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Redox regulation of PSI nuclear genes through photosynthetic electron flow.

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1 Introduction

Increasing evidences have demonstrated that the redox signals generated through photosynthesis play important roles in the regulation of photosynthesis genes in cyanobacteria, green alga and higher plants (Murakami *et al*. 1993, Escoubas *et al*. 1995, Maxwell *et al*. 1995, Pfannschmidt *et al.* 1999). Recently we found that the photosynthetic electron transport induces the Photosystem I (PSI) subunit mRNAs in *Chlamydomonas reinhardtii* (Matsuo and Obokata, manuscript submitted). In order to test if this light induction is regulated by the redox state of plastoquinone and/or cytochrome b_6f complex, here we designed special LED (light emitting diode) panels with which we can activate PSII and PSI, selectively. In this manuscript, we describe the properties of these monochromes in terms of chromatic response of *Chlamydomonas* cells, then address above questions using these LED panels.

2 Materials and Methods

2.1 Growth condition of *Chlamydomonas* cells

Chlamydomonas reinhardtii cc125 (+) cells were grown in HS medium containing 14.6 mM sodium acetate under a photoperiod of 16-hour white light (10 W / m^2) and 8-hour dark at 25°C. When monochrome was irradiated, light intensity was adjusted to 5 W / m^2.

2.2 LED panels

We designed two LED panels, which preferentially activate PSII and PSI, respectively. PSI-light panel was made with the LED, TLRH190P (Toshiba, Tokyo) whose peak wave lenghts was 644 nm with a band width of 18nm. PSI-light panel was made with 695 nm LED, NR312 (Stanley Electric Ltd.,Tokyo) with bandwidth of 30nm.

2.3 Detection and monitoring Chlorophyll fluorescence

Chlorophyll fluorescence of *Chlamydomonas* cells were monitored with PAM 101 / 103 Walz-apparatus (Heinz Walz GmbH, Effeltrich, Germany).

2.4 Isolation of total membrane fraction and LDS PAGE

Chlamydomonas cells were harvested, then suspended in ice-cold 0.1M Na_2CO_3 containing 0.1M DTT. After freezing and thawing of the cell suspension, total membrane fraction was recovered by centrifugation at 15,000 g for 10min. The obtained membranes were dissolved by 2% LDS (Lithium Dodecyl Sulfate), and electrophoresed in 15% polyacrylamide gel at 4 °C according to Delepelaire and Chua (1982). Chlorophyll concentration was measured accordeding to Harris (1989).

3 Result and Discussion

3.1 Illumination of PSII-light and PSI-light induced the state transition

First, we examined the short-term effect of our LED panels (PSII-light and PSI-light) on the photosynthetic electron transport. For this sake, we monitored the influences of these monochromes on the chlorophyll fluorescence of PSII. Fig.1 shows that the chlorophyll fluorescence (Fm) by saturating pulses was decreased by irradiation of PSII-light (Fm' II), and subsequent irradiation of PSI-light up-shifted the fluorescence signal (Fm' I). When the PSI-light was turned off, the transient chlorophyll fluorescence (*) was observed and fluorescence level decreased once again. Based on these, it was confirmed that the monochrome irradiation by our LED panels caused state transition in *Chalmydomonas* cells (Allen 1992, Lunde *et al.* 2000, Bissati and Kirilovsky 2001).

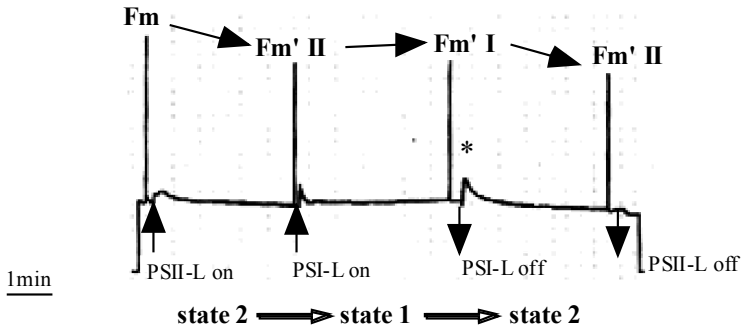

Fig.1 The effect of the irradiation of PSII-light (PSII-L) and PSI-light (PSI-L) on the chlorophyll fluorescence signal. The light intensity of each LED is 10 W / m^2. Scale bar is 1 min.

3.2 Composition of the chlorophyll protein complexes was altered by the chromatic treatment

Next, we examined the effects of those monochromes on the photosynthetic electron transport after a long time of illumination. When *Chlamydomonas* cells were irradiated with the monochromes for 24 hours, the chlorophyll content was altered in response to the illuminated light sources. As shown Fig. 2A, the cells irradiated with PSII-light has chlorophyll content twice of that of the PSI-light irradiated cells. In Fig. 2B, we compared chlorophyll protein complexes between PSI-light irradiated cells and PSII-light irradiated cells. These electrophoretic profiles demonstrate that the abundance of CPI and LHCPs were drastically changed by the irradiation of monochromes. These changes in the chlorophyll content and in the chlorophyll protein complexes are in good agreement with the phenomena previously reported for the chromatic adaptation of *Chlamydomonas* cells (Melis *et al.* 1996).

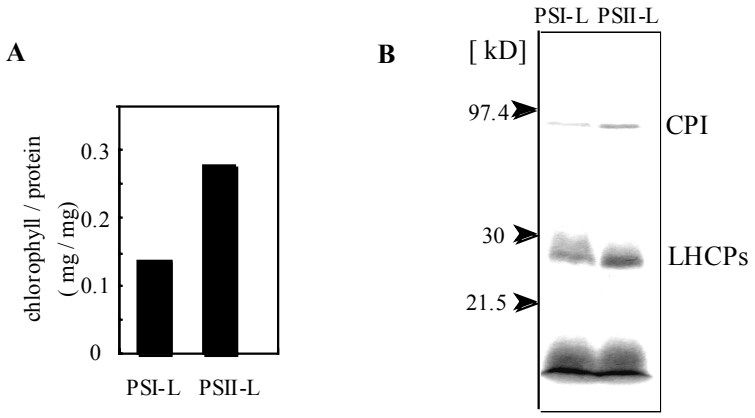

Fig. 2 The effect of PSI-light and PSII-light on the abundance of chlorophyll-protein complexes. (A) Chlorophyll contents of the membrane fraction, (B) Chlorophyll protein complexes in monochrome treated cells. Total membrane fractions containg 20 mg chlorophyll were separated by 15% LDS PAGE, and green bands of chlorophyll are discerned on the gel. The monochromes were irradiated for 24 hours.

3.3 The change of the redox state of the intersystem electron transport has no effect on PSI mRNA accumulations

As described above, the monochromes of LED panels induced the state transition and chromatic adaptation. This indicates that the monochromes altered the redox state of plastoquinone pool and/or cytochrome b_6f complex (Allen 1992, Vener *et al.* 1997, Pfannschmidt 1999). With the aid of the LED monochrome system, we examined whether the redox state of the electron carriers between two photosystems regulate the expression of PSI genes. When *Chlamydomonas* cells were irradiated with the PSI-light or PSII-light for 24 hours, the induction profiles of *psaE* mRNA were almost the same between two light sources (Matsuo and Obokata. manuscript submitted). From these, we concluded that the light induction of PSI subunit mRNA occurres irrespective of the redox state of intersystem electron carriers in *Chlamydomonas reinhardtii.*

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