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Minireview

Chlamydomonas, a model system for studying the assembly and dynamics of photosynthetic complexes

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Abstract **The green unicellular alga *Chlamydomonas reinhardtii* has emerged as a powerful model system for studying the biosynthesis of the photosynthetic apparatus and the acclimation of this system to changes in light conditions. The assembly of the photosynthetic complexes involves the coordinate interaction between the nuclear and chloroplast genetic systems. Many factors involved in specific chloroplast post-transcriptional steps have been identified and characterized. *Chlamydomonas* is able to adapt to changes in light quality and in cellular ATP content by performing state transition, a process that leads to a redistribution of light excitation energy between photosystem II and photosystem I and that involves the redox state of the plastoquinone pool, the cytochrome b_6f complex and one or several kinases specific for the light-harvesting system. Genetic approaches have provided new insights into this process.** © **2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Chloroplast; Photosynthesis; Photosystem I; State transition; *Chlamydomonas*

1. Introduction

The primary reactions of oxygenic photosynthesis occur on the thylakoid membrane and are catalyzed by several multimolecular complexes that include photosystem II (PSII), photosystem I (PSI), their associated light-harvesting systems, the cytochrome b_6f complex and the ATP synthase. Each of these complexes consists of multiple subunits, pigments and redox cofactors. Upon light absorption by the antennae, the excitation energy is channeled to the reaction centers to oxidize the chlorophyll dimers P680 and P700 in PSII and PSI, respectively (Fig. 1). The electron donor side of PSII acts as a strong oxidant capable of oxidizing water which, besides yielding molecular oxygen and protons in the thylakoid lumen, is the source of electrons for the photosynthetic electron transport chain. The electrons originating from $P680^+$ are rapidly transferred across the thylakoid membrane to the final PSII acceptor quinones Q_A and Q_B. Once it is doubly reduced, Q_B diffuses into the plastoquinone pool and transfers its electrons to the cytochrome b_6f complex after binding to the Q_o site. In a series of complex reactions some of the electrons are transferred from the cytochrome b_6f complex via soluble plastocyanin to PSI while other electrons follow a different route within the cytochrome b_6f complex and participate in the proton pumping activity of this complex. Upon charge separation within PSI, the electrons are transferred from plastocyanin to ferredoxin and ultimately to NADP. Photosynthetic electron transfer generates a proton gradient across the thylakoid membrane that is used by the ATP synthase to produce ATP, which together with NADPH drives the Calvin cycle for CO_2 fixation [1].

It is well established that the synthesis of the photosynthetic complexes depends on the concerted interaction between the nuclear and chloroplast genetic systems. Some of the polypeptides of these complexes are encoded by nuclear genes, synthesized on cytosolic 80S ribosomes, imported into the chloroplast and integrated into the thylakoid membrane together with their chloroplast-encoded partners that are synthesized on plastid 70S ribosomes (Fig. 1) [2]. The green unicellular alga *Chlamydomonas reinhardtii* has emerged as a powerful model system for analyzing this process for several reasons. (1) Photosynthetic function is dispensable when the cells are grown in the presence of acetate, a property that facilitates the isolation and maintenance of mutants deficient in photosynthetic activity. (2) Because chlorophyll synthesis also occurs in the dark in *C. reinhardtii*, the photosynthetic apparatus can be assembled in the absence of light and it is possible to study photosynthetic reactions in light-sensitive mutants. (3) Genetic analysis is well established in *C. reinhardtii* at the level of the nuclear, chloroplast and mitochondrial genomes [3]. (4) Efficient transformation methods for the nuclear [4] and chloroplast [5] compartments have been established. This allows one to isolate nuclear genes by genomic rescue of nuclear photosynthetic mutants with appropriate cosmid libraries. Because chloroplast transformation occurs through homologous recombination, disruption or site-directed mutagenesis of plastid genes is possible. (5) Powerful spectrophotometric methods have been developed for monitoring photosynthetic electron flow and the electrochemical gradient across the thylakoid membrane in vivo. Coupling of genetic, biophysical and biochemical approaches has been especially rewarding in *C. reinhardtii* [6]. (6) The *Chlamydomonas* genomic project has produced more than 130 000 ESTs that have greatly helped in the identification of genes and gene families in *Chlamydomonas* (http://www.biology.duke.edu/chlamy_genome/cgp.html; http://www.kazusa.or.jp/en/plant/chlamy/EST/).

Genetic analysis of numerous mutants of *C. reinhardtii* deficient in photosynthetic activity has revealed the existence of many nuclear genes that are involved in post-transcriptional

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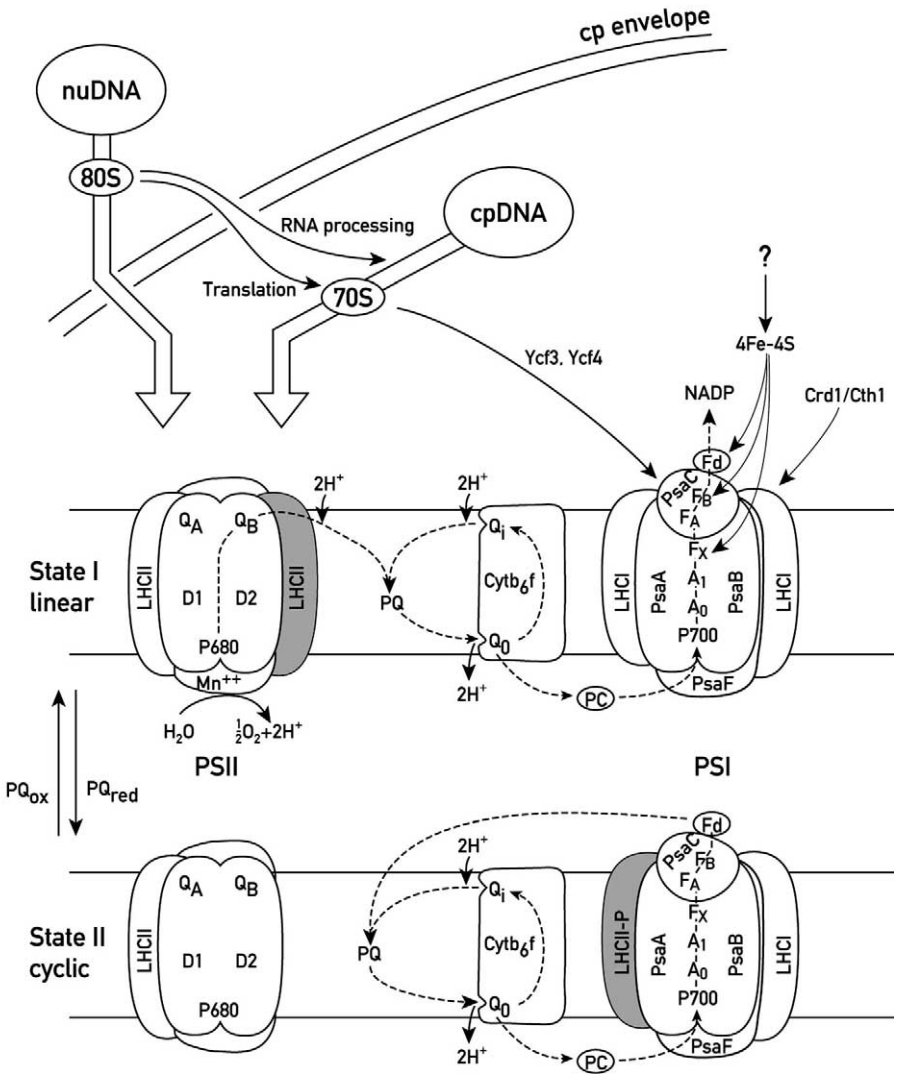

Fig. 1. Scheme of biosynthesis of the photosynthetic complexes in the thylakoid membrane. Upper part: several subunits of the photosynthetic complexes PSII, cytochrome b_6f and PSI are encoded by the nuclear genome (nuDNA), translated on cytosolic ribosomes (80S) and translocated across the chloroplast envelope through the plastid envelope. Others are encoded by the chloroplast genome (cpDNA), translated on chloroplast ribosomes (70S) and inserted into the thylakoid membrane together with their nucleus-encoded partners. Thick lines indicate synthesis of polypeptides from the photosynthetic complexes; thin lines indicate factors required for chloroplast post-transcriptional steps (RNA processing, translation, assembly). Lower part: photosynthetic complexes in the thylakoid membrane. Only the reaction center polypeptides are shown: D1 and D2 for PSII and PsaA, PsaB and PsaC for PSI. P680, chlorophyll dimer that acts as primary donor of PSII. Q_A and Q_B, primary and secondary acceptors of PSII. Qo, Qi binding sites of the cytochrome b_6f for pastoquinol and plastoquinone, respectively. LHC, light-harvesting complex; LHCII-P, phosphorylated form of LHCII; PC, plastocyanin. P700, chlorophyll dimer that acts as primary donor of PSI; Ao, A1 primary acceptors; F_X (liganded by PsaA and PsaB), F_A and F_B (liganded by PsaC), 4Fe–4S centers of PSI. Fd, ferredoxin; PsaF docking protein for plastocyanin. Ycf3, Ycf4, PSI assembly factors. Crd1, Cth1, di-iron enzymes required for accumulation of PSI and LHCI in the absence and presence of copper, respectively. Stt, factors identified by genetic means that are involved in state transition. In state I the mobile part of LHCII (indicated in gray) is associated with PSII; the photosynthetic electron transport chain operates in a linear mode and generates NADPH and ATP. In state II the mobile part of LHCII is associated with PSI in the phosphorylated form; the photosynthetic electron transport chain operates in a cyclic mode and produces only ATP.

steps of chloroplast gene expression. A striking feature is that most of the factors encoded by these genes appear to act specifically on the expression of individual chloroplast genes at the level of RNA stability, RNA processing and splicing, translation and assembly of the photosynthetic complexes (Fig. 1). Here I will first briefly review the biosynthesis of PSI that has been intensively studied in *Chlamydomonas*, from the level of gene expression to that of the assembly of the mature complex in the thylakoid membrane. In the second part, an approach for studying the dynamics of the light-harvesting system is described.

2. Assembly of PSI

The PSI complex acts as a light-driven plastocyanin-ferredoxin oxido-reductase that consists of at least five chloroplast- and eight nucleus-encoded subunits. The atomic structure of the PSI complex of the cyanobacterium *Synechococcus elongatus* has been established at a resolution of 2.5 Å [7]. The two major subunits of PSI, PsaA and PsaB, each consist of 11 transmembrane domains and they act as ligands for the redox cofactors P700 (chlorophyll *a* dimer), A_0 (chlorophyll *a*), A1, (phylloquinone) and F_X (4Fe–4S cluster) [8]. The terminal

electron acceptors F_A and F_B, which are also 4Fe–4S clusters, are bound to the peripheral PsaC subunit. This subunit, together with the PsaD and PsaE subunits, forms the docking site for ferredoxin on the stromal side of the thylakoid membrane. The analysis of PSI mutants of *C. reinhardtii* has revealed a surprisingly large number of factors required for the assembly of this complex. The chloroplast *psaA* gene of *C. reinhardtii* consists of three independently transcribed exons and the production of the mature *psaA* mRNA which occurs through two *trans*-splicing reactions requires at least 14 nucleus-encoded factors [2]. At least one factor is required for *psaB* mRNA stability and two additional factors are required for its translation. Several of these factors have recently been identified and characterized. Furthermore, there is an interesting asymmetry in the synthesis of the two reaction center polypeptides PsaA and PsaB [9]. Mutants deficient in PsaB synthesis are also deficient in PsaA synthesis whereas mutants deficient in PsaA synthesis are still able to synthesize PsaB. This suggests that PsaB has an important anchor function during the initial steps of PSI assembly. It is noteworthy that the dependence of the synthesis of a given subunit on the presence of other partners of the same photosynthetic complex has also been observed in other cases. In particular this process has been intensively studied for the cytochrome b_6f complex where repression of cytochrome *f* translation is mediated through an interaction of the 5′ untranslated region of its mRNA with the C-terminal domain of the protein [10]. This inhibitory interaction only occurs when cytochrome *f* cannot assemble with the other partner subunits of the cytochrome b_6f complex.

Given the complexity of PSI, it is not surprising that its synthesis requires several assembly factors. Two of these factors are encoded by the chloroplast genes *ycf3* and *ycf4*. These genes are present in all plastid genomes examined as well as in cyanobacteria. Inactivation of *ycf3* and *ycf4* in *C. reinhardtii* leads to a complete and selective loss of PSI [11]. Similar observations have been made in higher plants [12] and cyanobacteria [13] although in the latter case the inactivation of *ycf4* leads only to a partial loss of PSI. Ycf3 contains three tetratricopeptide-like domains that are thought to be involved in protein–protein interactions. A temperature-sensitive *ycf3* mutant of *C. reinhardtii* has been used in temperature shift experiments to show that Ycf3 is involved in the assembly, rather than in the stability of PSI [14]. Both Ycf3 and Ycf4 are located in the thylakoid membrane. Although they do not cofractionate with PSI upon solubilization of the membrane with non-ionic detergents, it is likely that they interact with some of the PSI subunits. Indeed, co-immunoprecipitations of solubilized thylakoid membranes with antisera raised against several PSI subunits indicate that Ycf3 interacts with a restricted set of PSI subunits, in particular with PsaD [14]. Recent experiments using the bacterial two-hybrid system have confirmed a direct interaction between PsaD and Ycf3 (D. Dauvillée and J.D. Rochaix, unpublished results).

Ycf4 is part of a high molecular weight complex that partially overlaps the PSI complex after sucrose density fractionation of solubilized thylakoid membranes [11]. Protein pulse-labeling reveals that newly synthesized PsaD is first associated with the Ycf4 complex before the production of the mature PSI complex suggesting that the Ycf4 complex may be involved in the formation of a PSI precomplex (Y. Takahashi and J.D. Rochaix, unpublished results).

With its three 4Fe–4S centers F_X, F_A and F_B, PSI constitutes an important iron sink in the chloroplast. Little is known on how the iron is recruited in the chloroplast and how these Fe–S centers are assembled. However, recently a set of Isc proteins involved in Fe–S center assembly have been identified in eubacteria that also appear to be present in the mitochondria of eukaryotic organisms [15]. IscS functions as a cysteine desulfurase and IscU and IscA are involved in the biosynthesis of the iron–sulfur clusters. In eukaryotic organisms these proteins are localized exclusively in the mitochondria. Thus a separate system must operate in the chloroplast unless one assumes that the iron–sulfur centers are imported from the mitochondria through the cytosol into the chloroplast. This possibility, however, appears unlikely, given the fact that Fe–S assembly has been reconstituted from chloroplast extracts [16]. An important challenge is to identify the factors involved in this chloroplast system. Recently a novel system, Suf, for the assembly of iron–sulfur clusters has been identified in bacteria [17]. Of special interest is that genes homologous to the Suf genes are present in plastids from red algae and in the nuclear genome of *Arabidopsis* with putative transit peptides for import into the chloroplast.

Once they have been reconstituted, iron–sulfur centers need to be specifically incorporated into PSI. It is likely that specific factors are required for this task. Indeed recent work in cyanobacteria has shown that inactivation of the gene of a membrane-associated rubredoxin leads to the loss of PSI activity [18]. The PsaA, PsaB and the other PSI subunits containing transmembrane domains are still present, but the peripheral proteins PsaC, PsaD and PsaE are absent. This rubredoxin protein appears to be required for the integration of the F_X center [19]. Similar rubredoxin proteins have been identified in the chloroplasts of vascular plants and green algae. Their exact role remains to be elucidated.

Although copper is not a component of PSI, *crd1*, a conditional mutant of *C. reinhardtii*, lacks PSI and its associated LHCI in copper-deficient cells, but accumulates normal levels of these complexes in copper-replete cells [20]. Crd1 accumulation is not only increased under copper limitation, but also under anaerobic conditions. The mutant phenotype of *crd1* is rather unusual because most PSI-deficient mutants accumulate normal levels of LHCI. *Crd1* encodes a protein with a di-iron motif and displays sequence similarity to a protein implicated in the cyclase reaction in the chlorophyll biosynthetic pathway [21]. However, the exact role of Crd1 remains unknown. A paralog of Crd1, called Cth1, displays a reciprocal pattern of expression, i.e. Crd1 is expressed under copper-deficient or anaerobic conditions whereas Cth1 accumulation is increased in copper-sufficient oxygenated cells [21]. This reciprocal relationship is similar to that of cytochrome c_6 and the copper protein plastocyanin that mediate electron transfer from the cytochrome b_6f complex to PSI [22]. Although mis-expression of Cth1 in copper-deficient cells can substitute for Crd1, slight differences in the peripheral LHCI-PSI antennae are detectable [21]. Thus Crd1 and Cth1 have overlapping, but non-identical functions.

3. Short-term adaptation of the photosynthetic apparatus to changing light conditions and intracellular ATP content

A remarkable feature of the photosynthetic apparatus is its ability to adapt to rapidly changing light conditions. In order

to grow optimally oxygenic photosynthetic organisms need to optimize their photosynthetic yield under low light conditions and to dissipate excess light excitation energy under high light to prevent oxidative damage. *C. reinhardtii* has proven to be especially attractive for studying these mechanisms that include non-photochemical quenching and state transition.

State transition involves an adaptive reorganization of LHCII, the light-harvesting complex of PSII within the thylakoid membrane [23,24]. PSII and PSI have distinct light absorption properties, but act in series to produce reducing power for CO_2 fixation (Fig. 1). Under changing light conditions state transition allows the photosynthetic apparatus to redistribute light excitation energy between PSII and PSI so as to optimize the quantum yield. This balance is governed by the redox state of the plastoquinone pool and a signal transduction chain involving the cytochrome b_6f complex and at least one kinase specific for LHCII. Although the activity of this LHCII kinase was identified nearly 25 years ago by Bennett [25], the isolation of this enzyme has proven to be rather difficult. However, recently genetic approaches in *Arabidopsis* and *Chlamydomonas* have yielded novel insights into this process. A LHCII kinase from *Arabidopsis*, called TAK kinase, has been identified and characterized and shown to be required for state transition [26,27]. Furthermore, loss of the PSI subunit PsaH in *Arabidopsis* prevents excitation energy transfer from LHCII to PSI under state II conditions [28].

C. reinhardtii has proven to be uniquely suited for analyzing state transition mainly because transition from state I to state II is accompanied by a large fluorescence decrease [29]. As much as 80% of LHCII is reconnected to PSI during a state I to state II transition [30]. In addition, state transition in *Chlamydomonas* also represents an adaptive response to the intracellular ATP content [31]. In particular, depletion of ATP induces a state I to state II transition. Screens based on fluorescence video-imaging have been used for isolating mutants deficient in state transition [32,33]. Several of these mutants are blocked in state I and deficient to a variable extent in LHCII phosphorylation. Most of these mutants grow photoautotrophically and their growth is not impaired by illumination with light of 600 $\mu E/m^2/s$. Thus in *Chlamydomonas* state transition does not appear to have an important role in the dissipation of excess light excitation energy, at least with light intensities below 600 $\mu E/m^2/s$. However, one mutant, stm1, is impaired in growth especially under low light [33].

Finazzi et al. [34] have made the interesting observation that DCMU, an inhibitor of linear electron flow acting on the acceptor side of PSII, has no effect on the electron flow through the cytochrome b_6f complex in *Chlamydomonas* cells in state II, suggesting that there is no linear, but only cyclic electron flow under these conditions. One of the state transition mutants isolated, *stt7*, has been used to examine the relationship between state transition and cyclic electron flow further [35]. Electron flow through the cytochrome b_6f complex in this mutant is affected to the same extent under state I and state II conditions thus indicating that the loss of DCMU sensitivity in the wild-type under state II conditions cannot be explained by an increased chlororespiratory electron flow into the plastoquinone pool, but is due to a switch from linear to cyclic electron flow. In addition to the *stt7* study, the analysis of mutants lacking the PSII outer antennae or accumulating low amounts of cytochrome b_6f complex reveals a strict correlation between the redistribution of the mobile photosynthetic antenna from PSII to PSI during a state I to state II transition and the onset of cyclic electron flow [35]. Thus state I promotes linear electron flow and the production of reducing power and ATP, whereas state II generates only ATP in *Chlamydomonas*. Whether the same holds for land plants remains to be investigated. We have recently cloned the gene defective in *stt7* by genomic complementation. This gene encodes an 80 kDa serine threonine kinase containing an N-terminal chloroplast targeting signal and a potential transmembrane domain (N. Depège, S. Bellafiore and J.D. Rochaix, unpublished results). It could thus specify the LHCII kinase or one of the kinases involved in state transition. The Stt7 kinase has homologs in *Arabidopsis*, but is unrelated to the TAK kinases that have been implicated in state transition in *Arabidopsis* [26]. Another mutant, *stt6*, is particularly interesting because it is completely deficient in phosphorylation not only of LHCII, but also of the other minor antenna complexes (S. Bellafiore and J.D. Rochaix, unpublished results). The analysis of these mutants is likely to provide important new insights into the components involved in state transition and adaptation to changing light conditions.

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