Hypothesis

CATION-TRANSLOCATING ADENOSINE TRIPHOSPHATASE MODELS: HOW DIRECT IS THE PARTICIPATION OF ADENOSINE TRIPHOSPHATE AND ITS HYDROLYSIS PRODUCTS IN CATION TRANSLOCATION?

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1. Introduction

The biochemical studies of the substrate-specific catalysis of solute transport through natural membranes that began to become popular about twenty years ago gave rise to the suggestion that permeation reactions of high specificity may occur by a process quite analogous to that of enzyme reactions, and that the thermal movement in an enzyme–substrate complex by which reactants are converted to resultants corresponds to the thermal movement in a catalytic carrier complex by which a passenger substrate moves from the phase on one side of a membrane to that on the other. This comparison between reactions catalysed by enzymes and by catalytic carriers led to the principle of enzyme-catalysed group translocation, in which the catalytic protein was conceived as the specifically mobile medium through which chemical groups could be translocated from a specific pathway of access for the group donor to a specific and spatially separate pathway of access for the group acceptor [1–4].

In a discussion of the application of the group-translocation principle to the mechanism of the Na^+/K^+-translocating ATPase, it was pointed out that the spatial flow of Na^+ and K^+ in this enzyme system should be related somehow to the spatial flow of ATP and its hydrolysis products as they pass through a cyclic system of transition states in the anisotropic ATPase complex. The coupling of the translocation of the cations Na^+ and K^+ to the metabolic reaction of ATP hydrolysis was accordingly attributed to a specifically articulated conformational mobility of the anisotropic ATPase complex designed (by natural selection) to link the translocation of Na^+ and K^+ across the ATPase complex to the translocation of ATP and its hydrolysis products along prescribed pathways interconnecting in phosphoryl group translocation through the active centre region of the complex [4]. Within this general concept, the notion that phosphoryl group translocation might occur through the ATPase complex, along a pathway closely related to that of the translocation of the cations, appeared to be particularly attractive because the ATP and its hydrolysis products might thus participate relatively directly in cation translocation. The possibility that the cations might be translocated across the membrane with the anionic reactants had, in fact, been suggested earlier [5] in view of the work of Lowenstein [6], who observed partially independent and additive catalytic effects of pairs of cation species, such as K^+ and Mn^{2+}, on the non-enzymic transfer of the terminal phosphoryl group of ATP to inorganic phosphate, yielding pyrophosphate. At that time there was no evidence for the production of a phosphorylated intermediate by the Na^+/K^+-translocating ATPase, and in one reading of the mechanisms proposed for this enzyme reaction [4], it was assumed, as illustrated in fig. 1, that phosphoryl-group translocation across the membrane might involve the specific mobility of ATP as a complex with Na^+, and that after conversion of the ATP to $ADP^- + P_i$ by the group-acceptor OH^- in the

Abbreviations:
Inorganic orthophosphate, usually abbreviated as P_i, is written as POH in contexts illustrating the addition of the elements of H_2O in hydrolysis.

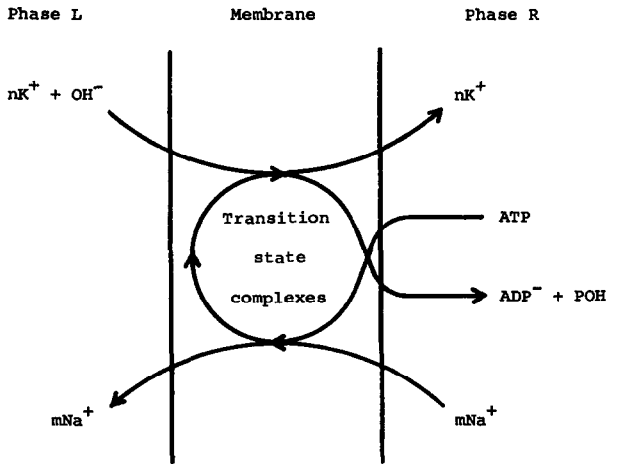

Fig. 1. Diagram of Na^+/K^+-translocating ATPase, illustrating a type of mechanism suggested previously (fig. 6A in [4]).

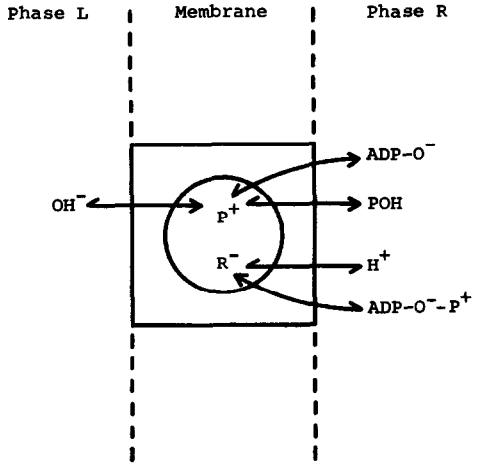

Fig. 2. Diagram of proton-translocating ATPase (after fig. 1 in [9]). The (reversible) hydrolysis is represented by the entry of ATP ($ADP–O^-–P^+$) from the right, the attack of the group R^- on the phosphorylium group (P^+) of ATP, followed by the attack of OH^- from the left on the phosphorylium group, and the exit of POH and $ADP–O^-$ to the right. It uses the same general principle of direct coupling between proton translocation and the translocation of ATP and ADP as that illustrated in figs. 3A and 5A, but with a stoichiometry of only one H^+ translocated per hydrolytic cycle instead of two.

outer phase, the $ADP^- + P_i$ might move inwards again as a complex with K^+.

The general group-translocation conception of the mechanism responsible for the coupling between Na^+ and K^+ transport and ATP hydrolysis has much in common with the more usual view of the attachment of the Na^+ and K^+ ions to specific carrier groups in the ATPase system, the affinity of which is supposed to depend on the progress of the ATPase reaction (see [7, 8]). However, there is a difference of emphasis because my object has been to attribute the *translocational movement* of the Na^+ and K^+ passengers to as direct a coupling as possible with the *diffusion* of the anionic reactants and resultants, ATP, ADP and P_i, catalysed by the group-translocating ATPase system [4].

The mechanism originally proposed for the proton-translocating ATPase of mitochondria [9] also included a direct coupling between the translocation of the proton and the translocation of the terminal phosphate groups of ATP into and out of the active centre region of the ATPase complex. As illustrated in fig. 2, the proton was represented [9] as being translocated from phase R to the active-centre region of the ATPase complex in a protonated form of ATP (written as $ADP–O^-–P^+$) which, after hydroxylation from phase L, was dephosphorylated and returned to phase R in the anionic form (written as $ADP–O^-$). The straight arrow with single barbs showed an additional pathway of equilibration of H^+ between the active centre and phase R, but, as indicated by the single barbs, no net transfer of H^+ was assumed to take place through this additional pathway and it could have been omitted without changing the stoichiometric or thermodynamic properties of the proposed mechanism. The type of mechanism shown in fig. 2 is clearly capable of more sophisticated development [10], as discussed further below.

A gate type of mechanism of the Na^+/K^+-translocating ATPase was proposed by Lowe [11], which depended on the opening of a cleft through the ATPase "molecule" alternately from either side, as in a phosphoryl-translocating enzyme proposed earlier [2]. The Na^+ and K^+ were not actually considered by Lowe [11] to be translocated together with the ATP, ADP and phosphate, but we might adapt this mechanism by assuming that the alkali-metal ions are primarily bonded to the reactants and resultants in the ATPase

complex with essentially the same result as in the examples given above.

2. Explicit description of model systems

In order to make further progress in exploring the possibility that ATP and its hydrolysis products may participate relatively directly in the carriage of ions by the cation-translocating ATPase, it is desirable to give a more explicit description of the fundamental requirements of this particular type of system.

We assume, in accordance with figs. 1 and 2, that the active centre region for the transfer of the phosphoryl group of ATP is accessible to the ATP, ADP and phosphate that is present in the aqueous phase R, but is also accessible to certain components of the aqueous phase L. The hydrolysis of ATP from the

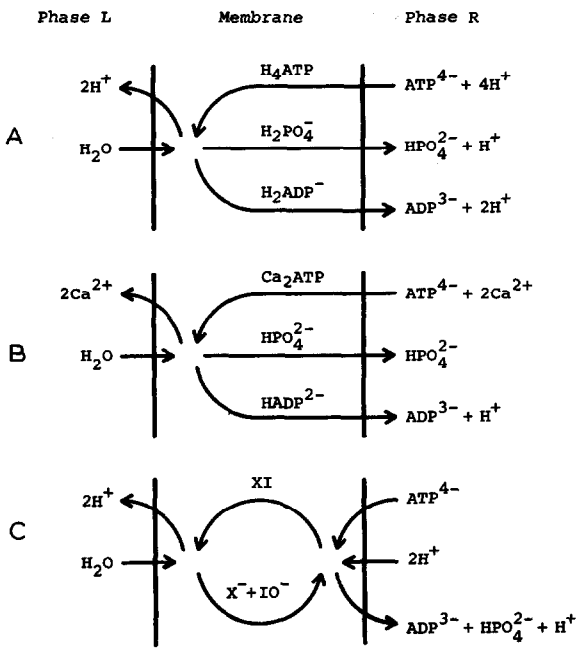

Fig. 3. Possible schemes for proton-translocating and Ca^{2+}-translocating ATPases, comparing mechanisms utilising ATP and its hydrolysis products as cation carriers (A and B), with a corresponding proton-translocating mechanism involving other specialised groups in the ATPase (X^- and IO^-) as proton carriers (C) as described previously [13]. For simplicity in representing the approximate ionisation states of the phosphate groups in phase R, the pH of that phase is taken as being near 8.

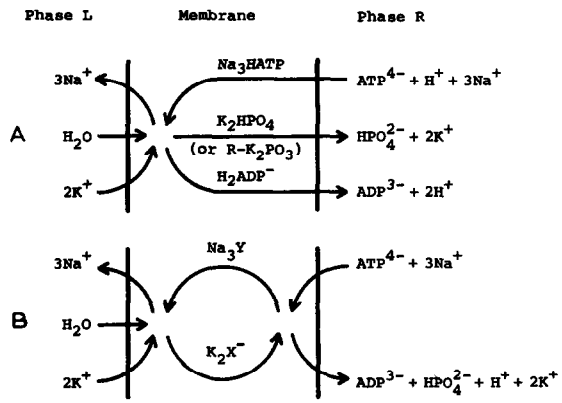

Fig. 4. Possible schemes for the Na^+/K^+-translocating ATPase, comparing a mechanism utilising ATP and its hydrolysis products as cation carriers (A), with a corresponding mechanism, analogous to that of Shaw (see [8]), involving the specialised carrier groups X and Y in the ATPase (B). The electrogenicity of scheme B represents a sophistication of the original scheme of Shaw. In scheme A, an alternative possibility for the translocation of phosphate as a derivative with the group R in the ATPase is shown in brackets, and the final hydrolytic step (not shown) would correspondingly occur in contact with phase R. The pH convention for phase R is as in fig. 3.

aqueous phase R is assumed to depend on the specific mobility of the ATPase complex that permits the terminal phosphate groups of the ATP *in a given state of protonation and salt formation* to enter and pass through the specific pathway leading to the active centre for phosphoryl transfer. In the region of this site, the phosphate groups of the ATP in the enzyme–nucleotide complex are supposed to be able to come into acid–base and ionic equilibrium with the aqueous phase L, but the acid–base and ionic dissociation coefficients would not generally correspond to those of free ATP, ADP and orthophosphate in aqueous solution. After transfer of the phosphoryl group from ATP, the reaction is supposed to be completed by the return of the ADP and of phosphate (possibly as a covalent derivative) *in given states of protonation and salt formation* through the specific pathways communicating with the aqueous phase R. The acid–base and ionic equilibration of the terminal phosphate groups of ATP and its reaction products with aqueous phase L may presumably be dependent on the same tightly articulated conformational changes of the system as are obligatory for the hydrolytic or phosphoryl-transfer reactions. The fundamental notion is

that the pathway of phosphoryl group translocation corresponding to the course of the ATPase reaction passes effectively across the osmotic barrier phase of the membrane and back again.

Possible translocation schemes for the mitochondrial proton-translocating ATPase, the Ca^+-translocating ATPase [12] and the Na^+/K^+-translocating ATPase are shown in fig. 3A, B and fig. 4A, respectively, the pH of phase R being taken to be near 8 for simplicity in representing the approximate ionisation states of the phosphate groups. These diagrams are intended to illustrate the general principle of the translocation-carrier participation of the adenine nucleotides, and are not supposed to be exclusive of a number of other possibilities accounting for the same overall translocation stoichiometries by means of different combinations of translocation specificities for salt and acid–base species of ATP, ADP and phosphate. For example, as indicated previously [10], in the proton-translocating ATPase, the ATP and ADP translocation channels might be specific for MgH_2ATP and $MgADP^-$, respectively; or in the Na^+/K^+-translocating ATPase, the phosphate and ADP translocation channels might be specific for $H_2PO_4^-$ and K_2HADP, respectively.

The specificities of the nucleotide and phosphate translocation channels for the given salt and acid–base species must, of course, be attributed to complementary structural features in the ATPase complexes. These features would correspond partially to the specific ion-carriers previously postulated, for example: the carriers X and Y supposed to be specific for K^+ and Na^+ respectively in the scheme for the Na^+/K^+-translocating ATPase originally suggested by Shaw (see [8]), as illustrated by comparing fig. 4B with fig. 4A; and the carriers XI and $X^- + IO^-$, suggested [13] for the ATPase translocating two H^+ ions [14], as illustrated by comparing fig. 3C with fig. 3A. There are, however, three main differences: i) The primary electrovalent or covalent bonding of the alkali-metal cation or H^+ ion responsible for coupling the flow of the passenger ion to the hydrolysis of ATP is directly between the passenger ion and the ATP, ADP or phosphate, the translocation of which is catalysed by the ATPase. ii) The passenger-specific sites are not X, Y, X^- or IO^-, but are complexes of ATP, ADP or phosphate with the specific translocation channels. iii) The hypothetical conversion of the K^+-carrier X to the Na^+-carrier Y by the hydrolysis of ATP, required in the scheme of Shaw (fig. 4B) and in most of the more sophisticated schemes derived from it (see [7, 8]), or the analogous hypothetical conversion of $X^- + IO^-$ to XI suggested for the proton-translocating ATPase (fig. 3C), is directly explained in the scheme of figs. 3A and 4A by the assumption that the complementary structural features of the channels for translocation of ATP, ADP and phosphate favour different combinations of passenger species (Na^+, K^+, Ca^{2+} or H^+) primarily bound to the ATP, ADP and phosphate.

The lines in figs. 3A, B and 4A (or figs. 1 and 2), showing the translocation channels for ATP, ADP and phosphate, are a very diagrammatic representation of the actual translocation pathways in the specifically mobile three-dimensional ATPase complex systems. They might be taken to represent, in principle, the mean pathways of the centres of gravity of the phosphate groups of the respective components. In reality such pathways might partially overlap because the same region of the ATPase complex might serve both as the inward translocation channel for ATP and the outward translocation channel for ADP (or ATP and phosphate). But the translocation channels should, nevertheless, be represented as distinct because the precise complementary structural configuration of the specifically mobile region of the ATPase catalysing the translocation would depend on whether ATP or ADP + P_i were undergoing translocation through this region. Of course, the pathways of translocation would inevitably be blurred to some extent by random thermal movements of the ATPase complex about mean minimum free energy positions in the continuous set of states corresponding to cation-translocation and ATP hydrolysis.

3. Evolutionary and functional properties of ATPase models directly involving ATP and its hydrolysis products in cation translocation

The possibility that the chemical constituents of ATP and H_2O might fulfil the function of ion-carrying groups in the Na^+/K^+-translocating, the Ca^{2+}-translocating and the proton-translocating ATPase systems, in some such way as I have suggested, is relevant both to the evolutionary origins of these systems and to their present functional properties.

As I have pointed out in a more general context

[15], owing to the way in which the catalytic systems of living organisms are likely to have evolved through stages of increasing complexity, there was presumably a tendency for the ordinary chemical reactivities of the small molecular weight components involved as metabolites and passengers in the biologically catalysed transformation and translocation reactions to be utilised initially. These ordinary reactivities have presumably become modulated subsequently by the secondary bonding and secondary and tertiary packing relationships in the enzyme and catalytic-carrier systems that have been evolved. Thus, rather unselective H^+ ion or alkali-metal ion carrier functions of ATP or its hydrolysis products in primitive membrane systems could conceivably have given rise to the present types of highly selective Na^+/K^+-translocating, Ca^{2+}-translocating or proton-translocating ATPase systems as a result of the evolution of sophisticated lipoprotein systems, the secondary and tertiary bonding and structural packing of which have come to determine the passenger specificity of the original nucleotide carriers without robbing them of their primary carrier function.

Certain diagnostic functional characteristics may be identifiable if ATP and its hydrolysis products have ion-carrying functions in the ion-translocating ATPase systems. In particular, the ion-exchange translocation reactions across the ATPase systems in the absence of net ATP hydrolysis (or synthesis) should be dependent on the ion-carrying nucleotides or phosphate. In this context, the use of non-hydrolysable analogues might be particularly rewarding.

In the case of the Na^+/K^+-translocating ATPase, the extensive literature includes some suggestive evidence. For example, the exchange of Na^+ for Na^+ across the Na^+/K^+-translocating ATPase system is dependent on the presence of ATP, while the exchange of K^+ for K^+ appears to be dependent on the presence of ADP and P_i. Also, unnatural substrates, such as acetyl phosphate can be hydrolysed by this ATPase system, but the transport of Na^+ has a specific requirement for ATP [see 7].

In the case of the Ca^{2+}-translocating ATPase of sarcoplasmic reticulum vesicle preparations, Ca^{2+} translocation occurs during hydrolysis of acetyl phosphate in the absence of added ATP, suggesting that ATP is not directly involved in Ca^{2+} translocation [16]. However, the Ca^{2+} affinity of the system (and possibly its conformation) is influenced by the binding of ATP

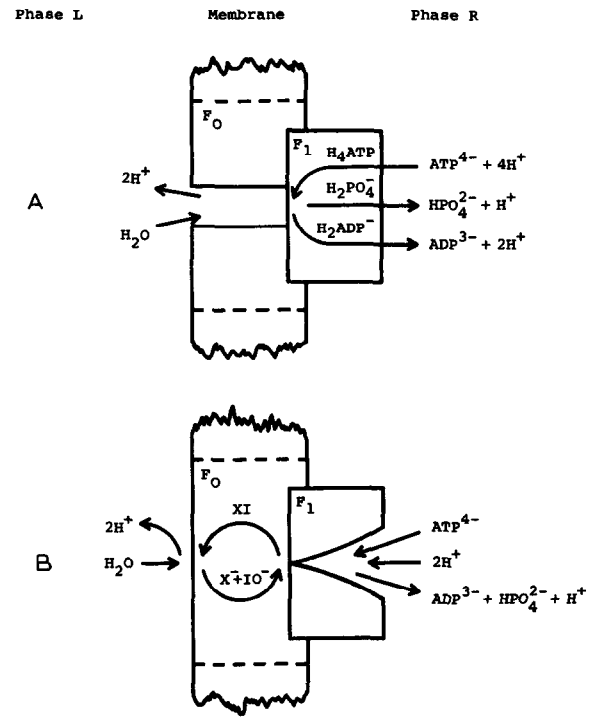

Fig. 5. Diagrams of proton-translocating ATPase, indicating possible functions of the F_1 component and the F_O complex in the alternatives A and B, corresponding to figs. 3A and C, respectively.

even when acetyl phosphate is used as substrate [17]; and Ca^{2+} translocation coupled to acetyl phosphate hydrolysis is comparatively inefficient [17]. Other observations in the literature [18] suggest that there may be two ATP-binding sites as well as two Ca^{2+}-binding sites in the ATPase system, although only one of the ATP molecules can be split at a time, giving rise to the translocation of the two Ca^{2+} ions. It would therefore appear to be worth investigating further the possible translocational role of ATP in this system.

In the case of the mitochondrial proton-translocating ATPase, as required by the type of mechanism shown in fig. 3A, the exchange of ^{18}O between water and inorganic phosphate is dependent on the presence of ADP [19, 20]. This does not eliminate the type of mechanism illustrated in fig. 3C because, even if XI were subject to phosphorolysis to give XH + $IO(H_2PO_3)$, the oxygen-bridge between phosphate

and the group I need not be exchangeable with water. Nevertheless, the lack of evidence for any intermediates, despite numerous attempts to identify them, has given rise to a growing body of opinion [21] against the type of mechanism shown in fig. 3C.

The proton-translocating ATPase system [22] can be separated into two structurally and functionally distinct parts: an ATPase protein, known as F_1, and a lipoprotein complex that is integral with the membrane that contains the complete ATPase system and confers oligomycin sensitivity and the function of trans-membrane proton translocation on the F_1 component [23, 24]. As discussed very briefly on a previous occasion [10], the possibility that ATP and its hydrolysis products might be involved in proton translocation appears to be especially relevant in view of this duplex composition of the proton-translocating ATPase system. The spatial relationship between F_1 and the rest of the complete proton-translocating ATPase system is represented crudely in the diagrams of figs. 5A and B which also show features corresponding to the alternative mechanisms represented in figs. 3A and C, respectively. In fig. 5A, the F_1 component is shown as having the enzymic and translocation properties of the ATPase of fig. 3A at the molecular level of dimensions, but the localisation and orientation of F_1 is represented as being due to the oligomycin-sensitivity-conferring complex F_0, which is integral with the membrane. In this case, the F_0 complex is depicted as having a translocation pathway through it, permitting access of H^+ and H_2O to the F_1 component; and it should be emphasised that, according to this formulation, the component F_1 has the same ATPase enzymic function in the complete (oligomycin-sensitive) ATPase system as it has in the isolated state (fig. 6A). On the other hand, according to the formulation of fig. 5B, the F_1 component has the enzymic properties of an X-I synthetase and the F_0 complex is represented as having the function of an XI hydrolase and of a translocator of XI and $X^- + IO^-$, as described previously [13]. In this case, whereas the XI synthetase reaction catalysed by F_1 in the complete ATPase system would be given by

$$ATP + XH + IOH \rightleftharpoons ADP + POH + XI \qquad (1)$$

the corresponding ATPase activity of isolated F_1 would be given by

$$ATP + HOH \rightarrow ADP + POH \qquad (2)$$

It was suggested that reaction 2 would not be catalysed by the F_1 component in the complete ATPase system because H_2O would be excluded from the active centre of F_1 by the presence of the F_0 complex containing XI and XH + IOH [13, 25]. However, although there is no objection, in principle, to the suggestion that F_1 might alternatively catalyse reactions 1 or 2 under appropriate conditions [13], the indirect type of mechanism illustrated in fig. 5B appears to be less realistic than the more direct type (fig. 5A) since there is not only a complete lack of evidence for intermediates such as those described by XH, IOH and XI in figs. 3C and 5B, but also, referring to figs. 5 and 6, the F_1 component has a somewhat greater turnover number as an ATPase when in the isolated state than when reincorporated in the F_0 complex [26, 27].

When the F_1 component is dissociated from the F_0 complex in membrane vesicles from beef heart mitochondria or from certain photosynthetic bacteria, it was discovered in my laboratory that the effective proton conductance of the membrane is relatively high, but can be decreased to a more normal level by an amount of oligomycin approximately sufficient to titrate the F_0 complex in the membrane preparations [25, 28]. This somewhat surprising observation (and its counterpart using *N,N'*-dicyclohexylcarbodiimide in the proton-translocating ATPase of chloroplasts) has been amply confirmed [29–33], and must be accounted for by appropriate features of mechanisms proposed for the complete proton-translocating ATPase system. As illustrated in fig. 6, the increased proton conductance observed when the F_1 component is dissociated from F_0, might be explained either (fig. 6A) in terms of an oligomycin-sensitive proton-conducting pathway through F_0, possibly involving proton-carrying intermediary groups, or alternatively (fig. 6B) in terms of an oligomycin-sensitivive XI hydrolase and XI and $X^- + IO^-$ translocator. In the latter case, it is necessary to assume [13] that XI and XH + IOH can come into rapid equilibrium with H_2O (in place of ATP and ADP + POH) in phase R when (and only when) the F_1 component is absent – a somewhat unattractive assumption. It is relevant to either mechanism A or B of fig. 6 that the leakiness of the membrane that occurs when the F_1 component

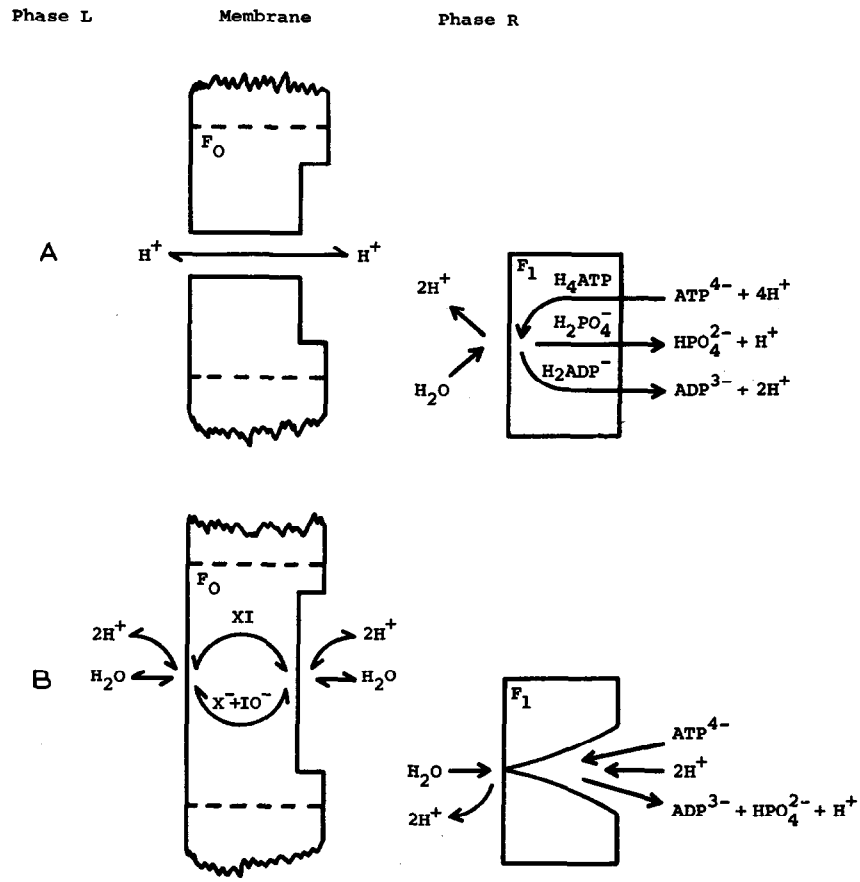

Fig. 6. Diagrams of proton-translocating ATPase, illustrating possible functional effects on F_1 and F_0 of dissociating F_1 from the F_0 complex in cases A and B, corresponding to figs. 5A and B, respectively. In these diagrams, the reactions catalysed by F_1 indicated in phase R are independent of the reactions catalysed across the membrane containing the F_0 complex.

is dissociated can be decreased not only by oligomycin, but also by F_1 that has been made enzymically inactive ([23] and see [33]).

4. Conclusions and research prospects

In the enzyme systems coupling the hydrolysis of ATP to the translocation of cations, the fundamental stoichiometric principle of coupling depends on an interrelationship between the relative movements of the chemical groups (i.e. group-translocations) during ATP hydrolysis and the movements of cations across the system. This interrelationship is presumably determined by the favourability (i.e. low free energy) of a particular set of transitional states through which the reaction may spontaneously proceed in the ATPase enzyme–substrate–cation complex. The question that the enzymologist and student of transport has to answer is: how direct are the interactions between the chemical constituents of ATP and H_2O and the cations during the overall hydrolytic and translocation process catalysed by the ATPase, and how closely related are the spatial pathways of the chemical groups and the cations?

The suggestion, discussed in this paper, that the relative movements of the cations and the constituents of ATP and H_2O, channelled by the cation-translocating ATPases, may be more closely related than has commonly been supposed is useful in relation to research

strategy because it focuses attention on the need to analyse the *relative movements* of the cations and the components involved in the hydrolytic "driving" reaction in order to understand the stoichiometric relationship between ATP hydrolysis and cation translocation. This type of approach is, I believe, more likely to provide insights into the fundamental molecular mechanisms by which ATP hydrolysis is coupled to cation translocation in the ATPase systems than the more customary approach in which particular phosphorylated or other intermediary "high-energy" or "low-energy" compounds or states, involved in the hydrolytic or cation-translocation reactions, tend to monopolise attention. The consequences of a relatively direct coupling of group translocation and cation translocation in the ATPases may be predicted with more certainty than would be possible in the case of a more complex and indirect mechanism. In particular, non-hydrolysable analogues should be capable of catalysing appropriate cation-translocation reactions independently of ATP hydrolysis.

It is a sound principle in the strategy of research to begin with relatively simple types of hypothetical model, such as that advocated here, and to proceed to use more complicated or less predictable models only when the relatively simple model is shown to be inadequate. This useful principle is especially appropriate in biological research because, as indicated above, it may follow an actual evolutionary trend from relatively simple to more evolutionarily sophisticated systems.

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