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Developmental, organ-specific, and light-dependent expression of the tomato ribulose-1,5-bisphosphate carboxylase small subunit gene family

(multigene family/differential expression)

MAMORU SUGITA* AND WILHELM GRUISSEM†

Department of Botany, University of California, Berkeley, CA 94720

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ABSTRACT **The tomato gene family for the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase [3-phospho-D-glycerate carboxy-lyase (dimerizing); EC 4.1.1.39] has five genes, designated *Rbcs-1*, *-2*, *-3A*, *-3B*, and *-3C*. We have measured the steady-state mRNA levels for each of the five genes in various tomato organs using gene-specific oligonucleotides. All five genes are highly expressed in leaves, and transcripts of two genes, *Rbcs-3B* and *Rbcs-3C*, account for ≈60% of the total leaf transcripts. The relative transcript levels in the stem, immature fruits, and etiolated seedlings (plants germinated and grown in the dark) correspond to 3.2%, 6.5%, and 4.6%, respectively, of the ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit mRNA level in leaves, and no transcripts have been detected in roots and ripe tomato fruits. Only *Rbcs-1* and *Rbcs-2* are expressed during the photosynthetically active phase of fruit development. Transcripts from these genes and from the *Rbcs-3A* locus are also present in etiolated seedlings. *Rbcs-3B* and *Rbcs-3C* transcripts, which are the most abundant mRNAs of the ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene family in the leaf, are undetectable in dark-grown seedlings and immature fruit. The selective expression of *Rbcs-1* and *Rbcs-2* in the dark and in the pericarp of green fruit and the induction and rapid mRNA accumulation for *Rbcs-3B* and *Rbcs-3C* after illumination may reflect different regulatory mechanism(s) that control the expression of individual members in the tomato ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene family.**

The synthesis of ribulose-1,5-bisphosphate carboxylase [RbuP_2Case; 3-phospho-D-glycerate carboxy-lyase (dimerizing); EC 4.1.1.39] in higher plants requires the coordinate expression of the nuclear genes for the small subunit of RbuP_2Case (*rbcS*) and the plastid gene for the large subunit of RbuP_2Case (*rbcL*) of the enzyme complex. The small subunit of RbuP_2Case is encoded by a multigene family of several members in higher plants (1–6), whereas the large subunit is encoded by a single gene in the chloroplast genome, which is present from a few to several hundred copies in the cell (7–9). The accumulation of RbuP_2Case small and large subunit mRNAs and/or proteins is modulated by light in several plant species (10–12). During the light-induced development of chloroplasts in seedlings, the *rbcL*-encoded mRNA level is primarily controlled at the posttranscriptional level (13), but large subunit synthesis can also be rapidly adjusted at the translational level (14). In contrast, transcriptional run-on experiments with isolated nuclei from *Lemna gibba*, pea, and soybean have demonstrated that the light-induced increase in *rbcS*-encoded mRNA levels is most likely the result of transcriptional activation of the *rbcS* genes, placing the primary mode of regulation for mRNA accumulation at the transcriptional level (15–17). The mutational analysis of *rbcS* genes in transformed plants has demonstrated that cis-acting DNA sequences in their 5′ regions are required to direct the light response at the transcriptional level (18). Individual members of the petunia and pea *rbcS* families that have been analyzed at the mRNA level are all expressed in organs containing chloroplasts, but their activity can vary (19, 20). The *rbcS* genes are not, however, expressed in roots (19, 20) or in ripe tomato fruits (21), organs that contain amyloplasts and chromoplasts, respectively. Except for the light-regulated cis-acting DNA sequences, no information is currently available for the mechanism(s) that control the developmental and/or light-dependent expression of *rbcS* genes during plastid differentiation in organs other than leaves.

During tomato fruit development and ripening, *rbcS* genes are expressed in the green pericarp tissue of developing fruit, most notably between 7 and 14 days after pollination, but the mRNAs are undetectable at the time of chromoplast differentiation (21). The *rbcS* mRNA levels in the pericarp tissue of immature fruit are significantly reduced as compared to leaf, however, even though the pericarp is morphologically related to leaves and the chloroplasts in this tissue are comparable in their photosynthetic function (22). In tomato, a significant *rbcS* mRNA level can also be detected in etiolated seedlings (21), which supports earlier reports of a comparable accumulation of this mRNA in dark-grown cotyledons of Amaranth (10).

We have shown that the tomato *rbcS* genes are encoded by a small nuclear gene family of five members (5, 23). Three of the genes (*Rbcs-3A*, *Rbcs-3B*, and *Rbcs-3C*) form a subfamily on chromosome 2, whereas *Rbcs-1* and *Rbcs-2* are located on chromosomes 2 and 3, respectively (24). The reduced *rbcS* mRNA levels in the tomato pericarp tissue and the accumulation in etiolated seedlings could result from differential expression of some members of the *rbcS* gene family, or result from a general decrease in the expression level of all five genes. As a first step in dissecting the molecular mechanisms that control the developmental and/or light-dependent transcription of the tomato *rbcS* gene family during plastid differentiation in seedlings, leaves, and fruit, we have analyzed the *in vivo* expression of all five members at the mRNA level. We report here that the expression of the *rbcS* gene family in tomato is primarily and differentially controlled by light in leaves, but this expression appears to be under organ-specific and/or developmental control in the fruit pericarp tissue and etiolated seedlings.

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Abbreviations: RbuP_2Case, ribulose-1,5-bisphosphate carboxylase; *rbcS*, genes encoding the RbuP_2Case small subunit; *rbcL*, gene encoding the RbuP_2Case large subunit.

*Present address: Department of Botany, Hokkaido University, Sapporo 060, Japan.
†To whom reprint requests should be addressed.

MATERIALS AND METHODS

Plant Material. Tomato plants, *Lycopersicon esculentum* VFNT LA1221 line, were grown under greenhouse conditions. Leaves and stems were harvested from 4- or 6-week-old plants. Immature leaves are defined as small, light-green leaves located at the tip of the tomato compound leaf. Immature, mature, and red fruits were harvested 14 days, 35 days, and 48 days, respectively, after pollination, and their pericarp tissues were isolated. Roots were harvested from 4-week-old hydroponically grown tomato plants. Seeds were germinated and grown for 7 days in absolute darkness to harvest etiolated seedlings.

DNA Clones. DNA fragments containing the tomato *rbcS* genes were isolated from tomato genomic libraries and subcloned into pUC19 and pUC119 as described (5). The DNA fragments used in this study are as follows: 1.5-kilobase (kb) *Sau*3A fragment (*Rbcs-1*); 2-kb *Eco*RI and 3.8-kb *Hin*dIII–*Eco*RI fragments, which overlap by 501 base pairs (bp) (*Rbsc-2*); 1.3-kb *Sau*3A fragment (*Rbcs-3A*); 2.9-kb *Hin*dIII–*Sau*3A fragment (*Rbcs-3B*); and 1.75-kb *Sau*3A fragment (*Rbcs-3C*). These DNA fragments contain the entire coding regions and 5′ and 3′ flanking sequences of the *rbcS* genes as described (5). The plasmid pTB1 contains a 1.2-kb *Bam*HI fragment of tobacco chloroplast DNA that contains 90% of the entire coding sequence of the *rbcL* gene (25).

Oligonucleotide Probes. The small subunit coding probe for detection of all *rbcS* transcripts is 5′ GTTGCATCAGTGCACCC 3′, which is a conserved DNA sequence of all five genes (5). The tomato *rbcS* gene-specific probes are as follows: 5′ CATTTTGATTATATGTTTGG 3′ (*Rbcs-1*); 5′ CAATTTTGTTCCTAATATGA3′(*Rbcs-2*);5′GCAATTAGTTTTTCTAGAAC 3′ (*Rbcs-3A*); 5′ GTCAGTTATACATTACATAA 3′ (*Rbcs-3B*); and 5′ TCACTGTTGACATATACACA 3′ (*Rbcs-3C*). Synthetic oligonucleotides were prepared as described previously (26). Probes were labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (27) and separated on a 20% polyacrylamide gel to determine the specific activity of each labeled probe. Unlabeled oligonucleotides were added to adjust the specific activities (0.5–1.8 × 10^6 cpm/pmol of probe), and the specific activities of the six oligonucleotides were the same for separate hybridization experiments. The relative hybridization efficiencies of the oligonucleotides were determined using gene-specific probes as described in Fig. 1.

RNA Blot Analysis and Quantitation of *RbcS* Transcripts. Total RNA was prepared from various tomato organs as described (21). Total RNA (20 μg) was separated in a 1.2% agarose gel containing formaldehyde, followed by transfer to nylon filters (Hybond, Amersham) for ≥20 hr. Filters were incubated for 5 hr in 0.9 M NaCl/50 mM NaH_2PO_4, pH 7.4/5 mM EDTA/0.1% $NaDodSO_4$/100 μg of denatured salmon sperm DNA per ml/5× Denhardt's solution (16) at 40°C (*Rbcs-1*, *-2*, and *-3B*), 42°C (*Rbcs-3A*), or at 44°C (*Rbcs-3C* and the small subunit coding probe) as described (28). End-labeled oligonucleotide probes (50 pmol) were added and hybridized for 20–48 hr. After hybridization, filters were washed twice at room temperature for 15 min in 6× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0) and then at 5°C above hybridization temperature for 30 min in 6× SSC. For detection of *rbcL* mRNA, a nick-translated 1.2-kb insert DNA of pTB1 was used in RNA hybridization experiments as described (21). After exposure of the filters to x-ray film, the films were scanned densitometrically to measure the *rbcS* transcript for each gene. Autoradiograms shown are the representative results of at least three independent RNA extraction and hybridization experiments.

RESULTS

Location of Tomato *RbcS* Gene-Specific Probes. The tomato *rbcS* gene family has five members (5), which, based on their chromosomal location, gene structure, and the deduced mature polypeptide sequences, have been assigned to three groups, *RbcS-1*, *RbcS-2*, and *RbcS-3* (5, 24, 29). The nucleotide sequences among the three groups diverge by 10–14% in the protein-coding region, and a number of nucleotide changes results in the replacement of a few amino acid residues in deduced mature polypeptide sequences. The three genes in the *Rbcs-3* locus have identical (3A and 3C) or nearly identical (3B and 3A or 3C) DNA sequences in the protein-coding regions. To measure the steady-state level of total mRNA for all *rbcS* genes, we took advantage of the homology in the protein-coding region to construct an oligonucleotide probe complementary to the mRNAs of all five genes described. In addition, we constructed gene-specific oligonucleotide probes to measure the mRNA level for each member in the *rbcS* gene family. Because tomato *rbcS* 5′ untranslated mRNA sequences vary in length from 8 to 75 nucleotides among the five genes and have moderate homology (5), we chose 20-base nucleotide sequences complementary to the 3′ nontranslated regions of the *rbcS* mRNAs. The sequences of the oligonucleotide probes are positioned within 35 nucleotides 3′ of the TAA stop codon and are within the transcribed 3′ portions of the genes (data not shown). Fig. 1 shows that the five gene-specific probes specifically hybridized to their respective gene and did not cross-hybridize with the other genes; hybridization efficiency was similar for all five oligonucleotides. In contrast, the coding probe, which has a sequence common to the protein-coding regions of all five genes, hybridized equally well to the insert DNAs of all clones. Thus the gene-specific oligonucleotide probes could be applied to detect and measure individual *rbcS* transcripts for each member of tomato *rbcS* multigene family.

Expression Levels of Total *rbcS* mRNA in Different Tomato Organs. To compare the steady-state mRNA levels of all five *rbcS* genes in different tomato organs, total RNAs isolated from these organs were subjected to RNA analysis using the *rbcS* coding probe (Fig. 2 and Table 1). The coding probe hybridized to a single RNA species of ≈0.8 kb. The *rbcS* mRNA transcripts were most abundant in leaves and were significantly lower (3.2%) in stem relative to leaves. The *rbcS* mRNA level increases during the early stages of fruit development (21); however, the maximum level of *rbcS* transcripts in immature fruit was only 6.5% of leaf transcript level. *RbcS* transcripts were undetectable in red fruit and root. In etiolated seedlings germinated and grown in the dark, the

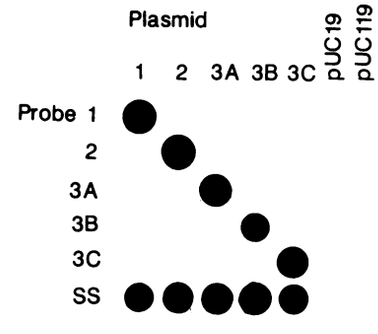

FIG. 1. Hybridization of *rbcS* 3′ gene-specific and coding region oligonucleotide probes with the five tomato *rbcS* genes. Equal moles (0.3 pmol, 0.8–1.4 μg of DNA) of plasmid DNAs containing the *rbcS* genes (1, 2, 3A, 3B, and 3C) and vector DNAs pUC19 and pUC119 were dot-blotted on nylon filter and hybridized with each gene-specific probe (1, 2, 3A, 3B, and 3C) or the coding probe (SS), which were labeled to the same specific activity. Hybridization and washing conditions were the same as described for the RNA analysis.

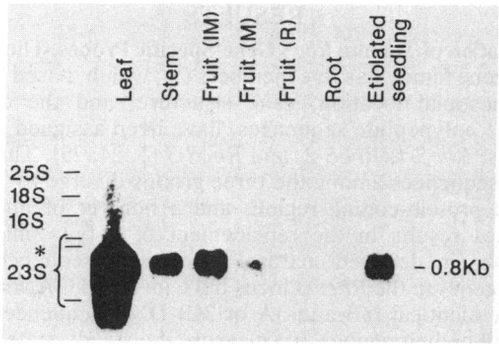

FIG. 2. Quantitative RNA hybridization analysis of total *rbcS* transcript levels in different tomato organs. Twenty micrograms of total RNA was fractionated in a 1.2% formaldehyde-agarose gel, blotted onto nylon filter, and hybridized with the tomato *rbcS* coding-region oligonucleotide probe. Positions of tomato cytoplasmic ribosomal RNA (25S and 18S) and chloroplast ribosomal RNA (16S) are indicated. Chloroplast 23S RNA (*) is present as a consistent pattern of smaller distinct RNA molecules. Relative amounts of *rbcS* transcript levels are summarized in Table 1.

steady-state *rbcS* mRNA level accumulated to 4.6% of the level in leaves.

Differential and Organ-Specific Accumulation of Individual Tomato *RbcS* mRNAs. We determined the relative amount of each mRNA in different tomato organs using the five gene-specific probes. *Rbcs-3B* and *Rbcs-3C* probes hybridized to 60% of the total leaf transcripts (Fig. 3 and Table 2). The transcripts of the three locus 3 genes account for 75% of the total *rbcS* mRNA level in mature leaves, but only account for 60% in immature leaves. The increase in mRNA level from this locus is primarily contributed by *Rbcs-3C*, which is characterized by a 2-fold increase in its mRNA level during leaf maturation. Expression levels of *Rbcs-3A* and *Rbcs-3B* remain nearly constant during this period, whereas the relative mRNA level of the *Rbcs-1* and *Rbcs-2* genes is reduced in mature leaves (Table 2). This result demonstrates that, in addition to light, the developmental stage of the tomato leaf has a fundamental effect on the relative expression of the genes in the *rbcS* family. All genes are expressed at a low level in stem, but *Rbcs-3B* contributes nearly half of the total *rbcS* transcript level in this organ, and transcript levels are significantly reduced for *Rbcs-3A* and *Rbcs-3C*. More interestingly, only *Rbcs-1* and *Rbcs-2* transcripts are detectable in immature tomato fruit, although the pericarp tissue is photosynthetically active, and all photosynthetic parameters of the chloroplasts in this tissue are comparable to those in leaves (22). The relative ratio of mRNA levels from these two genes found in leaves and stem is also maintained in the pericarp tissue of the fruit. No *rbcS* transcript could be detected in tomato root by either the five gene-specific oligonucleotide probes or the coding region probe.

Table 1. Distribution of total *rbcS* transcripts in tomato organs relative to the *rbcS* transcript level in fully expanded (mature) tomato leaves

Tomato organ RNA	*rbcS* transcript level, %
Leaves	100
Stem	3.2
Fruit, immature	6.5
Fruit, mature	1.6
Fruit, red	ND
Root	ND
Etiolated seedlings	4.6

Distribution and relative transcript levels of the 0.8-kb *rbcS* transcripts detected by RNA analysis were measured by densitometric tracing of autoradiograms exposed for various times. Percent values are the average of three to five experiments with RNA from different isolations. Total leaf RNA (20, 2, and 0.5 μg) was used to estimate the relative level of *rbcS* transcripts in other tomato organs. Representative results of the corresponding RNA analysis are shown in Fig. 2. ND, no *rbcS* transcript was detected.

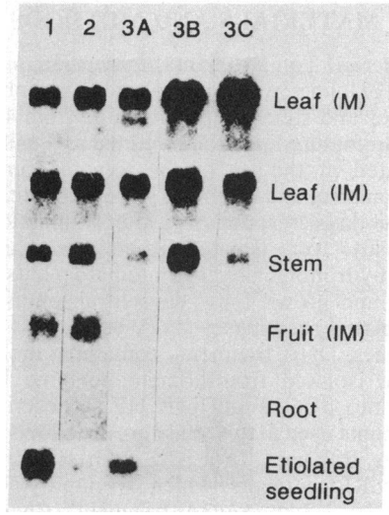

FIG. 3. Relative distribution of all five *rbcS* transcripts in different tomato organs. Total RNAs (20 μg) isolated from fully expanded (M, mature) and immature leaves (IM) of 4-week-old tomato plants—stem, root, immature fruit, and etiolated seedlings—were fractionated in 1.2% formaldehyde-agarose gels and blotted onto nylon filter. Filters were hybridized with the gene-specific 3′ oligonucleotide probes, labeled to the same specific activity. Relative amounts of specific *rbcS* transcript levels are summarized in Table 2.

Light-Control of Differential *RbcS* Gene Expression. *Rbcs-1*, *Rbcs-2*, and *Rbcs-3A* genes are selectively expressed in etiolated seedlings, with mRNAs from *Rbcs-1* accounting for 75% of the total *rbcS* transcript level at this developmental stage. *Rbcs-3B* and *Rbcs-3C* transcripts are undetectable in etiolated seedlings (Fig. 3). This suggests that the response of *rbcS* gene expression to light differs for each individual member of the tomato *rbcS* gene family. To substantiate this conclusion, the accumulation patterns of *rbcS* mRNAs were followed for each gene during the light-induced greening of etiolated tomato seedlings. As shown in Fig. 4, *Rbcs-1*, *Rbcs-2*, and *Rbcs-3A* transcripts accumulated as much as 2-fold over the etiolated seedling transcript level after 3 hr of greening, followed by an additional 2-fold increase during the next 6 hr of greening. In contrast, *Rbcs-3B* and *Rbcs-3C* transcripts are detectable only at a significantly lower level, even after the first 3-hr greening period, but then showed a rapid 6- to 8-fold increase between 3 and 9 hr of illumination

Table 2. Relative levels of specific transcripts for each member of the tomato *rbcS* gene family in different organs

Tomato organ RNA	Specific *rbcS* transcript level, %				
	-1	*-2*	*-3A*	*-3B*	*-3C*
Leaves	10	15	11	29	35
Immature leaves	14	25	13	32	16
Stem	18	21	7	45	9
Immature fruit	40	60	ND	ND	ND
Root	ND	ND	ND	ND	ND
Etiolated seedlings	76	6	18	ND	ND

Distribution and relative transcript levels for each individual gene were determined by RNA analysis and densitometric tracing as described in Table 1. Numbers are averages of three to five experiments with gene-specific oligonucleotides. Representative results of the corresponding RNA analysis are shown in Fig. 3. ND, no *rbcS* transcript was detected.

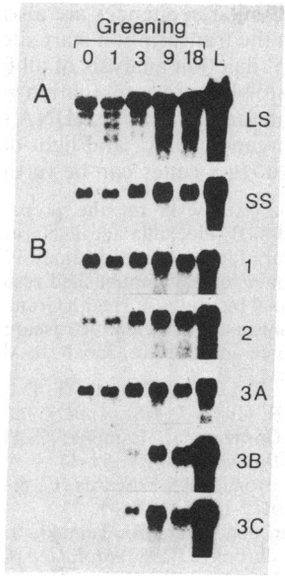

FIG. 4. Light-dependent accumulation of *rbcS* transcripts in etiolated tomato seedlings. Tomato seedlings grown for 7 days in the dark (0 hr) were exposed to white light for different times (1, 3, 9, and 18 hr) before RNA isolation. Total RNAs (20 μg) from the seedlings were hybridized (*A*) with the *rbcL* probe (LS) and tomato *rbcS*-coding region oligonucleotide probe (SS), and (*B*) with the five *rbcS* gene-specific 3′ oligonucleotide probes. Total leaf RNA (L) from 4-week-old plants is shown as a control.

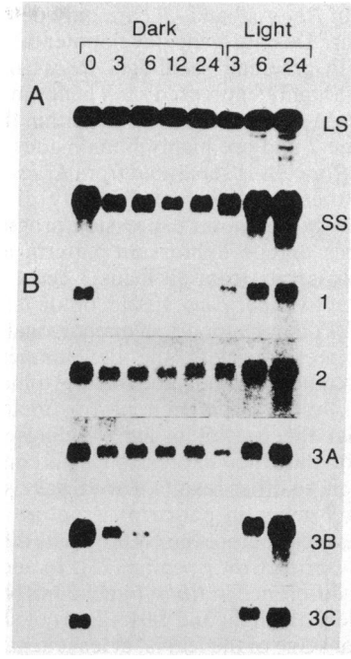

FIG. 5. Modulation of tomato *rbcS* transcript levels in light/dark-shifted tomato plants. Tomato plants were grown in the greenhouse for 6 weeks. At 10:00 AM plants were transferred to absolute darkness for 24 hr, after which they were transferred back to the greenhouse for 24 hr. Leaves were harvested 0, 3, 6, 12, and 24 hr after transfer of the plants to the dark and 3, 6, and 24 hr after transfer back to the greenhouse. Total RNAs (10 μg) were hybridized (*A*) with the *rbcL* probe (LS) and the *rbcS*-coding region oligonucleotide probe (SS), and (*B*) with the five *rbcS* gene-specific 3′ oligonucleotide probes.

with white light (Fig. 4). This result indicates that members of the tomato *rbcS* gene family can be assigned to at least two groups based on the differential, light-induced accumulation ratio of their transcripts in greening seedlings.

Although the *Rbcs-1* transcript dominates in etiolated seedlings, its level decreases significantly in mature leaves relative to transcripts from *Rbcs-3B* and *Rbcs-3C* (Fig. 3). Together with the results shown in Fig. 4, this indicates that the basal level of transcripts from *Rbcs-1*, *-2*, and *-3A* in tomato may be established by a developmental program. Our hypothesis is that this basal level of transcription can be modulated by light, whereas the expression of *Rbcs-3B* and *Rbcs-3C* is strictly dependent on light. To test this hypothesis, greenhouse-grown tomato plants were transferred to absolute darkness, and the level of each *rbcS* transcript was monitored over a 24-hr period. The *rbcS* transcript level in mature leaves rapidly decreased after 3 hr to <25% of the leaf transcript level in the light (Fig. 5*A*). This decrease is marked by the rapid decline of transcript levels for all five genes (Fig. 5*B*). Whereas transcripts from *Rbcs-1*, *-3B*, and *-3C* decreased to nondetectable levels after 12 hr in the dark, *Rbcs-2* and *Rbcs-3A* transcripts declined to 33% and 40%, respectively, of their light control level in the leaf after 3 hr. This level was approximately maintained throughout the remainder of the 24-hr dark period. Upon transfer of the plants into light, the transcripts of all five *rbcS* genes showed a rapid accumulation, with restoration to their light control level within 6 hr. In contrast to the stringent light regulation of the tomato *rbcS* gene family in leaves, the chloroplast *rbcL* transcript levels are less affected (Fig. 5*A*) and show a decrease of <2-fold over their light control level.

DISCUSSION

The comprehensive analysis of mRNA levels for the complete tomato *rbcS* gene family in different organs demonstrates that all members are expressed in leaves but show a differential and unique expression pattern in other tomato organs. This analysis was possible with the construction of gene-specific probes of equal length (20 nucleotides) and similar G + C content (25–35%) to distinguish individual *rbcS* transcripts. Because the oligonucleotide probes were labeled to similar specific activities and hybridized with nearly identical efficiencies, the hybridization results also provided a relatively accurate measurement of the mRNA levels for each gene. In our expression studies we were therefore able to correlate the individual mRNA level for each member of the *rbcS* gene family with four different plastid types—namely, chloroplasts in leaves, stem, and immature fruit; chromoplasts in mature fruit; amyloplasts in root; and etioplasts in dark-grown seedlings.

From these expression studies we conclude that the *rbcS* locus 3 genes account for 75% of the total leaf *rbcS* transcripts. Difference in the relative amount of transcript is <4-fold between the tomato *rbcS* genes that are expressed at the highest (*Rbcs-3C*) and lowest level (*Rbcs-1*) in this organ. This contrasts with the differences in transcript levels of *rbcS* genes in petunia leaves, estimated as great as 25-fold for individual members in this plant (19). Differences in transcript levels have also been reported for several genes in the pea and maize *rbcS* families (20, 30). In tomato, the *rbcS* transcript level is significantly reduced in stem as compared to immature and mature leaves, but all genes are expressed. In contrast, locus 1 and 2 genes are expressed in the pericarp tissue of tomato fruit, but no transcripts are detected from any of the locus 3 genes. This is an interesting observation, because chloroplasts in the pericarp tissue of developing tomato fruit show similar photosynthetic function, with ≈35% of the RbuP_2Case activity and 41% of photosynthetic electron-transport capacity of leaf tissue (22).

Of the five tomato *rbcS* genes, *Rbcs-1*, *-2*, and *-3A* are also expressed in etiolated seedlings. During greening of the etiolated seedlings in continuous white light, transcripts from these three genes start to accumulate rapidly, whereas

transcripts from *Rbcs-3B* and *-3C* are only detectable at low levels after 3 hr. Despite their developmental lag in mRNA accumulation in greening seedlings, *Rbcs-3B* and *-3C* are finally the most highly expressed *rbcS* genes in leaves. Thus, although *Rbcs-3A*, *-3B*, and *-3C* reside within the same locus on chromosome 2 and are highly homologous in their DNA sequence and *Rbcs-3A* is separated from *Rbcs-3B* and *-3C* by only ≈5 kb, the genes are significantly distinct in their developmental and temporal expression program.

Based on this unique expression pattern, as well as the absence of transcripts from all locus-3 genes in the photosynthetically active pericarp tissue of developing tomato fruit, our results do not support an unequivocal correlation of *rbcS* gene expression with the developmental stage of chloroplasts. Rather, we conclude that the tomato *rbcS* genes are controlled by developmental and/or organ-specific programs and that the prevailing light regimes in leaves and cotyledons modulate their expression. This conclusion is also supported by three other results. (*i*) We have shown that the expressed *rbcS* genes in pericarp tissue are inactivated in light at least 2 weeks before the chloroplasts differentiate into chromoplasts during fruit ripening (21). In addition, we find no significant difference in *Rbcs-1* and *-2* mRNA levels in the pericarp tissue in the light and dark (data not shown). (*ii*) The developmental stage of the leaf is, at least in part, responsible for the accumulation of mRNAs from individual *rbcS* genes. Although *Rbcs-1*, *-2*, *-3A*, and *-3B* have reached nearly their full relative expression level in immature leaves, *Rbcs-3C* shows the highest relative transcript level only in mature leaves. (*iii*) Leaf transcript levels for all five tomato *rbcS* genes decrease rapidly after the plants are transferred to absolute darkness. However, transcripts from *Rbcs-2* and *-3A* only decrease to relative basal levels, which are also detected in etiolated seedlings, whereas transcripts from *Rbcs-1*, *-3B*, and *-3C* decrease to undetectable levels after 12 hr. The expression pattern of the tomato *rbcS* genes allows us, therefore, to separate the multigene family, with the possible exception of *Rbcs-3A*, into two groups that differ in their developmental and/or light-dependent control mechanisms. Although light modulation of the developmental expression program appears to be the prevailing regulatory mechanism for the tomato *rbcS* genes in leaves, *Rbcs-1* may be an exception to this rule. We find that *Rbcs-1* is expressed at high levels in etiolated cotyledons, but the mRNA decreases to nondetectable levels in mature leaves after transfer to the dark. This indicates that, in addition to the programmed development and light modulation, the light-activated leaf development alters developmentally programmed *Rbcs-1* gene expression in cotyledons.

Our hybridization studies do not exclude that the observed differences in the transcript levels of the five tomato *rbcS* genes result from differential stabilities of the individual *rbcS* mRNAs. Several lines of experimental evidence support the conclusion, however, that the differential expression pattern of *rbcS* genes in higher plants is most likely controlled at the transcriptional level. First, transcription run-on experiments with nuclei isolated from etiolated and greening pea seedlings have demonstrated the increased transcriptional activity of *rbcS* genes in the light (16). Nuclei isolated from tomato leaves are active in the transcription of *rbcS* genes, but no *rbcS* transcripts have been detected in run-on experiments with root nuclei (L. Wanner and W. Gruissem, unpublished results). Second, the analysis of chimeric constructs of *rbcS* 5′ upstream regions with bacterial coding sequences in transgenic plants has revealed DNA sequences around the TATA box region and an enhancer-like sequence further upstream as cis-acting elements that are required for light-inducibility (18). Similar sequences are also present 140–160 bp upstream from the transcription start sites of tomato *rbcS* genes (5). Using 5′ deletion analysis of all five *rbcS* genes in transformed tomato plants in combination with DNA-binding protein assays, the function of these DNA sequences for the developmental, organ-specific, and light-dependent expression of the tomato *rbcS* genes can be further addressed.

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