The Deletion of petG in Chlamydomonas reinhardtii Disrupts the Cytochrome bf Complex*

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The 4-kDa protein encoded by chloroplast petG copurifies with the cytochrome bf complex of spinach and is found in a number of other photosynthetic organisms, including the eukaryotic alga Chlamydomonas reinhardtii. To determine whether petG is involved in the function or assembly of the cytochrome bf complex, the gene was cloned from C. reinhardtii, excised from the DNA fragment, and replaced with a spectinomycin resistance cassette. A petG deletion strain of C. reinhardtii was then obtained by biolistic transformation. The resulting homoplasmic petG deletion strains are unable to grow photosynthetically, and immunoblot analysis shows markedly decreased levels of cytochrome b, cytochrome f, the Rieske iron-sulfur protein, and subunit IV. To verify that this phenotype was due to the removal of petG, we also constructed a strain with a deletion in the open reading frame (ORF56), which is found 25 base pairs downstream of petG. The ORF56 deletion strain grew photosynthetically and had wild-type levels of the four major cytochrome bf subunits. We conclude that the absence of the PetG protein affects either the assembly or stability of the cytochrome bf complex in C. reinhardtii.

The cytochrome bf complex, found in green plants, eukaryotic algae, and cyanobacteria, serves to connect photosystem I to photosystem II in the chloroplastic or cyanobacterial electron transport chain. The complex functions as a plastoquinol/plastoquinone oxidoreductase, the rate-limiting step of the photosynthetic electron transport chain. These redox reactions are coupled to an efficient translocation of protons across the membrane; the resulting proton gradient provides a source of energy for the synthesis of ATP by the chloroplasm or cyanobacterial ATP synthase (1-3).

The cytochrome bf complex contains four large subunits. Three of these (cytochrome b6, cytochrome f, and the Rieske iron-sulfur protein) bind redox-active prosthetic groups. The remaining large subunit (subunit IV), together with cytochrome b6 forms the binding sites for plastoquinone oxidation and reduction. In addition to the four large subunits, a number of smaller polypeptides have been found to be associated with the cytochrome bf complex. A 4-kDa protein copurifies with the cytochrome bf complex in spinach, and antibodies raised to a synthetic decapetide derived from maize petG cross-react with preparations from spinach, tobacco, pea, wheat, and rice, although not to those from Chlamydomonas reinhardtii or Synechoystis 6803 (4). Several small proteins were found with the spinach and the C. reinhardtii cytochrome bf complex, among them the PetG protein (migrating at 4.8 kDa in spinach and at 4.1 kDa in C. reinhardtii) and another protein believed to be a nuclear gene product (migrating at 3.7 kDa in spinach and at 3.8 kDa in C. reinhardtii) (5, 6). The function of these small polypeptides is unknown.

Previously, the sequence and location of petG in the C. reinhardtii chloroplast genome were reported (7). To investigate the function of petG, we deleted the gene from the chloroplast, inserting in its place a spectinomycin resistance cassette containing a Chlamydomonas atpA promoter region and the bacterial aadA gene (8). Our results show that the petG deletion strains are incapable of photosynthetic growth, but that they grow heterotrophically on acetate. Components of the cytochrome bf complex are markedly diminished in these strains, indicating that the petG gene product is required for either the stability or assembly of the complex.

EXPERIMENTAL PROCEDURES

Cell Strains and Culture Conditions—C. reinhardtii wild-type strains CC-124, CC-125, and CC-1928 were obtained from the Chlamydomonas Culture Collection (Duke University), and wild-type strain 137 was obtained from M. Goldschmidt-Clermont (University of Geneva, Switzerland). Cells were grown on plates or in liquid culture at 20–25 °C under dim light in TAP or HSA medium or, where noted, in HS minimal medium (9).

Cloning petG—Oligonucleotides were synthesized by the DNA Synthesis Facility (Barker Hall, University of California, Berkeley, CA). End labeling of the multiply degenerate oligonucleotide and Southern hybridization procedures were performed according to Sambrook et al. (10). Chlamydomonas chloroplast DNA was isolated on a cesium chloride gradient using bisbenzimide dye (11). Plasmid DNA was sequenced using Sequenase following the manufacturer’s instructions (U. S. Biochemical Corp.) or a modification of this procedure (12). Chlamydomonas cell extracts were prepared, and polymerase chain reactions were performed as described previously (13). Sequencing of double-stranded polymerase chain reaction products involved removal of primers by washing the oligo-free 50-μl reaction mixture three times with 400 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) followed by concentration in a 100-kDa cutoff filter unit (Ultrafree-MC, Millipore Corp., Bedford, MA). The final volume was adjusted to 42 μl. 7 μl of polymerase chain reaction product was combined with 10 pmol of sequencing primer, 1 μl of dimethyl sulfoxide, and 2 μl of Sequenase buffer and annealed by heating at 95 °C for 3 min and immediately cooling on dry ice. Sequencing was then carried out following the modified Sequenase procedure (12).

The following degenerate oligonucleotide was designed from the N-terminal region of the 4.1-kDa protein isolated from C. reinhardtii (S): ATGTTG(T/A)GAAGCT(T/A)CTTCTGT(G/C)(T/A)GGTAT(T/C)GT. A size-specific library of PstI-HindIII-digested chloroplast DNA (from wild-type C. reinhardtii strain CC-125) was constructed in pBlueScript and probed, and a 3.6-kb insert containing petG was isolated. Sequencing of petG revealed a discrepancy in the N-terminal protein sequence at the

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§ The abbreviations used are kb, kilobase(s); bp, base pair(s); ORF, open reading frame.
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FIG. 1. Map of the region of the chloroplast DNA containing petG. A, shown is a diagram of plasmid pG35 containing pssl, petG, ORF56, and part of ORF712. The striped bars indicate the DNA fragments cloned to serve as probes for these four reading frames. B, petG is replaced with the aadA cassette to form plasmid pG14G. C, ORF56 is disrupted with the aadA cassette to yield p56A15. The sizes of the fragments that would be generated from a petG-aadA cassette are shown. The restriction sites depicted are not necessarily unique.

The position of the seventh amino acid; it is cysteine, in agreement with the report of Feng and Surzycki (7).

Construction of pG35 and p56A15—To construct the petG deletion, the 3.5-kb PstI-HindIII chloroplast DNA fragment containing petG was cloned into pBluescript SK+ to produce pG35 (Fig. 1A). pG35 was then cut with AflII, filled in, and recloned with PstI. The 1.2-kb fragment released was cloned into the PstI-Smal site of pUC19, destroying the Smal site, but regenerating the AflII site to form pUG. pUC-aptX-aadA was cut with HindIII and filled in to generate a 1.4-kb cassette containing the aptX promoter region followed by the bacterial aadA gene, which confers spectinomycin resistance when inserted into the C. reinhardtii chloroplast genome (8). pGA was partially digested with SspI to remove a 134-bp fragment containing the petG coding region, and blunt-ended aptX-aadA was ligated into the gap to create pG14. The orientation of the cassette in pG14 is such that aadA is transcribed in the same direction as for petG; pG35 was opened at the HindIII site in the multiple cloning site, and an EcoRI linker was inserted there to form pG35E. pG35E was cut with AflII and EcoRI to release a 2.4-kb fragment, which was inserted into the AflII and EcoRI sites in pG14 to create pG14G (Fig. 1B).

For the ORF56 deletion, pUC-aptX-aadA was cut with Clal and PstI, releasing a 1.5-kb fragment, which was cloned into pBS-KAS+, a modification of pBluescript in which the Smal site is replaced with AflII. This permitted the subsequent excision of the aptX-aadA cassette with Clal and AflII. pG35 was partially digested with KpnI, and the 3′-ends of the isolated single-cut fragment were blunted and religated to destroy the KpnI site in the multiple cloning region, creating pG35mK. pG35mK was cut with KpnI and AflII to generate a 462-bp fragment and a very large fragment that contained the vector. Both fragments were isolated. The 462-bp fragment was further digested with TaqI to release a 73-bp piece containing part of the ORF56 coding region. The remaining 391-bp KpnI-TaqI fragment was ligated to the isolated large KpnI-AflII vector-containing fragment and the Clal-AflII-cut cassette (see above) in a single reaction to create p56A15, taking advantage of the compatible cohesive ends generated by TaqI and Clal (Fig. 1C).

Transformation of Chlamydomonas—Plasmid DNA was isolated for transformation using QIAGEN columns, and 1–3 μg was precipitated onto either M10 tungsten particles (Analytical Scientific Instruments, Alameda, CA) or 1-μm gold particles (BioRad) by standard methods (14). Chlamydomonas cells were grown and plated for transformation as described by Boynton et al. (15). The transformation utilized a PDS-1000/Hebiologic device (DuPont NEN) using conditions described by Whitelegge et al. (16).

Hybridization Probes—To identify pssl, petG, ORF56, and ORF712 sequences, the probes pPSBL, pG5, pS6, and pO712, respectively, were used (Fig. 1A). In addition, the following fragments were isolated as probes: an 800-bp HindIII-AflII petA fragment, a 960-bp SacI-SstI fragment, a 300-bp PstI-HindIII petD-PstI fragment containing the petD coding region and ~40 bases of the S1-noncoding region, and a 580-bp ApaI-PvuI petB fragment. An 800-bp NcoI-PstI fragment containing the aadA gene from pUC-aptX-aadA was cloned into the EcoRV and PstI sites of pBluescriptII (eliminating all Chlamydomonas sequence) to form pAADA-NP. The 800-bp fragment was then cut from pAADA-NP to use as a probe for the aadA sequence. All probes were labeled using [32P]-dCTP by the random primer method (10).

Northern Hybridization—Total RNA was isolated from 500 μl of mid-log phase Chlamydomonas cells by a method modified from Merchant and Bogorad (17). The cells were pelleted and resuspended in 10 μl of TEN buffer (50 mM Tris-HCl, 150 mM NaCl, 15 mM EDTA, pH 7.5) containing 2% SDS and 40 μg/ml proteinase K by shaking at room temperature for 20 min. 10 μl of TEN buffer-saturated phenol was added, and the bottle was returned to the shaker for 20 min. The suspension was transferred to a sterile capped polypropylene tube and centrifuged at 20°C for 10 min at 11,000 × g. Organic extractions of the aqueous phase and LiCl precipitation of RNA were as described (17). The final RNA pellet was resuspended in 0.1 M sodium acetate, 5 mM magnesium acetate, pH 5.2, and digested with RNase-free DNase for 30 min at 37°C. The RNA was then extracted once with phenol and once with chloroform and precipitated with 2.5 volumes of ethanol.

RNA was electrophoresed in a 1.5% agarose gel containing 50 μM HEPES, 1 mM EDTA, pH 7.8. The running buffer contained in addition, 16% formaldehyde. Samples were prepared by denaturing in 50% formamide, 16% formaldehyde, 50 mM HEPES, 1 mM EDTA, 10% glycerol, pH 7.8, plus a small amount of tracking dye. Following electrophoresis, RNA was transferred to Hybond-N (Amersham Corp.) by a standard capillary method (10). The bands were UV-cross-linked and then visualized with 0.02% methylene blue in 0.3 M sodium acetate, pH 5.5. The RNA blot was probed with 10–50 ng of [32P]-dCTP-radiolabeled DNA at 65°C overnight in 10 μl of GM buffer (0.3 mM NaH2PO4, pH 7.2, 1 mM EDTA, 1% SDS). The blots were then washed four times; twice at 22°C for 15 min with 2 × SSC, 0.1% SDS and twice at 65°C for 30 min with 0.2 × SSC, 0.1% SDS.

Southern Hybridization—Chlamydomonas DNA either was isolated using a DNA mini prep (18) or was recovered from the LiCl supernatant of the RNA preparation described above. The latter method utilized a standard ethanol precipitation and RNase treatment to purify the DNA (10). Conditions for electrophoresis, transfer, and hybridization were conventional (19).

Antibody Production—A peptide (SKVYDFEEERLE-C-) was de-
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RESULTS

Sequencing of petG cloned from C. reinhardtii strain CC-125 revealed a transposition of a guanosine and a thymidine corresponding to the N-terminal region of C. reinhardtii cytochrome b6 with an added cysteine. It was synthesized and coupled to keyhole limpet hemocyanin at the University of Kentucky Macromolecular Structure Analysis Facility (Lexington, KY). This peptide conjugate (0.25 mg/ml) was used with Ribi adjuvant to inoculate rabbits at several subcutaneous sites using a standard regimen (20).

Immunoblotting—Protein samples were run on denaturing 15% polyacrylamide gels by the method of Laemmli (21). Samples for electrophoresis were prepared by extracting concentrated Chlamydomonas cells (5–15 mg/ml) with 4 volumes of acetone and centrifuging for 5 min to pellet the protein. The pellet was rinsed with acetone and allowed to air-dry briefly. The pellet was then solubilized with 30 μl of sample buffer (1% SDS, 12.5 mM Tris, pH 6.8, 100 mM dithiothreitol) and incubated at 65°C for 3 min. Loading dye (6 μl; 60% glycerol, 10 mg/ml bromphenol blue) was added, and the sample was loaded immediately. Lanes were loaded on an equal chlorophyll basis (22). Following electrophoresis, protein was transferred to nitrocellulose and detected using enhanced chemiluminescence (ECL, Amersham Corp.). Incubation, washes, and development were carried out following the manufacturer’s instructions. Rabbit sera containing polyclonal antibodies generated against spinach cytochrome f, spinach subunit IV, maize Rieske iron-sulfur protein (from Dr. A. Barkan, University of Oregon), or a synthetic peptide conjugate (corresponding to C. reinhardtii cytochrome b6) were used at a 1:10,000 dilution. The horseradish peroxidase-coupled goat anti-rabbit antibody was used at a 1:5000 dilution.

Amino acid sequence homology of PetG polypeptides. Identical amino acids are represented by dashes. Arginine in the C. reinhardtii sequence is underlined. The sequences for Beta vulgaris (sugar beet) (23), Oryza sativa (rice) (24), and Nicotiana tabacum (common tobacco) (25) are also known and are identical to that for Zea mays (maize) (4). The Spinacia oleracea (spinach) sequence was submitted to the SwissProt Data Bank by R. Oelmüller (1993). The sources for the remaining sequences are as follows: E. gracilis, Ref. 26; C. reinhardtii; this work; C. eugametos, Ref. 27; C. paradoxa, Ref. 28; Pinus thunbergii, Ref. 29; M. polymorpha, Ref. 30; and Cuscuta reflexa, Ref. 31.

Euglena gracilis
Chlamydomonas reinhardtii
Chlamydomonas eugametos
Chlamydomonas paradoxa
Pinus thunbergii
Marchantia polymorpha
Spinacia oleracea
Zea mays
Cuscuta reflexa

Signs confirmed this sequence (Fig. 2).

Immunoblot analysis of the petG deletion strains. Total DNA from each strain was digested with PstI and HindIII, run on an agarose gel, and hybridized with a psbl probe (A) (see Fig. 1) or an aadA probe (B). Lane 1, wild-type CC-125; lane 2, GF-3; lane 3, GF-6; lane 4, 56-2A; lane 5, 56-6A.

Immunoblotting—Immunoblot analysis of the petG deletion strains GF-3 and GF-6 showed substantially reduced levels of cytochrome b6, subunit IV, cytochrome f, and the Rieske iron-sulfur protein relative to the wild-type levels of these proteins (Fig. 5). One level of protein was estimated to be 5–20% of the wild-type level for cytochrome b6 and subunit IV and 10–20% of the wild-type level for cytochrome f and the Rieske iron-sulfur protein, with some variation between the two petG deletion strains. There is an obvious difference in the level of protein found in GF-6 versus GF-3, with GF-3 having consistently lower levels of cytochrome bf complex proteins (Fig. 5). Other independent GF- transformants were observed to fall between...

Fig. 2. Amino acid sequence homology of PetG polypeptides.

Fig. 3. Southern analysis of petG and ORF56 deletion strains. Total DNA from each strain was digested with PstI and HindIII, run on an agarose gel, and hybridized with a psbl probe (A) (see Fig. 1) or an aadA probe (B). Lane 1, wild-type CC-125; lane 2, GF-3; lane 3, GF-6; lane 4, 56-2A; lane 5, 56-6A.
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These extremes. The ORF56 strains appeared as wild type in the levels of the subunits of the cytochrome bf complex (Fig. 5).

Northern Blots—The loss of subunits of the cytochrome bf complex in the absence of petG, as determined by immunoblotting, either could be due to instability of the assembled complex, leading to degradation of the individual components, or could be caused by a problem in transcription. To rule out the latter possibility, total RNA was isolated from each of the strains and hybridized with DNA probes for each of the cytochrome subunits IV (subIV) or Rieske iron-sulfur protein (Rieske). Lane 1, GF-3; lane 2, GF-6; lane 3, 56-2A; lane 4, 56-6A; lane 5, CC-125. The arrow indicates the band corresponding to the Rieske iron-sulfur protein; the lower band, in this blot, is a nonspecific cross-reaction of the anti-maize antibodies with an unidentified Chlamydomonas protein. The source and dilution of the antibodies are described under “Experimental Procedures.”

In addition to transcript levels of pet genes, we analyzed transcript from genes and reading frames in the neighborhood of petG and ORF56 to see if these were affected by the removal of petG or ORF56 and the insertion of the aadA cassette. The petD gene (upstream from petG; see Fig. 1) generated transcript levels that varied among the independent isolates of each deletion: in GF-6 and 56-2A, the transcript level was diminished relative to the wild-type strain, but in GF-3 and 56-6A, the level was normal (Fig. 6). The situation with ORF56 and ORF712, however, was still more complex. In the wild-type strain, neither reading frame yielded a single defined transcript, but rather, very low levels of several large, possibly unprocessed, precursors (Fig. 7A). In the GF- and 56-strains, the size of several of these large transcripts increased, suggesting that there was initiation at the atpA promoter portion of the inserted aadA cassette. This was verified by hybridization with an aadA probe (Fig. 7B). Although it is unclear whether these large low-level transcripts have any function, their levels are undiminished in the GF- and 56-strains, and we have concluded that it is unlikely that they have any role in the observed nonphotosynthetic phenotype of the petG deletion strains.

DISCUSSION

C. reinhardtii petG was cloned from chloroplast DNA of strain CC-125 using an oligonucleotide probe to identify the gene. Sequencing of petG from three C. reinhardtii strains showed a transposition of guanosine and thymidine in codon 30, resulting in an arginine rather than a leucine at this position, in contrast to a previous report (7). This arginine is present in the derived PetG amino acid sequence from Marchantia polymorpha, and all higher plants for which the
sequence is known (Fig. 2). It is absent only in Euglena gracilis among all the PetG sequences determined to date.

To determine whether the PetG protein is essential for the functioning of the cytochrome bf complex, petG was deleted and replaced with a spectinomycin resistance cassette. This resulted in the loss of photosynthetic function and decreased levels of all of the cytochrome bf complex subunits. In contrast, Chlamydomonas mutants that contained a disruption of the open reading frame immediately (25 bp) downstream of petG, ORF56, were fully photosynthetic and showed normal levels of cytochrome bf subunits. Therefore, the loss of cytochrome bf activity and protein was not due to an indirect effect on an adjacent reading frame, but rather was the result of the loss of petG itself.

The diminished level of cytochrome bf subunits could result from an aberration in transcription or processing of RNA transcripts or may be a result of a problem with the assembly or stability of the assembled complex in the absence of the PetG protein. However, Northern blots of total RNA from the petG deletion mutants showed that the transcript level and size for the two chloroplast-encoded genes, petB and petD, and the nuclear-encoded PetC gene were all normal. The transcript level for petA was diminished, but this was unlikely to be the cause of the reduced level of petA protein or the inability of this strain to grow photosynthetically because strain 56-2A had even greater loss of petA transcript and yet had a wild-type level of cytochrome f and normal photosynthetic capability. It appears then that the absence of petG directly affects either the assembly or stability of the cytochrome bf complex.

The altered level of petA transcript in the two petG deletion strains may be a direct consequence of the deletion of petG, or it may also be a random effect similar to the variability in the levels of psbL transcripts or the variability in transcript levels between the two ORF56 deletion strains. In the latter two cases, the variability is most likely accounted for by secondary mutations in the Chlamydomonas genome possibly induced by the growth of the cells in fluorodeoxyuridine as part of the transformation protocol. On the other hand, it was noted by Kuras and Wollman (32) in a 5-min pulse labeling of ΔpetB and ΔpetD Chlamydomonas strains, there is an extensive decrease in the synthesis of cytochrome f relative to the wild-type strain. This is not observed with cytochrome b₅ in ΔpetA or ΔpetD strains or with subunit IV in ΔpetA or ΔpetB strains. However, they attributed this decrease in synthesis to either a cotranslational or early post-translational regulation (32), not as a result of a decrease in the level of petA transcripts, as observed here with the petG deletion strain.

Other photosynthetic complexes have been observed to be destabilized in the absence of a small polypeptide component. In Chlamydomonas photosystem II, disruption of psbl, which encodes a 4.8-kDa polypeptide, causes photosensitivity of the organism and an 80–90% loss of photosystem II complex relative to wild-type levels, although photosynthetic growth is still possible (33). In contrast, a psbK disruption strain of C. reinhardtii cannot grow photosynthetically; this 4.1-kDa protein is apparently required for the assembly and/or stability of photosystem II (34).

With the cytochrome bf complex, it has been observed that the deletion of any one of the subunits causes a large decrease in the level of the remaining subunits. In Chlamydomonas, the deletion of petA results in 5% cytochrome b₆ remained, 5% subunit IV, trace amounts of the Rieske iron-sulfur protein, and no detectable PetG protein (32). With a petD deletion, 10% of cytochrome f remained, and again, 5% of cytochrome b₆ trace amounts of the Rieske protein, and no detectable PetG protein. The deletion of petB follows the same pattern, except that subunit IV is found in barely detectable amounts (32). When a full complement of subunits was not present, the levels of cytochrome b₆ and subunit IV were found to be regulated by degradation; cytochrome f, as noted above, is believed to be regulated by cotranslation or at early post-translational. Therefore, it is not surprising to find that in the absence of the PetG protein, the remaining subunits of the cytochrome bf complex are found at a markedly decreased level.

The cytochrome bf complex has both structural and functional similarities to its bacterial and mitochondrial respiratory counterpart, the cytochrome bc₁ complex. However, the total number of subunits varies extensively among the cytochrome bc₁ complexes (35). The bacterium Paracoccus denitrificans has the minimal three subunits necessary to contain the redox centers, whereas the yeast and bovine mitochondrial complexes have 10 subunits each. In addition to the three subunits containing the prosthetic groups, these cytochrome bc₁ complexes have two large core subunits essential for assembly and five small subunits with a molecular mass of <15 kDa.

The effect of the deletion of each of the five small subunits has been studied extensively in yeast. Deletion of subunit 7 or 8 causes a full loss of activity and the spectral loss of cytochrome b, suggesting that the entire complex is absent (35). In contrast, deletion of subunit 6, 9, or 10 affects only the activity of the complex; in the case of a subunit 9 deletion, the loss of activity is >95%, but the complex is fully assembled (35–37). This provides a strong contrast to our results with the cytochrome bf complex, where the deletion of petG results in a large loss of every other subunit.

Although the sequence of the PetG protein is not homologous to any of the yeast small subunits, it is tempting to speculate that the PetG protein is functionally homologous to one of the two yeast small subunits (subunit 9 or 10) that likewise contains a single transmembrane helix. This type of homology has been proposed for bovine subunit 11, which in spite of a very minimal sequence identity to yeast subunit 10, shares the structural motifs of a transmembrane helix, a charge distribution of basic amino acids at the N terminus and both acidic and basic amino acids at the C terminus (36). Although PetG shares with yeast subunits 9 and 10 the feature of having both acidic and basic amino acid residues at the C terminus, the sole charged residue at the N terminus is a glutamate, rather than 3 or 4 basic residues. This difference in charge would be expected to confer upon the PetG protein the opposite orientation in the membrane relative to the yeast subunits. This prediction is in agreement with experiments on spinach and maize thyla-
koid membranes, where the hydrophilic C terminus of PetG was found to be sensitive to stroma-accessible protease activity (4). Because of this inverse orientation, it seems unlikely that PetG and the yeast subunits have any functional homology.

We have shown that the presence of the PetG polypeptide is essential to the Chlamydomonas cytochrome bf complex for either assembly or stability of the complex. Work is underway to determine whether, in addition, it may have a function in catalysis.

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REFERENCES

1. Malkin, R. (1992) Photosynth. Res. 33, 121-136
2. Hope, A. B. (1993) Biochim. Biophys. Acta 1143, 1-22
3. Cramer, W. A., Martinez, S. E., Furibacher, P. N., Huang, D., Smith, J. L. (1994) Curr. Opin. Struct. Biol. 4, 536-544
4. Haley, J., and Bogorad, L. (1989) Science 240, 1534-1538
5. Schmidt, C. L., and Malkin, R. (1993) Photosynth. Res. 38, 73-81
6. Pierre, Y., and Popot, J.-L. (1993) C. R. Acad. Sci. Paris Ser. III 316, 1404-1409
7. Fong, S. E., and Surzycki, S. J. (1992) Curr. Genet. 21, 527-530
8. Goldschmidt-Clermont, M. (1991) Nucleic Acids Res. 19, 4083-4089
9. Harris, Elizabeth H. (1989) The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use, Academic Press, New York
10. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
11. Ruffey, R. A., Galbec, J. H., Hille, C. R., and Sayre, R. T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9122-9126
12. Redston, M. S., and Kern, S. E. (1994) BioTechniques 17, 286-288
13. Berthold, D. A., Best, B. A., and Malkin, R. (1993) Plant Mol. Biol. Repr. 11, 138-144
14. Sanford, J. C., Smith, F. D., and Russel, J. A. (1993) Methods Enzymol. 217, 483-509
15. Boynton, J. E., Gillham, N., Harris, E., Hosler, J., Johnson, A., Jones, A., Randolph, B., Robertson, D., Klein, T., Shank, K., and Sanford, J. (1988) Science 240, 1534-1538
16. Whitelegge, J. P., Koo, D., Diner, B., Domian, I., and Erickson, J. M. (1995) J. Biol. Chem. 270, 225-235
17. Merchant, S., and Bogorad, L. (1988) Mol. Cell. Biol. 6, 462-469
18. Newman, S. M., Boynton, J. E., Gillham, N. W., Randolph-Anderson, B. L., Johnson, A. M., and Harris, E. H. (1990) Genetics 126, 875-888
19. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York
20. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Laemmli, U. K. (1970) Nature 227, 680-685
22. Arnon, D. (1949) Plant Physiol. (Bethesda) 24, 1-15
23. Ran, Z., and Michaelis, G. (1995) Theor. Appl. Genet. in press
24. Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, C. R., and Meng, B. Y. (1989) Mol. & Gen. Genet. 217, 185-191
25. Shinozaki, K., Ohme, M., Tanaka, M., Wakisugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinazzi, K., Ohto, C., Torazawa, K., Meng, B. Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdo, N., Shimada, H., and Sugiyama, M. (1986) EMBO J. 5, 2043-2049
26. Hallick, R. B., Hong, L., Drager, R. G., Favreau, M. R., Montfort, A., Orsat, B., Spielmann, A., and Stutz, E. (1993) Nucleic Acids Res. 21, 3537-3544
27. Turmel, M., and Otis, C. (1994) Curr. Genet. 27, 54-61
28. Stirewalt, V. L., and Bryant, D. A. (1989) Nucleic Acids Res. 17, 10095
29. Wakisugi, T., Tsuzuki, J., Ito, S., Nakashima, K., Tsuzuki, T., and Sugiyama, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9794-9798
30. Fukuzawa, H., Kôchi, T., Sano, T., Shirai, H., Umesono, K., Inukuchi, H., Ozeki, H., and Ohyama K. (1988) J. Biol. Chem. 263, 333-351
31. Haberhausen, G., Valentin, K., and Zetzsche, K. (1992) Mol. & Gen. Genet. 232, 154-161
32. Kuras, R., and Wolfman, F.-A. (1994) EMBO J. 13, 1019-1027
33. Kuras, R., Guardiola, A., Takahashi, Y., and Rochaix, J.-D. (1995) J. Biol. Chem. 270, 9651-9654
34. Takahashi, Y., Matsumoto, H., Goldschmidt-Clermont, M., and Rochaix, J.-D. (1994) Plant Mol. Biol. 24, 779-788
35. Trumpower, B. L. (1990) Microbiol. Rev. 54, 101-129
36. Brandt, U., Uribe, S., Schagger, H., and Trumpower, B. L. (1994) J. Biol. Chem. 269, 12947-12953
37. Phillips, J. D., Graham, L. A., and Trumpower, B. L. (1993) J. Biol. Chem. 268, 11727-11736
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