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Communication

Effect of Temperature Alterations on the Diurnal Expression Pattern of the Chlorophyll *a/b* Binding Proteins in Tomato Seedlings[1]

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ABSTRACT

In the leaves of plants that are grown in the natural environment, the accumulation of mRNAs encoding the chlorophyll *a/b* binding proteins (CAB) follow a circadian rhythm. It is generally accepted that the day/night (sunset, light/dark) or night/day (sunrise, dark/light) transitions play an important role in the synchronization of the rhythm and the determination of the accumulation amplitude. As the results of the experiments presented in this paper indicate, temperature alterations also support the setting and the arrangement of the rhythm. Apparently, simulating "day/night" temperature alternations influences the tomato (*Lycopersicon esculentum*) plants to express a typical circadian oscillation pattern of *cab* mRNAs. This rhythm was sustained in the plants after long-term exposure to an alternating temperature regime. In constant conditions, *e.g.* continuous illumination at either 18°C or 24°C or in continuous darkness at 24°C, this diurnal fluctuation pattern with a period of about 24 hours remained present for at least 2 days.

In addition to the influences of light, developmental program, and organ-specificity, the expression of the *cab*[2] protein genes is regulated by an endogenous rhythm (relevant citations ref. 6). As a result of the controlling function of an unknown, uncharacterized endogenous oscillator, the steady-state *cab* mRNA levels change during the day: high levels are reached about 5 h after the dark to light transition (sunrise), while decreasing levels are detected in the afternoon and night (8). Several experiments have shown that the mRNA oscillations continue to be present in constant environmental conditions (illumination and temperature constant) (relevant citations in ref. 6). These results are the strongest indications that the expression of *cab* protein genes at the transcript level is regulated by a "biological clock."

As shown for other photoperiodic phenomena, such as opening and closing of flowers and diurnal leaf movement (for review 3, 5, 9), the *cab* mRNA oscillations are synchronized by light/dark and/or dark/light transitions (12). In the natural environment this synchronization process is performed by sunset and sunrise, respectively. During our studies of the diurnal/circadian expression of the *cab* protein genes, we started to wonder which role daily temperature alterations may play in the manifestation of the *cab* mRNA accumulation pattern and level. Alternating temperature changes occur in nature, with higher temperature usually correlated with the day time and lower temperatures related to the night time. To answer at least part of the question, tomato seedlings were grown in varied temperature conditions to simulate natural temperature oscillations.

MATERIALS AND METHODS

Plant Material and RNA Isolation

Tomato plants (*Lycopersicon esculentum*, cv VFNT LA 1221, cherry line) were grown in vermiculite in a growth chamber and watered with water or Hoagland nutrient solution. The plants were grown in white light (76 W/m^2, 30 neon bulbs, Philips TLM, 115 W, 33RS double lux) and at indicated time points temperature shifts occurred (8 AM–8 PM: 24°C; 8 PM–8 AM: 18°C). Leaves of tomato seedlings were harvested at 7 AM, 9 AM, noon, 4 PM, 8 PM, and midnight, and the tissue was immediately frozen in liquid nitrogen and stored at −50°C until further use. The isolation and quantitation of the *cab* mRNA was performed by the method described elsewhere (11, 12).

RESULTS

In a previous publication (12), it was documented that tomato seedlings grown in continuous illumination and at constant temperature (24°C) express an oscillating accumulation pattern of the *cab* mRNAs. Under such experimental conditions a characteristic amplitude and a period length of 30 to 32 h was observed (12). To determine which influence temperature alternations have on this endogenous rhythm, two sets of experiments were performed. In the first set, tomato plants were grown at continuous illumination and constant temperature of 24°C and were transferred into alternating temperature conditions (Fig. 1). In the second experiment, the tomato seedlings were germinated and grown at alternating temperatures and in LL before they were shifted to constant temperatures either to 18°C or 24°C (Fig. 2).

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[2] Abbreviations, *cab* chlorophyll *a/b* binding; LL, continuous light; LD, light/dark alterations; DD, continuous darkness.

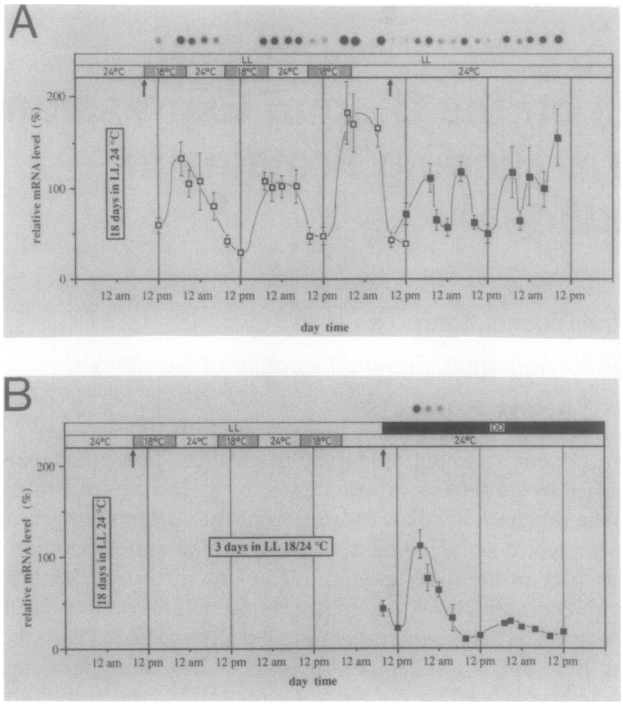

Figure 1. Diurnal fluctuation of Chl *a/b* binding protein mRNA levels at different temperature regimes. Tomato seedlings were grown in continuous light and constant temperature of 24°C before they were transferred to alternating temperatures of 12 h 18°C and 12 h 24°C for 3 d. Transitions from low to high and high to low temperatures were at 8 AM and 8 PM, respectively. After 3 d several seedlings were shifted either to 24°C in continuous light (panel A) or to 24°C in continuous darkness (panel B). Leaves of tomato seedlings were harvested at indicated time points. Equal amounts of total RNA were spotted onto dot blots, hybridized with the specific gene probe, and dots were cut out and counted. Data are based on three hybridizations of one experiment, error bars represent m SE.

Shift from Constant Conditions to Alternating Temperatures

Eighteen d-old tomato seedlings were transferred from a constant environment to "day/night" temperature simulated conditions (18°C/24°C). After this shift no obvious change of the phenotypical appearance of the seedlings was observed. The Chl contents and Chl *a/b* ratios of plants grown in LL at 24°C were 1.47 mg Chl/g fresh weight and 2.82, respectively, whereas at alternating temperatures and constant illumination, these values were determined to be 1.59 mg Chl/g fresh weight and 2.45, respectively. However, at the transcript level, a reduction of the period length from 30 to 32 h to about 24 h and an approximate 0.5-fold increase of the *cab* mRNA amplitudes were observed (Fig. 1A). Since a circadian rhythm was expressed, it seems likely that the 12 h 18°C and 12 h 24°C temperature program had a synchronizing effect on the endogenous *cab* mRNA oscillation pattern. The maximum of mRNA accumulation was reached at or within 2 h after the transition from low to higher temperatures.

Rhythmic expression of the *cab* protein genes in alternating temperatures was further analyzed in tomato plants that were transferred into constant environmental conditions, either LL at 24°C (Fig. 1A) or DD at 24°C (Fig. 1B). In the first case (Fig. 1A), the steady-state *cab* mRNA levels fluctuated but no rhythmic pattern with a constant period was observed, indicating that the circadian rhythm was not sustained after 3 d of synchronization by alternating "day/night" simulating temperatures. On the other hand, in darkness and at 24°C the oscillations continued, but with gradually decreasing amplitudes. Characteristically, the time of the appearance of the maximum and minimum as well as the period of approxi-

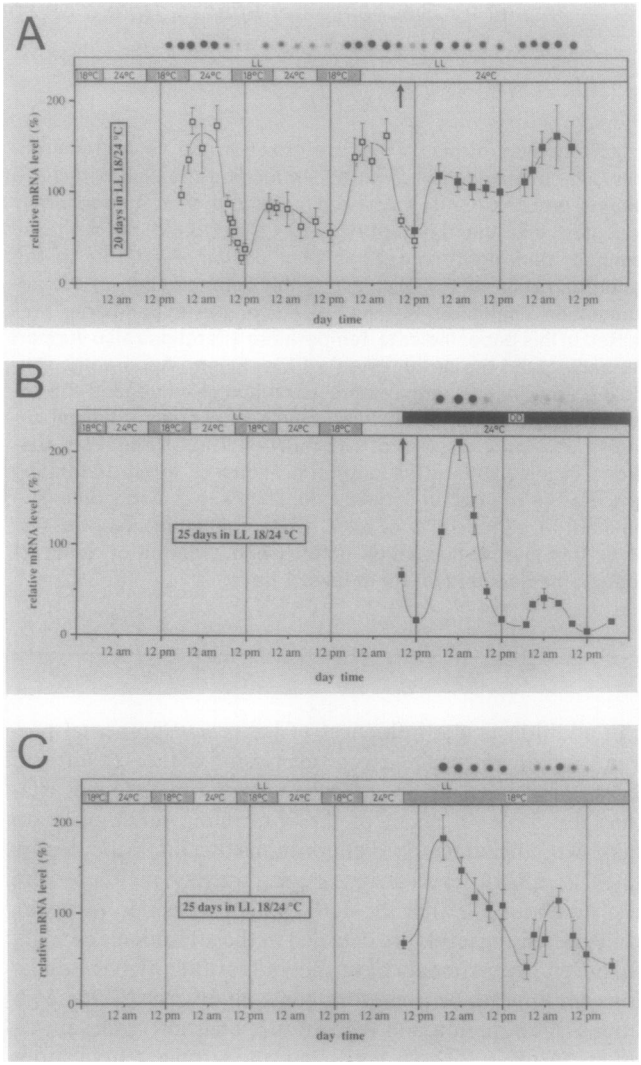

Figure 2. Diurnal fluctuations of Chl *a/b* binding protein mRNA levels at different temperature regimes. Tomato seedlings were germinated and grown in continuous light and in an alternating temperature regime of 12 h 18°C and 12 h 24°C. Transitions from low to high and high to low temperatures were at 8 AM and 8 PM, respectively. Several seedlings were shifted either to 24°C in continuous light (panel A), to 24°C in continuous darkness (panel B), or to 18°C in continuous light (panel C). leaves of tomato seedlings were harvested at indicated time points. Equal amounts of total RNA were spotted onto dot blots, hybridized with the specific gene probe, and dots were cut out and counted. Data are based on three hybridizations of one experiment, error bars represent ± SE.

mately 24 h remained. It seems that in the dark the circadian rhythm of *cab* gene expression was continued; however, it should be noted that the transition from light to darkness itself has a synchronizing effect which is known in other cases to have a stronger effect than temperature variations (5).

From these experiments it can be concluded that as long as such environmental conditions are applied, "day/night" simulating temperature variations are able to synchronize the *cab* mRNA rhythm and to elevate the amplitude of the rhythm, but without synchronization the circadian rhythm disappears.

Shift from Alternating Temperature Conditions to Constant Conditions

One reason the circadian rhythm was not manifested in the experiment described above may be the short "training" period. Because of this argument, we germinated and grew tomato seedlings for about 20 d in LL and the "day/night" temperature simulating regime, before they were shifted to constant environmental conditions (Fig. 2). In alternating temperature conditions the leaves of the seedlings contained 1.6 mg Chl/g fresh weight and a Chl *a*/*b* ratio of 3.15. In respect to the expression of the *cab* genes, a rhythm with a period length of approximately 24 h was observed. After transfer to LL at 24°C (Fig. 2A), DD at 24°C (Fig. 2B), and LL at 18°C (Fig. 2C), it seems that the period of the *cab* mRNA oscillations remained the same. Characteristic differences were documented for the accumulation level and the amplitudes of the fluctuation pattern. A small amplitude, however, at a high level of transcript accumulation was monitored in seedlings transferred to LL and 25°C, while higher *cab* mRNA amplitudes were present at DD at 24°C and LL at 18°C. In the latter cases, the amplitudes were reduced at the second day in constant conditions. Again, as observed in the first experiment (Fig. 1B), the L to D transition has a strong effect on the synchronization and amplitude of the oscillation.

Based on the results of these experiments we conclude that the long term exposure to "day/night" simulating temperature conditions synchronized the endogenous *cab* transcript accumulation rhythm so that a circadian oscillation is memorized and can be expressed in constant conditions for at least 2 d.

DISCUSSION

One reason for the existence of circadian rhythms in plants is speculated to be related to the anticipation of the "next day." In the case of the CAB proteins, it seems important to put plants into optimal conditions for the synthesis and assembly of thylakoid membranes, which are necessary for well functioning photosynthetic reactions. It is worth while to note that the maximum of the *cab* mRNA accumulation pattern is shortly after the transition from lower to higher temperatures, which supports this hypothesis. In *Oedogonium cardiacum* it was demonstrated that the maximum of the sporulation rhythm was also correlated with the beginning of the higher temperature (2). Interestingly, the increase of steady-state *cab* mRNA levels in tomato coincides also with the elevation of transcript accumulation after dark/light or night/day (sunrise) transitions (8). Furthermore it is important to point out that in alternating light/dark conditions and at different constant temperatures, neither a phase shift nor an alteration of the period of the *cab* mRNA rhythm occurred between 10 and 30°C (12). Only the amplitudes of the oscillation pattern of *cab* transcripts were altered, high levels were measured at 24°C, intermediate levels at 30°C, and low levels were detected at 10°C.

The goal of the experiments presented here was not to measure the rhythmic mRNA changes at different constant temperatures, but to focus on the accumulation patterns of the *cab* mRNAs which are due to the influence of diurnal alternating temperature alterations. To eliminate possible effects due to "heat shock" or "chilling" stress, the temperatures were varied in a physiological range between 18 and 24°C. It was reported in *Kalanchoë blossfeldiana, Gonyaulax polyedra, Lemna gibba,* and *Bryophyllum fedtschenkoi* that physiologically extreme temperatures inhibit circadian rhythms by driving the basic oscillator to, and holding it at, a fixed phase point of the cycle (relevant citations in 1). Additionally, a more pronounced petal movement was monitored in *K. blossfeldiana* grown at 15°C/20°C temperature variations than in 15°C/25°C or 15°C/30°C temperature regimes (10).

The results of the tomato *cab* mRNA accumulation presented in this paper (Figs. 1A and 2A) support the idea that diurnal cycles of high and low temperatures are an effective "Zeitgeber" for the synchronization of rhythms in plants. A similar hypothesis was proposed based on experiments performed with cold-blooded animals (5). In *Phaseolus multiflorus* a 12 h/12 h temperature alternation between 25 and 30°C in LL synchronizes the leaf movement, and the sporulation of the algae *O. cardiacum* is synchronized by 15 to 25°C temperature shifts. In the later case a 2.5°C variation is sufficient for the induction of the rhythm (reviewed in 5). In *K. blossfeldiana* the opening and closing of the flowers was synchronized and induced by 1°C temperature differences (5). In contrast, other examples with altered periods due to temperature variations have been pointed out, *e.g.:* the period of sporulation of *O. cardiacum* was 20 h at 17.5°C and 25 h at 27.5°C (2), and in *P. multiflorus* at 22°C and 14.5°C the periods of the leaf movement were determined to be 27.5 h and 23.2 h, respectively (4). Based on the results in *P. multiflorus,* Leinweber (7) argued for the existence of a regulating system that organizes—at least within a certain temperature range—the phenomenon of temperature independent period. However, the underlying mechanism was not investigated in further detail.

Based on all the results describing the phenomenon of rhythmic accumulation of *cab* mRNAs in tomato (relevant citations in 6), we conclude that the alterations of illuminating conditions (darkness and light) as well as the alternating low and high temperature regimes play an important role in the synchronizing process of the oscillations. Our data support the notion that L/D or D/L transitions are a stronger "Zeitgeber" than temperature variations. This indication coincides with the conclusions reported by Bünning (5).

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