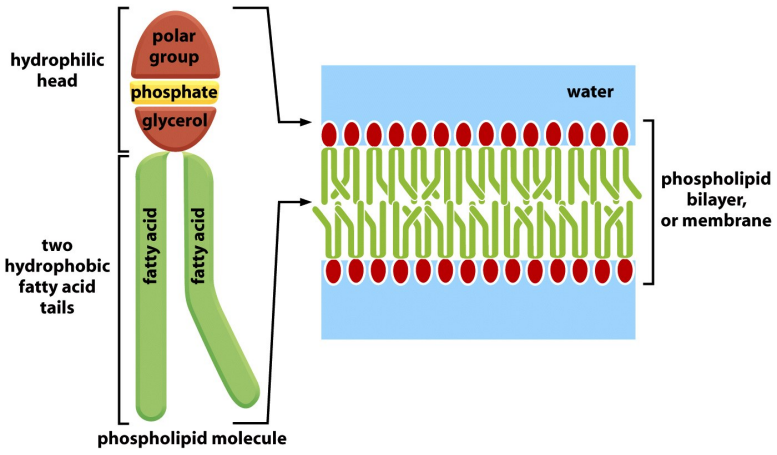
Membrane Transport

Core Curriculum II

Spring 2015

Cell Membrane



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- Two ways to cross the membrane:
  - "dissolve" in the hydrophobic barrier and go through
  - cross with the aid of a transmembrane protein

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- Gases (O_2, CO_2, N_2)

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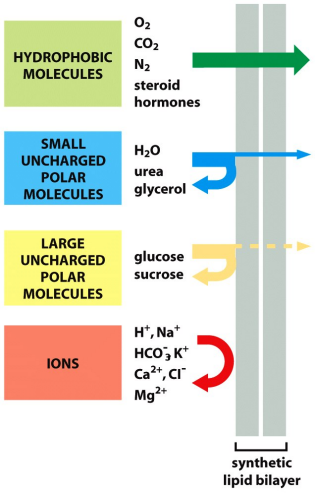
- Small nonpolar molecules (*e.g.*, diethylurea)

Molecules that need "help"

- Charged molecules
  - Ions (*e.g.*, Na^+, Ca^{++}….)
  - ATP
  - Amino acids



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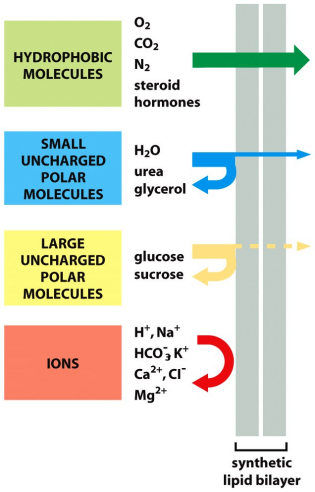

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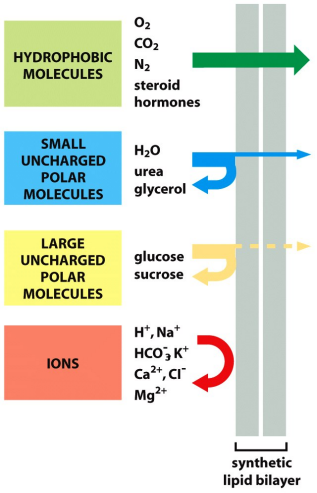
  Basically, everything of biological interest…..



Diffusion

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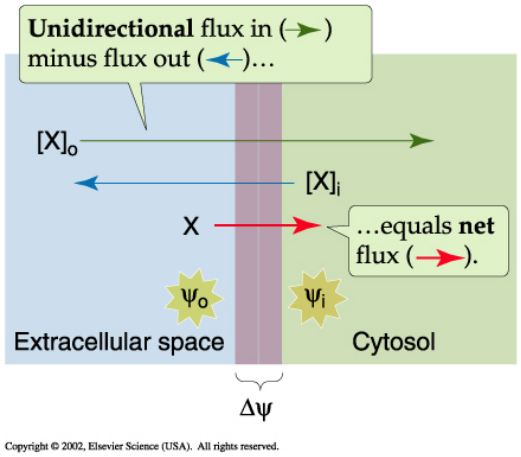
- Measured as FLUX (J)[=]moles/sec-area

Diffusion



Fick's Law

- Relates flux to concentration gradient
- D=diffusion coefficient, ΔC=concentration gradient, *x*=membrane thickness
- D depends on the nature of the solute, the solvent, and the bilayer
- Concentration refers to the concentration at the membrane surfaces; not always the same as bulk solution

$$J = \frac{D}{x}\Delta C$$

Slight complication: We don't know $C_{membrane}$

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$$C_{aqueous} \neq C_{membrane}$$

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  - $K>1$: $C_{membrane} > C_{water}$ (hydrophobic)
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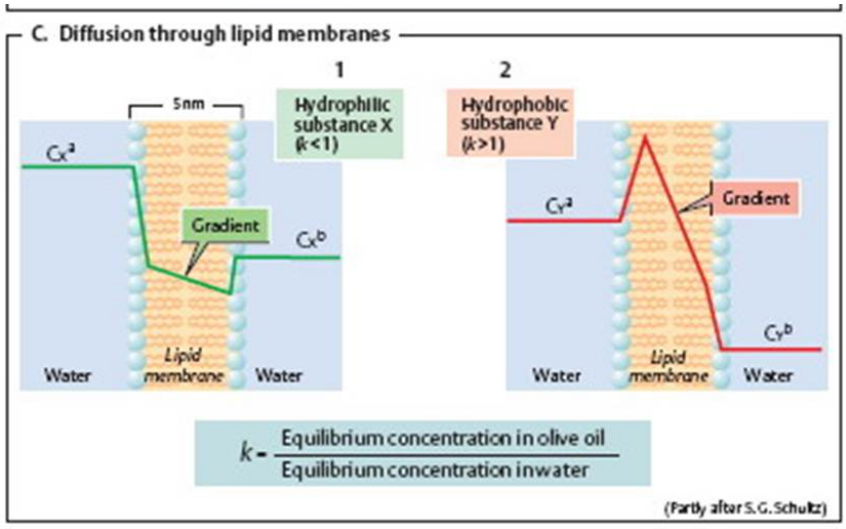
- $\Delta C_{membrane}=K\Delta C_{water}$

Partition coefficient, K



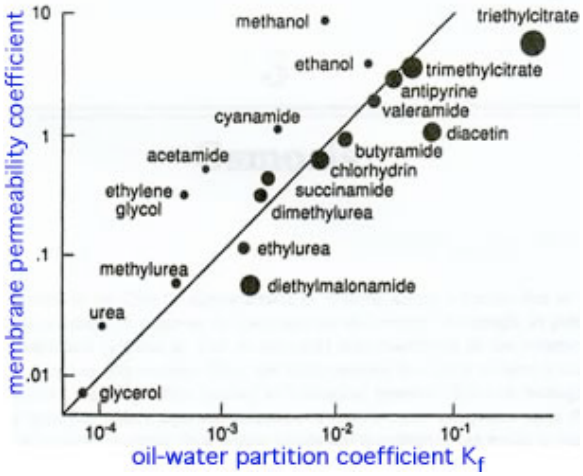
Modified (useful) form of Fick's Law

$$J = \frac{DK}{x}\Delta C = P\Delta C$$

P= permeability coefficient; a measurable parameter

Overton's Law:

The higher the lipid solubility, the higher the permeability



Data from Collander (1949)

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- Most "interesting" molecules (K<<1) have very low fluxes w/o help TRUE
- *Transporters* are the proteins that help the interesting molecules cross

Classes of Transport Proteins

*(*substrates travel down concentration gradient)*

- Transporters (10^2–10^4/sec)*
  - Uniports
  - Symports/cotransporters
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- Channels (10^8/sec)*
- ATP-driven pumps (10^2/sec)

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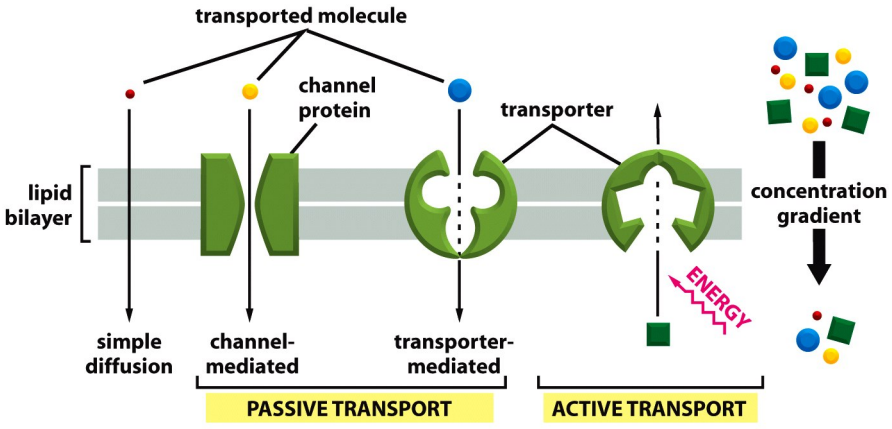

Common Properties

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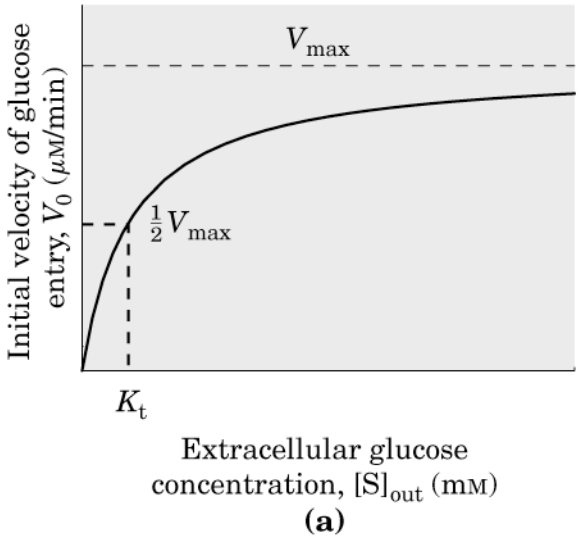
- Faster than simple diffusion through the membrane

Concentration Dependence



Uniporters aka "facilitated diffusion"

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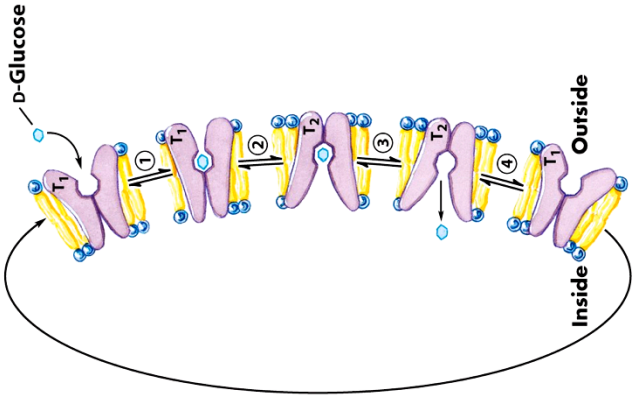
- Allow movement of molecules down concentration gradient
- Is bi-directional: direction of transport depends upon the gradient

Uniporters

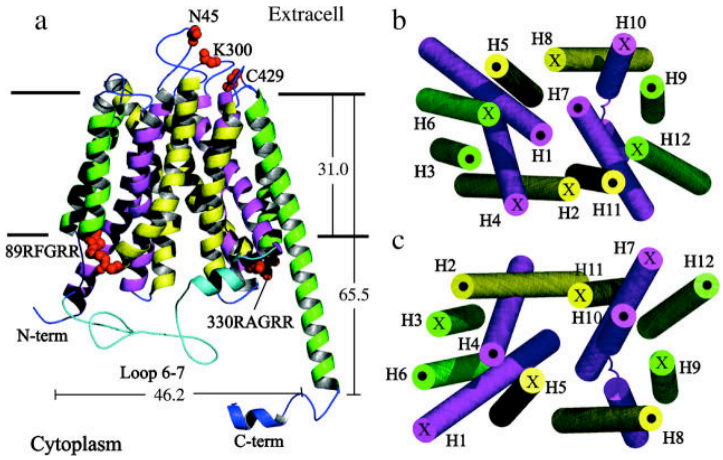
- Classic example: glucose carrier of red blood cells (GLUT1)
- Specificity
  - Glucose, mannose, galactose
  - Low affinity: K_m= 1.5 mM for glucose
  - Stereospecificity: D-glucose, not L-glucose (K_m>3 M)

"Alternating Access" *aka* "Rocking Banana" Model for uniporters



From Lehninger Principles of Biochemistry, 3rd edition

GLUT1:Proposed Structure



Salas-Burgos et al (2004) Biophys J 87:2990

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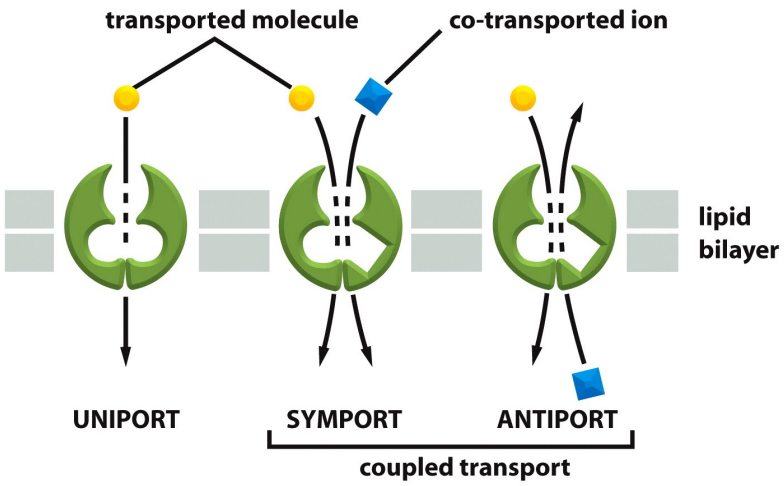
- Sometimes referred to as "secondary active transport"

Symports/Co-transporters



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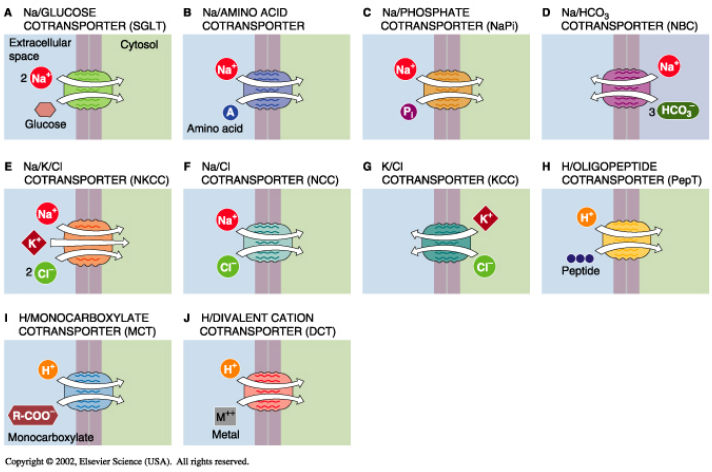
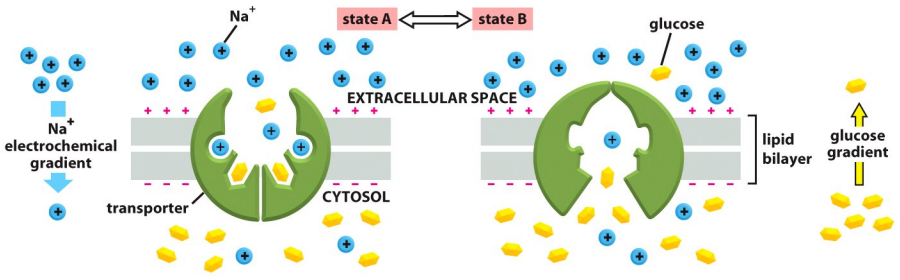
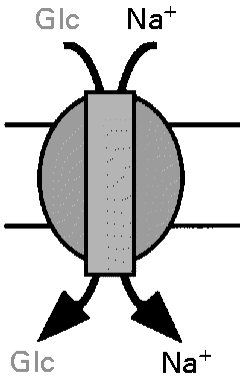


Coupled Transport



Na^+-dependent Glucose Uptake (SGLT family)

- Involved in carbohydrate uptake by the digestive system



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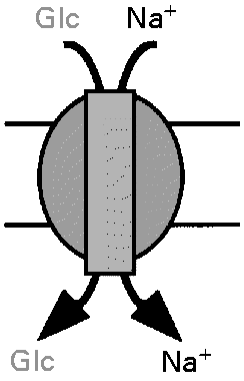
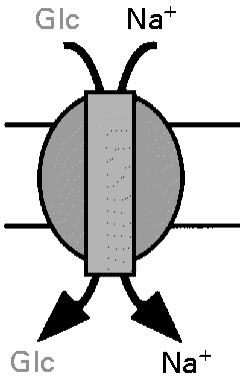
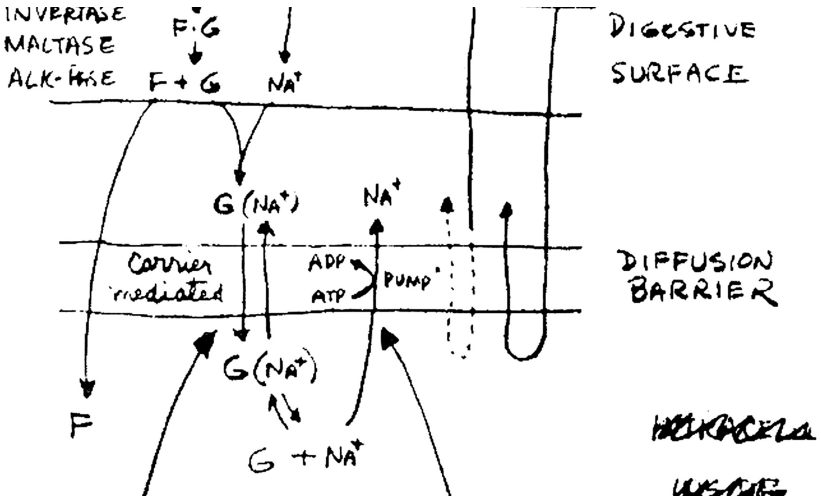


Na^+-dependent Glucose Uptake (SGLT family)

- Involved in carbohydrate uptake by the digestive system
- Movement of glucose requires concurrent Na^+ movement (obligatory coupling)
- Provided inspiration for the notion of coupled transport



Crane, 1960

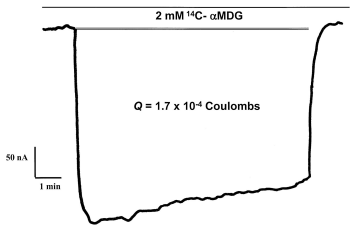


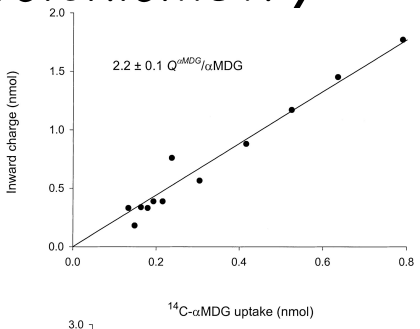
SGLT Family

TABLE 1. *SGLT substrates and expression in the human body*

Gene	Substrate	$K_{0.5}$, mM	Distribution
SGLT1 (SLC5A1)	Glucose, galactose	0.5	Intestine, trachea, kidney, heart, brain, testis, prostate
Cotransporter		0.5	
SGLT2 (SLC5A2)	Glucose	6	Kidney, brain, liver, thyroid, muscle, heart
Cotransporter		NI	
SGLT3 (SLC5A4)	Glucose	20	Intestine, testis, uterus, lung, brain, thyroid
Glucosensor		NI	
SGLT4 (SLC5A9)	Glucose, mannose	2	Intestine, kidney, liver, brain, lung, trachea, uterus, pancreas
Cotransporter		0.15	
SGLT5 (SLC5A10)	Glucose	ND	Kidney cortex
Cotransporter	Galactose	ND	

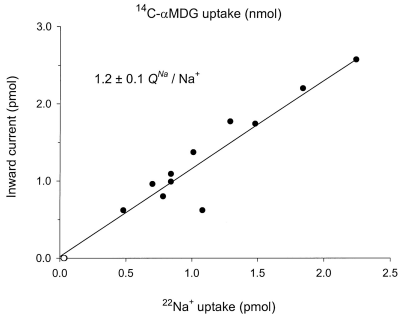
SGLT Stoichiometry





2 Na^+ per glucose

Diez-Sampredo *et al Am J Physiol* **280**:F272-F282



SGLT1 Topology

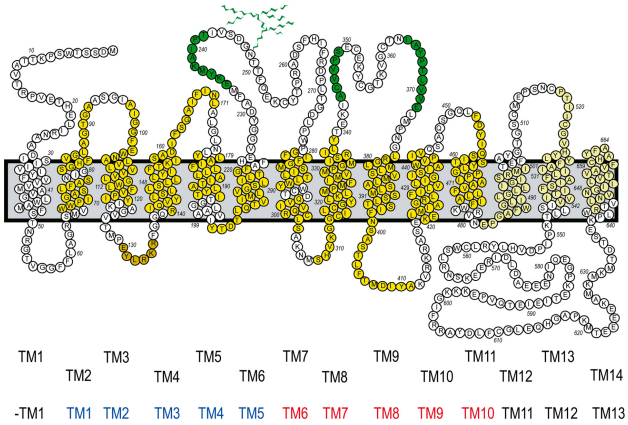


FIG. 13. Secondary structure model of hSGLT1 (217). This model shows the sequence of the 664 residues arranged in 14 transmembrane helices with both the NH_2 and COOH termini facing the extracellular side of the plasma membrane. A single *N*-glycosylation site occurs at Asn (N) 248. Highlighted are the locations of the helical domains based on the vSGLT structure (48). The numbering of the TMs has been revised to conform with the LeuT structural fold to allow easy comparisons between structural family members, i.e., TM1 through TM13 (1).

Structure of vSGLT

- Prokaryotic homolog from *Vibrio parahaemolyctus*

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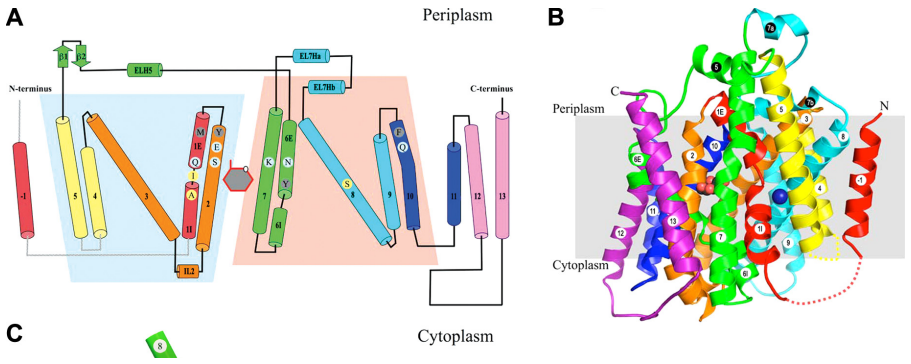
- Prokaryotic homolog from *Vibrio parahaemolyctus*
- Member of the so-called prokaryotic LeuT family- a family of Na^+-dependent cotransporters
- Structures are available for a number of the LeuT transporters

Structure of vSGLT



LeuT Family Structural Conservation

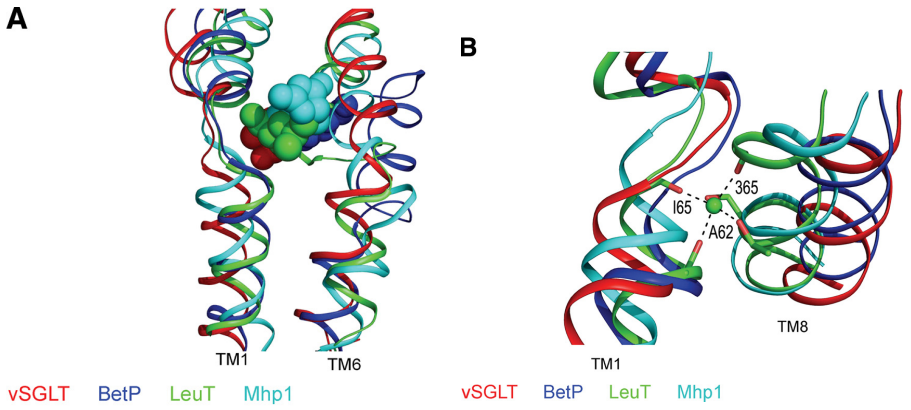
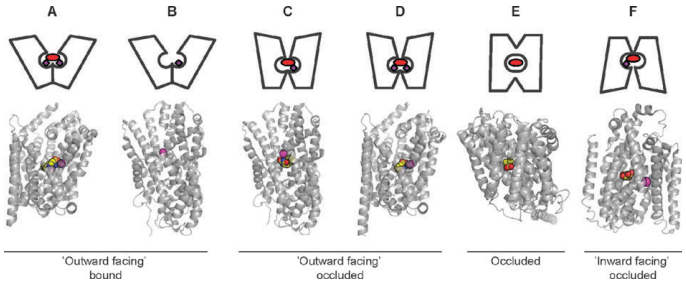


FIG. 16. The LeuT structural family. *A*: a structural alignment of TM1 and TM6 and the substrate binding sites for vSGLT (red, galactose), BetP (glycine betaine, blue), LeuT (leucine, green), and Mhp1 (benzylhydantoin, cyan). *B*: alignment of the Na2 sites on TM1 and TM8. [Redrawn from Abramson and Wright (1).]

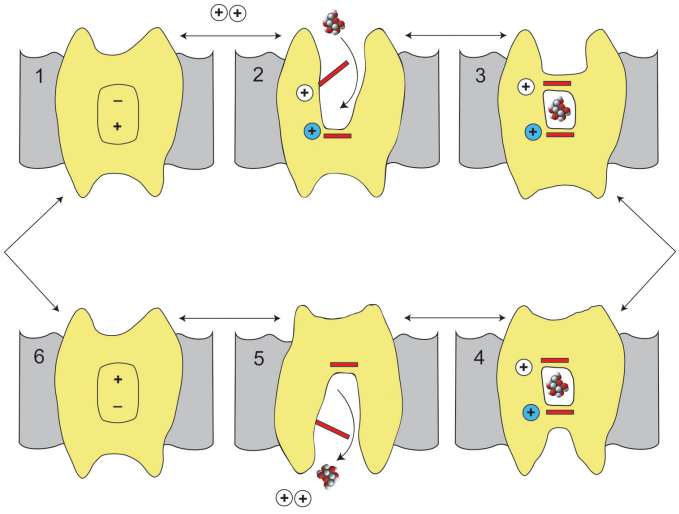
LeuT Family: Alternating Access



12 Crystal structures of LeuT and structurally homologous transporters. **A–F**: *Top*: Schematic representation of the conformations tallized transporters. Co-crystallized substrate/inhibitor and Na^+-ions are shown in red and magenta, respectively. *Bottom*: Sub: pitors are shown in yellow and Na^+-ions as magenta spheres in the crystal structures. **A**: LeuT co-crystallized with tryptophan a: -ions (PDB code 3F3A). **B**: Mhp1 co-crystallized with Na^+ (PDB code 2JLN). **C**: Mhp1 co-crystallized with benzyl-hydantoin a -ion (PDB code 2JLO). **D**: LeuT co-crystallized with leucine (PDB code 2A65). **E**: BetP co-crystallized with betaine (PDB code 2 SGLT co-crystallized with galactose (PDB code 3DH4).

5 | *Chem. Commun.*, 2009, 3677–3692

Model of SGLT1 Transport



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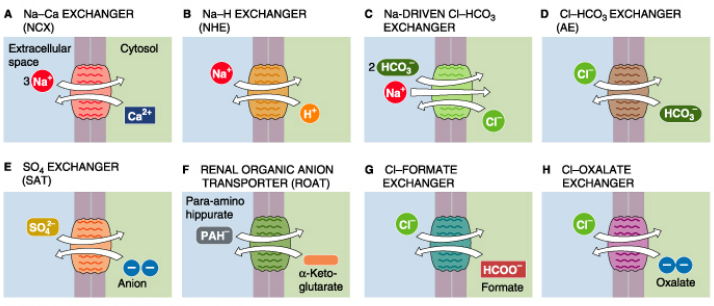

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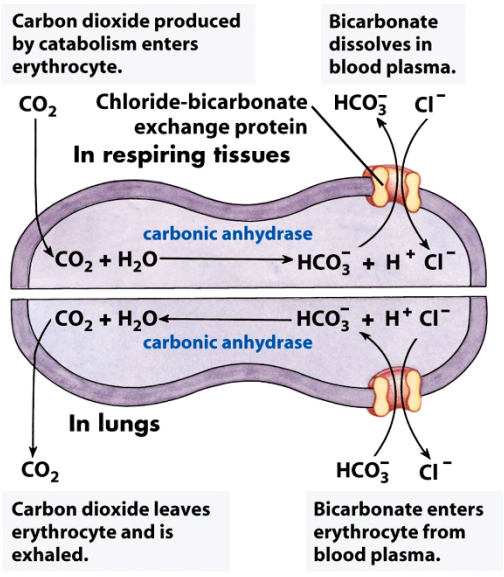
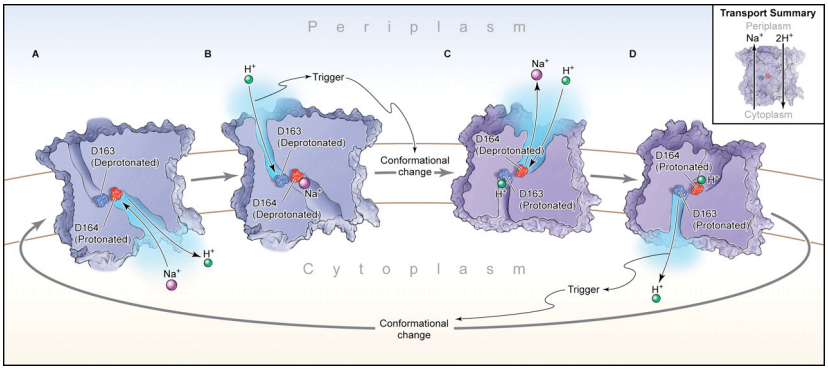
  - Help maintain intracellular pH and volume homeostasis
- Cl^-/HCO_3^- exchanger
  - Involved in transport of CO_2 from tissues to blood and to lung

Cl^-/HCO_3^- Exchanger from RBCs



Na^+/H^+ Exchanger NhaA from *E. coli*



Arkin et al (2007) Science 317:799

pH-driven conformational changes

ATP-driven Pumps

- *aka* primary active transporters

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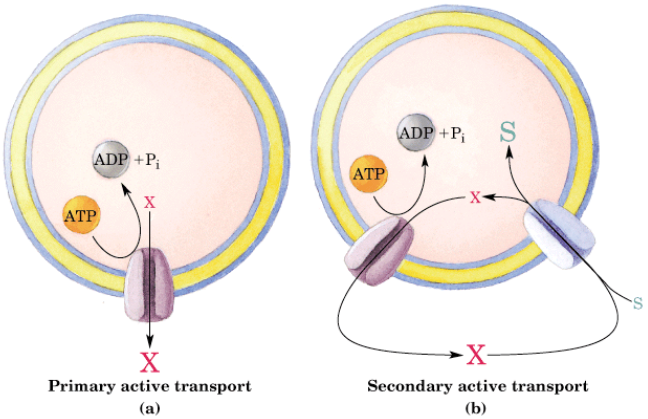
- Directly couples ATP hydrolysis to the movement of a substrate <u>against</u> its concentration gradient

Primary vs secondary active transport



- From Lehninger Principles of Biochemistry, 3rd Ed.

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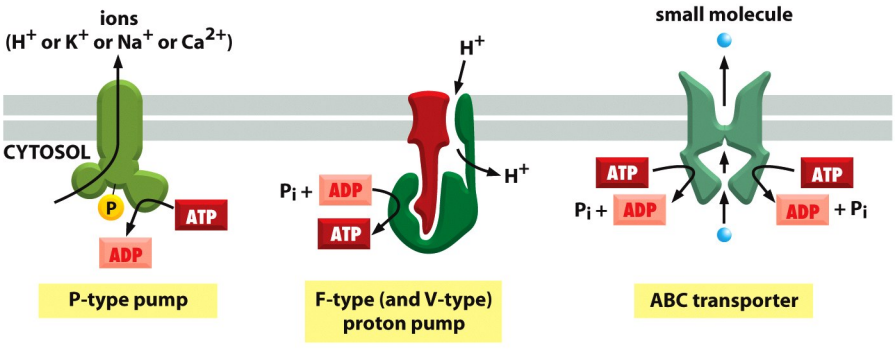
  - ABC superfamily
    - **A**TP-**B**inding **C**assette
      - >100 types (MDR, CFTR, etc.)

ATP-driven Pumps



P-class Pumps

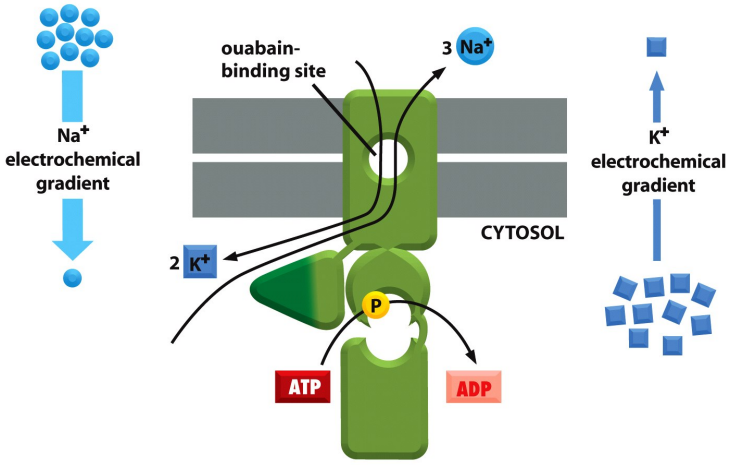
- All involve E~P intermediate
- Examples
  - Na^+/K^+ ATPase: set up ion gradients
  - Ca^{++} ATPase: maintain low cytosolic $[Ca^{++}]$
  - H^+/K^+ ATPase: acidify stomach
  - Menkes ATPase: pumps Cu^+ out of cells and into intracellular compartments

Na^+/K^+ ATPase

3 Na^+ move out and 2 K^+ move in per ATP hydrolyzed

Transport is said to be *electrogenic*- there is net charge movement

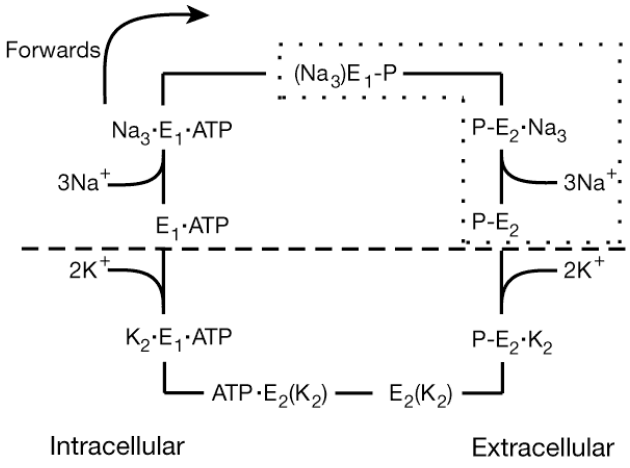
This pump is the conceptual model for how all P-type pumps work



Na^+/K^+ ATPase Cycle

E_1: opening faces cytoplasm

E_2: opening faces extracellular solution



Ca^{++} Pump aka SERCA

- SERCA: sarco/endoplasmic reticulum Ca^{++} ATPase

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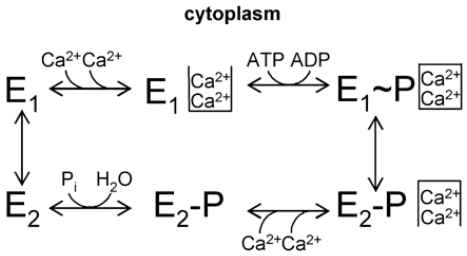
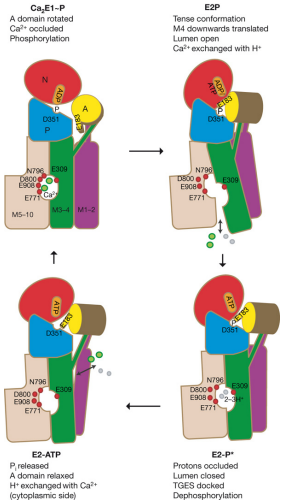
- The crystal structure of the SERCA pump has been determined in several different conformations, allowing one to examine various stages of the catalytic cycle

SERCA Pump Cycle





Olesen et al (2007) Nature 450:1036

F- & V-class pumps

- F-class
  - Bacteria, mitochondria, chloroplasts
  - Normally run "backward"
    - Make ATP from downward movement of H^+

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  - Normally run "backward"
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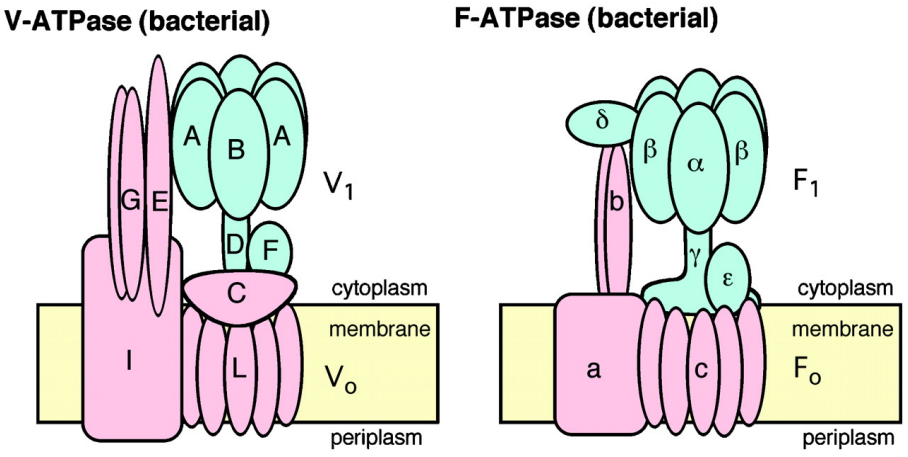
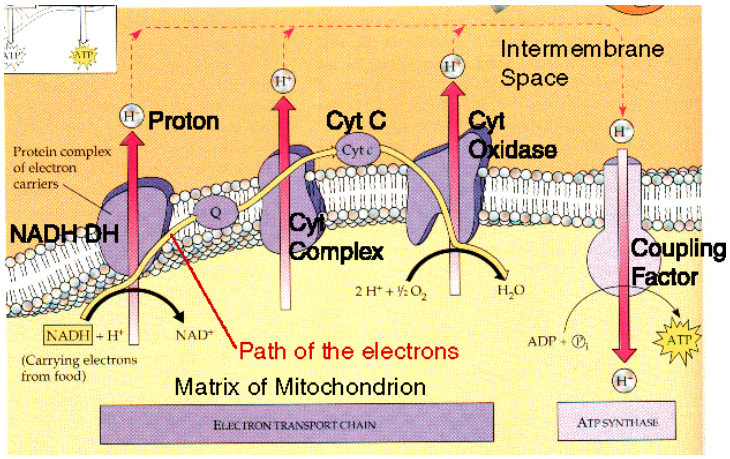
  - Used to acidify internal compartments such as lysosomes
- Similar structures
- No E~P intermediate

F- & V-class pumps



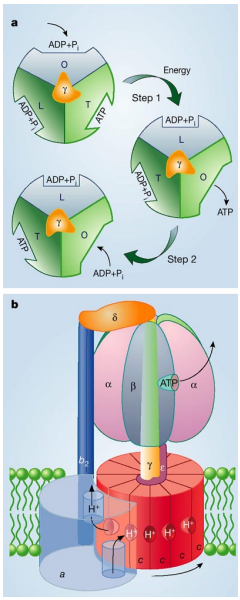
Mitochondrial F_1 ATPase

Generates ATP through proton movements

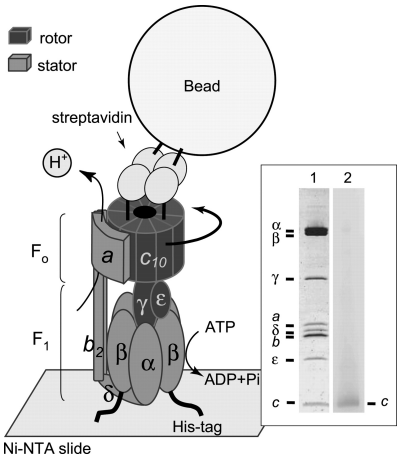


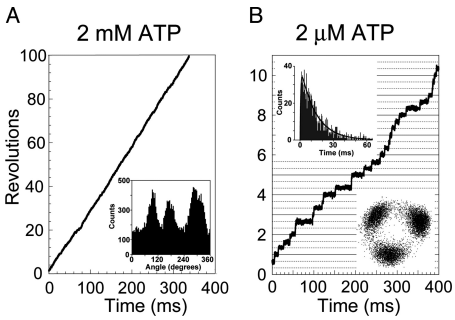
F_1 ATPase: A Molecular Motor

Cross Nature **427**:421 (2004)

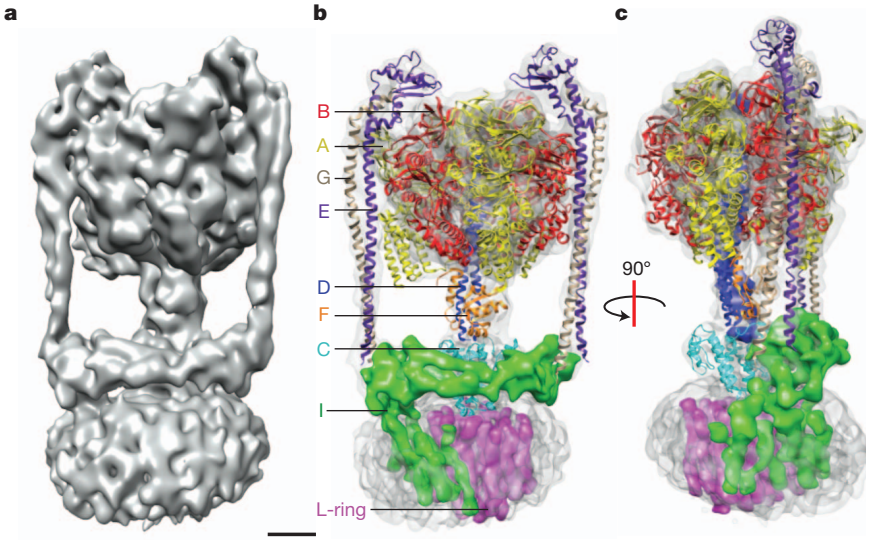


F_1 ATPase: A Molecular Motor





T. Thermophilus H⁺ pump



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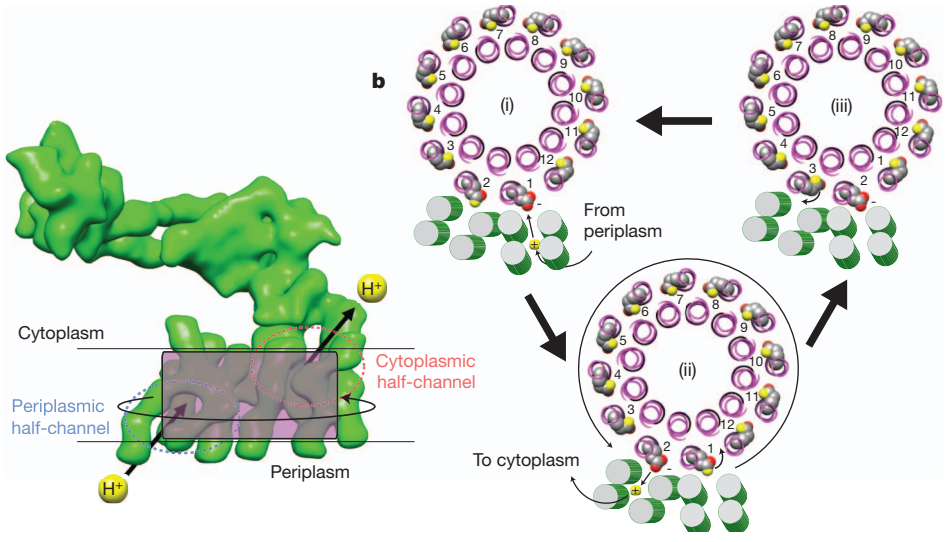

ABC Superfamily

- > 100 members

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- Each type is selective for a particular substrate or class of compounds

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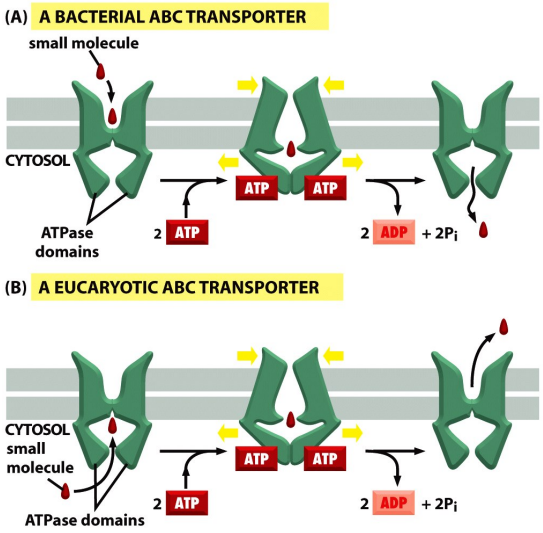
- Compounds transported include metabolites, lipids, sterol, and xenobiotics

ABC Superfamily



ABC Superfamily

Table 1. Clinically relevant and atypical ABC proteins

ABC Protein	Psuedonym	Ligand(s)/Function	Associated Disease(s)
ABC1	ABCA1	Cholesterol	Tangier disease
ABCR	ABCA4	Retinal	Various eye diseases
TAP1/2	ABCB2/B3	Peptides	Bare lymphocyte syndrome
ABC7	ABCB7	Iron	Anemia and XLSA
MRP6	ABCC6	?	Pseudoxanthoma elasticum
ALD	ABCD1	vlcFA	Adrenoleukodystrophy
Sterolin1/2	ABCG5/G8	Sterols	Sitosterolemia
PGY3/MDR3	ABCB4	Phosphatidylcholine	Liver disease: PFIC3, OC
BSEP/SPGP	ABCB11	Bile acids	Liver disease: PFIC2
MRP2	ABCC2	Conjugated bilirubin	Liver disease: D-J syndrome
MDR1	ABCB1	Hydrophobic drugs	Failure of chemotherapy
BCRP/MXR	ABCG2	Hydrophobic drugs	
MRP1	ABCC1	Conjugated drugs	
MRP4	ABCC4	Conjugated nucleosides	
		Atypical ABC proteins	
CFTR	ABCC7	Chloride ion channel	Cystic fibrosis
SUR	ABCC8	Regulation of K_{IR} channel	PHHI
SMC1-6		Chromosome maintenance	
Rad50		DNA, telomere repair	
Elf1p		mRNA trafficking	

XLSA, X-linked sideroblastomic anemia; PFIC, progressive familial intrahepatic cholestasis; OC, obstetric cholestasis; D-J, Dubin-Johnson syndrome; PHHI, persistent hyperinsulinemic hypoglycemia of infancy.

Linton (2007) Physiology 22:122

ABC Superfamily

- Proteins have two transmembrane domains and two nucleotide binding domains

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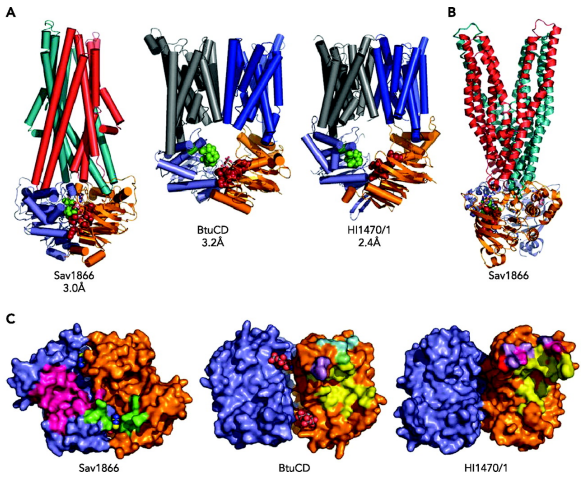
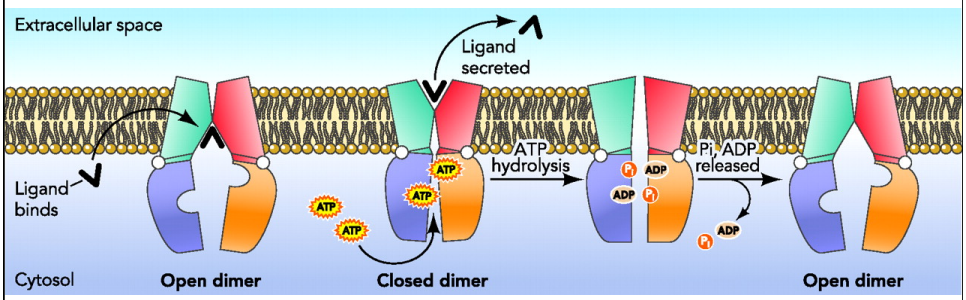
- ATP binding and hydrolysis drive the pumping
- Importers usually have an associated binding protein

ABC Superfamily

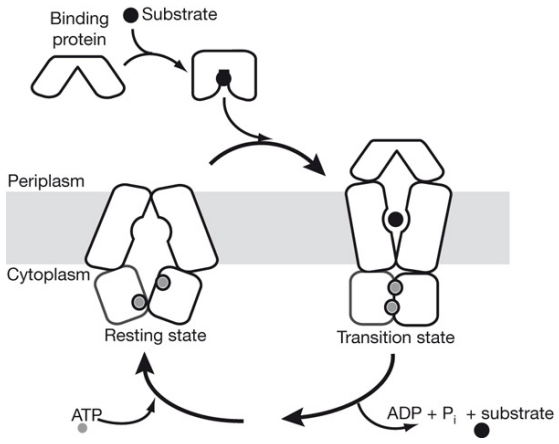


ABC Superfamily: Exporter



- Linton (2007) Physiology 22:122

ABC Superfamily: Importer



ABC Superfamily: Multidrug Resistance

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- Probable function is the transport of natural and metabolic toxins out of cells
- Similarity of drugs to "normal" substrates means that they're also pumped out, lessening their effectiveness
- Responsible for multidrug resistance seen in tumors

Multidrug Resistance

A number of MDR transporters are found in liver, and their role is to get rid of small hydrophobic compounds

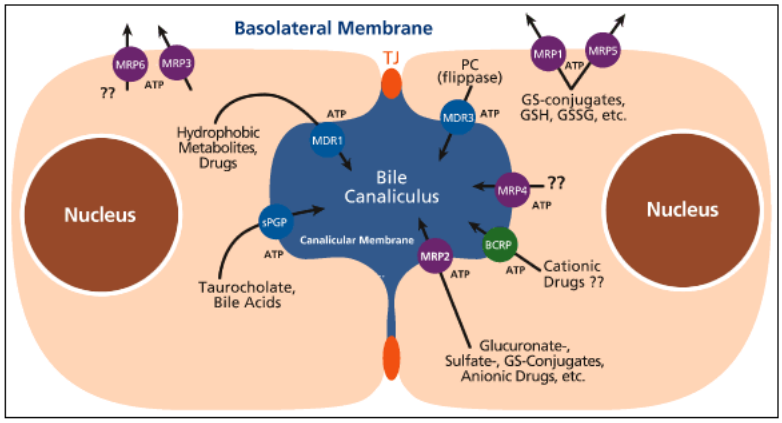
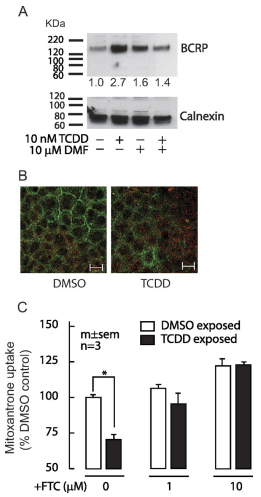


Figure 4. Multi-drug transporters in the human liver hepatocytes.
Abbreviations: TJ, tight junction

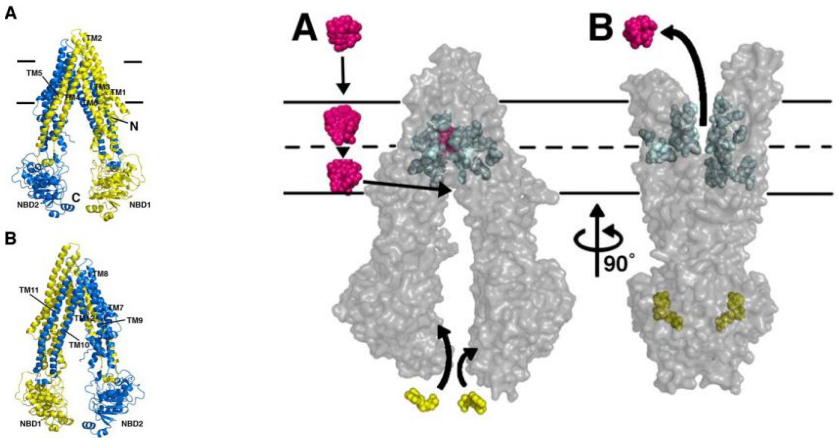
Multidrug Resistance

- MDR pumps can be induced by a variety of compounds, including drugs, meaning that drug treatment can result in greater transport
- Ex: Breast cancer resistance pump (BCRP) induction by aryl hydrocarbons

Tan et al (2010) Mol. Pharmacol. 78:175



P-glycoprotein



Aller, SG *et al* (2009) Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **323**:1718-1722.

ABC Superfamily: CFTR

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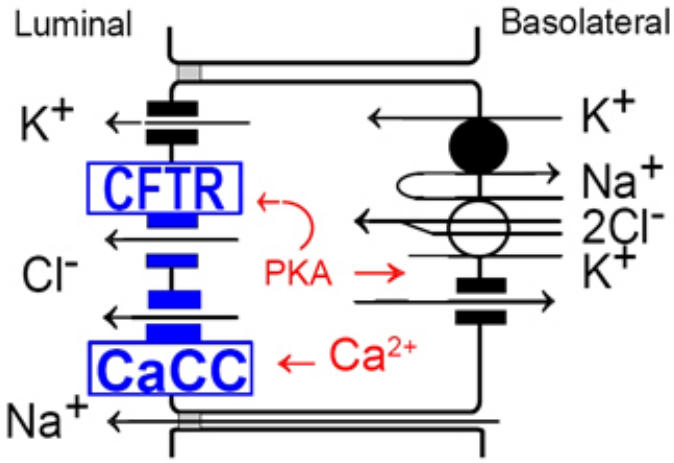
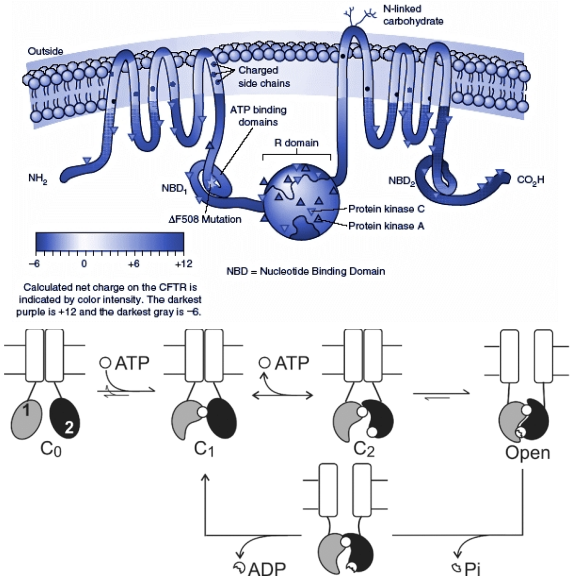
- CFTR: **C**ystic **F**ibrosis **T**ransmembrane **R**egulator
- cAMP-dependent Cl^- channel
- Movement of Cl^- helps move H_2O across epithelial cells
- Defect in Cl^- transport results in reduced H_2O movement, leading to viscous mucous or secretions

CFTR


Luminal
Basolateral
K+
CFTR
Cl-
PKA
CaCC
Ca2+
Na+
K+
Na+
2Cl-
K+

CFTR


N-linked carbohydrate
Outside
Charged side chains
ATP binding domains
R domain
NH2
NBD1
NBD2
CO2H
ΔF508 Mutation
Protein kinase C
Protein kinase A
−6
0
+6
+12
NBD = Nucleotide Binding Domain
Calculated net charge on the CFTR is indicated by color intensity. The darkest purple is +12 and the darkest gray is −6.
ATP
ATP
1
2
C0
C1
C2
Open
ADP
Pi

Everything Together