The Nuclear-encoded Chlorophyll-binding Photosystem II-S Protein Is Stable in the Absence of Pigments*

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The 22-kDa chlorophyll a/b-binding protein (CAB) (psbS gene product) is associated with photosystem II and related to the CAB gene family. Here we report that the PSII-S protein unlike other chlorophyll-binding proteins is stable in the absence of pigments. It is present in etiolated spinach plants and accumulates in the dark progressively with the cellular development of the seedlings. Furthermore, it is present in several pigment-deficient mutants. Analysis of the pigment composition of the PSII-S protein isolated from etiolated plants suggests that neither carotenoids nor chlorophyll precursors are involved in its stabilization in the dark. Exposure of etiolated spinach to light leads to further accumulation of the PSII-S protein, which appears more early than other chlorophyll-binding proteins. Accumulation of the PSII-S protein in green plants is developmentally regulated and restricted to photosynthetic tissues. It is suggested that the function of the PSII-S protein may not be light-harvesting but it could act as a ligand chaperone required for transient binding of pigments during biogenesis or turnover of chlorophyll-binding proteins. Such function would be essential for coordination between pigment biosynthesis and ligation as well as avoiding toxic effects of non-bound chlorophyll molecules.

Photosystem II (PSII)1 of higher plants and green algae is composed of more than 25 different proteins including several pigment binding subunits (1–3). Essentially two classes of such proteins can be distinguished. One class is comprised of plastid encoded chlorophyll a-binding proteins which are essential for exciton trapping and transformation of electrochemical Gibbs energy (1). This group includes the D1 (psbA)/D2 (psbD gene product) heterodimer which binds 6 chlorophyll a molecules, 2 pheophytins, 1–2 β-carotenes, and the two redox active plastoquinones (QA and QB) that are required for stable charge separation (2). In addition, CP47 (psbB) and CP43 (psbC gene product) each containing 12–15 chlorophyll a molecules form the inner antenna of PSII (2).

The second class of pigment-binding proteins comprise the family of chlorophyll a/b polypeptides encoded by the nuclear CAB genes (Lhcb1–6) which form the peripheral antenna system of PSII. These include (i) the major light-harvesting complex (LHCCI) (4, 5), containing about half of the total chlorophyll of the thylakoid membranes, and (ii) the minor antenna complexes consisting of CP29, CP26, CP24, and CP14 (5).

Recently a new type of chlorophyll a/b protein has been identified based upon genetic and biochemical characterization of the psbS gene and its gene product. The PSII-S protein was first detected as a 22-kDa subunit of PSII by immunoprecipitation experiments (6). When isolated by different protocols via chromatography (7, 8) or washing procedures (9), the PSII-S protein was never found to bind pigments or any other cofactors. Different possibilities of functional and/or stabilizing structural roles on either the donor- or the acceptor site of PSII were discussed (7–14). Later it was shown after sequencing the psbS gene that the PSII-S protein is related to the CAB gene family (15, 16). Moreover in accordance to its gene structure it has recently been shown to be able to bind both chlorophyll a and chlorophyll b (17, 18). However, the PSII-S protein possesses several distinct properties compared to the known chlorophyll a/b proteins which are all considered to have a light-harvesting antenna function. It contains an additional transmembrane spanning helix in addition to the three helices of the CAB proteins (15, 16). This fourth membrane helix is homologous to the so-called C-helix of LHCCI. The PSII-S protein binds less pigment molecules and the chlorophylls show a lower excitonic coupling compared to the other chlorophyll a/b-binding proteins (18).

The greening of etiolated plants provides a suitable experimental system for studies of the biogenesis, accumulation, and stability of chlorophyll-binding proteins (see Ref. 19). With respect to the formation and assembly of PSII some non-chlorophyll binding proteins like the extrinsic PSII-O, PSII-P, PSII-Q proteins, and cytochrome b559 are already detectable in the dark (see 20). In contrast, both the chlorophyll a-binding proteins and the chlorophyll a/b-binding proteins are stabilized by their pigments and therefore absent in etiolated tissue (21–23), even when their mRNA can be detected.

In this work we have followed the presence and accumulation of the PSII-S protein in etiolated spinach leaves and in various pigment-deficient mutants. The results show that in contrast to all previously characterized chlorophyll a/b-binding proteins this protein is unique in being stable in the absence of photosynthetic pigments or their precursors. These results and previous biophysical observations (18) suggest that the PSII-S...
protein represents a new type of chlorophyll a/b binding protein that may not have a major role as a traditional light-harvesting protein but could serve as a chlorophyll storage protein during biogenesis and turnover of chlorophyll-binding proteins.

MATERIALS AND METHODS

Plant Material—Seeds of spinach plants (Spinacia oleracea) were germinated on moistened vermiculite and grown at 20–23 °C under different light conditions.

For experiments on etiolated seedlings, spinach plants were grown in complete darkness for 14 days. Cotyledons of 20 plants were harvested after 5, 6, 7, 8, 12, and 14 days. Plants in liquid nitrogen were stored at −80 °C prior to isolation of proteins. All manipulations of dark-grown plants were performed in complete darkness without a safety light.

For experiments with green plants, seedlings were grown in complete darkness for 8 days and then transferred to white light of an intensity of 100 μE/m2/s for 48 h. Cotyledons with the upper part of the hypocotyl were collected at different stages of greening.

For experiments with green plants, spinach seedlings were grown for 2–3 weeks at 25 °C at a light intensity of 100 μE/m2/s provided by white fluorescent lamps, under a light regime of 10 h light/14 h dark (light from 08:00 to 18:00).

Seeds of wild type barley (Hordeum vulgare cv. Apex) were grown on vermiculite for 5 days at 25 °C at a light intensity of 100 μE/m2/s under a light regime of 12 h light/12 h dark (light from 08:00 to 20:00).

Seedlings from the barley mutant chlorina-f2, which lacks chlorophyll b (24), were grown under the same conditions. The nuclear barley mutants viridis-m29 and -269 are partially deficient in PSII components and LHClI. The chlorophyll content in these mutants is reduced to 25% as compared to the wild-type (25) and they exhibit a strongly reduced PSII activity. The stocks are maintained in the heterozygous condition. The mutant progeny arising from heterozygous plants were identified by their paler green color and separated from the wild-type phenotype after growing for 7 days under light/dark conditions as described for the wild-type.

Wild-type tobacco plants (Nicotiana tabacum cv. Virginia) were grown on soil for 4 weeks at 25 °C and at a light intensity of 100 μE/m2/s under a light regime of 12 h light/12 h dark. The tobacco plastome mutant SR1V35 (26), which lacks all photosynthetic pigments, was kept on agar plates with MS medium (27) supplemented with gerit (0.2%), sucrose (2%), kentin (0.2 mg/liter), and β-iodoacet acid (2 mg/liter) under the same light conditions as the wild-type. All thylakoid membranes were isolated as described previously (28).

Protein Analysis—SDS-polyacrylamide gel electrophoresis was performed according to Ref. 29; gels were stained with silver nitrate. Immunoblotting was performed according to Ref. 30 using polyvinylidene difluoride membranes; the antigen-antibody complexes were detected by enhanced chemiluminescence (Amersham Corp.). X-ray films with signals linear in intensity with exposure time (Amersham) were scanned at 600 nm for quantification using a Molecular Dynamics Personal Densitometer. Polyconal antibodies were raised against the PSI-S protein purified from spinach plants (7).

Isolation of the PSI-S Protein—For pigment analysis the PSI-S protein was isolated by the procedure of Ref. 18 either from mature green spinach plants or etiolated seedlings at different stages of greening using the preparative isoelectric focusing method (IEF) according to Ref. 31 in the presence of 1% octyl glucoside. For experiments on mature green plants, PSI-II enriched grana membranes (32) were isolated prior to octyl glucoside solubilization and IEF separation (18). Isolation of the PSI-S fraction from etiolated seedlings or seedlings for 7 days stages of greening was achieved by direct solubilization of isolated plastid membranes with octyl glucoside followed by the IEF separation.

Pigment Analysis—Total pigments were extracted from 5 g of etiolated or green spinach plants with acetone in the presence of CaCO₃ (33).

For analysis of pigments bound to the PSI-S protein, thin layer chromatography (TLC) was performed according to Ref. 34. IEF fractions were dialyzed at 4 °C overnight against 0.1% octyl glucosynanoside and 15 mM NaCl in 20 mM Mes-NaOH, pH 6.5, and total pigments were extracted with 80% acetone (final concentration). The pigment extract was purified by centrifugation at 17,000 × g for 10 min, transferred to diethyl ether, dried over anhydrous Na₂SO₄, and redissolved in chloroform. The concentrated pigments were separated on silica gel (TLC Silica Gel 60, Merck), using as developing solvent petroleum ether/diethyl ether/chloroform/methanol/acetone (40:10:10:5:5, v/v).

For analysis of pigments bound to the PSII-S protein, thin layer chromatography (TLC) was performed according to Ref. 35 using oligid(T)-cellulose chromatography (36). Dot blot hybridization was performed using a 32P-labeled random-primed homologous cDNA insert of psbS (15) or Lhcb1 clones (37) according to the Amersham protocol. The probes recognized specifically the mRNA as verified by Northern analysis (15).

RESULTS

Accumulation of the psbS Transcript and Gene Product during Greening of Etiolated Spinach Plants—Etiolated spinach seedlings grown in the dark for 7 days were exposed to continuous white light (100 μE/m²s) for induction of greening. After 0, 1, 2, 4, 8, 12, 16, 20, 28, 36, and 48 h cotyledons with part of the hypocotyl were harvested and used for mRNA (Fig. 1) and protein (Fig. 2) analysis.

Steady-state level of psbS transcripts visualized by dot blot hybridization of isolated poly(A+) RNA with a 32P-labeled insert of the psbS clone demonstrate that only a small amount of transcript is present in etiolated spinach (Fig. 1, upper panel). During exposure of the seedlings to light the abundance of the psbS transcript increases 20–30 times and its maximal level is reached during 12–16 h of illumination. Thereafter the abundance of psbS transcripts decreases (Fig. 1), but a low level is still present in mature green plants (not shown).

The maximal abundance of this CAB transcript was reached on a somewhat later stage, between 16 and 20 h of greening.

The accumulation of the PSII-S protein during exposure of etiolated spinach seedlings to light was assayed by immunoblotting (Fig. 2, a and b). An important observation was the divergence in appearance of the PSII-S protein and its corresponding mRNA. There was an increase in PSII-S protein level during the entire greening period in contrast to the transient appearance of the transcript. In sharp contrast to the other members of the CAB family like LHCII, CP29, CP24, as well as the light-stress proteins ELIPs, the PSII-S protein is present in relatively high levels already in etiolated seedlings. Quantification of the immunoblot data showed that 15–30% of the maximal amount of the PSII-S protein, which is reached between 12 and 16 h of greening, is present already in the dark, even if there is neither chlorophyll a nor chlorophyll b to stabilize the protein (see Table I). Chlorophyll a as well as chlorophyll b are detectable only after 4 h of illumination. At that time the PSII-S protein is the only potential chlorophyll-binding protein which is present in the plastid membranes in higher amounts. The amount of both, chlorophyll a and b, then raise constantly during illumination.

The other members of the CAB family like LHCII, CP29, and...
CP24 can be detected in thylakoid membranes only between 8 and 12 h of greening and are absent in etiolated seedlings or during the early stages of greening (Fig. 2, a and b). The ELIP proteins are short-lived and appear transiently during the greening of etiolated plants (maximum at 8 h) as was previously shown for pea (38) and barley (39) plants. Since it was previously reported that the PSII-S protein is a permanent, integral component of the PSII complex (7, 18, 40) it was also of interest to compare the accumulation of other PSII subunits during the greening process in spinach. Fig. 3 shows the accumulation of different proteins of the PSII reaction center (Fig. 3A) and the three extrinsic polypeptides of the oxygen evolving complex (Fig. 3B). The D1 and D2 proteins are not present in etiolated tissue and appear in the thylakoid membranes only after 4–8 h of illumination (Fig. 3A). The same pattern of appearance was seen for the chlorophyll a-binding proteins CP47 and CP43 (not shown). Cytochrome b$_{559}$, which is known to be present in etiolated spinach seedlings in the absence of other PSII components (21), accumulates almost linearly during the early stages of greening (Fig. 3A). However, the relative level of the PSII-S protein in etiolated tissue is even higher than the level of cytochrome b$_{559}$. The three extrinsic components of the oxygen evolving complex, the polypeptides PSII-O, PSII-P, and PSII-Q are also present in etiolated seedlings (Fig. 3B) and accumulate during greening.

Developmental and Organ-specific Regulation of the PSII-S Protein Accumulation in Plastid Membranes—To investigate whether the developmental stage of the plastids influences the accumulation of the PSII-S protein in the etiolated membranes, the amount of the PSII-S protein was assayed over a 14-day period in spinach plants grown in complete darkness (Fig. 4). After 4 days the cotyledons had partially emerged from the seed coat and during the period of 14 days the hypocotyls lengthened. The cotyledons were closed and unexpanded until day 8, when they partially started to unfold. The PSII-S protein, assayed by immunoblotting, was present in substantial amounts already in 4-day-old seedlings (Fig. 4, top panel). During subsequent days the PSII-S level increased steadily in the etiolated seedlings and the amount reached a maximum at day 8, which remained for the next 6 days. The a-subunit of cytochrome b$_{559}$ and the three extrinsic proteins of the oxygen evolving system exhibit a similar accumulation pattern in the dark (Fig. 4), their amounts increased with the age of the etiolated seedlings. Similar results were also found for cytochrome f. In contrast no traces of the D1 and D2 proteins, CP47, CP43, and the chlorophyll a/b-binding proteins LHCII, CP29, CP26, and CP24 could be detected in any of the etiolated samples (not shown).

Taking advantage of the fact that the polyclonal antibody

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Accumulation of the PSII-S protein in the plastid membranes during greening of etiolated spinach seedlings. Spinach seedlings were grown in complete darkness for 8 days and then exposed to light of 100 μE/m$^2$/s for 28 h. Plastid membranes were isolated at different stages of greening and the relative abundance of the PSII-S protein and related CAB family members including ELIPs were assayed by immunoblotting (A) and quantitated by densitometry (B). Equal amounts of protein were loaded in each lane.

| Hours of greening | Chl a | Chl b/ Chl a | a/b |
|------------------|-------|--------------|-----|
| 0                | ND    | ND           | ND  |
| 1                | ND    | ND           | ND  |
| 2                | 0.28  | 2.5          |      |
| 4                | 1.20  | 1.2          |      |
| 8                | 1.75  | 1.6          |      |
| 12               | 2.30  | 2.3          |      |
| 16               | 3.60  | 3.0          |      |
| 20               | 5.40  | 5.4          |      |
| 28               | 6.10  | 6.1          |      |
| 36               | 8.10  | 8.1          |      |

$^a$ ND, not determined.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Accumulation of the PSII-S protein in plastid membranes in relation to appearance of other PSII subunits during greening of etiolated spinach seedlings. Eight-day-old etiolated spinach seedlings were exposed to light of 100 μE/m$^2$/s for 28 h. Plastid membranes were isolated at different stages of greening and the abundance of various subunits of PSII was estimated by immunoblotting. Immunoblots were scanned at 600 nm and the maximal values reached at 28 h of greening were set as 100%. A, accumulation of the PSII-S protein and PSII the reaction center polypeptides D1 and D2 and the a-subunit of cytochrome b$_{559}$. B, accumulation of polypeptides of the oxygen evolving complex, PSII-O, PSII-P, and PSII-Q.
against the spinach protein cross-reacts with the PSII-S protein from barley, we studied the accumulation of PSII-S in plastids of different ages representing various stages of differentiation using the barley leaf gradient system (41, 42). In 6-day-old barley leaves grown under light/dark conditions the cells at the basal leaf end are at an early stage of development, whereas cells at the leaf tip are differentiated to a greater extent. Immunoblot analysis (Fig. 5) shows that the accumulation of the PSII-S protein is very low in the basal section of the leaf (containing meristematic tissue with proplastids) and increases toward the tip of the leaf which contains completely differentiated chloroplasts. In contrast, the other CAB- and chlorophyll a-binding proteins are totally absent in the basal segment. However, the non-chlorophyll-binding proteins PSII-O and small amounts of cytochrome b659 could be detected in the proplastids of this segment (Fig. 5).

Organ-specific accumulation of the PSII-S protein was investigated in 2-week-old green spinach plants. The plants were divided into cotyledons, upper hypocotyl, lower hypocotyl, and roots and the plastid membranes were isolated from each section and finally the PSII-S protein was detected by immunoblotting (Fig. 6). The PSII-S protein is present only in photosynthetic tissue, mainly in cotyledons, but the protein (25–30%) is also detectable in the upper hypocotyl. Other chlorophyll a-binding proteins, such as LHCII and CP24, exhibit a similar distribution although only traces of these proteins are present in the upper hypocotyl (Fig. 6). The same result is found for the chlorophyll a-binding proteins D1 and D2, whereas non-chlorophyll-binding proteins such as the extrinsic PSII-Q, PSII-P, PSII-O, and cytochrome b659 are present also in non-photosynthetic tissue such as the lower hypocotyl and the roots (not shown).

Pigment Composition of the PSII-S Protein Isolated from Etiolated and Green Spinach Plants—The question arises whether in etiolated plants the stabilization of PSII-S protein could be achieved by binding of chlorophyll precursors or carotenoids and at which stage during the greening process the chlorophyll finally becomes bound to the PSII-S protein. In order to address these questions the composition of pigments specifically bound to PSII-S was analyzed by thin layer chromatography after isolation of this protein by preparative isoelectric focusing throughout the greening process (18) (Fig. 7, upper panel).

Isolelectric focusing of solubilized plastid membranes isolated from etiolated plants gave two greyish bands, the first one focusing at the sample application site at pH 6.0 and the second one focusing at pH 5.9. After collection the band at pH 5.9 gave a turbid uncolored solution, which contained mainly the PSII-S protein weakly contaminated with some other unidentified proteins (Fig. 7, upper panel). When solubilized plastid membranes from the samples illuminated for 4 and 12 h were applied to the IEF separation, the PSII-S protein could be immunodetected in greenish bands focusing at pH 6.1. The same result was obtained after IEF isolation of the PSII-S protein from thylakoids of mature green spinach plants (Fig. 7, upper panel). The pigment composition of the various PSII-S protein fractions was analyzed spectrophotometrically and by thin layer chromatography (TLC) after precipitation with acetone (Fig. 7, lower panel). Notably, the PSII-S fraction isolated from the etiolated plants contained neither carotenoids nor chlorophyll precursors. After 4 or 12 h of greening the pigments bound to the PSII-S protein did not differ from that obtained for this protein isolated from completely green plants. The PSII-S protein contains chlorophyll a and b as well as δ-carotene, lutein, violaxanthin, neoxanthin, and some other unidentified pigments with low chromatographic mobility. These results taken together with those of Fig. 2 indicate that after 4 h of illumination the PSII-S protein is the only or major chlorophyll a/b-binding protein in the plastid membranes.

Presence of the PSII-S Protein in Pigment-deficient Mutants—The presence of the PSII-S protein was investigated in
both the ambient light (100 mE/m²/s) conditions, although the amount is reduced to only 15% in the mutant as compared to the wild-type (Fig. 9). The sequence similarity between the PSII-S protein and the other members of the CAB family, the LHCII, CP24, and the two CAB proteins (45, 46) and chlorophyll a/b-binding proteins (47–52) are not clear. Since PSII-S has four transmembrane helices and shorter loops between helices, it could fold as a membrane spanning helix (15, 16), make the PSII-S protein distinct from all the other known chlorophyll a/b-binding proteins. The present results from etiolated tissue and various mutants give further experimental data in relation to the unique properties of the PSII-S protein since it is shown to be the first chlorophyll-binding protein that can be stably integrated into the membrane in the absence of pigments.

It could also be shown that there is no binding of carotenoids or chlorophyll precursors under etiolated conditions showing that such pigments are not responsible for stabilization of the PSII-S protein. The molecular properties of the PSII-S protein that keep it stable without bound pigments, in contrast to the other CAB proteins (45, 46) and chlorophyll a-binding proteins (47–52) are not clear. Since PSII-S has four transmembrane helices and shorter loops between helices, it could fold as a classical 4-helix bundle with helix-helix interactions giving it additional stability.

During greening the PSII-S protein appears at an earlier stage than the other CAB proteins and many PSII subunits with the exception of cytochrome b₅₅₉. There is also an early appearance of the three extrinsic proteins (PSII-O, -P, and -Q) that keep it stable without bound pigments, in contrast to the other CAB proteins (45, 46) and chlorophyll a-binding proteins (47–52) are not clear. Since PSII-S has four transmembrane helices and shorter loops between helices, it could fold as a classical 4-helix bundle with helix-helix interactions giving it additional stability.

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After 4 h of greening, when chlorophyll accumulation is starting to occur the PSII-S protein was found to bind pigments, both chlorophylls and carotenoids. In fact under this stage of development the PSII-S protein appears to be the only chlorophyll a/b-binding protein in the membranes (Fig. 2) since there’s no trace of the conventional CAB proteins and ELIPs, which are present in minor amounts, have not been shown to be chlorophyll-binding.

The previous biochemical and biophysical data (18) suggest that the PSII-S protein may not be a conventional light-harvesting protein despite its ability to bind pigments and other functions should consequently be considered. The present results which demonstrate a stability of the protein without pigments and its appearance during very early stages of development suggest that it may play a role in biosynthesis and assembly of the other chlorophyll-binding proteins of PSII. During early stages of development, when the PSII-S protein appears to be the only pigment-binding protein, it could be a “ligand chaperone” serving as a transient chlorophyll storage protein. Newly synthesized chlorophyll molecules would appear to be the only pigment-binding protein, it could be a “ligand chaperone” serving as a transient chlorophyll storage protein. Newly synthesized chlorophyll molecules would thereby not be free in the membrane but kept in association with \( \beta \) carotene to avoid toxic oxidative damage via triplet formation. Moreover, the chlorophylls would be present in a ligation competent state in the PSII-S protein prior to the subsequent accumulation of D1, D2, CP47, CP43 and the various chlorophyll a/b-binding proteins. Such a function would be in line with the quite exclusive localization of the PSII-S protein to the appressed thylakoid region where ligation of chlorophyll is thought to occur (22).

In mature thylakoid membranes the PSII-S protein could be involved in transient chlorophyll binding during high-light induced turn-over of PSII reaction centers (see Ref. 53) and acclimative degradation of LHCCI (54). It is also known that photosynthetic reaction centers can acquire chlorophyll molecules from the antenna proteins (55) and the PSII-S protein could possibly mediate such a process. This would be consistent with its suggested location between the PSII core and the outer chlorophyll a/b antenna (14).

The PSII-S protein is present in chlorophyll-deficient mutants lacking the conventional CAB proteins. This observation is of interest with respect to the violaxanthin-zeaxanthin cycle which has been shown to take place in the barley chlorina-f2 mutant grown under intermittent light (56) despite that this mutant material lacks all the conventional CAB proteins. However, as this mutant contains the PSII-S protein (56), which has been shown to contain these carotenoids (18) under intermittent light conditions, a role of this protein in violaxanthin/zeaxanthin interconversion and non-photochemical quenching should therefore also be considered.

The regulation of the PSII-S expression appears to be distinct from the other CAB proteins. The accumulation of the PSII-S protein in etiolated membranes of spinach seedlings grown in complete darkness indicates that the psbs gene undergoes an endogenous, light-independent regulation which controls not only its transcript level but also influences the level of the protein. This is the first observation of a control of the protein level for a pigment-binding protein by the state of cellular development of the etiolated seedlings. An internal, developmental regulation of the transcript level in dark grown Arabidopsis (57), maize (58), or barley seedlings (59) was reported for other members of the CAB gene family, like the Lhcb1, Lhcb2, and Lhcb3 or the rbcS gene coding for the small subunit of the ribulose-bisphosphate carboxylase/oxygenase. However, in all these cases the accumulation of the corresponding protein was strictly light-dependent and did not follow the accumulation of their transcripts in the dark. Thus, the endogenous regulation of the PSII-S protein in etiolated plants seems to be unique and so far specific only for this protein.

In addition to an internal regulation of PSII-S expression in etiolated seedlings, the accumulation of this protein and its transcript is positively regulated by external stimuli like light. Illumination of etiolated seedlings resulted in a transient accumulation of the psbs transcript. Such a transient accumulation of transcripts during greening of etiolated plants is a common feature for genes which products are utilized for synthesis of the photosynthetic apparatus. Once a mature chloroplast population is formed the transcription of these genes declines (42). In contrast to other CAB family members, which proteins can be detected in the thylakoid membranes only a few hours after accumulation of the correspondent transcript, the transient increase of the psbs transcript is immediately followed by an accumulation of the PSII-S protein.

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