Down-regulation of the PSI-F Subunit of Photosystem I (PSI) in Arabidopsis thaliana

THE PSI-F SUBUNIT IS ESSENTIAL FOR PHOTOAUTOTROPHIC GROWTH AND CONTRIBUTES TO ANTENNA FUNCTION*

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Anna Haldrup, David John Simpson, and Henrik Vibe Scheller

From the *Plant Biochemistry Laboratory, Department of Plant Biology, the Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C and the ¶Department of Physiology, Carlsberg Laboratory, Gamle Carlsbergvej 10, DK-2500 Valby, Copenhagen, Denmark

The PSI-F subunit of photosystem I is a transmembrane protein with a large lumenal domain. The role of PSI-F was investigated in Arabidopsis plants transformed with an antisense construct of the psaf cDNA. Several plant lines with reduced amounts of the PSI-F subunit were generated. Many of the transgenic plants died, apparently because they were unable to survive without the PSI-F subunit. Plants with 5% of PSI-F were capable of photoautotrophic growth but were much smaller than wild-type plants. The plants suffered severely under normal growth conditions but recovered somewhat in the dark indicating chronic photoinhibition. Photosystem I lacking PSI-F was less stable, and the stromal subunits PSI-C, PSI-D, and PSI-E were present in lower amounts than in wild type. The lack of PSI-F resulted in an inability of light-harvesting complex I-730 to transfer energy to the P700 reaction center. In thylakoids deficient in PSI-F, the steady state NADP+ reduction rate was only 10% of the wild-type levels indicating a lower efficiency in oxidation of plastocyanin. Surprisingly, the lack of PSI-F also gave rise to disorganization of the thylakoids. The strict arrangement in grana and stroma lamellae was lost, and instead a network of elongated and distorted grana was observed.

The photosystem I (PSI) complex of higher plants, algae, and cyanobacteria is a light-driven plastocyanin:ferredoxin oxidoreductase, which mediates electron transfer from reduced plastocyanin in the thylakoid lumen to oxidized ferredoxin in the stroma. The PSI core in higher plants contains 13–14 different subunits denoted PSI-A to PSI-N. One copy of each subunit is present per PSI reaction center. Angiosperm plants do not contain the PSI-M subunit. In addition to the PSI core, plants have a peripheral antenna associated with PSI. The peripheral antenna is known as light-harvesting complex I (LHCI) and is composed of four different subunits denoted Lhca1 to Lhca4. P700, the primary electron donor of PSI, is bound to the two larger reaction center polypeptides, PSI-A and PSI-B, near the lumenal side of the thylakoid membrane and thus is accessible to plastocyanin, the secondary electron donor.

Two low molecular mass subunits, PSI-F and PSI-N, have been implicated in the interaction between PSI and plastocyanin. PSI-F is a transmembrane protein with a large lumenal domain. Regions of PSI-F are highly conserved between species, but eukaryotic PSI-F has an N-terminal extension (1). The 18 extra residues in eukaryotic PSI-F appear to form an amphoteric helix located on the lumenal side of the thylakoid membrane (2). In addition to providing positive charges, this helix may serve to bring plastocyanin into the proper orientation for efficient electron transfer (3). Results of cross-linking experiments in maize and spinach suggest that PSI-F is involved in docking soluble plastocyanin to the PSI core complex (4–6). Cross-linked plastocyanin interacts functionally with and reduces P700+ (7). Strong evidence for the involvement of PSI-F in plastocyanin docking has been obtained in a gene knockout study in Chlamydomonas (1). In Chlamydomonas PSI lacking PSI-F, plastocyanin, and PSI did not form the stable complex that is normally seen, and the second order rate constant for electron transfer was 20–100 times slower. Despite the change in kinetic constants, the mutant algae could grow photoautotrophically almost as well as the wild type (1).

In cyanobacteria, the PSI-F protein appears to have a completely different function as a PSI-F-less mutant of Synechocystis PCC 6803 was unaffected in docking of plastocyanin and cytochrome c553 (8, 9). In the psaF deletion mutant, the PSI-A, PSI-B, and PSI-E subunits were more easily degraded by thermolysin. Thus, the PSI-F subunit has a dispensable accessory role in the function and organization of the Synechocystis PSI complex (8). Apparently, the N-terminal region of eukaryotic PSI-F mediates the efficient binding of plastocyanin and fast electron transport kinetics that are characteristic of eukaryotic PSI. The introduction into Synechococcus elongatus of a modified PSI-F containing the N-terminal part of Chlamydomonas PSI-F led to a large increase in the rate of reaction with plastocyanin and cytochrome c553 (10).

A few reports have suggested that the PSI-F polypeptide is associated with LHCI (11, 12). PSI contains about 300 antenna chlorophyll molecules per P700 reaction center (13). Most of the chlorophyll antenna molecules are localized in LHCI, which is specific to PSI, and LHClII, which functions as light-harvesting complex for both photosystems. The remaining about 90 chlorophyll molecules per P700 are bound to the PSI core. The pigment proteins that carry the peripheral antenna chlorophylls can be separated from a core of proteins carrying the reaction center, the electron transfer components, and tightly bound antenna chlorophylls. The frequently observed absence
of PSI-F in isolated core complexes devoid of LHCl suggests that in plants this subunit is bound to LHCl (5, 14).

There are 10 distinct types of LHCl proteins encoded by the different lhc genes (15), which have been highly conserved for at least 345 million years of evolution (16). This means that all 10 must have specific functions within the light-harvesting apparatus, since random genetic drift would otherwise have eliminated some of the corresponding genes without positive selection pressure for their preservation. The lhca1-4 genes encode the antenna proteins of LHCl. The lhcb1 and lhcb2 genes encode the most abundant proteins of the LHClI trimers, which can associate with either PSI or PSII, and lhcb3-6 genes encode PSI-specific antenna proteins (17). The Lhca proteins are organized as dimers (18). Lhca1 and Lhca4 form heterodimers, LHClI-730, characterized by a 77 K fluorescence emission peak at 730 nm (19). Lhca2 and Lhca3 appear to be organized as homodimers forming LHClI-680, which can be fractionated into LHClI-680a (Lhca3) and LHClI-680b (Lhca2) both fluorescing at 680 nm at 77 K (20). In Arabidopsis, two additional genes were identified and named lhca5 and lhca6, but their expression is very low, so the proteins may not be normal subunits of LHClI (21). The stoichiometry of Lhca subunits in PSI is not clear, but eight Lhca subunits per reaction center have been suggested (15).

The PSI-N subunit has only been found in PSI of higher plants. It is still uncertain whether PSI-N is present in green algae. PSI-N is the only extrinsic PSI subunit on the lumenal side of the thylakoid membrane. Recently, PSI-N was shown to be important for efficient electron transfer from plastocyanin to PSI-N, Lhca1, Lhca2, Lhca3, Lhca4, Lhcb1, and Lhcb2. Plastocyanin antibodies and PSI-E antibodies were raised against Arabidopsis plastocyanin and PSI-E. The NDH-I antibody was raised against a fusion protein from tobacco (34), and antibodies against plastothreonine were obtained from New England Biolabs. In each lane in the SDS gels, 0.1-1 μg of protein loaded depending on the linear range for each of the primary antibodies. All antibodies were detected using a chemiluminescent detection system (SuperSignal™, Pierce) according to the instructions of the manufacturer. Antibodies were the kind gift of Dr. Stefan Jansson, University of Umeå, Umeå, Sweden (Lhca and Lhcb), Dr. Peter Nixon, Imperial College of Science, London, UK (NDH-I), and Dr. Olivier Vallon, Institut de Biologie Physico-Chimique, Paris, France (PSI-E).

**Immunoblot Analysis**—Plants lacking the PSI-F subunit were identified by immunoblotting. Crude leaf extracts were prepared as described in Haldrup et al. (23). Each sample loaded on the gels represented 1 μg of Chl. Immunoblotting was carried out by transferring electrophoresed proteins to nitrocellulose membranes followed by incubation with polyclonal rabbit antibodies raised against barley PSI-F protein (33) and visualization with the use of either horseradish peroxidase-conjugated secondary antibodies or alkaline phosphatase-conjugated secondary antibodies (DAKO, Copenhagen, Denmark). Isolated thylakoids were analyzed in similar immunoblotting procedures using antibodies raised in rabbits against barley PSI-A, PSI-B, PSI-H, PSI-K, PSI-L, Lhca1, Lhca2, Lhca3, Lhca4, Lhcb1, and Lhcb2. Plastocyanin antibodies and PSI-E antibodies were raised against Arabidopsis plastocyanin and PSI-E. The NDH-I antibody was raised against a fusion protein from tobacco (34), and antibodies against plastothreonine were obtained from New England Biolabs. In each lane in the SDS gels, 0.1-1 μg of protein loaded depending on the linear range for each of the primary antibodies. All antibodies were detected using a chemiluminescent detection system (SuperSignal™, Pierce) according to the instructions of the manufacturer. Antibodies were the kind gift of Dr. Stefan Jansson, University of Umeå, Umeå, Sweden (Lhca and Lhcb), Dr. Peter Nixon, Imperial College of Science, London, UK (NDH-I), and Dr. Olivier Vallon, Institut de Biologie Physico-Chimique, Paris, France (PSI-E).

**Low Temperature Fluorescence Measurements**—The fluorescence spectrum at 77 K was recorded for thylakoids from dark-adapted plants using a bifurcated light guide connected to a Perkin-Elmer LS50B fluorospectrophotometer. The excitation light had a wavelength of 435 nm, and emission was detected from 650 to 800 nm.

**Functional Antenna Size of PSI**—Functional PSI antenna size was determined from light-induced P700 absorption changes at 810 nm using the Dual Wavelength Emitter Detector Unit ED-P700D-W-E connected via a PAM 101 Fluorometer (Walz, Effeltrich, Germany) to a Tektronix TDS420 oscilloscope. A leaf from a dark-adapted plant was fixed to the light fiber. After recording for 5 s, the leaf was illuminated by far-red light (actinic light from a Walz 102-90R source (λmax = 735 nm)) to excite P700 and the induced P700 absorption changes were measured over a 20-s period. Then the actinic light was switched off, and re-reduction of P700 was followed for another 25 s. For each leaf, four traces were averaged, and three leaves from each plant were analyzed. Antenna function is expressed as the maximal absorption change at steady state.

**P700 Flash Absorption Spectroscopy**—Flash-induced P700 absorption change was measured at 834 nm, essentially as described previously (35, 36). The saturating actinic pulse (532 nm, 6 ns) was produced by a Nd:YAG laser. Thylakoids (32 μg Chl ml⁻¹) were dissolved in 250 μl of 20 mM Tricine (pH 7.5), 40 mM NaCl, 8 mM MgCl₂, 0.1% 4-decyl-β-d-maltopyranoside, 2 mM sodium ascorbate, and 60 μM 2,6-dichloro-phenolindophenol. The solution was centrifuged three times for 20 s at 200 × g to remove starch grains prior to measurement. The sample (200
μl) was transferred to a cuvette with 1 cm path length. A diode laser provided the measuring beam, which was detected using a photodiode. A total of 32 flash-induced decay curves were collected and averaged for each sample. The recorded absorption changes were resolved into exponential decay components by a Levenberg-Marquardt non-linear regression procedure.

**NADP⁺ Photoreduction Measurements**—NADP⁺ photoreduction activity of PSI was determined from the absorbance change at 340 nm as described by Naver et al. (35) using thylakoids equivalent to 5 μg of Chl. Thylakoids were solubilized in 0.09% decylmaltoside prior to the measurement. Saturating light was used during the measurement.

**Room Temperature Fluorescence Measurements**—Determination of conventional fluorescence quenching parameters was performed with a PAM 101-103 fluorometer (Walz, Effeltrich, Germany) by using a standard set up as reported previously (23). Measurements were performed under growth light conditions, and a leaf was fixed with tape to the end of the light fiber. Steady state fluorescence (Fₛ) was obtained, and a saturating pulse (0.8 s) of white light (6000 μmol photons m⁻² s⁻¹) was applied allowing determination of maximum fluorescence (Fₘ max). After the first flash, aluminum foil was wrapped around the fiber to determine Fₛ and to dark-adapt the leaf. A saturating pulse was subsequently applied every 10 min. PSII quantum yield (Fₘ / Fₘ max) was calculated as ((Fₛ - Fₛ') / Fₘ max) and excitation pressure (1 - qₑ) as (Fₛ - Fₛ'') / (Fₛ' - Fₛ′) for the first flash, aluminum foil was wrapped around the fiber to determine Fₛ and to dark-adapt the leaf. A saturating pulse was subsequently applied every 10 min. PSII quantum yield (Fₘ / Fₘ max) was calculated as ((Fₛ - Fₛ') / Fₘ max) and excitation pressure (1 - qₑ) as (Fₛ - Fₛ'') / (Fₛ' - Fₛ′).

**RESULTS**

**Plants That Are Deficient in PSI-F Grow Poorly**—The original transformed lines were self-pollinated, and the seeds produced were plated on media containing kanamycin or gentamicin.

A large number of plants died after being moved from tissue culture to soil, and others were too small for use in any experiments. These plants were most likely completely lacking PSI-F and not capable of photoautotrophic growth. Plants that were sufficiently healthy to be further analyzed were screened by immunoblotting. Different levels of down-regulation were detected. The detection limit was about 3% of wild-type PSI-F content.

Plants with 0–3% PSI showed a significantly decreased growth rate compared with wild type (Fig. 1) and usually died after few weeks in soil. Small, yellowish plants struggling to survive could be rescued by placing them under low light conditions or in the dark for 2–3 days. The plants subsequently started to produce new shoots and looked healthier. Plants with 5–40% PSI-F also had a changed phenotype being smaller than wild-type plants. This has not been observed in plants lacking PSI-N, PSI-H, or PSI-K (23, 37, 38).

**Pigment Composition**—In plants with 5% PSI-F, the Chl a/b ratio was 1.76 ± 0.20 (±S.D.), and in wild-type plants the ratio was 2.16 ± 0.01. The difference was significant (t test, p < 0.001). A decreased Chl a/b ratio indicates a decreased PSI/PSII ratio or an increased antenna size. The Chl/P700 ratio was 988 ± 120 (±S.D.) for transgenic plants with 5% PSI-F and 849 ± 237 for wild-type plants. The PSI-F-deficient plants thus have 10–20% less PSI compared with wild-type plants. However, the difference was not statistically significant. In contrast, transgenic Arabidopsis plants without PSI-N (23), PSI-H (37), or PSI-K (38) all compensate for a poorly functioning PSI by making 18–20% more PSI.

**The Thylakoids Have Altered Structure in PSI-F-deficient Plants**—The grana of plants with 3% PSI-F have a larger diameter (0.86 ± 0.13 μm) than those of wild-type (0.48 ± 0.09 μm) and fewer discs per granum (3.9 ± 1.2) compared with wild-type (8.8 ± 2.8) (Fig. 2). Stroma lamellae were essentially absent. Plants with 40% PSI-F showed thylakoid structure similar to those with 3% PSI-F.

**The Deficiency in PSI-F Leads to Changes in Abundance of Several Other Subunits**—PSI-F-deficient plants were analyzed with a range of antibodies (Fig. 3). For this investigation thylakoids were prepared from plants with about 5% PSI-F and from plants with about 40% PSI-F. Because of the postulated involvement of PSI-F in LCHI function (11, 12, 21), we investigated if the absence of PSI-F would lead to a secondary loss of LHCI proteins (Fig. 3a). Surprisingly, the content of Lhca4 in thylakoids was increased, whereas the content of Lhca1, Lhca2, and Lhca3 was decreased. LHCII is normally down-regulated in plants grown under high light, i.e. with a high excitation pressure, but we found increased amounts of Lhcb1 and Lhcb2. LHCII and D1 proteins showed high levels of phosphorylation, as shown by reaction with the phosphothreonine antibody which detected 120–700% more phosphorylation in plants with 5% PSI-F compared with wild type (Fig. 3b). Compared with wild type, the amount of PSI-A and PSI-B was higher in thylakoids with 40% PSI-F but lower in thylakoids with 5% PSI-F, and PSI-B was partly degraded (Fig. 3c). Thus, plants with 40% PSI-F seemed to be capable of up-regulating the amount of PSI based on PSI-A/B levels, in the same way as plants without PSI-N, PSI-H, and PSI-K (23, 37, 38). Partial degradation of the PSI-A and PSI-B proteins is a typical symptom of light-
induced PSI damage (39). The extrinsic subunits PSI-C, PSI-D, and PSI-E were all present at 15–60% of wild-type levels (Fig. 3c). PSI-N was almost absent and PSI-K was not detectable in thylakoids with 5% PSI-F. The level of PSI-H was normal.

The amount of plastocyanin was increased in PSI-F-deficient plants. Plastocyanin content in barley is positively correlated with growth irradiance (40), indicating that the PSI-F-deficient plants in some respects behave like plants exposed to high light conditions. The increased amounts of NDH-I and D1 are consistent with the enhanced levels observed in light-stressed plants (39, 41).

**LHCII-730 Is Non-functional in the Absence of PSI-F**—Leaves with 3–5% PSI-F showed a 7-nm blue shift in the fluorescence emission maximum to 727 nm (Fig. 4). This blue shift resembles the 6 nm shift observed in *Arabidopsis* plants with reduced levels of Lhca4 (42). Likewise, Knoetzel et al. (43) found a similar blue shift in barley mutants lacking Lhca4. When PSI is excited with far-red light, most of the photons are absorbed by the long wavelength chlorophylls in Lhca1 and Lhca4 (44). The very small absorbance change at 810 nm in leaves with reduced amounts of PSI-F shows that only a small amount of P700 became oxidized (Fig. 5). Thus, excitation energy transfer from Lhca1 and Lhca4 to P700 was severely impaired in the absence of PSI-F.

**P700 Absorption Decay**—The lack of steady state P700 photooxidation in far-red light could also result from a non-functional acceptor chain. To investigate this possibility, P700 flash measurements were performed. The time constant (τ) for charge recombination between $F_A/F_B^-$ and P700$^+$ is known to be $>30$ ms. With damage to $F_A/F_B$, the charge recombination
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**Fig. 4.** 77 K fluorescence emission spectra of thylakoids from wild-type (WT) and PSI-F-deficient plants.

**Fig. 5.** Antenna function in PSI-F-deficient and wild-type plants. Antenna function of PSI was measured by light-induced P700 absorption changes in leaves at 810 nm after excitation of LHCl-730 at 735 nm. Representative traces of wild-type (WT) leaves and leaves with different degree of down-regulation are shown. a, 15% PSI-F; b, 5% PSI-F; c, 0–3% PSI-F.

will proceed from $F^-_S$ with a $\tau \approx 1$ ms. With further damage to the electron transport chain, the charge recombination from either $A_{1}^-$ or $A_{0}^-$, via the P700 triplet state, takes place in 3–5 $\mu$s. The back reaction from $A_{0}^-$ to P700$^-$, with a $\tau$ of approximately 30 $\mu$s, is below the 1-$\mu$s time resolution of our experimental system. For wild-type PSI the decay time was >30 ms, demonstrating the presence of an intact PSI and antenna system (Fig. 6). In PSI with 40% PSI-F, the amount of PSI with an intact electron acceptor chain was about 60% of the wild-type level (Fig. 6). In samples with only 5% PSI-F, about 15% of the observed absorbance change decayed within about 5 $\mu$s (Fig. 6), indicating a back reduction from $A_{1}$ in the absence of the subsequent acceptors. On the average, the total amplitude of the signal in PSI with 5% PSI-F and 40% PSI-F was 36% lower than in the wild type. About 10% of the difference can be explained by the different Chl/F700 ratios. However, about 25% of the P700$^-$ from PSI-F-deficient plants decayed faster than the time resolution of the measurement, indicating damage to $A_{1}$. Although some damage to the electron acceptors was apparent in PSI, this cannot explain the inability of 735 nm light to oxidize P700. Thus, the flash measurements confirm that excitation energy transfer from LHCl to P700 is impaired in the absence of PSI-F.

**PSI Electron Transport Is Restricted in the Absence of PSI-F**—In order to determine the role of PSI-F in electron transfer from plastocyanin to PSI-F, NADP$^+$ photoreduction was determined with thylakoids isolated from plants with 5% PSI-F, plants with 40% PSI-F, and wild-type plants (Table I). From P700 decay measurements (Fig. 6), we know that about 40% of the electron acceptor chain was not functional and therefore not able to perform NADP$^+$ photoreduction. The residual PSI-F in thylakoids with 5% PSI-F is presumably located in the active PSI centers. Thus, the PSI complexes containing the residual 5% PSI-F can be estimated to account for 2.8 $\mu$mol of NADPH mg$^{-1}$ Chl h$^{-1}$. However, the determined activity of 5.1 $\mu$mol of NADPH mg$^{-1}$ Chl h$^{-1}$ is significantly higher. Therefore, we can estimate that the 95% PSI complexes devoid of PSI-F account for the remaining 2.3 $\mu$mol of NADPH mg$^{-1}$ Chl h$^{-1}$. Disregarding the PSI complexes with an incomplete acceptor chain, the NADP$^+$ reduction by PSI complexes without PSI-F can be calculated to be about 13 times lower than in the presence of PSI-F.

**Deficiency in PSI-F Leads to Photoinhibition**—Since plants deficient in PSI-F seemed to recover under low light conditions and in the dark, fluorescence measurements under growth light conditions were performed to check the state of the photosynthetic apparatus during normal growth (Table II). The results indicate a difference in the regulation of photosynthesis in plants without PSI-F. The reduction state of $Q_A$ ($1 - q_p$) was much higher in PSI-F-deficient plants (0.45) than wild-type (0.01) indicating that electron flow between PSII and PSI was restricted. The plants with less PSI-F also exhibited a lower efficiency of PSII photochemistry, $\Phi_{PSII}$. Plants with 5–10% PSI-F obtained a normal $\Phi_{PSII}$ after 10 min in the dark (Fig. 7). Thus, the lower $\Phi_{PSII}$ in these plants reflects the increased level of reduced $Q_A$. In contrast, $\Phi_{PSII}$ remained low in plants with less than 3% PSI-F after 40 min of dark adaptation. Thus, these plants exhibit signs of photoinhibitory damage to PSI. After 16 h of dark adaptation, all plants had normal $\Phi_{PSII}$ of about 0.8.

**DISCUSSION**

Arabidopsis Plants Lacking PSI-F Are Severely Inhibited in Growth Rate and Have an Altered Thylakoid Organization—We have made transgenic Arabidopsis plants with de-
TABLE II
Steady state chlorophyll fluorescence parameters in leaves of wild-type and PSI-F-deficient plants

| Condition                        | $\Phi_{\text{PSII}}$ | $1 - q_p$ |
|----------------------------------|-----------------------|-----------|
| Wild type                        | 0.76 ± 0.01           | 0.01 ± 0.01|
| PSI-F-deficient plants           | 0.55 ± 0.03           | 0.45 ± 0.04|

FIG. 7. Efficiency of PSII photochemistry. $\Phi_{\text{PSII}}$ was measured in plants removed from growth light (120 $\mu$mol photons m$^{-2}$ s$^{-1}$) and placed in the dark for 40 min. A saturating flash was given every 10 min.

creased levels of the PSI-F subunit (from 0 to 40% of wild-type levels), thereby obtaining a tool for investigating the role of the PSI-F polypeptide in vivo as well as in vitro in plants. Plants totally lacking PSI-F died, and plants with very low amounts of PSI-F developed slowly and struggled to survive. This differs markedly from the results with PSI-F-deficient Chlamydomonas and Synechocystis (1, 8, 9). Chlamydomonas reinhardtii without PSI-F grew photoautotrophically, although the electron transfer from plastocyanin to P700$^+$ was dramatically reduced. In Synechocystis sp. PCC6803, deletion of the psaF gene did not affect the rate of P700$^+$ re-reduction by cytochrome c$_o$ or plastocyanin (8). This PSI-F mutant was able to grow photoautotrophically and possessed a fully active PSI complex (9).

The PSI-F down-regulated plants had a significantly changed thylakoid organization with distorted grana lamellae and no stroma lamellae. We do not know why the lack of PSI-F causes the thylakoids to reorganize. The two photosystems of higher plants are not homogeneous, but different populations of PSI and PSII centers exist, which are unevenly distributed in the thylakoid membrane. Most notably, PSI is enriched in the appressed regions (the grana stacks) and PSI in the non-appressed, stroma-exposed regions of the thylakoids as follows: the stroma lamellae, the grana margins, and the end membranes (45). Changes in thylakoid organization may result in response to changes in irradiance or spectral composition of light (see review by Anderson (46)). Obviously the redox conditions are altered in plants lacking PSI-F, and this might be perceived by the plant as a change in the light environment. Plants can respond to excess light by increasing energy dissipation, e.g. as heat in a process dependent on the xanthophyll cycle and the acidification of the thylakoid lumen. Horton (47) suggested that grana stacking hinders energy quenching and that destacking is associated with high quenching. The redistribution of the excitation energy is regulated via the redox state of the plastoquinone pool (reviewed in Ref. 48). Phosphorylation of LHClII by a redox-regulated kinase is correlated with state transitions caused by movement of LHClII from PSII to PSI. Fig. 3b clearly shows that LHClII is heavily phosphor-ylated in PSI-F-deficient plants. In addition to state transition, an unbalanced excitation distribution between the two photosystems may cause a movement of PSI into the grana stacks. A permanent state transition with PSI movement into the grana stacks might explain the different organization of thylakoids in PSI-F-deficient plants with low number of discs per granum and the lack of stroma lamellae.

Plants without PSI-F Cannot Transfer Excitation Energy from LHCI-730 to P700—A change in the size and function of the PSI antenna was observed by 77 K fluorescence spectroscopy and immunoblot analysis of PSI-F-deficient plants. Three Chl spectral forms are known from PSI with 77 K fluorescence maxima at 720, 730, and 742 nm. Two Chl molecules that fluoresce at 720 nm are present in the isolated core (44). In Fig. 4 the peak at 734 nm results from two long wavelength emission maxima at 730 and 742 nm from Lhca1 and Lhca4, respectively. The 7-nm shift in fluorescence emission (77 K) in PSI-F-deficient plants (maximum at 727 nm) is similar to that in Arabidopsis plants down-regulated in Lhca4 (42) and the clof2101 barley mutant (43) which is also deficient in Lhca4. Based on the 77 K fluorescence emission blue shift and the low amplitude of P700 absorption changes induced by 735 nm excitation, we conclude that PSI-F-deficient plants cannot transfer excitation energy from Lhca1/Lhca4 heterodimers, i.e. LHCI-730, to the reaction center P700. In contrast, Chlamydomonas lacking PSI-F does not show significant changes in the efficiency of binding or excitation transfer between the antenna and the PSI centers (1, 49). Plants and green algae all have LHCI complexes but apart from the N-terminal region involved in plastocyanin docking, PSI-F in these organisms does not contain a common motif that differs from PSI-F of other species. A likely explanation is that the role of PSI-F in LHCI function is specific for plants and involves plant-specific features of PSI-F and LHCI. In agreement with this, LHCI has a somewhat different composition in Chlamydomonas than in plants (12, 50). Bassi et al. (12) found that, in contrast to higher plants, the long wavelength fluorescence emission typical of LHCI (705 nm) in Chlamydomonas, could not be correlated with the presence of specific polypeptides but rather with the changes in the aggregation state of LHCI components.

The secondary loss of PSI-K in the absence of PSI-F (Fig. 3c) is surprising since the two proteins do not appear to interact directly (18). Possibly, the effect on PSI-K is related through a perturbation of LHCI.

Arabidopsis plants lacking PSI-K have about 20–30% less Lhca2 and 30–40% less Lhca3, clearly indicating that PSI-K also has a role in organizing LHCI (38). Since PSI-K is not bound to PSI in the absence of PSI-F (Fig. 3c), it is important to distinguish between primary and secondary effects of the lack of PSI-F. Thus, the reduced amounts of Lhca2 and Lhca3 in the absence of PSI-F can be explained as a result of the missing PSI-F in the PSI-F deficient plants (Fig. 3a). However, plants without PSI-K exhibit only a minor fluorescence blue shift of 2 nm and have functional Lhca1/Lhca4 (38). Thus, the inactivation of LHCI-730 appears to be the primary result of the missing PSI-F. The inactivation of LHCI-730 is also not a result of photoinhibition as photoinhibited plants show neither fluorescence blue shift nor less of LHCI subunits.3 Because of the many secondary effects of the missing PSI-F, it can be difficult to conclude unequivocally that PSI-F actually binds LHCI-730. However, our interpretation is independently confirmed with the recent paper of Boekema et al.3 who found LHCI to be in close contact with PSI-F.

3 J. Knoetzel, unpublished data.
3 E. J. Boekema, P.-E. Jensen, E. Schlodder, J. F. L. van Breen, H. van Roos, H. V. Scheller, and J. P. Dekker, submitted for publication.
Inefficient Energy Transfer in Plants Lacking PSI-F

**PSI-F Is Necessary for Efficient Oxidation of Plastocyanin**—The inefficiency of excitation energy transfer from the peripheral antenna in PSI-F-deficient plants does not explain the severe phenotype. Under optimal conditions with sufficient light, plants lacking LHCl function would still be expected to grow reasonably well. In PSI-F-deficient *Chlamydomonas*, fast electron transfer from plastocyanin to PSI requires PSI-F (1, 5). Hippler *et al.* (2, 3) have shown that the N terminus of the PSI-F subunit of PSI cross-links to plastocyanin. The N-terminal α-helix has six lysine residues on one side, which may facilitate rapid one-dimensional diffusion of plastocyanin and provide electrostatic attraction at the attachment site. This interaction is likely to increase the electron transfer rate by more than 2 orders of magnitude in plants compared with cyanobacteria (2). Plastocyanin is up-regulated in response to PSI-F deficiency (Fig. 3c). This seems as an appropriate response to a poorer docking of plastocyanin to PSI. The signal mediating increased plastocyanin production is not known, but high irradiance will also increase plastocyanin production (40). Presumably, the increased plastocyanin production is related to the more reduced state in the inter-system chain.

The 13-fold decrease in NADP⁺ photoreduction by thylakoids lacking PSI-F is consistent with the 20-fold decrease in the rate constant for the electron donation from plastocyanin to PSI-F (1). Steady state levels of reduced Q₅ in PSI F, which reflect the redox state of the plastoquinone pool, were much higher in PSI-F-deficient plants (Table II) consistent with restricted electron flow between PSI and PSI-F.

**PSI Is Unstable and Susceptible to Photodamage in the Absence of PSI-F**—The PSI electron transport chain in PSI-F-deficient plants was partly degraded, and only about 55% of the PSI had an intact electron acceptor chain. PSI-F is known to interact with PSI-E (18), and the lack of PSI-F may destabilize the stromal side of PSI, as evidenced by the partial loss of PSI-C, PSI-D, and PSI-E (Fig. 3c). Very recently, it was shown that an *Arabidopsis* mutant with reduced amounts of PSI-E also has less PSI-C and PSI-D (52). PSI-E is in close contact with PSI-C (53) and could be directly involved in binding of PSI-C. However, reconstitution experiments with plant PSI have not indicated any requirement of PSI-E for the binding of PSI-C and PSI-D (54). The dissociation of PSI-C and PSI-D as well as the damage to the earlier electron acceptors resembles the pattern seen in response to severe photoinhibition of PSI (39) and may be the indirect effects of the low levels of PSI-E, itself an indirect effect of the lack of PSI-F. Reduced levels of PSI-E may limit the reduction of ferredoxin and lead to over-reduction of the iron-sulfur centers F₄₅₀/F₄₅₃. This will in turn lead to O₂ reduction by PSI, generating superoxide anion radical and other reactive oxygen species, causing degradation of the extrinsic proteins PSI-D and PSI-C. Initial damage to the iron-sulfur clusters results in recombination between the radical pair P₇₀₀⁺/A₅₅₃⁻ with the possible formation of excited triplet chlorophyll. Shuvalov *et al.* (55) showed that when electron transfer from P₇₀₀ to the iron-sulfur clusters was blocked, as found in PSI-F deficient plants (Fig. 6), P₇₀₀⁺/A₅₅₃⁻ can recombine with 30% triplet yield. The triplet state of P₇₀₀ can then react with molecular oxygen creating singlet oxygen that is very toxic and may be involved in further photoinhibitory damage of PSI (39). Simultaneously with the photoinhibitory damage to the PSI electron acceptors, some break down of proteins, particularly PSI-B, takes place (39, 56). The same pattern was observed in PSI-F-deficient plants (Fig. 3c). In contrast to the instability of plant PSI lacking PSI-F, no effects of PSI photoinhibition have been reported for *Chlamydomonas* (57). Possibly, this difference is not due to a different role of PSI-F in this organism but to the presence of alternative quinol-oxidizing systems that limit the formation of reactive oxygen species (58–60).

**Plants without PSI-F Exhibit Chronic PSII Photoinhibition**—Instability of PSI suggests that the PSI complex is more susceptible to photodamage in the absence of PSI-F. However, plants with PSI-F deficiency also show several signs of photoinhibition of PSII. PSII photoinhibition is evidenced by the lower ɸPSII, which reverts to normal levels in the dark over several hours. The photoinhibition of PSII may be an expected result of the increased excitation pressure, which will increase the risk of chlorophyll triplet formation in PSII. These data are consistent with recent results found in *Chlamydomonas* lacking PSI-F that were photo-oxidized under high light (400 μmol photons m⁻² s⁻¹) (57), but the effect is much stronger in plants. During photoinhibition, the D1 protein is phosphorylated, and this prevents degradation of D1 until the repair process can take place (61). Damaged PSII is transferred from the grana stacks to exposed regions of the thylakoid membrane, partly disassembled and reassocitated with *de novo* synthesized D1. The damaged D1 protein is removed in this process, and the repaired PSII is transferred back to the grana stacks (62). Therefore, the high phosphorylation level of D1 found in PSI-F-deficient plants is consistent with the observed PSII photoinhibition.

*Arabidopsis* mutants where only one of the two *psaE* genes is translated have reduced levels of PSI-C, -D, and -E that are very similar to the levels found in the plants deficient in PSI-F, and the mutants also show clear signs of photoinhibition (52). However, the excitation pressure is much higher in the PSI-F-deficient plants, and the growth of plants devoid of PSI-F is much more severely affected. Therefore, the phenotype seen in the absence of PSI-F must be a result of the combined effect of the photoinhibition and low electron transport rates.

Another indication of photo-oxidative stress in PSI-F-deficient plants is the higher levels of NAD(P)H dehydrogenase (NDH-I) detected by immunoblotting (Fig. 3c). These findings agree with a function for the NDH complex in cyclic electron transport in response to photo-oxidative stress (15, 41, 51, 63).

**Conclusion**—The PSI-F protein is essential for photoautotrophic growth in higher plants, which probably is the reason why no knockout mutants have been detected in plants. Low amounts of PSI-F cause the plants to grow more slowly, and the thylakoids lack stroma lamellae. The severity of the phenotype is markedly different from green algae and cyanobacteria without PSI-F and shows that PSI-F has additional roles in plants. In plants, PSI-F is involved in electron transport from plastocyanin and in energy transfer from LHCl-730. Furthermore, plants without PSI-F are severely photoinhibited in both PSI and PSII at normal growth conditions. PSII photoinhibition is a result of increased excitation pressure, whereas PSI photoinhibition may be due to a destabilization of the reducing side of PSI. The severe phenotype caused by the absence of PSI-F in plants is a result of the combined negative effects of photoinhibition, poor light-harvesting capability, and low rates of plastocyanin oxidation.

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