PratA is involved in D1 processing

PratA, a periplasmic tetratricopeptide repeat protein involved in biogenesis of photosystem II in *Synechocystis* sp. PCC 6803

Birgit Klinkert*, Friedrich Ossenbühl†, Martha Sikorski*, Stefan Berry‡, Lutz Eichacker† and Jörg Nickelsen*§

*Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany; ‡Biochemie der Pflanzen, Ruhr-Universität Bochum, 44780 Bochum, Germany; †Botanisches Institut, Ludwig-Maximilians Universität, Menzinger Str. 67, 80638 München, Germany

§ corresponding author: Tel.: 0049 234 3225539, FAX: 0049 234 3214184, email: joerg.nickelsen@ruhr-uni-bochum.de

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Summary

The light reactions of oxygenic photosynthesis are mediated by multisubunit pigment-protein complexes situated within the specialized thylakoid membrane system. The biogenesis of these complexes is regulated by trans-acting factors that affect the expression of the respective subunit genes and/or the assembly of their products. Here we report on the analysis of the PratA gene from the cyanobacterium Synechocystis sp. PCC 6803 which encodes a periplasmic tetratricopeptide repeat (TPR)\(^1\) protein of formerly unknown function. Targeted inactivation of PratA resulted in drastically reduced photosystem II (PSII) content. Protein pulse labeling experiments of PSII subunits indicated that the C-terminal processing of the precursor of the reaction center protein D1 (pD1) is compromised in the pratA mutant. Moreover, a direct interaction of PratA and pD1 was demonstrated by applying yeast two-hybrid analyses. This suggests that PratA represents a factor facilitating D1 maturation via the endoprotease CtpA. The periplasmic localization of PratA supports a model which predicts the initial steps of PSII biogenesis to occur at the plasma membrane of cyanobacterial cells.

\(^1\)Abbreviations used are: TPR, tetratricopeptide repeat; PSII, photosystem II; ORF, open reading frame; aa, amino acid
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Introduction

The light driven reactions of photosynthesis take place at a specialized membrane system - the thylakoids - that contains the essential protein complexes mediating photosynthetic electron transport, which include photosystem II (PSII), the cytb6f complex, PSI and the ATPase. Whilst the structural and functional features of these complexes have been analyzed in great detail, to date, relatively little is known about their regulated biogenesis during cell development.

Recent work on vascular plants, eukaryotic algae and cyanobacteria revealed that numerous regulatory factors exist that are involved in the expression of genes encoding subunits of the photosynthetic membrane complexes and/or their assembly (1, 2). These factors apparently constitute an intracellular communication system that mediates the controlled biogenesis of thylakoids. Amongst these, one subgroup is represented by so-called tetratricopeptide repeat (TPR) proteins, which share a degenerate protein-protein interaction motif consisting of multiple repeats of 34 amino acids (3, 4). For instance, TPR motifs have been found in factors, which are involved in the stabilization of chloroplast mRNAs (5, 6, 7), whereas, the chloroplast TPR-protein Ycf3 is involved in the assembly of PSI (8, 9). In cyanobacteria, the structurally related Ycf37 protein was also shown to affect PSI levels (10). Furthermore, the open reading frame (ORF) sll0886 from *Synechocystis* sp. PCC 6803 (*Synechocystis* 6803) encodes a TPR protein, which is required for light-activated heterotrophic growth (11). These data suggest that the TPR motif might play an important role in the organization/function of regulatory units in both cyanobacteria and chloroplasts of eukaryotic cells.

Beside structure and function of this regulatory machinery, the localization of the different biogenesis steps remains an intriguing, still unresolved matter. Current evidence supports a model of an intraorganellar vesicle transport system for chloroplasts (12, 13). The idea of a specialized region where the biogenesis of photosynthetic membrane complexes
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takes place, and which is spatially separated from the thylakoid membrane was further strongly supported by the recent observation that significant amounts of some PSII and PSI subunits are detectable in the plasma membrane of the cyanobacterium *Synechocystis* 6803 where they form chlorophyll-containing precomplexes (14). These data led to the hypothesis that the initial steps of cyanobacterial photosystem assembly may occur at the plasma membrane and not at the thylakoids (14).

One of these complexes, namely PSII, contains more than 25 subunits, which assemble in a highly coordinated fashion. The initial steps of this assembly include the formation of a dimer of the reaction center proteins D2 and D1 and the subsequent binding of Cytb559, PsbI, CP47 and CP43 (15). Much attention has been focussed on the D1 protein (16), which is synthesized as a precursor (pD1) with an extension of 8 – 16 amino acids (aa) at its C-terminus. The removal of this extension by a specific endoprotease called CtpA (carboxyl-terminal processing protease) represents an essential step of PSII biogenesis (17). In *ctpA* mutants, the unprocessed pD1 precursor, though it is incorporated into a PSII complex, impairs the assembly of the manganese cluster of the oxygen-evolving complex of PSII (18, 19) leading to a photosynthesis deficient phenotype. Since site-directed mutants of the corresponding *psbA* gene lacking the C-terminal extension were shown to grow at wild-type rates (20), the precise function of the C-terminal extension still remains elusive. However, long-term growth of mutant and wild-type strains of *Synechocystis* 6803 in mixed culture conditions indicated that the C-terminal extension might provide a selective advantage on cells, possibly by protecting D1 during integration into the membrane or PSII (21).

In this report, we show that ORF *slr2048* from *Synechocystis* 6803 encodes a periplasmic TPR-protein, named PratA (processing associated TPR protein), which is involved in the C-terminal processing of the pD1 protein. The finding that PratA directly interacts with pD1 supports the recently proposed model of a plasma membrane-localized biogenesis of cyanobacterial photosystems (14).
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Experimental Procedures

Strains and growth conditions

*Synechocystis* sp. PCC 6803 wild-type and mutant strains were grown on solid or in liquid BG 11 medium (22) at 30°C at a continuous photon irradiance of 100 µE m⁻² s⁻¹.

Plasmid constructions, transformations and complementation

The *PratA* gene was cloned after its PCR-amplification with oligonucleotides 2048-5 CCATGGCAAGCCGTCCCTTGG and 2048-3 CTAGAGATTATCCAGCTT and insertion of the resulting fragment into the bluescript pKS vector (Stratagene). A *Xba*I fragment from plasmid pBSL86 (23) containing a kanamycin resistance cartridge was inserted into the single *Nhe*I site within *PratA*. This construct was transformed into *Synechocystis* 6803 as described (10). For complementation of the *pratA* mutant, the *PratA* gene including its own promoter was PCR-amplified with oligonucleotides 2048a CTCGAGTGGAAAGCTACGAACGTTCCG and 2048b GCCAGACCAATGCTTGCTGACTCGAG and cloned into the single *Xho*I site of the vector pVZ321 that is capable of autonomous replication in *Synechocystis* 6803 (24). Conjugal transfer of this construct into *pratA* was performed as described (24).

Southern-, Northern-, Western-blot analysis

Total cyanobacterial DNA was isolated from 50 ml cultures by using the QIAGEN DNeasy-Plant Kit and Southern-blot analaysis was carried out using standard protocols. Isolation of total RNA, electrophoresis and gel blotting was performed as described (10). The *PratA* gene was inserted into the expression vector pCAL-n, recombinant *PratA* protein was expressed in *E. coli* BL21 and purified following the instructions of the Affinity Protein Expression and Purification System from Stratagene. The α*PratA* antiserum was raised in...
PratA is involved in D1 processing rabbits (Biogenes, Germany). Protein preparation from *Synechocystis* 6803 and Western analysis was performed as previously reported (10). Isolation of periplasmic proteins was performed as described (25).

**Measurements of chlorophyll fluorescence and oxygen evolution**

The ratio of maximum and minimum chlorophyll fluorescence (Fₘ/Fₒ) was assayed as described (26). Fluorescence emission spectra at 77 K were measured with a SLM-Aminco Series 2 Luminescence Spectrometer (excitation at 435 nm, slit width 4 nm for excitation and detection). Cell suspensions in BG11 medium were mixed with 30 % (v/v) glycerol.

Pigment content was determined with a Beckmann DU 7400 Spectrometer. Measurements of oxygen evolution during illumination with saturating white light were done with a Clark-type oxygen electrode (Bachofer, Germany) at 30°C. Cells were suspended at an OD₇₅₀ = 1 in 2 ml of BG11 medium, supplemented with 10 mM NaHCO₃.

**Pulse-chase labeling of cyanobacterial cells, preparation of total membranes and gel electrophoresis.**

Cells grown at 100 µEm⁻²s⁻¹ for 15 h in the late-exponential growth phase (2-4 µg chlorophyll/ml) were harvested by centrifugation, washed and resuspended in fresh BG11 to a concentration of 0.3 µg/µl chlorophyll ³⁵S-methionine (> 1000 Ci/mmol, Amersham) was added (final activity 750 µCi/ml) and the cell suspension was incubated at 60 µE m⁻²s⁻¹ for 20 min at 30°C. Thereafter, harvested cells were immediately frozen in liquid nitrogen. Cells were thawed on ice, washed twice in TMK-buffer (10 mM Tris/HCl, pH 6.8; 10 mM MgCl₂; 20 mM KCl) and resuspended in 250 µl TMK-buffer. 0.3 g of glass beads were added and cells were broken by vortexing two times for 1 min with an intermediate cooling on ice for at least 2 min. Unbroken cells were removed by differential centrifugation. Sedimented total membranes were resuspended in ACA-buffer (750 mM ε-aminocaproic
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acid; 50 mM Bistris, pH 7.0; 0.5 mM EDTA). Total membranes corresponding to 10 µg Chl.
in ACA-buffer were solubilized by adding of β-dodecylmaltoside to a final concentration of
1.0 % for 10 min on ice. Non-solubilized material was removed by centrifugation and 5 µl of
loading buffer (750 mM ε-aminocaproic acid; 5 % Coomassie brilliant blue G-250) was added
to the supernatant prior to loading the blue native (BN) 6 % to 12 % polyacrylamide (PA)
gradient gels. BN gel electrophoresis was performed at 12 mA constant current as described
(27). For the second dimension (so called 2D-BN/SDS gels), a single lane of the BN gel was
solubilized in solubilization buffer (66 mM Na₂CO₃; 100 mM β-mercaptoethanol; 2 % sodium
dodecylsulfate (SDS) and loaded on a 12.5 % SDS polyacrylamide gel containing
4 M urea. The 2D BN/SDS gels were run overnight at 16 mA constant current and 15°C. The
gels were stained with Coomassie Blue R, dried and then exposed to phosphor imaging plates
(Fuji Photo Film GmbH, Germany). The imaging plates were read out with a FLA3000-image
reader and quantified with the supplied AIDA software (raytest Isotopenmessgeräte GmbH,
Germany).

For the measurement of chlorophyll concentrations, cells were sedimented by
centrifugation and extracted with 100 % methanol. The concentration of chlorophyll was
calculated from the absorbance values of the extract at 666 and 720 nm (28).

**Yeast two hybrid analysis**

For the two hybrid analysis, the System MATCHMARKER LexA (Clontech) with the
Saccharomyces cerevisiae strain EGY48 (MATα, ura3, trp1, his3, lexAop(at) -LEU2)
transformed with the plasmid pSH18-34 (29), and the plasmids pEG202 and pJG4-5 (30)
were used. The PratA gene fragment was amplified by the oligonucleotides TH2048a2:
GAATTCATGAATTTACGCTACCTCTG

and

TH2048b:
CTCGAGCTAGAGATTATCCAGCTTTTCTTG and cloned into the yeast shuttle vector
pJG4-5 via the oligonucleotide-derived restriction sites EcoRI and XhoI. This construct did
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not contain the signalpeptide sequence for the localization in the periplasm. The pD1 C-
terminus was amplified with the oligonucleotides THpsbAa:
GAATTCATGGCGTTCAACCTGAACGG and THpsbAb:
CTCGAGTTAACCGTTGACAGCAGGAG and cloned into pEG202 via the oligonucleotide-
derived restriction sites EcoRI and XhoI. The pD1 C-terminus comprises 68 aa including its
16 aa-extension.

Results

Generation and characterization of a pratA mutant

Computer-assisted similarity searches revealed that the genome of Synechocystis 6803
harbors a total of 22 ORFs encoding putative TPR proteins. During the course of a systematic
mutagenesis study of these ORFs, we analyzed slr2048 that encodes a protein of 398 aa,
which is capable of forming nine TPR repeats. Based on this study, the gene was named
PratA (Fig. 1A). Interestingly, a transit sequence was predicted at the N-terminus of the
derived protein, which would allow secretion of the protein via the Sec system (Fig. 1A).
Indeed, PratA was identified within the periplasm of Synechocystis 6803 by Fulda and
coworkers during a proteomic analysis (31).

We disrupted the cloned PratA gene by inserting a kanamycin resistance cassette into
the single NheI site 379 bp downstream of its translation start codon (Fig. 1B). After
subsequent transformation of wild-type cells with this construct, resulting transformants were
tested for complete segregation of the mutation by applying Southern blot hybridizations (Fig.
1C) and PCR analyses (data not shown). Northern analysis confirmed that no PratA mRNA
was present in the segregated pratA mutant strain (Fig. 2A). Moreover, the detection of only
one single signal at 1,300 nt indicated a monocistronic transcription of the gene, since
cotranscription with either of the adjacent reading frames slr2047 or slr2049 would have
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resulted in transcripts with a minimum size of 2,400 nt or 1,900 nt, respectively. Thus, it is likely that disruption of the PratA gene has no polar effects on the expression of these genes. The absence of the PratA protein in the mutant was confirmed by Western analysis using an αPratA antiserum raised against the recombinant protein expressed in E. coli (Fig. 2B). Finally, the periplasmic localization of PratA was verified after analyzing cellular subfractions of Synechocystis 6803. As shown in Fig. 2B, a signal at the expected size of 35 kDa (31) was detected in proteins from total cells and in the periplasmic subfraction (Fig. 2B: T and P). No signal was obtained in the protein fraction from cells which had been separated from their periplasm (Fig. 2B: -P). In contrast, the cytoplasmic ribosomal protein S1 was found in the -P but not in the P fraction indicating that the periplasmic proteins are free of substantial cytoplasmic contaminants. Taken together, these data demonstrate that the vast majority of PratA is located in the periplasm.

The photoautotrophic growth rate of pratA was reduced by more than fourfold when compared to the wild-type, whereas the chlorophyll content was found to be similar in both strains after comparative analysis of absorption spectra (data not shown). When fluorescence emission spectra at low temperature (77K) from both wild-type and pratA were measured, a drastic decrease of chlorophyll emission peaks from PSII at 685 and 695 nm was observed in the mutant suggesting a lower relative PSII content (Fig. 3). This reduced PSII amount was confirmed by the finding that the ratio of maximum and minimum chlorophyll fluorescence (FM/F0) was significantly reduced in pratA compared to the wild type (data not shown). Moreover, photosynthetic oxygen production in pratA was reduced to 25 % of that of the wild-type rate, again, suggesting a considerable effect on PSII content/activity.

To calculate the PSII amount in pratA, Western analysis was performed. As shown in Fig. 4A, the steady state levels of the PSII subunits PsbB, D1 and PsbO in pratA were reduced to ca. 45 %, 15 % and 10 % of that of the wild-type amount, respectively. This suggests a more direct effect of PratA on D1 and PsbO accumulation. In contrast, both the level of the
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PSI subunit PsaD and the Rubisco enzyme were not or only slightly affected indicating that only the amount of PSII is affected in the mutant. In chloroplasts of eukaryotic algae and vascular plants, TPR proteins have been shown to be required for gene specific RNA stabilization processes. Thus, we tested whether the RNA levels of various genes encoding PSII subunits are compromised in pratA (Fig. 4B). Obviously, this was not the case for either psbA and psbO mRNAs or for psbB, psbC, and psbD transcripts. These data allow the conclusion that PratA functions in the synthesis and/or the stability of PSII subunits.

**PratA is involved in D1 maturation**

To distinguish between these two possibilities of PratA function, protein pulse labeling experiments with radioactive $^{35}$S methionine were carried out. Radiolabeled proteins were first separated by blue native (BN)-PAGE to resolve native protein complexes and then, in a second dimension, by SDS-PAGE, to elucidate their subunit composition (Fig. 5A). The identity of different proteins was determined through comparison of 2D-BN/SDS-PAGE analyses of pulse labeling experiments using deletion mutants of the PSII subunits D1, D2, CP43 and CP47 (32). During pulse labeling of *Synechocystis* 6803 proteins, PSII subunits and especially the reaction center protein D1 were predominantly labeled independent of the growth conditions used (33). This reflects the active synthesis of PSII and highlights in particular the D1 protein, which is subject to extensive photo-damage and, thus, must be constantly repaired via the PSII repair cycle. In wild-type cells, several different intermediates of the PSII assembly process could be visualized. Both the precursor (pD1) and the mature D1 protein were detected in the early PSII intermediates RCa, RCb and RC47 (Fig. 5A), whereas the PSII monomer RCC (1) and bigger complexes contained the C-terminally processed, mature D1 form alone. In the mutant pratA, the synthesis rates of the PSII components were the same as those of the wild-type indicating that the mutation affects the stability and not the synthesis of the PSII complex. Furthermore in pratA, PSII appears to be assembled since most
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intermediate PSII complexes were detectable except for the early precomplexes RCa and RCb (Fig. 5A). However, the main difference between wild-type and pratA was the almost complete absence of mature D1 protein in the mutant. This was verified by performing one-dimensional gel electrophoresis of pulse-labeled proteins (Fig. 5B).

As a control, the strain repratA was generated, in which via conjugation, the wild-type PratA gene including its promoter was reintroduced into the mutant on an autonomously replicating plasmid (24). The resulting revertant displayed a rescued phenotype with a photoautotrophic growth rate nearly at the wild-type level. In addition, 77 K fluorescence emission spectra indicated a significantly higher PSII/PSI ratio in repratA compared to the mutant (Fig. 3). The incomplete restoration of the wild-type phenotype is probably a result of the extrachromosomal expression of PratA in the revertant.

Consistent with the restored phenotype, efficient C-terminal D1 processing occurred in repratA (Fig. 5B). The ratio of D1 maturation, though, appeared to be slightly reduced. In contrast to the wild type, in the intermediate PSII monomer, most D1 was still present in the precursor form, which was processed only during later PSII assembly steps (Fig. 5A). Taken together, the pulse labeling data indicate that the PratA protein is involved in the C-terminal processing of the D1 protein. As a consequence, the water-splitting apparatus would not be able to correctly dock to PSII, thereby explaining the observed phenotype of the pratA mutant.

**PratA interacts with the C-terminus of D1**

To this end, the obtained results direct us to the question whether PratA interacts directly with the D1 protein and/or the D1 C-terminal protease CtpA. To test this, we applied a yeast two-hybrid analysis, which is based on the LexA system. PCR-fragments containing the PratA and CtpA genes as well as a fragment encoding the C-terminal 68 aa of pD1 were cloned into shuttle vectors either pEG202 or pJG4-5. This resulted in translational fusions of
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the proteins with the LexA-DBD (DNA binding domain) or the B42-AD (activation domain), respectively. It is important to note that the cloned pD1 C-terminus did not harbor any hydrophobic transmembrane regions, which could have possibly disturbed the two-hybrid assay by affecting nuclear import of the fusion protein in the yeast system. As shown in Fig. 6, indeed, an interaction between PratA fused to the AD and the pD1 C-terminus fused to the DBD was detected by analyzing the expression of the lacZ and LEU2 reporter genes. No reporter gene activity was observed in a negative control experiment in which either of the constructs was assayed alone (Fig. 6). This indicated that both PratA and pD1 are required for complex formation.

A putative interaction of PratA with the protease CtpA could not be analyzed in this system, because CtpA expressed in yeast was shown to impair the import of fusion proteins into the nucleus of yeast cells where reporter gene activity is regulated (data not shown). This might be due to the fact that CtpA, though its nature appears hydrophilic, might be membrane associated. Indeed, it has been reported to be tightly associated with the plasma membrane in Synechocystis 6803 (14). Hence, future work will focus on the establishment of alternative two-hybrid systems, which should allow the analysis of membrane proteins (34) to answer the question whether PratA also recognizes CtpA or not. Nevertheless in conclusion, the sensitive two-hybrid LexA system enabled us to identify a direct interaction of the TPR protein PratA with the C-terminus of the PSII reaction center protein pD1.

Discussion

In this study, we report on the characterization of the ORF slr2048 from Synechocystis 6803, which encodes the periplasmic TPR protein PratA. The insertion of a kanamycin resistance cassette into the PratA gene resulted in a significant reduction of the PSII content in the mutant and an apparent absence of the C-terminal processing of the pD1 protein. This phenotype resembles the one of a CtpA deletion mutant (ΔctpA), which contains no C-
PratA is involved in D1 processing terminal processing D1 protease CtpA (17). However, in contrast to ΔctpA, pratA was still able to grow photoautotrophically though with a fourfold reduced rate. This might explain, why no significant accumulation of pD1 was detectable in pratA in Western analysis, but only in pulse labeling experiments. (Fig. 4A). Since N-terminal parts of the PratA gene providing partial activity might still be expressed in the mutant, we recently constructed a site-directed, completely segregated mutant in which the complete ORF slr2048 is deleted. In this mutant, the photoautotrophic growth rate incurred even a tenfold reduction when compared to the wild-type, but residual photosynthetic activity was still observed. This suggests that PratA is involved, but not mandatory for D1 maturation. Most likely, it facilitates the CtpA-mediated processing event. Interestingly, such an assisting, additional component for pD1 processing has recently been proposed for spinach chloroplasts based on the finding that CtpA exhibits a relatively weak affinity to its substrate in vitro (35). Thus, it is tempting to speculate whether PratA might represent an assisting factor in Synechocystis 6803.

Computer-assisted BLASTP homology searches revealed several ORFs with similarity to PratA in various Cyanobacteria. Amongst these, ORF tlr2360 encoding a hypothetical TPR protein from Thermosynechococcus elongatus BP-1 and a hypothetical ORF from Trichodesmium erythraeum IMS 101 (ZP 00072573) showed the highest alignment scores of 138 and 137, respectively. In Arabidopsis thaliana, PratA shares the highest similarity (alignment score of 92) with an O-linked N-acetylglucosamine transferase (OGT; At3g04240). However, the homologous region is restricted to the N-terminal TPR domain of the enzyme and not to its C-terminal transferase domain (36), thus indicating that PratA is not an OGT. The absence of an obvious PratA homologue in higher plants might reflect the difference in the molecular details of the processing reaction between these organisms: In Synechocystis 6803, the removal of the C-terminal extension of 16 aa has been shown to be a sequential two-step proteolytic process with a transient accumulation of a processing intermediate (37). In contrast, the nine aa comprising C-terminal extension of pD1 from
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higher plants is excised by a single proteolytic step. However, since the TPR motif is highly
degenerate (4), further reliable conclusions concerning PratA homologues in other organisms
can only be drawn from functional analysis.

The two-hybrid data strongly suggest that PratA directly interacts with the C-terminus
of pD1, most likely through its TPR domain. Repeated attempts to detect this complex
formation in vitro by applying Far Western or pull-down experiments with recombinant PratA
and pD1 proteins were not conclusive. Most probably, this reflects the short-lived character of
the PratA/pD1 interaction, a feature which one would expect when the rapid C-terminal
processing of pD1 in the wild-type is taken into account.

However, even a transient interaction indicates that the periplasm-located PratA (31)
directly contacts pD1 implying that PratA must be present in close spatial vicinity to pD1
within the cell. Interestingly, it has been recently hypothesized that the initial steps of
photosystem biogenesis take place at the plasma membrane of Synechocystis 6803 (14). Consistent with this is the finding that, by immunological means, the D1-protease CtpA has
been detected exclusively in the plasma membrane and not in the thylakoids (14). In
conclusion, these data would favor a scenario in which newly synthesized pD1 is inserted into
the plasma membrane where it assembles into precomplexes. The C-terminus of pD1 would
be oriented to the periplasm and is processed by CtpA with the assistance of the periplasmic
PratA. The preformed PSII complexes would then migrate to the thylakoids by lateral
membrane fusion or membrane vesicle transport (38). In this model, a still unresolved matter
concerns the maturation of D1 protein which is inserted into preexisting PSII complexes
during the PS II repair cycle. Analogous to the situation in chloroplasts from eucaryotes, one
would expect that this process takes place at the cyanobacterial thylakoid membrane and not
the plasma membrane. Hence, a D1 maturation pathway at thylakoids might exist which
operates independently from the periplasmic PratA factor. The existence of an additional,
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spatially separated, D1 processing machinery might explain why the inactivation of PratA still allows residual photosynthetic activity and, thus, D1 maturation.

Of course, the proposed model requires further confirmation. Nevertheless, we succeeded to provide first genetic evidence that a periplasmic factor is involved in PSII biogenesis. Furthermore, our data identify the TPR-protein PratA that is involved in the C-terminal processing of the D1 reaction center protein of PSII. Future work will be focused on the elucidation of the precise working mode of PratA and, concurrently, on the spatial organization of thylakoid membrane biogenesis.

Acknowledgements

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References

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**Figure legends**

**Fig. 1.** Inactivation of *PratA*. (A) The aa sequence of PratA is given with positions of TPR repeats 1-9 and the TPR consensus motif (TPR-CS). The signal sequence is underlined by dots and the predicted cleavage site (31) is marked by an arrow. The highly conserved amino acids of the TPR motif are bordered of gray boxes. The corresponding amino acids of PratA are printed in white, other aa congruent with the motif are underlined. (B) The strategy for constructing donor plasmids with inactivated *PratA* is shown. The direction of transcription is indicated by horizontal arrows. The solid line delineates a 0.6 kb *Hinc*II fragment that represents the *PratA*-specific probe used in C. Km<sup>r</sup>, kanamycin-resistance cassette (shaded). (C) Southern analysis of wild-type (WT) and *pratA* with total DNA after restriction with *Bal*II.

**Fig. 2** Expression of *PratA* (A) Northern analysis of wild-type and *pratA* with 10 µg total RNA. Sizes of radioactively labeled bands are given at the margins (B) Western analysis of wild-type and *pratA*. Total proteins (T), proteins from sedimented cells after separation from periplasm (-P), and periplasmic proteins (P) were electrophoretically separated in a 12% SDS gel and decorated with an αPratA polyclonal antiserum or an αS1 polyclonal antiserum derived against the *E. coli* ribosomal protein S1. Proteins were loaded on a per cell basis corresponding to 4 x 10<sup>7</sup> cells in each lane.

**Fig. 3.** Low temperature fluorescence emission spectra. 77 K fluorescence emission spectra of whole cells of indicated strains are shown after excitation of chlorophyll at 435 nm. Spectra were normalized to the emission maxima at 725 nm.

**Fig. 4.** Expression of PSII subunits. (A) Western analysis of wild-type and *pratA* using total proteins after decoration with different indicated antibodies. (B) Northern analysis of wild-type and *pratA* with the indicated gene probes.
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**Fig. 5.** In vivo Pulse-labeling of proteins. Isolated and solubilized membranes were separated by (A) 2D-BN/SDS-PAGE or (B) 1D-SDS-PAGE and pulse-radiolabeled proteins were visualized by autoradiography. Different PSII subunits are marked at the left margins. Three non-characterized PSII assembly intermediates between RCC(1) and RCC(2) are marked by asterisks. Sizes of protein standards are given in kDa. RCa and RCb: reaction center complex a and b; RC47: reaction center complex with CP47; RCC (1 and 2): monomeric and dimeric reaction center core complexes, respectively.

**Fig. 6.** PratA/pD1 interaction in a yeast two-hybrid assay. Three independent transformants expressing the fusion proteins indicated at the right margin were analyzed for (A) *LEU2* reporter gene activation by growth on medium containing no leucine or (B) *lacZ* reporter gene activity by growth on medium containing X-Gal.
Figure 4

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Birgit Klinkert, Friedrich Ossenbuehl, Martha Sikorski, Stefan Berry, Lutz Eichacker and Joerg Nickelsen

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