The Plant Cell, Vol. 1, 437–445, April 1989

Tissue-Dependent Plastid RNA Splicing in Maize: Transcripts from Four Plastid Genes Are Predominantly Unspliced in Leaf Meristems and Roots

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Most plastid gene products do not accumulate to high levels in meristem proplastids or in the specialized plastids of roots. To assess whether a modulation of plastid splicing activities might play a role in this tissue-dependent expression of the plastid genome, the ratio of spliced to unspliced transcripts from the *atp*F, *pet*B, *pet*D, and *rpl*16 genes was compared between several tissues of maize. Although these transcripts are predominantly spliced in green leaf tissue (both bundle sheath and mesophyll cells), spliced *atp*F, *pet*B, and *pet*D transcripts are underrepresented relative to their unspliced precursors in roots and leaf meristems. The ratio of spliced to unspliced *rpl*16 transcripts varies in a similar fashion, but the magnitude of the differences between tissues is not as great. The proportion of RNA that is spliced reflects the tissue of origin and not photosynthetic competency, chlorophyll content, or exposure to light since the leaves of photosynthetic mutants and of seedlings grown in the absence of light contain spliced and unspliced transcripts in normal ratios. These results raise the possibility that low RNA splicing activities are in part responsible for the limited expression of the plastid genome in meristematic and root tissue.

INTRODUCTION

During plant development, several differentiated plastid forms arise from a common progenitor organelle called a proplastid (Kirk and Tilney-Bassett, 1978). Proplastids in the leaf meristem develop into chloroplasts during leaf cell differentiation in the presence of light, or into etioplasts in the absence of light. Proplastids in the root meristem develop during root cell differentiation into one of several unpigmented plastid forms, such as amyloplasts or leucoplasts. The concerted differentiation of plastids and cells implies the existence of mechanisms that coordinate the response of the nuclear and plastidic genetic compartments to environmental and developmental signals.

The development of proplastids into chloroplasts involves the elaboration of the thylakoid membrane, accompanied by large increases in the levels of protein complexes that function in photosynthesis and in plastid translation (Leech et al., 1973; Baker and Leech, 1977; Dean and Leech, 1982; Mayfield and Taylor, 1984; A. Barkan, unpublished results). The specialized, nonphotosynthetic root plastids resemble proplastids in that they have little internal membrane or pigment, few ribosomes, and low levels of those photosynthetic proteins that have been examined (Kirk and Tilney-Bassett, 1978; de Boer et al., 1988). Although it has been established that the plastid genome encodes many of the proteins that accumulate primarily during chloroplast development, little is known about the mechanisms that regulate their accumulation.

Identification of the rate-limiting steps in plastid gene expression will help to define the mechanisms that regulate plastid differentiation. Regulation of any of several processes, including DNA replication, transcription, RNA processing and stability, translation, and protein turnover, could potentially affect the abundance of plastid-encoded proteins. For many plastid genes, transcription rates do not solely determine expression level. For example, the relative rates of transcription of several plastid genes are similar in etiolated and green leaves despite the fact that the transcripts are found in different ratios in etioplasts and chloroplasts (Deng and Gruissem, 1987; Mullet and Klein, 1987). Furthermore, the levels of some plastid mRNAs do not determine the rates of synthesis of their protein products (Fromm et al., 1985; Inamine et al., 1985; Berry et al., 1986; Klein and Mullet, 1986).

Because many plastid RNAs are generated through a series of processing steps (splicing, endonucleolytic cleavage, and end-trimming), the possibility that RNA processing is regulated during plastid differentiation needs to be considered. Several examples exist of tissue- and stage-specific splicing activities that regulate the expression of nuclear genes (reviewed in Bingham et al., 1988). A similar

mechanism seems especially plausible in the case of plastid gene expression because splicing rates tend to be slow relative to transcription rates: many chloroplast RNAs containing intervening sequences accumulate to levels approaching those of their spliced products (Hollingsworth et al., 1984; Koller et al., 1985; Shinozaki et al., 1986; Hudson et al., 1987; Rock et al., 1987; Westhoff and Herrmann, 1988). In these cases, increasing the rate of splicing will certainly increase the rate of mature mRNA production, whereas increasing the rate of transcription may not. Similarly, other types of RNA processing such as intercistronic cleavage and end-trimming are also slow relative to transcription, since intermediates in processing accumulate (Hanley-Bowdoin et al., 1985; Westhoff, 1985; Kohchi et al., 1988). However, it is not yet known whether such processing events affect translational yield.

To determine whether plastid RNA splicing activities might be subject to developmental regulation, the transcripts derived from four plastid genes, *atp*F, *pet*B, *pet*D and *rpl*16, were examined in several tissues of maize. These genes are each interrupted by a single class II intervening sequence and are encoded within complex polycistronic transcription units that give rise to substantial levels of unspliced transcripts in chloroplasts (Gold et al., 1987; Hudson et al., 1987; Rock et al., 1987; Westhoff and Herrmann, 1988; A. Barkan, unpublished results). Results presented below indicate that the fraction of these transcripts that is spliced is similar in all leaf tissues examined: bundle sheath strands, mesophyll cells, etiolated leaves, and the leaves of photosynthetic mutants. However, spliced transcripts from all four genes are depleted relative to their unspliced precursors in root tissue, and in the proplastid-enriched tissue within and surrounding the leaf meristem. Thus, low RNA splicing activities or specific destabilization of spliced transcripts may be responsible in part for the limited expression of the plastid genome in meristematic and root tissue.

RESULTS

The Population of Transcripts from Two Plastid Gene Clusters Changes during the Differentiation of Proplastids into Chloroplasts

Maize can be used conveniently to study chloroplast differentiation because, like other grasses, its leaves form a natural developmental gradient. New cells arising from the meristem at the leaf base push older cells toward the tip, so that the cells are arranged in a linear array according to their age. There is a corresponding gradient of plastid development, with proplastids occurring in the meristematic cells at the leaf base and progressively more mature chloroplasts toward the tip (Leech et al., 1973). To assess whether plastid RNA processing activities might change during chloroplast differentiation, the population of transcripts in successive leaf sections was examined. Figure 1 shows the pattern of transcripts from the *pet*D gene in different leaf sections. The *pet*D gene lies within a transcription unit (the *psb*B gene cluster) encoding four genes. The primary transcript from this gene cluster includes the *psb*B and *psb*H genes, encoding components of photosystem II (PSII), and the *pet*B and *pet*D genes, encoding components of the cytochrome f/b_6 complex (Westhoff et al., 1986; Rock et al., 1987; Tanaka et al., 1987; Kohchi et al., 1988). More than 20 distinguishable transcript classes from these genes accumulate in maize leaves, differing with regard to the presence of introns within the *pet*B and *pet*D coding sequences, and the locations of their termini (Barkan, 1988).

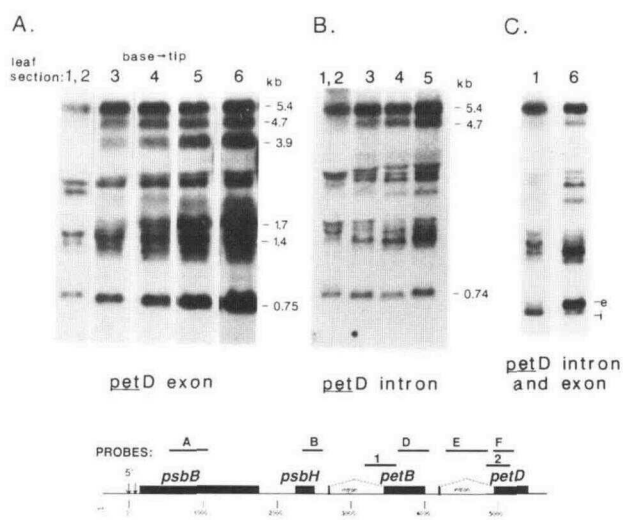

Figure 1. RNA Gel Blot Hybridizations Showing Transcripts from the *psb*B Gene Cluster in Successive Leaf Sections.

A map of the *psb*B gene cluster and the regions used as hybridization probes are shown. For details of gene organization and a diagram of transcript structures, see Barkan (1988). Leaf sections measured (starting from the base) 0 to 0.5 cm (section 1), 0.5 to 1 cm (section 2), 1 to 2 cm (section 3), 2 to 3 cm (section 4), 3 to 5 cm (section 5), and 5 to 8 cm (section 6). Sections from 15 leaves were pooled for each RNA extraction. Approximately 20 μg of RNA from basal tissue, 3 μg of RNA from apical tissue, and intermediate amounts of RNA from the intervening leaf sections were analyzed.

(A) Filter hybridized with a probe specific for the *pet*D coding region (probe F).

(B) Filter hybridized with a probe specific for the *pet*D intron (probe E).

(C) Filter hybridized with probes for the *pet*D coding region and intron.

Bands representing the free intron and the spliced, monocistronic *pet*D mRNA are designated "i" and "e," respectively. The exon probe was of a lower specific activity than the intron probe in order that band "e" not obscure band "i."

RNA isolated from successive leaf sections was first hybridized with a probe specific for the *pet*D exon (Figure 1A). The amounts of RNA analyzed in each lane were adjusted to give similar hybridization signals, to illustrate changes in the relative abundance of different transcripts rather than their overall abundance. Because plastid RNAs become an increasing fraction of the total cellular RNA during leaf development (Dean and Leach, 1982), approximately 10 times more RNA from the base than from the tip of the leaf was analyzed. As development proceeds, several transcripts (e.g., the 4.7-kb, 3.9-kb, and 0.75-kb RNAs) increase in abundance relative to the primary (5.4-kb) transcript. From previous studies of the maize *psb*B gene cluster (Rock et al., 1987; Barkan, 1988), it is known that these bands represent spliced derivatives of the primary transcript. A similar filter was hybridized with sequences specific for the *pet*D intron (Figure 1B). The ratio of the free intron (0.74-kb) to the primary transcript remains relatively constant during development, despite the fact that the ratios of some spliced products to the primary transcript increase dramatically. To visualize this in another way, RNA prepared from the leaf base and tip was hybridized sequentially with probes specific for the *pet*D coding region and the *pet*D intron (Figure 1C). The level of spliced monocistronic *pet*D transcript (band "e") increases approximately sixfold relative to that of the free intron (band "i") between the leaf base and tip.

To determine whether transcript populations from other plastid genes also change during chloroplast development, the expression of a second set of genes, the *atp*A gene cluster, was examined. This region includes four genes encoding components of the chloroplast ATP synthase: *atp*A, *atp*F, *atp*H, and *atp*I (Cozens et al., 1986; Hennig and Herrmann, 1986). Like the *psb*B gene cluster, these genes also give rise to multiple, overlapping transcripts (Bird et al., 1985; Rodermel and Bogorad, 1985; Westhoff et al., 1985; Cozens et al., 1986; Hudson et al., 1987). A class II intron interrupts the *atp*F coding sequence (Bird et al., 1985; Hennig and Herrmann, 1986), and both spliced and unspliced *atp*F transcripts accumulate in chloroplasts (Hudson et al., 1987). The pattern of *atp*A-specific transcripts in successive leaf sections is shown in Figure 2A. These results resembled those obtained with *pet*D probes: whereas a transcript of the correct size (5.3-kb) to be unspliced and span all four genes is detected in all leaf sections, a 4.5-kb transcript found in mature leaf cells is depleted in the leaf base, relative to the 5.3-kb putative primary transcript. The 4.5-kb transcript is of the appropriate size to be a spliced derivative of the 5.3-kb precursor, lacking the *atp*F intron. These results suggest that, similar to the situation with the *psb*B gene cluster, spliced transcripts of *atp*F are underrepresented in the meristematic tissue at the leaf base.

To examine whether the 4.5-kb transcript depleted from the basal leaf tissue is in fact spliced, transcripts arising from the maize *atp*A gene cluster were further characterized. Filters containing total leaf RNA were hybridized with probes for *atp*A, *atp*F intron, or *atp*F coding sequences (Figure 2B). Whereas the 5.3-kb transcript hybridizes with all three probes, the 4.5-kb transcript that accumulates during leaf cell differentiation hybridizes with *atp*A and *atp*F coding sequences but not with the *atp*F intron probe. It is therefore very likely that the 5.3-kb transcript is an unspliced RNA spanning the gene cluster, which gives rise to the 4.5-kb transcript upon splicing out of the *atp*F intron. These results support the idea that a spliced *atp*F transcript (the 4.5-kb RNA) is depleted from leaf meristem tissue, relative to its unspliced precursor (the 5.3-kb RNA).

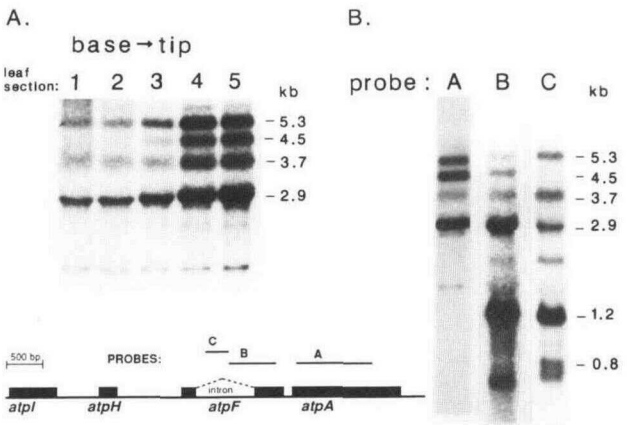

Figure 2. RNA Gel Blot Hybridizations Showing Transcripts from the *atp*A Gene Cluster.

A map of the maize *atp*A gene cluster (Rodermel and Bogorad, 1988) and the regions used as hybridization probes are shown. The direction of transcription is from left to right.

(A) RNA samples isolated from successive leaf sections hybridized with probe A, specific for *atp*A. Different RNA preparations were used in Figures 1 and 2.

(B) Total leaf RNA from greenhouse-grown seedlings hybridized with probe A, B, or C. The filter hybridized with probe C was washed at reduced stringency (0.5 × SSC, 65°C) relative to the filters hybridized with probes A or B (0.2 × SSC, 65°C), to avoid melting off the extremely A-T-rich probe. Intron-specific sequences do not appear strongly in lane B since this filter was washed at the higher stringency.

The Ratio of Spliced to Unspliced *atp*F, *pet*B, *pet*D, and *rpl*16 RNAs Is Reduced in Leaf Meristem and Roots

Results presented above, together with results of RNA gel blot hybridizations to other sequences within these gene clusters (not shown) suggested that the abundance of certain spliced RNAs relative to their unspliced precursors increases during chloroplast development. However, unambiguous interpretation of such experiments is not pos-

sible due to the complexity of the RNA patterns and to the distortion of band migration in the basal leaf samples resulting from large amounts of rRNA. Therefore, an S1 nuclease protection assay was used to quantify the ratio of spliced to unspliced *atp*F, *pet*B, *pet*D, and *rpl*16 RNAs in different tissues. Radiolabeled, single-stranded probes spanning each 3′-splice junction were hybridized in excess to total RNA prepared from the base or tip of leaves of greenhouse-grown seedlings, from the apical half of etiolated leaves, and from seedling roots. Two radiolabeled fragments of each probe are protected from S1 nuclease, corresponding to spliced and unspliced RNAs. This assay simplifies the quantification of the ratio of spliced to unspliced RNAs by representing the multiple transcripts from each gene with two bands. Although this assay cannot distinguish between a 5′ terminus and a 3′-splice junction in the RNA, it is very likely that the S1 nuclease cleavage sites detected correspond only to splice junctions since the sites of the discontinuities between probes and transcripts match exactly the 3′-splice junctions mapped by sequencing cDNAs (Hudson et al., 1987; Tanaka et al., 1987) or predicted by the nucleotide sequence (Gold et al., 1987), and since no transcript 5′ termini have been detected that coincide with these 3′-splice junctions (Hudson et al., 1987; Westhoff and Herrmann, 1988). The amount of RNA analyzed from each tissue was chosen to give a similar signal intensity, rather than to reflect the overall abundance of the RNAs.

Figures 3 and 4 show S1 nuclease analyses of RNA isolated from different tissues. For three genes encoding components of the photosynthetic apparatus, *atp*F, *pet*D, and *pet*B, the proportion of spliced transcripts is much lower in roots and leaf meristems than in mature leaf cells (Figure 3). Although the proportion of transcripts that is spliced is similar in etiolated and green leaf tips, the ratio of spliced to unspliced RNAs is substantially reduced in basal leaf tissue, and still more in root tissue. Based upon quantification of several independent assays, the ratio of spliced to unspliced *atp*F and *pet*B transcripts is approximately fivefold lower in the leaf base and 10-fold lower in roots than in fully differentiated leaf tissue. The proportion of *pet*D transcripts that is spliced is reduced approximately sevenfold and 10-fold in the leaf base and roots, respectively. However, some plastids within all of these tissues are competent for splicing, as a band corresponding to spliced RNA can be detected in each case. Because the RNA prepared from the leaf base is not derived from a pure population of meristematic cells, including also cells at early stages of leaf development, the possibility remains that proplastids have even lower levels of spliced RNAs than shown here. The results were similar when RNA was isolated from the roots or etiolated leaves of seedlings grown for periods of time between 8 and 13 days, demonstrating that the relative accumulation of spliced and unspliced RNAs has reached steady state in these tissues.

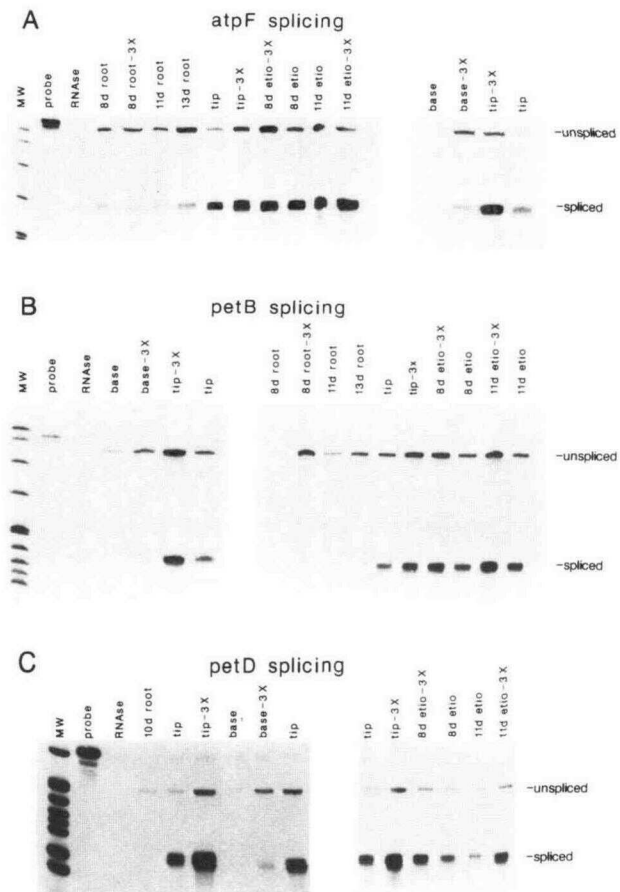

Figure 3. S1-Nuclease Protection Analysis of *atp*F, *pet*B, and *pet*D RNA Splicing in Different Tissues.

(A) *atp*F 3′-splice junction (probe B in Figure 2).
(B) *pet*B 3′-splice junction (probe 1 in Figure 1).
(C) *pet*D 3′-splice junction (probe 2 in Figure 1).
Radiolabeled, single-stranded probes spanning the three junctions were hybridized with total RNA prepared from the base of greenhouse-grown seedling leaves ("base"), the tip of greenhouse-grown seedling leaves ("tip"), the apical half of etiolated leaves ("etio"), or roots. Etiolated leaves and root tissues were harvested 8 days, 10 days, 11 days, or 13 days after planting, as indicated. The predicted migrations of probe fragments protected from S1 nuclease digestion by spliced and unspliced RNAs are shown. Amounts of RNA analyzed were approximately 0.2 μg (tip), 4 μg (base), 2 μg (etio), and 30 μg (roots). Reactions designated 3× contained 3 times as much maize RNA as the adjacent sample from the same tissue. These lanes were included to demonstrate that the assay was quantitative. Total amounts of RNA in each reaction were brought to 30 μg by the addition of tRNA. Results of reactions in which leaf base RNA samples were treated with RNase A prior to hybridization are shown in lanes labeled "RNase." All RNA samples treated with RNase gave a similar result (not shown).

A similar analysis of *rpl*16 expression is shown in Figure 4. The *rpl*16 gene encodes a protein component of the large ribosomal subunit. Although the proportion of *rpl*16 transcripts that is spliced is also reduced in nonleaf tissue, the differences between tissues are not as great as those shown above for three photosynthetic genes. The ratio of spliced to unspliced *rpl*16 RNA is reduced approximately twofold in the leaf base, and threefold in roots, relative to leaves. This contrasts with the 10-fold differences observed for *atp*F, *pet*B, and *pet*D. It is interesting that the overall abundance of *rpl*16 transcripts also varies much less between tissues than do the transcripts analyzed in Figure 3 (see Figure 3 and Figure 4 legends for the amounts of RNA analyzed in each lane). This relatively constant level of spliced *rpl*16 mRNA correlates with the presence of its translation product (a ribosomal protein) in all plastid types.

Transcript Populations in Separated Bundle Sheath and Mesophyll Cells

Maize is a C4 plant, containing within its mature leaf tissue two functionally distinct photosynthetic cell types. Bundle sheath and mesophyll cells have specialized chloroplasts that differ in ultrastructure and biochemistry. These two chloroplast types can first be distinguished several centimeters above the base of seedling leaves, at a position corresponding approximately to leaf section 4 in Figures 1 and 2 (Leech et al., 1973). It was of interest to determine whether the two chloroplast types differ with regard to the accumulation of processed RNA species. This question is particularly pertinent to expression of the *psb*B gene cluster, since components of PSII and the cytochrome f/b_6 complex are found in different ratios in bundle sheath cell and mesophyll cell chloroplasts (Broglie et al., 1984; Schuster et al., 1985). The fact that genes encoding components of these two complexes are co-transcribed in the *psb*B gene cluster raises questions about how the expression of these genes is uncoupled.

To determine whether the primary *psb*B transcript is subject to different patterns of processing in the chloroplasts of bundle sheath and mesophyll cells, total RNA purified from the separated cell types was analyzed by RNA gel blot hybridization. Control experiments shown in Figure 5 demonstrate the success of the cell separation in two ways. First, the *psb*B/*pet*D polypeptide ratio is much greater in the mesophyll than in the bundle sheath preparation (Figure 5A), as expected. In addition, *rbc*L transcripts are enriched in the bundle sheath fraction (Figure 5B), whereas *atp*B,E RNA (Figure 5B) and 16S rRNA (not shown) are equally distributed between the two fractions, as has been reported by others (Jolly et al., 1981; Sheen and Bogorad, 1985). Therefore, the fractionation has successfully separated bundle sheath and mesophyll cell components, although a small degree of cross-contamination is likely.

Figure 6 shows the results of RNA gel blot analyses of transcript populations in these bundle sheath and mesophyll cell preparations. Filters containing equal amounts of total RNA from bundle sheath strands or mesophyll cells were hybridized with probes specific for *psb*B, *psb*H, or *pet*D. An RNA gel blot assay was used rather than an S1 protection assay to visualize a variety of alterations in RNA populations, not just those due to splicing differences. This method is less quantitative but gives a more comprehensive picture than the S1 nuclease assay. By comparing the results obtained from a one-fourth dilution of total RNA to that from bundle sheath strand RNA, it can be deduced that the overall level of transcripts from the *psb*B gene cluster is approximately threefold lower in bundle sheath than in mesophyll cells. However, the transcript patterns are qualitatively similar. Thus, the one-fourth dilution of total RNA gives rise to a pattern similar to that seen in the bundle sheath sample, whereas the undiluted total RNA sample gives rise to a pattern similar to that seen in the mesophyll sample. Filters probed with *pet*B sequences gave similar results (not shown). It is unlikely that the minor differences in ratios between bands would alter the ultimate protein level, considering that all of the correctly spliced RNAs are translationally active (Barkan, 1988). Differential processing of RNA from the *psb*B gene cluster is therefore unlikely to be responsible for the differential

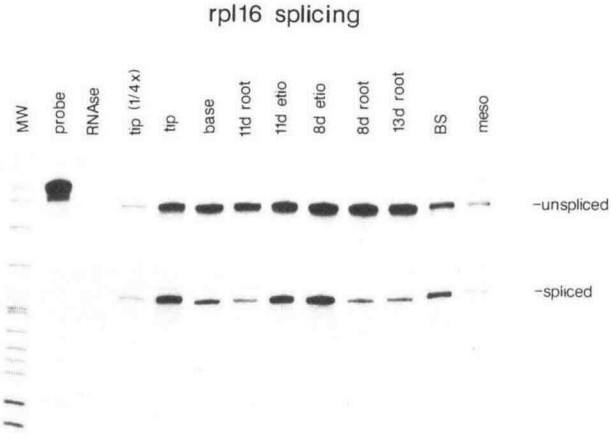

Figure 4. S1 Nuclease Protection Analysis of *rpl*16 RNA Splicing in Different Tissues.

RNA samples were analyzed as in Figure 3, except that they were hybridized with a probe spanning the *rpl*16 3′-splice junction. Amounts of RNA analyzed were approximately 2 μg (tip), 3 μg (base), 40 μg (root), or 6 μg (etio). In addition, 2 μg of bundle sheath strand (BS) and mesophyll cell (meso) RNA were analyzed.

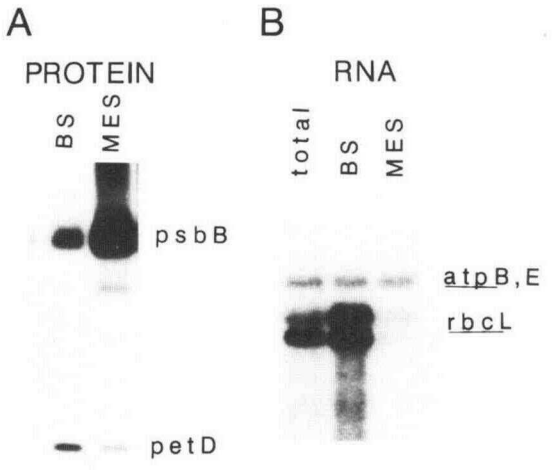

Figure 5. Control Experiments Demonstrating Successful Separation of Bundle Sheath and Mesophyll Cells.

(A) Immunoblot of membrane proteins isolated from bundle sheath strands (BS) or mesophyll protoplasts (MES). Membrane proteins were fractionated on an SDS-polyacrylamide gel, transferred to nitrocellulose, and reacted with a mixture of antisera for *psb*B and *pet*D proteins. Proteins were isolated and analyzed as described previously (Barkan et al., 1986). The *psb*B antibody was a gift of Nathan Nelson (Roche Institute).

(B) Filter hybridization of RNA prepared from whole seedling leaves (total), bundle sheath strands, or mesophyll protoplasts. Equal amounts of total RNA were applied to each lane. RNA was hybridized with the maize chloroplast BamHI 9 fragment (Larrinua et al., 1983), encoding *rbc*L and *atp*B,E.

accumulation of its protein products in bundle sheath and mesophyll cells.

An S1 nuclease protection assay was used to confirm that the ratio of spliced to unspliced *pet*B, *pet*D, and *rpl*16 transcripts is similar in bundle sheath and mesophyll cells (Figure 4 and not shown).

DISCUSSION

The accumulation of RNA processing intermediates in plastids indicates that certain chloroplast RNA processing reactions are slow relative to the transcription rate. Since RNA splicing is necessary to create translatable mRNAs, splicing rather than transcription must limit the rate of synthesis of those mRNAs for which unspliced precursors accumulate significantly. Thus, a modulation of splicing activities will have immediate effects on the rate of production of such mRNAs. Results presented here suggest that plastid splicing activities increase in excess of plastid transcriptional activity during leaf development. The ratio of spliced to unspliced transcripts from four plastid genes varies in different tissues. Mature leaf tissue grown in the presence or absence of light contains predominantly spliced transcripts. However, only a low proportion of transcripts from the *atp*F, *pet*B, *pet*D, and *rpl*16 genes is spliced in roots and meristem-proximal leaf tissue. Variation in the proportion of transcripts that is spliced correlates with the tissue of origin rather than with photosynthetic competency, since mutants blocked in photosynthetic electron transport can yield RNA populations similar to those in normal green leaves (Barkan et al., 1986). Similarly, mutations blocking chlorophyll accumulation (data not shown) and growth of seedlings in the absence of light (Figures 3 and 4) do not alter the proportion of these transcripts that is spliced, indicating that chlorophyll and light do not modulate the relative accumulation of spliced and unspliced RNAs.

These results indicate either that the rate of splicing relative to transcription or the relative stabilities of spliced and unspliced transcripts differ in the plastids of leaves, leaf meristems, and roots. The fact that the level of spliced *pet*D RNA increases relative to the free *pet*D intron during chloroplast development (Figure 1B and 1C) is consistent with both models. Considering that the free intron and spliced exons are products of the same reaction, the increasing ratio of the spliced exons relative to the intron between the leaf base and tip suggests that the spliced RNAs are specifically stabilized during leaf development.

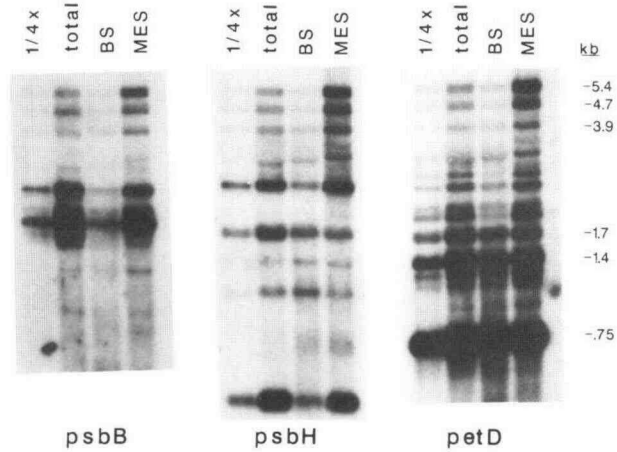

Figure 6. RNA Gel Blot Hybridizations Showing Transcripts from the *psb*B Gene Cluster in Separated Mesophyll Cells and Bundle Sheath Strands.

Equal amounts of total RNA from whole seedling leaves (total), bundle sheath strands (BS), or mesophyll protoplasts (MES) were hybridized with probes specific for *psb*B, *psb*H, or *pet*D (probes A, B, or F, respectively, in Figure 1). Lanes designated 1/4× contain a fourfold dilution of the adjacent total RNA sample. The apparent enrichment of the 1.1-kb and 1.7-kb *psb*H transcripts in bundle sheath cells was not reproducible.

However, regulation of splicing alone could give a similar result under the following scenario. If splicing rates increase substantially early in leaf cell differentiation, and if the rate of turnover of the free intron is constant and much greater than that of the spliced RNAs, then the ratio of the spliced RNAs to the free intron (both products of the same reaction and therefore synthesized at the same rate) would increase with time, as was observed. This is because a short-lived component (e.g., the free intron) will reach its steady-state level sooner than a long-lived component (e.g., spliced RNAs). Only after both classes of RNA have reached their steady-state levels will their ratio remain constant with time.

There is a large increase in the number of 70S ribosomes per plastid and per cell early during leaf cell differentiation (Dean and Leech, 1982). It is possible that this proliferation of the translation machinery is causally related to the concomitant increase in the ratio of spliced to unspliced transcripts. Several possible mechanisms linking plastid translation and the accumulation of spliced transcripts can be envisioned, including specific stabilization of spliced (i.e., translated) sequences by polysomes, the involvement of a plastid-encoded protein in splicing, and a modulation of splicing rate by traversing ribosomes. These possibilities are being investigated. It is interesting that the smallest differences between tissues were observed for *rpl*16 RNAs, since it has been suggested that mRNAs encoding ribosomal proteins may be preferentially translated in root plastids (Deng and Gruissem, 1988). Thus, ribosomal protein mRNAs may be both preferentially spliced and preferentially translated in root plastids, extending the correlation between splicing and translation. It remains to be seen, however, whether it is generally true that plastid genes encoding components of the translation machinery show smaller tissue-specific differences in spliced RNA accumulation than photosynthetic genes.

Differences in the population of transcripts were observed only between different tissues. No significant differences were found among three mature leaf cell populations (etiolated, bundle sheath, and mesophyll cells). The pattern of transcripts from the *psb*B gene cluster is similar in bundle sheath and mesophyll cells despite the fact that the relative accumulation of proteins encoded by this gene cluster is different in the two cell types. Although the overall level of all of the transcripts is lower in bundle sheath cells, the ratio between the different RNAs remains similar. Therefore, cell-type specific RNA processing reactions do not serve to uncouple the expression of the two sets of genes within the *psb*B transcription unit. These results are consistent with those of Sheen and Bogorad (1988), who also report a similar pattern but reduced quantity of transcripts from this region of the plastid genome in bundle sheath strands relative to mesophyll cells. Results presented here extend these previous findings by resolving transcripts corresponding to the different genes with gene-specific probes. To address the question of differential processing, the use of gene-specific probes is important in that RNAs of similar size arise from different regions of this gene cluster (Barkan, 1988; Westhoff and Herrmann, 1988).

Light-induced changes occur in the ratios of transcripts with different 5′ termini from the *rbc*L gene in maize (Crossland et al., 1984) and the *psb*C/*psb*D genes in barley (Gamble et al., 1988). Results presented here demonstrate that populations of overlapping transcripts can also change in a tissue-dependent rather than light-dependent fashion. These changes involve differences in splicing rather than terminal processing. Because the removal of introns from coding sequences is required for translation, the generation of spliced derivatives from unspliced precursors is crucial for biological function. Thus, the regulation of spliced mRNA levels will alter the ultimate level of gene product, except where translational and posttranslational events are rate-limiting. Whether variations in the ratio of spliced to unspliced transcripts result from differences in the relative rates of splicing and transcription or from different relative stabilities of spliced and unspliced RNAs remains to be determined.

METHODS

Plant Material

The maize inbred line B73 (Pioneer Hi-Bred) was the source of all tissue analyzed here. However, similar results were obtained with the normal siblings of mutant *hcf*38 (data not shown), which is in a mixed hybrid background (D. Miles, personal communication). Leaf sections were collected from the third photosynthetic leaf of 12-day-old seedlings grown in the greenhouse. Outer and inner leaves were carefully removed. Leaf "base" RNA was prepared from the basal 0.5 cm and leaf "tip" RNA from tissue 6 to 10 cm from the base. Intermediate sections were as described in the legend to Figure 1. Etiolated seedlings were germinated and grown in total darkness. RNA was extracted from the apical half of etiolated leaves. Root RNA was prepared from the roots of seedlings grown on water-saturated paper towels in foil-covered trays. The 10-day-old root RNA was generously provided by Kelly Dawe. Bundle sheath strands and mesophyll protoplasts were prepared from leaf midsections of seedlings grown in the greenhouse for 4 weeks, as described by Sheen and Bogorad (1985).

Hybridization Probes

DNA fragments used as hybridization probes for RNA gel blots were excised from plasmid clones of maize plastid DNA, gel-purified, and radiolabeled by the random hexamer priming method (Feinberg and Vogelstein, 1984). Probes containing sequences from the *psb*B gene cluster have been described (Barkan, 1988). A 1080-bp BamHI-SalI fragment of the maize *atp*A gene (Rodermel and Bogorad, 1987) was used to detect *atp*A transcripts. Nucleotide sequence analysis of DNA flanking the *atp*A fragment was performed to locate the *atp*F coding and intron sequences (A.

Barkan, unpublished results). A 172-bp EcoRI-DraI fragment from within the *atp*F intron was used to detect intron-containing transcripts. A 650-bp SstI-EcoRI fragment containing both 3′-exon and intron sequences of *atp*F was used to identify exon or intron containing *atp*F RNAs. The positions within the plastid gene clusters of these hybridization probes are diagrammed in Figures 1 and 2.

Probes for S1 nuclease protection experiments were synthesized by second-strand synthesis from recombinant M13 templates, restriction enzyme digestion in the distal polylinker site, and gel purification of the single-stranded radiolabeled insert fragment. Radioisotope was incorporated primarily near the 5′-end of each probe by performing the second-strand synthesis with limiting amounts of ^{32}P-dCTP, followed by a chase with excess unlabeled dCTP, as described previously (Barkan, 1988). Clones used for these experiments contained maize plastid sequences corresponding to the 240-nucleotide XbaI-XhoI fragment spanning the *pet*D 3′-splice junction (probe 2 in Figure 1) (Rock et al., 1987), the 480-nucleotide HaeIII fragment spanning the *pet*B 3′-splice junction (probe 1 in Figure 1) (Rock et al., 1987), the 650-nucleotide SstI-EcoRI fragment spanning the *atp*F 3′-splice junction (probe B in Figure 2) (A. Barkan, unpublished results), or the 500-nucleotide BamHI-BglII fragment spanning the *rpl*16 3′-splice junction (Gold et al., 1987). Primer and polylinker sequences at the 5′-end of each probe were removed by S1 nuclease digestion of RNA-DNA hybrids.

RNA Preparation and Analysis

Total RNA was prepared from each tissue by a guanidinium thiocyanate extraction procedure, as described previously (Barkan et al., 1986). After RNA was precipitated once with LiCl, traces of residual DNA were removed by digestion with 20 μg/mL RNase-free DNase (Bethesda Research Laboratories) for 15 min at room temperature. RNA was then further purified by phenol extraction and ethanol precipitation. RNA was electrophoresed in formaldehyde gels, transferred to nylon membranes, and hybridized to radiolabeled probes, as described (Barkan, 1988).

S1 nuclease protection experiments were performed as described previously (Barkan, 1988) by using single-stranded probes labeled primarily near their 5′-ends. To ensure that residual plastid DNA did not contribute detectably to the level of protected probe, control reactions were performed in which each RNA sample was treated with heat-treated RNase A (20 μg/mL) for 15 min at 37°C prior to hybridization. In each case, this treatment eliminated the bands corresponding to spliced and unspliced RNAs, giving a result identical to that observed when tRNA was substituted for maize RNA.

Results were quantified by scintillation spectroscopy of excised radiolabeled bands, or, when necessary due to insufficient signal, by densitometry of autoradiograms exposed without intensifying screens.

ACKNOWLEDGMENTS

I thank Kris Kallan for technical assistance, Wilhelm Gruissem and Bill Taylor for useful discussions and for providing laboratory space, and Wilhelm Gruissem, Thianda Manzara, Julia Bailey-Serres, Rob Martienssen, David Stern, and Richard Jefferson for helpful comments on the manuscript. This work was supported by National Institutes of Health grant GM38504.

Received January 4, 1989; revised February 2, 1989.

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