LOOKING FOR A NOVEL DOUBLE MUTANT: SINGLE DELETION OF GALECTIN-1 AND GALECTIN HOMOLOGUE IN *Drosophila melanogaster*

An Undergraduate Research Scholars Thesis

By

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ABSTRACT

Looking for a Novel Double Mutant: Single Deletion In *Drosophila melanogaster* of Galectin-1 and Galectin Homologue. (May 2013)

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Galectins represent a group of proteins that bind β-galactosyl-containing glycoconjugates and share primary structural homology in their carbohydrate recognition domains. In mammals, galectins contribute to cell-cell interactions, cell surface signaling, regulation in immune responses, and embryo development. With little still known about the lectin-ligand associations concerning galectin, the Galectin-1 and galectin homologue in *Drosophila melanogaster* would make Drosophila a good model system for studying gelectin interactions. A novel Drosophila mutant with a deletion of both Galectin-1 and its homologue may serve to be important in analyzing phenotypes in the study of galectin functions. Several crosses were carried out using FLP mediated mutagenesis while following curly wings and bristle phenotypic markers. DNA extraction, PCR purification, and genomic sequencing were carried out on the last cross's final product. Further experiments to remove an unnecessary bristle marker and cross the novel mutant with an L14 balancer stock are underway in order to investigate the lethality of the galectin deletion through Drosophila development.

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CHAPTER I

INTRODUCTION

Lectins are sugar-binding proteins that are highly specific towards their sugar counterparts. A vast majority of lectins have a role in biological recognition while involving other cells and proteins.[1] Many lectins are non-enzymatic in function and do not originate from the immune system, yet lectins occur ubiquitously in nature. They may bind to a soluble carbohydrate or to a specific carbohydrate domain that is a part of a bigger glycoprotein or glycolipid in order to aid in recognition. They can be found agglutinating certain animal cells and precipitating glycoconjugates.[2]

The S-type lectins, more recently termed galectins, represent a group of proteins that bind β-galactosyl-containing glycoconjugates and share primary structural homology in their carbohydrate recognition domains. Galectins are widely involved and distributed throughout the animal kingdom. Most galectins are soluble proteins which are secreted by an uncommon pathway and require specific reducing conditions to maintain their activity in the absence of ligands.[3] Certain members of the galectin family in humans can promote cell-cell adhesion and cell signaling through cross-linking of cell surface glycans. Some even have potent biological activities, such as the ability to induce apoptosis and metabolic changes, such as cellular activation and mitosis.[4, 5]

A putative Galectin-1 was identified in *Drosophila melanogaster*. Structurally, the Drosophila galectin is a tandem repeat galectin containing two carbohydrate recognition domains connected

by a unique peptide link. This structure suggests that like galectin found in mammals, Drosophila galectin may take part in cell-cell signaling and recognition. During embryogenesis, Drosophila galectin has a specific tissue distribution as it is seen in somatic and visceral musculature and in the central nervous system. Since Drosophila galectin is similar to other insect lectins and has a unique distribution in development, Drosophila galectin may function in both embryogenesis and in host defense. However, little is still known about lectin-ligand interactions in Drosophila. Furthermore, a BLAST analysis of the Berkeley Drosophila Genome Project with consensus sequences from various lectin families has identified a possible galectin homologue.[6]

The purpose of this project is to create a novel mutant, one without the known Galectin gene and without the possible galectin homologue gene. The function of galectin in Drosophila will be examined as both galectin and the possible homologue are simultaneously deleted. If the deletion is lethal then new crosses using heterozygotes will need to be carried out; however, if the deletion is not lethal, a novel phenotype should be expected and examined. Furthermore, it would be interesting to see if the galectin interacted with the sialylation process. The function of the galectin could then be investigated through many different experiments thereafter.

CHAPTER II

MATERIAL AND METHODS

Crosses for FLP-mediated mutagenesis to obtain a Galectin-1 & galectin homologue deletion

Males carrying one transposable element with an FRT site and a Bristle (Bl) marker were mated with females carrying an FLP recombinase transgene. Before progeny females carrying both the element with the marker and FLP recombinase could be mated to males carrying the second element, a stable stock of heterozygous heat shock (hs) inducible flippase (FLP) with Bristle marker females needed to be created (Table 1) . Heat shock induction of flippase activity was used to induce recombination.[7] There were two transposable elements flanking the region to be deleted. The curly wings and bristle phenotypes were used as markers to follow through the crosses and make sure the correct genotypes were being produced. The goal was to eliminate transposable elements 1 and 2 along with Galectin-1 (Gal-1) and the galectin homologue between the two elements, creating a single deletion of two genes. Y, w, hs-FLP; f07544 and i2: gal[c03082]/Cyo were generated in our lab using information obtained from a Drosophila genomic map, seen in figure 1, and stocks obtained from the Bloomington Drosophila Stock Center at Indiana University and Exelixis collection at Harvard University. Stocks were kept at the room temperature of 21˚C with constant food.

Table 1: Crosses leading to a stable Drosophila stock with FLP and two phenotypic markers

♀ y, w, hs-FLP/y, w, hs-FLP; f07544/f07544	X	♂ y, w/Y; f07544,bl/cyo
Collect ♂ y, w ; f07544, bl		
♀ y, w, hs-FLP/ y, w, hs-FLP; f07544/f07544	X	♂ y, w/Y; f07544,bl/f07544
Collect ♀ y, w, hs_FLP/ y, w, hs-FLP; f07544, bl		
♀ y, w, hs_FLP/ y, w, hs-FLP; f07544, bl/f07544	X	♂ y, w/Y; f07544,bl/cyo
Collect ♂ y, w, hs-FLP; f07544,bl/ cyo		
♀ y, w, hs-FLP/y, w, hs-FLP; f07544, bl/f07544	X	♂ y, w, hs-FLP/Y; fb7544, bl/cyo
Collect ♂ y, w, hs-FLP; f07544, bl/cyo		
♀ y, w, hs-FLP/y, w ,hs-FLP; f07544, bl/f07544,cyo	X	♂ y, w, hs-FLP/Y;f07544, bl/cyo
Gives stable stock		

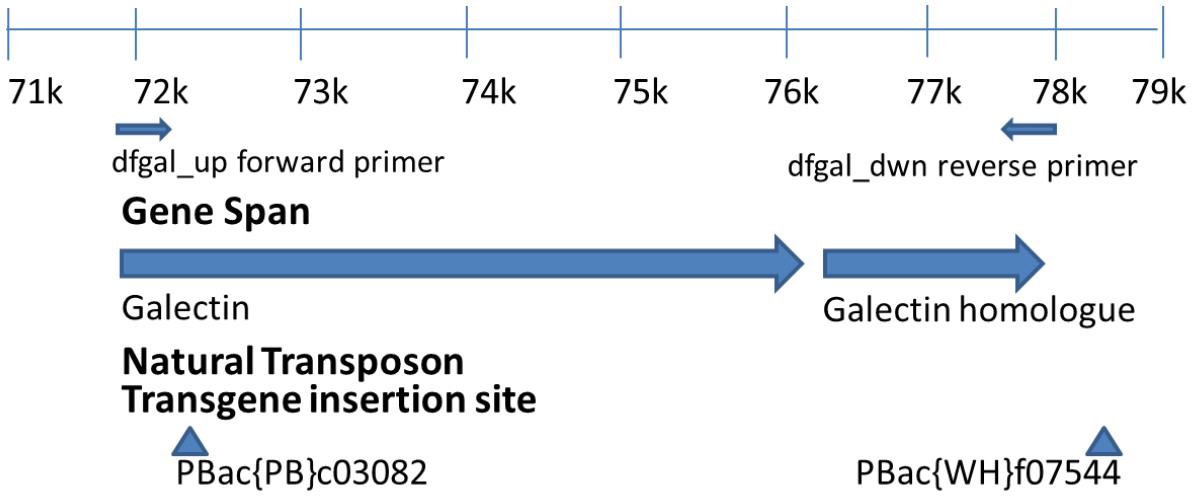

Figure 1: Genomic map of 71kb to 79kb span of Drosophila second chromosome. The map was generated from flybase.org and illustrates a visual representation of galectin and its homologue (Galectin-2) with transgene insertion sites of PBac{PB}c03082b and PBac{WHH}f07544 and the primers used throughout our crosses.

Parental crosses (P1) for FLP-mediated mutagenesis of galectin and its homologue, one parent with once transgene insertion site and the Bristle marker and the other parent with the other transgene insertion site and the Curly marker, were then set up using 7-10 virgins from y, w, hs-FLP; f07544, bl/cyo stock (Table 2). After 2 days, the parents and progeny in the vial of food were heat-shocked separately at 37°C for 1 hour. Before adult flies were heat-shocked, they were transferred into an empty vial with a piece of kim wipe that had been lightly soaked with 10% sucrose solution. After heat-shock was complete, adult flies were placed back into the original heat-shocked vial with the progeny. After one more day, 72 hours of egg-laying, P1 flies were transferred to a new vial and this vial repeated the previous steps of heat-shocking. The remaining vial with progeny (the vial with food still in it) was heat-shocked for an hour once a day for four more days. At the same time as the non-curly males with the Bristle marker were collected from the F1 generation (♂ y, w, hs-FLP / Y; f07544, Bl / c03082), virgins from the wild type stock (♀ w- [cs/2j]) were collected. The F1 flies were crossed en masse with 6-12 flies per vial. Males with white eyes and a Bristle marker were collected from the F2 generation and crossed individually to 3-4 virgin females of a different strain (♀ w; Sp / CyO). The F2 progeny was then crossed to each other to make a stable mutant F3 generation as seen in Table 2.[6]

Table 2: FLP-mediated mutagenesis of Galectin 1 & Homologue

P1	♀y,w,hs-FLP/ y ,w hs-FLP; f07544/f07544,Bl	X	♂ w;c03082/cyo
F1	♀ w- [cs/2j]	X	♂ y, w, hs-FLP / Y; f07544, Bl / c03082
F2	♀ w; Sp / CyO	X	♂ w / Y; Δgal1-2, Bl / +
F3	♀ w; Δgal1-2, Bl / Cyo	X	♂ w / Y; Δgal1-2, Bl / Cyo

PCR confirmation of Galectin-1 and homologue deletion

All PCR reactions were carried out using the primers dfgal_dwn and dfgal_up and purified genomic DNA from the F3 progeny fly lines (for genomic DNA preparations, see Appendix A). Two different PCR condition products of the i5 strain from the F3 progeny, a line used as a negative control, i2:gal[c03082]/cyo, and a 1kb Invitrogen ladder went through electrophoresis on a 0.8% TBE agarose gel to confirm band size. We assumed this band to be the segment of DNA in the F3 lines which did not contain the two galectin genes and was in fact the genomic deletion product. The i5 strain was also test by genomic PCR and electrophoresis. This strain was compared with a parent strain, y,w,hs-FLP/y,w,hs-FLP; f07544/f07544,Bl, and a wild type strain with both markers y,w;Bl/cyo, to confirm that the newly acquired band did not come from a parent or wild type strain but from the expected crossing over event.

DNA extraction and DNA sequencing on PCR product of i5w; Δgal1-2, Bl / Cyo

A Qiagen kit was used for extracting DNA from the 5-6 kb band of the PCR confirmation. 2 µl of this DNA extract was run through electrophoresis on a 0.8% TBE gel to confirm DNA extraction was successful. 56 µl of 30ng/µl DNA extract were sent off for complete genome sequencing.

CHAPTER III

RESULTS

Initially, several crosses were carried out in order to obtain a stable Drosophila stock with FLP and two phenotypic markers as seen in Table 1. Using the created stable stock, a second round of crosses took place using an FLP-mediated mutagenesis approach to delete Galectin 1 and its homologue as seen in Table 2. Many individual fly lines, 1-60, were collected from the FLP-mediated mutagenesis crosses with the resulting genotype of w; Δgal1-2, Bl / Cyo. One of the lines, line 5, was chosen as the primary sample (sample 5) due to its high penetrance of the curly phenotype. After genomic DNA preparations, PCR was carried out on sample 5. The PCR results of sample 5, i5w; Δgal1-2, Bl / Cyo, were run against a 1kb Invitrogen DNA ladder and one of the fly lines, i2:gal[c03082]/cyo, used in previous crosses that still contained the Gal-1 and homologue genes with the c03082 insertion site, seen in figure 2.

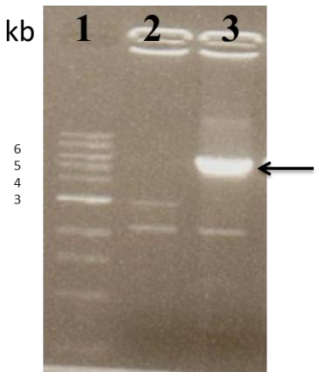

Figure 2: Gel electrophoresis of sample 5 and negative control on a 0.8% TBE gel. From left to right, lane is 1 kb DNA ladder from Invitrogen, lane 2 is the PCR product of a negative control of i2:gal[c03082]/cyo, lane 3 is the PCR product of sample 5, i5w; Δgal1-2, Bl / Cyo.

The band indicated by the arrowhead is the 5-6 kb band of interest, the sequence of which should not include the two galectin genes, although a shorter band of 3-4 kb was expected when the two galectin genes are subtracted from the wild-type genome. The gel image did prove that the band of interest did not come from the insertion site c03082. In order to make sure that the brightest band, the band of interest, of sample 5 did not come from the FLP insertion or either of the two phenotypic markers used during the crosses, sample 5 was run alongside the same 1kb Invitrogen DNA ladder, a fly line of Bl/Cyo, and a fly line of y, w, hs_FLP/ y, w, hs-FLP; f07544, bl/f07544, as seen in figure 3. This gel image did prove that the band of interest did not originate from an FLP insertion or either phenotypic marker. After gel electrophoresis, the DNA band was excised and prepared for DNA sequencing. Genome sequencing proved that the 5-6 kb band did, however, still include Galectin-1 and the galectin homologue.

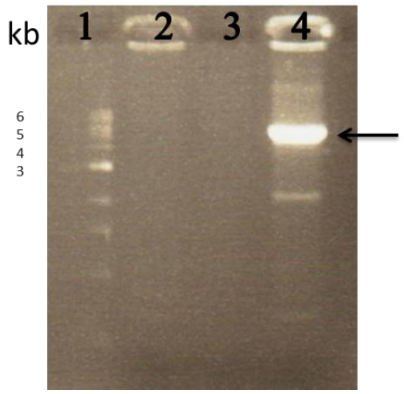

Figure 3: Gel electrophoresis of sample 5 and 2 negative controls on a 0.8% TBE gel. From left to right, lane 1 is 1kb DNA ladder from Invitrogen, lane 2 is the PCR product of the negative control of Bl/Cyo, lane 3 is the PCR product of the negative control of y, w, hs-FLP/ y, w, hs-FLP; f07544, bl/f07544, and lane 4 is the PCR product of sample 5, i5w; Δgal1-2, Bl / Cyo.

CHAPTER IV

DISCUSSUION

Over 60 different lines were generated of galectin mutants supposedly missing the Galectin-1 and galectin homologue genes. After sample 5, i5w; Δgal1-2, Bl / Cyo, of one of these mutant lines was run through electrophoresis, it was found that the 3-4kb band that was expected actually measured in the 5-6 kb range. Due to the recombination between the two p-elements, there may have been an unusual rearrangement in the galectin gene region which the experiment focused on. Certain duplications of sequences may have occurred within the p-elements themselves, while leaving the galectin and homologue region completely unaltered and successfully deleted. However, the DNA sequencing results showed that the Galectin-1 gene and its homologue were not deleted from the genome. These results were slightly expected due to the fact that deleting galectin genes from the Drosophila genome could lead to lethality. If the desired deletions are indeed lethal, then only heterozygotes of the mutation would survive through embryogenesis to become mature Drosophila flies.

Although the genome sequencing of the 5-6 kb band of interest, with the supposed deletion of Gal-1 and its homologue, confirmed that both genes were still present, this does not disprove that the lines which were created did in fact include galectin mutants. Further crosses will be carried out to remove the unnecessary Bl marker and to cross the novel galectin mutant with an L14 balancer stock in order to investigate the lethality of the galectin deletion through Drosophila development . PCR amplification, DNA extraction , and genome sequencing can then be carried out on homozygous mutant larva before the stage of lethality to confirm a deletion of both

Galectin-1 and its homologue. Depending on the stage at which the galectin deletions are lethal, this experiment can shed light on galectin's role in neural and physiological development in Drosophila. Furthermore, a double mutant which carries a galectin mutation and a DsiaT mutation could be used to study whether or not a galectin mutation could suppress the phenotype that a DsiaT mutation brings. It is known that Sia masks some proteins from recognition by galectin. If there is no sialic acid present, then protein is recognized by galectin and can be retained in a wrong location within the cell.[8] This hypothesis predicts that in a galectin mutant there will be no mislocalization due to galectin binding; therefore, the galectin mutation is expected to suppress Sia mutants. In the future, this mutant could be used to study and further knowledge in galectin's role in embryogenesis, neural development, sialylation, and lectin-ligand interactions. Hence, if the experiments carried out do not lead to a true galectin mutant, than none of the discussed studies can take place. If a true galectin mutant will not be generated through FLP-mediated mutagenesis, then an alternative approach, such as a HOBO method, may be more promising in fully deleting Gal-1 and its homologue.

REFERENCES

1. Cummings, R.D. and F.T. Liu, *Galectins*, in *Essentials of Glycobiology*, A. Varki, et al., Editors. 2009: Cold Spring Harbor (NY).

2. Ni, Y. and I. Tizard, *Lectin-carbohydrate interaction in the immune system.* Vet Immunol Immunopathol, 1996. **55**(1-3): p. 205-23.

3. Varki, A., *Essentials of glycobiology*. 1999, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. xvii, 653 p.

4. Kobata, A., *Glycobiology in the field of aging research - introduction to glycogerontology.* Biochimie, 2003. **85**(1-2): p. 13-24.

5. Taylor, M.E. and K. Drickamer, *Introduction to glycobiology*. 3rd ed. 2011, Oxford ; New York: Oxford University Press. xix, 283 p.

6. Pace, K.E., et al., *Characterization of a novel Drosophila melanogaster galectin. Expression in developing immune, neural, and muscle tissues.* J Biol Chem, 2002. **277**(15): p. 13091-8.

7. Schlake, T. and J. Bode, *Use of Mutated Flp Recognition Target (Frt) Sites for the Exchange of Expression Cassettes at Defined Chromosomal Loci.* Biochemistry, 1994. **33**(43): p. 12746-12751.

8. Zhuo, Y. and L. Bellis, Emerging Role of2,6-Sialic Acid as a Negative Regulator of Galectin Binding and Function*. J Biol Chem, 2011. **286**(8): p. 5935-41.

APPENDIX A

Genomic DNA Preparations

20 flies were collected and put into a 3 well glass plate containing 1 ml of 1X PBS. The flies were swished around in the well with a paintbrush that had been prewashed with 70% EtOH. The 1X PBS was sucked up and ~500 µL of 70% EtOH was placed into the well. The ethanol was quickly removed. The well was washed 2 more times with 1 ml of 1X PBS. The flies were transferred to a clearly labeled eppendorf tube. 180µL of Grind Buffer was added to the flies and they were homogenize for roughly 2 minutes. Debris was rinsed from the pestle into the tube with 525 µL of Grind Buffer. The sample was vortexed for 10 seconds and placed immediately in the 65°C water bath for 10 minutes. 3 µL of RNaseA was added and the sample was briefly vortexed. The sample was placed at room temperature and nutated for 5 minutes. 113 µL of 8 M KAc was added and the sample was vortexed. The sample was placed on ice for at least 15 minutes thereafter. 750 µL of Phenol/Chloroform (1:1) was added to the sample and then the sample was vortexed for 30 seconds and spun at a high speed for 5 minutes.The supernatant was transferred into a new eppendorf tube containing 1 ml of ice cold 100% EtOH and the sample inverted several times. The sample was then centrifuged for 10 minutes at high speed in order to pellet the DNA. The supernatant was carefully removed making sure not to detach the DNA pellet from the tube's bottom and 1.5ml of ice cold 70% EtOH was slowly added. Quickly after, the sample was inverted once. The sample was spun at high speed for 3 minutes and then the supernatant was removed. The sample was spun again at top speed for 0.5-1 minute and the remaining liquid was removed. The sample was allowed to air dry for 20 minutes. Once the pellet was dry, 1X TE (2µL/fly) was added. The sample was flicked 7-10 times and placed in a

centrifuge at high speed for 15 minutes. The DNA extract (liquid) was removed and placed in a 1.5 ml epi-tube. DNA extractions were stored at 4°C for at least 2 hours prior to use.