Nobel Prize in Chemistry – 1997

The Story of Two Extra-ordinary Enzymes

S Ranganathan

The modes of action of two enzyme ensembles that perform critical life operations are described.

There is a perfection, not commonly seen, in the award of the Nobel Prize in Chemistry this year to P D Boyer, University of California, Los Angeles and J E Walker, Medical Research Council, Laboratory of Molecular Biology, Cambridge, for their elucidation of the mechanism of ATP formation in environments of a proton gradient and to J C Skou, Aarhus University, Denmark for the first discovery of an Na^+/K^+ ion transporting enzyme, now called 'Na^+-K^+ ATPase'.

The trinity, *information – function – energy*, manifests in all forms of life. Sun is the primary energy source and plants directly use this in forming energy rich bonds like glucose, starch, cellulose etc by forcing a combination of carbon dioxide with water. In bacteria, plants and animals such energy rich compounds are 'burned' and the energy released by this combustion of such nutrients is captured universally in the form of ATP (adenosine triphosphate), a 'broker–extraordinary' in consummating union between reluctant partners, in the generation of nerve impulses, in swimming against osmotic pressure and several others. The transformation of nutrients to ATP occurs, in all life forms, largely by similar pathways and in ours by a subsystem called mitochondria (generally used as a plural, since this organ is present in numbers in all cells). Energy rich bonds are transformed to water and carbon dioxide, in mitochondria, generating almost all the ATP in our system. For example, glucose ($C_6H_{12}O_6$) when degraded to $6CO_2 + 6H_2O$ generates 38ATP of which 36 are synthesised in mitochondria. The shuffling of carbon bonds in mitochondria results in the formation of energy rich NADH (nicotinamide adenine dinucleotide phosphate) at

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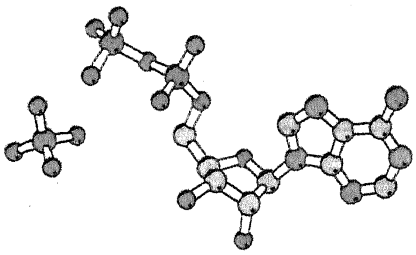


Courtesy: The Royal Swedish Academy of Sciences.

the expense of energy poor CO_2. In the process called 'respiration' NADH is oxidized to NAD^+ in the cascade, resulting in the reduction of oxygen to water:

$$NADH + 1/2\,O_2 + H^+ \rightarrow NAD^+ + H_2O = -53 \text{ kcal/mole} \quad (1)$$

The overall reaction here leads to the formation of 3ATP :

$$ADP + Pi + H^+ \rightarrow ATP + H_2O = 7.3 \text{ kcal/mole} \quad (2)$$

Thus part of the energy in NADH is captured in the formation of ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi).

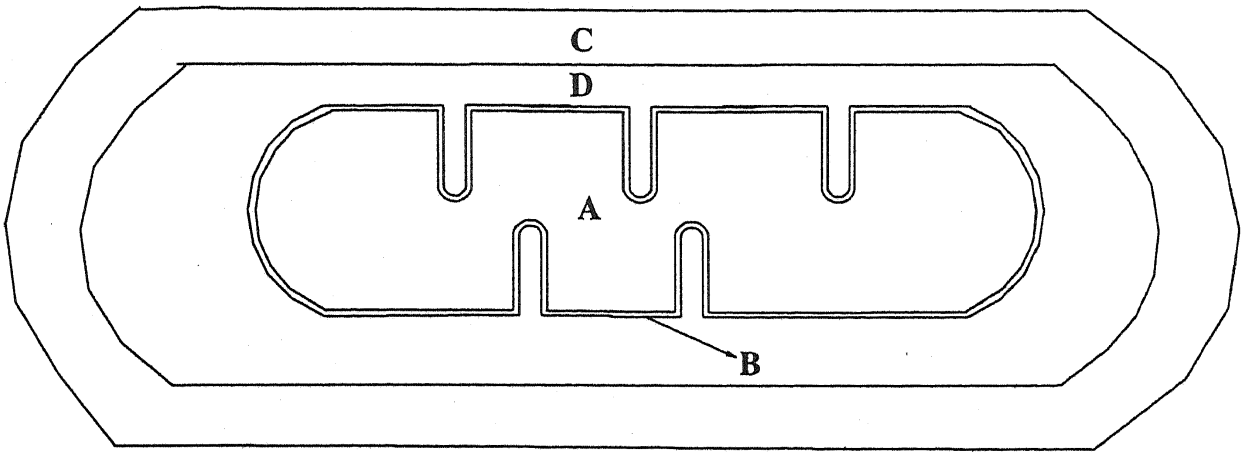
The deciphering of pathways by which the combustion of NADH results in ATP formation was not easy. Eventually novel concepts linking the electron gradient in respiration with a proton gradient and the identification of the latter as responsible for ATP formation was established.

Such unusual phenomena are possible due to the extraordinary architecture of the mitochondria (*Figure 1*).

A general picture of the major events taking place in mitochondria is presented in *Figure 2*.

The generation of a proton gradient concomitant with respiration was demonstrated in the early sixties. Simultaneous endeavours with the enzyme ensemble ATP synthase led to the understanding of pathways by which the proton gradient can result in the generation of ATP. The bold prediction of Boyer based on enzymatic studies was subsequently verified by Walker on the basis of structural studies including X-ray crystallography.

The heart of ATP synthase lies largely in the heart itself, an organ which works every moment of our life using ATP. Thus when horse heart muscle yielded a homogeneous enzyme complex which promoted ATP generation in the presence of a proton gradient many surprises were in store!

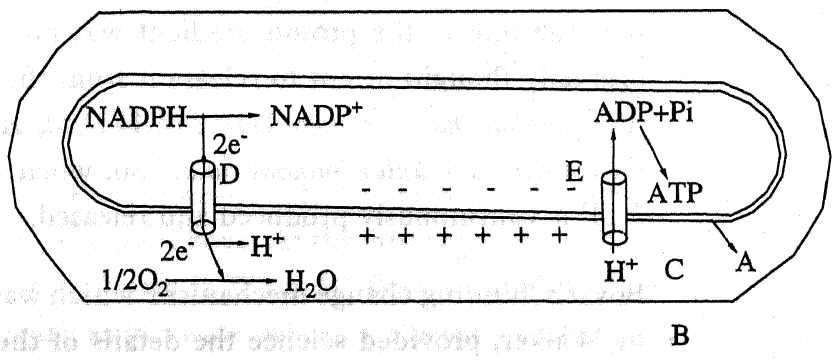


Organelle	*Remarks*
A	*Matrix*: Autonomous in some information, site for NADH/$FADH_2$ production by Acetyl Co-A, fatty acid degradation.
B	*Inner Membrane*: Highly selective; impermeable to ions and polar molecules; site for respiration and ATP synthesis ; responsible for proton gradient.
C	*Outer Membrane*: Permeable to ions and small molecules.
D	*Inter Membrane Space*: Links respiration and ATP synthesis.

Figure 1.

ATP synthase (*Figure 3*) consists of two sub units F_0 and F_1. F_0 is hydrophobic and spans the mitochondrial inner membrane and is the proton channel for the synthase. It consists of 6 proteins of ~ 8 kD each. F_1 is the catalytic site. It is placed beyond the mitochondrial inner membrane facing the matrix (*Figure 4*). F_1 consists of 9 protein chains designated as $\alpha_3\beta_3\gamma\delta\varepsilon$, each ~30 kD in size.

Figure 2.



A – Inner Membrane ; B – Outer Membrane ; C – Inter Membrane space; D – Site for respiration; E – Profile of ATP synthase

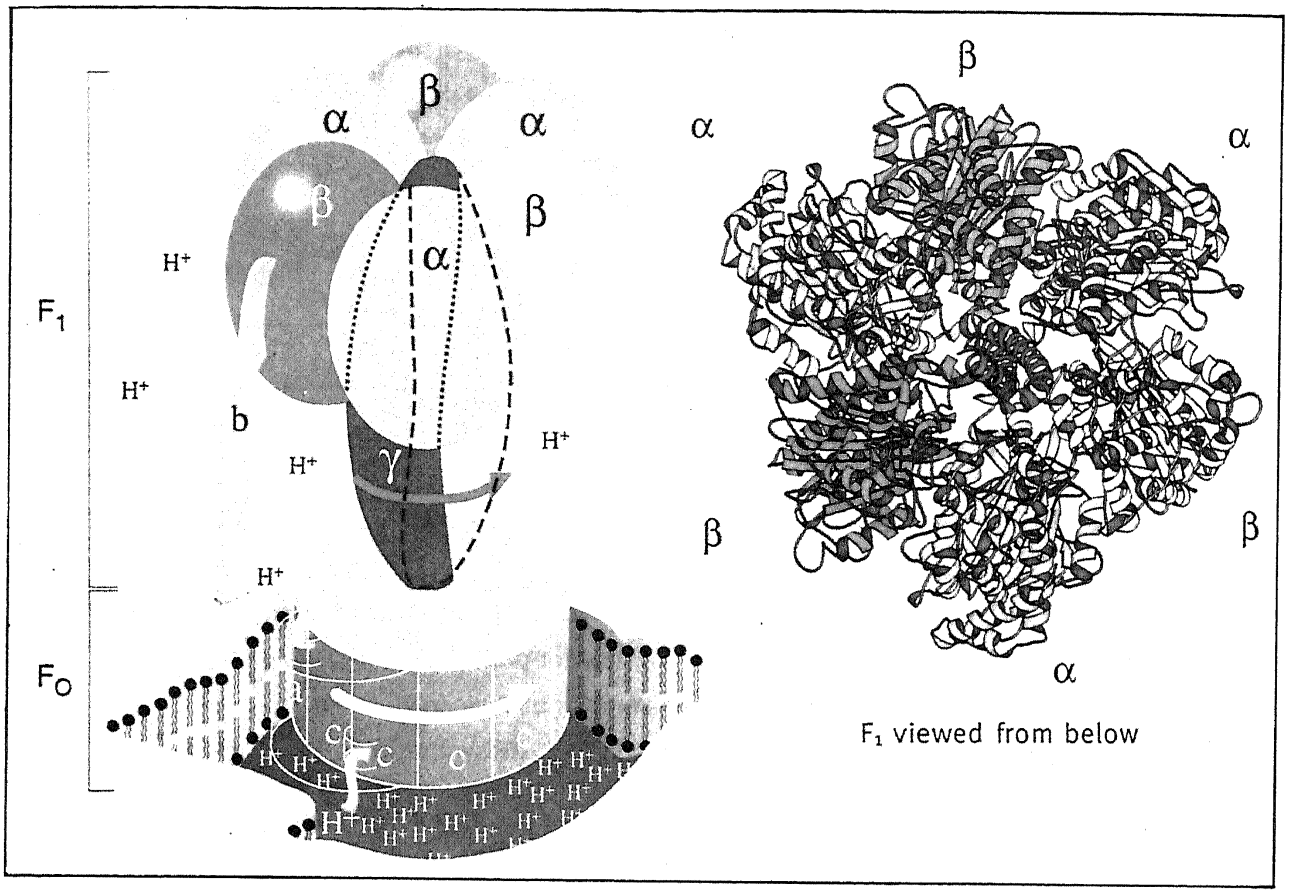


Figure 3. A schematic view of the enzyme ATP synthase. *(Courtesy: The Royal Swedish Academy of Sciences).*

ATP Synthase (*Figure 4*) in construction is a marvel. The alternating α, β proteins in a hexagonal arrangement generate a hollow channel into which the γ unit fits snugly!

Sustained efforts by Boyer and his colleagues over decades have unravelled how ATP synthase generates ATP. Boyer showed that the role of the proton gradient was not to form ATP as orginally thought of but to release it from the enzyme. *Further ATP synthase harbours both ADP and ATP, but ATP does not leave the catalytic site unless protons flow*! So, when the protons flow ATP is continuously produced and released.

Boyer's 'binding change mechanism' which was verified largely by Walker, provided science the details of the working of the ATP generating machine, ATP-synthase (*Figure 3*), the salient features of which are summarized below.

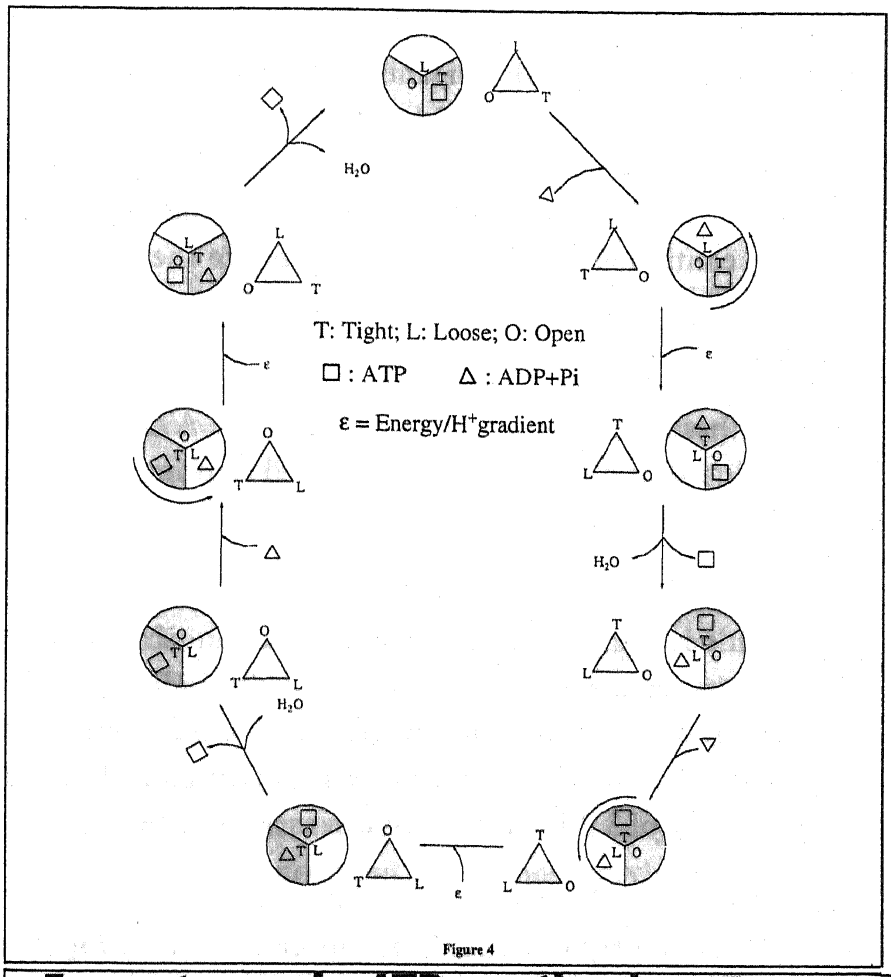


Figure 4

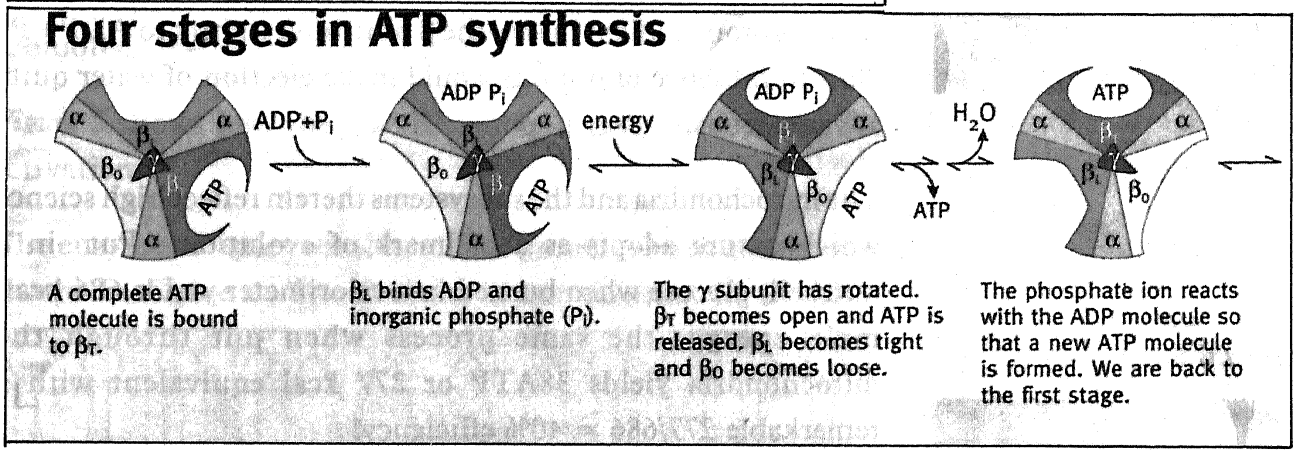


Figure 4. A complete cycle of operation of the ATP synthase machine.

A colourful representation of the first four stages of ATP synthesis is shown below (Courtesy: The Royal Swedish Academy of Sciences).

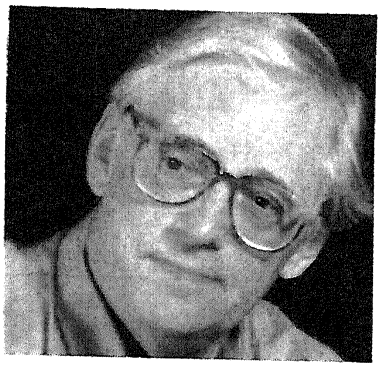
The $\alpha_3\beta_3$ cap of ATP synthase provides a channel into which the γ unit fits snugly, as noted above. At the other end γ is anchored to the subunits of F_0 (*Figure 3*). When H^+ flows through F_0, these subunits are twisted as in a water wheel. Since the γ unit of F_1 is attached to these, it also gets twisted. Two events of importance arise from twisting of the γ unit. The $\alpha_3\beta_3$ cap is stationary and

Paul D Boyer

normally they have identical conformations. The catalytic site, harbouring substrate (ADP+Pi) and product (ATP), residing in the β unit, due to the twisting of the γ unit, becomes asymmetric and unequal with respect to changing interactions with γ. *This results in the 3 catalytic sites in the $\alpha_3\beta_3$ cap becoming non-equivalent.* Second, a continuous proton push would rotate the γ unit and this, as in the transmission system in a car, will lead to continuous ATP production.

Based on these clues, Boyer suggested that the ATP synthase operates similar to a water gradient operated minting press and that for every rotation of γ, three fresh and bright ATP will be released!

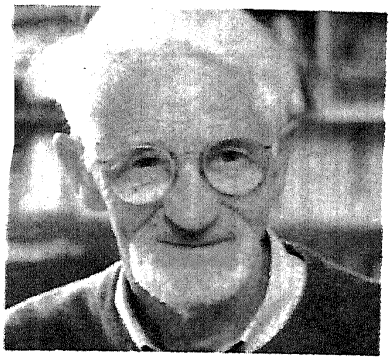
John E Walker



Basic to this is the assumption that the rotation of γ produces catalytic sites having tight binding (T), loose binding (L) and open (O) profiles, which naturally interchange on rotation. A complete rotation involving these sites is presented in *Figure 4*. The generation of 3 ATP as a result can easily be seen.

Finally how does the proton gradient run the unfavourable $ADP+Pi \rightarrow ATP+H_2O$ reaction? It can be seen from *Figure 4* that a proton gradient makes the β unit bind tightly to ADP+Pi. This in presence of protons would make ejection of water quite advantageous.

Jens C Skou



The mitochondria and the subsystems therein reflect high science which nature adopts as a hallmark of evolution. Put in a nutshell, glucose when burned in a calorimeter yields 686 kcal/mole energy; the same process when put through the mitochondria yields 38ATP or 277 kcal equivalent with a remarkable 277/686 = 40% efficiency!

In the living cell, the sodium ion concentration (Na^+) is lower than that outside, whilst that of potassium ions is higher. The Na^+/K^+ gradient controls cell volume, nerve and muscle excitation, transportation of sugars and amino acids and several others. In cells, the so called Na^+/K^+ pump operates in the forward direction by pumping out Na^+ and pumping in K^+.

Since both these events work against the concentration gradient, energy in the form of ATP has to be supplied. This activity, being basic for nerve function, is important. Indeed, more than a third of ATP consumed by a resting animal is involved in the pump. The reverse flow is passive.

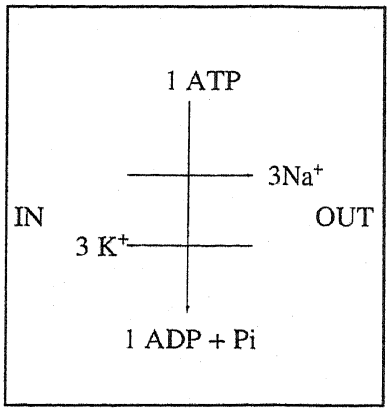


Figure 5.

Skou isolated from finely ground crab nerve membranes, a homogenous enzyme complex which promoted controlled transport of Na^+/K^+ across the membrane at the expense of ATP. The enzyme was inactive in the absence of any of the key players, namely ATP, Na^+ and K^+. This was the first enzyme complex that promoted the vectoral transport of substances across the cell membrane.

Several additional observations conclusively proved that the Na^+ -K^+ ATPase is responsible for the operation of the pump. First, the maximum efficiency of the enzyme matched with that corresponding to the inflow/outflow of ions in the cell. Further, sodium and potassium ions bind to the enzyme with great affinity *at different sites*. Also, ATP phosphorylation of enzyme becomes viable only when complexed with Na^+ ions. In sharp contrast, when complexed with K^+ ions, dephosphorylation is promoted.

Figure 5 shows how the Na^+/K^+ pump is associated with ATP activation.

The enzyme complex consists of four protein chains arranged as shown in *Figure 6*. The β chain is not essential for Na^+/K^+

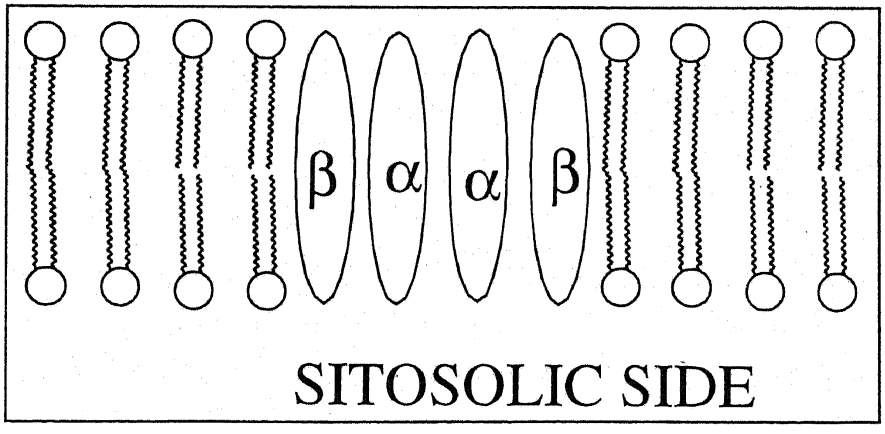


Figure 6.

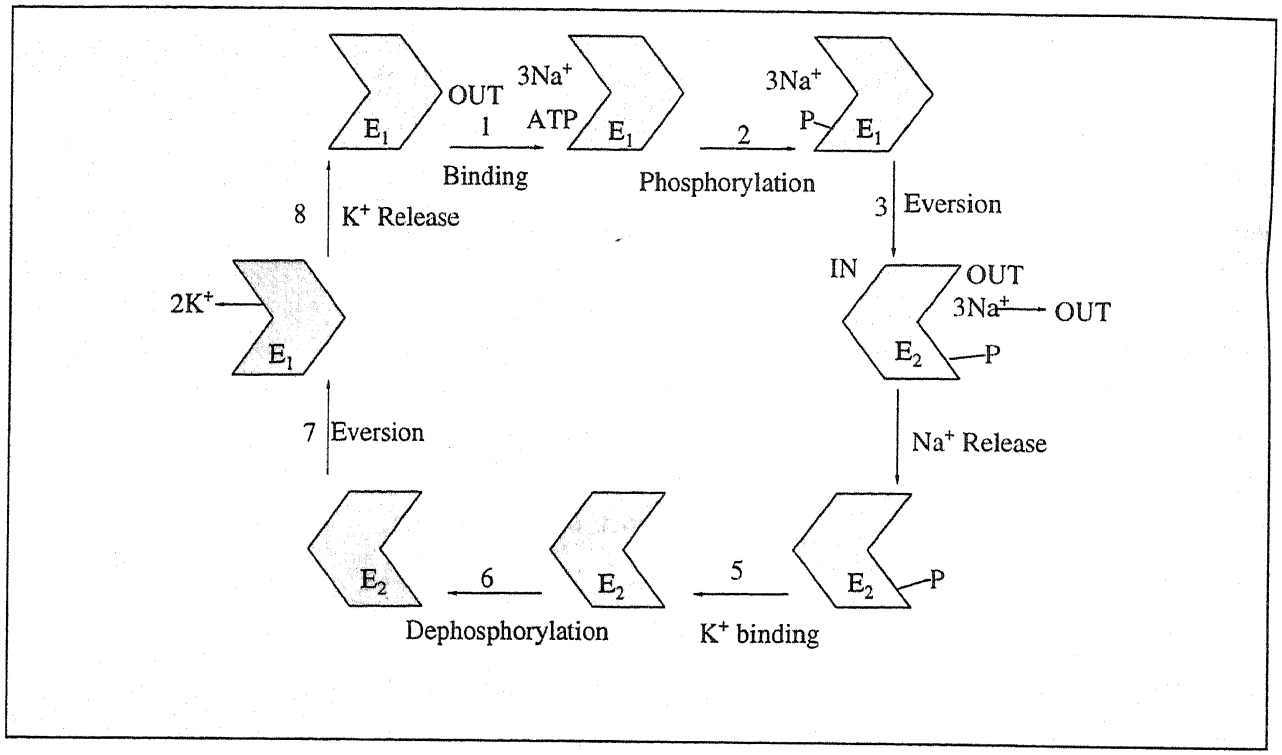


Figure 7. The mechanism of transport of ions by Na^+–K^+ ATPase.

transport, but plays a role in the stabilization of $\beta\alpha\alpha\beta$ ensemble, which is largely buried in the membrane.

The Na^+–K^+ ATPase promotes an unusual series of changes in bringing about Na^+/K^+ transport, the salient features of which, presented in *Figure 7*, describe a full cycle illustrating, in sum, the outflow of $3Na^+$ and the inflow of $2K^+$.

The E_1 conformation has high affinity for sodium ions. The resulting Na^+ uptake triggers phosphorylation. The phosphorylated E_1 conformation is unstable and everts to E_2 conformation which having little affinity for Na^+, releases it outside. Conformation E_2 having great affinity for K^+, picks up this ion. The uptake of K^+ triggers dephosphorylation and in this state conformation E_2 is unstable and everts to E_1. E_1 having little affinity for K^+ releases it inside, thus completing this marvellous cycle!

In sum, we have here a story of two enzyme ensembles, that perform critical life operations by molecular architecture based machines, whose copying by humans, one can only dream of at this juncture!

Suggested Reading

- L Stryer. *Biochemistry*. 3rd Edition. Freeman and Company. New York, 1988. Chapters 17 and 37.

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