PsbR, a Missing Link in the Assembly of the Oxygen-evolving Complex of Plant Photosystem II*

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The oxygen-evolving complex of eukaryotic photosystem II (PSII) consists of three extrinsic nuclear-encoded subunits, PsbO (33 kDa), PsbP (23 kDa), and PsbQ (17 kDa). Additionally, the 10-kDa PsbR protein has been found in plant PSII and anticipated to play a role in water oxidation, yet the physiological significance of PsbR has remained obscure. Using the Arabidopsis psbR mutant, we showed that the light-saturated rate of oxygen evolution is strongly reduced in the absence of PsbR, particularly in low-light-grown plants. Lack of PsbR also induced a reduction in the content of both the PsbP and the PsbQ proteins, and a near depletion of these proteins was observed under steady state low light conditions. This regulation occurred post-transcriptionally and likely involves a proteolytic degradation of the PsbP and PsbQ proteins in the absence of an assembly partner, proposed to be the PsbR protein. Stable assembly of PsbR in the PSII core complex was, in turn, shown to require a chloroplast-encoded intrinsic low molecular mass PSII subunit PsbJ. Our results provided evidence that PsbR is an important link in the PSII core complex for stable assembly of the oxygen-evolving complex protein PsbP, whereas the effects on the assembly of PsbQ are probably indirect. The physiological role of the PsbR, PsbP, and PsbQ proteins is discussed in light of their peculiar expression in response to growth light conditions.

Photosystem II (PSII) is a supramolecular pigment-protein complex embedded in the thylakoid membrane. It catalyzes light-induced electron transfer from water to plastoquinone, with concomitant evolution of oxygen occurring on the luminal side of PSII. The oxygen-evolving complex (OEC) of eukaryotic PSII is composed of three extrinsic nuclear-encoded subunits, PsbO (33 kDa), PsbP (23 kDa), and PsbQ (17 kDa), which protect the CaMn4 cluster bound to D1 and CP43 proteins (1). Cyanobacterial OEC, on the other hand, is composed of the PsbO protein, together with PsbU and PsbV (the cytochrome c550) (2). Despite these differences in the protein composition of OEC, the functional features of the manganese cluster and the requirement of two inorganic ions, calcium and chloride, in photosynthetic water oxidation have remained similar during the evolution of higher plants.

Recent genome and proteome analyses have, however, revealed that homologs of both PsbQ and PsbP are present in cyanobacteria (3–5) and are essential for regulation of PSII assembly and/or activity, particularly under calcium or chloride deficiency (5, 6). The PsbQ homolog was found to be present as one molecule for each cyanobacterial PSII core, whereas the PsbP homolog was suggested to be present only in substoichiometric amounts (5). On the other hand, it was recently found that in total membrane preparations and in whole cell extracts, the PsbP homolog is present in amounts comparable with other PSII proteins, suggesting that the protein becomes depleted by detergents used during the isolation of PSII complexes (7). Moreover, it has been reported that the 20-kDa extrinsic protein, which belongs to the OEC proteins of red algae, shows sequence similarity with the PsbQ protein of green algae and higher plants as well as with the PsbQ homolog of cyanobacteria, and it was proposed that the red algae 20-kDa protein is an ancestral form of the PsbQ protein (8).

The assembly process of the 33-kDa PsbO protein to the luminal side of the PSII core complex has been reported to take place instroma-exposed thylakoid membranes of higher plants (9). Structural studies have revealed that the luminal loops of the PSII core proteins D2 and CP47 are essential for docking the PsbO protein (10), and the presence of CP43 is also necessary since no stable assembly of the PsbO protein was found in the PSII monomer lacking CP43 (11). The 23- and 17-kDa OEC proteins PsbP and PsbQ have been found to associate with PSII only in the grana thylakoids (9). Moreover, the low molecular mass (LMM) PSII core protein PsbW was recently reported to be a prerequisite for the association of PsbP with the PSII core (11, 12). It has been suggested that PsbO provides a docking site for PsbW, and both PsbO and PsbP together are needed for the binding of PsbQ (13, 14). Unlike in higher plants, the PsbP and PsbQ homologs in cyanobacteria contain a lipid moiety in their N terminus, which probably anchors these proteins to the thylakoid membrane (5).

Besides the OEC proteins, the PSII core complex in higher plants and cyanobacteria also differs with respect to the necessity of several intrinsic LMM proteins to the function and assembly of the complex (15, 16). PsbP is a representative example of such proteins. In cyanobacteria, the deletion of PsbP allows slow photoautotrophic growth, whereas in tobacco plants, the corresponding deletion leads to a complete heterotrophy (11, 12, 17–19). Moreover, higher plant PSII contains the nuclearr-encoded LMM subunits PsbW and PsbR, which are completely lacking from cyanobacterial PSII.

The functional role and properties of PsbR, also known as the 10-kDa PSII polypeptide, have remained poorly characterized. It has been proposed that PsbR is located on the luminal side of PSII and is involved in binding the OEC proteins (20), although no direct in vivo experimental proof is available. Here we have characterized the Arabidopsis T-DNA insertion mutant psbR and demonstrated the requirement of the PsbR protein for stable association of the OEC subunit PsbP with the PSII core.

1 The abbreviations used are: PSII, photosystem II; PSI, photosystem I; Chl, chlorophyll; LHC, light-harvesting complex; LMM, low molecular mass; OEC, oxygen-evolving complex; WT, wild type; LL, low light; HL, high light; GL, growth light.

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2 The abbreviations used are: PSII, photosystem II; PSI, photosystem I; Chl, chlorophyll; LHC, light-harvesting complex; LMM, low molecular mass; OEC, oxygen-evolving complex; WT, wild type; LL, low light; HL, high light; GL, growth light.
**Materials and Methods**

**Plant Material and Growth Conditions—*Arabidopsis thaliana* ecotype Columbia T-DNA insertion mutant (SALK_114469) was obtained from the Salk collection (the Salk Institute Genomic Analysis Laboratory) (21) via the Nottingham Arabidopsis Stock Centre. Three plants were obtained lacking the PsbR protein, as analyzed by Western blotting. Furthermore, PCR analysis of these three plants and the wild type (WT) (ecotype Columbia) with primers specific to the flanking sequences of both the left (LP, 5′-ACCCGATCTTCTCTCCCTGT-3′) and the right (RP, 5′-TCTTGTGCATGGAAATCATAAAAGGA-3′) sides of the PsbR gene together with a primer inside the T-DNA insert (LB, 5′-TGTTTCACGTAGTGGAACCTCG-3′) confirmed the three plants to be homozygous mutants. PCR amplification of homozygous psbR mutant DNA resulted in a 780-bp product, whereas amplification of the WT DNA with the same primers produced a 960-bp fragment. Both psbR and WT plants were grown in a growth chamber under a photon flux density of 125 μmol of photons m⁻² s⁻¹ in 8-h light/16-h dark cycles at a temperature of 25 °C. For some experiments, plants were also grown under very low (15 μmol of photons m⁻² s⁻¹), low (50 – 60 μmol of photons m⁻² s⁻¹), and high (450 μmol of photons m⁻² s⁻¹) light. Fully grown rosettes were used for studies. For short-time regulation studies, WT plants were, after turning the lights on, first kept under growth light for 2 h and then exposed for 5 h to darkness, very low light (15 μmol of photons m⁻² s⁻¹), growth light (125 μmol of photons m⁻² s⁻¹), and high light (450 or 750 μmol of photons m⁻² s⁻¹).

The details of transformation, selection, and growth of the tobacco (*Nicotiana tabacum*) psbEFLJ operon mutants were described earlier (11, 18, 19). WT “RV” (which has an aadA gene cassette in a neutral EcoRV site in the 3′-untranslated region of the operon) plants, grown under the same conditions as the mutants, were used as controls.

**Northern Blotting**—Total RNA was isolated with TRIzol (Invitrogen) according to Tri reagent total RNA isolation protocol (Sigma), and Northern blotting was performed with standard techniques (22).

**Isolation of Thylakoid Membranes**—Leaves were briefly homogenized with a blender in 50 mM HEPES-KOH, pH 7.5, 330 mM sorbitol, 2 mM EDTA, 1 mM MgCl₂, 5 mM ascorbate, 0.05% bovine serum albumin, and 10 mM NaF, filtered through Miracloth, and centrifuged for 4 min, 2500 × g, and at 4 °C. The pellet was resuspended in 50 mM HEPES-KOH, pH 7.5, 5 mM sorbitol, 10 mM NaF, and centrifuged and resuspended at the same conditions as the mutant. The thylakoid pellet was resuspended in 50 mM HEPES-KOH, pH 7.5, with 100 mM sorbitol, 10 mM MgCl₂, and 10 mM NaF, centrifuged for 3 min at 2500 × g, 4 °C, and finally resuspended in the same buffer. Chlorophyll (Chl) was extracted in 80% (v/v) buffered acetone (2.5 mM HEPES-NaOH, pH 7.5) and quantified as described (23).

**Protein Analysis**—Proteins were separated in SDS-PAGE (15% acrylamide, 6 μM urea) (24). After electrophoresis, proteins were electroblotted to a polyvinylidene fluoride membrane (Millipore). Western blotting with chemiluminescence detection was performed with standard techniques using protein-specific antibodies or an antibody raised against the PSI complex. The PsbR antibody was produced against synthetic peptides (Eurogentec). Antibodies against the Lhcb and Lhca proteins were purchased from Agrisera, and the phosphothreonine antibody was from New England Biolabs. 0.5 μg of Chl was loaded, except for the detection of the Lhca and Lhcb subunits (0.3 μg), PsbQ (2 μg), and the phosphothreonine antibody (1 μg). These amounts of protein have been tested in our laboratory to give a linear immunoreponse with respective antibodies. Immunoblots were quantified with FluorChem Image Analyzer (Alpha Innotech Corp.). Blue native gel electrophoresis was performed as described earlier (25).

**Results**

We have characterized the T-DNA insertion mutant line SALK_114469, having an insert located in the third exon of the *PsbR* gene (At1g79040). *psbR* mutant plants showed no phenotype when grown under normal light conditions (125 μmol of photons m⁻² s⁻¹), under high light (450 μmol of photons m⁻² s⁻¹), or under low light (15 or 50 μmol of photons m⁻² s⁻¹). The Chl a/b ratio was slightly lower in the mutant thylakoids as compared with WT (Table 1), although no statistically significant difference could be detected.

**Functional Properties of PSI in the Absence of the PsbR Protein**—The steady state oxygen evolution was markedly lower in the thylakoids of the *psbR* mutant as compared with WT. 30% reduction was measured under low light conditions (Table 1). PSII excitation pressure was measured under 125 μmol photons m⁻² s⁻¹ (GL) and 50 μmol photons m⁻² s⁻¹ (LL). The oxygen evolution rate of *psbR* as a percentage value of that measured in WT is given in parentheses. For PSI excitation pressure, the plants were grown under 125 μmol photons m⁻² s⁻¹ (GL), and the measurements were performed under the same light and temperature conditions. The values are means ± S.D.

**Discussion**

**Conclusion**

We conclude that the PsbR protein is involved in the light-dependent regulation of PSI phosphorylation. The lack of PsbR did not make plants more susceptible to donor site photoinhibition (28) during high light illumination of the leaves (data not shown). On the other hand, the *psbR* mutant exhibited over 2-fold higher PSI excitation pressure as compared with WT plants (Table 1). Such defects in the electron transfer chain were reflected as reduced steady state phosphorylation of both the PSII core and the LHCII phosphoproteins as compared with WT (Fig. 1A).

**PSII/PSI Ratio in the psbR Mutant and WT Plants**—By using room temperature EPR measurements of the dark stable Y_D radical and the
The cytochrome b$_{6}$f complex seemed to be unaffected by the lack of PsbR, deduced from the equal presence of the cytochrome subunit (Fig. 2B). It was also tested by blue native gel electrophoresis that the oligomeric structures of the membrane protein complexes in psbR thylakoids were not altered (data not shown).

Expression of the OEC Proteins and the PsbR Protein in Mutant and WT Plants Grown under Low Light Intensity—When WT and the psbR mutant plants were grown under low light (50 μmol of photons m$^{-2}$ s$^{-1}$), extremely low amounts of both the PsbP and the PsbQ proteins were present in the psbR mutant as compared with WT. PsbP being only 6 ± 2% (n = 3) and PsbQ 31 ± 8% (n = 3) of that in WT plants as determined by immunoblotting (Fig. 3A). Similar low amounts of PsbP and PsbQ were evident from Coomassie Brilliant Blue-stained gels of WT and psbR thylakoids (Fig. 3B). Interestingly, the PsbO protein was present in equal amounts in the psbR mutant and WT, independently of the growth light conditions (Fig. 3A).

When WT plants grown under medium light (125 μmol of photons m$^{-2}$ s$^{-1}$) and low light (50 μmol of photons m$^{-2}$ s$^{-1}$) conditions were compared, it was observed that the amounts of the OEC proteins PsbP and PsbQ were much higher in the thylakoids from low light-grown plants (Fig. 3A). Also, the amount of the PsbR protein was somewhat higher in low light-grown than in control light-grown WT plants, although not as much increased as the PsbP and PsbQ proteins. No low light-induced increase was detected for the PsbO protein (Fig. 3A).

Because drastic differences were found in the amounts of the OEC proteins PsbP and PsbQ between the psbR mutant and the WT, we next investigated whether the regulation takes place at the level of transcription. Northern blots revealed that PsbP$_{2}$ and PsbQ$_{2}$ as well as PsbO$_{1}$ transcripts were present in both the WT and the psbR mutant plants in more or less equal amounts (Fig. 3C). Moreover, albeit clear increases at the protein level were observed in WT plants grown under low light, the

**FIGURE 1.** Steady state phosphorylation of PSII-associated proteins and the PSI/PSII ratio in psbR and WT thylakoid membranes. A, a phosphothreonine immunoblot demonstrating a steady state phosphorylation of the PSII core and LHCl proteins in WT and the psbR mutant thylakoid membranes. Proteins were separated by SDS-PAGE, electroblotted onto a polyvinylidene fluoride membrane, and probed with phosphothreonine antibody. 1 μg of Chl was loaded. B, EPR spectra recorded from the WT and the psbR mutant thylakoid membranes. EPR spectra were recorded in the dark after preillumination of the sample to oxidize Y$_{d}$ (1 spin per PSII reaction center, solid line) and after oxidation of the sample with 5 mM ferricyanide, representing both Y$_{d}$ and P700$^{+}$ radicals (dashed line). Ferricyanide-oxidized minus dark difference spectra representing pure P700$^{+}$ (1 spin per PSII reaction center) are indicated by dotted lines. The EPR conditions are as follows: room temperature, microwave frequency 9.78 GHz, microwave power 8 milliwatt, modulation amplitude 5 G. Plants grown under 125 μmol of photons m$^{-2}$ s$^{-1}$ were used for the experiments.

**FIGURE 2.** Immunobots of thylakoid membrane proteins in the WT plants and the psbR mutant. A, the presence of the PsbR and the OEC proteins PsbO, PsbP, and PsbQ, D1, CP43, Lhcb1, PsaaA/B, cytochrome f (Cyt f), and the CF1 α/β subunits. Proteins were separated by SDS-PAGE, electroblotted on to a polyvinylidene fluoride membrane, and probed with antibodies against different thylakoid membrane proteins. The equivalent of 0.5 μg of Chl (100%) was loaded, except for the detection of PsbQ (2 μg = 100%) and Lhcb1 (0.3 μg = 100%). Plants grown under 125 μmol of photons m$^{-2}$ s$^{-1}$ were used for the experiments.
amounts of PsbP and PsbQ mRNAs in low-light- and growth-light-grown plants were nearly equal.

Short Term Light Regulation of the OEC Proteins and the PsbR Protein—Short-term light regulation of genes encoding the OEC proteins and PsbR was also tested by exposing WT plants for 5 h to darkness, low light (LL) (here 15 μmol of photons m⁻² s⁻¹), two different intensities of high light (HL), 450 μmol of photons m⁻² s⁻¹ and 750 μmol of photons m⁻² s⁻¹, and as a control, growth light (125 μmol of photons m⁻² s⁻¹). As shown in Fig. 4A, the PsbR transcripts were clearly up-regulated in LL as compared with growth light (GL). The same trend was detected for the expression of PsbP and, to a lesser extent, also for PsbO and PsbQ. Moreover, a slight decrease occurred in these mRNAs at high light, especially for intensities ≥450 μmol of photons m⁻² s⁻¹ (Fig. 4A). For comparison, the amounts of the psbA transcripts were recorded under similar conditions, and contrary to the OEC transcripts, a slight up-regulation under high light was detected for the psbA mRNA.

In parallel with mRNA studies, we addressed the possible protein changes of PsbR after the 5-h exposure of plants to different light quantities. PsbR protein contents of the WT plants treated for 5 h at different light conditions were more stable than the respective transcripts. Nevertheless, a decrease in the PsbR protein content was observed after exposing the plants to high light for 5 h (Fig. 4B), being 82 ± 7% and 70 ± 5% of the control light values after the 5 h of treatment at 450 and 750 μmol of photons m⁻² s⁻¹, respectively. No decrease in the quantity of the D1 protein occurred during the 5-h treatment of plants under elevated irradiances (Fig. 4B).

Occurrence of the PsbR Protein in psbEFLJ Operon Mutants of Tobacco—To gain insights into the interaction of PsbR with the PSII core proteins, we tested the presence of PsbR in the thylakoid membranes of tobacco psbEFLJ mutant plants (11, 18, 19). PsbR was missing from the thylakoid membranes of the ΔpsbE and ΔpsbF mutants (Fig. 5), which do not show assembly of PSII core complexes (11). In the absence of PsbL, only a small amount of the PsbR protein was accumulated in the thylakoid membranes, corresponding to the contents of the D1 protein and the amount of assembled PSII complexes in this mutant. This straightforward comparison between the D1 protein and assembled PSII core complexes is possible because the D1 protein was never detected as a free protein in the thylakoid membrane. Thylakoid membranes of the ΔpsbL mutant, on the contrary, completely missed the PsbR protein, whereas these thylakoids have high amounts of assembled PSII complexes, although inactive in oxygen evolution (11). Intriguingly, the OEC protein PsbB was also completely missing from the ΔpsbF thylakoids, whereas PsbO was present in amounts comparable with those in WT thylakoids. Moreover, although the ΔpsbL mutant had only traces of assembled PSII centers, we also found traces of PsbP protein in ΔpsbL thylakoids (Fig. 5). An immunoblot of the D1 protein is included in Fig. 5 to indicate the overall contents of the PSII complexes in psbEFLJ mutants (Fig. 5) (for details, see Ref. 11).

**DISCUSSION**

PsbR has long been regarded as a “mystery” subunit of PSII implicated to reside on the luminal side of PSII in a close association with the OEC proteins. Since the PsbR gene is missing from cyanobacterial genomes, the high resolution structures of PSII complexes have not been helpful in elucidation of the location and the functional roles of PsbR in the PSII complex. As we have shown here, the absence of PsbR clearly diminishes the oxygen evolution capacity of thylakoid membranes, indicating that PsbR is essential for optimization of photosynthetic water splitting and electron transfer in PSII. Based on the high PSII excitation pressure,
which is an indicator of a high redox state of QA, together with a low phosphorylation state of both the PSII core and the LHCII proteins in the psbR mutant; it can be deduced that the electron transfer from PSII to the plastoquinone pool is hampered in the psbR mutant. Moreover, the higher PSI/PSII ratio in psbR is likely to keep the plastoquinone pool more oxidized than in WT under the steady state growth conditions.

The PsbR Protein Enables the Stable Assembly of the OEC Protein PsbP to the PSII Core—Among plants grown under low light intensity (50 μmol of photons m⁻² s⁻¹), the psbR mutant showed a near complete loss of the OEC protein PsbP and extremely low amounts of PsbQ. Under moderate growth light (125 μmol of photons m⁻² s⁻¹), the PsbP and PsbQ proteins were clearly observable yet in much smaller quantities as compared with WT thylakoids. It is thus conceivable that in higher plants, the PsbR protein is a prerequisite for a stable assembly of these two OEC proteins. The exact location of PsbR in the PSI core is not known, and it remains to be investigated whether PsbR offers a direct docking site for PsbP (and probably PsbQ, in turn, for PsbQ, see below) or whether PsbR only fine-tunes the structure of PSI on the luminal side, making the association of PsbP and PsbQ more feasible. Cyanobacteria lack the PsbR protein and also the proper PsbP and PsbQ proteins. However, homologs of PsbP and PsbQ are found in cyanobacterial genomes, and their gene products have been localized to PSI complexes. Anchoring of cyanobacterial PsbP and PsbQ homologs to the thylakoid membrane, however, takes place via a lipid moiety (5) and probably does not need docking to a protein, like PsbR in higher plants.

Our experimental results corroborate with an early suggestion of PsbR being important for the assembly/docking of PsbP to the PSI complex (20, 29). On the other hand, the characterization of PsbR antisense mutants of potato resulted in a contradictory conclusion of the PsbR protein having no effect on OEC proteins (30). However, a closer look at the Western blots of this latter study reveals a high variation in the amounts of PsbP, particularly depending on the extent of silencing these two OEC proteins. The exact location of PsbR in the PSI core is not known, and it remains to be investigated whether PsbR offers a direct docking site for PsbP (and probably PsbQ, in turn, for PsbQ, see below) or whether PsbR only fine-tunes the structure of PSI on the luminal side, making the association of PsbP and PsbQ more feasible. Cyanobacteria lack the PsbR protein and also the proper PsbP and PsbQ proteins. However, homologs of PsbP and PsbQ are found in cyanobacterial genomes, and their gene products have been localized to PSI complexes. Anchoring of cyanobacterial PsbP and PsbQ homologs to the thylakoid membrane, however, takes place via a lipid moiety (5) and probably does not need docking to a protein, like PsbR in higher plants.

Low amounts of the PsbP and PsbQ proteins in the psbR mutant under steady state growth conditions were apparently due to post-transcriptional regulation since the PsbP and PsbQ transcripts were present in WT levels. In theory, the low amounts of the PsbP and PsbQ proteins in the psbR mutant could result from malfunctions of polyribosome loading, translation initiation or elongation, processing of the preproteins in cytosol, protein import into chloroplasts and to the lumen, and finally, from the capability of proteins to stably associate with the PSI core. Although at present we do not have experimental data to exclude any of these phases, it is reasonable to conclude that the primary reason for low steady state amounts of the PsbP and PsbQ proteins in the psbR mutant is likely to be found from improper assembly of these proteins to PSII in the absence of PsbR.

PsbP, a PSII Core Protein, Is Required for the Assembly of Both the PsbR and the PsbP Proteins—The location of PsbR in the PSI core complex has been difficult to study because it easily drops out from the thylakoid membrane together with the oxygen-evolving proteins. The requirement of specific PSI proteins for the assembly of PsbR is, however, possible to investigate using the knock-out plants of various PSI LMM subunits. Strong in vivo association of PsbR with the PSI core is emphasized by the lack of PsbR from thylakoid membranes in the absence of stable PSI assembly in the ΔpsbE and ΔpsbF mutants. PsbR was also found to be practically missing in a tobacco ΔpsbJ mutant (Fig. 5). Interestingly, this mutant has a complete set of all other PSI core proteins studied so far and also the PsbQ protein (19), but it specifically lacks the PsbP protein (Fig. 5) (11,12). These results provide evidence that the association of these three proteins, PsbP, PsbR, and PsbJ, to the PSII core is highly dependent on each others.

PsbP has one putative transmembrane helix (31) and is stably located in PSII core in a close vicinity to cytochrome b₅₅₉ and CP43 (1). It is important to note that PsbP is an evolutionarily conserved PSII protein and present in both cyanobacteria and higher plants. PsbR, which also has one putative transmembrane helix and is missing from cyanobacterial PSI, as well as from higher plant PSI if the PsbR protein is knocked out, apparently requires the Psb1 protein for proper assembly to the PSI core. PsbP, on the other hand, is an extrinsic protein located on the luminal side of PSI and it seems that a proper assembly of both PsbP and PsbR to the PSII core is a prerequisite for docking of PsbP. Our recent studies on sequential assembly steps of PSI polypeptides corroborate this conclusion. PsbR was found to assemble to the PSI core monomer complex before the internal core antenna protein CP43 (25), whereas PsbP assembles only later in the grana thylakoids (9), probably after a PSI-LHCII supercomplex is formed.

The PsbR Protein Is Not Required for the Assembly of the PsbO Protein and Only Indirectly for the Assembly of the PsbQ Protein—Although no differences in the content of the PsbO protein was observed between psbR and WT, the amount of the 17-kDa OEC protein PsbQ was much lower in psbR thylakoids. It has been previously suggested that the presence of both PsbO and PsbP is needed for the binding of PsbQ (14). At present, we cannot make a distinction whether the significant decrease in the amount of PsbQ in psbR is a direct consequence from the loss of PsbR or whether it is due to the loss of the PsbP protein, an assumed docking site for PsbQ.

Higher plant PsbP and PsbQ have several features in common, which distinctively differ from those of PsbO. PsbO is the only subunit of the OEC complex that is present in all oxyphototrophs and directly involved in water splitting. The recently found PsbP and PsbQ homologs of cyanobacteria most probably have a regulatory role in PSII assembly (5). Functionally, PsbO stabilizes the manganese cluster, whereas PsbP and PsbQ, as well as PsbU and PsbV in cyanobacteria, are involved in optimizing the calcium and chloride binding to the OEC. In addition, PsbO already associates with the PSII core in the stroma lamellae via a Sec-dependent pathway, whereas PsbP and PsbQ utilize the ΔPH-dependent pathway for translocation (Ref. 9 and references therein) and assemble to PSII only after migration to grana thylakoids. PsbO is located toward the D2/CP47 side of the PSI core, whereas PsbP and PsbQ are positioned toward the N terminus of the D1 protein (10). It has also been reported that virus infection of tobacco (Nicotiana benthamiana) plants typically reduces the contents of only the PsbP and PsbQ proteins, whereas PsbO remains unaffected (32, 33).

Peculiar Regulation of the OEC Proteins and the PsbR Protein, Acclimation to Low Light Enhances the Expression of the PsbR, PsbP, and PsbQ Proteins but Not of the PsbO Protein—To elucidate the possible role of light-induced chloroplast signals in the expression of nuclear genes encoding the OEC proteins, we applied short-time light treatments of varying light intensity to WT plants. Shift of plants to low light induced a clear up-regulation in PsbR, PsbP, and PsbQ transcripts, which is consistent with our recent report on enhanced synthesis of nuclear encoded thylakoid proteins upon shift of plants to low light (25). It is highly conceivable that these short term processes demonstrate the trigger phenomena, which in long term low light acclimation lead to accumulation of the PsbP, PsbQ, and PsbR proteins in thylakoids of WT plants (Fig. 3A). At high light, on the contrary, the synthesis and steady state accumulation of these proteins declined (Fig. 4B). Intriguingly, PsbO did not respond to changes in light intensity at the protein level,
suggesting that a stoichiometric relationship between PsbO and the PSII core is maintained independently of light conditions.

It is known that the general rule of "quality control," i.e., a rapid degradation of subunits of the membrane protein complexes not capable for assembly (34–36), does not always hold true for the OEC proteins. Indeed, a pool of free, unassembled OEC proteins capable for assembly has been found in the thylakoid lumen (9, 37, 38). It was suggested that these unassembled subunits function as a store to ensure rapid reassembly of functional, oxygen-evolving PSII complexes during the PSII repair cycle (9, 37, 38). This explanation is, however, very unlikely considering that, in addition to their role in water splitting and oxygen evolution, the higher plant OEC proteins might also have structural roles, PsbP and PsbQ possibly being important for the stacking of grana thylakoids (41), which is known to become more pronounced upon acclimation to constant low light conditions. This explanation is, however, very unlikely considering that, in addition to their role in water splitting and oxygen evolution, the higher plant OEC proteins might also have structural roles, PsbP and PsbQ possibly being important for the stacking of grana thylakoids (41), which is known to become more pronounced upon acclimation of plants to low light (42). In line with this suggestion, the PSII complexes in non-grana bundle sheet chloroplasts of maize and Sorghum are depleted in PsbR, PsbP, and PsbQ proteins as compared with mesophyll chloroplasts with differentiated grana and stroma thylakoids (43). From different light treatments of Arabidopsis WT and psbR plants, it is clear that the stoichiometry of the PsbR, PsbP, and PsbQ proteins with respect to the PSII core and the OEC protein can be highly variable, and novel functions for these proteins are likely to be discovered.

Concluding Remarks—Taken together, our results have provided the first direct experimental proof for the role of the PSII LMM protein PsbR in binding the OEC protein PsbQ. Moreover, another LMM protein PsbP is, in turn, required for the assembly of the PsbR protein, thus explaining the earlier reported importance of PsbP for the assembly of PsbQ (11, 12). An interesting adaptation to constant low light conditions was detected; in WT plants, the OEC proteins PsbP and PsbQ as well as PsbR were up-regulated, whereas the PsbQ amounts were not dependent on light intensity. This is hypothesized to be connected with enhanced grana stacking at low light.

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