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Psb30 is a photosystem II reaction center subunit and is required for optimal growth in high light in *Chlamydomonas reinhardtii*

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ABSTRACT

The *psb30* (*ycf12*) gene is conserved in a wide variety of oxygenic-photosynthetic organisms except angiosperms and some marine cyanobacteria. Psb30 protein is found in cyanobacterial photosystem II (PSII) core complexes and is dispensable for PSII structure and function. The most recent three-dimensional structure of cyanobacterial PSII core complex has revealed that Psb30 is located in proximity of PsbJ, PsbK, and PsbZ. However Psb30 has not yet been detected in PSII complexes from eukaryotic photosynthetic organisms. Here we found the expression of the chloroplast *psb30* gene in the green alga *Chlamydomonas reinhardtii* by immunoblotting and Psb30 is exclusively co-purifies with PSII core complex and is significantly reduced in PSII-deficient mutants. Partial disintegration of PSII core complex and subsequent fractionation of the resulting subcomplexes revealed that Psb30 is exclusively associated with PSII reaction center. We have generated chloroplast transformants in which the *psb30* gene is disrupted and the resulting ΔPsb30 cells showed decreased oxygen evolution activity by 15%, grew photosynthetically under moderate light, and displayed increased sensitivity to high light relative to wild type. We conclude that Psb30 is a PSII reaction center subunit and is required for optimal PSII function under high light environments.

1. Introduction

In oxygenic photosynthesis, two photosystems, photosystem I (PSI) and photosystem II (PSII), drive the electron transfer from water to $NADP^+$ using light energy. PSII is responsible for the production of molecular oxygen and PSII core complex consists of over 20 different subunit polypeptides [1–4]. The redox components involved in photochemical and subsequent electron transfer reactions reside in the reaction center (RC) consisting of two homologous reaction center subunits, D1 and D2, encoded by the *psbA* and *psbD* genes, respectively. The RC intimately binds cytochrome b_{559} (Cyt b_{559}) of which apoproteins are encoded by the *psbE* and *psbF* and two core antenna complexes, CP43 and CP47, of which apoproteins are encoded by the *psbC* and *psbB* genes, respectively, to form PSII core complex. On the lumenal side of the core complex, several extrinsic subunits, such as PsbO, PsbP, and PsbQ in eukaryotes and PsbO, PsbU, PsbV in cyanobacteria and red algae, are present [1,5]. In addition to these subunits, a number of small hydrophobic polypeptides (PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbT, PsbX, PsbY, PsbZ and Psb30) with one or two transmembrane helices are bound to PSII core complexes [3,4].

Of the small hydrophobic polypeptides, Psb30 has most recently been identified in PSII preparations from *Thermosynechococcus elongatus* [6]. The gene *psb30* was originally designated as *ycf12*, one of the hypothetical chloroplast open reading frames, and is conserved in a wide variety of photosynthetic organisms except some marine cyanobacteria and angiosperm [7]. The expression of the *psb30* gene was also confirmed in *Synechocystis* sp. PCC 6803 by affinity purification of PSII complex containing Psb30 of which C-terminus is genetically fused with a hexa-histidine tag [8].

The three-dimensional structures of this highly integrated PSII core complex have been determined by three groups [9–11] using thermophilic cyanobacteria, *T. elongatus* and *T. vulcanus*. The latest structure at resolution of 2.9-Å has assigned all the 20 protein subunits in the cyanobacterial PSII core complex [2]. The structure reveals that Psb30 is located in periphery of the core complex and in proximity of PsbJ, PsbK, and PsbZ. PsbJ is associated with D1 and α-subunit of Cyt b_{559}, suggesting its intimate association with the RC. In contrast, PsbK and PsbZ are bound to CP43. It has been reported that PsbK consistently co-purifies with CP43 subcomplex purified from partially dissociated PSII core complex with KSCN [12]. PsbK is essential for the stability of PSII complex in the green alga *Chlamydomonas reinhardtii* [13] and is required for the stability of PsbZ and Psb30 in *T. elongatus* [14]. PsbZ is not essential for PSII stability and function but is required for the stability of PSII-LHCII supercomplex in tobacco [15]. The absence of PsbZ also affects the stable

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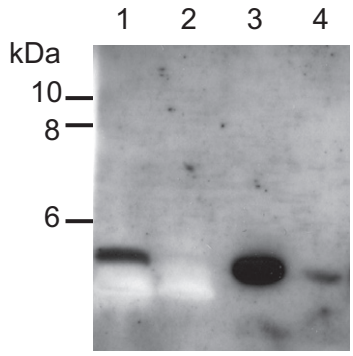

Fig. 1. Immunodetection of Psb30 in wild type and a PSII-deficient mutant. Thylakoids of 1, wild type; 2, the PSII-deficient mutant (Fud7; ΔD1); 3, de-lipidized wild type; 4, de-lipidized Fud7. Samples equivalent to 3 μg Chl were applied to the electrophoresis gel containing 22% acrylamide and 6 M urea [23]. The sizes and positions of the molecular mass standards are shown on the left.

association of PsbK and Psb30 with cyanobacterial PSII core complexes, suggesting structural interaction of PsbZ with PsbK and Psb30 [16]. Contrary to ΔPsbK and ΔPsbZ mutants, cyanobacterial ΔPsb30 displays no apparent phenotype; the cells grow photosynthetically and show oxygen-evolving activity comparable to wild-type cells [8]. However, the purified PSII complex lacking Psb30 shows a lower oxygen-evolving activity in high light [8], suggesting a regulatory role of Psb30 under high light conditions.

The expression of the *psb30* gene and the association of Psb30 with PSII complexes have not yet been determined in eukaryotes thus far. Since Psb30 is located in periphery of PSII core complex and in proximity of PsbZ which may have interaction with light harvesting chlorophyll (Chl) *a*/*b* complex II (LHCII) [15], it is of interest to characterize the function of Psb30 in PSII core complexes in green alga and plants. Accordingly, the green alga, *C. reinhardtii*, is a suitable model organism to study Psb30 characteristics because this organism contains the *psb30* gene in the chloroplast genome and is amenable to genetic manipulation of the chloroplast genes and to biochemical characterization of PSII complexes.

In the present study, we have generated an antibody against Psb30 of *C. reinhardtii* to localize this polypeptide in the thylakoids and generated ΔPsb30 mutants by chloroplast transformation to clarify its function/roles in PSII complex. We demonstrated that ΔPsb30 is stably bound to PSII RC but is not required for the structural stability of PSII complexes. In addition, we found that ΔPsb30 is more photosensitive than wild type and shows a slightly reduced

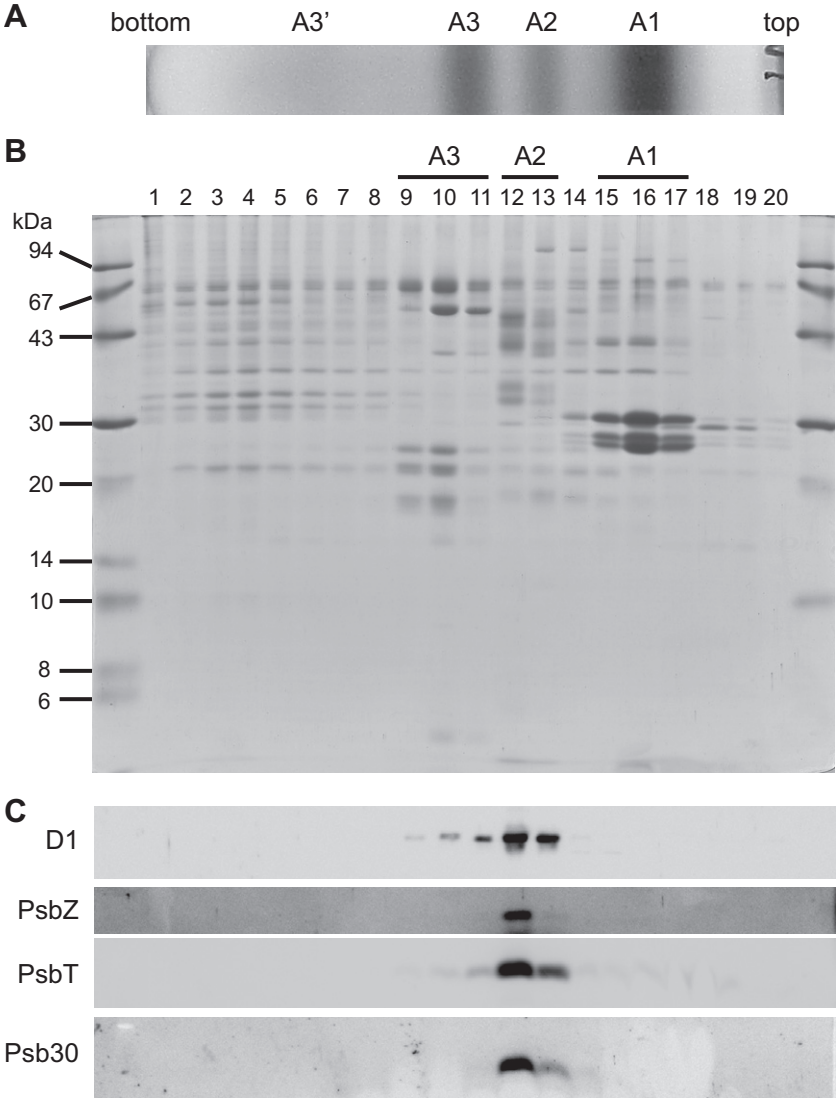

Fig. 2. Distribution of Psb30 on sucrose density gradient. Wild-type thylakoids solubilized with DM were separated by sucrose density gradient ultracentrifugation. The resulting gradient was fractionated from bottom to top. (A) Separation profile of chlorophyll proteins. (B) Polypeptide profile of each fraction. Polypeptides were separated by SDS–PAGE and were stained with Coomassie blue. (C) Immunoblots using antibodies against D1 (PsbA), PsbZ, PsbT, and Psb30.