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## **Molecular characterization of the nuclear-encoded photosystem II subunit PSII-W in *Chlamydomonas reinhardtii***

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### **Introduction**

PSII-W is encoded for by the nuclear gene *psbW*. This gene appears to be present throughout photosynthetic eukaryotes, but is absent from the fully sequenced genome of *Synechocystis* 6803 and from all other prokaryote examined. An unrelated gene, also termed 'psbW', is present in the cyanelle of *Cyanophora paradoxa* and the plastid genome of the non-green algae *Odontella sinensis* and *Porphyra purpurea*.

Compositional and topological studies indicate that the polypeptide is located close to the core of the PSII complex, in close proximity to the D1-D2 heterodimer (Shi and Schröder, 1997). Analysis of a *psbW* antisense *Arabidopsis thaliana* has suggested a role for PSII-W in the biogenesis and regulation of the PSII complex (Shi *et al.*, 2000). Recently a small protein of 5-6kDa, with high levels of N-terminal sequence homology with PSII-W, was isolated with PSI preparations from a number higher plant sources (Hiyama *et al.*, 2001). This work called into question the long held view that the PSII-W protein is found solely within the PSII complex.

In this work, both genomic and cDNA sequence data have been produced for the *psbW* gene of *C. reinhardtii*, together with analysis of the amino acid sequence of the PSII-W protein. Antibodies have been raised to the mature portion of the PSII-W protein. We examine the location of the PSII-W protein in the photosystem complexes and show that the PSII-W protein is present only in PSII complex in *C. reinhardtii*.

### **Materials and methods**

#### *Sequencing of the psbW gene of C. reinhardtii*

A cosmid library prepared by Purton and Rochaix (Purton and Rochaix, 1995) using the vector pARG7-8cos was screened. Two *Bam*HI fragments of 1.4kb and 2.2kb, both containing a portion of the *psbW* gene, were isolated and sequenced. RT-PCR was employed to isolate a 620bp cDNA product from wild type (WT) CC-1021 *C. reinhardtii* cells grown under photoautotrophic conditions, which was also sequenced.

#### *Antibody production to mature PSII-W and western analysis*

The coding region for the mature PSII-W protein was isolated and cloned into the expression vector pMAL-c2. Antibodies were raised to the complete MBP~PSII-W fusion protein. Western analysis was performed using a LiDS-Urea gel system.

## Results and Discussion

### *Structure of the psbW gene*

We have sequenced both genomic and cDNA clones from *C. reinhardtii*. Screening of a genomic cosmid library (Purton and Rochaix, 1995) isolated positive clones containing the *psbW* gene. Analysis of the clones with *Bam*HI identified fragments of 1.4kb and 2.2kb that contained portions of the *psbW* gene. Both fragments were cloned and used to determine the genomic sequence. The sequence obtained from a 620bp RT-PCR product confirmed the intron exon boundaries with the gene. The coding region of the *psbW* gene spans 1000 nucleotides and contains 6 exons and 5 introns (Accession No. AF170026).

### *psbW is a single copy gene*

Southern analysis of the genome of *C. reinhardtii* confirms that there is only a single copy of the *psbW* gene in the nuclear genome of *C. reinhardtii* (not shown).

### *Characterisation of the C. reinhardtii PSII-W sequence and comparison with other PSII-W sequences*

The *psbW* gene encodes a protein of 115 residues, containing a 56 amino acid mature protein with a predicted molecular mass of 6.1kDa. Amino acids one to 30 are proposed to constitute the first portion of the bipartite transit peptide, which enable import of the pre-protein across the chloroplast envelope. The second part of the bipartite transit peptide, residues 31 to 59, enables the insertion of the pre-protein into the thylakoid membrane, via a spontaneous insertion mechanism. The terminal 56 amino acids of the protein constitute the mature protein, with a molecular mass of 6.1kDa. The mature PSII-W protein is predicted to have a single transmembrane span with the N-terminus located in the thylakoid lumen. There is an overall high level of sequence similarity between the mature PSII-W proteins of *C. reinhardtii*, *Arabidopsis thaliana* (Accession No. X90769), and *Spinacia oleracea* (Accession No. Q41387). The sequence identity of the mature protein with that of the spinach sequence is 52%.

### *Western Analysis of PSII-W*

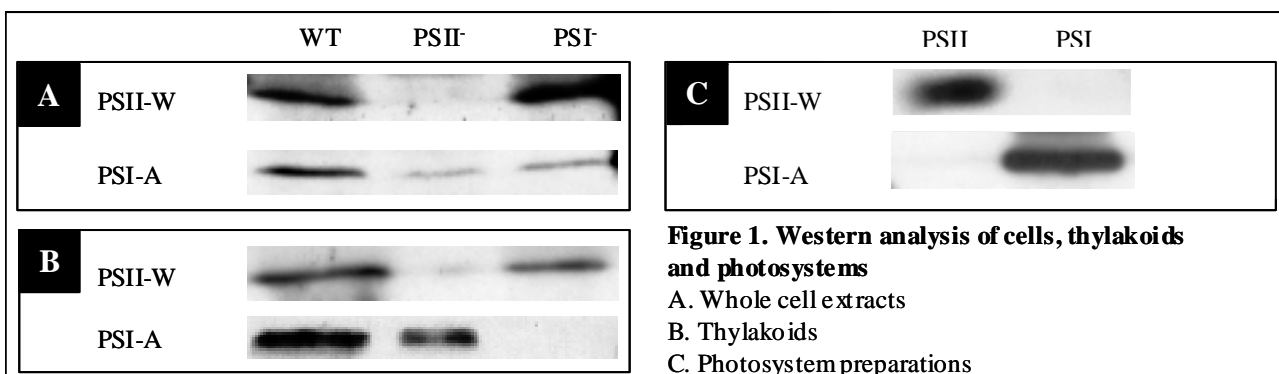
Antibodies were raised to a MBP~PSII-W fusion protein. To confirm the location of the PSII-W protein within the thylakoid membrane of *C. reinhardtii* western analysis initially performed on whole cell extracts and thylakoid preparations from WT CC-1021, PSII<sup>-</sup> (O'Connor *et al.*, 1998) and PSI<sup>-</sup> cells (Hallahan *et al.*, 1995) These results are shown in Figure 1.

Equivalent levels of PSII-W could be detected in the WT and PSI<sup>-</sup> cells. Although PSII-W could be detected in cells lacking PSII, the levels were considerably lower than those of the WT and PSI<sup>-</sup> samples. When thylakoid preparations from the same strains were investigated, the PSII-W could again be identified in the WT and PSI<sup>-</sup> thylakoids. However, PSII-W could not be detected in thylakoid samples from cells deficient in PSII. Western analysis of photosystem preparations resulted in detection of the PSII-W protein in PSII preparations. An equivalent band could not be detected in pure PSI preparations.

The blots containing thylakoid samples and the photosystem preparations were also probed with antibodies to the PSI subunit PsaA. The presence of this protein, and by inference the PSI complex, could be detected in WT thylakoids, PSII<sup>-</sup> thylakoids and PSI preparations. As

expected this protein could not be detected in PSI<sup>-</sup> thylakoids or preparations of PSII, the later confirming the purity of the PSII preparation.

It is probable that the PSII-W protein is expressed in the PSII<sup>-</sup> cells, this statement is supported by the detection of a weak band in the PSII<sup>-</sup> cell extract. One possibility for the absence of a band in the thylakoid samples is that, although the pre~PSII-W protein is expressed, its import into the chloroplast or its insertion into the thylakoid membrane is inhibited in the absence of an assembled PSII complex, and its degradation is rapid enough to avoid detection.



Our explanation of the detection of PSII-W in PSI preparations by Hiyama *et al.*, is that this represents protein released from PSII that has adventitiously bound to their PSI. The PSI preparations presented were crude preparations, which lends further weight to this argument. Furthermore, no western analysis was undertaken on these PSI preparations to confirm a lack of PSII contamination. From our results it can be concluded that the PSII-W protein is present when PSII is present and PSI absent in a sample. The protein could not be detected in samples with PSI solely or when PSII was lacking. These results support the view that PSII-W is present exclusively in the PSII complex of *C. reinhardtii*.

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