The photosystem II reaction center chlorophyll protein D2, is encoded by the chloroplast gene psbD. PsbD is transcribed from at least three different promoters, one which is activated by high fluence blue light. Sequences within 130 base pairs (bp) of the psbD blue light-responsive promoter (BLRP) are highly conserved in higher plants. In this study, the structure of the psbD BLRP was analyzed in detail using deletion and site-directed mutagenesis and in vitro transcription. Deletion analysis showed that a 53-bp DNA region of the psbD BLRP, from −57 to −5, was sufficient for transcription in vitro. Mutation of a putative prokaryotic −10 element (TATTCT) located from −7 to −12 inhibited transcription from the psbD BLRP. In contrast, mutation of a putative prokaryotic −35 element, had no influence on transcription. Mutation of a TATA-like element located between the barley psbA −10 and −35 elements significantly reduced transcription from this promoter. However, site-directed mutation of sequences located between −35 and −10 had no effect on transcription from the psbD BLRP. Transcription from the psbD BLRP was previously shown to require a 22-bp sequence, termed the AAG-box, located between −36 and −57. The AAG-box specifically binds the protein complex AGF. Site-directed mutagenesis identified two different sequence motifs in the AAG-box that are important for transcription.

The photosystem II contains at least four plastid-encoded chlorophyll apoproteins (D1, D2, CP47, CP43). Among these, D2 and D1 form a heterodimer, which houses the photosystem II reaction center chlorophyll P680. D1 and D2 are relatively unstable in illuminated plants (1–5). Therefore, synthesis of D1 and D2 is selectively elevated in mature barley chloroplasts in order to maintain the levels of these subunits and PSII function (5, 6). Maintenance of high rates of D1 and D2 synthesis in mature barley chloroplasts is paralleled by the retention of elevated levels of psbA and psbD mRNAs, which encode these proteins (6–8). D1 mRNA levels remain high in mature barley chloroplasts primarily due to the high stability of its mRNA, although transcription from psbA is also increased by light (9–12). Maintenance of high levels of psbD mRNA results primarily from the activation of psbD transcription by blue light combined with a small increase in RNA stability (5, 13).

The chloroplast genome in most higher plants is circular and ranges in size from 120 to 217 kilobase pairs (reviewed in Refs. 14–17). The genome encodes approximately 135 genes including genes for rRNAs, tRNAs, subunits of the plastid 70 S ribosome, subunits of an RNA polymerase (rpoA, rpoB, rpoC1, and rpoC2), and proteins that comprise the photosynthetic apparatus. Transcription of the chloroplast genome is complex and highly regulated (reviewed in Refs. 17 and 18). Plastid genes are transcribed by at least two different RNA polymerases (RNAPs). The catalytic subunits of one RNA polymer are encoded by the chloroplast genes rpoA, rpoB, and rpoC1/C2 (reviewed in Ref. 19). This RNAP recognizes prokaryotic −10 and −35 promoter elements (reviewed in Ref. 18). Other types of plastid promoters have been identified. For example, the promoter for the rps16 gene contains only a −35 element (20). Other genes, such as trnS, trnR, rpoB (22), rpl32 (23), and rpl23 (24) are not preceded by typical prokaryotic promoter consensus elements. Many of these genes are transcribed by a nucleus-encoded RNAP (Refs. 22, 25, and 26; reviewed in Ref. 17). This polymerase is likely to be encoded by the nuclear gene rpoZ, which shows sequence similarity to the bacteriophage T7 and SP6 RNA polymerases (27). Plastid transcription is also regulated via multiple σ-factors (28–30), which may be phosphorylated (31, 32). Other DNA binding complexes, such as CDF2 and AGF, have been identified, which modulate transcription of rrr (33), and psbD-psbC (34), respectively.

In barley, psbD is located in a complex operon that also contains psbC, psbK, psbI, orf62, and trnG (35). The psbD operon is transcribed from at least three different promoters (13). One of the psbD promoters is activated when plants are illuminated by high fluence blue light but not by red or far-red illumination (5, 36). Transcripts arising from the blue light-responsive promoter (BLRP) become the most abundant psbD transcripts in chloroplasts of mature barley leaves (13, 37). Light-induced accumulation of psbD transcripts has been observed in a wide variety of plants (37–39). A 130-bp region surrounding the psbD BLRP is conserved among cereals, dicots, and black pine (34, 37) despite DNA rearrangements

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upstream of the psbD BLRP in some plants (37). The conserved psbD BLRP contains sequences with significant similarity to typical prokaryotic −10 and −35 promoter regions (13). In addition, two conserved regions, termed the AAG-box and PGT-box, are located upstream of the putative −35 element (34). Previously, we showed that the AAG-box and its cognate DNA-binding protein complex, AGF, are required for transcription from the barley psbD BLRP in vitro (34). Furthermore, the DNA region containing the PGT and AAG-boxes was shown to be important for transcription from the tobacco psbD BLRP in vivo (40). In the present study, we define a minimal DNA region required for transcription of the barley psbD BLRP and further dissect the architecture of the promoter using deletion, insertion, and point mutation analyses.

**EXPERIMENTAL ANALYSES**

**Plant Growth**—Barley (Hordeum vulgare var. Morex) seedlings were grown in controlled environmental chambers at 23 °C as described by Kim et al. (12). Seedlings were germinated and grown in complete darkness. After 7.5 days, the dark-grown seedlings were either harvested or transferred to a continuously illuminated chamber (fluorescence both in vitro and darkness). After 7.5 days, the dark-grown seedlings were either harvested or transferred to a continuously illuminated chamber (fluorescence both in vitro and darkness).

**Preparation of Plasmid Extracts for In Vitro Transcription Experiments**—The plasmid resulting DNA templates used for the transcription experiments in this study were prepared according to Kim and Mullet (34). Protein extract from 5.2 × 10⁶ plastids obtained from approximately 7.5-day-grown barley plants was used in each in vitro transcription assay.

**In Vitro Transcription and Primer Extension Analyses**—Transcription of exogenous DNA templates in vitro and primer extension analyses of in vitro transcribed DNA were performed as described by Kim and Mullet (34). The minus 40 primer was used to analyze transcripts of the barley psbD BLRP region required for transcription of the barley psbD BLRP

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RESULTS

Minimal DNA Region Required for Transcription from the psbD BLRP—The structure of the barley chloroplast psbD BLRP is shown in Fig. 1A. Comparisons of the psbD BLRP region among numerous plants showed several stretches of sequence conservation from approximately 130 to 210. In particular, sequences surrounding the AAG-box (236 to 264) and PGT-box (271 to 310) are highly conserved (34, 37).

Previous analysis of the psbD BLRP demonstrated that the region from 164 to 276 was sufficient to activate transcription in vitro (34). In this study, sequences important for transcription in vitro were further delineated using a series of deletions of pLRP140 (Fig. 1A).

Recombinant plasmids pLRP97, pLRP80, and pLRP69 contain a series of 3'-end deletions of the psbD BLRP (Fig. 1A). Each of the recombinant plasmids was added to chloroplast in vitro transcription extracts obtained from 8-day-old barley plants that had been illuminated for 16 h. The psbD transcripts produced from the plasmids were assayed using primer extension analysis. No psbD transcript 5' termini were observed when mock transcription reactions were analyzed (data not shown). However, as shown in Fig. 1B (lanes 1–4), all of the 3' deletion recombinant plasmids and pLRP140 were equally good templates. Similar results were observed with plastid extracts from 7.5-day-old, dark-grown barley plants (data not shown).

Previous analyses demonstrated that the sequence AAAGTAAG (−54 to −47) in the AAG-box (see Fig. 1A) was required for transcription from the BLRP (34). To examine the influence of sequences upstream of this sequence, pLRP121 was constructed, which contains a 5' deletion to −57 (Fig. 1A). This deletion caused no loss of transcription activity from the psbD

**Fig. 1.** In vitro transcription from the psbD BLRP and modified psbD BLRP domains using barley plastid extracts. A, schematic representation of the barley chloroplast psbD BLRP and psbD BLRP constructs used for in vitro transcription. The boxed regions identify conserved sequences including the AAG-box, the PGT-box, and sequences homologous to *E. coli* −35 and −10 promoter elements (34). The site of psbD transcription initiation is designated by an arrow, and labeled as +1. Repeated sequences in the AAG-box are underlined. pLRP140 is a recombinant plasmid containing 140 bp of DNA (−76 to +64) from the psbD BLRP (34). pLRP185 was used as a template to construct recombinant plasmids, pLRP97, pLRP80, pLRP69, and pLRP 121. B, in vitro transcription of the recombinant plasmids shown in A using barley plastid extracts. Transcripts were analyzed using primer extension analysis. The arrows designate primary transcripts produced from the recombinant plasmids. The asterisk marks the position of a signal produced from plastid extracts in the absence of template. DNA size markers in base pairs are indicated to the right.
promoter elements, which are separated by 18 bp, have previously been identified upstream of the sites of transcription initiation in the rbcL and psbA promoters (reviewed in Ref. 16).

In addition, a TATA sequence located between the psbA −10 and −35 promoter elements contributes to promoter activity in mustard (46).

The function of the putative −10 and −35 prokaryotic promoter elements present in the psbD BLRP was analyzed by site-directed mutagenesis. As a control, the influence of modifying the −10 and −35 elements in the rbcL and psbA promoters was examined to ensure the in vitro transcription extract was faithfully replicating previous results. Our general approach was to introduce point mutations in potential −35 and −10 sequences at sites that show the highest conservation in both plastid and bacterial promoters (−35, TTGaca; −10, TAtaαT) (Ref. 47; reviewed in Refs. 16 and 48–51). The first and the third nucleotides (T and G) in the potential −35 promoter element of each promoter were switched to A and C, respectively (Figs. 3A and 4A). In the case of potential −10 promoter elements, the first and the sixth nucleotides, T and T, were both switched to A (Figs. 3A and 4A). The point mutations described above did not create any other potential −35 or −10 promoter elements. Each of the recombinant plasmids containing the point mutations was added to plastid transcription extracts, which were obtained from either 7.5-day-old, dark-grown barley plants, or similar plants that had been further illuminated for 16 h.

Transcription from the psbA and rbcL promoter constructs is shown in Fig. 3, B and C. Transcription from the wild type psbA or rbcL promoters was active in extracts of etioplasts isolated from dark-grown plants or plastoplasts isolated from illuminated plants (Fig. 3, B and C, lanes 1 and 2). Modification of the −35 sequences in these two promoters caused transcription to decrease to very low levels (Fig. 3, B and C, lane 3). Similarly, modification of the −10 sequences also caused transcription to decrease significantly (Fig. 3, B and C, lane 4). When the TATA sequence located between the −35 and −10 elements in psbA was modified, transcription was reduced although not eliminated (Fig. 3B, lane 5). Not surprisingly, modification of both the −35 and TATA sequence in the psbA promoter reduced transcription to nondetectable levels (Fig. 3B, lane 6).

The results in Fig. 4 show the influence of mutation of putative −10 and −35 promoter elements found in the psbD BLRP. Mutation of the −35 sequences did not alter transcription from the psbD BLRP (Fig. 4B, lane 2 versus lane 3). In contrast, mutation of the prokaryotic −10 element, TATTCT, reduced transcription from the psbD BLRP to very low levels (Fig. 4B, lane 2 versus lane 4). As expected, point mutations in both of the −10 and −35 sequences also abolished transcription (Fig. 4B, lane 5). These results indicate that the −10 sequence is required for transcription from the psbD BLRP in vitro, whereas the −35 and −10 elements are both required for transcription from the rbcL and psbA promoters. In addition, the psbA TATA sequence is important for transcription from the psbA promoter in barley. Transcription of all constructs was greater in extracts of plastids from illuminated plants compared with extracts from etioplasts of dark-grown plants, although the influence of illumination was greatest on the psbD BLRP (−6.5-fold versus 4-fold (psbA) and 2-fold (rbcL) (Figs. 3 and 4).

Role of the Sequence and Spacing between the AAG-box and −10 Sequence in the psbD BLRP—In the psbA promoter, a TATA sequence located between the −10 and −35 elements contributes to promoter activity. Therefore, to determine if additional motifs in the psbD BLRP confer promoter activity, we tested the influence of five point mutations in the sequences

![Architecture of a Chloroplast Blue Light-responsive Promoter](image_url)
located between the AAG-box and the prokaryotic \(-10\) element (see Fig. 4A, nt Switch). As shown in Fig. 4B, these substitutions did not alter transcription from the \(psbD\) BLRP, suggesting the absence of important sequence motifs between the AAG-box and the \(-10\) promoter element (Fig. 4B, lane 6).

The AGF, which binds to the AAG-box, may stabilize and orient the RNA polymerase relative to the \(-10\) element of the \(psbD\) BLRP. Therefore, spacing between the AAG-box and the \(-10\) element may be important to maintain alignment of AGF and the RNA polymerase on the same face of the \(psbD\) BLRP. The AAG-box and the \(-10\) motif are separated by 23 bp in the \(psbD\) BLRP. In contrast, most plastid \(-35\) and \(-235\) elements are separated by 18 bp (reviewed in Ref. 16). Therefore, the influence of altering the spacing between the AAG-box and the prokaryotic \(-10\) element was investigated. Nucleotide deletions (5 and 10 bp) or insertions (3, 7, and 10 bp) were introduced between the AAG-box and the \(-10\) element in plasmid pLRP140 (Fig. 4A). When a 5-bp deletion was introduced to reduce spacing between the AAG-box and the \(-10\) element to 18 bp, transcription from the \(psbD\) BLRP was undetectable (Fig. 4B, lane 7). Deletion of 10 bp, which represents one helical turn, resulted in low but detectable levels of transcription (Fig. 4B, lane 8). Insertion of 3, 7, or 10 bp between the AAG-box and the \(-10\) element also reduced transcription to very low levels (Fig. 4B, lanes 9–11). These results indicate the importance of the 23-nucleotide spacing between the AAG-box and the \(-10\) element for transcription from the \(psbD\) BLRP.

Further Analysis of Sequences in the AAG-box—The AAG-box, shown in Fig. 5A, was defined in past experiments as a 22-bp DNA region (\(\text{nt} \, 236 \, \text{to} \, 257\)) containing two motifs designated aa\(^9\) and bb\(^9\) (37). We have previously shown by point mutation analyses that the aa\(^9\) motif is important for both AGF binding and transcription from the \(psbD\) BLRP (34). To test the importance of the bb\(^9\) motif for transcription from the \(psbD\) BLRP, we introduced point mutations in this sequence (GAC-CTGACT) in plasmid pLRP140 (see Fig. 5A). Transcription analysis showed that mutation of GACC to GTAG inhibited transcription from the \(psbD\) BLRP (Fig. 5B, lane 1 versus lane 2 and lane 4 versus lane 5). Furthermore, mutations of GAC-CTGACT to GTAGTGTGA abolished transcription from the \(psbD\) BLRP (Fig. 5B, bb\(^9\) mt, lanes 3 and 6). To determine whether the bb\(^9\) sequences within the AAG-box also influence binding of AGF, gel retardation and competition binding experiments were carried out (Fig. 5C). As observed previously (34),
AGF binds to radiolabeled pLRP140 in the absence of specific competitor DNA fragments (Fig. 5C, lane 1). The addition of unlabeled pLRP140 to the binding assays greatly reduces the amount of AGF gel shift complex (Fig. 5C, lane 2). As described previously, LRP140 DNA fragments containing modified aa9 sequences (AAAGTAAG to AAATTCAT) do not compete well with native LRP140 (lane 3) (34). Modification of bb9 sequences in pLRP140 (b mt and bb9 mt) reduces the ability of the resulting DNAs to bind AGF to some extent (Fig. 5C, lanes 4 and 5), indicating that the bb9 sequence contributes to AGF binding either directly or indirectly.

**DISCUSSION**

**Delineation of a 53-bp Core psbD BLRP Promoter Domain**—The psbD BLRP is located approximately 570 bp upstream of the psbD translational start codon in cereals and even further upstream of the psbD open reading frame in dicots (37). In higher plants, a DNA region of approximately 130 bp surrounding the site of transcription initiation from the psbD BLRP is highly conserved (~60%) relative to sequences more than 100 bp upstream of the promoter or sequences between the promoter and the psbD open reading frame (9%) (37). At least 25 bp of the conserved region extends downstream of the site of transcription initiation. In this study, we determined that deletion of sequences from −5 to +64, relative to the site of transcription initiation, had no influence on transcription from the psbD BLRP in vitro. This result indicates that the conserved sequences downstream of the initiation site are probably not important for transcription. Previous analysis of changes in psbD transcription and RNA levels during leaf and chloroplast development indicated that psbD transcripts become more stable during light-mediated leaf maturation (8, 13). Therefore, the conserved sequences immediately downstream from the site of transcription initiation, which are present in the 5′-untranslated region of transcripts produced from the psbD BLRP, may be important for RNA stability.

The 100-bp DNA region immediately upstream of the psbD BLRP initiation site contains several stretches of sequence that are conserved among psbD genes from higher plants (37). Deletion of sequences from −107 to −55 in the tobacco psbD BLRP reduced transcription activity in vivo ~5-fold without altering light-stimulated transcription following dark adaptation of plants (40). In barley, this region of the psbD BLRP specifically binds a protein complex (PGTF) present in chloroplasts (34). In the current study, however, deletion of sequences...
upstream of −57 in the psbD BLRP had minimal effect on in vitro transcription. This suggests that this region of the psbD BLRP and the PGTF complex that binds in this region are not modulating transcription from the psbD BLRP in vitro. Mutations of sequences immediately downstream of −57 (34) or upstream of −5 (Fig. 5) reduce transcription from the psbD BLRP. These experiments define a 53-bp region that is required for transcription from the psbD BLRP in vitro.

Transcription from the psbD BLRP Requires a Prokaryotic −10 Element but Not a −35 Promoter Element or the psbA TATA-TATA Element—The psbD BLRP contains the sequence TATTCT, located between −7 and −12, which resembles a prokaryotic −10 promoter element. Mutation of this sequence to AATTCA reduced transcription from the psbD BLRP to very low levels. Similarly, mutation of −10 sequences found in the psbA (TATATCT to AATACAA) and rbcL (TACAAAT to AACAAA) promoters rendered these promoters inactive. In Escherichia coli, −10 promoter elements are recognized via interaction with σ-factors that are associated with the RNAP (reviewed in Refs. 52–54). These results are consistent with in vitro transcription of the psbD BLRP by a chloroplast RNAP containing a σ-like subunit that interacts with the −10 promoter element (29, 31, 55–57). Transcription from mustard psbA is stimulated by a TATA element located between the −10 and −35 promoter elements (46). The TATA sequence might be involved in the recruitment of RNA polymerase or in the isomerization from the “closed” to “open” complex formation (Refs. 58 and 59; reviewed in Refs. 60 and 61). Moreover, in mustard, this sequence may allow transcription in dark-grown plants that is not dependent on a −35 element from the psbA promoter (31, 46). Mutation of a similar sequence present in the barley psbA promoter decreased transcription in plastid extracts from dark-grown and illuminated plants (Fig. 3). In contrast, the psbD BLRP lacks the TATA sequence, and mutation of sequences located between −10 and −35 in the psbD BLRP had little influence on transcription activity.

The chloroplast-encoded RNAP’s ability to transcribe rbcL and psbA depends on a prokaryotic −35 promoter element (Figs. 3 and 4) (reviewed in Refs. 16 and 49). In contrast, mutation of the −35 sequence in the psbD BLRP had little effect on transcription in vitro (Fig. 5). The function of the −35 sequence in the psbD BLRP appears to be replaced by the action of AGF, an activating complex that binds immediately upstream of the −35 sequence (Ref. 34; see below).

Two Different Sequences in the AAG-box Are Involved in psbD BLRP Transcription—The sequence from −36 to −64 in the psbD BLRP was previously reported to be required for transcription from the psbD BLRP in vitro (34). In the current study, this region was further truncated to −57 without loss of activity. The corresponding sequence in the tobacco psbD BLRP was also found to be important for activity in vitro (40). The region from −36 to −57, termed the AAG-box, was previously reported to contain two conserved motifs (aa’ and bb’) (37). A protein complex, designated AGF, was found to specifically interact with sequences within the AAG-box. Footprint analysis indicated that AGF binding protected sequences from at least −40 to −63 (34). In a previous study, site-directed mutagenesis of the aa’ sequence (AAAGTAAGT to AAATTCAT) caused loss of AGF binding and eliminated transcription from the psbD BLRP (34). In the current study, site-directed mutagenesis of the bb’ sequence located immediately downstream from the aa’ motif and upstream of −35 caused a reduction in control binding assay in the absence of competitor DNA is shown in lane 1 (No Comp.). The arrow designates the AGF-binding complex consistent with previous analysis (34). The migration of free probe is indicated.
transcription as well as a reduction in the ability of DNA in this region to bind to AGF (Fig. 5). These results suggest that proteins in AGF interact with the bb' sequence. It is also possible that some other currently undetected protein binds to the bb' sequence and that this modifies AGF binding. In tobacco, proteins also bind specifically to the bb' sequence (40). Unfortunately, the relationship between the barley and tobacco AGA-box binding complexes could not be established.

**Model for AGF Activation of the psbD BLRP**—A model of the barley psbD BLRP is shown in Fig. 6 along with diagrams of the rbcL and psbA promoters. All three genes are shown being transcribed by the chloroplast-encoded RNAP with an associated σ-factor. This is consistent with several lines of evidence. First, light-induced transcription from the psbD BLRP in *vivo* is inhibited if plants are pretreated with tagetitoxin (13). The chloroplast-encoded RNAP and *E. coli* RNAP are sensitive to tagetitoxin, whereas the chloroplast-localized, nucleus-encoded RNAP and the homologous bacteriophage RNA polymerases, T7 or SP6, are not inhibited by tagetitoxin (62, 63). Second, plants that lack the chloroplast-encoded RNAPs do not accumulate transcripts from the psbD BLRP (or from rbcL, psbA), although they accumulate transcripts from many genes involved in transcription and translation that lack prokaryotic −10 and −35 promoter elements (22, 64). Third, mutation of sequences surrounding the psbD BLRP site of transcription initiation (*) from TTCTGATATAT*AAAT to TTCTGAGGAT−factor did not affect transcription initiation (Fig. 1 and 2). The nucleus-encoded chloroplast RNAP has been proposed to use a promoter sequence located in the 10 bases immediately adjacent to the site of transcription initiation (64). Based on comparative alignments, a rather variable promoter consensus sequence, ATAGAAT(A/G)AA, has been proposed for this polymerase (24, 64). This sequence is somewhat different from both the native and mutated psbD BLRP promoters that are active in *vivo*. Fourth, mutation of the prokaryotic −10 element, located between −7 and −12, dramatically reduced transcription from this promoter. Finally, the chloroplast-encoded RNAP preferentially transcribes genes encoding proteins involved in photosynthesis; therefore, transcription from the psbD BLRP is consistent with this tendency. However, further biochemical analysis of the nucleus-encoded RNAP will be needed to definitively eliminate a role for this RNAP in psbD BLRP transcription.

The RNAPs in Fig. 6 are shown associated with a generic σ-factor. However, there are several reasons to think that the σ-factor involved in transcription of the psbD BLRP may be different from σ-factors involved in transcribing *rbcL* and *psbA*. First, in the case of the *rbcL* and *psbA* promoters, σ-factors are likely to interact with both −10 and −35 promoter elements, based on analysis of bacterial σ-factor binding (reviewed in Refs. 52 and 65). An additional interaction may occur between the σ-factor and the TATA sequence in the psbA promoter. In contrast, the psbD BLRP lacks functional −35 and TATA elements, and the sequence of its −10 element differs from those of *rbcL* and *psbA*. Second, the psbD AAG-box did not activate transcription when fused upstream of a derivative of the *rbcL* promoter shown in Fig. 3, which lacks an active −35 element (data not shown). This could mean that AGF interacts with an RNA polymerase containing a σ-factor that is incompatible with the rbcL promoter. Third, utilization of a different σ-factor for transcription of the psbD BLRP would allow blue light-specific regulation of this promoter via the σ-factor. Recently, genes encoding three chloroplast σ-factors have been cloned (29, 30). Moreover, the expression of at least one σ-factor gene is regulated by light (56, 66), and previous work showed that these factors are the target of light-mediated regulation of chloroplast transcription (31).

The function of the −35 promoter element in the psbD BLRP is likely to be replaced by an activating complex bound to the AAG-box (Fig. 6, AGF/BB'). The AAG-box contains two binding domains, aa' and bb', which bind AGF. The AGF, unlike σ-factors, binds to DNA in the absence of the RNAP (34). A subunit of AGF or perhaps a separate protein, noted in Fig. 6 as BB', binds specifically to the bb' motif. The AGF/BB' could activate the psbD BLRP by recruiting the RNA polymerase to the psbD BLRP, by stabilizing the binding of the RNAP to the BLRP, or by changing RNAP recognition of the −10 element, thus promoting transcription (reviewed in Ref. 67).

The structure of the psbD BLRP shown in Fig. 6 resembles a class of bacterial promoters that use activating proteins to stimulate transcription (reviewed in Refs. 52 and 65). The activating sequences in one class of these promoters (type I; *i.e.* cAMP receptor protein binding site in *lacP1*) can be moved various distances upstream of the promoter (68). In type II promoters such as *galP1*, the site of activator binding must be immediately upstream of −35 (68). In both cases, the σ-subunit of RNAP interacts with the activating complex (Refs. 68 and 69; reviewed in Ref. 70), although in different ways (71). In this regard, the psbD BLRP is similar to a type II bacterial promoter. The addition of 3, 7, or 10 bp between the −10 element and the AAG-box dramatically inhibited transcription, indicating that the AGF factor needs to be approximately 23 bp from the −10 element. Moving the AAG-box closer to the −10 element by removal of five nucleotides between the −10 and AAG-box also inhibited transcription. However, constructs with deletion of 10 bp still showed a low level of activity. Deletion of 10 bp, or one helical turn, would keep the AAG-box and the −10 element in the same relative orientation along the DNA helix. Therefore, a low level of transcription from this
template is possible, although packing of the RNAP and AGF on the template must be tight.

**Regulation of the psbD BLRP—**Illumination of 7.5-day-old, dark-grown barley with white light caused a 10-fold increase in transcription from the psbD BLRP and a 4-fold increase in transcription from rbcL in vivo (72). Surprisingly, in vitro transcription of the 53-bp psbD BLRP in plastid extracts from 7.5-day-old, dark-grown plants that had been illuminated for 16 h, was approximately 6.5-fold higher than in extracts of dark-grown plants (Fig. 4). Transcription from the rbcL promoter was also approximately 2-fold greater in extracts from illuminated plants (Fig. 3). This suggests that light-induced modifications that activate transcription in vivo are retained in vitro. Light could induce the accumulation of a transcription factor and/or cause modification of the RNAP, a σ-factor, or the AGF during the illumination period. Inhibitor studies have implicated the involvement of protein kinases and phosphatases in blue light modulation of transcription from the psbD BLRP (73). Future experiments will be directed toward identification of the potential targets of these protein kinases/phosphatases and an understanding of their role in blue light modulation of the psbD BLRP.

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