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Genetics

Mutations in nine chloroplast loci of *Chlamydomonas* affecting different photosynthetic functions

(chloroplast genetics/chlorophyll-protein complex 1/chloroplast coupling factor/chloroplast ribosomes)

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ABSTRACT Chloroplast components known to be coded by chloroplast DNA include chloroplast rRNAs, tRNAs, and the large subunit of ribulose-bisphosphate carboxylase. Because these components comprise less than 3% of the estimated coding capacity of the chloroplast genome, most chloroplast gene functions have yet to be identified. One approach to this problem is the isolation and characterization of mutations in the chloroplast genome affecting specific photosynthetic functions. Recently we have found that such mutations can be preferentially recovered by using arsenate selection on cells previously grown in 5-fluorodeoxyuridine. Sixteen mutants thus isolated have been localized into nine chloroplast loci, based on their ability to recombine and produce photosynthetically competent progeny. Mutants at two loci show the characteristic syndrome of photosynthetic defects that results from a deficiency in chloroplast protein synthesis. These have been found to lack chloroplast ribosome monomers. Mutants at three loci are missing chlorophyll-protein complex 1 in their thylakoid membranes. Mutants at three other loci are deficient in membrane polypeptides known to be associated with the chloroplast coupling factor.

Chloroplast DNA molecules in all green algae and higher plants so far studied, with the exception of *Acetabularia*, are circles ranging in size from 40 to 60 μm and have the capacity to code for more than 250 proteins of M_r 20,000 (1). DNA·RNA hybridization has revealed that chloroplast rRNAs and tRNAs are coded by the chloroplast genome in tobacco, maize, and *Euglena*, and chloroplast rRNAs have been found to be chloroplast gene products in spinach and *Chlamydomonas reinhardtii* (1–4). However, only a small proportion of the coding capacity of chloroplast DNA can be accounted for by the stable RNAs (1). The only polypeptide so far shown by physical mapping studies to be a product of chloroplast DNA is the large subunit of the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase (5, 6).

Mutations have been used extensively to probe chloroplast DNA functions in *Chlamydomonas* and to a lesser extent in higher plants (1, 2). Mutations in *Chlamydomonas* that are transmitted in a uniparental manner are thought to be localized in chloroplast DNA (1, 7) but, until recently, only those mutations conferring antibiotic resistance on chloroplast ribosomes have been characterized (1, 2, 8, 9). Genetic analysis has shown that the antibiotic-resistance mutations fall in seven loci that map close together in the chloroplast genome and may comprise a ribosomal region comparable to ribosomal regions found in bacteria (9). One would predict that chloroplast mutations affecting specific photosynthetic functions should also occur in *Chlamydomonas* because many Mendelian mutations with photosynthetic defects are known (2). However, only three chloroplast mutations affecting photosynthetic functions have been reported to date. One of these causes the loss of chlorophyll-protein complex 1 (CP1) from the thylakoid membranes (10), the second alters the electrophoretic mobility of a different thylakoid membrane polypeptide (11), and the third appears to affect photophosphorylation (12, 13).

This paper describes the isolation and characterization of 16 nonphotosynthetic chloroplast mutations by using 5-fluorodeoxyuridine (FdUrd) which we have found increases specifically the frequency of all types of chloroplast gene mutations (14). These nonphotosynthetic mutants fall into nine distinct loci in the chloroplast genome on the basis of recombination analysis. Because somatic segregation of chloroplast genes prevents the assessment of allelism by the usual complementation tests, one cannot determine genetically whether each recombinationally separate locus specifies a different chloroplast gene function. Hence, the term "locus," defined here on the basis of recombination, is not necessarily equivalent to a gene defined by complementation tests in Mendelian genetics. Individual mutants representing each locus have been assayed for defects in specific photosynthetic functions. Mutants at two loci were found to block assembly of the chloroplast ribosomes and show the syndrome of defects associated with mutants deficient in chloroplast protein synthesis (8, 15). Mutants at three loci are missing CP1 from their thylakoid membranes, and mutants at three other loci are lacking thylakoid membrane polypeptides associated with the chloroplast coupling factor. Such photosynthetically defective mutants offer the same potential for probing chloroplast gene function as do the recently described *mit*⁻ and *syn*⁻ mitochondrial mutants in *Saccharomyces* (16, 17) which are deficient in specific respiratory functions and in mitochondrial protein synthesis, respectively.

MATERIALS AND METHODS

Isolation of Mutants and Stock Maintenance. *C. reinhardtii* wild-type strain 137c *mt*⁺ (GB-125) was pregrown to a concentration of 1 × 10^6 cells per ml in Tris/acetate/phosphate (TAP) liquid medium (18). These cells were used to inoculate 250-ml cultures at 1 × 10^4 cells per ml in TAP containing 0.1 mM FdUrd, and the cells were grown for approximately nine doublings to a density of 6 × 10^6 cells per ml. Cultures were shaken under continuous cool white fluorescent light (1.5 × 10^4 lux) at 25°C. Aliquots of these cultures containing 1 × 10^6 cells were plated on TAP plates (1.5% agar) containing 2 mM sodium arsenate for positive selection of nonphotosynthetic mutants (14, 15). Part of each colony that grew on the TAP/arsenate plates was transferred to plates of

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Abbreviations: FdUrd, 5-fluorodeoxyuridine; CP1, chlorophyll-protein complex 1; CF_1, chloroplast coupling factor; TAP, Tris/acetate/phosphate liquid medium (18); PSII, photosystem II; HS, high-salt medium; HSA, HS containing 15 mM sodium acetate; HSHA, HS containing 30 mM sodium acetate.

high-salt medium (HS) (19) and of HS containing 15 mM sodium acetate HSA. Only those mutants which died on the HS plates were selected for further analysis. Mutants were isolated in two separate experiments (distinguished by the first digit of their identification numbers). The stringent acetate-requiring mutants isolated were maintained routinely on HS medium with twice the normal amount of sodium acetate (HSHA) to avoid the necessity of frequent transfer. Because some of the mutants were sensitive to high light intensity, all stocks were kept in dim light. The CP1-deficient mutant *C1* was the gift of Pierre Bennoun.

Genetic Analysis. Crosses were made between each mutant and wild-type strain 137c mt^- (GB-126). Gametes of both mutant and wild-type cells were induced by suspending 7-day-old cultures from HSHA plates in nitrogen-free medium (HSHA without NH_4Cl) overnight. Equal numbers of gametes of the two mating types were mixed in the light for 2 hr, and the mating mixture was then plated onto HS containing 4% agar. After 18–24 hr in cool white fluorescent light (5.5×10^3 lux), the plates were placed in the dark for 6 days at 25°C. Zygotes were then transferred to HSHA plates and placed in the light for germination. Between 10 and 20 tetrads were analyzed for each mutant by using the methods of Ebersold and Levine (20). Once the meiotic products had formed colonies, they were tested for acetate dependence as described above. Normally, chloroplast mutations are transmitted to the meiotic progeny almost exclusively by the mt^+ parent (1, 7) and each of the 16 mutants studied here showed the expected 4 ac^-:0 ac^+ segregation for acetate requirement. Nuclear mutations could be identified by their 2 ac^-:2 ac^+ segregation pattern. When a chloroplast mutant had been identified by its inheritance pattern, mt^+ and mt^- segregants were selected for use in allele tests.

Chloroplast acetate mutants were grouped into recombinationally distinct loci by making pairwise matings as described above except that the mating mixture was spread onto HS plates that were kept in continuous light (cool white fluorescent, 5.5 $\times 10^3$ lux). Although zygotes are routinely matured in the dark, Van Winkle-Swift (21) has shown that they will germinate with high efficiency when matured in the light. Total zygotes per plate were estimated by counting three or four sectors covering approximately 5% of the plate. Zygote germination, which was greater than 70%, was determined on a sample of 100–200 zygotes on a sector of each plate. Mutants falling in the same locus were identified by their failure to recombine to yield photosynthetically competent progeny.

Reversion frequencies were determined by growing mutants in HSHA liquid medium to a density of 5×10^6 cells per ml and plating 3–5 $\times 10^7$ cells on HS medium at a density of 1×10^6 cells per plate. The number of colonies observed on HS plates was divided by the total number of cells plated as determined by hemocytometer counts to obtain a reversion frequency.

Biochemical Assays. Cells were grown in HSHA liquid medium to 1–2 $\times 10^6$ cells per ml in darkness and then transferred to continuous light (1.5×10^4 lux, cool white fluorescent) for 24 hr prior to harvest. Ribulose-1,5-bisphosphate carboxylase, chlorophyll, photosystem II (PSII) activity, whole cell protein, and whole cell ribosome profiles were analyzed by the methods of Harris *et al.* (15). Thylakoid membrane polypeptides were isolated by flotation and separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis using the methods of Chua and Bennoun (22). Membrane polypeptides were identified by consecutive numbers starting from the high molecular weight region, according to the nomenclature of Chua and Bennoun (22).

For analysis of the amount of *P*700, a modification of the method of Alberte *et al.* (23) was used. The cells (1×10^8) were harvested by centrifugation at 12,100 $\times g$ for 5 min and then were washed and resuspended in 4 ml of 50 mM Tris·HCl, pH 8.0/2 mM $MgCl_2$/0.5 M sucrose/0.1 M NaCl. The cells were broken at 5000 psi (34.5 MPa) in a French pressure cell and spun at 480 $\times g$ for 2 min to remove cells and cell debris. The supernatant was collected and spun at 27,000 $\times g$ for 10 min. The green pellet from this centrifugation was solubilized overnight in Triton X-100 at a Triton/chlorophyll ratio (wt/wt) of 75:1. Spectrophotometric measurements were carried out on the solubilized membranes in an Aminco DW-2 spectrophotometer according to the procedure of Alberte *et al.* (23). Total *P*700 (μmol per sample) was calculated as: absorbance change at 697 nm/(64×10^3) (24).

RESULTS

Mutant Isolation, Allele Testing, and Reversion of Mutants. The key to efficient isolation of acetate-requiring chloroplast mutants is growth of the cells in FdUrd which increases the recovery of all detectable classes of chloroplast mutations without a concomitant increase in nuclear mutants of similar phenotype (14). In this respect we should note that the arsenate method for positive selection of nonphotosynthetic cells is far from ideal because less than 2.0% of the cells forming colonies on arsenate are acetate requiring (15). All of the mutants obtained from our control cultures exhibited Mendelian inheritance whereas 56% of the mutants obtained from the FdUrd-grown cells showed the uniparental pattern of inheritance characteristic of chloroplast mutations. Nonphotosynthetic mutations in the chloroplast genome arise at a frequency of about 0.8×10^{-6} mutations per viable cell in FdUrd-grown cultures (14). Of the 19 stable chloroplast mutations obtained so far, 16 have now been characterized genetically.

Mutants found by tetrad analysis to be inherited uniparentally were tested for their ability to recombine in pairwise matings. All crosses were done at least twice, with between 50,000 and 100,000 zygotes plated each time. Although chloroplast markers normally exhibit maternal inheritance, 1–10% of the zygotes (biparental zygotes) spontaneously transmit both maternal and paternal chloroplast markers and can be assayed for recombination of chloroplast mutants (1, 7). In the tests reported here, assuming 50,000 zygotes screened, an average of 70% germination, and 1% biparental zygotes, there should be at least 350 biparental zygotes in which recombination could occur. In crosses of antibiotic-resistant mutants with similar phenotypes, progeny of 100 or fewer biparental zygotes are scored (9). A large number of zygotes can be analyzed conveniently in the case of the acetate mutants because photosynthetically competent recombinants are positively selected on the HS plates. With antibiotic-resistant mutants, positive selection of antibiotic-sensitive recombinants is not possible.

The 16 acetate-requiring mutants characterized to date were found to fall in nine distinct loci within which recombination was not detectable (Table 1). Photosynthetically competent progeny were produced in all crosses between mutants in different loci, which were designated *ac-u-a* through *ac-u-i*. Both mutants at the *ac-u-b* locus were isolated in a single experiment. Mutants at the *ac-u-c* locus were isolated in two separate experiments. The *C1* mutant of Bennoun *et al.* (10) was found to map in the *ac-u-i* locus.

Experiments were carried out to ascertain whether any of the mutants were reverting to photosynthetic competence at a rate that would give spurious results in either the genetic or physiological analyses. Uniparentally inherited reversions or suppressors conferring wild-type phenotype were found at frequencies of about 1×10^{-7} per mutant cell for specific

Table 1. Recombination matrix of nonphotosynthetic chloroplast mutants

Locus	Mutant	1-15	1-10	1-5	2-21	2-13	2-9	1-7	1-20	2-29	2-43	2-12	1-24	2-31	2-3	2-17	2-25	C1	Revertant frequency/10^7 cells
ac-u-a	1-15	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.2
ac-u-b	1-10		−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.2
	1-5			−	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	0.4
ac-u-c	2-21				−	−	−	−	−	−	−	+	+	+	+	+	+	+	0
	2-13					−	−	−	−	−	−	+	+	+	+	+	+	+	ND
	2-9						−	−	−	−	−	+	+	+	+	+	+	ND	0
	1-7							−	−	−	−	+	+	+	+	+	+	ND	ND
	1-20								−	−	−	+	+	+	+	+	+	ND	0
	2-29									−	−	+	+	+	+	+	+	ND	ND
	2-43										−	+	+	+	+	+	+	ND	ND
ac-u-d	2-12											−	+	+	+	+	+	+	98.0
ac-u-e	1-24												−	+	+	+	+	+	2.2
ac-u-f	2-31													−	+	+	+	+	0.33
ac-u-g	2-3														−	+	+	+	0.25
ac-u-h	2-17															−	+	+	122.0
ac-u-i	2-25																−	−	1.25
	C1																	−	ND

+, Occurrence of photosynthetically competent recombinants; −, absence of photosynthetically competent recombinants; ND, not determined.

mutants at most loci (Table 1). At the ***ac-u-d*** and ***ac-u-h*** loci, Mendelian suppressor mutations were recovered at frequencies of around 1×10^{-5} per mutant cell.

Biochemical Defects in Mutants Falling at the Nine Loci. Mutants at seven of the nine loci were found to have near wild-type levels of ribulose-bisphosphate carboxylase activity, PSII activity, and chlorophyll (Table 2). Mutants falling in the two remaining loci, *ac-u-d* and *ac-u-h*, were deficient in both ribulose-bisphosphate carboxylase and PSII activities, a syndrome characteristic of mutations affecting chloroplast ribosome assembly and defective in chloroplast protein synthesis (8, 15). Analysis of ribosomes from mutants at both loci on sucrose gradients showed that they lacked chloroplast ribosome monomers (70S) and accumulated 54S particles that may represent large subunits of the chloroplast ribosome (Fig. 1). Sodium dodecyl sulfate/polyacrylamide gels of thylakoid membrane polypeptides isolated from these mutants revealed

Table 2. Biochemical characterization of nonphotosynthetic chloroplast mutants

Genotype	Photosynthetic functions, % of wild type: Carboxylase activity	PSII activity	Chlorophyll	*P*700
Wild type	100	100	100	100
ac-u-a 1-15	110	90	113	ND
ac-u-b 1-5	85	77	97	ND
1-10	76	86	113	98
ac-u-c 1-20	80	58	90	ND
2-21	82	48	86	24
2-9	75	65	100	ND
1-7	94	ND	78	ND
ac-u-d 2-12	7	3	92	ND
ac-u-e 1-24	117	94	103	ND
ac-u-f 2-31	76	95	96	ND
ac-u-g 2-3	97	100	48	12
ac-u-h 2-17	2	11	94	ND
ac-u-i 2-25	85	76	90	17

Wild-type values: ribulose-bisphosphate carboxylase, 2.9 μmol of CO_2 fixed per hr per mg of whole cell protein; PSII, 2.1 μmol of 2,6-dichloroindophenol reduced per hr per mg of whole cell protein; chlorophyll, 93 μg/mg of whole cell protein; *P*700, 6.9 nmol/10^9 cells; whole cell protein, 44.5 mg/10^9 cells. ND, not determined.

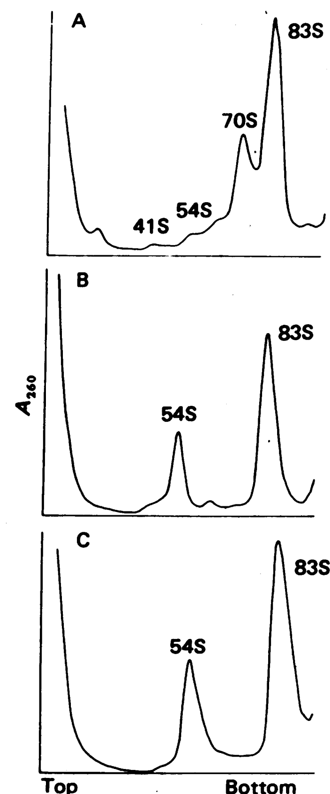

FIG. 1. Sucrose gradient analysis of total cell ribosomes from wild type (*A*) and mutants at the *ac-u-d* (*B*) and *ac-u-h* (*C*) loci. The 83S peak contains cytoplasmic ribosome monomers, the 70S peak contains chloroplast ribosome monomers, and the 54S and 41S peaks represent the large and small subunits of the chloroplast ribosomes, respectively (8, 15). Both mutants lack 70S chloroplast ribosome monomers but accumulate 54S particles.

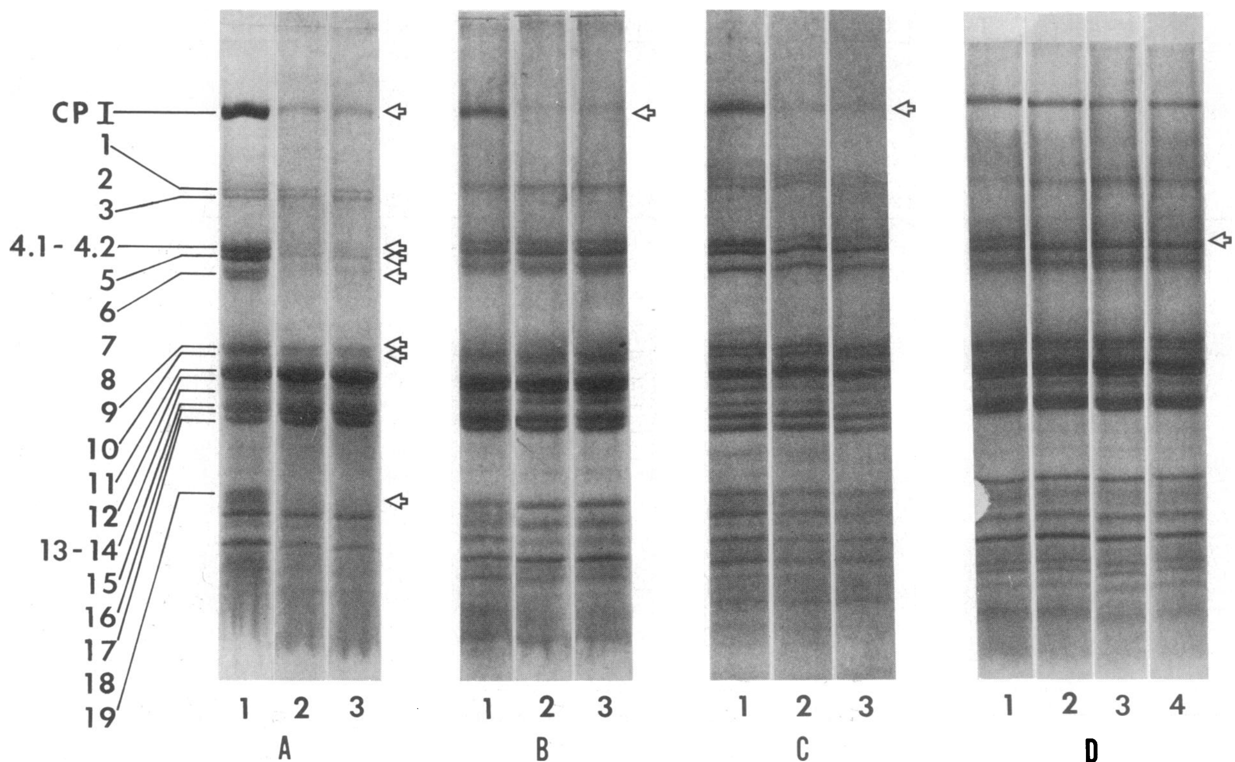

FIG. 2. Sodium dodecyl sulfate/gradient gels of thylakoid membrane polypeptides of wild type and mutants at eight chloroplast loci stained with Coomassie blue. The bands are numbered according to Chua and Bennoun (22). Apparent M_r: polypeptide 2, 68,000; polypeptides 4.1–4.2, 52,000; polypeptide 8, 40,000; polypeptide 15, 29,000; polypeptide 19, 21,000. Polypeptide 2, which forms part of the CP1 complex, is only seen upon heat dissociation of CP1. (*A*) Comparison of chloroplast ribosome-deficient mutants at the *ac-u-d* and *ac-u-h* loci. Lanes: 1, wild type; 2, *ac-u-d-2-12;* 3, *ac-u-h-2-17.* In both mutants, CP1 and polypeptides 4.1–4.2, 5, 6, 9, 10, and 19 are greatly reduced (arrows). Essentially the same phenotype is seen in wild type cells of *C. reinhardtii* grown for long periods in the presence of chloramphenicol which inhibits chloroplast protein synthesis (25). (*B*) Comparison of two allelic CP1-deficient mutants at the *ac-u-i* locus. Lanes: 1, wild type; 2, *ac-u-i-2-25* mutant; 3, *C1* mutant studied by Bennoun *et al.* (10). (*C*) Comparison of two nonallelic CP1-deficient mutants at the *ac-u-c* and *ac-u-g* loci. Lanes: 1, wild type; 2, *ac-u-g-2-3;* 3, *ac-u-c-2-21.* Together, *B* and *C* demonstrate that mutants in at least three chloroplast loci can cause the loss of CP1 (arrows). (*D*) Comparison of three nonallelic mutants at the *ac-u-a, ac-u-b,* and *ac-u-e* loci deficient in polypeptides 4.1–4.2 (arrow) which are part of the CF_1 ATPase. Lanes: 1, wild type; 2, *ac-u-a-1-15;* 3, *ac-u-b-1-10;* 4, *ac-u-e-1-24.* All lanes in a given panel are from the same slab gel.

that CP1 and polypeptides 4.1–4.2, 5, 6, 9, 10, and 19 were drastically reduced in amount (Fig. 2*A*). Representative mutants from the other loci had normal amounts of 70S chloroplast ribosomes (data not shown).

Isolated thylakoid membranes from mutants in three of the seven other loci, *ac-u-c, ac-u-g,* and *ac-u-i,* almost totally lacked CP1 when analyzed on sodium dodecyl sulfate/polyacrylamide gels (Fig. 2 *B* and *C*). No other thylakoid membrane polypeptides were markedly affected in the membrane preparations of the two mutants at the *ac-u-i* locus (Fig. 2*B*). In the case of mutants at the *ac-u-c* and *ac-u-g* loci, we also observed that polypeptides 4.1–4.2 varied in amount in different preparations (Fig. 2*C*). However, N.-H. Chua (personal communication) has independently analyzed thylakoid membrane polypeptides from the same *ac-u-c* and *ac-u-g* mutants shown in Fig. 2*C* and has confirmed that they specifically lack CP1 but have normal amounts of polypeptides 4.1–4.2. Because only about 5% of the total chlorophyll of thylakoid membranes is tightly associated with polypeptide 2 in the CP1 complex analyzed in sodium dodecyl sulfate gels (26), we were not surprised to find that most of the mutants at these three loci had nearly normal levels of chlorophyll (Table 2).

Thylakoid membranes isolated from mutants in the *ac-u-a, ac-u-b,* and *ac-u-e* loci were missing only polypeptides 4.1–4.2 (Fig. 2*D*) which are thought to be part of the chloroplast coupling factor (CF_1)–ATPase complex (see *Discussion*). Mutants at the remaining locus, *ac-u-f,* had no defects in any of the photosynthetic functions analyzed.

DISCUSSION

This paper reports an efficient protocol for the isolation and genetic characterization of numerous chloroplast mutants affecting essential photosynthetic functions. Although many nuclear acetate-requiring mutants have been isolated and characterized (2), the methods used have not yielded chloroplast acetate-requiring mutants. Even when diploid cells are used to mask recessive nuclear acetate mutants (27), arsenate selection (15) has proved unsuccessful in our hands. However, FdUrd, which decreases the amount of chloroplast DNA (28), increases the recovery of chloroplast mutations resistant to various antibiotics as well as nonphotosynthetic chloroplast mutations requiring acetate (14).

The nine chloroplast loci reported here more than double the number of chloroplast loci defined by mutations in *Chlamydomonas.* Recombination analysis has shown that mutants at the *ac-u-a, ac-u-b,* and *ac-u-i* loci are loosely linked to the chloroplast antibiotic-resistance loci (data not shown).

The chloroplast ribosome deficient mutants at the *ac-u-d* and *ac-u-h* loci have the syndrome of defects characteristic of similar Mendelian (*cr*) mutants described by Harris *et al.* (8, 15). Mutants at both chloroplast loci and at several of the nuclear gene loci (8, 15) accumulate what appear to be the large (54S)

subunits of the chloroplast ribosome but are deficient in the small (41S) subunits. Thus, several loci in both the nuclear and chloroplast genomes may affect the synthesis of the small subunit of the chloroplast ribosome. Mutants at the *ac-u-d* and *ac-u-h* loci as well as certain of the *cr* loci have a strong tendency to be suppressed by nuclear mutations (see Table 1 and ref. 15).

The chloroplast membrane polypeptide pattern of the *ac-u-d* and *ac-u-h* mutants is similar to that seen in wild-type cells of *Chlamydomonas* after long-term growth in chloramphenicol, an inhibitor of chloroplast protein synthesis (25). In both cases, CP1 and polypeptides 4.1–4.2, 5, 6, 9, 10, and 19 are affected (Fig. 2A).

The results presented here show that at least three chloroplast loci specifically affect CP1, including locus *ac-u-i* which contains the *C1* mutant described by Bennoun *et al.* (10). Both Mendelian and non-Mendelian mutations are now known to cause loss of the CP1 complex and its principal protein component (polypeptide 2) from thylakoid membranes (10, 29).

Mutants at the *ac-u-a, ac-u-b,* and *ac-u-e* loci lack polypeptides 4.1–4.2. Bennoun and Chua (29) have shown that these two polypeptides as well as polypeptide 8.1 are missing in thylakoid membranes of the Mendelian mutant *F54* described by Sato *et al.* (30) as being defective in photophosphorylation. Because Sato *et al.* (30) reported that the mutant had an active, but nonlatent, CF_1-ATPase, Bennoun and Chua (29) speculated that these missing polypeptides are associated with the membrane sector and interact with CF_1. However, H. R. Piccioni and N.-H. Chua (personal communication) recently have found that polypeptides 4.1–4.2 comigrate in sodium dodecyl sulfate gels with the largest CF_1 subunits α and β, whereas the γ and ϵ subunits comigrate with polypeptide 8.1 and with one of a group of less-well-resolved low molecular weight polypeptides, respectively. On the basis of these recent findings, the *ac-u-a, ac-u-b,* and *ac-u-e* mutants are likely blocked in the synthesis, assembly, or integration of CF_1 because they lack polypeptides 4.1–4.2. At least three subunits of CF_1 are known to be synthesized in isolated chloroplasts of pea (31) and spinach (32) and polypeptides 4.1–4.2 are known to be products of chloroplast protein synthesis in *Chlamydomonas* (25). If the CF_1 complex must be assembled before association with the thylakoid membrane, any defect that causes failure of proper assembly would result in the absence of CF_1 polypeptides from membrane preparations. The presence of these polypeptides in the cytoplasm might be detected by using antibodies to the specific subunits. The chloroplast photophosphorylation-deficient mutant reported by Hudock *et al.* (12, 13) may well be an allele at one of these three loci.

Although the results presented here are not proof that the chloroplast genome codes for the aforementioned chloroplast components, they show that chloroplast gene functions are necessary for the assembly of CP1 and CF_1 as well as chloroplast ribosomes. If these recombinationally separate loci correspond to conventional cistrons, then at least three chloroplast gene products are involved in the assembly of CP1 and three in the case of CF_1. When thylakoid membrane polypeptides from mutants at each of these loci are fractionated and characterized further, our genetic approach, in contrast to the molecular hybridization approach, should allow identification of both structural genes for CP1 and CF_1 polypeptides and also genes involved in the processing and assembly of these polypeptides into the thylakoid membrane.

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