Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7870–7873, December 1985
Biochemistry

Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*

(bioluminescence/*Photinus pyralis*/antibody screening/expression vector/recombinant DNA)

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Communicated by W. D. McElroy, July 26, 1985

ABSTRACT **A cDNA library was constructed from firefly (*Photinus pyralis*) lantern poly(A)$^+$ RNA, using the *Escherichia coli* expression vector λgt11. The library was screened with anti-*P. pyralis* luciferase (*Photinus* luciferin:oxygen 4-oxidoreductase, EC 1.13.12.7) antibody, and several cDNA clones expressing luciferase antigens were isolated. One clone, λLuc1, contained 1.5 kilobase pairs of cDNA that hybridized to a 1.9- to 2.0-kilobase band on a nitrocellulose blot of electrophoretically fractionated lantern RNA. Hybridization of the cloned cDNA to lantern poly(A)$^+$ RNA selected an RNA that directed the *in vitro* synthesis of a single polypeptide. This polypeptide comigrated with luciferase on NaDodSO$_4$/PAGE and produced bioluminescence upon the addition of luciferin and ATP. A 1.8-kilobase-pair cDNA was isolated by probing the firefly cDNA library with the cDNA from λLuc1. This cDNA contained sufficient coding information to direct the synthesis of active firefly luciferase in *E. coli*.**

Luciferases are the enzymes that catalyze the light-producing chemical reactions of bioluminescent organisms. Insect luciferases require ATP, an organic molecule called luciferin, and oxygen as substrates (1). The absolute requirement for ATP as a substrate is a characteristic unique to insect luciferases, and this property has been used to develop bioluminescence assays for ATP (1).

The only insect luciferase that has been purified and extensively characterized was isolated from the North American firefly, *Photinus pyralis* (*Photinus* luciferin:oxygen 4-oxidoreductase, EC 1.13.12.7) (1, 2). This enzyme catalyzes the conversion of chemical energy into light with a very high efficiency; the quantum yield (photons emitted per molecule of luciferin oxidized) is 0.88 (3). Purified *P. pyralis* luciferase migrates in NaDodSO$_4$/polyacrylamide gels as a single band at an apparent molecular weight of 62,000 (4). The luciferases from other firefly species migrate at a similar position, and all show extensive crossreactivity with antibody raised against *P. pyralis* luciferase (26). *P. pyralis* emits yellow-green light with peak emission occurring at 560 nm (3). Other species of fireflies emit different colors of light ranging from yellow (582 nm) to green (552 nm), the particular color being a characteristic of a given species (5). Since all firefly luciferases use the same substrates (5), these observed differences indicate that the color of light emission is dependent on enzyme structure. Comparative studies of insect luciferases may yield information on how changes in protein structure affect the light-emission properties of these enzymes.

In an earlier paper we showed that poly(A)$^+$ RNA isolated from the lanterns of adult *P. pyralis* fireflies contained mRNA that directed the synthesis of luciferase *in vitro* (4). We have used this poly(A)$^+$ RNA to construct a cDNA library in λgt11, an *Escherichia coli* expression vector (6, 7). The library was screened with anti-*P. pyralis* luciferase antibody, using a chromogenic detection technique (8), and several cDNA clones were isolated and characterized. These clones were found to be homologous to the mRNA that encodes luciferase. The largest luciferase cDNA clone that was isolated was able to direct the synthesis of active luciferase in *E. coli*.

MATERIALS AND METHODS

Enzymes and Strains. Restriction endonucleases and *E. coli* DNA polymerase I were purchased from New England Biolabs. RNase H and bacteriophage λ *in vitro* packaging extracts were obtained from Bethesda Research Laboratories, and avian myeloblastosis virus reverse transcriptase was from Boehringer Mannheim. λgt11 and *E. coli* strains Y1088 [*supE supF metB trpR hsdR hsdM*$^+$ *tonA21* Δ*lacU169 proC*::Tn*5*(pMC9)] and Y1090 [Δ*lacU169 proA*$^+$ Δ*lon araD139 strA supF trpC22*::Tn*10*(pMC9)] were obtained from R. Young (6, 7). *E. coli* strain TB1 [*ara* Δ(*lac-proA,B*) *strA* φ80d*lacZ*ΔM15 *hsr*$^-$ *hsm*$^+$] was obtained from T. Baldwin (Texas A & M University). The plasmid pUC13 has been described (9). The *E. coli* expression plasmid pKJB824.17, consisting of pBR322 carrying the temperature-sensitive λ repressor gene *c*I857 and the λ promoter P_R was obtained from K. Buckley (10).

Construction and Screening of the λgt11 cDNA Library. Live fireflies (*P. pyralis*) were obtained from W. Biggley (Johns Hopkins University). Isolated lanterns were homogenized in guanidinium thiocyanate, and total lantern RNA was isolated from the homogenate by sedimentation through a CsCl cushion (11). Poly(A)$^+$ RNA was isolated by chromatography on oligo(dT)-cellulose (12). The construction of the λgt11 cDNA library was as described (8), except that RNase H and DNA polymerase I were used to synthesize the second strand of the cDNA (13). Packaging of 1 μg of λgt11 DNA ligated to cDNA yielded ≈10^5 recombinant phage, and after amplification of the library on Y1088, the recombinants represented ≈10% of the total phage population. The amplified library was screened with rabbit anti-luciferase antibody (2 μg/ml) as described (8).

Hybridizations. All hybridizations were performed with isolated restriction fragments used as probe. Restriction fragments were isolated from agarose gels by electrophoresis onto DE81 paper (14), and the DNA was labeled with [α-^{32}P]dCTP (≈800 Ci/mmol, Amersham; 1 Ci = 37 GBq) by nick-translation (15) to a specific activity of ≈10^8 cpm/μg. Phage were plated on Y1088 cells and screened by plaque hybridization (16). Southern blots were prepared essentially as described (17). RNA samples were electrophoresed in formaldehyde/agarose gels and blotted onto nitrocellulose (Schleicher & Schuell, BA85) (18). Hybridizations of probe to filters were at 37°C in 55% (vol/vol) formamide/5× SSPE (1×

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Abbreviations: bp, base pair(s); kb, kilobase(s).

SSPE is 0.18 M NaCl/10 mM sodium phosphate, pH 7.7/1 mM EDTA)/heparin (200 μg/ml)/0.1% $NaDodSO_4$ containing 2×10^5 cpm of nick-translated probe per ml (19). Filters were washed in 0.1× SSPE/0.1% $NaDodSO_4$ at 37°C and autoradiographed with Kodak XAR-5 film and Cronex Lightning-plus intensifying screens at −70°C.

Hybridization-Selection and *in Vitro* Translation. Hybridization-selection of mRNA with plasmids was performed as described (20). Twenty micrograms of each plasmid was linearized with *Hin*dIII and bound to 3-mm nitrocellulose squares. *P. pyralis* lantern poly(A)$^+$ RNA (30 μg) was hybridized to the filters in one tube at a final concentration of 200 μg of RNA/ml. Bound RNA was eluted from the filters by boiling. Nuclease-treated rabbit reticulocyte lysates were purchased from Bethesda Research Laboratories and were used according to the supplier's recommended conditions for optimal protein synthesis. One microgram of total lantern poly(A)$^+$ RNA and 1/10th of the hybridization-selected RNAs each were translated *in vitro* in a final volume of 20 μl. Translation mixtures contained L-[^{35}S]methionine (600 Ci/mmol, Amersham) at 2 mCi/ml. Five-microliter samples of the translation products from hybridization-selected RNAs and a 1-μl sample of the translated lantern poly(A)$^+$ RNA were analyzed by $NaDodSO_4$/PAGE (21) followed by fluorography (22). Four microliters of each translation mixture was assayed for the presence of active firefly luciferase.

Expression of Firefly Luciferase in *E. coli*. TB1, TB1(pKJB824.17), and TB1(pKW101) cells were grown in 10 ml of Luria–Bertani medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter, pH 7.4) at 30°C to OD_{650} = 0.5. The cells were heat-induced at 45°C for 30 min and then shifted to 37°C for 1 hr. Cells were pelleted for 5 min at 3000 × *g*, and the supernatant was decanted. Pellets were resuspended in 200 μl of 10 mM Tris Cl, pH 8.0/1 mM EDTA/lysozyme (1 mg/ml) and were incubated on ice for 10 min. The cells were then frozen on dry ice and thawed. Fifty microliters of each cell lysate was assayed for luciferase activity.

Assay of Luciferase Activity. Samples to be assayed for luciferase activity were added to 300 μl of 25 mM glycylglycine, pH 7.8/5 mM $MgCl_2$/0.1 mM luciferin. Each test tube was placed in an LKB luminometer equipped with a chart recorder, and 100 μl of 20 mM ATP (pH 7.0) was injected. The time course of light emission was recorded.

RESULTS

Isolation of Luciferase cDNA Clones. We screened ≈150,000 phage (≈15,000 recombinant phage) from the λgt11 firefly lantern cDNA library with anti-*P. pyralis* luciferase antibody. Sixteen clones expressing luciferase antigens were detected, and eight of these were purified to homogeneity through repeated rounds of screening. All of the clones contained inserts that were released upon digestion of isolated phage DNA with *Eco*RI, and the inserts ranged from 400 base pairs (bp) to 1200 bp in length. Careful analysis of one clone, λLuc1, showed that it contained two *Eco*RI fragments: Luc1A (1200 bp) and Luc1B (270 bp). The 1200-bp Luc1A fragment hybridized to the other seven clones but was not homologous to the 270-bp Luc1B cDNA fragment.

The coding sequence required for the ≈62-kDa luciferase protein was estimated to be 1.6 kilobases (kb), whereas the total length of the cDNA in λLuc1 was 1.5 kb. To be certain that the Luc1A and Luc1B fragments represented contiguous sequences in the luciferase mRNA, isolated Luc1A and Luc1B restriction fragments were labeled with [α-^{32}P]dCTP by nick-translation and then used to probe identical lanes of a blot of *P. pyralis* poly(A)$^+$ RNA after electrophoresis in a denaturing gel (Fig. 1). Luc1A and Luc1B both hybridized to

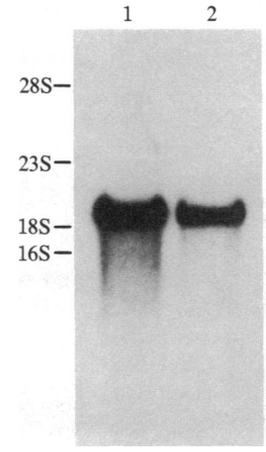

FIG. 1. Blot hybridization analysis of firefly lantern poly(A)$^+$ RNA. Total *P. pyralis* lantern poly(A)$^+$ RNA (1 μg) was electrophoresed in each lane of a formaldehyde/1.3% agarose gel and then blotted onto a nitrocellulose filter. The individual lanes were cut apart before hybridization. After hybridization to ^{32}P-labeled DNA probe, the filters were autoradiographed. Ribosomal RNAs from human (HeLa) and *E. coli* cells were run as size standards in an adjacent lane, and their positions are indicated at left. Lane 1: Hybridization with Luc1A cDNA, the 1200-bp *Eco*RI fragment isolated from λLuc1. Lane 2: hybridization of Luc1B cDNA, the 270-bp *Eco*RI fragment isolated from λLuc1.

a single species of RNA that was 1.9–2.0 kb long; thus, both of the *Eco*RI cDNA fragments in λLuc1 most likely resulted from the reverse transcription of a single species of mRNA.

Luc1A and Luc1B Hybrid-Select Luciferase mRNA. The *Eco*RI cDNA fragments Luc1A and Luc1B were inserted into the *Eco*RI site of the plasmid pUC13 to generate pLuc1A and pLuc1B, respectively. Plasmids pLuc1A, pLuc1B, and pUC13 were used to hybrid-select mRNA from total firefly lantern poly(A)$^+$ RNA. The selected RNAs then were translated *in vitro*, and the translation products were analyzed by gel electrophoresis (Fig. 2) and assayed for luciferase activity. When translated *in vitro*, the mRNA selected by pLuc1A and pLuc1B produced a single species of protein that comigrated with *P. pyralis* luciferase in a $NaDodSO_4$/polyacrylamide gel. No protein bands other than those endogenous to the rabbit reticulocyte extract were detected when pUC13-selected RNA was translated. The *in vitro* translation of total *P. pyralis* poly(A)$^+$ RNA and of pLuc1A- and pLuc1B-hybrid-selected RNA produced active firefly luciferase as assayed by the production of light in the presence of luciferin and ATP (data not shown). No active luciferase could be detected in the reticulocyte translation extract or an extract that contained RNA selected by pUC13 alone.

Isolation of Larger Luciferase cDNA Clones. The sum of the two cDNA fragments from λLuc1 (1.5 kb) was less than the size of the mRNA detected by Luc1A and Luc1B (1.9–2.0 kb). Therefore, the λgt11 lantern cDNA library was rescreened, using the Luc1B fragment labeled with [α-^{32}P]dCTP by nick-translation as probe, to isolate a longer cDNA clone. Ten clones were purified to homogeneity, and the DNA isolated from these phage was cut by *Eco*RI endonuclease and examined by DNA blot analysis (Fig. 3). All of the clones contained an *Eco*RI fragment that was detectable by hybridization to nick-translated Luc1B cDNA. These fragments ranged in size from 200 bp to 600 bp. Subsequent hybridization of the blot to nick-translated Luc1A fragment showed that all but one of these clones also contained the large *Eco*RI A fragment (1.2–1.3 kb). The clone with the largest insert, λLuc23, carried 1.8 kb of cDNA composed of a 1.2-kb *Eco*RI A fragment (Luc23A) and a

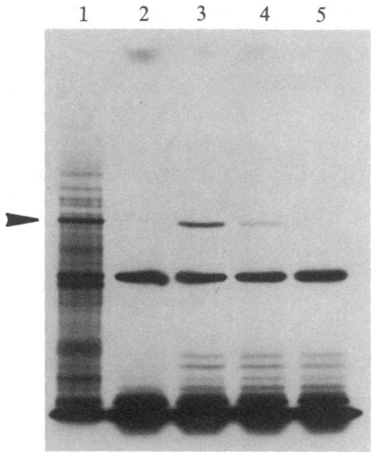

FIG. 2. Hybridization-selection and *in vitro* translation of luciferase mRNA. Plasmid DNAs bound to nitrocellulose filters were hybridized to total *P. pyralis* lantern poly(A)$^+$ RNA. Selected RNA was eluted from the filters and assayed by *in vitro* translation in rabbit reticulocyte lysates in the presence of [^{35}S]methionine. Samples from each *in vitro* translation were resolved in a NaDodSO$_4$/7.5% polyacrylamide gel, and labeled proteins were visualized by fluorography. The arrowhead indicates the position at which luciferase migrates. The RNAs used to direct the *in vitro* synthesis of proteins in the reticulocyte lysates were total *P. pyralis* lantern poly(A)$^+$ RNA (lane 1), no added RNA (lane 2), RNA selected by hybridization to pLuc1A (lane 3), RNA selected by hybridization to pLuc1B (lane 4), and RNA selected by hybridization to pUC13 (lane 5).

600-bp *Eco*RI B fragment (Luc23B). This clone is nearly full-length, especially if one takes into account that some portion of the 1.95-kb mRNA is composed of a poly(A) tract and the unlikely possibility that the full poly(A) tail was copied into the cDNA. Since all but one of the λLuc clones detected by probing with Luc1B contain the same sized A fragment and variable B fragments, it is likely that the A fragment represents the 3′ end of the luciferase gene.

Expression of Firefly Luciferase in *E. coli*. We used the expression plasmid pKJB824.17 in an attempt to express the luciferase cDNA in *E. coli*. This plasmid carries the temperature-sensitive repressor gene *c*I857 of bacteriophage λ and the λ promoter P_R followed by a truncated *cro* gene with an *Eco*RI site. Preliminary DNA sequence analysis of the Luc23B fragment showed that it had only one open translational reading frame (unpublished results). The *Eco*RI linker at the 5′ end of Luc23 was located so that insertion of Luc23 into the *Eco*RI site of pKJB824.17 would fuse the reading frame of the truncated *cro* gene to that of the luciferase cDNA. λLuc23 was partially digested with *Eco*RI, and the 1.8-kb complete cDNA fragment was isolated from an agarose gel. This fragment was ligated into the *Eco*RI site of pKJB824.17 to produce pKW101 (Fig. 4). When cells harboring this plasmid are heat-induced, the λ repressor is inactivated and transcription from P_R proceeds through the partial *cro* gene into the luciferase cDNA. Translation of this RNA should produce a fusion protein with the eight N-terminal amino acids derived from vector sequence and the *Eco*RI linker, with the remainder of the amino acids determined by the luciferase cDNA. Since the N-terminal amino acid sequence of native luciferase is unknown, it is not possible at this time to predict what portion of luciferase, if any, is absent from the fusion protein. Luciferin and ATP were added to an extract prepared from 10 ml of heat-induced TB1(pKW101) cells. A flash of yellow-green light was observed that rapidly decayed to a lower level of luminescence. The reaction was repeated in a luminometer equipped with a chart recorder (Fig. 5). When luciferin alone was added to extract from heat-induced *E. coli* cells carrying pKW101 a low level of light production could be detected, presumably because of endogenous levels of ATP in the cells. A flash of light was recorded upon the injection of excess ATP into the reaction tube, and this light emission decayed rapidly to a lower level. The amount of light measured corresponded to at least 10 pmol of active luciferase in the extract obtained from 10 ml of cells (≈3 × 10^8 cells per ml). No luciferase activity was detected in extracts of heat-induced TB1 cells or

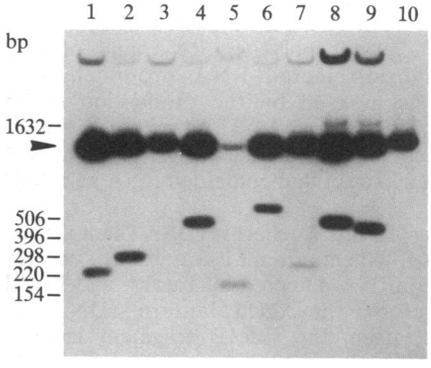

FIG. 3. Southern blot of λLuc clones isolated by probing the library with Luc1B cDNA. DNA was isolated from 10 λ clones that contained sequences homologous to the *Eco*RI cDNA fragment Luc1B. The DNAs were digested with *Eco*RI, electrophoresed in a 1% agarose gel, and blotted onto nitrocellulose. The filter was first probed with ^{32}P-labeled Luc1B fragment. This probe hybridized only to fragments ≤600 bp in the *Eco*RI digests of the λLuc clones. ^{32}P-labeled Luc1A fragment was then hybridized to the filter. Luc1A hybridized to 1200- to 1300-bp *Eco*RI bands (arrowhead) in all but one of the clones. The clone containing the largest cDNA insert (1.8 kb), λLuc23, is in lane 6. A *Hin*fI digest of pBR322 was run as a size standard.

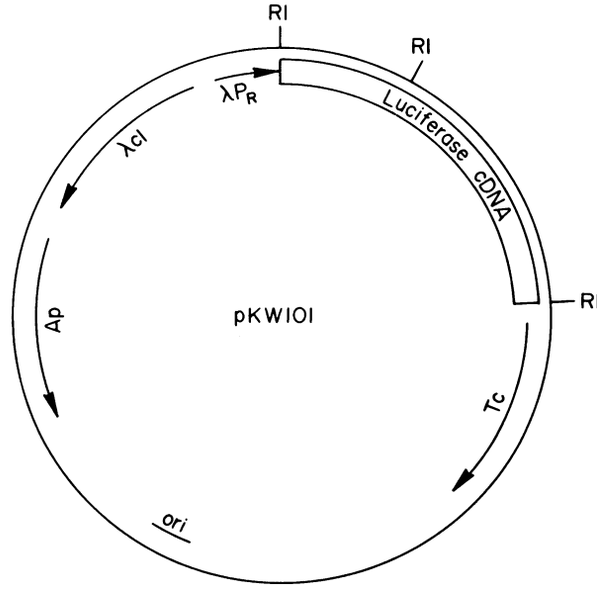

FIG. 4. Structure of pKW101. The cDNA insert in λLuc23, isolated from a partial *Eco*RI digest of the phage DNA, was inserted into the *Eco*RI site of the *E. coli* expression plasmid pKJB824.17. pKJB824.17 is the plasmid pBR322 carrying a segment of bacteriophage λ DNA containing the temperature-sensitive λ repressor gene *c*I857 and the λ promoter P_R plus the beginning of the λ *cro* gene. Upon heat-induction, transcription initiates at P_R and proceeds through the partial *cro* gene sequence and into the luciferase cDNA. Labeled on the diagram are the *Eco*RI restriction sites (RI), the ampicillin-resistance gene (Ap), the tetracycline-resistance gene (Tc), and the pBR322 origin of replication (ori).

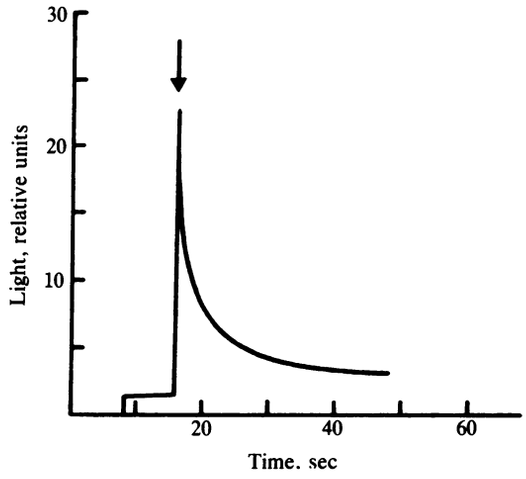

FIG. 5. Active firefly luciferase is synthesized in *E. coli* cells. TB1 cells containing the plasmid pKW101 were heat-induced and incubated to allow expression of the luciferase cDNA under the control of λ P_R. Cells were pelleted and lysed, and the lysate was assayed for luciferase activity in the presence of luciferin and ATP. Light emitted by the luciferase was monitored in an LKB luminometer equipped with a chart recorder. The time course of light emission is shown. The arrow indicates the time at which ATP was injected into the reaction mixture.

TB1 cells carrying the vector pKJB824.17, even with the sensitivity of the luminometer set 500 times greater than the sensitivity used to obtain the recording shown in Fig. 5.

DISCUSSION

We have isolated several cDNA clones that are homologous to the mRNA encoding the luciferase of the firefly *P. pyralis*. The longest of these cDNA clones, Luc23, is 1.8 kb long, which is greater than the 1.6 kb required to encode a 62-kDa protein. This cDNA is, however, shorter than the 1.95-kb luciferase mRNA that is detected on RNA blots. Further analysis is necessary to determine whether or not Luc23 contains the entire coding sequence for luciferase. Luc23 does contain the coding information necessary for catalytic activity of luciferase: when inserted in an *E. coli* expression plasmid, this cDNA directs the *in vivo* synthesis of an enzyme that produces light in the presence of luciferin and ATP. On the basis of nucleotide-sequence information the luciferase produced in *E. coli* is a fusion protein in which the eight N-terminal amino acids are determined by the expression vector and synthetic restriction-site sequences. The activity of the fusion protein indicates that the N-terminus of *P. pyralis* luciferase can be altered without complete loss of its catalytic activity.

Insect luciferases have great potential as an experimental system for investigating the structural basis of enzyme-catalyzed light emission. The demonstration of the synthesis of active firefly luciferase in *E. coli* will greatly facilitate the isolation of mutant enzymes that may have altered light emission properties. Although the colors of the light emitted by firefly luciferases range from yellow to green, it should be possible to modify luciferase so that light of even longer wavelengths will be produced, particularly in view of the observation that treating *P. pyralis* luciferase with heat, Zn^{2+}, or low pH causes it to emit red (610 nm) light (5, 23). Furthermore, the railroad worm, which is the larva of a South American beetle (*Phrixothrix* sp.), has anterior light organs that naturally produce red light in addition to abdominal yellow-green light organs (24).

In a recent paper, the use of the cloned *lux* operon of *Vibrio fischeri* as an indicator of promoter activity in bacteria was described (25). The luciferase gene of the firefly, *P. pyralis*, could be used in a similar fashion as an indicator gene and may have more extensive applicability. Unlike the bacterial luciferase, the firefly enzyme requires a single subunit for activity, and its synthesis could therefore be placed under the control of a single eukaryotic promoter. In addition, the exceptionally high quantum yield of light characteristic of the firefly luciferase may make this luciferase gene particularly suitable for use as an indicator of transcriptional activity.

This work was supported by National Science Foundation Grant PCM8305446 and National Institutes of Health Grant AI07194.

1. DeLuca, M. & McElroy, W. D. (1978) *Methods Enzymol.* **57**, 3–15.
2. Green, A. A. & McElroy, W. D. (1956) *Biochim. Biophys. Acta* **20**, 170–178.
3. Seliger, H. H. & McElroy, W. D. (1960) *Arch. Biochem. Biophys.* **88**, 136–141.
4. Wood, K. V., de Wet, J. R., Dewji, N. & DeLuca, M. (1984) *Biochem. Biophys. Res. Commun.* **124**, 592–596.
5. Seliger, H. H. & McElroy, W. D. (1964) *Proc. Natl. Acad. Sci. USA* **52**, 75–81.
6. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
7. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
8. de Wet, J. R., Fukushima, H., Dewji, N. N., Wilcox, E., O'Brien, J. S. & Helinski, D. R. (1984) *DNA* **3**, 437–447.
9. Messing, J. (1985) *Methods Enzymol.* **101**, 20–78.
10. Buckley, K. J. (1985) Dissertation (Univ. of California, San Diego).
11. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
12. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
13. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
14. Dretzen, G., Bellard, M., Sassone-Corsi, P. & Chambon, P. (1981) *Anal. Biochem.* **112**, 295–298.
15. Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
16. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
17. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–517.
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 202–203.
19. Singh, L. & Jones, K. W. (1984) *Nucleic Acids Res.* **12**, 5627–5638.
20. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2253–2257.
21. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
22. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
23. McElroy, W. D. & DeLuca, M. (1978) in *Bioluminescence in Action*, ed. Herring, P. J. (Academic, London), pp. 109–127.
24. Lloyd, J. E. (1978) in *Bioluminescence in Action*, ed. Herring, P. J. (Academic, London), p. 256.
25. Engebrecht, J. M., Simon, M. & Silverman, M. (1985) *Science* **227**, 1345–1347.
26. Weinhausen, G. & DeLuca, M. (1985) *Photochem. Photobiol.*, in press.