J. Plant Physiol. Vol. 144. pp. 259–264 (1994)

A New Type of Cytochrome *c* from *Synechocystis* PCC6803*

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Received January 24, 1994 · Accepted March 8, 1994

Summary

A gene encoding a *c*-type cytochrome, designated *cytM*, was discovered 3.4 kbp downstream from the *desA* gene in the genome of *Synechocystis* PCC6803. The amino acid sequence derived from the nucleotide sequence exhibits about 35% similarity to the amino acid sequences of cytochromes *c*-553 from other cyanobacteria and eukaryotic algae, but it also includes regions typical of soluble *c*-type cytochromes from eukaryotic mitochondria. The molecular mass of the putative mature protein was estimated to be 8.3 kDa, and the isoelectric point was calculated to be 7.3. The amino-terminal region of the putative product of the *cytM* gene is hydrophobic, suggesting that this domain may be a transit peptide or a membrane anchor. Both photosynthesis and respiration in a mutant with a disrupted *cytM* gene were as efficient as they were in the wild-type strain.

Key words: Electron carrier; Cytochrome c; Cyanobacterium.

Introduction

Cyanobacteria and algal chloroplasts contain *c*-type cytochromes, such as cytochromes *c*-553 and *c*-550 (Pettigrew and Moore, 1987; Krogmann, 1991). Cytochrome *c*-553 is a monoheme monomer of a polypeptide with a molecular mass of about 10 kDa, and it has a high redox potential of about 350 mV. Biosynthesis of cytochrome *c*-553 is induced in cells grown under conditions of copper deprivation, and this cytochrome can substitute for plastocyanin in the donation of electrons to photosystem I (Sandmann, 1986; Merchant and Bogorad, 1986). Cytochromes *c*-553 in algal chloroplasts are acidic (Kamimura et al., 1977; Krogman, 1991) and those in filamentous cyanobacteria are basic (Lockau, 1981; Alpes et al., 1984). Unicellular cyanobacteria contain either an acidic or a basic cytochrome *c*-553 (Bovy et al., 1992). The amino acid sequences of these cytochromes *c*-553 are at least 32% identical. It is likely that the variations in net charge among the cytochromes originate from a small region, namely, residues 62–69, in the amino acid sequence of each mature protein (Ho and Krogmann, 1984).

In the filamentous cyanobacteria *Anabaena variabilis* (Lockau, 1981) and *Nostoc muscorum* (Sturzl et al., 1982; Alpes et al., 1984), a basic cytochrome *c*-553 donates electrons to both respiratory and photosynthetic electron-transport systems. In the unicellular cyanobacterium *Anacystis nidulans*, an acidic cytochrome *c*-553 of 8.9 kDa (Laudenbach et al., 1990) is active only in the photosynthetic electron-transport system (Kienzl and Peschek, 1982), and a basic cytochrome *c*-552 with a molecular mass of 9.0 kDa (Holton and Myers, 1967 a, b; Omata and Murata, 1984) probably participates in respiratory electron transport (Pettigrew and Moore, 1987).

Another soluble *c*-type cytochrome, cytochrome *c*-550, has been found in cyanobacteria (Holton and Myers, 1967 a, b; Alam et al., 1984) and in red and green algae (Evans

* This paper is dedicated to Prof. Dr. Hartmut K. Lichtenthaler, University of Karlsruhe, on the occasion of his 60th birthday.

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and Krogmann, 1983; Kamimura et al., 1977). This cytochrome is acidic (pI 4.1 to 4.8) and is a monoheme monomer with a redox potential of −260 mV. Shen et al. (1992, 1993) found that the low-potential cytochrome *c*-550 is associated with the core complex of photosystem II in the thermophilic cyanobacterium *Synechococcus vulcanus*. Our recent study (Nishiyama, Y. et al., 1994) indicates that the low-potential cytochrome *c*-550 stabilizes the oxygen-evolving photosystem II complex against heat inactivation in *Synechococcus* PCC7002. Hoganson et al. (1990) reported two types of low-potential cytochrome *c*-550 from *Anacystis nidulans*. Both cytochromes have a molecular mass of 15 kDa, but they differ in their electron paramagnetic resonance spectra and in their ability to bind to an anion exchanger.

Here we report the nucleotide sequence and the deduced amino acid sequence of a new type of cytochrome *c* from the unicellular cyanobacterium *Synechocystis* PCC6803. The gene for this cytochrome, designated *cytM*, was found 3.4 kb downstream from the *desA* gene for the Δ12 desaturase (Wada et al., 1990, 1993).

Materials and Methods

Strains and culture conditions

Anabaena variabilis M-3 was obtained from the algal collection of the University of Tokyo (Tokyo, Japan), and *Synechococcus* PCC7002, *Synechocystis* PCC6714 and *Synechocystis* PCC6803 from the Pasteur Culture Collection (Paris, France). *Anacystis nidulans* R2-SPc (*Synechococcus* PCC7942) was originally obtained from Dr. W. E. Borrias, University of Utrecht. All the cyanobacteria were cultured at 34 °C under photoautotrophic conditions with continuous aeration with air that contained 1% CO_2, as described previously (Wada and Murata, 1990). Medium A was used for growth of *Synechococcus* PCC7002 (Stevens et al., 1973), and BG-11 medium was used for the strains of *Synechocystis, Anabaena* and *Anacystis* (Stanier et al., 1971). In some experiments the *cytM* mutant was grown in BG-11 medium depleted of Cu^{2+} ions.

Determination of nucleotide sequence

A plasmid clone with an 8-kbp insert that included the *desA* gene of *Synechocystis* PCC6803, designated pTZ19R/8-kbp, was obtained as described previously (Wada et al., 1990). The nucleotide sequence of the region downstream from the *desA* gene in pTZ19R/8-kbp was determined by the dideoxy chain-termination method (Sanger et al., 1977) using deletions generated by exonuclease III/mung bean exonuclease and synthetic oligonucleotides as sequencing primers. For the search in the NBRF and PIR databases, we used the BLAST program of Altschul et al. (1990). The amino acid sequence was aligned by application of the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970) with some manual corrections.

Southern blot analysis

For Southern blot analysis, the chromosomal DNAs were isolated from the cyanobacterial cells by the method of Williams (1988) and were purified by centrifugation in a CsCl gradient. The chromosomal DNA was digested with an appropriate restriction endonuclease, subjected to electrophoresis on a 0.8% agarose gel, and fragments were transferred to a nylon membrane (GeneScreen Plus; Biotechnology Systems, NEN Research Products, Boston, MA, USA). The probe was derived from the region between position 15 and position 711 of the nucleotide sequence (see Fig. 1) and was labeled with [α-^{32}P]dCTP using a nick translation kit (Takara, Kyoto, Japan). Hybridizations were carried out in accordance with the protocol of the manufacturer of GeneScreen Plus with the exception that different concentrations of formamide (10%, 20%, 30%, 40%, and 50%, v/v) were used. Washing of the membranes after hybridization was carried out at 42 °C with buffers that corresponded to different stringencies.

Inactivation of the cytM *gene*

A plasmid that contained the *cytM* gene and its flanking regions (232 bp upstream from the initiation codon and 598 bp downstream from the stop codon) was constructed by delection with exonuclease III/mung bean exonuclease. It was digested with *Nco* I restriction endonuclease, and the excised *Nco* I – *Nco* I fragment (from position 180 to position 397 of the nucleotide sequence in Fig. 1) was replaced by a kanamycin-resistance (Km^r cartridge of 1.2 kbp, derived from plasmid pUC4KIXX (Pharmacia LKB Biotechnology, Pharmacia Biosystems, Tokyo). The resultant plasmid, containing the disrupted *cytM* gene, was used for the transformation of the wild-type strain of *Synechocystis* PCC6803 by homologous recombination (Williams, 1988). Screening of the Δ*cytM* mutants was performed by PCR analysis of the total genomic DNA and verified by Southern blot analysis of the chromosomal DNA with a probe that corresponded to the region from nucleotide 393 to nucleotide 562 in the sequence shown in Fig. 1.

After purification of homozygous mutants, the chromosomal DNAs from two independent kanamycin-resistant clones were isolated. The DNAs were digested with restriction enzymes, and analyzed by Southern blot hybridization. These analysis indicated that the two independent mutants had the same gene structure and, therefore, one of them was selected for further experiments.

Measurements of growth rate and oxygen exchange

The growth rates of the wild type and the Δ*cytM* mutant of *Synechocystis* PCC6803 were estimated by counting numbers of cells per unit volume with Goryaev's camera. The uptake and the evolution of oxygen by intact cells suspended in BG-11 medium were monitored at 34 °C with a Clark-type oxygen electrode (Hansatech Instruments Ltd., Kings Lynn, England). Cells were collected by centrifugation for 10 min at 3,000 × g and resuspended at a chlorophyll concentration of 2–5 μg mL^{-1} in BG-11 medium. The measurements of oxygen exchange were repeated at least 5 times each for the wild type and the Δ*cytM* mutant.

Results and Discussion

DNA sequence

The nucleotide sequence of 6 kbp in the downstream region of the *desA* gene in plasmid pTZ19R/8-kbp was determined. An open reading frame (ORF) was identified 3.4 kbp downstream from the *desA* gene. As described below, this ORF corresponds to the gene for a new type of cytochrome *c*, designated *cytM*. Figure 1 shows the nucleotide sequence of the gene and the deduced amino acid sequence.

Two possible sites of initiation of translation are found at positions 181 and 250 in the nucleotide sequence. The second methionine, marked by dots in Fig. 1, but not the first

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CCGACCTAGGAAAATTCTGGGTATCGATATCGGGGGAGTTTTAGTTTGTCAACAGGGTTA   60
ATCCGGACTTCAATCTGGGACTGAGCAAAGACAGGAATGCCCGGCATCAATACAATAGAT  120
TAAGTTAGATTAAGTTTCTGAGTGGTGGTACTGGCCACCTTGACCAATCGTTCTTAACCC  180
ATGGCCCCTGTAATCGAAAAAAGTCCAACTGTTGCCACGGTAAATGCTTCCCCCACTGGG  240
M  A  P  V  I  E  K  S  P  T  V  A  T  V  N  A  S  P  T  G    20
         ...
ATATGGATTATGGCAGGCATTGTTTCCCTGGTGATATTGGCAGTGGCTTTGTTTAGTTTT  300
I  W  I  M  A  G  I  V  S  L  V  I  L  A  V  A  L  F  S  F    40
                                     ▼
ATGAACTTTGACCCCTATGTAAGCCAGGTACTCGCTCTCAAAGGAGATGCGGATAGGGGT  360
M  N  F  D  P  Y  V  S  Q  V  L  A  L  K  G  D  A  D  R  G    60
CGGGCCATTTTTCAAGCTAACTGTGCAGTTTGCCATGGCATCCAAGCTGACGGTTACATT  420
R  A  I  F  Q  A  N [C  A  V  C  H] G  I  Q  A  D  G  Y  I    80
GGCCCCAGTCTTTGGGGGGTATCCCAACGGCGGTCCCAGAGCCACATCATTCATCAGGTG  480
G  P  S  L  W  G  V  S  Q  R  R  S  Q  S  H  I  I  H  Q  V   100
GTGAGTGGTCAAACTCCTCCCATGCCCCAGTTTGAACCCAATCCCCAGGAGATGGCGGAT  540
V  S  G  Q  T  P  P  M  P  Q  F  E  P  N  P  Q  E  M  A  D   120
TTATTGAATTACCTCAAAACCCTCTGAAGGGATAAGCCCAGATTGGTTTGGATAGACGTT  600
L  L  N  Y  L  K  T  L  *                                     128
TTTAGCCATGTTCTTAACCATCTGGTTTTAGGCCATTTGTTGGATGCCACTGCCAACCAA  660
CCCCACACCTATTGATCTCGATTGATCCCGCTGGAGTGAGATTAACCCCATTTTTAACTG  720
ACTCAAGCCTTGCCGTTTTA                                          740
```

Fig. 1: The nucleotide sequence and the deduced amino-acid sequence of the *cytM* gene of *Synechocystis* PCC6803. The putative site of initiation of translation is marked with dots, and the putative processing site for removal of the transit peptide is marked with a triangle. Amino acid residues 68–72, which resemble a heme-attachment motif, are boxed. The sequencing data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries under the accession number D10716.

methionine, is preceded by a GA-rich motif, and this motif resembles a ribosome-binding site. Therefore, it seems likely that the second methionine represents the actual site of initiation of translation. Based on this assumption, the molecular mass of the product of the *cytM* gene was calculated to be 11.4 kDa.

Figure 2 shows the hydropathy profile deduced for the product of the *cytM* gene. The hydrophobic domain near the amino terminus may be a transit peptide or a membrane anchor. A region of the polypeptide chain near the hydrophobic domain (amino acids 42–47 in Fig. 1) contains amino acids that might form a β-turn, as well as the amino

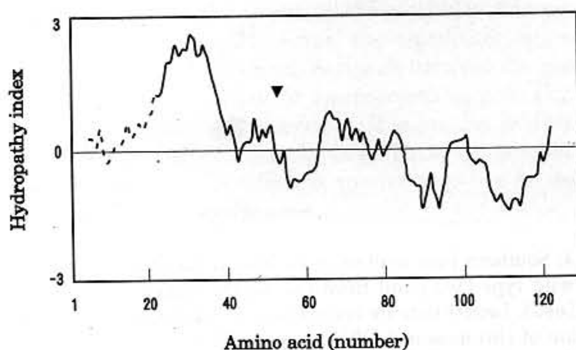

Fig. 2: The deduced hydropathy profile of the product of the *cytM* gene of *Synechocystis* PCC6803. The dashed line indicates amino acid residues that precede the second site of initiation of translation. The putative processing site for removal of the transit peptide is marked with a triangle. The hydropathy profile was calculated by the method of Kyte and Doolittle (1982) with a window size of 11 amino acid residues.

acid motif VLA (amino acid residues 50–52 in Fig. 1). This structure is the same as that found at the site at which the transit peptide is cleaved from the 18-kDa polypeptide of the oxygen-evolving complex of spinach (Bartling et al., 1990). These findings suggest that the product of the *cytM* gene is processed at a site 30 residues from the second methionine (position 54 in the amino acid sequence in Fig. 1) after translocation to the lumen or to the periplasmic space and, moreover, that the putative mature protein is hydrophilic. Thus, the molecular mass of the mature protein is estimated to be 8.3 kDa. The isoelectric point of the mature protein, as estimated from the amino acid composition, is 7.3.

Similarities between the product of the cytM *gene and other c-type cytochromes*

The deduced amino acid sequence of the product of the *cytM* gene was compared with the entries in the NBRF and PIR databases in an attempt to identify homologous protein sequences (Altschul et al., 1990). The highest degree of similarity was observed in comparisons to various mitochondrial cytochromes *c* and to cytochrome *c*-550 from *Bacillus subtilis.* Some of these sequences were extracted from the databases and used for more detailed comparisons.

Figure 3 shows a comparison of the amino acid sequence of the product of the *cytM* gene of *Synechocystis* PCC6803 with amino acid sequences of cytochromes *c* from a variety of organisms. The extent of sequence similarity is 35 % with respect to the basic cytochrome *c*-553 from *Anabaena* PCC7937 (Bovy et al., 1992), 18 % with respect to the acidic cytochrome *c*-550 from *Microcystis aeruginosa* (Cohn et al., 1989), 26 % with respect to the acidic cytochrome *c*-550 from *B. subtilis* (von Wachenfeldt and Hederstedt, 1990) and 26 % with respect to the mitochondrial cytochrome *c* from *Tetrahymena pyriformis* (Tarr and Fitch, 1976). The highest degree of similarity among all five proteins is found when the respective heme-binding sites are compared, and this sequence is CAVCH in the product of the *cytM* gene (box 1 in Figs. 1 and 3). Another domain in the product of the *cytM* gene, GPSLWGV (box 2 in Fig. 3), is similar to the respective domains in the mitochondrial cytochrome *c* and in the cytochrome *c*-550 from *B. subtilis*, but it is unlike the corresponding region in the cytochrome *c*-553 of *Anabaena* PCC7937. The sequence of the carboxy-terminus of the product of the *cytM* gene is similar to that of the mitochondrial cytochrome of *T. pyriformis* (Fig. 3). The carboxy-termini of cytochrome *c*-553 of *Anabaena* PCC7937 and cytochrome *c*-550 of *B. subtilis* differ from the carboxy-terminus of the product of the *cytM* gene. The putative transit peptide of the product of the *cytM* gene is similar to the transit peptide of the cytochrome *c*-553 from *Anabaena* PCC7937 (Bovy et al., 1992; underlined in Fig. 3) and to the membrane anchor of cytochrome *c*-550 from *B. subtilis* (von Wachenfeldt and Hederstedt, 1990).

The petI gene for an acidic cytochrome *c*-553 was recently isolated from *Synechocystis* PCC6803 (Zhang et al., 1994). The amino acid sequence deduced from the petI gene is only 19 % homologous to the amino acid sequence deduced from the *cytM* gene. This finding demonstrates that *cytM* is not a

```
                                                              1
CytM                     MAGIVSLVILAV-ALFSFMNFDPYVSQVLALKGDAD-RGRA  IFQANCAVCH  49
                         *  *.***.*..  ***.*     ..  *  **    .*     *  **.**** **
c-553 A.                 MKKIFSLVLLGI-ALFTF----AFSSPALA--ADV-ANGAK  IFSANCASCH  43
                              *    *    * *.  * *     .*     *  .* ***   **
c-550 M.a.                      LELDEKTLTITLN-DAGES-VTLTSEQAT-EGQK  LFVANCTKCH  41
                   **     ..*.* .*     ..  .   * * **.    ..  *..***   **
c-550 B.s.  MKWNPLIPFLLIAVLGIGLTFFLSVKGLDDSREIASGGESKSAEKKDANASPEE  IYKANCIACH  64
                                           *  ..*    ***    **  **...*. **
Mit-c T.p.                                GPKEPEVTVPEGDAS-AGRD  IFDSQCSACH  29

                              2
CytM        G-----IQADGYI  GPSLWGVS  QRRSQSHIIHQVVSGQTP------------------------  83
            .    .**.  .              *   ** **..*
c-553 A.    AGGKNLVQAQKTL  KKEDLEKF  GMYSAEAIIAQVTNGKNA------------------------  82
                  *.            **   ..        .
c-550 M.a.  L------QGKTKT  NNN---VS  LGLGDLAKAEPPRDNLLALIDYLEHPTSEDGEDGLSELHPNV  95
               . .*.    **** **.   ...  ..* ...  * ..
c-550 B.s.  G-----ENYEGVS  GPSLKGVG  DKKDVAEIKTKIEKGGNG------------------------ 109
            .    *..*.     .* * **    *.. ..   .  *  .
Mit-c T.p.  A-----IEGDSTA  APVLGGVI  GRKAGQE-KFAYSKGMKGSGITWNEKHLLKFLKFPSKHVPGT  86

CytM        PMPQFE-PNPQEMADLLNYLKTL                                           105
             ** *.   .  .  .  .
c-553 A.    -MPAFKGRLKPDQIEDVAAYÖLGQADKSWK                                    111
             *..   .  .  *
c-550 M.a.  SRPDIFPELRNLTEDDVYNVAAYMLVAPRLDERWGGTIYF                          135
             **.    ....      . *
c-550 B.s.  -MPSGLVPADKLDDMAEWVSKIK                                           119
             *.    ...  ***. ***..
Mit-c T.p.  KMAFAGLPADKDRADLIAYLKSV                                           109
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Fig. 3: Alignment of the amino acid sequence of the product of the *cytM* gene with those of basic cytochrome *c*-553 of *Anabaena* PCC7937 (Bovy et al., 1992), acidic *c*-550 of *Microcystis aeruginosa* (Cohn et al., 1989), acidic *c*-550 of *Bacillus subtilis* (von Wachenfeldt and Hederstedt, 1990), and mitochondrial cytochrome *c* of *Tetrahymena pyriformis* (Tarr and Fitch, 1976). Asterisks indicate amino acid identify and dots indicate homologous amino-acid replacements. The transit peptide of cytochrome *c*-553 of *Anabaena* PCC7937 and a putative transit peptide of the product of the *cytM* gene are underlined. Abbreviations: CytM, amino acid sequence predicted from the *cytM* gene of *Synechocystis* PCC6803; *c*-553 A., cytochrome *c*-553 of *Anabaena* PCC7937; *c*-550 M.a., cytochrome *c*-550 of *M. aeruginosa; c*-550 B.s., membrane-bound cytochrome *c*-550 of *B. subtilis;* Mit-c T.p., soluble cytochrome *c* of mitochondria of *T. pyriformis.* The sites with the highest degree of homology are boxed.

gene for cytochrome *c*-553 of *Synechocystis* PCC6803 and that it encodes another *c*-type cytochrome.

Physico-chemical characteristics, such as the molecular mass and the pI value, of the product of the *cytM* gene were also compared with those of other cyanobacterial cytochromes for which complete nucleotide and/or amino acid sequences have not yet been determined. Cytochromes *c*-550 have much higher molecular masses and lower pI values (Hoganson et al., 1990) than the product of the *cytM* gene. Thus, the probability that this product is a cytochrome *c*-550 seems rather slight. The basic cytochrome *c*-552 of *Anacystis nidulans,* with a molecular mass of 9.0 kDa and a pI of 7.8 (Holton and Myers, 1967 b), is the most similar to the mature protein predicted from the *cytM* gene, which has a molecular mass of 8.3 kDa and a pI of 7.3.

Inactivation of the cytM *gene*

For the functional characterization of the product of the *cytM* gene we applied the cartridge mutagenesis technique to the wild type of *Synechocystis* PCC6803 to produce a disruption mutant, Δ*cytM* (*cytM*-deficient). Southern blot analysis showed that digestion of the chromosomal DNA from the wild-type cells with *Kpn* I, *Eco* 0109 I and *Ava* I produced fragments of 3.8 kbp, 2.0 kbp and 3.0 kbp, respectively, which hybridized with the *cytM* probe (Fig. 4). The digestion of the chromosomal DNA from the Δ*cytM* mutant with *Kpn* I, *Eco* O109 I and *Ava* I generated hybridizable fragments of 5.0 kbp, 3.2 kbp, and 1.1 kbp, respectively (Fig. 4). The increase in the size of the DNA fragments generated by *Kpn* I and *Eco* O109 I reflects the insertion of the Km^r-cartridge (Km^r, kanamycin resistance gene), which is 1.2 kbp in size, into the *cytM* gene. The hybridizable fragment of 1.1 kbp produced by digestion with *Ava* I is regarded as a result of the creation of an additional *Ava* I site in the chromosome of the Δ*cytM* mutant upon fusion of the internal *Nco* I site of *cytM* with the terminal *Sma* I site of the Km^r, followed by a spontaneous mutation, which occurred during the construction of the recombinant DNA molecule. Wild-type co-

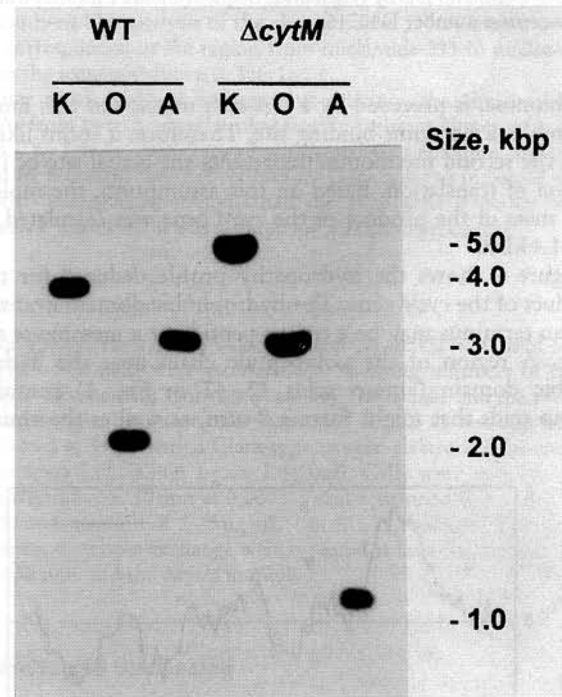

Fig. 4: Southern blot analysis of chromosomal DNA isolated from the wild type (WT) and from the Δ*cytM* mutant of *Synechocystis* PCC6803. Letters indicate restriction endonucleases used for the digestion of chromosomal DNA: lanes K, *Kpn* I; lanes O, *Eco* O109; and lanes A, *Ava* I. Chromosomal DNAs isolated from the wild type and the Δ*cytM* mutant were loaded on the 0.8 % agarose gel at 3 μg per lane.

pies of the *cytM* gene were not detected in the DNA of the Δ*cytM* mutant cells, an indication that the mutant clones had been segregated as stable cell lines that lacked the native *cytM* gene. An independent analysis of the clones by the polymerase chain reaction confirmed the absence of wild-type copies of the *cytM* gene in the DNA of the Δ*cytM* mutant (data not shown).

The Δ*cytM* mutant grew photoautotrophically in BG-11 medium at the same rate as the wild-type cells. Elimination of Cu^{2+} ions from BG-11 medium did not affect the growth rate of the Δ*cytM* mutant. No significant differences between the wild type and the Δ*cytM* mutant were found in the rate of photosynthetic evolution of oxygen in the light: rates were 354 ± 22 and 360 ± 22 µmol O_2 per mg of chlorophyll per hour in wild-type and the Δ*cytM* mutant cells, respectively. The respiratory activities, as measured by monitoring the uptake of oxygen in the dark, were 11 ± 1 and 10 ± 1 µmol O_2 per mg of chlorophyll per hour in the wild-type and the Δ*cytM* mutant cells, respectively. Although the addition of 1 mM glucose accelerated the respiratory uptake of oxygen, no differences were detected between the wild-type and the mutant cells: rates were 36 ± 4 and 38 ± 4 µmol O_2 per mg of chlorophyll per hour, respectively.

Presence of the cytM *gene in other cyanobacteria*

Figure 5 shows the results of Southern blot analysis of the genomic DNAs of five species of cyanobacteria with the *cytM* gene as probe. Only one band appeared upon digestion of the genomic DNA from *Synechocystis* PCC6803 with restriction endonucleases *Kpn* I, *Hinc* II and *Eco* RI (lanes 1). This result suggests that the *cytM* gene is present as a single copy on the chromosome of *Synechocystis* PCC6803. Using different conditions for the hybridization and subsequent washing procedure, we found that the probe hybridized with the genomic DNA from *Synechocystis* PCC6714 (lanes 2), but not with genomic DNAs from *Anacystis nidulans* R2-SPc (lanes 3), *Anabaena variabilis* (lanes 4), and *Synechococcus* PCC7002 (lanes 5). These observations may suggest that the *cytM* gene is present in the genomes of two species of *Synechocystis*, but not in the genomes of unicellular *Anacystis* or filamentous *Anabaena*. However, the significant difference in the intensities of hybridization signals between the genomic DNAs of the two strains of *Synechocystis* suggests that the extent of the homology between *cytM* sequences in different strains of cyanobacteria may be low. Thus, the presence of the analogous genes in different species may not be detectable by Southern hybridization.

Conclusion

A novel gene, *cytM*, was discovered in *Synechocystis* PCC6803, and identified as a gene for a new type of cytochrome *c*. The product of this gene contains a heme-binding motif and is to some extent homologous to cytochromes *c* from cyanobacteria and mitochondria. Although we created and characterized a mutant that lacked an active *cytM* gene, the product of *cytM* was not identified.

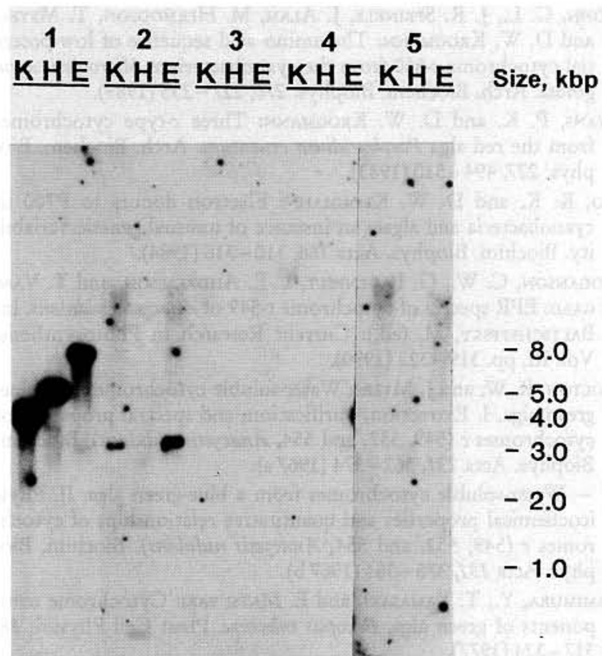

Fig. 5: Southern blot analysis of the *cytM* gene in the chromosomes of cyanobacteria. Lanes 1, chromosomal DNA isolated from *Synechocystis* PCC6803; lanes 2, from *Synechocystis* PCC6714; lanes 3, from *Anacystis nidulans* R2-SPc; lanes 4, from *Anabaena variabilis* M-3; lanes 5, from *Synechococcus* PCC7002. Letters indicate restriction endonucleases used for the digestion of chromosomal DNA: K, *Kpn* I; H, *Hinc* II; E, *Eco* RI. The DNA isolated from *Synechocystis* PCC6803 was loaded on a 0.8 % agarose gel at 0.3 µg per lane. The DNAs isolated from the other cyanobacteria were loaded on the gel at 3 µg per lane.

Acknowledgements

We thank Prof. R. Douce and Dr. M. Mamedov for stimulating discussions, and Mr. T. Ohkawa of the Technical Department of this Institute for his kind assistance with computers. D.A.L. was supported by a Postdoctoral Fellowship from the Japanese Society for the Promotion of Science.

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