Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5460–5464, August 1985
Genetics

Nonsense mutations in the *Chlamydomonas* chloroplast gene that codes for the large subunit of ribulosebisphosphate carboxylase/oxygenase

(chloroplast intergenic suppression/chloroplast genetics/organelle biogenesis/photosynthesis/plant productivity)

ROBERT J. SPREITZER*, MICHEL GOLDSCHMIDT-CLERMONT, MICHELE RAHIRE, AND JEAN-DAVID ROCHAIX

Department of Molecular Biology, University of Geneva, 1211 Geneva 4, Switzerland

Communicated by Myron K. Brakke, May 1, 1985

ABSTRACT **The *Chlamydomonas reinhardtii* chloroplast mutants 18-5B and 18-7G lack both the chloroplast-encoded large subunit and nuclear-encoded small subunit of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39). A chloroplast intergenic-suppression model has been postulated to account for the genetic instability of 18-5B revertants. Here, we have determined the molecular basis of the 18-5B and 18-7G mutants. They contain nonsense mutations close to the 3′ and 5′ ends of their large-subunit genes, respectively. Pulse–chase experiments revealed that the 18-5B mutant produces a truncated large subunit that is unstable. In connection with previous experiments, this work identifies nonsense suppression in the chloroplast. Small subunits are also synthesized but then degraded in the mutants. Thus, the coordinated absence of subunits is achieved through degradation of the small subunit in the specific absence of the large subunit.**

The chloroplast-localized ribulose-1,5-bisphosphate (Rbu-P_2) carboxylase/oxygenase (EC 4.1.1.39) holoenzyme is assembled from eight copies of two different subunits (1). The large subunit (55,000 daltons) is encoded by a chloroplast gene in the green alga *Chlamydomonas reinhardtii* (2), whereas the small subunit is encoded by two or more nuclear genes (3). The small subunit is synthesized as a 20,000-dalton precursor that is processed to 16,500 daltons during entry into the chloroplast (4). Synthesis of the holoenzyme is light-inducible and may be under phytochrome control in higher plants (5, 6). Holoenzyme assembly appears to require a series of discrete steps (7).

The large subunit contains the catalytic site (8) at which CO_2 and O_2 are competitive inhibitors of each other in the carboxylation and oxygenation of Rbu-P_2 (9). Carboxylation is the essential, limiting reaction of photosynthetic CO_2 fixation, but oxygenation is nonessential and inhibitory (10). The enzymology of Rbu-P_2 carboxylase/oxygenase is further complicated by a large-subunit CO_2/Mg^{2+}-activation process (11) that can be regulated by phosphorylated effectors (12).

We have been recovering and analyzing light-sensitive, acetate-requiring chloroplast mutants of *C. reinhardtii* that lack photosynthesis due to defective Rbu-P_2 carboxylase/oxygenase (13–16). These mutants may be useful for gaining new information about the synthesis, assembly, and function of the enzyme. Revertants have been recovered in an attempt to increase the carboxylase/oxygenase ratio and thus increase the efficiency of photosynthesis (14, 17). Furthermore, well-characterized mutations in a single chloroplast gene may be important for understanding chloroplast genetics in general. For example, physical and genetic mapping of the 10-6C large-subunit gene mutation has provided an initial point of correlation between the physical and genetic maps of the *C. reinhardtii* chloroplast genome (15, 18).

Mutant 10-6C is well characterized. It results from a large-subunit gene mutation that alters the large-subunit isoelectric point and greatly reduces carboxylase and oxygenase activities (13–15). More recently, two chloroplast mutants (18-5B and 18-7G) were recovered that, unlike 10-6C, lack the Rbu-P_2 carboxylase/oxygenase holoenzyme as well as both large and small subunits (16). Biochemical/genetic analysis of photosynthetic-competent revertants of 18-5B has led to the development of a model for chloroplast intergenic suppression (17). In this model, a mutation in the large-subunit structural gene is assumed to be present in every copy of the polyploid chloroplast genome of the revertants, but suppressor and wild-type alleles of a second gene are maintained in an appropriate balance by constant selection for photosynthetic competence. In the present investigation, we characterized the holoenzyme-deficient mutants at the molecular level. The mutants make normal amounts of large- and small-subunit mRNAs and small-subunit protein, but this protein is degraded in the chloroplast. One of the mutants synthesizes a truncated large-subunit protein, which is also degraded. DNA sequence analysis has revealed that both mutants have a nonsense mutation in the large-subunit gene. Thus, the molecular basis for the suppressible mutation and the coordinated loss of subunits has been resolved.

MATERIALS AND METHODS

Strains and Culture Conditions. Light-sensitive, acetate-requiring chloroplast mutants of *C. reinhardtii*, which have defective Rbu-P_2 carboxylase/oxygenase, were recovered previously (13, 16). These photosynthesis-deficient mutants (10-6C mt^+, 18-5B mt^+, 18-7G mt^+) and wild type (2137 mt^+) were grown on a rotary shaker in the dark at 25°C with 10 mM acetate medium (19). Bacteriophage λ NM1149 (20) was obtained from N. Murray. It contains a single *Eco*RI site in the *c*I gene. *Escherichia coli* strains C600 and Y1073 *hfl 150* (21) were used as hosts. Strains containing recombinant plasmids were screened on 50 μg of chloramphenicol per ml and maintained on 100 μg of ampicillin per ml.

Purification and Analysis of mRNA. Dark-grown cells were washed by centrifugation and lysed by extensive mixing in a Vortex in 6 M guanidine hydrochloride/0.1 M sodium acetate, pH 5.0, in the presence of glass beads. RNA was prepared by centrifugation through a cesium chloride cushion (22). The RNA level was measured spectrophotometrically, separated by electrophoresis in formaldehyde/agarose gels (23), and blotted to nitrocellulose filters (24). The filters were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: Rbu-P_2, ribulose 1,5-bisphosphate.

*Present address: Department of Agricultural Biochemistry, East Campus, University of Nebraska, Lincoln, NE 68583-0718.

pretreated for 4 hr and then hybridized with [^{32}P]DNA in 5× concentrated SSPE buffer (SSPE buffer = 180 mM NaCl/10 mM sodium phosphate/1 mM EDTA, pH 7.0), 50% formamide, 0.2 mg of denatured salmon DNA per ml, and 0.1% each of bovine serum albumin, Ficoll 400, and polyvinylpyrrolidone (25) for 16 hr at 42°C. They were washed with 0.5× concentrated SSPE buffer/0.25% $NaDodSO_4$ at room temperature and exposed to x-ray film.

Pulse Labeling, Immunoprecipitation, and Gel Electrophoresis. Cells were grown to exponential phase in sulfate-free acetate medium in the dark, concentrated to 1.5×10^7 cells per ml, and pulse-labeled for 5 min with carrier-free $H_2{}^{35}SO_4$ (0.5 mCi/ml; 1 Ci = 37 GBq) in the dark. Samples were removed at the end of the pulse and after a 1-hr chase with 10 mM Na_2SO_4 in acetate medium. The cells were pelleted at 0–4°C and boiled in 4% $NaDodSO_4$/5 mM EDTA/50 mM Tris·HCl, pH 7.4. The extracts were diluted to 2% $NaDodSO_4$/5 mM EDTA/1 mM phenylmethylsulfonyl fluoride/50 mM Tris·HCl, pH 7.4, and clarified by centrifugation. Incorporation was measured by liquid scintillation spectroscopy.

For gel electrophoresis of total cell proteins, the samples were mixed with an equal volume of 2.5% $NaDodSO_4$/10% glycerol/0.001% bromophenol blue/100 mM dithiothreitol/125 mM Tris·HCl, pH 6.8, heated at 65°C for 3 min, and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis as described by Chua (26). After staining and destaining, the gel was exposed to x-ray film.

Immunoprecipitation was performed as described by Westhoff and Zetsche (27). Labeled samples were diluted to the same level of radioactivity in a final buffer of 0.1% $NaDodSO_4$/300 mM NaCl/5 mM EDTA/1% Triton X-100/1 mM phenylmethylsulfonyl fluoride/50 mM Tris·HCl, pH 7.4. *C. reinhardtii* holoenzyme antibody (obtained from N.-H. Chua) was added at 0.1 mg/ml for 1.5 hr at 25°C. Pansorbin cells (*Staphylococcus aureus*, Calbiochem) were added at 10 mg/ml for 1 hr at 25°C. The cells were washed and then extracted with 2.5% $NaDodSO_4$/50 mM dithiothreitol/10% glycerol/0.001% bromophenol blue/125 mM Tris·HCl, pH 6.8, for 3 min at 65°C. The samples were centrifuged, and the supernatants were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis (26) followed by gel fluorography (28).

Large-Subunit Gene Cloning and Sequencing. Cells were grown in 500 ml of acetate medium and broken in a Yeda press, and DNA was isolated (29). Total cell DNA and λ NM1149 DNA was digested with *Eco*RI, ligated, and packaged (30). Phage were plated on Y1073 and recombinants were screened (31) with a nick-translated internal R15.4 fragment of the *Chlamydomonas* large-subunit gene (32). DNA was isolated and the large-subunit R15 fragment (32) was subcloned into pBR328 (33), which was then used to transform C600 (34). Plasmid DNA was purified (35) and the R15 fragment was isolated. Subfragments were labeled at the 5′ (36) or 3′ end (37) and, when necessary, strand separated (36). DNA sequencing was performed by the method of Maxam and Gilbert (36).

RESULTS

Analysis of Subunit mRNAs. Coordinated regulation at the level of transcription may be one possible reason for the absence of large and small subunits in the 18-5B and 18-7G mutants. To test this possibility, total cellular RNA was purified from wild type and mutants 10-6C, 18-5B, and 18-7G. The RNA was quantitated and equal amounts were separated by electrophoresis on a formaldehyde/agarose gel. The RNA was transferred to nitrocellulose and the same filter was probed with a ^{32}P-labeled, internal R15.4 fragment of the *Chlamydomonas* large-subunit gene (32) and a 194-base-pair *Taq* I fragment of the *Chlamydomonas* small-subunit gene specific for the 5′ end of the mRNA (unpublished data). As

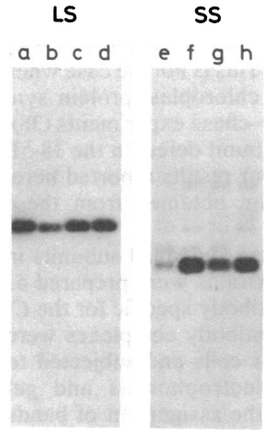

FIG. 1. RNA transfer analysis of large- and small-subunit mRNAs. Total RNA from dark-grown cells was fractionated by electrophoresis through a formaldehyde/agarose gel, transferred to nitrocellulose, and hybridized to [^{32}P]DNA fragments specific for the large-subunit (LS) mRNA (lanes a–d) or the small-subunit (SS) mRNA (lanes e–h). Lanes: a and e, wild type; b and f, mutant 18-5B; c and g, mutant 18-7G; d and h, mutant 10-6C.

shown in Fig. 1, both the 1.6-kilobase-pair large-subunit mRNA (32) and small-subunit mRNA were found to be present in all of the strains. The mutants appeared to have elevated levels of small-subunit mRNA, but this increase was variable in different experiments, requiring further analysis. The amount of large-subunit mRNA was reduced in 18-5B (lane b). Despite these differences, all of the Rbu-P_2 carboxylase/oxygenase mutants were able to transcribe the large- and small-subunit genes.

Pulse Labeling and Immunoprecipitation of Large and Small Subunits. Schmidt and Mishkind (38) had shown that in the absence of chloroplast protein synthesis in *C. reinhardtii*, and thus in the absence of large-subunit synthesis, small subunits were synthesized but rapidly degraded in the chloroplast. To determine if this was the case for the holoenzyme-deficient 18-5B and 18-7G mutants, we performed similar pulse-labeling experiments.

Cells were pulse-labeled with $^{35}SO_4^{2-}$ for 5 min and chased with Na_2SO_4 for 1 hr. Cell proteins were extracted with boiling 4% $NaDodSO_4$, and samples of equal radioactivity were separated on gradient $NaDodSO_4$/polyacrylamide gels. Because Rbu-P_2 carboxylase/oxygenase is an extremely abundant protein, enzyme subunits can be readily observed on these gels (16). As shown in Fig. 2 in relation to wild type, small subunits were synthesized during the pulse in the 18-5B and 18-7G mutants (lanes a–c) and then degraded during the chase (lanes d–f). Since these small subunits were identical in size when compared with wild type (lanes a–c), this indicated that the small subunits were being transported into the chloroplast and processed prior to degradation.

Neither of the holoenzyme-deficient mutants produced a large subunit comparable to the wild-type large subunit during the pulse or chase (Fig. 2). However, a band of lower molecular mass and approximately equal intensity was ob-

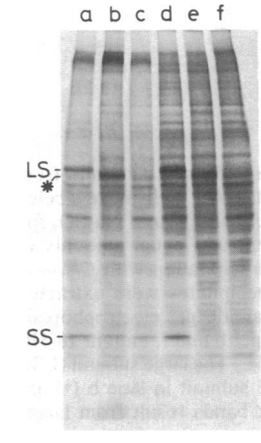

FIG. 2. Pulse labeling of soluble cell proteins in wild type, 18-5B, and 18-7G. Cells were labeled with $^{35}SO_4^{2-}$ for 5 min (lanes a–c) and chased with 10 mM Na_2SO_4 for 1 hr (lanes d–f). Samples were extracted, and equal amounts of radioactivity were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis, followed by autoradiography. Lanes: a and d, wild type; b and e, mutant 18-5B; c and f, mutant 18-7G. The 55,000-dalton large subunit (LS), 16,500-dalton small subunit (SS), and a labile band present in lane b (*) are indicated.

served in 18-5B during the pulse (lane b). No other protein bands were missing when the mutants were compared with wild type during the pulse or chase. This is not the case when wild-type cells and cells that lack chloroplast protein synthesis are compared in similar pulse–chase experiments (38). Thus, the specificity of the large-subunit defect in the 18-5B and 18-7G mutants, suggested by our results reported here, is in agreement with previous data obtained from these mutants (16).

To confirm the assignment of large and small subunits in the pulse–chase experiment, cell extracts were prepared as before and allowed to react with antibody specific for the *C. reinhardtii* holoenzyme. Antigen/antibody complexes were isolated by absorption to *S. aureus* cells and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and gel fluorography. As shown in Fig. 3, the assignment of bands was confirmed and the lower molecular mass band in 18-5B is, in fact, an altered large subunit (lane b). This truncated large-subunit protein was degraded during the chase (lane f). By estimating with the aid of molecular mass marker proteins (data not shown), the truncated large-subunit protein was between 2000 and 4000 daltons smaller than the wild-type large subunit. No putative large subunit was precipitated from 18-7G (lane c). Mutant 10-6C was included in this experiment as an additional control (lanes d and h).

Large-Subunit Gene Sequencing. The 18-5B and 18-7G mutants are known to result from mutations at the same recombinationally defined chloroplast locus as the 10-6C, large-subunit mutation (16). In the present investigation we found that the 18-5B mutant synthesized a truncated large-subunit protein (Figs. 2 and 3). The 18-5B mutant may have resulted from a deletion mutation in the large-subunit gene. However, because 18-5B suppresses at a high frequency (17), it seemed unlikely that a deletion was present. Alternatively, 18-5B may have resulted from a nonsense mutation. It can be calculated from the size of the truncated protein that not more than 35 amino acids would be absent from the carboxyl terminus of the large-subunit protein. Therefore, we decided to sequence the 3′ region of the 18-5B large-subunit gene corresponding to the carboxyl-terminal region of the large-subunit protein. As for 18-7G, large-subunit mRNA was present (Fig. 1) but the large-subunit protein was absent (Figs. 2 and 3). This result would be consistent with a mutation that interfered with the initiation of translation or a nonsense mutation that caused early termination of large-subunit protein synthesis. We did not expect that a protein of much less than 10,000 daltons would have been visible on our $NaDodSO_4$/polyacrylamide gels. Therefore, we decided to sequence the 5′ region of the 18-7G large-subunit gene.

Chloroplast DNA from 18-5B and 18-7G was cloned in bacteriophage λ and the R15 fragment that contains the large-subunit gene (32) was isolated. Fragment R15 from 18-5B and 18-7G was subcloned in pBR328 to produce plasmids pLS18-5B and pLS18-7G, respectively. Restriction-enzyme analysis of these plasmids showed that deletions of >30 base pairs were not present in the large-subunit genes (data not shown).

A *Hin*dIII–*Hin*dIII fragment from positions 2468 to 4037 in the R15 fragment (32) was isolated from pLS18-5B (Fig. 4). This fragment was labeled at the 3′ or 5′ end, cut asymmetrically with *Hin*fI, and sequenced as indicated in Fig. 4. As noted above, a termination mutation was expected in this region. A single transition of G·C to A·T was found at position 2546 (Fig. 4). This mutation changes a UGG (tryptophan) to UGA (opal) in the 18-5B large-subunit mRNA and would produce a protein 25 amino acids shorter at the carboxyl terminus than the wild-type large-subunit protein. The mutant protein would be 450 amino acids long with a molecular mass of ≈52,000 daltons.

An *Eco*RI–*Hin*dIII fragment from positions 169 to 1575 in the R15 fragment (32) was isolated from pLS18-7G (Fig. 4). This fragment was digested with *Hpa* II, and the resulting fragments were labeled at their 5′ ends. Following strand separation, these fragments were sequenced as indicated in Fig. 4. As noted above, a mutation causing early termination or absence of translation initiation was expected in this region. A single transition of G·C to A·T was found at position 1390 (Fig. 4). This mutation changes a UGG (tryptophan) to UAG (amber) in the 18-7G large-subunit mRNA and would produce a protein of only 65 amino acids with an approximate molecular mass of 7500 daltons.

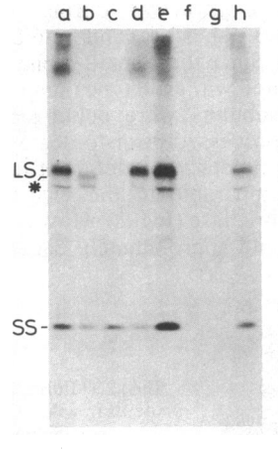

FIG. 3. Immunoprecipitation of large and small subunits from pulse-labeled wild type, 18-5B, 18-7G, and 10-6C. Cells were labeled with $^{35}SO_4^{2-}$ for 5 min (lanes a–d) and chased with 10 mM Na_2SO_4 for 1 hr (lanes e–h). Samples were extracted, and equal amounts of radioactivity were subjected to immunoprecipitation with *Chlamydomonas* holoenzyme antiserum. The precipitates were extracted and resolved with $NaDodSO_4$/polyacrylamide gel electrophoresis and fluorography. Lanes: a and e, wild type; b and f, mutant 18-5B; c and g, mutant 18-7G; d and h, mutant 10-6C. The large subunit (LS), small subunit (SS), and a truncated large subunit in lane b (*) are indicated. Bands below the large-subunit bands result from large-subunit protein that is displaced by IgG monomer.

DISCUSSION

Holoenzyme Assembly and Posttranslational Regulation of Small-Subunit Synthesis. The 18-5B and 18-7G mutants have specific lesions in the Rbu-P_2 carboxylase/oxygenase large subunit (Fig. 4). The large subunit would contain 450 amino acids in 18-5B and 65 amino acids in 18-7G, whereas the wild-type large subunit is 475 amino acids long (32). Even though the holoenzyme is absent in these mutants, the mutants have normal levels of chlorophyll, thylakoid membrane proteins, and photosynthetic electron-transport activity (16). All other soluble cell proteins observed on $NaDodSO_4$/polyacrylamide gels are present (ref. 16; Fig. 2, lanes d–f), and these proteins appear to be synthesized at essentially normal rates (Fig. 2, lanes a–c). The small subunit is synthesized in the 18-5B and 18-7G mutants and processed upon entry into the chloroplast (Fig. 2, lanes a–c). However, in the absence of normal large subunits, the small subunits are degraded (Figs. 2 and 3). In experiments similar to ours, Schmidt and Mishkind (38) observed that small subunits were synthesized and degraded when chloroplast protein synthesis was inhibited, either with chloramphenicol or a chloroplast ribosome-deficient strain of *C. reinhardtii*. However, other proteins were also lost under these experimental conditions (38). Although it could be suggested that the absence of large subunits was responsible for the degradation of small subunits, a possible relationship between the absence of chloroplast protein synthesis and small-subunit instability

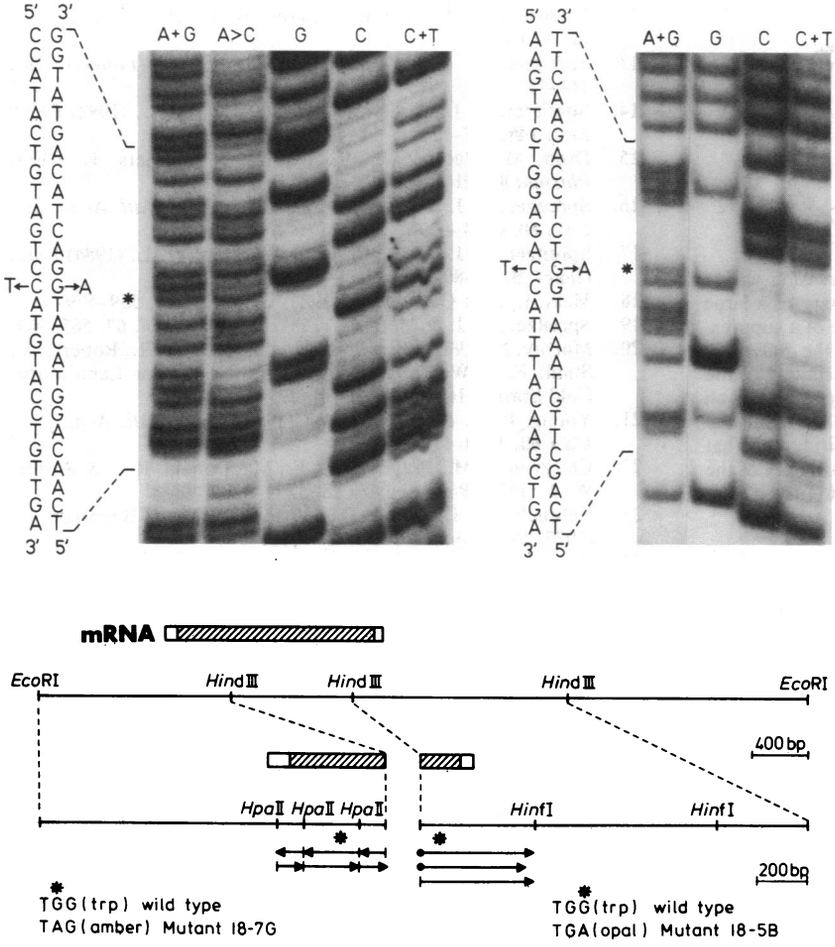

FIG. 4. Large-subunit gene sequencing in 18-5B and 18-7G. The large-subunit genes were cloned and sequenced from mutant 18-5B (*Right*) and mutant 18-7G (*Left*). In the case of both mutants, a single transition from G·C to A·T is indicated on the sequencing gels (*). Below the gels is shown a restriction map of the *Eco*RI–*Eco*RI R15 fragment with the position of the large-subunit gene indicated by the 5′ to 3′ large-subunit mRNA. Within the mRNA, translated regions are designated by slash marks (32). The sequencing strategy is shown in a blowup of the map. Fragments were ^{32}P-labeled at their 5′ (|→) or 3′ (●→) ends. In the 18-5B large-subunit gene (*Right*) a single base substitution at position 2546 (*) changes UGG (tryptophan) to UGA (opal) in the mRNA. In the 18-7G large-subunit gene (*Left*) a single base substitution at position 1390 (*) changes UGG (tryptophan) to UAG (amber) in the mRNA. bp, Base pairs.

could not be ruled out. This is an important point when one considers that reports have been made in higher plants of small-subunit accumulation in the absence of large subunits (39, 40). Our results clearly show that small subunits do not accumulate in *C. reinhardtii* when large subunits are specifically absent, and the amount of small-subunit protein is posttranslationally regulated under this condition.

Mutant 18-5B synthesizes a large-subunit protein only 25 amino acids shorter than the wild-type protein, as determined from the mutant gene sequence (Fig. 4), but holoenzyme does not accumulate (Fig. 3, lane f). Though we cannot conclude from our experiments that holoenzyme does not form prior to subunit degradation, it is clear that these amino acids are essential for holoenzyme stability. Furthermore, these amino acids are encoded by a conserved region of the large-subunit gene (32). This result is in contrast to the 10-6C large-subunit gene mutant, which lacks Rbu-P_2 carboxylase and oxygenase activities but maintains normal levels of holoenzyme (13–15).

It is well established that mRNA is degraded more rapidly when protein synthesis is terminated prematurely at codons introduced by nonsense mutations (41–44). This may explain the reduced level of the large-subunit mRNA in 18-5B (Fig. 1, lane b). However, although large-subunit protein synthesis terminates 385 amino acids sooner in the 18-7G mutant, this mutant produces a normal amount of mRNA (Fig. 1, lane c).

Genetics and Chloroplast Intergenic Suppression. By performing recombination screening between the large-subunit structural gene mutant 10-6C (13, 15) and mutants 18-5B and 18-7G, Spreitzer and Ogren (16) suggested that these latter mutants were also large-subunit structural gene mutants. In the present report we have confirmed the utility of the recombination screening procedure by showing that 18-5B and 18-7G both contain a mutation in the large-subunit gene. All *C. reinhardtii* Rbu-P_2 carboxylase/oxygenase-deficient mutants so far recovered are genetically related at a single, chloroplast genetic locus.

Spreitzer *et al.* (17) recovered photosynthetic-competent revertants from the acetate-requiring 18-5B mutant at a high spontaneous frequency (6×10^{-6}). These revertants were unstable and displayed heteroplasmicity. They segregated out both wild-type (revertant) and acetate-requiring phenotypes in crosses or during vegetative growth under conditions that permitted acetate-requiring mutants to survive (acetate medium in the dark). Although acetate-requiring segregants were stable (homoplasmic) and indistinguishable from the original 18-5B mutant, wild-type (revertant) segregants were always unstable (heteroplasmic). Acetate-requiring segregants had a selective advantage over wild-type segregants when grown in the absence of photosynthesis in the dark. Spreitzer *et al.* (17) presented a model for stable heteroplasmicity to account for the chloroplast suppression of the 18-5B mutation. In this model (Fig. 5), all chloroplast genomes within the single *C. reinhardtii* chloroplast would contain a mutation in the large-subunit gene, whereas only some percentage of genomes would contain a second mutation in a suppressor (*sup*) gene (Fig. 5*a*). The mutant *sup* gene would restore normal large subunit, and the wild-type allele (Fig. 5*b*) would be required to carry out the normal function of the *sup* gene. Homoplasmicity of the wild-type allele of the *sup* gene may provide a selective advantage for cells grown in the absence of photosynthesis (acetate medium in the dark). Homoplasmicity of either allele would be lethal under photosynthetic conditions. Thus, heteroplasmicity would be maintained by constant selection for photosynthetic ability.

In the present investigation, we have resolved two aspects of this heteroplasmic intergenic-suppression model. (*i*) 18-5B

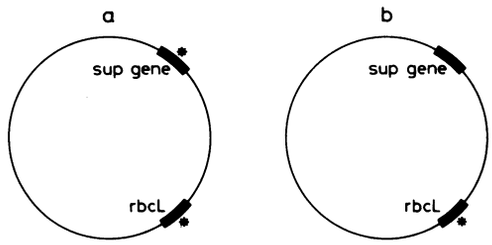

FIG. 5. Model for heteroplasmic intergenic suppression within the single chloroplast of *C. reinhardtii*. Homoplasmicity of a large subunit gene (*rbcL*) mutation (*) and heteroplasmicity of a suppressor (*sup*) gene mutation (*) were presumed within the polyploid genome of revertants of mutant 18-5B (17). Homoplasmicity of the *sup* mutation (genome in *a*) would be lethal if the *sup* gene has an essential function. Homoplasmicity of the wild-type allele of the *sup* gene (genome in *b*) would result in an acetate-requiring cell that would die under photosynthetic conditions. Heteroplasmicity of the genomes in *a* and *b* is maintained by constant selection for photosynthetic growth.

does, in fact, result from a mutation in the large-subunit gene and (*ii*) this mutation is a nonsense mutation (Fig. 4) that may be suppressed easily. The mutation in 18-5B produces a UGA that is 25 codons away from the normal UAA codon in the large-subunit mRNA (Fig. 4), and the mutant mRNA produces a truncated large-subunit protein with the expected molecular mass (Fig. 3). Nonsense suppression is clearly occurring in the chloroplast, but the exact mechanism of this suppression remains to be determined.

It is of interest to note that the 18-7G mutant also reverts, but at a considerably lower frequency than 18-5B (unpublished data). Since UAG appears to occur at a higher frequency than UGA in chloroplast mRNA (45–53), it is possible that UAG suppression would have more severe effects on chloroplast protein synthesis than suppression of UGA. Whether the UAG present in the 18-7G mRNA (Fig. 4) may be subject to heteroplasmic suppression remains to be explored. Further analysis of Rbu-P_2 carboxylase/oxygenase mutants and their revertants will define the range of chloroplast mutations that can be suppressed. Suppression based upon the polyploidy of the chloroplast genome may be extremely useful for investigating genetic suppression in general and may serve as a new tool for introducing variation into the agronomically important Rbu-P_2 carboxylase/oxygenase enzyme.

We thank Dr. S. J. Surzycki for helpful discussions and Mr. O. Jenni for assistance with the figures. This work was supported by Grant 3.258.0.82 from the Swiss National Science Foundation.

1. Baker, T. S., Eisenberg, D., Eiserling, F. A. & Weissman, L. (1975) *J. Mol. Biol.* **91,** 391–399.
2. Malnoe, P., Rochaix, J.-D., Chua, N.-H. & Spahr, P.-F. (1979) *J. Mol. Biol.* **53,** 159–162.
3. Goldschmidt-Clermont, M. (1984) *Experientia* **40,** 624 (abstr.).
4. Chua, N.-H. & Schmidt, G. W. (1979) *J. Cell Biol.* **81,** 461–483.
5. Tobin, E. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4749–4753.
6. Tobin, E. M. (1981) *Plant Mol. Biol.* **1,** 35–51.
7. Bloom, M. V., Milos, P. & Roy, H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1013–1017.
8. Norton, I. L., Welch, M. H. & Hartman, F. C. (1975) *J. Biol. Chem.* **250,** 8062–8068.
9. Laing, W. A., Ogren, W. L. & Hageman, R. H. (1974) *Plant Physiol.* **54,** 678–685.
10. Ogren, W. L. (1984) *Annu. Rev. Plant Physiol.* **35,** 415–442.
11. Miziorko, H. M. & Lorimer, G. H. (1983) *Annu. Rev. Biochem.* **52,** 507–535.
12. Jordan, D. B., Chollet, R. & Ogren, W. L. (1983) *Biochemistry* **22,** 3410–3418.
13. Spreitzer, R. J. & Mets, L. (1980) *Nature (London)* **285,** 114–115.
14. Spreitzer, R. J., Jordan, D. B. & Ogren, W. L. (1982) *FEBS Lett.* **148,** 117–121.
15. Dron, M., Rahire, M., Rochaix, J.-D. & Mets, L. (1983) *Plasmid* **9,** 321–324.
16. Spreitzer, R. J. & Ogren, W. L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6293–6297.
17. Spreitzer, R. J., Chastain, C. J. & Ogren, W. L. (1984) *Curr. Genet.* **9,** 83–89.
18. Mets, L. J. & Geist, L. J. (1983) *Genetics* **105,** 559–579.
19. Spreitzer, R. J. & Mets, L. (1981) *Plant Physiol.* **67,** 565–569.
20. Murray, N. (1983) in *Lambda II*, eds. Hendrix, R., Roberts, J., Stahl, F. & Weisberg, R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 395–432.
21. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1194–1198.
22. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
23. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16,** 4743–4751.
24. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
25. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–643.
26. Chua, N.-H. (1980) *Methods Enzymol.* **69,** 434–446.
27. Westhoff, P. & Zetsche, K. (1981) *Eur. J. Biochem.* **116,** 261–267.
28. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
29. Rochaix, J.-D. (1980) *Methods Enzymol.* **65,** 785–795.
30. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
31. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
32. Dron, M., Rahire, M. & Rochaix, J.-D. (1982) *J. Mol. Biol.* **162,** 775–793.
33. Soberon, X., Covarrubias, L. & Bolivar, F. (1980) *Gene* **9,** 287–305.
34. Mendel, M. & Higa, A. (1970) *J. Mol. Biol.* **53,** 159–162.
35. Katz, L., Kingsburg, D. T. & Helinski, D. R. (1973) *J. Bacteriol.* **114,** 577–591.
36. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
37. Levis, R., Dunsmuir, P. & Rubin, G. M. (1980) *Cell* **21,** 581–588.
38. Schmidt, G. W. & Mishkind, M. L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2632–2636.
39. Feierabend, J. & Wildner, G. (1978) *Arch. Biochem. Biophys.* **186,** 283–291.
40. Barraclough, R. & Ellis, R. J. (1979) *Eur. J. Biochem.* **94,** 165–177.
41. Hiraga, S. & Yanofsky, C. (1972) *J. Mol. Biol.* **72,** 103–110.
42. Schneider, E., Blundell, M. & Kennel, D. (1978) *Mol. Gen. Genet.* **160,** 121–129.
43. Losson, R. & Lacroute, F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5134–5137.
44. Pelsy, F. & Lacroute, F. (1984) *Curr. Genet.* **8,** 277–282.
45. Krebbers, E. T., Larrinua, I. M., McIntosh, L. & Bogorad, L. (1982) *Nucleic Acids Res.* **10,** 4985–5002.
46. Zurawski, G., Bottomley, W. & Whitfeld, P. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6260–6264.
47. Shinozaki, K., Deno, H., Kato, A. & Sugiura, M. (1983) *Gene* **24,** 147–155.
48. Zurawski, G. & Clegg, M. T. (1984) *Nucleic Acids Res.* **12,** 2549–2559.
49. Zurawski, G., Perrot, B., Bottomley, W. & Whitfeld, P. R. (1981) *Nucleic Acids Res.* **9,** 3251–3270.
50. Willey, D. L., Auffret, A. D. & Gray, J. C. (1984) *Cell* **36,** 555–562.
51. Heinemeyer, W., Alt, J. & Herrmann, R. G. (1984) *Curr. Genet.* **8,** 543–549.
52. Alt, J. & Herrmann, R. G. (1984) *Curr. Genet.* **8,** 551–557.
53. Alt, J., Morris, J., Westhoff, P. & Herrmann, R. G. (1984) *Curr. Genet.* **8,** 597–606.