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Phytochrome-controlled expression of a wheat Cab gene in transgenic tobacco seedlings

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We have monitored changes in the chlorophyll a/b-binding protein (Cab) mRNA levels in etiolated wheat leaves exposed to light of different wavelengths by Northern blot hybridizations and 5′ S1 nuclease protection assays. Accumulation of the Cab mRNA and the specific *Cab-1* transcript is regulated by phytochrome. Transcript levels are elevated by red light and the red enhancement can be partially reversed by far-red light. Moreover, far-red light alone can elicit a small increase in the transcript levels. These characteristic features of the wheat *Cab-1* phytochrome response can be recapitulated in etiolated seedlings of transgenic tobacco containing the *Cab-1* gene. Analyses of a chimeric construct revealed that a 1.8-kb 5′-flanking fragment (−1816 to +31) of the *Cab-1* gene can confer phytochrome response as well as leaf-specific expression on a heterologous coding sequence.

Key words: phytochrome regulation/chlorophyll a/b-binding protein gene/Ti-mediated gene transfer/transgenic tobacco/chimeric gene

Introduction

The chlorophyll a/b-binding protein (Cab) is the major protein of chloroplast thylakoids, accounting for up to 30% of the total thylakoid membrane polypeptide. This protein is found in association with chlorophylls a and b to form the light-harvesting chlorophyll–protein complex whose function is to harvest light energy for photosynthetic electron transport (cf. Glazer, 1983). Although the Cab protein is localized within the chloroplasts, it is first synthesized on free cytoplasmic ribosomes as a larger precursor (Apel and Klopstech, 1978; Schmidt *et al.*, 1981). After synthesis, the precursor is imported into the chloroplast by an energy-dependent process (Grossman *et al.*, 1980) and processed correctly to the mature form. The mature polypeptide is integrated into the thylakoids where it binds chlorophylls a and b to form the light-harvesting chlorophyll–protein complex (Schmidt *et al.*, 1981).

mRNA encoding the Cab protein is a major transcript in leaf mesophyll cells, consistent with the abundance of the protein in this cell type (Broglie *et al.*, 1981). In both monocots and dicots, the Cab mRNA is transcribed from a multigene family in the nucleus (Coruzzi *et al.*, 1983; Dunsmuir *et al.*, 1983; Cashmore, 1984; Lamppa *et al.*, 1985a; Dunsmuir, 1985; Karlin-Neumann *et al.*, 1985). Several laboratories have reported that the Cab mRNA level is very low in etiolated barley (Apel, 1979), *Lemna* (Tobin, 1981) and peas (Thompson *et al.*, 1983; Jenkins *et al.*, 1983). The mRNA level can be increased considerably after a brief illumination with red light but the stimulating effect of red light can be reversed by far-red illumination (Apel, 1979; Tobin, 1981; Thompson *et al.*, 1983; Jenkins *et al.*, 1983; Gollmer and Apel, 1983). In fact, far-red light alone can also elicit a small increase in the Cab mRNA level (Kaufman *et al.*, 1984). These modulating effects of red and far-red light provide strong evidence that the Cab gene family is regulated by the photomorphogenic pigment, phytochrome (Shropshire and Mohr, 1983; Tobin and Silverthorne, 1985). An important point of phytochrome regulation is the transcriptional activation of the Cab genes as shown by transcript run-off experiments using isolated nuclei (Silverthorne and Tobin, 1984; Mosinger *et al.*, 1985).

Our laboratory is interested in the identification and manipulation of *cis*-acting regulatory elements of nuclear genes that respond to phytochrome. To this end we have isolated and characterized a wheat Cab gene, designated *Cab-1* (previously referred to as whAB1.6), whose expression is induced by light (Lamppa *et al.*, 1985a). We have used Ti-mediated gene transfer techniques to introduce this monocot gene into the tobacco genome and showed that the *Cab-1* gene retains light-inducible expression in leaves of transgenic tobacco (Lamppa *et al.*, 1985b). Chimeric constructs containing 5′-flanking fragments of pea and petunia Cab genes have also been transferred into tobacco and petunia plants, respectively (Jones *et al.*, 1985; Simpson *et al.*, 1985). Both chimeric constructs are active in transgenic plants (Jones *et al.*, 1985; Simpson *et al.*, 1985) and, in addition, expression of the construct containing the pea Cab-AB80 5′-flanking sequence is stimulated by light (Simpson *et al.*, 1985). However, it is not known whether the monocot (Lamppa *et al.*, 1985b) or the dicot Cab gene (Simpson *et al.*, 1985) would be regulated by phytochrome in transgenic plants.

In this paper we have extended our previous investigations on the wheat Cab-1 gene (Lamppa *et al.*, 1985a, 1985b). By Northern blot hybridizations and 5′ S1 nuclease protection assays we show here that the expression of the wheat Cab gene family and of the *Cab-1* gene in particular is regulated by phytochrome in etiolated wheat leaves. Moreover, phytochrome control of the wheat *Cab-1* gene can be reconstructed faithfully in etiolated seedlings of transgenic tobacco. Analysis of a chimeric construct reveals that a 1.8-kb 5′-flanking sequence of the Cab-1 gene can confer phytochrome responsiveness on a heterologous coding sequence.

Results

Expression of the Cab-1 gene is under phytochrome control

We first investigated the responses of the wheat Cab mRNA to various light treatments by Northern blot hybridizations. Figure 1A shows that etiolated wheat leaves contain negligible amounts of Cab mRNA (lane 1). After 24 h of continuous illumination with white light the mRNA level is elevated by at least 20 times (lane 2). The same level of induction can be obtained with just a 3 min pulse of red light (lane 3). The red induction is largely but not completely reversed by far-red illumination (lane 4). This is consistent with the finding that far-red light alone also elicits

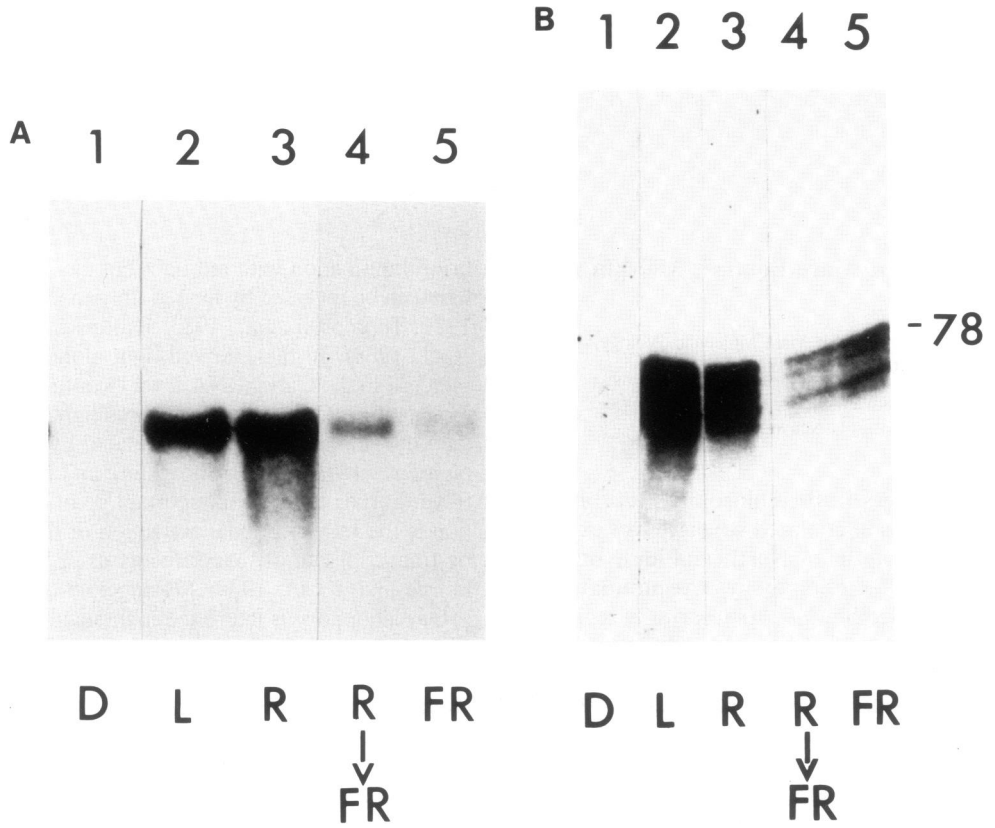

Fig. 1. Phytochrome regulation of wheat Cab transcript accumulation. Wheat seedlings were germinated in the dark for 7 days before the various light treatments **(1)** D, dark control; **(2)** L, 24 h continuous white light (1000 $\mu E/M^2/s$); **(3)** R, 3 min red light (100 $\mu E/M^2/s$); **(4)** R/FR, 3 min red light (100 $\mu E/M^2/s$) followed by 10 min far-red light (35 $\mu E/M^2s$); **(5)** FR, 10 min far-red light (35 $\mu E/M^2/s$). After these light treatments seedlings were returned to the dark and RNA was extracted 16 h later as described in Materials and methods. Panel **(A)** Northern blot hybridizations using a ^{32}P-labeled *PstI – SmaI* fragment of the wheat *Cab-1* gene as a probe (Lamppa *et al.*, 1985a) 10 μg of total RNA were used for each lane. Panel **(B)** 5′ S1 nuclease protection assays. Details are given in Materials and methods. 10 μg of total RNA were used for each lane. The position of the 78-nucleotide protected fragment is indicated.

a small increase in the Cab gene level (lane 5). Taken together, these results provide clear evidence that the wheat Cab mRNA is under phytochrome control, as has been reported for several other higher plants (Apel and Kloppstech, 1978; Tobin, 1981; Jenkins *et al.*, 1983; Thompson *et al.*, 1983; Gollmer and Apel, 1983; Kaufman *et al.*, 1984).

In the experiments presented in Figure 1A, a Cab-coding sequence, which hybridizes to all members of the Cab gene family, was used as a probe. To examine the expression pattern of the wheat *Cab-1* gene which we have characterized previously (Lamppa *et al.*, 1985a) a probe derived from the 5′ end of the gene was used to detect gene-specific transcripts by S1 nuclease protection assays. Figure 1B shows that the wheat *Cab-1* gene is indeed regulated by phytochrome; moreover, its transcript level is also increased slightly by far-red light alone.

Phytochrome regulation in transgenic tobacco

As a first step toward identifying *cis*-acting elements for phytochrome regulation of the *Cab-1* gene, the latter was transferred into tobacco using a Ti-mediated gene transfer vector, pMON145 (Figure 2). The transgenic tobacco plants were selfed and the seeds were germinated on MS medium in the dark for 7 days. The etiolated F_1 seedlings were subjected to various light treatments and the RNAs were analyzed by Northern blot hybridization and S1 nuclease protection assays. Figure 3A shows that the endogenous tobacco Cab mRNA is subjected to phytochrome regulation in a way similar to the wheat Cab mRNA. As a control, the mRNA for the β subunit of mitochondrial ATPase (*atp2-1*), a constitutively expressed gene (Boutry and Chua, 1985), remains approximately at the same concentration with the various light treatments (Figure 3B). To examine the response of the introduced wheat gene, we analyzed the RNA samples for *Cab-1*-specific transcripts by 5′ S1 nuclease protection assays. Figure 4 shows that the wheat *Cab-1* gene retains phytochrome regulation in transgenic tobacco seedlings. The multiple bands comprising the 5′ S1 (Figures 1b and 4b) or the 3′ S1 (Figures 6 and 7) signal represent a spread of a few nucleotides. This is likely due to the 'chewing in' of the DNA–RNA hybrids by S1 nuclease.

Phytochrome response of chimeric constructs

The retention of phytochrome regulation of the wheat *Cab-1* gene in transgenic tobacco renders the latter a useful system to investigate *cis*-acting elements required for the phytochrome response. In another light-inducible gene (*rbcS*), encoding the small subunit of ribulose-1,5-bisphosphate carboxylase, the phytochrome-responsive elements are located 5′ to the transcription start site (Fluhr and Chua, 1986). Accordingly, we isolated a 1.8-kb fragment from the 5′-flanking region (position −1816 to +31) of the *Cab-1* gene. This fragment was fused to the coding sequence of the bacterial chloramphenicol acetyl transferase (CAT) followed by a 3′ non-coding region of the pea *rbcS-E9* gene (Figure 2b). The chimeric construct was introduced into tobacco using the Ti-mediated vector, pMON145 (Broglie *et al.*, 1984; Lamppa *et al.*, 1985b). The transgenic plants were selfed and the F_1 seedlings were analyzed. Similar F_1 seedlings con-

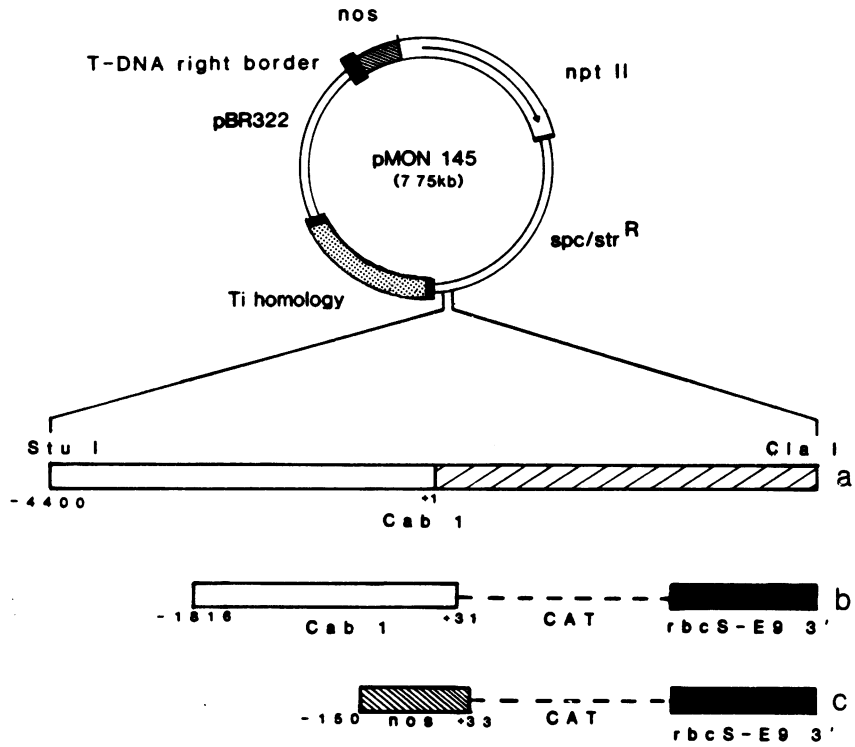

Fig. 2. A schematic diagram of the pMON145 intermediate cloning vector with the inserted genes. (**a**) 6.5-kb wheat genomic fragment containing the entire wheat *Cab-1* gene including 4.4 kb 5′ upstream sequences and 800 bp 3′ sequences beyond the TAA stop codon (Lamppa *et al.*, 1985a, 1985b). (**b**) A 5′-flanking fragment (−1816 to +31) of the wheat *Cab-1* gene was fused to the coding sequence of the bacterial CAT gene followed by the 3′-non-coding region of the pea *rbcS-E9* gene. Details of the CAT−*rbcS-E9* construction were given in Morelli *et al.* (1985). (**c**) Same as (**b**), except that the wheat *Cab-1* fragment was replaced by a truncated promoter (−150 to +33) of the NOS gene. The chimeric gene was inserted into the unique *StuI*−*ClaI* sites and the orientation relative to the NOS-neomycin phosphotransferase II (nptII) gene is shown. The intermediate cloning vector pMON145 has been described in detail by Broglie *et al.* (1984).

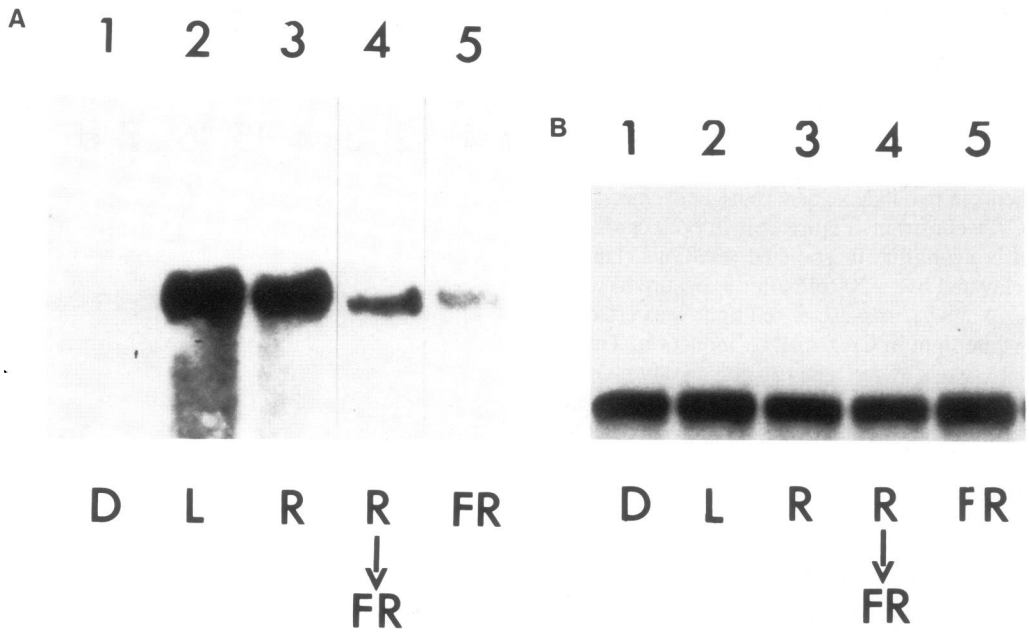

Fig. 3. Expression of the tobacco Cab gene but not of the *atp2* gene is controlled by phytochrome. F_1 seedlings of selfed tobacco were germinated on MS medium in the dark for 7 days. For abbreviations and light treatments, see legend to Figure 1. Northern blot hybridizations were performed using 10 μg of total RNA for each lane. The probes were (**A**) the *PstI*−*SmaI* fragment of the wheat *Cab-1*gene (Lamppa *et al.*, 1985a) which under non-stringent conditions (37°C, 50% formamide, 6 × SSC) cross-hybridizes to the endogenous tobacco Cab mRNA and (**B**) the *atp2-1* cDNA clone (Boutry and Chua, 1985).

taining a nopaline synthase (NOS)−CAT hybrid construct (Figure 2c) were used as controls.

The F_1 seedlings were analyzed for CAT activity after various light treatments. Etiolated transgenic seedlings containing the Cab−CAT construct possess low levels of CAT activity in the dark (Figure 5, lane 4); however, the CAT activity can be increased by ~5−10 times with a pulse of red light (Figure 5, lane 5). The effect of red light can be completely abrogated by

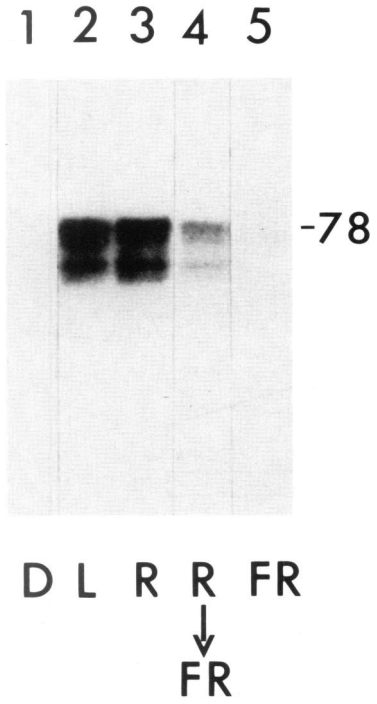

Fig. 4. The wheat *Cab-1* gene retains phytochrome response in transgenic tobacco. F_1 seedlings of selfed transgenic tobacco containing the wheat *Cab-1* gene were germinated in the dark for 7 days. RNA samples were analyzed for *Cab-1* specific transcripts by 5′ S1 nuclease protection. Each lane contained 10 μg of total RNA. For abbreviations see Figure 1. The position of the 78-nucleotide protected fragment is indicated.

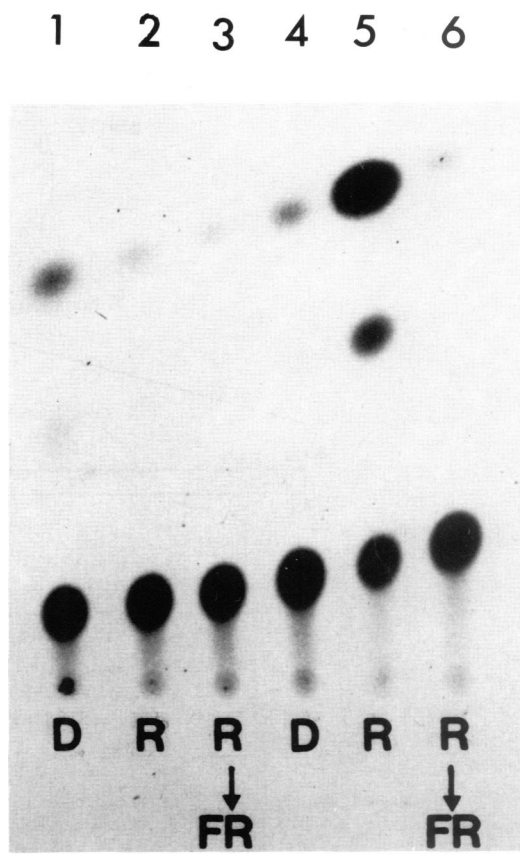

Fig. 5. Effects of red and far-red light on CAT activities in transgenic tobacco seedlings. **Lanes 1–3**, NOS-CAT chimeric construct (Figure 2c); **lanes 4–6** Cab–CAT chimeric construct (Figure 2b). For abbreviations and light treatments see legend to Figure 1. CAT activities were assayed according to Gorman *et al.* (1982) except that the protein concentration was 5 μg/40 μl.

far-red light (Figure 5, lane 6). By contrast, the CAT activity of seedlings containing the NOS–CAT construct is not increased by red or red/far-red illumination (Figure 5, lanes 1–3). Considered together, these results strongly suggest that the 5′-flanking fragment of the *Cab-1* gene is sufficient to confer phytochrome regulation on a heterologous coding sequence.

To see whether the stimulation of Cab–CAT activity by red light in Figure 5 is reflected at the transcript level, we determined the CAT mRNA concentrations by 3′ S1 nuclease protection. Figure 6 shows the changes in the CAT mRNA levels with various light treatments in two independent transgenic clones containing the Cab–CAT construct (Figure 2b). In both cases, the CAT mRNA level is negligible in etiolated seedlings (lanes 5 and 6) but can be elevated by ~20-fold after exposure to white light for 24 h (lanes 4 and 7). A pulse of red light is as effective (lanes 3 and 8) as white light in CAT mRNA induction. The increase of CAT mRNA levels by red light can be partially reversed by far-red light (lanes 2 and 9) and far-red light alone also promotes a small response (lanes 1 and 10). Since the CAT mRNA stability is not sensitive to phytochrome (Fluhr and Chua, 1986) these results demonstrate clearly that the 5′-flanking sequence of the *Cab-1* gene is sufficient to confer phytochrome-induced transcription.

Organ-specific expression of the Cab–CAT chimeric construct

Leaves, stems and roots were collected from transgenic plants containing the Cab–CAT chimeric construct and analyzed for CAT activity. Figure 7A shows that the enzymatic activity is present in leaves but hardly detectable in stems and roots. Similar organ-specific distribution of the CAT mRNA was obtained when RNA samples were analyzed by 3′ S1 nuclease protection (Figure 7B). These results provide evidence that the *Cab-1* 5′-flanking fragment is sufficient to confer organ-specific expression on the bacterial CAT coding sequence.

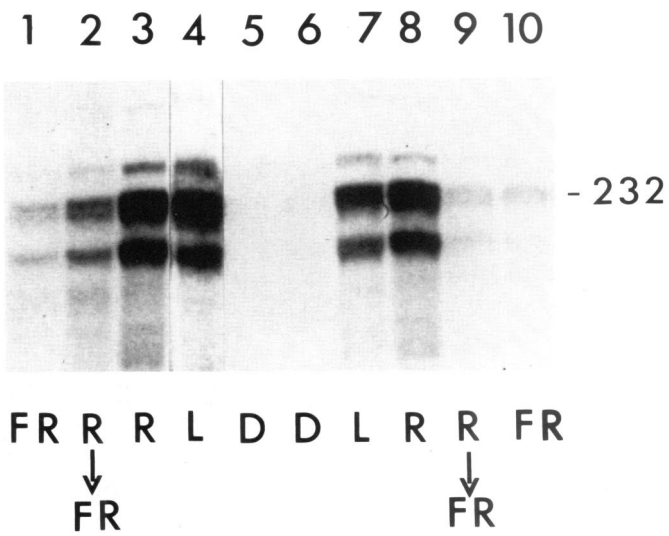

Fig. 6. A 5′-flanking fragment of the *Cab-1* gene is sufficient for phytochrome responsiveness. F_1 seedlings of selfed transgenic tobacco (two independent clones) containing the Cab–CAT construct (Figure 2b) were germinated on MS medium in the dark for 7 days. For abbreviations and light treatments, see Figure 1. Total RNA (50 μg) was assayed by S1 nuclease protection using a 3′-specific probe from the *rbcS-E9* gene (Morelli *et al.*, 1985). **Lanes 1–5**, clone 1; **lanes 6–10**, clone 2. The position of the 232-nucleotide protected fragment is indicated.

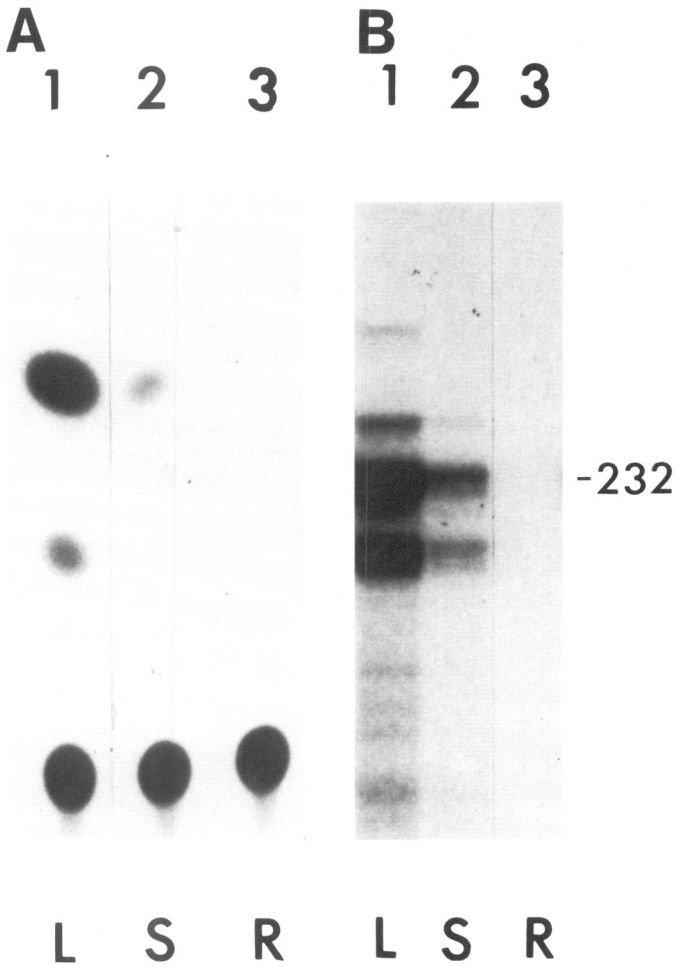

Fig. 7. Organ-specific expression of the Cab−CAT chimeric construct. **(A)** CAT activities were determined as described in legends to Figure 5. **(B)** CAT mRNA levels were analyzed by 3′ S1 nuclease protection using a 3′-specific probe from the *rbcs-E9* gene (Morelli *et al.*, 1985), 50 μg total RNA were used for each sample; **lanes 1, 2** and **3** contained samples from leaves, stems, and roots respectively.

Discussion

The regulation of Cab mRNA levels by phytochrome has been reported for two monocots (Apel, 1979; Tobin, 1981) and two dicots (Thompson *et al.*, 1983; Jenkins *et al.*, 1983). However, in all these previous experiments, the mRNA levels were monitored either by *in vitro* translation (Apel, 1979; Tobin, 1981) or by hybridization to a cDNA probe (Thompson *et al.*, 1983; Jenkins *et al.*, 1983; Stiekema *et al.*, 1983; Gollmer and Apel, 1983). Neither method was able to discriminate related Cab transcripts and therefore, phytochrome regulation of specific Cab genes has never been addressed. We have approached this question by using a probe derived from the 5′ terminus of the *Cab-1* gene in S1 nuclease protection assays. Here, we show that the *Cab-1* transcript level is modulated by red and far-red light in a manner expected of a phytochrome response. These results confirm and extend previous work.

A distinctive feature of the Cab gene regulation by phytochrome in peas is its very low fluence (VLF) response, which is characterized by sensitivity to very low fluences of red light, an incomplete reversal of the red enhancement by far-red light and the induction by far-red light alone (Kaufman *et al.*, 1984, 1985). While we have not carried out detailed fluence-response experiments, the general features of the VLF response appear to apply to the wheat Cab gene family as a whole (Figure 1A) and the *Cab-1* gene in particular (Figure 1B). More significantly, the VLF response of the *Cab-1* gene is faithfully recapitulated in etiolated seedlings of transgenic tobacco (Figure 4). These results constitute the first report of phytochrome-regulated expression of any Cab gene in transgenic plants. Retention of phytochrome response of two pea *rbcS* genes in transgenic petunia has been demonstrated recently (Fluhr and Chua, 1986).

Immunological experiments have revealed some similarities as well as differences in the structure of phytochrome between monocots and dicots (Vierstra and Quail, 1985). Our reconstruction of phytochrome regulation of the wheat *Cab-1* gene in transgenic tobacco strongly suggests that the mechanisms for phytochrome-mediated gene expression are conserved between monocots and dicots. Since these two groups of flowering plants are thought to have diverged in the Cretaceous, the experiments reported here have bridged a gap of 110×10^6 years in evolutionary time (Dahlgren *et al.*, 1985). Moreover, the experiments also provide us with confidence to use the transgenic tobacco system as an assay for *cis*-regulatory elements that are responsive to phytochrome.

Phytochrome regulation of Cab gene expression may involve transcriptional and/or post-transcriptional events. Experiments using isolated nuclei have demonstrated that the rate of transcription initiation of Cab genes is modulated by red and far-red light (Silverthorne and Tobin, 1984; Mosinger *et al.*, 1985). These results are confirmed by our experiments here using a different approach. We have used two different 5′-flanking fragments, one from NOS and the other from *Cab-1*, to express the bacterial CAT coding sequence. Under NOS control, there are no significant changes in the CAT activity between dark and the different light treatments. By contrast, under *Cab-1* control, the CAT activity and mRNA level show the classical phytochrome response. Considered together, these results provide strong evidence that the transcription rate of the *Cab-1* gene is modulated by phytochrome. We note that a single red light pulse (Figure 6, lanes 3 and 8) is as effective, or almost as effective, as 24 h of continuous white light (Figure 6, lanes 4 and 7) in eliciting an increase in transcription rate. Therefore, in etiolated tissues, light is needed to trigger the Cab gene transcription but not to maintain it, at least within the time span (16 h) of our experiments. Experiments are in progress to define critical sequences for phytochrome induction of the *Cab-1* gene.

Materials and methods

Ti-mediated gene transfer

pMON145 intermediate cloning vectors containing different chimeric genes (Figure 2) were transferred by triparental crosses into a 'disarmed' *Agrobacterium tumefaciens* (GV3111SE) and recombined into the modified pTiB6S3SE plasmid, in which all phytohormone biosynthetic genes and the TL-DNA right border have been deleted (Fraley *et al.*, 1985). These *A. tumefaciens* cells were co-cultured with leaf disks of *Nicotiana tabacum* SR1 medium containing 100 μg/ml kanamycin (Horsch *et al.*, 1985). After root formation, transgenic plantlets were transferred to soil and grown to flowering in a greenhouse.

Preparation of RNA and Northern blot analysis

RNA was extracted from the appropriate plant organs using guanidium thiocyanate as a protein denaturant (Chirgwin *et al.*, 1979) and further purified by centrifugation through CsCl cushions (Glisen *et al.*, 1974). For small amounts of tissue, 0.5−1.0 g, RNA was extracted according to Kirk and Kirk (1985) except that 0.5 mM aurin tricarboxylic acid was added as an inhibitor of RNase. Aliquots of RNA were denaturated in glyoxyl at 50°C (Carmichael and McMaster, 1980), electrophoresed on 1% agarose gels, and transferred onto nitrocellulose filters (Thomas, 1980). The filters were hybridized with ^{32}P-labeled probes using the conditions described earlier (Nagy *et al.*, 1985). The probes used are given in the figure legends.

5' and 3' S1 nuclease analysis

The transcript level of the wheat *Cab-1* gene was determined by 5' S1 nuclease protection assay (Berk and Sharp, 1977; Weaver and Weissmann, 1979). An *Eco*RI – *Cla*I fragment (~3 kb) containing the *Cab-1* gene was subcloned into pEMBL12. The complementary strand was synthesized by polymerase I Klenow large fragment in the presence of labeled nucleotides using an 18-mer oligonucleotide as primer. After chasing with cold nucleotides the DNA was digested with *Pst*I and the labeled strand (205 bp) was separated on a 6% urea – polyacrylamide gel. For the sequence of the labeled (205-bp) strand see Lamppa *et al.* (1985a). The isolated strand was hybridized with 10 μg RNA in a solution (10 μl) containing 80% formamide, 0.4 M NaCl, 2 mM EDTA and 20 mM Pipes (pH 6.8) for 12 h at 52°C. After hybridization the reaction mixture was diluted to 150 μl with a solution containing 0.5 M NaCl, 30 mM NaOAc (pH 4.6), 1 mM $ZnSO_4$, 20 μg/ml of denatured salmon sperm DNA, 1000 U/ml S1 nuclease (BRL) and incubated at room temperature for 90 min. DNA fragments (~78 bases) protected from S1 nuclease digestion were sized in 6% sequencing gels and visualized by autoradiography. 3' S1 nuclease protection assays were performed according to Morelli *et al.* (1985) using a *Hin*dIII – *Cla*I fragment (690 bp) from the *rbcS-E9* gene as a probe. Transcripts containing the *rbcS-E9* 3' termini give a cluster of protected fragments centered around 232 bases. For details see Nagy *et al.* (1985).

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References

Apel,K. (1979) *Eur. J. Biochem.*, **97**, 183–188.
Apel,K. and Kloppstech,K. (1978) *Eur. J. Biochem.*, **85**, 581–588.
Berk,A.J. and Sharp,P.A. (1977) *Cell*, **12**, 721–732.
Boutry,M. and Chua,N.-H. (1985) *EMBO J.*, **4**, 2159–2165.
Broglie,R., Coruzzi,G., Fraley,R.T., Rogers,S.G., Horsch,R.B., Neidermeyer, J.G., Fink,C.L., Flick,J.S. and Chua,N.-H. (1984) *Science*, **224**, 838–843.
Carmichael,G.G. and McMaster,G.R. (1980) *Methods Enzymol.*, **65**, 380–391.
Cashmore,A.R. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2960–2964.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294–5304.
Coruzzi,G., Broglie,R., Cashmore,A.R. and Chua,N.-H. (1983) *J. Biol. Chem.*, **258**, 2399–2402.
Dahlgren,R.M.T., Clifford,H.T. and Yeo,P.F. (1985) *The Families of the Monocotyledons*. Springer-Verlag, Berlin.
Dunsmuir,P. (1985) *Nucleic Acids Res.*, **13**, 2503–2518.
Dunsmuir,P., Smith,S.M. and Bedbrook,J. (1983) *J. Mol. Appl. Genet.*, **2**, 285–300.
Fluhr,R. and Chua,N.-H. (1986) *Proc. Natl. Acad. Sci. USA*, in press.
Fraley,R.T., Rogers,S.G., Horsch,R.B., Eicholtz,D.A., Flick,J.S., Fink,C.L., Hoffmann,N.L. and Sanders,P.R. (1985) *Biotechnology*, **3**, 629–635.
Glazer,A.N. (1984) *Annu. Rev. Biochem.*, **52**, 125–157.
Glisen,V., Crkvenjakov,R. and Byus,C. (1974) *Biochemistry*, **13**, 2633–2637.
Gollmer,I. and Apel,K. (1983) *Eur. J. Biochem.*, **133**, 309–313.
Gorman,C.M., Moffat,L.F. and Howard,B.H. (1982) *Mol. Cell Biol.*, **2**, 1044–1051.
Grossman,A.R., Bartlett,S. and Chua,N.-H. (1980) *Nature*, **258**, 625–628.
Horsch,R.B., Fry,J.E., Hoffman,N.L., Eicholtz,D., Rogers,S.G. and Fraley,R.T. (1985) *Science*, **227**, 1229–1231.
Jenkins,G.I., Harltley,M.R. and Bennett,J. (1983) *Phil. Trans. R. Soc. Lond. (Biol.)*, **303**, 419–431.
Jones,J.D.G., Dunsmuir,P. and Bedbrook,J. (1985) *EMBO J.*, **10**, 2411–2418.
Karlin-Neumann,G.A., Kohorn,B.D., Thornber,J.P. and Tobin,E.M. (1985) *J. Mol. Appl. Genet.*, **3**, 45–61.
Kaufman,L.S., Thompson,W.F. and Briggs,W.R. (1984) *Science*, **226**, 1447–1449.
Kaufman,L.S., Briggs,W.R. and Thompson,W.F. (1985) *Plant Physiol.*, **78**, 388–393.
Kirk,M.M. and Kirk,D.L. (1985) *Cell*, **41**, 419–428.
Lamppa,G., Morelli,G. and Chua,N.-H. (1985a) *Mol. Cell Biol.*, **5**, 1370–1378.
Lamppa,G., Nagy,F. and Chua,N.-H. (1985b) *Nature*, **316**, 750–752.
Morelli,G., Nagy,F., Fraley,R.T., Rogers,S.G. and Chua,N.-H. (1985) *Nature*, **315**, 200–204.
Mosinger,E., Batschauer,A., Schafer,E. and Apel,K. (1985) *Eur. J. Biochem.*, **147**, 137–142.
Nagy,F., Morelli,G., Fraley,R.T., Rogers,S.G. and Chua,N.-H. (1985) *EMBO J.*, **12**, 3063–3068.
Schmidt,G.W., Bartlett,S.G., Grossman,A.R., Cashmore,A.R. and Chua,N.-H. (1981) *J. Cell Biol.*, **91**, 468–478.
Shropshire,W. and Mohr,H. (1983) *Photomorphogenesis*. Springer-Verlag, Berlin.
Silverthorne,J. and Tobin,E.M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1112–1116.
Simpson,J., Timko,M.P., Cashmore,A.R., Schell,J., VanMontagu,M. and Herrera-Estrella,L. (1985) *EMBO J.*, **4**, 2723–2729.
Stiekema,W.J., Wimpee,C.F., Silverthorne,J. and Tobin,E.M. (1983) *Plant Physiol.*, **72**, 717–724.
Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5201–5205.
Thompson,W.R., Everett,M., Polans,N.O., Jorgensen,R.A. and Palmer,J.D. (1983) *Planta*, **158**, 487–500.
Tobin,E.M. (1981) *Plant Mol. Biol.*, **1**, 35–51.
Tobin,E.M. and Silverthorne,J. (1985) *Annu. Rev. Plant Physiol.*, **36**, 569–593.
Vierstra,R.D. and Quail,P.H. (1985) *Plant Physiol.*, **77**, 990–998.
Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.*, **7**, 1175–1193.

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