A profile of adenosine triphosphate

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An attempt is made to draw a profile of adenosine triphosphate (ATP) and to project its many actions. The amazing versatility of its participation in a number of synthetic reactions lies in the oligophosphate structure. Many proteins that use ATP have conserved binding 'P-loop' but this gives no clue what makes it so special. The energy transducing reactions leading to synthesis of the terminal phosphodiester had at least three strategies. Of these, direct dehydration and transfer of inorganic phosphate using respiratory energy operate through mechano-coupling in a multisubunit protein. This tripartite, knob–stalk–base structure provides a novel mechanism of rotational catalysis and the tiniest molecular motor. All the reactions occur in concert with no sign of energized chemical intermediate. With the new knowledge on the crystal structure of F_1-ATPase, proton translocation needs a relook. An alternative perspective is emerging on energy being received and stored in polypeptide structure by breaking hydrogen bonds. Membrane serves the purpose of mobilizing the constituent proteins and also as a potential energy carrier of proteins with little loss of energy.

IN nature, energy is transformed from the primary chemical storage as carbon–hydrogen (C–H) bonds to chemical, electrical, osmotic, mechanical and thermal forms to support functions of biosystems. Biological energy transduction systems are fine examples of design and engineering perfected over the long evolutionary period. Basically, there are two underlying processes: the production of the unique energy currency of the cell, the magic molecule adenosine triphosphate (ATP), and the energization of the membrane, for performing energy transactions. Here lies the secret of the design backed by accumulated experience of three billion years of existence of life on earth. Two key questions are: What makes ATP the most suited energy-dollar? What happens in cellular membranes which are the site of key tranduction processes?

The ATP molecule and its energy

Fiske and SubbaRow[1], and Lohmann[2] both independently discovered ATP in 1929. Narrating the historical event, Maruyama[3] reported that SubbaRow presented his paper at the New York Academy of Sciences much earlier on the isolation from muscle extracts a compound of adenosine with three phosphates, one of them linked to ribose. But the abstract was not printed in the proceedings. Lohmann later found that ATP is a derivative of adenosine-5-phosphate with two more phosphates attached in phosphodiester linkages (Figure 1) and this was later confirmed by chemical synthesis by Todd[4].

Requirement of energy for cellular processes such as membrane transport and chemical synthetic reactions is self-evident. With a commendable foresight, Lipmann[5] postulated that some compounds act as intermediate carriers of energy, designated by the famous swiggle (~) to indicate their 'energy-rich' character, and suggested that hydrolysis of ATP to ADP+P_i could be the major provider of cellular energy currency, the equivalent of a metal coin, a paper note or a plastic card.

Of the main phosphate compounds, only those with phosphodiester–acyl phosphate, enol phosphate and N-phosphate bonds – are 'energy-rich' or more appropriately described as having 'high group transfer potential', (Table 1). The expression 'energy-rich' implies that on hydrolysis of such bonds sufficient energy is available for formation of bonds of useful compounds. In general, ATP provides for much of the energy requirement of a biosystem, but the pool size of adenosine phosphates is limited. Therefore hydrolysis of ATP must be continuously compensated by its synthesis. The awesome magnitude of this demands a turnover of ATP in a day equal to half the body weight of a lethargic person!

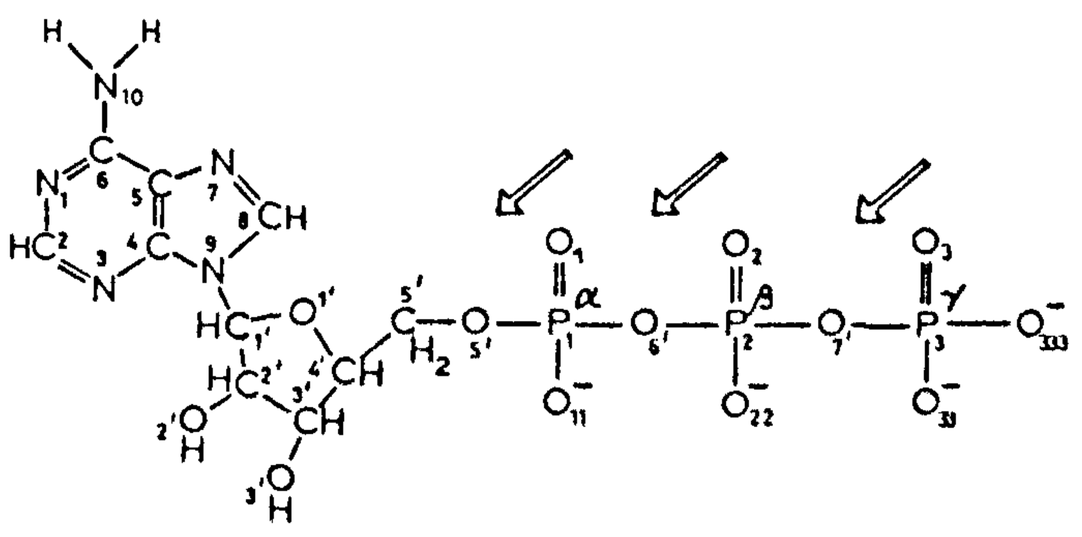

Figure 1. The structure of ATP. The structure and numbering is adapted from ref. 10 except for showing the three phosphates in linear chain. Arrows are given on top to indicate the splits that occur before α, β and γ phosphates in many reactions ATP undergoes.

Table 1. High group transfer potential phosphates. The atoms attached to phosphate are arranged to bring a common basis of three 'energy-rich' phosphates: –(O=C–O–P), –(H_2C=C–O–P) or –(HN=C–N–P) indicated by (X=Y–Z–P). The values of energy released on hydrolysis of the phosphate bond ΔG_0 are given in kcal/mole (1 kcal is about 4.2 joules/mole). All the O and H are not shown. R is attached to Y

Compound	ΔG_0 kcal/mole	R	X=Y–Z–P
ATP	7.3	AMP–O–	O=P–O–P
Pyrophosphate	8.0	–	O=P–O–P
Creatine phosphate	10.3	CH_3–N– | $HOOC–CH_2$	HN=C–N–P
Acetyl phosphate	10.3	CH_3–	O=C–O–P
Phosphoenol pyruvate	14.8	–COOH	H_2C=C–O–P

Structuring three phosphates with two phosphodiester linkages is part of an overall strategy of selection of ATP as the crucial molecule for bioenergetic functions. This unfolds as we learn about the multiple hydrolytic and synthetic reactions that depend on ATP molecule. Although the single phosphodiester of ADP should make it equally competent, however, ADP substitutes poorly, if at all, in most ATP-requiring reactions. A rare exception was found in our laboratory[6] wherein ADP was equally effective as ATP for supporting low rates of mevalonate kinase, only when used at sub-saturation concentration.

Why so many ATPases?

Hydrolysis of ATP by the enzyme, ATPase cleaves the terminal γ-phosphate, releasing 7 kcal of energy which can be made available to support a variety of cellular activities. A number of ATPases associated with specific functions are identified by single alphabets (Table 2). Broadly they form two groups, which are characterized by formation of enzyme intermediates either of E–P type with covalently linked phosphate or of E_P^{ADP} with

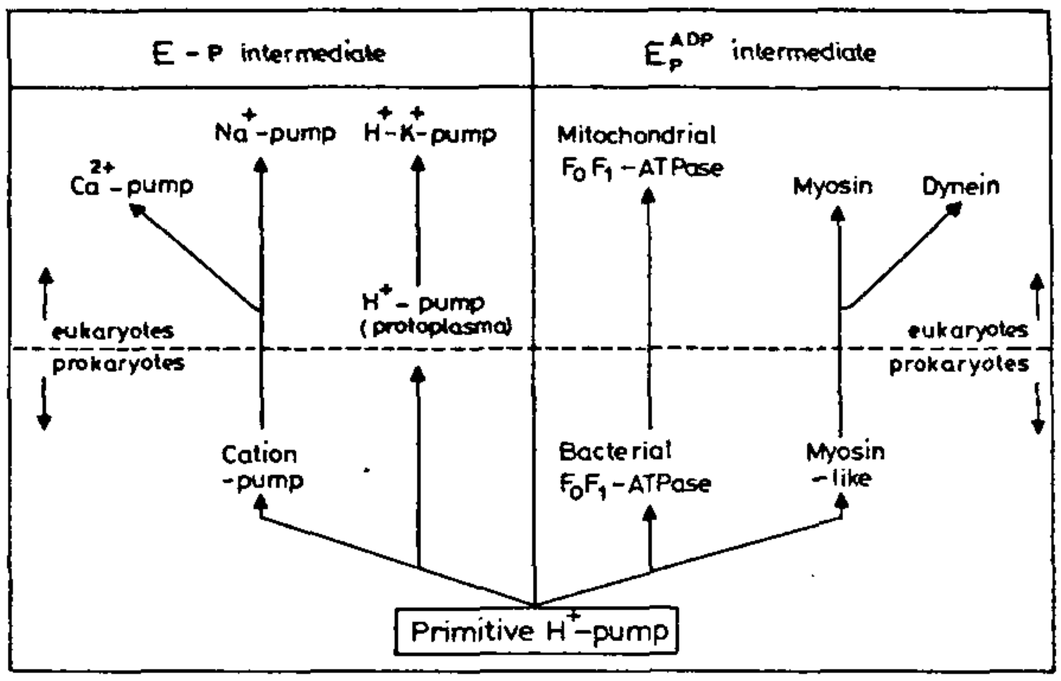

Figure 2. Schematic representation of evolution of two branches of ATPases from a primitive H^+-pump. Those with E–P-intermediate developed into pumps. Those with E_P^{ADP} intermediate formed the contractile proteins and ATP synthase indicating mechano-coupling as the basis of their action (adapted from *Energy Transducing ATPases* (ed. Tonomura, Y.), Cambridge Univ. Press, 1986).

noncovalently bound ADP and inorganic phosphate (P). These two groups have evolved from a primitive H^+-ATPase (Figure 2). One branch developed into vanadate-sensitive transport forms; P-type for cations and M-type for efflux of drugs (also known as ABC transporter or traffic ATPases). Discovery in 1957 by Skou[7] of Na^+, K^+-ATPase, the first among this class, was rewarded by half share of the 1997 Nobel Prize in Chemistry. The second group of ATPases are not sensitive to vanadate as they carry no E–P intermediate, and are utilized for functions that depend on mechano-coupling of energy. Among these are the C-type, which are involved in contractile proteins such as myosin, and the F-type present in mitochondria, chloroplasts and bacterial membranes, whose real function is synthesis of ATP in the reverse. The first of these F_1-ATPase in

Table 2. Classification of some ATP-hydrolysing proteins. The list is not complete. Some information on the size and subunits is given. The single alphabet code is shown. The P and M type activities are vanadate-inhibited

Alphabet code	Name	Mol. wt (kDa)	Subunits (kDa)
P	Na^+, K^+-ATPase	500	100 (4α), 40 (4β), 11 (1γ)
M	P-Glycoprotein (multidrug resistant)	130–170	–
C	Myosin (contractile)	480	200 (2HC), 15–25 (2LC)
	Dynein (contractile)	1300	330 (A), 122, 90, 76, 24–14 (LC)
V	Vacuolar ATPase	172	89(a), 64 (b), 19.5 (c)
F	F_0F_1 (mitochondria)	370	54 (3α), 50 (3β), 33 (3γ) (other minor ones, variable)
H	Heat-shock proteins (hsp)	70,90	–
	Grp	78	–
	Gro E	60+10	60 (EL), 10 (ES)
N	Rec B, Rec A (DNA-dependent ATPase)	140, 40	–
E	Ectonuclease (outer surface ATPase)	–	–

Table 3. Multiple synthetic reactions dependent on ATP. The molecule ATP is used as source of energy and its components in multiple enzyme reactions. These involve split at α–, β–, and γ–phosphate positions and transfer of phosphate, pyrophosphate and AMP groups to acceptors

ATP reaction and type of overall reaction	Examples
A. ATP → ADP+P_i	
(source of energy; β–γ phosphate bond split)	
1. Carboxylation, addition to CO_2 to CH_3–C	Pyruvate carboxylase
2. Decarboxylation, removal of CO_2 from CH_2COOH	Mevalonate–5–PP decarboxylase
3. R.NH_2–N transfer to carboxyl–C	Glutamine synthetase
	Formyl glycinamide ribonucleotide glutamine aminotransferase
4. Transfer of whole amino acid:	
– amino–N to a carboxyl–C	5-Aminomidazole-4-(N-succinylocarboxamide ribonucleotide) (SACAIR) synthetase
– carboxyl–C to an amino–N	Glycinamide ribonucleotide (GAR) synthetase
5. N–C cyclization	5-Aminoimidazole ribonucleotide (AIR) synthetase
B. ATP+X → ADP+X–P	
(γ–P transfer to acceptors, kinases)	
1. Hydroxyl group - sugar	Hexokinase
- primary	Choline kinase
- secondary	Pyruvate kinase
2. Carboxylate–OH	3-phosphoglycerate kinase
3. Phosphate–OH	Adenylate kinase
4. Guanidine–N	Creatine kinase
5. Protein–Ser/Thr–OH	Protein kinase A (or C)
6. Protein–Tyr–OH	Protein tyrosine kinase
7. Pyruvate+P_i (→ PEP + PP)	Pyruvate: P_i dikinase
C. ATP→AMP+PP	
(source of energy, α–β phosphate bond split)	
1. Acyl group activation	Fattyacyl CoA synthetase
(Adenylate intermediate)	Aminoacyl-tRNA synthetase
2. Cyclization of phosphate to cyclic AMP	Adenyl cyclase
D. ATP+X→AMP+X–PP	
(α–β phosphate bond split with PP transfer)	
1. ATP + R–5–P → AMP + PRPP	5-Phosphoribosyl-1-pyrophosphate synthetase
E. ATP+X→X-AMP+PP	
(α–β phosphate bond split with AMP transfer)	
1. ATP + NMN → NAD^+ + PP	NAD^+ synthetase
2. ATP + FMN → FAD + PP	FAD synthetase
3. ATP + Phosphopantetheine → Dephospho–CoA	3-Dephospho-CoA synthetase
4. ATP + Sulphate → 5′–Adenosyl phosphosulphate	5-APS synthetase (sulphate activation)
5. ATP + $(NTP)_n$ → RNA + PP_n	RNA polymerase
F. ATP + X → Adenosine-X + PP + P_i	
(Splits all three phosphates)	
1. ATP + Methionine → S–Adenosyl–methionine + PP+P_i	S-Adenosyl-methionine synthetase (methionine activation)

mitochondrial membranes was discovered by Racker[8] who should have shared the 1997 Nobel Prize in Chemistry had he been alive.

Other ATPases with specific functions are also recognized: vacuole-located V-type sequesters some metabolites within the cell; the H-type, representing heat shock proteins (hsp), act as chaperones and protect vital proteins under stress conditions; the N-type are dependent on DNA and help in their unwinding (e.g. Rec B) or restriction enzymes (e.g. *Eco*R1); the E-type ecto-ATPase, residing on the outside surface of the cell, is related to purinergic receptor system and needs endogenous ATP to be brought out for its activation.

Multiple synthetic reactions dependent on ATP

ATP is a versatile molecule, both as a source of energy and as a donor of its own constituents – phosphate, pyrophosphate and AMP. It can be cleaved at the ester linkages of all the three phosphate groups and transfer part of the split molecule to the acceptors (Table 3). Some reactions which are supported by split in γ-phosphate of ATP with both ADP and P_i released are: carboxylation, decarboxylation, transfer to carboxyl-C of ammonia-N, amino-N of glutamine and amino-N along with the rest of asparagine, transfer to amino-N of glycine carboxyl, and cyclization of formyl-C and amino-N.

Kinases form the major group of enzymes among the ATP-utilizing reactions. Other nucleotide triphosphates do not substitute ATP, or do so only poorly. A large number of compounds are activated by phosphorylation using γ-phosphate of ATP. There are different phosphate acceptor groups – primary hydroxyl-O, carboxylate-O, phosphate-O, guanidino-N (creatine and arginine), and also protein-bound serine, threonine and tyrosine (protein kinases).

No example exists which shows transfer of ADP from ATP to an acceptor. There does exist a sole example of a kinase which uses both γ- and β-phosphates of ATP in a coupled reaction of phosphorylation of two substrates. Exclusive to plants, pyruvate-P_i dikinase phosphorylates pyruvate to phosphoenol pyruvate and inorganic phosphate to pyrophosphate thereby releasing AMP from ATP.

The significance of ATP having three phosphates is revealed by a set of reactions that use energy derived by splitting between α- and β-phosphates. The two products, AMP and PP, are released, each of which can be transferred to suitable acceptors and in the process release the other one, and AMP portion released can go into polymerization or cyclization reactions.

One unique reaction that cleaves all the three phosphates of ATP is in the activation of methionine. The synthesis of the biological source of methyl groups, S-adenosylmethionine, occurs by transfer of the adenosyl group of ATP to methionine sulphur with simultaneous release of PP and P_i.

Structure of ATP molecule

The oligomeric phosphates make ATP a highly flexible molecule. The P–O bonds can freely rotate and can keep

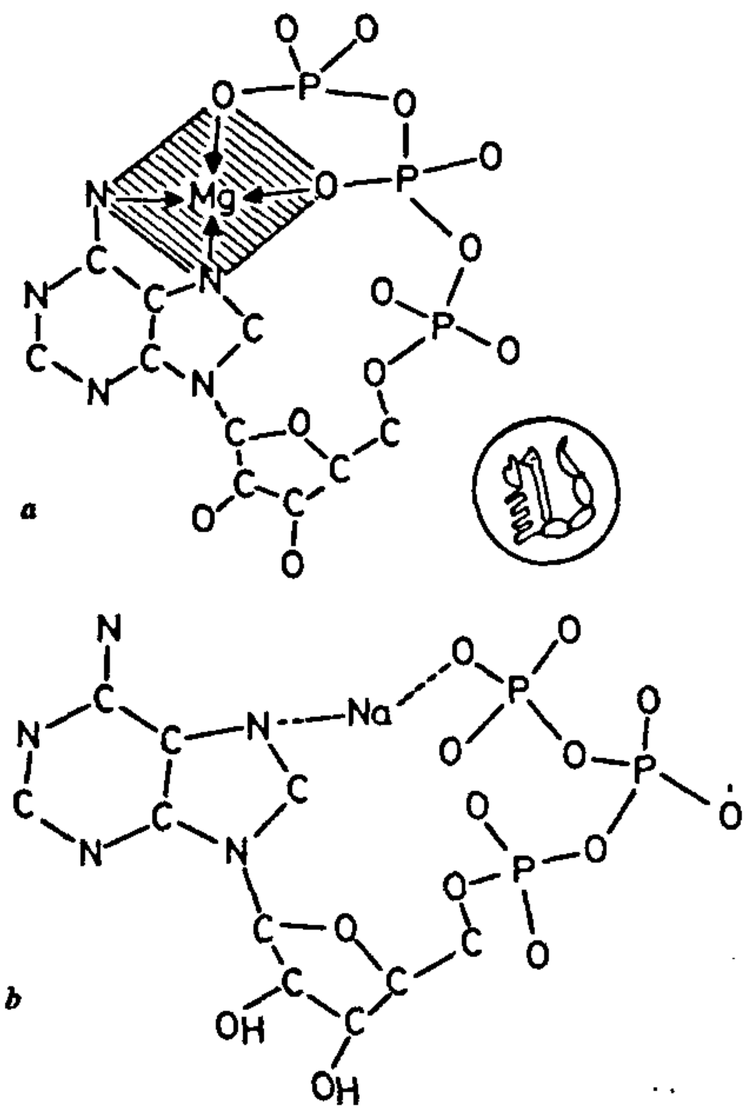

Figure 3. Structures of ATP and Mg^{2+} and Na^{+} complexes. *a*, Mg.ATP based on the proposal of Szent-Gyorgyi[13] with the phosphate tail bent over the base to form a quadridentate chelate. *b*, The crystal structure of disodium ATP of Kennard *et al*[10] also shows bent tail over base but coordination of cation occurs only with N_7 of adenosine and an oxygen of γ-phosphate. Only C, N, O and P atoms are shown along with Mg and Na.

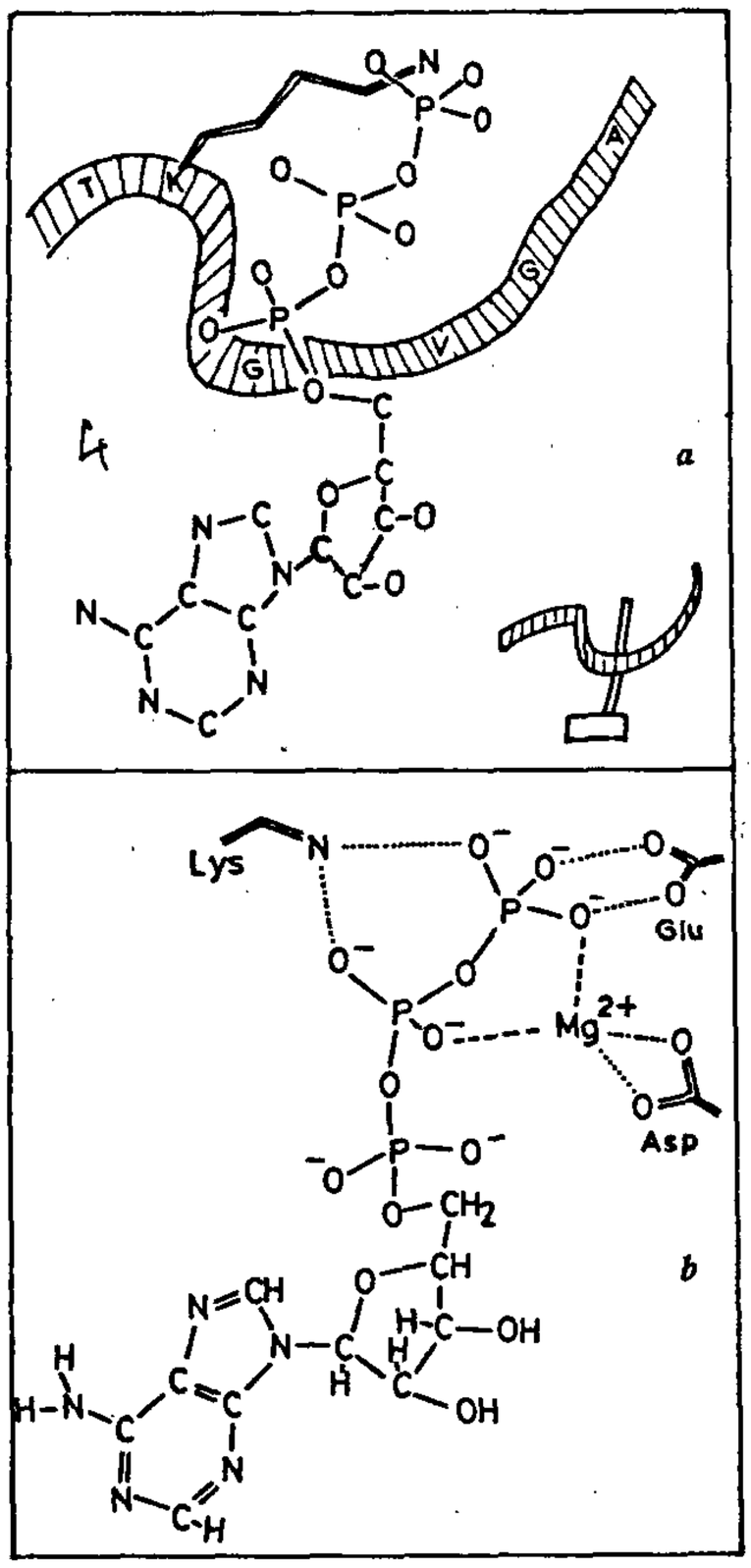

Figure 4. Structure of protein-bound ATP. *a*, The molecule of ATP bound to β-subunit of F_1–ATPase as seen in the crystal structure, redrawn from the data provided by Abrahams *et al.*[17]. Only the atoms C, N, O and P are shown. The 'P-loop' (AGVGKT) is shown as a hatched band surrounding the phosphate chain as a sickle and hammer. Only lysine (K)-N comes close to γ-phosphate–O. Note the phosphate chain is linear and away from the base unlike the metal complexes. *b*, Common topology of Mg-dependent ATP hydrolysing enzymes at the binding site in protein (Adapted from refs 17 and 19).

the chain in linear extended form as in the case of inorganic triphosphate[9], or fold it back over the adenine base[10]. The requirement for Mg^{2+} for most ATP reactions led to the recognition that Mg–ATP complex is the actual substrate, particularly for kinases[11]. Increase of either Mg^{2+} or ATP concentration over 1:1 ratio produced inhibition of choline kinase[12]. Intramolecular quadridentate complex of Mg–ATP was therefore proposed as the active species by Szent-Gyorgyi[13]. In this two oxygens of β- and γ-phosphates and two nitrogens of adenine base (C_6 amino and N_7) are bridged by Mg^{2+}, as though the tail is bent over the body (Figure 3). In view of this shape we refer to this as a 'scorpion model'. Initial evidence for this model was provided by the appearance of a minor band at 1683 cm^{-1} in infrared

spectrum representing C–N^+ when Mg^{2+} was complexed with ATP or ADP, but not with AMP + PP (ref. 14).

The crystal structure of disodium ATP confirmed that the phosphate chain is indeed folded back on the adenine base[10]. The bent conformation is stabilized by Na^+ by bridging γ-phosphate-O and adenine N_7 (Figure 3). This is facilitated by the unusually large torsion angle of 224° about C5′–O5′ between ribose and α-phosphate, in contrast to 180° or less in other AMP derivatives. The complex Mg–ATP has not been crystallized so far and, consequently, it remains to be seen whether two phosphates and two nitrogens are involved as indicated in the 'scorpion model'. In such a folded structure the N–N–O–O, with approximate 2.8 Å distance, appears as a parallelogram with Mg at the centre. It is a coincidence that two amino-N and two carboxyl-O of peptide bonds in adjacent hydrogen bonds in α-helix or β-sheet provide a similar parallelogram[15].

Structure of protein-bound ATP

It is known for some time that a common nucleotide-binding motif, called 'P-loop' also 'Walker motif A', is present in a number of nucleotide-binding proteins[16]. This has a consensus sequence of $G(X)_4GKT/S$. The proteins include adenylate kinase, Rec A, efTu, tranducion-α and α- and β-subunits of F_1-ATPase[17] (see ref. 16 and 17 for others).

It is clear from the above-mentioned reports that the phosphate chain is linear and away from the base, a common pattern with ATP and other nucleotides bound to proteins. This is illustrated by the disposition of ATP in β-subunits of mitochondrial F_1-ATPase redrawn from the coordinates provided by the crystal structure data[17] (C. Ramakrishnan, unpublished) (Figure 4 *a*). The phosphate chain is not in the bent conformation in the protein-bound state. The phosphate-O are within 5 Å distance from the side chain amino acids of the 'P-loop' (AGVGKT) and also of Glu-188, Arg-189, Gln-192 and Try-311. It is the latter polar amino acids which bind with phosphate through charges or hydrogen bonding. The conserved 'P-loop' identifies the location for fitting ATP-phosphate chain (a 'sickle–hammer' model). Absence of side chain (glycines positioned here) probably facilitates movement of phosphates, if required. The adenine base itself is in company with hydrophobic amino acids in the β-subunit, leaving no chance for negatively charged phosphate chain to bend over the base.

The essential Mg^{2+} is bound near the terminal phosphate position and obviously plays a role in the release or addition of γ-phosphate. In the absence of Mg^{2+} all three β-subunits, in EF_1-ATPase, bind ATP with same affinity, and it is the presence of Mg^{2+} that imposes cooperativity and high affinity at the catalytic site with Kd's being 0.2, 500, 25000 nM in the three states of the β-subunit[18].

In both F_1-β-subunit and Rec A protein, ATP forms bidentate complex with Mg^{2+} at the binding site[19]. This common topology may exist with proteins catalysing ATP-triggered reactions (Figure 4 *b*). More than the ability of Mg^{2+} to partially neutralize negative charges of ATP and to stabilize the chelate with bent conformation, it seems to have a crucial function in quaternary structure, and also in tightening and loosening the protein structure around ATP and ADP.

Strategies of synthesis of ATP

A high turnover of ATP characterizes an active metabolism in a cell, and poisoning a step thereof is a sure way of killing it. Cellular synthesis of ATP from ADP and P_i is vital.

In glycolysis, glyceraldehyde-3-phosphate (GAP) is earmarked for oxidation and part of the energy realesed is captured by transferring the resultant acyl group to inorganic phosphate. The product 1,3-diphosphoglycerate transfers the 'energy-rich' 1-acyl-phosphate to ADP in the next step. The enzyme GAP dehydrogenase, catalysing this oxidation, is a soluble protein, a tetramer of 37 kDa subunit. Interestingly, this enzyme uses NAD^+ both as a structural component and as an oxidant (parallel ATP binding to structural and catalytic sites is found in ATP synthases). At the active site of this enzyme is a highly oxidant-prone Cys-149 sitting at the edge of an α-helix[20]. A key role is played by this sulphhydryl group in anchoring the GAP–aldehyde group for oxidation by NAD^+ followed by phosphorolysis of the thioester bond. Note that oxidation of this cysteine by H_2O_2, known to inactivate the enzyme, leads to a large loss of helical content of the protein[21].

Intramolecular redistribution of energy through dehydration is another method of obtaining 'energy-rich' phosphate. Enolase, the only such reaction in glycolysis, removes the elements of water, –H from C_2 and –OH from C_3 of 2-phosphoglycerate (2-PGA), yielding phosphoenolpyruvate (PEP). This enzyme is a dimer of 48 kDa subunits. It has a distinct barrel topology with two β-strands followed by two α-helices and then a six-fold repeat of alternating β- and α-units $[\beta_2\alpha_2(\beta\alpha)_6]$ (ref. 22). Mg^{2+} is essential for activity of this enzyme as it is necessary for dimerizing the protein, for coordinating with the substrate–phosphate at the conformational site, and for extracting –OH of the substrate $-C_3$ at the active site cavity, which consists of Glu-168, Asp-321, Lys-345 and Lys-396. It will be seen later that Mg^{2+} has a similar role in F_0F_1-ATP synthase.

It is instructive to note that soluble proteins catalyse oxidation and dehydration reactions for energizing inorganic phosphate to give 'energy-rich' phosphates. The

phosphate of 2-PGA is originally from inorganic pool, derived in phosphorolysis of glycosidic bond. In both cases the proteins use NAD^+ and Mg^+ as structural units to tighten their structures which subsequently help in binding as well as in breaking of C–H bond from C_1 of GAP or C_2 of 2-PGA. Although soluble proteins undergo conversions from C–H to O=C–O^- to O=C–OP to derive ATP, this is not enough to meet requirements of the various cellular processes. Consequently, either devising extra amounts of enzyme protein (already GAPD is about 10% of total cellular proteins) or enhancing the reaction rate could well have been developed. However, secondary problems arise with increased formation of the product, pyruvate. Making ethanol, as yeast does, is not only impractical in all cells but also wasteful energetically.

Relative ATP yield is 15 times more per carbon on oxidation of pyruvate in mitochondria than its formation from glucose in cytosol. NADH is produced in the soluble matrix in three steps of oxidative decarboxylation of pyruvate, isocitrate and α-ketoglutarate. Further oxidation of NADH, also by removal of a proton and an electron from C–H bond, is carried out by membrane-bound electron transport chain of flavoproteins, Fe–S proteins, ubiquinone, and cytochromes assembled in the form of distinct complexes. In contrast to soluble enzymes, these complexes I–IV (Figure 5) contain a large number of subunits of about 25, 4, 20 and 13, respectively and are integrated into the membrane by several transmembrane passes.

The strategy adopted for energy transduction is by electron transfer at three sites in complexes I (NADH $\rightarrow$ Q), III (Q $\rightarrow$ cyt. c) and IV (cyt. c $\rightarrow$ O_2) with large enough energy drop ($\Delta E_0' = 150$ mV or above) which is sufficient to make one ATP. By implication, their action together as a respiratory chain is an interactive transfer of electrons directly through proteins. These redox–protein complexes in the purified soluble state are able to function in the absence of Mg^{2+} and lipid bilayer. Then why membrane localization? Attempts to identify 'energy-rich' intermediate with any of the components or proteins proved futile.

Withdrawal of electrons by acceptors (e.g. by O_2 from cytchrome oxidase) on the matrix side releases protons on the cytosolic side of the membrane. Experimentally this was demonstrated in mitochondria from liver, and led to formulation and chemiosmotic hypothesis by Mitchell[23], showing existence of a proton gradient separated by membranes as a general mechanism for energy coupling and ATP synthesis. The energy made available during electron transfer ejects protons (ΔpH), generates transmembrane potential ($\Delta\psi$) and builds proton electrochemical potential ($\Delta\mu_{H^+}$), commonly referred to as proton gradient. It can also be built during hydrolysis of ATP and is discharged by uncouplers of energy transduction.

It is generally believed that this process of formation and discharge of proton gradient in membrane systems forms the core of energy transduction. A mechanism is proposed for thermogenesis wherein a 32 kDa protein, called uncoupler protein, exclusively found in mitochondria of brown adipose tissue (BAT) and implicated in cold adaptation, discharges proton gradient releasing heat instead of ATP formation[24]. Work in this laboratory found that BAT-generated heat is relatively minor whereas major source of heat is through peroxidation of polyunsaturated fatty acids[25]. As already discussed above, ATPases have evolved from a primitive H^+-ATPase to perform energy-dependent functions. Why not adapt this capacity in the reverse and utilize proton gradient to generate ATP? This is indeed the third strategy adopted in nature to synthesize ATP.

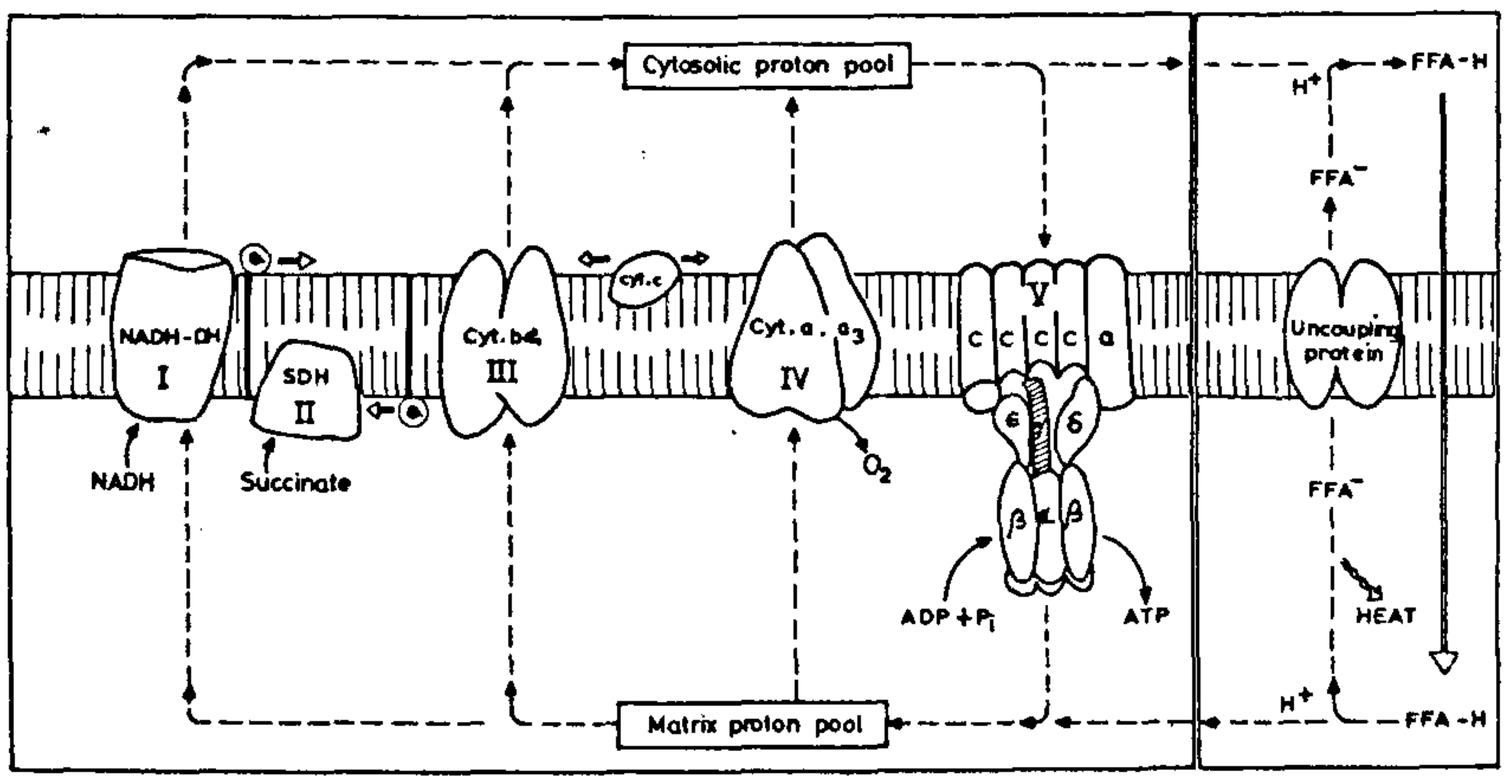

Figure 5. Complexes I–IV of electron transport chain. These are shown as transferring protons for matrix pool to cytosolic pool. According to Mitchell's chemiosmotic hypothesis, protons returning to matrix through F_0F_1 ATP synthase (complex V) synthesize ATP. Those returning through uncoupling protein in brown adipose tissue mitochondria generate heat in which free fatty acid shuttle is proposed to participate.

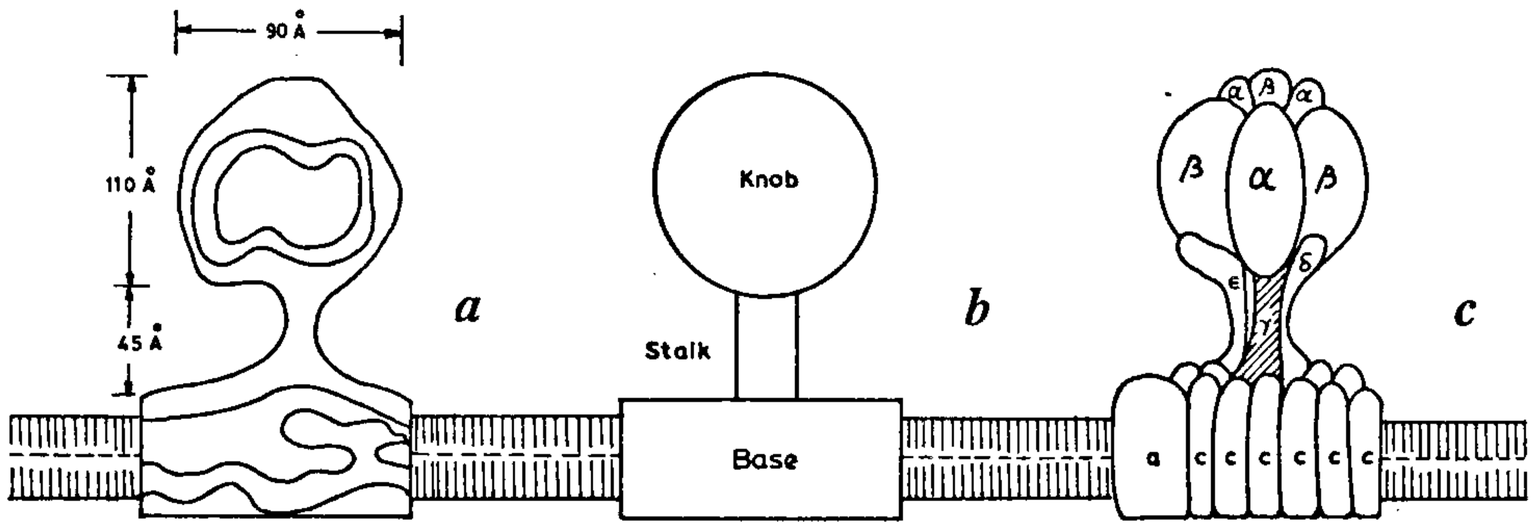

Figure 6. The tripartite structure of F_0F_1-ATPase. ***a***. A typical diagram obtained from a large number of studies on electron microscopy and image processing of F_0F_1-ATPase. ***b***. The dimensions vary to a small extent with different sources. The parts of knob, stalk and base are shown schematically. ***c***. The present understanding of the distribution of subunits. The γ-subunit, known to act as a rotor in the two stator units of knob and base, is shown hatched at the centre of the stalk (Adapted from refs 29 and 34).

The phenomenon of oxidative phosphorylation

The credit for discovery of oxidative phosphorylation is shared by Belitzer and Kalckar who independently showed in 1937 occurrence of respiration-dependent ATP synthesis. The ratio of phosphate esterified to oxygen consumed (P/O) for NADH-linked substrates was calculated by Ochoa to be three which showed an efficiency of nearly 50%. The cellular site of this process was identified in 1948 by Lehninger as the mitochondrion.

Oxidation of NADH and succinate and accompanying phosphorylation occur in protein complexes in the inner membrane of mitochondrion which can be separated into submitochondrial particles. A tripartite structure, consisting of a base embedded in the membrane linked by a stalk to a knob projecting into the matrix, was observed in the membrane by electron microscopic studies[26–28] (Figure 6). This is identified as the structure for ATP synthase, part of which also shows ATP hydrolysis. The soluble form of ATPase consisting of the knob and stalk portions was first isolated by Racker[8] in 1960 and designated as F_1-ATPase (F for factor). Work in the last three decades by many scientists all over the world brought out significance of F_1-ATPase which actually synthesizes ATP in the native state when associated with the base portion called F_0. Together F_0F_1-ATPase acts as ATP synthase, also known as complex V.

F_0F_1-ATPase

It is known that the tripartite structure of ATP synthase is ubiquitous in mammalian mitochondria, plant chloroplasts and bacterial plasma membranes. These are identified by prefixing an alphabet to indicate the source (Mitochondria (M), Chloroplasts (C), *E. coli* (E), Thermophilic bacterium (T)). ATP synthases are isolated and studied as F_0F_1-ATPase and its components. Interestingly, presence of Mg^{2+} is necessary to hold the two parts together. F_1 is soluble and shows ATPase activity after chelating with Mg^{2+} and extracting from the membrane, and the process is reversed by replacing Mg^{2+}.

It is now recognized that F_0F_1-ATPase possesses many subunits, a characteristic of these membrane-associated complexes[29]. Table 4 gives comparative information of the subunits of M, C and E derived from F_0F_1-ATPase. It is also known that the knob is formed by three units of dimer of the two major subunits $(\alpha\beta)_3$. This and its complex with γ-subunit, $(\alpha\beta)_3\gamma$ show ATPase activity. Both α- and β-subunits bind ATP but catalysis occurs only in β-subunits. The stalk has atleast three subunits. The γ-subunit provides link between F_0 and F_1 and thereby supports proton transfer. The δ- and ε-subunits, and the oligomycin-sensitivity conferring protein (OSCP) in M. F_1-ATPase, and in some cases subunit **b** seems to link the knob and base. These have a role in suppressing ATP hydrolysis in association with another protein IF_1 inhibitor factor-1 (IF_1) which is shown to be effective in non-helical form at lower pH (ref. 30). The MF_0 consists of large number of subunits compared to other sources: **a**, **b**, **c**, **d**, **e**, **f**, **g**, F_6, A6L (Table 4). Of these, **a b** $\mathbf{c}_n$ complex is of common occurrence. It will be of interest to know if the additional subunits found in MF_0 have a role in interacting with electron transfer complexes and this is discussed later. Together $\alpha_3\beta_3\gamma\delta\varepsilon$ (OSCP, IF_1 in MF_1) form F_1-ATPase, and $\mathbf{abc}_{9-12}$ (other subunits in MF_0) form F_0-ATPase. In the native state F_0 and F_1 form an interesting architecture as a unit and constitute ATP synthase.

The binding change mechanism

A new concept for energy coupling and ATP synthesis, introduced by Boyer[31] in 1973, perceives that energy is utilized for release of tightly-bound product, ATP, from the catalytic site. This hypothesis was based on the

Table 4. Subunit composition of F_0F_1–ATPases. The distribution of subunits in mitochondria, chloroplasts and *E. coli* is shown. The subunits of F_1 are given Greek letters in decreasing order of mol. wt. and that of F_0 in lower case alphabet. The mol. wt. are shown in parentheses as kDa

Main location	Mitochondria	Chloroplasts	*E. coli*	Function
Knob	α (55)	α (57)	α (55)	Structural
	β (52)	β (54)	β (50)	Catalytic
Stalk	γ (20)	γ (38)	γ (32)	Link between knob and base, rotates between β-subunits
	OSCP (21)	δ (21)	δ (21)	Structural, stator link between knob and base
	δ (15)	ε (15)	ε (15)	Associates with β-action
	ε (6)	–	–	–
	IF_1	–	–	Inhibits ATPase activity
Base	**a** (25)	**a**	**a** (30)	Helps H^+ movement
	b	**b**	**b** (17)	Not clear (one or two present), part of stator?
	c	**c**	**c** (8)	Multiple number, H^+ transfer
	d, e, f, g	–	–	Not known
	F_6, A6L, Factor B	–	–	H^+ conduction (?)

observation that exchange of ^{18}O between Pi-H_2O, an ADP-dependent reaction, was less sensitive to inhibition by uncouplers than Pi-ATP or ATP-H_2O reactions. This led to the proposal that ADP and Pi bind to the catalytic subunit of F_1 where spontaneous dehydration occurs to form ATP ($K_{eq} = 1$) with no energy input. ATP thus formed remains in a tightly bound state in the protein and is released consequent to energy-induced conformational change (Figure 7). This prompted Boyer[31] to make a fervent plea to 'overcome a limitation in our own thinking... that in net oxidative phosphorylation, as in substrate level phosphorylation, use of energy was limited to event occurring before or during but not after the formation of each ATP molecule'. A most unusual proposition.

Decades of study went on to explain how pyrophosphate in ATP is 'energy-rich' and its formation is now dismissed as energetically inconsequential[32], though, from the standpoint of teaching it is not fair to confuse a student of bioenergetics[33]. Undoubtedly, energy is needed to loosen tightly bound ATP. As will be seen later, all the reactions occur in concert – the binding of ADP + P_i, their dehydration to ATP and the release of the product – with energy used simultaneously by the protein complex. There is no way to infer that the binding and dehydration steps needed no energy as the active sites in these subunits are already prepared for action.

It is known that three β-subunits exist in three different conformations. Two have ATP and ADP bound and one has no nucleotide. All the three forms, viz. β-ATP, β-ADP and βE coexist. Negative cooperativity between the three sites in ATP binding was observed with tight binding of one with Kd in the picomolar range and the second in micromolar range and the third one hardly binding at all. These are also referred to as tight (T), loose (L) and open (O) states. Based on these binding changes of three states, a mechanism had been proposed with three β-subunits participating in the reaction in sequence[32]. On input of energy to F_0F_1-ATP synthase, the

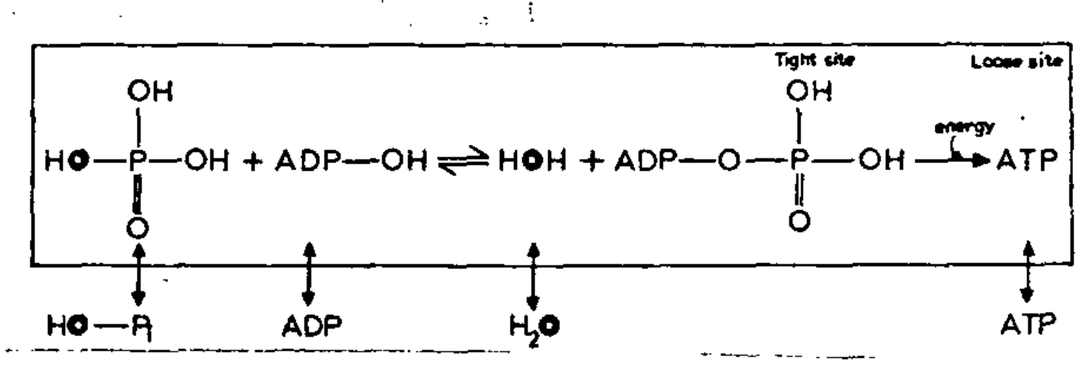

Figure 7. Conformation coupling hypothesis of ATP synthesis and release. The scheme proposed by Boyer[31] implies that P_i, ADP, H_2O and ATP can freely exchange with those bound in mitochondrial-ATP synthase. Thus ^{18}O (shown by bold O) can readily exchange between P_i and H_2O provided ADP is present. This does not involve loosely bound ATP and therefore does not depend on energy and is not sensitive to uncouplers. As shown, energy is needed for moving ATP from a tight-site to loose-site in the net synthesis of ATP. Note the dehydration reaction is spontaneous with no energy input.

evidence favours changes in the conformation at the three sites, from O to L, L to T and T to O, which will allow in concert binding of ADP + P_i, formation of ATP and release of ATP[34]. A cyclic conversion of $O \rightarrow L \rightarrow T$ and then $T \rightarrow O$ states occurs at each β-subunit, and three states are always present in the three β-subunits at any one time as shown in Figure 8.

Thus, there is elegance of the binding change mechanism which though drives a flux through a rotating system, maintains an overall constant structure. The same process occurs in the reverse direction for ATP hydrolysis by F_1-ATPase. Translocation of atleast three protons is necessary for net formation of ATP molecule[35], and requires energy input which not only releases ATP from T-state but also modifies the other two states. Boyer[36] himself is aware of this and expressed thus (p. 737): 'The concomitant change of a second site with bound ADP and P_i to the tight conformation may... provide part of the driving force of ATP release. The balance of the energy input from the proton translocation may be needed to change the conformation of the third site and to provide for modulation of the covalent bond-formation step.' The question is how to assess how

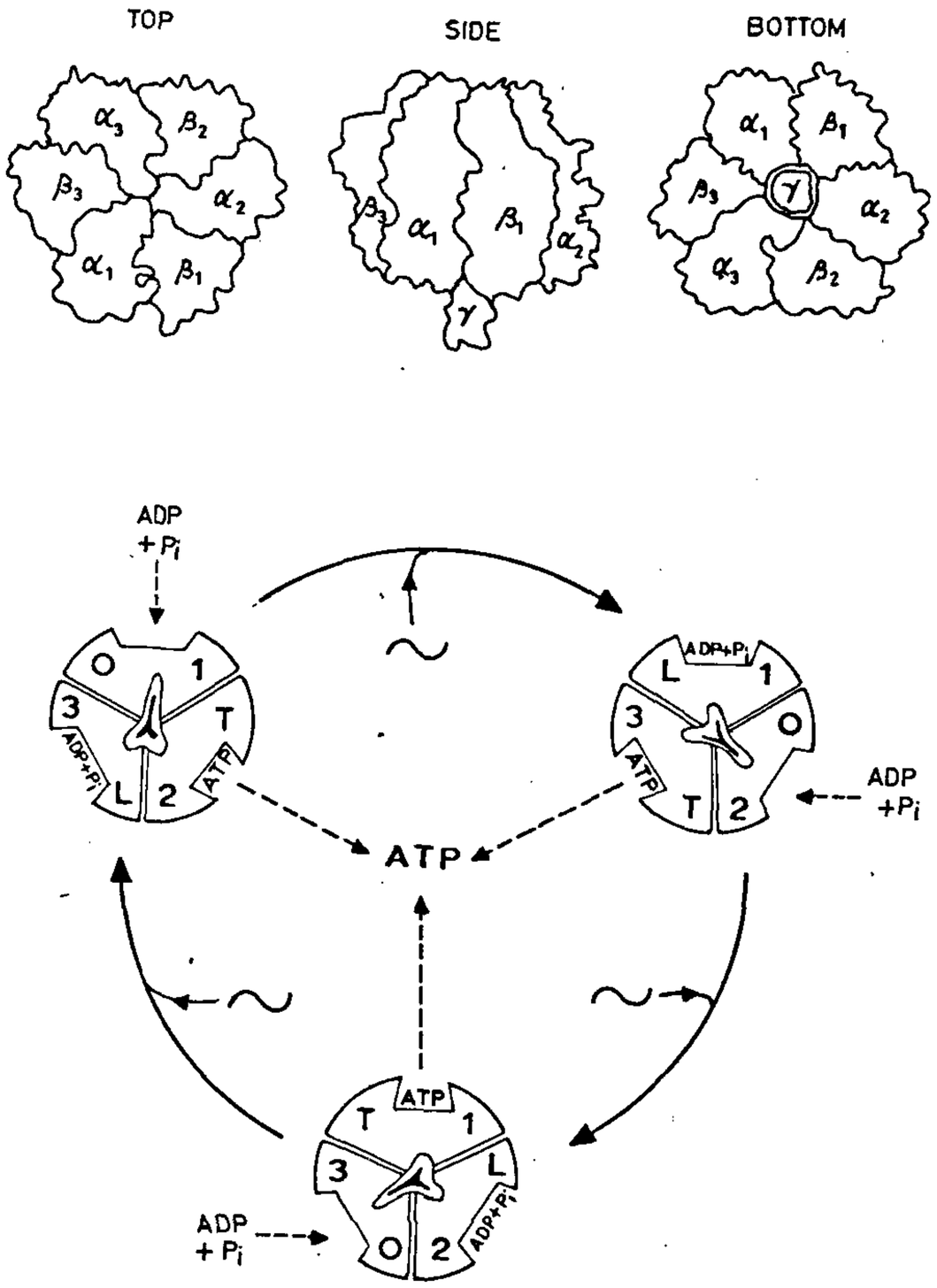

Figure 8. Binding change mechanism for ATP synthase action. The upper section shows top, side and bottom views of the three pairs of $\alpha\beta$ subunits and the central 'shaft' of γ-subunit, derived from the crustal structure of Walker[17]. The bottom section shows each of 1, 2 and 3 $\alpha\beta$ subunits undergoing open (O), loose (L) and tight (T) states in the cycle receiving energy (~) to convert ADP + P_i to ATP (Adapted from refs 32 and 34).

much energy is used in each part of L → T → O → L transitions.

Three-dimensional structure of F_1-ATPase

Bovine F_1-ATPase is the largest asymmetric structure solved by X-ray crystallography[16]. This achievement by the group in Cambridge (UK) earned Walker a share in 1997 Nobel Prize in Chemistry. The protein ($\alpha_3\beta_3\gamma$) was crystallized from a solution containing Mg^{2+}, ADP and a non-hydrolysable ATP derivative AMPPNP. Like 'segments of an orange' the major subunits, α and β are arranged alternately around a central shaft-like extended α-helical domains of C- and N-terminal portions of the γ-subunit. The three α-subunits had all AMPPNP bound, an essential structural requirement albeit they do not participate in catalysis or exchanges. Of the β-subunits, one was empty, another had ADP, and the third AMPPNP bound in the interior of the middle portion of the molecule as already mentioned. This supported the existence of three states of β-subunits, as proposed from binding and catalysis studies, and the cyclical binding change mechanism[32].

The γ-subunit is unique in more than one sense. Its C-terminus (64 amino acids 209–272) and N-terminus (45 amino acids, 1–45) form α-helices of about 90 Å and 60 Å respectively. These are unusually long helices in proteins and apparently designed for their shaft-like function. About half of this subunit is not clearly seen in crystal structure, and appears to fill the central space within the ring of C-subunits in the base. This along with a fitting in a sleeve-like circular assembly of middle portion of $(\alpha\beta)_3$ makes an ideal setting for its rotation.

The asymmetry of the entire molecule arises out of interactions of the γ-subunit by its positions in relation to β-subunit at 'catch' interaction of a loop of polar amino acids of sequence DELSEED at the lower portion in β-ADP and β-ATP which moves away in α–E (empty), and this brings another loop between G-helix and 7-sheet in the middle portion of β–E into 'catch' position with C-terminus of γ-subunit (Figure 9).

It is interesting to see that all the three α-subunits and the two β-subunits that bind a nucleotide have

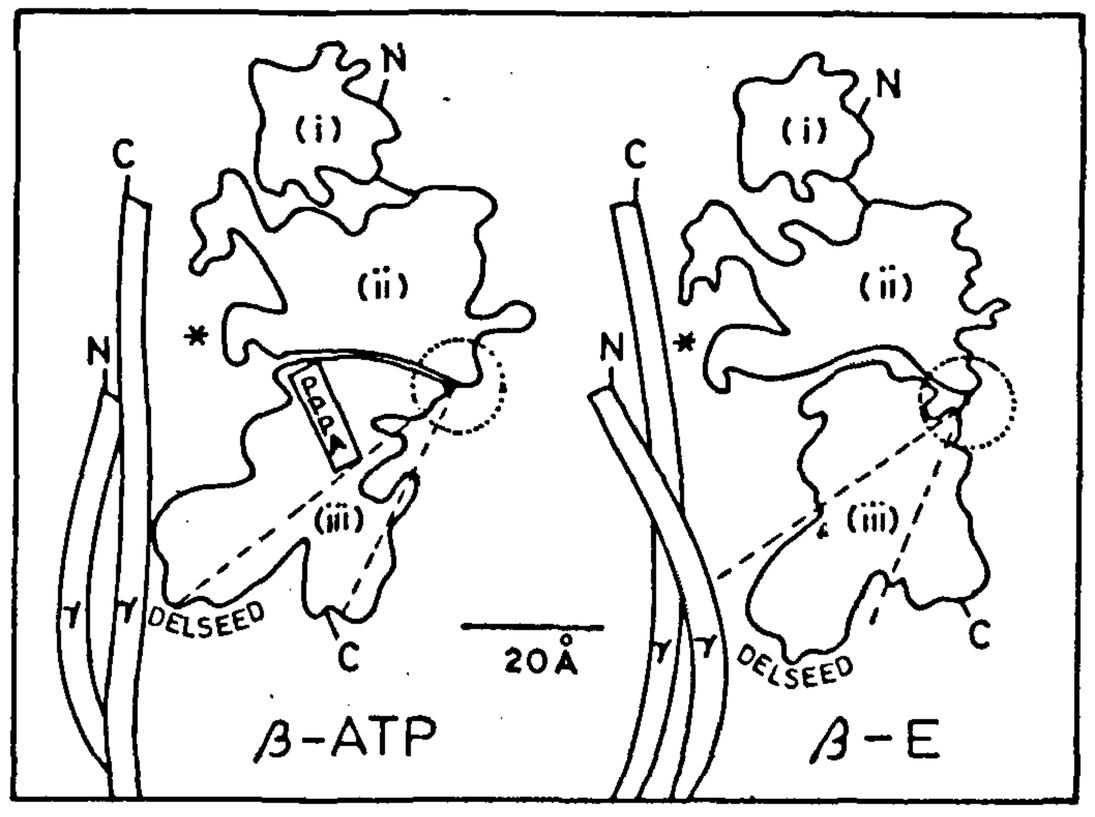

Figure 9. Gross differences in structure of β-subunit in β-ATP and β–E (empty) forms. In this stylized diagram adapted from ref. 17, the lines do not represent polypeptide chains but the boundaries within which the helices, sheets and coil of the polypeptide are located. The β-subunit is divided into three sections: (i) the N-terminal β-barrel with undetermined function; (ii) the domain just above the ATP-binding area, having the loop that makes a 'catch' with γ-subunit shown by *; (iii) the C-terminus domain with ATP-binding site in β-ATP indicated by a block, and having a second 'catch' loop at the bottom with the conserved sequence of DELSEED. This domain moves by about 30° from a hinge marked by broken circle at the right side between (ii) and (iii) in β-E compared to β-ATP, with consequent loss of structure for binding ATP and separation of DELSEED loop from γ-subunit by 20 Å. The broken lines starting from the hinge show positions of first D in DELSEED and C-terminus in β-ATP and these move away from these positions by several degrees.

essentially similar superimposable structures. The exception, however, is the β-subunit that has the catalytic site empty (β–E) in which the 'P-loop' and its holding sheet and helix are displaced and along with this the whole domain consisting of nucleotide-binding site and DELSEED-catch loop moves by 20 Å away from the γ-subunit. The bottom-half of the protein appears to have flipped by 30° from a point on the surface, corresponding to β-helix (159–178) that now gains an extra turn of helix and accompanying hydrogen bonds. This acts more like a hinge moving this domain upwards on losing the hydrogen bonding (Figure 9, encircled). Such a significant conformational change needs a guide. The 'noncatalytic' α-subunits, known to have sequence homology to heat shock protein (hsp-60) family[37], may assist in this process.

A great deal of information is awaited to understand how various parts of the protein work to achieve these changes. Could it be that breaks in hydrogen-bonds are propagated from the γ-subunits helix though this catch and helix G to helix B that results in 'hinge-like' action?

Rotational catalysis, a new concept in enzymology

The presence of multiple subunits in ATP synthase brings out a new meaning in having three identical β-subunits participate cooperatively in catalysis in sequence. Asymmetric interaction between γ-subunits with each of the three β-subunits alternately at the two catch sites, one above (DLTD) and one below (DELSEED) the nucleotide binding loop, confers a rotational action (Figure 9). Evidence for such a rotational change of γ-subunit came from many experiments listed in Table 5. Electron microscopy pictures showed movement of central mass relative to the knob structure[38,39]. Crosslinking of β-subunit with γ-subunit[40–43] as well as ε-subunit[44] inhibited the activity which could be reversed by removing the crosslink. However, no inhibiting effect was observed if the crosslinking was between δ-subunit and β-subunit[45] or α-subunit[46].

By using an elegant method of introducing labelled β-subunits[41,47], γ-subunit was shown to exchange partner of β-subunit during catalytic turnover. The first attempt at actually seeing the rotation of γ-subunit was made by attaching to it a fluorescent label and observing its polarized absorption–relaxation after photobleaching[48]. In 1997 Yoshida's laboratory[49] convincingly showed the actual rotation of γ-subunit with attached fluorescent actin filament on adding ATP. This justified calling F_0F_1-ATPase a 'splendid molecular machine' by Boyer[36], the smallest known molecular motor, and also a share for Boyer in 1997 Nobel Prize in Chemistry.

The $\alpha_3\beta_3\gamma$-subcomplex had to be attached to the F_0 portion for coupling to energy and proton translocation. Unlike F_1-subcomplex, composition of F_0 varies with

Table 5. Evidence for rotational catalysis in ATP synthesis

Experimental studies	Observations	Ref.
A. *Electron microscopy*		
1. EF_1 labelled with antibodies to α- and ε-subunits	Central mass moved in response to Mg.ATP	38
2. CF_1-similar study	Movement of central mass	39
B. *Cross-linking of subunits*		
1. MF_1 β–γ	Reversible inhibition	40
2. MF_1 β–γ	Inhibited, reversed by S–S reduction	41
3. EF_1 β–γ	Reversible inhibition	42
4. EF_1 β–γ	Inhibited, reversal by S–S reduction	43
5. EF_1 β–ε	Reversible inhibition	44
6. EF_1 β–δ	No effect, membrane attachment lost	45
7. EF_1 α–δ	No effect (part of stator)	46
C. *Exchange of radiolabelled subunits*		
1. Introduce radiolabelled β-subunits along with β–γ cross-linked subunits reduce, go through catalytic cycle (MF_1)	Position of γ-subunit randomized relative to labelled β-subunit	41
2. MF_0F_1 ATP synthase, similar experiment	Similar change in β-subunit blocked by DCCD indicating involvement of F_0-c-subunit	47
D. *Modification of γ-subunit*		
1. CF_1-subunit labelled with eosin-5-maleimide on a cysteine, polarized absorption relaxation after photobleaching	Rotation of γ-subunit relative to immobile β- and α-subunits	48
2. TF_1-$\alpha_3\beta_3\gamma$ attached to glass plate through β-subunits and a fluorescent actin filament (about 2 µm long) attached through streptavidin to γ-subunit (a classical experiment)	Rotation of actin filament on adding ATP visualized under a microscope	49

different sources (see Table 4). The common fixtures are one unit of **a** and multiple units (9–12) of **c**. One unit of **b** is present in several but not all. These subunits are transmembrane proteins and are embedded in the base. In the absence of crystal structure, models of F_0-subcomplex are based on electron microscopic pictures. Width seems to be same as the knob (about 100 Å). It is probably spherical, at least partially, with 75 Å diameter composed of multiple subunits of **c** (9–12) arranged as a wheel that is attached to a single **a**-subunit (see Figure 6).

To fit the rotational catalysis, Vick[50] proposed a model with conserved, DCCD-sensitive Asp 61 residues on the outside of the wheel at the centre of the transmembrane helix of **c**-subunits, with some of these hydrogen-bonded to His-245, Glu-219 and Arg-210 of **a**-subunit to form a 'proton wire'[51,52]. Such an arrangement places the charged residues in the middle of hydrophobic lipid bilayer. If these are inverted, these Asp-61 residues can form spokes inside the wheel, as in ion channels, which can be used to interact and hold in their centre the other half of γ-subunit, not seen beyond the stalk portion in the crystal structure. The torque is generated by 'energy input' which gets transmitted via the stalk to the knob. An electrostatic mechanism for gating of proton transport through a channel formed in the interface of subunits **a** and **c** had been proposed[53]. Reversible protonation of Asp-61 on **c**-subunits forms the core of this gating and moving the rotor (**c**$\gamma\varepsilon$) relative to stator (**ab**$\delta\alpha_3\beta_3$). This model implies a rotor role for **c**-wheel put to revolving motion by protons slipping past the **a**-subunit. However, evidence is awaited to establish this.

The entire complex network of proteins undergoes a simple dehydration reaction by using the energy presumably in the form of discharge of proton gradient. Conveniently, text-books show arrows for H^+ entering from the cytosolic side into the F_0 and returning to the matrix through the stalk as though it is a channel, and then to the F_1 where it exchanges energy and synthesizes ATP from ADP + P_i. But how are the events linked? With a mind-set benumbed to accept that proton gradient is the form of energy, the proposals are intuitive attempts to fit the available structures with the expected travel of protons. To an extent, these satisfy the objective, but are not sufficient to explain the overall process of energy transfer and chemical bond formation. It is noticed that all the changes occur within the polypeptides of the protein complex, including those based in the lipid bilayer. Whither membranes?

Role of membrane in energy transfer

One of the least appreciated assets of membrane is its ability to mobilize protein complexes and not merely anchor them. Membrane lipids have a high content of unsaturated fatty acids which confers low viscosity, thereby allowing diffusion of proteins and lipids. The relative proportion of lipid in mitochondrial inner membrane is strangely small, with a lipid : protein ratio of about 1:3. With such an obvious high protein density, most of it must be extending beyond the lipid bilayer. It is known that in some cases removal of bulk lipid by solvent extraction does not collapse the vesicle structure. The large protein complexes and the mobile electron carriers, cytochrome *c* and ubiquinone, are distributed randomly in the membranes. Gupte *et al.*[54] found that these can move freely by lateral diffusion with frequencies of collision higher than their turnover numbers.

The electron transfer complexes and ATP synthase do seem to have a direct 'cross-talk'. This unique facility can be employed for transfer of energy without dissipation throughout intermediate mobile 'energy carrier proteins'. These can shuttle between 'generators' and 'utilizers' in the overall energy transduction. Chargerins, which are proteolipids (8 and 13 kDa) isolated from mitochondria, have properties that fit this role[55]. Their binding to anisotropic inhibitor monoazide ethidium is enhanced 4-fold on oxidation-dependent membrane energization, and their antibodies inhibit ATP synthesis. One of these is identified as the A6L protein[56] among the many subunits now found in F_0 of mammalian mitochondria. Is it that some of these, with unassigned function, act as such energy carriers and shuttle between redox complexes and ATP synthase, possibly associating with both? Do they carry back energy to redox carriers in the reverse from ATP? Mobility and interaction between the components will be far more efficient than if they are designed to do the same as soluble enzymes. Is this a vignette of the wisdom of membrane architecture?

Lest we forget water and hydrogen bond

Water is the smallest and most abundant molecule in living cells. Water would not be in liquid form without its ordered hydrogen bond network, and with its large specific heat it is a veritable sink of heat. To vaporize one molecule of water requires breaking of four hydrogen bonds and energy equivalent of 10 kcal. This process accounts for 20% of solar energy captured on earth. In the reverse the energy is lost into space and water comes down as rain and source of fresh water. Involved in this magnificent phenomenon is the basic process of energy exchange through reversible hydrogen bond formation. An unnoticed statement of Guttfreund[57] in 1972 is most appropriate in this context, 'All biological processes are either directly or indirectly under the influence of some of the characteristic properties of water.' More so the dictum Pauling[58] made in his cele-

brated book on *The Nature of Chemical Bond*, 'It will be found that the significance of hydrogen bond for physiology is far greater than that of any other single structural feature.' An emphatic prophecy. Can hydrogen bond, a characteristic of water, perform such energy exchange in the secondary structure of proteins? Based on these thoughts a proposal is developed for linking hydrogen bond and energy exchange. Folding into α-helix form is a natural tendency of a polypeptide, especially in the hydrophobic environment. Having been placed in membrane the complexes I–V have a high percentage of helical content. Even the $(\alpha\beta)_3$ domain in ATP synthase projected into the cytosol has a large hydrogen-bonding structure with the peptide bonds and side-chains. The amazing C-terminus of γ-subunit is probably the longest known α-helix. And this subunit is the key player in energy transfer.

Based on water–steam analogy we proposed in 1974 that a polypeptide has the potential for holding and transferring of energy (4–8 kcal in hydrophobic membrane) by reversibly breaking a pair of hydrogen bonds in α-helix[59]. Thermalization of this energy is probably avoided in hydrophobic environment and membrane localization will thereby give an advantage. The process is visualized as follows: An energy carrier protein in the membrane acquires oxidation energy from an electron transfer complex by breaking a pair of its own hydrogen bonds, keeps mobile in the membrane until it reaches F_0-base and exchanges the energy in the same way[60]. Illustrated in Figure 10, this provides an alternative extraordinary way of energy transfer that a membrane could do. One could see the underlying potential of the accompanying small conformation change. A set of such changes together become amplified into a large structural change at the domain level. Walker[16] and Kagawa[61] described a disruption in the β-sheet and formation of an additional turn in β-helix close to the position of nucleotide-binding 'P-loop', when ATP is released. It will be interesting to see if a correlation of such changes and energy transfer exists.

Magnesium – the water (O) diviner

Synthesis of ATP superficially is a reversal of hydrolysis and thereby a simple dehydration is shown in Figure 7. The elements of water ($H^+ + {}^-OH$) have to be removed from the hydroxyl groups of ADP and P_i. It is known for a long time from ^{18}O exchange studies[62] that ADP retains its oxygen and P_i loses its oxygen to water. It is the inorganic phosphate that becomes activated by attaching first to 'handles' as in GAPD and enolase reactions

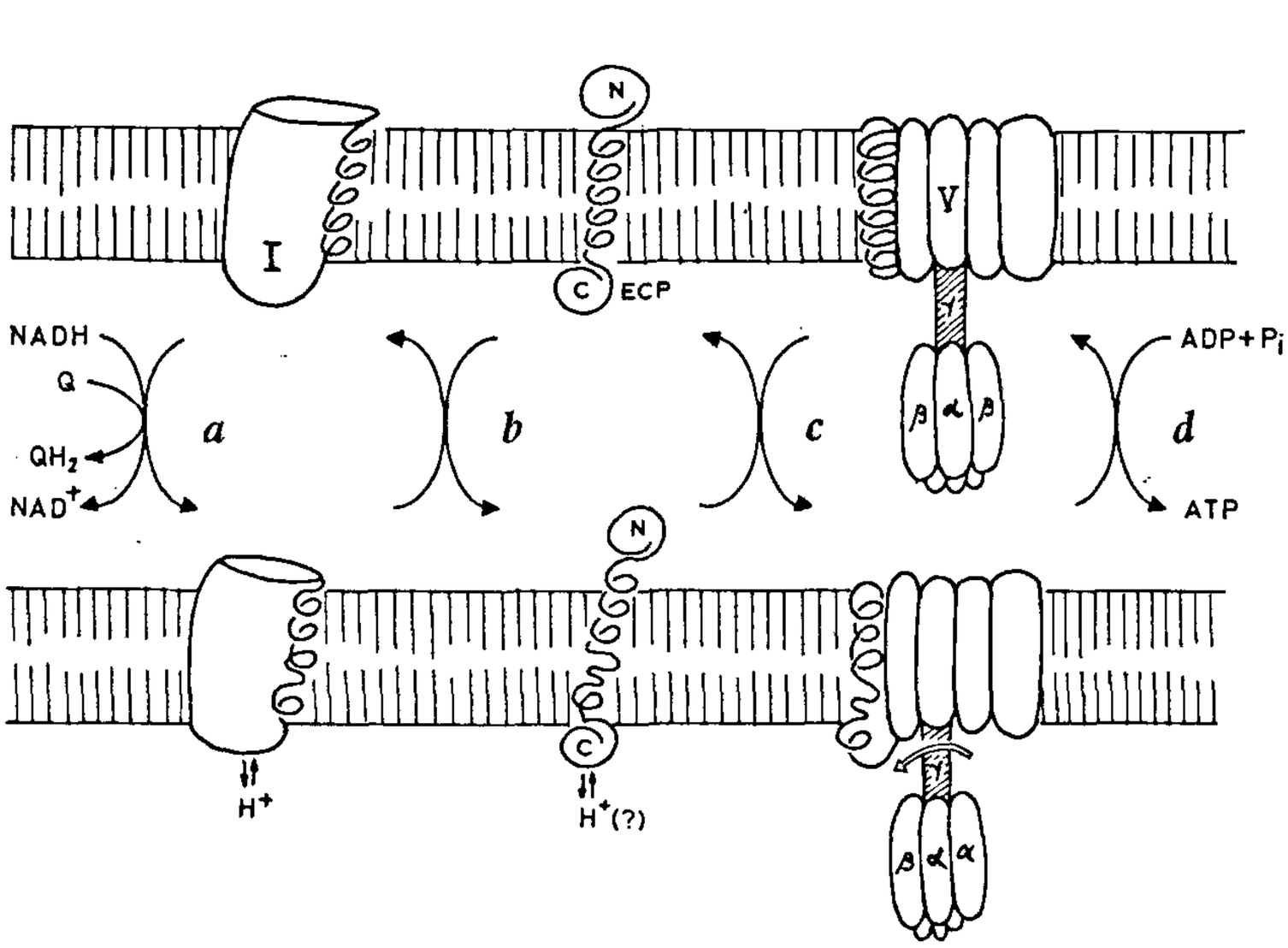

Figure 10. Alternative way of energy transfer using hydrogen bond exchange. A highly speculative proposal of linking energy of oxidation of NADH by complex I and ATP synthesis by complex V through a hypothetical energy carrier protein (ECP). ***a***. On oxidation of NADH by ubiquinone, energy is used to break hydrogen bonds in a segment of α-helix in complex I. ***b***. The mobile ECP in the membrane picks up the energy by breaking its own hydrogen bonds. The possibility of release and reuptake of H^+ in these steps is also indicated. ***c***. The energy is passed on to subunits in F_0 in the same way, which in turn is used to rotate the highly helical γ-subunit. ***d***. Coupled to this mechanical change, dehydration of ADP + P_i and ATP synthesis occurs (Adapted from refs 59 and 6).

and then forms an electrophile, P^+, accepted readily by ADP–O^-. The energy requirement is ultimately for processing the active P^+ form.

The crucial step in ATP synthesis then is labilization of P–O bond. Mitchell[63] even proposed that 'energized' protons (whatever they may be) can strip an oxygen from phosphate, an unlikely event. The role of Mg^{2+} although recognized as absolutely essential had always remained an undercurrent of its obvious chelation. An extended basis of action of Mg^{2+} was conjectured that the energy available on restoring a pair of hydrogen bonds in the protein will help Mg^{2+} pull away the oxygen from P–O and shed it as ^-OH in the medium[15]. An equally vague scheme except for implication of Mg^{2+} is oxygen extraction. There are indications that Mg^{2+} helps in tightening structures of these energy transducing proteins. We surmised Mg^{2+} may cause pulling of N–N into proper position with O–O of the adjacent hydrogen bonds in α-helix[15]. In some step, Mg^{2+} becomes partially electron-deficient which it will make up by an electron shift from P–O bond followed by break to give P^+ and Mg–OH (Figure 11). Cross[34] elaborated on this and on Mg^{2+} coordination with α- and β-phosphate oxygens of ADP as well as carboxyl oxygens of aspartate or glutamate of the protein. Electron withdrawal by Mg^{2+} from phosphate, possibly held by lysine, facilitates OH^- elimination from P–OH which is then transferred to the negative-charged ADP–O^- to make ATP.

An interesting common mechanism for all polymerases involving transfer of α-phosphorus of dNTP to 3′-OH of ribose was suggested by Tietz[64], involving two divalent metal ions coordinated to two aspartate–carboxyls with a split of α–P–O bond. Actions of Mg^{2+} will include chelation to carry ADP in and ATP out of the protein, change separation on N- and O-groups of protein and nucleotides, and as an intermediate in oxygen transfer from P–O to water. These actions of Mg^{2+} (equivalent to a water diviner) to find a P–OH or C–OH and remove it as water may form the crux in the dehydration reactions in ATP synthase and enolase as both use Mg^{2+} and aspartate, glutamate and lysine in the sites of action.

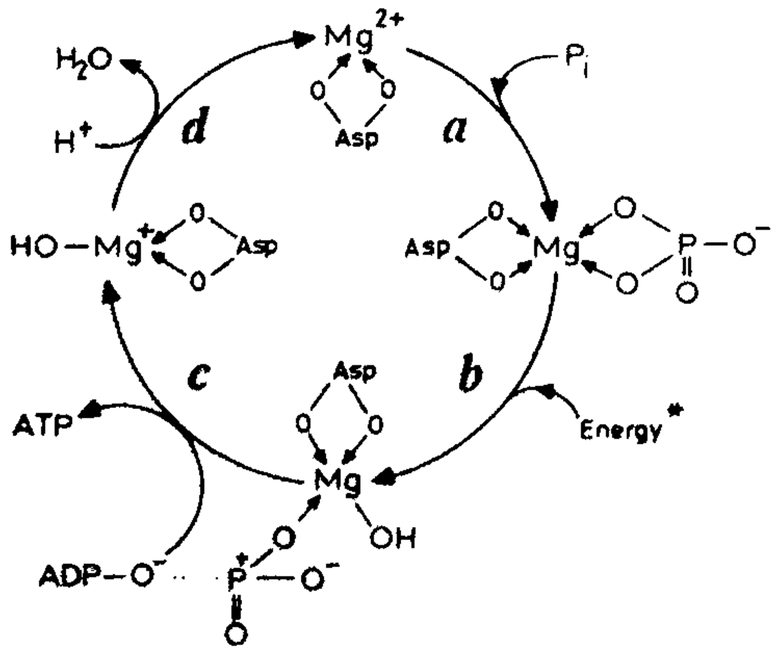

Figure 11. A role of Mg^{2+} in labilization of phosphate oxygen. The scheme proposes the essential role of Mg^{2+} in extracting an 'O' from P–O. *a*. Coordination complex of Asp–Mg–O. *b*. Energy is used in Mg pulling an 'O' leaving a P^+. *c*. The P^+ is transferred to ADP O to make ATP. *d*. Mg–OH discards OH in medium to make water (Adapted from refs 15 and 34).

Conclusion

Over the last four decades, energized state of membranes and oxidative phosphorylation have been the subjects of much speculation and intense investigations. Some of the questions remain unanswered namely: what is membrane for, what is happening therein and what forms the basis of energy transduction. A recent review on rotary chemiosmotic machines by Khan[65] brought out interesting comparison of flagellar and F_0F_1-ATP synthase motor proteins. We may find some answers by visualizing a mechanical basis for bringing molecules together for bond formation. Should some of the proposals made be proved right, 'hydrogen bond' will once again prove to be the very foundation of life processes.

1. Fiske, C. H. and SubbaRow, Y., *Science*, 1929, **70**, 381–382.
2. Lohmann, K., *Naturwissenschaften*, 1929, **17**, 624–625.
3. Maruyama, K., *J. History Biol.*, 1991, **24**, 145–154.
4. Baddiley, J., Michelson, A. M. and Todd, A. R., *J. Chem. Soc.*, 1949, 582–590.
5. Lipmann, F., *Adv. Enzymol.*, 1941, **1**, 99–162.
6. Lalitha, R., Kalpana, G. V. and Ramasarma, T., *Indian J. Biochem. Biophys.*, 1986, **23**, 204–207.
7. Skou, J. C., *Biochim. Biophys. Acta*, 1957, **23**, 394–401.
8. Penefsky, H. S., Pullman, M. E., Datta, A. and Racker, E., *J. Biol. Chem.*, 1960, **235**, 3330–3336.
9. Corobridge, D. E. C., *Acta Crystallogr.*, 1971, **B27**, 291–296.
10. Kennard, O., Isaacs, N. W., Motherwell, W. D. S., Coppala, J. C., Wampler, D. L., Larson, A. C. and Watson, D. G., *Proc. R. Soc. London*, 1971, **A325**, 401–436.
11. Hers, H. G., *Biochim. Biophys. Acta*, 1952, **8**, 416–432.
12. Ramasarma, T. and Wetter, L. R., *Can. J. Biochem. Physiol.*, 1957, **35**, 853–863.
13. Szent-Gyorgyi, A., in *Enzymes: Units of Biological Structure and Function* (ed. Gaebler, O. H.), Academic Press, New York, 1956, p. 393.
14. Epp, A., Ramasarma, T. and Wetter, L. R., *J. Am. Chem. Soc.*, 1958, **80**, 724–727.
15. Ramasarma, T., *Trans. Bose Res. Inst. Calcutta*, 1982, **45**, 1–18.
16. Walker, J. E., Saraste, M., Runswick, M. J. and Gay, N. Y., *EMBO J.*, 1982, **1**, 945–951.
17. Abrahams, J. P., Leslie, A. G. W., Lutter, R. and Walker, J. E., *Nature*, 1994, **370**, 621–628.
18. Weber, J. and Senior, A. E., *Biochim Biophys. Acta*, 1997, **1231**, 223–246.
19. Yoshida, M. and Amano, T., *FEBS Lett.*, 1995, **359**, 1–5.
20. Harris, J. I. and Waters, M., *The Enzymes*, 1974, **13**, 1–49.
21. Vaidyanathan, V. V., Sastry, P. S. and Ramasarma, T., *Biochim. Biophys. Acta*, 1993, **1203**, 34–36.
22. Lebioda, L., Stec, B. and Brewer, J. M., *J. Biol. Chem.*, 1989, **264**, 3685–3692.
23. Mitchell, P., *Nature*, 1961, **191**, 144–148.
24. Lin, C. and Klingenberg, M., *Biochemistry*, 1982, **21**, 2950–2956.

25. Ramasarma, T., Seshadri Sekhar, B. and Ramakrishna Kurup, C. K., in *Bioenergetics: Structure and Function of Energy Transducing Systems* (eds Ozawa, T. and Papa, S.), Japan Sci. Soc. Press, Tokyo, 1987, pp. 225–233.
26. Kagawa, Y., Sone, N., Hirata, H. and Okamoto, H., *J. Biochem.*, 1976, **30**, 141–151.
27. Lunsdorf, H., Ehrig, K., Freidl, P. and Schaierer, H. U., *J. Mol. Biol.*, 1984, **173**, 131–136.
28. Boekema, E. J., Berden, J. A. and Von Heel, M. G., *Biochim. Biophys. Acta*, 1986, **851**, 353–360.
29. Senior, A. E., *Physiol. Rev.*, 1988, **68**, 177–231.
30. Sah, J. F., Kumar, C. and Mohanty, P., *Biochem. Biophys. Res. Commun.*, 1993, **194**, 1521–1529.
31. Boyer, P. D., Cross, R. L. and Momsen, W., *Proc. Natl. Acad. Sci. USA*, 1973, **70**, 2837–2839.
32. Boyer, P. D., *Biochim. Biophys. Acta*, 1993, **1140**, 215–250.
33. Ramasarma, T., *Biochem. Educ.*, 1982, **10**, 153.
34. Cross, R. L., *Am. Rev. Biochem.*, 1981, **50**, 681–714.
35. van Walraven, H. S., Strotmann, H., Schwarz, O. and Rumberg, B., *FEBS Lett.*, 1996, **379**, 309–313.
36. Boyer, P. D., *Annu. Rev. Biochem.*, 1997, **66**, 717–747.
37. Alaconda, A., Flores, A. I., Blanco, L. and Cuezra, J. M., *J. Biol. Chem.*, 1994, **269**, 13670–13679.
38. Gogol, E. P., Johnson, F., Aggeler, R. and Capaldi, R. A., *Proc. Natl. Acad. Sci. USA*, 1990, **86**, 9585–9589.
39. Boekema, E. G. and Bottcher, B., *Biochim. Biophys. Acta*, 1992, **1098**, 131–143.
40. Kandapal, R. P. and Boyer, P. D., *Biochim. Biophys. Acta*, 1987, **890**, 95–105.
41. Duncan, T. M., Bulygin, V., Zhou, Y., Hutcheon, M. L. and Cross, R. L., *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 10964–10968.
42. Aggeler, R., Cai, S. X., Keana, J. F., Koike, T. and Capaldi, R. A., *J. Biol. Chem.*, 1993, **268**, 20831–20837.
43. Aggeler, R., Haughton, M. A. and Capaldi, R. A., *J. Biol. Chem.*, 1995, **2720**, 9185–9191.
44. Aggeler, R. and Capaldi, R. A., *J. Biol. Chem.*, 1996, **271**, 13888–13891.
45. Tozer, R. G. and Dunn, S. D., *Eur. J. Biochem.*, 1987, **161**, 513–518.
46. Ogilvie, I., Aggeler, R. and Capaldi,, R. A., *J. Biol. Chem.*, 1997, **272**, 16652–16656.
47. Zho, Y., Duncan, T. M., Bulygin, V. V., Hutcheon, M. L. and Cross, R. L., *Biochim. Biophys. Acta*, 1996, **1275**, 96–100.
48. Sabbert, D., Engelbrecht, S. and Junge, W., *Nature*, 1996, **381**, 623–625.
49. Noji, H., Yasuda, R., Yoshida, M. and Kinosita, J. K., *Nature*, 1997, **386**, 299–302.
50. Vick, S. B. and Antonio, B. J., *J. Biol. Chem.*, 1994, **269**, 30364–30369.
51. Nagle, J. and Tristram-Nagle, S., *J. Mem. Biol.*, 1983, **74**, 1–14.
52. Hoppe, J. and Sebald, W., *Biochim. Biophys. Acta*, 1984, **768**, 1–27.
53. Elston, T., Wang, H. and Oster, G., *Nature*, 1998, **391**, 510–513.
54. Gupte, S., Wu, E. S., Hoechli, L., Jacobsen, K., Sowers, A. E. and Hackenbrock, C. R., *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 2606–2610.
55. Higuti, T., Takigawa, M., Kotera, Y., Oka, H., Uchida, J., Arakaki, R., Fujita, T. and Ogawa, T., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 1331–1335.
56. Higuti, T., Negamo, T., Takigawa, M., Uchida, J., Yamane, T., Asai, T., Tani, I., Oeda, K., Shimizu, M., Nakamura, K. and Ohkawa, H., *J. Biol. Chem.*, 1988, **263**, 6772–6776.
57. Gutfreund, H., in *Enzymes: Physical Principles*, Wiley-Interscience, London, 1972.
58. Pauling, L., in *The Nature of Chemical Bond*, Cornell Univ. Press, Ithaca, New York, 3rd edition, 1960, p. 450.
59. Ramasarma, T. and Susheela, L., in *Biomembranes: Architecture, Biogenesis, Bioenergetics and Differentiation* (ed. Packer, L.), Academic Press, New York, 1994, pp. 261–277.
60. Ramasarma, T., in *Trends in Bioenergetics and Biotechnological Processes* (eds Singhal, G. S. and Ramasarma, T.), Today & Tomorrow's Printers and Publishers, New Delhi, 1991, pp. 5–17.
61. Kagawa, Y. and Hamamato, T., *Biochem. Biophys. Res. Commun.*, 1997, **240**, 247–256.
62. Boyer, P. D., Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto, Maruzen Publishers, London, 1957, pp. 301–307.
63. Mitchell, P., *FEBS Lett.*, 1974, **43**, 189–194.
64. Tietz, T. A., *Nature*, 1998, **391**, 231–232.
65. Khan, S., *Biochim. Biophys. Acta*, 1997, **1322**, 86–105.

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