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Regulation of the chloroplast *psbD* blue-light responsive promoter (BLRP) in higher plants

Minkyun Kim

Department of Agricultural Chemistry, Division of Applied Biology and Chemistry, Center for Plant Molecular Genetics and Breeding Research, College of Agriculture and Life Sciences, Seoul National University

Introduction

Photosystem II (PS II) contains at least four plastid-encoded chlorophyll-apoproteins (D1, D2, CP47, CP43). Among these, D2 and D1 form a heterodimer, which houses the PS 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 kbp (rev., 14-17). The genome encodes approximately 135 genes including genes for rRNAs, tRNAs, subunits of the plastid 70S 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 (rev., 17, 18). Plastid genes are transcribed by at least two different RNA polymerases (RNAPs). The catalytic subunits of one RNAP are encoded by the chloroplast genes rpoA, rpoB, rpoC1/C2 (rev., 19). This RNAP recognizes prokaryotic -10 and -35 promoter elements (rev., 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 (21), rpoB (22),

rpl32 (23), and rpl23 (24) are not preceded by typical prokaryotic promoter consensus elements. Many of these genes are transcribed by a nuclear-encoded RNAP (22, 25, 26 ; rev., 17). This polymerase is likely to be encoded by the nuclear gene RpoZ which shows sequence similarity to the bacteriophage RNA polymerases T7 and SP6 (27). Plastid transcription is also regulated via multiple sigma 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 rrn (33), and *psbD-psbC* (34), respectively.

Structure and expression of the psbD operon

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 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). It was shown 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).

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. It was recently shown 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* (41). 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'-UTR 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, 41). In most recent study, however, deletion of sequences upstream of -57 in the *psbD* BLRP had minimal effect on *in vitro* transcription (42). This suggests that this region of the *psbD* BLRP and the PGTF complex which binds in this region, are not modulating transcription from the *psbD* BLRP *in vitro*. Mutation of sequences immediately downstream of -57 (34) or upstream of -5 (42) 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 TATATA 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 (TATACT to AATACA) and rbcL (TACAAT to AACAAA) promoters rendered these promoters inactive. In *E. coli*, -10 promoter elements are recognized via interaction with sigma factors that are associated with the RNAP. These results are consistent with *in vitro* transcription of the *psbD* BLRP by a chloroplast RNAP containing a sigma-like subunit which interacts with the -10 promoter element (29, 31).

Transcription from mustard psbA is stimulated by a TATATA sequence located between the -10 and -35 promoter elements (43). The TATATA sequence might be involved in the recruitment of RNA polymerase or in the isomerization from the 'closed' to 'open' complex formation. Moreover, in mustard, this sequence may allow transcription in dark-grown plants which is not dependent on a -35 element from the psbA promoter (31, 43). Mutation of a similar sequence present in the barley psbA promoter decreased transcription in plastid extracts from dark-grown and illuminated plants. In contrast, the *psbD* BLRP lacks the TATATA sequence and mutation of sequences located between -10 and -35 in the *psbD* BLRP had little influence on transcription activity (42).

The chloroplast-encoded RNAP's ability to transcribe rbcL and psbA depends on a prokaryotic -35 promoter element (42). In contrast, mutation of the -35 sequence in the *psbD* BLRP had little effect on transcription *in vitro* (42). The function of the -35 sequence in the *psbD* BLRP appears to be replaced by the action of AGF, an activating complex which binds immediately upstream of the -35 sequence (34).

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). Most recently, this region was further truncated to -57 without loss of activity (42). The corresponding sequence in the tobacco *psbD* BLRP was also found to be important for activity *in vivo* (40). The region from -36 to -57, termed the AAG-box, was previously reported to contain two conserved motifs (aa', 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).

Site-directed mutagenesis of the aa' sequence (AAAGTAAGT to AAATTCAT) caused loss of AGF binding and eliminated transcription from the *psbD* BLRP (34). Site-directed mutagenesis of the bb' sequence located immediately downstream from the aa' motif, and upstream of -35, also caused a reduction in transcription (42). In tobacco, proteins bind specifically to the bb' sequence (40). Unfortunately, the relationship between the barley and tobacco AAG-box binding complexes could not be established. Therefore, experiments are underway to determine if mutations in the bb' region modify binding of the AGF or some other protein complex to the *psbD* BLRP.

Model for AGF activation of the psbD BLRP.

A model of the barley *psbD* BLRP is shown in Figure 1 along with diagrams of the rbcL and psbA promoters. All three genes are shown being transcribed by the chloroplast-encoded RNAP with an associated sigma 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, nuclear-encoded RNAP and the homologous bacteriophage RNA polymerases, T7 or SP6, are not inhibited by tagetitoxin (44, 45). Second, plants that lack the chloroplast-encoded RNAP 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 (22,46). Third, mutation of sequences surrounding the *psbD* BLRP site of transcription initiation (*) from TTCTGATATAT*AAAT to TTCTGAGGATC*CCCC had no influence on transcription *in vitro* (42). The nuclear-encoded chloroplast RNAP has been proposed to use a promoter sequence located in the 10 bases immediately adjacent to the site of transcription initiation (46). Based on comparative alignments, a rather variable promoter consensus sequence, ATAGAAT(A/G)AA, has been proposed for this polymerase (24, 46). This sequence is somewhat different from both the native and mutated *psbD* BLRP promoters that are active *in vitro*. 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 nuclear-encoded RNAP will be needed to definitively eliminate a role for this RNAP in *psbD* BLRP transcription.

The RNAPs in Figure 1 are shown associated with a generic sigma factor. However, there are several reasons to think that the sigma factor involved in transcription of the *psbD* promoter may be different from sigma factors involved in transcribing rbcL and psbA. First, in the case of the rbcL and psbA promoters, sigma factors are likely to interact with both -10 and -35 promoter elements, based on analysis of bacterial sigma factor binding. An additional interaction may occur between the sigma factor and the TATATA sequence in the psbA promoter. In contrast, the *psbD* BLRP lacks functional -35 and TATATA 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, which lacks an active -35 element. This could mean that AGF interacts with an RNA polymerase containing a sigma factor that is incompatible with the rbcL promoter. Third, utilization of a different sigma factor for transcription of the *psbD* BLRP would allow blue light specific regulation of this promoter via the sigma factor. Recently, genes encoding three chloroplast sigma factors have been cloned (29, 30). Moreover, the expression of at least one sigma factor gene is regulated by light, 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. 1, AGF/BB'). The AAG-box contains two binding domains, aa' and bb', which bind AGF and a putative factor, BB', respectively. The AGF, unlike sigma factors, binds to DNA in the absence of the RNAP (34). The nature and association of the factor (BB') which binds to bb' motif is not clear at present. This factor could be part of the AGF or a separate subunit. 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.

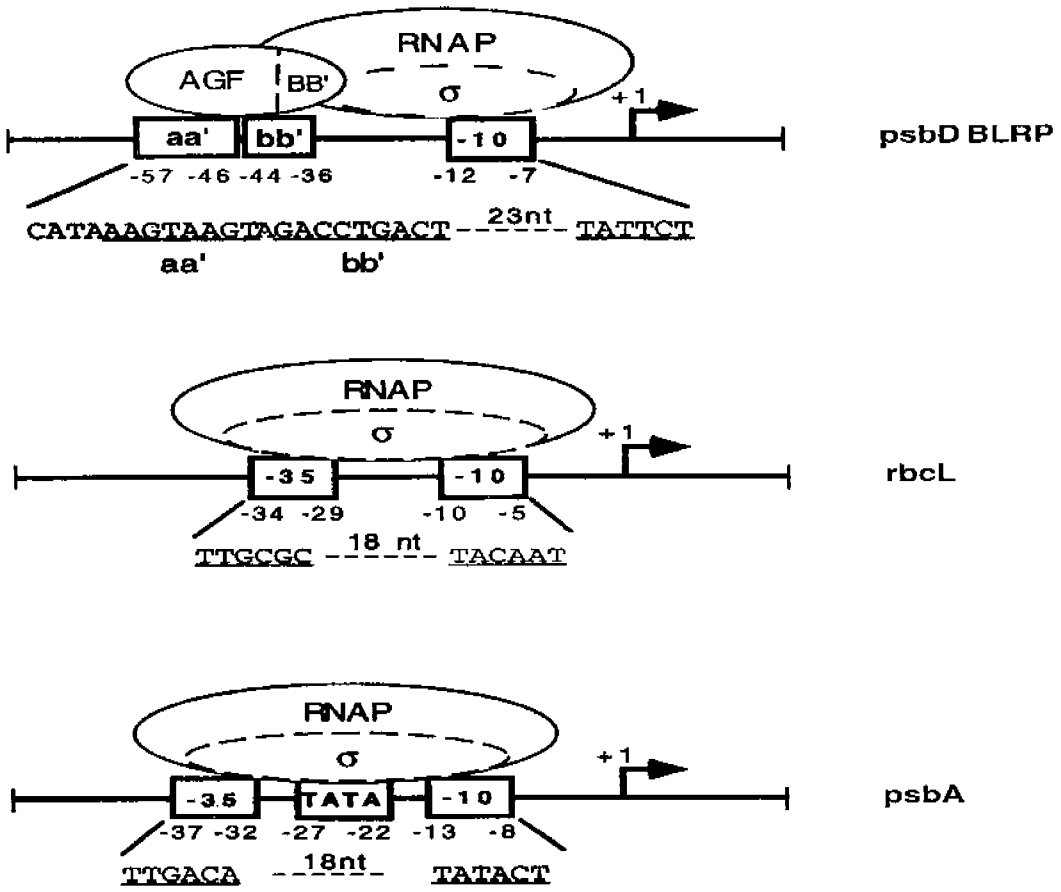

Fig. 1. Models of transcription complexes associated with the *psbD* BLRP, rbcL and psbA promoters. Arrows (+1) indicate the site and direction of transcription initiation. Important transcription cis-elements (-10, -35, TATA, and aa'/bb') are boxed and the sequences and spacing between elements is indicated. A chloroplast RNAP and an associated sigma factor is shown interacting with each promoter. In addition, the AGF/BB' complex, which binds to the AAG-box sequences aa'/bb', is shown interacting with the RNAP to promote transcription from the *psbD* BLRP.

The structure of the *psbD* BLRP shown in Figure 1 resembles a class of bacterial promoters that use activating proteins to stimulate transcription (47). The activating sequences in one class of these promoters (type I; i.e., CRP binding site in lacP1) can be moved various distances upstream of the promoter (48). In type II promoters such as galP1, the site of activator binding must be immediately upstream of -35 (48). In both cases, the alpha subunit of RNAP interacts with the activating complex, although in different ways. In this regard, the *psbD* BLRP is similar to a

type II bacterial promoter. Addition of 3, 7 or 10 bp between the -10 element and the AAG-box dramatically inhibited transcription indicating the AGF factor needs to be approximately 23 bp from the -10 element (42). Moving the AAG-box closer to the -10 element by removal of 5 nucleotides between the -10 and AAG-box also inhibited transcription (42). 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, even though 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* (49). 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 (42). Transcription from the rbcL promoter was also approximately 2-fold greater in extracts from illuminated plants (42). 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 sigma 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 (50). Future experiments will be directed towards 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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