the cytosol by HCO_3^- import to alkalinize (pH goes up!)



- **Na^+ -driven Cl^- - HCO_3^- exchanger:**
coupled influx of Na^+ and HCO_3^- to efflux of Cl^- and H^+
 - usually, NaCO_3 goes in and HCl goes out.
 - **twice as effective** as the **Na^+ - H^+ exchanger** because for each imported Na^+ , **one H^+ is neutralized** and a second H^+ is **exported**.
 - Regulation: activity of the **Na^+ -driven Cl^- - HCO_3^- exchanger** is increased by **low** cytosolic pH.
- **Na^+ -independent Cl^- - HCO_3^- exchanger** acidify (pH goes down)
coupled export of NaHCO_3 to influx of HCl
 - Regulation: activity of the **Na^+ -independent Cl^- - HCO_3^- exchanger** is increased by **high** cytosolic pH.

4. Transcellular transport

Transcellular transport: solute is transferred from one cell to the other cell.
Reason: Transporters are distributed nonuniformly in the plasma membrane



IV. ATP pumps - short overview

ATP pumps are classified in four groups:

- **P-type pumps:** Ca^{2+} pump
 Na^+-K^+ pump
- **F-type pumps:** ATP-synthase/ATPase
- **V-type pumps:** VHA ATPase
- **ABC transporters:** Multidrug resistance protein (MDR)

Common feature of all pumps:

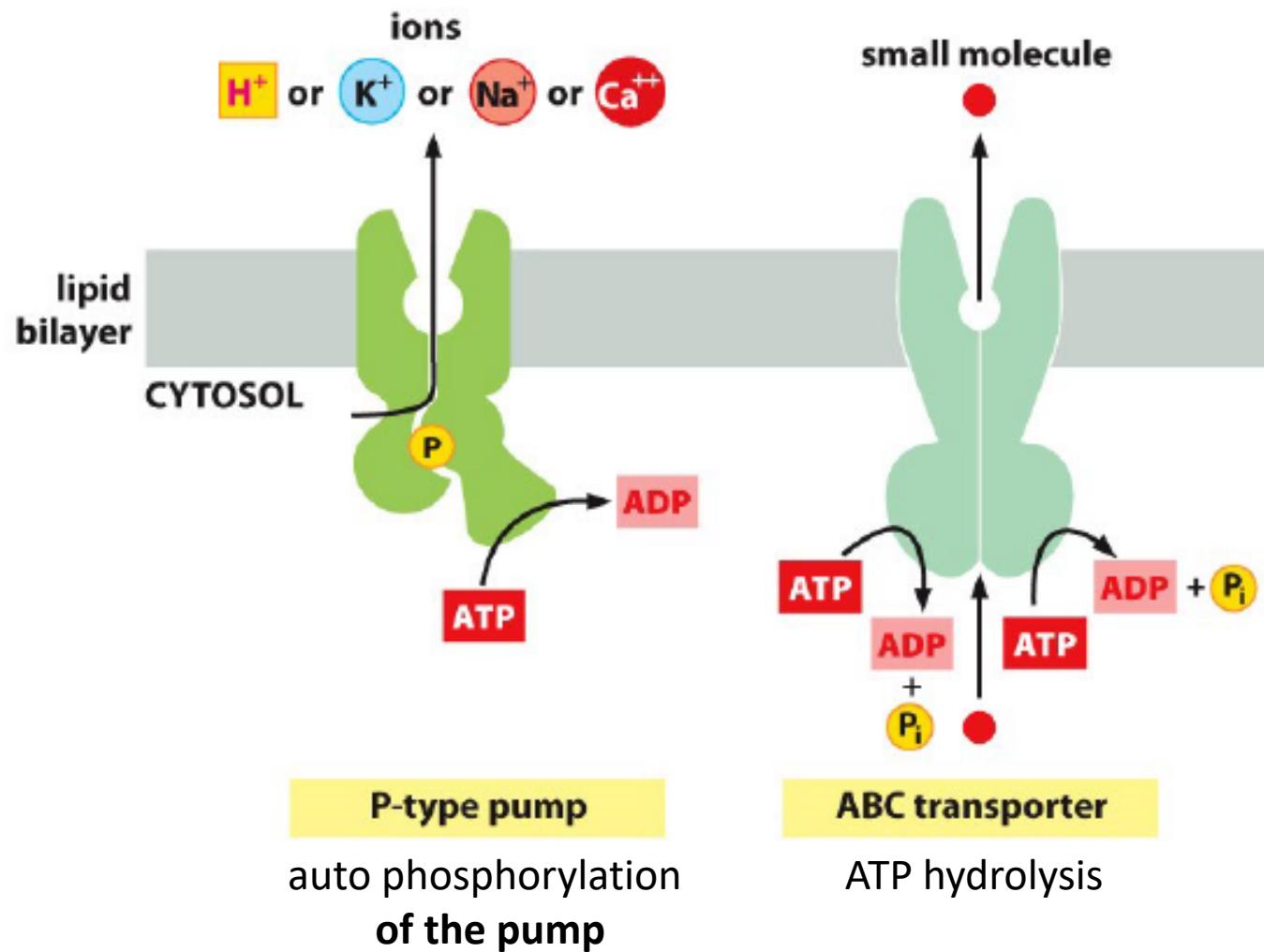
One or more ATP binding sites on the **cytosolic portion** of these transporters

ATP pumps: what do they transport?

- P-type, F-type and V-type pumps **transport ions**,
(as do **some** ABC transporters)
- **but most ABC transporters** transport **small molecules**:
 - amino acids
 - sugars
 - peptides
 - lipids
 - drugs, etc.
- ALL pumps use **ATP** as energy source for the pumping,
the ATP is not transported!

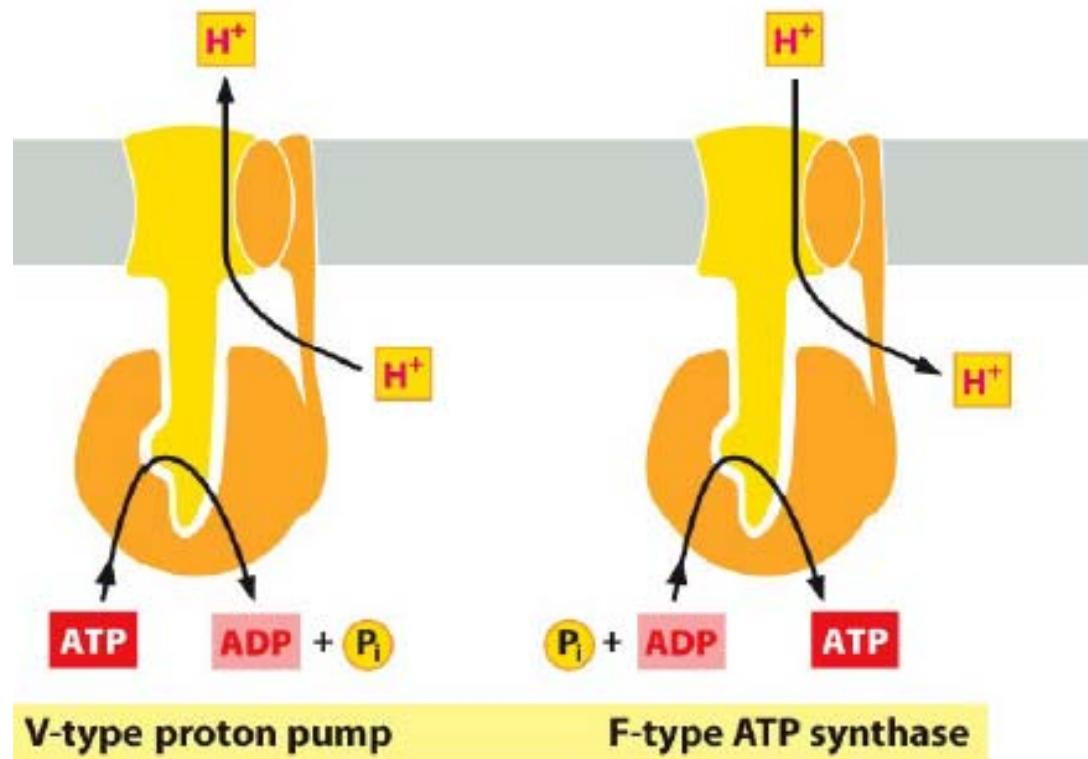
Overview: comparing different ATP pumps

P-type pumps versus ABC transporters:



Overview: comparing different ATP pumps

V-type pumps versus F-type pumps



ATPase types: 1. P-type pumps

Features:

- Structurally and functionally related multipass transmembrane protein
- They **phosphorylate themselves** during the pumping cycle.
- Many P-type pumps are responsible for generating and maintaining gradients of

Na⁺, K⁺, H⁺ and Ca²⁺ across cell membranes:

H⁺ pump in plants and fungi

Na⁺/K⁺ pump in higher organism

H⁺/K⁺ pump in stomach

Ca²⁺ pump (endoplasmic reticulum in **all** eukaryotic cells)

Example 1: The Ca^{2+} pump or Ca^{2+} ATPase

Ca^{2+} is an important messenger in signal transduction.

What is the cytosolic concentration of Ca^{2+} ?

What about extracellular levels?

Features:

- Locate in the membrane of the endoplasmic reticulum (ER) in all eukaryotic cells
- Represents up to 90% of the membrane protein in the sarcoplasmic reticulum (ER) of skeletal muscle cells
- Possesses 10 transmembrane α -helices (for pumping)

Phosphorylation of the Ca^{2+} pump induces the conformational change that drives pumping

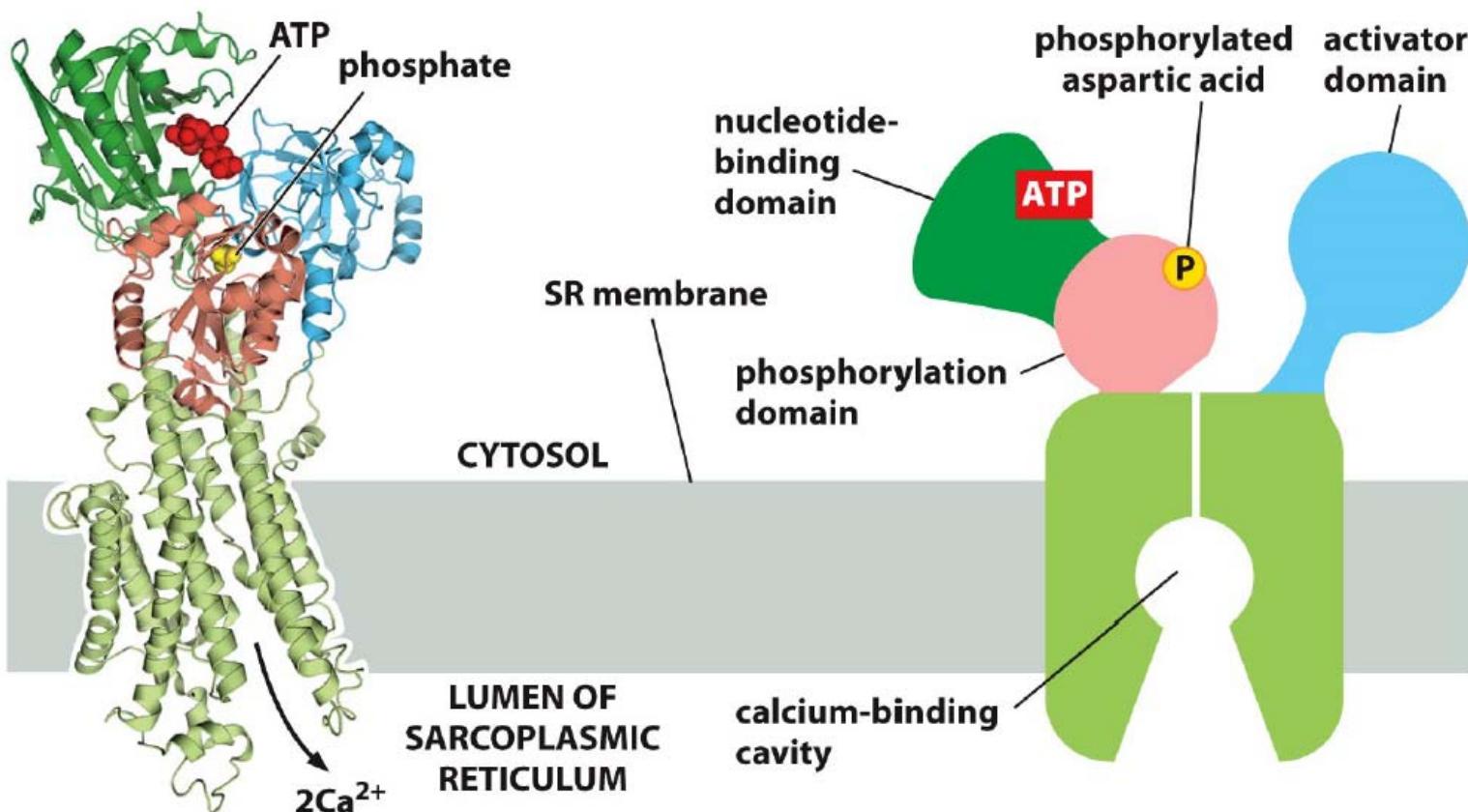
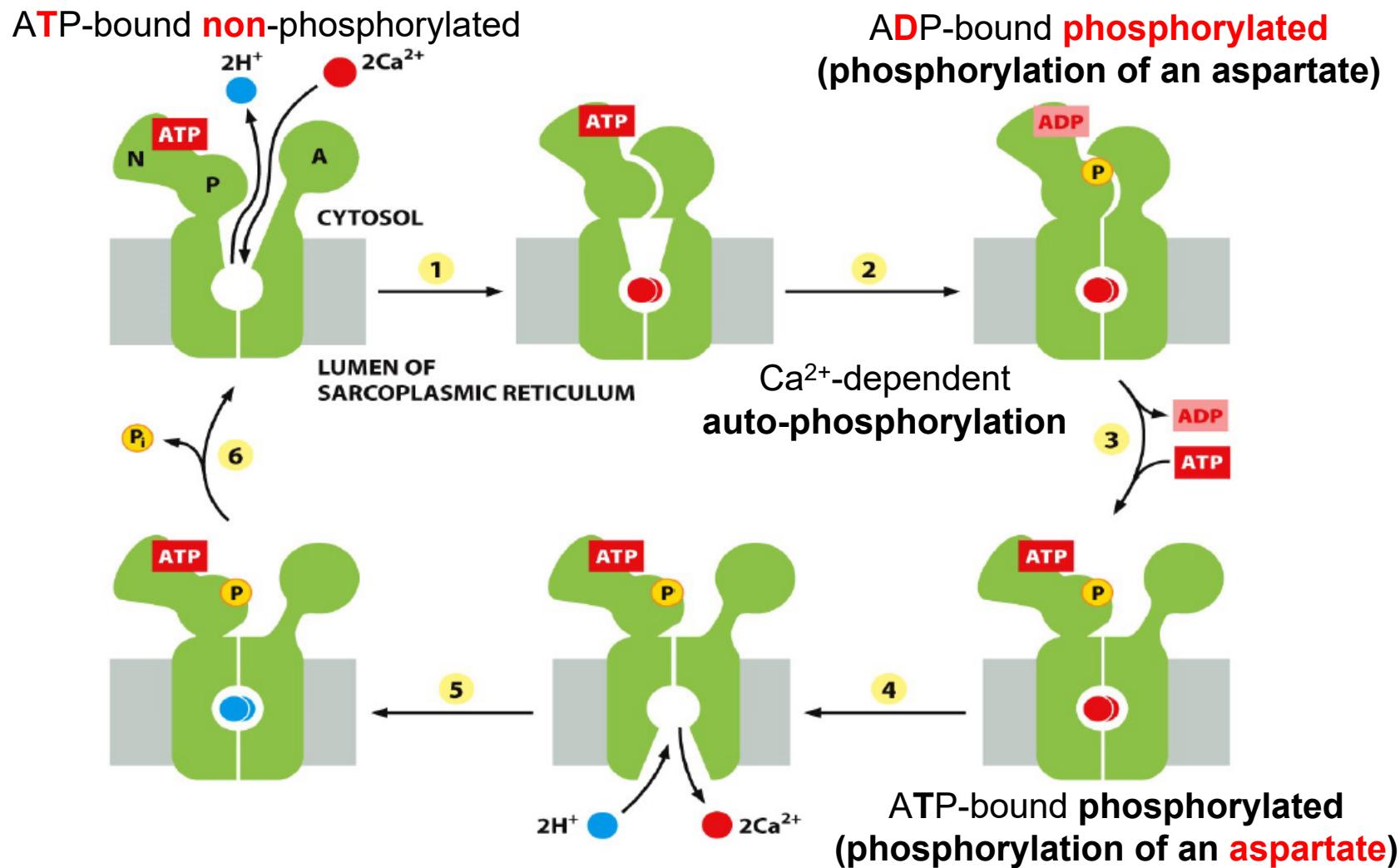


Figure 11-13 Molecular Biology of the Cell 6e (© Garland Science 2015)

“pumping” is due to conformational changes and movement of α -helices

The pumping cycle of the Calcium pump

(ATP-driven antiport of $\text{Ca}^{2+}/\text{H}^+$)



Transient “self-phosphorylation” is characteristic for all P-type pumps!

Example 2: The Na^+-K^+ pump (Na^+-K^+ ATPase) of the PM

The Na^+-K^+ ATPase is a major regulator of **osmolarity** and it is an **electrogenic pump**: export of **3 Na^+** vs. import of **2 K^+**

Generation of an electrical current across the membrane and builds electric potential (negative inside, positive outside)

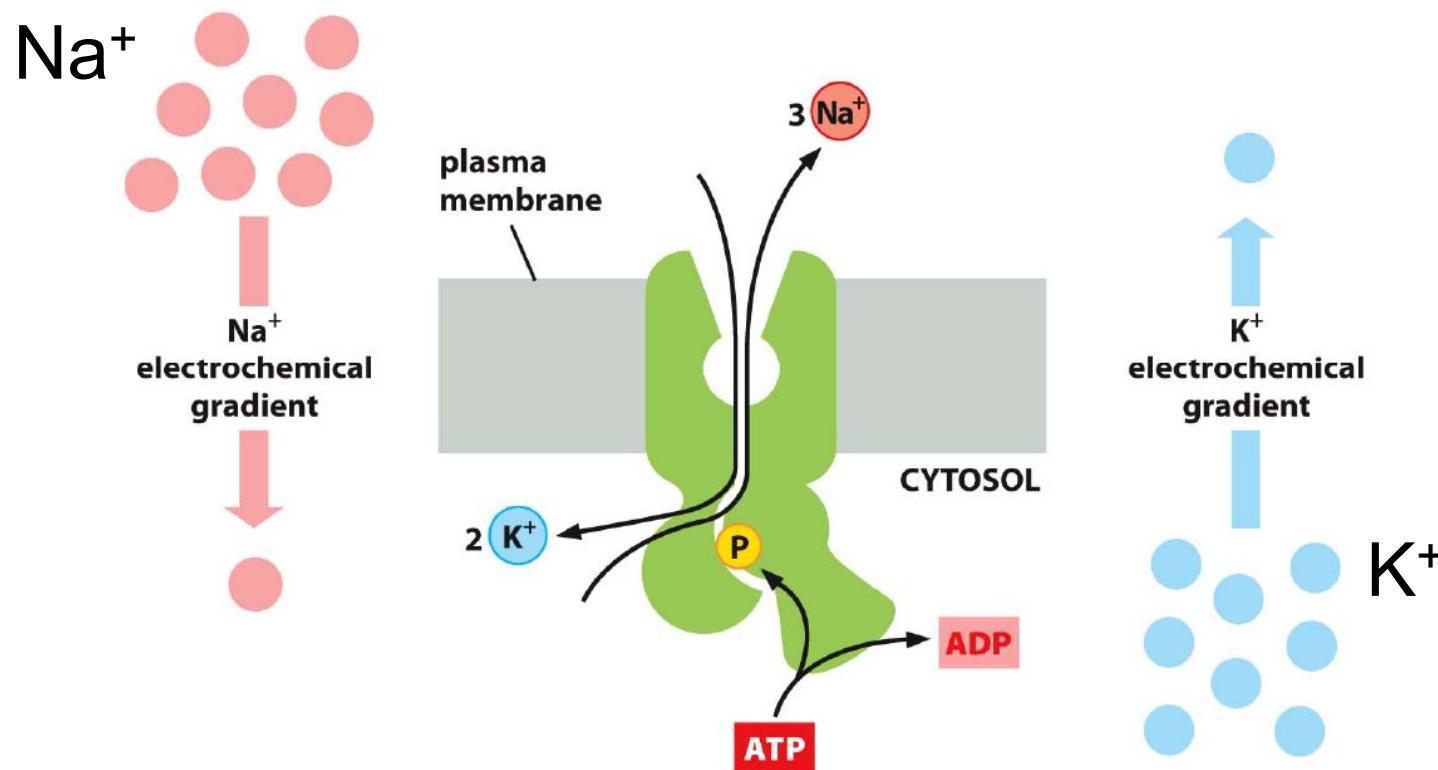


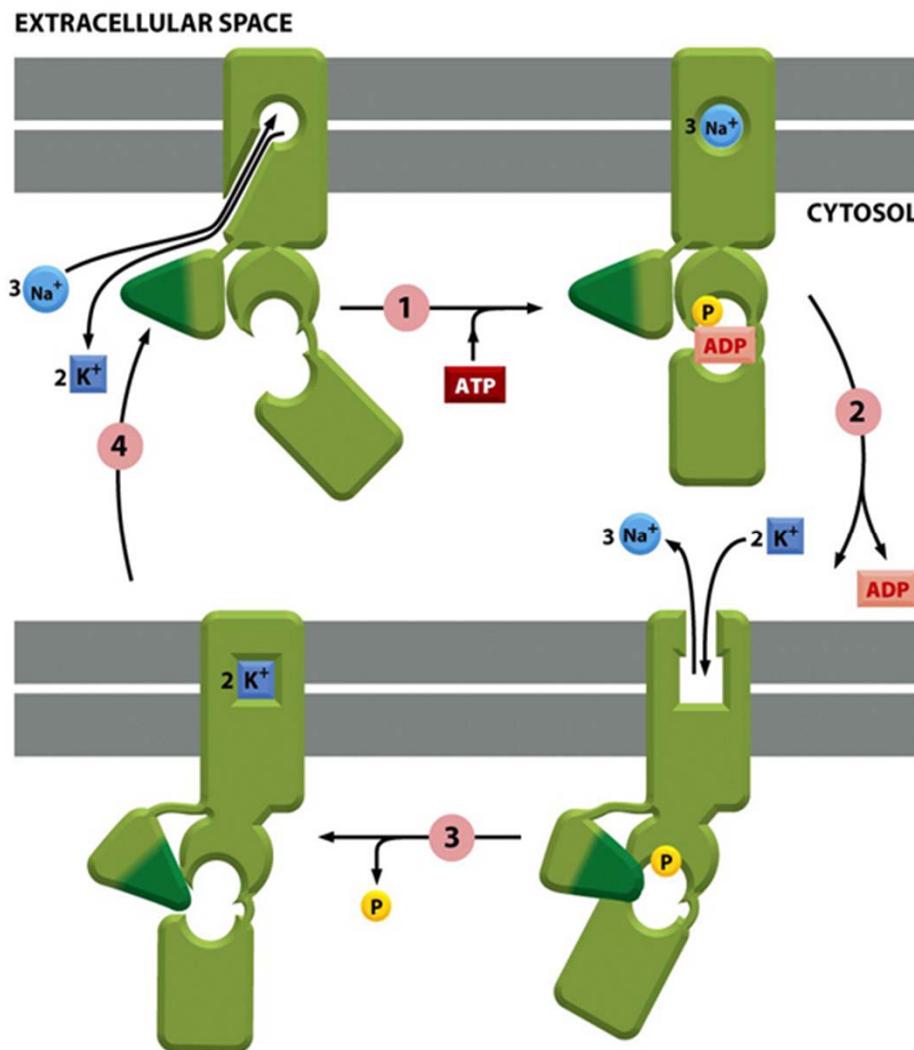
Figure 11-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

The Na⁺-K⁺ pump (Na⁺-K⁺ ATPase)

Features & facts:

- Structurally homologous to the Ca²⁺ ATPase
- It has the conserved **aspartate** (Asp) phosphorylation site
- For each hydrolysed ATP:
3 Na⁺ ions are pumped **outside** and 2 K⁺ ions are pumped **inside** the cell: both against their gradients.
- Phosphorylation of aspartate (Asp) is Na⁺ dependent.
- Dephosphorylation of aspartate (Asp) is K⁺-dependent
- Produces a net charge of the PM
- It is a hetero **tetramer** of subunit composition $\alpha 2\beta 2$

The pumping cycle of the Na⁺-K⁺ ATPase



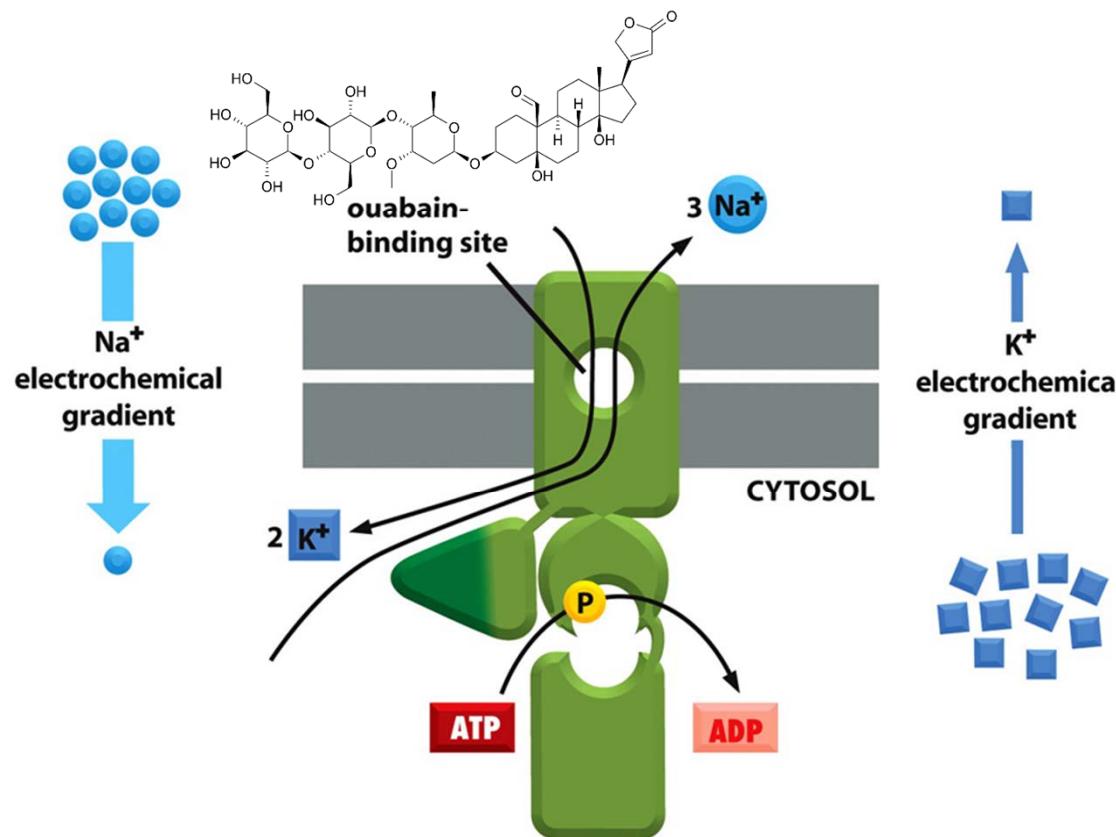
First step: in the cytosolic surface the non-phosphorylated transporter has high affinity for Na⁺ ions but only low affinity for K⁺ ions

Second step: ATP binding and hydrolysis causes conformational change and the release of 3 Na⁺ ions outside.

Third step: the phosphorylated transporter has now high affinity for K⁺ ions but only low affinity for Na⁺ ions in the exoplasmic surface.

Fourth step:
K⁺-dependent dephosphorylation causes conformational change and release of K⁺ inside.

The cardio glycosides ouabain and digoxin are drugs that target the Na⁺-K⁺ ATPase



Ouabain (from *Strophanthus gratus*) :

- specific inhibitor of the Na⁺-K⁺ ATPase from plants.
- Was used as arrow poison for hunting/warfare
- At low concentration: drug against hypotension

Digoxin (from *Digitalis lanata*):

Both bind to exoplasmic domain specific inhibitors of ATPase activity.

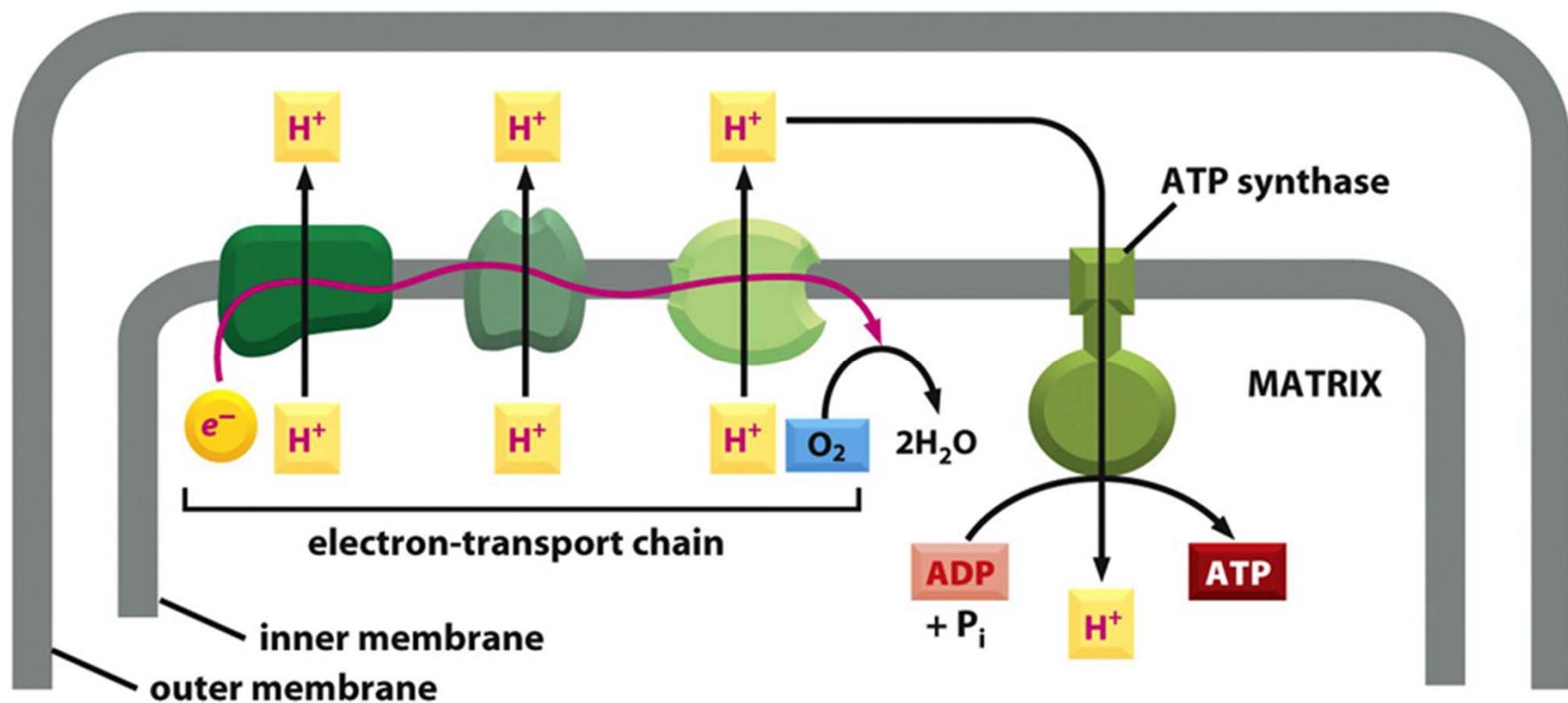


ATPase types: 2. F-type pumps (ATP synthases)

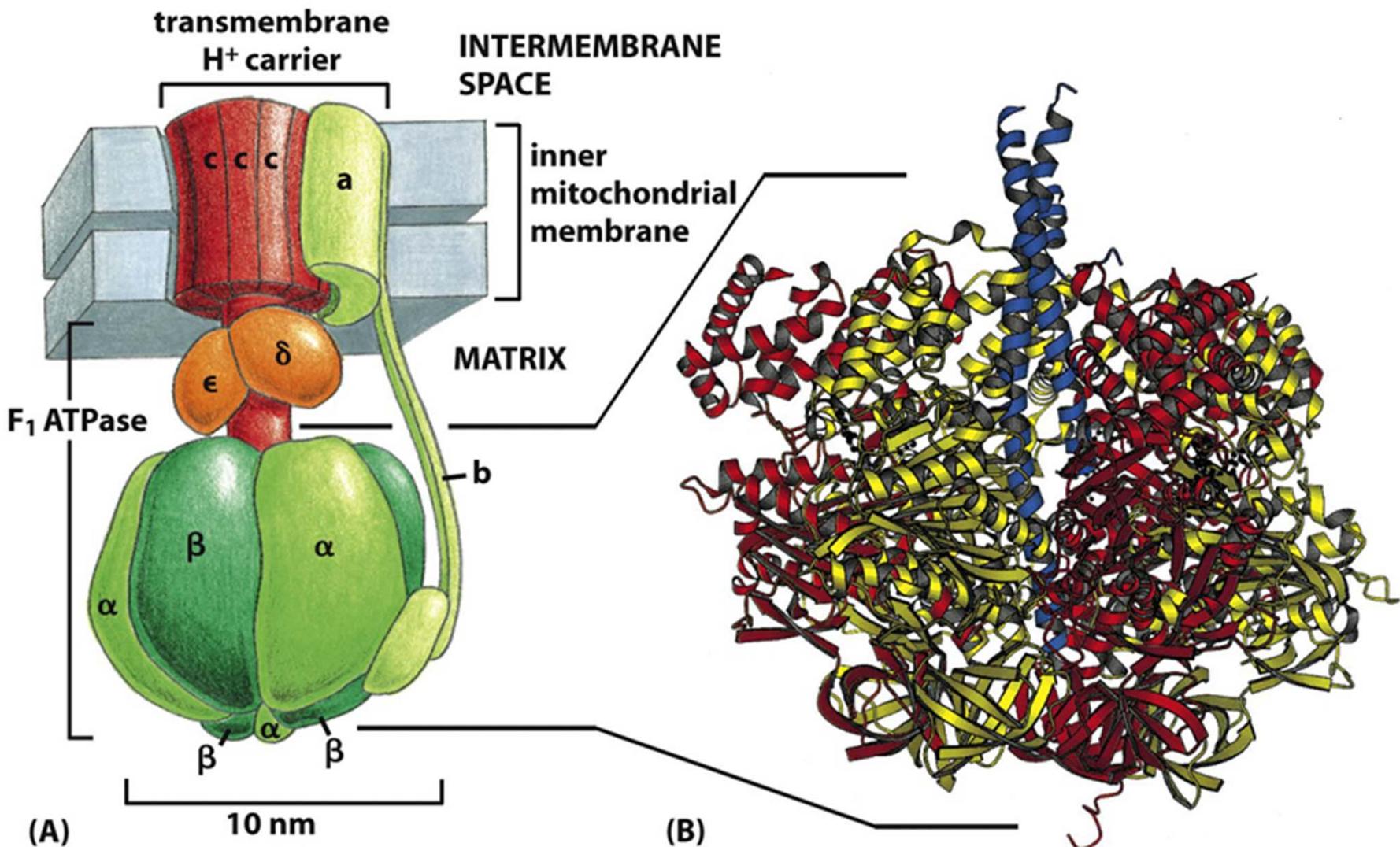
Facts & Features:

- Turbine-like proteins, consist of **multiple different subunits**.
- Found in the **plasma membrane of bacteria**, the **inner membrane of mitochondria**, and in the **thylakoid membrane of chloroplast**.
- Called ATP synthase because they can catalyze **both**, **synthesis and hydrolysis** of ATP.
- They use a **H⁺ gradient** across the membrane **to drive the synthesis of ATP** from ADP and P.
- They are **NOT** controlled by **auto-phosphorylation**.

ATP synthesis is driven by a proton gradient



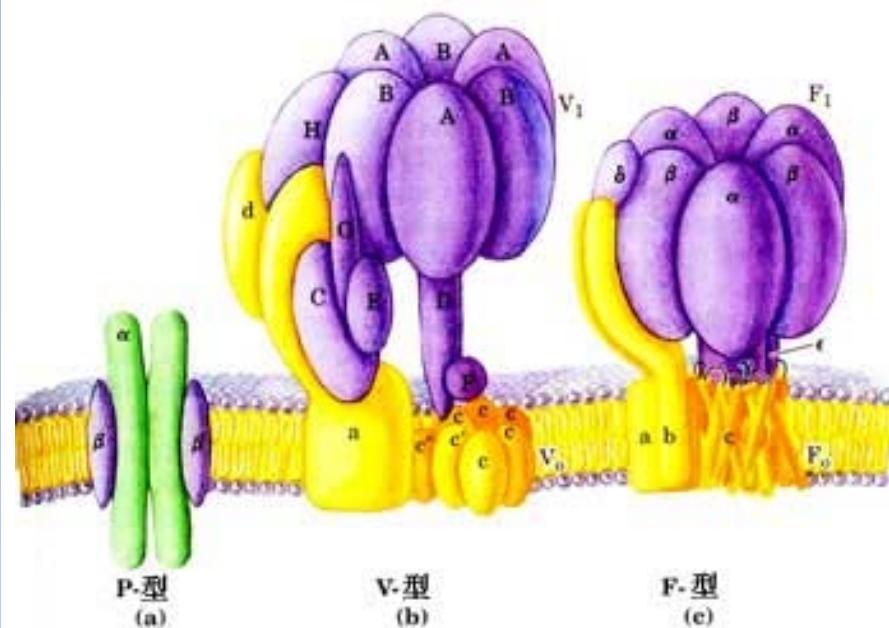
Crystal structure of ATP synthase



ATPase types: 3. vacuolar (V)-type transporter V-ATPase

Facts & features:

- Transport **only** protons, but **do not synthesize ATP**
(purpose: acidification and not energy production)
- Present in all **acidic** compartments: **lysosomes, endosomes** and **vacuoles** in mammals, plants and yeasts.
- **Pump in protons** in the organelle to maintain **low pH**.
- **Structurally similar** to F-type transporters.



ATPase types: 3. ABC transporters

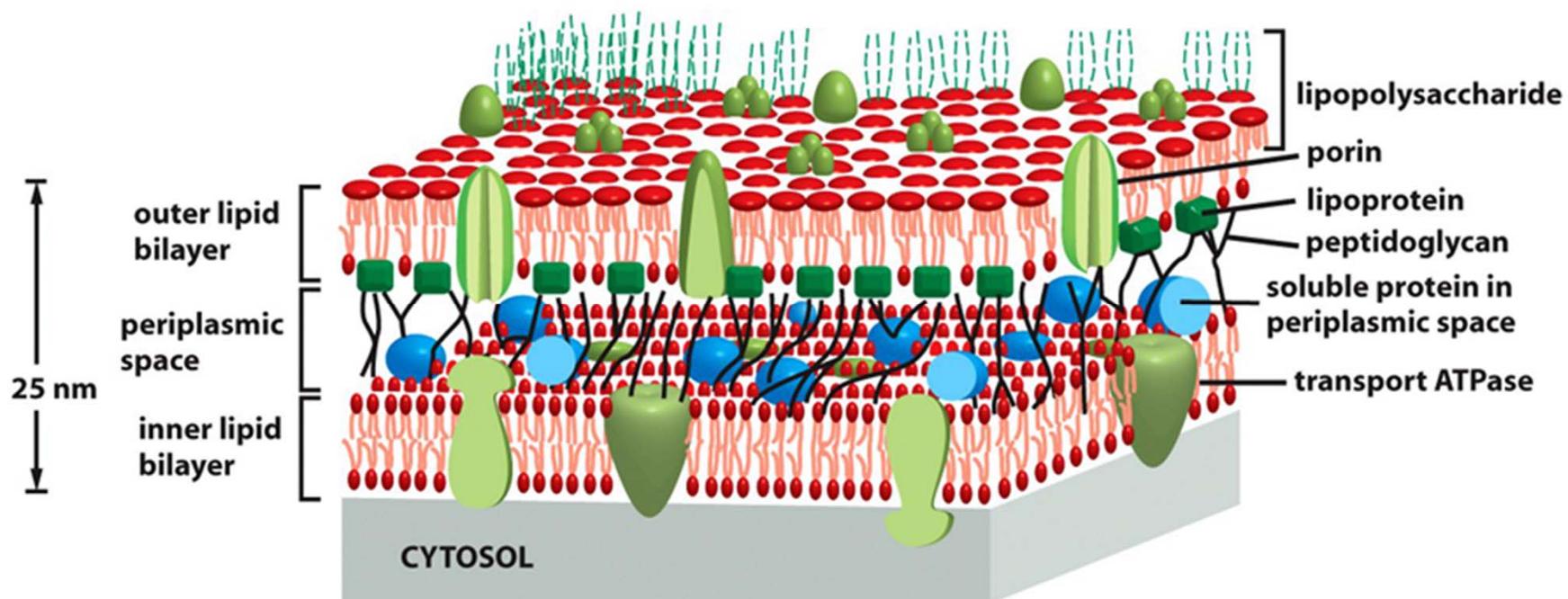
Facts & features:

- Pump **small molecules** across cell membranes.
- Contain **two transmembrane domains** and **two cytosolic ATP binding domains** (**ATP-Binding Cassettes**)
- **ATP binding** leads to **dimerization of ATP–cassettes**.
- **ATP hydrolysis** leads to the **dimer dissociation**.
- **Conformational changes** leads to **transport of small molecules**.
- ABC transporters are important: In *E. coli*, 5% of all bacteria genes encode for ABC transporter
- ABC transporters are **clinically important drug targets**.

The function of ABC transporter in prokaryotes (*E. coli*)

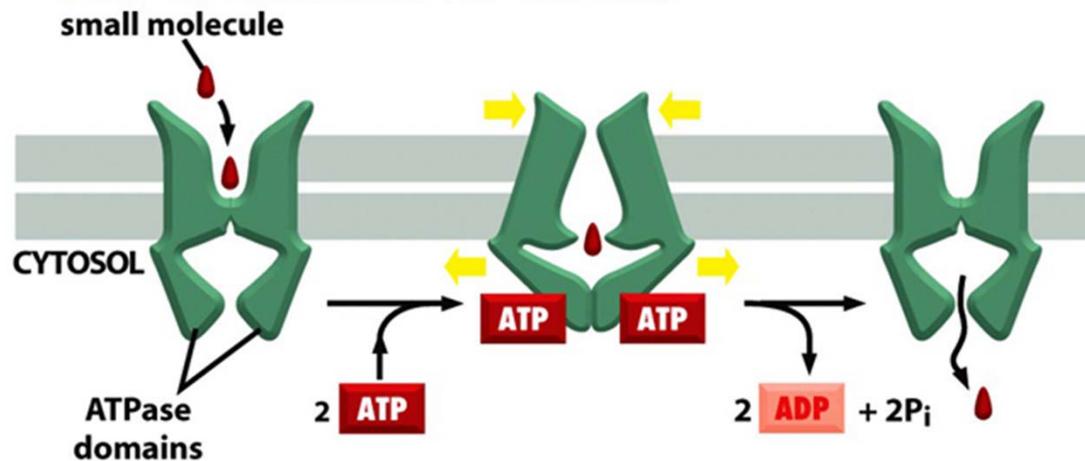
Gram (-) bacteria

inward transport / uptake



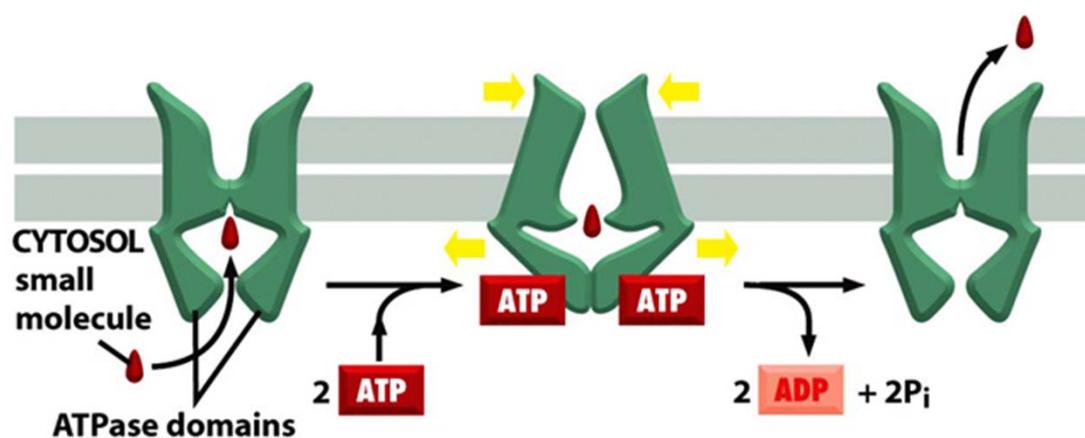
The transport cycle of a typical ABC transporter

(A) A BACTERIAL ABC TRANSPORTER



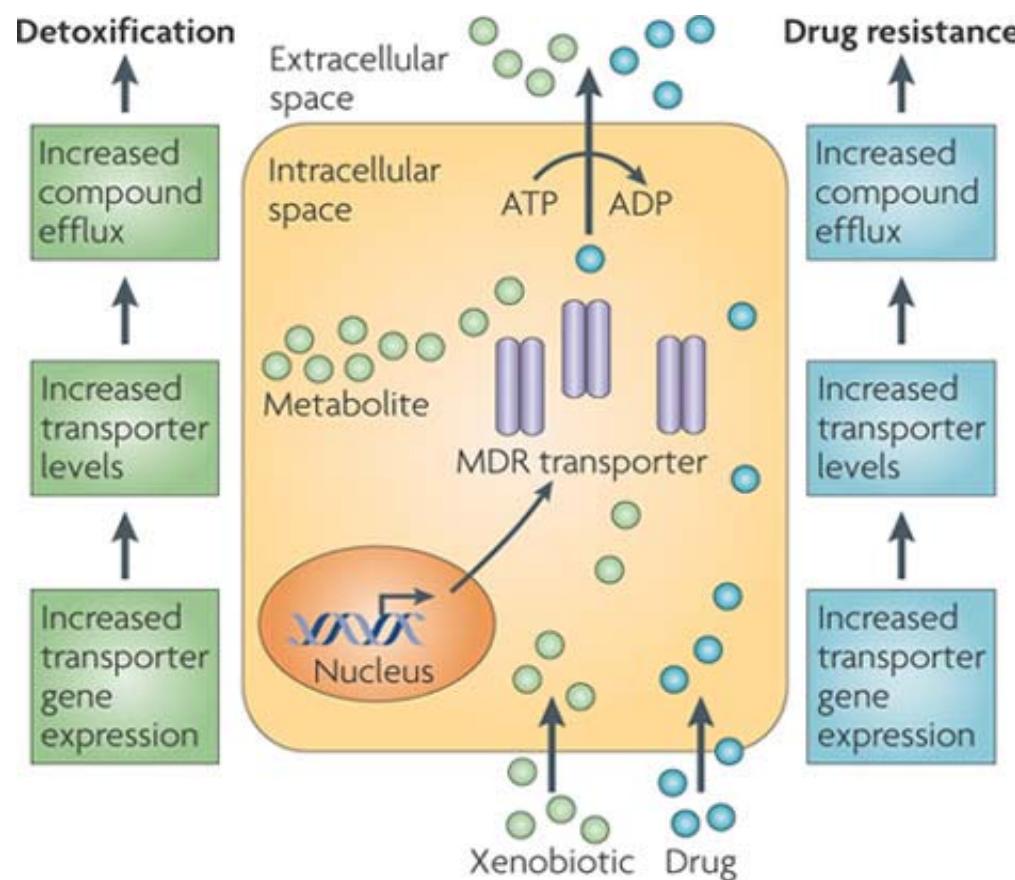
inward transport
(uptake of cool molecules?)

(B) A EUKARYOTIC ABC TRANSPORTER



outward
transport/secretion
(send cool molecules to
neighboring cells?)
&
import in intracellular
compartments!
(send cool precursors in
compartments?)

Example 1: Multidrug resistance (MDR) protein -the first eukaryotic ABC transporter identified-



Nature Reviews | Cancer

Cancer cells with overexpression for MDR are resistant to cancer drugs

Example 2: Cystic fibrosis transmembrane conductance regulator (CFTR)

CFTR is an **ABC transporter**

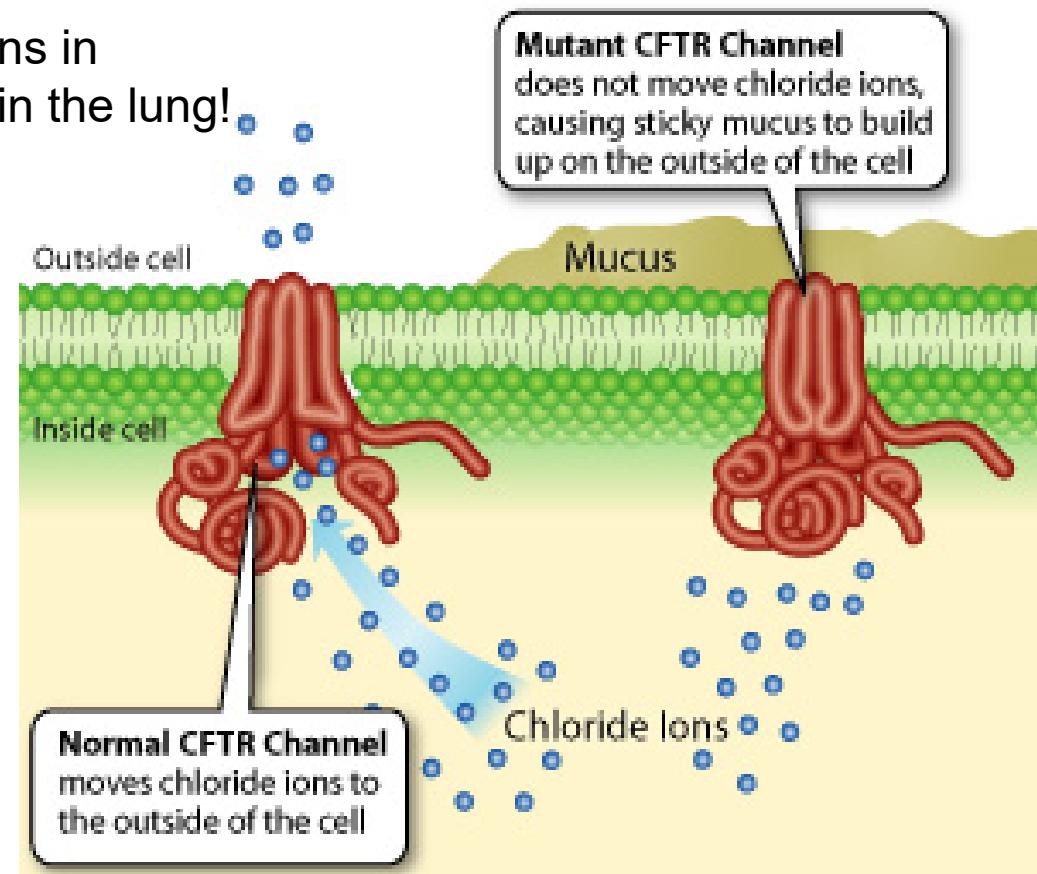
CFTR regulates **open** and **close** of Cl⁻ channel and mutation in CFTR leads to cystic fibrosis

CFTR regulates ion concentrations in the extracellular fluid, especially in the lung!

ATP binding and hydrolysis
do not drive the transport.

They **control opening/closing** of a **continuous channel**!
(passive conduit for Cl⁻ to move down its electrochemical gradient)

Thus, **some** ABC proteins can **function as transporters** and **others as gated channels !!!**



Example 3: Chloroquine resistance in malaria-causing *Plasmodium falciparum*

- The protozoa has **enhanced level** of chloroquine **ABC transporters** and therefore has **resistance to chloroquine**.
- The resistant *P. falciparum* have amplified the gene encoding an **ABC transporter** that pumps out the chloroquine.

V. Neuron electric potential

1. Membrane potential
2. K^+ channel and membrane potential
3. Action potential
4. Transmitter-gated ion channels
5. Chemical synapses
6. Transmitter-gated ion channels as drug targets

1. Membrane potential

+ - + - + - +
- + - + - + -
+ - + - + - +
- + - + - + -
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- + - + - + -
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- + - + - + -
+ - + - + - +
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- + - + - + -
+ - + - + - +

exact balance of charges on each side of the membrane; membrane potential = 0

+ - + - + - +
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a few of the positive ions (*red*) cross the membrane from right to left, leaving their negative counterions (*red*) behind; this sets up a nonzero membrane potential

Membrane potential:

Difference in the electrical charge on the **two sides** of the membrane

Resting membrane potential:

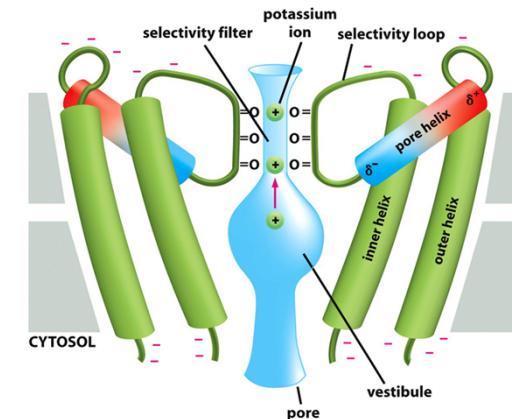
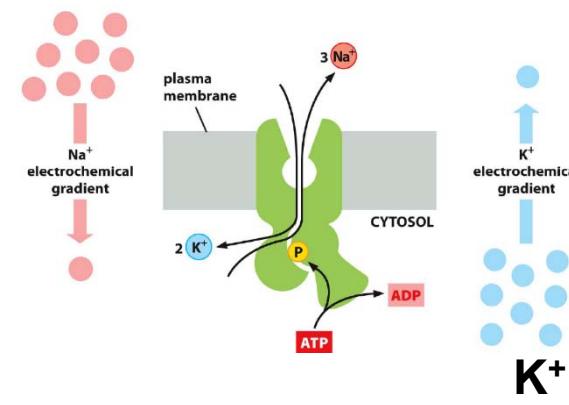
Electric charge difference in the **equilibrium conditions**

when there is **no** net flow of ions across the plasma membrane.

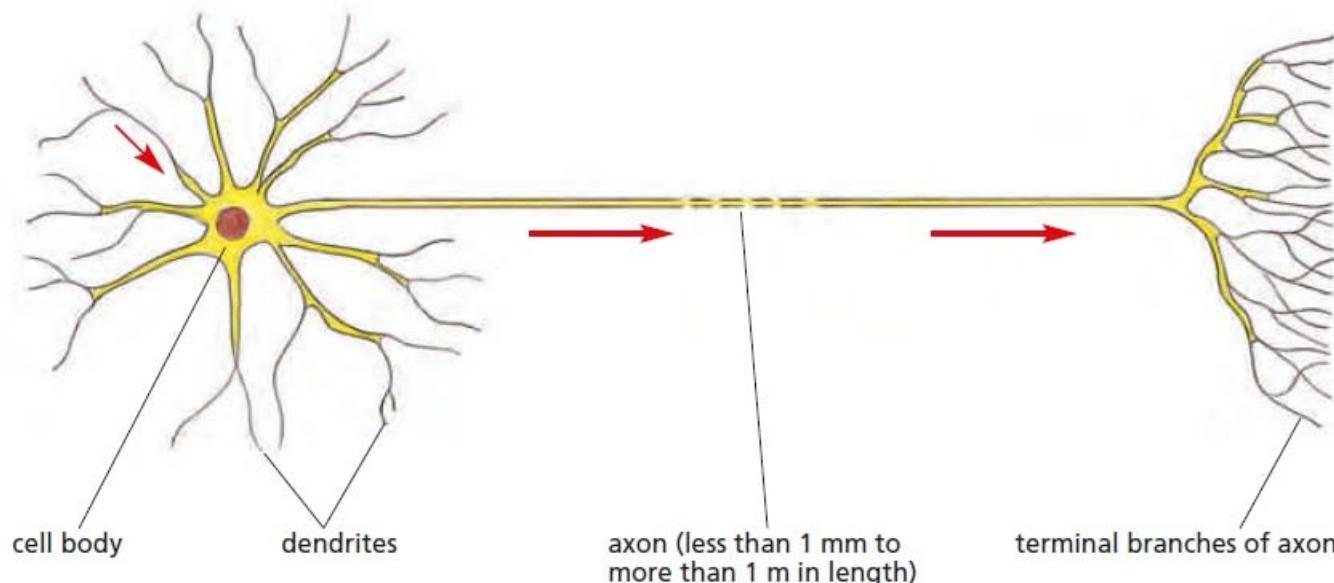
usually between -20mV to -120mV

2. The K⁺ channel is an important factor in causing membrane potential

- Na⁺-K⁺ pumps leave a charge difference in and out of membrane, with high K⁺ inside and low K⁺ outside.
- K⁺ channels allow flow in and out to balance the difference.
- Due to **high K⁺ concentration inside the cell:**
- **Each K⁺ that is transported out by K⁺ channel will leave a negative charge inside.**
(even in resting cells called K⁺ leak channels)
- **THIS results in the build up of a membrane potential.**
(rather than only the action of the Na⁺-K⁺ pump)



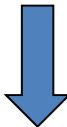
3. Signaling in nerve cells



- Nerve cells receive, conduct and transmit signals: always the **same** signal: **changes in electrical potential** across the neuron's PM (**action potential, AP**)
- The AP is a traveling wave of electrical excitation
- Speed of an AP 100 meters per second or faster
- The action potential is a direct consequence of the property of **voltage-gated channels**

3. Action potential: traveling wave of electrical excitation

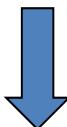
Start: a depolarization signal on the plasma membrane.



Voltage-gated Na^+ channels open, cause influx of Na^+



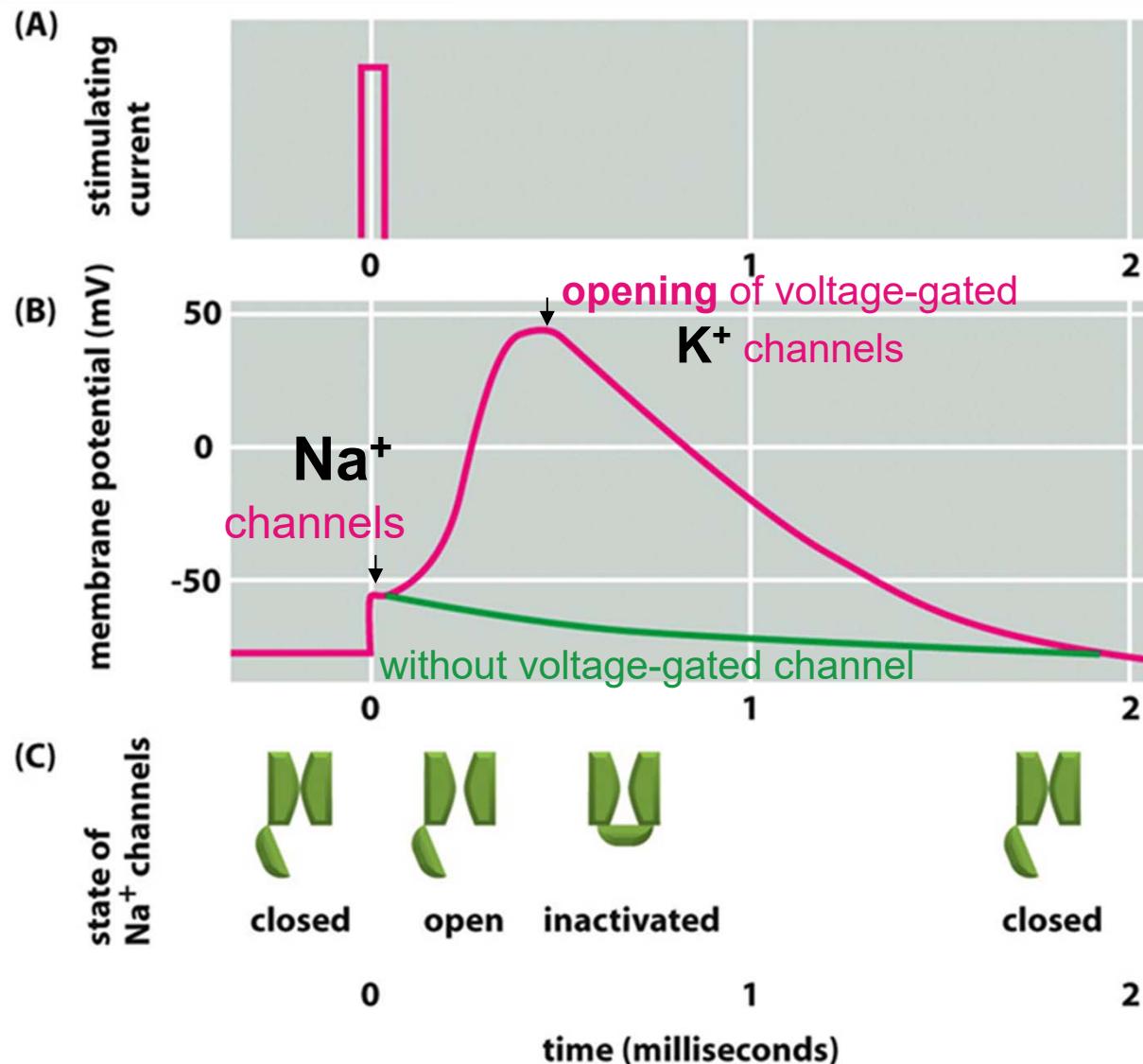
Opening of more Na^+ channels, positive feedback



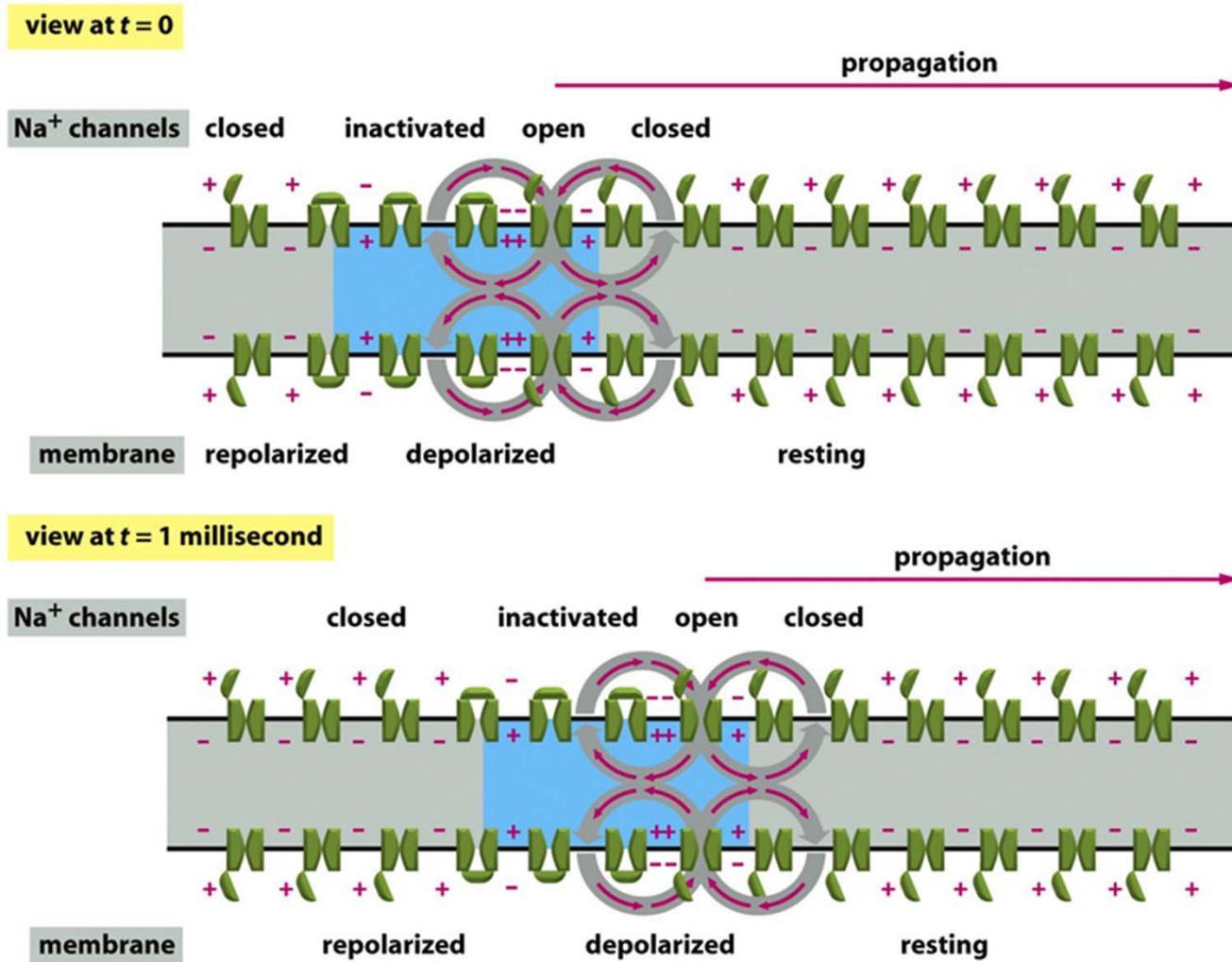
When **electrochemical driving force** is zero,
 Na^+ channels becomes inactivated and **voltage gated K^+ channels open**

Voltage-gated cation channels generate action potentials in electrically excitable cells

1. Nerve cells
2. Muscle cells



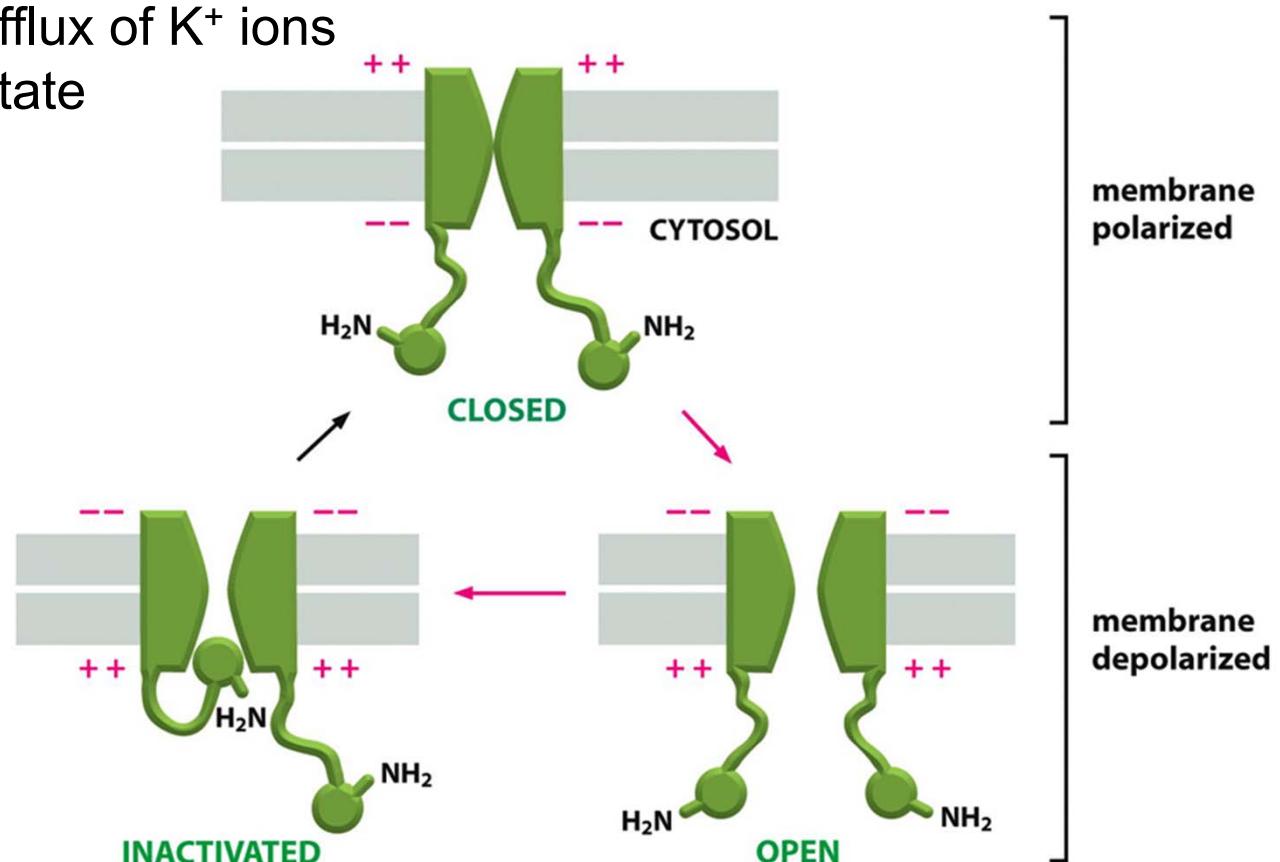
The propagation of an action potential along an axon



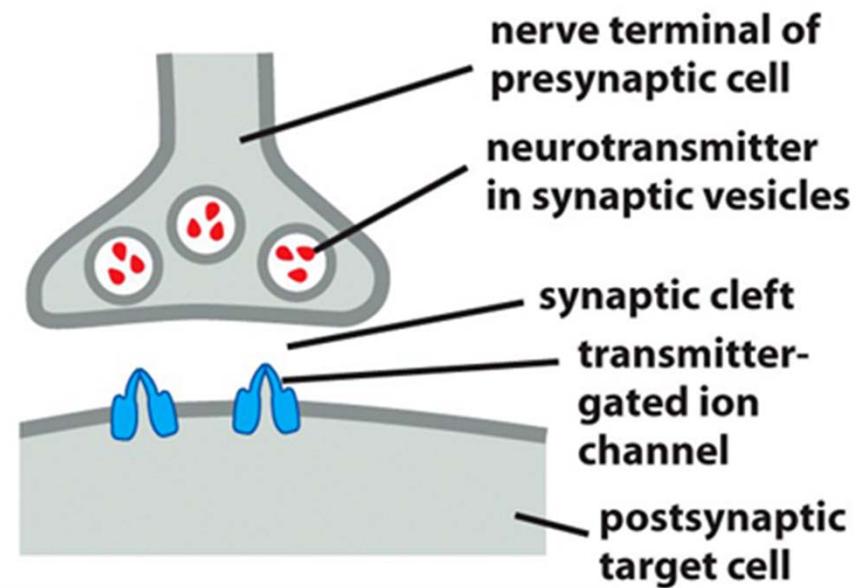
Voltage gated K⁺ channels brings back the membrane potential

Features:

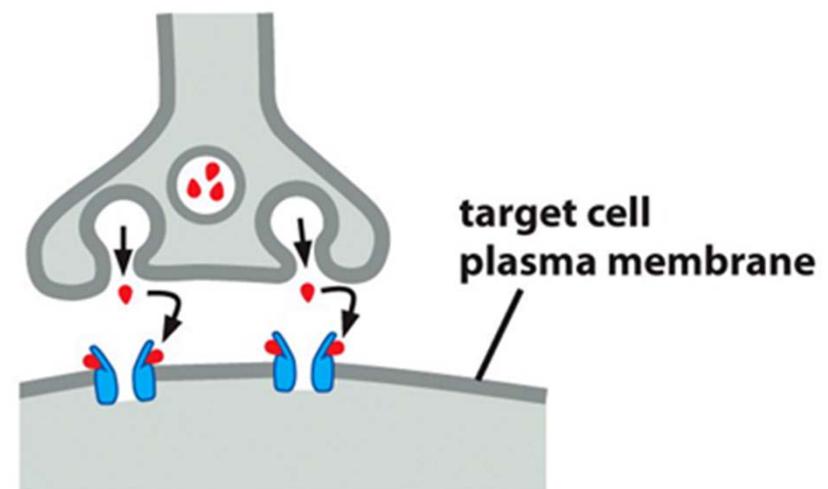
- Slower kinetics than the Na⁺ channel
- Rapidly causes efflux of K⁺ ions
- Has inactivated state



4. Transmitter-gated ion channels convert chemical signals into electrical ones at chemical synapses



RESTING CHEMICAL SYNAPSE



ACTIVE CHEMICAL SYNAPSE

5. Types of chemical synapses

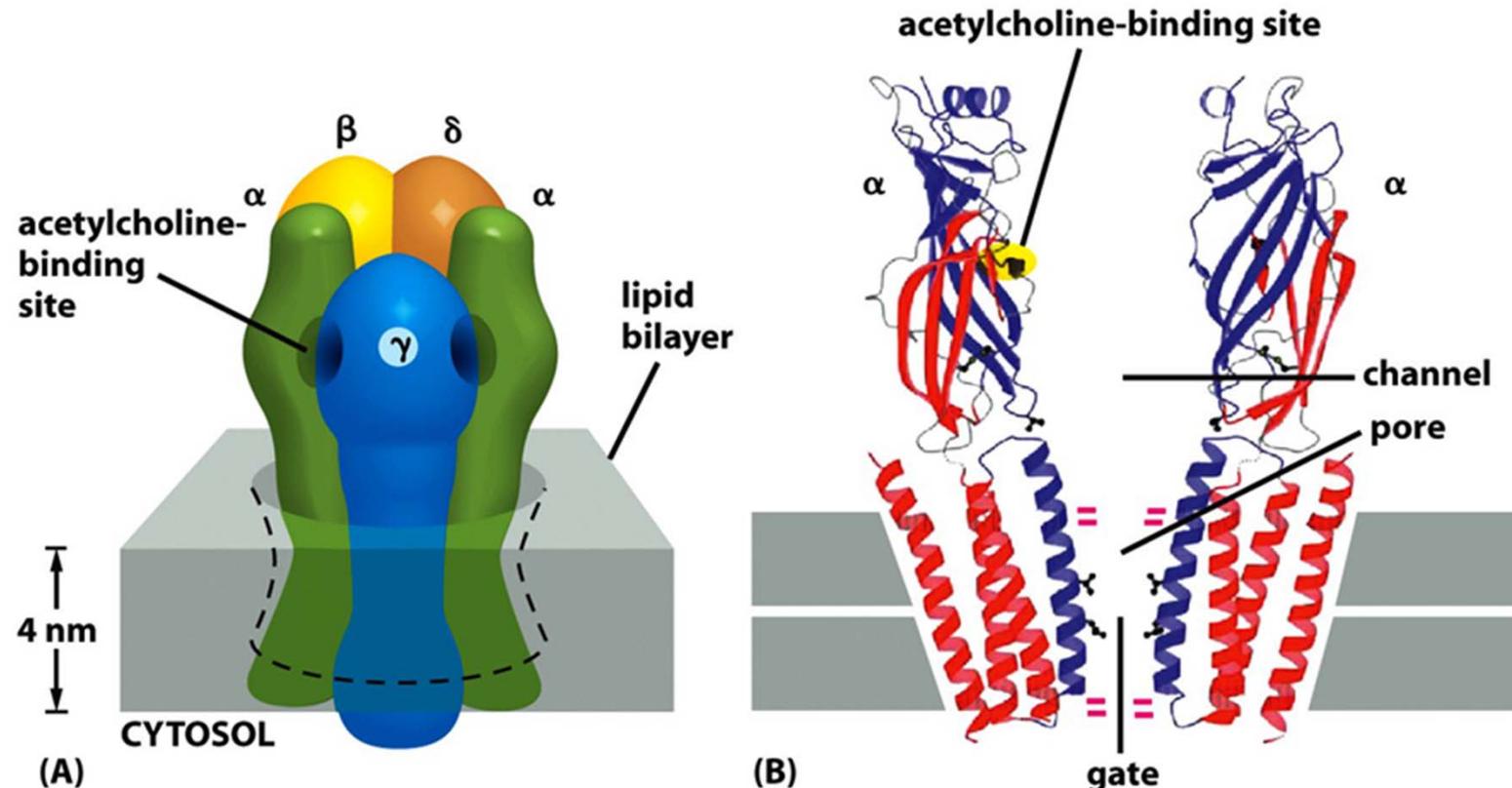
- **Excitatory neurotransmitters:**
open cation channels, causing **influx** of Na^+ and **firing** of action potential
- **Inhibitory neurotransmitters:**
open Cl^- or K^+ channels, causing **delays** of action potential.

Usually : **excitatory transmitters:** **acetylcholine, glutamate, serotonin**

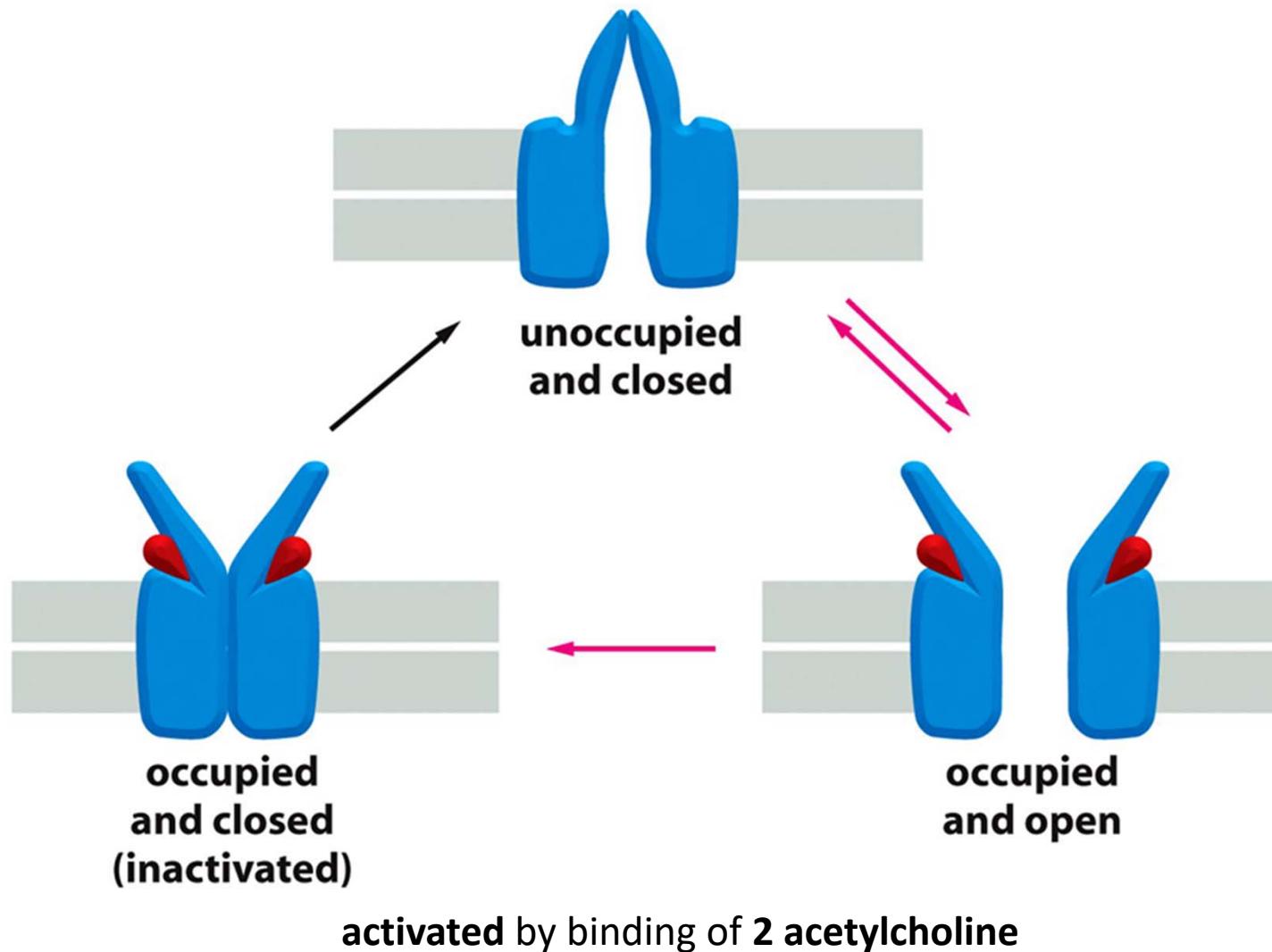
inhibitory transmitters: **γ -aminobutyric acid (GABA), glycine**

Acetylcholine receptor ---the first purified ion channel

- Transmitter-gated cation channel
- 20,000 receptors/ μm^2
- not discriminative for Na^+ , K^+ or Ca^{2+}
- activation by 2 molecules acetylcholine
- inactivation by degradation of acetylcholine (acetylcholinesterase)

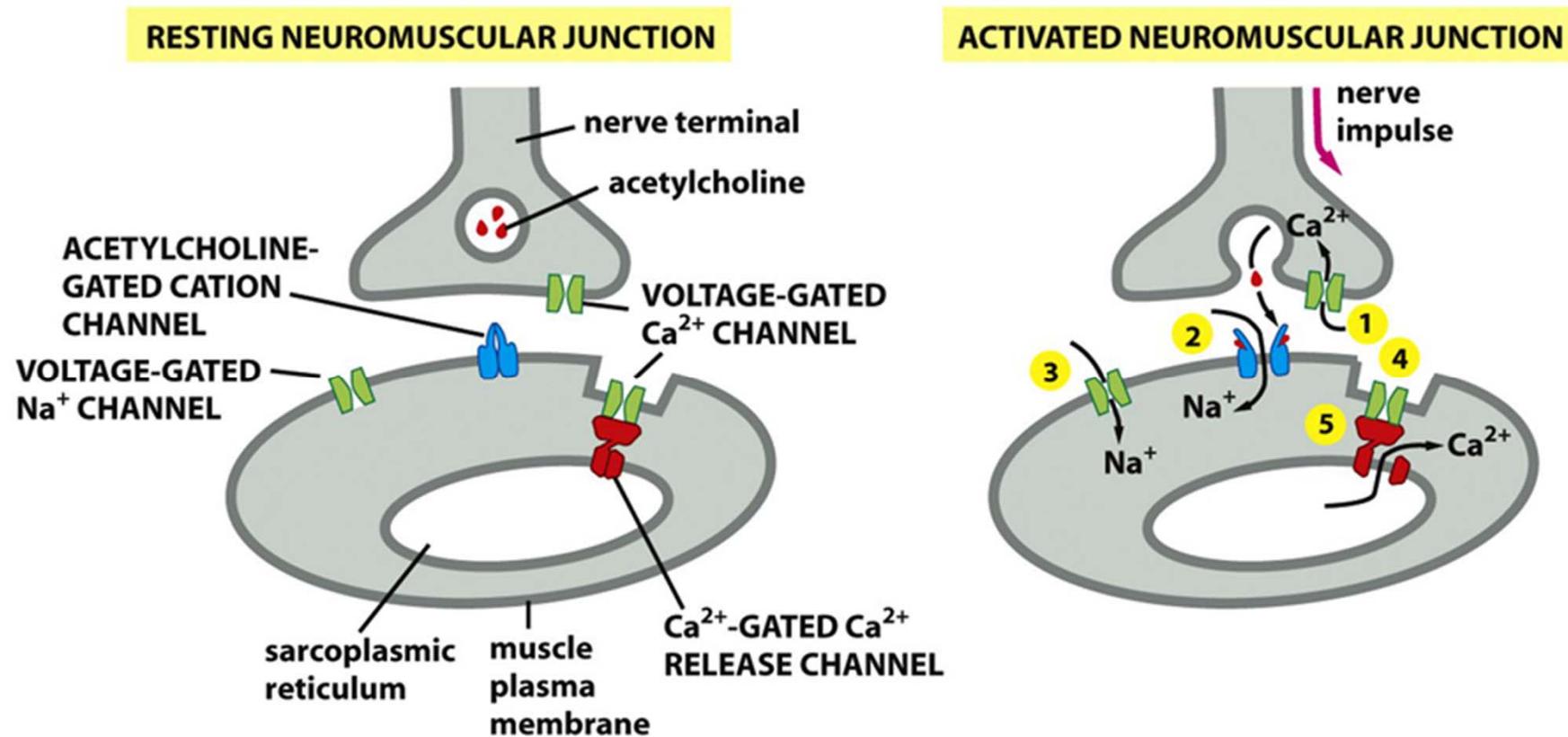


Three states of acetylcholine receptor



Neuromuscular signal transmission

Sequential activation of five different sets of ion channels:



6. Transmitter-gated ion channels as drug targets

- Snake toxins: bind to **acetylcholine receptors** and inhibit them.
- Curare: bind to **acetylcholine receptors**
- Barbiturates tranquilizers: bind to **GABA receptors** (valium, Librium etc.)
- Prozac: inhibits uptake of serotonin

Many drugs to treat insomnia, anxiety, depression, schizophrenia , etc. target receptor function at the synaptic cleft