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Transcript levels of two adjacent chloroplast genes during mustard (*Sinapis alba* L.) seedling development are under differential temporal and light control

Jonathan E. Hughes, Heike Neuhaus and Gerhard Link
University of Bochum, Plant Cell Physiology, P.O. Box 102148, D-4630 Bochum, FRG

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Abstract

Transcript levels of two plastid genes were investigated during early seedling development of mustard (*Sinapis alba* L.) until 96 h after sowing. The two genes, which are closely linked and have the same polarity, are the *psbA* gene encoding the M_r 32–35000 herbicide-binding Q_B-protein of photosystem II and the *trnK* gene encoding plastid $tRNA^{Lys}$ (UUU) and potentially an intron-derived maturase-related protein. By using Northern and dot blot hybridization techniques with sensitive RNA probes, the 1.2 kb *psbA* transcript was found to be present in low amounts during the initial phase of seed germination. Thereafter, it increases in concentration both in light- and dark-grown seedlings until approximately 48 h after sowing. A further increase in *psbA* transcript concentration during the subsequent phase until 96 h was observed in light-grown, but not in dark-grown seedlings. The 2.8 kb *trnK* transcript is one to two orders of magnitude less abundant than the *psbA* transcript throughout the time period investigated. The concentration of this transcript is light-independent and shows a transient peak level at around 48 h, i.e. at the onset of light-enhanced accumulation of the *psbA* transcript.

Introduction

Chloroplast formation in higher plants is a complex developmental process which involves the coordinated expression of both nuclear and chloroplast genes and is dependent on the presence of light (for reviews, see [7, 18, 27]). Light regulation of plastid gene expression seems to include multiple control levels, e.g. changes in plastid DNA copy number [23], translational control [8, 9], as well as changes in the concentration of plastid transcripts, indicating control at the level of transcription and/or RNA processing [1, 11, 12, 26, 21, 22, 28]. The extent to which each of these mechanisms contributes to the control of plastid gene expression seems to vary, depending on the plant species and the developmental state of the organs and tissues investigated (see e.g. [27]).

Differential plastid gene expression at the RNA level has been established in work with seedlings which were usually grown in the dark for several days and then transferred to the light (e.g. [1, 26, 12, 22, 28]). As such seedlings are etiolated and have undergone considerable development already, critical molecular events might be missed in such an experimental system. In attempts to define the onset and extent of developmental control by light during seedling development, we have now investigated transcript levels of two plastid genes in seeds and cotyledons of young mustard seedlings up to 96 h after sowing.

In mustard [11] as well as in other plants [1, 26, 22,

28], a major light-regulated transcript originates from the plastid *psbA* gene [30], which codes for D1, the M_r 32–35000 herbicide-binding Q_R-protein of photosystem II [16, 24]. The map position on mustard chloroplast DNA, nucleotide sequence, and transcriptional organization of the mustard *psbA* gene have been determined previously [10, 14, 13].

Immediately upstream of the *psbA* gene on the same DNA strand lies the *trnK* gene [25, 4, 19] specifying plastid $tRNA^{Lys}$ (UUU). A unique feature of this tRNA gene is the presence of a 2574 kb (class II) intron, which contains a long open reading frame. The derived intron-encoded protein might be structurally related to mitochondrial maturases known to be involved in RNA splicing [19], although it is not yet known whether it exists as a functional chloroplast protein *in vivo*.

The results of our present experiments establish that, despite the physical proximity of the *psbA* and *trnK* genes, their transcript levels show substantial differences: (i), transcript levels of the *psbA* gene are one to two orders of magnitude higher than those of the *trnK* gene throughout mustard seedling development; (ii), the kinetics of *psbA* and *trnK* transcript accumulation are grossly different; (iii), the effect of light on *psbA* transcript levels is large, but is insignificant in the case of *trnK*. Transcript levels of the two genes might not, however, be unrelated.

Materials and methods

Plant material

Mustard seedlings (cv. "Albatros") were grown on moist filter paper in plastic boxes at 25 °C in darkness or under white light from mercury discharge lamps (250 μmol m^{-2} s^{-1} PAR; approximately 10000 lux). Cotyledons were harvested at twelve-hour intervals and stored in liquid N_2.

Nucleic acid extraction

Frozen cotyledons were ground in a mortar with liquid N_2. The powder was suspended in 50 mM Tris-HCl pH 7.6, 2.5 mM $MgCl_2$, 100 mM NaCl, 2% (w/v) sodium triisopropylnaphthalenesulfonate and extracted three times with phenol/chloroform as described [11]. Nucleic acids were precipitated with isopropanol and dissolved in 5 mM Tris-HCl pH 8.0, 0.25 mM EDTA. Following precipitation with ethanol, nucleic acids were resuspended in the Tris/EDTA buffer and their concentration was determined at 260 nm (1 OD = 40 μg/ml). DNA was removed by digestion with 100 μg/ml DNase I (Worthington DPFF) in the presence of 250 units/ml human placental RNase inhibitor (Amersham). Following extraction with phenol/chloroform and precipitation with ethanol, RNA was resuspended in the Tris/EDTA buffer and the final concentration determined. Purified RNA samples were stored at −80 °C in small portions. Chloroplast, etioplast and mitochondrial RNA was prepared from sucrose gradient-purified organelles [3, 20] of five-day-old mustard cotyledons.

Northern blots

RNA samples were heat-denatured and then separated electrophoretically on 1.2% (w/v) formaldehyde/agarose gels. RNA was transferred to nitrocellulose filters (Schleicher & Schuell, BA85 or BA83) in 10 × SSC (1 × SSC = 0.15 M NaCl, 0.025 M sodium citrate) [15].

Dot blots

RNA samples were heat-denatured in 10 × SSC, 7.5% (v/v) formaldehyde at 65 °C for 15 min [5]. Serial dilutions were applied to nitrocellulose (BA85), using the Schleicher & Schuell Minifold I apparatus.

Cloned DNA

Plasmid pSA364a-05, containing the 5′ portion of the mustard chloroplast *psbA* gene and the 3′ portion of the *trnK* gene, has been described [14]. Plasmid pSA452a contains the 3′ portion of the *psbA* gene (the 1.0 kb PstI/EcoRI fragment of the paren-

tal plasmid pSA452 in pBR322) [10]. Plasmid pSPT452a contains the same fragment in the transcription vector pSPT18 (Pharmacia) [17]; pSPTB0.5 contains a 0.5 kb BamHI fragment representing intron-specific *trnK* sequences in pSPT18 [19] (H. Neuhaus and G. Link, manuscript in preparation). Plasmid pSA05/B1, derived from pSA364a-05 [14], contains the 3′ half of the $tRNA^{Lys}$ (UUU) coding region on a 0.47 kb PstI/HincII fragment in pUC13 (3′ *trnK* exon probe); pSPTS0.2 contains a 0.2 kb Sau3A fragment representing the 5′ half of the $tRNA^{Lys}$ coding region and adjacent sequences in pSPT18 (5′ *trnK* exon probe) [19] (H. Neuhaus and G. Link, in preparation). All plasmids were purified by the alkaline procedure [2], followed by two cycles of cesium chloride centrifugation.

Preparation of radioactive probes

DNA fragments were isolated by preparative gel electrophoresis and labelled with ^{32}P by nick translation [15]. Labelled RNA probes were prepared by transcription of linearized pSPT18-based plasmids with SP6 and T7 RNA polymerase (BRL) as described [17]. Unlabelled transcripts used for calibration of dot blots were prepared by the same procedure, except that the UTP concentration was raised to 400 μM, and their concentration was determined photometrically. The integrity of all probes used was checked by gel electrophoresis.

Hybridization

For hybridizations with DNA probes [15], filters containing blotted RNA samples were pretreated with 5 × SSC, 10 × Denhardt's solution, 150 μg/ml denatured sonicated salmon sperm DNA, 0.1% (w/v) SDS at 68 °C for 5 h. Hybridization was in 5×SSC, 1× Denhardt's solution, 50 μg/ml salmon sperm DNA, 0.1% (w/v) SDS and heat-denatured nick-translated DNA probe (10^6 to 10^7 cpm) in 10 ml at 68 °C for 12 h. Blots were then washed successively for 30 min each three times in 2 × SSC with 0.1% (w/v) SDS at 63 °C, and once in 0.1 × SSC at 37 °C. Hybridizations with RNA probes were carried out essentially as described [29]. The washed and dried blots were exposed to Kodak XAR-5 X-ray film, using intensifying screens.

For quantitative assessment of transcript levels following dot blot hybridization, discs representing invidual RNA dots were stamped out from the nitrocellulose sheet and radioactivity was measured by liquid scintillation counting. A linear relationship was found between the amount of radioactivity hybridized and the applied total amount of RNA up to 5 μg. Dots containing 0.5 to 5 μg RNA were used for quantitation. Each dot blot contained reference dots representing known amounts of unlabelled *in vitro* transcripts of either *psbA* (plasmid pSPT452a) or *trnK* (plasmid pSPTB0.5) sequences. Corrections were made to account for the different lengths of the *in vivo* and *in vitro* transcripts (see Fig. 1A).

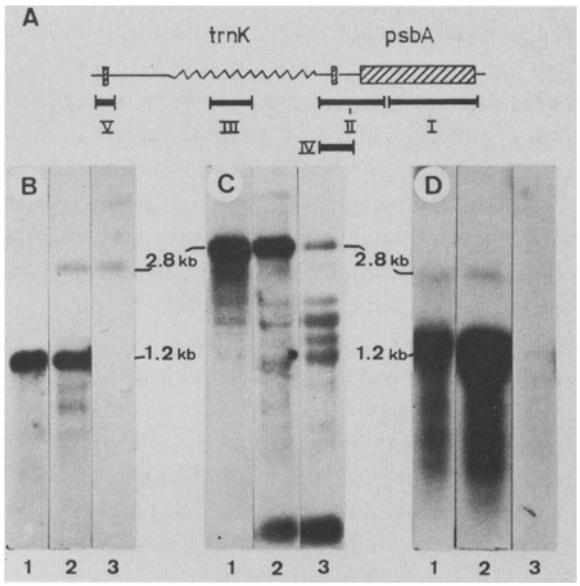

Fig. 1. A, Position and structure of mustard *psbA* and *trnK* genes. Hatched bars, *psbA* coding region and *trnK* 5′ (left) and 3′ (right) exons; zigzag line, open reading frame within *trnK* intron. Heavy black bars, location of hybridization probes: I, *psbA* 3′ probe (pSA452a and pSPT452a); II, *psbA* 5′/*trnK* 3′ probe (pSA364a-05); III, *trnK* intron-specific probe (pSPTB0.5); IV, *trnK* 3′ exon probe (pSA05/B1); V, *trnK* 5′ exon probe (pSPTS0.2) (for details, see Materials and methods). B – D, Hybridization bands of electrophoretically separated RNA (10 μg) of five-day-old mustard seedlings with *psbA* and *trnK*-specific probes. B, Etioplast RNA, hybridized with nick-translated DNA probes I (Lane 1), II (Lane 2) and III (Lane 3). C, Chloroplast RNA, hybridized with *trnK* intron-specific RNA probe III (Lane 1); with *trnK* 3′ exon probe IV (Lane 2); and with 5′ exon probe V (Lane 3). D, Total cellular RNA from light-grown seedlings (Lane 1), chloroplast RNA (Lane 2), and mitochondrial RNA (Lane 3), all hybridized with DNA probe II.

Results

Transcriptional organization of the psbA/trnK region on mustard chloroplast DNA

Figure 1A gives details on the location of the *psbA* [14] and *trnK* [19] genes as well as the positions of hybridization probes used in the present study. Region I covers the *psbA* 3′ portion, region II overlaps both the 5′ portion of the *psbA* gene and the 3′ portion of the *trnK* gene, and region III provides intron-specific *trnK* probes. The results obtained with DNA probes of regions I – III following Northern hybridization with mustard etioplast RNA are shown in Fig. 1B. Probes I and II both reveal a predominant signal at the position of the full-size 1.2 kb *psbA* transcript [10] (Fig. 1B, lanes 1 and 2) as well as several minor signals in the lower molecular-weight region that are more prominent in lane 2 than in lane 1 and might represent degradation products of the *psbA* transcript [10], while only probe II detects an additional minor transcript 2.8 kb in size (Fig. 1B, lane 2). This high molecular-weight RNA species is virtually the only transcript detected by probe III (Fig. 1B, lane 3), indicating that it might represent the full-length *trnK* transcript [19].

This is further supported by the results of hybridization experiments with chloroplast RNA and probes covering three different *trnK* regions (Fig. 1C). A high specific-activity RNA probe III (equivalent to region III in Fig. 1A) gives rise to a major hybridization signal at 2.8 kb as well as several minor bands, which together account for less than 10% of the total radioactivity (Fig. 1C, lane 1). These additional bands are likewise detected following hybridization with either a 3′ exon DNA probe (Probe IV; Fig. 1C, lane 2) or a 5′ exon probe (Prove V; Fig. 1C, lane 3), which contain the entire 3′ and 5′ half of the $tRNA^{Lys}$ coding region, respectively. They are more prominent with the 5′ exon probe. Although the origin of these minor bands is not clear, at least some of them might represent RNA processing intermediates [25]. Both the 3′ and 5′ *trnK* exon probes generate a strong low molecular-weight RNA signal (Fig. 1C, lanes 2 and 3; bottom band) which is not found with the intron-specific probe III (Fig. 1C, lane 1) and is likely to represent mature tRNA. These data suggest that the intron-specific probe III is suitable for assessment of the 2.8 kb *trnK* transcript with minimal cross-hybridization to other RNA species.

As shown in Fig. 1D (Lane 2), both the 2.8 kb *trnK* and 1.2 kb *psbA* transcripts are detected in chloroplast RNA by using DNA probe II (Fig. 1A), but the intensity of the 2.8 kb *trnK* signal relative to the 1.2 kb *psbA* signal is much lower than with etioplast RNA (Fig. 1B, lane 2). Following hybridization with an equal amount of total cellular RNA from light-grown seedlings (Fig. 1D, lane 1), both the *psbA* and *trnK* signals are found with reduced intensity, but in comparable ratio (estimated 100:1) as with chloroplast RNA. No hybridization signals are seen with mitochondrial RNA (Fig. 1D, lane 3), indicating that hybridization signals at 2.8 kb obtained with total cellular RNA can be specifically assigned to plastid *trnK* transcripts.

Transcript levels of plastid psbA *and* trnK *genes during mustard seedling development*

To estimate transcript levels, RNA was extracted from seeds and from cotyledons of seedlings up to 96 h after sowing, separated on gels, and blotted for Northern hybridization. As shown in Fig. 2 (left panel), the pattern, intensity and integrity of rRNA bands revealed by ethidium bromide staining did not change substantially among the loaded RNA samples representing various stages of seedling development in the dark and in the light. The autoradiographs (Fig. 2) following hybridization reveal that the 1.2 kb *psbA* transcript is present in low amounts in seed RNA (Fig. 2A, lane 0) and in cotyledon RNA of seedlings until approximately 24 h of growth either in the dark (Fig. 2A, lanes 1 and 2) or in the light (Fig. 2B, lanes 1 and 2). Thereafter, the *psbA* transcript rapidly becomes more abundant and accumulates to high levels between 48 h and 96 h after sowing (Fig. 2B, lanes 3 – 8). Accumulation of the *psbA* transcript between 24 h and 48 h is also found with RNA from dark-grown seedlings (Fig. 2A, lanes 2 – 4). However, transcript levels appear lower than those found with RNA from light-grown seedlings (Fig. 2B) and not to accumulate further be-

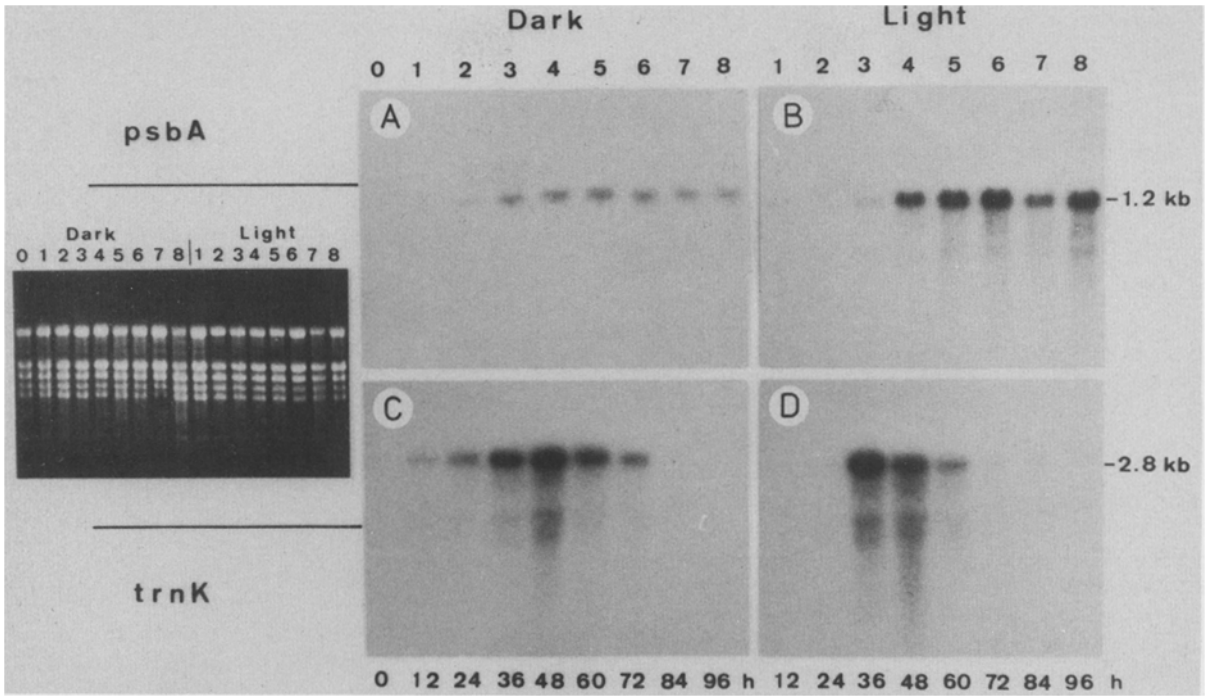

Fig. 2. Northern hybridization analysis of mustard *psbA* and *trnK* transcript levels. RNA was prepared from seeds (Lanes 0) and cotyledons of mustard seedlings 12 to 96 h after sowing (Lanes 1 to 8) and 10-μg portions were electrophoretically separated. Ethidium bromide staining (left panel) shows that each lane contains approximately the same amount of RNA of comparable integrity. Following blotting, *psbA* (A and B) and *trnK* (C and D) transcripts were detected by hybridization with RNA probes I and III, respectively (see Fig. 1A). Hybridization bands with RNA from dark-grown (A and C) and light-grown (B and D) seedlings. Film exposure times were 16 h for *psbA* and 5 days for *trnK* transcripts.

tween 48 h and 96 h (Fig. 2A, lanes 4–8), in accordance with previous findings on the enhancement of *psbA* transcript levels by light [1, 11, 12].

Based on Northern blot analysis, the 2.8 kb *trnK* transcript (Figs. 2C and 2D) is likewise present at low levels both in seeds (Fig. 2C, lane 0) and in cotyledons of either light-grown seedlings (Fig. 2D, lanes 1 and 2) or dark-grown seedlings (Fig. 2C, lanes 1 and 2) up to approximately 24 h after sowing. As for the *psbA* transcript, the intensity of the *trnK* hybridization signal increases until approximately 36 h–48 h after sowing both in the light (Fig. 2D, lanes 2–4) and in the dark (Fig. 2C, lanes 2–4), but thereafter decreases again, indicating rapid transient accumulation of the *trnK* transcript (Figs. 2C and 2D, lanes 4–8). Unlike that of *psbA*, the *trnK* transcript does not appear to accumulate to higher levels in light-grown seedlings (Fig. 2D) as compared to dark-grown seedlings (Fig. 2C).

For a more quantitative assessment of transcript levels, dot blot hybridization was carried out with RNA samples representing various stages of seedling development. Figure 3 shows the autoradiographs of representative dot blots involving hybridization of RNA samples from light-grown (Figs. 3B and 3D) and dark-grown seedlings (Figs. 3A and 3C) with either the *psbA*-specific RNA probe I (Figs. 3A and 3B) or the intron-specific *trnK* RNA probe III (Figs. 3C and 3D) (see Fig. 1A for position of probes). Visual inspection and densitometric analysis (data not shown) revealed relative transcript levels generally consistent with the results of Northern hybridization (Fig. 2), except for an apparent difference in relative signal intensity between Figs. 2D and 3D, at 36 h, respectively. To account for such variability, multiple dot blots were prepared. Transcript levels were quantified by liquid scintillation counting of radioactive dots, using measured amounts of

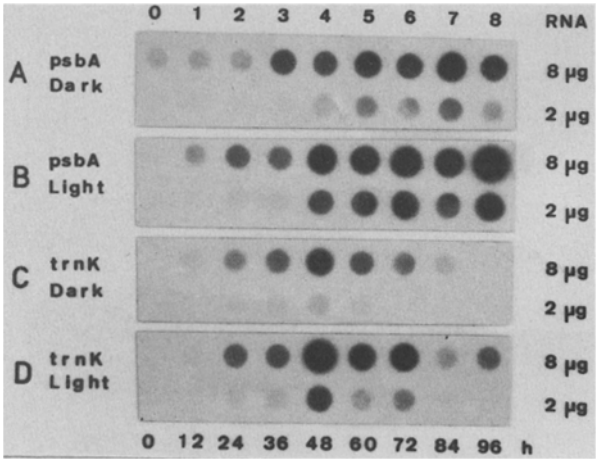

Fig. 3. Dot blot hybridization analysis of *psbA* and *trnK* transcript levels during mustard seedling development. Autoradiographs showing hybridization signals for mustard seed RNA (0 h) and cotyledon RNA from seedlings grown for 12–96 h in the dark (A and C) or in the light (B and D). The RNA probe I (Fig. 1A) was used for detection of *psbA* transcripts (A and B) and RNA probe III for *trnK* transcripts (C and D). Dots represent 8 µg (upper series) or 2 µg of total RNA. No RNA samples were applied in panels B and D at 0 h and in panel C at 96 h.

in vitro psbA transcripts (from pSPT452a) and *trnK* transcripts (from pSPTB0.5) for comparison (Fig. 3A, lane 8), taking into account the *in vitro* and *in vivo* transcript size difference (see Fig. 1A). The resulting graphs (Figs. 4B and 4C) further substantiate the estimates of *psbA* and *trnK* transcript levels during seedling development obtained by Northern hybridization (Fig. 2). Three major features are evident: (i), light-independent initial accumulation of both the *psbA* and *trnK* transcripts between approximately 24 h and 40 h; (ii), subsequent further accumulation of *psbA* transcript levels in the light, but constant, even slightly decreasing levels in the dark (Fig. 4B); (iii), transient peak levels of the *trnK* 2.8 kb transcript between approximately 36 h and 48 h, with no significant difference in extent and time-course detectable between light-grown and dark-grown seedlings (Fig. 4C).

The *psbA* mRNA represents approximately 2.1 mg/g of total cotyledon RNA from 96-h light-grown seedlings, 0.4 mg/g of RNA from dark-grown seedlings of the same age, and 0.6 mg/g of RNA at 48 h. The 2.8 kb *trnK* transcript represents less than 0.01 mg/g of total cellular RNA from either light-

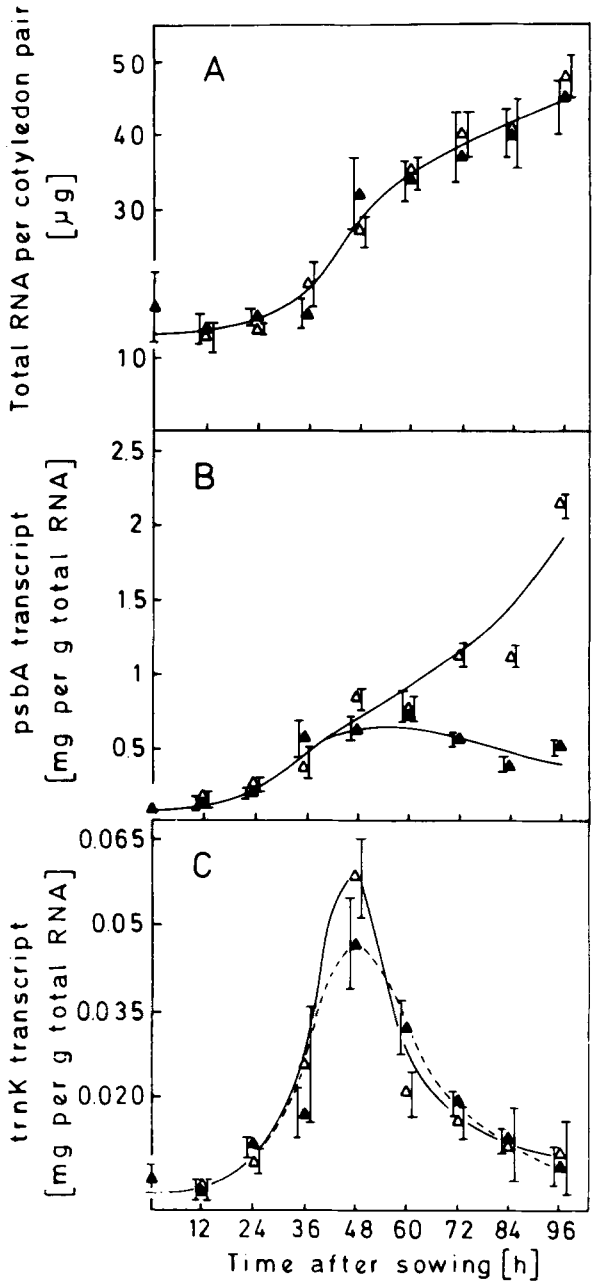

Fig. 4. Quantification of total cotyledon RNA and relative *psbA* and *trnK* transcript levels. A, Total RNA yield of mustard seeds and cotyledons at 12 h–96 h. Relative *psbA* (B) and *trnK* (C) transcript levels in equal amounts of total RNA. Open symbols denote mean values for light-grown seedlings (error bars on the right) and closed symbols those for dark-grown seedlings (error bars on the left). Total RNA levels of light-grown and dark-grown cotyledons were found insignificantly different and were thus presented as one single curve (A). Dark levels of *trnK* transcripts in (C) are shown by the dashed curve. Mean values (standard error of 5–20%) are based on two to three independently extracted RNA samples at each time-point; each RNA sample was dot-blotted two to four times.

Table 1. Absolute *psbA* and *trnK* transcript levels during mustard seedling development.

Time (h)	Transcript (ng) per cotyledon pair			
Time (h)	*psbA*		*trnK*	
	Dark	Light	Dark	Light
0 (Seed)	1.5		0.012	
24	3.1	3.4	0.18	0.12
48	21	22	1.5	1.6
72	22	44	0.72	0.62
96	20	103	0.35	0.47

Relative transcript levels in equal amounts of total cellular RNA were determined by dot blot analysis (Figs. 4B and 4C) and used for calculation of absolute levels by multiplying values with the total amounts of RNA per seed and cotyledon pair (Fig. 4A) at the time-points indicated.

grown or dark-grown seedlings at 96 h and 0.05 mg/g RNA at the peak concentration 48 h after sowing, i.e. 1/10 of *psbA* mRNA at 48 h and 1/200 at 96 h (light) on a weight basis.

For calculation of absolute transcript levels during seedling development on a "per cotyledon pair" basis, the total amount of RNA extracted per cotyledon pair was determined. RNA levels were found to be approximately 12 μg át 12 h and 24 h, 25–30 μg at 48 h, and 40–50 μg at 96 h (Fig. 4A), i.e. they increase three- to fourfold over the period investigated The curves obtained for light-grown and dark-grown seedlings did not differ significantly with regard to the extent and time-course of total RNA accumulation.

Based on the amounts of total cotyledon RNA and relative transcript levels (Fig. 4), absolute *psbA* and *trnK* transcript levels, expressed as ng transcript per cotyledon pair, were calculated and are summarized for several time-points in Table 1. By using the estimate of 5.6×10^5 cells per cotyledon [18], our data suggest that approximately 130000 *psbA* transcripts per cell exist at 96 h in the light, compared to 28000 in the dark, while there appear to be only 900 *trnK* 2.8 kb transcripts per cell at peak levels around 48 h.

Discussion

The present results of Northern and dot blot hybridizations extend our previous findings [11, 12] that the *psbA* mRNA is a major light-enhanced plastid transcript in mustard seedlings. It is interesting to note that *psbA* transcript levels during early mustard seedling development appear to be independent of light until approximately 40 to 48 h after sowing and thus are exclusively related to the endogenous developmental program. Thereafter, light becomes necessary for the *psbA* transcript to accumulate to high levels, while the transcript remains at a lower level and eventually decreases slightly in dark-grown seedlings. A similar time-course with an initial light-independent increase and subsequent light-dependent further accumulation has also been observed for transcripts of several other mustard plastid genes, e.g. the genes encoding RuBPCase (LS), P700 and P680 apoproteins of the two photosystems, CF_0-alpha, and cytochrome b_6 and f as well as several unidentified genes [6] (G. Dietrich and G. Link, unpublished data). We have found no examples of plastid transcripts that are light-inducible before 36 to 48 h after sowing. Thus, it seems likely that photocontrol of plastid gene expression emerges only subsequently to a light-independent "competence" phase [18], which might be characterized by the initial increase in transcript levels between 24 h and 40 h.

The observed light-enhanced levels of the *psbA* transcript during mustard seedling development do not appear to be related to a light-dark difference in gene copy number, as has been found in pea seedlings [23]. Approximately 15% (w/w) of total nucleic acids extracted from cotyledons of various age represents DNA, indicating a three- to fourfold increase of total DNA per cotyledon pair over the period investigated. Southern hybridization analyses (H. Neuhaus and G. Link, unpublished data) have shown that the fraction of chloroplast DNA among total DNA remains constant in both light- and dark-grown seedlings. Hence, the increase in plastid DNA copy number per cotyledon pair appears to be independent of light.

The *trnK* transcript similarly accumulates between 24 and 48 h after sowing. The subsequent

time-course differs however from that found for the *psbA* transcript (and for other plastid transcripts investigated; G. Dietrich and G. Link, unpublished data) in that the *trnK* transcript level is scarcely affected by light and peaks transiently around 48 h after sowing. Obviously, this transient expression of the *trnK* gene might be completely unrelated to the observed light-enhanced expression of the *psbA* gene (and other plastid genes) during the subsequent phase of seedling development and purely coincident in time. On the other hand, the 2.8 kb *trnK* transcript (or one of its products) might be functionally involved in processes required for plastid gene expression to become light-responsive, i.e. in the development of competence for light regulation. In addition to the low abundance of the 2.8 kb transcript and its transient accumulation around 48 h, two othr aspects of the *trnK* gene would be consistent with a regulatory role. First, it lies physically close to *psbA*, the plastid gene whose expression in the mustard seedling is most dramatically affected by light. Second, the structural organization of the *trnK* gene is unusual in that the two tRNA coding regions are split by an exceptionally long intron containing an open reading frame [25, 19]. This might code for a protein with structural features related to maturases [19]. It will be interesting to investigate whether this protein is expressed *in vivo* and then test its possible physiological role.

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References

1. Bedbrook JR, Link G, Coen DM, Bogorad L, Rich A: Maize plastid gene expressed during photoregulated development. Proc Natl Acad Sci USA 75: 3060–3064 (1978).
2. Birnboim HC, Doly J: A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7: 1513–1523 (1979).
3. Bottomley W, Smith HJ, Bogorad L: RNA polymerases of maize: Partial purification and properties of the chloroplast enzyme. Proc Natl Acad Sci USA 68: 2412–2416 (1971).
4. Boyer SK, Mullet JE: Characterization of *P. sativum* chloroplast *psbA* transcripts produced *in vivo, in vitro* and in *E. coli*. Plant Mol Biol 6: 229–243 (1986).
5. Cheley S, Anderson R: A reproducible microanalytical method for the detection of specific RNA sequences by dot-blot hybridization. Anal Biochem 137: 15–19 (1984).
6. Dietrich G, Link G: Transcriptional organization and possible function of mustard plastid DNA regions expressed *in vivo*. Curr Genet 9: 683–692 (1985).
7. Ellis JR: Chloroplast proteins: Synthesis, transport, and assembly. Annu Rev Plant Physiol 32: 111–137 (1981).
8. Fromm H, Devic M, Fluhr R, Edelman M: Control of *psbA* gene expression: In mature chloroplasts, light regulation of 32 kDa protein synthesis is independent of transcript level. EMBO J 4: 291–295 (1985).
9. Inamine G, Nash B, Weissbach H, Brot N: Light regulation of the synthesis of the large subunit of ribulose-1,5-bisphosphate carboxylase in peas: Evidence for translational control. Proc Natl Acad Sci USA 82: 5690–5694 (1985).
10. Link G: Cloning and mapping of the chloroplast DNA sequences for two mRNAs from mustard (*Sinapis alba* L.). Nucleic Acids Res 15: 3681–3694 (1981).
11. Link G: Phytochrome control of plastid mRNA in mustard (*Sinapis alba* L.). Planta 154: 81–86 (1982).
12. Link G: Hybridization study of developmental plastid gene expression in mustard (*Sinapis alba* L.) with cloned probes for most plastid DNA regions. Plant Mol Biol 3: 243–248 (1984).
13. Link G: DNA sequence requirements for the accurate transcription of a protein coding plastid gene in a plastid *in vitro* system from mustard (*Sinapis alba* L.). EMBO J 3: 1697–1704 (1984).
14. Link G, Langridge U: Structure of the chloroplast gene for the precursor of the Mr 32000 photosystem II protein from mustard (*Sinapis alba*). Nucleic Acids Res 12: 945–957 (1984).
15. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning, a Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory (1982).
16. Mattoo AK, Pick U, Hoffmann-Falk H, Edelman M: The rapidly metabolized 32000-dalton polypeptide of the chloroplast is the "proteinaceous shield" regulating photosystem II electron transport and mediating diuron herbicide sensitivity. Proc Natl Acad Sci USA 78: 1572–1576 (1981).
17. Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K, Green MR: Efficient *in vitro* synthesis of biologically active RNA and DNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res 12: 7035–7057 (1984).

18. Mohr H: Phytochrome and chloroplast development. In: Baker NR, Barber J (eds) Chloroplast Biogenesis, pp 307–347. Amsterdam: Elsevier Science Publishers (1984).
19. Neuhaus H, Link G: The chloroplast $tRNA^{Lys}$ (UUU) gene from mustard (*Sinapis alba*) contains a class II intron potentially coding for a maturase-related polypeptide. Curr Genet 11: 251–257 (1987).
20. Reiss T, Link G: Characterization of transcriptionally active DNA-protein complexes from chloroplasts and etioplasts of mustard (*Sinapis alba* L.). Eur J Biochem 148: 207–212 (1985).
21. Richter G: Blue light control of the level of two plastid mRNAs in cultured plant cells. Plant Mol Biol 3: 271–276 (1984).
22. Rodermel SR, Bogorad L: Maize plastid photogenes: Mapping and photoregulation of transcript levels during light-induced development. J Cell Biol 100: 463–476 (1985).
23. Sasaki Y, Tomada Y, Kamikubo T: Light regulates the gene expression of ribulosebisphosphate carboxylase at the levels of transcription and gene dosage in greening pea leaves. FEBS Lett 173: 31–35 (1984).
24. Steinback KE, McIntosh L, Bogorad L, Arntzen CJ: Identification of the triazine receptor protein as a chloroplast gene product. Proc Natl Acad Sci USA 78: 7463–7467 (1981).
25. Sugita M, Shinozaki K, Sugiura M: Tobacco chloroplast $tRNA^{Lys}$ (UUU) gene contains a 2.5-kilobasepair intron: An open reading frame and a conserved boundary sequence in the intron. Proc Natl Acad Sci USA 82: 3557–3561 (1985).
26. Thompson WF, Everett M, Polans NO, Jorgensen RA, Palmer JD: Phytochrome control of RNA levels in developing pea and mung-bean leaves. Planta 158: 487–500 (1983).
27. Tobin EM, Silverthorne J: Light regulation of gene expression in higher plants. Annu Rev Plant Physiol 36: 569–593 (1985).
28. Zhu YS, Kung SD, Bogorad L: Phytochrome control of levels of mRNA complementary to plastid and nuclear genes of maize. Plant Physiol 79: 371–376 (1985).
29. Zinn K, DiMaio D, Maniatis T: Identification of two distinct regulatory regions adjacent to the human beta-interferon gene. Cell 34: 865–879 (1983).
30. Zurawski G, Bohnert HJ, Whitfeld PR, Bottomley W: Nucleotide sequence of the gene for the M_r 32000 thylakoid membrane protein from *Spinacia oleracea* and *Nicotania debneyi* predicts a totally conserved primary translation product of M_r 38950. Proc Natl Acad Sci USA 79: 7699–7703 (1982).