Genetic Dissection of Photosynthesis

Gene mutations can be used to delineate aspects of the photosynthetic electron transport chain.

R. P. Levine

One way to understand a normal system is to alter it by dissecting out its component parts individually and then to examine its operation in the absence of a given part. Such an approach makes it possible to deduce the nature and function of the individual components of the system and thus to understand the operation of the system. Once a gene mutation has removed a given component or altered its function, the properties of the system can be studied in its absence. In other words, it is possible to carry out the genetic dissection of a biological system.

We have been engaged in the genetic and biochemical analysis of mutant strains of the unicellular, green alga *Chlamydomonas reinhardi* that cannot carry out normal photosynthesis (*1–4*). Genetic dissection of photosynthesis has led to an understanding of the nature, function, and sequence of action of some of the components of this alga's photosynthetic apparatus.

Series Model for Electron Transport in Photosynthesis

Photosynthesis provides reducing power and energy for the reduction of carbon dioxide to carbohydrate. In algae and higher plants, reducing power (reduced nicotinamide adenine dinucleotide phosphate, NADPH) and energy (adenosine triphosphate, ATP) are generated as a consequence of light-induced transport of electrons from water, the electron donor, to nicotinamide adenine dinucleotide phosphate, NADP, the terminal electron acceptor. The generally accepted model for photosynthetic electron transport involves two photochemical reactions that act in series (*5*) and that occur in two different photochemical systems (Fig. 1). Each system contains a reaction center at which an oxidant and a reductant are produced. In green plants, red light, absorbed by photochemical system II (PS II), sensitizes a reaction that results in the oxidation of water coupled to the formation of a weak reductant; far-red light, absorbed by photochemical system I (PS I), sensitizes a reaction that yields a weak oxidant and a strong reductant that eventually reduces NADP. Photochemical systems I and II are linked in series by several electron carriers, and the reductant produced by PS II is oxidized by the oxidant produced by PS I. There is at least one site for ATP formation during the course of electron transport from water to NADP, and there is evidence that this site lies between the two photochemical systems (*4, 6*). Cyclic electron flow sensitized by PS I has been postulated, and it may also be coupled to ATP formation (*7*).

Although the series formulation for photosynthetic electron transport has gained wide support, the nature, function, and sequence of action of different components of the electron transport chain have not yet been fully elucidated. Among the possible components of the chain are *b*-type cytochromes (*8*), a *c*-type cytochrome often known as cytochrome *f* (*9*), plastoquinones (*10*), the copper protein plastocyanin (*11*), a specialized form of chlorophyll a called P700 (*12*), pteridines (*13*), ferredoxin (*14*), and a ferredoxin-NADP reductase (E.C. 1.6.99.4) (*15*). The function of certain of these potential components is well established; for example, P700 is known to be the reaction center chlorophyll of PS I (*16*), and ferredoxin and the reductase function in the terminal steps of NADP reduction (*17*). The respective roles of the other components are not as clear, even though they may have been shown to participate in electron transport.

One investigator (*18*) has suggested that there is only one photochemical system and that, except for chlorophyll, ferredoxin, and the reductase, the remaining components are not involved in the main chain of electron transport from water to NADP. This position is extreme in view of a vast amount of evidence to the contrary (*19*).

We have examined the photosynthetic process in mutant strains of *C. reinhardi* and have concluded that this process is driven by two photochemical reactions linked in series, and that at least six different electron carriers are required for electron transport from water to NADP in this alga.

Mutant Strains and Their Genetic Analysis

Acetate-dependent mutant strains are easily obtained in *C. reinhardi* (*20*), and among them are some that cannot fix carbon dioxide by photosynthesis at a rate comparable to that of the wild type. None of these mutant strains differs markedly in chlorophyll content from the wild type. Two questions can now be asked: (i) what is the mode of inheritance and the genetic relationship between these strains, and (ii) what has occurred to impair their photosynthesis?

It has been found that all of the mutations affecting photosynthesis in *C. reinhardi* are single gene mutations and that they exhibit Mendelian inheritance. Many of them have been mapped to specific sites in the alga's genome (*21*); that is, they appear to be mutations in nuclear genes. The independent occurrence of more than one mutation at a given site has been observed in several instances, and in certain others, phenotypically identical mutations have been found to occupy different sites in the genome. To date there appears to be nothing unusual, or particularly noteworthy, regarding the inheritance of the mutations that affect photosynthesis. However, genetic analysis has been essential in order to demonstrate that we are not dealing with large deletions that might encompass

The author is professor of biology and chairman of the department of biology, Harvard University.

several chromosomal sites involved in photosynthesis. This analysis has not yet been possible in *Chlorella* and *Scenedesmus*, other algae in which mutations affecting photosynthesis have been found (*22*).

Nature of the Affected Components

The inability of a mutant strain of *C. reinhardi* to fix carbon dioxide by photosynthesis can be attributed to one of several possibilities. First, mutation may have caused some structural change in the chloroplast which in turn impairs photosynthesis. Second, a mutant strain may have lost the capacity to synthesize, at least in an active form, an enzyme that is essential for the dark reactions of carbon dioxide fixation. Third, a mutant strain may have lost the ability to generate the ATP whose formation is coupled to the photosynthetic electron transport chain. Fourth, a mutant strain may be unable to synthesize, at least in an active form, a component of the photosynthetic electron transport chain. With two exceptions (*23*) the mutant strains of *C. reinhardi* studied to date appears to fall into the last category.

Chloroplast fragments from these mutant strains have lost the capacity to photoreduce NADP, the terminal acceptor of the photosynthetic electron transport chain (*1, 3, 4*). This observation is important because it establishes that photosynthetic electron transport is blocked in each mutant strain. It has been established for several of the strains that electron transport is blocked because a specific chloroplast component has been affected by mutation (*3, 4*) (Table 1). Having shown that photosynthesis does not occur in the mutant strains and having demonstrated that certain components are either missing or inactive, it is reasonable to infer that each of the affected components is essential for photosynthesis. Thus, we conclude that a *b*-type cytochrome (cytochrome 559), a *c*-type cytochrome (cytochrome 553) analogous to cytochrome *f* from higher plants, plastocyanin, and P700 most probably lie in the main chain of photosynthetic electron transport and that they are essential for the transport of electrons from water to NADP. There is, in addition, a mutant strain of C. *reinhardi* in which the affected component of electron transport has not yet been identified (Table 1). Since this possesses cytochrome 559, cytochrome 553, plastocyanin, and P700, it is assumed that there is at least one additional essential component for photosynthetic electron transport. This component is given the notation M.

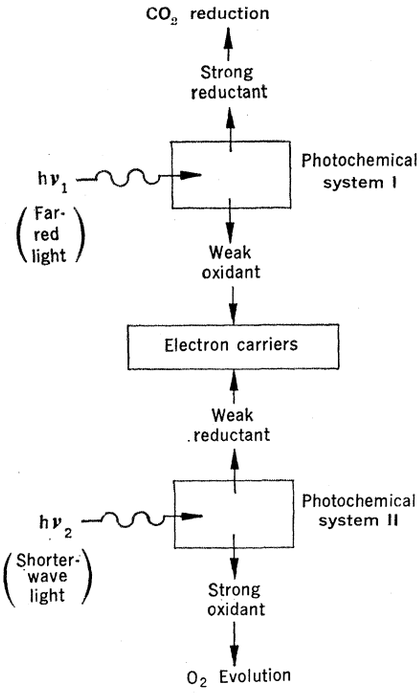

Fig. 1. Series formulation for photosynthetic electron transport [after Clayton (*5*)].

One criterion for the proof of function of a given component would be the restoration of photosynthetic reactions in chloroplast fragments of a given mutant strain upon the addition of the component derived from the wild type. Such proof has been obtained in the case of the mutant strain lacking plastocyanin, for upon the addition of purified plastocyanin obtained from the wild type, chloroplast fragments of the mutant strain carry out the photoreduction of NADP with either water or ascorbate and reduced dichlorophenolindophenol dye (the ascorbate-DPIP couple) as the electron donor, and the light-induced oxidation of cytochromes 559 and 553 is restored (*3*).

Table 1. Affected components in mutant strains of *Chlamydomonas reinhardi*.

Mutant strain	Component
ac-115	Cytochrome 559
ac-141	Cytochrome 559
ac-21	Unidentified
ac-206	Cytochrome 553
ac-208	Plastocyanin
ac-80a	P700

Partial Photosynthetic Reactions of Mutant Strains

Although the mutant strains cannot complete normal photosynthesis, they can carry out certain partial reactions. One such reaction, the Hill reaction, involves the photoreduction of an added oxidant, such as an indophenol dye or ferricyanide, with water as the reductant. Because the Hill reaction is absent in strains lacking cytochrome 559, we may conclude that electron flow from water to the Hill oxidants must pass through cytochrome 559 but not necessarily through an M component, cytochrome 553, plastocyanin, or P700.

In another partial reaction, the photoreduction of NADP can be achieved without water as the electron donor if a substitute reductant, the ascorbate-DPIP couple, is provided (*24*). According to the series formulation, this reaction bypasses PS II, and electrons from the ascorbate-DPIP couple enter the transport chain at a site between PS II and PS I. This reaction occurs in mutant strains in which cytochrome 559, M, and cytochrome 553 are the affected components but not in those strains in which plastocyanin and P700 are affected. Therefore, electrons from the ascorbate-DPIP couple must enter the electron transport chain at the level of plastocyanin but after cytochromes 559 and 553 and after M.

Sequence of Photosynthetic Electron Transport

These observations reveal that several components are required for electron transport from water to NADP and that the absence of these components, at least in active form, blocks electron transport at different sites. The sequence in which these components act has been determined by comparison of wild-type and mutant strains with respect to light-induced absorbance changes. Upon illumination of whole cells, chloroplasts, or chloroplast fragments, light-induced absorbance changes, attributable to the oxidation or reduction of any one of several chloroplast components, can be detected (*6, 16, 25, 26*). For example, photosynthetic cytochromes are oxidized by far-red light absorbed by PS I, and reduced by shorter wavelengths of light that are absorbed by PS II (*25*).

In wild-type *C. reinhardi*, cyto-

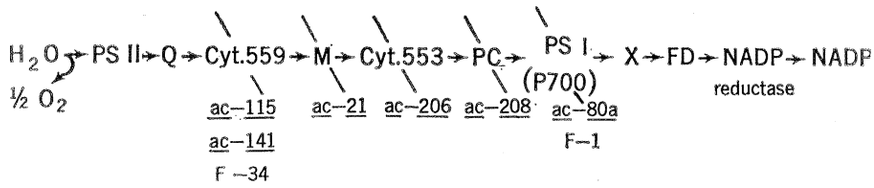

Fig. 2. Diagram of a series formulation for the photosynthetic electron transport chain of *C. reinhardi* [modified from Levine and Gorman (*2*)]. The dashed lines refer to the components affected in the different mutant strains; Q is the quencher of fluorescence of system II (*31*); Cyt. and PC refer to cytochrome and plastocyanin, respectively; P700 is the reaction center chlorophyll of system I (*16*); X is the postulated electron acceptor of system I; and FD is ferredoxin. Plasto- and tocopherylquinones are known to be present, and they probably function in the photosynthetic electron transport chain, but their site of action has not yet been rigorously established.

chromes 559 and 553 are oxidized by far-red light (720 nanometers) and reduced by red light (650 nanometers) (*2*). According to the series formulation, the former reaction is sensitized by PS I and the latter by PS II; therefore, we examined the absorbance changes in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor that blocks electron flow from water. Under conditions of inhibition, light induces the oxidation of the two cytochromes but not their reduction, as shown for the cytochrome oxidation-reduction changes in whole cells of the red alga *Porphyridium cruentum* by Duysens, Amesz, and Kamp (*5*), and in spinach chloroplasts (*6*). In terms of the series formulation, the formation of the reductant by PS II is blocked by DCMU, but PS I still produces its oxidant and reductant, and it is the oxidant, presumably P700, that ultimately oxidizes the cytochromes.

If this interpretation is correct, then in the absence of an active P700 both cytochromes should be reduced by light but not oxidized. Although there is no inhibitor which will specifically inhibit PS I without affecting the normal operation of PS II, PS II activity independent of PS I can be studied with a mutant strain that lacks an active P700. This mutant strain indeed shows only the light-induced reduction of cytochromes 559 and 553 (*4*).

When the mutant strains lacking either cytochrome 559 or 553 were studied, it was seen that in the absence of cytochrome 559, cytochrome 553 can be oxidized by light but not reduced, and that in the absence of cytochrome 553, cytochrome 559 can be reduced by light but not oxidized (*2, 3*). These observations are in accord with the series formulation and they reveal that cytochrome 559 is on the PS II side of cytochrome 553, and conversely, that cytochrome 553 is on the PS I side of cytochrome 559.

In the mutant strain lacking M, cytochrome 559 can be reduced by light but not oxidized, and cytochrome 553 can be oxidized by light but not reduced (*2*). This means that electron flow is blocked at some site between the two cytochromes, and accordingly an essential component (M) must lie between them, that is, on the PS II side of cytochrome 553, but on the PS I side of cytochrome 559.

In studies of a mutant strain lacking plastocyanin, it was found that the copper protein is required for the photoreduction of NADP (*3*). This stands in contradiction to the suggestion (*27*) that plastocyanin and cytochrome *f* lie in parallel rather than in series between PS I and PS II. If this were the case, one would expect that electron flow to NADP could still occur along the alternate parallel routes in strains lacking plastocyanin or cytochrome 553, respectively. In fact, neither of these alternatives occurs, suggesting that the two components lie in a single electron transport chain.

When the electron donor is the ascorbate-DPIP couple, the strain lacking cytochrome 553 can photoreduce NADP, but the strain lacking plastocyanin cannot (*3*). Again, a scheme involving parallel paths for the two components does not explain this observation. In an entirely different system, spinach chloroplasts, treated with a detergent, yielded particles that contained neither cytochrome *f* nor plastocyanin (*28*). Similarly, these particles cannot photoreduce NADP with either water or the ascorbate-DPIP couple as electron donors. However, photoreduction of NADP with the ascorbate-DPIP donor system can be restored in the particles if plastocyanin is added, but the addition of cytochrome *f* alone has no effect (*28*).

In the mutant strain of *C. reinhardi* lacking plastocyanin, the reduction of cytochromes 559 and 553 was observed but not their oxidation (*2, 3*). However, upon the addition of purified plastocyanin from the wild type, their light-induced oxidation was restored. Therefore, according to the series formulation, plastocyanin is on the PS I side of cytochrome 553. It has been suggested that plastocyanin is on the PS II side of cytochrome *f* on the basis of experiments in which light-induced cytochrome reduction was inhibited in the presence of a relatively high concentration of salicylaldoxime (*29*), an agent affecting the action of copper-containing proteins. Although the inhibitor can affect photosynthetic electron transport, its inhibitory action is not limited specifically to plastocyanin, and its effect on plastocyanin does not occur until after a considerable time of treatment (*30*).

Other steps and components of the photosynthetic electron transport chain may be postulated on the basis of the data derived from the mutant strains. As stated earlier, DCMU inhibits the light-induced reduction of cytochrome 559 but not its light-induced oxidation. This means that the site of DCMU action is on the PS II side of cytochrome 559. However, this inhibitor does not affect the light-induced reduction of Q, the quencher of fluorescence of PS II (*31*). This observation indicates that Q lies on the PS II side of cytochrome 559. The chemical nature of Q remains to be defined.

In common with other algae and higher plants, the chloroplasts of *C. reinhardi* contain ferredoxin and the ferredoxin-NADP reductase, and both function in the reduction of NADP. It has been suggested (*18*) that ferredoxin is the primary electron acceptor of PS I. Ferredoxin is a soluble component of the photosynthetic apparatus, and it is absent from chloroplast fragments that have been isolated in aqueous media. In its absence, however, it is still possible to obtain the light-induced oxidation and reduction of the cytochromes and of P700 (*2, 4*). Also, PS I has the capacity to reduce compounds having redox potentials that are significantly lower than that of ferredoxin (*32*), and thus there may be at least one electron carrier between PS I and ferredoxin.

Evidence for Two Light Reactions in Series

The study of photosynthesis with mutant strains of *C. reinhardi* has clearly indicated that photosynthetic electron transport is driven by two light reactions. It will be recalled that in the strain in which cytochrome 553 is affected, the light-induced reduction of cytochrome 559 can be obtained with red light, but the cytochrome cannot be photooxidized by far-red light as it is in the wild type. Thus the mutation blocks electron flow between two photochemical systems.

The same effect is seen in the mutant strain lacking the unknown component M, in which cytochrome 559 is reduced by light but not oxidized, and cytochrome 553 is oxidized by light but not reduced. In all of these mutant strains, there is light-induced electron flow from water only up to that point in the chain where a component is lacking, but NADP, the terminal acceptor, is not photoreduced. However, when reductant is supplied in the form of DPIP and ascorbate, light will drive the reduction of NADP, indicating that a second photochemical system lies beyond the block. Further support for the participation of two light reactions can be obtained by examining the properties of the mutant strain lacking an active P700. In this case, light-induced electron flow is detected as the photoreduction of Hill oxidants, such as indophenol dye and potassium ferricyanide, and the light-induced reduction of cytochromes 559 and 553. However, the mutant strain does not have the capacity to photoreduce NADP with the ascorbate-DPIP couple as the electron donor, nor is there the light-induced oxidation of the two cytochromes. Thus, a light reaction involving P700 is a prerequisite for NADP reduction.

The photosynthetic electron transport chain of *C. reinhardi* begins with water and ends with NADP, and the data obtained with the different mutant strains show conclusively that the photoreduction of NADP requires two light reactions and that cytochromes 559 and 553, plastocyanin, P700, and one or more additional but as yet unidentified electron carriers be present. A scheme for photosynthetic electron transport in *C. reinhardi* is shown in Fig. 2.

The study of photosynthesis with mutant strains of algae is a unique approach toward identifying the nature and function of components of the photosynthetic electron transport chain (*33*). The deletion of a component of the photosynthetic electron transport chain by mutation is perhaps a more natural way of affecting electron transport than one involving the use of inhibitors or other agents which destroy part of the action of the chain in wild-type organisms. It should be emphasized, however, that the two approaches have led to similar conclusions: higher plant and algal photosynthesis involves the action of two photochemical systems and these systems are linked in series by several electron carriers.

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