TRANSMEMBRANE TRANSPORT OF ION AND SMALL MOLECULES

Lecture 4

Lecture 4 – 11.1 (Overview),

11.2 (Uniport of glucose and water)

and 11.5 (Symporters and Antiporters)

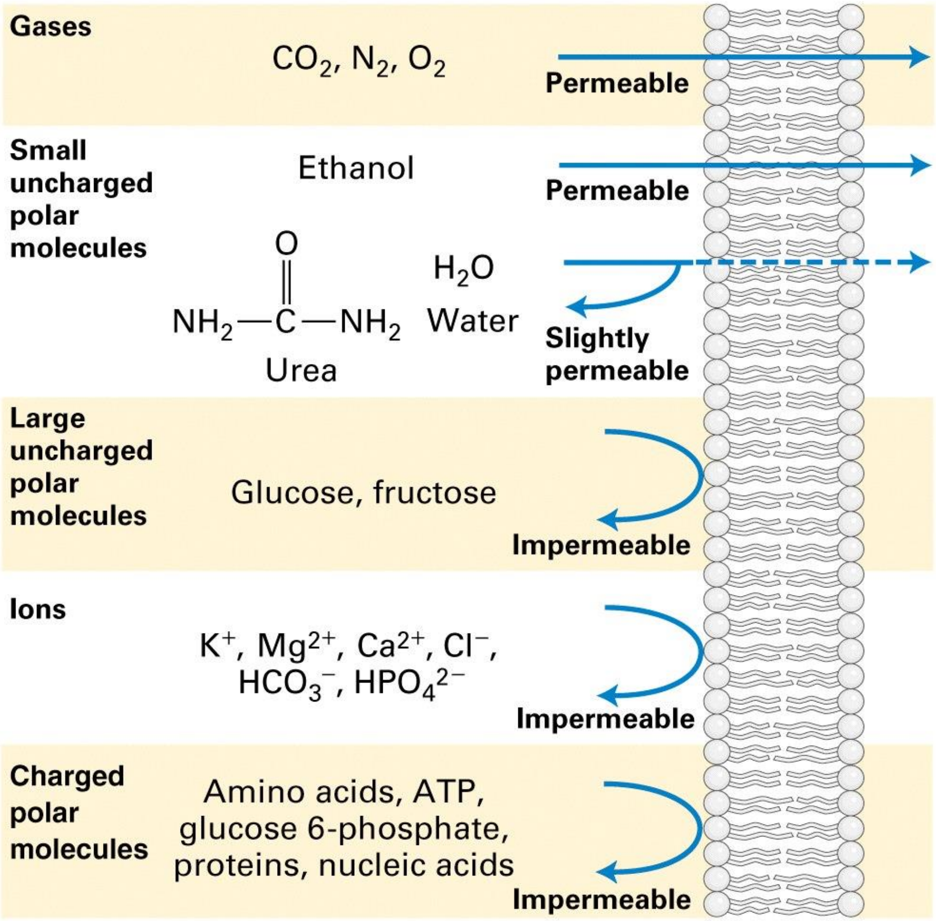
Overview

- The lipid bilayer plays two roles – a barrier preventing the movement of almost all molecules except a few and at the same time must allow the movement of material between the cell’s exterior and interior. How do they do this?

  Transport proteins

PERMEABILITY OF VARIOUS MOLECULES

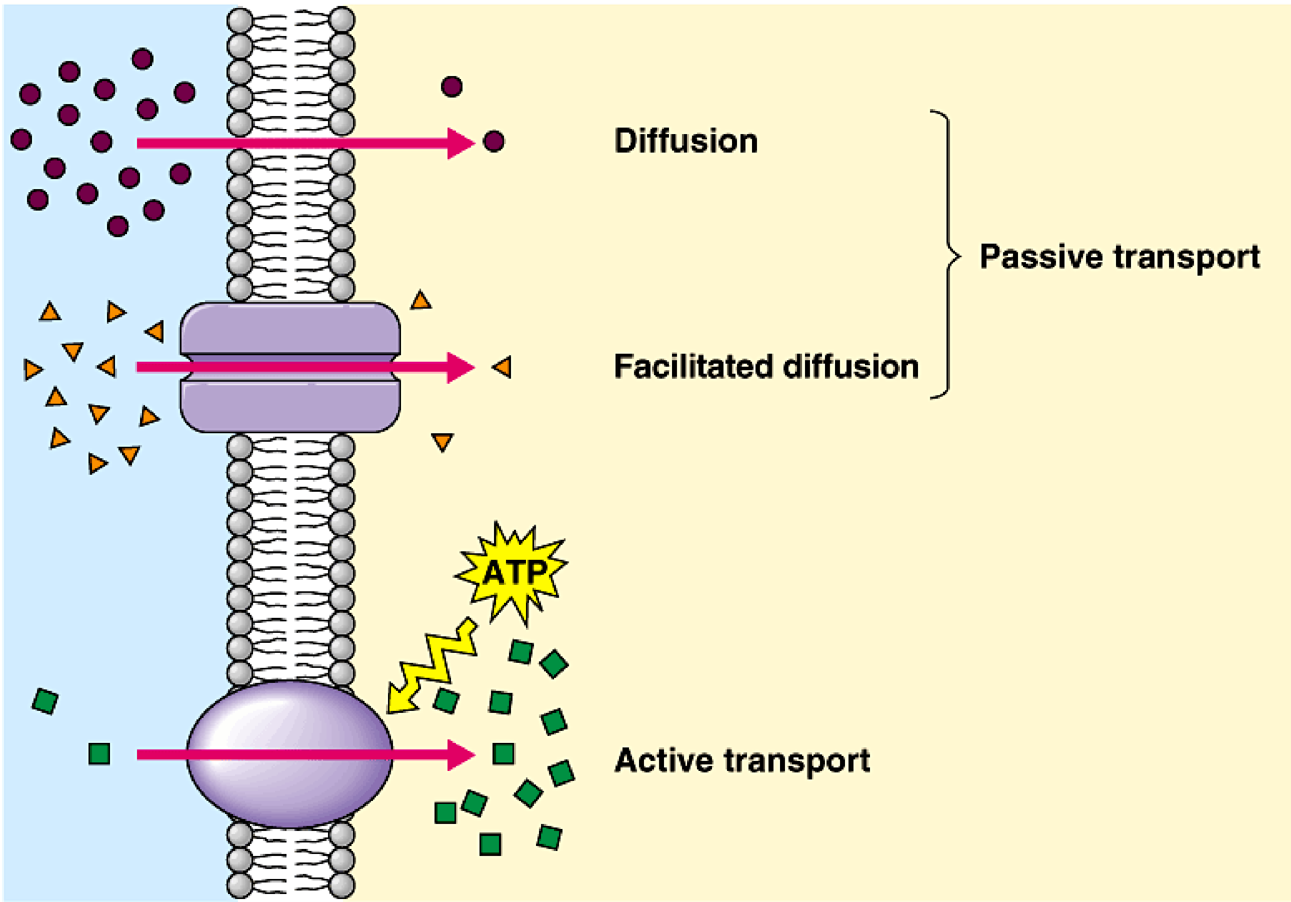
The bilayer is permeable to small hydrophobic and small uncharged polar molecules and slightly permeable to water and urea



TYPES OF TRANSPORT

- SIMPLE DIFFUSION
- FACILITATED TRANSPORT – Down the concentration gradient or electro-potential gradient, does not require ATP
- ACTIVE TRANSPORT – against a chemical concentration gradient, an electrical potential or both and it requires ATP
- SECONDARY ACTIVE TRANSPORT or COTRANSPORT – two substrates move in opposite or same direction and use the energy stored in the electrochemical gradient

SCHEMATIC OF DIFFERENT TYPES OF TRANSPORT



What does diffusion depend on?

CONCENTRATION GRADIENT ACROSS THE BILAYER

HYDROPHOBICITY

SIZE

IF THE MOLECULE IS CHARGED IT DEPEND NOT ONLY ON THE CONCENTRATION GRADIENT BUT ALSO ON THE ELECTRIC POTENTIAL ACROSS THE MEMBRANE

TRANSPORT MECHANISMS

TABLE 11-1 Mechanisms for Transporting Ions and Small Molecules across Cell Membranes

PROPERTY	SIMPLE DIFFUSION	FACILITATED TRANSPORT	ACTIVE TRANSPORT	COTRANSPORT*
Requires specific protein	−	+	+	+
Solute transported against its gradient	−	−	+	+
Coupled to ATP hydrolysis	−	−	+	−
Driven by movement of a cotransported ion down its gradient	−	−	−	+
Examples of molecules transported	O_2, CO_2, steroid hormones, many drugs	Glucose and amino acids (uniporters); ions and water (channels)	Ions, small hydrophilic molecules, lipids (ATP-powered pumps)	Glucose and amino acids (symporters); various ions and sucrose (antiporters)

*Also called *secondary active transport.*

Table 11-1
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THREE CLASSES OF MEMBRANE PROTEINS

- ATP- Powered pumps
- Ion channels – channel proteins are either gated or non-gated
- Transporters – symporters, antiporters and uniporters

ATP-driven active transport

Use energy of ATP hydrolysis

Against a chemical gradient or electric potential or both

Active transport

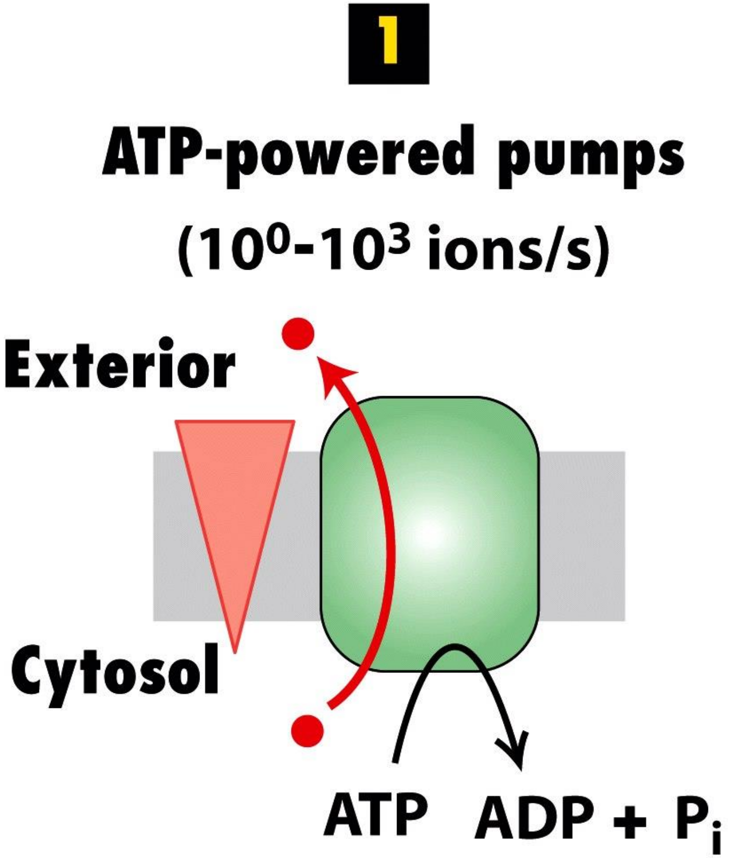

Figure 11-3 part 1
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FACILITATED TRANSPORT

Channel proteins transport water or specific ions down their concentration or electrical gradient. This is facilitated transport

Some channels are open all the time and are called “nongated” Example: Plasma membrane K+ channel

Most ion channels open only in response to specific chemical or electrical signals – “gated”

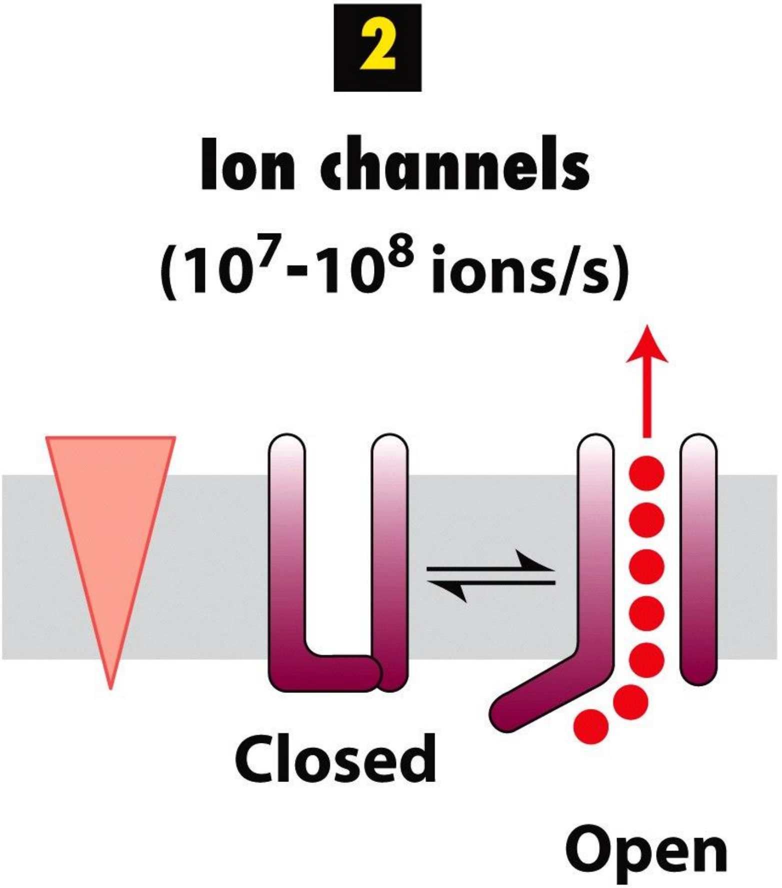

Figure 11-3 part 2
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What is the advantage of Facilitated diffusion over simple diffusion?

- Rate of facilitated diffusion is higher than simple diffusion
- Transport only via the uniporter and not all across the membranes
- Transport is specific
- The transported molecule never enters the hydrophobic core
- V_{max}- maximum transport rate

FACILITATED DIFFUSION vs. SIMPLE DIFFUSION

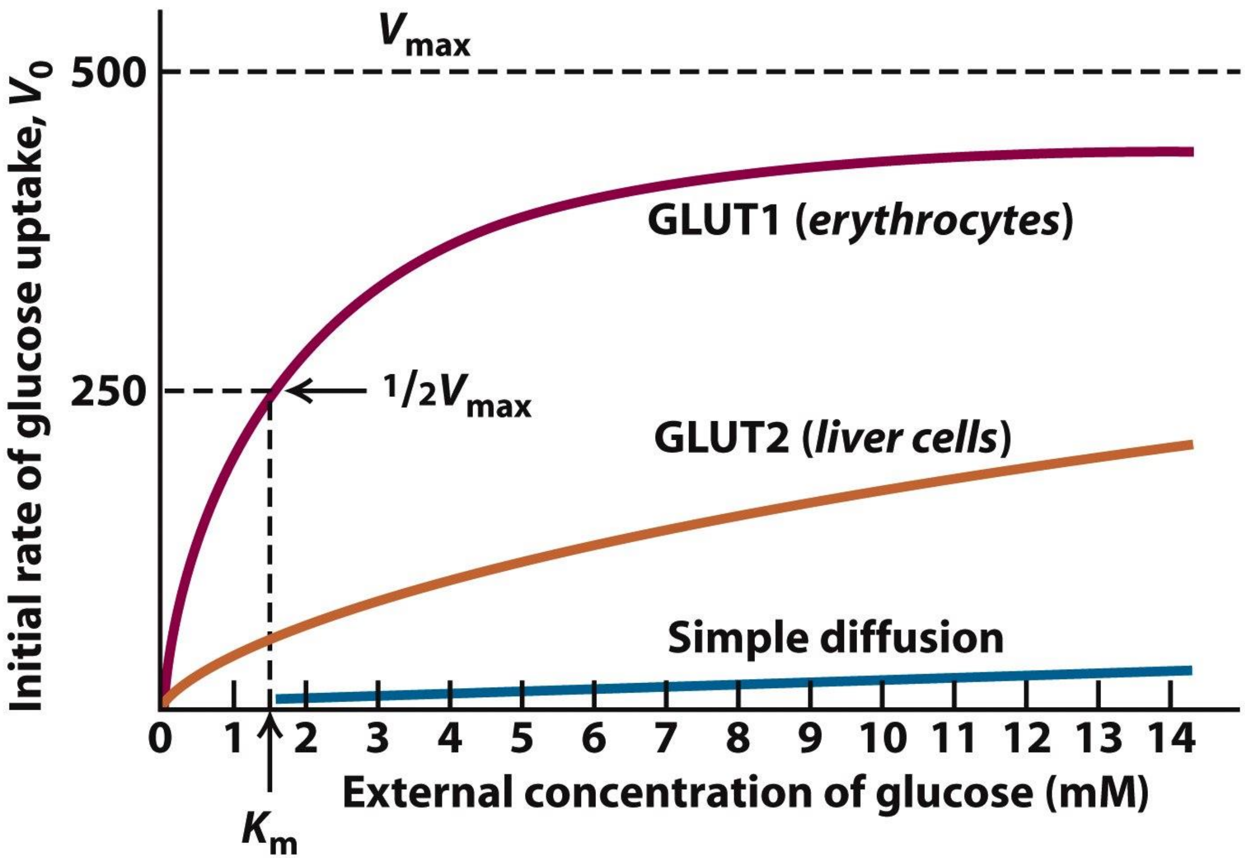

Figure 11-4
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SECONDARY ACTIVE TRANSPORT or COTRANSPORT

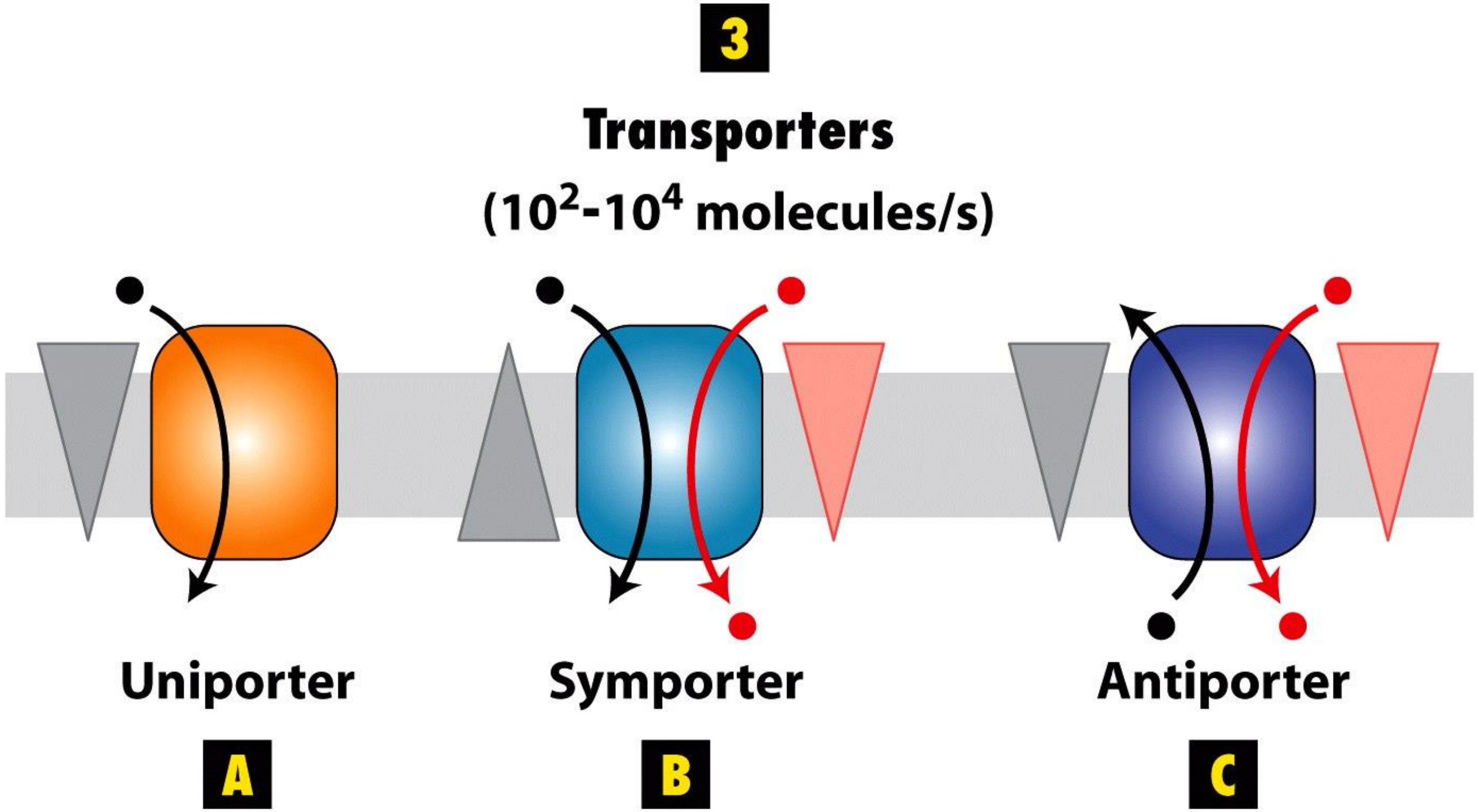

Figure 11-3 part 3
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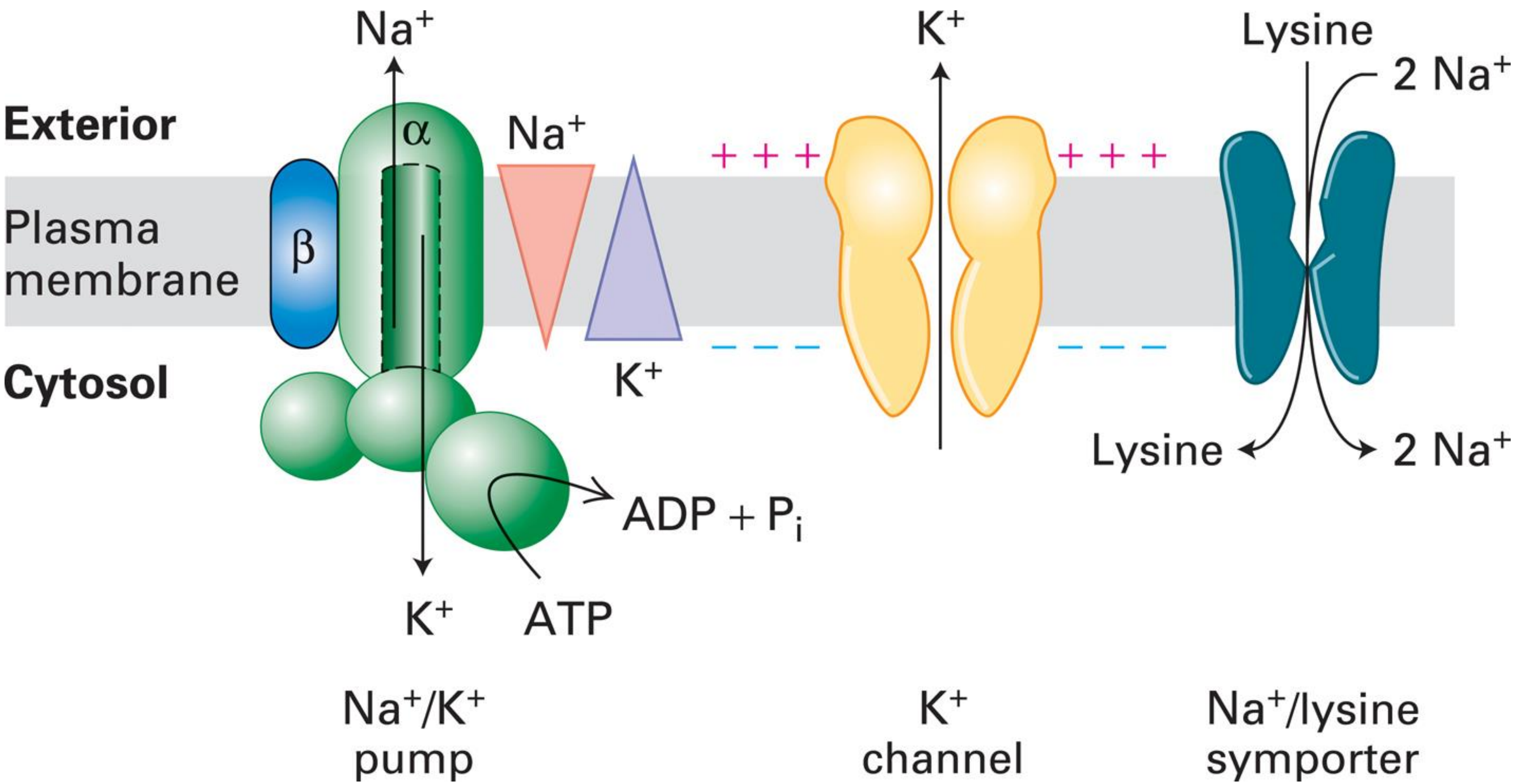
Transporters

- Uniporters
  - Transport a single type of molecule down its concentration gradient
- Antiporters and Symporters

  Couple the movement of one type of ion against its concentration gradient with the movement of one or more ions down its concentration gradient in the same direction (symporter) and different directions (antiporter).

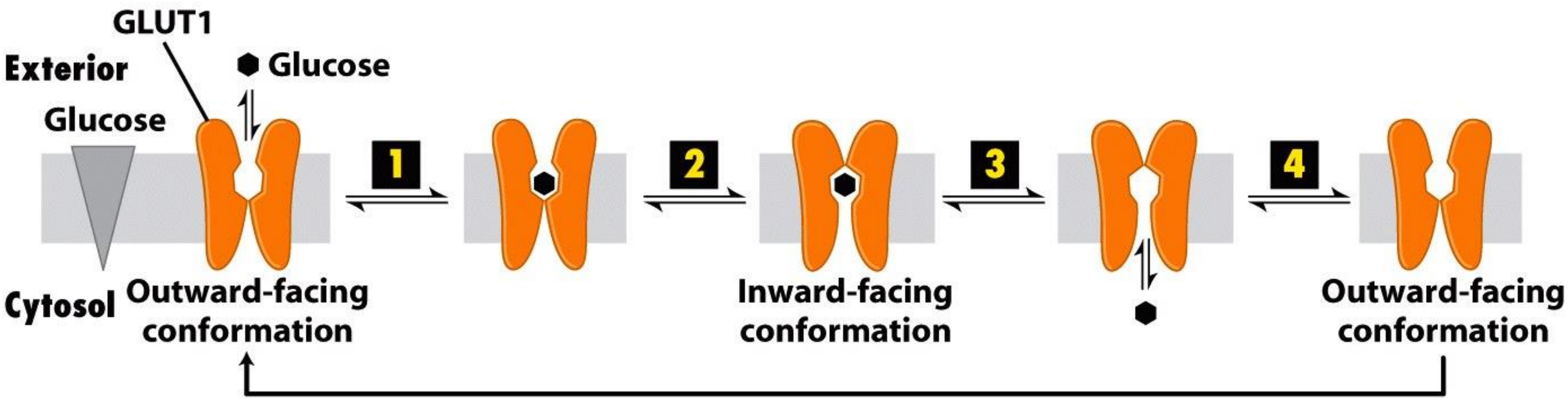
Figure 11.3 Multiple membrane transport proteins function together in the plasma membrane of metazoan cells.



MODEL OF UNIPORTER, GLUT1

GLUT 1 - Glucose Transporter 1

GLUT1 transports glucose from higher concentration to lower concentration



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Km for GLUT1 is 1.5mM

D-mannose and D-galactose are also taken up by GLUT1. Km for D-Mannose is 20mM

And D-galactose is 30mM.

Thus GLUT1 has a higher affinity for the substrate glucose. 2% of the protein in the plasma membrane of erythrocytes is GLUT1.

Glucose is converted to glucose – 6-phosphate rapidly once inside the cell.

GLUT FAMILY OF PROTEINS

- Human genome encodes about 12 different isoforms of the GLUT proteins
- They all have 12 membrane spanning alpha- helices. The amino acids in these helices are predominantly hydrophobic; several helices contain amino acids whose side chains can form hydrogen bonds with the OH groups of glucose
- Differential expression in various cell types

  GLUT 3 (Km is same as GLUT 1)- neuronal cells

  GLUT2 (Km = 20mM)- liver cells and beta cells of the pancreas (rise in blood glucose after a meal)

  GLUT 4 – fat and muscle cells (GLUT 4 is present in intracellular membranes and not on plasma membrane. In the presence of insulin, these GLUT4 rich membranes fuse with the plasma membranes and increases glucose uptake).

EXPERIMENTAL SYSTEMS TO STUDY TRANSPORTERS

- LIPOSOMES CONTAINING A PURIFIED TRANSPORT PROTEIN
  - The protein is first extracted using non-ionic detergent and then purified (using affinity chromatography). This purified protein is then reincorporated into phospholipid bilayer as liposomes.
- TRANSFECT CELLS with a gene encoding a particular transport protein

OSMOSIS

Water moves across membranes from regions of lower solute concentration to regions of higher solute concentration . This is termed osmosis. Osmotic pressure is the hydrostatic pressure required to stop the net flow of water.

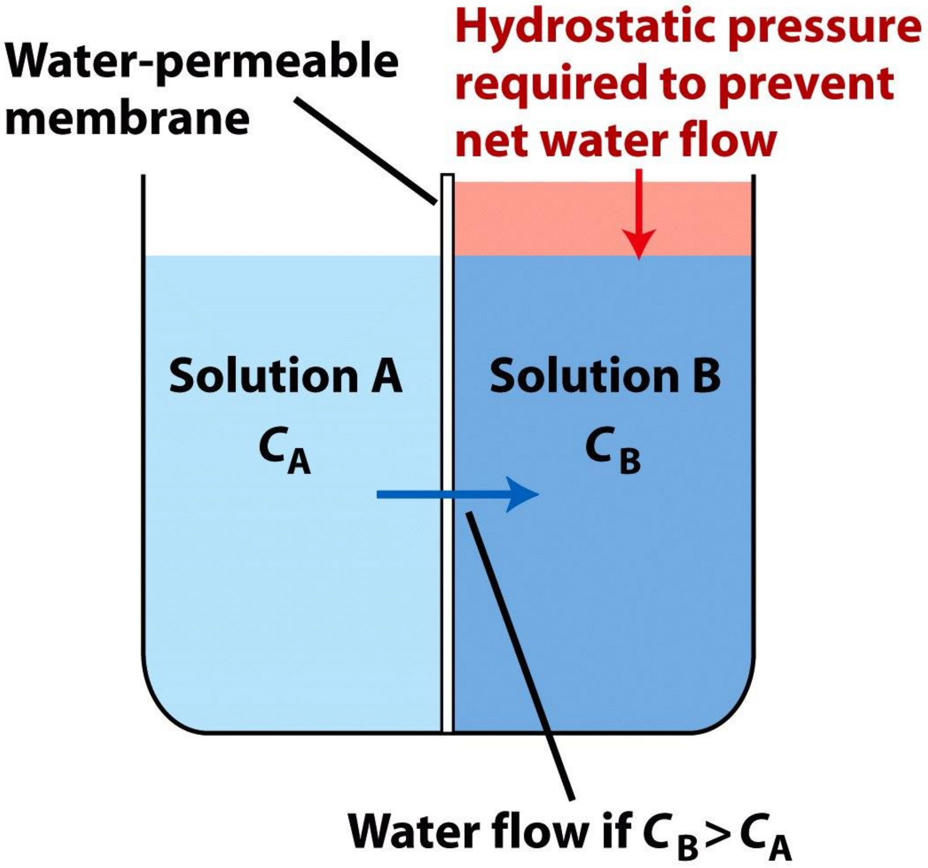

Figure 11-6
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OOCYTES MICROINJECTED WITH mRNA encoding Aquaporin

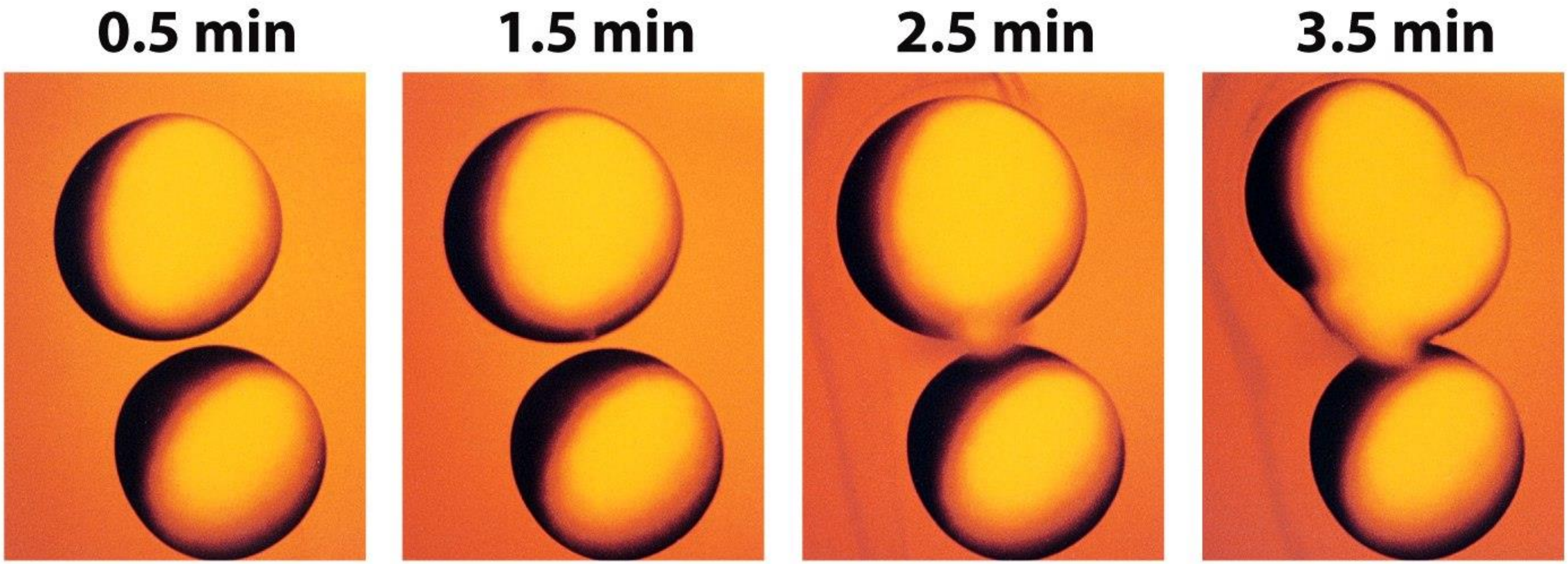

Figure 11-7
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Animal cells tend to swell or shrink when placed in hypotonic or hypertonic solution. Frog eggs did not exhibit this property. What did the animal cells possess that the frog eggs did not have? **AQUAPORINS**

AQUAPORINS

- Water-channel proteins that increase the permeability of bio-membranes to water. They allow water and few other uncharged molecules such as glycerol to pass through
- Tetramer of identical subunits – 28KDa each
- Each subunit contains 6 membrane spanning helices that form a central pore that allows the movement of water
- Central core is 0.28 nm in diameter (slightly larger than a water molecule)
- The central pore is lined with hydrophilic amino acids whose side chains and carbonyl groups form hydrogen bonds with water

AQUAPORINS

- 11 known aquaporins in human
  - Aquaporin 1 – eryhtrocytes
  - Aquaporin 2 – resting Kidney cells (water reabsorption from urine)
    - Activity of aquaporin 2 is controlled by the hormone, vasopressin (antidiurectic hormone). Aquaporin 2 in localized in intracellular vesicular membranes until vasopressin binds to its receptor on the cell surface. Mutations in either vasopressin or aquaporin 2 lead to diabetes insipidus.
  - Aquaporin 3 - transports glycerol

STRUCTURE OF AQUAPORIN

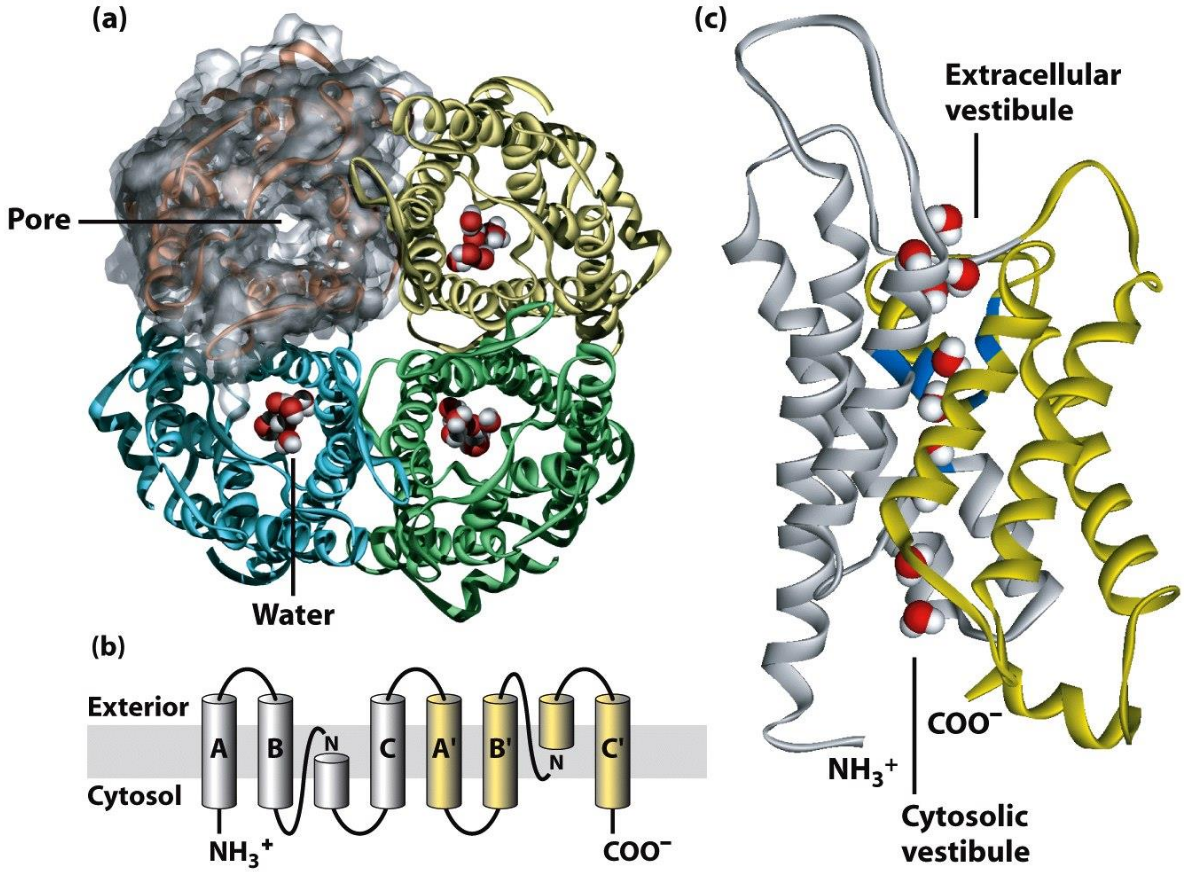

Figure 11-8
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SYMPORTERS

The energetically favorable movement of a ion (the cotransported ion) into a cell across the plasma membrane driven by both its concentration gradient and the transmembrane voltage gradient can be coupled to the movement of the transported molecule against its concentration gradient. Both molecules move in the same direction

2Na+/Glucose SYMPORTER

Cells lining the small intestine or kidneys need to import glucose against a concentration gradient.

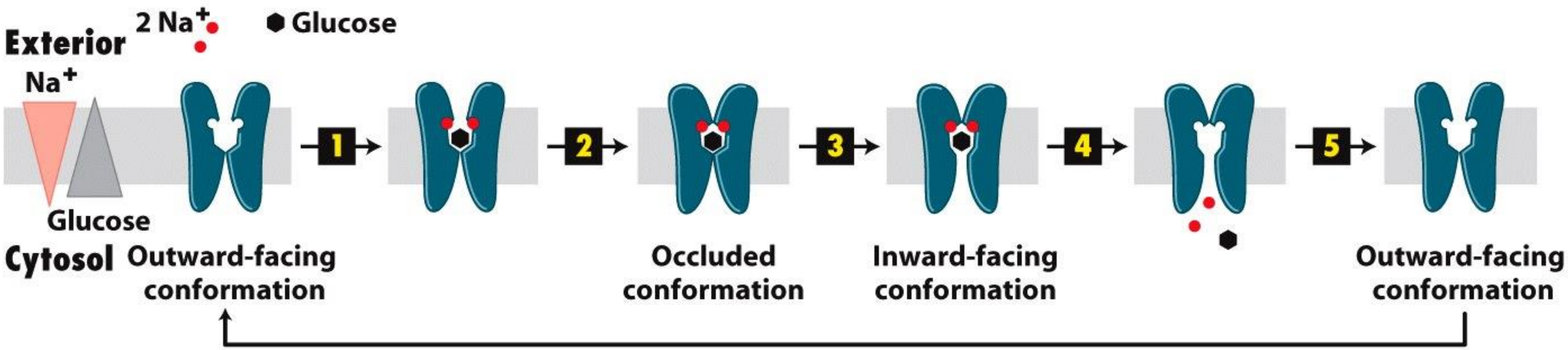

Figure 11-25
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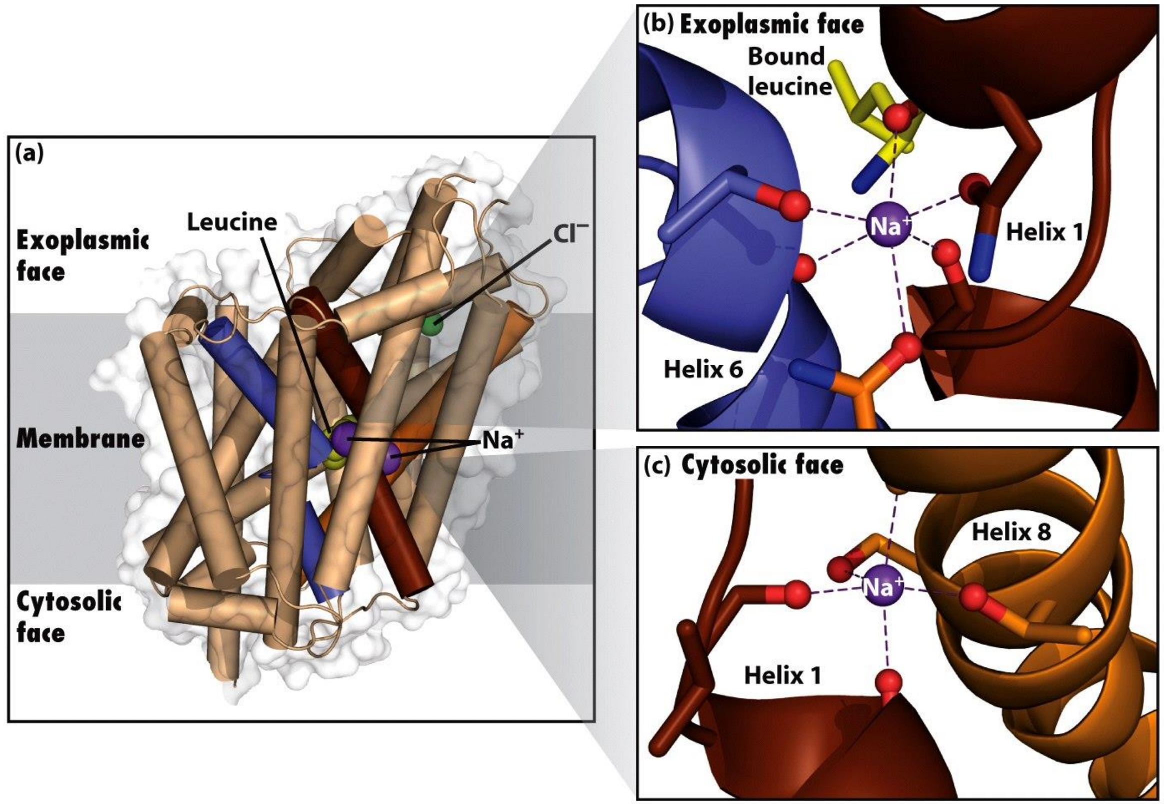
$$2Na^{+}_{out} + Glucose_{out} \rightleftharpoons 2Na^{+}_{in} + glucose_{in}$$

2Na +/one-glucose SYMPORTER

- 14 transmembrane helices with both its N and C terminii in the cytosol
- Truncated protein containing only five C-terminal transmembrane helices – transports glucose down its concentration gradient independent of Na+
- N-terminal portion (transmembrane helices 1-9) of the protein is required to couple Na+ binding and transport of glucose against a concentration gradient

Two-Na+/one-Leucine symporter in Bacteria

Each of the two sodium atoms is bound to six oxygen atoms.
One of the sodium atoms is also bound to the carboxyl group of the transported Leucine (yellow).



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ANTIPORTERS

- Three Na+/one Ca2+ antiporter works to reduce the intracellular calcium ion concentration – three sodium ions are pumped into the cell for every calcium ion that is exported
- Increase in calcium ion concentration triggers cardiac muscle contraction
- This antiporter works to reduce the strength of the heart muscle contraction
- Na+/K+ ATPase and its link to this antiporter

P-class pumps

- 2 alpha subunits and 2 beta subunits
- Each alpha subunit has an ATP binding site
- During transport at least one of the alpha subunits is phosphoryltaed
- Na^+/K^+ ATPase in the plasma membrane
- $Ca2^+$ ATPase pump

Na+/K+ ATPase pump

3 Na+ ions are pumped out for every 2K+ ions that are pumped in. When you inhibit this pump with drugs such as ouabain or digoxin, the cytosolic K+ concentration decreases and the Na+ ion concentration increases. This causes the Na+/Ca+ antiporter to function less efficiently. This causes the Ca2+ concentration in the cytosol to increase and causes the muscle to contract strongly.

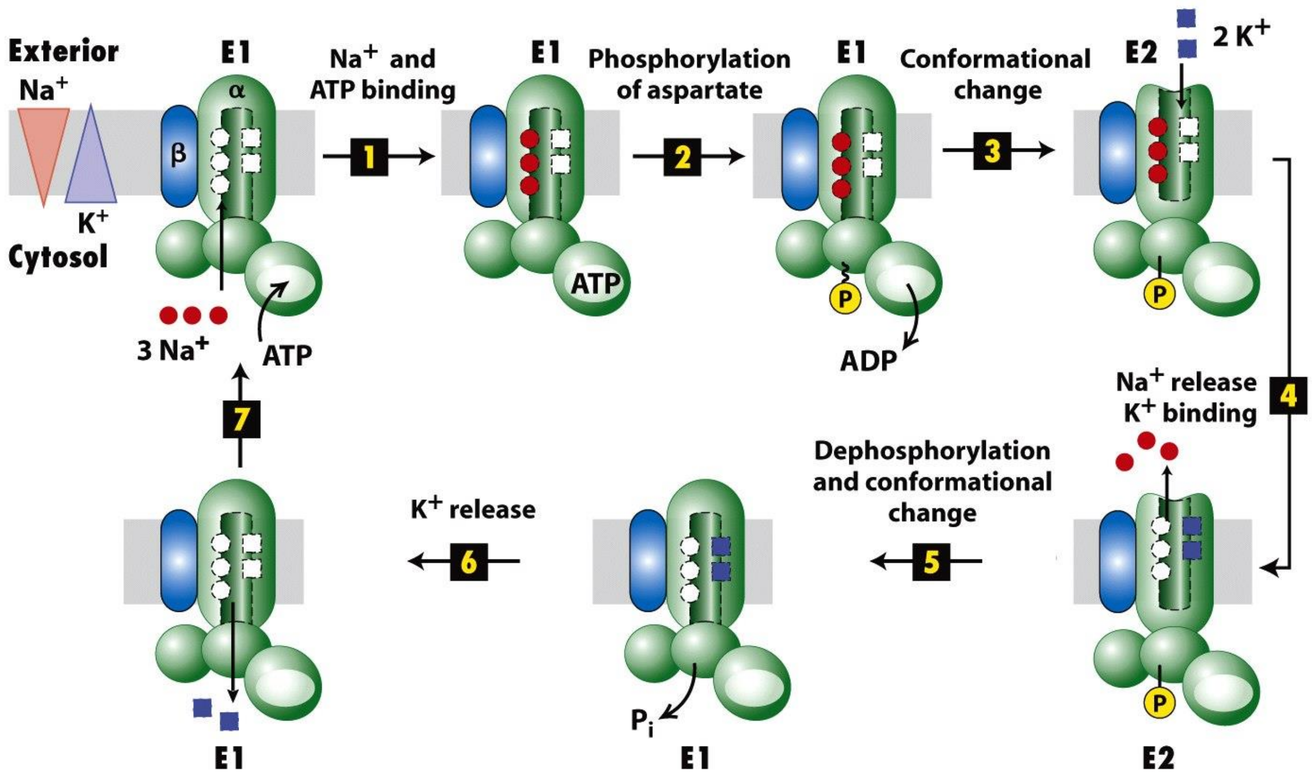

Figure 11-12
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Alpha2 Beta 2 tetramer
The beta subunits help the newly synthesized alpha subunits to fold properly but are not involved in the pumping of ions.

N domain (nucleotide binding), P (phosphorylation)domain and A (actuation) domain

Ions are pumped in both directions

Protein synthesis requires a high K+ concentration

Na+/K+ ATPase pump

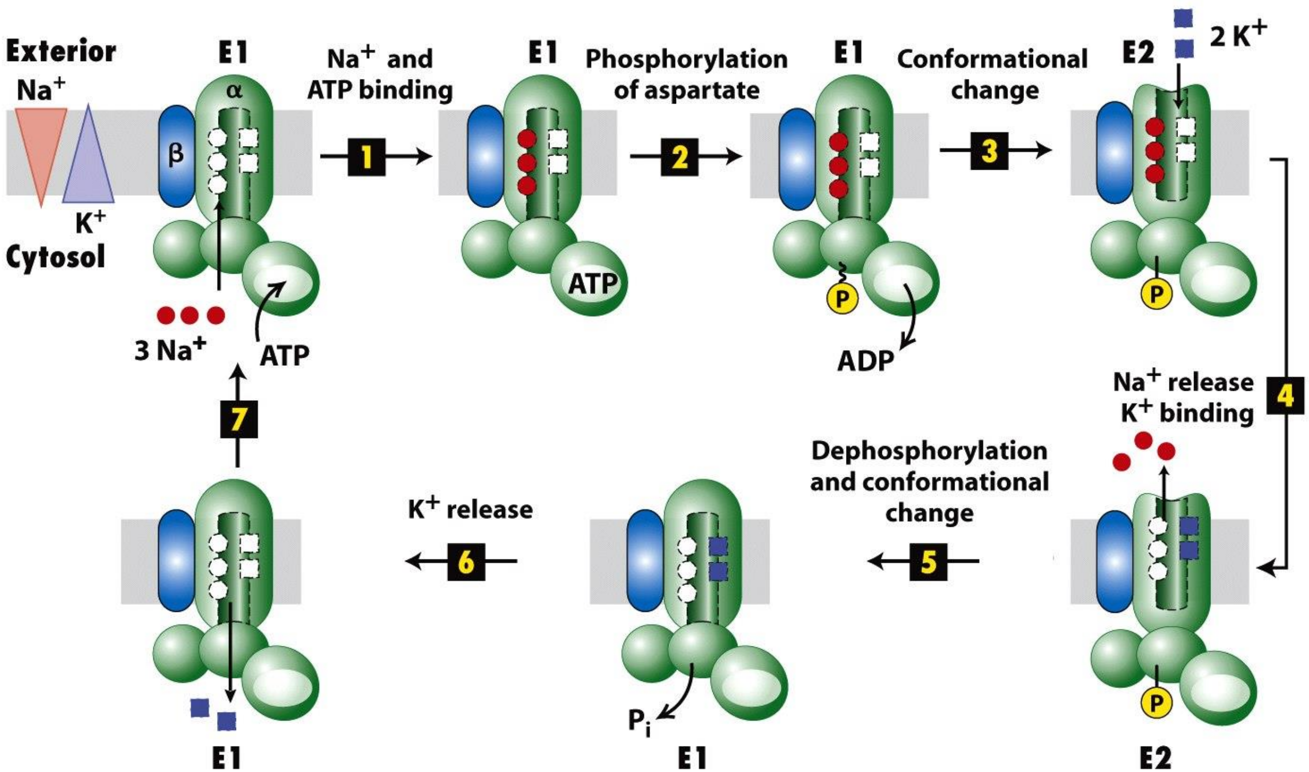

Figure 11-12
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How cytosolic pH is regulated?

- Several antiporter systems are involved
- $Na^+HCO_3^-/Cl^-$ antiporter increases cytosolic pH – imports one Na^+ ion and a HCO_3^- ion in exchange for the export of one Cl^-ion. The HCO_3^- ion dissociates into CO_2 and OH^-. The OH^- combines with the H^+ to form water. This raises the overall pH of the cytosol.
- Na^+/H^+ antiporter also increases cytosolic pH – this couples entry of one Na^+ ion with the export of one H^+ ion.
- At high pH(>7.5) anion antiporter Cl^-/HCO_3^- comes into play. This protein exports one HCO_3^- ion for import of every Cl^- ion.
- Cytosolic pH is maintained at 7.4

How do different transport proteins help accumulate metabolites in the vacuoles?

- V-class ATP powered proton pump and pyrophosphate-powered pump maintain acidity in plant vacuoles
- Contains Cl^- and NO_3^- channels that transport these anions against their concentration gradients due to the positive potential generated by the protons
- Sucrose/H+ antiporter that transports sucrose into the vacuole against its concentration gradient

CONCENTRATION OF IONS AND SUCROSE BY THE VACUOLE

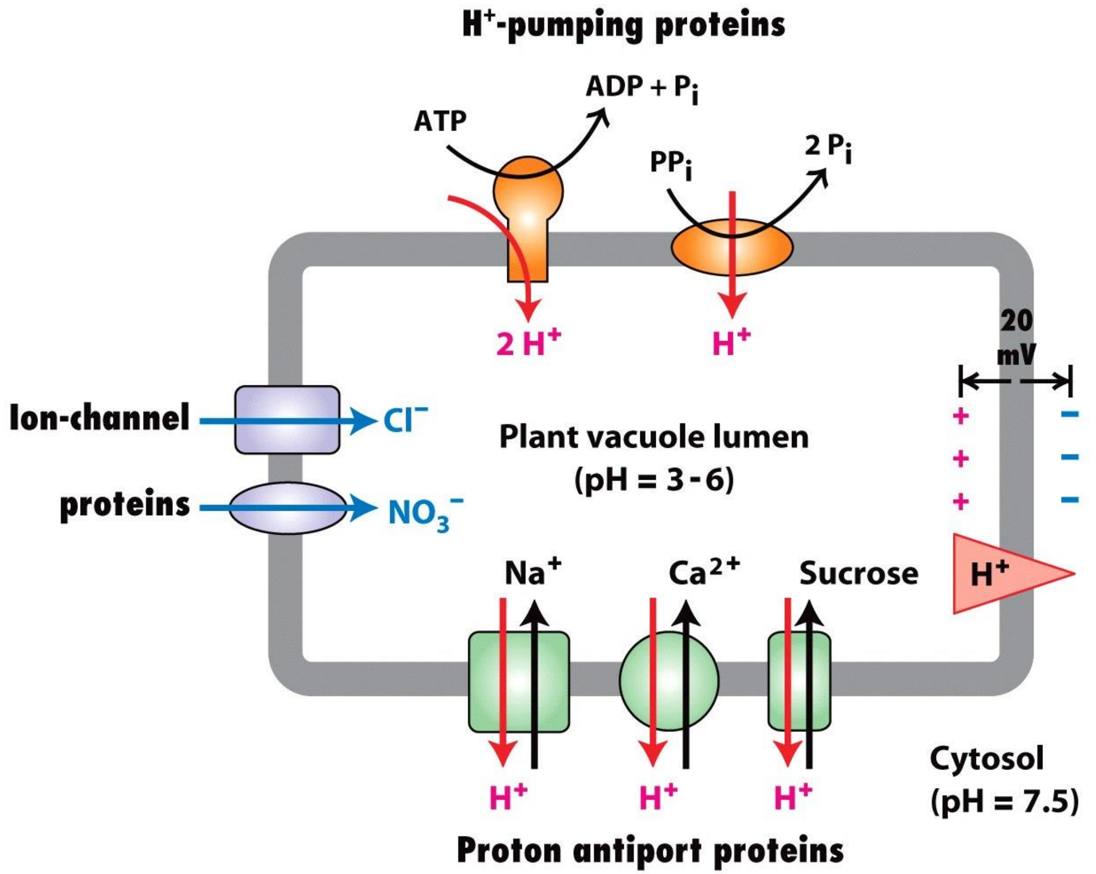

Figure 11-28
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