A Small Chloroplast-encoded Protein as a Novel Architectural Component of the Light-harvesting Antenna

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Abstract. A small conserved open reading frame in the plastid genome, ycf9, encodes a putative membrane protein of 62 amino acids. To determine the function of this reading frame we have constructed a knockout allele for targeted disruption of ycf9. This allele was introduced into the tobacco plastid genome by biolistic transformation to replace the wild-type ycf9 allele. Homoplasmic ycf9 knockout plants displayed no phenotype under normal growth conditions. However, under low light conditions, their growth rate was significantly reduced as compared with the wild-type, due to a lowered efficiency of the light reaction of photosynthesis. We show that this phenotype is caused by the deficiency in a pigment–protein complex of the light-harvesting antenna of photosystem II and hence by a reduced efficiency of photon capture when light availability is limiting. Our results indicate that, in contrast to the current view, light-harvesting complexes do not only consist of the classical pigment-binding proteins, but may contain small structural subunits in addition. These subunits appear to be crucial architectural factors for the assembly and/or maintenance of stable light-harvesting complexes.

Key words: chloroplast • LHCII • light-harvesting complex • photosynthesis • ycf9

Introduction

During the past decade, the plastid genomes from a number of higher and lower plant species have been completely sequenced. The picture that has emerged from these analyses is that plastid genomes show a remarkably conserved structure and coding capacity across large phylogenetic distances. The vast majority of plastid genome-encoded genes can be grouped into two classes: genetic system genes (rRNAs, tRNAs, ribosomal proteins, subunits of an Escherichia coli-like RNA polymerase) and photosynthesis genes (subunits of the photosystems I and II, the cytochrome b6f complex, the ATP synthase, large subunit of RuBisCO). The few remaining genes have functions other than gene expression and photosynthesis and specify, for example, components of certain biosynthetic pathways (e.g., an acetyl-CoA-carboxylase subunit).

In addition to the >100 identified plastid genes, the chloroplast genomes of higher plants carry a number of open reading frames of unknown function designated ycf (hypothetical chloroplast reading frames; for an overview see Maier et al., 1995). These reading frames usually display strong interspecific conservation and therefore are assumed to be genuine genes. Recent methodological progress has opened new avenues to the functional characterization of plastid genome-encoded open reading frames. The successful development of transformation technologies for chloroplast genomes (Boynton et al., 1988; Svab et al., 1990; Svab and Maliga, 1993) has facilitated reverse genetics approaches in plastids. The presence of an efficient homologous recombination system in chloroplasts allows the targeted inactivation of plastid-encoded reading frames by deletional or insertional mutagenesis (for review see Rochaix, 1997; Bock et al., 1999). The resulting transplastomic plants can be purified to homoplasmy: all wild-type plastid genome copies are eliminated through continuous antibiotic selection and the plants eventually carry a homogeneous population of transformed plastid genomes. Such homoplasmic plants will be deficient for the gene product encoded by the open reading frame of interest and detailed analysis of their phenotype will allow conclusions about the function(s) of the reading frame in the wild-type. Reverse genetics experiments in the unicellular green alga Chlamydomonas reinhardtii (Monod et al., 1994; Takahashi et al., 1996) and, more recently, in the higher plant tobacco (Ruf et al., 1997; Burrows et al., 1998) have contributed substantially to the advancement of our understanding of both photosynthesis and chloroplast gene expression.
In this work, we have analyzed by reverse genetics a small conserved chloroplast open reading frame, ycf9. Generation of knockout tobacco plants revealed that ycf9 is a photosynthesis-related gene which, however, is not essential for photoautotrophic growth. We show that ycf9 encodes a novel structural component of a light-harvesting complex (LHC)
1 of photosystem II (PSII). This component appears to be required for the stable integration of the pigment-binding LHC protein CP26 into the antenna of PSII.

Materials and Methods

Plant Material and Growth Conditions

Sterile tobacco plants (Nicotiana tabacum cv. Petit Havana) were grown on agar-solidified MS medium containing 30 g/liter sucrose (Murashige and Skoog, 1962). Homoplasmic transplastomic lines were rooted and propagated on the same medium. For protein isolation and physiological measurements, wild-type and transformed plants were planted in pots and kept under greenhouse conditions. Plants grown under standard light (4,000–7,000 lux), high light (27,000 lux), or low light (100–300 lux) conditions were used for physiological measurements.

Construction of a Plastid Transformation Vector for Inactivation of ycf9

The region of the tobacco chloroplast genome containing the ycf9 reading frame was excised from a SalI pTDNA clone as a BamH1/Kpn1 fragment (Shinozaki et al., 1986; Fig. 1 A). The fragment was ligated into a similarly cut Bluescript vector (Stratagene). The ycf9 knockout allele was created by digestion with MunI, followed by a fill-in reaction of the recessed ends with Klenow DNA polymerase. A chimeric aadA gene conferring resistance to aminoglycoside antibiotics (Svab and Maliga, 1993) was inserted into the blunt MunI site to disrupt ycf9 and to facilitate selection of plastid transformants. A plasmid clone carrying the aadA gene in the same orientation as ycf9 yielded transformation vector pycf9 (Fig. 1 B).

Chloroplast Transformation and Selection of Homoplasmic Transplastomic Tobacco Lines

Young leaves from sterile tobacco plants were bombarded with plasmid pycf9-coated tungsten particles using a biolistic gun (PD1000000; Bio-Rad). Primary spectromycin-resistant lines were selected on R MOP regeneration medium containing 500 mg/liter spectinomycin (Machold et al., 1979). To analyze the association of the Ycf9 protein with the thylakoid membrane, purified thylakoids from wild-type and mutant plants (75 μg chlorophyll per 300 μl wash solution) were washed for 20 min at room temperature in 2 M NaCl, 2 M KJ, 0.1 M Na2CO3, 0.1 M NaOH, or 4 M urea. After centrifugation for 20 min at 40,000 g, pelleted thylakoids were washed once with a solution of 20 mM EDTA and 50 mM Hepes, pH 8.0. Washed thylakoids and supernatants were subsequently analyzed by PA GE.

Purification of LHC complexes and other protein complexes of the thylakoid membrane was carried out according to published protocols (Feng and McCarty, 1990; Eshaghi et al., 1999).

SDS-PAGE and Western Blot Analyses

Isolated thylakoid or soluble proteins were separated on tricine-SDS polyacrylamide gels (Schägger and von Jagow, 1987) and stained with Coo massie blue or silver, according to standard protocols (Coligan et al., 1995). For Western blot analyses, electrophoretically separated proteins were transferred to Hybond ECL nitrocellulose membranes (Amersham) using the Trans-Blot SD semidry transfer cell (BioRad) with a standard transfer buffer (182 mM glycine, 20 mM Tris, 20% methanol, 0.05% SDS). Immunoblot detection was performed using the enhanced chemiluminescence system (ECL; Amersham).

Mass Spectroscopy

Matrix-assisted laser desorption/ionization mass spectroscopy (MALDI MS) was performed with a Voyager STR DE mass spectrometer (PE Biosystems). LHCs purified from wild-type and ycf9 knockout plants were crystallized with dihydroxybenzoic acid and analyzed in the linear mode with an accelerating voltage of 20 kV. All measurements were externally calibrated for the low molecular weight range. D ata were confirmed by several independent measurements.

Physiological Measurements

Determination of PSII activity was performed on dark-adapted leaves from wild-type and mutant plants as described previously (Ruf et al., 1997). The redox state of the reaction center chlorophyll P700 of photosystem I (PSI) was monitored by following the apparent changes in absorbance of dark-adapted leaves from wild-type and mutant plants at 830-nm wavelength (Schreiber et al., 1988) using a PA M fluorometer with a modified emitter/detector unit. For reruduction of PSI, leaves were exposed to white light pulses of 4,000 μE/m2/s.

Net oxygen evolution was measured at room temperature with a Clark electrode (H ansatech) in suspensions of isolated chloroplasts lysed in a hypotonic medium (Laech, 1987). Phenyl-1,4-benzoquinone (1 mM) was used as an electron acceptor. A halogen lamp (K L1500; Schott) served as a light source and was connected to the reaction vessel through fiber optics. Different light intensities were adjusted with neutral density filters.

Results

Targeted Inactivation of the Tobacco Chloroplast ycf9 Reading Frame

The chloroplast genomes of higher plants harbor in their large single-copy region a small conserved open reading frame designated ycf9 (Fig. 1 A). The putative gene product of ycf9 is a protein of 62 amino acids (Fig. 2 A) consisting of two potential membrane-spanning helices that are highly hydrophobic (Fig. 2 B). A hydrophilic domain separates the two hydrophobic stretches from each other suggesting that the Y c9 protein spans twice either the thylakoid membrane or the inner membrane of the chloroplast
Active transcription of ycf9 (Yao et al., 1989), together with the presence of strongly homologous reading frames in cyanobacteria (Kaneko et al., 1996) and algae (Evrard et al., 1990), suggest that ycf9 is a genuine gene the product of which participates in an evolutionarily conserved cellular function. To determine the function of ycf9, we have taken a reverse genetics approach and constructed a knockout allele for targeted disruption of the tobacco ycf9 (Fig. 1). The knockout allele was introduced into the tobacco plastid genome by particle bombardment-mediated chloroplast transformation (Svab et al., 1990; Svab and Maliga, 1993). From 30 bombarded leaf samples, altogether seven chloroplast transformants were selected as verified by PCR and DNA gel blot analyses (Fig. 3). Three independent transplastomic lines (Nt-pIycf9-1, Nt-pIycf9-2, and Nt-pIycf9-3) were subjected to additional rounds of regeneration on spectinomycin-containing medium to obtain homoplasmic tissue.

Presence of Promiscuous ycf9 Copies and Homoplasmy of ycf9 Knockout Plants

According to our and others’ experience (Maliga and Nixon, 1998), homoplasmy of plastid transformants typically is achieved after subjecting the primary transformant to one to two additional rounds of regeneration on antibiotic-containing medium. We were surprised that in RFLP analyses performed after the fourth round of regeneration, all transformants carrying the ycf9 knockout allele showed, in addition to a strong band for the transplastomic fragment, a faint hybridization signal that corresponds in size to the restriction fragment from the wild-type genome (Fig. 3 A). However, we believed that the wild-type–like hybridization signal was not caused by true heteroplasmy of the chloroplast transformants, but rather by the presence of promiscuous plastid DNA in one of the other two genomes of the plant cell. It is well established that during evolution large fragments of chloroplast DNA integrated in the nuclear and mitochondrial genomes (Stern and Lonsdale, 1982; Ayliffe and Timmis, 1992; Nakazono and Hirai, 1993; Ayliffe et al., 1998). Such promiscuous plastid DNA fragments can exhibit a remarkably high degree of sequence conservation at the DNA level (Ayliffe and Timmis, 1992). If a promiscuous origin of the wild-type–like hybridization signals (Fig. 3 A) were indeed the case, these signals should be absent in Southern blot analyses using purified chloroplast DNA instead of total cellular DNA. Alternatively, if the transplastomic plants were still heteroplasmic, the wild-type signal should also persist upon analysis of isolated chloroplast DNA. When we repeated the RFLP analysis using the same restriction enzyme, but purified chloroplast DNA, no hybridization signal for the wild-type could be detected anymore (Fig. 3 B), strongly suggesting that our ycf9 knockout plants are homoplasmic and that promiscuous copies of the ycf9 region are present in tobacco cells.

H homoplasmy of ycf9 knockout plants was further confirmed by our subsequent biochemical analyses (see below) and by the lack of segregation of both phenotype and antibiotic resistance in the F1 generation.
Thylakoid Membranes from ycf9 Knockout Plants Lack a Small Protein

When transferred to the soil, ycf9 knockout plants grew photoautotrophically and displayed no obvious mutant phenotype under standard greenhouse conditions. This indicates that disruption of ycf9 does not abolish any essential function in plastid gene expression or cellular metabolism. Moreover, wild-type-like photosynthetic activity suggests that all of the major protein complexes of the thylakoid membrane (PSII, PSI, cytochrome b6f complex, and ATP synthase) are functional in ycf9 knockout plants. Intact photosynthetic electron transfer in ycf9 knockout plants was further confirmed by fluorescence measurements of PSII and PSI activities (data not shown).

Figure 3. RFLP analyses as test for homoplasmy of ycf9 knockout plants. A, Restriction analysis of total cellular DNA with BamHI. Three independently generated transplastomic lines (designated 9-1, 9-2, and 9-3; capital letters mark individually regenerated plants from one and the same line) and two wild-type controls (WT) are shown. Digestion with BamHI and hybridization to a radio-labeled ycf9-specific probe yields a 4.5-kb fragment for the wild-type genome and a 5.6-kb fragment for the transplastome (see Fig. 1). Note presence of a weak band of wild-type size in all transplastomic lines in addition to the 5.6-kb band characteristic of transformed plastid genomes. B, Restriction analysis of purified chloroplast DNA. Digestion with BamHI and hybridization to the same probe as in A detects only the 5.6-kb fragment diagnostic of the transplastome. Absence of the weak band of wild-type size detected in the total DNA samples from all transplastomic lines indicates homoplasmy and suggests the presence of promiscuous ycf9 copies DNA in an extraplastidic cellular compartment.

Figure 4. Analysis of thylakoid proteins from wild-type (WT) and ycf9 knockout plants. A, Silver staining of thylakoid proteins separated by electrophoresis in a 16.5% polyacrylamide (PA A) gel. For comparison, a mutant lacking PSI (PSI−; Ruf et al., 1997), a mutant lacking the cytochrome b6f complex (b6f−; Hager et al., 1999) and a wild-type in tissue culture (WT-tc) are also shown. The putative Y cf9 protein is marked by an arrow and is clearly present in all samples except for the thylakoids from the ycf9 knockout (9-2). Note that, as expected for a hydrophobic protein, the putative Y cf9 protein migrates faster than a protein of the calculated molecular weight of Y cf9 (6.58 kD). The asterisk marks a protein specifically reduced in ycf9 knockout plants (see part B). M1, M2: molecular weight markers. B) Coomassie staining of thylakoid proteins from the wild-type and three independently generated ycf9 knockout plants (9-1, 9-2, and 9-3). Thylakoid proteins were electrophoresed in a 10% PA A gel. Two different protein concentrations were loaded. All ycf9 knockout plants clearly show specifically a drastic reduction of a thylakoid protein with an approximate molecular weight of 25 kD (asterisk). Selected prominent thylakoid proteins are marked (AtpA / AtpB, LHCII proteins). M, molecular weight marker.
Since the computer analyses had predicted membrane localization of the putative Ycf9 gene product (Fig. 2), we suspected the Ycf9 protein to be present in thylakoid membranes. Therefore, we compared the protein patterns of thylakoid membranes from wild-type and ycf9 knockout plants. Indeed, in high-resolution polyacrylamide gels, we detected a single band among the low molecular weight thylakoid proteins that was completely absent from ycf9 knockout plants (Fig. 4 A) suggesting that this band could correspond to the Ycf9 gene product. The protein is not stained by Coomassie, but stains well with silver. Comparison of the thylakoid protein patterns of ycf9 knockout plants with those of tobacco mutants lacking PSI or the cytochrome b_{6}f complex (Fig. 4 A) revealed that the putative Ycf9 protein is present also in the absence of PSI or cytochrome b_{6}f. Presence of wild-type levels of all major protein complexes of the thylakoid membrane in ycf9 knockout plants was confirmed by Western blot analyses with antibodies against key proteins of PSII, PSI, the cytochrome b_{6}f complex, and the chloroplast ATP synthase (data not shown).

The Putative Ycf9 Protein Is Tightly Associated with Thylakoid Membranes

The hydrophobic properties of the potential Ycf9 protein (Fig. 2), as well as its coisolation with thylakoid membranes (Fig. 4 A), suggest that Ycf9 is a genuine thylakoid protein. To unambiguously prove the tight association of the Ycf9 gene product with the thylakoid membrane and to exclude possible isolation artifacts, we applied washes of purified thylakoids with chaotropic salts, alkali treatment of thylakoid membranes, and treatment with urea. All these conditions are known to remove proteins that are only loosely associated with membranes, as well as peripheral membrane proteins (e.g., Boudreau et al., 1997). Even upon the most stringent treatments (2 M KJ, 0.1 M Na_{2}CO_{3}, or 0.1 M NaOH) the potential Ycf9 protein remained exclusively in the membrane fraction, whereas peripheral thylakoid membrane proteins (e.g., the water-splitting complex of PSII and the CF_{1} part of the ATP synthase) were effectively released from the membranes (Fig. 5). These data confirm the tight association of the putative Ycf9 protein with photosynthetic membranes and suggest that Ycf9 is an integral thylakoid protein.

Lack of Ycf9 Gene Product Is Associated with Reduced Amounts of the LHC Protein CP26

When we analyzed the thylakoid protein patterns of wild-type and ycf9 knockout plants, we noticed that the absence of Ycf9 gene product is accompanied by a significant reduction in the amount of a single thylakoid protein of ~25 kD (Fig. 4 B). As it seemed reasonable to us to assume that the function of the Ycf9 gene product is related to this 25-kD protein, we were interested in identifying the association of this protein with a specific protein complex in the thylakoid membrane. Therefore, we fractionated thylakoid membranes and isolated all major protein complexes. All fractions were subsequently assayed for the presence of the 25-kD protein that could be identified easily, making use of the quantitative difference between the wild-type and the ycf9 knockout (Fig. 4 B). In these analyses, the 25-kD protein was detected exclusively in purified thylakoid membranes, and treatment with urea, KJ, Na_{2}CO_{3}, or NaOH effectively dissolves peripheral thylakoid proteins formed in the supernatants (Sup) are labeled: AtpA/B, CF_{1}γ/β subunits of the ATP synthase; PsbO, 33-kD manganese-stabilizing protein of the oxygen-evolving complex of PSII. The putative Ycf9 protein is marked with an arrow and clearly remains membrane-associated upon all treatments. M1 and M2, molecular weight markers.
LHCs of PSII (Fig. 6 A), but not in any of the other purified thylakoidal protein complexes (PSI, PSII, or ATP synthase; data not shown). The isolated LHCs showed the typical pattern of pigment-binding LHC proteins (e.g., Boekema et al., 1999) and readily identified the 25-kD protein as being the LHC protein CP26 (Fig. 6 A). The molecular identity of the 25-kD protein was confirmed by Western blotting with antibodies against selected subunits of the LHCs of PSI and PSII (Fig. 6 B and data not shown).

The Ycf9 Protein Copurifies with PSII LHCs

To establish a clear link between the absence of Ycf9 gene product and the reduction of the LHC protein CP26, we set out to test whether the putative Ycf9 protein is also part of the LHCs of PSII. As Ycf9 stains well with silver (Figs. 4 A and 5), we separated purified thylakoidal protein complexes from the wild-type and the ycf9 knockout mutant on high-percentage polyacrylamide gels that were subsequently subjected to silver staining. Like CP26, the putative Y cf9 protein was detected exclusively in purified LHC (Fig. 6 C and data not shown). No difference between the wild-type and the ycf9 knockout plants was detectable with either Coomassie or silver staining in any of the other protein complexes of the thylakoid membrane (not shown).

To unambiguously demonstrate that the single small thylakoid protein missing in the knockout plants is indeed the Ycf9 protein, we comparatively analyzed purified LHCs from the wild-type and the mutant by MALDI-TOF mass spectroscopy, a technique suitable to determine molecular masses with extraordinarily high accuracy. These analyses detected a single low molecular weight protein present in wild-type LHCs, but absent from mutant LHCs. The molecular mass of this protein was determined to be 6,581 D, which exactly corresponds to the theoretical molecular mass of the Ycf9 protein (including the NH2-terminal formyl group of the chloroplast initiator amino acid N-formyl methionine). This confirms that the single protein present in the wild-type, but missing from thylakoid preparations, as well as LHC preparations of ycf9 knockout plants (Figs. 4 A, 5, and 6 C), is indeed the Ycf9 gene product.

In sum, presence of the Ycf9 and CP26 proteins in one and the same complex confirms that ycf9 knockout plants have a specific defect in the light-harvesting antenna of PSII and, moreover, suggests that the reduced amounts of CP26 in the knockout plants are directly correlated with the absence of the Ycf9 protein.

Our finding that the ycf9 gene product functions in photosynthesis is consistent with the absence of the ycf9 reading frame from the plastid genome of the nonphotosynthetic holoparasitic plant Epifagus virginiana, which has lost practically all plastid-encoded photosynthesis-related genes during evolution (Wolfe et al., 1992).

The Ycf9 Protein Is Likely to Act as Stability Factor for CP26

Several possible reasons can be envisaged for the reduced amounts of CP26 in the absence of Y cf9 protein. Reasonable hypotheses would be, for example, that Y cf9 assists in the incorporation of CP26 into the PSII antenna or, alter-
The CP26 with PSII. To assess the amount of CP26 present in
natively, that Ycf9 is required for the stable association of
CP26 in mature leaf tissue of ycf9 knockout plants. The CP29
protein cross-reacting with this antibody (Di Paolo et al., 1990;
Fig. 6 B) is also indicated.

Discussion
LHCs act as antennae for the absorption of light energy in
photosynthetic membranes. They are pigment-rich protein
fraction of significant amounts of free CP26 in the stroma nor
presence of any CP26 degradation products was detected
in ycf9 knockout plants (Fig. 7 B and data not shown).
This is consistent with the idea that CP26 is only stable
when incorporated in the thylakoid membrane, whereas
unassembled or disassembled CP26 is degraded rapidly.

ycf9 Knockout Plants Have a Reduced
Photosynthetic Capacity

CP26 is part of the light-harvesting antenna of PSII and
forms a subcomplex also referred to as LHCIIc (for review
see, e.g., Wollman et al., 1999). CP26 builds one of the
three so-called minor LHCs. Together with the other
minor complexes, CP24 (LHCId) and CP29 (LHCIIa),
and the trimeric major light-harvesting complex LHCII
(LHCIIb), CP26 forms the chlorophyll a/b-containing
antenna of PSII. Having established that the knockout of the
ycf9 reading frame results in a deficiency in the LH Cs of
PSII, we were interested in the phenotypic consequences
of this defect. This was of particular interest, since data on
specific functions of any of the subcomplexes of the PSII
antenna are largely lacking (Flachmann, 1997).

As mentioned above, ycf9 knockout plants displayed no
obvious mutant phenotype under standard growth condi-
tion indicating that, in spite of the lowered amounts of
CP26, the plants are still capable of capturing photons
quite efficiently. We also did not observe significant differ-
ences to the wild-type when plants were grown under light
stress conditions (27,000 lux), suggesting that the reduc-
tion in the CP26 LHC does not affect appreciably the pho-
toprotective function suggested for the PSII antenna.
However, when plants were kept under low light condi-
tions (100–300 lux) we noticed reduced growth of the
ycf9 knockout plants as compared with the wild-type control
plants. These analyses revealed that the ycf9
knockout plants produce significantly less oxygen than the
wild-type (Table I). This effect was particularly pro-
going to the wild-type when plants were grown under light
condition in the CP26 LHC does not affect appreciably the pho-
toprotective function suggested for the PSII antenna.
To confirm that this phenotype was caused by a reduced
efficiency of light harvesting, we measured photosynthetic
oxygen evolution of chloroplasts isolated from wild-type
and mutant plants. These analyses revealed that the ycf9
knockout plants produce significantly less oxygen than the
wild-type (Table I). This effect was particularly pro-
ounced when oxygen evolution was measured under low
light conditions (Table I), indicating that photosynthetic
water splitting is less efficient in the mutant, most proba-
ably as the result of inefficient light harvesting.

Table I. Photosynthetic Oxygen Evolution of Chloroplasts
from Wild-type and ycf9 Knockout Plants

| Photon flux density | 14 µE | 55 µE | 192 µE | 1,150 µE | 1,530 µE |
|--------------------|------|------|-------|---------|---------|
| Wild-type          | 2.91 | 13.6 | 40.8  | 116.6   | 127.5   |
| Nt-plyc9           | 1.05 | 7.4  | 28.7  | 96.0    | 118.1   |
| Nt-plyc9 (% of wild-type) | 36% | 54%  | 70%   | 82%     | 93%     |

Chloroplasts were isolated from young plants adapted to low light conditions. Oxygen
evolution values are given in µmol oxygen per hour and mg chlorophyll.
complexes that trap photons and transfer them rapidly to the reaction centers in the two photosystems. In addition to their light-harvesting function, LHCs may be involved in photoprotection and proton channeling in PSII.

The largest portion of the photosynthetic pigments present in both prokaryotic and eukaryotic photoautotrophic organisms is found associated with the LHCs. Pigment molecules are bound by LHC proteins either noncovalently (carotenoids and chlorophylls) or covalently via thioester bonds (phycobilins). The presence of many different pigment types (e.g., chlorophyll a, chlorophyll b, violaxanthin, and β-carotene) ensures optimal utilization of the sunlight by the photosynthetic apparatus, in that a wide range of spectral qualities can be absorbed.

Sequence alignments have clearly demonstrated that all pigment-binding LHC proteins exhibit significant similarity to each other. Moreover, all classical LHC proteins show a characteristic topology (three α-helical transmembrane domains with stromal-exposed NH$_2$ termini), strongly suggesting that they are evolutionarily related and that all LHC genes arose from a common ancestor. The LHC genes form a multigene family in all higher plant nuclear genomes with at least 30 members in Arabidopsis (Jansson, 1999).

The light-harvesting complex of PSII is the most abundant pigment-protein complex in plants. It consists of four subcomplexes: the trimeric major LHC and three minor complexes, CP24, CP26, and CP29. A pool of the trimeric LHC plays a crucial role in the light energy distribution between the two photosystems. This regulation of light harvesting occurs through reversible redox-controlled LHCl protein phosphorylation resulting in lateral migration of LHCl away from PSII (state transition; for review see Allen and Nilsson, 1994; Gal et al., 1994).

As all pigment-binding LHC proteins are nuclear-encoded, they are synthesized as precursor proteins in the cytosol and subsequently imported into the chloroplast compartment. Although in vitro experiments have provided some information about the assembly of LHCs inside the chloroplast (including the targeting of LHC proteins to the thylakoid membrane, pigment attachment to apo-LHC proteins, supercomplex formation with photosystem cores), many steps in antenna biogenesis and turnover are still elusive (for review see Wollman et al., 1999).

In the course of this work, we have identified a first LHC protein lacking the conserved pigment-binding motifs. This protein is encoded by the chloroplast ycf9 reading frame. As it is rather small (6.58 kD) and obviously lacks pigment-binding domains, the Ycf9 protein is unlikely to be directly involved in light harvesting. Instead, it appears to be a structural component in the absence of which the CP26 complex is unstable and tends to be lost from the PSII antenna. In accordance with the nomenclature conventions for plastid-encoded genes, we propose to rename the ycf9 reading frame lhbA (LHC of PSII associated factor 1).

Upon transfer to low light conditions, plants undergo a low irradiance-induced increase in PSII antenna size. An efficient light harvesting becomes particularly important when light availability is limiting, it is not surprising that our ycf9 knockout plants displayed a phenotype of mild growth retardation only under low light conditions (Fig. 8). This suggests that the Ycf9/LhbA protein (and hence CP26), while being of limited importance under optimal growth conditions, confer a selective advantage by contributing to maximum light harvesting under unfavorable environmental conditions.

As the lack of Ycf9/LhbA leads to destabilization of CP26, the ycf9 knockout plants provide an excellent experimental system to study the function of one of the minor LHCs, the CP26 complex. Detailed electron transfer studies by fluorescence measurements have indicated that, in the absence of CP26, energy transfer from the major PSII LHC (LHClII) to the PSII reaction center is less efficient in ycf9 knockout plants, confirming the phenotype of the mutant plants and suggesting that the CP26 complex is involved in the transmission of energy absorbed by the LHClII trimer to the PSII reaction center (Biehler, K., S. Ruf, and R. Bock, manuscript in preparation).

The LhbA/Ycf9 protein is unlikely to be the only structural protein of the light-harvesting antenna. In fact, in all LHC preparations we detected several other small proteins by silver staining (Fig. 6 C and data not shown). This may suggest that LHCs contain, in addition to the...
classical pigment-binding proteins, a second class of LHC proteins that serve important functions in the assembly and/or maintenance of stable antenna complexes. The purification and characterization of potential other members of this new class of LHC proteins is currently underway.

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