CHRONOBIOLOGY INTERNATIONAL, 16(2), 115–128 (1999)

Minireview

CIRCADIAN EXPRESSION OF THE LIGHT-HARVESTING COMPLEX PROTEIN GENES IN PLANTS

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ABSTRACT

Photosynthesis is one of the important processes that enable life on earth. To optimize photosynthesis reactions during a solar day, most of them are timed to be active during the light phase. This includes the components of the thylakoid membranes in chloroplasts. Prominent representatives are the proteins of the light-harvesting complex (LHC). The synthesis of both the *Lhc* mRNA and the LHC protein occurs during the day and is regulated by the circadian clock, exhibiting the following pattern: increasing levels after sunrise, reaching a maximum around noon, and decreasing levels in the afternoon. To elucidate the involved control elements and regulatory circuits, the following strategies were applied: (1) analysis of promoters of *Lhc* genes, (2) analysis of DNA binding proteins, and (3) screening and investigation of mutants. The most promising elements found so far that may be involved in mediating the circadian rhythmicity of *Lhc* mRNA oscillations are a myb-like transcription factor CCA1 (Wang et al. 1997) and the corresponding DNA binding sequence (Piechulla et al. 1998). (*Chronobiology International, 16(2), 115–128, 1999*)

Key Words: Algae—Angiosperms—Circadian oscillation of mRNA and protein—Ferns—Gymnosperms—Light-harvesting complex proteins and genes (*Lhc*, LHC)—Moss—Promoter analysis—Sequence motif—Transcription factor.

Received August 31, 1997; returned for revision September 2, 1997; accepted September 28, 1998.

INTRODUCTION

The ability to anticipate upcoming events, particularly repetitive changes with 24h or annual periodicities, is apparently advantageous for survival in a natural environment since the mechanisms that allow organisms to foresee these changes are conserved during evolution. One of the numerous processes that need endogenously controlled precise daily timing is photosynthesis. The realization begs for the presence of an optimized photosynthetic apparatus. Prominent components of the photosynthetic machinery are the light-harvesting complexes (LHC), composed of chlorophyll a and b bound to several distinct LHC proteins. These pigment-binding proteins are localized in the thylakoid membranes of the chloroplasts, surrounding photosystem I and II reaction centers (LHCa and LHCb, respectively). They are responsible for the initial step of photosynthesis: capturing of sunlight and transfer of energy to the reaction centers to facilitate conversion into chemical energy. To execute this central function properly, a precise coordination of protein accumulation is required. Plants master this task partially by establishing a timing system (circadian clock) that determines the daily time point of accumulation of LHC proteins, as well as other thylakoid membrane proteins (Riesselmann and Piechulla 1992; Beator and Kloppstech 1994; Oelmüller et al. 1995). This circadian clock influences the expression of the *Lhc* genes at the level of transcription, as well as at the level of translation.

TRANSCRIPT OSCILLATIONS AND CONTROL AT THE TRANSCRIPTIONAL LEVEL

The surprising observation of diurnally oscillating steady-state mRNA levels of the *Lhc* genes (formerly *cab* genes) was independently made in three laboratories working with three different plant species: pea (Kloppstech 1985), tomato (Piechulla and Gruissem 1987), and wheat (Nagy et al. 1988, reviewed in Piechulla 1993). The phenomenon was identical: increasing steady-state mRNA levels during the morning hours, reaching a maximum around noon (approximately 4h after sunrise), decreasing levels in the afternoon, reaching undetectable levels during night. Plant species that have been investigated for this characteristic expression pattern to date are summarized in Table 1. Oscillations of mRNA accumulations continue to be present in constant illumination and in constant darkness; however, in the latter case, continuously decreasing amplitudes occur, usually resulting in undetectable levels after 3 days (Piechulla 1988). Since RNA preparations and detections cannot be automated, a continuous recording of mRNA content is impossible. With the help of reporter gene constructs and assays, the period length in wild-type plants under free-running conditions (continuous dark, DD) was determined to be 30–36 hours (Millar et al. 1992, 1995). Therefore, the endogenous clock is apparently slow and is synchronized to 24h by external zeitgebers.

It is generally accepted that dark-light (sunrise) or light-dark (sunset) transitions are dominant zeitgeber(s) that synchronize the endogenous rhythm, which is also the case for the *Lhc* mRNA oscillations (Piechulla 1993). Under natural conditions, other stimuli, such as periodic temperature changes, also may entrain the oscillations. Indeed, simulation of the day and night temperature profile (e.g., 18°C and 24°C) synchronizes the free-running oscillations (period length 32h) in 16-day-old tomato plants to 24h, with a maximum in the 24°C phase (Piechulla and Riesselmann 1990; Riesselmann and Piechulla 1990). On transfer to constant temperature conditions (24°C), the oscillations re-

Table 1. Plant Species Exhibiting Diurnal and/or Circadian *Lhc* Expression

Plant species	Gene	Steady-state mRNA		Transcription run on/off	Reference
		Diurnal	Circadian[a]		
Angiosperm					
Arabidopsis thaliana	*Lhc*b1*1	+	+	+	Millar and Kay 1991; Kolar et al., 1998
	*Lhc*b1*2	+	+		
	*Lhc*b1*3	−	−	+	
Bryophyllum fedschenkoi	*Lhc*	+	+		Anderson-Jones et al.. 1994
Glycine max	*Lhc*	+			Meyer et al. 1989
Hedera helix	*Lhc*	+			Jäschke et al.[b]
Helianthus annuus	*Lhc*	+			Jäschke et al.[b]
Hordeum vulgare	*Lhc*	+			Meyer et al., 1989; Beator et al. 1992; Nevo et al. 1993
Lycopersicon esculentum	*Lhc*a1,2,3,4	+	+		Kellman et al. 1993
	*Lhc*b1,2,3,5,6	+	+	+	Giuliano et al. 1988; Wehmeyer et al. 1990; Kellman et al. 1993
Nicotiana tabacum	*Lhc*	+		+	Wehmeyer et al. 1990
	*Lhc*b1	+	+		Kolar et al. 1995
Oryza sativa	*Lhc*	+	+		Kay et al. 1989
Petunia hybrida	*Lhc*b1*5,1*6	+	+		Stayton et al. 1989
	*Lhc*b2*1,1*9	+	+		Stayton et al. 1989
	*Lhc*a(cab15)	+	+		
Phaseolus aureus	*Lhc*	+			Meyer et al. 1989
P. coccineus	*Lhc*	+			Meyer et al. 1989
P. vulgaris	*Lhc*	+	+		Meyer et al. 1989; Tavladoraki et al. 1989
Pisum sativum	*Lhc*	+	+		Kloppstech 1985; Spiller et al. 1987; Otto et al. 1988; Adamska et al. 1991
Rhododendron sp.	*Lhc*	+			Jäschke et al.[b]
Secale cereale	*Lhc*	+	+		Ernst et al. 1990
Sinapis alba	*Lhc*	+			Meyer et al. 1989
Solanum tuberosum	*cab* 14[c]	+	+		Im et al. 1994
Sorghum bicolor	*Lhc*b	+	+		Childs et al. 1995
Spinacea-oleracea	*Lhc*b1,6	+			Oelmüller et al. 1995
Triticum aestivum	*Lhc*b1	+	+		Nagy et al. 1988
Vicia faba	*Lhc*	+			Meyer et al. 1989
Zea mays	*Lhc*	+			Taylor 1989

(*continued*)

Table 1. Continued

Plant species	Gene	Steady-state mRNA		Transcription run on/off	Reference
		Diurnal	Circadian[a]		
Gymnosperm					
Abies alba	*Lhc*	–			Oberschmidt et al. 1995
Dioon spinolosum	*Lhc*	+			Oberschmidt et al. 1995
Ephedra campylopoda	*Lhc*	–			Jäschke et al.[b]
Ginkgo biloba	*Lhc*	–			Jäschke et al.[b]
Gnetum gnemon	*Lhc*	+			Jäschke et al.[b]
Larix decidua	*Lhc*	–			Oberschmidt et al. 1995
Picea abies	*Lhc*	–			Oberschmidt et al. 1995
Pinus sylvestris	*Lhc*	–			Oberschmidt et al. 1995
Pseudotsuga menziesii	*Lhc*	+	+		Alosi et al. 1990
Taxus baccata	*Lhc*	–			Jäschke et al.[b]
Welwitschia mirabilis	*Lhc*	–			Jäschke et al.[b]
Fern					
Ceratopterisrichardii	*Lhc*	+			Oberschmidt et al. 1995
Equisetum myriochaetum	*Lhc*	+			Oberschmidt et al. 1995
Moss					
Conocephalum conicum	*Lhc*	–			Oberschmidt et al. 1995
Physcomitrella patens	*Lhc*	+			Oberschmidt et al. 1995
Algae					
Dunaliella					
D. bioculata	*Lhc*	+			Oberschmidt et al. 1995
D. tertiolecta	*Lhc*	+	+		LaRoche et al. 1991
Chlamydomonas					
C. eugametos	LI818	+	+		Gagne and Guertin 1992
C. reinhardtii	*Lhc*a1	+	+	+	Hwang and Herrin 1994
	*Lhc*b1	+	+		Nikaido et al. 1994; Jacobshagen et al. 1996
Scenedesmus obliquus	*Lhc*b2	+			Hermsmeier et al. 1994

[a]Several days in continuous darkness or light.
[b]K. Jäschke, H. Menzel, M. Brinker, and B. Piechulla, unpublished results.
[c]*cab* 14 promoter fused to the cat gene in transgenic tobacco plants.

main present (Piechulla and Riesselmann 1990; Kloppstech et al. 1991), indicating that temperature shifts can act as true zeitgebers.

Besides acting as a zeitgeber, extreme temperatures (4°C, 10°C, 30°C, 40°C) seem to have additional effects on the circadian clock. At low temperatures, the circadian clock loses its oscillatory function partially or completely, as demonstrated by reduced amplitudes (Kreps and Simon 1997), nonmeasurable diurnal or circadian *Lhc* mRNA accumulation (Riesselmann and Piechulla 1990), or an arrest of the clock during the time of cold temperature exposure (Martino-Catt and Ort 1992). In contrast, high temperatures, either continuously applied or as heat-shock pulses (40°C), do not disturb the circadian *Lhc* expression completely, but only modify the amplitude (Riesselmann and Piechulla 1990; Kloppstech et al. 1991). Together, these observations clearly indicate the zeitgeber function of temperature changes, but the interplay of light and temperature signals (and even other signals) as different zeitgebers remains presently unknown.

Detailed investigations demonstrated that, at least in higher plants, a set of individual *Lhc* genes is localized in the genome. When individual members of the *Lhc* gene family were investigated, in most cases all members expressed a circadian transcript pattern. The mRNA accumulation of 19 genes of the tomato *Lhc* gene family, for example, oscillates in LD (light-dark), DD, and LL (continuous light)—all in phase (Kellmann et al. 1990, 1993; Piechulla et al. 1991). An exception to this rule is the *Lhc*b1*3 (*cab*1) gene of *A. thaliana*, which shows constant transcript levels throughout several days (Millar and Kay 1991; Brusslan and Tobin 1992). This observation suggests additional regulatory mechanisms involved in *Lhc* gene expression, such as posttranscriptional processes or a lack of circadian control. This possibility is supported by our observation that 8 of 10 gymnosperm plant species investigated exhibit constant levels of *Lhc* mRNA (summarized in Table 1). This result was unexpected since a previous investigation showed circadian, or at least diurnal, *Lhc* mRNA expression in various algae, moss, and fern (Oberschmidt et al. 1995). This noncircadian/nondiurnal *Lhc* mRNA synthesis/degradation may suggest that a precise timing of *Lhc* synthesis is not required in gymnosperm species. This agrees well with the fact that gymnosperms are able to synthesize chlorophyll in the dark and thus may not need a precise daily timing of synthesis of chlorophyll and binding proteins. As a result of this, other regulatory components or circuits are required for the *Lhc* gene expression in plants of these two divisions of the plant kingdom.

The phenomena of diurnal and circadian *Lhc* transcript oscillations are usually based on measurements of steady-state mRNA levels, which may be the result of phase-dependent variations of transcript synthesis or transcript stability. Some authors performed transcription run on/off experiments to determine RNA synthesis at different phases of the day (Giuliano et al. 1988; Wehmeyer et al. 1990; Millar and Kay 1991; Meyer 1993; Hwang and Herrin 1994). Based on these experiments, it was concluded that diurnal changes of the transcription rate are the primary reason for the changes observed in the amount of RNA. RNA stability seems to play a minor role, as determined in *Chlamydomonas reinhardtii* (Hwang and Herrin 1994).

PROTEIN OSCILLATIONS AND CONTROL AT THE TRANSLATIONAL LEVEL

It may be assumed that daily or circadian changes of mRNA have consequences for the synthesis of LHC proteins. Determinations of diurnal steady-state LHC protein

levels during a LD cycle were different at the mature and seedling stages: western blot analysis did not reveal differences of LHC protein levels between day and night in full-size green tomato fruits (Piechulla and Gruissem 1987), while differences were observed in greening barley cotyledons (Beator et al. 1992; Anandan et al. 1993; Beator and Kloppstech 1993). Furthermore, the synthesis and the steady-state levels of chlorophyll oscillated together with the LHC apoprotein accumulation in seedlings (Busheva et al. 1991; Beator et al. 1992; Beator and Kloppstech 1993).

Although only approximately 1% of the total LHC protein is renewed every day, a precise timing is apparently indispensable: 35S methionin incorporation studies by Riesselmann and Piechulla (1992) clearly demonstrated that protein synthesis was limited in growing tomato leaves to the light phase (Martino-Catt and Ort 1992; Riesselmann and Piechulla 1992). The same synthesis pattern was also observed for the plastid-encoded D1 protein (psbA gene product), another photosynthetic protein localized in the reaction center of the thylakoid membrane (Riesselmann and Piechulla 1992). Both synthesis rates continue to oscillate in complete darkness, with a maximum at 14:00. This approach allowed the registration of the dominant LHC proteins of photosystem II (LHCb), but not the synthesis of each individual LHC protein. It remains to be investigated whether all LHC proteins show a circadian oscillation of synthesis that may be deduced from the mRNA oscillations of the individual *Lhc* mRNAs (Kellmann et al. 1993).

PHYSIOLOGICAL SIGNIFICANCE

The expenditure performed in each cell to control gene expression is enormous. Many regulatory circuits and elements are required to allow the existence and optimal adaptation of organisms. Here, we describe a control system that enables precise timing. The reason for oscillatory changes of mRNA levels rather than levels at a constant steady state may be seen in the energetic conditions and/or ribonucleotide supply in photosynthetically active (day) versus inactive (night) cells. This argument holds, in principle, for all transcripts, but we know that not all transcript species in a plant cell accumulate with a diurnal/circadian pattern (Piechulla 1993). The characteristic distinction of *Lhc* mRNAs versus other transcripts lies in the very short half-life time of approximately 2.5h (Piechulla and Klaff unpublished results). This high turnover makes the daily synthesis of the *Lhc* mRNA possible. Surprisingly, based on our calculations, only 1% of the LHCII protein underlies a daily turnover (degradation: half-life of the protein, damage due to high irradiance). The turnover of each individual LHC protein type is not known. The discrepancy of high mRNA synthesis in contrast to a small amount of protein synthesis is striking and begs for a plausible explanation. The synthesis of *Lhc* mRNA and LHC protein at a precise time window under the control of a circadian clock in contrast to continuous synthesis is one possibility to minimize the synthesis expenses. However, limitation of costs should not result in a loss of flexibility and adaptation of the plants. Since the plants are sessil, they have to cope with the changes that occur in their environment. Changes at dawn are, for example, an increase of illumination and temperature; however, each dawn is specific in respect to light quality, light quantity, rate of temperature alterations, moisture conditions, and so on. These differing, but repetitive, conditions each day ask for a system that maintains flexibility, as well as preparedness, of the plants. Therefore, the synthesis of all individual *Lhc*, mRNAs as well as a certain amount of

each, is necessary every morning. Apparently, it is more advantageous to develop and maintain a regulatory network that includes a circadian clock than to synthesize mRNA and protein continuously. The universal appearance of the circadian clock and the conservation through evolution support this idea.

MOLECULAR COMPONENTS INVOLVED IN CIRCADIAN *Lhc* GENE EXPRESSION

The main interest in elucidating circadian *Lhc* gene expression focuses on the regulatory mechanisms involved in gene transcription. Two major components need to be identified: (1) DNA sequences (motifs, boxes, *cis* elements) and (2) DNA binding proteins (*trans* elements, e.g., transcription factors). DNA binding proteins can be identified by analysis of mutants or by gel shift experiments. The latter procedure was performed by Carre and Kay (1995), who investigated the -142 to -38 5′ upstream sequence of the *Lhc*1*1 gene of *Arabidopsis*. At least six proteins/protein complexes were shown to bind to this part of the *Lhc* promoter. Three of these proteins, CUF-2, CUF-3, and TAC, bind under these in vitro conditions to the promoter region sufficient to mediate circadian expression (-111 and -74). Coincidentally, this promoter region contains the novel 10-nucleotide motif identified by Piechulla et al. (1998) (described below), indicating that this motif, as well as the three proteins, may play a role in the circadian control of *Lhc* gene expression.

The alternative way to identify components of the circadian clock machinery is a screening of mutants. Kay and coworkers obtained *Arabidopsis* mutants with aberrant cycling patterns. Some of the mutants (toc = timing of *cab*) were found to be short-period mutants. The toc-1 mutation maps to chromosome 5 (Millar et al. 1995), and Somers et al. (1998) concluded that TOC1 is not a part of the input pathway, but is central to the proper function of the oscillator since a variety of clock-controlled processes are altered by the mutation (stomatal conductance, flowering time, temperature entrainment). However, Somers et al. (1998) admit that full confirmation of their conclusion awaits further experimental evidence. Cloning of the *toc* gene and its functional analysis, as well as the analysis of other mutants, may contribute to the understanding of the complete circadian clock circuit.

The main progress was reached by analyzing the promoters of *Lhc* genes of different plant species. Since all members of the *Lhc* family display circadian mRNA accumulation patterns, it was hypothesized that common *cis* or *trans* elements should be involved in the regulation of each individual gene. The I-box (GATA motif) was considered as a possible candidate for a sequence motif: The respective binding protein (IBF-2a) binds during the light phase, but not during darkness, and it was suggested that it may be involved in the regulation of the genes by the circadian clock (Borello et al. 1993). However, detailed investigations of the homologue from *Arabidopsis*, the CGF-1 factor, did not support this notion (Anderson and Kay 1995; Teakle and Kay 1995; Anderson et al. 1997). Furthermore, a general key function in mediating circadian rhythmicity cannot be attributed to the I-box since several tomato *Lhc* gene promoters do not contain this motif (Piechulla et al. 1991).

With the help of promoter reporter gene constructs and the method of plant transformation, it was possible to identify short 5′ upstream sequences of several *Lhc* genes (Table 2) that are capable of inducing mRNA oscillations in transgenic plants. Promoter

Table 2. Analysis of 5′ Upstream Regions of *Lhc* Genes

	Construct	Rhythmic expression	Reference
A. thaliana			
–734/+462	*Lhc*b1*1::cat	+	Millar and Kay 1991
–249/+6	*Lhc*b1*3::5′UT(wheat)::cat	+	Millar and Kay 1991
–319/+3	*Lhc*b1*1::5′UT(wheat)::cat	+	Millar and Kay 1991
–319/+3	*Lhc*b1*1::5′UT(wheat)::luc::E9	+	Millar et al. 1992
–111/–33	*Lhc*b1*1::TMVomega::luc::E9	+	Anderson et al. 1994
–111/–74	*Lhc*b1*1::TMVomega::luc::E9	+	Carre and Kay 1995
T. aestivum			
–244/+1110	*Lhc*b1	+	Fejes et al. 1990
–357/+31	*Lhc*b1::gus::nos	+	
–357/–90	*Lhc*b1::–90/+8 35S::gus::nos	+	
S. tuberosum			
–920	*Lhc*b::cat::terminator	+	Im et al. 1994
L. esculentum			
–701	*Lhc*b1*1	+	Piechulla et al. 1998
–159	*Lhc*b1*1	–	Piechulla et al. 1998
–152	*Lhc*b1*2	+	Piechulla et al. 1998
–278	*Lhc*a3	+	Piechulla et al. 1998
–231	*Lhc*a3	–	Piechulla et al. 1998
–119/+4	*Lhc*a4::gus::nos	+	Piechulla et al. 1998

DNA fragments of 268 bp of the wheat *Lhc*b1 gene (Fejes et al. 1990), 36 bp of the *Arabidopsis Lhc*b1*1 (Carre and Kay 1995), 152 bp of the tomato *Lhc*b 1*2, 119 bp of the tomato *Lhc*a4 gene, and 47 bp of the tomato *Lhc*a3 gene (Piechulla et al. 1998) are sufficient for mediating transcript oscillations. The extensive investigation of the 5′ upstream regions of tomato *Lhc* genes included genes that encode different LHC protein types, as well as proteins affiliated with both photosystems, and resulted in the identification of a 10-nucleotide sequence CAANNNNATC (Piechulla et al. 1998). This motif is present in 81% of all *Lhc* promoters collected in the database. Incidentally, Wang et al. (1997) published a myb-like transcription factor (CCA1) involved in phytochrome regulation of the *Arabidopsis Lhc*b1*3 gene that binds to a DNA region that includes the above-mentioned motif. It turned out that the abundance of this transcription factor oscillates during 24h, which may indicate that it underlies the control of the circadian clock as well (Wang and Tobin 1998). Such a rhythmic change in quantity was not observed in the case of TAC, CUF-2, and CUF-3 proteins/protein complexes identified by Carre and Kay (1995). Therefore, it is possible that the *Arabidopsis* factor CCA1 is a more likely candidate for a role in mediating the circadian control of *Lhc* gene transcription. Investigations of other factors isolated from other plant species (e.g., tomato) are under way and may confirm and extend this hypothesis.

The identification of a DNA sequence motif by means of independently designed and performed experiments for (1) light induction and (2) circadian regulation, for instance, strongly indicates that phototransduction, as well as circadian clock control, of *Lhc* genes is transmitted to the same promoter position. The notion that both processes are inextricably tied together is supported by altered damping of the amplitude and period

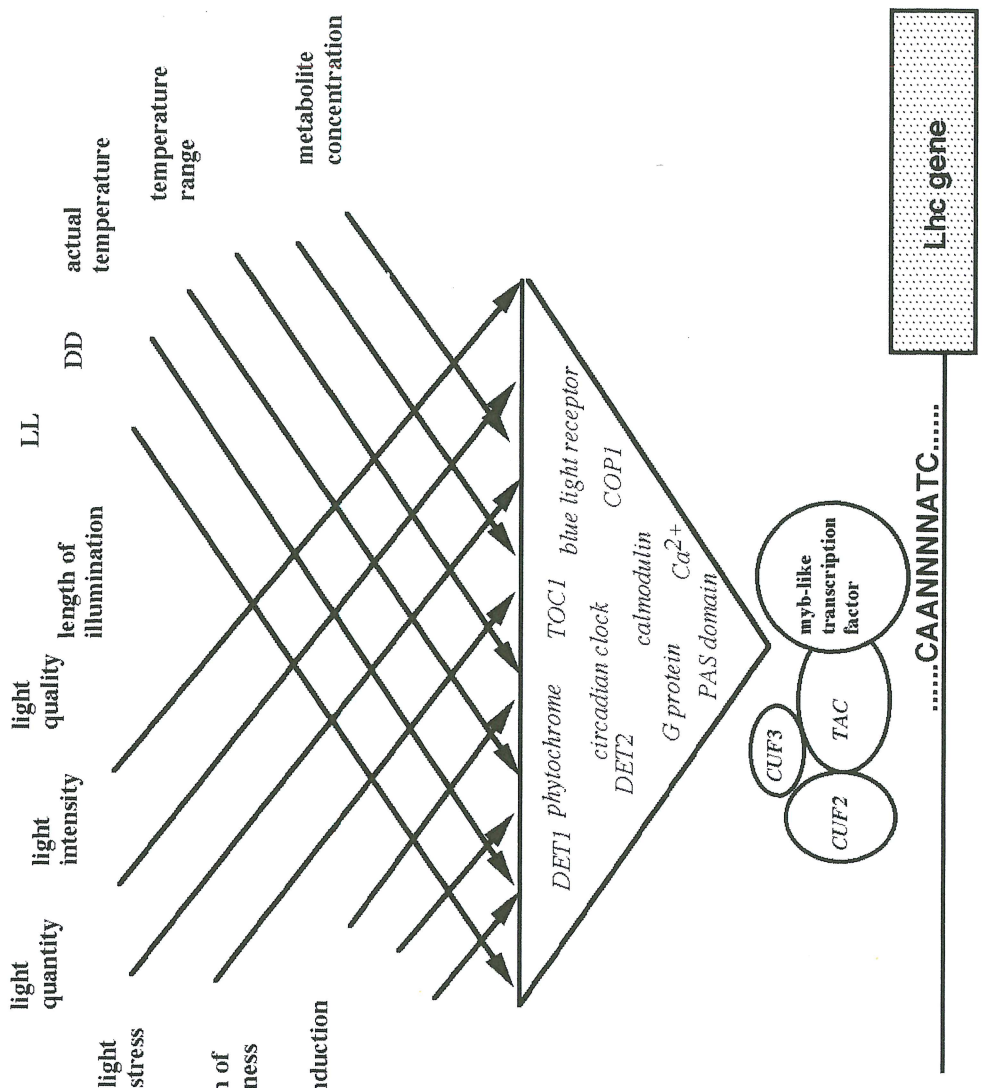

FIGURE 1. Network of parameters and components involved in the regulation of *Lhc* genes.

length in photomorphogentic mutants such as *det*1, *det*2, and *cop*1 (Anderson and Kay 1996) and by involvement of proteins in circadian control (e.g., PER, WC1, and WC2) and light perception (e.g., phytochrome, photoactive yellow protein [PYP]), which share a common motif (PAS domain). This domain is apparently responsible for protein-protein interactions (e.g., homo- and heterodimer formation) (Kay 1997). The question whether the presence of the PAS domain is really an evolutionary link between photosensory processes and circadian clocks can only be answered by continued identification and sequencing of more clock genes.

Apart from this protein domain and the 10-nucleotide DNA motif, other parts of the regulatory mechanisms may be shared by these two processes (e.g., DNA binding proteins, transcription factors, cellular molecules). Therefore, it seems likely that instead of individual linear signal transduction chains, rather a network is the fundamental structure combining regulatory circuits. This idea, schematically presented in Fig. 1, has the consequence that the input pathway of the basic clock model (input, central oscillator, and output) is not a linear, straightforward, one-way structure, but includes several cross points and most likely also several feedback loops. The principal possibility of feedback loops within the input pathway, central oscillator, or output pathway was recently put forward by Roenneberg et al. (1998). In the case of the *Lhc* gene expression, published results show the involvement of many components (such as phytochrome, G protein, calmodulin, etc.) (Fig. 1) responsible for supporting the transcription. While feedback loops have not yet been demonstrated, investigations exist that clearly demonstrate cross-talk between signal transduction chains (Bowler et al. 1994). The exact interplay of all these components and signal transduction pathways within this network remains to be elucidated in the future.

ACKNOWLEDGMENT

Work from this laboratory has been supported by the German Research Foundation (DFG) and the Fonds of the Chemical Industry to B. P. The data by Jäschke et al. were summarized by permission prior to publication.

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