Expression Dynamics of the Tomato \textit{rbcS} Gene Family during Development

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The tomato \textit{rbcS} gene family is composed of five genes (\textit{rbcS1}, \textit{2, 3A, 3B}, and \textit{3C}) that are differentially expressed during tomato development. Nuclear run-on transcription assays and RNA analysis were used to determine the contribution of transcriptional and post-transcriptional regulation to the accumulation of mRNA from the five \textit{rbcS} genes in tomato seedlings, leaves, and fruit. We found that the qualitative pattern of mRNA accumulation is regulated at the transcriptional level and that, in general, there is a correlation of rates of \textit{rbcS} transcription with overall \textit{rbcS} mRNA abundance in fruit and leaves. Although transcriptional control is a primary determinant for \textit{rbcS} gene expression in tomato, examination of relative transcription rates and mRNA accumulation of each \textit{rbcS} gene demonstrated that there is also significant post-transcriptional control of \textit{rbcS} gene expression during organ development. Individual \textit{rbcS} mRNAs, which have highly conserved coding sequences and differ only in their 5' and 3' untranslated sequences, have different stabilities. We showed that both transcription and stability of individual \textit{rbcS} mRNAs are altered in different organs and by the developmental program within these organs as well as by exposure to light. Together, the results provide a comprehensive analysis of the extent of transcriptional and post-transcriptional control that operates within the \textit{rbcS} gene family during plant development.

\textbf{INTRODUCTION}

Ribulose-1,5-bisphosphate carboxylase (Rubisco) is a critical enzyme that comprises approximately 50% of the soluble protein in leaves and is present in much lower levels in other photosynthetic organs of the plant (Bonner and Varner, 1976; Ellis, 1979). The enzyme catalyzes the initial fixation of atmospheric carbon dioxide into carbohydrates. Rubisco is composed of eight identical large subunits (LSU) and eight identical small subunits (SSU). It has been of considerable interest to understand how accumulation of each of the subunits is regulated because the LSU and the SSU proteins are products of the chloroplast \textit{rbcL} gene and the nuclear \textit{rbcS} genes, respectively, and the subunits are both required (and found) in chloroplasts in stoichiometric amounts. Excess amounts of either SSU or LSU are apparently rapidly degraded (Schmidt and Mishkind, 1983; Inamine et al., 1985). LSU is probably made constitutively in most plants, although it accumulates only when SSU is synthesized to form the holoenzyme. Thus, regulation of formation of this enzyme is dependent on the control of \textit{rbcS} gene expression (Rodermel et al., 1988).

All known plant \textit{rbcS} genes occur in families of four to 13 genes, but there is no known functional difference between mRNAs or proteins from each of the \textit{rbcS} genes within plant species. All genes within a family produce proteins of similar or identical sequence that presumably function with equal efficiency. Many \textit{rbcS} genes are, however, expressed in qualitatively and quantitatively differing patterns in different organs and at different developmental stages (for review, see Manzara and Gruissem, 1988; Dean et al., 1989). Previous reports have shown that regulation of \textit{rbcS} gene expression is primarily exerted at the level of transcription (Gallagher and Ellis, 1982; Silverthorne and Tobin, 1984; Berry-Lowe and Meagher, 1985), although recent work has also demonstrated control at the level of mRNA stability (e.g., Shirley and Meagher, 1990; Shirley et al., 1990; Silverthorne and Tobin, 1990). The molecular mechanisms of transcriptional regulation, primarily during light induction, have been dissected for selected \textit{rbcS} genes, and cis-regulatory elements and corresponding trans-acting proteins that have a role in their transcriptional control are currently under investigation (for review, see Gilchrist et al., 1990). No information is available on cis- or trans-acting components that are critical to the differential stability of \textit{rbcS} mRNAs. Also, most of the previous studies on the transcriptional and post-transcriptional regulation of \textit{rbcS} gene expression have either focused on overall \textit{rbcS} mRNA or protein accumulation without differentiating between members of

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We are investigating the molecular mechanisms that control transcription, mRNA accumulation, and protein accumulation of all members of the tomato rbcS gene family to understand differential expression of rbcS genes in plants. The tomato rbcS gene family consists of five members, which are arranged into three loci that are located on two chromosomes (Pichersky et al., 1986; Sugita et al., 1987). Three of the five genes (rbcS3A, rbcS3B, and rbcS3C) are linked within a 12-kb region and encode identical mature SSU proteins, whereas rbcS1 and rbcS2 are located on different chromosomes and encode SSU proteins that differ from each other and from the locus 3 proteins (Sugita et al., 1987). A preliminary analysis with cDNA probes and gene-specific oligonucleotides showed that the accumulation of the tomato rbcS mRNAs is controlled by unique developmental and organ-specific programs, as well as by light and diurnal cycles (Piechulla and Gruissem, 1987; Sugita and Gruissem, 1987). For example, rbcS1, 2, and 3A mRNAs accumulate in cotyledons in the absence of light, whereas accumulation of rbcS3B and 3C mRNAs is light dependent. In contrast, all five mRNAs are found at high but different levels in illuminated leaves, and transfer to darkness significantly decreases the level of rbcS1 mRNA as well as of rbcS3B and 3C mRNAs (Sugita and Gruissem, 1987). In photosynthetically active young fruit, only rbcS1 and 2 mRNAs are found, and they decrease to undetectable levels during fruit development (Piechulla et al., 1986; Sugita and Gruissem, 1987). The five mRNAs do not accumulate in roots. Consequently, effectors of tomato rbcS gene expression must integrate different developmental, organ-specific, and environmental signals to establish the distinct mRNA accumulation patterns.

We used nuclear run-on transcription assays and steady-state mRNA quantification in combination with single-stranded DNA probes to investigate the transcriptional and post-transcriptional components of differential rbcS mRNA accumulation in response to the developmental program and light induction in tomato. In this paper, we showed that there is a strict correlation between the transcription of individual rbcS genes and the presence of their cognate mRNAs, indicating that transcriptional regulation acts as a primary control within the gene family. In the accompanying paper (Manzara et al., 1991), we showed that the differences in transcriptional activities during development correlate with changes in DNA-protein interactions in the promoter regions of the five genes. We also demonstrated in the report here that differential rbcS mRNA stability contributes to the quantitative changes in mRNA accumulation that occur during light activation and development. Because the DNA coding sequences of the five tomato rbcS genes are highly conserved, differences in rbcS mRNA stability must be controlled by differences in 5' and/or 3' untranslated regions.

RESULTS

Construction of rbcS Gene-Specific Probes

Gene-specific probes longer than the 20-nucleotide oligomers used previously (Sugita and Gruissem, 1987; Sugita et al., 1987) were constructed to assay rbcS transcript levels at high stringency. Protein-coding and intron DNA sequences of tomato rbcS genes are highly conserved, and the 5' untranslated regions are only 8 to 73 nucleotides in length (Figure 1; Sugita et al., 1987). The DNA sequences in the 3' untranslated regions of the five tomato rbcS genes are longer and divergent (Sugita et al., 1987), therefore allowing construction of gene-specific probes.
from these regions. The polymerase chain reaction (PCR) was used to amplify precise regions of maximum hybridizable length, utilizing sequence information from tomato rbcS genomic clones (Sugita et al., 1987; Wanner, 1990), because the DNA sequences in the 3' untranslated regions are AT rich and provide few restriction sites. Sequences of PCR fragments were determined to confirm their identity. The locations of the probes are shown in Figure 1.

Polyadenylation sites for the three genes were mapped. Single sites of polyadenylation occur in rbcS1, 2, 3A, and 3B, and confirm the previously established polyadenylation sites in rbcS1 and 2 (McKnight et al., 1986). The polyadenylation sites in rbcS3A and 3B genes are located 156 and 158 nucleotides 3' to the stop codon, respectively, and are within the cloned PCR DNA fragments used in this study. In rbcS3C, two polyadenylation sites are located 137 and 140 nucleotides 3' to the stop codon and are also within the cloned PCR DNA fragment (Figure 1). Although the lengths of hybridizable sequences in the gene-specific DNA fragments vary between 133 and 164 nucleotides, the GC content and the calculated Tm values are very similar (see Table 3 and Methods). The rbcS gene-specific probes hybridized with approximately equal efficiencies to the cognate DNA fragments from the five genes and did not cross-hybridize (Wanner, 1990). The rbcS gene-specific probes were used to compare the relative rates of transcription in isolated nuclei with the corresponding levels of rbcS mRNAs detected by RNA blot analysis in response to light and to the developmental program in tomato.

Light Affects rbcS Gene Transcription and mRNA Stability in Developing Seedlings

Tomato seedlings grown in the dark for 7 days differ in development from seedlings of the same age grown in light/dark cycles. Etiolated seedlings had 5-cm-long white hypocotyls and light yellow cotyledons that were just emerging from the seed coats. Seedlings grown in 14-hr light/10-hr dark cycles had 2.5- to 3-cm anthocyanin-containing hypocotyls, their seed coats were shed, and they had dark green cotyledons with the first pair of true leaves appearing between them. The rbcS gene-specific probes were hybridized to total RNA from cotyledons of 7-day-old seedlings grown in the dark or grown in light/
dark cycles. Figure 2 shows that the abundance of the five rbcS mRNAs differed significantly between etiolated and green seedlings. In etiolated seedling cotyledons, rbcS1 and 3A mRNAs provided most of the rbcS mRNA. rbcS2 mRNA was present at low but detectable levels, but mRNAs from rbcS3B and 3C were not detected. In contrast, all five rbcS mRNAs were abundant in light-grown seedlings.

Etiolated seedlings that had been exposed to light for 6 hr were darker yellow to yellow-green. The rbcS mRNAs that were not detected in etiolated seedlings were readily detected only 3 hr after light exposure (Figure 2). Although exposure to light enhanced the accumulation of all five rbcS mRNAs, light was required for accumulation of rbcS3B and 3C mRNAs.

Nuclei were isolated from the same seedlings used in preparation of RNA shown in Figure 2. Nuclear run-on transcripts from etiolated or green seedlings were hybridized to filter-bound, single-stranded DNA probes for each rbcS gene and to DNA probes for ribosomal RNA, phytochrome (phyA), and a proline-rich protein (FR2; Salts et al., 1991), as shown in Figure 3. FR2 mRNA accumulates to high levels in young tomato fruit and to lower levels in all other tomato organs (Salts et al., 1991), and transcription of this gene was similar in etiolated and green cotyledons. In addition, there was no significant difference in rRNA transcription between etiolated and green seedlings. Accumulation of phytochrome mRNA is reduced by light, and this decrease is regulated at the level of transcription (Lissemore and Quail, 1988). Transcription of the tomato phytochrome gene that cross-hybridizes to the Arabidopsis phyA DNA probe was, as expected, detectable only in etiolated seedling cotyledons. Nuclear run-on transcripts from all five rbcS genes were readily detectable in green seedlings (Figure 3B). In etiolated seedlings, rbcS1, 2, and 3A were actively transcribed, although transcripts from 3A were near the limits of detection. Transcription was not detected from rbcS3B and 3C, suggesting that the transcription of these two genes in seedlings is light dependent.

Single-stranded DNA probes of the same sense as rbcS mRNAs [(-)-strand DNA] were used as controls in
hybridizations with run-on transcripts. As shown in Figures 3A and 3B, a low level of hybridization was detected with the (−)-strand DNA for FR2, rbcS3A, and 3B. Although these hybridization signals were reproducible in independent experiments, it is not known whether this signifies the presence of stable antisense RNA from these genes.

Quantification of the hybridization results (Figure 3C) confirmed that rbcS1, 2, and 3A are transcribed at twofold to eightfold higher levels in green seedling cotyledons than in etiolated seedling cotyledons, demonstrating that light enhances rbcS transcription in tomato seedlings. It is likely that light enhancement of rbcS3A transcription is underestimated because (−)-strand DNA hybridization was subtracted as background in the quantitative data in Figure 3C. Light enhancement of transcription is specific to the rbcS genes because significant differences were not detected in the transcription of the ribosomal RNA genes or FR2.

Comparison of the rates of transcription among the tomato rbcS genes with their respective mRNA levels provided evidence for post-transcriptional control. In etiolated seedlings, rbcS3A mRNA was most abundant, but the rbcS1 gene was more highly transcribed than was rbcS3A (Figures 2B and 3C). Therefore, it appears that there are differences in stability among the three rbcS mRNAs that accumulate in etiolated seedlings; rbcS3A mRNA was more stable in the dark than were either rbcS1 or rbcS2 mRNAs. The rbcS1 mRNA level was at least fivefold higher in light-grown seedlings than in etiolated seedlings, but rbcS1 transcription rates differed by only 2.5-fold. A similar discrepancy between transcriptional activity and mRNA abundance was detected for rbcS2. Thus, light had a stabilizing effect on rbcS1 and rbcS2 mRNAs but not rbcS3A mRNA, suggesting that light-dependent post-transcriptional differences in rbcS mRNA stability play a significant role in defining rbcS mRNA levels during tomato seedling development.

**Post-Transcriptional Differences in rbcS mRNA Stability during Leaf Development**

The above results raised the possibility that both transcriptional and post-transcriptional control mechanisms coordinate the differential expression of the tomato rbcS gene family at other developmental stages as well. For this reason, we examined the relative rbcS run-on transcription rates and rbcS mRNA levels in tomato leaves at two developmental stages. Immature, light green, incompletely expanded leaves were collected from apical regions of the plant. Fully expanded, green leaflets were collected from mature leaves. RNA and nuclei were prepared from portions of the same collection of leaves. Figure 4A shows that the five rbcS mRNAs were found at similar levels in immature and mature leaves. The quantitative data in Figure 4B show that rbcS mRNAs were either similar (rbcS3C) or reduced in mature leaves as compared with immature leaves. Although the relative rates of run-on transcription of ribosomal DNA and the FR2 gene were similar in mature and immature leaves, as shown in Figure 5A, the relative rates of transcription of the five rbcS genes were twofold to threefold higher in mature leaves than in immature leaves. The discrepancy between increased rates of rbcS transcription in mature leaves and the decreased rbcS mRNA abundance suggests that the overall stabilities of rbcS mRNAs decrease during leaf development.

In addition to these general changes, the results shown in Figures 4 and 5 reveal changes in the relative stability of individual rbcS mRNAs during leaf development. The approximate changes in relative rates of rbcS transcription and in rbcS mRNA abundance between immature and mature leaves are summarized in Table 1, together with the calculated relative increase in mRNA turnover required to accommodate the differences in mRNA abundance. From the results in Table 1, it appears that the decay rates for rbcS1 and 3A mRNA increase relative to their transcription rates, thus resulting in a lower "steady-state" level of these mRNAs. The increase in rbcS2, 3B, and 3C mRNA decay in mature leaves was paralleled by an increase in rates of transcription; thus, the "steady-state" levels of these mRNAs did not decline significantly during leaf maturation. We conclude that both transcriptional and post-transcriptional mechanisms regulate the differential expression of the members of the rbcS gene family during tomato leaf development.

**rbcS Gene Expression in Developing Fruit is Controlled by Temporal Changes in Transcriptional Activity and mRNA Stability**

Previous results have shown that only rbcS1 and 2 mRNAs accumulate early during tomato fruit development and that their mRNA levels decrease rapidly during fruit ripening (Sugita and Gruissem, 1987). To understand the molecular basis for this rbcS gene expression pattern, we determined the transcriptional activity of rbcS genes in developing fruit. Total RNA and nuclei were prepared from young fruit (3 to 5 and 5 to 8 mm diameter) and fully expanded, mature green fruit. Fruit of 3 to 5 mm diameter are approximately 3 to 6 days postpollination and are growing primarily by cell division. Fruit of 5 to 8 mm diameter are approximately 7 to 10 days postpollination. During this time, cell division stops and growth continues by cell expansion until the mature green stage is reached 35 to 40 days postpollination. At the mature green stage, fruit are fully enlarged but have no visible color change resulting from the degradation of chlorophyll and the concomitant accumulation of carotenoids, which are characteristic phenotypic markers of the ripening process.
Figure 4. *rbcS* mRNA Accumulation in Immature and Mature Leaves.

(A) Slot blots containing 2 \( \mu g \) of total RNA from immature (I) and mature (M) leaves were hybridized to each *rbcS* gene-specific DNA probe. Specific activities (SA \( \times 10^7 \) Cerenkov cpm/\( \mu g \) template) in this particular experiment are indicated next to the *rbcS* probe designations.

(B) Average cpm hybridizing in slot blots as shown in (A) and similar sets of slot blots/2 \( \mu g \) total RNA. Hybridized cpm were corrected for the specific activities of the individual *rbcS* gene probes. Numbers are the averages of separate hybridizations to three independent RNA preparations, with the range in quantification between blots indicated by the error bars.

Figure 6 shows that *rbcS1* and *rbcS2* mRNAs can be readily detected throughout development of the fruit until the mature green fruit stage. There was a small increase in the relative levels of both mRNAs from 3 to 5 mm diameter to 5 to 8 mm diameter fruit (Figure 6B), which correlates with an increase in chlorophyll content and photosynthetic activity (Piechulla et al., 1987). The significant reduction of *rbcS* mRNA levels in mature green fruit (Figure 6B), however, is temporally uncoupled from the loss of chlorophyll and photosynthetic activity during the ripening phase (Piechulla et al., 1987). Of the locus 3 *rbcS* mRNAs, only *rbcS3A* was found near the limit of detection in early fruit development. These results are consistent with the failure to detect any SSU isoform 3 protein in young fruit (D. Somers and W. Gruissem, unpublished data).

To determine the extent to which the *rbcS* expression pattern in fruit was controlled at the transcriptional level, the activity of all five genes was assayed by nuclear run-on transcription. Figure 7 shows that only *rbcS1* and 2 genes were transcribed at significant levels in developing and mature green fruit and that transcription of locus 3 *rbcS* genes was at or below the level of detection. The hybridization to the \((-\rangle\)-strand DNA probes for *rbcS3A* and 3B was pronounced in run-on transcripts from developing fruit. Therefore, the average counts per minute hybridizing to the \((-\rangle\)-DNA strand of *rbcS3C* were used as background levels in the quantification of transcription of *rbcS3A* and 3B. The quantitative data in Figure 7D confirmed that the transcriptional activity of *rbcS1* and 2 increases early in fruit development but decreases sixfold to eightfold by the time fruit reach the mature size. The transcriptional activation/inactivation during fruit development was specific to the *rbcS* genes and not a general property of developing fruit because other genes (e.g., FR2 and FR5) show a different temporal transcription activity profile. We conclude from these results that the unique *rbcS* gene expression pattern in fruit is established at the transcriptional level.

Comparison of the relative rates of transcription of both *rbcS1* and 2 with the accumulation of their respective mRNAs indicated that differences in mRNA stability are superimposed on the transcriptional control in fruit, as in seedling and leaves. The approximately threefold increase
in transcriptional activity of \( rbcS1 \) and 2 was not accompanied by a corresponding increase in their mRNA levels, indicating that \( rbcS \) mRNA turnover rates, similar to leaves, increase during fruit development. In addition, transcriptional activity of \( rbcS2 \) was only approximately 1.5-fold higher than transcriptional activity of \( rbcS1 \), but \( rbcS2 \) mRNA increased to an approximately threefold higher level, indicating that \( rbcS2 \) mRNA is more stable than \( rbcS1 \) mRNA in developing fruit. Additional evidence for increased \( rbcS2 \) mRNA stability was also found in mature green fruits, where \( rbcS1 \) and \( rbcS2 \) transcriptional activities have declined between sixfold and eightfold, but \( rbcS2 \) mRNA levels have only declined approximately twofold.

**DISCUSSION**

In this report, we showed that the developmental, organ-specific, and light-dependent differential expression of the \( rbcS \) gene family in tomato is regulated both by overlapping and complex transcriptional and post-transcriptional controls. This is particularly striking because even though they show significant differences in their regulation, the five genes are highly conserved at the DNA and protein levels (Sugita and Gruissem, 1987) and the locus 3 genes are clustered within a 10-kb region. Our results demonstrated that the qualitative pattern of \( rbcS \) mRNA

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**Table 1.** Estimation of the Change in \( rbcS \) mRNA Stabilities during Leaf Development

<table>
<thead>
<tr>
<th>( rbcS ) Gene</th>
<th>A</th>
<th>B</th>
<th>A/B</th>
</tr>
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<tr>
<td></td>
<td>trx* Mature RNA</td>
<td>Mature RNA</td>
<td>Increased Turnover Required (Decrease in mRNA ( t_0 ))</td>
</tr>
<tr>
<td>1</td>
<td>2.7</td>
<td>0.55</td>
<td>4.9 X</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>1.0</td>
<td>2.3 X</td>
</tr>
<tr>
<td>3A</td>
<td>2.6</td>
<td>0.67</td>
<td>3.9 X</td>
</tr>
<tr>
<td>3B</td>
<td>4.5</td>
<td>0.92</td>
<td>4.9 X</td>
</tr>
<tr>
<td>3C</td>
<td>2.3</td>
<td>1.07</td>
<td>2.1 X</td>
</tr>
</tbody>
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* trx, transcription as measured in nuclear run-on assays.

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**Figure 5.** Nuclear Run-On Transcription Activities in Immature and Mature Leaves.

(A) and (B) Labeled transcripts were hybridized to membranes containing 2 \( \mu \)g of single-stranded DNA with sequences either complementary to (+) or the same (−) as mRNA, as indicated in the key. The amounts of single-stranded DNA containing sequences complementary to ribosomal RNA were 2, 0.2, and 0.02 \( \mu \)g, respectively. Abbreviations for gene probes shown in the key are as in Figure 3 (except that the phytochrome cDNA probe was not included). Total transcript cpm of \( 4.5 \times 10^7 \) and \( 1.75 \times 10^7 \) were hybridized in (A) and (B), respectively.

(C) Quantitative results of run-on transcription assays are plotted as cpm hybridizing to each \( rbcS \) probe/10^7 total transcript cpm, as described in Methods. Columns are averages of two (immature leaf) or three (mature leaf) run-on assays in independent nuclear preparations. Error bars show the range of cpm between experiments.
accumulation in tomato cotyledons, leaf, and fruit is strictly correlated with the transcriptional activity of the corresponding genes. Comparison of relative rates of transcription in seedlings (Figure 3C), leaves (Figure 5C), and fruit (Figure 7D) to rbcS mRNA abundance in these organs (Figures 2B, 4B, and 6B, respectively) showed that there is a general correlation between rbcS transcription rates and mRNA accumulation. Both transcription rates and mRNA accumulation from each rbcS gene are 10-fold to 20-fold higher in leaves than at any stage of fruit development. As would be expected, when an rbcS mRNA is not detected in an organ (e.g., rbcS3B and rbcS3C in fruit and etiolated seedlings), it is not transcribed. Thus, our results confirmed that a primary determinant of rbcS mRNA accumulation is the rate of transcription of each gene. In addition, we found that regulation of temporal and organ-specific differences in rbcS mRNA stability is superimposed on the transcriptional control.

The rbcS transcription and mRNA accumulation patterns in tomato differ from those in pea and petunia, for which it has been suggested that rbcS gene expression and chloroplast function are tightly coupled (Fluhr and Chua, 1986; Fluhr et al., 1986). In tomato, rbcS transcriptional activities are primarily controlled by developmental and organ-specific programs. Light conditions (or chloroplast function) can modulate the quantitative expression patterns of all five genes in cotyledons and leaves, but light (or chloroplasts) does not activate the transcription of locus 3 genes in fruit to any significant extent. Transcription of rbcS1 and 2 is down regulated prior to chloroplast differentiation, and prior to differentiation of chloroplasts into photosynthetically inactive chromoplasts, providing evidence that rbcS transcription can be uncoupled from plastid differentiation. In dark-grown seedling cotyledons, rbcS1, 2, and 3A are transcribed and all three SSU isoforms accumulate (D. Somers and W. Gruissem, unpublished data), although chloroplast development has not been initiated. Only rbcS3B and 3C are strictly light dependent for transcription activation and coordination with chloroplast development in cotyledons. We conclude, therefore, that rbcS transcription in tomato can be uncoupled from light induction and chloroplast development/differentiation, as well as the presence of photosynthetically active chloroplasts.

Since the first report that light activates transcription of pea rbcS genes (Gallagher and Ellis, 1982), most of the work on regulation of rbcS gene expression in plants has focused on defining sequence elements and protein factors involved in rbcS gene transcription. cis-acting sequences mediating light-responsive and organ-specific expression of rbcS genes have been identified in several plants,
including pea, tobacco, Arabidopsis, and tomato (reviewed in Manzara and Gruissem, 1988; Dean et al., 1989; Gilmartin et al., 1990). In the accompanying report, we showed that differences in the organ-specific rbcS gene transcription pattern can be correlated with specific changes in DNA-protein interactions in the promoter regions of all five genes (Manzara et al., 1991). Most strikingly, we found that in tomato roots and ripe fruit, where we were unable to detect transcription of any of the five rbcS genes (data not shown), DNA-protein interactions do not occur at promoter sites that are generally protected in cotyledons, leaves, or young fruit. The differential transcriptional control is also striking for the tightly linked locus 3 genes in tomato, for which the developmental regulation of rbcS3A transcription in dark-grown cotyledons contrasts with the light-dependent transcriptional regulation of the rbcS3B and 3C genes. It is interesting to note that the rbcS3B and 3C genes share several, but not all, conserved DNA sequence motifs in their promoter regions with the rbcS3A promoter, which may account for their differential regulation (Manzara et al., 1991).

The contribution of post-transcriptional regulation to rbcS gene expression in plants has been less extensively examined. Recent reports have indicated that the expression of individual rbcS genes in soybean, petunia, and Lemna gibba is also regulated by changes in the stability of their corresponding mRNAs (Shirley and Meagher, 1990; Shirley et al., 1990; Silverthorne and Tobin, 1990). Our results demonstrated that the quantitative patterns of rbcS mRNA accumulation in tomato are produced by post-transcriptional regulation of mRNA levels from specific members in the gene family. We found that mRNA stability within the tomato rbcS gene family is altered by organ-specific and developmental programs. rbcS3A mRNA is more stable than rbcS1 mRNA in etiolated seedlings. The stabilities of rbcS1 and rbcS2 mRNAs, but not rbcS3A

Figure 7. Nuclear Run-On Transcription Activities in Developing Fruit.

(A) Run-on transcription assays were carried out in nuclei from 3- to 5-mm green fruit. Labeled transcripts were hybridized to filters containing 2 µg of single-stranded DNA with sequences either complementary to (+) or the same (−) as mRNA, as indicated in the key. Two, 0.2, and 0.02 µg of single-stranded DNA containing sequences complementary to ribosomal RNA were loaded on the filter. Abbreviations for gene probes shown in the key are as in Figure 5; FR5, a cDNA expressed at high levels in immature fruit. The run-on transcript cpm were $1.3 \times 10^8$ in the hybridization solution.

(B) Run-on transcription assays were carried out in nuclei from 5- to 8-mm green fruit. Hybridization conditions and DNA probes on the filter were as described in (A). The run-on transcript cpm were $2.1 \times 10^8$ in the hybridization solution.

(C) Run-on transcription assays were carried out in nuclei from mature green fruit. Hybridization conditions and DNA probes were as described in (A), except that only 2.0 and 0.02 µg of single-stranded DNA containing sequences complementary to ribosomal RNA were loaded on the membrane. The run-on transcript cpm were $4.4 \times 10^7$ in the hybridization solution.

(D) Quantitative results are plotted as cpm hybridizing to each rbcS probe per $1 \times 10^7$ total transcript cpm, as described in Methods. The column for 3- to 5-mm fruit nuclei represents the average of three independent nuclear run-on assays. Columns for 5- to 8-mm fruit and mature green fruit are derived from single experiments.
mRNA, are apparently enhanced in green cotyledons as compared with etiolated cotyledons. Differences in stabilities of rbcS1 and rbcS2 mRNAs were also observed during fruit development. The transcription rates for both rbcS1 and rbcS2 increased approximately threefold during early fruit development, but there was no corresponding increase in mRNA levels. Furthermore, the stability of rbcS2 mRNA was increased relative to rbcS1 mRNA during fruit maturation, as indicated by the rapid decline in rbcS2 transcription that was not accompanied by a similar rapid decline in rbcS2 mRNA. These results, together with two related reports (Shirley et al., 1990; Silverthorne and Tobin, 1990), establish that individual members of the rbcS gene family in plants show different patterns of transcriptional and post-transcriptional regulation.

Sequences in the 3' (and 5') untranslated regions of the tomato rbcS genes are not highly conserved, in contrast to the high degree of sequence similarity in the rbcS coding regions (Sugita et al., 1987). It is therefore possible that RNA sequences in the 3' and 5' untranslated regions could regulate the quantitative accumulation of the five tomato rbcS mRNAs. A recent study of the role of 3' untranslated regions showed that 3' sequences from different genes can alter the expression of a reporter gene by as much as 60-fold (Ingelbrecht et al., 1989), but in that study no distinction was made between effects on transcriptional activity by 3' enhancer elements and mRNA stability.

No information is available on specific cis-elements or trans-acting molecules that regulate cytoplasmic mRNA stability in higher plants. There are no sequence motifs in the 3' untranslated regions of chloroplast rbcL mRNA and other mRNAs. These chloroplast mRNA 3' inverted repeat sequences function as cis-regulatory elements for stability (Stern and Gruissem, 1987; Stern et al., 1991) and interact with specific proteins (Stern et al., 1989). The expression of at least one of the 3' inverted repeat RNA binding proteins (28RNP) correlates with the accumulation of chloroplast mRNAs (Schuster and Gruissem, 1991). Like most cytoplasmic mRNAs, rbcS mRNAs in plants have a 3' poly(A) tail. In animals and yeast, there is evidence for a role of the poly(A) tail in mRNA stability and translation (Jackson and Standart, 1990). Poly(A) tail lengths were not examined for the tomato rbcS mRNAs in this study, but based on the discrete size of tomato rbcS mRNAs on RNA blots, we can exclude substantial length heterogeneity. Poly(A) tail lengths were also found to be similar in two soybean rbcS mRNAs that differ significantly in their stability (Shirley et al., 1990).

Alternatively, the 5' untranslated regions of the tomato rbcS mRNAs could be determinants for mRNA stability. We have shown previously that the sequences in the 5' untranslated regions differ in size from 8 to 75 nucleotides and are not conserved. The length of the 5' untranslated region could influence the rate of translation initiation of rbcS mRNAs, and this in turn may affect their stability. We have found, however, that there is no significant difference in binding of individual rbcS mRNAs to ribosomes in mature leaves (L. Harper and W. Gruissem, unpublished data), suggesting that ribosome binding does not appear to be dependent on the length of the 5' untranslated regions. Similarly, no differences in ribosome binding were detected between two soybean rbcS mRNAs that differ eightfold in their relative stabilities (Shirley et al., 1990). It is also possible that the rate of intron splicing affects the differential rbcS mRNA accumulation. Intron-exon boundaries are conserved in all tomato rbcS genes, however, and only rbcS2 differs from the other four tomato rbcS genes in intron number and structure. We conclude, therefore, that the rbcS 3' untranslated regions are the most likely sites of regulation of the differential rbcS mRNA stabilities in different organs and at different developmental stages.

It is not known whether there is any functional difference between the three different Rubisco SSU protein isofoms encoded by the five tomato rbcS genes. The individual patterns of developmental and organ-specific expression suggest, however, that the multiple rbcS genes in tomato do not serve simply to amplify the expression of their abundant and important protein product in photosynthetic organs. Rather, tomato appears to have evolved precise and multiple mechanisms to coordinate the activity of rbcS genes to maximize expression in different light environments and at different stages in development. These mechanisms operate at both the transcriptional and post-transcriptional levels and are specific for different members within the rbcS gene family.

METHODS

Plant Material

All plant material was from the VFNT cherry tomato cultivar LA 1221. Mature leaf material consisted of leaflets from fully expanded, dark green leaves of 6-week-old to 5-month-old greenhouse-grown plants. Immature leaves were collected as entire light green shoot tips of 0.5 to 1.5 cm in length from plants grown either hydroponically for 3 to 5 weeks, in flats of soil in a greenhouse for 3 weeks, or from 6-week-old to 5-month-old greenhouse-grown plants. Supplemental lighting was provided to greenhouse-grown plants between September and May.

To grow seedlings in the dark, seeds were sterilized for 20 min in 5% bleach and rinsed thoroughly in distilled water. They were then spread one layer thick on three layers of presoaked, sterile Whatman 3MM filter paper in aluminum pans, which were covered with aluminum foil. Seedlings were grown for 7 days at 25°C, after which cotyledons were collected into liquid nitrogen under green safelight. For light induction studies, 7-day-old dark-grown seedlings were exposed to a combination of fluorescent/ incandescent light of 250 µE·m⁻²·sec⁻¹ for 1, 3, or 6 hr, after
which cotyledons were collected into liquid nitrogen. For seedlings grown with a light/dark cycle, sterilized seeds were spread thickly from sepals and frozen in liquid nitrogen. Frozen fruits were later separated by size using sieves with hole diameters of 3, 5, and 8 mm for immature stages. Fruits of less than 3, 3 to 5, and 5 to 8 mm diameter were approximately 3, 5, and 7 days postpollination, respectively. Mature green fruit stages were of the same average diameter (2.5 to 3 cm) as fully mature red fruits but showed no external or internal signs of lycopene accumulation. POLLINATION efficiency in greenhouse-grown plants was increased by gently shaking branches with mature flowers every 3 days; this also served to produce batches of staged fruit. All plant material was harvested between 10:00 AM and 1:00 PM directly into liquid nitrogen and stored at −80°C.

Oligonucleotides

Twenty nucleotide synthetic DNA oligomers used as primers in the PCR synthesis of gene-specific probes were isolated from a 20% acrylamide/7 M urea/TBE gel (Maniatis et al., 1982), eluted from the gel into 0.3 M sodium acetate, desalted on Bio-Gel P6-DG columns (Bio-Rad, Inc.) in water, dried down, and stored dry at −20°C until needed. The sequences of the oligonucleotides used to make rbcS gene-specific probes are found in Table 2.

DNA Probes

The rbcS gene-specific probes used in this study are described in detail in Wanner (1990). Briefly, DNA fragments corresponding to approximately the first 160 nucleotides 3' to the stop codon of each rbcS gene were synthesized using PCR. The 100-μL reaction mixtures contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM MgCl2, 0.02% gelatin, 0.2 mM each dATP, dCTP, dGTP, and TTTP, approximately 200 pmol each of one pair of oligonucleotide primers specific to an rbcS gene (see Table 2), 50 ng of the appropriate rbcS plasmid subclone (or 200 ng of genomic phage clone ϕ17 for rbcS3B), and 3 units of Taq DNA polymerase (Stratagene). Amplification was obtained by 25 cycles of denaturation at 94°C for 2 min, reannealing at 45°C for 2 min, and extension at 72°C for 2 min. Amplified fragments were purified by electrophoresis in a 3% NuSieve agarose (FMC BioProducts)/1% agarose gel in TAE buffer (Maniatis et al., 1982), 5' phosphorylated, and blunt-end ligated into EcoRV cut and dephosphorylated pBluescript KS+ vector (Stratagene). Clones in both orientations were identified by sequencing phagemids containing inserts.

The probe for cytoplasmic rRNA was either pH2A, an 8.7-kb subgenomic DNA clone encompassing the entire rDNA repeat from pea (Jorgenson et al., 1982), or a derivative of pH2A designated pH2A2.3, which contains a 1.2-kb BamHI fragment from the 28S region of the rDNA intron in pH2A2 cloned into the BamHI site of the pBluescript KS+ vector. Cloned genes from an early fruit cDNA library (constructed by Dr. Y. Saltz in the laboratory of W. Gruissem) were used to verify that fruit nuclei actively transcribe RNA other than ribosomal RNA. The cDNA clones used were FR2+ and FR2−, which are opposite orientations of a 750-bp cDNA for a proline-rich protein (Saltz et al., 1991), and FR5+ and FR5−, which are opposite orientations of a 400-bp cDNA for an unknown protein (Y. Saltz and W. Gruissem, unpublished data). Both cDNAs were cloned with EcoRI linkers into the EcoRI site of the pBluescript KS+ vector. Orientation of these pairs of clones was determined by sequencing from both ends. Although both of these cDNA probes showed maximum hybridization to early fruit RNA, neither was completely fruit specific. FR2 hybridizes most highly to a 1.15-kb RNA on blots of RNA from 8- to 10-mm fruit and at 1% to 2% of the maximum fruit level to RNA from leaves (Saltz et al., 1991). FR5+ hybridizes to a 1-kb RNA, with hybridization most strongly to RNA from 4- to 8-mm fruit RNA, and at lower levels to root and leaf RNA (Y. Saltz and W. Gruissem, unpublished data).

Two additional DNA clones were obtained by subcloning the approximately 900-bp PstI fragment from the Arabidopsis type A phytochrome clone pSP18-8 (courtesy of Dr. Peter Quail, UCB-USDA Plant Gene Expression Center, Albany, CA), in both orientations into the PstI site of the plasmid pBluescript KS+. Orientation of the clones was determined by use of an asymmetric HindIII site within the phytochrome insert sequence.

Mapping of Polyadenylation Sites

The 3' ends of each rbcS gene were cloned directly from total leaf RNA by cDNA synthesis using the poly(dT) primer 5'TCTGAGAATTCGGCGGCCGC3', followed by amplification of the rbcS sequences using PCR with DNA primers corresponding to the first 20 nucleotides of the 3' untranslated region of each gene (X-PCR 1 in Table 2) and the complement to the unique sequence of the poly(dT) primer below 5'GGCGGCCGACTTTCTGAG3'. Products of the PCR reaction were isolated from 3% NuSieve agarose/1% agarose gel, phosphorylated, and ligated into either EcoRV-digested (rbcS51, 2, 3A, and 3B) or Smal-digested (rbcS3C), dephosphorylated

<table>
<thead>
<tr>
<th>Table 2. Primer Oligonucleotide DNAs for Synthesis of rbcS Gene-Specific DNA Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligonucleotide</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>X-PCR 1</td>
</tr>
<tr>
<td>X-PCR 2</td>
</tr>
<tr>
<td>X-PCR 3</td>
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<tr>
<td>X-PCR 4</td>
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<td>X-PCR 6</td>
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<td>X-PCR 7</td>
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<td>X-PCR 8</td>
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<td>X-PCR 9</td>
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<td>X-PCR 10</td>
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<tr>
<td>X-PCR 18</td>
</tr>
<tr>
<td>X-PCR 19</td>
</tr>
<tr>
<td>X-PCR 20</td>
</tr>
</tbody>
</table>

*Oligonucleotides designated X PCR-1 and X PCR-2 (X = 1, 2, 3A, 3B, 3C) demarcate the sequence used to synthesize gene-specific PCR DNA fragments and correspond to the first 20 nucleotides of the 3' nontranslated coding strand (X PCR-1) and the complement to a 20-nucleotide sequence approximately 160 nucleotides downstream of the last codon (X PCR-2).
RNA Preparation, Hybridization, and Quantification

Total RNA was prepared from frozen plant material by the method of Prescott and Martin (1987) and quantified by measuring absorbance at 260 nm in triplicate dilutions. For RNA slot blots, total RNA was either diluted into 100 μL of 6 x SSC (1 x SSC is 0.3 M NaCl/0.03 M sodium citrate, pH 7.0) or denatured in 50% formamide/6% formaldehyde/6 x SSC before dilution into 6 x SSC, and then applied to Hybond N membranes under weak vacuum. No differences in hybridization efficiency were detected whether or not the RNA was formamide/formaldehyde-denatured prior to blotting. RNA was fixed to nylon membranes using a Stratalinker (Stratagene) on auto-crosslink.

Slot blots were prehybridized in 0.1 mL/cm² of filter area in 5 x SSC/5% Denhardt’s (Maniatis et al., 1982)/1% sodium sarcosine/10 mM sodium phosphate, pH 7.0/0.1 mg/mL denatured herring sperm DNA for 2 hr at 65°C. Prehybridization solution was replaced with 0.5 volume of the same solution containing 0.5 to 5 x 10⁷ Cerenkov cpm radioactively labeled DNA probe prepared from 1 μg of single-stranded DNA templates as described below. Filters were hybridized for 24 hr at 65°C. After removal of probe solution, filters were washed at room temperature for 5 min in 2 x SSC/0.8% sodium sarcosine, then for 30 min at 65°C in 2 x SSC/0.8% sodium sarcosine, and 45 min at 65°C in 1 x SSC/0.8% sodium sarcoside. Following autoradiography, individual hybridized slots were excised, and relative levels of hybridization were determined by scintillation counting. rbcS mRNA hybridization signals were normalized to hybridization of the random primer-labeled rDNA probe isolated from PHA2.3 to minimize errors in quantification by measurement of absorbance at 260 nm and to account for the much higher contribution of plastid RNA to total RNA in leaves than in other organs.

Preparation of Single-Stranded DNA

M13K07 helper phage was prepared according to Vieira and Messing (1987), except that XL-1 Blue (Stratagene) JM101 were used as host bacterial strains. For preparation of single-stranded DNA, Bluescript phagemids were transformed into competent TG2 or XL-1 Blue host cells. Fifty milliliters of 2x yeast tryptone or Luria broth containing 50 μg/mL ampicillin and 4 x 10⁹ to 4 x 10¹⁰ plaque-forming units of M13K07 were inoculated with a single colony of phagemid and incubated at 37°C with aeration for 2 hr. Kanamycin was added to a final concentration of 70 μg/mL, and phagemids were incubated for 18 hr. Single-stranded DNA was prepared from the supernatant of these cultures by PEG precipitation and phenol extraction (Sambrook et al., 1989), and DNA was quantified by measurement of absorbance at 260 nm (1 OD₂₆₀ = 40 μg/mL single-stranded DNA). The accuracy of quantification and extent of contamination with single-stranded helper phage were monitored by electrophoresis in 1% agarose/TBE gels.

Preparation of Radioactively Labeled DNA Probes

Radioactively labeled DNA probes to specific rbcS genes were prepared from single-stranded pBluescript DNA containing rbcS inserts and the appropriate oligonucleotide primer, shown in Table 3, using standard conditions. Incorporation of α-³²P-dATP was monitored using DE81 paper (Maniatis et al., 1982). The labeled DNA was digested with XhoI, which cleaves in the polylinker near the site of rbcS sequence insertion. The labeled rbcS DNA region was isolated by gel electrophoresis, precipitated with ethanol, and dissolved in hybridization solution.

Preparation of Nuclei

Plant material used for nuclear preparations was collected into liquid nitrogen and stored frozen at ~80°C until needed. Nuclei were prepared as rapidly as possible in a cold room (4°C), and all solutions and filtrates were kept on ice. Two to 10 g of frozen plant material were powdered in a coffee grinder together with a single colony of phagemid and incubated at 37°C with aeration for 2 hr. Kanamycin was added to a final concentration of 40 μg/mL single-stranded DNA. The accuracy of quantification and extent of contamination with single-stranded DNA fragments is shown.

Table 3: Lengths and Theoretical Melting Temperatures (Tₘ) of the rbcS Gene-Specific PCR DNA Fragments

<table>
<thead>
<tr>
<th>3' End Clone</th>
<th>L (bp)</th>
<th>(G + C)%</th>
<th>Tₘ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbcS2 +/-</td>
<td>162</td>
<td>29.0</td>
<td>77.2</td>
</tr>
<tr>
<td>rbcS3A -</td>
<td>164</td>
<td>30.5</td>
<td>77.8</td>
</tr>
<tr>
<td>rbcS3A +</td>
<td>134</td>
<td>32.8</td>
<td>77.8</td>
</tr>
<tr>
<td>rbcS3B +/-</td>
<td>157</td>
<td>28.7</td>
<td>76.9</td>
</tr>
<tr>
<td>rbcS3C +/-</td>
<td>134</td>
<td>28.3</td>
<td>76.0</td>
</tr>
<tr>
<td>137</td>
<td>28.5</td>
<td>76.2</td>
<td></td>
</tr>
</tbody>
</table>

*+, a clone from which the single-stranded DNA produced will be the antisense strand; -, a clone from which the single-stranded DNA produced will be the sense strand.

L, number of base pairs in the homologous sequence.

Tₘ (in degrees centigrade), 69.3 ± 0.41 (G + C)% - 650/L.

rbcS3C has two polyadenylation sites 3 nucleotides apart; Tₘ for both DNA fragments is shown.
Quantification of DNA in Nuclei

Duplicate 10-µL aliquots of resuspended nuclei were added to 290 µL of 10 mM Tris-HCl, pH 8.0/1 mM EDTA. A 100-µL aliquot of 0.2 M Tris-HCl, pH 8.0/2 M NaCl/100 mM EDTA/2% sodium sarcosine and 2 µL of 10 mg/mL heat-treated RNase A were added, and nuclei were incubated at room temperature for 60 min. Ten microliters of 10 mg/mL proteinase K were added for a further incubation at 37°C for 1 hr. The digest was then extracted once with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), and a second time with chloroform:isoamyl alcohol (24:1), and DNA was precipitated by 2 volumes of 95% ethanol. The DNA pellet was washed with 70% ethanol, dried briefly, and dissolved in 0.5 mL of 10 mM Tris-HCl, pH 8.0/10 mM EDTA. DNA concentrations were calculated from the absorbance measured at 260 nm.

Nuclear Run-On Transcription Assay

Typically, the DNA concentration in nuclear preparations was 0.5 to 1.0 mg/mL. To reinitiate transcription, 2 mL of RNasin (Promega, 40 units/mL), and 250 µCi (for leaf) or 1 mCi (for fruit) α-32P-UTP (3000 Ci/mmol) dissolved in one-quarter final volume 10 mM Tris-HCl, pH 8.0/375 mM KCl/25 mM MgCl2/1 mM EDTA/5 mM dithiothreitol/5 mM ATP/2.5 mM GTP/2.5 mM CTP/0.05 mM UTP. Transcription reactions were incubated at 25°C for 20 min. To terminate the transcription reaction, CaCl2, proteinase K, and RNase-free DNase I (Bethesda Research Laboratories, 2 µg/250 µL) were added to a final concentration of 2 to 3 mm, 0.02 µg/µL, and 40 mg/mL, respectively, and the mixture was incubated at 37°C for 30 min. An equal volume of 0.1 M Tris-HCl, pH 8.0/0.05 M EDTA/1 M NaCl/2% sodium sarcosine, and 0.25 mg/mL proteinase K was added and incubation was continued for 30 min at 37°C. The nuclear lysate was extracted once with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). Two micrograms of RNA as carrier were added, and transcripts were precipitated by addition of 2 volumes of 95% ethanol. The pellet was washed with 70% ethanol and dried. Transcripts, dissolved in 100 µL of 10 mM NaCl/10 mM Tris-HCl, pH 8.0/1 mM EDTA, were chromatographed on a Sephadex G-50 spin column (Maniatis et al., 1982) to remove unincorporated α-32P-UTP. Incorporation of α-32P-UTP into run-on transcripts was estimated from duplicate aliquots of the aqueous phase after phenol/chloroform extraction by binding to DE81 filter discs (Maniatis et al., 1982). Typical incorporation was 2 to 10 x 10^6 cpm/µg of DNA. The rate of UTP incorporation was approximately 0.1 pmol/µg of DNA/min, which is comparable with the rate of 0.1 to 0.2 pmol of GTP incorporated/µg of DNA/min in mammalian cell nuclei reported by Marzluff and Huang (1985).

The tomato nuclear run-on transcription system has similar monovalent and divalent cation optima and sensitivity to inhibition by α-amanitin (Wanner, 1990), as previously established run-on transcription systems from many tissues, including other plants (Gallagher and Ellis, 1982; Marzluff and Huang, 1985; Walling et al., 1986).

Run-On Transcript Hybridization and Quantification

Membrane filters containing rbcS DNA probes were prepared by slot-blotting 2 µg of sense and antisense single-stranded DNA for each rbcS transcript onto Hybond N in a volume of 100 µL of 6 x SSC. Slots were rinsed with an additional 200 µL of 6 x SSC, and DNA was cross-linked using a Stratalinker. Slots were prehybridized in 0.1 mL of hybridization solution/cm² of filter area for 6 hr at 65°C. Hybridization solution was 5 x SSC (Maniatis et al., 1982)/5 x Denhardt’s (Maniatis et al., 1982)/1% sodium sarcosine/10 mm sodium or potassium phosphate, pH 7/0.1 mg/mL sheared, denatured salmon or herring sperm DNA. Prehybridization solution was replaced with hybridization solution containing run-on transcripts, and hybridization was continued at 65°C for 24 hr. Hybridized filters were rinsed in 2 x SSC/0.8% sodium sarcosine at room temperature for 5 min, followed by washes in 2 x SSC/0.8% sodium sarcosine at 65°C for 30 min, and 1 x SSC/0.4% sodium sarcosine at 65°C for 45 min.

Quantitative results of run-on transcription assays were obtained by scintillation counting of individual hybridized slots. The hybridization to the (+)DNA strand rbcS3 probes was corrected for hybridization to the (−)DNA strand rbcS3 probes, except in fruit, where significant hybridization to the (−)DNA strands of rbcS3A and 3B was seen. Transcription data are expressed as cpm hybridizing to rbcS or other gene probes per 10^7 transcript cpm to adjust for differences in transcript specific activities between experiments. This method of quantifying run-on transcripts resulted in reproducible numbers of transcript cpm from a particular organ. The variability between experiments was generally less than 10%.

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