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*Communication*

# Missense Mutation in the *Chlamydomonas* Chloroplast Gene that Encodes the Rubisco Large Subunit[1]

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## ABSTRACT

**The 69-12Q mutant of *Chlamydomonas reinhardtii* lacks ribulose-1,5-bisphosphate carboxylase activity, but retains holoenzyme protein. It results from a mutation in the chloroplast large-subunit gene that causes an isoleucine-for-threonine substitution at amino-acid residue 173. Considering that lysine-175 is involved in catalysis, it appears that mutations cluster at the active site.**

The chloroplast-encoded large-subunit of Rubisco[3] contains the active site of the holoenzyme (10). $CO_2$ and $O_2$ compete for RuBP at this site, and the ratio of carboxylation and oxygenation determines net $CO_2$ fixation in photosynthesis (13). Attempts have been made to increase carboxylation by exploring the effects of Rubisco mutations generated by directed mutagenesis *in vitro* (6–8, 12, 23). However, this approach has been limited to the large-subunit-like Rubisco holoenzyme of *Rhodospirillum rubrum* which can be expressed in *Escherichia coli* (15). We have instead been investigating Rubisco-deficient chloroplast mutants of *Chlamydomonas reinhardtii* that are recovered randomly by screening collections of photosynthesis-deficient, acetate-requiring mutants (16, 19, 21). In this manner, *Chlamydomonas* Rubisco mutants may define previously unrecognized sites that are important in the function, expression or assembly of a Rubisco holoenzyme that is similar to the Rubisco holoenzyme found in higher plants. In the present study, we have identified a missense mutation that replaces threonine with isoleucine at residue 173 in the large-subunit protein. This amino-acid substitution is close to the active-site lysine at position 175 (10, 22).

## MATERIALS AND METHODS

**Strains and Culture Conditions.** The 69-12Q *mt*$^+$ mutant was recovered as a light-sensitive, acetate-requiring mutant at 25°C in a previous study (16). Wild-type strain 2137 *mt*$^+$ (20) was used for biochemical comparisons. Genetic analysis was performed with the *pf*-2 *mt*$^-$ centromere-marker strain following standard procedures (20). All strains were maintained in the dark at 25°C on acetate medium (20) containing 1.5% Bacto agar. Liquid cultures, containing acetate medium without agar, were grown on a rotary shaker in the dark at 25°C.

**Biochemical Analysis.** A Hansatech oxygen electrode was used to measure oxygen evolution and whole-chain electron-transport activity (methyl viologen reduction) as described previously (21). Chl (24) and total soluble protein (1) were determined in sonicated cell lysates. Rubisco carboxylase activity was assayed (16) in clarified lysates, and the amount of holoenzyme was estimated from the absorbance at 280 nm of the lysates fractionated on sucrose gradients (19). All values are the average of three independent determinations.

**Molecular Biology.** Total cell DNA was isolated, cloned in bacteriophage lambda, and the R15 fragment (4) containing the Rubisco large-subunit gene was subcloned in pBR329 (3), all by previously described methods (17). The recombinant plasmid, constructed with the 69-12Q large-subunit gene, was designated pLS69-12Q. A *Hae*III fragment, containing the large-subunit gene, was isolated from the plasmid, digested with *Hin*dIII and *Hin*cII, and the fragments were cloned in both orientations in bacteriophage M13 vectors (9). DNA sequencing was performed with the dideoxy chain-termination method (14).

DNA transfer from 0.8% agarose gels to nylon membrane was performed by standard methods (2). The 891-bp R15.4 *Hin*dIII-*Hin*dIII fragment within the protein-coding region of the large-subunit gene was used as a probe (4, 17).

## RESULTS AND DISCUSSION

**Genetics and Biochemistry.** The 69-12Q *mt*$^+$ mutant was recovered from a large collection of acetate-requiring mutants (16) as a strain that failed to recombine in crosses with the 10-6C *mt*$^-$ large-subunit gene mutant (5, 19). This result suggested that 69-12Q also resulted from a mutation in the chloroplast large-subunit gene, and subsequent crosses with the *pf*-2 *mt*$^-$ centromere-marker strain verified its uniparental (maternal) mode of inheritance.

The 69-12Q mutant is a light-sensitive, acetate-requiring strain. Biochemical analysis (Table I) showed that it had greatly reduced photosynthetic ability and Rubisco carboxylase activity, but it had normal levels of photosynthetic electron-transport activity and Chl. Thus, the 69-12Q mutant is similar to other Rubisco large-subunit mutants that have photosynthesis defects confined to the Rubisco enzyme (5, 17, 19, 21). The 69-12Q mutant also retained a near-normal level of Rubisco holoenzyme protein (Table I), suggesting that it resulted from a missense mutation within the large-subunit gene.

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[3] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; mt, mating type; pf, paralyzed flagella; bp, base pair.

Table I. *Biochemical Analysis of Mutant 69-12Q mt⁺ and Wild Type 2137 mt⁺*

| Strain | Oxygen Evolution | Whole-Chain $e^-$ Transport Activity | Chl | Rubisco | |
|---|---|---|---|---|---|
| | | | | Holoenzyme | Carboxylase activity |
| | *μmol $O_2$/h · mg Chl* | | *μg/mg protein* | | *μmol $CO_2$/h · mg protein* |
| 69-12Q | 7 | 13 | 92 | 38 | 0 |
| 2137 | 66 | 12 | 101 | 45 | 3.6 |

**Molecular Genetics.** About 70% of the protein-coding region of the 69-12Q large-subunit gene was sequenced, and only a single base change was found when compared with the wild-type sequence. A transition was observed at nucleotide position 1708 within the R15 fragment (4), changing an ACA threonine codon to an AUA isoleucine codon in the large-subunit mRNA. Since this mutation would eliminate an *Rsa*I site (GTAC) in the large-subunit gene, we were able to verify the presence of the mutation by identifying the expected 543-bp fragment in an *Rsa*I digest of 69-12Q total-cell DNA (Fig. 1). Thus, it is likely that the 69-12Q mutant has isoleucine in place of threonine at amino-acid position 173 in the Rubisco large-subunit protein (4).

Only one other missense mutation (10-6C) has been identified previously within the chloroplast large-subunit gene (5), and this mutation occurred in a *Chlamydomonas* strain that was recovered with procedures identical to the procedures used to recover the 69-12Q mutant (16, 20). The 10-6C mutation causes aspartic acid to be substituted for glycine at residue 171 in the large-subunit protein. This change is only two residues away from the isoleucine-for-threonine substitution found at position 173 in the 69-12Q mutant. Since both mutations are very close to a lysine residue at position 175 that appears to be involved in catalysis (22), this could account for the near absence of Rubisco function in the two mutant strains (Table I) (18, 19). However, it was somewhat unexpected to find both of these mutations clustered near the active site. Considering that the original mutant strains were recovered randomly from collections of photosynthesis-deficient, acetate-requiring mutants (16, 20), one may have expected the mutations to be distributed randomly within the highly conserved large-subunit gene. Thus, the nonrandom distribution of missense mutations supports our previous suggestion that many mutations occur in the large-subunit gene, but few of them give rise to a mutant, acetate-requiring phenotype (16). It may be possible to identify mutations in other regions of the large-subunit gene by recovering conditional Rubisco mutants. For example, Rubisco-deficient mutants have recently been recovered that display an acetate-requiring phenotype only at elevated temperature (16).

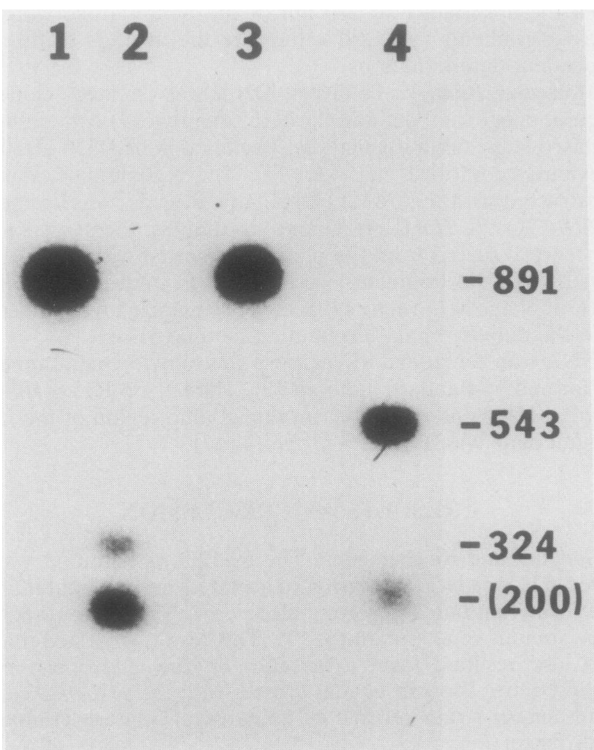


FIG. 1. DNA hybridization of total-DNA restriction-enzyme digests. The 891-bp R15.4 *Hin*dIII-*Hin*dIII fragment (4, 17) was used as the probe. Wild-type DNA was digested with *Hin*dIII (lane 1) and *Rsa*I (lane 2). Mutant DNA was digested with *Hin*dIII (lane 3) and *Rsa*I (lane 4). *Hin*dIII fragments of identical size (891 bp) were expected and observed in both wild type and the mutant. *Rsa*I fragments were expected to differ in size between wild type (*324*, *219*, 50, 202, 228, 66 bp) and the mutant (*543*, 50, 202, 228, 66 bp). Only fragments greater than about 100 bp were retained on the filter.

**Structure-Function Relationship.** The fact that missense mutations appear to cluster near lysine 175 suggests that this region exerts strong structural or functional constraints on Rubisco. It is known to be one of the most highly conserved regions between *R. rubrum* Rubisco and the Rubisco large-subunit of higher plants and *Chlamydomonas* (4, 11). It is interesting to note that *R. rubrum* Rubisco normally has isoleucine at the site where the 69-12Q mutation would cause an isoleucine-for-threonine substitution in the *Chlamydomonas* large-subunit. However, the mutant *Chlamydomonas* enzyme has greatly reduced carboxylase activity (Table I). This observation emphasizes the fact that a number of amino-acid residues must complement each other to maintain a functional enzyme. Perhaps these interactions can be investigated by selecting for photosynthesis-competent revertants of the 69-12Q mutant.

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