

# Assembly, Function, and Dynamics of the Photosynthetic Machinery in *Chlamydomonas reinhardtii*

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The green unicellular alga *Chlamydomonas reinhardtii* occupies a unique position among photosynthetic organisms. Although its photosynthetic function is very similar to that of vascular plants, it combines the advantages of unicellular organisms, which include fast growth under controlled environmental conditions with highly sophisticated genetics of the nuclear, chloroplast, and mitochondrial compartments. The aim of this article is to provide an overview of this powerful algal model system and to show that *C. reinhardtii* is ideally suited for studying the biogenesis of the photosynthetic apparatus, its structure-function relationship, and its remarkable ability to adapt to changing environmental conditions such as light quality and quantity and nutrient limitation.

## BASIC GROWTH PROPERTIES

*C. reinhardtii* cells are oval shaped, approximately 10  $\mu\text{m}$  in length and 3  $\mu\text{m}$  in width, with two flagella at their anterior end. The cells contain a single chloroplast occupying 40% of the cell volume and several mitochondria. There are two mating types,  $\text{mt}^+$  and  $\text{mt}^-$ , determined by two structurally distinct alleles of the mating type locus. The haploid vegetative cells multiply through mitotic divisions. However, upon nitrogen starvation the vegetative cells differentiate into gametes, and cells of opposite mating type fuse to give rise to a zygote, which will undergo meiosis under appropriate light-dark conditions and produce four haploid daughter cells that can resume vegetative growth. It is also possible to recover mitotically dividing diploid cells after the mating reaction, a property that is useful for determining whether a mutation is recessive or dominant and for testing whether mutations with the same phenotype belong to the same complementation group (see Harris, 1989).

In the presence of acetate in the growth medium, the photosynthetic function of *C. reinhardtii* cells is dispensable. This feature has been exploited extensively for isolating and maintaining mutants deficient in photosynthetic function. Cells can thus be grown under three different conditions: in minimal

medium with light and  $\text{CO}_2$  as the sole carbon source (phototrophic growth), in acetate-containing medium with light (mixotrophic growth), or without light (heterotrophic growth). The growth of the cells can easily be synchronized by light-dark cycles.

## THREE TRANSFORMABLE GENETIC SYSTEMS

Like plants, *C. reinhardtii* contains three genetic systems located in the nucleus, chloroplast, and mitochondria. Mutations in the genomes of these three compartments can be easily recognized by their unique segregation patterns during crosses. Whereas nuclear mutations segregate according to the classical Mendelian rules, chloroplast and mitochondrial mutations are normally transmitted uniparentally from the  $\text{mt}^+$  and  $\text{mt}^-$  parents, respectively (see Harris, 1989).

The complexity of the nuclear genome has been estimated at 100 Mbp (Harris, 1989). Currently, the genetic map includes 148 loci distributed over 17 linkage groups. In addition, approximately 240 RFLP and short tagged sequence markers have been mapped to all linkage groups with an average spacing of 4 to 5 centiMorgan or 0.4 to 0.5 Mbp (Silflow, 1998). The nuclear transformation yield is sufficiently high to allow for genomic complementation of nuclear mutants (see Kindle, 1998). In addition, because nuclear transformation of this alga occurs mainly through non-homologous recombination, the transforming DNA integrates randomly into the nuclear genome. Thus, transformation can be used as a mutagen for tagging genes.

The complete sequence of the chloroplast genome of *C. reinhardtii* has been recently completed (J. Maul, J. Lilly, and D. Stern, unpublished data). It consists of circular molecules, 204,210 bp in length, and contains 34 genes involved in photosynthesis, 31 genes involved in chloroplast transcription and translation, one protease gene, 29 tRNA genes, and nine genes of unknown function. Chloroplast transformation can be achieved routinely in *C. reinhardtii* by bombarding cells with DNA-coated tungsten particles (Boynton and Gillham, 1993). In contrast to nuclear transformation, chloroplast transformation occurs exclusively through homologous recombination. Because foreign selectable marker genes are available, e.g. *aadA* (Goldschmidt-Clermont, 1991) and *aphA-6*

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(Bateman and Purton, 2000), conferring resistance to specific antibiotics in the chloroplast, it is possible to inactivate specific genes or to perform site-directed mutagenesis on any plastid gene of interest (see below). This reverse genetics approach has been rather successful, especially for elucidating the function of conserved open reading frames, also called *ycfs*, present in the plastid genomes of several plants, algae, and cyanobacteria (see Rochaix, 1997). Chloroplast transformation has also been extremely useful for studying chloroplast gene expression, especially when it is combined with classical genetic analysis. It has been possible to dissect regulatory elements such as promoters and 5' and 3' untranslated regions, and to use chimeric genes consisting of chloroplast regulatory elements fused to reporter genes for identifying the target sites of specific nucleus-encoded factors required for chloroplast gene expression. This analysis has revealed that some of these factors act on the 5' untranslated region of specific mRNAs and that they are required for mRNA stability or translation (see Goldschmidt-Clermont, 1998).

The 15.8-kbp linear mitochondrial genome of *C. reinhardtii* encodes seven proteins involved in respiration, one protein resembling a reverse transcriptase, two ribosomal RNA genes that are fragmented and interspersed with other coding sequences, and three tRNA genes (see Remacle and Matagne, 1998). The other tRNAs have to be imported from the chloroplast or cytosol. Although mitochondrial transformation has been reported for *C. reinhardtii*, the yield is rather low. However, the availability of several drug-resistant mitochondrial mutations offers promising new selectable markers for mitochondrial transformation (Remacle and Matagne, 1998).

#### MOLECULAR CROSS TALK BETWEEN NUCLEUS AND CHLOROPLAST

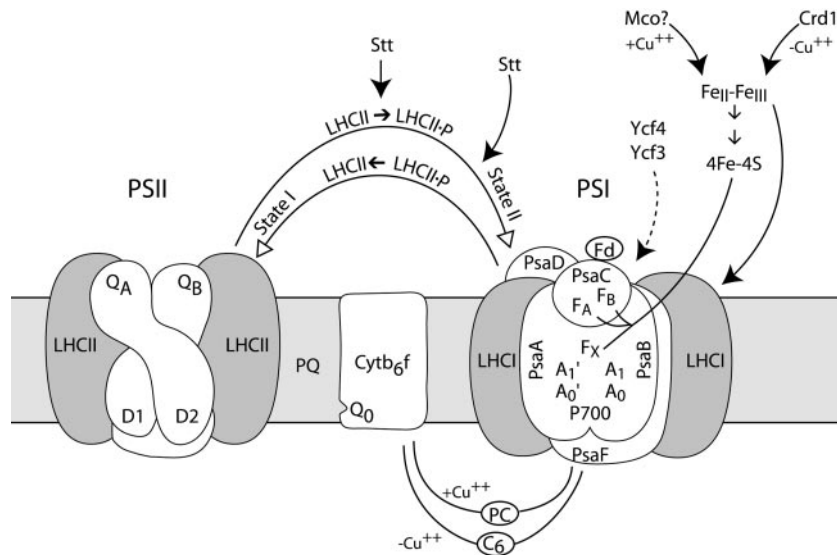
An area in which *C. reinhardtii* is especially powerful as model system is the biosynthesis of the photosynthetic apparatus that occurs through the concerted interactions between the chloroplast and nuclear genomes. An extensive analysis of nuclear mutants deficient in photosynthesis has revealed that besides the mutations that directly affect the genes of the components of the photosynthetic apparatus, the vast majority of the mutations are in genes encoding factors that are required for several chloroplast post-transcriptional steps, including RNA stability, RNA processing, translation, and the assembly of photosynthetic complexes (see Goldschmidt-Clermont, 1998). The number of these genes is surprisingly high, and their products act in a gene-specific manner. Several of these genes have recently been isolated through genomic complementation of the mutants with genomic cosmid libraries or by gene tagging. The phenotypes of some of these photosynthetic mutants resemble those of

mutants of *Arabidopsis* and maize (*Zea mays*), indicating that a similar complex nuclear-chloroplast network may exist in higher plants (Barkan and Goldschmidt-Clermont, 2000). The sequencing of the *Arabidopsis* genome has indeed revealed that as many as 3,000 nuclear genes encode chloroplast proteins (Abdallah et al., 2000). Many of these factors may be involved in chloroplast gene expression.

#### GENETIC DISSECTION OF PHOTOSYNTHESIS

The primary reactions of photosynthesis take place in the thylakoid membranes within the chloroplast. An important advantage of *C. reinhardtii* is that the functional state of its photosynthetic system can be monitored *in vivo* with noninvasive techniques such as chlorophyll fluorescence transients, absorption spectrophotometry using detecting flashes, and photoacoustic techniques (Joliet et al., 1998). The availability of a homogenous cell population grown under controlled environmental conditions is especially important for this type of analysis. Because of the large number of mutants of *C. reinhardtii* obtained both by random and site-directed mutagenesis, this alga has emerged as one of the best model systems for this functional *in vivo* analysis. The alga is also suited for *in vitro* studies because it can be grown in large amounts, making the purification of the photosynthetic complexes relatively easy. An additional important feature of *C. reinhardtii* is its ability to synthesize chlorophyll both in a light-dependent and light-independent manner. The cells are thus able to fully assemble the photosynthetic apparatus in the dark in marked contrast to higher plants. Many mutants deficient in photosynthesis are light sensitive and need to be grown in the dark. It is thus possible to isolate photosynthetic complexes from these mutants and to study their properties.

An example of the power of coupling genetics, chloroplast transformation and *in vivo* and *in vitro* kinetic spectrophotometry in *C. reinhardtii* is provided by the analysis of the structure-function relationship of photosystem I (PSI). The PSI complex acts as light driven oxidoreductase that transfers electrons from plastocyanin or cytochrome  $c_6$  in the thylakoid lumen to ferredoxin in the stroma (Fig. 1). The crystal structure of the complex from a cyanobacterium has been determined (Jordan et al., 2001) and revealed in particular the location of the redox components P700, the primary electron donor,  $A_0$ , a chlorophyll  $a$ ,  $A_1$ , a phylloquinone, and the 4Fe-4S center  $F_X$  that is liganded by PsaA and PsaB. The structure of the complex is symmetrical in this region with two potential electron transfer branches between P700 and  $F_X$  (Fig. 1). The *in vivo* analysis of the kinetics of electron transfer from the quinone to  $F_X$  revealed two kinetic components (Joliet and Joliet, 1999). By mutating quasi symmetrical residues of PsaA and PsaB near the quinone, it was found that a particular mu-



**Figure 1.** Schematic view of the photosynthetic complexes within the thylakoid membrane. The photosynthetic complexes PSII, PSI, and the cytochrome *b<sub>6</sub>f* complex are shown. PSII: D1 and D2, Main reaction center polypeptides; Q<sub>A</sub> and Q<sub>B</sub>, primary and secondary electron acceptors of PSII. PQ, Plastoquinone pool; Q<sub>O</sub>, binding site of the cytochrome *b<sub>6</sub>f* complex for plastoquinol. LHC, Light-harvesting complex; LHCII-P, phosphorylated form of LHCII. PC, Plastocyanin; C<sub>6</sub>, cytochrome *c<sub>6</sub>*. PSI: PsaA and PsaB, Reaction center subunits with the ligands P700, a Chl dimer that acts as primary electron donor, and the electron acceptors A<sub>0</sub>, A<sub>1</sub>, and their homologs A<sub>0</sub>', A<sub>1</sub>' in the other active branch, and the 4Fe-4S center F<sub>X</sub>. F<sub>A</sub>, F<sub>B</sub>, 4Fe-4S centers, Terminal electron acceptors of PSI liganded by the PsaC subunit. Fd, Ferredoxin. PsaF, Docking protein for PC (plastocyanin) and *c<sub>6</sub>* (cytochrome *c<sub>6</sub>*). Ycf3 and Ycf4, PSI assembly factors. Crd1, Di-Fe enzyme required for PSI accumulation under Cu deficiency; Mco, hypothetical multi-Cu oxidase. Stt, Genetically identified factors involved in state transition. In State I, the mobile part of LHCII is associated with PSII, whereas in State II it is phosphorylated and associated with PSI.

tation in one branch affected the faster component, whereas an analogous mutation in the other branch affected the slower component, indicating that in striking contrast to photosystem II (PSII), both branches are active in PSI (Guergova-Kuras et al., 2001). It is not possible within this short review to mention the numerous studies performed with *C. reinhardtii* on the structure-function relationship of the other parts of PSI and on the other complexes PSII, the cytochrome *b<sub>6</sub>f* complex, the ATP synthase, and Rubisco (for details, see Hippler et al., 1998).

### DYNAMICS OF THE PHOTOSYNTHETIC APPARATUS

Plants and algae have the remarkable ability to adapt and to modulate the operation of the photosynthetic apparatus in response to changes in light quality and quantity. Too much light can be harmful and excess light energy can be dissipated as fluorescence or heat (non-photochemical quenching [NPQ]). At least part of this non-radiative energy dissipation occurs through reversible covalent modifications of the thylakoid xanthophylls and involves the reductive de-epoxidation of violaxanthin to zeaxanthin (xanthophyll cycle) that is triggered by the pH gradient produced by photosynthetic electron flow (see Muller et al., 2001). Among *C. reinhardtii* mutants deficient in NPQ, *npq1* was found to be defective in

the xanthophyll cycle and provided direct genetic evidence for the importance of zeaxanthin in NPQ (Niyogi et al., 1997). This genetic analysis also revealed that the pigments of the xanthophyll cycle derived from  $\beta$ -carotene, and most likely lutein derived from  $\alpha$ -carotene are required both for NPQ and for protection against oxidative damage in high light (Muller et al., 2001).

*C. reinhardtii* has proven to be uniquely suited for analyzing state transition, a process that involves the reversible distribution of excitation energy from PSII to PSI through a reorganization of the antennae (State I to State II transition; Wollman, 2001). In this way the two photosystems that are serially linked by the photosynthetic electron transfer chain operate at the same pace and the quantum yield is optimized. Key factors in this process are the redox state of the plastoquinone pool, the cytochrome *b<sub>6</sub>f* complex, in particular the state of occupancy of the quinol binding site Q<sub>O</sub>, and the LHCII kinase(s). In State I, the mobile part of LHCII is associated with PSII. Preferential excitation energy flux to PSII reduces the plastoquinone pool and leads to the activation of the LHCII kinase and phosphorylation of LHCII that is laterally displaced from PSII in the grana to PSI in the stroma lamellae of the thylakoid membranes (Vallon et al., 1991; Delosme et al., 1996). Such a State I to State II transition is associated with a significant fluorescence decrease in *C. reinhardtii* because as

much as 80% of LHCII is displaced. In contrast, only 15% to 20% of LHCII is mobile in higher plants. The large decrease in fluorescence during a State I to State II transition has been used for screening mutants deficient in this process by fluorescence video imaging (Fleischmann et al., 1999; Kruse et al., 1999). Some of these mutants are deficient in LHC phosphorylation, whereas others are still able to phosphorylate the antenna. It should be noted that the distribution of excitation energy to the two photosystems is 0.45 for PSII and 0.55 for PSI in State I and 0.15 for PSII and 0.85 for PSI in State II. Thus, the cross sections of the PSII and PSI antennae are nearly balanced in State I and considerably uneven in state II in *C. reinhardtii*. It has indeed been shown that linear electron transfer is active in State I, whereas cyclic electron transfer operates in State II (Finazzi et al., 1999). Interestingly, the state transition mutants have been used for demonstrating that in *C. reinhardtii* state transition acts as a switch from linear electron transfer in State I to cyclic electron flow in State II (G. Finazzi and F. Rappaport, unpublished data). Mutants blocked in State I are unable to switch to cyclic electron flow under State II conditions. State transition in *C. reinhardtii* serves not only as a light adaptation mechanism, but also for rerouting photosynthetic electron flow, thereby allowing the organism to adapt to changes in cellular demand for ATP (Wollman, 2001).

#### RESPONSE OF THE PHOTOSYNTHETIC SYSTEM TO NUTRIENT LIMITATION

A genetic analysis of the response to nutrient limitation in *C. reinhardtii* has been especially rewarding. Upon depletion of sulfate or phosphate, the cells respond by arresting cell division, by decreasing photosynthetic electron flow, by inducing a highly efficient sulfate or phosphate uptake system, and by secreting arylsulfatase or phosphatases that are able to metabolize other sources of sulfate or phosphate (see Grossman and Takahashi, 2001). *SacI*, a mutant unable to synthesize arylsulfatase upon sulfur starvation, fails to diminish photosynthetic electron flow and dies in the light unless photosynthesis is blocked by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of PSII (Wykoff et al., 1998). The *SacI* protein contains 12 transmembrane domains and resembles ion transporters.

Limitation in micronutrients such as Cu or Fe also induce specific responses in *C. reinhardtii*. The best known example is the replacement of plastocyanin by cytochrome  $c_6$  under Cu starvation (Wood, 1978; Merchant and Bogorad, 1987). Both proteins transfer electrons between the cytochrome  $b_6f$  complex and PSI. Recent work has revealed a novel role of Cu in photosynthesis through a search for mutants that are conditionally deficient in photosynthetic activity under Cu deprivation (Moseley et al., 2000). One of the

mutants obtained, *crd1*, lacks PSI and LHCI under Cu limitation, but not in the presence of nutritionally adequate Cu. This phenotype is rather unusual, as the analysis of numerous mutants deficient in PSI or LHC accumulation has shown that either of these complexes can accumulate in the absence of the other. The phenotype of the *crd1* mutant reveals an unrecognized role for Cu in the maintenance of PSI and LHCI. With its three 4Fe-4S clusters, PSI is a major Fe sink in the chloroplast. Because the chlorotic phenotype of the *crd1* mutant in the absence of Cu is recapitulated in Fe-deficient wild-type cells, it is likely that the observed effect is mediated through interactions between Cu and Fe metabolism. *Crd1*, which contains a dicarboxylate di-Fe binding site, could possibly facilitate the mobilization of Fe in the chloroplast when a multi-Cu oxidase is necessarily ineffective (Fig. 1; S. Merchant, unpublished data).

#### PERSPECTIVES

Since the isolation of the first chloroplast mutants of *C. reinhardtii* nearly 50 years ago, the genetic and molecular analysis of this unicellular alga has greatly progressed. Among eukaryotic photosynthetic organisms, *C. reinhardtii* has emerged as one of the most powerful model systems for analyzing the cooperative interplay between the nuclear and chloroplast genetic systems in the biogenesis of the photosynthetic apparatus. Because chloroplast transformation is easy to perform with *C. reinhardtii*, it has become the organism of choice for the structure-function analysis of the components of the photosynthetic apparatus. Thanks to the impressive advances in the determination of the atomic structures of PSI, PSII, the cytochrome  $b_6f$  complex, LHC, and the ATP synthase, it is possible, using chloroplast site-directed mutagenesis and transformation, to selectively alter residues for addressing specific questions about photosynthetic electron transfer processes. Perhaps the most exciting area concerns the remarkable dynamics and flexibility of the photosynthetic apparatus in response to changes in light conditions and in nutrient limitation, which has only recently become accessible to experimental investigation. We still have a very limited understanding of the molecular identity of the components of the signal transduction pathways that are used in these processes. The coming years should provide novel insights and many surprises.

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