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Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit development and ripening

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Summary

The expression of plastid and nuclear genes coding for photosynthesis-specific proteins has been studied during tomato fruit formation. The steady-state transcript levels for the large (*rbc*L) and small (*rbc*S) subunit of RuBPC/Oase, as well as the thylakoid membrane proteins, the 32 kD Q_B-binding protein of PS II (*psb*A), the P700 reaction center protein of PS I (*psa*A) and the chlorophyll a/b-binding protein (*cab*) vary at different time points during fruit development and ripening. Messenger RNA levels of plastid-encoded photosynthesis-specific genes (*rbc*L, *psb*A) are at least several fold higher, relative to respective nuclear-encoded genes (*rbc*S, *cab*). The transcript levels for the large and small subunit of RuBPC/Oase are highest in approximately 14-day-old tomato fruits, while the chl a/b-binding protein, the P700 reaction center protein and the 32 kD Q_B-binding protein reach their maxima in approximately 7-, 14- and 25-day-old tomato fruits, respectively. The inactivation of the photosynthesis-specific genes occurs during the first period of fruit formation. In addition, there is considerable variation in the mRNA levels of these photosynthesis-specific genes in four organs of tomato (leaves, fruits, stems, roots).

Introduction

The development and ripening of tomato fruit is characterized by a number of morphological and physiological changes. These include the increase in fruit size due to cell division during the first 10 days after pollination, and subsequent cell enlargement to the mature size of the fruit (8). Based on measurements of photosynthetic activity in mature green fruit (34) it is most likely that during fruit development chloroplasts in the pericarp tissue are photosynthetically active. At the onset of fruit ripening chlorophyll and the thylakoid membrane system disappear rapidly and lycopene starts to accumulate as a major carotenoid in the differentiating chloroplast (3, 17, 18, 37). This is accompanied by a decrease in the level of mRNA for several chloroplast-encoded, photosynthesis-specific polypeptides (33), although the chromoplast in the red fruit retains the DNA complement present in the chloroplast (22, 33).

Chloroplast development requires a coordinated expression of photosynthesis-specific nuclear and plastid genes to achieve a stoichiometric accumulation of polypeptides for photosynthetic enzymes or membrane complexes, respectively. Light is known to be involved in the regulation of expression of several nuclear genes in leaf tissues, including the small subunit (SSU) of ribulose-1.5-bisphosphate carboxylase (RuBPC/Oase, *rbc*S) and the light harvesting chlorophyll a/b-binding proteins (LHCP, *cab*) (15, 24, 28, 38, 39, 41, 42, 45, 47). In addition, developmental regulation has been demonstrated in leaves of monocots (30) and in cotyledons of a C4-dicotyledonous plant (4). Very little is known about the developmental and/or light regulation of photosynthetic genes in other tissues, including the photosynthetically active pericarp tissue of tomato

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fruit. In specialized tissues other than leaf it is unknown to what extend light and/or developmental factors are responsible for the regulation of photosynthetic nuclear and plastid gene expression.

We report here the expression of the large (LSU) and small (SSU) subunit of RuBPC/Oase genes (*rbc*S, *rbc*L), LHCP genes (*cab*), and genes coding for polypeptides of photosystem I (*psa*A, P700 reaction center protein) and II (*psb*A, 32 kd Q_B-binding protein) in pericarp tissue during the development, maturation, and ripening of tomato fruit. Changes in mRNA steady-state levels for these genes were compared with changes in protein accumulation and photosynthetic activity in mature and ripening tomato fruits. It appears that the inactivation of photosynthesis-specific genes (with the exception of *psb*A) occurs within two weeks after pollination. At the molecular level, these events precede the cease of chlorophyll synthesis and the disassembly of the thylakoid membrane system by 2–3 weeks. Our results also indicate that the SSU and LSU genes of RuBPC/Oase might be simultaneously inactivated. The transcript levels in the developing and ripening tomato fruit have been compared with the expression of the photosynthesis-specific genes in other organs of tomato.

Materials and methods

Plant material and tissue preparation

Tomato plants (*Lycopersicon esculentum,* cv. VFNT LA 1221, cherry line) were grown under greenhouse conditions. Fruits were harvested at different developmental stages after pollination: 3 days, 7 days, 14 days, 30 days, 40 days, 44 days (orange) and 48 days (red). Whole fruits (3 days, 7 days) and pericarp tissue (14–48 days) were isolated, immediately frozen in liquid nitrogen and stored at −70 °C. Roots and stems of hydroponically grown plants (approximately 4 weeks), seedlings grown in the dark for 5 days and leaves were prepared and stored under identical conditions.

Isolation of RNA

Fifty g of frozen tissue was ground in a mortar, and the resulting tissue powder was resuspended in 50 ml buffer (0.35 M sorbitol, 50 mM Tris-HCl, pH 8.0, 25 mM EDTA, 15 mM 2-mercaptoethanol, 2 mM dithiothreitol, 0.1% polyvinylpyrrolidone, 5 mM aurintricarboxylic acid). One-tenth volume of chloroplast lysis buffer (5% sodium lauroylsarcosinate, 50 mM Tris-HCl, pH 8.0, 25 mM EDTA) was added, and the homogenate was incubated for 15 min at room temperature. Protein was removed by three phenol:chloroform:isoamyl alcohol (25:25:1) extractions. One-tenth volume of 5 M ammonium acetate (pH 5.2) and 2 volumes of ethanol were added to the recovered aqueous phase to precipitate total nucleic acids. The nucleic acid pellet was resuspended in a small volume of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA), and 10 M lithium chloride was added to a final concentration of 2 M to selectively precipitate high-molecular-weight RNA (14). The RNA pellet was resuspended in diethylpyrocarbonate-treated H_2O (27) and stored in aliquots at −20 °C. This method typically yielded 10–1000 μg total RNA/g tissue depending on the developmental stage of fruits or pericarp tissue.

Preparation of hybridization probes

Specific gene probes were used for the hybridizations. The plasmid pTB1 contains a 1.2 kb BamHI fragment of the tobacco chloroplast genome inserted into pBR322 with almost the entire coding region of the *rbc*L gene (41). pTB8-P has a 0.8 kb PstI-XbaI fragment which contains the internal region of the *psb*A gene. This region was subcloned from the 4.8 kb BamHI fragment in pTB8 (46). Plasmid pSoc1080 contains the entire *psa*A1/A2 gene region on a 8.2 kb PstI fragment in pBR322 (E. Orozco, unpublished). Plasmid p3-91 has a 0.7 kb cDNA fragment encompassing the entire coding sequence of the tomato *rbc*-S2A gene (31), inserted into the PstI site of pUC9. Plasmid pIA27 contains a 2.0 kb EcoRI fragment of tomato nuclear DNA inserted into pUC9, on which the 0.55 kb PvuII-HincII fragment is specific for the internal sequence of the *cab*-1B gene (32). pHA2 was derived from plasmid pHA1 and contains a 8.7 kb HindIII fragment of pea nuclear DNA inserted into pBR322 that contains the genes for the 18S and 25S rRNA (23). Plasmid DNA was prepared as described by Piechulla *et al.* (33). The cloned inserts, coding for the gene of interest, were isolated

by preparative digestion of the plasmid DNA with the appropriate restriction enzymes. DNA fragments were separated by gel electrophoresis and the fragments were isolated by electroelution (100 V in 0.1×TBE buffer, ref. 27). The reisolated DNA was purified by phenol:chloroform:isoamyl alcohol (25:25:1) extractions and used for nick translation reactions (specific activities of the probes: *cab* 4.6×10^6 cpm/μg; *rbc*S 8.8×10^6 cpm/μg; *psa*A 2.0×10^7 cpm/μg; *psb*A 3.5×10^6 cpm/μg; *rbc*L 6.1×10^6 cpm/μg).

Analysis of RNA

Electrophoresis of RNA on formaldehyde containing agarose gels was performed as described by Maniatis *et al.* (27). The RNA was transferred to nylon filter (Hybond, Amersham) and fixed to the filters by exposing it 4–5 min to UV light. Nylon filters were then prehybridized for 4 h at 65 °C in 2×SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 1×Denhardt's solution (0.1 g Ficoll, 0.1 g polyvinylpyrrolidone 40, 0.1 g BSA per 500 ml). Hybridizations with specific probes were carried out at 65 °C for 12–16 h in 2×SSC, 1×Denhardt's solution, 0.5% SDS. Filters were washed at 65 °C in 1 liter 2×SSC (3 times, 15 min) and 1 liter 1×SSC (3 times, 10 min) and exposed to X-ray film (−70 °C, with intensifying screen).

The autoradiograms obtained from northern blot hybridizations were scanned with a Joyce Loebl densitometer. Relative amounts of mRNAs were determined by peak-area measurements.

Results

The genes described in this study encode polypeptides which participate in the assembly of stromal enzymes (LSU and SSU of RuBPC/Oase) and photosystem I and II membrane complexes (LHCP, P700 reaction center protein, 32 kD Q_B-binding protein). They were chosen as representative genes expressed in the nucleus and chloroplasts of the photosynthetically active pericarp tissue during tomato fruit development, maturation and ripening. Our previous results have indicated that the transcript levels for a number of plastid encoded proteins decline during the differentiation of chloroplast into chromoplast (33). These results are correlated with a measureable decrease in photosynthetic activity and the disappearance of photosynthesis-specific proteins (34). In order to evaluate the coordination of nuclear and plastid gene expression during the photosynthetically active period of tomato fruit development and maturation and their role during plastid differentiation, we have examined the levels of mRNAs for the above genes. Total RNA was extracted from whole fruit (days 3 and 7) and pericarp tissues (days 14, 30, 40, 44, 48), as well as from etiolated seedlings, roots, stems, and leaves. The amounts of RNA applied to the gels in all of the following experiments were standardized upon spectrophotometric quantitation, quantitation of the fluorescence intensity of cytoplasmic rRNA in ethidium bromide-stained gels, and relative levels of hybridization with heterologous cytoplasmic rDNA. To ensure a more precise RNA quantitation, normalization of RNA levels to cytoplasmic ribosomal RNA was routinely applied in all experiments, since cytoplasmic ribosomal RNA represents a large (larger than 90%) portion of the total cellular RNA and does not change significantly in different tissues or during fruit ripening (32). To precisely correlate changes in mRNA levels, two or three repeats of the experiments were completed with the results in each case corresponding to those described below.

RuBPC/Oase large and small subunit mRNA levels

Northern analysis of total RNA from fruit at different developmental stages using a DNA fragment from the *rbc*L coding region as a probe reveals a discrete band of 1.7 kb (Fig. 1). Similar transcript sizes have been observed for the *rbc*L transcripts from several other plant species (12, 41, 51), although a heterogeneity in the length of the 5' end of the *rbc*L mRNA has been reported in other species (9, 12, 29, 36). LSU mRNA can be detected in 3 day old tomato fruits, and the level of the LSU transcript reaches a maximum in approximately 14 days old tomato fruits (Fig. 1). After this stage and before maturation, the LSU transcript level declines, but transcripts are still detectable during fruit ripening (Fig. 1 and 3).

The presence of the LSU mRNA in mature green fruit (35–40 days) corresponds with the presence of the LSU protein and a measureable RuBPC/Oase activity (approximately 35% of the

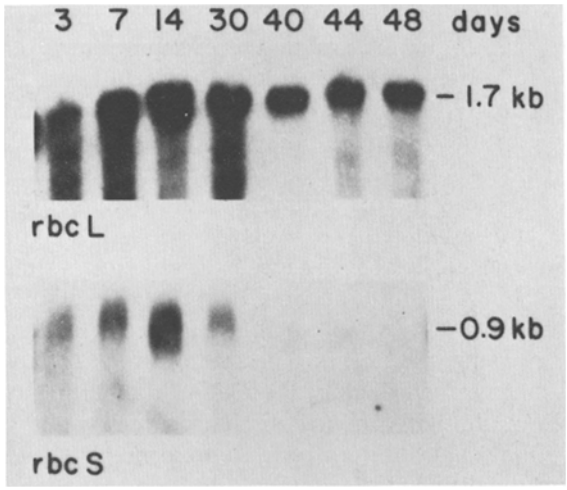

Fig. 1. Identification of transcripts for the LSU (*rbc*L, upper panel) and the SSU (*rbc*S, lower panel) of RuBPC/Oase in total RNA preparations from 3, 7, 14, 30, 40, 44 (orange), and 48 (red) day old tomato fruit pericarp. 3 μg aliquots of total RNA from the different developmental and ripening stages were separated on a denaturing formaldehyde gel. Northern blot hybridization conditions and specific gene probes are described in materials and methods. Autoradiograms were exposed for 63 h with intensifying screens at −70 °C. Transcript sizes of the tomato *rbc*L and *rbc*S genes are indicated.

activity determined in tomato leaves; ref. 34). However, decreasing amounts of immunologically detectable LSU protein, together with decreased levels of RuBPC/Oase activity, have been observed during fruit ripening, although the LSU mRNA level does not decrease significantly (34).

The level of SSU mRNA was determined by Northern analysis using the tomato p3-91 cDNA clone as a probe. The SSU is encoded in the tomato nuclear genome by a small multigene family of five genes, which have been cloned and sequenced (M. Sugita, T. Manzara and W. Gruissem, manuscript in preparation). DNA sequences of *rbc*S-1 (locus#1), *rbc*S-2A (locus#2) and *rbc*S-3A (locus#3) have been reported previously (31). Since the DNA sequence of all genes is highly conserved, the cDNA clone therefore will detect transcripts from all SSU genes under our hybridization conditions. The cDNA probe hybridizes to transcripts approximately 800–900 nucleotides in size (Fig. 1). This is in good agreement with previously published sizes of SSU mRNAs from other plants (5, 6, 10) and the size of the tomato SSU cDNA. The observed variability of transcript length can be attributed to length polymorphisms at the 5' and 3' non-coding regions (1). SSU mRNA is present in 3 day old tomato fruit at low levels, and the increase parallels the LSU mRNA levels during the early stages of development (Fig. 1 and 3). However, SSU mRNA levels decrease rapidly before fruit maturation, and are no longer detectable in mature fruit. Although the changes in LSU and SSU mRNA levels appear to be coordinated during early fruit development, it is interesting to note that the nuclear SSU mRNAs disappear more rapidly than the chloroplast LSU mRNA later in fruit development and ripening.

Thylakoid membrane protein mRNA levels

Eight genes have been identified in the chloroplast genome that code for polypeptides of photosystem I and II complexes (*psa*A1/A2 of PS I and *psb*A, B, C, D, E, and F of PS II, ref. 20, 33, 35, 49). The nuclear location has been demonstrated for genes encoding LHCPs (11, 25, 32, 47) and plastocyanin (44), and other PS I and II polypeptides are also thought to be encoded in the nuclear genome. We have analyzed the expression of plastid genes (*psa*A, P700 reaction center polypeptide; *psb*A, the 32 kD Q_B-binding protein) and nuclear genes (*cab,* LHCP) at different stages during tomato fruit development and ripening. Northern blot analysis of total RNA from fruit with a probe consisting of the internal *psb*A sequence reveals a discrete RNA band of 1.2 kb (Fig. 2). The size of the tomato *psb*A mRNA is in good agreement with previously published sizes for *psb*A from mustard, tobacco and spinach (26, 46, 50). The *psb*A mRNA rises continuously between day 3 and 30, after which the level declines, but the mRNA is still detectable in significantly higher amounts in ripe fruit than in the earliest developmental stage (Figs. 2 and 3). The observed mRNA levels correlate with data from Western blot analysis which demonstrate that the decrease of the 32 kD Q_B-binding protein levels is concomitant with the decrease in mRNA, but the protein is still present in chromoplasts of ripe fruits (34).

The *psa*A gene is transcribed into an approximately 6.0 kb transcript (Fig. 2), which is similar in size to that reported for the spinach *psa*A mRNA (48). The mRNA levels found in pericarp tissue are significantly lower when compared to the respective mRNA levels in leaf tissue (Table 1). During fruit

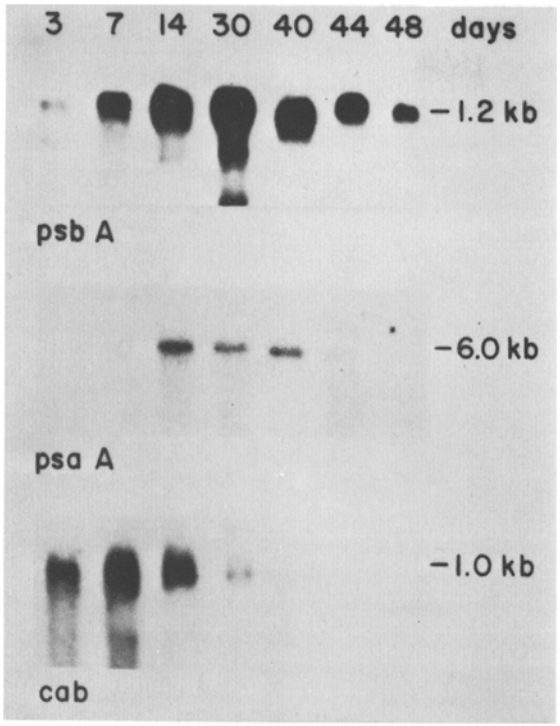

Fig. 2. Identification of mRNAs for the 32 kD Q_B-binding protein (*psb*A, upper panel), the P700 reaction center protein of PS I (*psa*A, middle panel), and the LHCPs (*cab*, lower panel) in total RNA preparations from 3, 7, 14, 30, 40, 44 (orange), and 48 (red) day old tomato fruit pericarp. 3 μg aliquots of total RNA from different developmental and ripening stages were separated on denaturing formaldehyde gels and analyzed as described in materials and methods. The filters hybridized with the *psa*A and *cab* DNA probe were exposed for 63 h, the filter hybridized with the *psb*A DNA probe was exposed for 6 h at −70 °C with intensifying screen. The length of the transcripts identified in the tomato RNA preparations are indicated.

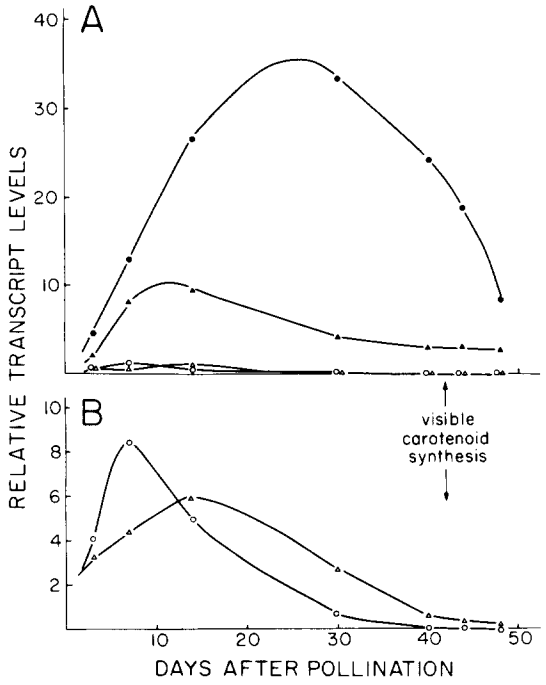

Fig. 3. Correlation of relative transcript levels for the 32 kD Q_B-binding protein (*psb*A, ●), the LSU of RuBPC/Oase (*rbc*L, ▲), the LHCP (*cab*, ○), and the SSU of RuBPC/Oase (*rbc*S, △) during tomato fruit development, maturation and ripening. Densitometer scanning of the autoradiograms (6 h exposure, panel A) and peak area measurements indicate the relative mRNA levels of plastid (*psb*A and *rbc*L) and nuclear (*cab* and *rbc*S) encoded genes. In panel B the relative amounts of *rbc*S (△) and *cab* (○) mRNAs during tomato fruit formation are presented on an expanded scale.

development the *psa*A mRNA reaches a maximum in 14 days old tomatoes, and then declines to non-detectable levels at the maturation stage and in ripe fruit tissue (Figs. 2 and 3). The P700 polypeptide can still be detected with specific antibodies in mature green fruit, but not in chromoplasts of ripe pericarp tissue (34).

Similar results were obtained for the expression of the nuclear cab genes. Tomato LHCPs are encoded by a multigene family of at least 13 genes (32). The hybridization probe pIA27 from the coding region of the *cab*-1B gene identifies transcripts of approximately 1 kb, which is similar to the size reported for *cab* mRNAs from petunia, pea, and wheat (7, 11, 25). Although members of the gene

Table 1. Relative transcript levels (%) of photosynthesis-specific genes in different plant organs.

Gene	Leaf	Fruit*	Root	Stem	Etiolated seedlings
*psb*A	100	63.0	5.2	46.6	9.2
*rbc*L	100	24.3	0.8	23.9	24.0
cab	100	11.0	n.d.	39.8	n.d.
*rbc*S	100	2.0	n.d.	3.0	13.3

* Values given for *psb*A, *rbc*L, *rbc*S, and *cab* represent the level of maximum hybridization detected during fruit development (30, 14, 14, and 7 days, respectively).
n.d. = not detectable.

family show significant divergence in their DNA sequence for the transit sequence and N-terminus of the mature polypeptide, the sequence of the rest of the mature polypeptide is essentially identical (90% homology at the DNA sequence level of at least seven genes, whose sequences have been determined, ref. 32), and thus we would expect to detect mRNAs for all these genes, and possible others, under our hybridization conditions. The mRNA level for the LHCPs peaks early during fruit development (day 7) and then declines to non-detectable levels in premature fruits (day 30 to 48, Figs. 2 and 3). However, cross reaction with LHCP-specific antibodies demonstrates that the proteins are still present in photosynthetically active mature green fruit (34).

Transcript levels in different organs of tomato

To evaluate the role of photosynthetic gene expression in tomato fruit, we compared the peak steady-state mRNA levels for *rbc*L, *rbc*S, *psa*A, *psb*A and *cab* in fruit with their respective levels in other organs of tomato. Northern blots of total RNA isolated from leaves, fruit pericarp, stems, roots and etiolated seedlings were hybridized with the specific probes for the above nuclear and plastid genes. To allow a direct comparison, the identical set of RNAs on four different filters were hybridized separately with the probes for *rbc*L, *rbc*S, *psb*A and *cab*, and then all filters were washed and exposed under the same conditions. The expression of the *psa*A gene was analysed in a separate set of experiments. All quantitations are based on normalization of RNA preparations to cytoplasmic rRNA. We cannot exclude, however that changes occur in the ratio of rRNA:mRNA in RNA isolated from the different organs or etiolated seedlings. In addition, the total RNAs isolated from leaves, roots, stems and etiolated seedlings represent RNAs from different cell types, thus allowing only to indicate average values for *rbc*L, *rbc*S, *psa*A, *psb*A and *cab* expression in these organs.

All genes are expressed in stems, but little or no mRNA can be detected in roots (Table 1, Fig. 4). It is interesting to note that a low level of *psb*A mRNA (5%) can be detected in root, although amyloplasts in root tissue are photosynthetically inactive. This result correlates with the presence of *psb*A mRNA in fruit pericarp chromoplasts

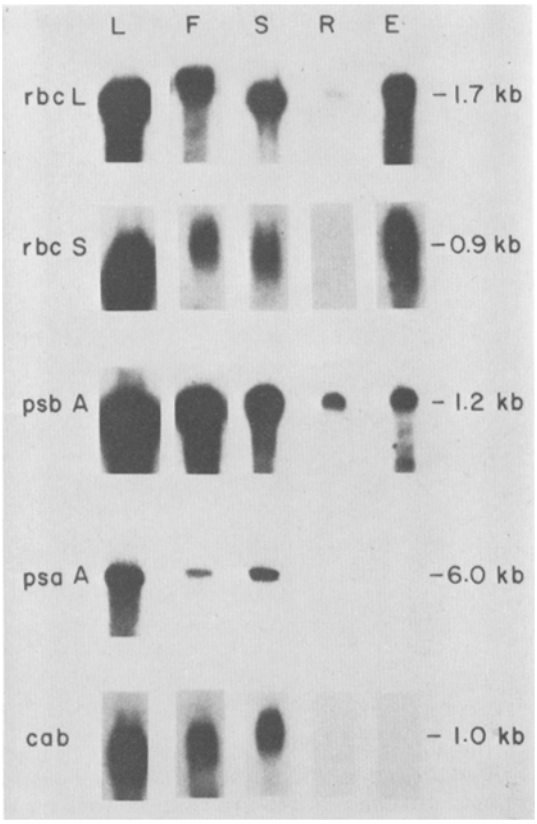

Fig. 4. Identification of transcripts of the LSU (*rbc*L) and SSU (*rbc*S) of RuBPC/Oase, the 32 kD Q_B-binding protein (*psb*A), the P700 reaction center protein of PS I (*psa*A), and LHCP (*cab*) in total RNA preparations from leaf (L), fruit (F), stem (S), root (R), and etiolated seedlings (E). 3 μg aliquots of total RNA from different organs were separated on formaldehyde gels. Northern blot hybridization conditions and specific gene probes are described in materials and methods. Autoradiograms were exposed for 63 h at −70 °C with intensifying screen. The hybridization with RNA from fruit pericarp represent to the highest levels detected during fruit development (see Figs. 1 and 2). The length of the transcripts identified in tomato RNA are indicated.

(Fig. 2), and might indicate the stability and/or light-independent transcription of this plastid mRNA. We find that SSU and LSU mRNAs accumulate to significant levels in etiolated seedlings, which has been previously observed for other plants (24, 45). The level of LSU mRNA is very similar in fruit pericarp (day 14), stem and etiolated seedlings, whereas SSU mRNA levels are reduced 4.5- and 6.5-fold in stem and fruit pericarp, respectively, compared to SSU mRNA levels in etiolated seedlings. No mRNA expression can be detected

from the *cab* and *psa*A genes in root and etiolated seedlings, and from the *rbc*S genes in root (Fig. 4, Table 1).

Discussion

The expression of genes encoding photosynthetic polypeptides in leaves and other organs has been under intensive study in a variety of plant species. It has been shown that light and/or developmental regulation in leaves is always correlated with the development of the photosynthetically active chloroplasts (4, 15, 28, 30, 38, 39, 41, 45), but the mechanisms of regulation vary among different plant groups. We report here a study of the expression of photosynthetic genes in pericarp tissue of tomato fruit. During fruit formation, development and function of chloroplasts in pericarp tissue is similar to that of chloroplasts in leaf tissue. At the onset of fruit ripening, the photosynthetically active pericarp chloroplasts differentiate into carotenoid-producing chromoplasts (3, 17, 18, 37). We are interested to understand the interaction of nuclear and plastid genomes during the development and differentiation process, as well as the coordination of gene expression in the two compartments. In addition, since plastid differentiation during fruit formation occurs in the presence of light, it will be important to evaluate the role of light as a factor in the regulation of photosynthetic gene expression. As a first step we have investigated the expression of nuclear and plastid genes for proteins which participate in the assembly of photosystem I and II complexes (*psa*A, *psb*A and *cab*) and the ribulose-1.5-bisphosphate carboxylase (*rbc*L and *rbc*S). In tomato fruit, active growth due to cell division only occurs for 7–10 days after pollination (8), and further growth is due to cell expansion. Thus, the number of cells in the tissue does not change significantly at most of the time points in our developmental study. Although the physiology of tomato fruit development and ripening has been well characterized, it is unknown if the plastid number per cell changes during cell growth and expansion. However, earlier electron microscopic observations (17, 18, 37) indicate that the existing cellular chloroplast population differentiates into chromoplasts during fruit ripening. In addition we find that the plastid/nuclear DNA ratio is constant throughout the fruit development and ripening process (data not shown). Consequently, we can exclude that the observed changes in the expression of photosynthetic genes are due to an increase or decrease of plastid DNA copies.

The analysis of photosynthetic gene expression revealed that most plastid (*psa*A and *rbc*L) and nuclear mRNAs (*rbc*S, *cab*) reach their highest levels early during fruit development at or shortly after the cease of mitotic activity in the pericarp tissue. Since no immunological characterization of photosynthesis-specific proteins is available, we lack the information if this correlates with maximum protein synthesis at these stages. We have shown, however that the mature (green) fruit pericarp tissue is active in photosynthesis, with a photosynthetic electron transport capacity of approximately 41% as compared to leaf tissue (34). At this stage, the mRNA levels for *psa*A, *rbc*L, *rbc*S and *cab* have declined already, and no or very little mRNA is detectable for the nuclear *cab* and *rbc*S genes, respectively. It is possible, therefore that the photosynthetic capacity is higher in pericarp tissue earlier during fruit development. Also, it is still unknown to what extent photosynthetic activity is required for normal fruit development.

After the onset of ripening mRNAs of *psa*A and *cab* are undetectable in pericarp tissue and only low levels of *rbc*L mRNA can be detected in chromoplasts. This is in good agreement with the observed decrease in photosynthetic activity, RuBPC/Oase activity, P700 and LHCP protein levels (34), and the disintegration of grana stacks and thylakoid membranes (17, 18, 37). In contrast, *psb*A shows a different expression pattern during fruit development and ripening. The mature mRNA reaches its highest level late in fruit development and is still present at a significant level in chromoplasts of ripe fruit. In addition, the 32 kD protein can still be detected immunologically at this stage (34). Since we have demonstrated that plastid mRNAs for other components of photosystem II (i.e. *psb*B, *psb*C, *psb*D) decline to non-detectable levels in chromoplast (33), we can at present only speculate about the continued expression of 32 kD Q_B-binding protein in ripe fruit. Using cloned promoter fragments for spinach *atp*B, *rbc*L and *psb*A, it has been shown that the *psb*A promoter directs transcription with the highest efficiency (13). Thus, the observed high *psb*A mRNA levels could be a

combination of promoter strength and/or mRNA stability in the light.

Although *rbc*L and *rbc*S mRNA levels are similar in leaf (Fig. 4), comparison of mRNA levels of photosynthesis-specific plastid and nuclear genes in fruit demonstrates a several fold excess of *rbc*L and *psb*A transcripts over *rbc*S and *cab* mRNA throughout fruit development (Fig. 3). These significant differences in the fruit mRNA levels may be attributed to a different regulation of nuclear gene expression in the specialized pericarp tissue. However, the consequences of such imbalances of the respective mRNA levels are not well understood. Also, it is unknown if all copies of the respective plastid genes are transcribed, and the relative contribution of each member of multigene families in plants has only been addressed recently for the nuclear SSU genes in petunia (10). The existence of stringent translational control mechanisms have been proposed to operate during leaf development for the expression of SSU and LSU of RuBPC/Oase (4, 21), and it has been demonstrated that the synthesis and/or transport rate of SSU limits the final assembly of RuBPC/Oase in the chloroplast (40).

Studies of a variety of monocots and dicots have indicated that accumulation of LSU and SSU, but not necessarily their mRNAs, is tightly coordinated in developing leaves (16, 42, 45). At least at the level of their mRNAs it appears that tomato *rbc*L and *rbc*S expression is coordinated during fruit development (Fig. 3). The mRNA levels for both *rbc*S and *cab* genes decline during fruit development in the presence of light (Fig. 3). In leaves the expression of these genes appear to be under light control (2, 19, 28), and reduced or no expression can be detected for *rbc*S and *cab*, respectively, in the dark. Northern analysis of RNA from etiolated tomato seedlings confirms these observations (Table 1). Since we observe activation and inactivation of these genes during tomato fruit development and ripening in the presence of light, we conclude that the expression of nuclear photosynthetic genes is at least in part, controlled by a developmental programm in tomato pericarp tissue. The concomitant decrease in plastid expression may then occur as a consequence of regulatory events at the nuclear DNA level, although the mechanisms and direct consequences of such events are poorly understood. This aspect of gene regulation is currently under investigation.

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