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Diurnal mRNA fluctuations of nuclear and plastid genes in developing tomato fruits

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Steady-state transcript levels of nuclear (*rbc*S, *cab*) and plastid (*rbc*L, *psb*A) encoded photosynthesis-specific genes were determined at noon and 05.00 h in different developmental stages of tomato fruits (7–35 days after anthesis). Small alterations are observed in mRNA levels for the small subunit (ssu) and large subunit (lsu) of RuBPC/Oase and the Q_B-binding protein of photosystem II at these two time-points, while significant steady-state transcript level fluctuations are detectable for the light harvesting complex protein. LHCP II transcripts accumulate during the day, and decline to low levels during the night. In contrast, the LHC II protein levels remain constant during the same period of development. A detailed analysis of transcript levels of the nuclear and plastid genes at 4-h intervals throughout a 38-h period demonstrates that LHCP II mRNA accumulation is highest at noon and lowest at 04.00 h. The transcripts of the ssu and lsu of RuBPC/Oase, photosystem I and II reaction center proteins, as well as the β-subunit of the mitochondrial ATPase and the β-subunit of tubulin, accumulate during the night and decrease to low levels in the afternoon. The transcript levels of the genes examined in this study fluctuate with certain periodicities. We suggest that gene expression in developing tomato fruits is at least partially controlled by diurnal rhythms, which are therefore also operational in other organs, besides leaves, of higher plants.

Key words: tomato (*Lycopersicon esculentum*) fruit development/LHCP gene expression/mRNA levels fluctuations/diurnal cycles

Introduction

Several morphological and physiological alterations are involved in the development and ripening process of tomato fruits (Harris and Spurr, 1969a,b). Detailed analysis at the molecular level demonstrates that a number of specific mRNAs appear and disappear in correlation with morphological and physiological changes (Grierson *et al.*, 1985; Manssen *et al.*, 1985; Piechulla *et al.*, 1985; 1986; Piechulla and Gruissem, 1986; Smith *et al.*, 1986; B.Piechulla, in preparation). During early stages of fruit development elevated mRNA levels are detectable for several proteins, e.g. the α- and β-subunit of tubulin, and the chloroplast and mitochondrial ATPase subunits. In contrast, other mRNAs and proteins, such as polygalacturonase and the fructose-1,6-bisphosphate aldolase, appear only at the onset of tomato fruit ripening. The mRNA levels for several proteins of photosystem I and II, as well as mRNAs for stromal enzymes which are required for photosynthetic function, are present at their highest levels 2–3 weeks after anthesis, but decrease to low or non-detectable levels before or during the fruit ripening. The expression of photosynthesis-specific nuclear and plastid genes is correlated with the presence of the respective proteins and the photosynthetic activity of the organelle in green tomato fruits (Piechulla *et al.*, 1987).

We wish to determine the mechanisms that control gene expression during the early phase of fruit development. Since this period is characterized by the presence of photosynthetically active chloroplasts, we have primarily focused on the study of genes for photosynthesis-specific proteins. The expression of two sets of genes was characterized in more detail. These include genes for thylakoid membrane proteins (Q_B-binding protein, *psb*A; P700 reaction center protein of PS I, *psa*A; PS II protein, *psb*B; light-harvesting chlorophyll *a/b* binding protein, *cab*) and genes encoding the stromal RuBPC/Oase [large subunit (lsu), *rbc*L; small subunit (ssu), *rbc*S]. Light is known to be involved in the regulation of ssu of RuBPC/Oase and LHCP gene expression in several plants. Several *cis*-acting DNA sequences have been identified that may participate in the light-regulated expression of these genes (Coruzzi *et al.*, 1984; Fluhr *et al.*, 1986; Herrera-Estrella *et al.*, 1984; Morelli *et al.*, 1985; Simpson *et al.*, 1986b; Timko *et al.*, 1985). In addition to light, gene activation and inactivation is also affected by developmental program in leaves of monocots (Nelson *et al.*, 1984) and cotyledons of dicotyledonous plants (Berry *et al.*, 1985; Sugita and Gruissem 1987).

However, the molecular mechanisms that control the developmental expression of photosynthesis-specific genes is less well understood. In addition, little information is currently available about the extent to which light and/or developmental control regulate the expression of these genes in other organs of higher plants besides leaves, or if the controls operational in leaves are also conserved in other photosynthesis-active plant organs. The tomato fruit is a well-suited organ to study these questions in more detail. We report here that the accumulation of tomato mRNAs for photosynthesis-specific proteins is regulated by a developmental programme during a defined period of tomato fruit formation. However, fluctuations in the mRNA levels during day/night cycles are superimposed on this developmental program. Such fluctuations are characteristic for mRNAs encoding different photosynthesis-specific proteins, and allow us to categorize the genes into two classes. Based on our results we suggest that light and/or diurnal cycles also control the mRNA levels of photosynthesis-specific genes in other organs of higher plants besides leaves.

Results

We have shown in earlier experiments that the mRNAs for photosynthesis-specific proteins follow a characteristic pattern of accumulation and decrease during tomato fruit development (Piechulla *et al.*, 1986). It appears that the expression of these genes is regulated by a developmental programme which causes gene activation and inactivation at specific developmental stages.

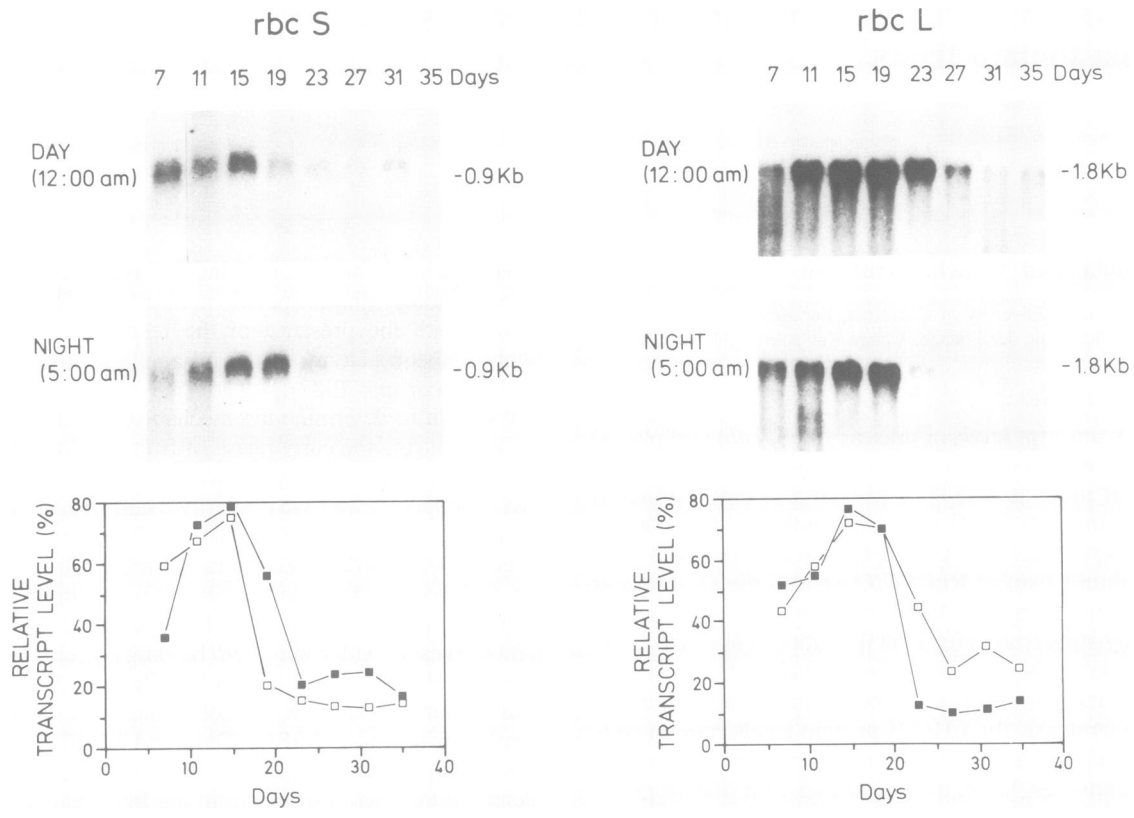

Fig.1. Upper panels: mRNA levels of the small (*rbc*S, **left panel**) and large (*rbc*L, **right panel**) subunits of RuBPC/Oase in total RNA preparations from different developmental stages of tomato fruits collected at noon and 05.00 h. The filters were exposed for 5 days (*rbc*S) and 20 h (*rbc*L) with intensifying screens at −70°C. The lengths of the transcripts are indicated. **Lower panels:** Correlation of relative transcript levels of the small (*rbc*S) and large (*rbc*L) subunit of RuBPC/Oase during tomato fruit development at day (□) and night (■). Relative mRNA levels were calculated based on densitometer scannings of six autoradiograms.

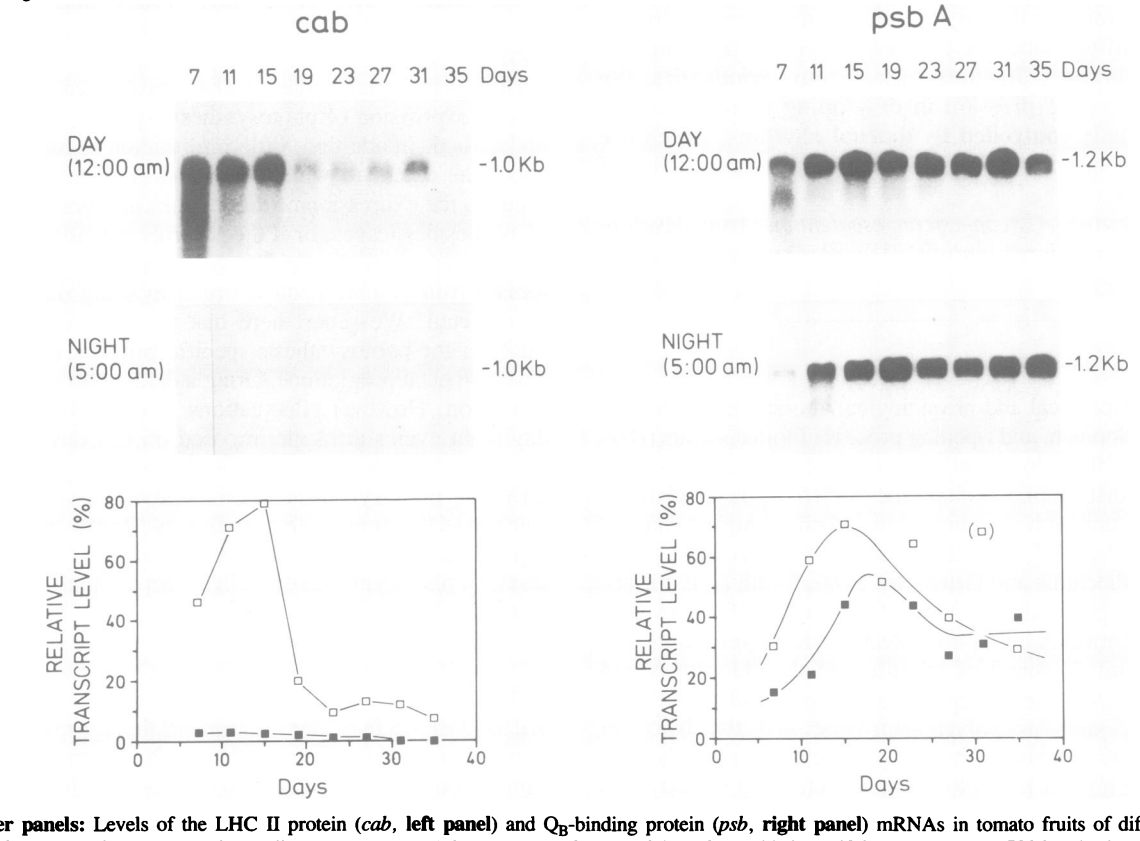

Fig. 2. Upper panels: Levels of the LHC II protein (*cab*, **left panel**) and Q_B-binding protein (*psb*, **right panel**) mRNAs in tomato fruits of different developmental stages and two time-points. Filters were exposed for 4 days (*cab*) and 10 h (*psb*A) with intensifying screens at −70°C. The lengths of the transcripts are indicated. **Lower panels:** Correlation of relative transcript levels of the LHCP II (*cab*) and Q_B-binding protein (*psb*A) during tomato fruit development at day (□) and night (■). Relative mRNA levels were calculated based on densitometer scannings of four autoradiograms.

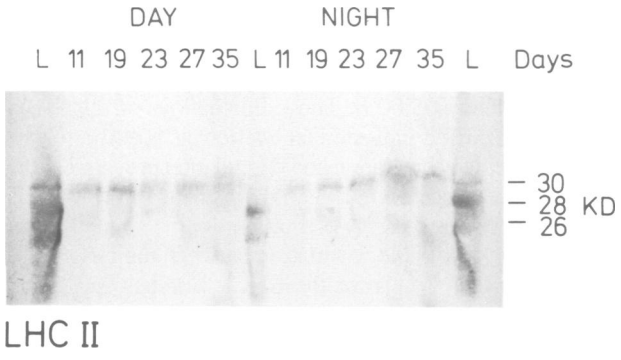

Fig. 3. Identification of the LHC II proteins in leaves (L) and fruits. Twenty micrograms of protein extracts from leaves and whole fruits of different developmental stages harvested at day and night were separated on a SDS–polyacrylamide gel (12.5%), blotted to nitrocellulose and analyzed with homologous LHC II-specific antibodies. The relative molecular masses of the identified proteins are indicated.

However, it is possible that during the photosynthetically active phase of fruit development most or all photosynthesis-specific genes are also controlled by light. To distinguish between a developmental programme and a possible superimposed light regulation, we applied two different experimental strategies. In the first series of experiments we analyzed the effect of light on the steady-state mRNA levels throughout the period of tomato fruit development during which most of the photosynthesis-specific mRNAs are detectable. In the second set of experiments the role of light in the control of photosynthetic gene expression was evaluated for one stage of fruit development over a 38-h period. We have included two non-photosynthesis-specific nuclear genes in these experiments to determine the specificity of modulations at the mRNA levels for photosynthesis-specific genes that may be controlled by light. The photosynthesis-specific genes analyzed in these studies include the lsu (*rbc*L) and ssu (*rbc*S) of ribulose-1,5-bisphosphate carboxylase (RuBPC/Oase), the light-harvesting chlorophyll a/b binding proteins (LHCP, *cab*), the reaction center protein of photosystem I (*psa*A), the Q_B-binding protein of photosystem II (*psb*A) and the reaction center protein of photsystem II (*psb*B).

Light/dark modulation of photosynthesis-specific mRNA levels during tomato fruit development

Northern blots were prepared from total RNA isolated from fruit of different developmental stages. Fruits were picked at noon and 05.00 h and used for RNA isolations as described in Materials and methods. Hybridization of the Northern blots with the *rbc*L-coding region and the *rbc*S2A cDNA clone reveal the characteristic transcripts of 1.8 and 0.9 kb respectively (Figure 1; Piechulla *et al.*, 1986). Relative transcript levels of ssu and lsu of RuBPC/Oase at day and night are shown in Figure 1 (lower panels). The transcripts are present at relatively high levels in early stages in fruit development, reach maximum levels ~15 days after anthesis and decline during further growth and ripening. Only small differences of the steady-state mRNA levels at noon (day) and 05.00 h (night) at different stages of fruit development are observed for both the *rbc*L gene and *rbc*S gene families, although the *rbc*L transcript level is consistently higher compared with the *rbc*S (Piechulla *et al.*, 1986).

In addition to the mRNA levels of the stromal RuBPC/Oase, the transcript levels of two thylakoid membrane proteins, the nuclear-encoded light-harvesting chlorohyll *a/b* binding protein (LHCP II) and the plastid encoded Q_B-binding protein were determined. It has been suggested the LHCP mRNA levels in

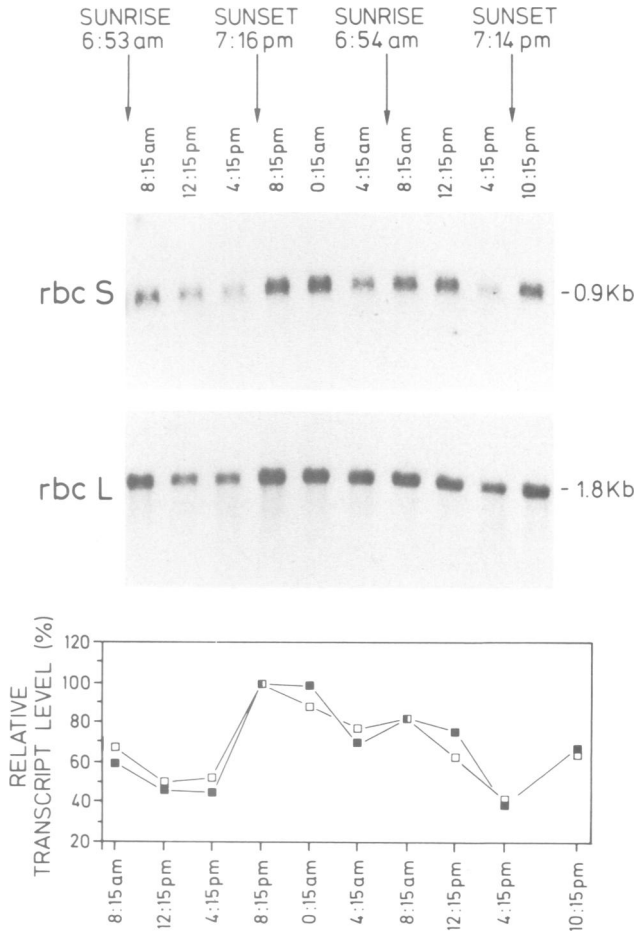

Fig. 4. Identification of transcripts for the small (*rbc*S, **upper panel**) and large (*rbc*L, **middle panel**) subunits of RuBPC/Oase in 14- to 15-day-old tomato fruits at different time-points during a 38-h period. Filters were exposed with intensifying screens at −70°C for 2 days (*rbc*S) and 18 h (*rbc*L). Transcript sizes are indicated. **Lower panel:** Relative transcript levels of the small (*rbc*S, ■) and large (*rbc*L, □) subunit of RuBPC/Oase were calculated based on densitometer scannings. The relative transcript levels are expressed as percent of the maximum level for each individual transcript. The graph does not reflect the relative steady-state levels of different transcripts detected in total RNA.

several plant species are strictly controlled by light in cotyledons and leaf tissue, since no mRNAs can be detected in the dark (for review see Tobin and Silverthorne, 1985). However, the LHC II proteins do not follow this pattern and are present in both light and dark. This suggests a stringent control of LHCP II gene expression by transcriptional or post-transcriptional mechanisms. In contrast, the expression of the Q_B-binding protein appears to be controlled at the translational level, since significant differences have been reported for the synthesis of this protein in the dark and light, which do not correlate with changes at the mRNA level (Fromm *et al.*, 1985). Hybridization of probes specific for the LHCP II and the Q_B-binding protein genes to total RNA from tomato fruit of different developmental stages collected at noon and 05.00 h reveal characteristic transcripts of 1.0 and 1.2 kb respectively (Figure 2; Piechulla *et al.*, 1986). The highest mRNA levels for the *cab* and psbA transcripts are observed in 11- to 15-day-old tomato fruits which were harvested at noon. In older tomato fruits the mRNA levels decrease and remain low during further fruit development. The transcript levels of both the LHCP II gene family and the Q_B-binding protein gene were compared with mRNA levels in fruits of comparable developmen-

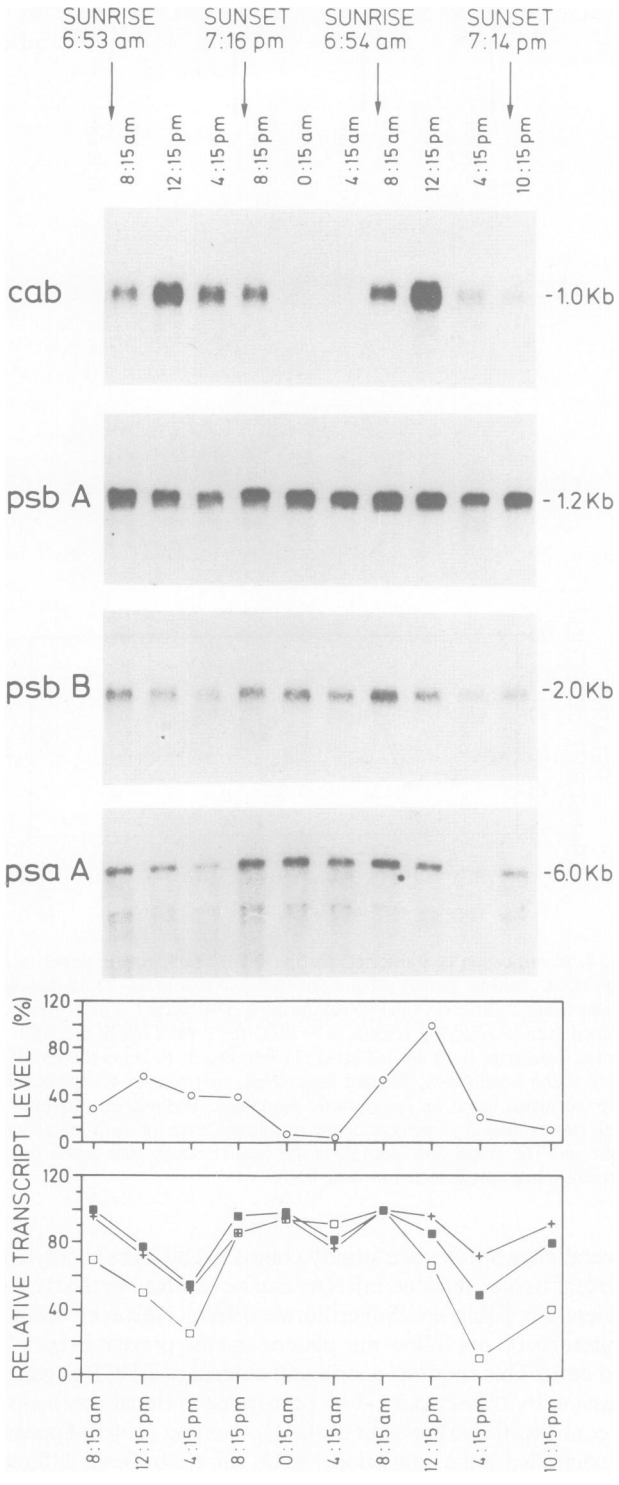

Fig. 5. Identification of transcripts for the LHCP II (*cab*, **upper panel**), the Q_B-binding protein (*psb*A), the P680 reaction of PS II (*psb*B), and the P700 reaction center of PS I (*psa*A) in 14- to 15-day-old tomato fruits at different time-points during a 38-h period. Filters were exposed with intensifying screens at −70°C for 2 days (*cab*), 18 h (*psb*A), 11 h (*psb*B), 11 h (*psa*A). Transcript sizes are indicated. **Lower panel**: relative transcript levels of the LHCP II protein (*cab*, ○), Q_B-binding protein (*psb*A, +), the P680 reaction center protein of PS II (*psb*B, ■), and the P700 reaction center protein of PS I (*psa*A, □) were calculated based on densitometer scannings. The relative transcript levels are expressed as percent of the maximum level for each individual transcript. The graph does not reflect steady-state levels of different transcripts detected in total RNA.

tal stages harvested at 05.00 h. Significant differences in transcript levels between these two time-points are observed for LHCP II. High mRNA levels are present at noon and mRNAs are not detected at 05.00 h. These fluctuations of mRNA levels within a 24-h period indicate that transcripts specific for LHCP II underlie rapid accumulation and degradation processess. In contrast, no significant differences in the *psb*A transcript levels are detectable between these two time-points.

To establish a possible correlation between the observed fluctuations in mRNA levels for the LHCP II genes and their protein products, we determined the protein levels of LHC II in tomato fruits of the same developmental stages harvested at noon and 05.00 h. Total protein preparations from leaves and fruits were separated on a SDS−polyacrylamide gel and analyzed on a Western blot (Figure 3). Homologous antibodies against LHC II proteins of tomato react with three proteins of relative molecular masses 30, 28 and 26 kd in tomato leaf preparations. In fruit protein preparations only the 30-kd protein is present. The concentration of this protein does not vary significantly in fruits of different developmental stages harvested at noon and 05.00 h. Taken together, these results suggest that the expression of LHCP II genes in tomato fruits is most likely controlled at the transcriptional and/or post-transcriptional level, and that this control mechanism is maintained at different developmental stages. Since LHCP II mRNA accumulation is highest during the day, it appears that light-dependent changes of LHCP II mRNA levels are superimposed on the developmental control of LHCP II gene activation and inactivation during fruit formation.

Transcript levels in tomato fruit during a diurnal cycle

The differences observed for the LHCP II mRNA level at noon and 05.00 h during fruit development may reflect the light-regulated expression of this gene class, which is also operational in tomato fruit pericarp. However, we can not exclude the possibility that the changes in LHCP II mRNA levels are part of a more complex expression pattern that is established during a diurnal cycle, which also regulates the expression of other photosynthesis-specific genes. To characterize more precisely the increase and decline in LHCP II mRNA levels, and to compare these fluctuations with potential changes in mRNA levels of other photosynthesis-specific plastid and nuclear genes and gene-families, we collected 14- to 15-day-old tomato fruits at 4-h intervals over a 38-h period. As shown above, at this developmental stage the mRNA levels are highest for most photosynthesis-specific genes, and maximal differences are observed for LHCP II mRNA levels between noon and 05.00 h.

Photosynthesis-specific genes

Northern blots were hybridized with gene probes specific for ssu and lsu of RuBPC/Oase and relative mRNA concentrations were determined (Figure 4). Fluctuations of *rbc*S and *rbc*L transcript levels are detected throughout a 24-h period. Both mRNA levels decrease to low levels during the afternoon (16.15 h), but accumulate within 4 h to high levels (20.15 h), after which mRNA levels continuously decrease to low levels in the afternoon of the next day. These experiments also demonstrate that the accumulation and degradation pattern of ssu and lsu mRNAs is very similar within a 24-h period, which supports the notion of a closely coordinated regulation of these mRNAs in developing tomato fruits.

In addition to the subunits of the RuBPC/Oase, the steady-state mRNA levels of several thylakoid membrane proteins, the nuclear

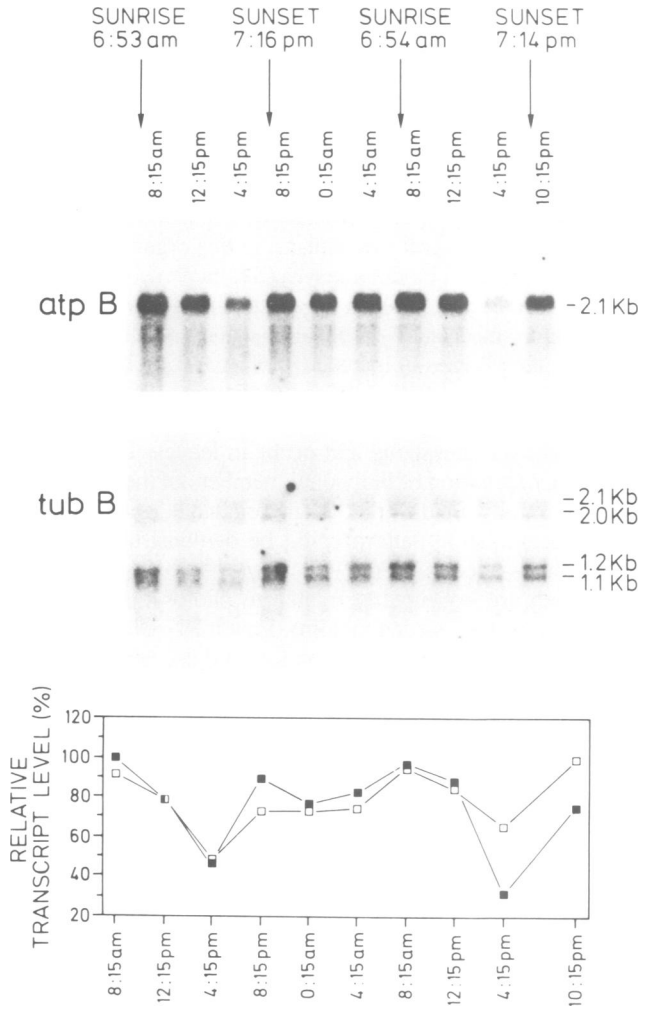

Fig. 6. Identification of transcripts for the β-subunit of the mitochondrial ATPase (*atp*B, **upper panel**) and the β-subunit of tubulin (*tub*B, **middle panel**) in 14- to 15-day-old tomato fruits at different time-points during a 38-h period. Filters were exposed with intensifying screens at −70°C for 4 days (*atp*B and *tub*B). Transcript sizes are indicated. **Lower panel:** Relative transcript levels of the β-subunit of the mitochondrial ATPase (*atp*B, ■) and β-subunit of tubulin (*tub*B, □) were calculated based on densitometer scannings. The relative transcript levels are expressed as percent of the maximum level for each individual transcript. The graph does not reflect the relative steady-state levels of different transcripts detected in total RNA.

encoded LHCP II (*cab*), and plastid encoded Q_B-binding protein (*psb*A), the P680 reaction center of PS II (*psb*B) and the P700 reaction center of PS I (*psa*A) were monitored during a 38-h period (Figure 5). Significant changes are again detected for the LHCP II transcripts, while fluctuations of the plastid encoded transcripts are only marginal. The highest LHCP II mRNA levels are present at noon, after which they continuously decrease during the evening and night until no mRNA can be detected at 04.15 h. During the following 8-h period the mRNAs accumulate to their highest levels. These results indicate that LHCP II mRNAs are degraded and newly synthesized over a 24-h period, suggesting a high turnover rate of < 12 h in developing tomato fruits. In contrast, a different expression pattern is observed for the three plastid genes encoding photosynthesis-specific thylakoid membrane proteins. Accumulation of transcripts is detectable between 16.15 and 20.15 h, after which the levels remain nearly constant during the night, and then decrease to the lowest levels in the early afternoon. It is interesting to note that within this pattern a small, but consistent, increase in mRNA levels for *psa*A, *psb*A and *psb*B is observed at 08.15 h. In general, however, the diurnal changes of transcript levels for these genes are comparable with the pattern found for the *rbc*S/*rbc*L genes.

Genes for non-photosynthesis-specific proteins

In addition to photosynthesis-specific genes we were interested in determining whether genes coding for other cellular functions are also subject to diurnal mRNA fluctuations. Specific DNA fragments of two nuclear encoded genes, the β-subunit of the mitochondrial ATPase and the β-subunit of tubulin, were used as hybridization probes. The ATPase β-subunit probe hybridizes to transcripts of 2.1 kb (B.Piechulla, in preparation), while four transcripts of 2.1, 2.0, 1.2 and 1.1 kb are observed with the tubulin B hybridization probe (Figure 6). The two transcripts of smaller mol. wt (1.2 and 1.1 kb) are more abundant than the two larger transcripts. The mRNA levels of the β-subunit of tubulin and the β-subunit of the mitochondrial ATPase were determined in tomato fruits harvested at 4-h intervals over a 38-h period, and relative mRNA levels were calculated and plotted (Figure 6). The calculation of relative mRNA levels for tubulin B are based on the measurements of the 1.1- and 1.2 kb transcripts. Similar to the expression pattern of *psb*A, *psb*B, *psa*A, *rbc*L and *rbc*S, we detected reduced mRNA levels in the afternoon, after which the levels increase over the next 4-h period, and only small fluctuations occur during the following night and morning hour. These results suggest that mRNAs encoding proteins of non-photosynthetic function also fluctuate in their levels and thus may be controlled by a general diurnal cycle in tomato fruits.

Discussion

The influence of light on the expression of plastid- and nuclear-encoded photosynthesis-specific genes is often examined by applying the following strategies to plants: (i) seedlings are grown for 7−10 days in darkness prior to illumination or (ii) plants or transgenic plants are kept under continuous dark conditions for 4−5 days and are then transferred into light or vice versa. The latter treatment is artificial, and one has to expect that such experimental conditions are also reflected in the expression behavior of genes. We were interested in evaluating the dark/light effect in tomato plants grown under physiological conditions in the greenhouse. The conditions are defined by a natural day/night cycle, no supplemental light conditions or extended dark periods. Under these conditions, mRNA accumulation was compared in tomato fruits harvested at various time-points during the day and night. The analysis of steady-state mRNA levels in the work reported here does not distinguish between transcriptional and post-transcriptional regulatory events. The observed fluctuations of mRNA levels could result from: (i) a constant transcription initiation/elongation rate and differences in specific RNAase activities or RNA decay kinetics, (ii) there being no differences in RNA stability but variations in transcription initiation/elongation rates or (iii) a combination of the above regulatory mechanisms. Although none of the mechanisms can be discounted, it is still possible to define periods during the day/night cycle in tomato fruits in which genes are transcribed or RNA decay exceeds its accumulation.

Diurnal cycle I

LHCP II transcripts are not detectable in fruit pericarp and locular

tissue in the dark, but accumulate to high levels in the light. Previous experiments also demonstrated the absence of these transcripts in etiolated tomato cotyledons (Piechulla *et al.*, 1986). These results, together with studies in several other plant species (Tobin and Silverthorne, 1985), suggest that expression of LHCP II genes in tomato is regulated by light. This conclusion is further substantiated by our results obtained from a detailed analysis of mRNA level alterations within a 24-h period (Figure 5). Concomitant with the time-point of sunrise, high levels are reached ~6 h after sunrise. During the afternoon hours, LHCP II mRNAs rapidly decline and are not detectable during the night. In addition to the described results with tomato fruits, similar mRNA level fluctuations are detected in tomato leaves (data not shown), demonstrating that this is not an organ-specific phenomenon. Similarly, Kloppstech (1985) finds LHCP mRNA present at higher levels in pea and maize seedlings during the morning, followed by a decline in the evening. However, LHCP transcripts of maize and pea are already present at elevated levels 2 h prior to illumination and do not decrease to undetectable levels. The reason(s) why light does not continue to stimulate the expression of LHCP II genes in the afternoon—in tomato fruits and leaves, and in leaves of other plants (Kloopstech, 1985)—are presently unknown. It is possible that diurnal alterations of the light composition cause such effects, since red wavelength light components are more abundant in the morning than the evening, when the blue wavelength light components increase (Holmes and McCartney, 1976; Holmes and Smith, 1977). Phytochrome has been demonstrated to influence LHCP gene expression in several plants at the transcription level (Tobin and Silverthorne, 1985). Therefore, it is possible that the activation of LHCP II genes in tomato fruits and leaves is due to the lower P_{R660}/P_{FR730} ratio in the morning. Also, other experiments indicate that three LHCP II genes (*cab*1, 4, 5; Pichersky *et al.*, 1985; 1987a) and one LHCP I (*cab*6a; Pichersky *et al.*, 1987b) gene of the *cab* multigene family follow the same expression pattern, and therefore might be controlled by a similar mechanism (B.Piechulla, unpublished results).

In contrast to the mRNA level fluctuations no significant alterations of the LHC II protein concentrations (Figure 3) or visible loss of chlorophyll were observed when tomato fruits collected during the day and at night were compared. Since the antibodies used to detect LHC proteins in tomato protein extracts are specific for proteins associated with PS II, it is still possible that LHC I protein concentrations alter during the day/night cycle.

Diurnal cycle II

In contrast to the fluctuations of the LHCP II mRNA levels, a different pattern is observed for most of the other genes encoding photosynthesis-specific components (Figures 4 and 5; *rbc*S, *rbc*L, *psa*A, *psb*A, *psb*B), as well as non-photosynthesis-specific genes (Figure 6; β-subunit of the mitochondrial ATPase and β-subunit of tubulin). First, their mRNA level fluctuations are only marginal compared with the LHCP mRNA level alterations; second, the time-points of maximum and minimum mRNA levels are different; and finally, the time-point of mRNA level increase is different from the time-point of sunrise. The latter is particularly interesting, since light has been demonstrated to modulate the *rbc*S gene expression in tomato leaves and etiolated seedlings after illumination (Piechulla *et al.*, 1986; Sugita and Gruissem, 1987). Similar effects were observed in leaves in wild-type (soybean, pea, tobacco, lemna) and transgenic plants (tobacco) (Morelli *et al.*, 1985; Timko *et al.*, 1985; Fluhr *et al.*, 1986; Simpson *et al.*, 1986a,b), while in barley and mung bean light does not cause increased mRNA accumulation (Tobin and Silverthorne, 1985). The discrepancy that sunlight does not appear to stimulate the accumulation of *rbc*S mRNAs in tomato fruits, but *rbc*S gene expression is clearly under light-control in leaves, may be explained as follows. First, the light effect on the expression of *rbc*S genes in fruits may be delayed for several hours; second, sunlight may induce different responses than artificial light sources; and third—and this explantation seems most likely—the two genes expressed in tomato fruits (*rbc*S1 and *rbc*S2) underlie different control mechanisms in this organ as compared with their expression mode in leaves (Sugita *et al.*, 1987; Sugita and Gruissem, 1987). Such an organ-specific and light-independent regulatory mechanism would also be consistent with our finding that all three genes of locus 3 (*rbc*S3A, 3B, 3C) are inactive in tomato fruit pericarp (Sugita and Gruissem, 1987). This may also account for the small mRNA fluctuations in fruits versus more significant alterations that occur in leaves. In addition to the specific expression of individual members of the tomato *rbc*S multigene family in different plant organs, a general diurnal mRNA accumulation pattern could be demonstrated for other photosynthesis-specific genes (*rbc*L, *psa*A, *psb*A, *psb*B) and genes which are not directly related to photosynthesis activity (mitochondrial ATPase and tubulin). Low mRNA levels are consistently measured in the afternoon for all these genes, followed by increased mRNA accumulation in the evening. The role of light, if any, in this expression pattern is still unclear and requires additional analysis.

Diurnal and circadian rhythms in plants, such as flower and stomata closing and opening, and leaf movements, have been observed since 1729 (Salisbury and Ross, 1977). More recently changes in enzyme activities (e.g. RuBPC/Oase in tomato fruits and leaves; Laval-Martin and Farineau, 1977; Farineau and Laval-Martin, 1977) and other plants (Servaites *et al.*, 1984), and metabolite concentrations (e.g. fructose-2,6-bisphosphate and sucrose; Stitt *et al.*, 1984; 1986), were observed during a day/night cycle. However, a diurnal or circadian cycle at the molecular level has only been demonstrated in pea for the mRNAs of the ssu of RuBPC/Oase, LHCP and a protein of unknown function (elip) (Kloppstech, 1985). The results presented in this report support a model of diurnal rhythms for six photosynthesis-specific and two non-photosynthesis-specific genes in developing tomato fruits. Nuclear- and plastid-encoded genes follow a similar mRNA fluctuation pattern, indicating that a general control mechanism may be responsible for regulating the expression of genes in different cellular compartments. A divergent pattern is observed for LHCP II mRNA accumulation, which may indicate a stringent control of this gene class by light superimposed on diurnal changes. The mechanisms that are involved in the control of diurnal and circadian rhythms at the molecular level are unknown. Our experiments demonstrate, however, that diurnal cycles are established in different organs of higher plants. The regulatory mode(s) that may be operational as part of such cycles are superimposed on developmental decisions that control the activity of different gene classes in tomato fruits.

Materials and methods

Plant material and tissue preparation

Tomato plants (*Lycopersicon esculentum*, cv. VFNT LA 1221, cherry line) were grown at UC Berkeley under greenhouse conditions without additional light and at temperatures between 20 and 25°C. Fruits for the 'day/night' experiments were harvested at different developmental stages after anthesis (7, 11, 15, 19, 23, 27, 31, 35 days) at noon (7.5 h after sunrise) and at 05.00 h (8.5 h after sunset) in June/July 1986. For the 'diurnal rhythm' experiments fruits at the developmental stage 14/15 days were harvested at 4-hour intervals 08.15, 12.15, 16.15, 20.15, 00.15, 04.15, 08.15, 12.15, 16.15 and 22.15 h (sunrise 06.54 h; sunset 19.14 h). During the night, when fruits were harvested (September 1986), a full moon was

shining. Intact fruits were immediately frozen in liquid nitrogen and stored at −70°C.

Isolation of RNA

Two to four grams of tissue, a mixture of tissue pieces from approximately five different tomato fruits, was used to isolate total RNA. The volume of buffers were reduced with respect to the amount of tissue according to the isolation procedure described previously (Piechulla *et al.*, 1986). For the 'day/night' experiments, RNA from pericarp and locular tissue was isolated separately. Since no significant differences were detected in the expression pattern of different genes between the two tissue types, the data in Figures 1 and 2 therefore reflect average values of relative transcript levels. For the 'diurnal cycle' experiments, RNA was extracted from whole fruits, and RNA was prepared from each time-point within the 38-h period. The method typically yielded 100−800 μg total RNA/g tissue.

Preparation of hybridization probes

Specific gene probes were used for the hybridizations. The plasmids pTB1 (*rbc*L), pTB8-P (*psb*A), pSoc1080 (*psa*A_1/A_2), p3-41 (*rbc*S2A), pIA27 (*cab*1B), pHA2 (rDNA) have been described previously (Piechulla *et al.*, 1986). The plasmid pATB4 has a 1.2-kb *Eco*RI−*Hin*dIII nuclear DNA fragment of *Arabidopsis thaliana* inserted into pUC19, on which a 1.0-kb *Kpn*I−*Bam*HI fragment is specific for an internal sequence of the tubulin B gene (Marks *et al.*, 1987). The plasmid cNp10 has a 1.5-kb cDNA inserted into EMBL 12, which codes for the nuclear-encoded β-subunit of the tobacco mitochondrial ATPase (Boutry and Chua, 1985). A 1.3-kb *Sal*I−*Eco*RI fragment of the spinach chloroplast genome coding for the Q_B-binding protein of PS II was cloned into pUC8 (D.Stern and W.Gruissem, unpublished results). The cloned inserts were isolated by preparative digestion of the plasmid DNA with the appropriate restriction enzyme(s). DNA fragments were separated by gel electrophoresis and the fragments were isolated by electroelution. The reisolated DNA was purified by phenol/chloroform/isoamyl alcohol extractions and used for nick translations. The specific activity was 10^6-10^7 c.p.m./μg DNA.

Analysis of RNA

RNA (3 μg) was separated in formaldehyde agarose gels as described by Maniatis *et al.* (1982). The amounts of RNA applied to the gel were standardized by spectrophotometric quantitation, quantitation of the ethidium bromide fluorescence of cytoplasmic rRNA in stained gels, and relative levels of hybridization with heterologous cytoplasmic rDNA (Piechulla *et al.*, 1986). The RNA was transferred to a nylon filter (Amersham, Hybond N) and fixed by exposing the membrane to UV light for 4−5 min. 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 polyvinylpyrollidone 40, 0.1 g BSA in 500 ml H_2O). 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 litre 2 × SSC (three times, 15 min) and 1 litre 1 × SSC (three times, 10 min) and exposed to X-ray film (−70°C, with intensifying screen).

Autoradiograms for different exposure times, obtained from Northern blot hybridizations, were scanned with a Joyce Loebl densitometer. Relative amounts of mRNAs were determined by peak-area measurements. Relative mRNA levels shown in Figure 1 and 2 are an average of 4−6 hybridizations. In Figures 4, 5 and 6 data are based on one hybridization.

SDS-PAGE and immunreaction

To isolate proteins from tomato fruit tissue the procedure described by Coruzzi *et al.* (1984) was followed. Frozen tissue from tomato fruits collected at different developmental stages (11, 19, 23, 27, 35 days after anthesis) at noon and 05.00 h was disrupted with a mortar and pestle in liquid nitrogen. One half volume of buffer (50 mM Tris, pH 7.5; 5 mM EDTA; 20 μM PMSF) and 1/10 volume 20% SDS was added to the tissue powder. The suspension was shaken at room temperature for 10 min, heated to 65°C for 10 min and filtered through miracloth. The proteins from this extract were precipitated with 70% $(NH_4)_2SO_4$ (w/v). The precipitate was collected by centrifugation (10 min, 5000 *g*), resuspended in a small volume of TE-buffer (10 mM Tris, pH 7.5; 1 mM EDTA), dialyzed against TE-buffer and TE-buffer with 20% glycerol, and stored frozen at −20°C. Proteins were separated in SDS−polyacrylamide gels (12.5%), and LCH II proteins were identified using specific antibodies as described previously (Piechulla *et al.*, 1987).

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