PetC1 Is the Major Rieske Iron-Sulfur Protein in the Cytochrome $b_6f$ Complex of Synechocystis sp. PCC 6803*

Many of the completely sequenced cyanobacterial genomes contain a gene family that encodes for putative Rieske iron-sulfur proteins. The Rieske protein is one of the large subunits of the cytochrome $bc$-type complexes involved in respiratory and photosynthetic electron transfer. In contrast to all other subunits of this complex that are encoded by single genes, the genome of the cyanobacterium Synechocystis PCC 6803 contains three $petC$ genes, all encoding potential Rieske subunits. Most interestingly, any of the $petC$ genes can be deleted individually without altering the Synechocystis phenotype dramatically. In contrast, double deletion experiments revealed that $petC1$ and $petC2$ cannot be deleted in combination, whereas $petC3$ can be deleted together with any of the other two $petC$ genes. Further results suggest identical functional features for three of the Rieske proteins. Whereas PetC2 can partly replace the dominating Rieske isoform PetC1, PetC3 is unable to functionally replace either PetC1 or PetC2 and may have a special function involving a special donor with a lower redox potential than plastoquinone. A predominant role of PetC1, which is (partly) different from PetC2, is suggested by the mutational analysis and a detailed characterization of the electron transfer reactions in the mutant strains.

In 1997 the complete DNA sequence of the mesophilic cyanobacterium Synechocystis PCC 6803 was published (1). About 3200 open reading frames were identified within the genome, and for about 55% of them a function was inferred by similarity and homology searches, whereas the function of the remaining open reading frames remained unclear (2). Although computational analysis indicated the occurrence of many gene families, the function of these is elusive so far. The best investigated example at present is the D1 protein of photosystem II, which is encoded by three different $psbA$ genes (3, 4). It was shown that all three genes encode functional D1 proteins, although only $psbD1$ and $psbD2$ are expressed at a significant level. Similarly, three potential $petC$ genes are present encoding for the Rieske subunit of the cytochrome $b_{6f}$ complex (5).

The Rieske protein is an essential subunit of all cytochrome $bc$ complexes, where it is involved in quinol binding and electron transfer within the complex. In addition to the Rieske protein, cytochrome $bc_{1}$ complexes contain the cytochrome $b$ subunit carrying two $b$-type hemes and a $c$-type cytochrome with one covalently bound heme. Although in the cytochrome $b_{6f}$ complex the $b$-type cytochrome is split into the two subunits cytochrome $b_{6}$ and subunit IV (6), the overall structure is conserved, and both complexes can transfer electrons from a quinol to the $c$-type cytochrome (7). The structures of the cytochrome $bc_{1}$ complexes as well as of the cytochrome $b_{6f}$ complex have been determined by X-ray crystallography recently (8–10). Although the sequence similarities between the Rieske proteins from both complexes are quite low (11), structural analysis of the water-soluble parts of these proteins revealed a highly conserved three-dimensional structure (11, 12).

In the genomic sequence of the cyanobacterium Synechocystis PCC 6803, no genes encoding subunits of a cytochrome $bc_{1}$ complex were found, i.e. the cytochrome $b_{6f}$ complex is an essential component of both the respiratory and the photosynthetic electron transfer chain. In general, the thylakoid membrane of cyanobacteria harbors a mixed photosynthetic and respiratory electron transport chain (13) with the plastoquinone pool receiving electrons from both photosystem II and from respiratory dehydrogenases; these are then transferred via the cytochrome $b_{6f}$ complex and soluble carriers (plastocyanin or cytochrome $c_{6}$) to photosystem I and the terminal respiratory oxidase. Due to this central function of the cytochrome $b_{6f}$ complex, it is impossible to delete crucial subunits of this complex (14). Within the cyanobacterial cytochrome $b_{6f}$ complex only for the Rieske subunit a gene family has been found. Besides Synechocystis, which contains three potentials Rieske genes, multiple Rieske genes have also been shown to exist in other cyanobacteria like Synechococcus PCC 7002 and Anaabaena PCC 7120. However, in contrast to the $psbA$ gene family, up to now no specific physiological function is known for the $petC$ gene family encoding for the Rieske subunit.

In this study the function of the three $petC$ genes in Synechocystis PCC 6803 was investigated by deletional mutagenesis of $petC$ genes and by characterization of the generated mutants. The presented results show that all $petC$ genes can be deleted individually without altering the Synechocystis phenotype dramatically. Although $petC1$ and $petC2$ can be deleted individually, it was not possible to delete the genes in combination in Synechocystis wild type (wt).\(^1\) This indicates that $petC2$ and $petC1$ have at least partly overlapping functions and that $petC2$ can complement for the $petC1$ deletion. Further detailed characterization of the $\Delta petC1$ strain suggested that the function of $petC1$ can only to some degree be substituted by $petC2$, and the deletion of $petC1$ leads to a partial loss of the cytochrome $b_{6f}$ complex function.

\(^*\) This study was supported by the Deutsche Forschungsgemeinschaft Grant SFB 480 and the Human Frontier Science Program. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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This paper is available on line at http://www.jbc.org

Received for publication, June 7, 2004
Published, JBC Papers in Press, July 15, 2004, DOI 10.1074/jbc.M406288200
**MATERIALS AND METHODS**

**Growth Conditions**—Synechocystis PCC 6803 wt and mutant strains were grown at 30 °C in BG11 media (15) under cool-white fluorescent illumination at a light intensity of 40 μm−2 s−1.

Transformation of Synechocystis with plasmids was carried out as described previously (16). Because Synechocystis contains several identical genome copies, numerous rounds of selection on BG11 plates with increasing amounts of spectinomycin, kanamycin, or chloramphenicol (5–100 μg/ml) were necessary to obtain fully segregated mutants. For completeness of the segregation, individual clones were tested by PCR.

Growth of the wt and mutant strains was monitored at 730 nm (OD730) under photoautotrophic, photomixothrophic (S. elongatus PCC 6803 was grown at 30 °C in BG11 media, with or without further additions, as indicated in the protocol of Ref. 16. Because the level of minimum fluorescence, F0, of dark incubation, each sample was illuminated again to determine the variable chlorophyll fluorescence (Fv) from photosystem II depends on the redox state of Qo, the primary quinone acceptor, and, indirectly, also on the state of the plastoquinone pool, which thus can be monitored by using chlorophyll fluorescence measurements (20). Light-induced changes of chlorophyll fluorescence were measured at 686 nm in a laboratory-built set up at room temperature (23 °C), using a 1×1 cm cuvette with a 2-ml sample volume. Cells were incubated for 2 min in the dark at a chlorophyll concentration of 2.5 μg/ml in BG11 medium, with or without further additions, as indicated in the figure legends. After the dark incubation, the cells were illuminated with blue light (center wavelength of 445 nm, intensity of 205 μW m−2 s−1). Illumination was controlled by an electronic photoshutter; the duration of the light pulse was 250 ms or 1 s, respectively, as indicated.

**Spectroscopic Methods**—77 K fluorescence emission spectra were recorded in an Amino fluorimeter with a chlorophyll concentration of 5 μg/ml (19). Cells were diluted by BG11 medium and frozen in liquid nitrogen. Monochromators were set to a slit width of 4 nm.

The yield of the variable chlorophyll fluorescence (Fv) from photosystem II was determined from the initial fast phase of fluorescence (20). The oxygen evolution was measured after addition of 0.5 mM 2,6-dichloro(5-iodo)-1,1-dimethylurea (DCMU).

**DNA Techniques**—Molecular cloning was carried out by using standard techniques according to Ref. 17. Enzymes used for PCR and cloning were obtained from MBI Fermentas. PCR was carried out using a Bio-Rad Thermocycler. DNA sequencing was performed by MWG Biotech (Ebersberg, Germany). For PCR, genomic DNA of Synechocystis PCC 6803 was prepared as described recently (18), and inactivation of the various petC genes in Synechocystis PCC 6803 was done following the protocol in Ref. 16.

**Oxygen Evolution**—Oxygen evolution rates were measured at 30 °C using a Clark-type oxygen electrode under actinic light (600 μW m−2 s−1), Synechocystis cells were harvested in the mid-log phase and diluted to a chlorophyll concentration of 20 μg/ml in BG11 medium. Oxygen evolution was measured after addition of 0.5 mM 2,6-dichloro-p-benzoquinone or of 10 mM NaHCO3.

**Results**

**Insertional Deletion of the Three Putative petC Genes**—The three open reading frames of Synechocystis PCC 6803 encoding putative Rieske proteins were assigned petC1 (sll1316), petC2 (sll1185), and petC3 (sll1182) (5, 21) with the numbers given in parentheses showing the deposited name in the Cyanobase database (22).

The gene encoding the PetC1 protein was amplified with flanking regions by PCR and cloned into the plasmid pUC18 (New England Biolabs, Beverly, MA) after restriction of the PCR product using restriction sites that had been introduced by the primers. A 260-bp fragment from the petC1 encoding and upstream region was replaced by a chloramphenicol resistance cassette, which was amplified by PCR without a transcription termination signal (Fig. 1). By this method the promoter of the chloramphenicol acetyltransferase (cat) cassette can be used for the transcription of petC1 encoding for the cytochrome f subunit in Synechocystis. A wild type Synechocystis PCC 6803 strain was used for transformation of the deletion construct, and transformants were grown on plates containing increasing amounts of chloramphenicol to select for complete segregation and to obtain a homozygous genotype.

For the inactivation of petC2, the gene was amplified by PCR with flanking regions, and after ligation of the PCR product to the plasmid pBluescript II SK (MBI Fermentas, St. Leon-Rot, Germany), a 640-bp fragment was replaced by a spectinomycin resistance cassette from pH451 (23). Wild type, ΔpetC1, and ΔpetC3 strains of Synechocystis PCC 6803 were transformed with this construct to yield the appropriate single or double deletion strains.

For deletion of petC3, the gene was amplified by PCR with flanking regions and cloned into the plasmid pASK-IBA4 (IBA GmbH, Gottingen, Germany). To inactivate the petC3 gene, a 215-bp DNA fragment was replaced by a kanamycin resistance cassette obtained from the plasmid pBSL14 (24). The resulting...
constrct was transformed into the wt, the ΔpetC1, and the ΔpetC2 strain to obtain the single and double deletion strain. For complete segregation of the ΔpetC3 cells, mutants were selected on BG11 plates containing increasing amounts of kanamycin.

PCR was used to test whether the various strains were homozygous for the deletion of the different petC genes (Fig. 1). Although the PCR fragments of the DNA regions containing the wt gene were less than 1000 bp in size, the introduction of the respective resistant cassettes increased the size of these PCR fragments by more than 500 bp (Fig. 1). Fig. 1 also shows that the individual deletion of all three petC genes is possible. In neither case was a fragment corresponding to the size of the wt DNA fragment amplified. The simultaneous deletion of petC2 with petC3 was possible as well as the deletion of petC1 in combination with petC3. All attempts to delete petC2 completely in the ΔpetC1 strain failed (Fig. 1) as well as attempts to delete petC1 in the ΔpetC2 strain. These results indicate an overlapping function of these two genes, whereas the function of petC3 does not seem to overlap directly with the possible roles of the other two petC genes.

Effect of the petC Deletions on Cell Growth—To test whether the deletions have any effect on cell viability under different growth conditions, growth rates of the completely segregated mutant strains relative to the wt were measured under photoautotrophic, mixotrophic, and photoheterotrophic growth conditions, respectively.

As can be seen in Fig. 2, strains carrying the single deletions ΔpetC2 or ΔpetC3, as well as the ΔpetC2Δ3 double deletion do not alter significantly in their growth properties under any of the tested growth conditions when compared with wt cells; this suggests a nonessential function of PetC2 and PetC3 under the chosen growth conditions. Although the ΔpetC1 strain shows similar growth rates as the wt strain under photomixotrophic, and photoheterotrophic conditions (Fig. 2) the growth rate is slowed down under photoautotrophic conditions. It should be noted that growth of this mutant in the presence of chloramphenicol, i.e. the antibiotic used for obtaining a completely segregated deletion strain, resulted in highly impaired growth under photomixotrophic conditions (data not shown); this indicates a considerable stress of the ΔpetC1 strain caused by the gene deletion. Most interestingly, although the single deletion of petC3 did not cause any observable growth defect and the single deletion of petC1 had a somewhat stronger impact on the growth properties, the combination of both in the double deletion strain ΔpetC1Δ3 resulted in a distinctly increased doubling time under all tested growth conditions when compared with the wt strain.

Chlorophyll Fluorescence as a Probe for the Reduction and Reoxidation of the PQ Pool—To examine the effect of the petC1 deletion on the redox activity of the cytochrome b₅f complex, the photooxidation of the PQ pool by photosystem I was monitored by measuring the chlorophyll fluorescence of photosystem II, which is dependent on the redox state of Q₁ and thus indirectly reflects the redox state of the PQ pool. In order to obtain a reduced PQ pool in darkness (due to PQ reduction by respiratory dehydrogenases), wt and ΔpetC1 cells were incubated with 1 mM KCN, which blocks the reoxidation by the terminal oxidases. During a subsequent illumination of wt cells, the yield of variable fluorescence (Fᵥ) increases quickly at the onset of illumination but returns to a low level after a short lag phase due to the fast photooxidation of PQH₂ by photosystem I (Fig. 3). In contrast, the reoxidation of PQH₂ is drastically slowed down in the ΔpetC1 cells, and the transient increase of Fᵥ is much stronger with about 60% of the maximum yield of Fᵥ. This is about three times higher than in wt and indicates a decreased rate of electron transport between the photosystems, indicating a severely impaired activity of the cytochrome b₅f complex in ΔpetC1 cells.

In addition to the cytochrome b₅f complex, plastoquinol can also be oxidized by a cytochrome bd-type oxidase in Synechocystis PCC 6803 (20, 25–27). If PQH₂ oxidation could only occur via the cytochrome b₅f complex, illumination of the cyanobacterial cells in the presence of DBMIB, a cytochrome bd complex inhibitor, should result in a completely reduced PQ pool. For this reason, the partial quenching of Fᵥ in the presence of saturating concentrations of DBMIB, as shown in Fig. 4A, is evidence for such an alternative route of PQH₂ oxidation (20). On average, photoheterotrophically grown wt cells (Fig. 4A) show a quenching of 28 ± 10% (n = 10) of the total yield of Fᵥ. ΔpetC1 cells show a similar effect (Fig. 4B), but here a significantly higher amount of Fᵥ quenching (52 ± 3%, n = 4) is observed, indicating a distinctly higher oxidation rate of the PQ pool via the cytochrome bd-type complex in this mutant. As a control, the cells were illuminated in the presence of both DBMIB and KCN. In this case, a rapid reduction of the PQ pool during illumination is expected, as neither respiratory nor light-dependent oxidation of plastoquinol is possible. Indeed, this effect could be observed for both wt (Fig. 4C) and mutant cells (Fig. 4D).

Identical measurements have also been performed with the ΔpetC2 and ΔpetC3 strains; however, there are no significant differences in comparison to wt. Fᵥ quenching in the presence of DBMIB (conditions as in Fig. 4, A and B) was 18 ± 1% (n = 4) in ΔpetC2 and 19 ± 7% (n = 4) in ΔpetC3 (not shown).

Photosynthetic Activity—In order to test the photosynthetic
activities of the generated deletion strains, the rate of oxygen evolution was determined for all \( \Delta \text{petC} \) strains using phenyl-p-benzoquinone as electron acceptor at photosystem II. These measurements, which were normalized to the total chlorophyll concentration, indicate an \(~60\%\) reduced amount of active photosystem II in the \( \Delta \text{petC}1 \) and \( \Delta \text{petC}1\beta3 \) strains in comparison with wt (Fig. 5). Photosystem II activity in the other mutant strains was also impaired, although to a lesser extent as in the case of the \( \Delta \text{petC}1 \) strain. Using HCO\(_3\) as electron acceptor allows the determination of the activity of the whole electron transport chain. With this acceptor the \( \Delta \text{petC}1 \) mutant cells showed only 40% of the activity of wt cells (175 \( \pm \) 5 versus 57 \( \pm \) 9 \( \mu \)mol O\(_2\)/mg of chlorophyll-h). The strain carrying the double deletion \( \Delta \text{petC}1\beta3 \) showed similar photosystem II activity as the \( \Delta \text{petC}1 \) strain as can be seen in Fig. 5. In contrast, all other mutant strains, i.e. \( \Delta \text{petC}2 \), \( \Delta \text{petC}3 \), and \( \Delta \text{petC}2\beta3 \), were similar active as the wt.

To study further the reason for the decreased oxygen evolution rate in the \( \Delta \text{petC}1 \) strain, wt and \( \Delta \text{petC}1 \) cells were analyzed by 77 K fluorescence spectroscopy. The relative content of photosystem I and II in the thylakoid membrane of cyanobacteria can be determined from their fluorescence emission spectra at 77 K. Upon chlorophyll excitation at 435 nm, photosystem II shows a characteristic emission spectrum with maxima at 695 and 685 nm, and photosystem I shows a characteristic emission maxima at 725 nm. The emission spectra shown in Fig. 6 indicate an increased photosystem I content relative to photosystem II in the \( \Delta \text{petC}1 \) mutants, whereas the photosystem I to photosystem II ratio in the \( \Delta \text{petC}2 \) and \( \Delta \text{petC}3 \) mutants was identical to wt (data not shown). A decreased photosystem II content rather than an increased photosystem I content in the \( \Delta \text{petC}1 \) strain is supported by the observation that the concentration of chlorophyll per cell (cell density measured as OD\(_{750}\)) is decreased by more than 7% in the \( \Delta \text{petC}1 \) mutant strain.

In summary, the oxygen evolution measurements as well as the 77 K fluorescence measurements indicate a reduced photosystem II content in the \( \Delta \text{petC}1 \) strain.

**DISCUSSION**

Presence of Multiple Genes for Rieske Proteins—To investigate the specific role of three potential Rieske proteins in *Synechocystis* PCC 6803, their genes have been deleted individually and in combination. In general, the deletion of subunits of the cyanobacterial cytochrome \( b_{\beta}f \) complex is difficult because this complex has a central role for both the photosynthetic and the respiratory electron transport chain of cyanobacteria (14). Up to now, only the successful deletion of the gene encoding the small subunit PetM was reported (19), which, however, does not seem to have an essential function. Here we show that the complete deletion of each of the three petC genes alone is possible. These results are in contrast to a recent report (28) on deletion of the petC1 gene in the cyanobacterium *Synechococcus* PCC 7002. In this study, deletion of petC1 did not lead to a viable \( \Delta \text{petC}1 \) segregant, and the authors concluded that petC2 and petC3 are silent with respect to a possible function in the cytochrome \( b_{\beta}f \) complex. However, the authors did not perform deletions of the two additional petC genes, and potentially overlapping function of any of the petC genes was not studied. In addition, it is possible that the function of the two additional petC genes is less important in *Synechococcus* than in *Synechocystis* making a deletion of petC1 impossible in *Synechococcus*.

Our attempts to delete petC1 in *Synechocystis* completely, using a resistance cassette with a transcription termination signal, also failed, most likely because of an interruption of the petA transcription. Only the use of a resistant cassette without a transcription termination signal resulted in a successful deletion of petC1. Most likely in this case the cat promoter is also used for the transcription of the petA gene. The deletion experiments also indicate that petC2 does not have any special function in the *Synechocystis* cytochrome \( b_{\beta}f \) complex. Although the single deletion of petC2 did not result in a signifi-
cantly altered phenotype, the ΔpetC2/3 double deletion strain was also unaffected in the stoichiometry or the activity of the photosynthetic protein complexes and in the growth rate (doubling time) of the strain. Although the single deletion of petC3 did not result in significantly altered growth properties of the mutant strain, the results obtained with the ΔpetC1/3 double deletion strain indicate that petC3 shares some function with petC1. Because the double deletion was possible, the functions of these two genes seem to be different and not replaceable as in the case of petC1 and petC2. Whereas the phenotype of the ΔpetC1/3 mutant was basically identical to the ΔpetC1 strain, the combined deletion of petC1 and petC2 turned out to be impossible. From these data we conclude that the Rieske protein PetC2 can indeed replace the Rieske protein PetC1 in the cytochrome $b_{6f}$ complex to some extent. This is consistent with the relative high sequence similarity and especially with the similar midpoint potential of these two proteins (5). Also the fact that the petC2 gene is functionally expressed in Synechocystis supports the view of various co-existing Rieske proteins in the cytochrome $b_{6f}$ complex of this organism (5). Because it was already shown that petC3 is also functionally expressed in Synechocystis and that the encoded protein is associated with the cytochrome $b_{6f}$ complex, although to rather low extent (5), the results presented in this study indicate a function of PetC3 that is different from the function of PetC1. We showed recently (5) that the petC3 gene encodes for a truncated Rieske protein with a highly lowered midpoint potential. The observation of this study that PetC3 seems to have some function in vivo together with the observed highly lowered midpoint potential argue for the existence of different cytochrome $b_{6f}$ complexes in Synechocystis as has been suggested.

PetC1 Is the Main Rieske Protein in Synechocystis PCC 6803—As the cytochrome $b_{6f}$ complex is a central component of the energy-transducing system of Synechocystis PCC 6803, it seemed unlikely that the deletion of an essential subunit such as the Rieske protein would be possible. However, our results show that the petC1 gene can be deleted and that its function can at least partly be replaced by another petC gene. Nevertheless, in contrast to the ΔpetC2 and ΔpetC3 strains, the ΔpetC1 mutant strain showed some new characteristics when compared with the wt, indicating an important and specific role of the petC1 gene. The electron transfer through the cytochrome $b_{6f}$ complex was still possible in this mutant, although the activity of the complex seemed to be impaired, and chlorophyll fluorescence measurements showed a significant reduction of the PQ reoxidation rate by the cytochrome $b_{6f}$ complex (Fig. 3). In addition, an increased activity of the cytochrome $bd$-type complex was indicated in the ΔpetC1 mutant strain (Fig. 4) by fluorescence measurements. Synechocystis PCC 6803 contains three different respiratory terminal oxidases (25, 27). Two of them are homologous to the mitochondrial cytochrome $c$ oxidase, and the third one, the cytochrome $bd$-type complex, is a quinol oxidase that can function in parallel to the cytochrome $b_{6f}$ complex. Its function in thylakoid membranes of Synechocystis PCC 6803 has been elucidated recently (20). It was shown that the activity of the cytochrome $bd$-type complex increases in particular when the rate of plastoquinol oxidation via the cytochrome $b_{6f}$ complex appears to be insufficient. The changes in the electron transport system as well as the decreased photosystem II content of the ΔpetC1 mutant strain can be interpreted as adjustments in response to the partially defective cytochrome $b_{6f}$ complex and more reduced PQ pool. On the other hand, the fact that photosystem I seems to be assembled at wild-type levels (as judged from the 77 K fluorescent spectra) and that photosynthetic electron transfer through the whole electron transport chain is still operative indicates rather strongly that the cytochrome $b_{6f}$ complex is still active. Finally, the diminished growth of the ΔpetC1 strains under photoautotrophic conditions is in agreement with the observation that the photosystem II content is lowered in this mutant resulting in less provided energy by the photosynthetic light reaction.

But why does the ΔpetC1 strain show characteristics indicating a less active cytochrome $b_{6f}$ complex? The presented mutational data indicate that petC2 can partly compensate for the petC1 deletion, although the activity of the complex is lowered to some degree. In two recent studies the electron transfer activity of the cytochrome $b_{6f}$ complex Rieske protein with mutations in the flexible hinge region was analyzed. This glycine-rich region connects the transmembrane domain with the soluble domain, and it was shown in the cytochrome $bc_1$ complex that the activity of the complex is very sensitive to perturbations of the sequence and structure of that hinge region (29–34). Most interestingly, changes in the flexible regions of the cytochrome $b_{6f}$ complex Rieske proteins did not alter the complex function as dramatically as observed with the cytochrome $bc_1$ complex (28, 35). Nevertheless, even in these studies changes were observed indicating an important role of this region for proper function of the Rieske protein. In contrast to PetC1 and other Rieske proteins, PetC2 contains one glutamine and one lysine residue in the hinge region that can highly diminish the flexibility of that region, and electrostatic interactions to other subunits are possibly favored. Because the electron transfer within the cytochrome $bc$ complex Rieske proteins is accomplished by a movement of the soluble Rieske domain (10, 36), the observed differences in the amino acid sequence in the hinge region could cause less flexibility resulting in impaired electron transfer by domain movement.

petC Gene Families in Cyanobacteria—A recent genome-wide expression analysis in the cyanobacterium Anabaena sp. PCC 7120 showed the functional expression of multiple petC genes (37). The aim of that study was to investigate the up-regulation of genes after nitrogen deprivation, and the authors could show that the two putative Rieske genes all0606 and all11512, which encode for proteins with a high degree of homology to Synechocystis PetC3 and PetC2 proteins, respectively, were up-regulated more that 5.0-fold under the chosen growth conditions. Because petC1, which is cotranscribed with petA in cyanobacteria (38), is the mainly transcribed petC gene, these results indicate the functional expression of three Rieske proteins both in Anabaena and in Synechocystis.

Although in many of the cyanobacterial genomes, such as in Synechococcus PCC 7002 and Anabaena PCC 7120, a petC gene family can be found, only the main petC gene, which is cotranscribed with petA, is present in the genomes of the thermophilic cyanobacterium Thermosynecochoccus elongatus (39, 40) and of the mesophilic cyanobacterium Gloeobacter violaceus PCC 7421 (41). Therefore, the occurrence of a petC gene family seems to be common but not obligatory in cyanobacteria. This is in good agreement with our observation that the additional petC copies petC2 and petC3 can be deleted without any significant effect. In higher plants, the functional expression of two almost identical chloroplast Rieske proteins is also reported (14). However, the similar expression pattern of the two genes and their high degree of sequence identity argue rather for the simultaneous expression of two petC genes encoded by both parental chromosomes. In this case no special function of the different Rieske proteins is expected.

As outlined in the Introduction, petC is not the only gene for which a gene multiplication has been found in Synechocystis PCC 6803. Best characterized is psbA encoding the D1 subunit of photosystem II with three gene copies. In principle, the
expression of all three genes leads to a functional D1 protein (4), although under standard growth conditions more than 90% of the total psbA transcripts originate from the psbA2 gene and the remaining part from the psbA3 gene. In contrast to the petC genes, the psbA2/3 genes share 99% nucleotide identity and encode identical proteins. The function of these various D1 proteins is not yet completely clear although increased synthesis of psbA3 has been observed under stress conditions (42). As all three Rieske proteins in Synechocystis are sufficiently different (5), various properties of the resulting proteins can also be expected in their physiological context, although the physiological functions of the petC gene family are elusive so far.

In conclusion, our results show that PetC1 has a predominant function in the cytochrome b6f complex of Synechocystis PCC 6803, which can partly be taken over by PetC2. Although PetC3 also seems to have a specific function, the protein cannot replace PetC1 or PetC2 and may only operate in parallel to one of the two other Rieske proteins. Based on the presented data, it can be speculated that under normal physiological conditions PetC1 is the dominant Rieske isofrom that can, under certain conditions, be replaced by PetC2, although at the expense of activity loss or decreasing cytochrome b6f complex activity. Independent of PetC1 and PetC2, PetC3 may have a specific physiological function within a small community of cytochrome b6f complexes, which is especially indicated by its much more negative redox potential. Although difficult due to its small amount in the membrane, this special role of PetC3 is still to be elucidated.

Acknowledgments—We thank Sven Skrzyzczak for help and advice and Ursula Altenfeld for excellent technical assistance.

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