Nuclear Suppressors of the Photosensitivity Associated with Defective Photosynthesis in *Chlamydomonas reinhardii*

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**ABSTRACT**

Several nuclear mutations were recovered that suppress the photosensitivity associated with the *Chlamydomonas reinhardii* chloroplast mutant *rcl-u-1-10-6C*, which is defective in ribulose-1,5-bisphosphate carboxylase/oxygenase. Two of the suppressor mutations affect other components of photosynthesis. These results show that suppressors of photosensitivity are sufficiently common to permit the recovery of photosensitive, photosynthesis-deficient mutants in bright light, and indicate that photosynthesis-deficient mutants selected and maintained in the light may accumulate suppressors which can confuse the biochemical analysis of lesions in photosynthesis. One of the suppressor mutations inhibits photosystem II activity, indicating that photosensitivity can be mediated by partial reactions of the photosynthetic electron transport chain.

Mutants of the green alga *Chlamydomonas reinhardii* that lack photosynthesis can be maintained on acetate as an exogenous carbon source. These mutants are frequently sensitive to light, growing better in the dark than in the light when supplied with acetate. Several *C. reinhardii* mutants, selected as pigment-deficient strains, were found to be photosensitive (5, 12, 20, 21), while others, selected as light-sensitive, included mutants with altered pigmentation (18, 19). Some degree of photosensitivity was also associated with PSI and photosophorylation-deficient mutants (14) even though these mutants were recovered in the light. More recently, Spreitzer and Mets (16) found a direct correlation between deficiency of photosynthesis and photosensitivity in a wide range of mutants recovered from dark-grown cells. This collection includes mutants that lack normal pigmentation, PSI activity, PSII activity, or Calvin cycle activity. Selman-Reimer et al. (13) have shown that several of these mutants also lack photosophorylation activity. Spreitzer and Mets (16) suggested that some photosynthesis-deficient mutants may not have been recovered previously because they were photosensitive and would not survive the bright light commonly present in mutant isolation experiments. For example, they recovered a chloroplast (uniparental) mutant, *rcl-u-1-10-6C*, that lacks normal function of the Calvin cycle enzyme RuBPICO\(^2\) (15, 16). Nevertheless, the majority of photosynthesis-deficient *C. reinhardii* mutants have been selected in bright light and have not been described as photosensitive (e.g. 4, 8).

In experiments designed to recover revertants of *rcl-u-1-10-6C*, we sought strains with partially restored RuBPCO activity using a non-light-sensitive acetate-requiring phenotype as the selection criterion. We reasoned that this type of revertant might carry out enough photosynthesis to reduce photosensitivity but not enough to survive photoautotrophically. Because non-light-sensitive strains were recovered at a high frequency, we reasoned that perhaps many of these, as well as previous photosynthesis mutants capable of growth in the light on an exogenous carbon source, were actually double mutants, the second mutation relieving the light sensitivity caused by a primary mutation blocking photosynthesis. To explore this possibility, genetic and physiological analyses were conducted on non-light-sensitive strains derived from the light-sensitive RuBPCO mutant. All of the strains examined appeared to result from nuclear ( mendelian) gene mutations that suppressed photosensitivity without affecting RuBPCO. When separated from *rcl-u-1-10-6C*, these suppressors had either wild-type or photosynthesis-deficient phenotypes.

**MATERIALS AND METHODS**

Strains. Several photosynthesis-deficient mutants of *Chlamydomonas reinhardii* have been partially characterized (16). In all selection experiments reported here, the uniparentally inherited RuBPCO mutant *rcl-u-1-10-6C* mt+ (15) was used. Because of associated photosensitivity, this mutant cannot grow on acetate medium at 4,000 lux. Wild-type strain 2137 mt+ (16) and centermore marker strain pf-2 (paralyzed flagella) mt- were used for genetic analysis.

Culture Conditions. All strains were maintained on 10 mm sodium acetate medium (16) containing 15 g/l Bacto agar at 25°C. Photosynthesis-deficient mutants were cultured in the dark. The 2137 mt+ and pf-2 mt- strains were cultured under cool-white fluorescent light at 2,000 lux. Liquid cultures consisted of 50 ml of acetate medium and were grown on a rotary shaker in the dark. Cells were counted with a hemacytometer.

Mutagenesis and Selection. Clones of *rcl-u-1-10-6C* mt+ were isolated and used in separate experiments to maximize the independence of revertants. Independent cultures within a single experiment were prepared containing 3 × 10\(^5\) cells/ml in 1 mm 5-fluorodeoxyuridine in acetate medium and grown in the dark. The frequency of recovery of uniparental mutants is increased by 5-fluorodeoxyuridine treatment (23). When the cultures reached stationary phase (5 × 10\(^5\) cells/ml), they were subjected to mutagenesis with 0.27 mm ethyl methanesulfonate in 0.1 mm phosphate buffer (pH 6.9) (10). After 2 h in the dark, the cells were washed with 0.02 mm phosphate buffer and resuspended in acetate liquid cultures at 3 × 10\(^5\) cells/ml. When the cells reached stationary phase, they were plated at 2 × 10\(^4\) or 2 × 10\(^5\) cells per 100-mm Petri dish on acetate medium at 4,000 lux. Colonies that grew under these conditions were picked from independent Petri dishes.

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\(^{2}\) Abbreviations: RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; mt, mating type; PD, parental ditype; NPD, nonparental ditype; T, tetratype; DCP, dichlorphenolindophenol.

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Genetic Analysis. Each non-light-sensitive strain was crossed to pf-2 mt− to determine if its phenotype was uniparental or mendelian. From these crosses, non-light-sensitive acetate-requiring pf-2 mt− strains were recovered and crossed to 2137 mt+. Since uniparental mutants are not transmitted by the mt− parent, these backcrosses eliminated rcl-u-1-10-6C and allowed the phenotypes of mendelian suppressors to be visualized. Gamete induction, mating, zygote maturation, and germination were performed as described previously (16). Phenotypes of progeny in tetrads were determined by replica-plating tetrads to minimal medium (not containing acetate) at 4,000 lux, acetate medium at 4,000 lux, and acetate medium in the dark. In contrast to the photosensitive acetate-requiring rcl-u-1 strain, non-light-sensitive acetate-requireers are able to grow on acetate medium at 4,000 lux, while neither is able to grow on minimal medium. The pf-2 and mt markers were scored as described previously (16). For mendelian traits in crosses which included pf-2, tetrads were scored as PD, NPD, or T, and tetrad analysis was performed to determine gene-centromere distance as described previously (16).

Spot Tests. Phenotypes of strains were compared with regard to photosensitivity, acetate requirement, and pigmentation as described previously (16).

Biochemical Analysis. PSII activity was measured spectrophotometrically at 590 nm as the rate of reduction of DCPIP (9). Dark-grown cells were sonicated at 0°C in buffer (2.5 mM MgCl₂, 20 mM KCl, in 1 mM phosphate buffer, pH 7.0) for 2 min at a setting of 150 on an Insonator sonicator (model 500; Savant Instruments, Inc.). An aliquot of this lysate was added to 0.01 mM DCPIP in the same buffer. The reaction was allowed to proceed for 1 min at 25°C at a light intensity of 1500 μE m⁻² s⁻¹. All values were recorded as the percentage of the wild-type rate per unit Chl measured from the same lysate.

CO₂ fixation was measured in whole cells as the rate of incorporation of NaH¹⁴CO₃ into acid-stable material as described previously (15).

RuBP carboxylase activity was measured in dark-grown cells. Cells were sonicated at 0°C for 5 min at a setting of 150 on an Insonator sonicator in 1 mM DTT, 5 mM MgCl₂, 10 mM NaHCO₃, 50 mM Tris Base (pH 7.5). The lysate was centrifuged at 27,000g for 15 min and the supernatant was retained. To start the reaction, equal volumes of supernatant and reaction mix (1 mM DTT, 15 mM MgCl₂, 90 mM NaH¹⁴CO₃ (0.5 μCi/μmol), 1 mM RuBP, 150 mM Tris Base (pH 7.5) were combined at 25°C. Samples were removed at 0, 1, 3, and 5 min and mixed with an equal volume of HCl-acetic acid (4:1) to stop the reaction. The samples were dried in scintillation vials and acid-stable dpm determined by liquid scintillation counting.

Chl was measured spectrophotometrically in a clarified supernatant of a 95% ethanol extract (22).

RESULTS

Recovery of Non-Light-Sensitive Revertants. Non-light-sensitive colonies arose from mutagenized cells of rcl-u-1-10-6C mt+ plated on acetate medium at 4,000 lux. The phenotypes of these colonies ranged from small and pale to large and dark green. Many colonies bleached during the experiment. Some colonies had altered pigmentation, but these could not be enumerated because of bleaching. When plates originally containing 1 × 10⁶ cells were scored after 11 d, the average frequency of green colonies per cells plated was 2.7 × 10⁻⁴ (Table I). In comparison, cells not subjected to mutagenesis gave an average frequency of 0.6 × 10⁻⁴ (Table I). Cells plated at a lower density of 1 × 10⁵ per plate yielded higher average frequencies for both mutagenized (5.0 × 10⁻⁴) and nonmutagenized (0.4 × 10⁻⁴) cells (Table II).

These results suggested that the majority of non-light-sensitive colonies did not result from true reversion of the rcl-u-1 mutant gene, because the forward recovery frequency of rcl-u-1 mutants (16) is lower than the frequency of recovery of non-light-sensitive colonies. The reversion frequency would be expected to be much lower. Therefore, the non-light-sensitive colonies most likely represented mutations which suppressed either photosensitivity or the rcl-u-1 mutation.

Description of Non-Light-Sensitive Strains. Several colonies which grew on acetate medium at 4,000 lux were maintained for further investigation. Characteristics of 13 of these strains are listed in Table III. Spot tests showed that there was some variation in the level of resistance to light among the strains. All strains still displayed some photosensitivity, but they could be readily differentiated from rcl-u-1-10-6C at 2,000 or 4,000 lux. The rcl-u-1-10-6C strain and many other photosynthesis-deficient mutants cannot survive at 4,000 lux (16). All of the non-light-sensitive strains maintained a stringent requirement for acetate. Strain NLS6-1A was pale-green in the dark and yellow-green on acetate at 4,000 lux. Otherwise, all of the other strains had normal green pigmentation in the light and dark.

Biochemical analysis of the non-light-sensitive rcl-u-1 strains (Table III) revealed that all were incapable of CO₂ fixation, a characteristic of rcl-u-1-10-6C. Thus, the strains had not become non-light-sensitive by regaining any degree of photosynthetic competence. PSII activity was found to be completely absent in one strain, NLS6-3A. Since PSII activity is normal in rcl-u-1-10-6C, this finding suggested that a single mutation both reduced photosensitivity in NLS6-3A and inhibited PSII activity.

Genetic Analysis. Each non-light-sensitive rcl-u-1 mt+ strain was crossed to the centromere marker pf-2 mt−. Since rcl-u-1-10-6C is uniparentally inherited, all of the progeny from these crosses are acetate-requiring. If the non-light-sensitive phenotype resulted from a nuclear (mendelian) mutation, there would be 2:2 segregation of non-photosensitivity and photosensitivity. Mendelian inheritance of the non-light-sensitive phenotype, as well as pf-2, was found for each of the 13 strains (Table III). This indicated that nuclear gene mutations were acting as suppressors of the photosensitivity associated with RuBPCO deficiency in rcl-u-1-10-6C. Although in several cases only a small number of tetrads could be recovered, the calculated gene-centromere distances (Table III) suggest that there are several loci for non-light-sensitive suppressors.

Test crosses were carried out on several of the suppressors. Nonlight-sensitive rcl-u-1 mt− progeny were recovered and crossed to 2137 mt+. Since the rcl-u-1-10-6C mutation is not transmitted by the mt− parent, tetrads from these crosses contain the non-light-sensitive suppressor mutation against a wild-type genetic background. Of the strains analyzed, three types of suppressors were found. In all cases, pf-2 was scored to verify meiotic tetrads. The first suppressor type, represented in strains NLS1-3A, NLS2-5A, NLS4-1B, and NLS6-4B, gave rise to progeny indistinguishable from wild-type. Another type, found in strain NLS6-1A, showed
2:2 segregation of wild-type and a pale-green leaky acetate-requiring phenotype. These segregants were pale-green on minimal medium or on acetate medium in the light or dark. Growth on minimal medium was only slightly reduced. We assigned the collection name SLS6-1A to this suppressor of light sensitivity. The final suppressor type, in strain NLS6-3A, yielded stringent acetate-requiring progeny in a 2:2 ratio to wild-type progeny. We assigned the collection name SLS6-3A to this suppressor mutant.

Tetrad analysis of the NLS6-1A and NLS6-3A test crosses, which included the pf/2 centromere marker, yielded gene-centromere distances of 23 (PD = 10, NPD = 12, T = 18) for NLS6-1A and 33 (PD = 5, NPD = 8, T = 25) for NLS6-3A. Contingency chi-square analysis of first and second division segregation tetrads in the forward crosses (Table III) and the test crosses (above) indicated that the gene-centromere distances were not significantly different for NLS/SLS6-1A (P > 0.70) nor NLS/SLS6-3A (P > 0.30). These data indicate that, in the case of both NLS6-1A and NLS6-3A, a single mutation (SLS6-1A and SLS6-3A, respectively) both altered photosynthetic properties and suppressed the photosensitivity usually associated with rcl-u-1-10-6C.

To demonstrate clearly that suppression of light sensitivity, requirement for acetate, and absence of PSII activity were all characteristics of a single mutation in NLS6-3A, several forward and test-cross tetrads were analyzed biochemically. Table IV shows that the absence of RuBP carboxylase activity was associated with the inheritance of rcl-u-1-10-6C. In forward crosses, non-light-sensitive was linked to the absence of PSII activity, as was the acetate-requiring phenotype in the test-cross tetrads.

Suppression of Other Photosynthesis-Deficient Mutants. Nonlight-sensitive colonies were recovered from other types of photosensitive, photosynthesis-deficient mutants when plated on acetate medium at 4,000 lux (Table V). These mutants have been described previously (16). Strains 11-2B and 11-4D were the least light sensitive, but non-light-sensitive colonies could still be observed against a faint background lawn of surviving cells. Several of the other strains grew slightly on acetate medium at 4,000 lux. After about 5 d, this growth bleached, leaving behind non-light-sensitive colonies.

We have not analyzed the nonphotosensitive strains observed in this experiment, but they did not arise by reversion to photoautotrophy. The frequency of recovery of photoautotrophic revertants was very low, as shown by the absence of colonies on minimal medium (Table V). Only for one mutant, w-7, which is an obligate heterotroph that lacks almost all pigmentation, were no non-light-sensitive colonies observed.

DISCUSSION

Mutations which suppress the photosensitivity usually associated with photosynthesis-deficient mutants may explain how previous investigators succeeded in selecting such mutants in bright light. In the present study, we have found that these suppressors occur at several loci (Table III) and at a high frequency (Tables I and II). Because some suppressors exert apparently no deleterious effect in an otherwise wild-type strain, it is possible that wild-type strains used in mutant isolation experiments may contain suppressors of photosensitivity. Although we have investigated suppression in only one photosynthesis-deficient mutant, rcl-u-1-10-6C, spontaneous non-light-sensitive colonies can be obtained in many other mutant types (Table V). In addition, mutations which suppress the photosensitivity of some pigment-altered mutants have been described (7) and characterized in C. reinhardtii (1, 2, 3). Double mutations, one suppressing photosensitivity and another blocking photosynthesis, would be readily maintained in mutant stocks grown on acetate in the light. This association would also be maintained following a cross, since the least photosensitive progeny would be retained in culture collections.

Although suppressors of photosensitivity may appear to be useful in the general handling of photosensitive photosynthesis-deficient mutants, our results show that some can exert their own influence on photosynthesis. In our collection of suppressor mutations, one lacks PSII activity (SLS6-3A) while another alters pigmentation (SLS6-1A). The presence of these types of suppressors in a mutant strain with a primary photosynthetic lesion may confuse the biochemical analysis of defective photosynthesis. For example, the NLS6-3A isolate, which carries separate mutations affecting RuBPCO activity (rcl-u-1-10-6C) and PSII activity (SLS6-3A), could be mistaken for the chloroplast protein synthesis-deficient mutants described by others (6, 14). For this reason, biochemical analysis should be conducted only on the original photosensitive strains, and to prevent the subsequent appearance of suppressor mutations, all mutants should be maintained in the dark. It is essential that conclusions regarding the biochemical or physiological lesion of photosynthesis-deficient mutants be verified by analyzing tetrads from an appropriate cross.

The mechanism of light sensitivity in C. reinhardtii photosynthesis-deficient mutants is not known, but may be related to the
Table IV. Reciprocal Crosses of NLS6-3A

<table>
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<tr>
<th>Tetrads</th>
<th>Progeny</th>
<th>Phenotype</th>
<th>PS II activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RuBP carboxylase activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tetrads</th>
<th>Progeny</th>
<th>Phenotype</th>
<th>PS II activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RuBP carboxylase activity&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup> Abbreviations: ac, acetate-requiring; nls ac, non-light-sensitive, acetate-requiring; wt, wild-type.

<sup>b</sup> Values are from single experiments. Control values were: PSII activity, 52 μmol DCPIP·mg<sup>-1</sup> Chl·h<sup>-1</sup>; RuBP carboxylase activity, 6 μmol CO<sub>2</sub>·1 X 10<sup>6</sup> cells<sup>-1</sup>·h<sup>-1</sup>.

Table V. Spontaneous Recovery of Non-Light-Sensitive and Photoautotrophic Colonies from Photosynthesis-Deficient C. reinhardtii Mutants

<table>
<thead>
<tr>
<th>Mutant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lesion</th>
<th>Frequency of Colonies at 4,000 Lux&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Acetate Medium&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Minimal Medium&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>× 10&lt;sup&gt;6&lt;/sup&gt;</td>
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<sup>a</sup> These mutants have been characterized previously (16).

<sup>b</sup> In this experiment, 5 × 10<sup>6</sup> cells were plated on acetate medium and 5 × 10<sup>6</sup> cells were plated on minimal medium from the same dark-grown, nonmutagenized cultures at 1 × 10<sup>6</sup> cells/100-mm Petri dish.

<sup>c</sup> This mutant lacks PSII activity in vitro but has wild-type fluorescence induction (16).

<sup>d</sup> Selman-Reimer et al. (13) found these mutants to be deficient in photosophorylation (PP).

The phenomenon of photoinhibition observed in higher plants. In higher plant photosynthesis, an inability to dissipate absorbed light energy initially leads to damage of PSII, and subsequently the photosynthetic apparatus (11). Although the absence of photosynthesis in higher plants is lethal, it is not lethal in C. reinhardtii. Thus, in C. reinhardtii, photodestruction must be the result of damage to additional essential cellular functions. In mutants that have defects in photosynthesis, reductant generated by partial photosynthetic reactions may be channeled to many other cellular constituents which, when reduced, are lethal to the cell. Suppression of the photosensitivity associated with defective photosynthesis in C. reinhardtii could occur in at least two ways. First, electrons from the photosynthetic electron transport chain could be redirected to acceptors which can be reduced without damage to other cellular components. Perhaps the suppressors represented by secondary photosynthesis defects SLS6-1A and SLS6-3A accomplish this, for they relieve the photosensitivity associated with defective RubPcO. Also, Chunaev and Maslov (2) have described a PSI-deficient mutant which suppresses the photosensitivity associated with a pigment-deficient mutant. The existence of these types of suppressors also demonstrates the requirement of photosynthetic reactions in the mechanism of photosensitivity. A second mechanism for suppressing photosensitivity could be increased levels of acceptors that produce minimal damaging effects when reduced by photosynthetic reactions. For example, a mutant strain of the cyanobacterium Plectonema boryanum has been described that is resistant to levels of light that inhibit wild-type growth (17). This mutant has elevated levels of a H<sub>2</sub>O<sub>2</sub>-insensitive isoenzyme of superoxide dismutase. The suppressors of photosensitivity described in our experiments, that have wild-type phenotypes when separated from _rcu-1<sub>-1</sub>-10-6C, may act in a similar fashion. The relatively high frequency at which suppressors are recovered (Tables I and II) and the existence of several loci (Table III) suggest that additional mechanisms of this type of suppression exist.

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LITERATURE CITED

SUPPRESSORS OF PHOTOSENSITIVITY IN C. REINHARDII