Photo-oxidative Stress in a Xanthophyll-deficient Mutant of Chlamydomonas

When there is an imbalance between the light energy absorbed by a photosynthetic organism and that which can be utilized in photosynthesis, photo-oxidative stress can damage pigments, proteins, lipids, and nucleic acids. In this work we compared the wild type and a xanthophyll-deficient mutant of Chlamydomonas reinhardtii in their response to high amounts of light. Wild-type Chlamydomonas cells were able to acclimate to high amounts of light following transfer from low light conditions. In contrast, the npq1 lor1 double mutant, which lacks protective xanthophylls (zeaxanthin and lutein) in the chloroplast, progressively lost viability and photosynthetic capacity along with destruction of thylakoid membrane protein-pigment complexes and accumulation of reactive oxygen species and membrane lipid peroxides. Loss of viability was partially rescued by lowered oxygen tension, suggesting that the high sensitivity of the mutant to light stress is caused by the production of reactive oxygen species in the chloroplast. Cell death was not prevented by the addition of an organic carbon source to the growth medium, demonstrating that the photo-oxidative damage can target other essential chloroplast processes besides photosynthesis. From the differential sensitivity of the mutant to exogenously added pro-oxidants, we infer that the reactive oxygen species produced during light stress in npq1 lor1 may be singlet oxygen and/or superoxide but not hydrogen peroxide. The bleaching phenotype of npq1 lor1 was not due to enhanced photodamage to photosystem II but rather to a less localized phenomenon of accumulation of photo-oxidation products in chloroplast membranes.

Carotenoids are tetraterpenoid (40-carbon isoprenoid) pigments synthesized by plants and algae and by some fungi and bacteria. They give many flowers and leaves their distinctive red, orange, and yellow colors. Carotenoids also play a role in human and animal nutrition as provitamin A and as potential anti-cancer agents. The chloroplasts of all photosynthetic cells contain β-carotene and some array of oxygenated carotene derivatives, the xanthophylls. In green tissue, the xanthophylls are located in the light-harvesting antennae of the membrane-bound photosystem I (PSI) and photosystem II (PSII) complexes. They aid in light harvesting and are essential for maintaining the condition of the photosynthetic apparatus by participating in energy dissipation under excessive light and by preventing photo-oxidative damage to the thylakoid membrane through quenching of excited intermediates, such as triplet chlorophylls and singlet oxygen (1–3).

The contents of β-carotene and xanthophylls in the chloroplasts of plants and algae are tightly regulated in response to the environment and to the developmental stage of the organism, and their accumulation is coordinated with the biogenesis and assembly of the photosynthetic apparatus. Carotenoid deficiency, caused either by mutation or by the application of herbicides, has devastating effects on chloroplast integrity, interferes with thylakoid membrane biogenesis, and influences the expression of nuclear genes that encode the protein components of the chloroplast (4).

In plants and green algae, the presence of either zeaxanthin or lutein in the chloroplast is necessary for protection against photo-oxidative stress. Zeaxanthin is the only xanthophyll that accumulates exclusively under light stress. Work with mutants impaired in the accumulation of lutein and zeaxanthin has shown that when plants and algae are under high light stress, these pigments are required for the efficient transition of the light-harvesting antenna from a conformation that favors light harvesting to one that allows for thermal dissipation of part of the excess excitation energy (5–7). This thermal dissipation of excess light energy is known as nonphotochemical quenching (NPQ) (8). The process of NPQ is accompanied by the de-epoxidation of existing violaxanthin to zeaxanthin in the so-called xanthophyll cycle (9). However, zeaxanthin may also prevent photo-oxidative stress by a mechanism separate from NPQ, such as through direct quenching of excited intermediates and/or scavenging of free radicals (10, 11). It has been
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suggested as well that zeaxanthin may exert its protective effect by making the thylakoid membrane less permeable to oxygen (12).

Two xanthophyll-deficient mutants of Chlamydomonas reinhardtii are defective in the development of NPQ when exposed to high light (HL), but neither mutation has a lethal effect on growth in HL conditions (5). The npq1 mutant is impaired in the HL-induced de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin, such that zeaxanthin accumulation in HL is prevented, but the mutant can survive in HL treatment (19). The lor1 mutation prevents the constitutive accumulation of lutein and its derivative loroxanthin in Chlamydomonas (15). The double mutant npq1 lor1 has a normal xanthophyll cycle, it is partially defective in NPQ, and it can survive in HL (5). The double mutant npq1 lor1, which accumulates violaxanthin and neoxanthin as the only xanthophylls irrespective of light conditions, shows a severe lack of NPQ and undergoes irreversible bleaching at a photon flux density (PFD) of 500 μmol of photons m⁻² s⁻¹, a condition in which the wild type and the single mutants grow normally (5). Suppressors that restore growth of npq1 lor1 in HL include npq2 mutants that cause constitutive accumulation of zeaxanthin, demonstrating that zeaxanthin is sufficient for survival and growth in HL (11).

In this work, we analyzed the behavior of the low-light-grown npq1 lor1 double mutant under conditions of oxidative stress and during exposure to HL leading to photobleaching. We monitored the changes in growth, photosynthetic activity, and oxidative stress status in the double mutant in comparison with the wild-type strain. Our results suggest that photobleaching of npq1 lor1 in HL is because of excessive formation of reactive oxygen species (ROS) in the thylakoid membrane due to the absence of the antioxidants, zeaxanthin and lutein. Our results also rule out the possibility that photobleaching of npq1 lor1 is initiated by an enhanced susceptibility to photodamage of the PSII reaction center.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—The npq1 lor1 double mutant has been described previously (5). The wild-type strain CC125 was obtained from the Chlamydomonas Genetics Center (Duke University, Durham, NC). To score the amount of growth under different conditions, the cells were grown photoautotrophically on plates in minimal HS medium or mixotrophically in acetate-containing TAP medium (15). The strain stocks were maintained at a PFD of 10 μmol of photons m⁻² s⁻¹ on TAP agar medium. For light transition experiments, the cells were grown photoautotrophically to the early exponential phase (fewer than 1.5 x 10⁶ cells/ml) in 100 ml of HS at 25°C with shaking in air in sterile beakers under continuous low light (LL, 50 μmol of photons m⁻² s⁻¹). Illumination was provided from the top by cool white fluorescent lights, 1.5 ml medium (freshly adjusted to pH 5.7 with acetic acid) to bring the total volume to 1 ml. 1.5 μm of CM-H₂DCFDA (freshly dissolved in Me₂SO; final concentration 10 μM) or a Me₂SO-only control were added to the cell samples, which were incubated at room temperature for 15 min in the dark. The cells were then centrifuged and resuspended as before, and fluorescence of 10⁶ cells was measured on an EPICS XL-MCL flow cytometer (Beckman-Coulter, Miami, FL).

The extent of lipid peroxidation in cells was estimated by measuring the formation of thiobarbituric acid-reactive substances (TBARS) and by thermoluminescence. For TBARS determination, 10-ml culture aliquots were taken before and at time intervals after exposure to HL. Butylated hydroxytoluene was added to the samples at a final concentration of 0.01% (w/v) to terminate lipid peroxidation chain reactions, and the content of TBARS was measured as described by Baroli et al. (11). Thermoluminescence measurements were performed with a custom-built apparatus (17). The cells were deposited on 2.5-mm diameter 12-μm pore size nitrocellulose filters (Millipore), which were placed in the copper chamber of the apparatus. The samples were heated from 25°C to 150°C at a rate of 6°C min⁻¹. Temperature was controlled using a thermocouple mounted between the sample and the heating element. The luminescence signal was recorded during sample heating with a compact red extended photomultiplier (H5701-50, Hamamatsu, Bridgewater, NJ) shielded with an RG665 glass filter (Schott, Yonkers, NY).

Both the sample temperature and thermoluminescence were recorded by a computer using a DaqPad-1200 data acquisition system (National Instruments, Austin, TX). The amplitude of the 35°C thermoluminescence band was used as an index of lipid peroxidation (10, 18).

Preparation of Protein Extracts, SDS-PAGE, and Immunoblotting—To prepare whole-cell protein extracts for SDS-PAGE, cells were harvested from 15-ml culture aliquots by centrifugation at 3200 x g for 4 min at 4°C. Protein sample preparations, SDS-PAGE, and immunoblotting were performed as described in Ref. 11. The anti-D1 and anti-LHCII antibodies were kindly provided by A. Melis (University of California, Berkeley, CA) and F.-A. Wollman (Institut de Biologie Physico-Chimique, Paris, France), respectively.

RESULTS

Growth under Photo-oxidative Stress Conditions—The npq1 lor1 double mutant was unable to grow photoautotrophically on agar medium at light intensities higher than 250 μmol of photons m⁻² s⁻¹ (Fig. 1). The growth impairment at 250 μmol of photons m⁻² s⁻¹ (ML) could be rescued if the agar plates were kept under anaerobic conditions (Fig. 1). However, rescue of the light sensitivity phenotype by anaerobiosis was partial and was not seen at higher light intensities (data not shown).
The addition of acetate as an organic carbon source was unable to rescue the sensitivity of npq1 lor1 to HL (Fig. 1).

Cell Viability and Photosynthesis during Exposure to HL—To characterize the photo-oxidative stress experienced by npq1 lor1 cells, we examined liquid cultures of the double mutant and the wild type at various times after transfer of LL-grown cells to HL. When grown photoautotrophically in liquid cultures under continuous LL, both the wild type and the double mutant npq1 lor1 had a similar doubling time of ~14 h. Fig. 2A shows that, when exposed to HL, the rate of increase in cell density slowed down but persisted in both strains. The wild-type culture had an initial slight decrease in cell viability, as assayed by colony forming units, after transfer to HL (Fig. 2B). The apparent viability of wild-type cells then increased rapidly between 3 h and 6 h, after which viability was maintained at a level that was consistent with the total cell density. In contrast to the wild type, the npq1 lor1 cells showed a substantial decline in viability that was already evident after 6 h in HL. By 24 h of HL treatment, only 20% of the initial cells were able to form colonies (Fig. 2B), but there was a slight increase in cell viability between 24 and 48 h.

As with the doubling time, photosynthetic parameters were similar in the mutant and wild type when cells were grown in LL, except that npq1 lor1 showed a significantly elevated Chl a:Chl b ratio of ~3.5 compared with a value of ~2.6 for the wild type (see also Ref. 5). The Chl a:Chl b ratio did not change significantly in either strain during the HL treatment (data not shown). The cellular total Chl content of LL-grown cells was ~25% lower in the double mutant compared with the wild type (Fig. 3A). During the first 6 h of HL treatment, the Chl content in the wild type initially decreased ~20% and then stabilized at the lower value. In the double mutant, the Chl content decreased progressively upon exposure to HL until it reached only 12% of the initial value after 48 h of HL treatment. At this point, the npq1 lor1 culture contained a mixed population composed of bleached cells, which were devoid of thylakoid membranes when observed with transmission electron microscopy (data not shown), and cells containing at least some Chl. These two kinds of cells could be distinguished by flow cytometry (see below).

The decrease in Chl in the double mutant was preceded by a loss of photosynthetic activity, measured either as the maximal photochemical efficiency of PSII in the dark-adapted state, Fv/Fm (Fig. 3B), or as oxygen evolution in whole cells (Fig. 3C). On a per cell basis, photosynthetic oxygen evolution in npq1 lor1 was only 15% of the initial value after 48 h in HL (Fig. 3C). As with the cell viability, the Fv/Fm value showed a partial recovery in the double mutant, which was also evident when photosynthetic oxygen evolution was calculated on a per mol of Chl basis (Fig. 3D). In the wild type, there was an initial decrease in Fv/Fm and the culture stabilized at this lower value of ~0.65 for the rest of the treatment. The decline in PSII efficiency was not accompanied by a decrease in photosynthetic oxygen evolution, which remained the same during the first 6 h of treatment and then showed a progressive increase followed by stabilization after 24 h in HL (Fig. 3C).

Loss of Photosynthetic Proteins during Exposure to HL—The loss of Chl and photosynthetic activity in the double mutant was indicative of damage to the thylakoid membrane. To characterize the kinetics of this photodamage, we monitored the content of specific photosynthetic proteins. Fig. 4 shows the contents of the PSII reaction center D1 protein and the peripheral LHCII as a function of time in HL as determined by immunoblotting. The wild type showed no significant change in the cellular level of either protein during exposure to HL. In contrast, after a lag of ~6 h, the cellular content of the D1 protein decreased dramatically (half-time ~6 h) in the double mutant such that only 30% of the initial D1 content remained after 48 h in HL (Fig. 4A). LHCII proteins in npq1 lor1 cells
were also lost in HL, but more gradually, with a half-time of ~24 h.

**Effect of Lack of Zeaxanthin and Lutein on the Rate of D1 Photodamage**—The reaction center of PSII has been reported to produce singlet oxygen under conditions that lead to photoinhibition (19, 20). Because the npq1 lor1 double mutant is defective in the rapidly induced thermal dissipation process of NPQ in PSII, the loss of D1 protein (Fig. 4A) could be due to enhanced sensitivity of the PSII reaction center to photodamage and concomitant generation of reactive singlet oxygen in its proximity. Alternatively, singlet oxygen or other reactive oxygen species could be formed at higher levels in the chloroplast of npq1 lor1 as a consequence of the lack of the protective xanthophylls, zeaxanthin and lutein. To address the question of whether PSII and thylakoid destruction in the double mutant was caused by lack of thylakoid membrane antioxidant capacity or whether it was induced by increased photoinhibition of PSII, we monitored photodamage by following the turn-over rate of the D1 protein of PSII after a short exposure to HL in the presence of the chloroplast protein translation inhibitor lincomycin. Fig. 5 shows that the D1 protein in the double mutant had a significantly longer half-life than in the wild type (2.2 h and 1.4 h, respectively). A similar trend was observed when \( F_{v}/F_m \) was measured in the same experiment (data not shown). These results with D1 protein turnover were consistent with measurements of the PSII excitation pressure as a function of light intensity in the two strains (data not shown). Taken together, these results rule out the possibility of thylakoid photobleaching in npq1 lor1 being caused primarily by increased photodamage to PSII and point to a more generalized damage in the thylakoid membrane itself.

**Lipid Peroxidation during HL Exposure**—We also examined the kinetics of accumulation of damaged membrane lipid products in npq1 lor1. A well known effect of ROS in the thylakoid membrane is the peroxidation of unsaturated lipids through the initiation of radical chain reactions. Lipid peroxidation, measured as TBARS, increased ~9-fold during exposure of the double mutant to HL, whereas the wild type showed only a slight increase in lipid peroxidation (Fig. 6A). The oxidation of lipids in the proximity of Chl can also be monitored by the luminescence signal emanating from cells heated to temperatures higher than 70 °C (18, 21). Fig. 6B shows that, compared with that of the wild type, in the double mutant, the thermoluminescence signal peaking at 135–145 °C increased considerably during HL illumination, consistent with the TBARS results (Fig. 6A).
ROS Production during HL Exposure—The fact that the light sensitivity of the npq1 lor1 double mutant was diminished in the presence of a lowered oxygen tension (Fig. 1) suggested that ROS might be the cause of bleaching in HL. We measured the presence and relative quantity of ROS in npq1 lor1 and wild-type cells during exposure to HL (Fig. 7A). The measurements were performed by flow cytometry using the cell-permeant indicator dye CM-H₂DCFDA. This dye diffuses passively into the cell where its acetate groups are cleaved by intracellular esterases, trapping it in the cell and exposing the CM-H₂DCF, which, when oxidized, fluoresces brightly. This dye thus indicates levels of ROS in live cells only. The use of flow cytometry allowed us to measure separately the fluoresce emitted from Chl and from the ROS indicator dye in each individual cell. The Chl fluorescence measurements (Fig. 7B) yielded results that were very similar to the measurements of Chl per cell (Fig. 3A). Significant variability was present in the ROS measurements at all time points, particularly the 0 and 48 h time points, but the overall trend was consistent (Fig. 7A). The mean fluorescence in both strains after 3 h in HL was consistently lower (and less variable) than at 0 h. The average ROS level in live cells increased significantly in the npq1 lor1 double mutant after 24 and 48 h of HL exposure, whereas in the wild type, it remained steady at a low level. After 24 and 48 h of HL, two populations of mutant cells were distinguishable. One exhibited increased ROS and normal Chl fluorescence, whereas the other population, presumably dead cells unable to retain or activate the dye, showed little or no ROS or Chl fluorescence (Fig. 7C). Only the populations of presumed live cells (i.e., cells that could take up the dye) were used to calculate the average ROS fluorescence (Fig. 7A).

Growth in the Presence of Exogenous Pro-oxidants—To gain insight into which ROS might be produced in the npq1 lor1 double mutant when exposed to excess light, we examined sensitivity to different ROS in LL (Fig. 8). Concentrations of pro-oxidants were selected that were just below what was necessary to inhibit growth of wild-type cells completely. The npq1 lor1 double mutant was more sensitive than the wild type to rose bengal, a photosensitizing dye that generates singlet oxygen upon illumination. npq1 lor1 was also more sensitive than the wild type to the superoxide generators methyl viologen and metronidazole but not to hydrogen peroxide. The addition of methyl viologen to agar plates significantly retarded the growth of npq1 lor1 relative to the wild type, and the double mutant bleached more rapidly than the wild type on both methyl viologen and metronidazole plates.

**DISCUSSION**

A previous study showed that the npq1 lor1 mutant of *Chlamydomonas* bleaches when grown on agar plates at HL (5). Here we extend that study and present data on the response of wild-type cells to HL and the temporal sequence of events that leads to photo-oxidative damage and cell death in the mutant.

**Response of Wild-type Chlamydomonas Cells to a Transition from LL to HL**—A light intensity of 500 μmol of photons m⁻² s⁻¹ did not appear to be excessive for growth of wild-type *Chlamydomonas*, but it caused some initial symptoms of light stress. When grown in LL and transferred to HL, the wild type responded with a slowing down of the cell duplication rate (doubling time of 14 h in LL versus >24 h after transfer to HL, Fig. 2A) and an initial slight decrease in cell viability. The number of cells able to form colonies then rose substantially.
Accumulation of ROS during exposure to HL. A, average intensity of the 525 nm fluorescence emitted from cells excited at 488 nm, corresponding to the amount of oxidized ROS reporter dye CM-H$_2$DCF. Data shown are the means ± S.D. from three independent experiments, except in the mutant at the times 6 and 12 h, where n = 2 and the error equals the difference from the means. The means were taken from live and actively fluorescing cells as shown by region M2 in the inset histograms. The insets represent one example each of the wild type and mutant for the 48 h in the HL time point. B, percent of the cell population retaining Chl fluorescence during the time in HL. Chl fluorescence was measured using a 675-nm bandpass filter and was taken as that above the arbitrary fluorescence intensity of 1.3; this is indicated as region M1 in the inset histograms. Data shown are the means ± S.D. from five independent experiments. Insets are the same 48-h samples as shown for CM-H$_2$DCF fluorescence (panel A), and represent 100,000 cells each. C, scatter plot of ROS-induced CM-H$_2$DCF fluorescence versus Chl fluorescence in one representative sample of 100,000 npq1 lor1 cells after 48 h in HL showing coincidence of elevated ROS and normal Chl fluorescence. Open circles, wild type; black circles, npq1 lor1 double mutant; a.u., arbitrary units.
between 6 and 12 h in HL (Fig. 2B), despite the more gradual increase in cell density (Fig. 2A). The discrepancy between cell density and apparent cell viability might be explained by an increase in the plating efficiency of wild-type cells occurring within the first 6 h after transfer from LL to HL.

After the initial stress period in which the Chl/cell and Fv/Fm decreased slightly but significantly (Fig. 3, A and B, respectively), the wild-type cells stabilized at lower values of both parameters. In contrast, the light shift did not have any negative effect on the rate of photosynthetic electron transport, measured as oxygen evolution in intact cells (Fig. 3, C and D), or on the cellular content of the PSII D1 protein (Fig. 4A) consistent with the notion that the HL treatment is not photo-inhibitory to the wild type.

The LHCII protein level showed a transient decrease of ~10% during the first 12 h in HL, but it stabilized at a level almost identical to the initial one (Fig. 4B). A similar response of the major LHC proteins has been observed in the green alga Dunaliella salina (22). The fact that the Chl α/Chl b ratio remained constant after the shift from LL to HL suggests that there was no preferential down-regulation of the LHC proteins relative to the content of reaction centers within the first 48 h in HL. A similar lack of LHC down-regulation has been observed in wild-type Arabidopsis immediately following transfer from LL to HL (23).

Photo-oxidative Stress in npq1 lor1 Cells—Why does the npq1 lor1 double mutant bleach in HL? Upon transfer from LL to HL, symptoms of light stress in npq1 lor1 cells were first manifested within 3 h as declines in PSII efficiency, measured as Fv/Fm, and photosynthetic oxygen evolution (Fig. 3). The addition of acetate as an organic carbon source did not rescue growth of npq1 lor1 in HL (Fig. 1), however, which would indicate that essential processes other than photosynthesis are being affected by the HL treatment. Cell viability began to decrease between 3 and 6 h (Fig. 2B) followed by loss of D1 and LHCII proteins after 6 h in HL (Fig. 4) and accumulation of lipid peroxidation products after 12 h in HL (Fig. 6). The fact that lowered oxygen tensions reduced the extent of bleaching in npq1 lor1 at least partially (Fig. 1) suggests the involvement of ROS, yet significantly higher levels of ROS were detected only after at least 12 h in HL (Fig. 7A). Because the intracellular CM-H2DCF probe for ROS is a soluble molecule, it is conceivable that ROS are generated initially in chloroplast membranes where photosynthesis and other processes would be subject to photo-oxidative damage and are not readily detected by the probe until later time points when antioxidant defenses are overwhelmed.

The lack of NPQ in the mutant might be expected to increase ROS levels in thylakoids, primarily resulting in photodamage to PSII that leads to a more general photo-oxidative bleaching.

However, npq1 lor1 was not more susceptible than the wild type to PSII photodamage, as indicated by turnover of the D1 protein (Fig. 5). In addition, the recently described npq5 mutant of Chlamydomonas, which has normal xanthophyll composition but lacks NPQ, survives excess light (24). Taken together, these results show that lack of NPQ and enhanced rates of photodamage to PSII are not the primary underlying cause of the HL sensitivity of npq1 lor1, and they point instead to a more general deficiency in antioxidant capacity as the causative factor.

Formation of ROS in the chloroplast is consistent with the widespread damage to the thylakoid membrane that we observed when npq1 lor1 was exposed to HL (see particularly Figs. 4 and 6). The lack of NPQ could increase the yield of singlet oxygen in the Chl antenna of PSII. In addition, higher levels of superoxide and hydrogen peroxide could be produced at PSI due to lack of NPQ. We hoped to provide insights into these possibilities by examining the effect on npq1 lor1 of pro-oxidants that produce different ROS. The double mutant was considerably more sensitive than the wild type to the singlet oxygen generator rose bengal and to the superoxide generators metronidazole and methyl viologen but not to H2O2 (Fig. 8). Thus, we can exclude formation of H2O2 as a cause of enhanced oxidative damage in the mutant. Toxicity of metronidazole and methyl viologen is the result of the ability of these molecules to accept electrons from ferredoxin and subsequently donate electrons to oxygen, generating superoxide. Metronidazole is toxic to Chlamydomonas only in the light, and PSI-deficient mutants are more resistant to the inhibitor, supporting the idea that toxicity occurs because of superoxide generation at the acceptor side of PSI (25). Methyl viologen is capable also of killing Chlamydomonas cells in the dark (25), indicating that its toxicity does not derive entirely from photochemical superoxide production in the chloroplast. Nevertheless, in LL-grown cells, the presence of lutein in the chloroplast of wild-type cells is apparently sufficient to protect against toxicity of methyl viologen (Fig. 8). The ROS indicator dye that we used, CM-H2DCFDA, does not allow a distinction between singlet oxygen and superoxide in vitro and attempts to measure singlet oxygen with a specific probe (DanePy) (26) in intact cells failed because of interfering background fluorescence of Chlamydomonas cells. Both singlet oxygen and superoxide could be produced by the mutant in HL.

However, other results reported here indicate that singlet oxygen could be the main ROS produced in HL-exposed npq1 lor1. For example, HL treatment of the mutant led to extensive formation of lipid peroxides (Fig. 6A), a known product of singlet oxygen attack on membrane lipids (27). In addition, it is likely that some of the high-temperature thermoluminescence signal that was detected in npq1 lor1 at wavelengths of >665 nm (Fig. 6B) is attributable to singlet oxygen. This red luminescence can be because of both singlet oxygen and excitation energy transfer from triplet carbonyls to Chl (28). Given that the Chl content of the mutant was strongly reduced in HL (Fig. 3A), the high thermoluminescence signal might be due, at least in part, to singlet oxygen. These results, along with the fact that zeaxanthin and lutein are well known as efficient quenchers of singlet oxygen (reviewed in Ref. 3), are consistent with the hypothesis that higher levels of singlet oxygen are produced in npq1 lor1 in HL. To test this hypothesis, experiments will be performed to examine singlet oxygen-mediated lipid peroxidation (29) in npq1 lor1 in response to HL.

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