Micb 688L Cell Biol. Feb. 3, 2005

Membranes & Transport

1. All organisms need to take up nutrients, translocate and sort them to the right cells and compartments.

2. Organisms remove toxic or waste materials.

3. Transport depends on signals received. Organisms sense changes in environment and respond to the stimuli. E.g. adapt to nutrient starvation or osmotic extremes.

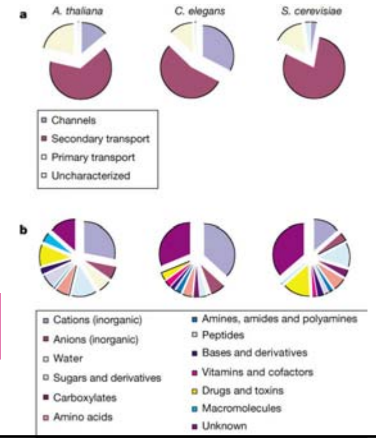
Q? How do cells/organisms coordinate all this?

Transport is integral to growth, movement, signal transduction, development and adaptation.

5-10% of genome encode transporters.

Comparison of the transport capabilities of Arabidopsis, C. elegans and S. cerevisiae. Pie charts show the percentage of transporters in each organism according to bioenergetics (a) and substrate specificity (b).
TAGI 2000. Nature 408, 796

Predicted functions only: Biological role is unknown for most



Arabidopsis genes with similarities to human disease genes

Human Disease	Arabidopsis hit
Darier-White SERCA	Ca-pumping ATPase
Renal tubul acidosis ATP6B1	Vac H+-ATPase, subunit B
Menkes ATP7A	ATP-dep Cu transporter
Bare Lymphocytes ABCB3	ABC transporter

TAGI 2000, Nature 408, 796

Harmful bacteria use transporters to nullify antibiotics

Antibiotic-resistant bacteria present a major problem in public health in the United States and worldwide.

One of the ways that bacteria resist antibiotic drugs is by using membrane transporters.
E.g. ABC transporters

MOCB 639 **All cells generate and maintain electrical & chemical gradients across membranes. How?**

1. Gen. Concepts

1.1 p-lipids bilayer is a remarkable barrier
1.2 Three classes of proteins catalyze transport: pumps, cotransporters, channel
1.3 Transport can be active or passive

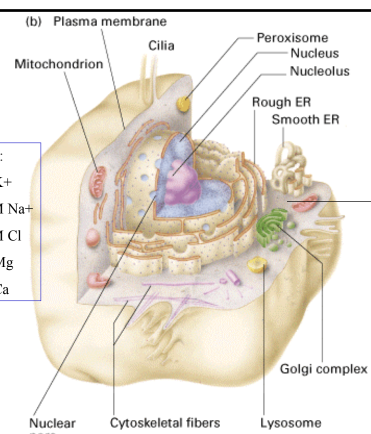
2. Proteins move solutes with high specificity & affinity (like enzymes)

3. Passive and active transport of ions result in electric potential difference across membranes

3-1. Movement of an uncharged mol Is dependent on conc. gradient alone.
3-2. Movement of an ion depends on the electric gradient and the conc. gradient.
3-3. Primary vs 2nd active transport

4. Examples of pumps and cotransporters
R esearch approaches and methods

4-1. Glucose
4-2. ATP driven pumps
4-3. Na+ or H+- coupled cotransporters
4-4. Ion Channels

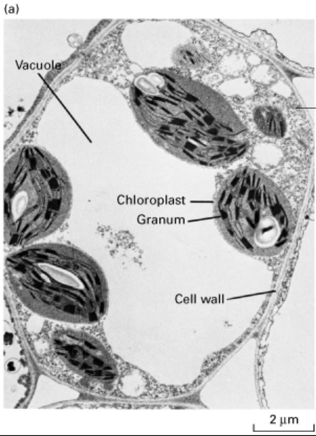


Outside:
4 mM K+
140 mM Na+
116 mM Cl
1 mM Mg
1 mM Ca

Cytosol: pH 7.3
130 mM K+
12 mM Na+
4 mM Cl-
1 mM Mg
< 1µM Ca

-70 mV

Fig. 5-42
Tab 15-1



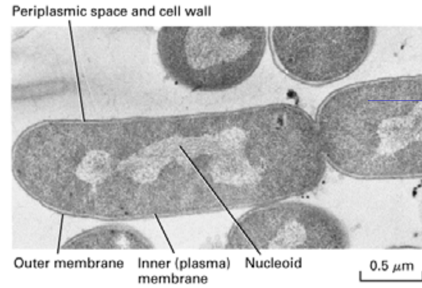
Outside
pH 5.5
1 mM K
1 mM Na
1 mM Cl
0.25 mM Mg
1 mM Ca

Plant Cell
Cytosol: pH 7.3
100 mM K
8 mM Na
10 mM Cl
3 mM Mg
< 1 μM Ca

-140 mV

Fig. 5-43

Fig. 1-7. E coli

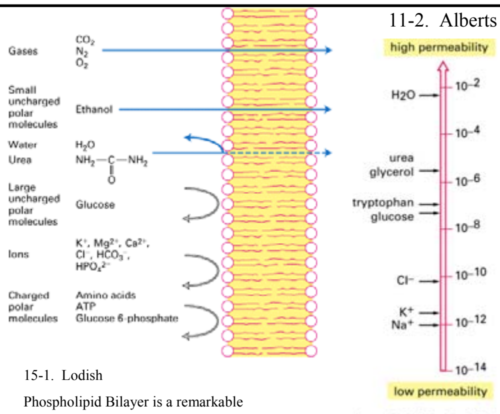


Inside: pH 7.4
100-200 mM K
< 1 μM Ca
-100 mV

Outside: pH ~5.5 (acidic)
trace ions ~ 1 mM K, Ca, Na
other ions < 1 mM

How do cells generate electrical potential and maintain ion homeostasis?

11-2. Alberts



15-1. Lodish

Phospholipid Bilayer is a remarkable barrier to ions and metabolites

Figure 11-2. Molecular Biology of the Cell, 4

Two classes of transport proteins-
two mechanisms of transport

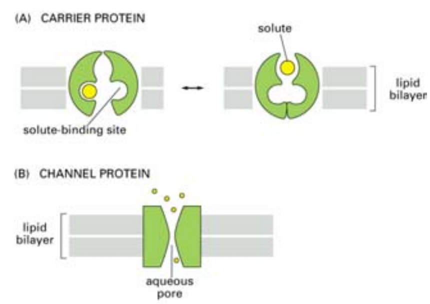


Figure 11-3. Molecular Biology of the Cell, 4th Edition.

. Transport via Carriers can be active or passive.

Transport through Channels are always passive

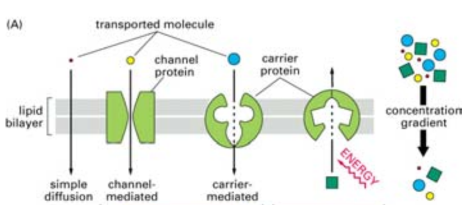


Figure 11-4 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Transport of a charged solute depends on the ***electrochemical gradient***

Passive- down an electrochemical gradient
Active- against an electrochemical gradient

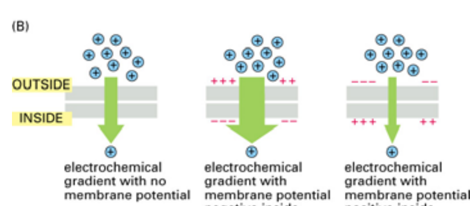
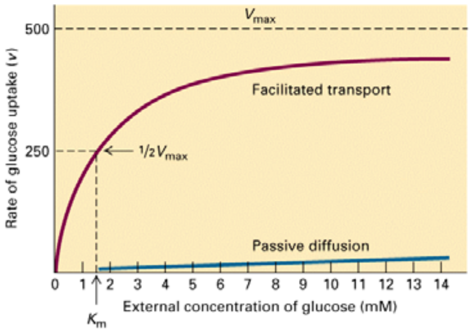


Figure 11-4 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Transport proteins act like enzymes

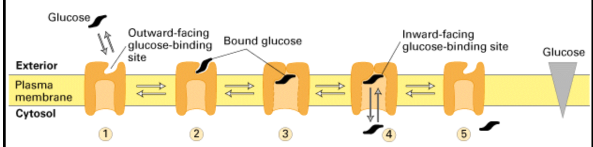
Fig. 15-5



Enzyme rx. E + S --> ES--> EP --> E + P

Transport: E + Si --> ESi --> ESo--> E + So

Fig. 15-7. Mechanism of glucose transport depends on two conformational states of the transporter.



Transport: E_1 + Si --> E_1Si --> E_2So--> E_1 + So

3 ways of Active transport

secondary

Primary

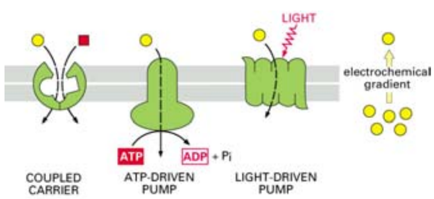


Figure 11-8. Molecular Biology of the Cell, 4th Edition.

Secondary active transport of S uses energy generated by the primary pump

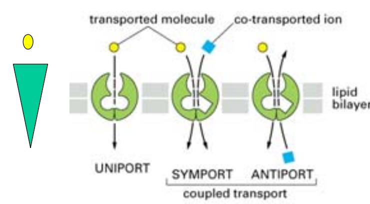
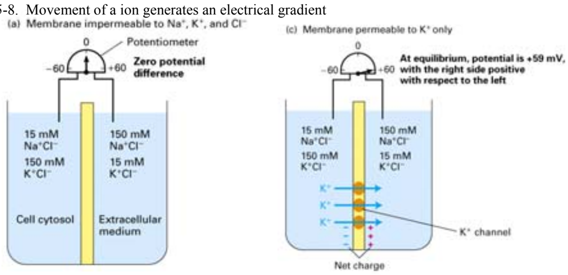


Figure 11-9. Molecular Biology of the Cell, 4th Edition.

How do cells form a membrane potential? 2 ways: diffusion of ion ; active ion pump

15-8. Movement of a ion generates an electrical gradient



At equilibrium: electrical energy is balanced by the energy of the conc gradient

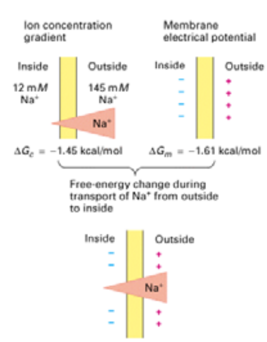
$zF\Delta E_{(2\text{-}1)} = -RT \ln [K^+]_2/[K^+]_1$

$\Delta E = -RT/zF \ln [K= 15]/[K =150] = +0.059$ V   Nernst Equation

Nernst equation predicts the ΔE when an ion is at equilibrium.

Nernst equation predicts the ion conc. at equilibrium when ΔE is known.

15-9. Forces acting on Na in animal cells



Na is pumped out by Na/K-ATPase.

Na will diffuse down conc and electrical gradient into the cell.

The driving force for diffusion of an ion is dependent on the conc.(chemical) gradient and the electrical gradient.

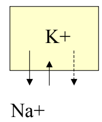
$\Delta G = zF\Delta E + RT \ln [A_2]/[A_1]$

$\Delta E = -70$ mV

Animal:

How are Na and K gradients across the PM generated and maintained? Na out /Kin pump.

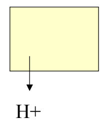
How is the membrane potential formed? K+ efflux from the cell via K+ channels generate the -70 mV.



Plants/ fungi

1. Electric potential of -140 mv is generated and maintained by a H+ extrusion pump.

2. How is K+ gradient maintained? K+ comes into cell down electrical gradient via K+ channels.



Bacteria:

Electric potential of -100 mV is generated by H+ extrusion.

Roles of primary H^+ or Na^+ pumps
Central theme of bioenergetics is ion coupling.

-Generate and maintain electric and chemical gradient.
•provide the driving force for transport of various ions and metabolites.
In plants, pH gradient e.g.ion/ H+-cotransporters
In animal cells, the Na^+ gradient. E.g. ion/Na+ cotransport
•Generate electric and ion changes that serve as signals/ stimuli.

Active transporters move solutes energetically uphill across the membrane or against the electrochemical gradient.

Primary active transport is coupled directly to an energy yielding chemical or photochemical reaction. e.g. ATP hydrolysis. Pumps generate a voltage and chemical gradient.

Secondary active transporters utilize the electrochemical gradient to drive solute transport. e.g. symport , antiport

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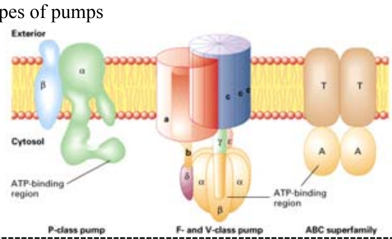
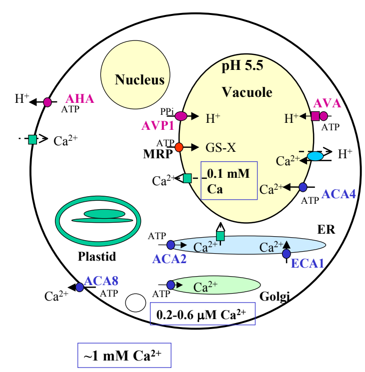
15-10. 4 types of pumps



	P-type	F-type	V-type	ABC
Subunit Mass	1-2 100 kD	8 subunits 450	>12 subunits 800 kD	1 or 4 170 kD
Phosphorylated	E-P (α)	no	none	?
Inhibitors	vanadate	oligomycin	bafilomycin	vanadate
Cell location	PM, endo	mito, chloro	endom, PM	PM, vac
	H^+, K^+, Na^+, Ca^+	H^+	H^+, Na^+	organic, GS-X

Pumps in plants: H+, Ca, and ABC



Methods to study a transporter and determine its cellular roles

1. Assay Transport activity- in whole cell or isolated membrane/organelle (in vitro)
   a. isotope flux,
   b. current (channel)
   c. Flourescence dye (pH, electrical, Ca)
2. Distinguish one type of transporter from another using
   a. Specific inhibitors
   b. Energy source: is it a pump or cotransporter. Ionophores dissipate ion gradient
3. Identify protein and the functional domains
   a. Biochemical- purify and reconstitute in simple system-
   b. Molecular- Clone gene- express cDNA/gene in heterologous system
   Mutate or delete functional domains or residues
4. Determine role of transporter in whole organism (in vivo)
   a. Localize it to membrane, cell-type, and tissue
   Immuno-detection, epitope Tag , GFP-tag

   b. Reverse genetics: What cellular activity is changed in a mutant?
   E.g. signal transduction pathways
5. Application . Test if its overexpression or reduced expression in a organism or cell gives desired property. E.g. tolerance to toxic compounds, bone remodeling.

How do you determine if a transporter
- is active or passive?

-is a carrier or a channel?

15-4.

Biochemical method to study transporters.

-Purify protein:
solubilize Membrane

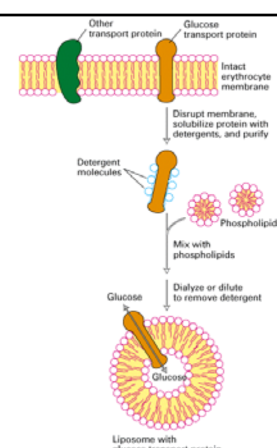
purify by chromatography

-Reconstitute with p-lipids

-Measure transport as uptake of

14C-glucose.

-if passive, [glu]in =[gluc]out



Q? You find a eukaryote gene that encodes a P-type ATPase.

How would you determine if it is for H+, Na+, or Ca?

1. ??

2. Let's say you think it pumps Ca.

Distribution of Ca in cells.

Where do you expect to find Ca pumps in cells?

Clue: [Ca]cytosol = 0.2 µM

outside [Ca] = 1 mM

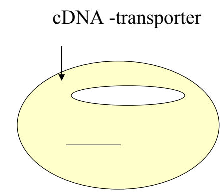
ER lumen or Golgi lumen Ca = 0.1-1 mM

Test function after expression in a heterologous system.

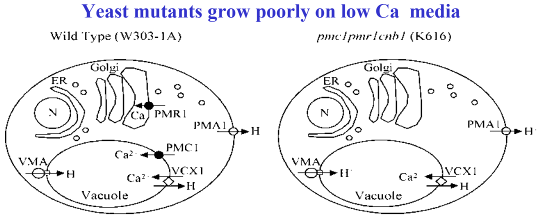
1. Obtain cDNA encoding a putative transporter
2. Express in a heterologous system

e.g. a suitable yeast mutant, COS cell

3. Test for rescue of wild-type phenotype
4. If transporter is localized on PM, measure uptake of radioactive S into yeast.
5. If transporter is localized on endomembrane, isolate membrane vesicle to measure transport.

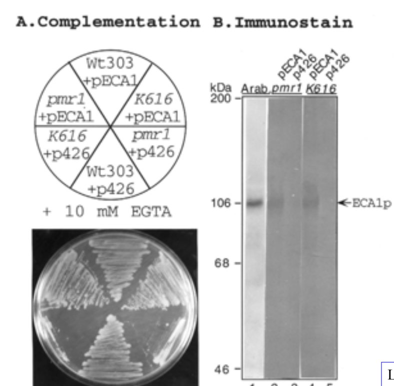


Yeast mutants grow poorly on low Ca media



Strain	Relative Growth on Various Media		
	1 mM Ca	10 mM EGTA	3 mM Mn
Wild type	+++++	+++++	+++++
pmr1 AA542	+++++	+	+
K616	+++++	+	+

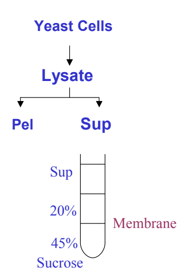
ECA1 restores K616 and pmr1 mutant growth on 1 µM Ca



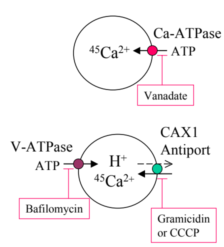
Liang F. et al 1997. PNAS

Test activity directly in isolated membrane

Isolation of Membrane Vesicles



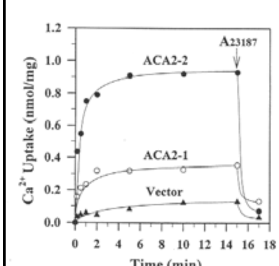
^{45}Ca transport



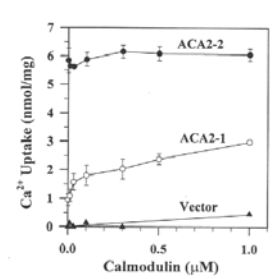
Vesicles are collected on filters, and ^{45}Ca is counted.

Determine functional or regulatory domains of protein

N-terminal truncated ACA2-2 is an active Ca^{2+} pump



Calmodulin stimulated the full-length ACA2-1 pump but not the N-terminal truncated ACA2-2



ACA2-1 ______________________________

ACA2-2 ______________________________

Hwang et al 2000. Plant Physiol

15-12. Model of Ca pump- a P-type ion pump

H^+, K^+, Na^+, Ca^+

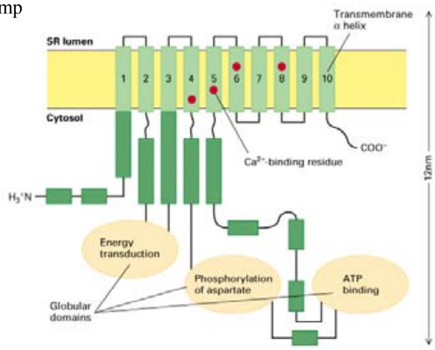
100 kda

1-2 subunits

P = phosphorylated

Recently crystallized, 3D structure available

Toyoshima C. 2000-4 (Nature)



15-11. Mechanism of sarcoplasmic/endoplasmic reticulim Ca ATPase (SERCA)

Carrier me

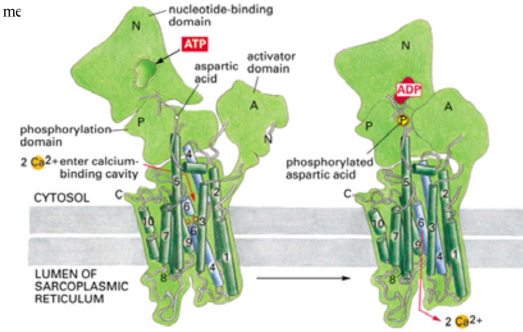


Figure 11–15. Molecular Biology of the Cell, 4th Edition.

E1 and E2 are two alternate conformational states

Crystallized

V-ATPase: Vacuolar H+-pumping ATPase acidifies the vacuole

Many roles

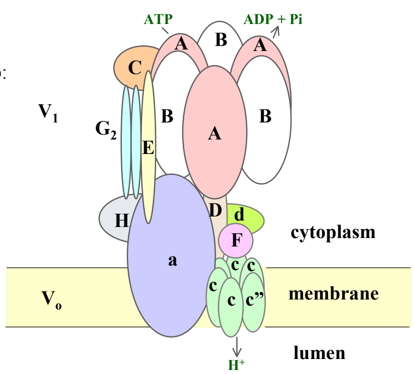
Complex pump:

8 subunits

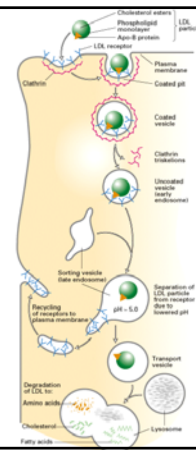
A-H

4 subunits:

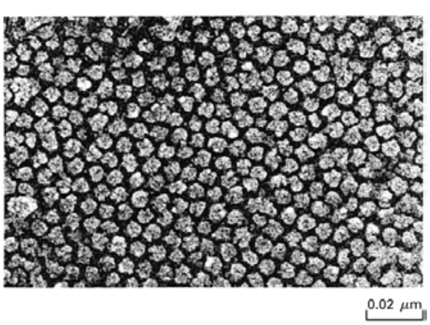
a, c, c", d



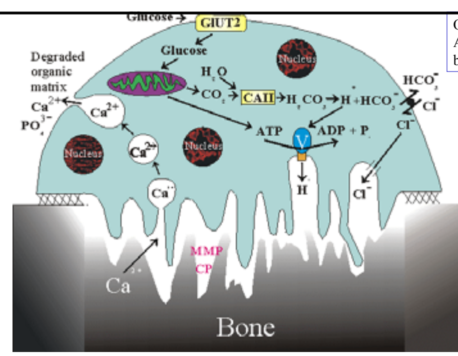
17-46. Role of V-ATPase in recycling receptors and sorting internalized proteins



15-14. Plasma membrane of acid secreting cells (bladder epithelial) is filled with Vacuolar type H+-pumping ATPase complexes



Osteoclast V-ATPase remodel bone



M Manolson web site

The acid pumped onto the bone dissolves the matrix by removing calcium from the structure. We are trying to develop therapeutic reagents that will specifically stop the osteoclast V-ATPases in order to prevent the pathological bone loss associated with inflammatory types of arthritis, such as rheumatoid arthritis, psoriatic arthritis, reactive arthritis, juvenile rheumatoid arthritis and Lupus. We hope first to identify portions of the acid pump that stick out of the cell and thus would be accessible to reagents. Once this external and accessible domains have been identified, we will create reagents that should specifically bind to hopefully inhibit the acid pump and hence reduce the bone resorption in osteoporosis and arthritic joints.

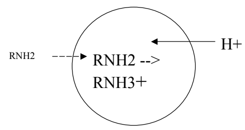
How do you measure H+ pumping?

1. Intact cells: yeast vacuoles accumulate fluorescent weak bases RNH3+.

pH-sensitive GFPs

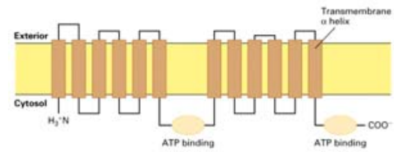
2. Isolated membrane vesicles: accumulate pH-sensitive probes

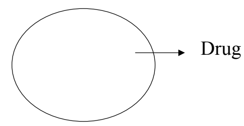
Verify there is a pH gradient by destroying gradients with an ionophore or detergent.



15-16. Mammalian MDR protein- remove toxic cpds from cells

ABC transporters: ATP-binding cassette are found in bacteria, animals and plants



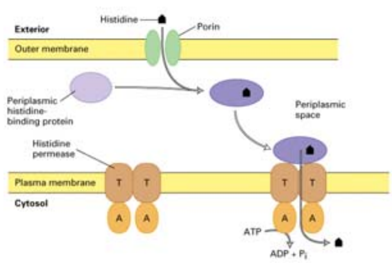


Plant has >100 genes encoding ABC-like

Human has 51

15-15. ABC protein imports amino acids in bacteria

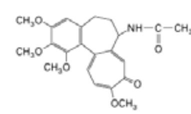
[ATP-binding cassette]



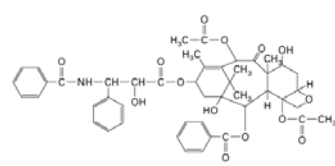
2 TMD (transmembrane domains) , and

2 NBD (nucleotide binding domains)

19-16. Drugs that interfere with normal assembly and disassembly of microtubules are used as anti-cancer agents. They inhibit rapidly dividing cells.



Colchicine



Taxol

15-17. ABC protein as pump