

The TATA-Box Sequence in the Basal Promoter Contributes to Determining Light-Dependent Gene Expression in Plants^{1[W]}

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A prototype 13-bp TATA-box sequence, TCACTATATATAG, was mutated at each nucleotide position and examined for its function in the core promoter. Specific nucleotides in the first TATA, the second TATA, as well as the flanking sequences influenced promoter function in transient transformation of tobacco (*Nicotiana tabacum* var Petit Havana) leaves. The effect of a given mutation on reporter gene expression in light versus dark was variable and sometimes contrasting. Some mutations, like T₇ or A₈ → C or G, completely inactivated the expression of the minimal promoter in light but not in dark. In general, the sequence requirement for dark expression was less stringent than that for light expression. The selective effect of TATA-box mutations on light versus dark expression was exerted on core promoter function in the chromatin-integrated state also. Even in the presence of an upstream light response activator element, TATA-box mutations influenced modulation of the promoter by light. An A at the eighth position was specifically involved in the red light response of the promoter. Selectivity in gene expression was associated with a high level of transcript initiation from a site that was not active in the dark. Nuclear proteins from dark- and light-grown seedlings showed that the sequence variation within the TATA-box governs the formation of alternative transcriptional complexes. The experiments give direct evidence for the role of a core TATA-box sequence in determining the level as well as selectivity of gene expression in plants.

The minimal or core promoter, by definition, is the sequence located between the -35 to +35 region with respect to transcription start site (Smale, 2001). Eukaryotic promoters of protein-coding genes have one or more of three conserved sequences in this region (i.e. the TATA-box, initiator region, and downstream promoter element). Computational analysis of the core promoter of plants (Shahmuradov et al., 2003) suggests that core promoters are quite heterogeneous in composition for different conserved cis-regulatory elements. The TATA-box, found commonly in eukaryotic promoters, is typically a T/A-rich sequence, located about 25 to 30 bp upstream of the transcription start site. Breathnach and Chambon (1981) analyzed a number of eukaryotic genes, most of those from invertebrates and vertebrates, and identified TATAAA as the consensus TATA-box. Joshi (1987) did computational analysis of 79 plant promoters and suggested TCACTATATATAG as the consensus sequence in the

TATA region. Our analysis (Sawant et al., 1999) attributed this sequence preferentially to highly expressed plant genes rather than plant genes in general. By examining the effect of mutations on in vitro transcription, a duplicated TATA sequence in the TATA-box was reported to enhance transcription (Mukumoto et al., 1993). Mutational studies (Zhu et al., 1995) have also established the importance of the TATA-box in the correct selection of a transcription initiation site.

Although the minimal promoter is generally believed to determine the rate of transcription and the point of its initiation (for review, see Roeder, 1996), there is some evidence suggesting its role in promoter selectivity also. Two different TATA-boxes in the human globin gene determine the regulation of γ -globin gene expression in embryogenesis and adult stage (Duan et al., 2002). Human myoglobin enhancer activates TATAAAA and not TATTTAT, although the latter sequence functions in the SV40 early promoter (Wefald et al., 1990). Heterogeneous composition of TFIID complexes (Martinez, 2002) associated with different promoters also suggests that sequence variations within the core promoter may govern alternative transcription complexes needed in different physiological settings. The diversity observed in the sequence of minimal promoters can also be interpreted as related to combinatorial regulation by activators bound to distal sites because such transcription factors often function through contacts with factors associated with specific TATA-box sequences (Butler and Kadonaga, 2001). However, the effect of specific mutations in the core TATA-box on the in vivo selectivity in

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eukaryotic gene expression has not been examined in detail.

Mukumoto et al. (1993) and Yamaguchi et al. (1998) carried out mutational analysis and examined the effect of changes at each position in a consensus plant TATA element on *in vitro* transcription, using the HeLa- and tobacco (*Nicotiana tabacum*)-based transcription systems, respectively. This article presents results on the effect of mutations at each position in a 13-nucleotide-long prototype TATA-box on promoter function in leaf tissue. The effect of various mutations in light-regulated expression was examined in tobacco leaves after transient, as well as stable, transformation using β -glucuronidase (*gusA*) as the reporter gene. The results suggest that the sequence architecture in the TATA region may be important not only in modulating the level of gene expression, but also in determining selectivity of promoter function in response to the absence or presence and quality of light.

RESULTS

Several Single Mutations in the TATA-Box Region Are Tolerated in *In Vivo* Gene Expression

The effect of single mutations in the TATA-box on transient gene expression from reporter gene constructs was observed by determining the GUS activity after incubating the leaf discs for 48 h in continuous light. The GUS activity expressed by the prototype TATA-box, TCACTATATATAG, was compared with the activity of the mutated derivatives following incubation of the excised leaves in light. As seen in Figure 1, although several mutations had no significant effect on the activity of the basal promoter, a majority of them resulted in a decrease or an increase in promoter expression as compared to the prototype promoter that contained the parent TATA sequence. For example, a 5-fold increase in light was obtained when G at the thirteenth position was mutated to C ($G_{13} \rightarrow C$). In some cases, the results are in contradiction to those published earlier on plant promoter mutants in HeLa-based (Mukumoto et al., 1993) and tobacco-based (Yamaguchi et al., 1998) *in vitro* transcription systems. A decline in transcription was reported in the case of most of the mutations in those studies. Our experiments on transient transformation of tobacco leaves show that, in contrast to *in vitro* transcription, mutations in the core TATA sequence are more tolerated *in vivo*. In the case of mutations like $T_9 \rightarrow A$, no significant effect was observed by us, which is in agreement with that reported in an *in vitro* tobacco-based transcription system (Yamaguchi et al., 1998). Several mutations, especially those at the fifth, seventh, eighth, and tenth positions, and others like $A_3 \rightarrow G$, $C_4 \rightarrow G$, $A_{12} \rightarrow C$, etc., decreased transcription substantially. However, others, especially at the eleventh and thirteenth positions, had a substantial increase in activity in light. The effect of mutations on gene expression

suggested the importance of both TATATATA as well as the flanking sequences (i.e. the TCAC and G) located, respectively, before and after it, in determining the level of *in vivo* transcription.

Mutations that were not tolerated at all in gene expression in leaves exposed to continuous light were the substitutions to C and G at the seventh and eighth positions (i.e. the latter TA of the first TATA sequence). The promoters containing a TATA-box with these mutations failed to activate *gusA* expression in leaves exposed to continuous light (Fig. 1). The results suggest that certain positions in the prototype TATA-box have very little or no sequence preference for *in vivo* promoter function, although others could be critical to the level of gene expression.

Nucleotide Sequence in the TATA-Box Determines Promoter Selectivity in Gene Expression

We examined whether specific mutations in the TATA-box sequence influenced promoter strength in general or whether the mutated promoters behaved differently, depending upon other physiological cues. In other words, is the sequence requirement of the TATA-box the same for all promoters or only if the TATA-box sequence reflects specific sequence signatures related to the needs of gene expression specificity? To address this question, the expression of *gusA*, driven by each of the above-mentioned mutated promoters, was compared between the leaf discs incubated in light or dark. The dark versus light data are included in Figure 1. In general, the expression in dark was affected less substantially and varied from 41% to 214% of the expression driven by the prototype promoter. The results suggest that a given TATA-box cannot be considered as the optimal signal for transcription initiation. Depending upon the physiological cues, fine differences in the TATA-box sequence may modulate promoter expression differently.

The effect of some of the mutations in the first TATA (i.e. $T_7 \rightarrow C$, $T_7 \rightarrow G$, $A_8 \rightarrow C$, and $A_8 \rightarrow G$) were most noticeable in light-activated promoter expression. Whereas these mutations made the promoter completely nonfunctional in light, they continued to express at a high level in dark. In fact, dark activity was not reduced to zero in any of the 40 promoters. In some cases, as in the case of $C_2 \rightarrow T$ and $A_6 \rightarrow T$, dark activity declined by about 3-fold as compared to the prototype TATA-box sequence. In other cases, such as $T_1 \rightarrow G$, $A_8 \rightarrow T$, $A_{10} \rightarrow G$, $A_{10} \rightarrow T$, and $G_{13} \rightarrow A$, expression from the core promoter in dark increased 2- to 3.6-fold. The mutations that stimulated dark transcription did not necessarily give a similar effect in light. For example, among the aforementioned five mutations that increased promoter activity in dark, only $G_{13} \rightarrow A$ gave a comparable increase, whereas the others had little effect or resulted in a decline in light-modulated promoter function. Other mutations, like $T_{11} \rightarrow A$, $T_{11} \rightarrow C$, and $G_{13} \rightarrow C$, gave a 3- to 5.5-fold increase in light-dependent activity but not in dark.

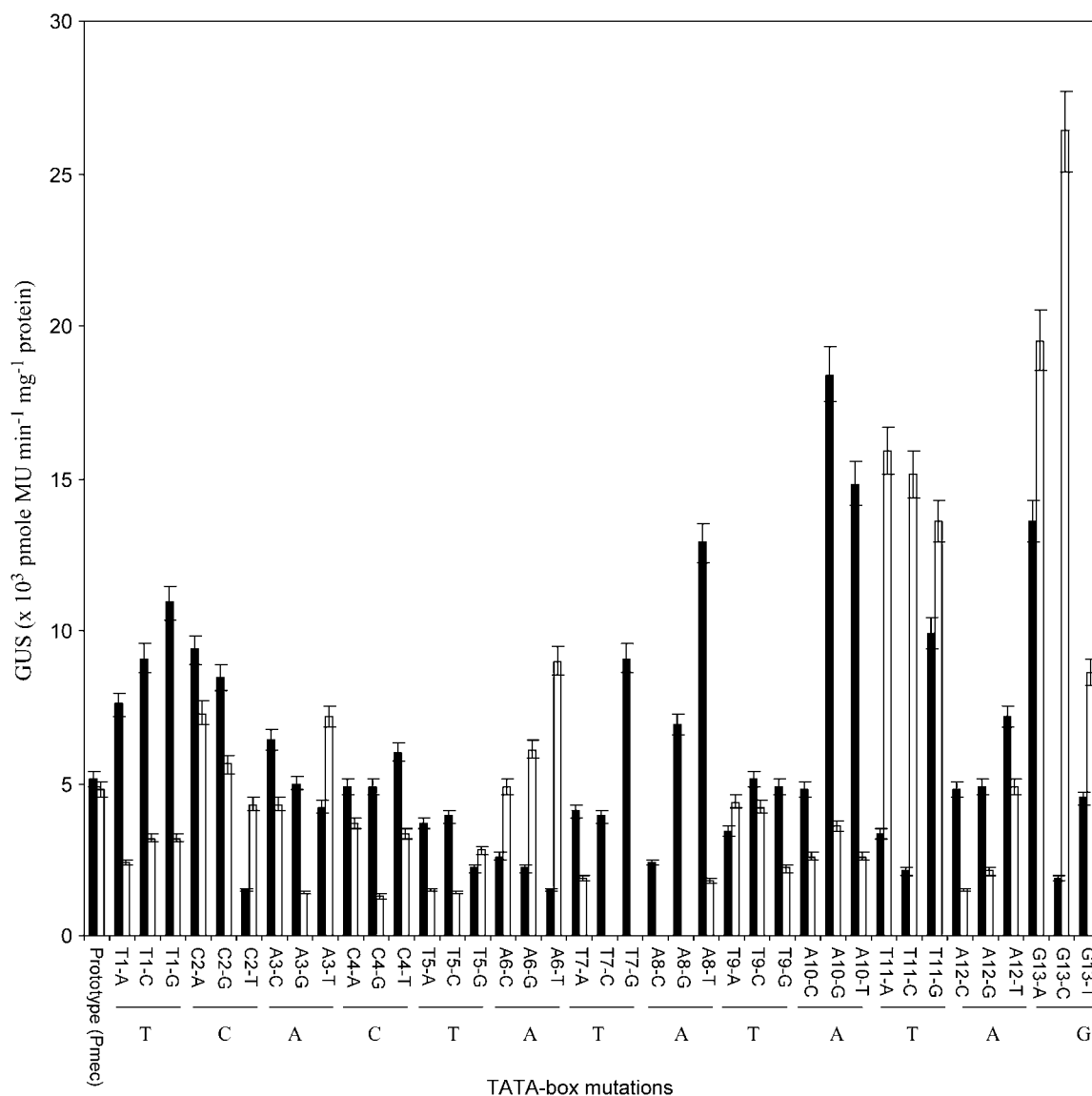


Figure 1. Light-related effect of the TATA-box mutations on promoter function. Each bar represents reporter gene (*gusA*) expression in the absence (black bars) and presence (white bars) of light. The x axis identifies the mutations for which GUS activities are shown. Each reading is an average of 18 to 24 leaf disc bombardments over three to four independent experiments.

Multiple Mutations in the TATA-Box Cause General Loss of Promoter Function

To examine the extent to which sequence distortions could be tolerated by the transcription machinery in dark, double mutations were constructed in a few selected cases. Table I shows that dark transcription, although less sensitive to single mutations, fell off completely in some of the double mutations. The $A_6 \rightarrow G$ mutation in the first TATA reduced dark-mediated expression by about 2-fold, whereas it gave a small increase in light-mediated promoter function. The second accompanying mutation at the third, seventh, or eighth position resulted in near-complete or complete failure of promoter function in both light and dark. The results suggest that expression from the promoter used

in this study is dependent on the TATA-box for expression in both light and dark. The results also point to the role of the previously unreported flanking motif TCAC in promoter function and its selectivity in combination with the TATA-box sequence. As noted in Table I, the $A_3 \rightarrow G$ mutation in TCAC by itself had no effect on dark expression, although it inhibited light expression. However, when accompanied by the $A_6 \rightarrow G$ mutation, the $A_3 \rightarrow G$ resulted in 10-fold lower and no expression, respectively, in dark and light.

The Seventh and Eighth Positions in the TATA-Box Are Critical to Transcription in Light

The actual level of the transcript was measured in a few representative cases by real-time PCR. The $T_7 \rightarrow C$,

Table I. Synergistic effect of the nucleotides at different positions in the TATA-box on the specificity of promoter expression

GUS values are averages \pm SD of data from three independent experiments comprising six to nine replicates each. The variation in GFP values expressed from the 35S promoter in the internal control were $34.05 \pm 4.56 \times 10^3$ RFU mg protein⁻¹.

Promoter Mutation	GUS Expression		
	Dark	Light	Ratio (Dark/Light)
	$10^3 \text{ pmol min}^{-1} \text{ mg protein}^{-1}$		
<i>Pmec</i>	5.40 \pm 1.10	5.00 \pm 1.10	1.08 \pm 0.22
A ₆ →G	2.40 \pm 0.45	6.49 \pm 1.29	0.36 \pm 0.06
A ₃ →G	4.87 \pm 1.11	1.52 \pm 0.33	3.20 \pm 0.62
T ₇ →G	9.15 \pm 1.92	0	∞
A ₈ →C	2.60 \pm 0.50	0	∞
A ₆ →G; A ₃ →G	0.48 \pm 0.09	0	∞
A ₆ →G; T ₇ →G	0	0	0
A ₆ →G; A ₈ →C	0	0	0

T₇→G, A₈→C, and A₈→G mutations were examined for the level of *gusA* transcript in light and dark vis-à-vis that for ubiquitin as an internal control. As seen in Table II, in light-incubated leaves, nearly no *gusA* transcript was formed in the case of promoters with any of the four single mutations. On the other hand, none of these mutations led to nonexpression of the *gusA* transcript when the leaf was incubated in dark. The results on transcript quantitation in the above four representative mutations therefore confirm that the relative levels of glucuronidase activity measured after microprojectile bombardment (Fig. 1) are an authentic reflection of the actual transcription.

Modulation of Gene Expression in Light by a TATA-Box Sequence in the Chromatin-Integrated State

It was important to establish whether the TATA-box sequence regulated gene expression selectively under light versus dark conditions even when the promoter was integrated in the chromatin. For this purpose, stable transgenic lines of tobacco were developed using minimal promoter-reporter constructs representing the selected mutations.

Pmec-Mediated Transcription in Light as Well as Dark Is Primarily TATA Dependent

The minimal promoter sequence *Pmec* used in this study is a typical core promoter having both the consensus TATA and a putative initiator sequence PyTCANTPyPy (Nakamura et al., 2002). To establish the contribution of these elements in transcription regulation of *Pmec*, we mutated both the TATA sequences in TCACTATATAG to GAGA in one case, and the initiator-like motif TTGTCATTCTC to AAGACAAAACAC (*inr*^m) in another case. The constructs *Pmec*-GAGA and *Pmec-inr*^m were then transformed in tobacco to obtain several independent transgenic lines. *gusA* expression was monitored in transgenic seed-

lings grown either continuously in dark or in a 16-h-light/8-h-dark cycle for a period of 11 d. As compared to the prototype promoter *Pmec*, transgenic lines with *Pmec*-GAGA showed almost no activity either in light- or dark-grown seedlings (Fig. 2). In contrast to the GAGA mutation, transgenic seedlings expressing *Pmec-inr*^m showed about a 36% decline in *gusA* activity in light-grown seedlings. Dark-grown seedlings with *inr*^m showed promoter activity similar to that of *Pmec*. The results indicate that *Pmec* used in this study is primarily a TATA-dependent promoter, although the *inr*-like element also contributes to light-mediated transcription.

Light-Specific Influence of the TATA-Box Sequence on Transcription Is Maintained in the Chromatin-Integrated State

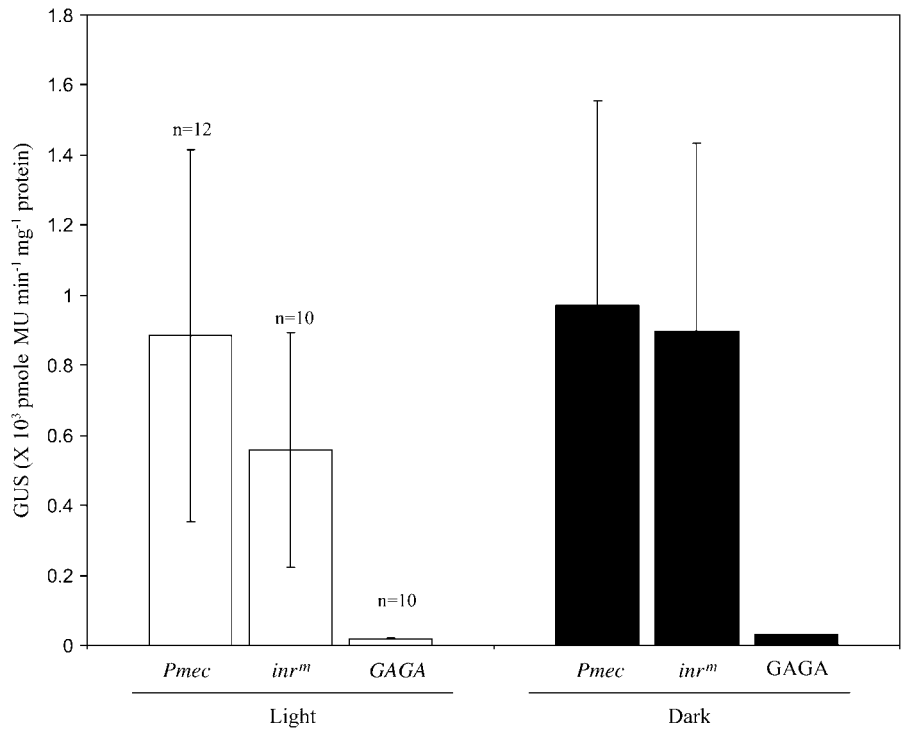
Figure 3 presents data on the expression of *gusA* in the leaves of several independent transgenic lines transformed with constructs carrying the prototype promoter *Pmec* or promoter mutations (i.e. T₇→G or A₈→C). Transgenic seedlings were grown either in continuous dark or a 16-h light cycle before estimation of GUS activity. Whereas *Pmec* showed no difference in the expression of *gusA* in light and dark, the mutants gave strikingly different results. In a majority of transgenic plants, the T₇→G as well as T₈→C mutations resulted in complete inhibition of promoter function in light. On the other hand, dark expression of the promoter was not affected significantly by any of the two mutations (Fig. 3). The results show that light-specific regulation of transcription by the TATA-box sequence was not overridden by the neighborhood genomic sequences in any of the 12 integration events. Thus, selective regulation of gene expression by TATA-box sequence per se exerts

Table II. Relative levels of *gusA* transcript in selected promoter mutations

Ratios of the transcripts are the average of three independent experiments, where *gusA* expression was normalized by internal ubiquitin gene expression. *gfp* gene expression was taken as a control for the bombardments. The values in the column on relative enzyme level have been derived from the data in Figure 1.

Serial No.	Promoter Mutation	Incubation Condition	Relative Transcript Level	Relative Enzyme Activity
Expression in Mutations at T ₇ in the TATA-Box				
1	<i>Pmec</i>	Light	1.00	1.00
		Dark	1.04 \pm 0.20	1.06
2	T ₇ →C	Light	0.01 \pm 0.00	∞
		Dark	0.79 \pm 0.16	0.81
3	T ₇ →G	Light	0.02 \pm 0.00	∞
		Dark	2.04 \pm 0.40	1.89
Expression in Mutations at A ₈ in the TATA-Box				
1	<i>Pmec</i>	Light	1.00	1.00
		Dark	0.91 \pm 0.18	1.06
2	A ₈ →C	Light	0.01 \pm 0.00	∞
		Dark	0.42 \pm 0.08	0.5
3	A ₈ →G	Light	0.02 \pm 0.00	∞
		Dark	1.68 \pm 0.32	1.44

Figure 2. Role of TATA-box and *inr*-like element in light-regulated expression of *Pmec* GUS activity in 11-d-old light-grown (white bars) or dark-grown (black bars) transgenic seedlings containing minimal promoter *Pmec* with the prototype sequence and its derivatives mutated in the *inr* (*inr^m*) and TATA-box (GAGA) regions. The values above the bars indicate the SD of three independent experiments. The numbers of independent transgenic lines (*n*) used in the analysis are indicated.



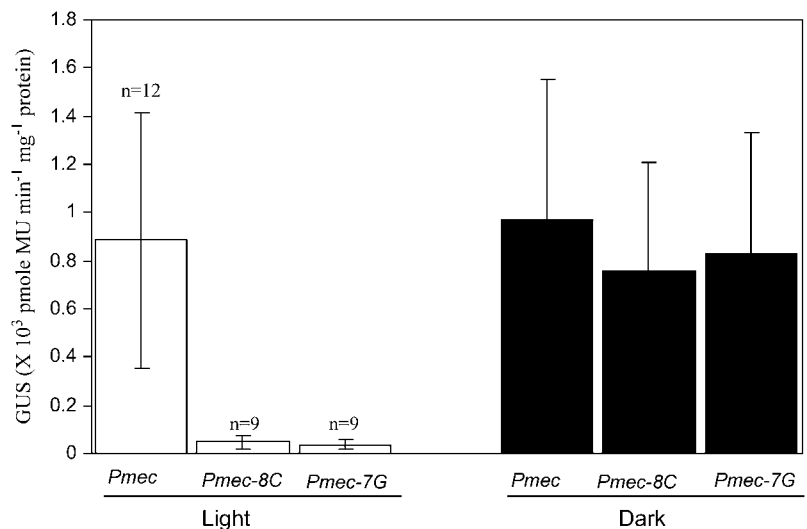
itself in spite of the chromatin structure and nonspecific sequences in the neighborhood in the integrated state. The above results suggested that certain sequences should preferentially be present in the TATA-box of promoters in genes that express in light.

TATA-Box-Dependent Modulation of Transcription by Light Is Not Overridden by the Light-Responsive Activator Element

To evaluate the contribution of the TATA-box sequence in the presence of the light-specific activator motif, we placed the 52-bp light-responsive activator

element (*lre*) fragment of the small subunit of Rubisco (*rbcS*) 8B gene of tobacco (Martinez-Hernandez et al., 2002) upstream of *Pmec* and some selected mutants. The 52-bp *lre* has been reported to confer light-specific activation of transcription to even heterologous promoters (Martinez-Hernandez et al., 2002). As expected, the *lre* fragment conferred typical light-dependent activation on the *Pmec* promoter in transgenic lines. The *lre* increased expression from *Pmec* severalfold in both light and dark, the increase being greater in light (compare Figs. 3 and 4A). *lre-Pmec* transgenic lines showed 2- to 3-fold higher expression in light as compared to the seedlings grown in dark (Fig. 4A).

Figure 3. Role of the seventh and eighth positions in the TATA-box in light-mediated expression of *Pmec*. GUS activities in 11-d-old light-grown (white bars) or dark-grown (black bars) transgenic seedlings containing the minimal promoter with prototype TATA (*Pmec*), mutation A8C (*Pmec-8C*), or T7G (*Pmec-7G*). The values above the bars indicate the SD of three independent experiments.



The relationship between the activation by *lre* and the sequence of the TATA-box was further analyzed by placing the *lre* fragment upstream of *Pmec* with the 7G and 8C mutations in transgenic tobacco lines. Expression studies were carried out on dark etiolated seedlings and those grown for 11 d in a 16-h light cycle. As depicted in Figure 4A, both mutations resulted in a 7- to 14-fold decrease in the expression of *gusA* specifically in light-grown seedlings. However, expression of *gusA* was unaffected in dark-grown seedlings regardless of the two mutations. Semiquantitative reverse transcription (RT)-PCR was performed on several independent transgenic lines carrying *lre-Pmec* with the 7G or 8C mutations to establish a correlation between reporter gene expression and the level of transcripts. The results in Figure 4B show that the *lre-Pmec* (lanes 2–7) results in

formation of the *gusA* transcript, whose levels are in proportion to reporter enzyme activity in the seedlings. In contrast, the mutant *lre* lines showed no transcript in semiquantitative PCR (Fig. 4B, lanes 8–12) in agreement with the very poor GUS activity. Thus, the seventh and eighth positions in the canonical TATA-box sequence influence transcription in light even in the presence of *lre*. In contrast, these positions do not significantly affect transcription from *Pmec* in the dark.

Light-Related Transcription Regulation by TATA-Box Sequence Is Mainly Phytochrome Specific

Specificity of TATA-box sequence-dependent transcription regulation was examined further in monochromatic light. The results for one transgenic line of

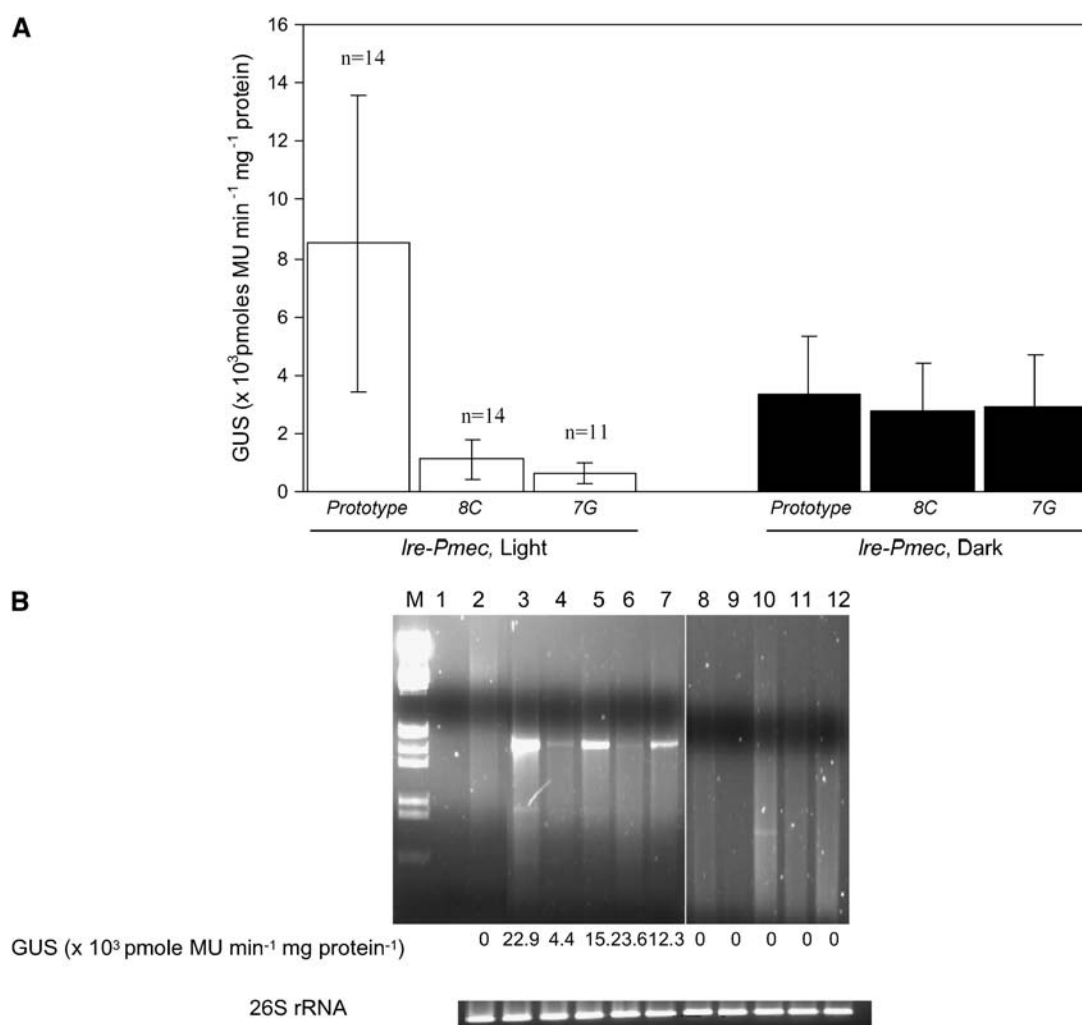


Figure 4. Role of the seventh and eighth positions in the TATA-box in light-dependent, *lre*-activated transcription from *Pmec*. **A**, GUS activities in 11-d-old light-grown (white bars) or dark-grown (black bars) seedlings containing *lre-Pmec* with the prototype TATA-box (*lre-Pmec*) and its variants with mutations A8C and T7G. The values above the bars indicate the SD from the means of three independent experiments. **B**, Semiquantitative RT-PCR carried out on five light-grown transgenic lines of *lre-Pmec* (lanes 3–7) and *lre-Pmec-8C* (lanes 8–12). Lanes 1 and 2 represent nontemplate control and semiquantitative RT-PCR of non-transformed tobacco, respectively. The values below the lanes indicate GUS activities in respective transgenic lines. The 26S rRNA levels detected by semiquantitative PCR are shown for comparison.

lre-Pmec and of *lre-Pmec* 8C are shown in Figure 5. A typically contrasting expression was noticed in light and dark (Fig. 5, L/D). Seedlings were germinated either in continuous dark or in a 16-h light regime. For the monochromatic study, plants were germinated in dark for 9 d and exposed to specific monochromatic light, namely, red, far-red, or blue light for 2 d before determining GUS activity. As expected (Martinez-Hernandez et al., 2002), *lre* responds to various monochromatic light by showing activation (Fig. 5). As compared to the mutant *lre-Pmec* 8C transgenic line, the *lre-Pmec* transgenic line showed 55-, 4.7-, and 3.3-fold higher expression in white, red, and far-red light, respectively. However, expression of both lines was comparable in dark (0.87-fold) and blue (1.27-fold) light. Activation by red light was most inhibited in the presence of the 8C mutation in *Pmec*. The inhibitory effect was not seen in blue light. Thus, TATA-box sequence-dependent transcription regulation is more specific to red/far-red light and thus indicative of phytochrome-specific transcription regulation.

Light-Dependent Nucleoprotein Complex Formation on the Core Promoter Is Inhibited by Specific TATA-Box Mutations

To study the mechanism by which mutations at the seventh and eighth positions may inhibit transcription in light (and not in dark), electrophoretic mobility shift assays were carried out using nuclear protein extracted from either light-grown (NE^L) or dark-grown (NE^D) seedlings. At the optimal DNA to NE^L ratio, the oligonucleotide containing the prototype TATA-box sequence made a discrete nucleoprotein complex (Fig. 6A). The discrete complex formation occurred as we increased the protein concentration by 5-fold more than the starting protein concentration (lanes 6–8). A

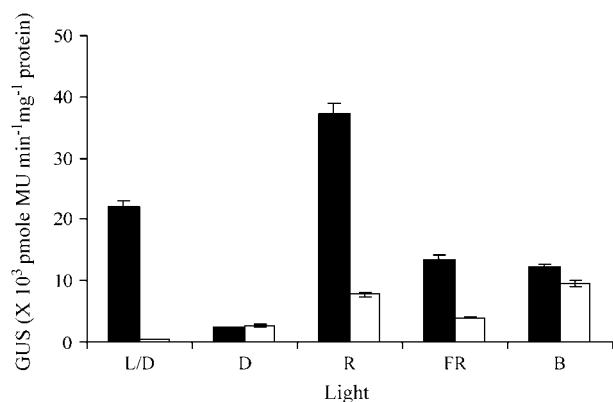


Figure 5. Effect of quality of light in TATA-box-dependent gene expression from *Pmec* GUS activities in the transgenic line of *lre-Pmec* (black bars) and *lre-8C* (white bars) constructs grown in dark for 9 d and then exposed for 2 d of different light conditions (i.e. 16 h white light/8 h dark [L/D], continuous dark [D], continuous red [R], continuous far-red [FR], and continuous blue [B] light). Intensities of the WL, R, FR, and B irradiation were 80, 10, 0.86, and 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively.

further increase in NE^L resulted in the disappearance of the specific complex because, under such conditions, larger nonspecific complexes that did not enter the agarose gel were formed. That the discrete complex seen was a functional transcriptional complex was established by various approaches. First, under incubation conditions, in vitro transcription gave a single major transcript (Supplemental Fig. 1). Second, the distinct promoter-protein complex disappeared on competition with molar excess of *Pmec* oligo (Fig. 6B), resulting in the reappearance of the free oligonucleotide. Titration with random nucleotides, poly(dG-dC), poly(dA-dC), or salmon sperm DNA did not result in such dissociation (data not shown). To establish that the complex formed was indeed TATA-box dependent, we completed the complex with the oligo containing only the canonical TATA-box sequence (multimer of TCACTATATAG). Somewhat unexpectedly, the concentration of the complex increased with the increase in competitor TATA from 5- to 50-fold (Fig. 6C). However, when the competitor oligo was increased further, it completely abolished the complex at 500-fold molar excess (Fig. 6C). The results established that the complex formed on *Pmec* was a TATA-box-dependent complex. The addition of polyclonal anti-TATA-binding protein (TBP) antibodies to the incubation mixture resulted in a supershift (Fig. 6B, lanes 9–11) of the complex. Thus, the nucleoprotein complex seen on the agarose gel represented a transcriptionally relevant TATA-box/TBP-containing complex.

The results of titration of the NE^L and NE^D with the promoter containing the prototype TATA-box are shown in Figure 7, A and B, respectively. In both cases, a discrete TBP-TATA complex was formed and maintained because the nuclear extract was increased by about 5-fold. However, contrasting results on the complex formation were obtained when promoter mutations were used. Nuclear extract from light-grown seedlings showed weak complex formation with the TATA-box oligonucleotide containing the $T_7 \rightarrow C$ mutation (Fig. 7C). A diffused complex was formed, which disappeared into nonspecific larger complexes that do not enter the agarose gel at higher concentrations of NE^L . With the oligo containing the $A_8 \rightarrow G$ mutation, the NE^L failed to form a specific complex (Fig. 7E). On the other hand, in the case of the dark-grown extract NE^D , a specific nucleoprotein complex was formed with the TATA-box containing either of the two mutations (Fig. 7, D and F). The results suggest that the nuclear preparations from the seedlings grown in the absence or presence of light have different sequence requirements for the formation of transcriptional complexes on the TATA-box.

Transcription Activation by Light Operates from a Novel Initiation Site

To probe further into mechanistic differences operating on the minimal promoter during transcription in light and dark, we mapped the transcription start site.

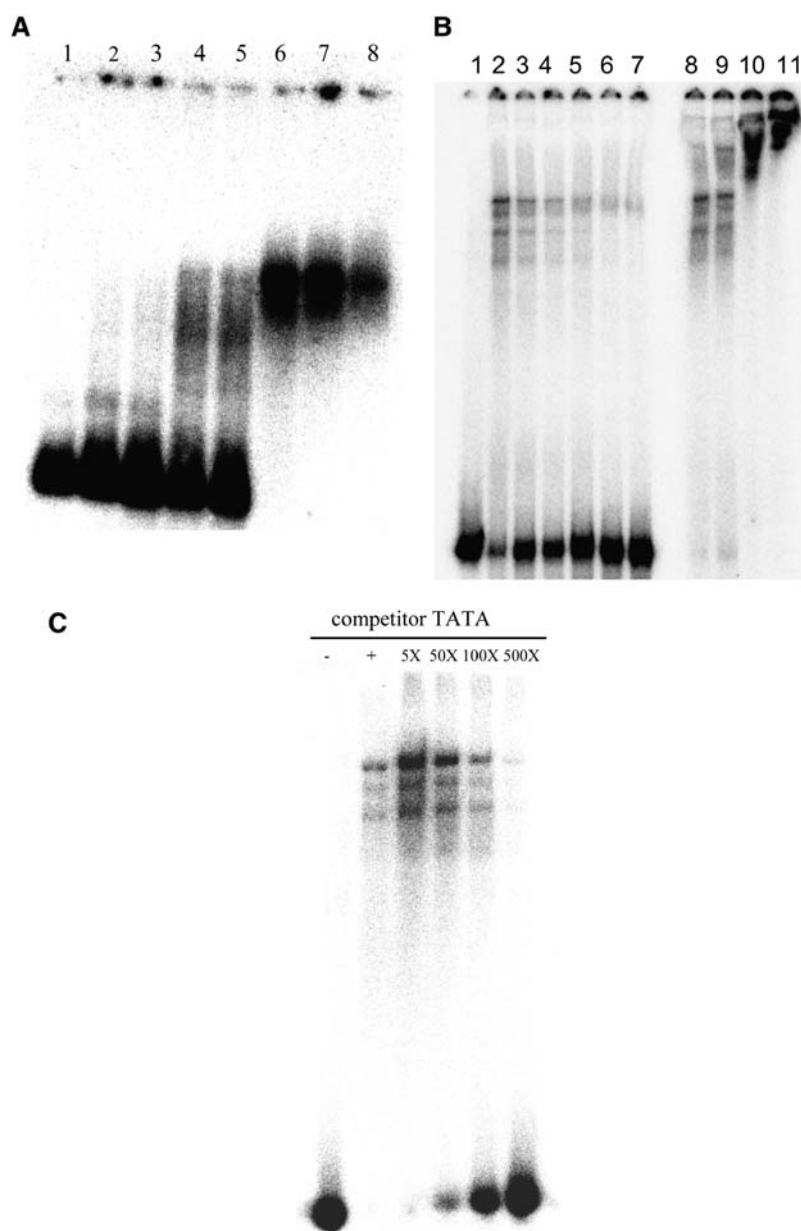


Figure 6. Autoradiograph of 2% agarose (A) and 5% PAGE (B and C) showing nucleoprotein complex formation on ^{32}P -labeled 120-bp *Pmec* oligo. A, In the presence of 0, 5, 10, 50, 100, 500, and 1,000 ng of NE^{I} protein per 20 μL of incubation mixture (lanes 1–8). B, In the presence of 500 ng of NE^{I} at 0-, 2-, 5-, 10-, 50-, and 100-fold molar excess of unlabeled 120-bp *Pmec* oligo (lanes 2–7). Lanes 8 to 11 show a supershift of the complex following addition of 0.5, 1.0, 2.0, and 5.0 μL of anti-TBP antibody. C, In the presence of 500 ng of NE^{I} at 0-, 5-, 50-, 100-, and 500-fold molar excess (lanes 2–6) of unlabeled multimerized TATA-box sequence. Lane 1 shows the position of the free ^{32}P -labeled 120-bp *Pmec* oligo.

We carried out RNA ligation-mediated (RLM)-RACE to map the transcription start site in several *lre-Pmec* transgenic lines grown in light or dark. A major transcription start site (Fig. 8, arrow) was obtained in light-grown seedlings. In the case of etiolated seedlings, we obtained another transcript two nucleotides downstream of transcription site (*) obtained in light-grown seedlings.

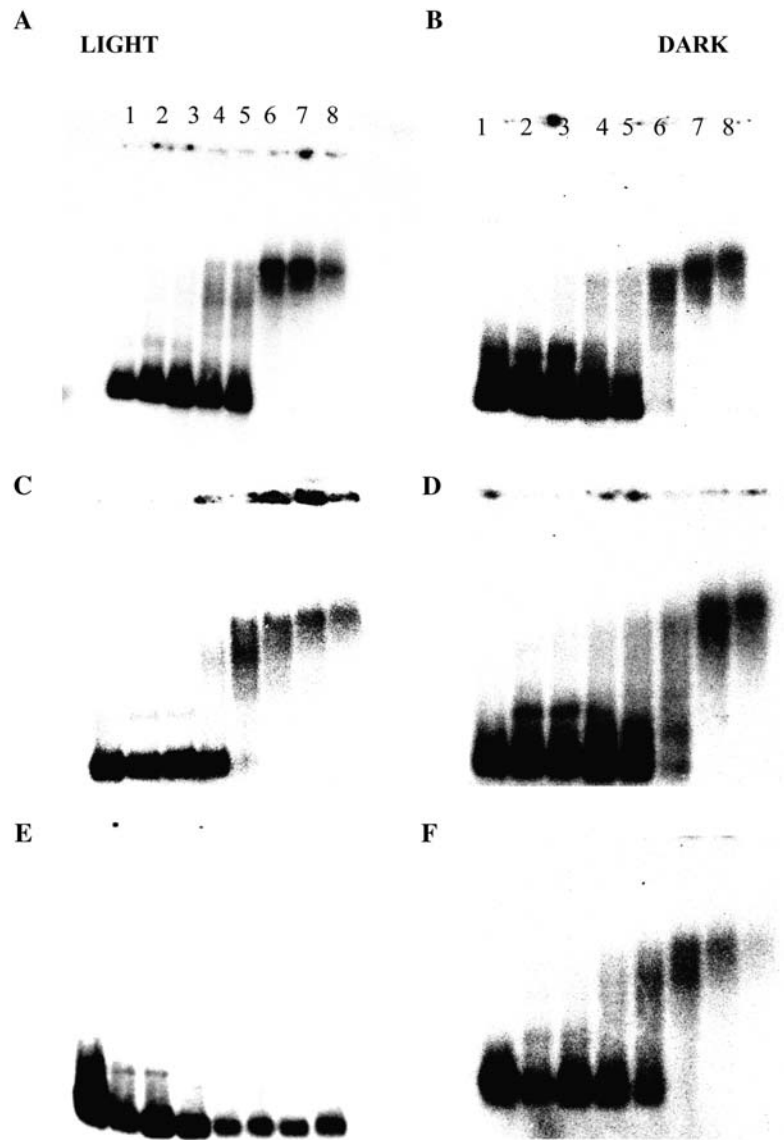
To evaluate whether differences in transcription site in the case of light and dark conditions is a general mechanism operating on light-activated promoters, we mapped a transcription start site in the *rbcS 8B* gene of tobacco from light-grown and etiolated seedlings. In contrast to the results obtained on *lre-Pmec*, the *rbcS 8B* gene showed a single major transcript in light and a weak transcript at the same position in

etiolated seedlings (data not shown). The results suggest that the mechanism that operates during light and dark might be context dependent and might selectively operate on some, but not all, light-regulated promoters.

DISCUSSION

Our study shows that several single mutations in the prototype TATA-box (inclusive of the immediately flanking sequences) are largely tolerated in vivo by leaf transcriptional machinery. Transcription in the dark was particularly more tolerant to several mutations that resulted in substantial to complete failure in reporter gene expression in light. This is in contrast

Figure 7. Light-related specificity of the TATA-box sequence in complex formation with nuclear extract. The formation of complexes using 0 to 1,000 ng of nuclear protein (lanes 1–8) prepared from light-grown (left) and dark-grown (right) seedlings is shown. Shown is the mobility shift in the promoter oligo containing the prototype TATA-box without T7C (A and B) and with T7C (C and D) and A8G (E and F) mutations.



to earlier studies based on *in vitro* transcription (Mukumoto et al., 1993; Yamaguchi et al., 1998), which suggested that the function of the TATA-box region was highly vulnerable to single mutations. Differences in the results may be because certain factors involved in the stabilization of TATA-TBP interaction *in vivo* may be absent in *in vitro* transcription systems. Intracellularly, TBP is maintained in a dynamic state of equilibrium with other protein complexes, like TFIID (Coleman et al., 1999), where it is associated with a variety of TBP-associating factors (TAFs). Some of the TAFs stabilize TATA-TBP interactions directly or indirectly in association with other general transcription factors (Wassarman and Sauer, 2001). Complexes like TFIID-TATA are rate limiting for transcription *in vivo* (Colgan and Manley, 1992) and are likely to be limiting or absent in nuclear extracts used for *in vitro* transcription. Hence, the results on *in vitro* and *in vivo* transcription could be different, as noted in this study.

The results establish that the prototype minimal promoter *Pmec* is a typical TATA-box-dependent promoter. None of the 10 transgenic lines with the TATA-box mutated to GAGA showed significant GUS activity. Thus, GUS activity expressed from the variants containing the TATA-box mutations reflects true behavior of the TATA-box in the promoters. The *inr*-like sequence also contributed to light-mediated expression of *Pmec* but failed to activate transcription significantly in the absence of a TATA-box. This is in agreement with Nakamura et al. (2002), who reported the importance of the *inr* region in light-mediated regulation of *psaDb* in tobacco.

High vulnerability of transcription in light-incubated leaf to mutations in the first TATA-box largely agrees with the above *in vitro* studies and with other mutational and TATA-TBP interaction studies in yeast (*Saccharomyces cerevisiae*; Steven et al., 2002). Light-dependent transcription from the core promoter

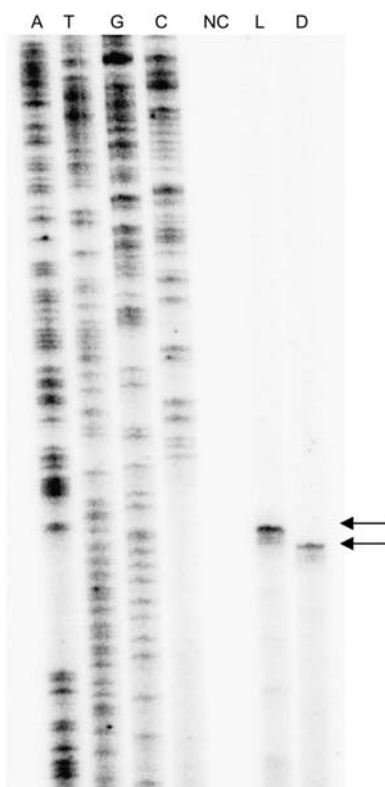


Figure 8. Mapping of point of transcript initiation from *Ire-Pmec*. Shown is primer extension analysis conducted with ^{32}P -labeled PE1 primer using RLM-RACE. Lanes A, T, G, and C represent sequencing ladders with the primer. Lanes L and D represent primer extension of the transcripts formed in the *Ire-Pmec* transgenic line grown in light and dark, respectively. Lane NC represents the control lane (i.e. nontransgenic plant).

appears to require TATA-box architecture different from that required by the transcriptional machinery that functions in the dark. Some of the substitutions ($T_{11}A$, $T_{11}C$, $G_{13}C$) in the flanking positions showed light-specific stimulation of transcription. Others, like $G_{13}A$, resulted in general improvement in promoter function. Footprinting studies using *in vitro* and *in vivo* systems in eukaryotes have shown that the points of contact of the TFIID complex extend well beyond the core TATA region (Nakajima et al., 1988; Purnell and Gilmour, 1993). Some of the TAFs have been reported to interact with the sequences flanking TATA and thus stabilize transcription. For example, TFIIB interacts with the TATA-box 5'-flanking sequences (Wolner and Gralla, 2001) and stabilizes the TBP-TFIIA complex on the TATA-box (Pan et al., 2000).

Our results suggest that the TATA-box flanking sequences are involved in *in vivo* stability of the pre-initiation complex (PIC) as well as specificity of gene expression. Double mutations, including the sequences flanking the TATA-box, reduced transcription synergistically. Hence, a larger context in the TATA-box region determines the performance of the core promoter. For example, as seen in Table I, TGTA in a

sequence context, TCGCTGTA, is more deleterious to promoter function than that in a different context, like TCACTGTA. Earlier, TGTA has been reported to inactivate promoter function in *Arabidopsis* (*Arabidopsis thaliana*; Pan et al., 2000). In that study, the TATA-box of the cauliflower mosaic virus 35S promoter was mutated from CCTCTATA to CCTCTGTA. However, a mutated TATA-box binding protein, TBPm3, was reported to recognize the promoter containing the TGTA motif (Strubin and Struhl, 1992). Our results show that a change in the context of the TATA-box can also alter the specificity and functional interactions of the core promoter with components of the transcriptional complex. Thus, given a different context, the core promoter containing TGTA can also be functionally active.

Light-specific failure in expression of the minimal promoter *Pmec* with C and G at the seventh or eighth positions was neither affected by chromatin context nor the light-specific activator motif placed upstream. The results were completely reproducible in independent transgenic lines. Although GUS activity varied significantly in different transgenic lines due to the positional effect, the promoter-specific differential transcription in light versus dark conditions was noticed in all cases. A similar case occurred when we cloned the *lre* from the *rbcS 8B* promoter upstream of either the prototype *Pmec* or the mutants 7G and 8C. As expected, the *lre* rendered light activation to *Pmec*. The activation was abolished when the *lre* was cloned upstream of the TATA-box with 7G or 8C mutations. Our results suggest that the expression of *Pmec* in light and dark happens by different mechanisms operating on the minimal promoter.

The light-specific response of the TATA-box mutations seems to be phytochrome A (PhyA) and phytochrome B (PhyB) dependent because expression from the 8C mutation was substantially compromised in red and far-red light, but not in blue light. The results reflect that the TATA-box-specific expression obtained with *Pmec* is primarily PhyA and PhyB dependent and may involve synergistic action because inhibition was greater in white light (composed of both red and far-red components) as compared to the individual monochromatic light. Such cooperative inhibition due to *PhyA* and *PhyB* has been reported for light-dependent promoters like *TUB1* and *lhcb* in the *phyA phyB* double-mutant background in *Arabidopsis* (Leu et al., 1995).

Our results suggested that certain sequences containing a G or a C at the third and fourth positions in the TATA-box of promoters with double TATA may preferentially be present in genes whose expression is suppressed in light. To test this hypothesis, we generated an *Arabidopsis* minimal promoter database containing 30,975 entries. This database was searched for the occurrence of TATA variants TAGATATA, TACA-TATA, TATGTATA, and TATCTATA within the first 50 nucleotides upstream of the reported transcription start site. The above variants in TATATATA were present in 166 *Arabidopsis* genes (Supplemental Sequence Data 1). However, only 44 of these 166 genes

RNA Extraction and RT

Total RNA was isolated from the bombarded leaves by TRIzol LS reagents (Invitrogen Life Technologies). After DNase treatment, the integrity of RNA was tested by electrophoresis. Total RNA (1 μg) was used for first-strand cDNA synthesis. A 20- μL reaction mixture consisting of 2 μL 10 \times incubation buffer, 2.5 μM primer [poly(dT)], 500 μM dNTPs, 5.5 mM MgCl_2 , 0.4 units RNase inhibitor, and 1.25 units Multiscribe reverse transcriptase (TaqMan reverse transcription reagents kit; PE Biosystems) was incubated at 42°C for 1 h, heat inactivated at 70°C for 10 min, and used for real-time PCR.

Real-Time PCR

Real-time PCR was carried out using an ABI Prism 7000 sequence detection system (TaqMan; Perkin-Elmer/PE Biosystems). Each multiplex PCR mixture contained 12.5 μL of TaqMan master mixture, an amount of cDNA equivalent to 200 ng total RNA, 20 μM *gusA* forward and reverse primers, 12.5 μM ubiquitin forward and reverse primers, 5.6 μM *gusA*, and 0.7 μM ubiquitin probes. The oligonucleotides and probes were supplied by PE Biosystems. The relative values for quantitation of RNA are expressed as $2^{-\Delta\Delta\text{Ct}}$, where ΔCt represents the difference between the threshold cycle (Ct; reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold) value of a sample and that of ubiquitin (endogenous control) in the same sample, and $\Delta\Delta\text{Ct}$ is the difference between the ΔCt value of a sample and that of the prototype promoter.

Plant Material and Transient Transformation Assay to Study Promoter Expression

Leaves from the third or fourth node of 9- to 10-week-old tobacco plants were used to study expression of the reporter *gusA* gene from different promoter cassettes. These were surface sterilized with 0.1% HgCl_2 , washed, and incubated on Murashige and Skoog agar medium (Murashige and Skoog, 1962) at 25°C in dark or continuous light (irradiance 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) provided by cool-white fluorescent tube lights for 24 h before bombardment with DNA-coated gold microprojectiles. The DNA was bombarded at 1,100 lbs cm^{-3} pressure using a PDS 1000 He machine (Bio-Rad). Ten micrograms of DNA were coated on 1.5-mg gold particles and used for the bombardment of three discs of tobacco leaves taken as replicates for each treatment. Each experiment was repeated at least three times. After bombardment, the leaves were placed on fresh Murashige and Skoog agar medium and incubated in continuous light or dark as desired for a total period of 48 h before estimating the expression of GUS using the fluorogenic substrate MUG (Jefferson and Wilson, 1991). Total soluble protein in leaf extracts was quantified using Bradford reagent (catalog no. 500-0006; Bio-Rad Laboratories). Enzyme activity was expressed as pmol MU released $\text{min}^{-1} \text{mg}^{-1}$ soluble protein.

Tobacco Transformation

The desired TATA-box-*gusA* cassettes or their corresponding counterparts with *lre* were cloned at the *Bam*HI site in T-DNA in the binary vector pBI101. The recombinant vector plasmids were mobilized into *Agrobacterium tumefaciens* strain LBA4404 by electroporation. Leaf-disc explants were transformed following the protocol of Horsch et al. (1985). Several independently transformed plants were developed with each construct. Transgenic plants were grown in a 16-h light/dark cycle. The number of independent transgenic lines (*n*) tested in a given experiment are specified in the figures.

Growth Conditions and GUS Assays

After transformation, seeds of the R_0 generation were collected and homozygous lines were obtained through kanamycin selection. Seeds of homozygous lines from the R_1 generation were used for further study. For dark versus light experiments, seeds were grown in a 16-h-dark/8-h-light cycle or in complete darkness for 11 d. For light induction experiments, 9-d-old dark-grown seedlings were exposed to white light (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$), far-red light (0.86 $\mu\text{mol m}^{-2} \text{s}^{-1}$), red light (10 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and blue light (15 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 2 d before measurement of GUS activity.

Nuclear Protein Preparation

Nuclear protein from tobacco leaves was prepared from plants grown in a 16-h light/dark cycle. The plants were then incubated for 72 h in continuous

light (NE^{L}) or dark (NE^{D}). Following isolation of intact nuclei, protein was extracted in high ionic strength buffer and concentrated by ammonium sulfate precipitation. A procedure modified from Green et al. (1987) was used.

Tobacco leaf tissue was washed thoroughly with chilled distilled water. It was powdered in a mortar and pestle in liquid nitrogen and extracted in homogenization buffer (1 M hexylene glycol, 10 mM PIPES/KOH, pH 7.0, 10 mM MgCl_2 , 0.5% [v/v] Triton X-100, 5 mM 2-mercaptoethanol, and 0.8 mM phenylmethylsulfonyl fluoride [PMSF]). The homogenized extract was filtered sequentially through four layers of muslin cloth, followed by 80-, 60-, 40-, and 20- μm nylon mesh. Nuclei were pelleted at 3,000g for 10 min and resuspended gently with a soft paintbrush in wash buffer (0.5 M hexylene glycol, 10 mM PIPES/KOH, pH 7.0, 10 mM MgCl_2 , 5 mM 2-mercaptoethanol, 0.5% [v/v] Triton X-100, and 0.8 mM PMSF). Nuclei were pelleted at 3,000g for 15 min, the pellet was resuspended in 5% Percoll buffer (0.5 M Suc, 25 mM Tris, pH 8.0, 10 mM MgCl_2 , 0.8 mM PMSF, 0.5% Triton X-100, 5 mM 2-mercaptoethanol, 5% Percoll) and loaded on to 40% to 80% Percoll step gradient containing a 2 M Suc pad at the bottom. The nuclei were collected from the 80% Percoll-2 M Suc interface, washed in the wash buffer without Triton X-100, and resuspended in lysis buffer (110 mM KCl, 15 mM HEPES/KOH, pH 7.5, 5 mM MgCl_2 , 1 mM dithiothreitol [DTT], and 5 $\mu\text{g mL}^{-1}$ leupeptin). One-tenth volume of 4 M ammonium sulfate was added and placed on a rocker for 30 min. Chromatin and particulate matter were pelleted at 100,000g for 45 min. Ammonium sulfate powder was added at 0.3 g mL^{-1} to the supernatant to precipitate the protein. The protein pellet was suspended in nuclear extract buffer (40 mM KCl, 250 mM HEPES/KOH, pH 7.5, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 5 $\mu\text{g mL}^{-1}$ leupeptin) and subjected to dialysis for 2 h in the same buffer.

Electrophoresis Mobility Shift Assay

Electrophoresis mobility shift assays with nucleoprotein prepared from tobacco leaves were performed using 120-bp-long double-stranded oligonucleotides (*Pmec* without the coding sequence of *gusA*, as shown in the Construction of Cassettes section). The binding reactions were performed in a buffer containing 20 mM HEPES/KOH, pH 7.5, 40 mM KCl, 1 mM EDTA, 10% glycerol, and 0.5 mM DTT. As nonspecific carrier DNA, 5 μg of poly(dG-dC) was included in the binding reaction. Seventy-three fmol of the desired ^{32}P -labeled double-stranded oligonucleotide were incubated with 5 ng to 1 μg of tobacco nucleoprotein at 25°C for 1 h, loaded onto 2% agarose gel or 4% PAGE, and electrophoresed in 0.5 \times Tris-borate/EDTA buffer at 200 V or 25 W, respectively, at 4°C.

Generation of Polyclonal Anti-Tobacco TBP Antibody

Total RNA was isolated from tobacco leaves, reverse transcribed using SuperScript II reverse transcriptase (GIBCO-BRL) to obtain cDNA, which was used to amplify the tobacco TBP gene. The TBP was cloned in pET 19b expression vector and expressed in *Escherichia coli* strain BL-21 as per standard protocol (Novagen PET manual). Most of the TBP expressed in this way formed inclusion bodies. The protein was solubilized using 8 M urea in the buffer. After solubilization, the protein was loaded onto nickel-nitrilotriacetic acid agarose column. The eluted protein was dialyzed to remove urea using urea step gradient. After the final step of dialysis, some protein still remained soluble and was removed by centrifugation. Two milligrams of purified protein were given to Bangalore Genei Pvt. Ltd. for generation of polyclonal antibodies in rabbit. The recognition of tobacco-TBP by antibody was confirmed through western blotting and ELISA assay using tobacco nuclear extract (data not shown).

RLM-RACE to Map *gusA* Transcripts of *lre-Pmec* Transgenic Lines in Light and Dark Conditions

Total RNA of *lre-Pmec* transgenic seedlings was used to perform RLM-RACE using a kit from Ambion. PCR was performed on cDNAs using the gene-specific primer GSP₁ (5'-TAGTCTGCCAGTTCAGTTCGFT-3') and the 5'-RACE outer primer supplied with the kit. A second round of PCR was performed with the product of the first PCR using a nested gene-specific primer (5'-GCTCCATCACTTCCTGATTATT-3') coupled with the 5'-RACE inner primer supplied with the kit. The products of RACE reaction were confirmed with three sets of primers. Primer extension was then performed using PCR products of the second RACE reaction with ^{32}P -labeled primer

(from +94 to +1 of the *gusA* gene). The 5' ends were identified by electrophoresis of the primer extension product alongside sequencing fragments obtained with the same primer.

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LITERATURE CITED

- Bertrand C, Benhamed M, Li YF, Ayadi M, Lemonnier G, Renou JP, Delarue M, Zhou DX (2005) *Arabidopsis* HAF2 gene encoding TATA-binding protein (TBP)-associated factor TAF1 is required to integrate light signals to regulate gene expression and growth. *J Biol Chem* **280**: 1465–1473
- Breathnach R, Chambon P (1981) Organization and expression of eukaryotic split genes coding for proteins. *Annu Rev Biochem* **50**: 349–383
- Butler JEF, Kadonaga JT (2001) Enhancer-promoter specificity mediated by DPE or TATA core promoter motifs. *Genes Dev* **15**: 2515–2519
- Coleman RA, Taggart AK, Burmam S, Chicca JJ II, Pugh BF (1999) TFIIA regulates TBP and TFIID dimers. *Mol Cell* **4**: 451–457
- Colgan J, Manley JL (1992) TFIID can be rate limiting in vivo for TATA-containing, but not TATA-lacking, RNA polymerase II promoters. *Genes Dev* **6**: 304–315
- Dikstein R, Zhou S, Tjian R (1996) Human TAFIII105 is a cell type specific TFIID subunit related to hTAFIII130. *Cell* **67**: 137–146
- Duan ZJ, Fang X, Rohde A, Han H, Stematoyannopoulos G, Li Q (2002) Developmental specificity of recruitment of TBP to the TATA-box of the human γ -globin gene. *Proc Natl Acad Sci USA* **99**: 5509–5514
- Freiman RN, Albright SR, Chu LE, Zheng S, Hong-Ert L, Liang Sha WC, Tjian R (2002) Redundant role of tissue-selective TAFII 105 in lymphocytes. *Mol Cell Biol* **22**: 6564–6572
- Green PJ, Kay SA, Chua N-H (1987) Sequence-specific interactions of pea nuclear factor with light-responsive elements upstream of the *rbcs-3A* gene. *EMBO J* **6**: 2543–2549
- Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT (1985) A simple and general method for transferring genes into plants. *Science* **227**: 1229–1231
- Jefferson RA, Wilson KJ (1991) The GUS gene fusion system. In S Gelvin, R Schilperoot, eds, *Plant Molecular Biology Manual*, Vol B 14. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–33
- Joshi CP (1987) An inspection of the domain between putative TATA-box and translation start site in 79 plant genes. *Nucleic Acids Res* **15**: 6643–6653
- Leu W, Cao X, Wilson T, Snustad DP, Chua N-H (1995) Phytochrome A and phytochrome B mediate the hypocotyl-specific downregulation of TUB1 by light in *Arabidopsis*. *Plant Cell* **7**: 2187–2196
- Martinez E (2002) Multi-protein complexes in eukaryotic gene transcription. *Plant Mol Biol* **50**: 925–947
- Martinez-Hernandez A, Lopez-Ochoa L, Arguello-Astroga G, Herrera-Esterella L (2002) Functional properties and regulatory complexity of a minimal RBCS light-responsive unit activated by phytochrome, cryptochrome, and plastid signals. *Plant Physiol* **128**: 1223–1233
- Metsis M, Branhorst A, Neuman T (2001) Cell-type-specific expression of the TFIID component TAF(II)135 in the nervous system. *Exp Cell Res* **269**: 214–221
- Mukumoto F, Hirose S, Imaseki H, Yamazaki KI (1993) DNA sequence requirement of a TATA element binding protein from *Arabidopsis* for translation in vitro. *Plant Mol Biol* **23**: 995–1003
- Murashige T, Skoog F (1962) A revised method for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* **15**: 473–497
- Nakajima N, Horikoshi M, Roeder RG (1988) Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA-box-promoter interactions of TFIID. *Mol Cell Biol* **8**: 4028–4040
- Nakamura M, Tsunoda T, Obokata J (2002) Photosynthesis nuclear genes generally lack TATA-boxes: a tobacco photosystem I gene responds to light through an initiator. *Plant J* **29**: 1–10
- Pan S, Verner EC, Gurley WB (2000) Role of the TATA binding protein transcriptional factor IIB interaction in supporting basal and activated transcription in plant cells. *Plant Cell* **12**: 125–136
- Perera LP (2000) The TATA motif specifies the differential activation of minimal promoter by Varicella zoster virus immediate-early regulatory protein IE62. *J Biol Chem* **275**: 487–496
- Purnell BA, Gilmour DS (1993) Contribution of sequences downstream of the TATA element to protein-DNA complex containing the TATA-binding protein. *Mol Cell Biol* **13**: 2593–2603
- Roeder RG (1996) The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem Sci* **21**: 327–335
- Sawant SV, Kiran K, Singh PK, Tuli R (2001) Sequence architecture downstream of the initiator codon enhances gene expression and protein stability in plants. *Plant Physiol* **126**: 1630–1636
- Sawant SV, Singh PK, Gupta SK, Madanala R, Tuli R (1999) Conserved nucleotide sequence in highly expressed genes in plants. *J Genet* **78**: 123–131
- Sawant SV, Singh PK, Tuli R (2000) Pretreatment of microprojectiles to improve the delivery of DNA in plant transformation. *Biotechniques* **29**: 246–248
- Shahmuradov IA, Gammerman AJ, Hancock JM, Bramley HP, Solovvey YV (2003) Plant Prom: a database of plant promoter sequences. *Nucleic Acids Res* **31**: 114–117
- Smale ST (2001) Core promoters: active contributors to combinatorial gene regulation. *Genes Dev* **15**: 2503–2508
- Steven LS, Krassimira AG, Weil PA (2002) Molecular characterization of *Saccharomyces cerevisiae* TFIID. *Mol Cell Biol* **22**: 6000–6013
- Stewart JJ, Stargell LA (2001) The stability of the TFIIA-TBP-DNA complex is dependent on the sequence of the TATAAAA element. *J Biol Chem* **276**: 30078–30084
- Strubin M, Struhl K (1992) Yeast and human TFIID with altered DNA-binding specificity for TATA elements. *Cell* **68**: 721–730
- Wassarman DA, Sauer F (2001) TAF(II) 250: a transcription tool box. *J Cell Sci* **114**: 2895–2902
- Wefald FC, Devlin BH, Williams RS (1990) Functional heterogeneity of mammalian TATA-box sequences relieved by interaction with a cell specific enhancer. *Nature* **344**: 260–262
- Wolner BS, Gralla JD (2001) TATA-flanking sequences influence the rate and stability of the TATA-binding protein and TFIIB binding. *J Biol Chem* **276**: 6260–6266
- Yamaguchi Y, Itoh Y, Takeda Y, Yamazaki KI (1998) TATA sequence requirements for the initiation of transcription for an RNA polymerase II in vitro transcription system from *Nicotiana tabacum*. *Plant Mol Biol* **38**: 1247–1252
- Zhang DPT, Morris PL, Roeder RG (2001) Cell- and stage-specific high level expression of TBP-related factor 2 (TRF2) during mouse spermatogenesis. *Mech Dev* **106**: 203–205
- Zhu Q, Dabi T, Lamb C (1995) TATA-box and initiator functions in the accurate transcription of a plant minimal promoter in vitro. *Plant Cell* **7**: 1681–1689