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Diurnal rhythms of the chlorophyll a/b binding protein mRNAs in wild emmer wheat and wild barley (*Poaceae*) in the Fertile Crescent

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Key words: *Poaceae, Triticum dicoccoides, Hordeum spontaneum.* – Cab (Chlorophyll a/b binding proteins), circadian rhythms.

Abstract: The mRNAs encoding the chlorophyll a/b binding (*cab*) proteins of the light harvesting system were monitored in the wild cereals, wild emmer wheat, *Triticum dicoccoides*, and wild barley, *Hordeum spontaneum*, the progenitors of all cultivated wheats and barley, respectively. Significantly different mRNA levels were detected at different time points during the day, with generally low levels around sunrise, sunset and midnight, and maximum levels around noon. These results indicate that a diurnal control of the *cab* gene expression is present in these ancient species.

Biological rhythms in nature are entrained to a 24 hour period (circadian) by environmental cues designated "Zeitgeber". Circadian clocks are abundant in both plants and animals. In plants morphological (BUNNING 1956, SATTER & GALSTON 1981), cellular (JEREBZOFF 1986), biochemical, physiological (VINCE-PRUE 1983), and photosynthetic (BRITZ & al. 1987) oscillations have been observed. However, the molecular basis of these rhythms is still largely unknown (JEREBZOFF 1986).

Recently, molecular studies revealed that the steady-state transcript levels of the chlorophyll a/b binding proteins oscillate (PIECHULLA & GRUISSEM 1987, PIECHULLA 1989, reviewed in KAY & MILLAR 1992). The chlorophyll a/b binding proteins play a central role in photosynthesis, since chlorophyll molecule, associated with the *cab* protein, are responsible for capturing the light energy, converting and transferring the energy to the respective reaction centers of photosystem I and II. The molecular organization of these proteins within the thylakoid membranes of the chloroplast has been the interest of several investigations in the past (reviewed in GREEN 1988, HOYER-HANSEN & al. 1988, GREEN & al. 1991). In higher plants the appearance of several structurally and functionally related *cab* proteins is genetically determined, e.g., in *Lycopersicon esculentum* up to now 19 *cab* genes have been characterized. In addition, investigations were focused on the regulation of gene expression. Several exogenous (light, temperature; KUHLEMEIER & al. 1987,

RIESSELMANN & PIECHULLA 1990) and endogenous (developmental program, tissue-specificity, circadian rhythm; MEYER & al. 1989, reviewed in KAY & MILLAR 1992) stimuli are known that influence the expression of these genes. Particular interest was focused on the accumulation of *cab* transcripts due to the control by an endogenous oscillator (biological clock). In several higher plants, monocots as well as dicots, the typical oscillation pattern of *cab* mRNA transcripts was detected (MEYER & al. 1989, reviewed in KAY & MILLAR 1992).

In the present study we wanted to assess additional information to the *cab* mRNA oscillations present in monocots. Therefore, wild cereals, wild emmer wheat, *Triticum dicoccoides,* and wild barley, *Hordeum spontaneum*, both from the Near East Fertile Crescent, were investigated. Diurnal *cab* mRNA fluctuations similar in pattern to that of the tomato are demonstrated.

Material and methods

Sixteen genotypes, seven of *Triticum dicoccoides*, and eight of *Hordeum spontaneum*, were tested for the potential rhythmicity of *cab* mRNA accumulation. The wild emmer genotypes represented seven populations, six across the range of the species in Israel (NEVO & BEILES 1989), and one Turkish population from Siverek (NEVO & al. 1988), as well as a cultivar control (*T. durum* SPERBER) (Table 1). The wild barley genotypes represented eight populations, five from Israel (NEVO & al. 1979), two from Turkey (50 km west of Gaziantep and 60 km south of Bitlis) (NEVO & al. 1986 a), and one from Iran (5 km west of Kubuasht) (NEVO & al. 1986 b) (Table 1). The ecogeographical background of all eight genotypes are given in NEVO & al. 1986 c.

The plants were grown in a greenhouse at the University of Göttingen. The seeds used in the first phase of the experiment were embedded on 17-07-89 and harvested on 1-08-90 (sunrise 4:46 am, sunset 8:10 pm), the seeds used in the second phase of the experiment were embedded on 15-05-90 and harvested on 30-05-90 (sunrise 4:32 am, sunset 8:56 pm). The plants were exposed to the natural day/night regime, with temperatures of 24 °C during the day and 19 °C during the night. Leaves of approximately two-week old plants were harvested at indicated time points during the day, and the total RNA was extracted as described in PIECHULLA & al. (1986). RNA from different preparations was standardized and analyzed by spectrophotometric quantitation and ethidium bromide fluorescence of cytoplasmic rRNA. RNA was separated by formaldehyde gel electrophoresis and its high quality is apparent from the distinct ribosomal RNA bands observed after electrophoresis (Figs. 1 and 2). RNA was either transferred to a nylon filter (Northern blot) (Amersham Buchler, Hybond N) and hybridized or directly spotted onto a filter, using a dot blot apparatus. The hybridization probe (1.5 kb Bgl I-Hind III fragment) was isolated from the plasmid (pHvLF 2) containing a *cab* gene from *Hordeum vulgare*. One major band of expected mRNA length was obtained after exposing filters to x-ray films.

The autoradiograms were scanned with a densitometer (Desaga "Quick Scan" Heidelberg, Federal Republic of Germany). Relative amounts of mRNAs were determined by peak-area measurements of autoradiograms of different exposure times.

Results

The *cab* mRNAs of all genotypes from different sites in Israel and Turkey of both wild cereals clearly accumulate to different levels at different time points during the day. Representative comparisons between the transcript levels are presented in Figs. 1 and 2. Generally, the highest levels of the Israeli and Turkish genotypes of both cereals are detected around noon, while in the morning, afternoon and night

Table 1. The tested genotypes of wild emmer wheat, *Triticum dicoccoides, T. durum* cv. 'Sperber', and wild barley, *Hordeum spontaneum* from the Near East Fertile Crescent, with their country and population origin, identification number and testing years

	Species	Country	Population*		Genotype	Year of Testing
			No.	Name		
1.	*Triticum dicoccoides*	Israel	11	Tabigha	H-5	1989
2.	*Triticum dicoccoides*	Israel	18	Gitit	18–46	1990
3.	*Triticum dicoccoides*	Israel	1	Mt Hermon	G-13	1989
4.	*Triticum dicoccoides*	Israel	7	Yehudiyya	B-13	1990
5.	*Triticum dicoccoides*	Israel	30	Bat Shelomo	I-4	1990
6.	*Triticum dicoccoides*	Israel	24	Amirim	14–34	1990
7.	*Triticum dicoccoides*	Turkey	7	Siverik	T-7-7	1989
8.	*Triticum durum* cv. 'Sperber' (control)					
9.	*Hordeum spontaneum*	Israel	7	Tabigha	P-46	1989
10.	*Hordeum spontaneum*	Israel	1	Mt Hermon	A-24	1989
11.	*Hordeum spontaneum*	Israel	23	Wadi Qilt	23–41	1989
12.	*Hordeum spontaneum*	Israel	9	Mt Meron	9–53	1990
13.	*Hordeum spontaneum*	Israel	20	Sede Boqer	L-12	1990
14.	*Hordeum spontaneum*	Turkey	20	Bitlis	T-20-16	1989
15.	*Hordeum spontaneum*	Turkey	1	Gaziantep	T-1-47	1990
16.	*Hordeum spontaneum*	Iran	11	Kubuasht	I-11-8	1989

* Population numbers of *Triticum dicoccoides* are from NEVO & BEILES (1989). Population numbers of *Hordeum spontaneum* from Israel are from NEVO & al. (1979); Turkey from NEVO & al. (1986a); Iran from NEVO & al. (1986b).

cab mRNAs accumulate to lower levels. In contrast, the Iranian *H. spontaneum* genotype peaked at 5:00 pm.

The differences of the *cab* mRNA between two time points of a day are in most cases more than two-fold, and rise up to five-fold. Due to the limited number of samples during the day, it is uncertain that the maximum and minimum time points were used for this comparison. Therefore, the true extremes of steady-state mRNA levels may be even more distant. In addition, one must consider the fact that the 6:00 am sample point was already 1.5 hours after sunrise, and therefore, already accumulated mRNAs were monitored.

Discussion

The most obvious result of this study is that a diurnal expression of the chlorophyll a/b binding proteins, with a maximum level around noon, was displayed in most wild emmer wheat and wild barley genotypes. Only the Iranian genotype peaked in the late afternoon. This result may indicate genetic polymorphism, although further verification will be necessary for this general statement. Additionally, differences in the height of the amplitudes were monitored in the different genotypes, indicating that each genotype may individually express a variable control system for *cab* mRNA accumulation. However, before speculating about the significance of differences in amplitudes as well as of the shapes of the oscillation patterns, it

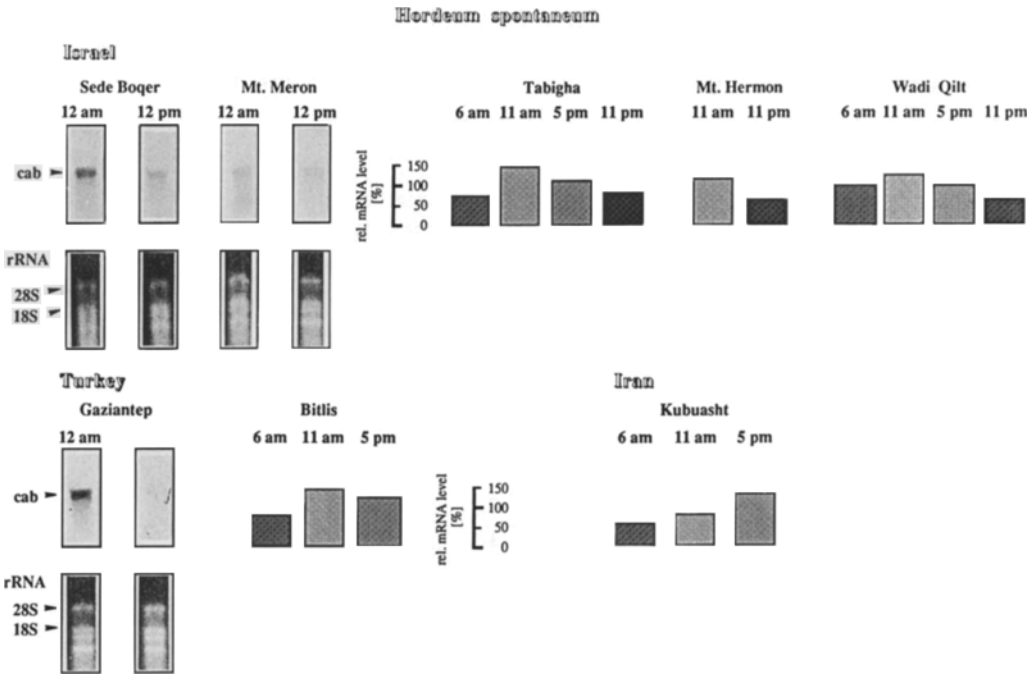

Fig. 1. Diurnal fluctuations of chlorophyll a/b binding protein steady-state mRNA levels in wild barley genotypes. Total RNA extracts from different time points during the day were analyzed on formaldehyde agarose gels and cytoplasmic rRNAs (18S and 28S) were stained with ethidium bromide; examples are given from Sede Boqer, Mt Meron (Israel), and Gaziantep (Turkey) genotypes. The autoradiogram of respective Northern blots hybridized with a specific *cab* probe from *Hordeum vulgare* are presented, the respective bands representing the *cab* transcripts are indicated. The length of *cab* transcripts is approximately 1 kb. Quantitative analysis of *cab* probe hybridization is presented for Israel (Tabigha, Mt Hermon, Wadi Qilt), Turkey (Bitlis), and Iran (Kubuasht) genotypes. Scanning of the autoradiogram and peak area determination, as well as scintillation counting of dot blots were used for the determination of relative mRNA levels

is necessary to determine the steady-state mRNA levels at more time points during the day, e.g., at intervals of two to three hours. In addition, a comparison of the mRNA levels between the different genotypes is critical, since the amount of mismatches between the hybridization of the probe-*cab* gene of *H. vulgare* and the *cab* genes of various genotypes may be different, and therefore, distinct hybridization signals are expected in each case. Because of these precautions, we did not extensively discuss the issue of quantitative comparison, and potential genetic polymorphism of *cab* circadian rhythmicity in both wild cereals. Despite these difficulties the diurnal fluctuations of *cab* transcript accumulation that were found in the progenitors of wheat and barley confirm the antiquity of the circadian pattern.

In a series of multidisciplinary studies we have shown multiple genetic polymorphisms in wild emmer wheat (Nevo & Beiles 1989, Nevo & al. 1988) and in wild barley (Nevo & al. 1979, 1986 a, b, c) in the Near East Fertile Crescent. These genetic polymorphisms include morphological, biochemical, physiological, immunological, and agronomic traits. The results presented in this paper show the

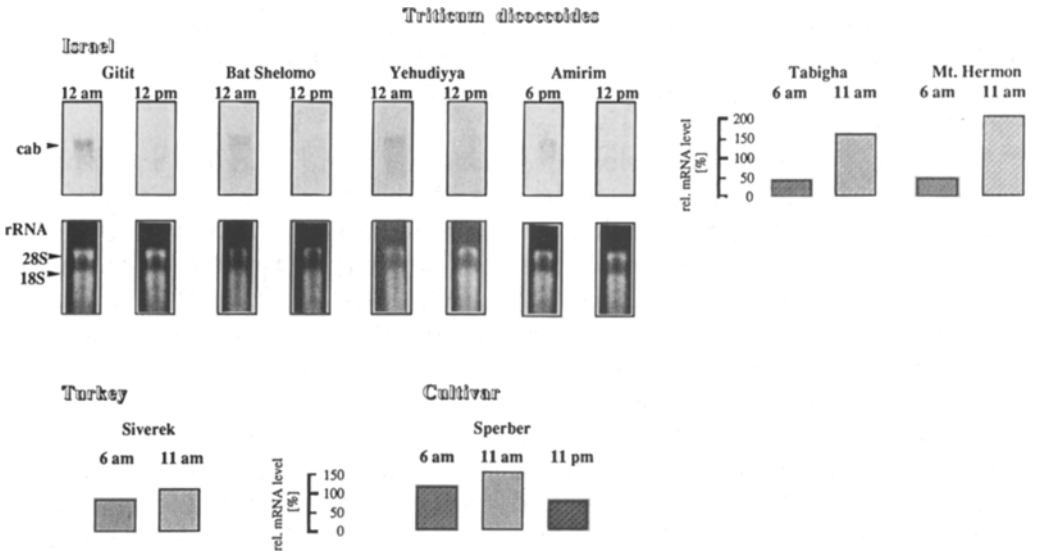

Fig. 2. Diurnal fluctuations of chlorophyll a/b binding protein steady-state mRNA levels in wild wheat genotypes. Total RNA extracts from Gitit, Bat Shelomo, Yehudiyya, and Amirim (Israel) genotypes were analyzed on formaldehyde agarose gels. The autoradiogram of respective Northern blots are presented and the respective bands representing the *cab* transcripts are indicated. Quantitative analysis of *cab* probe hybridization is presented for the Tabigha, Mt Hermon (Israel), and Siverek (Turkey) genotypes. The cultivar *T. durum* cv. "Sperber" was used as a control. Further details see Fig. 1

presence of the phenomenon of diurnal, and most likely also circadian, *cab* mRNA rhythmicity in all tested genotypes. A variation of the *cab* mRNA accumulation pattern of the Iranian *H. spontaneum* pop. 11 (Kubuasht) may indicate a possible genetic polymorphism. To verify this result further studies on the genotypes of this locality are necessary. It should be added that a variation of the *cab* mRNA accumulation patterns in different plant species has been noticed (reviewed in PIECHULLA 1988). In pea and maize seedlings the *cab* mRNAs are present at elevated levels two hours prior to illumination, while in tomato leaves and fruits increasing mRNA levels are measured after the transition from darkness to light. The significance of this discrepancy is not yet understood, but it may indicate genetic polymorphism between species in the otherwise very similar mechanisms involved in the *cab* gene expression of different plant species. Our results, if substantiated, may suggest genetic polymorphism in *cab* circadian patterns also within and between populations of the same species.

The history of the study of circadian clocks in plants was reviewed by FELDMAN (1982). The first demonstration of diurnal and circadian rhythms at the level of transcription of the chlorophyll a/b binding protein genes was exhibited in the pea (KLOPPSTECH 1985). Later on, circadian mRNA accumulation patterns were also shown in other plant species, e.g., tomato (PIECHULLA & GRUISSEM 1987), wheat (NAGY & al. 1988), petunia (STAYTON & al. 1989), maize (TAYLOR 1989), soybean, mustard, and bean (MEYER & al. 1989). The mechnism of *cab* rhythmicity seems to be highly conserved in higher plants (MEYER & al. 1989) and genetically determined (BUNNING 1935, 1936; RIESSELMANN & PIECHULLA 1990). NAGY & al. (1988)

have suggested that phytochrome is involved in the signal transduction chain between light and the measurable steady-state mRNA levels. *Cab* gene expression appears adaptable to variations in the light regime and appears to be under the control of diversifying natural selection.

The diurnal/circadian expression of *cab* genes in tomato and other higher plants is characteristic for this multigene family. This is highlighted by the fact that mRNAs of other genes encoding proteins involved in photosynthetic reactions such as the small subunit of ribulose biophosphate carboxylase/oxygenase do not accumulate rhythmically with a constant period (MEYER & al. 1989). At present the significance of the control of gene expression by an endogenous rhythm can only be speculated. It is possible that the expression of *cab* genes is very closely correlated to the light dependent chlorophyll synthesis.

Our present study suggests that a circadian clock is involved in *cab* gene expression in wild cereals, indicating that this regulatory mechanism represents an ancient process. Genetic polymorphism in diurnal *cab* gene expression is suggestive though not yet conclusive. Remarkably however, such polymorphisms in photosynthetic performance were recently found both within and between populations of wild emmer wheat in Israel (CARVER & NEVO 1990). These genetic polymorphisms are predictable by ecology and isozyme markers (NEVO & al. 1991), indicating adaptive photosynthetic patterns generated by natural selection.

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