Spring 2006

Generation of Crz-MCD8-GFP Transgenic Fly Strain for the Use of Green Fluorescent Protein (GFP) as a Reporter Protein in the Analysis of Corazonin-Expressing Neurons (Crz)

Mitchell Ryan Allgyer
University of Tennessee-Knoxville

Follow this and additional works at: https://trace.tennessee.edu/utk_chanhonoproj

Recommended Citation
Generation of Crz-MCD8-GFP Transgenic Fly Strain for the Use of Green Fluorescent Protein (GFP) as a Reporter Protein in the Analysis of Corazonin-Expressing Neurons (Crz)

A Senior Honors Project
In Partial Fulfillment of Bachelor of Science with University Honors in Biochemistry and Cellular and Molecular Biology
The University of Tennessee, Knoxville

Mitchell Ryan Allgyer
May 2006

Senior Project Advisor: Dr. Jae H. Park
Biochemistry and Cellular and Molecular Biology
Abstract

Corazonin (Crz) is a neuropeptide composed of eleven amino acid residues and is expressed in many insects. It has been shown to have different functions and effects in various insects, and not one consensus function has been displayed. Corazonin as a neuropeptide family appears to have evolved alongside adipokinetic hormones (AKH), and its function and structure depend on exactly what ligand is needed for its energy needs. Corazonin is expressed in *Drosophila melanogaster* although its exact function is not known. It, however, can be inferred that it acts as both a neurohormone and a neuromodulator based on the expression patterns of neurons expression corazonin. In order to shed more light on the function of corazonin, a transgenic fly line was established – Crz-GFP. Corazonin was directly coupled with green fluorescent protein (GFP) using mouse lymphocyte marker CD8 (mCD8) as a fusion protein. GFP being directly fused to the membrane of corazonin neurons has many advantages, including easier staining protocols, better visualization in neural regions, and greater ability to directly study mutations. Through microinjections of the DNA construct pCrz-mCD8-GFP-pCasper4, at least two transgenic fly lines have been established in which Crz is signaled by GFP.

Key Words: corazonin, green fluorescent protein, *Drosophila*
Introduction

Neuropeptides are important messenger molecules, which influence a significant amount of physiological processes. In insects, they function as neuromodulators being released within the central nervous system (CNS) and also as neurohormones being released into the hemolymph (Predel et al, 2004). The adipokinetic hormones (AKHs) of insects are a large family of neuropeptides that mobilize lipids and sugar from fat during energy-requiring activities. About 80 different adipokinetic hormones have been isolated from more than 30 insect species with many producing several structurally and functionally different hormones (Cazzamali et al, 2002). The structural evolution of adipokinetic hormones throughout the different insect species is related to its functional evolution, suggesting that differences in the energy substrates mobilized by the different hormones cause adaptations in the structure of each individual peptide (Veenstra, 1994). Interestingly, adipokinetic hormones and corazonin are evolutionarily related displaying both structural and functional similarities, as well as a co-evolution of ligands and receptors (Cazzamali et al, 2002; Veenstra, 1994).

Corazonin (CRZ) is a neuropeptide composed of eleven amino acid residues – Glu-Thr-Phe-Gln-Tyr-Ser-Arg-Gly-Trp-Thr-Asn – and found in a variety of insect species (Veenstra, 1989; Veenstra 1994). Corazonin has different physiological effects in various insect species and seems to affect functions in a species-specific manner (Choi et al, 2005). It was originally isolated from the corpora cardiaca of the American cockroach, Periplaneta Americana, where it functioned as a cardiostimulatory peptide (Veenstra, 1989). Corazonin, however, demonstrates no other cardioactive functions in other insect which it has been discovered, including grasshoppers, crickets, and moths.
Drosophila CRZ-GFP

Mitchell Allgyer

(Nassel, 2002; Veenstra, 1991). It plays crucial roles in a variety of regulatory mechanisms in different insects, such as pigmenting, molting, morphogenically changing, and even possibly circadian pacemaking. In the migratory locust, Locusta migratoria, corazonin induces dark pigmentation in the cuticle, and when injected into the albino mutant, it causes the development of the dark color (Tanaka, 2000; Tawfik et al., 1999). It also initiates ecdysis in the moth, Manduca sexta, and reduces the rate of silk spinning in the silkworm, Bombyx mori, prior to the pupal stage (Kim et al., 2004; Tanaka et al., 2002). Corazonin is expressed in Drosophila melanogaster during the embryonic, larval, pupal, and adult stages although its exact function of is not known. However, it can be inferred that it acts as both a neurohormone and a neuromodulator since its immunoreactive axons terminate at the neurohemal releasing sites and within the central nervous system, respectively (Choi et al., 2005). Furthermore, it may possibly serve as a clock-output gene whose functional levels are posttranslationally controlled by central clockworks (Choi et al., 2005; Park et al., 2000).

Because the function of neuropeptide genes is often elicited by the anatomical characteristics of peptidergic neurons, new strategies must be employed in order to visualize and analyze the expression pattern of the corazonin gene in Drosophila melanogaster (Choi et al., 2005). Since its discovery, green fluorescent protein (GFP) has become an important and popular reporter protein in many model systems, including Drosophila because it is expressed and thus can be monitored in intact living cells and tissues. In 1962 green fluorescent protein was first discovered in the jellyfish, Aequorea victoria, as a companion protein to aequorin, the chemiluminescent protein, and described as a protein exhibiting a bright green fluorescence upon excitation with
ultraviolet (UV) or blue light (Shimomura et al, 1962). The originally discovered green fluorescent protein is a small protein of approximately 27kDa and 238 amino acid residues in length and is present as a monomer in solution (Brand, 1995; Rizzuto et al, 1995). However, the major breakthrough in green fluorescent protein research occurred when the gene was cloned and when it was demonstrated that the protein caused fluorescence in other organisms, meaning that the gene contained all the information required for the synthesis of the chromophore and no jellyfish-specific enzymes were necessary (Chalfie et al, 1994; Prasher et al, 1992). Green fluorescent protein was shown to be a vital reporter for gene expression in both bacteria and Caenorhabditis elegans in which it was under the transcriptional control of the mec-7 promoter, which is activated in certain C. elegans mechanosensory neurons (Chalfie et al, 1994). The expression of green fluorescent protein, which is nontoxic in organisms other than the jellyfish, does not interfere with normal cellular development and function; in addition, it can be viewed with fluorescein filter sets without becoming photobleached (Yeh et al, 1995). Also in order to make green fluorescent protein more useful, much of the research being conducted in flies with it utilizes the GAL4-UAS enhancer trap technique, which helps target the expression of green fluorescent protein (Brand and Perrimon, 1993). This technique is so beneficial because it allows for the controlled expression of the reporter protein only in cells containing the GAL4 transgene, since the reporter’s transcription is under the control of the GAL4 upstream activation sequence (UAS) which is activated by the GAL4 transcription factor (Brand and Perrimon, 1993; Giniger et al, 1985).

In this study, green fluorescent protein was employed as a reporter protein for the analysis of corazonin-expressing neurons. However, the GAL4-UAS system was not
used for this study. A transgenic line was established through the formation of a DNA construct, pCrz-mCD8-GFP, and its subsequent microinjection into embryos in order to generate the Crz-GFP transgenic fly strain. Microinjection of the DNA construct leads to stable germ line transformation, which is achieved by utilizing the *Drosophila* transposon – P element (Rubin and Spradling, 1982; Spradling and Rubin, 1982). The DNA construct just formed is inserted between the terminal repeats of a P element lacking the transposase gene, and so it requires a helper plasmid which contains the transposase gene but missing the terminal repeats. This method of germ line transformation leads to random insertion of the DNA into the genome generally at a frequency of one insertion per diploid genome. This transgenic fly strain has green fluorescent protein expression directed to the cell surface of corazonin-expression neurons because of mCD8-GFP (a fusion protein between mouse lymphocyte marker CD8 and green fluorescent protein, Lee and Luo, 1999). As a reporter, mCD8-GFP is extremely effective because mCD8 is a transmembrane protein so it labels the cell surface and axons and dendrites have a high surface/volume ratio so it is highly concentrated in these neuronal processes (Lee and Luo, 1999).

**Materials and Methods**

**PCR of mCD8 and GFP genomic DNA**

Primers were designed and manufactured in order to amplify mCD8-GFP genomic DNA by polychain reaction (PCR). The primers were designed based on the published sequences for mouse lymphocyte marker CD8 and green fluorescent protein but with the substitution of restriction sites so that the PCR products could be effectively
Drosophila CRZ-GFP

subcloned into various vectors. Four primers were ultimately designed so that mCD8 and GFP could be amplified separately – (1) mCD8-NotI-f, (2) GFP-KpnI-r, (3) BamHI-f, and (4) BamHI-r (Table 1).

TABLE 1: PCR Primer Sequences for Amplifying mCD8 and GFP

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCD8-NotI-f</td>
<td>GCGGGCGGCCGCAAAATGGCCTC</td>
</tr>
<tr>
<td>GFP-KpnI-r</td>
<td>GCGGTACCTATTATTTGTATAGTTCATCCATGC</td>
</tr>
<tr>
<td>BamHI-f</td>
<td>CACAGCCGCCGGATCCATGAGTAAAGG</td>
</tr>
<tr>
<td>BamHI-r</td>
<td>CCTTTACTCATGGATCCGCGCTGTG</td>
</tr>
</tbody>
</table>

Genomic DNA was isolated from UAS-mCD8-GFP transgenic flies using DNAzol reagent (Gibco BRL) according to the manufacturer’s protocol. Full-length mCD8 and GFP genomic products were obtained by PCR using UAS-mCD8-GFP genomic DNA as a template and Pfu Turbo as the DNA polymerase. For mCD8, the forward primer is mCD8-NotI-f and the reverse primer is BamHI-r, while the forward primer for GFP is BamHI-f and the reverse primer is GFP-KpnI-r. During the PCR reaction, the genomic DNA progressed through 30 cycles of a six step procedure consisting of denaturation, annealing, and extension. The two PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA).

Next, mCD8 and GFP PCR products were subcloned into separated pBluscript SK vectors. Not I and BamH I were used to digest mCD8 and its SK vector, while GFP and another sample of the SK vector were digested using BamH I and Kpn I. The mCD8 and
GFP digestion products were ligated into the SK vector T4 ligase. The mCD8-SK and GFP-SK plasmids were transformed using DH5alpha competent cells. Blue/white screening was utilized in order to distinguish between colonies which contained inserted DNA sequence; eight white colonies containing inserted DNA from each plasmid were collected and inoculated. The inoculated colonies were purified using QI Pep Spin Miniprep Kit (Qiagen, Valencia, CA) and analyzed by gel electrophoresis to ensure the correct size insert. At least three colonies from each plasmid containing the correct size insert were sequenced to detect any nucleotides mismatched during PCR (Table 3).

**TABLE 3: Sequencing Reactions for mCD8 and GFP**

<table>
<thead>
<tr>
<th>Colony</th>
<th>Direction</th>
<th>Primer</th>
<th>Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCD8</td>
<td>forward</td>
<td>T3</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>#1</td>
<td>reverse</td>
<td>T7</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>mCD8</td>
<td>forward</td>
<td>T3</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>#2</td>
<td>reverse</td>
<td>T7</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>mCD8</td>
<td>forward</td>
<td>T3</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>#8</td>
<td>reverse</td>
<td>T7</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>GFP #</td>
<td>forward</td>
<td>T3</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>reverse</td>
<td>T7</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>GFP #</td>
<td>forward</td>
<td>T3</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>#7</td>
<td>reverse</td>
<td>T7</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

The genomic sequences were too large to sequence using just one primer, so the forward and reverse primers for each colony were used to ensure the whole gene was sequenced accurately.

**Subcloning of pCrz-mCD8-GFP**

Once the sequences of mCD8 and GFP were verified and matched with the published sequence, mCD8 and GFP were subcloned together into a single pBluescript SK vector. Two digestion reactions were initially set up – (1) digest GFP out of its SK vector and ligate into mCD8-SK plasmid and (2) digest mCD8 out of its SK vector and ligate into GFP-SK. For reaction 1, mCD8-SK and GFP-SK were digested with BamH I
and Kpn I, while in reaction 2, mCD8-SK and GFP-SK were digested with Not I and BamH I. The digestion products from both reactions were separated by gel electrophoresis, and the bands corresponding to mCD8 and GFP were extracted from the gel. These extracted bands were subsequently purified by QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). A ligation reaction was performed for each one of the digestion reactions, and the mCD8-GFP-SK plasmids were transformed with DH5-alpha competent cells. Five colonies were inoculated and subsequently purified using the QIIPrep Spin Miniprep Kit (Qiagen, Valencia, CA). A small portion of the purified product was digested with Not I and Kpn I to verify the correct ligation of mCD8-GFP.

Once mCD8 and GFP were successfully ligated together, a triple digestion and ligation was performed in order to form the final DNA construct pCrz-mCD8-GFP-pCasper4. First, mCD8-GFP was digested from the SK vector with Not I and Kpn I; second, the 0.8kb corazonin promoter (pCrz) was digested from the pGEM-T-Easy vector (Promega, Madison, WI) with Spe I and Not I; and finally, the pCasper4 vector was digested with Spe I and Kpn I. mCD8 and pCrz were separated from their vectors using gel electrophoresis, and the corresponding bands were extracted and purified. The pCasper4 digestion product was also purified. Simultaneously, mCD8-GFP and pCrz were subcloned into the pCasper4 vector, and the plasmid was transformed with DH5-alpha competent cells. Eight colonies of pCrz-mCD8-GFP-pCasper4 were inoculated and purified; a small volume of the purified plasmid was digested and verified by gel electrophoresis. The ligation sites between pCrz, mCD8-GFP, and pCasper4 were verified to contain the correct sequence through three sequencing reactions.
TABLE 4: Sequencing Reactions of pCrz-mCD8-GFP-pCasper4

<table>
<thead>
<tr>
<th>Ligation Site</th>
<th>Primer Description</th>
<th>Denaturation °C</th>
<th>Denaturation sec</th>
<th>Denaturation °C</th>
<th>Denaturation sec</th>
<th>Annealing °C</th>
<th>Annealing sec</th>
<th>Extension °C</th>
<th>Extension sec</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCasper4-pCrz</td>
<td>pCasper4 forward primer</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
<td>15</td>
<td>60</td>
<td>240</td>
<td>30</td>
</tr>
<tr>
<td>pCrz-mCD8</td>
<td>pCrz 250bp upstream primer</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
<td>15</td>
<td>60</td>
<td>240</td>
<td>30</td>
</tr>
<tr>
<td>GFP-pCasper4</td>
<td>pCasper4 reverse primer</td>
<td>94</td>
<td>120</td>
<td>94</td>
<td>30</td>
<td>50</td>
<td>15</td>
<td>60</td>
<td>240</td>
<td>30</td>
</tr>
</tbody>
</table>

The pCasper4 forward and reverse primers corresponding to the known sequences of pCasper4 vector on both sides of the multiple cloning site, and the pCrz 250bp upstream primer recognizing the corazonin promoter.

Figure 1: Select gel electrophoresis images from formation of pCrz-mCD8-GFP-pCasper4 construct.
A: Digestion of mCD8-SK colonies to identify correct insert; wells 2, 3 and 9 show the correct size band for mCD8 at ~700bp. B: Digestion of GFP-SK colonies to verify inserts; wells 2, 6, 7, 8 and 9 all show the correct size band at ~700bp. C: Digestion of both mCD8-SK and GFP-SK for the ligation of mCD8 and GFP. D: Digestion of pCrz, mCD8-GFP, and pCasper4 in preparation of triple ligation to form pCrz-mCD8-GFP-pCasper4. E: Digestion of pCrz-mCD8-GFP-pCasper4 transformed colonies to verify the correct insert by size; wells 2, 4, 6 and 8 have bands of the appropriate size around 2.2kb. F: Digestion of pCrz-mCD8-GFP-pCasper4 transformants prior to microinjection to test insert.
Microinjection of pCrz-mCD8-GFP-pCasper4 Construct

The prepared pCrz-mCD8-GFP-pCasper4 construct was injected into w+ embryos in order to generate the transgenic line. Approximately ten bottles of w+ flies were prepared for the injection process to ensure an ample number of flies. Two days before the injections begun, three laying bottles outfitted with grape juice plates containing a little yeast paste in the center were prepared and approximately 200 flies were transferred to each laying bottle. The grape juice plates were changed each day prior to the injections to cause the female flies to discard their hold embryos, and then on the days of injections, the grape juice plates were changed every 40 minutes to ensure that the embryos were in the correct stage of embryogenesis for the injection. The injection DNA was prepared by mixing 15 micrograms of plasmid DNA (final 5 micrograms/microliter) and 10 micrograms of delta2-3 helper plasmid (2 micrograms/microliter). The DNA is then vacuum dried and redissolved in 14 microliters of distilled water, 2 microliters of 10x injection buffer, and 4 microliters of green food dye. The injection DNA was placed dropwise into the injection needles – pulled microcapillary tubes. The embryos were collected from the laying bottles by replacing the grape juice plate and scraping the embryos from the grape juice and agarose medium with a paintbrush. The embryos were spread evenly on a slide and coverslip covered by double-sided tape. The embryos were dechorionated by placing two slides with double-sided tape together and then pulling them apart. The dechorionated embryos were carefully transferred to a smaller strip of double-sided tape with the embryos posterior end facing the near edge of the slide. Embryos were then selected for injection based on age (i.e. a stage prior to the pole cells being visible) and damage; embryos suited for injection have a darker color and the
granules appear evenly distributed throughout the embryo. The embryos were then punctured in the posterior end by the injecting needle using the micromanipulator controls; the DNA was carefully injected just inside the posterior end where the germ cells are located. The non-injected embryos were discarded, and the slide holding the injected ones was transferred to a moist container and incubated at 25°C. Once the larvae have hatched between 24 and 48 hours after injection, they were transferred to vials filled with food—made with dextrose, cornmeal, agar, yeast, and methyl paraben—and yeast dust and moved to a humid environment in the 25°C incubator.

Approximately 10 to 14 days after the larvae were transferred, they eclosed into adult flies, compiling the G₀ generation. All male and virgin G₀ flies were crossed with w⁺; Bl/cyo flies; males were separately mated with three virgin females, while females were separately crossed with three males. The progeny of this cross was the G₁ generation, and once they became adults, they were examined for the transformant by eye color. The transformant males and females were separated as to prevent mating, and then the four of the male transformants were individually crossed with w⁺; Bl/cyo virgin females. The progeny of this cross, G₂, were used as a means to determine chromosomal location based on the genetic markers.

**GFP Staining**

To determine the expression pattern of Crz-GFP, the central nervous system (CNS) of larvae was dissected in 1x PBS. The brains and ventral nerve cords (VNC) were placed on ice in wells containing PBS. The CNS was stained for green fluorescent protein expression using 20% PFA as a fixative. The GFP-expressing CNS’s were cleared of unneeded tissues in 60% glycerol and mounted on slides in Vectashield
medium (Vector Laboratories, Burlingame, CA). The images of the CNS’s were obtained using an Olympus BX-61 microscope equipped with a camera and processing software.

Results

Crz-GFP Microinjection

The microinjection of pCrz-mCD8-GFP-pCasper4 was a difficult process, but it lead to positive results. The injections were performed over a six day period in which injections were being done for around five hours per day. During each round of injections, approximately 15 embryos were injected so around six or seven slides of injected embryos were generated a day. Of those embryos that were injected, only an average of two per slide developed into larvae, so out of the nearly 540 injected, only 70 or so hatched into larvae. However, these numbers are slightly skewed because they were lower the first few days and higher the later days as the process of microinjection became more familiar and easy. Of the embryos that actually developed into larvae, around 25 developed into G₀ adults.

These G₀ adults were the embryos that survived the microinjection although it can not be determined yet if the DNA construct was transformed. These adults were crossed with w⁺; Bl/cyo flies in order to determine this fact by examining the presence of transgenics in the G₁ progeny. Of the 25 G₀ adults crossed, twelve of these flies were infertile and sterile as examined by the lack of reproduction over a two week period, and so only thirteen G₀ adults were able to reproduce. Of these thirteen flies, two of the crosses produced transgenic progeny as determined by the presence of reddish eyes.
These reddish eyes are caused by the presence of the w⁻ marker gene, which produces a dominant red phenotype, on the inserted P element. Because the DNA is injected into the germ cells, the developing embryos retain the white phenotype of its parents, but because the w⁻ marker phenotype is in the germ cells, it will be seen in a portion of the progeny. Four transgenic male G₁’s from one G₀ and two from another G₀ were crossed with virgin w⁺; Bl/cyo in order to determine chromosomal location. Although it is not clear yet whether the Crz-GFP DNA was inserted on the second or third chromosome, it has been inferred that it’s neither on the X chromosome or the fourth chromosome because male G₂’s had the red-eye phenotype and because not seen in all genetic marker phenotypes.

**Crz-GFP Expression Patterns**

The expression pattern of Crz-GFP transgenic flies was consistent with previously published accounts of corazonin expression. Expression signals were detected in both brain lobes and also in the VNC. In the brain lobes, a strong signal is detected the DL neurons, a cluster of three neurons in the dorsolateral region of the protocerbrum, and also a weaker signal is seen in the DM neurons, located more medially in the dorsomedial region (Choi et al, 2005). Crz-GFP signaling is also located in eight pairs of bilateral neurons in the VNC.

![Figure 2: GFP staining in larval CNS. A: Schematic representation of corazonin expression in larval CNS. B: Larval CNS from Crz-GFP 2-1. C: Larval CNS from Crz-GFP 1-1.](image_url)
Not only are the Crz-expressing neurons signaled by GFP, but the neuronal processes (i.e. axons and dendrites) containing corazonin are also signaled by GFP. From the dorsolateral neurons, projections extend to the medial brain region and then bifurcate into two branches. One branch continues to the midline and then turns and extends ventrally, while the other branch travels ventrally, turning dorsally, making a loop, and finally terminates at the ring gland and aorta. DM neurons move contralaterally across the midline. The neurons in the VNC have several projections; one crosses the midline and travels towards the symmetrical cells. Another process projects dorsally, bifurcates, and extends both anteriorly and posteriorly (Choi et al, 2005).

Discussion

In this project, the transgenic fly line Crz-GFP was generated. This particular fly strain couples the corazonin promoter (Crz) with the fusion protein mouse CD8 (mCD8) and the reporter protein green fluorescent protein (GFP). This construct is will be an extremely advantage in additional experimental studies because the corazonin promoter is directly labeled with GFP and no additional crossings are needed. In *Drosophila*, the role of corazonin is not known and so the analysis of its expression patterns may help to elucidate its function.

This construct will be of particular use for determining corazonin function because it combines the reporter gene with the neuropeptide promoter by means that do not involve crossings. Genetic crossings have shown tremendous ability to display expression patterns within the model organism *Drosophila* using the GAL4-UAS transcription factor activation system, but genetic crossings have their shortcomings.
When genetic crosses are being used as a means of showing expression, mutations have to be indirectly introduced into the model system and then crosses still have to be carried out for the mutation to be expressed. However, with corazonin neurons being directly labeled with GFP, mutations can be entered into the system and no further crosses have to be performed.

Also green fluorescent protein shows great attributes as a reporter protein, and it has many advantages over other reporter proteins. GFP allows for the visualization of the global static and dynamic patterns in living tissues and organisms. This is a particularly huge advantage of GFP, considering all other reporter proteins use damaging staining procedures so expression is always in fixed tissues. So in order to examine temporal characteristics of neurons, extraction and staining of many cells from different times is required, but with GFP, temporal characteristics can be examined dynamically in living cells. GFP also does not need the elaborate staining procedures and can be fixed in about an hour, while other reporter proteins require antibody staining and sometimes takes overnight to complete. And of particular interest to the field of Drosophila neurogenetics, GFP is most readily observed in neural and cuticular tissues, and it displays spatially complex patterns in larval brains where beta-galactosidase staining was absent (Timmons et al, 1997).

**Acknowledgements**

I would like to thank J. Park, my faculty mentor and PI, for his guidance on this project and for also giving me the opportunity to work in his lab. I would also like to thank G. Lee for her tremendous help and input on microinjections, as well as my other fellow lab members for their help and support on this endeavor.
References


