The Plant Cell, Vol. 18, 159–175, January 2006, www.plantcell.org © 2005 American Society of Plant Biologists

Chloroplast Biogenesis of Photosystem II Cores Involves a Series of Assembly-Controlled Steps That Regulate Translation

Limor Minai,[1] Katia Wostrikoff,[2] Francis-André Wollman, and Yves Choquet[3]

Unité Mixte de Recherche 7141, Centre National de la Recherche Scientifique/Université Pierre et Marie Curie (Paris VI), Institut de Biologie Physico-Chimique, 75005 Paris, France

The biogenesis of photosystem II, one of the major photosynthetic protein complexes, involves a cascade of assembly-governed regulation of translation of its major chloroplast-encoded subunits. In *Chlamydomonas reinhardtii*, the presence of the reaction center subunit D2 is required for the expression of the other reaction center subunit D1, while the presence of D1 is required for the expression of the core antenna subunit apoCP47. Using chimeric genes expressed in the chloroplast, we demonstrate that the decreased synthesis of D1 or apoCP47 in the absence of protein assembly is due to a genuine downregulation of translation. This regulation is mediated by the 5′ untranslated region of the corresponding mRNA and originates from negative feedback exerted by the unassembled D1 or apoCP47 polypeptide. However, autoregulation of translation of subunit D1 is not implicated in the recovery from photoinhibition, which involves an increased translation of *psbA* mRNA in response to the degradation of photodamaged D1. De novo synthesis and repair of photosystem II complexes are independently controlled.

INTRODUCTION

Photosystem II (PSII) is a multimeric protein-pigment complex embedded in the thylakoid membrane, where it initiates linear photosynthetic electron flow. It comprises >20 different subunits, most of which are integral membrane proteins, and binds numerous cofactors. In photosynthetic eukaryotes, PSII subunits, assembled in a 1:1 stoichiometry, are encoded in two different genetic compartments. In the green alga *Chlamydomonas reinhardtii*, six subunits are nuclear encoded, while 15 subunits are encoded by the chloroplast.

The functional assembly of this highly complex oligomeric protein should require some control mechanisms that set the expression of the constitutive subunits to their proper level. Indeed, PSII assembly is a stepwise process, in which the level of accumulation of PSII subcomplexes increases with the number of their constitutive subunits. The presence of cytochrome b_{559}, which accumulates in the membrane even in the absence of other PSII subunits, is a prerequisite for PSII assembly (Morais et al., 1998). It interacts at a very early step with the reaction center (RC) subunit D2, encoded by the chloroplast gene *psbD*, to form a precomplex, detected as a high molecular mass species (45 to 200 kD) in etioplasts (Müller and Eichacker, 1999). This precomplex probably serves as a receptor for the assembly of the RC subunit D1, the product of the chloroplast *psbA* gene. Cytochrome b_{559} and the heterodimer D1/D2, together with subunits PsbI and PSBW, form the PSII RC that binds all essential redox cofactors of PSII and is the smallest protein complex able to perform light-induced charge separation (Nanba and Satoh, 1987). In subsequent steps, core antenna subunits that bind chlorophyll *a*, CP47 then CP43, encoded by the *psbB* and *psbC* chloroplast genes, respectively, are recruited to form the PSII core complex, allowing the further binding of the oxygen evolving enhancer (OEE) proteins, PSBO, P, and Q, on the lumenal side of the membrane.

Strikingly, studies of PSII mutants, mainly performed in *C. reinhardtii*, revealed that not only the assembly but also the expression of some chloroplast-encoded PSII core subunits is a sequential process. Mutants lacking D1 show a reduced synthesis of CP47 but not of D2 (Bennoun et al., 1986; Jensen et al., 1986; de Vitry et al., 1989). Mutants lacking D2 show a reduced synthesis of both D1 and apoCP47 subunits (Bennoun et al., 1986; Erickson et al., 1986).

This process, whereby the presence of one subunit is required for sustained synthesis of another chloroplast-encoded subunit from the same protein complex, has been termed control by epistasy of synthesis (CES) (Wollman et al., 1999; Choquet and Vallon, 2000; Choquet and Wollman, 2002). The molecular mechanism responsible for CES has been best characterized for cytochrome *f* from the cytochrome b_6f complex. In the absence of its assembly partners, subunit IV or cytochrome b_6, the synthesis of cytochrome *f* decreases 10-fold (Kuras and Wollman, 1994) as a result of an autoregulation of translation initiation (Choquet et al., 1998) mediated by the C-terminal domain of unassembled cytochrome *f* (Choquet et al., 2003). A similar mechanism

[1] Current address: Inserm U528, Institut Curie, 26 rue d'Ulm, F-75248 Paris Cedex 05, France.
[2] Current address: Boyce Thompson Institute for Plant Research at Cornell University, Tower Road, Ithaca, NY 14853.
[3] To whom correspondence should be addressed. E-mail choquet@ibpc.fr; fax 33-1-58415022.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Yves Choquet (choquet@ibpc.fr).
Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.105.037705.

controls the synthesis of PsaA and PsaC, two CES subunits from the photosystem I (PSI) complex (Wostrikoff et al., 2004).

PSII is by far the photosynthetic complex whose biogenesis has been studied in greatest detail, in particular because it is the major target for photodestructive processes in the canopy. Under bright illumination, charge separation in PSII generates highly reactive radical species that damage PSII cores, specifically the D1 polypeptide, resulting in a decreased PSII activity described as photoinhibition (reviewed in Ohad et al., 1994). Recovery from photoinhibition involves migration of damaged PSII complexes from stacked to stroma thylakoids, degradation of the damaged subunit D1, and its replacement by neosynthesized D1. During recovery from photoinhibition, the synthesis of D1 is strongly increased (Kettunen et al., 1997).

Although regulation of D1 expression has been extensively studied (reviewed in Baena-Gonzalez and Aro, 2002; Zhang and Aro, 2002), some key aspects of PSII biogenesis remain poorly understood. Whether similar mechanisms control *psbA* expression during de novo synthesis of PSII complexes or repair of photodamaged D1 remains to be determined. The mechanisms linking assembly of PSII to the rate of production of its individual subunits have not been addressed either. The contribution of the CES process to the high turnover of D1 has not been studied up to now to our knowledge. Here, we used reporter genes translated under the control of the 5′ untranslated regions (UTRs) of the CES genes *psbA* or *psbB* to demonstrate that biogenesis of PSII involves a cascade of translational autoregulation mediated by the unassembled D1 and CP47 subunits. We show that recovery from photoinhibition relies on a molecular mechanism distinct from the translational regulation that controls de novo synthesis of PSII cores.

Table 1. Transformation Experiments

Transformants	Recipient Strains	Transforming Plasmid
Δ*psbD*[a]	WT	pΔ*psbD*::K_r
Δ*psbB*[a]	WT	pΔ*psbB*::K_r
{Δ*psbA*, Δ*psbD*}[a]	Δ*psbA* (Fud 7)	pΔ*psbD*::K_r
WT::*bA*K	WT	p*fbA*K
Δ*psbD*::*bA*K	Δ*psbD*[b]	p*fbA*K
Δ*psbA*::*bA*K	Δ*psbA* (Fud 7)	p*fbA*K
bAf	WT	pK*bAf*
{*bAf*, Δ*psbD*}	Δ*psbD*[b]	pK*bAf*
{*bAf*, Δ*psbA*}	Δ*psbA* (Fud 7)	pK*bAf*
{*bAf*, Δ*psbB*}	Δ*psbB*[b]	pK*bAf*
bAfbA	WT	pK*bAfbA*
$bA_{2H}f$	WT	p$KbA_{2H}f$
$D1_{Tr}$[a]	WT	pbA_{259}StK_r
{$D1_{Tr}$, *bAf*}	$D1_{Tr}$[b]	pK*bAf*
bBf	WT	pK*bBf*
{Δ*psbA*, *bBf*}	Δ*psbA* (Fud 7)	p*f*bBK
$CP47_{Tr}$[a]	WT	p$K_r$$bB_{164}$St
{$CP47_{Tr}$, *bBf*}	$CP47_{Tr}$[b]	pK*bBf*

All recipient strains were *mt*+ and spectinomycin sensitive. Transformants were selected for resistance to spectinomycin (100 μg·mL^{-1}). WT, wild type.

[a] Strains, initially selected for spectinomycin resistance due to the presence of the recycling spectinomycin resistance cassette (K_r), were screened by fluorescence induction kinetics for impaired PSII activity. Once homoplasmic with respect to the PSII mutation, they were grown on TAP medium for several generations to allow the spontaneous loss of the recycling cassette according to Fischer et al. (1996) but not that of the mutated PSII allele.

[b] Strains, which became spectinomycin sensitive again, could then be used as recipient strains in a new round of transformation experiments based on selection for spectinomycin resistance.

RESULTS

Hierarchy in the Synthesis of PSII Core Subunits in *C. reinhardtii*

To investigate the tight coupling in the synthesis of PSII core subunits, we compared various PSII mutants specifically deleted for one of the chloroplast genes encoding major PSII subunits. To this end, we generated the Δ*psbB* and Δ*psbD* deletion strains, unable to express the core subunit apoCP47 and the RC subunit D2, respectively. We also used the Fud7 chloroplast mutant strain, hereafter referred to as Δ*psbA*, lacking expression of the other RC subunit D1 because it carries a deletion of the entire *psbA* gene (Bennoun et al., 1986), from which we generated a double deletion strain {Δ*psbD*, Δ*psbA*} (Table 1).

The wild-type and deletion strains were pulse labeled with ^{14}C acetate for 5 min in the presence of cycloheximide, an inhibitor of cytoplasmic translation. In the Δ*psbD* mutant, the synthesis of D1 and CP47 are strongly reduced, compared with the wild-type strain. The diffuse band (asterisk in Figure 1A) most likely corresponds to a newly synthesized form of D1, since it is absent in *psbA* deletion strains {Δ*psbD*, Δ*psbA*} and Δ*psbA*. The Δ*psbA* mutant, lacking D1, still expressed wild-type levels of D2 but poorly synthesized apoCP47. In the Δ*psbB* mutant, lacking apoCP47, the synthesis of D1 or D2 remained unaltered.

The decreased synthesis of the CES subunits, D1 in the absence of D2 and CP47 in the absence of D1 (and therefore in the absence of D2), does not result from a decreased accumulation of *psbA* or *psbB* mRNAs that remained unchanged in all strains (Figure 1B), suggesting a translational or early posttranslational regulation.

The *psbB* 5′UTR Is Sufficient to Confer a CES Behavior to a Reporter Gene

The mechanism that couples synthesis of apoCP47 to the presence of D1 could be due to an actual regulation of translation, possibly at the step of initiation, as observed for PSI or cytochrome b_6f biogenesis (Choquet et al., 1998; Wostrikoff et al., 2004). Thus, we constructed a chimeric gene made of the promoter and 5′UTR of the *psbB* gene, fused in frame to the *petA* coding region that encodes cytochrome *f*, previously shown to be a convenient reporter gene (Wostrikoff et al., 2004). We associated this *psbB*-driven chimeric gene with an antibiotic resistance cassette (Goldschmidt-Clermont, 1991) that allows one to select transformed cells on Tris-acetate-phosphate (TAP) medium supplemented with spectinomycin (TAP-Spec) (Figure 2A, Table 1). Using the wild-type strain as a recipient, spectinomycin-resistant

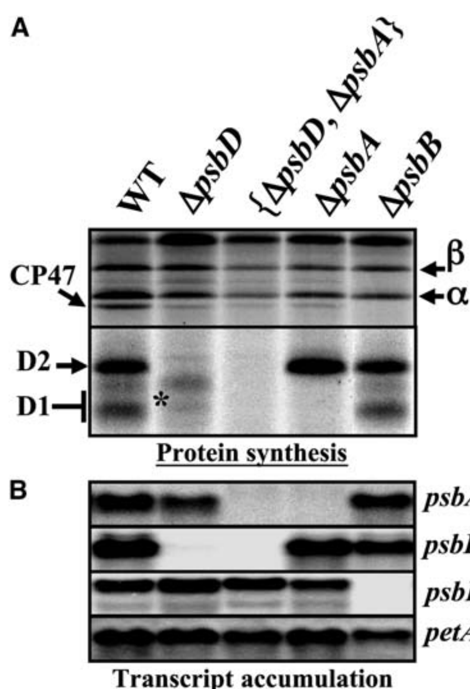

Figure 1. Hierarchy of Synthesis among PSII Core Subunits.

(A) Rate of synthesis of the major PSII subunits, determined by 5-min pulse-labeling experiments in wild-type and deletion strains of *C. reinhardtii.* Analysis on 8% acrylamide-urea gels. An asterisk marks a neosynthesized form of D1.

(B) Accumulation of *psbB*, *psbD*, *psbA*, and *petA* (loading control) transcripts in the same strains.

transformants, hereafter referred to as *bBf* because they express a 5′*psbB*-driven cytochrome *f*, also were found to be phototrophic. Expression of the chimeric cytochrome *f* is high enough to sustain photosynthetic growth. Pulse-labeling experiments showed that it was made at the same rates in the wild-type and *bBf* transformants (Figure 2B).

To assess the hypothesis of an assembly-dependent regulation of translation initiation of *psbB*, we examined the susceptibility of translation of the 5′*psbB*-driven reporter gene to the presence or absence of D1. The *bBf* strain was crossed to the nuclear mutant strain *tba2*-F35, defective for *psbA* translation (Girard-Bascou et al., 1992; Figure 2B, lane *tba2*). All offspring carried the chloroplast chimeric gene, which was uniparentally transmitted from the mt^+ parent *bBf*. Expression of *petA* from the chimeric gene is distinguishable from that of the endogenous *petA* gene as its shorter transcript migrates ahead of the regular *petA* mRNA (Figure 2D). Half of the progeny inherited the mutant *tba2* allele and lacked expression of D1, while the other half inherited the wild-type *TBA2* allele. Therefore, analysis of cytochrome *f* expression among tetrad members allowed comparing the expression of the chimeric gene in the presence or absence of D1. While offspring expressing D1 translated the chimeric cytochrome *f* to a level similar to that observed in the parental strain *bBf*, *tba2* progeny, lacking D1, displayed a dramatic decrease in cytochrome *f* synthesis (lanes *bBf*, *TBA2* versus *bBf*, *tba2* in Figure 2B). Cytochrome *f* is a stable protein whose accumulation reflects the rate of synthesis. Accordingly, cytochrome *f* accumulation was drastically reduced in the *bBf*, *tba2* progeny (Figure 2C).

To exclude that the decreased translation of the 5′*psbB-petA* chimeric gene in *tba2* progeny resulted from a pleiotropic effect of the *tba2* mutation on the expression of both 5′*psbA*- and 5′*psbB*-driven genes, we introduced by chloroplast transformation the 5′*psbB-petA* chimera in the Δ*psbA* strain that lacks expression of D1 but has a wild-type nuclear genome (Table 1). Transformed strains, hereafter called {Δ*psbA*, *bBf*}, selected for

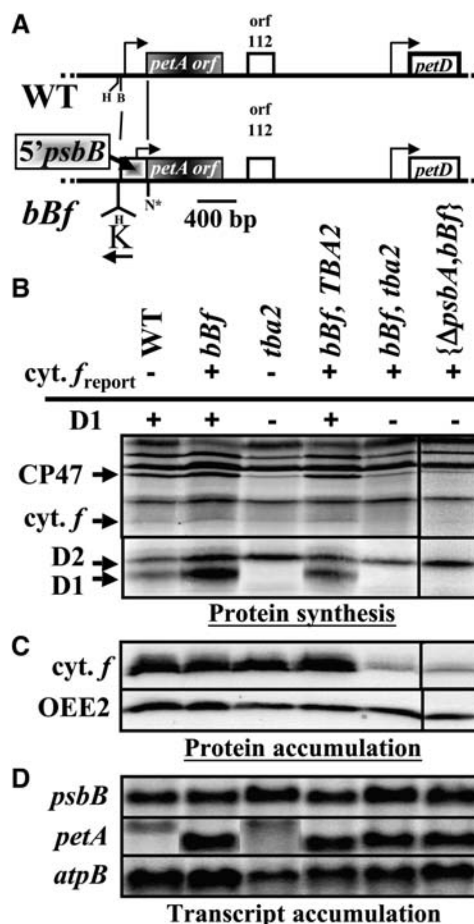

Figure 2. Expression of the 5′*psbB-petA* Chimeric Gene Decreases in the Absence of D1.

(A) Map of the chloroplast *petA* gene in wild-type and *bBf* strains. Relevant restriction sites are indicated: B, *Bgl*II; N*, an *Nco*I site engineered around the *petA* initiation codon for cloning purposes; H, *Hinc*II. The bent arrow indicates transcription start sites. K stands for the spectinomycin resistance cassette, in opposite orientation with respect to *petA*.

(B) Translation of the chimeric gene in a representative half tetrad from *bBf* × *tba2* crosses and in parental and wild-type strains. Translation of the chimeric gene in the Δ*psbA* chloroplast mutant is also shown.

(C) and **(D)** Protein **(C)** and transcript **(D)** accumulation of the chimeric gene in the same strains. Accumulation of OEE2 and of *atpB* transcript provide loading controls.

resistance to spectinomycin, showed the same reduced expression of the chimera (Figures 2B and 2C, lane {Δ*psbA*, *bBf*}) as the *tba2* progeny from the cross *bBf* × *tba2*.

The reduced expression of the reporter cytochrome *f* did not result from a reduced accumulation of the chimeric mRNA in strains *bBf*, *tba2* or {Δ*psbA*, *bBf*} (Figure 2D). Thus, the decreased synthesis of the CES subunit apoCP47 in the absence of its assembly partner D1 is governed by the *psbB* 5′UTR and, hence, is most likely controlled at the level of translation initiation.

The Regulation of Translation Initiation of *psbB* mRNA Is Reflected by Changes in Its Association with Polysomes

In order to confirm that conclusion, we compared the polysome loading of *psbB* mRNA by sedimentation of cell extracts on sucrose gradients in the wild type and in strains lacking expression of D1: *tba2*-F35 and Δ*psbA*. The bulk of free mRNA as well as dissociated 50S and 30S ribosome subunits are found in fractions 6 to 10 (as can be seen from the sedimentation pattern of *rrnS* and *psbB* RNAs in extracts from wild-type cells treated with EDTA that dissociates ribosomes; Figure 3, WT panel). Therefore, in untreated extracts, transcripts found in fractions 5 to 1 and in the pellet correspond to polysomes of increasing sizes. We compared the distribution of three RNAs: *psbD*, *psbB*, and *atpB*, the latter serving as a control for a chloroplast transcript unrelated to PSII biogenesis. In the wild type, the three mRNAs present roughly a similar distribution: ribosome-free mRNAs peak at fractions 7 and 8, but the maximum in fractions 4 and 5 indicates polysome loading (Figure 3). In the *tba2* or Δ*psbA* strains, *psbD* and *atpB* mRNAs were still associated with

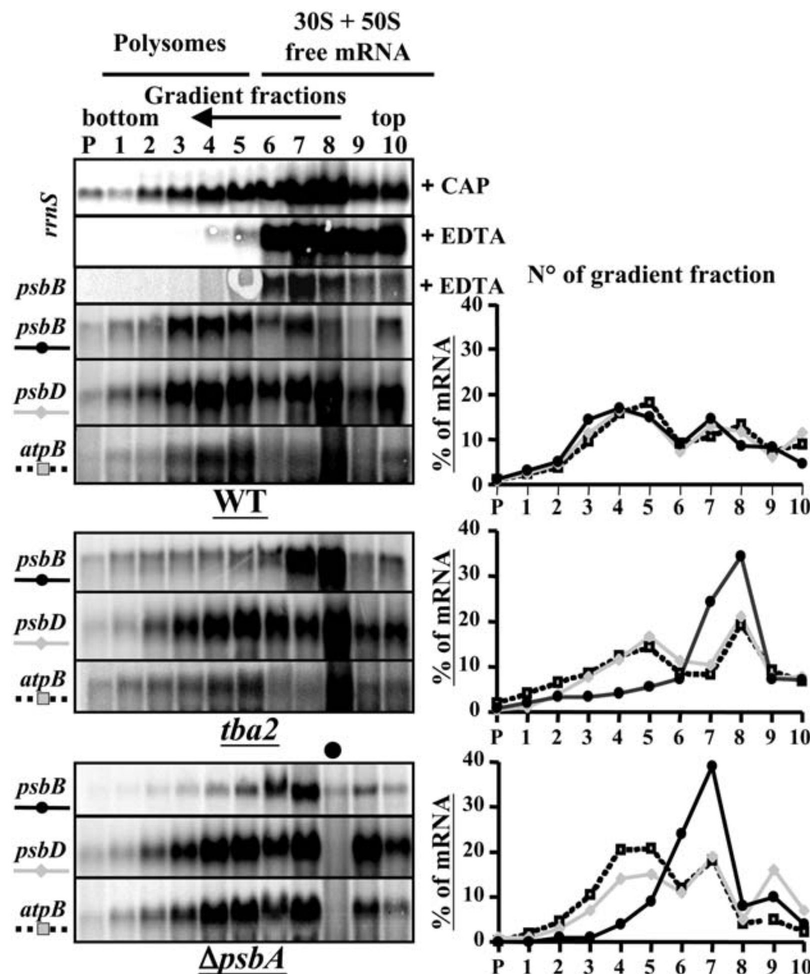

Figure 3. *psbB* mRNA Is Poorly Translated in the Absence of D1.

Left panels: Distribution of *psbB*, *psbD*, and *atp*B mRNAs on sucrose gradients in the wild type and in Δ*psbA* and *tba2*-F35 mutants lacking expression of D1. The distribution of *rrnS* and *psbB* RNAs in extracts from wild-type cells, in conditions preserving polysomes (+CAP) or disrupting polysomes (+EDTA), is shown to substantiate the assignment of polysome and free subunits to specific fractions. Fraction P contains the material found as a pellet at the bottom of the tube. The black circle in panel Δ*psbA* indicates RNA degradation in that specific fraction. Right panels: mRNA distributions were quantified by PhosphorImager scan of ^{33}P labeling bound to the membrane in the three strains and expressed as the percentage of total transcript found in each fraction.

polysomes (Figure 3). By contrast, the *psbB* mRNA was mostly found in fractions 7 and 8, with no peak in fractions P to 5 and <10% of total *psbB* mRNA in heavy fractions versus almost 40% in the wild type. These observations confirm a defect in the initiation of translation of the *psbB* mRNA in the absence of D1.

Downregulation of apoCP47 Expression in the Absence of D1 Is Due to an Autoregulation of Translation

The control on the translation of apoCP47 subunit can be achieved in either of two ways: either D1 per se is required to activate *psbB* mRNA translation or apoCP47, which remains unassembled because of the absence of D1, exerts a negative feedback on the translation of *psbB* mRNA. Since the *psbB* 5′UTR was found sufficient to confer the CES regulation to a reporter gene, we could distinguish between these two possibilities by studying the expression of the 5′*psbB*-driven cytochrome *f* in mutant strains lacking both the CES subunit (i.e., apoCP47) and its assembly partner, D1. If the CES process results from a positive control mediated by D1, translational activation should be abolished in the double mutant strain, resulting in low expression of the cytochrome *f* reporter. By contrast, if the CES protein apoCP47 negatively controls its own translation, the negative feedback will be lost in the double mutant strain, thereby causing a high expression of the cytochrome *f* reporter.

A possible pitfall in such experiments would be the facilitated recruitment of *psbB* translational activators by 5′*psbB*-driven genes in strains lacking the endogenous *psbB* mRNA, such as Δ*psbB*. This may artifactually increase expression of reporter genes. Thus, we generated by transformation another chloroplast mutant, $CP47_{Tr}$, still able to accumulate and translate the *psbB* mRNA but unable to accumulate the apoCP47 protein.

This was done by introducing a stop codon in the *psbB* coding sequence, at position +165 with respect to the initiation codon, in the connecting loop between transmembrane helices III and IV (Figure 4A). The mutated *psbB* gene was associated with the recycling spectinomycin resistance cassette (Fischer et al., 1996), enabling the selection of transformants on TAP-Spec plates.

Pulse labeling of the $CP47_{Tr}$ strain showed the absence of neosynthesized apoCP47 but the presence of a new translation product with an apparent molecular mass of ~16 kD (Figure 4B, lane $CP47_{Tr}$). The assignment of this new band to truncated apoCP47 was confirmed by crossing the $CP47_{Tr}$ strain with the nuclear mutant *mbb1*-222E, deficient for CP47 expression because of the absence of *psbB* mRNA (Vaistij et al., 2000). In the resulting tetrad, the two *mbb1* members that lacked the *psbB* mRNA (data not shown; indicated by an asterisk in Figure 4B) also lacked translation of the 16-kD polypeptide. This truncated apoCP47 was found to be very short-lived by pulse-chase experiments (*t*1/2: 10 min) (Figure 4C). Thus, it is not expected to accumulate to significant levels in the thylakoid membrane, fulfilling our aim to create a strain that accumulates a translatable *psbB* mRNA, but not its protein product, even in the truncated form.

The $CP47_{Tr}$ strain was then transformed with the 5′*psbB*-*petA* reporter gene, associated with the spectinomycin resistance cassette for selection of transformants on TAP-Spec

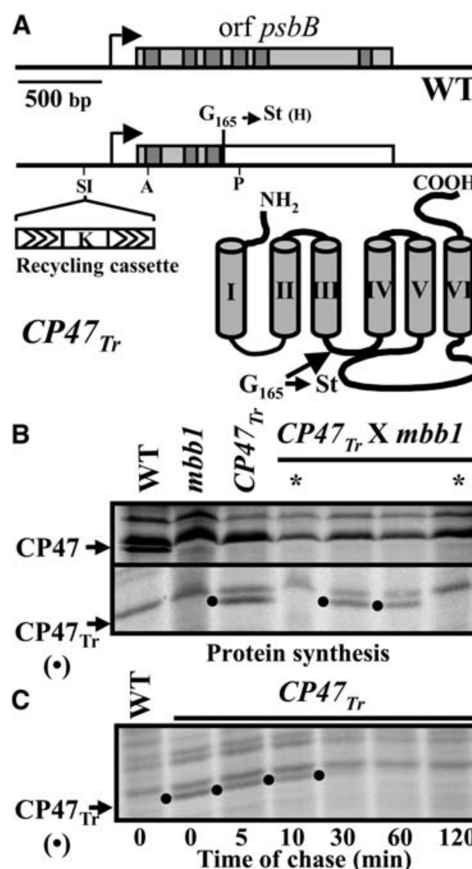

Figure 4. Characterization of a Strain Expressing a Truncated apoCP47 Polypeptide.

(A) Top line: Map of the *psbB* gene. Transmembrane helices are represented as gray boxes. The bent arrow indicates transcription start site. Bottom line: Schematic map of the construct used to introduce a stop codon (linked to a *Hin*dIII restriction site) into the *psbB* coding sequence as well as a recycling spectinomycin resistance cassette, schematically depicted by the boxed K with chevrons on either side (not to scale), for selection of transformants. Restriction sites used are as indicated: A, *Apa*LI; P, *Pvu*II; S, *Stu*I; H, *Hin*dIII. The position of the mutation with respect to the protein sequence is indicated below.

(B) Level of translation of the *psbB* mRNA (either endogenous or mutated) in the wild type, in the $CP47_{Tr}$ strain expressing the truncated apoCP47 (marked by a black circle), in a representative tetrad progeny from the cross $CP47_{Tr}$ × *mbb1*-222E, and in the parental strain *mbb1*-222E. Asterisks mark the tetrad members carrying the *mbb1* mutation.

(C) Stability of the truncated apoCP47 polypeptide determined by pulse labeling of the $CP47_{Tr}$ strain (time 0) followed by a chase for the indicated times in the presence of an excess of nonradioactive acetate and 200 $\mu g \cdot mL^{-1}$ chloramphenicol. Pulse-labeled wild type is shown for comparison.

medium (Table 1). The resulting strain {$CP47_{Tr}$, *bBf*} expressed the chimeric gene at a rate ~1.7 higher than a strain expressing full-length apoCP47 (Figure 5A, cf. lanes *bBf* and {$CP47_{Tr}$, *bBf*}). We then compared the expression of 5′*psbB*-driven cytochrome *f* in the absence of both D1 and apoCP47 among a tetrad progeny from the cross between transformants

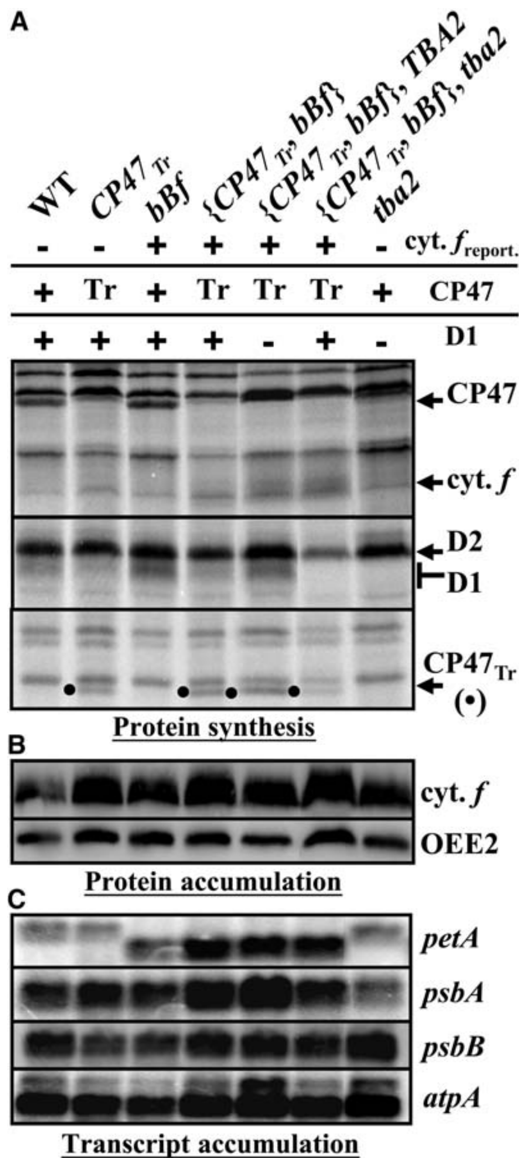

Figure 5. Cytochrome *f* Reporter Driven by the *psbB*-5′UTR Is Overexpressed in the Absence of Both the Assembly Partner (D1) and the CES (CP47) Subunits.

(A) Chloroplast translation products in representative tetrad progeny from {$CP47_{Tr}$, *bBf*} × *tba2*-F35 crosses and in the wild-type and parental strains. Cells expressing only the chimeric gene 5′*psbB-petA* or the truncated *psbB* allele are shown for comparison. The black circles indicate the position of the truncated apoCP47.

(B) Accumulation of cytochrome *f* and OEE2 (loading control) in the same strains, detected using specific antibodies.

(C) Accumulation of *petA*, *psbA*, *psbB*, and *atpA* (loading control) transcripts in the same strains.

{$CP47_{Tr}$, *bBf*} and mutant *tba2*. Half of the progeny inherited the *tba2* nuclear mutation, whereas all inherited both the truncated *psbB* allele (Figure 5A) and the chimeric *petA* gene, whose presence can be distinguished from the wild-type *petA* gene by the higher mobility of its transcript in mRNA gel blots (Figure 5C). As shown in Figures 5A and 5B for a representative half tetrad, the level of translation of the cytochrome *f* reporter remained high and independent of the presence or absence of D1. Thus, in contrast with what was observed when the full-length *psbB* gene was expressed, the translation and accumulation of the *psbB*-driven cytochrome *f* in the presence of the truncated version of apoCP47 was no longer dependent on the presence of D1. We also note that the rate of synthesis of truncated apoCP47 is insensitive to the absence or presence of D1 (Figure 5A).

Since the expression of both the chimeric 5′*psbB-petA* gene and the truncated *psbB* gene product became independent of the presence of D1, we ruled out a positive control of D1 on the translation of apoCP47. Rather, CP47, when unassembled

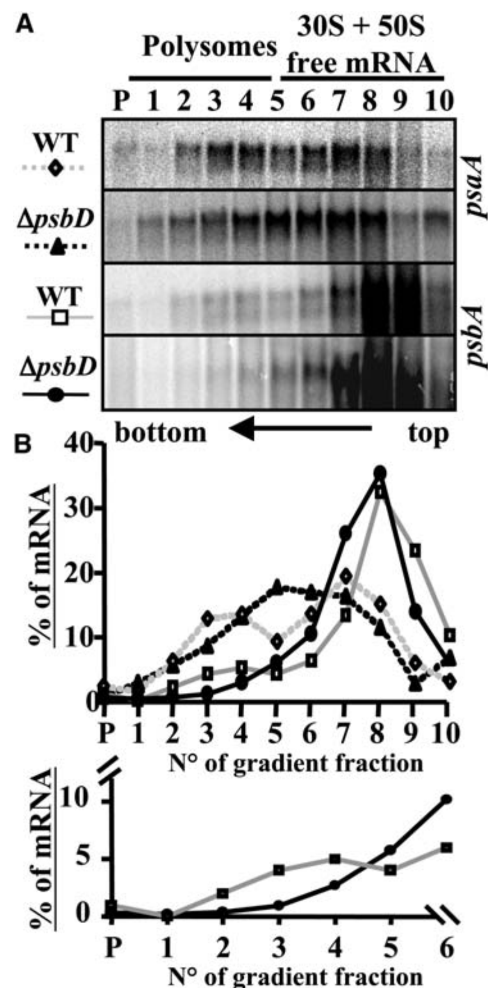

Figure 6. *psbA* mRNA Association with Polysomes

(A) Distribution of *psaA* (as a control) and *psbA* mRNAs on sucrose gradients in the wild-type and Δ*psbD* strains. Fraction P contains the pellet, rinsed out from the bottom of the tube after collection of fractions 1 to 10.

(B) mRNA distribution was quantified from a PhosphorImager scan of the ^{33}P labeling and expressed as the percentage of total transcript found in each fraction. The bottom panel shows an enlarged view of the distribution of *psbA* mRNA in the heavy fractions.

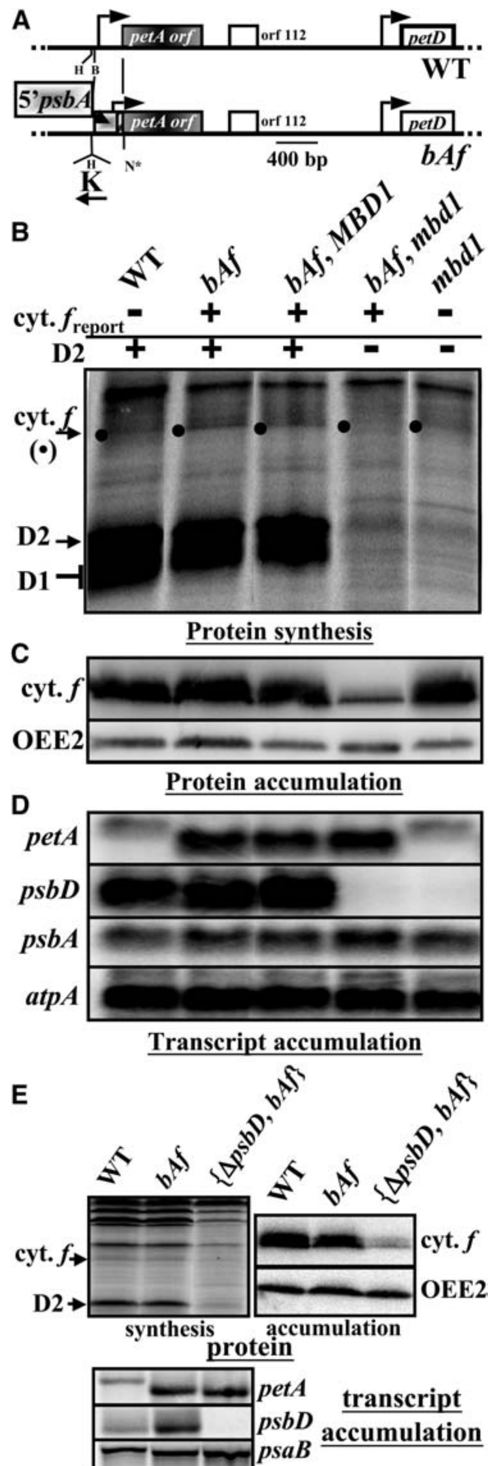

Figure 7. The CES Behavior of D1 Corresponds to a Translational Regulation Mediated by the *psbA*-5′UTR.

(A) Schematic representation of the chimeric gene 5′*psbA-petA*, using conventions of Figure 2A. The small rectangle upstream of the N* site depicts the first 60 nucleotides of the *psbA* coding sequence.

(B) Chloroplast translation products in a representative half tetrad of *bAf* × *mbd1*-nac2 crosses and in parental and wild-type strains.

(C) Accumulation of cytochrome *f* and OEE2 (loading control) in the same strains, immunodetected with specific antibodies.

(D) Accumulation of *petA*, *psbD*, *psbA*, and *atpA* (loading control) transcripts in the same strains.

(E) Expression of the chimeric gene 5′*psbA-petA* in the deletion strain Δ*psbD*. The wild-type and *bAf* strains are shown as controls.

because of the absence of D1, exerts a negative feedback on the translation of its own mRNA.

The CES Behavior of D1 Reflects a Genuine Regulation of Translation of 5′UTR-Driven *psbA* Transcripts

Evidence for a decreased translation of D1 in the absence of D2 can be found in a comparative analysis of the *psbA* polysome loading in the wild type and Δ*psbD* strains. In the wild type, there is a great contrast between the extent of polysome loading for *psaA* and *psbA* mRNAs: while >40% of *psaA* mRNAs were loaded on polysomes (see also *psbB*, *psbD*, and *atpB* mRNAs in Figure 3, WT panel), <15% of the *psbA* mRNAs were found in heavy fractions in our culture conditions (10 $\mu E \cdot m^{-2} \cdot s^{-1}$), most of *psbA* mRNAs being found in lighter fractions as free mRNA (Figure 6A). This observation correlates well with the poor level of D1 translation observed in pulse-labeling experiments when performed with cells grown in low light (Figure 1A). Still, the limited loading of *psbA* mRNA on polysomes in the wild type, with a maximum found in fraction 4, was no longer observed in the Δ*psbD* strain (Figure 6B).

The significant but limited change in polysome loading of *psbA* transcripts in the absence of D2 prompted us to seek stronger evidence that translation of *psbA* is genuinely dependent on the presence of D2. We used a *psbA*-driven reporter gene approach, similar to the one described above for the regulation of apoCP47 synthesis. We constructed various chimeric genes made of the coding region for cytochrome *f*, translated under the control of the *psbA* 5′UTR, that extended to some degree into the *psbA* coding region. These chimeric 5′*psbA-petA* genes, associated with the spectinomycin resistance cassette to allow selection of the transformed strains (Figure 7A), were introduced into the chloroplast genome of *C. reinhardtii*. Transformants, selected on TAP-Spec plates, were assayed for phototrophic growth on minimum medium. Only when at least 60 nucleotides of the *psbA* coding sequence were added in frame, upstream of the reporter gene, did the chimeric mRNA accumulate, enabling the phototrophic growth of the transformants (data not shown). These results point to the existence of *cis*-acting element stabilizing *psbA*-driven transcripts within the coding sequence of *psbA*, as already described for other chloroplast genes in *C. reinhardtii* (Singh et al., 2001) or for *psbA* in cyanobacteria (Kulkarni and Golden, 1997). Thus, we used for the rest of our study transformed strains expressing cytochrome *f* translated under the control of the *psbA* 5′UTR, followed by the 60 first nucleotides of the *psbA* coding sequence, hereafter called *bAf* because they express a 5′*psbA*-driven cytochrome *f*.

We compared the expression of this reporter gene in the presence or absence of D2 in progeny from crosses between *bAf*

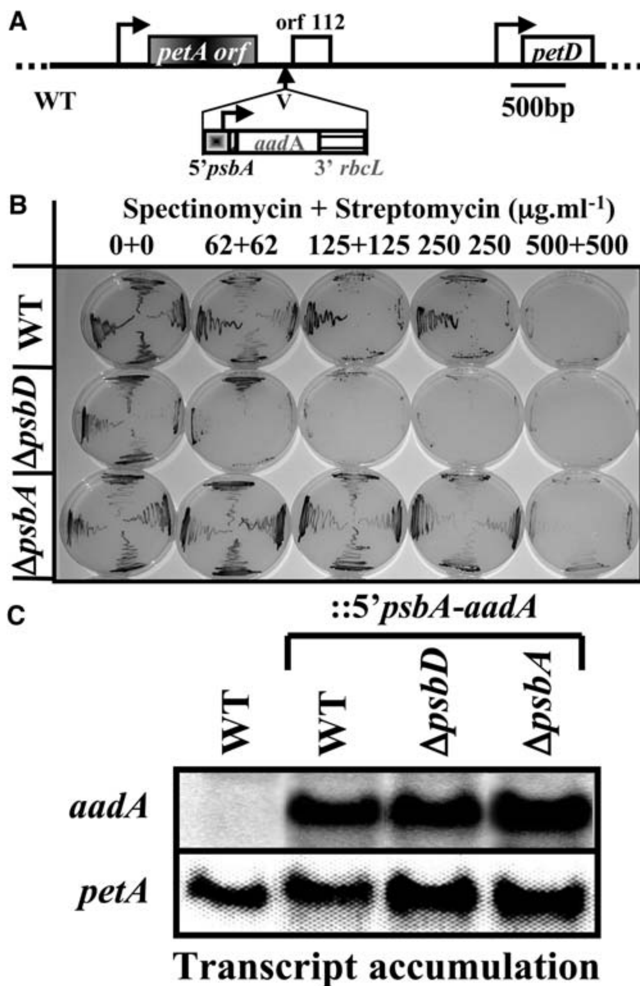

Figure 8. The *psbA* 5′UTR Confers a D2-Dependent Expression to the Reporter Gene *aadA*.

(A) Schematic map of the *petA*-*petD* region where the 5′*psbA*-*aadA* chimeric gene was inserted in direct orientation with respect to the *petA* gene, at the neutral *Eco*RV (V) site. The bent arrows indicate transcription start sites. The small rectangle upstream of *aadA* coding sequence depicts the 60 nucleotides from the *psbA* coding sequence.

(B) Growth of four independent transformants, derived from the recipient strains listed on the left, in the presence of increasing concentrations of spectinomycin plus streptomycin.

(C) Accumulation of the chimeric *aadA* (and *petA* as a loading control) transcripts in the wild type and in one representative transformant for each genetic background presented in **(B)**.

transformants with the nuclear mutant strain *mbd1*-nac2. This mutant is defective in D2 protein expression because it lacks stable accumulation of *psbD* mRNA (Kuchka et al., 1989). The chloroplast 5′*psbA*-*petA* chimeric gene is easily identified by the faster migration of its transcript (Figure 7D). We observed that the level of translation and accumulation of this chimeric cytochrome *f* was much lower in the absence of D2 than in its presence (cf. lanes *bAf, MBD1* and *bAf, mbd1* for a representative half tetrad in Figures 7B and 7C). We could exclude a pleiotropic effect of the *mbd1* mutation on the expression of 5′*psbA*-driven genes since the chimeric gene presented a lower expression when expressed in the Δ*psbD* context as well (Figure 7E, lane {Δ*psbD*, *bAf*}). No reduction in the accumulation of the chimeric mRNA correlated with the absence of the *mbd1* allele (Figure 7D). Thus, the absence of D2 causes a decreased translation of *psbA* as well as of chimeric *petA* gene translated under the control of the *psbA* 5′UTR.

As an independent assay for expression changes of a 5′ driven *psbA* reporter in conditions where D1 can assemble or not with D2, we constructed a 5′*psbA*-*aadA* cassette, in which the bacterial *aadA* reporter gene was translated under the control of the promoter and 5′UTR of the *psbA* gene, followed by the first 60 nucleotides of the *psbA* coding sequence. This 5′*psbA*-*aadA* chimeric gene was inserted downstream of the *petA* gene, in plasmid p*fbA*K, used to transform the chloroplast genome of wild-type and deletion strains Δ*psbD* and Δ*psbA* (Figure 8A, Table 1). Transformants were recovered on TAP medium supplemented with 100 $\mu g \cdot mL^{-1}$ of spectinomycin.

We analyzed the level of expression of the 5′*psbA*-*aadA* chimeric gene through the changes in antibiotic resistance (to spectinomycin and streptomycin) in the transformed strains according to Choquet et al. (1998). Four independent transformants, derived from each recipient strain transformed with plasmid p*fbA*K, were plated on TAP medium supplemented with increasing concentrations of both spectinomycin and streptomycin. As shown in Figure 8B, cells expressing the chimeric gene in a wild-type context demonstrated significant resistance up to 250 $\mu g \cdot mL^{-1}$ of both antibiotics. Cells lacking D2, the assembly partner of D1, were barely resisting 62 $\mu g \cdot mL^{-1}$ of both antibiotics, whereas clones originating from Δ*psbA* were resistant up to 500 $\mu g \cdot mL^{-1}$ of both antibiotics. The latter observation demonstrates that the decreased resistance observed in Δ*psbD* strains is not a mere consequence of PSII deficiency, as it is not observed in the Δ*psbA* strain.

Hence, cells lacking D2, the assembly partner of D1, are unable to express the 5′*psbA*-driven *aadA* gene to the same level as the control that expresses D2. Since the 5′*psbA*-*aadA* mRNA accumulated to the same level in all transformed strains (Figure 8C), these results point to a specific downregulation of the expression of the chimeric gene operating at the level of translation.

Unassembled D1 Represses Translation of 5′*psbA*-Driven Genes

To address the mechanism of this translational regulation and determine whether D2 stimulates the expression of D1 or whether unassembled D1 exerts a negative feedback on the translation of 5′*psbA*-driven mRNAs, we studied the expression of the 5′*psbA*-driven cytochrome *f* in the absence of both the assembly partner D2 and the CES subunit D1. Using a strategy similar to that described above for the *psbB* gene, we generated a strain still able to accumulate and translate the *psbA* mRNA but lacking accumulation of the D1 protein.

To that end, we substituted by site-directed mutagenesis the codon for residue I_{259} in the stromal loop connecting helices IV and V by a stop codon, causing synthesis of a truncated D1 protein (Figure 9A). This mutation, associated with a recycling

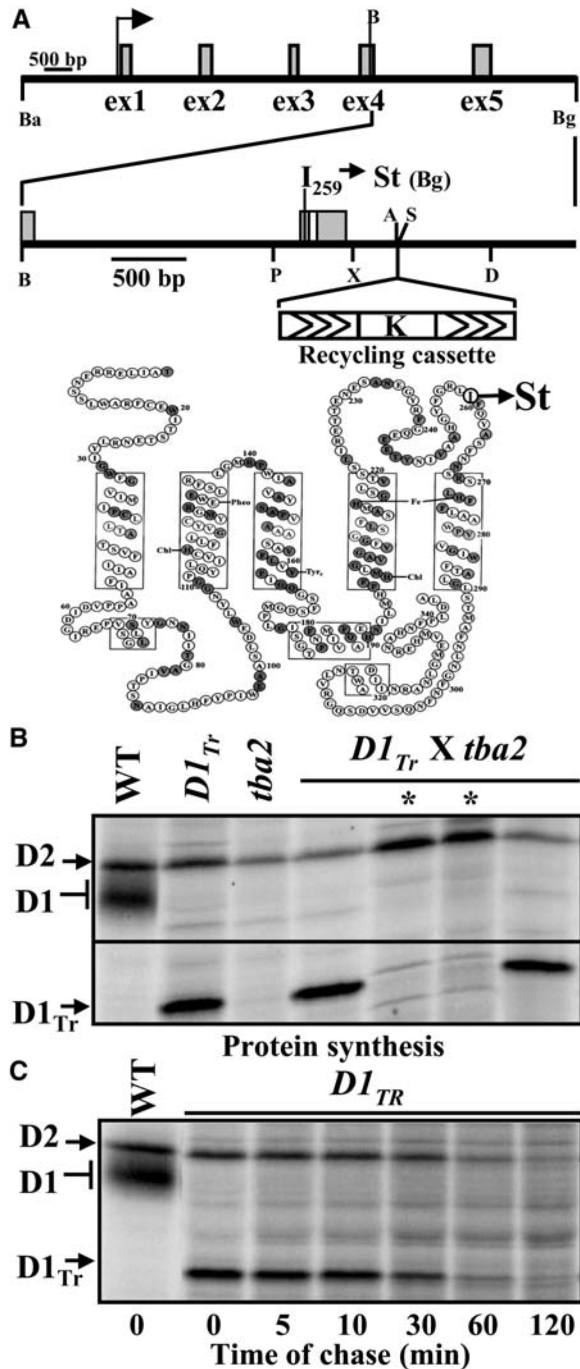

Figure 9. The $D1_{Tr}$ Strain Expresses a Truncated and Short-Lived *psbA* Gene Product.

(A) Top: Map of the *psbA* gene. Exons are indicated by gray boxes. The bent arrow indicates transcription start site. Bottom: schematic enlarged view of the insert in p-157BS, were a stop codon (linked to a *Bgl*II restriction site) was introduced in the 5th exon of the *psbA* gene (in the IV to V connecting loop) and associated with the recycling spectinomycin resistance cassette, schematically depicted by the boxed K with chevrons on either side (not to scale). The sequence coding for the fifth transmembrane helix is shown as a white box. Relevant restriction sites are as follows: P, *Pst*I; X, *Xba*I; A, *Apa*I; D, *Dra*III; S, *Sac*II; B, *Bst*EII; Bg, *Bgl*II; Ba, *Bam*HI. The position of the mutation with respect to the protein sequence is indicated in the schematic structure below.

(B) Expression of the truncated D1 polypeptide: *psbA* translation products were analyzed by pulse-labeling experiments in the wild type, in a transformed strain expressing the truncated *psbA* allele $D1_{Tr}$, in a representative tetrad progeny from the cross $D1_{Tr} \times$ *tba2*-F35, and in the parental strain *tba2*-F35. Asterisks mark tetrad members carrying the *tba2* mutation.

(C) Stability of the truncated protein assessed by pulse-chase experiments. Pulse-labeled wild type is shown for comparison. The positions of D2 and D1 and of the truncated D1 polypeptide are indicated.

spectinomycin cassette (Figure 9A), was introduced by transformation in the chloroplast genome of a wild-type strain of *C. reinhardtii*. Spectinomycin-resistant transformants, hereafter called $D1_{Tr}$, were screened by fluorescence for PSII deficiency.

The profile of chloroplast translation products in the $D1_{Tr}$ strain lacked D1 but showed an additional polypeptide with an apparent molecular mass of ~24 kD that we tentatively assigned to truncated D1 polypeptide (Figure 9B, lane $D1_{Tr}$). This was confirmed by crossing the $D1_{Tr}$ strain with the nuclear mutant strain *tba2*-F35, deficient for translation of the *psbA* mRNA. As expected from a Mendelian segregation of *tba2*, only two members of the resulting tetrads expressed the 24-kD truncated form of D1 (Figure 9B).

Pulse-chase experiments showed that truncated D1 was rapidly turning over, with a half-life of ~20 min (Figure 9C), as already observed for another, larger truncation of the D1 polypeptide (Preiss et al., 2001). Thus, this truncated and unstable version of the D1 protein cannot accumulate in the thylakoid membrane.

The $D1_{Tr}$ strain was then transformed with the 5′*psbA-petA* chimeric gene associated with the spectinomycin resistance cassette. Transformants, hereafter referred to as {$D1_{Tr}$, *bAf*}, were selected on TAP-Spec plates. They expressed the 5′*psbA*-driven cytochrome *f* but could not accumulate D1 even in the truncated form. In those strains, the expression of the chimeric gene was tremendously increased: it was overexpressed ~12 times when compared with a wild-type background (Figure 10A). A closer examination of its electrophoretic properties showed that instead of migrating as a single sharp band, as in a wild-type context, cytochrome *f* migrated as a doublet with the major band being of slightly faster mobility than regular cytochrome *f*. This corresponds to the behavior of apocytochrome *f* (Kuras et al., 1995) and indicates that such a high level of translation overwhelms the capability of the cytochrome *c* synthesis machinery required for apocytochrome to holocytochrome *f* conversion (Xie and Merchant, 1998). Apocytochrome *f* is highly unstable (Kuras et al., 1995), and indeed most of the newly synthesized polypeptide rapidly turned over in pulse-chase experiments (data not shown). As a result, the increased accumulation of cytochrome *f* in the $D1_{Tr}$ context, instead of being ~12, was only 4 times that observed in the wild type (Figure 10B).

Double mutants lacking both D2 and full-length D1 subunits were then generated by crossing the {$D1_{Tr}$, *bAf*} strains with the *mbd1* mutant. The expression of the 5′*psbA-petA* reporter gene, uniparentally transmitted to all tetrad members together with the truncated *psbA* allele, was compared in representative progeny

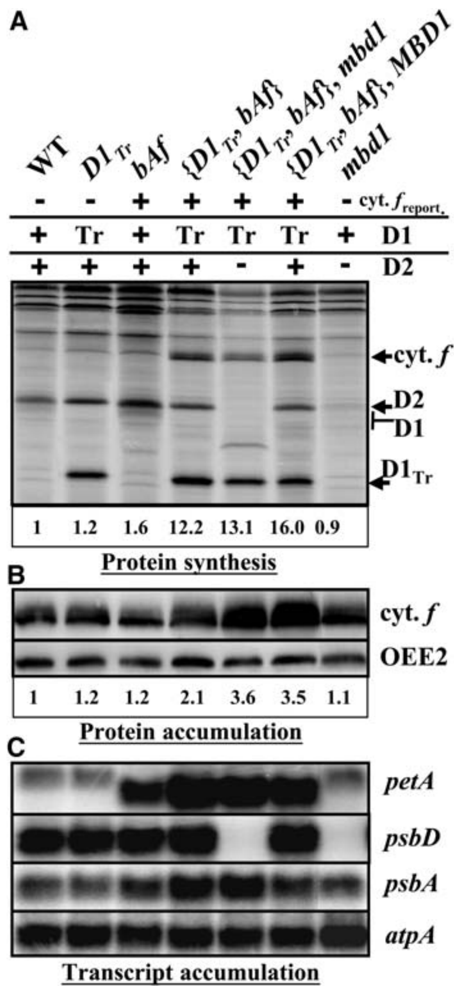

Figure 10. The 5′*psbA-petA* Reporter Gene Is Overtranslated in Strains Expressing Truncated D1.

(A) Chloroplast translation products in representative offspring from the cross {$D1_{Tr}$, *bAf*} × *mbd1*-nac2 in wild-type, *bAf*, $D1_{Tr}$, and parental strains. Lack of D2 synthesis indicates the *mbd1* progeny. PhosphorImager quantification of cytochrome *f* synthesis, relative to that observed in the wild type (after normalization to the level of synthesis of the β-subunit from the ATP synthase complex to correct for variations in the efficiency of ^{14}C incorporation) is indicated below the figure.
(B) Accumulation of cytochrome *f* and OEE2 (loading control) in these strains. Quantification of cytochrome *f* accumulation (relative to the wild type) is shown below the panel.
(C) Transcript accumulation for *petA*, *psbA*, *psbD*, and *atpB* (loading control) in the same strains.

that inherited the wild type or the mutated allele of *MDB1* and hence accumulated or not the *psbD* mRNA (Figure 10C). As can be seen in Figure 10A, the level of translation of truncated D1 is identical in a representative half-tetrad, irrespective of D2 synthesis capability. At variance with full-length D1, the synthesis of its truncated version did not depend on the presence of D2. Moreover, the increased translation (Figure 10A) and accumulation (Figure 10B) of the cytochrome *f* reporter remained similar regardless of the presence or absence of D2. Thus, when full-length D1 protein fails to accumulate in the thylakoid membrane, the translation of the *psbA*-driven cytochrome *f* (as well as that of truncated D1) is no longer repressed in the absence of D2, excluding the D2 positive control hypothesis. Rather, translation of the D1 CES subunit is autoregulated through a negative feedback mechanism mediated by its unassembled state.

Assessment of the Role of CES in the Recovery from Photoinhibition

During recovery from photoinhibition, the synthesis of D1 is enhanced (Kettunen et al., 1997). The underlying mechanism is triggered by the process of D1 degradation itself rather than by the high light conditions (Zer et al., 1994; Krieger-Liszkay et al., 2000). Apparently, a D1-less complex is a prerequisite for productive D1 translation, as would be expected for a CES protein. This is illustrated in Figure 11 by the strong increase in the rate of D1 synthesis in the wild type but not in strains defective for PSII assembly (Figures 11A, Δ*psbD*, or 11B, {Δ*psbB, bAf*}) upon a light intensity switch from 7 to 2000 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$. We thus investigated whether the CES process could account for the high turnover of D1 in high light conditions. The expression of the

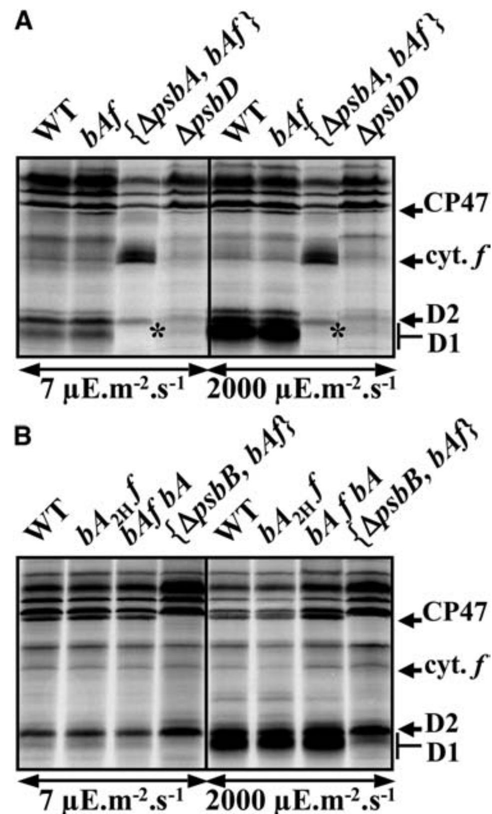

Figure 11. The CES Process Does Not Take Part in the Recovery from Photoinhibition.

Wild type, *bAf*, {Δ*psbA*, *bAf*} and Δ*psb*D **(A)** or wild type, $bA_{2H}f$, *bAfbA* and {Δ*psbB*, *bAf*} strains **(B)** were pulse labeled under normal light (left) or photoinhibition conditions (right). Asterisks indicate the neosynthesized form of D1 described in Figure 1.

5′*psbA*-*petA* reporter gene was compared in pulse-labeling experiments using *bAf* strains grown under dim light (7 $\mu E \cdot m^{-2} \cdot s^{-1}$) or placed in photoinhibition conditions (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$). Although, in the latter condition, the rate of D1 synthesis was strongly increased, that of chimeric cytochrome *f* did not increase significantly (Figure 11A, lane *bAf*, right part). This lack of stimulation of synthesis is not due to specific limitations in the expression of the chimeric gene since it can be very efficiently overexpressed by more than an order of magnitude in strain {Δ*psbA, bAf*} placed either in low or strong light conditions (Figure 11A).

Since the *psbA* 3′UTR may participate in the efficient translation of *psbA* mRNA (Katz and Danon, 2002), we constructed a 5′*psbA*-*petA*-3′*psbA* reporter gene. We also constructed a chimeric gene expressing cytochrome *f* translated under the control of the *psbA* 5′UTR, followed by the first 144 codons of *psbA* (i.e., up to the 3rd residue of the third transmembrane helix of D1). Both chimeras, yielding strains *bAfbA* and $bA_{2H}f$ after chloroplast transformation in *C. reinhardtii*, allowed cytochrome *f* expression that sustained phototrophic growth. Surprisingly enough, the introduction of >140 residues (and two transmembrane helices) upstream of the lumen targeting peptide of *petA* did not prevent protein translocation and cleavage of the targeting sequence. However, we failed to observe any significant increase in the expression of the two cytochrome *f* reporters when the transformed strains were subjected to photoinhibition (Figure 11B). Two hours after transfer to high light, cytochrome *f* expression was even reduced in the $bA_{2H}f$ strain, while it was only moderately stimulated (less than twofold, relative to the other chloroplast translation products) in the *bAfbA* strain. A weak stimulation of the 5′*psbA*-driven *petA* gene was also most often observed in the {*bAf*, Δ*psbB*} strain exposed to strong light. In the latter strain, no photoinhibition-induced stimulation of D1 synthesis occurs since it is defective for PSII assembly. We attribute this marginal increase to a limited light effect on the rate of D1 translation.

Thus, the expression of the very same reporter gene that is under CES control when assembly of PSII cores is prevented is not sensitive to photoinhibition conditions, leading to the conclusion that D1 expression involves widely different mechanisms during PSII biogenesis or repair.

DISCUSSION

The Synthesis of D1 and CP47 Is Regulated at the Level of Translation Initiation

The biogenesis of PSII involves a CES cascade, where the RC subunit D2 is required for high levels of translation of the RC subunit D1, whose presence is, in turn, necessary for high levels of translation of the core antenna subunit CP47. Whether other chloroplast-encoded PSII subunits participate to this cascade is not known yet. While the synthesis of CP43 appears independent of PSII assembly (de Vitry et al., 1989), the phosphoprotein PsbH is clearly a CES protein since its rate of translation is strongly reduced in the absence of the D1 subunit (K. Wostrikoff and Y. Choquet, unpublished results). It has been proposed that cytochrome b_{559} could also be part of this cascade, as it would be required for the efficient translation of *psbD* and *psbC* mRNAs (Morais et al., 1998). However, we were not able to observe significant alterations in the rate of translation of D2 and CP43 in a *psbE*-null mutant (L. Minai and Y. Choquet, unpublished results). Therefore, the status of cytochrome b_{559} in the CES cascade is still pending. Further studies will be required to determine the CES behavior of the other PSII subunits.

To assess the importance of translation initiation in the decrease of D1 translation in absence of D2, we used the *petA* coding sequence, encoding the endogenous cytochrome *f* as a reporter gene. Although cytochrome *f* is by itself a CES subunit in the course of cytochrome b_6f biogenesis, we have previously demonstrated that the regulation of its synthesis requires the *petA* 5′UTR (Choquet et al., 1998), which is absent in the chimeric constructs. Thus, when expressed from another 5′UTR, cytochrome *f* is a faithful reporter protein (Wostrikoff et al., 2004). Here, we showed that the *psbA* 5′UTR (together with the first 60 nucleotides just downstream of the *psbA* initiation codon) was sufficient to confer a D2-dependent rate of synthesis to the *petA* and *aadA* reporter genes. Their lower expression in the absence of D2 was not accompanied by decreased mRNA levels, excluding significant contribution of a pretranslational step to the D2-dependent regulation of *psbA* expression. The specificity of D2 in this process was confirmed by the preserved expression of the 5′*psbA*-*petA* chimera in other strains deficient for PSII assembly, such as {Δ*psbB, bAf*}. The CES control of *psbA* expression occurs at a step earlier than translation elongation or cotranslational degradation of the nascent D1 polypeptide, since both should depend on the *psbA* coding sequence rather than on the *psbA* 5′UTR. Either an activation step prior to initiation or translation initiation itself CES regulates D1 translation. This regulatory mechanism is in contrast with the proposed role of D2 in driving efficient translation of D1, which occurs at the level of translation elongation (Zhang et al., 1999): nascent D1 polypeptides, more specifically the 17- and 25-kD translation intermediates that correspond to the escape of the second and fourth transmembrane helices of D1 from the ribosome tunnel, interact cotranslationally with D2, probably through a transient disulfide bridge. When this interaction is impaired, in an in organello translation system, these intermediates are stabilized, while formation of full-length D1 is decreased (Zhang et al., 2000). Several observations argue for independent participation of translation initiation and translation elongation in the fine-tuning of D1 expression. Besides recovery from photoinhibition, most likely controlled at the level of translation elongation (see below), the elongation, but not the initiation of D1 translation, is altered in *C. reinhardtii* mutant strains *mf1* and *mf2*, deficient in the synthesis of Δ^3-*trans* hexadecenoic-phosphatidylglycerol (Pineau et al., 2004).

Similarly, the *psbB* 5′UTR is sufficient to confer a D1-dependent regulation to the translation of the cytochrome *f* reporter, suggesting a regulation of translation initiation, as confirmed by the marked decrease in polysome loading of *psbB* mRNA in strains deficient for the expression of D1.

Translation of D1 and CP47 Is Autoregulated

Two possibilities might explain the decreased translation of D1 and CP47 in the absence of their assembly partners. The latter

could behave as dominant subunits (D2 for D1 or D1 for CP47) that transactivate translation of the CES subunits. We exclude this possibility since the expression of 5′CES-driven *petA* genes remains high in the combined absence of the dominant and CES subunits. This behavior fully supports a negative feedback inhibition on translation of their own mRNA by the CES subunits D1 or CP47 when unassembled because of the absence of their assembly partners.

An efficient translational autoregulation requires that unassembled D1 or CP47 accumulate to some extent in the absence of their assembly partners. Indeed, D1 and CP47 remained stable, although synthesized in markedly reduced amounts, in the absence of D2 and D1, respectively (de Vitry et al., 1989). Paradoxically, they are normally translated but rapidly degraded in mutants lacking expression of CP43 (or of CP47 for polypeptide D1) (de Vitry et al., 1989). Most likely, assembly of the CES subunits in their target complex induces a protease-sensitive conformation that allows their proteolysis if PSII assembly cannot proceed further. Similarly, cytochrome *f*, a stable protein when unassembled (Kuras and Wollman, 1994; Choquet et al., 2003), becomes susceptible to proteolytic degradation upon nitrogen starvation once assembled within cytochrome b_6f complexes (Bulte and Wollman, 1992).

As we showed here for PSII and in previous studies of the biogenesis of PSI and cytochrome b_6f complexes, the 5′CES-driven reporter genes were even more translated in the absence of the CES proteins than in a wild-type genetic context, regardless of the presence of their assembly partners. This argues for the presence of a fraction of unassembled CES subunits in wild-type chloroplasts that downregulates their own expression in order to tune their production to the availability of their assembly partners. This feedback inhibition is released when the neo-synthesized CES subunits assemble with their partners (i.e., only when all upstream steps in the sequential assembly pathway of PSII, including an efficient expression of those assembly partners, have been completed). It is thus not a surprise that the CES hierarchy reflects the order of polypeptide assembly (Figure 12), the core antenna subunit CP47 being made only when D1 has successfully assembled with D2.

That a similar process is at work in the biogenesis of PSII in higher plant chloroplasts is supported by the phenotype of the *vir*[115] mutant of barley (*Hordeum vulgare*) that is primarily affected, in a developmentally regulated manner, in the translation of D1 (Kim et al., 1994) but also in the expression of CP47 (Gamble and Mullet, 1989). The molecular mechanism of this translational coupling between these two PSII subunits in higher plants, however, remains to be studied. By contrast, there is no evidence for a CES process in the expression of PSII subunits in cyanobacteria, where mutations preventing PSII assembly increase the proteolytic disposal of the remaining subunits, with no apparent effect on their rate of transcription or translation (Yu and Vermaas, 1990). The biogenesis of the multimeric photosynthetic proteins obeys widely different rules in photosynthetic prokaryotes and in chloroplasts (Wollman et al., 1999).

Ternary Effectors Involved in the CES Process?

The true nature of the interaction between unassembled CES proteins and the 5′UTR of their mRNAs is not known. However, considering the lack of CES process in cyanobacteria but the high conservation of D1 and CP47 sequences from cyanobacteria to higher plants, it seems unlikely that chloroplast CES subunits, D1 and apoCP47, have evolved a specific repressor domain capable of sequence-specific interactions with the 5′UTR of their mRNAs. We note that an active RNA binding domain has recently been proposed for another conserved CES protein of the photosynthetic apparatus, the large subunit of

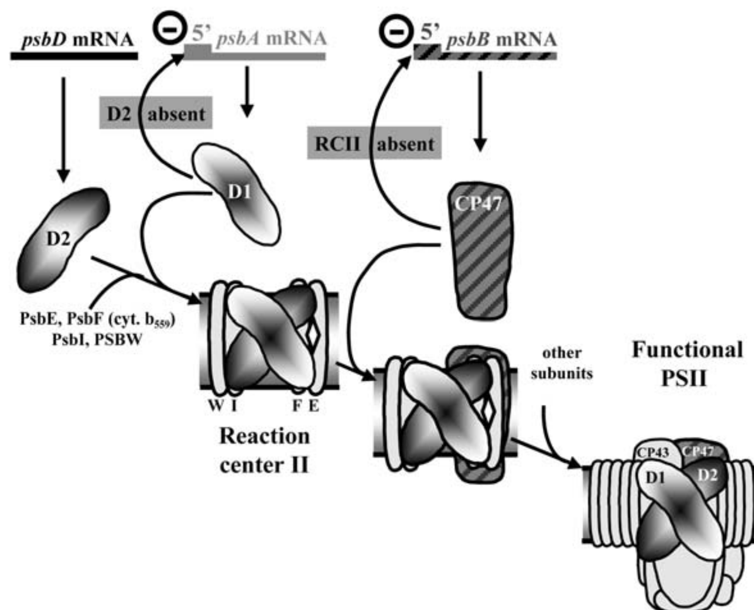

Figure 12. Model of the CES Cascade Involved in PSII Biogenesis.

Table 2. Oligonucleotides Used for This Work

Name of Primer	Sequence (5′/3′)	Features[a]
bA_{prom}	CGCATCGATGGATCCTGCCACTGACGTCCTATTTTAATACTCC	ClaI, BamHI
bA_{60}	CGCGGATCCATGGTCCACTCACAAAAACGAGCCCATAGG	BamHI, NcoI
bA_{2H}	CGCCCA TGGCAGCGATCCATGGACGC	NcoI
$bA_{5'}$	GGCAACCTGCAGCGAAGCTAGGG	**PstI**
$bASt_{Dir}$	GGTCGTCTATAGATCTTCCAATACGCTTCTTTCA	**Stop**, **BglII**
$bASt_{Rev}$	GTATTGGAAGATCTAGAGACGACCAAAGTAACCCA	**BglII**, **Stop**
bA_{Dir}	TTCCCTCTAGACTTAGCTTCAACT	**XbaI**
bA_{Rev}	AGTTGAAGCTAAGTCTAGAGGGAA	
bA_{SacII}	ACCACCAGGGCCCAGCCCGCGGCTGGCTCCGCAGTATTAACATCCT	**ApaI**, **SacII**
bA_{ApaI}	GGAGCCAGCCGCGGGCTGGGCCCTGGTGGTACCACTGCCTCC	**SacII**, **ApaI**
$bA_{3'}$	TGTAAGCCCAGGCTGTCACGAAGTG	**DraIII**
cA_{Cod}	GTTGTTGTAGACAAAATCCCAGCAGGTCCTG	**AccI**
$cASt_{Rev}$	AAAAAAAACTGCAGTTAGAAGTTCATTTCTGCTAATTGAACT	**PstI**, **Stop**
$bA3'_{Dir}$	TCAATTAGCAGAAATGAACTTCTAACTGCAGTTTTTTTTTAAACTAAAATAA	**PstI**, **Stop**
$bA3'_{Rev}$	GCGGATATCGGCAGTTGGCAGGATATTTATATA	**EcoRV**
bB_{prom}	CGCATCGATCTGCAGTCGTTTACAGGCCTTAACATATGGAT	**ClaI**, **PstI**
bB_{ATG}	CGCGGATCCATGGCCATAAAAAATTTTTTACTGATTTTTTTACTTAATTATT	BamHI, NcoI
$bB5'$	ATCTCTGTGCACTTAATGCACACAGC	**ApaLI**
$bBSt_{Dir}$	GTCCTGGTTAAGCTTTGGGTTTCAGACCCTTACGGT	**HindIII**, **Stop**
$bBSt_{Rev}$	TCTGAAACCCAAAGCTTAACCAGGACCAAAAACACC AGT	**HindIII**, **Stop**
$bB3'$	GAAAGCTGCCCAGAATACTGCTG	

[a] Stop codons and/or restriction sites (bold or underlined) introduced into the sequence of the primers for cloning or restriction fragment length polymorphism (RFLP) analysis purposes.

ribulose-1,5-bis-phosphate carboxylase/oxygenase. However, its RNA binding property was not sequence specific (Yosef et al., 2004), a requirement for its contribution to the CES process. Therefore, as discussed for the biogenesis of other photosynthetic proteins (Choquet et al., 2003; Wostrikoff et al., 2004), this sequence specificity would rather be born by a ternary translational activator capable of competitive binding to both the unassembled CES subunit and the 5′UTR of its target mRNA. This model sets translational efficiency to the rate of assembly of a CES subunit within a (sub)complex along the biogenesis pathway of a particular photosynthetic protein. Such a role has recently been shown for the COX14p/Mss51p proteins, involved in the control of the expression of the CES protein COXIp in mitochondria of yeast (Perez-Martinez et al., 2003; Barrientos et al., 2004). Gene-specific translation activators are major features of organelle gene expression (Grivell, 1995; Zerges, 2000). For the CES protein D1, translational activators have been identified through genetic analysis (Girard-Bascou et al., 1992; Somanchi et al., 2005). Additional proteins involved in the initiation of *psbA* mRNA translation have been characterized biochemically, although some of them may be less specific (reviewed in Bruick and Mayfield, 1999).

The CES Process Plays No Role in the Recovery from Photoinhibition

The PSII repair mechanism following photoinhibition involves an increase in the rate of D1 translation, apparently triggered by the degradation of photodamaged D1 and by the formation of D1-less complexes (Adir et al., 1990; Bailey et al., 2002). Such a mechanism is consistent with an autoregulation of D1 synthesis. This prompted us to investigate the contribution of the CES process to the recovery from photoinhibition. The use of reporter constructs carrying variable parts of the *psbA* gene fused in frame with the coding region of cytochrome *f* did not allow us to observe any significant stimulation in the expression of the reporter protein upon photoinhibition, although this construct can be highly overexpressed in other conditions. This seems conflicting with the 100-fold increase in the expression of a 5′*psbA-uidA* reporter gene, when tobacco (*Nicotiana tabacum*) etiolated cells are transferred to light (Staub and Maliga, 1994). However, biogenesis of PSII and translation of *psbA* mRNA are arrested in tobacco etioplasts, whereas *C. reinhardtii* has retained the capability to develop fully differentiated chloroplast and to synthesize D1 in the dark.

Thus, translation initiation, although being a main regulatory step in *psbA* gene expression during PSII biogenesis, does not play a significant role in the rapid D1 synthesis during recovery from photoinhibition. Rather, translation elongation represents the key control point of *psbA* gene expression during recovery from photoinhibition: under high light stalled *psbA* polysomes are associated with the thylakoid membrane. The presence of D1-less PSII complexes would release this translational arrest (Kettunen et al., 1997).

It is tempting to attribute the two widely different mechanisms for the control of *psbA* gene expression to different spatial localizations. During the recovery from photoinhibition, the synthesis of D1 takes place in stromal thylakoids, as required for cotranslational interactions with D2 and assembly within D1-less PSII complexes (Kettunen et al., 1997). By contrast, PSII

biogenesis may develop in a specialized membrane compartment, the low density membranes, biochemically related to the chloroplast inner envelope (Zerges and Rochaix, 1998). Indeed many nuclear encoded factors, required for posttranscriptional steps of chloroplast gene expression, including RB47 involved in *psbA* translation initiation, are associated with this low density membrane fraction but not with the bulk of thylakoid membrane (Zerges and Rochaix, 1998). Similarly, in cyanobacteria, de novo biogenesis of PSII complexes is likely to occur in plasma membranes (Zak et al., 2001), while repair of photodamaged complexes takes place in the thylakoid membrane. Most interestingly, an RNA binding protein with a high affinity for the *psbA* 5′UTR (RB63) has been identified by a biochemical approach in *C. reinhardtii* (Ossenbuhl et al., 2002). This protein is associated with thylakoids but not with the low density membrane fraction and could be specifically required for *psbA* expression during the high turnover of D1.

Our study demonstrates that the molecular mechanism for the CES process in PSII shares similar features to those operating in the biogenesis of cytochrome b_6f and PSI complexes (Choquet et al., 1998; Wostrikoff et al., 2004). This assembly-dependent autoregulation of translation is then central to the biogenesis of photosynthetic proteins in the chloroplast of *Chlamydomonas*. Given the similar features of the biogenesis of cytochrome oxydase in yeast mitochondria, it is likely that the CES process is a key signature of protein assembly in situations in which interactions between two genetic intracellular compartments prevail (i.e., in the biogenesis of organellar protein complexes).

METHODS

Strains and Growth Conditions

Wild-type, mutant, and transformed strains of *Chlamydomonas reinhardtii* were grown in TAP medium, pH 7.2 (Harris, 1989), under continuous low light (5 to 10 $\mu E \cdot m^{-2} \cdot s^{-1}$). *mt*− parental strains *mbd1*-nac2 (Kuchka et al., 1989), *tba2*-F35 (Girard-Bascou et al., 1992), and *mbd1*-222E (Monod et al., 1992; Vaistij et al., 2000) were used for crosses performed as described by Harris (1989). The Fud7 strain (*mt*+) (Bennoun et al., 1986) is referred to as Δ*psbA*.

Constructs and Nucleic Acid Manipulations

Standard nucleic acids manipulations were performed according to Sambrook et al. (1989).

DNA Constructs

Construction of psbB and psbD Deletions

A plasmid carrying a partial deletion of the *psbB* gene was created by ligation of vector p38.NcoI (Vaistij et al., 2000), digested with *Nco*I and *Pvu*I, with the 2.95-kb SacI-*Kpn*I fragment from plasmid pKS-aadA-483 (Fischer et al., 1996), both fragments being treated with T4 DNA polymerase to generate blunt ends. In the resulting plasmid, pΔ*psbB*::K_r, the first 614 bp of the *psbB* coding region were replaced by the recycling *aadA* cassette, in a reverse orientation with respect to *psbB*.

To create a vector carrying a deletion of the *psbD* gene, a 5827-bp *Hin*dIII-*Pst*I fragment from the chloroplast genome, containing the whole *psbD* gene (Erickson et al., 1986), was cloned into the pUC9 vector to yield plasmid pH3P. This vector, cut with *Bst*1107 and *Pml*I to remove most *psbD* coding sequence, was ligated to the 2.95-kb recycling *aadA* cassette, in reverse orientation with respect to *psbD* to form the Δ*psbD*::K_r plasmid.

Construction of Chimeric Genes

Plasmid P-157, obtained from the Chlamydomonas Genetic Center (www.biology.duke.edu/chlamy/), contains a 10-kb *Bam*HI-*Bgl*II fragment of the chloroplast genome from strain DCMU4 (Erickson et al., 1984) subcloned into pUC18 vector. It was used as a template with oligonucleotide primers bA_{prom} and bA_{60} (Table 2) in PCR reaction. The 324-bp amplicon contained the promoter and 5′UTRs of the *psbA* gene, followed by the first 20 codons of *psbA*, as well as two restriction sites, *Cla*I, upstream of the *psbA* promoter, and *Nco*I, downstream of *psbA* coding sequences (Table 2, 3rd column).

Similarly, plasmid pBA158 bearing an intronless *psbA* gene (Sugiura et al., 1998), kindly provided by A.R. Crofts, was used as template with primers bA_{Prom} and bA_{2H} in PCR reactions. The 716-bp amplicon contained the *psbA* promoter and 5′UTRs, followed by the first 144 codons of *psbA* (up to the 3rd residue from the 3rd transmembrane helix of D1).

A 356-bp DNA fragment carrying the promoter and 5′UTRs of the *psbB* gene, but no *psbB* coding sequence, was amplified by PCR with primers bB_{prom} and bB_{ATG} from the template vector p38.NcoI.

The three fragments, digested with *Cla*I and *Nco*I, were ligated into vector pAFFF (Choquet et al., 1998), digested with the same enzymes, to yield plasmids p*bAf*, p$bA_{2H}f$, or p*bBf*, in which the *Eco*RV-*Sma*I 1.9-kb *aadA* cassette (Goldschmidt-Clermont, 1991) was inserted, in reverse orientation with respect to the *petA* gene, at the unique restriction site *Hin*cII to form plasmid pK*bAf*, pK$bA_{2H}f$, or pK*bBf*.

The 324-bp amplicon (5′*psbA*), digested by *Cla*I and *Nco*I, was also cloned into the corresponding sites of vector PUC-ATPX-AAD (Goldschmidt-Clermont, 1991) to yield plasmid p*bA*K, from which the 5′*psbA*-*aadA* chimeric gene was removed by digestion with *Sma*I and *Eco*RV. It was then inserted downstream of the *petA* gene, at the *Eco*RV site from plasmid pWF (Kuras and Wollman, 1994), in direct orientation with respect to the *petA* gene to create plasmid p*fbA*K.

Generation of Truncated Alleles of psbA and psbB Genes

psbA. Plasmid p-157, cut with *Bst*EII and *Sph*I, was treated with T4 DNA polymerase and religated on itself to yield plasmid p-157BS. Codon I_{259}, in the fifth exon of *psbA*, was substituted by a stop codon associated with the restriction site *Bgl*II for RFLP analysis by a two-step PCR procedure (Higuchi, 1990). Two pairs of oligonucleotides (*bA*5′ and $bASt_{Rev}$ on one hand and $bASt_{Dir}$ and bA_{Rev} on the other hand) were used to amplify two partially overlapping fragments from template plasmid p-157BS. The purified fragments were mixed and used as templates in another PCR reaction with the external primers *bA*5′ and *bA*Rev. The final amplicon, carrying the mutation, was digested with *Pst*I and *Xba*I, two restriction sites, upstream and downstream of the introduced mutation, and cloned into plasmid p-157BS digested with the same enzymes to create plasmid pbA_{259}St.

Using *bA*Dir/*bA*apaI and *bA*SacII/*bA*3′ oligonucleotide pairs, the same two-step strategy allowed amplification from the template vector P-157BS of a 1013-bp DNA fragment corresponding to regions downstream of the *psbA* coding sequence where two restriction sites, *Sac*II and *Apa*I, were introduced after the *psbA* 3′UTR. This fragment, digested with *Xba*I and *Dra*III, was cloned into the corresponding sites of plasmid pbA_{259}St to yield plasmid pbA_{259}StR. The recycling cassette excised with *Apa*I and *Sac*II from plasmid pKS-aadA-483 was then inserted into these sites within plasmid pbA_{259}StR to create plasmid p$bA_{259}StK_r$.

psbB. Similarly, a stop codon, associated with a *Hin*dIII restriction site for RFLP analysis, was introduced in the *psbB* coding sequence, 495

nucleotides after the initiation codon, using the two oligonucleotide pairs *bB*5′/*bB*St_{rev} and *bB*St_{dir}/*bB*3′ and the template plasmid p38.NcoI. The resulting 742-bp amplicon was digested with *Apa*LI and *Pvu*II, two unique restriction sites within the coding sequence of *psbB* and ligated with the 5266-bp fragment obtained from vector p38.NcoI by *Pvu*II digestion and *Apa*LI partial digestion to yield plasmid p*bB*$_{164}$St. The recycling *aadA* cassette was then introduced in the unique *Stu*I site of this plasmid, in the same orientation as *psbB*, to create plasmid pK_r*bB*$_{164}$St.

Creation of a 5′psbA-petA-3′psbA Chimeric Gene

Partially overlapping fragments were obtained using the oligonucleotide pairs cA_{Cod}/$cASt_{Rev}$ with vector pWF as template and $bA3'_{Dir}$/$bA3'_{Rev}$ with the template vector P-157. The resulting 269- and 349-bp amplicons were mixed together with primers cA_{Cod} and $bA3'_{Rev}$ to yield a 579-bp PCR product that contains the C-terminal part of the *petA* gene, fused to the *psbA* 3′UTR. This amplicon, digested with *Acc*I and *Eco*RV, was cloned into the pK*bAf* vector digested with the same enzymes to yield plasmid p*bAfbA*.

All constructs were sequenced to assess the presence of appropriate mutations before transformation of *C. reinhardtii*. RNA gel blot analyses were performed as described by Wostrikoff et al. (2004).

Analysis of Polysomes

The polysome purification protocol was adapted from Rott et al. (1998).

Preparation of Polysome Fractions

One hundred milliliters of cell culture (2×10^6 cell·mL^{-1}), preincubated for 10 min with chloramphenicol (CAP; 100 μg·mL^{-1}), were harvested by gentle centrifugation. Cells were resuspended in 2.5 mL of resuspension buffer (0.2 M Tris-HCl, pH 8.0, 0.2 M KCl, 25 mM $MgCl_2$, 25 mM EGTA, 0.2 M sucrose, 1% Triton X-100, 2% polyoxyethylene-13-tridecylehter, 50 mM β-mercaptoethanol, 0.5 mg·mL^{-1} heparin, and 100 μg·mL^{-1} CAP), broken with a French press (13 kg·cm^{-2}), and centrifuged at 1000*g* for 5 min to pellet unbroken cells and large cell debris. Supernatant, after addition of deoxycholate (0.5% final), was kept on ice for 5 min and centrifuged at 10,000*g* for 15 min. Two milliliters of this new supernatant were loaded on 9 mL of continuous sucrose gradients poured from bottom to top by continuous mixing of 4 mL of a 15% sucrose (w/v) solution (40 mM Tris-HCl, pH 8.0, 20 mM KCl, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.5 mg·mL^{-1} heparin, and 100 μg·mL^{-1} CAP) with 5 mL of a 55% sucrose (w/v) solution. Gradients were centrifuged at 38,000 rpm in a Beckmann SW41 rotor for 2 h and 30 min.

Ten fractions of 1.1 mL were collected, out of which a 100-μL aliquot was removed to determine the sucrose percentage by refractometry. To assess the presence of pelleted material, the bottom of the tube was rinsed out after collection of the fractions with 200 μL of 5 mM EDTA, 0.1% SDS and then treated as the other fractions (fraction P). For EDTA treatment, $MgCl_2$ was omitted in the resuspension buffer, and 20 mM EDTA was added to the deoxycholate supernatant prior to loading onto the sucrose gradients that contained 1 mM EDTA instead of $MgCl_2$.

RNA Preparation and Analysis

From these fractions, nucleic acids were precipitated overnight at −20°C by addition of 400 μL of 2 M KCl and 3 mL absolute ethanol and centrifuged for 15 min at 16,200*g*. Pellets, resuspended in 400 μL of 5 mM EDTA, 0.1% SDS, were extracted once with 400 μL of phenol/chloroform/IAA and ethanol precipitated. After centrifugation, pellets were washed with 70% ethanol, dried under vacuum for 5 min, resuspended in 25 μL of 5 mM EDTA, 0.1% SDS, and stored at −80°C. Five microliters of these RNA solutions were analyzed by RNA gel blot experiments.

Identification of the ribosome populations present in the sucrose gradient fractions resulted from the analysis of the sedimentation properties of cell extracts treated with EDTA that dissociates ribosome subunits (Figure 3, WT panel).

Transformation Experiments

Cells were transformed by tungsten particle bombardment (Boynton and Gillham, 1993) as described by Kuras and Wollman (1994). Transformants were selected on Tap-Spec (100 μg·mL^{-1}) under low light (5 to 6 μE·m^{-2}·s^{-1}) and subcloned on selective medium to reach homoplasmy. Proper insertion of transforming DNA and homoplasmy were checked by RFLP analysis of specific PCR amplification products. At least three independent transformants were analyzed for each transformation.

Protein Analysis

Pulse and pulse-chase experiments, protein electrophoresis, and immunoblotting were performed according to Kuras and Wollman (1994). For pulse labeling, we used illumination of 50 μE·m^{-2}·s^{-1}, except for experiments in Figures 2 and 9, where an illumination of 200 μE·m^{-2}·s^{-1} increased ^{14}C incorporation for a better visualization of the decreased level of cytochrome *f* synthesis. Cells were photoinhibited by transfer for 2 h under 2000 μE·m^{-2}·s^{-1} illumination before pulse labeling in the same conditions (Figure 11). Cell extracts were analyzed on 8% (Figure 1) or 12 to 18% acrylamide SDS-PAGE in the presence of 8 M urea after loading on equal chlorophyll basis. All pulse-labeling experiments were repeated twice and performed on three independent transformed strains. Quantification of rates of translation was done on PhosphorImager scans of pulse-labeled proteins from dried gels using the program ImageQuant (Molecular Dynamics). Values for the level of synthesis of cytochrome *f* (either endogenous or chimeric) were corrected for background by measuring in each lane an empty window of the same area below cytochrome *f*. Synthesis of other polypeptides (including the unrelated β-subunit from the ATP synthase complex) was similarly quantified. The rates of translation of cytochrome *f* reporter were normalized to that of the β-subunit to correct for variations in the uptake and incorporation of radiolabeled acetate in the various strains. Quantification of the accumulation of cytochrome *f*, determined from PhosphorImager scans of immunoblots revealed with ^{125}I protein A, was normalized to that of the OEE2 protein from the PSII complex. The accumulation of this protein is not affected by mutation preventing the assembly of the PSII core (de Vitry et al., 1989).

ACKNOWLEDGMENTS

We thank A.R. Crofts for his kind gift of the pBA158 plasmid and D. Drapier and S. Bujaldon for help during polysome analysis and critical reading of the manuscript. This work was supported by Unité Mixte de Recherche, Centre National de la Recherche Scientifique, Université Pierre et Marie Curie 7141. L.M. was supported by a Marie Curie individual fellowship from the European Community (HPMF CT 2001 01376) and by Chateaubriand fellowship 386409K. K.W. was Attaché Temporaire d'Enseignement et de Recherche at Collège de France.

Received September 6, 2005; revised November 4, 2005; accepted November 14, 2005; published December 9, 2005.

REFERENCES

Adir, N., Shochat, S., and Ohad, I. (1990). Light-dependent D1 protein synthesis and translocation is regulated by reaction center II. Reaction center II serves as an acceptor for the D1 precursor. J. Biol. Chem. **265,** 12563–12568.

Baena-Gonzalez, E., and Aro, E.M. (2002). Biogenesis, assembly and

turnover of photosystem II units. Philos. Trans. R. Soc. Lond. B Biol. Sci. **357,** 1451–1459.

Bailey, S., Thompson, E., Nixon, P.J., Horton, P., Mullineaux, C.W., Robinson, C., and Mann, N.H. (2002). A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo. J. Biol. Chem. **277,** 2006–2011.

Barrientos, A., Zambrano, A., and Tzagoloff, A. (2004). Mss51p and Cox14p jointly regulate mitochondrial Cox1p expression in *Saccharomyces cerevisiae.* EMBO J. **23,** 3472–3482.

Bennoun, P., Spierer-Herz, M., Erickson, J., Girard-Bascou, J., Pierre, Y., Delosme, M., and Rochaix, J.-D. (1986). Characterization of photosystem II mutants of *Chlamydomonas reinhardtii* lacking the PSBA gene. Plant Mol. Biol. **6,** 151–160.

Boynton, J.E., and Gillham, N.W. (1993). Chloroplast transformation in Chlamydomonas. Methods Enzymol. **217,** 510–536.

Bruick, R.K., and Mayfield, S.P. (1999). Light-activated translation of chloroplast mRNAs. Trends Plant Sci. **4,** 190–195.

Bulte, L., and Wollman, F.-A. (1992). Evidence for a selective destabilization of an integral membrane protein, the cytochrome b_6f complex, during gametogenesis in *Chlamydomonas reinhardtii.* Eur. J. Biochem. **204,** 327–336.

Choquet, Y., Stern, D.B., Wostrikoff, K., Kuras, R., Girard-Bascou, J., and Wollman, F.-A. (1998). Translation of cytochrome *f* is autoregulated through the 5′ untranslated region of *petA* mRNA in Chlamydomonas chloroplasts. Proc. Natl. Acad. Sci. USA **95,** 4380–4385.

Choquet, Y., and Vallon, O. (2000). Synthesis, assembly and degradation of thylakoid membrane proteins. Biochimie **82,** 615–634.

Choquet, Y., and Wollman, F.-A. (2002). Translational regulations as specific traits of chloroplast gene expression. FEBS Lett. **529,** 39–42.

Choquet, Y., Zito, F., Wostrikoff, K., and Wollman, F.-A. (2003). Cytochrome *f* translation in Chlamydomonas chloroplast is autoregulated by its carboxyl-terminal domain. Plant Cell **15,** 1443–1454.

de Vitry, C., Olive, J., Drapier, D., Recouvreur, M., and Wollman, F.-A. (1989). Posttranslational events leading to the assembly of photosystem II protein complex: A study using photosynthesis mutants from *Chlamydomonas reinhardtii.* J. Cell Biol. **109,** 991–1006.

Erickson, J.M., Rahire, M., Bennoun, P., Delepelaire, P., Diner, B.A., and Rochaix, J.-D. (1984). Herbicide resistance in *Chlamydomonas reinhardtii* results from a mutation in the chloroplast gene for the 32-kilodalton protein of photosystem II. Proc. Natl. Acad. Sci. USA **81,** 3617–3621.

Erickson, J.M., Rahire, M., Malnoe, P., Girard-Bascou, J., Pierre, Y., Bennoun, P., and Rochaix, J.-D. (1986). Lack of the D2 protein in a *Chlamydomonas reinhardtii psbD* mutant affects photosystem II stability and D1 expression. EMBO J. **5,** 1745–1754.

Fischer, N., Stampacchia, O., Redding, K., and Rochaix, J.-D. (1996). Selectable marker recycling in the chloroplast. Mol. Gen. Genet. **251,** 373–380.

Gamble, P.E., and Mullet, J.E. (1989). Translation and stability of proteins encoded by the plastid *psbA* and *psbB* genes are regulated by a nuclear gene during light-induced chloroplast development in barley. J. Biol. Chem. **264,** 7236–7243.

Girard-Bascou, J., Pierre, Y., and Drapier, D. (1992). A nuclear mutation affects the synthesis of the chloroplast *psbA* gene product in *Chlamydomonas reinhardtii.* Curr. Genet. **22,** 47–52.

Goldschmidt-Clermont, M. (1991). Transgenic expression of aminoglycoside adenine transferase in the chloroplast: A selectable marker of site-directed transformation of Chlamydomonas. Nucleic Acids Res. **19,** 4083–4089.

Grivell, L.A. (1995). Nucleo-mitochondrial interactions in mitochondrial gene expression. Crit. Rev. Biochem. Mol. Biol. **30,** 121–164.

Harris, E.H. (1989). The Chlamydomonas Source Book: A Comprehensive Guide to Biology and Laboratory Use. (San Diego, CA: Academic Press).

Higuchi, R. (1990). Recombinant PCR. In PCR Protocols: A Guide to Methods and Applications, M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds (London, New York: Academic Press), pp. 177–183.

Jensen, K.H., Herrin, D.L., Plumley, F.G., and Schmidt, G.W. (1986). Biogenesis of photosystem II complexes: Transcriptional, translational, and posttranslational regulation. J. Cell Biol. **103,** 1315–1325.

Katz, Y.S., and Danon, A. (2002). The 3′-untranslated region of chloroplast *psbA* mRNA stabilizes binding of regulatory proteins to the leader of the message. J. Biol. Chem. **277,** 18665–18669.

Kettunen, R., Pursiheimo, S., Rintamaki, E., van Wijk, K.J., and Aro, E.M. (1997). Transcriptional and translational adjustments of *psbA* gene expression in mature chloroplasts during photoinhibition and subsequent repair of photosystem II. Eur. J. Biochem. **247,** 441–448.

Kim, J., Klein, P.G., and Mullet, J.E. (1994). Vir-115 gene product is required to stabilize D1 translation intermediates in chloroplasts. Plant Mol. Biol. **25,** 459–467.

Krieger-Liszkay, A., Kienzler, K., and Johnson, G.N. (2000). Inhibition of electron transport at the cytochrome b_6f complex protects photosystem II from photoinhibition. FEBS Lett. **486,** 191–194.

Kuchka, M.R., Goldschmidt-Clermont, M., van Dillewijn, J., and Rochaix, J.-D. (1989). Mutation at the Chlamydomonas nuclear *NAC2* locus specifically affects stability of the chloroplast *psbD* transcript encoding polypeptide D2 of PS II. Cell **58,** 869–876.

Kulkarni, R.D., and Golden, S.S. (1997). mRNA stability is regulated by a coding-region element and the unique 5′ untranslated leader sequences of the three Synechococcus *psbA* transcripts. Mol. Microbiol. **24,** 1131–1142.

Kuras, R., Buschlen, S., and Wollman, F.-A. (1995). Maturation of pre-apocytochrome *f* in vivo. A site-directed mutagenesis study in *Chlamydomonas reinhardtii.* J. Biol. Chem. **270,** 27797–27803.

Kuras, R., and Wollman, F.-A. (1994). The assembly of cytochrome b_6f complexes: An approach using genetic transformation of the green alga *Chlamydomonas reinhardtii.* EMBO J. **13,** 1019–1027.

Monod, C., Goldschmidt-Clermont, M., and Rochaix, J.-D. (1992). Accumulation of chloroplast *psbB* RNA requires a nuclear factor in *Chlamydomonas reinhardtii.* Mol. Gen. Genet. **231,** 449–459.

Morais, F., Barber, J., and Nixon, P.J. (1998). The chloroplast-encoded alpha subunit of cytochrome b_{559} is required for assembly of the photosystem two complex in both the light and the dark in *Chlamydomonas reinhardtii.* J. Biol. Chem. **273,** 29315–29320.

Müller, B., and Eichacker, L.A. (1999). Assembly of the D1 precursor in monomeric photosystem II reaction center precomplexes precedes chlorophyll *a*-triggered accumulation of reaction center II in barley etioplasts. Plant Cell **11,** 2365–2378.

Nanba, O., and Satoh, K. (1987). Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome *b*-559. Proc. Natl. Acad. Sci. USA **84,** 109–112.

Ohad, I., Keren, N., Zer, H., Gong, H., Mor, T.S., Gal, A., Tal, S., and Domovich, Y. (1994). Light-induced degradation of the photosystem II reaction centre D1 protein *in vivo*: An integrative approach. In Photoinhibition from the Molecule to the Field, N. Baker, ed (Oxford, UK: Bios Scientific Publishers), pp. 161–177.

Ossenbuhl, F., Hartmann, K., and Nickelsen, J. (2002). A chloroplast RNA binding protein from stromal thylakoid membranes specifically binds to the 5′ untranslated region of the *psbA* mRNA. Eur. J. Biochem. **269,** 3912–3919.

Perez-Martinez, X., Broadley, S.A., and Fox, T.D. (2003). Mss51p promotes mitochondrial Cox1p synthesis and interacts with newly synthesized Cox1p. EMBO J. **22,** 5951–5961.

Pineau, B., Girard-Bascou, J., Eberhard, S., Choquet, Y., Tremolieres, A., Gerard-Hirne, C., Bennardo-Connan, A., Decottignies, P., Gillet,

S., and Wollman, F.-A. (2004). A single mutation that causes phosphatidylglycerol deficiency impairs synthesis of photosystem II cores in *Chlamydomonas reinhardtii.* Eur. J. Biochem. **271,** 329–338.

Preiss, S., Schrader, S., and Johanningmeier, U. (2001). Rapid, ATP-dependent degradation of a truncated D1 protein in the chloroplast. Eur. J. Biochem. **268,** 4562–4569.

Rott, R., Levy, H., Drager, R.G., Stern, D.B., and Schuster, G. (1998). 3′-Processed mRNA is preferentially translated in *Chlamydomonas reinhardtii* chloroplasts. Mol. Cell. Biol. **18,** 4605–4611.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Singh, M., Boutanaev, A., Zucchi, P., and Bogorad, L. (2001). Gene elements that affect the longevity of *rbcL* sequence-containing transcripts in *Chlamydomonas reinhardtii* chloroplasts. Proc. Natl. Acad. Sci. USA **98,** 2289–2294.

Somanchi, A., Barnes, D., and Mayfield, S.P. (2005). A nuclear gene of *Chlamydomonas reinhardtii*, *Tba1*, encodes a putative oxidoreductase required for translation of the chloroplast *psbA* mRNA. Plant J. **42,** 341–352.

Staub, J.M., and Maliga, P. (1994). Translation of *psbA* mRNA is regulated by light via the 5′-untranslated region in tobacco plastids. Plant J. **6,** 547–553.

Sugiura, M., Inoue, Y., and Minagawa, J. (1998). Rapid and discrete isolation of oxygen-evolving His-tagged photosystem II core complex from *Chlamydomonas reinhardtii* by Ni^{2+} affinity column chromatography. FEBS Lett. **426,** 140–144.

Vaistij, F.E., Goldschmidt-Clermont, M., Wostrikoff, K., and Rochaix, J.D. (2000). Stability determinants in the chloroplast *psbB/T/H* mRNAs of *Chlamydomonas reinhardtii.* Plant J. **21,** 469–482.

Wollman, F.-A., Minai, L., and Nechushtai, R. (1999). The biogenesis and assembly of photosynthetic proteins in thylakoid membranes. Biochim. Biophys. Acta **1411,** 21–85.

Wostrikoff, K., Girard-Bascou, J., Wollman, F.A., and Choquet, Y. (2004). Biogenesis of PSI involves a cascade of translational autoregulation in the chloroplast of Chlamydomonas. EMBO J. **23,** 2696–2705.

Xie, Z., and Merchant, S. (1998). A novel pathway for cytochromes *c* biogenesis in chloroplasts. Biochim. Biophys. Acta **1365,** 309–318.

Yosef, I., Irihimovitch, V., Knopf, J.A., Cohen, I., Orr-Dahan, I., Nahum, E., Keasar, C., and Shapira, M. (2004). RNA binding activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit from *Chlamydomonas reinhardtii.* J. Biol. Chem. **279,** 10148–10156.

Yu, J., and Vermaas, W. (1990). Transcript levels and synthesis of photosystem II components in cyanobacterial mutants with inactivated photosystem II genes. Plant Cell **2,** 315–322.

Zak, E., Norling, B., Maitra, R., Huang, F., Andersson, B., and Pakrasi, H.B. (2001). The initial steps of biogenesis of cyanobacterial photosystems occur in plasma membranes. Proc. Natl. Acad. Sci. USA **98,** 13443–13448.

Zer, H., Prasil, O., and Ohad, I. (1994). Role of plastoquinol oxidoreduction in regulation of photochemical reaction center II D1 protein turnover in vivo. J. Biol. Chem. **269,** 17670–17676.

Zerges, W. (2000). Translation of mRNAs encoded by chloroplast genomes. Biochimie **82,** 583–601.

Zerges, W., and Rochaix, J.-D. (1998). Low density membranes are associated with RNA-binding proteins and thylakoids in the chloroplast of *Chlamydomonas reinhardtii.* J. Cell Biol. **140,** 101–110.

Zhang, L., and Aro, E.M. (2002). Synthesis, membrane insertion and assembly of the chloroplast-encoded D1 protein into photosystem II. FEBS Lett. **512,** 13–18.

Zhang, L., Paakkarinen, V., van Wijk, K.J., and Aro, E.M. (1999). Co-translational assembly of the D1 protein into photosystem II. J. Biol. Chem. **274,** 16062–16067.

Zhang, L., Paakkarinen, V., van Wijk, K.J., and Aro, E.M. (2000). Biogenesis of the chloroplast-encoded D1 protein. Regulation of translation elongation, insertion, and assembly into photosystem II. Plant Cell **12,** 1769–1782.

Chloroplast Biogenesis of Photosystem II Cores Involves a Series of Assembly-Controlled Steps That Regulate Translation
Limor Minai, Katia Wostrikoff, Francis-André Wollman and Yves Choquet
PLANT CELL 2006;18;159-175; originally published online Dec 9, 2005;
DOI: 10.1105/tpc.105.037705

This information is current as of November 30, 2009

References	This article cites 55 articles, 23 of which you can access for free at: http://www.plantcell.org/cgi/content/full/18/1/159#BIBL
Permissions	https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&issn=1532298X&WT.mc_id=pd_hw1532298X
eTOCs	Sign up for eTOCs for *THE PLANT CELL* at: http://www.plantcell.org/subscriptions/etoc.shtml
CiteTrack Alerts	Sign up for CiteTrack Alerts for *Plant Cell* at: http://www.plantcell.org/cgi/alerts/ctmain
Subscription Information	Subscription information for *The Plant Cell* and *Plant Physiology* is available at: http://www.aspb.org/publications/subscriptions.cfm

© American Society of Plant Biologists

ADVANCING THE SCIENCE OF PLANT BIOLOGY