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# The Rice Light-Regulated Gene *RA68* Encodes a Novel Protein Interacting with Oxygen-Evolving Complex PsbO Mature Protein

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**Abstract** The oxygen-evolving complex (OEC), which is located on the luminal side of photosystem II, plays an important role in water oxidation. It is generally considered that OEC consists of the $Mn_4Ca$ cluster and three extrinsic proteins, PsbO, PsbP, and PsbQ. In this study, we report that a novel rice protein RA68 interacts with PsbO. *RA68* is expressed preferentially in seedlings and encodes a novel protein without significant homology with any other proteins. Northern analysis demonstrates that *RA68* is a light-regulated gene with a diurnal oscillation pattern under different light conditions. Yeast two-hybrid screening reveals that RA68 interacts with PsbO and PsbP. Further experiments demonstrate that RA68 has specific interaction with PsbO mature protein rather than its precursor form. Moreover, in situ hybridization shows that *RA68* and *PsbO* have similar expression patterns in seedlings.

**Keywords** Light-regulated gene · *Oryza sativa* · Oxygen-evolving complex · PsbO · RA68 · Protein interaction

**Abbreviations**

| | |
|---|---|
| DDO | double dropout |
| LD | long day |
| LL | constant light |
| LREs | light-regulatory elements |
| OEC | oxygen-evolving complex |
| PSII | photosystem II |
| QDO | quadruple dropout |
| SD | short day |
| TDO | triple dropout |

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## Introduction

Photosystem II (PSII) is a multisubunit pigment–protein supercomplex embedded in thylakoid membrane and catalyzes electron transfer from water to plastoquinone by light producing molecular oxygen. The PSII supercomplex consists of three subcomplexes, light harvesting complex, core complex, and oxygen-evolving complex (OEC). Like other membrane complexes involved in the light reactions, the protein subunits of PSII are encoded by both chloroplast and nuclear genomes with each genome contributing to nearly half of the components of PSII (Gray 2004). Light is an essential environmental factor for plant growth and development, especially for the light reactions. Through the perception of photoreceptors, light signal regulates the expression of numerous photosynthesis genes to produce suitable amounts of proteins for assembly of functional photosynthetic complexes at the right time. Under light–dark cycles, the accumulations of nuclear photosynthesis genes exhibit diurnal oscillation (Gray 2004; Thompson and White 1991).

OEC, comprised of the $Mn_4Ca$ cluster and several extrinsic proteins, is located on the luminal side of PSII and plays an important role in water oxidation (Suorsa and Aro 2007). The extrinsic protein components of OEC are

different from cyanobacteria to higher plants. In green algae and higher plants, the extrinsic proteins include PsbO, PsbP, and PsbQ, whereas in cyanobacteria, PsbP and PsbQ are substituted by PsbU and PsbV, respectively (Seidler 1996; Suorsa and Aro 2007). Recently, homologs of PsbP and PsbQ have also been found in cyanobacterium *Synechocystis* 6803 and were proved to function in optimizing PSII water oxidation activity (Thornton et al. 2004). PsbO is present in all photosynthetic organisms and responsible for the stabilization of manganese cluster (Bricker and Ghanotakis 2004; Miyao and Murata 1984). In addition, PsbR, a 10-kDa protein, is also considered to be a component of OEC in green algae and higher plants (Roose et al. 2007). Being encoded by the nuclear genome, the extrinsic OEC proteins are synthesized in the cytoplasm as precursors with N-terminal transit peptides, translocated into the thylakoid lumen, processed to mature forms by cleavage of transit peptides, and then assembled into functional OEC (Chia and Arntzen 1986; Seidler 1996; Westhoff et al. 1985). With respect to the assembly of OEC in higher plants, one model suggests that only PsbO attaches the PSII core complex and provides a binding site for PsbP, which then forms a binding site for PsbQ (Miyao and Murata 1989). Based on recent publications, another model has been proposed, which suggests that PsbO and PsbP are independently bound to the PSII core complex and both of them provide docking sites for PsbQ (Suorsa and Aro 2007).

Recent studies have identified numerous low-molecular-mass proteins relating to PSII (Hankamer et al. 2001; Shi and Schroder 2004; Suorsa and Aro 2007). A loss-of-function mutation of *psbJ*, a tobacco chloroplast *psbEFLJ* operon gene, leads to accumulation of incompletely assembled OEC devoid of PsbP and dramatic reduction in photosynthetic oxygen evolution rate, indicating that PsbJ is required for the stable assembly of PsbP into OEC in tobacco (Hager et al. 2002). The other three proteins encoded by the *psbEFLJ* operon genes are also essential for the proper assembly of the OEC (Suorsa et al. 2004). Nuclear-encoded PsbR from *Arabidopsis* is essential for the stable assembly of PsbP and probably plays an indirect role in the stability of PsbQ (Suorsa et al. 2006). Chloroplast-encoded PsbY was reported to be important for the stable binding of OEC proteins PsbU and PsbV, even participate in the stability of PsbO in *Synechocystis* 6803 (Ban et al. 2006). These studies indicate that multiple low-molecular-mass proteins may be involved in the OEC assembly, stability, and function maintenance; however, the mechanisms of these processes remain largely unknown. Further identification of novel proteins interacting with the OEC proteins and illumination of exact protein constitution of functional OEC will be important to elucidate the mechanisms.

In this work, we found a novel protein RA68 which interacts with PsbO in rice (*Oryza sativa*). *RA68* is a nuclear gene showing a diurnal expression pattern under light–dark cycles and encodes a protein specifically interacting with PsbO mature protein rather than its precursor form. Additionally, *RA68* and *PsbO* have similar spatial expression patterns in seedlings.

## Materials and Methods

### Plant Materials and Growth Conditions

Seeds of rice cultivar Zhonghua 10 (*O. sativa* L. ssp. *japonica*) were soaked in water at 30°C in darkness for 3 days until germination. Germinated seeds were spread on soaked filter paper and transferred into the plant growth chamber (E8, Conviron, Canada) at 12-h light 30°C/12-h dark 25°C cycle for additional 4 days. At the eighth hour after dawn, aerial parts of 7-day-old seedlings were collected and immediately frozen in liquid nitrogen for extraction of total RNA.

For light period entrainment, germinated seeds were spread on soaked filter paper and transferred into plant growth chambers (E8, Conviron, Canada) at 30°C under short day (SD, 8-h light/16-h dark) or long day (LD, 16-h light/8-h dark) conditions for additional 4 days. For constant light (LL) experiment, seedlings were grown under SD for 5 days and then transferred to LL condition at dawn. At dawn (time 0), aerial parts of seedlings were collected at 4-h intervals within 24 h for SD and LD experiments and within 72 h for LL experiment, respectively.

### Northern Analysis of *RA68* Expression

Total RNA was extracted using Trizol Reagent (Invitrogen, USA). Forty micrograms of total RNA was separated by 1.5% denatured formaldehyde agarose gel and then transferred onto Hybond-N+ nylon membrane (Amersham Biosciences, UK). A *RA68* cDNA fragment amplified by primer pair P1F (5′-AGA TGA GTA GCA AGG TTC-3′) and P1R (5′-GTA GTG ATA ATC ATT TGC C-3′) was labeled with $^{32}$P using Prime-a-Gene® Labeling System (Promega, USA) and used as a probe. Hybridization was carried out overnight at 65°C in 6× SSC, 0.5% SDS, and 0.1 mg/ml denatured salmon testes DNA. The membrane was washed twice with 2× SSC, 0.1% SDS for 20 min and twice with 0.1× SSC, 0.1% SDS for 10 min at 65°C and then autographed at −80°C. rRNA was used as a loading control. Images were scanned into a computer and band intensities were quantified using the Scion Image 4.0.3.2 software (http://www.scioncorp.com). *RA68*/rRNA values

represented the relative values of *RA68* to the lowest value of the experiment after normalization to the rRNA control.

### Yeast Two-Hybrid Screening and Assays

A yeast two-hybrid screening of proteins interacting with RA68 was performed according to BD Matchmaker™ Library Construction and Screening Kits User Manual (BD Biosciences, USA). The open reading frame (ORF) of *RA68* (AY568677 in DDBJ database) was amplified with primer pair P2F (5′-CGG *GAA TTC* ATG AGT AGC AAG GTT CTT-3′, *Eco*RI site italicized) and P2R (5′-CCT *GTC GAC* GTA GTG ATA ATC ATT TGC-3′, *Sal*I site italicized), digested, and cloned into pGBKT7 vector (BD Biosciences, USA) as yeast two-hybrid bait construct BD-RA68. Rice 7-day-old seedling cDNA library was constructed and cotransformed into *Saccharomyces cerevisiae* strain AH109 with pGADT7-Rec and BD-RA68. Transformants were grown on triple dropout (TDO, SD/-His/-Leu/-Trp) medium. White or light pink clones which appeared within 3 to 5 days and grew larger than 2 mm in diameter were picked and streaked on quadruple dropout (QDO, SD/-Ade/-His/-Leu/-Trp) medium for an additional 4 days. Positive clones on QDO medium were chosen for β-galactosidase colony-lift filter assays as described in the Yeast Protocols Handbook (BD Biosciences, USA). The prey plasmids in clones which displayed β-galactosidase activity were rescued in *Escherichia coli* and recotransformed into yeast strain AH109 with bait construct BD-RA68 to check one-on-one interaction. All positive candidates showing good growth condition on QDO medium and β-galactosidase activity were sequenced for a later BLASTX in the National Center for Biotechnology Information (NCBI) *O. sativa* database.

### Verification of Interaction Between RA68 and PsbO

Primers P3R (5′-GGA AGG CAA ATG TTC GTG AAG ATA GGC A-3′), P4F (5′-GGC CAC ACC ACC CAC CAA CTG AGA G-3′), and P4R (5′-CGA GAA CAG AGG AAG AAA CAA AAG C-3′) were designed according to the full-length sequence of *PsbO* cDNA (NM_001049669 in NCBI database). Seven-day-old seedling total RNA was reverse transcribed to synthesize first-strand cDNA by primer P3R. The first-strand cDNA was used as a template for polymerase chain reaction (PCR) amplification of *PsbO* cDNA spanning the ORF with primer pair P4F and P4R. The PCR product was cloned into pMD19-T vector (TaKaRa, Japan) to generate pMD19-T-PsbO and sequenced.

Two cDNA fragments *PsbO-P* and *PsbO-M* were amplified from pMD19-T-PsbO with primer pairs P5F (5′-CGA *GAA TTC* ATG GCA GCA TCG CTC CAA G-3′, *Eco*RI site italicized) and P5R (5′-GTT *GGA TCC* CTA CTC GAG CTG CGC GTA C-3′, *Bam*HI site italicized) and P6F (5′-TAT *GAA TTC* GAG GGC GTG CCG AGG AGG C-3′, *Eco*RI site italicized) and P5R, respectively. *PsbO-P* encoded all 333 amino acid residues of PsbO precursor protein (1–333), whereas *PsbO-M* encoded 247 amino acid residues of PsbO mature protein (87–333). The two cDNA fragments were double digested by *Eco*RI and *Bam*HI, cloned into pGADT7 vector (BD Biosciences, USA) as prey constructs AD-PsbO-P and AD-OsPsb-M, respectively, and further confirmed by sequencing.

The interaction between RA68 and PsbO was verified by yeast two-hybrid assays. AD and BD empty vectors were used as negative controls, while murine p53 and SV40 large T-antigen (BD Biosciences, USA) as a positive control. To check the interaction between two individual proteins, different combinations of bait and prey constructs were cotransformed into yeast strain AH109. Transformants were grown on double dropout (DDO, SD/-Leu/-Trp) medium and then streaked on fresh QDO medium to grow 4 days to check the reporter genes *HIS3* and *ADE2*. To check the third reporter gene *LacZ*, transformants were streaked on fresh DDO medium then performed β-galactosidase colony-lift filter assays.

### In Situ Hybridization of *RA68* and *PsbO*

Digoxigenin (DIG)-labeled antisense and sense RNA probes for *RA68* or *PsbO* were synthesized in vitro with SP6 polymerase (Roche, Germany) using cDNA fragments spanning nt 364–560 of *RA68* and nt 542–737 of PsbO as templates, respectively. Seven-day-old seedlings were fixed and further processed following the previous methods (Ding et al. 2002).

## Results

### *RA68* is a Light-Regulated Gene with a Diurnal Expression Pattern

Our previous study has shown that *RA68* is a single-copy gene located in rice chromosome 2 and the full-length of its cDNA is 902 bp, consisting of a 5′ untranslated region (UTR) of 64 bp, a 3′ UTR of 178 bp, and an ORF of 660 bp (AY568677 in DDBJ database). The gene encodes a protein of 219 amino acid residues with molecular mass of 22.8 kDa. RA68 contains a putative signal peptide of 22 amino acid residues; the mature RA68 protein consists of hydrophilic N-terminal and hydrophobic C-terminal domains. BLASTP in GenBank shows that the RA68 protein has no significant homology with any known proteins except 38% overall sequence similarity with *Arabidopsis* protodermal factor 1. Expression analysis

shows that *RA68* is expressed preferentially in seedlings and flowers (Wu and Wang 2004).

To illuminate transcription regulation of *RA68*, we analyzed the organization of *cis*-acting regulatory elements in the *RA68* promoter (a region of 1.5 kb upstream from the putative transcription initiation site) using the PLACE (Higo et al. 1999; Prestridge 1991) and PlantCARE (Lescot et al. 2002) databases. As shown in Table 1, the region comprised many putative *cis*-acting light-regulatory elements (LREs), including some representative LREs such as G-boxes, GATA-boxes, and GT1-motifs (Castresana et al. 1988; Gilmartin et al. 1990; Giuliano et al. 1988; Green et al. 1988; Terzaghi and Cashmore 1995). Some of these LREs were presented in multiple copies: GATA-boxes and GT1-motifs showed eight copies, while SORLIPs (Hudson and Quail 2003), Inr (Nakamura et al. 2002), and G-boxes LREs showed five, three, and two copies, respectively. The GATA-boxes and GT1-motifs were evenly distributed throughout the whole promoter region, whereas the G-boxes were grouped in the flanking region far from the transcription initiation site (Fig. 1). The existence of numerous LREs in *RA68* promoter implied that *RA68* might have a light-regulated expression pattern.

In addition, we examined *RA68* expression pattern in seedlings under distinct light regimes by Northern hybridization. Under LD, *RA68* mRNA accumulation showed a clear 24-h rhythm with a broad peak in light and the highest point at dawn, whereas *RA68* mRNA accumulation was hardly detected in dark (Fig. 2a). There was a 33-fold change comparing the highest level to the lowest level of *RA68* mRNA accumulation (Fig. 2c). Similarly, under SD, *RA68* mRNA also showed high accumulation in light with a peak at 4 h and low accumulation in dark (Fig. 2b, d). However, when the seedlings entrained under SD were transferred into LL, *RA68* mRNA accumulation lost its 24-h rhythm accompanying that the oscillation amplitude became lower as time went by (Fig. 2e, f), indicating that *RA68* expression was not regulated by circadian clock. Taken together, the results indicated that *RA68* was a light-regulated gene and had a diurnal expression pattern under light–dark cycles.

## RA68 Interacts with PsbO and PsbP in Yeast

In order to further reveal the function of RA68, we screened RA68 interactors using a yeast two-hybrid method. By screening a rice 7-day-old seedling cDNA library with bait construct BD-RA68, 375 clones were picked from TDO medium and restreaked on fresh QDO medium. After growing condition test and β-galactosidase assays, only 50 clones were left. Finally, 15 clones were identified as positive ones by the examination of one-on-one interaction between the prey plasmids rescued from the 50 clones and RA68 bait construct. Sequencing and BLASTX analyses revealed that, among the 15 clones, nine represented rice OEC protein PsbO and one corresponded to another OEC protein PsbP.

## RA68 Specifically Interacts with PsbO Mature Protein

The nuclear-encoded PsbO precursor protein is processed to mature form in chloroplast and assembled into functional OEC (Seidler 1996). In rice, PsbO precursor protein of 333 amino acid residues (BAF05048 in the NCBI database) is processed to mature protein of 247 amino acid residues (A38889 in the PIR-PSD database). In yeast two-hybrid screening results, inserted sequences in the nine PsbO clones all encoded truncated proteins lacking the N-terminal compared to the rice PsbO precursor sequence. To verify whether RA68 interacts with PsbO precursor protein or mature one, two constructs, AD-PsbO-P and AD-PsbO-M, encoding PsbO precursor protein (1–333aa) and mature protein (87–333aa), respectively, were generated by fusing the respective *PsbO* cDNA fragments to the GAL4

**Table 1** The LREs in the *RA68* 1.5-kb promoter

| LRE | Copy numbers | Species | Spatial specificity | Databases |
|---|---|---|---|---|
| AE-box | 1 | At | | PlantCARE |
| Circadian | 1 | Le | Leaf, shoot | PLACE, PlantCARE |
| G-box | 2 | St, Zm | | PlantCARE |
| GAG-motif | 1 | At | | PlantCARE |
| GATA-box | 8 | At, Os, Ph | Leaf, shoot | PLACE |
| GT1-motif | 8 | As, At, Nt, Os, Ps, So | Leaf, shoot | PLACE, PlantCARE |
| I-box | 1 | Monocots, dicots | Leaf, shoot | PLACE, PlantCARE |
| Inr | 3 | Nt | | PLACE |
| SORLIPs | 5 | At | | PLACE |
| SORLREPs | 1 | At | | PLACE |
| T-box | 1 | At | | PLACE |
| TCT-motif | 1 | At | | PlantCARE |

The copy numbers of each LRE in the *RA68* 1.5-kb promoter are listed. Their spatial specificities and species origin are also listed. The databases represent where the LREs were predicted

*At Arabidopsis thaliana*, *As Avena sativa*, *Le Lycopersicon esculentum*, *Nt Nicotiana tabacum*, *Os Oryza sativa*, *Ph Petunia hybrida*, *Ps Pisum sativum*, *So Spinacia oleracea*, *St Solanum tuberosum*, *Zm Zea mays*

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-1500   AATCCTATGTAAAACATCTTAGGTCGAGTATTAGGACTGTGCCAACAGAAATACTATGTG
-1440   CTAGCCCAGCTTTCAGGGGCCCAACAATCACCAAAGTAACTCTGCCCTTCGGAAAATTAA
-1380   AGGCAGCCACTCAGACACACAGTGATATCCAGCAAATGCACGACGGATCACATGGAGTTT
-1320   CAGCCCCAGGGCCAATCACATCTTGCGCAAGTACAGCCGTTGCTGGTATCTTCAACCATT
-1260   CCTCTATTATCTCCTGGCCTGGTCCTGCTCAGCTGCAACTGTAACGGTTAGTTCAGTCCC
-1200   ATACTTCTTCCGTTTCACAATGCAAGTCATTCTACCATTTTTCACATTTATATTGATATT
-1140   AATGAATCTAGATATATATATTTGTCTAGATTCATTAACATCAATATAAATGTAGGAAAT
-1080   GTTAGAATGACTTATATTGTGAAATGGAGGGAGTATGTACGTATGGTTTCAGAGAGTTCG
-1020   ACTGTTCCAGAAGTTAAGGGCAATAGACAGTTGGATGCAGGCTACTAGTCATTACCTGGT
-960    AATTTTCTGCCTTTTTTTGTGGTCATTGTAAGTAATTCTTGACAGGAGATCACACATGCA
-900    TAGCTCCATTTCATCTGCAATGTCAGACGCTGTCAAGATTAAGGAGTAGGAGTTTTGTTT
-840    ATAGGAGGGACGAAAAATGTGAGTGATGGATAGCATGATGAAATCGCAGGTGCGATATTGA
-780    TGAATGACTGCAGCAGCATTACACTAATTGCTATATATATTTTTTATTTAAGAGCAGCAA
-720    GGTCTGTTTGTAACTAAAACAGAAATGTTTTCAATTAAAAAAAACTAACAGCACAAGGTC
-660    TACAGCATACCAAGTAAACTAACGCTAGCTAGAAGCAGCCAGATAGATCAGAAAAACTCG
-600    AAAACTTTGCAGCACAATTCTGTAATGTAATGTGCTGATAATGTATAGCATGAAAGACTG
-540    CGGCACAAATCAGTTCCAAGAAACTTACAAGAATTAACAAGCGGAATTAGACCAAAGCAT
-480    CGCTTTTTACACAAAAACAATTGTAAAATCAAATTTGGTGCTTCCACATAACAACTGGGC
-420    CAGGGAATCTTACCATACTGTGCTTCCATTAAATTGAGAAATTCAATTTTGCCACCACAG
-360    CATGAAATCCAGCTCGAGGCATGGTTAAGTGTACTGAGTAGGTGGCAAGCACCGATCCCC
-300    ATTAAATCAGCAGACTTTCGGGGAGGAAATCCATTCTGCATGGAAAGAAGACTGCCAATA
-240    TCCATGGAAGCTAAAAGTGCCATGGCCTCGCTCTAACCTACAAATTTGGACACAGATTTG
-180    AGTTAAAGAACTCACAATGGAAGCTTCATCATGTGAATTGAAAGCGAGCAGATCACCGTC
-120    ATGCTGAACTAGCTGGACGAATCAAACCATTAAATGATAGACAACTACTAACAGTGTCAA
-60     TGGAACTGTAGAGCATCTTTCGCATGTATATATAGGCGCACAAAGGCAATTCAGTTCATC
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**Fig. 1** The distribution of three representative LREs in the *RA68* 1.5-kb promoter. Nucleotide acids represent the *RA68* 1.5-kb promoter sequence and the putative transcription initiation site is zero. Potential LREs are represented as follows: *white box* G-boxes, *gray box* GATA-boxes, *underlined* GT1-motifs

activation domain (AD). Different dual combinations of AD-PsbO-P, AD-PsbO-M, BD-RA68, BD, and AD empty vectors were cotransformed into yeast strain AH109. Interaction was tested by activating three reporter genes. As shown in Fig. 3a, all transformants grew well on DDO medium. Positive control showed good growth condition on QDO medium and strong β-galactosidase activity due to the activation of all the three reporter genes (Fig. 3b, c).

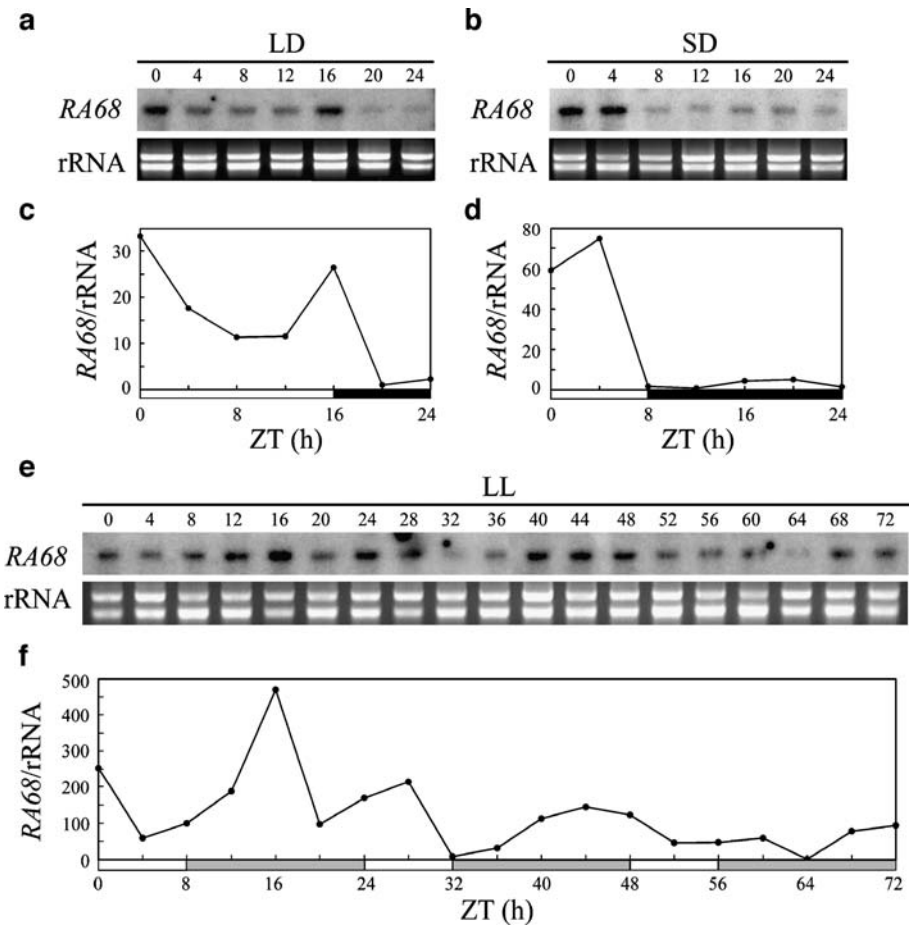


**Fig. 2** Diurnal oscillations of *RA68* transcript in seedlings under distinct light regimes. **a**, **b**, **e** *RA68* mRNA accumulation under LD (16-h light/8-h dark), SD (8-h light/16-h dark), and LL (constant light), respectively. **c**, **d**, **f** Quantification of *RA68* mRNA for the experiments under LD (**a**), SD (**b**), and LL (**e**), respectively. *ZT* zeitgeber time

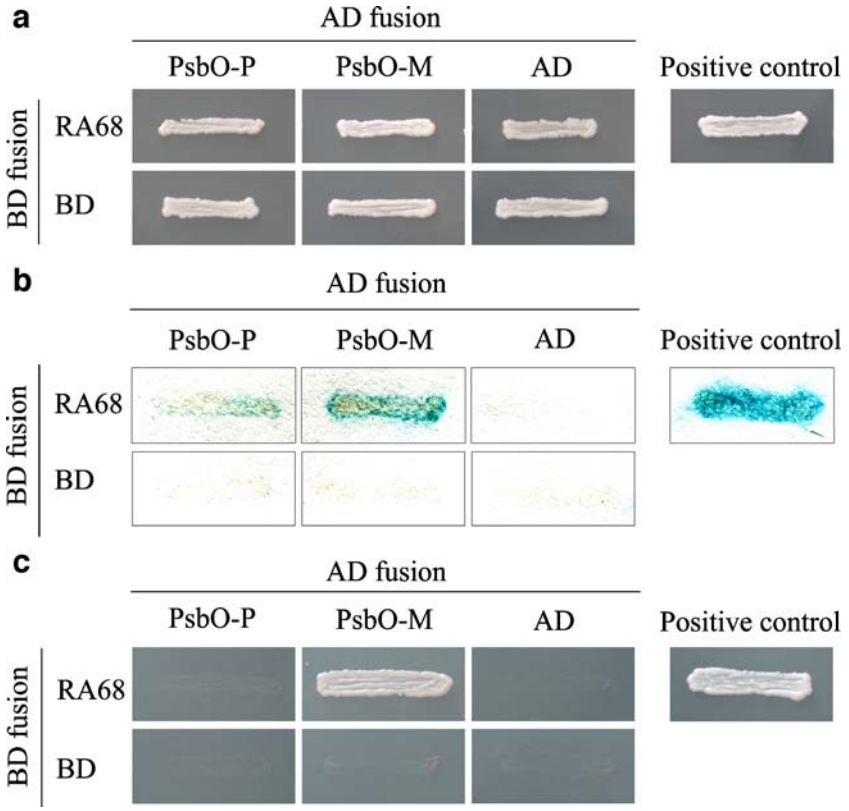


**Fig. 3** RA68 specifically interacts with PsbO mature protein rather than its precursor form in yeast. Different dual combinations of prey (AD fusion) and bait (BD fusion) constructs were cotransformed into yeast strain AH109. **a** Growth on DDO (SD/-Leu/-Trp) medium lacking leucine and tryptophan. **b** β-galactosidase activity of colonies grown on DDO medium. **c** Growth on QDO (SD/-Ade/-His/-Leu/-Trp) medium lacking adenine, histidine, leucine, and tryptophan. *PsbO-P* PsbO precursor protein, *PsbO-M* PsbO mature protein, *AD* pGADT7 empty vector, *BD* pGBKT7 empty vector

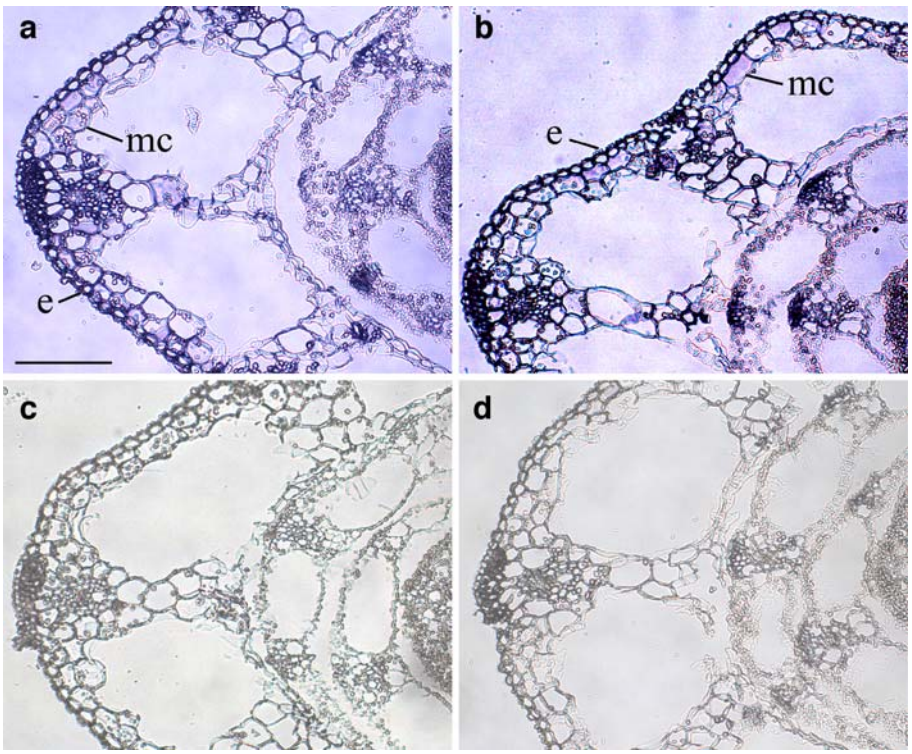


**Fig. 4** In situ hybridization analyses of *RA68* and *PsbO* mRNA in seedlings. Traverse sections of 7-day-old seedlings were hybridized with DIG-labeled antisense RNA (**a**, **b**) and sense RNA (**c**, **d**) probes of *RA68* (**a**, **c**) or *PsbO* (**b**, **d**). Signals were detected with anti-DIG antibody conjugated alkaline phosphatase and visualized with substrates nitro blue tetrazolium and 5-Bromo-4-chloro-3-indolyl phosphate (Roche, Germany). *mc* mesophyll cell, *e* epidermis. Bar in **a**=100 μm for **b**–**d**

The transformants containing either AD or BD empty vectors could not activate any reporter genes, indicating that any single protein did not have transcriptional activation (Fig. 3b, c). Interestingly, only the combination of RA68 and the PsbO mature protein showed strong β-galactosidase activity and made yeast grow well on QDO medium, whereas the combination of RA68 and the PsbO precursor protein could not activate any reporter genes (Fig. 3b, c). The results indicated that RA68 specifically interacted with the PsbO mature protein rather than its precursor form.

### Both *RA68* and *PsbO* are Expressed in Mesophyll Cells of Seedlings

Furthermore, to identify whether *RA68* and *PsbO* have similar spatial expression patterns in seedlings, we performed RNA in situ hybridization in 7-day-old seedlings using DIG-labeled antisense and sense RNA probes for *RA68* or *PsbO*. In seedlings, signals were detected in mesophyll cells of outer leaf sheath using both *RA68* (Fig. 4a) and *PsbO* (Fig. 4b) antisense RNA probes. No signals were detected in epidermis or other parts of leaf sheath (Fig. 4a, b). Neither *RA68* (Fig. 4c) nor *PsbO* (Fig. 4d) sense RNA probe detected any signals in seedlings. These results gave a direct proof that the *RA68* and *PsbO* mRNA have similar spatial expression patterns in seedlings.

## Discussion

The mRNA levels of components that belong to the same photosynthetic complex show parallel diurnal oscillation patterns. For example, under a 12-h light/12-h dark cycle, the mRNA levels for the three OEC proteins (genes *PsbO*, *PsbP*, and *PsbQ*) show parallel oscillation trends with peaks in light (Oelmüller et al. 1995). Our results showed that *RA68* mRNA accumulated at high level in light and at low level in dark with quick change during light–dark switch under both LD and SD conditions. The diurnal oscillation pattern of *RA68* is comparable with that of the OEC genes described previously (Oelmüller et al. 1995). In addition, both *RA68* and *PsbO* are expressed in mesophyll cells of seedlings. The similar spatiotemporal expression patterns of *RA68* and *PsbO* are consistent with their protein interaction in yeast, indicating that RA68 and PsbO may have interaction in planta. The data that RA68 specifically interacts with PsbO mature protein rather than its precursor form in combination with the fact that the nuclear-encoded OEC precursor proteins are translocated into chloroplast and processed to their mature forms there (Seidler 1996) suggest that the interaction between RA68 and PsbO might take place in chloroplast.

RA68 is a nuclear-encoded protein (Wu and Wang 2004), but no typical transit peptide is found in RA68 protein sequence by different program predictions. Psb27, another nuclear-encoded protein, was identified in the PSII complex of *Synechocystis* 6803 (Kashino et al. 2002) and predicted to be targeted to the thylakoid lumen by a signal peptide II (Roose et al. 2007). Analysis of RA68 protein sequence using the same programs SignalP (Dyrlov Bendtsen et al. 2004) and LipoP (Juncker et al. 2003) predicted that RA68 also contained a putative signal peptide II. It is likely that RA68 may be targeted to the thylakoid lumen by the same mechanism as that of Psb27.

In summary, our results in this study indicate that *RA68* is a novel light-regulated gene with spatiotemporal expression pattern similar to the OEC gene *PsbO*. Besides, RA68 interacts specifically with PsbO mature protein rather than its precursor form. However, elucidation of the function of RA68 for OEC needs further experiments by use of the RA68 mutation line. As mentioned in the introduction, many novel proteins have been found to be involved in the OEC assembly, stability, and function maintenance. Our results potentially provide new insight into the understanding of the biological process about rice OEC.

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