CARRIER-MEDIATED TRANSPORT

Voet & Voet Chapter 20
Channels and Carriers

There are two classes of protein-mediated transport systems:

1) channels
2) carriers
The channels form membrane-spanning pores that allow molecules to diffuse down the electrochemical gradient into or out of the cell.

Some channels are gated. They are opened or closed by binding of a ligand or by altered membrane potential.
The carriers are an altogether different class of transport mechanism. The carriers appear to present either an import or an export site to the transported molecule but not both sites simultaneously.
## Carriers

### Passive

<table>
<thead>
<tr>
<th>Sugar Transporters</th>
<th>Amino acid transporters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleoside transporters</td>
<td>Purine transporter(s)</td>
</tr>
<tr>
<td>Pyrimidine transporter(s)</td>
<td>Nucleotide transporter(s)</td>
</tr>
<tr>
<td>Amine Transporter(s)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Na/Ca exchanger(s)</th>
<th>Na/H exchangers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl/HCO₃ exchanger(s)</td>
<td></td>
</tr>
</tbody>
</table>

### Active

<table>
<thead>
<tr>
<th>Na,K,Cl co-transporter(s)</th>
<th>Phosphate, H co-transporter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotransmitter/cation co-transporter(s)</td>
<td></td>
</tr>
<tr>
<td>Sugar/cation co-transporters</td>
<td></td>
</tr>
<tr>
<td>Amino acid/cation co-transporters</td>
<td></td>
</tr>
</tbody>
</table>

| Cation ATPase(s) (Na/K, Ca, H) | |

| Drug resistance transporter (P-glycoprotein(s)) | |
Passive

Uniport
(simple carrier)

- bidirectional fluxes
- net flux from high [S] to low [S]
- "relaxation" operational

Examples
- sugar transport in most cells
- nucleoside transport
- amino acid transport

Antiport
(exchange only carrier)

- bidirectional fluxes
- net flux from high [S] to low [S]
- "relaxation" absent

- anion exchange in most cells
- Na/Ca exchange
- ADP/ATP exchange in mitochondria
Using radiotracers in transport studies, we assume isotopes are chemically equivalent.

 tracer                      parent molecule

\[ ^{14}\text{C} \quad \text{H} = \quad ^{12}\text{C} \]

\[ t_{1/2} = 5730 \text{ yr} \quad \text{stable} \]

\[
\begin{align*}
^{22}\text{Na} & \quad \equiv \quad ^{23}\text{Na} \\
t_{1/2} = 2.6 \text{ yr} & \quad \text{stable}
\end{align*}
\]

\[
\begin{align*}
^{45}\text{Ca} & \quad \equiv \quad ^{40}\text{Ca} \\
t_{1/2} = 162.7 \text{ days} & \quad \text{stable}
\end{align*}
\]
α-particle emitters
Produced during alpha decay, an alpha particle is a fast moving helium nucleus. Alpha particles carry a charge of +2 and strongly interact with matter. They travel only a few inches through air and can easily be stopped with a sheet of paper.

Examples of alpha emitters are radon-222, plutonium 236 and uranium 238.

β-particle emitters
Beta particles are sub-atomic particles ejected from the nucleus of some radioactive atoms. They are equivalent to electrons.

Examples of beta emitters are phosphorous-31, tritium (H-3), carbon-14, phosphorous-32, calcium-45, strontium-90 and lead-210.

γ-ray emitters
Often, gamma ray emission accompanies the emission of a beta particle. When the beta particle ejection doesn't rid the nucleus of the extra energy, the nucleus releases the remaining excess energy in the form of a gamma photon. Gamma rays are highly penetrating.

Examples of gamma emitters are Na-22, Cr-51 and iodine-125,
Pathway of energy transmission in scintillation counting

After emission of a beta particle, the solvent is the first molecule to be excited (being present in large excess). This excitation occurs within 10-100 ns. Subsequently, the energy is transferred to the primary scintillant such as PPO (2,5-diphenyloxazole, see below). The primary scintillant emits the absorbed energy as light with a characteristic wavelength.

Sometimes, secondary scintillants (such as POPOP, see below) are also added to the scintillation cocktail. These molecules emit light at a different wavelength than the primary scintillant.
Radioisotopes are much easier to detect and quantitate than specific molecules which may require chromatography for separation and quantitation.

Radioisotopes and parent compound compete for interaction with a common substrate binding site. 

e.g. \([^{14}C]-D\text{-glucose}\) and \([^{12}C]-D\text{-glucose}\) compete for binding to the glucose transporter GluT1.

Uptake of extracellular \([^{14}C]-D\text{-glucose}\) by cells is competitively inhibited by increasing levels of extracellular \([^{12}C]-D\text{-glucose}\).
dpm of $[^{14}\text{C}]-\text{D-glucose}$ can be expressed as mol glucose

\[ 10 \, \mu\text{L} \, 100 \, \mu\text{M} \, \text{D-glucose} = 20,000 \, \text{dpm} \]
\[ 1 \times 10^{-6} \times 100 \times 10^{-6} \, \text{mol D-glucose} = 20,000 \, \text{dpm} \]
\[ 1 \, \text{dpm} = 100 \times 10^{-12}/20,000 \, \text{mol glucose} = 5 \times 10^{-15} \, \text{mol} \]

Thus if $10^6$ cells take up 5000 dpm $[^{14}\text{C}]-\text{D-glucose}$ in 1 min

the rate of D-glucose uptake is

\[
\frac{5000 \times 5 \times 10^{-15}}{60 \times 10^6} \, \text{mol.cell}^{-1}\cdot\text{s}^{-1}
\]

\[
= 4.2 \times 10^{-19} \, \text{mol.cell}^{-1}\cdot\text{s}^{-1}
\]
**What are the properties of carrier-mediated transport systems?**

With a channel, the presence of substrate on one side of the membrane inhibits unidirectional flux from the other side of the membrane. Thus extracellular Rb or K inhibits unidirectional Rb\textsuperscript{86} efflux through voltage-gated K channels in squid giant axons.

For carrier systems, the presence of transported substrate at the interior of the cell can be without effect on, inhibit or even stimulate substrate influx! Let’s examine this with a classic carrier-mediated transport mechanism – the sugar transporter.

See Figures 2, 3 and 4.
COLLECT & COUNT intracellular perfusate

COLLECT & COUNT extracellular perfusate

Extracellular flow

giant axon

transport

diffusion

Intracellular flow
Fig 2

![Graph showing concentration of 3-OMG (mm) over time. The top graph displays a stepwise increase in concentration from 0 to 100 mm, with a horizontal line at 5 mm-3 OMG. The bottom graph shows the influx of 3-OMG (pmol cm⁻² s⁻¹) over time from 0 to 3 hours.]
Fig 3

A

Fraction of 3 OMG lost per min

Time (h)

B

Inhibition of 3 OMG efflux (%)

[Sugar]₀ (mM)
isotonic saline

hypotonic saline (4 °C)

isotonic saline (4 °C)

substrate

substrate

substrate

isotonic saline 37 ° C

transport substrate and artificial cytosol
Figure 4

Exchange 3OMG exit

3OMG exit $\mu$mol/L/min

[S]₀ mM

0 10 20 30 40 50 60 70 80 90 100 110

Curves 1 and 2 with error bars
Cytochalasin B is an inhibitor of these sugar transport systems shown in Figs 2 - 4. A number of studies suggest that cytochalasin B acts at a cytosolic surface of the sugar transporter. These data include:

1) $K_{i(app)}$ for cytochalasin B inhibition of sugar efflux increases with increasing cytosolic sugar levels.

2) $K_{m(app)}$ for sugar efflux increases with increasing [cytochalasin B].

These data suggest that cytochalasin B and intracellular sugar compete for binding to a common site.

3) Cytochalasin B binding to erythrocyte glucose transporter is inhibited by intracellular D–glucose but not by extracellular D–glucose (Fig 5).

4) Cytochalasin B binding to erythrocyte glucose transporter is inhibited by extracellular maltose and by extracellular ethylidene glucose (Fig 5 and 6). Maltose and ethylidene glucose are cell impermeant inhibitors of erythrocyte sugar transport that compete with D–glucose for binding to the sugar import site.
Figure 5

Time course of cytochalasin B binding to red cells and red cell ghosts and the effects of extracellular and intracellular sugars on binding. Ordinate, pmols of cytochalasin B bound to red cells (O, ●, □, ■) and ghosts (△, ▲). Abscissa, time in minutes. Binding to red cells was measured in the absence of D-glucose (O), the presence of 100 mM intracellular D-glucose (■), the presence of 100 mM extracellular D-glucose (●), and the presence of 100 mM D-glucose in both the intra- and extracellular media (□). Binding in ghosts was measured in the absence (△) and presence (▲) of 100 mM D-glucose. Temperature, 4 °C. Centrifugation for 1 min. Cytochalasin D (10 μM) was present throughout. Each point represents the mean of duplicate assays. Free [³H]cytochalasin B, 100 nM. Number of ghosts/ghost assay, 8.1 x 10⁷. Number of red cells/red cell assay, 5.4 x 10⁷. Red cell data have not been corrected for cytochalasin B binding to hemoglobin.
Time course of sugar-induced inhibition of cytochalasin B binding to intact red cells. Ordinate, free/bound [cytochalasin B] ($\omega$). Abscissa, time in hours. Binding was measured at 5 and 10 min (at 24 °C) in the absence of added sugar (▽) and then, at 10 min (see arrow), D-glucose (○), maltose (△), or ethyldene glucose (□) was added from a stock of 1 M sugar to give an initial extracellular concentration of 100 mM. Data are also shown for the effects of addition of 100 mM D-glucose on binding at 4 °C (●). Each point represents the mean ± S.D. of two measurements made in duplicate. Centrifugations were for 1 min. Data have been corrected for nonspecific binding to hemoglobin. Number of cells/assay, 3.9 × 10^8; free [cytochalasin B], approximately 40 nM.
A cell-impermeant photoreactive bis-mannose derivative competitively inhibits galactose uptake by erythrocytes (Fig 7) but its labeling of erythrocyte glucose transporter is abolished by cytochalasin B (Fig 8).

**Figure 7**

**Inhibition of d-galactose uptake by ATB-BMPA**

The erythrocyte uptake of 100 μM-d-galactose was measured at a range of ATB-BMPA concentrations. The reciprocal of the uptake rate constant ($s/v$) was plotted against the ATB-BMPA concentration. The $K_i$ was determined by non-linear regression fitting to the Michaelis-Menten equation. The $K_i$ was $297 \pm 53 \mu M$ (from two experiments with triplicate observations at each inhibitor concentration); results are means and the bars represent the S.E.M. of the six observations at each concentration.
These data suggest that the cytochalasin B (export) and bis-mannose (import) sites are mutually exclusive.

Widdas guessed this in 1952 when he proposed the simple–carrier hypothesis for erythrocyte sugar transport. He surmised that because intracellular sugar fails to inhibit sugar uptake and extracellular sugar fails to inhibit sugar exit, exit and import pathways must either exist independently of each other or exist alternately. He chose the simplest hypothesis – exit and import sites exist alternately on the transporter.

**Figure 8**

*Photolabelling of the glucose transporter*

(a) ATB-BMPA photolabelling of the glucose transporter in intact erythrocyte cells. The labelling by ATB-[2-3H]BMPA was compared in the absence of D-glucose (●) and at 1 mm (○), 5 mm (△), 25 mm (■) and 100 mm (▲) D-glucose. Binding and labelling was completely displaced by 50 μM cytochalasin B (□). Membranes were prepared and the labelled proteins were analysed by electrophoresis. Only the glucose-transporter region of the electrophoresis gel is shown for clarity. There were no other labelled peaks in other regions of the gel.
One simple mechanism that can account for this result is called the simple carrier which is shown in schematic and in King-Altman forms below.

The key to this mechanism is that in the absence of substrate, the carrier, e, can exist in one of two forms - e₁ or e₂ - that present a substrate binding site to either side 1 or to side 2 of the membrane (where the subscripts 1 and 2 refer to intracellular and extracellular sides respectively).

Cytochalasin B (CCB) binds only to e₁ and bismannose (M) binds only to e₂.
How can this model account for transacceleration of fluxes?

Consider an influx cycle in the absence of intracellular substrate (S1). The cycle is the following:

A subunit transport cycle

\[ e_2 \rightarrow e.S2 \rightarrow_{\text{FAST}} e.S1 \rightarrow e_1 \rightarrow_{\text{slow}} e_2 \]
If the reverse translocation occurs more rapidly than does forward relaxation (e.g.)

then uptake of extracellular sugar (S2) will be stimulated by the presence of intracellular sugar (S1).
If translocation and relaxation reactions occur at identical rates, the presence of S1 will have no effect on S2 uptake.

No trans-acceleration
If translocation is slower than relaxation, the presence of S1 will inhibit S2 uptake.
Examples of passive transport systems that are consistent with this model include:

Sugar transport, nucleoside transport and amino acid transport.
A variation on the simple carrier mechanism ANTIPORT

While the simple carrier model can account for the properties of a large number of passive transport systems, a number of passive transporters cannot transport substrate in the absence of substrate at the opposite (trans) side of the membrane. With a slight modification, the simple carrier model can account for these transport systems.

Consider the following model:
The difference between this model and the simple carrier is that unoccupied carrier isoforms e1 and e2 cannot isomerize in the absence of substrate. This is known as the *exchange-only simple carrier*. In order for multiple rounds of substrate influx to occur, e1 must be converted back to e2 by transporting S1 out of the cell.

Since transport is passive, the following must be true:

\[ k_3 \cdot k_{-3} \cdot k_4 = k_4 \cdot k_1 \cdot k_{-4}. \]

You might suspect that this transport system would lead to futile cycling of substrate. However, what normally occurs is that one substrate is transported into the cell in exchange for a different substrate being transport out.
An example is Na:Ca exchange. Here 1 Ca\(^{2+}\) is extruded from the cell in exchange for 3 Na\(^+\). The inwardly directed Na electrochemical gradient is used to do useful work - pump Ca out of the cell!

What is the equilibrium distribution of Na and Ca produced by such a carrier?

If the membrane potential were 0, then at equilibrium, passive transport requires

\[
Na_i^3Ca_o = Na_o^3Ca_i \quad \text{or} \quad \frac{Na_i^3}{Na_o^3} = \frac{Ca_i}{Ca_o}
\]

In other words, Ca\(^{2+}\) is accumulated at one side of the membrane to an extent proportional to Na\(^+\) accumulation at the same side. If Na\(_o\)/Na\(_i\) = 10 then Ca\(_o\)/Ca\(_i\) = 10\(^3\) = 1000.

The generic solution (where \(m\) molecules of S are exchanged for \(n\) molecules of P) is:

\[
S_i^mP_o^n = S_o^mP_i^n \quad \text{or} \quad \frac{S_i^m}{S_o^m} = \frac{P_i^n}{P_o^n}
\]
Let us examine this Ca:Na exchanger in a little more detail. It seems obvious that if 3 Na\(^+\) are exchanged for 1 Ca\(^{2+}\), the transport system must carry one net positive charge during the transport cycle. This must mean that the rate of transport is affected by membrane potential (\(\Delta \Psi\)). How can we analyze such a system?

Let us make the following assumptions:

1. The net charge on the Ca-occupied carrier, \(Z_{E\text{Ca}} = 0\)
2. The net charge on the Na-occupied carrier, \(Z_{E\text{Na}} = +1\).

When \(\Delta \Psi = 0\) mV, the net charge carried by E3Na\(_i\) or E3Na\(_o\) will have no effect on the rates of carrier isomerizations (k2 and k-2).
When $\Delta \Psi = -60$ mV (inside), $k-2$ will be decreased to some extent (the positive charge will be attracted by the negative potential inside the cell) and $k2$ will be increased to the same extent (the positive charge will be attracted by the negative potential inside the cell). Because $ZECa= 0$, $k1$ and $k-1$ are unaffected.

When $\Delta \Psi = +60$ mV (inside), $k-2$ will be increased to some extent and $k2$ will be decreased to the same extent. Because $ZECa= 0$, $k1$ and $k-1$ are unaffected.

Thus we would predict:

1. When $\Delta \Psi$ is negative (inside), $Na_o:Ca_i$ exchange (external Na dependent Ca efflux) is stimulated. Thus hyperpolarization stimulates $Na_o:Ca_i$ exchange.

2. When $\Delta \Psi$ is negative (inside), $Na_i:Ca_o$ exchange (external Ca dependent Na efflux) is inhibited. Thus hyperpolarization inhibits $Na_i:Ca_o$ exchange.

3. When $\Delta \Psi$ is positive (inside), $Na_o:Ca_i$ exchange (external Na dependent Ca efflux) is inhibited. Thus depolarization inhibits $Na_o:Ca_i$ exchange.

4. When $\Delta \Psi$ is positive (inside), $Na_i:Ca_o$ exchange (external Ca dependent Na efflux) is stimulated. Thus depolarization stimulates $Na_i:Ca_o$ exchange.

These predictions are observed experimentally (See Figures 9 and 10).
Fig. 14. Investigation of the sensitivity to membrane potential of the external Ca-dependent Na efflux into choline sea water. 0 Ca-choline (open circles), 20 mM-Ca-choline (filled circles). Axon prepared by the preinjection of (final concentration in mM): MOPS, 32; HEPES, 32; TEA, 64; pH 7.3. External solutions also contained $10^{-4}$ M-ouabain and 0.6 $\mu$m-TTX. Axon diameter, 850 $\mu$m; temperature, 18°C.
Fig. 12. Sensitivity of the Na-dependent Ca efflux at external pH 9 to changes in membrane potential. Ca-free sea water. Axon had been preinjected with (final concentration in mM), MOPS, 49; TEA, 49; pH 7.3. External solutions also contained 5 mM-TAPS, $10^{-5}$ m-ouabain and 0.6 μM-TTX. Axon diameter, 686 μm; temperature, 17 °C.
As a final note, we can adjust our equations describing the equilibrium distributions of Na and Ca across the cell membrane to take into account the effects of membrane potential.

\[
\frac{Ca_i}{Ca_o} = \frac{Na_i^3}{Na_o^3} e^{(ZENa - ZECa) u}
\]

where \[
u = \frac{F}{RT}
\]

and F is the Faraday, R the gas constant and T the absolute temperature.

\[\Delta\Psi\] refers to the membrane potential, positive at the outside.

At room temperature, \(u\) is equal to 2.303/58 mV.
Examples of exchange only systems:

a. ATP/ADP exchanger of mitochondria
b. Anion exchanger of red cells
c. Na/H exchanger of most cells
d. Na/Ca exchanger of neurons.
**Active**

**Symport** (co-transport)
Net uphill transport of solute driven by net downhill flow of cation.

**Uniport** (ATPase)
relaxation operational

**Antiport** (ATPase)
"relaxation" absent or very slow

**Examples**
sugar and amino acid transport in absorptive epithelia
Neurotransmitter transport in synaptic clefts

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ATP

ADP

H-ATPase
Ca-ATPase
Na,K-ATPase
Co-transport Systems: Two substrates are carried on a single transporter

These transport systems are exemplified by active sugar transport by epithelial cells. Sugar transport in these cells is complicated by the additional presence of a facilitated diffusion mechanism for sugars e.g. In mammalian cells, active transport of 3-O-methylglucose and α–methyl glucoside can occur and this is inhibited by phloridzin. Passive transport is selective, only 3-O-methylglucose is transported and this is inhibited by cytochalasin B. The passive transporter cannot transport α–methyl glucoside and is relatively insensitive to phloridzin, while the active transporter is relatively insensitive to cytochalasin B.
Types of transmembrane sugar transport

basal or apical, mucosal or
Serosal surface Absorptive surface

Passive carrier protein

Cytosol

plasma membrane

Glucose

Cation

Active carrier protein

Non-Stokesian transbilayer diffusion
A typical uptake experiment with cultured epithelial cells would show the following:

In the absence of cytochalasin B, 3-O-methylglucose is transported out of the cells by the passive transporter and is thus not accumulated to the same extent as α-methyl glucoside.

Phloridzin blocks accumulation of both 3-O-methylglucose and α-methyl glucoside.

Active transport of sugar (S2) is critically dependent upon the presence of Na ions at side 2 of the membrane.
Uptake of S2 is more precisely dependent upon the transmembrane Na electrochemical gradient. Accumulation of S is driven by the downhill flow of Na.

What is the free energy available in the transmembrane Na gradient? This is computed in the usual way:

\[
\Delta G = RT \log_e \frac{Na_{in}}{Na_{out}} + ZF\Delta \Psi
\]

where \( Z \) is +1; \( F \) (the Faraday) = 23,062 cal V\(^{-1}\)mol\(^{-1}\); \( Na_0/Na_i \approx 10; \Delta \Psi = -70 \text{ mV}. \)
\( \Delta G \) is approximately \(-2.98 \text{ kcal per mol at } 25 \text{ °C}. \) This is equivalent to an equilibrium constant of:

\[
K_{eq} = 10^{-\Delta G^*/(2.303RT)} = 153
\]

Thus the inwardly directed Na electrochemical gradient would permit D-glucose levels inside the cell 153-fold greater than those present outside.
Effective design minimizes slippage

What will make a real system deviate from the ideal relation $S_1/S_2 = A_2/A_1$? - two types of non ideal behavior: slippage and leakage. Slippage is refers to movements on the transporter of either substrate, cosubstrate or both along a path that is not coupled to the movement of the complementary substrate. e.g. movements of S during a conformational change of the ES form of the carrier will represent slippage. A co-transporter in which all possible slippage paths are present is shown below.
How far away from ideal behavior this system functions depends upon the extent to which slippage dissipates the (accumulating) gradient of the driving cosubstrate (A) and the (accumulated) gradient of driven substrate (S).

In order to minimize slippage, the protein must evolve in such a way as to minimize isomerizations of the binary complexes ES and EA. How this is achieved remains a mystery.
Effective design minimizes leakage

We saw above (Fig 1) how leakage can affect the gradient of the driven substrate. In this figure-methyl glucoside achieves a 10-fold higher accumulation than does 3-O-methylglucose. Blocking 3-O-methylglucose leakage via the passive transport system using cytochalasin B results in a 10-fold increase in 3-O-methylglucose accumulation!

Leakage would be minimized if we could reduce all parallel pathways in the cell. However, this would defeat the function of the co-transport system which is to deliver substrate from one surface of the cell to the opposite, trans-surface.

Leakage will be minimized, however, when the co-transport system evolves so as to operate at the maximal achievable rate. Here, the system has an opportunity to approach the concentrations reached by the strictly coupled system.

How can we achieve this state?
The order of substrate and co-substrate binding is critical

Kinetic analyses of the effects of the order of substrate and cosubstrate binding to the carrier have indicated that maximal pumping efficiency is achieved when:

a. S binds first at the cis (whence) side
b. Na binds first at the trans (whither) side.

Why should this be so?

Consider a case of high substrate concentrations (S × Na is high) at both sides of the membrane (S₁ > S₂, Na₂ > Na₁). Here what limits further rounds of pumping is the rate of return of unloaded carrier at the whither side to the whence side.

If the driven substrate (S) binds first at the whither side, it will lock up carrier in the ES configuration, reducing the amount of carrier available for return to the whence side and, therefore, the rate of pumping up the gradient.

If the driven substrate can only bind second at the side at which it is at high concentration, it cannot trap carrier at this surface.
Next consider a situation where the product (S \times Na) is low overall, although there is a concentration gradient of driving and, therefore, driven substrate. Here, what limits pumping is the rate of forward movement of the already loaded carrier.

At the whence side of the membrane, the concentration of driving substrate is high. If it binds second, it can pull the equilibrium between E, S and itself (Na) over into the ENaS form and thus achieve the maximal velocity of cotransport. If it binds first, no matter how much is present, the maximal velocity is never achieved.

This would be further assisted if the affinity of carrier for the driven substrate is high at the whence side and low at the whither side (& vice versa).
Examples of cotransport systems

a. Sugar/Na in mammalian epithelial cells.
b. Amino acid/Na in mammalian cells.
c. Neurotransmitter precursor/H symport into synaptic vesicles
d. Neurotransmitter/Na symport brain
e. Sugar/H⁺ and amino acid/H⁺ in bacteria.
A PRIMARY ACTIVE CARRIER MECHANISM

ATP Hydrolysis Drives the Transport of Sodium and Potassium Ions Across the Plasma Membrane

Most cells have a high concentration of K and a low concentration of Na relative to the external medium. These transmembrane ionic gradients are generated by a specific transport system that is called the Na,K pump because the movement of these ions is linked.

The active transport of Na and K is of great physiologic importance. Indeed, more than a third of the ATP consumed by a resting animal is used to pump these ions. The Na K gradient in animal cells controls cell volume, renders nerve and muscle cells electrically excitable and drives the active transport of sugars an amino acids.
In 1957 Jens Skou discovered an enzyme that hydrolyzes ATP only if Na and K are present in addition to Mg, which is required by all ATPases. This enzyme was named the Na\(^+\)K\(^+\)ATPase.
Thus the reaction scheme was viewed as

\[ \text{ATP + H}_2\text{O} \rightarrow \text{ADP + P}_i + \text{H}^+ \]

\[ \text{Na}^+, \text{K}^+, \text{Mg}^{2+} \]

It was proposed that the Na\(^+\)K\(^+\)ATPase is an integral part of the Na K pump and that the splitting of ATP provides the energy needed for the active transport of these cations. This is important because in order to move Na against an electrochemical gradient, the cell requires an input of energy. Similarly, for the transporter to move K from the outside of the cell to the inside of the cell against an electrochemical gradient, an input of energy is needed.

This hypothesis has been supported by the finding that the level of ATPase’s activity is correlated with the level of pump activity. Also, variations in Na and K levels have parallel effects on the ATPase activity in transport. Finally both the Na\(^+\)K\(^+\)ATPase and the NaK pump activities are specifically inhibited by cardiotonic steroids.
The isolation of the Na\textsuperscript{+}K\textsuperscript{+}ATPase and its reconstitution into artificial lipid bilayers confirms that the ATPase and the pumping activity are mediated by the same molecular complex, the NaK pump. The results of these various studies have confirmed the identity of the Na\textsuperscript{+}K\textsuperscript{+}ATPase and the NaK pump and have revealed new insight into the class of transport mechanisms called carriers.

The NaK pump consists of two types of subunits: \( \alpha \) and \( \beta \) subunits that are associated in the membrane as an \( \alpha_2\beta_2 \) tetramer. Hydrophobicity analyses and membrane localization studies suggest that the \( \alpha \) chain contains at least eight transmembrane helices.

Much of the \( \alpha \) chain plus its ATPase activity is located on the cytosolic side of the membrane. The small portion of the \( \alpha \) chain on the extracellular side contains the binding site for cardiotonic steroid inhibitors. The \( \beta \) chain which contains a single membrane helix does not appear to be essential for ATPase or transport function.
What is the catalytic mechanism of Na and K pumping? An important clue is the discovery that the ATPase is phosphorylated by ATP in the presence of Na and Mg. The site of phosphorylation is the side chain of a specific aspartate residue. This $\beta$-aspartylphosphoryl intermediate or EP is then hydrolyzed if K is present. Phosphorylation does not require K whereas dephosphorylation does not require Na or Mg.

The reaction scheme can thus now be viewed as:
Na-dependent phosphorylation and K-dependent dephosphorylation are not the only critical reactions. Because the pump serves to extrude Na and to import K, it is reasonable to conclude that Na binds at the cytosolic surface of the transporter and dissociates at the exoplasmic surface of the ATPase while K binds at the exofacial surface and is released at the endofacial surface of the transporter.

Kinetic evidence also suggests that the pump interconverts between two states: e1 and e2. Na binds to the e1 or intracellular state while potassium binds to the e2 or extracellular state of the carrier. The catalytic cycle may now be represented by:

![Enzymatic cycle of the Na⁺-K⁺ ATPase.](image)
The binding cavity alternately faces the inside and outside of the cell in each transport cycle

Insufficient data exist to form a detailed structural model for NaK pumping. However, the following model was proposed by Jardetzky and by Glynn.

1) The pump must contain a cavity large enough to permit entry of a small molecule.

2) The pump must be able to assume 2 conformations such that the cavity is open to the inside in one form and to the outside in the other form.

3) The affinity for the transported species must be different in the 2 conformations.
Schematic diagram of a proposed mechanism for the Na\(^+\)-K\(^+\) pump. The upper sequence of reactions depicts the extrusion of three Na\(^+\) ions, whereas the lower reactions show the entry of two K\(^+\) ions. The E\(_1\) (light) and E\(_2\) (dark) forms are shown here as having very different conformations. The actual conformational differences may be quite small.
In skeletal muscle, calcium ions are transported (pumped) against a concentration gradient from the cytoplasm into the sarcoplasmic reticulum, an intracellular organelle. This causes muscle cells to relax after cytosolic calcium increases during excitation. The Ca\textsuperscript{2+} ATPase that carries out this pumping is a representative P-type ion-transporting ATPase.

P-type ion transporting ATPases, (e.g. Na\textsuperscript{+}K\textsuperscript{+}-ATPase and gastric H\textsuperscript{+}K\textsuperscript{+}-ATPase), are fundamental in establishing ion gradients by pumping ions across biological membranes. Ca\textsuperscript{2+}-ATPase (SERCA1a) from skeletal muscle sarcoplasmic reticulum (SR) is structurally and functionally one of the best-studied members. SR Ca\textsuperscript{2+}-ATPase pumps Ca\textsuperscript{2+} from the cytoplasm into the reticulum, thereby causing the relaxation of muscle cells. Two Ca\textsuperscript{2+} ions can be transported per ATP hydrolysed and two or three H\textsuperscript{+} ions are counter-transported. Active transport of Ca\textsuperscript{2+}-ATPase is achieved, according to the E1-E2 model by changing the affinity of Ca\textsuperscript{2+}-binding sites from high (E1) to low (E2). The release of Ca\textsuperscript{2+} ‘occluded’ in the transmembrane binding sites takes place during the transition from E1P to E2P ('P' indicating that the enzyme is phosphorylated). Autophosphorylation of an aspartyl residue in the reaction cycle is a characteristic feature of the P-type ATPases. However, the residues constituting the phosphorylation site are shared by the members in the haloacid dehalogenase superfamily and by many bacterial response regulators, despite the differences in the folding patterns.
The structure of SR Ca\(^{2+}\)-ATPase with two bound Ca\(^{2+}\) in the transmembrane (M) region, which consists of ten a-helices (Protein Data Bank, PDB, code 1EUL) has been determined. The cytoplasmic part of Ca\(^{2+}\)-ATPase consists of three domains (A, actuator or anchor; N, nucleotide; and P, phosphorylation), well separated in this Ca\(^{2+}\)-bound (E1Ca\(^{2+}\)) form. The phosphorylation residue, Asp 351, is located on the P domain, and the adenosine moiety of ATP binds to the N domain. Modelling the structures (PDB codes 1FQU and 1KJU) based on low-resolution maps of the tubular crystals, in which the enzyme is in a state similar to E2P showed large movements of the three cytoplasmic domains to form a compact headpiece. These rearrangements of the cytoplasmic domains must be associated with the changes in the transmembrane binding sites, but the molecular mechanism was far beyond what might be imagined.

Here the authors describe the crystal structure of Ca\(^{2+}\)-ATPase in the absence of Ca\(^{2+}\) and in the presence of thapsigargin, a potent inhibitor that fixes the enzyme in a form analogous to E2, abbreviated as E2(TG). The structure determined to 3.1 A resolution, is very different from that of E1Ca\(^{2+}\), yet can be compared directly, because no ATP or phosphorylation is involved in the transition between them. The movements of cytoplasmic domains are even larger than we described for the tubular crystals. Transmembrane helices undergo drastic rearrangements that involve shifts normal to the membrane. These movements have clear mechanistic implication in the release and binding of Ca\(^{2+}\). Knowing the second structure in the reaction cycle, we can now begin to understand how ion pumps work.
Figure 1 Ribbon representation of SR Ca\(^{2+}\) - ATPase in the Ca\(^{2+}\) -bound form (E1Ca\(^{2+}\)) and that (E2(TG)) in the absence of Ca\(^{2+}\) but in the presence of thapsigargin (TG). Inset, a simplified reaction scheme (showing only the forward direction). Colours change gradually from the amino terminus (blue) to the carboxy terminus (red). Two purple spheres (circled) in E1Ca\(^{2+}\) represent bound Ca\(^{2+}\). Red circles in E2(TG) indicate extra hydrogen bonds in E2(TG). Large arrows in E1Ca\(^{2+}\) indicate the direction of movement of the cytoplasmic domains during the change from E1Ca\(^{2+}\) to E2(TG). PrtK, proteinase-K digestion site (around Glu 243; ref. 27); T2, trypsin digestion site at Arg 198 (ref. 41); ATP, binding pocket for the adenine moiety of ATP. Principal residues are marked: E183 (A domain), F256 (thapsigargin-binding site), D385 (P domain, phosphorylation site), K400 (N domain, phosphoholamban-binding site\(^{47}\)) and R751 (linking M5 and the loop (L67) connecting M6 and M7). Prepared with Molscript\(^{42}\).
Rearrangement of transmembrane helices viewed from the rear (a), and a diagram illustrating the shift of M4 normal to the membrane by the tilting of M5 (b). The models for E1Ca\(^{2+}\) (violet) and E2(TG) (light green) are superimposed. The M5 helix lies along the plane of the paper. M8 and M9 are removed in a. Double circles show pivot positions for M2 and M5. Arrows indicate the directions of movements during the change from E1Ca\(^{2+}\) to E2(TG). In b, M4 and M5 are linked in the P domain (ovals) and move as a rigid body. Tilting of M5 around the pivoting point (Gly 770) generates a vertical shift of \(d_1\) for M4 without a large horizontal shift at the level of the pivoting point. For \(L_1 = 10\) Å and \(\phi = 30^\circ\), \(d_1\) becomes 5 Å.
Pump Stoichiometry and thermodynamics

Measurements of electrogenic Na and K movements (net current movement is generated during transport) support the view that 3 Na and 2 K are transported with each full cycle of the pump. In the red cell, the free energy change for Na transport from 15 mM inside the cell to 140 mM outside the cell at a membrane potential (V) of –30 mV inside is given by:

\[ \Delta G = RT \ln \frac{140}{15} + zFV \]

{where \( R \) is the gas constant (1.987 cal/deg/mol), \( T \) is absolute temperature (25°C = 298°C), \( F \) is the Faraday (23060 cal/volt/mol), \( z \) is the valence of Na (+1), \( V = 0.03 \) (Na moved to the outside which is +ve relative to inside)}

For Na, \( \Delta G = 2.0 \) kcal/mol (at 25 °C) x 3 (3 mol Na are transported per cycle)

\[ \Delta G = 6 \text{ kcal per cycle.} \]

Similarly for K (120 mM inside, 5 mM outside)

\[ \Delta G = 2.57 \text{ kcal/mol} \times 2 = 5.14 \text{ kcal per cycle.} \]

Total \( \Delta G = 5.14 + 6 \) kcal per cycle = 11.14 kcal
Cost of Transport $= 11.14 \text{ kcal}$

In the red cell at 25 °C,

$[\text{ATP}] = 5 \times 10^{-3} \text{ M}$

$[\text{ADP}] = 0.125 \times 10^{-3} \text{ M}$

$[\text{Pi}] = 1.25 \times 10^{-3} \text{ M}$

$\Delta G^\circ$ for ATP hydrolysis $= -7300 \text{ cal per mol}$

$\Delta G$ for ATP hydrolysis $= -13443 \text{ cal per mol}$

Thus sufficient free energy is released upon hydrolysis of 1 molecule of ATP to move 3 Na and 2 K against the prevailing electrochemical gradients.
A Medical Moment

The cardiotonic steroids obtained (originally) from the foxglove *Digitalis purpurea* have been used as cardiac stimulants for centuries.

Their application at low doses results in increased force of contraction of heart muscle. It is, therefore, a drug of choice in treating congestive heart failure. It is thought that inhibition of the Na,KATPase results in diminished transmembrane Na gradients. This in turn produces less Ca efflux via the Na/Ca exchange transporter (an antiporter), raising sarcoplasmic Ca levels and thereby enhancing contraction of the contractile apparatus.

Inhibition of Na reabsorption by the renal tubule results in diuresis (water loss). This can be achieved by application of NaKATPase inhibitors which block active Na transport out of the epithelial cell into the interstitium or by application of substances such as furosemide which inhibit Na and Cl symport from the renal tubule lumen into the epithelial cell tubule.
Several other findings have been obtained:

1) N-ethylmaleimide inhibits the pump and traps Na\(^{2+}\) in an occluded (nonreleasable) state.

2) Intracellular vanadate inhibits the pump and traps Rb\(^{86}\) (a substitute for K) in an occluded (nonreleasable) state.

3) In the absence of K\(_o\) and K\(_i\) and in the presence of high Na\(_o\) and low Na\(_i\), the pump synthesizes ATP from ADP and Pi. When the Na gradient is reversed in the absence of K, ATP is hydrolyzed. Under both conditions, the pump catalyzes Na/Na exchange.

4) In the absence of Na but in the presence of Pi, the pump catalyzes K/K exchange.

5) E2.P dephosphorylation is inhibited by cardiotonic steroids
These allow further refinement of the model: