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# Isolation and characterization of a maize chlorophyll a/b binding protein gene that produces high levels of mRNA in the dark

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**Summary.** A cDNA library prepared using mRNA isolated from red-light irradiated maize seedlings was screened by a difference procedure for clones that represent red-light regulated mRNA. Two such clones were found to represent mRNA for a chlorophyll a/b binding protein (CAB), and one of these (pAB1084) was used to screen a maize genomic library. One positive genomic clone (λAB1084) was isolated and sequenced. The gene represented by λAB1084, which we designate maize *cab-1*, contains extensive nucleotide homology within its protein coding region to CAB genes from other species. The boundaries of the transcribed region of the *cab-1* gene were determined by S1 nuclease mapping. The 5′ terminus of *cab-1* mRNA is located 52–54 nucleotides (nt) upstream of the translation start site and 34 nt downstream of a TATA box. As in the case of petunia CAB genes, several poly(A) addition sites are present in mRNA from the *cab-1* gene. The 5′ flanking DNA of *cab-1* contains sequences related to elements that have been implicated in the light-regulated expression of CAB and *rbcS* genes in other plant systems. Quantitative Northern blot hybridization analysis using a gene specific probe for *cab-1* indicates that the mRNA for this gene is present at 0.4% of the total mRNA and up to 80% of the total CAB mRNA in the leaves of dark-grown seedlings. In consequence, although the degree of up-regulation by white light is only moderate (3- to 6-fold), *cab-1* transcripts account for approximately 2% of the mRNA in the leaves of light-grown seedlings.

**Key words:** *Zea mays* – Phytochrome – Light-regulated gene expression – Light harvesting chlorophyll a/b binding protein – Chlorophyll a/b binding protein

## Introduction

The regulatory photoreceptor, phytochrome, plays an important role in mediating the effect of light on plant development (Shropshire and Mohr 1983). The first step in the action of phytochrome is the photoconversion of the Pr form (absorbance maximum at 660 nm) of the photoreceptor to the conformationally distinct Pfr form (absorbance maximum at 730 nm). The steps between Pfr formation and changes in plant development are largely unknown. It was postulated some time ago that the effects of phytochrome involve transcriptional changes in the expression of various genes (Mohr 1966), and recent experiments indicate that this is indeed the case (Harpster and Apel 1985; Kuhlemeier et al. 1987b; Quail et al. 1986; Thompson et al. 1985; Tobin and Silverthorne 1985).

Studies of animal and viral genes indicate that *trans*-acting factors can bind to specific DNA sequences in the promoter region of genes, and in so doing influence the rate of transcription from that promoter (reviewed in Sassone-Corsi and Borelli 1986). Therefore, work has begun in many laboratories to identify plant promoter sequences and protein factors responsible for transcriptional control of gene expression by light (Kuhlemeier et al. 1987a, b; Timko et al. 1985; Fluhr and Chua 1986; Green et al. 1987; Morelli et al. 1985; Nagy et al. 1986, 1987; Simpson et al. 1986; An 1987; Castresana et al. 1988; Manzara and Gruissem 1988). Thus far, most attention has been focussed on the genes for the chlorophyll a/b binding proteins (CAB) and the small subunit of ribulose bisphosphate carboxylase (rbcS).

The CAB polypeptides are major constituents of the light harvesting complex of thylakoid membranes (Anderson 1986). They are nuclear encoded by a multigene family and are synthesized on cytoplasmic ribosomes as precursor polypeptides containing a block of amino acids, called the transit peptide, at the N-terminus. The transit peptide is removed during or after translocation of the CAB polypeptide into the chloroplasts (Schmidt et al. 1981). The amounts of CAB polypeptide and of CAB mRNA increase in response to light treatment (Tobin and Silverthorne 1985). The increase in CAB mRNA is at least partially phytochrome mediated (Apel 1979; Tobin 1981) and is transcriptionally regulated (Gallagher and Ellis 1982; Kuhlemeier et al. 1987b; Quail et al. 1986).

As part of a project to isolate phytochrome-regulated genes from maize, we have isolated and characterized two cDNA clones and one genomic clone (λAB1084) representing one of the maize CAB genes. In this report we present the DNA sequence and the deduced amino acid sequence of the cloned CAB gene (maize *cab-1*) and the structure of the mRNA derived from this gene. We have also determined the amounts of *cab-1* mRNA as well as total CAB mRNA present in dark-grown and in light-treated maize

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seedlings. The results show that the *cab-1* gene transcripts are present at high levels in the dark and increase in abundance in response to either white- or red-light treatment.

## Materials and methods

*Plant growth and RNA isolation.* Maize seedlings (*Zea mays*, inbred line B73, Jacques Seed Co or Pioneer Hybrid Seed Co) were grown at 25° C in complete darkness from the start of germination or in fluorescent white light (250 μeinsteins/$m^2$ per sec at 400–700 nm) as indicated. Total cell RNA was isolated from seedlings as previously described (Colbert et al. 1983).

*cDNA and genomic cloning.* Double stranded cDNA synthesis and cloning were done as described (Hershey et al. 1984). $^{32}$P-labeled cDNA probes were synthesized with reverse transcriptase from poly(A)$^+$ RNA isolated from shoots of dark-grown plants (day 5 post-germination) or from shoots harvested 3 h after such plants were given a pulse of red-light (R3) (Hershey et al. 1984). Clones that gave different hybridization signals with the dark and R3 cDNA were analyzed further. A genomic library was produced using λ charon 35 (Loenen and Blattner 1983) from a partial *Sau*3A digest of maize DNA, as described by Murray et al. (1984). A total of $2 \times 10^6$ phage were screened for sequences homologous to the maize CAB cDNA clone pAB1084 (Benton and Davis 1977). One λ recombinant with CAB homology, λAB1084, was isolated.

*Sequencing.* The 4 kbp internal *Eco*RI fragment of λAB1084 was subcloned in both orientations into the *Eco*RI site of M13mp18 (Yanisch-Perron et al. 1985). For each orientation, a series of overlapping exonuclease III deletions was produced and cloned (Yanisch-Perron et al. 1985). These deletion clones were used for sequencing by the dideoxy chain-termination method (Biggin et al. 1983) with an M13 "universal primer" (New England Biolabs). DNA sequence analyses were performed using programs provided by the University of Wisconsin Genetics Computer Group (Devereux et al. 1984).

*S1 nuclease mapping.* S1 nuclease mapping was done according to Carlson and Ross (1983) with some modifications. The probe for mapping of the 5′ terminus was produced by 5′ end-labeling subcloned *cab-1* DNA at *Nco*I (Fig. 1). After secondary digestion with *Eco*RI the 900 bp (Fig. 1) fragment was gel purified. The fragment ($2 \times 10^4$ cpm, $10^6$ cpm/pmole) was denatured and incubated with RNA in hybridization buffer containing 80% formamide at 50° C. S1 nuclease (New England Nuclear) digestion (in 280 mM NaCl, 50 mM sodium acetate, 4.5 mM $ZnSO_4$, pH 4.6, and 20 μg/ml sonicated, heat denatured salmon testes DNA) was at 37° C for 1 h. Digestion products were separated in 8% acrylamide gels containing 8 M urea (Maxam and Gilbert 1980). For mapping 3′ termini, a single-stranded, uniformly-labeled probe was produced by synthesizing complementary $^{32}$P-labeled DNA from single-strand M13 phage DNA of deletion clone ΔK20 followed by digestion with *Nsi*I, and isolation of a 265 nucleotide (nt) fragment (Figs. 1 and 2) as described by Myers et al. (1985). The single-strand fragment ($10^4$ cpm, $2.7 \times 10^7$ cpm/pmole) was incubated at 45° C with RNA in hybridization buffer containing 50% formamide, and treated as above.

*Northern and Southern blot analysis.* The probes used for Northern and Southern blot hybridization analysis were uniformly-labeled single-stranded fragments synthesized as

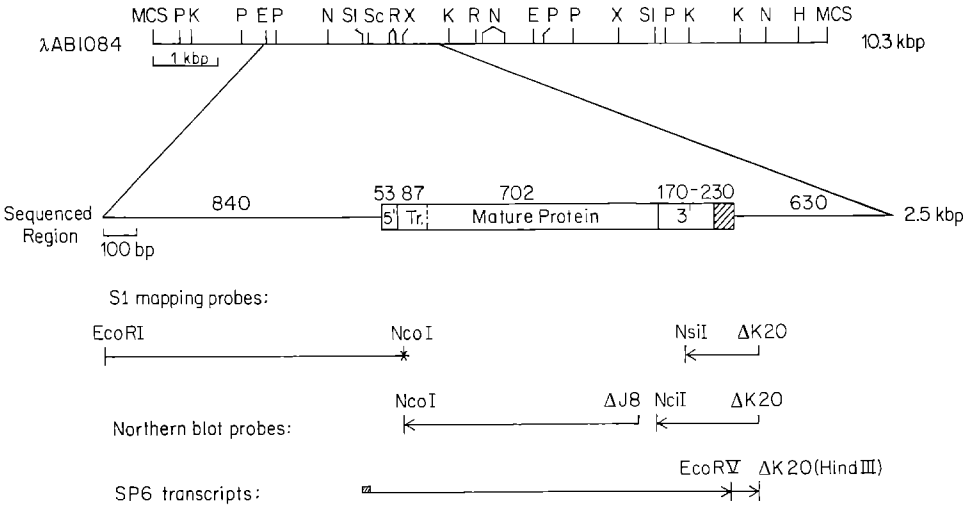


**Fig. 1.** Restriction endonuclease map of maize *cab-1* gene clone λAB1084 and restriction fragments used for transcript analysis. The top line shows the restriction enzmye map of the entire 10.3 kbp genomic DNA insert: MCS, multiple cloning site of λphage Charon 35 (Loenen and Blattner 1983); P, *Pst*I; K, *Kpn*I; E, *Eco*RI; N, *Nco*I; S1, *Sal*I; Sc, *Sac*I; R, *Eco*RV; X, *Xba*I; H, *Hin*dIII. The second line shows a summary of the sequenced region including the block indicating the transcribed region of the gene: 5′, 5′ untranslated region of mRNA; Tr, region encoding the transit peptide; 3′, 3′ untranslated region of mRNA. The *hatched region* represents the 60 nucleotide region where several 3′ termini are found in the transcribed mRNA. The number above the boxes are the length in bp. The various restriction fragments from subclones of λAB1084 that were used in the RNA analyses are shown below: ΔJ8 and ΔK20 are the end points of deletion subclones. The sequences transcribed into RNA by SP6 polymerase in vitro for use as hybridization standards are indicated at the bottom

above. The coding region probe was synthesized from clone ΔJ8, cut at *Nco*I, and a 720 nt fragment was purified (ΔJ8/*Nco*I, $8 \times 10^7$ cpm/pmole, Fig. 1). The 3′ untranslated region probe was synthesized from deletion clone ΔK20, cut at *Nci*I, and a 350 nt fragment was purified (ΔK20/*Nci*I, $2 \times 10^7$ cpm/pmole, Fig. 1). Northern blot analyses were performed as described (Lissemore et al. 1987). Hybridization buffers were prepared with either 50% (v/v) or 30% (v/v) formamide as indicated. After hybridization at 42° C membranes were washed at either 42° C or 65° C in buffer (5 mM EDTA, 25 mM sodium phosphate buffer, pH 6.8, 1.5 mM sodium pyrophosphate, 0.5% w/v SDS) containing either 0.5× or 0.1× SSC as indicated. Autoradiography was at −80° C with an intensifying screen. Southern blot analysis was performed as described by Maniatis et al. (1982) using Gene Screen Plus membrane (New England Nuclear). Prehybridization and hybridization were performed as for Northern blots (above) and as indicated.

*SP6 polymerase transcription.* The CAB mRNA region and some flanking DNA from deletion clone ΔK20 were subcloned into pSP19 (BRL). This plasmid, pSPCAB, was linearized with either *Eco*RV, which cuts 212 bp beyond the translation stop codon, or *Hin*dIII, which cuts in the pSP19 polylinker beyond the *cab-1* insert (305 bp beyond the stop codon of the *cab-1* gene, Fig. 1), and transcribed with SP6 polymerase (Promega Biotec). After transcription, the samples were extracted with phenol and the RNA was precipitated with 2M LiCl. This LiCl precipitation step removed most of the DNA template and the resulting RNA contained less than 5% DNA (data not shown).

## Results

### *Sequence analysis of* cab-1 *cDNA and genomic clones*

A cDNA library was produced from mRNA obtained 3 h after a red-light treatment of 4.5-day-old maize seedlings. Two cDNA clones were identified that hybridize to an approximately 1100 nt RNA, which increases in amount in response to the red-light treatment. Upon sequence analysis, these cDNAs were found to share sequence homology (data not shown) with a pea CAB gene (Cashmore 1984). The longer of these cDNAs (pAB1084, with a 700 bp insert) was used as a probe to screen a maize genomic library in λ phage charon 35 (Loenen and Blattner 1983). One clone containing a 10.3 kbp insert with homology to pAB1084 was isolated (λAB1084, see Fig. 1). Since this is the first maize CAB gene isolated, we will refer to it as *cab-1*. The 4 kbp internal *Eco*RI fragment of λAB1084 contains the region of homology to pAB1084 and it was subcloned into pBR322 and M13mp18 (pAB41 and mCAB41, respectively) for use in making hybridization probes and for sequencing.

The sequence of the entire polypeptide coding region, 5′ and 3′ untranslated regions, and several hundred bases of flanking DNA is reported, along with the deduced amino acid sequence, in Fig. 2. The sequences of the two CAB cDNA clones are identical to each other and to the comparable sequences in the genomic clone λAB1084 [except for a single base change in one cDNA at position 670 (Fig. 2), which probably represents a reverse transcriptase error (Battula and Loeb 1974)]. The sequence of the longest cDNA extends from position 428 to 1033 of the *cab-1* gene where it ends in a poly(A) tract (Fig. 2). As found for most CAB genes that have been analyzed, *cab-1* does not contain an intron. On the basis of comparison with other CAB proteins, the N-terminal 29 amino acids of the deduced amino acid sequence represents the transit peptide (Karlin-Neumann and Tobin 1986).

### *S1 nuclease mapping of mRNA termini*

S1 nuclease mapping of the CAB mRNA was used to delineate the transcribed sequences of the *cab-1* gene (Berk and Sharp 1977; Weaver and Weissmann 1979). The 5′ terminus was mapped using a probe 5′ end-labeled at the *Nco*I site, 15 nt downstream from the translation start codon (Figs. 1 and 2). This probe was hybridized to RNA from white-light grown maize leaves and treated with S1 nuclease, and several fragments about 70 nt long were produced (Fig. 3). S1 nuclease treatment of RNA:DNA hybrids often results in such a series of protected fragments differing by one nucleotide (Murray 1986). On the basis of the size of the protected fragments, the cap site for the *cab-1* gene mRNA is 52–54 nt upstream of the translation start codon (indicated by arrows in Fig. 3). The site most resistant to S1 nuclease digestion (Fig. 3) was chosen as the likely mRNA cap site and designated +1 for numbering the *cab-1* gene sequence (Fig. 2). Neither longer nor shorter S1 resistant fragments were detected in experiments with this probe, and the same size fragments were detected with RNA from dark-grown seedlings (data not shown).

S1 nuclease protection experiments were also performed with a probe extending to an *Xho*II site at position +453 of Fig. 2 (data not shown). Several protected fragments were obtained, the most prominent and longest of which maps the 5′ terminus of *cab-1* mRNA to the same position as the *Nco*I probe (+1, Fig. 2). Shorter fragments map between positions +52 and +220, with predominant bands at +125, +178 and +220, and minor bands at +52, +83, +143, +148, +158 and +208 (Fig. 2). These positions are dispersed across the 5′ untranslated region, the transit peptide region and the N-terminal 30 amino acids of the mature protein and may represent protected fragments from transcripts of divergent CAB genes.

The mRNA derived from individual CAB genes in petunia contain multiple poly(A) addition sites (Dean et al. 1986). Evidence for multiple 3′ termini in maize *cab-1* mRNA was obtained by S1 nuclease mapping. Initial experiments with 3′ end-labeled probes (labeled at *Xho*I, position 759; or *Hpa*II, position 841) indicated multiple 3′ ends in the region 170–230 nt downstream from the translation stop codon (data not shown). These sites are in the region of the poly(A) tract found in the sequence of the *cab-1* cDNA clone, pAB1084, which maps to 192 nt downstream of the stop codon (Fig. 2).

The 3′ termini were mapped more precisely using a uniformly-labeled probe synthesized from the deletion clone ΔK20. This probe includes the sequences from 82 to 305 nt downstream from the stop codon (Materials and methods; Fig. 1). When it was used in an S1 nuclease protection experiment, four groups of protected fragments were produced (A–D, Fig. 4; Fig. 2). These fragments map 190–240 nt downstream of the stop codon. Fragments A map to the region identified as the poly(A) addition site of cDNA clone pAB1084. Each of the other sets of fragments (B–D) represent poly(A) addition sites downstream

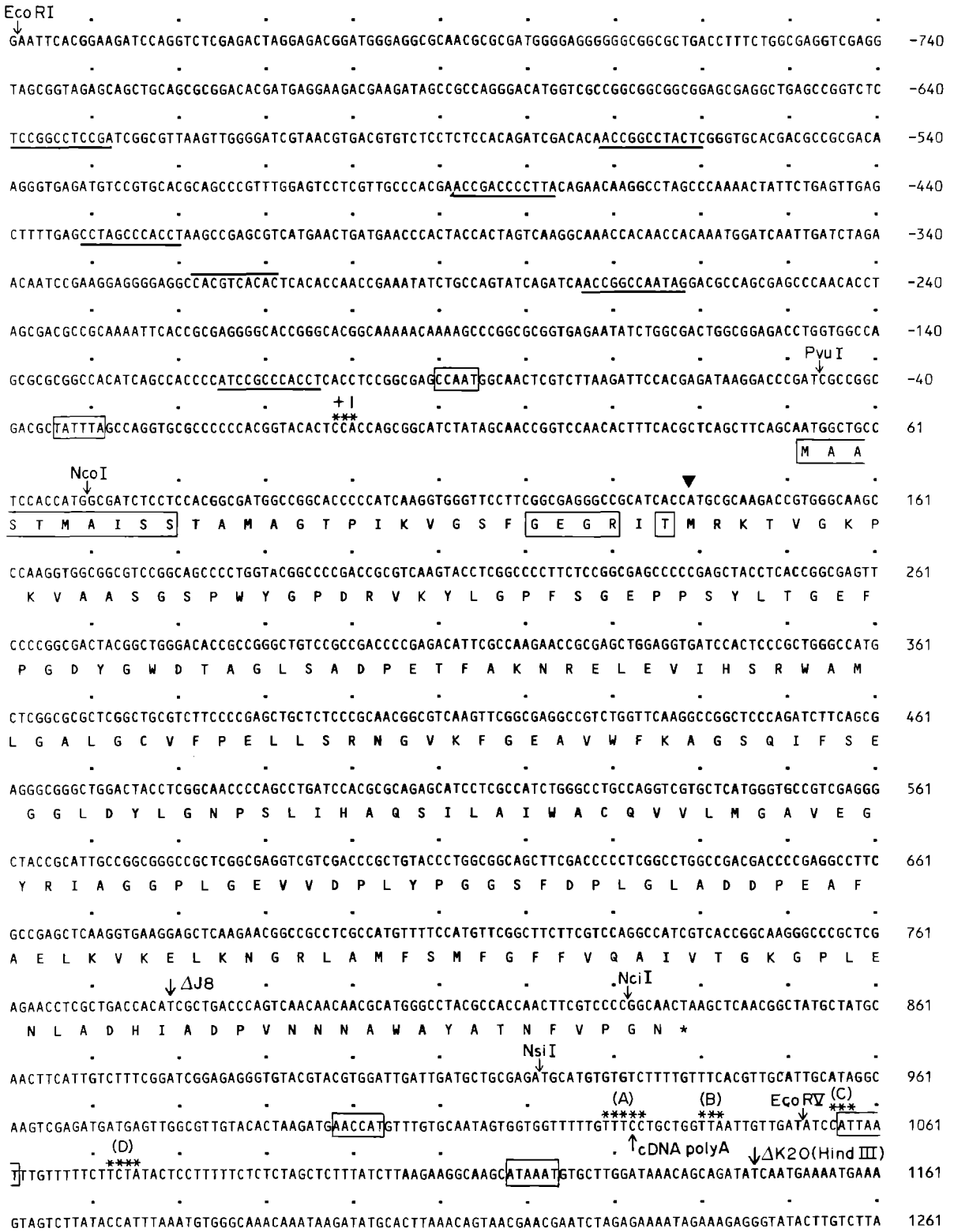


**Fig. 2.** Sequence of *cab-1* transcribed region plus flanking DNA. The nucleotide sequence for both DNA strands was determined from a series of overlapping deletion subclones. +1 is the cap site; the deduced amino acid sequence is shown below the nucleotide sequence starting at +53. The start site of the mature peptide is indicated by a *filled triangle*; (A) (B) (C) and (D) mark sites of 3′ termini found in *cab-1* mRNAs. *Boxed* nucleotide sequences include a CAAT box at −90, a TATA box at −35, putative poly(A) addition signals at +999, +1057 and +1119. *Overlined* nucleotides at −319 represent sequences similar to the *rbc*S Box 3 sequence (Manzara and Gruissem 1988). *Underlined* sequences are similar to sequences repeated several times in the *Nicotiana plumbaginifolia CAB-E* positive regulatory element sequence (Castreasana et al. 1988). The amino acid sequences with homology to the consensus transit peptide for chlorophyll a/b binding proteins are also *boxed* (Karlin-Neumann and Tobin 1986). The site of the poly(A) tail in cDNA clone pAB1084 is indicated at +1033. Restriction enzyme sites used in subcloning, probe preparation, and for SP6 transcription are shown. *Δ*J8 and *Δ*K20 mark the 3′ end points of deletion clones used for probe preparation and SP6 transcription

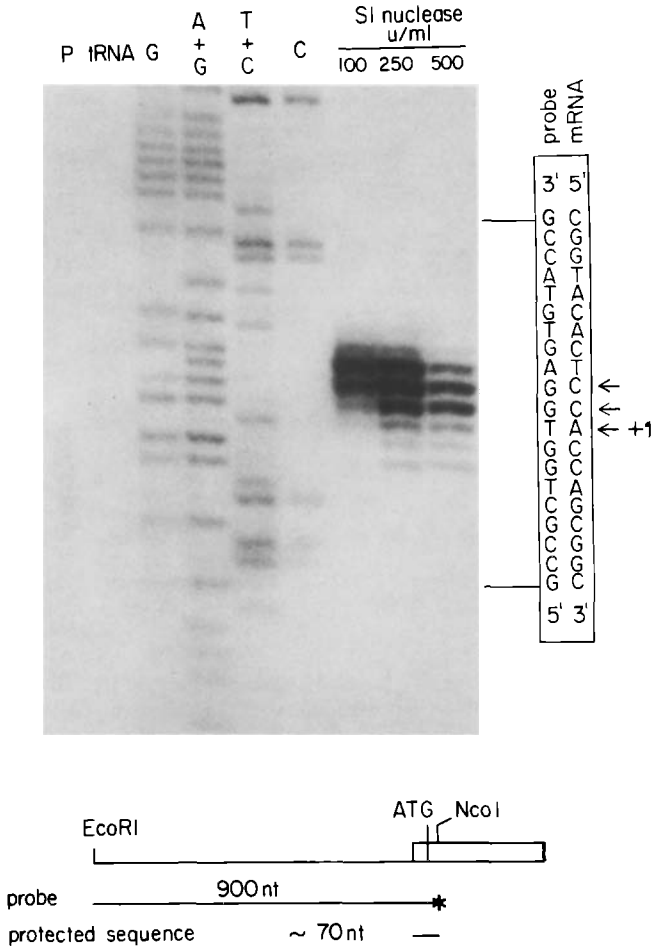


**Fig. 3.** S1 nuclease protection analysis of the 5′ terminus of *cab-1* mRNA. The probe used was 5′ end-labeled at the *Nco*I site 15 bp downstream of the ATG translation start codon. Only the portion of the gel with protected fragments is shown. P, input probe; tRNA, control hybridization and S1 digestion in the absence of maize RNA. G, A+G, T+C, and C lanes are Maxam and Gilbert (1980) reactions of the probe fragment. The last three lanes contain protection products from 40 µg of total RNA (from white-light grown maize leaves) hybridized to the probe and digested with the indicated amounts of S1 nuclease. The nucleotide sequence in the region of the protected fragments is shown on the right. A schematic of the probe and the protected sequences are shown below. *Arrows* indicate the cap site for the *cab-1* gene mRNA

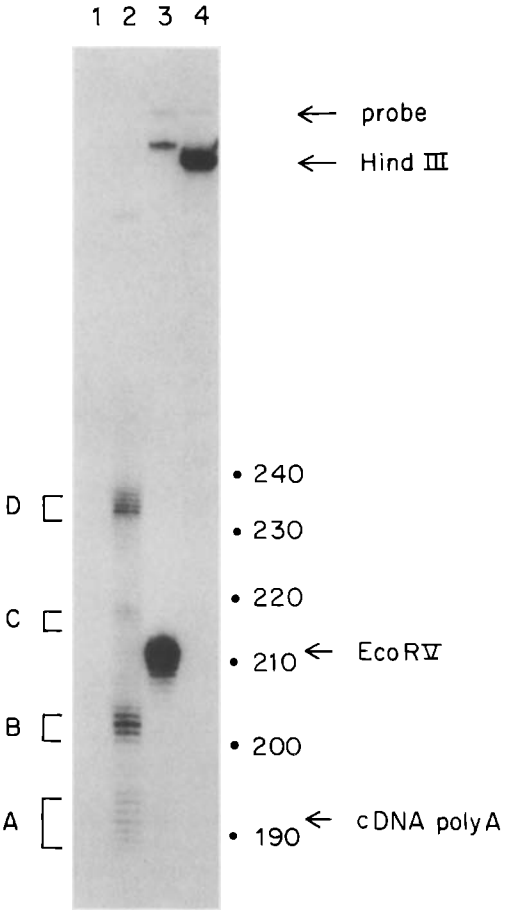


**Fig. 4.** S1 nuclease protection analysis of *cab-1* mRNA 3′ termini. The probe used was a uniformly-labeled single-stranded fragment 265 nucleotides long (*ΔK20/Nsi*I). Lane 1, tRNA control; lane 2, 2 µgm total RNA (from white-light treated maize leaves); lane 3, 2 ng SP6 polymerase transcribed RNA from pSPCAB template cut at *Eco*RV; lane 4, 2 ng SP6 transcribed RNA from template cut at the *Hin*dIII site of pSPCAB (in pSP19 polylinker). All hybridizations were made up to 10 µg of RNA with tRNA. S1 nuclease treatment was at 50 units/ml for 30 min. Protected fragments in lane 2 labeled A–D on the left are indicated in Fig. 2. The expected location of protected fragments for the *Eco*RV and *Hin*dIII cut pSPCAB RNAs, and RNAs with the same poly(A) site as *cab-1* cDNA clone pAB1084 are shown on right. The numbers on the right indicate the distance in nucleotides from the translation stop codon

of site A. It is unlikely that any of these protected fragments are the result of hybridization of the probe from the *cab-1* gene with mRNA from the other CAB genes since a probe containing these sequences reacts only with DNA from the homologous gene on genomic Southern blots (see below, Fig. 5).

The DNA sequence in the region of the 3′ termini is AT-rich. Therefore, some of the fragments generated by S1 nuclease treatment may result from digestion of regions of "breathing" in the RNA/DNA duplex (Maquat et al. 1981). This possibility was tested by using in vitro transcribed RNA from the cloned *cab-1* gene in parallel S1 nuclease digests (Materials and methods). In vitro transcribed SP6/CAB RNA with two different 3′ termini (*Eco*RV or *Hin*dIII; Figs. 1 and 2) protected the predicted sequences of the probes (Fig. 4). No extra protected fragments resulting from digestion at internal sites were detected. These data support the conclusion that transcripts from the maize *cab-1* gene have different 3′ termini.

### *Maize CAB gene expression*

The amount of CAB mRNA produced and the degree of light-regulation of CAB mRNA abundance was determined by Northern blot analysis of RNA from plants grown in the dark or in white-light. Two different probes were made to measure separately total CAB mRNA and *cab-1* specific mRNA (a coding region probe, *ΔJ8/Nco*I and a 3′ untranslated region probe *ΔK20/Nci*I; see Fig. 1, Materials and methods). The specificity of each probe was tested by

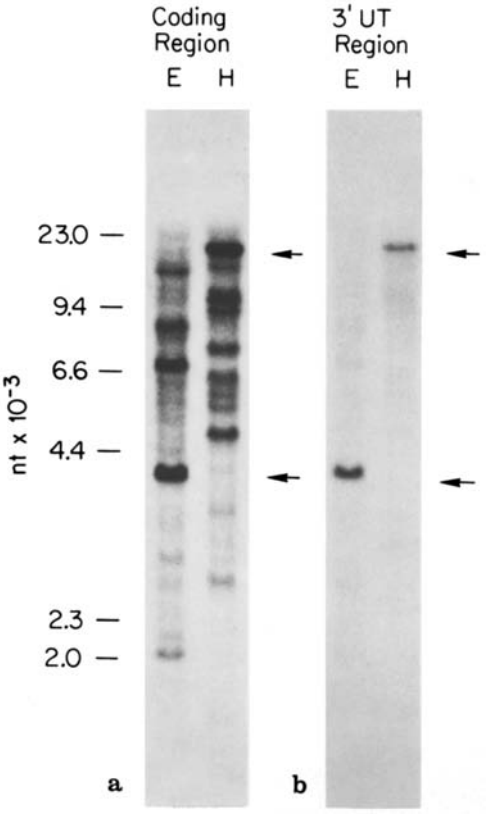


**Fig. 5a and b.** Specificity of hybridization probes determined by Southern blot analysis. Maize genomic DNA was digested with *Eco*RI (E) or *Hin*dIII (H) as indicated and duplicate samples were run in parallel lanes. **a** Filter hybridized with the coding region probe (*ΔJ8/Nco*I, $8 \times 10^7$ cpm/pmol, $3 \times 10^{-15}$ moles/ml) in hybridization solution with 30% formamide and washed with buffer containing 0.5 × SSC at 65° C. Autoradiography was for 64 h. **b** Filter hybridized with the 3′ untranslated region probel (*ΔK20/Nci*I, $2 \times 10^7$ cpm/pmole, $3 \times 10^{-15}$ moles/ml) in buffer with 30% formamide and washed with buffer containing 2 × SSC. Autoradiography was for 4 days. The location of molecular size standards is indicated on the left. The *arrows* on the right show the location of the bands unique to *cab-1*. The 4.0 kb *Eco*RI fragment is predicted from the restriction map of clone λAB1084

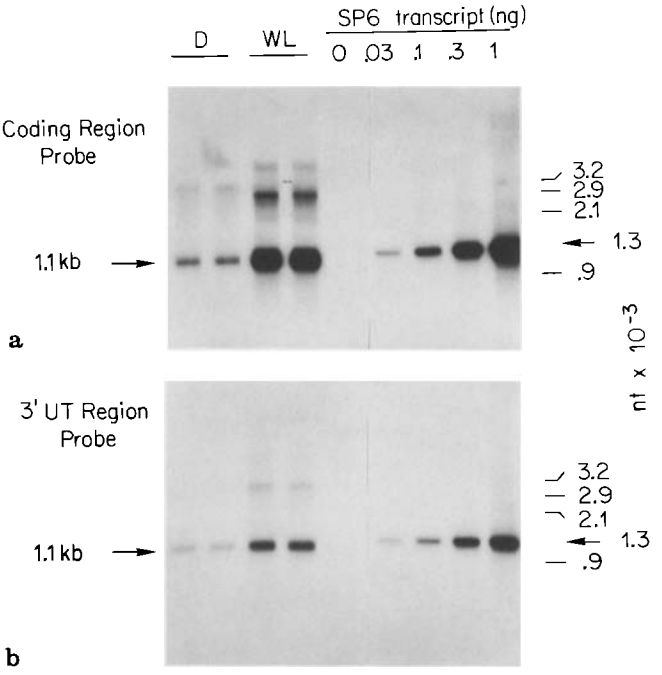


**Fig. 6a and b.** Northern blot analysis of chlorophyll a/b binding protein (CAB) mRNA in dark and white-light treated seedlings. Total cell RNA (1 μg) from dark-grown (D) and 24 h white-light (WL) treated 8-day-old seedlings was loaded in duplicate lanes as indicated. The right side of the gel contains in vitro transcribed SP6/CAB RNA (pSPCAB, *Hin*dIII cut template) which was included as a concentration standard for the hybridization signal. Parallel gels were run, blotted and hybridized with the probes indicated. **a** Coding region probe (*ΔJ8/Nco*I) was hybridized in hybridization buffer with 30% formamide and washed with buffer containing 2 × SSC at 42° C. **b** The 3′ untranslated region probe (*ΔK20, Nci*I) was hybridized in buffer containing 50% formamide and washed with wash buffer containing 0.1 × SSC at 65° C. Autoradiography was for 11 h at room temperature. Brome mosaic virus RNA was used as molecular size markers as indicated on the right. The size of the SP6/CAB transcript (1.3 kb) is also indicated on the right

Southern blot hybridization of maize genomic DNA (Fig. 5). The coding region probe, *ΔJ8/Nco*I, reacts with multiple bands on the blot (Fig. 5a), indicating that the probe cross-hybridizes to the other CAB genes and is therefore useful for analyzing total CAB mRNA. In contrast, even under low stringency hybridization conditions (30% formamide, 42° C), the 3′ untranslated region probe, *ΔK20/Nci*I, is specific for *cab-1* (Fig. 5b). It reacts with only one size fragment in either *Eco*RI and *Hin*dIII digested DNA. For Northern blot analysis with the *ΔJ8/Nco*I probe, hybridization and wash conditions were set to allow cross-hybridization with other CAB mRNAs, whereas *ΔK20/Nci*I was used under more stringent conditions to ensure that the probe hybridized only with *cab-1* mRNA.

We have measured the effect of 24 h of continuous white-light treatment on the amount of both total CAB mRNA and *cab-1* mRNA 8 days after germination. In RNA from both dark-grown and white-light treated maize leaves the predominant RNA detected by Northern blot analysis was about 1100 nt long (Fig. 6). This size matches well with the predicted size of the *cab-1* mRNA based on S1 mapping the 5′ and 3′ termini (1032–1072 nt from the cap site to the poly(A) addition sites with the additional nucleotides presumably provided by a poly(A) tail, Figs. 1 and 2).

The amount of either total CAB mRNA or *cab-1* mRNA in the dark or white-light treated samples was quantitated by densitometer scans of the bands and comparison with the SP6/CAB RNA signals. A 13-fold increase in total CAB mRNA following a 24 h white-light treatment was detected with the coding region probe (Fig. 6a, D vs WL). In contrast, only a 6-fold increase in *cab-1* mRNA was observed in response to the white-light treatment (detected with the 3′ untranslated region probe, *ΔK20/Nci*I, Fig. 6b). This difference was seen in repeated experiments, including those in which the poly(A)$^+$ fraction of the cell RNA was tested. The range for the white-light increase in mRNA abundance was 6- to 15-fold for total CAB mRNA and 3- to 6-fold for *cab-1* (data not shown).

It is possible to estimate the absolute amount of *cab-1* mRNA in these samples by comparison of the signals produced from maize seedling RNA with the signals produced

from in vitro transcribed *cab-1* RNA (SP6/CAB). Quantitation of signal intensities from Fig. 6b indicates that there is 0.04 ng *cab-1* mRNA per μg of total cellular RNA in the leaves of dark-grown maize seedlings, and 0.24 ng per μg in samples from white-light treated seedlings. Given that the poly(A)$^+$ RNA fraction isolated from such seedlings constitutes approximately 1% of the total RNA (data not shown), these values are equivalent to approximately 0.4% and 2.4% of the poly(A)$^+$ fraction of RNA in these samples.

On the basis of similar quantitations of signals obtained with the coding region probe under low stringency hybridization conditons (Fig. 6a), the amounts of total CAB gene transcripts are estimated to be about 0.5% of the poly(A)$^+$ RNA in the dark and 6% of the poly(A)$^+$ RNA after the 24 h white-light treatment. Estimates from the data in Fig. 6 suggest that CAB gene transcripts other than *cab-1* (non-*cab-1* transcripts) increase, on average, approximately 40-fold in response to 24 h of white-light treatment. If some of the non-*cab-1* gene transcripts hybridize less efficiently than the SP6/CAB RNA to the *cab-1* coding region probe, then the values for the absolute amount of total CAB mRNA and the induction of non-*cab-1* transcripts will be underestimated. The variable intensity of the signals obtained on Southern blot analysis with the *cab-1* coding region probe (Fig. 5) is consistent with the possibility that individual members of the CAB gene family may hybridize with different efficiencies to this probe.

To determine whether *cab-1* is a phytochrome-regulated gene, the response of *cab-1* to red-light, and to red followed by far-red light was tested. RNA samples were analyzed by blot hybridization with the *cab-1* gene specific probe (*ΔK20/NciI*). The induction of *cab-1* by a pulse of red-light was low (less than 3-fold after 3 h) but was reversible by far-red light to the level of mRNA observed following a far-red treatment alone (data not shown). Therefore, the level of *cab-1* mRNA does appear to be regulated by phytochrome.

## Discussion

We have isolated and characterized a gene encoding a chlorophyll a/b binding protein from a maize genomic library. This is, to our knowledge, the first report of the isolation of a CAB gene from maize, although several maize CAB cDNA clones have been isolated and characterized (Sheen and Bogorad 1986; Nelson et al. 1984; Matsuoka et al. 1987).

Both the nucleotide and deduced amino acid sequences of *cab-1* share extensive homology with the coding sequence of other published CAB genes. For example, in the 233 amino acid mature protein region there is 83% nucleotide and 90% amino acid homology with a wheat CAB gene (Lamppa et al. 1985), 76% nucleotide and 94% amino acid homology with a pea CAB gene (Cashmore 1984), and 92% nucleotide and 94% amino acid homology with a recently reported maize CAB cDNA clone (Matsuoka et al. 1987). Also, there is $\geq$90% amino acid homology with reported CAB sequences from petunia, *Lemna, Arabidopsis*, cucumber, and tomato (Dunsmuir 1985; Kohorn et al. 1986; Leutwiler et al. 1986; Greenland et al. 1987; Pichersky et al. 1985). The least conserved sequences are near the N-terminus of the mature peptide. For example, a block of 10 amino acids (from amino acids 4–14 in *cab-1*) contains 7 substitutions from the reported wheat sequence, 6 differences from pea, and 5 substitutions and 1 amino acid deletion relative to the other reported maize CAB sequence. The remainder of the amino acid differences are scattered throughout the protein (data not shown). The N-terminal portion of the mature CAB protein may play an important role in CAB function in thylakoid stacking and in the control of the interaction between photosystems in the thylakoid membrane (reviewed in Anderson 1986). The amino acid differences in the N-terminal region of different CAB proteins have been postulated to reflect functional differences, but the significance of specific amino acid changes in this region is unknown (Karlin-Neumann et al. 1985; Pichersky et al. 1985).

The amino acid sequence of the transit peptide in the recently published maize CAB cDNA clone is only 61% homologous to the transit peptide of *cab-1* (Matsuoka et al. 1987). This is consistent with results from other species which indicate that the sequence of the transit peptide is less conserved than the mature CAB protein (Dunsmuir 1985; Pichersky et al. 1985). In *cab-1*, the sequence of the transit peptide N- and C-termini fits the consensus sequence derived by Karlin-Neumann and Tobin (1986) for transit peptides of CAB proteins from several species. There is a 9 out of 10 agreement at the N-terminus, and a 5 out of 6 match at the transit/mature protein junction but no recognized homology with the consensus sequence in the less conserved internal portion of the transit peptide (Fig. 2).

Pichersky and coworkers (1987) have proposed that the CAB genes encoding polypeptides for photosystem II (PSII) in *Lycopersicon esculentum* (tomato) and *Lemna gibba* can be divided into two groups, designated Type I and Type II, based on amino acid sequence homology. Within each group there is high sequence homology (>90%) which is conserved between species. Between the two polypeptide types the sequence homology is less. In addition, the three known CAB genes that contain an intron belong to the Type II group (Karlin-Neumann et al. 1985; Stayton et al. 1986; Pichersky et al. 1987). The maize *cab-1* gene appears to be a Type I, PSII gene. It contains no introns and has 93% amino acid homology with a tomato Type 1 gene (*cab-1A*) and only 86% homology to a tomato Type II gene (*cab-4*). A recently published maize CAB cDNA sequence appears to represent a Type I CAB gene that is different from maize *cab-1* (Matsuoka et al. 1987).

The *cab-1* mRNA was mapped to the gene sequence by S1 nuclease protection analyses. The 5′ terminus was mapped 52–54 bases upstream of the translation start codon using a probe 5′ end-labeled at the *Nco*I site (Fig. 3) as well as with a probe labeled at the *Xho*II site (+453, data not shown). The 5′ flanking reegion contains the sequences TATTTA at position −34 and CCAAT at −90 (Fig. 2). Similar sequences are often found in the 5′ flanking region of many eukaryotic genes and appear to be important for efficient transcription initiation (Benoist et al. 1980; Breathnach and Chambon 1981; Myers et al. 1984).

In S1 nuclease protection experiments using the *Xho*II probe, in addition to the predominant band corresponding to the cap site, several bands of considerably lower intensity representing smaller protected fragments were detected (data not shown). These fragments may represent partial protection of the *cab-1* gene probe by CAB mRNA produced from CAB genes that diverge in sequence at various

points in this region, as suggested by Lamppa et al. (1985) for wheat CAB mRNAs. The sequences of the maize *cab-1* gene and the CAB cDNA reported by Matsuoka et al. (1987) differ substantially throughout this region (data not shown). Therefore, at least one other transcribed CAB gene contains enough sequence divergence in the region such that fragments representing partial protection of the *cab-1* *Xho*II probe would be expected. Sequence divergence in the region surrounding the *Nco*I site, which is located in the transit peptide region only 15 bp from the translation start codon, may account for the failure to detect any shorter fragments produced with the *Nco*I probe (Fig. 3, data not shown). Since we do not have any sequence information for the other maize CAB genes, we cannot definitively exclude the possibility that some of the maize CAB genes contain identical sequences in the region covered by the probes out to the cap site. However, it is unlikely that the predominant CAB transcripts induced by light are identical to *cab-1* in this region since *cab-1* mRNA constitutes about 80% of the total CAB transcript in unirradiated tissue (Fig. 6), and both *cab-1* mRNA detected by Northern blot analysis (Fig. 6, data not shown) and the signal for the longest fragment protected in S1 nuclease experiments (data not shown) increase 3- to 6-fold following light treatment.

Four different sites of poly(A) addition in maize *cab-1* mRNA were identified by S1 nuclease protection assays in the region 170–230 bases beyond the translation stop codon (Figs. 2 and 4). One of the identified poly(A) sites (Site A, Figs. 2 and 4) corresponds to the position of the poly(A) tract in a cDNA clone of mRNA from *cab-1*. The poly(A) addition signal in plant mRNA is less well conserved than in animal mRNA (Dean et al. 1986). There are several sequences in the *cab-1* 3′ untranslated region that agree with the consensus plant poly(A) addition signal determined by Dean et al. (1986) from the sequence of 15 cloned cDNAs (Fig. 2). The sequence AACCAT located 158 bp 3′ of the stop codon precedes the A, B and C termini by 29–52 bp. The sequence ATTAAT (221 bp from stop codon) precedes the D terminus by 12 bp (Fig. 2). It does not appear that there is a unique 3′ terminus for each poly(A) addition signal, since sites A, B, and C all follow one putative poly(A) addition signal. The relative abundance of the different 3′ termini of *cab-1* mRNA is not affected by white-light treatment (data not shown).

Several factors influence CAB gene expression in maize and in other plant species. These include tissue and development specific factors, as well as the response to light treatment (Mayfield and Taylor 1984; Nelson et al. 1984; Broglie et al. 1984; Batschauer et al. 1986). Individual CAB genes respond differently to these factors (Lamppa et al. 1985; Sheen and Bogorad 1986). In many species including maize, both CAB polypeptides and CAB mRNA are present at low levels in dark-grown plants and dramatically increase in the plant leaves in response to either white-light or red-light treatment (reviewed in Tobin and Silverthorne 1985).

Our results indicate that maize *cab-1* gene transcripts are present at high levels in 8-day-old, dark-grown seedlings, comprising approximately 80% of the CAB gene family transcripts and about 0.4% of the total leaf mRNA pool (Fig. 6). Even though the level of *cab-1* mRNA increases only 3- to 6-fold in response to the 24 h white-light treatment (compared to 6- to 15-fold increase in total CAB mRNA), the *cab-1* gene still accounts for a large proportion of the total CAB mRNA and 2% of the total mRNA pool in the light-treated seedlings (Fig. 6, data not shown). Estimates based on the data in Fig. 6 suggest that *cab-1* may account for as much as 35% of the total CAB mRNA after irradiation. However, since it is possible that the *cab-1* coding region probe hybridizes less efficiently to the non-*cab-1* mRNA than to the SP6/CAB RNA used as a hybridization standard, this may be an overestimate.

From the data in Fig. 6, it may be deduced that CAB gene transcripts other than *cab-1* mRNA are increased approximately 40-fold on average by the light treatment. The extent of the light-induced increase in the intensity of signals for shorter protected fragments observed in the S1 nuclease experiments with the *Xho*II probe discussed above (data not shown) is consistent with the notion that the shorter protected fragments represent strongly responsive CAB genes. These data are also consistent with those of Sheen and Bogorad (1986) who have examined CAB mRNA levels in maize seedlings with gene-specific probes made from six different CAB cDNA clones and have found that the clones differ in the amount of mRNA detected in dark-grown seedlings and after exposure of the seedlings to white-light.

Some of the properties of *cab-1* gene expression appear to be similar to those of the maize gene represented by the CAB cDNA clone Δpmc6 of Sheen and Bogorad (1986). The RNA homologous to the Δpmc6 clone is present at relatively high levels in dark-grown seedlings, is not highly induced by light treatment and comprises 8% of the total CAB mRNA after a 24 h white-light treatment (Sheen and Bogorad 1986). However, the sequence of Δpmc6 has not been reported and, therefore, we are unable to determine if the cDNA clone Δpmc6 does represent the *cab-1* gene.

Our results and those of Sheen and Bogorad (1986) differ somewhat from those of Nelson et al. (1984), who report that total CAB mRNA is at very low levels in dark-grown maize seedlings and increases 200-fold following a 24 h white-light treatment. At present we have no explanation for these differences, especially in the level of CAB mRNA detected in dark-grown seedlings. Since we find that total CAB mRNA comprises at least 0.4% of the total mRNA pool in dark-grown seedlings, we would clearly not expect a 200-fold induction of CAB mRNA by white-light treatment under our conditions.

Reports from a number of laboratories indicate that the light induced increase in CAB transcript abundance is mediated by phytochrome (Harpster and Apel 1985; Kuhlemeier et al. 1987b; Thompson et al. 1985; Tobin and Silverthorne 1985). Our preliminary data also indicate that the white-light enhanced abundance of the maize *cab-1* gene mRNA is also at least partially phytochrome mediated (data not shown).

The recent identification of promoter segments that are responsible in *cis* for phytochrome-mediated stimulation of gene expression in both CAB and *rbcS* genes suggests that the 5′ flanking region of light-regulated genes will contain such *cis*-acting light-regulated elements (LREs) (Kuhlemeier et al. 1987a, b; Simpson et al. 1985; Fluhr and Chua 1986; Nagy et al. 1986, 1987; Green et al. 1987; Castresana et al. 1988; Manzara and Gruissem 1988). A search of the maize *cab-1* 5′ flanking region for sequences found in the promoters of other light-regulated genes detected two types of sequences similar to previously reported elements (Fig. 2). An 8 of 10 match with the *rbc*S Box 3 sequence element, CACGTGG/TCAC, described by Manzara and

Gruissem (1988) is located at −319 in the maize *cab-1* promoter. The Box 3 sequence is found in the promoter of *rbc*S genes from soybean, pea, tobacco and petunia (Manzara and Gruissem 1988). A similar sequence is also found in the *Nicotiana plumbaginifolia Cab-E* gene (Castresana et al. 1988). Interestingly, it is also found in two tomato *rbc*S genes that are expressed at high levels in the dark but not in the promoters of three other tomato *rbc*S genes with low expression in the dark (Manzara and Gruissem 1988). It will be of interest to determine whether the high level of expression of maize *cab-1* is related to the presence of this motif. *cab-1* also contains multiple elements similar to a sequence repeated several times within the positive regulatory elements (PREs) of a *N. plumbaginifolia* CAB gene described by Castresana et al. (1988). These *cab-1* sequence elements have between 7 and 10 out of 12 agreement with the consensus sequence, ACCGGCCCACTT, for the repeated motif in the PREs, and are located between −639 and −116 in the maize *cab-1* sequence (Fig. 2). The PRE elements have been shown to confer maximal light-regulated expression on CAB-chloramphenicol acetyl transferase chimeric constructs in transgenic tobacco (Castresana et al. 1988). Thus, these sequence elements may also be important in regulating expression of the *cab-1* gene. However, functional assays of the *cab-1* promoter will be required to assess this possibility. Other sequence elements, notably the box II and III LREs, reported to be important for light-regulated expression of *rbc*S genes (Nagy et al. 1986, 1987; Green et al. 1987) were not detected in the *cab-1* 5′ flanking sequence. There are several possible explanations of why such homologous sequences were not found. CAB and *rbc*S genes may be regulated differently. Nagy et al. (1987) report finding no obvious homology in 5′ flanking DNA of a wheat CAB gene and a pea *rbc*S gene. There may be differences between plant species and especially between C3 and C4 plants in the *cis*-acting LREs. Functionally equivalent sequences may be present but they may have poor nucleotide homology. Alternatively, the amount of sequence similarity between LREs may reflect the functional activity of the elements (i.e. the ability to stimulate expression). Since the maize *cab-1* gene is only moderately light-regulated, it is possible that it may contain *cis*-acting elements that are quite dissimilar to LREs of highly regulated genes. The results of sequence analysis of different CAB gene promoters and functional assays of CAB promoters in transgenic plants should eventually resolve many of these questions.

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