continuously the three-dimensional distribution of temperature and pressure (and, therefore, of wind) over the entire globe, will undoubtedly play a major role in providing much of the required meteorological information.

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Oxidative and Photosynthetic Phosphorylation Mechanisms

The chemistry reflects a possible evolutionary pattern for the driving force of life.

Jui H. Wang

To maintain life, all living systems must feed on free energy from their surroundings either directly in the form of light or in the form of stored chemical free energy converted from light. During respiration, the free energy stored in the substrate or food is released by the controlled aerobic oxidation in mitochondria. Part of this free energy is utilized to synthesize adenosine 5′-triphosphate (ATP) (*1*) for growth and reproduction according to genetically predetermined codes and for adaptation to the environment as required by circumstantial factors.

The biosynthesis of ATP from adenosine 5′-diphosphate (ADP) and inorganic phosphate (P_i), coupled to the oxidation of substrates, is known as oxidative phosphorylation and takes place (*2*, *3*) mainly in the respiratory chain embedded in the mitochondrial membrane. But by what molecular mechanism is the free energy liberated in an oxidation process utilized to drive a phosphorylation reaction in which P_i and ADP condense to form ATP and water? There is not yet a universally accepted answer.

Model Reactions

The only simple electron transfer reaction that has been reported to cause P_i to condense with adenosine 5′-monophosphate (AMP) or ADP to form ADP or ATP, respectively, is the aer-

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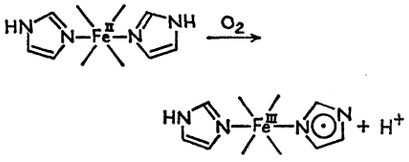
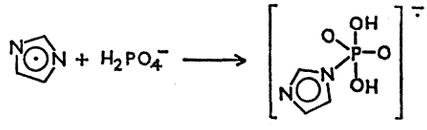
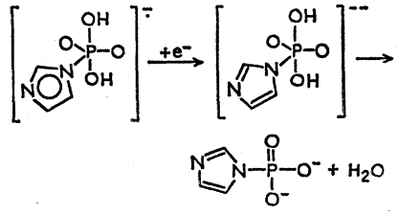
obic oxidation of diimidazolyl-ferrohemochrome in *N,N*-dimethylacetamide solution containing imidazole, P_i, and AMP or ADP (*4*). In this model reaction, 1-phosphoimidazole was first formed, and then it transferred its phosphoryl group to AMP or ADP to form ADP or ATP. But how was the 1-phosphoimidazole formed? Studies with substituted imidazoles suggest that molecular oxygen first extracts two electrons from the ferrohemochrome to produce a complex of ferriheme and the reactive imidazolyl radical ($C_3H_3N_2$).



This radical can then react with P_i to form an unstable phosphoimidazolyl radical,



which is subsequently reduced by another ferrohemochrome molecule to produce 1-phosphoimidazole and water (*5*) as shown below.



The formation of the above trigonal-bipyramidal intermediate compound 1-orthophosphoimidazole ($C_3H_5N_2PO_4^{-2}$) by the usual nucleophilic attack at the P-atom is a very slow process. But since radical reactions generally require a much lower activation free energy, the above trigonal-bipyramidal phosphoimidazolyl radical ($C_3H_5N_2PO_4^{-}$) can be formed much more rapidly. In the subsequent step driven by the oxidation-reduction free energy, this phosphoimidazolyl radical is reduced to the unstable 1-orthophosphoimidazole which then spontaneously eliminates H_2O to form 1-phosphoimidazole. In this way, oxidation can be coupled to phosphorylation.

Oxidative Phosphorylation

With these model reactions as a guide, a molecular mechanism for mitochondrial oxidative phosphorylation has been proposed (*6*). This mechanism is summarized in a slightly modified form in Fig. 1, where the direction of electron transport under normal conditions is represented by the bold-faced arrows between the respiratory electron-carriers, nicotinamide-adenine dinucleotide (NAD), flavin, quinones, and the cytochromes (b, c_1, c_1, a, and a_3).

The oxidative phosphorylation scheme (Fig. 1) utilizes a coupling mechanism similar to that in the model reactions, with the important difference that in mitochondria the imidazolyl radical cannot diffuse away to cause biological damage but is covalently bonded to the protein, for example, cytochromes. Furthermore, if the responsible cytochrome is adjacent to a suitable phospholipid, (RO)(R'O) PO_2^-, or phosphoprotein molecule, the imidazolyl radical can rapidly react with the latter to form a phosphoimidazolyl radical that can subsequently be reduced to the "energy rich" intermediate **4**, **5**, or **6**. Each of these intermediates can likewise react with P_i in the presence of a specific enzyme or coupling factor to give **7**; compound **7** can then transfer its phosphoryl group to ADP in the presence of another enzyme or coupling factor to give ATP and regenerate the phospholipid **8** for the next round of oxidative phosphorylation. Since the phosphoryl group in **4**, **5**, or **6** is not from P_i, each of these "energy rich" compounds should behave as a "non-phosphorylated" intermediate according to the conventional criterion (*7*).

Although the midpoint reduction potentials in vivo of the respiratory electron-carriers are not precisely known, it is generally believed that the value increases steadily from NAD to O_2 for efficient electron transport along the respiratory chain (Fig. 1). That electron transport is necessary for phosphorylation is expected not only for thermodynamic reasons but also from a mechanistic point of view, because it is the transfer of electrons that gives rise to the radical intermediates and then changes them to the "energy rich" intermediates **4**, **5**, and **6**. The more puzzling requirement of phosphorylation for electron transport becomes understandable if we recognize that the 1-phosphoimidazole group is a much weaker coordinating ligand for the Fe(II) state than the original imidazole group and hence should lower considerably the midpoint reduction potential of the corresponding electron carrier. Therefore unless the concentrations of P_i and ADP are sufficiently high and that of ATP sufficiently low to discharge the intermediates **4**, **5**, and **6** rapidly and continually through the enzyme-catalyzed equilibria, these electron carriers will be left in their "energy rich" states, each with a considerably lower midpoint reduction potential than its neighboring carrier on the substrate side of the respiratory chain. Such a modified sequence of midpoint potentials could retard electron transport in the normal direction. For example, if the midpoint reduction potential of cytochrome c_1 in its "energy rich" state **5** is considerably lower than that of cytochrome b, then, thermodynamically, cytochrome c_1 will be unable to extract electrons from cytochrome b unless cytochrome c_1 is almost completely in the Fe(III) form or cytochrome b is almost completely in the Fe(II) form. But in either of these latter cases the steady-state rate of electron transport would approach zero, because in a respiratory chain at steady state the rate of oxidation of the reduced form of every carrier is equal to the rate of reduction of the oxidized form of every carrier. It is for these reasons that regulation of the ratio of the concentrations [ADP] $[P_i]$/[ATP] regulates the rate of respiration (*3*, *8*). Indeed, since all the enzyme-catalyzed equilibria in Fig. 1 are reversible, it is even possible to reverse the normal direction of electron transport by large doses of ATP (*9*).

Thermodynamic data show that O_2 serves more efficiently as a two- or four-electron acceptor than as a one- or three-electron acceptor. Likewise at the substrate end of the respiratory chain, NADH (reduced NAD) serves more efficiently as a two-electron donor than as a one-electron donor. Thus it would seem kinetically undesirable to employ a single chain of one-electron carriers such as the cytochromes to transport electrons from NADH to O_2. However, from the standpoint of the mechanism of Fig. 1, the oxidation of a one-electron carrier by a two-electron acceptor could facilitate the formation of the imidazolyl radical which is needed for coupling oxidation to phosphorylation. For example, once the bound O_2 has extracted an electron from the Fe(II) of cytochrome c_1 (or c), it has a strong tendency also to extract a second electron from the coordinating imidazole group, thereby converting the latter to the highly reactive imidazolyl radical that could immediately attack the neighboring phospholipid to form

the phospholipid-imidazolyl radical. In the subsequent reduction by cytochrome b, the phospholipid-imidazolyl radical is first reduced to compound **5** with the elimination of OH^-, followed by the formation of compound **7** and the reduction of the Fe(III) back to Fe(II). Consequently, one ATP is formed at cytochrome c_1 (or c) for every pair of electrons transferred. The same ratio of phosphorus to oxygen is expected at the heme a (or a_3) site and at the nonheme iron (*10*) or at the flavin plus quinone site. Therefore an overall P : O ratio of 3 is expected from the mechanism in Fig. 1.

Because the triesters of phosphoric acid are generally much more reactive than the monoesters and 1-phosphoimidazole rapidly hydrolyzes in even weakly acidic solutions (*11*), one may infer by analogy that "energy rich" intermediates such as compound **5** should be even more susceptible to hydrolysis and hence unsuitable to function as a free energy transducer at an exposed site. Protecting this reactive group by a second lipid layer is hardly satisfactory, because the latter would also hinder the elimination of water or OH^- or prevent the subsequent reaction with P_i. The only conceivable protection which satisfies all the requirements is to combine the reactive part of compound **5** with a specific enzyme or coupling factor that catalyzes the transphosphorylation of P_i by compound **5** in preference to the hydrolysis of compound **5**. Similarly, the enzyme that catalyzes the transphosphorylation of ADP by compound **7** may also be regarded as a dual function coupling factor.

If any of these endogenous coupling factors is leached out during the preparation of the so-called electron-transport particles from mitochondria (*12*), the corresponding "energy rich" intermediate will be susceptible to rapid hydrolysis. As a result, compounds **4**, **5**, and **6** will be spontaneously discharged to restore the midpoint reduction potentials of the respective electron carriers to their normal values for efficient electron transport without phosphorylation. When the electron transport particles form complexes with coupling factors, the phosphorylating properties of these particles are restored (*13*). Figure 1 also predicts the last transfer enzyme to exhibit adenosine triphosphatase activity when it forms a complex with a partial complement of the phospholipids.

According to the mechanism (Fig. 1), inhibitors of electron transport could be molecules which form complexes with the electron carriers and thereby prevent electron transport either by increasing the energy barrier for electron transfer between neighboring carriers or by drastically changing the midpoint reduction potential of a carrier, or both. But inhibitors of the specific transferring enzymes or coupling factors should behave as inhibitors of phosphorylating oxidation, such as oligomycin and aurovertin. In addition, molecules such as 2,4-dinitrophenol and dicoumarol may, because of their high solubility in lipids, penetrate the mitochondrial membrane and uncouple the oxidative phosphorylation mechanism there either by reducing the unstable radical intermediate **1**, **2**, or **3** and regenerating the imidazole group, or by displacing the imidazole group from compound **4**, **5**, or **6** and forming the phenolic ester of the corresponding phospholipid. In either case, the midpoint reduction potential of the cytochrome will have been restored to its normal value for further efficient electron transport without phosphorylation having taken place. In other words, the mechanism is uncoupled. It is of interest also to note that according to this interpretation, P_i is not necessary for the uncoupling of respiratory chain by dinitrophenol, an inference which is in agreement with the work of Slater (*14*).

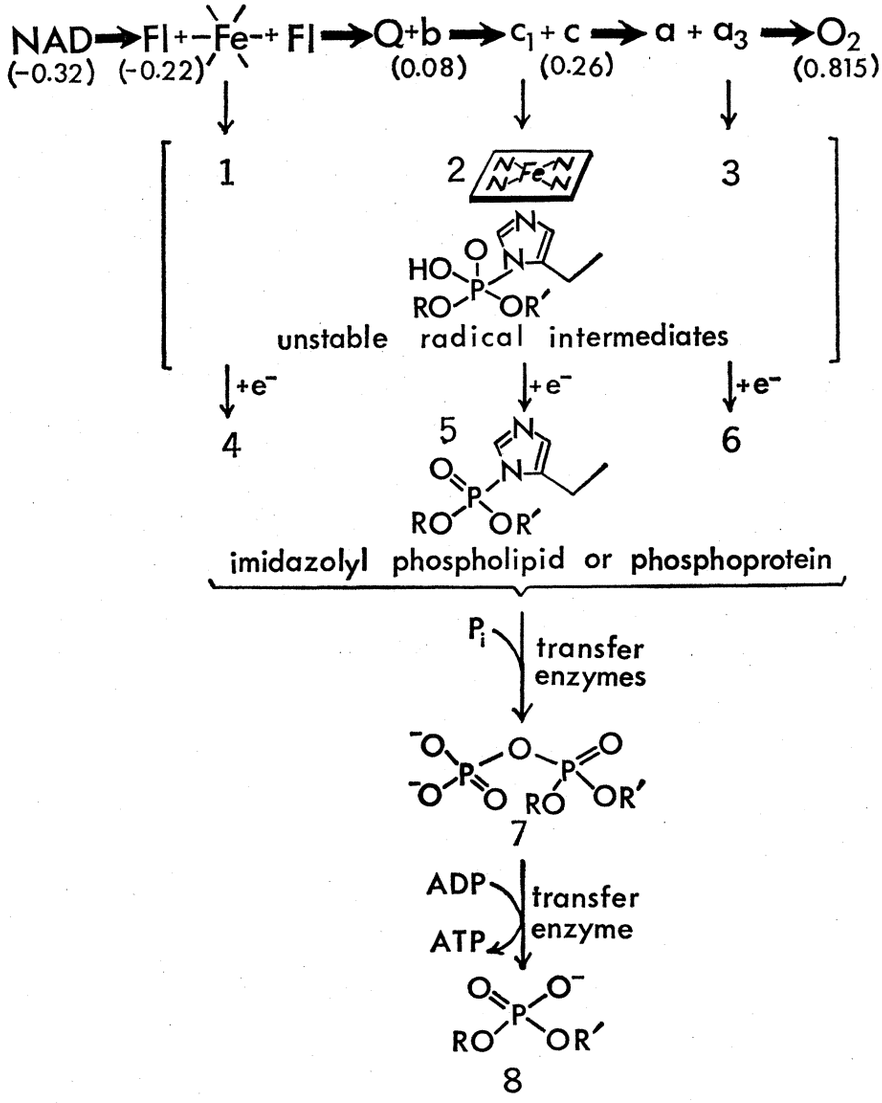


Fig. 1. A proposed molecular mechanism of mitochondrial oxidative phosphorylation. The intermediates in brackets, **1**, **2**, and **3**, represent unstable, covalently bonded radicals. Structures **4**, **5**, and **6** represent "energy rich" states formed through the oxidation of nonheme iron, c_1 (or c) and a (or a_3), respectively. In each case, the transition from normal state to the "energy rich" state is triggered by the formation of a new N-P bond.

Other Free-Energy Terms

The most obvious weakness of any chemical coupling mechanism of respiratory oxidative phosphorylation is that, in spite of the intensive efforts during the last 25 years, no "energy rich" intermediate has ever been isolated from mitochondria. The failure to isolate such an intermediate could either be due to the fact that the intermediate does not exist or that it cannot be isolated by conventional methods. The mechanism in Fig. 1 illustrates the second possibility, because the "energy rich" N-P bond in compound **4, 5**, or **6** is between a hydrophilic protein molecule and a phospholipid molecule with bulky hydrophobic side chains. Consequently, compound **4, 5**, or **6** should be insoluble in most solvents, and any successful attempt to separate the hydrophilic and the hydrophobic moieties of such an intermediate would invariably rupture the crucial N-P bond.

The newly formed N-P bond in compound **5** between the cytochrome and the phospholipid could trigger conformational change in either macromolecular subunit. The cumulative effect of such conformational rearrangements could be morphological changes in mitochondrial as well as in chloroplastic membranes in a manner similar to the oxygenation of hemoglobin which is known to cause tertiary structural changes at the molecular level (*15*) and morphological changes at the cellular level (*16*).

Inasmuch as the formation of such a new N-P bond and the conformational changes are thermodynamically linked processes (*17*), they both affect the free energy of the "energy rich" state. Consequently, competing interpretations based on the "energy rich" intermediate and on the conformationally (*18*) or morphologically (*19*) energized state are not really mutually exclusive, but may reflect different features of the same molecular process.

The free energy of oxidation may also be stored in the form of concentration gradients, because the electron transport chain may take up protons by reduction and release protons by oxidation at different locations in space. Application of Gibbs-Donnan equilibrium to membranes of selective permeability allows the calculation of the concentration gradients of other ions from this proton gradient (*20*). Moreover, since the chemical changes **5** + $P_i \rightleftarrows$ **7**, **7** + ADP $\rightleftarrows$ ATP + **8**, and **8** $\rightleftarrows$ **5** + H_2O involve changes in the net charge of membrane lipids, they may cause changes in local cation distribution.

In general, differences in local concentration lower the entropy and hence also raise the free energy of the system. Since this generation of concentration gradients and electron transport are also linked processes, their reciprocal thermodynamic relation allows the reversal of electron transport, with or without coupled phosphorylation, by externally imposed sudden concentration changes (*21*).

A proposal has indeed been made (*22*) to consider such concentration gradients as the primary form of converted free energy with which ATP is formed from ADP and P_i. Hypothetical models based on this premise are in general unattractive because they are electrochemically all equivalent to the inefficient concentration cells. For example, at *p*H 7 the net reaction

$$H^+ + NADH + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$

has a midpoint potential of 1.14 volts. To build an equivalent concentration cell we would need a concentration (or activity) ratio of $e^{1.14/(RT)} \approx 10^{19}$. In other words, if the reacting solute concentration is 1*M* on one side of the membrane, the concentration of this solute on the other side would have to be $10^{-19}M$ or less. With such a low concentration of the reacting solute responsible for one side of the membrane, it would be impossible for the system to attain the steady-state metabolic rates of terrestrial life. Attempts to remedy this chemiosmotic hypothesis by introducing loops in the electron transport path (*23*) are unsatisfactory, because at steady state the net production or consumption of protons can only occur at the source or sink of electrons but not at any other point or junction along the electron transport path.

On the other hand, in view of the wide range of midpoint potentials of oxidation-reduction couples in the chemical world, it seems much more promising to use net chemical reactions as a primary concept and build upon it a theory of reaction-induced conformational and concentrational changes to account for the cellular regulatory processes, such as permeability control and active transport (*24*). Likewise, reaction-induced conformational and morphological changes could be used as a working hypothesis to explore developmental processes at the molecular level.

Harvesting Light

Since the pioneering work of Priestley, Ingen-Housz, and Senebier (*25*), great strides have been made in our progress toward understanding photosynthesis (*26*). It is now generally accepted that, in the primary energy conversion reaction of photosynthesis, electrons are driven by light from molecules of higher reduction potential to those of lower reduction potential, and that in photosynthesis ATP is produced by essentially the same mechanism which couples phosphorylation to electron transport in the respiratory chain.

Experiments with various models demonstrate that it is even possible to convert light to chemical free energy through rather simple photoredox reactions (*27*). In general, molecules in their excited electronic states have quite different oxidation-reduction properties than in their ground states. An electronically excited pigment molecule can be a stronger reductant, because the removal of an electron from its antibonding molecular orbital requires less energy. It can also be a stronger oxidant because the positive hole left in the bonding or nonbonding orbital could be filled by abstracting an electron from a donor molecule. For this reason, certain pigment molecules can utilize the absorbed light energy to drive electrons up a free-energy hill (*28*). As an example, in one of the model systems, electrons were driven by red light from cytochrome c via aggregated chlorophyllin a and the flavin group of nicotinamide-adenine dinucleotide phosphate (NADP) reductase to NADP against an electrochemical potential of 0.36 volt (*29*).

This observation suggests that the energy converter of green plants, often called *P*700 because of its absorption maximum at 700 nm (*30*), may be a molecular complex of a particular chlorophyll a molecule and a particular flavin group. This possibility was also supported by the observation that flavin and chlorophyll a indeed form a complex with an absorption maximum near 700 nm and photochemical properties resembling those of *P*700 (*31*). However, our experiments (*31*) indicate that the NADP reductase itself does not form a complex with chlorophyll a. Consequently it is necessary to modify the previously suggested mechanism (*31*) by assuming *P*700 to be a molecular complex of chlorophyll a and another flavin group or its equivalent. The latter accepts the photoelectron from

chlorophyll a and subsequently transfers it to either ferredoxin or the NADP reductase.

With these model experiments as a guide, a mechanistic scheme has been proposed for the primary energy conversion reactions of photosynthesis (*32*). A slightly modified version of this scheme is given in Fig. 2, which represents a synthesis of several earlier formulations (*33*) based on a wide range of experimental data (*26*). This scheme is also substantiated by the demonstration that, by connecting two model systems electrically in series, it is possible to produce a photoelectromotive force of more than 2 volts and use it to drive electrons from water to NADP to generate O_2 and NADPH (reduced NADP) (*32*). For clarity, let us explore separately the interpretation of cyclic photophosphorylation, photosynthesis, and their relation to respiration in terms of this scheme.

Cyclic Photophosphorylation

In 1954, Arnon and co-workers made the important discovery that, when deprived of CO_2 and exogenous NADP, illuminated chloroplasts can sustain cyclic photophosphorylation, that is, generation of ATP and H_2O from ADP and P_i with no other net chemical change (*34*). Although this process produces no net oxidation-reduction, they observed that the system required a catalytic amount of molecular oxygen or another electron acceptor such as flavin or vitamin K. Shortly afterward, Frenkel discovered that cyclic photophosphorylation also takes place in photosynthetic bacteria (*35*). The data of Arnon and co-workers on cyclic photophosphorylation were questioned, but later experiments of improved yield supported their earlier conclusion (*36*).

According to the scheme in Fig. 2, the light energy absorbed by the assembly of pigment molecules in photosystem I is first transferred by Förster's type of mechanism (*37*) to a particular pigment molecule that has formed a complex with a particular flavin group. This electronically excited chlorophyll or bacteriochlorophyll molecule can then transfer the electron from its antibonding orbital to its acceptor flavin group, which can subsequently pass it on along the cyclic electron-transport chain with coupled phosphorylation by the radical mechanism. Phosphorylation can take place at the coupling sites

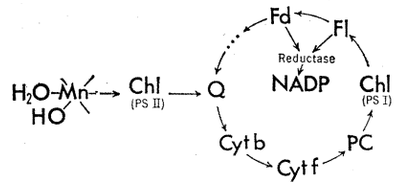


Fig. 2. A scheme for the mechanism of primary energy conversion reactions in photosynthesis. Abbreviations are: Fl, flavin group; Fd, ferredoxin; PC, plastocyanin; Cyt f, c-type cytochrome; Cyt b, b-type cytochrome; Chl, chlorophyll a; Q, quinone; PSI, photosystem I; and PSII, photosystem II.

between ferredoxin and plastoquinone and between plastoquinone and plastocyanin, respectively. Since for a given number of electrons that this particular chlorophyll molecule supplies to its acceptor flavin group, exactly the same number is recovered from plastocyanin or its equivalent, cyclic photophorylation does not produce net redox change.

Because at steady state the rate of oxidation of the reduced form of every carrier in an electron-transport chain is equal to the rate of reduction of its oxidized form, every carrier has to maintain a healthy proportion of its oxidized and reduced forms for the chain to function efficiently in the kinetic sense. In the event that all carriers in a particular preparation are completely in the reduced form, electron transfer from the chlorophyll of photosystem I to its reduced flavin neighbor cannot occur. Consequently, in such cases, a small amount of oxygen gas, oxidized flavin, or vitamin K is needed to oxidize a sufficient fraction of the endogenous acceptor flavin groups of photosystem I to start the light-driven electron pump.

Photosynthesis

Van Niel (*38*) proposed in 1931 that the net photosynthetic reactions of both bacteria and green plants may be represented by the equation

$$CO_2 + 2H_2A + \text{light} \rightarrow \{CH_2O\} + H_2O + 2A$$

in which CO_2 is reduced by a general donor H_2A to a carbohydrate represented by $\{CH_2O\}$. In the case of purple sulfur bacteria (*39*), the donor is hydrogen sulfide which is oxidized to sulfur. In the case of green plants, the donor is water which is oxidized to oxygen gas (*40, 41*). Recognizing that the photo-oxidation of a suitable donor (*39–41*) and the dark reduction of carbon dioxide (*42*) are separate events, one may divide the above overall reaction into two separate reactions

$$2H_2A + 2X \xrightarrow{\text{light}} 2A + 2H_2X$$

$$2H_2X + CO_2 \xrightarrow{\text{dark}} 2X + CH_2O + H_2O$$

where X represents an endogenous acceptor. During the two decades after van Niel's proposal, when the elucidation of the path of carbon in photosynthesis was in active progress (*43*) and the concept of photosynthetic units was being developed (*44*), the molecular identity of X remained a mystery. Then, unexpectedly, within a period of 6 weeks in 1951, three groups of investigators independently identified NADPH as the H_2X of chloroplasts (*45*). Others later reported that NADH (*46*) played the same role in bacterial photosynthesis as NADPH played in green plant photosynthesis. With this information, the basic problem of energy conversion in green plant photosynthesis now becomes the elucidation of the mechanisms by which light is utilized to produce ATP from ADP and P_i and to reduce NADP to NADPH by water.

According to the Calvin-Bassham cycle (*43*), the reduction of 1 mole of CO_2 to the oxidation level of carbohydrate involves the oxidation of 2 moles of NADPH and the hydrolysis of 3 moles of ATP. Because of the withdrawal of electrons at the NADP in Fig. 2 for CO_2 reduction, fewer electrons are returned to quinone than are taken away from it by photosystem I through plastocyanin and the cytochromes. Consequently photosynthesis cannot continue unless the electron deficit at quinone is compensated by an exogenous donor. In bacterial photosynthesis with a strong reductant such as H_2S or H_2 as the donor, a single photosystem (photosystem I) should be sufficient, since each quantum of light at 870 nm is equivalent to 1.43 volts. But in the photosynthesis by green plants with H_2O as the donor, kinetic complications arise because of the high free energies of activation of the necessary chemical mechanisms for oxidizing H_2O to O_2. The development of a second photosystem in green plants, represented by the side chain in Fig. 2, with a manganese complex (*47*) as the depolarizer, overcomes these complications.

Thus in the presence of CO_2, the withdrawal of electrons at the NADP by reactions of the Calvin-Bassham cycle creates a deficit of electrons at quinone. This electron deficit makes

quinone a better acceptor, thereby activating the light-driven electron transfer from the chlorophyll of photosystem II to quinone, and the chlorophyll$^+$ thus produced in photosystem II can continue to raise the oxidation state of the manganese complex until the latter starts to produce O_2 by oxidizing its own water of hydration. Extraneous electron acceptors which are capable of removing electrons rapidly from quinone are also expected to activate photosystem II and generate O_2 (*41*, *48*).

Emergence of Respiration

As photosynthesis continued to produce organic matter and oxygen gas on earth, conceivably heterotrophs could emerge by deleting the light-harvesting equipment from the scheme in Fig. 2 and by further developing the electron-transport chain to improve the yield of coupled phosphorylation. As an example, by substituting cytochrome oxidase for plastocyanin and by replacing the NADP for CO_2 reduction with the NAD attached to the Krebs cycle, this sequence of electron carriers is made formally almost identical with the respiratory chain of aerobes. Thus the scheme in Fig. 2 is also consistent with the general assumption of the common origin of life.

Summary

A molecular mechanism based on simple model reactions is proposed for coupling phosphorylation to electron transport in both mitochondria and chloroplasts. The reaction schemes seem to offer a unified interpretation of a large number of relevant facts which have been reported. They also reflect a possible evolutionary pattern for the driving force of life at the molecular level.

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