Characterization of non-coding alpha, beta, and gamma heat shock tran

Lauren Meyer

Follow this and additional works at: https://repository.lsu.edu/honors_etd

Part of the Biology Commons

Recommended Citation
https://repository.lsu.edu/honors_etd/1028

This Thesis is brought to you for free and open access by the Ogden Honors College at LSU Scholarly Repository. It has been accepted for inclusion in Honors Theses by an authorized administrator of LSU Scholarly Repository. For more information, please contact ir@lsu.edu.
Characterization of non-coding alpha, beta, and gamma heat shock transcripts in the polytene chromosomes of *Drosophila melanogaster*

by

Lauren Meyer

Undergraduate honors thesis under the direction of

Dr. Patrick DiMario, PhD

Department of Biological Sciences

Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of the Upper Division Honors Program.

April, 2022

Louisiana State University
& Agricultural and Mechanical College
Baton Rouge, Louisiana
Abstract

Heat shock, a stress condition referring to the exposure of cells to excessive heat, inhibits normal gene transcription within the nuclei of cells and induces the transcription of specific heat shock genes. This project focuses on the heat shock genes α, β, and γ which are found within polytene cytological regions 87B-87C of Drosophila melanogaster. RNA-binding proteins with RGG/RG motifs such as Nopp140-RGG and Fibrillarin bind to the non-coding α, β, and γ RNA transcripts and form condensates at these loci. Similar condensates are believed to form through a process referred to as liquid phase condensation. As the RGG/RG proteins bind these ncRNA transcripts, they are prevented from binding transcripts encoding a heat shock protein of 70 Kda (Hsp70) which has a primary function of protecting the cell from excess heat.

The primary objective of this thesis is to ligate heat shock genes α, β, and γ into a pBlueScript vector and transform the resulting plasmid into Eschrechia coli for in vivo production of these RNA transcripts. While a ligation was accomplished between alpha, beta, and gamma genes and a pBlueScript vector, a successful E. Coli transformation was not achieved, and further progress on this project is needed to purify and characterize alpha, beta, and gamma ncRNA molecules. A secondary objective of the thesis was to better characterize how the RNA-binding proteins bind to alpha, beta, and gamma ncRNA transcripts as this is not well understood. While we were able to generate a secondary structure of a portion of the alpha-gamma ncRNA repeats using Vienna RNAfold software, further work is needed to describe where the RNA-binding proteins bind on the alpha, beta, and gamma RNA transcripts.
**Introduction**

Heat shock is a stress condition in which cells are exposed to excessive heat. When heat shock occurs, normal transcription is inhibited, and is replaced by the transcription of heat shock genes, which are found in all organisms. This project specifically focuses on the heat shock genes which lie within the cytological regions 87B-87C on the right arm of the 3rd chromosome in the polytene cells of *Drosophila melanogaster*. This heat shock locus, residing within the polytene chromosomes, becomes transcriptionally active and swells upon heat shock. Genes within the 87C region encode for a heat shock protein of 70 Kda, also known as Hsp70, which functions to protect the cell from excess heat (Lis et al., 1981). Other repeated genes found within the 87B-87C region produce repeats *alpha*, *beta*, and *gamma* transcripts, which are non-coding RNA molecules (ncRNAs) (Lis et al., 1981). Upon heat shock, RNA-binding proteins Nopp140-RGG and Fibrillarin redistribute from the nucleolus into the nucleoplasm of the nucleus. Each of these RNA-binding proteins contain a RGG/RG motif, which is responsible for the RNA-binding property. Nopp140-RGG and Fibrillarin could potentially lead to problems as these RNA-binding proteins can bind to the RNA transcripts encoding heat shock proteins, such as Hsp70, and inhibit the heat shock proteins from their original function of protecting the cell from excess heat.
Localization of RNA-binding proteins to the cytological region 93D during heat shock was demonstrated earlier by immunoprecipitation and fluorescence RNA:RNA in situ-hybridization studies (Prasanth et al., 2000). The result of the binding phenomenon between RNA-binding proteins and RNA transcripts within the nucleus of *D. melanogaster* under both stressed and unstressed conditions is referred to as omega speckles (Prasanth et al., 2000). In these earlier studies, antibodies were used against several RNA-binding proteins, and in the absence of stress conditions, these RNA-binding proteins were observed to bind to cytological regions which are undergoing active transcription uniformly (Prasanth et al., 2000, Fig. 1). In unstressed conditions, omega speckles are more numerous and smaller in size within the *D. melanogaster* nucleus (Prasanth et. al., 2000, Fig. 1). However, when cells were exposed to excessive heat, these RNA-binding proteins relocated almost exclusively to the 93D cytological region which encodes for non-coding ω RNA transcripts (Prasanth et. al. 2000, Fig. 1). When the cell was under heat shock stress, omega speckles became larger in size and less numerous over time until the speckles exclusively accumulated on the 93D cytological region (Prasanth et. al. 2000, Fig. 1). While the functioning of the ncRNA omega transcripts are not well understood.

![Diagram](image-url)
outside of its sequestration of RNA-binding proteins during heat shock, studies show that these transcripts are essential for survival of *D. melanogaster* (Prasanth et al., 2000).

**Figure 2:** Phase-contrast and fluorescence microscopy methods localizing Nopp140-RGG and Fibrillarin to the 87C and 93D cytological regions on the polytene chromosomes after heat shock, but not the 87A cytological region. Nopp140-RGG was exposed as a fusion protein with the Green Fluorescent Protein (GFP). Antibodies against Fibrillarin co-localized Fibrillarin to the same heat shock loci 87C and 93D, but not 87A.

Fluorescence work by the DiMario lab has shown the RNA-binding protein Nopp140-RGG and Fibrillarin locate exclusively to the 87C and 93D cytological regions or “puffs” within polytene chromosomes in response to heat shock. Heat shock genes within cytological region 87A produce only *Hsp70* mRNA transcripts, but Nopp140-RGG and Fibrillarin did not localize to this region. Binding between the *alpha, gamma, beta* ncRNAs at 87C and *omega* ncRNAs at 93D and the RNA-binding proteins can prevent the RNA-binding proteins from binding heat shock protein transcripts such as Hsp70 (Prasanth et al., 2000). As the RNA-binding proteins Nopp140-RGG and Fibrillarin are prevented from binding transcripts encoding Hsp70, the function of Hsp70 is no longer inhibited. Other work within the DiMario lab has utilized Nopp140-RGG to characterize the distribution of nucleolar proteins during oocyte and early embryonic developmental stages of *D. melanogaster* (McCain et al., 2006). Their observations showed that Nopp140-RGG had a uniform distribution throughout oocyte nuclei which lacked transcriptional activity entirely. This suggests that aggregates formed by Nopp140-RGG within
D. melanogaster nuclei require the presence of the alpha, beta, gamma and omega ncRNA transcripts encoded by genes within 87C and 93D. This study also emphasizes that RGG-containing proteins do not form aggregates on their own. The exact structural biochemistry behind the binding of the alpha, beta, and gamma ncRNA transcripts and the RNA-binding proteins is unknown, and the alpha, beta, and gamma transcripts are not well characterized. The major goal of this thesis is to better characterize these specific alpha, beta, and gamma heat shock transcripts, and to further describe how the RGG-containing proteins bind these transcripts through methods of in vivo transcription and outside computer work.

Figure 3: Sequence of the lncRNA gene based on the CR32865 clone described at FlyBase. The sequence denotes separate alpha, beta, and gamma units within 87B-C based on Hackett and Lis (1981). The alpha sequence is denoted in orange, the beta sequence is denoted in pink, and the gamma sequence is denoted in blue (Hackett and Lis, 1981). Red sequences denote HindIII restriction enzyme sites (Hacket and Lis, 1981). Each alpha, beta, and gamma segment begins...
and ends with highlighted segments in a different color than the segment itself based on the right and left junctions denoted in the Hackett and Lis (1981) article. The segments which are not highlighted at the 5’ and 3’ ends are BamHI and EcoRI restriction enzyme sites, respectively.

**Alpha-gamma transcript:**

![Transcript Sequence](image)

**Figure 4:** An alpha-gamma transcript sequence based on clone CR32865 at cytological region 87B13 found on FlyBase. The yellow highlighted regions denote HINDIII sites which flank either end of the region.

In-situ hybridization in past studies demonstrates the mapping of alpha-beta and alpha-gamma units to the 87C cytological region (Lis et al., 1981). Alpha-beta and alpha-gamma elements are arranged in tandem-repeats throughout this cytological region (Lis et al., 1981). The individual functions of each of these elements are not well understood, but the level of transcription of each of these elements is confirmed to increase in response to heat shock along with heat shock gene, *Hsp70* (Lis et al 1981). A portion of the gamma element lying upstream the 5’ end of the alpha-beta unit shares approximately 98% homology with a portion of the
sequence lying at and upstream the 5’ end of the Hsp70 gene (Hacket and Lis, 1981). This may be an explanation for the synchronous upturn in the level of transcription in these regions of 87C (Hacket and Lis, 1981).

**Liquid Phase Condensation**

Current research on liquid phase condensation demonstrates the medical relevance of the process of RNA molecules assembling with other proteins into membraneless organelles such as the assembly of alpha, beta, and gamma ncRNA transcripts with RNA-binding proteins (Boeynaems et al., 2018). A defining feature of these membraneless organelles is “adhesive motifs”, or a portion of the RNA molecules which encourage the binding between the RNA molecule itself and the RNA-binding protein (Boeynaems et al., 2018). Research on liquid phase condensation also notes that RNA-binding proteins participating in liquid phase condensation often contain intrinsically disordered regions (IDRs) which are often repetitive in their amino acid sequence (Boeynaems et al., 2018). The “adhesive motif” is one focus of our studies within this project as we inquire which folded region within the secondary structure of the non-coding alpha, gamma, and beta RNA transcripts binds with the RGG/RG region, or IDR, of the RNA-binding proteins Fibrillarin and Nopp140-RGG. The arginine repeats within the repeating RGG motifs are important as arginine interacts with the nucleotide bases within RNA molecules by hydrogen bonding and π-stacking (Chong et al., 2018). π-stacking refers to the noncovalent attractive force between two pi bonds in which the two structures align in a parallel orientation due to the distribution of electrons within pi bonds. RGG/RG motifs are closely associated with the formation of membraneless organelles such as stress granules (SGs), and have been identified in many human proteins (Chong et al., 2018). For example, nucleolar protein nucleolin found in humans contains RGG/RG motifs, and often forms nucleolar aggregates. While D. melanogaster
does not produce nucleolin, it does produce Nopp140-RGG with a similar RGG/RG domain and predicted nucleolar function.

Liquid phase transition becomes applicable to medicine in the focus of SGs, which form reversibly when cells are exposed to stress conditions (Boeynaems et al., 2018). Ongoing research on SGs shows that missense mutations in IDRs of these assemblies can cause Amyotrophic Lateral Sclerosis (ALS) (Boeynaems et al., 2018). The exact mechanism behind the formation of the mutant SG aggregates and how it relates to the transition of wild type SGs to mutant ALS-causing aggregates is not well understood (Boeynaems et al., 2018). As the importance behind RGG/RG proteins surfaces within the realm of liquid phase condensation and medicine, researchers strive to better characterize the exact roles and processes behind the assembly of the membraneless organelles (Boeynaems et al., 2018).

The objective of this thesis is to better characterize the alpha, beta, and gamma ncRNA transcripts expressed and located within the cytological regions 87B-87C of D. melanogaster polytene chromosomes. This objective would be achieved by successfully cloning the genes encoding the transcripts into an E. coli plasmid vector, resulting in the transcription of the ncRNA alpha, beta, and gamma transcripts in vitro. This would allow for the resulting non-coding alpha, beta, and gamma RNA transcripts to be purified and further characterized. Another objective of this thesis is to identify the secondary structure of the ncRNA transcripts, and to relate this secondary structure to the RGG/RG region of specific RNA-binding proteins in order to better understand how the RNA-binding proteins, such as Nopp140-RGG and Fibrillarin, bind to the ncRNA alpha, beta, and gamma transcripts.
Materials and Methods

Polymerase Chain Reaction (PCR) Amplification of “alpha-gamma” unit

All DNA oligonucleotide segments were ordered from Integrated DNA Technologies (IDT) and were based on the clone CR32865 which can be found on FlyBase (Fig. 4). PCR technology was used to amplify the “alpha-gamma” segment of interest from genomic *D. melanogaster* DNA into many copies. 1 μL of each oligonucleotide segment (forward and reverse primers) at 10 μM were added to 35.5 μL of Milli-Q water, 1 μL of template *D. melanogaster* genomic DNA, 10 μL GC 5X buffer, 1 μL dNTPs at 10 μM, and 0.5 μL Phusion enzyme. The GC buffer, dNTPs, and Phusion enzyme solutions were all ordered from New England Biolabs. The conditions of the PCR included 40 cycles of 98 °C for 5 minutes, 98 °C for 1 minute, 64 °C for 30 seconds, and 72 °C for 1 minute and 30 seconds.

Analytical Agarose Gel Electrophoresis

We used analytical agarose gel electrophoresis in order to ensure the DNA product amplified in the prior PCR reaction is the proper length based on the “alpha-gamma transcript” (Fig. 4). Aliquots consisting of 5 μL PCR product, 5 μL Milli-Q water, and 1 μL blue dye were loaded into one well of the prepared agarose mini gel. A 1 Kb DNA ladder (New England Biolabs) was loaded into an adjacent well for size reference. 1X TAE buffer was used to flood the agarose gel prior to the start of running the electrophoresis. The electrophoresis was run for about 30 minutes to an hour in order to allow the DNA to migrate across the gel. The gel was soaked in a shaking container of 0.001% ethidium bromide in TAE for 5 minutes before viewing under UV light.
**Gel Purification of “alpha-gamma” PCR Product**

After observing the length of our “alpha-gamma” PCR product by analytical agarose gel electrophoresis, we loaded the same PCR product into wells of a larger 0.7% agarose gel so that the PCR product could be excised for further purification of the DNA. A sample consisting of the remaining “alpha-gamma” PCR product and blue dyes was divided evenly among wells, maximizing the amount of PCR product in each well. A 1 kb ladder was loaded into an adjacent well for length reference. The gel was flooded with a 1X TAE buffer, and then the gel electrophoresis was run for approximately 1 hour. The gel was subsequently soaked in ethidium bromide for 5 minutes prior to viewing under UV light. Using UV light guidance, we used a razor blade to excise the bands of DNA from each lane, and placed the bands into clean eppendorf tubes. We further extracted the *alpha-gamma* DNA from the agarose gel by using a freeze thaw extraction procedure. After we froze the eppendorf tubes overnight at -20 °C, we thawed the bands by placing the eppendorf tubes in a 37 °C water bath for approximately 2 minutes. Then, we removed the bands (and aqueous portion) from the eppendorf tubes and placed them on parafilm. With the edge of a razor blade, we chopped the gel into a paste and evenly divided this paste back into the tubes. Once the gel was in a paste, we placed the eppendorf tubes into a -80 °C freezer for 5 minutes, then immediately removed the tubes and placed them into a 37 °C water bath to thaw for approximately 2 minutes. We repeated this freeze-thaw method, and let the tubes sit in the 37 °C water bath for approximately 2.5 to 3 hours. After the freeze-thaw process was complete, the gel-DNA solution was placed in a -20°C freezer for storage prior to phenol-chloroform extraction.
Phenol-chloroform Extraction of DNA Product

Beginning with the gel-DNA solution which has undergone the previously mentioned “freeze-thaw” process, we determined the total volume of the solution in the eppendorf tube. After determining the total volume, we added an equal volume of phenol to the gel-DNA solution, vortexed this vigorously for around 30 seconds, and then centrifuged the tube at 4°C for 3 minutes. We then extracted the aqueous top layer of DNA from the phenol-DNA solution, and added this aqueous layer into a new eppendorf tube. Into the new eppendorf tube with the aqueous product of the previous extraction, we added an equal volume of chloroform (24 parts chloroform and 1 part isoamyl alcohol). We then vortexed this mixture vigorously for approximately 30 seconds, and then centrifuged this tube for around 2-5 minutes at 4 °C. After centrifuging, we extracted the top aqueous layer and added this aqueous product into a new, clean eppendorf tube. We then repeated the extraction process with chloroform.

Ethanol Precipitation of DNA Product

After extracting DNA using the phenol-chloroform extraction, we added enough 5 M NaCl solution to our aqueous DNA product to produce a 0.2 M NaCl solution. To this, we added 2.5 times the total volume of the sample of 100% ethanol, and let this sit at -20°C overnight. After storing the ethanol-DNA solution overnight, we centrifuged this solution for 15 minutes at 4°C. Being careful not to expel the DNA pellet, if visible, we poured out this solution and added around 750 μL of 70% ethanol. After ensuring that the ethanol contacted all parts of the eppendorf tube, we poured out the solution carefully once again, being careful to not expel the DNA pellet. After letting the eppendorf tube partially dry on a paper towel, we placed the tube in the Speedvac machine for 5 minutes to vacuum dry the pellet of DNA.
After placing the alpha-gamma DNA product from the previous step in the Speedvac for drying, we added 10 μL of Milli-Q water and pipetted this mixture up and down to dissolve the DNA. We then added 1 μL of the DNA sample to the Nanodrop Spectrophotometer for measurement of the concentration and purity of the DNA present.

Figure 5: Plasmid map sent by Thermo Fisher Scientific based on the Gene Block template ordered. The IncRNA gene denoted in this figure refers to the sequence described in Figure 3. “KanR” refers to the Kanamycin resistance gene found within this plasmid.

Transformation of IncRNA gene into E. Coli

As a separate project in an effort to efficiently transform our alpha, beta, and gamma heat shock genes of interest, we ordered a IncRNA Gene Block template from Thermo Fisher Scientific based on the sequence denoted in Figure 3 (Fig. 5). We prepared Kanamycin agarose plates (0.625 g NaCl, 0.625 g yeast extract, 1.25 g tryptone, 2.5 g agar, 50 μg/mL Kanamycin, and 125 mL distilled H2O) to culture E. coli for transformation. Next, we added 1 μL of the
synthetic Gene Block containing the IncRNA gene and a Kanamycin resistance gene to approximately 100 μL of competent *E. coli* cells. We let this plasmid transform into the *E. coli* cells on ice for 30 minutes, shaking the eppendorf tube every 10 minutes to ensure the plasmid and the *E. coli* cells were thoroughly mixed. We then heat-shocked the *E. coli* cell mixture in a 42 °C water bath for 2 minutes. Next, we aseptically added 1 mL of sterile Luria (LB) broth to the *E. coli* cells, and placed the eppendorf tube containing this mixture into a shaker for 45 minutes at 37 °C. We then plated both a high-density and a low-density plate by first plating 100 μL of the resulting *E. coli* solution and creating a bacterial lawn on the plate aseptically (low-density). After centrifuging and disposing of most of the supernatant, we plated the remainder (about 100 μL) of the high concentration *E. coli* cells on a separate Kanamycin agarose plate (high-density). The high-density and low-density plates were both incubated at 37 °C overnight.

Following incubation overnight, we selected 3 colonies to inoculate into 3 sterile tubes containing 1 μL each of LB broth and 50 ng/μL Kanamycin using a sterile toothpick. We then incubated these three tubes in a 37 °C shaking incubator overnight. Following incubation, we took 40 μL from each tube and incubated it into a new sterile test tube containing 10 mL LB broth and 50 ng/mL Kanamycin. Once again, these tubes were incubated overnight in a 37 °C shaking incubator.

Following overnight incubation, we started the preparation of mini-preps. We began by taking 1.5 mL aliquots from the tube and added this into clean eppendorf tubes. These samples were then spun down in a centrifuge for 30 seconds, and then most of the supernatant was disposed of. We then resuspended the *E. coli* cells within each of the samples in 100 μL AlkI solution (0.9 g glucose, 0.37 g EDTA, 2.5 mL 1 M Tris-HCl) and incubated this solution for 5 minutes at room temperature. We then added 200 μL of Alk II (4 mL NaOH, 2 mL 10% SDS, 14 mL dd H₂O) to each of the eppendorf tubes, mixed the samples, and incubated on ice for 5
minutes. Next, we added 150 μL of precooled Alk III solution (7 mL glacial acetic acid, 3 mL dd H2O, 15 mL 5 M Potassium acetate) to each of the samples and incubated this on ice for 5 minutes. We centrifuged the samples for 5 minutes, and poured off the supernatant into a new, clean eppendorf tube. We ethanol-precipitated the DNA within the tube using the same technique as mentioned earlier, and vacuum dried the DNA pellet in the Speedvac for 10 minutes. Lastly, we added 50 μL of Milli-Q water to each of the remaining pellets to resuspend the DNA product.

**Ligation of the PCR product into pBlueScript Vector and Restriction Enzyme Cleavage**

We first had to cleave the plasmids from each project with the appropriate restriction enzymes. Restriction enzyme sites were built into the oligos ordered from IDT (for the gene in Fig. 4 to be ligated into a pBlueScript vector), and were built into the synthetic gene sequence within the Gene Block template as denoted by Figure 3. All restriction enzymes and buffers were ordered from New England Biolabs. In one eppendorf tube, we added 1 μL pBluescript vector in the K-S orientation, 5 μL Cutsmart buffer, 2.5 μL BamHI endonuclease, 2.5 μL EcoRV endonuclease, and 39 μL Milli-Q water. In another eppendorf tube, we added 5 μL of our mini-prep solution, 2 μL Cutsmart buffer, 1 μL BamHI endonuclease, 1 μL EcoRI endonuclease, and 11 μL Milli-Q water.

For the ligation of our alpha-gamma PCR product into pBlueScript, we added 1 μL pBluescript vector (100 ng/μL), 5.3 μL PCR product insert (150 ng/μL), 2 μL 10X buffer, 2 μL T4 ligase, and 9.7 μL Milli-Q water. Prior to this ligation, we had performed phenol-chloroform extraction on the pBlueScript vector cut with restriction enzymes to ensure purity of the DNA. Due to time constraints, we were unable to further transform the pBlueScript ligation into E. coli for transcription of the alpha-gamma ncRNA product.
Results

Figure 6: Two predicted secondary structures of alpha-gamma RNAs transcribed from the alpha-gamma element CR32865 found on FlyBase. The structure pictured to the left is minimum free energy secondary structure, and the structure pictured to the right is the centroid secondary structure. A centroid secondary structure is one in which there is minimum distance between base-pairs (Ding et al., 2005). Both structures were generated by inputting the reverse compliment of the “alpha-gamma” element found in Fig. 4 into the Vienna RNAFold computer program.
Analytical gels were used throughout the length of the thesis work in order to verify the length of PCR products following PCR amplification. The middle lane containing visible bands depicts the length of the alpha-gamma PCR sample to be around 1600 nucleotides (nt) (Fig. 7). The top lane containing a visible smear across its length contains another alpha-gamma PCR sample using the same synthetic primers, but this sample did not amplify efficiently (Fig. 7).
Large 0.7% agarose gels were utilized for gel extraction of PCR DNA products. The bottom-most lane with visible bands contains a 1 DNA Kb marker set for reference in order to determine the length of the alpha-gamma PCR product, which was once again found to be around 1600 nt in length (Fig. 8). The top-most three lanes with visible bands contain the remainder of the alpha-gamma PCR product which was successfully run out on the middle lane of Figure 7 (Fig. 8).
Figure 9: UV image of analytical gel in which the lncRNA Gene Block template and pBlueScript vectors were both run following restriction enzyme cleavage. The lncRNA Gene Block template was run in the middle lane. The pBlueScript vector was run in the top lane. The 1 kb marker was run in the bottom lane for reference.

As shown by the top lane of the agarose gel, the pBlueScript vector was confirmed to be around 3000 base pairs following the cleavage of the plasmid into a linear DNA product using EcoRV and BamHI restriction enzymes (Fig. 9). As shown by the middle lane of the gel, the synthetic lncRNA gene and the remaining Gene Block vector both localized to around 2300 base pairs in length following restriction enzyme digestion by EcoRI and BamHI (Fig. 9). The lncRNA gene and the remaining length of the plasmid following cleavage were the same length, which is why there is co-localization in this region of the gel.
Discussion

Due to time constraints, we were unable to finish cloning the alpha-gamma D. melanogaster gene into the E. coli vector for further transcription and characterization. Further progress is also needed within the second project regarding the lncRNA Gene Block transcript to allow for transcription of alpha, beta, and gamma transcripts within E. coli. We were able to amplify what we think is the alpha-gamma gene segment of interest (Figure 4). However, further sequencing may be needed to verify this PCR product is the alpha-gamma segment of interest as the PCR product localized around 1600 nt on the agarose analytical gel. The expected length of the alpha-gamma transcript would be 1464 nt including the synthetic primers used for PCR amplification. We were also able to extract the DNA successfully from the agarose gel as denoted from our Nanodrop measurements.

Future work in regards to this research project would include completing the transformation of heat-shock genes alpha, beta, and gamma into E. coli for the transcription of non-coding RNA transcripts in vitro. Using pBlueScript vector and our PCR product of the alpha-gamma gene, we were able to produce a ligation product. However, due to time constraints we were unable to transform this ligation into E. coli. Transforming this ligation into E. coli in future work would allow for RNA polymerase within E. coli cells to transcribe the non-coding RNA transcripts of interest for further purification and characterization of these transcripts.

The lncRNA Gene Block template was pre-ligated, and after cleavage with the proper restriction enzymes, we were able to run a small aliquot of the Gene Block on an analytical gel next to the cleaved pBluescript vector. Gel analysis showed that the cleavage of the lncRNA Gene Block template with EcoRI and BamHI, with proper sites built into the lncRNA gene, yielded equivalent lengths of the remaining vector and lncRNA gene. Future work would include
cleaving the \( \text{ln}cRNA \) Gene Block with a separate restriction enzyme (BglII) to separate the \( \text{ln}cRNA \) from the remaining Gene Block vector on the analytical gel. Future progress within the DiMario lab will focus on the transcription of this Gene Block vector within \( E. \text{coli} \) for transcription and future purification of the heat shock transcripts \( \text{alpha, beta, and gamma}. \) Further work is also needed to identify where the RGG/RG proteins bind the secondary structure of the \( \text{alpha-gamma} \) element as denoted in Figure 6 using clues from the purification of heat-shock transcripts.

In conclusion, heat-shock genes \( \text{alpha, beta, and gamma} \) found within polytene cytological regions 87B-87C are important to the survival of \( D. \text{melanogaster} \) and the sequestration of RGG/RG RNA-binding proteins Nopp140-RGG and Fibrillarin during heat shock. Current research on liquid phase condensation highlights the interaction of RGG/RG proteins such as Nopp-140-RGG and Fibrillarin with ncRNA molecules in the formation of membraneless organelles molecules including stress granules, which have a role in the neurodegenerative disease ALS. The objective of this thesis was to transform heat shock genes \( \text{alpha, beta, and gamma} \) within a vector into \( E. \text{coli} \) in order to induce transcription of the non-coding RNA transcripts in vitro for further characterization of the heat shock transcripts, which are poorly understood. While we were unable to achieve this in vitro transcription due to time constraints, we were able to successfully amplify and purify the DNA segment and begin a ligation with the vector pBlueScript. Future work within the DiMario lab on this project is needed to complete this ligation and transformation and purify the non-coding RNAs produced for further studies on the transcripts themselves. Lastly, a second objective of this thesis was to generate a secondary structure of the \( \text{alpha, beta, and gamma} \) RNA transcripts to be able to observe where RGG/RG RNA-binding proteins bind within this structure. While we were able to
generate a secondary structure, further work is needed to determine where RGG/RG proteins
bind (Fig. 6).

Literature Cited


