Plant Physiol. (1994) 104: 997–1006

Promoters from Genes for Plastid Proteins Possess Regions with Different Sensitivities toward Red and Blue Light[1]

Thomas Lübberstedt, Cordelia E. H. Bolle, Sudhir Sopory, Karin Flieger, Reinhold G. Herrmann, and Ralf Oelmüller*

Botanisches Institut der Ludwig-Maximilians-Universität, Menzingerstrasse 67, 80638 München, Germany

The light-regulated expression of eight nuclear-encoded genes for plastid proteins from spinach (*Spinacia oleracea*) (*RBCS*-1 and *CAB*-1; *ATPC* and *ATPD*, encoding the subunits γ and δ of the ATP synthase; *PC* and *FNR*; *PSAD* and *PSAF*, encoding the subunits II and III of photosystem I reaction center) was analyzed with promoter/β-glucuronidase (GUS) gene fusions in transgenic tobacco (*Nicotiana tabacum* and *Nicotiana plumbaginifolia*) seedlings and mature plants under standardized light and growth conditions. Unique response patterns were found for each of these promoters. GUS activities differed more than 30-fold. Strong promoters were found for the *PC* and *PSAD* genes. On the other hand, the *ATPC* promoter was relatively weak. Expression of the *CAB*/GUS gene fusion in etiolated material was at the detection limit; all other chimeric genes were expressed in the dark as well. Light stimulation of GUS activities ranged from 3- (*FNR* promoter) to more than 100-fold (*CAB*-1 promoter). The *FNR* promoter responded only to red light (RL) and not significantly to blue light (BL), whereas the *PC* promoter contained regions with different sensitivities toward RL and BL. Furthermore, different RNA accumulation kinetics were observed for the *PSAF*, *CAB*, *FNR*, and *PC* promoter/GUS gene fusions during de-etiolation, which, at least in the case of the *PSAF* gene, differed from the regulation of the corresponding endogenous genes in spinach and tobacco. The results suggest either that not all *cis* elements determining light-regulated and quantitative expression are present on the spinach promoter fragments used or that the spinach *cis*-regulatory elements respond differently to the host (tobacco) regulatory pathway(s). Furthermore, as in tobacco, but not in spinach, the *trans*-gene hardly responds to single light pulses that operate through phytochrome. Taken together, the results suggest that the genes have been independently translocated from the organelle to the nucleus during phylogeny. Furthermore, each gene seems to have acquired a unique set of regulatory elements.

Light plays a crucial role for plant growth and development. It is absorbed by at least three photosystems (phytochromes, blue/UV-A light photoreceptors, and a UV-B photoreceptor; Kendrick and Kronenberg, 1986; Gilmartin et al., 1990; Thompson and White, 1991; Ahmand and Cashmore, 1993). The perceived light signals are transduced to responsive genes via largely unknown signal pathways. Four *Arabidopsis thaliana* mutants (*det*-1, *det*-2, *cop*-1, *cop*-9) and one pea mutant (*lip*1) have been isolated in which signal perception is uncoupled from the expression of normally light-regulated genes (Chory et al., 1986, 1989; Deng et al., 1991; Frances et al., 1992; Wei and Deng, 1992). Since these mutations are pleiotropic, important functions in early steps of the transduction chain(s) must have been impaired. At the other end of the pathway(s), a variety of *cis*-regulatory elements have been identified that specifically interact with families of related proteins (cf. Dehesh et al., 1990; Gilmartin et al., 1990; Schindler and Cashmore, 1990; Lübberstedt et al., 1994). GT-1-binding sites, GPu(T/A)AA(T/A), for example, have been found in the upstream regions of various light-regulated genes (Stockhaus et al., 1987; Manzara and Gruissem, 1988; Kay et al., 1989; Dehesh et al., 1990; Lübberstedt et al., 1994). The core element of the G-box sequence (CACGTG), in turn, is not only present in promoters of light-regulated genes (Giuliano et al., 1988; Schulze-Lefert et al., 1989; Staiger et al., 1989; Donald and Cashmore, 1990; Weisshaar et al., 1991), but also in promoters that are regulated by other stimuli (cf. Marcotte et al., 1989; DeLisle and Ferl, 1990). Weisshaar et al. (1991) and Armstrong et al. (1992) have shown that four of the nucleotide residues of the G-box element, ACGT, are found in several diversely regulated plant, viral, and bacterial promoters and bind to conserved homodimeric and heterodimeric leucine zipper proteins. Even though several other protein factors have been described or isolated that interact with upstream elements in light-regulated gene promoters (cf. Datta and Cashmore, 1989; Gilmartin et al., 1990; Sarokin and Chua, 1992), the current view is that the linkage between the early steps in signal transduction and responsive genes rests on only a limited number of conserved *cis*- and *trans*-acting elements.

This view is not easily reconciled with observations that the expression of light-responsive genes can differ substantially with regard to light requirement, light intensity, time course, induction, and phytochrome escape kinetics (Thompson and White, 1991). Even closely related genes in the same organisms can exhibit different responses to light. Pea appears to possess at least two classes of *CAB* genes, one with a strong and fast response to RL, another with only little or no transcript accumulation within the first 24 h after light

[1] This paper is dedicated to Prof. W. Rüdiger on the occasion of his 60th birthday. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 184). S.S. was supported by the Alexander-von-Humboldt-Stiftung, Bonn.

* Corresponding author; fax 89–1782–274.

Abbreviations: BL, blue light; CAB, chlorophyll *a*/*b*-binding protein of photosystem II; CaMV, cauliflower mosaic virus; FNR, ferredoxin-NADP$^+$-oxidoreductase; GUS, β-glucuronidase; 4-MU, 4-methylumbelliferone; PC, plastocyanin; P_{tot}, total phytochrome; RBCS, small subunit of Rubisco; RL, red light; WL, white light.

treatment (White et al., 1992). Furthermore, major *cis* determinants for qualitative (light regulated) and quantitative expression are found not only upstream of the transcription start site but also within or downstream of transcribed regions. The promoter of the pea *Fed*-1 gene, for instance, confers organ specificity and contributes to the light response in etiolated material, whereas elements located within the transcribed region play a more dominant role in modulating expression when the gene is active (Gallo-Meagher et al., 1992). The eight petunia *RBCS* genes, in turn, show a high degree of nucleotide sequence similarity within their 5′ and 3′ flanking sequences, but their transcription rates vary by 2 orders of magnitude (Dean et al., 1985, 1987, 1989a, 1989b). Important regulatory elements for the quantitative expression of these genes are located downstream of the transcription start site, in contrast to a pea *RBCS* gene, in which downstream sequences do not affect steady-state transcript abundance (Kuhlemeier et al., 1988). These examples illustrate that nuclear gene transcription is probably more diverse and complex than currently anticipated and that elements determining not only the quantitative but also the qualitative (e.g. light regulated) expression may be spread out in quite different gene regions.

Photophysiological studies with etiolated spinach seedlings have suggested that the steady-state mRNA levels of nuclear genes for thylakoid membrane proteins seem to be under comparable photocontrol, i.e. their levels increase with almost identical kinetics during de-etiolation (Herrmann et al., 1991, 1992; Bolle et al., 1992; Flieger et al., 1993). The observed similarities in mRNA accumulation appear to find no equivalent in the organization of the corresponding promoters (Oelmüller et al., 1992). In fact, promoter architectures differ substantially as judged from an analysis of chimeric GUS gene fusions in transgenic tobacco. Taking advantage of transgenic tobacco seeds with various spinach promoter/GUS gene fusions, we have analyzed GUS gene expression in developing seedlings and adult plants grown under standardized light conditions. Our data indicate that the eight promoters selected differ substantially in strength, that important *cis* elements for their quantitative expression are located in different regions upstream of the respective transcription start sites, and that light-responsive elements with different sensitivities toward RL and BL can be distinguished.

MATERIALS AND METHODS

Most methods used for gene manipulation and plant transformation have been described (Flieger et al., 1993; Oelmüller et al., 1993) or are standard techniques (Sambrook et al., 1989). These include construction of promoter/GUS gene fusions in the binary vector pBI 101.2 (Jefferson, 1987; Jefferson et al., 1987), leaf disc transformation of *Nicotiana tabacum* Samsun NN, nucleotide sequencing, oligonucleotide synthesis, and primer extension analysis. For the results shown in Table II, *Nicotiana plumbaginifolia* was transformed with the −1126- to +60-bp *PC* promoter/leader fragment.

Plant Growth

Transgenic F_0 and F_1 plants (*Nicotiana tabacum*, var Samsun NN, or *Nicotiana plumbaginifolia*) were kept in a greenhouse under conditions specified in the German "Gentechnikgesetz." Seeds were collected from individual plants and stored at 4°C for at least 2 months. Between 25 and 100 seeds were plated onto half-strength Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with agarose (0.6%) and Suc (1%) in sterile Petri dishes and kept in RL for 12 h in temperature-controlled growth chambers (25.0 ± 0.5°C) to synchronize germination. The growth chambers were equipped with cool-white fluorescent lamps (Osram L18W/30 and Osram L18W/25) and RL or BL Plexiglas filters (6 mm thick, gs 502 for RL and gs 602 for BL; Röhm GmBH, Darmstadt, Germany). Except for the greening experiment (Fig. 5), for which 30 W m^{-2} was used, the light intensities for all other experiments were 5 W m^{-2}. After 12 h in RL, the dishes were transferred to the specified light sources. Under these conditions the germination rate was between 70 and 90%. "Etiolated" seedlings were exposed to the same pretreatment but were then kept in darkness.

In pilot experiments it was shown spectrophotometrically that a 5-min RL pulse suffices to convert the maximum amount of Pr to Pfr (Pfr/P_{tot} = 0.83) and that reversion of Pfr to Pr by a 10-min exposure to far-red light establishes a Pfr/P_{tot} ratio of less than 0.02 in etiolated tobacco seedlings. The Pfr/P_{tot} ratio in BL and WL was 0.43 and 0.46, respectively (data not shown).

Determination of GUS Activities

Standardized conditions were used for quantitative determination of GUS activities in transgenic F_1 plants and seedlings following in principle the protocol of Jefferson et al. (1987). Twenty pairs of cotyledons or 1 cm^2 of leaf material was harvested and immediately used for the enzyme assay. The material was homogenized on ice in 1 mL of extraction buffer (50 mM Na_3PO_4 [pH 7.0], 10 mM EDTA, 0.1% Triton X-100, 1 mM mercaptoethanol) and quartz sand. The homogenate was clarified by two centrifugations (each 10 min; 20,000*g*, 4°C). Supernatants (2 μL) were used for the determination of protein concentrations (Bradford, 1976). The enzyme reaction was performed at 37°C for 15 min with 70 μL of extraction buffer, 10 μL of 4-methylumbelliferyl glucuronide (10 mM) dissolved in extraction buffer, and 20 μL of extract. The reaction was stopped by adding 400 μL of Na_2CO_3 solution (0.2 M) and further diluted between 100- and 10,000-fold with the same solution prior to fluorometric determination of the reaction product, 4-MU, in a total volume of 1 mL using the LS lumenescense spectrophotometer (Perkin-Elmer Cetus, Überlingen, Germany). Emission was measured at 455 nm after fluorescense excitation at 365 nm. Under these conditions accumulation of the reaction product was linear with regard to enzyme amount and time. Furthermore, determination of GUS activity in leaves (6–10 cm long) from the same plant gave comparable results. No change in the enzyme activity was noted if the crude extract was kept on ice for 3 h.

Quantitation of RNA Levels: Dot Blot and Northern Analysis

Poly(A)$^+$ RNA was isolated from tobacco and spinach seedlings, and an equal amount of RNA was used for north-

ern and dot blot analysis. DNA fragments, cloned into pBSC$^+$ (Stratagene), were isolated from agarose gels after restriction of plasmid DNA and labeled by random priming (Feinberg and Vogelstein, 1983). Hybridization was performed as described previously (Oelmüller and Briggs, 1990). The specificity of the probe was checked by northern analysis; only single mRNA species with correct electrophoretic mobilities hybridized to the respective radiolabeled DNA fragments. For all dot blot filters, the low level of background hybridization was monitored individually by the addition of control RNA that did not hybridize to the probe, and the sample values were corrected accordingly.

Determination of Segregation Ratios and Gene Copy Numbers

Primary transformants were selfed and their seeds collected individually. The seeds were surface sterilized and germinated on half-strength Murashige and Skoog medium with 0.6% agar in the presence of 300 μg mL^{-1} of kanamycin. After 30 d of growth, the segregation ratios could be readily determined. Kanamycin-sensitive seedlings were completely bleached. Analysis of segregation ratios revealed that the majority of the primary transformants contained more than one transgenic locus (data not shown).

Isolation of Tobacco DNA and PCR

Plant material was harvested and immediately frozen in liquid nitrogen. The material was then ground with a mortar and pestle and resuspended in 2 volumes (w/v) of extraction buffer (90°C, 50 mM LiCl, 0.5% SDS, 50 mM Tris-NaOH [pH 9.0], 2 mM EDTA, 50% [w/v] phenol). After phenol-chloroform extraction, RNA was precipitated in the presence of 3 M LiCl at 4°C overnight and collected by high-speed centrifugation (20,000*g*, 30 min). The DNA was precipitated from the supernatant with isopropanol (0.6 volume) and removed from the solution with a sterile glass tip, redissolved in a small volume of buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA), precipitated again in the presence of 2.5 volumes of ethanol, washed once with 70% ethanol, and solubilized in the above buffer with 1 mM $MgCl_2$. DNA aliquots, together with gene- or promoter-specific primers, were used for the PCR following the protocol supplied by Perkin-Elmer Cetus (Überlingen, Germany).

The −298- to −78-bp *RBCS*-1 promoter fragment was amplified by PCR from spinach DNA. The promoter-specific primers were designed according to Tittgen (1987). The PCR product was cloned into the *Sma*I site of pBSC$^+$ (Stratagene) before insertion into pUC-GUS-90, a vector in which the −90- to +3-bp 35S RNA CaMV minimal promoter was cloned in front of the GUS reporter gene (Oelmüller et al., 1993). Two oligonucleotides with the *RBCS*-1 G-box sequence were synthesized, annealed, and cloned as a blunt-end fragment into *Sma*I-digested pBSC$^+$ before being cloned in front of the 35S RNA CaMV minimal promoter. All plasmid constructs were sequenced with commercially available or synthesized primers.

RESULTS

Promoters Differ in Strength in Adult Tobacco Plants

Promoter fragments from eight nuclear genes for plastid proteins that were analyzed in this study are summarized in Table I. The GUS activities in tobacco plants (F_1 generation) directed by the promoters from six nuclear genes for thylakoid membrane proteins are compared in Figure 1. Four of them, *ATPC* and *ATPD*, encoding the γ and δ subunits of the plastid ATP synthase, and the *FNR* and *PC* genes are single-copy genes in spinach (Bichler and Herrmann, 1990; Herrmann et al., 1991; Bolle et al., 1992; Oelmüller et al., 1992, 1993). Two truncated promoter fragments from the spinach multicopy *RBCS* and *CAB* gene families with 300- to 400-bp segments upstream of the respective transcription start sites were included as well. All promoter fragments used contained the complete or almost complete 5′ untranslated leader sequence (Table I). Except for the *CAB*-1, *RBCS*-1, and CaMV promoter fragments, for which only one fragment was analyzed, 5′ extension of the promoter fragments did not result in higher GUS levels (data not shown). Longer *PSAF* promoter fragments, however, resulted in decreased GUS activities (Flieger et al., 1993). In spite of the variation of GUS levels in individual plants harboring the same *trans*-gene, the GUS

Table I. *Promoter/leader fragments analyzed in this study*

The positions are given relative to the transcription start site. CG → TA, the −296- to +79-bp *RBCS*-1 gene promoter, in which the CG nucleotides of the G-box (5′-TCCACGTGGT-3′) were replaced by TA.

Gene	Gene Copy No. in Spinach	Length of Leader	GUS Gene Fusions[a]
		bp	
PC	1	65	−1126 to +60
			−259 to +60
			−202 to +60
			−174 to +60
			−168 to +60
FNR	1	238	−753 to +231
			−313 to +231
			−118 to +231
			−28 to +231
ATPC	1	173	−992 to +173
			−172 to +173
ATPD	1	69	−1137 to +62
PSAD	2–3	50	−1802 to +55
PSAF	2–3	188	−712 to +163
			−573 to +163
			−220 to + 163
CAB-1	>8	71	−377 to +71
			−77 to +71
RBCS-1	>7	80	−296 to +79
			CG → TA
35S RNA CaMV			−1583 to +3
			−90 to +3

[a] Chimeric fusions cloned 5′ to the 35S RNA CaMV minimal promoter (−90 to +3): (a) *RBCS*-1 (−296 to −77); (b) trimer of the *RBCS*-1 G-box sequence: $(TCCACGTGGT)_3$; (c) trimer of the mutant *RBCS*-1 G-box sequence: $(TCCAATTGGT)_3$.

activity in the leaves, as well as in the cotyledons of tobacco seedlings (see below), differs substantially among promoters (Fig. 1). This could imply that the upstream regions of these genes are different in strength or so divergent that they respond differently to tobacco regulatory pathways or that essential *cis* elements that determine quantitative expression are not present in the chosen promoter fragments. Finally, since the GUS mRNAs contain different 5′ untranslated leaders that derive from the spinach genes (Table I), posttranscriptional mechanisms cannot be excluded (see "Discussion").

The Effect of RL, BL, and WL on GUS Activity in 12-d-Old Tobacco Seedlings

The effect of RL, BL, and WL on the accumulation of GUS activity in transgenic tobacco seedlings is compared in Figure 2. For these experiments seeds were germinated in RL for 12 h before transfer to darkness or to the indicated light regimes for an additional 12 d.

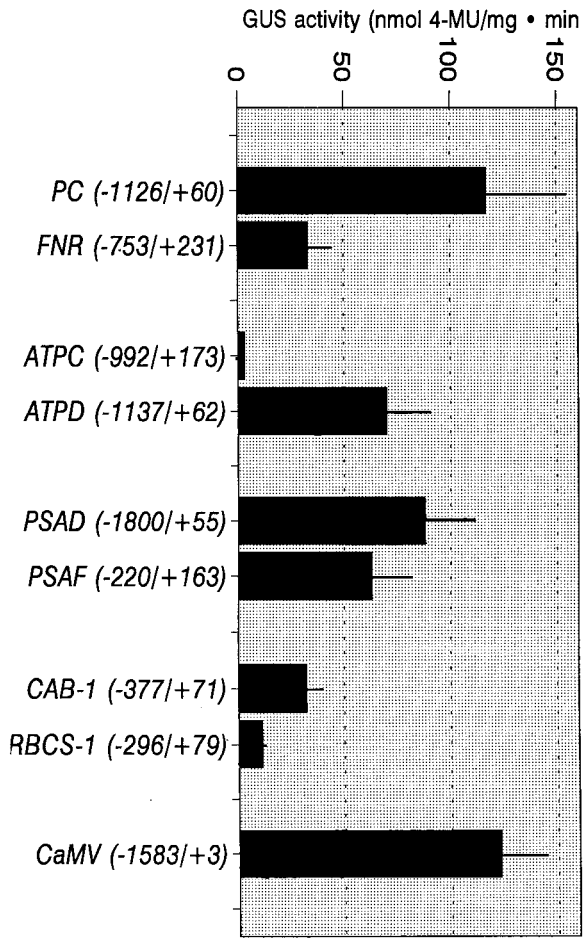

Figure 1. Comparison of GUS activities under the control of eight spinach and the 35S RNA CaMV promoters in leaves (6–10 cm long) of 6- to 7-week-old F_1 tobacco plants kept in the greenhouse. Each column represents the mean GUS activity from at least 15 independent primary transformants for each promoter construct. Bars represent SE.

With the exception of chimeric GUS fusions with the 35S RNA CaMV (−1583 to +3), *FNR* (−28 to +231), and *CAB*-1 (−77 to +71) promoter fragments and the G-box sequences (see below), light-grown seedlings express higher GUS levels than material kept in darkness. However, the extent of light stimulation and effectiveness of the three light qualities differ. The values in Figure 2 are averages from the analysis of 20 seedlings that derive from 10 to 15 primary transformants for each promoter construct. The offspring of each primary transformant were analyzed separately before the data were combined for the presentation. Because of the enormous variation in absolute expression levels of the reporter gene, the SES of the mean values in Figure 2 are often too high to demonstrate significant differences between two physiological conditions. For the following conclusions, an effect was considered specific if it was observed in the seedling populations from *all* independent primary transformants with the same construct.

Expression in Etiolated Seedlings

GUS activities were already detectable in etiolated material and were stimulated 2- to 5-fold (average values) upon illumination. The only exception is the *CAB*-1 promoter/GUS gene fusion (−377 to +71), which was not, or not significantly, expressed in the dark and was stimulated at least 100-fold by light.

Response to RL and BL

No *FNR* promoter constructs that responded to RL and WL (−118 to +231; −313 to +231; −753 to +231) responded significantly to BL, in contrast to the results obtained for the *PC*, *ATPC*, *PSAF*, *CAB*-1, and *RBCS*-1 constructs, which respond to all three light qualities. This indicates a promoter-specific response pattern to different wavelengths (see "Discussion"). In addition, significant changes in the sensitivities toward RL and BL were observed when the *PSAF* and *PC* promoters were deleted from their 5′ ends. All three *PSAF* promoter fragments tested respond comparably to BL, but the shortest fragment is more sensitive to RL compared to the longer fragments (Fig. 2). This is consistent with the finding that upstream sequences in this promoter appear to exert RL-dependent silencing functions (Flieger et al., 1993). In the *PC* promoter, the −168 to +60 promoter fragment is less effective than the −259 to +60 fragment, a result that is consistent with previous observations that essential elements determining the quantitative expression of the *PC* gene are located between −259 and −169 bp (Lübberstedt et al., 1994). However, if relative effects are compared, the −1126- to +60- and −259- to +60-bp fragments respond equally to BL, RL, and WL, whereas all independent primary transformants harboring the −168- to +60-bp fragment respond more strongly to BL and WL as compared to RL (Fig. 2).

A more detailed analysis for this promoter is shown in Figure 3. If only relative values are considered, RL was slightly more effective in the cases of the −259- to +60- and −202- to +60-bp fragments, equally as effective as BL in the case of the −174- to +60-bp fragment, and approximately 3 times less effective in case of the −168- to +60-bp fragment.

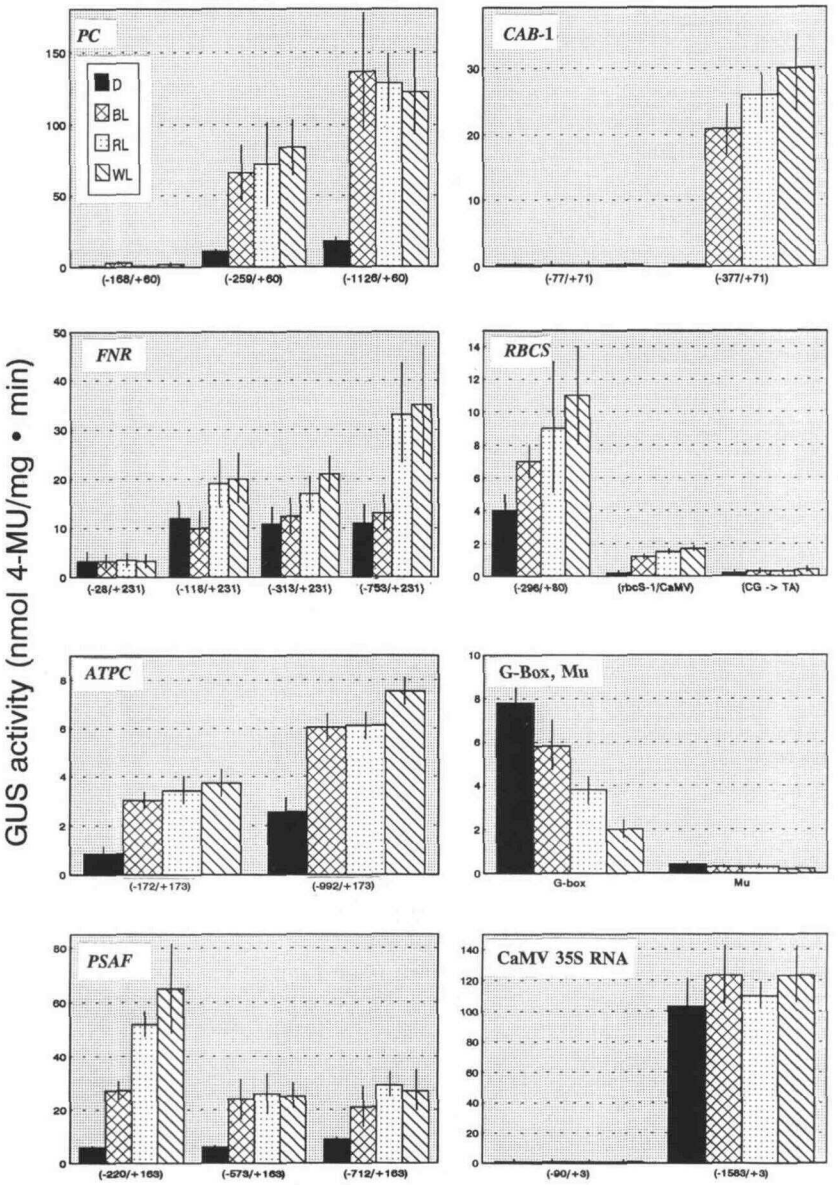

Figure 2. GUS activity in the cotyledons of 12-d-old transgenic tobacco seedlings that were kept in darkness (D), BL, RL, or WL. The numbers in parentheses refer to nucleotide positions relative to the transcription start site. G-box, A trimer of the G-box motif found in the spinach *RBCS*-1 promoter (5′-TCCACGTGGT-3′) fused to the 90-bp 35S RNA CaMV minimal promoter; Mu, a mutant trimer (5′-TCCAatTGGT-3′) of the G-box motif. CG → TA, The −296- to +80-bp *RBCS*-1 promoter fragment, in which the central CG nucleotides of the G-box sequence were replaced by TA. *RBCS*-1/CaMV, the −296- to −77-bp *RBCS*-1 promoter fragment, fused to the 90-bp CaMV minimal promoter. For each GUS measurement, 20 pairs of cotyledons were harvested. Bars represent SE.

The −146- to +60-bp fragment was inactive (T. Lübberstedt, R. Oelmüller, G. Wanner, R.G. Herrmann, unpublished data). This indicates that the −168- to +60-bp region is more responsive to BL than to RL, whereas sequences located between −259 and −169 contain *cis* elements, which interact more specifically with the RL-dependent signal pathway.

A Defined Segment in the RBCS Promoter (−296 to −77) Is Sufficient to Confer Light-Regulated Expression to the −90- to +3-bp CaMV Minimal Promoter

Light regulation directed by the −296- to −77-bp *RBCS* promoter fragment fused to the 90-bp CaMV minimal promoter is shown in Figure 2 (*RBCS*-1/CaMV). This region contains a G-box motif (5′-TCCACGTGGT-3′, located at position −219 to −212; for sequence see EMBL accession No. X73236) that is known to be essential for the expression of various light-regulated genes. The importance of this region has been verified by exchanging the two central CG to TA residues, which leads to an almost complete loss of GUS gene expression (Fig. 2, *RBCS*, CG → TA). A trimer of the authentic motif, fused to the 90-bp CaMV minimal promoter, is highly active in roots (Fig. 4) and cotyledons of etiolated seedlings (Fig. 2, G-box), and this activity is inhibited by WL, RL, and BL. The mutant G-box sequence (5′-TCCAatTGGT-3′) is hardly active (Figs. 2 and 4). Furthermore, it is worth noting that the GUS activity directed by the G-box trimer in etiolated material and roots is comparable to that of the entire −296- to +80-bp *RBCS*-1 promoter fragment in green tissue and to the levels obtained with the "full-length" *ATPC* promoter construct in light (Fig. 2).

Taken together, these results suggest that light-regulated gene expression can be wavelength dependent. At least in part, this is based on elements with different sensitivities toward RL and BL. The response to BL, RL, and/or WL is promoter-specific. Light regulation requires the arrangement

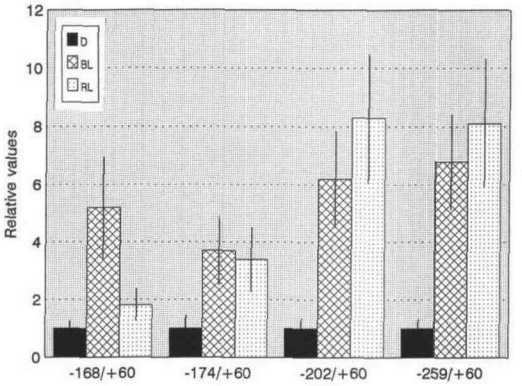

Figure 3. The effect of RL and BL on GUS gene expression directed by the −259 to +60-, −202- to +60-, −174- to +60-, and −168- to +60-bp PC promoter fragments. For comparison, dark values (D) were taken as 1.0 and the other values are expressed relative to it. The average absolute values in BL are: −259 to +60, 61.2 ± 19.0 nmol 4-MU mg^{-1} min^{-1}, −202 to +60, 19.2 ± 5.3 nmol 4-MU mg^{-1} min^{-1}, −174 to +60, 4.0 ± 1.1 nmol 4-MU mg^{-1} min^{-1}, −168 to +60, 0.097 ± 0.015 nmol 4-MU mg^{-1} min^{-1}. Bar, SE.

of relevant *cis* elements within a given promoter context (cf. *RBCS* and G-box data in Fig. 2). An isolated *cis* element can be functional in plants but exhibits quite a different expression pattern in the context of its promoter.

De-Etiolation Experiments and Phytochrome Response

To compare GUS gene expression directed by the various promoter fragments during de-etiolation, 10-d-old etiolated tobacco seedlings were transferred to WL and the accumulation of GUS activities was followed for 72 h. Again, in spite of the variation in absolute GUS levels, the basic kinetics were identical for seedlings harboring the same construct (data not shown). However, Figure 5A demonstrates that the GUS accumulation kinetics for the different promoter fusions differ. GUS levels under the control of the two *PSAF* promoter fragments −220 to +163 and −850 to +163 increase rapidly within 12 to 24 h after the onset of light and decrease thereafter, whereas those for the *CAB*-1, *ATPC*, and *PC* promoters increase steadily within the first 72 h of illumination, although to different extents. The accumulation kinetics

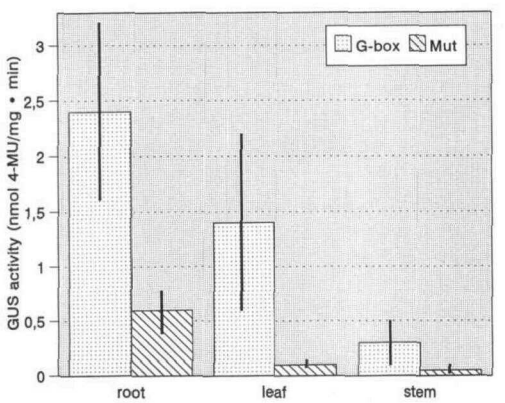

Figure 4. The G-box trimer of *RBCS*-1 confers organ-specific expression to the 35S RNA CaMV minimal promoter. GUS activity was determined in roots, leaves, and stems of 6- to 7-week-old tobacco plants kept in the greenhouse. Mut, Mutated G-box sequence, in which the central CG residues were replaced by AT. Bars, SE.

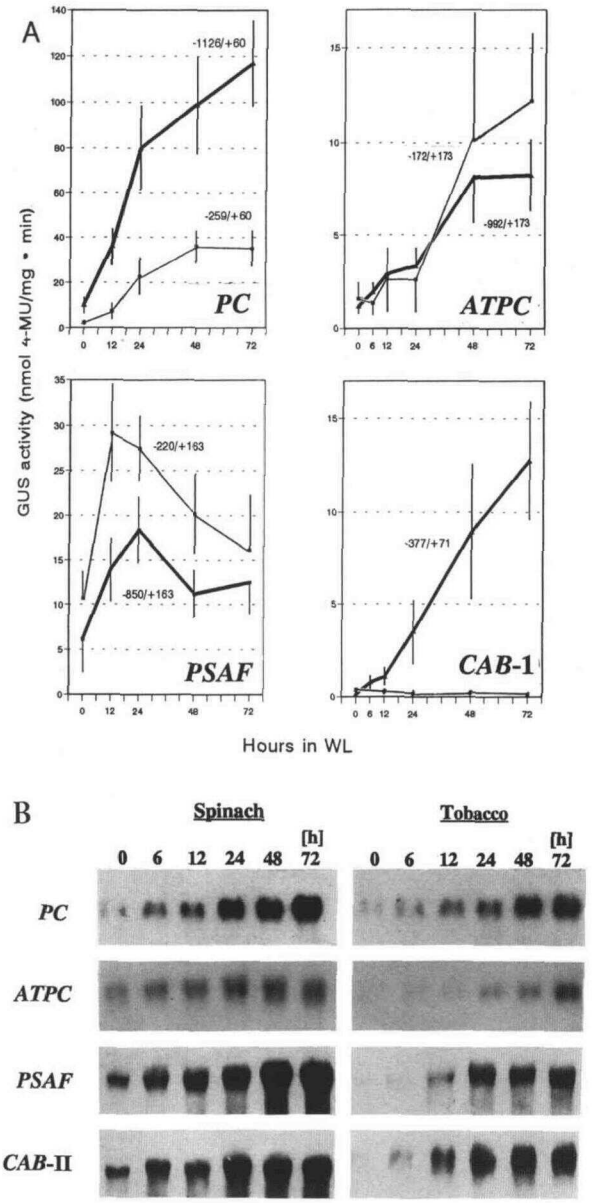

Figure 5. A, Accumulation of GUS activity after transfer of 10-d-old, etiolated tobacco seedlings to WL (30 W m^{-2}). Seedlings from six to nine independent primary transformants were analyzed individually. To synchronize germination, seeds received a 12-h RL treatment directly after sowing before transfer to darkness. B, Northern blots with spinach and tobacco RNA. For this experiment, seedlings were grown in the same way, and RNA was extracted at the times indicated. The low level of hybridization of the tobacco *ATPC* transcripts is probably due to the heterologous (spinach) probe. For the other filters, the respective homologous probes were used.

of the GUS activities for the *PSAF* promoter fusions in transgenic tobacco seedlings differ from the mRNA accumulation kinetics of the corresponding genes in spinach and tobacco, which all increase within the first 72 h of illumination in both spinach and tobacco (Fig. 5B). Therefore, the response pattern observed for the *PSAF*/GUS gene fusions is not representative for either spinach or tobacco seedlings.

No significant stimulation of GUS gene expression by single light pulses operating through phytochrome were observed in transgenic tobacco seedlings harboring the spinach *PC*, *PSAF*, *CAB*, and *ATPC* promoters (data not shown). The only exception observed was the spinach *FNR* promoter, which did respond to a single RL pulse (Oelmüller et al., 1993).

Taking advantage of the availability of homologous cDNA probes for the spinach and tobacco *PC* genes, phytochrome-controlled expression of these genes was analyzed in *N. plumbaginifolia* and compared with that in spinach. Both species contain only a single copy of the *PC* gene per haploid genome (Bichler and Herrmann, 1990; Lübberstedt et al., 1994). The transcript levels in spinach increase 2- to 3-fold after a saturating RL pulse, and the effect is fully reversible by a subsequently given far-red light pulse (Table II). In contrast, no, or no significant, stimulation was observed for the *N. plumbaginifolia PC* transcript level. Seedlings from three independent primary *N. plumbaginifolia* transformants harboring the −1126- to +60-bp spinach *PC* promoter fused to a GUS gene also fail to respond to an RL pulse (Table II). Identical results were obtained with *N. tabacum* seedlings: neither the *trans*-gene nor the endogenous PC transcript level responded to light pulses operating through phytochrome (data not shown). Kinetic studies confirmed that the lack of the phytochrome response is not caused by different accumulation kinetics of the GUS and *PC* messages (data not shown). These results demonstrate that the response of the *trans*-gene to a single light pulse activating the phytochrome system is different from that in spinach but normal in comparison to the regulation in *N. plumbaginifolia* and *N. tabacum* (see "Discussion").

Table II. *Steady-state mRNA levels in the poly(A)$^+$ RNA fraction from 10-d-old spinach and N. plumbaginifolia seedlings*

Etiolated seedlings received either no light (D), a 5-min RL (RL) or far-RL (far-RL) pulse, or a RL pulse followed by a far-RL pulse (RL/far-RL) 12 h before harvest. Hybridization was performed with a *PC* cDNA from spinach or tobacco (*N. tabacum*) or the bacterial GUS gene probe (transformants 1, 2, and 3). RNA was isolated from the offspring of three independent primary transformants harboring the −1126 to +60-bp *PC* promoter or from spinach seedlings. For hybridization with the tobacco *PC* gene, RNA from the transgenic material was pooled.

Plant Material	Light Treatment	Relative mRNA
		cpm
Spinach (*PC*)	D	87
	RL	289
	Far-RL	90
	RL/far-RL	98
N. plumbaginifolia (*PC*)	D	198
	RL	255
	Far-RL	221
	RL/far-RL	211
Transformant 1 (GUS)	D	56
	RL	43
	Far-RL	65
	RL/far-RL	61
Transformant 2 (GUS)	D	311
	RL	302
	Far-RL	298
	RL/far-RL	312
Transformant 3 (GUS)	D	235
	RL	229
	Far-RL	234
	RL/far-RL	219

DISCUSSION

The aim of the present study was to compare the quantitative and qualitative (light regulated) expression of the GUS reporter gene under the control of different upstream regions from nuclear genes for plastid proteins from spinach. The results demonstrate that these regions direct a gene-specific and unique response pattern to the GUS gene, which can also be different from the regulation of the endogenous genes in spinach and tobacco. This could imply that (a) not all regulatory elements are located within the promoter fragments or that (b) *cis*-regulatory elements that are probably essential for promoter function in spinach respond differently to tobacco regulatory signals or that (c) posttranscriptional mechanisms operate in concert with transcriptional mechanisms to synchronize the mRNA accumulation for plastid proteins. Examples for all three explanations have been reported in the literature.

That not all regulatory elements are located within promoter fragments is shown by the fact that various animal and plant genes contain nucleotide sequences located downstream of the transcription unit that act as enhancer elements and determine quantitative expression (Choi and Engel, 1986; Owen and Kuhn, 1987; Fischer and Maniatis, 1986; Bodine and Ley, 1987; Callis et al., 1987; Sanchez-Serrano et al., 1987; Thornberg et al., 1987; Trainor et al., 1987; Dean et al., 1989a, 1989b; Dietrich et al., 1992). These can include light-responsive elements (Gallo-Meagher et al., 1992). In addition, introns have been shown to increase the transcription of genes (Callis et al., 1987; Brinster et al., 1988). Similarly, a far-upstream promoter element has been identified that modulates the quantitative expression of the tomato *RBCS*-3A gene in a developmentally regulated manner (Ueda et al., 1989). Therefore, it is conceivable that the different expression levels of the chimeric gene fusions in transgenic tobacco arise because regulatory elements determining quantitative and/or the qualitative expression are not located within the promoter fragment used.

An example of a *cis*-regulatory element that responds differently in different species is provided by Sun et al. (1992), who have shown that the *Arabidopsis CAB*140 gene contains a sequence that is identical with the GT-1 box II-binding site in the pea *RBCS* promoter but is not involved in the expression of the *Arabidopsis* gene. Also, the *Fed*-1 gene from pea exhibits different light responses in pea and tobacco

(Gallo-Meagher et al., 1992) because not only promoter sequences but also internal regulatory elements control the expression of the *trans*-gene in tobacco. Elements with slight similarity to box II and III of the pea *RBCS*-3A promoter are also found within the spinach *ATPD* promoter, but they are located in regions that do not appear to contribute to light responsiveness (Bichler and Herrmann, 1990). Comparably, Luan and Bogorad (1992) have shown that a rice *CAB* gene promoter contains different *cis*-acting sequences that regulate expression in dicots and monocots. An octamer repeat that lies within the −269- to −170-bp promoter region is essential for photoregulated expression of the chimeric GUS gene in leaf cells of maize and rice but is not required for expression in illuminated tobacco leaves. Conversely, boxIII*- and G-box-like sequences are necessary for high levels of expression in tobacco leaves but not in monocots. These observations can have, in part, a phylogenetic explanation. The spinach *ATPC* and *ATPD* genes, which have probably been transferred independently from the organelle chromosome to the nucleus (Herrmann et al., 1991; Pancic and Strotmann, 1993), have acquired *cis* elements that respond to the light-induced signal pathways in both spinach and tobacco (Fig. 3), but these elements could otherwise be so divergent that the GUS activities directed by their promoter regions differ by more than 1 order of magnitude (Fig. 1).

Most evidence for light-regulated posttranscriptional controls has been deduced from discrepancies between in vitro (run-on) transcription and in vivo mRNA levels, which, for instance, have been reported for the oat phytochrome (Colbert, 1988) and the oat Pchlide-oxidoreductase (Mösinger et al., 1988). The mechanisms and control steps are unknown. However, differences in the mRNA abundance reported for various members of the *RBCS* and *CAB* gene families (Dean et al., 1985, 1987) seem to be caused predominantly by transcriptional effects (Dean et al., 1989a, 1989b) rather than posttranscriptional events.

The Effect of Light Quality on GUS Gene Expression

Although various short promoter regions have been identified that are capable of conferring light-regulated GUS gene expression to heterologous minimal promoters and a growing number of regulatory proteins that specifically interact with upstream regions of light-regulated genes have been identified, their mode of interaction with the light-dependent signal transduction pathway is not yet understood (see below). In addition, the expression of light-regulated genes can be modulated by more than one wavelength (Tobin and Silverthorne, 1985; Marrs and Kaufman, 1989; Oelmüller et al., 1989; Oelmüller and Kendrick, 1991). The different light responses are mediated through distinct photoreceptors. This raises the basic question of whether genes that respond differently to different wavelengths operate with distinct *cis* elements or whether the signal transduction pathways converge to act on the same regulatory sequence. Fluhr and Chua (1986) have shown that 0.4-kb *RBCS*-3A and 2.0-kb *RBCS*-3C upstream regions are sufficient for phytochrome response and BL induction in transgenic petunia plants. In a series of 5′ promoter deletions from the spinach *PSAF* and *PC* genes in tobacco, changes in sensitivity to RL and BL were observed (Figs. 2 and 3), indicating that these promoters contain regions with different responses to the BL- and RL-dependent signal transduction pathway(s) even within short promoter segments. Of course, this does not necessarily imply that such elements are exclusively sensitive to either RL or to BL. Furthermore, since 5′ deletions from different promoters respond differently to RL and BL (cf. *PC* and *PSAF* in Figs. 2 and 3), the arrangement of these elements seems to be promoter specific. As expected, WL, which activates both photosystems, operates like RL in the cases of the *PSAF* and *FNR* promoters and like BL in case of the *PC* promoter.

An additional complexity derives from the observation that not only the light quality but also the intensity seems to be an important parameter. For instance, under the chosen light conditions (5 W m^{-2}), no significant BL response was detectable in seedlings transformed with the *FNR* promoter fragments, whereas the same BL intensity suffices to direct GUS gene expression under the control of other chimeric gene fusions (Fig. 2). However, GUS activity under the control of the *FNR* promoter fragments can be induced by higher BL intensities (data not shown). Therefore, the effectiveness of RL and BL in inducing GUS gene expression is dependent on the light intensity, and this dependency can be promoter specific.

Differences in the Light Response in Spinach and Tobacco

Table II and Figure 2 demonstrate that all *trans*-genes in 10-d-old etiolated tobacco seedlings fail to respond to light pulses but are induced after transfer of the seedlings to WL. This is comparable to the regulation of the endogenous genes in tobacco but different from that in spinach, for which a response to a single light pulse is detectable. The spinach promoters in transgenic plants seem to respond normally to tobacco regulatory pathways. Since endogenous tobacco genes (Wehmeyer et al., 1990; Palomares et al., 1991), as well as chimeric GUS gene fusions in tobacco (Oelmüller et al., 1993), can be activated by RL pulses in etiolated tobacco seedlings, it remains to be determined why the promoter constructs analyzed in this study fail to respond to such light pulses.

The different accumulation kinetics after transfer of etiolated transgenic tobacco seedlings to WL does not always reflect the situation in either donor or host plant. This points to the limitation of this approach and to the mechanisms that coordinate the expression of these genes in spinach. During evolution, the interaction between quite different *cis* elements caused by an independent transfer of originally organelle-encoded genes to the nucleus and conserved signal pathways must have been optimized, probably separately for each promoter (cf. Wedel et al., 1992). This could explain, at least in part, why each promoter functions optimally in its environment but responds differently, although not necessarily qualitatively differently, to regulatory pathways from other organisms. Deciphering the adaptation of regulatory sequences (and of sequences for import of the cytosol-synthesized proteins into the organelle; Wedel et al., 1992, Cai et al., 1993) to light-dependent signal pathways as well as of processes that led to controlled/coordinated expression of

nuclear genes and of their plastid partner genes represents one of the principal challenges of modern cell biology.

G-Box Element

Among the nine spinach promoters studied so far, only the *RBCS*-1 promoter contains a fully conserved G-box palindrome, 5′-CACGTG-3′. Salinas et al. (1992) have recently shown that a tetramer of the element 5′-GCCACGTGGC-3′, which is sufficient for binding the tobacco transcription activator TAF-1 in vitro, confers relatively high levels of GUS gene expression to the 90-bp 35S RNA CaMV minimal promoter in roots. The same result has been obtained with a trimer of the a G-box sequence with flanking regions from the spinach *RBCS*-1 promoter (5′-TCCACGTGGT-3′). Furthermore, although the element is part of a light-responsive segment, alone it confers negative light regulation to the 35S RNA CaMV minimal promoter in cotyledons of developing tobacco seedlings (Fig. 2). This result, as well as the absence of this element in the promoters of other light-regulated genes from spinach, suggests that this palindrome is probably more involved in quantitative expression rather than as an essential component of light-induced signal transduction pathways.

Received September 20, 1993; accepted December 6, 1993.

LITERATURE CITED

Ahmad M, Cashmore AR (1993) HY4 gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature **366:** 162–166

Armstrong GA, Weisshaar B, Hahlbrock K (1992) Homodimeric and heterodimeric leucine zipper proteins and nuclear factors from parsley recognize diverse promoter elements with ACGT cores. Plant Cell **4:** 525–537

Bichler J, Herrmann RG (1990) Analysis of the promoters of the single-copy genes for plastocyanin and subunit delta of the chloroplast ATP synthase from spinach. Eur J Biochem **190:** 415–426

Bodine DM, Ley TJ (1987) An enhancer element lies 3′ to the human gamma globin gene. EMBO J **6:** 2997–3004

Bolle CEH, Kusnetsov V, Michl D, Cai D, Klösgen RB, Oelmüller R, Herrmann RG (1992) The genes for the three nuclear-coded subunits of chloroplast ATP synthases: characterization of the promotors from the single-copy genes *atp*C and *atp*D from spinach. *In* N Murata, ed, Research in Photosynthesis, Vol III. Kluwer Academic Publishers, Boston, MA, pp 377–380

Bradford MJ (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem **72:** 248–254

Brinster RL, Allen JM, Behringer RR, Gelinas RE, Palmiter RD (1988) Introns increase transcriptional efficiency in transgenic mice. Proc Natl Acad Sci USA **85:** 836–840

Cai D, Herrmann RG, Klösgen RB (1993) The 20 kDa apoprotein of the CP24 complex of photosystem II: an alternative model to study import and intra-organellar routing of nuclear-encoded thylakoid proteins. Plant J **3:** 383–392

Callis J, Fromm M, Walbot V (1987) Introns increase gene expression in cultured maize cells. Genes Dev **1:** 1183–1200

Choi OR, Engel JD (1986) A 3′ enhancer is required for temporal and tissue-specific transcriptional activation of the chicken adult beta-globin gene. Nature **323:** 731–734

Chory J, Nagpal P, Peto CA (1986) Phenotypic and genetic analysis of *det*2, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell **3:** 445–459

Chory J, Peto C, Feinbaum R, Pratt L, Ausubel F (1989) An *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58:** 991–999

Colbert JT (1988) Molecular biology of phytochrome. Plant Cell Environ **11:** 305–318

Datta N, Cashmore AR (1989) Binding of a pea nuclear protein to promoters of certain photoregulated genes is modulated by phosphorylation. Plant Cell **1:** 1069–1077

Dean C, Favreau M, Bedbrook J, Dunsmuir P (1989a) Sequences 5′ to translation start regulate expression of petunia *rbc*S genes. Plant Cell **1:** 209–215

Dean C, Favreau M, Bond-Nutter D, Bedbrook J, Dunsmuir P (1989b) Sequences downstream of translation start site regulate quantitative expression of two *rbc*S genes. Plant Cell **1:** 201–208

Dean C, Favreau M, Dunsmuir P, Bedbrook J (1987) Confirmation of the relative expression levels of the *Petunia* (Mitchell) *rbc*S genes. Nucleic Acids Res **15:** 4655–4668

Dean C, van den Elzen P, Tamaki S, Dunsmuir P, Bedbrook J (1985) Differential expression of eight genes of the petunia ribulose bisphosphate carboxylase small subunit multi-gene family. EMBO J **4:** 3055–3061

Dehesh K, Bruce WB, Quail P (1990) A *trans*-acting factor that binds to a GT-motif in a phytochrome gene promoter. Science **250:** 1397–1399

DeLisle AJ, Ferl RJ (1990) Characterization of the *Arabidopsis Adh* G-box binding factor. Plant Cell **2:** 547–557

Deng XW, Caspar T, Quail PH (1991) *cop*1: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev **5:** 1172–1182

Dietrich RA, Radke SE, Harada JJ (1992) Downstream DNA sequences are required to activate a gene expressed in the root cortex of embryos and seedlings. Plant Cell **4:** 1371–1382

Donald RGK, Cashmore AR (1990) Mutation in either G box or I box sequences profoundly affects expression from the *Arabidopsis thaliana rbc*S-1 promoter. EMBO J **9:** 1717–1726

Feinberg AR, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem **132:** 2–13

Fischer JA, Maniatis T (1986) Regulatory elements involved in *Drosophila Adh* gene expression are conserved in divergent species and separate elements mediate expression in different tissues. EMBO J **5:** 1275–1289

Flieger K, Tyagi A, Sopory S, Cseplö A, Herrmann RG, Oelmüller R (1993) A 42 bp promoter fragment of the gene for subunit III of photosystem I (*psa*F) is crucial for its activity. Plant J **4:** 9–17

Fluhr R, Chua N-H (1986) Developmental regulation of two genes encoding ribulose-bisphosphate carboxylase small subunit in pea and transgenic petunia plants: phytochrome response and blue-light induction. Proc Natl Acad Sci USA **83:** 2358–2362

Frances S, White MJ, Edgerton MD, Jones AM, Elliott RC, Thompson WF (1992) Initial characterization of a pea mutant with light-independent photomorphogenesis. Plant Cell **4:** 1519–1530

Gallo-Meagher M, Sowinski DA, Elliott RC, Thompson WF (1992) Both internal and external regulatory elements control expression of the pea *Fed*-1 gene in transgenic tobacco. Plant Cell **4:** 389–395

Gallo-Meagher M, Sowinski DA, Thompson WF (1992) The pea ferredoxin I gene exhibits different light responses in pea and tobacco. Plant Cell **4:** 383–388

Gilmartin PM, Sarokin L, Memelink J, Chua N-H (1990) Molecular light switches for plant genes. Plant Cell **2:** 369–378

Giuliano G, Pichersky E, Malik VS, Timko MP, Scolnik PA, Cashmore AR (1988) An evolutionary conserved protein binding sequence upstream of a plant light-regulated gene. Proc Natl Acad Sci USA **85:** 7089–7093

Herrmann RG, Oelmüller R, Bichler J, Schneiderbauer A, Steppuhn J, Wedel N, Tyagi AK, Westhoff P (1991) The thylakoid membrane of higher plants: genes, their expression and interaction. *In* RG Herrmann, BA Larkins, eds, Plant Molecular Biology. Plenum Press, New York, pp 411–427

Herrmann RG, Steppuhn J, Herrmann GS, Nelson N (1993) The nuclear-encoded polypeptide CF_0II from spinach is a real, ninth subunit of chloroplast ATP synthase. FEBS Lett (in press)

Herrmann RG, Westhoff P, Link G (1992) Biogenesis of plastids in

higher plants. *In* RG Herrmann, ed, Plant Gene Research, Vol 6: Cell Organelles. Springer, New York, pp 275–349

Jefferson RA (1987) Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep **5:** 387–405

Jefferson RA, Kavanagh TA, Bevan MW (1987) Gus-fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Kay SA, Keith B, Shinozaki K, Chye M-L, Chua N-H (1989) The rice phytochrome gene: structure, autoregulated expression, and binding of GT-1 to a conserved site in the 5′ upstream region. Plant Cell **1:** 351–360

Kendrick RE, Kronenberg GHM (1986) Photomorphogenesis in Plants. Martinus Nijhoff, Dordrecht, The Netherlands

Kuhlemeier C, Cuozzo M, Green P, Goyvaerts E, Ward K, Chua N-H (1988) Localization and conditional redundancy of regulatory elements in *rbc*S-3A, a pea gene encoding the small subunit of ribulose-bisphosphate carboxylase. Proc Natl Acad Sci USA **85:** 4662–4666

Luan S, Bogorad L (1992) A rice *cab* gene promoter contains separate *cis*-acting elements that regulate expression in dicot and monocot plants. Plant Cell **4:** 971–981

Lübberstedt T, Delmüller R, Wanner G, Herrmann RG (1994) Interacting cis-elements in the plastocyanin promoter from spinach ensure regulated high-level expression. Mol Gen Genet (in press)

Manzara T, Gruissem W (1988) Organization and expression of the genes encoding ribulose-1,5-bisphosphate carboxylase in higher plants. Photosynth Res **16:** 117–139

Marcotte WR, Russel SH, Quatrano RS (1989) Abscisic acid-responsive sequences from the EM gene of wheat. Plant Cell **1:** 969–976

Marrs KA, Kaufman LS (1989) Blue-light regulation of transcription for nuclear genes in pea. Proc Natl Acad Sci USA **86:** 4492–4495

Mösinger E, Batschauer A, Apel K, Schäfer E (1988) Phytochrome regulation of greening in barley. Effects of mRNA abundance and transcriptional activity of isolated nuclei. Plant Physiol **86:** 706–710

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497

Oelmüller R, Bolle C, Tyagi AK, Niekrawietz N, Breit S, Herrmann RG (1993) Characterization of the promoter from the single-copy gene encoding ferredoxin-$NADP^+$-oxidoreductase from spinach. Mol Gen Genet **237:** 261–272

Oelmüller R, Briggs WR (1990) Intact plastids are required for nitrate- and light-induced accumulation of nitrate reductase activity and mRNA in squash cotyledons. Plant Physiol **92:** 434–439

Oelmüller R, Kendrick RE (1991) Blue light is required for survival of the tomato phytochrome-deficient *aurea* mutant and the expression of four nuclear genes coding for plastidic proteins. Plant Mol Biol **16:** 293–299

Oelmüller R, Kendrick RE, Briggs WR (1989) Blue-light mediated accumulation of nuclear-encoded transcripts coding for proteins of the thylakoid membrane is absent in the phytochrome-deficient *aurea* mutant of tomato. Plant Mol Biol **13:** 223–232

Oelmüller R, Lübberstedt T, Bolle C, Sopory S, Tyagi A, Cseplö A, Flieger K, Herrmann RG (1992) Promotor architecture of nuclear genes for thylakoid membrane proteins from spinach. *In* N Murata, ed, Research in Photosynthesis, Vol III. Kluwer Academic Publishers, Boston, MA, pp 219–224

Owen D, Kuhn LC (1987) Non-coding 3′ sequences of the transferring receptor gene are required for mRNA regulation by iron. EMBO J **6:** 1287–1293

Palomares R, Herrmann RG, Oelmüller R (1991) Different blue-light requirement for the accumulation of transcripts from nuclear genes for thylakoid membrane proteins in *Nicotiana tabacum* and *Lycopersicon esculentum*. J Photochem Photobiol B Biol **11:** 261–272

Pancic PG, Strotmann H (1993) Structure of the nuclear encoded gamma subunit of CF_0CF_1 of the diatom *Odontella sinensis* including its presequence. FEBS Lett **320:** 61–66

Salinas J, Oeda K, Chua N-H (1992) Two G-box-related sequences confer different expression patterns in transgenic tobacco. Plant Cell **4:** 1485–1493

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sanchez-Serrano J, Keil M, Pera-Cortes M, Rocha-Sosa M, Willmitzer L (1987) Wound-induced expression of proteinase inhibitor II in potato and transgenic tobacco. *In* J Key, L McIntosh, eds, Plant Gene Systems and Their Biology. Alan R Liss, New York, pp 331–338

Sarokin LP, Chua N-H (1992) Binding sites for two novel phosphoproteins, 3AF5 and 3AF3, are required for *rbc*S-3A expression. Plant Cell **4:** 473–483

Schindler U, Cashmore AR (1990) Photoregulated gene expression may involve ubiquitous DNA binding proteins. EMBO J **9:** 3415–3427

Schulze-Lefert P, Becker-Andre M, Schulz W, Hahlbrock K, Dangl J (1989) Functional architecture of the light-responsive chalcone synthase promoter from parsley. Plant Cell **1:** 707–714

Staiger D, Kaulen H, Schell J (1989) A CACGTG motif of the *Antirrhium majus* chalcone synthase promoter is recognized by an evolutionary conserved nuclear protein. Proc Natl Acad Sci USA **86:** 6930–6934

Stockhaus J, Eckes P, Rocha-Sosa M, Schell J, Willmitzer L (1987) Analysis of *cis*-acting sequences involved in the leaf-specific expression of a potato gene in transgenic plants. Proc Natl Acad Sci USA **84:** 7943–7947

Sun L, Doxsee RA, Harel E, Tobin EM (1992) CA-1, a novel phosphoprotein, interacts with the promoter of the cab140 gene in *Arabidopsis* and is undetectable in *det*1 mutant seedlings. Plant Cell **5:** 109–121

Thompson WF, White MJ (1991) Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 423–466

Thornberg RW, An G, Cleveland TE, Johnson R, Ryan C (1987) Wound-inducible expression of a potato inhibitor II-chloramphenicol acetyl transferase gene fusion in transgenic tobacco plants. Proc Natl Acad Sci USA **84:** 744–748

Tittgen J (1987) Die Klonierung von genomischer und *copy*-DNA: Untersuchungen an nukleären Genen für plastidäre Polypeptide aus *Spinacea oleracea*. PhD thesis, Ludwig-Maximilians-University, Munich

Tobin E, Silverhtorne J (1985) Light-regulation of gene expression in higher plants. Annu Rev Plant Physiol **36:** 569–593

Trainor CD, Stamler SJ, Engel JD (1987) Erythroid-specific transcription of the chicken histone H5 gene is directed by a 3′ enhancer. Nature **328:** 827–830

Ueda T, Pichersky E, Malik VS, Cashmore AR (1989) Level of expression of the tomato *rbc*S-3A gene is modulated by a far upstream promoter element in a developmentally regulated manner. Plant Cell **1:** 217–227

Wedel N, Klein R, Ljungberg U, Andersson B, Herrmann RG (1992) The single-copy gene *psb*S codes for a phylogenetically intriguing 22 kDa polypeptide from photosystem II. FEBS Lett **314:** 61–66

Wehmeyer B, Cashmore AR, Schäfer E (1990) Photocontrol of the expression of genes encoding chlorophyll *a*/*b*-binding proteins and small subunit of ribulose-1,5-bisphosphate carboxylase in etiolated seedlings of *Lycopersicon esculentum* (L.) and *Nicotiana tabacum* (L.). Plant Physiol **93:** 990–997

Wei N, Deng X-W (1992) *cop*9: a new genetic locus involved in light-regulated development and gene expression in *Arabidopsis*. Plant Cell **4:** 1507–1518

Weisshaar B, Armstrong GA, Block A, da Costa e Silva O, Hahlbrock K (1991) Light-inducible and constitutively expressed DNA-binding proteins recognizing a plant promoter element with functional relevance in light responsiveness. EMBO J **10:** 1777–1786

White MJ, Fristensky BW, Falconet D, Childs LC, Watson JC, Alexander L, Roe BA, Thompson WF (1992) Expression of the chlorophyll-*a*/*b*-protein multigene family in pea (*Pisum sativum* L.). Planta **188:** 190–198