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Post-Transcriptional Regulation of Organ-Specific Expression of Individual *rbc*S mRNAs in *Lemna gibba*

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Many studies of nuclear genes encoding chloroplast proteins have focused on the transcriptional regulation of their expression. The genes (*rbc*S) encoding the small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase/oxygenase, a major stromal protein, comprise one such group. We have examined the role played by post-transcriptional events in determining the relative levels of individual *rbc*S mRNAs in different organs of the aquatic monocot *Lemna gibba*. *L. gibba* is unusual among angiosperms in that its roots are normally exposed to light during growth and contain chloroplasts. We have found that such roots transcribe *rbc*S genes and contain *rbc*S mRNA. We have used sequence-specific probes from the 3′-untranslated region of six *rbc*S genes from *L. gibba* to analyze the expression of the individual genes in different organs. All six genes were expressed in steady-state mRNA in fronds grown in constant white light. However, only five of these were easily detectable in steady-state mRNA isolated from roots of the same plants, and the relative expression of each gene varied between the roots and the fronds. In steady-state mRNA, SSU1 was found to be highly expressed in both roots and fronds, whereas SSU40B was expressed at low levels in the roots as compared with the fronds, and SSU5B RNA was barely detectable in the roots. The extremely low level of SSU5B RNA in steady-state root mRNA is likely to be a consequence of post-transcriptional events because this gene was transcribed at comparable rates in vitro in nuclei isolated from either roots or fronds. Localization of individual gene transcripts by in situ hybridization showed that SSU1 and SSU5B are expressed in the same cells in the fronds. Thus, the mechanism of differential expression is likely to involve an organ-specific post-transcriptional mechanism.

INTRODUCTION

The enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, which catalyzes carbon dioxide fixation, is the most abundant stromal protein of the chloroplasts of higher plants (Ellis, 1981). It comprises eight large subunits (LSU) and eight small subunits (SSU) that are encoded in different genomes within the cell (reviewed by Jensen and Bahr, 1977). The LSU is encoded by a single gene in the chloroplast genome (*rbc*L), whereas the SSU is encoded by a small multigene family (*rbc*S) in the nucleus. The size of the *rbc*S gene families varies in size from four members in *Arabidopsis thaliana* to at least 12 in *Lemna gibba* (reviewed by Dean et al., 1989c).

Expression of *rbc*S genes can be controlled by multiple factors. In *Lemna*, as in several other plants, *rbc*S gene expression is transcriptionally regulated by light by way of the phytochrome system (reviewed by Tobin and Silverthorne, 1985; Kuhlemeier et al., 1987). In addition, expression of *rbc*S genes has also been shown to be altered by blue light and by dark treatments or white light (e.g., Fluhr and Chua, 1986). The photoreceptor for these responses has not been determined. Furthermore, expression of these genes is organ specific in a number of plants, for example tomato (Sugita and Gruissem, 1987), petunia (Dean et al., 1987), and pea (Corruzi et al., 1984). Transformation experiments have shown that transcriptional regulation can play a role in the organ-specific expression of *rbc*S genes (reviewed by Kuhlemeier et al., 1987). A further level of regulation of *rbc*S expression is the requirement for mature, green chloroplasts to be present in cells for expression of these nuclear genes. In tobacco callus transformed with a reporter gene (chloramphenicol acetyltransferase) driven by *rbc*S promoter sequences, chloramphenicol acetyltransferase activity was only detected in light-grown tissue in which chloroplast development had been induced by treatment with cytokinin (Herrera-Estrella et al., 1984). Similarly, mustard seedlings bleached by treatment with the herbicide norfluorazon do not express *rbc*S mRNA (Oelmüller et al., 1986) and transgenic tobacco plants transformed with a reporter gene driven by *rbc*S sequences from pea do not express the construct

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Table 1. Chlorophyll Content of *Lemna* Roots and Fronds

Culture Age (days)	μg Chlorophyll/g, Fresh Wt[a]		Root % of Total Chlorophyll in Colony[a]
	Fronds	Roots	
11	572.7 ± 6.5	244.6 ± 4.1	29.6 ± 0.2
21	535.0 ± 66.0	289.3 ± 21.4	35.2 ± 1.1

[a] Values are means ± SD of three replicates.

after treatment with this herbicide (Simpson et al., 1986). Such regulatory events could occur at the transcriptional and/or post-transcriptional level. In the case of the *ghost* mutant of tomato, the mechanism has been shown to involve transcription. In sectors of these plants that are deficient in carotenoids, the chloroplasts photobleach and nuclei isolated from these cells transcribe *rbc*S genes at a lower rate than those from normal, unbleached sectors (Giuliano and Scolnik, 1988). Similarly, transcription of *cab* (Batschauer et al., 1986; Burgess and Taylor, 1988) and *rbc*S (Ernst and Schefbeck, 1988) genes has been shown to be affected by carotenoid deficiency. In the work presented here, we show that post-transcriptional processes can also play a role in the regulation of *rbc*S mRNA levels. A preliminary account of some of this work has been presented (Tobin et al., 1987).

L. gibba, an aquatic monocot, is a member of the family Lemnaceae. The structure and physiology of these plants have been reviewed extensively by Landolt (1986). The structure of the plant is simplified compared with many terrestrial angiosperms and comprises colonies of small leaf-like structures, fronds, which float by means of gibbous air sacs located on the lower side. Fronds contain few differentiated cell types, having a single cell layer epidermis and between one and 10 layers of parenchymatous cells inside. In the lower portion of the frond, these cells surround the air-filled sacs and are referred to as aerenchyma. Chloroplasts have been observed to be located mainly in the upper epidermis and parenchyma cells. Vegetative growth occurs by way of the production of a daughter frond from a meristematic pocket at the base of each frond. Each frond has a single root, which does not develop lateral roots. The root cap is separated from the inner cell layers by a water-filled space and because it is not renewed during growth, it eventually falls off. In a previous study (Silverthorne et al., 1990), we isolated six *rbc*S genes from *L. gibba* genomic libraries and a cDNA clone representing a seventh distinct gene. Whereas the coding regions of these genes are highly conserved, the 3′-untranslated regions are sufficiently divergent to allow their use as gene-specific probes. By using such probes, we were able to show that six of the genes are expressed in total RNA isolated from light-grown *Lemna*, although at widely differing levels, and that their expression is under phytochrome control. Here we show that expression of individual *rbc*S genes in *Lemna* differs between organs in light-grown plants, and that this difference cannot be accounted for by transcriptional differences. By using in situ hybridization we showed that *rbc*S gene expression occurs in chloroplast-containing cells of both fronds and roots and may be determined by an organ-specific mechanism that involves post-transcriptional events.

RESULTS

*Lemna rbc*S Gene Expression in Steady-State mRNA Differs between Roots and Fronds

In contrast to the roots of many terrestrial angiosperms, *Lemna* roots are normally green when grown in the light. Although the whole root is green, the majority of the chloroplasts are confined to the tip in the cortical and endodermal cells. Table 1 shows the chlorophyll distribution in the plants. The chlorophyll content of the roots is less on a per gram fresh weight basis than fronds and accounts for between about 30% and 35% of the chlorophyll in the whole colony, depending on the culture age. The age of the culture influences the relative chlorophyll content of roots and fronds, primarily because the roots elongate during development and the fronds become more gibbous. At 11 days, *Lemna* cultures contain a monolayer of colonies under the growth conditions used. A 21-day culture is packed with colonies, but is not senescent. Because *Lemna* roots contain plastids and a significant proportion of the chlorophyll in a colony, it was of interest to see whether the relative expression of individual *rbc*S genes was similar to that observed in the fronds.

RNA gel blot analysis of *Lemna* total RNA with an *rbc*S coding region probe and gene-specific probes is shown in Figure 1. Because the coding regions of *Lemna rbc*S genes were highly conserved at the nucleotide level, a coding region probe would measure the expression of all family members. These data confirmed the previous observation (Silverthorne et al., 1990) that all the *rbc*S genes for which we probed were expressed in *Lemna*, although at widely differing levels. In addition, it is clear that the relative expression of an individual gene between roots and fronds also differed. The most abundant *rbc*S mRNA in both roots and fronds was SSU1. By contrast, whereas SSU5B and SSU26 were expressed at comparable levels in the fronds, SSU5B was almost completely absent in the roots. Thus, the relative order of expression of individual *rbc*S genes in the fronds differed from that seen in the roots.

Expression of SSU5B Is Regulated Post-Transcriptionally

The apparent near absence of SSU5B mRNA in roots could arise by several mechanisms. For example, the gene

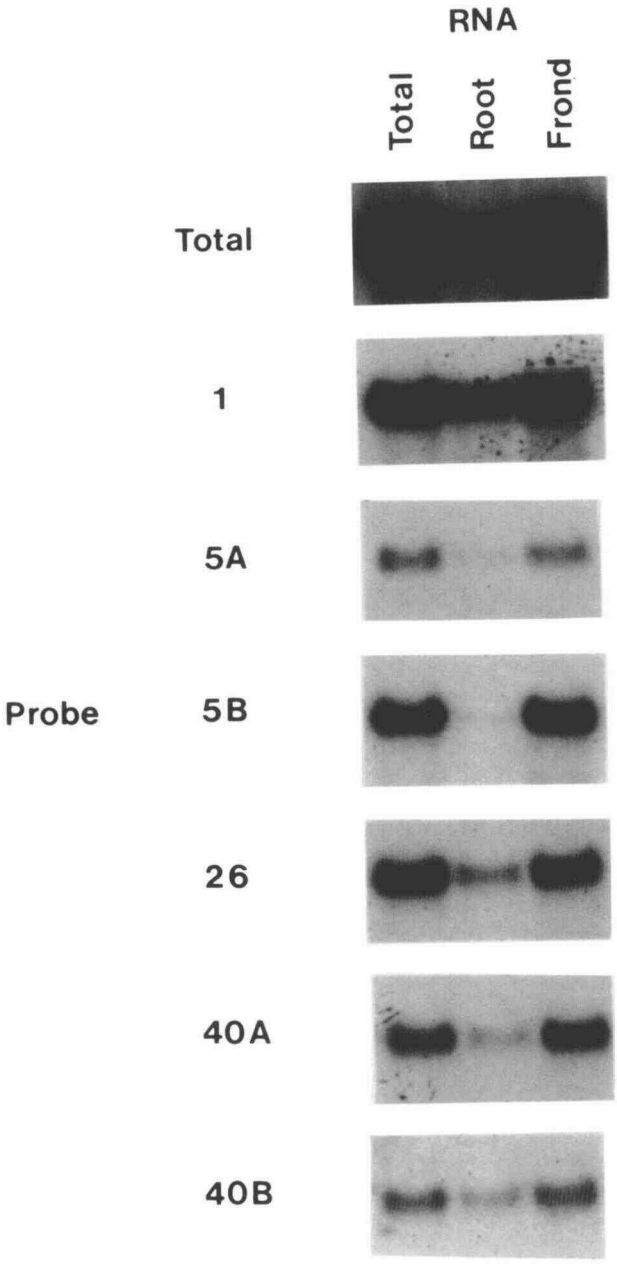

Figure 1. RNA Gel Blot Analysis of *Lemna* Root and Frond RNA.

RNA gel blots of equal aliquots (5 μg) of root or frond RNA or of RNA isolated from whole plants (Total) were probed with α-^{32}P-labeled single-stranded antisense RNA to the SSU coding region (Total) or to individual 3′-untranslated regions (1, 5A, 5B, 26, 40A, and 40B). The film was exposed for 72 hr.

may not be transcribed in roots or, alternatively, there may be transcription of a subsequently unstable mRNA. To distinguish between such transcriptional and post-transcriptional mechanisms, nuclei were isolated from root and frond tissue and allowed to continue RNA synthesis in an in vitro run-on transcription assay (Silverthorne and Tobin, 1984). Because this assay measures transcripts already initiated in vivo, it indicates whether SSU5B genes are being transcribed in root nuclei. The results of such an analysis with each gene-specific probe are shown in Figure 2. The data clearly show that the amount of transcription of all of the *rbc*S genes tested, including SSU5B, was similar between roots and fronds. Exact quantitative comparisons could not reliably be made because of background associated with all preparations of root nuclei (see Methods). A similar pattern of transcription was obtained in each of three separate experiments and whether young or old (cf. Table 1) *Lemna* cultures were used (data not shown). No detectable background with pBR322 was ever observed. We also note that the relative transcription rate of each gene in roots or fronds did not parallel the pattern of mRNA accumulation shown in Figure 1. SSU1 RNA was more abundant than SSU26 RNA in both roots and fronds, although SSU26 was the most heavily transcribed *rbc*S gene in both organs. Furthermore, these data are unlikely to be an artifact caused by the use of detergent during nuclear extraction because omission of detergent during

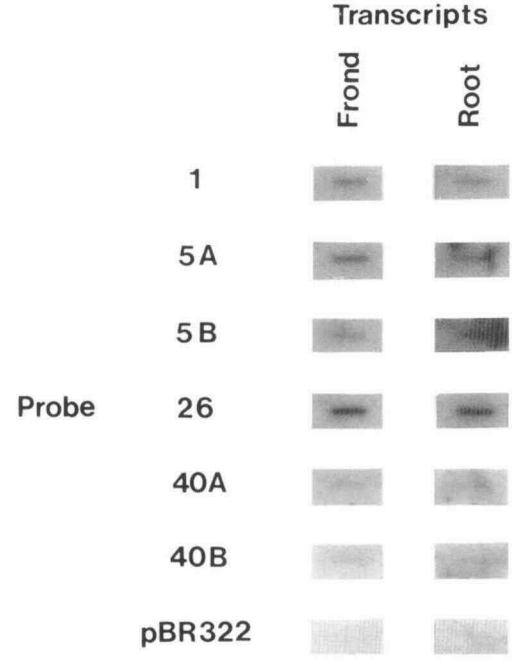

Figure 2. DNA Gel Blot Analysis of RNA Transcribed in Vitro in Nuclei Isolated from *Lemna* Roots and Fronds.

Slot blots of linearized individual SSU 3′-untranslated region subclones (1, 5A, 5B, 26, 40A, and 40B) or pBR322 DNA were hybridized to in vitro labeled transcripts from root and frond nuclei as described in Methods and exposed to film for 72 hr.

extraction did not alter the results (data not shown). Therefore, we conclude that transcriptional differences cannot account for the relative differences in *rbc*S mRNA levels between roots and fronds and that some post-transcriptional event(s) must determine *rbc*S mRNA levels.

SSU1 and SSU5B Are Expressed in the Same Cell Types in the Fronds

One possible mechanism for this apparent organ-specific, post-transcriptional regulation of *rbc*S mRNA abundance in *Lemna* could be cell-specific expression. It is possible that the relative stabilities of SSU1 and SSU5B are determined by the type of cell in which the sequences are expressed. We have tested this hypothesis using in situ hybridization because if these mRNAs are stable in different cell types, the pattern of hybridization will differ between sections hybridized with gene-specific probes. Figures 3, 4, and 5 show the results of in situ hybridization experiments performed using a total *rbc*S coding region probe (to follow expression of all family members) or gene-specific probes for SSU1 and SSU5B mRNA, the most and least abundant *rbc*S mRNAs in roots, respectively. Hybridizations were carried out using antisense RNA probes to detect expression of specific *rbc*S sequences, or sense RNA probes to control for nonspecific hybridization.

Figure 3 shows the expression pattern of the *rbc*S genes using a coding region probe. Total *rbc*S expression was seen to occur primarily in the upper epidermis and parenchyma, and to a lower extent in the aerenchyma cells, daughter frond, and root (Figures 3A, 3B, 3E, and 3F). Figure 4 shows the expression patterns of two individual *rbc*S genes, SSU1 and SSU5B. It is clear that the two gene-specific probes hybridized the same cell types (Figures 4A, 4B, 4G, and 4H) as the coding region probe in the fronds (Figure 3). In fronds, the majority of the signal for all three probes was detected in the upper epidermal and parenchymatous cells. In addition, there was hybridization to the aerenchyma cells that bound the air sacs and to the daughter fronds. Because the expression levels of the individual genes were lower than that of the whole family, the signal:background ratios were also lower. The upper cell layers of *Lemna* fronds have been reported to contain chloroplasts by Landolt (1986), and expression of total *rbc*S sequences, as well as SSU1 and SSU5B, correlated well with this observation. However, the location of cells maximally expressing *rbc*S mRNA on the upper surface of the frond did not simply reflect the orientation of the fronds with respect to the light because daughter fronds that were buried inside the parent frond often contained more signal than the parental cells surrounding them (for example, see Figures 4A and 4B). In addition, all three probes hybridized these cell types in both the mature fronds and the daughter fronds. There was no evidence for specific expression of either SSU1 or SSU5B in a particular cell type (for example, aerenchyma or epidermis) within the frond.

Hybridizations to root cross-sections are shown in Figure 5. The *rbc*S coding region probe (SSU560) hybridized primarily to cells in the cortex and epidermis, but not to the root cap or to the central cylinder tissue, which functions as the vascular tissue (Figures 5A and 5B). The SSU1 probe (Figures 5E and 5F) hybridized the same pattern of cells as the coding region probe, although with less signal. As expected, no hybridization over background was seen using the SSU5B probe (Figures 5I and 5J). Furthermore, it was evident that there is developmental regulation of *rbc*S gene expression in roots as well as fronds. For example, root tips showed much higher signals with the *rbc*S coding region probe than did sections from older root tissue (data not shown).

DISCUSSION

The mechanisms by which a plant controls the differentiation of different organs is a central problem in the developmental biology of plants. Organ-specific expression of *rbc*S genes in petunia (Dean et al., 1987; 1989a, 1989b), tomato (Sugita and Gruissem, 1987; Ueda et al., 1989), and pea (Simpson et al., 1986) involves transcriptional regulation. Specific sequences from *rbc*S genes can confer organ-specific expression on a reporter gene in transgenic plants transformed with such constructs (reviewed by Kuhlemeier et al., 1987; Silverthorne and Tobin, 1987; Dean et al., 1989c). However, the roles and mechanisms of post-transcriptional regulation are not as well understood.

Generally, post-transcriptional regulation has been postulated to occur when a disparity between the results of run-on transcription experiments and steady-state mRNA measurements is observed (Thompson, 1988). In addition to *rbc*S genes (see also Shirley et al., 1990), such observations have been made in plants for seed storage protein genes (Walling et al., 1986). In the case of ferredoxin, in vivo transformation experiments have indicated that sequences within the transcribed region of the gene are responsible for light regulation and that these may act at a post-transcriptional level (Elliott et al., 1989). In addition, messenger RNA stability has also been postulated to play a major role in regulation of chloroplast gene expression (Deng and Gruissem, 1987; Mullet and Klein, 1987).

Specific regions of plants' mRNAs can be postulated to play a role in the post-transcriptional regulation of organ-specific gene expression. In a recent study of the role of the 3′ end of a reporter gene [neomycin phosphotransferase (*nptII*)], Ingelbrecht et al. (1989) found that optimal expression required a functional polyadenylation signal and a GT-rich sequence downstream from this. Although all the constructs tested gave rise to similar NPTII activities

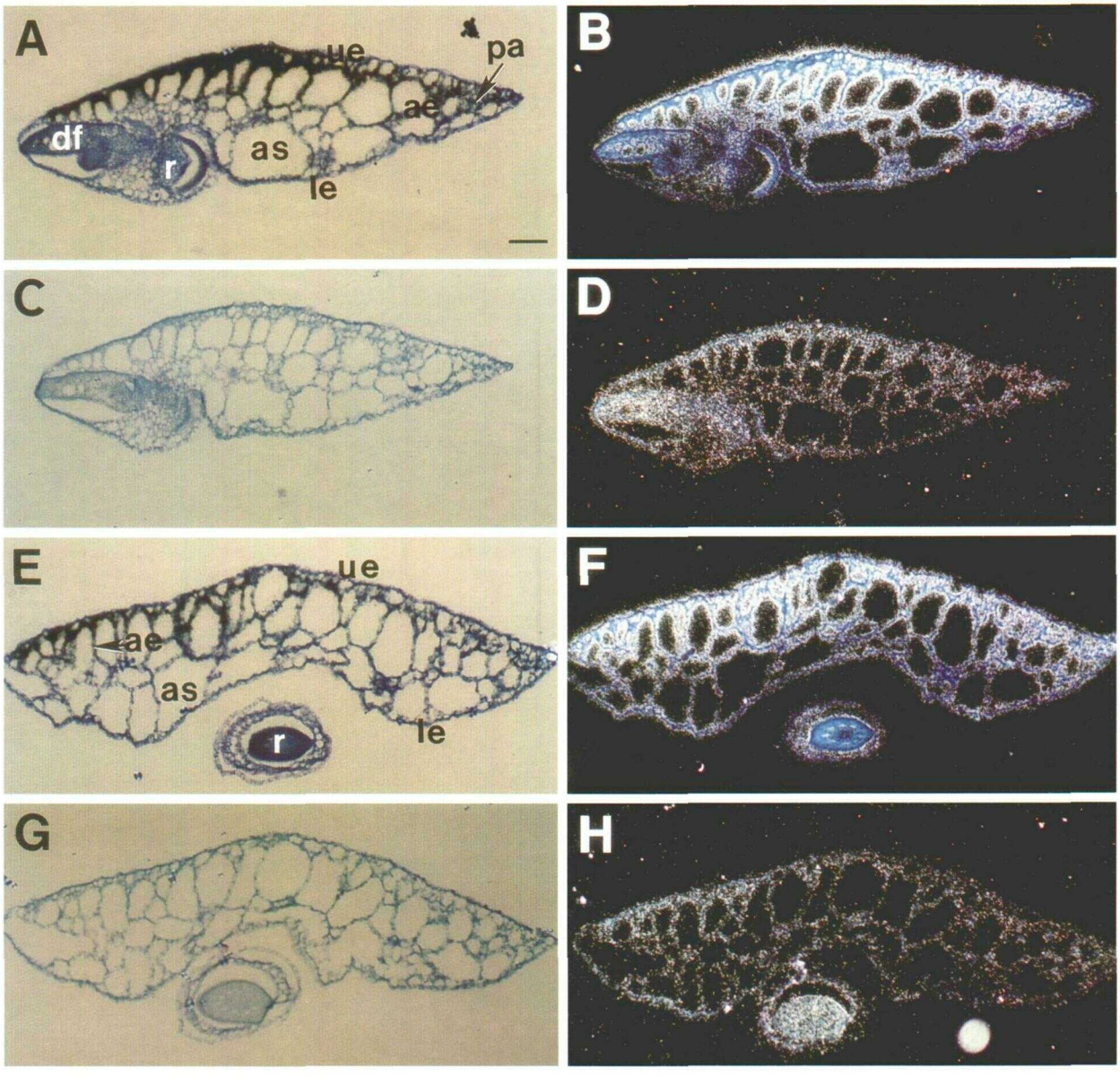

Figure 3. In Situ Hybridization Analysis of *Lemna* Sections Using the *rbc*S Coding Region Probe.

The bar in **(A)** represents 0.1 mm, and the scale is the same for all the panels. Frond cross-sections were hybridized in situ with single-stranded ^{35}S-RNA probes derived from SSU560.

(A) and **(E)** Bright-field photographs of sections hybridized with the antisense probe showing upper epidermis (ue), parenchyma (pa), aerenchyma (ae), air spaces (as), daughter fronds (df), root (r), and lower epidermis (le).

(B) and **(F)** Dark-field photographs of **(A)** and **(E)**. The silver grains are visible as white dots, indicating the presence of *rbc*S mRNA.

(C) and **(G)** Bright-field photographs of sections hybridized with the sense probe.

(D) and **(H)** Dark-field photographs of **(C)** and **(G)**.

in transient expression assays, stably transformed tissue levels varied by as much as 60-fold. Thus, the 3′ end of a gene may influence the efficiency of processing and/or stability of its transcript. Addition of a poly(A) tail of as few as 25 residues in length can also increase the stability of β-glucuronidase mRNA compared with a poly(A)$^-$ form after electroporation into monocot or dicot protoplasts in a transient expression assay (Gallie et al., 1989). Similarly,

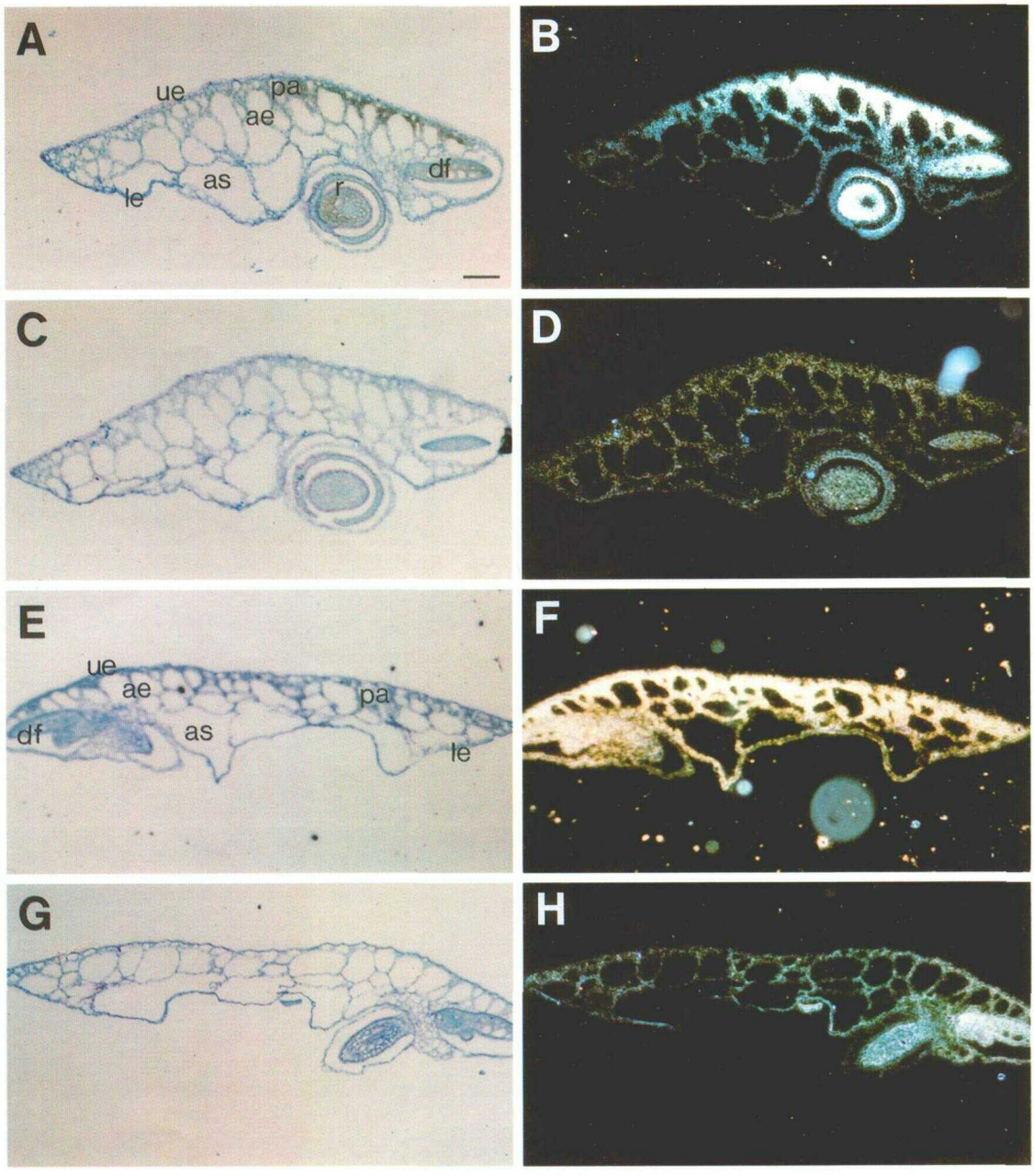

Figure 4. In Situ Hybridization Analysis of *Lemna* Sections Using Gene-Specific *rbc*S Probes.

Details marked and magnification are as in Figure 3. Frond cross-sections were hybridized in situ with single-stranded ^{35}S-RNA probes as indicated.

(A) Bright-field photograph of section hybridized with the SSU1 antisense probe.

(B) Dark-field photograph of **(A)**. The silver grains are seen as white dots, indicating the presence of SSU1 mRNA.

(C) Bright-field photograph of section hybridized with the SSU1 sense probe.

(D) Dark-field photograph of **(C)**.

(E) Bright-field photograph of section hybridized with the SSU5B antisense probe.

(F) Dark-field photograph of **(E)**. The silver grains are seen as white dots, indicating the presence of SSU5B mRNA.

(G) Bright-field photograph of section hybridized with the SSU5B sense probe.

(H) Dark-field photograph of **(G)**.

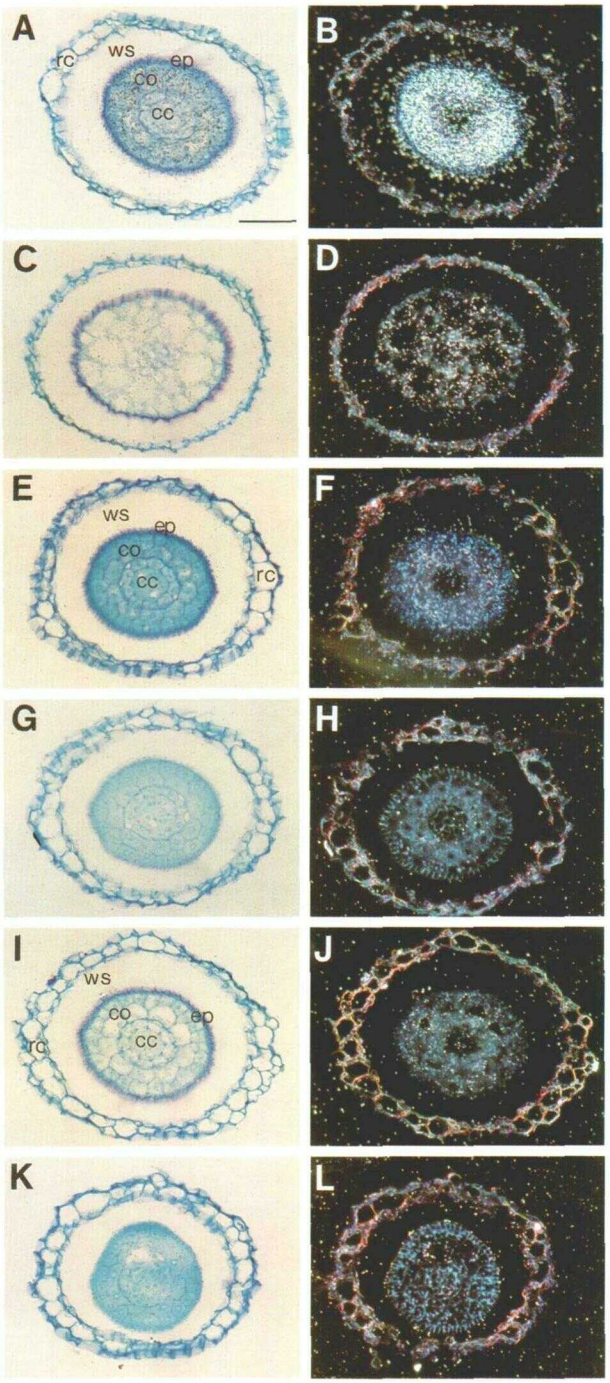

Figure 5. In Situ Hybridization of *Lemna* Root Cross-Sections.

Root cross-sections were hybridized with single-stranded ^{35}S-RNA probes as indicated. The size bar in **(A)** represents 0.1 mm, and the scale is the same in all panels.

(A) Bright-field photograph of section hybridized with the *rbc*S 560 antisense probe. The root cap (rc), water-filled space (ws), epidermis (ep), cortex (co), and central cylinder (cc) are indicated.

(B) Dark-field photograph of **(A)** showing the presence of *rbc*S mRNA.

(C) Bright-field photograph of section hybridized with the 560 sense probe.

(D) Dark-field photograph of **(C)**.

(E) Bright-field photograph of section hybridized with the SSU1 antisense probe. Details as in **(A)**.

(F) Dark-field photograph of **(E)** showing the presence of SSU1 mRNA.

(G) Bright-field photograph of section hybridized with the SSU1 sense probe.

(H) Dark-field photograph of **(G)**.

(I) Bright-field photograph of section hybridized with the SSU5B antisense probe. Details as in **(A)**.

(J) Dark-field photograph of **(I)**.

(K) Bright-field photograph of section hybridized with the SSU5B sense probe.

(L) Dark-field photograph of **(K)**.

the presence of introns can influence the expression of different mRNAs. Dean et al. (1989b) found that removal of the intron sequences from the petunia *rbc*S gene SSU301 resulted in an approximately fivefold reduction in steady-state mRNA levels from this construct in tobacco compared with the gene-containing introns. These findings are consistent with the observations of Callis et al. (1987), showing that addition of a heterologous intron sequence to gene fusions increased the levels of steady-state mRNA expressed after electroporation into maize cells. Thus, there are multiple levels at which post-transcriptional events could operate to regulate *rbc*S mRNA levels.

In *Lemna*, we have found that both the roots and fronds transcribe and accumulate *rbc*S mRNA. However, individual *rbc*S genes were found to be transcribed at rates that did not correlate with the amount of RNA accumulated either in the roots or the frond. In particular, SSU1 transcripts accumulated to the highest levels in both roots and fronds, although this gene was not found to be the most highly transcribed. In addition, SSU5B was transcribed at comparable rates in root and frond nuclei, yet the mRNA did not accumulate significantly in roots. We infer from these observations that *rbc*S transcripts are differentially stable in root cells as compared with frond cells. The results of the in situ hybridization analysis did not support the idea that this is because each gene is only expressed in a specific cell type in the fronds or roots, but indicated that the mechanism involves organ-specific processes. The precise mechanism(s) determining *rbc*S transcript stability in different *Lemna* organs has yet to be determined. Although the coding regions of individual *rbc*S genes are almost identical at the nucleotide level, the intron and the 5′-untranslated and 3′-untranslated regions are divergent (Silverthorne et al., 1990). Thus, differential expression of individual *rbc*S genes could result from variable splicing efficiencies, polyadenylation, export from the nucleus, stability in the cytoplasm, or a combination of these. Given

the in situ hybridization data for SSU5B, the mechanism must involve steps specific for each gene because SSU1 accumulated in root cells that did not accumulate SSU5B. Either a specific sequence is involved in accumulation of SSU5B transcripts in fronds and/or prevents their accumulation in roots. These possibilities are currently under investigation.

METHODS

Plant Material

Duckweed (*Lemna gibba* G3) was grown aseptically as described previously (Tobin, 1981). Roots and fronds were separated manually using a sterile razor blade. Tissue for RNA isolation was frozen in liquid nitrogen before use.

Chlorophyll Estimations

Roots and fronds were dissected from 0.03g to 0.3g of colonies for chlorophyll measurements. Separated roots and fronds were ground in a Ten-Broeck homogenizer in a solution containing 10 mM Tris, pH 8.0. Aliquots were made 80% (v/v) in acetone (Fisher, spectral quality) and spun at 8160*g* in an Eppendorf microcentrifuge. The supernatant fraction was read against 80% (v/v) acetone containing 2 mM Tris, pH 8.0, at 647 nm and 664 nm in a Shimadzu UV160 spectrophotometer. Chlorophyll concentrations were estimated using the formula of Zeigler and Egle (1965).

RNA Isolation and Analysis

RNA was isolated from 10-g aliquots of frozen roots or fronds by a modification of the method of Loening (1969) as described by Silverthorne et al. (1990). RNA gel blots were prepared as described by Thomas (1980). Individual SSU 3′-untranslated region subclones were purified by the procedure described in the Promega-Biotec Protocols and Applications guide. Isolated plasmid DNA was banded on cesium chloride step gradients (Garger et al., 1983) in a Beckman TL-100 tabletop ultracentrifuge. High-specific activity radioactive, single-stranded RNA antisense probes were prepared by transcription of linearized plasmids with SP6 polymerase (Melton et al., 1984) in the presence of α-^{32}P-UTP (ICN; 3000 Ci/mmol). Before use, probes were passed over Bio-Gel P-60 columns (Bio-Rad) equilibrated in 10 mM Tris, pH 7.5/10 mM EDTA/0.1% SDS and the excluded fractions collected. Hybridization conditions were as described by Silverthorne et al. (1990). Hybridized blots were exposed to XAR 5 film (Kodak) with screens (Lightning-Plus, Du Pont-New England Nuclear) for 72 hr at −70°C.

*Lemna rbc*S Probes

The *rbc*S coding region probe SSU560 was prepared using the 0.56-kb SalI/HindIII fragment of pLgSSU1 (Stiekema et al., 1983) cloned into pGEM3Z. This clone contains the coding region of the mature SSU polypeptide from amino acid number 5 to the termination codon, a region highly conserved between all the *Lemna rbc*S genes sequenced. The 3′-untranslated regions of SSU1, SSU5B, SSU26, SSU40A, and SSU40B were subcloned into pGEM3Z as described in Silverthorne et al. (1990). These clones contain only portions of the 3′-untranslated regions and do not contain coding sequences or sequences downstream of the gene as determined by S1 protection mapping. These clones contain inserts of almost uniform size.

Isolation of Nuclei and Analysis of in Vitro Labeled Run-On Transcripts

Nuclei were prepared from 5-g to 10-g aliquots of freshly dissected roots or fronds and in vitro labeled run-on transcripts prepared and isolated by the methods described in Silverthorne and Tobin (1984). Slot blots of linearized 3′-untranslated region plasmids (1 μg DNA/slot) on Nytran (Schleicher & Schuell) were used to probe equal radioactive aliquots of transcripts (2.25×10^6 cpm/filter) from root or frond nuclei. Filters were washed at 55°C in 0.1 × SSC/0.1% SDS before exposure to x-ray film for 72 hr as described above. The preparations of nuclei from roots gave rise to a higher background on blots than those from the fronds, but we do not know the reason for this observation. However, background hybridization with pBR322 was never observed.

In Situ Hybridization

A modification of the protocols of Cox and Goldberg (1988) was used. In brief, complete fronds (1 mm to 2 mm across) or portions of larger fronds were used such that the tissue was smaller than 3 mm square. Root segments corresponding to the terminal 3 mm were cut using a fresh blade. Selected tissue was fixed in a solution containing 1% (v/v) glutaraldehyde in 0.05 M sodium cacodylate, pH 7.0, embedded in Paraplast Plus, and sliced into 10-mm sections on a Reichert microtome using a Reichert knife. Tissue was prestained for 1 min in 1% (w/v) eosin in 70% (v/v) ethanol during dehydration to facilitate visualization during the embedding and sectioning steps. Sections were attached to slides using a 2% (v/v) solution of 3-aminopropyltriethoxysilane (Aldrich) in acetone as described in Angerer et al. (1987). Single-stranded ^{35}-S-labeled RNA probes were synthesized from 3′-untranslated region plasmids using the pGEM transcription system (Promega-Biotec). The ^{35}-S labeled probes were hydrolyzed to between 0.15 kb and 0.2 kb in length and then hybridized to slides containing both root and frond sections for 14 hr at 42°C in 0.5 M Na^+ and 50% formamide. After hybridization, the sections were incubated with RNase A at 50 μg/mL, washed at 50°C in 0.2 M Na^+ and coated with photographic emulsion (NTB 2, Kodak) diluted 1:1 with 0.6 M ammonium acetate. Developed sections were stained in 0.5% toluidine blue in 50% (v/v) ethanol and visualized using both dark-field and bright-field microscopy as appropriate.

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