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Plastid Genes Encoding the Transcription/Translation Apparatus Are Differentially Transcribed Early in Barley (*Hordeum vulgare*) Chloroplast Development[1]

Evidence for Selective Stabilization of *psb*A mRNA

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Chloroplast genomes encode rRNAs, tRNAs, and proteins involved in transcription, translation, and photosynthesis. The expression of 15 plastid genes representing each of these functions was quantitated during chloroplast development in barley (*Hordeum vulgare*). The transcription of all plastid genes increased during the initial phase of chloroplast development and then declined during chloroplast maturation. RNAs corresponding to *rpo*B-*rpo*C1-*rpo*C2, which encode subunits of a plastid RNA polymerase, and *rps*16, which encodes a ribosomal protein, reached maximal abundance early in chloroplast development prior to genes encoding subunits of the photosynthetic apparatus (*rbc*L, *atp*B, *psa*A, *pet*B). Transcription of *rpo*B as well as 16S rRNA, *trn*fM-*trn*G, and *trn*K was high early in chloroplast development and declined 10-fold relative to *rbc*L transcription during chloroplast maturation. RNA hybridizing to *psb*A and *psb*D, genes encoding reaction center proteins of photosystem II, was differentially maintained in mature chloroplasts of illuminated barley. Differential accumulation of *psb*D mRNA relative to *rbc*L mRNA was due to light-stimulated transcription of *psb*D. In contrast, enhanced levels of *psb*A mRNA in mature chloroplasts were due primarily to selective stabilization of the *psb*A mRNA. These data document dynamic modulation of plastid gene transcription and mRNA stability during barley chloroplast development.

Barley (*Hordeum vulgare*) leaf mesophyll cells are highly specialized for photosynthesis and contain an average of 60 chloroplasts (Baumgartner et al., 1989). During barley leaf development, most mesophyll cells are derived from an intercalary meristem located in the leaf base. Cells located in the leaf basal meristem are active in cell division and contain approximately 10 proplastids that are small (0.5 μm diameter), are nonphotosynthetic, contain few ribosomes, and exhibit low transcription activity (Baumgartner et al., 1989). Early in barley mesophyll cell and chloroplast development, plastid DNA content increases, followed by activation of plastid transcription (Baumgartner et al., 1989). These events are followed by accumulation of plastid RNA, activation of protein synthesis, and assembly of the photosynthetic apparatus (Klein and Mullet, 1987; Mullet and Klein, 1987; Rapp and Mullet, 1991). Once a full complement of mature chloroplasts is established, overall plastid transcription and translation activities decline (Mullet and Klein, 1987; Baumgartner et al., 1989), although the synthesis of some plastid proteins remains elevated in mature chloroplasts to replace proteins damaged due to photochemistry (Gamble et al., 1988; Ruf and Kössel, 1988; Greenberg et al., 1989; reviewed by Mattoo et al., 1989). Similar global changes in plastid transcription activity and mRNA levels occur during chloroplast biogenesis in other plants (i.e. Rodermel and Bogorad, 1985; Dietrich et al., 1987; Deng and Gruissem, 1987; Schrubar et al., 1990).

Plastid genes encode rRNAs, tRNAs, and proteins. The plastid-encoded proteins include subunits of an RNA polymerase (encoded by *rpo*A, B, C1, C2), ribosomes (encoded by 12 *rps* and 8 *rpl* genes in rice) (Hiratsuka et al., 1989), a putative NADH oxido-reductase complex (*ndh*A, B, C, D, E, F, G), and proteins involved in photosynthesis. This latter group of genes includes the large subunit of Rubisco (*rbc*L), subunits of PSI (*psa*A, B, C), PSII (*psb*A, B, C, D, E, F, G, H, I, K, L), ATP synthase (*atp*A, B, E, F, H, I), and the Cyt b_6/f complex (*pet*A, B, D) (Shinozaki et al., 1986; Hiratsuka et al., 1989). These genes are often organized in cotranscribed gene clusters that encode proteins involved in transcription/translation or photosynthesis. For example, the *psb*B-*psb*H-*pet*B-*pet*D and *psb*I-*psb*K-*psb*D-*psb*C operons encode proteins involved in photosynthetic electron transport, whereas the *rrn* (16S-*trn*I-*trn*A-23S-4.5S-5S), *rps*23-*rpl*2-*rps*19-*rpl*22-*rps*3-*rpl*16-*rps*8-*inf*A-*rpl*36-*rps*11-*rpo*A, and *rpo*B-*rpo*C1-*rpo*C2 operons encode proteins involved in transcription and translation (Shinozaki et al., 1986; Ohyama et al., 1988; Hiratsuka et al., 1989).

This organization of genes, in principle, offers the opportunity to differentially express genes involved in transcription/translation relative to genes encoding proteins involved in photosynthesis. Until the present study, however, there was little direct evidence to support this possibility. In fact, it has been suggested that plastid gene expression may be optimized for rRNA synthesis and that other plastid genes are transcribed in excess (Bendich, 1987). However, a recent quantitative analysis of plastid RNA levels and transcription rates at one stage of barley chloroplast development revealed an over 300-fold variation in plastid gene transcription activity and mRNA levels (Rapp et al., 1992).

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In general, genes encoding abundant proteins were transcribed at high rates (*psb*A, *rbc*L), whereas genes encoding low abundance proteins were transcribed at low rates (*rpo*B, *rpo*A). Correlation between transcription rate and mRNA accumulation was observed for most genes, indicating that transcription activity is an important determinant of mRNA level (Rapp et al., 1992). Moreover, light-induced differential transcription of *psb*A and *psb*D is observed in barley (Klein and Mullet, 1990; Sexton et al., 1990a) and for *pet*G in maize (Haley and Bogorad, 1990). In contrast, in spinach (*Spinacia oleracea*), differential transcription of plastid genes during chloroplast biogenesis is limited and differential accumulation of mRNA has been attributed to modulation of RNA stability (Deng and Gruissem, 1987).

In this study, we have quantitated transcription and mRNA levels for 15 plastid genes during chloroplast development in barley. Overall plastid transcription rates and mRNA levels increased early in chloroplast development and then declined during chloroplast maturation. In addition to this global regulation of transcription activity, several genes encoding the transcription/translation apparatus were differentially transcribed early in chloroplast development, whereas genes encoding proteins involved in photosynthesis showed highest expression later in development. Two plastid genes, *psb*A and *psb*D, were differentially expressed in mature chloroplasts. Differential accumulation of *psb*D mRNA was attributed to light-induced transcription, whereas elevated *psb*A mRNA levels in mature chloroplasts were due primarily to selective stabilization of *psb*A mRNA.

MATERIALS AND METHODS

Plant Growth and Plastid Isolation

Barley (*Hordeum vulgare* L. var Morex) seedlings were grown in controlled environmental chambers for 4 d in darkness or for 2 d in darkness followed by continuous illumination for 2 or 4 d (Baumgartner et al., 1989). Chloroplasts from various sections of barley leaves (Fig. 1) were isolated on Percoll gradients as described in Baumgartner et al. (1989).

Extraction of RNA from Isolated Plastids and Analysis of Plastid RNA

RNA was extracted from isolated plastids as described in Orozco et al. (1985). To ensure maximum recovery of plastid RNA, no more than 2×10^8 plastids were used per 500-μL volume of extraction buffer. RNA extracted from isolated plastids was fractionated on formaldehyde-agarose gels, blotted onto GeneScreen Plus nylon membranes, and probed with in vitro-generated antisense RNA probes as described (Rapp et al., 1992). RNA abundance was quantitated using RNA dot blots as described (Rapp et al., 1992).

Chloroplast Gene Probes

Fragments of chloroplast DNA containing portions of plastid genes were prepared from restriction digests of barley chloroplast DNA or by the polymerase chain reaction procedure (Rapp et al., 1992). DNA fragments were cloned into pBluescript plasmid expression vectors (Stratagene). DNA restriction fragments included a 0.99-kb *Sac*I-*Sma*I fragment of 16S rDNA and a 1.0-kb *Bam*HI fragment of *psa*A. Additional chloroplast gene fragments were isolated by polymerase chain reaction, including portions of the coding sequences of *rps*16 (1.0 kb), *rpl*16 (0.35 kb), *rpo*A (1.0 kb), *rpo*B (2.3 kb), *trn*fM-*trn*G (0.9 kb), *trn*K (2.3 kb, which also contains open-reading frame 542), *pet*B (0.65 kb), *ndh*A (0.47 kb), and *atp*B (1.5 kb). The remaining genes used in this study included a 1.3-kb *Hin*dIII-*Pst*I fragment of *rbc*L (Zurawski et al., 1984), a 1.4-kb *Hin*cII-*Eco*RI fragment of *psb*A (Boyer and Mullet, 1988a), a 0.87-kb *Eco*RI fragment of *psb*D (Gamble et al., 1988), and a 1.9-kb *Bam*HI fragment of *psa*B (Berends et al., 1987).

Plastid Run-On Transcription Assays and Quantitation of Plastid Gene Transcription

Plastid run-on transcription assays (Mullet and Klein, 1987) were carried out as modified in Rapp et al. (1992). Radiolabeled transcripts generated in 5-min lysed plastid run-on transcription assays were extracted as described (Klein and Mullet, 1990). The transcripts were hybridized to dot blots containing saturating levels (1 pmol) of in vitro-synthesized antisense RNA transcripts for each of the 15 genes analyzed.

RESULTS

Chloroplast Development and Overall Transcription Activity

Barley leaves grow from a basal intercalary meristem, which creates a gradient of cell and chloroplast development with undifferentiated cells and proplastids located in the leaf base and more developed cells and chloroplasts in the leaf apex (Fig. 1, top) (Baumgartner et al., 1989). To assay transcription and mRNA levels in plastids throughout chloroplast development, we isolated plastids from the leaf sections shown in Figure 1. Plastids in leaf section 1 are small, contain little Chl, and exhibit low transcription activity (Fig. 1, bottom). Once cells stop dividing and enter the zone of cell elongation (section 2), plastid transcription increases rapidly. In dark-grown seedlings, chloroplast development reaches its end point in sections 4/5 of 4-d-old seedlings (fully developed etioplasts) (Baumgartner et al., 1989). However, in illuminated plants, plastids develop past the etioplast stage, accumulate Chl, and become photosynthetically active (sections 4, 5, and 7 of illuminated plants) (Baumgartner et al., 1989). Chloroplast transcription activity declines during the latter phase of chloroplast maturation.

Plastid RNA Populations Change during Chloroplast Development

Plastid RNA from sections 4/5 of dark-grown barley was previously analyzed by northern analysis (Rapp et al., 1992). This analysis was extended in this paper to include earlier and later stages of chloroplast development. RNA from the plastid populations described in Figure 1 was loaded on northern gels on an equal plastid number basis and probed with 14 gene-specific probes. Figure 2 shows the genes

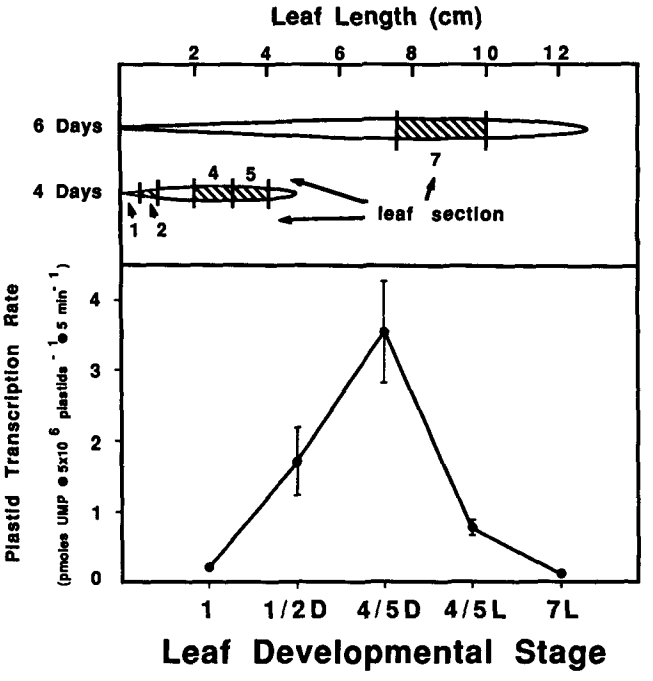

Figure 1. Plastid transcription rates as a function of barley leaf and chloroplast development. Barley seedlings were grown 4 d in darkness or 2 d in darkness and then exposed to continuous light. Primary leaves from 4- or 6-d-old seedlings were excised and then divided into the sections shown at the top of the figure. Plastids were isolated from various leaf sections and overall transcription activity in each plastid population assayed using 5-min run-on transcription assays. These data are shown in the lower portion of the figure, as pmol UMP incorporated per 5 × 10^6 plastids per 5 min. Leaf development stage refers to the leaf sections from which plastids were isolated (1, 1/2D, 4/5D from leaf sections of 4-d-old dark-grown plants; 4/5L from leaf sections of plants grown 2 d in darkness and then illuminated for 2 d; 7L from leaf section 7 of plants grown 2 d in darkness then illuminated for 4 d).

analyzed (boxed), a small portion of the barley genome where each gene is located, the location of the probe used for analysis (bracketed region), and northern blots. The abundance of RNA among the genes analyzed varied over 400-fold. Therefore, northern blots were exposed to x-ray film for different periods of time so that RNA populations corresponding to all of the genes could be visualized.

rRNA and tRNAs

The 16S rRNA probe detected an abundant 1.6-kb RNA at all stages of plastid development (Fig. 2A). This result is consistent with the observation that ribosomes are present in all plastid types, although ribosome abundance varies greatly (Smith, 1970; Thien and Schopfer, 1975; Deng and Gruissem, 1988; Rapp et al., 1992). Several genes that encode tRNAs were analyzed, including *trn*fM-*trn*G, which are cotranscribed, and *trn*K (Oliver and Poulsen, 1984). The *trn*fM-*trn*G probe detected RNAs of 0.86 kb and less than 0.1 kb, which most likely correspond to the unspliced precursor RNA and processed tRNAs, respectively. The ratio of large to small RNAs decreased during development. In addition, the level of the 0.86-kb RNAs was high early in development and decreased with chloroplast maturation. The third tRNA gene analyzed, *trn*K, is located upstream from *psb*A in barley. The *trn*K probe detected a 2.6-kb RNA that represents the unspliced precursor for tRNA(Lys) (Sexton et al., 1990b). The abundance of the unspliced RNA was high early in chloroplast development, but low in mature chloroplasts.

RNAs Encoding Subunits of the RNA Polymerase, Ribosomes

The plastid genome contains four genes that encode subunits of the RNA polymerase (*rpo*A, B, C1, C2). Three of these genes are organized in the *rpo*B-*rpo*C1-*rpo*C2 operon (Hudson et al., 1988). In barley, an *rpo*B probe detected a 6.0-kb RNA (Fig. 2) that probably results from cotranscription of *rpo*B and *rpo*C1 as previously shown in spinach (Hudson et al., 1988). The abundance of the *rpo*B RNA was highest in section 1/2 from dark-grown seedlings and declined rapidly in abundance during chloroplast development. *rpo*A, which encodes the α subunit of the RNA polymerase, and *rpl*16, which encodes a ribosomal protein, are part of a cluster of genes encoding additional ribosomal proteins and *inf*A (Hiratsuka et al., 1989). In some plastids, these genes are cotranscribed (Ruf and Kössel, 1988; Christopher and Hallick, 1990), although the complex mRNA profiles arising from this gene cluster could result from the action of multiple promoters and RNA processing. Northern analysis using *rpo*A and *rpl*16 probes revealed a complex RNA pattern for both of these genes (Fig. 2B). RNA abundance for both genes was maximal in section 4/5 from dark-grown plants. The expression of a second ribosomal protein-encoding gene, *rps*16, which is located between *trn*Q and *trn*K in barley, was also analyzed. The *rps*16 probe detected mRNAs of 1.4, 1.2, and 0.6 kb that correspond to introns containing precursor RNAs (1.2 and 1.4 kb) and processed RNAs (0.6 kb) (Sexton et al., 1990b). The precursor *rps*16 RNAs were in greatest abundance in sections 1/2 and decreased thereafter. The processed *rps*16 mRNA was more abundant in sections 1/2 and 4/5 of dark-grown plants compared with illuminated plants.

RNAs Encoding the Photosynthetic Apparatus, NADH Oxidoreductase

The gene, *ndh*A, which encodes a subunit of the putative NADH oxidoreductase complex (Matsubayashi et al., 1987) is located in the small single-copy region of the barley genome. This gene has not been sequenced in barley, so the rice *ndh*A gene organization is shown in Figure 2C (Hiratsuka et al., 1989). The *ndh*A northern probe detected RNAs 4.0, 2.3, and 1.2 kb in size. If the barley gene, like rice *ndh*A, contains an intron, the larger RNAs could be unprocessed precursor RNAs, whereas the 1.2 kb RNA could be the mature *ndh*A mRNA. RNA levels for *ndh*A were low in plastids isolated from the leaf base (sections 1/2), high in sections 4/5, and low again in mature chloroplasts, similar to the genes that encode proteins involved in photosynthesis described below.

Six genes that encode proteins involved in photosynthesis

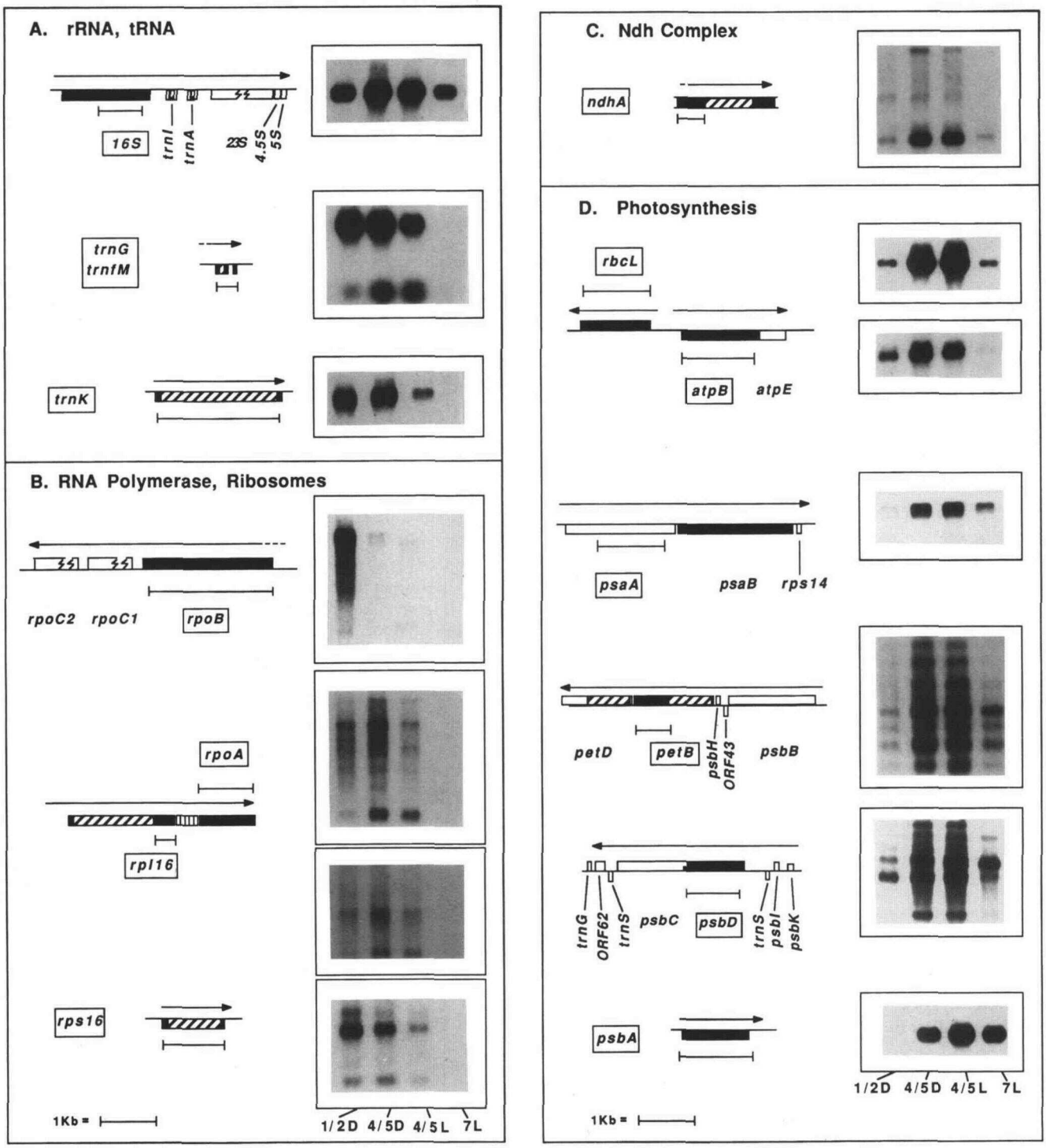

Figure 2. Northern blots of RNA from developing barley chloroplasts. Plastids were isolated from the leaf sections of dark-grown (1/2D, 4/5D) or illuminated plants (4/5L, 7L) described in Figure 1 and denoted at the bottom of the figure. RNA from an equal number of plastids was loaded in each lane and probed with radiolabeled antisense RNA from the gene shown at the left (gene-specific probes are designated by brackets). A portion of the barley chloroplast genome corresponding to each gene analyzed (gene name boxed) is shown with gene exons represented by solid boxes, introns by cross-hatched boxes, and the direction of gene transcription by an arrow over the DNA region. Genes encoding rRNA and tRNAs are shown in A (16S rRNA, *trn*fM/G, *trn*K); genes encoding subunits of the plastid-encoded RNA polymerase and ribosomal proteins are shown in B (*rpo*B, *rpo*A, *rpl*16, *rps*16); a gene from the *ndh* complex is indicated in C (*ndh*A); and genes encoding proteins of the photosynthetic apparatus are shown in D (*rbc*L, *atp*B, *psa*A, *pet*B, *psb*D, *psb*A).

Table I. *RNA levels in developing barley chloroplasts*

Plastid RNA from leaves of 4-d-old dark-grown barley (sections 1/2D and 4/5D), or barley grown 2 d in darkness then illuminated for 2 d (sections 4/5L), or 4 d (section 7L) was serially diluted and bound to a nylon filter. After hybridization to excess gene-specific probes, radioactivity hybridizing to in vivo RNA and in vitro RNA standards was quantitated and the data expressed as fmol RNA per 5×10^6 plastids. The mean ± SE of two separate experiments done in duplicate is shown.

Gene	Chloroplast Developmental Stage			
	1/2D	4/5D	4/5L	7L
16S rRNA	266 ± 20	1183 ± 136	800 ± 32	185 ± 316
*trn*fM/G	14 ± 0.6	52 ± 7	19 ± 0.8	5.8 ± 0.7
*trn*K	2.3 ± 0.3	3.7 ± 0.1	1.4 ± 0.2	0.2 ± 0.04
*rpo*B	0.14 ± 0.01	0.05 ± 0.01	0.02 ± 0.002	0.02 ± 0.01
*rpo*A	1.2 ± 0.16	1.6 ± 0.2	0.6 ± 0.03	0.07 ± 0.02
*rpl*16	1.15 ± 0.08	2.4 ± 0.5	1.4 ± 0.2	0.4 ± 0.07
*rps*16	0.35 ± 0.05	0.31 ± 0.08	0.11 ± 0.002	0.07 ± 0.02
*ndh*A	0.09 ± 0.04	0.26 ± 0.04	0.33 ± 0.12	0.03 ± 0.009
*rbc*L	4.1 ± 0.2	45.1 ± 2.5	35.1 ± 4.3	4.3 ± 0.7
*atp*B	0.9 ± 0.1	3.9 ± 0.5	1.9 ± 0.2	0.2 ± 0.03
*psa*A	1.5 ± 0.3	8.5 ± 0.9	3.7 ± 0.4	0.6 ± 0.1
*pet*B	1.4 ± 0.3	12.5 ± 4.2	6.9 ± 2.0	0.5 ± 0.1
*psb*D	3.0 ± 0.3	13.0 ± 1.6	8.1 ± 1.1	4.6 ± 0.6
*psb*A	1.7 ± 0.2	38.1 ± 2.8	52.0 ± 6.1	26.6 ± 3.2

were analyzed. They include *rbc*L, which encodes the large subunit of Rubisco; *atp*B, which encodes the β subunit of the ATP synthase; *psa*A, a PSI reaction center protein-encoding gene; *pet*B, which encodes Cyt b_6 of the Cyt b_6/f complex; and *psb*D and *psb*A, encoding D1 and D2, respectively, which are reaction center subunits of PSII (Fig. 2D). The *rbc*L probe detected a 1.4-kb RNA, which increased to maximal abundance in sections 4/5 of illuminated plants and then declined in more mature chloroplasts. The *atp*B probe detected a 2.2-kb RNA, which showed maximal abundance in sections 4/5 of dark-grown seedlings. The *psa*A probe detected a 5.6-kb RNA, which, as previously described (Berends et al., 1987), also encodes the *psa*B gene product, a second subunit of the PSI reaction center. The abundance of *psa*A-*psa*B mRNA was low early in chloroplast development, increased to a maximum in sections 4/5, and declined again in mature chloroplast populations.

A similar change in mRNA abundance was observed for *pet*B, although the *pet*B mRNA population was complex, as previously described (Westhoff and Herrmann, 1988). The *psb*D gene is also located in a complex plastid operon (Sexton et al., 1990a). Consistent with earlier studies (Berends et al., 1987), the *psb*D probe detects RNAs ranging in size from 1.5 to 5.7 kb. The abundance of *psb*D-*psb*C RNAs increased to a maximum in sections 4/5, then decreased in chloroplasts of section 7. In addition, a shift in *psb*D mRNA population is observed during development. The change in *psb*D mRNA population was previously shown to be due to activation of a specific light-responsive promoter in this operon (Sexton et al., 1990a). The final gene of this class analyzed was *psb*A (Fig. 2D). *psb*A mRNA abundance was low in sections 1/2, increased to a maximum in sections 4/5 from illuminated plants, and remained high in mature chloroplasts isolated from section 7.

Quantitation of Plastid RNA Levels

The northern blots shown in Figure 2 revealed that changes in plastid RNA abundance occurred during chloroplast development. To obtain information on the levels of each RNA and an accurate estimate of the abundance changes, quantitative analysis of mRNA levels was carried out according to Rapp et al. (1992), and the data are displayed in Table I. Not surprisingly, 16S rRNA was the most abundant RNA analyzed. Consistent with previous studies, rRNA levels increase during early chloroplast biogenesis and then decline in mature chloroplast populations. Levels of mRNAs varied over 400-fold from a high for *psb*A in sections 4/5 of illuminated plants (52 fmol/5×10^6 plastids) to a low of 0.02 fmol for *rpo*B. *rpo*B and *rps*16 mRNA levels were maximal in sections 1/2 and declined thereafter. *trn*K, *rpo*A, and *rpl*16 RNA levels were over 50% of their maximum values in sections 1/2, suggesting that the expression of these genes is active early in chloroplast development. In general, mRNA levels for genes encoding proteins of the photosynthetic apparatus increased 5- to 10-fold between sections 1/2 and 4/5, then declined in mature chloroplasts. *psb*D and *psb*A mRNA levels declined the least during light-induced chloroplast maturation.

Quantitation of Plastid Gene Transcription

Quantitative analysis of specific plastid gene transcription on plastids from section 4/5 of dark-grown plants was previously carried out by Rapp et al. (1992). In the present study, this analysis was extended to include four stages of chloroplast development (Table II). Genes such as 16S rRNA, *trn*fM/G, *trn*K, and *psb*A were transcribed at the highest rates, followed by genes encoding proteins of the photosynthetic apparatus, ribosomes, and the RNA polymerase, re-

Table II. *Transcription rates in developing barley chloroplasts*

Radioactively labeled run-on transcripts from plastids of different developmental stages (described in Fig. 1) were hybridized to gene-specific antisense RNAs. Radioactivity hybridizing to each antisense RNA was quantitated and the data were corrected for percent hybridization and probe size and expressed as fmol UMP incorporated per 5×10^6 plastids per kb per 5 min. The mean ± SE of two separate experiments done in duplicate is shown.

Gene	Chloroplast Developmental Stage			
	1/2D	4/5D	4/5L	7L
16S rRNA	128 ± 18	98 ± 8	18 ± 2	2.5 ± 0.1
*trn*fM/G	97 ± 2	174 ± 27	29 ± 2	4.2 ± 0.7
*trn*K	17 ± 2	31 ± 4	5 ± 1	0.4 ± 0.2
*rpo*B	0.5 ± 0.2	0.5 ± 0.1	0.1 ± 0.01	0.004 ± 0.002
*rpo*A	0.7 ± 0.1	1.2 ± 0.2	0.4 ± 0.05	0.08 ± 0.03
*rpl*16	1.2 ± 0.04	2.4 ± 0.3	1.0 ± 0.1	0.3 ± 0.1
*ndh*A	1.1 ± 0.2	2.4 ± 0.3	1.0 ± 0.7	0.1 ± 0.1
*rbc*L	4.9 ± 0.7	26 ± 2	9.0 ± 0.1	1.4 ± 0.1
*atp*B	2.3 ± 0.5	14 ± 4	3.3 ± 0.5	0.4 ± 0.1
*psa*A	3.8 ± 0.8	16 ± 3	4.4 ± 0.5	1.0 ± 0.1
*pet*B	1.8 ± 0.4	4.3 ± 0.5	1.6 ± 0.2	0.3 ± 0.1
*psb*D	4.6 ± 0.1	14 ± 3	6.1 ± 0.2	3.5 ± 0.4
*psb*A	66 ± 2	153 ± 25	38 ± 3.1	14 ± 0.5

spectively. Transcription activity for all of the genes increased during the early phase of chloroplast biogenesis and then decreased once mature chloroplast populations were established. However, depending on the gene, maximal transcription rates were observed in sections 1/2 or 4/5 in dark-grown plants. In addition, changes in the relative ratios of gene transcription occurred during development. For example, transcription of 16S rRNA was greater than *psb*A early in chloroplast development but less than that of *psb*A in mature chloroplasts. Changes in relative rates of transcription among plastid genes as a function of chloroplast development were analyzed by calculating the ratio of the transcription rate for each gene to that of *rbc*L (Table III). Changes in the ratio of transcription as a function of development were then calculated and expressed in Table III. This analysis showed that transcription of 16S rRNA, *trn*fM/G, *trn*K, and *rpo*B decreased 7- to 33-fold relative to *rbc*L as a function of chloroplast development. In contrast, transcription of *psb*D increased 3-fold relative to *rbc*L in illuminated plants.

Modulation of Relative Plastid RNA Stability

RNA levels are determined by rates of transcription and rates of RNA turnover. If RNA stability increases, then RNA levels will increase for a given transcription rate. Therefore, the ratio of RNA accumulated divided by the transcription rate provides a way of estimating relative RNA stability. Furthermore, changes in this ratio indicate that a change in RNA stability has occurred, assuming a steady state has been reached. The ratio of RNA abundance divided by transcription rate was calculated using the quantitative transcription and RNA abundance data in Tables I and II and the results are shown in Table IV. Ratios were calculated for plastids from leaf sections 1/2 and 4/5 from dark-grown plants and for mature chloroplast populations from sections 7 or 4/5 of illuminated plants. For some genes, expression in section 7 was very low so data from sections 4/5 from illuminated plants were used to provide more accurate estimations. The data in Table IV show that predicted RNA stabilities in sections 1/2 varied over 100-fold with 16S rRNA being the most stable and *psb*A being the least stable RNA. The predicted stability of most of the RNAs did not change during chloroplast development. However, a few RNAs showed dramatic increases in predicted stability. For example, 16S rRNA stability increased 35-fold during development. Smaller changes in calculated relative mRNA stability were observed for *rbc*L (4-fold) and *pet*B (3-fold). The greatest increase in predicted RNA stability, a 100-fold increase, occurred for the *psb*A mRNA.

DISCUSSION

Modulation of Overall Plastid Transcription Activity

The conversion of proplastids to chloroplasts in barley is accompanied by an increase in plastid transcription activity during early chloroplast biogenesis followed by a decrease once mature chloroplasts are established (Mullet and Klein, 1987; Baumgartner et al., 1989). In barley, where leaf development occurs in the absence of light, activation of plastid transcription during chloroplast development is light independent and occurs when cells stop dividing and enter the zone of cell elongation (Baumgartner et al., 1989; also see Fig. 1). In plants that require light for leaf development (i.e. spinach, sorghum, pea, and mustard), plastid transcription (Deng and Gruissem, 1987; Schrubar et al., 1990) and mRNA accumulation (Thompson et al., 1983; Dietrich et al., 1987; Woodbury et al., 1989) are light stimulated. The molecular mechanisms controlling these global changes in plastid transcription are unknown. DNA levels per plastid increase early in chloroplast biogenesis and decline in mature chloroplasts (Lawrence and Possingham, 1986; Baumgartner et al., 1989).

Table III. *Ratio of plastid gene transcription relative to rbcL transcription during chloroplast development*

Ratio of transcription of the plastid genes listed on the left relative to *rbc*L from leaf sections 1/2 and 4/5 of 4-d-old dark-grown seedlings (1/2, 4/5D) and sections 4/5 and 7 of illuminated plants (4/5L, 7L). The fold change in the ratio of the transcription of each specific gene to *rbc*L was calculated using data from sections 1/2D and section 7L. The data used for the ratios shown were derived from Table II.

Gene	Chloroplast Developmental Stage				Fold Change in Ratio
	1/2D	4/5D	4/5L	7L	
16S rRNA	26 ± 5.2	3.9 ± 0.4	2.0 ± 0.2	1.9 ± 0.1	↓13 ×
*trn*fM/G	19.8 ± 2.9	6.7 ± 1.2	3.2 ± 0.2	3.0 ± 0.5	↓7 ×
*trn*K	3.5 ± 0.6	1.2 ± 0.2	0.6 ± 0.1	0.3 ± 0.1	↓12 ×
*rpo*B	0.1 ± 0.04	0.02 ± 0.001	0.01 ± 0.001	0.003	↓33 ×
*rpo*A	0.1 ± 0.03	0.05 ± 0.1	0.04 ± 0.04	0.06 ± 0.02	
*rpl*16	0.2 ± 0.04	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.07	
*ndh*A	0.2 ± 0.05	0.1 ± 0.01			
*rbc*L	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.02	1.0 ± 0.1	
*atp*B	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.06	0.3 ± 0.07	
*psa*A	0.8 ± 0.2	0.5 ± 0.1	0.5 ± 0.06	0.7 ± 0.1	
*pet*B	0.4 ± 0.1	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.07	
*psb*D	0.9 ± 0.1	0.5 ± 0.1	0.7 ± 0.02	2.5 ± 0.3	↑3 ×
*psb*A	13.5 ± 1.9	5.9 ± 1.0	4.2 ± 0.3	10.6 ± 0.8	

However, transcription activity per DNA template varies over 10-fold during chloroplast biogenesis (Deng and Gruissem, 1987; Baumgartner et al., 1989) and the kinetics of change in plastid DNA level and transcription are different (Dietrich et al., 1987; Baumgartner et al., 1989). Furthermore, in sorghum, light-induced increases in plastid transcription were paralleled by increased abundance of the plastid-encoded β subunit of the RNA polymerase (Schrubar et al., 1990). Therefore, the increase in plastid transcription activity during chloroplast development must involve enhanced expression of *rpo*B, which encodes the β subunit.

Differential Accumulation of Plastid mRNAs during Chloroplast Development

In this study, we show that all of the plastid genes examined are influenced by the global changes in plastid transcription activity that occur during barley leaf and chloroplast

Table IV. *Predicted relative plastid RNA stabilities and fold change in stability during chloroplast development*

Relative RNA stability was calculated for each plastid gene as the ratio of RNA level to transcription rate, which is expressed in arbitrary units. Data for the calculations were obtained from Tables I and II. Plastids were derived from dark-grown plants (1/2D, 4/5D) or illuminated plants (4/5L, 7L) as described in Figure 1.

Gene	Chloroplast Developmental Stage			Fold Change in RNA Stability
	1/2D	4/5D	7L (or 4/5L)	
16S rRNA	2.0 ± 0.3	12 ± 2	76 ± 13	↑35 ×
*trn*fM/G	0.1 ± 0.02	0.3 ± 0.06	1.3 ± 0.3	↑13 ×
*trn*K	0.1 ± 0.02	0.1 ± 0.01	0.3 ± 0.06 (4/5L)	↑3 ×
*rpo*B	0.3 ± 0.1	0.1 ± 0.02	0.2 ± 0.03 (4/5L)	
*rpo*A	1.6 ± 0.2	1.4 ± 0.2	1.0 ± 0.4	
*rpl*16	1.0 ± 0.1	1.0 ± 0.2	1.5 ± 0.8	
*ndh*A	0.2 ± 0.08	0.1 ± 0.02	0.3 ± 0.2 (4/5L)	
*rbc*L	0.8 ± 0.1	1.7 ± 0.1	3.1 ± 0.4	↑4 ×
*atp*B	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	
*psa*A	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	
*pet*B	0.7 ± 0.2	2.9 ± 1.0	2.0 ± 1.3	↑3 ×
*psb*D	0.6 ± 0.1	1.0 ± 0.3	1.3 ± 0.2	
*psb*A	0.02 ± 0.002	0.2 ± 0.04	1.9 ± 0.2	↑100 ×

development. However, the kinetics of accumulation and decline of specific plastid mRNAs varied depending on the gene analyzed. For example, *rpo*B and *rps*16 mRNAs reached maximal abundance per plastid early in chloroplast development in sections 1/2 of dark-grown seedlings. Lower levels were observed when plastids from section 1 were analyzed (data not shown). At this stage of chloroplast development, *rpo*A and *rpl*16 mRNAs were approximately 50% of their maximal accumulation, whereas *rbc*L and *psb*A mRNAs had accumulated to less than 10% of their maximal levels. In general, genes that encode proteins of the photosynthetic apparatus showed delayed accumulation of their mRNAs relative to *rpo*B or *rps*16. *rbc*L, *atp*B, *psa*A, *pet*B, and *psb*D, as well as *rpo*A and *rpl*16, all showed maximal accumulation of mRNA in plastids from leaf sections 4/5 of dark-grown plants. *psb*A mRNA levels reached their maximum per plastid even later in chloroplast development (sections 4/5 of illuminated plants). In addition, *psb*A and *psb*D mRNA levels remained differentially elevated in mature chloroplast populations relative to other plastid mRNAs. *rbc*L, *pet*B, and *atp*B mRNA levels declined 10-fold between sections 4/5 and 7 in illuminated plants, whereas *psb*A and *psb*D mRNA levels declined only 2-fold.

Differential early activation of genes encoding components of the plastid RNA polymerase and ribosomes indicates that a build-up of transcription and translation capacity is given high priority early in chloroplast development. The differential accumulation of *rpo*B mRNA early in chloroplast development is consistent with increases in plastid RNA polymerase levels during sorghum chloroplast biogenesis (Schrubar et al., 1990). Ribosome number per plastid also increases during chloroplast biogenesis (Smith, 1970; Thien and Schopfer, 1975; Bisanz-Seyer et al., 1989) and differential accumulation of 16S rRNA, *rps*19, and *rpl*23 mRNA relative to *rbc*L mRNA was observed during an early phase of chloroplast biogenesis in spinach (Bisanz-Seyer et al., 1989) and for *rpl*2 in pea (Nagano et al., 1991). Differential early activation of genes encoding the transcription/translation apparatus during chloroplast development is followed by maximal expression of genes encoding the photosynthetic apparatus.

Differential Transcription of *rpo*B, 16S rRNA, *trn*fM/G, and *trn*K Early in Chloroplast Biogenesis

Differential accumulation of *rpo*B and *rps*16 mRNA relative to *rbc*L mRNA early in chloroplast development could result from differential transcription or RNA stability. Run-on transcription assays indicate that *rpo*B as well as 16S rRNA, *trn*K, and *trn*fM/G are differentially transcribed early in chloroplast development relative to *rbc*L (Tables II and III). Unfortunately, information on *rps*16 could not be obtained because transcription assays of *rps*16 are influenced by read-through from *trn*Q (Rapp et al., 1992). Nevertheless, *rpo*B and 16S rRNA reached maximal transcription prior to *rbc*L and the ratio of transcription of *rpo*B, 16S rRNA, *trn*K, and *trn*fM/G to *rbc*L declined 10-fold as a function of chloroplast biogenesis. In contrast, the ratio of *rbc*L transcription to *atp*B, *pet*B, and *psa*A remained constant during development. The promoters of these latter genes contain −10 and −35 elements homologous to *cis*-elements in prokaryotic sigma-70 promoters (for a review, see Hanley-Bowdoin and Chua, 1987). In contrast, the presumptive promoters of *rpo*B (Hudson et al., 1988) and *rps*16 (Neuhaus et al., 1989) are AT-rich and lack these elements. On the other hand, the *trn*K (Boyer and Mullet, 1988b) and 16S rRNA (Sun et al., 1986; Baeza et al., 1991) promoters contain −10 and −35 elements. It is possible that the *trn*K and 16S rRNA promoters have elements for early and late transcription, whereas *rpo*B and *rps*16 have only early transcription determinants.

Differential transcription of *rpo*B versus *rbc*L could be due to the action of multiple RNA polymerases (Greenberg et al., 1984), modulation of DNA conformation (Lam and Chua, 1987; Thompson and Mosig, 1990), DNA methylation (Gauly and Kössel, 1989; Ngernprasirtsiri and Akazawa, 1990), or protein factors that alter promoter selection by the plastid RNA polymerase (Baeza et al., 1991; Tiller et al., 1991). Multiple RNA polymerase activities have been reported in plastids (Greenberg et al., 1984; Zaitlin et al., 1984). Several subunits of a prokaryotic-like RNA polymerase are encoded by plastid DNA (encoded by *rpo*A, B, C_1, C_2) (Little and Hallick, 1988; Purton and Gray, 1989; Hu and Bogorad, 1990).

Other studies indicate that a plastid RNA polymerase is nuclear encoded (Lerbs et al., 1985). The strongest evidence for a nuclear-encoded RNA polymerase comes from studies of albostrians, a barley mutant that lacks plastid ribosomes (Siemenroth et al., 1981) and *Epifagus*, which lacks *rpo*B, *rpo*C_1, and *rpo*C_2 (Morden et al., 1991). In both of these cases, 16S rRNA synthesis occurs in plastids that lack the ability to synthesize the plastid-encoded RNA polymerase. Furthermore, selective transcription of 16S rRNA has been associated with a transcriptionally active complex that has a different protein composition than the soluble plastid RNA polymerase (Narita et al., 1985). It is possible, therefore, that the initial activation of plastid transcription as well as differential expression of *rpo*B observed in this study are mediated by increased levels of a nuclear-encoded RNA polymerase.

Increased expression of the *rpo*B-*rpo*C_1-*rpo*C_2 transcription unit could then lead to a build-up of the plastid-encoded RNA polymerase and enhanced transcription of genes with −10 and −35 promoter elements. Expression of *rpo*A, which is somewhat delayed relative to *rpo*B-*rpo*C_1-*rpo*C_2, may further modulate RNA polymerase specificity. However, whereas *rpo*A mRNA levels reach maximal abundance later in chloroplast development than *rpo*B, *rpo*A mRNA levels are always 5- to 20-fold greater than *rpo*B. Protein factors may also modulate promoter selection and thus be responsible for the observed heterogeneity of transcription (Lam et al., 1988; Tiller et al., 1991). For example, the 16S rRNA promoter selectively binds a 33- to 35-kD protein that may regulate the transcription of the 16S rRNA gene in spinach (Baeza et al., 1991). In addition, putative sigma-like factors have been identified in mustard, which bind to the *Escherichia coli* core RNA polymerase and modify its binding to plastid promoters (Tiller et al., 1991).

Differential Expression of *psb*D and *psb*A in Mature Chloroplasts

Differential synthesis of D1 and D2, the reaction center proteins of PSII, is observed in chloroplasts (Gamble et al.,

1988; Schuster et al., 1988; Greenberg et al., 1989; Mattoo et al., 1989). These proteins, encoded by *psb*A and *psb*D, respectively, are damaged by photochemistry and must be degraded and replaced to maintain PSII activity (reviewed in Mattoo et al., 1989). Thus, differential accumulation of the *psb*A and *psb*D mRNAs in mature chloroplasts (Link, 1982; Rodermel and Bogorad, 1985; Deng and Gruissem, 1987; Klein and Mullet, 1987; Sexton et al., 1990a) is consistent with the need to maintain the capacity to synthesize D1 and D2 in these plastids.

In this study, *psb*D and *psb*A mRNA levels decreased only 2-fold from maximum levels in sections 4/5 to mature chloroplasts of section 7 (Table I). In contrast, most other mRNAs (*rbc*L, *atp*B) decreased over 10-fold during this phase of chloroplast development. Differential maintenance of *psb*D mRNA levels is correlated with a light-induced increase in the ratio of *psb*D to *rbc*L transcription (Table III). Differential light-induced accumulation of *psb*D mRNA has been previously documented in barley (Gamble et al., 1988; Krupinska, 1992) and found to be due to a special blue light-responsive promoter (Gamble and Mullet, 1989; Sexton et al., 1990a). The blue light-activated *psb*D promoter lacks −10 and −35 promoter elements but is highly conserved among monocot members of the grass family and dicots (Christopher et al., 1992). Light-induced activation of *psb*D requires protein synthesis in the cytoplasm, suggesting that blue light stimulates synthesis of a nuclear-encoded plastid-localized protein that activates *psb*D transcription (Gamble et al., 1988).

Differential accumulation of *psb*A mRNA in mature chloroplasts observed in this study has been reported in mustard (Link, 1982), maize (Rodermel and Bogorad, 1985), spinach (Deng and Gruissem, 1987), and other plants (reviewed by Mattoo et al., 1989). The accumulation of *psb*A mRNA in chloroplasts can be partially explained by differential light-activated transcription (Eisermann et al., 1990; Klein and Mullet, 1990). In mustard, differential transcription of *psb*A was attributed to a TA-rich element located between the −10 and −35 promoter elements (Link, 1984). However, the 2-fold increase in transcription of *psb*A relative to *rbc*L, observed when 4-d-old barley seedlings are illuminated (Klein and Mullet, 1990), is insufficient to account for the differential accumulation of *psb*A mRNA observed.

Quantitative analysis of transcription and mRNA levels for four stages of chloroplast development allowed prediction of changes in relative RNA stabilities as a function of development (Table IV). For most mRNAs, only small changes in predicted stability occurred during chloroplast development (*atp*B, *psa*A, *psb*D, *rpo*B, *rpo*A, *rpl*16, *ndh*A). These genes had relative RNA stabilities ranging from 0.4 to 1.5 (arbitrary units). *rbc*L and *pet*B mRNAs showed a 3- to 4-fold increase in predicted stability between sections 1/2 and sections 4/5 of illuminated plants. This increase in relative RNA stability may help to elevate the expression of these genes during assembly of the photosynthetic apparatus. Among the RNAs analyzed, *psb*A mRNA showed the largest change in predicted stability. The stability of this RNA increased from 0.02 in sections 1/2 of dark-grown plants to 1.9 in section 7 of illuminated plants. The increase in stability was not strictly light dependent because an increase in predicted stability occurred between sections 1/2 and sections 4/5 of dark-grown plants. A 2-fold increase in *psb*A mRNA stability during chloroplast development has also been documented in spinach (Klaff and Gruissem, 1991). RNA stability in spinach was measured by following RNA decay in actinomycin D-treated plants. The change in *psb*A mRNA stability in spinach (2-fold) is much less than the change in stability predicted in barley (100-fold).

In the present study, data were collected on plastids very early in chloroplast development through mature chloroplast populations. In contrast, the study in spinach compared young leaves that already contained over 50% of their final *rbc*L mRNA levels to chloroplasts from fully expanded spinach leaves. Therefore, it is possible that analysis of a greater range of chloroplast developmental stages in spinach would reveal a greater change in *psb*A mRNA stability. It is also possible that changes in the ratio of RNA to transcription used in the present study overestimates changes in RNA stability. Overestimation will occur with stable RNAs that have degradation rates that are less than the rate of change in transcription during development. In spinach, the half-life of *psb*A mRNA ranged from 5 to 12 h (Klaff and Gruissem, 1991). In barley, transcription rates increase 10-fold over a 48-h period when cells move from the leaf base into and through the zone of cell elongation (Baumgartner et al., 1989). Likewise, transcription activity in plastids declines 5-fold over a 36-h period during chloroplast maturation (Mullet and Klein, 1987). Because the predicted stability of *psb*A mRNA is similar to that of other mRNAs that do not show large changes in stability during development, we believe that the changes observed are significant, although the magnitude of the change in stability will need to be verified by other assays. Similarly, 16S rRNA shows a 35-fold increase in predicted stability during chloroplast biogenesis. Because 16S rRNA is relatively stable, our analysis most likely overestimates the actual change in stability of this RNA. In summary, although differential light-activated transcription of *psb*D accounts for elevated levels of *psb*D mRNA in chloroplasts, differential accumulation of *psb*A mRNA is due primarily to selective stabilization of the *psb*A mRNA.

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LITERATURE CITED

Baeza L, Bertrand A, Mache R, Lerbs-Mache S (1991) Characterization of a protein binding sequence in the promoter region of the 16S rRNA gene of the spinach chloroplast genome. Nucleic Acids Res **19:** 3577–3581

Baumgartner BJ, Rapp JC, Mullet JE (1989) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol **89:** 1011–1018

Bendich AJ (1987) Why do chloroplast and mitochondria contain so many copies of their genome? Bioessays **6:** 279–282

Berends T, Gamble PE, Mullet JE (1987) Characterization of the barley chloroplast transcription units containing *psa*A-*psa*B and *psb*D-*psb*C. Nucleic Acids Res **15:** 5217–5239

Bisanz-Seyer C, Li Y-F, Seyer P, Mache R (1989) The components of the plastid ribosome are not accumulated synchronously during

the early development of spinach plants. Plant Mol Biol **12:** 201–211

Boyer SK, Mullet JE (1988a) Sequence and transcript map of barley chloroplast *psb*A gene. Nucleic Acids Res **16:** 8184

Boyer SK, Mullet JE (1988b) Pea chloroplast $tRNA^{Lys}$(UUU) gene: transcription and analysis of an intron-containing gene. Photosynth Res **17:** 7–22

Christopher DA, Hallick RB (1990) Complex RNA maturation pathway for a chloroplast ribosomal protein operon with an internal tRNA cistron. Plant Cell **2:** 659–671

Christopher DA, Kim M, Mullet JE (1992) A novel light-regulated promoter is conserved in cereal and dicot chloroplasts. Plant Cell **4:** 785–798

Deng X-W, Gruissem W (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. Cell **49:** 379–387

Deng X-W, Gruissem W (1988) Constitutive transcription and regulation of gene expression in non-photosynthetic plastids of higher plants. EMBO J **7:** 3301–3308

Dietrich G, Detschey S, Neuhaus H, Link G (1987) Temporal and light control of plastid transcript levels for proteins involved in photosynthesis during mustard (*Sinapis alba* L.) seedling development. Planta **172:** 393–399

Eisermann A, Tiller K, Link G (1990) *In vitro* transcription and DNA binding characteristics of chloroplast and etioplast extracts from mustard (*Sinapis alba*) indicate differential usage of the *psb*A promoter. EMBO J **9:** 3981–3987

Gamble PE, Mullet JE (1989) Translation and stability of proteins encoded by the plastid *psb*A and *psb*B genes are regulated by a nuclear gene during light-induced chloroplast development in barley. EMBO J **8:** 2785–2794

Gamble PE, Sexton TB, Mullet JE (1988) Light-dependent changes in *psb*D and *psb*C transcripts of barley chloroplasts: accumulation of two transcripts maintains *psb*D and *psb*C translation capability in mature chloroplasts. EMBO J **7:** 1289–1297

Gauly A, Kössel H (1989) Evidence for tissue-specific cytosine-methylation of plastid DNA from *Zea mays*. Curr Genet **15:** 371–376

Greenberg BM, Gaba V, Canaani O, Malkin S, Mattoo AK, Edelman M (1989) Separate photosensitizers mediate degradation of the 32-kDa photosystem II reaction center protein in the visible and UV spectral regions. Proc Natl Acad Sci USA **86:** 6617–6620

Greenberg BM, Narita JO, DeLuca-Flaherty C, Gruissem W, Rushlow KA, Hallick RB (1984) Evidence for two RNA polymerase activities in *Euglena gracilis* chloroplasts. J Biol Chem **259:** 14880–14887

Haley J, Bogorad L (1990) Alternative promoters are used for genes within maize chloroplast polycistronic transcription units. Plant Cell **2:** 323–333

Hanley-Bowdoin L, Chua N-H (1987) Chloroplast promoters. Trends Biochem Sci **12:** 67–70

Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun C-R, Meng B-Y, Li Y-Q, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M (1989) The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. Mol Gen Genet **217:** 185–194

Hu J, Bogorad L (1990) Maize chloroplast RNA polymerase: the 180-, 120-, and 38-kilodalton polypeptides are encoded in chloroplast genes. Proc Natl Acad Sci USA **87:** 1531–1535

Hudson GS, Holton TA, Whitfeld PR, Bottomley W (1988) Spinach chloroplast *rpo*BC genes encode three subunits of the chloroplast RNA polymerase. J Mol Biol **200:** 639–654

Klaff P, Gruissem W (1991) Changes in chloroplast mRNA stability during leaf development. Plant Cell **3:** 517–529

Klein RR, Mullet JE (1987) Control of gene expression during higher plant chloroplast biogenesis. J Biol Chem **262:** 4341–4348

Klein RR, Mullet JE (1990) Light-induced transcription of chloroplast genes. *Psb*A transcription is differentially enhanced in illuminated barley. J Biol Chem **265:** 1895–1902

Krupinska K (1992) Transcriptional control of plastid gene expression during development of primary foliage leaves of barley grown under a daily light-dark regime. Planta **186:** 294–303

Lam E, Chua N-H (1987) Chloroplast DNA gyrase and *in vitro* regulation of transcription by template topology and novobiocin. Plant Mol Biol **8:** 415–424

Lam E, Hanley-Bowdoin L, Chua N-H (1988) Characterization of a chloroplast sequence-specific DNA binding factor. J Biol Chem **263:** 8288–8293

Lawrence ME, Possingham JV (1986) Microspectrofluorometric measurement of chloroplast DNA in dividing and expanding leaf cells of *Spinacia oleracea*. Plant Physiol **81:** 708–710

Lerbs S, Bräutigam E, Parthier B (1985) Polypeptides of DNA-dependent RNA polymerase of spinach chloroplasts: characterization of antibody-linked polymerase assay and determination of sites of synthesis. EMBO J **4:** 1661–1666

Link G (1982) Phytochrome control of plastid mRNA in mustard (*Sinapis alba* L.). Planta **154:** 81–86

Link G (1984) 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

Little MC, Hallick RB (1988) Chloroplast *rpo*A, *rpo*B, and *rpo*C genes specify at least three components of a chloroplast DNA-dependent RNA polymerase active in tRNA and mRNA transcription. J Biol Chem **263:** 14302–14307

Matsubayashi T, Wakasugi T, Shinozaki K, Yamaguchi-Shinozaki K, Zaita N, Hidaka T, Meng B-Y, Ohto C, Tanaka M, Kato A, Maruyama T, Sugiura M (1987) Six chloroplast genes (*ndh*A–G) homologous to human mitochondrial genes encoding components of the respiratory chain NADH dehydrogenase are actively expressed: determination of the splice sites in *ndh*A and *ndh*B pre-mRNA's. Mol Gen Genet **210:** 385–393

Mattoo AK, Marder JB, Edelman M (1989) Dynamics of the photosystem II reaction center. Cell **56:** 241–246

Morden CW, Wolfe KH, dePamphilis CW, Palmer JD (1991) Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo genes. EMBO J **10:** 3281–3288

Mullet JE, Klein RR (1987) Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. EMBO J **6:** 1571–1579

Nagano Y, Ishikawa H, Matsuno R, Sasaki Y (1991) Nucleotide sequence and expression of the ribosomal protein L2 gene in pea chloroplasts. Plant Mol Biol **17:** 541–545

Narita JO, Rushlow KE, Hallick RB (1985) Characterization of an *Euglena gracilis* chloroplast RNA polymerase specific for ribosomal RNA genes. J Biol Chem **260:** 11194–11199

Neuhaus H, Scholz A, Link G (1989) Structure and expression of a split chloroplast gene from mustard (*Sinapis alba*): ribosomal protein gene *rps*16 reveals unusual transcriptional features and complex RNA maturation. Curr Genet **15:** 63–70

Ngernprasirtsiri J, Akazawa T (1990) Modulation of DNA methylation and gene expression in cultured sycamore cells treated by hypomethylating base analog. Eur J Biochem **194:** 513–520

Ohyama K, Kohchi T, Sano T, Yamada Y (1988) Newly identified groups of genes in chloroplasts. Trends Biochem Sci **15:** 19–22

Oliver RP, Poulsen C (1984) Structure of a heavily transcribed region of barley chloroplast DNA. Transfer RNA genes for serine (UGA), glycine (GCC,UCC), formyl-methionine and theronine (GGU). Carlsberg Res Commun **49:** 647–673

Orozco EM Jr, Mullet JE, Chua N-H (1985) An *in vitro* system for accurate transcription initiation of chloroplast protein genes. Nucleic Acids Res **13:** 1283–1301

Purton S, Gray JC (1989) The plastid *rpo*A gene encoding a protein homologous to the bacterial RNA polymerase alpha subunit is expressed in pea chloroplasts. Mol Gen Genet **217:** 77–84

Rapp JC, Baumgartner BJ, Mullet JE (1992) Quantitative analysis of transcription and RNA levels of fifteen barley chloroplast genes: transcription rates and mRNA levels vary over 300-fold: predicted mRNA stabilities vary 30-fold. J Biol Chem **267:** 21404–21411

Rapp JC, Mullet JE (1991) Chloroplast transcription is required to express the nuclear genes *rbc*S and *cab*. Plastid DNA copy number is regulated independently. Plant Mol Biol **17:** 813–823

Rodermel SR, Bogorad L (1985) Maize plastid photogenes: mapping

and photoregulation of transcript levels during light-induced development. J Cell Biol **100:** 463–476

Ruf M, Kössel H (1988) Structure and expression of the gene coding for the α-subunit of DNA-dependent RNA polymerase from the chloroplast genome of *Zea mays*. Nucleic Acids Res **16:** 5741–5754

Schrubar H, Wanner G, Westhoff P (1990) Transcriptional control of plastid gene expression in greening sorghum seedlings. Planta **183:** 101–111

Schuster G, Timberg R, Ohad I (1988) Turnover of thylakoid photosystem II proteins during photoinhibition of *Chlamydomonas reinhardtii*. Eur J Biochem **177:** 403–410

Sexton TB, Christopher DA, Mullet JE (1990a) Light-induced switch in barley *psb*D-*psb*C promoter utilization: a novel mechanism regulating chloroplast gene expression. EMBO J **9:** 4485–4494

Sexton TB, Jones JT, Mullet JE (1990b) Sequence and transcriptional analysis of the barley ctDNA region upstream of *psb*D-*psb*C encoding *trn*K(UUU), *rps*16, *trn*Q(UUG), *psb*K, *psb*I and *trn*S(GCU). Curr Genet **17:** 445–454

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamagashira T, Yamada K, Kusuda J, Fakaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J **5:** 2043–2049

Siemenroth A, Wollgiehn R, Neumann D, Börner Th (1981) Synthesis of ribosomal RNA in ribosome-deficient plastids of the mutant "albostrians" of *Hordeum vulgare* L. Planta **153:** 547–555

Smith H (1970) Changes in plastid ribosomal-RNA and enzymes during the growth of barley leaves in darkness. Phytochemistry **9:** 965–975

Sun E, Shapiro DR, Wu BW, Tewari KK (1986) Specific *in vitro* transcription of 16S rRNA gene by pea chloroplast RNA polymerase. Plant Mol Biol **6:** 429–439

Thien W, Schopfer P (1975) Control by phytochrome of cytoplasmic and plastid rRNA accumulation in cotyledons of mustard seedlings in the absence of photosynthesis. Plant Physiol **56:** 660–664

Thompson RJ, Mosig G (1990) Light affects the structure of *Chlamydomonas* chloroplast chromosomes. Nucleic Acids Res **18:** 2625–2631

Thompson WF, Everett M, Polans NO, Jorgensen RA, Palmer JD (1983) Phytochrome control of RNA levels in developing pea and mung-bean leaves. Planta **158:** 487–500

Tiller K, Eisermann A, Link G (1991) The chloroplast transcription apparatus from mustard (*Sinapis alba* L.). Evidence for three different transcription factors which resemble bacterial σ factors. Eur J Biochem **198:** 93–99

Westhoff P, Herrmann RG (1988) Complex RNA maturation in chloroplasts. The *psb*B operon from spinach. Eur J Biochem **171:** 551–564

Woodbury NW, Dobres M, Thompson WF (1989) The identification and localization of 33 pea chloroplast transcription initiation sites. Curr Genet **16:** 433–445

Zaitlin D, Hu J, Bogorad L (1989) Binding and transcription of relaxed DNA templates by fractions of maize chloroplast extracts. Proc Natl Acad Sci USA **86:** 876–880

Zurawski G, Clegg MT, Brown AHD (1984) The nature of nucleotide sequence divergence between barley and maize chloroplast DNA. Genetics **106:** 735–749