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A Regulatory Role of the PetM Subunit in a Cyanobacterial Cytochrome b_6f Complex*

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To investigate the function of the PetM subunit of the cytochrome b_6f complex, the *petM* gene encoding this subunit was inactivated by insertional mutagenesis in the cyanobacterium *Synechocystis* PCC 6803. Complete segregation of the mutant reveals a nonessential function of PetM for the structure and function of the cytochrome b_6f complex in this organism. Photosystem I, photosystem II, and the cytochrome b_6f complex still function normally in the *petM*$^-$ mutant as judged by cytochrome *f* re-reduction and oxygen evolution rates. In contrast to the wild type, however, the content of phycobilisomes and photosystem I as determined from 77 K fluorescence spectra is reduced in the *petM*$^-$ strain. Furthermore, whereas under anaerobic conditions the kinetics of cytochrome *f* re-reduction are identical, under aerobic conditions these kinetics are slower in the *petM*$^-$ strain. Fluorescence induction measurements indicate that this is due to an increased plastoquinol oxidase activity in the mutant, causing the plastoquinone pool to be in a more oxidized state under aerobic dark conditions. The finding that the activity of the cytochrome b_6f complex itself is unchanged, whereas the stoichiometry of other protein complexes has altered, suggests an involvement of the PetM subunit in regulatory processes mediated by the cytochrome b_6f complex.

The cytochrome b_6f complex is an essential component of the electron transport chain in oxygenic photosynthetic organisms. In green algae and higher plants it is located in the thylakoid membrane of chloroplasts and mediates electron transport from PS[1] II to PS I (for reviews see Refs. 1 and 2). In cyanobacteria (3), it is located both in thylakoid and cytoplasmic membranes (4). Because of the absence of a cytochrome bc_1 complex, the cytochrome b_6f complex fulfills additional functions in these organisms. It is involved in both photosynthetic and respiratory electron transport, acting as a plastoquinol-cytochrome c_6-plastocyanin oxidoreductase and playing a role in electron transfer from PS II or NAD(P)H dehydrogenase to PS I or cytochrome oxidase, respectively (5).

In all organisms capable of oxygenic photosynthesis the cytochrome b_6f complex consists of four major proteins and additional small subunits. The 25-kDa cytochrome b_6 protein contains two b-type hemes and, together with the 17-kDa protein subunit IV, is homologous to cytochrome *b* of the cytochrome bc_1 complex (6). Cytochrome b_6 and subunit IV are integral membrane proteins with four and three predicted transmembrane α-helices, respectively. Cytochrome *f* is a 31-kDa c-type cytochrome with a covalently bound heme c in the large lumen-exposed domain; it is anchored by a single C-terminal α-helix in the membrane. Similarly, the Rieske iron-sulfur protein has a large hydrophilic lumenal domain attached to a single transmembrane α-helix at the N terminus.

Whereas the three-dimensional structure of the whole cytochrome b_6f complex has not yet been resolved at high resolution, the structure of some fragments is known. The lumenal domain of cytochrome *f* of *Chlamydomonas reinhardtii* (7) and *Phormidium laminosum* (8) has been resolved up to 1.9 Å, and the structure of the lumenal domain of the Rieske protein from spinach has been resolved up to 1.83 Å (9). Analysis of two-dimensional crystals of the whole complex has led to a projection map of only 8-Å resolution to date (10). However, the structure of the cytochrome bc_1 complex from mitochondria has been determined to about 3-Å resolution (11–13), and this structure is a useful guide for predicting the general structure of homologous subunits of the cytochrome b_6f complex (14).

Because the core structures of bc_1 and b_6f complexes consist of similar proteins and are functionally nearly identical, their three-dimensional structures are likely to be very similar. However, the additional subunits, PetG (15), PetL (16), and PetM (17) in b_6f complexes and up to eight additional subunits in eukaryotic bc_1 complexes, have no obvious relation with each other and are likely therefore to be structurally quite different. The functions of the additional small b_6f subunits is not yet known. Interestingly, a gene encoding the PetL protein cannot be found in the completely sequenced genome of the cyanobacterium *Synechocystis* PCC 6803 (18). Recently the open reading frame *ycf6* of chloroplasts, for which a cyanobacterial homolog exists, was shown to encode an essential subunit of the cytochrome b_6f complex that is now called *petN* (19). Whereas inactivation of *petG*, *petL*, and *petN* indicate an essential role of these subunits for the cytochrome b_6f complex, such experiments have not yet been performed with *petM*. This may be due to the fact that PetM is nuclear encoded in *C. reinhardtii* (20) and higher plants (21), making its deletion more difficult.

Little information is available on the function of PetM, and its orientation in the membrane remains controversial. Ketcher and Malkin (17) argue for a lumenal orientation of the C terminus because of its more positive character. However, de Vitry *et al.* (20) concluded from trypsin treatment experiments with thylakoid membranes that the C terminus had a stromal orientation.

In this report the *petM* gene of *Synechocystis* PCC 6803 was

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[1] The abbreviations used are: PS, photosystem; WT, wild type; PCR, polymerase chain reaction; LL, low light; HL, high light; PQ, plastoquinone.

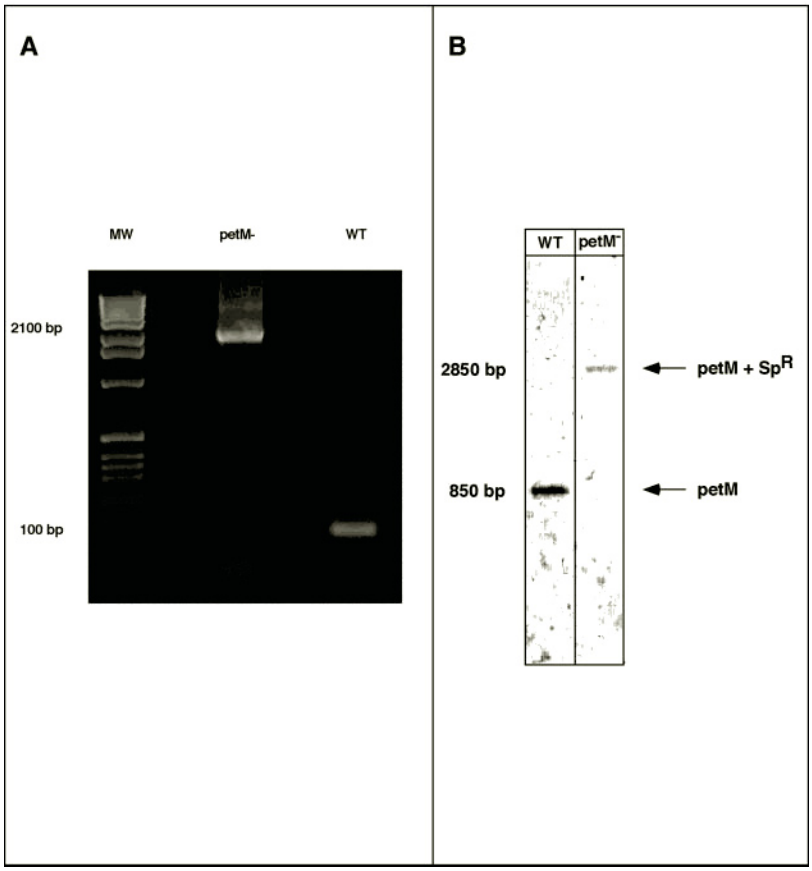

FIG. 1. **Analysis of DNA from the wild type and the *petM*$^-$ mutant.** *A*, PCR products of the *petM* gene from both strains; in the case of *petM*$^-$ DNA, only a fragment corresponding to the interrupted *petM* gene can be amplified. *B*, Southern blot of *Nco*I-restricted DNA using the labeled *petM* gene as a probe. For the *petM*$^-$ strain only a 2.85-kilobase fragment is visible, which corresponds to the interrupted *petM* gene. *MW*, molecular weight; *bp*, base pairs; *Sp*^{R}, spectinomycin resistance.

deleted. Analysis of the resulting mutant revealed characteristic differences from WT that suggest that PetM is not essential for b_6f complex activity but instead plays a regulatory role.

MATERIALS AND METHODS

Growth Conditions—Synechocystis PCC 6803 wild type and the *petM*$^-$(*petM* interruption) mutant strains were grown at 30 °C in BG11 medium (22) aerated with air enriched in CO_2 and at light intensities of 20 or 100 μmol photons m^{-2} sec^{-1}. For selection of spectinomycin-resistant mutants, BG11 medium was supplemented with increasing concentrations of spectinomycin (5–100 μg/ml).

Cloning, DNA Sequencing, and Gene Inactivation—Cloning was carried out using standard techniques as described in Ref. 23. Enzymes used for PCR and cloning were obtained from MBI Fermentas and New England Biolabs. PCR was done in a Bio-Rad Thermocycler for 30 cycles under the following conditions: 95 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min. The following primers were used for amplification of the *petM* gene alone and the *petM* gene with flanking regions: CT PetM, TTATTCTTCGCTACCTTGGAGTTT; NT PetM, ATGACCGCTGAAAGCATGTTGGCC; CT PetM+, AGCACCATTTGGGCTTGGGTGTCC; NT PetM+, TTTGAGCGGAATATGGGTGGTGGC. A 1.0-kilobase pair DNA fragment encoding the *petM* gene and flanking regions from *Synechocystis* PCC6803 was amplified by PCR. The *Nhe*I-restricted DNA fragment was cloned into the *Nhe*I side of the pDMI.1 (24) plasmid, resulting in the plasmid pPMF. DNA sequencing was performed by MWG Biotech (Ebersberg, Germany).

For Southern hybridization the amplified *petM* gene was labeled with digoxygenin using the instructions of the non-radioactive labeling kit (Roche Molecular Biochemicals). Genomic *Synechocystis* DNA was prepared according to Ref. 25. Insertional inactivation of the *petM* gene in *Synechocystis* was carried out according to Ref. 26.

Spectroscopic Techniques—Absorbance spectra of cell lysates in the visible range were recorded with a Beckman spectrophotometer as described in Ref. 27, and fluorescence emission of cells was recorded with an Aminco Bowman Series 2 fluorimeter as described in Ref. 39. For fluorescence measurements, cells were diluted in BG11 medium containing 20% glycerol to a final chlorophyll concentration of 5 μg/ml and frozen in liquid nitrogen. Fluorescence induction measurements were carried out as described previously.[2]

Single turnover kinetics of cytochrome *f* and P_{700} (the photosynthetic reaction center of photosystem I) were monitored at room temperature using an in-house-constructed single beam spectrophotometer as described in Ref. 29. *Synechocystis* cells were suspended to a final chlorophyll concentration of 12 μg/ml in BG11 medium. Cytochrome *f* and P_{700} kinetics were determined by analysis of flash-induced absorbance changes at 551, 556, 561, and 703 nm. Flashes of 6-ms half peak width were provided to both sides of the sample with a xenon flashlamp (20 millifarad capacitance) filtered with BG635 filters. Cytochrome *f* redox changes were calculated from the absorbance difference at 556 nm minus the average of 551 nm and 561 nm and with an assumed extinction coefficient of 18 mM^{-1} cm^{-1}. Redox changes of P_{700} were monitored at 703 nm with an assumed extinction coefficient of 64 mM^{-1} cm^{-1}. To obtain anaerobic conditions, 10 mM glucose, 400 units/ml catalase, and 24 units/ml glucose oxidase were added, and samples were incubated for 5 min prior to the measurement.

Data Analysis—Protein sequence information was analyzed using the public domain CuraTools protein analysis software (CuraGen). Similarity searches were performed using the BLAST program provided by the National Center for Biotechnology Information.

RESULTS

Generation of a petM$^-$ *Strain*—For the generation of a *petM*$^-$ *Synechocystis* strain, the 5.7-kilobase pair plasmid pPMF was cleaved with *Bsp*HI, which has a unique site in the *petM* gene, and the resulting termini of the plasmid were filled in using T4 DNA polymerase. A spectinomycin/streptomycin resistance cassette containing the *aadA*$^+$ gene was obtained by restricting the plasmid pHP45Ω (30) with *Sma*I and ligated to the linearized plasmid pPMF. The resulting 7.8-kilobase pair plasmid pPMS was used for transformation of the *Synechocystis* wild-type cells. A completely segregated *petM*$^-$ mutant was con-

[2] S. Berry, D. Schneider, W. F. J. Vermaas, and M. Rögner, submitted for publication.

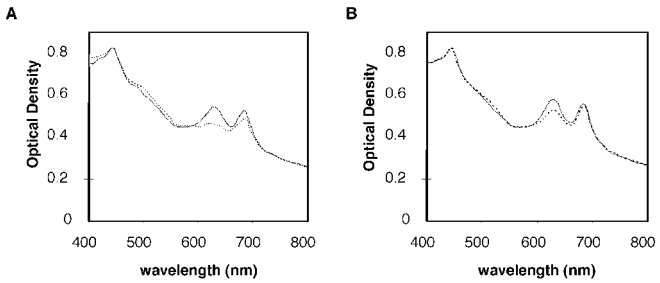

FIG. 2. **Absorbance spectra of wild-type (–) and *petM*$^-$ (- -) strains of *Synechocystis* grown under different conditions.** Peaks represent PS I (675 nm), PS II (435 nm), and phycobilisomes (620 nm). *petM*$^-$ cells always show a decreased level of phycobilisomes, which is stronger under HL conditions (*A*) than under LL conditions (*B*).

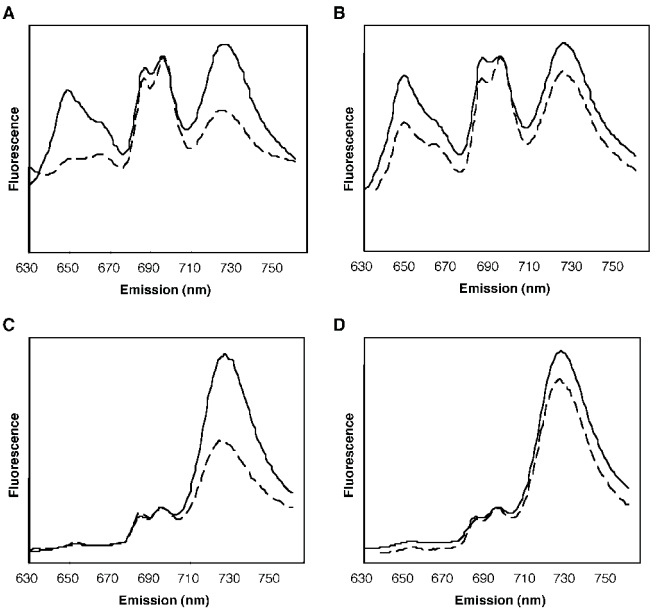

FIG. 3. **77 K fluorescence spectra of *Synechocystis petM*$^-$ (- -) and wild-type strain (–) grown under HL (*A* and *C*) and LL (*B* and *D*) conditions with excitation at 580 nm (*A* and *B*) or 435 nm (*C* and *D*).** The decreased absorbance at 420 nm can be correlated with a decreased amount of phycobilisomes. The lower fluorescence emission at 730 nm additionally indicates a decreased level of PS I in the cells. The spectra were normalized at 695 nm.

firmed by PCR after several subcultures in BG11 medium with increasing amounts of spectinomycin. Fig. 1*A* shows a fragment of about 100 base pairs carrying the *petM* gene, which was amplified from wild-type DNA, whereas a fragment of about 2.2 kilobase pairs was obtained with DNA from the *petM*$^-$ mutant corresponding to the size of the *petM* gene interrupted by the resistance cassette. No 100-base pair fragment was detectable in the mutant under various PCR conditions, indicating a complete segregation, which was confirmed by Southern blotting experiments (Fig. 1*B*).

According to the *Synechocystis* genome sequence, an open reading frame of unknown function (smr0003) is located directly upstream of the interrupted gene. To check for polar effects on this possibly cotranscribed gene, an alternative inactivation strategy for the *petM* gene was also carried out. The pPMF plasmid was restricted with *Bsp*HI, and the 4-nucleotide overhang was filled in by T4 polymerase. Religation of the plasmid caused a frameshift in the *petM* gene. After insertion of a chloramphenicol resistance cassette at the *Bsp*E I site of the plasmid, the construct was used for transformation of *Synechocystis*. The resulting mutant was checked for complete segregation by restricting the PCR-amplified *petM* gene with *Bsp*HI. Only the wild-type gene could be restricted with this enzyme, because the frameshift introduced into the mutated gene caused the loss of this restriction site. The completely segregated mutant showed the same phenotype as the mutant with the interrupted gene in all measurements carried out. This indicated that either the interruption of the *petM* gene did not affect the expression of this putative gene or that the additional inactivation of this open reading frame did not yield any phenotype.

Pigment Composition—Fig. 2 shows absorbance spectra of *Synechocystis* wild-type and mutant cells grown under low (20 μmol photons m^{-2} sec^{-1}; LL) and medium high light intensity (100 μmol photons m^{-2} sec^{-1}; HL). Under both conditions, particularly at higher light intensity, the level of phycobilisomes (absorption peak at 620 nm) is decreased in the *petM*$^-$ strain. This is reflected in a yellow-green color of the mutant in comparison with the blue-green color of the wild type.

To test for additional effects of the *petM* disruption on the photosynthetic pigment composition, fluorescence emission spectra of whole cells were recorded at 77 K upon excitation of chlorophyll at 435 nm or of phycobilisomes at 580 nm (Fig. 3). Peaks at about 650 and 665 nm can be attributed to phycobilisome pigments, and the peak at 685 nm can be attributed to the phycobilisome terminal emitter allophycocyanin B and the small antenna of PS II. The peak at 695 nm is associated with CP47 of PS II, and the peak at 725 nm originates from PS I (31). In the mutant, a decreased amount of phycobiliproteins relative to chlorophyll is obvious from Fig. 3. Fig. 3, *A* and *C*, illustrates a decreased emission peak at 725 nm in the mutant, particularly under HL conditions. Because the chlorophyll content per cell is decreased by up to 20% in the mutant relative to the wild type (as assessed from the ratio of chlorophyll to OD_{730}), the amount of PS I apparently is decreased in the mutant. In addition, because PS I binds about 90% of the total cellular chlorophyll, and the relative decrease of the emission peak at 725 nm is more than 20%, an increase in the amount of PS II per cell is also likely.

Electron Transport Activity—To check the activity of the remaining PS I complexes, the primary donor P_{700} of PS I was oxidized by a single turnover flash, and the re-reduction by plastocyanin was monitored. As shown in Fig. 4, the kinetics of the wild type (*A*) and *petM*$^-$ strain (*B*) are very similar, indicating that the intrinsic activity of the remaining PS I complexes is unaffected. For a more quantitative analysis, the amount of P_{700} was determined as outlined under "Materials and Methods." The wild type yielded 3.27 mmol of P_{700}/mol of chlorophyll, and the mutant yielded only 2.48 mmol of P_{700}/mol of chlorophyll; this clearly confirms the increased PS II:PS I ratio in the *petM*$^-$ strain observed by 77 K fluorescence spectroscopy.

In contrast to higher plants, the PQ pool of the cyanobacterium *Synechocystis* PCC 6803 is mainly reduced in the dark,[2] indicating that the intersystem components cytochrome *f*, the Rieske protein, plastocyanin, and P_{700} will also be reduced under these conditions. Photooxidation of P_{700} by a single saturating xenon flash is followed within a millisecond by oxidation of intersystem components as P_{700} is re-reduced. This in turn is followed by re-reduction of the intersystem components by plastoquinol, catalyzed by the cytochrome b_6f complex and occurring at a rate governed by the degree of reduction of the plastoquinone pool. The re-reduction kinetics of cytochrome *f*, monitored as described above, is shown in Fig. 5 under aerobic conditions for WT (*A*) and the *petM*$^-$ strain (*B*). Although both exhibit oxidation followed by re-reduction on this timescale, a considerable part of cytochrome *f* in the *petM*$^-$ mutant remains oxidized in comparison with the wild type. This effect could be caused either by a malfunctioning fraction of the cytochrome

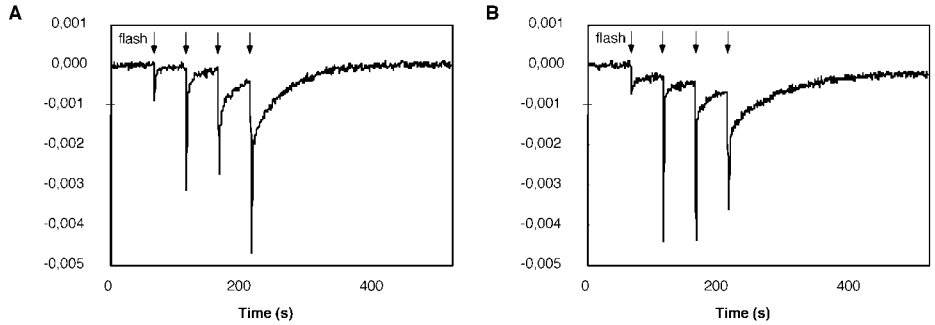

FIG. 4. **P_{700} turnover kinetics of PS 1.** Wild-type (*A*) and *petM*$^-$ strains (*B*) show the same kinetics of re-reduction. Identical chlorophyll concentrations were used for both types of cells, which were flashed four times with far red light while absorbance changes were monitored at 703 nm.

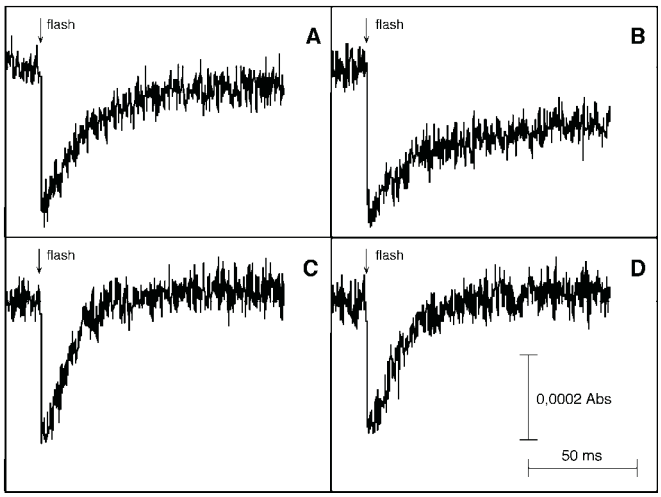

FIG. 5. **Transient kinetics of the cytochrome *f* turnover in wild-type (*A* and *C*) and *petM*$^-$ (*B* and *D*) cells under aerobic (*A* and *B*) and anaerobic conditions (*C* and *D*).** Cells were illuminated with far red light, and the cytochrome *f* re-reduction was determined as indicated under "Materials and Methods." *Abs*, absorption.

b_6f complex or by a more oxidized redox state of the PQ pool in the mutant. Interestingly, under anaerobic conditions, the re-reduction kinetics of cytochrome *f* in the *petM*$^-$ mutant are almost identical to those of the wild type (Fig. 5, *C* and *D*). This indicates that the aerobic difference arises because the plastoquinone pool is more oxidized in the mutant, rather than because of any difference in the b_6f function itself. Moreover, quantification of the extent of the cytochrome *f* transient shows that the content of the cytochrome b_6f complex in the mutant is similar to that of the wild type (Fig. 5).

Status of the PQ Pool—The redox state of the PQ pool in the mutant was monitored by fluorescence induction measurements. This is possible because the redox state of Q_A depends on the redox state of the PQ pool.[2] Fig. 6, *A–D*, shows fluorescence induction curves of wild-type and *petM*$^-$ cells grown under LL and HL conditions and in the presence of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, an inhibitor of cytochrome b_6f oxidoreductase activity. For comparison, the maximum fluorescence yield of cells from different strains was measured by blocking the reoxidation of Q_A^- with 3-(3,4-dichlorophenyl)-1,1-dimethylurea at the Q_B site of PS II. Interestingly, the variable fluorescence (F_V) in the *petM*$^-$ strain (Fig. 6*D*) did not reach the maximal level obtained by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, in contrast to the behavior of the wild-type strain (Fig. 6*B*). This effect is even stronger under HL (Fig. 6, *A* and *C*), although under these conditions WT also does not reach the maximum level. Even an enhanced illumination of 4 s could not help the mutant strain to reach the maximum fluorescence yield (Fig. 6*E*), leaving the PQ pool partly oxidized.

This suggests that another component besides the cytochrome b_6f complex is capable of oxidizing the PQ pool. Fig. 6*F* shows that a combination of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone and pentachlorophenol, an inhibitor of quinol oxidases, yields similar kinetics of variable fluorescence in the mutant and the wild type, strongly indicating an increased activity of a quinol oxidase in the mutant that is capable of keeping the PQ pool in a more oxidized condition.

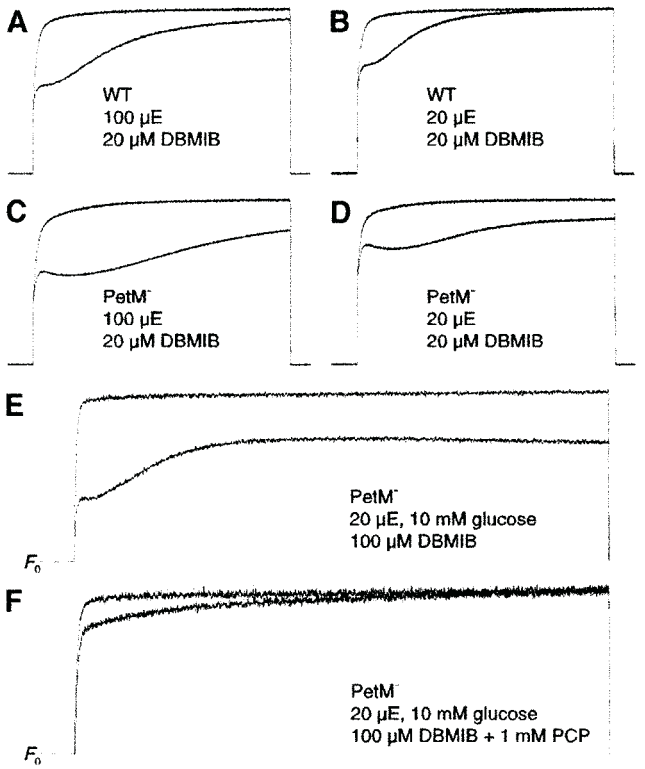

FIG. 6. **Fluorescence induction curves from wild-type (*WT*) (*A* and *B*) and *petM*$^-$ cells (*C* and *D*) grown under HL (*A* and *C*) and LL (*B* and *D*) conditions after blocking the cytochrome b_6f complex (*A–E*) and the cytochrome *bd* oxidase (*F*).** Illumination for 1 s (*A–D*) or 4 s (*E* and *F*). *E* and *F* have an enlarged ordinate scale, starting at the F_O level, to show the variation of F_V more clearly. The *upper curve* in each case was always recorded in the presence of 10 µM 3-(3,4-dichlorophenyl)-1,1-dimethylurea. *DBMIB*, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; *PCP*, pentachlorophenol.

DISCUSSION

The Role of PetM in the Cytochrome b_6f Complex—Previous inactivation experiments of PS II (32), PS I (33, 34), the NADH dehydrogenase (35), and the terminal oxidases (24) resulted in fully segregated mutants of *Synechocystis* PCC 6803 and indicate that these complexes are not essential for the survival of the cells. In contrast, inactivation of genes encoding the main cytochrome b_6f subunits have failed so far to produce a viable organism (36); this is due to the absence of a cytochrome bc_1 complex in *Synechocystis* PCC 6803 and the essential function

of the cytochrome b_6f complex in both respiratory and photosynthetic electron transport. For this reason, inactivation or site-directed mutagenesis leading to inactivation of this complex is not possible, in contrast to other organisms such as the green alga *C. reinhardtii*, where the b_6f complex is nonessential (37).

In *C. reinhardtii* deletion of the gene encoding the small cytochrome b_6f subunit PetG (38), as well as the inactivation of the *petL* gene (16), resulted in no, or impaired, photoautotrophic growth; in addition, the amount of other cytochrome b_6f subunits is strongly reduced. Additionally, a new small subunit of the cytochrome b_6f complex, PetN, was recently identified, the inactivation of which also led to a photosynthetically incompetent phenotype (19).

In contrast, *petM* can be fully deleted in *Synechocystis*, resulting in only minor effects on the cytochrome b_6f complex. This is the first time that a complete deletion of a cytochrome b_6f subunit in *Synechocystis* has been described. Inactivation of *petM* in *Synechocystis* did not affect the amount of assembled b_6f complex in the membranes as quantified from the extent of the cytochrome *f* kinetic transients. Under all tested conditions growth is normal (data not shown, but see Ref. 39), and there is also not a very obvious phenotype under various light intensities. These results clearly show that the PetM subunit has no essential role in b_6f electron transfer function in the cyanobacterium *Synechocystis* PCC 6803; however, the absence of this subunit apparently affects the levels of other protein complexes involved in energy transduction.

Effects on Other Protein Complexes—Our results clearly show an impact of PetM on some protein complexes of *Synechocystis* that are involved in the electron transport chain. Whereas the amount of the cytochrome b_6f complex is similar in mutant and wild-type strains, both PS I and phycobilisome content are reduced in the mutant strain. Furthermore, the re-reduction kinetics of cytochrome *f* are different under aerobic conditions; this could result from an altered cytochrome b_6f complex activity or a more oxidized redox state of the PQ pool. Because the activity of the complex was the same in the mutant and WT under anaerobic conditions, the different aerobic re-reduction behavior probably arises from an increased oxidation state of the PQ pool in the aerobic state. Furthermore, the fluorescence induction measurements indicate that electrons can leave the PQ pool of the $petM^-$ mutant even when the cytochrome b_6f complex is inhibited.

A strong candidate for this alternative route of plastoquinol oxidation under aerobic conditions is the cytochrome *bd* oxidase, especially because pentachlorophenol, a specific inhibitor of the cytochrome *bd* oxidase in *Synechocystis* (25), is able to restore the mutant fluorescence induction kinetics to resemble that of WT. The comparison with wild-type cells suggests an increased activity of this oxidase in the thylakoid membrane of the $petM^-$ mutant. Whereas the spectroscopic data indicate that the subunit PetM is not required for the stability, assembly, or redox activity of the cytochrome b_6f complex, changes in the activity of other protein complexes in the membrane imply a specific regulatory role of the cytochrome b_6f complex in general and the PetM subunit in particular.

Model for a Regulatory Role of the Cytochrome b_6f Complex—A regulatory function of the redox state of the PQ pool has been postulated for a long time, and analysis of this phenomenon is an active field of research. In some cases the redox state of the PQ pool itself seems to be the signal for further regulatory steps (40), whereas in other cases the cytochrome b_6f complex seems to be directly involved in signaling.

It has been shown that the cytochrome b_6f complex can regulate the PS I/PS II stoichiometry in cyanobacteria (41). Changing light conditions or cytochrome b_6f complex inhibitor

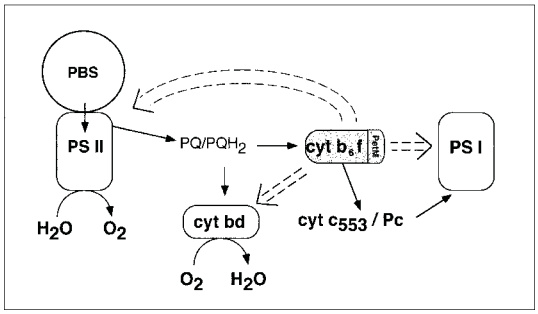

FIG. 7. **Model for a regulatory function of the cytochrome b_6f complex.** *Small arrows* indicate electron pathways, and *large arrows* indicate possible regulatory effects of the cytochrome b_6f complex on various protein complexes. *PBS*, phycobilisome; *PQH_2*, plastoquinol; *cyt*, cytochrome; *Pc*, plastocyanine.

treatment affects the amount of PS I, whereas the content of PS II and the cytochrome b_6f complex remains constant. These observations are in good agreement with our results and support the conclusion that the PS I content has changed in the $petM^-$ mutant without, however, having any effect on its re-reduction kinetics.

In addition, the data presented in this work indicate an increased activity of a quinol oxidase. This could be due either to an activation of this complex or to accumulation of more quinol oxidase in the membrane. In the case of PS I it seems already clear that the cytochrome b_6f complex controls the assembly process of this complex (42).

A working model based on the data presented here is shown in Fig. 7. The cytochrome b_6f complex seems to control the activity or assembly of the phycobilisomes, of PS I, and of the cytochrome *bd* oxidase. The relative stoichiometries of these complexes, as gained from the presented data, indicate that the regulatory function of the cytochrome b_6f complex is impaired; *i.e.* the inactivation of *petM* yields a cytochrome b_6f complex with altered regulatory properties. Because the redox activity of the complex seems unaffected, PetM itself must have a role in the accumulation of other protein complexes.

PetM is extremely hydrophobic and of relatively small size. Both facts argue against a role of PetM in the expression or post-translational modification of other cytochrome b_6f subunits. Hydropathy analysis of PetM suggests an intrinsic protein with one transmembrane helix.

A new small intrinsic subunit (PetO) of the cytochrome b_6f complex of *C. reinhardtii* with a possible regulatory function has recently been identified (28). Because there exists no homologous protein in *Synechocystis* and because the function of PetM is completely unknown, it seems possible that this small subunit is involved in a signal transduction process involving the cytochrome b_6f complex in this cyanobacterium. The inactivation of the *petM* gene may effect a signal transduction cascade with concomitant influence on protein stoichiometries in the thylakoid membrane of *Synechocystis*.

In summary, the presented data indicate that the cytochrome b_6f subunit PetM is not essential for the redox activity of this complex. Although the mutant strain shows no apparent phenotype, PetM seems to be important for regulatory circuits including a quinol oxidase in which the cytochrome b_6f complex may be involved.

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