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Changes of the diurnal and circadian (endogenous) mRNA oscillations of the chlorophyll a/b binding protein in tomato leaves during altered day/night (light/dark) regimes

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Abstract

Characteristic steady-state mRNA level oscillations were monitored for the chlorophyll a/b-binding (cab) protein in tomato plants grown under the natural day/night (light/dark) regime as well as under constant environmental conditions. This typical expression pattern was altered when plants were transferred to different light/dark regimes. For example, by shifting the light phase by six hours, a change of the time points of maximum and minimum of expression level was monitored, while the principal oscillation pattern remained the same. It appeared that the transition from dark to light is involved in determining the time points of minima and maxima of mRNA accumulation.

After exposing tomato plants to an abnormal light/dark periodicity (e.g. six hours of alternating light/dark) an altered oscillation pattern was determined: within 24 hours two maxima of cab mRNA levels were detected. However, this 'entrained' abnormal rhythm was not manifested at the molecular level and the circadian pattern reappeared under constant environmental conditions (e.g. darkness). This result favours the hypothesis that the oscillation pattern of the cab mRNA in tomato plants is not only endogenous but also hereditary.

Introduction

Biological rhythms are believed to exist in all eukaryotic organisms (e.g. cycles of activity and rest, body temperature, evoked responses) [6, 8]. In higher plants, several biochemical and physiological oscillations are the result of endogenous rhythms (for review, see [30]). 'Endogenous rhythm' is used to describe periodic processes that occur even when all periodic environmental cues associated with the solar day are excluded [8]. In nature, these rhythms are entrained to a 24-hour period (circadian) by environmental cues, the so-called 'Zeitgeber'. The best studied 'Zeitgeber' is the light/dark cycle directly associated with the movement of the sun. Examples of circadian rhythms in plants are the periodic up- and downward movement (nyctinastic motion) of leaves in *Phaseolus* and certain other legumes (for reviews, see [4, 22]), the diurnal motions of the petals of *Kalanchoe blossfeldiana* [5], the clock-controlled synthesis and degradation of luciferase in *Gonyaulax polyedra* [9], or the changes of net photosynthesis and accumulation rates of starch

and soluble sugars in *Sorghum* [1]. During the past 20 years considerable information about 'circadian clocks' in plants at the physiological and organismic level has been collected [6, 9]. However, the molecular basis and the mechanisms involved in controlling circadian rhythmicity are still not understood [9].

Recent investigations of gene expression patterns in tomato fruits and leaves revealed that the steady-state transcript levels of the chlorophyll a/b-binding (cab) proteins (LHCP II) follow characteristic oscillation patterns: low levels before sunrise, high levels approximately at noon and declining levels during the afternoon and night [17, 18]. In addition, it has been demonstrated that this typical fluctuation pattern remains present for 3–4 days with gradual damping of the amplitude in continuous darkness [18]. This 'free-running' rhythm was the first indication that cab gene expression is controlled and/or influenced by an endogenous rhythm at the level of transcription in tomato plants.

For better understanding, it was necessary to characterize the rhythmic responses of cab gene expression in more detail. It is well established that light is the most effective external factor in resetting the phase or modulating the frequency of a circadian clock [20]. In the experiments described here, the effect of modified light/dark regimes on the typical oscillation pattern of the cab transcripts was monitored. Tomato plants, grown under the normal (natural) light/dark (day/night) pattern, were exposed to altered dark/light regimes, a) the illumination period was shifted from 07.00–18.00 to 13.00–24.00 ('phase-shift experiment'), and b) the length of the light and dark periods were reduced from approximately 12 hours to 6 hours ('training experiments').

The results obtained from these experiments demonstrate that the typical oscillation pattern of cab transcript levels remains present under altered light/dark periodicites and during adaptation to new environmental circumstances. However, the time points of maximum and minimum amplitudes and the maximum expression level changed under the modified light situation.

Materials and methods

Plant material and light regimes

Tomato plants (*Lycopersicon esculentum* cv. VFNT LA 1221, cherry line) were grown in a growth chamber at different light regimes (hydroponically, Hoagland's solution). Terminal leaflets 4–5 cm long were harvested from 4–5 week-old vegetative tomato plants at indicated time points during the day. The plants were kept at 25 °C and 15 °C during light (76 W/m^2) and dark periods, respectively, and at 80% relative humidity.

For the 'phase-shift experiment' plants were grown in an 11 hours light/13 hours dark regime, starting the light phase at 07.00. Leaflets were harvested at 06.50, 12.50, 17.50, and 22.50. To shift the dark and light phases, the dark phase was prolonged for 6 hours, while the length of light and dark periods were kept the same. After four days the dark phase was shortened by 6 hours, and from day 8 on the plants were kept in continuous darkness for 5 days. The light and dark periods are indicated in Fig. 1.

For the 'training experiment' plants were grown in a growth chamber with 18 hours light/6 hours darkness (temperature and relative humidity as described above), kept for 6 days in continuous darkness, then exposed to 6 hours of alternating light and dark period (light was switched on at 06.00 and 18.00), followed by a continuous dark period for 4 days. Terminal leaflets of 4–5 cm length were harvested at 05.50, 11.50, 17.50 and 23.50. The light and dark periods are indicated in Fig. 2.

In the third series of experiments germination, seedling and plant growth occurred in continuous light (76 W/m^2) in the growth chamber (70% relative humidity and 24 °C permanently). After four weeks plants were exposed to a 6 hours light/6 hours dark regime for three days. The first dark period was initiated at 12.30. The dark/light cycles were followed by a period of continuous darkness. Leaves were harvested at 06.20, 12.20, 18.20, and 00.20 (Fig. 3).

Tissue preparation and RNA isolation

At indicated time points terminal leaflets of approximately 4–5 cm of at least 3 individual plants (Fig. 1 and 2) or leaves of several plants (Fig. 3) were harvested and immediately frozen in liquid nitrogen and stored at −80 °C. About 0.5 g leaf tissue was used to isolate total RNA according to the isolation procedure described previously for tomato fruits [16]. RNA from different preparations were standardized and analyzed by spectrophotometric quantitation and ethidium bromide fluorescence of cytoplasmic rRNA. RNA was separated by formaldehyde agarose gel electrophoresis and transferred to nylon filter (Amersham Buchler, Hybond N) or directly spotted onto filter using a dot blot apparatus [13].

Analysis of RNA and quantitation of specific mRNAs

DNA fragments specific for the chlorophyll a/b binding proteins of tomato (1 kb DNA HpaI-PstI fragment carrying the internal part of the coding sequence of cab 1B) and the small subunit of Rubisco (0.7 kb fragment of a cDNA clone coding for rbcS2) were used for hybridization. To determine the relative transcript levels of specific mRNAs autoradiograms were scanned with a densitometer (Desaga 'Quick scan', Heidelberg, FRG) and mRNA was quantitated by peak area measurements using the Apple 2E 'graphics tablet' software. Relative mRNA levels are based on two or three hybridizations.

Results

Tomato plants were exposed to altered light/dark regimes and mRNA levels were determined. Total RNA was extracted from leaflets harvested at four time points during the day (at 6 hour intervals). Northern and dot blot hybridizations were used to identify and quantify the chlorophyll a/b-binding protein mRNA levels.

Effect of a light/dark phase-shift on the expression level of cab genes

Tomato plants were grown in an 11 hours light/13 hours dark regime, with a light phase from 07.00 to 18.00. RNA was extracted from leaves, which were harvested at 06.50, 12.50, 17.50, and 22.50, and hybridized with a probe encoding the cab 1B gene of the tomato multigene family. The characteristic diurnal oscillation pattern of cab mRNA was observed, low levels at 06.50, highest levels approximately at noon and decreasing levels in the afternoon and night (Fig. 1A). After shifting the light phase to 13.00–24.00 by extending the dark phase by 6 hours, a fluctuation pattern that displays the typical increase and decrease of cab mRNA accumulation becomes apparent (Fig. 1A), reaching slightly reduced or the same maximum expression levels as determined under the normal dark/light cycle. However, the time points of maxima and minima of mRNA level amplitudes were shifted by approximately 6 hours, indicating that plants are able to respond to the changed light/dark phase situation within a short time by modulating their cab gene expression pattern.

After shifting the light/dark phases, the time point 12.50 – which usually exhibits the time point of highest expression level – is placed into the dark period. Although no light was present until 13.00, increasing mRNA levels were detected between 07.00 and 13.00, indicating that cab genes were activated approximately at a similar day time and probably by the same principal mechanism (e.g. circadian clock) as under the normal light/dark regime. It should be noted that after the prolonged dark period the mRNA level at noon was reduced by 30%. In the afternoon, when transcript levels under the normal light/dark situation or under extended dark conditions typically decrease by approximately 60–70% within six hours (Fig. 1A and 4A, respectively), mRNA accumulation after the phase-shift was only reduced by approximately 5%. Apparently, the illumination period 13.00–24.00 is able to antagonize the usual mRNA degradation process. During the light period of the following days mRNA

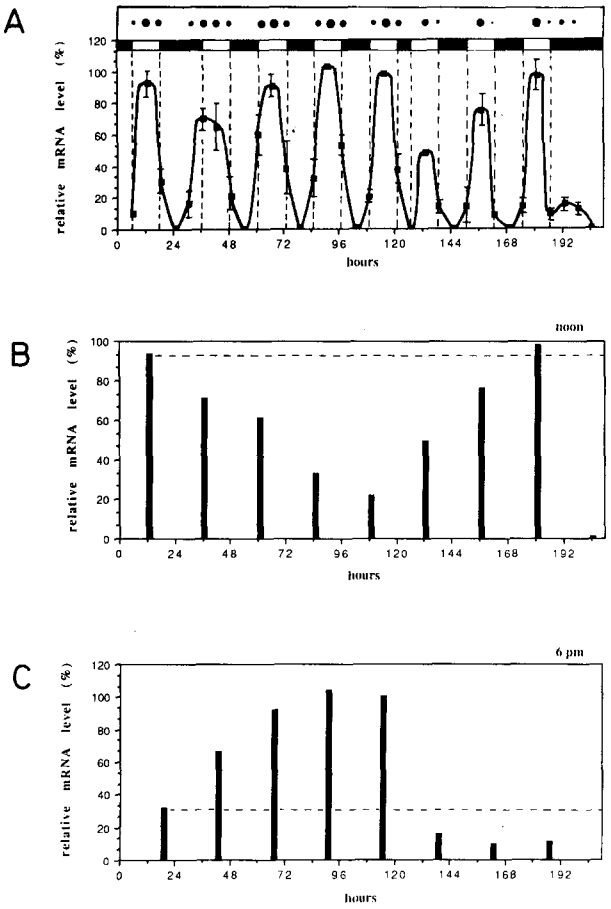

Fig. 1. Phase-shift experiment A. mRNA levels of the chlorophyll a/b-binding proteins (cab 1B) were determined at 06.50, 12.50, 17.50 and 22.50 during a period of 6 days. Total RNA was isolated from terminal leaflets. The dark (18.00–07.00) and light (07.00–18.00) periods of the normal day, and the dark (13.00–24.00) and light (24.00–13.00) periods of the phase shifted light/dark regime are indicated by filled and open bars, respectively. Phase-shifts were at 30 h and 126 h. Relative mRNAs are based on two hybridizations. Bars represent standard errors. B. Comparison of relative cab transcripts at 12.00 analysed for 8 days. C. Comparison of relative cab transcripts at 18.00 analysed for 8 days.

levels increased in the afternoon and maximum levels were reached approximately at 18.00. The results presented here and results of previous experiments [17, 18] demonstrate that approximately 6 hours after the beginning of the illumination period maximum steady-state mRNA levels for the chlorophyll a/b-binding proteins could be monitored. This observation suggests that the role of light/dark alternations is not the induction of the oscillations, but determining the time points of minima and maxima of mRNA accumulation.

After resetting the light phase to the initial time points (07.00–18.00), by shortening one night phase by 6 hours, the original oscillation pattern, characterized by the time point of maximum mRNA level amplitude around noon, appeared within one day after the phase-shift. However, the steady-state transcript level at noon was reduced by approximately 50% after the short dark period (six hours darkness).

The analysis of the steady-state expression levels at noon throughout the phase-shift experiment is depicted in Fig. 1B. Despite the fact that transcript levels decrease permanently at noon, four days after the shift 20% of the normal expression level could be monitored. This indicates that the original gene activation mechanisms (endogenous rhythm, light induction) becomes less pronounced the longer the plants were kept in the altered light/dark regime. While mRNA levels decreased at noon, cab transcripts increased continuously at 18.00 until the maximum expression level was reached three days after the phase-shift (Fig. 1C). It seems likely that the 18.00 time point under the altered light/dark regime corresponds to the 12.00 time point under normal conditions. Immediately after plants were transferred back to the normal light regime (light phase from 07.00 till 18.00) the mRNA level at 18.00 dropped by approximately 85–90%, while the levels at noon increased gradually until after three days of adaptation the maximum amplitude was reached.

Expression levels in tomato plants exposed to a 6 hours light/dark regime

This experiment was designed to investigate the mRNA expression pattern of the chlorophyll a/b-binding proteins during unusual light/dark regimes. Furthermore the results may give first indications whether a new periodic expression pattern can be 'entrained' after exposing plants to alternating 6 hours light/6 hours darkness.

Plants were first grown at an 18 hours light/6 hours dark regime in a growth chamber (light phase from 06.00 till midnight), leaves were harvested at 05.50, 11.50, 17.50 and 23.50, and cab mRNA levels were determined. The typical circadian oscillation pattern was observed (Fig. 2A). After five days in continuous darkness, when no cab transcripts can be detected by Northern or dot blot analysis, plants were exposed to a 6-hour dark/light periodicity, and mRNA levels were determined at 6-hour intervals. The first light phase was initiated at 06.00. After 6 hours of illumination the mRNA level increased to approximately 15% compared to the maximum level determined under the original dark/light cycle. During the following 6 hours darkness (12.00–18.00) cab transcripts decreased to undetectable levels. Only 1–2% cab mRNA was detected after the second light phase (18.00–24.00). After the third light phase (06.00–12.00) the mRNA level reached approximately 30%, but decreased to undetectable levels during the following 6 hours of darkness, increased again to about 20% after the fourth light phase. On the third day mRNA accumulation was 80% and 50% at noon and at midnight, respectively, compared to the level during the initial light/dark cycle. The mRNA amplitudes at noon and at midnight are depicted in Fig. 2B and 2C, respectively. mRNA accumulation was higher when light was applied between 06.00 till 12.00, lower steady-state levels were measured when plants were illuminated from 18.00 till 24.00. However, in both cases continuously increasing mRNA amplitudes were detected during the 6-hour light/dark regime. Decreasing transcript levels at noon and midnight were monitored when plants were transferred into complete darkness.

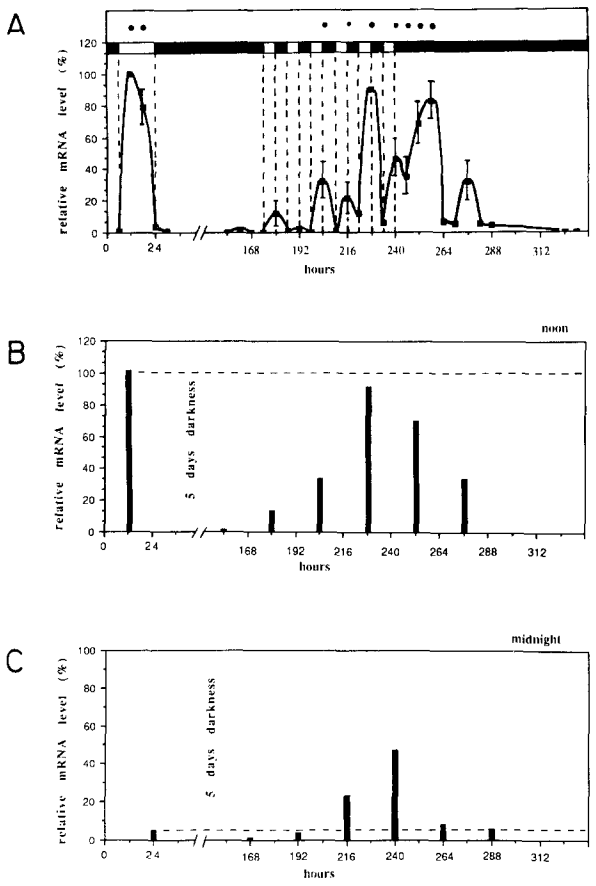

Fig. 2. Training experiment A. Transcript levels of the chlorophyll a/b-binding proteins (cab 1B) were monitored at 05.50, 11.50, 17.50 and 23.50 during the normal and altered light/dark regime. DNA was isolated from terminal leaflets. During the altered regime light was on from 06.00 to 12.00 and from 18.00 to 24.00. Light and dark periods are indicated by open and filled bars, respectively. Relative mRNA levels are based on four hybridizations. Bars represent standard errors. B. Comparison of relative cab transcripts at 12.00 analysed for 7 days. C. Comparison of relative cab transcripts at 24.00 analysed for 7 days.

To evaluate whether it was possible to 'entrain' the cab gene expression mechanism during the altered dark/light regime, plants were transferred into continuous darkness and mRNA levels were monitored at 6-hour intervals. During the first 24 hours in continuous darkness elevated mRNA levels were detected at 12.00 and 18.00, and at 12.00 the second day. Based on these results, the mRNA accumulation pattern appears to resemble the oscillation pattern under normal light/dark conditions more than the pattern monitored under the abnormal light/dark periodicity. Thus, an 'entrainment' at the level of cab gene expression does not occur under such experimental conditions, but rather the original 24-hour endogenous rhythm reappears suggesting that the circadian clock is hereditary.

Transcript levels in tomato plants grown in continuous light and exposed to a 6-hour light/dark regime

In the previous experiment it was demonstrated that in tomato plants, which were exposed to an abnormal light/dark periodicity, the cab gene expression pattern cannot be 'entrained', but rather the plants reorganize the original expression pattern. Since the tomato plants were germinated and grown approximately for 4 weeks in the natural occurring day/night (light/dark) pattern it cannot be excluded that a characteristic pattern was manifested during early plant development. A prolonged dark phase (e.g. six days) reduces cab mRNA accumulation (Fig. 2A), but apparently does not eliminate an initially memorized dark/light alternation. To evaluate whether a certain oscillation pattern of cab mRNA levels is manifested during germination and/or early seedling and plant development, tomato plants were permanently exposed to continuous light from the time point of sowing. This procedure should exclude a recognition of certain day/night (light/dark) phases before transferring plants to the abnormal light/dark regime. Similar to the previous experiment, leaves were harvested at 6-hour intervals during the time of 'entrainment' and during continuous darkness (Fig. 3A).

During 29 days of light the tomato plants developed similarly to the control plants, except for the dark green-blue color of the leaves. The total chlorophyll content in light-exposed tomato leaves was determined to be 1.42 mg/mg protein compared to 2.07 mg/mg protein in control plants. However, the chl a/b ratio remained approximately the same, viz. 3.71 and 3.55, respectively. In addition, a highly alkalized extract was obtained during RNA isolation. However, the significance of this observation is not yet understood. After four weeks of illumination only low mRNA levels of the chlorophyll a/b-binding proteins and no or not significantly pronounced oscillation pattern was detected (Fig. 3A). After applying the abnormal light/dark rhythm of 6 hours light and 6 hours darkness to the plants, several changes occurred: a) the chlorophyll content increased to

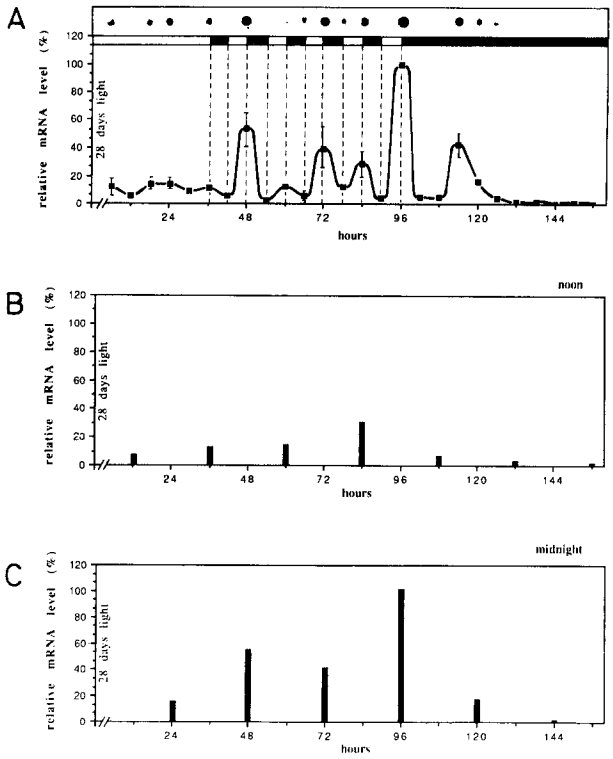

Fig. 3. Training experiment A. Relative mRNA levels were determined in tomato leaves harvested after germination and growth in continuous light, during a 6-hour light/dark regime, and during continuous darkness. RNA was extracted from leaves at 06.20, 12.20, 18.20 and 00.20. The first dark phase was initiated at 12.30. Dark and light phases are indicated by filled and open bars, respectively. Relative transcript levels are based on two hybridizations. Bars represent standard errors. B. Relative transcript levels at 12.00 analysed for 7 days. C. Relative transcript levels at 24.00 analysed for 7 days.

1.77 mg/mg protein (chl a/b ratio 3.6) cab gene expression increased significantly, and c) a fluctuation pattern became apparent. After one dark/light phase (18.00–24.00) cab transcripts accumulated to 50%, while after the second light phase (06.00–12.00) can mRNA accumulation was less pronounced (approximately 10%). Continuously increasing mRNA level amplitudes were detected at noon and midnight (Fig. 3B, 3C, respectively). It should be noted that under these experimental conditions the amplitudes at midnight were always higher than at noon. To investigate, whether the applied light regime was mani-

fested in the molecular mechanism, the cab transcript levels were monitored under constant environmental conditions (e.g. continuous darkness). Surprisingly, elevated mRNA levels (approximately 45%) were not detected at noon or at midnight, but at 06.00. This result demonstrates again that cab gene expression cannot be 'entrained' by an abnormal 6-hour light/dark periodicity.

Analysis of transcript level patterns during adaptation processes

When plants are transferred from one light/dark regime to another, they have to adjust or respond to the 'new' environmental situation. The response at the molecular level was analyzed for the transcript levels of the chlorophyll a/b-binding proteins in tomato leaves. A compilation of six expression patterns is depicted in Fig. 4 A–F. A general picture emerged from this analysis: the characteristic cab mRNA fluctuation pattern continued to be present during the alteration of the light/dark regimes but the amplitudes were altered. Decreasing amplitudes were observed when plants were transferred into continuous darkness (Fig. 4A). The damping of the amplitude exhibits the typical pattern of a 'free running cycle', indicating most strongly that an endogenous (circadian) rhythm influences cab gene expression in tomato leaves [18].

Permanently increasing maximum amplitudes and a typical oscillation pattern were detected when plants are transferred from several days of darkness back to the normal day/night cycle (Fig. 4E) or to 6-hour light/dark alternations (Fig. 4C). An increase of the transcript amplitude was also observed after exposure of plants to continuous light for several days and transfer to the normal dark/light regime (Fig. 4D). Additionally, after a short dark period of 6 hours, tran-

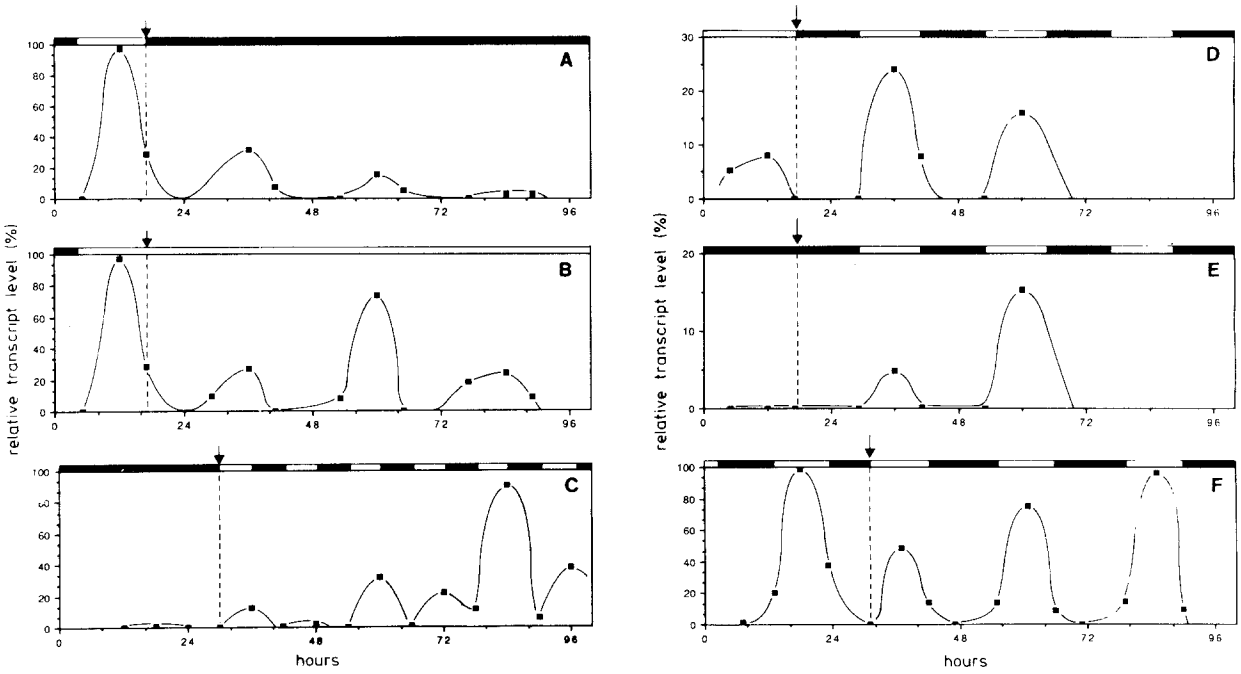

Fig. 4. Adaptation Transcript levels of the chlorophyll a/b-binding proteins were analysed during alterations of the light/dark regimes. A) 12 h day/12 h night regime → continuous darkness. B) 12 h day/12 h night regime → continuous light. C) Continuous darkness → 6 h light/6 h dark regime. D) Continuous light → 12 h day/12 h night regime. E) Continuous darkness → 12 h day/12 h night regime. F) 6 h dark phase → 12 h light/12 h dark regime.
RNA was extracted from tomato leaves at indicated time points and relative transcript levels were based on maximum hybridization within one series of experiments. Filled and open bars indicate the periods of darkness and light, respectively. The arrow indicates the time point when plants were transferred to another light/dark regime.

script amplitudes at noon increased during the following days (Fig. 4F). The maximum expression level, which is manifested under the normal light/dark alternations, was usually reestablished approximately after three days of adjustment (Fig. 4C, E, F). To date, it is not understood which mechanisms are involved in controlling these adaptation processes. Based on the results presented here, it is suggested that not only a simple 'feedback mechanism' can regulate the adaptation of cab gene expression, since cab mRNA levels fluctuate regularly with a periodic pattern and do not increase continuously until a saturation level is reached. This result supports the hypothesis that an endogenous (circadian) rhythm is involved in the cab gene activation/inactivation system in tomato leaves. However, such typical saturation pattern was detected for the mRNA levels of the small subunit (ss) of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco). The rbcS transcript level increased continuously for more than 48 hours (Fig. 5A–D). It is possible that a 'feedback mechanism' controls the adaptation processes of ss mRNA accumulation. Clearly, the examples of the chlorophyll a/b-binding proteins and the ss of rubisco demonstrate that the regulation of adaptation processes are different for nuclear encoded photosynthesis-specific genes in tomato leaves.

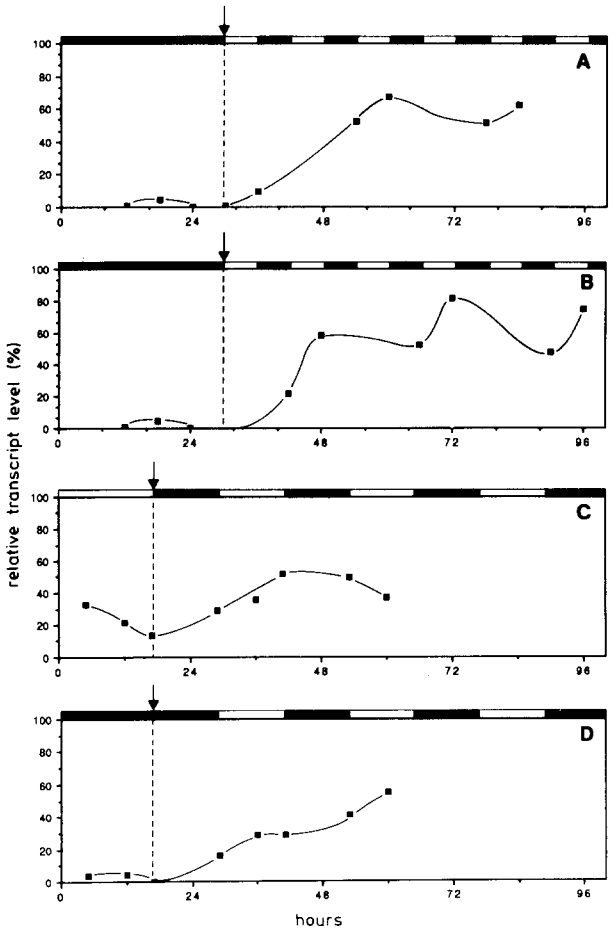

Fig. 5. Adaptation Transcript levels of the small subunit of the rubisco were analyzed during alterations of the light/dark regimes. A, B. Continuous darkness → 6 h light/6 h darkness. C. Continuous light → 12 h day/12 h night. D. Continuous darkness → 12 h day/12 h night.
RNA was extracted from tomato leaves at indicated time points and relative transcript levels were based on maximum hybridization within one series of experiments. Filled and open bars indicate the periods of darkness and light, respectively. The arrow indicates the time point when plants were transferred to another light/dark regime.

Discussion

Reports on the phenomenon of circadian clocks at the biochemical, physiological and organismic level have been published approximately since 1900. Genetic evidence was provided by investigations of so-called 'clock-mutants' such as *Acetabularia* [25], *Neurospora* [21], *Drosophila* and *Chlamydomonas* (for a review, see [6]). Apart from the observation in pea [11], we were the first to demonstrate the occurrence of diurnal and circadian rhythms at the level of transcription [17, 18]. In tomato fruits and tomato leaves oscillations of the steady-state mRNA levels coding for the chlorophyll a/b-binding proteins were monitored. In the meantime, similar fluctuation patterns and circadian oscillations have been observed in other plant species such as petunia [26], pea [24], maize [27], and wheat [15]. A detailed analysis of ten different monocotyledonous and dicotyledonous plants supports the notion that this mechanism of cab gene expression is highly conserved [14]. Furthermore, the analysis of several members of the cab multigene family of tomato (cab 1B, 3, 4, 5, 6, 7, 8, 9) exhibits the

same principal cyclic variation pattern at the steady-state transcript level (B. Piechulla and E. Pichersky, unpublished results). Similarly, comparison of the total steady-state level with the expression level of a single cab 1B gene of the wheat multigene family reveals the same fluctuation pattern [15]. In contrast, out of three genes investigated in petunia extracts, the mRNA level of one gene revealed a slightly different pattern [26].

Despite the fact that a circadian clock controls the expression of the cab gene family in a variety of plant species, an interesting difference has been recognized. While in tomato fruits and leaves increasing cab transcripts are detected after sunrise or after the onset of illumination, increasing steady-state mRNA levels were detected prior (at least two hours) to illumination in pea [11], petunia [26] and wheat [15]. This result seems notable since it may indicate that different mechanisms are involved in cab gene expression in different plant species. Nagy *et al.* [15] concluded in their recent publication that phytochrome rather than light plays a role in regulating the gene expression level. Here it was demonstrated that the length of the dark phase (Figs. 1A, 4F) as well as the time point when plants receive light (Figs. 2A and 4C), and how far along they are in an adaptation process (Figs. 1A, 2A, 3A and 4C) are critical parameters for reaching a maximum and balanced gene expression level.

It appeared that the first illumination period within 24 hours had a more dramatic effect on mRNA accumulation then the second light phase (Figs. 2A and 3A). This preference of the first light phase over the second one was, however, independent of the actual time of the day. To date it can only be speculated why such a different response occurred. It is possible that a) a factor of the signal chain (e.g. phytochrome) has not recovered or been reactivated to full capacity, b) a 'circadian factor' (negative regulator) exists which prevents transcription of the DNA, or c) a 'circadian factor' (positive regulator) has to be synthesized or accumulated to support transcription. From the data presented here it appears that the amplitudes of the first as well as of the second illumination period increase continuously, indicating that whatever the underlying mechanism is, gene expression is adaptable to the new and unusual light/dark regime. Such adaptive responses at the transcript level of the chlorophyll a/b-binding protein are summarized in Fig. 4. It is interesting to note that during the adaptation to an altered photoperiod the mRNA levels continue to oscillate. This excludes that cab gene expression is controlled by a simple 'feedback mechanism' as may be the case for the gene activation/inactivation mechanism of the small subunit of rubisco (Fig. 5). Despite the fact that both genes/multigene families are nuclear-encoded and both code for proteins which function during photosynthetic reactions, the mechanisms controlling the gene activation and inactivation processes are significantly different in tomato leaves. While a circadian rhythm is involved in cab gene expression, the results indicate that rbcS genes are not under the regulation of such a 'biological clock' in tomato leaves.

With the detailed characterization of the cab transcript oscillations under altered light/dark regimes (Fig. 2A and 3A) it was possible to demonstrate that the rhythm is not only endogenous but also hereditary, i.e., not induced at early stages of individual development. Bünning [2, 3] found that circadian rhythms persist in *Phaseolus* and *Drosophila* even though generation after generation had been raised in environments completely lacking cues to the passage of time. Thus, the function of light/dark alternations is not inducing the rhythm, but determining the times of maxima and minima. In addition, the rhythm cannot be modified by preceding abnormal light/dark periodicities. This observation agrees with the data established for higher plants [10] and for ripening of sporangia of *Pilobolus* [23, 29] and *Oedogonium cardiacum* [29].

The effect of light in inducing cab gene expression has been investigated quite intensively (for reviews, see [12, 28]. Nagy *et al.* [15] concluded from their experiments that the action of phytochrome appears to be secondary because it can be overridden by the regulatory mechanism of the circadian clock. It is interesting to note that two

photoreceptors are postulated to be involved in controlling the circadian clock in the unicellular alga *Gonyaulax polyedra* [20]. Beside the critical effect of light quality [20] and quantity in influencing cab gene expression some expression patterns documented in this paper demonstrate clearly the importance of the dark phase. In plants exposed to continuous light (Fig. 4D) or after a short dark phase of 6 hours (Fig. 4F) a reduced cab mRNA amplitude was reached. It is plausible that a certain length of the dark phase may be necessary for recovering the full functional capacity of all components involved in the activation and accumulation process of chlorophyll a/b-binding proteins. Therefore, light as well as the dark phase are important for maximum and balanced cab gene expression.

Endogenous rhythms with periods of 24 hours are common to all eukaryotic organisms, but it is not yet well understood how such oscillations have arisen during evolution. Pittendrigh [19] suggested that in earlier times organisms which perform their DNA replication and cell division overnight may have an advantage because of the high UV irradiation during the day. Based on this hypothesis it should be possible to identify other genes with oscillation patterns similar to those of the cab genes. However, an analysis with ten different types of genes revealed that this typical transcript level fluctuation was only detected for the chlorophyll a/b-binding proteins [14]. This leads to the question: 'What is special with the cab multigene family that such oscillations are present in a 'variety of plants?' It should be pointed out that LHC proteins have the function to bind chlorophyll. Chlorophyll synthesis itself is strictly dependent on the presence of light, particularly the conversion of protochlorophyllide to chlorophyllide catalyzed by NADPH-protochlorophyllide oxidoreductase [7]. It is possible that the LHC protein and mRNA synthesis is correlated with the synthesis of chlorophyll in the photosynthesis-active cell. This supports the notion that the circadian clock operating at the transcript level is a specific rather than a general process in plants. However, this does not exclude that other genes (which have not been investigated by the author) may exhibit circadian rhythmicity at the transcipt level.

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