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Paired Moving Charges in Mitochondrial Energy Coupling

(paired charge separation/charge-separated state/paired charge flow)

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ABSTRACT **A model of mitochondrial energy coupling has been proposed based on the principles of paired charge separation and vectorial paired charge flow. The unique role of the electron transfer chain and ionophores in mediating charge separation is emphasized.**

The present model evolved in three stages. The study of configurational transitions in isolated mitochondria led to the concept of a conformationally strained energized state (1) generated by an exergonic center and discharged by an endergonic center (the conformational model). The study of energized proton ejection in turn led to the concept of the energized state as a charge-separated state (2, 3) with the protein acting as an electromechanochemical transducer (the electromechanochemical model). Finally, the study of respiratory control in liposomal vesicles of cytochrome oxidase (4) led to the recognition of the primary role of moving charges in energy coupling. From the conformational model, the principle of direct coupling between exergonic and endergonic centers was deduced. From the electromechanochemical model was derived the principle that energy coupling requires the charge-separated state in both exergonic and endergonic centers. Thus, the two earlier models prepared the ground for the development of the moving charge model (5, 6).

THE PAIR MOVING CHARGE MODEL

Exergonic and Endergonic Centers. Coupling invariably involves two reaction centers vectorially aligned with respect to the membrane (see Fig. 1)—one in which an exergonic reaction (the driving reaction) takes place and the other in which endergonic reaction (the driven reaction) takes place. The two centers are separated by a linkage system to be defined later which facilitates the coupling of the two respective chemical reactions. We are assuming complete separation in space of the exergonic and endergonic centers and no mixing of any of the reactants or products.

The exergonic and endergonic reactions proceed in a vectorial fashion (see Fig. 2) so that the initial reactants are on one side of the membrane and the final products are on the other side of the membrane (7–9).

The Essentiality of the Charge-Separated State for Energy Coupling. Chemical energy may be defined as the energy intrinsic to the system of paired electrons and protons in atoms and molecules. The utilization of chemical energy requires, as a first preliminary, the tearing out of charges beyond orbital constraints. We are thus postulating that the charge-separated state of the reactants in a chemical reaction is a prerequisite for energy coupling.

Consider the energy of an electron and a proton in a H atom (the valency state) and the energy of the separated electron and proton at a critical distance at which orbital interactions no longer apply. We are assuming that this separation is achieved by the appropriate conditions (paired charge separation) and with the necessary catalysts, e.g., an electron transfer chain or an ionophore, to satisfy the requirements for reversibility. According to Kemeny (10), the valency state and the charge-separated state for a pair of charges can be isoenergetic or nearly so (see Fig. 3) under these conditions. Note that the energy of the charge-separated state increases as the distance between the charges increases beyond the critical separation distance (d_s). This resistance to extended charge separation is a crucial factor in energy coupling.

We are postulating that coupling between two chemical reactions proceeding independently with no common intermediates, and taking place in vectorially aligned reaction centers which are separated in space, must involve interactions between the charges generated in the chemical reactions in the two respective centers (see Fig. 4).

Paired Charge Separation. In Fig. 5, the coupling of electron flow in an electron transfer complex to the ionophore-mediated transmembrane flow of K^+ in an endergonic center is depicted according to the model. The charge separation in the electron transfer complex is paired to charge separation in the cation-transporting center. But note that charge separation is followed by charge substitution (K^+ replaces the proton as the partner for the electron, and the electron replaces the anion as the partner for the K^+) and finally charge flow. The flow of the electron is coupled to the flow of K^+ contained within an ionophore and there is consequently no net transfer of charge across the membrane. Charge separation, charge substitution and charge flow are concerted processes which require specialized catalysts and molecular devices. We shall be considering these devices in a later section.

The principle of paired charge separation is basic to our model of energy coupling, and it needs further definition. In the electron transfer complex (the exergonic center) the electron and the proton in the primary electron donor (SH_2) are separated via the electron transfer chain. The electron moves through the chain in the membrane, whereas the proton is ejected into the aqueous medium on one side of the membrane. The movement of the electron through the chain from one oxidation-reduction component to the next is energetically favorable, but the separation of the electron from its proton is energetically more unfavorable. Thus, unpaired charge separation is essentially excluded. More energy has to be expended in charge separation than is gained by transit of the electron to a lower energy level. When, however, the separation of the electron and proton in the exergonic center is paired to the ionophore-mediated separation of K^+ and Cl^-

Abbreviation: PMC, paired moving charge.

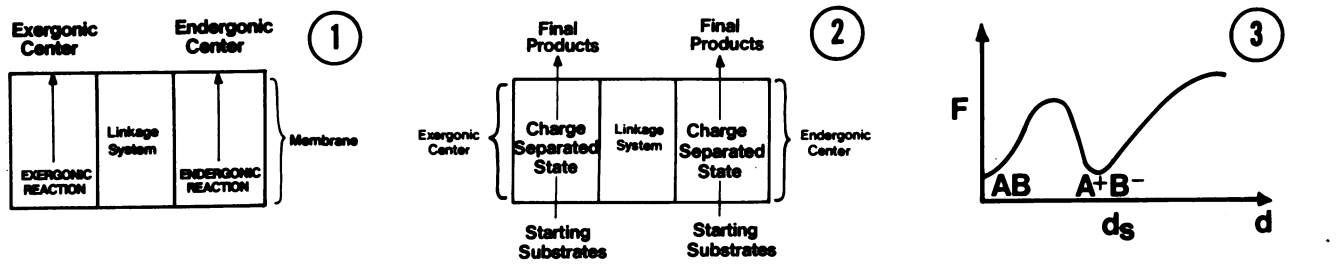

FIG. 1. Coupling as an interaction between two reaction centers (exergonic and endergonic) separated in the membrane phase by a linkage system.

FIG. 2. Vectorial nature of the paired reactions involved in energy coupling.

FIG. 3. The free energy (F) in the valency and the charge-separated state. Figure reproduced from the article of G. Kemeny (10).

in the endergonic center, the energetic disadvantage of separating charge is eliminated. Now the energetic advantage of moving an electron down the potential gradient becomes paramount. This will be true even if the K^+ is moving up the potential gradient, providing there is no net gain of energy.

In respiratory control (11) electron flow in coupled mitochondria is arrested unless this flow is coupled to some other charge flow such as active transport of cations or synthesis of ATP. The pairing principle may be considered to be the basis for the phenomenon of respiratory control.

The profound difference in the energetics of paired versus unpaired charge separation is readily appreciated by comparing electrolysis of water and the interaction of Na_2SO_4 and $BaCl_2$ in water leading to the deposition of $BaSO_4$. Electrolysis would correspond to unpaired charge separation, and double substitution to paired charge separation. The former requires a large input of energy; the latter proceeds spontaneously.

Gradient Generation During Active Transport. Electron flow automatically generates a proton gradient. Protons are ejected on one side of the membrane and taken up on the other side (see Fig. 5). Similarly, cation flow automatically generates a gradient equal in magnitude and opposite in direction for the cation. The concentration of K^+ will be higher on one side of the membrane and lower on the other side of the membrane. Note, however, that the K^+ gradient is generated by actual transmembrane movement of the cation, whereas the H^+ gradient is generated *without* transmembrane movement of protons. In coupled particles the membrane is impermeable to protons.

Symport Versus Antiport Coupling. Kemeny has enunciated the principle (12) that coupling requires the interaction of a moving negatively charged species with a moving positively charged species (symport charge flow). Yet mitochondria can effectuate a set of processes which involve coupling between two negatively charged species moving in opposite directions (antiport coupling) (13–15). Such coupling is possible only when antiport charge flow is a composite of two symport flows which are proceeding in opposite directions (see Fig. 6). A linkage system involving two connected positive charges which move in opposite directions can catalyze antiport coupling. Note that the charges of the linkage system circulate within the membrane and never leave the membrane phase.

In oxidative phosphorylation, electron flow is coupled to the antiport flow of P_i^- and $ADPO^-$ (see Fig. 7) and such coupling requires the intervention of the linkage system. The ATP synthesizing system has the capability of separating P_i into P_i^- and a proton, and ADP into $ADPO^-$ and a proton (we are assuming that both P_i and ADP enter the catalytic cavity of the synthetase in their fully protonated forms). The separation of P_i^- and H^+ or of $ADPO^-$ and H^+ in the synthetase is synchronized with the separation of an electron and a proton in the electron transfer complex. P_i^- reacts with an internal bound phosphate acceptor (ROH) to form RO*P;* in turn, $ADPO^-$ reacts with RO*P* to form ATP and ROH. ROH has been identified by Roy and Moudrianakis (16) as adenosine monophosphate. Note that in antiport coupling, no proton gradient is formed, since the protons are expelled and taken up in equal amounts on the two sides of the membrane.

Chemical Versus Gradient Energy. In oxidative phosphorylation, electron flow is coupled to a chemical synthesis (synthesis of ATP). In active transport, electron flow is coupled to the transmembrane movement of a cation. In oxidative phosphorylation, the released energy is conserved as chemical energy, whereas in active transport the released

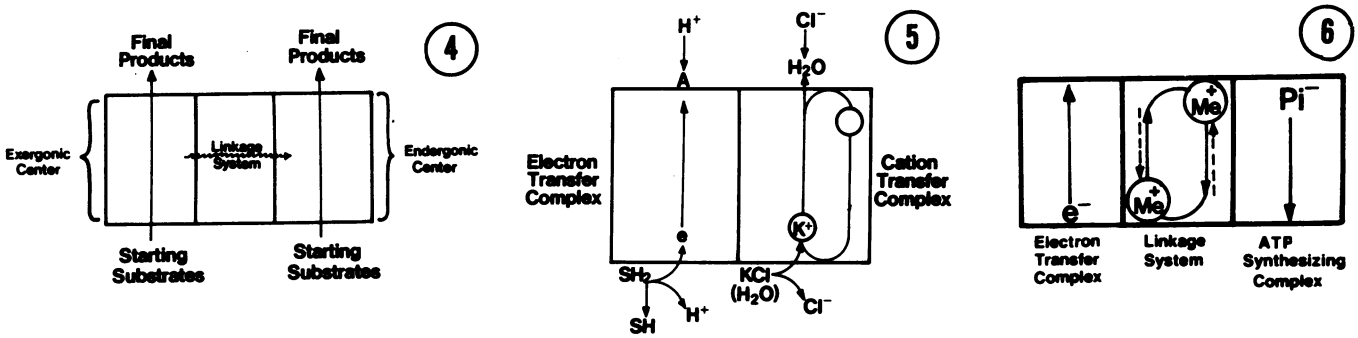

FIG. 4. Coupling as an interaction between an uncompensated charge in an exergonic center with an uncompensated charge in an endergonic center.

FIG. 5. Coupling of electron transfer to active transport of K^+ (mediated by an ionophore, ○).

FIG. 6. Antiport coupling the sum of two symport charge flows.

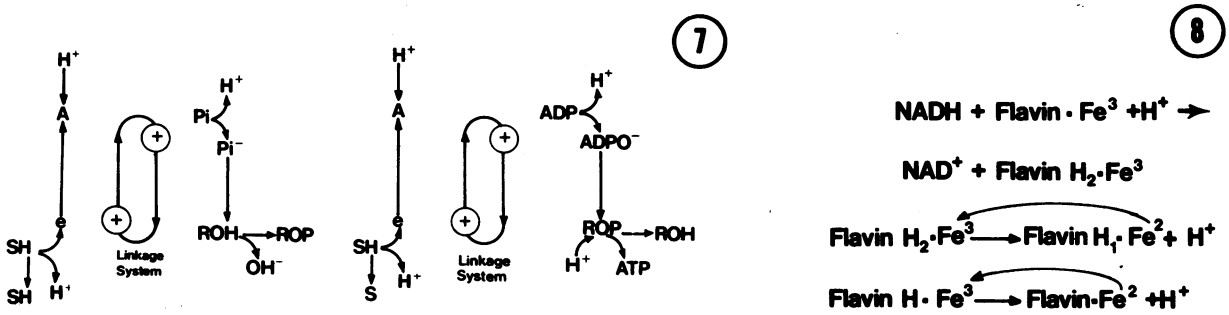

FIG. 7. Oxidative phosphorylation according to the paired moving charge model.

FIG. 8. Charge separation in the electron transfer chain (Complex I). Flavin represents the flavin prosthetic group of NADH dehydrogenase and Fe represents the iron–sulfur center with which the flavoprotein is intimately associated. Since Flavin $H_2 \cdot Fe^{+3}$ would immediately be converted to Flavin $H \cdot Fe^{+2}$, we have included this species as an intermediate only for reasons of clarity.

energy is conserved as gradient energy (the equal and opposite gradients of protons and K^+). Symport coupling leads to the generation of gradient energy, whereas antiport coupling leads to the generation of chemical energy.

Charge-Separating Devices. The electron transfer chain and ionophores provide two alternative molecular devices for effectuating charge separation, charge substitution, and charge movement. Each of these devices provides the means for separating a negatively charged species from a positively charged species (the electron from the proton or the cation from its anion) and for inducing the movement of one of the charged species. Thus, the electron transfer chain facilitates the controlled movement of the electron to lower energy levels, whereas the ionophore facilitates the movement of the cation away from its anion. In the case of the ionophore, charge separation and charge movement are essentially one synchronized and concerted process. However, these two events take place in sequence in the electron transfer chain. For example, in the oxidation of NADH by ubiquinone in Complex I, the electron and proton from NADH are first transferred to flavin and there separated in a virtual fashion (17). That is, the reduced flavin is still electroneutral, but the electron and the proton are separated within the molecule. It is only in the next step (the transfer of the electron to nonheme iron) that *de facto* separation of the electron from the proton takes place (18, 19) (see Fig. 8). The electron moves through the chain, whereas the proton is ejected into the medium on one side of the membrane.

Charge substitution is a consequence of two paired charge separations. The new pairing of, say, the electron with the K^+ depends upon the fact that both charges can undergo synchronous charge flow given the preliminary paired charge separation. The essence of coupling is this charge substitution, for now the movement of the electron can be coupled to the movement of K^+ or to the movement of P_i^-.

The *sine qua non* for charge substitution is charge separation on one side of the membrane, with one charge moving into the membrane and the other charge being expelled from the membrane on the same side. In the case of the electron transfer chain, the charge-separating species (nonheme iron, cytochrome *b*, cytochrome *a*) must be asymmetrically aligned within the membrane, i.e., oriented on one side of the membrane.

At the terminus of the charge movement trajectory, the reversal of charge separation must take place. The electron is reunited with a proton in the final acceptor for the complex, but this proton is taken from the medium on the other side of the membrane. In an analogous fashion, the cation is dislodged from the ionophore when it reaches the other side of the membrane and is reunited with an anion provided by the medium on the side of the membrane opposite that from which the original proton was ejected. The ionophore is both a charge-separating and a charge-reuniting device, whereas in the electron transfer chain, these two functions require separate devices. Ubiquinone, cytochrome *c*, and oxygen, the acceptors for complexes I, III, and IV respectively, are the charge-reuniting devices in the electron transfer chain.

Effective Distance for Charge–Charge Interaction During Paired Charge Movement. Since the movement of the driving charge has to be coupled to the movement of the driven charge for energy coupling to be realized, it is important to know the distance which separates the two coupled charges and whether the electrostatic interaction between the two charges over this distance is sufficiently intense to compel coupling.

The dimensions of cytochrome oxidase (Complex IV of the electron transfer chain) are accurately known from electron micrographs of crystalline preparations (20). Each through-membrane unit is 60 × 60 × 85 Å; the key dimensions (60 × 60 Å) refer to the dimensions in the plane of the membrane. Electron flow in cytochrome oxidase under appropriate conditions can be coupled to the ionophore-mediated flow of K^+ (21, 22). This coupling involves an interaction between the oxidase and K^+ in the presence of externally added valinomycin. Since the oxidase in this reconstituted system is diluted to the point that each unit is completely surrounded by lipid, we can be reasonably sure that the distance separating the electron from K^+ will not exceed 30 Å. When the oxidase is reduced by substrate, it undergoes distortion to a parallelogram which we estimate from the electron micrographs to have the dimensions 40 × 90 × 85 Å (23), and this compression of the oxidase would reduce the critical distance separating the two charges to about 20 Å. We can now pose the question of whether the charge–charge interaction across 20 Å in a hydrocarbon-like medium (dielectric constant of about 2) would be sufficiently intense to permit direct coupling. According to Coulomb's law, the interaction energy between two opposite charges in a medium with a relative dielectric constant of 2 and a separation distance of 20 Å would be about 10 kilocalories (42 kilojoules) per mole (24). This energy would be ample for purposes of coupling‡.

‡ D. J. Boone and A. Kowalsky [(1974) *Biochemistry* **13**, 731–737] find ion pair coupling in polar media (ϵ = 13, d = 10 Å); strong interactions in the 20–30 Å range in less polar media may be inferred.

The Energetics of Coupled Charge Flow. As a first approximation, we may say that the energy of a charge is inversely proportional to the polarity of the medium (10). In a polar medium, the energy of the charge is less than the energy in a nonpolar medium. During the movement of an electron from NADH to oxygen via the three complexes, the electron is moving stepwise from a nonpolar to a polar environment. We are referring here to the very local environment of the electron and not to that of the molecule as a whole. The electron in the pyridine ring of DPNH is in a hydrophobic environment, whereas the electron in O_2 is in a highly polar environment. Thus, the energy is declining stepwise during this transition and in this sense, the electron is moving down the electrochemical gradient (25, 26). The opposite would be true for the transfer of K^+ from the aqueous medium to the ionophore in the membrane phase. This transfer would involve a transition from a highly polar phase (water) to a relatively nonpolar phase (the interior of the ionophore). Thus, the electron is moving down an electrochemical gradient, whereas K^+ is moving up an electrochemical gradient§. The downhill flow of the electron can thus drive the uphill flow of K^+, given the necessary coupling of the two processes.

The principle of energy minimization underlies the coupling of two charge flows. If an electron and a K^+ which are coupled are separated by a given distance (say 20 Å) and the electron can move through the chain across the membrane, what will be the energetic consequence if the electron moves without a concomitant movement of K^+? The distance between the two charges would necessarily increase and the energy of the system would increase correspondingly. If the two charges move together so that the separating distance remains constant, the energy of the system will remain constant. In solid-state physics, several examples are known (28, 29) of two ions moving in a cooperative manner. This cooperativity of ion movement could also play a role in establishing a minimal energy configuration.

Two additional features of the energetics of paired moving charges need some comment. The separated charges may be considered to be in the ground state of a potential well, i.e., at minimal energy (10). Thus, the problem of thermalization inherent in excited states is nonexistent. Moreover, the energetics of coupled charge movements eliminate the problem of energy transfer.

Although we have not considered explicitly the endergonic synthesis of ATP from P_i^- and $ADPO^-$, we may consider the energetics of this process as analogous to that for ionophore-mediated transmembrane movement of K^+. P_i^- and $ADPO^-$ are moved from the aqueous medium to the interior of the membrane. Thus, the charges move from a highly polar environment in the aqueous phase to a less polar environment in the membrane. In oxidative phosphorylation, the downhill movement of the electron is coupled to the uphill movement of P_i^- and $ADPO^-$. In the coupled hydrolysis of ATP, the position is reversed. P_i^- and $ADPO^-$ now move down the electrochemical gradient and can thus drive active transport or reversed electron flow.

§ The partition coefficient for the distribution of K^+ between the aqueous phase and the ionophore in the membrane phase is greatly in favor of K^+ remaining in the aqueous phase (27)—a token of the uphill character of the transition.

We have said nothing about the molecular mechanisms by which P_i is separated into P_i^- and H^+ or by which ADP is separated into $ADPO^-$ and H^+. Elsewhere the experimental thesis will be developed that specialized ionophores are required for these charge separations (30).

There is a basic difference between ATP synthesis and active transport of cations which needs defining. The end result of the coupled movement of P_i^- and $ADPO^-$ into the membrane is the synthesis of ATP. The energy released by electron transfer is conserved as the chemical energy of a pyrophosphate bond in ATP. In active transport, electron transfer drives the uphill movement of a cation from the aqueous phase to the membrane phase, but at the end of the trajectory, the cation is unloaded from the ionophore into the aqueous phase on the other side of the membrane. The cation is moved uphill and then downhill to the original energy level, but on the other side of the membrane. The free energy released by electron transfer is thus conserved as a potential energy of K^+ (increased electrochemical potential on one side of the membrane and decreased electrochemical potential on the other side). Given a sufficient electrochemical potential, K^+ can reverse roles and serve as a driving ion in the synthesis of ATP achieved by the reversal of ATP-energized active transport of K^+ (31).

The Electron Transfer Chain and Charge Flow. Electrons are transferred from one oxidation-reduction component to another within a complex. Since in each such transfer the electron is incorporated into a valency system, would such transfer lead to an electrically neutral species? We may assume the electroneutrality of each oxidation-reduction component within a complex, and thus the movement of the electron, would necessarily lead to a wave of negativity traversing the chain. The introduction of an electron within the valency shells of a previously neutral electron carrier, such as a cytochrome in the ferric state, would release a negatively charged anion locally, but this temporary substitution of a negatively charged anion for a negatively charged electron would not affect the argument.

There is accumulating evidence that the electron as such may not be transferred to the heme center of cytochrome *c*, but rather that there is entry of a hydrogen and exit of a proton [retrogressive electron flow, according to Harrison (32)]. The net effect of such flow would still be the movement of a negative charge into the heme center and, therefore, there would be no difficulty in accommodating the phenomenon of retrogressive electron flow to the fundamental postulates of the paired moving charge model.

Is there in the ATP synthesizing or hydrolyzing complex a transfer chain for P_i^- and $ADPO^-$ analogous to the electron transfer chain? The complexes concerned in ATP synthesis and hydrolysis contain a series of proteins which could constitute a chain for transfer of P_i^- and $ADPO^-$. One of these proteins, the Beechey protein (33), has been shown to be an ionophoroprotein capable of binding P_i when supplemented with a divalent metal (Mg^{++} or Ca^{++}) (unpublished studies of R. Kessler).

Chemiosmotic Versus Paired Moving Charge (PMC) Model. The two models, though resembling one another in respect to a few features (charge separation, vectorial arrangement of complexes, gradient generation via electron flow and active transport), are profoundly different in respect to other features. The chemiosmotic model requires a membrane for coupling (34); the PMC model does not (35, 36).

The chemiosmotic model assumes that charge separation does not have to be paired, whereas the PMC model is based on the essentiality of paired charge separation. The chemiosmotic model invokes a membrane potential as a consequence of charge separation. The PMC model explicitly forbids the generation of a transmembrane potential, since coupling depends on the avoidance of a membrane potential by complementary charge flow. Finally, according to the chemiosmotic model, the proton is the driving ion in oxidative phosphorylation and in the synthesis of ATP by reversal of the active transport of K^+. According to the PMC model, the driving ion in oxidative phosphorylation is the electron, and in the synthesis of ATP by reversal of active transport, the driving ion is K^+. The proton plays no direct role in either of these two coupled processes.

The Linkage System. The two interconnected charges in the linkage system which are required for antiport coupling are probably ionophore-associated charges. This conclusion is based on the fact that under conditions which support antiport coupling, the K^+ ionophore becomes latent, whereas under conditions which support symport coupling, the activity of this ionophore is readily demonstrable (unpublished studies of J. H. Southard and D. E. Green). Latency is equated with the encapsulation of ionophores within a protein system to form a linkage system of charges.

Supporting Evidence. The PMC model in its present form is rooted in a large body of experimental evidence and thus may be expected to be generally compatible with the phenomena of mitochondrial energy coupling. Elsewhere we have considered this compatibility (6). The predictions are the most crucial tests of the model. We have predicted the nonessentiality of the membrane state for energy coupling and this has been verified (35, 36). We have predicted the role of intrinsic ionophores not only in active transport, but also in energy coupling and this prediction is now being borne out (unpublished studies of J. H. Southard and D. E. Green). We have predicted P_i^- and $ADPO^-$ as moving charges in ATP synthesis and hydrolysis and we have found an ionophoroprotein in the ATP-Pi exchangease complex capable of binding phosphate (unpublished studies of R. Kessler). We have predicted that the individual complex is the unit of energy coupling, and this prediction has been verified by the demonstration of respiratory control and energy coupling in liposomal preparations of cytochrome oxidase (4, 21, 22).

Catalysis and Charge Separation. What is perhaps surprising is the infrequency with which catalysis intervenes in the charge separation process. In the initial stages of the hydrolysis of ATP and the final stages of the synthesis of ATP and in the interaction of NADH with the first acceptor of Complex I, catalysis plays a role, but in all other charge separations no catalysis by an enzyme is involved. This means that charge separation does not require an enzyme. Specialized molecular structures are the instruments of charge separation. Enzymes come into the picture only in a preparatory role—generating the species which undergo charge separation. In the earlier version of the moving charge model, catalysis was made an integral part of charge separation in coupled systems. We no longer hold this position.

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