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# Light-stimulated transcription of genes for two chloroplast polypeptides in isolated pea leaf nuclei

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**Nuclei isolated from both light-grown and dark-grown leaves of *Pisum sativum* by Percoll density gradient centrifugation incorporate labelled UTP into RNA when supplemented with the other three nucleoside triphosphates. The RNA is heterodisperse, with transcripts up to at least 25S in size. Among these transcripts are sequences hybridizing to cloned DNA probes for wheat rRNA and two abundant chloroplast polypeptides of *Pisum*, viz. the small subunit of ribulose bisphosphate carboxylase and a polypeptide of the light-harvesting chlorophyll a/b binding complex. Transcription of small subunit and light-harvesting complex sequences is greater (18-fold and 9-fold, respectively) in nuclei from light-grown leaves than in nuclei from dark-grown leaves. Transcription of ribosomal genes, by contrast, is only doubled by growth in the light. Small subunit and light-harvesting complex sequences transcribed in dark-grown nuclei are not degraded in a 120 min chase. These results suggest that the stimulation of accumulation of small subunit and light-harvesting complex mRNAs by exposure of *Pisum* seedlings to light is mediated by an increase in transcription rather than by a decrease in RNA degradation.**

*Key words:* chloroplast polypeptides/hybridization analysis/isolated nuclei/light-stimulation/transcription

## Introduction

Chloroplast development requires the integrated activities of both the plastid and nuclear genetic systems (Ellis, 1981). Some chloroplast polypeptides are encoded in the nucleus and synthesized as higher mol. wt. precursors by cytoplasmic ribosomes; examples are the small subunit (SSU) of ribulose bisphosphate carboxylase (EC 4.1.1.39) and the polypeptides of the light-harvesting chlorophyll a/b binding complex (LHC). Precursors of the SSU and LHC polypeptides are post-translationally transported into isolated chloroplasts and processed to their mature size (Highfield and Ellis, 1978; Chua and Schmidt, 1979; Smith and Ellis, 1979; Grossman *et al.*, 1980).

Exposure of young seedlings to light causes an increase in the accumulated amount of many chloroplast polypeptides. In the case of ribulose bisphosphate carboxylase and the LHC polypeptides, this effect is mediated by the pigment phytochrome (Graham *et al.*, 1968; Apel, 1979). Correlations have been found between light-induced changes in the accumulation of SSU and LHC polypeptides and the concentrations of their mRNAs which are translatable in cell-free protein-synthesizing systems (Tobin, 1978, 1981a; Apel, 1979; Bedbrook *et al.*, 1980; Cuming and Bennett, 1981; Sasaki *et al.*, 1981). Hybridization analysis, using cloned cDNA probes for SSU mRNA of the pea *Pisum sativum*, has shown that the stimulation of SSU accumulation by light is reflected in changes in the total content of RNA sequences for this polypeptide in both the cytoplasmic and nuclear compartments (Smith and Ellis, 1981). This latter study used 'Northern blot' analysis which measures only the steady-state concentration of SSU transcripts and not their rate of synthesis. Thus, the light-stimulated accumulation of SSU RNA in the nucleus and cytoplasm may result from either an increase in the rate of transcription of SSU genes or a decrease in the rate of breakdown of nuclear SSU transcripts. One way to distinguish between these two mechanisms is to study transcription by isolated nuclei, since it is commonly observed that transcription in isolated nuclei represents elongation of already-engaged RNA polymerases (Tsai *et al.*, 1978; Derman *et al.*, 1981; McKnight and Palmiter, 1979). Here we describe the use of nuclei isolated from light- and dark-grown leaves of *P. sativum* to show that light increases the transcription of the genes for both SSU and LHC polypeptides.

## Results

### *Characteristics of transcription by isolated Pisum nuclei*

Nuclei isolated from *Pisum* leaves appear devoid of contamination by other cellular organelles when sections are examined by electron microscopy (Figure 1). The nuclei are intact but lack envelope membranes, possibly due to the use of detergent to remove thylakoids. These isolated nuclei incorporate [$^3$H]UMP into RNA when incubated with nucleoside triphosphates (Table I). Transcription is reduced by 90% when unlabelled nucleoside triphosphates are omitted, and by 75% when 10 μg/ml actinomycin D is added. The addition of α-amanitin at 10 μg/ml inhibits RNA synthesis by 36%; this concentration is reported to inhibit completely transcription by isolated wheat germ RNA polymerase II (Jendrisak and Guilfoyle, 1978). The rate of transcription is comparable with those reported for nuclei isolated from some plants (Luthe and Quatrano, 1980b; Wilson and Bennett, 1976; Slater *et al.*, 1978), but is lower than those reported for nuclei from tobacco (Hamilton *et al.*, 1972; Mennes *et al.*, 1978). Nuclei isolated from dark-grown pea leaves show closely similar rates of transcription and α-amanitin sensitivity to those isolated from light-grown pea leaves.

RNA polymerase II activity in nuclei from turnip leaves is stimulated by high (150–400 mM) concentrations of monovalent cations (Guilfoyle, 1980). This is also true for pea leaf nuclei (data not shown), but at cation concentrations above 125 mM the pea nuclei lyse, and no longer appear as discrete organelles by phase contrast microscopy. It is possible that conditions which cause lysis of nuclei might alter the rates of transcription of particular genes (Garigilio *et al.*, 1981). Therefore, we use a monovalent cation concentration of 75 mM at which the nuclei remain intact during the incubation. Transcription in such nuclei is linear for ~15 min, and then continues at a lower rate for at least 60 min (data not shown).

RNA synthesized by isolated pea leaf nuclei during a 20 min incubation is heterodisperse (Figure 2). There are

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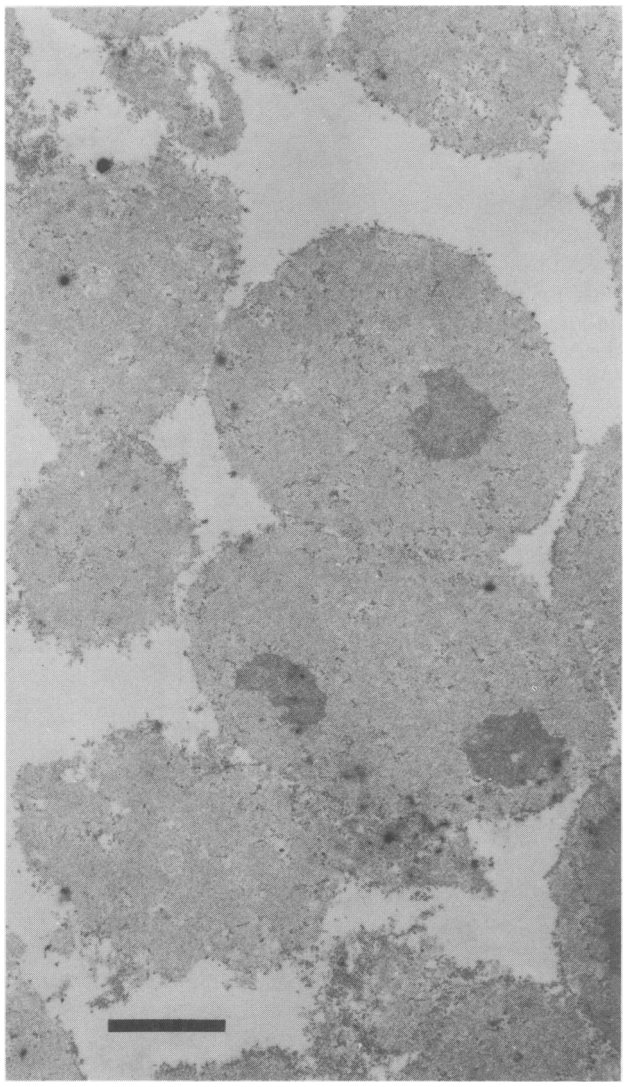

**Fig. 1.** Electron micrograph of a section through a pellet of Percoll gradient-purified *Pisum* leaf nuclei. Bar = 2 μm.

**Table I.** Characteristics of transcription by isolated *Pisum* leaf nuclei[a]

| Incubation mixture | [$^3$H]UMP incorporated into TCA-insoluble fraction | | |
|---|---|---|---|
| | c.p.m./5 μl | pmol/100 μg DNA | % |
| Complete | 19 689 | 52.1 | 100 |
| Minus unlabelled nucleoside triphosphates | 1784 | 4.7 | 9 |
| Minus nuclei | 170 | – | 0.9 |
| Plus 10 μg/ml actinomycin D | 4887 | 12.9 | 25 |
| Plus 10 μg/ml α-amanitin | 12 545 | 33.2 | 64 |

[a]Details of the assay are given in Materials and methods.

discrete bands among the transcripts that are of similar size to the mature 25S rRNA. Unlike earlier methods of nuclear isolation, the procedure of Luthe and Quatrano (1980b) produces nuclei which synthesize RNA of high mol. wt. A decrease in mean size of the transcripts occurs when the nuclei are incubated for a further 60 min in the presence of unlabelled UTP and actinomycin D (Figure 2, lane 2), but during this chase there is no loss of TCA-precipitable counts. If 10 μg/ml α-amanitin is added at the beginning of the incubation, the

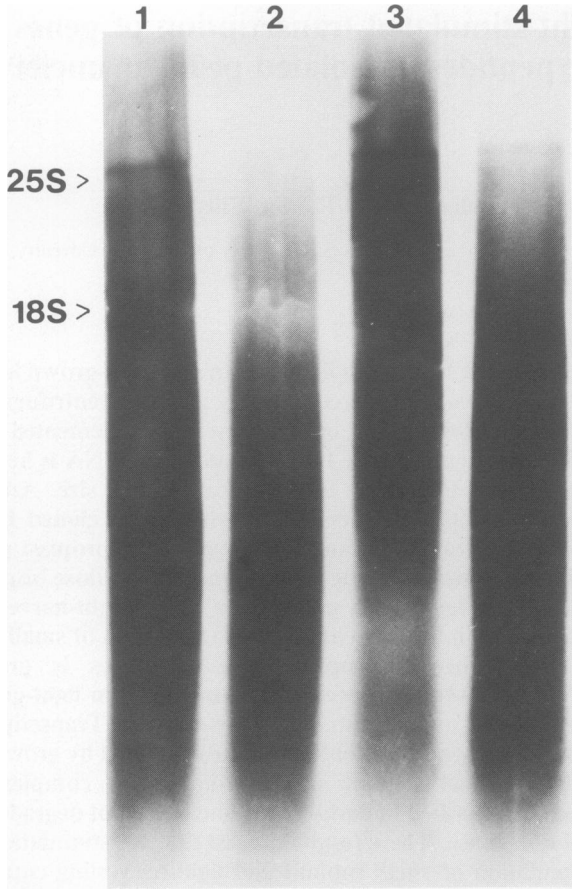


**Fig. 2.** Size of RNA synthesized by isolated leaf nuclei. Nuclei isolated from dark-grown pea leaves were incubated with [$^3$H]UTP for 20 min (**lanes 1** and **3**), or for 20 min followed by a further 60 min in the presence of 10 mM UTP and 10 μg/ml actinomycin D (**lanes 2** and **4**). Incubations for **lanes 3** and **4** contained, in addition, 10 μg/ml α-amanitin. RNA was isolated and resolved on 1.1% agarose formaldehyde gels at $10^5$ c.p.m. per lane; the gels were fluorographed. Arrows indicate the position of 25S and 18S rRNA markers.

size distribution of transcripts is unaltered, both at 20 min and after a 60 min chase (Figure 2, lanes 3 and 4).

### *Transcription of specific genes*

Specific gene transcripts can be detected among the RNA molecules synthesized by isolated pea leaf nuclei. Recombinant plasmids carrying inserts for specific gene sequences were digested with appropriate restriction enzymes to excise the inserts. The DNA fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose sheets. $^{32}$P-labelled transcripts from light-grown nuclei were incubated with these sheets under hybridization conditions. Figure 3 shows that transcripts hybridize to the excised inserts but not to the plasmid fragments. There is intense hybridization to an insert containing the major 9-kb ribosomal DNA repeat from wheat (Figure 3, lane 5). Weaker hybridization is seen to two inserts which cover most of the *Pisum* SSU mRNA (Figure 3, lanes 1 and 2), and to one insert covering part of the mRNA for one of the LHC polypeptides (Figure 3, lane 3). No hybridization is seen to an insert which carries a histone H4 gene from *Xenopus* (Figure 3, lane 4). The strong hybridization to the ribosomal DNA probe is consistent with the data in Table I which suggest that the bulk of the nuclear transcripts is produced by polymerases which are insensitive to α-amanitin.

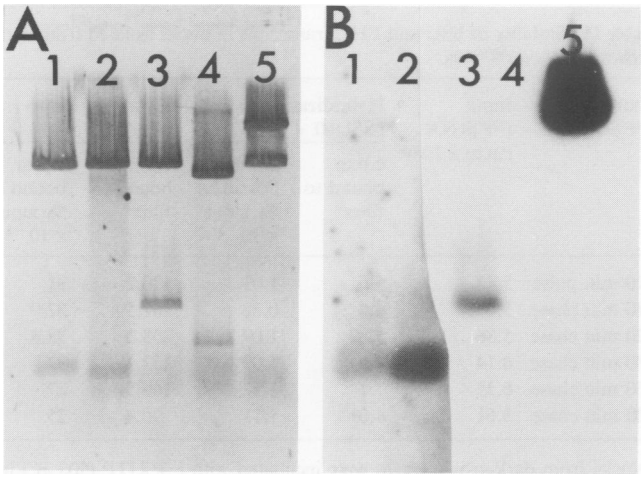


**Fig. 3.** Hybridization of RNA synthesized by isolated light-grown pea leaf nuclei to specific DNA probes. Plasmid DNAs containing inserts for pea SSU (pSSU60 and pSSU160), pea LHC (pFa/b31), wheat ribosomal DNA repeat unit (pTA250), and *Xenopus* histone H4 (pcXlH4W1) were digested with restriction enzymes to excise the inserts. The enzymes used were - *Hind*III for pSSU60 and pSSU160, *Bam*HI for pFa/b31 and pcX1H4W1, and *Eco*RI for pTA250. The restricted DNAs were separated on 1% agarose gels, transferred to nitrocellulose, and hybridized to $^{32}$P-labelled transcripts (20 x $10^6$ c.p.m.). **(A)** U.v. picture of restricted DNAs; **lane 1**, pSSU60; **lane 2**, pSSU160; **lane 3**, pFa/b31; **lane 4**, pcX1H4W1; **lane 5**, pTA250. Each lane was loaded with 5 μg DNA. **(B)** Autoradiograph of RNA hybridized to **(A)**; **lanes 1** and **2** were exposed for a longer period than **lanes 3–5**. **Lane 4** showed no hybridization even when exposed for 24 days.

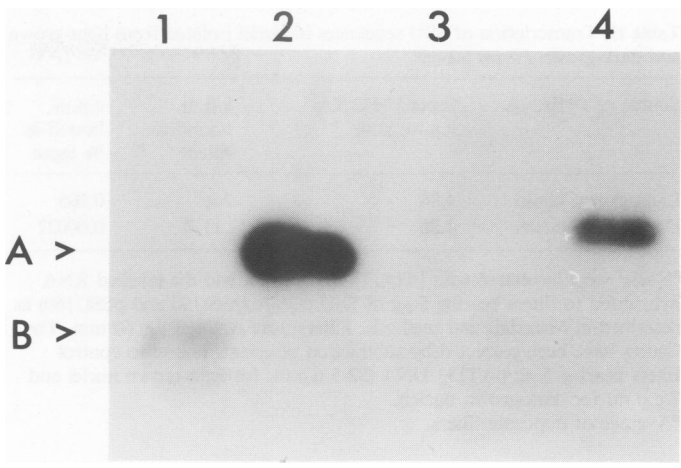


**Fig. 4.** Comparison of hybridization to SSU and LHC probes of labelled RNA synthesized by nuclei isolated from light-grown and dark-grown pea leaves. Southern transfers were carried out as described in Figure 3, except that pSSU60 and pSSU160 DNA (2.5 μg each) were run in the same lane. Source of labelled RNA: **lanes 1** and **2**, light-grown nuclei; **lanes 3** and **4**, dark-grown nuclei. DNA probes: **lanes 1** and **3**, pSSU60 and pSSU160; **lanes 2** and **4**, pFa/b31. Equal amounts (15 x $10^6$ c.p.m.) of [$^{32}$P]RNA from both light- and dark-grown nuclei were used in the hybridization. **A**, insert of pFA/b31; **B**, insert of pSSU60 and pSSU160.

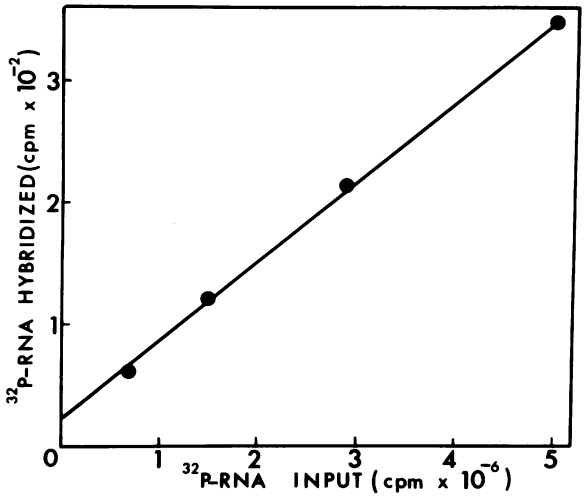


**Fig. 5.** Quantitative hybridization of RNA synthesized by nuclei isolated from light-grown pea leaves to SSU cDNA. Increasing amounts of [$^{32}$P]RNA were hybridized to filters bearing 2.5 μg each of pSSU60 and pSSU160 DNA. Counts bound to control filters bearing 5 μg of pAT153 DNA were subtracted.

*Comparison of transcripts from light- and dark-grown nuclei*

When the labelled RNA transcripts synthesized by nuclei from either light-grown or dark-grown pea leaves are compared, differences between the amount of hybridization to the SSU and LHC DNA probes are seen. Hybridization to the LHC probe is stronger than to the SSU probe for RNA from both dark- and light-grown nuclei (Figure 4: compare lanes 2 and 4 with lanes 1 and 3). Transcripts for both SSU and LHC polypeptides are more abundantly synthesized by light-grown nuclei than by dark-grown nuclei (Figure 4: compare lanes 1 and 2 with lanes 3 and 4). Hybridization of transcripts from dark-grown nuclei to the SSU probe is just above the level of detection by fluorography.

The rate of transcription of genes can be measured quantitatively by filter hybridization of RNA synthesized by isolated nuclei to a DNA probe (Tsai *et al.*, 1978; Palmiter and Lee, 1980). Isolated pea leaf nuclei were incubated with [α-$^{32}$P]UTP, and the labelled RNA hybridized to plasmid DNA bound to nitrocellulose filters under conditions of DNA excess. Figure 5 shows that there is a linear relationship between the amount of labelled RNA used and the amount of hybridization to the SSU probe.

Quantitative measurement of transcription by filter hybridization shows that both the SSU and LHC polypeptide genes are much more actively transcribed in nuclei isolated from light-grown pea leaves than from dark-grown ones (Tables II and III). The stimulatory effect of growth in the light on transcription of these genes has been observed in eight separate experiments. The percentage of the input RNA bound to the filters was the same when either [$^{32}$P]UTP or [$^{3}$H]UTP was used as the labelled precursor. Equal numbers of nuclei from light-grown and dark-grown plants were used to synthesize the RNA bound to the filters, so the observed changes in hybridization cannot be due to changes in the complexity of the RNA. The increase in transcription of the SSU gene due to growth in the light is ~18-fold, while that for the LHC gene is ~9-fold. There is also substantial transcription of rRNA genes in both types of nuclei. In this case, growth of plants in the light produces only a 2-fold stimulation in the rate of transcription (Table III).

*Stability of SSU and LHC transcripts in isolated nuclei*

The results presented so far suggest that the difference in the steady-state concentrations of SSU and LHC transcripts between light- and dark-grown pea leaves is produced by differences in the rates of transcription of their genes. However, it is possible that the genes are transcribed at equal rates in both dark- and light-grown leaves, but that in the dark tissue

**Table II.** Transcription of SSU sequences in nuclei isolated from light-grown and dark-grown *Pisum* leaves[a]

| Source of [$^3$H]RNA | Input [$^3$H]RNA c.p.m. x $10^{-6}$ | c.p.m. bound to filter[b] | c.p.m. bound as % input |
|---|---|---|---|
| Light-grown nuclei | 4.86 | 246 | 0.005 |
| Dark-grown nuclei | 4.36 | 11.7 | 0.00027 |

[a]Nuclei were incubated with [$^3$H]UTP for 10 min and the labelled RNA hybridized to filters bearing 5 μg of SSU cDNA (pSSU60 and pSSU160) as described in Materials and methods. Filters were counted for 60 min. The figures have been corrected by subtraction of counts bound to control filters bearing 5 μg pAT153 DNA (32.5 c.p.m. for light-grown nuclei and 7 c.p.m. for dark-grown nuclei).
[b]Average of duplicate filters.

**Table III.** Transcription of LHC and rRNA sequences in nuclei isolated from light-grown and dark-grown *Pisum* leaves[a]

| Source of [$^{32}$P]RNA | DNA probe | Input [$^{32}$P]RNA c.p.m. x $10^{-6}$ | c.p.m. bound to filter[b] | c.p.m. bound as % input |
|---|---|---|---|---|
| Light-grown nuclei | pFa/b31 | 2.29 | 744 | 0.032 |
| Dark-grown nuclei | pFa/b31 | 2.21 | 75 | 0.0034 |
| Light-grown nuclei | pTA250 | 0.192 | 25 825 | 13.4 |
| Dark-grown nuclei | pTA250 | 0.203 | 12 719 | 6.2 |

[a]Nuclei were incubated with [$^{32}$P]UTP for 10 min and labelled RNA hybridized to filters bearing 5 μg of DNA probe as described in Materials and methods. Filters were counted for 60 min. The figures have been corrected by subtraction of counts bound to control filters bearing 5 μg pAT153 DNA (89 c.p.m. for light-grown nuclei and 25 c.p.m. for dark-grown nuclei).
[b]Average of duplicate filters.

the transcripts are rapidly degraded, so that the observed hybridization is due to a residual amount of transcript. It is difficult to test this possibility by *in vivo* experiments, but the stability of SSU and LHC transcripts synthesized by nuclei isolated from dark-grown leaves can be measured. Nuclei were incubated for 10 min with [$^{32}$P]UTP, and aliquots incubated for up to 120 min in the presence of excess unlabelled UTP and actinomycin D. Table IV shows that the low amount of hybridization to both SSU and LHC probes seen in RNA made by dark-grown nuclei persists during the chase period. The small number of counts hybridizing to the SSU probes is not seen if α-amanitin is added at the start of the incubation, confirming that these counts are produced by the activity of RNA polymerase II (data not shown).

## Discussion

This is the first report of the transcription of specific polypeptide-encoding genes in nuclei isolated from a higher plant tissue. Pea leaf nuclei give reproducible results, and their transcriptional activity is unimpaired on storage at −80°C for at least 2 months. The use of cloned cDNA probes for SSU and LHC RNA sequences shows that genes for these chloroplast polypeptides are transcribed in nuclei isolated from both dark-grown and light-grown *Pisum* leaves. Previous studies have detected translatable LHC and SSU mRNAs in dark-grown *Pisum* leaves (Cuming and Bennett, 1981; Sasaki *et al.*, 1981). Although SSU and LHC mRNAs are the most abundant mRNAs in light-grown pea leaves, as measured by translatability in a cell-free protein-synthesizing

**Table IV.** Stability of SSU and LHC transcripts in nuclei isolated from dark-grown pea leaves[a]

| Source of [$^{32}$P]RNA | Input [$^{32}$P]RNA c.p.m x $10^{-6}$ | Hybridization to pSSU60 + pSSU160 | | Hybridization to pFa/b31 | |
|---|---|---|---|---|---|
| | | c.p.m. bound to filter[b] | c.p.m. bound as % input x $10^{-4}$ | c.p.m. bound to filter[b] | c.p.m. bound as % input x $10^{-4}$ |
| 10 min pulse | 5.42 | 5.6 | 1.03 | 170.2 | 31 |
| 10 min chase | 5.096 | 4.2 | 0.82 | 167.5 | 32.9 |
| 20 min chase | 5.36 | 5.85 | 1.09 | 208.3 | 38.8 |
| 30 min chase | 4.14 | 4.5 | 1.09 | 132.3 | 32 |
| 60 min chase | 6.35 | 7.1 | 1.12 | 169.5 | 27 |
| 120 min chase | 3.61 | 4.0 | 1.11 | 90.4 | 25 |

[a]Nuclei from dark-grown leaves were incubated with [$^{32}$P]UTP (500 μCi in 300 μl) for 10 min (pulse), and 10 mM unlabelled UTP and 10 μg/ml actinomycin D were then added. Aliquots (60 μl) were removed at once and after incubation for the indicated times (chase). Labelled RNA was hybridized to filters bearing 5 μg DNA probe and the filteres counted for 60 min. The figures are corrected for counts bound to control filters bearing 5 μg pAT153 DNA (3.2 c.p.m.).
[b]Average of duplicate filters.

system (Broglie *et al.*, 1981), the percentage of RNA synthesized by isolated nuclei that is represented by these sequences is very small (Tables II and III). However, comparison of these values with corresponding values for the transcription of genes for abundant mRNAs in nuclei isolated from animal cells indicates that higher values should not be expected (McKnight and Palmiter, 1979; Tsai *et al.*, 1978; Colbert *et al.*, 1980).

The SSU gene occurs in one or a very few copies per haploid *Pisum* genome (Cashmore, 1979). In contrast, the genes for rRNA occur at a frequency of ~4000 copies per haploid genome in this species (Ingle and Sinclair, 1972), so the high percentage of rRNA in the nuclear transcripts is expected (Table III).

Growth of leaves in the light has a pronounced stimulatory effect on the rate of transcription of genes for both SSU and LHC polypeptides in isolated nuclei (Figure 4, Tables II and III). This stimulation is a true increase in the rate of transcription, and is not due to a decrease in the rate of RNA degradation (Table IV). These results suggest that a major contribution to the light-induced increase in the steady-state concentrations of hybridizable SSU RNA sequences (Smith and Ellis, 1981) and translatable LHC RNA sequences (Cuming and Bennett, 1981) is mediated by an increase in the rate of transcription. It must not be assumed from this conclusion that these RNA sequences are necessarily stable in the cytoplasm; in other species there is evidence for breakdown of SSU and LHC mRNAs when light-grown plants are transferred to darkness (Tobin, 1981b; Tobin and Suttie, 1980).

There is an increasing body of evidence that phytochrome is involved in the light regulation of the translatable mRNAs for SSU and LHC polypeptides (Apel, 1979; Tobin, 1981a). The mechanism of phytochrome action is unknown, because most studies have been confined to the physiological level. Our demonstration that the transcription of chloroplast genes in isolated nuclei retains the rate changes induced by light treatment of the seedlings opens up the possibility of a biochemical analysis of the difference in transcription produced by the phytochrome system.

## Materials and methods

*Growth of pea seedlings*

Seeds of *P. sativum* (var. Feltham First) were sown in J.Arthur Bowers potting compost and grown in the light with a 12 h photoperiod at 20 – 22°C for 9 days. Illumination was provided by 'warm white' fluorescent tubes (Phillips) at 20 – 40 μeinsteins/m²/s of photosynthetically active radiation. Dark-grown seedlings were grown in total darkness at 22°C for 9 days.

*Isolation of nuclei*

Nuclei were isolated by a modification of the method of Luthe and Quatrano (1980a). All procedures were carried out at 0°C. Pea leaves (10 g) were soaked in ice-cold ether under a slight vacuum for 30 – 60 s. The leaves were drained and washed twice in 0.44 M sucrose, 25 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol (Buffer A). The leaves were washed once in buffer A containing 2 mM spermine, 2.5% Ficoll F400, 5% Dextran T40, and 0.5% Triton X-100 (buffer B). The leaves were then suspended in 20 ml of buffer B and homogenized using a Polytron PT 10-20 blender at speed setting 2 – 3 for 15 s. Buffer B (20 ml) was added to the homogenate, and the whole filtered through eight layers of muslin. The residue was re-extracted with 20 ml of buffer B, and the combined filtrates centrifuged at 2500 *g* for 5 min. The pellet was resuspended in a small volume (2 – 3 ml) of buffer B with spermine omitted, and layered onto a Percoll gradient prepared according to Luthe and Quatrano (1980a) except that the 2 M sucrose pad was replaced by one of 85% (w/v) sucrose. The gradient was centrifuged at 4000 *g* for 30 min. The grey band at the sucrose pad:Percoll interface was aspirated, diluted with buffer B with spermine omitted and washed twice with the same buffer by pelleting twice at 2500 *g* for 5 min. The pellet was washed a third time in buffer C (0.44 M sucrose, 50 mM Tris-HCl pH 7.8, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol and 20% (v/v) glycerol). The final pellet was resuspended in buffer C at a concentration of $1 – 2 \times 10^8$ nuclei/ml, and stored at −80°C. The yield of nuclei was in the range $1 – 2 \times 10^7$ nuclei/g fresh weight of leaves.

*Incubation of isolated nuclei and isolation of RNA*

A standard transcription assay mixture (25 μl) contains $10^6$ nuclei in 50 mM Tris-HCl pH 7.8, 75 mM $NH_4Cl$, 10 mM $MgCl_2$, 0.5 mM ATP, GTP CTP, 10% (v/v) glycerol and 2 μCi of either [5-6 $^3$H]UTP (42 Ci/mmol) or [α-$^{32}$P]-UTP (400 Ci/mmol). Transcription is started by the addition of nuclei and the assay mixture incubated at 27°C for either 10 or 20 min. Transcription is terminated by spotting aliquots onto filter disks. The disks are washed three times in 5% (w/v) TCA over a period of 20 min and then washed once in ethanol and ether. The dried filters are counted by liquid scintillation spectrometry at a counting efficiency of 20% for $^3$H. When inhibitors are used, the nuclei are incubated with the inhibitors for 10 min on ice before addition to the assay medium. Preparative scale assays (0.2 – 1.0 ml) contain $10^8$ nuclei and 100 μCi of either [$^3$H]UTP or [α-$^{32}$P]UTP. Transcription is stopped by the addition of 10 μg/ml DNase 1 (RNase-free). Incubation is continued at 27°C for a further 10 min and TCA-precipitable counts determined.

Nuclear RNA was isolated by the procedure of McKnight and Palmiter (1979). The final ethanol precipitate is dissolved in distilled water and stored at −80°C.

*Preparation of plasmid DNA*

Recombinant plasmids containing sequences specific for the small subunit of *P. sativum* ribulose bisphosphate carboxylase, pSSU60 and pSSU160 (Bedbrook *et al.*, 1980), one LHC polypeptide of *Pisum,* pFa/b31 (S.M.Smith, personal communication), the wheat ribosomal DNA repeat unit, pTA250 (Gerlach and Bedbrook, 1979) and *Xenopus* histone H4 pcX1H4W1 (Turner and Woodland, 1982) were prepared according to Clewell (1972). Purified plasmids were restricted with *Eco*RI according to the suppliers instructions. The restricted DNA was isolated from the reaction mixture by extraction with phenol and chloroform-isoamylalcohol, and precipitated with ethanol. Plasmid DNA was attached to nitrocellulose filters (Schleicher and Schull BA 85 0.45 μm) according to McKnight and Palmiter (1979).

*DNA-excess hybridization*

Filters bearing cloned probes were prehybridized overnight at 41°C in 50% formamide, 40 mM Pipes-NaOH pH 6.5, 0.5 M NaCl, 1 mM EDTA, 0.4% SDS, 100 μg/ml poly(A) and 100 μg/ml tRNA *(Escherichia coli).* Hybridization of labelled nuclear RNA was performed in the same buffer. The solution was heated to 100°C for 90 s before hybridization. Each reaction mixture (60 μl) contained a filter bearing 5 μg of a cloned probe and a control filter bearing 5 μg of pAT153 DNA. Plasmid pAT153 is a derivative of pBR322 (Twig and Sherratt, 1980). The filteres were overlaid with 2 ml of paraffin oil and hybridized at 41°C for 20 h. After the hybridization period, filters were washed in chloroform, and then 5 times with 1.4 x SSC, 0.2% SDS for 30 min each at 60°C, twice with 0.1 x SSC for 30 min each at 60°C, once with 2 x SSC containing 10 μg/ml RNase A for 1 h at room temperature, once with 2 x SSC, 10 μg/ml proteinase K for 20 min at room temperature, and twice with 2 x SSC, 0.2% SDS for 30 min each at room temperature. The filters were treated with 250 μl of 40 mM NaOH for 1 h, followed by 100 μl of 0.1 M acetic acid, and counted after the addition of 4 ml of Triton X-100-xylene scintillant (McKnight and Palmiter, 1979). Filters were counted for 60 min. The efficiency of hybridization was measured by hybridizing [$^3$H]RNA synthesized on pSSU60 and pSSU160 templates by the method of McKnight and Palmiter (1979), and found to be ~50%.

*Gel electrophoresis and blotting of RNA and DNA*

Purified cDNA-containing plasmids were digested with restriction endonucleases according to the suppliers instructions. Digests were electrophoresed on 1% agarose gels, and DNA transferred to nitrocellulose sheets by the procedure of Southern (1975), as modified by Thomas (1980).

Hybridization of nuclear transcripts to Southern transfers of plasmid DNA was performed in the same buffer used for DNA-excess hybridization. Sheets were prehybridized at 41°C for 2 h, and hybridized for 48 – 60 h at the same temperature. The sheets were washed 4 times with 1.4 x SSC, 0.2% SDS at 60°C, twice with 0.1 x SSC at 60°C, once with 2 x SCC containing 10 μg/ml RNase A at room temperature for 1 h, and twice with 2 x SSC, 0.2% SDS at room temperature. The dried sheets were wrapped in plastic film and fluorographed at −80°C using DuPont Cronex intensifying screens.

RNA was resolved on 1.1 or 1.2% agarose gels in 3% formaldehyde, 10 mM phosphate buffer pH 7.0 (Lehrach *et al.*, 1977). Gels were fluorographed at −80°C.

*Electron microscopy*

Nuclear pellets were fixed by the procedure of Ryter and Kellenberger (1958). Ultrathin sections were cut on a Reichert OMU2 microtome and examined in a Jeol JEM 100S instrument operating at 60 kV.

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