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Paired Moving Charges in Mitochondrial Energy Coupling. II. Universality of the Principles for Energy Coupling in Biological Systems*

(charge-separating devices/electron transfer chain/intrinsic ionophores/ionophore-mediated processes)

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ABSTRACT **The thesis is developed that an acceptable model of biological energy coupling must have universal application. The paired moving charge model of mitochondrial energy coupling is examined from the standpoint of this thesis. Fundamental to this model is the notion that energy coupling involves interaction between paired uncompensated charged species in two vectorially aligned and spatially separated reaction centers. The two charge-separating devices are assumed to be the electron transfer chain (in chloroplast and mitochondria) and intrinsic ionophores (in all transducing organelles and kinases). The universality of the ionophore principle becomes then the crucial test of the validity of the paired moving charge model. The multiple facets of ionophore-mediated coupled processes are explored, e.g., coupled hydrolysis of ATP, hormonal control of ion movements, and active transport.**

The design and performance of living systems reflect to a profound degree two sets of principles—the principles of heredity and the principles of energy. The double helix principle of nucleic acid construction enunciated by Watson and Crick two decades ago (1) has been shown by overwhelming evidence to apply across the board to the hereditary process in all types of cells and in all forms of life. Moreover, all the events and systems that translate the hereditary process reflect with great fidelity both the centrality and the uniqueness of the double helix principle. The systems that execute replication (ribosomes, messenger RNA, transfer RNA, chromosomes, and the mitotic apparatus) and that control replication (genes, cistrons, and operons) are in fact molecular devices for the precise translation of the double helix principle. The point to be made is that, given a universal principle of heredity, then all the agents, systems, and tactics that underlie the execution of this principle must also be universal in nature. The ribosome and transfer RNA are two such examples in the structural domain. The mechanisms of protein and nucleic acid synthesis are examples in the functional domain. The universality of the genetic code is an example in the domain of molecular strategy.

We have considered elsewhere the compelling nature of the case for the proposition that the principles of energy, like those of heredity, must be universal in character in biological systems (2, 3). We shall restrict our treatment of energy to the molecular tactics by which two processes are coupled one to another. Energy coupling subtends the most crucial aspects of energy in biological systems, and in our view, the understanding of all other aspects of energy, such as catalysis, is derivative from the fundamental principles of energy coupling.

In the previous communication of this series, we have proposed and developed a model for mitochondrial energy coupling (4). Since the principles that underlie the model were arrived at by the fitting of theory and experiment, the ability of the model to rationalize mitochondrial energy coupling is in large measure a reflection of the accuracy of the fitting process. The crucial test of the paired moving charge model, as well as of all other proposed models, is then the capacity to rationalize energy coupling in bioenergetic systems generally. In this extension of the model, the universal applicability of the postulated principles of energy transduction is being tested.

The present communication is addressed primarily to the delineation of the mechanistic, molecular, structural, and functional features of the paired moving charge model which should have universal applicability. If these features are shown experimentally to be intrinsic to energy coupling mechanisms, then the model can be assumed to be validated. We are not attempting in the present communication to develop fully the experimental case in support of these features, although mention will be made of specific experiments that have guided us in these projections. The systematic marshalling of the supporting experimental evidence will be undertaken in future communications.

UNIVERSAL PRINCIPLES OF ENERGY COUPLING

Energy coupling is carried out both by organelles (mitochondria, chloroplast, sarcoplasmic reticulum, muscle, cell membrane, etc.) and by specialized enzymes known as kinases (acetate kinase, hexokinase, enolase, etc.). We shall consider the principles of energy coupling in organelles before attempting to extend these principles to energy coupling in kinases.

In all energy coupling, two chemical reactions or processes taking place in separate centers are coupled. In one center, the exergonic center, the chemical reaction or process is the driving reaction. In the complementary center, the endergonic center, the chemical reaction or process is driven by coupling to the driving reaction in the exergonic center. The two centers are vectorially aligned across a membrane, as in the mitochondrion, or in an assembly of parallel filaments, as in muscle. In each center, charge separation takes place (for example, the separation of the electron from its proton in the exergonic center, and the separation of K^+ from its anion in the endergonic center). The two respective charge separations are paired and coupled so that in a net sense, there is no separation of charge, but rather substitution of one charged partner by another partner of the same charge. By virtue of the charge separation in each of the two reaction centers

* Paper I in this series is ref. 4.

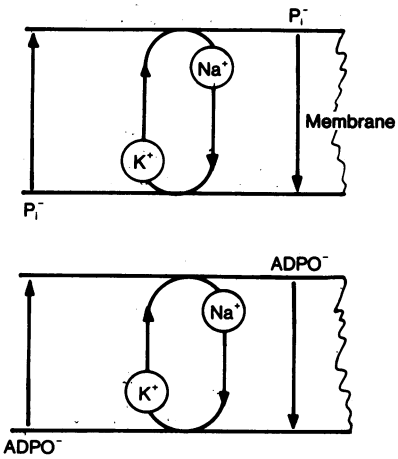

FIG. 1. The mechanism of the Na^+/K^+ ATPase. ATP generates two negatively charged ions (P_i^- and $ADPO^-$), each of which drives in an ionophore-bound K^+. Since the ionophore for K^+ is linked to the ionophore for Na^+ in cog-wheel fashion, the influx of K^+ is automatically geared to the efflux of Na^+. Thus, ATP hydrolysis drives P_i and ADP across the membrane and this is coupled to the efflux of P_i and ADP. In effect, the only net change is then the influx of K^+ and the efflux of Na^+. It is to be noted that the antiport movement of two plus charges (K^+ and Na^+) is mediated by the antiport movement of two negative charges (P_i^- and $ADPO^-$).

(ejection of the proton from the exergonic center, and of the anion from the endergonic center), a moving charge in the exergonic center (e.g., the electron) drives a moving charge in the endergonic center (e.g., an ionophore-linked cation). The movement of the driving charge is energetically downhill; the movement of the driven charge is energetically uphill. By virtue of electrostatic and other interactions and the principle of respiratory control, the movement of the driven charge is inextricably tied in to the movement of the driving charge.

If the moving charge in the endergonic center is a cation contained by coordination bonds within an ionophore, coupling leads to active transport. If the moving charge in the endergonic center is an anion stabilized by coordination with a metal–ionophore complex in an anion transfer chain, coupling leads to a chemical reaction such as synthesis of ATP from ADP and P_i. If the moving charge in the endergonic center is an electron stabilized by an electron transfer chain, coupling leads to reverse electron flow or to energized transhydrogenation. Finally, if the moving charge in the endergonic center is a filament (positively charged by virtue of paired charge separation), coupling leads to muscular contraction. These four options cover all of the coupling alternatives found in energy-coupling organelles. The ATP-energized antiport movement of Na^+ and K^+ in the nerve membrane (5) may appear at first sight to be incompatible with the moving charge model. Fig. 1 provides a mechanism derivable from the model which provides a simple explanation for the movement of K^+ and Na^+ in equal amounts and in opposite directions. The propagation of an electrical impulse by the nerve membrane is, strictly speaking, not a coupled process, but rather a perturbation by a triggering mechanism (electric current or hydrolysis of acetylcholine) of an already primed system, i.e., a system with osmotic gradients that controls the directional movements of ions. How the moving charge model can be extended to cover this special case lies outside the scope of the present article.

In energy coupling in kinase systems, the same principles are assumed to apply except that the two centers are so close together that the movement of separated charges is virtual and, thus, the extent of charge separation is limited (2). Paired charge separation in the two centers of the kinase corresponds to paired charge separation in the two centers of a transducing organelle. Moreover, the driving charge (usually generated from ATP) drives the uphill synthesis accomplished by the driven charge (acetylation of CoASH, phosphorylation of glucose, etc.).

UNIVERSAL MOLECULAR DEVICES FOR CHARGE SEPARATION

The essence of the paired moving charge model is that charge separation in energy coupling is always paired (2, 4, 6). This is the condition that has to be met for charge separation to be isoenergetic or nearly so and for charge separation to be a ground state phenomenon (7). Thus, in describing the molecular tactics by which one pair of charge can be separated, it should be implicit that this separation of one pair in one center is coupled to the separation of another pair in another center. In essence then, charges are being substituted by the pairing technique, although in each center the original two partners of the charged pair are indeed physically separated.

In biological systems, two molecular devices are known that can achieve charge separation. These are the electron transfer chain and the ionophores. The electron transfer chain, as in the mitochondrion, the chloroplast, and respiration-dependent bacterial transducing systems, separates active hydrogen into an electron (which traverses the chain in the membrane) and a proton (which is ejected from the membrane). At the other side of the membrane, the electron and proton are reunited in the terminal acceptor molecule. The proton is taken up from the aqueous medium on the side of the membrane opposite from that in which the original charge separation took place. Ionophores are the molecular devices par excellence for achieving charge separation, and these are found in all the transducing organelles as well as the kinases. Ionophores not only separate cations from anions, but also anions from cations (8). Thus, valinomycin will separate K^+ from, say, Cl^- (by transfer of K^+ from the aqueous phase to the interior of the ionophore in the membrane phase). But by the same token, the valinomycin–K^+ complex can separate the dinitrophenolate anion from its cation (by transfer of the anion from the aqueous phase to the exterior of the ionophore in the membrane phase). Anions, like cations, can be transported by ionophores across membranes. There is circumstantial evidence for a chloride ionophore in mitochondria that mediates both the passive and energized movements of chloride (9–11).

The electron transfer chain and the ionophores achieve charge separation in the ground state (7). Moreover, they achieve net charge separation, i.e., separation beyond orbital constraints. Thus, in the electron transfer chain, the electron is separated from its proton by a distance of 50–70 Å (the thickness of a membrane); similarly, ionophore systems can separate cations from anions by comparable distances across a membrane (11).

DUAL FUNCTIONS OF INTRINSIC IONOPHORES

Ionophores play key roles, not only in transport of ions, but also in catalysis. Ionophores must therefore be placed in a larger context than that of merely a charge-separating device. An ionophore may be defined as a molecule with a polar in-

terior containing a constellation of groups capable of coordinating a metal ion, and with a nonpolar exterior that enables passage through a nonpolar medium such as a membrane phase. The charge of the metal ion is sufficiently screened by the ionophore that the ionophore-bound metal ion can move freely across a membrane phase. The maneuver by which the ionophore becomes accessible to the metal ion in the aqueous phase and closes down on the metal ion in the nonpolar phase has been thoroughly documented in a long series of systematic investigations [see the reviews of Eisenman *et al.* (12) and Eigen and De Maeyer (13)]. But this containment of the metal ion within the polar interior of the ionophore is relevant not only to the tactics of transmembrane ion movements, but also to the activation of groups such as phosphate, ADP, etc. We may think of the interior of an ionophore as a highly protected local environment that insulates the charged species contained therein against random interactions. In view of this dual role of ionophores, it would be simpler to examine each role separately and consider the multiple facets of ionophores relevant to each of these roles.

The antibiotic ionophores provide the usual stereotype of ionophores concerned in transport of cations. These are small molecules, soluble in nonpolar organic solvents, which can readily shuttle monovalent or divalent metal ions across a membrane (14). However, the intrinsic ionophores of biological energy coupling systems are not free species (15); these are contained within proteins and become free only when released from their protein containers. This is not to say that the intrinsic ionophores are covalently linked to the proteins with which these are associated. They may be freely moving, perhaps within the lipid interior of a protein canister spanning the membrane, but the fact of their association with protein means that the control of the transport of ions by intrinsic ionophores can be exercised at the protein level. In mitochondria, the control of the coupling options is known to be exercised via the control of ionophore function (J. H. Southard and D. E. Green, unpublished studies).

Whether ionophores move freely across a membrane within the lipid phase, as in the case of antibiotic ionophores, or move freely across a membrane within the protein phase, as in the case of intrinsic ionophores concerned in transport, the rationale of ionophore-mediated ion flow remains unchanged. Ionophores collectively provide unique devices for ferrying cations across biological membranes.

Synthetic iodonium and organo-tin compounds are known which can ferry anions (Cl^-, OH^-) across biological membranes (9, 10). These undoubtedly have their counterparts in intrinsic ionophores, since mitochondria and other organelles show, to a high degree, the capability for transporting chloride ions (11). In such ionophores, a positively charged heavy metal ion intrinsic to the ionophore coordinates the anion, and the transported species is then the anion rather than the cation.

Only the merest beginnings have been made in the exploration of ionophores that play a role in catalysis. All such catalytic ionophores are associated with protein, but in a different way from that which applies to the transport ionophores. The catalytic ionophores are more like the prosthetic groups of enzymes. They are fixed in position within the protein, and their links with the protein are crucial to their catalytic function. But whether concerned with catalytic or transport functions, the ionophore in both cases has the same fundamental set of properties—polar interior, nonpolar exterior, coordinating groups for metal binding, etc. The catalytic ionophore extracted from its protein system is indistinguishable from the ionophores concerned in transport with respect to its ability to induce movement of cations in the usual assay systems for ionophores (R. J. Kessler, G. A. Blondin, and D. E. Green, unpublished studies).

The catalytic ionophores have the property of forming composite ions between a metal (usually Mg^{++}) and some anion such as phosphate or ADP (R. J. Kessler and D. E. Green, unpublished studies). The protein is an absolute requirement for generating such composite ions. We may think of phosphate coordinated to Mg^{++} within a catalytic ionophore as equivalent to active phosphate, i.e., phosphate capable of phosphorylating some acceptor hydroxyl group. The ionophore principle, in effect, allows the containment of a metal ion within a protein without the necessity for the positive charge or charges of the metal ion to be committed to the linkage with the protein. The catalytic ionophore thus serves as a molecular device not only for separating charge (in this case the separation of the divalent metal from its anion and the separation of the phosphate group from its cations), but also as a catalytic device for activation of the phosphate group.

The activated group in the catalytic ionophores is transferred to an acceptor group, but the metal ion is not. Thus, catalytic ionophores participate in group transfer (be it the phosphoryl, acetyl, or whatever group). The ionophore is thus not a mobile species. It is the repository of a transferrable and activated charged group. In that sense, catalytic ionophores correspond to the oxidation-reduction proteins in the electron transfer chain.

There are basically three kinds of ionophore structural modules—closed or doughnut-shaped ionophores like valinomycin (16), hairpin-shaped ionophores like avenaciolide (17), which are closed at one end and open at the other, and finally ionophores like X537A, which have the shape of two apposed platforms and which are open at both ends (18). These descriptions of the shape and geometry of the ionophore apply to the state of the ionophore when linked to a cation in a hydrophobic phase. The closed-type ionophores are exclusively concerned in transport functions, are highly selective with respect to the entering atoms (19), and usually show a high degree of preference for monovalent cations. In general, there is a severe limit to the size of the atom or molecule that the closed type of ionophore will accommodate. The open types of ionophores usually can accommodate both divalent and monovalent metals (20). The catalytic ionophores probably will be drawn exclusively from this structural class.

ENERGY PULSING BY ARRAYS OF CHLOROPHYLL, RHODOPSIN, ETC.

In the moving charge model, we have considered thus far only the coupling of two chemical reactions. How can this framework of energy coupling accommodate photochemical processes such as chlorophyll-mediated photosynthetic phosphorylation and the rhodopsin-mediated generation of a nerve impulse? We may think of these excited state phenomena as reflections of events that precede or prepare the way for energy coupling; the principles of energy coupling still apply even when energy coupling is dependent upon prior photochemical events. The chlorophyll arrays (21) provide a device by which the energy of a photon can be transmitted with no

loss from one chlorophyll molecule to another within the array and then delivered to a reductant in the photosynthetic electron transfer chain. The light-activated reductant thereby acquires a highly negative reducing potential that then triggers the coupled downhill flow of an electron through the transfer chain (22). The chlorophyll chain is thus considered to be a device for transmitting electromagnetic energy absorbed by chlorophyll directly to the primary reductant. We are postulating that arrays of rhodopsin (23) fulfill a similar function in the visual cycle. These arrays transmit electromagnetic energy absorbed by a particular molecule of rhodopsin to some as yet undefined component in the nerve transmission mechanism. This component becomes photoactivated and triggers the train of events that lead to nerve transmission. Thus, photochemical phenomena are adjuncts of, but are not intrinsic to, energy coupling.

UNIVERSAL MECHANISM FOR COUPLED ATP HYDROLYSIS

In transducing organelles, the mechanism for coupled ATP hydrolysis is assumed to involve the following sequence of reactions mediated by the ATPase complex and its complement of ionophores. The fundamental and initial event is the enzyme-catalyzed charge separation of ATP into P_i^+ and $ADPO^-$ (§). $ADPO^-$ reacts with a divalent metal ionophore, which we shall symbolize as (Me^{++}). This interaction leads to a composite $(Me^{++}\bullet ADP)$ ion, which we will symbolize as $(Me^{++}\bullet ADP)^{-1}$. The charge of this composite ion is denoted by the notation on the right-hand side of the symbol. Not all the groups that will contribute charge to the ionophore complex are shown in the formulation, e.g., a possible carboxyl group in the ionophore or protein. P_i^+ reacts with a phosphoryl acceptor (ROH) to generate ROP with liberation of a proton (24). After the $ADPO^-$ group is transferred through a chain of ionophoroproteins finally to the aqueous medium, ROP is charge-separated in the presence of a second divalent metal ionophore, which we will symbolize as (Mg^{++}). ROP, in effect, separates into R^+ and P_i^-; P_i^- then reacts with ionophore-bound Mg^{++} to generate $(Mg^{++}\bullet P_i)^{-1}$. The P_i^- group is then transferred via a chain of ionophoroproteins to water. We are of course assuming that the hydrolysis of ATP is coupled and that the charged species generated during ATP hydrolysis undergo coupled movement with charge species generated in the endergonic center. The actual charge movements are not being considered in the present context, which is concerned only with the chemical intermediates.

In energy coupling in kinase systems, the initial resolution of ATP by charge separation is assumed to follow the same pattern as for the ATPase of organelles:

$$ATP \rightleftarrows P_i^+ + ADPO^-$$

In acetate kinase, the cleavage point is between the second and third phosphate groups (28):

$$ATP \rightleftarrows PP_i^+ + AMPO^-$$

Only the negatively charged species interacts with the divalent metal ionophore:

$$Mg^{++} + AMPO^- \rightleftarrows (Mg^{++}\bullet AMP)^{-1}$$

PP_i^+ reacts with an OH^- group to generate PP_i, and $(Mg^{++}\bullet AMP)^{-1}$ reacts with CoASH to generate AMPS-CoA and Mg^{++}. Finally, acetic acid displaces AMP from AMPSCoA to generate acetyl-SCoA and $AMPO^-$, which is then protonated to form AMP (25).

In the hexokinase system (26), ATP is hydrolyzed into ADP^+ and P_i^-, and it is the latter that reacts with a divalent metal ionophore:

$$P_i^- \rightleftarrows (Mg^{++}\bullet P_i)^{-1}$$

$$(Mg^{++}\bullet P_i)^{-1} + \text{glucose} \rightleftarrows \text{glucose-6-}P + (Mg^{++})$$

Not all kinases react exclusively with Mg^{++}-requiring ionophores. Some, like enolase, have requirements for both monovalent (K^+) and divalent (Mg^{++}) ionophores (27). Some show a specific requirement for a Mn^{++}-binding ionophore (28). Similarly, not all kinases are energized by ATP (29). The nucleotide could be GTP, UTP, or ITP; moreover, the nucleotide could be the diphosphoester rather than the triphosphoester (30).

Apart from the elimination of paired charge movement, the chemical mechanism of coupled ATP hydrolysis in kinase systems is indistinguishable from that in transducing organelles. Both products of ATP hydrolysis are activated by ionophores in organelles, whereas usually only one product is activated in kinase systems. There is, of course, greater variety with respect to the two products of ATP hydrolysis in kinase systems (e.g., AMP + PP_i, ADP + P_i, adenosine + PPP_i), but this is not a fundamental distinction.

UNIVERSAL MECHANISMS FOR CONTROL OF ENERGY COUPLING

Mitochondria have a control mechanism that regulates the coupling mode of the transducing units (J. H. Southard and D. E. Green, unpublished studies). In one mode, the transducing units carry out oxidative phosphorylation, reverse electron flow, and energize transhydrogenation. In the other mode, the transducing units carry out active transport of K^+ and Mg^{++}. The control of the transition from one coupling mode to the other is apparently exercised at the level of the ionophores. The K^+ and Mg^{++} ionophores are turned on and off during a cycle of this transition in the coupling mode. Oxidative phosphorylation is tied in to the latency of the K^+ and Mg^{++} ionophores and the loss of the capability for oxidative phosphorylation goes parallel with the emergence of functional K^+ and Mg^{++} ionophores. The point to be emphasized is that the mitochondrial system for control of energy coupling exercises that control by regulation of the state of the ionophores. On the basis of the known rationale of the mitochondrial control systems, we are suggesting and predicting that control of energy coupling in organelle transducing systems will generally be exercised at the level of the ionophores.

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§ P_i^+ denotes H_3PO_4 stripped of an OH^- group. P_i^- denotes H_2PO_4 stripped of a proton; P_i^{2-} denotes H_3PO_4 stripped of two protons; and $ADPO^-$ denotes adenosine diphosphoric acid stripped of one proton.

There is, in fact, a large body of evidence in the older literature to support this thesis. Hormones, such as thyroxin (31, 32), parathyroid hormone (33, 34), and the corticosteroids (35), exert a profound influence on ionophore-controlled ion movements. The control by vitamin D of the movements of Ca^{++} in kidney and intestinal mucosa is another clear example of the exercise of control at the ionophore level (36). Then finally, we have innumerable examples of how the level of Ca^{++} profoundly affects a wide variety of physiological processes—muscular contraction (37), blood coagulation (38), bone deposition (39), osteoporosis (40), complement fixation (41), regulation of glycogenolysis (42), secretion of neurohormones (43), gluconeogenesis (44), histamine release (45). It is to be noted that we are assuming that all movements of cations, such as those of Na^+, K^+, Mg^{++}, and Ca^{++}, are ionophore-mediated and that perturbation of cation movements is *ipso facto* evidence of ionophore participation.

Cyclic AMP has been shown to be a key component in the regulation of a large number of metabolic processes (46, 47). It is noteworthy that cyclic AMP is generated from ATP in a Mg^{++}-requiring kinase reaction and that the action of cyclic AMP on enzymes such as phosphorylase kinase is also a Mg^{++}-requiring reaction (48, 49). We interpret these observations in terms of the intimate relation between Mg^{++}-dependent ionophores and cyclic AMP, and we are suggesting that cyclic AMP may exercise in part its control functions via the ionophores of kinase systems.

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