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Effect of dark phases and temperature on the chlorophyll a/b binding protein mRNA level oscillations in tomato seedlings

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Abstract

External parameters (e.g. light/dark alterations and temperature) were tested for their ability to influence the expression pattern of the chlorophyll a/b binding protein (*cab*) genes and the small subunit of RuBPC/Oase (*rbcS*) genes. Particular interest was focused on the alterations of diurnal/circadian oscillation patterns of *cab* mRNA levels.

Chlorophyll a/b binding protein and small subunit of RuBPC/Oase mRNA oscillations were observed in tomato seedlings grown without environmental perturbations (constant illumination and temperature), indicating that these genes are controlled by an endogenous rhythm. The rhythmic fluctuation patterns revealed a period of about 32 hours and a weakly expressed amplitude. A several-fold increase of the *cab* mRNA amplitude and a reduction of the period to about 24 hours (circadian) was monitored after exposure of the tomato seedlings to 3, 6 or 9 hours of darkness. The elevated amplitude disappeared at consecutive days if seedlings were not exposed to further dark phases. A circadian rhythm with clearly expressed *cab* mRNA amplitudes was also present after the plants had been transferred to darkness at various circadian times. However, under those circumstances the time points of maximum and minimum transcript levels were shifted by respective hours.

Alteration of the growth temperature from 24 °C to 10 °C or 30 °C at constant illumination or in a light/dark cycle resulted in a reduction of the amplitudes or of the steady-state mRNA levels. Such extreme temperature changes do not induce or enhance the diurnal *cab* mRNA oscillations.

Introduction

The chlorophyll a/b binding (*cab*) proteins play a primary and central role in capturing and distributing the light energy. During the course of investigations regarding the biogenesis of these polypeptides, the respective nuclear-encoded genes were isolated and the organization at the molecular level was determined in several higher plant species (e.g. tomato; see for relevant citations [8]). Beside the structural information, the expression of these *cab* genes have been charac-

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terized. Several factors or parameters have been identified that are involved in the regulation of *cab* gene expression (for reviews [10, 24]). To date, the complex control mechanism and the hierarchic organization of the expression are not completely understood.

Early experiments with etiolated seedlings demonstrated a light-dependent *cab* mRNA accumulation. Particularly the involvement of phytochrome and the effect of the red and far-red photoconversion have been extensively studied with respect to chlorophyll a/b binding protein and the small subunit of RuBPC/Oase (*rbcS*) gene expression for a variety of plant species (for reviews [10, 24]). Beside the influences of light acting as an external factor it has also been shown that internal parameters such as the 'developmental programme' and the tissue and organ specificity contribute to a particular *cab* mRNA level [15, 22]. Additionally, another intrinsic factor has been documented to be involved in the expression of *cab* genes in tomato fruits [16]. Analysis of steady-state *cab* mRNA levels at different time points during the day exhibits a typical diurnal expression pattern, maximum mRNA levels being measured approximately at noon and continuously decreasing levels during the afternoon and night. In consecutive investigations it has been shown that the *cab* mRNA oscillations in tomato leaves are due to an endogenous rhythm with a period of aproximately 24 hours (circadian rhythm) [17].

In the past two or three years daily mRNA fluctuations of the *cab* mRNA levels have been demonstrated in several other plant species, including monocots and dicots (relevant citations in [8]). In addition, other genes such as the nitrate reductase [7] and other genes of the photosynthetic apparatus in spinach (R. Herrmann, pers. communication) showed diurnal alterations of their mRNA levels, but evidence for the involvement of a true endogenous rhythm in these cases is lacking to date. It is notifiable that the phenomenon of circadian mRNA accumulation of *cab* genes has been demonstrated first in 1985 [9], but since then only little progress has been made with respect to further characterization of the 'biological clock' or the oscillator involved in *cab* gene expression.

Recently, the importance of the circadian phase and duration of light as well as the duration of darkness has been demonstrated in adult vegetative tomato plants [11], suggesting the involvement of photoperiodic time keeping processes for the expression of *cab* genes in tomato leaves. Furthermore, after shifting the dark/light regime by several hours and reduction of the light/dark cycle to 12 hours, the oscillating expression pattern remained present and the expression in the plants was adjusted to the altered external conditions within 3 days [18]. The results of all these experiments do not allow a conclusion regarding the origin of this circadian rhythm. It is possible that the pattern of a circadian oscillation is entrained or that the 'biological clock' is induced by events early during seed germination and/or plant development, while at later developmental stages this pattern is synchronized by the actual environmental conditions.

Based on the limitations interpreting these results with respect to the origin of the rhythm we started a series of experiments in young tomato seedlings which were germinated and grown in constant light and temperature conditions. Growth in a constant environment was speculated to give some information about the origin of the circadian rhythm of the chlorophyll a/b binding proteins. Secondly, we asked the quesiton, which external parameters may be able to induce or enhance the characteristic *cab* mRNA oscillation pattern. As a first step to investigate this topic, tomato seedlings were first kept in constant conditions and then exposed to various external stimuli such as darkness and temperature. The results of these experiments are reported and discussed in this paper.

Methods

Plant material and tissue preparation

Tomato seeds (*Lycopersicon esculentum* cv. VFNT LA 1221, cherry line) were germinated and

seedlings were grown on clay beads (average diameter of 0.5 cm) in growth chambers at 24 °C and white light (20 W/m^2). For one week the seedlings were watered with tap water and then with Hoagland's nutrient solution.

Exposure to dark phases
The seedlings were grown in continuous white light and at 24 °C. Sixteen-day-old seedlings were exposed to 3 hours (07.00–10.00), 6 hours (07.00–13.00) and 9 hours (07.00–16.00) of darkness. One set of seedlings were kept in darkness from 10.00 to 19.00. Growth was then continued in constant light conditions. Approximately 0.5 g leaf material from 6–7 seedlings were harvested at various time points (LL): 07.00, 10.00, 12.00, 18.00, 22.00, 02.00; 3 hours darkness: 07.00, 10.00, 14.00, 17.00, 20.00, 24.00; 6 hours darkness: 07.00, 13.00, 17.00, 20.00, 24.00; 9 hours darkness: 07.00, 13.00, 16.00, 20.00, 24.00; 9 hours darkness, shifted: 07.00, 10.00, 19.00, 23.00, 24.00. Samples were immediately frozen in liquid nitrogen and kept at −50 °C until further analysis.

Temperature alterations (A)
Seedlings were grown in continuous white light and at 24 °C. Sixteen-day-old seedlings were transferred to 10 °C and 30 °C, a tray with the control seedlings was kept at 24 °C. After three days all seedlings were shifted back to 24 °C. Samples were harvested at 07.00, 10.00, 12.00, 18.00, 22.00 and 02.00, immediately frozen in liquid nitrogen and kept at −50 °C.

Temperature alterations (B)
Seedlings were grown in a 12 hours light/12 hours dark cycle (light 08.00–20.00) at 24 °C. Sixteen-day-old seedlings were transferred to 10 °C and 30 °C, and a control tray was kept at 24 °C. After three days the seedlings were shifted back to 24 °C. Leaves were harvested at 07.30, 10.00, 13.00, 16.00, 18.00 and 22.00, immadiately frozen in liquid nitrogen and stored at −50 °C.

RNA isolation and mRNA level quantitation

RNA was isolated from approximately 200 mg leaf tissue according to the method described elsewhere [15]. RNA of different preparations were standardized and analysed by spectrophotometric quantitation, ethidium bromide fluorescence of the cytoplasmic rRNA in stained formaldehyde gels and hybridization with a heterologous probe encoding the cytoplasmic rRNA genes from pea. Total RNA samples were spotted onto nylon filters (Hybond N, Amersham Buchler, Braunschweig, FRG) using the dot blot apparatus. Specific gene probes labelled by 'random priming' were used for hybridizations. Plasmid pTSS-4 has a 0.35 kb DNA fragment encompassing part of exon 2 and the complete exon 3 sequence of the tomato *rbcS* 3A gene, inserted into the *Eco* RI and *Xba* I site of pUC9 [14]. Plasmid pIA27 contains a 2.0 kb *Eco* RI fragment of tomato nuclear DNA inserted into pUC9, on which the 0.55 kb *Pvu* II-*Hinc* II fragment is specific for the coding region of *cab* 1B [13].

To determine transcript levels of specific mRNAs, dots of the hybridized dot blot were cut out and counted in a scintillation counter ('Cerenkov counting'). The average of all counts of one set of experiment was equal to 100%. The relative mRNA levels within one experiment were calculated on the basis of this value.

Chlorophyll determination

Leaf tissue was ground in liquid nitrogen and chlorophyll was extracted in 80% acetone [2]. The chlorophyll a and b contents were calculated:

chlorophyll a:
$(E_{663nm} \times 12.7) - (E_{645nm} \times 2.69)$
chlorophyll b:
$(E_{645nm} \times 22.9) - (E_{663nm} \times 4.68)$
total: $(E_{645nm} \times 20.2) + (E_{663nm} \times 8.02)$

Results

To test the question about the origin of the rhythmic *cab* mRNA oscillations in tomato leaves, seeds were germinated and seedlings were grown in a growth chamber with a constant environment or at specifically altered growth conditions. Total RNA was extracted from leaves at indicated time points during a time course of 3–4 days and hybridized with probes encoding the chlorophyll a/b binding protein (*cab*) and the small subunit of RuBPC/Oase (*rbcS*) genes. The hybridization signal was used as a measure to quantitate the relative amounts of transcripts.

Continuous light conditions

Approximately 100 seeds were germinated and seedlings were grown in constant illumination and at 24 °C. To ensure that no dark-to-light transition affected the growth or sets the internal clock during germination, the seeds were layered on top of the clay beads. Seed germination occurred approximately 3 days after embedding and cotyledons appeared at the 5th or 6th day, while the first true leaves became visible approximately at the 10th day. The leaves of these seedlings exhibited a blue-green colour. However, chlorophyll/g FW (1.47) and the chlorophyll a/b ratio (2.82) had similar values as measured in tomato seedlings and adult vegetative plants grown in light/dark or day/night regimes, 1.27–1.52 and 2.75–3.0, respectively. The dark blue-green colour suggests a high content of antocyanins and carotenoids which may be necessary during adjustment to the unphysiological light conditions.

First true leaves (16 days after embedding) of about 5–7 plants were harvested at indicated time points throughout a course of 3 days. Steady-state mRNA levels of the chl a/b binding proteins were determined by hybridization with the coding region of the tomato *cab* 1B gene. The results of a typical example of this set of experiments are depicted in Fig. 1A. In continuous illumination a rhythmic accumulation pattern of *cab* mRNA was

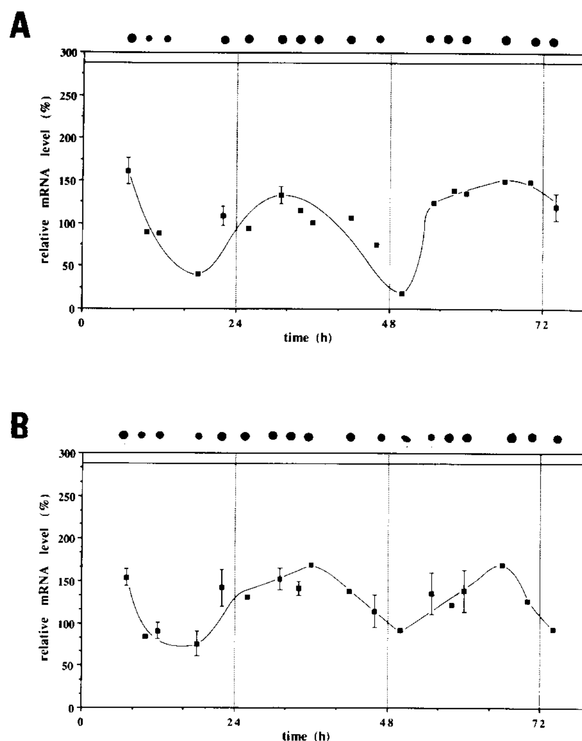

Fig. 1. Relative changes of the transcript levels in leaves of tomato seedlings germinated and grown in continuous light and at constant temperature conditions (24 °C). Sixteen days after embedding, total RNA was extracted from first true leaves at indicated time points. Equal amounts of total RNA were spotted onto dot blots, hybridized with a specific gene probe, and dots were cut out and counted (100% relative mRNA level equals 91 cpm (panel A) and 203 cpm (panel B)). mRNA level calculations were based on 3 hybridizations. Error bars represent SE. A. Hybridization with the tomato chlorophyll a/b binding protein gene probe, *cab* 1B. B. Hybridization with the tomato gene coding for the small subunit of RuBPC/Oase, *rbcS* 3A.

apparent. The length of the period was approximately 32 hours and the amplitude varied between 50 and 150% relative transcript level. However, in comparison to the results obtained in previous experiments [11, 17, 18], where plants were grown in light/dark cycles, the amplitude was reduced about 8-fold and the period length prolonged by approximately 8 hours.

In addition to the chl a/b binding protein mRNA levels another nuclear encoded photosynthesis-specific gene, coding for the small subunit of the RuBPC/Oase (*rbsS* 3A), was hybrid-

ized to the RNA extracts (Fig. 1B). A similar fluctuation pattern as monitored for the mRNAs of the chlorophyll a/b binding protein was detected for the *rbcS* transcript levels. The oscillation appeared with a small amplitude and a period of approximately 32 hours.

From these results it was concluded that a basic oscillation pattern for the expression of these photosynthesis-specific genes follows an intrinsic mode in tomato seedlings.

Exposure to a single dark phase

Seedlings exclusively grown in continuous light for 16 days were exposed to 3 or 6 hours of darkness. In both cases the dark period began at 07.00 and was terminated at 10.00 or 13.00, respectively. The resulting mRNA accumulation patterns for the *cab* genes and the *rbcS* genes are documented in Fig. 2A–D. During 3 hours of darkness the *cab* transcript levels first decreased, but increased after the dark/light transition within 6 hours to 140% relative transcript level (Fig. 2A). The mRNA levels were monitored during the following two days in continuous light. An oscillation pattern with a periodic length of approximately 24 hours was manifested, and remained present for at least 2 days.

Similarly, a 6 hour dark phase enhanced the oscillation amplitude and a circadian rhythm was expressed. The first amplitude after the dark

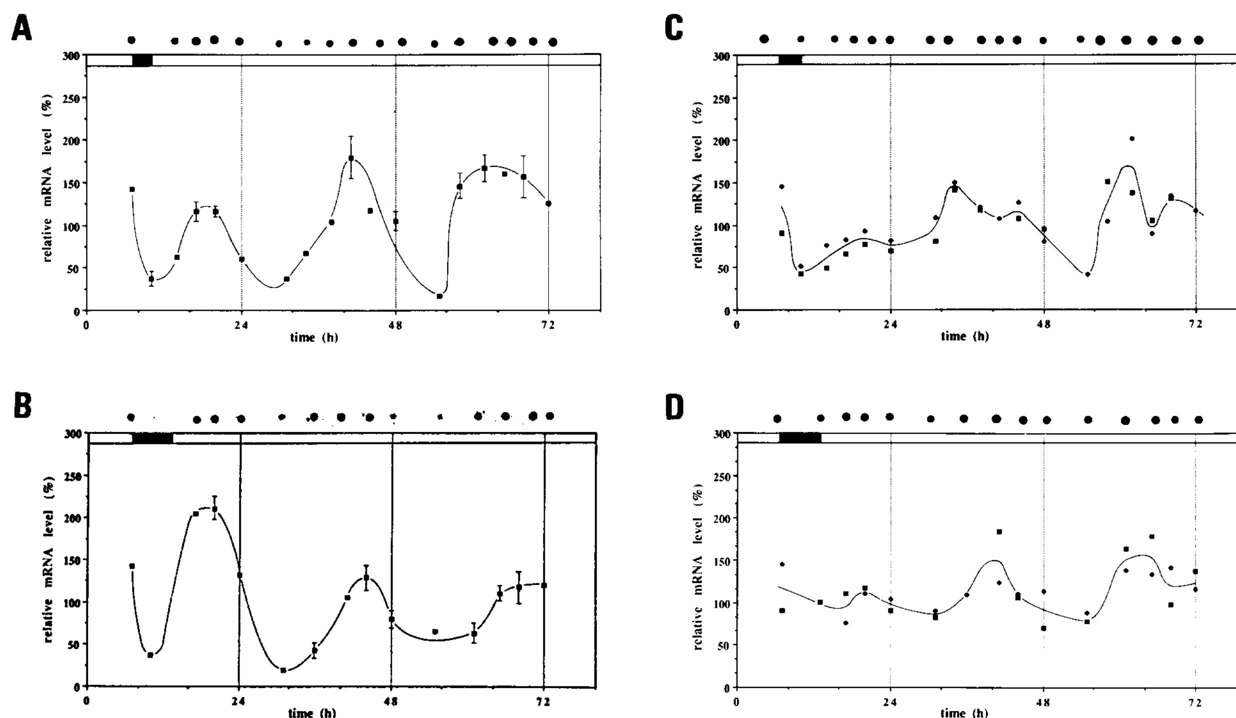

Fig. 2. Relative changes of the transcript levels in leaves of tomato seedlings germinated and grown in continuous light and at constant temperature conditions (24 °C). Sixteen days after embedding seedlings were exposed to 3 or 6 hours of darkness, panels A, C and B, D, respectively. Phases of darkness (07.00–10.00 and 07.00–13.00) are indicated by filled bars at the top of the panel. Total RNA was extracted from first true leaves at indicated time points. Equal amounts of total RNA were spotted onto dot blots, hybridized with a specific gene probe, and dots were cut out and counted (100% relative mRNA level equals 393 cpm (panels A, B) and 348 cpm (panels C, D)). mRNA level calculations were based on two independent experiments and 2–3 hybridizations (panels A, B; error bars represent SE) or two experiments and two hybridizations (panels C, D; range of values are shown). A and B, hybridization with the chl a/b binding protein gene, *cab* 1B. C and D, hybridization with the ssu of RuBPC/Oase gene, *rbsS* 3A.

phase reached 220% relative transcript level (Fig. 2B). After two periodic cycles in continuous illumination the amplitude was reduced to approximately similar relative transcript levels as determined in continuous light conditions in the previous experiment (Fig. 1A). It is interesting to note that the maximum levels of the periods remained almost constant, while the minimum levels gradually increased. In summary, exposure to various dark phases suggested that the length of the dark phase is correlated with the resulting height of the *cab* mRNA amplitude. Interestingly, the maximum and minimum of the periods of these two experiments appeared at the same circadian time, indicating that the transition from light to darkness rather than the transition from dark to light resets the time points of maximum and minimum of mRNA accumulation.

In contrast to the rhythmic *cab* mRNA accumulation pattern, the oscillations of the *rbcS* transcript levels were not enhanced after the transfer of the seedlings to darkness (Fig. 2C, D). Apparently, in this group of photosynthesis-specific genes 'darkness' equalized rather than promoted mRNA level fluctuations.

In two additional experiments, seedlings were transferred to darkness for 9 hours at different circadian time points (Fig. 3). In both cases the typical circadian rhythm of *cab* mRNA was observed. The heights of the amplitudes reached similar levels, but the time points when maximum and minimum appeared were separated by three hours. This result again seemed to indicate that the time point of transition from light to darkness was a measure for synchronizing the phase of the periodic cycle.

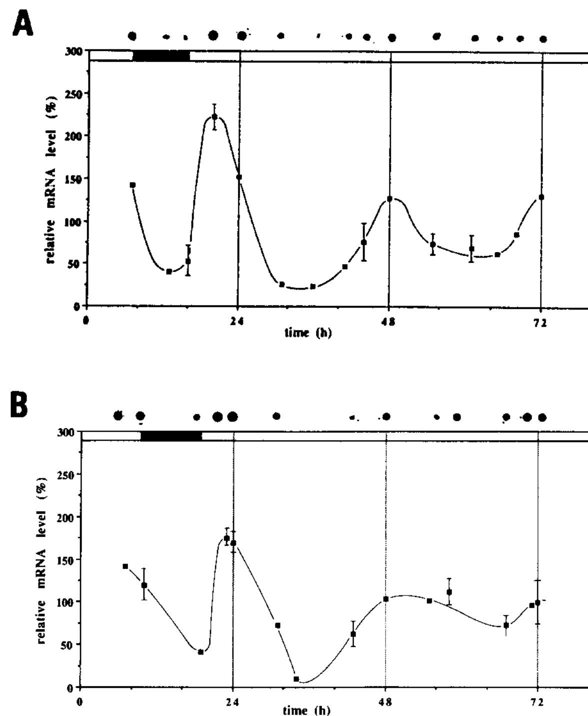

Fig. 3. Relative changes of the transcript levels in leaves of tomato seedlings germinated and grown in continuous light and at constant temperature conditions (24 °C). Sixteen days after embedding seedlings were exposed to 9 hours of darkness (panel A, darkness from 07.00–16.00; panel B, darkness from 10.00–19.00). Dark phases are indicated by filled bars at the top of the panel. Total RNA was extracted from first true leaves at indicated time points. Equal amounts of total RNA were spotted onto dot blots, hybridized with the *cab* 1B gene probe, and dots were cut out and counted (100% relative mRNA level equals 393 cpm (panels A,B)). mRNA level calculations were based on two independent experiments and three hybridizations. Error bars represent SE.

Influence of temperature shifts on the cap *expression level*

In the previous sections it was demonstrated that a short dark phase of 3 hours was sufficient to express a characteristic circadian mRNA accumulation pattern of the chl a/b binding protein. It was of interest to find other external parameters which may influence the *cab* expression pattern. An additional variable factor which is also present during plant development in the natural environment is the 'temperature'. Several different experiments were designed to answer the question whether temperature or temperature shifts induce or enhance a rhythmic fluctuation pattern of the *cab* transcript levels.

Chlorophyll a/b binding protein expression in seedlings grown at continuous illumination and different temperatures

In the first series of experiments tomato seedlings were grown in constant light and at 24 °C (Fig. 4B) and then shifted either to 10 °C (Fig. 4) or 30 °C (Fig. 4C). After these alterations no periodic oscillation pattern was observed. While at 10 °C the *cab* transcript levels in leaves of seedlings decreased continuously to 20% relative transcript level, an almost constant level of about 100% relative mRNA level was monitored in seedlings kept at 30 °C. After increasing the temperature from 10 °C to 24 °C the *cab* expression level increased continuously, without mani-

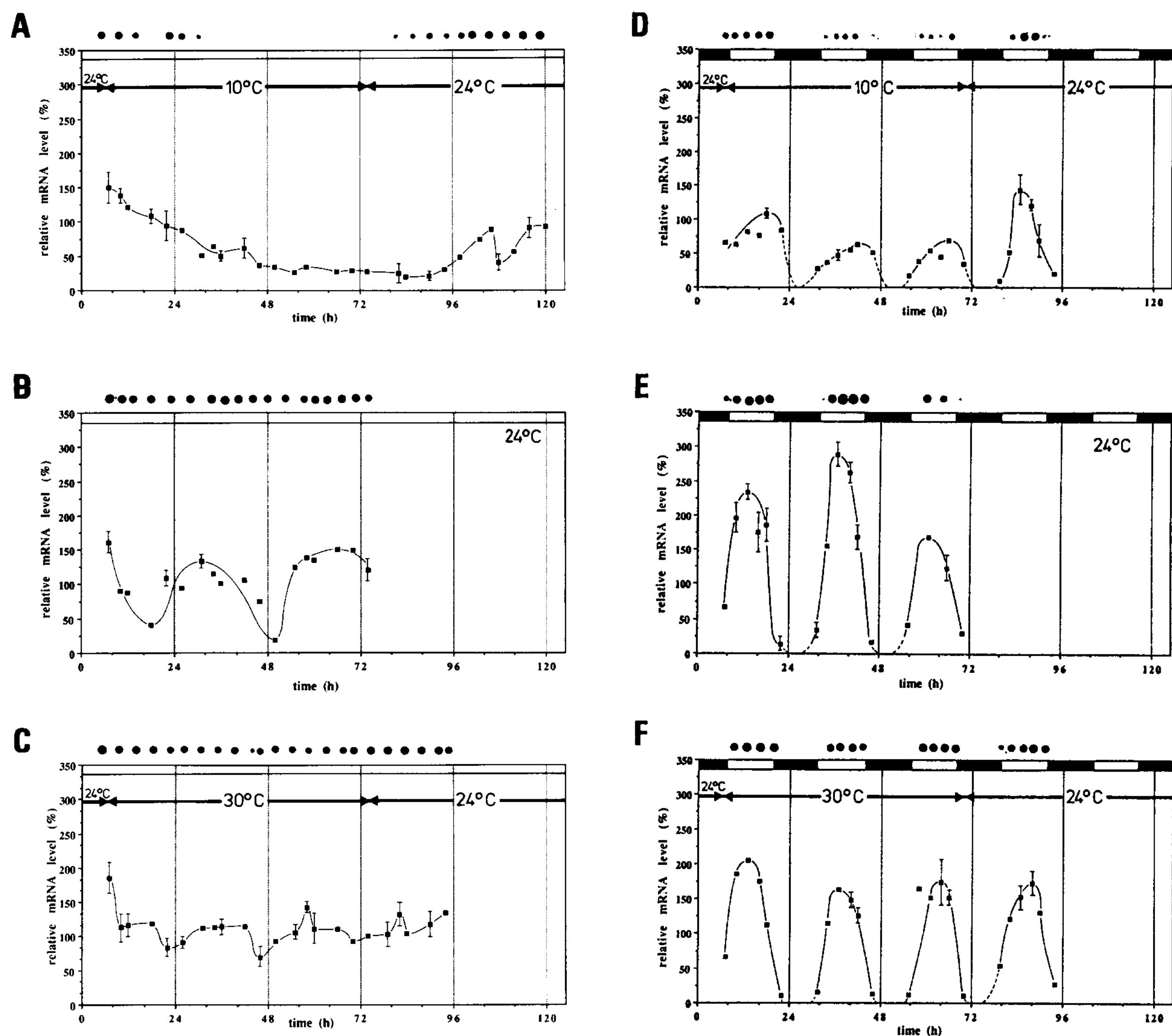

Fig. 4. Relative changes of the chl a/b binding protein transcript levels in leaves of tomato seedlings germinated and grown in continuous illumination (panels A–C) or in a 12 hours light/12 hours dark regime (panels D–F) at different temperatures. Light/dark alterations are indicated at the top of the panels, temperature shifts are indicated by arrows. Equal amounts of total RNA were spotted onto dot blots, hybridized with the *cab* 1B gene probe, and dots were cut out and counted (100% relative mRNA levels equals 149 cpm (panels A, C), 91 cpm (panel B) and 139 cpm (panels D, E, F). mRNA level calculations were based on two hybridizations, error bars represent SE.

festing an oscillation pattern within 2 days. In contrast, after reduction of the temperature from 30 °C to 24 °C the *cab* transcript levels remained the same.

Similar results were obtained in the case of the small subunit of RuBPC/Oase mRNA expression levels (Fig. 5A–C). A decrease of the temperature by 14 °C resulted in a gradual reduction of the *rbcS* mRNA levels, while at 30 °C the levels remained fairly constant. Again, no rhythmic expression pattern was enhanced or induced.

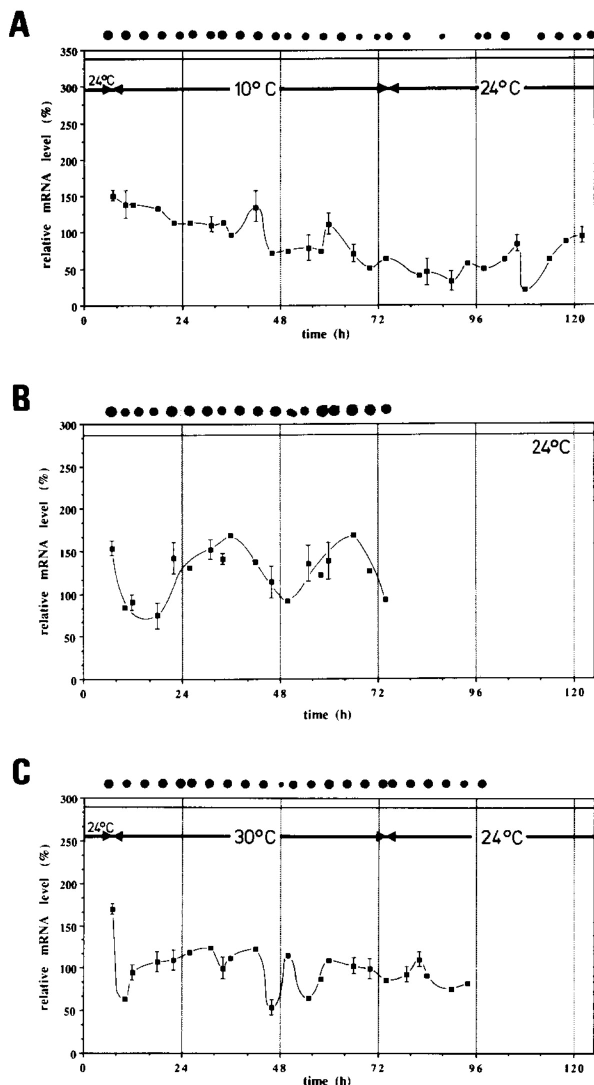

Fig. 5. Relative changes of the ssu of RuBPC/Oase transcript levels in leaves of tomato seedlings germinated and grown in continuous illumination (panels A–C) at different temperatures. Equal amounts of total RNA were spotted onto dot blots, hybridized with the *rbs* 3A gene probe, and dots were cut out and counted (100% relative mRNA level equals 203 cpm (panels A, B, C)). mRNA level calculations were based on three hybridizations. Error bars represent SE.

Chlorophyll a/b binding protein expression levels in seedlings grown in a light/dark regime and at different temperatures

This set of experiments was initiated to investigate which effect temperature alterations have on the typical mRNA oscillation pattern of the chlorophyll a/b binding protein in tomato plants grown in a light/dark cycle. For this purpose seedlings were germinated and grown in a 12 hours light/12 hours dark regime at 24 °C and then shifted to different temperatures. At 24 °C the *cab* mRNA levels exhibited a characteristic oscillation pattern, with a period of approximately 24 hours and an amplitude of 300% relative transcript level (Fig. 4E). Maximum amplitude was measured 18 hours after the light/dark transition. After a temperature shift to 10 °C a 3-fold reduction of the amplitude was immediately observed. At the consecutive two days the oscillation pattern with small amplitudes remained present (Fig. 4D). The period length was not altered, but the time point of maximum mRNA accumulation of the period was delayed by approximately 6 hours (24 hours after the light/dark transition). After transfer of the seedlings back to 24 °C, the maximum of the period occurred at its original phase, and the amplitude was enhanced 2–3-fold. In contrast, after transfer of tomato seedlings from 24 °C to 30 °C the *cab* mRNA amplitude gradually dampened from 300% to about 150% within 3 days (Fig. 4F) and the period length and the phase of maximum mRNA accumulation remained constant.

The results of these experiments indicated clearly that the most pronounced mRNA level variations were expressed at 24 °C, while at lower or higher temperatures only smaller *cab* mRNA amplitudes were observed. This result supports the ability of temperature compensation, a characteristic feature of circadian rhythms [6].

Discussion

Evidence for the diurnal/circadian rhythms acting at the molecular level in plants has been presented in several publications since 1985 (relevant citations in [8]). The research in most laboratories has been focused on the characterization of the expression of the chlorophyll a/b binding proteins in a variety of monocots and dicots. Several experiments, such as the transfer in constant environmental conditions and the adjustment to several altered light/dark regimes were performed to analyse the rhythmic oscillations of the *cab* mRNA accumulation in more detail [11, 17, 18, relevant citations in 8]. However, a central question regarding the origin of the diurnal rhythm remained open. Since most investigations were based on seedlings or adult, vegetative plants germinated and grown in a certain light/dark regime (natural or artificial) prior to the conditions used for the particular expriment designed to investigate aspects of the circadian rhythm, the interpretation with respect to the origin or inducibility of the rhythm was difficult. To overcome this problem of possible entrainment and synchronization by signals or impulses present during early plant development, we used tomato seedlings germinated and grown in a growth chamber in a constant environment. However, even under such growth conditions it cannot be excluded that i) the induction/initiation of these oscillations occurred at the time point of seed embedding, or ii) that light/dark cycles have an effect on the dry seeds themselves.

Beside this criticism, a rhythmic expression pattern of the chlorophyll a/b binding protein with a defined period length of approximately 32 hours and a small amplitude was present in tomato seedlings grown in constant illumination and at constant temperature. Based on this pattern it is likely that the circadian rhythm of *cab* gene expression is inherited. Although the amplitude of the rhythm is several-fold lower than a light/dark cycle, the fact that it was observed in three independently performed experiments argues against an artefact of the measurements. Similar results were obtained for the *cab* 1 gene in wheat seedlings [12], but the amplitude reached in continuous illumination was substantially higher than as in light/dark regimes (Steve Kay, personal communication). The significance of this difference between the amplitude of the expression levels of the chlorophyll a/b binding proteins in these two plant species can only be speculated about at this time. It is possible that i) different growth conditions (e.g. light quality or quantity) were used or ii) characteristically different mechanisms (e.g. strength of the oscillator) underly the circadian rhythms of *cab* gene expression in dicots (tomato) and monocots (wheat). Other plant species have to be investigated for this aspect to allow of a conclusive interpretation. Consistent with the possible inherited origin of the oscillations of the chlorophyll a/b binding protein mRNA rhythm were the results of Bünning [3, 4] demonstrating that the circadian rhythms of flowering and eclusion persist in *Phaseolus* and *Drosophila*, respectively, even though generation after generation had been raised in environment completely lacking cues to the passage of time.

To our knowledge, this is the first report showing that a single dark phase of 3, 6 or 9 hours is sufficient to enhance and synchronize a rhythm of the chlorophyll a/b binding protein transcript accumulation in tomato seedlings grown in continuous illumination. The amplitude of the oscillation was dependent on the duration of the dark phase, while the time point of maximum and minimum level was determined by the light-to-dark transition phase. During the following days in continuous illumination the elevated amplitude decayed, indicating that the dark phase was important for high *cab* mRNA accumulation. Similar effects of the duration of darkness in combination with the circadian time were recently reported for adult, vegetative tomato plants [11]. At present it is not understood which action the dark phase exhibits at the transcriptional level during *cab* gene expression. In this context it is interesting to note that critical durations of day and/or night length also have an important function in the flowering response of plants [19, 25, for review 26]. The photoperiodic time keeping in short day plants as *Xanthium* and

Glycine was essentially a question of measuring the duration of the darkness. Flowering occurred only when the dark phase was in excess of 8.5 hours or 10 hours, respectively. In *Pharbitis nil* (cv. Violet) seedlings, grown in continuous white light flowered in response to a single dark phase longer than 8.5 hours. It should be pointed out that the inducive dark phase for flowering was only effective after preceding illumination [25, for review 26].

Beside the significance of the duration of the light and dark phases, the timing of light/dark or dark/light transitions suggested an interesting aspect, when the photoperiodic time keeping mechanism of flowering was studied. The duration of the darkness was precisely timed and the trigger for the beginning of the time measurement is most likely the transfer from light to darkness. It appeared that a similar interpretation was applicable in the case of the expression mechanism for the chlorophyll a/b binding protein genes in tomato seedlings. The time points of maximum and minimum of the transcript accumulation patterns were correlated to the time points of the light/dark transitions (Figs. 2, 3). This statement seems to contradict the finding that dark-to-light transisitons determine the time points of maximum and minimum of the period in tomato plants grown in altered and unusual light/dark regimes [18]. However, experiments regarding flowering responds in *Pharbitis nil* demonstrated that time measurement can be coupled either to the light-on or light-off signal, depending on the duration of illumination or dankness [25] or circadian time [11]. Thus, the function of 'light/dark alterations is not inducing the rhythm, but determining the time of maxima and minima' [5].

The hypothesis of the involvement of a photoreceptor such as photochrome in sensing the light to dark transition at dusk was supported by several examples in plants. The photoconversion of photochrome is involved in resetting the circadian rhythm of the nyctinastic closure of leaflets of *Albizzia julibrissin* and *Samanea saman* [20, 21], the flowering responds in *Xanthium, Pharbitis* and *Kalanchoe* [26], and probably the accumulation of the chlorophyll a/b binding protein mRNAs. Recently, it was documented that a 2 min flash of red light was sufficient to induce the circadian rhythm of the chlorophyll a/b protein mRNAs in wheat and bean seedlings [12, 23].

The expression of the two gene families encoding the chlorophyll a/b binding proteins and the small subunit of RuBPC/Oase were investigated in parallel in several laboratories. Interesting similarities of the expression patterns were observed with respect to their light dependence, red and far-red responsiveness and tissue specificity, suggesting that similar or even the same regulating mechanisms apply for both photosynthesis-specific nuclear-encoded gene families. In contrast to this coherence apparently a photoperiodic time measurement system regulates *cab* gene expression but interestingly the *rbcS* genes seem to be unaffected [11, 17, 18]. It was therefore concluded that transcriptional and/or post-transcriptional processes involved in the expression of the *cab* genes but not of the *rbcS* genes are at least partially governed by a circadian clock. In this paper however, we demonstrated that weakly expressed oscillations, which are due to an endogenous rhythm, occurred for both gene families in tomato seedlings grown without environmental perturbations. It seems possible that the superimposed signals such as light/dark cycles, dark phases, light quantity etc. effect the transcript levels of the *cab* and *rbcS* genes differentially in tomato leaves.

Beside the effect of light/dark transistions as a basis of a photoperiodic time measurement system in plants, the parameter 'temperature' was tested for its ability to influence the oscillations of *cab* mRNA levels. Tomato seedlings grown either in continuous light or in a 12 hours light/12 hours dark cycle accumulated less *cab* mRNAs at 10 °C and 30 °C as in comparison at 24 °C. Similarly, the rhythm of CO_2 fixation in the crassulacean plant *Bryophyllum fedtschenkoi* was absent in leaves exposed to low temperature [1]. These data suggest that at these extreme temperatures in general an inactivation or reduction of activity of metabolic and/or enzymatic reactions occurred. Interestingly, the periodic length of the rhythm of *cab* mRNA accumulation in light/dark cycles was

not altered at higher or lower temperatures while in other plants (e.g. *B. fedtschenkoi*, [1]) a significantly lengthened period was accompanied by decreasing temperatures.

Several physiological and morphological functions (flowering, nyctinastic leaf movement, accumulation of sugar compounds and the accumulation of specific mRNAs) are precisely regulated and synchronized in anticipation of the next day and adaptation to the variations of the environment suggesting that the photoperiodic time measurement involves a circadian oscillator in higher plants. However, at present no evidence exists for explanations how and why particularly the transcript levels of the chlorophyll a/b binding proteins are under the control of a circadian pacemaker. The present model that emerges from the collected information regarding the *cab* gene expression pattern includes an inherited rhythm as a basis on which superimposed different signals such as light, organ type, developmental stage etc. manifest their specific effects. Further investigations are necessary to refine the *cab* expression mechanism.

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References

1. Anderson CM, Wilkins MB: Period and phase control by temperarure in the circadian rhythm of carbon dioxide fixation in illuminated leaves of *Bryophyllum fedtschenkoi*. Planta 177: 456–469 (1989).
2. Arnon DI: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15 (1949).
3. Bünning E: Insight into the hereditary diurnal periodicity of the primordial leaves of *Phaseolus multiflorus*. Jahrb Wiss Bot 81: 411–418 (1935).
4. Bünning E: Endogenous daily rhythms as the basis of photoperiodism. Ber Deut Bot Ges 54: 590–607 (1936).
5. Bünning E: Endogenous rhythms in plants. Ann Rev Plant Physiol 7: 71–90 (1956).
6. Feldman JF: Genetic approaches to circadian clocks. Ann Rev Plant Physiol 33: 583–608 (1982).
7. Galangau F, Daniel-Vedele F, Moureaux T, Dorbe M, Leydecker M-TH, Coboche M: Expression of leaf nitrate reductase genes from tomato and tobacco in relation to light-dark regimes and nitrate supply. Plant Physiol 88: 383–388 (1988).
8. Kellmann JW, Pichersky E, Piechulla B: Analysis of the diurnal expression pattern of the tomato chlorophyll a/b binding protein genes. Influence of light and characterization of the gene family. Photochem Photobiol, manuscript submitted (1989).
9. Kloppstech K: Diurnal circadian rhythmicity in the expression of light-induced plant nuclear messenger RNAs. Planta 165: 502–506 (1985).
10. Kuhlemeier C, Green PJ, Chua NH: Regulation of gene expression in higher plants. Ann Rev Plant Physiol 38: 221–255 (1987).
11. Meyer H, Thienel U, Piechulla B: Molecular characterization of the diurnal/circadian expression of the chlorophyll a/b binding proteins in leaves of tomato and other dicotyledonous and monocotyledonous plant species. Planta, 180: 5–15 (1989).
12. Nagy F, Kay SA, Chua NH: A circadian clock regulates transcription of the wheat cab-1 gene. Genes and Dev 2: 376–382 (1988).
13. Pichersky E, Bernatzky R, Tanksley SA, Breidenbach RW, Kausch AP, Cashmore AR: Molecular characterization and genetic mapping of two clusters of genes encoding chlorophyll a/b-binding proteins in *Lycopersicon esculentum* (tomato). Gene 40: 247–258 (1985).
14. Pichersky E, Bernatzky R, Tanksley SD, Cashmore AR: Evidence for selection as a mechanism in the concerted evolution of *Lycopersicon esculentum* (tomato) genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. Proc Natl Acad Sci USA 83: 3880–3884 (1986).
15. Piechulla B, Pichersky E, Cashmore AR, Gruissem W: Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit development and ripening. Plant Mol Biol 7: 367–376 (1986).
16. Piechulla B, Gruissem W: Diurnal mRNA fluctuations of nuclear and plastid genes in developing tomato fruits. EMBO J 6: 3593–3599 (1987).
17. Piechulla B: Plastid and nuclear mRNA fluctuations in tomato leaves – diurnal and circadian rhythms during extended dark and light periods. Plant Mol Biol 11: 345–353 (1988).
18. Piechulla B: 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. Plant Mol Biol 12: 317–327 (1989).
19. Salisbury FB: Time measurement and the light period in flowering. Planta 66: 1–26 (1965).

20. Satter RL, Galston AW: Mechanisms of control of leaf movements. Ann Rev Plant Physiol 32: 83–110 (1981).
21. Simon E, Satter RL, Galston AW: Circadian rhythmicity in excised *Samanea pulvini.* II. Resetting the clock by phyotochrome conversion. Plant Physiol 58: 421–425 (1976).
22. Simpson J, Van Montagu M, Herrera-Estrella L: Photosynthesis-associated gene families: Differences in response to tissue-specific and environmental factors. Science 233: 34–38 (1986).
23. Tavladoraki P, Kloppstech K, Argyroudi-Akoyunoglou J: Circadian rhythm in the expression of the mRNA coding for the apoprotein of the light-harvesting complex of photosystem II. Plant Physiol 90: 665–672 (1989).
24. Tobin E, Silverthorne J: Light regulation of gene expression in higher plants. Ann Rev Plant Physiol 36: 569–593 (1985).
25. Vince-Prue D: The perception of light-dark transitions. Phil Trans R Soc Lond 303: 523–536 (1983).
26. Vince-Prue D: The duration of light and photoperiodic responses. In: Kendrick RE, Kronenberg GHM (eds) Photomorphogenesis in Plants, pp. 269–305. Martinus Nijhoff/Junk Publishers, Dordrecht (1986).