Number 28

PROMEGA NOTES

December 1990

Firefly Luciferase: A New Tool for Molecular Biologists

Keith V. Wood, Ph.D.
Promega Corporation

Measurements of gene expression and regulation are fundamental to studies in cell biology and molecular genetics. Reporter genes, which code enzymes with readily detectable activities, are commonly used for such measurements. In mammalian cells, the gene coding chloramphenicol acetyltransferase (*cat*) is most commonly used. Both *cat* and a gene coding glucuronidase activity (*gus*) are used in plant cells. However, in the past few years, the cDNA coding firefly luciferase (*luc*) has gained much interest as a reporter gene for both animal and plant systems. A recent review of reporter genes states that "the firefly luciferase cDNA and gene may have provided the most versatile reporter gene yet (1)." The major advantages provided by *luc* are a rapid and sensitive assay for gene expression with relatively little cost.

Greater Sensitivity

Users of the *luc* reporter benefit primarily from the extreme sensitivity with which luciferase activity can be measured. Compared to the *cat* reporter, the assay for expression of *luc* is roughly 100-fold more sensitive (1). The increased sensitivity especially aids in studies of low-level genetic activity, such as that of weak promoters (2). Studies may also be done using smaller amounts of biological materials. Enzymatic activity of luciferase is easily measured because of the high energy content in photons. One photon (560nm) carries six times the energy of phosphate bond hydrolysis in ATP. Furthermore, catalysis by luciferase is approximately 90% efficient in photon production. Because endogenous luminescence is essentially absent from biological samples, there is no interference with the signal generated by luciferase.

Luciferase is a single polypeptide (62kDa) which requires no post-translational modifications for enzymatic activity. It contains no prosthetic groups, bound metals, nor disulfide bonds. Because of this structural simplicity, it can be expressed actively in virtually any host organism or cell type. Experiments have been done using bacteria (3, 4), yeast (5), *Dictyostelium* (6), insect cells (7), mammalian cells (8), and plant cells (9), as well as transgenic animals (10) and plants (9, 11). Luciferase is useful also in *in vitro* biochemical experiments (12, 13).

The luminescent reaction of luciferase is an oxidation of beetle luciferin, a heterocyclic organic acid. In nature, luciferin is found only in luminous beetles and presumedly has evolved specifically for efficient light production. Today, luciferin is prepared synthetically for research and commercial use. The enzyme uses ATP to catalyze formation of an acyl-AMP intermediate of luciferin, which is subsequently oxidized by O_2 to yield oxyluciferin. The oxyluciferin emits a photon as its electronic structure converts to the ground state (14, 15). As noted above, only a small portion of the photon energy comes from the ATP consumed in the reaction; the majority of the energy derives from direct conversion of molecular oxygen.

Faster Results

Another major advantage of firefly luciferase in genetic experiments is the rapidity of its assay. Light intensity of the luminescence reaction can be measured immediately upon addition of substrates to cell extracts containing the luciferase. Usually measurements are made for 10 seconds to 1 minute. With efficient cell lysis procedures developed for measurements of luciferase activity (16), the entire procedure from cell culture to result requires less than 10 minutes.

Measurements of luminescence are most easily made using a luminometer. These instruments are highly sensitive and easy to operate, can measure luminescence over several decades of intensity, and are relatively inexpensive. Due to growing interests in luminescence for many applications, luminometers have become more available to researchers. However, scintillation counters also are generally suitable for measuring luciferase activity. Their sensitivity is comparable with that of luminometers (17). Optimal results are usually achieved by using the counters in manual mode with the coincidence circuit turned off. (Some counters may have additional features that interfere with measurements of chemiluminescence; it is recommended that a titration of luciferase activity be done to verify the linear range of the instrument.) Photographic film also should prove useful for some applications (18).

The benefits of increased sensitivity and ease of assay were demonstrated in a study using the interleukin-2 (IL2) gene promoter (2). This promoter, derived from human T-lymphocytes, could be induced with a mixture of phytohemagglutinin (PHA-P) and phorbol ester (TPA). When coupled to *luc*, induction of the promoter appeared to be 7-fold greater than when coupled to *cat*. The greater apparent induction resulted from more precise measurement of the low-level genetic activity prior to induction. The greater sensitivity and relative induction

(continued next page)

allowed increased transcription to be detected within 7 hours of induction rather than 3 days as required for the *cat* reporter. The authors claimed that quantitative results using *luc* were obtained 24 hours after cell transfection, compared to greater than 100 hours required when using *cat*. Furthermore, the results using *luc* were obtained using 10-fold fewer cells than required with *cat*.

The time saved in experiments using the *luc* reporter reduces cost. The cost of materials is also less since the assay for expression of *cat* uses radioisotope-labeled substrates. These are more expensive than the substrates of luciferase and require additional expense in protective supplies for radioactive handling. There are also administrative costs in the handling and disposal of radioactive material. Moreover, reduced use of radioactivity is desired by many researchers for health and environmental reasons. Further cost savings may be achieved in the maintenance and manipulations of cell cultures, as fewer cells are typically needed for experiments using *luc*.

Applications of Luciferase

The combined benefits of luciferase as a genetic reporter have fostered its increased popularity as a tool of molecular biology. Most applications of *luc* have been in the analysis of genetic regulation. These experiments generally involve analysis of gene promoter or enhancer activity. The above example of IL2 promoter activity is typical. Another example was presented in an analysis of the promoter regulating expression of human tumor necrosis factor α/cachectin (19). This promoter is induced by the phorbol ester, PMA. When coupled to *luc*, induction of the promoter would cause a 12-fold increase of luciferase expression. Analysis of deletions to the promoter allowed identification of the smallest DNA region still capable of inducing luminescence. Many other examples of similar analysis are presented in the literature. It has been shown using mammalian cells that *luc* responds like *cat* to effectors of gene activity (8).

A dramatic example of luciferase as a genetic reporter was depicted in a photograph of a luminous tobacco plant (9). The *luc* reporter was introduced into the plant cells using an *Agrobacterium tumefaciens* vector system, then selected cells were induced to grow into plants. Luminescence in the plants was initiated by watering with a solution of luciferin. The distribution of luminescence in extracts from different tissues of the plants was dependent upon the promoter regulating luciferase expression (11). The distribution of luminescence also correlated to the levels of mRNA coding luciferase in the different tissues.

Luciferase expression has also been used to examine the structure and processing of mRNA in cells. A study in maize showed that intron processing could increase expression of the luciferase gene 6- to 16-fold depending upon the source of promoter and intron used (20). Another study showed that the 5′ untranslated sequence of mRNA could greatly affect luciferase expression both *in vitro* and *in vivo* (13). In mammalian cells, analogous results were obtained, as well as results showing the requirement for a 5′ cap structure (21). Because of the sensitivity afforded by luminescence in these experiments, expression from the mRNA could be measured within 30 minutes of introduction into cells.

Structure and function relationships of proteins that act upon genetic material can also be examined with the *luc* reporter. An example of this is a study of genetic regulation by estrogen receptor (22). A system was established where estrogen receptor expressed from one plasmid could regulate expression of *luc*, which was coupled to an estrogen responsive element (ERE) on another plasmid. With this arrangement, a 3- to 7-fold increase in luminescence was caused by the presence of estrogen. Amino acid substitutions in the DNA binding region of the receptor were shown to abolish the induction of luminescence. Another experiment showed that truncation of the steroid binding region caused constitutively high level expression of luminescence. These results help to map functional domains within the structure of the receptor.

A rapid method to screen for biological activity of estrogen and antiestrogen inhibitors was developed with a similar strategy (23). The *luc* reporter was coupled to an ERE and transfected into cells expressing estrogen receptor. Selection was used to establish a stable cell line. Addition of estrogen to the cell line caused increased expression of luminescence within 24 hours. Conversely, induction of luminescence could be reduced by addition of an antiestrogen. As described above for tobacco plants, luminescence *in vivo* could be observed in transfected cells by addition of luciferin to the culture media. This was used as a non-destructive means to select a well behaved cell line for these studies.

Some studies have used expression of luciferase to reveal the presence of other genes or organisms. For example, the propagation of vaccinia virus in mice could be easily monitored by inserting *luc* into the viral genome (24). By using this method an attenuated strain of virus was shown to grow more slowly than wild type, but with the same tissue distribution. A different strategy was used to assay growth of HIV in cell culture (25). The *luc* reporter was coupled to cloned HIV LTR and transformed into cells prior to viral infection. Upon infection, the *tat* gene product of HIV acted in trans on the LTR to cause up to 50-fold induction of luciferase. Protective action of antiviral drugs could be tested rapidly with this system.

Reporters from Promega

Because of its widespread popularity, Promega has supported the *cat* reporter system with convenient vectors and assay reagents. However, it is believed at Promega that the firefly luciferase system offers some substantial advantages for measurements of genetic activity. To make this new technology easily accessible, we soon will be offering also a complete line of vectors and reagents for the *luc* reporter system. For more information contact Promega Technical Services, 1-800-356-9526.

References:

1. Alam, J., *et al.* (1990) *Anal. Biochem.* **188**, 245–254.
2. Williams, T. M., *et al.* (1989) *Anal. Biochem.* **176**, 28–32.
3. de Wet, J. R., *et al.* (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7870–7873.
4. Palomares, A. J., *et al.* (1989) *Gene* **81**, 55–64.
5. Tatsumi, H., *et al.* (1988) *Agric. Biol. Chem.* **52**, 1123–1127.
6. Howard, P. K., *et al.* (1988) *Nucl. Acids. Res.* **16**, 2613–2623.
7. Hasnain, S. E., *et al.* (1990) *Gene* **91**, 135–138.
8. de Wet, J. R., *et al.* (1987) *Mol. Cell. Biol.* **7**, 725–737.
9. Ow, D. W., *et al.* (1986) *Science* **234**, 856–859.
10. DiLella, A. G., *et al.* (1988) *Nucl. Acids. Res.* **16**, 4159.
11. Schneider, M., *et al.* (1990) *Plant Mol. Biol.* **14**, 935–947.
12. Wood, K. V., *et al.* (1984) *Bioch. Biophy. Res. Commun.* **124**, 592–596.
13. Baughman, G., *et al.* (1988) *Virology* **167**, 125–135.
14. White, E. H., *et al.* (1980) *J. Am. Chem. Soc.* **102**, 3199–3208.
15. McElroy, W. D., *et al.* (1985) In: *Chemi- and Bioluminescence* (ed. J.G. Burr) Marcel Dekker, Inc., New York, 387–400.
16. Brasier, A. R., *et al.* (1989) *BioTechniques* **7**, 116–1122.
17. Nguyen, V. T., *et al.* (1988) *Anal. Biochem.* **171**, 404–408.

(continued next page)

18. de Wet, J. R., *et al.* (1986) In: *Methods in Enzymology*, Vol. 133 (eds. M. DeLuca, and W. McElroy) Academic Press, New York, 3–14.
19. Economou, J. S., *et al.* (1989) *J. Exp. Med.* **170**, 321–326.
20. Callis, J., *et al.* (1987) *Genes and Development* **1**, 1183–1200.
21. Malone, R. W., *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6077–6081.
22. Waterman, M. L., *et al.* (1988) *Mol. Endocrin.* **2**, 14–21.
23. Pons, M., *et al.* (1990) *BioTechniques* **9**, 450–459.
24. Rodriguez, D., *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1287–1291.
25. Schwartz, O., *et al.* (1990) *Gene* **88**, 197–205.

Functional Expression and Detection of Shaker K⁺ Channels in a Baculovirus-Infected Insect Cell Line

Kimberly Klaiber
Howard Hughes Medical Institute
Graduate Department of Biochemistry
Brandeis University
Waltham, Massachusetts

Three years ago, the gene defective in the *Drosophila* behavioral mutant *Shaker* was cloned and shown to code for a voltage-dependent potassium (K^+) channel (1-4). Most ion channels are extremely rare membrane proteins; except in a few special cases, natural tissues are inadequate sources for purification of ion channels. The present work investigates the applicability to ion channels of a high-level expression system previously used for production of post-translationally processed proteins from higher eukaryotes.

We constructed a recombinant baculovirus, *Autographa californica* nuclear polyhedrosis virus, containing the *Drosophila Shaker* H4 K^+ channel cDNA under control of the polyhedrin promoter. The basic approach was to use a cell line, Sf9, from the army-worm caterpillar *Spodoptera frugiperda*, as host for expression of *Shaker* K^+ channels. *Shaker* cDNA was delivered to the Sf9 cells via infection with a recombinant baculovirus, *A. california* nuclear polyhedrosis virus (AcMNPV), which has a circular double-stranded DNA genome of 128kb (5). This virus encodes a gene for a cytoplasmic protein, polyhedrin, which is normally expressed late in the infection cycle at such high levels that the protein precipitates in the interior of the host cell; polyhedrin can account for as much as 50% of the total cell protein in Sf9 cells infected with wild-type AcMNPV. To utilize this virus for heterologous expression of *Shaker* K^+ channels, we employed the conventional strategy of positioning the coding sequence of *Shaker* cDNA downstream from the polyhedrin promoter in the viral genome.

The large size of the viral genome makes it impossible to construct this engineered viral DNA by standard *in vitro* methods. Instead, the desired virus is obtained by homologous recombination in Sf9 cells into which wild-type viral DNA and *Shaker* cDNA are introduced together. Using *Shaker* H4 cDNA (provided by Dr. M. Tanouye), we constructed a baculovirus transfer vector, pKK101, containing *Shaker* cDNA flanked on the 5′ end by the polyhedrin promoter sequence and on the 3′ end by part of the polyhedrin coding sequence. Sf9 cells were cotransfected with wild-type viral DNA and transfer vector. Virus particles were harvested from the supernatants of these transfected cells and were then added at low density to a lawn of Sf9 cells. About 1% of the resulting viral plaques resulted from *Shaker*-recombinant virus, as judged visually by the absence of polyhedrin occlusion in the cells. Four independent recombinant plaques were selected and purified for subsequent *Shaker* expression (6).

Infected Sf9 cells express fully functional *Shaker* transient K^+ currents, as assayed by whole-cell recording. Figure 1 illustrates a family of outward currents elicited by a train of depolarizing pulses from a holding potential of –85 millivolts, in a cell observed 35 hours after infection by a recombinant virus. The voltage-dependent currents show the characteristic properties of *Shaker* A-type K^+ currents. They activate at potentials positive to –20 millivolts and spontaneously inactivate upon maintained depolarization. Further characteristics of these currents are described in reference 6.

(continued next page)

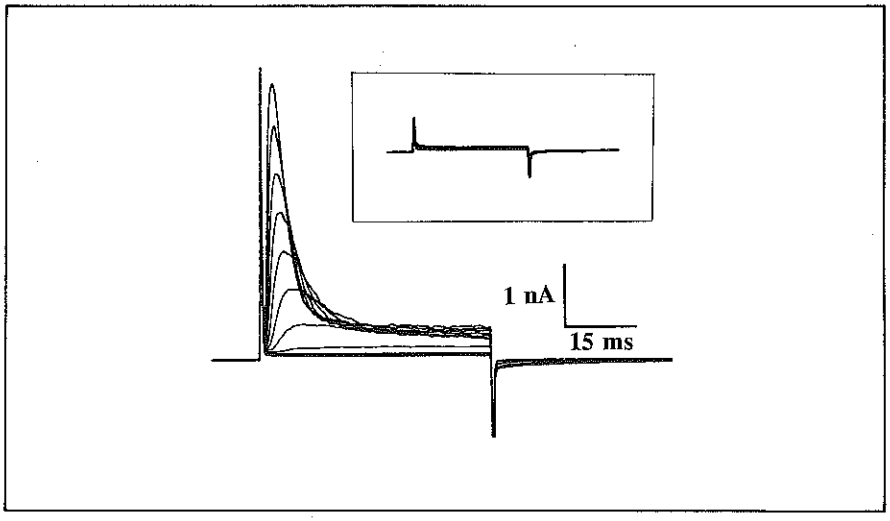

Figure 1. Baculovirus-expressed *Shaker* currents in Sf9 cells. A series of 50 millisecond voltage pulses from a holding voltage of –85 millivolts was applied to an Sf9 cell 35 hours after infection by *Shaker* recombinant baculovirus. Raw current records shown are in response to a voltage pulse of –40 to +60 millivolts (10 millivolt increments). Holding potential was applied for 2 seconds between successive pulses. Inset: current response to an identical series of 50 millisecond voltage pulses applied to uninfected Sf9 cells, with electronic compensation to remove the bulk of the initial capacitance transient. The vertical scale is identical to that in the main figure. For complete electrophysiological methods, see reference 6.

Reprinted with permission of Cell, *first appeared August, 1990.*