DNA RESEARCH 3, 277–285 (1996)

Nucleotide Sequence of the *psbP* Gene Encoding Precursor of 23-kDa Polypeptide of Oxygen-Evolving Complex in *Arabidopsis thaliana* and its Expression in the Wild-Type and a Constitutively Photomorphogenic Mutant

Anju KOCHHAR, Jitendra P. KHURANA, and Akhilesh K. TYAGI*
Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi-110021, INDIA

(Received 5 August 1996)

Abstract

The *psbP* gene encoding the precursor of 23-kDa polypeptide of the oxygen-evolving complex of photosystem II has been isolated from *Arabidopsis thaliana* genomic library and sequenced. The gene harbors three introns and encodes a mature polypeptide of 186 amino acid residues and a transit peptide of 77 amino acid residues. The deduced molecular mass of the mature polypeptide is 23.5-kDa and it contains 22.6% charged amino acid residues which may contribute to the hydrophilic nature of the protein. The transcript encoded by *psbP* gene of *Arabidopsis* is approximately 1.3-kb long. In wild-type *Arabidopsis* seedlings, its expresssion is organ-specific and is regulated by endogenous developmental cues, light and sucrose. In a constitutively photomorphogenic mutant of *Arabidopsis*, designated as *pho1*, the *psbP* gene is partly derepressed in young, dark-grown seedlings, resulting in a slightly higher level of the transcript. Additionally, the *pho1* mutant shows slow accumulation of *psbP* transcript upon illumination of young, dark-grown seedlings. However, the derepression is not markedly displayed on dark-adaptation of *pho1* plants grown in continuous light. These studies, therefore, define the activity of at least one cellular effector involved in regulation of *psbP* expression.

Key words: *Arabidopsis thaliana*; gene expression; oxygen-evolving complex; photomorphogenic mutant; *psbP* gene

1. Introduction

The oxygen-evolving complex associated with photosystem II of the chloroplast thylakoid membrane is a vital component of the photosynthetic machinery. This complex consists of three major polypeptides (33-, 23- and 16-kDa) encoded by nuclear genome, which are synthesized on cytoplasmic ribosomes and translocated into thylakoid lumen with the help of a bipartite transit peptide.[1] Mutational analysis in *Chlamydomonas* has demonstrated that 23-kDa polypeptide is necessary for PSII to be fully functional *in vivo*.[2] Although the precise role of this polypeptide in oxygen evolution is not understood, *in vitro* studies have shown that it modulates chloride requirement for maximal oxygen evolution by increasing the affinity of water-oxidation site for chloride.[3] Additionally, it provides conditions for high-affinity binding of calcium.[4,5] This is perhaps accomplished by serving as a physical barrier between a high-affinity calcium-binding site and the medium resulting in restricted calcium exchange with the environment and not by increasing its affinity for calcium *per se*.[6] Recently, genes for the polypeptide components of oxygen-evolving complex have also been investigated.[7] Since the first report on the isolation of a cDNA clone for the 23-kDa polypeptide from spinach,[8] cDNA clones from a few other plants (tomato,[9] tobacco,[10–12] wheat,[13] pea,[14] rice[15]) and complete genomic counterpart from *Sinapis alba*[16] and an incomplete gene from tobacco,[11] have been isolated. The expression of the *psbP* gene (encoding 23-kDa polypeptide) has been shown to be regulated by phytochrome in *S. alba*[17] and the spatio-temporal pattern of its light-regulated expression has also been studied during tobacco seedling development.[18]

Recently, as a result of extensive studies on a class of photomorphogenic mutants, it has become clear that light signal is transduced through a hierarchy of cellular effectors to finally affect various cellular processes and gene expression patterns.[19–21] At least eleven such loci

Communicated by Masahiro Sugiura

* To whom correspondence should be addressed. Tel. +91-11-4671208, 600669, Fax. +91-11-6886427

† The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X98108

designated as either *det* (*de-etiolated*), *cop* (*constitutively photomorphogenic*) or *fusca* have been identified. The corresponding wild-type alleles defined by these loci encode putative repressors of photomorphogenesis, and a mutation in any of them leads to manifestation of a range of light-dependent processes in dark. A similar class of mutants designated as *pho* (for *plumular hook open*, in dark) is being characterized by us. The *pho* mutants display a number of photomorphogenic features in dark such as plumular hook opening, cotyledon expansion, hypocotyl growth suppression, leaf differentiation, bolting and floral bud formation.[20,22] In addition, they undergo slow greening when transferred from dark to light and flower earlier than wild-type when grown in continuous light .

In the present investigation, the gene encoding the 23-kDa component of the oxygen-evolving complex in *Arabidopsis thaliana* has been isolated, sequenced and characterized for its expression in response to intrinsic and extrinsic factors in the wild-type *A. thaliana*. Since photomorphogenic mutant, *pho1*, is likely to define an important cellular effector mediating light-regulated gene expression, the effect of this mutation on *psbP* gene expression has also been examined.

2. Materials and Methods

2.1. Isolation of gene and sequencing

A genomic library of *A. thaliana*, ecotype Columbia, in EMBL3 replacement vector, was screened with a heterologous cDNA clone from spinach[8] at a density of 50,000 pfu per 22.5 × 22.5 cm^2 culture dish. Prehybridization and hybridization of the replica filters were done as described for analysis of eukaryotic genomic DNA in Sambrook et al.[23] Phage DNA was isolated from positive clones by procedure given by Santos.[24] Restriction analysis and cloning was done using standard protocols. pBluescript KS^- or SK^+ (Stratagene) vector was employed as the cloning vehicle.

For sequencing, plasmids were prepared by the PEG precipitation method.[23] Double-stranded plasmids were subjected to sequencing by the dideoxy chain termination method[25] using T_3 and T_7 primers and Sequenase version 2.0 kit (USB, USA), following the manufacturer's specifications.

2.2. Plant material and growth conditions

Seeds of *A. thaliana*, ecotype Estland, and its photomorphogenic mutant, *pho1*, were treated with 5% sodium hypochlorite containing 0.02% Triton X-100 for 5 min, washed thrice with sterile distilled water, followed by three washings with mineral medium.[26] Seeds were finally suspended in 0.1% agar solution prepared in the mineral medium and inoculated in 9.0 cm Petri dishes containing gelled (0.8% agar) mineral medium. Sucrose was added at the 2% level only in the experiment involving the study of its effect on *psbP* gene expression. Seeds were subjected to chilling treatment (at 4°C) for 24–48 h and then illuminated with white light for 6–8 h. Following this light treatment for potentiating seed germination, Petri dishes were either shifted to dark or maintained in continuous light depending upon the requirement. For studying organ-specific expression and extracting total plant DNA, plants were grown in clay pots containing 'Soilrite mix' (a mixture of vermiculite, perlite and *Sphagnum* moss in 1:1:1 ratio; Kelperlite, Bangalore), saturated with mineral medium, under continuous light and at 23 ± 2°C.

2.3. DNA extraction and Southern analysis

Total plant DNA was extracted by the method of Dellaporta et al.[27] and digested separately with several restriction enzymes. Digested DNA samples (8 μg each) were electrophoresed on a 1% agarose gel in 1X TAE buffer. The gel was treated with depurination (0.15 N HCl; 15 min), denaturation (1.5 M NaCl, 0.5 M NaOH; 45 min) and neutralization (1 M Tris-Cl, pH 8.0, 1.5 M NaCl; 45 min) solutions, respectively, and blotted on Hybond-C membrane (Amersham International Inc.). Prehybridization (in 50% formamide, 5X SSC, 5X Denhardt's solution, 5% dextran sulfate, 1% SDS, 200 μg ml^{-1} herring sperm DNA) and hybridization (in 50% formamide, 5X SSC, 0.1% SDS, 5X Denhardt's solution, 10% dextran sulfate, 1% SDS, 250 μg ml^{-1} herring sperm DNA) of the blot were performed at 37°C. The 6.9-kb *Bam*HI fragment from *Arabidopsis* was used as probe after labelling with Multiprime DNA Labelling System (Amersham International Inc.). Subsequently, the blot was washed, in sequence, with 50% formamide, 5X SSC, 0.1% SDS; 5X SSC, 0.1% SDS; 2X SSC, 0.1% SDS, and 1X SSC, 0.1% SDS, each time for 10 min, at room temperature and exposed to X-ray film for autoradiography.

2.4. RNA extraction and northern analysis

RNA was extracted from the tissue following the procedure of Nagy et al.,[28] and quantified spectrophotometrically at 260 nm. Denatured RNA (10–20 μg) was electrophoresed on 1.2% agarose-formaldehyde gel using 1X MOPS buffer.[29] Capillary blotting was done on Hybond-C membrane using 20X SSC. Prehybridization was performed in a solution containing 50% formamide, 5X SSC, 10X Denhardt's solution, 50 mM sodium phosphate buffer (pH 6.5) and 250 μg ml^{-1} herring sperm DNA, at 37°C. The 6.9-kb *Bam*HI fragment from *Arabidopsis*, as above, was used for hybridization in a solution containing 10% dextran sulfate in addition to all other components of the prehybridization solution. The hybridization was performed for 48 h at 37°C. Washing of

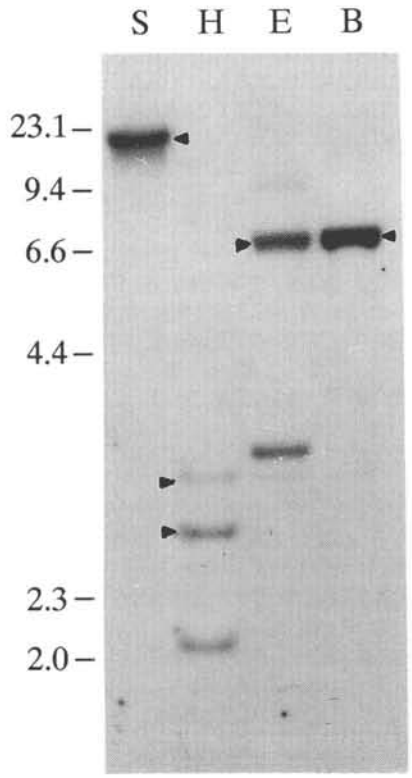

Figure 1. Southern blot analysis of *Arabidopsis* genomic DNA digested with *Sal*I (S), *Hin*dIII (H), *Eco*RI (E) and *Bam*HI (B) using a 6.9-kb *Bam*HI fragment that harbors complete *psbP* gene of *Arabidopsis* alongwith extensive 3′ neighbouring sequences. The genomic fragments which bear complete or a part of the *psbP* gene, are marked by arrowheads. Other fragments appear due to homology with the region downstream of the gene. The uppermost band in lane H is the extended genomic version of the 1.6-kb fragment present on the 5′ end of the probe and contains N-terminus coding region of the gene and more than 2.0-kb of the upstream region (see text). The small *Hin*dIII fragments of 0.3-kb and 0.15-kb became apparent only after very long exposure and were not visible in this autoradiograph. Numerals on left indicate size markers (λDNA *Hin*dIII digest).

blots was done under the conditions described above for washing Southern blot. The blots were also hybridized under similar conditions with a cDNA clone encoding 25S ribosomal RNA[30] to ensure equal loading and integrity of the RNA samples being used.

3. Results and Discussion

3.1. Isolation and characterization of the gene

A genomic library of *Arabidopsis* DNA in EMBL3 vector was screened using a cDNA clone encoding a 23-kDa polypeptide component of the oxygen-evolving complex of photosystem II from spinach.[8] Twenty plaques giving a signal were replated, out of which only 8 yielded overlapping signals in the secondary screening. The number of positive clones was reduced to only two after tertiary screening and their purity was confirmed in quaternary screening. Phage DNA isolated from the two purified clones (designated as 'A' and 'B') was characterized by restriction digestion and Southern analysis using the heterologous probe from spinach. On digestion with *Sal*I, clones 'A' and 'B' revealed single inserts of *ca.* 13- and 15-kb, respectively, which hybridize with the heterologous spinach probe. In *Eco*RI-digested clones, a 7-kb fragment binds the probe. The smallest single hybridizing fragment (*ca.* 6.9-kb) was obtained with *Bam*HI in both the clones. This is also obvious in genomic Southern blots hybridized with the 6.9-kb *Bam*HI fragment of clone 'A', where signals with *Sal*I-, *Hin*dIII-, *Eco*RI-, and *Bam*HI-restricted DNA reflect the presence of single or identical gene(s) within the limit of detection, for 23-kDa polypeptide (Fig. 1). A single *psbP* gene has also been reported earlier in pea[14] and spinach,[8] but multigene families are reportedly present in *Sinapis* (4 genes[16]) and tobacco (4 or more genes[12]). The 6.9-kb *Bam*HI fragment from clone 'A' was subcloned in pBluescript KS$^-$ vector for further characterization. Restriction and Southern analysis of this recombinant clone revealed that three fragments (approximate sizes 0.3-kb, 1.6-kb and 2.7-kb) obtained after digestion with *Hin*dIII give positive signals. These three fragments were further subcloned in pBluescript KS$^-$ vector and sequenced. Another *Hin*dIII fragment of 152 bp was revealed during sequencing of the overlapping region between the 1.6- and 0.3-kb fragments with the help of a oligonucleotide primer. For obtaining complete sequence information of the gene, cloning and sequencing of *Sau*3AI subfragments of the 1.6-kb fragment were also undertaken. Thus, the C-terminus coding region was located on the 2.7-kb fragment, the N-terminus was located on the 1.6-kb fragment, while the 0.15- and 0.3-kb fragments encoded the middle region of the protein.

The nucleotide sequence of the gene alongwith 289 bases of the 5′ region and 146 bases of the 3′ untranslated region is shown in Fig. 2. The gene consists of four exons and three introns as is the case with *psbP* genes of *Sinapis*[16] and tobacco.[11] Length of introns and their sequences show considerable variation in these species with tobacco having particularly longer introns I (516 bases) and II (487 bases) but they share a common feature of all plant introns in being AT-rich.[31] Introns I (111 bases), II (90 bases) and III (255 bases) of *Arabidopsis psbP* gene have 73%, 62.2% and 66.2% of AT base complement, respectively. The GT-AG consensus, found at the borders of most introns[32] has helped in defining the boundaries of all three introns. Also, certain other features of yeast and vertebrate introns[31] such as a branch point sequence (CYRAY, Y = pyrimidine and R = purine), nearly 30 bases upstream from the 3′ splice site, is present in in-

```
   1          GATCATGGAATGGCTTTAGAGTAAAATACTAAAATAGATGGAATCGAAGGCTT
  54  ATGAATCTGACGTGGAACAAGAAGAAAGCGTATCTGCCACGTGGCACAATACAACACCT
 113  CAACAGCCTTATCTGGACATCAACAGAGAGATCCTCCAATATCCGAAACCAACCAATCA
 172  GCTTCACTCTTGGTTTCACCTCCATACATTAACACACCAACAATCCTCAAAAAACACTT
 231  TCATAACTTAATCACACACAAGGAGAGAAGAGAGAAAGAGAGAAAGAGAGACAGAGATA
 290  ATG GCG TAC AGT GCG TGT TTC CTA CAC CAG AGC GCA TTG GCT TCA
       M   A   Y   S   A   C   F   L   H   Q   S   A   L   A   S
 335  TCA GCC GCA CGA TCA TCA TCT TCC TCC TCA TCC CAG CGT CAC GTG
       S   A   A   R   S   S   S   S   S   S   S   Q   R   H   V
 380  TCG CTC TCC AAA CCT GTT CAG ATC ATC TGT AAA GCT CAA CAG TCT
       S   L   S   K   P   V   Q   I   I   C   K   A   Q   Q   S
 425  CAT GAA GAC GAT AAC TCC GCC GTC TCC CGC CGT CTT GCT CTC ACT
       H   E   D   D   N   S   A   V   S   R   R   L   A   L   T
 470  CTC CTC GTC GGC GCC GCT GCT GTT GGT TCC AAA GTA TCT CCT GCT
       L   L   V   G   A   A   A   V   G   S   K   V   S   P   A
 515  GAT GCC GCC TAC GGT GAA GCT G GTGCGTTTATAAAATAAAATAACCTTGGA
       D   A * A   Y   G   E   A
 566  TCATTTCATTTTCTTGAAGAAGAATCTTGAAGAACATATTGTGTTTTTTTCATCCTATT
 625  TTTATTTTTTCGGTTTGTTGCAG CA AAC GTG TTT GGG AAG CCA AAG ACG
                              A   N   V   F   G   K   P   K   T
 674  AAC ACA GAC TTC TTG CCA TAC AAT GGA GAT GGG TTC AAA GTG CAG
       N   T   D   F   L   P   Y   N   G   D   G   F   K   V   Q
 719  GTT CCA GCA AAA TGG AAC CCA AGC AAA GAG ATT GAG TAT CCA GGA
       V   P   A   K   W   N   P   S   K   E   I   E   Y   P   G
 764  CAA GTC CTT AGG TTC GAA GAC AAC TTC GAT GCT ACT AGC AAT CTC
       Q   V   L   R   F   E   D   N   F   D   A   T   S   N   L
 809  AAT GTC ATG GTC ACT CCT ACC GAC AAG AAG TCC ATC ACT GAT TAC
       N   V   M   V   T   P   T   D   K   K   S   I   T   D   Y
 854  GGT TCT CCC GAA GAG TTC CTC TCT CAG GTCTTTGCTTTACTCCATCATCT
       G   S   P   E   E   F   L   S   Q
 904  GTAAAGGTATTGATGTTACAAAATCTAATGAAGTGTGAGACTGAGTGGTGATTTGGGCT
 963  TTATGCAG GTT AAT TAC CTC CTA GGG AAA CAA GCT TAC TTC GGT GA
                V   N   Y   L   L   G   K   Q   A   Y   F   G   E
1009  G ACT GCC TCT GAG GTAAACTTCCCATTGTTATTTGTTATAAGTAAACCAGAAGC
        T   A   S   E
1063  TGATAGAATAGAATAAGAGAATCAAACACTAAAGAGAGAACACATTGTCTCCCTCGTTG
1122  TTAACTGATAAAACAAAACTCAAAAGCTTTTTTAACTGATAACCTTCAAAGACCTCTCT
1181  AGTAGTCGATGATTATTACAAGAAAGATTCTTACCTTAGGTGGAGTGAGTTATCAAGTT
1240  CTAACTCTGTGTGTGTGTGAATCTGATGTTCTTACAG GGA GGC TTT GAC AAC A
                                          G   G   F   D   N
1293  AT GCA GTG GCA ACA GCA AAC ATT CTG GAG TCA TCA TCT CAG GAA
      N   A   V   A   T   A   N   I   L   E   S   S   S   Q   E
1337  GTT GGT GGG AAA CCC TAC TAT TAC TTG TCT GTG TTG ACA AGA ACG
       V   G   G   K   P   Y   Y   Y   L   S   V   L   T   R   T
1382  GCT GAT GGA GAC GAA GGT GGG AAG CAT CAG CTG ATC ACA GCA ACC
       A   D   G   D   E   G   G   K   H   Q   L   I   T   A   T
1427  GTG AAT GGA GGG AAG CTT TAC ATC TGC AAA GCA CAA GCT GGA GAC
       V   N   G   G   K   L   Y   I   C   K   A   Q   A   G   D
1472  AAG AGG TGG TTC AAG GGA GCC AGG AAA TTT GTC GAG AGC GCA GCC
       K   R   W   F   K   G   A   R   K   F   V   E   S   A   A
1517  ACT TCT TTC AGT GTT GCT TGA GTGAAAGCAACACAACGTAACAATGCTCTGC
       T   S   F   S   V   A   Z
1569  TTGCTTTCTTCATTTGTCTCTTGTAAAAAATGGAAAATGAAACTGAGCTTTTGAGAACT
1628  ATCAAGATGATGTTACCTTTTCGGCCATCACTTGTGTACCTATGATAACAGACTCG
```

Figure 2. Nucleotide and deduced amino acid sequence of the *Arabidopsis psbP* gene encoding precursor of 23-kDa polypeptide of oxygen-evolving complex associated with photosystem II. Nucleotides are numbered on the left. The branch point sequences in introns II and III are underlined. Amino acid residues implicated in forming β-pleated sheets, hydrophobic domain and terminal processing site are shown in bold. The asterisk marks the site of terminal processing of the precursor polypeptide.

tron II and intron III, and a 3′ polypyrimidine tract is present in intron I of the *Arabidopsis psbP* gene. The intron/exon boundaries have been further confirmed by comparison of the *Arabidopsis psbP* gene with the *psbP* gene sequence from *Sinapis alba*, a member of the same family (Cruciferae), which share 88.8% homology in the coding region.

The *psbP* gene of *Arabidopsis* codes for a precursor polypeptide of 263 amino acid residues comprising a 77-amino acid-long transit peptide and a 186-amino acid-long mature polypeptide (Fig. 2). Genomic and cDNA sequences of this gene known from other plants also encode a mature polypeptide of almost the same length and length of the transit peptide varies from 72 in tomato[9] to 82 amino acid residues in tobacco.[11,12] The transit peptide (77 amino acid residues) encoded by the *Arabidopsis psbP* gene is three amino acids longer than that from *Sinapis*,[16] both sharing 85.7% homology. Despite differences in the primary sequence of the transit peptide of the 23-kDa polypeptide from divergent species, certain features suggested to be important for the import process of thylakoid lumen proteins[8,33] are clearly present. These include a preponderance of hydroxylated and charged amino acids, a β-sheet-forming region (amino acids 40–43), a hydrophobic domain (amino acids 57–69) and presence of short chain residues at -3 and -1 positions with respect to the terminal cleavage site.[34] Homology at the mature polypeptide level between *Arabidopsis* (present investigation) and *Sinapis*[16] is 92.5%. The deduced size of the mature polypeptide encoded by the *Arabidopsis psbP* gene is 23.5-kDa, while that of the transit peptide is 9.3-kDa. The presence of 22.6% charged residues in the mature polypeptide suggests that the polypeptide is hydrophilic in nature. The context of the AUG start codon consists of an A at -3 position and a G at $+4$ position and thus fulfils the requirement for the optimal context suggested by Kozak[35] which is found only in $\sim$ 35% of eukaryotic mRNA species. Additional AUG codons in the optimal context are also present at nucleotide positions 5 and 38 but are followed by in-frame stop codons after a few bases.

3.2. *Development-dependent expression*

The *psbP* gene encodes a transcript of 1.3-kb in *A. thaliana*. Its expression was examined in 1- to 7-day-old seedlings grown in continuous light or darkness to understand how the gene is regulated by light and endogenous developmental cues. Surprisingly, the pattern of *psbP* gene expression is similar in both dark- and light-grown seedlings although the steady-state transcript level is always higher in light-grown plants (Fig. 3A, B). The level of transcript gradually increases with development and attains a maximum in 3-day-old seedlings, whether grown in dark or light, and thereafter it declines remarkably in dark-grown seedlings, but is maintained at a higher steady-state level in light-grown seedlings. The expression pattern can be correlated with the early seedling development as the third day marks the time when hypocotyl elongates considerably (in dark) and cotyledon expansion also takes place in seedlings exposed to light. Therefore, it is the time when photosynthetic machinery is being established. A certain steady-state level of *psbP* transcript maintained in dark-grown seedlings perhaps allows rapid attainment of photosynthetic competence with the onset of light. The development-dependent accumulation of *psbP* transcript was also checked in the *pho1* mutant displaying light-grown phenotype in dark. In comparison to wild-type, slightly higher level of *psbP* transcript was recorded in 2- to 3-day-old *pho1* seedlings thereby indicating that the product of the wild-type gene (*PHO1*) defined by this mutation is involved in repression of the *psbP* gene during very early stages of seedling growth.

3.3. *Light-induced changes in transcript abundance*

The seedlings grown in dark for 4 days were illuminated with white light for different durations and analyzed for *psbP* transcript abundance. In the case of wild-type *Arabidopsis* seedlings, a prominent increase in transcript level was detectable with even 4 h of illumination, and a steady rise continued thereafter for at least 24 h, before stabilizing at a high steady-state level (Fig. 3C). However, *pho1* seedlings displayed a delay in accumulation of *psbP* gene transcript, as did several other photosynthesis-related nuclear- as well as chloroplast-encoded genes;[20,22] the significant increase in *psbP* transcript level was apparent only with 24 h of illumination, and thereafter increased rather slowly, leading eventually to a considerable delay in attainment of levels comparable to wild-type. This delayed accumulation of photosynthesis-related gene transcripts is paralleled by a delayed accumulation of chlorophyll and carotenoid pigments in similar sets of experiments with *pho1* mutant (data not shown). The data indicate that mutation in the *PHO1* gene causes a general delay in the attainment of photosynthetic competence when dark-grown seedlings are exposed to light and, therefore, defines at least one component of the light signal transduction chain which modulates the chloroplast biogenesis by altering expression levels of genes encoding chloroplast proteins.

3.4. *Dark-adaptation and reinduction kinetics*

Two-week-old plants of *Arabidopsis* grown in continuous light were dark-adapted for various durations and the transcript levels for *psbP* gene were estimated by northern hybridization. Figure 3D shows that the level of *psbP* transcript is reduced significantly after 2 h of dark-adaptation and is virtually undetectable after 24 h in

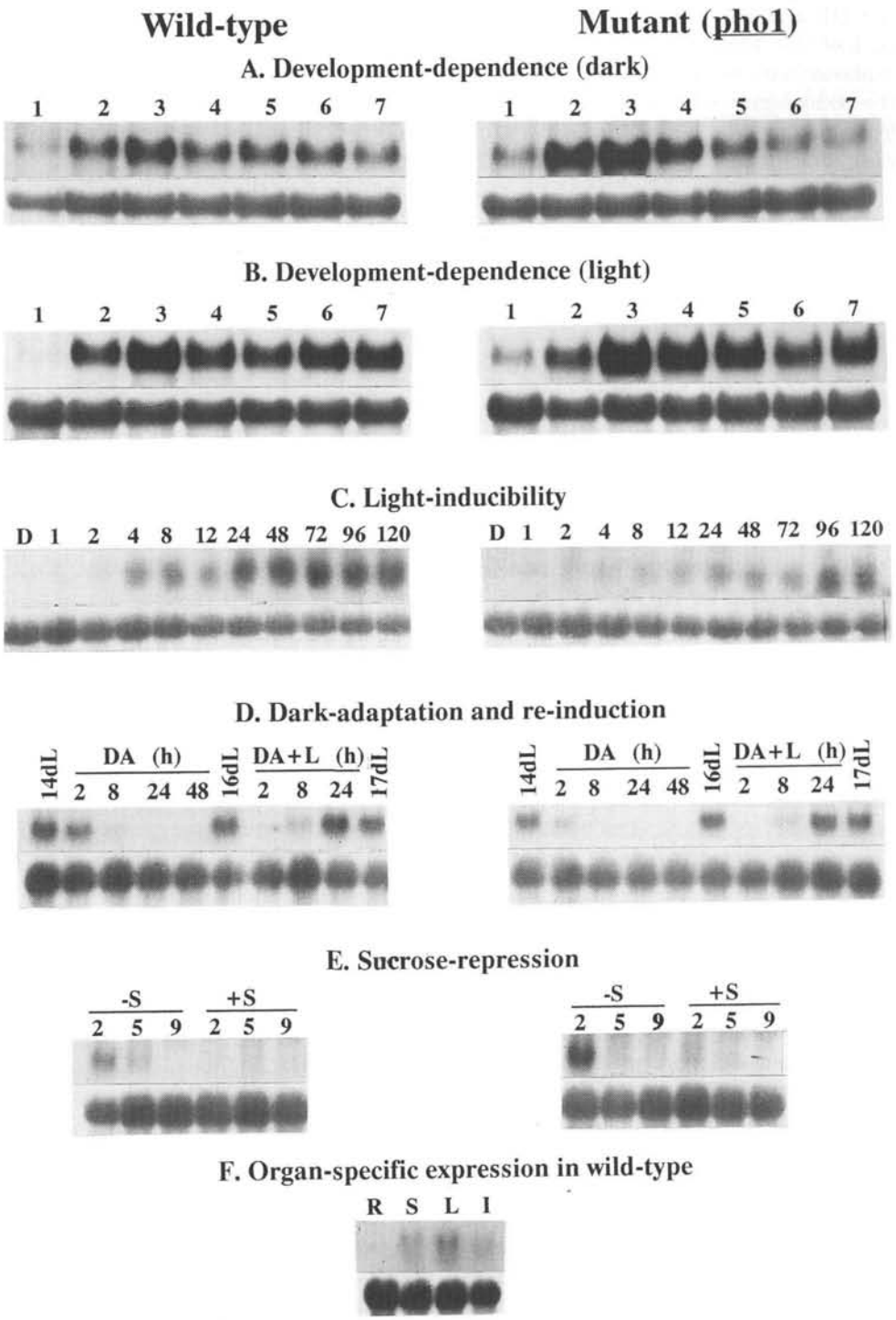

Figure 3. Regulated expression of *psbP* gene (upper row in each set) in *Arabidopsis thaliana* wild-type and a photomorphogenic mutant *pho1* in relation to the control gene for 25S ribosomal RNA (lower row in each set). **A, B**. Effect of temporal development of the seedling in dark (A) or light (B). The numerals represent age of seedlings in days. **C**. Inducibility of gene by light after transfer of 4-day-old dark-grown seedlings (D) to light. Numerals represent hours of illumination. **D**. Gene expression during dark-adaptation (DA) of 14-day-old light-grown plants (14 dL) for various durations (h). After dark-adaptation for 48 h, plants were again exposed to light, DA + L (h). 16 dL and 17 dL represent controls grown in light for 16 and 17 days. **E**. Sucrose-dependent regulation of gene expression in seedlings grown in dark for varying days (see numerals), S for sucrose. **F**. Organ-specific expression of *psbP* gene in root (R), stem (S), leaf (L), and inflorescence (I) of 36-day-old plants grown in continuous light. Variation in transcript levels for similar treatments in different vertical panels is due to development of autoradiographs for different period in order to show the influence of most effective treatment.

dark. Re-irradiating these plants with white light causes an increased accumulation of *psbP* transcript within 8 h and the steady-state transcript level, as observed in continuous light-grown plants of the same age (17-day-light), is attained within 24 h. The *pho1* plants when examined in this perspective show similar pattern of *psbP* transcript accumulation except that the transcript level in general is relatively lower than wild-type which could be due to reduced greening of leaves of the mutant. Another interesting observation is that the level of *psbP* transcript after 24 h of illumination of the dark-adapted plants is higher than the continuous light-grown plants of the same age (17-day-light). This could perhaps be to enable the plants to quickly regain its optimal photosynthetic ability. The results of the dark-adaptation experiment also suggest that the *PHO1* product may not be important in down-regulation of *psbP* expression in dark-adapted plants.

3.5. Effect of sucrose

Metabolizable sugars like sucrose and glucose have been shown to repress expression of photosynthetic genes including *cab*[36,37] and *rbcS*.[38,39] This has been suggested as the mechanism for feedback regulation of photosynthesis. The effect of sucrose on *Arabidopsis psbP* gene was analyzed in 2-, 5- and 9-day-old wild-type and *pho1* seedlings grown in dark in a medium with or without sucrose. As shown in Fig. 3E, sucrose does repress distinctly the accumulation of *psbP* transcript in 2-day-old wild-type as well as *pho1* seedlings. A similar effect has also been reported earlier for *cab* gene of *Arabidopsis*.[40] Despite repression by sucrose, the level of *psbP* transcript is higher (though marginally) in 2-day-old *pho1* seedlings, thereby indicating that the feedback regulation by this metabolite occurs by a pathway independent of the one which represses photomorphogenic development in the dark-grown seedlings.

3.6. Organ-specific expression

Total RNA was isolated from roots, bolting stalks (without the cauline leaves), rosette leaves and inflorescence of 36-day-old wild-type plants grown under continuous light conditions and analysed for steady-state level of *psbP* transcript. The highest expression was detectable in leaves, followed by bolting stalks and inflorescence, respectively (Fig. 3F). The *psbP* transcript was almost undetectable in roots. The expression can thus be correlated with the photosynthetic competence of the tissue; in the case of inflorescence, sepals and pedicels fit into this criteria. This kind of expression pattern has also been reported for *psbP* gene of pea.[14]

In conclusion, *A. thaliana psbP* gene isolated and sequenced in this investigation shows an intron/exon arrangement similar to the genes from *Sinapis*[16] and tobacco.[11] Several important features of the gene and its product have also been described. The expression of *psbP* gene of *Arabidopsis* is organ-specific and is regulated by several intrinsic and extrinsic factors. Therefore, it provides a tool to study how different regulatory signals act together to affect the expression of a particular gene. In this context, we have studied the expression of this gene in a photomorphogenic mutant of *Arabidopsis* and the studies reveal the activity of at least one cellular effector, PHO1, which is partly responsible for dark-repression of *psbP* gene in 2- to 3-day-old seedlings and also participates in the rapid induction of this gene (and several other photosynthesis-related genes) on illumination of young dark-grown seedlings. It is worth mentioning here that genetic allelism has been found between a constitutively photomorphogenic mutant *cop3*[41] and *pho1*[20] but differences are still evident at the phenotypic level, particularly in flowering time,[20] which could be due to mutations at different sites on the gene. Moreover, the effect of *cop3* mutation on photosynthetic gene expression has not been reported in detail and differences between *pho1* and *cop3* may be revealed in this regard as well on further investigation. This approach, if extended to other possible combinations of mutants and the responses evoked by the affected pathways, will help resolve the components of intricate molecular circuitry driving temporal/spatial/inducible expression of various genes as well as other downstream responses.

Acknowledgements: We thank Professor Dr. R. G. Herrmann and Dr. R. Oelmuller (Munich) for kindly providing us cDNA probe from spinach and the genomic library of *Arabidopsis thaliana*, ecotype Columbia, respectively, and Prof. S. C. Maheshwari for encouragement and keen interest in this work. This research was funded by the Department of Science and Technology and the University Grants Commission, New Delhi. AK acknowledges the award of Senior research fellowship by the Council of Scientific and Industrial Research, New Delhi.

References

1. James, H. E., Bartling, D., Musgrove, J. E., Kirwin. P. M., Herrmann, R. G., and Robinson, C. 1989, Transport of proteins into chloroplasts. Import and maturation of precursors to the 33, 23 and 16 kDa proteins of the oxygen-evolving complex, *J. Biol. Chem.*, **264**, 19573–19576.
2. Mayfield, S. P., Rahire, M., Frank, G., Zuber, H., and Rochaix, J.-D. 1987, Expression of the nuclear gene encoding oxygen-evolving enhancer protein 2 is required for high levels of photosynthetic oxygen evolution in *Chlamydomonas reinhardtii*, *Proc. Natl. Acad. Sci. USA*, **84**, 749–753.
3. Andersson, B., Critchley, C., Ryrie, I. J., Jansson, C., Larsson, C., and Anderson, J. M. 1984, Modification of

the chloride requirement for photosynthetic O_2 evolution. The role of 23 kDa polypeptide, *FEBS Lett.*, **168**, 113–117.
4. Ghanotakis, D. F., Topper, J. N., Babcock, G. T., and Yocum, C. F. 1984, Water-soluble 17 and 23 kDa polypeptides restore oxygen evolution activity by creating a high-affinity binding site for Ca^{2+} on the oxidizing side of photosytem II, *FEBS Lett.*, **170**, 169–173.
5. Homann, P. H. 1988, The chloride and calcium requirement of photosynthetic water oxidation: effects of pH, *Biochim. Biophys. Acta*, **934**, 1–13.
6. Adelroth, P., Lindberg, K., and Andreasson, L.-E. 1995, Studies of Ca^{2+} binding in spinach photosystem II using $^{45}Ca^{2+}$, *Biochemistry*, **34**, 9021–9027.
7. Herrmann, R. G., Oelmuller, R., Bichler, J. et al. 1991, The thylakoid membrane of higher plants: genes, their expression and interaction, In: Herrmann, R. G., Larkins, B. A. (eds), Plant Molecular Biology 2, Plenum Press, New York, pp. 411–427.
8. Jansen, T., Rother, C., Steppuhn, J. et al. 1987, Nucleotide sequence of cDNA clones encoding the complete '23 kDa' and '16 kDa' precursor proteins associated with the photosynthetic oxygen-evolving complex from spinach, *FEBS Lett.*, **216**, 234–240.
9. Betts, S. and Pichersky, E. 1992, Nucleotide sequence of cDNA encoding the precursor of the 23 kDa photosystem II protein of tomato, *Plant Mol. Biol.*, **18**, 995–996.
10. Hua, S.-B., Dube, S. K., Barnett, N. M., and Kung, S.-D. 1991, Nucleotide sequence of a cDNA clone encoding 23 kDa polypeptide of the oxygen-evolving complex of photosystem II in tobacco, *Nicotiana tabacum* L., *Plant Mol. Biol.*, **16**, 749–750.
11. Hua, S.-B., Dube, S. K., Barnett, N. M., and Kung, S.-D. 1991, Nucleotide sequence of gene *oee2-A* and its cDNA encoding 23 kDa polypeptide of the oxygen-evolving complex of photosystem II in tobacco, *Plant Mol. Biol.*, **17**, 551–553.
12. Hua, S.-B., Dube, S. K., Barnett, N. M., and Kung, S.-D. 1992, Photosystem II 23 kDa polypeptide of oxygen-evolving complex is encoded by a multigene family in tobacco, *Plant Mol. Biol.*, **18**, 997–999.
13. James, H. E. and Robinson, C. 1991, Nucleotide sequence of cDNA encoding the precursor of the 23 kDa protein of the photosynthetic oxygen-evolving complex from wheat, *Plant Mol. Biol.*, **17**, 179–182.
14. Wales, R., Newman, B. J., Rose, S. A., Pappin, D., and Gray, J. C. 1989, Characterization of cDNA clones encoding the extrinsic 23 kDa polypeptide of the oxygen-evolving complex of photosystem II in pea, *Plant Mol. Biol.*, **13**, 573–582.
15. Yoshiba, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., and Harada, Y. 1995, Characterization of a cDNA clone encoding 23 kDa polypeptide of the oxygen-evolving complex of photosystem II in rice, *Plant Cell Physiol.*, **36**, 1677–1682.
16. Merkle, T., Krenz, M., Wenng, A., and Schafer, E. 1990, Nucleotide sequence and deduced amino acid sequence of a gene encoding the 23 kDa polypeptide of the oxygen-evolving complex from mustard (*Sinapis alba* L.), *Plant Mol. Biol.*, **14**, 889–890.
17. Wenng, A., Ehmann, B., and Schafer, E. 1989, The 23 kDa polypeptide of the photosynthetic oxygen-evolving complex from mustard seedlings (*Sinapis alba* L.). Nucleotide sequence of cDNA and evidence for phytochrome control of its mRNA abundance, *FEBS Lett.*, **246**, 140–144.
18. Kretsch, T., Emmler, K., and Schafer, E. 1995, Spatial and temporal pattern of light-regulated gene expression during tobacco seedling development: the photosystem II-related genes *Lhcb* (*cab*) and *psbP* (*Oee2*), *Plant J.*, **7**, 715–729.
19. Chory, J. 1993, Out of darkness: mutants reveal pathways controlling light-regulated development in plants, *Trends Genet.*, **9**, 167–172.
20. Khurana, J. P., Kochhar, A., and Jain, P. K. 1996, Genetic and molecular analysis of light-regulated plant development, *Genetica*, **97**, 349–361.
21. McNellis, T. W. and Deng, X.-W. 1995, Light control of seedling morphogenetic pattern, *Plant Cell*, **7**, 1749–1761.
22. Kochhar, A., Jain, P. K., Sharma, R. P., Tyagi, A. K., and Khurana, J. P. 1994, Isolation and characterization of a new class of constitutively photomorphogenic mutants of *Arabidopsis*, XVI Internatl. Cong. Biochem. Mol. Biol., New Delhi, Abst. No. P1-111.
23. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
24. Santos, M. A. 1991, An improved method for the small scale preparation of bacteriophage DNA based on phage precipitation by zinc chloride, *Nucl. Acids Res.*, **19**, 5442.
25. Sanger, F., Nicklen, S., and Coulson, A. R. 1977, DNA sequencing with chain terminating inhibitors, *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
26. Somerville, C. R. and Ogren, W. L. 1982, Isolation of photorespiration mutants in *Arabidopsis thaliana*, In: Edelman, M., Hallick, R. B., Chua, N.-H. (eds), Methods in Chloroplast Molecular Biology, Elsevier Biomedical Press, New York, pp. 129–139.
27. Dellaporta, S. L., Wood, J., and Hicks, J. B. 1983, A plant DNA minipreparation: version II, *Plant Mol. Biol. Rep.*, **1**, 19–21.
28. Nagy, F., Kay, S. A., and Chua, N.-H. 1988, Analysis of gene expression in transgenic plants, In: Gelvin, S. B., Schilperoort, R. A., Verma, D. P. S. (eds), Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordrecht, pp. B4/1–B4/29.
29. Ausubel, F. M., Brent, R., Kingston, R. E. et al. 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York.
30. Silverthorne, J. and Tobin, E. M. 1984, Demonstration of transcriptional regulation of specific genes by phytochrome action, *Proc. Natl. Acad. Sci. USA*, **81**, 1112–1116.
31. Goodall, G. J. and Filipowicz, W. 1989, The AU-rich sequences present in the introns of plant nuclear pre-mRNAs are required for splicing, *Cell*, **58**, 473–483.
32. Breathnach, R. and Chambon, P. 1981, Organization and expression of eucaryotic split genes coding for proteins,

Ann. Rev. Biochem., **50**, 349–383.
33. Tyagi, A., Hermans, J., Steppuhn, J., Jansson, C., Vater, F., and Herrmann, R. G. 1987, Nucleotide sequence of cDNA clones encoding the complete "33 kDa" precursor protein associated with the photosynthetic oxygen-evolving complex from spinach, *Mol. Gen. Genet.*, **207**, 288–293.
34. von Heijne, G., Steppuhn, J., and Herrmann, R. G. 1989, Domain structure of mitochondrial and chloroplast targeting peptides, *Eur. J. Biochem.*, **180**, 535–541.
35. Kozak, M. 1986, Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes, *Cell*, **44**, 283–292.
36. Harter, K., Talke-Messerer, C., Barz, W., and Schafer, E. 1993, Light- and sucrose-dependent gene expression in photomixotrophic cell suspension cultures and protoplasts of rape (*Brassica napus* L.), *Plant J.*, **4**, 507–516.
37. Sheen, J. 1990, Metabolic repression of transcription in higher plants, *Plant Cell*, **2**, 1027–1038.
38. Cheng, C.-L., Acedo, G. N., Cristinsin, M., and Conkling, M. A. 1992, Sucrose mimics the light induction of *Arabidopsis* nitrate reductase gene transcription, *Proc. Natl. Acad. Sci. USA*, **89**, 1861–1864.
39. Krapp, A., Hofmann, B., Schafer, C., and Stitt, M. 1993, Regulation of the expression of *rbcS* and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis?, *Plant J.*, **3**, 817–828.
40. Brusslan, J. A. and Tobin, E. M. 1992, Light-independent developmental regulation of *cab* gene expression in *Arabidopsis thaliana* seedlings, *Proc. Natl. Acad. Sci. USA*, **89**, 7791–7795.
41. Hou, Y., von Arnim, A. G., and Deng, X.-W. 1993, A new class of *Arabidopsis* constitutive photomorphogenic genes involved in regulating cotyledon development, *Plant Cell*, **5**, 329–339.