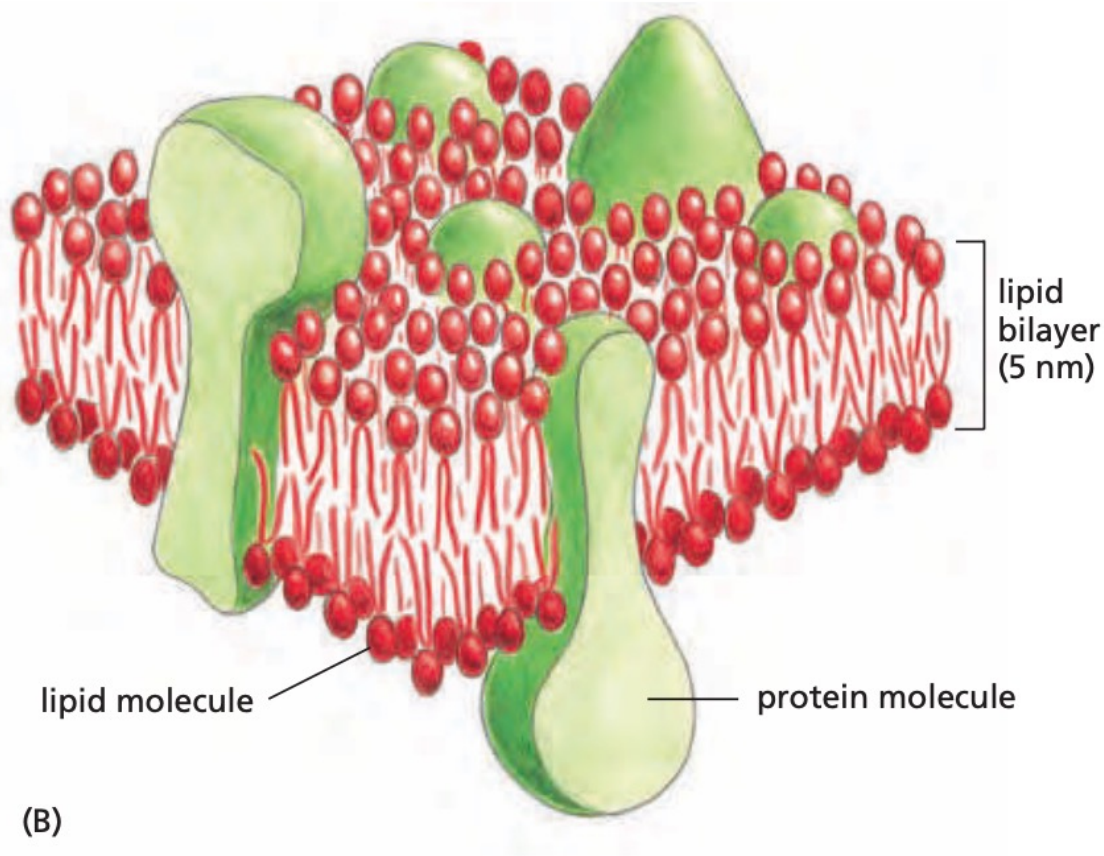
Molecular biology of the cell
BIO 207

Prof Wouter R. Karthaus PhD
EPFL-SV-ISREC

BIO207@EPFL.CH

Last weeks: Discussed the membrane structure



Topic of the day: membrane transport

Membrane Transport of Small Molecules and the Electrical Properties of Membranes

CHAPTER 11

IN THIS CHAPTER

PRINCIPLES OF MEMBRANE TRANSPORT

TRANSPORTERS AND ACTIVE MEMBRANE TRANSPORT

CHANNELS AND THE ELECTRICAL PROPERTIES OF MEMBRANES

Topic of the day: membrane transport

Proteins involved in membrane transport
Active vs Passive transport
ATP-driven pumps and Na+/K+ ATPase - ABC transporters
Channels
- Aquaporin
Introduction to ion channels
- Ion selectivity
- Ion current measurements

Examples of the importance of these transporters

ion concentrations inside and outside a cell

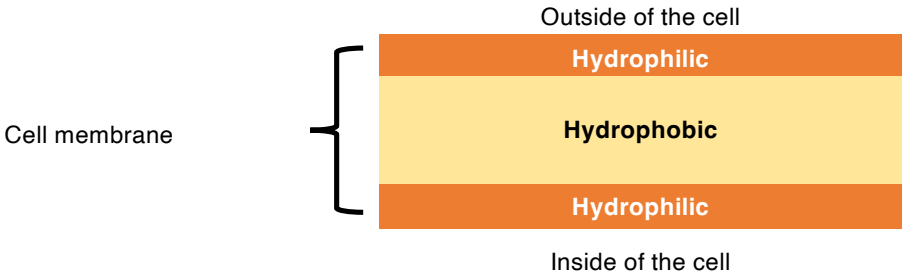
TABLE 11–1 A Comparison of Inorganic Ion Concentrations Inside and Outside a Typical Mammalian Cell*

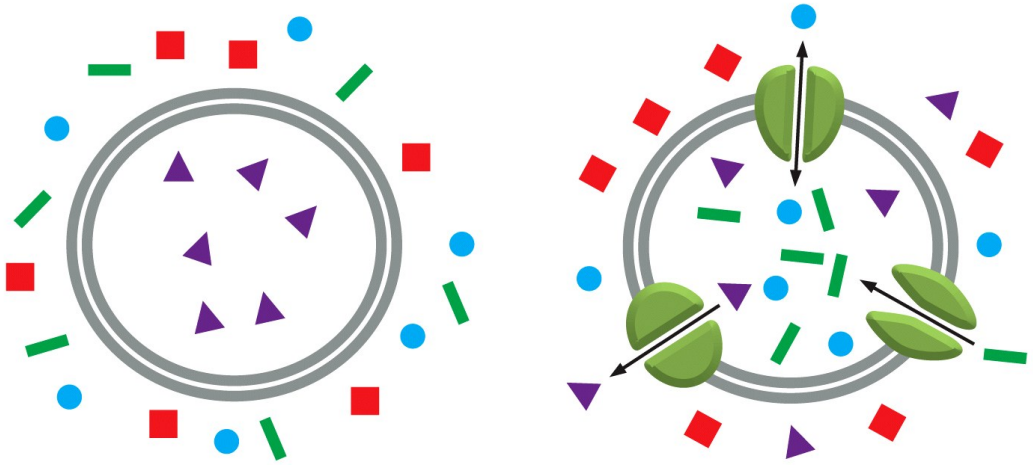
Component	Cytoplasmic concentration (mM)	Extracellular concentration (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions		
Cl^-	5–15	110

*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions: although there is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, both ions are mostly bound to other substances (such as proteins, free nucleotides, RNA, etc.) and, for Ca^{2+}, stored within various organelles.

The lipid bilayer is impermeable

- The lipid bilayer of cell membranes is a barrier that prevents water-soluble molecules and small ions to pass through.
- This allows to have different concentrations of solutes in and out. - Cells use specialized transmembrane proteins for transport

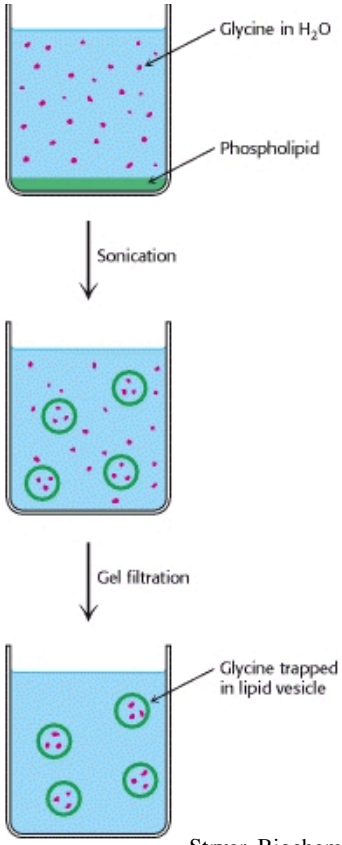




highly impermeable to ions

Figure 12-1 *Essential Cell Biology* (© Garland Science 2010)

Using a synthetic lipid bilayer one can study permeability for different classes of molecules.



Stryer, Biochemistry, 5th edition

Figure 12.13 Preparation of Glycine-Containing Liposome

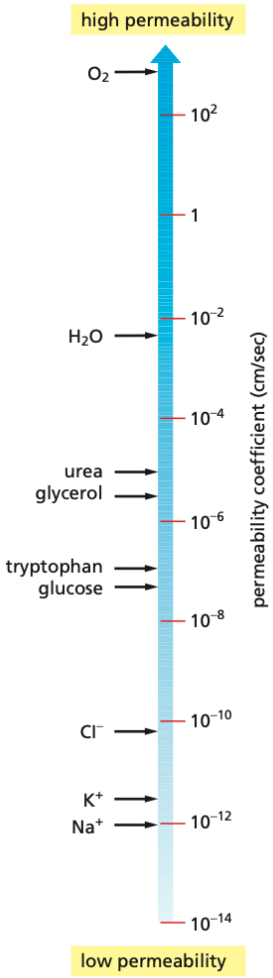
Basic chemical principles for this experiment

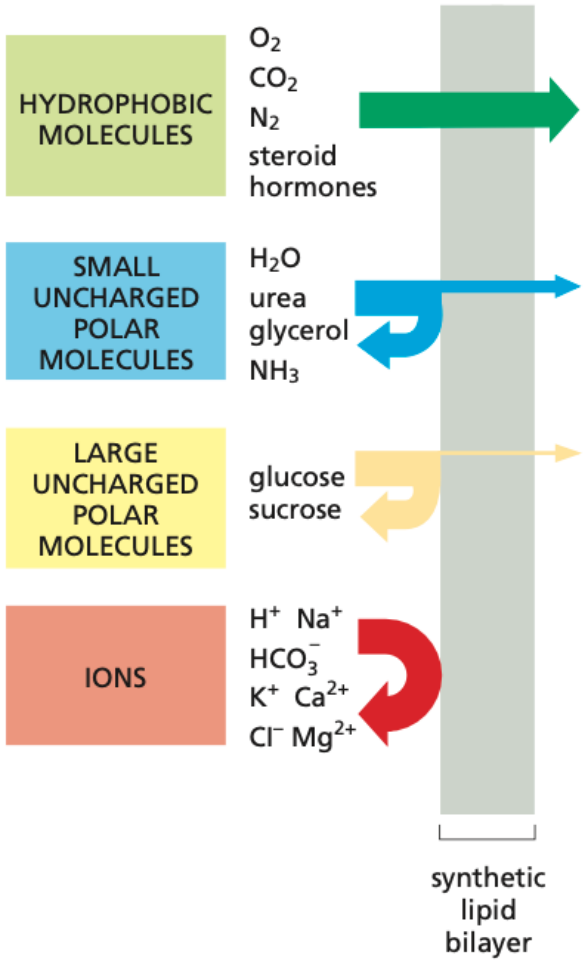
Phospholipids form liposomes with an amphiphilic nature

Molecules will diffuse over the lipid bilayer down its concentration gradient
High concentration -> Low concentration

The features of the compound tested will define diffusion rate

Using a synthetic lipid bilayer one can study permeability for different classes of molecules.

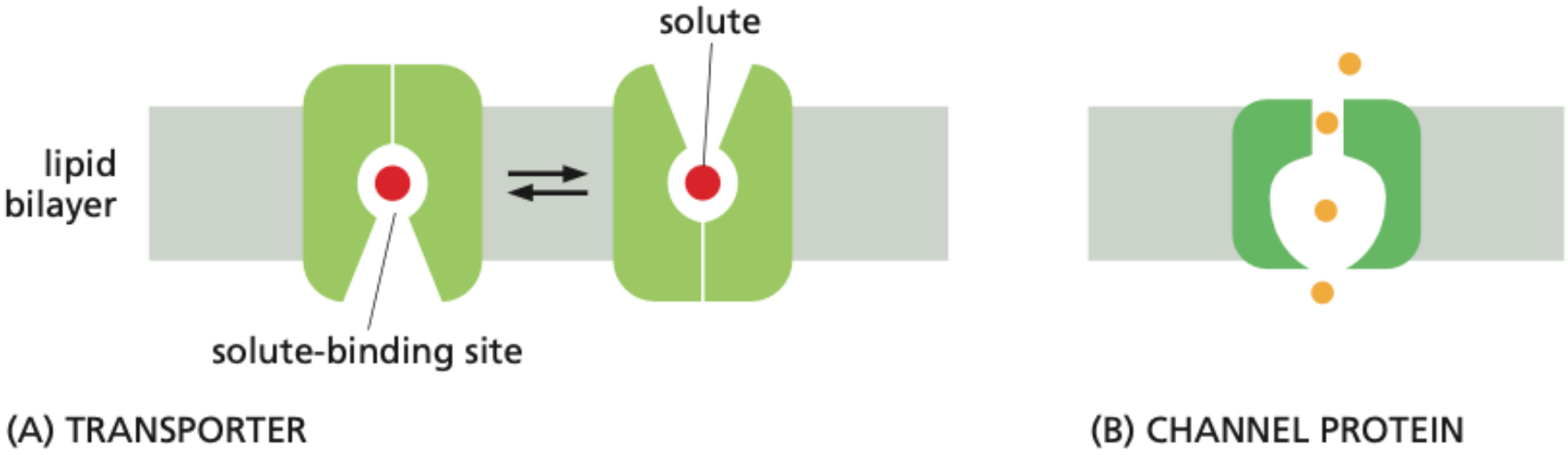




Using this information, we can predict which groups of molecules require membrane transport

Molecules can be transported across the membrane by proteins
What are the types and features of these proteins?

Transporters and channel proteins



Transporters are also called carriers or permeases

Channel proteins

-Form hydrophilic, often highly selective pores

-No change in conformation

-The pores can usually open and close rapidly

-No energy coupled, only passive transport

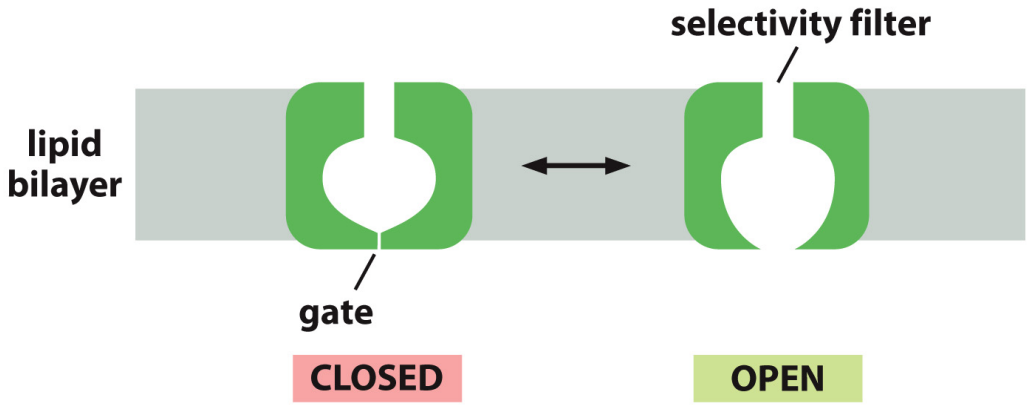


Figure 11-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

Transporters and channel proteins

	transporter	channel
Interaction with the solute	strong	weak
Conformational change	yes	no
Transport speed	slow	fast
Transport type	active or passive	passive

Transporters are found in most or all biological membranes

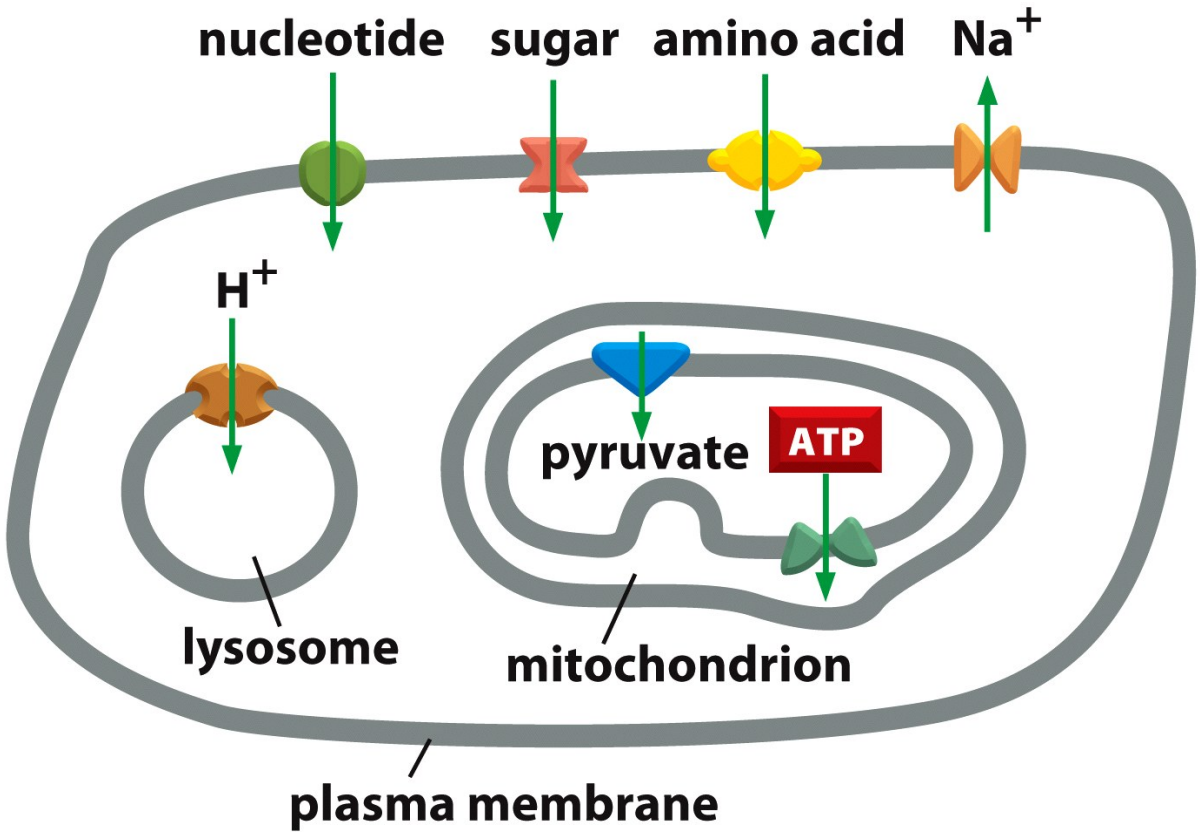
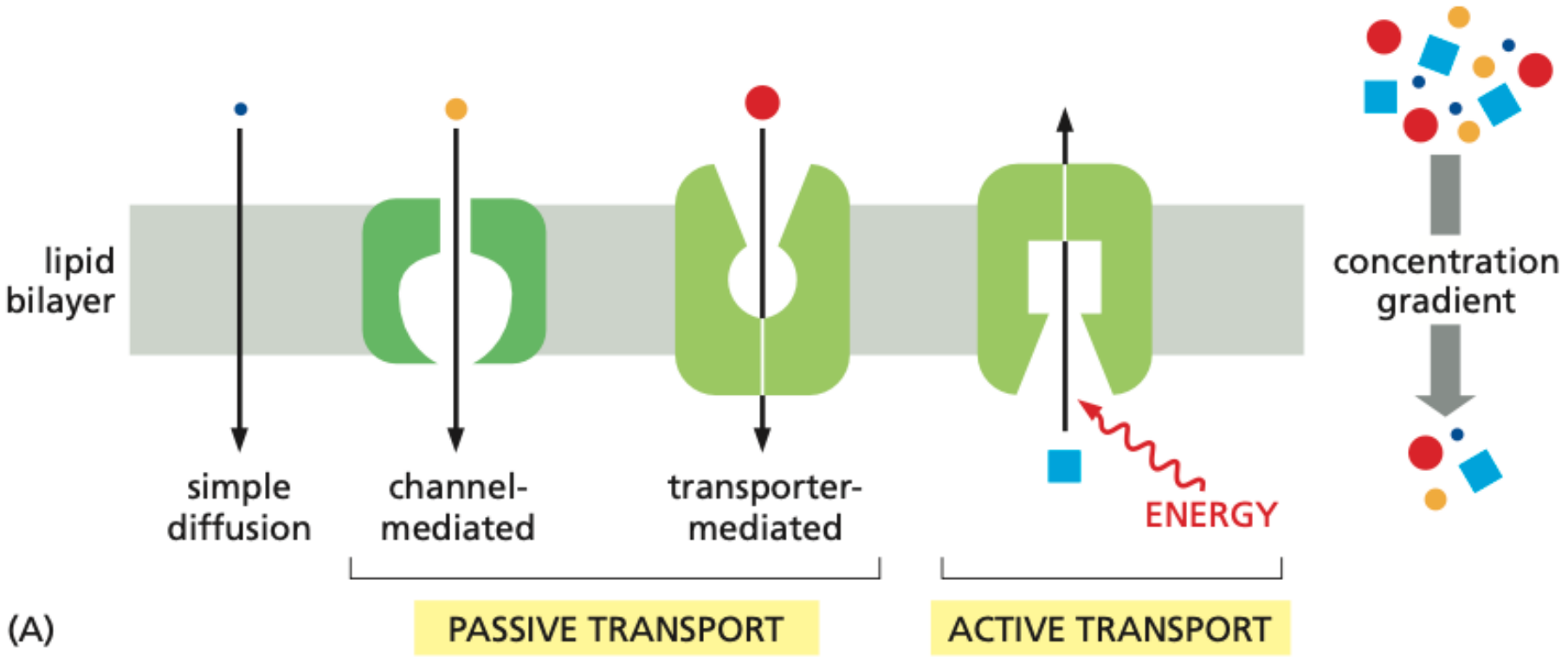


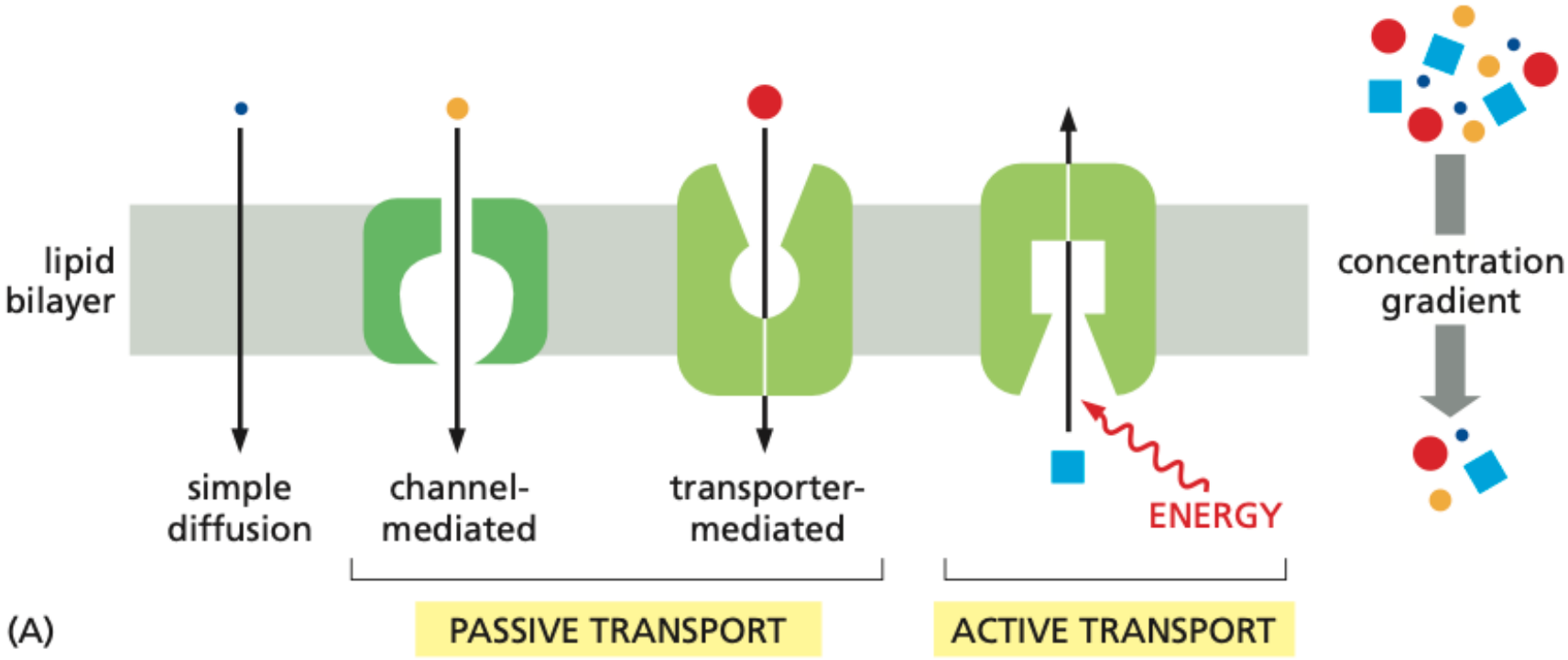
Figure 12-5 *Essential Cell Biology* (© Garland Science 2010)

Passive versus Active transport



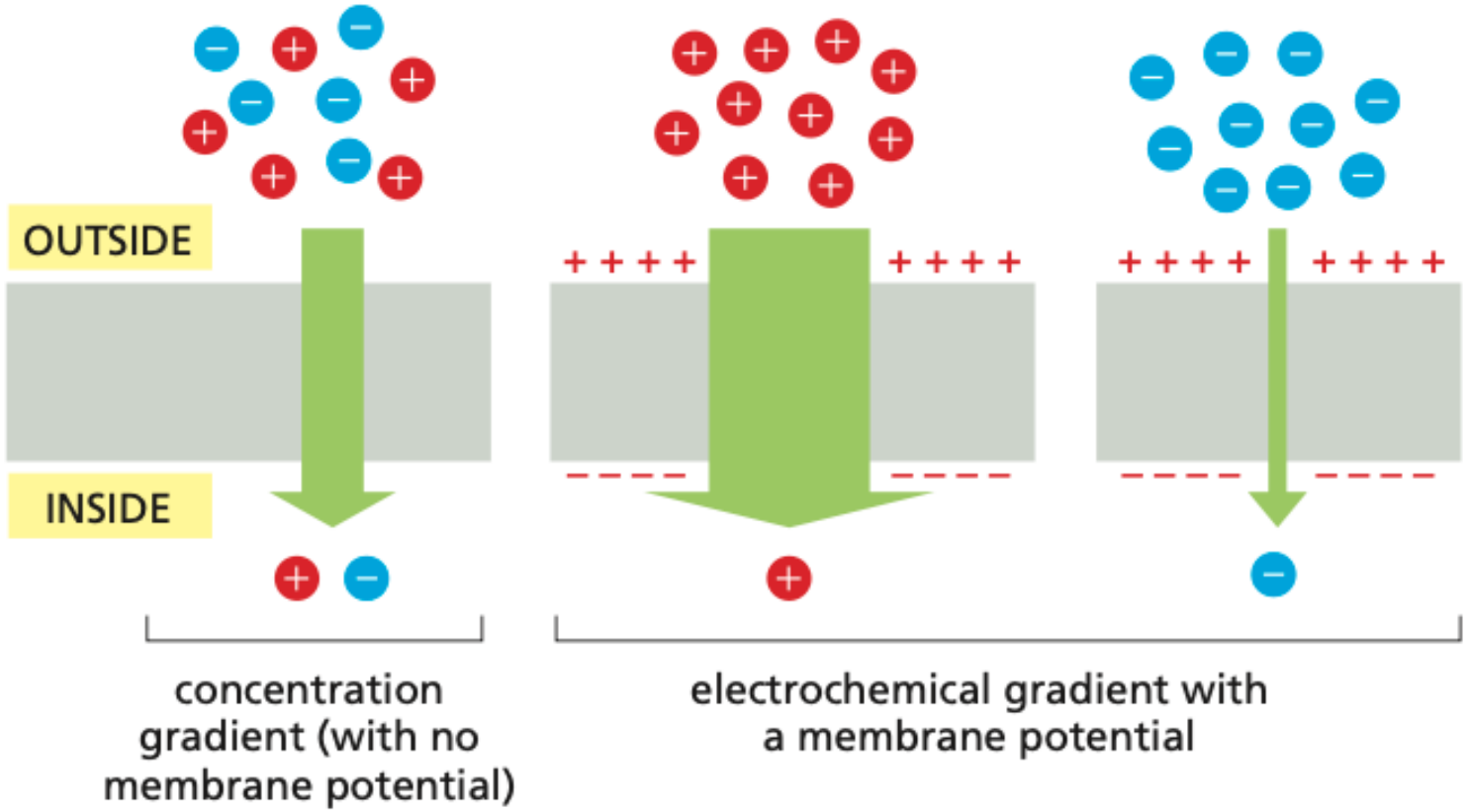
Passive transport relies on concentration gradients

Passive versus Active transport



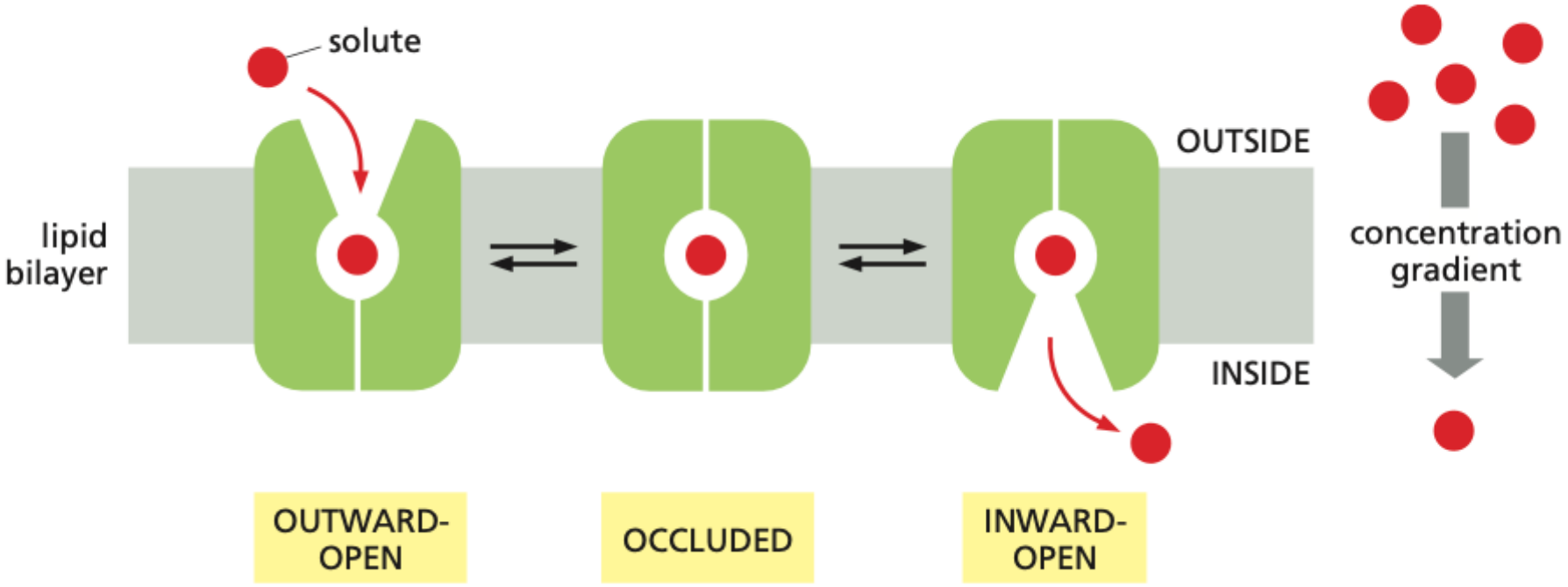
The basic structures and amino acid sequences of passive transporters and active transporters are very similar.

Charge of the transported molecule influences transport

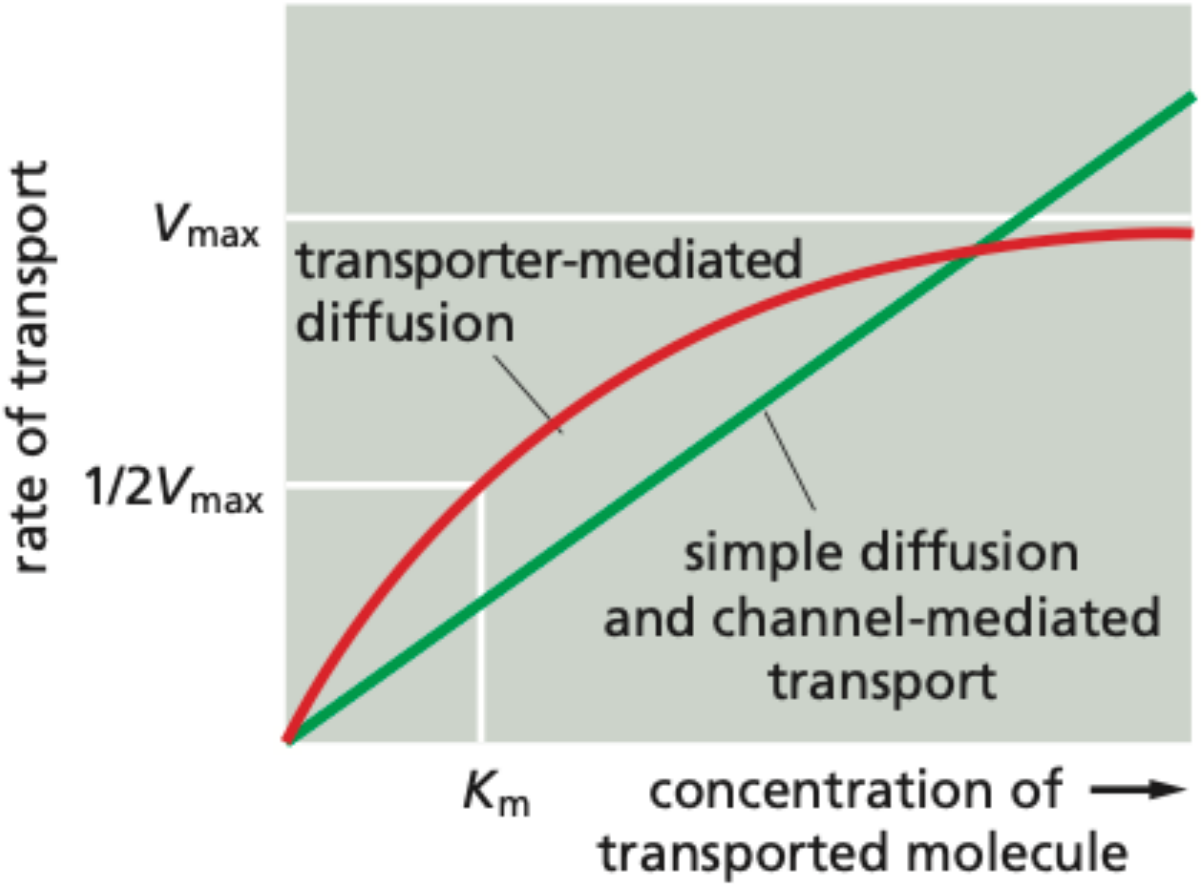


(B)

A model of how a conformational change in a transporter mediates the passive movement of a solute

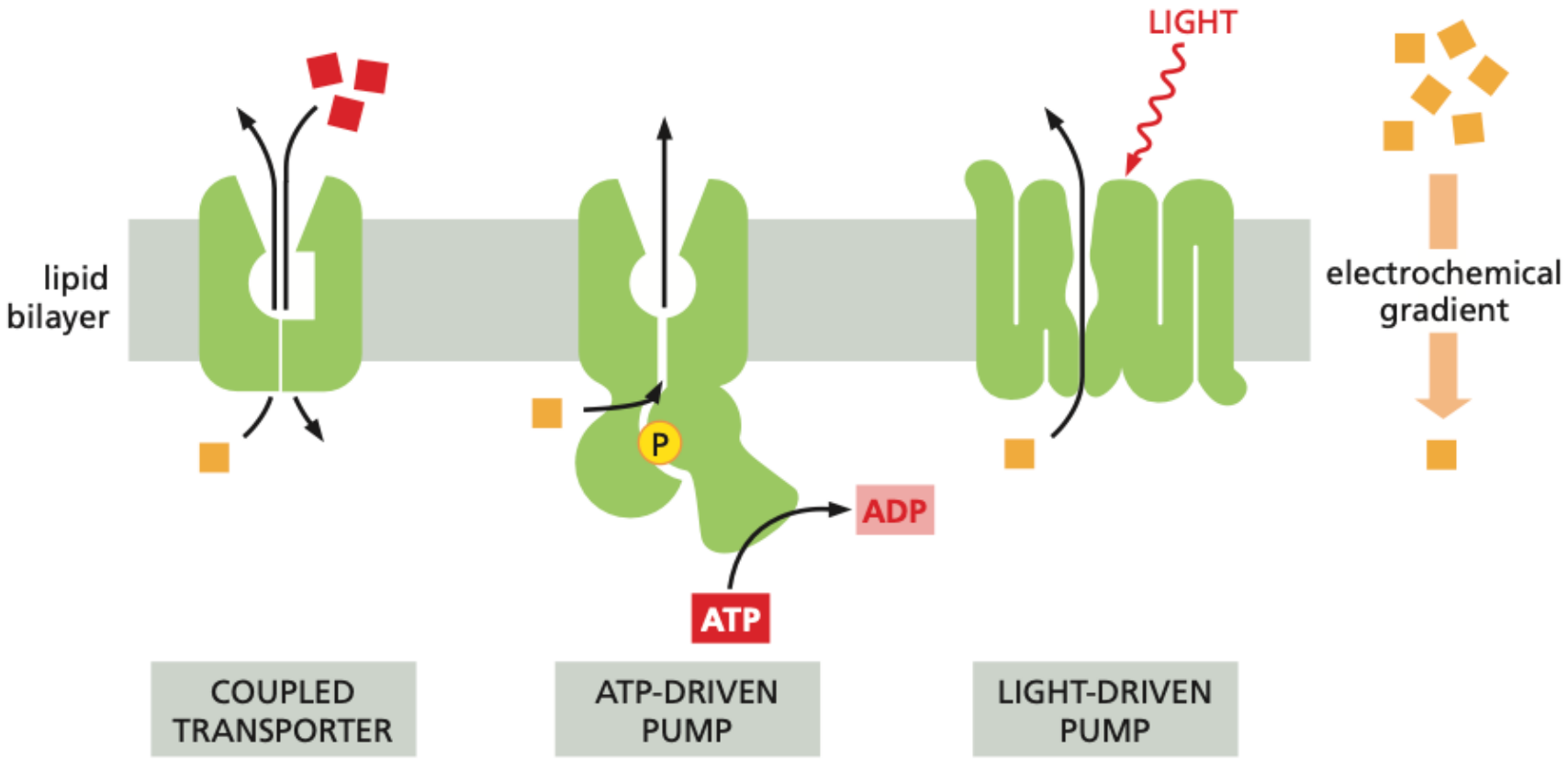


The kinetics transporters resemble a enzymatic reaction



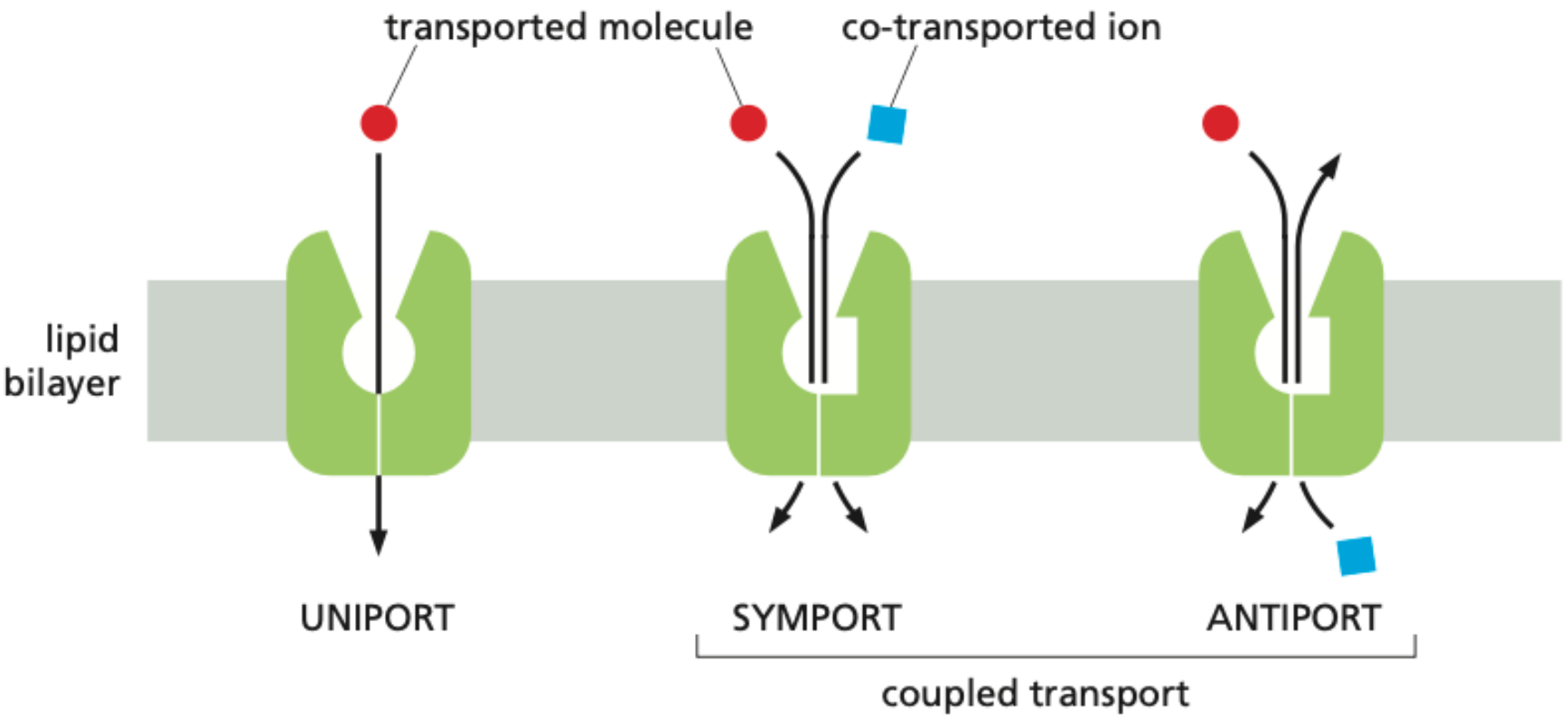
-Each carrier has a defined maximal flipping rate (V_{max})
-V_{max} is reached when the transporter is saturated
-The affinity (Km) equals the concentration of solute at 1⁄2 V_{max}

Three ways of driving active transport

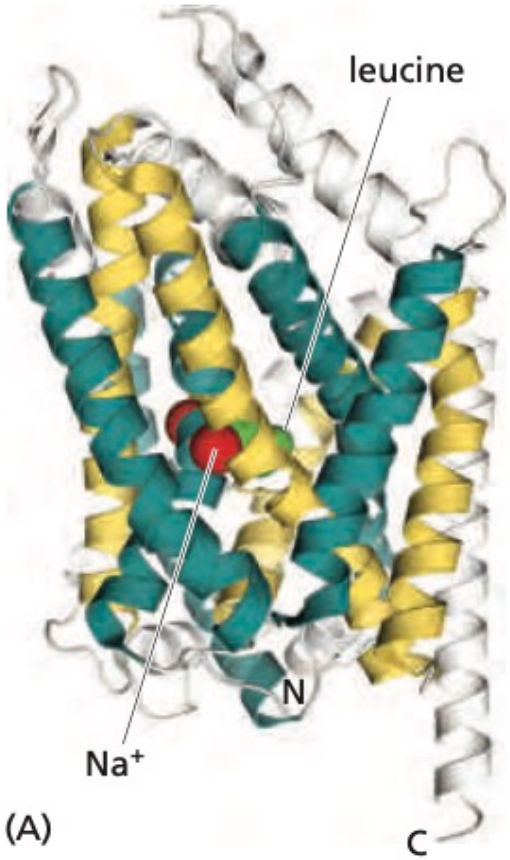


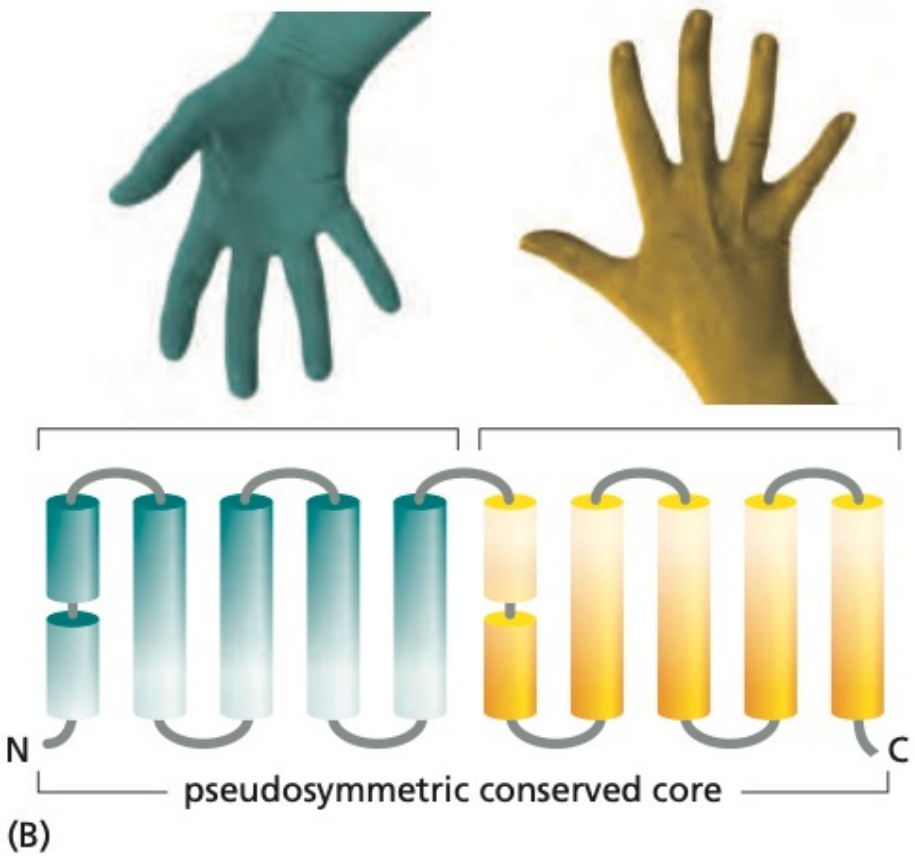
Active transport uses energy to drive transport against the electrochemical current

Different modes of coupled transport. uniporters, symporters, and antiporters



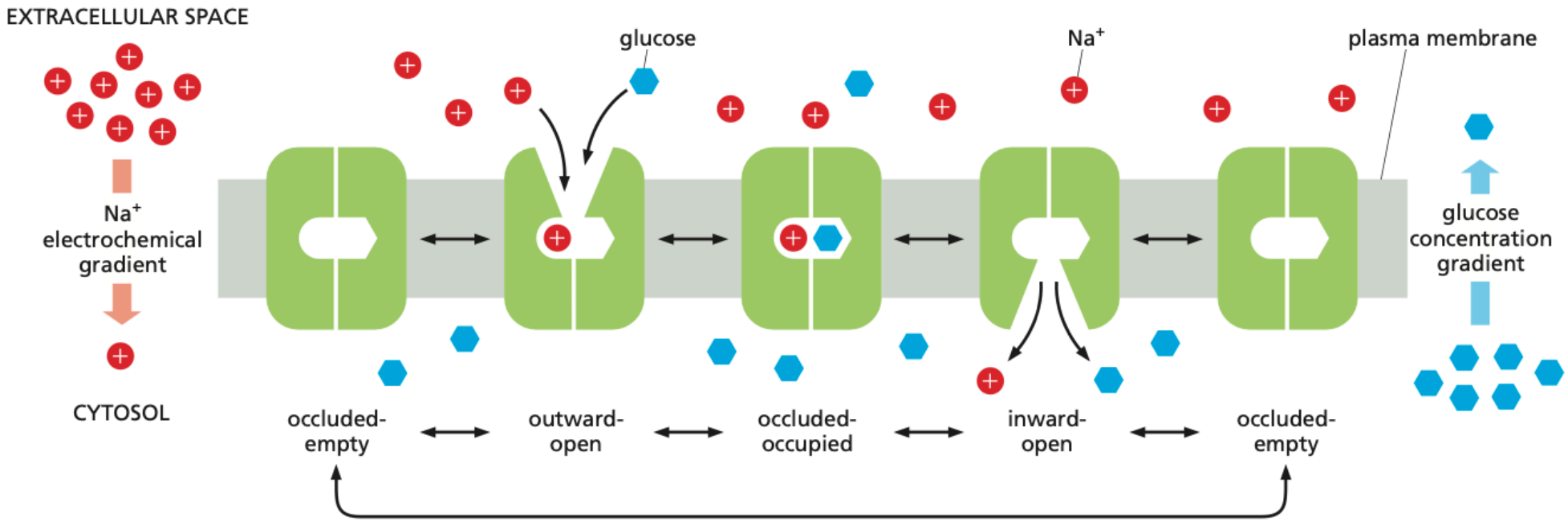
Transporters are built from inverted repeats





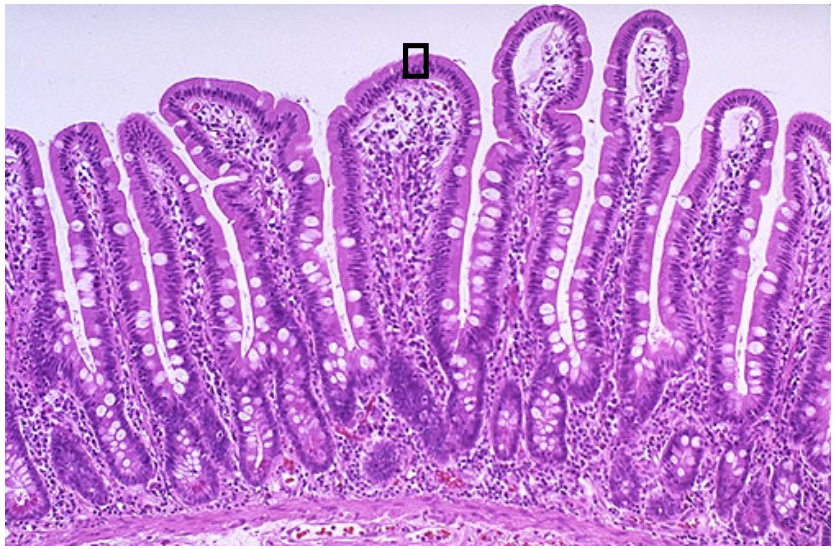
Active coupled transport driven by a Ion concentration

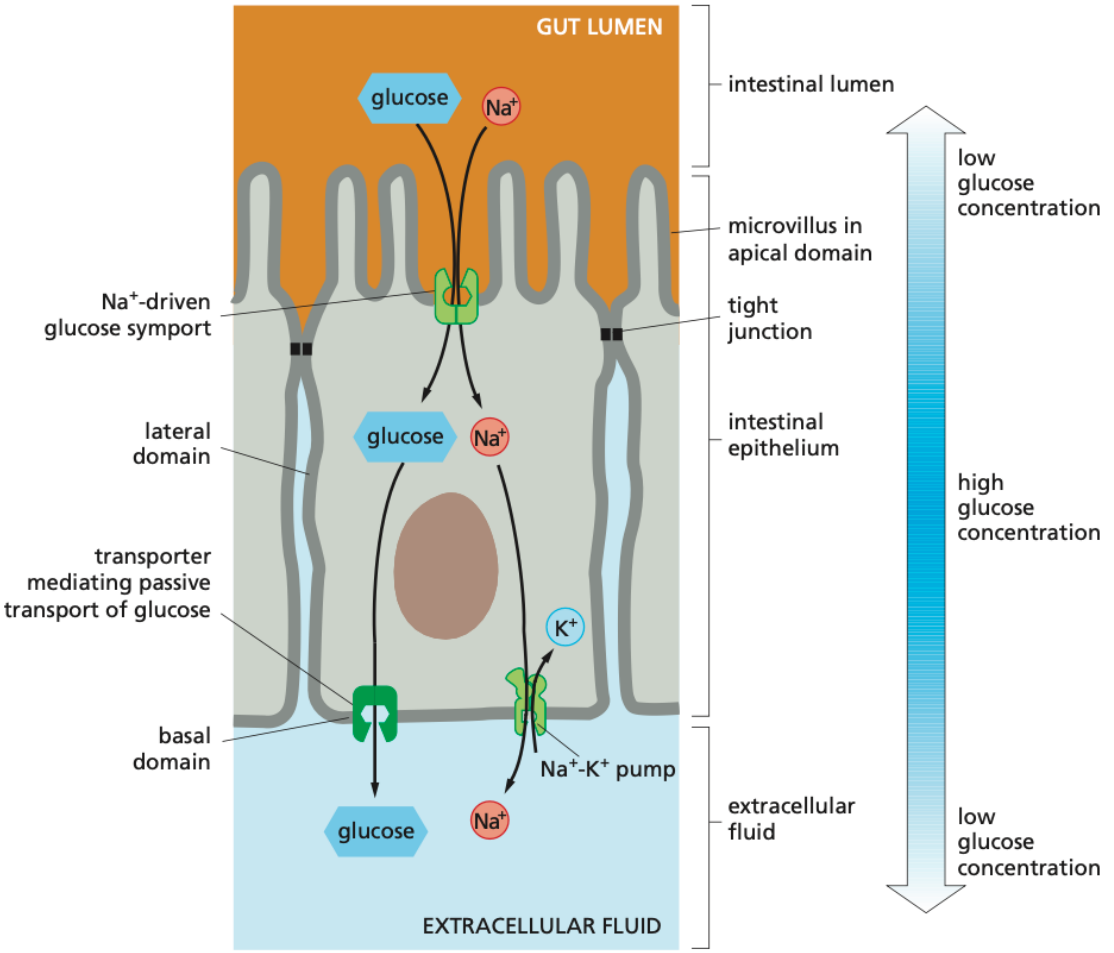
Mechanism of glucose transport fuelled by a Na+ gradient.



These glucose transporters are called the sodium glucose linked transport (SGLT) family of transporters. There are several with SGLT1 and SGLT2 the most well known

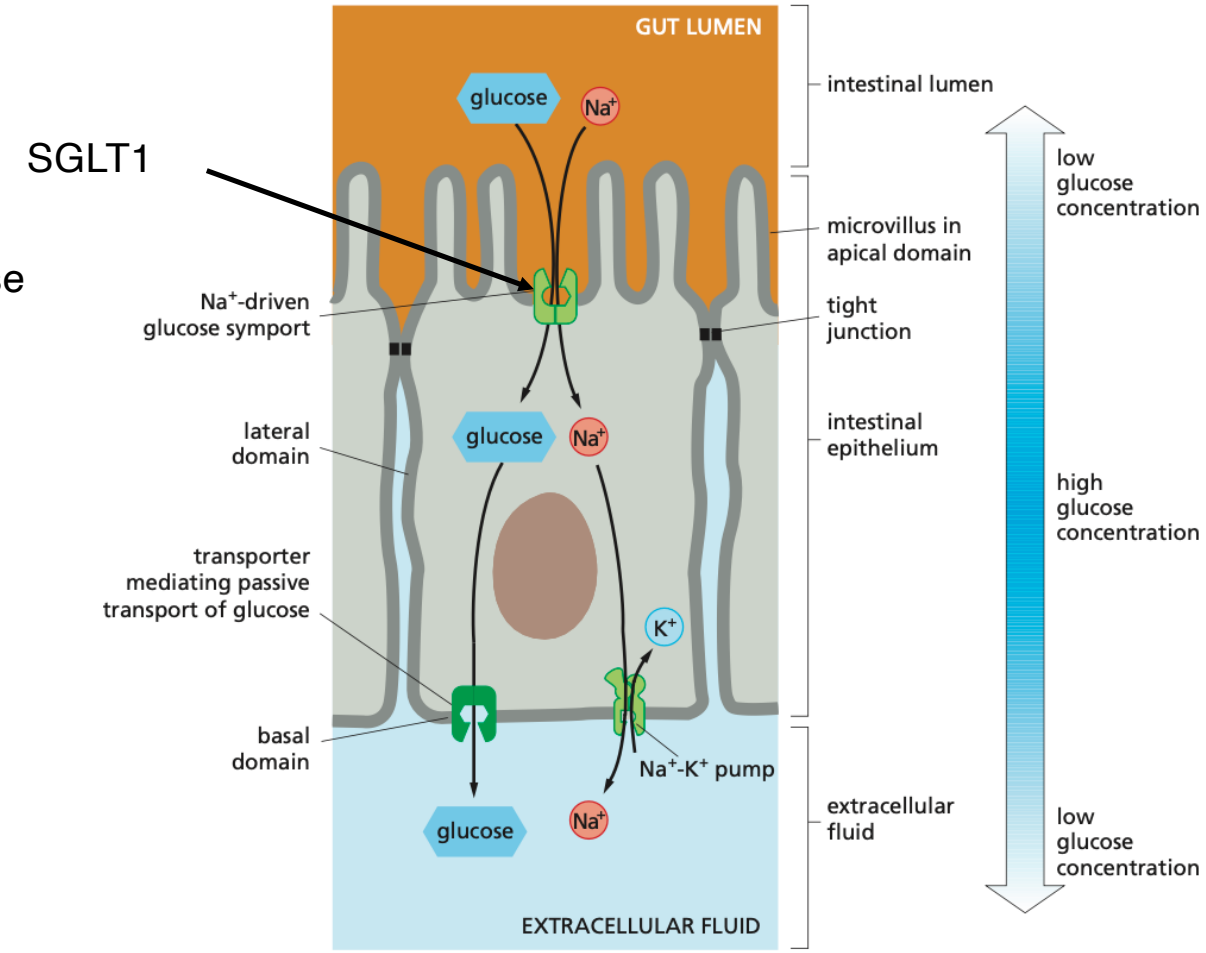
Glucose absorption in the small intestine





Transcellular transport

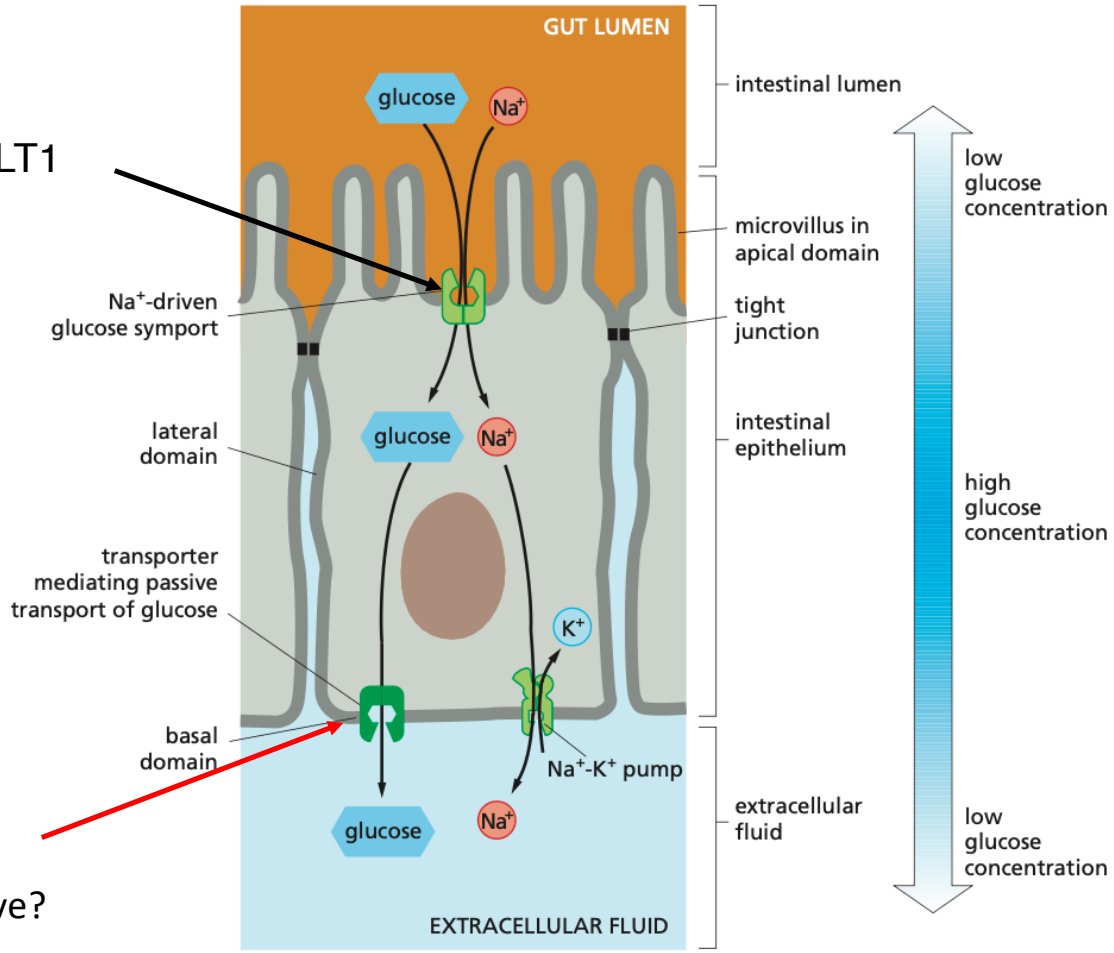
Co transport of glucose



Transcellular transport

SGLT1

Co transport of glucose



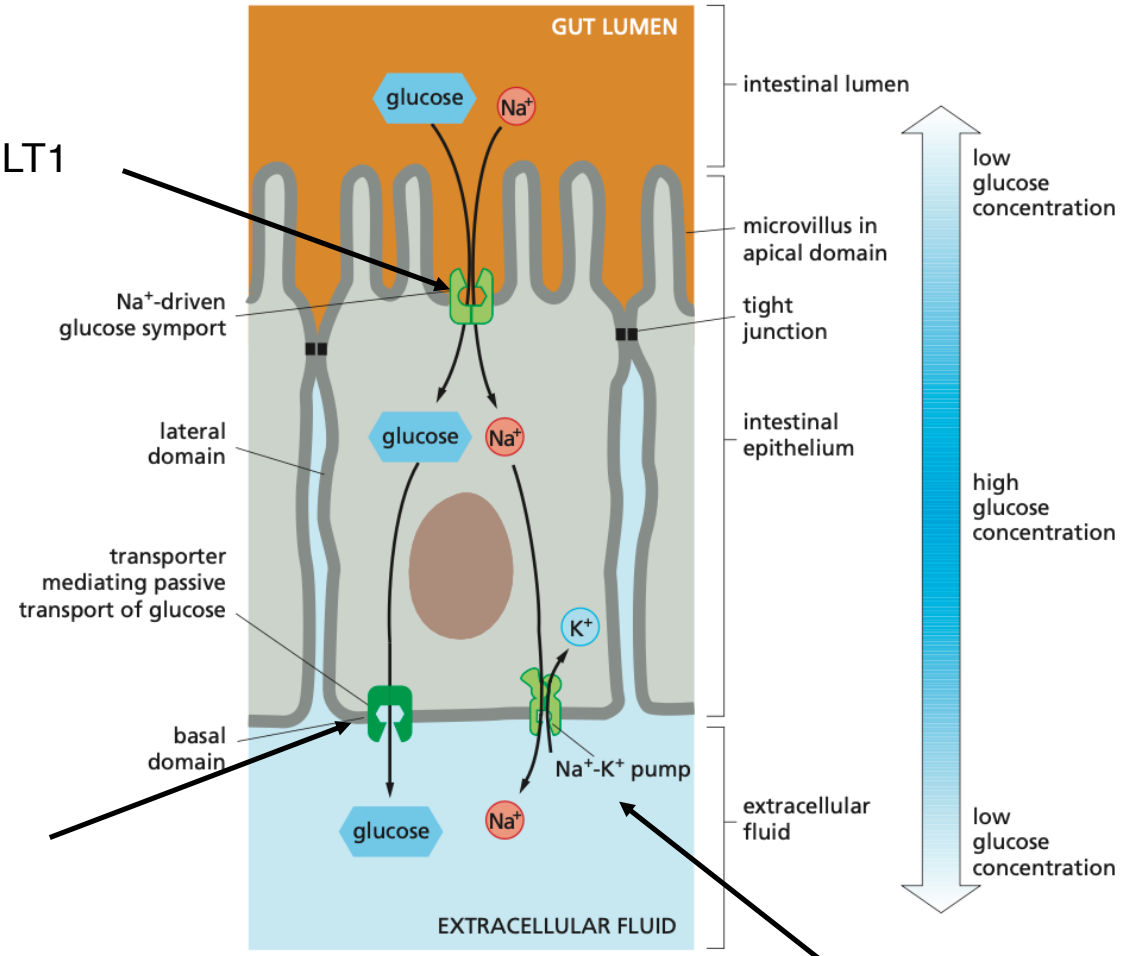
What is this transporter?

Would it be active of passive?

Transcellular transport

SGLT1

Co transport of glucose

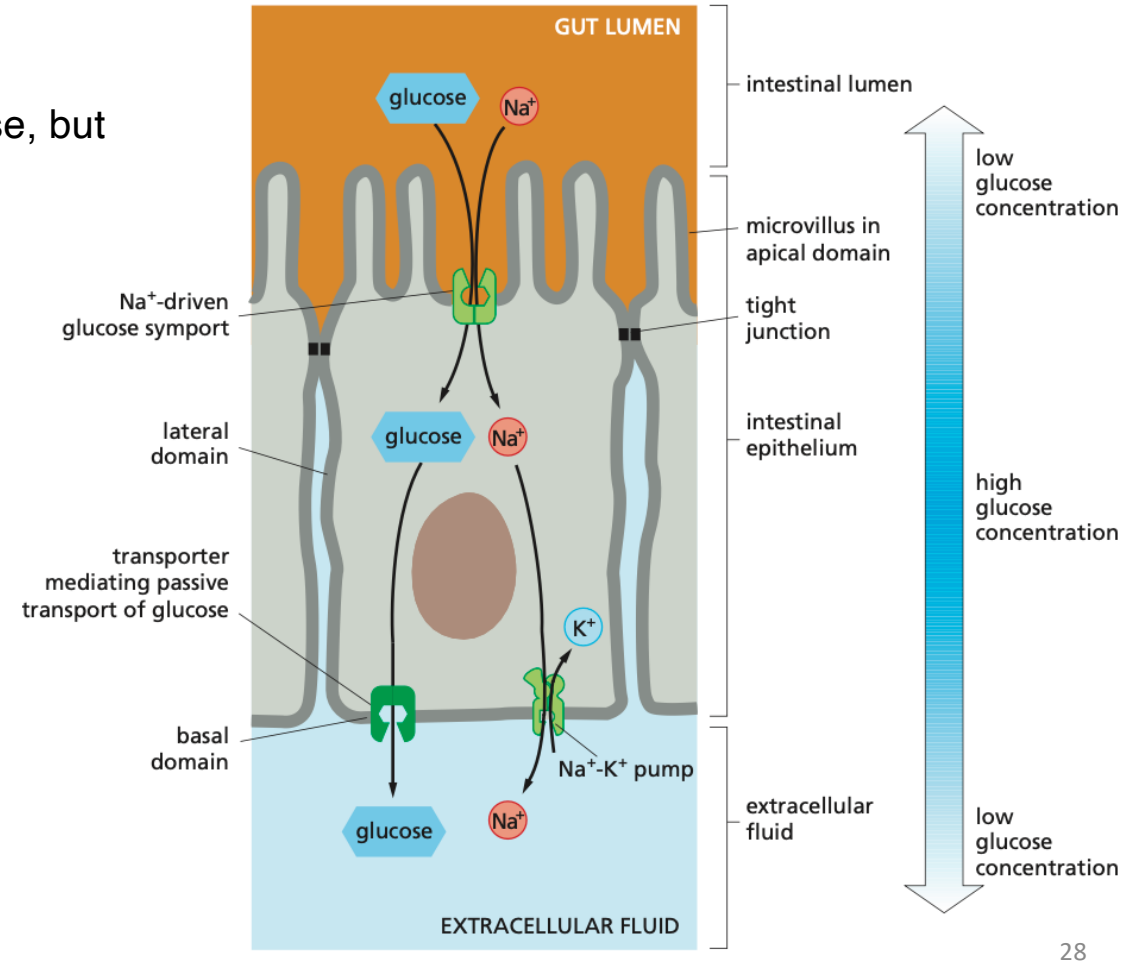


A passive glucose transporter GLUT

Discussed later!

Transcellular transport

There are 14 members in the GLUT family.
• Transport different substrates. Not only glucose, but fructose (GLUT5), urate (GLUT9).
• Have different K_M and V_{max}
• Are expressed in different tissues



Three types of ATP driven pumps

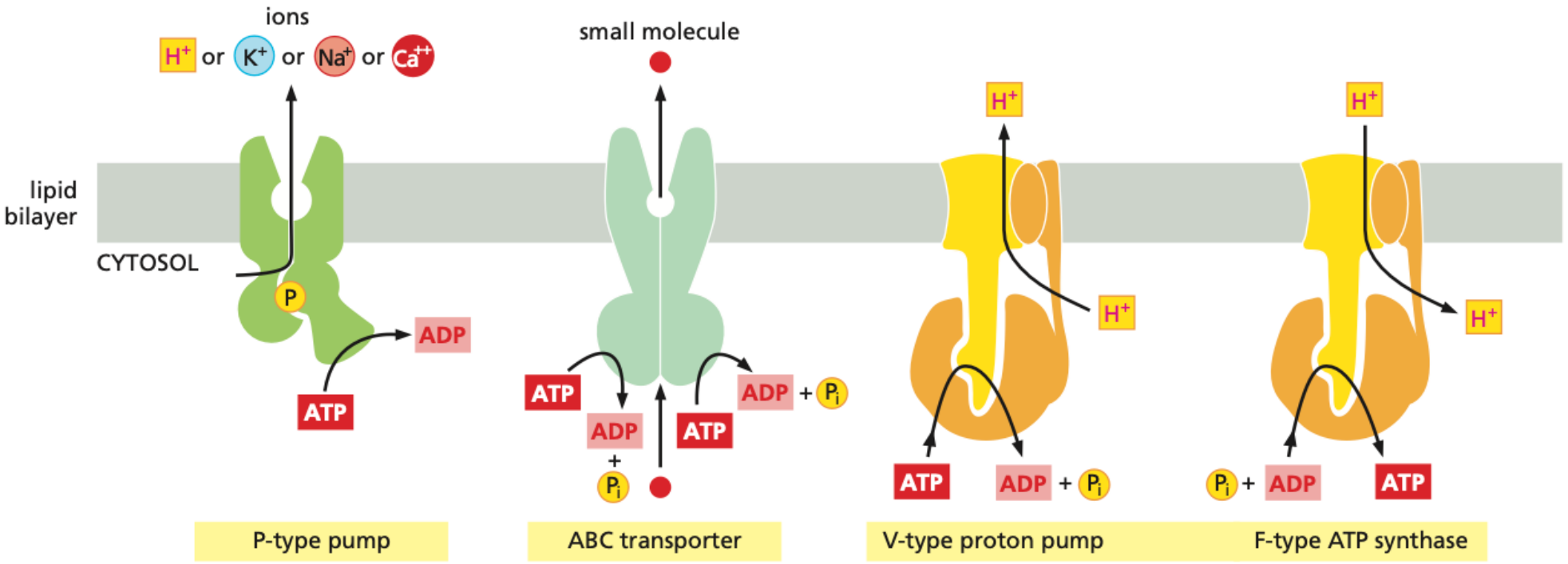


Figure 11–12 Three types of ATP-driven pumps. Like any enzyme, all ATP-driven pumps can work in either direction, depending on the electrochemical gradients of their solutes and the ATP/ADP ratio. When the ATP/ADP ratio is high, they hydrolyze ATP; when the ATP/ADP ratio is low, they can synthesize ATP. The F-type ATPase in mitochondria normally works in this "reverse" mode to make most of the cell's ATP.

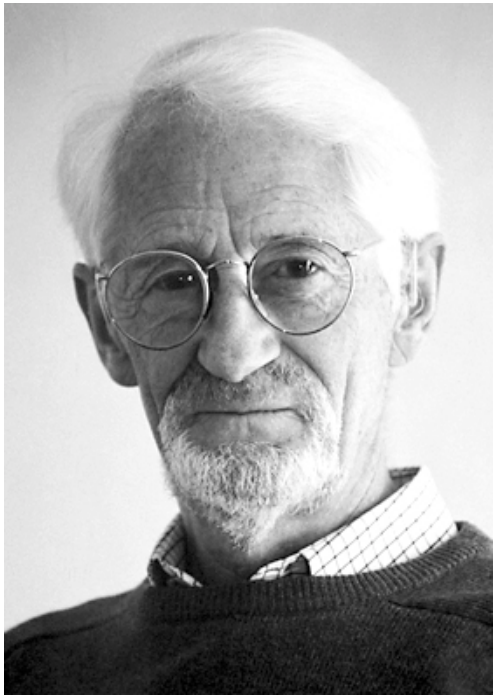
Examples of ATP driven pumps

Transporter type	function
Na^+/K^+ pump (P-type)	To maintain gradient of Na^+ and K^+
Ca^{++} ATPase (P-type)	To keep [Ca^{++}] low (for signaling)
Gastric H^+/K^+ pump (P-type)	To secrete HCl

The Na+/K+ ATPase

- ATP-driven antiporter
  P-type ATPase
  Maintains the Na+ gradient important for:
- transport of nutrients into cells - osmotic balance
- 1/3 of the cell's energy is devoted to this pump!

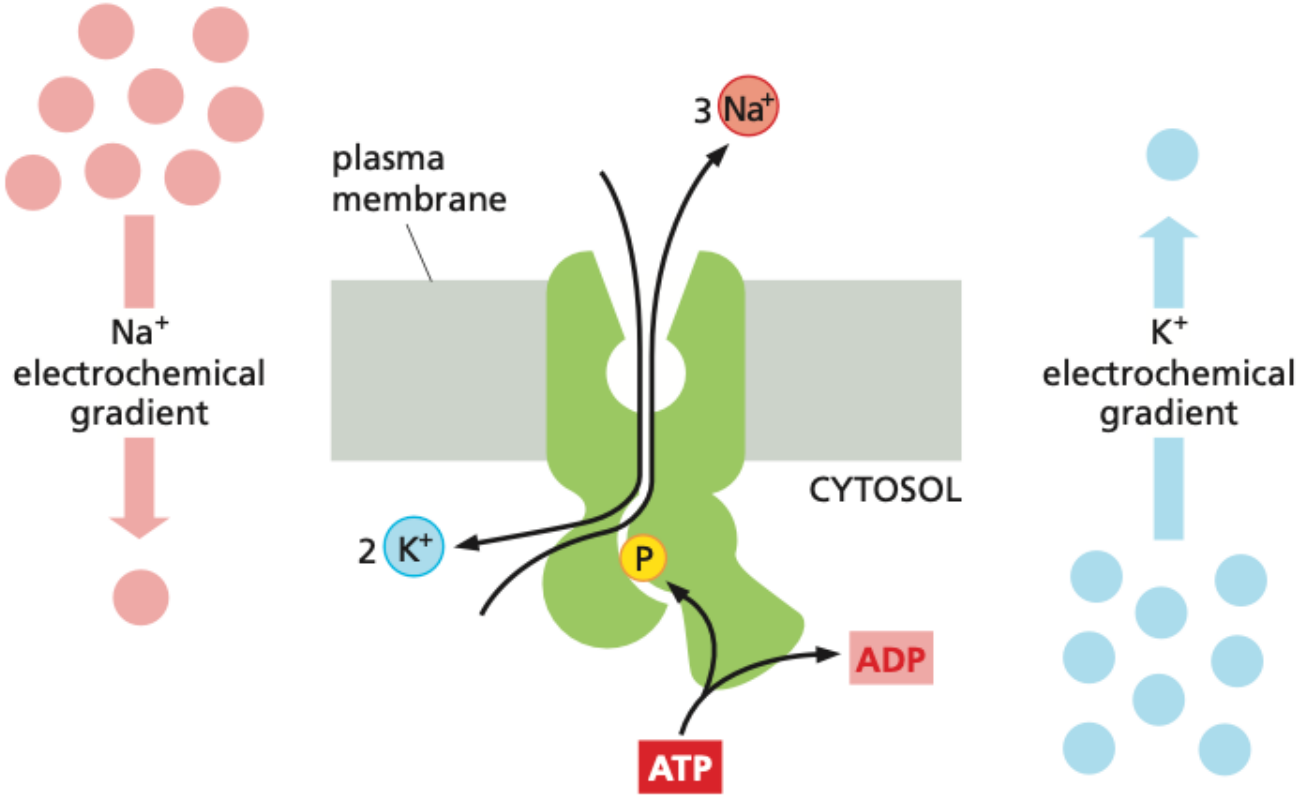
Jens C. Skou



Nobel prize in chemistry, 1997

"for the first discovery of an ion-transporting enzyme, Na^+, K^+ -ATPase"

The function of the Na^+-K^+ pump



The cycle of the Na^+-K^+ pump

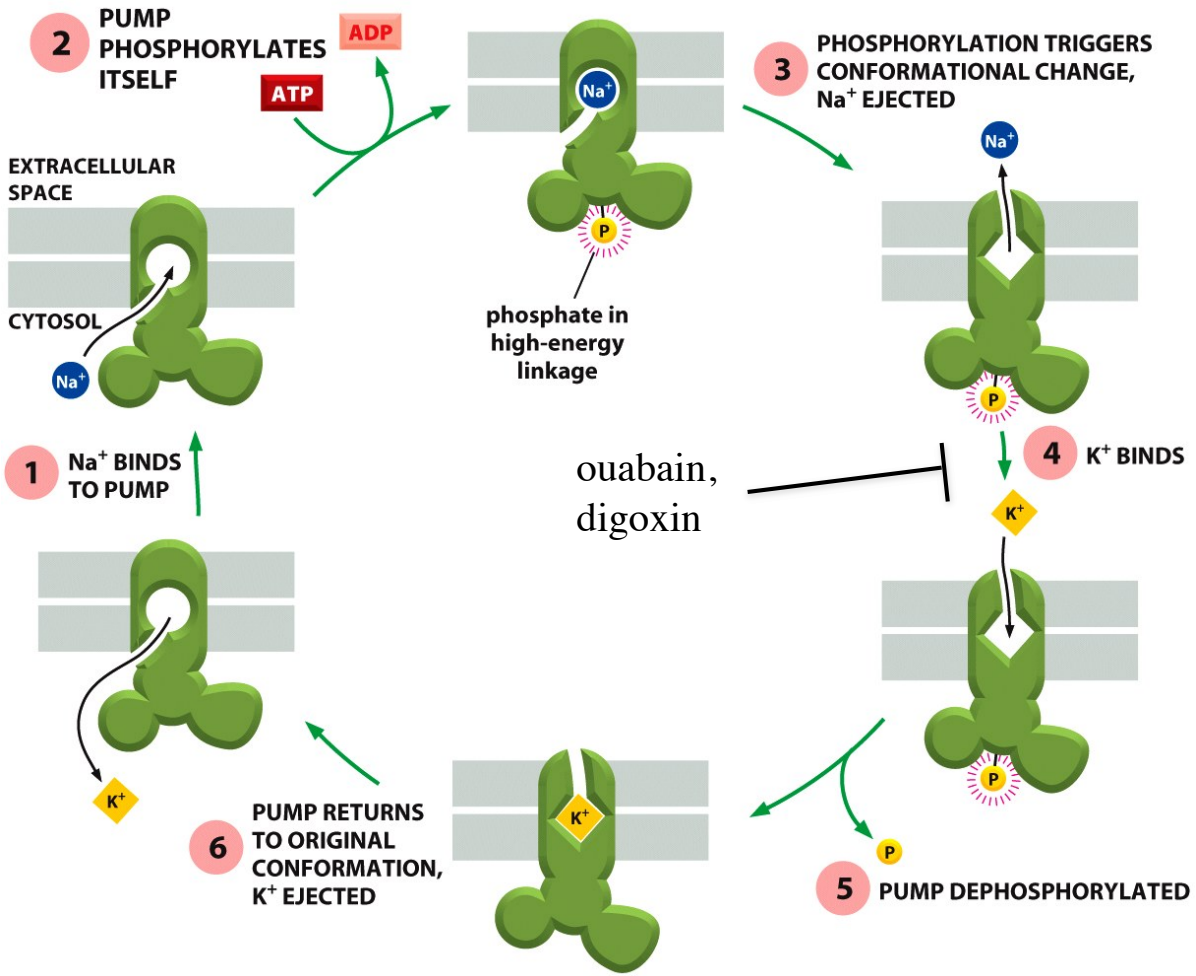


Figure 12-11 *Essential Cell Biology* (© Garland Science 2010)

The Na+/K+ ATPase is important to regulate cellular osmolarity

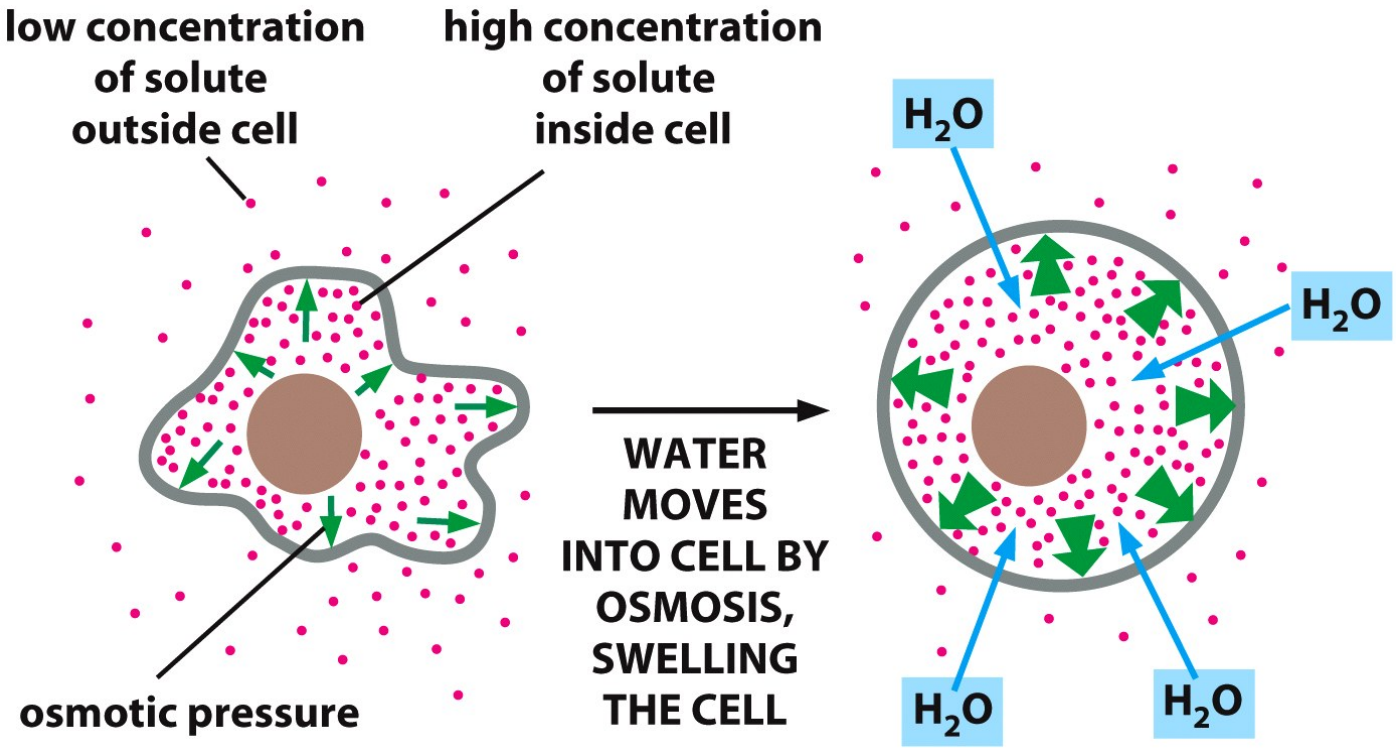
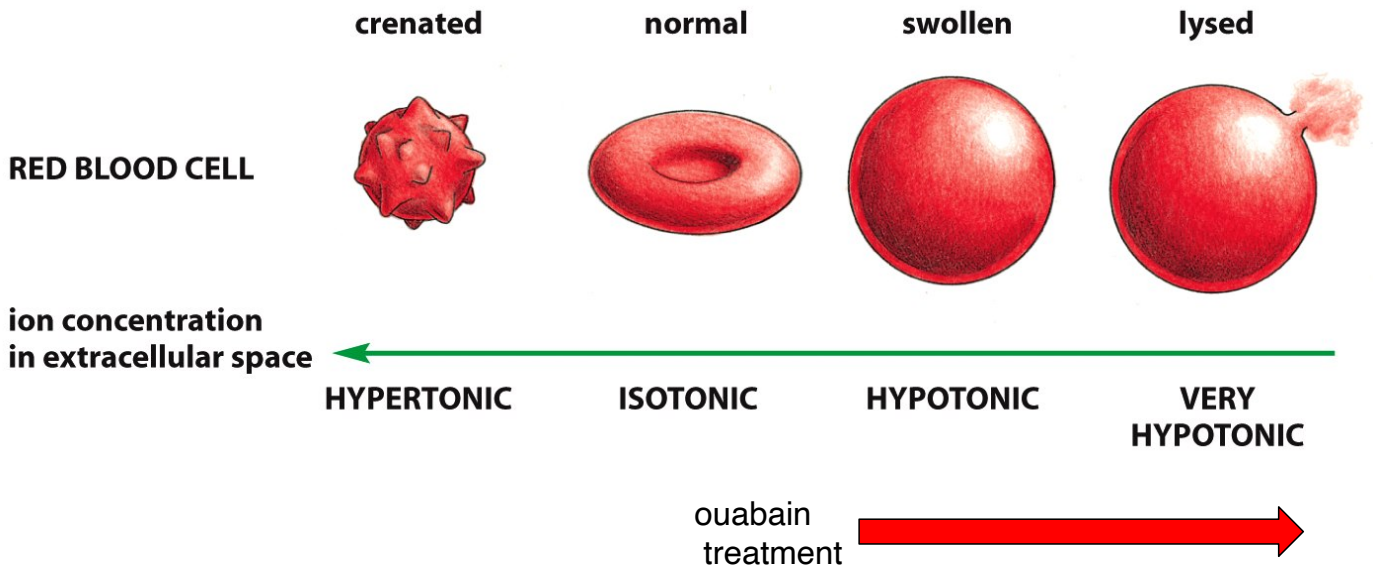


Figure 12-12 *Essential Cell Biology* (© Garland Science 2010)

Osmolarity in red blood cells

- Plasma membrane has high permeability to H_2O
- Na^+/K^+ pump is important to keep red blood cell volume

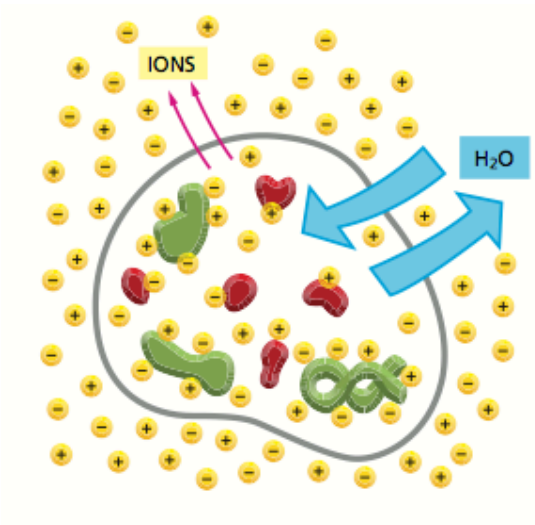


11

Figure 11-16 *Molecular Biology of the Cell* (© Garland Science 2008)

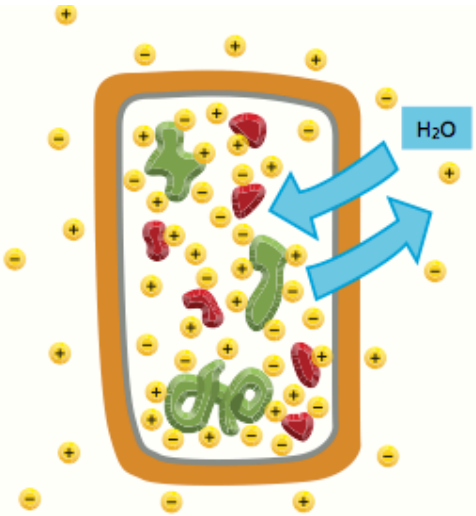
Osmolarity across life

Animal cells and bacteria



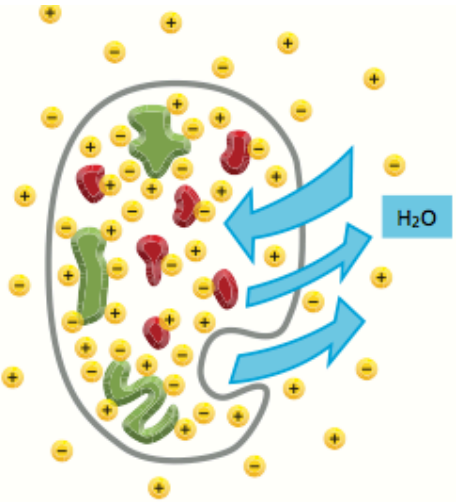
Active pumping out
To have lower intracellular [ion], to compensate the excess of organic solutes

Plant cells



Can tolerate an osmotic difference, due to rigid wall

Protozoa



Extrude water from vacuoles

10

Panel 11-1, Molecular Biology of the Cell (© Garland Science 2008)

the sarcoplasmic reticulum Ca2+ pump.

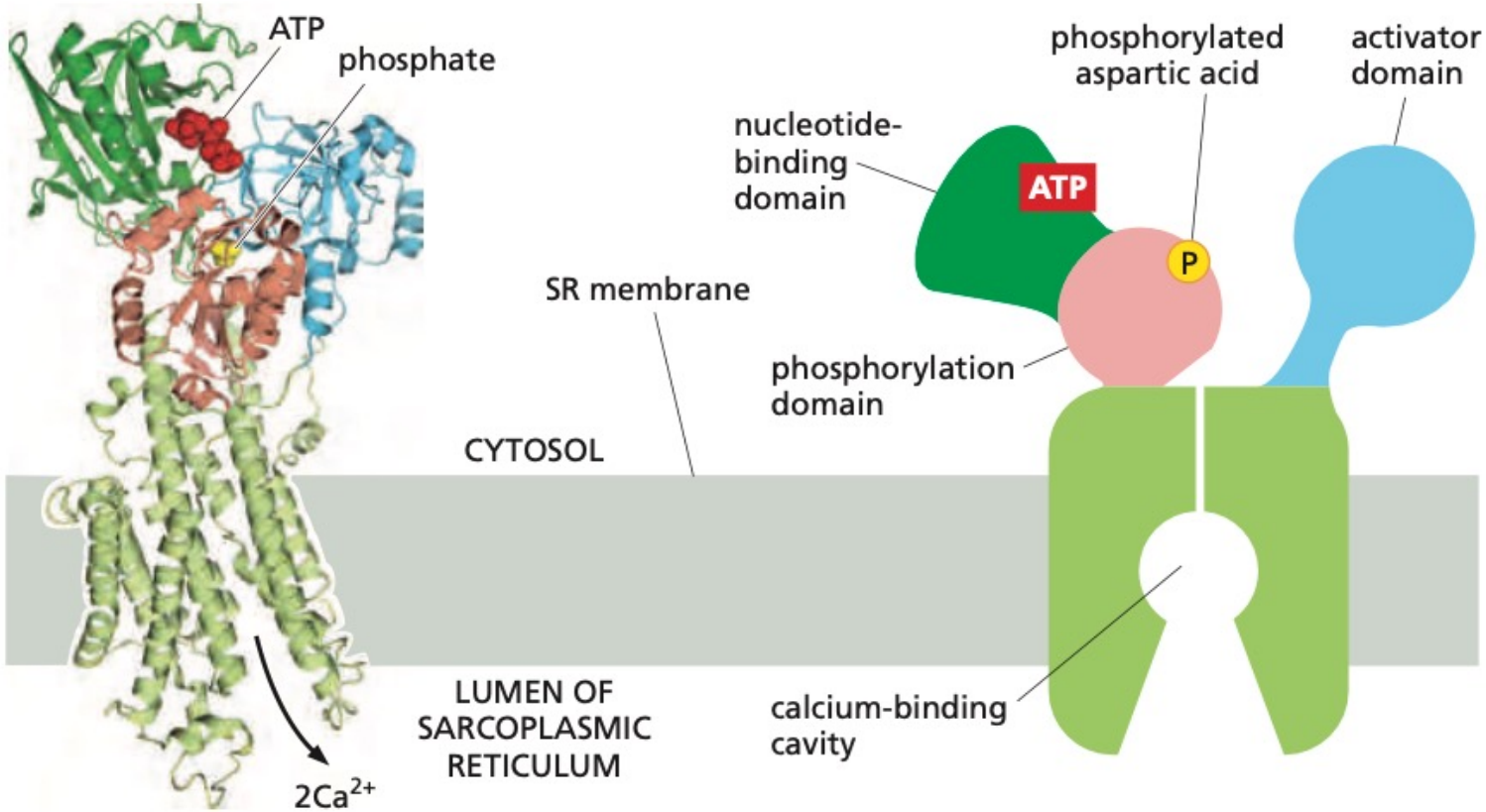


Figure 11–13 The structure of the sarcoplasmic reticulum Ca^{2+} pump. The ribbon model (*left*), derived from x-ray crystallographic analyses, shows the pump in its phosphorylated, ATP-bound state. The three globular cytosolic domains of the pump—the nucleotide-binding domain (*dark green*), the activator domain (*blue*), and the phosphorylation domain (*red*), also shown schematically on the *right*—change conformation dramatically during the pumping cycle. These changes in turn alter the arrangement of the transmembrane helices, which allows the Ca^{2+} to be released from its binding cavity into the SR lumen (Movie 11.3). (PDB code: 3B9B.)

The pumping cycle of the sarcoplasmic reticulum Ca2+ pump.

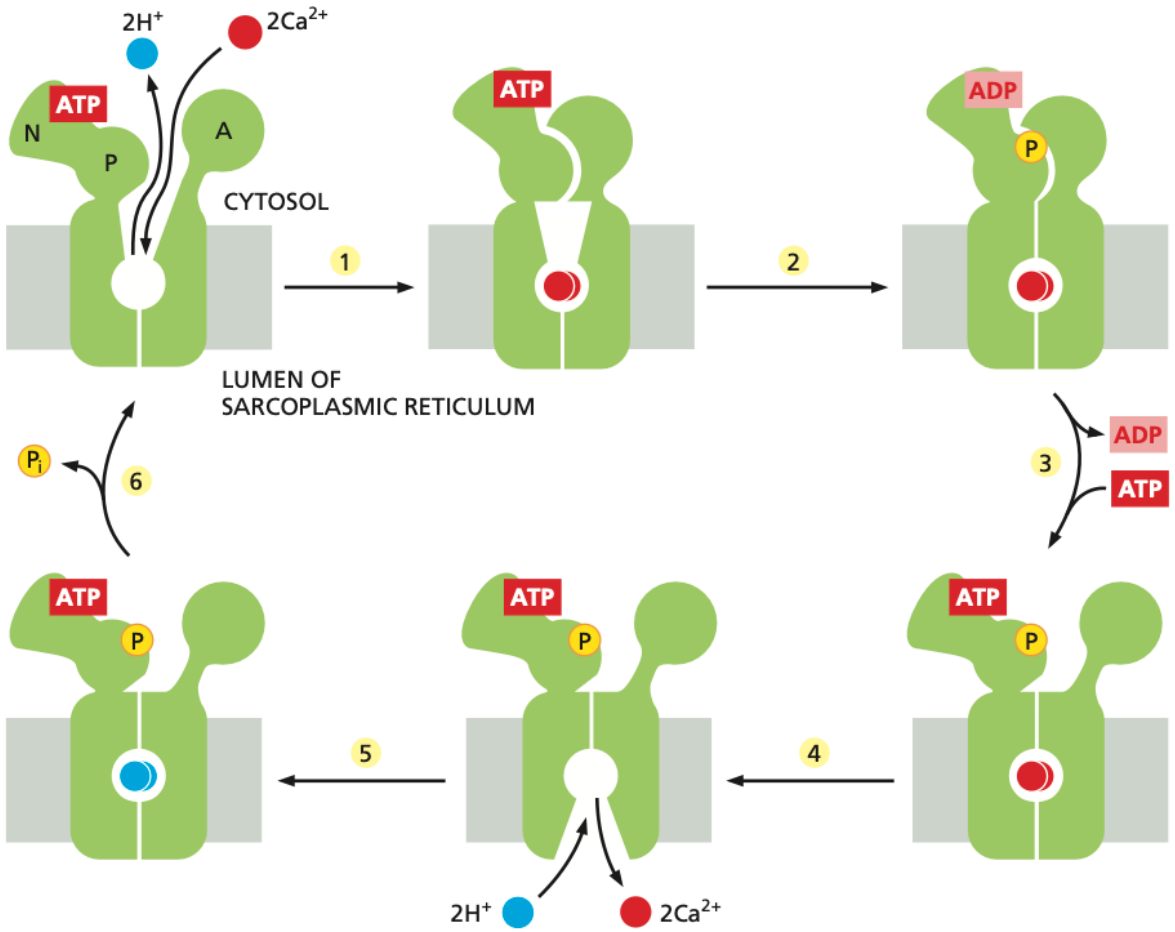
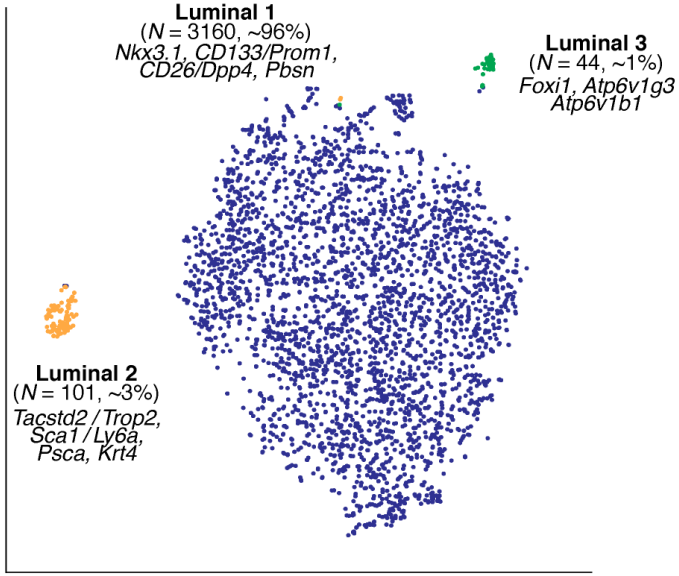
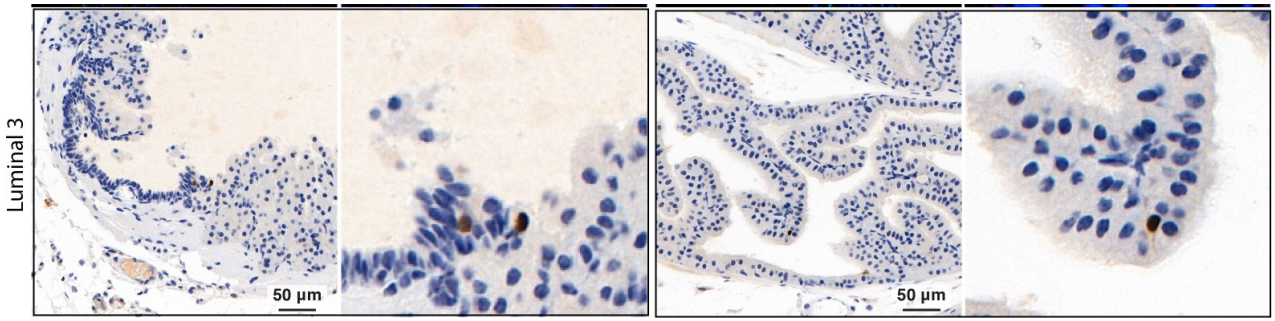


Figure 11–14 The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump. Ion pumping proceeds by a series of stepwise conformational changes in which movements of the pump's three cytosolic domains [the nucleotide-binding domain (N), the phosphorylation domain (P), and the activator domain (A)] are mechanically coupled to movements of the transmembrane α helices. Helix movement opens and closes passageways through which Ca^{2+} enters from the cytosol and binds to the two centrally located Ca^{2+} binding sites. The two Ca^{2+} then exit into the SR lumen and are replaced by two H^+, which are transported in the opposite direction. The Ca^{2+}-dependent phosphorylation and H^+-dependent dephosphorylation of aspartic acid are universally conserved steps in the reaction cycle of all P-type pumps: they cause the conformational transitions to occur in an orderly manner, enabling the proteins to do useful work. (Adapted from C. Toyoshima et al., *Nature* 432:361–368, 2004 and J.V. Møller et al., *Q. Rev. Biophys.* 43:501–566, 2010.)

Cell type Luminal 3 in the prostate (Karthaus lab)

A cell expressing vacuolar-type H^+-ATPase (V-ATPase) identified in the male prostate
Regulates the pH of reproductive fluids
Necessary for reproduction!



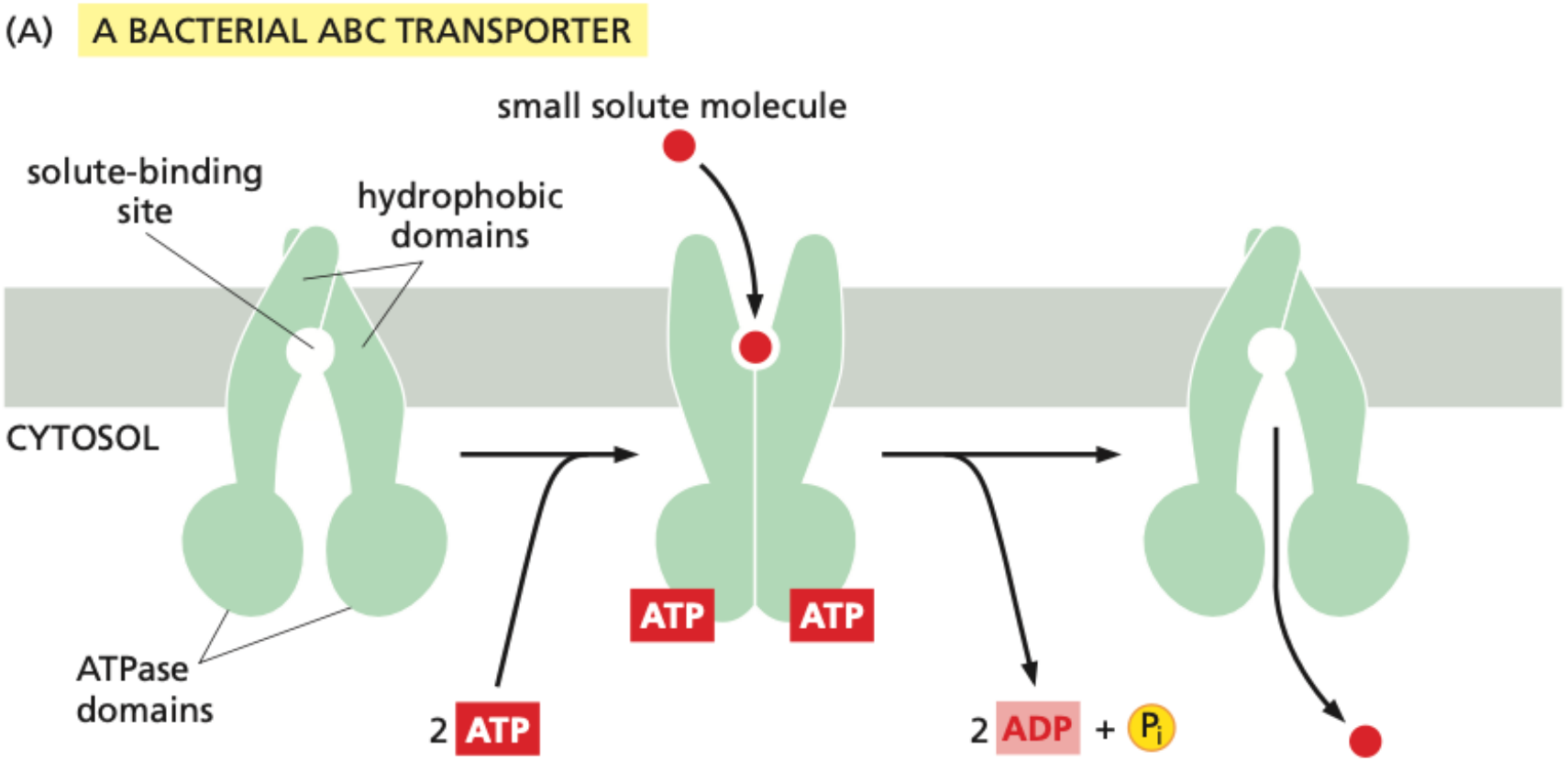


ABC transporters

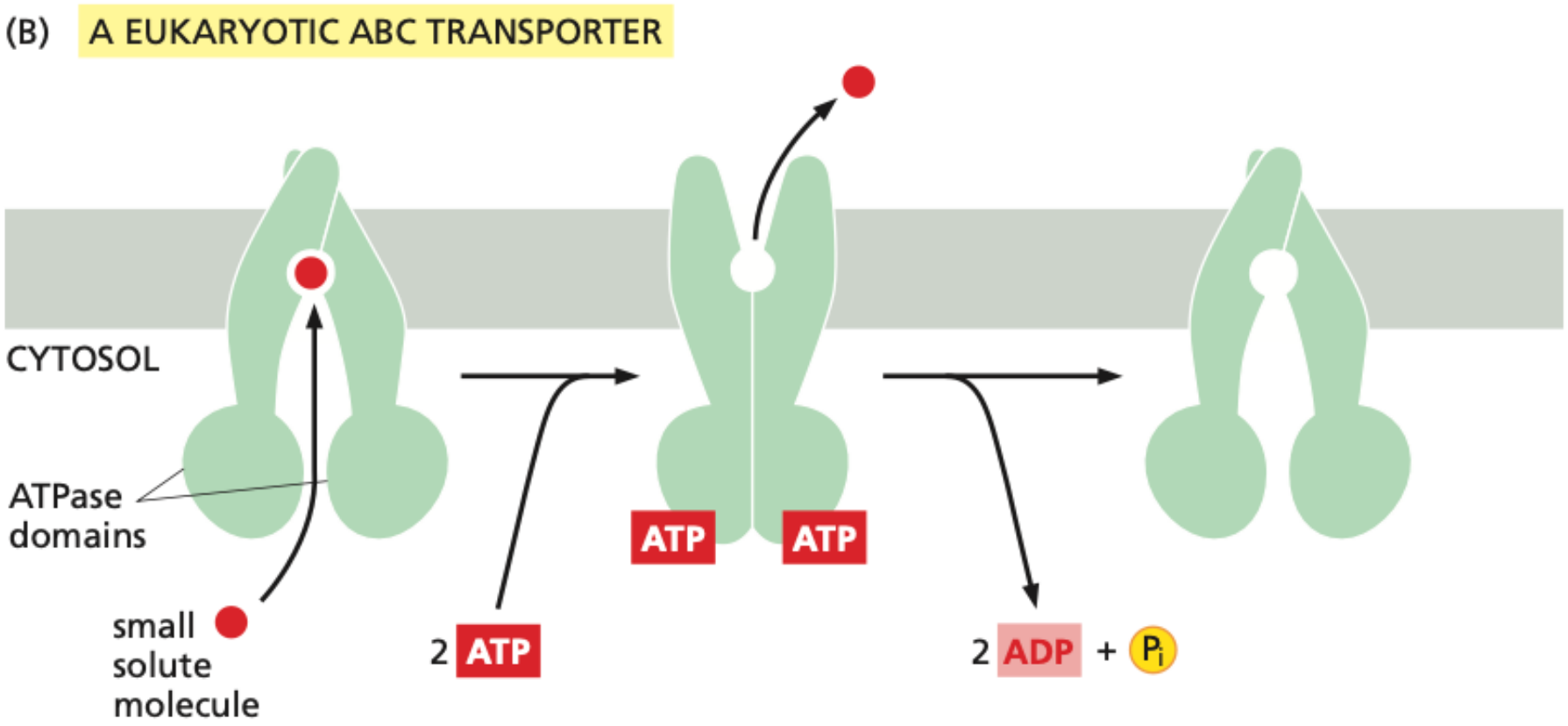
ABC transporters

- Large family of membrane transport proteins (ex: 5% of *E. coli* genome). Exist in all phyla. 48 proteins in human.
- 2ATPase domains (ATP-binding cassettes) involved
- ATP binding leads to dimerization of the ATP-binding domains
- ATP hydrolysis leads to their dissociation
- Transport a wide variety of substrates: sugars, amino acids, drugs, antibiotics, toxins, lipids, peptides, nucleotides and more

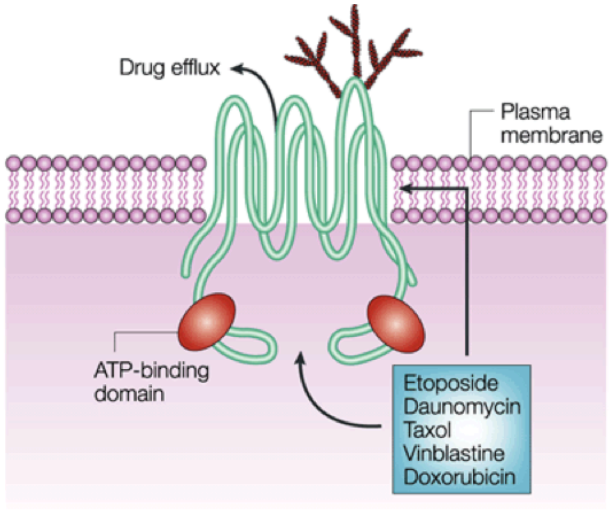
Small-molecule transport by typical ABC transporters



Small-molecule transport by typical ABC transporters



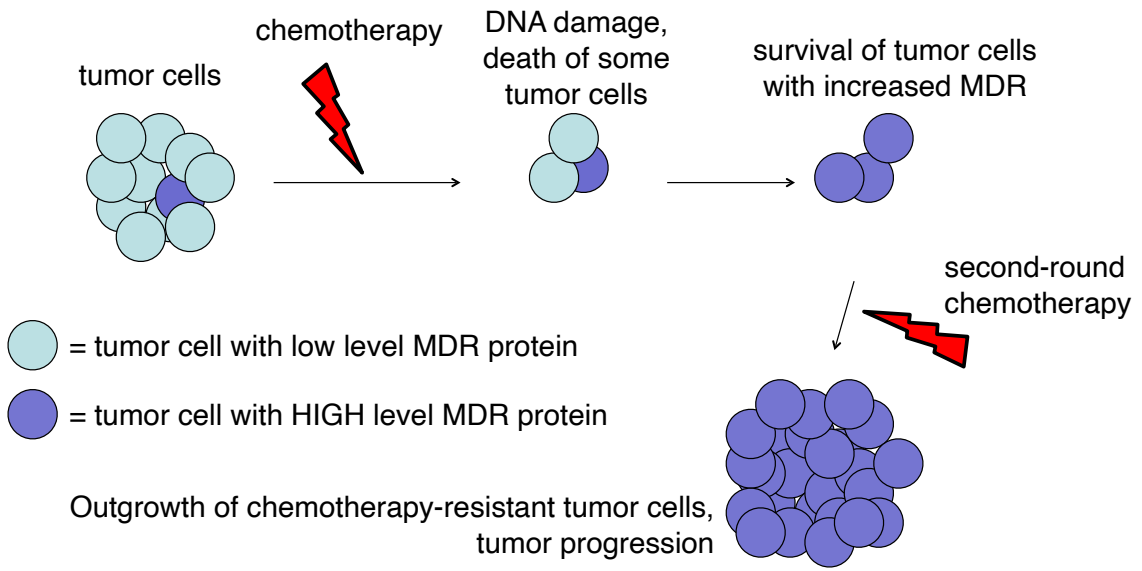
The multidrug resistance (MDR) efflux pumps.
The expression of ABC drug transporters has been linked to chemotherapy resistance and poor prognosis.



Brian P. Sorrentino
Nature Reviews Cancer 2, 431-441 (2002)

Some eukaryotic ABC transporters promote resistance to chemotherapies

The multidrug resistance (MDR) proteins in cancer promote drug efflux



19

An example of a transporter dependency in cancer

nature cancer

ARTICLES

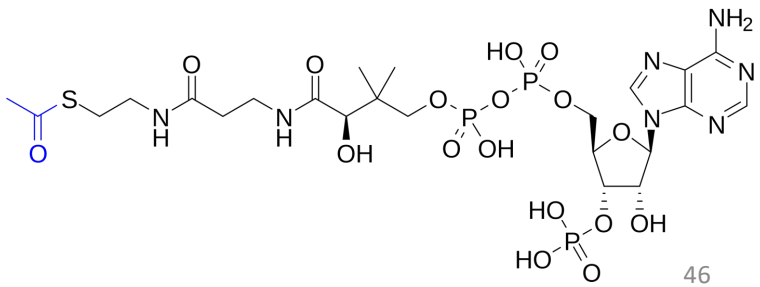
https://doi.org/10.1038/s43018-020-0071-1

Check for updates

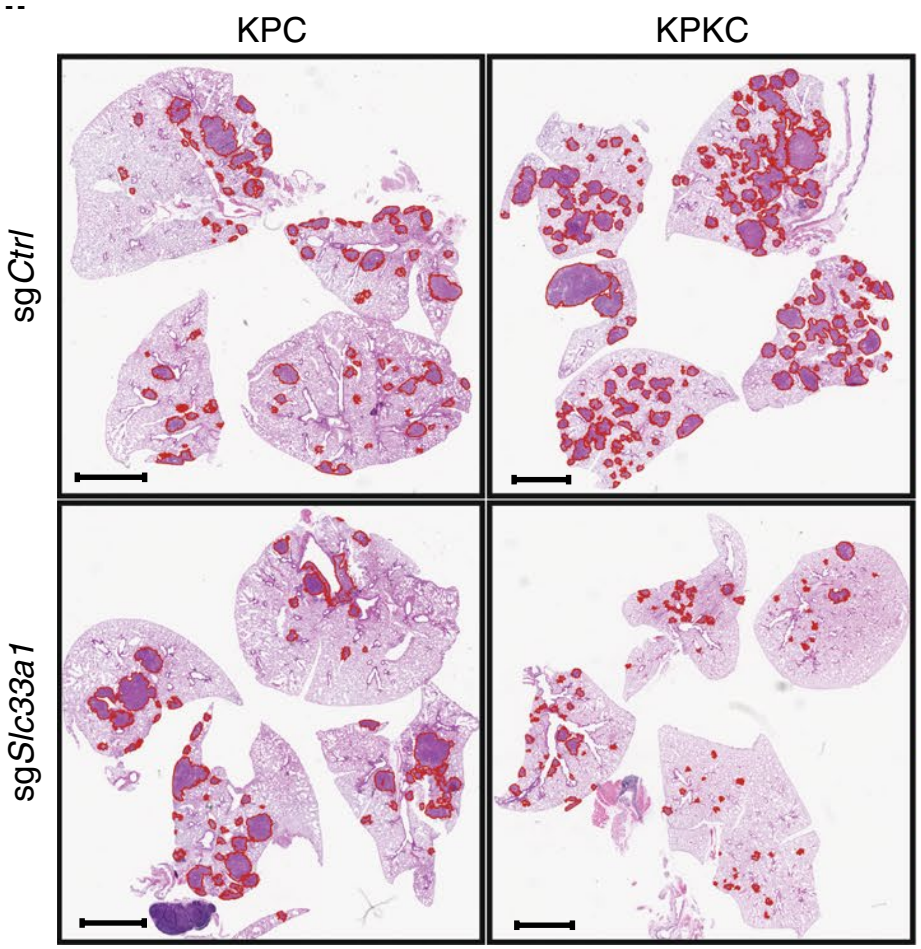
Keap1 mutation renders lung adenocarcinomas dependent on *Slc33a1*

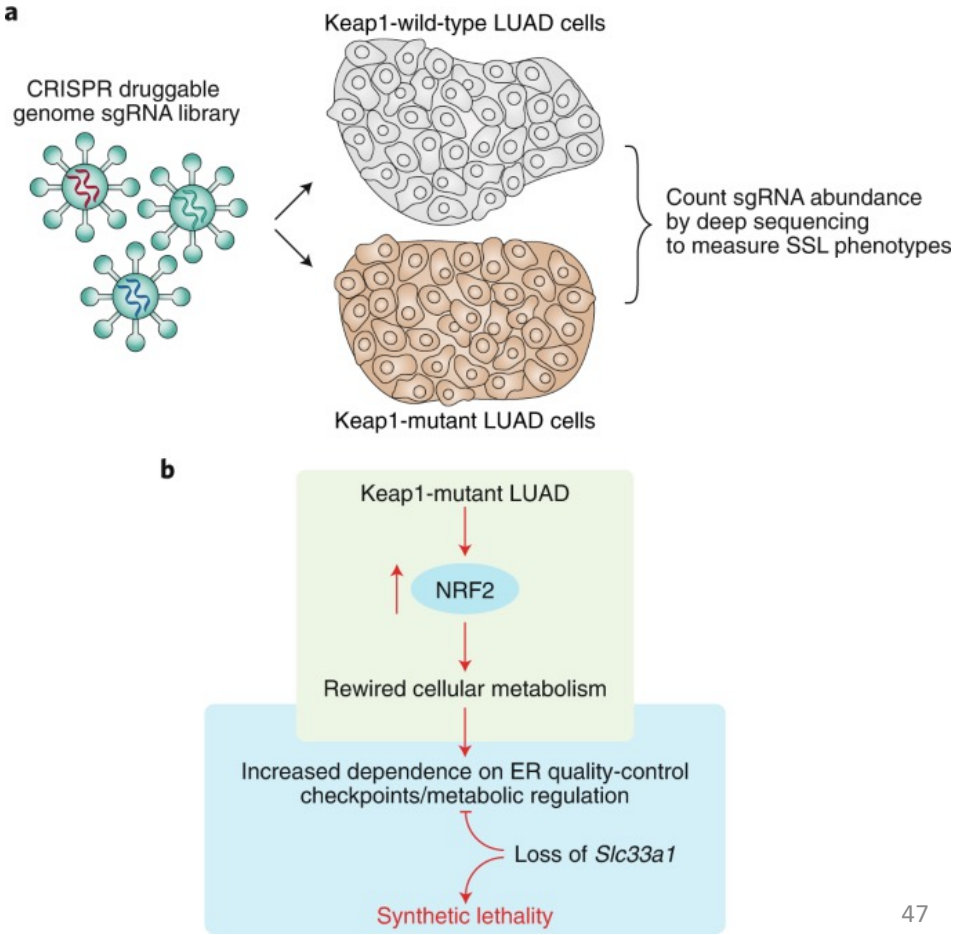
Rodrigo Romero[1,2,11], Francisco J. Sánchez-Rivera[1,2,9,11], Peter M. K. Westcott[1], Kim L. Mercer[1,3], Arjun Bhutkar[1], Alexander Muir[1,10], Tania J. González Robles[1], Swanny Lamboy Rodríguez[2], Laura Z. Liao[2], Sheng Rong Ng[1,2], Leanne Li[1], Caterina I. Colón[1], Santiago Naranjo[1,2], Mary Clare Beytagh[2], Caroline A. Lewis[4], Peggy P. Hsu[1,5,6], Roderick T. Bronson[7,8], Matthew G. Vander Heiden[1,2,6] and Tyler Jacks[1,2,3] ✉

Acetyl-coenzyme A transporter 1 also known as **solute carrier family 33 member 1** (SLC33A1) is a protein that in humans is encoded by the SLC33A1 gene.[5]



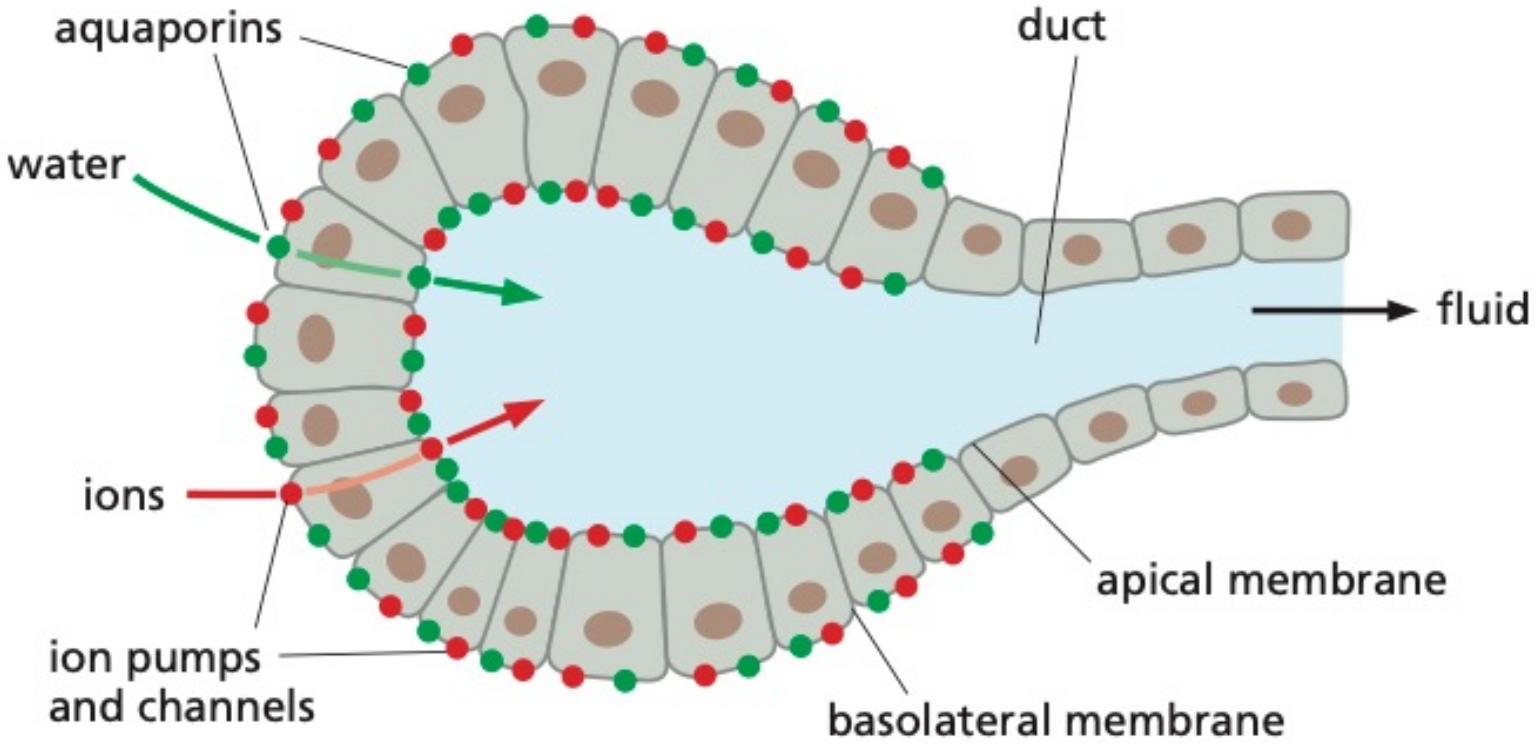
An example of a transporter dependency in cancer





Aquaporins

The role of aquaporins in fluid secretion



Aquaporins

There are thirteen known types of aquaporins in mammals, and six of these are located in the kidney
Transport water at high rates
Ions cannot pass

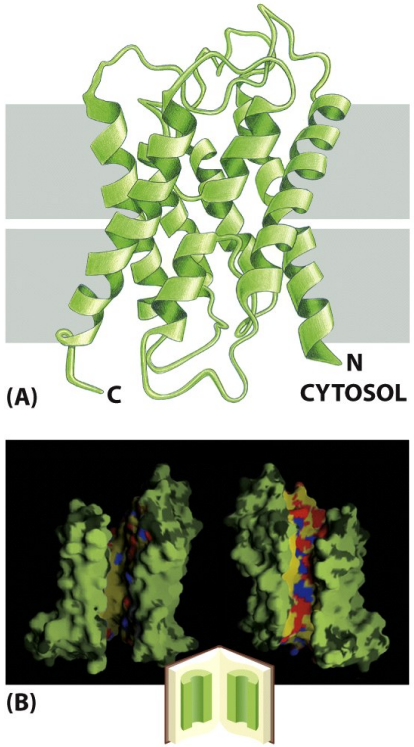
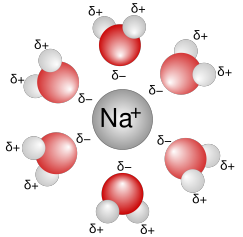


Figure 11-27 *Molecular Biology of the Cell* (© Garland Science 2008)



Pore is very thin

↓

Ions need to be dehydrated

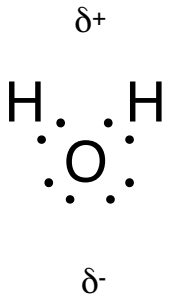
↓

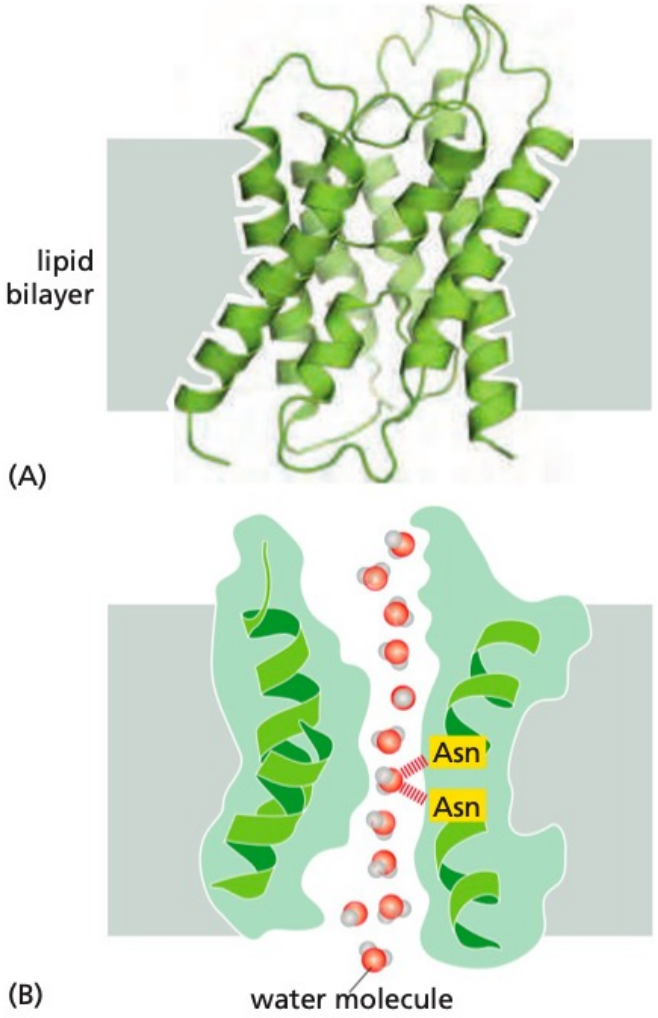
Energy loss not compensated because the hydrophobic wall of the pore cannot interact with dehydrated ion

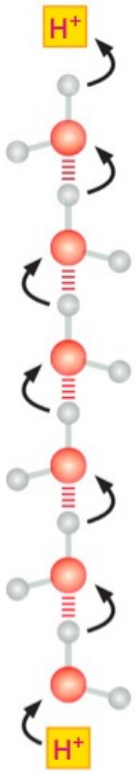
↓

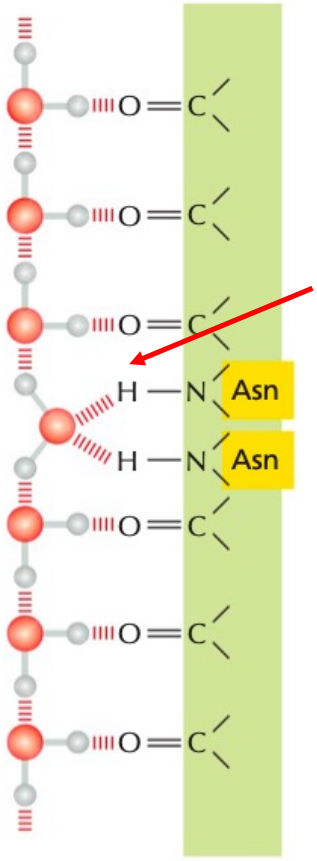
Na^+, K^+, Ca^{++}, Cl^- excluded

The structure of aquaporins







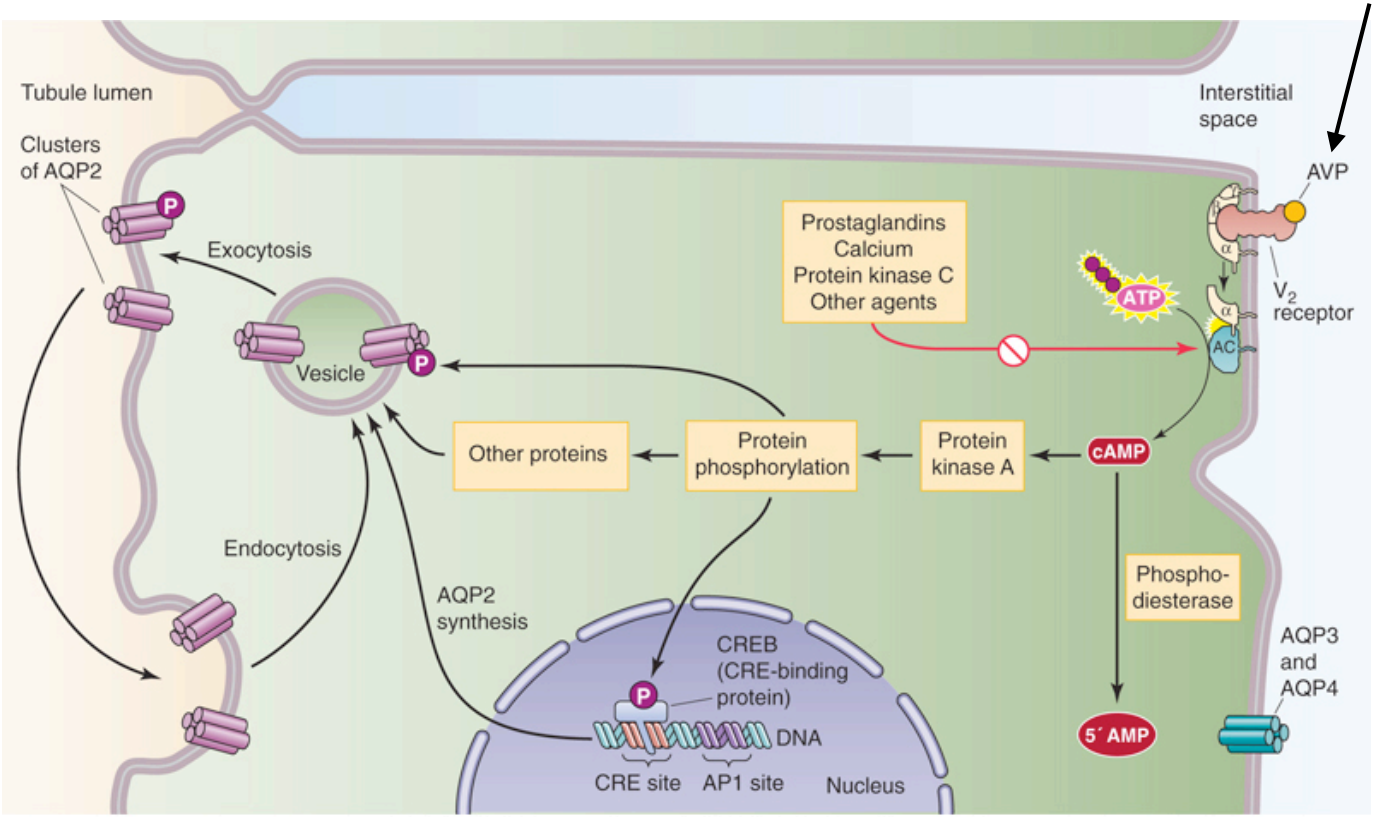


Valence of oxygen is fully occupied, hence water cannot act as proton acceptor here

The response to dehydration

Effect of vasopressin on aquaporin-2 (AQP2)expression in kidney epithelial cells, in response to dehydration

hypothalamus: vasopressin



Ion channels

a typical ion channel, fluctuates between closed and open conformations

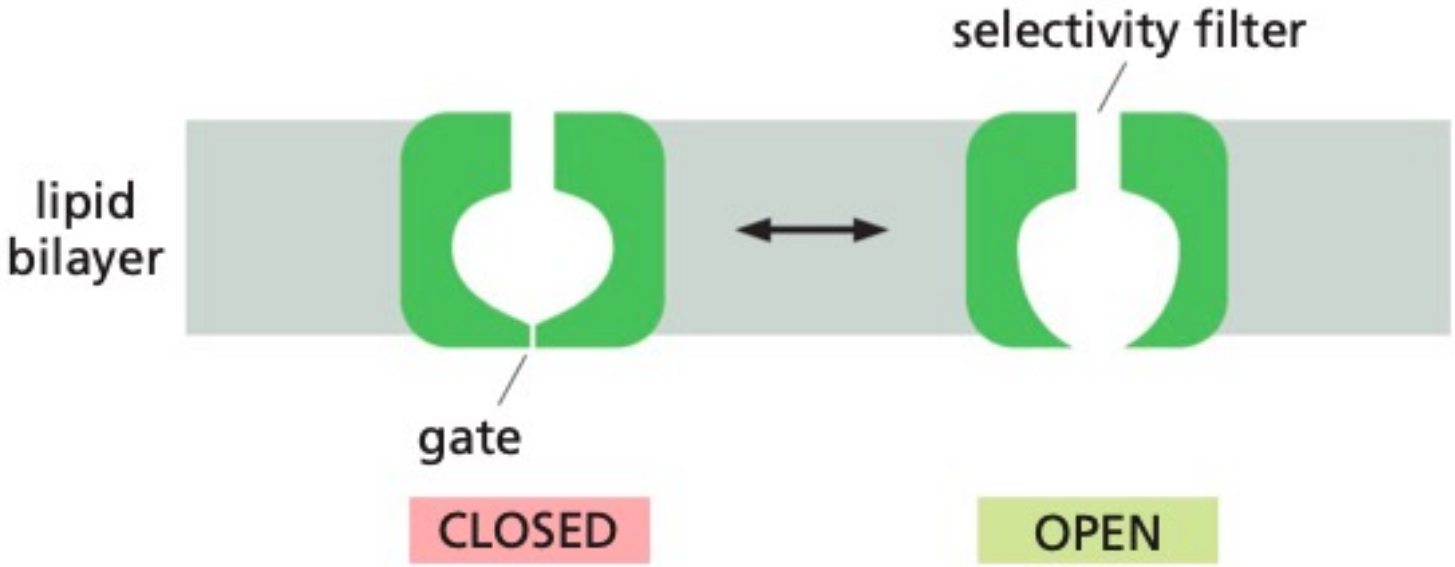


Figure 11–21 A typical ion channel, which fluctuates between closed and open conformations. The ion channel shown here in cross section forms a pore across the lipid bilayer only in the "open" conformational state. The pore narrows to atomic dimensions in one region (the selectivity filter), where the ion selectivity of the channel is largely determined. Another region of the channel forms the gate.

The gating of ion channels can be regulated in many ways

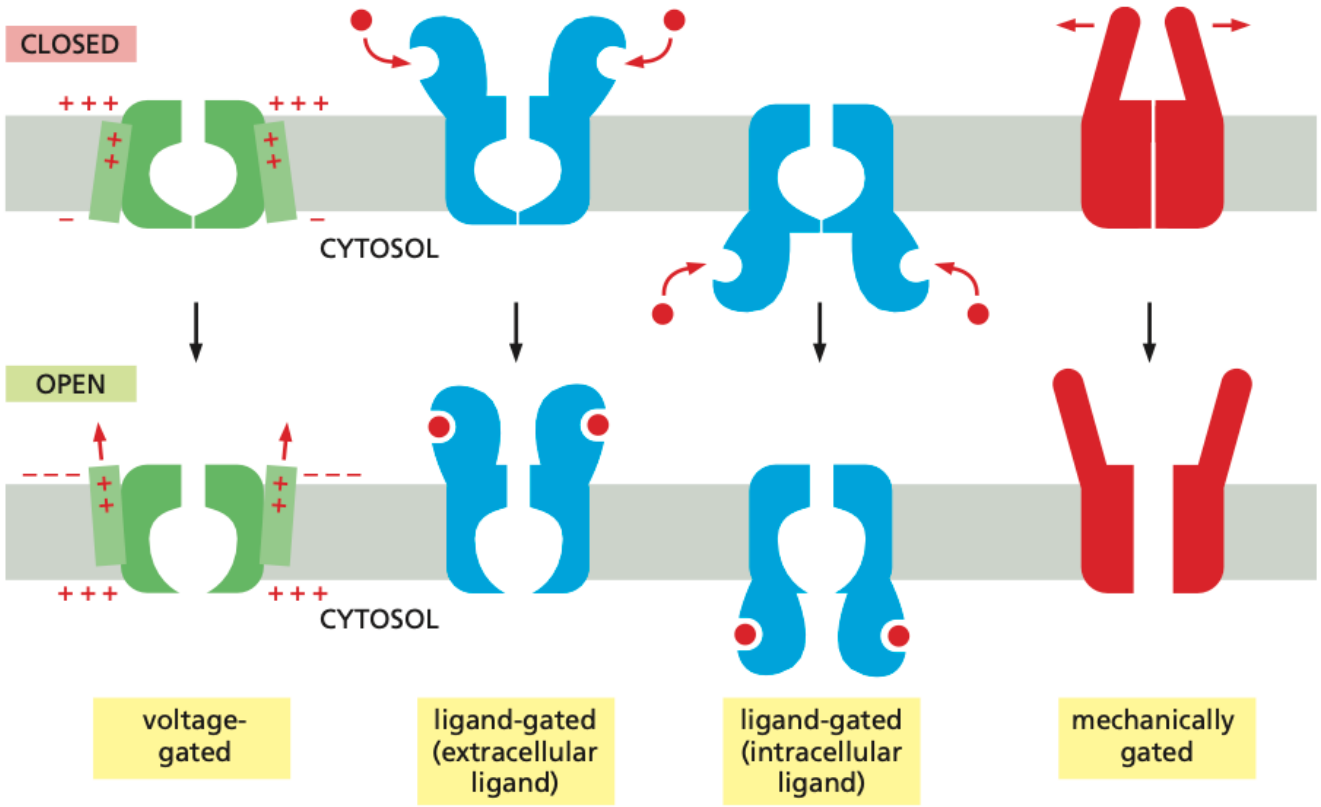


Figure 11–22 The gating of ion channels. This schematic drawing shows several kinds of stimuli that open ion channels. Mechanically gated channels often have cytoplasmic extensions (not shown) that link the channel to the cytoskeleton.

Ion channel selectivity

Ion channels

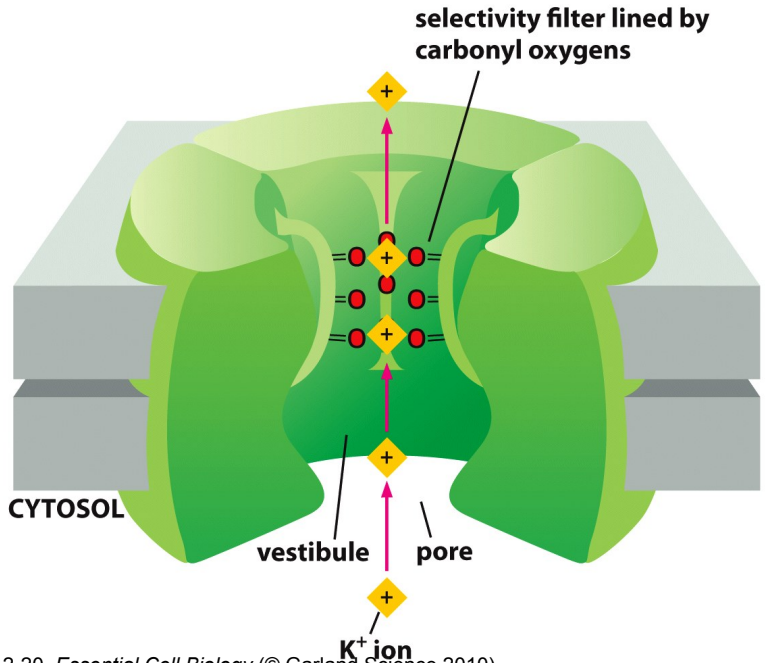


Figure 12-20 *Essential Cell Biology* (© Garland Science 2010)

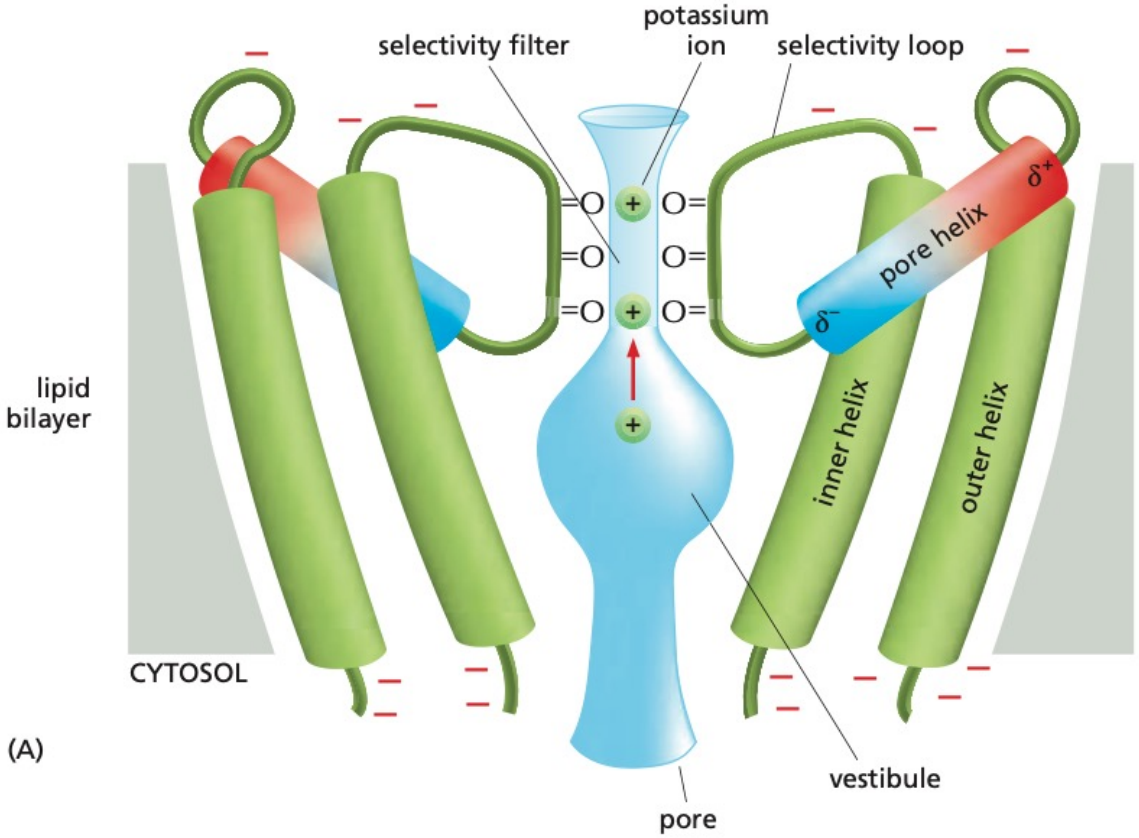
To ensure that only ions of appropriate size and charge can pass

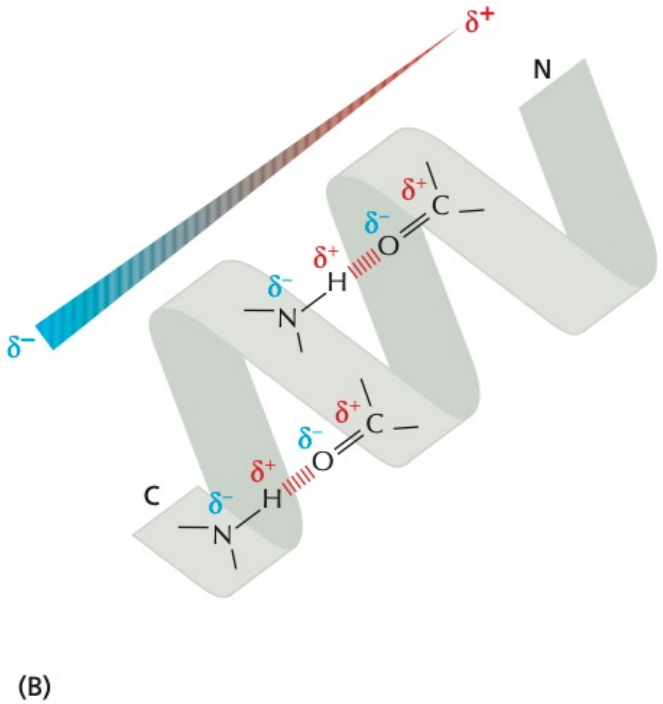
The ions shed their associated H_2O molecules to pass

Ion channel selectivity

Why don't K_+ channels let Na_+ pass through ? Not the pore size: Na_+ is smaller.
High affinity binding to K_+ is not the answer. Otherwise speed would be greatly affected.
The answer came from the crystal structure of a bacterial K_+ channel

The structure of a bacterial K^+ channel





The structure of a bacterial K^+ channel

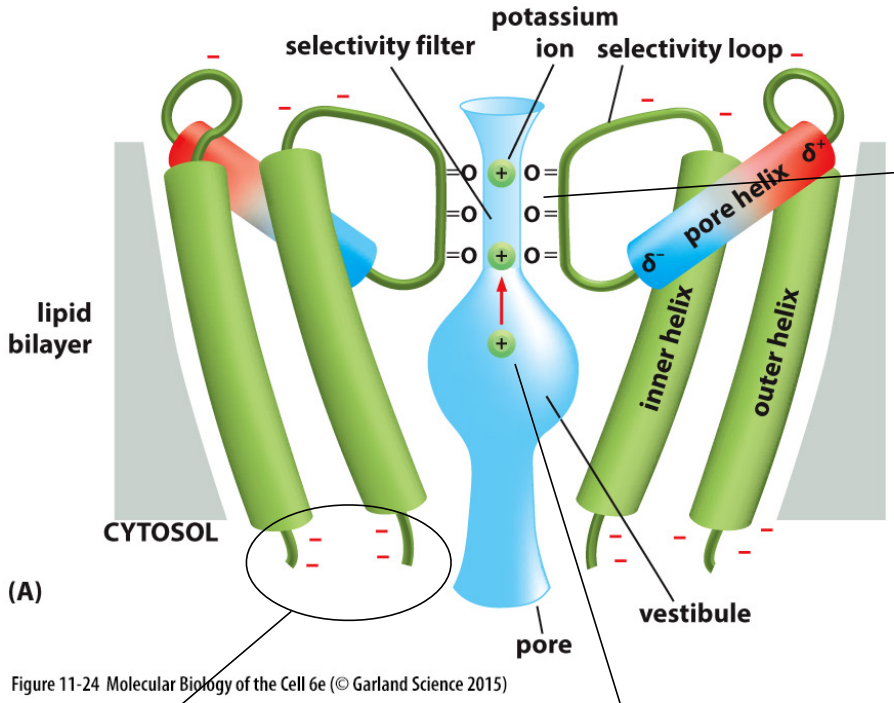


Figure 11-24 Molecular Biology of the Cell 6e (© Garland Science 2015)

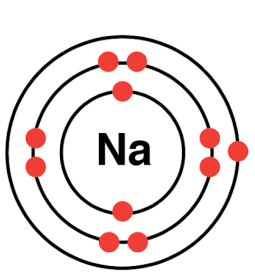
Coordination between carbonyl oxygens and dehydrated K^+

Cation attraction by negatively charged amino acids

In the vestibule, there can still be Na^+ and K^+

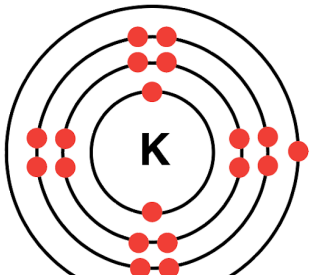
45

K^+ specificity of the selectivity filter in a K^+ channel.



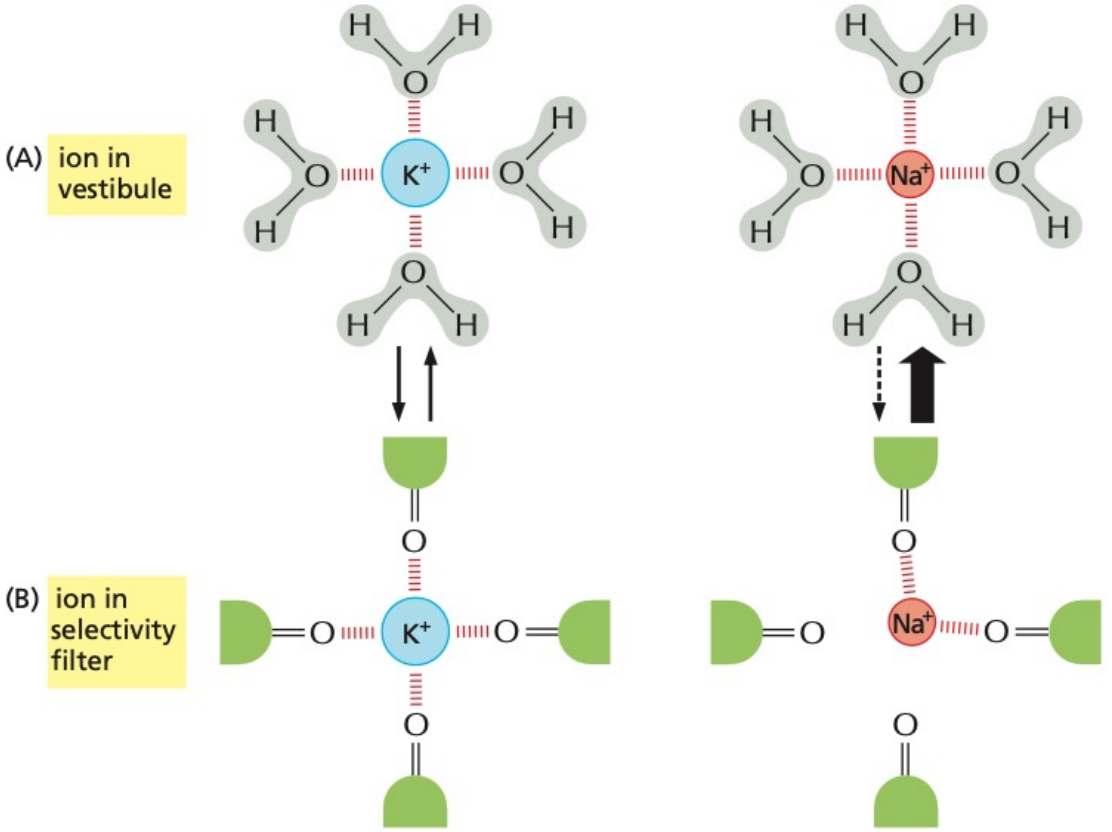
Sodium

$1s^2\ 2s^2\ 2p^6\ 3s^1$

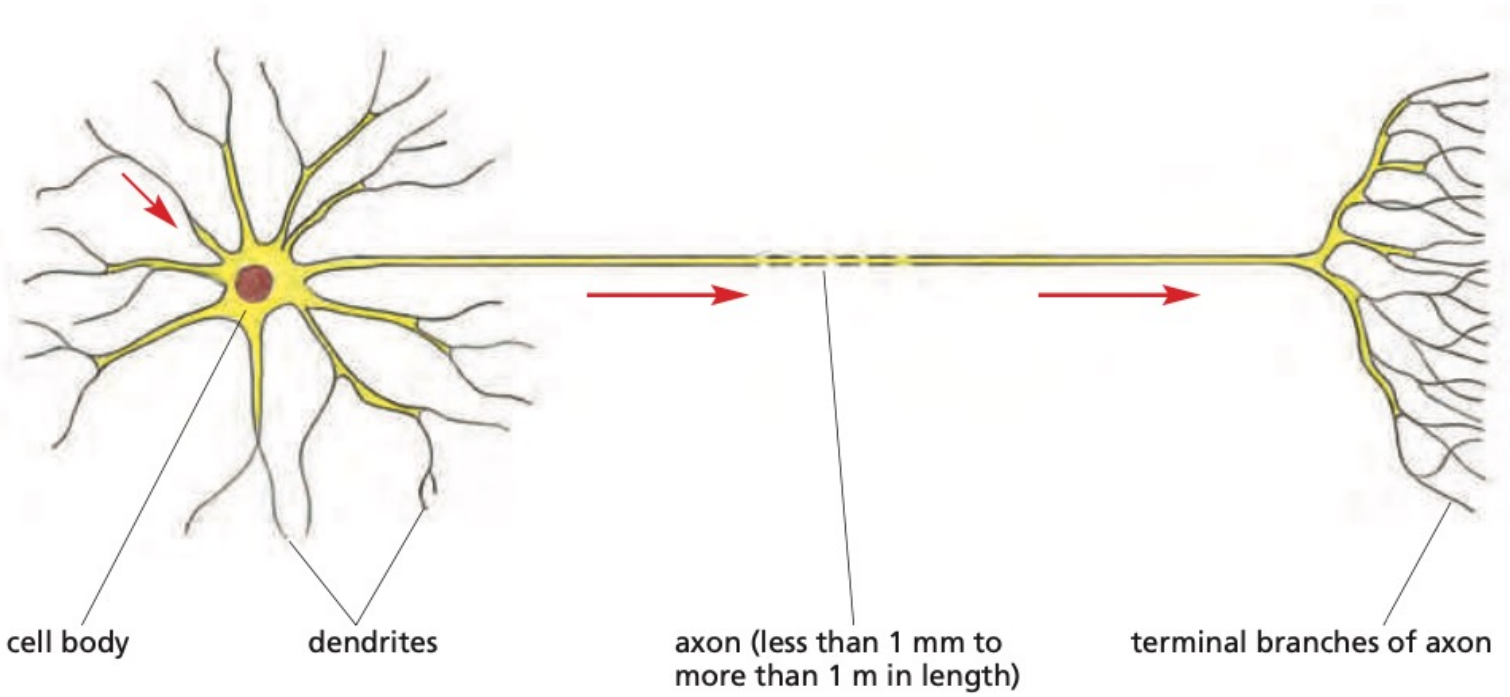


Potassium

$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^1$



Importance of Ion channels in neurons



In your future

The technique of patch-clamp recording

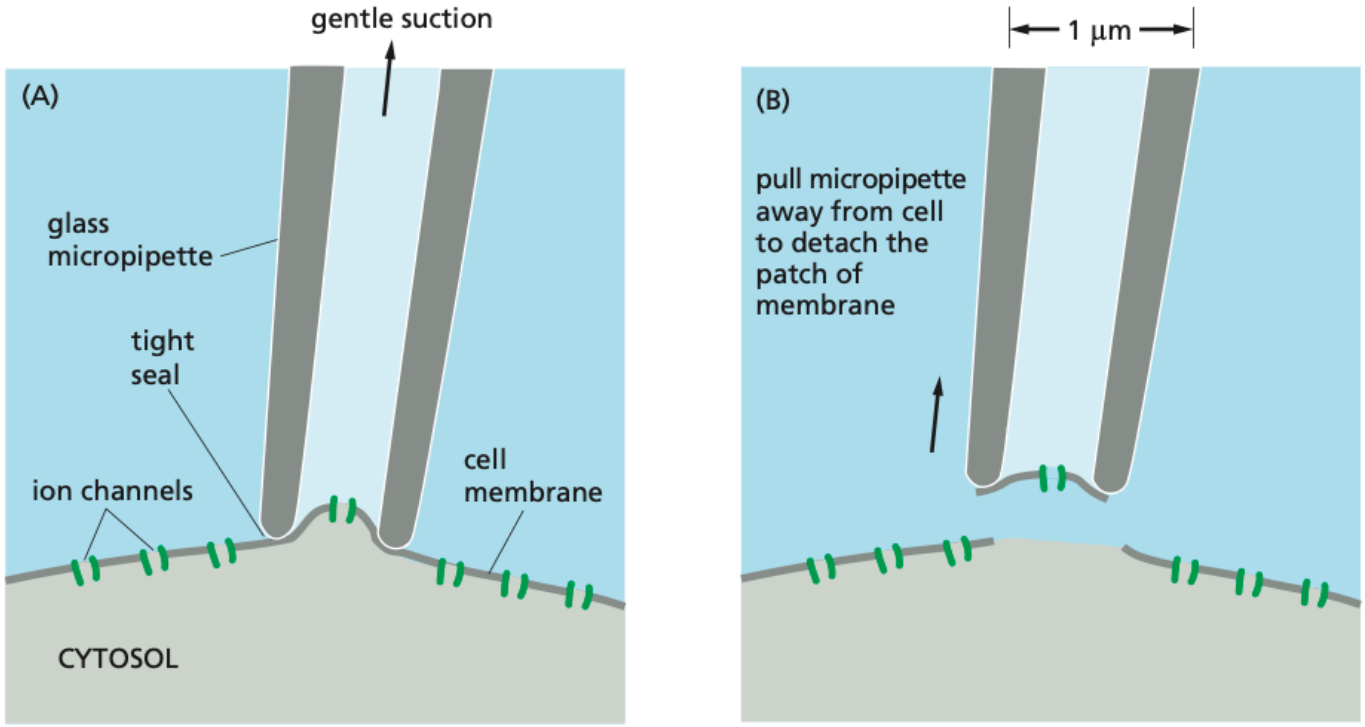


Figure 11–34 The technique of patch-clamp recording. Because of the extremely tight seal between the micropipette and the membrane, current can enter or leave the micropipette only by passing through the ion channels in the *patch* of membrane covering its tip. The term *clamp* is used because an electronic device is employed to maintain, or "clamp," the membrane potential at a set value while recording the ionic current through individual channels. The current through these channels can be recorded with the patch still attached to the rest of the cell, as in (A), or detached, as in (B). The advantage of the detached patch is that it is easy to alter the composition of the solution on either side of the membrane to test the effect of various solutes on channel behavior. A detached patch can also be produced with the opposite orientation, so that the cytoplasmic surface of the membrane faces the inside of the pipette.

The technique of patch-clamp recording

The Nobel Prize in Physiology or Medicine 1991

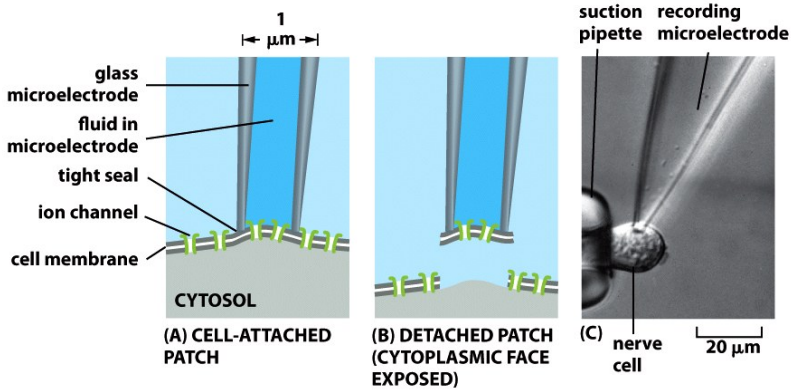
Erwin Neher

Bert Sakmann

The Nobel Prize in Physiology or Medicine 1991 was awarded jointly to Erwin Neher and Bert Sakmann *"for their discoveries concerning the function of single ion channels in cells"*

The technique of patch-clamp recording

To record ionic current flow through individual channels, while membrane potential is clamped



Current passes exclusively through the channels

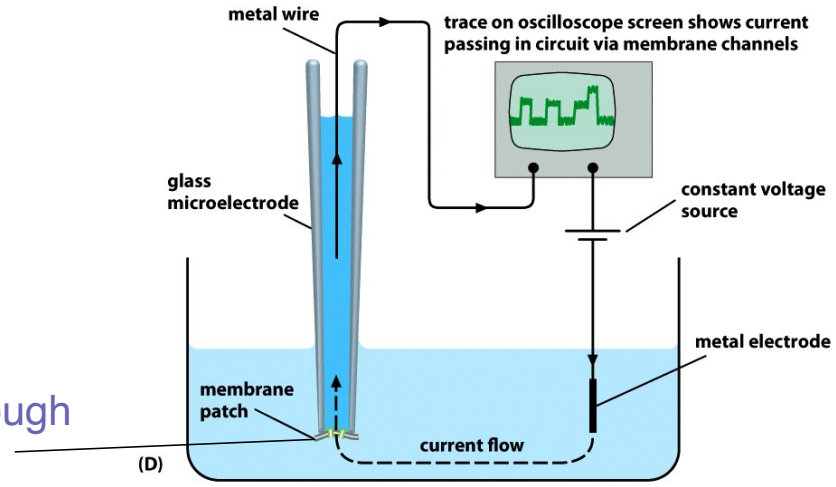


Figure 12-23 *Essential Cell Biology* (© Garland Science 2010)

The technique of patch-clamp recording

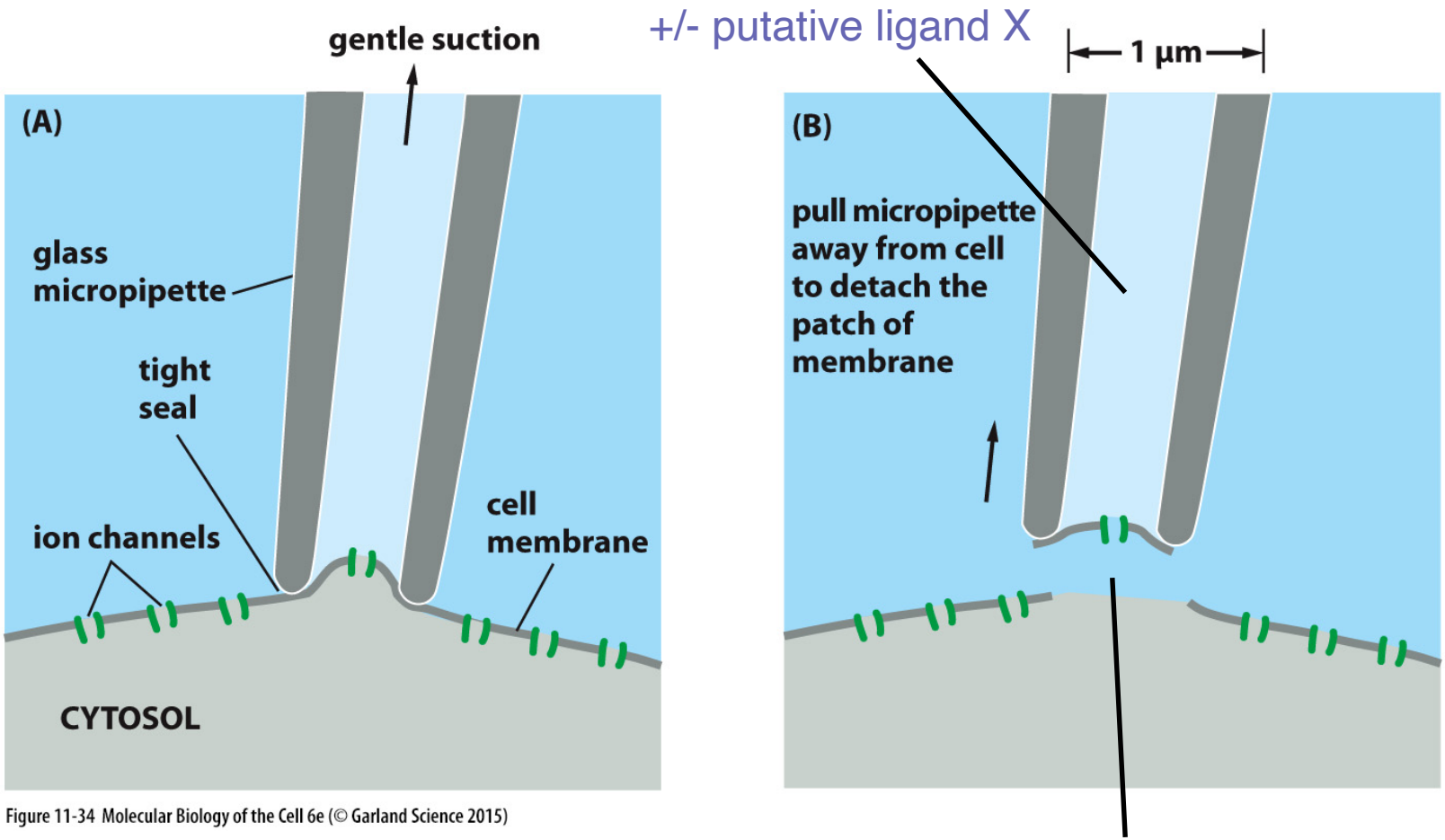
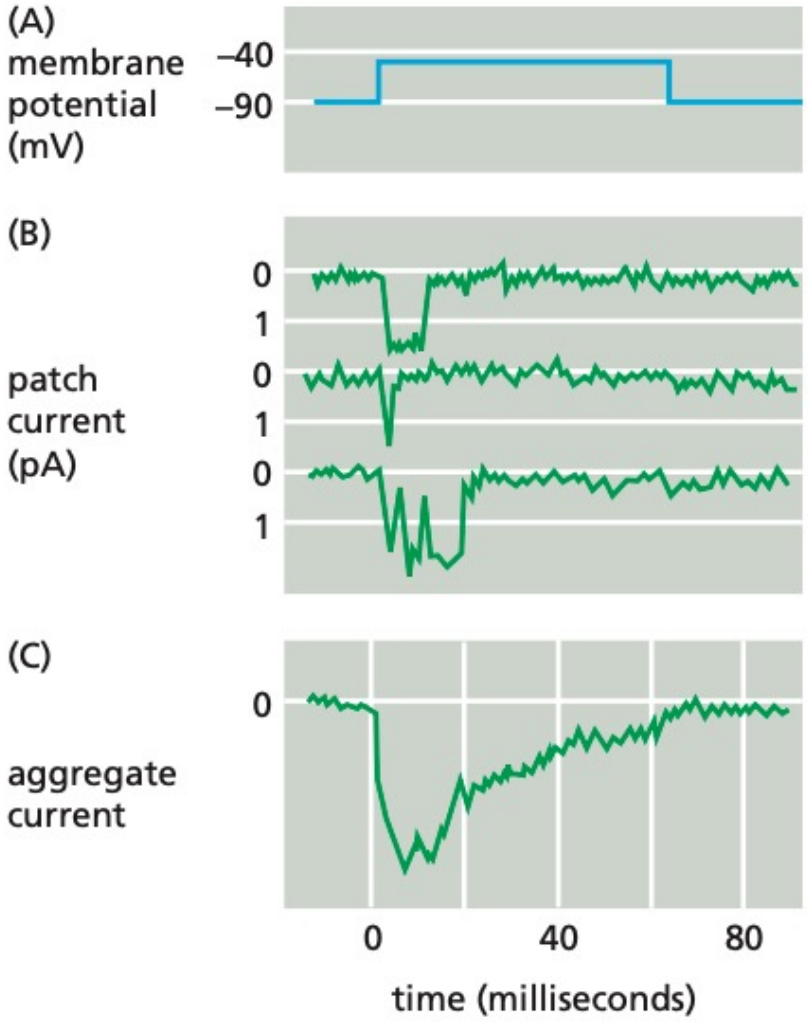


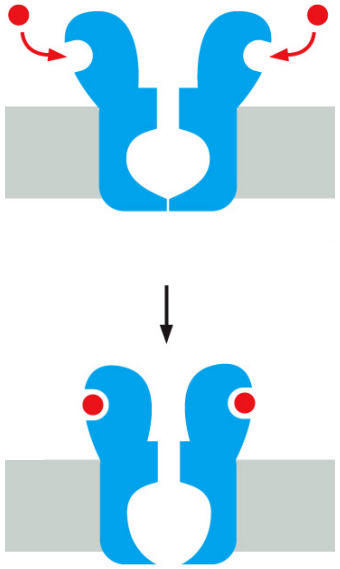
Figure 11-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

The patch-clamp enables to determine which molecule(s) activate(s) the channel, and on which side

Patch-clamp measurements for a single voltage-gated Na^+ channel



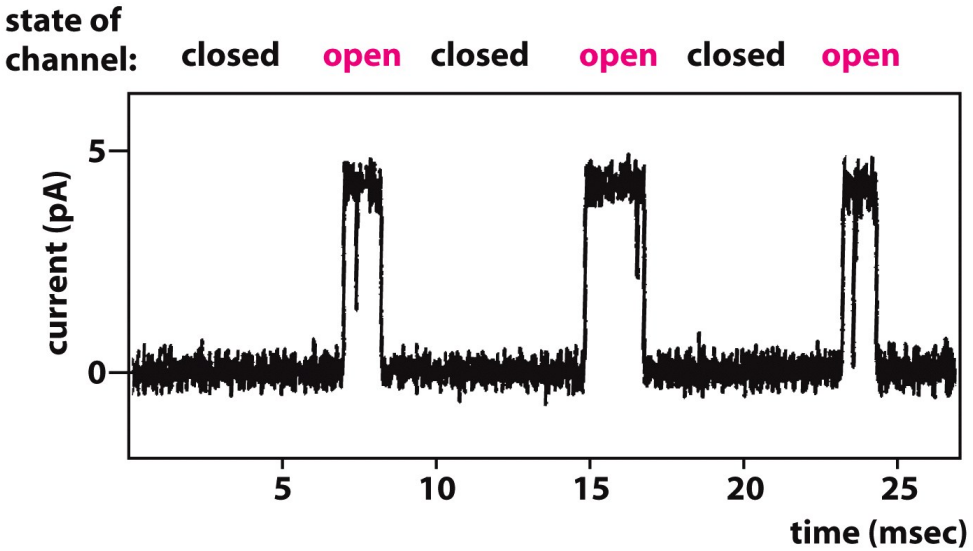
Ex: acetylcholine bound to an ion channel in muscle cells



ligand-gated (extracellular ligand)

and Science 2015)

Although acetylcholine is always present, the channel does not stay open



When open, the channel shows the same conductance

Figure 12-24 *Essential Cell Biology* (© Garland Science 2010)

Done for the day!

Proteins involved in membrane transport
Active vs Passive transport
ATP-driven pumps and Na+/K+ ATPase - ABC transporters
Channels
- Aquaporins
Introduction to ion channels
- Ion selectivity
- Ion current measurements

Examples of the importance of these transporters