Planta (1988) 174:505–512

Planta

Differential expression of nuclear- and organelle-encoded genes during tomato fruit development

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Abstract. Steady-state mRNA levels of nuclear- and organelle-encoded genes were determined during fruit development and ripening. Transcripts specific for subunits of the mitochondrial and chloroplast ATPase complexes appear simultaneously and reach high levels two to three weeks after anthesis, but follow a different expression pattern during the ripening period. While the chloroplast-specific mRNA levels continuously decrease to low levels in ripe tomato fruits, the transcripts specific for two mitochondrial ATPase subunits continue to be present at relative high levels in red fruits. Transcript levels for the fructose-1,6-bisphosphate aldolase increase significantly during ripening. Structural proteins such as the alpha-subunit of tubulin and the hydroxyproline-rich glycoprotein extensin are expressed during maximal fruit growth. In addition, comparisons of mRNA levels of different genes in several plant organs (leaf, fruit, stem, and root) show characteristic differences. The results presented in this paper demonstrate that changes at the transcriptional or post-transcriptional level during fruit development can be correlated with morphological and physiological alterations.

Key words: Fruit (development, ripening) – *Lycopersicon esculentum* (fruit development) – Gene expression (nuclear, organelle) – mRNA (steady-state level).

Introduction

The development and ripening process in tomato fruits (*Lycopersicon esculentum*) is characterized by a number of physiological and morphological changes that occur after pollination and fruit set. Initially, growth proceeds by cell division for 7–10 d after anthesis, followed by cell elongation (Asahira et al. 1968; Davies and Cocking 1965). Recently, it was demonstrated that chloroplasts in pericarp tissue of mature green fruit are capable of photosynthesis (Piechulla et al. 1987). This is consistent with the finding that photosynthetic polypeptides and their mRNAs (small and large subunit of ribulose-1,5-bisphosphate carboxylase (RuBPCase), P700 reaction-center protein of photosystem I, Q_B-binding protein of photosystem II, light-harvesting chlorophyll a/b-binding protein) can be detected during this developmental stage (Piechulla et al. 1985, 1986, 1987). Photosynthates of the fruit chloroplast and imported sucrose subsequently convert into starch (Ho 1984) and accumulate during tomato fruit development. Shortly before ripening a rapid decrease of starch and a significant increase of reducing sugars are detectable (Davies and Cocking 1965). During this phase, when chloroplasts differentiate into chromoplasts (Rosso 1968; Raymundo et al. 1976), chlorophyll and thylakoid-membrane complexes disappear, and accumulation of carotenoids increases (Thomas and Yen 1975; Raymundo et al. 1976). During the same period a climacteric rise in mitochondrial respiration occurs (Biale and Young 1981), and fruit softening is initiated as a result of increased levels of polygalacturonase (Goodenough et al. 1982; Crookes and Grierson 1983; Grierson et al. 1985; Biggs et al. 1986). This series of physiological changes requires a coordinated interaction of nuclear, plastid and mitochondrial genomes to control the expression of genes for several proteins of different compartments.

As a first step in understanding the regulation of nuclear and organelle genes involved in the complex series of physiological and structural changes during tomato fruit development and ripening, a wide spectrum of gene probes has been used in

the present study to measure the relative mRNA levels for various proteins. The mRNA levels of polypeptide subunits for the mitochondrial and chloroplast ATPase, which are functionally related in both organelles, have been monitored. In addition, the steady-state transcript levels for the glycolytic enzyme fructose-1,6-bisphosphate aldolase were determined. As examples of proteins which are not involved in photosynthetic (Piechulla et al. 1986), glycolytic or respiratory functions, it was decided to study the regulation of genes coding for structural components (tubulin and the hydroxyproline-rich cell-wall glycoprotein, extensin) during fruit development and ripening. Furthermore, the relative mRNA levels of these genes in four organs of tomato plants are presented.

Material and methods

Tissue preparation and isolation of RNA. Lycopersicon esculentum cv. VFNT LA 1221 fruits of various developmental stages (3, 14, 20, 30, 40, 44, 48 d after anthesis), and stems, leaves, roots and etiolated seedlings were harvested and prepared as described elsewhere (Piechulla et al. 1986). A detailed protocol for the isolation of total RNA from tomato pericarp and other plant tissues is also given in the same publication.

Preparation of hybridization probes and analysis of RNA. Specific heterologous and homologous probes were used for the hybridizations. The plasmid pHA2 was derived from plasmid pHA1 and contains an 8.7-kb HindIII fragment of pea nuclear DNA inserted into pBR322, which contains the genes for the 18S and 25S rRNA (Jorgensen et al. 1981/1982). The plasmid pZM1154 has a 1.0-kb PstI fragment which contains a nuclear gene for maize fructose-1,6-bisphosphate aldolase (Hake et al. 1985). pDC5A1 has a 1.5-kb PstI – XbaI fragment which carries the nuclear gene for carrot extensin (Chen and Varner 1985a, b). The plasmid pTA12 has a 0.5-kb EcoRI – BamHI fragment which codes for pea alpha-subunit of tubulin (Webster and Long, pers. communication; Dept. of Biological Sci., Stanford University, Stanford, Calif., USA). The plasmid pATB4 contains a 1-kb KpnI – BamHI fragment coding for an *Arabidopsis thaliana* beta-subunit tubulin gene (Marks et al. 1987). The cNp10 plasmid has a 1.6-kb PstI – EcoRI fragment that contains parts of the nuclear gene coding for the beta-subunit of the tobacco mitochondrial ATPase (Boutry and Chua 1985). The plasmid pTA22 has a 0.7-kb EcoRI – BamHI mitochondrial DNA fragment coding for the alpha-subunit of the mitochondrial ATPase from maize (Braun and Levings III 1985). pTB4 has a 0.8-kb HindIII – BstEII chloroplast DNA fragment which contains an internal part of the alpha-subunit of the chloroplast ATPase from tobacco (Shinozaki et al. 1986). Plasmid DNA and nick-translated isolated DNA fragments were prepared as described elsewhere (Piechulla et al. 1986). Three µg of total RNA from fruits of different developmental stages and various plant organs were analysed by Northern-blot hybridizations. In order to guarantee the comparison of equal amounts of RNA from different preparations, the RNA preparations were standardized by spectrophotometric quantitation, and quantitation of the ethidium bromide fluorescence of cytoplasmic rRNA in stained gels. To ensure a precise quantitation, normalization of RNA levels by hybridization with heterologous cytoplasmic rRNA (Jorgensen et al. 1981, 1982) was routinely applied in all experiments, since cytoplasmic rRNA represents a large (larger than 90%) portion of the total cellular RNA and does not change significantly in different tissues and during fruit ripening. Northern blots were prehybridized in 2 × SSC, 1 × Denhardts at 65° C for 4 h, hybridized in 2 × SSC, 1 × Denhardts, 0.5% sodium dodecyl sulfate (SDS) at 65° C for 16 h, and washed with 2 × SSC and 1 × SSC (Piechulla et al. 1986). Specific activities of all probes used for hybridizations were $1–10 \cdot 10^6$ cpm · µg^{-1}. Filters were exposed at −70° C. To correlate changes in mRNA levels precisely, two or three repeats of each experiment were completed.

The autoradiograms obtained from Northern-blot hybridizations were scanned with a densitometer (Joyce Loebl & Co. Ltd., Gateshead-on-Tyne II, GB). Relative amounts of mRNAs were determined by peak-area measurements of autoradiograms of different exposure times. For hybridizations with a particular gene probe, values at all timepoints during fruit development and in different plant organs were calculated relative to the highest peak-area value.

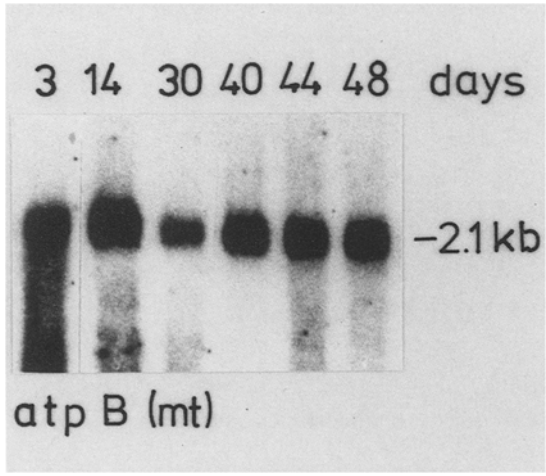

Fig. 1. Northern-blot analyses of mRNA levels in developing tomato fruits. RNA was extracted from 3-, 14-, 30-, 40-, 44-, and 48-d-old tomato fruits. The Northern blot was incubated with nick-translated DNA fragments specific for the beta-subunit of the mitochondrial ATPase, washed and autoradiographed (3 d)

Results

Identification of transcripts and relative changes of steady-state mRNA levels during fruit development and ripening. It was demonstrated previously that significant alterations of steady-state mRNA levels of plastid- and nuclear-encoded photosynthesis-specific genes appear throughout tomato fruit development and ripening (Piechulla et al. 1986). In this study the work has been extended to genes which are not directly involved in photosynthesis. Changes of mRNA levels were documented by Northern-blot analysis. A typical result from this assay is shown in Fig. 1 and the data from a series

of transcript-level determinations are plotted in Figs. 2–4.

Subunits of the mitochondrial and chloroplast ATPase complex. When the 8-kb BamHI fragment coding for the subunits alpha and III of the chloroplast ATPase as well as several other open reading frames of the tobacco chloroplast genome (pTB4; Deno et al. 1984; Shinozaki et al. 1986) is hybridized to total RNA preparations from tomato leaves, a multiband pattern becomes apparent (data not shown). More than 15 RNA species including ten major components of approx. 0.9, 1.25, 1.4, 1.8, 2.6, 3.15, 3.85, 4.15, 5.25, and 6.45 kb were detected. To identify some of these transcripts, a restriction fragment encoding only the alpha ATPase subunit (HindIII – BstEII) was used as a hybridization probe (Deno et al. 1984). This specific probe detects a major transcript of 3.15 kb and two minor mRNAs of approx. 4.15 and 1.8 kb in leaf RNA preparations. In developing tomato fruits, increasing steady-state levels of the 3.15-kb transcript were measured, and highest levels were determined in 14-d-old tomato fruits (Fig. 2a). During further growth the amount of the 3.15-kb mRNA decreases to very low levels (lower than 10%) in red fruits. A similar pattern was observed for the 4.15-kb transcript, although some fluctuations were detected in 30-d-old fruits (data not shown).

In addition to the steady-state mRNA levels for the chloroplast ATPase complex, the expression of the alpha- and beta-subunits of the mitochondrial ATPase were determined in developing and ripening tomato fruits (Fig. 2b). Fragments of DNA specific for the alpha-subunit, which is encoded by the mitochondrial genome of maize and broad bean (Braun and Levings 1985), cross-hybridize with a distinct transcript of approx. 2.8 kb in RNA preparations from tomato fruits. The transcript length is similar to the 2.6-kb mRNA reported for maize (Braun and Levings 1985). The steady-state mRNA level increases continuously during early fruit development and reaches high levels in 14- to 30-d-old tomato fruits (Fig. 2b). Thereafter, the level declines, but significant mRNA amounts (approx. 50% of the maximum level) were measured in orange and red tomato fruits. A similar expression pattern was observed when a DNA fragment encoding the beta-subunit of the mitochondrial ATPase (Boutry and Chua 1985) was hybridized to tomato-fruit RNA from different developmental stages (Fig. 2b). Maximum transcript levels of the beta-subunit-specific mRNA were determined in 14-d-old fruits and approx. 40% remained in ripe tomato fruits. The transcript size in tomato is 2.1 kb, which is the same size as reported for the tobacco mRNA.

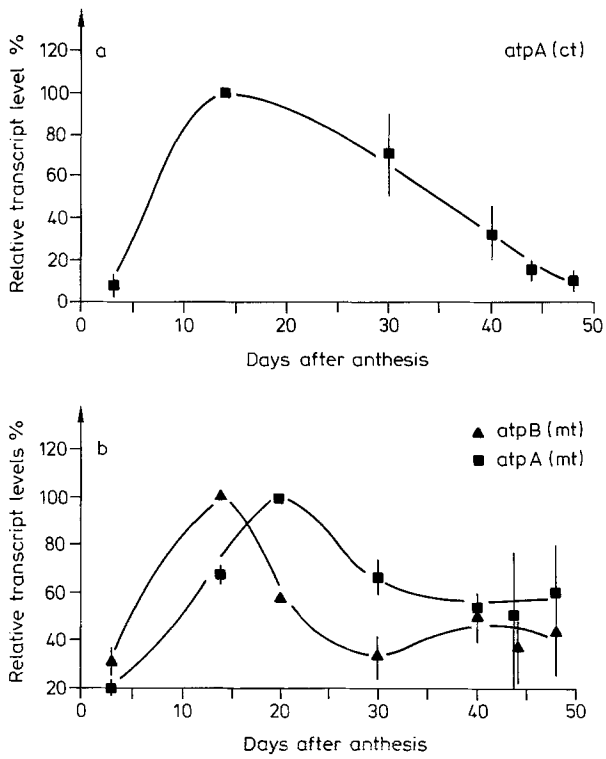

Fig. 2a, b. Relative changes of mRNA levels of the chloroplast (**a**) and mitochondrial (**b**) ATPase subunits during tomato fruit development. The calculations are based on two and three Northern blots, analyzed for the alpha-subunit of the chloroplast ATPase and the alpha- and beta-subunits of the mitochondrial ATPase, respectively. The gene for the chloroplast ATPase subunit is located on the chloroplast genome, the alpha-subunit of the mitochondrial ATPase is encoded by the mitochondrial genome, and the beta-subunit of the mitochondrial ATPase is encoded by a small nuclear multigene family. *Error bars* represent ± SE

Glycolytic enzyme. To determine the steady-state mRNA levels of cytoplasmic fructose-1,6-bisphosphate aldolase, the final enzyme of the first step of glycolysis, a specific cDNA clone from maize (Hake et al. 1985) was nick-translated and hybridized to RNA preparations from tomato fruit pericarp (Fig. 3). Transcripts of approx. 1.5 kb in length were identified in tomato RNA isolates, similar to the mRNA size reported for maize (Hake et al. 1985). The mRNA levels accumulate significantly in orange and red tomato fruits; however, it is not known when aldolase mRNAs reach maximum levels during ripening. Only very low aldolase-mRNA levels, less than 10% compared to the levels in red fruits, were detected throughout fruit development (Fig. 3).

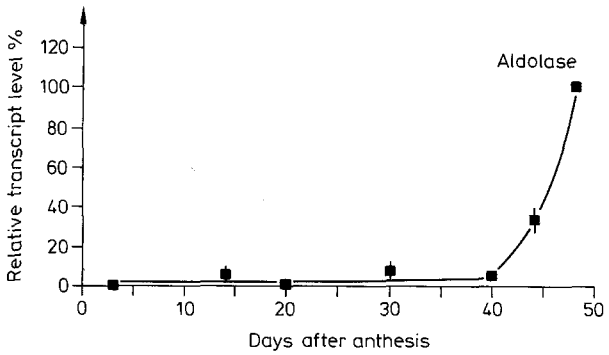

Fig. 3. Relative changes of transcript levels of fructose-1,6-bisphosphate aldolase during tomato fruit development. Calculations are based on two Northern-blot experiments. *Error bars* represent ± SE

Structural components of the cell. Heterologous probes specific for the alpha-subunit of tubulin were used to measure the steady-state mRNA levels in developing and ripening tomato fruits (Fig. 4a). The tubulin-A transcripts have a length of approx. 1.85 kb in tomato fruits. The mRNA levels for the alpha-subunit of tubulin increase continuously during early fruit development and reach maximum levels in 15-d-old tomato fruits, respectively. During further growth the transcript level decreases to non-detectable levels in orange and red fruits. A minor transcript with a molecular weight of approx. 1.6 kb becomes apparent in 30-d-old tomato fruits; its function is presently unknown.

Hybridizations with a heterologous clone coding for the cell-wall hydroxyproline-rich glycoprotein extensin identified three major transcripts of 1.1, 1.45, and 4.6 kb length in RNA preparations from stems and roots of tomato (Fig. 5). In tomato fruits only the two smaller mRNAs of 1.1 and 1.45 kb were detected, which accumulate to highest levels in 14- and 40-d-old tomato fruits, respectively (Fig. 4b).

Levels of mRNA of nuclear and organelle genes in various tomato organs. To evaluate the role of functionally unrelated genes, we compared the peak steady-state mRNA levels of the subunits of mitochondrial and chloroplast ATPase, fructose-1,6-bisphosphate aldolase, the alpha-subunits of tubulin, and extensin in fruits with their respective levels in other organs of tomato. Northern blots of total RNA isolated from leaves, fruit pericarps, stems, roots and etiolated seedlings were hybridized with the above-mentioned nuclear- and organelle-encoded genes. All quantitations are based on normalization of RNA preparations of cytoplasmic rRNA. We cannot exclude that changes occur

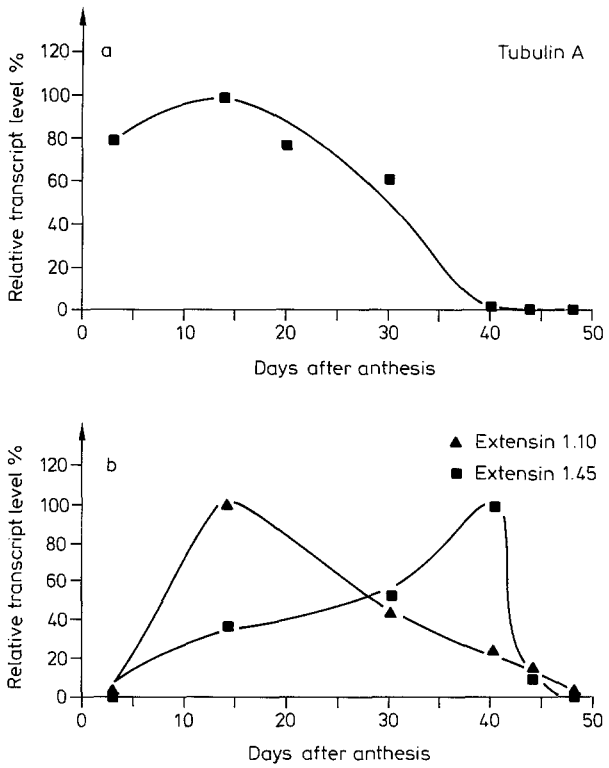

Fig. 4a, b. Relative changes of mRNA levels of the alpha-subunit of tubulin (**a**) and the hydroxyproline-rich glycoprotein extensin (**b**). The calculations represent average values from two Northern-blot experiments.

in the ratio of rRNA:mRNA in RNA isolated from different organs and etiolated seedlings.

The mRNA of the alpha-subunit of the chloroplast ATPase accumulates to its higher level in leaf tissue to moderate levels in fruit and stems, and to its lowest level in roots (Table 1, Fig. 5). A similar expression pattern has been previously observed for photosynthesis-specific proteins (Piechulla et al. 1986). Both results together support the notion that genes coding for proteins involved in photosynthesis such as RuBPCase, thylakoid membrane proteins as well as chloroplast ATPase are predominately expressed in green-tissue plant organs. Although we lack the information of protein concentrations and/or activities, the high levels of alpha- and beta-subunits of the mitochondrial ATPase in root tissue indicate that oxidative phosphorylation plays an important role in this particular plant organ. Appreciable levels of the mitochondrial ATPase subunits were also observed in green organs of the plant, perhaps indicating that oxidative phosphorylation occurs concomitant with photophosphorylation. We find that mRNAs of the cytoplasmic fructose-1,6-bisophosphate aldolase accumulate to appreciable levels in tomato fruits (48-d-old fruits), while the level is reduced

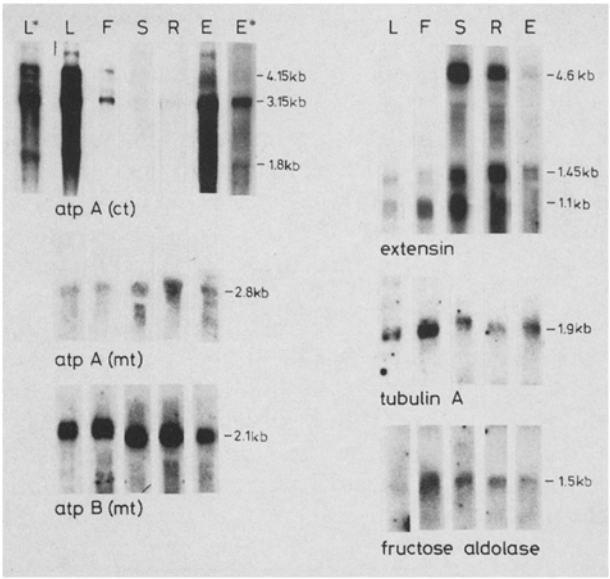

Fig. 5. Determination and comparison of steady-state mRNA levels in different plant organs, leaves (*L*), fruits (*F*), stems (*S*), roots (*R*) and etiolated seedlings (*E*). Three-µg aliquots of total RNA preparations were hybridized with specific probes coding for the alpha- and beta-subunit of the mitochondrial ATPase, the alpha-subunit of the chloroplast ATPase, the alpha-subunit of tubulin, fructose-1,6-bisphosphate aldolase, and the hydroxyproline-rich glycoprotein extensin. Northern blots were exposed with intensifying screens: atpA(mt), 5 d; atpA(ct), 6 d and 7 h (*); atpB(mt), 3 d; fructose-1,6-bisphosphate aldolase, 2 d; alpha-subunit of tubulin, 3 d; and extensin, 3 d. The level of maximum hybridization during fruit development is depicted (see **Fig. 2–4**)

Table 1. Relative transcript levels in different organs of tomato

Gene	Leaf[a]	Fruit[b]	Stem[a]	Root[a]	Etiolated[c] Seedlings
Aldolase	n.d.	100	18.0	39.6	14.7
atpA(ct)	100	22.7	24.1	3.4	45.9
atpA(mt)	34.3	42.7	81.5	91.0	18.1
atpB(mt)	37.8	66.9	91.7	69.6	16.4
Tubulin A	30.0	100	60.0	29.3	60.0
Extensin					
1.45 kb	28.2	23.3	74.4	100	20.4
1.1 kb	25.0	27.5	100	32.3	10.7

[a] Tissue from hydroponically grown plants (approx. 4 weeks old)
[b] Values represent the level of maximum hybridization detected during fruit development, see **Fig. 2–4**
[c] Seedlings were grown for 5 d in darkness

2.5-fold in roots and approx. fivefold in stems and etiolated seedlings. No transcripts coding for this enzyme were detected in leaf tissue. A similar expression pattern was observed for alpha-amylase (Piechulla and Gruissem 1986) using a heterologous hybridization probe from wheat (Lazarus et al. 1985). The mRNAs coding for the hydroxyproline-rich glycoprotein extensin are preferentially expressed in stems and roots; particularly high levels are present of the 4.6-kb mRNA species. This RNA species is not detected in leaves and fruits, while in these organs the 1.45- and 1.1-kb transcripts accumulate to 25–35% of the to high levels found in roots and stems, respectively. Another important structural component of the cell, the alpha-subunit of tubulin, is highly expressed in developing tomato fruits and to a lower extent approx. 30–60% in leaves, stems, roots and etiolated tissue (Table 1, Fig. 5).

Discussion

A wide spectrum of different genes was used to investigate their expression pattern at different stages during tomato fruit development. The results demonstrate differential gene expression, since certain nuclear and organelle genes are transcribed in young green tomato fruits (tubulin A, extensin, alpha-subunit of the chloroplast ATPase), others are expressed preferentially during late developmental stages (fructose-1,6-bisphosphate aldolase), while the alpha- and beta-subunit of the mitochondrial ATPase transcripts can be detected throughout fruit development and ripening. It was of interest whether the appearance and disappearance of specific mRNA species can be correlated with changes that occur in morphology and physiological status during tomato fruit formation.

During the early stages of fruit development (approx. 10 d after anthesis), growth of tomato fruits is the result of cell division, followed by cell enlargement (Davies and Cocking 1965; Asahira et al. 1968) until the fruit reaches the mature green stage. During this period, microtubules are involved in many essential cellular functions, such as the formation of the spindle apparatus, movement of particles and organelles, and the maintenance of cell shape (Dustin 1984). The major protein component of the microtubules in a variety of organisms is tubulin, which is a heterodimer composed of an alpha- and beta-subunit each having a molecular weight of approx. 50 kDa. Tubulin subunits isolated from distantly related organisms copolymerize in vitro, indicating a relative high homology of the protein in different organisms. This homology is also reflected at the nucleotide level (Marks et al. 1987). With a specific probe for the alpha-subunit of tubulin we were able to detect a specific transcript of 1.85 kb in tomato fruits. This size is similar to the transcript length reported for *Chlamydomonas* (Silflow et al. 1985). Tubulin-

specific mRNA is predominately detected in young green tomato fruits, and its appearance thus correlates with the stage of development when cell division and cell enlargement occur. Since no information is available on the actual amounts of the polypeptides and their polymerization behaviour during fruit formation, the level of microtubule involvement in cellular functions at different phases during fruit development cannot be predicted. However, the steady-state mRNA data indicate that less tubulin can be synthesized in older fruits, indicating that the components for cell division and cell structure have been established early during development and, presumably, do not continue to be synthesized in fruits older than 40 d.

The other important structural component extensin, which appears to form networks to stabilize the cell wall, is synthesized as a soluble monomer which is subsequently polymerized into an insoluble matrix (Lamport and Catt 1981). There is evidence that the level of extensin is developmentally regulated (Varner and Cooper 1983). During tomato fruit development the steady-state mRNA levels of the 1.1- and 1.45-kb transcripts alter, and maximum levels are measured in 14- and 40-d-old tomato fruits, respectively. This result indicates that extensin protein is synthesized in growing tomato fruits until the mature green stage is reached. With the onset of ripening, when degradation of the cell wall by polygalacturonase occurs, the mRNAs for extensin as well as tubulin disappear. The question remains open whether the alternating appearance and disappearance of the 1.1-kb and 1.45-kb transcripts during tomato fruit development is based on a mechanism similar to that observed after elicitor treatment of carrot slices. There, the shift from a 1.5-kb to a 1.8-kb transcript is the consequence of the utilization of a transcription start site which is of the same gene but is located further upstream (Chen and Varner 1985b, J. Varner, Dept. of Biology, Washington University, St. Louis, Mo., USA, personal communication). A third transcript of approx. 4.6 kb, which is most likely encoded by different gene(s) (J. Varner, personal communication), is preferentially detected in tomato roots and stems (Fig. 5), suggesting that the structural components deposited in stems and roots are different from those in tomato fruits. Similarly in beans, three transcripts of 1.6, 2.7 and 5.6 kb were identified after elicitor treatment (Lamport and Catt 1981; Chen and Varner 1985a, b; Showalter et al. 1985).

The degradation of the photosynthetic apparatus during ripening is accompained by a decrease of photosynthetic capacity and the disappearance of mRNAs encoding photosynthesis-specific proteins (Piechulla et al. 1987). This is also reflected in decreasing levels of the subunit of the chloroplast ATPase. Here we analysed the steady-state mRNA levels of the chloroplast ATPase subunit during tomato fruit development (green fruit stages). A major transcript of 3.15 kb and two minor mRNAs species of 4.15 kb and 1.8 kb were detected with a probe specific for the alpha-subunit of the chloroplast ATPase. In RNA preparations of leaves of the closely related tobacco plant a single 3.0-kb transcript was identified to code for the alpha-subunit (Deno et al. 1984). To determine the changes of relative transcript levels during tomato fruit development, the measurements were based on the amount of the 3.15-kb transcript (Fig. 2a). The pattern of relative changes of the 4.15-kb and 1.8-kb transcripts parallels the pattern of the 3.15 kb transcript. The function of the two minor RNA species is presently unknown. In spinach, two major mRNA species with 2.6 and 2.3 kb and three minor transcripts of approx. 3.5, 1.6 and 1.4 kb were detected (Westhoff et al. 1985). Together with results of hybrid-selection experiments, Westhoff et al. (1985) concluded that a long precursor, which undergoes stepwise modifications, is transcribed from this region of the spinach chloroplast genome. These mRNA species have not been characterized in more detail and their functions are presently unknown. In developing tomato fruits the transcripts encoding the alpha-subunit ATPase follow an expression pattern similar to that observed for other photosynthesis-specific proteins (Piechulla et al. 1986). Although we do not have information about the protein levels and/or the specific activities of the chloroplast ATPase during fruit formation, these results indicate that photosynthetic activity and photophosphorylation probably reach maximum levels in two- to three-week-old fruits. During ripening, when chloroplasts differentiate into non-photosynthetically active chromoplasts, energy for the de-novo synthesis of enzymes (i.e. polygalacturonase, Grierson et al. 1985; enzymes for lycopene synthesis, Raymundo et al. 1976) and other metabolic reactions (starch and sugar degradation) are required. Relatively high steady-state mRNA levels for the subunits of the mitochondrial ATPase complex during ripening indicate that continuation of ATP synthesis by oxidative phosphorylation during this period. These results are in agreement with the dramatic increase of respiratory activity at the onset of ripening in tomato fruits (Biale and Young 1981).

Concomitant with the respiratory climacteric, decreasing starch content and increasing amounts

of reducing sugars are present in tomato fruits (Davies and Cocking 1965; Ho 1984). During this period, significantly elevated levels of fructose-1,6-bisphosphate aldolase transcripts (Fig. 3) were detected. This enzyme plays a crucial role in glycolysis. It is likely that a certain amount of the reducing sugar will subsequently be converted into fructose-1,6-bisphosphate, the substrate for the aldolase. In roots of maize the aldolase mRNAs appear to be highly inducible under anaerobic conditions, and the induction pattern is similar to other anaerobiosis-inducible genes (e.g. alcohol dehydrogenase; Hake et al. 1985).

As demonstrated in this paper, specific mRNAs appear and disappear at defined stages during fruit formation, indicating that differential expression may be controlled by a developmental program that operates for nuclear and organelle genes at the level of transcription. However, we cannot exclude the possibility that other control mechanisms function at different levels. Presently, it is also not known whether genes which apparently appear and disappear simultaneously during certain growth phases are regulated by identical or different mechanisms.

In the case of photosynthesis-specific genes it has been demonstrated in several plant species that in addition to a developmental control mechanism (Berry et al. 1985; Nelson et al. 1984) a regulation by light is superimposed (Simpson et al. 1986; Fluhr et al. 1986). In addition, the developmental and metabolic states of the chloroplasts play a crucial role in the regulation of nuclear genes involved in photosynthesis. It is also postulated that nuclear and plastid gene expression is coordinated to such extent that not only nuclear gene products enter the chloroplast but the plastids also produce factors which enter and effect the regulation of nuclear photosynthesis-specific genes (Simpson et al. 1985). The question remains open as to the role which all these regulatory factors play in the activation and inactivation of genes that are not directly involved in photosynthetic reactions (i.e. tubulin, extensin, aldolase, mitochondrial ATPase). It seems possible that, for these genes, other factors or levels, for example metabolites, nucleotides, polyamines and hormones, may cause alterations at the level of transcription or translation.

This work was performed in the laboratory of Dr. W. Gruissem, and supported by a grant from National Institute of Health (GM 33813) to him and a fellowship from the Deutsche Forschungsgemeinschaft to B.P. (Pi 153/1-1). For generously providing me with clones, I thank Dr. E. Penhoet (Dept. of Biochemistry, University of California, USA) for the fructose-1,6-bisphosphate aldolase clone, Dr. M. Boutry and Dr. N.H. Chua (Laboratory of Plant Molecular Biology, The Rockefeller University, New York, NY, USA) for the mitochondrial ATPase beta-subunit clone, P. Webster and Dr. S. Long (Department of Biological Sciences, Stanford University, Stanford, Calif., USA) for the clone specific for the alpha-subunit of tubulin, Dr. C. Braun and Dr. S. Levings III. (Department of Genetics, North Carolina State University, Raleigh, NC, USA) for the mitochondrial ATPase A clone, and Dr. J. Varner (Department of Biology, Washington University, St. Louis, MO., USA) for the extensin clone.

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Received: 15 September; accepted 24 November 1987