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Nuclear-encoded chloroplast RNA polymerase sigma factor SIG2 activates chloroplast-encoded phycobilisome genes in a red alga, *Cyanidioschyzon merolae*

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ABSTRACT

The phycobilisome (PBS) is a photosynthetic light-harvesting complex in red algae, whose structural genes are separately encoded by both the nuclear and chloroplast genomes. While the expression of PBS genes in both genomes is responsive to environmental changes to modulate light-harvesting efficiency, little is known about how gene expression of the two genomes is coordinated. In this study, we focused on the four nuclear-encoded chloroplast sigma factors to understand aspects of this coordination, and found that SIG2 directs the expression of chloroplast PBS genes in the red alga *Cyanidioschyzon merolae*.

1. Introduction

The phycobilisome (PBS) is a photosynthetic antennae pigment complex found in cyanobacteria and red algae for the efficient harvesting of light energy. The PBS is a macromolecular complex on the thylakoid membrane composed of core allophycocyanin (APC) proteins and stacked rod proteins such as phycocyanin (PC) and phycoerythrin (PE), and mainly transfers captured light energy to the reaction center of photosystem II (PSII) [1]. The abundance and composition of PBSs is modulated by a number of environmental parameters such as light intensity, light wavelength and nutrient availability [2–5]. As the underlying mechanisms, the expression of PBS genes has been suggested to respond to relevant environmental changes in cyanobacteria [4,6], while little information is available for red algae.

In red algae, PBS genes are complementarily encoded by both the nuclear and chloroplast genomes (see Table S1 for gene distribution in *Cyanidioschyzon merolae*). This architecture is considered to be a result of the endosymbiotic origin of the chloroplast and the subsequent transfer of chloroplast genes to the nuclear genome. However, encoding by two separate genomes requires fine-tuning of the two independent gene expression systems, the mechanism of which still remains largely unknown. Nuclear-encoded PBS genes are transcribed by the nuclear RNA polymerase II as in other eukaryotes, while chloroplast genes are transcribed by a bacterial-type chloroplast RNA polymerase descended from the cyanobacterial endosymbiont. This bacterial-type chloroplast RNA polymerase is likely responsible for all chloroplast transcription in unicellular algae, since there are no evidence of other types of chloroplast RNA polymerase in *C. merolae* as was found in seed plants [7]. In general, the subunits of the chloroplast RNA polymerase core enzyme are encoded by the chloroplast genome, whereas the specificity and transcription initiation factor, sigma, is encoded by the nuclear genome [8]. Thus, the nucleus controls chloroplast transcription through modulation of the expression and/or activity of sigma factor(s), which could be the coordination mechanism between the two genomes for PBS gene expression.

Abbreviations: bp, base pair(s); EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase(s); kDa, kilodalton(s); OD, optical density; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; SD, standard deviation; SDS, sodium dodecyl sulfate

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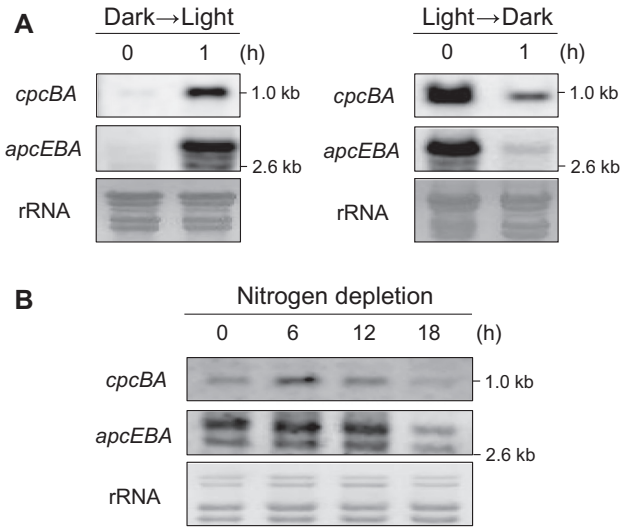

Fig. 1. Expression of chloroplast PBS genes under light/dark shifts and nitrogen depletion. (A) Changes of *apcEAB* and *cpcBA* transcript levels under light/dark shifts. *C. merolae* cells were harvested at the indicated times after dark-to-light or light-to-dark shift, and total RNAs were prepared. The RNAs (3 μg) were then subjected to Northern blot analysis with specific probes. The positions of molecular weight markers are shown on the right. The electrophoretic patterns of rRNAs stained with methylene blue are shown below as loading controls. (B) Changes of *apcEAB* and *cpcBA* transcript levels during nitrogen depletion. RNAs (1.5 μg) were subjected to Northern blot analysis as in (A).

C. merolae is a unicellular red alga that lives in acidic high-temperature environments (pH 1–3, 40–50 °C) and was isolated from an Italian hot spring [9]. Because of the extremely primitive features of the cell, *C. merolae* has been the subject of various basic analyses including elucidation of the complete genome sequences of the nucleus, chloroplast and mitochondrion [10–13], and the development of tools and systems for molecular genetics analyses [14–16]. Based on the nuclear genome sequence, four chloroplast sigma factor genes have been identified and designated *SIG1–4* (http://merolae.biol.s.u-tokyo.ac.jp/) [17]. In this study, we focused on and analyzed these nuclear-encoded sigma factors to understand the nuclear control of chloroplast PBS gene expression in red algae, and found that SIG2 directs the expression of chloroplast PBS genes.

2. Materials and methods

2.1. Strain and growth conditions

C. merolae 10D was cultivated in liquid MA2 medium [14] under continuous white light (50 μmol m^{-2} s^{-1}) at 40 °C, bubbled by air supplemented with 2% CO_2. For the M4 strain, 0.5 mg/mL uracil was added to the medium. Nitrogen depletion condition was as described in [15].

2.2. Construction of SIG overexpression and underexpression strains

Details of the construction of the *SIG2* overexpression and underexpression plasmids are described in the Supplementary methods. Transformation of the M4 strain was performed as described previously [14–16].

2.3. Immunoblot analysis

Immunoblot analysis was performed as described previously [18]. Antibodies against SIG1–4 were as described [19].

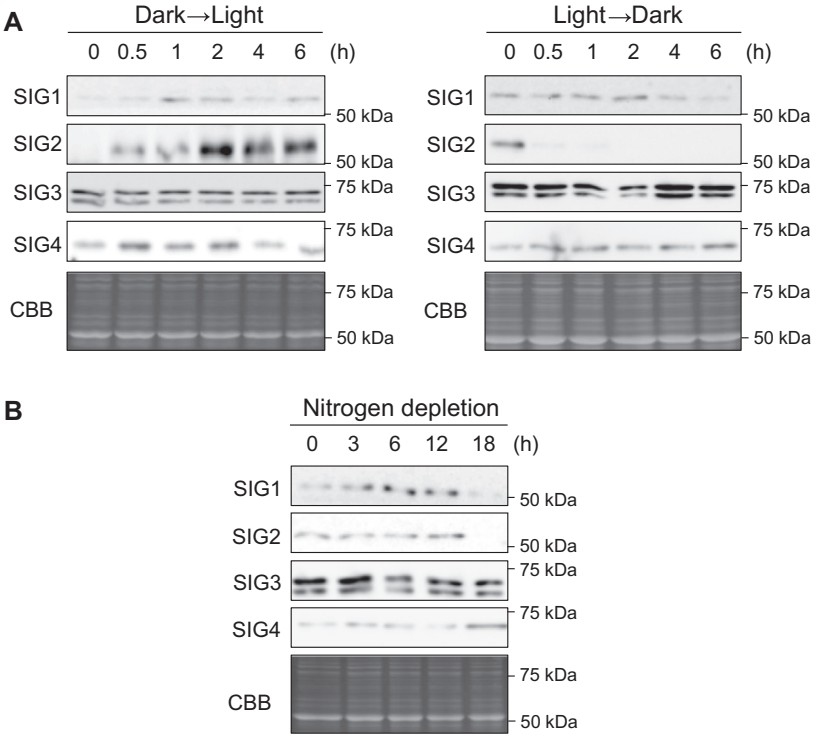

Fig. 2. Accumulation of sigma factor proteins under light/dark shifts and nitrogen depletion. (A) Protein levels of SIG1–4 during dark-to-light or light-to-dark shifts. *C. merolae* cells were harvested at the indicated times after dark-to-light or light-to-dark shifts. Aliquots containing 10 μg soluble protein from *C. merolae* cells were separated by 10% SDS–polyacrylamide gel electrophoresis (PAGE) and analyzed by immunoblot analysis with antibodies specific for each sigma (SIG1–4). The positions of molecular weight markers are shown on the right. Loaded proteins stained Coomassie Brilliant Blue (CBB) are shown below as a loading control. (B) Protein levels of SIG1–4 under nitrogen depletion. All other figure elements are as described in A.