Circadian Synthesis of a Nuclear-Encoded Chloroplast Glyceraldehyde-3-Phosphate Dehydrogenase in the Dinoflagellate *Gonyaulax polyedra* Is Translationally Controlled[†,‡]

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ABSTRACT: The circadian clock has previously been shown to restrict synthesis of several proteins in the dinoflagellate *Gonyaulax polyedra* to only a few hours each day. We have identified one of these proteins as glyceraldehyde-3-phosphate dehydrogenase. Two nuclear genes encoding the enzyme have been cloned, one corresponding to a cytoplasmic isoform and the other to a plastid targeted protein. On the basis of protein microsequence data, we conclude that the synthesis of the plastid isoform is clock-regulated. This regulation is not related to mRNA levels, which remain constant throughout the cycle, suggesting a translational control mechanism, in contrast to the transcriptional regulation of GAPDH that has been demonstrated in *Neurospora*. Although the rhythm of synthesis has a high amplitude, the abundance and activity rhythms are greatly attenuated, which is attributed to the long half-life of the protein.

By biochemical mechanisms not yet understood, circadian control in cells and organisms may be exerted over a considerable number of different biochemical and physiological processes, each with its own phase of maximum expression (*1*, *2*). Key components of the underlying circadian oscillators have been identified in organisms ranging from bacteria and fungi to insects and mammals (*3*−*7*), but little is known about the molecular identity of other circadian elements, notably the "input" and "output" pathways. In the marine dinoflagellate *Gonyaulax polyedra*, it has been shown that circadian control of bioluminescence, which peaks during the night phase, correlates with the daily synthesis and destruction of two proteins involved in the light-emitting reactions, luciferase (LCF)[1] and the luciferin binding protein (LBP), and that this regulation is controlled at the level of translation (*8*, *9*). In the case of LBP, synthesis occurs during the late day and early night phase, and is postulated to be repressed by a protein that binds to the 3′ UTR of the *lbp* transcript during the day phase (*10*).

No studies of how regulation may occur for proteins and processes that peak at other circadian phases have been reported for *Gonyaulax*. However, it was previously shown that the synthesis of many proteins is translationally controlled, with maxima occurring at different circadian times for different proteins (*11*, *12*). This paper reports the identification of one such protein as the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the synthesis of which occurs preferentially during the late night and early day phase. We show that while the amplitude of the circadian rhythm of GAPDH synthesis is high, amplitudes of protein abundance and enzyme activity are only modest.

EXPERIMENTAL PROCEDURES

GAPDH Activity Measurements. GAPDH activity was measured by following the reduction of $NADP^+$ to NADPH at 25 °C in the presence of glyceraldehyde 3-phosphate and sodium arsenate, which prevented the back reaction (*13*). Assay mixtures contained 60 mM Tris-HCl (pH 8.5), 0.1 M KCl, 0.01 M Na_2AsO_4, 7 mM L-cysteine, 1 mM $NADP^+$, and ∼100 mM glyceraldehyde 3-phosphate, in a final volume of 1 mL. Assays were started by adding 10 μL of *Gonyaulax* crude extract, and the reduction of the dinucleotide was followed by measuring the increase in the absorbance at 340 nm (*14*). The initial velocity was calculated on the basis of an extinction coefficient of 6400 M^{-1}.

Preparation of Gonyaulax Crude Extract. Cultures of *G. polyedra* were grown photoautotrophically in Fernbach flasks in f/2 medium (*15*). Unless otherwise stated, cells were grown in a 12 h light:12 h dark (12L:12D) regimen with illumination from below by cool fluorescent bulbs at a light intensity of ∼150 μE m^{-2} s^{-1} (*16*). When cells were grown under constant dim light, they were first subjected to the 12L:12D regimen and then transferred to an environment with constant dim light at the dark to light transition and maintained at a light intensity of ∼50 μE m^{-2} s^{-1}. Cells were harvested by filtration on Whatman 541 paper and either used immediately or frozen in liquid nitrogen and stored at −70 °C. Crude

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[1] Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LBP, luciferin binding protein; LCF, luciferase; DTT, dithiothreitol.

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extracts were prepared by lysing cells using a mini bead-beater (Biospec Products, Bartlesville, OK). Typically, 0.1−0.3 g of filtered cells was placed in a mini bead-beater tube containing 2.4 g of 1 mm zirconium beads and filled with cold extraction buffer (*17*) [0.1 M potassium phosphate (pH 9.0), 0.03 M EDTA containing 1 mM PMSF, and 10 mM E-64 to prevent proteolytic activity]. The mixture was agitated for 1 min, then transferred to a microcentrifuge tube, and clarified by centrifugation at 30000*g* for 20 min at 4 °C. The clear orange supernatant was removed and used immediately for enzyme activity and protein assays. For polyacrylamide gel electrophoresis, samples were diluted into denaturing buffer [75 mM Tris-HCl (pH 6.8), 0.1 M DTT, 2.5% SDS, 6.25% glycerol, and 0.001% bromophenol blue], boiled for 4 min, and then frozen at −20 °C.

Cloning. Clones for GAPDH were obtained by polymerase chain reaction and by screening of a *G. polyedra* cDNA library. Sequences were determined using the dideoxy chain termination method (*18*), and their identities were determined by BLAST searches of the NCBI sequence data banks (*19*). The forward degenerate primer GAPP 5a (TCSAACGCNTC-STGYACBAC), which encodes the conserved peptide sequence SNASCTT, surrounding the GAPDH catalytic cysteine residue, was used with the vector primer T7 to amplify a 765 bp fragment from a *Gonyaulax* cDNA library directionally cloned into the λ Zap vector. From this fragment, the nondegenerate antisense primer GAPP 27 (CACAAAA-CAAGCGAGCTTCACTC) was designed, which together with the vector primer T3 amplified a 1.3 kb product from the same library, later named GAPC.

GAPP 5a was also used with the reverse degenerate primer GAPP 4a (RATSGGGTTVGTCTCSARSTC), the complement of which encodes the peptide DLETNPI (*12*), to amplify a 430 bp fragment. This was used to create the reverse primer GAPP 4b (TAGTCTCGAAGTCTGTGGA-CAC), which together with the vector primer T3 amplified a 1.1 kb clone. This was used to isolate a 1.4 kb cDNA clone from the library, later designated GAPCp.

Western Analysis. For one-dimensional analysis, samples of denatured crude extract were electrophoresed on 12% denaturing polyacrylamide gels (*20*) and then transferred electrophoretically onto nitrocellulose (Schleicher and Schuell, BA85). The membrane was rinsed in distilled water and stained for 5 min in Ponceau red (0.5% w/v Ponceau S and 1% v/v glacial acetic acid in distilled water). The membrane was then rinsed with water, and the positions of the molecular mass standards were marked and the lanes checked visually to confirm that protein had been loaded equally before completely destaining. For two-dimensional analysis, blots were prepared as previously described (*12*). Briefly, samples of crude extract were first isoelectric focused, then equilibrated in denaturing buffer, and run in the second dimension on a denaturing gel (*20*). Proteins were then electrophoretically transferred to nitrocellulose membranes. Before incubation with antibody, membranes were blocked overnight in 5% nonfat dried milk in TBST buffer [20 mM Tris-HCl (pH 7.6), 0.14 M NaCl, and 0.1% Tween 20] at 4 °C. After being thoroughly rinsed, they were incubated for 1 h at room temperature in a dilution (1:7000 to 1:15000) of rabbit anti-trypanosome glycosomal GAPDH serum; the serum was determined by Western blotting to react with equal affinity with histidine-tagged fusion proteins of GAPCp and GAPC isoforms expressed in *Escherichia coli*. Blots were then washed with TBST and incubated with a donkey anti-rabbit $F(ab')_2$ horseradish peroxidase-linked fragment (Amersham) for 1 h at room temperature. Following extensive washing, the bound second antibody was visualized by chemiluminescence (ECL, Amersham). After scanning, densitometry was carried out using NIH Image 1.60.

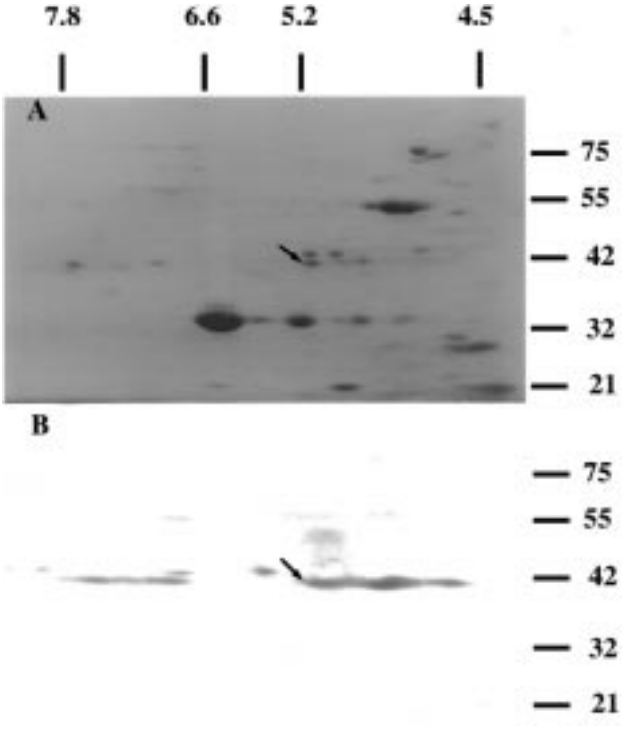

FIGURE 1: Two-dimensional gel electrophoresis of total *Gonyaulax* protein visualized by Coomassie blue staining (A) and immunoluminescence with anti-trypanosome GAPDH (B). The arrows point to the protein previously shown to have a robust rhythm of synthesis, which was eluted from gel A for digestion and peptide sequencing. The ordinate is the molecular mass in kilodaltons; the abcissa is p*I*.

Northern Analysis. Total RNA was isolated from *Gonyaulax* as previously described (*8*), run on 1% agarose/formaldehyde gels, and then transferred to nylon membrane (genescreen plus, NEN DuPont), probed with antisense transcripts, washed under stringent conditions, and autoradiographed. Under these conditions, *gapc* and *gapcp* probes yielded single bands of 1.4 and 1.3 kbp, respectively, indicative of their specificities. *lbp*, which has previously been shown to occur in a constant amount over the circadian cycle, was used as a control.

RESULTS

The Circadian-Regulated p45 Is a Nuclear-Encoded Chloroplast GAPDH. Markovic et al. (*12*) demonstrated by two-dimensional polyacrylamide gel electrophoresis and autoradiography that in vivo incorporation of [^{35}S]methionine into numerous *Gonyaulax* proteins depended on the circadian phase. One of these proteins, p45 (Figure 1, arrow), exhibited a robust synthesis peak in the late night and/or early morning phase of cells maintained in constant light. This indicates regulation by the circadian clock, so we undertook the identification of this protein to determine its physiological role and its abundance over the circadian cycle and to elucidate the mode of its regulation.

Table 1: Amino Acid Sequences of Peptides from Gel-Purified GAPDH Compared with the Deduced Amino Acid Sequences from *Gonyaulax* GAPC, GAPCp, and GAPDH Sequences from *Giardia* and *Chlamydomonas*[a]

PEPTIDE A	ATTYEEICAEIK	Identities
Gonyaulax Cp	.....	12/12
Gonyaulax C	.AK.DD.V.A..	6/12
Giardia	KD..D.....	9/12
Chlamydomonas	.....D.MKAL.	7/12
PEPTIDE B	AGIMLDPTFVK	
Gonyaulax Cp	.....	11/11
Gonyaulax C	...S.NDN...	7/11
Chlamydomonas	.....S.....	10/11
PEPTIDE C1	GFLGYTDEPLVSTDFETNPIS	
Gonyaulax Cp	.....T..	20/21
Gonyaulax C	.V.DW...EV.....VSCKS.	11/21
Chlamydomonas	.V.A....DV..S..V.D.A.	13/21
PEPTIDE C2	VPTIDVSVVDLTTELEK	
Gonyaulax Cp	.....C....	16/17
Gonyaulax C	...P.....CRI..	13/17
Chlamydomonas	...N.....VT...	14/17

[a] The fractions of identical residues are indicated on the right. The sequences of peptides C1 and C2 have been corrected, as described in the text, on the basis of the deduced amino acid sequence of GAPCp.

The p45 protein, abundant enough to be visible after Coomassie staining (Figure 1), was electrophoretically transferred from the two-dimensional gel onto a PVDF membrane. Tryptic peptide fractions were generated and separated by HPLC for microsequencing (Harvard Microchemistry, Cambridge, MA). Unambiguous sequences were obtained from two fractions (peptides A and B, Table 1), which, when compared to known genes in the GenBankTM/EMBL nonredundant database with the BLAST algorithm (*19*), were 90% identical to GAPDH from both *Giardia lamblia* and *Chlamydomonas reinhardtii*. Peptide A also was 58% identical to human protein kinase C.

A third fraction was found to be a nearly equimolar mixture of two peptides, and thus, two amino acids were identified during each sequencing cycle. The original interpretation of the sequence data yielded peptides C1 (GFLIYVDEPDVTTDLETNPIS), which was 61% identical with GAPDH from *Bacillus subtilis*, and C2 (VPTGDTSVVLLSEFK), which was 58% identical with glucon endo-1,3-β-glucosidase from *Hordeum vulgare*. However, when the sequence of cloned *Gonyaulax* GAPDH became available, it was observed that the peptide sequences could be reinterpreted as being nearly identical to the sequence of the GAPDH (see Table 1).

To aid in the identification of the sequenced protein, a heterologous antibody raised against a trypanosome glycosomal GAPDH, and shown to react with both GAPC and GAPCp, was tested with the two-dimensional gel immunoblots. The antibody recognized a protein (arrow in Figure 1B) having the same isoelectric point and molecular mass as the protein that was microsequenced, and also several other proteins with the same molecular mass but with different isoelectric points. These other proteins are considered to be additional isoforms of GAPDH (see below).

To identify and characterize possible GAPDH isoforms, *Gonyaulax* GAPDH genes were cloned from a *Gonyaulax* cDNA library by PCR, using GAPDH-specific primers together with vector primers. Degenerate primer sequences derived from the peptide microsequence data (Table 1) and from the conserved GAPDH active site motif SNASCTNCLAP yielded two GAPDH sequences, which were about 50% identical to one another in their region of overlap.

The full-length coding sequences for both of these were obtained, one by screening the cDNA library and the second by PCR, as described in Experimental Procedures. The two clones, termed GAPCp and GAPC, are 1.433 and 1.290 kb long and encode proteins having molecular masses of 45 and 36.8 kDa, respectively (Figure 2) and have sequences that are significantly similar to those of GAPDHs from other organisms. The longer GAPCp cDNA has an N-terminal extension whose deduced protein sequence has two hydrophobic regions, similar to the plastid targeting sequences of PCP (*21*, *22*). It is thus likely that the GAPCp isoform is destined for the chloroplast. This is supported by the examination of three key amino acid residues, corresponding to Asp32, Gly187, and Pro188 in the numbering of Biesecker et al. (*23*) for *Bacillus* GAPDH, which are required to confer specificity for NAD^+ as a cofactor instead of a dual specificity for both NAD^+ and $NADP^+$ (*24*). These key amino acids are found in the *Gonyaulax* GAPC protein as Asp34, Gly194, and Pro195 (boldface type in Figure 2) and are substituted in the GAPCp sequence with Ala125, Ser280, and Ala281, suggesting that this form of the enzyme can function with either NAD^+ or $NADP^+$, as is true for all other chloroplast GAPDHs. With the putative signal peptide removed, the deduced protein sequences of the two cDNAs predict similar molecular masses (36.8 and 36.4 kDa). In the region of overlap (Figure 2), the two clones share 55 and 53% sequence identity at the nucleic acid and deduced protein levels, respectively.

Of the two clones, the one encoding GAPCp is more likely to correspond to the protein microsequenced from the two-dimensional gel. When the sequence of GAPCp is compared to those of all the peptides that were microsequenced (Table 1), 59 of 61 residues are identical (97%), while in GAPC, only 37 of 61 (61%) are identical. The few mismatches in GAPCp occur near the C-terminal end of the peptides, where sequencing errors are more likely.

Small Circadian Fluctuations in GAPCp Activity and Protein Levels Are Accompanied by a Robust Synthesis Rhythm. Although the synthesis rate of GAPCp exhibits a robust circadian rhythm (*12*), the amplitude of the enzyme activity rhythm was found to be quite small. Crude extracts assayed with $NADP^+$ as a cofactor exhibited an only ~1.5-fold difference between peak and trough activity, with the peak occurring in the middle of the day phase (Figure 3A). Similarly, changes in the cellular amounts of GAPDH protein at different times over the circadian cycle were found to be modest. Equal amounts of crude *Gonyaulax* extract, prepared at intervals throughout a light:dark (12L:12D) cycle, were electrophoresed on SDS−PAGE before being transferred to nitrocellulose, and probed with an anti-trypanosome glycosomal GAPDH antibody (Figure 3B). A low-amplitude rhythm peaking during the day phase was observed, the density values for which are plotted in Figure 3A.

No Circadian Changes with NAD^+ as a Cofactor. Even though chloroplast forms are reported to have dual specificity for $NADP^+/NAD^+$ (*24*), no circadian changes in activity were observed when NAD^+ was used as a cofactor. This

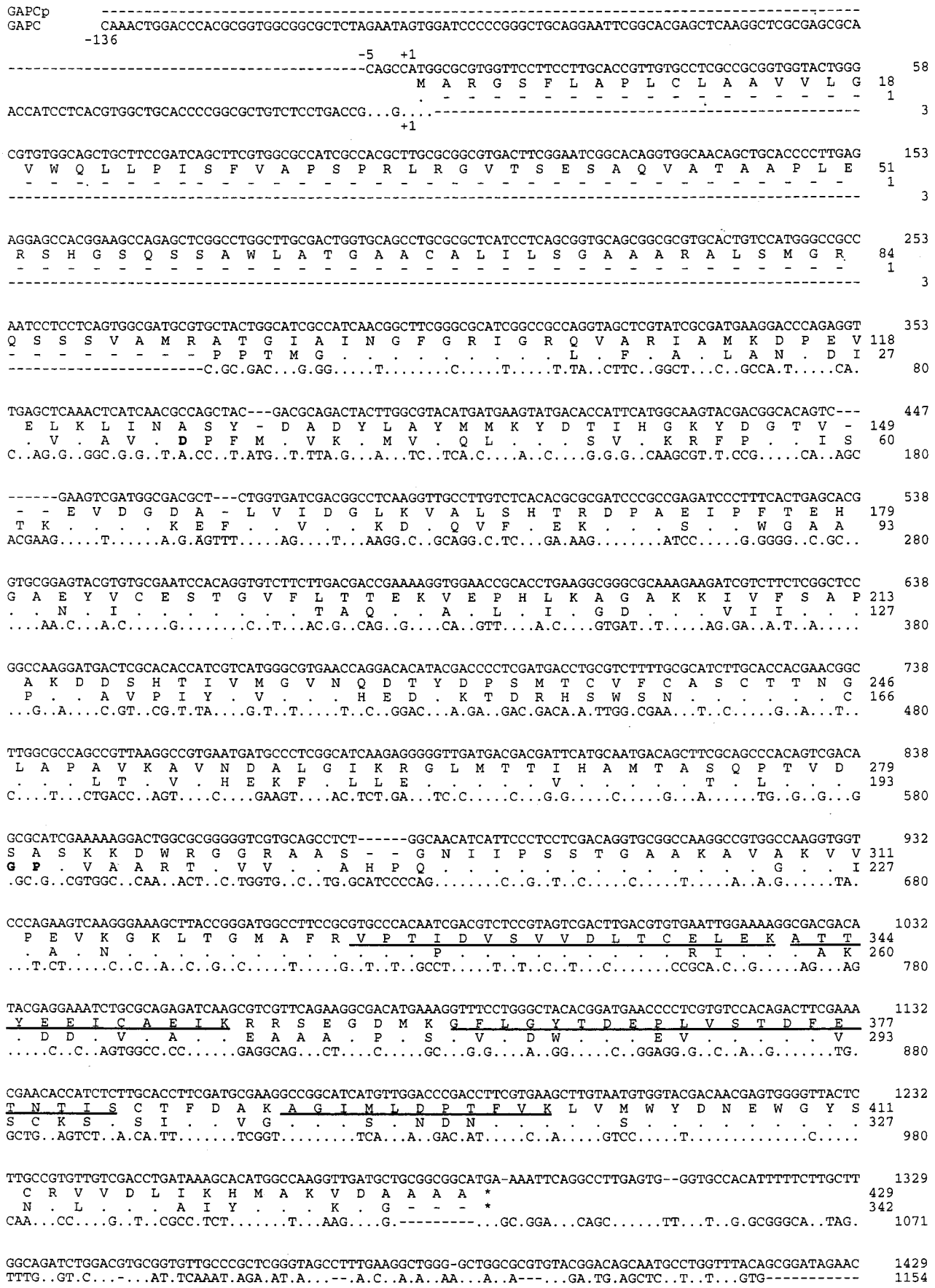

FIGURE 2: Alignment of nucleotide and derived amino acid sequences of the two GAPDH isoforms, GAPCp (429 amino acids, upper two lines) and GAPC (342 amino acids, lower two lines). Identical nucleotides and amino acid residues are indicated by dots (.) and gaps in the alignments by dashes (−). The locations of sequences corresponding to the isolated peptides are underlined. The bold amino acid residues in GAPC correspond to those which confer specificity for NAD^+ as a cofactor.

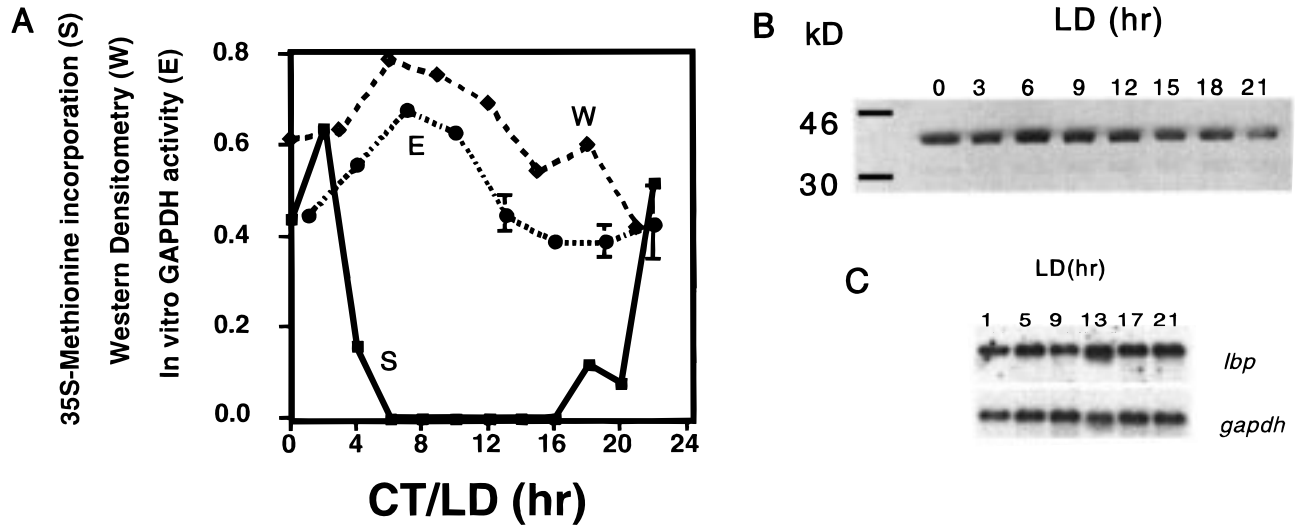

FIGURE 3: (A) Synthesis rate (S), specific enzyme activity (E), and protein levels estimated by Western analysis (W) for GAPDH. Synthesis rates taken from Marcovic et al. (*12*) were determined by the extent of ^{35}S incorporation in relative units. Specific activities are in units of nanomoles of NADPH formed per minute per milligram of protein. Data for Western blots in relative units are taken from scans of gels in Figure 3B. Error bars show the standard deviations of duplicate experiments. (B) Western blots of *Gonyaulax* crude extracts prepared at different times throughout the 24 h light:dark cycle. (C) Northern blots of *lbp* and *gapcp* mRNA levels at different times throughout the 24 h light:dark cycle.

suggests either that the NAD^+-specific cytosolic form is nonrhythmic and more active in the crude extracts, thus obscuring the rhythm of GAPCp activity, or that it cycles out of phase with the chloroplast form.

Two-Dimensional Gels Reveal Several Isoforms Exhibiting Rhythms of Abundance. To resolve and examine the rhythmicity of isoforms, we ran crude extracts, isolated at different circadian times, on a two-dimensional gel, blotted it onto nitrocellulose, and probed it with the antibody. Five distinct isoforms were resolved (Figure 4A), all of which exhibited circadian rhythmicity peaking between mid- and late-day phase, at the time of maximum enzyme activity with $NADP^+$. As judged by its isoelectric point, isoform 4 was the protein identified as having the robust synthesis rhythm (see Figure 1). We found no isoforms cycling out of phase which could explain the lack of activity rhythm with NAD^+, suggesting that a nonrhythmic cytosolic form with a high specific activity is present in crude extracts. A definitive evaluation of this matter cannot be provided without knowledge of the K_m and V_{max} values for NAD^+ and $NADP^+$ for the cytosolic and chloroplast forms.

GAPCp Synthesis Is Controlled at a Translational Level. To determine if the rhythm of GAPCp synthesis correlated with a change in its mRNA level, an antisense *gapcp* transcript was used as a probe in Northern blot analysis of the total RNA extracted at intervals over the circadian cycle (Figure 3C). No such changes were found; an antisense luciferin binding protein (LBP) transcript was used to probe *lbp* RNA levels as a control, since *lbp* mRNA levels are known to be constant in *Gonyaulax* (*8*). Similarly, no mRNA changes were observed with a *gapc* probe. Thus, regulation of GAPDH synthesis takes place at the translational level, contrasting with a recent report of circadian regulation of GAPDH activity in *Neurospora*, where *gapdh* (CCG-7) transcript levels exhibit a robust circadian rhythm (*25*).

Circadian regulation of *Gonyaulax* luciferase and LBP also occurs at the translational level, and for *lbp*, a putative regulatory protein has been shown to bind to a specific UG repeat sequence in the 3′ UTR (*9*, *10*). This sequence is not present in the *gapcp* 3′ UTR, indicating that a different signal or control mechanism is involved in the regulation of GAPDH synthesis. Indeed, in gel retardation analysis, no specific interaction between *gapcp* 3′ UTR and *Gonyaulax* cytosolic extracts was observed. However, there may be some

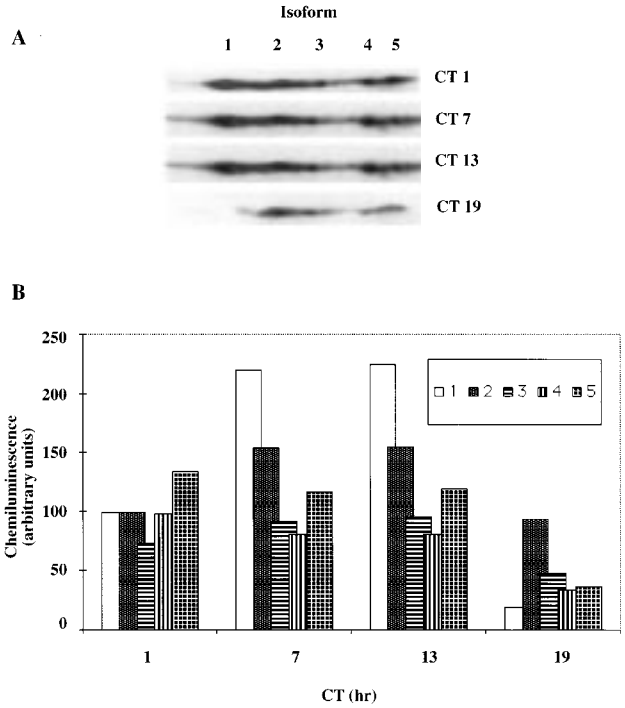

FIGURE 4: (A) Two-dimensional Western analysis of extracts obtained at four times during the circadian cycle. Cultures of *G. polyedra* entrained to a 12:12 light:dark cycle were transferred to an environment with constant dim light ($\sim$30 $\mu E\ m^{-2}\ s^{-1}$). Samples were taken at the indicated circadian times, run on two-dimensional gels, blotted, and probed with anti-GAPDH antibody. Isoforms thus visualized were numbered from left to right. (B) After scanning and densitometry, the relative amounts of the five isoforms were plotted vs circadian time.

factor(s) that binds to the 5′ UTR, which has not yet been determined. In an attempt to elucidate at what step in translation the regulation occurs, we have analyzed polysomes from extracts at LD 0 and LD 12. In both cases, the polysome fraction contained RNA that hybridized to an antisense *gapcp* transcript (data not shown). This would seem to indicate that the control of synthesis does not occur at initiation but at the elongation phase of protein synthesis. Similar observations have been made for the light-regulated translation of nuclear-encoded chloroplast proteins in Euglena (*26*) and higher plants (*27*). Alternatively, there may be some *gapcp* isoforms which are less tightly regulated and are present in the polysome fraction throughout the circadian cycle.

DISCUSSION

In the marine dinoflagellate *G. polyedra*, many biological processes exhibit circadian rhythmicity. Such processes can be measured at a physiological level, for example, bioluminescence, photosynthesis, cell division, and cell motility. Alternatively, they can be tracked biochemically, for example, by the activity or amount of a given protein, as for LBP, luciferase, nitrate reductase, and superoxide dismutase (*8*, *28*−*30*). Such rhythms persist under constant conditions, being controlled by an endogenous biological clock (*31*, *32*). As with circadian-controlled processes in organisms generally, different rhythms may have different phases. For example, the flashing and glow bioluminescent rhythms have peak values in the middle and end of the night, respectively (*33*), while photosynthetic (*34*) and superoxide dismutase activities (*29*) peak during the day phase.

In this study, we have unambiguously identified a nuclear-encoded chloroplast isoform of GAPDH termed GAPCp as one of the "day phase" proteins in *Gonyaulax* previously shown to exhibit a strong circadian rhythm of synthesis (*12*). However, its cellular abundance shows no more than a 1.5-fold difference between peak and trough (isoform 4, Figure 4B). This is readily explained if the half-life of the protein is long (e.g., 24 h), since even if the amplitude of the synthesis rhythm is great (e.g., ∼100 times), the amplitude of the rhythm of protein abundance (and activity) will be very modest (e.g., ∼1.5). Interestingly, we have observed four other isoforms which exhibit circadian rhythmicity in abundance of varying amplitudes, one of which had a higher amplitude than the other four (isoform 1, Figure 4A). The apparent greater abundance of this isoform may be due to a greater affinity for the heterologous antibody, while the higher amplitude may be due to a shorter half-life. In vivo pulse chase experiments indicate that the synthesis of this isoform is also regulated by the circadian clock; however, its absolute levels, as judged by Coomassie staining, are much lower than for isoform 4, and it has not been studied in detail. The molecular basis for the observed rhythms for isoforms 2, 3, and 5 has not been determined.

The pulsed synthesis of isoform 4 represents about 30−50% of its maximum cellular quantity, and serves to replace the protein degraded over the course of a circadian cycle, in a process that might correspond to "normal" protein turnover. The daily pulse of synthesis serves only to replace the protein lost by turnover during the preceding 24 h. If the protein half-life is long, so that not much is lost, then the amplitude of the abundance rhythm will not be high, whereas the synthesis rhythm may have a high amplitude no matter what the half-life is, since it could involve no synthesis at one time of day and some discrete amount at another. The different amplitudes of abundance rhythms of the several GAPDH isoforms may thus reflect their different half-lives. On the basis of these considerations, the fact that both *gapdh* mRNA and enzyme activity in *Neurospora* exhibit robust circadian rhythms (*25*) suggests that both have short half-lives in that organism.

The two circadian-controlled proteins involved in the bioluminescence reaction, luciferase and luciferin binding protein, also undergo daily synthesis pulses. However, there is also a daily destruction of essentially all (>95%) of these two proteins, resulting in a high-amplitude rhythm of protein abundance (*8*, *28*). Synthesis in this case occurs in the late day and/or early night phase, which is appropriate for the occurrence of enzyme activity and luminescence at night. In contrast, GAPDH synthesis peaks during late night and/or early morning, resulting in a maximum enzyme activity during midsubjective day. This is when the photosynthetic activity and the mobility of the cells are at their highest, and one would expect the requirement for GAPDH activity to be at its highest also. But the quantitative difference seems scarcely large enough to be physiologically significant.

Indeed, what functional importance can be ascribed to such rhythms? With some, such as bioluminescence, the value of circadian regulation of the luciferase and related proteins seems to be evident. But for proteins and enzymes that are viewed as having housekeeping functions, the need for circadian regulation is not at all evident. Why synthesis should occur as a pulse rather than as a constant replacement is also not obvious, especially since the resulting rhythm in enzyme activity may not be large. A similar question has emerged from studies showing circadian control of transcription but a greatly attenuated downstream effect on the levels of the corresponding proteins (*35*). The observations presented here suggest that pulsed processes, some of which are circadian-controlled, may represent a quite general biological phenomenon, whose mechanism and functional importance remain to be understood.

Northern analysis showed that the *gapcp* transcript levels are constant, indicating that the control of GAPCp synthesis occurs in *Gonyaulax* at the level of translation. Translational control of protein synthesis in *Gonyaulax* has been shown for luciferase (*9*), LBP (*8*), PCP (*21*), and RuBisCo (D. Morse, unpublished). Indeed, earlier experiments indicated that many other as yet unidentified proteins are regulated translationally (*11*).

Many different specific mechanisms for translational regulation have been identified (for recent reviews, see refs *36* and *37*). However, other than a protein that binds to the 3′ UTR of the *lbp* transcript, which is postulated to function as a repressor of translation during subjective day (*10*), little is known about how translational control is achieved in *Gonyaulax*.

The *gapcp* transcript is found in the polysome fraction at LD12, at a time when GAPCp synthesis is halted, indicating that regulation occurs at the elongation step. In this regard, it is worth noting that GAPCp, synthesized on cytoplasmic ribosomes, must be transported to the chloroplast. How this is achieved in dinoflagellates is uncertain. It is known that

such transport occurs via the ER in Euglena (*38*), and similarities between the signal peptide structures of Euglena GAPA and *Gonyaulax* GAPCp (*22*) suggest a common mode of transport to the chloroplast. Indeed, the ER itself may mediate the elongation phase of protein synthesis; microsomal membranes have been shown to relieve signal recognition particle-mediated arrest of translational elongation of secretory proteins (*39*). Elongation arrest may also contribute to the circadian regulation of synthesis of other nuclear-encoded chloroplast proteins, including peridinin-chlorophyll *a* binding protein and RuBisCO (*12*).

In fact, it is also possible that the chloroplast itself may play a role in the regulation of the cytoplasmic synthesis of chloroplast proteins. It has been shown previously that *Gonyaulax* chloroplasts migrate between the outer plasma membrane of the cell and the cell interior and that this migration is regulated by the circadian clock (*40*). Chloroplasts are closest to the center of the cell late in the subjective night (CT 23) (*40*), the time at which the rate of GAPCp synthesis is at its peak. Thus, one can speculate that the elongation of the GAPCp polypeptide is halted unless the chloroplasts are in the vicinity of the ribosomal machinery, suggesting that the chloroplasts themselves, together with the ER, may act to relieve blocked elongation.

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