Cell Membrane Transport:
Active Transport
(part 1 of 2)

Lecture 10

Objectives

Understand/know/focus on/note

- the fundamental concept of active transport and how ATP is involved
- direct versus coupled (indirect) active transport
- what substances are transported this way
- types of pumps (ATPases): where they are and what role they play
- what the ABC-type ATPases are & what they do
- the mechanism of the Na/K ATPase (P-type protein) and what regulates it

Kinds of Transport

Passive transport or diffusion

Active transport

Exocytosis & Endocytosis

discussed later

"Transport" With The Concentration Difference

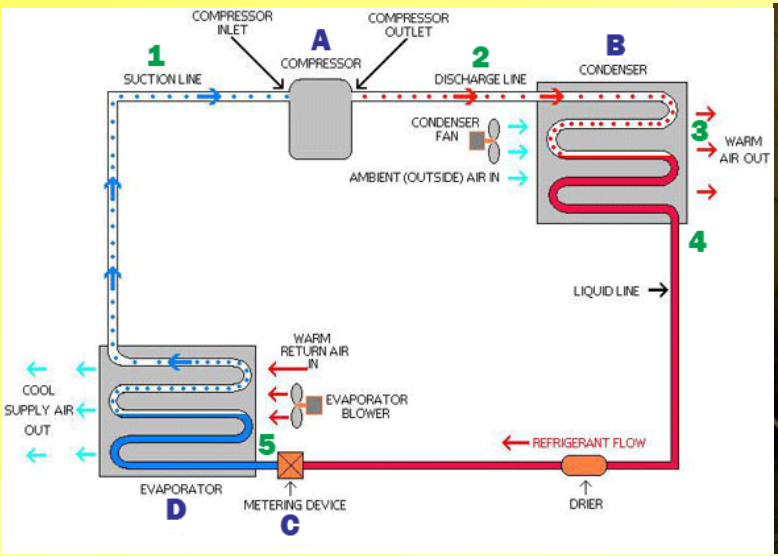
Passive Transport

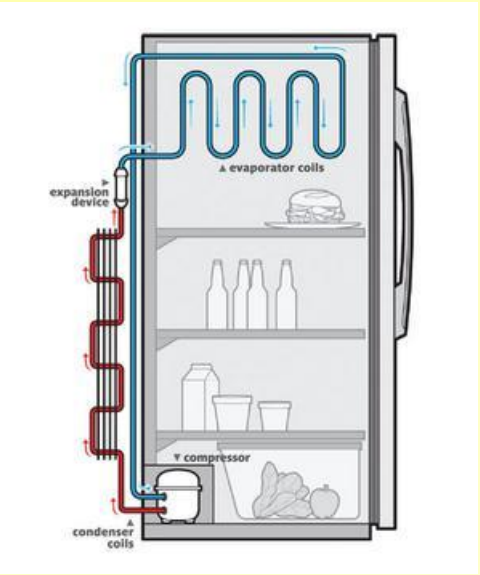
- hydrophobic/lipophilic substances
  - soluble in plasma membrane
  - pass through membrane without any pore
- polar hydrophilic substances
  - impermeable to plasma membrane
  - pass through only with pore-forming transmembrane protein
- "Transport" is really diffusion caused by a concentration difference: substance with a high concentration one one side of membrane diffuses to achieve maximal dispersal (even spacing of substance molecules) within the diffusible space/volume it has

2nd law of thermodynamics

Heat Pumps & Refrigerators

- Refrigerators utilize electrical energy to operate a pump (compressor) that compresses a gaseous refrigerant through coils on back of refrigerator
- the cooler kitchen air causes the gas in coils to cool into liquid
- this liquid refrigerant passes into the refrigerator inside
- an expansion valve causes the liquid to form into a gas, drawing its heat for the phase change from the inside of the refrigerator, and thus cooling down the inside
- the cycle starts over as the gas is compressed again back into a liquid





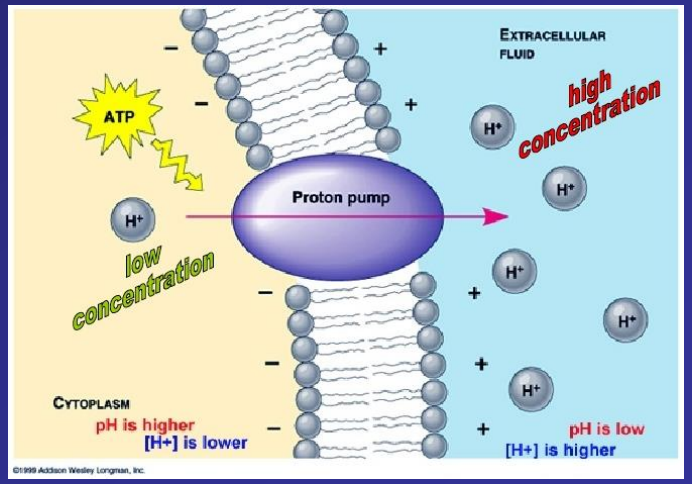
Transport Against The Concentration Difference

The refrigeration example shows how work is done to counter the dispersion of matter and energy

The cell uses its energy to counter

diffusion: high concentration → low concentration

active transport: low concentration → high concentration



The Energy Source

- Adenosine Triphosphate (ATP)
  the gas for the engine
- High energy substances (glucose) generate ATP from usual metabolic pathways
- ATP will interact with a domain or subunit on the protein with the transport role
- Interaction will use the ATP → ADP + P_i reaction energy to drive a change in conformation (3°/tertiary structure) that results in ion pumping

Direct Active Transport

- The term direct means that the ATP interacts with the transporting protein
- This interaction usually involves ATP transferring the high energy phosphate (forming ADP as a product) to some part of the protein on one of its amino acids
- This may put the protein into another physical state (conformational change)
- When the phosphate is removed (hydrolyzed) from the protein, the protein returns to its original shape or conformational state, and the cycle can be started again

Coupled Active Transport

- Also called secondary active transport
- The transport protein does not derive its energy directly from ATP
- It still requires energy, and the energy ultimately comes from the ATP → ADP + P_i reaction, but transport is driven by a chemical and/or electrical potential created across the membrane by an ATP-driven process (thus it is "coupled")

Coupled Transport Types

- Transporters basically couple ATP hydrolysis (utilization of energy) to the movement of a substance (usually ion)
- Cotransporters couple the energy in the concentration difference AND/OR the voltage across the cell membrane to the transport of a 2nd ion in the same direction of movement as the driving substance/ion
- Cotransporters are called symporters
- Exchangers couple energy to concentration differences AND/OR membrane voltage differences to the transport of a 2nd ion but in the opposite direction: they are called antiporters

Substances Transported Actively

directly or coupled

- metal ions: Na^+, K^+, Ca^{2+}, H^+
- organic cations /
- lipophilic agents

- toxic substances, drugs, xenobiotics: these can be a variety of water-soluble substances which are organic ions (cations or anions) or polar molecules, as well as lipophilic substances

  anti-cancer chemotherapeutics especially

Active Transport Pumps (Animal Cells)

- Plasma Membrane Transporters
  - Na^+/K^+ ATPase
  - Multidrug Resistance Efflux Transporters
  - H^+/K^+ (used to pump H^+ into stomach to acidify)
  - Ca^{2+} ATPase: used by muscle to pump out Ca^{2+} into sarcoplasmic reticulum to stop contraction

Organelle Membrane Pumps

- Mitochondrial H^+/ATPase
- Lysosomal H^+

ATPase Types

- P-Type (phosphorylation)

  these include variety of transporters, cotransporters, exchangers
- V-Type (vacuole)

  these pump H^+ into the space of vacuoles to acidify them
- F-type (factor)

  these actually do the reverse: make ATP using H^+ gradients
- ABC-type (ATP-binding cassette)

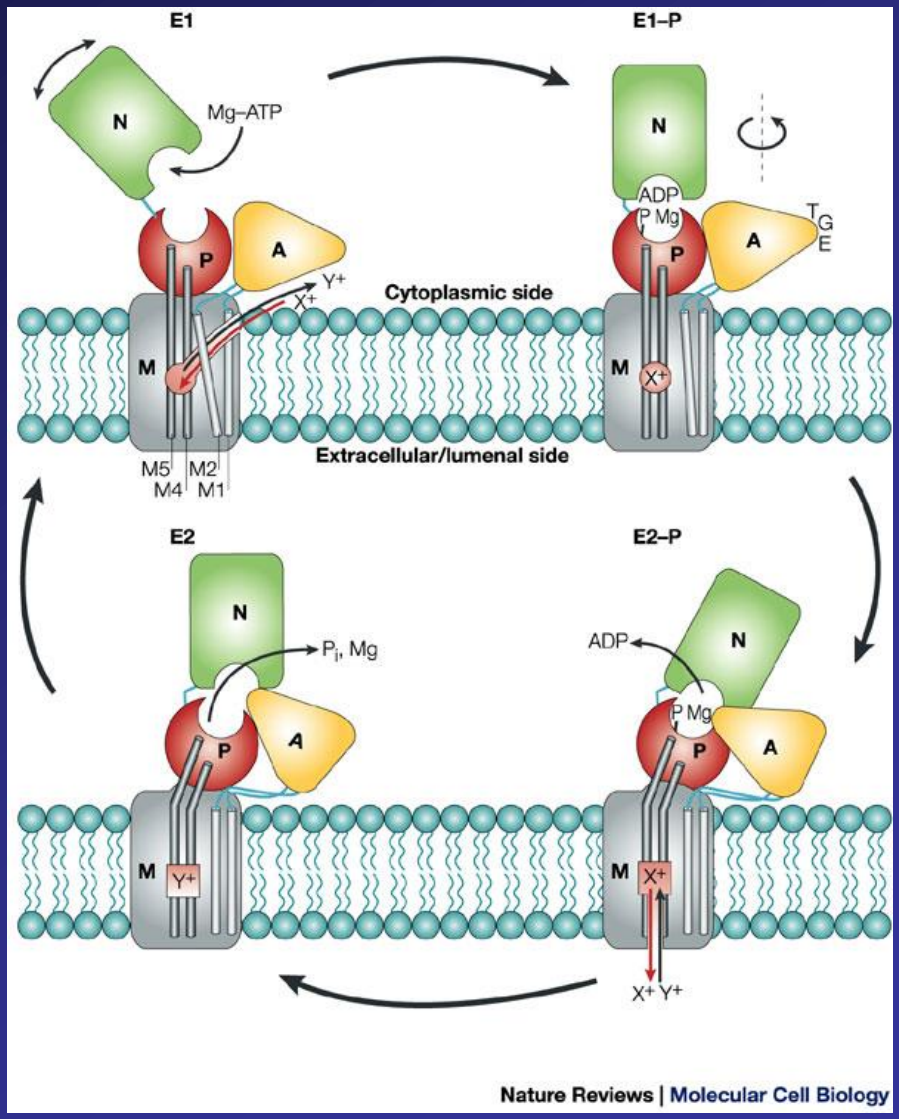
  these pump poisons/toxicants back out to where they tried to enter

P-Type ATPases

- These are often single polypeptide transmembrane proteins
- 8-10 TM segments forming a pore wall in membrane
- They work by ions loading from inside into a cavity of one subunit in the membrane, which triggers ATP transferring a phosphate to an aspartate side chain on one subunit, which triggers a twist in the protein to open to the outside, releasing the ions
- Ions from outside now load into the cavity, triggering phosphate to be hydrolyzed (released), and this triggers a twist back to open the cavity to the inside, moving the ions in the cavity in

P-Type ATPase Mechanism

1. In upper left (E1 state), ion X^+ moves from cytoplasm to bind to the membrane domain (M) high affinity site
2. This causes the phosphorylation domain (P) to move to its E1 conformation
3. Mg-ATP binds to the nucleotide-binding domain (N), then phosphorylates P on a key amino acid side chain, aspartate (Asp), now changing the protein to the E1-P state
4. The actuator (A) domain rotates causing a change from E1-P → E2-P, releasing the ADP.
5. A sequence of three amino acids, TGE (threonine-glycine-glutamic acid) on A domain is used to prevent the ATP from being reformed by a reverse of the phosphorylation step)



http://www.nature.com/nrm/journal/v5/n4/fig_tab/nrm1354_F6.html

Reusability of Good Designs

- Many proteins show homology (similarity, identity) in structure and function
- This is particularly true of ATPases
- This re-usability keeps a good function & makes slight changes to use new molecules / substrates

the figure at right shows three different P-Type ATPases

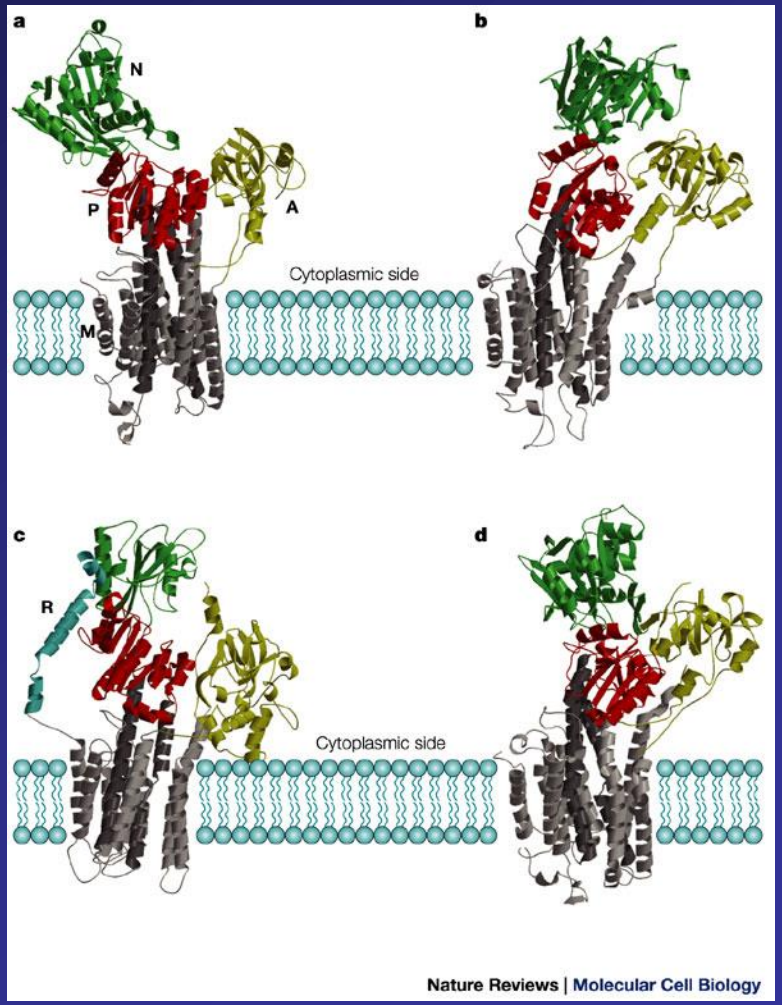
- upper two are structures of a muscle Ca^{2+}-ATPase in E1 (left) and E2 states
- lower left is a yeast H^{+}-ATPase in E1
- lower right is duck Na^{+}/K^{+}-ATPase in E2

green (N) = ATP-binding domain

red (P) = phosphorylation domain

yellow (A) = actuator domain

grey (M) = transMembrane domain



ABC-Type ATPases

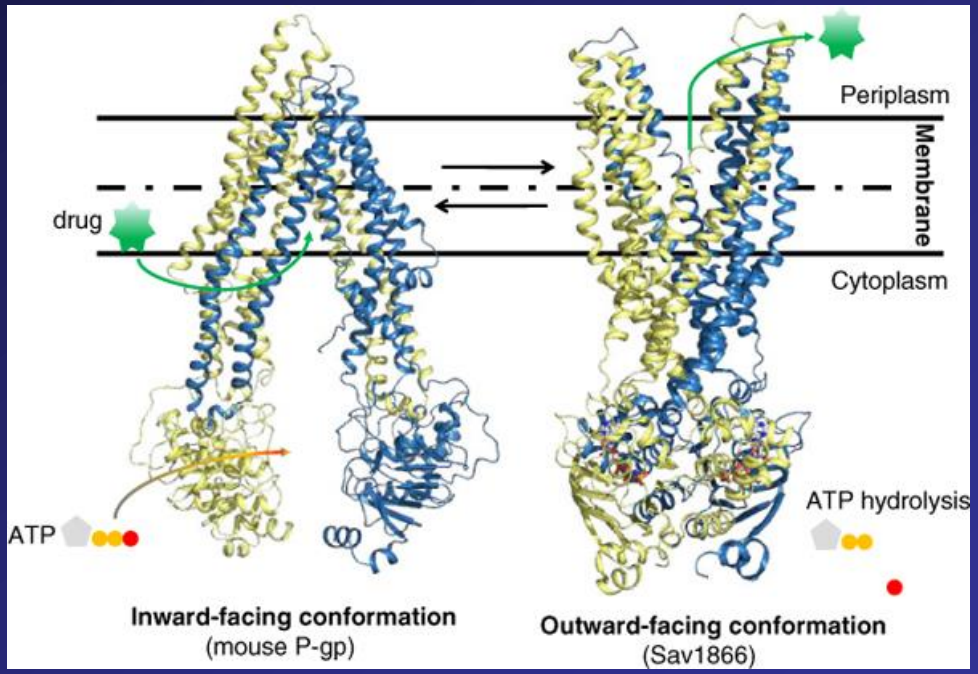
- ABC = ATP-these serve a vital function in transporting out toxicants (poisons, xenobiotics) out of the cell

  thus they are called
  multidrug-resistant (MDR) efflux transporters
- multiple ABC-types are present in cells that have a primary function in detoxification
  - liver hepatocytes
  - GI tract mucosal epithelial cells
- at least 150 different types in nature, with 48 different genes in humans
- these can be single polypeptides with 4 domains or can be 4 separate polypeptides that have single functions

  in bacteria, these proteins usually function as importers, not exporters (of nutrients)

Structure-Function Similarities

- While not exactly like P-type, ABC-type still have large parts of the protein on cytoplasmic side that bind and release energy from ATP
- Additionally, they get the protein to twist to open a "mouth" from inside and outside to emit the drug/toxin
- The transmembrane pore is formed by alpha helices just as in P-type, with about a half dozen helices making up the wall



V-Type/F-Type

V-Type

- have two subunits: embedded membrane pore and peripheral subunit that has ATPase activity
- these embed in the membranes of lysosomes, vacuoles, vesicles, endosomes, and Golgi complex
- These pump protons (H^+) into space to acidify it

F-Type

- these have similarities in structure to other ATPases but have a different function
- to be discussed later

The Na^+/K^+ ATPase

- The sodium-potassium pump is perhaps the most significant ion pump in the cell
- Its discovery and characterization were worthy of a Nobel Prize
- A large fraction of a cell's energy goes to operating this antiporter, because it also drives other processes by coupled transport; over half the cell energy of a neuron is used to maintain sodium and potassium across the membrane so that it can transmit electrical impulses
- Because it is so important, the details of the pump mechanism should be understood to some depth
- Na/K pump is P-Type

MAJOR NOTE

- The textually detailed step-by-step mechanism of Na^+/K^+-ATPase described in the slides that follow is the mechanism described by Horisberger in a 2004 review
- This differs in one very important aspect from discussions found in biology & biochemistry textbooks:
  - The E2→E1 conformational change occurs according to Horisberger when ATP binds the protein
  - In textbooks, the E2→E1 conformational change occurs when the phosphate is hydrolyzed from the protein

Na/K ATPase Mechanism

At the start of the cycle, the Na^+/K^+ ATPase has an intracellular opening. This is called the E1 state of the pump. ATP is bound to the protein.

1. $Na^+ \times 3$ Binding. THREE (3) Na^+ ions enter the pore space/cavity from the intracellular side and nestle inside the cavity.
2. ATP Phosphorylation. Na^+ cations binding triggers the bound ATP molecule to transfer a phosphate to an amino acid side chain on an intracellular segment or loop of the protein. [That amino acid happens to be an aspartate (Asp), and it forms a acyl phosphate ester ($-C(=O)-PO_3^{2-}$), a quite common chemical modification.] The ADP product is released into the cytosol. This is the E1-P state of the protein.

Na/K ATPase Mechanism

3. Conformation Change. The act of transferring the phosphate to the polypeptide causes the transmembrane polypeptide to twist & turn. This twisting & turning shuts the intracellular "door" and opens the extracellular "door". The enzyme is now in the E2-P state.
4. Na^+ ions exit. Because they have low affinity for the protein in the E2-P conformation, the 3 Na^+ ions leave the cavity into the extracellular fluid
5. K^+ ions enter. TWO (2) K^+ ions now enter into the pore from the extracellular side, and occupy the cavity with high affinity

Na/K ATPase Mechanism

6. Dephosphorylation of protein. With the two K^+ ions binding inside, this triggers a reaction that causes the acyl phosphate to be hydrolyzed: that is, a reaction in which H_2O eliminates the phosphate. The dephosphorylated protein is now in the E2 state
7. ATP binds. ATP binds (but does not transfer a phosphate!) to the protein.
8. Conformation change. With ATP binding, the protein twists and turns, going from the E2 to E1 state: the extracellular door is closed and the intracellular door opened.

Na/K ATPase Mechanism

9. K^+ ions exit. With the intracellular door now open, the two K^+ ions exit to the cytosol.

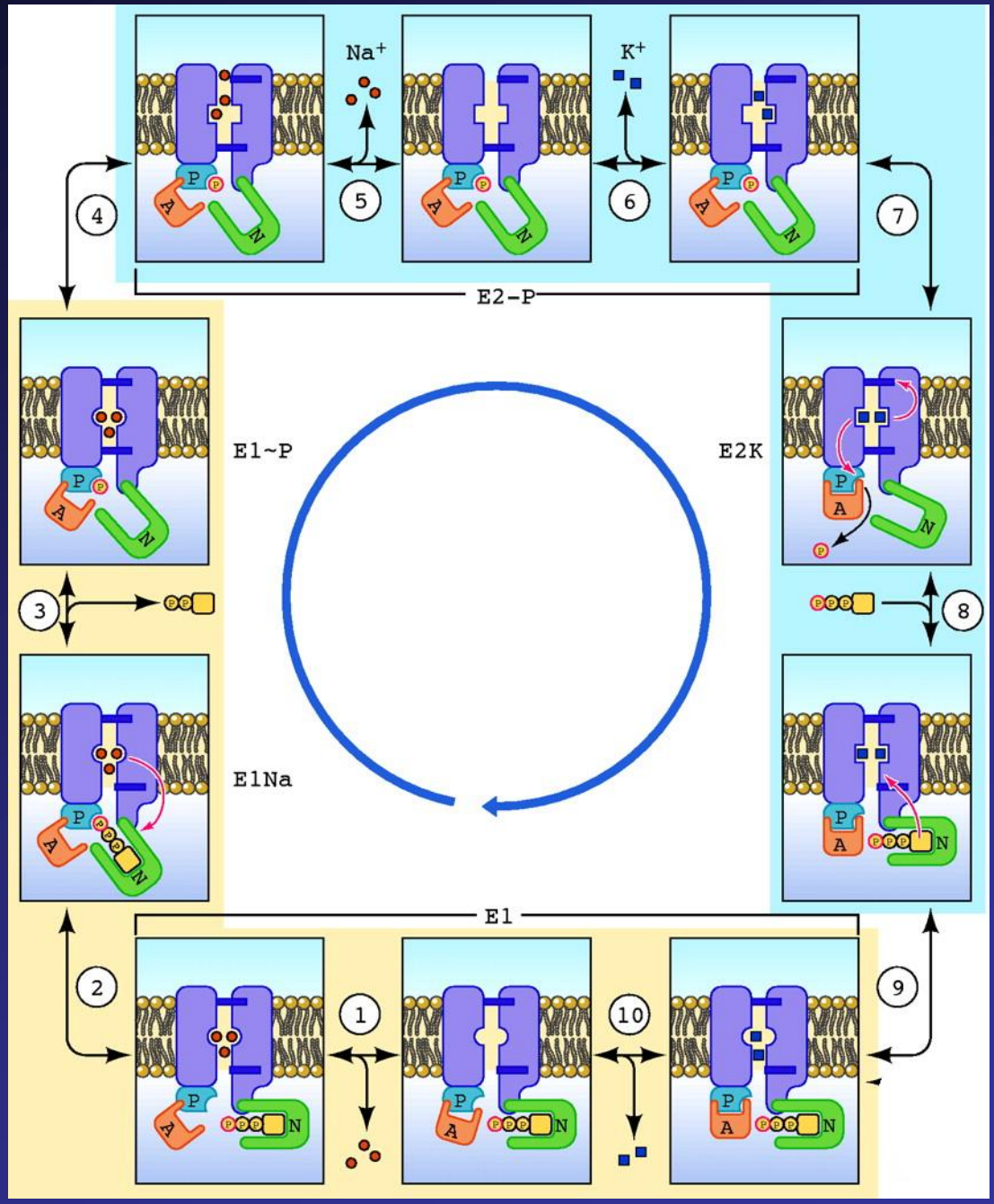
The cycle is complete, and can loop back to step 1.

The pump has achieved in one cycle the pumping out of 3 Na^+ ions and pumping in 2 K^+ ions, using one ATP molecule.

Na/K ATPase Mechanism

The steps textually describing the cycle in the previous slides are illustrated here to the right, published in 2004

Note that these steps are at odds with illustrations and descriptions in the biology texts and the animations.



Regulation of Na/K ATPase

- The membrane protein can have its activity upregulated by increases in intracellular cAMP
- cAMP is made by adenyl cyclase, the enzyme that was discussed as a downstream target of G-protein coupled receptor signaling. Thus G protein signaling that causes increases in cAMP concentration make the Na/K pump work more

- There is a separate G protein signaling pathway that also decreases cAMP levels, so stimulating that pathway can downregulate Na/K pump activity

More Regulation of Na/K ATPase

- Digoxin and ouabain are "cardiac glycosides" used to stimulate the pumping (contractility) of the heart
- This happens because Ca^{2+} levels in heart muscle cells remain high instead of being pumped out quickly so that muscle can relax
- Ca^{2+} is pumped out by a Na/Ca exchanger, which does not work unless the Na/K pump is working
- These drugs/poisons actually affect the Na/K pump, and thereby affect the Na/Ca exchanger: slowing down or stopping the Na/K pump also does the same to the Na/Ca exchanger

Reading (Sources)

Na/K pump flash animation ← click here

http://highered.mheducation.com/sites/0072495855/student_view0/chapter2/animation__how_the_sodium_potassium_pump_works.html

- Becker's WotC: Chapter 8 pp 208-216
- Raven: Chap 5.5
- Marieb: p 79