Balanced regulation of expression of the gene for cytochrome c_M and that of genes for plastocyanin and cytochrome c_6 in *Synechocystis*

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Abstract The *cytM* gene for cytochrome c_M was previously found in *Synechocystis* sp. PCC 6803. Northern blotting analysis revealed that the *cytM* gene was scarcely expressed under normal growth conditions but its expression was enhanced when cells were exposed to low temperature or high-intensity light. By contrast, the expression of the genes for cytochrome c_6 and plastocyanin was suppressed at low temperature or under high-intensity light. These observations suggest that plastocyanin and/or cytochrome c_6, which are dominant under non-stressed conditions, are replaced by cytochrome c_M under the stress conditions.

Key words: Cytochrome c_6; Cytochrome c_M; Photosynthetic electron transport; Plastocyanin; Stress-induced regulation of gene expression; *Synechocystis*

1. Introduction

We previously discovered the *cytM* gene for cytochrome c_M in the chromosome of *Synechocystis* sp. PCC 6803 [1]. The deduced amino acid sequence of this cytochrome is about 40% homologous to that of cytochromes c_6 from cyanobacteria [2] and about 30% homologous to that of mitochondrial cytochrome *c* from *Tetrahymena pyriformis* [3]. The molecular mass of the predicted mature form of cytochrome c_M was estimated to be 8.3 kDa, and the isoelectric point was calculated to be 7.3 [1]. In cells grown under normal conditions, the level of cytochrome c_M is very low (K.K. Ho, personal communication).

In cyanobacteria and some eukaryotic algae, two electron carriers, plastocyanin and cytochrome c_6, transport electrons from the cytochrome b_6/f complex to the photosystem I complex or to cytochrome *c* oxidase [4–6]. The levels of these components are regulated by the amount of copper in the growth medium [7,8]. Cytochrome c_6 is dominant under copper-depleted conditions, whereas plastocyanin becomes dominant when the medium contains an adequate concentration of copper.

Since the size and solubility of predicted mature cytochrome c_M are similar to those of cytochrome c_6 and plastocyanin, it seems likely that cytochrome c_M might play a role that is similar to that of cytochrome c_6 and plastocyanin. However, inactivation of the *cytM* gene by targeting had no effect on growth, photosynthesis and respiration when cells were grown either in the standard medium or in Cu^{2+}-depleted medium [1].

In this work we studied expression of the *cytM* gene in comparison with that of the *petE* and *petJ* genes, that encode plastocyanin and cytochrome c_6, respectively, in cyanobacterial cells which had been subjected to stress conditions. We detected only very low levels of the *cytM* transcript under normal conditions but the level was increased upon a decrease in temperature or an increase in light intensity.

2. Materials and methods

2.1. Culture conditions

Synechocystis sp. PCC 6803 was obtained from the Pasteur Culture Collection (Paris, France) and cultured photoautotrophically at 34°C with continuous aeration with sterile air that contained 1% CO_2, as described previously [9]. Illumination was provided by incandescent lamps at an intensity of 70 μmol quanta m^{-2} s^{-1} (referred to as normal light) or 2000 μmol quanta m^{-2} s^{-1} (referred to as high-intensity light). The growth medium was BG-11 [10] supplemented with 20 mM HEPES-NaOH (pH 7.5). $CuSO_4$ was either omitted from the BG-11 medium or supplemented to give the final concentration of 1 μM. In the latter case a solution of $CuSO_4$ (Analytical Grade, Wako Pure Chemicals, Osaka, Japan) was added after sterilization by passing through a filter. For incubation in darkness, culture bottles were wrapped in aluminum foil.

2.2. Isolation of RNA and Northern blotting analysis

Isolation of RNA and Northern blotting analysis were carried out as previously described [11], with the exception that cell cultures were mixed with an equal volume of chilled 5% phenol in ethanol before harvest of cells by centrifugation. Probes for Northern blotting analysis, which covered the entire coding sequences of the corresponding genes, were amplified by polymerase chain reaction from plasmids that carried corresponding genes in the case of the *rplI* and *cytM* genes or from chromosomal DNA in the case of *petE* and *petJ* genes. Nucleotide coordinates in CyanoBase (*http://www.kazusa.or.jp/cyano/*): *cytM*, 1750681–1751067; *rplI*, 1751306–1751740; *petE*, 2525827–2526207; *petJ*, 845966–846328. Sizes of mRNAs were assessed by reference to mobilities of RNA molecular size markers (Life Technologies, Rockville, MD, USA). Data were quantified with a BAS2000 image-analyzing system (Fujitsu Co. Ltd., Tokyo, Japan).

3. Results and discussion

3.1. Stress-regulated cotranscription of the rplI and cytM genes

In Northern blotting analysis with a probe derived from the *cytM* gene (Fig. 1), the transcript of the *cytM* gene was barely detectable under normal growth conditions. When cells were exposed to a low temperature (22°C) and high-intensity light (2000 μmol m^{-2} s^{-1}), levels of the *cytM* transcripts of 1.3, 0.9 and 0.5 kb increased rapidly. The transcript of 0.5 kb corre-

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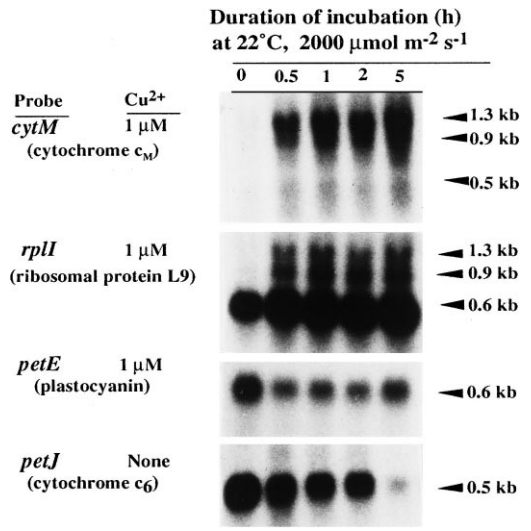

Fig. 1. Northern blotting analysis of transcripts of the *cytM*, *rplI*, *petE* and *petJ* genes in cells of *Synechocystis* sp. PCC 6803 at low temperature and under high-intensity light. Cells grown under normal conditions (34°C and 70 μmol quanta m^{-2} s^{-1}) in the presence of 1 mM Cu^{2+} ions or in their absence were transferred to 22°C and 2000 μmol quanta m^{-2} s^{-1}. At designated times, total RNA was extracted from the cells and subjected to Northern blotting analysis. Ten μg of RNA were loaded in each lane. Molecular sizes of transcripts were assessed by reference to mobilities of RNA molecular size standards.

sponded in size to that expected from the *cytM* gene, whereas the transcripts of 0.9 and 1.3 kb were longer than expected.

When we used a probe for transcription of the *rplI* gene, which is located upstream of the *cytM* gene [11], we detected only one transcript of 0.6 kb that corresponded to the *rplI* gene under normal conditions. When cells were exposed to the low temperature and the high-intensity light, the level of the 0.6-kb transcript increased 2.5-fold and additional transcripts of 0.9 and 1.3 kb were also detected (Fig. 1). The latter two transcripts appeared to be the same as those that had been detected with the *cytM* probe.

The structure of the genome, as shown in Fig. 2, suggested that the 1.3-kb transcript corresponded to the *rplI-cytM* cotranscript and the 0.9-kb transcript corresponded to the prematurely terminated *rplI-cytM* cotranscript. It was assumed that, under normal conditions, transcription of the *rplI* gene started at the promoter P_{rplI} and terminated at the terminator T_1, yielding a 0.6-kb transcript. Under stress conditions, the terminator T_1 became leaky and a substantial fraction of transcripts was extended to terminators T_2 and T_3, yielding the transcripts of 0.9 and 1.3 kb.

Some operons in prokaryotes produce multiple transcripts of different lengths according to environmental conditions. Under given conditions the transcription stops at a terminator which forms a stable stem loop structure, whereas under modified environment an antiterminator protein interacts with the respective terminator and allows reading through. Such operons include the amidase operon of *Pseudomonas aeruginosa* which is essential for this bacterium to grow with short-chain aliphatic amides [13], the *bgl* operon in *E. coli* [14] and the *sac* operon in *B. subtilis* [15] both for the utilization of aryl-β-glucosides and sucrose, and the *rrn* operon for ribosomal proteins in *E. coli* [16]. The *rbpA1-rpsU* operon for an RNA-binding protein and ribosomal protein S21 in *A. variabilis* yields transcripts of different lengths in response to changes in temperature [17,18].

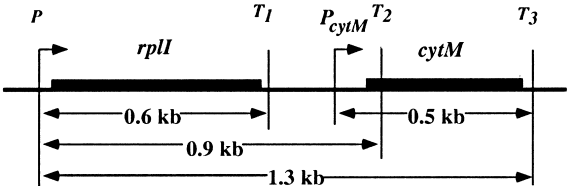

Fig. 2. A genetic map of the *cytM* and *rplI* genes with predicted sites of the initiation and termination of transcription.

3.2. Expression of the cytM, petE and petJ genes under stress conditions

The *petE* gene for plastocyanin [12] was expressed at a maximum level in the presence of Cu^{2+} ions under normal conditions (Fig. 1). The expression of the *petE* gene was down-regulated when cells were exposed to the low temperature and the high-intensity light. In the absence of Cu^{2+} ions, no *petE* transcript was detected under normal or stress conditions. The *petJ* gene for cytochrome c_6 was not expressed in the presence of Cu^{2+} ions but was maximally expressed in their absence under normal conditions. When cells grown in the absence of Cu^{2+} ions under normal conditions were exposed to low temperature and high-intensity light, the level of the *petJ* transcript decreased (Fig. 1). Thus, regulation of expression of the *petE* and *petJ* genes was the mirror image of that of the *cytM* and *rplI* genes.

Fig. 3 shows that multiple transcripts of the *cytM* gene were also detected when cells that had been grown in the absence of Cu^{2+} ions were exposed to a low temperature (22°C) or high-intensity light (2000 μmol quanta m^{-2} s^{-1}). The low temperature was more effective than the high-intensity light in inducing the 1.3-kb and 0.9-kb transcripts (the *rplI-cytM* cotranscripts), whereas the converse was true for the expression of the 0.5-kb transcript (the *cytM* transcript). After exposure of cells to either stress for 16 h, levels of the transcripts declined. In particular, the *rplI-cytM* cotranscripts disappeared 16 h after the start of exposure to high-intensity light.

Fig. 4 shows effects of low temperature and high-intensity light on the expression of *cytM* (i.e. the sum of the levels of

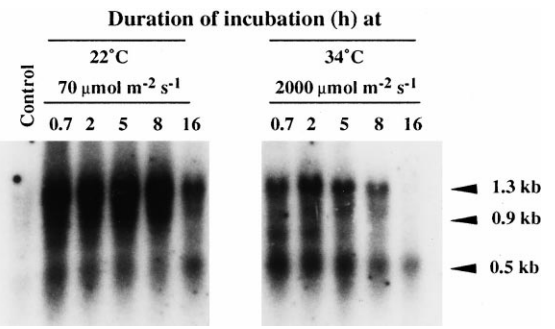

Fig. 3. Northern blotting analysis of *cytM* transcripts in cells of *Synechocystis* sp. PCC 6803 at low temperature or under high-intensity light. Cells grown in the absence of Cu^{2+} ions at 34°C and 70 μmol quanta m^{-2} s^{-1} (control) were transferred to 22°C and 70 μmol quanta m^{-2} s^{-1} or to 34°C and 2000 μmol quanta m^{-2} s^{-2}. At designated times, total RNA was extracted from cells and subjected to Northern blotting analysis with a probe derived from the *cytM* gene. Ten μg RNA were loaded in each lane.

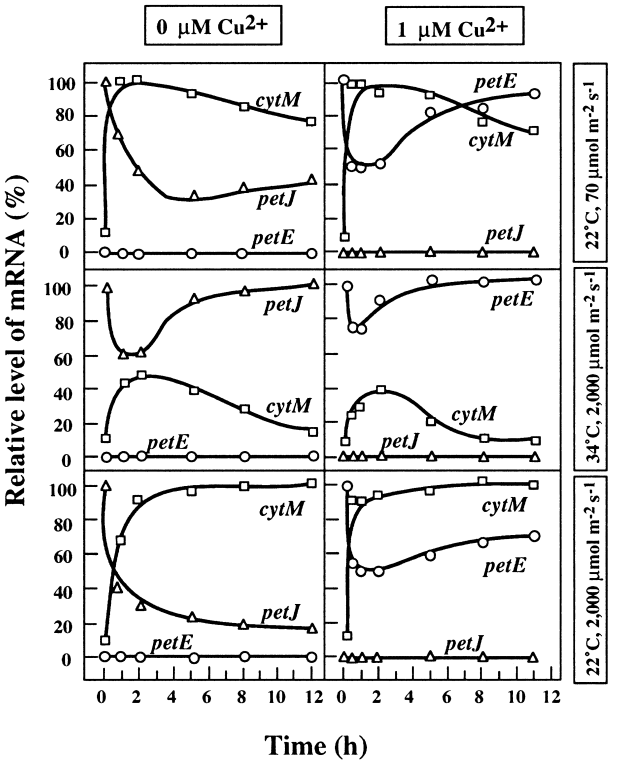

Fig. 4. Changes in levels of transcripts of the *cytM*, *petE* and *petJ* genes in cells of *Synechocystis* sp. PCC 6803 after a decrease in temperature and/or an increase in light intensity. Cells that had been grown under normal conditions (34°C, 70 μmol quanta m^{-2} s^{-1}) in the presence or absence of Cu^{2+} ions were exposed to 22°C or high-intensity light. Signals from all three fragments that hybridyzed with the *cytM* probe (0.5, 0.9 and 1.3 kb) were taken into account. Values shown are averages from at least two independent experiments.

the 0.5-kb, 0.9-kb and 1.3-kb transcripts), *petE* and *petJ* genes. When cells were grown in the absence of Cu^{2+} ions, only the *petJ* gene for cytochrome c_6 was expressed under normal growth conditions (i.e. 34°C, 70 μmol quanta m^{-2} s^{-1}). After the temperature had been shifted to 22°C, the level of the *cytM* transcript increased rapidly, reaching a maximum in one hour, and then it decreased gradually. The *petJ* transcript decreased to 30% of the original level in 5 h and then gradually increased. When high-intensity light (2000 μmol quanta m^{-2} s^{-1}) was applied, the level of the *petJ* transcript decreased transiently, whereas the level of the *cytM* transcript increased transiently. When low temperature and high-intensity light were applied together, the level of the *cytM* transcript reached a maximum level and the *petJ* transcript declined to 30% of the original level within 2 h.

When cells were grown in the presence of 1 mM Cu^{2+} ions under normal conditions (34°C, 70 μmol quanta m^{-2} s^{-1}), the level of the transcript of the *petE* gene for plastocyanin was maximum, whereas the transcript of the *petJ* gene for cytochrome c_6 was absent, as observed previously by Zhang et al. [2]. When cells were transferred to 22°C, the level of the *cytM* transcript increased rapidly to reach the maximum level in one hour and that of the *petE* transcript decreased to 50% of the original level. These changes were followed by a slow decline in the level of the *cytM* transcript and by a slow increase in the level of the *petE* transcript. When cells were transferred to high-intensity light (2000 μmol quanta m^{-2} s^{-1}), the level of the *petE* transcript decreased transiently and that of the *cytM* transcript increased transiently. When cells were exposed to low temperature and high-intensity light (i.e. 22°C, 2000 μmol m^{-2} s^{-1}), the level of the *cytM* transcript reached a maximum in one hour and remained high, whereas the level of the *petE* transcript decreased to 50% of the original level and then increased slowly.

In the presence and in the absence of Cu^{2+} ions, low temperature was much more effective in influencing the expression of the *cytM* gene than was high-intensity light. The combination of low temperature and high-intensity light had the maximum effect and, under such conditions, the level of the *cytM* transcript remained maximal for more than 12 h. However, high-intensity light and low temperature together were lethal if applied for longer than 12 h. The expression of the *petJ* gene in the absence of Cu^{2+} ions and that of the *petE* gene in the presence of Cu^{2+} ions responded to low temperature and high-intensity light in the opposite way to the expression of the *cytM* gene.

We also performed experiments in which cells grown under normal conditions in the presence of Cu^{2+} ions were transferred to 22°C in darkness. The level of the *cytM* transcript increased initially, whereas the level of the *petE* transcript decreased, as it did also after illumination at 70 μmol quanta m^{-2} s^{-1} (data not shown). However, the induction of the *cytM* gene was transient; the level of the *cytM* transcript returned to the original undetectable level within 4 h.

A similar form of stress-induced balanced regulation of gene expression has been demonstrated in the case of expression of the *psbAI* gene and *psbAII/III* genes for the D1:1 and the D1:2 isoforms of the D1 protein in the photosystem II complex in *Synechococcus* sp. PCC 7942 [19–21]. When cells are grown under normal conditions, the D1:1 isoform is dominant. Upon a decrease in temperature [21] or an increase in light intensity [22,23], the D1:1 isoform is replaced by the D1:2 isoform as a result of altered gene expression [20,24]. Overproduction of the D1:2 protein increases the resistance of the photosystem II complex to photo-induced inactivation [25]. Note, also, that repression of the *psbAI* gene is transient [20], resembling the transient repression of the *petE* and *petJ* genes.

The biological role of the balanced expression of cytochrome c_M versus cytochrome c_6 and plastocyanin is not yet clear. Replacement of cytochrome c_6 or plastocyanin by cytochrome c_M might be advantageous under stress conditions, such as low temperature and high-intensity light.

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