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In order to better understand the enzymatic structure and function of firefly luciferase, the cDNA encoding this protein was recently cloned. In this report we present the extension of our earlier work with the cloning of several luciferases from a bioluminescent click beetle (Pyrophorus plagiophthalamus). The luciferases of this beetle produce bioluminescence of different colors. We isolated 13 cDNA clones which were capable of producing luminescence in E. coli of four different colors: green (546nm), yellow-green (560nm), yellow (578 nm), and orange (593 nm). The amino acid sequence of these luciferases are approximately 95% identical with each other, but are less than 50% identical with the firefly luciferase.

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Final Report: Firefly Luciferase–Structure and Function

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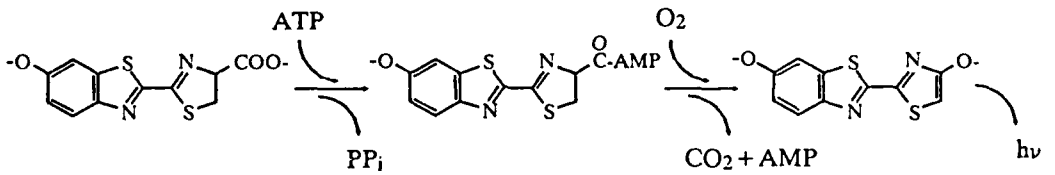
In 1985 the cDNA encoding firefly luciferase was cloned from *Photinus pyralis* (1). It was found that because the primary translation product of the cDNA did not require any post-translational modifications, it could be used to express luminescent activity in a wide range of hosts. To date active luciferase has been expressed in bacteria, yeast, slime molds, insect and mammalian cell culture, plant tissue culture, and both transgenic plants and animals. This, combined with the extreme sensitivity with which luminescence can be detected, has made the cDNA encoding firefly luciferase useful as a reporter gene. Compared with the widely used gene encoding bacterial chloramphenicol acetyl-transferase (CAT), the cDNA encoding luciferase provides more sensitive and faster assay of gene activity, and does not require the use of radioisotopes. In many cases, it can be detected without destruction of the exogenous host.

The cloning of this cDNA has also been instrumental in understanding some of the basic features of firefly luciferase. As stated above, it was found that there are no post-translational modification required for activity. In fact, none are evident by SDS gel electrophoresis, though this does not rule out the possibility of minor structural alterations. (For example, we have evidence that the N-terminus of the enzyme is modified.) The cDNA clone has allowed us to demonstrated conclusively that the enzyme is active as a single type of subunit. Previously some evidence suggested that the active enzyme may be a heterodimer. We have also shown that the enzyme contains a peroxisomal targeting sequence which has been conserved since the divergence of insects and mammals.

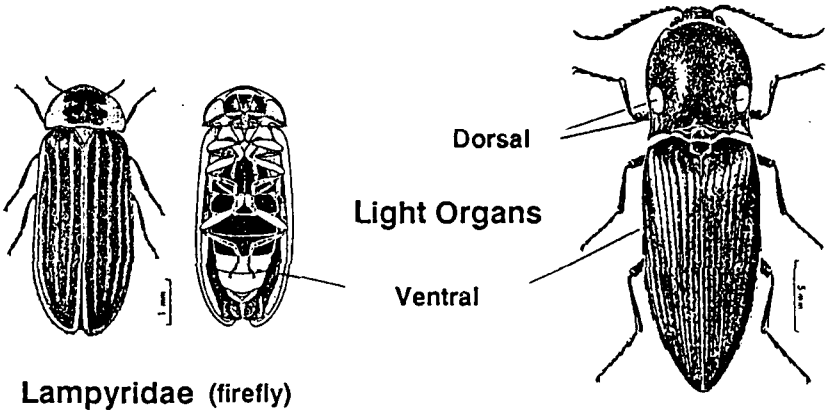
However, we are still not able to identify which portions of the primary structure contribute to catalysis. It was hoped that sequence similarity could be found among the DNA and protein sequences in available databases. By this method we might be able to relate the structure and function of luciferase to other enzymes. Our attempts to identify such homologies have not been successful. In a related approach, we hoped that comparison of this luciferase with evolutionarily distant luciferase would be instructive. Our best choice for this were the luciferases from luminescent elaters.

Elateroidae is the only other superfamily of beetles containing luminescent members. It is known that luciferases from these beetles utilize the same substrates and catalyze the same reaction as the firefly. This reaction is shown below.



Other bioluminescent insect systems are possibly related to the beetle system but very little is known about them. In those tested it is known that they can not utilize the same luciferin. Of the other commonly studied luminescent systems there is no evidence for evolutionary relatedness.

One of the most studied of the bioluminescent elater is the Jamaican click beetle *Pyrophorus plagiophthalamus*. Like other elaters, its body shape and placement of light organs is quite different from the firefly. In particular, it has a pair of dorsal light organs and a single ventral light organ that is centrally located. In *Pyrophorus plagiophthalamus*, the dorsal light organs can emit light of green to yellow in color. The ventral organ can emit light from green to orange, though in the majority of cases it is yellow or orange. The specific color varies between individual beetle but has not been observed to change within a single specimen. Also, in contrast to fireflies, these click beetle do not flash their light. Instead, the luminescence is displayed in long continuous pulses.



Lampyridae (firefly)

Elateridae (click beetle)

Because of these unique characteristics, the decision was made to clone the cDNA's encoding luciferases from these beetles. Approximately 1000 beetles were collected from Jamaica and frozen live in liquid N_2 to preserve their mRNA. The ventral light organ of about 60 specimen were used to construct a cDNA library in a lambda cloning vector. From this library 13 clones were isolated that contained full length coding sequences capable of directing the synthesis of active luciferase in E. coli. These clones fell into four groups based on the color of light generated by their respective gene products. Three clones produced orange light (594 nm), eight produced yellow light (578 nm), one produced yellow-green light (563 nm), and one produced green light (546 nm). The spectra of four representative members of these clones are shown on the following page. The green represents the shortest wavelength measured in beetle bioluminescence, either *in vitro* or *in vivo*. The orange is the longest wavelength measured with one exception found in a rare beetle in South America. (The firefly luciferase produces light at 562 nm). It is interesting to note the regularity with which the spectra are separated. The significance of this is not yet clear.

Protein sequence comparison of the click beetle luciferases with the firefly luciferase shows about 47% identity. There are no long contiguous stretches of sequence identity, though the C-terminal half of the protein in more conserved than the N-terminal half. The sequence alignment resulted in six gaps of one to two amino acids each. Overall the lengths are quite similar (543 amino acids for the click beetle luciferase, and 550 amino acids for the firefly luciferase). The sequence shown to be important for peroxisomal targeting in the firefly luciferase is also present in the click beetle luciferase, so it is likely that these luciferases are also targeted to the peroxisome.

Between the different click beetle clones, sequence similarity is very high, varying from 94% to 99%. Since the only difference between the *E. coli* expressing the various click beetle luciferases is the sequence of the cDNA, the determinants of color must be within these sequences. By comparison of the sequences producing each of the four colors, it is found that in no position does the identity of an amino acid vary between more than two types. Thus, a gradation of chemical properties at a single position cannot be responsible for the emitted colors. Further, the positions that do vary are globally located in the primary structure. It is additionally not known whether all of the variable positions can alter the color emission. At present, then, it is not even apparent whether the determinants lie within a specific region of the primary structure.

The order of evolution of the different luciferases can be estimated from their sequence similarities. It appears that the genes evolved sequentially in the order of their emission maxima, i.e. the green emitting luciferase is the oldest and the orange emitting luciferase is the most recent. Presently we are engaged in basic characterization of the cloned click beetle luciferases. We are also attempting to better identify the sequence determinants of color emission.