S6-010

A molecular characterisation of PsaN: a nuclear-encoded subunit of the photosystem I complex of *Chlamydomonas reinhardtii.*

K. Ali and S. Purton

Photosynthesis Research Group, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT, U.K. Fax: +44 (0) 20 7679 7096. E-mail: k.ali@ucl.ac.uk.

Keywords: Chlamydomonas reinhardtii, Photosystem I, PsaN, nuclear-encoded subunits

Introduction

Photosystem I (PSI) uses light energy to transfer electrons across the thylakoid membrane from plastocyanin (PC) to ferredoxin (Fd) (Chitnis, 2001). The PSI complex is a membrane protein complex composed of a large number of polypeptide subunits, designated PsaA to PsaN. There are distinct differences in the subunit composition between prokaryotic and eukaryotic PSI complexes. For example, PsaG, PsaH and PsaN are all absent from cyanobacterial PSI. In eukaryotes, the genes for the PSI subunits are distributed between the nuclear and chloroplast genomes, with the core subunits encoded by chloroplast genes. The core subunits PsaA and PsaB form the heterodimeric reaction centre and bind the electron donor P700, electron acceptors A_0, A_1 and the Fe-S centre F_X. The stromal subunit PsaC binds the terminal electron acceptors F_A and F_B. The remaining subunits do not bind any redox cofactors and have accessory functions.

The function of many of the small nuclear-encoded subunits is not fully understood. We are investigating the role of the subunit PsaN, which is unique to eukaryotic PSI complexes. PsaN is an extrinsic subunit 9-10 kDa in size and located entirely on the lumenal side of the thylakoid membrane. The PsaN protein is synthesised as a precursor protein with a transit peptide sequence to import the protein into the chloroplast and across the thylakoid membrane to the lumen. The protein is processed to the mature size by a thylakoid processing peptidase. The translocation of the protein across the thylakoid membrane is believed to be ΔpH dependant (Nielsen *et al*., 1994). The PsaN protein is predicted to have no membrane spanning regions and is assumed to be held to the PSI complex by electrostatic interactions (He and Malkin, 1992). Initial work with PsaN from spinach showed that it was easily dissociated from the PSI complex by salt washing. The loss of PsaN from the PSI complex had no significant effect on electron transfer from PC to $NADP^+$ (He and Malkin, 1992). Recent work on transgenic *Arabidopsis* plants devoid of PsaN has shown that the plants grow photoautotrophically and have functional PSI complex. However, the rate of electron transfer from PC to $P700^+$ is only 55% of wild-type, indicating that PsaN is required for the efficient interaction with PC (Haldrup *et al*., 1999). Furthermore, the down-regulation of PsaF in *Arabidopsis* has shown to cause a decrease in PsaN (Haldrup *et al*., 2000). This raises the question as to whether PsaN interacts with PC directly or indirectly via PsaF.

PsaF is an integral membrane subunit of ~18 kDa with a transmembrane domain and an additional lumenal domain found only in eukaryotes. The nuclear *psa*F gene has been successfully inactivated in *C. reinhardtii* (Farah *et al*., 1995). The mutant strain (3bF) assembles functional PSI and grows photoautotrophically but the rate of electron transfer from PC to $P700^+$ is considerably reduced. In eukaryotes, the N-terminal domain of PsaF is

involved in docking PC and PsaF is required for efficient electron transfer from PC to $P700^+$ (Farah *et al*., 1995; Hippler *et al*., 1997).

In this paper, we report the cloning and characterisation of the *psa*N gene from the unicellular green alga *C. reinhardtii*. We have raised antibodies to the PsaN protein and shown that the 3bF mutant lacking the PsaF subunit has undetectable levels of PsaN, suggesting that the mutant fails to assemble the PsaN subunit in the PSI complex in the absence of PsaF. These findings highlight a possible interaction between PsaN and PsaF. Furthermore, since the 3bF mutant is capable of photoautotrophic growth under moderate light conditions (Hippler *et al*., 2000), then PsaN appears to be a non-essential component of PSI.

Materials and Methods

Isolation and analysis of the psaN gene. A cosmid library (Purton and Rochaix, 1995) was screened using the *psa*N cDNA as a probe to isolate the *psa*N gene. A positive clone was identified and characterised. In order to locate the *psa*N gene in the genomic clone, DNA was isolated and digested with restriction enzymes. Southern analysis was carried out as described in Sambrook *et al.* (1989). A positive 2.2 kb *Sac* I-*Sal* I fragment was isolated and cloned into pBluescript. The subclone was then sequenced.

Copy number determination. Genomic DNA was isolated using the DNeasy plant mini kit (Qiagen) from wild-type *C. reinhardtii* cells (CC-1021). 3 μg of genomic DNA was digested with restriction enzymes and electrophoresed on a 1% agarose gel. DNA was transferred to Hybond N+ membrane (Amersham). Southern analysis was carried out using the *psa*N cDNA as a probe.

Raising antibodies to PsaN. PsaN protein was over-expressed using the pMAL-c2 expression vector system in *E. coli* (New England Biolabs). The coding sequence of the mature PsaN protein was cloned downstream from the *malE* gene (which encodes a maltose-binding protein-MBP) in the pMAL-c2 vector. The PsaN protein was expressed as a fusion protein. The MBP-PsaN fusion protein was used to raise polyclonal antibodies in rabbits.

Western analysis. Western analysis was carried out using whole cell extracts of *C. reinhardtii*. Cells were grown to mid-log phase ($2x10^6$cells/ml) in TAP medium, pelleted and re-suspended in 0.8M Tris-HCl, pH 8.3; 0.2M sorbitol; 1% β-mercaptoethanol and 1% SDS. 50μl samples were boiled for 1 min and separated on a 17.5% SDS-polyacrylamide gel and electroblotted to polyvinylidene fluoride (PVDF) membrane (Immobilon-P, Millipore). Antibodies to PsaN were used at a dilution of 1:5000 and detected with the ECL detection kit (Amersham).

Results

The *psa*N gene of *C. reinhardtii* was cloned and sequenced (Accession number: AF323725). The gene contains 7 exons with a remarkably small ‘micro’- exon (exon 6) which is only 22 bp in size (Fig. 1.).

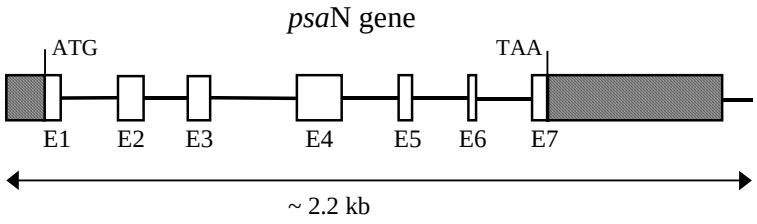

Fig.1. *psa*N gene map. White boxes represent exons and shaded areas represent untranslated regions. Introns are represented as horizontal lines.

To determine the copy number of this gene in *Chlamydomonas,* Southern analysis was performed and the results indicated that the *psa*N gene is a single copy gene in the *Chlamydomonas* nuclear genome (Fig. 2.).

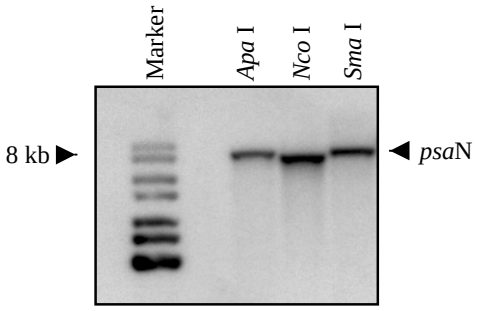

Fig.2. Southern blot analysis of WT genomic DNA digested with *Apa* I, *Nco* I and *Sma* I. Probed with ^{32}P labelled *psa*N cDNA.

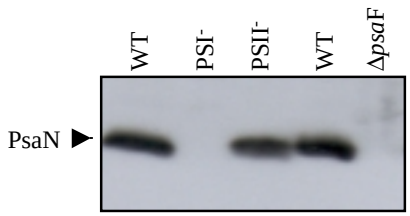

Fig.3. Western analysis on whole cell extracts of WT, PSI$^-$ (lacking PSI), PSII$^-$ (lacking PSII) and Δ*psa*F mutant using antibodies to PsaN. Equal volumes of whole cell extracts were loaded in each lane.

To investigate whether PsaN is present in the absence of PsaF, western analysis was conducted on whole cell extracts from WT and the mutant strain lacking the *psa*F gene (3bF). Western analysis showed that the PsaN protein is detectable in whole cell extracts from WT, but in the absence of PsaF the PsaN subunit is undetectable in the mutant (Fig.3).

Discussion

We have cloned and characterised the *psa*N gene, which encodes for the 14.8 kDa precursor PsaN protein in *Chlamydomonas*. We have shown for the first time that the PsaN protein is present in the unicellular green alga *C. reinhardtii*.

In order to gain an insight into the role of PsaN in the PSI complex, we analysed a mutant strain of *Chlamydomonas* deleted in the *psa*F gene. In the absence of the PsaF subunit, the PsaN subunit is not assembled in the PSI complex. We propose a possible interaction of PsaN with PsaF. We postulate that PsaF is required for the assembly of PsaN into the PSI complex and that the lumenal domain of PsaF holds PsaN to the PSI complex by electrostatic interactions. Our findings are consistent with recent data where transgenic *Arabidopsis* plants lacking PsaF have dramatically reduced levels of PsaN (Haldrup *et al*., 2000). The fact that the mutant devoid of PsaF in *Chlamydomonas* grows photoautotrophically in the absence of PsaN indicates that the function of PsaN is also dispensable in photosynthesis.

Acknowledgements

We would like to thank BBSRC for funding this research.

References

Chitnis, P.R. (2001). Photosystem I: function and physiology. *Annu. Rev. Plant. Physiol. Plant. Mol.*, **52:** 593-626.

Farah, J., Rappaport, F., Choquet, Y., Joliot, P. and Rochaix, J.-D. (1995). Isolation of a *psa*F-deficient mutant of *Chlamydomonas reinhardtii*: efficient interaction of plastocyanin with the photosystem I reaction centre is mediated by the PsaF subunit. *EMBO J*., **14**: 4976-4984.

Haldrup, A., Naver, H. and Scheller, H.V. (1999). The interaction between plastocyanin and photosystem I is inefficient in transgenic *Arabidopsis* plants lacking the PSI-N subunit of photosystem I. *Plant J*., 17: 689-698.

Haldrup, A., Simpson, D.J. and Scheller, H.V. (2000). Down-regulation of the PSI-F subunit of photosystem I (PSI) in *Arabidopsis thaliana*. *J. Biol. Chem.*, **275**: 31211-31218.

He, W.Z., Malkin, R. (1992). Specific release of a 9-kDa extrinisic photosystem I from spinach chloroplasts by salt washing. *FEBS Lett.*, **308**: 298-300.

Hippler, M., Drepper, F., Farah, J. and Rochaix, J.-D. (1997). Fast electron transfer from cytochrome c_6 and plastocyanin to photosystem I of *Chlamydomonas reinhardtii* requires PsaF. *Biochemistry*, **36**: 6343-6349.

Hippler, M., Biehler, K., Krieger-Liszkay, A., van Dillewjin, J and Rochaix, J.-D. (2000). Limitations in electron transfer in photosystem I donor side mutants of *Chlamydomonas reinhardtii*. *J. Biol. Chem*., **275**: 5852-5859.

Nielsen, V.S., Mant, A., Knoetzel, J., Möller, B.L. and Robinson, C. (1994). Import of barley photosystem I subunit N into the thylakoid lumen is mediated by a bipartite presequence lacking an intermediate processing site. *J. Biol. Chem.*, **269**: 3762-3766.

Purton, S. and Rochaix, J.-D. (1995). Characterisation of the *ARG*7 gene of *Chlamydomonas reinhardtii* and its application to nuclear transformation. *Eur. J. Phycol*. **30**: 141-148.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York, USA.