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Chemiosmotic Coupling in Energy Transduction: A Logical Development of Biochemical Knowledge

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Introduction

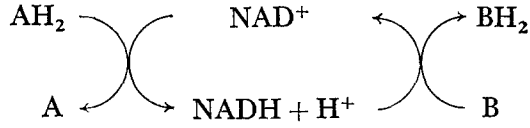
At the invitation of the Editors, this paper gives a summary sketch of my position regarding some metabolic aspects of energy transduction and describes some present and anticipated perspectives from my point of view. To maintain as broad a horizon as possible, however, I have used this opportunity to describe how my views, and the rationale that I have developed to express them, have been derived from accepted or acceptable physicochemical theory and biochemical knowledge stemming from the creative and painstaking observations of my progenitors and colleagues.

My interest in the conceptual and functional relationships between chemical and osmotic reactions was first seriously stimulated when I was studying the specific exchange and uptake of inorganic phosphate and arsenate through the plasma-membrane of staphylococci.[1,2,3] The remarkably high specificity of the phosphate translocation reaction, its susceptibility to specific inhibitors including SH-reactors, its high entropy of activation which indicated a large conformational change in the translocator system, and the tight coupling of phosphate translocation against arsenate translocation,[1,2,3] indicated how closely osmotic translocation reactions could resemble (or be functionally related to) enzyme catalysed group-transfer reactions. Further, the observation that the plasma-membrane material isolated from staphylococci contained the cytochrome system and associated enzyme activities[4,5] suggested that certain of the group-transfer reactions catalysed by the enzyme and catalytic carrier systems in the plasma-membrane might actually be vectorial group-translocation reactions because of the spatial orientation of the catalytic systems. These were the circumstances that led me to remark at a symposium nineteen years ago (see ref. 2) that "in complex biochemical systems, such as those carrying out oxidative phosphorylation (e.g. Slater &

Cleland, 1953),[6] the osmotic and enzymic specificities appear to be equally important and may be practically synonymous". The same circumstances drew my attention to certain related conceptual and factual knowledge from the fields of enzymology and membrane transport which, at that time, tended to be pursued by separate schools of thought. In this paper I show how the bringing together of this knowledge opened up some new perspectives and provided a logical foundation for the development of the chemiosmotic coupling concept.

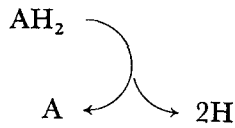
Primary Chemical Coupling

The general principle of primary chemical coupling was already recognized in the nineteen thirties when Green, Stickland and Tarr[7] described the coupling between the oxidation of one substrate (AH_2) and the reduction of another (B) by a solution of the appropriate AH_2 and BH_2 dehydrogenases in the presence of the specific coupling factor (NAD) which was alternately reduced and oxidized in the following type of reaction (see also Green[8]):



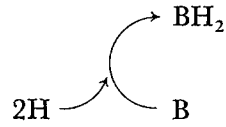
(1)

According to the elegant rationale and terminology subsequently introduced by Lipmann,[9] this type of system effectively coupled the group-transfer reactions,



(2)

and

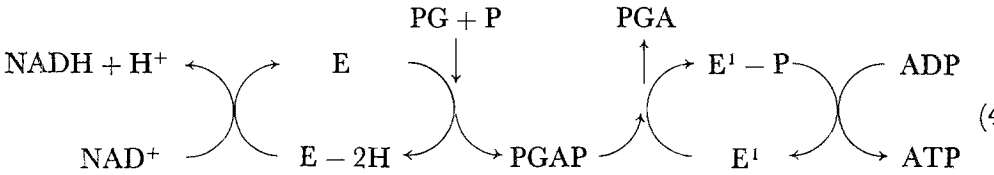


(3)

by facilitating the transfer of the hydrogen groups, and equilibrating the hydrogen group potential, between the donor and acceptor redox couples. Thus, "energy-coupling" actually corresponded to the material transfer of hydrogen groups via the substrate-specific enzymes and the NAD coenzyme mediating between the chemical components A and B; and Lipmann's view enabled one to imagine

the reaction going forward because of a "thrust" transmitted down the group-potential gradient—although, of course, there was no *net* vector component of this "thrust" in aqueous "homogeneous" enzyme solutions.

In the case of the soluble enzyme systems catalysing substrate-level phosphorylation by a coupling between redox reactions and phosphorylation or dehydration reactions, a more complex but fundamentally similar coupling scheme was found to be required. It was difficult to see, at first, how the "driving" redox reaction could involve a component in common with the "driven" phosphorylation or dehydration reaction so that the "thrust" of a group-potential gradient could be transmitted between the two reactions. But this apparent difficulty was overcome when it was shown by Warburg[10] and by Racker[11] that the oxidation of 3-phosphoglyceraldehyde (PG) to 3-phosphoglycerate (PGA) could be coupled to ADP phosphorylation through an appropriate series of group transfers because both the hydrogen and phosphoryl group transfer reactions were channelled through the intermediate 1,3-diphosphoglycerate (PGAP), as summarised in the following equation:



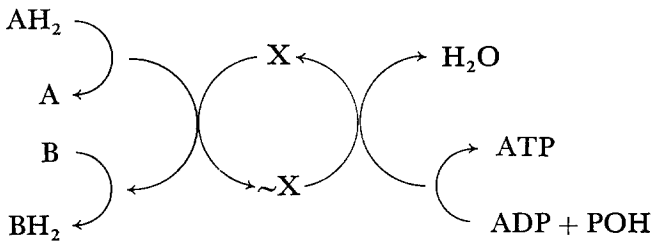
(4)

where E and E^1 stand for 3-phosphoglyceraldehyde dehydrogenase and 3-phosphoglycerate kinase enzyme complexes respectively.

Thus, in the nineteen fifties it was evident that the transfer of one type of chemical group could be "energetically coupled" to the transfer of another type of chemical group by means of a series of appropriate chemical intermediates that would enable the thermodynamic driving force to be transmitted through the corresponding group-potential gradients—as described most eloquently by Lipmann.[12] This type of mechanism implicitly attributed the transmission of the thermodynamic force through the system to interaction of chemical groups across covalent bonds.

The success and elegance of this "substrate-level" type of coupling mechanism led to the belief that it would be generally applicable, and that even in the particulate systems catalysing oxidative and photosynthetic phosphorylation, the coupling mechanism would be explained in this way when the pathways of group transfer were elucidated by isolating and identifying the covalent "energy-rich" chemical intermediates.

The emphasis placed on the covalent intermediates in "substrate level" metabolic coupling tended to obscure the fact that, while the changes of group potential accompanying covalent bond interchanges between certain standard states could properly be regarded as the source of metabolic energy, the transmission of this energy through a given region of a metabolic pathway by the "thrust" of chemical particles diffusing through it should be attributed not only to forces along covalent bonds but also to concentration-dependent osmotic forces and to forces along ionic and secondary bonds. The relevance of this important fact to "energy coupling" in metabolism was indicated by Glasstone, Laidler and Eyring's "theory of absolute reaction rates" in which chemical transformation and viscous fluid flow were treated as proceeding through fundamentally similar thermally activated rearrangements of chemical particles via transitional states of appropriately low free energy.[13] Likewise, the suggestion by Pauling[14] that enzymes have a higher affinity for the transitional configurations of their substrates than for the normal reactant and resultant species—and thus catalyse group transfer by lowering the free energy (and increasing the probability) of the transition state—illustrated the role of secondary bonding relationships in catalysing or channelling the transfer of chemical groups from donors to acceptors, and therefore in coupling group transfer processes. In the case of the transfer of a given type of chemical group between acceptor and donor systems, as illustrated by equations 1–3, it was evident that the coupling should be attributed to non-covalent bonding interactions inasmuch as these were involved in catalysing the flow of the specific group through the prescribed pathway, and minimizing energy loss through side reactions. In the case of systems like that of equation (4), where the transfer of one type of chemical group is coupled to that of another, it followed that the osmotic and non-covalent bonding interactions might play a major part in linking the two different group-transfer processes as well as being involved in the direct catalytic role described above. This is illustrated by the following equation, representing the overall process of oxidative phosphorylation:

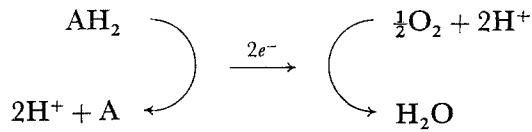


(5)

In this system, the intermediate process, represented as the transition between X and ~X, might mainly represent a change of covalent bonding of X—as in the orthodox chemical coupling hypothesis—but might, equally well, mainly represent a change of osmotic status or ionic or secondary bonding of X—providing a basis for alternative coupling hypotheses.

Electron Translocation and Group Translocation

The idea of spatially orientated chemical reactions in biochemistry stemmed from the work of Lundegardh[15] who suggested that cytochrome pigments might provide an electron-conducting pathway across plant cell membranes so that oxygen could be reduced (and H^+ ions consumed) on one side while hydrogenated substrates were oxidized (and H^+ ions were produced) on the other side, as represented by the following equation:



(6)

This idea of electron conduction or translocation, accompanied by redox and acid-base changes across natural membranes, was further developed, notably by Davies and Ogston,[16] by Conway,[17] and by Robertson[18] (see Robertson[19]).

Since Curie[20] pointed out that effects cannot be less symmetric than their causes, it obviously followed that, when a (vectorial) transport or osmotic process across a membrane was caused by a chemical reaction, the group-potential gradient acting as the driving force[9] must be directed in space. The beautifully simple thermodynamic treatment of chemicomotive cells and circuits by Guggenheim[21] likewise showed how the transport of a chemical component is determined by gradients of chemical potential representing what he called the "chemicomotive forces" directed across (artificial) membranes or phase boundaries; and a somewhat similar treatment of biological transport was also given by Rosenberg.[22] Thus, it was logical to introduce the concept of group translocation[23,24] as a generalization of the idea of electron translocation, so that one could more readily recognize and describe, not only electromotive forces, but also other chemicomotive forces (such as phosphorylmotive forces or protonmotive forces) as prime movers in metabolically-coupled translocation reactions. In this way it was possible to consider all biological transport reactions as arising from the thermally activated diffusion of solutes and chemical groups down the (vectorial or higher order tensorial) electrochemical potential gradients of their

various compounds and complexes in the local aqueous and lipid-membrane media, including conformationally mobile "active centre" and "carrier centre" regions of enzymes and catalytic carriers.[2, 25, 26]

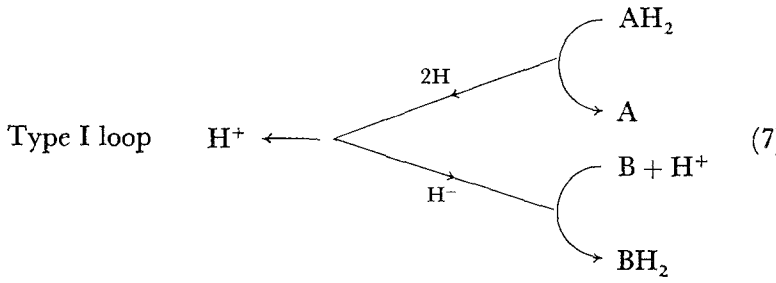
As shown by my mobile enzyme model for phosphoryl translocation,[25] I was initially encouraged to regard the idea of specific conformational flexibilities or articulations in the condensed complex of the enzymes or catalytic carriers catalysing group translocation as acceptable by analogy with the notion of conformational mobility in membranes, which originated from Langmuir's elegant idea of the turning over of lipid molecules in artificial monolayers.[27, 28] This view implied that such group-translocation proteins or protein complexes (including, for example, prosthetic groups or lipid) would undergo cyclic conformational changes synchronous with the group transfer reaction; and it also followed that in such three-dimensional articulated complexes the translocation of the group undergoing chemical transfer along one pathway might be tightly coupled to the translocation of the acceptor or donor substrate species or of other solute species in the same or in different directions in space.[29] The point to be stressed is that according to this view "energy coupling" is attributed to the transmission of "thrusts" along interacting trains of chemical particles including the macromolecular components. In this type of system, the osmotic "thrust" or potential gradient of a small molecular weight solute can generally be exerted only along the translational degree of freedom across an osmotic barrier or into a group-transfer reaction centre, but the osmotic "thrust" of a group translocation catalyst is exerted through the complex degrees of freedom defined by the permitted articulations, as in a macroscopic mechanical engine. This development of ideas was consistent with contemporary developments in the fields of classical enzymology and protein structure, such as the "induced fit" interpretation of enzyme kinetic data by Koshland,[30] the suggestion by Hammes[31] that the activation energy for group transfer may be lowered by the balancing of stress-strain relationships in mobile enzyme-substrate complexes, and the observations of Muirhead and Perutz[32] on conformational changes in haemoglobin molecules during oxygenation. Subsequent experimental work on translocation catalysis has served to confirm my view that the conformational mobility of the translocation catalysts is associated with their normal group-translocation and solute-translocation functions.[33–36]

Proton Translocating Respiratory Chain and Photoredox Chain

The development of my working hypothesis for the chemiosmotic type of coupling mechanism in oxidative and photosynthetic phosphorylation[37] depended on a logical sophistication of the concept of

group translocation, because, in order to account for "energy coupling" by the transmission of an osmotic "thrust" through a train of protons between the redox chain and reversible ATPase systems, it was necessary to formulate group-translocation mechanisms by which the electron and hydrogen transfer reactions through the redox chain, and the H_2O transfer through the ATPase system, could each result in effective proton translocation.

Proton translocation in the redox chain was attributed to paired electron translocation and hydrogen atom (or hydride ion) translocation reactions[37–39] which could conveniently be represented by so-called redox loops of type I or type II, according to whether one or two H^+ ions were translocated respectively per divalent redox equivalent transferred, as represented in the following equations:



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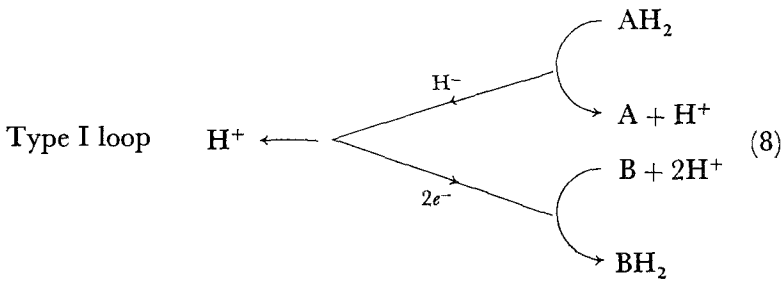
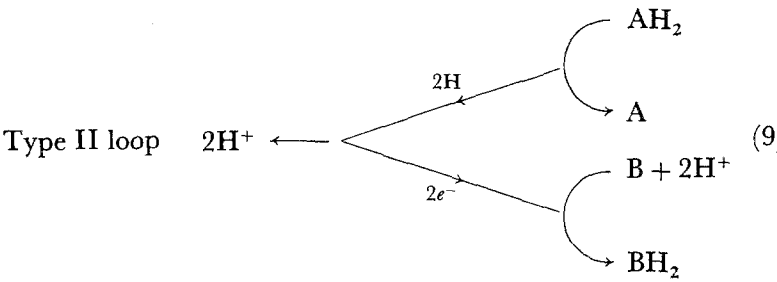


(8)



(9)

The essential point to be noted is that these proposals depicted proton translocation as occurring, not by the movement of protons as such, but by the stoichiometrically coupled translocation of hydrogen

atoms to the left and of electrons to the right through the duplex catalytic carrier system represented abstractly by the redox loop. Thus, this redox loop type of group translocation complex was considered to transform the (scalar) redox potential difference between the A/AH_2 and B/BH_2 couples present on the right into a hydrogenmotive force to the left and an electromotive force to the right, adding up to a protonmotive force (and a corresponding *net* flow of protons) to the left.[38–41]

In the case of the photoredox chain, it was assumed that the electron-carrying arm of the redox loop could include a photoelectric reaction so that the photoelectric potential could be transformed into the protonmotive force.[38–40]

There was a danger that the need to present the concept of the redox loop in a somewhat abstract form for the sake of explicitness and simplicity in defining general principles might detract from the realization of the functional detail involved. Therefore it was emphasized that, in accordance with fundamental observations by Chance, Holmes, Higgins and Connelly[42] on the bimolecular characteristics of respiratory carrier interactions, it was necessary to attribute both hydrogen and electron translocation largely to the specific mobilities of hydrogen-carrying and electron-carrying groups in the catalytic carrier complexes.[38,39]

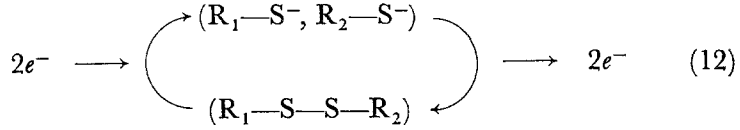
The idea of specific mobility in the group-translocation complex was related to the requirement that the coupling between the redox reaction and the translocation process in a redox loop must be specified on the one hand by the uniqueness of the primary oxido-reduction changes, and on the other hand by the uniqueness of the translocation reactions, channelled by the specific packing and secondary articulations in or between the enzymic and/or catalytic-carrier components of the redox loop complex.[38,39] For example, it was pointed out that the redox couple $NAD^+/(NADH + H^+)$ could act as a carrier of hydrogen groups (2H) or of hydride groups (H^-), depending upon whether the essentially cyclic translocation process catalysed was that of $(NADH + H^+$ minus $NAD^+)$ translocation, as follows:

$$2H \longrightarrow \underset{NAD^+}{\overset{(NADH + H^+)}{\circlearrowright}} \longrightarrow 2H \qquad (10)$$

or (NADH minus NAD^+) translocation, as follows:

$$H^- \longrightarrow \underset{NAD^+}{\overset{NADH}{\circlearrowright}} \longrightarrow H^- \qquad (11)$$

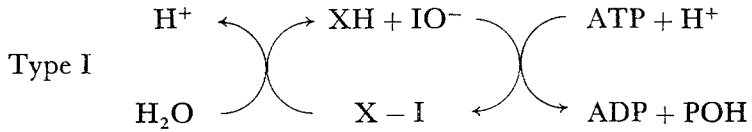
Likewise, it was pointed out[38,39] that a dithiol (R_1–SH, R_2–SH) could act as an electron carrier if the translocational specificities were selective for the disulphide and deprotonated dithiol species only, as follows:



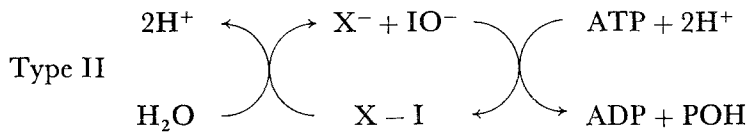
(12)

Proton Translocating ATPase

In the ATPase systems of mitochondria, chloroplasts and bacteria, the effective translocation of protons was attributed to the specific translocation of OH^- or O^{2-} groups.[37–40] It was suggested that this could occur by the following type of cyclic translocation reaction involving cyclically mobile groups X–I and XH + IO^- or X^- + IO^- in the translocation system, which would come reversibly into equilibrium with water on the left but with ATP (and not with water) via a kinase on the right:



(13)



(14)

By analogy with the case of the redox loops, the proposed ATPase systems could be of types I or II, translocating one or two protons respectively per ATP hydrolysed, depending on the specificity of the translocation channel for XH + IO^- (corresponding to OH^- translocation) or for X^- + IO^- (corresponding to O^{2-} translocation).

Chemiosmotic Coupling

Considerations of the conditions required to enable enzyme-catalysed group translocations to produce thermodynamically macroscopic metabolic effects led to the recognition of two possible types of chemiosmotic coupling, one applying to molecular-level enzyme associations, and the other applying to associations of enzymes in subcellular vesicles.[24] In the molecular-level (or microscopic) type of

chemiosmotic coupling, described by Dr. Moyle and me,[24] enzyme molecules or sub-units catalysing two consecutive reactions were supposed to be associated in pairs so that a microscopic internal phase was enclosed between them. A product of the first reaction was supposed to be translocated through the active centre of the first enzyme (or sub-unit) into the internal phase so that it could gain access to and react via the active centre of the second enzyme (or sub-unit) faster than it escaped into the neighbouring medium. In this case, chemiosmotic coupling would be achieved through the osmotic "thrust" of the common intermediate sequestered in the microscopic internal phase—as we suggested might, for example, be the case in the coupling between isocitrate oxidation to oxalosuccinate and oxalosuccinate decarboxylation to ketoglutarate in isocitrate dehydrogenase.[24]

In the subcellular vesicle (or macroscopic) type of chemiosmotic coupling, proposed as the direct means of coupling redox reactions to dehydration reactions in oxidative and photosynthetic phosphorylation systems,[37] the topological arrangement was supposed to be fundamentally similar to that of the molecular-level type, but on a larger scale. Thus, the membrane-bound respiratory chain systems of mitochondria and bacteria, and the membrane-bound photoredox chain systems of chloroplasts and bacteria, were supposed to consist of assemblies of proton-translocating redox loops; and several such assemblies, present in the topologically closed membrane vesicles of mitochondrial cristae, bacterial protoplasts, chloroplast grana or derived "particles" were taken to contribute to the establishment of the total effective proton flux and to the protonmotive force across the so-called coupling membrane of the vesicles. The resulting "thrust" of protons through the reversible proton-translocating ATPase assemblies, also present in the coupling membrane, was supposed to give rise to ATP synthesis by reversal of the ATPase reaction.[37–40]

The effect of scale in the macroscopic type of chemiosmotic coupling introduced a more fundamental difference from the microscopic type than might have been expected, because it meant that there need be no direct physical (or chemical) contact between the redox chain assemblies and the ATPase assemblies in systems catalysing oxidative and photosynthetic phosphorylation. It also meant that the coupling membrane, of low proton conductance, in which the proton-translocating assemblies were assumed to be situated, would be an essential component of the normal coupled oxidative and photosynthetic phosphorylation systems.[37] Further, it followed that specific proton-coupled solute-translocation systems would be required in the coupling membrane to maintain osmotic

stability[37] and to facilitate the entry and exit of appropriate substrates.[29, 38, 41]

In this brief paper it is not possible to give a meaningful survey of the experimental work that has been carried out with the object of defending or refuting one or another of the proposed mechanisms of coupling in oxidative and photosynthetic phosphorylation systems; but the review by the late G. D. Greville[43] provides an excellent main source of information, which can readily be supplemented by more recent reviews.[44–49] The wealth of detail has tended, however, to divert attention from some rather general considerations about the biochemical relationships between the parts of the different feasible coupling mechanisms that should, I believe, make it rather easier than has commonly been supposed to distinguish the macroscopic chemiosmotic coupling mechanism from other possible mechanisms.

Comparisons between Chemical, Conformational and Chemiosmotic Coupling Mechanisms

Referring back to equation (5), the fundamental question of so-called "energy transduction" can be answered in biochemically realistic terms only when we can specify how the flow of redox particles (electrons and hydrogen atoms) in the redox chain system is connected by actual physicochemical interactions with the flow of the elements of water (electrons, hydrogen atoms and oxygen atoms) through the reversible ATPase system. In other words, what are X and ~X in equation (5), and how do they participate in the redox-chain and reversible ATPase systems?

As illustrated by equation (4), in substrate-level oxidative phosphorylation, the flow of redox particles through the redox system on the left is coupled to the flow of phosphoryl into (or H_2O out of) the phosphorylation system on the right because the phosphorylated intermediate (PGAP) that passes between the two systems contains both the redox functional acyl group and the phosphoryl group linked together covalently in the same small molecule. The pattern of events is not quite the same as that illustrated by equation (5), because the dehydration and oxidoreduction systems overlap in equation (4); but the fundamental coupling principle, depending on the actual coupling between the flows of the chemical particles, is identical.

It is noteworthy that in substrate-level phosphorylation, the redox system and the ADP phosphorylation system, which are linked by the diffusion of the intermediate substrate, are physically separate from one another, although they are both present in the same aqueous phase. Likewise, in the macroscopic type of chemiosmotically coupled

oxidative phosphorylation, the proton-translocating redox chain or photoredox chain system, and the proton-translocating ATPase system, which are linked by the diffusion of the intermediate H^+ ion, are physically separate from one another, although they are both present in the same non-aqueous coupling membrane between the two aqueous phases. In this case, X and ~X of equation (5) correspond to the H^+ ion at different electrochemical potentials in the two aqueous phases.

In the other conceivable types of coupling mechanism that have seriously been advocated, a direct interaction is required, at the molecular level of dimensions, between certain of the redox chain or photoredox chain components and components of the reversible ATPase. For example, in the microscopic chemiosmotic mechanism[24] advocated by Williams,[50] protons, trapped in microscopic domains, are assumed to mediate between redox activity and ADP phosphorylation at various sites along the respiratory chain. Similarly, in versions of the chemical coupling hypothesis, where it is assumed that non-diffusable derivatives of catalytic components of the redox chain act as intermediates between redox activity and ADP phosphorylation,[51] direct contact is required between the appropriate redox chain components and components of the reversible ATPase. Likewise, according to the conformational coupling hypothesis originally conceived by Boyer[52] and by Green,[53] and developed in different ways by Slater[54] and by Green,[55] segments of the respiratory chain and components of the reversible ATPase are supposed to undergo related conformational and local electrical changes during redox and reversible ATPase activity, and coupling is attributed to the direct transmission of forces and flows by intimate contact between the respiratory chain segments and the reversible ATPase components.

In all the "direct interaction" mechanisms of coupling, the cyclic transition between X and ~X in equation (5) is represented by the cyclic change of chemical or physical state and configuration in the specific domain of contact assumed to exist between components of the redox (or photoredox) chain and the reversible ATPase. Thus, according to these mechanisms, the respiratory chain (and photoredox chain) should be chemically duplex, and in view of the intimate association assumed between the redox and reversible ATPase systems, it would be expected that normal biochemical fractionation methods should provide evidence for well-defined structural associations and functional interrelationships between the redox (or photoredox) and ATPase components. However, as I have pointed out before,[56] and re-emphasized in a recent review,[57] Keilin's chemically simple concept of the respiratory chain, as a hydrogen- and electron-carrying system, was almost universally rejected in the

nineteen fifties in favour of a chemically duplex type of system, although there was never any experimental support for this remarkable swing of opinion. Despite earlier claims (see ref. 56), there is no experimental evidence for a *direct* influence of the redox state of components of the respiratory chain (or photoredox chain) on the activity of the ATPase, and there is no evidence for a *direct* influence of the poise of the ATPase on redox activity through the chain. Nor is there any evidence of *direct* associations between components of the redox chain (or photoredox chain) and components of the reversible ATPase system, such as would be required by the "direct interaction" type of coupling mechanism; even though there were strong incentives for finding such associations, as illustrated, for example, by the work of Racker and co-workers.[58–62]

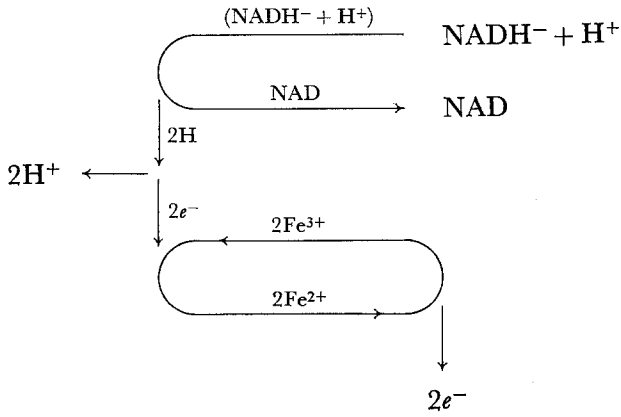
It is now generally acknowledged as a fact (see refs. 43–49) that coupling between the redox (or photoredox) chain system and the reversible ATPase system is dependent on the osmotic intactness of the topologically closed coupling membrane in which the two systems are situated, and that the classical uncoupling agents, such as 2,4-dinitrophenol, act by specifically equilibrating the electrochemical potential of H^+ ions across the coupling membrane—in accordance with a chemiosmotic type of coupling mechanism.[37] Slater[63] has sought to reconcile the "direct interaction" type of coupling mechanism with these facts by assuming the existence of a proton pump, separate from the redox and ATPase systems, but actuated by an energy-rich intermediate in equilibrium with the redox and ATPase systems (see Chappell and Crofts[64] and Lardy, Connelly and Johnson[65]). Thus, in complete oxidative or photosynthetic phosphorylation systems, the energy-rich intermediate (or state) would be discharged by activity of this superimposed proton pump if the coupling membrane were broken or made specifically permeable to H^+ ions. However, one would expect that, by the use of suitable inhibitors or by partial resolution of the system, it would be possible to eliminate the activity of the superimposed proton pump and thus obtain coupled systems that would be independent of the osmotic integrity of the coupling membrane and unaffected by the proton-conducting uncoupling agents.

At all events, one finds it hard to accept that it would have been beyond the wit of all the biochemists engaged in studying the coupling mechanism in oxidative and photosynthetic phosphorylation during the last forty years to demonstrate a single relevant *direct* functional interaction or structural complexation between components of the redox (or photoredox) and ATPase systems, if such direct interactions and complexations are actually responsible for "energy coupling" in the natural systems.

Some Perspectives on Future Developments: Design Principles for Redox Loops and Cation-Translocating ATPase Systems

At the end of their recent review on energy conservation in mitochondria, van Dam and Meyer[46] have remarked that it may be appropriate to caution the proponents of the different hypotheses with the words of the Greek philosopher Myson: "It should not be our aim to explain facts in the light of arguments, but to argue on the basis of facts." I would wholeheartedly agree with this sentiment as long as it is remembered that facts are not the same as randomly selected experiments, and that facts are formulated in words and other symbols and emerge from the raw experimental data only in the light of arguments. It is in this spirit that I have thought it useful to present the following comments on some possible interpretative applications of the chemiosmotic coupling rationale.

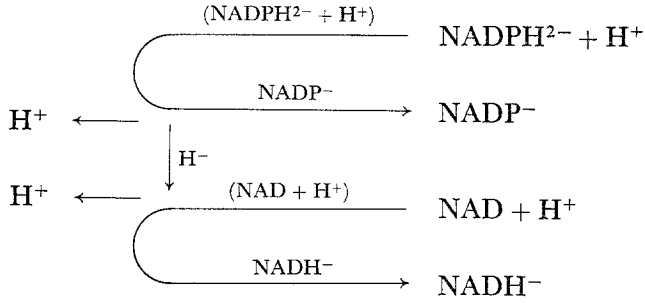
In my attempts to assign known carriers of the respiratory chain and photoredox chain to appropriate locations in the redox loop systems, I have begun with the assumption that natural electron acceptors, such as haem groups of cytochromes and iron–sulphide groups of non-haem iron proteins, would probably function as electron translocators, and that natural hydrogen acceptors such as ubiquinone, plastoquinone and the flavin groups of flavoproteins, would probably function as hydrogen translocators.[37–40] However, as indicated by equations (10) to (12), it was also explicitly recognized[38,39] that the translocational specificities could affect the overall translocation reaction by determining the degree of protonation of the species undergoing translocation; and that, as in equation (10), functional groups of the carrier (such as the phosphate groups of NAD), which were not directly involved in the redox changes, could participate in proton translocation. Thus, my suggestion for one possible mechanism for Loop I of the mitochondrial respiratory chain[38,39] may be represented as follows:



(15)

where $NADH^-$ and NAD represent the actual ionization states of the nicotinamide nucleotides in water near pH 7, $(NADH^- + H^+)$ represents $NADH^-$ with the phosphate group fully protonated in the translocation complex, and Fe stands for the iron atom of a non-haem iron protein in the NADH dehydrogenase.

It was not possible to account for proton translocation by the "energy-linked" nicotinamide nucleotide transhydrogenase (Loop O) in terms of a normal type II loop (see equation 9), because this was not compatible with the observed transfer of tritium between the nicotinamide nucleotides without dilution in the water hydrogen-pool.[38,39] However, I pointed out at a meeting of the Biochemical Society in 1969, during the discussion of a paper on the "energy-linked" transhydrogenase,[66] that the principle which I employed to account for translocation of 2H atoms by NAD in Loop I provided the basis for a mechanism of proton translocation in Loop O, according to the following type of scheme:

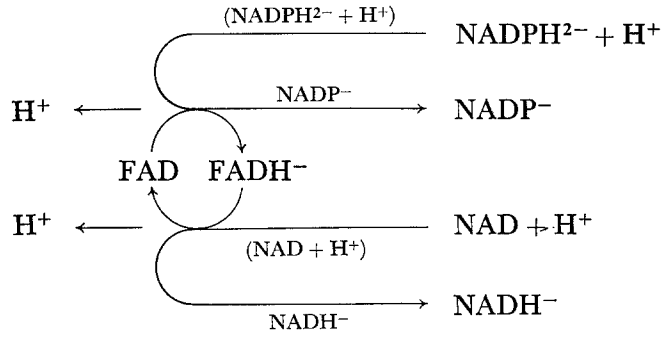


(16)

where $NADPH^{2-}$, $NADP^-$, $NADH^-$ and NAD represent the actual ionization states of the nucleotides in water near pH 7 and the bracketed forms represent the protonated species in the specific translocation complexes. The same general principle as that used above was also used by Skulachev[67] to suggest a proton-translocating transhydrogenase mechanism which was fundamentally similar to that of equation (16), but rather more complicated in detail. Incidentally, it is noteworthy[68] that the transhydrogenase system may contain a flavin nucleotide (FAD) and that this might possibly be included as shown in the scheme of equation (17) (see top of p. 20).

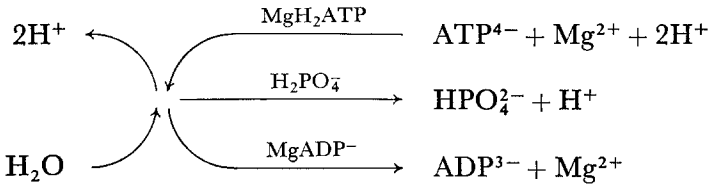
As suggested previously,[41] hydrogen translocation in Loop I might be mediated by FMN rather than by NAD, and likewise FAD could conceivably be involved rather than NAD or NADP in the type of Loop O mechanism represented by equation (16). The general principle of the proton-translocating mechanism would be the same.

Referring to equation (14), representing the proton-translocating ATPase of type II, since there are no known anhydrides formed prior



(17)

to ATP in oxidative and photosynthetic phosphorylation systems, and by analogy with the suggestions mentioned above for proton-translocating redox loops, it is possible that X–I, X^- and IO^- might be represented by ATP, ADP and inorganic phosphate. Accordingly, equation 14 might involve the adenine nucleotide and phosphate in proton translocation in some such way as the following:



(18)

where ATP^{4-}, ADP^{3-} and HPO_4^{2-} represent the actual states of ionization of ATP, ADP and phosphate in the right hand aqueous phase near pH 8 and MgH_2ATP and $MgADP^-$ represent the corresponding magnesium salts in the specific translocation complexes.

Figure 1A illustrates the suggested functions [in the process represented by equation (18)] of the biochemical components of the ATPase known as F_0 and F_1 in the terminology of Racker.[59] The The component F_1 is here represented as the natural ATPase, accessible to H^+ (and H_2O) in the complex with F_0, only from the left hand phase; and the region shown crudely as a hole through F_0 represents a specific proton-conducting pathway that can be blocked by oligomycin. Figure 1B illustrates, for comparison, my previous suggestion[38–40] according to which F_1 is an XI synthetase which only shows (artificial) ATPase activity when accessible to H_2O (in place of XI) on the left after dissociation from the F_0 component, as illustrated in Fig. 2.

It is interesting to compare equation (18) with a corresponding type of mechanism speculatively suggested for the $(Na^+ + K^+)$-activated

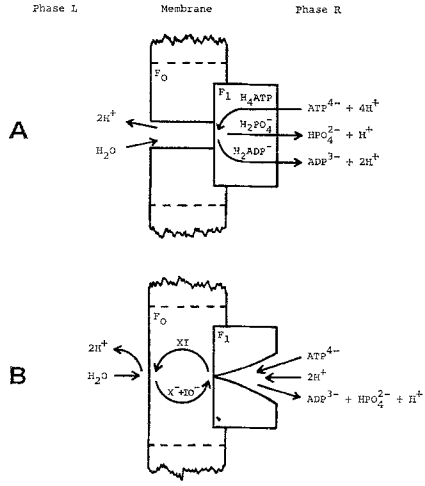


Figure 1. Diagrams of proton-translocating ATPase II: A, in which F_1 is represented as a natural ATPase catalysing proton translocation by the translocation of the adenine nucleotides and phosphate in appropriate protonation states; and F_0 is a locator of F_1, having a specific proton-conducting pathway through it. B, in which F_1 is represented as an XI synthetase and F_0 is a locator of F_1 having XI hydrolase activity and providing specific translocation channels for the functional groups XI and $X^- + IO^-$.

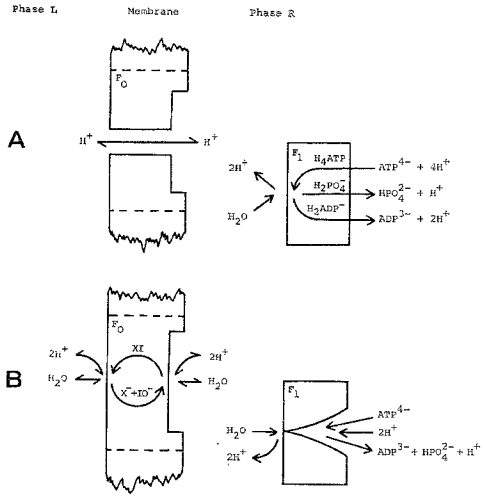
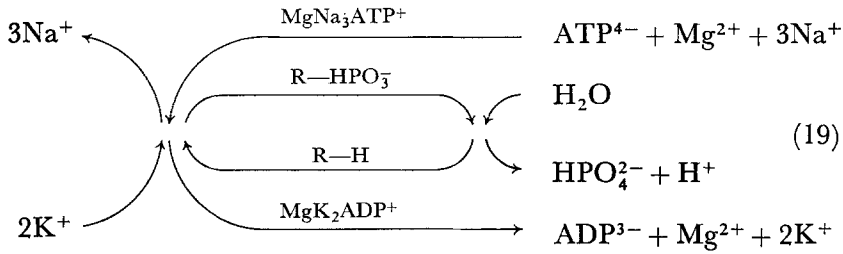


Figure 2. Diagrams of proton-translocating ATPase II, as in Fig. 1, but dissociated into separate F_0 and F_1 components, to illustrate the oligomycin-sensitive proton-conducting activity of F_0, and the natural (A) or artificial (B) ATPase activity of F_1 (see ref. 40).

ATPase, involving the adenine nucleotides directly in Na^+ and K^+ translocation,[29] in some such way as the following:



(19)

where $R–HPO_3^-$ represents the phosphorylated intermediate in the enzyme and the other conventions are as before.

The above comments, concerning the possible involvement of some nucleotide coenzymes in proton-translocation reactions, cannot be discussed fully here; but it is hoped that the general principles illustrated by the specific examples that I have given will help to provide new models for the interpretation of known experimental data, and also that they will help to enable new experiments to be designed so as to establish new unequivocal facts. In this context it is relevant to draw attention to the fact that the catalytic complexes in the systems catalysing oxidative and photosynthetic phosphorylation, which we may legitimately regard as "miniature engines", have actually been designed by the evolutionary process of natural selection.[35,36,42] Therefore, this brief discussion of some of the design principles that may possibly be involved in the redox loop and ATPase systems is not merely an academic exercise, but, if correct, should be related to the actual biochemical potentialities exploited by natural selection.

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