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Circadian regulation of bioluminescence in *Gonyaulax* involves translational control

(dinoflagellate/luciferin binding protein cDNA)

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ABSTRACT **A 10-fold circadian variation in the amount of luciferin binding protein (LBP) in the marine dinoflagellate *Gonyaulax polyedra* is reported. This protein binds and stabilizes luciferin, the bioluminescence substrate. In early night phase, when bioluminescence is increasing and LBP levels are rising in the cell, pulse labeling experiments show that LBP is being rapidly synthesized *in vivo*. At other times, the rate of LBP synthesis is at least 50 times lower, while the rate of synthesis of most other proteins remains the same. The LBP mRNA levels, as determined by *in vitro* translations and by RNA (Northern) hybridizations, do not vary over the daily cycle, indicating that circadian control of bioluminescence in this species is mediated by translation.**

In the unicellular marine alga *Gonyaulax polyedra*, many properties of the cells change from day to night, representing, in effect, a cyclic daily differentiation. Such circadian (daily) rhythms continue under constant conditions (e.g., light and temperature) and occur widely in eukaryotes from humans to microorganisms (1). Although believed to be of fundamental importance in the control of diverse processes, neither the biochemical components nor the cellular organization of the underlying oscillatory mechanism is known in any system.

Bioluminescence in *G. polyedra* is a circadian controlled process, occurring predominately during the organism's night phase (2). The cellular capacity for bioluminescence is directly correlated with the presence of the biochemical components involved in the *in vitro* light-producing reaction: dinoflagellate luciferin, a fluorescent oxidizable substrate noncovalently bound to a luciferin binding protein (LBP), and an enzyme (luciferase) catalyzing the light-producing reaction. Both the luciferin and the luciferase have been shown to undergo circadian variation in amounts with 10-fold higher concentrations of both present in the middle of the subjective night (3–6).

Gonyaulax is the only system known to date in which a circadian controlled process—namely, bioluminescence—has been shown to correlate with both changes in the activity and absolute levels of a protein—namely, the luciferase (4, 5). We have extended these observations to LBP with two important additions. First, we show that the increase in LBP levels results from a transient increase in the rate of synthesis of the protein and, second, that these changes in synthetic rate are apparently due to a translational control mechanism.

MATERIALS AND METHODS

Cell Culture. *G. polyedra* was grown in f/2 medium under a 12-hr light (150 microeinsteins·m^{-2}·sec^{-1})/12-hr dark (LD) cycle to a cell density of ≈8000 cells per ml (4, 7) and, for many experiments, was maintained under constant dim light (LL; 15 microeinsteins·m^{-2}·sec^{-1}). The beginning of the light period is defined as time zero. For biochemical analysis, cells were harvested by centrifugation at 3000 × *g* (2 min); cells for *in vivo* labeling and RNA preparations were isolated by filtration on 25-μm Nitex (8) and Whatman 541 filters, respectively.

LBP Purification and Anti-LBP. LPB, assayed by its ability to bind and release luciferin as a function of pH (9, 10), was purified with a 30% yield from night phase cells by a procedure involving ammonium sulfate precipitation, DEAE chromatography, gel filtration, and chromatography on hydroxyapatite, and it was estimated to be ≈95% pure by $NaDodSO_4$/PAGE (band at 72 kDa). Rabbit anti-LBP was isolated after a 1-mg sensitizing injection and two 3-mg booster injections of the purified protein emulsified in Freund's complete adjuvant.

$NaDodSO_4$/PAGE. After $NaDodSO_4$/PAGE (11), gels were stained with Coomassie blue, dried, and autoradiographed with Kodak X-AR Omat film at −70°C. Alternatively, gels were transferred electrophoretically to nitrocellulose (12) and stained with anti-LBP. Protein A labeled with ^{125}I (New England Nuclear) and autoradiography was used for detection. Molecular mass markers (Sigma) were as follows: myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; egg albumin, 45 kDa; carbonic anhydrase, 29 kDa.

***In Vivo* Labeling.** Fifty milliliters of cell culture (≈2.5×10^5 cells) was concentrated to 4 ml by filtration on a Nitex membrane and incubated for 20 min with 150 μCi of [^{35}S]methionine (800 mCi/mmol; 1 Ci = 37 GBq; New England Nuclear) and 100 μM chloramphenicol (13) under dim light. After pelleting, cells were washed twice in f/2 medium, resuspended in 100 μl of 100 mM Tris·HCl (pH 8.5) containing 1 mM EDTA, and lysed by sonication. The sample, with 50 μl of sample buffer added (11), was placed in a boiling water bath for 5 min, clarified by centrifugation, and electrophoresed.

RNA Purification and LBP cDNA Cloning. After filtration, cells were resuspended in 50 mM Tris·HCl (pH 9) containing 25 mM EDTA, 25 mM EGTA, 4 M guanidine isothiocyanate, 2% sodium lauryl sarcosine, and 10 mM 2-mercaptoethanol, broken in a cell disruption bomb (Parr Instruments, Moline, IL), and extracted four times with phenol/chloroform (1:1). Nucleic acids were precipitated with 0.2 M ammonium acetate and 70% ethanol at −70°C. The pellet was dissolved in water, and the RNA was precipitated by an overnight incubation with 2 M LiCl at 0°C. Poly(A) RNA was obtained by chromatography of the RNA on an oligo(dT)-cellulose column (Collaborative Research) (14).

The cDNA library was prepared from 10 μg of poly(A) RNA (isolated at CT 14) using 4 μg of the pARC7 vector

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Abbreviations: LBP, luciferin binding protein; LD, 12 hr light/12 hr dark; LL, constant dim light.

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primer system (15). After incubation with 114 units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim) for 90 min at 42°C (14), the reaction mixture was extracted with phenol/chloroform and the nucleic acids were precipitated with ammonium acetate and ethanol. The precipitate was redissolved in 10 μl of water and dG-tailed using terminal deoxynucleotide transferase (14). The protocol of Alexander *et al.* (15) was used to prepare and anneal a dC-tailed linker and to remove the RNA and synthesize the second DNA strand. The cDNA library was amplified by transformation into *Escherichia coli* MM294; cells were allowed to grow overnight on a selective medium and the plasmids were isolated and purified on a CsCl gradient (14).

The plasmid cDNA library (2.5 μg of DNA) was subcloned into the *Sst* I site of the λ phage expression vector Charon 16 (10 μg of DNA) for immunological screening (16). The phage *Sst* I site is located in the 5′ end of the β-galactosidase coding sequence, so that after ligation the cDNA insert is immediately downstream from the β-galactosidase promoter. The recombinant phage were packaged *in vitro* with Packagene (Promega Biotec) and used to infect *E. coli* BNN 45 cells. The infected cells were grown overnight in NZ top agarose spread on 135-mm plates at a density of ≈50,000 plaques per plate (14).

The plates were chilled at 4°C for 1 hr before being overlaid with dry nitrocellulose filters. The filters were keyed to the plates and, after several minutes, lifted and washed at 37°C in Tris-buffered saline (TBS; 30 min), TBS with 2% nonfat dry milk (2% DM; 30 min), 1/100 anti-LBP in TBS + 2% DM (60 min), TBS (five times, 10 min each), ^{125}I-labeled protein A (0.5 μCi per filter; New England Nuclear) in TBS (60 min), and TBS (five times, 10 min each). The filters were then dried and exposed to Kodak X-AR Omat film at −70°C with an intensifying screen. The positions corresponding to areas of positive signals were picked, replated, and rescreened with anti-LBP. This last procedure was repeated until only phage producing immunoreactive protein remained on the plate.

The cDNA-containing plasmid was excised from a phage DNA preparation (14) by digestion with *Sst* I, religated, and used to transform *E. coli* BNN 45. The plasmid was purified (14) and the cDNA was excised by digestion with *Sma* I and analyzed by agarose gel electrophoresis and Southern blotting (17). ^{32}P-labeled probes were prepared by the random primer method (18) and hybridized by the method of Church and Gilbert (19).

***In Vitro* Translation.** One microgram of poly(A) RNA, or mRNA obtained by hybrid selection (14) with the LBP cDNA, was translated in rabbit reticulocyte lysates (Bethesda Research Laboratories) (20) with 50 μCi of [^{35}S]-methionine (New England Nuclear). Protein products were analyzed by NaDodSO$_4$/PAGE and autoradiography.

Northern Hybridization. RNA was electrophoresed on a 1.2% agarose gel containing 2.2 M formaldehyde, transferred to Hybond nylon membranes (14, 17), and incubated with a ^{32}P-labeled probe prepared from the LBP cDNA (18, 19).

RESULTS

There Is a Circadian Rhythm in Cellular Levels of LBP. Sulzman *et al.* (9) reported a circadian rhythm in the binding capacity of LBP. Fig. 1 shows that these changes are due to variations in the absolute amounts of protein, as previously shown for luciferase (4, 5). In LD$_1$ the highest amounts of immunologically reactive protein are found during the night (Fig. 1*A*), and similar changes occur in LL (Fig. 1*B*). This shows that differences in amounts of protein are not directly related to changes in the environmental light conditions as observed in some systems (21, 22).

There is no apparent trivial explanation for these changes. For example, the possibility that LBP is sequestered in some cell compartment during the day, so that extraction of the protein becomes much more difficult, seems to be excluded. The samples shown in Fig. 1 represent protein that was extracted at 100°C with NaDodSO$_4$ (11), a procedure that should open up and solubilize the contents of all subcellular compartments.

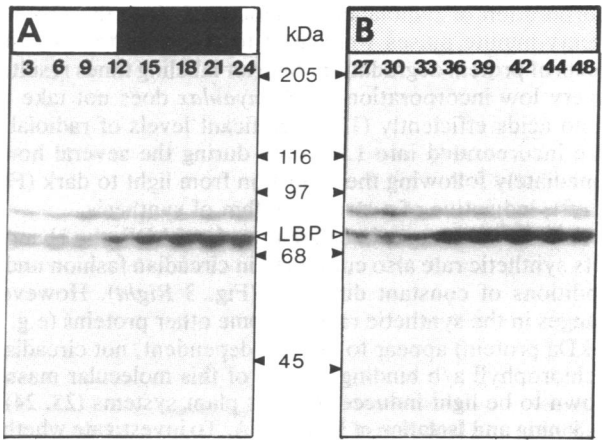

FIG. 1. Western blot analysis of circadian changes in anti-LBP reactive protein. Protein was isolated at 3-hr intervals from cells growing in either LD (*A*) (light, white; dark, black) or LL (*B*) (stippled) at the indicated times after lights on. Each lane contains an extract from 10^5 cells electrophoresed on a NaDodSO$_4$/polyacrylamide gel, blotted electrophoretically onto nitrocellulose, and probed with anti-LBP. The antibody, as visualized by reaction with ^{125}I-labeled protein A and autoradiography for 90 min, recognizes primarily the LBP (72 kDa) but also unidentified contaminants (85 and 70 kDa).

As shown in Fig. 2, the LBP (72 kDa) is a major protein component of the cell, and thus changes in LBP levels can be visualized directly by Coomassie blue staining. This figure also illustrates that most of the other major proteins of the cell do not appear to undergo circadian variations in amounts.

The Rate of LBP Synthesis Is Under Circadian Control. The circadian rhythm in the amounts of LBP could be due to changes in rates of synthesis, rates of degradation, or both. LBP levels do drop sharply at the end of the night phase, suggesting that circadian changes in the degradation rate occur, but no studies of this aspect have been completed.

We examined synthetic rates at different times over a 24-hr period, as these can be conveniently estimated by the *in vivo*

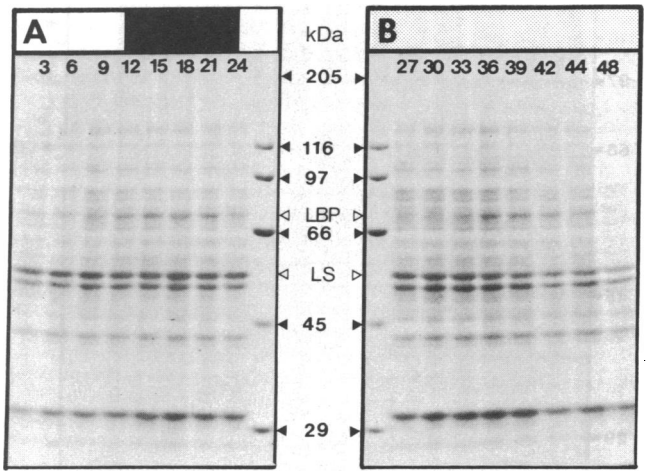

FIG. 2. Circadian changes in LBP visualized by Coomassie blue staining after NaDodSO$_4$/PAGE. Protein samples were obtained as described in Fig. 1; the positions of LBP and the large subunit of ribulose bisphosphate carboxylase (LS) are marked.

incorporation of radiolabeled amino acids into the protein. Twenty-minute pulses were used to minimize the possible effects of protein degradation. Shorter labeling times resulted in very low incorporations as *Gonyaulax* does not take up amino acids efficiently (13). Significant levels of radiolabel were incorporated into LBP only during the several hours immediately following the transition from light to dark (Fig. 3 *Left*), indicative of a strong rhythm of synthesis.

As with the changes in cellular levels of LBP, the changes in its synthetic rate also continued in circadian fashion under conditions of constant dim light (Fig. 3 *Right*). However, changes in the synthetic rates of some other proteins (e.g., a 32-kDa protein) appear to be light dependent, not circadian. A chlorophyll a/b binding protein of this molecular mass is known to be light induced in other plant systems (23, 24).

Cloning and Isolation of LBP cDNA. To investigate whether or not changes in the rates of LBP synthesis were attributable to differences in LBP mRNA levels, we first isolated a LBP cDNA from a cDNA library constructed in the pARC7 vector of Alexander *et al.* (15) and subcloned into the λ phage expression vector Charon 16. Two positive signals were identified by immunological screening, and the phages responsible were isolated. Both contained the same 1.1-kilobase (kb) cDNA insert, based on cross-hybridization in Southern blot analysis. The identity of the cDNA insert as corresponding to LBP was established by hybrid selection; *in vitro* translation of the mRNA specifically bound by the cDNA produced only one product, a 72-kDa protein immunoreactive with anti-LBP (Fig. 4).

The Rate of LBP Synthesis Is Not Controlled by the Amount of Its mRNA. With poly(A) RNA extracted from cells at different circadian times, there were no significant fluctuations in the amounts of LBP mRNA, as measured by Northern hybridizations with ^{32}P-labeled LBP cDNA as a probe (Fig. 5). Only one major RNA species was observed, and its size (2.5 kb) was slightly larger than required for a protein the size of the LBP monomer (72 kDa; ≈2 kb). Total cellular RNA, when analyzed in the same way, gave the same

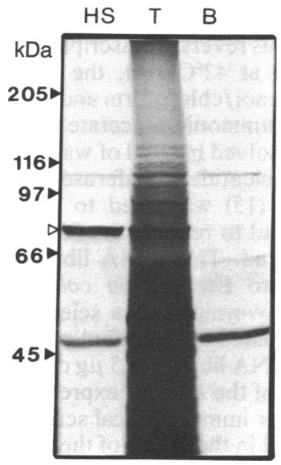

FIG. 4. *In vitro* translation in a rabbit reticulocyte lysate of mRNA hybrid selected by the LBP cDNA (lane HS), total poly(A) RNA (lane T), and a blank (in the absence of added message) (lane B). The protein products were electrophoresed on NaDodSO$_4$/polyacrylamide gel and autoradiographed.

results except that 8- to 10-fold higher amounts were required to give a band of the same intensity with the same probe and exposure times. There is no indication of a difference in size between the LBP mRNAs at the different time points and no indication that high molecular weight forms of the message (i.e., heterogenous nuclear RNA) may be present.

The observation that the levels of LBP mRNA remained constant over a 24-hr period, whereas the *in vivo* rate of LBP protein synthesis exhibited dramatic circadian changes against a background of otherwise constant cellular protein synthesis, strongly suggests some sort of specific translational control. One explanation could be that the mRNA observed on the Northern analyses, although the same in amounts, contained structural differences that altered its ability to be translated at different times. However, when the RNA samples isolated at

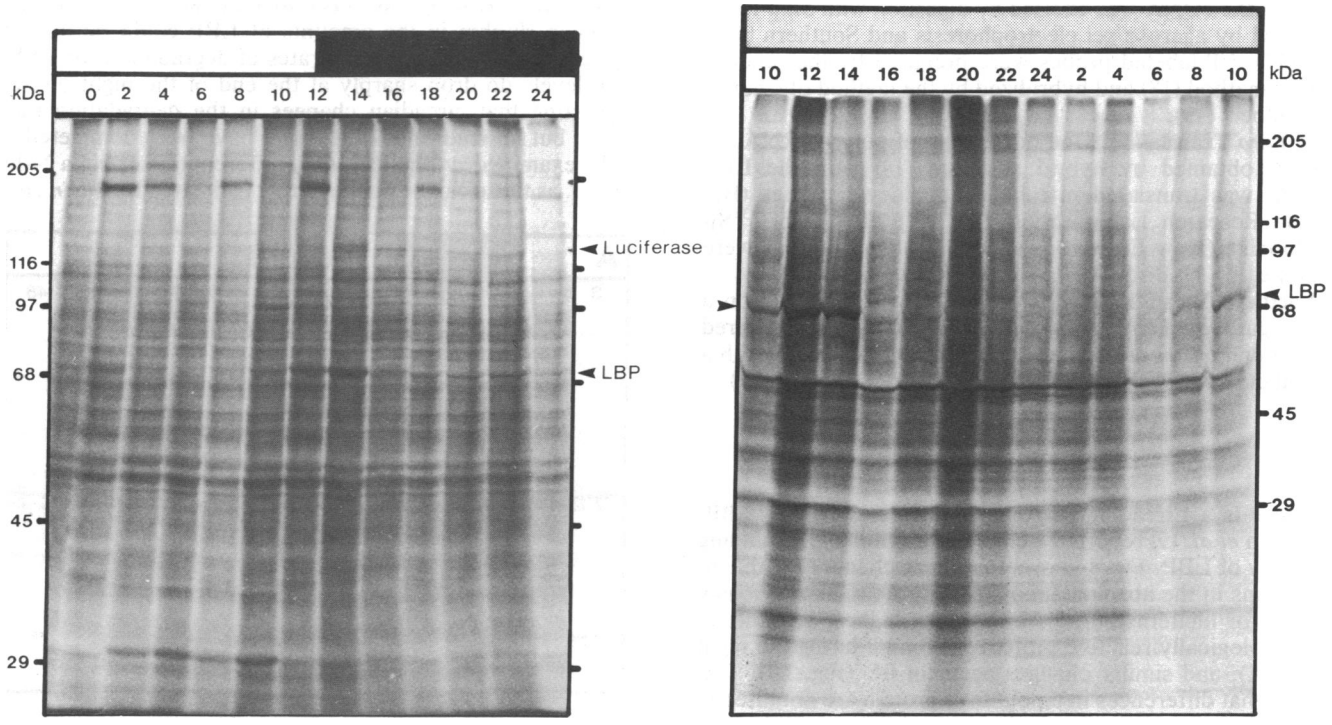

FIG. 3. *In vivo* pulse labeling of LBP. About 2.5 × 10^5 cells grown under LD (*Left*) or LL (*Right*) were pulse-labeled for 20 min every 2 hr with 150 μCi of [^{35}S]methionine in 4 ml of f/2 medium containing 100 μM chloramphenicol. Extracted proteins were subjected to NaDodSO$_4$/PAGE and autoradiographed for 4 days. The sampling hours under constant light are given as circadian time.

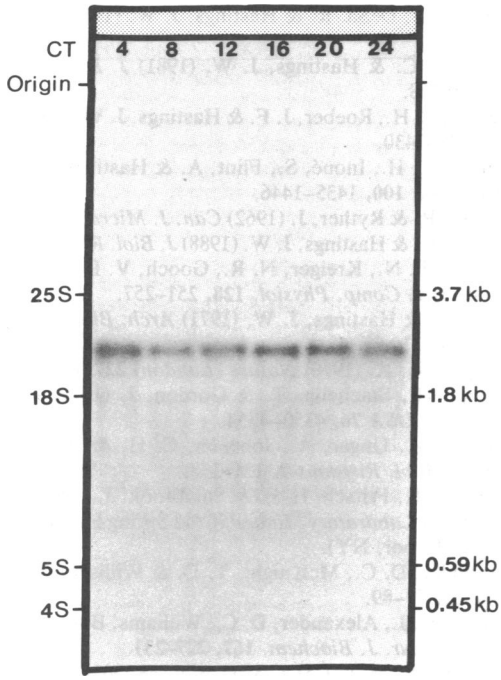

FIG. 5. Northern hybridizations with the LBP cDNA. Samples of poly(A) RNA (5 μg), purified from cells grown in LL and harvested at the times indicated, were electrophoresed on a 1.2% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to a nylon Hybond membrane, which was probed with a ^{32}P-labeled LBP cDNA and autoradiographed for 90 min.

different circadian times were translated in rabbit reticulocyte lysates, the amounts of LBP synthesized were the same (Fig. 6). Thus, a heterologous translational machinery does not recognize any qualitative differences in the mRNAs isolated at the different time points.

DISCUSSION

The nature of the cellular oscillatory mechanism responsible for the generation of circadian rhythms is unknown. However, the present study provides two important findings relating to the mechanism whereby the oscillator controls an observable rhythm. First, we demonstrate a circadian variation in the synthetic rate of LBP, a protein involved in the rhythmic bioluminescence of *G. polyedra*. Circadian changes in the synthetic rates of individual proteins have been detected by pulse labeling in other systems (25, 26), but, unlike LBP, their levels have not been shown to change over the circadian cycle, nor are their cellular functions defined. Second, we show that the control of this rhythmic synthesis is at the translational rather than the transcriptional level. A similar conclusion was reached in studies of daily variations of a rat liver sterol carrier protein, but the experiment was not performed under constant light (21).

Our findings, summarized in Fig. 7, show that the changes in LBP synthetic rates account very well for the rise and subsequent plateau of both bioluminescence and LBP levels. Despite the use of 20-min pulses, which may seem somewhat long, we believe that our results accurately reflect changes in synthetic rates, uncomplicated by possible protease activity. LBP levels remain high for 6 hr after synthesis has been shut off, so termination of synthesis can therefore not be attributed to protease activity. At the end of the 6-hr plateau period, however, both luminescence and LBP levels drop precipitously, suggesting that a protease specific for LBP does exist and that its activity is also rhythmic. Evidence for the existence of a protease whose activity is greater at one

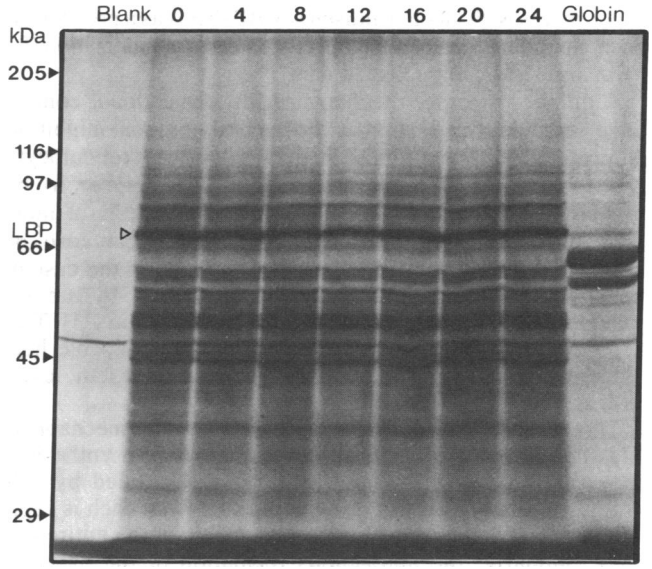

FIG. 6. *In vitro* translation in a rabbit reticulocyte lysate of 1 μg of each poly(A) RNA preparation isolated for the gel shown in Fig. 5. The products were analyzed by NaDodSO$_4$/PAGE and autoradiography. The positions of the LBP monomer and molecular mass markers are shown.

time than another is found in studies of cyclin in sea urchin eggs (27).

Independent of the time of extraction, the LBP mRNA is present in the same amount, has the same size, and is translated equally well in a heterologous translation system. This excludes both transcriptional control and posttranscriptional modification as possible mechanisms. Concurrent studies of *Gonyaulax* luciferase suggest that its mRNA also remains constant in the face of a clear circadian rhythm in its cellular levels. Thus, the same translational control mechanism may regulate the circadian synthesis of both proteins.

Translational control is now recognized as being of major importance in the expression of genetic material (28, 29). Such control might be exerted simply by cellular sequestration of message, as is the case for the *veg-1* gene in the developing frog oocyte (30). In *Gonyaulax*, preliminary *in situ* hybridization

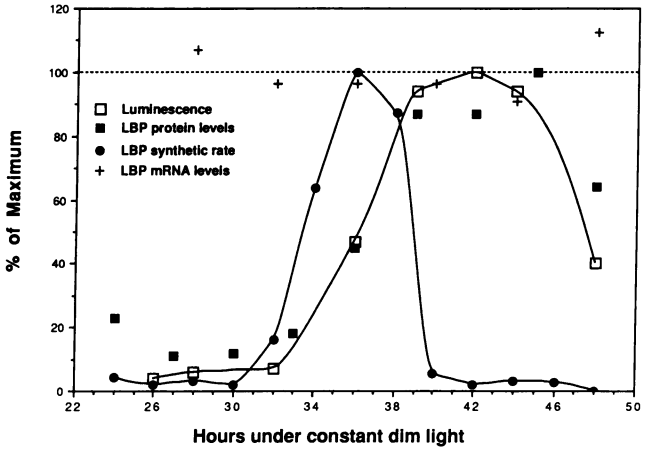

FIG. 7. Densitometric scans of Western blots (■, LBP levels), Northern blots (+, LBP mRNA levels), and pulse labeling autoradiographs (●, LBP synthetic rates) plotted with the bioluminescent capacity of cells in LL (2). Values were normalized relative to the band at 85 kDa in Western blots (Fig. 1) and the band at 55 kDa in pulse labeling (Fig. 3). RNA levels are taken from Fig. 5 without correction. The rates of LBP synthesis peak prior to LBP and bioluminescence, which rise concurrently to a maximum and remain high for ≈6 hr, while LBP mRNA remains constant.

experiments with labeled antisense LBP–RNA as a probe have given no indications of such mRNA compartmentalization (L. Fritz and D.M., unpublished data).

A number of other mechanisms for translational control have been identified, such as the general or global inhibition of protein synthesis found in hemin-deprived reticulocyte lysates (29). Such a mechanism does not appear to be involved in circadian control, since the synthesis of most proteins is constant over time. Translation can also be controlled by mRNA secondary structure, as in the case of negative retroregulation of RNA degradation by sib sequences in expression of λ phage integrase protein (31). This type of mechanism also appears not to be applicable here since the *in vitro* translation of mRNA shows constant levels of translated LBP (Fig. 6).

Direct feedback inhibition represents another mechanism, illustrated by the regulation of ribosomal protein synthesis in *E. coli*. The >50 ribosomal proteins are encoded by ≈20 transcriptional units; one protein product from each is capable of specifically inhibiting the translation of the entire unit (32). Similarly, the autogenous regulation of the bacteriophage T4 gene 32 protein (33) reflects the ability of feedback repression to maintain a constant level of free protein in the cell. In its straightforward form, this homeostatic type of mechanism would not appear to be involved in circadian control, since low levels of LBP are not followed by an immediate increase in LBP synthesis. Although feedback from the protein product to the translational machinery has not been rigorously excluded for LBP, feedback to the LBP gene itself cannot be accommodated by our results.

A more reasonable mechanism for regulation of circadian protein synthesis would involve the action of factors able to exert control over the translation of message but that are not direct products of that translation. This type of mechanism is exemplified by translational control of heat shock messages in *Drosophila*, where sequences, located in the 5′ untranslated region of the message under control, are recognized by factors that are products of other messages (34, 35). Such sequences are also involved in translational control of ferritin (36) and cytochrome *c* oxidase mRNAs (37, 38). Protein factors mediate control in both cases (38, 39).

If protein factors of this type are responsible for circadian translational regulation, then it is temping to speculate that they might also be part of the actual cellular clock mechanism. A "translational" clock would be expected to be especially susceptible to disruption by inhibitors of protein synthesis, as has been found in many different systems (40–44). It could involve a closed loop of many different regulatory proteins, each one of which controls the synthesis of the next in the series (the "clock" proteins); but each would also control the synthesis of certain cellular proteins involved in overt rhythms ("hands" of the clock, such as LBP). It seems plausible that any mRNA species whose translation is regulated by such factors should be identifiable by sequences in its 5′ untranslated region. The identification of these postulated regulatory sequences in these mRNAs and the factors that bind to them and control their translation may thus provide a first look at the molecules of the circadian clock.

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1. Johnson, C. H. & Hastings, J. W. (1986) *Am. Sci.* **74,** 29–36.
2. Hastings, J. W. & Sweeney, B. M. (1958) *Biol. Bull.* **115,** 440–458.
3. Bode, V. C., DeSa, R. & Hastings, J. W. (1963) *Science* **141,** 913–915
4. Dunlap, J. C. & Hastings, J. W. (1981) *J. Biol. Chem.* **256,** 10509–10518.
5. Johnson, C. H., Roeber, J. F. & Hastings, J. W. (1984) *Science* **223,** 1428–1430.
6. Johnson, C. H., Inoué, S., Flint, A. & Hastings, J. W. (1985) *J. Cell Biol.* **100,** 1435–1446.
7. Guillard, R. & Ryther, J. (1962) *Can. J. Microbiol.* **8,** 229–239.
8. Homma, K. & Hastings, J. W. (1988) *J. Biol. Rhythms* **3,** 49–58.
9. Sulzman, F. N., Kreiger, N. R., Gooch, V. D. & Hastings, J. W. (1978) *J. Comp. Physiol.* **128,** 251–257.
10. Fogel, M. & Hastings, J. W. (1971) *Arch. Biochem. Biophys.* **142,** 310–321.
11. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
12. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
13. Olesiak, W., Ungar, A., Johnson, C. H. & Hastings, J. W. (1987) *J. Biol. Rhythms* **2,** 121–138.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Alexander, D. C., McKnight, T. D. & Williams, B. G. (1984) *Gene* **31,** 79–89.
16. Schnell, D. J., Alexander, D. C., Williams, B. G. & Etzler, M. E. (1987) *Eur. J. Biochem.* **167,** 227–231.
17. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
18. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
19. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
20. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
21. McGuire, D. M., Olson, C. D., Towle, H. C. & Dempsey, M. E. (1984) *J. Biol. Chem.* **259,** 5368–5371.
22. Kirk, M. M. & Kirk, D. L. (1985) *Cell* **41,** 419–428.
23. Kloppstech, K. (1985) *Planta* **165,** 502–506.
24. Klein, R. R. & Mullet, J. E. (1986) *J. Biol. Chem.* **261,** 11138–11145.
25. Hartwig, R., Schweiger, M., Schweiger, R. & Schweiger, H. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6899–6902.
26. Strumwasser, F. (1987) in *Neurobiology Molluscan Models,* eds. Boer, H. H., Geraerts, W. P. M. & Joosse, J. (North Holland, Amsterdam), pp. 298–310.
27. Evans, T., Rosenthal, E. T., Youngbloom, J., Distel, D. & Hunt, T. (1983) *Cell* **33,** 389–394.
28. Mathews, M. B., ed. (1986) *Translational Control* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
29. Ochoa, S. (1983) *Arch. Biochem. Biophys.* **223,** 325–349.
30. Weeks, D. L. & Melton, D. A. (1987) *Cell* **51,** 861–867.
31. Echols, H. & Guarneros, G. (1983) in *Lambda II,* eds. Henderix, R., Roberts, J., Stahl, F. & Weisberg, R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 75–92.
32. Nomura, M., Gourse, R. & Baughman, G. (1984) *Annu. Rev. Biochem.* **53,** 75–117.
33. Lemaire, G., Gold, L. & Yarus, M. (1978) *J. Mol. Biol.* **126,** 73–90.
34. Storti, R. V., Scott, M. P., Rich, A. & Pardue, M. L. (1980) *Cell* **22,** 825–834.
35. McGarry, T. J. & Lindquist, S. (1985) *Cell* **42,** 903–911.
36. Hentze, M. W., Caughman, S. W., Rouault, T. A., Barriocanal, J. G., Dancis, A., Marford, J. B. & Klausner, R. D. (1987) *Science* **238,** 1590–1593.
37. Fox, T. D. (1986) *Trends Genet.* **2,** 97–100.
38. Poutre, C. G. & Fox, T. D. (1987) *Genetics* **115,** 637–647.
39. Leibold, E. A. & Munro, H. N. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2171–2175.
40. Karakashian, M. W. & Hastings, J. W. (1963) *J. Gen. Physiol* **47,** 1–12.
41. Feldman, J. F. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 1080–1087.
42. Rothman, B. S. & Strumwasser, F. (1976) *J. Gen. Physiol.* **68,** 359–384.
43. Karakashian, M. W. & Schweiger, H.-G. (1976) *Exp. Cell Res.* **98,** 303–312.
44. Taylor, W. R., Krasnow, R., Dunlap, J. C., Broda, H. & Hastings, J. W. (1982) *J. Comp. Physiol.* **148,** 11–25.