Circadian Synthesis of Light-Harvesting-Chlorophyll-Proteins in *Euglena gracilis* Is under Translational Control

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Z. Naturforsch. **53c,** 1017–1026 (1998); received July 7/August 3, 1998

Euglena gracilis, Circadian Rhythm, Translational Control, Protein Synthesis, Light-Harvesting-Chlorophyll-Proteins

Two proteins with apparent molecular masses of 17 and 24 kD that are synthesized in a circadian manner in the phytoflagellate *Euglena gracilis*, were recognized as proteins belonging to the family of light-harvesting-chlorophyll-proteins (LHCPs) of class I (17 kD) and of class II (24 kD). Identification was achieved by N-terminal sequencing of the proteins isolated from two-dimensional polyacrylamide gels and by detection with an anti-LHCP II serum. While it was found that the total amount of LHCPs remains almost constant, when *Euglena* is grown under diurnal conditions (12 h light and 12 h dark), we could show that the amount of newly synthesized 17 and 24 kD proteins varies about 20-fold with a maximum of synthesis in the light phase. In contrast, the analysis of the mRNA levels at different times revealed only minor differences in the stationary concentration of the LHCP specific mRNA, indicating that the control of LHCP synthesis is at the translational level. Principally, the same finding was obtained using inhibitors of transcription. Thus, it is concluded that the expression of LHCPs in *Euglena gracilis* in contrast to that of higher plants is primarily regulated at the translational level.

Introduction

Circadian rhythms have been demonstrated in a wide range of biochemical, physiological and behavioral parameters in different organism ranging from prokaryotes to man (Bünning, 1973; Hastings and Schweiger, 1976; Edmunds, 1988; Huang *et al.*, 1990). The synthesis of distinct proteins has been shown to play an essential role in the observed rhythms, and a number of such proteins and enzymes showing a circadian rhythm in their synthesis has been identified, such as e. g. luciferase and luciferin-binding-protein in *Gonyaulax*, the *per* gene product in *Drosophila* and the *frq* gene product in *Neurospora* (Morse *et al.*, 1990; Hardin *et al.*, 1992; Aronson *et al.*, 1994). Moreover, substantial information exists in the literature of whether the regulation of such rhythmically synthesized proteins occurs at the transcriptional or the translational levels. So far, in most organisms regulation has been shown to occur on transcriptional level (Kloppstech, 1985; Hardin *et al.*, 1992; Aronson *et al.*, 1994; Jacobshagen and Johnson, 1994), while regulation on translational level was less frequent (Hartwig *et al.*, 1986; Morse *et al.*, 1990).

Several proteins which follow a circadian rhythm in their synthesis have been identified in algae and higher plants, such as luciferase and luciferin-binding-protein in *Gonyaulax*, 3-phosphoglycerate kinase in *Chlorella* and LHCP in barley (Walla *et al.*, 1989, 1994; Morse *et al.*, 1990; Beator and Kloppstech, 1993). Among these proteins the LHCPs have been extensively studied. In most plants investigated to date the nuclear genes (*cab* genes) encoding the LHC proteins are transcribed only during the light phase (Kloppstech, 1985; Lam and Chua, 1989; Adamska *et al.*, 1991; Beator and Kloppstech, 1993; Kellman *et al.*, 1993). Only few exceptions have so far been described in the literature where these circadian controlled genes are also transcribed in darkness (Mukai *et al.*, 1992; Yamamoto *et al.*, 1993; Oberschmidt *et al.*, 1995). As stated above, it has been demonstrated for several plants, such as *Chlamydomonas*, wheat,

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0939–5075/98/1100–1017 $ 06.00

barley and tomato, that the genes coding for LHCPs are transcribed under circadian control. This rhythm continues when these organisms are kept in constant light (Kloppstech, 1985; Lam and Chua, 1989; Beator and Kloppstech, 1993; Kellman *et al.*, 1993; Jacobshagen and Johnson, 1994). In the phytoflagellate *Euglena*, synthesis of LHCPs appears to some extent different from that in other plants (Houlné and Schantz, 1993). In all other plant organisms the nuclear encoded LHCPs are synthesized as precursor proteins with a signal sequence for transport into the chloroplast and incorporation into the thylakoid membrane (Smeekens *et al.*, 1990). The signal sequence is cleaved off during transport. In contrast, in *Euglena* LHCPs are synthesized as polyprotein precursors which are translated from one mRNA (Houlné and Schantz, 1988; Muchal and Schwartzbach, 1992). These polyproteins are connected via small peptides of ten amino acids and are transported to the Golgi apparatus prior to chloroplast import and polyprotein processing (Houlné and Schantz, 1993; Sulli and Schwartzbach, 1995). Also the small subunit of the ribulose bisphosphate carboxylase/oxygenase is synthesized as a polyprotein, indicating that in *Euglena* the synthesis of proteins as polyprotein precursors might occur more frequently (Chan *et al.*, 1990). One of the few other examples for synthesis of a protein as a polyprotein precursor is the synthesis of ubiquitin in yeast (Özkaynak *et al.*, 1987).

In a previous paper we reported three protein bands with apparent molecular masses of 17, 24 and 60 kD which were synthesized in *Euglena gracilis* under circadian control. It was also shown that the 17 and 24 kD protein are synthesized on 80S ribosomes (Künne *et al.*, 1997). In the present paper we give evidence that the 17 and 24 kD protein bands represent LHC I and II proteins, and that regulation of the rhythmic synthesis of these proteins occurs at the translational level.

Materials and Methods

Cell culture

Cultures of *Euglena gracilis* no. 1224–5/25 obtained by the Algensammlung Universität Göttingen, Germany were grown as described previously (Künne and de Groot, 1996). The bleached *Euglena gracilis* mutant no. 1224–5/15f of the same supplier was grown under identical conditions, using a Cramer-Myers medium containing glucose (Blum and Wittels, 1967).

Inhibitor treatment of cells and [^{35}S]methionine labelling of proteins

Cells were handled as described previously (Künne and de Groot, 1996). In experiments with inhibitors of transcription (Bovarnick *et al.*, 1969; Falchuk *et al.*, 1976), α-amanitin (final concentration 50 μM) or actinomycin D (final concentration 0.4 mM) were added to subcultures 18 h before the beginning of constant light. Labelling of proteins with [^{35}S]methionine was performed for 2 h at three different time points starting at -6 h (in darkness), 6 h in the light, and 18 h in continuous light.

Cell fractionation

Euglena cells were broken by sonification and were subsequently separated into a soluble and membraneous protein fraction by centrifugation as described previously (Künne *et al.*, 1997). Polyadenylated mRNA was isolated according to published procedures (Pötter and Kloppstech, 1993).

Gel electrophoresis, fluorography and densitometric analysis

One-dimensional (1-D) and two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE) as well as fluorography were performed as described previously (Walla *et al.*, 1989; Künne *et al.*, 1997).

Blotting of proteins

For Western-blotting 10 μg protein of each probe were separated by PAGE and transferred onto nitrocellulose membranes (BA-S83, Schleicher & Schuell, Dassel, Germany) by pressure. The procedure was essentially the same as described by Specht *et al.* (1990). The gels as well as the membranes and filter papers (3 MM, Whatman, Maidstone, U. K.) were equilibrated in transfer buffer (10.0 mM Tris[hydroxymethyl]aminomethane, 2.0 mM ethylenediamine-N,N,N',N'-tetraacetic acid, 50.0 mM NaCl, 0.1 mM dithiothreitol at pH 8.8). Gels were covered with membranes and with three sheets of filter paper on each side. Blotting was performed at room temperature by placing this sandwich between two planes of glass

and applying a pressure of 1 kg/100 cm^2 for approximate 20 h.

For sequencing proteins separated by 2-D PAGE were electrotransferred to siliconized glass-fiber membranes by means of a semi-dry blotting apparatus (Pegasus, Phase, Mölln, Germany). Membranes were stained by Coomassie brilliant blue and dried at room temperature. The spots representing the rhythmically synthesized proteins were cut out of the membranes.

Sequencing of proteins

About twenty protein spots, cut out of the glass-fiber membrane were submitted to microsequence analysis by Dr F. Lottspeich (Max-Planck-Institut für Biochemie, München, Germany) according to standard protocols (Eckerskorn *et al.*, 1988).

Immunodetection

The nitrocellulose membranes containing *Euglena* proteins were handled according to Engvall (1980) and Specht *et al.* (1990). For detection of LHCPs an antiserum raised against LHCP II from *Euglena gracilis* in goat was used. The manganese-stabilizing-protein (MSP) was detected by an antiserum raised against oat derived protein in rabbit (Specht *et al.*, 1990).

Detection of primary antibodies was either achieved by a horseradish peroxidase conjugated antibody raised in pig against rabbit immunglobulins (DAKO-Immunglobulin a/s, Denmark) or by an alkaline phosphatase conjugated antibody raised in rabbit against goat immunglobulins (Sigma, Deisenhofen, Germany).

Dot blot hybridisation of mRNA

Dot blots of poly(A)$^+$RNA were performed as described (Thomas, 1980; Pötter and Kloppstech, 1993) and hybridized to a LHCP II mRNA probe of barley labelled with [^{32}P]ATP.

Results

Identification of the rhythmically synthesized 24 and 17 kD proteins in Euglena gracilis as LHCPs

Euglena gracilis cells (synchronized by cycles of 12 h light and 12 h darkness) were exposed to continuous light, and *in vivo* labelling of proteins with [^{35}S]methionine was performed as described previously (Künne and de Groot, 1996). Subsequently cells were harvested and separated by centrifugation into a soluble protein fraction and a membraneous fraction. The membraneous fraction was submitted to PAGE followed by autoradiography. Maximum synthesis of a 24 and 17 kD protein band occurred in the middle of the light phase. The corresponding minimum was observed in the middle of the subjective dark phase under constant light conditions as previously shown by Künne *et al.* (1997) (see also Fig. 4, *lanes A + B* of this paper). The corresponding protein samples were further submitted to 2-D PAGE, and the positions of the rhythmically synthesized proteins

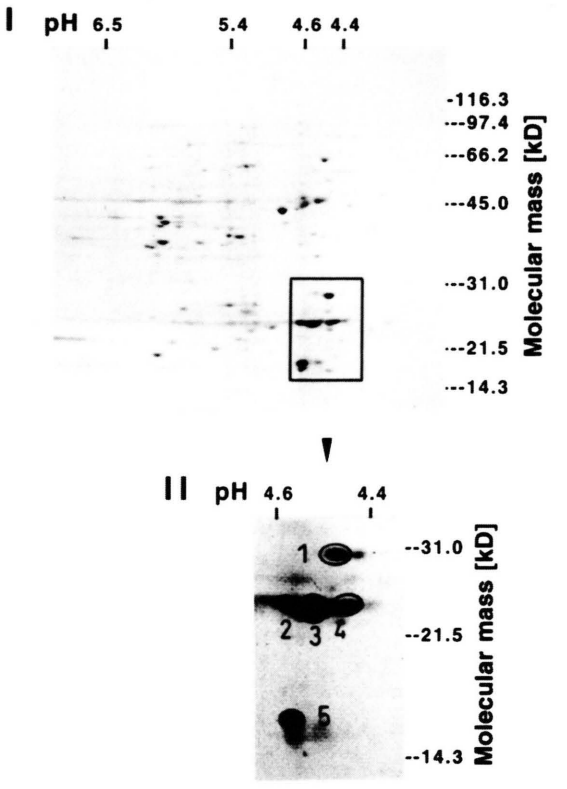

Fig. 1. Isolation of rhythmically synthesized proteins. 2-D PAGE of membraneous cell proteins of *Euglena gracilis* were stained by Coomassie brilliant blue. I, whole polyacrylamide gel (9–15%). II, enlarged, part of a similar gel, corresponding to the boxed part of the gel given under I, after electroblotting onto a glass-fiber membrane and subsequent staining with Coomasie brilliant blue. The pH and molecular mass indicate the positions of standard proteins. The proteins which were cut out for microsequencing are indicated by numbers.

were identified on the corresponding fluorographs (17 kD spot and the 24 kD band now giving 3 spots) (Künne *et al.*, 1997). A representative Coomassie brilliant blue stained 2-D polyacrylamide gel is shown in Fig. 1I. Proteins separated on similar gels were transferred electrophoretically on glass-fiber membranes and subsequently stained with Commassie brilliant blue for protein detection (Fig. 1II). For microsequencing about twenty spots of each protein were cut out of the membranes. In addition to the 17 kD (spot labelled No. 5 in Fig. 1II) and the 24 kD spots (No. 2, 3, and 4 in Fig. 1II) a 31 kD protein (No. 1 in Fig. 1II) which did not show a rhythmic change in its synthesis was also submitted to N-terminal sequencing.

The results obtained by N-terminal sequencing are presented in Fig. 2. For spot No. 1 and No. 5 sequences of 26 and 20 contiguous amino acids, respectively, were obtained. For the three 24 kD protein spots in each case one major N-terminal sequence (Fig. 2: 2a, 3a and 4a) with one subsequence (Fig. 2: 2b, 3b and 4b) was obtained. As the amino acid sequences No. 2a to 4b were very similar, a consensus sequence was derived from all six sequences (Fig. 2) and used for homology search in the ProteinDataBase.

The N-terminal sequence of spot 1 (protein with apparent molecular mass of 31 kD) has similarity to the manganese-stabilizing-protein (MSP, *psbO* gene product) of photosystem II. In the region of amino acids 3 to 22 it exhibited a 70% similarity to the corresponding proteins from *Chlamydomonas reinhartii* and from spinach (Oh-Oka *et al.*, 1986; *Mayfield et al.*, 1989) (data not shown). The molecular masses of known MSPs range from 30 to 34 kD which is in agreement with the molecular mass of 31 kD obtained for the protein from *Euglena*. The proteins of spots No. 2 to 4 (isoforms with apparent molecular mass of 24 kD) show the highest homology to LHC proteins of group II which are mainly associated with photosystem II (Fig. 3, *upper part*). A 100% similarity exists to one LHCP II isoform from *Euglena gracilis* var. *bacillaris* (Muchal and Schwartzbach, 1992). But there is also similarity to other known LHCP II sequences. The sequence of the 17 kD protein also shows similarity to LHCPs (Fig. 3, *lower part*), although in this case the highest similarity was to LHCPs of group I which are mainly associated with photosystem I. The two best fitting sequences (95 and 70% similarity) were obtained by translating a cDNA of *Euglena gracilis* Z coding for a polyprotein (Houlné and Schantz, 1988).

The identification of the rhythmically synthesized 17 and 24 kD proteins as LHCPs by N-terminal sequencing was verified in immunoblots using a polyclonal antiserum raised in goat against the LHCP II from *Euglena* (Fig. 4). The anti-LHCP serum recognized all three 24 kD isoforms with sequence similarity to LHCPs II and recognized to a lower degree also the 17 kD protein with sim-

Protein	Amino acids																									
	1				5					10					15					20					25		
1:	A	S	L	T	Y	D	E	L	Q	S	L	S	Y	L	E	V	K	S	(S)	G	I	A	(D)	T	T	(V)
2a:	?	A	S	G	R	K	S	A	P	P	K	S	D	N												
2b:	?	A	**T**	**S**	R	K	S	A	P	**A**	K	**L**	D	N												
3a:	?	A	S	G	R	K	S	A	A	P	K	S	D	N	L	(S)	Q	(W)	Y	G	P	D				
3b:	?	A	**T**	G	R	K	S	A	**P**	P	K	S	D	N	L	(S)	Q	(W)	Y	G	P	D				
4a:	?	A	S	G	R	K	S	A	A	P	K	S	D	N												
4b:	?	A	**T**	G	R	K	S	A	**P**	P	K	**L**	D	N												
5:	A	S	S	G	H	K	D	G	L	(W)	F	P	N	A	E	P	P	A	G	L						

Consensus sequence of proteins 2a to 4b

1				5					10					15					20		
?	A	S	G	R	K	S	A	A	P	K	S	D	N	L	(S)	Q	(W)	Y	G	P	D

Fig. 2. N-terminal amino acid sequences of the proteins marked in Fig. 1II. For protein 2, 3 and 4 a major amino acid sequence (a) and a minor subsequence (b) was obtained. Non-identical amino acids were printed in bold type. Brackets indicate positions where the determined amino acid is uncertain. The question marks stand for unidentified amino acids. The last line represents a consensus sequence derived of sequences 2a to 4b which was used for the homology search.

Comparison of the N-terminal amino acid sequence of the 24 kD proteins (consensus sequence shown in Fig. 2) with known LHCP II sequences

				1	2	3	4	5	6		7	8	9	10	11			12	13	14	15	16	17	18	19	20	21	22
A)				**?**	**A**	**S**	**G**	**R**	**K**	**-**	**S**	A	A	P	K	-	-	S	D	N	L	(S)	Q	(W)	Y	G	P	D
B)	**A**	**M**	**F**	**A**	**+**	**+**	**+**	**+**	**+**	**-**	**+**	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
C)	**A**	**M**	**L**	**A**	**T**	**+**	**+**	**+**	**+**	**A**	K	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
D)	G	A	A	R	F	T	M	+	+	-	+	+	T	T	+	K	V	A	S	S	G	+	P	+	+	+	+	+
E)	K	A	A	G	T	K	Q	T	+	-	A	+	P	A	+	-	-	+	A	G	I	-	E	+	+	+	+	+

Comparison of the N-terminal amino acid sequence of the 17 kD protein with known LHCP I sequences

				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
F)				**A**	**S**	**S**	**G**	**H**	**K**	**D**	G	L	(W)	F	P	N	A	E	P	P	A	G	L
G)	**A**	**M**	**F**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	+	+	+	+	+	+	+	+	+	+	+	+	+
H)	**A**	**M**	**F**	**+**	**T**	**T**	**+**	**+**	**+**	**+**	+	V	+	+	+	G	+	Q	+	+	+	H	+
I)	T	V	C	V	A	A	D	P	D	R	P	+	+	+	+	G	S	T	+	+	P	W	+
J)	T	S	A	V	A	A	D	P	D	R	P	+	+	+	+	G	S	T	+	+	E	W	+

Fig. 3. Comparison of the N-terminal amino acid sequences. Sequences of LHCP II and LHCP I of different organisms were compared with the sequences of the rhythmically synthesized proteins of 24 kD (upper part) and 17 kD (lower part) of *Euglena gracilis*. The N-terminal amino acid consensus sequence of the 24 kD protein isoforms (A) is compared to two LHCP II isoforms of *Euglena gracilis* var. *bacillaris* (Muchal and Schwartzbach, 1992) (B and C), one LHCP II of *Pisum sativum* (Cashmore, 1984) (D) and one LHCP II of *Chlamydomonas moewusii* (Larouce *et al.*, 1991) (E), while the sequence of the 17 kD protein is compared to two LHCP I isoforms of *Euglena gracilis* Z (Houlné and Schantz, 1988) (G and H), one LHCP I of *Lycopersicon esculentum* (Pichersky *et al.*, 1988) (I) and one LHCP I of *Petunia hybrida* (Stayton *et al.*, 1987) (J). The plus sign (+) mark identical amino acids to the determined *Euglena gracilis* sequences in this paper and minus signs (-) are positions where gaps were introduced to maximize the alignment. The question mark stands for an unidentified amino acid. Bold letters represent *Euglena* specific sequences belonging to the decapeptides connecting the LHCPs in the polyprotein precursor. They were used as recognition sequence for cleavage by a processing protease (Houlné and Schantz, 1993).

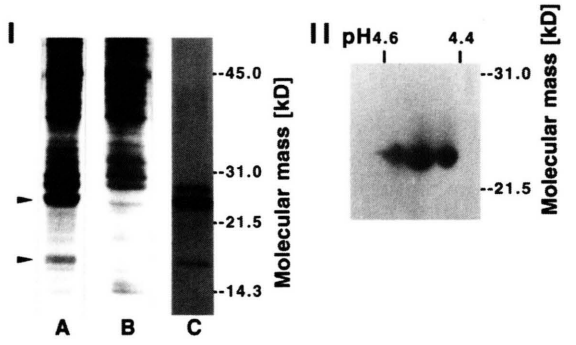

Fig. 4. Identification of the LHCPs of *Euglena gracilis* with an antiserum. *Part I*, comparison of 1-D PAGE (12%) separation of *Euglena* membraneous proteins. *Lane A*, and *B*, fluorographs of proteins labelled at the maximal (6 h in the light = *A*) or minimal (18 h in constant light = *B*) synthesis rate of the 24 and 17 kD proteins, respectively. Similar amounts of radioactivity were applied to the gel lanes. *Lane C*, corresponding Immunoblot: *Euglena gracilis* membraneous proteins after transfer on nitrocellulose. Immunostaining of proteins was performed with an antiserum raised against the LHCP II of *Euglena*. *Part II*, immunoblot: Part of a 2-D PAGE separation of membraneous proteins of *Euglena*. The part of the membrane containing the 24 kD isoforms is shown. Detection was performed by the LHCP II antiserum. The pH and molecular mass mark the positions of standard proteins.

ilarity to LHCPs I. In addition to recognizing the 17 and 24 kD proteins under investigation, the anti-LHCP serum showed a cross-reaction with a 28 kD protein which was not further investigated during this study. Most likely this protein represents an additional member of the LHCP family in *Euglena*. The identity of the 31 kD protein band which shares homology with MSPs of PS II and which did not show a rhythmic change in its synthesis, was also verified in immunoblots with a corresponding anti-MSP serum raised against the MSP from oat (not shown).

Investigation of the total LHCP amount in Euglena gracilis under diurnal and circadian conditions

Coomassie brilliant blue stained gels of the membraneous fraction of *Euglena gracilis* cells indicated that the total amount of the 24 and 17 kD protein band, now identified as LHCP II and I respectively, did not greatly vary during 12 : 12 h light : dark cycles (data not shown). This result was confirmed by immunoblot experiments with the anti-LHCP II serum (Fig. 5). Thus, hardly any variation occurs in the total LHCP amount under diurnal and circadian conditions, while in contrast

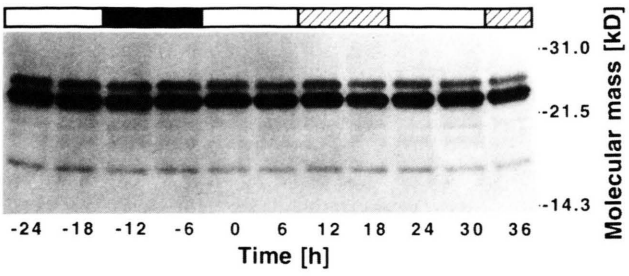

Fig. 5. Detection of the total amount of LHCPs in *Euglena gracilis* cells. Cells were harvested at different times in light-dark cycles of 12 : 12 h, as indicated below the gel lanes. On each lane 10 µg of *Euglena* protein were separated and detection of LHCPs was performed with the anti-LHCP serum. The illumination to which the cells were exposed at the time of harvest is indicated by the bar at the top: Black = darkness; white = light and hatched = theoretical darkphase in constant light. The position of standard proteins is indicated by their molecular mass.

the synthesis pattern determined by [^{35}S]methionine labelling results in 20-fold circadian oscillations as previously shown (Künne *et al.*, 1997).

Regulation of the synthesis of the 17 and 24 kD LHCP occurs at translational level

To determine whether the observed rhythm in the synthesis of the 17 and 24 kD LHCPs occurs on transcriptional or translational level, mRNA of *Euglena gracilis* was extracted by oligo-dT-cellulose at different times in a 12 : 12 h light-dark cycle followed by constant light. For quantification of the mRNA present at the various times, dot blots were performed in which the mRNA from *Euglena* was hybridized to an LHCP II probe from barley labelled with [^{32}P]ATP (Fig. 6). Analysis of the resulting fluorographs revealed that maximally a difference of about 40% in the LHCP II mRNA was measurable. The highest amounts of LHCP II mRNA were detected at the beginning and the lowest amounts at the end of the light phase.

In vitro translation of these mRNAs in a wheat germ cell-free system resulted in up to 50 protein bands resolved by PAGE. No time dependent intensity changes of specific protein bands were observed (data not shown).

To confirm these results, inhibitors of transcription were used (Fig. 7). These inhibitors were added 18 h before the onset of constant light. When α-amanitin was added, the synthesis pattern of the LHCP I and II was not altered in comparison to samples from cells without inhibitor. Optimal synthesis occurred at 6 h in the light, and minima at -6 h (darkness) and at 18 h in constant light (Fig. 7). When actinomycin D was added the amplitudes of synthesis were lowered, but the overall pattern with corresponding maxima and minima remained unchanged. Thus, it can be concluded

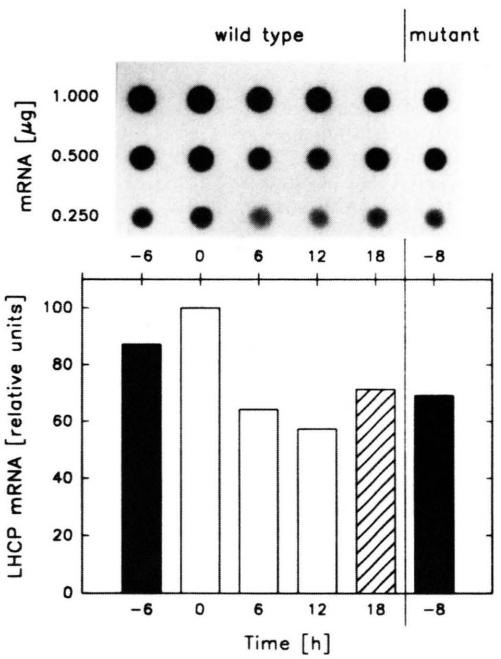

Fig. 6. Quantification of the LHCP mRNA in *Euglena*. Dot blot hybridization of mRNA from *Euglena* wild type cells and cells of a bleached *Euglena* mutant, extracted at different times in light-dark cycles and constant light, with a LHCP II message of barley. Time points of extraction (zero h is onset of constant light), amount of blotted mRNA and relative units of hybridizing LHCP mRNA are indicated. Black bars symbolize the relative LHCP mRNA in cells harvested in darkness, while white and coarse bars (18 h in constant light) mark values for cells harvested in the light. Bars are the result of the densitometric evaluation of the dot blot.

that the highest LHCP synthesis always occurred at the middle of the day phase, even though inhibitors of transcription were added 24 h before translation was measured. This result implies that the corresponding mRNAs are stable over prolonged time spans and that the regulation of the

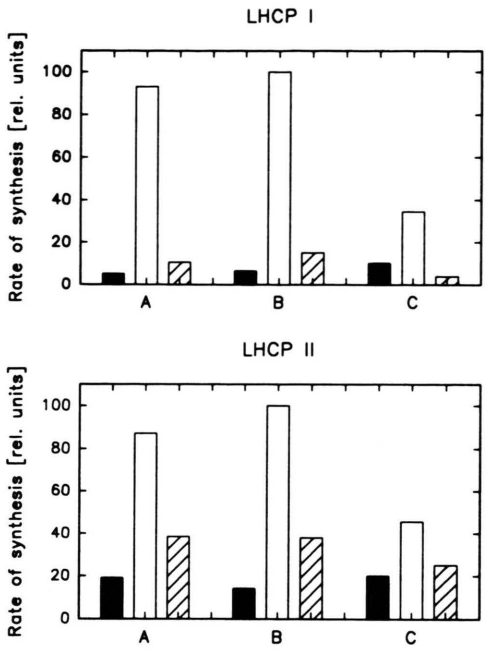

Fig. 7. Influence of transcription inhibitors on the rhythmic synthesis rate of LHCP I and LHCP II in *Euglena*. Cells were incubated with [^{35}S]methionine for two h starting at -6 h in the dark (black bars), 6 h in the light (white bars) and 18 h in constant light (striped bars). The amount of labelled LHCP I and II was measured by densitometric evaluation of fluorographs of one dimensional gels. Inhibitors were added to the cultures 18 h before the onset of constant light. Bars above (A) are values of a control culture, while α-amanitin (final concentration 50 μM) was added to culture (above B). Actinomycin D (final concentration 0.4 mM) was added to culture (bars above C).

rhythmic LHCP synthesis occurs at the translational level.

Further evidence that in *Euglena* the synthesis of the LHCPs is mainly regulated at the translational level came from the investigation of a bleached *Euglena gracilis* mutant. In the mutant cells we failed to detect the 17 and 24 kD LHCPs by means of Coomassie brilliant blue staining, radioactive labelling or immunoblot with anti-LHCP II serum (data not shown). Despite absence of the LHC proteins in this *Euglena* mutant the LHCP II mRNA could be detected in amounts comparable to that measured in wild type cells (Fig. 6, *lane -8*). Thus, it can be concluded that in *Euglena* the mRNA encoding the LHCPs is abundant even though no synthesis of LHC proteins can be observed.

Discussion

LHCPs of group I and II belong to a large gene family possibly all possessing a common ancestor (Green *et al.*, 1991). For example in tomato nineteen different LHCPs have so far been identified (Kellman *et al.*, 1993). In *Euglena gracilis* Z and *E. gracilis* var. *bacillaris* five isoforms of LHCP I and four isoforms of LHCP II have been detected on basis of cDNA and genomic sequencing respectively (Houlné and Schantz, 1988; Muchal and Schwartzbach, 1992). A circadian rhythm of the synthesis of LHCP mRNAs has been described for a great number of photosynthetic organisms. Such a rhythm was first described in pea and later in wheat, barley and tomato (Kloppstech, 1985; Lam and Chua, 1989; Beator and Kloppstech, 1993; Kellman *et al.*, 1993). Most recently a circadian transcription of LHCP genes was reported for the unicellular green algae *Chlamydomonas* (Jacobshagen and Johnson, 1994). In all these organisms transcription of the LHCP genes is regulated resulting in a circadian fluctuation of the corresponding mRNAs. Although only in a few cases a circadian change in the rate of synthesis or in the total amount of the corresponding LHCPs has been actually measured (Adamska *et al.*, 1991; Riesselmann and Piechulla, 1992; Beator and Kloppstech, 1993), it can be assumed that such an oscillation in protein level does exist for all these organisms in which a rhythm in the mRNA level was detected but does not become manifest due to the large amount of accumulated protein.

As known from the sequence of LHCP I and LHCP II coding mRNA, these proteins are translated as polyprotein precursors in *Euglena* (Houlné and Schantz, 1988; Muchal and Schwartzbach, 1992). This is explained assuming that some LHCPs are connected by decapeptides and are transported to the Golgi apparatus prior to chloroplast import and polyprotein processing (Houlné and Schantz, 1993; Sulli and Schwartzbach, 1995). Such high molecular polyprotein precursors have been described by Rikin and Schwartzbach (1988), who also showed that these polyprotein precursors are cleaved into native proteins within a time span of 20 min. Once synthesized *Euglena* LHCPs were stable in constant light and only very slowly degraded in constant darkness (Künne *et al.*, 1997) explaining the

failure to detect a rhythm in the total LHCP amount. In photoautotrophically growing cultures as used in our experiments, almost no LHC proteins were degraded. The newly sythesized LHCP's were divided between the daughter cells when cell division takes place.

By N-terminal sequencing we found that seven amino acids of the decapeptides connecting the single LHCPs in the polyprotein precursors were present in the mature LHCPs (Fig. 3). This finding is in contrast to the assumption that the decapeptides were removed during processing of the LHCP polyproteins (Houlné and Schantz, 1993), and explains the missing sequence similarity at the beginning of the N-terminus of *Euglena* LHCPs compared to LHCP sequences of other species (Fig. 3).

In the present paper we give evidence that in *Euglena gracilis* a circadian rhythm exists for the LHCP I and II. In contrast to the findings for other algae and higher plants, the regulation was found to be at the translational level. Therefore, it can be speculated that in *Euglena* the LHCP mRNA is quite stable and that it is even synthesized in darkness, while it is rapidly degraded in plants and other algae. This suggestion is supported by findings of Kishore and Schwartzbach (1992) who measured only a two fold reduced level of LHCP mRNA in *Euglena* cells grown for several years in darkness – a condition under which no LHC proteins are synthesized, compared to cells grown in the light. Even during light-induced chloroplast development protein synthesis is mostly regulated at the translational level (Bouet *et al.*, 1986).

Among the proteins having a circadian rhythm only a few proteins have so far been detected for which the regulation is at the translational level. This appears true for a protein of unknown function named P230 of *Acetabularia* and 3-phosphoglycerate kinase in *Chlorella vulgaris* (Hartwig *et al.*, 1986; Walla *et al.*, 1989, 1994). For *Gonyaulax polyedra* it has been shown that the circadian rhythm of bioluminescence depends on the rhythmic synthesis of luciferase and the luciferin-binding-protein and that regulation of the oscillation occurs at the translational level (Morse *et al.*, 1990). *In vitro* translation experiments revealed that the mRNA for luciferase and the luciferin-binding-protein is abundant at all times (Milos *et al.*, 1990). The mechanism responsible for the rhythmic translation of the mRNA has been investigated by sequencing the mRNA coding for luciferin-binding-protein (Lee *et al.*, 1993). These experiments gave evidence that extended untranslated regions at the 3' and 5' end of this mRNA are present and that binding of a protein to the 3' region may be responsible for the rhythmic translation (Mittag *et al.*, 1994). To investigate whether similar untranslated sequences might exist in the LHCP mRNA in *Euglena*, the sequence of *Gonyaulax* luciferin-binding-protein mRNA and of the LHCP mRNA sequences of *Euglena gracilis* var. *bacillaris* and *E. gracilis* Z were compared (Houlné and Schantz, 1988; Muchal and Schwartzbach, 1992; Lee *et al.*, 1993). However, we could not detect a similar sequence motif in the untranslated region of the *Euglena* LHCP mRNAs. Another means of translational regulation of *Euglena* protein synthesis may involve the transfer from small RNAs to the 5' end of premature mRNAs by *trans*-splicing (Tessier *et al.*, 1991).

Another relevant finding of our investigation is the confirmation of the presence of mRNA coding for LHCP II in a bleached *Euglena* mutant (Fig. 8, *lane -8*, and results in Kishore and Schwartzbach, 1992). This is remarkable in two aspects. Firstly, the translation of the LHCP mRNA must be inhibited or not be initiated in the mutant indicating that LHCP synthesis is regulated at the translational level. Secondly, it has to be assumed that there is a signal (or signals) sent from the chloroplasts for the regulation of cytoplasmatic translation of LHCP mRNA in *Euglena*.

Finally, it might be suggested that the rhythmically synthesized LHCP's in *Euglena* could be responsible for other circadian oscillations of physiological parameters like the rhythm in total protein synthesis (Feldman, 1968; Künne and de Groot, 1996) and at least partially explain the rhythm in photosynthesis (Lonergan and Sargent, 1978; Edmunds, 1988).

Acknowledgement

We would like to thank Dr. Lottspeich, Max-Planck-Institut of Biochemie, München, Germany, for performing the protein sequencing.

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