THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DIVISION OF SCIENCE

The Role of SR Splicing Protein 9G8 in Regulating Lipid Metabolism in Drosophila Intestine

ROMAN VOSKOBOYNIKOV
SPRING 2024

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry and Molecular Biology
with honors in Molecular and Cell Biology

Reviewed and approved* by the following:

Justin DiAngelo
Associate Professor of Biochemistry and Molecular Biology
Thesis Supervisor

Jeanne Marie Rose
Associate Professor of English
Honors Adviser

* Electronic approvals are on file.

ABSTRACT

For the last several decades, the rate of metabolic diseases in humans, such as obesity or diabetes (type 2), has been increasing drastically. These diseases arise from defects in the body's ability to take in and store nutrients such as carbohydrates and triglycerides. To better understand the pathways which our body uses to absorb and store nutrients, a simpler, yet similar organism, such as *Drosophila melanogaster*, can be used as a model system. Previous studies have shown splicing factors – proteins that assist alternative splicing of mRNA – to have an impact on lipid storage in the fly body. Specifically, adipose tissue knockdown of SR proteins, which are a group of splicing factors that regulate splice-site selection, revealed an increase in triglyceride storage in whole flies. However, whether SR proteins function in other tissues to regulate nutrient metabolism was not known. We focused on studying the role of SR proteins in fly intestines by decreasing their levels in the gut and measuring the relative concentrations of lipids and carbohydrates. In total, we explored eight such proteins, including *9G8*, *B52*, *SF2*, *SC35*, *Rbp1*, *Rbp1-Like*, *RSF1*, and *SRp54*. Among those, *RSF1* limits overall triglyceride levels, while *Rbp1* promotes the storage of triglycerides in both male and female flies. Other SR proteins, including *9G8*, *SC35*, *Rbp1-Like*, *SF2*, and *Srp54* also seem to alter whole fly triglyceride stores, in only one of the sexes. *RSF1* was also found to reduce overall glycogen storage, while *Rbp1* functioned to promote it in both sexes. Additionally, *SC35*, *SF2*, and *Srp54* were found to diminish overall available free glucose, while *B52* enhanced its production in female flies. We next further investigated *9G8*, an SR protein which displayed a prominent lipid phenotype in both the fat body and intestine. After using RNA-seq to identify differentially expressed genes, we utilized qPCR to confirm the differential expression of lipid processing enzymes, such as lipases and fatty acid synthases and fatty acid elongases. Overall, we determined most SR proteins to have a unique phenotype in the fly intestine and found *9G8* to regulate whole body, as well as intestinal, lipid homeostasis by altering the expression of genes involved in metabolism of lipids.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I would like to give my upmost gratitude to Dr. Justin DiAngelo for helping me design, plan, and execute nearly every part of this three-year long project. As my research coordinator, he helped me every step of the process and answered any questions I had along the way. I wish every Penn State student would have as caring and helpful an advisor in their journey as him.

I also wanted to thank Dr. Sandy Feinstein and Dr. Jeanne Marie Rose for their help throughout my undergraduate Schreyer journey, as well as review of my research (including this thesis).

Furthermore, I would like to thank my lab colleagues, including Louis Betz, Brian Truong, Erick Astacio, and Matt Kauffman for providing me with a wonderful research lab experience throughout these years.

Last, but not least, I wanted to say thank you to all of my past (and present) professors, as their guidance and expertise are one of the only things that got me this far in the field.

Chapter 1

Introduction

Research Involving Human Metabolic Diseases

Throughout the past century, the lifespan of an average American man and woman has grown drastically, rising from 46.3 and 48.3 years, respectively, in 1900, to 76.1 and 81.1 years, respectively, in 2019 (Malik and Hoenig, 2019). Many of the health advances implemented during this time are believed to have contributed to this change in lifespan, including declines in infant mortality, control of infectious diseases, safer foods, as well as recognition of the dangers of cigarette smoking. The health-related issues we face today are thus rather different, and more profound, compared to previous centuries. One such issue is maintaining a healthy body weight, which puts one at a lower risk of developing related diseases, such as severe obesity, type 2 diabetes, and cancer. However, despite decades of ongoing research, the age-adjusted prevalence of obesity has increased from 30.5% to 42.4% over the past 18 years (2000 - 2018), for an average American (Hales et al., 2020). Additionally, the prevalence of severe obesity has also nearly doubled since 2000 (Hales et al., 2020).

Many metabolic diseases, like obesity and type 2 diabetes, have been documented to be a direct result of excess triglyceride storage (Lin and Li, 2021). While excess caloric intake and a sedentary lifestyle are appreciated risk factors for developing obesity, genetic factors that regulate lipid storage and transport can also contribute to an individual's predisposition to weight gain (Lin and Li, 2021). Thus, understanding the genes involved in the synthesis, absorption, and storage of lipids will be helpful to allow us to better understand the pathogenesis of obesity and type 2 diabetes.

Drosophila as a System to Model Human Metabolic Function

Drosophila melanogaster has been shown to be an excellent model system to study metabolism, having a short generation time (10–12 days) and sharing over 60% orthologous genes with humans (Staats et al., 2018). A fly's food intake, body composition, locomotor activity, cognition, fertility, aging, and lifespan can be systematically measured, with minimal effort and time (Staats et al., 2018). *Drosophila* also contains several tissues similar to those involved in human obesity and associated metabolic diseases, such as the gut (intestines), oenocytes (liver) and the fat body (adipose tissue). *Drosophila* can develop an obesity-like state with its associated complications (hyperglycemia, reduced longevity, etc.) during caloric overload, similar to humans (Musselman and Kühnlein, 2018). Together, the genetic and physiological similarities between flies and humans make *Drosophila* an ideal system with which to study the genetic control of metabolism.

In previous genome-wide RNAi screens performed in adult flies and *Drosophila* cells, several candidate genes responsible for regulating lipid storage were identified (Guo et al., 2008; Pospisilik et al., 2010; Beller et al., 2008). For example, oenocyte- and fat-body-specific gene knockdown in adult flies was performed using RNA interference (RNAi). Triglyceride levels were then measured in the whole fly, and potential mediators of lipid and glucose metabolism were identified, such as homologs of the ADP/ATP symporter and a homolog of fatty acid elongase (Pospisilik et al., 2010). In addition, genome-wide RNAi screens in cultured Drosophila cells identified hundreds of genes that affected lipid-droplet morphology (Guo et al., 2008; Beller et al., 2008). One group of genes of particular interest to the DiAngelo lab were those that play a role in regulating RNA processing.

SR Protein Function in Regulating Gene Expression

During the process of gene expression to form functional proteins, genes are transcribed to form messenger RNAs (mRNA). During this transcription process, mRNA splicing occurs, a step where mRNAs are processed to remove noncoding introns from primary transcripts and to join expressed sequences called exons to create mature mRNA. mRNA splicing is performed by

the spliceosome, a dynamic RNA-protein enzyme complex, which recognizes splice sites at intron boundaries (Matera and Wang, 2014). Splicing regulatory RNA-binding proteins (SRBPs) are thought to regulate splicing by recognizing distinct RNA sequences. Various families of regulatory proteins will then bind to these sequences and enhance mRNA splicing by recruiting the spliceosome (Matera and Wang, 2014). Another important group of SRBPs is the Serine–Arginine (SR) family of proteins, which has primarily been shown to regulate splice-site selection (Shepard and Hertel, 2009). These proteins can interact with other splicing factors, which can either enhance or inhibit splice site selection. SR proteins have thus been implicated in all crucial aspects of mRNA metabolism including export, localization, translation, and nonsense-mediated decay (Bradley et al., 2015; Shepard and Hertel, 2009). SR proteins have been shown to regulate the processing of genes that function in various cellular processes, including nutrient metabolism. For example, in mouse liver cells, the SR protein *SRSF3* was found to be involved in the differential splicing of glucose-6-phosphate dehydrogenase (*G6PD*) (Walsh et al., 2013). This enzyme functions in the pentose phosphate pathway (PPP) to produce NADPH, an important co-factor for lipid synthesis. Another SR protein, *SFRS10*, was found to be downregulated in both liver and muscle of obese mice and regulates the splicing of LIPIN1, a key regulator of triglyceride esterification (Pihlajamäki et al., 2011). Whether additional SR proteins are important for regulating lipid storage, breakdown, or transport, however, is not known.

Known Role of SR Proteins in *Drosophila* Fat Body

Eight SR proteins have been identified to regulate splicing in *Drosophila* (*9G8*, *B52*, *SF2*, *SC35*, *Rbp1*, *Rbp1-Like*, *RSF1*, and *SRp54* (Bradley et al., 2015)). Previous studies from the DiAngelo lab have shown that some SR proteins function in fat tissue to regulate lipid storage in flies. Specifically, decreasing *9G8*, *tra2*, *SF2*, and *RBP1* in fly adipose tissue using RNAi resulted in an increase in overall triglycerides stored in these flies (Gingras et al., 2014; Mikoluk et al., 2018; Bennick et al., 2019). In addition, decreasing *9G8* specifically in fat tissue results in altered expression and splicing of several lipid metabolic genes (Weidman et al., 2022). For example, the splicing of *CPT1*, the gene responsible for producing an enzyme involved in the

breakdown of lipids, was altered during *9G8*, *SF2* and *tra2* knockdown in the fat body such that CPT1 enzyme activity was decreased (Gingras et al., 2014; Mikoluk et al., 2018; Bennick et al., 2019).

The splicing of the gene Zwischenferment (*Zw*), the fly homolog of *G6PD* in humans, was also significantly altered during *9G8* knockdown in the fly fat tissue. *G6PD* splicing is also regulated by SR proteins in humans suggesting conservation of the splicing of metabolic enzyme genes from flies to humans (Weidman et al., 2022; Walsh et al., 2013). While their role in regulating lipid storage has been studied in *Drosophila* fat tissue, whether SR proteins function in other metabolic tissues such as the fly intestine is still not known.

Exploration of SR Protein Function in the *Drosophila* Intestine

The *Drosophila* gut is the primary site for nutrient absorption, including that of triglycerides. In order for triglycerides to be absorbed into intestinal cells, they have to be emulsified first. The derived fatty acids, monoglycerides, and cholesterol are then packaged into chylomicrons, a type of lipoprotein that allows for lipid transport in the bloodstream (Miguel-Aliaga et al., 2018). The apoB-family lipoprotein Lipophorin (Lpp) is the major hemolymph lipid carrier in *Drosophila*, produced by the fat body. It acquires sterols and most diacylglycerol in the fly gut via the Lipid Transfer Particle (LTP), after which it is secreted into the bloodstream with the help of Microsomal TAG Transfer Protein (MTP) (Palm et al., 2012). However, the molecular control of lipid absorption, transport, and storage, and specifically the roles of SR proteins in regulating intestinal metabolic functions, are not well understood. Thus, the major goal of this study is to assess the intestine-specific functions of SR proteins to regulate carbohydrate and lipid processing and storage in *Drosophila*.

To first determine any changes in absorption and storage of these molecules in the fly body, we measured the effect of decreasing each SR protein in the intestine on overall triglyceride and glycogen levels. To better understand how the SR protein *9G8* regulates nutrient storage and transport, RNA sequencing was performed on RNA isolated from intestines from flies with decreased *9G8*. A number of lipases and genes involved in fatty acid elongation were identified as being upregulated through this analysis and qPCR was performed to confirm these

changes from the RNA sequencing experiments. Furthermore, to better understand the role of *9G8* in lipid transport, intestinal triglycerides were also assayed in flies with *9G8* decreased specifically in the intestines. These experiments will help us determine the extent of the role SR proteins in general and *9G8* in particular play on nutrient absorption, packaging, and transport in the *Drosophila* intestine.

Chapter 2

Methods

Fly Genetics

The following lines were used in this study (Bloomington (BL) and Vienna *Drosophila* Resource Center (VDRC), stock numbers included (Table 1)): *UAS-EGFP-RNAi-1, UAS-EGFP-RNAi-2, UAS-GFP-RNAi, UAS-SF2-RNAi, UAS-RBP1-RNAi, UAS-RBP1 like-RNAi, UAS-SRP54-RNAi, UAS-RSF1-RNAi, UAS-9G8-RNAi, UAS-B52-RNAi, UAS-SC35-RNAi*. Flies were grown at 25 °C on a 12 h:12 h light : dark cycle on a standard cornmeal-sugar yeast medium (9 g Drosophila agar (Genesee Scientific), 100 mL Karo Lite Corn Syrup, 65 g cornmeal, 40 g sucrose, and 25 g whole yeast in 1.25 L water).

Fly Strains

Fly Line	Genotype	Source
UAS-EGFP-RNAi-1	y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = VALIUM20-EGFP.shRNA.1}attP40	BL#41555
UAS-EGFP-RNAi-2	y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.GLV21067}attP2	BL#35702
UAS-GFP-RNAi	w [1118]; P{w [+mC] = UAS-GFP.dsRNA.R}142	BL#9330
UAS-SF2-RNAi	y [1] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HM05199}attP2	BL#29522
UAS-RBP1-RNAi	y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HMC03902}attP40	BL#55688
UAS-RBP1 like-RNAi	y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HMS02820}attP40	BL#44100
UAS-SRP54-RNAi	y [1] sc [*] v [1]; P{y [+t7.7] v [+t1.8] = TRiP.HM05224}attP2	BL#30533
UAS-RSF1-RNAi	w [1118]; UAS-IR:Rsf1/TM3	VDRC#22186
UAS-9G8-RNAi	w[1118]; attB:UAS-IR:9G8	VDRC#100226
UAS-B52-RNAi	w[1118]; attB:UAS-IR:B52	VDRC#101740
UAS-SC35-RNAi	w[1118]; attB:UAS-IR:SC35	VDRC#104978
Mex-Gal4	w[1118]; P{w[+mC]=mex1-GAL4.2.1}10-8	BL#91368

Table 1. *Drosophila* Strains and Respective Stock Numbers and Sources Used

Triglyceride, Glycogen, and Protein Measurements

Triglyceride, protein, and carbohydrate measurements were made as described previously (Gingras et al., 2014; Bennick et al., 2019). Briefly, pairs of one-week old males or females or groups of 15 intestines extracted from approximately one-week old adult females were homogenized in lysis buffer (140 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.1% Triton-X and 1X complete, EDTA-free protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA)). Triglycerides were measured using the Infinity Triglyceride Reagent (Thermo Fisher Scientific, Waltham, MA) and protein was measured using the BCA protein assay kit (ThermoFisher Scientific, Waltham, MA) according to manufacturer's instructions. To measure glycogen levels, homogenized samples were treated with 8 mg/ml amyloglucosidase (Sigma–Aldrich, St. Louis, MO) in 0.2M Citrate buffer, pH 5.0 for 2 h at 37 °C and then total glucose was measured using the Glucose Oxidase Reagent Set (Pointe Scientific, Inc., Canton, MI). Free glucose concentrations were also measured in samples that were not treated with amyloglucosidase. Free glucose was subtracted from total glucose to determine glycogen levels. Triglyceride, glycogen, and free glucose levels were normalized to total protein content.

Feeding assay

Food consumption was measured using the CAFÉ assay as previously described (Ja et al., 2007). Briefly, groups of three one-week old adult females were placed in 1% agar vials and the amount of 5% sucrose consumed over 24 hours was measured using 5xl capillary tubes (ThermoFisher Scientific, Waltham, MA). Vials without flies were used to control for evaporation.

RNA isolation

Groups of 25-30 intestines dissected from one-week old female flies were suspended in TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA) and homogenized briefly followed by a 5 min incubation at room temperature. Samples were chloroform extracted and nucleic acids

were precipitated by adding isopropanol. Pellets were washed with 70% ethanol, air dried for 5 minutes, and then resuspended in nuclease-free water.

RNA sequencing and Gene Expression analysis

RNA sequencing was performed by GeneWiz using procedures described previously (Weidman et al., 2022). Differentially expressed gene lists were analyzed using The Database for Annotation, Visualization, and Integrated Discovery (DAVID) software (Dennis et al., 2003) to identify any enrichment of genes in specific biological processes.

DNase treatment and cDNA synthesis

5µg of each RNA sample was DNase treated with the DNA-Free Kit (Ambion), according to manufacturer's instructions. 0.25 µg of DNased RNA samples were reverse transcribed using qScript Ultra cDNA Supermix (QuantaBio), according to manufacturer's instructions.

qPCR

qPCR reactions were made from 1µl of cDNA, 2x Perfecta SYBR Green (QuantaBio) and 200nM of the forward and reverse primer for each gene segment. The qPCR cycling conditions were as follows: 3 min at 95 °C; 40 cycles of: 30 s at 95 °C, 1 min at 60 °C, and 30 s at 72 °C, with a melt curve. The following genes were amplified as described: *9G8* (also known as *xl16*), *rp49* (*cg7939*), *apoltp* (*cg15828*), *mtp* (*cg9342*), *apolpp* (*cg11064*), *bmm* (*cg5295*), *FASN3* (*cg17374*), *eloF* (*cg16905*), *Fad2* (*cg7923*), *cg16904*, *cg30008*, *cg31089*, *cg10163*, *cg31091*, and *cg13562*. Forward and reverse primer sequences are shown in Table 2. Resulting expression levels for each gene were normalized to *rp49*.

Table 2. Forward and Reverse Primer Sequences Used to Quantify Gene Expression

Primer	Forward Sequence	Reverse Sequence
Rp49	GACGCTTCAAGGGACAGTATCTG	AAACGCGGTTCTGCATGAG
FASN3 (cg17374)	TGCTGGTACTGGAGGCATTG	CCCCTTTTCCGTTCGTCTCA
eloF (cg16905)	CACCGAAAGCCCTTCCATTTG	GATCCATCGGCAGGCTAACA
FAD2 (cg7923)	CAACGGTCGTGCTCTTTTGG	TTGCCCTTCTCCACAACCTC
cg16904	GAAGCCGTACAACCTGAGCT	ATGGAGGAACGTGATCTGGC
cg30008	GTAACGCTGGTCTACGCACT	CACCAAGGTCTTATCCGCCA
cg31089	TTCCTTGGTGCACATGTGGT	TCCATTCCGTATCCGCCATG
cg10163	GATGCCCGACTCCATGTGAT	ACGGAGAGATGATGACGCAC
cg31091	ATACGATGTTTGGCTGGGCA	CTGATCCTGTCCGTTCTCCG
cg13562	CTTCGTCTACTCCTGCCACC	CGAAGTTCTCCTCGTAGCCC

Chapter 3

Results

SR Proteins Affect Lipid Levels in Whole Flies

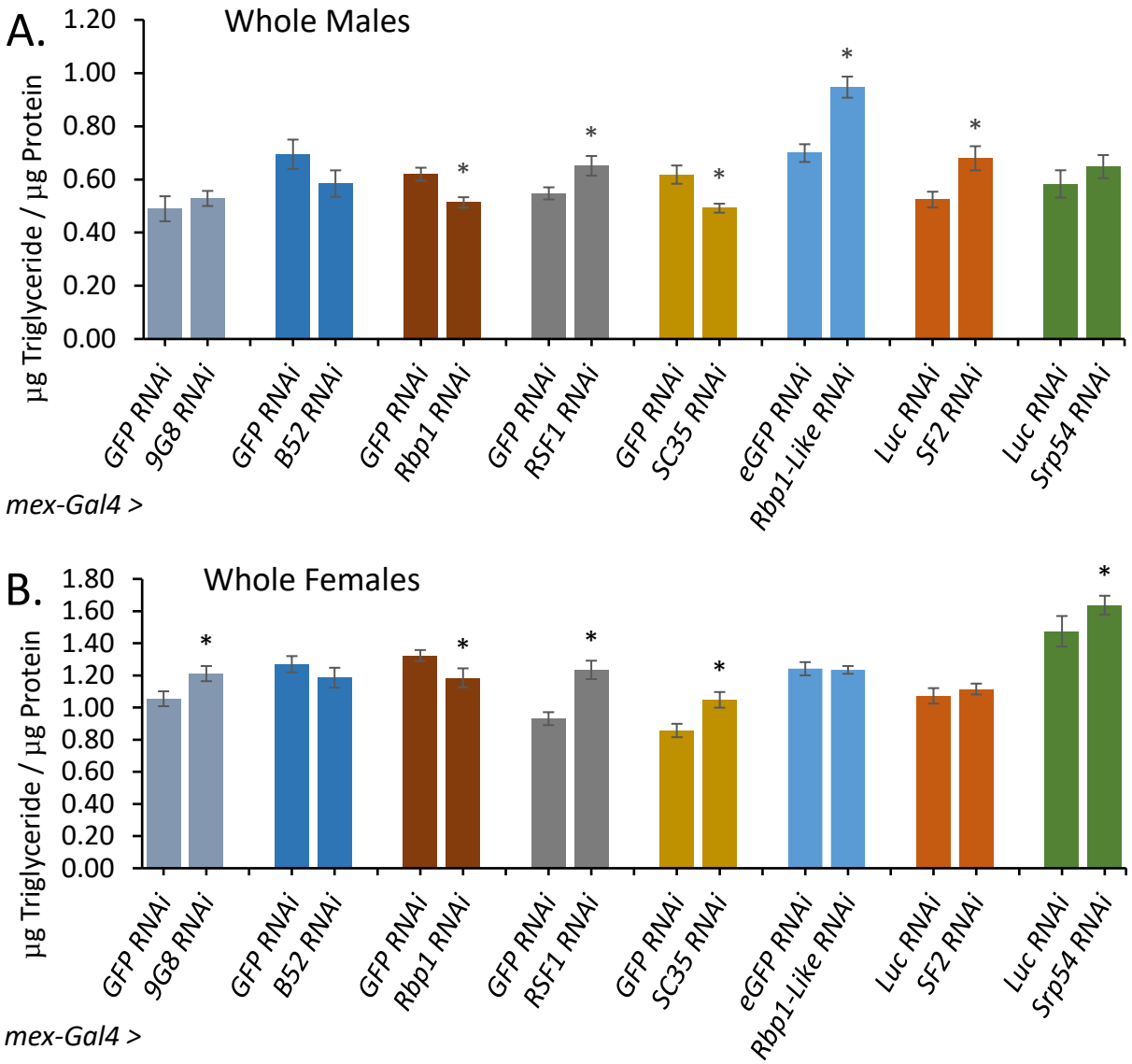

Figure 1. Decreasing SR proteins in the gut alters overall levels of triglycerides in male and female flies. Triglycerides were measured in one week-old male and female flies, then normalized to protein content. $44 \geq n \geq 20$. Bars indicate average normalized triglyceride concentrations in **A.** male and **B.** female flies, +/- standard error. *P<0.05 by a t-test comparing control and SR protein gene knockdown conditions in male and female flies.

To characterize the metabolic functions of SR proteins in the fly intestines, we used the intestine-specific *mexGal4* driver (Phillips and Thomas, 2006) to induce RNAi towards *9G8*, *B52*, *Rbp1*, *RSF1*, *SC35*, *Rbp1-Like*, *SF2*, and *Srp54* individually to lower their expression, specifically in the fly gut. Next, triglycerides normalized to total protein content was measured in males and females with the expression of a single SR protein reduced. In males, decreasing *RSF1*, *Rbp1-Like*, and *SF2* augmented triglyceride storage, while knockdown of *Rbp1* and *SC35* blunted it (Figure 1A). In female flies, decreasing *9G8*, *RSF1*, *SC35*, and *Srp54* resulted in triglyceride accumulation, while knockdown of *Rbp1* resulted in lean flies (Figure 1B). This suggests that *RSF1* limits overall triglyceride levels, while Rbp1 promotes the storage of triglycerides. Other SR proteins, including *9G8*, *SC35*, *Rbp1-Like*, *SF2*, and *Srp54* also seem to alter whole fly triglyceride stores, but only in female flies. Additionally, while *SC35* knockdown resulted in changes in triglyceride levels, the lipid storage phenotypes were opposite in the different sexes.

SR Proteins Alter Levels of Carbohydrates in Whole Flies

To further analyze the metabolic role of SR proteins in the fly intestines, we measured glycogen and free glucose in whole flies, with individual SR proteins decreased specifically in the intestine. In males, decreasing *RSF1* enhanced glycogen storage, while knockdown of *Rbp1* and *SF2* lowered it (Figure 2A). In female flies, decreasing *RSF1* and *Rbp1-Like* increased glycogen levels, while knockdown of *Rbp1* and *Srp54* diminished it (Figure 2B). This suggests that RSF1 reduces overall glycogen storage, while Rbp1 promotes it. In addition, while there was no effect on free glucose levels when RNAi was induced towards any SR protein in males (Figure 3A), decreasing *SC35*, *SF2*, and *Srp54* in female flies was found to increase free glucose levels, while knockdown of *B52* lowered them (Figure 3B). This finding suggests that at least in females, *SC35*, *SF2*, and *Srp54* diminish overall available free glucose, while *B52* enhances its production and/or its accumulation. Together, these triglyceride, glycogen and free glucose data suggest that SR proteins have distinct functions in the fly intestine to regulate lipid and

carbohydrate storage, and these varying phenotypes may arise from different metabolic splicing targets of each SR protein.

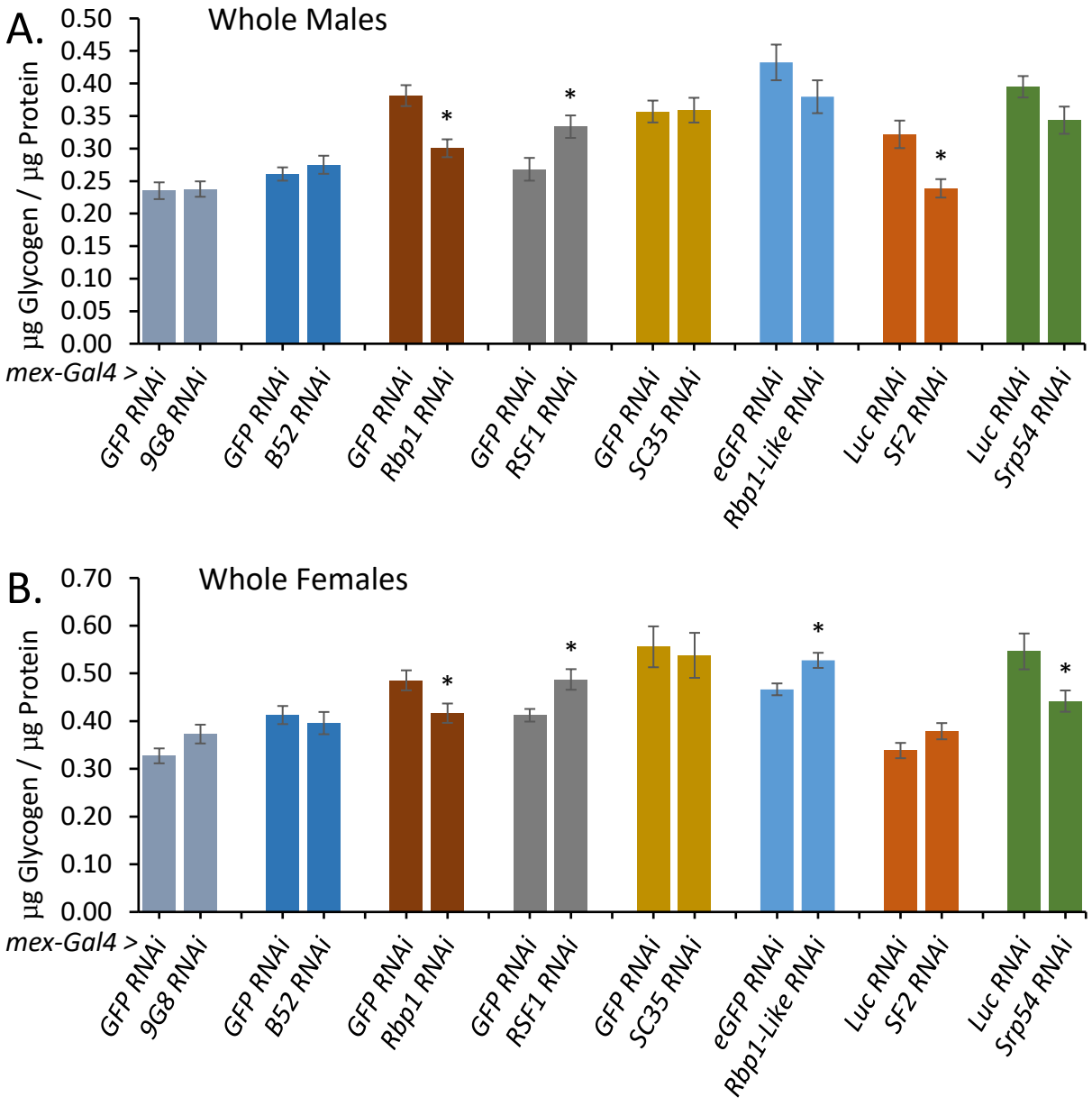

Figure 2. Decreasing SR proteins in the gut alters overall levels of glycogen in male and female flies. Glycogen levels were measured in one week-old male and female flies, then normalized to protein content. $44 \geq n \geq 20$. Bars indicate average normalized glycogen concentrations in **A.** male and **B.** female flies, +/- standard error. *P<0.05 by a t-test comparing control and SR protein gene knockdown conditions in male and female flies.

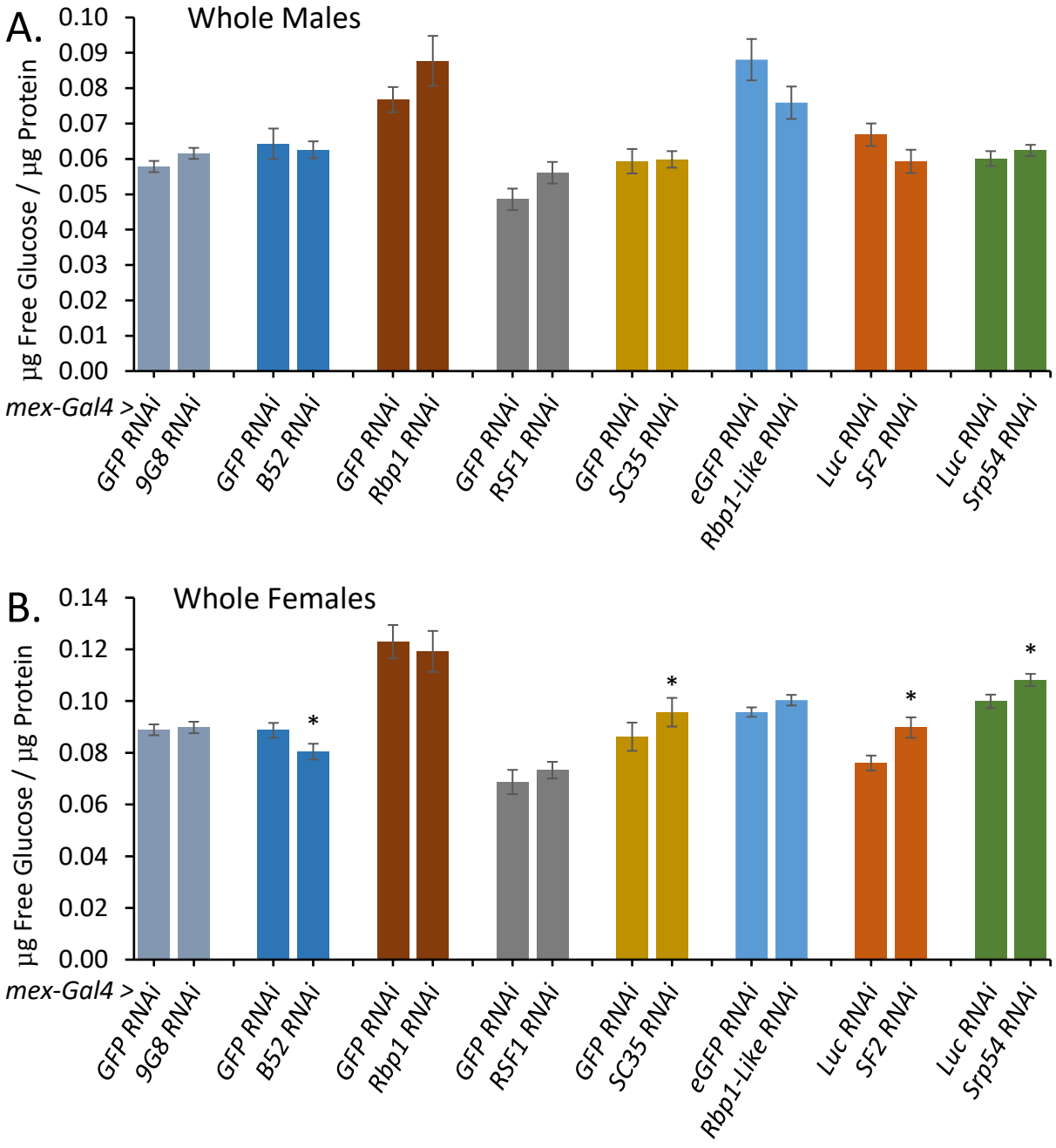

Figure 3. Decreasing SR proteins in the gut alters overall levels of free glucose in female flies. Free glucose levels were measured in one week-old male and female flies, then normalized to protein content. $44 \geq n \geq 20$. Bars indicate average normalized free glucose concentrations in **A.** male and **B.** female flies, +/- standard error. *P<0.05 by a t-test comparing control and SR protein gene knockdown conditions in female flies.

9G8 Functions on a Vast Array of Genomic Targets

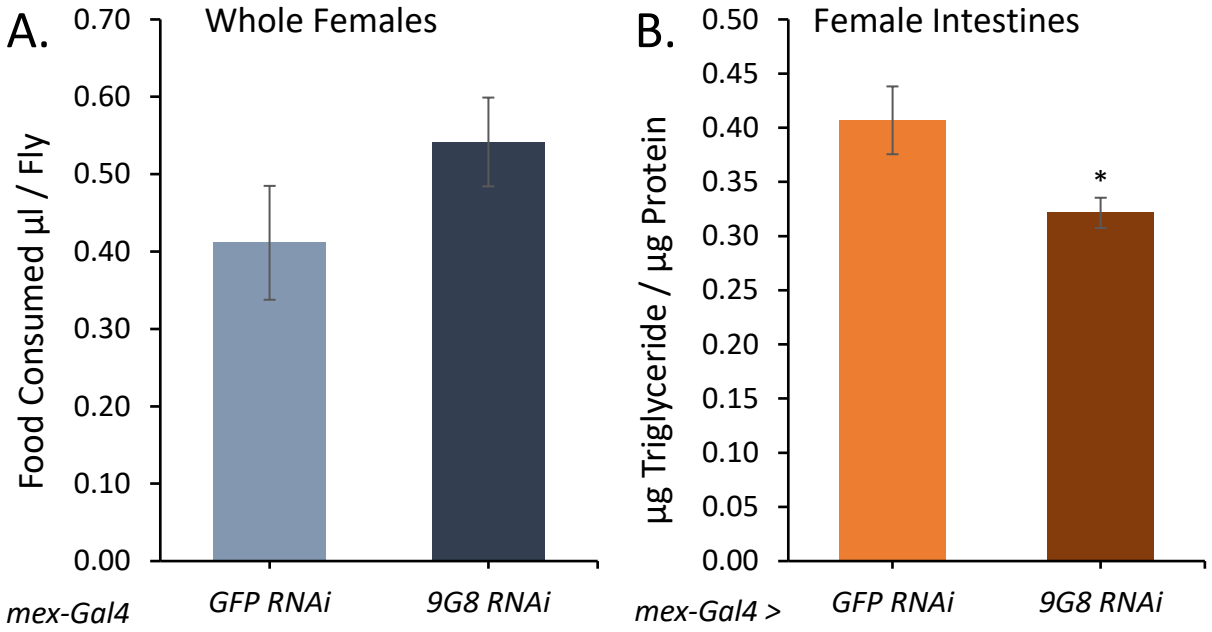

Figure 4. Decreasing *9G8* in the gut decreases gut levels of triglyceride storage in female flies. A. Feeding in approximately one-week old *mexGal4>GFPRNAi* and *mexGal4>9G8RNAi* flies was measured over 24 hours. n=36. **B**. Triglyceride levels of intestines, dissected from one-week old female *mexGal4>GFPRNAi* and *mexGal4>9G8RNAi* flies were measured and normalized to protein content. n=10. *P<0.05 by a t-test comparing control and 9G8 knockdown conditions.

In previous studies, decreasing *9G8* specifically in adipose tissue yielded a large increase in triglyceride levels (Gingras et al., 2014). In addition, *9G8* has also been shown to regulate the network of NADPH-producing enzymes, suggesting its potential in controlling cellular metabolism (Weidman et al., 2022). While the metabolic functions of *9G8* have been characterized in fly fat tissue, how 9G8 acts in the fly intestine to regulate metabolic homeostasis is not understood. Therefore, we decided to investigate further the function of *9G8* in the *Drosophila* intestine. Since triglycerides were higher in flies with *9G8* knockdown compared to controls (Figure 1B), we wanted to determine whether this increase was due to lipid accumulation in the intestine. To test this, intestines were dissected from *9G8RNAi* females and lipids were measured. Interestingly, intestines from *9G8* knockdown flies had decreased triglyceride levels (Figure 4A), and this finding was not due to any alterations in food

consumption (Figure 4B). These data suggest that *9G8* may be acting in the intestine to increase triglyceride storage locally and perhaps limit the amount of lipids transported to other tissues.

To better understand the genes regulated by *9G8* to control lipid absorption and metabolism within the fly gut, we performed RNA-Seq on RNA isolated from *9G8RNAi* fly intestines. Differential expression analysis was used to determine the difference in gene expression caused by the *9G8* knockdown. Of the 104 genes whose differential expression was identified in *9G8RNAi* intestines, 65 were upregulated and 39 were downregulated. Gene function was then analyzed using Gene Ontology analysis as described previously (Weidman et

al., 2022). The Gene Ontology terms resulting from this analysis that were significantly enriched in the upregulated and downregulated genes are shown (Table 3).

Table 3. Gene Ontology terms identified using RNA-seq during 9G8 knockdown

Upregulated		
Term	**# of Genes**	**P value**
fatty acid elongation, saturated fatty acid	3	2.60E-03
fatty acid elongation, monounsaturated fatty acid	3	2.60E-03
fatty acid elongation, polyunsaturated fatty acid	3	2.60E-03
lipid catabolic process	4	3.02E-03
transmembrane transport	6	3.04E-03
fatty acid elongation	3	3.15E-03
very long-chain fatty acid biosynthetic process	3	3.44E-03
sphingolipid biosynthetic process	3	4.39E-03
pheromone metabolic process	2	1.14E-02
intracellular cholesterol transport	2	2.65E-02
lipid metabolic process	3	3.33E-02
sterol transport	2	4.13E-02
O-glycan processing, core 1	2	4.13E-02
response to DDT	2	4.13E-02
Downregulated		
Term	**# of Genes**	**P value**
carbohydrate metabolic process	3	1.67E-02

9G8 Regulates Metabolic Gene Expression in *Drosophila* Gut

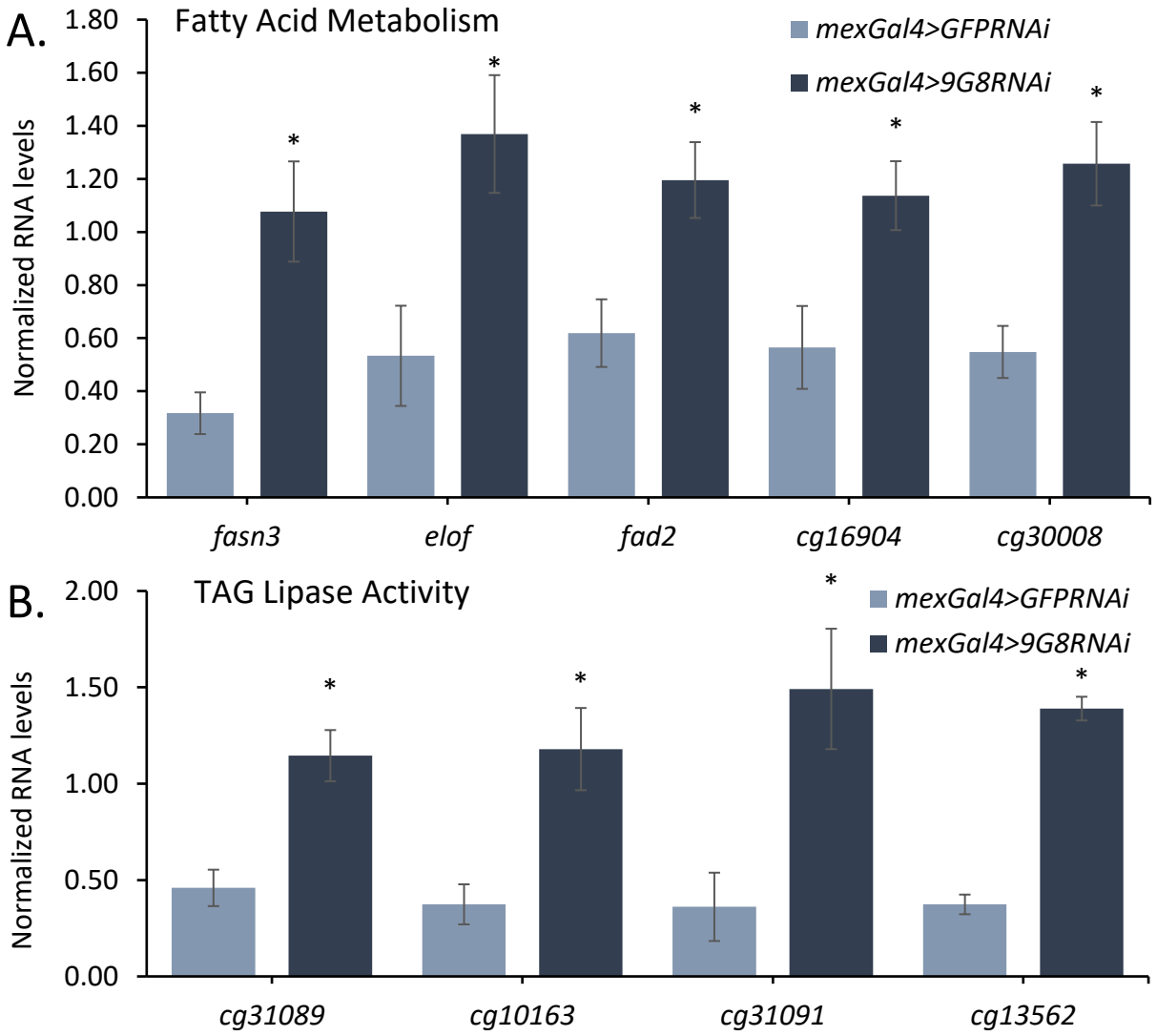

Figure 5. Intestinal knockdown of *9G8* increases TAG metabolism and TAG lipase gene expression. Levels of gene expression of **A.** fatty acid metabolism genes (*fasn3*, *elof*, *fad2*, *cg16904*, *cg30008*) and **B.** genes with predicted TAG lipase activity (*cg31089*, *cg10163*, *cg31091*, and *cg13562*) were measured using qPCR on RNA samples from intestines dissected from female *mexGal4>GFPRNAi* and *mexGal4>9G8RNAi* flies and normalized to *rp49* levels. n=8. Bars indicate average RNA levels, +/- standard error. *P<0.05 by a t-test comparing *mexGal4>GFPRNAi* and *mexGal4>9G8RNAi* conditions.

Most of the upregulated genes identified in the *9G8RNAi* fly intestines were in fact linked directly to lipid metabolism. Specifically, some (*cg16904, cg30008, Fad2,* and *eloF*) had a role in sphingolipid and fatty acid synthesis and elongation. Others (*cg31089, cg10163, cg31091,*

cg13562, cg17560, cg31089, and cg31091) were predicted to have triglyceride lipase activity or further assist in lipid breakdown. Interestingly, genes responsible for sterol transport (*NPC2F* and *NPC2H*), as well as transmembrane transporter activity (*cg9270, cg3168, cg12490, CG8028, CG2187, cg9717*) were also upregulated in *9G8RNAi* guts. Conversely, most of the downregulated genes were found to function in carbohydrate (*MAL-A6, MAL-A1,* and *CHT4*) or glutathione (*GSTD9* and *GSTD8*) metabolism.

Since intestinal triglyceride contents were lower in *9G8RNAi* flies, we decided to focus on many of the lipid metabolic genes that were identified during the RNA-seq analysis. We first focused on genes involved in fatty acid synthesis and elongation including *FASN3*, *eloF*, *fad2*, *cg16904*, and *cg30008*. All these genes had substantially higher expression in the intestines of *9G8RNAi* flies, compared to control flies using qPCR (Figure 5A). This suggests that *9G8* acts to limit the synthesis of fatty acids in the fly gut. In addition, we also examined the expression of putative triglyceride lipase genes shown to be upregulated in *9G8RNAi* intestines by the RNA-seq, including *cg31089*, *cg10163*, *cg31091*, and *cg13562*. All these genes had higher levels of expression in the *9G8RNAi* fly guts, compared to the control flies via qPCR (Figure 5B). This suggests that *9G8* limits the expression of acid lipases, the role of which is to aid in digesting triglycerides in the insect gut (Horne et al., 2009).

Chapter 4

Discussion

SR Proteins in the Intestine Influence Whole Fly Body Nutrient Levels

In this study, we explored the role various SR proteins play in the intestine to regulate *Drosophila* metabolism. Using the Gal4/UAS system to induce RNAi towards all eight SR proteins in the gut, we detected a variety of functions of each SR protein to regulate triglyceride and carbohydrate storage. For example, intestinal Rbp1 was shown to increase both triglycerides and glycogen in the whole fly. Previous studies of this SR protein in the fat body revealed it to decrease both whole fly lipid levels and the resistance to starvation (Bennick et al., 2019). This suggests that Rbp1 may play a different metabolic role in the fat body from that of the gut. Conversely, the SR protein *RSF1* acted in the gut to decrease triglyceride and glycogen levels in whole flies. Interestingly, *RSF1* has no effect on whole fly triglyceride and carbohydrate levels (Bennick et al., 2019), suggesting it has a unique function in the intestine. Another SR protein, *Srp54*, was shown here to function in the fly intestine to also lower total triglyceride content. Interestingly, it also increased free glucose, while lowering glycogen in whole flies. This suggests it may distinctly affect carbohydrate storage and metabolism in the fly gut. Similarly to *RSF1*, knockdown of *Srp54* in the fat body did not significantly alter whole fly nutrient storage (Bennick et al., 2019), implying its intestine-specific function.

We have also shown the SR protein *9G8* to function in the intestine to decrease whole fly lipid levels. This is consistent with triglyceride accumulation observed in previous experiments where *9G8* was decreased in the fat body (Gingras et al., 2014), implying the knockdown in both the gut and the fat body have a similar whole fly lipid storage phenotype. However, while triglyceride levels were also higher in isolated fat bodies during *9G8* knockdown (Gingras et al., 2014), decreasing *9G8* in the intestine led to decreased lipid levels in the gut, indicating 9G8 has varying organ-specific effects. In the fat body, *9G8* knockdown was also shown to downregulate fat body expression of several key NADPH-producing enzyme genes, including Zwischenferment (*Zw*), the homolog of human glucose 6-phosphate dehydrogenase (G6PD), phosphogluconate dehydrogenase, isocitrate dehydrogenase, and malic enzyme (Weidman et al.,

2022). *9G8* was also found to affect lipid breakdown by regulating the splicing of carnitine palmitoyltransferase I (*CPT1*), an enzyme responsible for downstream fatty acid catabolism, decreasing its activity (Gingras et al., 2014). These enzymes are responsible for breaking down lipid and carbohydrate derivatives, which is interesting, since these derivatives do not build up in the intestine, as they likely do in the fat body. This means *9G8* may serve a different role in the gut, where it may affect compensatory transport, leading to lower the levels of these derivatives.

The SR Protein 9G8 Affects Fly Metabolism by Altering Metabolic Gene Expression

To investigate the effect of *9G8* activity on metabolic gene expression in the gut, we performed RNA-seq in *9G8RNAi* intestines. Differential gene expression was analyzed using Gene Ontology, revealing numerous upregulated fatty acid (FA) synthesis, FA elongation, and lipid catabolism genes. This result is consistent with previous RNA-seq experiments in the *9G8RNAi* fat body, where oxidation-reduction and lipid catabolism genes were found to be upregulated as well (Weidman et al., 2022). However, the specific intestinal genes in question are completely distinct than those of the fat body, suggesting *9G8* invokes a similar phenotype by acting in different pathways in both organs. Moreover, many of the lipid metabolism related genes upregulated in the gut do not undergo alternative splicing upon expression, suggesting *9G8* may function to regulate their expression and not their splicing. It is possible that *9G8* simply alters the splicing of an intermediary molecule that regulates the expression of these genes. However, this could also be explained by *9G8* regulating transcription or mRNA transport, as both *9G8* and *Rbp1* have been previously shown to regulate nuclear mRNA export (Sanford et al., 2005). A possible future direction would thus be to determine whether 9G8 specifically interacts with the upregulated mRNAs identified here.

Several of the upregulated lipid metabolism genes (i.e., *fasn3, eloF,* and *fad2*) in the *9G8RNAi* intestines are involved in the synthesis and elongation of fatty acids. *fasn3,* commonly named FA Synthase, is known to synthesize methyl branched FAs in the presence of methylmalonyl-CoA and malonyl-CoA (de Renobales et al., 1986). *eloF* is known to be primarily expressed in female flies, where it elongates saturated and unsaturated FAs up to 30 carbons (Chertemps et al., 2007). Similarly, fad2, an enzyme primarily active in female flies, has

a non-specified role in pheromone diene production (Chertemps et al., 2006). We also identified several FA elongases, which have not been well characterized in the fly. To help us understand the possible roles these genes play, as well as to relate them to human metabolism, we identified their mammalian homologs. Two genes we identified (cg16904 and cg30008) share homology with ELOVL1 and ELOVL7. ELOVL1 is involved in the elongation of saturated and monounsaturated very long FAs (VLCFAs), while ELOVL7 regulates the elongation of polyunsaturated VLCFAs (Leonard et al., 2004; Jakobsson et al., 2006). Increased expression of these genes and the decreased intestinal triglycerides in *9G8RNAi* intestines may indicate that free FAs may accumulate in the intestine and these FAs may be very unsaturated. Experiments designed to measure the full lipid profile of *9G8RNAi* intestines would help to confirm this hypothesis.

Additionally, multiple genes identified in the RNA-Seq analysis that were predicted to have triglyceride lipase function were homologous to triglyceride lipases in mammals. cg31089, for instance, resembles (in sequence) human LIPA, LIPF, and LIPJ. LIPA is a lysosomal lipase known to hydrolyze triglycerides and diacylglycerols into free FAs and cholesterol (Bianco et al., 2023). LIPF, on the other hand, is a secreted gastric lipase that under acidic conditions, hydrolyzes triglycerides down to monoglycerides during fat emulsification (Armand et al., 1994). Lastly, LIPJ is a known intracellular lipase expressed in the testis (Holmes et al., 2010), the full function of which is yet to be characterized. The homologues of cg31091, however, include a set of minimally characterized epidermal cell lipases, including LIPM, LIPN, and LIPK (Toulza et al., 2007), which suggests some evolutionary conservation between different epidermal tissues of the fly. Finally, cg10169 and cg13562 both have homologous regions with LIPC, LIPH, and LIPG. LIPC is a secreted hepatic lipase that breaks down triglycerides and phospholipids in VLDL and LDL molecules (Connelly, 1999). Similarly, LIPG, secreted from vascular endothelial cells, functions to cleave FAs and other lipid metabolites from HDL complexes, for intracellular absorption (Jaye et al., 1999; Riederer et al., 2012). Lastly, LIPH is a membrane-bound intestinal lipase, that catalyzes the production of lysophosphatidic acid (LPA), a lipid mediator with diverse biological properties (Inoue et al., 2011).

This vast range of human lipase homologues demonstrates that *9G8* may regulate expression of a diverse group of genes, involved in gut lipid metabolism. Specifically, many of these lipases are known to break down triglycerides and phospholipids, supporting the previously

established low triglyceride phenotype in the gut. Therefore, it is possible that these lipases function to breakdown dietary lipids to promote their absorption into the fly intestine. Additionally, we hypothesize that these FAs may be delivered from the intestine to other organs, where they are utilized for triglyceride synthesis, leading to the observed (high triglyceride) whole fly phenotype. FA transport in and out of the intestine would thus be a useful direction to explore in the future.

Conclusions

In summary, we have characterized the metabolic phenotypes of eight SR proteins in the *Drosophila* intestine. These SR proteins are highly conserved in mammals (Shepard and Hertel, 2009) and some have been shown to have metabolic functions. For example, *SRSF3* and *SRSF7*, the mammalian homologs of *Rbp1* and *9G8*, respectively, are decreased in livers of obese patients (Pihlajamäki et al., 2011) and this is consistent with the triglyceride accumulation phenotypes when *Rbp1* and *9G8* are decreased in the *Drosophila* intestine and fat body (Bennick et al., 2019). However, whether these SR proteins function in the mammalian intestine to regulate lipid metabolism is not well understood. Together, the work described here advances our understanding of the metabolic roles of SR proteins in the fly intestine specifically and may have implications as potential therapeutic targets for the treatment of obesity and other metabolism-related diseases.

BIBLIOGRAPHY

Armand, M., et al., 1994. Characterization of emulsions and lipolysis of dietary lipids in the human stomach. The American journal of physiology, 266(3 Pt 1), G372–G381. https://doi.org/10.1152/ajpgi.1994.266.3.G372

Beller M., et al., 2008. COPI Complex Is a Regulator of Lipid Homeostasis. PLOS Biology 6(11): e292. https://doi.org/10.1371/journal.pbio.0060292

Bennick, R. A., Nagengast, A. A., & DiAngelo, J. R. 2019. The SR proteins SF2 and RBP1 regulate triglyceride storage in the fat body of Drosophila. Biochemical and biophysical research communications, 516(3), 928–933. https://doi.org/10.1016/j.bbrc.2019.06.151

Bianco, V., et al., 2023. Impact of (intestinal) LAL deficiency on lipid metabolism and macrophage infiltration. Molecular metabolism, 73, 101737. https://doi.org/10.1016/j.molmet.2023.101737

Bradley, T., Cook, M. E., & Blanchette, M. 2015. SR proteins control a complex network of RNA-processing events. RNA (New York, N.Y.), 21(1), 75–92. https://doi.org/10.1261/rna.043893.113

Chertemps, T., et al., 2006. A female-specific desaturase gene responsible for diene hydrocarbon biosynthesis and courtship behaviour in Drosophila melanogaster. Insect molecular biology, 15(4), 465–473. https://doi.org/10.1111/j.1365-2583.2006.00658.x

Chertemps, T., et al., C. 2007. A female-biased expressed elongase involved in long-chain hydrocarbon biosynthesis and courtship behavior in Drosophila melanogaster.

Proceedings of the National Academy of Sciences of the United States of America, 104(11), 4273–4278. https://doi.org/10.1073/pnas.0608142104

Connelly P. W. 1999. The role of hepatic lipase in lipoprotein metabolism. Clinica chimica acta; international journal of clinical chemistry, 286(1-2), 243–255. https://doi.org/10.1016/s0009-8981(99)00105-9

de Renobales, M. and Blomquist, G. J. 1984. Biosynthesis of medium chain fatty acids in Drosophila melanogaster. Archives of biochemistry and biophysics, 228(2), 407–414. https://doi.org/10.1016/0003-9861(84)90004-3

Dennis, G., et al., 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 4, R60. https://doi.org/10.1186/gb-2003-4-9-r60

Ding, S., et al., 2012. Regulation of alternative splicing of tau exon 10 by 9G8 and Dyrk1A. Neurobiology of aging, 33(7), 1389–1399. https://doi.org/10.1016/j.neurobiolaging.2010.11.021

Escudero-Paunetto, L., et al., 2010. SR proteins SRp20 and 9G8 contribute to efficient export of herpes simplex virus 1 mRNAs. Virology, 401(2), 155–164. https://doi.org/10.1016/j.virol.2010.02.023

Fu, Y., & Wang, Y. 2018. SRSF7 knockdown promotes apoptosis of colon and lung cancer cells. Oncology letters, 15(4), 5545–5552. https://doi.org/10.3892/ol.2018.8072

Gingras, M., et al., 2014. The control of lipid metabolism by mRNA splicing in Drosophila BBRC, 443(2): 672-676.

Guo, Y., et al., 2008. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. Nature 453, 657–661 https://doi.org/10.1038/nature06928

Hales, C. M., et al., 2020. Prevalence of Obesity and Severe Obesity Among Adults: United States, 2017-2018. *NCHS data brief*, (360), 1–8.

Holmes, R. S., Cox, L. A., and VandeBerg, J. L. 2010. Comparative studies of mammalian acid lipases: Evidence for a new gene family in mouse and rat (Lipo). Comparative biochemistry and physiology. Part D, Genomics & proteomics, 5(3), 217–226. https://doi.org/10.1016/j.cbd.2010.05.004

Horne, I., Haritos, V. S., & Oakeshott, J. G. 2009. Comparative and functional genomics of lipases in holometabolous insects. Insect biochemistry and molecular biology, 39(8), 547–567. https://doi.org/10.1016/j.ibmb.2009.06.002

Inoue, A., et al., 2011. LPA-producing enzyme PA-PLA$_1\alpha$ regulates hair follicle development by modulating EGFR signalling. The EMBO journal, 30(20), 4248–4260. https://doi.org/10.1038/emboj.2011.296

Ja, W. W., et al., 2007. Prandiology of Drosophila and the CAFE assay. Proceedings of the National Academy of Sciences of the United States of America, 104(20), 8253–8256. https://doi.org/10.1073/pnas.0702726104

Jakobsson, A., Westerberg, R., and Jacobsson, A. 2006. Fatty acid elongases in mammals: their regulation and roles in metabolism. Progress in lipid research, 45(3), 237–249. https://doi.org/10.1016/j.plipres.2006.01.004

Jaye, M., et al., 1999. A novel endothelial-derived lipase that modulates HDL metabolism. Nature genetics, 21(4), 424–428. https://doi.org/10.1038/7766

Jumaa, H., Wei, G., & Nielsen, P. J. 1999. Blastocyst formation is blocked in mouse embryos lacking the splicing factor SRp20. Current biology: CB, 9(16), 899–902. https://doi.org/10.1016/s0960-9822(99)80394-7

Labourier, E., et al., 1999. Recognition of exonic splicing enhancer sequences by the Drosophila splicing repressor RSF1. Nucleic acids research, 27(11), 2377–2386. https://doi.org/10.1093/nar/27.11.2377

Leonard, A. E., et al., 2004. Elongation of long-chain fatty acids. Progress in lipid research, 43(1), 36–54. https://doi.org/10.1016/s0163-7827(03)00040-7

Lin, X., & Li, H. 2021. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Frontiers in endocrinology, 12, 706978. https://doi.org/10.3389/fendo.2021.706978

Malik, A., & Hoenig, L. J. 2019. Can aging be slowed down?. *Clinics in dermatology*, *37*(4), 306–311. https://doi.org/10.1016/j.clindermatol.2019.04.003

Matera, A. G., & Wang, Z. 2014. A day in the life of the spliceosome. Nature reviews. Molecular cell biology, 15(2), 108–121. https://doi.org/10.1038/nrm3742

Miguel-Aliaga, I., Jasper, H., & Lemaitre, B. 2018. Anatomy and Physiology of the Digestive Tract of Drosophila melanogaster. Genetics, 210(2), 357–396. https://doi.org/10.1534/genetics.118.300224

Mikoluk, C., Nagengast, A. A., & DiAngelo, J. R. 2018. The splicing factor transformer2 (tra2) functions in the Drosophila fat body to regulate lipid storage. Biochemical and biophysical research communications, 495(1), 1528–1533. https://doi.org/10.1016/j.bbrc.2017.12.002

Musselman, L. P., & Kühnlein, R. P. 2018. Drosophila as a model to study obesity and metabolic disease. The Journal of experimental biology, 221(Pt Suppl 1), jeb163881. https://doi.org/10.1242/jeb.163881

Palm, W., et al., 2012. Lipoproteins in Drosophila melanogaster--assembly, function, and influence on tissue lipid composition. PLoS genetics, 8(7), e1002828. https://doi.org/10.1371/journal.pgen.1002828

Pan, Y. J., et al., 2022. Alternative splicing of HSPA12A pre-RNA by SRSF11 contributes to metastasis potential of colorectal cancer. Clinical and translational medicine, 12(11), e1113. https://doi.org/10.1002/ctm2.1113

Phillips, M. D., & Thomas, C. M. 2006. Brush border spectrin is required for early endosome recycling in Drosophila. Journal of cell science, 119(Pt 7), 1361–1370. https://doi.org/10.1242/jcs.02839

Pihlajamäki, J., et al., 2011. Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. Cell metabolism, 14(2), 208–218. https://doi.org/10.1016/j.cmet.2011.06.007

Pospisilik, J. A., et al., 2010. Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. Cell, 140(1), 148–160. https://doi.org/10.1016/j.cell.2009.12.027

Riederer, M., et al., 2012. Impact of endothelial lipase on cellular lipid composition. Biochimica et biophysica acta, 1821(7), 1003–1011. https://doi.org/10.1016/j.bbalip.2012.03.006

Sanford, J. R., Ellis, J., & Cáceres, J. F. 2005. Multiple roles of arginine/serine-rich splicing factors in RNA processing. Biochemical Society transactions, 33(Pt 3), 443–446. https://doi.org/10.1042/BST0330443

Schürch, C., et al., 2021. SRP54 mutations induce congenital neutropenia via dominant-negative effects on XBP1 splicing. Blood, 137(10), 1340–1352. https://doi.org/10.1182/blood.2020008115

Sen, S., Jumaa, H., & Webster, N. J. 2013. Splicing factor SRSF3 is crucial for hepatocyte differentiation and metabolic function. Nature communications, 4, 1336. https://doi.org/10.1038/ncomms2342

Sen, S., Langiewicz, M., Jumaa, H., & Webster, N. J. 2015. Deletion of serine/arginine-rich splicing factor 3 in hepatocytes predisposes to hepatocellular carcinoma in mice. Hepatology (Baltimore, Md.), 61(1), 171–183. https://doi.org/10.1002/hep.27380

Shepard, P. J., & Hertel, K. J. 2009. The SR protein family. Genome biology, 10(10), 242. https://doi.org/10.1186/gb-2009-10-10-242

Sieber, M. H., & Thummel, C. S. 2012. Coordination of triacylglycerol and cholesterol homeostasis by DHR96 and the Drosophila LipA homolog magro. Cell metabolism, 15(1), 122–127. https://doi.org/10.1016/j.cmet.2011.11.011

Staats, S., et al., 2018. Drosophila melanogaster as a versatile model organism in food and nutrition research. Journal of agricultural and food chemistry, 66(15), 3737–3753. https://doi.org/10.1021/acs.jafc.7b05900

Toulza, E., et al., 2007. Large-scale identification of human genes implicated in epidermal barrier function. Genome biology, 8(6), R107. https://doi.org/10.1186/gb-2007-8-6-r107

Walsh, C. M., et al., 2013. Serine arginine splicing factor 3 is involved in enhanced splicing of glucose-6-phosphate dehydrogenase RNA in response to nutrients and hormones in liver. The Journal of biological chemistry, 288(4), 2816–2828. https://doi.org/10.1074/jbc.M112.410803

Weidman, T., Nagengast, A. A., & DiAngelo, J. R. 2022. The splicing factor 9G8 regulates the expression of NADPH-producing enzyme genes in Drosophila. Biochemical and biophysical research communications, 620, 92–97. https://doi.org/10.1016/j.bbrc.2022.06.073

Wu, J. Y., et al., 2006. SRp54 (SFRS11), a regulator for tau exon 10 alternative splicing identified by an expression cloning strategy. Molecular and cellular biology, 26(18), 6739–6747. https://doi.org/10.1128/MCB.00739-06