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Structural and functional investigations on the Ycf3, Ycf4 and Ycf37 proteins in the cyanobacterium *Synechocystis sp.* PCC 6803

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Introduction

During recent years the entire nucleotide sequences of plastid genomes have been determined in several land plants. The most conserved genes found among sequenced plastid genomes of photosynthetic organisms are involved in either genetic system functions or in photosynthesis. Several hypothetical chloroplast open reading frames (*ycf*), which functions are still elusive, are conserved in algae, land plants and in the genome of the cyanobacterium *Synechocystis* sp. PCC 6803. In contrast to *ycf3* and *ycf4* which are ubiquitous in plastid genomes of all plants and algae (with the exception of the nonphotosynthetic parasitic plant *Epifagus virginiana*), a third ORF *ycf37* is encoded only in the plastid genome of the non green algae *C. paradoxa*, *P. purpurea*, *G. theta.* However, similar sequences to *ycf37* are found in the nuclear genome of *Arabidopsis thaliana*. Disruption of the *ycf3* gene in tobacco (Ruf et al., 1997) and *Chlamydomonas* (Boudreau et al., 1997) led to a deficiency in photosystem (PS) I activity and to the destabilization of the PS I complex. The inactivation of the *ycf4* gene similarly caused a complete loss of the PS I core components in *Chlamydomonas* (Boudreau et al., 1997). In the cyanobacterium *Synechocystis* sp. PCC 6803 disruption of *ycf4* only leads to reduced levels of PS I, which is still active and supports photoautotrophic growth on the wild-type level (Wilde et al., 1995). Inactivation of *ycf37* in *Synechocystis sp.* PCC 6803 leads to a decreased PS I content, a phenotype that is similar to *ycf4* mutants (Wilde et al., 2001). Ycf3 as well as Ycf37 contain three tetratricopeptide repeats (TPR), a structural motif that mediates protein-protein interactions. Most TPR-containing proteins are associated with multiprotein complexes and they are involved in processes such as chaperone function, cell cycle, transcription or protein transport (Blatch and Lässle, 1999). In this report the localizations of the *ycf3* and *ycf4* gene products and their association or interaction with subunits of PS I have been identified using cross-linking and Western blot analyses and the functions of the *ycf3*, *ycf4* and *ycf37* gene products in the cyanobacterium *Synechocystis* sp. PCC 6803 will be discussed.

Materials and methods

Bacterial strains and growth conditions

Liquid cultures of *Synechocystis* sp. PCC 6803 wild-type and mutants were grown at 30°C in BG-11 medium. Growth media for mutant strains contained the appropriate antibiotics (40 µg/ml kanamycin for *ycf4* mutant, 7 µg/ml chloramphenicol for *ycf37* mutants). Growth of the

wild-type and mutant cells was followed under photoautotrophic conditions (40 µmol photons m^{-2} s^{-1} white light).

77K fluorescence and P700 measurements

Low-temperature fluorescence emission spectra (77K) were recorded using a F-4500 spectrophotometer (Hitachi, Tokyo, Japan) as described by Wilde et al. (1995). Time resolved transient absorption changes at 703 nm were measured at a chlorophyll (Chl) concentration of 10 µg / ml of either purified trimeric PS I complexes or thylakoid membranes of wild-type (WT) and mutant cells. 5 mM Na-ascorbate and 10 µM phenazine methosulfate were used as artificial electron donors/acceptors. The time resolution of the experimental setup was 50 µs. Samples were excited by a saturating flash of a xenon lamp of about 15 µs duration filtered by coloured glases (Gerken et al., 1989). An extinction coefficient of 64000 $M^{-}1cm^{-1}$ has been used (Ke, 1972).

Cross-linking of thylakoid membranes

Thylakoid membranes from *Synechocystis* sp. PCC 6803 wild-type and mutant cells were prepared using the method described by Burnap et al. (1989). Isolated thylakoid membranes from the *Synechocystis* sp. PCC 6803 wild-type strain were treated with the cross-linker *o*-phthalaldehyde (OPA) at a Chl concentration of 1 mg/ml for 30 min at 25°C. OPA was added to a final concentration of 20 mM. The cross-linking reaction was quenched with 100 mM Tris-HCl, pH 6.5.

Results and discussion

In the *Synechocystis* sp. PCC 6803 genome there are several ORFs with similarity to putative chloroplast genes (Table 1). The conservation of *ycf3*, *ycf4* and *ycf37* across relatively large phylogenetic distances implies an important role of the gene products in chloroplast and cyanobacterial cell function.

Table 1 Comparison of the deduced amino acid sequences from *Synechocystis* sp. PCC 6803 *ycf* genes with sequences from several plastid genomes

Gene	Corresponding ORF in *Synechocystis* 6803	% identity (similarity) with plastid ORFs from other organisms		
		Porphyra purpurea	*Cyanophora paradoxa*	*Nicotiana tabacum*
ycf3	*slr0823*	69 (87)	72 (86)	62 (77)
ycf4	*sll0226*	55 (74)	50 (69)	47 (68)
ycf37	*slr0171*	30 (51)	37 (64)	-

Low-temperature (77K) fluorescence emission spectra of intact cells after Chl excitation are shown in Fig.1. The large emission peak at 725 nm was predominantly derived from PS I Chl a, whereas the two peaks at 685 and 695 nm reflected fluorescence emission from Chls of the PS II core complexes. When spectra are normalized to the PS I emission peak, a decrease in the PS I/PS II emission ratio became obvious in both mutants *ycf4*$^{-}$ and *ycf37*$^{-}$. However, it was more pronounced in the *ycf4* mutant. In the *ycf4* mutant the decrease in the PS I/PS II

ratio appeared to be a result of both a reduction of the PS I content as well as an increase in the PS II content (Wilde et al., 1995). In contrast, in the *ycf37* mutant only the PS I content was decreased (Wilde et al., 2001). PS I from wild-type and mutant cells was characterised by measuring the flash-induced absorption changes at 703 nm reflecting the photooxidation of its reaction centre P700. Within the experimental error the *ycf4* and *ycf37* mutants reveal virtually the same number of Chls/P700 as those from the corresponding wild-types. However, the Chl contents per cell of the mutants were found to be reduced. The *ycf4* and *ycf37* mutant cells contained merely 56% and 67 %. of the Chl of their wild-types. The origin of these decreased Chl contents remain to be analysed and are under current investigations.

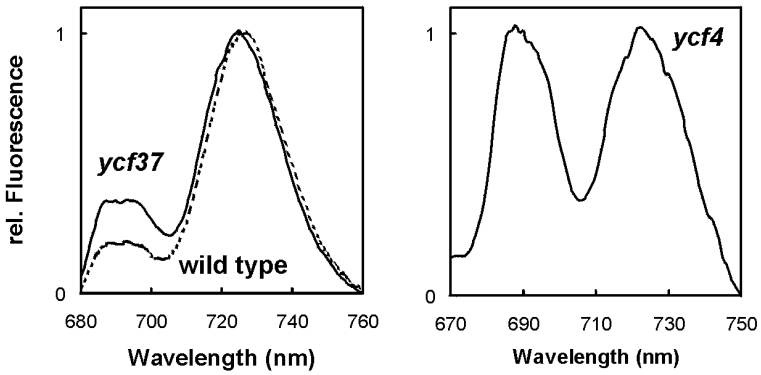

Fig. 1. 77 K Fluorescence emission spectra

The 77 K fluorescence emission spectra of whole cells of wild-type *Synechocystis sp.* PCC 6803, *ycf4* and *ycf37* mutants are shown after excitation of Chl *a* at 440 nm. The spectra were normalized to the emission maximum of PS I at 725 nm.

In order to localize the Ycf3 and Ycf4 proteins and to investigate the nature of their association or interaction with other components of PS I, thylakoid membrane from the cyanobacterium *Synechocystis* sp. PCC 6803 was treated with chemical cross-linker *o*-phthalaldehyde. Chemical cross-linking with OPA suggested that Ycf3 is in close proximity with PsaA in a distance of no more than 5Å, whereas Ycf4 and PsaC are closely associated with each other (≤ 5Å) (Miao et al., 2000). The cross-linking studies on Ycf37 are in progress. Fig. 2 shows a scheme of the association or interaction of Ycf3, Ycf4 and Ycf37 with components of photosystem I.

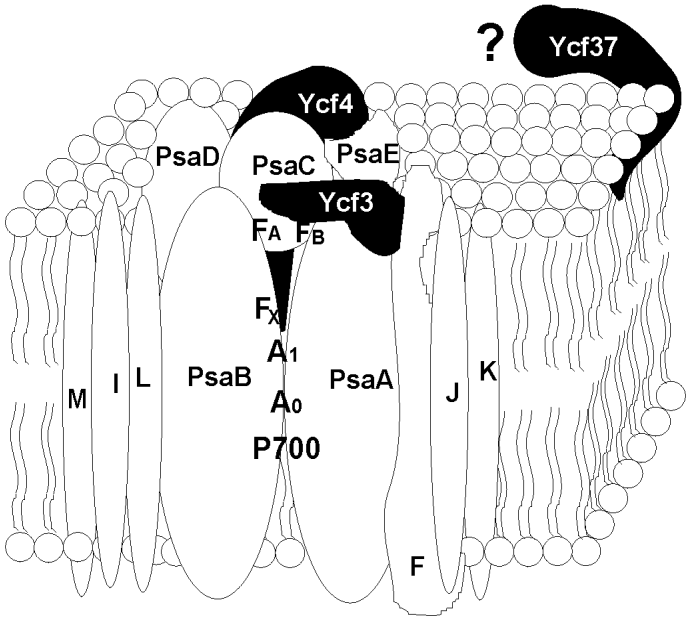

Fig. 2. Model of the interaction of Ycf3/Ycf4 with monomeric PS I from *Synechocystis sp.* PCC 6803

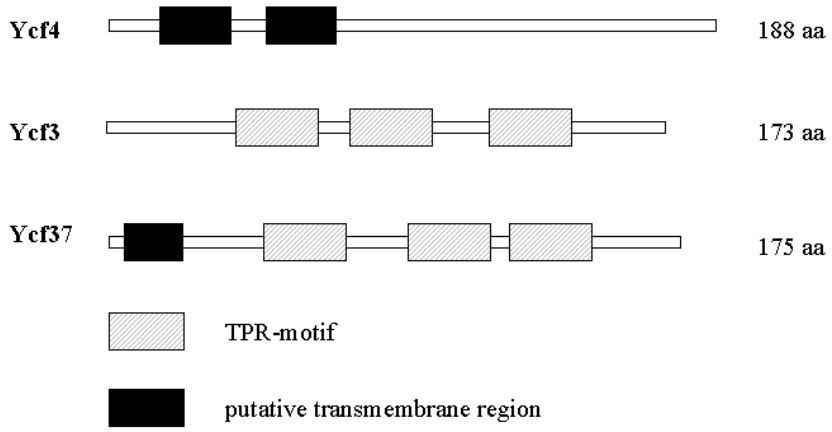

Fig. 3. Domain structure of putative *ycf3, ycf4* and *ycf37* gene products

According to sequence comparison Ycf3 and Ycf37 contain so-called tetratrico-peptide repeat (TPR) motifs (Fig. 3). The TPR consists of a degenerate 34 amino acid stretch present in a variety of structurally and functionally diverse proteins (Lamb et al., 1995). The presence of these motifs suggest that Ycf3 and Ycf37 may interact with other proteins and are involved in assembly or stability of the PS I proteins in the thylakoid membrane. The cross-linking studies also suggest a direct interaction between Ycf3 and Ycf4 and PS I complexes.

References

Blatch,G.L. and Lässle,M. (1999) *BioEssays* **21**, 932-939.
Boudreau,E., Takahashi,Y., Lemieux,C., Turmel,M. and Rochaix,J.-D. (1997) *EMBO J.* **16**, 6095-6104.
Burnap,R., Koike,H., Sotiropoulou,G., Sherman,L.A. and Inoue,Y. (1989) *Photosynth. Res.* **22**, 123-130.
Gerken,S., Dekker,J.P., Schlodder,E. and Witt,H.T. (1989) *Biochim Biophys. Acta* **977,** 52-61.
Ke,B. (1972) *Arch. Biochem. Biophys.* **152,** 70-77.
Lamb,J.R., Tugendreich,S. and Hieter,P. (1995) *Trends Biochem. Sci.* **20**, 257-259.
Miao,J. (2000) *Dissertation* (Technical University Berlin).
Ruf,S., Kössel,H. and Bock,R. (1997) *J. Cell Biol.* **139**, 95-102.
Wilde,A., Härtel,H., Hübschmann,T., Hoffmann,P., Shestakov,S.V. and Börner,T. (1995) *Plant Cell* **7**, 649-658.
Wilde,A., Lünser,K., Ossenbühl,F., Nickelsen,J. and Börner,T. (2001) *Biochem. J.* **357**, 211-216.