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Biochemistry

Circadian expression of the luciferin-binding protein correlates with the binding of a protein to the 3′ untranslated region of its mRNA

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ABSTRACT **The circadian-expressed luciferin-binding protein from the dinoflagellate *Gonyaulax polyedra* is regulated at the translational level. We detected a protein, apparently a dimer, that binds specifically to the 3′ untranslated region of its mRNA. Its binding site was localized within a 22-nt region in the 3′ untranslated region containing seven UG repeats. The binding activity of this protein cycles on a daily basis, decreasing at the beginning of the night when synthesis of luciferin-binding protein starts and increasing at the end of the night when synthesis of luciferin-binding protein stops. This suggests that it functions as a clock-controlled repressor, preventing the translation of lbp mRNA during the day.**

Circadian rhythms are defined as biological rhythms with a period of ≈24 h that persist under constant conditions; they occur widely in eukaryotes from humans to microorganisms, including prokaryotes (1). Although the existence of endogenous biological rhythms has been known for many years, very little is understood about the clock-like mechanism and its signaling pathways controlling cellular processes. Our approach toward an understanding of circadian mechanisms has focused mainly on the regulation of gene expression by the clock.

In the unicellular marine dinoflagellate *Gonyaulax polyedra*, many cellular processes are under circadian control, including photosynthesis, cell motility, and bioluminescence (2). The present experiments concern bioluminescence, which peaks during the night. The biochemical components of the bioluminescent system in *Gonyaulax* are the enzyme luciferase, its substrate luciferin, and a luciferin-binding protein (LBP); these are localized in small (≈0.4 μm) spherical organelles called scintillons. The entire system is under circadian control (2, 3). Both luciferase and LBP reach their maximum amount during the night phase and decrease to a minimum in the day phase (4, 5). The synthesis of LBP starts at the beginning of the night. However, LBP mRNA is constant throughout the day–night cycle, indicating that LBP synthesis is controlled at the translational level (5).

Translational regulation occurs in many systems and can be mediated by a variety of mechanisms (6). In some cases it is controlled by protein factors, acting as activators or repressors that bind to the 5′ untranslated region (UTR) of a mRNA.

At first the 3′ UTR was considered irrelevant for translational control. However, during the past 2 or 3 years an increasing number of examples reveal its importance (7). Thus, regulatory proteins binding to the 3′ UTR can either initiate (c-fos mRNA) or prevent (transferrin receptor mRNA) degradation of certain mRNAs (8, 9). In mouse male germ cells, the translation of the protamine 2 mRNA is controlled by a repressor binding to its 3′ UTR (10, 11) and so is the tra-2 mRNA in *Caenorhabditis elegans*, which controls sexual identity (12).

To understand the translational control of LBP synthesis, we have cloned and sequenced its complete cDNA including the 5′ and 3′ UTRs (13) and then looked for protein factors that might bind to the 5′ and/or 3′ UTR of its mRNA. Although we did not find any proteins that bind specifically to the 5′ UTR, we discovered a protein that appears to be a dimer and binds specifically to the lbp 3′ UTR and exhibits a circadian variation in its binding activity.

MATERIALS AND METHODS

Cell Culture. *G. polyedra* 70 was grown under a 12-h light/12-h dark cycle (LD 12:12) as described (5). For some experiments, cells were maintained under constant dim light [LL; 32 microeinsteins (μE) per m^2 per sec (1 E = 1 mol of photons)]. The beginning of the light period is defined as time zero (LD 0 or LL 0), and its end is LD 12.

Preparation of Crude Extracts. Cells were harvested by vacuum filtration on Whatman 541 filters, resuspended in binding buffer [40 mM NaCl/10 mM Tris·HCl, pH 7.4/0.1 mM EDTA, pH 8.0/2 mM dithiothreitol/5% (vol/vol) glycerol], and lysed for 55 sec with zirconium beads using a Bead-beater (Biospec Products, Bartlesville, OK). Cell debris was removed by centrifugation at 13,000 × *g* for 12 min for small-scale extracts (up to 1 ml) and at 17,000 × *g* for 60 min for large-scale extracts (>1 ml).

Preparation of Plasmid Constructs. pDLA2 was constructed by PCR amplification of genomic lbp DNA with the oligonucleotides Ol 1024 and Ol 1007 (13) and blunt-end ligation of the product into the *Sma* I site of pTZ19U, which includes the T7 promotor. pMM3 was made by subcloning an *Eco*RI–*Xba* I fragment from LBP 1.1 (13) into the *Eco*RI–*Xba* I sites of pTZ18R and by subsequently deleting an *Eco*RI–*Aat* II fragment. pMM4 was constructed by BAL-31 digestion of the *Eco*RI-digested pMM3.

Preparation of RNA Transcripts. The RNAs containing various lengths of the 5′ or 3′ UTRs of lbp mRNA were transcribed from the following pTZ plasmids: pDLA2 (5′ UTR transcript: *Eae* I, 145 nt), pMM3 (3′ UTR transcripts: *Xba* I, 263 nt; *Bgl* I, 145 nt; *Hin*dIII, 93 nt), and pMM4 (3′ UTR transcript: *Bst*NI, 138 nt). Transcripts *Bgl*I+Ol-1, *Bgl*I+Ol-2, and *Bgl*I+Ol-3 were *Bgl* I transcripts to which 22-nt antisense oligonucleotides, CTGCACACAACACACACAAAGC (Ol-1), TGGTCGTCCACCCCACTTGTAA (Ol-2), and TTAAAGAGATCGAAAGGCGCAG (Ol-3), had been attached by heating the samples at 70°C for 10 min and slowly cooling them to room temperature. The location of all

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Abbreviations: LBP, luciferin-binding protein; UTR, untranslated region; LD, light/dark; LL, constant dim light; CCTR, circadian-controlled translational regulator.

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3′ UTR transcripts is shown in Fig. 1*A*. The start site of the RNAs was determined by the T7 promotor, and the ends were determined by digestion with restriction enzymes. Transcripts were prepared by following the protocols of the suppliers (Promega and Ambion). For the preparation of ^{32}P-labeled transcripts, the concentration of UTP was decreased 2.4 times to obtain a higher specificity of radioactive transcripts. Radiolabeled full-length transcripts were isolated from urea/6% polyacrylamide gels. RNAs were heated at 70°C for 10 min and cooled slowly to room temperature.

Mobility Shift Assay. Binding assays were performed by a modified procedure of Giordano *et al.* (14). *Gonyaulax* crude extract (28 μg of protein) or various amounts of further purified protein were preincubated in binding buffer containing 28 μg of poly(G) in 16 μl (unless otherwise indicated) for 20 min at 23°C. After 20 min, the ^{32}P-labeled RNA (1–1.5 × 10^4 cpm) was added and the reaction mixture was incubated for another 20 min at 23°C. RNA–protein complexes were resolved in a nondenaturing 4% polyacrylamide gel containing 10% glycerol that was electrophoresed at 150 V for ≈3 h at 23°C. For specific competition experiments, various unlabeled competitor RNAs were added with the radiolabeled RNA.

UV-Crosslinking of RNA–Protein Complexes. Binding reactions were performed as described above. After binding was completed, the samples were put on ice, exposed for 30 min to strong UV-radiation (2000 μW/cm^2), and then incubated for 15 min at 37°C with 1.5 μl of RNase T1 (≈2000 units) and 2 μl of RNase A (10 μg/μl). The reaction products were denatured and resolved on a SDS/15% polyacrylamide gel (15).

Gel Filtration. All procedures were conducted at 4°C. Solid ammonium sulfate was added to the 17,000 × *g* supernatant fraction to 60% saturation. Precipitated proteins were collected by centrifugation (1 h, 17,000 × *g*), dissolved in 80 mM NaCl/10 mM Tris·HCl, pH 7.4/0.1 mM EDTA, pH 8.0/2 mM dithiothreitol, and again centrifuged at 30,000 × *g* for 1 h. The supernatant was loaded onto a Sephadex G-75 superfine column (95 cm high and 22 cm in diameter) and run at a flow rate of 11 ml/hr. Fractions (4.5 ml) were collected and tested for their activity to bind to the lbp 3′ UTR. The column was standardized with proteins of known molecular mass, thyroglobin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B_{12} (1.35 kDa).

Northwestern Blot Experiment. Total proteins were electrophoresed on a SDS/15% polyacrylamide gel and transferred onto a poly(vinylidene difluoride) membrane (Millipore). After preincubating the membrane for 1 h in binding buffer containing 3% (wt/vol) bovine serum albumin, an RNA probe (5 × 10^5 cpm) was added in fresh buffer. After a 3-h incubation, the membrane was washed several times with binding buffer and autoradiographed.

RESULTS

A Protein Binds Specifically to a 22-nt Region of the lbp 3′ UTR. Regulatory proteins that activate or repress translation often bind to the untranslated regions of a mRNA. To investigate the translational control of the circadian-expressed LBP, we therefore looked for protein factors that bind to the 3′ and/or 5′ UTRs of the lbp mRNA.

The ^{32}P-labeled *Xba* I transcript (Fig. 1*A*) that covers the entire 3′ UTR (158 nt) was incubated with crude extracts from both the light and dark periods, and RNA–protein complexes were resolved on a nondenaturing polyacrylamide gel. One major RNA–protein complex was detected with crude extracts from both the light and dark periods in the presence of the nonspecific competitor RNA poly(G) (data not shown). By using the *Bgl* I transcript that covers only the first 119 nt of the 3′ UTR and a crude extract from the light period (LD 6), this RNA–protein complex was resolved into two bands (called bands A and B) (Fig. 1*B*, lane 2). The *Bgl* I transcript, which resulted in a better resolution on the gel, was thus used for all further experiments.

To determine whether both complexes (A and B) are derived from the specific binding of a protein to the lbp 3′ UTR, further competition assays were performed using a specific competitor (unlabeled *Bgl* I transcript) (Fig. 1*B*, lanes 3–6). A *Gonyaulax* crude extract from the light period (LD 6) was incubated in the presence of poly(G) and increasing amounts of specific competitor RNA (0, 2, 20, 200, and 400× excess) and resolved on a native polyacrylamide gel. Protein–RNA complex A was diminished with 2× excess competitor, nearly gone with 20× excess, and absent with

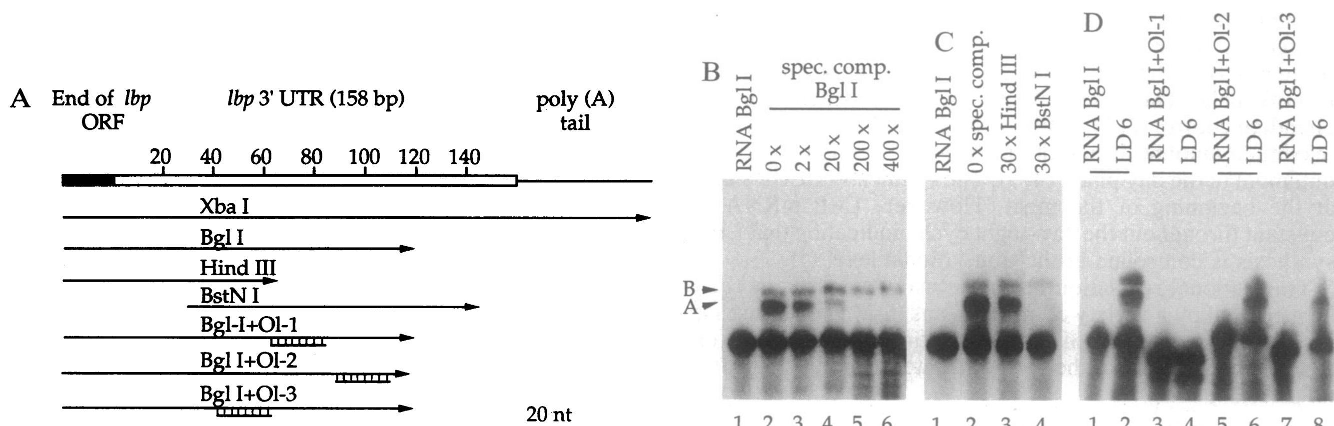

FIG. 1. Protein binds specifically to the middle of the lbp 3′ UTR. (*A*) Map of transcripts covering various parts of the lbp 3′ UTR. The ladders show 22-mer oligonucleotides (Ol-1, Ol-2, and Ol-3) hybridized to the *Bgl* I transcript. ORF, open reading frame. (*B–D*) Autoradiograms of mobility shift assays using the ^{32}P-labeled *Bgl* I transcript (*B* and *C*) or its hybrids (*D*) in the presence of poly(G) as nonspecific competitor RNA. For the binding reaction, the samples are incubated with *Gonyaulax* crude extracts from the light period (LD 6). One lane always demonstrates the mobility of the transcript alone. (*B*) Specific competitor (spec. comp.) RNA (unlabeled *Bgl* I transcript) is added in 0–400× excess to the binding reaction mixtures containing LD 6 extract (lanes 2–6), as indicated. RNA–protein complexes are called A and B. (*C*) Specific competitor RNAs covering various parts of the 3′ UTR (*Hin*dIII or *Bst*NI transcripts) are added in a 30× molar excess to the binding reaction mixtures containing LD 6 extract (lanes 3 and 4). A control without specific competitor RNA is included (lane 2). The competitor RNAs are indicated above each lane. (*D*) The hybrid transcripts *Bgl*I+Ol-1, *Bgl*I+Ol-2, and *Bgl*I+Ol-3 are used for the binding reaction mixtures containing LD 6 extract (lanes 4, 6, and 8) and a control with the *Bgl* I transcript (lane 2). Lanes 1, 3, 5, and 7 contain only the transcript indicated above the lane.

200× excess. This behavior is typical for a specific nucleic acid binding protein. In contrast, protein–RNA complex B could still be seen with a 400× excess, indicating that the binding of this particular protein to the lbp 3′ UTR was nonspecific.

To define the approximate region in the 3′ UTR of lbp mRNA that is protected by binding protein A, competition was done with transcripts covering different parts of the lbp 3′ UTR. As described above, the binding site of protein A is within the first 119 nt of the 158-nt lbp 3′ UTR, which is covered by the *Bgl* I transcript. The binding site was further localized by using unlabeled *Hin*dIII and *Bst*NI transcripts (Fig. 1*A*) as specific competitor RNAs (30× molar excess). The *Hin*dIII transcript covers the first 66 nt of the lbp 3′ UTR. It did not exhibit competition with the RNA–protein complex A (Fig. 1*C*, lane 3), in contrast to the *Bst*NI transcript, which spans the middle part of the lbp 3′ UTR (nt 30–nt 146; Fig. 1*C*, lane 4). Thus, the binding site of the protein A is localized within a 52-nt region in the middle of the lbp 3′ UTR.

The binding site was further delimited by hybridizing three 22-nt antisense oligonucleotides to the *Bgl* I transcript. One (Ol-1) covered the first 22 nt, the second (Ol-2) covered the next 22 nt of this region, and the third (Ol-3) covered the region just in front of Ol-1 (see Fig. 1*A*). The resulting transcripts were used in the presence of poly(G) in the mobility shift assay. Only the *Bgl*I+Ol-1 transcript was not able to form a complex with protein A (Fig. 1*D*, lanes 3–8). Thus, the binding site embraces a 22-nt region in the middle part of the lbp 3′ UTR with the sequence GCUUUGUGU-GUGUUGUGUGCAG.

In contrast to the results obtained with the 3′ UTR, no evidence was found for an RNA binding protein that interacts specifically with the lbp 5′ UTR. A transcript containing the 111-nt 5′ UTR (*Eae* I) was incubated with crude extracts from both the day and night periods (LD 2, 6, 10, 14, 18, and 22) in the presence of poly(G); no RNA–protein complexes could be detected (data not shown).

lbp 3′ UTR Binding Protein A Exhibits Circadian Changes in its Activity. The synthesis of LBP starts at the beginning of the night (5). By assuming that a regulatory binding protein acts as an activator or repressor of translation, one would expect that the rate of LBP synthesis would be reflected by its affinity to the lbp 3′ UTR. Cells were therefore collected at intervals throughout the light–dark cycle and immediately frozen in liquid nitrogen. Crude extracts were then prepared, incubated with the *Bgl* I transcript in the presence of poly(G), and examined in mobility shift assays.

Although the RNA–protein complex A could be detected during the entire cycle (Fig. 2*A*), its amount decreased to a low level after the beginning of the dark period (lanes 7, 12, and 13) and increased again to a high level at the end of the night (lane 3). Thus, the binding activity of protein A is low when lbp mRNA is being translated and high at the other times. The change in protein A binding was also evident with gel filtration fractions of light (LD 6) and dark (LD 13) period extracts that contained similar amounts of protein (see Fig. 4). The fractions from the light period contained much more binding activity than did the dark-period samples.

To determine whether the daily change of protein A activity is dependent only on the light–dark cycle or is driven by the circadian clock, cells were grown under constant dim light (LL) and crude extracts were prepared from various time points. During subjective day (lanes 1, 2, 6, and 8–10), RNA–protein complex A was present at a relatively high level (Fig. 2*B*). It decreased at the beginning of subjective night (lanes 3 and 10) and remained at this low level until the end of the night phase. Thus, the daily change of activity of the binding protein A occurs both in cells grown under LD and LL, showing that the activity change of protein A is regulated by the circadian clock.

The nonspecific RNA–protein complex B also cycles with a maximum during the day. The possible relevance of this finding has not been investigated.

lbp 3′ UTR Binding Protein Appears to Be a Dimer. To characterize the protein component of the RNA–protein complex A, we used UV-crosslinking to covalently bind the protein to RNA. The ^{32}P-labeled *Bgl* I transcript was incubated with a light-period crude extract (LD 5), UV-irradiated, RNase-treated, and then resolved on a denaturing polyacrylamide gel (Fig. 3). In the presence of poly(G), an ≈25-kDa RNA–protein complex appeared (lane 3). This complex was not detectable without UV treatment (lane 1) or when the sample was preincubated with proteinase K (data not shown). Even when poly(G) was increased from 700× (lane 3) to 900× (lane 4) excess, the 25-kDa RNA–protein complex was still present, but when 200× specific competitor was added, it was absent (lane 5). This indicates that the UV-crosslinked protein represents the specific lbp 3′ UTR binding protein A. The same results were obtained with a dark-period extract (LD 13) (data not shown). Since the 25-kDa RNA–protein complex contains the piece of RNA that is protected by the binding protein A (a contribution of as much as ≈7 kDa), the molecular mass of the binding protein A itself is in the range of 18–25 kDa under denaturing conditions.

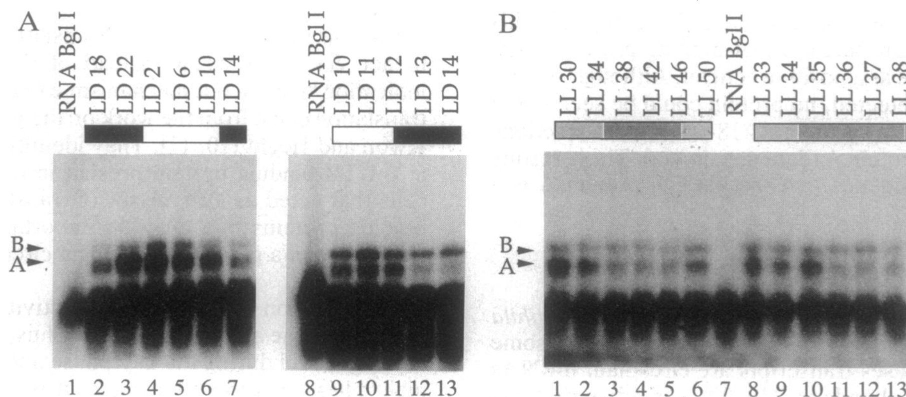

FIG. 2. Circadian changes in binding activity of protein A. Autoradiograms of mobility shift assays using the ^{32}P-labeled *Bgl* I transcript (Fig. 1*A*) in the presence of poly(G). One lane always demonstrates the mobility of the transcript alone (RNA *Bgl* I). (*A*) Crude extracts were prepared at the times indicated from cells grown in a 12-h light (open bar)/12-h dark (solid bar) cycle. Lanes: 2–7, 4-h intervals covering the entire light/dark cycle; 9–13, 1-h intervals covering the light/dark transition when translation of lbp mRNA starts. (*B*) Crude extracts were prepared at the times indicated from cells growing in constant dim light (LL: stippled). Times are given in hours after lights were switched on. Subjective day phase, less-dense stippling; subjective night phase, dense stippling. The 4-h intervals cover the entire day/night cycle (lanes 1–6); 1-h intervals are shown at the time spanning the subjective day/night switch (lanes 8–13), when translation of LBP starts.

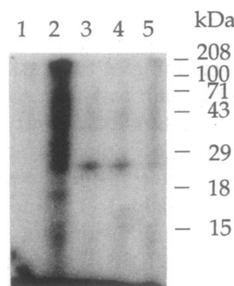

FIG. 3. Molecular mass of protein A under denaturing conditions is ≈25 kDa including the RNA binding site. The autoradiogram shows a UV-crosslinking experiment using ^{32}P-labeled *Bgl* I transcript with a *Gonyaulax* crude extract from the light period (LD 5) and poly(G) as nonspecific competitor RNA (lane 3). All samples were UV-irradiated (except for the negative control; lane 1), RNase-treated, and then electrophoresed on a denaturing SDS/15% polyacrylamide gel along with a prestained molecular mass marker. In lane 2, poly(G) was omitted; lane 4 shows the general binding reaction with 200× excess additional nonspecific competitor, and lane 5 shows a 200× excess specific competitor (the unlabeled *Bgl* I transcript).

The molecular mass of protein A was also estimated under native conditions using gel filtration. Proteins from two *Gonyaulax* crude extracts (LD 6 and LD 13) were precipitated by 60% ammonium sulfate. In both cases, only the specific binding protein A was precipitated, while the nonspecific one (protein B) remained in the supernatant. The two preparations were then subjected to gel filtration (GP75) calibrated with proteins of known molecular mass. The fractions were tested for binding the lbp 3′ UTR (*Bgl* I transcript) in the mobility shift assay. Activity, which was estimated by the amount of the *Bgl* I transcript that was shifted, peaked in fractions that contained proteins of 40–45 kDa in both light- and dark-period extracts (Fig. 4). As mentioned above, there is much more activity in the light-period extracts. The fact that the molecular mass of the binding protein was found to be 40–45 kDa under native conditions but 18–25 kDa under denaturing conditions (UV-crosslinking) suggests that the binding protein is a dimer (or possibly a multimer).

To determine whether the binding of protein A to the 3′ UTR of the lbp mRNA is dependent on its putative dimeric form, we performed a Northwestern blot analysis using the *Bgl* I transcript. For a dimer, the subunits would be expected to separate on a denaturing gel and, therefore, would not react in the Northwestern blot. We omitted poly(G) in this experiment so that nonspecific RNA binding proteins were able to react and serve as a positive control. Although several such proteins were detected, no protein could be seen in the molecular mass range of protein A (18–25 kDa) in fractions enriched in binding protein A (data not shown). These results provide additional evidence that the binding protein A is a dimer.

DISCUSSION

Genes affecting circadian period, such as *per* in *Drosophila* (16, 17) and *frq* in *Neurospora* (18), were discovered some years ago. In these cases transcripts are circadian: mRNAs exhibit 24-h cycles of abundance (19, 20). Circadian rhythms in the synthesis of mRNAs have also been reported in other systems, including cyanobacteria (1) and higher plants (21, 22).

For *Gonyaulax*, Northern blots have shown that the level of lbp mRNA remains constant over the course of the circadian bioluminescent cycle as the amount of bioluminescence proteins changes with time. Since pulse-labeling ex-

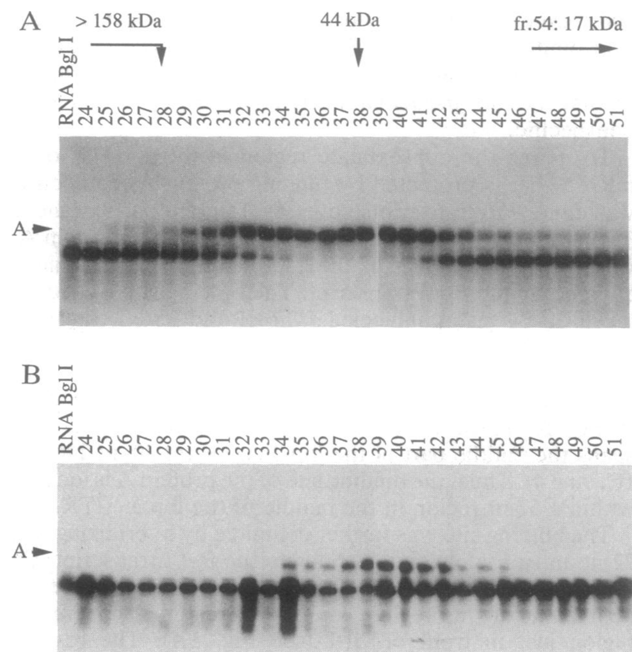

FIG. 4. Native molecular mass of protein A is 40–45 kDa. Autoradiograms of mobility shift assays using the ^{32}P-labeled *Bgl* I transcript (Fig. 1*A*) in the presence of poly(G) are shown. The lanes labeled RNA *Bgl* I demonstrate the mobility of the transcript alone. *Gonyaulax* crude extracts from the light (LD 6) (*A*) or dark (LD 13) (*B*) period were treated with 60% ammonium sulfate. Precipitated proteins were redissolved and separated on a gel filtration column standardized with proteins of known molecular mass. The fractions (20 μl per fraction; fractions 24–51, see above) were tested for binding to the 3′ UTR.

periments revealed that the rate of synthesis of LBP oscillates, it was concluded (5) that the circadian control of its expression occurs at the level of translation. The present experiments suggest that crude extracts of *Gonyaulax* cells contain a circadian-controlled translational regulator (CCTR) that binds specifically within a 22-nt region of the lbp 3′ UTR.

The amount of binding of CCTR exhibits a circadian change, which persists in cells grown under constant dim light. It decreases just when synthesis of LBP starts (beginning of the night phase) and increases again when it stops (end of the night phase), suggesting that the CCTR acts as a repressor, inhibiting translation of lbp mRNA during the day phase.

It seems rather unusual for a translational repressor protein to act via the 3′ UTR instead of the 5′ UTR. The first indication that such a mechanism exists in the regulation of translation came from the work on the protamine 2 mRNA by Kwon and Hecht (10, 11). They identified and characterized a 3′ UTR binding protein present in developing male germ cells that acted as a repressor of translation. Thus, 3′ UTR binding proteins may play an important role in repressing translation at a given time during a circadian cycle or during development.

The variation in CCTR binding activity could be caused by a change in the amount of CCTR. Thus, its amount would be at a high level during the day phase and at a low level in the night phase. On the other hand, it is also possible that the amount of CCTR remains constant in terms of protein throughout the circadian cycle and that the change in binding activity is caused by a modification of CCTR itself. For example, this might involve phosphorylation and dephosphorylation of protein, which is well known as a switch mechanism for several translation and transcription factors (23, 24). Inhibition of either protein kinases or phosphatases

has a large effect on the period and/or phase of the bioluminescence rhythm (29), suggesting a role for phosphorylation in the circadian rhythm of bioluminescence.

The specificity of the binding of CCTR to the 3′ UTR of the lbp mRNA is shown by the absence of its signal after addition of specific competitor RNA (unlabeled 3′ UTR transcript) and by the retention of its activity in the presence of nonspecific competitor RNA. Based on the effects of specific competitors representing different parts of the 3′ UTR and by the use of antisense oligonucleotides, we narrowed the binding site of CCTR to 22 nt located in the middle of the 3′ UTR. The sequence of this region, GCUUUGUGUGUGUUG-UGUGCAG, is rather unusual in regard to the occurrence of seven U(U)G repeats. A search in the EMBL databank (October 29, 1993) using the underlined sequence revealed only a few completely homologous sequences. In nearly all cases they were found to be in an untranslated region or an intron. We cannot yet be sure whether this sequence is involved in translational control in other organisms or of other *Gonyaulax* genes.

The binding of a regulatory protein to RNA can be dependent on both sequence and secondary structure, as, for the human ferritin mRNA, for example, in which a repressor protein was found to bind to a hairpin-loop structure called iron responsive element situated in the 5′ UTR (25). We looked for stable secondary structures that may be present in the middle of the lbp 3′ UTR by using an RNA folding program (26). This predicts several hairpin-loop structures with a total stability of $\Delta G = -87.9$ kcal/mol (1 cal = 4.184 J) (Fig. 5). The binding site embraces a 14-nt loop. By using the KNOTFOLD program of A. Gultayaev and D. E. Draper (Johns Hopkins University), five pseudoknots were predicted within the 3′ UTR. It will be of interest to verify the secondary structure and its importance for binding.

Under native conditions, CCTR was estimated by gel filtration to have a molecular mass of 40–45 kDa. However, under denaturing conditions a molecular mass of ≈25 kDa was determined. This involved a UV-crosslinking experiment in which a protein–RNA bond is formed, so the molecular mass includes the piece of RNA that is protected from RNase digestion. Subtracting the molecular mass of the bound RNA (up to 22 nt), the protein is in the range of 18–25 kDa. These data suggest that CCTR may be a dimer although there is also a possibility of a multimer. This postulate is supported by the fact that, as expected for a dimer, CCTR does not react in a Northwestern blot. Dimeric formation of RNA binding proteins is known and can be relevant for binding. Thus, the U small nuclear ribonucleoprotein, U2B″, shows specific high-affinity binding only in the presence of the U2A′ protein, with which it forms a heterodimer in solution (27). For the signal recognition particle (SRP), two proteins, SRP9 and SRP14, form a heterodimer in the absence of SRP RNA to which they bind (28).

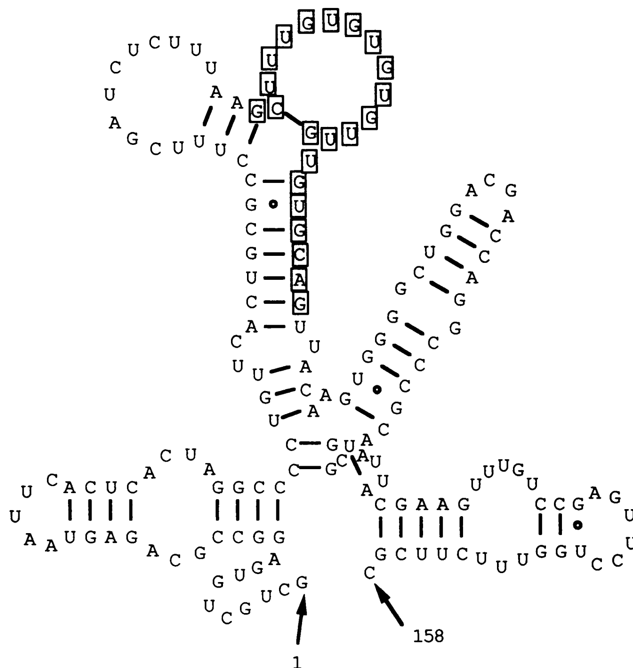

FIG. 5. Theoretical secondary structure of the lbp 3′ UTR. RNA folding of the entire lbp 3′ UTR (nt 1–158) was predicted by the computer program created by Abrahams *et al.* (26). The binding site of CCTR is located within 22 nt (boxed) of the middle of the 3′ UTR.

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