The EMBO Journal vol.8 no.4 pp.1013 - 1021, 1989

Nuclear and chloroplast mutations affect the synthesis or stability of the chloroplast *psbC* gene product in *Chlamydomonas reinhardtii*

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Communicated by J.-D.Rochaix

The *psbC* gene of *Chlamydomonas reinhardtii* encodes P6, the 43 kd photosystem II core polypeptide. The sequence of P6 is highly homologous to the corresponding protein in higher plants with the exception of the N-terminal region where the first 12 amino acids are missing. Translation of P6 is initiated at GUG in *C.reinhardtii*. The chloroplast mutant MA16 produces a highly unstable P6 protein. The mutation in this strain maps near the middle of the *psbC* gene and consists of a 6 bp duplication that creates a Ser-Leu repeat at the end of one transmembrane domain. Two nuclear mutants, F34 and F64, and one chloroplast mutant, FuD34, are unable to synthesize P6. All of these mutants accumulate wild-type levels of *psbC* mRNA. The FuD34 mutation has been localized near the middle of the 550 bp 5′ untranslated region of *psbC* where the RNA can be folded into a stem-loop structure. A chloroplast suppressor of F34 has been isolated that partially restores synthesis of the 43 kd protein. The mutation of this suppressor is near that of FuD34, in the same stem-loop region. These chloroplast mutations appear to define the target site of a nuclear factor that is involved in P6 translation.

Key words: Chlamydomonas/nuclear:plastid co-operation/nuclear:plastid mutation/*psbC* gene

Introduction

The synthesis and assembly of photosynthetic complexes in higher plants and algae require the close co-operation of both the chloroplast and nucleo-cytoplasmic compartments. These multimolecular complexes consist of subunits which are encoded by the nuclear and chloroplast genomes. While nuclear encoded proteins are translated on cytoplasmic ribosomes and post-translationally imported into the organelle, chloroplast encoded proteins are translated on chloroplast ribosomes. Subunits of both types associate together with chloroplast synthesized pigments and lipids to form functional photosynthetic complexes.

Photosystem II is one of the major complexes in the thylakoid membrane (for a review see Andreasson and Vänngard, 1988; Homann, 1988). Its core, which is embedded in the membrane, consists of several chloroplast encoded polypeptides and pigments. The polypeptides include D1 and D2, the apoproteins of cytochrome b559 and two chlorophyll binding proteins P5 (47 kd) and P6 (43 kd). The core of PSII is closely associated with the three nuclear encoded proteins OEE1, 2 and 3 involved in oxygen evolution and located on the lumen side of the thylakoid. The PSII core is also linked to the light harvesting system (LHCII). Several additional polypeptides have been found to belong to the PSII complex (Delepelaire, 1984; Ljungberg *et al.*, 1986a,b).

One approach towards understanding the co-ordinate assembly of this complex is to isolate and characterize mutants deficient in PSII activity. Several of these mutants are unable to assemble a stable PSII complex because they no longer synthesize one of the core polypeptides (Rochaix, 1987). In contrast, a PSII mutant lacking the OEE1 protein is still capable of assembling a partially stable core complex, while in a mutant lacking the OEE2 protein this complex is as stable as in the wild-type (Mayfield *et al.*, 1987a,b).

Genetic analysis of several nuclear PSII mutants indicates that besides structural genes coding for PSII components, a number of nuclear factors exist that are required for the synthesis and assembly of the PSII complex (Jensen *et al.*, 1986; Kuchka *et al.*, 1988). In order to obtain more insights as to how these factors act we have examined two nuclear mutants, F34 and F64, and two chloroplast mutants, FuD34 and MA16, that are unable to synthesize or stably accumulate the P6 protein, the product of the chloroplast *psbC* gene. All these mutants are affected at a post-transcriptional level since they accumulate wild-type levels of *psbC* mRNA. The analysis of a chloroplast suppressor of F34 which partially restores P6 synthesis has allowed us to uncover a short region in the 5′ untranslated region of *psbC* mRNA that may act as a target site for a nuclear factor required for the translation of P6. The same region is also altered in the chloroplast mutant FuD34.

Results

Lack of P6 in two chloroplast and two nuclear PSII mutants is due to a post-transcriptional deficiency

The nuclear mutants F64 and F34 (Chua and Bennoun, 1975; Bennoun *et al.*, 1980; Delepelaire, 1984) and the two chloroplast mutants FuD34 and MA16 (Materials and methods) are all deficient in photosystem II activity. The Coomassie blue staining patterns obtained after gel electrophoresis of the thylakoid membrane proteins of MA16 and FuD34 (Figure 1) reveal that these mutants are missing the core PSII polypeptides P5 and P6, the three OEE proteins (bands 12, 19 and 24) and the two low mol. wt polypeptides 34 and 36 which are known to be associated with PSII (Delepelaire, 1984). Similar results were obtained for F34 (Delepelaire, 1984) and F64 (data not shown). Analysis of proteins from mutant cells pulse-labelled in the presence of

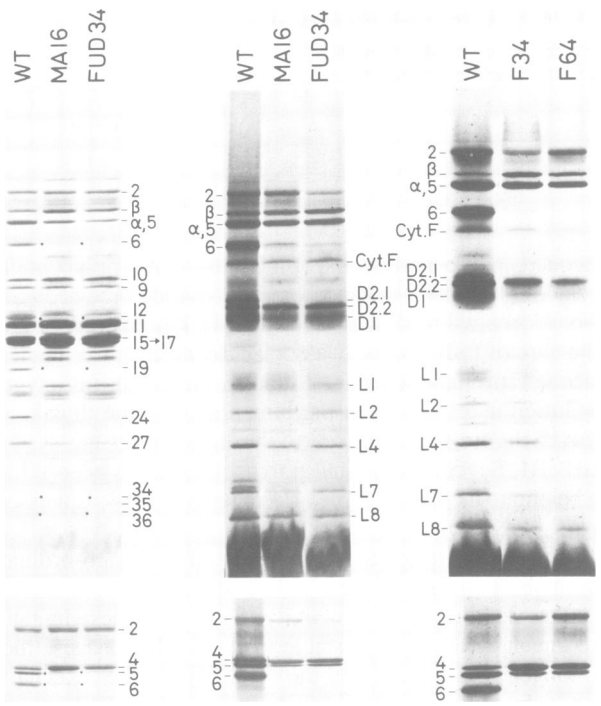

Fig. 1. Gel electrophoretic fractionation of thylakoid membrane polypeptides from wild-type cells (WT), chloroplast (MA16, FuD34) and nuclear (F34, F64) mutants of *C.reinhardtii*. Cells were labeled for 15 min wtih [^{14}C]acetate in the presence of 8 μg/ml cycloheximide. Coomassie blue staining of thylakoid membrane polypeptides is shown in the left panel. Bands that are missing or strongly reduced are marked with dots. Autoradiographs are shown in the two panels on the right. Upper part: 12–18% SDS–polyacrylamide gel with 8 M urea. Lower part: 7.5–15% SDS–polyacrylamide gels, which allow for a better resolution of polypeptides 4, 5 and 6. The nomenclature of thylakoid polypeptides is according to Chua and Gillham (1977) and Delepelaire (1984). Bands labelled 5 (P5), 6 (P6), D1, 12 (OEE1), 19 (OEE2) and 24 (OEE3) are referred to in the text.

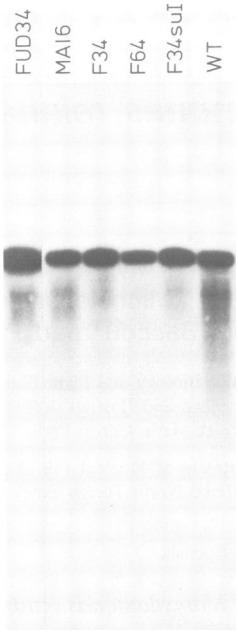

Fig. 2. Northern analysis of the mutants FuD34, MA16, F34, F64, the chloroplast suppressor F34suI and the wild-type. Total RNA was fractionated on denaturing agarose gels, blotted and hybridized with a probe specific for *psbC*. The *psbC* message has a size of 2 kb.

an inhibitor of cytoplasmic translation has proved to be a powerful screen for determining the primary lesion of photosynthetic mutations (Bennoun *et al.*, 1986; Erickson *et al.*, 1986; Jensen *et al.*, 1986). Accordingly, mutant and wild-type cells were pulse-labelled with [^{14}C]acetate for 15 min in the presence of cycloheximide. Thylakoid membrane polypeptides were fractionated by SDS–urea polyacrylamide gel electrophoresis (PAGE) and subjected to autoradiography. Figure 1 shows that the P6 band is missing in the mutants F64, F34 and FuD34. This can be seen more clearly at the bottom of the figure which displays the polypeptide pattern obtained by SDS–PAGE in the absence of urea allowing for better resolution of P6. The other chloroplast encoded thylakoid polypeptides are produced at levels comparable to those of the wild-type. A very faint band is detectable in the mutant MA16 at the position of P6. Similar results were obtained with total cell proteins (data not shown) indicating that the absence of P6 in the thylakoids of these mutants is indeed due to their inability to synthesize or stabilize the P6 protein.

To determine whether these mutants are affected at the transcriptional level for P6 synthesis, RNA was extracted from the mutants, separated on denaturing agarose gels, blotted and hybridized with a *psbC* probe. Figure 2 shows that P6 mRNA accumulates to wild-type levels in all the mutants indicating that these mutations act at a post-transcriptional level.

Absence of P6 synthesis leads to the destabilization of the PSII core complex

Proteins were extracted from the wild-type and from each of the PSII deficient mutants and separated into crude soluble and membrane fractions. The proteins of each fraction were separated on gradient SDS–polyacrylamide gels and either stained with Coomassie blue or transferred to cyanogen bromide (CNBr) paper as previously described (Mayfield *et al.*, 1987a). The protein blots were incubated with antisera specific to the PSII reaction centre polypeptides D1, D2, P5 and P6; to the proteins of the oxygen evolving complex OEE1, OEE2 and OEE3; and to the light harvesting chlorophyll *a/b* proteins (LHCII).

As expected from the pulse-labelling data, Figure 3 shows that mutants F34, F64, FuD34 and MA16 accumulate no detectable amounts of the P6 protein. The other PSII core polypeptides D1, D2 (data not shown) and P5 are greatly reduced or even undetectable in these mutants. For comparison we have included two other PSII deficient chloroplast mutants FuD7 and FuD47 which are defective in the *psbA* (D1) and *psbD* (D2) genes respectively (Bennoun *et al.*, 1986; Erickson *et al.*, 1986). Figure 3 shows that in these two mutants there is no detectable amount of D1 and P5 protein. However, the mutant FuD7 accumulates almost 20% of wild-type levels of P6 protein. The picture which emerges from these PSII mutants is that in the absence of PSII core polypeptides, no stable PSII complex assembles. The other PSII polypeptides whose synthesis is not affected in these mutants turn over and some accumulate in small amounts. Although no stable PSII core complex is formed in these PSII mutants the three OEE proteins accumulate to wild-type levels except for in F64 where the amount of OEE1 protein is reduced (Figure 4). The LHCII proteins also

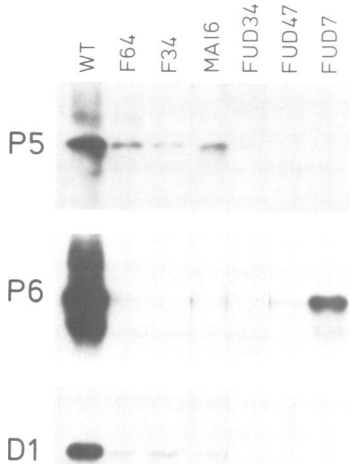

Fig. 3. Accumulation of PSII proteins in the wild-type and in PSII mutants. Total membrane protein from the wild-type and the mutants was electrophoresed on SDS−polyacrylamide gels (Mayfield *et al.*, 1987a) and transferred to CNBr-activated paper. Protein blots were reacted with antisera specific for PSII polypeptides P5, P6 and D1. Protein−antibody complexes were labelled with [^{125}I]Staph-A protein and visualized by autoradiography. Mutants FuD47 (Erickson *et al.*, 1986) and FuD7 (Bennoun *et al.*, 1986) have been described.

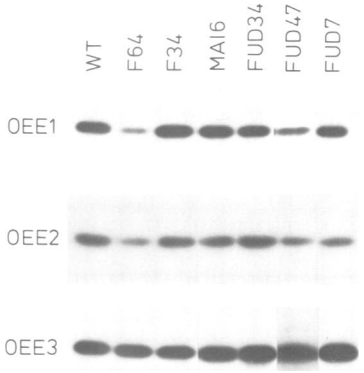

Fig. 4. Accumulation of the OEE proteins in wild-type cells (WT) and PSII mutants. Total soluble proteins were fractionated by electrophoresis on SDS−polyacrylamide gels (Mayfield *et al.*, 1987a) and transferred to CNBr-activated paper. The protein blots were reacted against antisera specific for OEE1, OEE2 and OEE3.

accumulate to wild-type levels in all these mutants (data not shown).

To obtain insights into the nature of the chloroplast mutations of MA16 and FuD34 it was first necessary to characterize the *psbC* gene of *C.reinhardtii*. Previous work indicated that this gene spans the chloroplast *Eco*RI fragments R9 and R23 (Rochaix, 1981). Its entire nucleotide sequence was determined by the strategy outlined in Materials and methods. S1 nuclease protection and primer extension experiments revealed that the *psbC* 5′ untranslated region contains 550 nt. Comparison of the 5′ ends of the coding sequences of *psbC* from *C.reinhardtii* and spinach revealed that the algal gene starts with GTG and is missing the first 12 amino acids of the spinach sequence (Figure 5). To ensure that there is no intron upstream of this putative initiation codon a 21mer oligonucleotide (oligo 107 in Figure 5) was constructed with a sequence complementary to the RNA-like strand ending 18 nt from the GTG. This oligonucleotide was annealed to total RNA and the cDNA sequence was established by the dideoxynucleotide sequencing method. The identity of the deduced RNA sequence with the DNA sequence upstream of the GTG codon clearly proves that there is no intron. Although the *C.reinhardtii* 43 kd protein has not been sequenced, it is very likely that its initiation codon is GTG because there is a termination site three codons upstream and the amino acid sequence to the first Met residue at position 35 is nearly identical to the corresponding spinach sequence (Alt *et al.*, 1984) with only three amino acid changes. A Shine−Dalgarno sequence is also present near the GTG codon (Figure 5). The overall homology between the algal and higher plant protein is 86%.

The P6 protein is destabilized by a two amino acid duplication in MA16

Comparison of the entire *psbC* sequences from wild-type and MA16 reveals a 6 bp duplication near the middle of the gene (Figure 6). This gene rearrangement results in the duplication of Leu−Ser in the protein sequence. This change explains why the faint P6 band of MA16 migrates slightly more slowly than the corresponding band in the wild-type (Figure 1).

The mutation of FuD34 maps in the 5′ untranslated region of psbC

Comparison of the *psbC* sequences from the wild-type and FuD34 revealed an alteration near the middle of the 5′ untranslated region. As shown in Figure 7, the change involves the insertion of two Ts and the removal of one C located 6 nt downstream in the RNA-like strand. To exclude a cloning artefact, the corresponding region of the RNA from both FuD34 and wild-type was sequenced using oligonucleotide 138 (Figure 5) as a primer for dideoxy sequencing. The deduced RNA sequences fully agree with the DNA sequences.

A chloroplast mutation specifically suppresses the nuclear mutation in F34

The nuclear mutations in F34 and F64 define factors that appear to be specifically involved in the expression of the *psbC* gene at a post-transcriptional level. In order to understand how these factors act, a search for chloroplast suppressors was undertaken. After mutagenesis of the cells with fluorodeoxyuridine (Materials and methods) a single chloroplast suppressor, F34suI, was isolated for F34 while only nuclear suppressors were found for F64. Only partial suppression occurs in F34suI, as can be seen in Figure 8A which shows that the rate of P6 synthesis as measured by 10 min pulse-labeling is significantly reduced in F34suI as compared to the wild-type. Western blotting (Figure 8B) also indicates that P6 accumulates between 20 and 25% of wild-type levels in F34suI. A genetic analysis of this suppressor was performed. In a cross of F34suI(mt+) × F34(mt−) all 12 tetrads segregated to produce 0 acetate-requiring progeny:4 wild-type progeny, suggesting that suppression was due to a chloroplast mutation. Such mutations are known to be inherited predominantly from the mating type + (mt+) parent. By crossing F34suI to the wild-type the suppressor mutation could be separated from the nuclear mutation in F34 and the phenotype of the isolated suppressor mutation

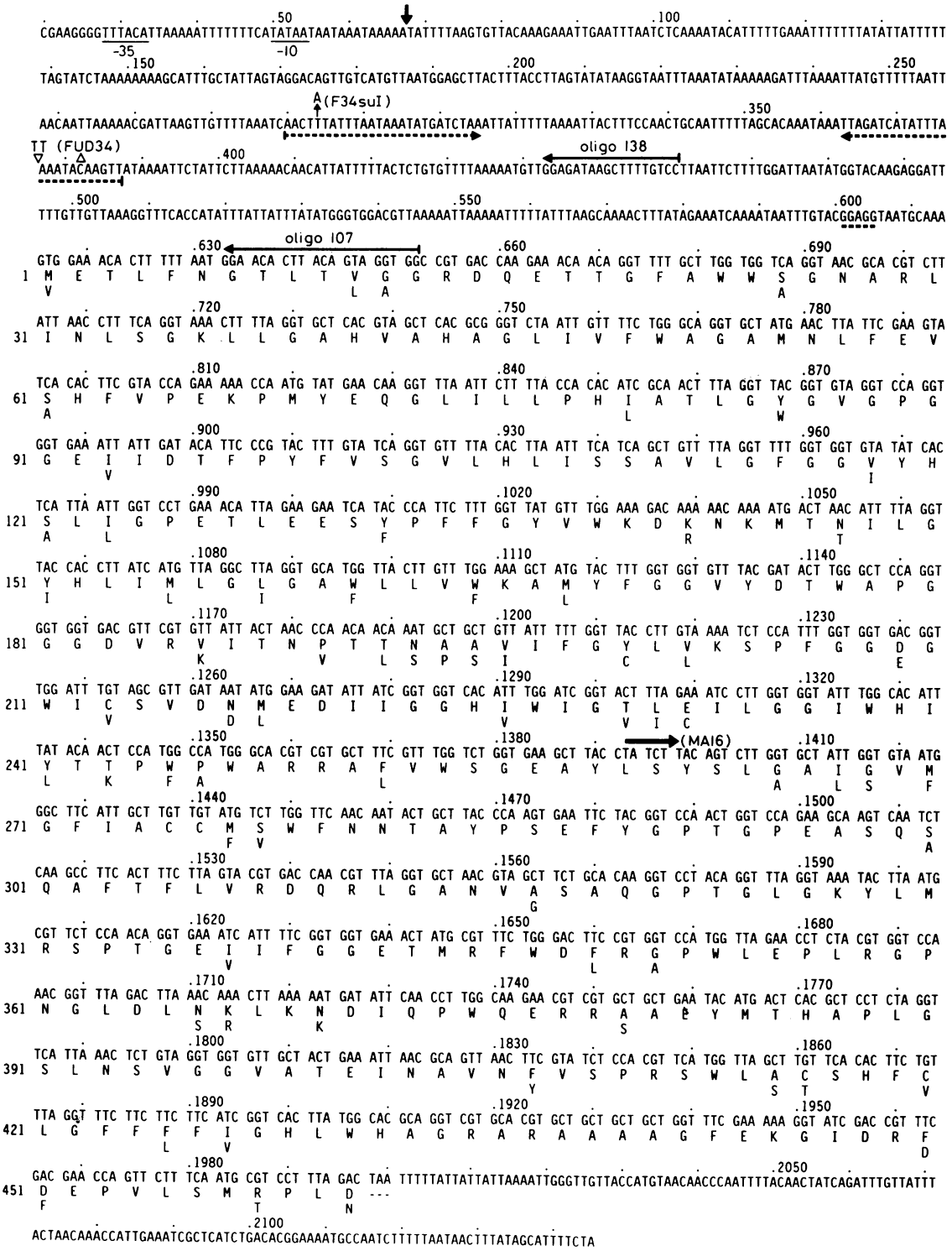

Fig. 5. Nucleotide sequence of the *psbC* gene of *C.reinhardtii* and of its flanking regions. The 5′ end of the *psbC* transcript is marked with a vertical arrow. Putative −35 and −10 regions are underlined. The 5′ untranslated region is located between positions 68 and 614. The two segments of the inverted repeat are underlined by broken arrows. The two oligonucleotides (oligo 138 and oligo 107) used for cDNA sequencing are shown as arrows. The mutations in F34suI and FuD34 are indicated (▽, insertion; △, deletion). The Shine−Dalgarno sequence is marked by a broken line. The protein sequence is indicated in the single letter amino acid code and is compared with the corresponding sequence of spinach. Only residues that are different have been indicated and the first 12 residues of the spinach protein have been omitted. The six bases that are duplicated in MA16 are marked by a horizontal arrow (positions 1393−1398).

was found to be indistinguishable from the wild-type. Crossing of suI(mt+) to F34(mt−) produced exclusively 0:4 tetrads as expected from a chloroplast suppressor mutation. The effect of the mutation of F34suI is rather specific since it does not suppress the mutation of F64.

To determine the mutation in suI, the *psbC* gene of F34suI was cloned and sequenced. In Figure 9 the DNA sequence of a portion of the 5′ untranslated region of *psbC* is compared

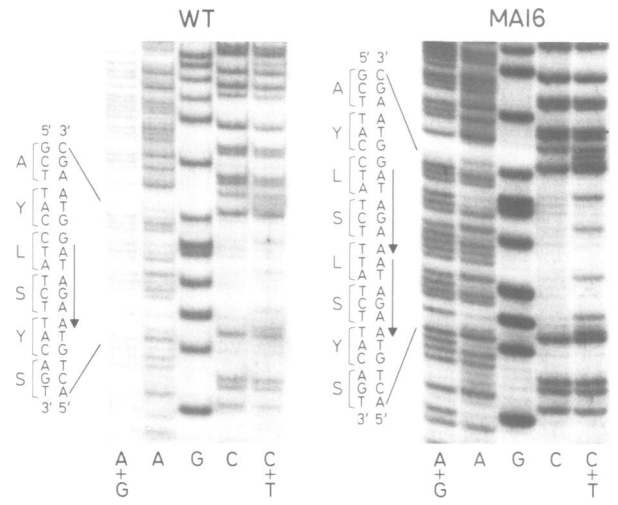

Fig. 6. Comparison of the DNA sequences of a portion of *psbC* from the wild-type and MA16. The Maxam–Gilbert sequencing reactions are indicated under each lane. The 6 bp that are duplicated are indicated by an arrow.

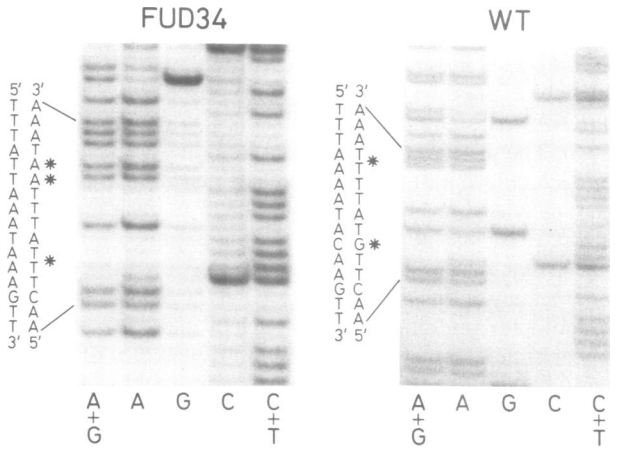

Fig. 7. Comparison of the DNA sequences of a segment of the *psbC* 5′ untranslated region from FuD34 and the wild-type. The altered bases are marked with stars. The Maxam–Gilbert sequencing reactions are indicated under each lane.

in F34suI and FuD34 (the FU34 sequence is identical with the wild-type in this region). Besides a single T to A transversion in this region, the remaining *psbC* sequences of F34suI and wild-type are indistinguishable. This single point mutation was verified (Figure 9) by comparing the corresponding suppressor and wild-type RNA sequences by dideoxy sequencing of cDNA using the oligonucleotide 138 as a primer and total RNA as template (Figure 5). As shown in Figure 5 the mutations found in F34suI and FuD34 are located within the central portion of the *psbC* 5′ untranslated region.

Discussion

Implications of the presence of the GUG initiation codon in the psbC gene of C.reinhardtii

The homology between the *C.reinhardtii* and higher plant *psbC* gene products is 85%, slightly lower than the homology observed for D1 and D2 (92%) (Erickson *et al.*, 1985). The most conspicuous difference is in the amino-terminal region:

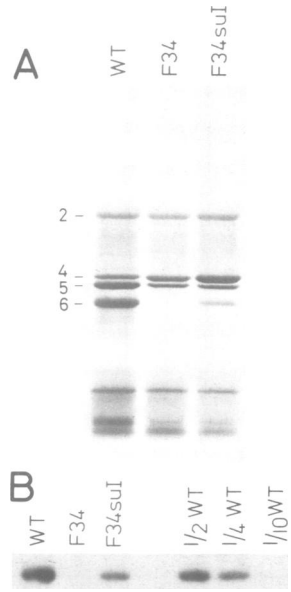

Fig. 8. (**A**) Gel electrophoretic fractionation of thylakoid polypeptides from WT, F34 and F34suI. Electrophoresis was performed on SDS 7.5–15% polyacrylamide gels. Cells were labelled for 15 min with [^{14}C]acetate in the presence of 8 μg/ml cycloheximide.
(**B**) Accumulation of the P6 protein in WT, F34 and F34suI. Following electrophoretic fractionation, proteins were transferred to nitrocellulose and reacted with an antiserum specific for P6. Lanes with smaller amounts of WT proteins (1/2, 1/4, 1/10) are shown for comparison.

the *C.reinhardtii* P6 protein lacks the first 12 amino acids predicted by the spinach *psbC* sequence (Alt *et al.*, 1984; Figure 10). The N-terminal sequence of the 43 kd protein of spinach has recently been determined by tandem mass spectrometry and found to start with *N*-acetyl-O-phosphothreonine at residue 15 of the amino acid sequence predicted by the *psbC* sequence (Michel *et al.*, 1988). One possibility is that the spinach protein is initiated at the AUG and made as a precursor which is processed to the mature form by removing the first 14 residues. Another possibility is that translation of the spinach protein initiates at GUG as in *C.reinhardtii*. This view is consistent with the presence of a ribosomal binding site upstream of the GTG in both *C. reinhardtii* and spinach (Figure 11). There is no such site upstream of the ATG in spinach. In this scheme the first two residues of the protein would be removed before it is acetylated.

It is well documented that GUG can serve as an initiation codon and is recognized by $tRNA^{met}$ in prokaryotes, but not in eukaryotic cells (Lewin, 1987). GUG initiation codons have also been found in the *rps*19 gene from all plants examined, with the exception of liverwort (Moon and Wu, 1988). Since *N*-acetylation is common in eukaryotes but rare in prokaryotes (Driessen *et al.*, 1985), it is interesting to note that the P6 protein and its gene share both eukaryotic and prokaryotic features.

Lack of P6 destabilizes the PSII core complex

All four mutants described in this study are unable to accumulate the P6 protein. Although the other PSII core polypeptides are synthesized at or near wild-type levels, in these mutants they accumulate to small or even undetectable

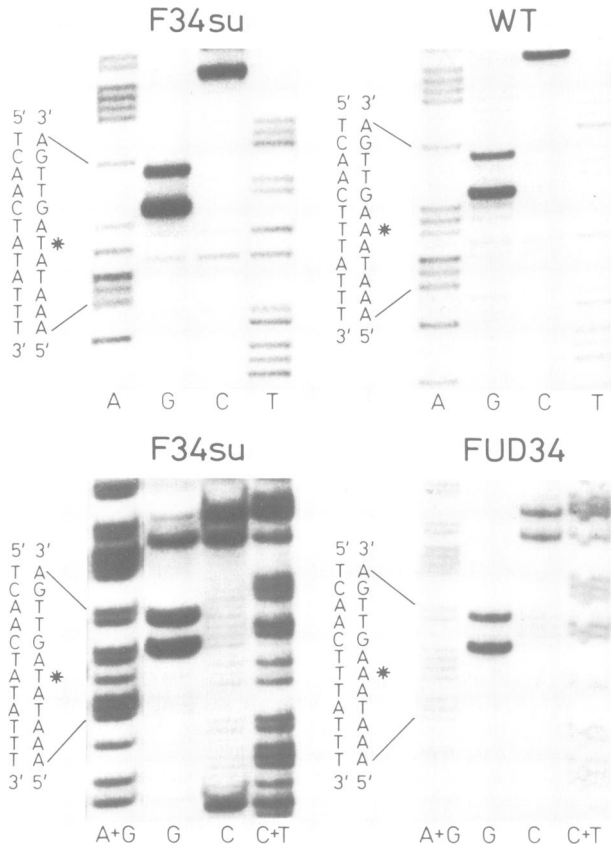

Fig. 9. Comparison of RNA (upper) and DNA sequences (lower) of a segment of the *psbC* 5′ untranslated region from F34su and the wild-type. The altered base pair is marked with a star. The RNA sequence was obtained by dideoxy sequencing using oligo 138 as a primer. The lower right panel displays the sequence of FuD34 which is identical to the wild-type in this segment.

levels. Similar findings have been reported for other PSII mutations affecting D1, D2 or P5 synthesis (Bennoun *et al.*, 1986; Erickson *et al.*, 1986; Jensen *et al.*, 1986; Kuchka *et al.*, 1988). It thus appears that each of these core polypeptides is required to form a stable complex.

While the amount of P6 as measured by pulse-labeling of cells remains largely undetectable in F34, F64 and FuD34, trace amounts of P6 are seen in MA16 under the same conditions (Figure 1). The mutation of MA16 is a 6 bp duplication which results in the duplication of two amino acids (Leu−Ser). The hydropathy plot of P6 shown in Figure 11 reveals several putative transmembrane domains. The amino acid duplication occurs near an end of one of these domains and strongly destabilizes the protein.

In contrast to the core PSII polypeptides the OEE proteins accumulate to wild-type levels in these mutants with the exception of the F64 mutant where the amount of OEE1 protein is reduced. It is important to note that the Western blots displayed in Figure 4 were performed on total soluble protein. The crude membrane fractions did not contain significant amounts of the OEE proteins in agreement with the Coomassie blue staining patterns of thylakoid polypeptides of these mutants, where the OEE proteins are missing (Figure 1). It appears that although the OEE proteins accumulate within the thylakoid lumen as inferred from their mature size in these mutants, they are not firmly associated with the thylakoid membrane in the absence of the PSII core.

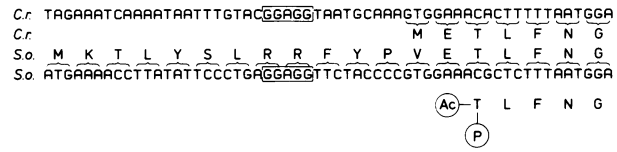

Fig. 10. Comparison of the 5′ ends of the *psbC* genes of *C.reinhardtii* (C.r.) and spinach (S.o) (Alt *et al.*, 1984). Amino acids are indicated in the single letter code. The mature protein of spinach begins with *N*-acetyl-O-phosphothreonine (Michel *et al.*, 1988). Putative Shine−Dalgarno sequences are framed.

At least two nuclear genes are involved in the expression of the psbC gene of C.reinhardtii

The two nuclear mutations in F34 and F64 which belong to two complementation groups (Bennoun *et al.*, 1980) specifically affect the synthesis of P6 at a post-transcriptional level. Other nuclear mutations in *C.reinhardtii* have been shown to block specifically the synthesis of D2 (Kuchka *et al.*, 1988), D1 (J.Girard-Bascou, unpublished results) and P5 (Jensen *et al.*, 1986). At least 10 nuclear complementation groups are involved in the maturation of the chloroplast *psaA* mRNA (Choquet *et al.*, 1988) and at least two have been uncovered for the synthesis of the *psaB* gene product (J. Girard-Bascou, unpublished results). Similar results have been reported in yeast where nuclear mutations specifically block the expression of mitochondrial genes both at the level of RNA maturation and translation (Dujon, 1981; Fox, 1986). The picture which emerges from these studies is that for each chloroplast or mitochondrial gene which has been thoroughly examined, between one and >10 nuclear encoded factors exist that are required for the expression of these genes. One can estimate that up to 2−3% of the nuclear genome may be involved in this task in *C.reinhardtii*. While this complex circuitry between nucleus and organelle may appear cumbersome it is affordable for genomes of limited size such as chloroplast and mitochondrial DNA. Some of the nuclear encoded factors may have a dual function as has been demonstrated for fungal tRNA synthetases that participate in both protein synthesis and splicing of mitochondrial RNAs (Akins and Lambowitz, 1987; Herbert *et al.*, 1988).

Does the mutation in F34 affect a specific translation factor for psbC?

The effect of the nuclear mutation in F34 is partially suppressed by the chloroplast mutation in F34suI. In this suppressor both P6 synthesis and accumulation are restored to about 20% of wild-type levels. The suppressor mutation maps near the middle of the 550 bp 5′ untranslated region of *psbC* where the RNA could fold into a stem−loop structure as shown in Figure 12. The mutation creates a mismatch in the stem region thereby lowering the free energy associated with this secondary structure from −23 kcal in the wild-type to −19 kcal. It should be noted that this chloroplast suppressor acts in a very different way from the mitochondrial suppressors of nuclear mutations described in yeast (Fox, 1986). In this case mitochondrial genome rearrangements generate deletions whereby the coding sequence of the affected gene is fused to the 5′ untranslated region of another mitochondrial gene. It is apparent from Figure 12 that the mutation in FuD34 affects the same stem region on the opposite strand by inserting two Ts and removing one C. It thereby creates a stem with 26

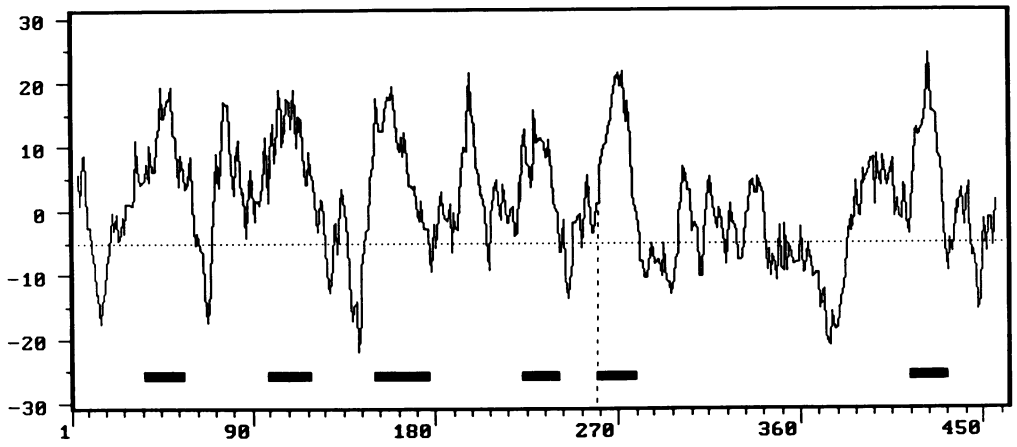

Fig. 11. Hydropathy plot of the 43 kd protein of *C.reinhardtii* according to Kyte and Doolittle (1982). Putative transmembrane domains are indicated by horizontal bars. The mutated region in MA16 is marked with a vertical dotted line.

complementary bases with a free energy of −33 kcal, considerably higher than in the wild-type. It is unlikely that the nucleotide changes found in FuD34 and F34suI reflect polymorphic changes. We have sequenced these modified regions in the wild-type, MA16, FuD34 and F34suI. In each case we find that two of the three mutants have the wild-type sequence. Although we have sequenced almost all of *psbC* and its flanking regions in these four strains we have not detected any polymorphic differences.

Our results can be explained by assuming that this stem region defines a target site for a nuclear factor N that is altered in F34. It is possible that the stem region interferes with initiation of protein synthesis and that the N factor interacts with the stem region, perhaps by destabilizing it. In F34 the N factor has been altered most likely by a mis-sense mutation and can no longer interact properly with the stem. In FuD34 the stability of the stem has been increased to such an extent that it cannot be disrupted or is not recognized by N. It is also understandable that the chloroplast suppressor mutation alone has no phenotype in a wild-type nuclear background because it changes the strength of base pairing in the stem only slightly. This may however be sufficient to allow the altered N in F34suI to either partially destabilize the stem or to restore its activity for translation in some other way. This model can be tested now that chloroplast transformation is feasible in *C.reinhardtii* (Boynton *et al.*, 1988). While this model emphasizes the specificity of N for *psbC* expression we cannot rule out the possibility that N may act non-specifically, i.e. as an enzyme involved in RNA maturation. In this view it is conceivable that as a result of the mutation in F34, N would no longer be active in *psbC* mRNA translation but it would still be able to perform its other functions that are required for cell growth.

Control of translation by mRNA-binding proteins has been demonstrated in prokaryotes. Several *Escherichia coli* ribosomal proteins act as translational repressors for ribosomal proteins and for transcription and translation factors (Lindahl and Zengel, 1986). Similarly some bacteriophage proteins repress their own synthesis by binding to their mRNA (Kozak, 1988). The target site for this repression is usually localized within the leader of the mRNA and consists of a stem−loop structure with unpaired nucleotides in the stem (Krisch and Allet, 1982; Climie and Friesen, 1987; Thomas and Nomura, 1987; Winter *et al.*, 1987).

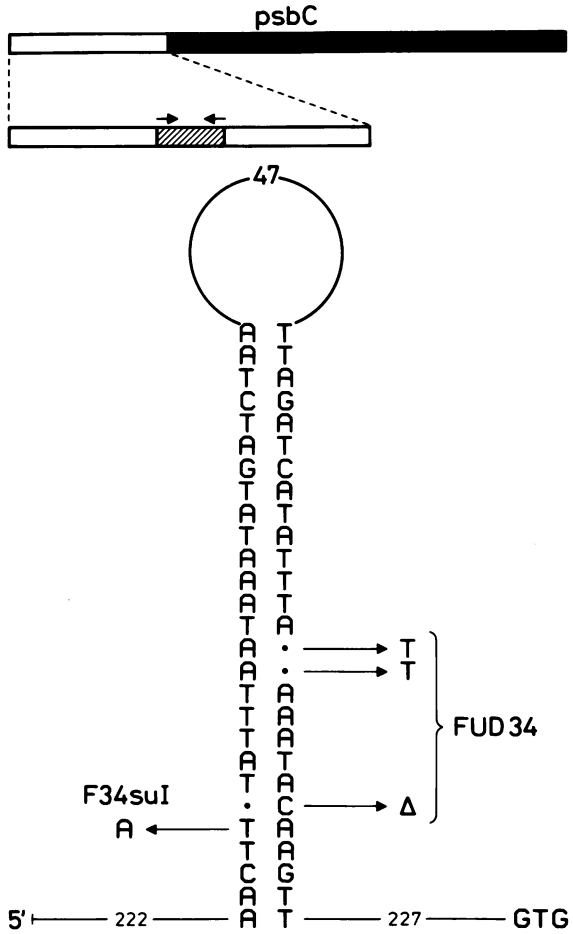

Fig. 12. The stem region in the *psbC* 5′ untranslated region is altered in FuD34 and F34suI. The altered nucleotides in these two mutants are indicated. Dots indicate missing nucleotides. In the upper part of the figure the two segments of the stem region are drawn as arrows within the 5′ untranslated region (white bar) of *pbsC*.

Subtle changes in these target sites can abolish the repression. Translational repression has also been demonstrated for human ferritin mRNA where a cytosolic protein binds to a specific iron-responsive element in the 5′ untranslated region (Rouault *et al.*, 1988). This element consists of a stem−loop structure and mediates regulation of ferritin translation by iron.

It is striking that all these examples point to the presence of translational repressors. A recent report, however, suggests that RNase III may act as a translational activator for the lambda cIII mRNA (Altuvia *et al.*, 1987). The evidence presented in this study favours the hypothesis that the synthesis of the chloroplast P6 protein of *C.reinhardtii* requires a translational activator although we cannot exclude the possibility that F34 produces a super-repressor that binds tightly to the stem region shown in Figure 12 and fails to come off unless the stem is mutated as in F34suI.

Materials and methods

Algal strains

C.reinhardtii wild-type strain 137c was used in this work. The PSII nuclear mutants F34 and F64 have been described previously (Chua and Bennoun, 1975: Bennoun *et al.*, 1980; Delepelaire, 1984). The MA16 mutant was a generous gift from G.Schmidt and was isolated following mutagenesis with azide by a method similar to that described by Girard *et al.* (1980). The FuD34 mutant was isolated following mutagenesis with 5-fluorodeoxyuridine according to Bennoun *et al.* (1978). The mutations in MA16 and FuD34 lead to a PSII deficiency. They are uniparentally inherited by the mt+ parent and are thus considered as chloroplast mutations. The strains were grown on Tris–acetate–phosphate (TAP) on a rotary shaker at 25°C under an illumination of 400 lux. To isolate the chloroplast suppressor F34suI, cells of F34 were inoculated at 10^6 cells/ml in the presence of 1 mM fluorodeoxyuridine and grown to the stationary phase. 20 ml of this culture was washed and diluted 10× in TAP medium to allow for expression of the mutation. After reaching the stationary phase the culture was diluted 10× in minimal medium under an illumination of 3000 lux. Mutant cells were cloned on solid minimal medium. One clone, F34suI, was obtained and analysed.

RNA isolation and Northern analysis

RNA was isolated, electrophoretically separated on denaturing formaldehyde agarose gels and electroblotted to nylon membranes as described previously (Kuchka *et al.*, 1988). The RNA was hybridized to a nick-translated 930 bp *Hin*dIII fragment that covers most of the *psbC* gene.

Protein isolation and immunoblot analysis

Protein isolation and sample preparation were performed as described by Mayfield *et al.* (1987a). Proteins were electrophoretically separated on 7.5–15% polyacrylamide gels as described (Chua, 1980) and electroblotted onto CNBr-activated paper (Clark *et al.*, 1979) or nitrocellulose paper (Towbin *et al.*, 1979). The filters were reacted with antisera directed against various PSII polypeptides. The antibodies against P5, P6, D2, OEE1, OEE2, OEE3 and LHC were obtained from N.H.Chua. The antibody against D1 from *Amaranthus hybridus* was obtained from L.McIntosh.

Pulse-labelling of proteins

Pulse-labelling was carried out in minimal medium with [^{14}C]acetate for 15 min under an illumination of 800 lux or 350 lux in the presence of cycloheximide (8 μg/ml). At the end of the labelling period, 200 ml of culture were directly used for thylakoid membrane purification (Chua and Bennoun, 1975) and 30 ml were used for the analysis of proteins from whole cells (Bennoun *et al.*, 1986). The extract was fractionated by electrophoresis on 7.5–15% SDS–polyacrylamide gels or 12–18% SDS–polyacrylamide gels containing 8 M urea as described (Chua and Bennoun, 1975) and the gels were then subjected to autoradiography.

Cloning

*Eco*RI digested DNAs from FuD34, MA16 and F34suI were cloned in plasmid pUN121 (Nilsson *et al.*, 1983). Clones containing the *Eco*RI fragments R23 and R9 were identified by colony hybridization (Grünstein and Hogness, 1975) using labelled R23 and R9 fragments as probes.

DNA sequence analysis

The *psbC* regions of the wild-type, FuD34, MA16 and F23suI were sequenced as outlined in Figure 13. Fragments were labelled at either the 3′ or 5′ end and sequenced by the chemical cleavage technique of Maxam and Gilbert (1980). Reaction products were separated by electrophoresis on 8 and 20% polyacrylamide gels containing 6 M urea and visualized by autoradiography with Kodak X-ray film XR5.

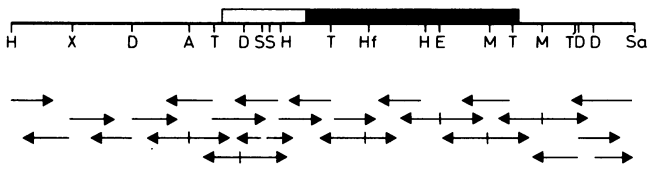

Fig. 13. Strategy used for sequencing *psbC* and its flanking regions in the wild-type, FuD34, MA16 and F34suI. Arrows indicate sequencing tracts. Restriction sites for *Hin*dIII (H), *Xba*I (X), *Dde*I (D), *Aav*I (A), *Taq*I (T), *Sau*3A (S), *Hin*fI (H), *Eco*RI (E), *Mae*I (M) and *Sal*I (Sa) are indicated. The *psbC* 5′ untranslated and coding regions are marked with a white and black bar, respectively.

RNA sequencing

100 μg of total mRNA were annealed to oligonucleotide primer, end-labelled to 10^7 c.p.m. with ^{32}P. The annealed primers (oligonucleotides 107 and 138; Figure 5) were extended separately with reverse transcriptase and the four dideoxynucleotides as described by Johanningmeier *et al.* (1987). Reaction products were fractionated and visualized by autoradiography as described for the DNA sequence analysis.

Acknowledgements

We thank Y.Pierre for excellent technical help, O.Jenni and F.Ebener for drawings and photography, N.H.Chua and L.McIntosh for antibodies and M.Goldschmidt-Clermont and A.Day for critical reading of the manuscript. The work was supported by grant 3.328-086 from the Swiss National Foundation and U.A.1187 from the Centre National de la Recherche Scientifique.

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Received on 22 December 1988

Note added in proof

These sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X13879.