Blue light specific and differential expression of a plastid σ factor, Sig5 in *Arabidopsis thaliana*

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Abstract The transcription of plastid gene *psbD* is under the control of the BLRP (blue-light-responsive promoter) recognized by plastid-encoded RNA polymerase, in which nuclear-encoded σ factors play a crucial role in the promoter recognition. We examined the effects of light on mRNA levels of six different *SIG* genes in *Arabidopsis* and found that blue light extensively induced the accumulation of *SIG5* transcripts, but red light did not. The blue light specificity was not observed in the accumulations of remaining five *SIG* genes. The blue light dependency of the *SIG5* expression well explains the light-dependent behavior of the *psbD* BLRP.

Key words: RNA polymerase; Blue light; σ factor; Sig5; *psbD*

1. Introduction

Expression of chloroplast *psbD* gene encoding the D2 subunit of the photosystem II reaction center is transcriptionally controlled by light. High-fluence blue light, but not red light, differentially activates *psbD* transcription from a blue light-responsive promoter (*psbD* BLRP) that is conserved among higher plants [1–3]. Even when the total plastid transcription declines as a consequence of chloroplast maturation, the *psbD* BLRP maintains high activity as to assist with maintaining the synthesis of D2, which is photo-damaged and undergoes rapid turnover in high light condition [1,4].

The *psbD* BLRP is recognized by the bacterial-type plastid-encoded RNA polymerase (PEP), but it is an unusual σ^{70}-type promoter that requires the '−10' element for transcription, but not the '−35' element. Instead, two well-conserved sequence elements, termed PGT box and AAG box, are located upstream of the '−35' position [5–8]. In general, bacterial RNA polymerases are regulated by σ factors mediating the promoter recognition and by DNA-binding proteins functioning as activator or repressor. Since the plastome of higher plants encodes neither gene for σ factors nor DNA-binding protein, it is assumed that the blue light-dependent activation of the *psbD* BLRP is mediated by nuclear-encoded regulatory factors.

There are several proteins interacting with the AAG and PGT boxes of the *psbD* BLRP. However, it is reported that the PGT box is not necessary to activate the *psbD* transcription in vivo [9]. While, the AAG-box binding proteins (AGF) are constitutively expressed independent of light in matured chloroplasts [6,7]. Recently, PTF-1 that specifically binds to the ACC repeat in the AAG box was cloned in *Arabidopsis* [10]. Inactivation of PTF-1 by T-DNA insertion partially reduced the *psbD* BLRP activity, but did not eliminate the light-dependent transcription. These evidences suggest that AGF and PTF-1 act as positive regulators that generally enhance the *psbD* BLRP activity rather than the blue light-responsive factors that switch on the *psbD* BLRP activity.

The subunits of the core enzyme of PEP are encoded in plastid genome [11], while plastid σ factors are encoded in nuclear genome. In *Arabidopsis thaliana*, there are six putative plastid σ genes, which are homologous to bacterial group 1 and 2 σ factors [12–14]. We previously showed that in wheat chloroplasts, PEP changes its promoter preference depending on the light condition and the developmental stage of leaves possibly through the differential usage of the heterogeneous σ factors [6,15]. These findings might indicate that the blue light-induced differential expression of a particular σ factor is involved in the mechanism of the blue light-dependent activation of the *psbD* BLRP.

In this work, we examined the effect of light on the accumulations of the transcripts from the six *SIG* genes in *A. thaliana* and compared them with the blue light-dependent behavior of the *psbD* BLRP. The results suggest that Sig5 is differentially expressed by blue light as to recognize the *psbD* BLRP and initiate the transcription from the promoter with the help of blue light-non-specific high-fluence irradiation.

2. Materials and methods

2.1. Plant materials, growth conditions and light treatment

A. thaliana, ecotype Columbia, was grown on vermiculites for 4 weeks at 22°C under continuous white light (10–20 μmol m^{-2} s^{-1}). Plants employed for Northern blot analysis were exposed to white or blue and/or red light after dark-adaptation for 16 h. LED panels (LED-B or LED-R, Eyela) were used as light sources for blue (470 nm, FWHM (full width at half maximum) 30 nm) or red (660 nm, FWHM 20 nm) light and their photon-fluence rates were measured using a quantum photometer (LI-250, Li-Cor). White light was provided by a fluorescence lump (FPL27AX, Mitsubishi).

2.2. Northern blot analysis

Total RNAs were extracted from rosette leaves of *Arabidopsis* by RNeasy Plant Mini kit (Qiagen). cDNA fragments (1030–1509 of

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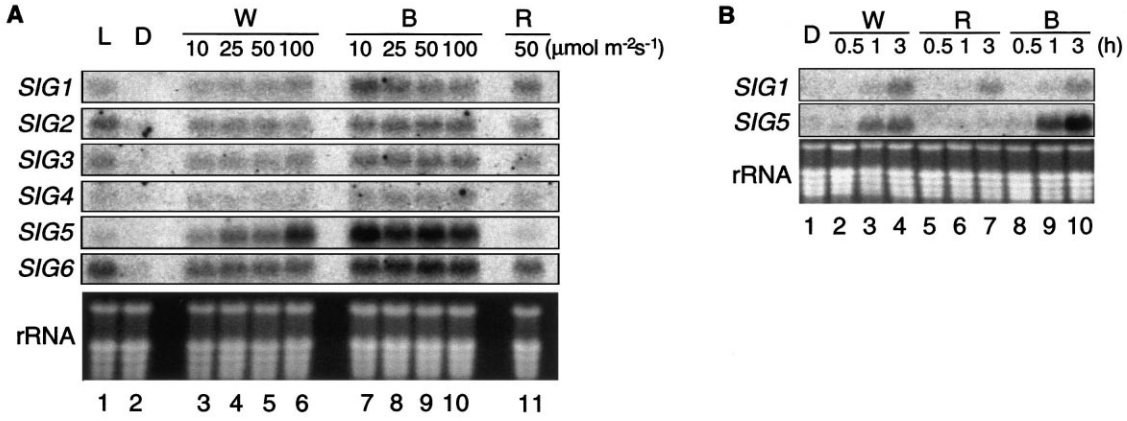

Fig. 1. Light-dependent accumulation of *SIG* transcripts in *Arabidopsis*. A: Effects of light quality and intensity on the expression of *SIG* genes. Light-grown plants (L) were dark-adapted for 16 h (D) and re-illuminated with white (W), blue (B) or red (R) light for 3 h at indicated fluences (μmol m^{-2} s^{-1}). Total cellular RNA (10 μg) was subjected to RNA gel-blot hybridization with six *SIG* probes. The ethidium bromide-stained rRNAs serve as gel-loading references. B: Time course of the induction of *SIG1* and *SIG5* transcripts by light. Dark-adapted plants (D) were re-illuminated with white (15 μmol m^{-2} s^{-1}), red (50 μmol m^{-2} s^{-1}) or blue (50 μmol m^{-2} s^{-1}) light for 0.5, 1 and 3 h. The amount of total cellular RNA per lane was 7 μg.

SIG1 (AB019942), 1048–1719 of *SIG2* (AB019943), 1049–1716 of *SIG3* (AB019944), 739–1260 of *SIG4* (AB021119), 1096–1554 of *SIG5* (AB021120), 1158–1644 of *SIG6* (AB029916)) were amplified by PCR and used as probes for Northern analyses. The CDR probe (+78 to +1002 of the *psbD* translational start codon) was designed to detect the transcripts produced from all of multiple promoters in the *psbD/C* operon, and the UTR probe (−1085 to −276) to detect specifically the transcripts from the *psbD* BLRP. DNA fragments were labeled with [α-^{32}P]dCTP using RTG DNA labeling beads (Amersham Pharmacia). 10 μg of total RNAs were separated on 1.0% agarose–formaldehyde denaturing gels, transferred onto a Hybond N+ membrane (Amersham Pharmacia), and hybridized at 60°C for 18 h with the labeled DNA probes. Final wash conditions were 0.1×SSC, 0.1% SDS at 60°C for 30 min.

3. Results

3.1. The effect of light on the transcript accumulation of six SIG genes in Arabidopsis

Light-dependent accumulation of the transcripts of *SIG* genes was reported in various plants including *Arabidopsis* [12–14,16–21], but the effects of the light quality and strength on the expression of each *SIG* gene remain unknown. Here we examined the effect of white, blue (470 nm) or red (660 nm) light on the accumulation of the transcripts of *SIG1* through *SIG6* genes in *Arabidopsis* by Northern blot analyses. As shown in Fig. 1A, the transcripts of all *SIG* genes were accumulated in rosette leaves of the plants grown under continuous white light for 4 weeks (Fig. 1A, L), although extents of their accumulation were dependent on the genes as *SIG6* > *SIG2* > *SIG3* > *SIG1* > *SIG5* = *SIG4*. The transcripts of all genes observed in the light-grown leaves virtually disappeared after dark-adaptation for 16 h (Fig. 1A, D), and recovered almost to the original levels by exposure to white light of 10 μmol m^{-2} s^{-1} (Fig. 1A, W) within 3 h except for the *SIG5* transcript. In the case of *SIG5*, accumulation of the transcript significantly exceeded the original level. Increase of the white light fluence to 100 μmol m^{-2} s^{-1} enhanced the accumulation of the *SIG5* transcript but scarcely affected to other genes. A dramatic light quality effect was observed in the accumulation of *SIG5* transcript. Blue light of 10–100 μmol m^{-2} s^{-1} extensively induced the *SIG5* transcript, but red light of 50 μmol m^{-2} s^{-1} never induced the *SIG5* transcripts. The *SIG6* transcripts were induced by both of blue and red lights, although the effect of blue light slightly exceeded that of red light. In the cases of the other *SIG* genes, blue and red lights of 50 μmol m^{-2} s^{-1} showed almost equal effects on the induction of their transcripts. Change of the blue light fluence from 10 to 100 μmol m^{-2} s^{-1} did not make a significant difference in the induction of transcripts from all *SIG* genes. Fig. 1B compares the time courses of the accumulation of the *SIG1* and *SIG5* transcripts induced by white, blue and red lights. The transcript of *SIG1* was observed after 3 h illumination but not 1 h irrespective of light quality, while the transcript of *SIG5* appeared after 1 h illumination with white or blue light.

3.2. Comparison of the effect of blue light on the accumulation of the SIG5 transcript and the psbD BLRP activity

It would be interesting to compare the effect of blue light on the induction of the *SIG5* transcript and that on the *psbD* BLRP activity. As shown in Fig. 2, the *psbD* CDR probe (see Section 2) detected four bands (4.5, 3.7, 2.8 and 2.6 kb) in the leaves illuminated with blue light of 100 μmol m^{-2} s^{-1} (lane 12). Among them, the 4.5 and 3.7 kb transcripts were

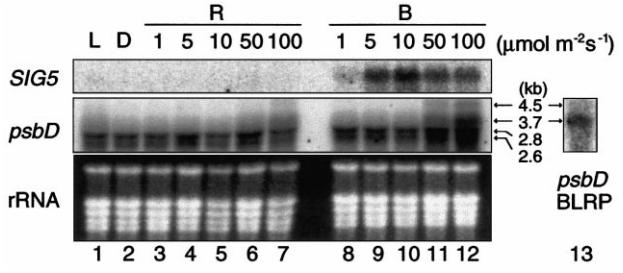

Fig. 2. Comparison of the light-dependent accumulation of *SIG5* and *psbD* transcripts. Light-grown plants (L) were dark-adapted for 16 h (D) and re-illuminated with red (R) or blue light (B) for 3 h at indicated fluences (μmol m^{-2} s^{-1}). Total cellular RNA (7 μg) was subjected to RNA gel-blot hybridization with the *SIG5* and *psbD* CDR probes (lanes 1–12). The *psbD* UTR probe was used to detect transcripts from the *psbD* BLRP in leaves illuminated 100 μmol m^{-2} s^{-1} of blue light for 3 h after dark-adaptation (lane 13). The ethidium bromide-stained rRNAs serve as gel-loading references.

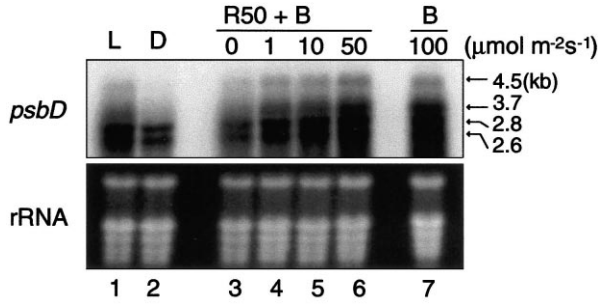

Fig. 3. The effect of blue light intensity on the *psbD* BLRP activity under the simultaneous illumination with an intensive red light (50 μmol m^{-2} s^{-1}). Light-grown plants (L) were dark-adapted for 16 h (D) and simultaneously re-illuminated with 50 μmol m^{-2} s^{-1} of red and blue light at indicated fluences (μmol m^{-2} s^{-1}) for 3 h following dark-adaptation. Total cellular RNA (7 μg) was subjected to RNA gel-blot hybridization with the *psbD* CDR probe. The ethidium bromide-stained rRNAs serve as gel loading references.

detected by the *psbD* UTR probe (lane 13), which was designed to detect specifically the transcripts from the *psbD* BLRP based on the 5′-ends mapping analysis of *psbD* transcripts in *Arabidopsis* [3], indicating that these two transcripts were originated from the *psbD* BLRP. These BLRP transcripts were induced by high-fluence of blue light (lanes 8–12), but not red light (lanes 3–7) irradiation. These results were in agreement with the previous findings [3,22]. These two transcripts strongly induced by 100 μmol m^{-2} s^{-1} blue light (lane 12) were reduced to less than one fifth when the blue light intensity was at 50 μmol m^{-2} s^{-1} (lane 11), and were undetectable at less than 10 μmol m^{-2} s^{-1} (lane 10). Contrary to the case of the *psbD* BLRP transcription, the *SIG5* transcript was detected at a low intensity such as 1 μmol m^{-2} s^{-1} of blue light (lane 8) and the transcript level increased with intensifying the light up to 5 μmol m^{-2} s^{-1} and saturated (lanes 9–12). This result seems to indicate that there is a great difference in the intensity requirement of blue light between the activation of the *psbD* BLRP and the expression of *SIG5*. As shown in Fig. 3, however, a weak blue light such as 1 μmol m^{-2} s^{-1} that induced the *SIG5* transcript was able to induce the accumulation of the 4.5 and 3.7 kb transcripts from the *psbD* BLRP to the original level (lane 1), when the leaves were simultaneously illuminated with 50 μmol m^{-2} s^{-1} red light. Simultaneous illumination with 50 μmol m^{-2} s^{-1} red light and 50 μmol m^{-2} s^{-1} blue light (lane 6) was able to enhance almost equivalently the accumulation of these two transcripts from the *psbD* BLRP to the illumination with 100 μmol m^{-2} s^{-1} blue light (lane 7). These evidences indicate that red light equally satisfies the requirement of high light intensity to the enhancement of the *psbD* BLRP activity, as long as a weak blue light is present.

4. Discussion

Phylogenetic analysis revealed that higher plant σ factors so far known were similar to the bacterial principal σ factors (group 1 or 2 σ factors) and formed a monophyletic group [14]. Among plant σ factors, *Arabidopsis* Sig5 appears isolated from other σ factors in a phylogenic tree constructed by analyzing different plant σ factors [14] and intron sites of *SIG5* are distinct among *Arabidopsis SIG* genes, possibly indicating an unique function of Sig5 in plastid transcription. In this work, we found that all of *Arabidopsis* σ factors are expressed light-dependently in matured leaves, but the expression of *SIG5* is significantly different from the others in the selectivity for blue light and the rapid response to the light. These evidences may suggest Sig5 to be a special σ factor, which contributes to a blue light-dependent and gene-specific transcription in plastids.

The accumulation of 4.5 and 3.7 kb *psbD* transcripts derived from the *psbD* BLRP are notably induced in *Arabidopsis* matured leaves by the illumination with high-fluence blue light (100 μmol m^{-2} s^{-1}) but not with monochromatic red light, in agreement with the previous work [1]. Inhibitor experiments had suggested that blue light regulated the expression of nuclear factors that comprise the pathway activating the *psbD* BLRP [23–25], but the key factor induced by blue light to activate the *psbD* BLRP has not been identified yet. The blue light-specific and differential response of the *SIG5* transcription seems to indicate that *SIG5* is the nuclear-encoded key factor, which mediates blue light signal from cytoplasm/nuclei to chloroplasts and functions as a molecular switch to activate the *psbD* BLRP.

The strength of monochromatic blue light required for activating the *psbD* BLRP was higher than that for inducing the transcription of *SIG5* by one order of magnitude. It has been a common understanding that the *psbD* BLRP requires high-fluence of blue or UV-A light to be activated, although the observation indicating that far-red light given together with high-fluence of blue light enhanced the *psbD* transcription in a synergistic manner in barley was reported [26]. In this work, however, we revealed that the requirement of high light intensity for the activation of the *psbD* BLRP was not confined to blue light, but red light was almost equally effective under simultaneous illumination with low-fluence of blue light such as 1 μmol m^{-2} s^{-1}, which can induce the *SIG5* transcription. These evidences strongly suggest that the effect of blue light and that of high light intensity on the activation of the *psbD* BLRP originate from the different molecular events mediated by at least two photo-sensory pathways. Comparing the blue light-dependent behaviors of the accumulation of the *SIG5* transcript and that of the transcripts from the *psbD* BLRP, it seems quite probable that blue light-dependent expression of Sig5 is essential for the *psbD* BLRP to be recognized by PEP. However, the accommodation of Sig5 to PEP is not enough to initiate the transcription from the *psbD* BLRP effectively. Other unknown events induced by the high-fluence light are also required. In vitro transcription analyses in wheat indicated that the deletion of two enhancer regions of the *psbD* BLRP resulted in a severe reduction of the transcription activity, although the light-responsive transcription was still observed [6,23]. High-fluence light might induce the full activity of PEP on the *psbD* BLRP transcription via the modifications of enhancer proteins and/or PEP. Thus, the blue light-induction of *SIG5* expression observed in this work well explains the light-responsive activation of the *psbD* BLRP, although the direct evidence has not been obtained. It will be interesting to examine whether over-expression of Sig5 results in the continuous activation of the *psbD* BLRP.

Blue light responses are mediated by at least two different types of photoreceptors, cryptochromes (cry1 and cry2) and phototropins (nph1 and npl1). Since the activity of the *psbD* BLRP was repressed in a *CRY1*/*CRY2* double mutant [27], it is likely that both cry1 and cry2 are involved in the blue light-

specific pathway, regulating the *psbD* BLRP activity possibly through the activation of *SIG5* gene. On the other hand, the BLRP activity was also repressed in phytochrome (*phy*A) mutants [27]. PhyA signaling pathway might be involved in the high-fluence light requirement of the *psbD* BLRP activation. Alternatively, the high-fluence response of the *psbD* BLRP might be related to the highly reduced redox state in plastids generated under the high light intensity.

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