Glutathione Redox Potential Modulated by Reactive Oxygen Species Regulates Translation of Rubisco Large Subunit in the Chloroplast*

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Previous work showed a transient but dramatic arrest in the synthesis of Rubisco large subunit (LSU) upon transfer of Chlamydomonas reinhardtii cells from low light (LL) to high light (HL). Using dichlorofluorescin, a short-term increase in reactive oxygen species (ROS) was demonstrated, suggesting that their excessive formation could signal LSU down-regulation. A decrease in LSU synthesis occurred at LL in the presence of methyl viologen and was prevented at HL by ascorbate. Interfering with D1 function by mutations or by incubation with DCMU prevented the increase in ROS formation at HL and the concomitant down-regulation of LSU synthesis. If the electron transport was blocked further downstream, by mutation in the cytochrome b6/f or by incubation with 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone, ROS formation increased, and LSU synthesis ceased. The elevation of ROS occurred concurrently with a change in the redox state of the glutathione pool, which shifted toward its oxidized form immediately after the transfer to HL and returned to its original value after 6 h. The decrease in the reduced/oxidized glutathione ratio at HL was prevented by ascorbate and could be induced at LL by methyl viologen. We suggest that excess ROS mediate a decrease in the reduced/oxidized glutathione ratio that in turn signals the translational arrest of the rbcL transcript.

Exposure of photosynthetic organisms to light intensities that exceed the limits of photosynthesis saturation can cause severe damage to the photosynthetic machinery, referred to as photoinhibition (1–3). Most plants and algae have the capacity to recover from light stress through photoacclimation, which normally involves a reduction in either the number or size of the light harvesting complexes and increased synthesis of the photodamaged D1, the core protein of photosystem II (PSII) (4, 5). Excess light energy generates reactive oxygen species (ROS), which in turn lead to the induction of antioxidative and protectophotoprotective mechanisms enabling the plant to combat the danger posed by the presence of ROS.

Ribulose biphosphate carboxylase-oxygenase (Rubisco) is the key enzyme in carbon assimilation during photosynthesis. In Chlamydomonas reinhardtii and in land plants the enzyme is composed of eight large subunits (LSU) encoded by the chloroplast rbcL gene and eight small subunits encoded by the nuclear rbcS gene family (6, 7). Assembly of the Rubisco holoenzyme is driven by the chloroplast Cpn60 and Cpn10, encoded by groEL and groES, respectively. Previously we observed unique and opposite patterns for translational regulation of the chloroplast LSU and D1 polypeptides in response to changes in light intensity. Within minutes of shifting cells of C. reinhardtii from low light to higher light intensities, LSU synthesis was down-regulated dramatically for a period that did not exceed 4–6 h, whereas that of D1 was gradually up-regulated. Translation of other genes was hardly affected, including photosynthesis-related genes such as the chloroplast encoded ATPase β-subunit, the nuclear encoded small subunit of Rubisco (small subunits), or nonphotosynthetic genes, such as tubulin. The observed changes in D1 and LSU synthesis could not be correlated with changes in the steady state levels of their corresponding mRNAs, implying that translational regulation was involved. Primer extension analysis of rbcL mRNA revealed two transcripts that differed in their 5′ ends and in their abundance at LL and after transfer to HL. The appearance of the longer transcript correlated with the down-regulation in LSU synthesis, but its involvement in arresting LSU translation was unresolved. These several distinct effects of temporary light stress were correlated with a rapid, sustained increase in the reduction state of QA, a transient decline in the photosynthetic efficiency, a less rapid drop in total chlorophyll content, and a delay in cell division (8).

This study attempts to decipher the mechanism that controls translation of LSU and signals the immediate and short-term down-regulation observed upon transferring low light grown C. reinhardtii cells to higher light intensities. We propose that modulation of the glutathione redox potential by changes in the level of ROS regulate the synthesis of Rubisco LSU in the chloroplast.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—C. reinhardtii wild type strain CC-125 was used in all experiments. Cultures in High Salt Reduced Sulfate (HSRS) (300 ml) (9) were grown with 5% CO2 bubbling and constant rotary shaking at 22 °C. Cultures were illuminated with low light (LL; 70 μmol m⁻² s⁻¹) using cool white fluorescent lamps. LL grown cells were adapted for low irradiance and were not permitted to attain densities greater that 0.2–0.3 A₅₇₀. The D1 mutant strains were CC-741 (FUD7), a D1 deletion mutant, and CC-3376 (A251R*), which carries a point mutation in D1 (10). The cytochrome b6/f-deficient mutant was CC-2910 (F2D8) (11).

In Vivo Pulse Labeling of Chloroplast Proteins—In vivo labeling of plastid and nuclear encoded proteins with [³⁵S]H₂SO₄ was performed essentially as described (12), with the following modifications. Cells were grown under LL in HSRS (13), until their biomass A₅₇₀ reached 0.2–0.3. The cells were then harvested by centrifugation for 5 min
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(4500 × g, 20 °C) and resuspended at 0.4 A450 in minimal medium lacking sulfate (HS-S; Ref. 13) and containing 10 mM bicarbonate to ensure carbon availability for photosynthesis. The cells were equilibrated for 1 h under LL in HS-S. Aliquots (4 ml) of the HS-S suspension were placed in 30-ml Corex tubes containing magnetic stirring bars and illuminated at LL or at HL for 5 h. 700 μmol m⁻² s⁻¹ for the designated time periods. Anisomycin (Sigma) was then added to a concentration of 250 μg ml⁻¹ for 15 min to reduce labeling of cytoplasmic proteins (14). [³⁵S]H₂SO₄ (125 μCi; carrier free; NEN Life Science Products) was then added to each aliquot of cells for additional 15 min. To terminate labeling, 3 ml of the cell aliquot were added rapidly to 10 ml of ice-cold acetone incubated on ice for 1-2 h and centrifuged for 10 min, and the protein pellets were dried. Samples were resuspended in 100 μl of H₂O and 100 μl of denaturing solution (4% SDS, 5 mM EDTA, 40 mM Tris-HCl, pH 7.4), briefly vortexed, and boiled for 5 min. Incorporation of the radiolabel was measured by trichloroacetic acid precipitation, and the protein content was determined with the BCA reagent (Pierce). Samples containing equal protein quantities were loaded and visualized by silver staining. The gels were dried, exposed to XAR5 film (Kodak), and also analyzed by a Fuji phosphorimager.

Labeling of Wild Type CC-125 Cells in the Presence of Herbicides or Ascorbic Acid—Wild type cells were grown photoautotrophically and processed for labeling as described above. Following transfer to HS-S for 1 h, 10 ml of ice-cold acetone incubated on ice for 1-2 h and centrifuged for 10 min, and the protein pellets were dried. Samples were resuspended in 100 μl of H₂O and 100 μl of denaturing solution (4% SDS, 5 mM EDTA, 40 mM Tris-HCl, pH 7.4), briefly vortexed, and boiled for 5 min. Incorporation of the radiolabel was measured by trichloroacetic acid precipitation, and the protein content was determined with the BCA reagent (Pierce). Samples containing equal protein quantities were loaded and verified by Coomassie staining. The gels were dried, exposed to XAR5 film (Kodak), and also analyzed by a Fuji phosphorimager.

Labeling of Wild Type CC-125 Cells in the Presence of MeV—Early log cells grown photoautotrophically at LL on HSRS with bubbling of 5% CO₂. Aliquots (4 ml) were transferred to HS-S medium for 1 h in LL in presence of MeV (10⁻² M), DBMIB (10⁻⁶ M), or ascorbic acid (5 and 10 mM) for 2 h and then labeled as described for wild type cells. Following cell harvest, proteins were extracted and analyzed as described above. Labeling of LSU and D1 was evaluated by phosphorimaging.

In Vivo Pulse Labeling of Nonphotosynthetic Mutants—The nonphotosynthetic mutant strains CC-741 (FUD7), CC-3376 (A251R), and CC-2910 (F2D8) were grown heterotrophically in TAP medium (9) under LL conditions to early log phase. Acetate was depleted by transfer of the cells to minimal growth conditions (HSRS/5% CO₂) 16 h before transfer to HL. The cells were then resuspended in HS-S for 1 h and transferred to LL and HL for 2.5 h. Labeling and SDS-PAGE analysis were performed as described for wild type cells.

Labeling of Wild Type CC-125 Cells in the Presence of MeV—Early log cells grown photoautotrophically at LL on HSRS with bubbling of 5% CO₂. Aliquots (4 ml) were transferred to HS-S medium for 1 h in LL in presence of MeV (10⁻⁶, 6 × 10⁻⁶, and 10⁻⁵ M) for 45 min and radioisotted.

DCF Fluorescence at LL and after the Shift to HL—Early log cells grown photoautotrophically on minimal medium with 5% CO₂ at LL were harvested by centrifugation at 5000 rpm for 10 min, washed once in phosphate buffer (10 mM, pH 7.0), and resuspended at a density of 0.4 A750 in HS-S. Cultures were then illuminated with LL or HL irradiance. Samples (2 ml) from LL and HL were removed after 0.5, 1.5, and 2.5 h and added to 8 ml of thawing buffer (10 mM Tris-HCl, pH 7.2, 50 mM KCl) containing 0.05 mM DCFH (5 μl from a 100 mM stock solution in Me₂SO; Ref. 15). The samples were then maintained 20 min in the dark, and relative fluorescence was measured using a Perkin-Elmer LS50B Spectrofluorometer. Excitation wavelength was chosen 488 nm and emission wavelength of 525 nm with a slit width of 7.5 nm. DCF fluorescence at LL and after the shift to HL was performed in wild type CC-125 and in the nonphotosynthetic mutants (CC-741, CC-3376, and CC-2910) as well as in wild type cells in the presence of DCMU (10⁻⁶ M), DBMIB (10⁻⁶ M), and ascorbic acid (10 mM). Measurement of DCF fluorescence at LL in the presence of MeV (10⁻⁴–10⁻⁶ M) was performed with samples that were collected after 0.5 and 1.5 h. Fluorometry measurements were performed in triplicate and expressed as relative fluorescence units.

Measurement of GSH/GSSG Ratio—GSH and GSSG were measured using a modified method for glutathione measurement in microtiter plates (16, 17). Cells were grown under LL in HSRS up to a density of 0.2–0.3 A750, harvested by centrifugation for 5 min (4000 × g, 20 °C), and resuspended at a density of 0.4 A750 in HS-S. Cultures were then illuminated at LL and at HL for different time periods, and cell samples (12 ml) were removed, washed once in phosphate buffer (10 mM, pH 7.0), and centrifuged. The wet weight of the washed cell pellet was measured, and 5% sulfosalicylic acid (Sigma) was added, 150 μl/50 mg pellet. The mixture was agitated on a Vortex mixer and stood at 4 °C for 20 min, at which time the cells were thawed, and the insoluble debris was removed by centrifugation (13,000 × g, 4 °C, 20 min). The supernatant was collected, and samples of 100 μl were diluted 1:1 with distilled water and distributed into two aliquots, for individually measuring GSSG and total glutathione. To conjugate GSH, 2-vinylpyridine (Fluka) was added to one of the aliquots to a final concentration of 0.35 M. The mixture was neutralized to pH 6.7–7 with triethanolamine (diluted 1:2 with double distilled H₂O). GSSG concentrations in these extracts were determined using the enzymatic recycling assay (16, 17) involving the color development at 412 nm of 0.15 mM 5,5-dithiobis-2-nitrobenzoic acid (Sigma) in the presence of 0.2 mM NADPH and 1 unit ml⁻¹ of GSH reductase (Fluka). Total glutathione (GSH and GSSG) was measured also in cells incubated with acetic acid at LL and at HL in cells incubated with MeV at LL.

RESULTS

Effect of Disrupting the Photosynthetic Electron Transport Chain on the Synthesis of Rubisco LSU Following Transfer from LL to HL.—To test whether the redox state of specific components along the electron transport chain affected the down-regulation of LSU, labeling experiments of wild type cells at LL and after transfer to HL were performed in the presence of herbicides that inhibit the electron transfer at different sites along the electron transport chain. DCMU inhibits the oxidation of the plastocyanine pool, and DBMIB reduces its oxidation by competitively binding to cytochrome b₆f complex. Wild type C. reinhardtii cells were labeled at LL and after transfer to HL (2 h) in the presence of DCMU (10⁻⁷ M) and DBMIB (10⁻⁶ M), which were added when the cells were transferred to HL. Although synthesis of LSU decreased 2-fold in control cells shifted to HL, it was almost unaffected in the presence of DCMU (Fig. 1A). However, addition of DBMIB did not prevent the down-regulation of LSU synthesis after transfer from LL to HL (Fig. 1B). In addition, LSU synthesis decreased already at LL in the presence of DBMIB (10⁻⁶ M).

To complement the experiments using herbicides, C. reinhardtii mutants in which D1 was completely absent (FUD7) or inactivated by a point mutation (A251R), and a mutant defective in cytochrome b₆f (F2D8) were labeled at LL and after shifting to HL (2 h). The FUD7 mutant does not synthesize any D1 and is thus deficient of functional PSII. In the D1 mutant A251R, Ala at position 251 was substituted with Arg. Ala251R is located in the quinone binding loop connecting the IV–V helices of D1 (10). This mutation does not alter the size (32 kDa) or amount of the D1 protein. The mutant synthesizes D1 up to 80% of its level in wild type when grown under HL but has a nonphotosynthetic phenotype because electron transfer between QA and QB is completely blocked (10). Labeling experiments of both D1 mutants indicate that in the absence of a functional D1, synthesis of LSU is unaffected by shifting the cells from LL to HL (Fig. 2, A and B). However, inactivation of cytochrome b₆f in the mutant strain F2D8 did not prevent the down-regulation of LSU synthesis (Fig. 2D).

The effect of mutations in the photosynthetic electron carriers that prevent oxidation or reduction of the plastocyanine (Fig. 2) correlated with the herbicide data (Fig. 1). In the presence of DCMU or in mutants defective in D1, the plastocyanine pool remained oxidized in wild type cells, despite transfer to HL. Thus LL conditions are mimicked, and LSU down-regulation was not observed. Preventing oxidation of the plastocyanine pool by incubation with DBMIB or by mutagenesis of the cytochrome b₆f complex did not alter the pattern of LSU down-regulation. Although these results could indicate that the redox state of the PQ has a regulatory role in LSU synthesis during LL to HL shifts, previous fluorometric measurements ruled out this possibility. The plastocyanine was reduced immediately after transfer from LL to HL and was maintained in a reduced state at HL (8), whereas LSU synthesis initially declined and then recovered.

The labeling pattern of D1 indicated that in control cells synthesis of this protein increased 9-fold upon transfer from LL.
FIG. 1. Labeling of wild type cells in the presence of DCMU and DBMIB. Early log cells grown photoautotrophically at LL on minimal medium with 5% CO₂ were transferred to LL or HL (2 h) with or without DCMU (10⁻⁷ M) (A) and DBMIB (10⁻⁶ M) (B) and metabolically labeled in the presence of anisomycin. Labeled proteins were subjected to 11% SDS-PAGE, autoradiography, and phosphorimaging. The HL/LL ratio of radiolabel incorporation into LSU and D1 is shown below each autoradiogram.

FIG. 2. Labeling of nonphotosynthetic mutants at LL and after the shift to HL. Mutant strains were grown heterotrophically in TAP medium to early log phase under LL conditions. Acetate was depleted by transfer of the cells to minimal growth conditions (HSRS/5%CO₂) 16 h before transfer to HL. The cells were then resuspended in HS-S for 1 h and transferred to LL and HL for 2 h. Labeling of wild type cells and SDS-PAGE were performed as described in the legend of Fig. 1. A, wild type cells; B, A251R⁺; C, FUD7; D, F2D8.

to HL, in line with previous observations (18). This increase was inhibited by DCMU, DBMIB, and a mutation in cytochrome b₆/f. These data are in line with the redox control of D1 synthesis in the chloroplast (19). However, it was difficult to explain why D1 synthesis increased (by 5.5-fold) in A251R⁺, because electron transport is blocked in this nonphotosynthetic mutant. Different forms of D1 that vary in their half lives were shown to coexist in the thylakoids of this mutant, and these could be reflected in the pattern of labeling (10).

Effects of Ascorbic Acid on Synthesis of LSU and on ROS Levels during LL to HL Shifts—Excess energy not trapped by the photosynthetic electron transport chain can increase the formation of ROS at specific sites of the PSII and PSI reaction centers. In PSII, singlet oxygen is formed by the reaction of the triplet state of P680 with oxygen. Hydrogen peroxide is generated both in PSII and in PSI, and can be converted to hydroxyl radicals by interaction with non-heme iron (20, 21). As photoprotective mechanisms come into play (4, 5), the free radical density should decline, and LSU synthesis would normalize once again. We therefore hypothesized that the increase in ROS could signal the down-regulation of LSU synthesis, either directly or indirectly. To test this possibility, ascorbic acid, which acts as an antioxidant by removing hydrogen peroxide (22) was added to cells labeled at HL. Synthesis of LSU was not interrupted if the cells were transferred to HL in the presence of ascorbic acid (5 and 10 mM), whereas in its absence, LSU translation decreased at HL (2 h). Addition of ascorbic acid increased the synthesis of D1 already at LL, thus reducing the difference between incorporation of radiolabel into D1 at LL and at HL (Fig. 3A).

To examine whether accumulation of excess ROS indeed signaled the translational arrest of LSU, their level was measured using the oxidatively sensitive DCFH (15). DCFH enters the cells in its diacetate form (DCFH-DA), becomes hydrolyzed and remains trapped intracellularly. Oxidation of DCFH by H₂O₂ generates DCF, a highly fluorescent compound. Although DCFH reacts mainly with H₂O₂, it is useful for monitoring other ROS species, because singlet oxygen is converted to superoxide anion resulting in the formation of H₂O₂ by superoxide dismutase activity (20, 23).

The results of DCF fluorescence indicate that the level of ROS increased by almost 2-fold at 0.5 and 1.5 h after transfer from LL to HL and decreased after 2.5 h back to the level measured at LL (Fig. 3B). Addition of ascorbic acid (5 and 10 mM) to wild type cells prevented the ROS increase at HL, in line with its antioxidant activity.

Monitoring ROS Levels with DCF in the Presence of Herbicides and in Mutant Cells—To establish the correlation between down-regulation in LSU synthesis and the increase in ROS levels, DCF fluorescence was measured at LL and after transfer to HL in the presence of DCMU (10⁻⁷ M) and DBMIB (10⁻⁶ M) and in mutants defective in D1 and in cytochrome b₆/f. DCF fluorescence in cells transferred from LL to HL hardly changed in the presence of DCMU and in mutants deficient or defective in D1 (Fig. 4, A–C), indicating that the level of ROS at HL did not increase. However, addition of DBMIB or inactivation of the cytochrome b₆/f complex did not prevent the increase in ROS at HL (Fig. 4, D and E). With DBMIB (10⁻⁶ M) the basal level of ROS was higher already at LL than that measured in the absence of this herbicide (Fig. 4D). This result was in correlation to the already reduced synthesis of LSU observed with DBMIB at LL (10⁻⁶ M, Fig. 1).

The Effect of MeV on ROS Formation and LSU Synthesis—To establish the direct involvement of ROS in signaling the down-regulation of LSU synthesis, cells were incubated at LL with MeV. MeV accepts an electron from ferredoxin and reacts with molecular O₂, forming a superoxide radical anion that is transformed in subsequent reactions to ROS such as hydrogen peroxide and hydroxyl radicals (24). The free radical pool can therefore be increased by MeV, similar to what occurs when cells are transferred from LL to HL. Cells were labeled in the presence of MeV (10⁻⁶–10⁻⁵ M). Labeling was performed in the absence of anisomycin to maintain the synthesis of cytoplasmic proteins and to ensure that MeV did not cause a general decrease in protein synthesis, which could be masked in the presence of anisomycin. Increasing concentrations of
MeV exclusively reduced the synthesis of LSU at LL (by 79% after 1 h), whereas synthesis of other proteins was unaffected.

DCF fluorescence at LL in the presence of MeV (10^{-6}–10^{-4} M) shows an increase in the level of ROS in a dose-dependent manner (Fig. 5B). Although the highest ROS level was measured with 10^{-4} M, this concentration was too high for labeling, because it inhibited protein synthesis nonspecifically (data not shown). These results in combination with the labeling data indicate the role of ROS in controlling the translation of Rubisco LSU.
Glutathione Redox State Regulates Translation of Rubisco LSU

Monitoring GSH/GSSG Ratios at LL and after Transfer to HL—Glutathione is a low molecular mass thiol that has a key regulatory role in plants and algae. Most of it is present in the reduced form (GSH), and only a minor fraction is oxidized and exists as two molecules of GSH linked by a disulfide bond (GSSG). An increase in ROS during stress could affect the GSH/GSSG ratio, shifting the balance toward oxidation. As shown in our DCF based fluorescence assay, a “light shock” causes a rapid increase in the level of ROS that subsequently returns to the original value measured at LL. We therefore examined whether the transient increase in ROS after the LL to HL shift is coupled to parallel changes in the GSH/GSSG ratio. Transfer of C. reinhardtii cells from LL to HL resulted in a transient decrease in the GSH/GSSG ratio (Fig. 6A) which dropped 2-fold after 1.5 h and recovered its original LL level within 6 h. The accumulation of excess ROS thus changed the redox state of glutathione, increasing its relative oxidized fraction. The changes in the GSH/GSSG ratio paralleled the down-regulation and the subsequent recovery of LSU synthesis. The translational arrest of LSU was maximal at 2 h and recovered after 4–6 h (8). The GSH/GSSG ratio returned to its original value also after 6 h, only after the level of ROS decreased. Modulation of the GSH/GSSG ratio by changes in the ROS level is further supported by the opposite effects observed for ascorbate and MeV. Addition of ascorbic acid to cells transferred to HL prevented the transient decrease in the GSH/GSSG ratio (Fig. 6B), and addition of MeV to cells grown at LL increased the relative fraction of oxidized glutathione decreasing the GSH/GSSG ratio, mimicking the transfer to HL (Fig. 6C).

DISCUSSION
Changes in light intensities play a key role in regulation of photosynthetic genes. Recent studies assigned a regulatory role for the redox state of components in the photosynthetic electron pathway in controlling expression of chloroplastic proteins (19, 25). Regulation of the nuclear encoded cab genes, and the chloroplast encoded psbA gene is thought to be controlled directly by the redox potential of specific components in the electron transport chain. Expression of cab genes is reversibly repressed by a phosphorylatable factor coupled to the redox status of PQ through a chloroplast protein kinase (25). Translation of D1 is subject to regulation by the redox state of thioredoxin and ferredoxin (19) by affecting the thiol groups on proteins that bind to the 5′-untranslated region (26). Unlike D1, whose synthesis gradually increases in response to elevation of light intensities, LSU synthesis follows a unique regulatory pattern, displayed by its transient arrest in cells transferred from LL to HL and a subsequent recovery upon photoacclimation (8). The mechanism that regulates the synthesis of these two proteins should therefore differ.

In this study we propose that translational arrest of rbcL is signaled by the increased generation of ROS that can modulate the redox potential of the glutathione pool and thus inhibit the translation of this protein. The labeling pattern of LSU in the presence of DCMU and DBMIB or in mutants defective at different sites of the electron transport chain could imply that the redox state of plastoquinone is involved in signaling the down-regulation in LSU synthesis. However, because the measured value of 1-qP (the index of QA reduction state) was high and remained unaltered during the first hours after transfer to HL, whereas LSU translation initially declined and then recovered (8), changes in the redox state of PQ were not likely to be the direct cause for the down-regulation of LSU, although indirect effects of the chloroplast redox state could be involved. Alternatively, these results could be explained on the basis of ROS formation upon transfer to HL. The down-regulation of the rbcL gene encoding LSU could occur in response to a signal generated by the imbalance that takes place when cells grown in LL, with their extensive chlorophyll antenna complexes, are shifted to HL. These antennae would trap more light quanta than can be processed by the photosynthetic electron transport system, resulting in the rapid elevation of ROS. As the antenna size is adjusted downward the imbalance would be dissipated, the level of ROS would decrease, and LSU synthesis would increase once again. Quantitative estimation of ROS correlates with this hypothesis, with their level transiently increasing upon transfer from LL to HL and then returning to the basal level. Incubation with ascorbic acid prevented the increase in ROS at HL and prevented the translational arrest of LSU. In accordance with these data, incubation of cells at LL in the presence of MeV, an inducer of ROS in the chloroplast, led to the increase of ROS and the down-regulation of LSU synthesis, whereas translation of other proteins was unaffected.

The results of labeling wild type cells in the presence of herbicides or labeling of mutants defective at different sites of the electron transport chain could also be interpreted by ROS formation. DCMU protects cells from the oxidative stress ex-
experienced during high intensity illumination by preventing the increase in ROS and the light induced breakdown of D1 (27, 28). The DCF-based measurements of ROS in the presence of DCMU confirmed this observation, which correlated with the continued synthesis of LSU at HL in the presence of DCMU. Likewise, ROS levels in nonphotosynthetic D1 mutants did not show a marked increase at HL. DBMIB or mutations in the cytochrome $b_6/f$ complex did not prevent the elevation of ROS levels nor the translational arrest of LSU. The increase in ROS in the presence of DBMIB is relatively short-term for reasons not completely clear to us; however, the basal level of ROS is high already at LL, possibly because of the block in the electron pathway.

Previous reports indicated that ROS can function as second messengers in mediating stress responses in plants. Plants attacked by pathogens respond by elevating ROS levels, leading to the induction of pathogen response genes (29, 30). Transcriptional activation of pathogen response genes can also be obtained by elicitors of ROS (31), by direct application of H$_2$O$_2$ (32), or by suppression of catalase activity resulting in increased levels of H$_2$O$_2$ (33, 34).

Modulation of gene expression by ROS can be mediated by changes in the redox state of the glutathione pool (23, 35). Elevation of reduced glutathione in transformed plants overexpressing glutathione reductase increased resistance to oxidative stress (36), and changes in the redox status of glutathione regulated the expression of copper, zinc-superoxide dismutase and of ascorbate peroxidase (37).

Here we show that transfer of cells from LL to HL causes a transient increase in ROS that correlates with the reduction in the GSH/GSSG ratio. Addition of ascorbic acid prevented the increase in ROS, and thus the GSH/GSSG ratio remained unaltered at HL, whereas addition of MeV at LL increased the formation of ROS and decreased the GSH/GSSG ratio. Concomitantly, ascorbic acid prevented the down-regulation of LSU translation at HL and MeV induced it at LL. Thus the decrease in the GSH/GSSG ratio in the chloroplast could serve as a signal for the translational arrest of LSU. We hypothesize that translational arrest of the $rbcL$ transcript could occur because of oxidation of sulfhydryl groups in one or more of the components of the translational initiation complex that assembles on the $rbcL$ 5'-untranslated region. During photoacclimation the intrachloroplastic glutathione pool shifts to its reduced form, oxidation of the sulfhydryl groups on the target protein is reversed, and translation of LSU can proceed. At this stage the...
nature of the putative protein that modulates translation of the \textit{rbcL} transcript possibly by oxidation of its sulfhydryl groups is yet unclear. In addition to its role in translational control, redox changes induced during photoinhibitory stress and se-nessence have been implicated in Rubisco breakdown (38, 39), and oxidation of sulfhydryl groups in critical Cys residues has been demonstrated to play a key role in LSU degradation (40).

The time period required for recovery of the GSH/GSSG ratio is similar to that observed for restoration of LSU translation (4–6 h), suggesting that the two processes are associated. However, the level of ROS returned to its original level already within 2.5 h. This difference can be explained by a lag period that is required for the cell to overcome the oxidative damage induced by the transfer to HL. The delay in restoring the GSH/GSSG ratio could be due to a requirement for synthesis of new proteins.

The synthesis of subunits that compose organellar multim-eric protein complexes is coordinated, even when they are en-coded by the different genomes of the cell (41, 42). Translation of the chloroplast-encoded Rubisco LSU was inhibited when the eric protein complexes is coordinated, even when they are en-

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