Hypothesis

Mosaic protonic coupling hypothesis for free energy transduction

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1. INTRODUCTION

Mitchell's [1] chemiosmotic coupling hypothesis is now generally accepted as the best model for membrane linked biological free energy transduction. Yet a number of reports have appeared which are seemingly at variance with the predictions of this hypothesis and which are considered as evidence for its necessary revision. Here we present an elaboration of the chemiosmotic hypothesis which is able to account for many of the published discrepancies. We have here taken the liberty of omitting all references. These will be included in a more extensive review, to be published elsewhere. An account of the relevant experimental evidence has recently been given by Ferguson and Sorgato [2].

2. POSTULATES OF THE CHEMIOSMOTIC HYPOTHESIS

The 'mosaic protonic coupling' model to be discussed below, maintains all the 4 basic postulates of the chemiosmotic hypothesis:

(i) Free energy transducing membranes contain 'primary proton pumps' which couple exergonic electron transfer or primary photochemical reactions to transmembrane proton movement.
(ii) Free energy transducing membranes also contain 'secondary proton pumps' which couple downhill transmembrane proton movement to endergonic ATP synthesis.
(iii) The membranes of energy transducing organelles have a finite H^+ permeability.
(iv) The membranes of energy transducing organelles contain anion and cation translocators.

3. THE ADDITIONAL POSTULATE OF THE MOSAIC PROTONIC COUPLING HYPOTHESIS

In the original formulation of the chemiosmotic coupling hypothesis, the nature of the aqueous phases, from which and into which protons are pumped, was left undefined (usually denoted as L and R phases). Slowly however, it has emerged that these two phases correspond to the (mitochondrial) M, or matrix, and C, cytosolic, spaces. Furthermore, these two aqueous phases have been assumed as being essentially homogeneous, i.e.,

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allowing complete free diffusion of protons. The extreme development of these ideas has led to the concept that the free energy transducing unit is identical to one entire energy transducing organelle: a chloroplast, or a mitochondrion would function as one vesicle, the free energy transduction of which could be uncoupled by making its single membrane permeable to protons. Thus, a single gramicidin channel should be sufficient to uncouple a single mitochondrion or chloroplast.

It is from this concept, and not from the coupling role of the proton, that most experiments digress. The mosaic protonic coupling hypothesis therefore takes a radical departure from these views and introduces a fifth postulate:

(v) In the native organelle, single primary (redox or light-driven) H^+ pumps do not share with other primary H^+ pumps the space (or domain) into which they pump protons but they do share these domains with other individual secondary ATP-driven H^+ pumps (or with a few of them).

This postulate has the fundamental implication that the proton spaces of the coupling units (the space from and into which the protons are pumped) are not at the same proton electrochemical potential. This also implies that a barrier exists to the free diffusion of protons between the proton spaces of the individual coupling units and the bulk phase of the organelle. As a consequence, the individual coupling units must act independently.

The idea of energy transducing organelles organized on the basis of coupling units is not entirely new and, from time to time, a close linkage between electron transfer and ATPase activity has been proposed. The most novel aspect of the present view lies however in the fact that, here, the concept of the pool function of protons is replaced by that of the coupling unit although the free energy is still being transduced by the protons. A fundamental role of localized protons in energy coupling has previously been proposed by Williams [3]. However in Williams' view the contribution of the electrical potential to the proton free energy is not considered predominant. In contrast, we retain the electrical potential term as providing a basic contribution to the proton free energy and we accept the possibility that some energy coupling can occur via the proton electrochemical potential in the bulk phase. We emphasize though that, in the intact native organelle, energy coupling operates mainly through small, independently operating, coupling units.

4. THE ELECTRICAL NETWORK ANALOGUE

In fig.1 we give an electrical network representation of the mosaic protonic coupling model.

Using electric network theory to simulate the expected behaviour of the mosaic protonic coupling, we have been able to generate equations, which account for those experimental results that are generally considered to conflict with the chemiosmotic hypothesis:

(i) At similar $\Delta\tilde{\mu}_H$ the respiratory rate is higher during ATP synthesis than during artificially induced proton (ion) leakage (uncoupling). The slope of the plot J_o *vs* $\Delta\tilde{\mu}_H$ is steeper when respiration is stimulated by ADP than when stimulated by uncouplers.

(ii) The rate of ATP synthesis, J_{ATP}, has no unequivocal relationship with the level of $\Delta\tilde{\mu}_H$. Although depression of $\Delta\tilde{\mu}_H$ by uncouplers is invariably accompanied by a reduction of J_{ATP}, respiratory inhibitors can inhibit J_{ATP} with little or no depression of $\Delta\tilde{\mu}_H$, while application of valinomycin + K^+ depresses $\Delta\tilde{\mu}_H$ but does not impair J_{ATP}.

(iii) The maximal attainable ΔG_p:$\Delta\tilde{\mu}_H$ ratio increases when $\Delta\tilde{\mu}_H$ is lowered by increased H^+ permeability.

(iv) In addition to these discrepant observations regarding the coupling between primary and secondary proton pumps, there is another experimental observation concerning the behaviour of the isolated proton pumps (either redox- or ATP-driven) which can only be accounted for by a sophistication of the delocalized coupling hypothesis. Addition of oxygen pulses to anaerobic mitochondria and bacteria results in negligible or non-appearance of protons in the bulk aqueous phase unless permeant ions are added. This is not due to proton backflow driven by $\Delta\tilde{\mu}_H$.

The simulations for the observations mentioned in points (i)–(iii), as well as for those reported in the next section will be presented elsewhere.

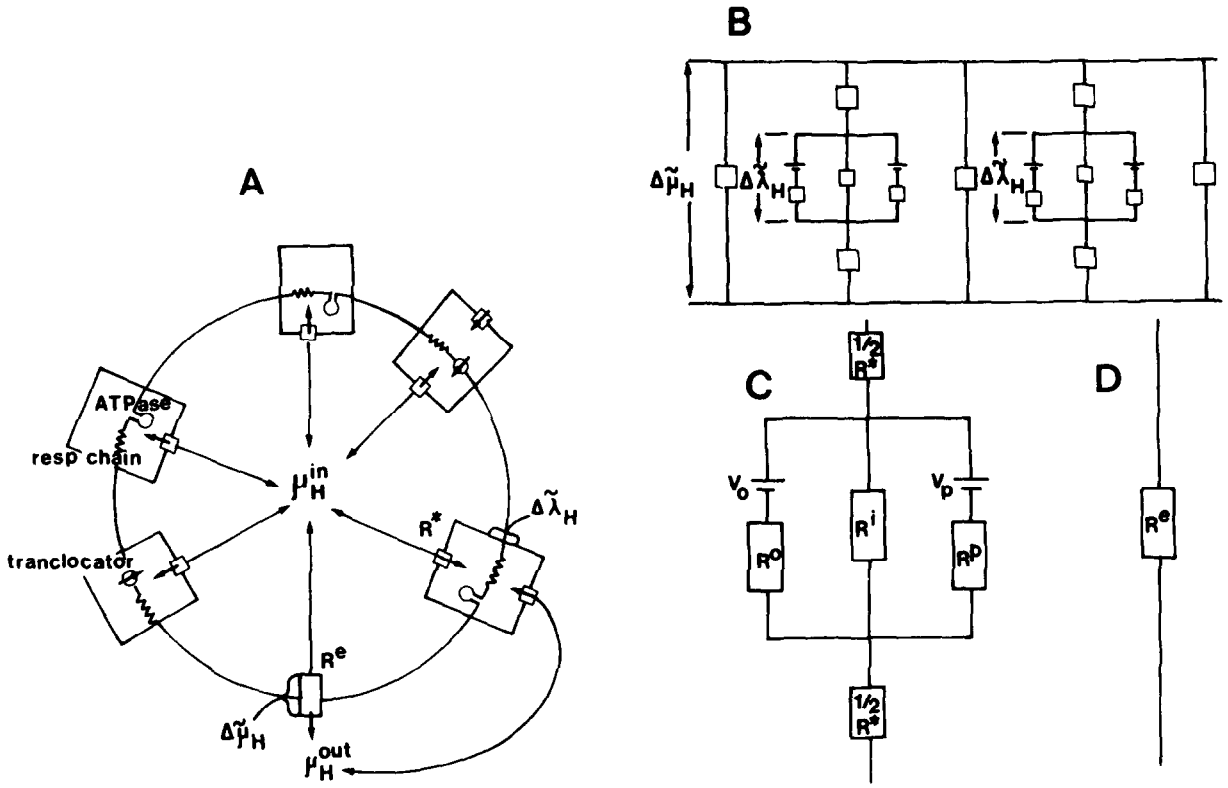

Fig.1. (A) The mosaic protonic coupling hypothesis for energy coupling (B). The electric (non-equilibrium thermodynamic) representation of (A). The scheme consists of the juxtaposition of two elements: n coupling units (C) and m bulk-phase-to-bulk-phase proton leaks (D). R, resistance, V, electromotive forces, and R^e, the resistance of each of the m proton leaks between the two bulk aqueous phases. Capacitances have been left out for simplicity. A coupling unit consists of two batteries (one 'O', for the redox H^+ pump and one, 'P', for the ATP H^+ pump), each with an internal resistance R^o and R^p respectively, a resistance with respect to proton back leakage (R^i) and connections to the bulk phases through finite resistances (R^*). It should be noted that the 'internal resistances' R^o and R^p do not refer to novel proton leakage pathways but rather to the enzymatic activity of the respective proton pump.

5. FURTHER CONSEQUENCES OF THE MOSAIC NATURE OF FREE ENERGY COUPLING

Some important observations still remain unexplained. Among these are:

(i) Inhibition of the primary proton pumps is accompanied by simultaneous inhibition of the secondary proton pump (and vice versa); upon such inhibition no excess capacity in the other pumps becomes apparent.

(ii) The exponential rise of the ΔG_p:$\Delta\tilde{\mu}_H$ ratio is also observed when $\Delta\tilde{\mu}_H$ is lowered with respiratory inhibitors.

(iii) During titrations with respiratory inhibitors there is a strict parallelism between the levels of $\Delta\tilde{\mu}_H$ in state 4 and state 3 mitochondria indicating that in state 3 abolition of the redox proton pump brings about the concomitant abolition of the ATPase proton pump.

All these phenomena indicate that there is more direct 'cross talk' between the primary and secondary proton pumps than through $\Delta\tilde{\mu}_H$. One proposed explanation for these cross talk observations has been that of a $\Delta\tilde{\mu}_H$ mediated allosteric control by the activity of the primary over the secondary proton pump: the 'local' $\Delta\tilde{\mu}_H$ would not only act as the high free energy substrate but also control an on-or-off switch at the secondary pump. The presence of such an on-or-off switch at the secon-

dary proton pump would then be an additional postulate. Here we wish to stress that such an additional postulate might not be strictly necessary to account for the 3 experimental observations mentioned in the preceding paragraph: the direct cross talk between the primary and secondary proton pumps might become a natural consequence of the fifth postulate of the mosaic protonic coupling hypothesis. This stems from the following analysis of the consequences of the fifth postulate.

The bulk phase proton electrochemical potential, $\Delta\tilde{\mu}_H$, represents the time and space average of the potentials of a large number of protons in a more or less homogeneous, aqueous phase. This is however not the case for the very small proton space of the coupling unit. In this case a very small number of protons needs to be pumped to raise the proton electrochemical potential to the level sufficient to drive ATP synthesis. Even if this local H^+ electrochemical potential were defined as an ensemble average or a time average (averaged over a time equal to many times the lifetime of the proton in the domain) the thermodynamic interpretation of this parameter would be problematic. Of course, if we assume that some domains contain a few energized protons and some do not, the average number of protons would still correlate with the rate of ATP synthesis although the overall process of ATP synthesis would then occur through a discontinuous distribution among the various coupling units. Yet, a single turnover of the redox- or light-driven H^+ pump will already generate a local proton potential driving ATP synthesis but only through that ATPase belonging to the same coupling unit. Furthermore, abolition of a primary pump will lead to abolition of ATP synthesis in its associated coupling unit. ATP synthesis will therefore depend on the number of active coupling units rather than on the average local proton potentials.

A visual description of the pattern of the proton potentials in an energy transducing organelle, organized in small and independent coupling units, can be obtained by considering the properties of a population of liposomes each possessing one (or a few) redox-driven and ATP-driven H^+ pumps. Activation of electron transfer will lead to formation of a proton electrochemical potential across the membrane of each liposome and this will drive ATP synthesis. Abolition of the individual redox H^+ pumps by suitable inhibitors will lead to depression of the local proton potential in each liposome and consequent abolition of ATP synthesis by that liposome, independently of the value of the proton potentials in the other liposomes. Thus the rate of ATP synthesis will be strictly a function of the number of active liposomes and not of the average local H^+ potentials.

If we compare the expected behaviour of such a population of liposomes to that expected for one large liposome containing all redox- and ATP-driven proton pumps in a single membrane, then we see that (points (i)–(iii) refer to the observations left unexplained, see above):

(i) In the large liposome, there will be no proportionality, in titrations with oligomycin, between abolition of the ATPase proton pumps and depression of the rate of ATP synthesis. This is because ATP synthesis would be stimulated in the uninhibited ATPases as a consequence of the rise of $\Delta\tilde{\mu}_H$ following the abolition of other ATPases. In the case of a population of small liposomes the increased $\Delta\tilde{\mu}_H$ would remain localized in those liposomes having inhibited ATPases and this would not be sensed by the uninhibited ATPases, situated in different liposomes. Also, in the large liposomes, the depressive effect of an inhibitor of the H^+-ATPase on oxidative phosphorylation would be reduced upon addition of a respiratory inhibitor. This is because such an addition would result in a relative excess of secondary over primary proton pumps. No such effect would be expected in the case of the population of small liposomes because there could be no such excess.

(ii) Elimination of redox proton pumps in the large liposome, though increasing the time needed to reach the static head (state 4), would not affect the ratio $\Delta G_p:\Delta\tilde{\mu}_H$ at static head when the condition has been achieved (the H^+-ATPase would still be in equilibrium). In the case of the population of small liposomes, elimination of redox proton pumps must cause a proportionate reduction in the number of liposomes having a proton gradient, $\Delta\tilde{\mu}_H$. Yet, the active liposomes retaining $\Delta\tilde{\mu}_H$ would still be competent at making ATP until the same ΔG_p is attained. Provided that the liposomes with an unenergized membrane (i.e., an in-

hibited respiratory chain) do not hydrolyze ATP at a high rate (which they would not be expected to do unless the ATP:ADP ratio really exceeds unity), then ΔG_p would be little affected by the addition of the respiratory inhibitor whereas the average $\Delta\tilde{\mu}_H$ would be significantly affected.

(iii) Titration with a respiratory inhibitor of the population of small liposomes would lead to the same percentage reduction of the average $\Delta\tilde{\mu}_H$ independently of whether the liposomes are in state 3 or in state 4. In the single large liposome one would expect the respiratory inhibitor to have a much stronger effect in the presence (state 3) than in the absence (state 4) of a free energy drain.

We find it of further interest to note that mosaic protonic coupling by itself also accounts for a phenomenon which is in keeping with delocalized coupling only if one makes the additional assumption of molecular slip in the proton pumps (or of non-ohmicity of the proton leaks): upon addition of respiratory inhibitors there is no proportionality between the reduction in the rate of electron transfer and the reduction in $\Delta\tilde{\mu}_H$.

The question may arise as to whether the electrical field formed by an active primary pump may extend also over the proton domain, situated in the vicinity of another coupling unit, whose primary pump is inactive. This would certainly be the case if:

(i) The translocation of protons were to occur from the inside to the outside of a topologically closed and homogeneous coupled membrane and

(ii) the aqueous phases at the two sides of the coupling membrane were analogous to the two metal phases of a spherical electric capacitor.

However, we visualize the coupling membrane as a mosaic structure containing locally coupled redox- and ATP-driven H^+ pumps and independent H^+ leaks. The electrical field through such a membrane will not be laterally constant but depend on the spacing between the coupling units, the dielectric constants of the barriers between proton domains and bulk aqueous phases and the resistances of the leak pathways. It might be predicted that the electrical potential difference across the coupling unit will be higher than that through the bulk to bulk phase or that corresponding to a leak pathway. It may also be predicted that the values of $\Delta\psi$ and ΔpH calculated on the distribution of suitable markers in the stationary state will be a composite mixture of the proton electrochemical potentials in the proton domains and in the bulk aqueous phases depending on the values of the interphase resistances and of the dimensions of the different osmotic spaces.

We are aware of the fact that the electric network analogue of fig.1 is not adequate to deal with the thermodynamic considerations outlined above. This is because the principles of electric circuits, based on steady state assumption, cannot be satisfactory applied to a system where the definition of the proton electrochemical potential has become so problematic. Certainly a model based on a statistical, rather than on a thermodynamic, satisfactorily applied to a system where the defini- to this purpose. This is currently under development in our laboratories.

In conclusion, here is a model, which we denote as the mosaic protonic coupling system, which constitutes a sophistication of the chemiosmotic hypothesis in that it assumes a functional organization of the primary and secondary proton pumps in small coupling units. This proposal has been shown to account for a large number of the observations which are believed to be in conflict with the delocalized version of the chemiosmotic hypothesis. In a forthcoming article we shall review and reference most of the relevant literature in the light of the delocalized and mosaic protonic coupling hypotheses and, by using electric network theory, we shall illustrate simulations of all the experiments listed in the present paper.

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