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Nuclear mutations specifically affect the synthesis and/or degradation of the chloroplast-encoded D2 polypeptide of photosystem II in *Chlamydomonas reinhardtii*

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To study the interaction of the nuclear and chloroplast genomes in the biogenesis of the photosynthetic apparatus, nuclear mutants of *Chlamydomonas reinhardtii* deficient in photosystem II (PSII) activity were analyzed. Two independently-isolated, allelic nuclear mutants show a pleiotropic reduction in a set of functionally related PSII polypeptides. Immunoblot analysis reveals that the two mutants, *nac-1-18* and *nac-1-11*, accumulate reduced amounts of the chloroplast-encoded polypeptides P5 and P6 and are completely deficient in polypeptides D1 and D2. Polypeptides of the oxygen-evolving and light-harvesting complexes associated with PSII, however, are present at wild-type levels. Analysis of mRNAs encoding PSII polypeptides from these mutants indicates that all messages are present, although some species, including the D2 message, are significantly elevated. When mutant cells are pulse-labeled for 10 min with [^{14}C]acetate, a greatly reduced amount of labeled D2 protein is observed, while all other PSII polypeptides are synthesized normally. These data indicate that the mutations present in *nac-1-18* and *nac-1-11* affect a nuclear gene whose product specifically controls the translation and/or degradation of the chloroplast-encoded D2 polypeptide.

Key words: nuclear-chloroplast interactions/*psb*D gene product/photosynthesis

Introduction

The light reactions of photosynthesis take place on several discrete multisubunit complexes located within the thylakoid membranes of the chloroplast. Each of these complexes is composed of both nuclear- and chloroplast-encoded polypeptides. The assembly of these complexes during chloroplast biogenesis requires the coordinate expression of proteins from both genomes, as the complexes will not accumulate in the absence of a single constituent component (reviewed in Rochaix, 1987). Although most of the proteins and several of the genes which encode them have been extensively characterized, little is known about the mechanism by which chloroplast and nuclear gene expression is coordinated during the formation of the photosynthetic apparatus. As a model for studying the regulation of synthesis and assembly of the thylakoid membrane complexes, we have focused our attention on the photosystem II (PSII) complex in the unicellular green alga *Chlamydomonas reinhardtii*.

The PSII complex is the site of the initial light-capturing charge-separating events of photosynthesis as well as the site of oxygen evolution. The PSII complex is composed of at least six chloroplast-encoded proteins, three nuclear-encoded proteins and an array of chlorophylls, carotenoids, quinones and lipids (Murata and Miyao, 1985). The PSII complex of *C.reinhardtii* is identical in both function and protein complement with those of higher plants (Chua and Bennoun, 1975). In *C.reinhardtii*, the chloroplast-encoded proteins known as D1, D2, P5, P6 and the apoproteins of cytochrome (Cyt) b_{559} are encoded by the *psb*A, *psb*D, *psb*B, *psb*C, *psb*E and *psb*F genes respectively. The nuclear-encoded proteins, which are all part of the oxygen-evolving complex, are known as oxygen-evolving enhancer (OEE) proteins 1, 2 and 3, and are encoded by the *psb*1, *psb*2 and *psb*3 genes respectively. The chloroplast-encoded genes for D1 and D2 have been cloned and characterized in *C.reinhardtii* (Erickson *et al.*, 1984, 1986; Rochaix *et al.*, 1984) as have the genes for P5, P6 and Cyt b_{559} in spinach (Morris and Herrmann, 1984; Alt *et al.*, 1984; Holschuh *et al.*, 1985; Herrmann *et al.*, 1984). The nuclear-encoded *psb*1, *psb*2 and *psb*3 genes have likewise been cloned and characterized in *C.reinhardtii* (Mayfield *et al.*, 1987a,b).

Several mutants of *Chlamydomonas* have been studied which carry mutations in the structural genes encoding PSII polypeptides. These include mutants affected in the gene *psb*A (Bennoun *et al.*, 1986), *psb*D (Erickson *et al.*, 1986), *psb*C (Mayfield *et al.*, in preparation), *psb*1 (Mayfield *et al.*, 1987b) and *psb*2 (Mayfield *et al.*, 1987a). The study of such mutants has proven to be a powerful approach towards understanding the assembly and function of the PSII complex. However, these mutants have provided little information concerning the events which regulate and coordinate the expression of the genes encoding the PSII complex proteins. Here we report an analysis of two nuclear mutants which show a pleiotropic deficiency in the PSII complex. These mutants are not affected in any known PSII structural gene, nor do they show effects outside the PSII complex. The reduction in accumulation of PSII proteins in these mutants is apparently due to the specific absence of translation and/or increased turnover of the chloroplast-encoded D2 protein. All PSII protein-encoding mRNAs including the mRNA for the D2 protein are present in the mutants and all of the proteins except for D2 are translated efficiently in these cells. These data suggest that a nuclear gene product regulates the expression of a chloroplast-encoded protein which itself is required for the stable accumulation of the entire PSII complex. The implication of these data in the regulation of the coordinate expression of nuclear and chloroplast genes is discussed.

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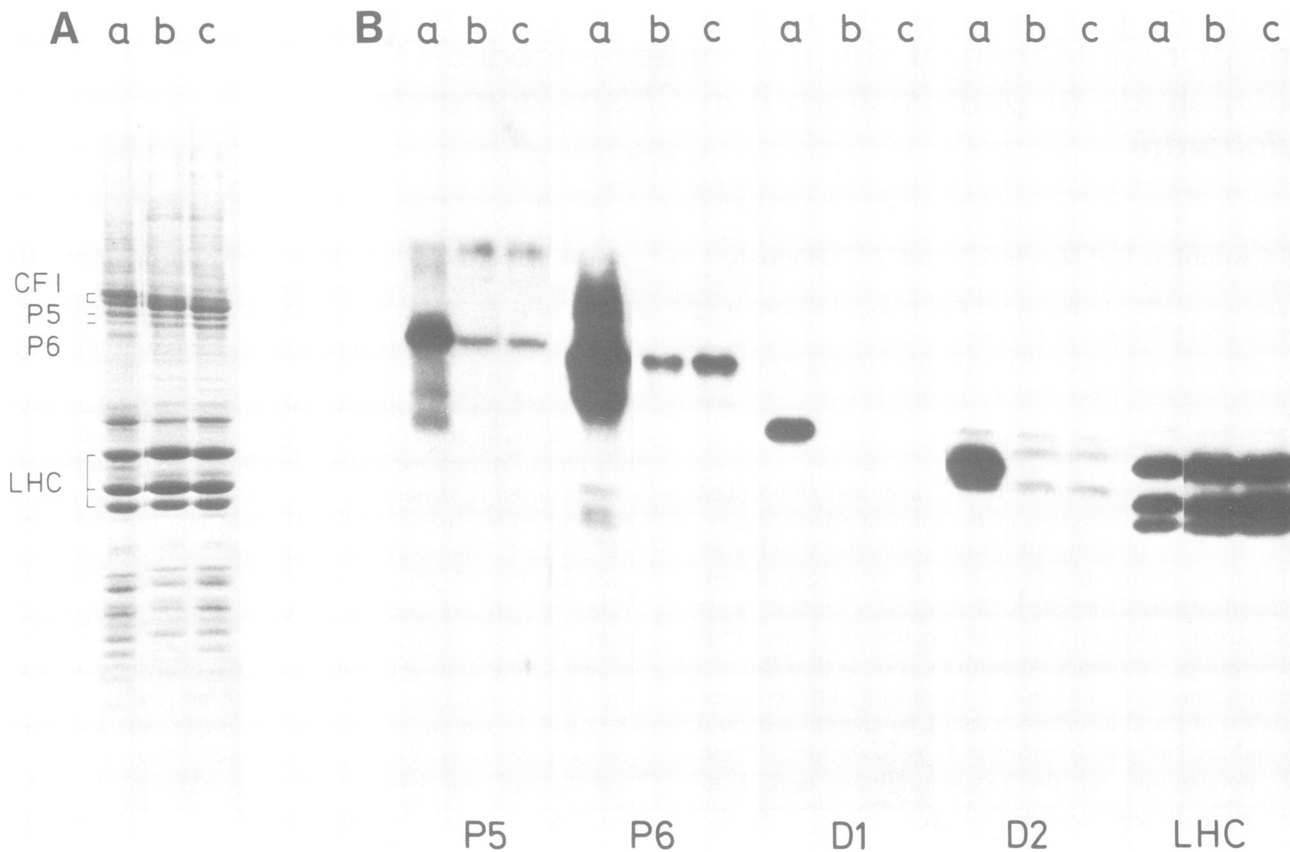

Fig. 1. Polyacrylamide gel electrophoresis of total membrane proteins from wild-type (**a lanes**), *nac-1-18* (**b lanes**) and *nac-1-11* (**c lanes**). Following electrophoresis, gels were either stained with Coomassie Blue (**A**) or electrophoretically transferred to CNBr-activated paper (**B**). The protein blots were subsequently reacted with antisera specific for PSII polypeptides, P5, P6, D1, D2 and the light-harvesting antenna chlorophyll binding proteins (LHC). Protein–antibody complexes were labeled with ^{125}I-*Staph-A* protein and visualized by autoradiography.

Results

Mutant isolation and initial characterization

Wild-type *C.reinhardtii mating-type*$^+$ cells were mutagenized with either UV light or by X-irradiation to a survival of ~10%. Photosynthetically deficient mutants were identified as high-chlorophyll fluorescent, acetate-requiring clones (Bennoun and Delepelaire, 1982). The fluorescence induction kinetics of certain of these mutant strains proved to be characteristic of a PSII-deficient phenotype (Bennoun and Delepelaire, 1982). Two independently-isolated strains, one of which was UV-induced (*nac-1-18*), the other X-ray-induced (*nac-1-11*), which both appeared to be altered in PSII by all phenotypic criteria, were chosen from among a collection of PSII mutants for comparative analysis.

To determine the genetic basis for the high-fluorescent, acetate-requiring phenotype in *nac-1-18* and *nac-1-11*, the two mutants were backcrossed to wild-type. In all 13 tetrads derived from the backcross of *nac-1-11* to wild-type, and in all 21 tetrads from the cross of *nac-1-18* to wild-type, the mutant phenotype segregated as a single Mendelian trait, i.e. as the result of a single nuclear mutation.

Photosynthetic mutant strains can be assigned to genetic complementation groups by measuring the fluorescence yield of young diploid zygotes, the immediate product of mating (Bennoun *et al.*, 1980). These zygotic cells possess a single nucleus and a single chloroplast and contain the genetic information of both parents. Mated zygotic cells form an adherent film which can be separated away from unmated cells. The fluorescence induction kinetics of these zygotic cells can then be used as a measure of genetic complementation. When the two mutant strains were backcrossed to wild-type and the fluorescence induction kinetics of zygotic cells was analyzed 48 h after mating, a wild-type pattern was observed, indicating that the mutant phenotype is recessive to wild-type. *nac-1-18* and *nac-1-11* were likewise crossed to three previously characterized nuclear mutants defective in PSII, *F64*, *F34* (Chua and Bennoun, 1975; Delepelaire, 1984) and *ac-115* (Levine and Goodenough, 1970) and the fluorescence induction kinetics of diploid zygotes was studied. Positive complementation is observed in zygotes derived from crosses between the two nuclear mutants and the strains *F64*, *F34* and *ac-115*, indicating that the mutations present in *nac-1-18* and *nac-1-11* must lie in complementation groups distinct from those affected in these other nuclear PSII mutants. However, when *nac-1-18* and *nac-1-11* were intercrossed, the fluorescence induction kinetics of zygotes produced from this mating showed a distinct PSII-deficient phenotype. This suggests that *nac-1-18* and *nac-1-11* carry mutations in the same complementation group, i.e. that the two mutations are allelic. The results of this complementation study were confirmed by the analysis of the meiotic products of the intercross between the two mutants. All segregants of the 45 tetrads derived from this

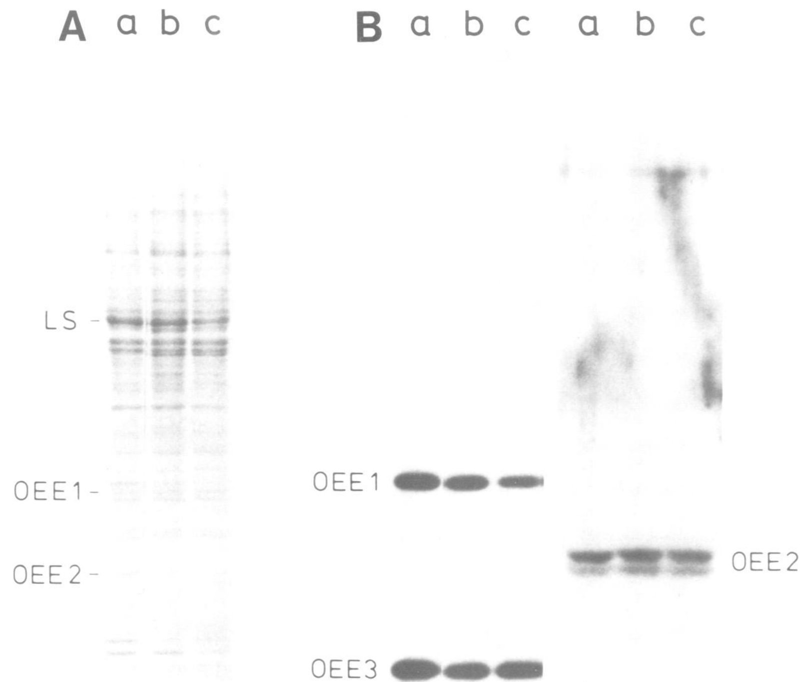

Fig. 2. Polyacrylamide gel electrophoresis of total cell soluble proteins either stained with Coomassie Blue (**A**) or electroblotted to CNBr-activated paper (**B**) and reacted with antisera specific for the polypeptides of the oxygen-evolving complex, OEE1, OEE2 and OEE3. Protein–antibody complexes were labeled with ^{125}I-*Staph-A* protein and visualized by autoradiography. Wild-type (**a lanes**), *nac-1-18* (**b lanes**) and *nac-1-11* (**c lanes**).

cross possessed an acetate-requiring phenotype, indicating tight linkage between the two mutations and suggesting that they affect the same nuclear gene.

nac-1-18 and nac-1-11 show a pleiotropic reduction in PSII polypeptides

The level of stable accumulation of PSII polypeptides in thylakoid membranes of the two mutants was assessed by immunoblot analysis. Total membrane and water-soluble protein fractions from the two mutants and the wild-type strain from which they were derived were electrophoretically separated on 7.5–15% polyacrylamide gels. The proteins were then transferred to CNBr-activated paper and reacted with antisera raised against individual PSII polypeptides. *nac-1-18* and *nac-1-11* membrane fractions show a pleiotropic reduction in all PSII core polypeptides (Figure 1). The mutants accumulate < 10% of polypeptides 5 and 6 and are completely deficient in D1 and D2. In contrast, the accumulation of the proteins of the light-harvesting complex (LHC) proceeds normally in the mutants (Figure 1), indicating that the presence of this complex is not dependent on the stable accumulation of the PSII core proteins. Water-soluble proteins were similarly analyzed with antibodies specific to the OEE1, OEE2 and OEE3 proteins of the oxygen-evolving complex to determine the extent of peripheral PSII polypeptide accumulation. The polypeptides of the oxygen-evolving complex are present at wild-type levels in the two mutants (Figure 2). Therefore, the *nac-1-18* and *nac-1-11* mutations result in the reduced accumulation of the PSII reaction center proteins only.

PSII polypeptide-encoding mRNAs are present in nac-1-18 and nac-1-11

In order to determine how the mutations present in *nac-1-18* and *nac-1-11* prevent the accumulation of PSII polypeptides, mRNAs encoding PSII proteins were measured by Northern analysis. Equal amounts of total cell RNA from wild-type and the two mutants were separated on denaturing agarose gels, electroblotted to nylon membranes and hybridized with nick-translated, gene-specific probes. The messages for P5, P6, D1, D2, OEE1, 2 and 3, and the large subunit (LSU) of ribulose bisphosphate (RuBP) carboxylase are all present in the two mutants (Figure 3). There is, however, some variability with respect to message abundance. The level of D2 message is elevated 2- to 3-fold over wild-type levels in *nac-1-18* and *nac-1-11* and similar increases are also observed for OEE1 and OEE3 mRNA. It is clear from these results though that the failure to accumulate PSII polypeptides in the two mutants is not caused by a block of transcription of PSII protein-encoding genes.

nac-1-18 and nac-1-11 fail to synthesize D2 protein

Since all PSII polypeptide-encoding mRNAs are present at least at wild-type levels in the two mutants, it was of interest to determine whether the reduction in accumulation of polypeptides P5 and P6 and the complete absence of D1 and D2

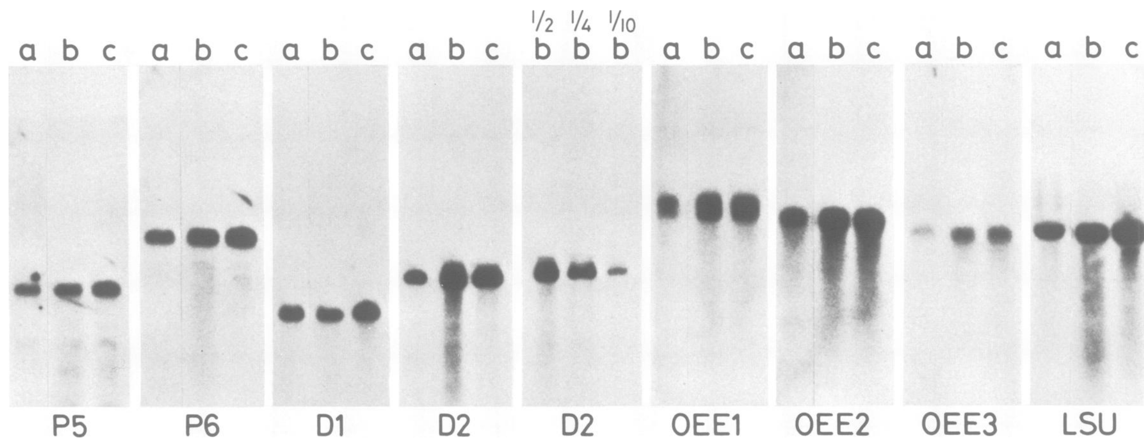

Fig. 3. Northern analysis of mRNA isolated from wild-type (**a lanes**), *nac-1-18* (**b lanes**) and *nac-1-11* (**c lanes**). Total RNA was separated on denaturing formaldehyde gels, electroblotted to nylon membranes and hybridized with nick-translated gene-specific probes. A dilution series of *nac-1-18* RNA (1/2b, 1/4b and 1/10b) was probed with a nick-translated chloroplast DNA fragment encoding the D2 protein to compare mutant RNA levels with wild-type.

was due to a block in the synthesis of any of these PSII polypeptides or whether this deficiency reflected an instability of the complex as a whole. Chloroplast protein translation was studied by pulse-labeling mutant cells with [^{14}C]acetate for 10 min in the presence of 10 μg/ml cycloheximide, an inhibitor of cytoplasmic protein synthesis. Membrane proteins were then electrophoretically separated and the resultant bands correlated to known PSII polypeptides, as shown in Figure 4. It appears that all of the major PSII core components are synthesized normally in the two mutants, with the single exception of the D2 polypeptide which is completely missing in both strains. The identity of the D2 protein and its absence in *nac-1-18* and *nac-1-11* can be verified by comparing the protein pattern of the two mutants with that of *FuD47*, a chloroplast mutant which carries a structural gene mutation in *psb*D (Erickson *et al.*, 1986). As in *FuD47*, the two nuclear mutants fail to synthesize D2 protein. It is also clear that although *nac-1-18* and *nac-1-11* fail to accumulate any D1 protein as evidenced by immunoblot analysis, the two strains are able to synthesize this protein. Therefore, the reduction in the extent of accumulation of core components P5 and P6 and the complete absence of D1 protein must be the result of an increased rate of protein turnover due to the apparent instability of the PSII complex. We conclude from these data that *nac-1-18* and *nac-1-11* carry mutations in a nuclear gene whose product in some way regulates the translation and/or turnover of the chloroplast-encoded D2 polypeptide.

Discussion

The products of nuclear genes are required for chloroplast gene expression, chloroplast development and the functioning of the photosynthetic apparatus. A powerful method to reveal levels of interaction and coordination between the nuclear and chloroplast genomes is to perturb the system by mutation, an approach we have used in this study of the PSII complex of *C.reinhardtii*. We have analyzed two mutants which, as a result of mutations within the same nuclear gene, appear to be specifically affected in the synthesis and/or degradation of the chloroplast-encoded D2 polypeptide. Other PSII core polypeptides are synthesized at near wild-type levels in the mutants although they do not accumulate, presumably because of increased turnover in the absence of the D2 polypeptide.

nac-1-11 and *nac-1-18* have been shown to be genetically distinct from three other nuclear PSII mutants, *ac-115* (Levine and Goodenough, 1970) and two others which are affected in the accumulation of P6 (*F34* and *F64*) (Chua and Bennoun, 1975; Delepelaire, 1984). Recently, Jensen *et al.* (1986) have identified a mutant of *C.reinhardtii* which is specifically affected in the synthesis of D1 and P5, as well as a mutant which fails to accumulate transcripts of *psb*B. Therefore, it is becoming increasingly apparent that one, or in some cases several, nuclear gene products are required for the synthesis of individual chloroplast-encoded proteins and that these distinct nuclear gene products affect different chloroplast proteins.

The pleiotropic reduction of all PSII core polypeptides in *nac-1-11* and *nac-1-18* is consistent with findings of previous mutant studies. The loss of a PSII core component, whether as a result of a structural gene mutation or because of a mutation in a gene regulating the synthesis of that polypeptide, has been shown to destabilize the entire PSII complex (Metz and Miles, 1982; Leto *et al.*, 1985; Bennoun *et al.*, 1986; Erickson *et al.*, 1986; Jensen *et al.*, 1986). Thylakoid membranes isolated from such mutants are deficient in all PSII core components as well as in the peripheral proteins associated with oxygen evolution, OEE1, 2 and 3. Typically, the loss of those proteins not directly affected by mutation occurs post-translationally, although there is some evidence for coordination at the level of translation between the D1 and D2 proteins (Erickson *et al.*, 1986) and between the D1 and P5 proteins in *C.reinhardtii* (Jensen *et al.*, 1986). Immunoblot analysis has shown that *nac-1-11* and *nac-1-18* have reduced levels of all integral PSII components, but stably accumulate the peripheral polypeptides OEE1, 2 and 3. Recent results from our laboratory have shown that mutants with primary lesions in *psb*A, *psb*C or *psb*D likewise accumulate wild-type levels of OEE2 and OEE3 and only in some cases have somewhat reduced amounts of OEE1 (Mayfield *et al.*, in preparation). Similarly, nuclear mutants of barley which lack PSII core polypeptides have also been shown to accumulate the OEE2 protein normally (Honberg, 1984). It is likely that the OEE proteins are not associated with the thylakoid membrane in a normal manner in these

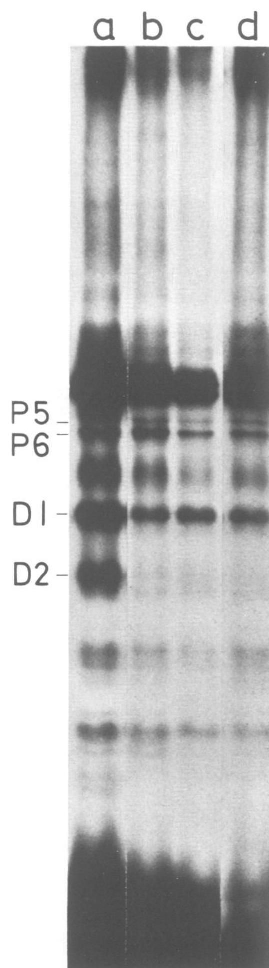

Fig. 4. Autoradiograph of pulse-labeled total cell protein. Cells were labeled for 10 min in the presence of 10 μg/ml cycloheximide (**lane a**—wild-type, **lane b**—*nac-1-18*, **lane c**—*nac-1-11*, **lane d**—*FuD47*). Strain *FuD47* is a chloroplast mutant which carries a 46-bp duplication in its *psb*D gene and fails to synthesize D2 polypeptide (Erickson *et al.*, 1986). Polypeptides P6 and D1 were identified by running pulse-labeled protein samples from strains *F34* and *FuD7*, mutants deficient in the synthesis of P6 (Delepelaire, 1984) and D1 (Bennoun *et al.*, 1986) respectively, as controls on this gel system. The band appearing between P6 and D1 is a degradation product of P5, a protein which is unstable in our preparations. The heavily labeled region above P5 is composed of LSU and the α and β subunits of coupling factor 1

mutants, which would explain why other authors have reported deficiencies in the OEE polypeptides from PSII mutant protein preparations.

All PSII polypeptide-encoding mRNAs are present in *nac-1-11* and *nac-1-18*. The only difference between the mutants and wild-type is that the levels of D2, OEE1 and OEE3 encoding messages are elevated several fold in mutant cells. It may be that the *psb*D mRNA level is controlled by D2 through some sort of feedback mechanism. However, a reduced level of *psb*D message was found in *FuD47*, a mutant containing a 46-bp duplication in its *psb*D gene and lacking the D2 protein (Erickson *et al.*, 1986). Possibly, the presence of this small insertion may destabilize the *psb*D message. Also, similar changes in *psb*C transcripts are not observed in nuclear mutants unable to synthesize P6, the product of *psb*C (Mayfield *et al.*, in preparation). The reason for the increase in mRNA encoding OEE1 and OEE3 is not clear. The analysis of other nuclear mutants affected in D2 translation as well as revertants derived from these strains may provide more insights into this problem.

The results of pulse-labeling experiments suggest that the primary effect of the mutations in *nac-1-11* and *nac-1-18* is a specific block in the translation of the D2 protein. However, we cannot rule out that the D2 protein is still synthesized in these mutants but that its turnover rate is vastly increased. This possibility seems unlikely though, as the half-life of D2 in PSII-deficient mutants has been estimated to be of the order of 1 h (Jensen *et al.*, 1986) and we see no D2 protein synthesized in the mutants in 10 min pulse-labelings. The protein pattern of *nac-1-11* and *nac-1-18* cells mimics that of *FuD47*, a mutant carrying a structural gene mutation in *psb*D: all chloroplast-encoded PSII polypeptides are synthesized normally with the single exception of D2. It is noteworthy that the synthesis of D1 is not markedly reduced in *FuD47* as compared with wild-type, an observation which is at variance with the previous characterization of this mutant (Erickson *et al.*, 1986). The pleiotropic reduction of PSII core polypeptides in these mutants must then reflect the general instability of the PSII complex in the absence of D2 and is probably due to the post-translational degradation of these proteins. *nac-1-11* and *nac-1-18*, therefore, fall into a general class of mutants which, as a result of nuclear mutation, are blocked in the accumulation of organellar-encoded gene products. Such mutants have been described in *Chlamydomonas* (Chua and Bennoun, 1975; Jensen *et al.*, 1986), as well as *Zea mays* (Metz and Miles, 1982; Leto *et al.*, 1985; Barkan *et al.*, 1986), *Hordeum vulgare* (Honberg, 1984) and *Saccharomyces cerevisiae* (Cabral and Schatz, 1978; Müller *et al.*, 1984; Dieckmann and Tzagoloff, 1985; Rödel *et al.*, 1985).

In the yeast *S.cerevisiae*, several nuclear *pet* mutants deficient in mitochondrial function have been characterized. Certain of these mutants fail to accumulate either individual mitochondrial gene products or small groups of mitochondrial-encoded proteins (Michaelis *et al.*, 1982). In some cases, it has been shown that nuclear gene products are required for the translation of specific mitochondrial-encoded mRNAs. For example, *pet494* mutants of yeast synthesize normal levels of all mitochondrial proteins with the exception of subunit III of cytochrome oxidase, *coxIII* (encoded by the mitochondrial gene *oxi2*) (Müller *et al.*, 1984). The synthesis of *coxIII* is blocked post-transcriptionally in *pet494* mutants, as *oxi2* mRNA is present at wild-type levels in these cells (Müller *et al.*, 1984). Two lines of evidence suggest that the *PET494* protein promotes the translation of the mitochondrial *oxi2* mRNA. First, the *PET494* protein can be found inside yeast mitochondria, suggesting that it could interact directly with *oxi2* message (Costanzo and Fox, 1986), and second, mitochondrial suppressors of *pet494* mutants have been found in which the normal 5′ noncoding region of the *oxi2* message is replaced with foreign mitochondrial sequences, thereby creating new translation initiation codons and circumventing the requirement for *PET494* (Müller *et al.*, 1984; Costanzo and Fox, 1986). Recently, it was shown that translation of *oxi2* message is dependent on at least two other nuclear genes which may function analogously to *PET494* (Costanzo *et al.*, 1986; Fox, 1986). The mitochondrial genes encoding cytochrome oxidase subunit II (*coxII*) and cytochrome *b* (*cob*) are similarly under the translational control of nuclear-encoded proteins (Cabral

and Schatz, 1978; Dieckmann and Tzagoloff, 1985; Rödel *et al.*, 1985).

It is possible that the *NAC-1* gene product functions in a manner analogous to *PET494* in *Chlamydomonas* to promote the translation of *psb*D mRNA. Alternatively, *NAC-1* may encode a protein which physically interacts with D2, stabilizing it within the thylakoid membrane. We have recently isolated photosynthetically competent revertants from *nac-1-11* and *nac-1-18*. Genetic analyses have revealed that reversion is due to the presence of second site suppressor mutations. The identification of the genetic basis for suppression and the biochemical analysis of these revertants will greatly aid our understanding of the specific role of *NAC-1* in the expression of *psb*D and, more generally, will help to explain the involvement of the nuclear genome in chloroplast gene expression.

Materials and methods

Strains, mutant isolation and genetic characterization

Wild-type *137C; mating type*$^+$ and mutant strains were typically maintained on Tris−acetate phosphate (TAP) medium at 25°C and 250 lux (Gorman and Levine, 1965). To isolate photosynthetic mutants, wild-type cells were grown to mid-log phase and mutagenized in liquid culture with either UV light (3 min of illumination, 48 cm from a germicidal lamp) or with X-rays (2500 rad) to a survival of 10%. Mutagenized cultures were then kept in complete darkness for 24 h to allow for mutational expression and to prevent photorepair. Photosynthetic mutants were identified on the basis of three criteria. First, following mutagenesis, colonies growing on agar plates containing TAP medium were screened for high or low fluorescence (Bennoun and Delepelaire, 1982). Mutant candidates were then transferred to a TAP master plate and replica-plated onto minimal medium (Sager and Granick, 1953) in order to identify clones which required acetate for growth. Finally, the fluorescence induction kinetics of putative mutant strains were determined as described (Bennoun and Delepelaire, 1982).

Gametes were made by starving cells for nitrogen overnight (Kates and Jones, 1964). Matings, tetrad dissection and the scoring of meiotic products was performed following standard genetic protocols for *C.reinhardtii* (Levine and Ebersold, 1960).

Complementation analysis in young zygotes was performed as described by Bennoun *et al.* (1980).

Protein isolation, polyacrylamide gel electrophoresis and immunoblot analysis

Protein isolation and sample preparation were performed as described in Mayfield *et al.* (1987a). Electrophoretic separation of proteins was carried out on 7.5−15% polyacrylamide gels according to the protocol of Chua (1980). Proteins were then either stained with Coomassie Blue or electroblotted onto CNBr-activated paper (Clark *et al.*, 1979) and reacted with antisera directed against individual PSII polypeptides (Nelson *et al.*, 1984). The antibodies against P5, P6, D2, OEE1, OEE2, OEE3 and LHC, obtained from N.H.Chua, were raised against gel purified thylakoid membrane polypeptides. The antibody against D1, obtained from L.McIntosh, was raised against D1 protein expressed from a recombinant plasmid in *Escherichia coli*.

RNA isolation, electrophoresis and Northern analysis

RNA was isolated with guanidinium hydrochloride as described (Nelson *et al.*, 1984). RNA was electrophoretically separated on denaturing formaldehyde agarose gels (Lehrach *et al.*, 1977) and electroblotted to nylon membranes (Khandjian, 1986). Prehybridization and hybridization were performed as in Johnson *et al.* (1984). RNA was hybridized to nick-translated gene-specific probes. Plasmids which carry the chloroplast genes from *C.reinhardtii* of D1, D2, P5, P5 and LSU (Rochaix, 1981) and nuclear sequences of OEE1, OEE2 and OEE3 (Mayfield *et al.*, 1987a,b) were used as a source of probes.

Pulse-labeling of proteins with [^{14}C]acetate

The procedures for cell culture, protein labeling and sample preparation were followed as described in Mayfield *et al.* (1987b).

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