Hcf101-1 is a high-chlorophyll-fluorescence (hc) Arabidopsis mutant that lacks photosystem I (1). Photosystem I subunits are synthesized in the mutant but do not assemble into a stable complex. hcf101 was isolated by map-based cloning and encodes an MRP-like protein with a nucleotide-binding domain. The protein is localized in the chloroplast stroma. In green tissue, the Hcf101 level is stimulated by light, and the protein is not detected in roots. Two independent knockout lines, hcf101-2 and hcf101-3, are also impaired in Hcf101 accumulation, although to different extents. Like hcf101-1, hcf101-2 and hcf101-3 are hcf mutants with impaired photosystem I. Our results indicate that Hcf101 is a novel component required for photosystem I biosynthesis.

Photosystem I (PSI) is a pigment-protein complex in the thylakoid membranes of cyanobacteria and chloroplasts that catalyzes the electron transfer from plastocyanin to ferredoxin. In higher plants, the PSI complex consists of 14 different subunits (Psaa-O) and four chlorophyll (Chl) α- and β-associated light-harvesting complex (LHC) proteins, LhcA-L. The subunits Psaa, Psab, Psac, Psal, and Psaj are chloroplast-encoded, whereas the remaining subunits, Psad-Psah, Psak, Psal, Psan, and Psao, are nuclear-encoded (cf. 2–5). The major proteins Psaa and Psab form a heterodimer, which binds most Chl α molecules, the primary electron donor P700, and the electron acceptors A0, A1, and Fx, a [4Fe-4S] cluster. The remaining two co-factors, Fx and Fb, both [4Fe-4S] clusters, are bound to Psac.

The initial process in PSI biogenesis is the formation of a heterodimeric core of Psaa and Psab and the correct association of the Fx cluster (6–9). Analysis of PSI-deficient mutants in Chlamydomonas reinhardtii showed that failure to synthesize any of the plastid-encoded polypeptides, Psaa, Psab, or Psac, results in a loss of PSI activity and the degradation of the other PSI subunits (10–12). In addition, Psad is essential for the stable binding of Psac to the reaction center (13, 14).

In the last few years, proteins without any structural function were identified that are essential to regulate different steps in PSI biogenesis (cf. 15–20). The chloroplast genes ycf3 and ycf4 are required for stable accumulation of the PSI complex in C. reinhardtii (15). Ycf3 seems to act as a PSI-specific chaperone, facilitating the assembly of the complex by interacting with Psaa and Psad (21). A ycf3 knock-out mutant of tobacco results in a loss of PSI and in a light-sensitive phenotype (16). Inactivation of the cyanobacterial ycf4 and ycf37 increases the PSI/PSII ratio without any influence on photoautotrophic growth (17, 18). Disruption of PsfH, a putative AAA-protease homolog in Synechocystis, and mutation of the btpA gene reduce the amount of functional PSI (22, 23). BtpA is a peripheral membrane-bound protein that stabilizes the PSI reaction center at low temperatures (24, 25). Ruba gene deletion prevents photoautotrophic growth of Synechococcus sp. PCC 7002. The protein interacts transiently with monomeric PSI complexes and seems to be directly involved in the assembly of Fx (8). EPR measurements revealed the absence of the iron-sulfur clusters Fx, Fx, and Fb in the mutant. However, the absence of Fx and Fb could be a secondary effect, because none of thestromal subunits, Psac, Psad, and Psae, could be detected in isolated PSI complexes of the mutant (9).

Although molecular genetics, spectroscopy, electron microscopy, and crystallography revealed many details about the organization of PSI (cf. 26–28), our knowledge about regulatory factors involved in PSI assembly, stabilization, and degradation is very incomplete. Here we report on the isolation of hcf101, a gene that is essential for the accumulation of PSI in Arabidopsis, and the initial characterization of mutants impaired in Hcf101 synthesis. Furthermore, possible biological functions of Hcf101, based on its modular structure, will be discussed in the context of the mutant phenotype.

**EXPERIMENTAL PROCEDURES**

**Growth Conditions and Plant Material—** Arabidopsis thaliana seedlings were grown in growth chambers under continuous white light with a light intensity of 80 μmol m⁻² s⁻¹ at 22 °C. For physiological experiments, seeds were sterilized with 33% (v/v) bleach and 0.08% N-laurylsarcosinate, washed four times with 1 ml of sterile water, and placed on Petri dishes with solidified half-strength Murashige and Skoog (29) media supplemented with 1.35% (w/v) sucrose. To ensure synchronized germination, the plates were kept in darkness at 4 °C for the first 48 h. The Arabidopsis mutant hcf101-1 was described (1). The T-DNA insertion line 296G02, named hcf101-2, was provided by Dr. B. Weisshaar (Max Planck Institute for Plant Breeding Research, Cologne, Germany). Seeds were germinated on a sulfadiazine-containing medium (15 μg/ml). Seedlings from a segregating population with a hcf phenotype were further analyzed. The T-DNA insertion site in hcf101-2 is located in the 5'-region of the gene (54 bp upstream of the ATG codon), as confirmed by PCR and sequence analyses. The T-DNA insertion line N54397 (Nottingham Arabidopsis Stock Center, Nottingham, Great Britain; 30), named hcf101-3, was propagated on kanamycin (50 μg/ml) plates. Segregation analyses confirmed that hcf phenotype is caused by a T-DNA insertion in the DNA for the 5'-untranslated leader region (66 bp downstream of the stop codon). For the experiment shown in Fig. 9, 12-day-old etiolated seedlings were illuminated for 4, 24, or 72 h with continuous white light.

**PAM101/PDA-100 Measurements—** In vivo Chl a measurements were performed with 18-day-old Arabidopsis seedlings using the pulse amplitude-modulated fluorometer PAM101 equipped with a PAM data.
acquisition system (PDA-100, Walz, Effeltrich, Germany). Prior to measurements, the fiber optic of the emitter/detector unit (101-ED) was positioned closely to the upper surface of the plants, which were dark-adapted for 7 min before the minimal fluorescence $F_{0}$ was recorded. A saturating white light pulse of 6000 $\mu$mol m$^{-2}$ s$^{-1}$ for 600 ms was used to determine the maximal fluorescence $F_{\text{m}}$ and the $F_{0}/F_{\text{m}}$ ratio. After 1 min, actinic red light (650 nm, 40 $\mu$mol m$^{-2}$ s$^{-1}$) emitted from a photodiode (102-L) was turned on, and the fluorescence parameter $F_{\alpha}$ of illuminated leaves was determined by the application of saturating flashes every 30 s until a stable fluorescence level ($F_{\alpha}$) was reached. Subsequently, the actinic light was switched off to determine the minimal fluorescence $F_{\text{r}}$ in the light-adapted state. The fluorescence quenching parameter $q_{P}$ (photochemical quenching) was calculated as $q_{P} = (F_{\alpha} - F_{\text{r}})/(F_{\text{m}} - F_{\text{r}})$, whereas $F_{\alpha}$ (steady state fluorescence) was determined as $F_{\alpha} = F_{\text{m}} - F_{\text{r}}$. The quantum yield of PSI ($\Phi$PSII) was calculated as $\Phi$PSII = $F_{\alpha}/(F_{\text{m}} - F_{\text{r}})$. $F_{\text{r}}$ Absorbance Changes—The light-induced in vivo absorbance changes of $F_{\text{r}}$ at 810 nm were measured using the PAM101/PDA-100 fluorometer connected to a dual wavelength emitter/detector unit (ED P700WD). Saturating far-red light (730 nm, 15 watts m$^{-2}$) emitted by a far-red diode (102-PR) for 1 min was applied to oxidize $F_{\text{r}}$. After 30 s of far-red light, a strong white light pulse of 6000 $\mu$mol m$^{-2}$ s$^{-1}$ was applied for 400 ms. The maximum signal difference ($\Delta A_{\text{10min}}$) between the reduced and the oxidized states of $F_{\text{r}}$ was used to estimate the photochemical capacity of PSI (31). 77 K Fluorescence Emission Spectra—Fluorescence emission spectra at 77 K were recorded from 650 to 800 nm after excitation at 480 nm using the spectrometer (LS50B; PerkinElmer Life Sciences). 18-day-old plants were homogenized in 0.53 M sorbitol, 50 mM Hepes, pH 8, 1 mM MgCl$_2$, and 2 mM N$_{2}$EDTA. The slurry was filtered through two layers of Miracloth and centrifuged at 10,000 $\times$ g for 15 min at 4°C. The pellet was resuspended in the same buffer, and the Chl concentration was adjusted to 10 $\mu$g/ml. Chl concentrations were determined according to Porra et al. (32). Photoreduction Measurements—NADP$^+$ photoreduction was measured as absorption changes of NADP$^+$ at 340 nm. The reductase activity was determined in a 450-$\mu$l volume containing a thylakoid membrane homogenate (33) equivalent to 10 mg of Chl. The measurement was performed in the presence of 0.5 mM NADP$^+$, 5 $\mu$m ferredoxin, 0.5 $\mu$m ferredoxin-NADP$^+$- oxidoreductase from spinach (Sigma), 5 $\mu$m plastocyanin, 15 mM tricine, pH 7.6, 5 mM MgCl$_2$, 2 mM sodium ascorbate, 0.5% B-demicylalcohol, and 3 $\mu$m 2,6-dichlorophenolindophenol. Ferredoxin and plastocyanin were isolated from C. reinhardtii as described (34–36). The concentrations of ferredoxin and plastocyanin were determined according to Wood (37) and Katoh et al. (38). Samples were illuminated with high intensity light-emitting diodes. Antiserum Production, Purification of Antibodies, and Immunoblot Analyses—A DNA fragment encoding the amino acid residues 206–250 of Hcf101 was amplified by PCR from an Arabidopsis cDNA library (39) using Pfu DNA polymerase (Invitrogen). The primers generated SacII and Sall sites (5'-GTCACCGGGCTCTAGAGACCTATCTATGATG-3' and 5'-GGCTGTCGACCTAGACTTCGACTGGAGAC-3'), respectively. The resulting fragment was cloned into a T vector. The plasmid was digested with HindIII and XhoI and cloned into the HindIII and XhoI sites of the E. coli-derived expression vector. The resulting plasmid was transformed into the E. coli strain XL1-blue. The Hcf101-GST fusion protein was expressed in E. coli strain XL1-blue and purified by affinity chromatography using glutathione-Sepharose 4B. The fusion protein was digested with thrombin, and the GST tag was removed. The purified Hcf101-GST fusion protein was used for immunization. Immunoprecipitations with protein A-Sepharose were described (41). Radiolabeling of Proteins—14-day-old mutant and wild type seedlings were incubated in reaction buffer containing 20 mM KH$_2$PO$_4$, pH 6.3, 0.1% (v/v) Tween 20, and 40 $\mu$g/ml cycloheximide at room temperature and ambient light. After 30 min, the medium was replaced by reaction buffer containing 25 $\mu$Ci/ml [methyl-3H]methionine (specific activity >1000 Ci/mmol) and 20 $\mu$g/ml cycloheximide. 30 min later, the leaves were harvested, washed twice with distilled water, and frozen at 70°C. Insoluble membrane proteins were resuspended in 100 mM Na$_2$CO$_3$ and 50 mM dithiothreitol. After trichloroacetic acid precipitation, the radioactivity in the pellet was determined by scintillation counting (Beckman LS6500, Munich, Germany). Immunoprecipitations with protein A-Sepharose were described (41). Isolation of Chloroplasts and Total Membrane and Soluble Proteins—Chloroplasts for immunolocalization analyses were isolated from 18-day-old plants. The chloroplast-enriched fraction was purified on a Percoll gradient. Intact chloroplasts were washed twice with isolation medium (0.3 M sorbitol, 5 mM MgCl$_2$, 5 mM EGTA, 5 mM Na$_2$EDTA, 20 mM Hepes-KOH, pH 8, and 10 mM MgCl$_2$). The stromal membrane fractions were separated by centrifugation (20,000 $\times$ g for 20 min). The soluble proteins from the supernatant were precipitated with trichloroacetic acid and resuspended in 100 mM Na$_2$CO$_3$, 10% (v/v) sucrose, and 50 mM dithiothreitol. The membrane proteins in the pellet were resuspended in breaking buffer. Positional Cloning of hcf101 A segregating F$_2$ progeny was generated by crosses of male pollen donor plants of heterozygous lines of hcf101-1 with Columbia background with female recipient plants of ecotype Landsberg, followed by selfing of the resulting F$_2$ plants. To assign the mutant locus to one of the Arabidopsis chromosomes, 28 F$_2$ plants homozygous for the wild type hcf101 locus were used to produce non-segregating F$_3$ families for restriction fragment length polymorphism (RFLP) analyses with the pARMS set (44). DNA for RFLP analysis was isolated according to Dellaporta et al. (45). For high resolution mapping, genomic DNA from single leaves of individual F$_2$ plants was isolated. The simple sequence length polymorphism marker nga126 and the cleaved amplified polymorphic sequence (CAPS) markers m255, m256, T3250, and g4711, which were used for fine mapping procedures, were described previously (46, 47). The newly developed CAPS markers mju8/IV (5'-GGCCGGTTATTTACGACCACT-3') and 5'-GACCTACATGACCTTGGTGC-5') on the P1 clone MUS8 (AB028821), K8 (5'-CGATCGAGCTAATCGGAT-
CAGC-3′ and 5′-GCCTCTACTTTCATGGACC-3′) and KpaI/V (5′-CA-
GAACTGCTAGTCGG-3′ and 5′-ATAACTAGATGACTGTTCCACCG-3′) on the TAC clone K7M2 (AP000382), and MXP5-X
(5′-CGAGTTTCTCTTGTTGAGCC-3′ and 5′-CGTTCGCTCACTCCAGC-3′) on the P1 clone MXP5 (AP002048) showed polymorphisms
between ecotypes Columbia and Landsberg for the restriction enzymes VspI, XhoI, HpaI, and EcoRI, respectively.

RT-PCR and Northern Blot Analyses—Total mRNA from 18-day-old wild type and mutant plants were isolated according to Ref. 1. RT-PCR
was performed with 5′-GCTGATGTCTATGGTCCAAGTCTACC-3′ and 5′-CAATTACCGCTGCTGTCAATGGCGC-3′ and the Titan One Tube
RT-PCR system (Roche Diagnostics). PCR fragments were separated on a 5% polyacrylamide gel. Northern blot analyses were performed as
The reaction center core subunit PsaA was reduced by any of the subunits PsaA, PsaC, PsaF, PsaG, and PsaH (Fig. 3). Primary electron acceptor QA is reduced and that the electrons (qP) could be measured in 

\[ \text{versus} \ 8.6 \pm 0.2 \ \mu \text{g}\text{mg}^{-1} \text{fresh weight}. \]

The decreased Chl a/b-ratio of the mutant (e.g. 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), 3.26 \pm 0.01 wild type \( \text{versus} \ 2.60 \pm 0.01 \text{hcf101-1} \) indicates a loss of Chl a-associated proteins.

The mutant hcf101-1 did not show any detectable absorbance changes of P\(_{700}\) at 810 nm, indicating that PSI is not functional (cf. Table I; Ref. 1). This was confirmed by fluorescence measurements at 77 K (Fig. 1A). The emission band at 733 nm in the wild type, characteristic for a functional PSI, was shifted to 727 nm in the mutant. This blue shift indicates an impaired transfer of excitation energy from Lhca1/Lhca4 to the PSI core. Because all four Lhca proteins are only slightly reduced under our growth conditions (\(-80\% \) compared with the wild type; Fig. 1B), the impairment is likely to be caused by lesions in those PSI proteins that are directly involved in electron transfer. In older seedlings or seedlings kept under high light intensities, the overall level of Lhca proteins decreases, with Lhca3 being the most sensitive protein (data not shown). Finally, no NADP\(^+\) photoreduction can be detected with thylakoid membranes of hcf101-1 (wild type value 18.9 \pm 1.5 \mu \text{mol NADPH mg}^{-1} \text{Chl h}^{-1}).

Measurements of the Chl a fluorescence induction showed a significantly reduced ratio of variable to maximum fluorescence (F\(_{v}/F\(_{m}\)), (cf. Table I; Ref. 1). No photochemical quenching (qP) could be measured in hcf101-1, suggesting that the primary electron acceptor QA is reduced and that the electrons released from PSII accumulate in the plastoquinone pool. Taken together, these data clearly indicate that the mutation primarily affects the electron transport through PSI (cf. also Ref. 1).

Northern analysis with representative genes for nuclear and plastid-encoded PSI subunits demonstrated that the mutation had no effect on the accumulation of transcripts (Fig. 2). Immunoblot analyses uncovered that hcf101-1 contains barely any of the subunits PsaA, PsaC, PsaF, PsaG, and PsaH (Fig. 3). The reaction center core subunit PsaA was reduced by \(>95\%\). Representative proteins of the PSII (PsbO) and the cytochrome b\(_{58}\) complex (PetB) were not affected in the mutant (Fig. 3). In contrast, in \textit{vivo} labeling experiments of wild type and hcf101-1 proteins with \[^{35}\text{S}\]methionine (Fig. 4) and the immunoprecipitation of PsaA (Fig. 4, \textit{bottom}) demonstrated that photosystem I subunits are synthesized in the mutant. This indicates that accelerated degradation rather than a block in protein synthesis is responsible for the reduced amounts of PSI reaction center polypeptides in hcf101-1.

Lack of PSI in hcf101-1 is accompanied by changes in the plastid ultrastructure (Fig. 5). No stoma lamellae and assimiatory starch can be detected in the mutant. Furthermore, the grana stacks, located in parallel bundles in wild type organelles, appear to be less organized in the mutant, presumably due to the lack of connecting stromal thylakoids.

Map-based Cloning of hcf101-1—The gene hcf101 has been mapped on chromosome 3 using the pARMS set (44) and four previously existing PCR-based molecular markers (nga126, m255, T32C9, and ab13; cf. “Experimental Procedures”). Finally, the CAPS markers T32C9 and ab13 were chosen for high resolution mapping of 1356 F\(_{2}\) individuals deriving from backcrosses to the ecotype Landsberg (Fig. 6A). These two markers enclose the mutant locus with 49 and 29 recombinations, respectively. The appearance of the hcf phenotype was examined by segregation analysis of the following progeny. Two newly developed CAPS markers, K8 and MXP5-X, could localize hcf101 on the TAC clone K7M2 (AP000382) with two recombinations each. No recombination event could be observed between the mutation and the CAPS marker K\(_{2}\)M\(_{2}\)V, which was found to be located within hcf101.

A \(\lambda\)-ZAP-cDNA library prepared from \textit{Arabidopsis} leaves was used to isolate the hcf101 full-length cDNA (39). Comparison to the genomic sequence uncovered a complex exon-intron structure (Fig. 6B). The mutant sequence contains a single G to A exchange of the last nucleotide of intron 7. RT-PCR confirmed the failure of proper splicing of this intron in the mutant (Fig. 6C). Sequence analyses uncovered that the mutant is utilizing an alternative splice site 30 bases upstream of the 3’-splice site. Within this 30 bases, two in-frame stop codons are responsible for the complete loss of function of Hcf101 in the mutant.

The open reading frame of hcf101 encodes a polypeptide of 532 amino acids with a predicted molecular mass of 57.8 kDa (Fig. 7). Analysis of the N-terminal sequence using the prediction programs ChloroP (49) and TargetP (50, 51) revealed a putative plastid-directing transit sequence of 63 amino acids, consistent with the high frequency of Ala and Leu residues in the N-terminal part of the polypeptide (52). Thus, the predicted mature protein possesses an estimated molecular mass of 50.5 kDa.

Localization of Hcf101—A polyclonal antiserum was raised

![Fig. 5. Electron micrographs of chloroplasts from wild type (WT) and hcf101-1 leaves.](http://www.jbc.org/).
against the recombinant Hcf101 protein (amino acids 206–532) and purified as described under "Experimental Procedures." The purified antiserum detects a polypeptide with an apparent molecular mass of ~110 kDa in the soluble fraction of wild type leaf extracts, slightly higher than the predicted molecular mass (Fig. 8A; cf. "Experimental Procedures"). No protein was detected in the mutant. Antisera against the integral protein PsaF of PSI and the extrinsic 33-kDa subunit of PSII were used as a control (Fig. 8A). Immunoblot analyses of soluble and membrane fractions from Percoll-purified chloroplasts revealed that the majority of Hcf101 is located in the stroma (Fig. 8B). The small amount of Hcf101 in the membrane fraction disappears after washing with 0.1M sodium carbonate (data not shown). The protein is already detectable in etiolated material and accumulates after transfer of the seedlings to white light (Fig. 9A). No Hcf101 is detectable in root protein extracts (Fig. 9B).

Hcf101 Encodes an MRP-like Protein—Analysis of the primary sequence of Hcf101 did not reveal any obvious function. The highest similarities were found to nucleotide-binding and MRP-like proteins from pro and eukaryotic organisms (cf. "Discussion"). This includes an unknown protein from Oryza sativa (NP_916683; 87% identity, 95% similarity) as well as open reading frames from Synechocystis sp. PCC 6803 (NP_442227; 42% identity, 59% similarity) and Anabaena sp. PCC 7120 (NP_484696; 43% identity, 61% similarity). Hcf101 also shares similarities to a hypothetical 30.2-kDa protein from Pseudomonas fragi (P72190; 46% identity, 60% similarity), an MRP protein from E. coli (P21590; 44% identity, 61% similarity), and a hypothetical protein from Homo sapiens (AAH24919; 40% identity, 58% similarity). Taken together, BLAST P searches identified 65 proteins with unknown functions. In addition, Hcf101 appears to contain epitopes known from other proteins, such as the “Walker A box” (53). This box contains a hydrophobic β-strand followed by a loop (P-loop, residues 181–192; Ref. 54) with conserved Gly residues, (AG)X4G(K/ST) (Walker A motif), and an β-helix as well as an additional motif, A (residues 205–215), adjacent to the Walker A box. It is notable that the first conserved Gly residue in the Walker A motif is exchanged to a Cys residue in Hcf101 and its rice homolog. Furthermore, a third conserved domain of unknown function 100 amino acids behind the N-terminal P-loop is present in all MRP proteins. Within this domain another conserved motif, B, is found (55) that consists of a hydrophobic β-strand ending with an Asp residue (amino acid residues 285–290 in Hcf101). These four motifs are clustered in an 120-amino acid-long segment (Fig. 7). In addition, the region 79–155 at the N terminus matches to the DUF59 domain. This domain is present in proteins of prokaryotic ring-hydroxylating multiprotein complexes (56).
both mutants (Table I). Although the $F_v/F_m$ of hcf101-2 resembled that of hcf101-1, the value of hcf101-3 was less affected (Table I). PCR analysis of homozygous plants with an hcf phenotype confirmed the data bank (TAIR; www.arabidopsis.org) information that the insertions of the foreign DNA are located in the hcf101 gene. In hcf101-2, the insertion is located 54 bp 5'/H11032 to the ATG codon, whereas in hcf101-3 the insertion is present in the 3'-untranslated DNA sequence, 66 bp downstream of the TAG codon. Based on the analysis of available cDNAs, the insertion in hcf101-2 is either located at the very 5'-end of the 5'-untranslated leader region or in the promoter region for the basal transcription apparatus. In hcf101-2, barely any PSI activity can be detected, consistent with the observations that Hcf101 as well as PsaA, PsaD, and PsaF are at their detection limits (Fig. 10). In contrast, significant amounts of Hcf101 and the PSI subunits tested, including PsaA, accumulate in hcf101-3, although the extent of the reduction of the photosystem I subunits differed. We found that the reduction of Hcf101 correlated with that found for PsaA. Whereas, the PsaD and PsaF protein levels were only moderately reduced in hcf101-3 (90% or more for PsaA versus 75% or more for PsaD and PsaF compared with the wild type) (Fig. 10; cf. "Discussion").

**DISCUSSION**

Several regulatory proteins involved in PSI biogenesis of higher plants, *Chlamydomonas*, and *Synechocystis* have been isolated and characterized (8, 9, 15–18, 21–23, 57, 58). Hcf101

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**Fig. 7. ClustalW alignment of Hcf101 from *A. thaliana* (A.t.; GenBank™ accession number CAD90253) with *O. sativa* (O.s.; GenBank™ accession number NP_916683); *Synechocystis* sp. PCC 6803 (Syn.; GenBank™ accession number NP_442227), and *Anabaena* sp. PCC 7120 (Anab.; GenBank™ accession number NP_484696). A deduced consensus sequence is indicated. Identical amino acids are marked with stars; conserved exchanges are dotted. The functional domains are green, and the motifs A' and B are boxed in yellow. The secondary structure indicated above was determined by Spro (promoter.ics.uci.edu/BRNN-PRED/). The predicted cleavage sites of the amino-terminal transit sequence are indicated as red lines.**
is a new protein that can be added to this list. Mutation of hcf101 affects almost exclusively PSI, whereas PSII and the cytochrome b_{6}f complex remain unaltered. This demonstrates a high degree of specificity of Hcf101 function.

Chl a fluorescence measurements demonstrate that the ratio of variable to maximum fluorescence is considerably reduced in the mutant (Table I). The increase in Q_{A} reduction (Table I) further indicates that the electron flow downstream of PSII is limiting. Determination of absorbance changes of P_{700} at 810 nm revealed that PSI in hcf101-1 is not functional (Table I; cf. Ref. 1). Western analysis confirmed that essential subunits of the reaction center are either absent or severely reduced in hcf101-1 (Fig. 3). Furthermore, the 6-nm blue shift in the 77 K fluorescence emission spectrum of the mutant (Fig. 1A) is in agreement with the notion that the transfer of excitation energy from Lhca1/Lhca4 to the PSI reaction center P_{700} is impaired. A comparable blue shift was also reported in plants lacking PsaF (59). The fluorescence data are consistent with the observation that the Lhca1-4 proteins are still detectable in the mutant although they cannot be associated with PSI. Under our growth conditions, Lhca3 is not specifically reduced in the mutant (Fig. 1B), although this antenna protein appears to be the most sensitive one under stress conditions (data not shown). Finally, the loss of PSI in hcf101 has strong effects on the organization of the thylakoid membrane (Fig. 5). Stacking of the grana thylakoids is enlarged whereas stroma thylakoids are barely detectable. Like other photosynthetic mutants, hcf101 does not accumulate assimilatory starch (59–61).

Inactivation of hcf101 leads to PSI deficiency and the inability of the mutant to grow photoautotrophically. Because transcripts for representative plastid and nuclear encoded subunits of the PSI reaction center are present in wild type amounts in the mutant, it is unlikely that Hcf101 plays a role in transcription and/or transcript accumulation (Fig. 2). Furthermore, in vivo labeling experiments of thylakoid proteins revealed that major subunits of PSI reaction center are synthesized in hcf101 (Fig. 4). The prediction of a plastid transit peptide suggests that Hcf101 is a chloroplast protein. This was confirmed by cell fractionation and immunoblot analyses (Fig. 8). These experiments also revealed that Hcf101 is a soluble protein and only loosely associated with membranes. The protein can easily be released from thylakoid membranes by salt treatments, suggesting that it is not involved in PSI-related functions in the membranes. Furthermore, we could not detect Hcf101 in highly purified PSI particles (data not shown). Comparison of hcf101-1, hcf101-2, and hcf101-3 suggests that proteins constituting the reaction center core complex are the major target site for Hcf101 function (Figs. 3 and 10). This is supported by the observation that reduced levels of Hcf101 result primarily in the loss of PsaA, whereas PsaD and PsaF are less affected
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(Fig. 10). Thus, Hcf101 appears to be required for the assembly and/or stabilization of the complex. This might be caused by a stabilizing effect on the PSI core subunits, on the availability and/or binding of any of the cofactors to these subunits, or on the assembly of the subunits or of the cofactors into a functional PSI complex. Because all these processes appear to be controlled from the stromal side of the membrane, it is tempting to speculate that Hcf101 is involved in delivering components from the stroma to the assembly site of PSI in the stroma thylakoids.

The cyanobacterial and plant PSI core complex can be assembled and remain functional even without the subunits E, F, H, I, J, K, L, and N (21). The assembly of PsaC, PsaD, and PsaE occurs without help of other proteins but is dependent on the formation of both PsaC iron-sulfur centers (3, 62). RubA, a rubredoxin-like protein of Synechococcus sp. PCC 7002, seems to be essential for the binding of F$_x$ to PsaA/B (8, 9). Therefore, it might be possible that Hcf101 assists in the assembly of PsaA and PsaB by folding them or by delivering co-factors to the heterodimer or PsaC. Because iron starvation strongly effects Hcf101 accumulation,$^2$ we are currently examining a possible role of Hcf101 in delivering iron to the reaction center.

Alternatively, Hcf101 might be involved in the degradation of the core. As a consequence, the absence of any of the core subunits destabilizes PSI (10, 11) and causes an increased turnover of the peripheral subunits. Considering individual PSI subunits, it is unlikely that Hcf101 affects the plastid-encoded subunits PsaI or PsaJ directly, because disruption of these two subunits does not affect photoautotrophic growth of Chlamydomonas (63) and Synechocystis (64) cells, respectively. Hcf101 exhibits sequence similarities to MRP-like proteins (INTERPRO entry IPR000808) that contain a characteristic role of Hcf101 in delivering iron to the reaction center. It might be possible that Hcf101 assists in the assembly of PsaA and/or binding of any of the co-factors to these subunits, or on the peripheral subunits are present in excess. It remains to be determined where these subunits are located in the thylakoid membrane and whether they are stable, even though they do not seem to be assembled into a functional PSI complex. This result also suggests that PsaA rather than the peripheral proteins are the major target site of Hcf101 function. Finally, it is interesting to note that the insertions in the flanking sequences of Hcf101 have different effects. Insertion in the PsaA region abolishes Hcf101 accumulation almost completely, whereas the insertion in the 3'-untranslated region in hcf101-3 is less effective (Fig. 10). More detailed studies are required to elucidate this scenario.

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