Disruption of Thylakoid-associated Kinase 1 Leads to Alteration of Light Harvesting in Arabidopsis*

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The current kinase activation model states that light absorption by PSII and PSI leads to energy transduction along the thylakoid membrane (Fig. 1A). Electron flow from PSII to PSI results in the reduction of the plastoquinone to plastoquinol. The Rieske Fe-S center, cytochrome (cyt) f, and plastocyanin are also reduced in the membrane (2). Binding of the plastoquinol (Qr; Fig. 1) to the cytochrome b6f complex (cyt b6f) quinol oxidation site (Qox) activates a kinase (Ref. 2 and 11; Fig. 1B). The model predicts that the kinase then phosphorylates LHCP (creating LHCPP), resulting in LHCPP migration from PSI to PSII (Fig. 1C). The association of LHCP with PSI requires the PSI-H subunit (12). There are several theories attempting to explain how LHCPP moves within the thylakoid membrane to associate with the less stimulated PS. Some but not all evidence points to a lateral migration of LHCP in the thylakoid membrane from stacked to stroma exposed membranes (2, 13, 14). Other investigators have proposed a limited unstacking of the appressed grana membranes to allow for movement of the LHCPP to PSI (14, 15). The binding of the plastoquinol at the Qox site occurs in the luminal side of the thylakoid membrane, but since phosphorylation occurs on the stromal face of the thylakoid, it is not clear how the signal is conveyed; the model suggests that the signal is transduced through the subunits of the cyt b6f complex. The simplest scenario would be for the kinase to interact with the activated cyt b6f complex, become activated itself, and then phosphorylate LHCP. In this case, the kinase would be in physical contact with these thylakoid components. The activated state of the kinase persists as long as the Qox is occupied with Qr (2). Withdrawal of a single electron from Qr, due to PSI activity, leads to reoxidation of Qr and deactivation of the kinase. Oxidation of the reduced quinol pool is proposed to trigger the activation of a membrane-bound phosphatase (16), resulting in the dephosphorylation of the LHCP. This results in the reassociation of LHCP with PSII. Recently, a luminal cyclophilin-regulated phosphatase that is involved in dephosphorylation and protein turnover during repair of PSII has been identified (17). The role of this phosphatase during photosynthesis and state transitions is still unknown.

We have previously identified and purified a family of three kinases, thylakoid-associated kinases (TAKs), responsible for the in vitro phosphorylation of LHCP in Arabidopsis (10). The in vitro phosphorylation of LHCP was redox-dependent, since DTT was required for this reaction. The role and mechanism of interaction of the TAKs with themselves and with other thylakoid membrane components were unknown. TAKs contain a glycine serine motif (GS motif) that is a key regulatory sub-strate in TGF-β receptor signaling (18).

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The abbreviations used are: PSI, PSII, photosystem I and II, respectively; LHCP, light-harvesting complex protein; cyt, cytochrome; Qr, plastoquinol; µE, microeinsteins; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; IP, immunoprecipitation; PAGE, polyacrylamide gel electrophoresis.

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We show here that TAKs interact with both cyt \( f \) and LHCP and that specific stimulation of PSI causes the accumulation of LHCP with PSI, as predicted by the state transition model. A reduction in the amount of TAK1 protein leads to a loss of LHCP with PSI, as predicted by the state transition model.

**MATERIALS AND METHODS**

**Arabidopsis Growth**—Arabidopsis thaliana Columbia and TAK1 antisense mutants were grown at 25 °C in growth chambers with constant humidity under 150 \( \mu \text{m} \text{m}^{-2} \text{s}^{-1} \) light in an 18-h light/6-h dark cycle unless stated otherwise. When grown at higher light levels, the temperature was kept at 25−26 °C.

**Generation of TAK1 Antisense Mutants and Recombinant TAK1 Expression in Escherichia coli**—Full-length TAK1 was PCR-amplified using genomic A. thaliana Columbia DNA as a template with the primers 5′-CGG GAA TTC AGA AGA AAA AAG-3′ and 5′-GGA GAT TTA AGC GTA TCC GGA GGT GGA GTT CTT CTT ATT CCA-3′, introducing a NcoI site at the 5′-end and a BglII site with a hemagglutinin tag at the 3′-end. This PCR product was digested, gel-purified, and ligated into calf alkaline phosphatase-treated pet 16B vector (Novagen). This construct was used to transform DH10b to screen for plasmids with insertions; correct orientation and sequence of the insert were determined by restriction analysis and sequencing. Several plasmids with inserts were used to transform BL21 RIL (Stratagene), and expression of TAK1 in M9 minimal medium was determined followed by purification according to Roche Molecular Biochemical hemagglutinin tag purification protocols.

**RT-PCR**—A. thaliana Columbia RNA was isolated according to van den Ven et al. (21). Using the ProSTAR™ UltraHF RT-PCR kit (with poly(T) or random primers supplied with the kit), cDNA was synthesized according to the manufacturer’s protocol. The following primers, also used with the Qiagen® One step RT-PCR kit, were used to amplify the genes of interest. Both kits gave similar results. Oligonucleotides were as follows: TAK1, 5′-CGG GAA TTC AGA AGA AAA AAG-3′ and 5′-GGA GAT TTA AGC GTA TCC GGA GGT GGA GTT CTT CTT ATT CCA-3′; TAK2, 5′-CGA GCT TGT CGG TGT-3′ and 5′-AAT TCC AGG AAA TCC GGG GTT ACC CC-3′; LHC, 5′-GGA ATT CCA TAT GGC GCC CTC TTC AAC AA AAG-3′ and 5′-GCG CTC GAG CTT TCC GGA AAC-3′; β-tubulin, as described in Ref. 22.

**Antibodies**—Arabidopsis purified TAK (10) and TAK1-hemagglutinin (see above) were used as antigen to generate antibodies in rabbits (Cocalico, Reamstown, PA). Anti-phosphothreonine antibodies were purchased from Zymed Laboratories Inc. D1 antibody was a generous gift from Dr. Charles Hauser (Duke University). αLHCP and αcyt \( f \) were described previously (23).

**Thylakoid Preparations and Fractionations**—Thylakoid preparations were as described previously (10) with the addition of phosphatase inhibitor mixture 1 and 2 (Sigma) at the start of the preparation. The phosphatase inhibitor mixtures were added to all subsequent buffers.

** SDS-PAGE and Western Procedures**—SDS-PAGE was performed according to Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24) in 12.5% gels. Samples were prepared by the method of Laemmli (24). Blots, used for detection with phosphothreonine antibody, were blocked in 6% (w/v) bovine serum albumin (Sigma) for 1.5 h. Blotted membranes were incubated with the primary antibody, followed by a secondary antibody purchase (Cocalico, Reamstown, PA). Anti-phosphothreonine antibodies were purchased from Zymed Laboratories Inc. D1 antibody was a generous gift from Dr. Charles Hauser (Duke University). αLHCP and αcyt \( f \) were described previously (23).

We show here that TAKs interact with both cyt \( f \) and LHCP and that specific stimulation of PSI causes the accumulation of LHCP with PSI, as predicted by the state transition model. A reduction in the amount of TAK1 protein leads to a loss of LHCP phosphorylation and state transitions. We also provide evidence that TAKs are involved in additional phosphorylation events in the chloroplast.
each reaction and further incubated on ice for at least 2–4 h, followed by centrifugation at 5,000 rpm in a benchtop centrifuge. The supernatant was removed by aspiration, and the pellet was resuspended in loading dye, boiled for 5 min, and loaded onto 12.5% SDS-PAGE gels. Samples used for subsequent probing with αTAK and αTAK1 were separated on 30-cm-tall gels so that the TAK bands could be separated from the major IgG band at 50 kDa.

Fluorescence Measurements— PSI1 fluorescence at all times was measured with a WALZ Chlorophyll Fluorometer PAM101 at the 1.5-kHz setting. A basal level with the detector light on was set so that further readings could be equated to that level. Fiberoptics was used to concentrate the light from different sources and detector on one single leaf. Wild type and mutant plants (16 days old) were dark-adapted for 30 min before being illuminated by 30 μE m⁻² s⁻¹ far red light (WALZ KL 1500 light source with filter) for 15 min. All light levels were measured using a Skye SKP light meter. White light, generated by a WALZ KL 1500 light source at 600 μE m⁻² s⁻¹, was used for 2 s to illuminate the leaf. This light treatment was followed by another 15-min exposure to red light (generated by a Hansatech L.S. 3 Light source box with Ultrabright red light at 660-nm wavelength), followed by a 2-s saturating white light flash. The resulting PSI1 fluorescence was recorded on a Kipp and Zonen chart recorder. PSI1 fluorescence after individual white light treatments was calculated for each treatment and expressed as a ratio. This ratio was determined for the same leaf for five successive treatments as well as for different leaves on the plants. There was no difference in ratios between different leaves of the same plant. There was also very little variation in average PSI1 ratios between different homozygous plants of the same mutant. An F-test was used to determine the significance in difference between the means to determine whether the PSI1 ratios were statistically significant (Microsoft Excel).

RESULTS

TAK Proteins Interact with LHCP and Cyt f—TAK proteins purified from Arabidopsis were detected by Coomassie stain as a doublet of 55 and 56 kDa (Fig. 2A, Coom), with the upper band preferentially phosphorylated on serine and threonine residues (10). The three TAKs are 90% identical, and TAK2 and TAK3 are shorter than TAK1 (10). The highest degree of divergence in identity between TAKs is in the amino-terminal sequences, especially in the region of the GS motif; TAK2 and TAK3 have significantly shorter GS motifs than TAK1 (10). We raised antibodies against purified Arabidopsis TAKs and to recombinant TAK1 expressed in E. coli. The two TAK antibodies recognize different epitopes as shown in Fig. 2. The TAK antibody raised to Arabidopsis protein (αTAK) interacts with both TAK bands purified from Arabidopsis (Fig. 2, αTAK), while the TAK1 antibody raised to recombinant TAK1 protein only recognizes the upper band (Fig. 2, αTAK1). Since TAK2 and TAK3 are similar in predicted molecular weight and smaller than TAK1, it is likely that they are represented by the lower band of the doublet. This is consistent with the observation that the upper TAK1 band contains more phosphoserine than the lower band, and TAK2 and TAK3 have smaller GS motifs. Thus, we suggest that the lower band contains TAK2 and -3.

TAK Expression Is Light-regulated— LHCP mRNA and protein levels in 16-day-old Arabidopsis decrease with increasing light levels (Fig. 3, αLHCP and LHCP RT-PCR). At 300 μE m⁻² s⁻¹, there is half the LHCP in the membrane compared with plants grown at 150 μE m⁻² s⁻¹. RT-PCR with gene-specific probes indicates that TAK1 but not TAK2 mRNA decrease with increasing light intensity. TAK3 mRNA increases with increasing light. The protein profiles appear to follow the TAK1 and TAK2 mRNA profiles, since TAK1 protein levels decrease significantly but TAK2/3 protein decreases only slightly with increasing light intensity (Fig. 3A, αTAK, αTAK1, and TAK1 RT-PCR). TAK1 but not TAK2/3 levels vary with the age of tissue and are slightly higher in younger plants (data not shown). This expression analysis was with 16-day-old leaves, and all subsequent analysis was with the same age plants.

TAKs Are Associated with the Cyt b6f Complex and LHCP—The binding of Q₉ to the cyt b6f complex is correlated with the activation of a kinase in the thylakoid membrane (11, 26) (Fig. 1). The current model predicts that the kinase(s) is close to or in contact with the cyt b6f complex. We therefore used the TAK antiserum to immunoprecipitate thylakoid complexes to determine what proteins are associated with TAK. The amount of antibody used for each immunoprecipitation was determined by the relative affinity of each antibody for known concentrations of purified Arabidopsis TAK protein immobilized on a nitrocellulose membrane (results not shown). From these blots, it was determined that αTAK was twice as strong as αTAK1 in recognizing the same concentration of purified TAK protein, so twice the amount of αTAK1 (compared with αTAK) was used for each IP. Thylakoid membranes from Arabidopsis rosette leaves were isolated and used for immunoprecipitations with αTAK1 and αTAK (see “Materials and Methods”). These immunoprecipitations were then separated on SDS-PAGE, transferred to nitrocellulose, and probed with antibodies raised against LHCP (αLHCP) and cyt f (αcyt f). Both of the TAK antibodies were able to co-immunoprecipitate LHCP and cyt f compared with preimmune serum and beads alone (Fig. 4A, beads and PI). αTAK co-immunoprecipitated more LHCP than
did αTAK1 (Fig. 4A, αTAK IP and αTAK1 IP), while both antibodies detected very similar amounts of cyt f (Fig. 4A, αTAK IP and αTAK1 IP). Both antibodies precipitated only a fraction of total LHCP and cyt f present in the thylakoid (Fig. 4A, total). We performed the converse experiment using αLHCP and αcyt f antibodies to immunoprecipitate thylakoid membranes to confirm that TAK co-localized. As shown in Fig. 4B (αLHCP IP and αcyt f IP), both αLHCP and αcyt f could co-immunoprecipitate TAK. Only a fraction of the total TAK present in the membrane (Fig. 4B, total) could be precipitated by these antibodies. These results show that both TAK antibodies immunoprecipitate both LHCP and cyt f, although with different affinities. TAK1 and TAK2/3 are also associated with each other, since they can be isolated as a complex from Arabidopsis leaves that is disrupted by high salt or denaturation (10). When αTAK1 is used to immunoprecipitate thylakoid proteins, both TAK1 and TAK2/3 are precipitated (data not shown). The association of TAK1 and TAK2/3 is further strengthened by the in vitro interaction between E. coli expressed TAK1 with the amino-terminal region of TAK2 but not TAK3 (results not shown). These results provide the possibility that TAKs are not only responsible for the phosphorylation of LHCP (10) but are also involved in transducing a signal from the cyt bf complex.

TAKs Are Required for State Transitions—TAKs are associated with cyt bf complex and LHCP, and we asked if they are required for state transitions. To determine the effect of depleting TAK1 in Arabidopsis, TAK1 antisense was expressed under the constitutive cauliflower mosaic virus 35S promoter by transforming A. thaliana Columbia. We were able to generate 14 TAK1 antisense mutant lines. Four TAK1 antisense mutant lines (assigned as Tk1-1, Tk1-2, Tk1-3, and Tk1-4) are shown in Fig. 5A. Some limited growth retardation and bleaching are seen. These phenotypes become more pronounced when the plants are grown at higher light levels (above 200 μE m⁻² s⁻¹; Fig. 5B). However, these mutants do survive under these light conditions, recover to nearly wild type phenotype, and bolt early to produce seed.

To see whether the antisense TAK1 construct affects TAK1 levels, we performed Western blots on thylakoid membranes of 16-day-old wild type and mutant plants grown at 150 μE m⁻² s⁻¹ and probed with αTAK, αLHCP, and α-phosphothreonine (α-Threo) antibodies. Compared with wild type, all of the mutants have lower TAK1 levels (Fig. 6A, αTAK). TAK2/3 levels are only slightly reduced. RT-PCR of TAK1 and TAK2 RNA from the most severely affected Tk1–4 mutant shows that TAK1 mRNA but not TAK2 is affected by TAK1 antisense (Fig. 6B). Since there are only slight effects on TAK2/3 proteins and none on TAK2 mRNA, we conclude that other less related protein kinases are unlikely to be affected by TAK1 antisense expression. The TAK1 antisense mutations do not alter the LHCP levels (Fig. 6A, αLHCP), but the lower TAK1 levels directly correspond to lower amounts of LHCPP (Fig. 6A, αLHCPP).

To determine how a reduction in TAK1 affects the distribution of LHCPP between PSII and PSI in plants grown from seed under 150 μE m⁻² s⁻¹ white light, we fractionated thylakoid membranes by sucrose gradient centrifugation. Equal amounts of protein from PSII and PSI preparations were run in a denaturing gel, and the Western blots were probed with LHCP, cyt f, and anti-phosphothreonine antiseraum (Fig. 6C). LHCPP was detected as a 28-kDa band (Fig. 6C) in isolated thylakoids (thy1) and in PSI and PSII particles. The illumination with 150 μE m⁻² s⁻¹ white light causes LHCPP to be almost evenly distributed between the wild type photosystems. LHCP and cyt f are also found in both PSII and PSI fractions, as expected for these
growth conditions (Fig. 6C), and D1 is detected predominately in the PSII fraction (data not shown). Tk1–4 mutant thylakoids, grown under 150 μE m⁻² s⁻¹ white light, also show an even distribution of LHCP between PSI and PSII, although levels of phosphorylation are much lower, as expected. The TK1–4 mutant is not a null, and some low level of TAK1 can be seen on longer exposures of Western blots. This may be the reason that some LHCP is still detected in the mutants. In reason that some LHCP is still detected in the mutants. In

To determine if the reduction of TAK1 levels influence state transitions, we directly measured the fluorescence emissions from PSII after light treatments that favor activation of either PSI or PSII. A single leaf from a dark-adapted plant (16 days old) was illuminated with far red light, which stimulates PSI and leads to the oxidation of plastoquinone. This leads to state transitions, we directly measured the fluorescence emissions from PSII after light treatments that favor activation of either PSI or PSII. A single leaf from a dark-adapted plant (16 days old) was illuminated with far red light, which stimulates PSI and leads to the oxidation of plastoquinone. This leads to state transitions due to exposure for 15 min of dark (D), far red (FR), or red (R) light. W, saturating 2-s pulse of actinic white light to measure PSI.

I, where the model predicts that unphosphorylated LHCP should be associated with PSI (Fig. 1A). We then measured the PSIII fluorescence after a short saturating white light pulse.

The same leaf was then exposed to red light to stimulate PSII (state II) and reduce the plastoquinone pool (Fig. 1B). The model predicts that LHCP should become phosphorylated and associate with PSI (Fig. 1C). PSII fluorescence was then measured again to see if it were lower, as would be expected if its antennae LHCP had moved away. The PSIII fluorescence measurements at state I and state II were then expressed as a ratio. Five successive light treatments and measurements were done on individual leaves from wild type and each of the four mutant plants, and the average of these measurements is shown in Fig. 7A. After successive treatments of the leaf with this light regime, wild-type Arabidopsis is still capable of state transitions, and the PSIII ratio is 1.38 ± 0.08; PSI activity is higher after far red than red light exposure (Fig. 7A). The ratios for the four antisense mutants are significantly lower than wild type (F-test). None of the ratios were below 1, indicating that PSI activity is not damaged by the successive light exposure. Thus, plants that have lower levels of TAK1 have low amounts of LHCP and have lost some capacity to undergo state transitions.

To determine how TAK1 levels influence the distribution of LHCP during state transitions, we used leaves from the same plants analyzed in Fig. 7A and froze these after each light treatment. Thylakoids proteins from each leaf were analyzed for protein phosphorylation and localization. Wild type and mutant thylakoid membranes were fractionated further into PSII- and PSI-enriched particles by sucrose density centrifugation. Protein profiles for Tk1–4 are shown as a representative of the four antisense mutants (Fig. 7B). The analysis was performed...
for all light conditions where state transitions had been directly measured: after dark adaptation (D), far red light treatment (15 min at 30 μE m⁻² s⁻¹ (FR), actinic white light (W; 2-s saturating pulse (600 μE m⁻² s⁻¹) to measure PSII activity), and red light (15 min at 30 μE m⁻² s⁻¹ (R)) treatment. D1 was found mostly in PSII fractions (data not shown). LHCP levels, which as expected are mostly in the PSI fraction due to the long dark preincubation, do not change significantly during the course of the experiment. However, a small amount of LHCP does accumulate in the wild type PSI fraction in the red light. The concomitant decrease in the PSI fraction is hard to detect due to the abundance of LHCP. Thus, red light stimulation of PSI does indeed lead to the accumulation of LHCP in PSI as predicted by the model. Analysis of the mutant Tk1–4, which has a decreased TAK1 and state transitions, shows that LHCP does not accumulate with PSI in red light. We then determined the phosphorylation state of LHCP. Anti-phosphothreonine serum detects LHCP⁺ in PSI of wild type leaves in the dark, and none is seen in PSI particles. However, PSII-associated LHCP⁺ declines and disappears upon subsequent far red and red illumination, and there is no accumulation of LHCP⁺ in PSI. This is in contrast to our findings with white light-grown plants, where LHCP⁺ is distributed between PSI and PSII. Thus, specific stimulation of one or the other photosystems leads to different steady state LHCP⁺ levels than does a more comprehensive thylakoid activation with complex white light. As seen before, LHCP⁺ is reduced in Tk1–4 and also disappears with light exposure.

We then analyzed TAKs during state transitions, and Fig. 7B (αTAK) shows the TAK levels after each light treatment for the wild type and mutant. TAK levels increase with light exposure of the wild type but not in the mutant. The red and far red light levels (30 μE m⁻² s⁻¹) are far below the light levels that cause a reduction in TAK1 mRNA (300 μE m⁻² s⁻¹). After red light treatment in wild type plants, there is a slight increase in the level of TAKs associated with PSI, and they are phosphorylated (Fig. 7B, αTAK⁺, PSI lane R). The increase of TAKs in PSI coincides with a decrease of TAK levels in PSII (Fig. 7B, αTAK, αTAK⁺ lane R). Analysis of TAKs in the Tk1–4 mutant shows that the total amount of TAKs in PSI and PSII particles does not increase after exposure to light, unlike wild type. In addition, the TAKs that are present do not preferentially accumulate in the PSI fraction in red light, and no phosphorylated TAKs are seen. Thus, TAKs are required for LHCP phosphorylation and state transitions. LHCP and not LHCP⁺ accumulates in PSI upon specific PSI stimulation, and TAKs themselves redistribute.

**TAK1 Is Required for Phosphorylation of Multiple Thylakoid Proteins**—We have shown that Arabidopsis with partial reductions in TAK1 levels have decreased amounts of LHCP⁺ and a decrease in state transitions. To see whether any other thylakoid proteins are affected in TAK1 mutants, we analyzed total thylakoid protein phosphorylation in the four mutant lines. Equal amounts of thylakoid membranes of antisense mutants and wild type were run in SDS-PAGE and probed with anti-phosphothreonine and LHCP antiserum (Fig. 8). LHCP levels appear not to vary appreciably. However, compared with wild type, the mutants have lower LHCP as well as D1, CP43, and 9-kDa phosphorylation levels.

**DISCUSSION**

**TAKs Are Required for State Transitions**—We have purified and characterized a family of Arabidopsis TAKs that phosphorylate LHCP in vitro under reducing conditions (10). We now find that TAKs interact with cyt f and LHCP in vivo. In plants grown under constant low white light, LHCP and LHCP⁺ appear to be distributed between PSI and PSII. Specific stimulation of PSI by red light (and thus induction of state transitions) causes a loss of LHCP⁺ and the new accumulation of LHCP with PSI. Plants that express TAK1 antisense have lower amounts of TAK1, a decrease in LHCP phosphorylation, and a reduced ability to undergo state transitions. In addition, TAK mutants have a lower amount of phosphorylation of multiple thylakoid proteins.

Our results are consistent with the model’s prediction that a thylakoid LHCP kinase is required for state transitions. Induction of state transitions does indeed lead to the accumulation of LHCP in PSI. Moreover, TAKs are associated with the b6f complex, which is thought to activate the phosphorylation of LHCP. However, our results do not support a scenario where specific stimulation of PSI causes an increase in LHCP⁺ levels that subsequently associate with PSI. The lack of LHCP⁺ in PSI cannot be explained by phosphatase activity during fractionation, because there was not an overall loss of phosphorylation during the thylakoid isolation, and extraction was performed in the presence of a number of phosphatase inhibitors. Moreover, the Western analysis with phosphothreonine antibodies is sensitive enough to detect low levels of LHCP⁺, for example during the change from dark to far red or low white light. Indeed, PSI- and PSII-associated LHCP⁺ is easily detected in plants grown in constant white light. It is possible that the red light-induced phosphorylation is short lived in vivo, shorter than the time that it takes for LHCP to migrate to PSI in the process of migration.

While red light is a component of the white light, it is likely that growth in white light exposes the thylakoids to multiple stimulations and thus more complex regulatory mechanisms. The results suggest that the distribution of LHCP between PSI and PSII could be controlled by factors in addition to TAK1, since in white light some LHCP is found with PSI in TAK1 antisense mutants. It is possible that the different TAKs are responsive to different light conditions, but this remains to be established, and null TAK alleles are required to resolve this question.

These results do not, in fact, contradict published reports concerning higher plant thylakoids, since there has not been a biochemical analysis of LHCP phosphorylation and location in the same leaves that have been induced to undergo state transitions by specific PSI or PSII stimulation. Numerous studies...
with land plants have evaluated the effect of dark, low, and high light on LHCP phosphorylation and movement (27), but these are not possible to equate to state transitions. The light treatments activate numerous parts of the electron transport chain, cause changes in the PQ pool, reductants, and ATP levels. Indeed, there are reports of LHCP phosphorylation that show an influence of thiol reductants, (28), ATP pool variation (29), and temperature (30). Thus, there are multiple influences generated by these previous treatments, and it has not been possible to dissect any one of the many mechanisms that are likely to be active at any one time.

Moreover, the literature that deals with the induction of LHCP phosphorylation by light is contradictory. In some reports, LHCP phosphorylation is correlated with the occupation of PQ, in the cytochrome b6f complex (31), while in others phosphate is lost from LHCP upon light exposure (27). These differences may have several sources. Many experiments assume but do not demonstrate that state transitions are induced by the imposed light regimes. Thus, the varying results could reflect the degree or absence of state transitions. Reports also differ in the species used, and it is possible that there are mechanistic differences between land plants as well as between algae (32). Perhaps as important a source of variation is the method by which LHCPP is detected. Some reports use an in vitro reaction and subsequent light exposure (2), and others isolate thylakoid membranes from plants exposed to light (27). The former lacks cell organization, but the latter may suffer from the presence of phosphatases during isolation. In contrast, these issues are well addressed in the Chlamydomonas experiments, and the emerging picture is clearer.

In Chlamydomonas, during state transitions, there is a concomitant induction of LHCP phosphorylation, and at the same time up to 80% of the antennae containing LHCP associates with PSI, leaving PSII (9). Fluorescent measurements of PSII activity also lose LHCPP and migration (32). While the results (32) suggest an influence of thiol reductants, (28), ATP pool variation (30). Thus, there are multiple influences generated by these previous treatments, and it has not been possible to dissect any one of the many mechanisms that are likely to be active at any one time.

TAKs Are Required for Other Phosphorylation Events—TAKs also appear to be required for the phosphorylation of other thylakoid proteins, and this is consistent with the suggestion that TAKs regulate processes in addition to state transitions. Our results do not determine if TAKs phosphorylate these substrates directly in vivo. In vitro assays have only detected LHCP as a substrate in reducing conditions (10), but in these experiments TAKs were not integral to the membrane, and the appropriate conditions may not have been provided such that phosphorylation of other substrates would occur. Genetic analysis and additional in vitro experiments with recombinant TAKs in a reconstituted system may help to resolve this issue. Nevertheless, TAKs do appear to be required for thylakoid protein phosphorylation, but it is possible that there are additional protein kinases present (33).

The loss of state transitions is correlated with both TAK reductions and loss of LHCP phosphorylation, but we are as yet unable to determine the role of the multiple other phosphorylation events in the thylakoid. It is unlikely that the reduction in general phosphorylation and slight growth effects in the TAK mutants would be the cause of the loss of state transitions. In the TAK antisense lines that lose state transitions, steady state protein levels of the reaction centers and the electron transport chain appear to be normal compared with wild type, and PSII does not lose activity over multiple rounds of stimulation. Moreover, we and others find no correlation between reaction center (D1/D2) phosphorylation and state transitions or photosynthetic activity (28, 32). D1 dephosphorylation is a prerequisite for damaged D1 degradation (34), but we do not see a change in D1 protein levels in TAK mutants despite a reduction in D1 phosphorylation. This indicates that the D1 kinase may regulate a separate process, perhaps via different phosphorylation sites. It remains possible, however, that an unidentified TAK-dependent phosphorylation is required for the modulation of the reaction center activity, but as yet there is no evidence for this. It is also unlikely that TAK1 antisense expression affects other non-TAK kinases, since only TAK1 mRNA, and not TAK2 mRNA, is affected. The slight reduction of TAK2/3 protein in TAK1 antisense mutants may be due to increased protein turnover in the absence of an appropriate complex.

Our results indicate that TAKs might regulate other aspects of the thylakoid in addition to state transitions. Future experiments models must take into account that there are three TAKs that are in a complex and appear to be phosphorylation. The role of the GS motif has a regulatory role in TGFβ signaling (18) in TAK function remains to be established, but it is possible that one of the TAKs act as a sensor of a signal and activates another TAK to phosphorylate a thylakoid substrate. In addition, the family members each may have different substrates that are used under specific conditions of the thylakoid membrane. These predictions can be tested using dominant negative alleles of TAKs, insertional mutations, and in vitro reconstitution. Since we have now established a requirement for TAKs in state transitions, future studies will also focus on the central role of TAKs in chloroplast and thus plant function.

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