Curr Genet (1990) 18:377–383

Current Genetics

The chloroplast *psbK* operon from mustard (*Sinapis alba* L.): multiple transcripts during seedling development and evidence for divergent overlapping transcription

Heike Neuhaus and Gerhard Link

Ruhr-Universität Bochum, Fakultät für Biologie, Arbeitsgruppe Pflanzliche Zellphysiologie und Molekularbiologie, Universitätsstrasse 150, W-4630 Bochum 1, Federal Republic of Germany

Received June 25/August 2, 1990

Summary. The mustard chloroplasts genes *psbK* and *psbI* are co-transcribed, giving rise to precursor transcripts of several size classes, which are processed to the monocistronic mature RNAs. The *psbK* and *psbI* coding regions are flanked by the two tRNA genes *trnS*-GCU and *trnQ*-UUG on the opposite DNA strand. Transcript mapping indicates that the (primary) *psbK-psbI* transcript overlaps the complete *trnS*-GCU and *trnQ*-UUG transcripts. The transcription start site of the *psbK* operon appears to overlap that of the *rps16* gene. During seedling development, the *psbK* and *psbI* precursors and mature transcripts all become detectable between 30 and 48 h after sowing and then remain at constant levels without much difference either in light or in darkness.

Key words: Plastid gene expression – Complex transcript maturation pathway – Chloroplast promoter – *psbK-psbI*-operon

Introduction

Chloroplast development during photomorphogenesis of angiosperm seedlings involves dramatic structural and biochemical changes (see e.g., Bogorad 1981; Mohr 1984) which are the result of the developmentally regulated and light-controlled expression of many nuclear and chloroplast genes (Bedbrook et al. 1978; Ellis 1981; Rodermel and Bogorad 1985; Tobin and Silverthorne 1985; Taylor 1989). Light affects chloroplast gene expression at multiple levels, including plastid DNA copy number, transcription, RNA maturation, translation, and protein stability (for recent reviews see Kuhlemeier et al. 1987; Jenkins 1988; Link 1988; Mullet 1988; Thompson 1988; van Grinsven and Kool 1988; Gruissem 1989). For instance, plastid development during mustard (*Sinapis alba*) seedling photomorphogenesis has been shown to involve changes in transcript levels of a number of chloroplast genes (Link 1982, 1984; Oelmuller et al. 1986; Dietrich et al. 1987; Hughes et al. 1987; Neuhaus et al. 1989). By extending this work, we have now investigated the transcriptional organization and expression of two genes for photosystem II-related proteins and of two closely-associated tRNA genes.

The sequenced chloroplast genomes of tobacco (Shinozaki et al. 1986) and *Marchantia polymorpha* (Ohyama et al. 1986) each reveal the existence of two small open reading frames that are located adjacent to each other, and which have subsequently been termed *psbK* and *psbI*. Downstream of *psbI* is the *trnS* gene, which is located on the opposite strand (Deno and Sugiura 1983; Ohyama et al. 1986). Upstream of *psbK*, and likewise on the opposite strand, are the genes *trnQ* and *rps16* (Shinozaki et al. 1986). The polypeptides encoded by *psbK* and *psbI* have been shown to exist in vivo and to represent photosystem II-related components of the photosynthetic membrane (Ikeuchi and Inoue 1988; Murata et al. 1988; Webber et al. 1989). The 4 kDa "K" polypeptide is possibly involved in PSII assembly (Murata et al. 1988; Koike et al. 1989). The 4.8 kDa "I" polypeptide appears to be a component of the PSII reaction center in higher plants, as well as in cyanobacteria, although its precise function remains to be established (Ikeuchi and Inoue 1988; Ikeuchi et al. 1989a; Webber et al. 1989).

In the present work, we have analyzed the transcriptional organization of a mustard chloroplast DNA region which contains the gene cluster *psbK-psbI* as well as several neighboring genes. Northern experiments, nuclease S1 mapping, and ribonuclease protection assays indicate that the *psbK* and *psbI* genes are co-transcribed into a dicistronic RNA precursor which overlaps with two tRNAs encoded by the opposite DNA strand and possibly also with the *rps16* primary transcript. In addition, the expression mode of the *psbK-psbI* cluster during photoregulated seedling development has been investigated and compared to that of other mustard chloroplast genes.

Offprint requests to: H. Neuhaus

Materials and methods

Plant material. Mustards seedlings (cv. "Albatros") were grown on moist filter paper in plastic boxes at 25 °C either in the dark or under white light from mercury discharge lamps (photon fluence rate 250 μmol·m^{-2}·s^{-1}; approximately 10000 lux).

Cloned DNA, RNA probes. All plasmids used for hybridization experiments are subclones of pSA364 (Link and Langridge 1984). Plasmid pSA364-EX0.9 contains the 950 bp *Eco*RI/*Xba*I fragment cloned into pSPT19. Transcription of the *Eco*RI-linearized plasmid with T7 RNA polymerase produces a *trnS*-specific probe (probe I in Fig. 1 B), whereas a *psbI*-specific probe is obtained by transcription of the *Xba*I-linearized plasmid with SP6 RNA polymerase (probe II in Fig. 1 B). Plasmid pSA364-XE0.6 contains the 590 bp *Xba*I/*Eco*RI fragment in pSPT19. RNA produced from the *Eco*RI-linearized plasmid with T7 RNA polymerase represents a *psbK*-specific probe (probe III in Fig. 1 B). Plasmid pSA364-ET0.2 contains a 210 bp *Eco*RI/*Taq*I fragment in pSPT19. Transcription of the *Eco*RI-linearized plasmid with T7 RNA polymerase produces a *trnQ*-specific probe (probe IV in Fig. 1 B). Plasmid pSA364-XX1.5 (1.5 kb *Xba*I/*Xho*I fragment in pSPT19) gives rise to a probe that covers the complete *psbK* and *trnQ* genes as well as the 5′ exon and intron sequences of the *rps16* gene (probe V in Fig. 2 E). Plasmid pSA364-T0.3 contains the 310 bp *Taq*I-fragment cloned into pSPT19. Transcription of the *Bam*HI-linearized plasmid with T7 RNA polymerase produces a probe complementary to the 5′ end of the *psbK* mRNA (probe VII in Fig. 2 E). Plasmid pSA364-E0.5 is based on pSPT19 and contains a 580 bp fragment generated from the insert of pSA364-EX0.9 by digestion with exonuclease *Bal*31 from the *Xba*I site and cloned into the *Sma*I site of the vector (Fig. 2 E, probe VIII). The 580 bp fragment contains the 3′ end of the *psbK* dicistronic transcript.

Isolation of RNA, nuclease S1 mapping. The procedure for extracting chloroplast RNA and total cellular RNA from mustard cotyledons was as described previously (Hughes et al. 1987). For S1 mapping the DNA fragments were labelled at their 5′ ends with gamma ^{32}P-dATP and T4 polynucleotide kinase, or at their 3′ ends using alpha ^{32}P-dATP and the Klenow fragment of DNA polymerase I (Gibco/BRL). Twenty ng of the fragment were hybridized to chloroplast RNA (20 μg) and the mixture was then digested with 2000 units of nuclease S1 (Boehringer Mannheim, FRG) essentially as described by Link and Langridge (1984).

RNase protection assay. To localize the 5′ end of the *psbK* operon, plasmid pSA364-T0.3 was linearized with *Bam*HI and transcribed with T7 RNA polymerase. To localize the 3′ end of the operon, plasmid pSA364-E0.5 was linearized with *Bam*HI and transcribed with SP6 RNA polymerase. RNA probes (5×10^5 cpm) were hybridized with 50 μg cpRNA at 50 °C and hybrids were treated with both RNase A and T_1 (5′ probe) or with RNase T_1 alone (3′ probe) according to Sambrook et al. (1989).

Northern analysis. RNA (10 μg) was heat-denaturated, separated electrophoretically on 1.7% (w/v) formaldehyde agarose gels and transferred to nylon membranes (Amersham UK, Hybond-N). RNA samples were hybridized with labelled RNA probes prepared by transcription of linearized pSPT18/19-based plasmids (Pharmacia) with T7 and SP6 RNA polymerase (Melton et al. 1984). All hybridization and washing steps were carried out as previously described (Zinn et al. 1983; Hughes et al. 1987).

Results

Transcriptional organization of the gene cluster psbK-psbI, trnQ, and trnS

The mustard chloroplast DNA region with the positions of the genes studied in the present work is depicted in Fig. 1 B. This scheme is based on nucleotide sequence analysis of the genes *trnQ*-UUG, *psbK* (Neuhaus 1989) and *psbI, trnS*-GCU, (Neuhaus et al. 1990). Gene positions are equivalent to those in tobacco (Shinozaki et al. 1986) and *Marchantia polymorpha* (Ohyama et al. 1986). Fig. 1 A shows chloroplast transcripts detected by strand-specific RNA probes from this region that are indicated in Fig. 1 B with their position and polarity.

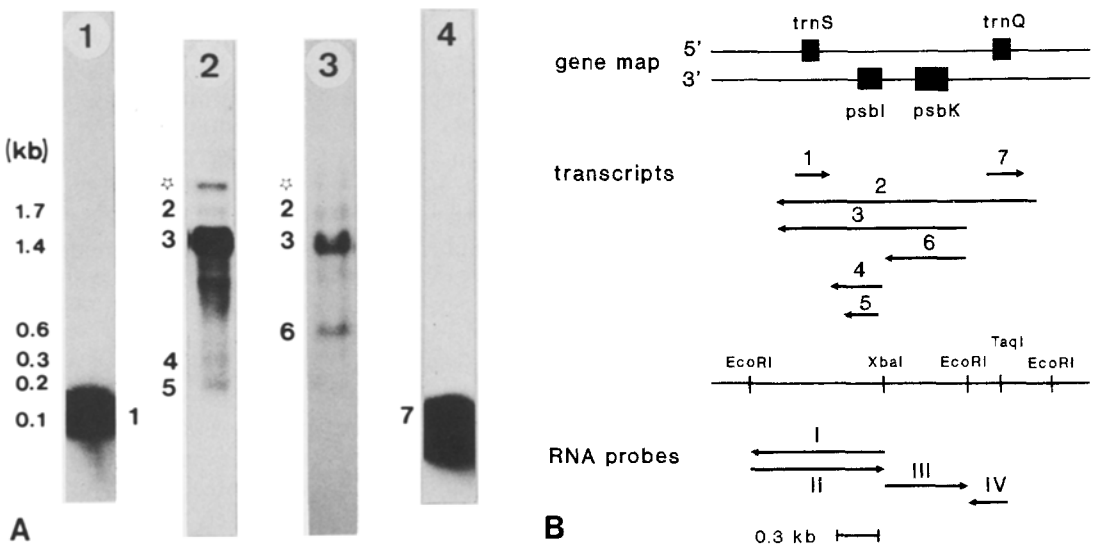


Fig. 1 A, B. Northern blot analysis of the mustard chloroplast DNA region containing the genes *trnS, psbI, psbK* and *trnQ*. **A** Chloroplast RNA, hybridized with specific RNA probes (I–IV, Fig. 1 B): *trnS*-specific probe I (*lane 1*); *psbI*-specific probe II (*lane 2*); *psbK*-specific probe III (*lane 3*); *trnQ*-specific probe IV (*lane 4*). The *asterisks* mark putative cross hybridization bands with 23S rRNA sequences. **B** Gene organization and transcripts detected in **A**. Coding regions are indicated by black bars. The scheme also depicts the putative position and polarity of transcripts (*central portion*) detected by the various probes (*lower portion*). *1*, tRNA^{Ser}; *2*, *3*, dicistronic precursors (*psbK, psbI*); *4*, *5*, processed *psbI* transcripts; *6*, processed *psbK* transcript; *7*, tRNA^{Gln}

The prominent 1.4 kb transcript "3" in Fig. 1 A, lane 2, is detected both by the *psbI*-specific probe II (Fig. 1 A, lane 2) and the *psbK*-specific probe III (Fig. 1 A, lane 3) and hence might represent a dicistronic transcript of these two genes. There are several bands above band "3", the most prominent of which co-migrates with 23S rRNA (asterisk) and appears to be the result of cross-hybridization (data not shown). However, the 1.7 kb transcript "2" might represent a longer precursor RNA of the *psbK* and *psbI* region. The *psbI*-specific probe II hybridizes to the 1.7 kb ("2") and to the 1.4 kb ("3") precursor RNAs and, in addition, to two smaller transcripts that are 0.3 kb and 0.2 kb in size (Fig. 1 A, lane 2, bands "4" and "5"). It was not possible with this probe to distinguish whether the 0.3 kb transcript represents a monocistronic intermediate for the 0.2 kb mature *psbI* mRNA or, alternatively, is an unrelated transcript. The labelled material between bands "3" and "4" (Fig. 1 A, lane 2) co-migrates with the major products of 23S rRNA degradation at hidden breaks (Kössel et al. 1985) and appears to reflect cross-hybridization (data not shown).

The *psbK*-specific probe III detects bands "2" and "3" as well as an 0.6 kb RNA species that might be the monocistronic *psbK* transcript (Fig. 1 A, lane 3, band "6"). Hybridization with the complementary-strand RNA probes I and IV each reveals a single strong signal in the low-molecular-weight range. These signals might represent $tRNA^{Ser}$ (Fig. 1 A, lane 1, band "1") and $tRNA^{Gln}$ (Fig. 1 A, lane 4, band "7"), both of which are encoded by the opposite DNA strand. The tentative map positions assigned to most transcripts detected in Fig. 1 A are summarized in Fig. 1 B.

5′ end of the psbK-psbI precursor transcript

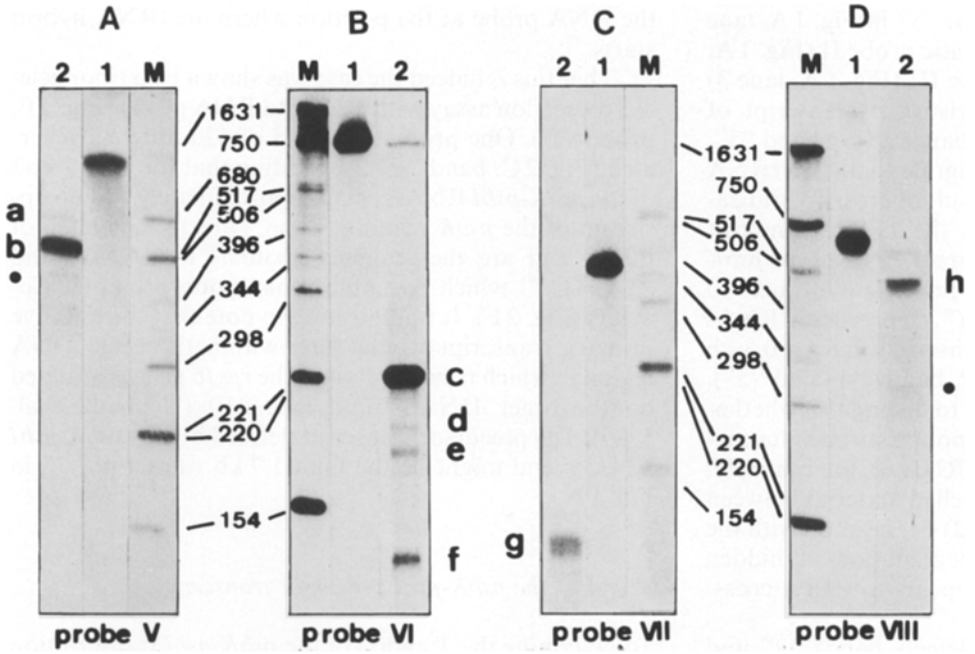
To determine the 5′ end(s) of the precursor(s), nuclease S1 mapping (Fig. 2) was carried out with a 1.5 kb *Xba*I/*Xho*I fragment which contains the *psbK* reading frame, the *trnQ* gene, and an extensive 5′ sequence up to the *Xho*I site within the *rps16* intron (Neuhaus et al. 1989). This probe was 5′-labelled at the *Xba*I site (probe V in Fig. 2 E). As shown in Fig. 2 A, lane 2, one prominent S1-resistant product with a size of approximately 570 ntd (band "b") and one minor product of 740 ntd (band "a") are generated. Considering that only one prominent band ("b") is formed, this band is likely to reflect a common 5′ end of both the abundant 1.4 kb dicistronic *psbK-psbI* transcript "3" (Fig. 1 A) and the monocistronic 0.6 kb *psbK* transcript (transcript "6", Fig. 1 A). To locate the 5′ end of the 740 ntd product "a" more precisely, S1 experiments were carried out with the 0.21 kb *Eco*RI/*Taq*I fragment (Fig. 1 B, probe IV) and the 5′-labelled S1-resistant DNA fragment was elecrophoresed alongside the products of dideoxy chain-termination reactions (Sanger et al. 1977) (data not shown). Based on the size of the resulting S1-resistant fragment, the RNA 5′ end correlates with the 5′ end of the *trnQ* coding region on the opposite DNA strand. The S1-resistant band "a" in Fig. 2 A might thus be due to hybridization of the *psbK* transcript(s) to $tRNA^{Gln}$ and subsequent displacement of the DNA probe at the position where the tRNA hybrid starts.

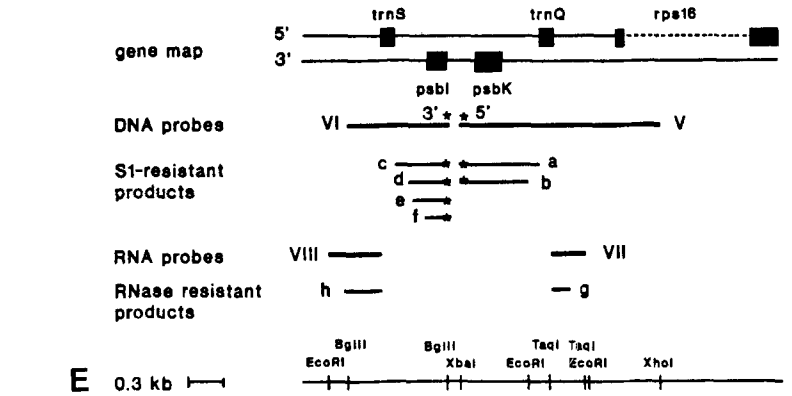
That this is indeed the case was shown by a ribonuclease protection assay with a 350 ntd RNA probe (Fig. 2 E, probe VII). One prominent band of 120 ntd was generated (Fig. 2 C, band "g"), suggesting that the true 5′ end of the *psbK-psbI* RNA(s) maps approximately 520 bp upstream of the *psbK* reading frame. Directly upstream of this 5′ end are the sequence motifs "TTGAAA" and "TATTCT" which resemble prokaryotic promoter elements (Fig. 2 F). It is interesting to note that the putative primary transcript would start within the same DNA region in which transcription of the *rps16* gene is initiated on the other DNA strand (Fig. 2 F) (Neuhaus et al. 1989). The precursor transcript defined by this *psbK-psbI* RNA 5′ end might be the faint 1.7 kb transcript "2" in Fig. 1 A.

3′ end of the psbK-psbI precursor transcript

To determine the 3′ end(s) of the *psbK-psbI* transcription unit, nuclease S1 mapping was carried out with a 3′-labelled 750 bp *Bgl*II fragment of the plasmid pSA364-EX0.9 (Fig. 2 E, probe VI). As shown in Fig. 2 B, one prominent band of 220 ntd ("c") and three minor products of 190 ntd ("d"), 180 ntd ("e") and 120 ntd ("f") were generated. Similar to the situation found for the *psbK* 5′ and *trnQ* region, the 220 ntd product ("c") seems to reflect the 3′ end of $tRNA^{Ser}$ transcribed from the opposite DNA strand and thus might be a hybridization artifact. The border of the 120 ntd S1-resistant product (band "f") roughly coincided with the end of the *psbI* reading frame, which might define the 3′ end of the 0.2 kb mature *psbI* transcript. The 180 ntd and 190 ntd S1 products (bands "e" and "d") correspond to 3′ end positions approximately 70 and 80 bp downstream of the *psbI* reading frame. These 3′ end(s) are in agreement with the size of the 0.3 kb RNA species that was detected in Northern experiments by the *psbI*-specific probe II (Fig. 1 A, lane 2), suggesting that the 0.3 kb RNA is indeed a monocistronic *psbI* processing intermediate.

To localize the primary 3′ end of the dicistronic 1.4 kb *psbK-psbI* transcript, a ribonuclease protection assay was carried out, using a 580 ntd RNA probe (Fig. 2 E, probe VIII). As shown in Fig. 2 D, one RNAse-resistant product of 480 ntd in length is generated, which places the 3′ end of the precursors 670 ntd downstream of the *psbI* reading frame. The distance between this 3′ end and the mapped 5′ end (Fig. 2 A and 2 C) matches the 1.4 kb of the precursor RNA determined by Northern hybridization (Fig. 1 A, band "3"). It appears likely that the same 3′ end is also shared by the 1.7 kb (primary) transcript, since the distance from the upper 5′ end (Fig. 2 A and 2 C) likewise reflects the size of this Northern band (Fig. 1 A, band "2").





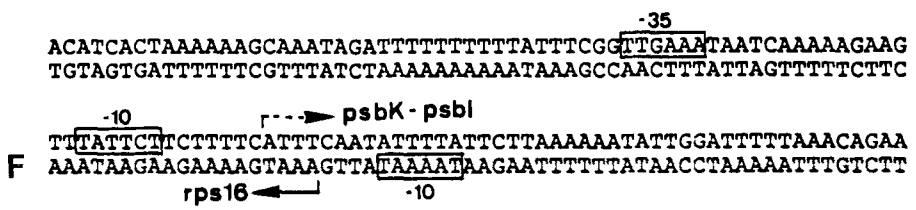


Fig. 2 A–F. Nuclease S1 mapping ribonuclease protection assays of the 5′ and 3′ *psbK-psbI* RNA termini. **A** *Lane 1*, the 1.5 kb *Xba*I/*Xho*I DNA fragment, 5′labelled at the *Xba*I site (Fig. 2E, probe V), without hybridization and S1 treatment; *lane 2*, S1-resistant products, following hybridization with chloroplast RNA and subsequent S1 treatment. Size markers (*M*) are *Hin*fI fragments of pBR322 and an additional 0.68 kb DNA fragment. **B** *Lane 1*, the 3′-labelled 750 bp *Bgl*II DNA fragment of plasmid pSA364-EX0.9 (Fig. 2E, probe VI); *lane 2*, S1-resistant products. Size markers (*M*) are *Hin*fI fragments of pBR322 and an additional 0.75 kb DNA fragment. **C** *Lane 1*, the 350 ntd ^{32}P-UTP labelled RNA probe (Fig. 2E, probe VII); *lane 2*, RNase-resistant product, following hybridization with chloroplast RNA and subsequent treatment with RNases A and T_1. **D** *Lane 1*, the 580 ntd-labelled RNA probe (Fig. 2E, probe VIII); *lane 2*, RNase-resistant product, following hybridization with chloroplast RNA and treatment with RNase T_1. The *dots* in Figs. 2A and 2B, panel 2, denote bands of unknown origin. **E** Scheme showing the positions of the DNA and RNA probes and products of nuclease mapping experiments. *Asterisks* indicate the labelled 5′ or 3′ ends of the DNA fragments. **F** Nucleotide sequence showing the transcription initiation site of the *rps16* gene and the putative start site of the dicistronic *psbK-psbI* primary transcript. (Nucleotide positions −170 to −289 in the published sequence; see Neuhaus et al. 1989). Putative '−10' and '−35' promoter elements are *boxed*. *Arrow* indicate the divergent orientation of the transcripts

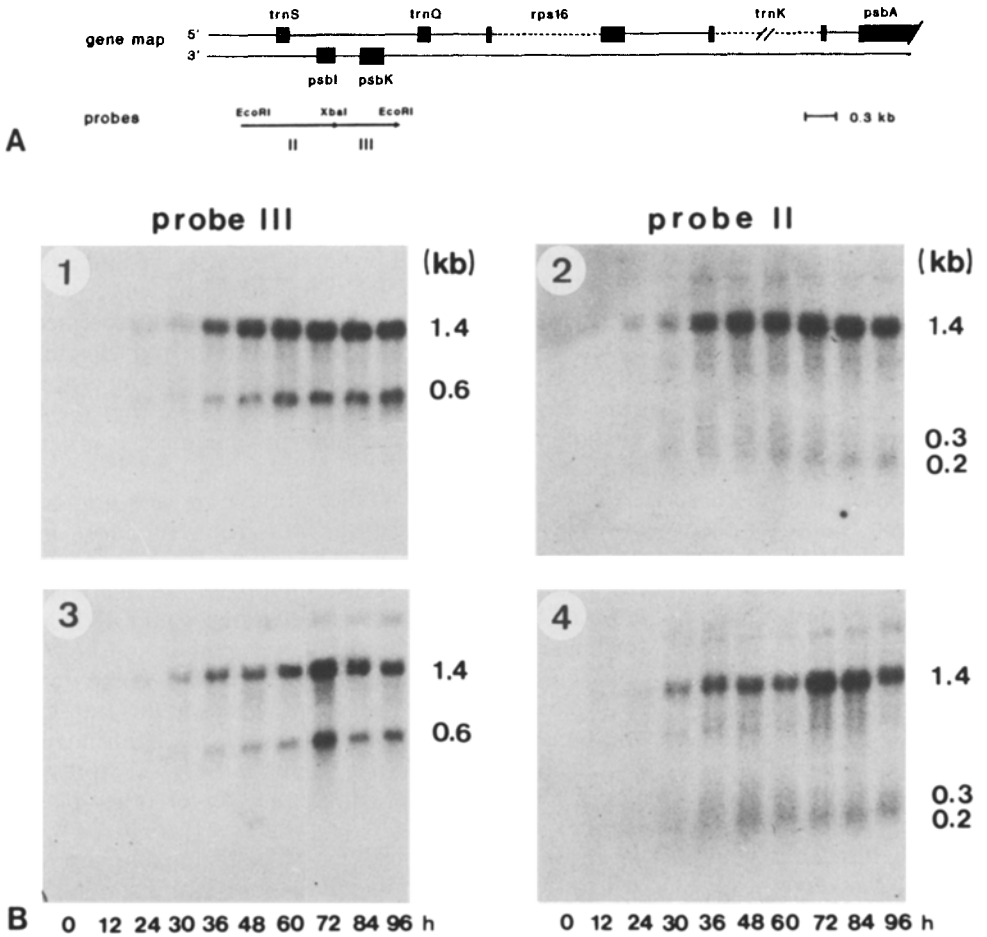


Fig. 3 A, B. Northern hybridization analysis of *psbK* and *psbI* transcript level in mustard seedlings. **A** Scheme of the position of the probes used in the hybridization experiments. **B** RNA was prepared from seeds and from cotyledons of light-grown (*1* and *2*) and dark-grown (*3* and *4*) mustard seedlings at 12 h to 96 h after sowing. Northern blots with 10 µg RNA per lane were hybridized with *psbK*-specific probe III (*1* and *3*) and with *psbI*-specific probe II (*2* and *4*)

Transcript levels of plastid psbK, psbI genes during mustard seedling development

To estimate transcript levels, total RNA was extracted from seeds and from cotyledons of seedlings up to 96 h after sowing. RNA samples, each from the same extraction series, were separated on gels and blotted for Northern hybridization.

The *psbK*-specific probe III (Figs. 1 B and 3 A) detects the 1.4 kb dicistronic *psbK-psbI* precursor and the 0.6 kb mature *psbK* mRNA (Fig. 3 B, panels 1 and 3). levels of both the 1.4 kb precursor and the 0.6 kb mature transcript begin to increase at approximately 30 h after sowing. At 48 h, plateau levels are reached, which are subsequently maintained until 96 h after sowing. Throughout this entire period, *psbK*-related transcript levels in light-grown seedlings (Fig. 3 B, panel 1) are not significantly different from those in dark-grown seedlings (Fig. 3 B, panel 3).

The *psbI*-specific probe II (Figs. 1 B and 3 A) detects the 1.4 kb dicistronic precursor and the two (monocistronic) *psbI*-related transcripts of 0.3 kb and 0.2 kb (Fig. 3 B, panels 2 and 4). Similar to the time-course of transcripts detected with the *psbK*-specific probe, both the 1.4 kb precursor and the two small (monocistronic) transcripts accumulate between 40 and 48 h in a light-independent way and then remain at constant levels throughout the later stages of development (light-grown seedlings, Fig. 3 B, panel 2; dark-grown seedlings, Fig. 3 B, panel 4).

Discussion

In the present work we have investigated the transcriptional organization and expression dynamics of a gene cluster on mustard chloroplast DNA, consisting of *psbK* and *psbI*, for the PSII-related "K" and "I" proteins, and of the *trnQ* and *trnS* genes, for $tRNA^{Gln}$ (UUG) and $tRNA^{Ser}$ (GCU). The largest transcripts from this region are approximately 1.7 kb and 1.4 kb in size and are hybridized by probes that cover either of these two genes, suggesting that they may represent dicistronic precursor RNAs of the entire *psbK-psbI* gene cluster. Several additional bands that also become visible with gene-specific probes indicate that processing steps are involved in the formation of the mature RNAs. The precursors are further cleaved to give the 0.6 kb *psbK* mRNA and a mono-

cistronic *psbI* precursor which undergoes extensive processing, leading to the 0.3 kb transcript and the 0.2 kb mature *psbI* mRNA.

It has been previously suggested (Murata et al. 1988) that the *psbK* gene might be co-transcribed with the adjacent *psbI* gene in tobacco (Shinozaki et al. 1986) as well as in *Marchantia* (Ohyama et al. 1986). In barley it has been shown that the *psbK* and *psbI* genes are co-transcribed with the *psbD* and *psbC* genes (Sexton et al. 1990). This appears to be the result of DNA rearrangements that have led to a different genomic organization in this monocotyledonous species.

Northern analysis, S1 mapping and ribonuclease protection assays have shown that the 1.7 kb *psbK-psbI* precursor transcript divergently overlaps with the *trnQ* and *trnS* transcripts and, in addition, the transcription start site of the *psbK* operon appears to overlap that of the *rps16* gene. A similar situation has been found for the gene clusters *psbE-psbF-psbL-ORF40* and *ORF31-petE-ORF42* from maize, which again reveals an extensive overlap of divergent transcription units (Haley and Bogorad 1990). Divergent overlapping transcription has also been detected in *Marchantia* within the DNA region containing the *psbB* operon (Kohchi et al. 1988). The *psbB-psbH-petB-petD* gene cluster (Rock et al. 1987; Tanaka et al. 1987; Westhoff and Herrmann 1988) encodes components of two different photosynthetic complexes, the expression of which is uncoupled in response to light and developmental signals (Herrmann et al. 1985). The *psbB-psbH-petB-petD* transcript divergently overlaps the transcript for the ORF43 encoded by the opposite DNA strand between the *psbB* and *psbH* genes. The levels of the *psbB* and *ORF43* transcripts (but not those of *psbH-petB-petD* transcripts) are increased in illuminated (greening) tissue. It was speculated that *ORF43* mRNA might act as a rho-independent terminator by forming double-stranded RNA, thus repressing transcription of the following *psbH, petB* and *petD* genes of the *psbB* operon in light-grown tissue (Kohchi et al. 1988). The *ORF43* product has been assigned to the PSII O_2-evolving core complex of the cyanobacterium *Synechococcus vulcanus* and has been designated *psbN* (Ikeuchi et al. 1989b). In contrast, the members of the *psbK-psbI* gene cluster do not show significant differences in their transcript levels in light-grown as compared to dark-grown seedlings. Transcription analysis with mutated DNA templates in chloroplast extracts could provide further clues to the possibility that anti-sense regulation might be involved in the expression of the *psbK* operon.

Another difference between the *psbB* and the *psbK-psbI* transcription unit is that in the latter we find a (processed) monocistronic RNA for each gene, whereas *petB* and *petD* transcripts of the *psbB* operon are processed to a dicistronic mature mRNA (Westhoff and Herrmann 1988). Thus, although intercistronic processing is not required for translation, it appears possible that production of monocistronic transcripts enhances translation efficiency (Barkan 1988).

The expression dynamics of the *psbK* and *psbI* genes have been investigated at the transcript level during mustard seedling development. The various RNA species (precursor and processed transcripts) from this genomic region all become detectable between 24 and 48 h after sowing. Thereafter, transcript levels remain constant and do not appreciably differ in light-grown as compared to dark-grown seedlings. Thus, both genes of this transcription unit are constitutively expressed in a light-independent manner, which adds to the variety of expression modes identified in previous analyses of other plastid genes (Table 1).

During mustard seedling development, three principally different modes of expression can be distinguished, each of which is exemplified by one of the three closely linked but independently transcribed genes, *psbA, trnK* and *rps16*, in the vicinity of the *psbK* operon. The levels of the *psbA* transcript (and of all other plastid transcripts investigated thus far) show an (endogenously programmed) light-independent increase between 30 and 40 h. Thereafter, *psbA* transcripts further accumulate to high levels in light-grown seedlings, while they remain at lower levels in dark-grown seedlings (Link 1982, 1984; Hughes et al. 1987). Variations of the "light-induced" mode of accumulation have also been observed for a number of other mustard chloroplast genes, including *psaA1/A2, atpA* and *petA* (Dietrich et al. 1987) (Table 1).

Table 1. Expression of plastid genes at the RNA level during mustard seedling development

Genes	Gene products	Intron	Transcription unit (cistronic)	Mode of expression/ peak RNA level (h after sowing)	References
psbA	PSII 32 kDa protein	–	mono	light-induced no late decrease	Hughes et al. 1987
trnk	tRNA Lys	+	mono	transient[1] (40–48 h) light-independent	Hughes et al. 1987
rps16	ribosomal protein S16	+	mono	constitutive light-independent[1,2]	Neuhaus et al. 1989
psbK	PSII K protein	–	di[a]	constitutive light-independent[1,2]	this work
psbI	PSII I protein	–	di[a]	constitutive light-independent[1,2]	this work
petA	cytochrome f apoprotein	–		light-induced, no late decrease[2]	Dietrich et al. 1987
atpA	ATPase alpha subunits	–	poly[b]	light-induced, late decrease (72 h)[2]	Dietrich et al. 1987
psaA	PSI P700	–	poly[c]	light-induced	Dietrich et al. 1987
psaB	apoproteins	–		late decrease (72 h)[1]	

[a] *psbK-psbI* (5′–3′)
[b] *rps2-atpI-atpH-atpF-atpA* (Woodbury et al. 1988)
[c] *psaA-psaB-rps14* (Meng et al. 1988)

[1] Precursor
[2] Processed transcript

Located directly upstream of *psbA* is the split *trnK* gene. Its intron contains an open reading frame for a derived polypeptide which appears structurally related to mitochondrial maturases (Sugita et al. 1985; Neuhaus and Link 1987). The *trnK* (precursor) transcript shows a transient light-independent peak-level at 40–48 h after sowing, i.e., at or before the onset of the light-enhanced transcript accumulation of *psbA* and other chloroplast genes (Hughes et al. 1987). The unique time-course of the *trnK* precursor might be related to the expression of the putative intron-encoded maturase (Neuhaus and Link 1987). The later has been suggested to be involved in changes in plastid RNA splicing (Hughes et al. 1987; Link 1988), which in turn might be related to the development of competence for light regulation of *psbA* and other genes (Mohr 1984; Dietrich et al. 1987; Link 1988).

The *rps16* gene encoding the ribosomal protein S16 lies in front of the *trnK* gene and represents the third expression mode. Like the members of the *psbK* operon (Table 1), this gene is constitutively expressed in a light-independent manner (Neuhaus et al. 1989). Its expression mode differs, however, from that of the *psbK* transcription unit by the earlier onset of the constitutive phase (24–36 h as compared to 36–48 h).

There is increasing evidence that post-transcriptional processes are important for establishing steady-state transcript levels and hence might also be responsible for light/dark differences (Herrmann et al. 1985; Barkan 1988; Mullet 1988; Deng et al. 1989; Gruissem 1989). The detailed analysis of the transcriptional organization and expression dynamics of chloroplast genes will aid in defining the molecular mechanisms that determine and regulate transcript levels.

Acknowledgements. We are grateful to U. Müller and C. Wittig for expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie, FRG.

References

Barkan A (1988) EMBO J 7:2637–2644
Bedbrook JR, Link G, Coen DM, Bogorad L, Rich A (1978) Proc Natl Acad Sci USA 75:3060–3064
Bogorad L (1981) J Cell Biol 91:256–270
Deng X-W, Tonkyn JC, Peter GF, Thornber JP, Gruissem W (1989) Plant Cell 1:645–654
Deno H, Sugiura M (1983) Nucleic Acids Res 11:8407–8413
Dietrich G, Detschey S, Neuhaus H, Link G (1987) Planta 173:393–399
Ellis RJ (1981) Annu Rev Plant Physiol 32:111–137
Gruissem W (1989) Cell 56:161–170
Haley J, Gogorad L (1990) Plant Cell 2:323–333
Herrmann RG, Westhoff P, Alt J, Tittgen J, Nelson N (1985) Thylakoid membrane proteins and their genes. In: an Vloten-Doting LV, Groot GSP, Hall TC (eds) Molecular form and function of the plant genome. Plenum Press, New York, pp 233–256
Hughes JE, Neuhaus H, Link G (1987) Plant Mol Biol 9:355–364
Ikeuchi M, Inoue Y (1988) FEBS Lett 241:99–104
Ikeuchi M, Koike H, Inoue Y (1989a) FEBS Lett 251:155–160
Ikeuchi M, Koike H, Inoie Y (1989b) FEBS Lett 253:178–182
Jenkins GI (1988) Photochem Photobiol 48:821–832
Kohchi T, Yoshida T, Komano T, Ohyama K (1988) EMBO J 7:885–891
Koike H, Mamada K, Ikeuchi M, Inoue Y (1989) FEBS Lett 244:391–396
Kössel H, Natt E, Strittmatter G, Fritzsche E, Gozdzicka-Jozefiak A, Przybyl D (1985) Structure and expression of rRNA operons from plastids of higher plants. In: van Vloten-Doting LV, Groot GSP, Hall TC (eds) Molecular form and function of the plant genome. Plenum Press, New York, pp 183–198
Kuhlemeier C, Green PJ, Chua N (1987) Annu Rev Plant Physiol 38:221–257
Link G (1982) Planta 154:81–86
Link G (1984) Plant Mol Biol 3:243–248
Link G (1988) Plant Cell Environ 11:329–338
Link G, Langridge U (1984) Nucleic Acids Res 12:945–957
Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR (1984) Nucleic Acids Res 12:7035–7057
Meng BY, Tanaka M, Wakasugi T, Ohme M, Shinozaki K, Sugiura M (1988) Curr Genet 14:395–400
Mohr H (1984) Phytochrome and chloroplast development. In: Baker NR, Barber J (eds) Topics in photosynthesis vol 5: Chloroplast biogenesis. Elsevier, Amsterdam, pp 305–348
Mullet JE (1988) Annue Rev Plant Physiol Plant Mol Biol 39:475–502
Murata N, Miyao M, Hayashida N, Hidaka T, Sugiura M (1988) FEBS Lett 235:283–288
Neuhaus H, Link G (1987) Curr Genet 11:251–257
Neuhaus H (1989) Nucleic Acids Res 17:444
Neuhaus H, Scholz A, Link G (1989) Curr Genet 15:63–70
Neuhaus H, Pfannschmidt T, Link G (1990) Nucleic Acids Research 18:368
Oelmuller R, Dietrich G, Link G, Mohr H (1986) Planta 169:260–266
Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki S, Takeuchi M, Chang Z, Aota S, Inokuchi H, Ozeki H (1986) Nature 322:572–574
Rock CD, Barkan A, Taylor WC (1987) Curr Genet 12:69–77
Rodermel SR, Bogorad L (1985) J Cell Biol 100:463–476
Sambrook J, Fritsch EF, Maniatis T (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Sanger F, Nicklen S, Coulson ARC (1977) Proc Natl Acad Sci USA 74:5463–5467
Sexton TB, Jones JT, Mullet JE (1990) Curr Genet 17:445–454
Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chungwongse J, Obokata J, Yamaguchi-Shinozaki K, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) EMBO J 5:2043–2049
Sugita M, Shinozaki K, Sugiura M (1985) Proc Natl Acad Sci USA 82:3557–3561
Tanaka M, Obokata J, Chunwongse J, Shinozaki K, Sugiura M (1987) Mol Gen Genet 209:427–431
Taylor WC (1989) Annu Rev Plant Physiol Plant Mol Biol 40:211–233
Thompson WF (1988) Plant Cell Environ 11:319–328
Tobin EM, Silverthorne J (1985) Annue Rev Plant Physiol 36:569–593
van Grinsven MQJN, Kool AJ (1988) Plant Mol Biol Reporter 6:213–239
Webber AN, Packman L, Chapman DJ, Barber J, Gray JC (1989) FEBS Lett 242:259–262
Westhoff P, Herrmann RG (1988) Eur J Biochem 171:551–564
Woodbury NW, Roberts LL, Palmer JD, Thompson WF (1988) Genet 14:75–89
Zinn K, DiMaio D, Maniatis T (1983) Cell 34:865–879

Communicated by H. Kössel