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Transients in chloroplast gene transcription

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Abstract

Transcriptional regulation of chloroplast genes is demonstrated by Quantitative Polymerase Chain Reaction (qPCR). These genes encode apoproteins of the reaction centres of photosystem I and photosystem II. Their transcription is regulated by changes in wavelength of light selectively absorbed by photosystem I and photosystem II, and therefore by the redox state of an electron carrier located between the two photosystems. Chloroplast transcriptional redox regulation is shown to have greater amplitude, and the kinetics of transcriptional changes are more complex, than suggested by previous experiments using only DNA probes in Northern blot experiments. Redox effects on chloroplast transcription appear to be superimposed on an endogenous rhythm of mRNA abundance. The functional significance of these transients in chloroplast gene transcription is discussed.

Keywords: Photosynthesis; Redox control; Plastoquinone; Plastid genome; Gene expression; Electron transport

Regulation at the level of transcription of DNA to RNA has long been recognized as a principal mechanism for modulating gene expression in biological systems [1]. Eukaryotic plants and algae contain chloroplasts, which perform photosynthesis [2] and also contain a functional genetic system [3]. Gene expression in chloroplasts has been viewed as an exception to the rule of transcriptional control [4,5]. The proponents of this view draw from the observation of relatively long-lived chloroplast mRNA transcripts, and argue that all gene regulation in chloroplasts is post-transcriptional. However, the notion of the non-regulation at the transcriptional level was challenged by a series of studies demonstrating specific light quality induced changes in the transcriptional pattern of chloroplast genes [6–8]. The exact nature of the action of light on chloroplast transcription was revealed by the use of various electron transport inhibitors—light-regulated transcriptional changes of chloroplast genes are, in fact, mediated by alterations in the reduction–oxidation (redox) state of a component of the photosynthetic electron transport chain. These studies employed the use of specific DNA probes and traditional nucleic acid blotting techniques to monitor transcript abundance and transcript initiation of chloroplast genes. Here we use quantitative PCR technique to follow changes in transcript abundance of two universally chloroplast-encoded reaction centre genes—*psaA*, which encodes the photosystem I reaction centre apoprotein PSI-A; and *psbA*, which encodes the photosystem II reaction centre apoprotein D1 (synonym "Q_B protein"). The changes in transcript abundance in the model plant *Arabidopsis thaliana* were initiated by changing the spectral quality of incident light. This is the first time to our knowledge that such a technique is used to study regulation of plastid gene transcription. The qPCR technique has been widely recognized for its wide linear dynamic range of detection, high sensitivity and reproducibility over the conventional transcript quantifying techniques. The problem of low amounts of starting material is especially important when working with young plants and the small leaves of *Arabidopsis*. Our results with qPCR confirm the earlier observations of transcriptional control of chloroplast genes [6–8] but the kinet-

Abbreviations: qPCR, Quantitative Polymerase Chain Reaction; PS I, photosystem I; PS II, photosystem II.

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ics of transcript accumulation is shown to be more complex than previously demonstrated.

Materials and methods

Plant material and growth conditions. Seedlings of *Arabidopsis thaliana* ecotype Columbia (Col-0) were grown for 12 days from germination in white light (100 $\mu E\ m^{-2}\ s^{-1}$) and then transferred to Light 1 (light condition favouring PS I) or Light 2 (favouring PS II). The plants were allowed to acclimatize to Light 1 or 2 conditions for 4 days. At the end of the 4th day, lights were exchanged. In this exchange or "light switch" light 1 was turned on while light 2 was turned off, and *vice versa*. Leaves from 2 to 3 plants were collected for RNA extraction before the light switch and at various time points extending to 32 h after the light switch. Light 1 was provided by two red fluorescent strip lamps (Osram L 18W/60 Red from Osram GmbH, Hellabrunner Straße 1, 81536 München Germany) each wrapped in red filter (Lee 027 medium red from Lee Filters, Andover, Hants, UK). Light 2 was provided by two white fluorescent strip lamps (Osram L 18W/827 Lumillux) each wrapped in orange filter (Lee 105 Orange). The photon flux density at the highest leaves was 12 $\mu E\ m^{-2}\ s^{-1}$. Light 1 and light 2 were present for a 16-h day (8 h dark period). Their selective actions on photosystem I and photosystem II, respectively, were confirmed by modulated chlorophyll fluorescence and state transition measurements in the growth cabinet (results not shown).

RNA isolation and quantitative real time PCR. Total RNA was isolated from the leaves of 15- to 17-day-old *Arabidopsis* plants with Qiagen RNeasy Plant mini kit. RNA was treated with RNase free DNase (Qiagen) to eliminate possible DNA contamination. Real time quantitative RT PCR was performed with Quantitech SYBR green kit from Qiagen, in a Chromo4 cycler (Bio-Rad). A ~150 bp long sequence was amplified from the psaA and psbA and the reference gene, Actin8 transcripts. For amplifying the psaA transcript, forward and reverse primers used are 5′ G GCACAAGCATCTCAGGTAA 3′ and 5′ AGCCCAAACAATGGATT CAA 3′ respectively, for psbA, 5′ GGTTACAGATTCGGGCAAGA 3′ and 5′ AATACCTACTACCGGCCAAGC 3′ and for Actin8, 5′ TTCCA GCAGATGTGGATCTCTA 3′ and 5′ AGAAAGAAATGTGATCCC GTCA 3′. The forward primer for the Actin8 transcript was designed as flanking an intron-exon boundary thus eliminating the chances of amplifying any contaminated DNA sequences. The optimum annealing temperature for the each primer pair was found out by a gradient PCR. The authenticities of the amplificates were confirmed by sequencing the PCR products. Amplification efficiency for each primer pair was calculated by a 16-fold serial dilution of the template and the R^2 value for each primer pair was found to be $\geqslant 0.99$. A non-template control reaction was carried out for each primer pair in order to check whether template-contamination or primer dimers contribute to the fluorescence signals observed. A slight fluorescence signal at the very late cycle numbers were seen in non-template control reactions for some primer pairs (results not shown). This signal is likely to have arisen from primer dimers. A non-RT (non-Reverse Transcriptase) control reaction was also included to check for amplification from any contaminated DNA. It was found that, as in non-template controls, a slight fluorescence signal showing off at very late cycles numbers (results not shown). For measuring the transcription kinetics of light switch samples, RNA was pooled from two to three plants and three technical replicates were used for each reaction. Expression values were normalized to total RNA. The quantitative real-time PCR technique used here is relative quantification based on comparative C_T method.

Results and discussion

When plants grown in light conditions favouring PS I (light 1) were switched to light conditions favouring PS II (light 2), the *psaA* transcript quantity increased, as shown by earlier studies [6,7], but the magnitude of the increase we detected is much greater than that demonstrated previously. We observed a 9-fold increase in *psaA* transcript quantity within 26 h as compared with plants maintained in light 1 (Fig. 1A). When the opposite light switch was performed, from light 2 to light 1, then the *psaA* transcript quantity fell steadily, decreasing up to 5-fold in 32 h compared with the quantity detected in light 2-adapted plants (Fig. 1B). The transcriptional patterns of *psbA* gene expression in response to light switches were found to be more complex and subtle than those of *psaA* gene expression. When plants were switched from light 1 to light 2, *psbA* transcript quantity fell by 2.5-fold, apparently with a single initial decay component (Fig. 2A). This decline in *psbA* transcript took only 2 h from the light switch. After this initial, rapid transient, the *psbA* transcript quantity oscil-

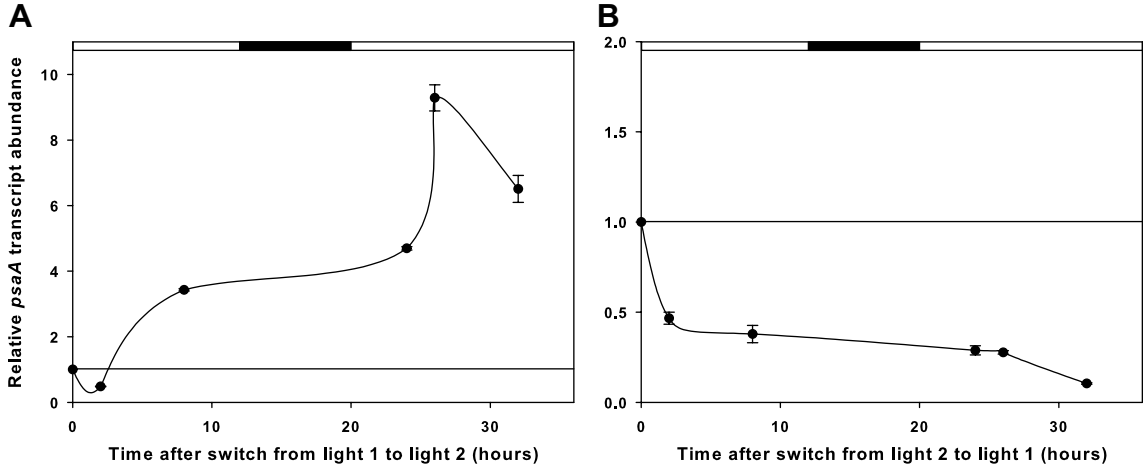

Fig. 1. Photosynthetic control of transcription of the chloroplast gene *psaA*. *psaA* (photosystem I reaction centre subunit A) gene transcription kinetics in *Arabidopsis thaliana* monitored by qPCR. Changes in gene expression are shown as fold change on a time scale. Experimental conditions are replacement of light 1 with light 2 and of light 2 with light 1. The time point at which the lights are switched is taken as zero time and the fold change that follows (up- or down-regulation) is calculated by taking the expression at the time of light switch (zero time) as baseline. Error bars represent ±SE from three technical replicates. An 8-h dark period is shown as the shaded rectangle on the *X*-axis.

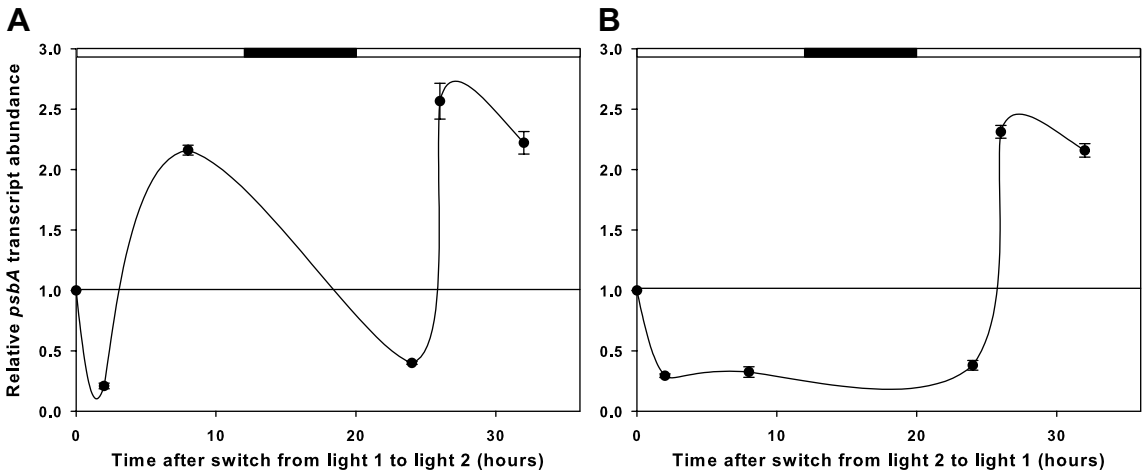

Fig. 2. Photosynthetic control of transcription of the chloroplast gene *psbA*. *psbA* (photosystem II reaction centre subunit D1) gene transcription kinetics in *Arabidopsis thaliana* monitored by qPCR. Changes in gene expression are shown as fold change on a time scale. Experimental conditions are replacement of light 1 with light 2 and of light 2 with light 1. The time point at which the lights are switched is taken as zero time and the fold change that follows (up- or down-regulation) is calculated by taking the expression at the time of light switch (zero time) as baseline. Error bars represent ±SE from three technical replicates. An 8-h dark period is shown as the shaded rectangle on the *X*-axis.

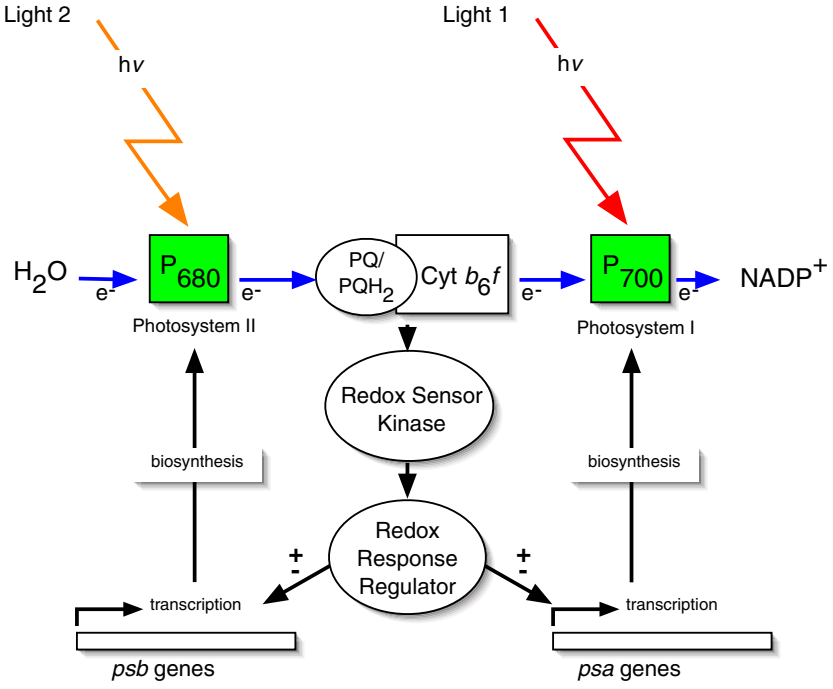

Fig. 3. The proposed role of a redox sensor and redox response regulator in transcriptional regulation in chloroplasts. Light reactions of photosynthesis are represented as electron transport from H_2O to $NADP^+$ via two photosystems connected by a cytochrome b_6f complex. The redox state of the plastoquinone pool is sensed directly, or indirectly via the cytochrome b_6f complex, and initiates the transcriptional response. The transcriptional response involves the activation of a DNA-binding response regulator protein through phosphorylation, and the activated response regulator then regulates the transcription of reaction centre genes for both photosystem I and II. After a switch between light 1 and light 2, the photosystem that becomes light-limiting is up-regulated, while the photosystem that becomes light-saturated is down-regulated.

lated between high and low values in the light and the dark, respectively. In response to the opposite light switch, i.e. from light 2 to light 1, it took 24 h before an increase in *psbA* transcript quantity was observed (Fig. 2B). As seen here (Figs. 1 and 2) the transcriptional patterns of *psaA* and *psbA* in response to light switches differ both qualitatively and quantitatively. It remains to be seen whether the more complex transcriptional response of the *psbA* gene results from regulation operating at more than one level of gene expression.

The resemblance of the kinetics of transcript accumulation to diel cycles is an unexpected observation in the transcriptional responses to light switches. *psaA* transcript quantity, from the second day of the light 1-to-light 2 switch, is seen to reach a maximum at midday, and then to fall off by the evening. *psbA* transcript quantity also

showed this oscillatory behaviour after the light 1-to-light 2 switch. Such a rhythmic behaviour in the kinetics of transcript accumulation was not observed in previous studies, which used light quality and electron transport inhibitors to induce changes in transcriptional pattern of chloroplast genes [6–8]. However, rhythmic changes in transcription have been documented for algal chloroplast genes in response to light–dark cycles [9]. This rhythmic behaviour of chloroplast transcription in response to light–dark cycles is shown to be independent of circadian rhythms and is postulated to be dependent on the activity of the photosynthetic electron transport chain [9]. The transcript accumulation kinetics shown here suggest that both the light quality–driven changes in transcriptional response and the oscillatory behaviour of transcript accumulation are driven by the activity of the electron transport chain. The two responses may therefore be governed by the same redox signal transduction pathways.

How is the activity of the electron transport chain connected to the transcription of chloroplast-encoded genes? In view of the bacterial ancestry of chloroplasts [10–15], it is interesting to consider whether a redox responsive two-component system akin to a bacterial signalling system [16–21] underlies the mechanism that couples electron transport to chloroplast transcription, as outlined in Fig. 3. A nuclear-encoded, bacterial-type sensor kinase has been identified in the *Arabidopsis* genome, and its disruption results in plants impaired in chloroplast transcriptional regulation [22,23]. A nuclear-encoded response regulator-like protein has also been reported in plant chloroplasts [24]. With robust transcriptional regulation and transcriptional on-off switches in the form two-component systems, the chloroplast may have inherited from its cyanobacterial ancestor both the hardware of oxygenic photosynthesis and the means of its genetic control [10,25,26].

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