The EMBO Journal vol.7 no.5 pp.1289-1297, 1988

Light-dependent changes in *psbD* and *psbC* transcripts of barley chloroplasts: accumulation of two transcripts maintains *psbD* and *psbC* translation capability in mature chloroplasts

Patricia E.Gamble, Tineke Berends Sexton and John E.Mullet

Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843-2128, USA

Communicated by J.D.Rochaix

The *psbD* and *psbC* genes encode two polypeptides of Photosystem II. These genes are adjacent in the barley chloroplast genome and are part of a 5.7 kbp transcription unit. In dark-grown barley, four large transcripts hybridize to *psbD* and *psbC*; two additional transcripts hybridize to *psbC*. Illumination of 4.5-day-old dark-grown seedlings causes a decrease in the six *psbD–psbC* transcripts found in etioplasts and the accumulation of two different transcripts of 4.0 and 3.2 kb which hybridize to *psbD* and *psbC*. The light-induced transcripts have a common 5′ end ~600 nt upstream of *psbD* and 3′ ends 1175 and 175 nt downstream of *psbC*. The shift in *psbD–psbC* transcript population occurs during a phase of chloroplast maturation when transcript levels and translation of chloroplast genes such as *psaA–psaB* and *psbB* decline ~3- to 5-fold. In contrast, translation of the *psbD* and *psbC* gene products declines to a lesser extent, suggesting that the light-induced accumulation of the 4.0 and 3.2 kb *psbD–psbC* transcripts is required to maintain *psbD* and *psbC* gene product translation in mature chloroplasts.

Key words: barley/chloroplast/light/RNA/translation

Introduction

Photosystem II (PSII) is one of four multisubunit complexes of chloroplast thylakoid membranes involved in photosynthetic electron transport (for review, see Gounaris *et al.*, 1986). The PSII complex, consisting of a reaction center core, a water-splitting apparatus and a light-harvesting antennae complex, functions as a water:plastoquinone oxidoreductase. PSII contains 12–18 different polypeptides which are encoded on either the chloroplast or nuclear genomes as well as bound cofactors such as chlorophylls, carotenoids, quinones and hemes. The PSII reaction center core consists of at least eight plastid-encoded polypeptides which include several chlorophyll- and/or quinone-binding proteins (gene products of *psbA*, *psbB*, *psbC* and *psbD*) and the two apoproteins of $cytb_{559}$ (*psbE* and *psbF* gene products). PSII also contains nuclear-encoded polypeptides including those involved in oxygen-evolution and the chlorophyll *a*/*b*-binding proteins of the light-harvesting complex (LHCII) (Gounaris *et al.*, 1986). Thus, the synthesis and assembly of PSII requires coordination between nuclear and chloroplast gene expression as well as coordination between cofactor and apoprotein synthesis.

The effect of light on chloroplast development and plant gene expression has been extensively investigated (for review, see Tobin and Silverthorne, 1985; Ellis, 1986). In dark-grown plants, proplastids develop into etioplasts which are photosynthetically incompetent since they lack chlorophyll and many of the polypeptides associated with light-harvesting and light-driven electron transport (Kirk and Tilney-Bassett, 1978). For instance, the nuclear-encoded chlorophyll *a*/*b*-binding proteins of the light-harvesting complex of PSII (*cab* gene products) are absent in dark-grown plants, and the levels of their corresponding transcripts are low (Apel, 1979; Bennett, 1981; Gollmer and Apel, 1983). Activation of the phytochrome system upon illumination results in an increase in *cab* gene mRNA and in the synthesis of the chlorophyll *a*/*b*-binding proteins. This increase in *cab* gene mRNA appears to be due to a phytochrome-induced increase in the rate of transcription, rather than to a change in transcript stability (Silverthorne and Tobin, 1984; Mosinger *et al.*, 1985; Steinmuller *et al.*, 1985). In maize, illumination has also been reported to increase the abundance of several plastid-encoded transcripts, particularly those of the *psbA* and *rbcL* genes (Bedbrook *et al.*, 1978; Nelson *et al.*, 1984; Rodermel and Bogorad, 1985; Zhu *et al.*, 1985). While these studies have indicated that light affects chloroplast gene expression at the level of transcription, other studies have shown that plastid gene expression is also regulated post-transcriptionally through RNA processing (Hanley-Bowdoin *et al.*, 1985; Mullet *et al.*, 1985; Westhoff, 1985) and at the level of translation (Altman *et al.*, 1984; Fromm *et al.*, 1985; Berry *et al.*, 1986; Klein and Mullet, 1986).

Klein and Mullet (1986) have reported the lack of synthesis of a select number of thylakoid polypeptides in dark-grown barley plastids. These include the 65–68 kd chlorophyll-apoproteins of PSI (*psaA–psaB* gene products), the 47 and 43 kd chlorophyll-apoproteins of PSII (*psbB* and *psbC* gene products), and a 32 kd polypeptide of PSII (*psbA* gene product). Despite the absence of these plastid-encoded polypeptides, transcripts which hybridize to the *psaA–psaB*, *psbA* and *psbC* genes are present in barley etioplasts (Oliver and Poulsen, 1984; Klein and Mullet, 1986; Berends *et al.*, 1987). Upon illumination of dark-grown barley seedlings, protochlorophyllide is reduced and esterified to chlorophyll and the chlorophyll-apoproteins of PSI and PSII and the *psbA* gene product are rapidly synthesized (Vierling and Alberte, 1983; Klein *et al.*, 1986; Klein and Mullet, 1986; Kreuz *et al.*, 1986). Based on pulse-label experiments and Northern blot analyses, it was concluded that synthesis of the chlorophyll-apoproteins of PSI and the *psbA* gene product is post-transcriptionally regulated and that the absence of these proteins in etioplasts likely results from inhibition of translation or rapid protein turnover (Klein and Mullet, 1986, 1987).

In a previous study, we localized the *psbD* and *psbC* genes on the barley chloroplast genome and characterized the transcripts which hybridize to these genes in plastids of dark-

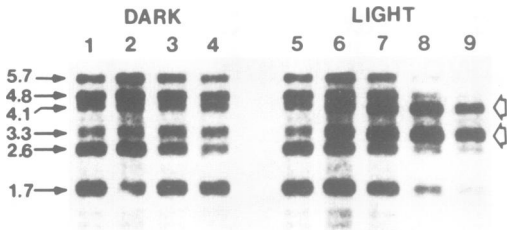

Fig. 1. Northern blot analysis of *psbD–psbC* mRNA. RNA was isolated from an equal number of plastids from 4.5-day-old dark-grown seedlings (**lanes 1** and **5**) or from seedlings kept in darkness for an additional 36 (**lane 2**), 72 (**lane 3**) or 108 h (**lane 4**). RNA was also isolated from 4.5-day-old dark-grown seedlings transferred to the light for 16 (**lane 6**), 36 (**lane 7**), 72 (**lane 8**) or 108 h (**lane 9**). Blots were hybridized with a nick-translated 1.27 kbp *Hin*dIII–*Eco*RI DNA probe from the *psbD–psbC* transcription unit. The numbers next to lane 1 refer to the sizes (kb) of the *psbD–psbC* transcripts observed in etioplasts. The large, open arrows next to lane 9 mark the two transcripts which accumulate during light-induced plastid development.

grown plants (Berends *et al.*, 1987). In spinach (Alt *et al.*, 1984; Holschuh *et al.*, 1984) and pea (Rasmussen *et al.*, 1984; Bookjans *et al.*, 1986), *psbD* (encoding a 32–33 kd polypeptide of PSII) and *psbC* (encoding the 43 kd chlorophyll-apoprotein of PSII) form a transcription unit whose open reading frames overlap by ~50 bp. It is likely that in barley the *psbD* and *psbC* genes are aligned in a similar way (Berends *et al.*, 1987). Barley etioplasts were found to contain at least six transcripts, ranging in size from 5.7 to 1.7 kb, that hybridize to the *psbD–psbC* transcription unit (Berends *et al.*, 1987). Although etioplasts contain transcripts which hybridize to *psbD* and *psbC*, synthesis of the *psbC* gene product was not detected in barley etioplasts (Klein and Mullet, 1986), while synthesis of a polypeptide which comigrates with the *psbD* gene product was detected (Klein and Mullet, 1987).

In this paper, we have examined *psbD* and *psbC* transcripts during plastid development in dark-grown and illuminated barley seedlings to determine the relationship between changes in transcript population and translation of the *psbD* and *psbC* gene products. The present results indicate that illumination of dark-grown barley causes a marked accumulation of two transcripts containing both *psbD* and *psbC* sequences which were barely detectable in etioplasts. Our results further indicate that accumulation of the two light-induced *psbD–psbC* transcripts is important for maintenance of *psbD–psbC* gene expression and PSII activity in mature chloroplasts.

Results

Levels of psbD–psbC transcripts in plastids of dark-grown and illuminated seedlings

In the present study, Northern blot analyses were performed to determine whether the induction in *psbC* gene product translation observed during light-induced plastid development (Klein and Mullet, 1986) was paralleled by a change in either the level or pattern of *psbD–psbC* transcripts. Plastids of 4.5-day-old dark-grown barley contained four transcripts (5.7–3.3 kb in length) which hybridized to *psbD* and *psbC*, and two additional transcripts (2.6 and 1.7 kb) which hybridized to *psbC* (Figure 1, lanes 1 and 5). After 6 days darkness, a slight increase in the level of all six transcripts was observed (lane 2), and thereafter *psbD–psbC* transcripts declined to levels close to those found in plastids of 4.5-day-old dark-grown plants (lanes 3 and 4).

Illumination of dark-grown seedlings for 16 or 36 h resulted in an increased level of all six *psbD–psbC* transcripts (Figure 1, lanes 6 and 7) similar to the level observed in plastids from 6-day-old dark-grown seedlings (lane 2). However, illumination of seedlings for 72–108 h resulted in a significant decline in the level of several *psbD–psbC* transcripts (lanes 8 and 9). Transcripts of 5.7, 4.8, 2.6 and 1.7 kb were barely detectable after 72 h of illumination (lane 8). In contrast, two transcripts of ~4.1 and 3.3 kb were abundant in plants illuminated for 72 and 108 h (lanes 8 and 9). Close examination of the autoradiogram revealed that the two abundant transcripts in illuminated plants were slightly smaller than the 4.1 and 3.3 kb RNAs present in etioplasts (compare lane 5 to 8). Therefore, illumination of dark-grown barley resulted in a decline in the level of all six *psbD–psbC* transcripts observed in etioplasts, and in the appearance of two different transcripts.

Mapping the boundaries of the light-induced transcripts by Northern analysis

To map the boundaries of the two *psbD–psbC* transcripts exhibiting light-induced accumulation, Northern blot analyses were performed using a series of nick-translated DNA fragments from the *psbD–psbC* transcription unit. Plastid RNA isolated from 4.5-day-old dark-grown seedlings (Figure 2, odd-numbered lanes) or from seedlings transferred to the light for an additional 72 h (Figure 2, even-numbered lanes) was hybridized to the nick-translated probes shown below the restriction map in Figure 2 (fragments A to I). The autoradiograms of each Northern blot are shown below the corresponding probes in panels A to I.

When fragment B was hybridized to etioplast RNA (Figure 2, lane 3) four transcripts of 5.7, 4.8, 4.1 and 3.3 kb were observed. These RNAs have previously been mapped by Berends *et al.* (1987) and extend beyond the 3′ end of the *psbC* open reading frame. After hybridization of fragment B to plastid RNA from seedlings illuminated for 72 h, a different *psbD–psbC* transcript pattern was obtained (lane 4). In addition to a decline in the level of the four transcripts observed in etioplasts, two new transcripts of 4.0 and 3.2 kb (marked by dashes) were observed (compare lane 3 to 4). These two RNAs were not detected when chloroplast RNA was hybridized with fragment A (lane 2) indicating that a terminus of the 4.0 and 3.2 kb light-induced transcripts lies within the 0.55 kbp *Cla*I–*Eco*RI fragment (probe B) just upstream of *psbD*. The larger of the two light-induced transcripts (4.0 kb) extended through fragment I (lane 18) but not beyond (data not shown). This placed the other terminus of the 4.0 kb RNA within the 0.52 kbp *Sal*I–*Xba*I fragment common to probes H and I. The smaller of the two light-induced transcripts (3.2 kb) extended through fragment F but not into fragment G (compare lane 12 to 14). Since probe G (*Bam*HI–*Eco*RI) completely overlaps with probe F (*Pst*I–*Eco*RI), one terminus of the 3.2 kb RNA lies within the 0.44 kbp *Pst*I–*Bam*HI fragment. Based on the Northern analyses the two light-induced transcripts contain both *psbD* and *psbC* sequences.

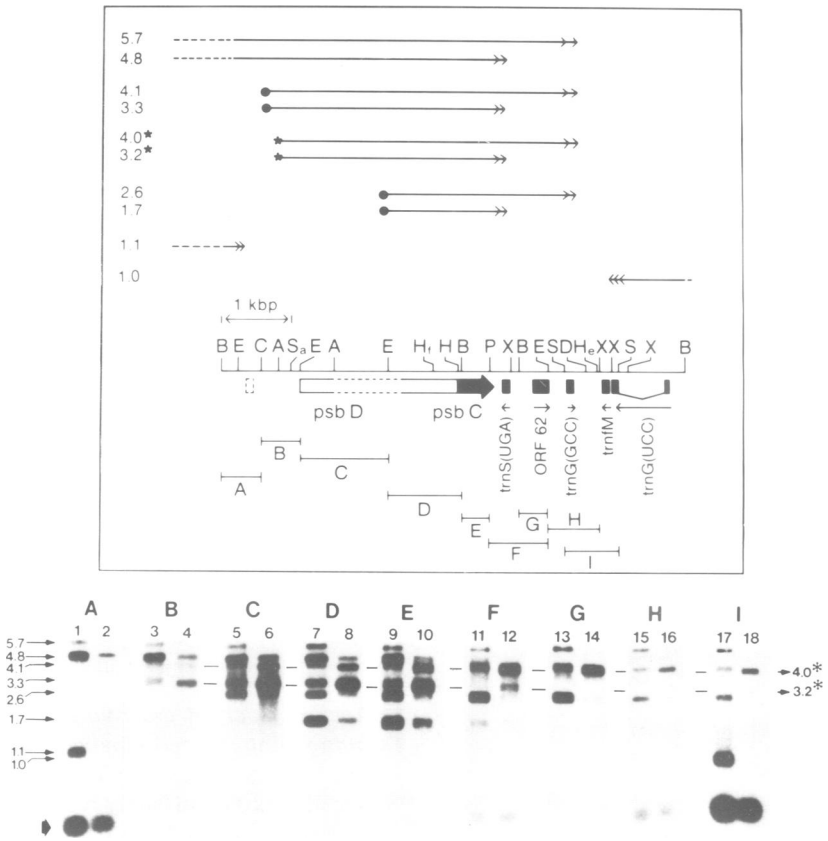

Fig. 2. Northern blot analysis of plastid RNA isolated from 4.5-day-old dark-grown seedlings (odd-numbered lanes) or from 4.5-day-old dark-grown seedlings which had been illuminated for an additional 72 h (even-numbered lanes). **Panels A–I** show RNAs which hybridized to DNA probes from the *psbD–psbC* transcription unit (probes A–I shown below the restriction map of *psbD–psbC*). The numbers next to lane 1 refer to the approximate sizes (kb) of the transcripts observed in etioplasts. The large, closed arrow next to lane 1 marks the position of tRNA-sized transcripts. The 4.0 and 3.2 kb RNAs which accumulate upon illumination are marked by the dashed line beside each panel of the autoradiogram. The location of RNAs which have been mapped in the DNA region containing *psbD* and *psbC* are shown at the top of Figure 2. Asterisks mark the 5′ end of the light-induced 4.0 and 3.2 kb RNAs. The solid circles indicate 5′ ends of transcripts observed in plastids of dark-grown plants.

Several other transcripts were observed when the nick-translated DNA fragments shown in Figure 2 were hybridized to plastid RNA. The previously characterized 2.6 and 1.7 kb *psbC* transcripts (Berends *et al.*, 1987) hybridized to fragments C to I and fragments C to F, respectively, and the level of both these transcripts declined significantly when plants were illuminated for 72 h (compare lane 7 to 8). In addition, the previously mapped 1.1 and 1.0 kb RNAs (Berends *et al.*, 1987) declined to almost undetectable levels when plants were illuminated for 72 h, while the level of putative tRNAs of 0.1 kb or less remained the same (lanes 1, 2, 11, 12, 15–18).

Determination of position and orientation of the 4.0 and 3.2 kb transcripts

From the Northern hybridizations presented in Figure 2 the approximate boundaries of the two light-induced *psbD–psbC* transcripts were identified. Using RNA size markers the two transcripts were estimated to be 4.0 and 3.2 kb in length. Therefore, these two light-induced transcripts are ~100–150 nt smaller than the 4.1 and 3.3 kb RNAs detected in etioplasts, respectively. S1 nuclease protection and primer extension analyses were conducted to map the 5′ terminus of the 4.0 and 3.2 kb transcripts (Figure 3). When etioplast RNA was hybridized to a doubly 5′ end-labeled 0.88 kbp *Eco*RI probe located just upstream of *psbD* (lane 5) and digested with S1 nuclease, a 480 bp protected fragment was generated (lane 4, marked by closed arrow). This band marks the 5′ terminus of the 4.1 and 3.3 kb transcripts (Berends *et al.*, 1987). Other protected fragments of ~800 and 465 bp were also detected in lane 4, but have been previously shown to be S1 nuclease artifacts (Berends *et al.*, 1987). The results obtained by the S1 nuclease protection experiment were confirmed by primer extension analysis (Figure 3, lanes 2 and 3). Primer extension from a 135 bp *Sau*96I–*Eco*RI probe (5′ end-labeled at the *Eco*RI site) (lane 2) verified the presence of the RNA 5′ terminus located ~480 bp upstream from the *Eco*RI site (lane 3).

To map the location of the 5′ end of the light-induced 4.0 and 3.2 kb transcripts, S1 nuclease protection and primer extension assays were done using plastid RNA isolated from seedlings illuminated for 108 h. S1 nuclease treatment of chloroplast RNA hybridized to the same doubly 5′ end-labeled *Eco*RI probe (lane 5) produced the 480 bp band corresponding to the 5′ end of the 4.1 and 3.3 kb RNAs as well as a protected fragment of ~320 bp (lane 6, marked by open arrow). The 320 bp fragment was not generated when etioplast RNA was used for the S1 nuclease protection experiment (lane 4). This result indicates the presence of a light-induced RNA 5′ terminus upstream of *psbD*. To determine the orientation of this 5′ end, a reverse transcription experiment was performed. Primer extension from the 135 bp *Sau*96I–*Eco*RI fragment (5′ end-labeled at the *Eco*RI site) (lane 8) confirmed the presence of a light-induced RNA

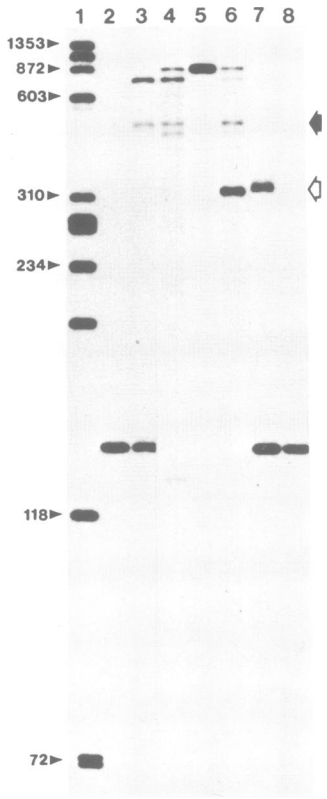

Fig. 3. S1 nuclease protection and primer extension assays mapping the 5′ terminus of the light-induced 4.0 and 3.2 kb transcripts. The numbers beside **lane 1** indicate the sizes (bp) of some of the end-labeled ϕX174/*Hae*III fragments used as mol. wt markers. **Lanes 2** and **8** show the 135 bp *Sau*96I−*Eco*RI probe (5′ end-labeled at the *Eco*RI site) which was used for reverse transcription in **lanes 3** and **7**. **Lane 5** shows the corresponding 0.88 kbp *Eco*RI−*Eco*RI probe (labeled at both 5′ ends) used for the S1 nuclease protection assays in **lanes 4** and **6**. For the reverse transcription and S1 nuclease protection assays shown in **lanes 3** and **4**, respectively, RNA was isolated from plastids of 4.5-day-old dark-grown seedlings. For the S1 nuclease protection and reverse transcription assays shown in lanes 6 and 7, respectively, RNA was isolated from plastids of 4.5-day-old dark-grown seedlings which had been illuminated for an additional 108 h. The large, black arrow marks the 480 bp fragment (lanes 3, 4, 6 and 7) corresponding to the 5′ terminus of the 4.1 and 3.3 kb RNAs. The large, open arrow marks the 320 bp fragment (lanes 6 and 7) corresponding to the 5′ end of the 4.0 and 3.2 kb RNAs which accumulate during light-induced plastid development.

5′ terminus located ~320 bp upstream from the *Eco*RI site of fragment B (lane 7). However, the 320 bp fragment produced by the S1 nuclease protection experiment did not co-migrate exactly with that produced by the primer extension assay. This could be due to anomalous migration of the fragment as a result of the different salt conditions used in the two assays. Alternatively, the slightly smaller S1 nuclease protected fragment might be due to digestion of the probe by S1 nuclease which would not occur in the primer extension assay (compare lane 6 to 7). In any case, the Northern blots, S1 nuclease protection experiment, and primer extension assay all indicated the presence of a light-induced 5′ end ~320 bp upstream from the *Eco*RI site of fragment B. We have assigned this 5′ terminus to the 4.0 and 3.2 kb transcripts which show light-induced accumulation.

The 3′ ends of the 4.0 and 3.2 kb RNAs were also mapped by S1 nuclease protection experiments. S1 nuclease mapping using either etioplast or chloroplast RNA indicated that illumination of dark-grown seedlings did not result in the appearance of any new light-induced 3′ ends (data not shown). The 3′ end of the 4.0 kb RNA was located ~1175 bp downstream of *psbC*, whereas the 3′ end of the 3.2 kb RNA was located ~175 bp downstream from *psbC*. It should be noted that 3′ end mapping of the 3.3 kb transcript observed in dark-grown seedlings and the 3.2 kb light-induced transcript indicated that these two transcripts have a common 3′ end (data not shown). However, the 3.2 kb transcript hybridized weakly to probe F in the Northern analysis shown in Figure 2 (lane 12), whereas hybridization of the 3.3 kb transcript was not detected (lane 11). The weak hybridization of the 3.2 kb transcript and the lack of hybridization of the 3.3 kb transcript to probe F may be the result of secondary structure within this region due to the presence of a *trnS* gene (Oliver and Poulsen, 1984). Furthermore, the difference in hybridization of the 3.3 and 3.2 kb transcripts to probe F may reflect the greater abundance of the 3.2 kb transcript in plastids of 72 h illuminated plants compared to the level of the 3.3 kb transcript in etioplasts (Figure 2, compare lane 9 to 10). The mapped positions of all the transcripts observed in both etioplasts and chloroplasts are shown in the top of Figure 2.

Accumulation of the two psbD−psbC transcripts during light-induced plastid development

The Northern blot shown in Figure 1 indicates that illumination of dark-grown barley results in the accumulation of two transcripts which after 72 h of illumination are the most abundant transcripts which hybridize to the *psbD−psbC* transcription unit. However, because the 4.1 and 4.0 kb RNAs and the 3.3 and 3.2 kb RNAs are not well separated on the gel, it is difficult to determine when the 4.0 and 3.2 kb transcripts first accumulate during light-induced chloroplast development. To overcome this difficulty, we have used primer extension assays to examine the accumulation of the 5′ terminus corresponding to the 4.0 and 3.2 kb RNAs during plastid development in dark-grown and illuminated seedlings (Figure 4A). When the 135 bp *Sau*96I−*Eco*RI probe (5′ end-labeled at the *Eco*RI site) (lane 2) was hybridized to etioplast RNA from 4.5-day-old seedlings and extended with AMV reverse transcriptase, a 480 bp fragment was produced (lane 3, marked by closed arrow). This fragment, which marks the 5′ end of the 4.1 and 3.3 kb RNAs, is a very prominent band in RNA extracted from plastids of 4.5- to 9-day-old dark-grown barley (lanes 3−6). A faint band of ~320 bp was also produced (lanes 3−6, marked by open arrow). This band corresponds to the 5′ terminus of the two RNAs which exhibit light-induced accumulation, indicating that plastids from dark-grown seedlings contain low levels of both the 4.0 and 3.2 kb transcripts. The abundance of the 320 bp fragment in etioplast preparations was variable (compare lane 3 to 9), but in all etioplast populations examined it was much less than the levels observed in plastids of illuminated plants.

When the reverse transcription probe (lane 8) was hybridized to plastid RNA isolated from 16 h illuminated seedlings and extended with reverse transcriptase, two fragments of 480 and 320 bp were generated (lane 10). With continued illumination the level of the 480 bp fragment declined gradually, while the level of the 320 bp band increased (lanes 10−13). These results show that the abundance of the 4.0 and 3.2 kb light-induced transcripts increased in plastids of plants illuminated for 16 h, and that continued illumination

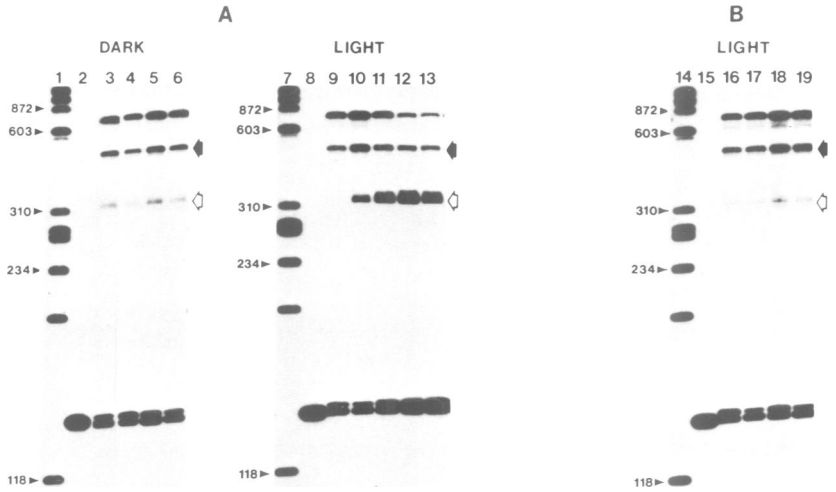

Fig. 4. Primer extension analysis of the 4.0 and 3.2 kb *psbD–psbC* transcripts in plastids of dark-grown and illuminated barley seedlings. Numbers next to **lanes 1, 7** and **14** refer to the sizes (bp) of some of the end-labeled ϕX174/*Hae*III fragments used as mol. wt markers. **Lanes 2, 8** and **15** show the 135 bp *Sau*96I–*Eco*RI DNA probe (5′ end-labeled at the *Eco*RI site) used for primer extension in **lanes 3–6, 9–13** and **16–19**. (**A**) The denatured DNA probe was hybridized with plastid RNA from 4.5-day-old dark-grown seedlings (lanes 3 and 9), or from seedlings kept in darkness for an additional 36 (lane 4), 72 (lane 5), or 108 h (lane 6), and extended with AMV reverse transcriptase. The denatured DNA probe was also hybridized to plastid RNA isolated from 4.5-day-old dark-grown seedlings transferred to the light for an additional 16 (lane 10), 36 (lane 11), 72 (lane 12) or 108 h (lane 13) and extended with AMV reverse transcriptase. (**B**) The 135 bp *Sau*96I–*Eco*RI DNA probe shown in lane 15 was used for primer extension on plastid RNA isolated from 4.5-day-old dark-grown seedlings (lane 16) or seedlings transferred to the light for 5 min (lane 17), 15 min (lane 18) or 30 min (lane 19). The large, closed arrow marks the 480 bp fragment corresponding to the 5′ terminus of the 4.1 and 3.3 kb RNAs. The large, open arrow marks the 320 bp fragment corresponding to the 5′ end of the 4.0 and 3.2 kb RNAs which accumulate upon illumination.

Table I. *psbD* RNA levels in dark-grown and illuminated barley seedlings

Treatment	RNA level (%)
4.5-days (dark)	100
7.5-days (dark)	89 ± 7
4.5-days (dark) + 72-h (light)	98 ± 19

Quantitation of RNA levels was conducted by Northern dot blot analyses. Plastids were isolated from 4.5-day-old dark-grown seedlings, 7.5-day-old dark-grown seedlings and 4.5-day-old dark-grown seedlings transferred to the light for an additional 72 h. Total nucleic acid was isolated from each plastid population by phenol extraction. Each sample was treated with DNase I and RNA from 0.25 × 10^6 plastids was applied to the individual wells. The blot was incubated with a nick-translated *psbD–psbC* probe (probe C, Figure 2), washed and autoradiographed. After exposure to X-ray film, the radioactive spots were excised from the filter and counted in a scintillation counter. 100% represents an average of 1263 ± 31.82 c.p.m. (n = 2). Each data point represents the mean of two observations ± SD.

of plants results in marked accumulation of both transcripts.

As previously stated, net synthesis of the *psbC* gene product is not observed in barley etioplasts, but synthesis is detected after 15 min of illumination and near maximum by 1 h (Klein and Mullet, 1986). To determine whether the light-induced accumulation of the 4.0 and 3.2 kb *psbD–psbC* transcripts correlates with the rapid induction in *psbC* gene product translation, primer extension analyses were performed with plastid RNA isolated from dark-grown seedlings (Figure 4B, lane 16) or from seedlings transferred to the light for 5, 15 or 30 min (lanes 17–19). Illumination of seedlings for 5 to 30 min (lanes 17–19) did not result in increased accumulation of the 4.0 and 3.2 kb RNAs, indicating that the rapid induction in *psbC* gene product synthesis occurs prior to the shift in *psbD–psbC* transcript population.

Quantitation of psbD transcript levels during plastid development

Our next objective was to determine if the light-induced shift in the *psbD–psbC* transcript pattern resulted in a change in *psbD* RNA levels. This was done by quantitating transcripts which contain *psbD* sequences in plastids from dark-grown and illuminated seedlings by Northern dot blots (Table I). The probe used in this analysis hybridizes to the four large transcripts found in etioplasts and the two light-induced transcripts which contain *psbD* sequences (probe C, Figure 2). This analysis showed that *psbD* transcript levels remained high during plastid development. When compared to 4.5-day-old dark-grown seedlings, *psbD* RNA levels declined 11% in 7.5-day-old dark-grown plants and only 2% in dark-grown plants illuminated for an additional 72 h. The transcripts detected by the probe used to quantitate *psbD* RNA also contain *psbC* sequences. However, this probe does not hybridize significantly to the 1.7 and 2.6 kb transcripts which contain the entire *psbC* open reading frame but not the entire *psbD* open reading frame. The 1.7 and 2.6 kb transcripts declined in plants illuminated for 72–108 h (Figure 1, lanes 8 and 9) indicating that *psbC* transcript levels decrease relative to *psbD* transcript levels during light-induced chloroplast development.

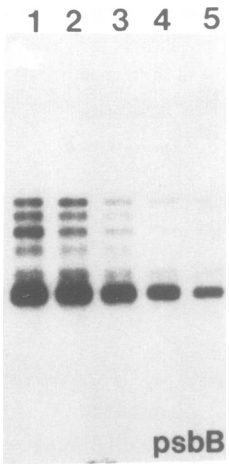

Fig. 5. Northern blot analysis of *psbB* mRNA. RNA was isolated from an equal number of plastids from 4.5-day-old dark-grown seedlings (**lane 1**) or from seedlings transferred to the light for 16 (**lane 2**), 36 (**lane 3**), 72 (**lane 4**) or 108 h (**lane 5**). The blot was hybridized with a nick-translated 1.1 kbp *Bgl*II−*Hin*dIII DNA fragment from the *psbB* gene.

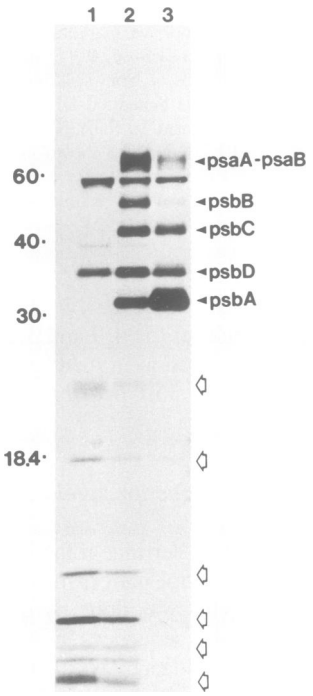

Fig. 6. *In vivo* labeled plastid polypeptides from 4.5-day-old dark-grown and illuminated barley seedlings. Barley leaves from 4.5-day-old dark-grown seedlings (**lane 1**), and dark-grown seedlings illuminated for 16 h (**lane 2**) or 72 h (**lane 3**) were incubated with [^{35}S]methionine and cycloheximide. Following incubation, isolated plastids were fractionated and the membrane polypeptides electrophoresed on an 8 M urea/SDS−PAGE gel (loaded on an equal c.p.m./lane basis) and autoradiographed. Numbers to the left of lane 1 indicate mobility of M_r standards (kd). Large open arrows to the right of lane 3 mark radiolabeled polypeptides whose synthesis declined by 72 h of illumination.

Transcript levels for psbB during chloroplast development

The above results show that during chloroplast development, *psbD* transcript levels remain high compared to dark controls (Table I). Recently, Mullet and Klein (1987) have shown that *psbA* (encoding a 32 kd polypeptide of PSII) transcript levels also remain elevated in plants illuminated for 72−108 h. To determine whether transcripts for another plastid-encoded PSII protein are maintained during chloroplast development, transcript levels for the *psbB* gene (encoding the 47 kd chlorophyll-apoprotein of PSII) were examined by Northern blot anlaysis. When a nick-translated probe from the *psbB* gene of pea was hybridized with etioplast RNA, six transcripts were observed (Figure 5, lane 1). The complex transcript pattern for the *psbB* transcription unit detected in barley is similar to that observed in spinach (Westhoff, 1985; Westhoff *et al.*, 1986) and pea (Berends *et al.*, 1986). As shown in Figure 5, *psbB* transcripts were present in chloroplasts of 16 h illuminated seedlings at levels similar to those found in etioplasts (compare lane 1 to 2). However, with continued plastid development in the light, the level of all six *psbB* transcripts declined over 3-fold (Figure 5, lanes 3−5). Therefore, *psbB* transcripts decline relative to *psbA* and *psbD* transcripts in plants illuminated for 72−108 h.

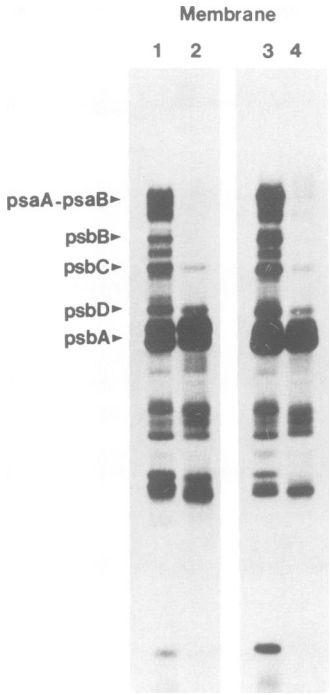

Fig. 7. Autoradiogram of membrane polypeptides synthesized by plastids isolated from dark-grown seedlings illuminated for 16 or 72 h. Plastids were isolated from 4.5-day-old dark-grown seedlings transferred to the light for 16 (**lanes 1** and **3**) or 72 h (**lanes 2** and **4**). Intact plastids were incubated for 10 min in the presence of [^{35}S]methionine and then chased for 0 (lanes 1 and 2) or 10 min (lanes 3 and 4) with excess unlabeled methionine. The plastids were fractionated and the membrane polypeptides loaded on an 8 M urea/SDS−PAGE gel on an equal plastid basis.

In vivo protein synthesis in plastids of dark-grown and illuminated seedlings

Our next objective was to determine if a correlation existed between *psbD* and *psbC* transcript levels and translation of the *psbD* and *psbC* gene products in chloroplasts of il-

luminated barley plants. When barley leaves of 4.5-day-old dark-grown seedlings and 4.5-day-old dark-grown seedlings illuminated for 16 or 72 h were incubated with [^{35}S]methionine and cycloheximide, the protein patterns shown in Figure 6 were obtained. As previously demonstrated (Klein and Mullet, 1986, 1987), synthesis of the chlorophyll-apoproteins of PSI (*psaA–psaB* gene products) and PSII (*psbB* and *psbC* gene products) and a 32 kd polypeptide of PSII (gene product of *psbA*) was not detected in plastids of dark-grown barley (Figure 6, lane 1). In contrast, synthesis of the *psbD* gene product and numerous other membrane proteins was detected in barley etioplasts (lane 1). After 16 h of illumination synthesis of the *psaA–psaB*, *psbB* and *psbC* chlorophyll-apoproteins as well as the *psbA* and *psbD* gene products was observed (Figure 6, lane 2). However, after 72 h of illumination synthesis of the *psaA–psaB* and *psbB* chlorophyll-apoproteins declined (lane 3), as did synthesis of several low M_r polypeptides (marked by open arrows). In contrast, translation of the *psbC*, *psbD* and *psbA* gene products showed no apparent decline in plastids from tissue illuminated for 72 h (lane 3).

Protein synthesis in isolated plastids from illuminated barley seedlings

Rates of polypeptide synthesis *in vivo* can be underestimated if rapid protein turnover occurs during the labeling period. Therefore, protein synthesis in plastids isolated from dark-grown seedlings illuminated for an additional 16 or 72 h was assayed using a 10 min pulse-labeling period (Figure 7). When expressed on an equal plastid basis, several changes in plastid polypeptide synthesis were observed in plastids isolated from 16 h illuminated tissue compared to plastids isolated from seedlings illuminated for 72 h (Figure 7, compare lane 1 to 2). After 16 h of illumination the *psaA–psaB*, *psbB* and *psbC* chlorophyll-apoproteins and the *psbA* and *psbD* gene products were the major membrane polypeptides synthesized by isolated intact plastids (Figure 7, lane 1). In seedlings illuminated for 72 h, synthesis of several membrane polypeptides including the *psaA–psaB* and *psbB* chlorophyll-apoproteins was barely detectable (Figure 7, lane 2). In contrast, intact plastids isolated from tissue illuminated for 72 h continued to synthesize the *psbA*, *psbD* and *psbC* gene products (lane 2). While the rate of *psbA* gene product synthesis was similar in plastids isolated from tissue illuminated for 16 or 72 h, the synthesis of both the *psbD* and *psbC* gene products had declined after 72 h of illumination (lane 2). However, the decrease in *psbC* gene product synthesis exceeded the decline in *psbD* gene product synthesis which correlates with the greater decline in *psbC* transcript levels relative to *psbD* transcript levels in these plastids (Figure 1 and Table I).

To determine whether polypeptides synthesized in isolated intact plastids were undergoing rapid turnover, plastids were pulse-labeled for 10 min then chased with excess unlabeled methionine for a further 10 min (lanes 3 and 4). As shown in Figure 7, the plastid polypeptide profiles obtained after the chase period were similar to those following the pulse-label (compare lane 1 to 3, and lane 2 to 4) indicating that protein turnover was minimal during the pulse-labeling period. Therefore, the decline in synthesis of the *psaA–psaB* and *psbB* chlorophyll-apoproteins observed in intact plastids isolated from plants illuminated for 72 h was not the result of protein turnover. Finally, the incorporation of [^{35}S]methionine into the *psbC* and *psaA–psaB* gene products relative to the *psbA* and *psbD* gene products was greater under *in vivo* labeling conditions than in the 10 min pulse label assay (compare Figure 6 lane 3 to Figure 7 lane 2). This difference in incorporation likely reflects more rapid turnover of the *psbA* and *psbD* gene products which would lead to an underestimation of the rate of *psbA* and *psbD* gene product synthesis given the long *in vivo* label period (2.5 h). Nevertheless, these results demonstrate that plants illuminated for 72 h continued to synthesize the *psbA*, *psbD* and *psbC* gene products while synthesis of the *psaA–psaB* and *psbB* chlorophyll-apoproteins declined.

Discussion

In this paper we have examined the expression of two genes which encode polypeptides of the PSII core (*psbD* and *psbC*). This analysis revealed that illumination of 4.5-day-old dark-grown barley seedlings caused a decline in six *psbD–psbC* transcripts, and the accumulation of two different transcripts which contain both *psbD* and *psbC* sequences. The change in *psbD–psbC* transcript population was detectable after 16 h of illumination and by 72–108 h the change in RNA population was nearly complete.

Light-induced modification of plastid gene transcript populations is not unique to the *psbD–psbC* transcription unit. Illumination of maize (Bedbrook *et al.*, 1978), *Spirodella* (Reisfeld *et al.*, 1978) or mustard (Link, 1982; Hughes *et al.*, 1987) causes *psbA* transcript levels to increase, however, the accumulation of *psbA* transcripts is not strictly light-dependent and high levels can accumulate in dark-grown plants (Klein and Mullet, 1986). The ratio of two transcripts which hybridize to *rbcL* has also been reported to change when plants are illuminated (Crossland *et al.*, 1984; Poulsen, 1984). This effect, which results from changes in the relative ratio of transcription and RNA processing (Hanley-Bowdoin *et al.*, 1985; Mullet *et al.*, 1985) can also be observed in dark-grown plants (Klein and Mullet, unpublished results). The processing of *psbB* transcripts also follows a complex pathway (Herrmann *et al.*, 1985; Westhoff *et al.*, 1986), but here again RNA processing is not strictly light-dependent. To our knowledge, the accumulation of the 4.0 and 3.2 kb *psbD–psbC* transcripts is the first example of a change in vascular plant plastid transcript population which is tightly regulated by light. The mechanism which controls this modification of the *psbD–psbC* transcript population in illuminated plants could involve RNA processing or a change in RNA transcription. These possibilities are presently under investigation.

The significance of the light-induced change in *psbD–psbC* transcript population may be related to the need for relatively high levels of *psbD* gene product translation in mature chloroplast populations as well as during rapid chloroplast biogenesis. During rapid chloroplast biogenesis, overall plastid transcription and translation activity are high (Klein and Mullet, 1987; Mullet and Klein, 1987). The plastids of 4.5-day-old dark-grown plants which had been illuminated for 16 h showed high rates of translation of numerous proteins (Figure 7, lane 1, gene products of *psaA–psaB*, *psbA*, *psbB*, *psbC* and *psbD*). However, by 72 h of illumination, mature populations of chloroplasts have been formed and the translation of many of the plastid proteins had declined significantly (Figure 7, lane 2, gene products of *psaA–psaB* and *psbB*). This result is consistent with

reduced transcription activity, ribosome content, and mRNA levels for *psaA–psaB* and *psbB* in plastids isolated from plants illuminated for 72 h (Klein and Mullet, 1987; Mullet and Klein, 1987; and Figure 5). In contrast to the genes described above, translation of the *psbA* and *psbD* gene products remained relatively high in plants illuminated for 72 h (Figure 7, lane 2).

High rates of *psbA* gene product translation in plastids of plants illuminated for 72 h is correlated with high levels of *psbA* mRNA (Klein and Mullet, 1987). The maintenance of *psbA* mRNA in this plastid population is due, in part, to increased stability of these transcripts (Mullet and Klein, 1987). High rates of *psbD* gene product synthesis, relative to *psaA–psaB* or *psbB* in barley plants illuminated for 72 h, is also correlated with the maintenance of *psbD* transcript levels (Figure 1 and Table I). In this case, illumination results in a decrease of transcripts containing *psbD* sequences found in plastids of dark-grown plants and in accumulation of two different transcripts which contain *psbD* sequences. This observation leads us to propose that the generation of the light-induced *psbD* transcripts is necessary for the maintenance of *psbD* gene product translation in illuminated plants.

The high rates of *psbA* gene product synthesis in chloroplasts of illuminated plants have been correlated with rapid turnover of this protein (Mattoo *et al.*, 1984; Ohad *et al.*, 1985). It is hypothesized that the *psbA* gene product becomes inactivated during PSII photochemistry which necessitates continued degradation and resynthesis in order to maintain PSII function (Kyle *et al.*, 1984). The requirement for *psbD* gene product translation in plants illuminated for 72 h may have a similar explanation. This possibility is consistent with studies on bacterial reaction centres (Deisenhofer *et al.*, 1984; Hearst and Sauer, 1984) and isolated PSII reaction center complexes (Danielius *et al.*, 1987; Nanba and Satoh, 1987) which indicate that the *psbA* and *psbD* gene products form a heterodimer which binds cofactors involved in primary charge separation. Therefore, it seems possible that the *psbD* gene product, like the *psbA* gene product, could become inactivated during PSII photochemistry. In addition, turnover of inactivated *psbA* gene product could destabilize the *psbD* gene product thereby leading to its turnover and resynthesis. This idea is consistent with increased turnover of PSII polypeptides in mutants which lack the *psbA* gene product (Bennoun *et al.*, 1986). In summary, our data suggest that in barley seedlings illuminated for 72 h, continued synthesis of the *psbA* and *psbD* gene products may be involved in the maintenance of PSII activity.

The *psbD* gene product was synthesized in barley etioplasts whereas translation of the *psbA* gene product was not detected (Figure 6). Furthermore, Klein and Mullet (1986) have shown that when dark-grown plants were illuminated for 15 min, the synthesis of the *psbA* gene product was greatly stimulated, whereas *psbD* gene product translation was unaffected. In *Chlamydomonas reinhardtii*, a mutant which lacks the wild-type *psbD* gene product does not synthesize the *psbA* gene product (Erickson *et al.*, 1986). This suggests that *psbA* translation is regulated by the presence of the *psbD* gene product. The translation of the *psbD* gene product in etioplasts, therefore, could be required for rapid activation of *psbA* translation when barley seedlings are illuminated.

PsbC gene product synthesis is also rapidly increased when dark-grown barley seedlings are illuminated (Klein and Mullet, 1986). However, the time course of accumulation of the light-induced *psbD–psbC* transcripts did not correlate with light-mediated activation of *psbC* gene product translation (Figure 4B; and Klein and Mullet, 1986). Instead, light-induced synthesis of the *psbC* gene product, like *psbA*, *psbB*, *psaA* and *psaB* gene product synthesis is correlated with photoreduction of protochlorophyllide to chlorophyll (Klein *et al.*, 1986). *PsbC* gene product synthesis could occur on transcripts which also contain *psbD* sequences. In addition, translation could occur on transcripts of 1.7 and 2.6 kb in size which do not contain the entire *psbD* sequence. The 1.7 and 2.6 kb *psbC* transcripts are abundant in etioplasts, but these transcripts show reduced levels in chloroplasts of plants illuminated for 72–108 h (Figure 1). Translation of the *psbC* gene product is also reduced in plants illuminated for 72 h relative to plants illuminated for 16 h (Figure 7). Therefore, reduced translation of the *psbC* gene product relative to the *psbD* gene product may be due, in part, to reduced abundance of the 1.7 and 2.6 kb transcripts after 72 h of illumination.

Materials and methods

Plant growth

Barley seeds (*Hordeum vulgare* L. var Morex) were planted in vermiculite, watered with full-strength Hoaglands nutrient solution, and maintained in controlled environment chambers at 23°C. Seedlings were grown for 4.5 days in a dark chamber located in a light-tight room. After 4.5 days in the dark, seedlings were either kept in the dark or were transferred to an illuminated chamber (light intensity of 123 $W/m^2/s$, fluorescent plus incandescent bulbs) for an additional 16, 36, 72 or 108 h. For one experiment, 4.5-day-old dark-grown seedlings were transferred to a lighted chamber for 5, 15 or 30 min prior to tissue harvest. When possible all manipulations of dark-grown plants were performed in complete darkness, however, when necessary, light was provided by a dim green safelight (Klein and Mullet, 1986).

Plastid isolation

Plastids were isolated from the apical 3 cm of the barley seedlings by Percoll gradient centrifugation as previously described (Klein and Mullet, 1986). All manipulations were performed at 2–4°C. For quantitation of plastid number (plastids/μl of suspension volume), intact plastids were diluted and counted in a hemocytometer with a ×20 phase contrast lens.

Isolation of plastid nucleic acid

Total nucleic acid was isolated from intact plastids by phenol extraction as desribed previously (Mullet *et al.*, 1985). The nucleic acid was resuspended in TE buffer (10 mM Tris–HCl, pH 8.0, 0.1 mM EDTA) on a per plastid basis. For isolation of plastid RNA, a portion of the total nucleic acid was treated with RQ1-DNase I (Promega Biotech), phenol extracted and precipitated twice.

Northern blot analysis

Northern blot analyses were performed by separating total nucleic acid on 0.8% agarose gels containing 6% formaldehyde according to Maniatis *et al.* (1982). Nucleic acid was transferred to Gene Screen membranes by NENs capillary blot procedure. Northern probes were nick-translated using a nick translation kit. Nick-translated λ/*Hin*dIII fragments, which did not hybridize to barley plastid nucleic acid, were used to visualize the RNA markers (data not shown). Methods used for hybridization and washing of the filters were as described previously (Berends *et al.*, 1987).

S1 nuclease and primer extension assays

5′ end DNA labeling was done according to Maniatis *et al.* (1982). S1 nuclease protection and primer extension assays were performed as described previously (Mullet *et al.*, 1985). Labeled fragments were analyzed on 8% polyacrylamide–8.3 M urea gels (Maxam and Gilbert, 1980).

Quantitation of psbD RNA

Quantitation of *in vivo* levels of *psbD* RNA was performed by blotting DNase-treated RNA extracted from 0.25×10^6 plastids onto a Gene Screen

Plus membrane using a Schleicher and Schuell minifold apparatus. Prior to transfer the RNA was denatured in 12% formaldehyde, 4 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate) at 60°C for 10 min and quenched on ice 2 min. The prehybridizaiton and hybridization conditions were as described previously (Berends *et al.*, 1987). The blot was analyzed by autoradiography followed by excision of the radioactive spots and counting in a scintillation counter. Methods for DNA labeling were as described (Berends *et al.*, 1987). The DNA probe for the *psbD* mRNA was a 1.26 kbp *Eco*RI fragment from the *psbD–psbC* transcription unit of barley (Berends *et al.*, 1987).

In vivo labeling

Barley leaves of 4.5-day-old dark-grown seedlings and 4.5-day-old dark-grown seedlings illuminated for an additional 16 or 72 h were incubated with [^{35}S]methionine and cycloheximide as previoulsy described (Klein and Mullet, 1986). Following the labeling period, intact plastids were isolated by Percoll gradient centrifugation and fractionated into membrane and soluble polypeptides as described previously (Mullet *et al.*, 1986). Measurements of trichloroacetic acid-insoluble radioactivity were obtained (Mans and Novelli, 1961) and the membrane protein samples were subsequently electrophoresed on an 8 M Urea/SDS–PAGE gel (Klein and Mullet, 1987). The gel was fixed, stained and fluorographed as previously described (Bonner and Laskey, 1974).

Protein synthesis in isolated intact plastids from illuminated tissue

ATP-driven protein synthesis by intact chloroplasts was conducted as previously described (Klein and Mullet, 1986; Mullet *et al.*, 1986) except the [^{35}S]methionine concentration was 140 μCi, and 7.5 mM ATP and 7.5 mM $MgCl_2$ was used for plastids isolated from seedlings illuminated for 72 h. Intact plastids were added at a final concentration of $\sim 1.375 \times 10^7$ plastids/75 μl. Plastids were incubated for 10 min at which time unlabeled methionine (8.5 mM final concentration) was added to block further incorporation of [^{35}S]methionine into protein (the chase period was 10 min).

Following the labeling period, intact plastids were fractionated into membrane and soluble phases and the membrane polypeptides electrophoresed as described above. Following electrophoresis the gel was cut into strips and either autoradiographed (Bonner and Laskey, 1974) or electrophoretically transferred to Gene Screen membranes according to the method of Towbin *et al.* (1979). The *psbB*, *psbC*, *psbD* and *psbA* gene products were identified by immunological detection after incubation of the protein blots with antisera specific for each protein followed by reaction with 5-bromo-4-chloro-3-indoylephosphate (BCIP) in the presence of Nitroblue tetrazolium (NBT) at pH 9.5 (data not shown).

Acknowledgements

We would like to thank Dr R.R.Klein for his help in conducting the plastid protein synthesis assays and for critical discussions during the preparation of this manuscript. We also thank Dr W.Vermaas for providing the antisera to the *psbA* and *psbD* gene products and Dr N.H.Chua for the antisera to the *psbB* and *psbC* gene products. This work was supported by grant no. GM37987-01 from the National Institutes of Health to J.E.M.

References

Alt,J., Morris,J., Westhoff,P. and Herrmann,R.G. (1984) *Curr. Genet.*, **8**, 597–600.

Altman,A., Cohen,B.N., Weissbach,H. and Brot,N. (1984) *Arch. Biochem. Biophys.*, **235**, 26–33.

Apel,K. (1979) *Eur. J. Biochem.*, **97**, 183–188.

Bedbrook,J.R., Link,G., Coen,D.M., Bogorad,L. and Rich,A. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 3060–3064.

Bennett,J. (1981) *Eur. J. Biochem.*, **118**, 61–70.

Bennoun,P., Spierer-Herz,M., Erickson,J., Girard-Bascou,J., Pierre,Y., Delosme,M. and Rochaix,J.-D. (1986) *Plant Mol. Biol.*, **6**, 151–160.

Berends,T., Kubicek,Q. and Mullet,J.E. (1986) *Plant Mol. Biol.*, **6**, 125–134.

Berends,T., Gamble,P.E. and Mullet,J.E. (1987) *Nucleic Acids Res.*, **15**, 5217–5240.

Berry,J.O., Nikolau,B.J., Carr,J.P. and Klessig,D.F. (1986) *Mol. Cell. Biol.*, **6**, 2347–2353.

Bonner,W.M. and Laskey,R.A. (1974) *Eur. J. Biochem.*, **46**, 83–88.

Bookjans,G., Stummann,B.M., Rasmussen,O.F. and Henningsen,K.W. (1986) *Plant Mol. Biol.*, **6**, 359–366.

Crossland,L.D., Rodermel,S.R. and Bogorad,L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4060–4064.

Danielius,R.V., Satoh,K., van Kan,P.J.M., Plijter,J.J., Nuijs,A.M. and van Gorkom,H.J. (1987) *FEBS Lett.*, **213**, 241–244.

Deisenhofer,J., Epp,O., Miki,K., Huber,R. and Michel,H. (1984) *J. Mol. Biol.*, **180**, 385–398.

Ellis,J.R. (1986) *Biosci. Rep.*, **6**, 127–136.

Erickson,J.M., Rahire,M., Malnoe,P., Girard-Bascou,J., Pierre,B. and Rochaix,J.-D. (1986) *EMBO J.*, **5**, 1745–1754.

Fromm,H., Devic,M., Fluhr,R. and Edelman,M. (1985) *EMBO J.*, **4**, 291–295.

Gollmer,I. and Apel,K. (1983) *Eur. J. Biochem.*, **133**, 309–313.

Gounaris,K., Barber,J. and Harwood,J.L. (1986) *Biochem. J.*, **237**, 313–326.

Hanley-Bowdoin,L., Orozco,E.M. and Chua,N.-H. (1985) *Mol. Cell. Biol.*, **5**, 2733–2745.

Hearst,J.E. and Sauer,K. (1984) *Z. Naturforsch.*, **39c**, 421–424.

Herrmann,R.G., Westhoff,P., Alt,J., Tittgen,J. and Nelson,N. (1985) In Van Vloten-Doting,L., Groot,G.S.P. and Hall,T.C. (eds), *Molecular Form and Function of the Plant Genome*. Plenum, New York, pp. 233–256.

Holschuh,K., Bottomley,W. and Whitfeld,P.R. (1984) *Nucleic Acids Res.*, **12**, 8819–8834.

Hughes,J.E., Neuhaus,H. and Link,G. (1987) *Plant Mol. Biol.*, **9**, 355–363.

Kirk,J.T.O. and Tilney-Bassett,R.A.E. (1978) *The Plastids: Their Chemistry, Structure, Growth, and Inheritance*. Elsevier/North Holland Biomedical Press, Amsterdam.

Klein,R.R. and Mullet,J.E. (1986) *J. Biol. Chem.*, **261**, 11138–11145.

Klein,R.R., Gamble,P.E. and Mullet,J.E. (1986) *Curr. Topics Plant Biochem. Physiol.*, **5**, 74–87.

Klein,R.R. and Mullet,J.E. (1987) *J. Biol. Chem.*, **262**, 4341–4348.

Kreuz,K., Dehesh,K. and Apel,K. (1986) *Eur. J. Biochem.*, **159**, 459–467.

Kyle,D.J., Ohad,I. and Arntzen,C.J. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4070–4074.

Link,G. (1982) *Planta*, **154**, 81–86.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mans,R.J. and Novelli,G.D. (1961) *Arch. Biochem. Biophys.*, **94**, 48–53.

Mattoo,A.K., Hoffman-Falk,H., Marder,J.B. and Edelman,M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1380–1384.

Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–560.

Mosinger,E., Batschauer,A., Schafer,E. and Apel,K. (1985) *Eur. J. Biochem.*, **147**, 137–142.

Mullet,J.E., Orozco,E.M. and Chua,N.-H. (1985) *Plant Mol. Biol.*, **4**, 39–54.

Mullet,J.E., Klein,R.R. and Grossman,A.R. (1986) *Eur. J.Biochem.*, **155**, 331–338.

Mullet,J.E. and Klein,R.R. (1987) *EMBO J.*, **6**, 1571–1579.

Nanba,O. and Satoh,K. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 109–112.

Nelson,T., Harpster,M.H., Mayfield,S.P. and Taylor,W.C. (1984) *J. Cell Biol.*, **98**, 558–564.

Ohad,I., Kyle,D.J. and Hirschberg,J. (1985) *EMBO J.*, **4**, 1655–1659.

Oliver,R.P. and Poulsen,C. (1984) *Carlsberg Res. Commun.*, **49**, 647–673.

Poulsen,C. (1984) *Carlsberg Res. Commun.*, **49**, 89–104.

Rasmussen,O.F., Bookjans,G., Stummann,B.M. and Henningsen,K.W. (1984) *Plant Mol. Biol.*, **3**, 191–199.

Reisfeld,A., Gressel,J., Jakob,K.M. and Edelman,M. (1978) *Photochem. Photobiol.*, **27**, 161–165.

Rodermel,S.R. and Bogorad,L. (1985) *J. Cell Biol.*, **100**, 463–476.

Silverthorne,J. and Tobin,E.M. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1112–1116.

Steinmuller,K., Batschauer,A., Mosinger,E., Schafer,E., Rasmussen,S.K. and Apel,K. (1985) In Van Vloten-Doting,L., Groot,G.S.P. and Hall,T.C. (eds), *Molecular Form and Function of the Plant Genome*. Plenum, New York, pp. 277–300.

Tobin,E.M. and Silverthorne,J. (1985) *Annu. Rev. Plant Physiol.*, **36**, 569–593.

Towbin,H., Staehelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350–4354.

Vierling,E. and Alberte,R.S. (1983) *J. Cell Biol.*, **97**, 1806–1814.

Westhoff,P. (1985) *Mol. Gen. Genet.*, **201**, 115–123.

Westhoff,P., Farchaus,J.W. and Herrmann,R.G. (1986) *Curr. Genet.*, **11**, 165–169.

Zhu,Y.S., Kung,S.D. and Bogorad,L. (1985) *Plant Physiol.*, **79**, 371–376.

Received on December 24, 1987; revised on February 2, 1988