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# Thermal Instability of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase from a Temperature-Conditional Chloroplast Mutant of *Chlamydomonas reinhardtii*[1]

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**Mutant 68–4PP of *Chlamydomonas reinhardtii* has only 10% of the normal level of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) holoenzyme when grown at 35°C. However, when grown at 25°C, the amount of holoenzyme is greater than 35% of the wild-type level, and the purified enzyme has a reduced $CO_2$/$O_2$ specificity factor. These mutant characteristics result from a chloroplast mutation that causes leucine-290 to be replaced by phenylalanine within the Rubisco large-subunit protein. A nuclear mutation (named S52–2B) was previously identified that can suppress both the in vivo instability and reduced $CO_2/O_2$ specificity of the mutant enzyme. However, the effect of this nuclear mutation on the in vitro stability of the holoenzyme was not resolved. In the present study, purified Rubisco from mutant 68–4PP was found to be less thermally stable than the wild-type enzyme, and it had maximal carboxylase activity at a lower temperature. When incubated at 35°C, the mutant enzyme lost carboxylase activity at a much faster rate than the wild-type enzyme. However, the nuclear S52–2B suppressor mutation improved the thermal stability of the mutant enzyme in all cases. These results indicate that structural changes in mutant 68–4PP Rubisco can account for its observed inactivation in vitro and degradation in vivo. Such structural alterations are alleviated by the function of a nuclear gene.**

Rubisco (EC 4.1.1.39) catalyzes both the carboxylation and oxygenation of RuBP within the chloroplast, thus linking photosynthetic $CO_2$ fixation with photorespiration in all photosynthetic organisms (reviewed by Ogren, 1984). Because $CO_2$ and $O_2$ compete at the same active site, the efficiency of carboxylation can be related to the $CO_2/O_2$ specificity factor ($V_cK_o/V_oK_c$, where $K_o$ and $K_c$ are $K_m$ values for $O_2$ and $CO_2$, respectively, and $V_o$ and $V_c$ are the $V_{max}$ values for $O_2$ and $CO_2$, respectively). The $CO_2/O_2$ specificity factor is a kinetic constant for a Rubisco enzyme at any given concentration of $CO_2$ and $O_2$ (Laing et al., 1974; Jordan and Ogren, 1981). In plants, the Rubisco holoenzyme is a complex hexadecamer, comprising eight chloroplast-encoded 55-kD large subunits and eight nuclear-encoded 15-kD small subunits (reviewed by Spreitzer, 1993). Despite this structural complexity, Rubisco is a poor catalyst with respect to turnover number, and its oxygenase activity ultimately lowers the overall rate of photosynthetic $CO_2$ fixation (Laing et al., 1974; Chen and Spreitzer, 1992). Consequently, cells must maintain a massive amount of Rubisco to achieve a reasonably high photosynthetic rate. Whereas there is strong interest in improving the enzyme's catalytic efficiency via genetic engineering (Hartman, 1992; Spreitzer, 1993), such changes may disrupt the complex structure of the holoenzyme and lead to a decreased level of Rubisco in vivo (Spreitzer, 1993). Thus, it is also important to investigate the assembly or stability of catalytically altered Rubisco enzymes.

A temperature-conditional, photosynthesis-deficient mutant of *Chlamydomonas reinhardtii*, named 68–4PP, was previously identified that has almost no RuBP carboxylase activity at the restrictive temperature of 35°C (Spreitzer et al., 1988). It results from a mutation in the *rbc*L that changes $Leu^{290}$ to Phe within the large-subunit protein (Chen et al., 1988). The amount of Rubisco holoenzyme is also greatly decreased in the mutant strain at the restrictive temperature, but pulse-chase experiments indicated that the holoenzyme is synthesized and assembled before its degradation (Chen et al., 1988). In contrast, there is about 35% of the normal level of Rubisco holoenzyme in cells grown at the permissive temperature (25°C), and the purified enzyme has a $CO_2/O_2$ specificity factor reduced by 13% (Chen et al., 1988, 1990). Nevertheless, the enhanced degradation of the mutant enzyme at 35°C would be primarily responsible for the mutant phenotype at this restrictive temperature.

A photosynthesis-competent revertant, named R52–2B, was selected from mutant 68–4PP at 35°C (Chen et al., 1990). Genetic analysis showed that the revertant arose from a mutation in a nuclear gene, named S52–2B, and this suppressor mutation restores the mutant holoenzyme to near-normal levels (Chen et al., 1988, 1990). The identity of the nuclear gene within which this mutation occurs is not known. No mutation has been found in either of the two Rubisco small-subunit genes (Chen et al., 1990). One possibility was that the suppressor eliminated a chloroplast protease that normally degrades structurally altered proteins. This possibility was supported by the fact that the mutant holoenzyme and its activity appeared to be stable when purified and analyzed in vitro (Chen et al., 1988, 1990; Chen and Spreitzer, 1991). However, the suppressor also influences the

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Abbreviations: *mt*, mating type; *rbc*L, Rubisco large-subunit gene; RuBP, ribulose 1,5-bisphosphate.

kinetic constants of the mutant enzyme, restoring the $CO_2$/$O_2$ specificity factor to a normal value (Chen et al., 1990; Chen and Spreitzer, 1991). Thus, the suppressor appears to alter the 68–4PP mutant holoenzyme directly. In the present study, we have performed a series of experiments to assess better the stability of the mutant and revertant enzymes in vitro and to determine whether the suppressor mutation might also improve the stability of the wild-type Rubisco enzyme.

## MATERIALS AND METHODS

### Strains and Culture Conditions

Wild-type *Chlamydomonas reinhardtii* 2137 *mt*$^+$ and mutant/revertant strains were maintained on medium containing 10 mM sodium acetate and 1.5% Select/Gibco agar at 25°C in darkness (Spreitzer and Mets, 1981). Liquid cultures consisted of the same medium without agar. The 68–4PP *rbc*L mutant was recovered as a temperature-conditional, acetate-requiring strain in a previous study (Spreitzer et al., 1988; Chen et al., 1988). Photosynthesis-competent revertant R52–2B contains both the 68–4PP *rbc*L mutation and the suppressor S52–2B nuclear mutation (Chen et al., 1990). By appropriate genetic crosses, the nuclear suppressor mutation was separated from the original 68–4PP *rbc*L mutation to generate the S52–2B strain containing only the suppressor mutation (Chen et al., 1990; Chen and Spreitzer, 1991). The S52–2B strain has a wild-type phenotype. Mutant 18–7G lacks Rubisco holoenzyme due to an *rbc*L mutation that changes the codon for $Trp^{65}$ of the large-subunit protein to a UAG termination codon (Spreitzer and Ogren, 1983; Spreitzer et al., 1985). Pulse-chase experiments have shown that small subunits are synthesized in the 18–7G strain, but they are rapidly degraded in the absence of the large subunit (Spreitzer et al., 1985).

### Preparations of Cell Extract and Purification of Rubisco

Cells were grown in 250 mL of liquid acetate medium in a 1000-mL Delong flask on a rotary shaker (220 rpm) at 25°C in darkness. When the cells reached a stationary phase of growth (about $5 \times 10^6$ cells $mL^{-1}$), they were concentrated and sonicated at 0°C in 1.5 mL of extraction buffer (1 mM DTT, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, and 50 mM Bicine, pH 8.2). The sample was then centrifuged at 30,000*g* for 15 min at 4°C, and the supernatant was retained as the cell extract. For the purification of Rubisco holoenzyme, 1 mL of cell extract was fractionated on a Suc gradient containing the same extraction buffer (Chen and Spreitzer, 1991). The peak of Rubisco protein was identified by scanning the gradient at 280 nm. Rubisco was dialyzed against extraction buffer to remove Suc, and the amount of protein was determined (Bradford, 1976).

### Thermal Properties of Rubisco

Optimum temperature for RuBP carboxylase activity was determined by incubating 5 μg of purified Rubisco in 0.5 mL of assay buffer containing 10 mM $MgCl_2$, 10 mM $NaHCO_3$ (2 Ci $mol^{-1}$), and 50 mM Bicine (pH 8.2) at temperatures ranging from 20 to 60°C. After 5 min of incubation, reactions were initiated by adding 20 μL of 10 mM RuBP. The reactions were terminated after 1 min by adding 0.5 mL of 3 M formic acid in methanol. The samples were then dried at 80°C, and acid-stable $^{14}C$ dpm were determined by liquid scintillation spectroscopy.

To determine the thermal stability of Rubisco, 5 μg of purified enzyme were incubated in 0.5 mL of assay buffer at temperatures ranging from 30 to 70°C for 10 min. After this time, the samples were cooled on ice for 5 min and then assayed for 1 min at 25°C by adding 20 μL of 10 mM RuBP as described above.

### Inactivation of Rubisco at Growth Temperatures

To determine the inactivation rate of Rubisco at 25 and 35°C, 0.5 mL of purified Rubisco (100 μg) was mixed with either 0.5 mL of incubation buffer (10 mM $MgCl_2$, 10 mM $NaHCO_3$, and 50 mM Bicine, pH 8.2) or with 0.5 mL of mutant 18–7G cell extract. This cell extract was prepared as described above, except that it was dialyzed against incubation buffer at 4°C and diluted to 5 mg of protein $mL^{-1}$. During a 10-h incubation at 25 or 35°C, samples were removed from the enzyme mixtures and assayed at 25°C by adding 50 μL of the enzyme mixture to 0.5 mL of assay buffer containing 10 mM $MgCl_2$, 1 mM $NaHCO_3$ (10 Ci $mol^{-1}$), 1 mM RuBP, and 50 mM Bicine (pH 8.2). After 1 min, the reactions were stopped with 3 M formic acid in methanol, and acid-stable $^{14}C$ dpm were determined by liquid scintillation spectroscopy.

### Electrophoresis and Western Blotting

After a 10-h incubation at 35°C with or without added cell extract (in the same experiments described immediately above), 25 μL of enzyme mixture, containing 2.5 μg of Rubisco, were separated by SDS-PAGE on 7.5 to 15% polyacrylamide gradient gels (Laemmli, 1970). Duplicate gels were either stained with Coomassie blue or electrophoretically transferred to nitrocellulose (Towbin et al., 1979). The latter were used for western blots and were probed with rabbit anti-tobacco Rubisco immunoglobulin G (5 μg $mL^{-1}$), and bound antibody was detected with goat anti-rabbit immunoglobulin G/horseradish peroxidase conjugate (3000-fold dilution; Bio-Rad) (Towbin et al., 1979). Antibody prepared against tobacco Rubisco was graciously provided by R. Chollet (University of Nebraska, Lincoln, NE).

## RESULTS

### Thermal Properties of Rubisco

To determine the optimum temperatures for RuBP carboxylase activity, purified wild-type and mutant Rubisco enzymes were assayed at temperatures ranging from 20 to 60°C (Fig. 1). RuBP carboxylase activity was maximal at 42°C for the enzymes from wild type and suppressor S52–2B (containing only the nuclear mutation). In contrast, Rubisco enzymes from mutant 68–4PP and revertant R52–2B (containing both 68–4PP and S52–2B mutations) had temperature optima for carboxylase activity at 36°C. To analyze thermal stability,

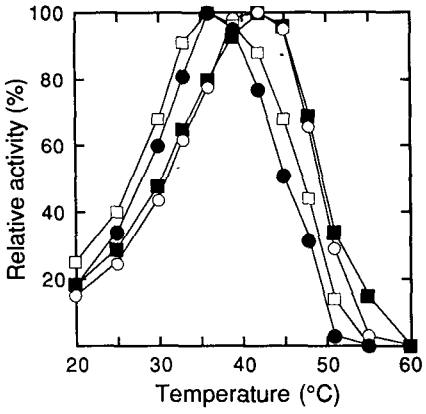


**Figure 1.** Effect of assay temperature on the RuBP carboxylase activity of purified Rubisco from wild type (○), mutant 68–4PP (●), revertant R52–2B (□), and suppressor S52–2B (■). Purified Rubisco (10 μg $mL^{-1}$) was incubated at each temperature for 5 min before initiating the reactions with RuBP. Activities were normalized against the maximal activity observed for each enzyme.

wild-type and mutant Rubisco enzymes were incubated at temperatures ranging from 30 to 70°C for 10 min but then assayed for RuBP carboxylase activity at 25°C (Fig. 2). Rubisco from wild type and suppressor S52–2B withstood temperatures as high as 55°C with no loss of enzyme activity. However, the S52–2B enzyme appeared to be more resistant than the wild-type enzyme at higher temperatures. For example, at 65°C, wild-type Rubisco retained only 2% of its initial carboxylase activity, but S52–2B Rubisco still retained 37% of its initial activity. In contrast to wild type and S52–2B, mutant 68–4PP and revertant R52–2B Rubisco enzymes began to lose activity at temperatures above 49°C, which is 6°C lower than that observed for the former enzymes. The thermal stability of the mutant 68–4PP Rubisco appeared to be significantly improved by the presence of the S52–2B mutation in revertant R52–2B. For example, at 60°C, Rubisco from R52–2B still retained about 45% of its initial carboxylase activity, but mutant 68–4PP Rubisco retained only 8% of its initial activity.

### Inactivation of Rubisco at Growth Temperatures

Although differences in optimum temperature and thermal stability were observed among the wild-type and mutant Rubisco enzymes (Figs. 1 and 2), it was important to see whether such differences could also be detected at the physiological temperatures of 25 and 35°C. Because purified mutant 68–4PP Rubisco displayed significant levels of carboxylase activity when assayed or incubated at 35°C (Figs. 1 and 2), it was difficult to explain why the amount and activity of the enzyme was substantially reduced in vivo when the temperature-conditional mutant strain was grown at 35°C (Chen et al., 1988, 1990).

When purified Rubisco enzymes were incubated at 25°C for 10 h and then assayed for carboxylase activity, the wild-type, S52–2B, and R52–2B enzymes each lost about 15% of its initial carboxylase activity, but mutant 68–4PP Rubisco lost about 20% of its initial activity (data not shown). When purified Rubisco enzymes were incubated at 35°C (Fig. 3A), the wild-type and S52–2B enzymes each lost less than 40% of its initial carboxylase activity after 10 h. Revertant R52–2B Rubisco lost slightly more than 40% of its initial activity, but the activity of mutant 68–4PP Rubisco was decreased by 50%. Thus, the 68–4PP mutant enzyme is less thermally stable than the wild-type enzyme in vitro, and the S52–2B suppressor improves the mutant enzyme's thermal stability (revertant R52–2B; Fig. 3A). However, the suppressor does not enhance the thermal stability of the wild-type enzyme under these conditions (suppressor S52–2B; Fig. 3A).

Because *C. reinhardtii rbc*L mutants are available that lack Rubisco holoenzyme (Spreitzer and Ogren, 1983; Spreitzer et al., 1985; Spreitzer, 1993), we attempted to mimic an in vivo condition by adding cell extract from one of these mutants (mutant 18–7G) to the purified Rubisco enzymes. As shown in Figure 3B, Rubisco enzymes from wild type and suppressor S52–2B lost 45% of their initial carboxylase activities after 10 h at 35°C in the presence of cell extract. This magnitude of loss is not much different from that observed in the absence of extract (about 40%; Fig. 3A). However, 68–4PP and R52–2B Rubisco enzymes had increased losses of carboxylase activity during the 10-h incubation in the presence of cell extract (Fig. 3B). The mutant 68–4PP enzyme lost about 80% of initial activity after 10 h, but revertant R52–2B enzyme lost only about 60% of its initial activity. It is clear that the S52–2B mutation, when present in R52–2B, dramatically improves the stability of the mutant Rubisco enzyme.

Because cell extract could accelerate the inactivation of mutant 68–4PP Rubisco relative to wild-type Rubisco at 35°C in vitro (Fig. 3), and, because the mutant protein is degraded at 35°C in vivo (Chen et al., 1988, 1990), it seemed possible that a protease in the extract might be responsible for further enhancing the inactivation of the mutant protein. As shown in Figure 4, wild-type and mutant Rubisco enzymes retained levels of carboxylase activities after 10 h at 35°C in the

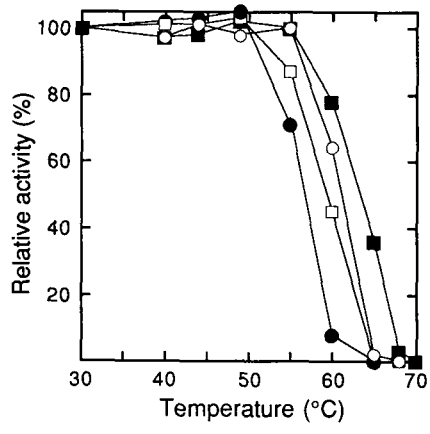


**Figure 2.** Thermal inactivation of purified Rubisco from wild type (○), mutant 68–4PP (●), revertant R52–2B (□), and suppressor S52–2B (■). Purified Rubisco (10 μg $mL^{-1}$) was incubated at each temperature for 10 min, cooled on ice for 5 min, and then assayed for RuBP carboxylase activity at 25°C. Activities were normalized against the level of each enzyme's activity observed from the 30°C incubation.

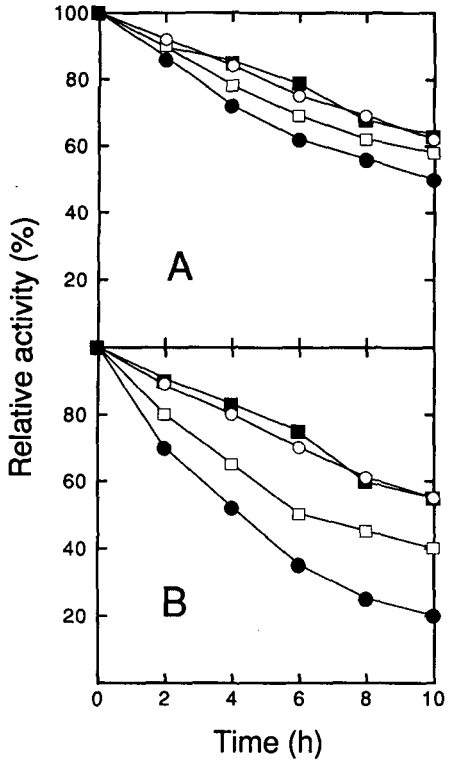


**Figure 3.** Effect of a 10-h incubation at 35°C on the RuBP carboxylase activity of Rubisco in the absence (A) or presence (B) of an added cell extract. Rubisco enzymes were purified from wild type (○), mutant 68–4PP (●), revertant R52–2B (□), and suppressor S52–2B (■). Enzymes (100 $\mu$g mL$^{-1}$) were continuously incubated with buffer or with a cell extract that had been dialyzed against the same buffer. The cell extract was prepared from an *rbc*L mutant strain that lacks Rubisco holoenzyme (Spreitzer and Ogren, 1983; Spreitzer et al., 1985). Samples were removed at the indicated times and assayed for RuBP carboxylase activity at 25°C. Activities were normalized against the level of each enzyme's activity measured after the first 10 min of incubation (time = 0 h). Two additional enzyme preparations from each of the strains were assayed with and without added cell extract at the 0- and 10-h times, and the percentage loss of activity after 10 h did not vary by greater than SD ±5 in any case.

presence of boiled extract that were similar to their activities in the absence of cell extract (Fig. 3A). Furthermore, when the Rubisco enzymes were incubated for 10 h in the presence of nonboiled cell extract and analyzed by SDS-PAGE and western blotting, a slight reduction in mutant 68–4PP and revertant R52–2B large subunits was detected (Fig. 5B, lanes 2 and 3). However, this difference was not apparent with Coomassie blue staining (Fig. 5A, lanes 6 and 7). Perhaps Coomassie blue staining is not as sensitive, or the antibody recognizes the wild-type large subunits better than the mutant large subunits. In either case, a substantial decrease in Rubisco subunits could not be documented, and no proteolytic products were detected after the 10-h incubation with nonboiled extract (Fig. 5).

## DISCUSSION

RuBP carboxylase activity of mutant 68–4PP Rubisco was found to have a lower optimum temperature (Fig. 1) and to be irreversibly inactivated at lower temperatures (Fig. 2) than the wild-type enzyme. However, these differences were apparent only at relatively high temperatures and could not directly account for the instability of the mutant protein in cells grown at 35°C (Chen et al., 1988, 1990). In contrast, the differences in thermal stability were also apparent when mutant 68–4PP and wild-type enzymes were assayed during a 10-h incubation at 35°C (Fig. 3A). Thus, the carboxylase activity of mutant 68–4PP Rubisco has an increased thermal instability in vitro, and this instability is likely to arise from structural alterations that are also responsible for the degradation of the mutant enzyme in vivo (Chen et al., 1988, 1990).

The difference in thermal stability between mutant and wild-type Rubisco was further amplified in the presence of a Rubisco-depleted cell extract (Fig. 3B). Because Rubisco large subunits are sensitive to trypsin in the absence of substrate RuBP in vitro (Houtz et al., 1989; Chen and Spreitzer, 1991), it was reasonable to consider that a structurally altered mutant Rubisco might be sensitive to a protease in the cell extract. In support of this possibility, boiled extract failed to enhance the thermal inactivation of carboxylase activity (Fig. 4). However, substantial proteolysis of Rubisco subunits could not be detected in the presence of the nonboiled extract when SDS-PAGE and Coomassie blue staining or western blotting were used (Fig. 5). Because the extract accounts for only a percentage of the overall decrease in activity of mutant 68–4PP Rubisco (Fig. 3; Chen et al., 1988), proteolysis may be difficult to detect. On the other hand, removal of only a single residue at the carboxyl terminus of the large subunit dramatically reduces Rubisco activity (Portis, 1990), but such limited proteolysis would also be difficult to detect with the methods used. Thus, additional experiments will be required to determine how the cell extract enhances the inactivation

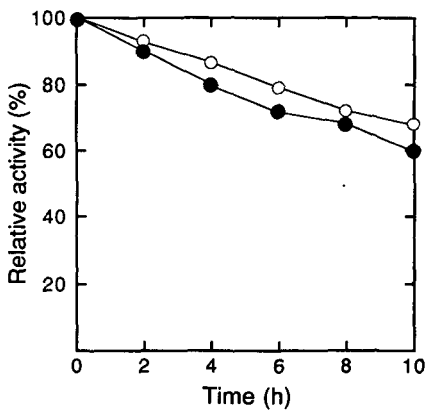


**Figure 4.** Effect of a 10-h incubation at 35°C on the RuBP carboxylase activity of Rubisco in the presence of boiled cell extract. Rubisco enzymes were purified from wild type (○) and mutant 68–4PP (●). Enzymes (100 $\mu$g mL$^{-1}$) were continuously incubated with a cell extract that had been dialyzed against buffer and then boiled for 5 min. The cell extract was prepared from an *rbc*L mutant strain that lacks Rubisco holoenzyme (Spreitzer and Ogren, 1983; Spreitzer et al., 1985). Samples were removed at the indicated times and assayed for RuBP carboxylase activity at 25°C. Activities were normalized against the level of each enzyme's activity measured after the first 10 min of incubation (time = 0 h).

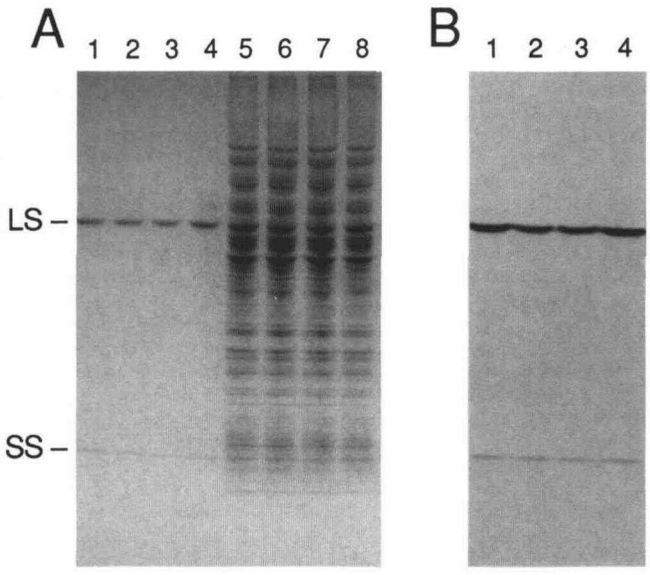


**Figure 5.** The effect of a 10-h incubation at 35°C on the level of Rubisco subunits. Purified Rubisco enzymes (100 $\mu$g mL$^{-1}$) were continuously incubated with buffer (A, lanes 1–4) or with a cell extract that had been dialyzed against the same buffer (A, lanes 5–8; B, lanes 1–4). The cell extract was prepared from an *rbc*L mutant strain that lacks Rubisco holoenzyme (Spreitzer and Ogren, 1983; Spreitzer et al., 1985). Samples containing 2.5 $\mu$g of Rubisco were removed after 10 h and subjected to SDS-PAGE. A, Coomassie blue staining: lanes 1 and 5, wild type; lanes 2 and 6, mutant 68–4PP; lanes 3 and 7, revertant R52–2B; lanes 4 and 8, suppressor S52–2B. B, Western analysis: lane 1, wild type; lane 2, mutant 68–4PP; lane 3, revertant R52–2B; lane 4, suppressor S52–2B. LS, Large subunit; SS, small subunit.

of the mutant enzyme. Nonetheless, the differences in thermal stability in the presence of cell extract likewise indicate that there are physical differences between mutant and wild-type enzymes in vitro.

Rubisco purified from the R52–2B revertant strain displayed levels of thermal stability that were intermediate to those of the mutant 68–4PP and wild-type enzymes (Figs. 2 and 3). After a 10-h incubation at 35°C in the presence of added cell extract, the revertant enzyme retained twice as much RuBP carboxylase activity as the mutant 68–4PP enzyme (Fig. 3B). It is apparent that the nuclear S52–2B mutation is responsible for altering the structure of the mutant enzyme in a way that protects it from thermal inactivation in vitro (as shown here, Figs. 1–3) or from degradation in vivo (as shown previously, Chen et al., 1990). We do not yet know the nature of the gene that contains the S52–2B mutation, but it is important to point out that the S52–2B gene product must act *prior* to the purification of the R52–2B holoenzyme (Chen et al., 1990). In other words, it is not a component of the purified Rubisco holoenzyme, nor is it present in the cell extract used for enhancing the observed thermal instability (Fig. 3). We had considered the possibility that the S52–2B mutation might also improve the thermal stability of wild-type Rubisco, and enzyme from the S52–2B strain did display a slight reduction in thermal inactivation relative to the wild-type enzyme (Fig. 2). However, this difference was observed only at temperatures much greater than those tolerated by *C. reinhardtii*.

Based on the x-ray crystal structure of spinach Rubisco (Knight et al., 1990), Leu$^{290}$ is the last residue of $\beta$-strand 5 at the bottom of the large-subunit $\alpha/\beta$-barrel active site. It is one of four Leu residues that surround Glu$^{158}$, which, in turn, contributes to a hydrogen-bond network within the $\alpha/\beta$-barrel hydrophobic core (Knight et al., 1990). The hydrogen-bond network extends to His$^{327}$ at the base of active-site loop 6, and the loop-6 region is known to influence the $CO_2/O_2$ specificity of *C. reinhardtii* Rubisco (Chen and Spreitzer, 1989; Chen et al., 1991; Spreitzer, 1993). Thus, it is not difficult to envision that changes in both structural integrity and $CO_2/O_2$ specificity can arise from the replacement of Leu$^{290}$ by the bulky Phe of mutant 68–4PP (Chen et al., 1988, 1990). It is also interesting to note that this Phe residue is normally present in *Rhodospirillum rubrum* Rubisco (Knight et al., 1990), which has a reduced $CO_2/O_2$ specificity factor relative to that of the *C. reinhardtii* enzyme (Jordan and Ogren, 1981). Perhaps the Phe residue of the bacterial enzyme would be a worthwhile target for exploration via directed mutagenesis (Hartman, 1992).

The S52–2B nuclear mutation improves both the thermal stability and $CO_2/O_2$ specificity of mutant 68–4PP Rubisco (Figs. 1–3; Chen et al., 1990; Chen and Spreitzer, 1991), further indicating that these elements can be directly connected. Although the nature of the S52–2B gene is not known (Chen et al., 1990), we favor the possibility that it plays a role in either the posttranslational modification or assembly of the holoenzyme (Houtz et al., 1989; Gatenby and Ellis, 1990). With regard to the latter possibility, it has been shown that overexpression of the *gro*E chaperons in *Salmonella typhimurium* can suppress a number of temperature-conditional mutant phenotypes (Van Dyk et al., 1989). It is not yet known whether posttranslational steps in holoenzyme synthesis and assembly can actually influence Rubisco catalysis. Nevertheless, the fact that a nuclear mutation can suppress detrimental alterations in both Rubisco holoenzyme stability and $CO_2/O_2$ specificity in vitro and in vivo (Figs. 1–3; Chen et al., 1988, 1990) indicates that subtle and related alterations in both structure and function will need to be considered as further attempts are made to improve Rubisco.

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