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Short Communication

Circadian and phytochrome control act at different promoter regions of the tomato *Lhca*3 gene

Marcella B. Pott, Jan W. Kellmann, Birgit Piechulla*

University of Rostock, Department of Molecular Physiology and Biotechnology, Gertrudenstraße 11a, 18051 Rostock

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Summary

The expression of the *Lhc* genes is influenced by external (e.g. light, temperature) and endogenous (e.g. circadian clock) factors and processes. Their signal transduction pathways are integrated in a complex regulatory network. The action of an endogenous clock is only useful if it can be synchronized by external factors, most importantly by light. Therefore, overlapping effects of the circadian system and light perception signal transduction chain are expected. Since exclusive phytochrome or circadian clock control has not been shown, it was hypothesized that both signal transduction pathways converge in one transcription complex using the same cis-regulatory element (Anderson et al. 1994). However, here we present for the first time evidence that different promoter regions are sufficient to mediate phytochrome or circadian control. While the Δ-278 and Δ-231 promoter deletion lines of the tomato *Lhca*3 gene can both be activated by red light and reversed by far red light pulses, only the Δ-278 deletion revealed transcript oscillations, indicating that 231 bp are sufficient mediating phytochrome control while 278 bp are needed for circadian expression.

Key words: circadian clock – *Lhc* gene expression – phytochrome – promoter – cis regulatory element – tomato (*Lycopersicon esculentum*).

The *Lhc* genes (formerly *cab*) encoding the light-harvesting complex proteins of photosystem I and II have been isolated and characterized in many plant species, usually comprising a large gene family. The regulation of the expression of these genes has been under investigation, revealing gene activation i) after illumination of etiolated and mature tissue; ii) in specified tissues or organs; iii) during plant development; and iv) gated to precise times during the day (circadian clock). Transcriptional or post-transcriptional events are responsible for the manifestation of such transcript accumulation patterns.

Previous investigations have demonstrated that all 19 *Lhc* genes from tomato exhibit very similar light responsive and diurnal/circadian oscillatory mRNA accumulation patterns (Kellmann et al. 1993, Piechulla 1999). Based on this con-

* E-mail corresponding author:
birgit.piechulla@biologie.uni-rostock.de

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formaty, we reasoned that similar molecular components should be involved; however, sequence alignment of promoter regions revealed low motif conservation. Therefore it was necessary to undertake a functional analysis of the tomato *Lhc* promoters. Using promoter deletion constructs transformed into tobacco, it was possible to define short promoter regions which are sufficient to mediate circadian expression patterns (Piechulla et al. 1998). For example, a promoter deleted upstream of position −278 of the transcription start site of the tomato *Lhc*a3 gene revealed a circadian transcript accumulation pattern in transgenic tobacco plants, while a shorter promoter fragment of −231 resulted in constant mRNA level in light/dark (LD) and continuous dark (DD) conditions. It was therefore concluded that a potential region interacting with molecular components of the circadian clock regulatory network lies between position −278 and −231.

The action of an endogenous clock is only useful if it can be synchronized by external factors. The most important zeitgeber is light, but temperature and other environmental conditions can also influence the clock. Therefore, overlapping effects of the circadian system and light perception may occur. The presence of the PAS domain (direct repeats in the *p*eriod protein of *Drosophila,* in the *a*ryl hydrocarbon receptor nuclear translocator of mammals, and the *s*im protein of *Drosophila*), in the period protein of *Drosophila,* white collar 1 and 2 of *Neurospora,* phytochrome and the photoactive yellow protein (Kay 1997, summarized in Taylor and Zhulin 1999) supports this notion. Furthermore, models presenting the interaction of phytochrome and the circadian oscillator have also been developed (Anderson et al. 1995), indicating a positive regulation by phytochrome and a negative control by the circadian clock revealing day-time gated expression of the *Lhc* genes. Since up to now no promoter element has been detected which allows solely the regulation by phytochrome or the circadian clock, it was hypothesized that the signal transduction pathways of both mechanisms merge, and the same cis- and trans-regulatory elements are present at the end of both chains (Anderson et al. 1994). This hypothesis was recently supported by two independent identifications of a *Lhc* promoter motive ($CAAN_{4-6}ATC$), present in 81 % of all published *Lhc* promoters. This sequence was found by Wang et al. (1997) investigating phytochrome regulation of the *Lhc*b1*3 gene of *Arabidopsis thaliana,* and by our group, screening for clock-controlled sequences in the tomato *Lhc* genes (Piechulla et al. 1998).

Using different transgenic tobacco plants bearing defined promoter deletions of the tomato *Lhc*a3 gene (Δ-278 and Δ-231), we could address the question of whether the endogenous clock and phytochrome signal transduction chains recruit the same or different regulatory elements, e.g. cis-regulatory element, by illuminating these transgenic lines with red and far red light.

To discriminate between the tomato *Lhc*a3 and the tobacco genes, a specific oligonucleotide was selected and hybridization conditions were established. Fig. 1 shows that

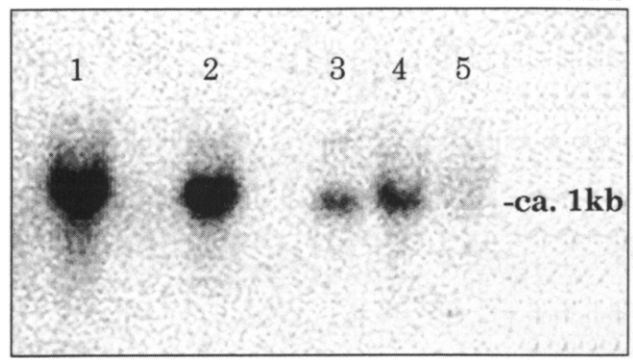
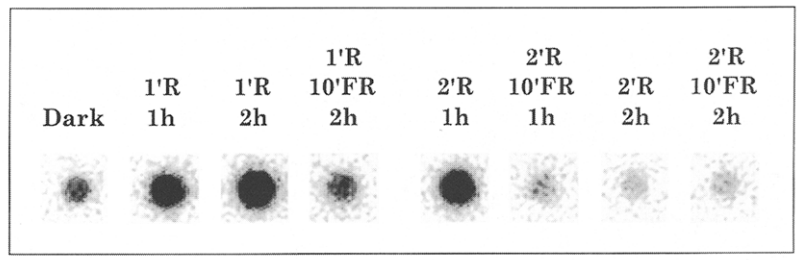


Figure 1. Specificity of the probe. Hybridization (Northern blot) of the tomato *Lhc*a3 oligonucleotide (CTTCTTCTCAAAAAAACAAAAA-CAAAC) with total RNA extracts from wild-type tomato (lane 1, 2, 3; 50, 20, and 2.5 µg RNA, respectively), transgenic tobacco (lane 4; 20 µg) and wild-type tobacco (lane 5; 20 µg).

the tomato *Lhc*a3 mRNA was detected in 50 µg (lane 1), 20 µg (lane 2), and 2.5 µg (lane 3) total RNA from tomato but not in 20 µg of tobacco total RNA (lane 5), but specifically hybridizes to the transgene mRNA in the transgenic tobacco plants (lane 4). Furthermore, the conditions for the red and far red light inductions and reversions were established and standardized with wild-type tobacco plants (Fig. 2). Pulses of one minute and two minutes of red light (1′ R or 2′ R) harvested one hour and two hours (1 or 2 h) after the light pulse were sufficient to increase total *Lhc* mRNA levels significantly. A ten minute far red pulse (10′ FR) was able to reduce the steady-state level to or below the dark control. To determine the phytochrome-mediated control on the Δ-278 and Δ-231 deleted promoter regions of the tomato *Lhc*a3 gene, transgenic tobacco plants were exposed to one minute red light (1′ R) as well as one minute red light followed by a ten-minute far red light pulse (1′ R/10′ FR) (Fig. 3). For both deletion constructs an increase of the steady-state mRNA levels was observed after red light illumination (R), while ten minutes of far red light (FR) led to a reduction as shown in Fig. 3 A. Such *Lhc*a3 hybridization signals were quantitated and normalized against ribosomal RNA, revealing relative mRNA levels under the various light conditions (Fig. 3 B). The experiments demonstrate that red light is able to activate *Lhc*a3 gene transcription of both deletion constructs (Δ-231 and Δ-278) in transgenic plants, and this process was reversible, to various degrees, after far red light illumination. The incomplete reversibility most likely has two reasons: i) the presence of several phytochrome genes/proteins responsible for light perception in higher plants may complement each other, and ii) the majority of the red light induction is based on the VLFR (very low fluence response) and only a smaller portion on the LFR (low fluence response). Only the LFR is reversible due to phytochrome B (Casal et al. 1998).

Conclusively, both plant deletions (Δ-278 and Δ-231) revealed increasing mRNA levels of the transgenes after red

Figure 2. *Lhc* gene regulation in red and far red illuminated wild-type tobacco plants. 5 day dark-adapted 3-week old tobacco plants were illuminated with red (R) and red/far red light (R/FR) for indicated times (1′, 2′ or 10′ = 1, 2 or 10 min). Overall light intensity of red light was 106 µmol/m^2*s with 76 µmol/m^2*s for 600–700 nm, of far red light 57 µmol/m^2*s (overall) with 54 µmol/m^2*s for 720–800 nm. Leaves and stems were harvested one hour and two hours (1 or 2 h) after the light pulses, RNA was extracted and the dot blot was hybridized with a probe from the tomato *Lhc*b1*2 coding region, which hybridizes to the majority of *Lhc* genes from tomato and tobacco.



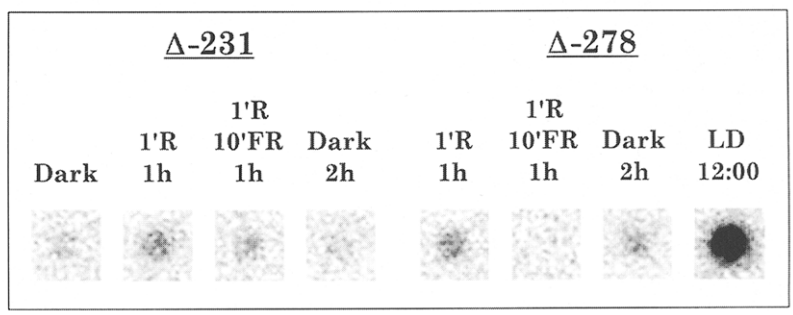


Figure 3 a.

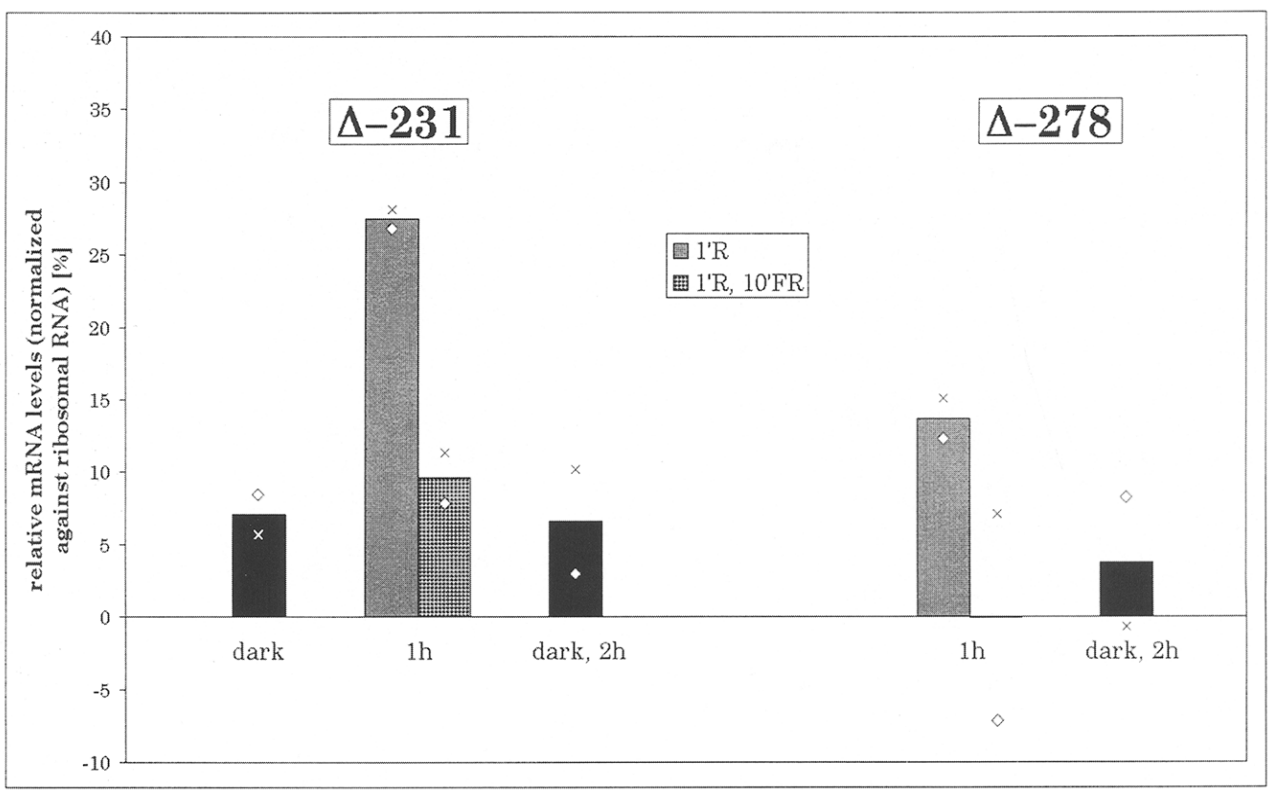


Figure 3. *Lhc*a3 gene regulation in red and far red illuminated transgenic tobacco plants. Δ-278 and Δ-231 promoter deletion lines were grown from seeds (F2 generation) for 3 weeks and were 5 days dark-adapted prior to the indicated illuminations (light conditions see Fig. 2). Leaves and stems were harvested one hour after the light pulses, RNA was extracted and the dot blot was hybridized with the specific *Lhc*a3 oligonucleotide (see Fig. 1). RNA isolated from LD (light/dark conditions) grown plants of the respective lines harvested at noon was used as a positive control. (A) shows a representative dot blot, (B) expresses the quantitated and normalized (against ribosomal RNA) relative mRNA levels of the promoter deletion lines Δ-278 and Δ-231, respectively. The column presents the average value of two experiments (stars and squares).

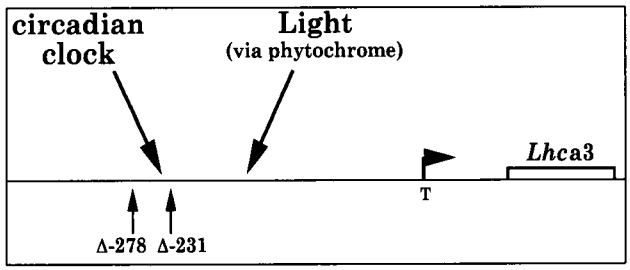


Figure 4. Schematic presentation: Circadian clock and light (via phytochrome) act on different tomato *Lhca*3 promoter regions. T: transcription start site.

light pulses as well as red/far red reversibility, indicating that both remaining short promoter fragments are able to mediate phytochrome-dependent gene activation. Together with the previous experiments, which revealed a loss of clock control in the Δ-231 transgenic plants (Piechulla et al. 1998), a separation of phytochrome and circadian clock control can be delineated from the results presented in this paper (Fig. 4). It becomes clear that at least for the tomato *Lhca*3 gene, neither signal transduction pathway converges by using an identical cis-regulatory element. The influences of the coding region and remaining 3′ UTR and 5′ UTR on RNA stability are not presently known. It would be interesting to further investigate the end points of the signal transduction chains of the other tomato *Lhc* genes to understand the complete phytochrome and circadian clock controlled regulatory network.

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