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Two phytochrome-mediated effects of light on transcription of genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase in dark-grown pea *(Pisum sativum)* plants

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Pytochrome mediates two effects of light on transcription of genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase in dark-grown pea *(Pisum sativum)* plants. One response is an initial transient increase in the rate of small subunit gene transcription which results in the accumulation of a small, but relatively stable, amount of small subunit mRNA. The second is the induction of the capacity for high rates of small subunit gene transcription when plants are subsequently illuminated with white light.

Ribulose-1,5-bisphosphate carboxylase-oxygenase; Plant gene; Transcriptional control; Photoregulation; Phytochrome; (Isolated nuclei)

1. INTRODUCTION

Many studies of the photoregulation of gene expression in higher plants have centred on the nuclear genes encoding the small subunit (*rbcS*) of ribulose-1,5-bisphosphate carboxylase-oxygenase [1,2]. Light induces a substantial increase in the *rbcS* mRNA content in leaf tissue [1,2] and this is primarily due to an increase in the rate of *rbcS* gene transcription [3–5]. In pea [6–9], and other species [4,5], the photoreceptor phytochrome is involved in mediating the effects of light on *rbcS* gene expression.

Gallagher et al. [10] demonstrated, using run-off transcription assays in isolated nuclei, that the light-induced increase in *rbcS* gene transcription in pea consisted of three distinct temporal responses. Here we show that two responses induced in dark-grown pea plants, namely the initial transient increase in *rbcS* gene transcription and the development of the capacity for subsequent high rates of *rbcS* gene transcription in white light, are both mediated by phytochrome.

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2. MATERIALS AND METHODS

2.1. *Plant material*

Pea (*Pisum sativum* cv. Feltham First) plants were grown from seed in darkness at 20°C for 6 days and illuminated as described in the text with either white light (400–700 nm; 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$), monochromatic red light (662 nm; 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or monochromatic far-red light (8 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as described [6].

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2.2. *Assay of* rbcS *gene transcription*

Nuclei were isolated from the shoot apical buds at the times indicated by gentle homogenisation and Percoll gradient centrifugation [3]. For plants which were in darkness the harvesting and initial stages of nuclear isolation were carried out in complete darkness. Labelled transcripts were synthesised using [^{32}P]UTP, extracted and hybridised under conditions of DNA excess to recombinant plasmids immobilised on nitrocellulose filters as in [3,10]. The plasmids used, pSSU160 and pSSU60 [11], contained cDNA inserts of a pea *rbcS* gene and 2.5 μg of each were bound to the filters [3,10]. RNase-resistant counts bound to the filters were determined and corrected for non-specific binding by subtracting counts bound to filters bearing a control plasmid, pAT153 [3,10]. Rates of *rbcS* gene transcription are expressed as counts hybridised as a fraction of total labelled transcripts applied to the nitrocellulose filters. It was previously shown [3] that under the hybridisation conditions employed there is a linear relationship between the number of counts hybridised and the amount of labelled RNA applied to the filters.

2.3. *Measurement of* rbcS *mRNA contents*

Total RNA was extracted from the shoot apical buds and used in quantitative dot-blot assays as described [6,12,13]. RNA samples (1 μg) were applied in triplicate to nitrocellulose using a manifold, along with a concentration series of 0–5 μg RNA from light-grown plants. The immobilised RNA was incubated under hybridising conditions with equimolar amounts of the recombinant inserts of pSSU60 and pSSU160 labelled with [^{32}P]dCTP by nick translation. After washing under stringent conditions the radioactivity bound to the dots was determined by scintillation counting and corrected for binding to control dots lacking RNA [12,13].

3. RESULTS

3.1. *Initial increase in* rbcS *gene transcription*

Dark-grown pea seedlings have extremely low rates of *rbcS* gene transcription and accumulate very small amounts of *rbcS* mRNA [3,6–10,13]. Fig.1A shows the change in rate of *rbcS* gene transcription in darkness following a brief illumination of dark-grown pea plants with red light. The red light treatment induces an increase in transcription which is detectable within 1 h of illumination and has a transient character similar to that observed in continuous white light [10]. Transcription remains slightly above the rate observed in non-illuminated plants 24 h after the brief red light treatment. The increase in *rbcS* gene transcription is greatly reduced if the red light treatment is followed immediately by far-red light (fig.1B), consistent with a photoreversible response mediated by phytochrome.

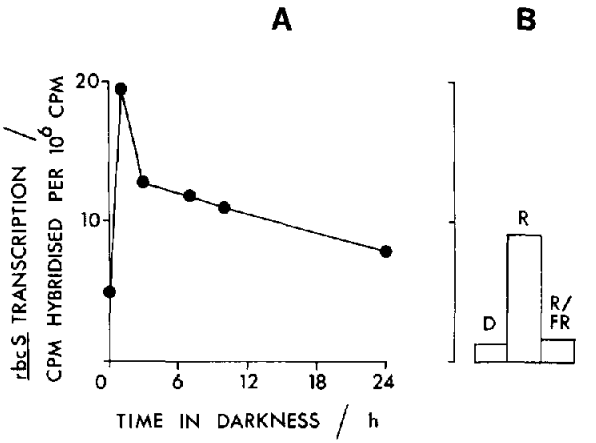

Fig.1. (A) Change in the rate of *rbcS* gene transcription in darkness following 3 min illumination of dark-grown pea plants with monochromatic red light. Transcription was assayed in isolated nuclei. (B) Far-red reversibility of the red light-induced increase in *rbcS* gene transcription: dark-grown plants (D); dark-grown plants illuminated with red light for 3 min followed by 3 h in darkness (R); as R but with 3 min under monochromatic far-red light immediately after the red light treatment (R/FR). The data in A and B are from different experiments.

The initial transient increase in *rbcS* gene transcription results in the accumulation of a small amount of *rbcS* mRNA which is detectable within 7 h of the red light treatment (fig.2). The *rbcS* mRNA content reaches a maximum and then remains roughly constant at a steady-state level approx. 10% of that observed in plants illuminated continuously with white light for 48 h. The effect of an immediate far-red light treatment in reversing the effect of red light on *rbcS* mRNA accumulation has been reported previously [6–8].

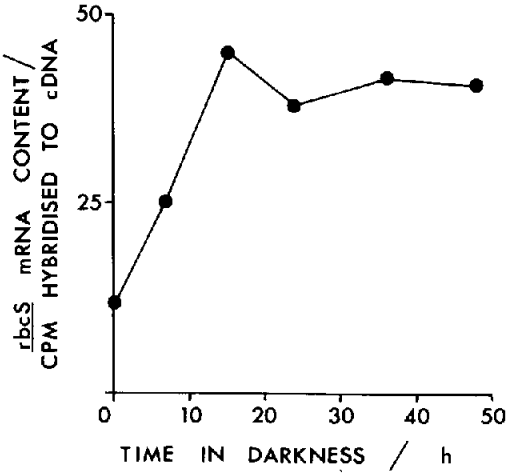

Fig.2. Increase in *rbcS* mRNA content in darkness following illumination of dark-grown pea plants with monochromatic red light for 3 min. *rbcS* mRNA content was measured by quantitative dot-blot hybridisation.

3.2. *Induction of the capacity to transcribe* rbcS *genes at maximal rates*

The largest increases in the rates of *rbcS* gene transcription and *rbcS* mRNA accumulation during greening of pea seedlings in continuous white light do not occur until approx. 20 h after the start of illumination ([10,13]; fig.3). We have found that the development of the capacity for high rates of *rbcS* gene transcription in white light is induced when dark-grown plants are first illuminated and that the response is mediated by phytochrome. Thus, if dark-grown plants which have received a brief red light treatment followed by 24 h in darkness are transferred to continuous white light, their rates of *rbcS* mRNA accumulation (fig.3A) and *rbcS* gene transcription (fig.3B) increase much more rapidly than in plants that have not been pre-illuminated. The greater rates of *rbcS* mRNA accumulation and *rbcS* gene transcription in the red-pretreated plants are apparent within 5–11 and 2–5 h, respectively, of the transfer to white light, and after 24 and 20 h the *rbcS* mRNA content and the rate of *rbcS* gene transcription are greater than in non-pretreated controls illuminated with white light for 48 h. It is interesting that the pre-illumination does not remove the transient character of the increase in *rbcS* gene transcription. The effect of red light in inducing the subsequent rapid accumulation of *rbcS* mRNA in white light is substantially reduced by an immediate far-red light treatment (fig.3A), consistent with a photoreversible response mediated by phytochrome.

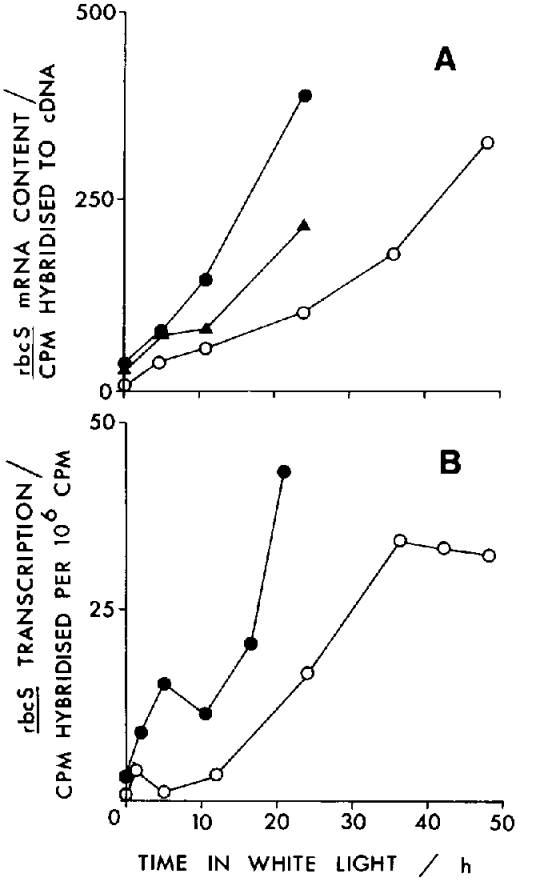

Fig.3. Effect of red light pretreatment on *rbcS* mRNA content and *rbcS* gene transcription. (A) *rbcS* mRNA contents measured by quantitative dot-blot hybridisation in: dark-grown pea plants illuminated with monochromatic red light for 3 min, returned to darkness for 24 h, and then transferred to continuous white light for the times indicated (●); as above but illuminated with monochromatic far-red light for 3 min immediately after the red light treatment (▲); dark-grown plants transferred directly to continuous white light (○). (B) *rbcS* gene transcription assayed in isolated nuclei for red pretreated (●) and dark control (○) plants as above.

4. DISCUSSION

Run-off transcription assays with isolated nuclei have been used extensively to study the rates of transcription of specific genes relative to the total

rate of transcript synthesis. Such transcripts are initiated in vivo prior to nuclear isolation [14,15]. Previous studies with pea have demonstrated the specificity and quantitative aspects of the assay and have shown that light has differential effects on the transcription of different genes [3,10].

It is clear that the light-induced increase of *rbcS* gene transcription in pea is much more complex than is implied by a simple 'on/off' mechanism, since distinct temporal responses are observed [10]. The data presented here show that illumination of dark-grown plants initiates two effects on *rbcS* gene transcription, both of which are mediated by phytochrome. The transient increase in *rbcS* gene transcription is a rapid response to the initial illumination of dark-grown plants and its kinetics resemble those for the red light-induced increase in transcription of light-harvesting chlorophyll *a/b*-binding protein genes in barley [16]. The slow increase in *rbcS* gene transcription during greening in white light can be interpreted as the product of two processes: the development of the capacity to transcribe the *rbcS* genes at high rates, which is shown here to be induced when dark-grown plants are first illuminated and is mediated by phytochrome, and the actual response to white light itself. This latter effect of light is not a simple inductive phytochrome response (in preparation).

The initial increase in *rbcS* gene transcription results in the accumulation of a small amount of *rbcS* mRNA. The steady-state level of *rbcS* mRNA is no greater in dark-grown plants that are illuminated continuously with red light for 24 h (not shown), indicating that the maximal possible response at this time is induced by the brief red light treatment. Since the steady-state *rbcS* mRNA content remains approximately constant for up to 48 h while the rate of transcription decreases to a low level within 24 h of the initial illumination, it seems that the *rbcS* mRNA produced is relatively stable in darkness. This contrasts with the substantial decrease in *rbcS* mRNA content which is observed when light-grown pea plants are transferred to darkness for an extended period ([9]; Jenkins, G.I., unpublished).

The molecular mechanisms underlying the two responses induced in dark-grown plants described here are not likely to be the same. The rapidity of the initial increase suggests some kind of activation mechanism, whereas development of the capacity to transcribe maximally, which occurs slowly even in continuous white light [10], is more consistent with such processes as the demethylation of DNA, conformational changes of chromatin or the synthesis of factors involved in signal transduction or the initiation of transcription. The activation of pre-existing factors may be responsible for the rapid modulation of *rbcS* gene transcription in green tissues described by Gallagher et al. [10]. Further studies are required to establish the molecular basis of these different transcriptional responses.

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