G-4

How the Chloroplast psbD Blue-Light Responsive Promoter is Activated in Higher Plants?

Minkyun Kim
Department of Agricultural Chemistry,
Center for Plant Molecular Genetics and Breeding Research,
Graduate School of Agricultural Biotechnology,
Seoul National University

The reaction center chlorophyll protein D2 in photosystem II (PSII) is encoded by the chloroplast gene psbD. psbD is transcribed from at least three different promoters, one of which is activated by high fluence blue light. Differential accumulation of light-induced *psbD-psbC* mRNAs in chloroplasts was due to transcription from a blue light-responsive promoter (*psbD* LRP). The light-induced mRNAs help to maintain levels of the D2 polypeptide, which is photodamaged and degraded in illuminated plants. The accumulation of light-induced *psbD-psbC* mRNAs was conserved in various plant species, despite differences in the structure and expression of the *psbD-psbC* operons. In addition, sequences within 130 base pairs (bp) of the *psbD* LRP are highly conserved in higher plants. Therefore, physiological and gene regulatory demands of the chloroplast are likely to act as constraints that preserve the linkage of the *psbD* LRP with *psbD*. The structure of the *psbD* LRP was analyzed using deletion and site-directed mutagenesis, *in vitro* transcription, gel shift assays, and DNAse I footprinting experiments. Deletion analysis showed that a 53-bp DNA region of the *psbD* LRP, from -57 to -5, was sufficient for transcription *in vitro*. Mutation of a putative prokaryotic -10 element located from -7 to -12 inhibited transcription from the *psbD* LRP. In contrast, mutation of a putative prokaryotic -35 element had no influence on transcription. Site-directed mutation of sequences located between -35 and -10 had no effect on transcription from the *psbD* LRP. Transcription from the *psbD* LRP required a 22-bp sequence, termed the AAG-box, located between -36 and -57. The AAG-box specifically bound the activating complex, termed AGF. Transcription from the *psbD* LRP is thus similar to type II bacterial promoters that use activating proteins to stimulate transcription. Transcription of the *psbD* LRP was ~6.5-fold greater in plastid extracts from illuminated *versus* dark-grown plants. This suggests that light-induced activation *in vivo* involves factors interacting with the 53-bp *psbD* LRP *in vitro*.