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Diurnal Lhc gene expression is present in many but not all species of the plant kingdom •

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Abstract

The diurnal and circadian expression of light-harvesting genes (Lhc) is well documented for many plant species of the 'Angiospermae' division. Here we present the diurnal mRNA levels of species of the Gymnospermae, Pteridophyta, Bryophyta and Phycophyta divisions. Except for four Coniferophytina species, diurnal Lhc mRNA accumulation is detected in fern, moss and algae, supporting the idea that the concept of 'circadian clock'-controlled gene expression is an ancient process. Possible reasons why plants need the 'circadian clock' control mechanism are discussed.

Introduction

In the past many examples of diurnal and/or circadian expression of plant genes have been published (summarized in [15]). Transcripts of genes encoding different classes and types of proteins (photosynthesis-related proteins, proteins involved in N or C-metabolism, etc.) accumulate with characteristic diurnal or circadian patterns. The majority of investigations deal with the light-harvesting complex proteins (LHC) present in the thylakoid membranes of chloroplasts of higher plants. These proteins accomplish the first step in photosynthesis, which is the capturing and conversion of light energy into chemical energy. The typical accumulation pattern of the Lhc mRNAs starts with increasing levels slightly before or after sunrise, reaching a maximum around noon, and decreasing mRNA levels in the afternoon and night. Similarly, the synthesis of LHC proteins is under the control of a 'circadian clock' [17].

Many investigations demonstrating the circadian expression pattern of Lhc genes were performed with plant species systematically organized in the Angiospermae division. Monocotyledonous and dicotyledonous species [10, 11, 15] exhibit basically the same characteristic transcript oscillations. Only a few species of different divisions of the plant kingdom have so far been investigated, such as *Pseudotsuga menziesii* (Gymnospermae [1]), *Ceratopteris richardii* (Pteridophyta [12]) and *Chlamydomonas eugametos* and *Dunaliella tertiolecta* (Phycophyta [4, 9]). Unexpectedly, the diurnal or circadian mRNA oscilla-

• Dedicated to Dr H. W. Heldt on the occasion of his 60th birthday.

tion was not present in the gymnosperms, while it was present in lower plants such as fern and algae. Since the current knowledge is very limited and does no legitimize a conclusion, we started a survey to investigate the Lhc expression in several species of different divisions and classes of the plant kingdom. In this paper we present and discuss the diurnal Lhc mRNA accumulations of *Picea abies, Pinus sylvestris, Abies alba, Larix decidua, Encephalartos altensteinii* (Gymnospermae), *Ceratopteris richardii* and *Equisetum myriochaetum* (Pteridophyta), *Conocephalum conicum* and *Physcomitrella patens* (Bryophyta) and *Dunaliella bioculata* (Phycophyta).

Material and methods

Plant material

Seeds of *P. sylvestris, P. abies, A. alba* and *L. decidua* were obtained from the Staatliches Forstamt (Munster-Oerrel, Germany), germinated and grown in the greenhouse (University of Göttingen, *P. sylvestris, P. alba*: 6 weeks old, April 1991, illumination between 06:00 and 20:00; *A. alba*: 9 weeks old, June 1993, sunrise 05:07, sunset 21:36, *L. decidua*: 5 weeks old, May 1993, sunrise 05:37, sunset 21:00). When the plants had their first whirl of cotelydons, leaves were harvested at indicated time points during the normal day (LD). *E. altensteinii, E. myriochaetum* (May 1993, sunrise 05:37, sunset 21:00) and *C. conicum* (February 1991, sunrise 07:25, sunset 17:47; July 1991, sunrise 04:10, sunset 20:41) grew in the greenhouses of the botanical garden of the University of Göttingen. Young leaves or parts of young leaves were harvested at different time points during the regular day. Spores of *C. richardii* were obtained from Dr E. Pichersky (Ann Arbor, MI), kept for a few days in sterile water and then plated [6] until sporophytes were visible. The sporophytes were transferred to soil and grow in the greenhouse. Young broad leaves were harvested at different time points during LD, DD and LL (see details in [12]). *P. patens* was grown in liquid medium, in growth chambers under a 16 h light/8 h dark regime [16]. Aliquots containing 6–10 g of cells (fresh weight) were withdrawn from a 5 l culture at different time points during the day, immediately filtered, rinsed with water and stored frozen at −20 °C. *Dunaliella bioculata* 39-1 (Algensammlung, University of Göttingen) was grown in 300 ml liquid medium (5 mM Na_2HPO_4/NaH_2PO_4 pH 7, 05 M NaCl, 10 mM KNO_3, 1 mM $MgSO_4$, 11 μM $CaCl_2$, 1 μM H_3BO_4, $MnSO_4$, $ZnSO_4$, 10 nM $CuSO_4$, $(NH_4)_6Mo_7O_{24}$, 2.5 nM $FeSO_4$/EDTA complex, 2% CO_2, 25 °C, 14 h light from 10:00 to 24:00, 10 h darkness, 160 μmol m^{-2} s^{-1} for three weeks to synchronize the culture. Plant material was harvested at indicated time points, 150 ml culture was filtered, washed with 1 M NaCl and frozen in liquid nitrogen and stored at −50 °C.

RNA extractions

For RNA extractions all plant tissues were ground in liquid nitrogen until a fine powder was obtained. The RNA of *P. sylvestris, P. abies, C. conicum, D. bioculata* was extracted according to the method described in Piechulla *et al.* [14], and for RNA isolation from *E. myriochaetum* the RP-buffer was 5 times concentrated, RNA of *C. richardii* was extracted with GTC buffer and centrifuged through a CsCl gradient as described by Sambrook *et al.* [18], and RNA from *L. decidua, A. alba* and *E. altensteinii* was extracted according to the method described by Chang *et al.* [3]. The RNA concentration was spectrophotometrically determined at 260 nm and the quality of the RNA was inspected on a denaturing formaldehyde gel. Only RNA that revealed distinct rRNA banding was used to determine Lhc mRNA levels.

Hybridizations

Formaldehyde gels (northern blots) or dot blots were loaded with equal amounts of total RNA (nylon membranes from Amersham Buchler, Braunschweig, Germany) as described by Sambrook *et al.* [18]. Lhcb1 genes from *L. esculentum*

[13], *P. patens* [16] or *D. salina* [8] were random-primed [18] and used as probes. Hybridization conditions were: 5 × or 6 × SSC, 55 °C or 60 °C; for *D. bioculata*: 2 × SSC, 65 °C. Nylon filters were exposed to X-ray films. RNA was quantitated by cutting the respective band or dot from

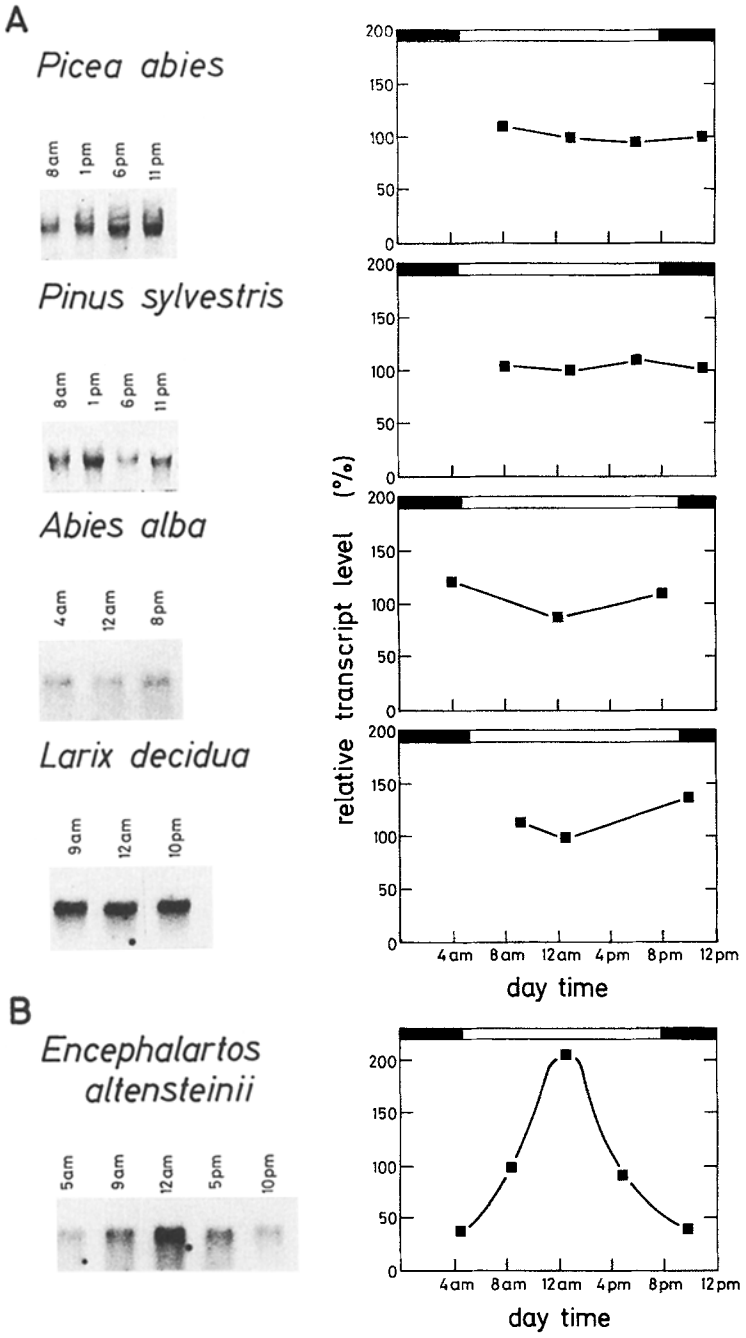

Fig. 1. Diurnal Lhc mRNA accumulation in Gymnospermae. Leaves of four Coniferophytina (A) and parts of leaves of a Cycadophytina (B) were harvested at different diurnal time points. RNA was extracted and hybridized with a Lhcb gene probe (northern blots, left panel). Quantitative determination of Lhc mRNA levels are presented in the right panel. Dark and light regime is presented by filled and open bars respectively.

the filter or autoradiograms were scanned (Desaga, Quick Scan densitometer, Heidelberg, Germany). Relative mRNA levels are based on at least three hybridizations.

Results and discussion

Diurnal Lhc mRNA fluctuations are present in *E. altensteinii* (Cycadophytina, Fig. 1B), *E. myriochaetum* (Articulatae, Fig. 2A), *C. richardii* (Leptosporangiatae, Fig. 2A), *P. patens* (Musci, Fig. 2B) and *D. bioculata* (algae, Fig. 2C). The Lhc transcripts accumulate significantly after sunrise, reach a maximum around noon and decline thereafter. A basal level is present in all species throughout the night. No rhythmic mRNA accumulation was detected in the four Coniferophytina species *Picea, Pinus, Abies* and *Larix* (Fig. 1A) and in the Hepaticae *C. conicum*, Fig. 2B).

The results of this survey and the previously published data are combined in Table 1. It can be demonstrated that the phenomenon of rhythmic Lhc mRNA accumulation is present in the different divisions of the plant kingdom. We believe that the control mechanism 'circadian clock' is an ancient process. However, it is striking that the species of the Gymnospermae subdivision Coniferophytina form the exception to the rule, apparently the machinery for the circadian expression was lost or eliminated during evolution. It is clear that the loss of this regulatory mechanism must have occurred after the pro-Gymnospermae divided into the different subdivisions Pinopsida, Gingkoopsida, Lyginopteridopsida (including Cycadopsida) [19], since the diurnal Lhc mRNA accumulation is present in Cycadopsida and not in Pinopsida. Unfortunately, this aspect has yet not been investigated in *Gingko biloba*. Why this sophisticated mechanism 'circadian clock' is needed or not is presently unclear and at this point of knowledge one can only speculate about the reasons.

It is possible that certain plant species live in ecological niches were a time-dependent synthesis of Lhc mRNA or protein is not useful. A possible scenario for the Coniferophytina may be based on their morphological/anatomical speciality to keep the leaves for many years, and not only for several months as in the case of many Angiospermae. A regulatory mechanism which

Table 1. Diurnal/circadian expression of Lhc genes.

Division	Plant species	LD	DD	LL	Reference
Angiospermae	monocots and dicots	+	+	+	15
Gymnospermae	*Picea abies*	−			
	Pinus sylvestris	−			
	Pseudostuga menziesii	−/+	−	−	1
	Abies alba	−			
	Larix decidua	−			
	Encephalartos altensteinii	+			
Pteridophyta	*Equisetum myriochaetum*	+			
	Ceratopteris richardii	+	+	+	12
Bryophyta	*Conocephalum conicum*	−			
	Physcomitrella patens	+			
Phycophyta	*Dunaliella bioculata*	+			
	D. tertiolecta	+	+		9
	Chlamydomonas eugametos LI818	+	+		4
	C. eugametos pMH32	+	−		

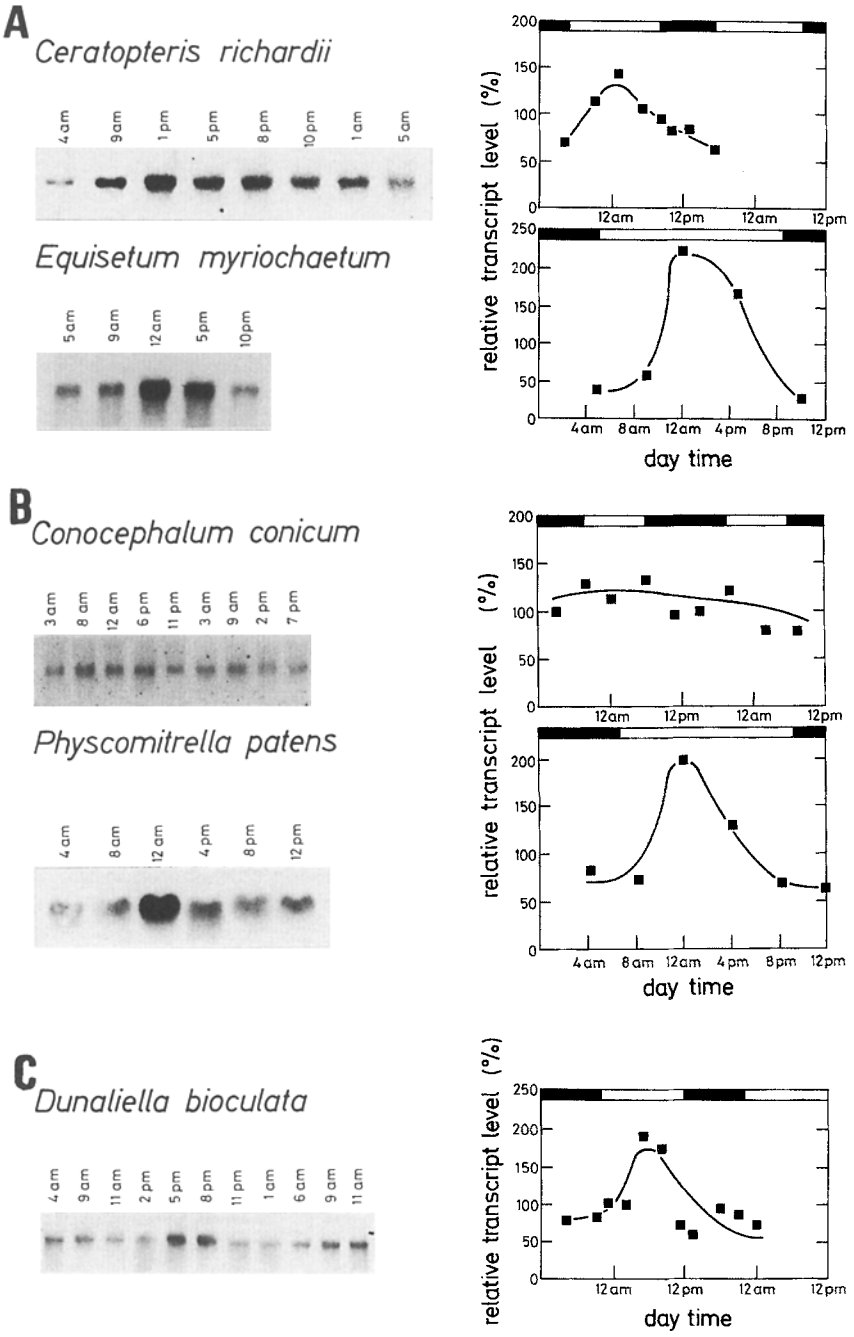

Fig. 2. Diurnal Lhc mRNA accumulation in Pteridophyta, Bryophyta and Phycophyta. Leaves of *C. richardii* (A), *E. myriochaetum* (A) and *C. conicum* (B) and cells of *P. patens* (B) and *D. bioculata* (C) were harvested at different diurnal time points. RNA was extracted and hybridized with a Lhcb gene probe (northern blots, left panel). Quantitative determination of Lhc mRNA levels are presented in the right panel. Dark and light regime is presented by filled and open bars respectively.

controls the expression on the *daily* basis may not be necessary for the 'long-term' development of the leaves of respective plants. In this context another aspect may also be important. In contrast to Angiospermae, Coniferophytina are able to synthesize chlorophyll independent of light [2, 7,

21]. In darkness chlorophylls accumulate to 20–50% of the levels reached in light-grown plants. Since LHC proteins bind chlorophyll *a* and *b* molecules in a particular stoichiometric relationship a coordinated expression of both components which construct the light-harvesting complexes in the thylakoid membrane is needed [5]. In Angiospermae, the chlorophyll and LHC protein synthesis is light-dependent. In addition, Lhc expression is controlled by a 'circadian clock' which directs and optimizes the majority of LHC protein synthesis to the time after sunrise (which correlates with the time point to synthesize chlorophyll after the dark/light transition). Since Coniferophytina are able to synthesize chlorophyll in the dark, respectively during the night, a circadian-controlled Lhc gene expression would be abundant. The same argument may also apply for some Bryophyta species, since Stahl [20] described species that are also able to synthesize chlorophyll in darkness and we demonstrated in this survey that one moss species, C. conicum, did not exhibit diurnal oscillations of Lhc mRNA levels. From this possible scenario we hypothesize that in plant species which have a light-dependent chlorophyll biosynthetic pathway need to control their Lhc expression by a 'circadian clock' to optimize synthesis while plant species that are able to synthesize chlorophyll in darkness do not need 'circadian clock'-mediated Lhc gene expression. Further experiments with different members of the plant kingdom and the correlation with the ability to synthesize chlorophyll in darkness or during the light period may indicate reasons why plant species need or need not the Lhc gene expression controlled by a 'circadian clock'.

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