THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 8, Issue of February 20, pp. 7229–7233, 2004
Printed in U.S.A.

The Efficient Functioning of Photosynthesis and Respiration in *Synechocystis* sp. PCC 6803 Strictly Requires the Presence of either Cytochrome c_6 or Plastocyanin*

Received for publication, October 22, 2003, and in revised form, November 21, 2003
Published, JBC Papers in Press, December 5, 2003, DOI 10.1074/jbc.M311565200

Raúl V. Durán, Manuel Hervás, Miguel A. De la Rosa‡, and José A. Navarro

From the Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y Consejo Superior de Investigaciones Científicas, Américo Vespucio s/n, 41092 Sevilla, Spain

In cyanobacteria, cytochrome c_6 and plastocyanin are able to replace each other as redox carriers in the photosynthetic and respiratory electron transport chains with the synthesis of one or another protein being regulated by the copper concentration in the culture medium. However, the presence of a third unidentified electron carrier has been suggested. To address this point, we have constructed two deletion mutants of the cyanobacterium *Synechocystis* sp. PCC 6803, each variant lacking either the *petE* or *petJ* gene, which respectively codes for the copper or heme protein. The photoautotrophic and heterotrophic growth rate of the two mutants in copper-free and copper-supplemented medium as well as their photosystem I reduction kinetics *in vivo* were compared with those of wild-type cells. The two mutant strains grow at equivalent rates and show similar *in vivo* photosystem I reduction kinetics as wild-type cells when cultured in media that allow the expression of just one of the two electron donor proteins, but their ability to grow and reduce photosystem I is much lower when neither cytochrome c_6 nor plastocyanin is expressed. These findings indicate that the normal functioning of the cyanobacterial photosynthetic and respiratory chains obligatorily depends on the presence of either cytochrome c_6 or plastocyanin.

In eukaryotic organisms, the components of the photosynthetic and respiratory electron transfer chains are well identified and located in chloroplasts and mitochondria, respectively, but in cyanobacteria, the two redox pathways occur in the same membrane and share a number of components such as the plastoquinone pool and cytochrome b_6f (1, 2). Because respiratory cytochrome *c* is lacking in cyanobacteria, it has been proposed that these organisms use the same *c*-type cytochrome, in particular cytochrome c_6 (Cyt),[1] to serve both in photosynthesis and respiration (3).

Cyt is a well known electron carrier between cytochrome b_6f and photosystem I (PSI) in cyanobacteria and some algae, but it can be replaced by plastocyanin (Pc) when the cells are grown in the presence of copper (*cf.* Ref. 4 for a recent review). In those species that are able to synthesize either Cyt or Pc, the expression level of the corresponding genes is controlled by the copper concentration in the growth medium (5, 6).

Despite their intrinsic different conformations, Cyt and Pc share a number of similar physicochemical and structural features that allow them to interact with the same redox partners with equivalent kinetic efficiency and replace each other inside the cells (7, 8). The two metalloproteins are thus presented as an excellent case study of biological evolution, which is not only convergent (two structures playing the same role) but also parallel (two proteins varying in a concerted way from one organism to another) (9). Such a interchangeability between Cyt and Pc in photosynthesis has also been proposed for the respiratory chain in which the two metalloproteins could act as alternative donors of electrons to cytochrome *c* oxidase (1, 10).

The observations by Zhang *et al.* (11) with Cyt- or Pc-deficient mutant strains of the cyanobacterium *Synechocystis* sp. PCC 6803 led these authors to conclude that electrons can be transferred from the cytochrome b_6f complex to PSI in the absence of both Cyt and Pc and that Cyt is not obligatorily required for respiratory electron transport. Metzger *et al.* (12) indeed proposed the presence of a third electron carrier as the mutant strains of *Synechocystis* were able to grow photoautotrophically and sustain normal rates of oxygen evolution and dark respiration in the absence of both Cyt and Pc. These authors also measured the kinetics of *in vivo* PSI reduction and found that the electron transfer rates only occurred 4–6 times slower in the mutants than in the wild-type (WT) cells.

The challenge is thus to understand how photosynthetic and respiratory electron transport chains operate in cyanobacterial cells in the absence of both Cyt and Pc. To answer this question, we herein report the construction of mutant strains of *Synechocystis* cells that lack either Cyt or Pc to further analyze their photoautotrophic and heterotrophic growth along with their *in vivo* PSI reduction kinetics. In contrast to previous proposals, our findings allow us to conclude that the normal functioning of the cyanobacterial photosynthetic and respiratory chains obligatorily depends on the presence of one of these proteins, thus discarding the existence of any third efficient electron carrier.

EXPERIMENTAL PROCEDURES

Strains and Culture Conditions—Synechocystis sp. PCC 6803 was grown photoautotrophically in liquid or solid mineral BG-11 medium (13) either in the presence or absence of copper under continuous white fluorescent illumination (50 microeinsteins m^{-2} s^{-1}) at 30 °C. Liquid cultures were bubbled with air supplemented with 1% (v/v) CO_2. When necessary, copper was added at 1 μM, whereas copper-depleted medium (BG-11-C) was supplemented with 300 μM bathocuproinedisulfonic acid as a chelating agent to eliminate any traces of copper (14). Cell cultures with or without copper were pre-adapted to the new conditions by

* This work was supported by the Spanish Ministry of Science and Technology (Grant BMC2003-458), European Union (Network HPRN-CT1999-00095), and Andalusian Government (CVI-0198). 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.

‡ To whom correspondence should be addressed. Tel.: 34-954-489-506; Fax: 34-954-460-065; E-mail: marosa@us.es.

[1] The abbreviations used are: Cyt, cytochrome c_6; k_F and k_S, observed rate constants for the fast and slower phases, respectively, of biphasic kinetics; k_M, observed rate constant for monophasic kinetics; Pc, plastocyanin; PSI, photosystem I; PSII, photosystem II; WT, wild-type; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

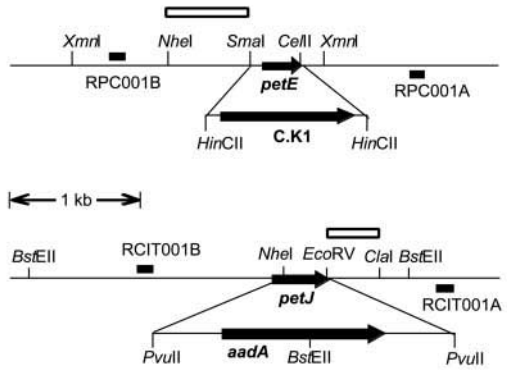

FIG. 1. **Strategy designed to delete the *petE* (*upper*) and *petJ* (*lower*) genes in *Synechocystis* cells.** *Solid arrows* denote open reading frames; *open segments* stand for probes used in Southern blot analysis; and *closed segments* correspond to primers for PCR cloning.

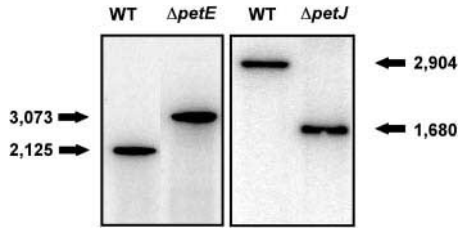

FIG. 2. **Southern hybridization of ΔpetE and ΔpetJ deletional mutants compared with the WT species.** Genomic DNAs were digested with appropriate restriction enzymes and hybridized with either the NheI-SmaI fragment of the [^{32}P]dCTP-labeled pPlaF plasmid (*left*) or the ClaI-EcoRV fragment of the [^{32}P]dCTP-labeled pCytF plasmid (*right*). The band length is expressed in number of bp. See "Experimental Procedures" for details.

repeated incubation cycles, and cell growth was monitored by spectrophotometrically measuring the chlorophyll content (15). Total protein concentration was determined as described elsewhere (16).

Light-activated heterotrophic growth experiments were carried out by adding 10 mM glucose to the standard BG-11 or BG-11-C solid medium. Cell cultures were incubated in darkness with 5-min pulses of white light (50 microeinsteins m^{-2} s^{-1}) every 24 h (17). Other conditions were as for the photoautotrophic growth.

Escherichia coli DH5α was grown in Luria-Bertani liquid or solid medium (18) supplemented, when required, with kanamycin (50 μg ml^{-1}), spectinomycin (100 μg ml^{-1}), or ampicillin (100 μg ml^{-1}).

Cloning and Deletional Inactivation of petE and petJ Genes—To amplify the *petE* gene (coding for Pc) and its flanking regions by PCR, the following two oligonucleotides were designed from the genomic sequence of *Synechocystis* (19) (NCBI accession number NC_000911): RPC001A (attggcacggcccatcttcccta) as the direct primer and RPC001B (atcgcccctccgttggcggtgtct) as the reverse primer. Genomic DNA from *Synechocystis* was used as a template (Fig. 1) (20). A 2.6-kb band resulting from PCR was purified by electrophoresis in 1% agarose gel and cloned into the pGEMT commercial vector (Promega) to obtain the pPlaF plasmid.

The SmaI-CelII fragment from pPlaF was replaced by a C.K1 kanamycin resistance cassette from pRL161 plasmid (21). A 5.2-kb band was obtained after pPlaF digestion with SmaI and CelII, and its ends were blunted with Klenow polymerase and ligated with a 1.3-kb fragment obtained after pRL161 digestion with HinCII. The resulting pPlaFΔCK1 plasmid was tested by restriction analysis. *Synechocystis* WT cells were transformed with this plasmid by standard procedures (22), and the transformants were selected and segregated in solid medium with increasing amounts of kanamycin from 50 to 500 μg ml^{-1}. The correct integration and segregation of the deleted copies was tested by Southern blot as follows. Genomic DNA of isolated mutants were digested with XmnI and separated in 0.7% agarose electrophoretic gels. DNA was then transferred to Z-probe nylon membranes (Bio-Rad). As a probe, the NheI-SmaI fragment from pPlaF was radioactively labeled with ^{32}P using the commercial kit Ready-to-Go (Amersham Biosciences). In a XmnI digestion of the genomic DNA, this probe hybridized with a 2,125-bp band in the case of WT and with a 3,073-bp band in the case of deletion mutant.

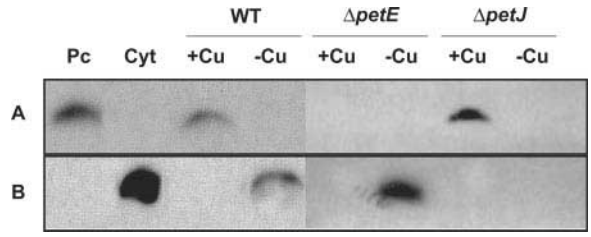

FIG. 3. **Immunodetection of Pc and Cyt.** WT, ΔpetE, and ΔpetJ *Synechocystis* cells were grown in the presence or absence of copper as indicated. 100 μg of total protein from cells were loaded into each gel lane with 0.1 μg of purified Cyt and Pc as controls. Polyclonal antibodies against Pc (*A*) and Cyt (*B*) were used.

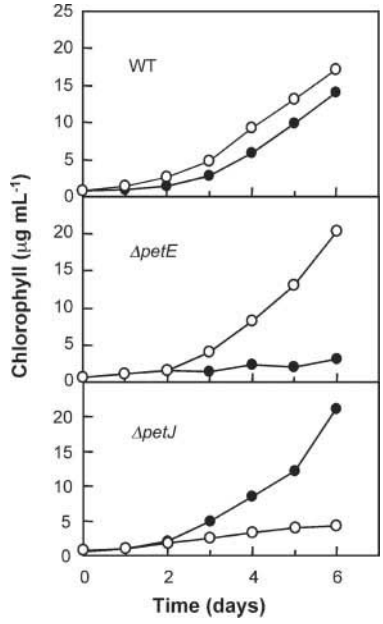

FIG. 4. **Photoautotrophic growth of WT, ΔpetE, and ΔpetJ *Synechocystis* strains.** In each case, the cells were cultured either in the presence (Δ*petJ*) or absence (Δ*petE*) of copper. See "Experimental Procedures" for further details.

Similarly, the RCYT001A (atggtgtggcggaaggtcgcaataa) and RCYT-001B (tagccaatagctctggccctgccgat) oligonucleotides were designed as direct and reverse primers, respectively, for PCR amplification of the open reading frame corresponding to the *petJ* gene (coding for Cyt) and its flanking regions (Fig. 1). A 2.4-kb band was purified and cloned into pGEMT to generate the pCytF plasmid.

The fragment NheI-EcoRV from pCytF was replaced by the Ω fragment from the pRL-SPE plasmid, which contains the *aadA* gene that confers spectinomycin resistance as follows. pCytF was digested with NheI and EcoRV, and the resulting 5.0-kb band was isolated by 1% agarose gel electrophoresis. The ends of this band were blunted with Klenow polymerase. The PvuII fragment from pRL-SPE corresponding to the Ω cassette was ligated to the 5.0-kb NheI-EcoRV fragment from pCytF, and the resulting pCytFΔSPE plasmid was tested by restriction analysis. The transformants were segregated and tested as for *petE* inactivation but using spectinomycin and streptomycin as selective antibiotics at concentrations ranging from 2.5 to 20 μg ml^{-1}. For the Southern blot analysis, genomic DNA was digested with BstEII using the ClaI-EcoRV fragment from pCytF as probe. In a BstEII digestion of the genomic DNA, this probe hybridized with a 2,904-bp band in the case of WT and with a 1,680-bp band in the case of deletion mutant.

SDS-PAGE and Immunoblotting—Proteins from *Synechocystis* WT cells and from the mutant strains were isolated and separated by 15% polyacrylamide gel electrophoresis using standard protocols (23). 100 μg of total protein were charged in each case. After separation, proteins were transferred to polyvinylidene difluoride Immobilon-P^{SQ} membranes (Millipore) and immunodetected with polyclonal antibodies raised against *Synechocystis* Pc or Cyt. Antibodies were obtained by

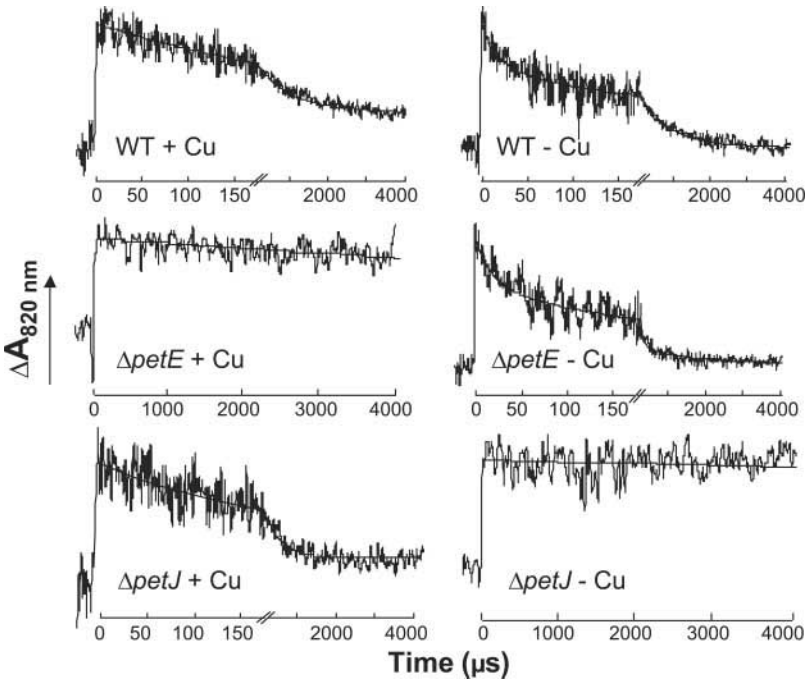

FIG. 5. **Kinetic traces showing *in vivo* PSI reduction in WT, ΔpetE, and ΔpetJ *Synechocystis* cells.** The cells were previously cultured in the presence or absence of copper as indicated. The reaction cell contained an amount of *Synechocystis* cells equivalent to total chlorophyll content of 150–300 μg ml^{-1}. Absorbance changes were recorded at 820 nm. The *continuous lines* correspond to theoretical fittings to monoexponential or biexponential kinetics. Other conditions were as described under "Experimental Procedures."

TABLE I
Observed rate constants for in vivo PSI reduction of WT, ΔpetE, and ΔpetJ Synechocystis cells

ND, not detected.

	+Cu	−Cu	
	$k_M \times 10^{-3}$	$k_F \times 10^{-4}$	$k_S \times 10^{-3}$
		s^{-1}	
WT	2.7 ± 0.2	4.5 ± 0.6	2.0 ± 0.2
Δ*petE*	<0.01	4.0 ± 0.3	3.1 ± 0.2
Δ*petJ*	3.6 ± 0.2	ND	<0.01

inoculating purified proteins (24, 25) into rabbits followed by extraction of serum blood at the Centro de Producción y Experimentación Animal (Seville, Spain). Binding of antibodies was visualized with peroxidase-conjugated antibodies and the commercial ECL-Plus kit (Amersham Biosciences).

Kinetics Analysis of in Vivo PSI Reduction—WT and mutant cells of *Synechocystis* were harvested by centrifugation at different growing phases and suspended in 20 mM Tricine-KOH buffer, pH 7.0, supplemented with 10% (w/v) Ficoll to avoid cell aggregation. The *in vivo* kinetics for electron transfer from either Pc (in copper-supplemented cultures) or Cyt (in copper-depleted cultures) to PSI were followed by laser flash absorption spectroscopy as previously described (26). The reaction cell (optical path length, 1 mm) contained whole *Synechocystis* cells at 150–300 μg ml^{-1} chlorophyll concentration and 1 mM ascorbate. The kinetics were recorded after excitation by laser flash both in the absence and presence of 20 μM phenazine methasulfate, which is a well known efficient donor of electrons to PSI. Interferences arising from photosystem II (PSII) were avoided by pre-illumination of the samples with white light in the presence of 10 μM DCMU (3-(3′,4′-dichlorophenyl)-1,1-dimethylurea) and 10 mM hydroxylamine (27).

RESULTS AND DISCUSSION

Deletion of the petE and petJ Genes in Synechocystis Cells—The cyanobacterial cells were transformed with the pPlaFΔCK1 or pCytFΔSPE plasmids, and the resulting ΔpetE and ΔpetJ strains were selected as transformants corresponding to *petE* and *petJ* null mutants, respectively. ΔpetE was selected in BG-11-C medium with 300 μM bathocuproinedisulfonic acid to ensure the complete removal of copper and kanamycin (500 μg ml^{-1}). ΔpetJ was selected in BG-11 medium supplemented with 1 μM copper in the presence of both spectinomycin and streptomycin (20 μg ml^{-1} each). The correct integration and segregation of both mutants were checked by Southern blot (Fig. 2) with the length of resulting bands and the absence of other bands being as expected for completely segregated mutants (see "Experimental Procedures"). Several attempts to segregate the double mutant were unsuccessful.

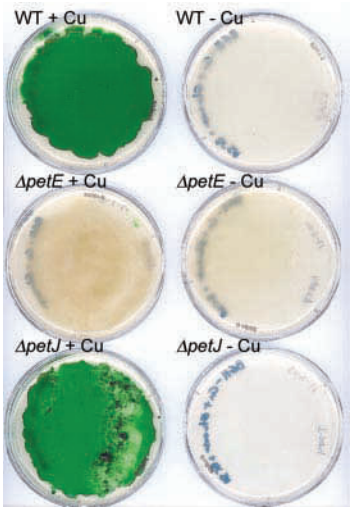

FIG. 6. **Light-activated heterotrophic growth of WT, ΔpetE, and ΔpetJ *Synechocystis* cells in copper-containing or copper-free culture medium.**

In agreement with the copper-dependent regulation of Pc and Cyt expression levels (see Introduction), WT *Synechocystis* cells synthesize either Pc or Cyt when cultured in the presence or absence of copper, respectively (Fig. 3). Accordingly, the ΔpetE strain produces Cyt when growing in copper-depleted medium but is completely unable to synthesize even traces of Cyt and/or Pc when the medium is supplemented with this metal. In a similar way, the ΔpetJ strain forms Pc in copper-supplemented medium but no detectable levels of Cyt and/or Pc were yielded in cells cultured in the absence of copper (Fig. 3).

Photoautotrophic Growth of WT and Mutant Strains—The ability of the ΔpetE and ΔpetJ mutants to grow under photoautotrophic conditions was determined in cultures with and without copper. As previous studies in the absence of a copper-chelating agent had led to controversial results (28) and we had

observed by Western blot analysis that residual copper levels (nanomolar range) in the culture medium were enough to induce Pc expression (data not shown), the copper-chelating agent bathocuproinedisulfonic acid was added to the cell cultures to completely remove metal traces and abolish residual Pc expression.

Growth curves show not only that the WT strain similarly grows either with or without added copper but also that the two deletion mutants grow at rates equivalent to that of WT when cultured in media that allow them to express one of the two electron donor proteins (Fig. 4). In fact, ΔpetE is only able to grow at a standard rate in the absence of copper, *i.e.* when Cyt is produced, whereas ΔpetJ grows normally when Pc is synthesized because of copper induction. When the ΔpetE and ΔpetJ strains are under conditions at which the expression levels of both Pc and Cyt are not detectable, their respective growth rate is much lower than that of WT cells.

In contrast to such a drastic decrease in the growth rates of the two mutant strains, Zhang *et al.* (11) reported a slightly diminished growth rate and a normal steady-state photosynthetic electron transport for their Pc- and Cyt-deficient mutants under similar photoautotrophic conditions. In view of these discrepancies, the kinetics for PSI reduction in whole cells of ΔpetE and ΔpetJ were analyzed by laser flash absorption spectroscopy.

In Vivo Reduction of PSI in WT, ΔpetE, and ΔpetJ Cells—In vitro PSI reduction by Cyt and Pc has been extensively investigated in a wide variety of photosynthetic organisms such as cyanobacteria (including *Synechocystis*), green algae, and plants (7, 25, 29). However, little is known on the kinetic behavior of the two metalloproteins serving as electron donors to PSI *in vivo* (30). In this work, the kinetics of *in vivo* PSI reduction by Cyt or Pc in *Synechocystis* were analyzed in whole cells by following the absorbance changes at 820 nm after flash-induced P_{700} photoxidation. From the resulting kinetics, the values for the observed rate constants of PSI reduction by either Pc or Cyt were estimated.

In WT *Synechocystis* cells growing in culture media supplemented with copper, PSI reduction was only ascribed to Pc because Cyt synthesis was repressed and the kinetics were well fitted to monoexponential curves (Fig. 5). The value for the observed rate constant of such a monophasic kinetic (k_M) was ~2,700 s^{-1} ($t_{½}$, 250 μs) (Table I). In WT *Synechocystis* cells growing in copper-depleted cultures in which Pc synthesis was repressed, Cyt-dependent PSI reduction followed a biphasic kinetic (Fig. 5): the first, fast phase yielded a rate constant (k_F) of ~45,000 s^{-1} ($t_{½}$, 16 μs), and the second, slower phase exhibited a rate constant (k_S) of ~2,000 s^{-1} ($t_{½}$, 350 μs) (Table I). The fast and slow phases accounted for 37 and 63%, respectively, of the total signal amplitude. These values for k_M, k_F, and k_S remained constant at the different culture stages but significantly increase when phenazine methasulfate, a well known artificial electron donor to PSI, was added to the reaction cell (not shown).

In vivo PSI reduction in both ΔpetE and ΔpetJ cells growing under conditions that allow the expression of one of the two electron donor proteins follows kinetics similar to those observed in the WT strain (Fig. 5). In fact, the kinetics with the ΔpetE mutant in the absence of copper and with the ΔpetJ mutant in the presence of copper were, respectively, biphasic and monophasic and their observed rate constants were equivalent to those attained with the WT cells (Table I), but no PSI reduction was detected in mutant cells in which neither Pc nor Cyt was expressed (Fig. 5).

These data not only explain our experimental observations on the photoautotrophic growth of WT and mutant *Synechocystis* strains (see above) but also suggest that either Cyt or Pc is obligatorily required for the efficient reduction of PSI. In contrast, Metzger *et al.* (12) observed *in vivo* PSI reduction even in the absence of Pc or Cyt, a finding that can be ascribed to one (or both) of the following facts: (i) optical interferences derived from PSII excitation, or (ii) Pc expression because of residual copper levels.

To avoid these difficulties and assure that the observed kinetic signals can only be assigned to changes in the oxidation state of PSI, we have used a 694-nm laser flash to specifically favor the excitation of PSI over PSII, have added DCMU-hydroxylamine to the reaction cell to block electron transfer from PSII, have confirmed that the kinetic signals are ascribed to PSI by adding phenazine methasulfate, and have completely removed copper from copper-free culture media by adding an efficient chelating agent.

Light-activated Heterotrophic Growth of WT and Mutant Cells—Taking into account that the cyanobacterial respiratory and photosynthetic electron transfer chains share a number of redox components like Cyt and Pc (1, 10), the ability of the WT, ΔpetE, and ΔpetJ strains to grow in glucose-supplemented culture media was investigated. Under such heterotrophic conditions, glucose was used as an organic carbon source (17) and the electrons were transported from the sugar molecule to dioxygen throughout the respiratory pathway.

Fig. 6 shows that none of the cell strains (including the WT) can grow in copper-free medium. This is as expected from the specific requirement for copper of cytochrome *c* oxidase to be correctly assembled and able to function (10). However, in copper-containing media, the WT and ΔpetJ strains synthesized Pc and showed a normal growth level but the ΔpetE strain produced neither Cyt nor Pc and was thus unable to grow because of the absence of a donor of electrons to cytochrome *c* oxidase.

Taken together, all of these data indicate that there is no any other alternative redox mediator as efficient as Cyt and Pc in the photosynthetic and respiratory electron transport chains and therefore *Synechocystis* cells strictly depend on the synthesis of either Cyt or Pc to grow not only photoautotrophically but also heterotrophically.

Acknowledgments—The pRL-SPE plasmid was kindly provided by Luis López-Maury (Instituto de Bioquímica Vegetal y Fotosíntesis, Seville, Spain). We thank Dr. Maria Feio for critically reading the paper.

REFERENCES

1. Schmetterer, G. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. G., ed) pp 409–435, Kluwer Academic Publishers, Norwell, MA
2. Peschek, G. A. (1999) in *The Photosynthetic Prokaryotes* (Peschek, G. A., Löffelhardt, W., and Schmetterer, G., eds) pp 201–209, Kluwer Academic Publishers, Norwell, MA
3. Binder, A., Hauser, R., and Krogmann, D. (1984) *Biochim. Biophys. Acta* **765,** 241–246
4. Hervás, M., Navarro, J. A., and De la Rosa, M. A. (2003) *Accounts Chem. Res.* **36,** 798–805
5. Zhang, L., McSpadden, B., Pakrasi, H. B., and Whitmarsh, J. (1992) *J. Biol. Chem.* **267,** 19054–19059
6. Malakhov, M. P., Malakhova, O. A., and Murata, N. (1999) *FEBS Lett.* **444,** 281–284
7. Hervás, M., Navarro, J. A., Díaz, A., Bottín, H., and De la Rosa, M. A. (1995) *Biochemistry* **34,** 11321–11326
8. Molina-Heredia, F. P., Hervás, M., Navarro, J. A., and De la Rosa, M. A. (2001) *J. Biol. Chem.* **276,** 601–605
9. De la Rosa, M. A., Navarro, J. A., Díaz-Quintana, A., De la Cerda, B., Molina-Heredia, F. P., Balme, A., Murdoch, P. S., Díaz-Moreno, I., Durán, R. V., and Hervás, M. (2002) *Bioelectrochemistry* **55,** 41–45
10. Pils, D., Gregor, W., and Schmetterer, G. (1997) *FEMS Microbiol. Lett.* **152,** 83–88
11. Zhang, L., Pakrasi, H. B., and Whitmarsh, J. (1994) *J. Biol. Chem.* **269,** 5036–5042
12. Metzger, S. U., Pakrasi, H. B., and Whitmarsh, J. (1995) in *Photosynthesis: From Light to Biosphere* (Mathis, P., ed) pp 823–826, Kluwer Academic Publishers, Norwell, MA
13. Rippka, R., Deruelles, J., Waterbury, J. B., Herman, M., and Stanier R. Y. (1979) *J. Gen. Microbiol.* **111,** 1–61

14. Tottey, S., Rich, P. R., Rondet, S. A. M., and Robinson, N. J. (2001) *J. Biol. Chem.* **276,** 19999–20004
15. Arnon, D. I. (1949) *Plant Physiol.* **24,** 1–15
16. Markwell, M. A. K., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) *Anal. Biochem.* **87,** 206–210
17. Anderson, S. L., and McIntosh, L. (1991) *J. Bacteriol.* **173,** 2761–2767
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirosawa, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., and Tabata, S. (1996) *DNA Res.* **3,** 109–136
20. Cai, Y., and Wolk, C. P. (1990) *J. Bacteriol.* **172,** 3138–3145
21. Elhai, J., and Wolk, C. P. (1988) *Gene* (*Amst.*) **68,** 119–138
22. Williams, J. G. K. (1988) *Methods Enzymol.* **167,** 766–778
23. Schägger, H., and von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379
24. Hervás, M., Navarro, F., Navarro, J. A., Chávez, S., Díaz, A., Florencio, F. J., and De la Rosa, M. A. (1993) *FEBS Lett.* **319,** 257–260
25. De la Cerda, B., Díaz-Quintana, A., Navarro, J. A., Hervás, M., and De la Rosa, M. A. (1999) *J. Biol. Chem.* **274,** 13292–13297
26. Hervás, M., Myshkin, E., Vintonenko, N., De la Rosa, M. A., Bullerjahn, G. S., and Navarro, J. A. (2003) *J. Biol. Chem.* **278,** 8179–8183
27. Soriano, G. M., Ponomarev, M. V., Tae, G. S., and Cramer, W. A. (1996) *Biochemistry* **35,** 14590–14598
28. Ardelean, I., Matthijs, H. C. P., Havaux, M., Joset, F., and Jeanjean, R. (2002) *FEMS Microbiol. Lett.* **213,** 113–119
29. Hervás, M., Ortega, J. M., Navarro, J. A., Díaz, A., De la Rosa, M. A., and Bottin, H. (1994) *Biochim. Biophys. Acta* **1184,** 235–241
30. Baymann, F., Rappaport, F., Joliot, P., and Kallas, T. (2001) *Biochemistry* **40,** 10570–10577