Plants protect themselves from excess absorbed light energy through thermal dissipation, which is measured as nonphotochemical quenching of chlorophyll fluorescence (NPQ). The major component of NPQ, qE, is induced by high transthylakoid ΔpH in excess light and depends on the xanthophyll cycle, in which violaxanthin and antheraxanthin are deepoxidized to form zeaxanthin. To investigate the xanthophyll dependence of qE, we identified suppressor of zeaxanthin-less1 (szl1) as a suppressor of the Arabidopsis thaliana npq1 mutant, which lacks zeaxanthin. szl1 npq1 plants have a partially restored qE but lack zeaxanthin and have low levels of violaxanthin, antheraxanthin, and neoxanthin. However, they accumulate more lutein and α-carotene than the wild type. szl1 contains a point mutation in the lycopene β-cyclase (LCYB) gene. Based on the pigment analysis, LCYB appears to be the major lycopene β-cyclase and is not involved in neoxanthin synthesis. The Lhcb4 (CP29) and Lhcb5 (CP26) protein levels are reduced by 50% in szl1 npq1 relative to the wild type, whereas other Lhcb proteins are present at wild-type levels. Analysis of carotenoid radical cation formation and leaf absorbance changes strongly suggest that the higher amount of lutein substitutes for zeaxanthin in qE, implying a direct role in qE, as well as a mechanism that is weakly sensitive to carotenoid structural properties.

INTRODUCTION

Light is required for photosynthesis in plants, but the quantity of light in natural environments is highly variable. Within a certain range of relatively low incident light intensities, photosynthetic carbon fixation increases linearly with increases in photon flux density. However, above a certain threshold, photosynthetic capacity is saturated, and a plant absorbs more light than it can actually use. Absorption of excess light can lead to overexcitation of chlorophyll and overreduction of the electron transport chain, which result in increased generation of reactive intermediates and harmful byproducts of photosynthesis (Niyogi, 1999). For example, overexcitation of chlorophyll would result in an increase in the lifetime of singlet-excited chlorophyll (1Chl*), which consequently increases the production of triplet-excited Chl (3Chl*) via intersystem crossing. 3Chl* interacts with molecular oxygen to generate singlet O2 (1O2*), which can damage proteins, pigments, and lipids in the photosynthetic apparatus (Niyogi, 1999; Asada, 2006).

Photosynthetic organisms have evolved a suite of short-term and long-term photoprotective mechanisms to cope with the absorption of excessive light and its consequences. Among these mechanisms, the thermal dissipation of excess absorbed light energy in photosystem II (PSII), which is commonly measured and referred to as nonphotochemical quenching (NPQ), is believed to play a key role in regulating light harvesting and preventing photooxidative damage to the photosynthetic apparatus. NPQ can be induced or disengaged in response to changes in light intensity on a time scale of seconds to minutes. Although there are several components of NPQ in higher plants, pH-dependent energy dissipation (also called qE) accounts for the major part of NPQ and results in deexcitation of 1Chl* and the thermal dissipation of excess absorbed light energy in the light-harvesting antenna of PSII (Müller et al., 2001). Because it involves the deexcitation of 1Chl*, qE can be easily measured as a decrease in the maximum yield of chlorophyll fluorescence in intact leaves or isolated chloroplast membranes (Müller et al., 2001).

qE is induced by a low thylakoid lumen pH (i.e., a high ΔpH) during illumination with excess light (Demmig-Adams and Adams, 1992; Horton et al., 1996; Müller et al., 2001). The low
thylakoid lumen pH plays dual roles, one of which is to activate the violaxanthin deepoxidase (VDE) enzyme, which converts violaxanthin into antheraxanthin and then zeaxanthin as part of a xanthophyll cycle (Figure 1) (Yamamoto et al., 1999; Jahns et al., 2009). The other role of the low thylakoid lumen pH is to protonate one or more PSII proteins that are involved in qE (Horton and Ruban, 1992). A light-induced absorbance change at 535 nm (ΔA535) is linearly correlated with qE (Ruban et al., 1993; Bilger and Bjorkman, 1994; Li et al., 2004). ΔA535 depends on both zeaxanthin and protonation and is thought to be due to a change in the absorption spectrum of zeaxanthin (Ruban et al., 2002).

Analysis of Arabidopsis thaliana mutants that lack qE has been a very useful approach to define factors that are necessary for qE, including xanthophylls, the PsbS protein, and light-harvesting complex (LHC) proteins (Niyogi, 2000). qE-deficient mutants were identified in forward genetics screens by video imaging of chlorophyll fluorescence yield during exposure of mutagenized Arabidopsis seedlings to excess light (Niyogi et al., 1998; Li et al., 2000). The nonphotochemical quenching1 (npq1) and lutein-deficient2 (lut2) mutants exhibit diminished levels of qE (Niyogi et al., 1998; Pogson et al., 1998), whereas the npq4 mutant is completely defective in qE and ΔA535 (Li et al., 2000; Peterson and Havir, 2000). The npq1 mutant is defective in VDE and therefore lacks zeaxanthin. Characterization of the npq1 mutant showed that zeaxanthin is necessary for most of the qE in vivo in Arabidopsis leaves (Niyogi et al., 1998). The lut2 mutant affects lycopene ε-cyclase (LCYE) activity (Pogson et al., 1998), so it is unable to synthesize either lutein or α-carotene (Figure 1). The npq1 lut2 double mutant is totally devoid of any qE, suggesting a possible role for lutein in qE (Niyogi et al., 2001). Complementary evidence was reported with LCYE-overexpressing transgenic Arabidopsis plants, which have elevated lutein and an increase in qE (Pogson and Rissler, 2000). It has been proposed that lutein might have a direct role in qE or, alternatively, that the change of lutein content could indirectly affect qE by disturbing the assembly and structure of the PSII antenna (Niyogi et al., 1997, 2001; Pogson et al., 1998; Lokstein et al., 2002).

Figure 1. Carotenoid Biosynthetic Pathway in Plants.

The block in xanthophyll metabolism in the npq1 mutant, which lacks zeaxanthin due to a defect in the violaxanthin deepoxidase gene, is indicated by the symbol “npq1.”
Despite these advances in identifying components of qE, the actual biophysical mechanism of 1Chl deexcitation in qE remains controversial. Two mechanisms, which are not mutually exclusive, have been proposed recently based on ultrafast transient absorption (TA) spectroscopy experiments (Holt et al., 2005; Ruban et al., 2007). Both mechanisms include a role for PsbS as a sensor of lumen pH that triggers conformational changes in the PSII antenna that result in efficient deexcitation of 1Chl* (Ahn et al., 2008; Avenson et al., 2008; Horton et al., 2008). In one model, zeaxanthin has a direct role in quenching 1Chl* through a charge-transfer (CT) mechanism (Holt et al., 2005) in the minor LHCs associated with PSII, CP29 (Lhcb4), CP26 (Lhcb5), and CP24 (Lhcb6) (Ahn et al., 2008; Avenson et al., 2008, 2009). The CT quenching mechanism involves energy transfer from chlorophyll to closely coupled chlorophyll and zeaxanthin molecules, followed by charge separation that transiently produces a zeaxanthin radical cation and chlorophyll radical anion. Subsequent charge recombination dissipates the excitation energy as heat (Holt et al., 2005). The second model proposes that qE occurs in the peripheral, trimeric antenna of PSII called LHCII (Pascal et al., 2005), and its molecular mechanism involves energy transfer from 1Chl* to a low-lying S1 excited state of a carotenoid (lutein 1) (Ruban et al., 2007). According to this model, zeaxanthin is not required for qE but functions indirectly as an allosteric regulator (Crouchman et al., 2006) by increasing the pH sensitivity of qE (Nocot et al., 1991).

To investigate the xanthophyll dependence of qE and, possibly at the same time, to identify new components of qE, we performed a screen for suppressors of the npq1 mutation that exhibit higher qE despite the absence of zeaxanthin. We isolated a suppressor that accumulates more lutein but has a very small xanthophyll cycle pool size. Molecular, physiological, and biophysical analysis of this mutant suggest that the higher amount of lutein can substitute for zeaxanthin to act directly as a quencher in qE.

RESULTS

Isolation of Suppressors of npq1

We used a chlorophyll fluorescence video imaging system (Niyogi et al., 1997) to isolate suppressors of the npq1 mutant. Ten potential suppressors that showed much higher NPQ level compared with that of the npq1 parent were isolated out of 15,000 M2 seedlings (Figure 2), and six suppressors were shown to lack zeaxanthin during the secondary screen by pigment analysis. One of these six suppressors with partially restored NPQ had very low levels of violaxanthin and neoxanthin but accumulated more lutein (Figure 3A). The suppressor was named suppressor of zeaxanthin-less1 (szl1), and the szl1 npq1 plant was backcrossed to the npq1 parent three times and then crossed to the wild type to isolate an szl1 single mutant, which was able to synthesize zeaxanthin upon exposure to high light (Figure 3B).

Pigment Content and NPQ in theSuppressor

The wild-type, npq1, szl1, and szl1 npq1 plants were grown under low light (LL) conditions (150 μmol photons m⁻² s⁻¹). In these growth conditions, the suppressor szl1 npq1 and single mutant szl1 exhibited a very similar whole-plant phenotype to that of the wild-type and npq1 plants, with the major difference being slightly smaller sizes (Figure 4A). The pigment composition and content of the four genotypes were measured before and after a short treatment of LL-grown plants with high light (HL; 1000 μmol photons m⁻² s⁻¹). All four genotypes had the same total chlorophyll content, chlorophyll a/b ratio, and total carotenoids under both conditions, although szl1 and szl1 npq1 had lower total carotenoids and correspondingly higher total xanthophylls (Table 1). Figure 4B compares the relative content of β-carotene–derived violaxanthin, antheraxanthin, and zeaxanthin and α-carotene–derived lutein in wild-type and mutant plants after HL treatment. Xanthophyll cycle pigment pool size (the sum of violaxanthin, antheraxanthin, and zeaxanthin) was the same in the wild type and npq1, but it was reduced nearly 75% in szl1 and szl1 npq1. Because the npq1 mutation affects the VDE gene, no zeaxanthin was detected in either npq1 or szl1 npq1. The concentration of lutein in the szl1 and szl1 npq1 mutants was nearly twice higher than in the wild type and npq1 mutant. The smaller xanthophyll cycle pool size and the greater lutein concentration in the suppressor szl1 npq1 and the szl1 single
mutant (relative to those in the wild type) indicated that the β-carotene branch of the carotenoid biosynthetic pathway is affected, and metabolic flux is redirected into the α-carotene branch from which lutein is synthesized.

The NPQ induction curves of the four genotypes plus the heterozygous szl1/SZL1 npq1/npq1 mutant were compared (Figure 5). When illuminated with 1200 μmol photons m⁻² s⁻¹, the wild type showed a rapid establishment of NPQ to a value of 2.2 within 5 min, whereas the npq1 mutant showed a slower rate of NPQ induction and an NPQ value of <1.2. In the szl1 npq1 plants, the NPQ induction had an even more rapid rise in the first 30 s compared with the wild type, which was probably due to the higher amount of lutein, and the total NPQ reached a value of 1.7, which was substantially higher compared with the npq1 mutant. By subtracting the residual NPQ in each genotype after relaxation in the dark, we estimated the qE component after 5 min of light induction to be 1.7, 0.8, and 1.4 in the wild type, npq1, and szl1 npq1, respectively. The szl1 single mutant showed the same NPQ induction as that of the suppressor szl1 npq1. The heterozygous szl1/SZL1 npq1/npq1 mutant had NPQ levels identical to the npq1 mutant, indicating that the szl1 mutation is recessive for the NPQ phenotype.

The SZL1 Gene Encodes LCYB

The pigment phenotype of the suppressor szl1 npq1 suggested that the activity of a carotenoid biosynthetic enzyme is impaired. Since szl1 npq1 had a very small xanthophyll cycle pool size but twice as much lutein, the most likely candidate for the enzyme affected by szl1 mutation was LCYB, which functions at the branch point in the carotenoid biosynthetic pathway (Figure 1).

In Arabidopsis, LCYB is encoded by a single-copy gene (Cunningham et al., 1996). The LCYB gene was amplified directly from the genomic DNAs of the wild type, npq1, and szl1 npq1. Sequence analysis revealed that LCYB from szl1 npq1 carries a point mutation from G to A at position 1352, which translates into

![Figure 3](image-url)  
**Figure 3.** HPLC Analysis of Pigments in the Wild Type, npq1, szl1, and szl1 npq1.
(A) Comparison of pigment profile of the wild type, npq1, and szl1 npq1 after exposure to high light (1000 μmol photons m⁻² s⁻¹) for 30 min. (B) Overlay of HPLC traces of a szl1 single mutant before (LL) and after (HL) treatment with high light. Neo, neoxanthin; Vio, violaxanthin; An, antheraxanthin; Zea, zeaxanthin; Chl a, chlorophyll a; Chl b, chlorophyll b; α-car, α-carotene; β-car, β-carotene.

![Figure 4](image-url)  
**Figure 4.** Characteristics of Wild-Type, npq1, szl1, and szl1 npq1 Plants. (A) Growth of the four Arabidopsis strains. Plants were grown in LL (150 μmol photons m⁻² s⁻¹) with a short-day photoperiod (10 h light and 14 h dark). Plants were photographed at an age of 4 weeks. (B) Xanthophyll cycle pigment pool size (V+A+Z) and lutein levels of the four Arabidopsis strains. Plants were grown as described in (A). At the end of the dark period, whole plants were exposed to HL (2000 μmol photons m⁻² s⁻¹) for 30 min, and leaf samples were taken and analyzed by HPLC. Data were normalized to chlorophyll a and shown as the means ± SD (n = 9).
Pigment measurements were performed before and after exposure of LL-grown plants to HL (2000 μmol photons m⁻² s⁻¹). This point mutation creates an antheraxanthin single recessive nuclear mutation. Antheraxanthin reconstitution of the recessive nuclear mutation. Different (gous szl1 (Figure 6B). Out of 48 F2 plants, 10 were identified as homozygous szl1/SZL1 npq1/npq1 mutants, which is not significantly different (χ², P > 0.5) from the expected number (12, or 25%) for a single recessive nuclear mutation.

Reconstitution of the szl1 Mutant Pigment Phenotype in Escherichia coli

To confirm that the LCYB point mutation found in the szl1 npq1 mutant was responsible for the mutant pigment phenotype (low xanthophyll cycle pool size and high lutein content), plasmids pAC-BETA-At and pAC-BETA-At-szl1 were constructed (see Methods). These plasmids contain bacterial genes for lycopene synthesis plus the wild-type or szl1 allele of LCYB, respectively. The composition of pigments accumulated by E. coli strains containing either of these two plasmids with or without an additional plasmid expressing the Arabidopsis LCYE gene was compared.

We first examined the products formed when either the wild-type or mutant copy of Arabidopsis LCYB was present in the lycopene-accumulating strain of E. coli. Figure 7A shows an HPLC profile of the carotenoid pigments accumulated in E. coli cells containing plasmid pAC-BETA-At. As expected from the previous work by Cunningham et al. (1996), these cells accumulated predominantly β-carotene and formed yellow colonies. When the wild-type LCYB gene was replaced by the szl1 mutant

![Figure 5. NPQ Induction Curves in the Wild Type, Homozygous npq1, szl1, and szl1 npq1, and Heterozygous szl1/SZL1 npq1/npq1.](Image)

NPQ was measured during 5 min of illumination with HL (1200 μmol photons m⁻² s⁻¹), followed by relaxation in the dark for 5 min. Data are presented as the means ± sd (n = 4).
allele of LCYB, E. coli cells containing plasmid pAC-BETA-At-
szl1 accumulated approximately one-third as much 
\(\beta\)-carotene and were paler yellow in color compared with cells containing 
plasmid pAC-BETA-At (Figure 7B). The lower level of 
\(\beta\)-carotene accumulation suggests that the 
LCYB gene from szl1 encodes a 
still functional but less active 
\(\beta\)-cyclase relative to the wild-type 
gene.

The addition of the Arabidopsis LCYE gene to cells containing 
pAC-BETA-At resulted in the production of both 
\(\alpha\)-and \(\beta\)-carotene, and the molar ratio of 
\(\alpha\)-carotene to \(\beta\)-carotene was 2:3 
(Figure 7C), which mirrors the accumulation of more \(\beta\)-branch carotenoids than \(\alpha\)-branch carotenoids found in both the wild type 
and npq1 Arabidopsis (Table 1). When the same LCYE gene was 
introduced into the E. coli strain containing pAC-BETA-At-szl1, the 
composition of the carotenoid pigments was altered, and the 
molar ratio of \(\alpha\)-carotene to \(\beta\)-carotene increased to 4:1 (Figure 7D). This 
change reflects the significant decrease of \(\beta\)-branch carotenoids and increase of \(\alpha\)-branch carotenoids observed in both the szl1 
single mutant and szl1 npq1 double mutant.

Effect of Pigment Alteration on the Composition of PSII and Photosystem I

To investigate how accumulation of lutein in the suppressor szl1 
npq1 suppresses the npq1 mutation, we first tested whether the 
pigment alteration in szl1 npq1 might indirectly suppress the low 
NPQ phenotype of npq1 by affecting the composition of the PSII antenna. Immunoblot analysis showed that the Lhcb4 (CP29) 
and Lhcb5 (CP26) protein levels were reduced in both szl1 npq1 
and szl1 compared with wild type and npq1, whereas the 
amounts of other LHC proteins were unchanged (Figure 8A). 
There were no differences in the levels of the PsbS protein and 
the PSII reaction center protein, D1 (Figure 8A). Further quanti-
tative analysis showed that Lhcb4 and Lhcb5 protein levels were 
decreased to 50% of the wild-type level, while Lhcb6, Lhca1, and 
PsaF protein levels were slightly reduced in both szl1 and szl1 
npq1 (Figures 8B and 8C). Because the pigment alteration in the 
suppressor affected only the levels of the Lhcb4 and Lhcb5 
proteins, and it was reported that a relatively minor effect on qE 
was observed in antisense or knockout plants that lack detect-
able Lhcb4 or Lhcb5 (Andersson et al., 2001; Betterle et al., 
2009), we then hypothesized that the accumulation of lutein in 
szl1 directly suppresses the npq1 mutation by replacing the role 
of zeaxanthin in qE at the molecular level.

TA Spectroscopy of szl1 npq1 Thylakoids

To determine if the spectroscopic signature of CT quenching, 
the formation of a carotenoid radical cation, was restored in szl1
npq1, we measured ultrafast time-resolved TA spectra and kinetics in isolated thylakoids in the presence or absence of light-induced qE. Figure 9A shows the near infrared (NIR) TA spectra of thylakoid membranes of the szl1 npq1 double mutant at 15 ps delay between pump and probe laser pulses. The TA signal in the qE state was measured every 20 nm from 880 to 1040 nm under an actinic light (\(~600\ \mu\text{mol photons m}^{-2} \text{s}^{-1}\) (red line). The spectrum without qE (black line) was measured after the sample was darkened for 10 min to relax the light-induced \(\Delta p\text{H}\). Because chlorophylls and carotenoids have no ground-state absorbance or emission in this NIR region, we selectively measured excited-state absorbance of transient species. The spectrum without qE exhibits a gradual increase with wavelength mainly due to chlorophyll excited-state absorbance (Polivka et al., 2002; Holt et al., 2005). The qE spectrum revealed an enhanced absorbance in the wavelength region from 880 to 1040 nm.

**Figure 7.** HPLC Analysis of Products Formed from Lycopene in *E. coli* Expressing the Arabidopsis Wild-Type \(\beta\)-cyclase and Wild-Type or Mutant \(\beta\)-Cyclase.

Carotenoid pigment composition was examined in cultures of *E. coli* containing the plasmids and genes indicated above and to the left. The Arabidopsis wild-type and mutant copy of \(\beta\)-cyclase were cloned directly in the pAC-LYC plasmid to give the plasmid pAC-BETA-At and pAC-BETA-At-szl1, respectively (see Methods). Carotenoids were extracted with acetone from equal numbers of cells (based on \(A_{600}\)), and pigments were separated by HPLC and detected by absorbance at 445 nm. \(\alpha\)-car, \(\alpha\)-carotene; \(\beta\)-car, \(\beta\)-carotene.

(A) pAC-LYC plus the Arabidopsis wild-type \(\beta\)-cyclase.
(B) pAC-LYC plus the Arabidopsis \(\beta\)-cyclase.
(C) pAC-LYC plus the Arabidopsis wild-type \(\beta\)-cyclase and \(\varepsilon\)-cyclase.
(D) pAC-LYC plus the Arabidopsis mutant \(\beta\)-cyclase and wild-type \(\varepsilon\)-cyclase.

**Figure 8.** PSII and PSI Protein Levels in LL-Grown Wild Type, npq1, szl1, and szl1 npq1.

Thylakoid protein samples were loaded on the basis of total protein (5 \(\mu\text{g} \text{ lane}^{-1}\)), and immunoblot analysis was performed with polyclonal antibodies directed against each of the indicated proteins. D1 is a PSII reaction center protein; PsbS is a PSII protein that is essential for qE; Lhcb1, Lhcb2, and Lhcb3 are components of LHCII trimers; Lhcb4, Lhcb5, and Lhcb6 (also called CP29, CP26, and CP24, respectively) are monomeric, minor antenna proteins of PSII; PsaF is a PSI reaction center protein; Lhca1 is a PSI antenna protein. For comparison to mutant samples, dilutions were made from wild-type samples.

(A) Immunoblot analysis of D1, PsbS, and Lhcb protein levels in the four genotypes.
(B) Quantification of Lhcb4, Lhcb5, and Lhcb6 protein levels in szl1 and szl1 npq1.
(C) Quantification of PsaF and Lhca1 protein levels in szl1 and szl1 npq1.
880 to 960 nm. To remove the chlorophyll excited-state absorption and to emphasize the difference, we subtracted the black trace under darkness from the red one under actinic light, resulting in the blue reconstructed spectrum (Figure 9A). The broad reconstructed spectrum provided evidence of CT quenching in szl1 npq1, but the spectrum was maximized at ~920 nm, which is substantially blue-shifted relative to the spectrum of a \( \beta \)-carotene cation radical (blue dotted line) or a zeaxanthin radical cation with a broad spectrum centered at ~980 to 1000 nm (Holt et al., 2005; Amarie et al., 2007). Instead, the spectrum observed in szl1 npq1 was consistent with the reported absorption spectrum of a lutein radical cation, which was centered at ~920 to 950 nm depending on the solvent used (Mortensen and Skibsted, 1997; Edge et al., 1998; Galinato et al., 2007).

Individual TA kinetic traces of the szl1 npq1 thylakoids at 950 and 1000 nm are shown in Figures 9B and 9C, respectively. The qE trace under actinic light at 950 nm (red line in Figure 9B) revealed distinctly slower kinetics than the trace without qE (black line in Figure 9B), whereas both the traces at 1000 nm were similar (Figure 9C). Thus, unlike the wild type, which has a difference in kinetics at 1000 nm but not 950 nm (Holt et al., 2005), the different kinetics in the szl1 npq1 thylakoids showed the characteristic pattern of a carotenoid radical cation at 950 nm (blue line in Figure 9B) but not at 1000 nm (blue line in Figure 9C).

**TA Spectroscopy of Isolated LHC Complexes**

To determine if the NIR absorption changes detected in szl1 npq1 thylakoids can be associated with LHC complexes, as in the case of zeaxanthin radical cation formation (Ahn et al., 2008; Avenson et al., 2008), we investigated the effect of substituting \( \beta \)-xanthophylls by lutein, as observed in the szl1 npq1 mutant, in reconstituted LHC complexes. CP24, CP26, and CP29 complexes were refolded in vitro from apoproteins expressed in *E. coli* and chlorophylls \( \alpha \) and \( \beta \) plus lutein only or a total carotenoid mix. The resulting complexes were characterized (Table 2) by having lutein in both carotenoid binding sites L1 and L2 (LL complexes), whereas in the control complexes, the L2 binding site could be occupied by lutein, violaxanthin, or neoxanthin (LNV complexes) (Pagano et al., 1998; Ruban et al., 1999; Ballottari et al., 2009), except for CP24, which cannot bind neoxanthin either in vivo or in vitro (Pagano et al., 1998). LHCII trimers were purified from the wild type or the chy1 chy2 lut5 mutant, which has lutein as the only xanthophyll (Fiore et al., 2006). Both LHCII samples have lutein in L1 and L2 xanthophyll binding sites; however, LHCII trimers from chy1 chy2 lut5 have lutein instead of violaxanthin bound in the external V1 binding sites, while site N1 remained empty (Liu et al., 2004; Mozzo et al., 2008). All these complexes were analyzed by NIR TA spectroscopy. LHCII-LL trimers did not show any TA difference compared with LHCII-LNV complexes; the TA kinetics in the lutein radical cation absorption region were characterized only by excited states absorption decay (see Supplemental Figure 1 online). This is similar to the case of CP26-LL, which has been previously analyzed (Avenson et al., 2009). As shown in Figure 10, CP24 and CP29 kinetics at 920 nm were instead characterized by the presence of an additional rise component only in the CP24-LL and CP29-LL complexes. The difference kinetics reported in Figures 10C and 10D clearly show the formation of a lutein radical cation in CP24 and CP29 binding lutein as the only xanthophyll, with a rise time of 5 ps and main decay of ~50 to 60 ps. Lutein radical cation formation in CP24 and CP29 complexes was confirmed by the reconstructed NIR TA spectra reported in Figures 10A and 10B, showing a peak at 920 nm, consistent with result obtained for szl1 npq1 thylakoids upon qE induction (Figure 9).

**Light-Induced Absorbance Changes**

A slower light-induced absorbance change, \( \Delta A_{535} \), depends on both zeaxanthin and the \( \Delta p \text{H} \) and is closely associated with the
induction and relaxation of qE (Ruban et al., 1993; Bilger and Björkman, 1994; Li et al., 2004). Figure 11 shows a typical qE spectrum for wild-type Arabidopsis, taken as the difference in absorbance changes between 10 and 60 s after illumination to eliminate contributions from the electrochromic shift (see Bilger and Björkman, 1994). The peak absorbance change occurred at ~530 to 535 nm. By contrast, peaks observed in the szl1, szl1 npq1, and npq1 mutants were blue-shifted by ~5 to 10 nm to ~525 to 530 nm (Figure 11). In each strain, the qE absorbance signals decayed in the dark with half times of 5 to 10 s, suggesting that all signals reflected similar processes.

**DISCUSSION**

Isolation of the suppressor mutant szl1 npq1, which contains a point mutation in the LCYB gene and thus results in a dramatic change in pigment profile and qE capacity, has provided insights into carotenoid biosynthesis and the role of lutein in qE.

**Regulation of the Carotenoid Biosynthetic Pathway at the Branch Point**

The plant carotenoid biosynthetic pathway branches at the cyclization reactions to produce carotenoids with either two β-rings or one β- and one α-ring. In brief, lycopene is either cyclized twice by LCYB to produce β-carotene and derivatives thereof or once each by LCYB and LCYE to produce α-carotene that is the precursor to lutein (Figure 1). It has been hypothesized that partition of flux into the β- and α-branched pathways is controlled by the relative activities of LCYB and LCYE (Cunningham et al., 1996; Pecker et al., 1996; Pogson et al., 1996). Indeed, transgenic manipulations to increase or decrease LCYE expression in Arabidopsis resulted in lutein levels ranging from 5 to 180% of the wild type (Pogson and Rissler, 2000). Similar results were reported recently with LCYE transgenic potato (Solanum tuberosum) and Brassica plants (Direito et al., 2006; Yu et al., 2007). It has also been shown that natural genetic variation at the LCYE locus in maize (Zea mays) changes the ratio of α- versus β-branches of the carotenoid pathway (Harjes et al., 2008).

Here, by affecting LCYB instead of LCYE, we provide complementary experimental evidence to support the above hypothesis. The suppressor szl1 npq1 has very low levels of violaxanthin, antheraxanthin, and neoxanthin, but it accumulates nearly twice as much lutein and approximately eight times more α-carotene compared with the wild type (Table 1).

| Pigment Composition of the CP24 and CP29 Recombinant Proteins |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Chl a | Chl b | Chl a/b | Car/Car | Car, No. | N | V | L | Z |
| CP24-LL | 6.07 ± 0.05 | 3.93 ± 0.05 | 1.54 ± 0.03 | 4.76 ± 0.09 | 2.08 ± 0.04 | ND | ND | 2.08 ± 0.03 | ND |
| CP24-LV | 5.99 ± 0.10 | 1.99 ± 0.01 | 1.50 ± 0.06 | 4.74 ± 0.10 | 2.11 ± 0.04 | ND | 0.90 ± 0.08 | 1.21 ± 0.06 | ND |
| CP29-LL | 6.01 ± 0.01 | 4.01 ± 0.10 | 3.02 ± 0.02 | 4.20 ± 0.10 | 1.90 ± 0.04 | ND | ND | 1.90 ± 0.02 | ND |
| CP29-LNV | 5.58 ± 0.06 | 2.42 ± 0.06 | 2.31 ± 0.08 | 3.69 ± 0.17 | 2.17 ± 0.11 | 0.37 ± 0.05 | 0.61 ± 0.04 | 1.19 ± 0.01 | ND |

Table 2. Pigment Composition of the CP24 and CP29 Recombinant Proteins

Induction of the carotenoid biosynthesis pathway branches at the cyclization reactions to produce carotenoids with either two β-rings or one β- and one α-ring. In brief, lycopene is either cyclized twice by LCYB to produce β-carotene and derivatives thereof or once each by LCYB and LCYE to produce α-carotene that is the precursor to lutein (Figure 1). It has been hypothesized that partition of flux into the β- and α-branched pathways is controlled by the relative activities of LCYB and LCYE (Cunningham et al., 1996; Pecker et al., 1996; Pogson et al., 1996). Indeed, transgenic manipulations to increase or decrease LCYE expression in Arabidopsis resulted in lutein levels ranging from 5 to 180% of the wild type (Pogson and Rissler, 2000). Similar results were reported recently with LCYE transgenic potato (Solanum tuberosum) and Brassica plants (Direito et al., 2006; Yu et al., 2007). It has also been shown that natural genetic variation at the LCYE locus in maize (Zea mays) changes the ratio of α- versus β-branches of the carotenoid pathway (Harjes et al., 2008).

Here, by affecting LCYB instead of LCYE, we provide complementary experimental evidence to support the above hypothesis. The suppressor szl1 npq1 has very low levels of violaxanthin, antheraxanthin, and neoxanthin, but it accumulates nearly twice as much lutein and approximately eight times more α-carotene compared with the wild type (Table 1).

Molecular genetic analysis demonstrated that the szl1 mutation affects the structural gene encoding LCYB by changing a Gly residue in a highly conserved predicted transmembrane helix to a Glu (Figure 6A). It is clear that szl1 is not a complete loss-of-function mutation affecting LCYB activity, as evidenced by the decreased but detectable levels of β-carotene when the szl1 allele of LCYB was expressed in a lycopene-accumulating strain of *E. coli* (Figure 7B). In *E. coli* cells that express both the szl1 allele of LCYB and the wild-type LCYE, the molar ratio of α-carotene to β-carotene increased to 4:1 (Figure 7D), which is in agreement with the high levels of α-branch carotenoids and low levels of β-branch carotenoids accumulated in the suppressor szl1 npq1.

**LCYB Is the Principal Lycopene β-Cyclase in Arabidopsis**

The enzyme encoded by the *Arabidopsis* LCYB gene belongs to the CrtL protein family, which includes the β- and κ-cyclases in some cyanobacteria and plants (Cunningham et al., 1994, 1996; Stickforth et al., 2003). Many bacteria, including the green sulfur bacterium *Chlorobium tepidum* and cyanobacteria, have an LCYB that is different from that of plants. The first member of this new family, CruA, was recently identified from *C. tepidum* in a complementation assay with a lycopene-accumulating strain of *E. coli* (Maresca et al., 2007). Two homologs of CruA, denoted CruA and CruP, were found and characterized in the cyanobacterium *Synechococcus* sp PCC 7002. A genome search revealed that Arabidopsis contains a CruP homolog, which is encoded by At2g32640 (Maresca et al., 2007). Whether the CruP homolog also has lycopene β-cyclase activity in *Arabidopsis* is not yet known, but the dramatic change of the pigment profile observed in the suppressor szl1 npq1 suggests that LCYB is the major lycopene β-cyclase in *Arabidopsis*.

**Does LCYB Have Neoxanthin Synthase Activity in Arabidopsis?**

Conversion of violaxanthin to neoxanthin is catalyzed by the enzyme neoxanthin synthase (NSY) (Figure 1). Genes encoding NSY activity have not yet been identified conclusively in *Arabidopsis*. Two homologous NSY genes have been cloned from either tomato (*Solanum lycopersicum*) or potato based on their ability to convert all-trans-violaxanthin to all-trans-neoxanthin in vitro or in transient expression systems (Al-Babili et al., 2000; Bouvier et al., 2000). Polypeptides encoded by NSY genes are homologous to LCYB, and the tomato NSY gene product has β-cyclase activity and accounts for the fruit-specific high
Based on these findings, it has been proposed that NSY has a dual function in converting both violaxanthin to neoxanthin and lycopene to $\beta$-carotene (Hirschberg, 2001). Because *Arabidopsis* LCYB is encoded by a single-copy gene and shows significant identity to tomato and potato NSYs, it is possible that *Arabidopsis* LCYB is also a bifunctional enzyme that has both LCYB and NSY activity. Isolation of the $szl1 npq1$ and $szl1$ mutants provides an opportunity to test this hypothesis. If LCYB does function as NSY, the $szl1$ mutation that impairs LCYB activity might also decrease NSY activity; thus, we would expect a preferential reduction of neoxanthin content compared with that of violaxanthin in the $szl1 npq1$ or $szl1$ mutant. On the other hand, if LCYB does not have NSY activity, then we would expect to observe a proportional reduction of neoxanthin and violaxanthin in the $szl1 npq1$ and $szl1$ was the same as that of $npq1$ (Table 1), indicating that LCYB does not have NSY activity. It is more likely that in *Arabidopsis* a novel type of NSY is responsible for the conversion of violaxanthin to neoxanthin, and the recently identified ABA4 protein would be a very good candidate (North et al., 2007).

**A Direct Role of Lutein in qE**

How does the increased lutein content caused by the $szl1$ mutation restore qE in the $npq1$ mutant that lacks zeaxanthin? Based on the very similar chemical structures of lutein and zeaxanthin, an analogy has previously been drawn with zeaxanthin when speculating about the functions of lutein in qE, and a possible role of lutein in qE has been proposed. Indeed, an effect of lutein on qE has been demonstrated in several mutants and

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**Figure 10. TA Spectroscopy of LHC Complexes.**

(A) Difference NIR-TA spectrum (from 880 to 1040 nm) between CP24 complexes binding lutein in both L1 and L2 sites (CP24-LL) and CP24 with violaxanthin in site L2 (CP24-LV). Each point represents the difference between the $\Delta A$ value obtained at 20 ps delay after pump pulse in CP24-LL and the corresponding value in CP24-LV. Data are presented as the means ± SE ($n = 5$).

(B) Difference NIR-TA spectrum (from 880 to 1040 nm) between CP29 complexes binding lutein in both L1 and L2 sites (CP29-LL) and CP29 with violaxanthin or neoxanthin in site L2 (CP29-LNV). Each point represents the difference between the $\Delta A$ value obtained at 20 ps delay after pump pulse in CP29-LL and the corresponding value in CP29-LNV. Data are presented as the means ± SE ($n = 5$).

(C) TA kinetics probed at 920 nm of CP24-LL (red trace) and CP24-LV (black trace). Difference kinetic trace is reported in blue with rise and decay times indicated.

(D) TA kinetics probed at 920 nm of CP29-LL (red trace) and CP29-LNV (black trace). Difference kinetic trace is reported in blue with rise and decay times indicated.

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**Figure 11. Light-Induced Spectral Absorbance Changes in Leaves.**

Intact leaves of wild type (squares), $szl1$ (circles), $szl1 npq1$ (triangles), and $npq1$ (inverted triangles) were illuminated with ~1150 $\mu$mol photons m$^{-2}$ s$^{-1}$ red light for 10 min to induce qE, and absorbance changes (from 460 to 563 nm) were measured during a 1-min dark interval. The qE spectrum was calculated as the difference in absorbance between 10 and 60 s after illumination to eliminate contributions from the electrochromic shift. The dashed lines indicate the peak positions of ~530 to 535 nm in the wild type and ~525 to 530 nm in the $szl1$, $szl1 npq1$, and $npq1$ mutants.
transgenic plants with altered lutein levels (Niyogi et al., 1997, 2001; Pogson et al., 1998; Pogson and Rissler, 2000; Lokstein et al., 2002), but whether and how lutein plays a direct or an indirect role in qE has been unclear.

Previously characterized mutants that accumulate extra lutein have only been obtained together with either the total depletion of β-carotophylls (Dall’Osto et al., 2007), which strongly affects antenna protein composition, or the normal accumulation of zeaxanthin (Pogson and Rissler, 2000), thus obscuring the specific lutein-associated qE phenotype. The szl1 npq1 mutant accumulates nearly double the wild-type amount of lutein, has lower total carotenoids (increased α-carotene but lower β-carotene), retains low levels of violaxanthin, antheraxanthin, and neoxanthin, and lacks zeaxanthin (Figure 4), but the total carotenoid (and chlorophyll) content does not change (Table 1).

Thus, the increase in α-carotenoids occurs at the expense of β-carotenoids, and the extra lutein and α-carotene are likely bound at sites that are normally occupied by β-carotenoids.

In principle, the enhancement of qE in the absence of zeaxanthin could result from an indirect effect of the substitution of lutein for β-carotophylls on the composition and/or structure of the PSII antenna (Lokstein et al., 2002) or by a direct role of lutein in qE (either by CT quenching or an S1 energy transfer mechanism). We did detect a 50% decrease in the levels of Lhcb4 and Lhcb5 in szl1 npq1 (Figure 8), but it seems unlikely that this could explain the increased level of qE because only a limited decrease of the qE amplitude was observed when a complete absence of Lhcb4 or Lhcb5 was induced in a wild-type background by antisense or knockout (Andersson et al., 2001; Betterle et al., 2009).

Therefore, we considered the possibility of a direct role of lutein in enhancing the qE mechanism. At present, it is not possible to test directly for the occurrence of the S1 quenching mechanism in isolated thylakoids (Ruban et al., 2007), but we were able to investigate formation of a carotenoid radical cation, a key molecular species in the CT quenching mechanism (Holt et al., 2005) by TA spectroscopy.

We hypothesized that lutein might be able to take the place of zeaxanthin in directly quenching "Chl" during qE through the CT mechanism via the formation of a lutein radical cation instead of a zeaxanthin radical cation, thus restoring qE to a higher level in the szl1 npq1 suppressor. Indeed, NIR TA traces revealed a carotenoid radical cation in szl1 npq1 by probing at 950 nm (Figure 9B) but not at 1000 nm (Figure 9C), in contrast with wild-type thylakoids (Holt et al., 2005). The TA spectrum of the suppressor thylakoids showed a maximum at ~920 nm (Figure 9A), which is blue-shifted relative to the spectrum of a β-carotene or zeaxanthin radical cation and is in agreement with the reported spectrum of a lutein radical cation (Mortensen and Skibsted, 1997; Edge et al., 1998; Galinato et al., 2007). Similarly, the ΔA255 leaf absorbance change associated with zeaxanthin-depentent qE was missing in npq1 (Niyogi et al., 1998) and replaced with a blue-shifted absorbance change in szl1 npq1 (Figure 11A). Thus, the results of the TA spectroscopy (Figure 9) and leaf absorbance (Figure 11) experiments strongly suggest that lutein can substitute for zeaxanthin in qE.

We suspect that the high amount of lutein accumulated in the suppressor probably magnifies a TA signal that is normally present at a relatively low level and is obscured by the stronger zeaxanthin radical cation signal in the wild type. Similarly, we propose that the residual qE in the npq1 mutant is lutein dependent, but the lutein radical cation signal is likely below the detection limit of the TA experiment. We are currently trying to improve the sensitivity and signal-to-noise ratio of our TA setup to test this idea. A basal level of CT quenching by lutein (that could be enhanced by PsbS overexpression) could explain previous observations of zeaxanthin-independent qE, which indicated that zeaxanthin (and antheraxanthin) is not required for qE (Crouchman et al., 2006). Indeed, a blue-shifted absorbance change very similar to that observed here (Figure 11A) was detected in npq1 leaves (Johnson et al., 2009) and in wild-type leaves treated with DTT to phenocopy the npq1 mutation by inhibition of VDE activity (Crouchman et al., 2006).

The spectroscopic signature of CT quenching has been detected recently in recombinant CP29 (Lhcb4), CP26 (Lhcb5), and CP24 (Lhcb6) (Ahn et al., 2008; Avenson et al., 2008, 2009). Each of these pigment-protein complexes can exhibit zeaxanthin radical cation formation through CT from zeaxanthin to a strongly coupled chlorophyll dimer. A lutein radical cation was so far detected only in CP26, but only when zeaxanthin is also present (Avenson et al., 2009). Because zeaxanthin is lacking in szl1 npq1, it is unlikely that lutein radical cation formation of the type observed in CP26 in vitro (Avenson et al., 2009) could explain the TA signal detected in szl1 npq1 thylakoids (Figure 9). Instead, we detected the formation of lutein radical cations in CP24 and CP29 complexes reconstituted with only lutein (Figure 10). Our results in vitro and in vivo thus highlight the possibility of lutein to substitute for zeaxanthin as a quencher of excess excitation energy through formation of radical cations. The fraction of lutein-only CP24 and CP29 complexes undergoing CT quenching in vitro, however, is rather low (~1%), as in the case of zeaxanthin binding complexes (Avenson et al., 2008), indicating the fundamental role of other factors, such as PsbS and ΔpH, for the induction of qE in vivo. In wild-type plants, CP24, CP26, and CP29 bind lutein and violaxanthin/zeaxanthin in carotenoid binding sites named L1 and L2, respectively. An increase in the lutein content of these complexes, substituting for violaxanthin/zeaxanthin in the L2 site as in the case of the szl1 npq1 mutant, was previously reported to induce a decrease of fluorescence quantum yield (Formaggio et al., 2001). Although we favor the hypothesis that the extra lutein bound in the L2 site of LHC complexes in the szl1 npq1 mutant is directly involved in CT quenching, we cannot at this point exclude the possibility that it acts as an allosteric factor, similar to zeaxanthin in CP26 (Avenson et al., 2009), inducing lutein radical cation formation in site L1. It is worth noting that carotenoid radical cation reactions have distinct patterns and dependence on xanthophyll composition in each Lhcb protein, thus contributing to explain the existence of multiple, conserved gene products in the plant antenna systems.

METHODS

Plant Material and Growth Conditions

All Arabidopsis thaliana plants were of the ecotype Columbia-0. The npq1 mutant is affected in VDE and lacks zeaxanthin under high light (Niyogi et al., 1997). The plant was grown for 2 weeks under continuous light of 200 μmol quanta m−2 s−1 at 22°C and 65% relative humidity.
et al., 1998). The szl1 npq1 double mutant was crossed with the wild type, and the szl1 single mutant was isolated from F2 progeny. Plants were grown on Sunshine Mix 4 potting mix (Sun Gro Horticulture) in controlled conditions of 10 h light, 22°C/14 h dark, 23°C, with a light intensity of 150 μmol photons m⁻² s⁻¹. For physiological studies, plants at the age of 5 to 6 weeks (prior to bolting) were used. For mutant screening and cosegregation analysis, plants were grown for 2 weeks on minimal plant nutrient agar medium (Haughn and Somerville, 1986) at 80 μmol photons m⁻² s⁻¹ (continuous light) at 23°C and then transferred to soil.

Isolation of Suppressors of the npq1 Mutant

M2 seedlings were derived from mutagenesis of npq1 seeds with 0.3% (v/v) ethyl methanesulfonate. Suppressors of npq1 were identified by chlorophyll fluorescence video imaging (Niyogi et al., 1998) for the NPQ phenotype and by HPLC for pigment composition after exposure to high light.

Chlorophyll Fluorescence Measurement

Chlorophyll fluorescence was measured at room temperature from detached, fully expanded rosette leaves using an FMS2 fluorometer (Hansatech). After an overnight dark period, leaves were exposed to actinic light (1250 μmol photons m⁻² s⁻¹ from an FMS2 fluorometer) for 5 min followed by 5 min of darkness. The maximum fluorescence levels after dark adaptation (Fₘₙ) and in the light-adapted condition (Fₐₙ) were recorded after applying a saturating pulse of light. NPQ was calculated as [Fₘₙ − Fₐₙ]Fₐₙ⁻¹.

Pigment Analysis

HPLC analysis of carotenoids and chlorophylls was done as previously described (Holt et al., 2005; Avenson et al., 2008). Briefly, the repetition rate was 250 kHz, and the pump pulses were tuned to ~650 nm (i.e., the chlorophyll b Qy transition). The maximum pump energy and full width half maximum of the pulse autocorrelation trace were ~48 nJ/pulse and ~0 fs, respectively. We chose 650 nm as our excitation wavelength because the output power of our optical parametric amplifier was higher than that at 680 nm, yielding higher signal-to-noise ratios. White light continuum probe pulses were generated in a 1-mm quartz plate. The cross-correlation function of the pump and probe overlap was ~5 fs. The diameters of the pump and probe beams at the sample holder were estimated to be 146 and 83 μm, respectively. The mutual polarizations of the pump and probe beams were set to the magic angle (54.7°). The time resolution of the TA measurements was 5 ps/point (~60 to ~10 ps), 0.5 ps/point (~10 to 60 ps), and 5 ps/point (~65 to 600 ps). A monochromator (Spectra Pro 300; Acton Research) with a spectral resolution of 2.7 nm, an InGaAs photodiode (DET410; Thorlabs), and a chopper-associated lock-in amplifier (SR830; Stanford Research Systems) were used to monitor transmission. The path length of the cuvette used for isolated thylakoids was 2 mm and was continuously translated during experiments to avoid sample degradation. NIR TA kinetic profiles were obtained during steady state actinic illumination and 10 min following a light-to-dark transition by photooxidation of the thylakoids at 650 nm and probing at 880 to 1040 nm. We obtained transient traces (~10 to 20 ps) at every 20 nm (880 to 1040 nm), averaged five times, and plotted the TA spectrum at 15 ps with standard error.

NIR TA Spectroscopy

Thylakoids for the NIR TA measurements were prepared as previously described (Gilmore et al., 1998). The NIR TA laser system has been previously described (Holt et al., 2005; Avenson et al., 2008). Briefly, the repetition rate was 250 kHz, and the pump pulses were tuned to ~650 nm (i.e., the chlorophyll b Qy transition). The maximum pump energy and full width half maximum of the pulse autocorrelation trace were ~48 nJ/pulse and ~650 nm, respectively. We chose 650 nm as our excitation wavelength because the output power of our optical parametric amplifier was higher than that at 680 nm, yielding higher signal-to-noise ratios. White light continuum probe pulses were generated in a 1-mm quartz plate. The cross-correlation function of the pump and probe overlap was ~5 fs. The diameters of the pump and probe beams at the sample holder were estimated to be 146 and 83 μm, respectively. The mutual polarizations of the pump and probe beams were set to the magic angle (54.7°). The time resolution of the TA measurements was 5 ps/point (~60 to ~10 ps), 0.5 ps/point (~10 to 60 ps), and 5 ps/point (~65 to 600 ps). A monochromator (Spectra Pro 300; Acton Research) with a spectral resolution of 2.7 nm, an InGaAs photodiode (DET410; Thorlabs), and a chopper-associated lock-in amplifier (SR830; Stanford Research Systems) were used to monitor transmission. The path length of the cuvette used for isolated thylakoids was 2 mm and was continuously translated during experiments to avoid sample degradation. NIR TA kinetic profiles were obtained during steady state actinic illumination and 10 min following a light-to-dark transition by photooxidation of the thylakoids at 650 nm and probing at 880 to 1040 nm. We obtained transient traces (~10 to 20 ps) at every 20 nm (880 to 1040 nm), averaged five times, and plotted the TA spectrum at 15 ps with standard error.

Leaf Absorbance Measurements

Absorbance spectra were measured using the diffused optics flash spectrophotometer (Kramer and Sacksteder, 1998) and the appropriate narrow band-pass interference filters (Omegam Optics). Detached leaves were gently clipped into the spectrophotometer and illuminated with ~1150 μmol photons m⁻² s⁻¹ red light, supplied by a high-flux, red light emitting diode (Luxeon LXHL-PD09; Phillips Lumileds) for 10 min, and leaf
absorbance changes (from 460 to 563 nm) were measured during a 1-min dark interval. Within this interval, spectral contributions from the xanthophyll cycle are negligible, since the conversion of zeaxanthin to violaxanthin is relatively slow. Typically, spectral changes attributable to the electrochromic shift (which peak at 518 to 520 nm) are most prominent during the first second of the dark interval, whereas beyond this window qE spectral contributions (which peak at ~535 nm) dominate. The qE absorbance spectra were partially corrected for the contributions of the residual fast electrochromic shift by subtraction of the 10-ms spectrum, which contains only electrochromic shift changes.

In parallel experiments, chlorophyll fluorescence was measured in a modified laboratory-built nonfocusing optics spectrophotometer (NoFOSpec) (Sacksteder et al., 2001), which used laboratory-built, small aperture compound parabolic concentrators (CPCs; both solid, clear-cast acrylic and hollow aluminum) designed specifically for work with intact Arabidopsis plants. Leaves from intact plants were gently clamped between the CPCs with the upper surface of the leaf against the solid acrylic CPC and the lower surface against the hollow CPC, through which water saturated air was pumped. The modified design uses two separate detectors to collect fluorescence and absorbance data. Surface fluorescence from the leaf returns through the acrylic CPC and is reflected, using a hot mirror (NT43-453; Edmund Optics), through a far-red cut-off filter (RG-710; Schott) onto the fluorescence detector. High-flux, red LEDs were used with collimating optics (Polymer Optics) for actinic and saturating light sources, and measuring pulses were supplied by a green LED (NSP5G005; Nichia). Maximum fluorescence values obtained from saturating flashes (1 s, >16,000 μmol photons m⁻² s⁻¹ red light) were measured at steady state (Fm′) (with actinic illumination of ~1150 μmol photons m⁻² s⁻¹ red light for 10 min) and during a 10-min dark recovery (Fm''). qE was calculated as (Fm'' - Fm'')/Fm''.

Isolation and Reconstitution of LHII Complexes
Native LHII trimers were isolated from the wild type and chy1 chy2 lut5 mutant (Fiore et al., 2006) as previously described (Caffarri et al., 2001). Recombinant apoproteins CP24 and CP29 were refolded in vitro in the presence of chlorophylls a and b and total carotenoids, or lutein as the only xanthophyll, as described previously (Pagano et al., 1998; Gastaldei et al., 2003).

Accession Number
Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under accession number U50739 (LCYB).

Supplemental Data
The following material is available in the online version of this article.

Supplemental Figure 1. Transient Absorption Spectroscopy of LHII Trimers.

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REFERENCES


