Rapid modulation of transcription of nuclear genes encoding chloroplast proteins by light

T.F Gallagher, G I Jenkins† and R.J. Ellis

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England

Received 7 May 1985

We have investigated the effects of light on transcription of the nuclear genes encoding the small subunit (SSU) of ribulose-1,5-biphosphate carboxylase-oxygenase, and the major apoprotein (LHCP) of the light-harvesting chlorophyll *a/b* complex in *Pisum sativum* Light treatments were given in vivo and transcription was assayed subsequently in isolated nuclei using specific cloned cDNA probes We have identified three different temporal effects of light on transcription of these genes an initial increase when dark-grown seedlings are first illuminated, a slow increase in the ability to transcribe these genes at maximal rates, and a rapid modulation of specific gene transcription in fully greened plants

Ribulose-biphosphate carboxylase-oxygenase *Light-harvesting chlorophyll* a/b *complex* *Transcriptional control* *Photoregulation* *Plant gene* *Isolated nucleus*

1. INTRODUCTION

The light-dependent development of chloroplasts in higher plants involves the synthesis and accumulation of numerous polypeptides of the photosynthetic machinery [1,2]. Notable examples are the abundant enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase [3], which consists of large (LSU) and small (SSU) subunit polypeptides, and the major apoprotein (LHCP) of the light-harvesting chlorophyll *a/b* complex LHC2 [4]. Both the SSU and LCHP polypeptides are encoded in small multigene families in nuclear DNA [5–7], and their accumulation following illumination is paralleled by increases in their respective steady-state transcript concentrations [2,3,8–11] Studies with isolated nuclei have shown that these light-induced increases in transcript content are primarily the result of an increase in transcription [12]. Here we report that light has different temporal effects on SSU and LHCP gene transcription in *Pisum sativum*, the type of control depending on the stage of development of the seedlings following illumination. One of these effects is a rapid modulation of specific gene transcription by light/dark transitions in fully greened plants.

2. MATERIALS AND METHODS

Pea (*Pisum sativum* L. cv. Feltham First) plants were grown from seed in darkness at 20°C for 6 days and then transferred to continuous white light (200 μmol·m^{-2}·s^{-1}; 400–700 nm) at 20°C for up to 48 h. Further dark and light treatments of plants greened for 42 h are described in the text.

Shoot apical buds were harvested from the seedlings at the times indicated, and nuclei were isolated by gentle homogenisation and Percoll gradient centrifugation [12]. For plants which were in darkness, the harvesting and initial stages of nuclear isolation were carried out in complete darkness. Labelled transcripts were synthesised ac-

† To whom correspondence should be addressed

Abbreviations. LSU, large subunit; SSU, small subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase, LHCP, the major apoprotein of the light-harvesting chlorophyll *a/b* complex LHC2

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cording to [12]; 2–5 × 10^7 nuclei were incubated at 27°C for 20 min in 400 μl containing 50 mM Tris-HCl, pH 7.8, 75 mM NH_4Cl, 10 mM $MgCl_2$, 0.2 mM aurin tricarboxylic acid, 10% (w/v) glycerol, 0.5 mM each of ATP, GTP and CTP and 3.7 MBq 5-6[^{3}H]UTP (1.7 TBq·mmol^{-1}) or 3.7 MBq [^{32}P]UTP (15 TBq·mmol^{-1}). The reaction was started by the addition of nuclei and stopped by the addition of 10 μg DNase I (RNase-free; Worthington). The reaction mixtures were then incubated for a further 10 min at room temperature. Labelled RNA was extracted [12] and dissolved in 50% formamide, 40 mM Pipes-NaOH pH 6.5, 0.5 M NaCl, 1 mM Na_2EDTA, 0.4% (w/v) SDS, 100 μg·ml^{-1} poly(A) and 100 μg·ml^{-1} *E. coli* tRNA. The labelled transcripts were hybridised under DNA excess to recombinant plasmids immobilised on 7 mm diameter nitrocellulose filter discs (Schleicher and Schull BA85, 0.45 μm) [13]; 5 μg DNA was bound to each filter. The plasmids contained cDNA inserts for either SSU (pSSU 60 and pSSU 160 [14]), LHCP (pFa/b31 [4]) or the wheat ribosomal DNA repeat unit (pTA250 [15]). The filters were prehybridised at 41°C for 24 h and hybridised at the same temperature for 64 h. The input of labelled transcripts was 2–3 × 10^6 cpm per filter ([^{3}H]UTP) or 7–14 × 10^6 cpm per filter ([^{32}P]UTP). RNase-resistant counts bound to the filters were then determined, and corrected for non-specific binding by subtracting counts bound to filters bearing 5 μg pAT153 [16] (approx. 10 cpm per 10^7 cmp input). Filters were counted for 30 min in a xylene-toluene scintillant [13] No corrections have been made for hybridisation efficiency (approx. 50%) or the size of the cDNA probes. Each point was determined in duplicate and is expressed as parts per million (ppm), that is cpm hybridised per 10^6 cpm applied to the filters. We have shown [12] that under the hybridisation conditions employed there is a linear relationship between the number of counts hybridised and the amount of labelled RNA applied.

3. RESULTS

Fig.1 shows changes in the rates of SSU and LHCP gene transcription following the transfer of dark-grown *Pisum* seedlings to continuous white light. As reported in [12], the rate of transcription in darkness is greater for the LHCP genes than the

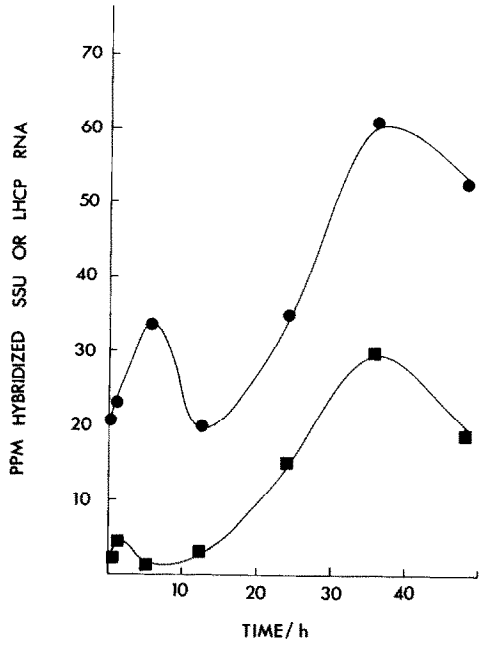

Fig 1 Changes in the rates of transcription of SSU (■) and LHCP (●) genes during greening of *Pisum sativum* seedlings. Plants were grown in darkness for 6 days and then illuminated with continuous white light. Nuclei were isolated from the shoot apical buds at the times indicated and transcription was assayed using [^{3}H]UTP as described in section 2

SSU genes, and large increases occur in the transcription of both genes, as a fraction of the total RNA synthesised, as a result of illumination. The major increase is observed between 12 and 36 h after transfer to light; this change parallels the increases in steady-state transcript content [2,8,9], and indicates that transcription is the principal level at which the abundance of these transcripts is controlled. We conclude that one effect of illumination is slowly to induce the ability to transcribe specific genes at maximal rates. This is not a general effect on the overall rate of transcription because differences are observed between different genes: rRNA genes show only a 2-fold increase in transcription when pea seedlings are grown in the light as opposed to darkness [12], whereas the increase in SSU gene transcription is 20-fold (fig.1). Moreover, Gallagher and Ellis [12] report that nuclei from dark- and light-grown pea seedlings show no difference in their overall rates of incorporation of UTP into RNA.

A notable feature of the results shown in fig.1 is that an initial, transient increase in SSU and LHCP gene transcription occurs shortly after the start of illumination. Similar results have been observed in 6 separate experiments. Although an increase in rate is always present 1 h after transfer to light, the maximum of the transient increase shows some variability in both its extent and timing. Despite being the most abundant mRNAs in light-grown plants [14], the SSU and LHCP transcripts still represent a relatively small proportion of the total RNA synthesised by isolated nuclei (SSU 26 ppm; LHCP 60 ppm), and so to further investigate the initial increase in transcription we increased the available counts hybridised by using [^{32}P]UTP instead of [^{3}H]UTP. Fig.2 shows an experiment where the initial increase was studied in more detail. Transcription of both SSU and LHCP genes increases within 1 h of transfer to light, reaches a maximum, and subsequently declines before exhibiting the slow increase described in fig.1.

A further distinct temporal effect of light on SSU and LHCP gene transcription is observed in seedlings that have attained the ability to transcribe these genes at maximal rates. Dark-grown plants which had received 42 h illumination were transferred to darkness for up to 5 h, and then returned to continuous white light for various times. As shown in fig.3, the rates of SSU and LHCP gene transcription decline by 75 and 50%, respectively, within 5 h of transfer to darkness, but the dark rates are always greater than those characteristic of dark-grown plants. Within 20 min of being returned to light the rate of SSU gene transcription is restored to its initial value while that of the LHCP genes 'overshoots' by 30%. The light-induced increase thus occurs more rapidly than the darkness-induced decrease. Changes in rRNA transcription were not found in these experiments and we have not detected any difference

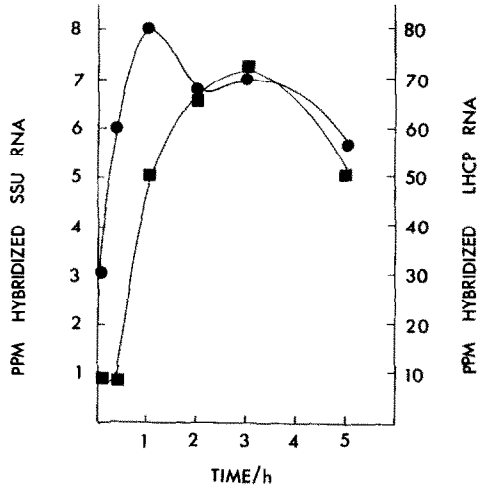

Fig 2. Changes in the rates of transcription of SSU (■) and LHCP (●) genes during the first 5 h of greening. Nuclei were isolated at the times indicated and transcription was measured using [^{32}P]UTP in the reaction mixtures

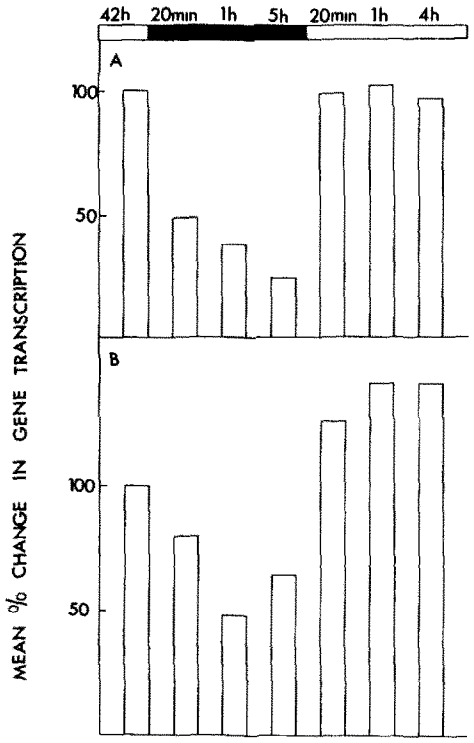

Fig.3 Modulation of SSU (A) and LHCP (B) gene transcription in greened *Pisum sativum* plants by light/dark transitions. Plants were grown in darkness for 6 days and then transferred to continuous white light for 42 h The illuminated plants were returned to darkness for 20 min, 1 h or 5 h (shaded bar). Plants left in darkness for 5 h were then transferred to white light for either 20 min, 1 h or 4 h. Apical buds were harvested from the plants at these times and nuclei were isolated Transcription was measured using [^{3}H]UTP in the reaction mixtures. Each point is the mean determined from 3 separate experiments

in the fraction of the total transcripts synthesised by RNA polymerase II (not shown) Thus this effect of light on transcription appears to be specific to certain genes. Such rapid modulations of specific gene transcription by light/dark transitions have not been reported previously. The phenomenon is not restricted to de-etiolated seedlings since we have observed similar effects in *Pisum* plants grown from seed under a 12 h photoperiod.

4. DISCUSSION

Isolated nuclei provide a convenient system in which to study changes in the rates of transcription of specific genes induced by particular light treatments. The transcripts synthesised in this system are initiated in vivo [17]. Here we have described 3 different temporal effects of light on SSU and LHCP gene transcription: an initial, transient increase when dark-grown plants are first illuminated, a slow increase in the ability to transcribe these genes at maximal rates, and a rapid modulation in fully greened plants. Clear differences are present in the degree of photoregulation between the SSU and LHCP genes, and their responsiveness to light is much greater than that of the rRNA genes [12]. Light-induced changes in the overall rate of transcription have been reported for isolated *Avena* nuclei [18], but we have found no evidence for such effects in our experiments with *Pisum* nuclei

The molecular mechanisms underlying these different temporal effects of light are not understood. The initial rapid increase could be explained in terms of a slow engagement of RNA polymerases onto the genes during dark growth, followed by a burst of transcription on illumination; the recruitment of polymerases onto non-transcribed genes has been observed in animal cells [19]. Other factors which are known to contribute to the control of eukaryotic transcription, such as changes in chromatin conformation, DNA methylation, and the binding of specific regulatory factors [20], may also be involved in the photoregulation of transcription of these genes. An additional complication is the possibility that different members of the SSU and LHCP multigene families are transcribed to different extents, some evidence for differential expression of SSU genes has been reported [7].

There is evidence that phytochrome is involved in controlling the steady state concentrations of SSU and LHCP transcripts in several species [2,8–11], and effects on transcription have been reported [21]. A brief red light treatment is sufficient to induce an increase in the contents of SSU and LHCP transcripts in dark-grown *Pisum* seedlings, but the magnitude of this increase is much smaller than that produced by exposing plants to continuous white light for 48 h [2,8,9]. We suggest that this phytochrome-mediated increase in transcript content following brief illumination of dark-grown plants is primarily effected through the initial increase in transcription described here. However, the rapid modulation of transcription in fully greened plants is difficult to explain in terms of inductive phytochrome control, since Pfr content does not decline rapidly in darkness in plants which have received prolonged illumination [22]. Some other type of photoregulation may thus be involved in this rapid response of specific gene transcription. The rapidity of this response should encourage attempts to unravel the molecular basis of the photocontrol of transcription by the establishment of soluble in vitro transcriptional systems which respond to light.

ACKNOWLEDGEMENT

This work was supported by funds from the UK Science and Engineering Research Council.

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