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Temperature-Sensitive, Photosynthesis-Deficient Mutants of *Chlamydomonas reinhardtii*[1]

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ABSTRACT

Mutants of the unicellular, green alga *Chlamydomonas reinhardtii* were recovered by screening for the absence of photoautotrophic growth at 35°C. Whereas nonconditional mutants required acetate for growth at both 25 and 35°C, the conditional mutants have normal photoautotrophic growth at 25°C. The conditional mutants consisted of two classes: (a) Temperature-sensitive mutants died under all growth conditions at 35°C, but (b) temperature-sensitive, acetate-requiring mutants were capable of heterotrophic growth at 35°C when supplied with acetate in the dark. The majority of mutants within the latter of these two classes had defects in photosynthetic functions. These defects included altered pigmentation, reduced whole-chain electron-transport activity, reduced ribulosebisphosphate carboxylase activity, or pleiotropic alterations in a number of these photosynthetic components. Both nuclear and chloroplast mutants were identified, and a correlation between light-sensitive and photosynthesis-deficient phenotypes was observed.

The unicellular green alga *Chlamydomonas reinhardtii* has proven to be a useful organism for the genetic dissection and analysis of photosynthesis in eukaryotes (16). Since *C. reinhardtii* maintains a functional photosynthetic apparatus when grown in the dark with acetate, photosynthesis-deficient mutants have been identified as light-sensitive, acetate-requiring strains (10). Genetic analysis can also be performed, and mendelian or uniparental (maternal) inheritance defines nuclear or chloroplast mutations, respectively (5).

We have been particularly interested in using chloroplast mutants and their revertants to investigate the structure/function relationships of Rubisco[3] (13). As in higher plants, Rubisco consists of eight nuclear-encoded small subunits and eight chloroplast-encoded large subunits, and the large subunit appears to contain the active-site domain (7). However, of three Rubisco mutants defined at the level of the large-subunit gene sequence (3, 15), two were found to result from nonsense mutations. Calculating from the large-subunit gene sequence (2), one would expect only about 5% nonsense mutations out of the total number of point mutations that could alter the large-subunit protein sequence. It thus appeared that many mutants with amino-acid substitutions in the large-subunit protein were not being recovered as acetate-requiring strains. Furthermore, several revertants of the nonsense mutants were found to contain much less than 50% of the normal amount of Rubisco holoenzyme, but they were able to grow photoautotrophically (in the absence of acetate) (9, 14). Therefore, mutants with significant alterations in Rubisco may not necessarily require acetate for growth.

Although there is no technique that would allow every amino-acid substitution to be identified by a mutant phenotype, we have decided to screen for temperature-conditional photosynthesis mutants for several reasons: (a) they would increase our yield of Rubisco mutants from a single experiment, (b) they would almost always be missense mutants, (c) they would allow biochemical analysis of partially-defective Rubisco at the permissive temperature, and (d) they would have a scorable phenotype at the restrictive temperature and thus permit genetic analysis and the selection of revertants.

Few attempts have been made at recovering temperature-sensitive mutants in plants. The most thorough study of temperature-sensitive, photosynthesis-deficient mutants was specifically concerned with Chl synthesis in *C. reinhardtii* (4). However, mutants were recovered by screening only for altered pigmentation. We have instead recovered and analyzed a group of temperature-sensitive, acetate-requiring mutants with regard to their general biochemical and genetic properties. Mutants of this type may be particularly useful for investigating partial lesions in a number of photosynthetic components.

MATERIALS AND METHODS

Strains and Culture Conditions. Wild type *C. reinhardtii* 2137 mt^+ (10) was used in mutagenesis experiments, and the *pf*-2 mt^- centromere-marker strain was employed for genetic analysis. All strains were maintained on solid medium containing 10 mM sodium acetate and 1.5% Bacto agar at 25°C in the dark (10), unless noted otherwise. Mutants were screened in the light on the same medium without the addition of sodium acetate (minimal medium). Liquid cultures consisted of 50 or 250 ml of acetate medium in a 250-ml or 1000-ml Delong flask. Cells were grown in liquid on a rotary shaker (220 rpm) at 35°C in the dark until the cells were in a late logarithmic phase of growth (2.5×10^6 cells/ml).

Mutagenesis and Screening. Independent cultures of wild-type mt^+ cells, within each separate experiment, were grown in the dark and treated with 1 mM 5-fluorodeoxyuridine at 25°C as described previously (10). This treatment reduces the ploidy of the chloroplast genome to about one copy and thus allows chloroplast mutants to be recovered (18). The cells from each culture were then concentrated to 2×10^7 cells/ml in 68 mM potassium phosphate buffer (pH 6.9), and mutagenized with 27 mM methyl methanesulfonate (Aldrich) for 1.5 h in the dark at 25°C. The cells were washed with phosphate buffer and used to inoculate a 50-ml acetate culture at 3×10^4 cells/ml. The cultures were grown to stationary phase in the dark at 25°C. The cells were

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[3] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; pf, paralyzed flagella; mt, mating type; PD, parental ditype; NPD, nonparental ditype; T, tetratype.

plated at 200 to 500 cells per 100-mm Petri dish of acetate medium and grown in the dark at 25°C. After 2 weeks, the plates were replica plated to minimal medium at 80 μE m^{-2} s^{-1} and 35°C. Colonies that failed to grow on minimal medium at the elevated temperature (35°C) were picked from the acetate-dark-25°C master plates and maintained on acetate medium in the dark at 25°C. Only mutants of independent origin were analyzed in the present study.

Spot Tests. The phenotype of every mutant strain was determined by plating a drop of cell suspension (~1 × 10^6 cells/ml) on minimal medium in the light (80 μE m^{-2} s^{-1}) and acetate medium in the dark, both at 25 and 35°C. Growth was compared with wild-type growth after 2 weeks. Characterized mutants were spot tested on minimal medium at 45 μE m^{-2} s^{-1} and 80 μE m^{-2} s^{-1}, acetate medium at 45 μE m^{-2} s^{-1} and 80 μE m^{-2} s^{-1}, and acetate medium in the dark, all at both 25 and 35°C.

Genetic Analysis. Mutant strains (mt^+) were crossed with a *pf*-2 mt^- strain to determine whether the mutant phenotypes were inherited in a mendelian (nuclear) or uniparental (chloroplast) pattern. Furthermore, since *pf*-2 is a centromere marker, it almost always segregates at the first division of meiosis. This allowed each mendelian mutation to be mapped relative to its centromere based on its frequency of segregation during the second division of meiosis (*i.e.* frequency of recombination between the mutation and its centromere) (10). Gene-centromere distance is thus calculated from the number of different tetrads observed: 100 × ½ T/(PD + NPD + T). Gamete induction, zygote maturation, and tetrad dissection were performed with acetate medium at 25°C as described previously (10). To score the mutant phenotype, tetrads were replica plated to minimal medium at 80 μE m^{-2} s^{-1} and acetate medium in the dark, each at 25 and 35°C.

Biochemical Analysis. The mt^+ progeny of mutant strains were maintained on acetate medium in the dark at 35°C for several weeks prior to investigating their biochemical properties. Acetate-medium liquid cultures were then grown in the dark at 35°C just prior to analysis.

Cells were harvested in a clinical centrifuge at 35°C and resuspended in 35°C minimal medium at 10 μg Chl/ml. Light-dependent oxygen evolution by whole cells was then measured at 35°C with a Hansatech oxygen electrode at saturating CO_2 and a light intensity of 800 μE m^{-2} s^{-1}. In the same way, whole-chain electron transport was measured as oxygen uptake in the presence of methyl viologen and gramacidin S (12). All values were measured as the percentage of the wild-type rates per unit Chl measured during the same experiment. The reported values are the average of three independent determinations.

To measure Rubisco carboxylase activity, 40 ml of a 35°C dark-grown culture were chilled to 0°C, pelleted at 3,000*g* for 10 min at 4°C, and sonicated for 3 min in 1.5 ml of 1 mM DTT, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 50 mM Bicine (pH 8.0) at 0°C. The cell extract was centrifuged at 30,000*g* for 15 min at 4°C. Then, 100 μl of supernatant were mixed with 200 μl of reaction mix (1 mM DTT, 10 mM $MgCl_2$, 0.6 mM RuBP, 10.22 mM $NaH^{14}CO_3$ [1.17 Ci/mol], 50 mM Bicine, pH 8.0) in a scintillation vial to start the reaction. After 2 min at 35°C, the assays were stopped with 150 μl of 3 M formic acid in methanol. The reactions were dried and acid-stable dpm were determined by liquid scintillation counting.

Sonicated cell extracts, prepared as described above, were used for determining the amount of Chl per unit of total protein. Chl was measured spectrophotometrically after extraction with 95% ethanol (17). Protein was assayed by the method of Bradford (1).

RESULTS

Recovery of Mutants. Mutagenized colonies were replica plated from acetate medium in the dark at 25°C to minimal medium in the light (80 μE m^{-2} s^{-1}) at 35°C. As shown in Table I, colonies that failed to grow on minimal medium at this restrictive temperature (nonphotoautotrophic) were recovered at a frequency of about 2 mutants per 100 colonies that were screened. Spot tests were performed to define the phenotypes of the mutants. As would be expected from the screening strategy, three general classes of mutants were identified.

The largest class of mutants (69%) consisted of temperature-sensitive, acetate-requiring strains. These mutants could grow photoautotrophically at the permissive temperature (25°C), but could not grow, or grew poorly, on minimal medium at the restrictive temperature (35°C). They could, however, grow equally well on acetate medium in the dark at 25 and 35°C. Whereas the majority of the temperature-sensitive, acetate-requiring mutants were stringent acetate-requirers at the restrictive temperature, about one-third of them were too 'leaky' for further biochemical or genetic analysis.

A second class of mutants was comprised of nonconditional acetate-requiring strains. They were unable to grow photoautotrophically at either 25 or 35°C, but they could grow on acetate medium in the dark. Of the mutants recovered in the present study (Table I), 27% had this 'standard' acetate-requiring phenotype (10).

About 4% of the mutants were unable to grow under all growth conditions at 35°C, but they could grow on minimal medium at 25°C. These mutants are thus temperature-sensitive mutants that may be defective in any one of the many metabolic functions of the cell.

Genetic Analysis. Twenty temperature-sensitive, acetate-requiring mutants were chosen for further analysis. Emphasis was placed on mutants that had a stringent acetate-requiring phenotype at the restrictive temperature (35°C), but were phenotypically indistinguishable from wild type at the permissive temperature (25°C). These mutants were also normally pigmented (green) in the dark at both temperatures. During initial analysis, four mutants were determined to be unsuitable for further study. Three of these would not produce viable progeny after a cross, and one appeared to result from a double-mutation event. This latter occurrence is not unexpected, considering the high mutant-recovery frequency (Table I). Of the 16 remaining mutants that were analyzed (Table II), three displayed uniparental (maternal) inheritance of the temperature-sensitive, acetate-requiring phenotype (67-12E, 68-4PP, 69-7M). The rest of the mutants showed 2:2 segregation of the mutant and wild-type phenotypes within tetrads, and thus followed a mendelian pattern of inheritance. The *pf*-2 centromere marker also segregated 2:2 with its wild-type allele in all of the crosses. The presence of this marker allowed the mendelian mutations to be mapped relative to their centromeres by determining the frequences of the observed PD, NPD, and T tetrads (10). As can be seen in Table II, many of the mutants have significantly different gene-centromere distances. Thus, it appears that a number of mutations can give rise to a temperature-sensitive, acetate-requiring phenotype.

About 3% of all of the temperature-sensitive, acetate-requiring mutants that were initially recovered had a pigment-deficient phenotype. They were green in the dark at 25°C, but at 35°C they were not pigmented normally. Three mutants of this type were analyzed genetically (Table III). Each of them displayed a

Table I. *Recovery of Nonphotoautotrophic Mutants at Restrictive Temperature*

Experiment No.	Colonies Screened	Mutants Recovered	Mutants/ 10^2 Colonies
67	15,203	236	1.6
68	30,746	647	2.1
69	15,506	278	1.8

Table II. *Genetic Analysis of Temperature-Sensitive, Acetate-Requiring Mutants*

Mutant *mt*$^+$	Cross to *pf*-2 *mt*$^-$			Gene-Centromere Distance
	PD	NPD	T	
67-4J	6	7	0	0
67-8X	12	13	3	5
67-12E	Uniparental inheritance			
68-3C	5	9	1	3
68-4PP	Uniparental inheritance			
68-5AN	2	3	24	41
68-6L	12	15	6	9
68-7AX	1	10	15	29
68-9AS	11	13	8	13
68-11AR	0	3	5	31
68-12F	2	3	5	25
69-1XA	6	7	6	16
69-2G	11	5	7	15
69-7M	Uniparental inheritance			
69-8H	0	2	11	42
69-12B	5	3	30	39

mendelian pattern of inheritance, and the pigment alteration cosegregated with the acetate-requiring phenotype in every case.

Three temperature-sensitive mutants, that died under all growth conditions at 35°C, were also analyzed (Table IV). All mutations of this type were inherited in a mendelian pattern.

Biochemical and Phenotypic Analysis. Progeny clones of the 16 temperature-sensitive, acetate-requiring mutants described in Table II were analyzed biochemically at the restrictive temperature (35°C). As shown in Table V, four of the mutants did not appear to be defective in photosynthesis (67-4J, 68-3C, 69-1XA, 69-8H). Nonconditional acetate-requiring mutants of this type have been observed previously (10). However, the 69-1XA and 69-8H mutants had greatly reduced growth on acetate medium in the light, and 67-4J died at the highest light intensity (80 μE m^{-2} s^{-1}).

The remaining 12 mutants had significant reductions in O_2 evolution, and thus appeared to result from lesions in photosynthesis (Table V). Most of these mutants had light-sensitive phenotypes (10). They grew better on acetate medium at 45 μE m^{-2} s^{-1} than at 80 μE m^{-2} s^{-1}. Three of the mutants (67-12E, 68-9AS, 69-12B) 'bleached' after growing on acetate medium for 1 week at either light intensity. However, two of the photosynthesis-deficient mutants (68-4PP, 68-12F) did not appear to be sensitive to light. The 68-12F mutant was the only leaky strain in the group of mutants; it grew slightly on minimal medium at 35°C. The presence of some photosynthetic activity in these non-light-sensitive mutants (Table V) may have contributed to their lack of photoinhibition.

Only one mutant (69-12B) was found that had a specific reduction in whole-chain electron-transport activity. However, other distinct types of mutants were identified. For example, two uniparental mutants (67-12E, 69-7M) were found to have reduced Chl, reduced oxygen evolution per unit Chl, and, in the case of 69-7M, reduced Rubisco carboxylase activity. These mutants are similar to chloroplast protein-synthesis mutants that have pleiotropic photosynthesis defects (6). However, mutants of this type were not found in our previous mutant-recovery experiments that were designed, as in the present experiments, to maximize the yield of stringent photosynthesis-deficient mutants (10). One other particularly unusual mutant was identified. The 68-7AX mutant displayed light-dependent O_2 uptake under conditions for assaying O_2 evolution in whole cells (Table V). However, reduction of methyl viologen occurred at a rate that was twice as fast as wild type. Further analysis will be necessary to determine the basis of this defect.

Several mutants had reduced Rubisco carboxylase activity. As noted above, the uniparental 69-7M mutant had reduced carboxylase as part of a syndrome of photosynthesis defects that may be caused by defective chloroplast protein synthesis. However, two other mutants (68-4PP, 68-11AR) also had reduced carboxylase activity, but their whole-chain electron transport and Chl levels were not markedly different from wild-type levels. The 68-4PP mutant displayed uniparental inheritance (Table II) and is thus likely to result from a mutation within the chloroplast-encoded Rubisco large-subunit gene (13). In contrast, the 68-11AR mutant was inherited in a mendelian pattern. Nuclear mutations that specifically affect Rubisco activity were not recovered in our previous experiments at 25°C (10, 12).

DISCUSSION

Temperature-sensitive mutants have generally been used to investigate proteins or biochemical pathways that are essential for cell function. Mutations in such cases would be lethal if they were not temperature-conditional. We have recovered temperature-sensitive mutants for a somewhat different reason. Although photosynthesis-deficient mutants can be maintained as acetate-requiring strains in *C. reinhardtii*, these mutants are usually devoid of photosynthetic function. They are useful as 'blocks' in the biochemical pathways of photosynthesis, but they may not provide much information about the function of individual components of the photosynthetic apparatus. However, temperature-sensitive, photosynthesis-deficient mutants may have only partially defective photosynthesis at the permissive temperature and thus allow more detailed biochemical analysis of photosynthesis. At the restrictive temperature, the gross biochemical defect could be easily defined because *C. reinhardtii* normally maintains full photosynthetic potential when grown heterotrophically (acetate in the dark). Furthermore, the mutants would have a scorable, acetate-requiring phenotype at the restrictive temperature, allowing detailed genetic analysis and manipulation.

Mutants were generated in the same way as nonconditional acetate-requiring mutants described in previous studies (10, 12). However, heterotrophically grown colonies were screened for the absence of photoautotrophic growth at 35°C instead of at 25°C. Thus, both nonconditional and temperature-sensitive, acetate-requiring mutants were recovered from the same experiments. Nonphotoautotrophic mutants were recovered at a frequency of about two mutants per 100 colonies (Table I). This frequency is about 10 times higher than the recovery frequency usually observed at 25°C ($\sim 2/1 \times 10^3$) (10), and about 73% of the nonphotoautotrophic mutants recovered at 35°C were temperature-conditional. Thus, the increase in recovery frequency appears to be directly related to the presence of the temperature-

Table III. *Genetic Analysis of Temperature-Sensitive, Pigment-Deficient Mutants*

Mutant *mt*$^+$	Color in Dark at 35°C	Cross to *pf*-2 *mt*$^-$			Gene-Centromere Distance
		PD	NPD	T	
67-2T	Yellow-green	6	10	9	18
68-5G	Yellow-green	7	4	8	21
68-9L	Pale-green	5	7	9	21

Table IV. *Genetic Analysis of Temperature-Sensitive Mutants*

Mutant *mt*$^+$	Cross to *pf*-2 *mt*$^-$			Gene-Centromere Distance
	PD	NPD	T	
68-9PP	11	10	0	0
69-9J	7	6	9	20
69-10C	11	10	7	13

Table V. *Biochemical Analysis of Temperature-Sensitive, Acetate-Requiring Mutants at Restrictive Temperature*

Mutant	Oxygen Evolution[a]	Whole-Chain e$^-$ Transport Activity[a]	Chl[a]	Rubisco Carboxylase Activity[a]
	Each value is the percentage of the wild-type level			
67-4J	92	130	86	134
67-8X	2	66	102	106
67-12E	20	112	26	108
68-3C[b]	86	59	89	99
68-4PP[b]	15	116	88	1
68-5AN	17	86	141	107
68-6L	1	68	130	71
68-7AX	−52	266	121	103
68-9AS	1	59	86	65
68-11AR	20	120	80	12
68-12F[b]	24	88	52	79
69-1XA	90	98	134	126
69-2G	41	101	87	61
69-7M	0	98	15	8
69-8H	74	88	82	91
69-12B	2	37	72	94

[a] Wild-type control values were: O_2 evolution, 156 μmol/h · mg Chl; whole-chain electron (e^-) transport, 27 μmol O_2/h · mg Chl; Chl, 55 μg/mg total protein; Rubisco carboxylase, 4 μmol CO_2/h · mg soluble protein. [b] Growth on acetate medium was not inhibited by light.

conditional mutants. This high recovery frequency indicates that the use of 'enrichment' techniques may be unnecessary for increasing the yield of photosynthesis-deficient mutants.

Whereas the majority of the nonphotoautotrophic mutants were able to grow heterotrophically at 35°C, about 4% were unable to survive on acetate medium in the light or dark at 35°C. This group of mutants is expected to consist of strains that primarily have defects in proteins that function outside of photosynthesis and that are required for cell growth and division in the dark. It is thus surprising that such a small number of these mutants was recovered relative to the number of temperature-sensitive mutants that could survive on acetate in the dark at 35°C. Perhaps these general temperature-sensitive mutants also grow slightly slower than wild type or photosynthesis mutants in the dark at 25°C. They may be under-represented in the mutagenized population of cells after extended growth in the dark prior to plating out the cells. In any case, the methods employed for recovering temperature-sensitive, photosynthesis mutants may not be appropriate for recovering general temperature-sensitive mutants.

We were primarily interested in identifying photosynthesis-deficient mutants from among the temperature-sensitive, acetate-requiring mutants. In general, the types of mutants in this group were similar to acetate-requiring mutants that were recovered at 25°C (10, 12). Several mutants did not appear to have defective photosynthesis (Table V), and these were all inherited in a mendelian pattern (Table II; 67-4J, 68-3C, 69-1XA, 69-8H). Temperature-sensitive, pigment-deficient mutants were also recovered. They displayed mendelian inheritance (Table III), as expected for pigment-deficient mutants in *C. reinhardtii* (10). Furthermore, normally pigmented, photosynthesis-deficient mutants were most common among the temperature-sensitive, acetate-requiring mutants. Both mendelian and uniparental mutants were found in this group (Table II). One mutant appeared to be specifically deficient in whole-chain electron transport (Table V; 69-12B), while two others had reduced Rubisco carboxylase activity (Table V; 68-4PP, 68-11AR). It thus appears that the temperature-sensitive, photosynthesis-deficient mutants have a range of lesions in photosynthesis. We expect that temperature-conditional mutants could be useful for investigating the function of a variety of photosynthetic components.

Although there were many similarities between nonconditional and conditional acetate-requiring mutants, two differences were apparent. The first concerns the photosensitivity of acetate-requiring mutants. In previous studies (10, 12), photosynthesis-deficient mutants were found to have light-sensitive phenotypes, but photosynthesis-competent, acetate-requiring mutants were not sensitive to light. Thus, photosynthesis-deficient mutants would be lost during a mutant recovery experiment if cells were grown or plated with acetate medium in the light prior to screening. We further showed that the photosensitivity of photosynthesis-deficient mutants could be suppressed by secondary mutations, some of which also produced lesions in photosynthesis (11). Therefore, photosynthesis-deficient mutants are maintained on acetate medium in the dark to guard against selection for suppressors of photosensitivity. In the present investigation, two photosynthesis-deficient mutants out of the 12 analyzed (Table V) were found to have nonlight-sensitive phenotypes. These mutants (68-4PP and 68-12F) had some photosynthetic activity, and this partial activity may confer some protection from light. On the other hand, three of the four photosynthesis-competent mutants (67-4J, 69-1XA, 69-8H; Table V) were sensitive to light. It thus appears that either different types of mutants are recovered as temperature-sensitive, acetate-requiring mutants, or the biochemical basis of photoinhibition is different at the elevated temperature. Nevertheless, a correlation was observed between photosensitivity and photosynthesis deficiency in the temperature-sensitive, photosynthesis-deficient mutants. Although not all of these mutants were obviously light sensitive, precautions will still be required to prevent the loss or suppression of the photosynthesis-deficient mutants in most instances.

The nonconditional and conditional acetate-requiring mutants differed in a second way. Temperature-sensitive, acetate-requiring mutants appeared to include new types or different proportions of mutants when compared with the nonconditional, acetate-requiring mutants recovered in a previous study (10). As discussed above, three light-sensitive mutants were recovered that did not have obvious defects in photosynthesis. Furthermore, five of the photosynthesis-deficient mutants (67-12E, 68-4PP, 68-5AN, 68-12F, 69-2G; Table V) retained some photosynthetic activity and two of these were not sensitive to light (68-4PP, 68-12F). Nonconditional photosynthesis mutants are usually light-sensitive and lack photosynthetic activity (10, 12). It thus appears that the collection of conditional photosynthesis mutants includes mutants that have not been recovered previously, primarily because they are recovered as conditional mutants. Whereas the retention of some photosynthetic ability may have been expected even at the restrictive temperature, several other unique mutants were recovered. Two uniparentally inherited mutants (67-12E, 69-7M) appeared to have a syndrome of photosynthesis defects (Table V). They had reduced Chl and thus, reduced whole-chain electron transport. One of them (69-7M) also had reduced Rubisco carboxylase activity. Nonconditional mutants of this type have been recovered previously in experiments performed with acetate medium in the light (6). They have defective chloroplast protein synthesis that causes the reduction of a num-

ber of photosynthetic components. We suspected that mutants of this type were not recovered in our previous experiments because they may have had reduced growth during growth and plating with acetate in the dark (10). If dark growth limited the recovery of the protein synthesis mutants, this may be less of a problem during the recovery of temperature-conditional mutants, which would have near-normal growth at the permissive temperature prior to screening.

We initially decided to screen for temperature-sensitive photosynthesis mutants as a method for recovering partially defective Rubisco mutants. One uniparentally inherited mutant (68-4PP) was recovered that is likely to result from a mutation in the chloroplast-encoded large-subunit gene (13). It has normal levels of Chl and whole-chain electron transport, and is thus not one of the putative chloroplast protein synthesis mutants described above. However, one other mutant (68-11AR) also had a specific reduction in carboxylase activity, but it was inherited in a mendelian pattern. Only one mendelian Rubisco mutant has ever been investigated in detail (8), and that *Chlamydomonas* mutant is no longer available. Therefore, the temperature-sensitive, photosynthesis-deficient mutants appear to have a rather high proportion of Rubisco-deficient mutants. In fact, the majority of the conditional photosynthesis mutants have lesions outside of photosynthetic electron-transport. Only one mutant was recovered that had reduced electron-transport activity (69-12B; Table V). In previous experiments (10), photosynthetic electron transport mutants were found in excess of other nonconditional photosynthesis mutants. Perhaps mutations that affect electron-transport components of the photosynthetic apparatus are especially lethal, which would limit their recovery as temperature-sensitive mutants. Nevertheless, Rubisco-deficient mutants were recovered at a high frequency among the temperature-sensitive, acetate-requiring mutants. Detailed analysis of these mutants at both the permissive and restrictive temperatures may define their precise biochemical lesions.

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