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Analysis of promoter selectivity of plastid sigma factors in *Arabidopsis thaliana*

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Introduction

There are multi-transcription apparatuses in the plastids of higher plants; the nuclear-encoded T7 bacteriophage-type RNA polymerase (NEP) and the plastid-encoded eubacteria-type RNA polymerase (PEP). The transcription of many photosynthesis genes is mediated by PEP. The subunits of the core enzyme of PEP are encoded by plastid genome. However, plastid sigma factors, which are considered to be the promoter recognition subunit of PEP, are encoded in nuclear genome. In *Arabidopsis thaliana*, there are six putative plastid sigma genes homologous to bacterial group 1 and 2 sigma factors. It is hypothesized that each sigma factor recognizes different sets of promoters and regulated the activity of PEP in response to the developmental stage and various environmental clues. To analyze the promoter selectivity of sigma factors, we over produced each of six sigma factors in *Arabidopsis* protoplasts using the transient expression method and measured the transcription activity of a set of plastid genes in each protoplast preparation by the run-on assay. Over-production of Sig1, Sig2 and Sig5 elevated the transcription activities of different sets of plastid genes, indicating differential promoter recognition property of each sigma factor. Interestingly, the over-production of Sig5 specifically enhanced the transcription of *psb*A and *psb*D genes, which encode D1 and D2 proteins of photosystem II respectively. The main promoter upstream of *psb*D is known as the blue light responsive promoter (psbD BLRP), since it is activated by high-fluence blue and UV-A light (Christopher and Mullet, 1994). The accumulation of *sig*5 transcripts was induced only by blue light, while blue and red light equally induced the accumulation of other *sig* genes transcripts. From these results, we concluded that Sig5 is involved in the blue light-dependent activation of *psb*D BLRP in illuminated mature

leaves.

Materials and methods

Plant materials, growth conditions and light treatment.

Arabidopsis thaliana, ecotype Columbia, was grown for 4 weeks at 22 ºC 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 16h. LED panels (LED-B or LED-R, Eyela) were used as light sources for blue (470nm) or red (660nm) light.

Construction of plasmids.

cDNAs of *sig* genes were amplified by PCR from *Arabidopsis* cDNA library based on their nucleotide sequence data taken from GeneBank (accession nos. AB019942-4, AB021119-20 and AB029916 for *sig*1-6 respectively). The PCR fragments were subcloned into the pTM vector, which is a pUC18-based plasmid that contains the 35S CaMV promoter and the nopaline synthase (NOS) polyadenylation region.

Run-on transcription assay.

Protoplasts were prepared from 10g of rosette leaves of *Arabidopsis*, basically according to Abel and Theologis's method. The isolated protoplasts ($7.5x10^6$ protoplasts) were transformed with 0.25mg of plasmid DNAs. The transformed protoplasts were incubated in the dark at 22 ºC for 16h. After the incubation, transformed protoplasts were added to the run on reaction mixture containing 10mM $MgCl_2$, 40mM KCl, 13.8mM Hepes-NaOH (pH7.9), 5mM ATP, 5mM CTP, 5mM GTP, 0.5mM UTP, 0.1mCi [α^{32}P] UTP and 1mg ml^{-1} heparin. The protoplasts were incubated for 10min at 25 ºC. Run-on transcripts were then hybridized with probes of 300ng each blotted onto a Hybond N^+ membrane (Amersham pharmacia). PCR fragments, with an average size of 900 nucleotides, covering *psa*A, *psb*A, *psb*B, *psb*D, *psb*E-F-L-J, *ndh*F, *rpo*A, *rpo*B, *rbc*L, *atp*B, *trn*E-Y-D, *rrn*16S and *sig*1 of *Arabidopsis*, and MspI digested pBR322, pUC18 and λDNA were used as run-on probes. Hybridized blots were autoradiographed after washing.

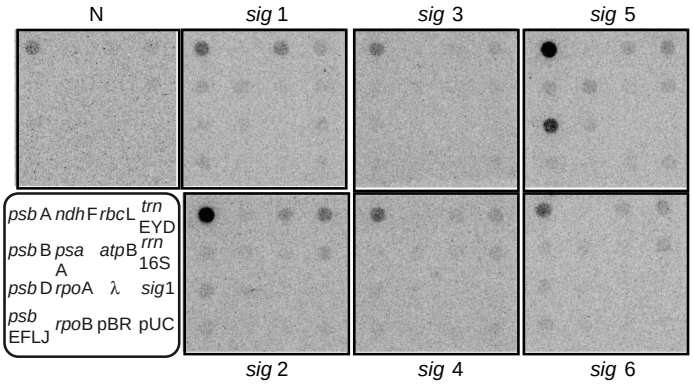
Northern blot analysis.

cDNA fragments (1030-1509 of *sig*1, 1048-1719 of *sig*2, 1049-1716 of *sig*3, 739-1260 of *sig*4, 1096-1554 of *sig*5, 1158-1644 of *sig*6) were amplified by PCR and used as probes for Northern analyses. 10 μg of total RNAs were separated on 1.0% agarose-formamid denaturing gels, transferred onto a Hybond N^+ membrane (Amersham pharmacia), and hybridized at 60C for 18h with the labeled DNA probes. Final wash conditions were 0.1x SSC, 0.1% SDS at 60C for 30min.

Results

To examine promoter preference of each sigma factor, we transformed the protoplasts prepared from *Arabidopsis* leaves with the expression vectors pTM-*sig*1, *sig*2, *sig*3,

*sig*4, *sig*5 and *sig*6 and submitted to transcriptional run-on assays for a set of plastid genes. A typical example of the transcription patterns in protoplasts expressing each plastid sigma factor is illustrated in Fig.1. Transformation with pTM-*sig*1 enhanced the transcription of *psa*A, *psb*B, *psb*EFLJ gene cassette and *rbc*L, but not *psb*A and *psb*D. Sig2 specifically enhanced the transcription of *psb*A and *trn*EYD, whose transcription was not activated by Sig1. Transformation of protoplasts with Sig3 and Sig4 expression vectors did not influence the transcription pattern of plastid genes. The most interesting results were obtained with the protoplasts transformed with pTM-*sig*5 to over produce Sig5. Sig5 dominantly and greatly enhanced the transcription of *psb*A and *psb*D, although some other photosynthesis genes were weakly enhanced. Although the enhanced transcription of *psb*A was also observed in the Sig2 over produced protoplasts, enhancement of *psb*D transcription was observed only in the Sig5 over produced protoplasts.



Light-dependent accumulation of the transcripts of *sig* genes was reported in various plants including *Arabidopsis*, but the effect of the light quality on the each *sig* gene accumulation remains unknown. We examined the effect of white, blue (470nm) or red (660nm) light on the accumulation of the transcripts of *sig* 1 through 6 genes by a Northern blot analysis. As shown in Fig.2, the transcripts of all *sig* genes were accumulated in rosette leaves of the plants grown under continuous white light for 4weeks (L), disappeared after dark adaptation for 16h (D), and recovered almost to the original levels by exposure to white light (W) within 3h except for *sig*5 transcripts. Accumulation of *sig*5 transcripts significantly exceeded the original level. Blue and red lights showed almost equal effects on the induction of the *sig* gene transcripts except for *sig*5. In the case of *sig*5, the transcript was tremendously accumulated in blue light, but not in red light.

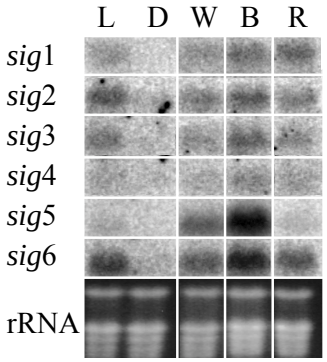


Fig.2 Effects of light quality on the expression of *sig* genes. Light-grown plants (L) were dark adapted for 16 h (D) and re-illuminated with white (W, 15 μmol m^{-2} s^{-1}), blue (B, 50 μmol m^{-2} s^{-1}) or red (R, 50 μmol m^{-2} s^{-1}) light for 3h. The ethidium bromide-stained rRNAs serve as gel loading references.

Discussion

We demonstrated that over production of Sig1, Sig2 and Sig5 enhanced the transcription of different sets of genes. From these results, it was revealed for the first time that there is the heterogeneity in promoter specificity among plastid sigma factors. Over production of Sig1 promotes the transcription of some photosynthetic genes. All photosynthesis genes enhanced by Sig1 over-production in this work share the well-conserved -10/-35 elements. Sig1 is supposed to be one of the general sigma factors recognizing typical σ^{70}-type PEP promoters. In mature chloroplasts, *psb*D and *psb*A genes are exclusively transcribed. These genes are transcribed from unique PEP promoters. The *psb*D BLRP possesses upstream enhancer region (AGT motif) but lacks the functional -35 elemnt. The TGn motif and TATA-like sequence in the core of *psb*A promoter can substitute for the -35 element. Interestingly, the over production of Sig5 selectively enhanced both *psb*D and *psb*A transcription. Thus, it appears that Sig5 recognizes uncanonical promoters, such as the *psb*D BLRP and the *psb*A promoter harboring unique core structures and supports the selective transcription of these two genes in mature leaves under illumination. Uniqueness of Sig5 is also detectable in phylogenetic relationship analysis. Sig5 falls into a subgroup, which is different from that of the other sigma factors and intron sites of *sig5* gene are distinct among all *Arabidopsi sig* genes (Fujiwara *et al*., 2000).

The blue light specific response of the *sig5* transcription and the selective enhancement of the *psb*A and *psb*D transcription by *sig5* found in this work lead us to a proposal that *sig5* is the nuclear encoded plastid factor which mediates blue light signal from cytoplasm/nuclei to chloroplasts and functions as a molecular switch to activate the *psb*D BLRP.

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