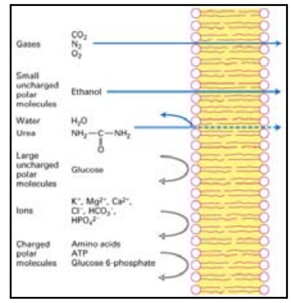
Transport across cell membranes
Pumps

A phospholipid bilayer acts as a selectively permeable barrier



A pure phospholipid membrane is permeable to

- Gases: oxygen, carbon dioxide
- Hydrophobic molecules: benzene, glycerol
- Small uncharged polar molecules: EtOH
- All above molecules translocate in the process of passive diffusion

Passive diffusion

- Migration of molecules from the area of high concentration to low concentration (down the gradient)
  - Reversible
- The molecule moves through the imperfections in the bilayer
- Diffusion rate is proportional to the concentration gradient and hydrophobicity

A pure phospholipid bilayer is impermeable to

- Water soluble molecules
- Charged molecules such as ions
- Sugars
- Amino acids

How do these molecules enter a cell?

- Membrane proteins form special "passages" through the membrane
  - Pumps
  - Carriers
  - Channels

Pumps

- Primary active transporters
  - Active = Use external energy
  - Primary = they generate chemical or electrical gradients and later transport against the gradient
- Are enzymes !!! (they have to break some bonds to produce energy)
  - ATP driven pumps are also called ATPases
- Transport ions and small molecules

Types of energy used by pumps

- ATP
- Light
- Redox potential
- Decarboxylation

Other transporters

- Carriers or secondary transporters
  - Use gradients created by pumps to transport other molecules
- Ion channels - passive transporters
  - Pores that open and close and allow passage of ions through the membrane

Back to pumps

- Active transport
- Require energy input
- Very specific
- Slow (~100 molecules/s)

Why do we need pumps?

- To survive cells must maintain unfavorable concentrations gradients across their membranes
- A third of the total energy supply of the cell is consumed by pumps

Diversity of membrane pumps

- Light driven
  - Proton pumping by bacteriorhodopsin
- ATP driven
  - F_0F_1 ATPases or F and V type pumps
  - E_1E_2 or P type pumps
  - ABC transporters

Diversity of membrane pumps

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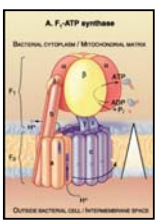
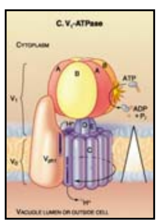
F_0F_1 ATPases or F and V-class ion pumps

- Similar structure for both classes
- Different function
  - V-class – pumps H^+ into lysosomes and other acidic vesicles and out of Archaea
  - F-class – works in opposite direction, uses H^+ to synthesize ATP – it is an ATP synthase in mitochondria
  - Purified F-class is reversible (that's why we still call it a pump)
- Both transport only H^+

Structure of F_0F_1 ATPases



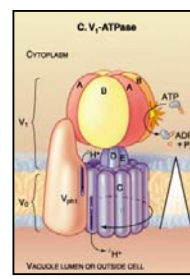


- Multiple transmembrane subunits
- Multiple cytosolic polypeptides

Structure of V type pump – V-ATPase

Two parts

- V_1 - cytoplasmic
  - Enzyme – breaks ATP
- V_0 - transmembrane
  - Proton channel



V class ion pumps

- Use ATP energy to move protons from cytosol into organelles (lysosomes)
- Generally function to maintain the low pH of acidic vesicles
- Protons flow against the gradient !!!

Expression of V class ion pumps

- Organelles
  - Lysosomes
  - Golgi
  - Secretory vesicles
  - Plant vacuoles
- Plasma membrane of cells that secrete H^+
  - Osteoclasts
  - Macrophages

P-class ion pumps or E_1E_2 ATPases

- Use ATP hydrolysis to create ion gradients required for function of ion channels and cation coupled carriers
- ATP hydrolysis provides the energy that drives the pump
  - Use high energy ATP intermediate

P-class ion pumps or E_1E_2 ATPases

- Examples:
  - Na^+/K^+ ATPase
  - Ca^{2+} ATPases
  - H^+ pump in stomach acid secreting cells

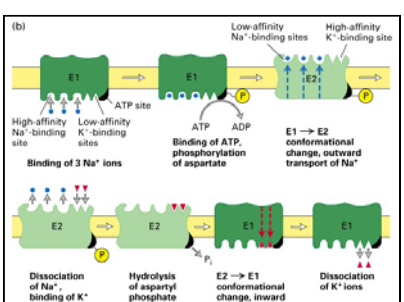
Na^+/K^+ ATPase

- Expressed in neurons and other excitable cells
- Catalyzes the exchange of 2 K^+ ions for 3 Na^+ ions
- Because of the uneven exchange in addition to ion gradient the pump produces voltage gradient = is **electrogenic**

Na^+/K^+ ATPase

- Binding of cytoplasmic Na^+ stimulates the phosphorylation of Asp by ATP and transport of Na^+ out
- Hydrolysis of Asp-phosphate provides energy for the transport of K^+ in together with returning the pump protein to the initial conformation

Na^+/K^+ ATPase



Muscle Ca^{2+} ATPase (SERCA1)

- 2 Ca^{2+}/2 H^+ pump
- Pumps Ca^{2+} ions from the cytosol into the sarcoplasmic reticulum
- Critical in muscle contraction and relaxation
- Two Ca^{2+} ions per molecule of ATP

Muscle Ca^{2+} ATPase

- Binding of cytoplasmic Ca^{2+} to calmodulin stimulates the phosphorylation of Asp and transport of Ca^{2+} out
- Hydrolysis of Asp-phosphate provides energy for the flip back and transport of H^+

ABC transport proteins

- ATP Binding Cassette
- Includes more than 100 different transport proteins
- Each ABC transport protein is specific for a single substrate

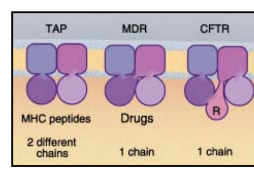
ABC transport proteins

- Transport ions
  - Cystic fibrosis transmembrane regulator
- Sugars, amino acids, peptides and proteins
- Also transport small lipid like drugs
  - Multidrug-resistance transport protein

ABC transport proteins

- Modular design
- Four “core” domains, two transmembrane and two cytosolic
  - Different number of peptides forming domains

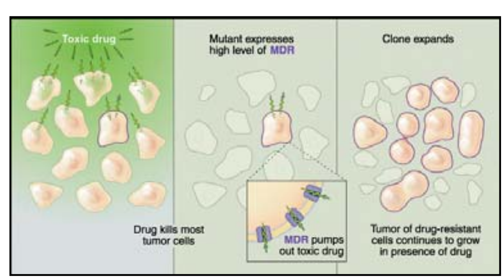
- Transmembrane domains form a passage



The MultiDrug - Resistance Transport Protein

- MDR1 and MDR2
- Transports hydrophobic molecules from the cell
- When overexpressed can “pump out” chemotherapy drugs

Multiple drug resistance in cancer therapy



Cystic Fibrosis Transmembrane Regulator - CFTR

- Acts like chloride channel
- Expressed in apical membranes of the epithelia in lungs, sweat glands, pancreas
- ATP opens and closes the channel
- Mutation of the gene causes cystic fibrosis

Cystic fibrosis

- CF is one of the most common human diseases caused by mutations in a single gene
- Overproduction of excessive, thick mucus that obstructs the lungs and the gastrointestinal system
- First disease to be “repaired” with gene therapy