FesM, a Membrane Iron-Sulfur Protein, Is Required for Cyclic Electron Flow around Photosystem I and Photoheterotrophic Growth of the Cyanobacterium Synechococcus sp. PCC 7002\textsuperscript{1}\textsuperscript{[w]}

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While it is known that cyclic electron flow around photosystem I is an important pathway of photosynthetic electron transfer for converting light energy to chemical energy, some components of cyclic electron flow remain to be revealed. Here, we show that fesM, encoding a novel membrane iron-sulfur protein, is essential to cyclic electron flow in the cyanobacterium Synechococcus sp. PCC 7002. The FesM protein is expected to have a cAMP-binding domain, an NtrC-like domain, a redox sensor motif, and an iron-sulfur (4Fe-4S) motif. Deletion of fesM (fesM-D) led to an inability for Synechococcus cells to grow to the presence of glycerol and 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Photoheterotrophic growth was restored by a complete fesM gene present on a replicable plasmid. A mutant fesM gene encoding a truncated FesM protein lacking the cAMP domain failed to restore the phenotype, suggesting this domain is important to the function of FesM. Measurements of reduction of P700 and the redox state of interphotosystem electron carriers showed that cells had a slower rate of respiratory electron donation to the interphotosystem electron transport chain, and cyclic electron flow around photosystem I in fesM-D was impaired, suggesting that FesM is a critical protein for respiratory and cyclic electron flow. Phosphatase fusion analysis showed that FesM contains nine membrane-spanning helices, and all functional domains of FesM are located on the cytoplasmic face of the thylakoid membranes.

In plants and cyanobacteria, there are two types of photosynthetic electron transfer. Linear electron transfer results in the oxidation of water and reduction of NADP\textsuperscript{+} while generating a proton gradient across thylakoid membranes for ATP synthesis. Cyclic electron flow around PSI also generates a proton gradient across the thylakoid membranes. In addition, thylakoid membranes have a respiratory electron transport system that plays important roles in both plants and cyanobacteria (Schmitterer, 1994; Peltier and Cournac, 2002; Munekage et al., 2004). While the linear and cyclic photosynthetic electron transport systems share some common electron carriers, cyclic electron flow around PSI has its uniqueness in transporting electrons from the acceptor of PSI to P700 through the plastoquinone (PQ)/cytochrome b\textsubscript{6}f (Cytb\textsubscript{f}) complex (Allen, 2002; Joliot and Joliot, 2002). Some of the electron carriers involved in cyclic electron flow around PSI are still unknown, and details of this electron transfer pathway remain to be elucidated.

There are several pathways for electron donation to PQ/Cytb\textsubscript{f} on thylakoid membranes. In the cyanobacterium Synechococcus sp. PCC 7002, electrons from PSII account for more than 90% of the total electron donation to the PQ/Cytb\textsubscript{f} complex while NAD(P)H dehydrogenase (NDH) and PsaE-dependent cyclic electron transfer account for less than 10% of electron donation to Cytb\textsubscript{f} (Myers, 1987; Yu et al., 1993). However, both NDH and PsaE are required for growth under photoheterotrophic conditions (Yu et al., 1993). In the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) that blocks the reduction of PQ by PSII, a double mutant lacking both ndhF and psaE genes showed a very slow rereduction of P700\textsuperscript{+}, suggesting that the pathways of NDH and PsaE-dependent electron transfer account for nearly all physiologically significant electron donation to P700\textsuperscript{+} under these conditions (Yu et al., 1993; Huang et al., 2003). The cyanobacterial NDH donates an electron to the interphotosystem chain from NAD(P)H and plays important roles in cellular activities. Cyanobacterial NDH is involved in cyclic electron transfer (Ogawa, 1991; Mi et al., 1992; Schluchter et al., 1993) and is required for adaptation to low CO\textsubscript{2} conditions (Maeda et al., 2002; Deng et al., 2003; Zhang et al., 2004). Recently, NDH-1 complexes from Synechocystis sp. PCC 6803 have been isolated and characterized. The results showed that the NDH-1 is present in multiple forms (Prommeenate et al., 2004; Zhang et al., 2004; Battchikova et al., 2005). The large NDH-1 complex contained more than 10 ndh gene products plus other proteins previously

\textsuperscript{1} This work was supported by the National Natural Science Foundation of China (30230040) and by the High Technology project from the Ministry of Science and Technology of China (2004AA626020).

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\textsuperscript{[w]} The online version of this article contains Web-only data.
unidentified. The mechanism of electron donation to NDH is currently unknown. The PsaE-dependent cyclic electron pathway has been suggested to share the same features of the ferredoxin:quinone reductase pathway (Bendall and Manasse, 1995; Scheller, 1996), which also remains to be elucidated. The three-dimensional structures of several cyanobacterial PsaE proteins have been determined, and they show that PsaE is not an electron carrier (Falzone et al., 1994; Mayer et al., 1999). However, its structure contains a SH3 domain, suggesting that it may interact with other proteins (Cesareni et al., 2002). Protein cross-linking studies show that PsaE strongly influences the interaction of proteins on the acceptor side of PSI (Muhlenhoff et al., 1996).

While many thylakoid membrane proteins are organized into complexes, a significant number of proteins on thylakoid membranes do not form complexes or may only loosely interact with other proteins. These proteins may play important roles in photosynthetic electron transport and membrane organization. For example, the rubA gene product in *Synechococcus* sp. PCC 7002 is important for PSI assembly, but it is not tightly associated with the PSI complex (Shen et al., 2002). In this article, we report a gene encoding a novel membrane iron-sulfur (Fe-S) protein that is required for photoheterotrophic growth of *Synechococcus* sp. PCC 7002. Our results show that this protein participates in cyclic electron transport around PSI.

**RESULTS**

*fesM* Encodes a Membrane Protein Required for Photoheterotrophic Growth

The complete genome sequence of *Synechococcus* sp. PCC 7002 has recently been determined (J. Zhao, unpublished data). In searching for proteins involved in cyclic electron flow, we constructed a mutant pool of *Synechococcus* sp. PCC 7002 through transposon mutagenesis and isolated a mutant that could not grow in the presence of DCMU and glycerol. The transposon was shown to be inserted into a previously unidentified open reading frame. To confirm that this phenotype was indeed due to the insertion, we reconstructed the mutation by deleting the entire coding region of the gene (*fesM*) and characterized the mutant strain.

Search of databases showed that cyanobacteria with genomes larger than 3 Mb, such as *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120, have *fesM*, while cyanobacteria with genomes smaller than 3 Mb, such as *Synechococcus* WH8102 and *Prochlorococcus marinus* SS120, do not have *fesM*. One exception is *Prochlorococcus marinus* MIT 9313, which also has a small genome. There is a *fesM* gene encoding an FesM-like protein with an incomplete cAMP domain at the C terminus. No homolog of *fesM* is found in *Arabidopsis thaliana* (Arabidopsis thaliana) and rice (*Oryza sativa*) genomes. A comparison of *FesM* sequences in various cyanobacteria shows that all domains of the protein are conserved, and the most conserved regions are the redox sensor and Fe-S motifs (Supplemental Fig. 1). The *FesM* protein from *Synechococcus* sp. PCC 7002 is predicted to be more closely related to *FesM* from heterocystous cyanobacteria than to *FesM* from unicellular cyanobacteria, based on analyses using the program ClustalW.

Figure 1 shows a physical map of the *fesM* gene, the result of Southern hybridization and predicted domains of the gene product. The *fesM* gene encodes a protein with a cAMP-binding domain, an NtrC-like domain, and several membrane-spanning helices with motifs that could coordinate Fe-S centers and a redox sensor (Fig. 1B). Deletion of *fesM* by replacement with a kanamycin-resistant cassette in *fesM*-D was confirmed by Southern hybridization (Fig. 1C), which shows that the 3.2-kb band of the wild type was missing and replaced by a 2.0-kb band in the mutant strain (*fesM*-D) as predicted (Fig. 1A).

To test if *fesM*-D could grow photoheterotrophically, growth of both the wild type and *fesM*-D was measured.

![Figure 1](http://www.plantphysiol.org/)

**Figure 1.** Physical map of the *fesM* gene from *Synechococcus* sp. PCC 7002, the domains of *FesM*, and Southern blotting results. A, A physical map showing the *fesM* gene, the replacement of *fesM* by kanamycin-resistant (*kan<sup>R</sup>*) cartridge, and the positions of *EcoRI* sites. The lengths of *EcoRI* fragments are shown at the bottom. The region of the gene shown in black was used as a template for synthesis of an isotope-labeled probe for Southern hybridization. B, Schematic drawing of *FesM* protein, showing the cAMP domain, the NtrC-like domain, and a membrane-spanning region containing both the redox sensor CX<sub>2</sub>CP metal binding motif and the Fe-S motif. C, Southern blot of genomic DNA that was isolated from wild-type cells (WT) and from *fesM*-D cells and digested with *EcoRI*. The DNA fragments were separated by agarose gel electrophoresis and then transferred onto a piece of nitrocellulose paper before hybridization.
growth with a doubling time.

Figure 2 shows that fesM-D could grow normally under photoautotrophic conditions. The inability of the fesM-D cells to grow in the absence of PSII activity indicated that cyclic electron flow around PSI might be impaired. Light-induced redox changes of P700 in both the wild type and fesM-D were measured by monitoring the absorption changes of 820 to 860 nm, and the results are shown in Figure 3A. In the presence of DCMU, which blocked electron donation from PSII to the PQ pool, the rereduction P700⁺ of fesM-D was slower than that of the wild type. The apparent half-times (t½) were 330 and 890 ms for the wild type and fesM-D, respectively. Examination of the kinetics of both curves suggested that the rereduction of P700⁺ in both strains involved more than one component. Curve fitting was then performed to further analyze the kinetics of the P700⁺ rereduction. Figure 3B shows that the P700⁺ rereduction of the wild type contained two exponential components with decay half-times of 248 and 445 ms. Figure 3C shows that P700⁺ rereduction in fesM-D also contained two exponential components with half-times of 478 and 2804 ms. It seems likely that one of the components in P700⁺ rereduction in the wild type was replaced by the slow component (2804 ms) in fesM-D. The rereduction of P700⁺ in 323C is virtually identical to that of the wild type (Fig. 3A), confirming that fesM located on a plasmid could complement the fesM mutation.

The slow rereduction of P700⁺ (Fig. 3) and lower respiratory rate (Table I) of fesM-D cells suggested that the reduction of PQ/Cytb/f was slower in fesM-D than that in the wild type. One method for determining the

| Table I. Parameters of wild-type and fesM-D cells |
|-------------------|-------------------|
| **Wild Type**     | **fesM-D**        |
| Chl:PBS (1% CO₂, FL) | 480 ± 18          | 492 ± 22          |
| Chl:P700 (1% CO₂, FL) | 131 ± 10          | 125 ± 12          |
| Chl:PSII (1% CO₂, FL) | 241 ± 18          | 231 ± 15          |
| PSI:PSII (1% CO₂, FL) | 1.91 ± 0.21       | 1.78 ± 0.17       |
| PSI:PSII (air, FL) | 3.62 ± 0.31       | 3.48 ± 0.29       |
| Glycogen⁺ (1% CO₂, FL) | 2.35 ± 0.19       | 2.30 ± 0.25       |
| O₂ evolution (1% CO₂, FL) | 4.68 ± 0.33       | 4.56 ± 0.38       |
| O₂ uptake (1% CO₂, FL) | 1.5 ± 0.1         | 1.7 ± 0.2         |
| O₂ evolution and uptake in μmol O₂ mg Chl⁻¹ h⁻¹ | 326 ± 21 | 342 ± 28 |
| O₂ uptake (1% CO₂, FL) | 12 ± 2            | 9 ± 2             |
| *Glycogen in mg mg Chl⁻¹. | *O₂ evolution and uptake in μmol O₂ mg Chl⁻¹ h⁻¹. |
reduction level of PQ/Cyt bf is the measurement of state transitions because state transitions are controlled by the redox state of interphotosystem electron carriers (Mullineaux and Allen, 1990). To measure the reduction of PQ/Cyt bf, cells were incubated in the dark in the presence of 10 μM DCMU for 2 min before actinic light (500 μmol m⁻² s⁻¹) was turned on to bring the cells to state 1. Then the actinic light was turned off for various periods of time before a pulse (2-s duration time) of actinic light was given to determine the level of chlorophyll (Chl) fluorescence at 682 nm (Finit; Huang et al., 2003). High and low Finit levels indicate that the cells are in state 1 and state 2, respectively. As shown in Figure 4A, the Finit levels declined with increasing dark times in both strains, suggesting a transition from state 1 to state 2 that reflects a gradual
reduction of PQ/Cyt$_b$. However, the $F_{\text{init}}$ in fesM-D was maintained at a higher level than that in the wild type, suggesting that there was impairment in the ability of fesM-D to reduce PQ/Cyt$_b$. Figure 4B shows P700 oxidation by phycobilisome (PBS)-absorbed actinic light (630 nm) in dark-adapted cells of both strains. Incubation of the wild type in the dark caused cells to reside almost completely in state 2. The oxidation of P700 by PBS-absorbing light was near its maximum value immediately after the actinic light was turned on, followed by a decrease in the level of P700$^+$ due to a transition from state 2 to state 1. In contrast, dark incubation of fesM-D did not drive cells completely into state 2, and oxidation of P700 by PBS-absorbing actinic light was not as complete as that in the wild type. However, a phase of rapid increase in the level of P700$^+$ was observed after the initial P700 oxidation when continuous illumination by PBS-absorbing light was provided to the fesM-D cells. This rapid phase was an indication that more PBS-absorbed light energy was transferred to PSI during illumination, probably because of a state 2 transition due to electron donation from PSI to PQ/Cyt$_b$.

Figure 5 shows percentages of the wild-type and fesM-D cells that survive when cultured in the dark in the absence of external organic carbon supply. The lack of a fesM gene apparently had a relatively strong effect on viability immediately after cells were transferred to darkness. The number of colony-producing cells in the culture of fesM-D started to decline within the first day after the culture was transferred to darkness. In contrast, the viability of wild-type cells only began to decline after 2 d in darkness. However, the rate of decline in viability was nearly the same for wild-type and fesM-D cells after the decline had begun.

**Localization of FesM and Determination of Its Membrane Topology**

Photosynthetic electron transfer in nearly all cyanobacteria, including cyclic electron flow around PSI, occurs on thylakoid membranes. Most components of respiratory electron transfer also occur on thylakoid membranes (Ohkawa et al., 2001; Zhang et al., 2004). To investigate the subcellular location of FesM, membranes from wild-type *Synechococcus* sp. PCC 7002 and fesM-D were isolated and fractionated. Plasma membranes and thylakoid membranes were separated by ultracentrifugation followed by two-phase separation. Figure 6 shows the results of an analysis of the proteins from the plasma membrane and of thylakoid membrane from both strains. The N-terminal domains of FesM (see Fig. 1) were overproduced in *Escherichia coli*, and antibodies were raised against the recombinant protein. The antibodies were used in immunoblotting assays to determine the subcellular location of FesM. The results (Fig. 6B) clearly show that the FesM protein is located on thylakoid membranes and is absent from plasma membranes. This correlates with the proposed function of FesM in cyclic electron transfer around PSI.

As shown in Figure 1, FesM is predicted to have multiple membrane-spanning helices. However, the exact number of the membrane spans could not be determined unambiguously with available computer programs. The FesM primary sequence that gives an ambiguous prediction of membrane spanning is the Fe-S motif region. Since this region is likely to be very important to the functions of FesM, we determined the topology of membrane-bound FesM using a phosphatase fusion system in *E. coli* (Manoil and Beckwith, 1986; Bibi et al., 1993). The *phoA* gene encoding *E. coli* phosphatase was fused to various parts of the fesM
gene encoding N-terminal portions of FesM and expressed in *E. coli*. Thirteen fusion genes were constructed. Immunoblotting results showed that all the fusion genes with one exception were expressed, and the molecular masses of the gene products were as predicted (Fig. 7). The lane that showed only a weak cross-reaction with the phosphatase antibody was the construction that would overproduce a fusion protein between the first 417 residues of FesM and PhoA. Because the first 417 amino acid residues contained no membrane-spanning region, the reason for the weak expression of this fusion gene was not examined. We measured the extracellular phosphatase activities from the *E. coli* strains expressing the fusion genes, both on plates and in liquid, to determine which fusion constructions could target the phosphatase to the periplasmic space, and the results are shown in Table II.

The results of on-plate assays and direct measurements of phosphatase activities agreed well: those fusion genes leading to blue bacterial colonies produced extracellular phosphatase activities and those leading to white colonies produced no extracellular phosphatase activity. Based on the information in Table II, the membrane topology of FesM should have the following features (Fig. 8): the N terminus of the protein, which includes the cAMP-binding domain and NtrC-like domain, is located on the stromal side of thylakoid membranes, while the C terminus of FesM is located on the luminal side. There are nine membrane-spanning helices. Both the redox sensor domain and Fe-S motifs are located on the stromal side of thylakoid membranes. These domains and motifs are probably in close contact with the thylakoid membranes because of their hydrophobic nature.

**DISCUSSION**

The results of this study show that the *fesM* gene, encoding an integral membrane protein with several distinct structural domains (Figs. 1 and 8), is required for photoheterotrophic growth of *Synechococcus* sp. PCC 7002 (Fig. 2). The inability of *fesM*-D to grow photoheterotrophically is a result of an impaired cyclic electron flow around PSI as demonstrated by the much slower rereduction of P700\(^{-}\) in the *fesM*-D strain (Fig. 3). Kinetic analysis of P700\(^{-}\) rereduction in the presence of DCMU (Fig. 3) reveals that there are two components in both the wild type and *fesM*-D. Previous measurements (Yu et al., 1993; Huang et al., 2003) suggest that in the wild type the fast reduction component may represent the NDH route, with the slow reduction component representing the PsbE-dependent route. In *fesM*-D, the fast and slow components in P700\(^{-}\) rereduction had half-times of 478 and 2804 ms, respectively. The similar P700\(^{-}\) reduction kinetics of the slow component of the wild type and the fast component of *fesM*-D (Fig. 3) suggest that the slow component in the *fesM*-D cells replaced the fast component of wild-type cells, although it cannot be ruled out that both components in P700\(^{+}\) rereduction were affected by the lack of FesM.

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**Table I. Analyses of FesM-PhoA fusion proteins produced in *E. coli***

<table>
<thead>
<tr>
<th>Fusion Site</th>
<th>Colony Color on XP Plate</th>
<th>PhoA Activity (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40Q</td>
<td>White</td>
<td>0</td>
</tr>
<tr>
<td>417S</td>
<td>White</td>
<td>0</td>
</tr>
<tr>
<td>455F</td>
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</tr>
<tr>
<td>510K</td>
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</tr>
<tr>
<td>537Y</td>
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</tr>
<tr>
<td>560R</td>
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**Figure 7.** Immunoblotting analysis of the expression of *fesM*-phoA fusion genes. Thirteen fusion genes were constructed and induced to express in *E. coli* as described in “Materials and Methods.” The numbers written above the gel correspond to specific fusion genes as described in Table II. Total cellular extracts were prepared and separated by SDS-PAGE before the proteins were transferred to a polyvinylidene difluoride membrane. A monoclonal antibody against the alkaline phosphatase from Sigma-Aldrich was used as primary antibody.
slower in fesM-D than in the wild type. These results agree with results obtained from an NDH mutant of *Synechocystis* sp. PCC 6803 (Mi et al., 1995; Schreiber et al., 1995) and an ndhF mutant of *Synechococcus* sp. PCC 7002 (Huang et al., 2003), which show that the interphotosystem carriers remain oxidized and the cells are in state 1 after dark incubation in the *ndh* mutant cyanobacterial strains. The results shown in Figure 4B demonstrate that dark incubation of the fesM-D cells resulted in an incomplete state 2 because less PBS-absorbed light was delivered to PSI, also suggesting that the reduction of interphotosystem electron carriers in fesM-D cells is impaired. This suggestion is supported by the observation that the number of living cells of the fesM-D started to decline immediately after the cultures were transferred to darkness in the absence of glycerol even though its intracellular glycogen content was similar to that of wild-type cells (Table I; Fig. 5). The ability to perform state transitions was not affected in fesM-D (Fig. 4). The fesM-D cells also retained the ability to regulate photosystem stoichiometry, as shown in Table I. As compared to cells bubbled with air enriched with 1% CO₂, those bubbled with air showed a much higher PSI.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Enzyme</th>
<th>Note</th>
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<tbody>
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<td>fesM-D construction</td>
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<tr>
<td>P2</td>
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</tr>
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<td>P3</td>
<td>AGATATCTGTTAACAACACCCAGGG</td>
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<tr>
<td>P4</td>
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Figure 8. Drawing of the topology and domains of FesM from *Synechococcus* sp. PCC 7002. A thylakoid membrane is shown in gray, and transmembrane helices are shown as vertical boxes. The numbers shown in diamond-shaped boxes are the positions where the *phoA* gene was fused.
to PSII ratio. A similar but smaller effect was observed when the cells were switched from fluorescent light, mostly absorbed by PBS, to tungsten light, mostly absorbed by Chl (Table I).

Except for FesM from *P. marinas* MIT 9313, all structural domains of FesM are conserved. The cAMP-binding domain located at the N terminus of FesM is required for photoheterotrophic growth of *Synechococcus* sp. PCC 7002, suggesting that it is important to the functions of FesM. Since cAMP participates in the regulation of many cellular processes, we speculate that this domain could play an important role in the regulation of FesM activity. It would be interesting to measure cyclic electron flow in *P. marinas* MIT 9313, since the cAMP domain is incomplete in FesM of *P. marinas* MIT 9313 (Supplemental Fig. 1). The role of the NtrC-like domain in FesM is unknown. NtrC is a key protein that interacts with other proteins in regulation of nitrogen metabolism in many bacteria. This domain might be important for FesM interactions with other proteins or dimerization (Yang et al., 2004). The FesM protein has a conserved helix-CX2-3CP-helix-helix-CX2-3CP motif. This motif has been found in a variety of integral membrane proteins and has electron transfer functions (Berk et al., 1995; McGuirt et al., 1998). It has been suggested that this motif could serve as a redox sensor that responds to changes in cellular redox potential (Berk et al., 1995; Kiley and Beinert, 2003). The motifs for two 4Fe-4S clusters are also well conserved in FesM proteins. The space between the two 4Fe-4S motifs in the primary structure strongly suggests that two 4Fe-4S clusters are close to each other in the native protein and could be important in electron transfer (Sticht and Rosch, 1998). The region containing the two 4Fe-4S clusters contains some hydrophobic amino acid residues and is predicted to be a membrane-spanning helix by some computer analyses. However, according the membrane topology shown in Table II and Figure 8, this region does not form a membrane-spanning helix, and both 4Fe-4S clusters are on the same side of the thylakoid membranes as the redox sensor. The hydrophobic residues could play a role in inserting of the 4Fe-4S clusters into the membrane by making a half-helix-membrane-spanning loop, a feature reported in some other membrane proteins (Czempinski et al., 1997).

The results presented in this study demonstrate that FesM plays an important role in respiratory and cyclic electron transport around PSI in *Synechococcus* sp. PCC 7002. The presence of a Fe-S motif and a redox sensor motif in FesM suggests that FesM could participate in the reduction of PQ/Cytb in vivo. The NDH-1 complexes of *Synechococcus* sp. PCC 7002 have not been characterized in detail, and it is not known if the NDH-1 complexes in *Synechococcus* sp. PCC 7002 are equivalent to those of *Synechocystis* sp. PCC 6803 (Prommeenate et al., 2004; Zhang et al., 2004). If the NDH-1L and the NDH-1M of *Synechocystis* sp. PCC 6803 are also present in *Synechococcus* sp. PCC 7002 and their activities remain similar, then FesM may be involved in the functions of both complexes, since P700+ reoxidation in fesM-D was much slower than that of the wild type and the photoheterotrophic growth of fesM-D was extremely slow, similar to the D1/D2 mutant of *Synechocystis* sp. PCC 6803, which could not grow photoheterotrophically (Zhang et al., 2004). The fact that the NDH complex (Ohkawa et al., 2001) and FesM are both located on thylakoid membranes (Fig. 6) supports this suggestion. Another possibility is that FesM regulates cyclic electron flow indirectly by regulating the activities of NDH complexes. Further study is needed to understand the mechanism of function of FesM in cyclic electron flow around PSI.

**MATERIALS AND METHODS**

**Strains, Culture Conditions, Mutagenesis, and DNA Cloning**

*Synechococcus* sp. PCC 7002 wild-type and mutant cells were grown in A medium (Stevens et al., 1973) at 38°C under either cool-white fluorescent light at 150 μmol m⁻² s⁻¹ or tungsten light at the same light intensity. The cultures were either bubbled with air or air plus 1% CO₂. Unless stated otherwise, the cells used for measurements were grown by bubbling with air plus 1% CO₂ and illuminated with fluorescent light. The photoheterotrophic growth of *Synechococcus* sp. PCC 7002 was carried out in the presence of 10 μM DCMU and 10 μM DCMU in A medium. Cultures were illuminated with cool-white fluorescent light at 50 μmol m⁻² s⁻¹ at 38°C. Growth was measured by monitoring optical density at 730 nm as described previously (Zhang et al., 1993). *Escherichia coli* strains were grown in Luria-Bertani medium supplied with appropriate antibiotics. *E. coli* strain DH5α was used for all routine cloning, and strain BL21(DE3) was used for overexpression of recombinant proteins. All enzymes were purchased from Promega (Beijing) and used according to instructions. The plasmid pRL592 used for transposon mutagenesis of *Synechococcus* sp. PCC 7002 was a generous gift from Dr. P. Wolk (Michigan State University, East Lansing, MI). Plates of A solid medium with or without glycerol (10 mEq) and DCMU (10 μM) were used in replicate to identify cells that could not grow phototrophically. For construction of a fesM mutant (*fesM-D*), a DNA fragment containing fesM was amplified by PCR with primers P1 and P4 (Table III) using total genomic DNA as template. The PCR-generated fragment was cloned in pGEM-T vector (Promega) to generate plasmid pTv-FesM. The plasmid was inversely amplified by PCR with primers P2 and P3 (Table III), and the generated fragment was ligated with a blunt-ended fragment containing a kanamycin-resistant cassette. The resulting plasmid pFesM-kan was digested with HindIII and used to transform wild-type *Synechococcus* sp. PCC 7002. Segregated mutant was confirmed by Southern hybridization as described by Zhao et al. (1993). Total genomic DNA of *Synechococcus* sp. PCC 7002 was isolated with the E.Z.N.A. Plant DNA miniprep kit (Omega Bio-Tek, Beijing) and digested with EcoRI. The generated fragments were separated by 1.0% agarose gel and transferred onto nitrocellulose paper before being hybridized with a DNA probe for the fesM gene. For complementary studies of the fesM-D strain, the plasmid pTv-FesM was digested with HindIII, and the fragment containing fesM was cloned into pAQE to generate pAQE-FesM. A mutant fesM gene that encodes a truncated FesM lacking the first 184 amino acid residues was generated by inverse PCR of pAQE-FesM with primers Pcom1 and Pcom2 (Table III) and self-ligation of the PCR fragment to obtain pAQE-FesMΔ. A gene encoding streptomycin resistance was cloned into pAQE-FesMΔ to generate plasmids pAQE-FesMΔ and pAQE-FesMΔc, respectively. These plasmids were then transformed into fesM-D.

**Spectroscopic Measurements**

Cells in exponential growth were diluted with A medium to a Chl concentration of 5 μg mL⁻¹ for room temperature fluorescence measurements. Room temperature fluorescence (682 nm) induction in the presence of DCMU was measured as follows according to Huang et al. (2003). The cell suspension...
was first incubated in the dark for 2 min before actinic light was turned on for 90 s to bring the cells to state 1. The actinic light was then turned off for various times before it was turned on again for 2 s to measure the levels of \( F_{m} \) that were used as indicators of the redox status of the interphotonsystem electron carriers (Huang et al., 2003). Chl fluorescence was measured with a fluorescence spectrophotometer from Photon Technology (Lawrenceville, NJ) with the emission monochromator set at 682 nm (slit width of 1 nm). The photomultiplier was also protected by an interference filter (685 nm, bandwidth 10 nm). The \( F_{m} \) was measured by monitoring the absorbance difference of 820 nm minus 860 nm with a Walz PAM-101 system (Walz, Effeltrich, Germany) equipped with an ED-P700DW dual-wavelength unit for P700 measurement as described by Huang et al. (2003). Actinic light absorbed by both Chl and PBS was provided by a 300-W halogen light source. The light was passed through a filter that allows light with wavelengths shorter than 600 nm to pass (\(<600\) nm). The light intensity of the actinic light was adjusted to 500 \( \mu \)mol m\(^{-2}\) s\(^{-1}\). An argon laser emitting at 630 nm (50 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) was used as PBS-absorbing actinic light. Curve fitting was performed with a custom-prepared computer program. PSI and PSII contents were determined according to Zhao et al. (2001).

**Determination of FesM Membrane Topology by Alkaline Phosphatase Fusion**

The \( \text{phoA} \) gene from \( E. coli \) was amplified by PCR with the primers phoA1 and phoA2 (Table III). The fragment was digested by \( B_{am}H_{II} \) and \( X_{ho}l \) and ligated into pET30a (Novagen, Madison, WI) digested by the same enzymes to obtain the plasmid pET-PhoA. To generate various fusion genes linking N-terminal portions of \( fesM \) with \( \text{phoA} \), DNA fragments encoding the N-terminal portions of \( fesM \) were amplified by PCR with the primer L1 and the primers R1 through R13 (Table III). A total of 13 fragments were generated and digested by \( N_{de}l \) and \( B_{am}H_{II} \). The digested fragments were then ligated into pET-PhoA digested with the same enzymes. The generated plasmids pFesM1-phoA through pFesM13-phoA were transformed into \( B_{2}L_{1}(DE3) \) for analysis of extracellular phosphatase activity. As a control, the entire \( \text{fesM} \) gene was amplified by PCR with primers LF and RF (Table III) and cloned into pET30a to generate pET-FesM. All constructions were verified by DNA sequencing. For analysis of the fusion gene expression, the transformed cells were first induced by 0.5 mM IPTG for 3 h at 37°C before centrifugation. The cell pellets were resuspended and treated with 2× SDS sample buffer for SDS-PAGE/immunoblotting analysis. A monoclonal antibody against \( E. coli \) alkaline phosphatase (Sigma-Aldrich, St. Louis) was used as primary antibody. The extracellular phosphatase activity of \( B_{2}L_{1}(DE3) \) containing pFesM-phoA was determined both on plates and in liquid. For on-plate assays, the cells on agar plates were used as indicators of the redox status of the interphotosystem electron transport. Biochim Biophys Acta 1229: 23–38.

**Other Biochemical Methods**

Chlorophyll concentration was determined according to MacKinney (1941). Membrane isolation was performed by ultracentrifugation (Omata and Murata, 1983), followed by fractionation in a PEG/Textran two-phase system that was adapted for cyanobacterial membrane isolation (Norling et al., 1998). Analysis of proteins with SDS-PAGE was performed as described previously (Li et al., 2002). Immunoblotting was performed as described by Zhou et al. (1998). Glycogen content was determined according to Ernst and Boger (1984). Oxygen evolution and uptake were measured with a Clark-type oxygen electrode as described by Zhao et al. (1993). Protein N-terminal amino acid sequence was determined with an ABI491 protein sequencer (Applied Biosystems, Foster City, CA) after the protein was blotted onto a polyvinylidene difluoride membrane.

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Sequence data from this article have been deposited with the EMBL/GenBank database under accession number AY849033.

**ACKNOWLEDGMENTS**

We thank Dr. C.P. Wolk from Michigan State University for providing the plasmid pRL592 and Dr. X. Xu (Institute of Hydrobiology, Wuhan, China) for his help in constructing the mutant library of *Synechococcus* sp. PCC 7002. We also thank Dr. J. Brand (University of Texas, Austin) for his critical reading of the manuscript and valuable suggestions. Skillful technical assistance by Ms. C. Dong (Peking University, Beijing) is greatly appreciated.

Received February 17, 2005; revised March 28, 2005; accepted April 6, 2005; published June 24, 2005.

**LITERATURE CITED**


