THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DIVISION OF SCIENCE

# The Regulation of Carnitine Palmitoyltransferase 1 (CPT1) mRNA Splicing by Nutrient Availability in *Drosophila* Fat Tissue

HUY GIA TRUONG
SPRING 2023

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

Reviewed and approved* by the following:

Justin DiAngelo
Associate Professor of Biochemistry and Molecular Biology
Thesis Supervisor

Sandy Feinstein
Professor of English
Honors Adviser

* Electronic approvals are on file.

**ABSTRACT**

After a meal, excess nutrients are stored within adipose tissue as triglycerides in lipid droplets. Previous genome-wide RNAi screens in *Drosophila* cells have identified mRNA splicing factors, genes that function in the expression and processing of mRNAs, as being important for lipid droplet formation. The DiAngelo lab has previously shown that a class of mRNA splicing factors called serine/arginine-rich (SR) proteins, which help to identify intron/exon borders, are important for triglyceride storage in *Drosophila* fat tissue, partially by regulating the splicing of the gene for carnitine palmitoyltransferase 1 (CPT1), an enzyme important for mitochondrial ß-oxidation of fatty acids. The *CPT1* gene in *Drosophila* generates two major isoforms, with transcripts that include exon 6A producing more active enzymes than ones made from transcripts containing exon 6B. To investigate whether nutrient availability regulates *CPT1* splicing in fly fat tissue, *CPT1* transcripts were measured under fed and fasted/starved conditions. During ad libitum feeding, control flies produce more *CPT1* transcripts containing exon 6B while fasting for 24 hours results in a shift in *CPT1* splicing to generate more transcripts containing exon 6A. Enzymes made from transcripts with exon 6A are more active, consistent with the need for triglyceride breakdown to generate energy under starvation. The SR protein 9G8 is necessary for regulating nutrient responsive *CPT1* splicing as decreasing 9G8 levels in fly fat tissue blocks the accumulation of *CPT1* transcripts including exon 6A during starvation. The role of protein kinase A (PKA), a mediator of starvation-induced lipid breakdown, in regulating *CPT1* splicing during starvation was also investigated. In control flies, starvation resulted in more transcripts including exon 6A, but transcripts including exon 6A did not accumulate when PKA was inhibited during starvation, suggesting that PKA responds to starvation to control *CPT1* splicing in fly fat tissue. Together, these results indicate that *CPT1*

splicing in adipose tissue responds to changes in nutrient availability contributing to the overall control of lipid homeostasis.

# TABLE OF CONTENTS

LIST OF FIGURES ........ iii

ACKNOWLEDGMENTS ........ v

Chapter 1 Introduction ........ 1

Chapter 2 Materials and Methods ........ 8

- Fly Genetics ........ 8
- Macromolecule Assays ........ 8
- Starvation Assay and RNA Isolation ........ 9
- DNAse Treatment, cDNA Synthesis, and qPCR ........ 9
- Statistical Analysis ........ 10

Chapter 3 Results ........ 11

- Regulation of *CPT1* Splicing by Starvation ........ 11
- The Role of SR Proteins in Starvation-induced *CPT1* Splicing ........ 12
- Confirmation of PKA Activation and Inhibition ........ 15
- PKA Regulates *CPT1* Splicing ........ 18

Chapter 4 Discussion ........ 21

BIBLIOGRAPHY ........ 27

# LIST OF FIGURES

Figure 1. *CPT1* Splicing. ........................................................................................................ 5

Figure 2. Metabolic signals that regulate lipid metabolism. ....................................... 6

Figure 3. Starvation regulates *CPT1* splicing. ............................................................ 11

Figure 4. The SR protein transporter Tnpo-SR and the SR protein 9G8 are necessary to regulate *CPT1* splicing during starvation. ............................................................ 14

Figure 5. Activating PKA decreases triglyceride levels while inhibiting PKA promotes fat and glycogen storage. ............................................................................... 18

Figure 6. PKA is necessary for the starvation-induced splicing of *CPT1*. .................. 20

Figure 7. Regulation of CPT1 splicing by SR proteins and SRPK.............................. 24

Figure 8. Regulation of SRPK activity and CPT1 splicing by nutrients. .................... 25

## ACKNOWLEDGMENTS

I am forever grateful to Dr. Justin DiAngelo for giving me the opportunity to perform my research, for being my Thesis Supervisor, and for his guidance, kindness, and patience throughout my time as a student at Penn State Berks.

Thank you to Dr. Sandy Feinstein, my Honors Advisor, for your wisdom and guidance throughout my thesis writing process, as well as for your honesty and compassion during my time as a Schreyer scholar.

Thank you to Dr. Holly Ryan who helped me to become the writer and student I am today, and for supporting me during my times here at Berks.

Lastly, thank you to my parents and sister for fostering my love for learning, as well as their unconditional support, nurture, and love.

# Chapter 1

## Introduction

Obesity and related issues of excess energy storage have been health concerns and the focus of decades of medical research. Defined as an increase in body weight as a result of excess fat storage, obesity remains an epidemic plaguing more than 39% of individuals across the globe, compromising their health and quality of life (Chooi et al 2019). Obesity has been linked to increased rates of diseases associated with higher mortality rates such as hypertension and coronary artery disease, type-II diabetes, and hepatic diseases (Upadhyay et al., 2018). More importantly, obese individuals diagnosed with cancer report higher mortality rates than obese people without cancer, making obesity a complex metabolic issue amongst high-risk patients (Pi-Sunyer, 2012). Therefore, obesity is a pervasive and expanding biomedical research area.

To identify the genes important in regulating the pathogenesis of obesity, scientists have employed genome-wide association studies (GWAS). GWAS specifically map genomes in people with a specific disease or phenotype, identify small genetic variants that are linked to the disease/phenotype, and then identify the genes associated with those genetic variants and, ultimately, those diseases (Cano-Gamez and Trynka 2020). For example, a single-nucleotide polymorphism (SNP) was first identified in the fat mass and obesity associated (*FTO*) gene of European young adults afflicted with type 2 diabetes mellitus (Loos and Yeo 2013). This SNP-containing *FTO* allele was correlated with increased body mass index (BMI) observed in these individuals in addition to cardiovascular issues (Loos and Yeo 2013). In addition, other GWAS of individuals with impaired metabolism found correlation between insulin response (IR) and

*FTO*. Genetic variations within the insulin receptor substrate 1 (IRS1) gene are linked to increased insulin resistance and fatty acid uptake in liver and muscle tissues, suggesting a correlation between insulin resistance and increased BMI (Fall and Ingelsson, 2014; Loos and Yeo 2013). Together, GWAS provide one way in which scientists can identify genes important to regulate obesity and obesity-related diseases such as diabetes.

A complementary approach to GWAS used to identify genes important for the development of metabolic diseases is genome-wide transcriptomic analysis. This approach uses microarrays and RNA sequencing to characterize the differential expression of crucial genes associated with obesity and other metabolic diseases in patients compared to normal individuals. For example, 41 to 43 out of 199 genes involved in RNA processing were found to be downregulated in human liver and muscle RNA isolated from obese individuals (Pihlajamäki et al., 2011). Interestingly, other transcriptomic studies focused on levels of DNA methylation to analyze gene expression and found that lipogenic genes were significantly underexpressed in adipose tissues of Caucasian individuals with impaired insulin sensitivity and triglyceride uptake (Keller et al 2017). While GWAS and transcriptomic analyses have increased our knowledge and understanding of the genetic underpinnings of obesity and other related metabolic diseases, there are limitations to these approaches to identify and then study novel genes associated with obesity and related diseases in human cells. The use of a genetic model organism can help expand our knowledge and understanding of the genes that regulate lipid metabolism.

In addition to mammalian model systems, *Drosophila melanogaster*, the common fruit fly, has been used extensively to study human diseases. Crucially, 75% of human disease genes and up to 80% of functional proteins are found in the *Drosophila* genome (Yamaguchi & Yoshida 2018). Furthermore, *Drosophila* have a fast life cycle, can produce large numbers of

offspring, and are economically advantageous to study in a laboratory setting (Yamaguchi & Yoshida 2018). Therefore, *Drosophila* genetics can be utilized to study obesity and related metabolic human diseases. Moreover, *Drosophila* adipose tissues function similarly to humans and other mammals, employing conserved lipogenic and lipolytic signaling pathways to use and metabolize fats (Zheng et al 2016; Musselman and Kuhnlein 2018), thus placing *Drosophila* in an excellent position to use to study the genetic control of lipid metabolism.

Previous genome-wide RNA interference (RNAi) screens have been performed on cultured *Drosophila* cells and intact flies to identify genes important for triglyceride storage and lipid droplet formation (Guo et al 2008; Beller et al 2008; Popsilik et al 2010). Genes-encoding members of the mRNA-processing spliceosome complex, were found to regulate lipid droplet size and number from these screens (Guo et al 2008; Beller et al., 2008). To regulate gene expression, the splicing machinery assembles at specific mRNA nucleotide sequences, the 5' and 3' splice sites, catalyzes splicing by removing non-coding regions of genes called introns, and connects selected protein coding regions of genes called exons, resulting in many different mRNA transcripts that code for different proteins all from a single gene (Keleman et al 2013; Will and Lürhmann 2011). General splicing factors such as the U1 and U2 small nuclear ribonucleoproteins (snRNPs) bind to specific mRNA sequences at the intron/exon junction to catalyze nucleotide excision, while regulatory splicing factors such as heterogeneous nuclear ribonucleoproteins (hnRNPs) inhibit splicing of introns, and serine-arginine rich (SR) proteins promote splice site usage (Will and Lürhmann 2011). The DiAngelo lab has identified many mRNA splicing factors that are necessary for regulating lipid metabolism in *Drosophila* by decreasing the expression of the genes encoding these factors using RNAi specifically targeted to fly fat tissue. For example, the general splicing factors U1 and U2 snRNPs were both found to

promote triglyceride storage and lipid droplet formation, consistent with the results of the RNAi screen data(Shabar et al 2022; Gingras et al 2014; Guo et al 2008; Beller et al., 2008). In contrast, the lab also identified that the regulatory splicing factors Hrb27c (an hnRNP), the SR proteins 9G8, SF2, and transformer 2 (tra2), all promote lipid breakdown, whose results are inconsistent with the RNAi screen described above (Bhogal et al 2020; Gingras et al 2014; Weidman et al 2022; Mikoluk et al., 2018; Bennick et al., 2019; Guo et al 2008; Beller et al., 2008). These results demonstrate the varied regulation of lipid metabolism by splicing factors to promote either fat accumulation or breakdown. However, the specific mechanisms of how splicing factors regulate genes encoding lipid metabolism enzymes are poorly understood.

An important lipid breakdown protein, the splicing of which is regulated by SR proteins, is carnitine palmitoylacyltransferase 1 (CPT1). Specifically, CPT1 catalyzes the transfer of long chain fatty acyl CoAs, which are impermeable to the mitochondrial membrane, across and into the mitochondrial matrix, where they are further broken down via beta oxidation (Schlaepfer and Joshi 2020). In *Drosophila* adipose tissue, the *CPT1* gene is alternatively spliced resulting in two major variants, differing by a single exon: 6A and 6B. Transcripts containing 6A exon produce a more active CPT1 enzyme, suggesting a higher rate of fatty acid metabolism, than exon 6B-containing transcripts (Price et al 2010) (Figure 1).

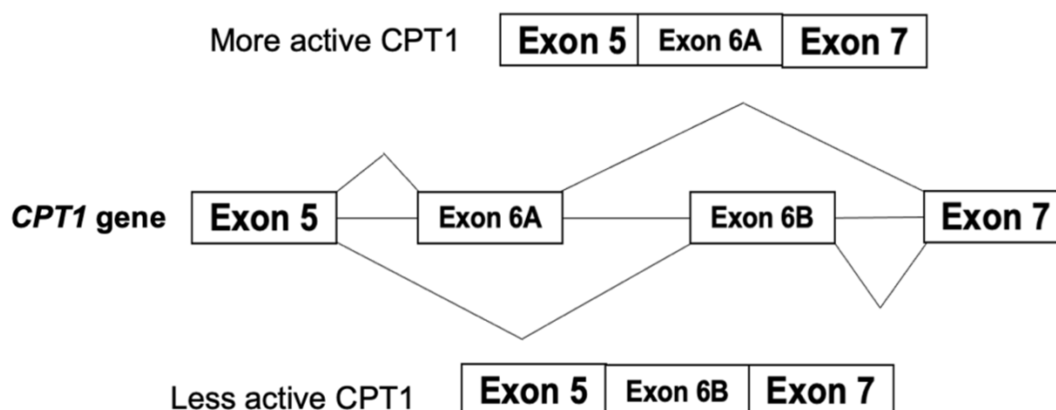



**Figure 1. *CPT1* Splicing.**

**Schematic diagram of *CPT1* splicing that displays two isoforms that include different versions of exon 6. Enzymes produced from transcripts including exon 6A are more active than enzymes produced from transcripts including exon 6B. (Adapted from Price et al 2010).**

Previous work from the DiAngelo lab has identified the SR proteins 9G8, tra2 and SF2 to regulate *CPT1* splicing functioning to limit triglyceride storage (Gingras et al 2014;Mikoluk et al., 2018; Bennick et al., 2019). In addition, the lab has also shown that the nuclear SR protein import protein Tnpo-SR, which shuttles splicing factors such as 9G8 in and out of the nucleus, alters *CPT1* splicing to limit lipid accumulation (Nagle et al 2022). Together, the data suggest that splicing factors play a key metabolic role in regulating lipid storage by modulating the splicing of the *CPT1* gene. However, the metabolic signals that control *CPT1* splicing are not fully understood.

A major signal that alters cellular metabolism is the presence or absence of food. The presence of glucose after a meal results in the secretion of the peptide hormone insulin, stimulating glucose uptake in response to the rise in blood glucose levels as well as *de novo* lipogenesis, leading to the conversion of acetyl-CoA to fatty acids (Sakers et al., 2022; Lewis et al 2002) (Figure 2B). By binding to the insulin receptor, insulin activates protein kinase B (PKB)

to promote glucose uptake and glycogen synthesis (Whiteman et al 2002; Soeters et al 2012; Sakers et al., 2022). Additionally, insulin promotes fatty acid uptake by adipose tissues and fatty acid esterification by suppressing hormone sensitive lipase (HSL), which functions to cleave fatty acids from triglycerides (Lewis et al 2002). Conversely, the lack of food (fasting or starvation) causes insulin levels to drop, leading to the inhibition of the PKB pathway in order to decrease glucose uptake and glycogen synthesis (Soeters et al 2012; Whiteman et al 2002). Consequently, glucagon levels rise, causing a rise in intracellular cyclic-AMP (cAMP) that activates protein kinase A (PKA). PKA then phosphorylates perilipin and HSL to promote lipid breakdown and the production of fatty acids, which are ultimately shuttled by CPT1 into the mitochondria for beta oxidation (Soeters et al 2012; Sakers et al., 2022; Schlaepfer and Joshi 2020 ) (Figure 2A). Previous studies in white adipose tissues of adult male rats found that an 8 hour fasting period is associated with increased levels of CPT1 and *CPT1* mRNA, suggesting that lipid breakdown and oxidation is increased in response to decreased nutrients (Palou et al 2008). However, the mechanisms of how nutrient availability affects *CPT1* splicing are unknown.

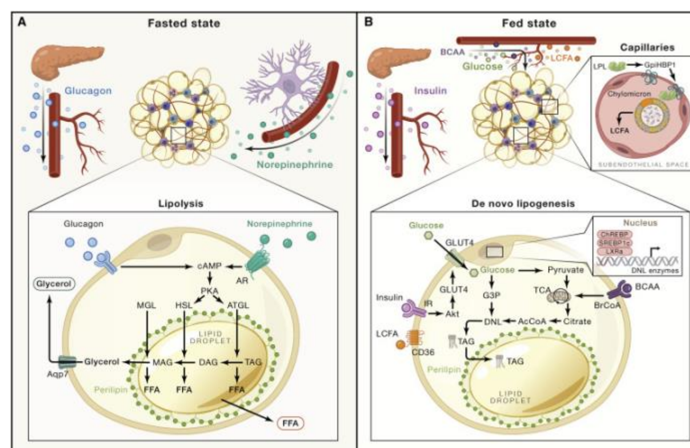



**Figure 2. Metabolic signals that regulate lipid metabolism.**

**(A) Lipolysis is activated during fasted state via glucagon and PKA signaling. (B) Lipogenesis is promoted during fed state via Akt/PKB signaling and glucose uptake. (Sakers et al., 2022)**

The following study sets out to identify and characterize the metabolic signals that regulate the splicing of *CPT1* in *Drosophila* fat tissue. While previous studies have summarized the effects of nutrient availability on lipid storage (Sakers et al., 2022; Doh et al 2005; Lewis et al 2002; Whiteman et al 2002; Soeters et al 2012), there is still limited information on whether the presence or absence of food affects *CPT1* splicing. More importantly, the role of splicing factors in regulating *CPT1* splicing under different nutrient conditions requires more understanding. The results show that a 24-hour starvation period promotes preferential inclusion of exon 6A in the *CPT1* gene in *Drosophila* fat tissue and that the SR protein 9G8 and the SR protein transporter Tnpo-SR are necessary for this to occur. In addition, PKA, a kinase known to be activated during fasting, is shown to mediate *CPT1* splicing under starvation to preferentially produce exon 6A-containing *CPT1* transcripts. Together, these data suggest that starvation promotes increased lipid breakdown by regulating *CPT1* splicing, thus increasing our understanding of how alternative splicing affects overall lipid homeostasis and perhaps the pathogenesis of obesity and associated metabolic disorders.

# Chapter 2

# Materials and Methods

## Fly Genetics

The following flies were used in this study: *yolkGal4* (Georgel et al., 2001); *y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=VALIUM20-EGFP}attP2* (BL#35782, referred to as *UAS-EGFP-RNAi); w[1118]; UAS-9G8RNAi* (VDRC #31203);*y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02010}attP2* (BL#25988, referred to as *UAS-Tnpo-SR-RNAi*); *w[*]; P{w[+mC]=UAS-GFP.S65T}Myo31DF[T2] (*BL#1521, referred to as *UAS-GFP*); *y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMC04936}attP40* (BL#57743, referred to as *UAS-PKA-C1-RNAi*); *w[*]; P{w[+mC]=UAS-PKA-C1.FLAG}1.1* (BL#35554, referred to as *UAS-PKA-C1); y[1] sc[*] v[1] sev [21]; P{y[+t7.7] v[+t1.8]=VALIUM20-EGFP.shRNA.1}attP40* (BL#41555, referred to as *UAS-EGFP-RNAi-2*); and *w[*]; P{w[+mC]=UAS-PKA-R1.BDK}35* (BL#35550, referred to as *UAS-PKA-R1-BDK*). Flies were grown on standard cornmeal-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) at 25° C in a 12h:12h light:dark cycle.

## Macromolecule Assays

Two one-week old female flies were homogenized in lysis buffer (140 mM NaCl, 50 mM Tris-HCl with pH 7.4, and 0.1% Triton-X) and 1X Complete Protease Inhibitor (Roche). Protein concentrations were determined using the Pierce BCA Assay kit (ThermoFisher Scientific), triglycerides were determined using Infinity Triglyceride Reagent (ThermoFisher Scientific), and

free glucose was determined using the Pointe Scientific Glucose Oxidase Kit (ThermoFisher Scientific) according to the manufacturer's instructions. Total glucose levels were determined by treating homogenized samples with 8 mg/mL amyloglucosidase diluted in 0.2M citrate buffer, pH. 5.0 and incubated for 2 hours at 37° C. Glycogen levels were then calculated by subtracting the free glucose from the total glucose levels. Triglyceride, free glucose, and glycogen levels were then normalized by total protein content.

**Starvation Assay and RNA Isolation**

For starvation experiments, one-week old female flies were starved for 24 hours before fat body dissection and compared to flies that had been given regular access to food. Cuticles with fat bodies attached were dissected from 15 one-week old adult female flies and homogenized in Ribozol RNA Extraction Reagent (AMRESCO). Samples were then incubated for 5 minutes (min) at room temperature and centrifuged for 15 min at 12,000 rpm at 4°C. The samples were chloroform extracted, incubated again at room temperature for 3 min and centrifuged for 15 min at 12,000 rpm at 4°C. Isopropanol was then added to the sample and centrifuged at 12,000 rpm for 15 min at 4°C to pellet the RNA. The pellet was washed with 75% ethanol, dried, and resuspended in nuclease-free water.

**DNAse Treatment, cDNA Synthesis, and qPCR**

5 µg of isolated RNA was DNase treated using the DNA-Free Turbo Kit according to the manufacturer's instruction (Ambion). 0.25 µg of DNase treated RNA was then reverse transcribed using qScript-XLT cDNA Supermix (Quanta Biosciences) according to the

manufacturer's protocol. The generated cDNA was amplified with 1 µL cDNA and 1X Perfecta SYBR Green Supermix (Quanta Biosciences). qPCR analysis was performed on samples in a Step-One Plus qPCR machine, with the following protocol: initial denaturation at 95°C for 3 min, followed by 40 cycles of 15 seconds (sec) at 95°C and 1 min at 60°C, followed by a melt curve. The primers used to amplify all *CPT1* isoforms were: (Forward: 5'-GCAAGTGCAAATTGAGGAAA-3', Reverse: 5'-AAGTGCTCCTCACCTTCCAC-3'), exon 6A-containing isoforms (Forward: 5'-CCGCTGGTTTGACAAGTG-3', Reverse: 5'-TCATCGACGATCAGGTTCTC-3'), and exon 6B-containing isoforms (Forward: 5'-AATGGTCGCGTTGGCTTC-3'; Reverse: 5'-TCCCAAAACCGGTGCATC-3'). The primers used for the *rp49* gene were (Forward: 5'-CCGCCACCAGTCGGATC-3', Reverse: 5'-TTGGGCTTGCGCCATT-3'). All primers sequences were obtained from Price et al, 2010. Relative expression of these genes was normalized by the *rp49* expression levels.

## Statistical Analysis

Standard deviation and standard error were calculated for all sample groups and a t-test was conducted to determine whether there were any differences between experimental and control groups. A P value $\leq 0.05$ was determined to be statistically significant.

# Chapter 3

# Results

## Regulation of *CPT1* Splicing by Starvation

To understand the signals that control fat storage, the regulation of *CPT1* splicing was first examined in *Drosophila* fat bodies. Based on previous studies, *CPT1* is shown to be alternatively spliced in a tissue-specific manner, resulting in two variants differing by a single exon 6 (Figure 1) (Price et al 2010).

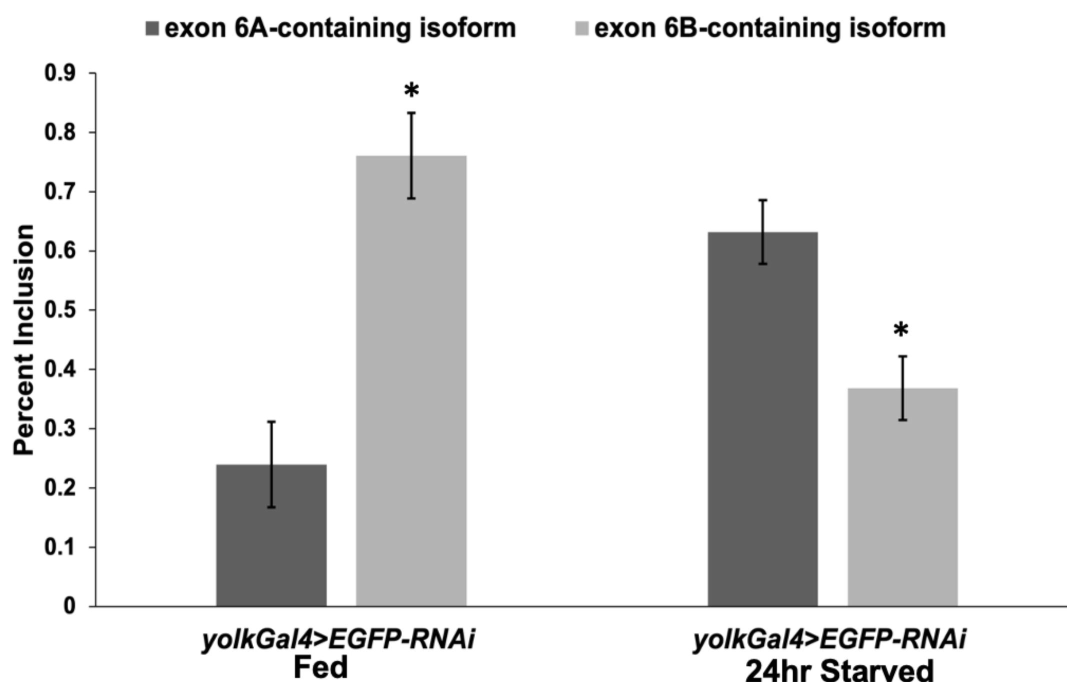



**Figure 3. Starvation regulates *CPT1* splicing.**

**RNA was isolated from fat bodies dissected from 1 week old *yolkGal4>EGFP-RNAi* females either fed or starved for 24 hours (n=5-10). qPCR was performed for *CPT1* isoforms and quantities were then normalized by *rp49,* and fractions of each transcript including either exon 6A or exon 6B that were expressed were calculated. Values are mean ± standard error. *, P < 0.05 by Student's *t* test comparing the percent inclusion of *CPT1* isoforms containing exon 6A to those containing exon 6B in each condition. Data generated by Jasleen Bhogal and Dr. DiAngelo.**

Since starvation induces lipolysis in adipocytes (Jaworski et al., 2007), it was hypothesized that starvation is a potential signal that alters the splicing of *CPT1* in *Drosophila* fat tissue. To test this hypothesis, *EGFP-RNAi* flies were starved for 24 hours, and RNA were isolated from fat bodies from these flies, and qPCR for *CPT1* isoforms was performed and compared to fed flies. The results confirm that a 24-hour starvation increases the relative amount of exon 6A-containing isoforms of *CPT1* compared to fed *EGFP-RNAi* flies (Fig. 1B). These data suggest that starvation serves as a signal that regulates *CPT1* splicing promoting the inclusion of exon 6A, thus producing a more active enzyme.

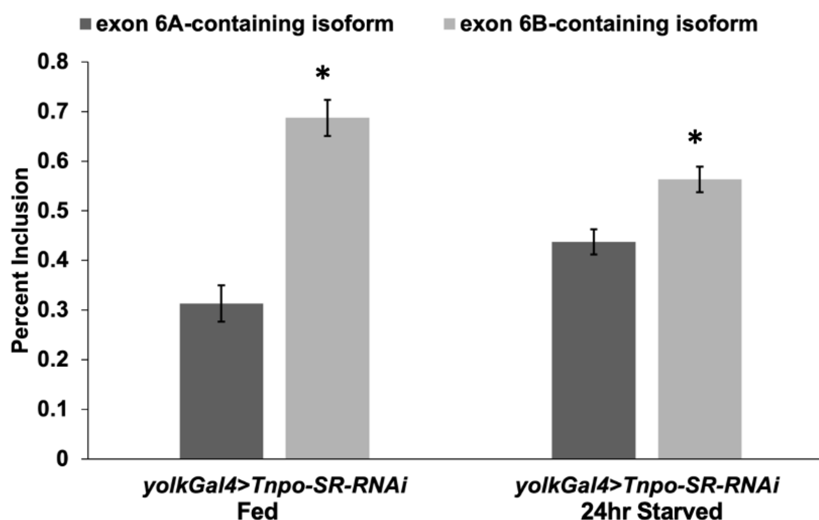
## The Role of SR Proteins in Starvation-induced *CPT1* Splicing

Since it was shown that starvation alters *CPT1* splicing, the next step was to investigate whether splicing factors are regulated by starvation to control the splicing of *CPT1*. Previous studies have characterized a nuclear protein transporter called transportin-SR, or Tnpo-SR, in *Drosophila* that shuttles a variety of serine/arginine (SR) domain-containing proteins in and out of the nucleus (Allemand et al., 2002). In addition, the DiAngelo lab has shown that Tnpo-SR regulates the splicing of *CPT1* transcripts to promote the inclusion of exon 6A (Nagle et al., 2022). Since starvation also seems to promote the inclusion of exon 6A, it was hypothesized that Tnpo-SR is necessary for starvation-induced splicing of *CPT1*. To test this hypothesis, *Tnpo-SR-RNAi* flies were starved for 24 hours, and their fat-tissue RNA was isolated and measured for *CPT1* splicing as described above. Similar to previous studies, fed *Tnpo-SR-RNAi* flies have more *CPT1* isoforms with exon 6B than isoforms with exon 6A in fat tissue (Fig 2A; Nagle et al., 2022). If Tnpo-SR is necessary for promoting the inclusion of exon 6A in *CPT1* during

starvation, then it is expected that starved *Tnpo-SR-RNAi* flies would accumulate *CPT1* isoforms with exon 6B. Consistent with this hypothesis, when *Tnpo-SR-RNAi* flies were starved, the amount of exon 6B-containing transcripts remained higher than exon 6A-containing transcripts (Fig. 2B) suggesting that Tnpo-SR regulates starvation-induced *CPT1* splicing.

Among the SR proteins that are shuttled by Tnpo-SR, 9G8 was identified to be an important splicing factor that promotes the usage of exon 6A in *CPT1* (Allemand et al., 2002; Gingras et al., 2014). Since 9G8 increases the levels of *CPT1* transcripts with exon 6A, whether 9G8 functions to promote exon 6A usage during starvation was also investigated. To address this question, *CPT1* splicing was measured from starved *9G8-RNAi* flies. As shown previously, fed *9G8-RNAi* flies have more exon 6B-containing isoforms of *CPT1* than exon 6A-containing isoforms in their fat bodies (Fig. 2A; Gingras et al., 2014). If 9G8 is important for exon 6A inclusion in *CPT1* during starvation, then it is expected that starving *9G8-RNAi* flies would decrease the amount of *CPT1* transcripts with exon 6A. Consistent with this hypothesis, when *9G8-RNAi* flies were starved, the amount of exon-6B containing isoforms remained higher than exon-6A containing isoforms (Fig. 2B). Together, the results suggest that 9G8 and Tnpo-SR are necessary proteins that regulate *CPT1* splicing under starvation to increase exon 6A-containing isoforms.

**A**




**B**

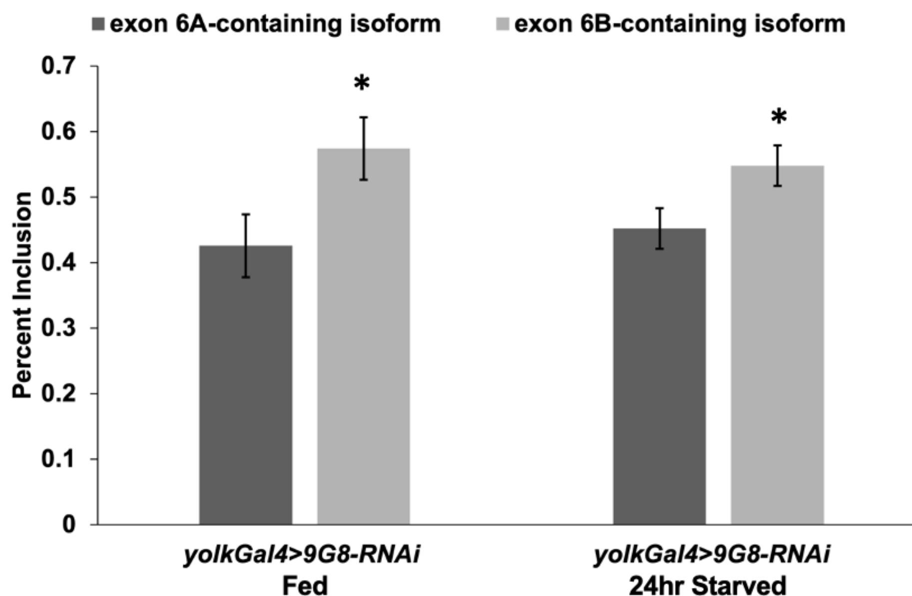



**Figure 4. The SR protein transporter Tnpo-SR and the SR protein 9G8 are necessary to regulate *CPT1* splicing during starvation.**

**RNA was isolated from fat bodies dissected from 1-week old (A) *yolkGal4>Tnpo-SR-RNAi* (n=8) and (B) *yolkGal4>9G8-RNAi* (n=9-14) females starved for 24 hours and compared to fed controls of the same genotypes. qPCR was then performed for *CPT1* isoforms including either exon 6A or 6B and quantities were normalized by *rp49,* and fractions of each transcript including either exon 6A or exon 6B that were expressed were calculated. Values are mean ± standard error. *, P < 0.05 by Student's *t* test comparing the percent inclusion of *CPT1* isoforms containing exon 6A to those containing exon 6B in each condition. Data generated by Annabella Kolasa and Dr. DiAngelo.**

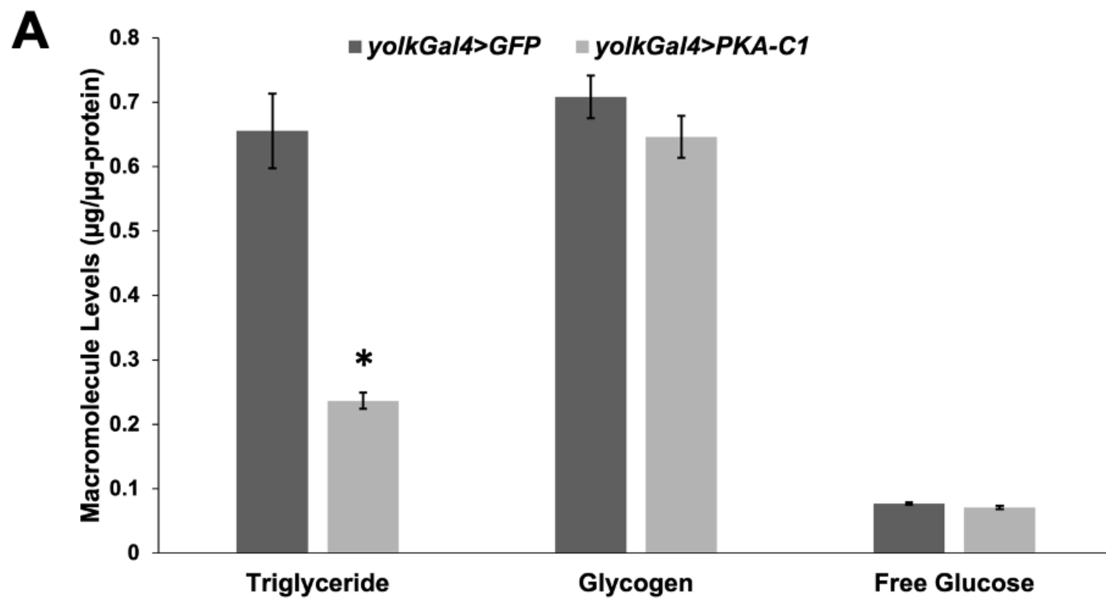
## Confirmation of PKA Activation and Inhibition

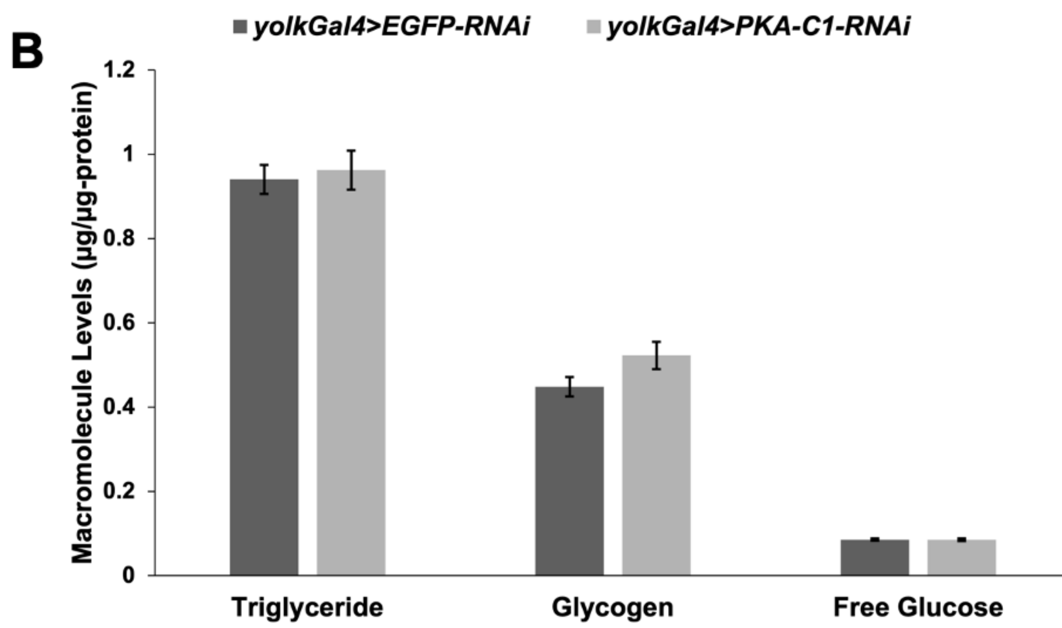
The data so far support the notion that starvation regulates *CPT1* splicing via SR proteins, but the signaling molecules and pathways linking starvation to splicing factors are not known. In response to starvation, glycogen and lipid breakdown are promoted in order to produce energy for cellular homeostasis (Soeters et al 2015) and the cyclic-AMP (cAMP)-dependent protein kinase A (PKA) is also activated to promote lipid breakdown (lipolysis) and glucose production (gluconeogenesis) (London et al 2020). Since *CPT1* is the rate-limiting step in the oxidation of fatty acids, a process activated during fasting (Schlaepfer  et al., 2020), it was hypothesized that PKA also plays a role in regulating fatty acid oxidation by controlling *CPT1* splicing.

PKA functions as a tetramer composed of two catalytic subunits, C1 and C2, which carry out kinase activities, and two regulatory subunits, R1 and R2, which bind to catalytic subunits and inhibit signaling (London et al., 2020). When cAMP levels rise as a result of a starvation or stress signal, the R subunit binds to cAMP and releases the C subunit, thereby activating PKA signaling (London et al., 2020). When active, PKA promotes lipid and glycogen breakdown in several types of mammalian cells such as adipocytes, liver cells, adrenal cells and neurons (London et al 2020).  In *Drosophila*, PKA has been manipulated in embryonic and muscle cells to study development and circadian rhythms (Ohlmeyer et al., 1997; Majercak et al., 1997); however, little has been done to characterize the metabolic functions of PKA in adult fly fat bodies. Therefore, the following experiments were performed to determine the best way to manipulate PKA activity in *Drosophila* fat tissue.

Since PKA promotes lipid and glycogen breakdown, then activating PKA is expected to decrease TAG and/or glycogen storage while inhibiting PKA is expected to result in an accumulation of TAG and/or glycogen. To confirm effective activation of PKA, overexpression

of the C1 subunit of PKA (*PKA-C1*) was performed and TAG and glycogen were measured. Overexpressing *PKA-C1* results in a significant decrease in TAG levels which suggests that PKA was successfully activated(Fig. 3A). Since overexpression of *PKA-C1* results in active PKA, it was expected that inhibiting *PKA-C1* would result in less active PKA. To do this, expression of *PKA-C1* was decreased using RNAi; however, it was found that there is no change in TAG and glycogen levels compared to *EGFP-RNAi* control flies (Fig. 3B), suggesting that targeting *PKA-C1* by RNAi is not sufficient to alter PKA-induced changes in lipid and carbohydrate metabolism. Previous studies have decreased PKA activity by engineering a mutated form of the PKA regulatory subunit 1 (known as R1-BDK) so that it cannot bind to cAMP; this mutation prevents the release of the catalytic subunits and decreases PKA signaling (Li et al., 1995). While this mutated R1 construct has been especially effective in studying larval growth and imaginal disc differentiation (Li et al., 1995; Kiger et al., 1999), it is not known how overexpressing the *R1-BDK* construct affects lipid and glycogen metabolism in adult *Drosophila* fat bodies. Overexpression of *PKA-R1-BDK* specifically in the adult fat body results in a significant increase of TAG and glycogen (Fig. 3C), suggesting less PKA activity consistent with previous larval growth and development studies (Li et al., 1995; Kiger et al., 1999). Together, these findings show that overexpression of *PKA-C1* and *PKA-R1-BDK* are effective ways to activate and inhibit PKA activity, respectively, in *Drosophila* fat tissues.


A
yolkGal4>GFP
yolkGal4>PKA-C1
Macromolecule Levels (µg/µg-protein)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
Triglyceride
Glycogen
Free Glucose


B
yolkGal4>EGFP-RNAi
yolkGal4>PKA-C1-RNAi
Macromolecule Levels (µg/µg-protein)
1.2
1
0.8
0.6
0.4
0.2
0
Triglyceride
Glycogen
Free Glucose

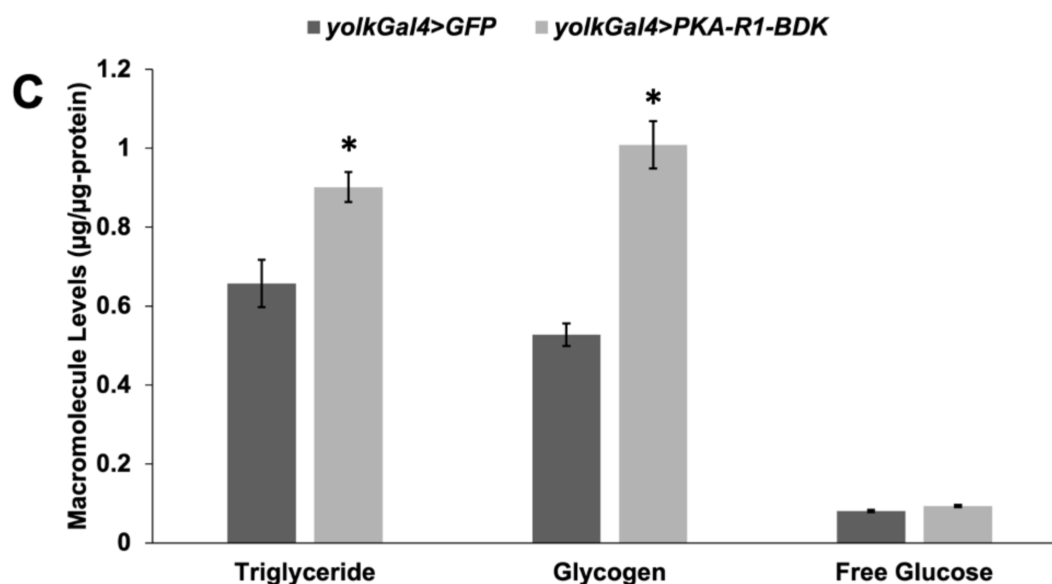



**Figure 5. Activating PKA decreases triglyceride levels while inhibiting PKA promotes fat and glycogen storage.**

**Triglyceride, glycogen, and glucose were measured from 1 week old female (A) *yolkGal4>PKA-C1* and (C) *yolkGal4>PKA-R1-BDK* flies compared to *yolkGal4>GFP* controls (n=29-30), and (B) *yolkGal4>PKA-C1-RNAi* flies compared to *yolkGal4>EGFP-RNAi* controls (n=58). TAG, glycogen, and free glucose were then normalized by protein levels. Values are mean ± standard error. **, P < 0.05 by Student's *t* test comparing macromolecule levels in control flies to flies with PKA genetically modified.**

## PKA Regulates *CPT1* Splicing

It was shown that starvation affects *CPT1* splicing to promote the inclusion of exon 6A; however, whether PKA is involved in starvation-induced *CPT1* splicing is not known. To determine whether PKA activity affects *CPT1* splicing in starved flies, PKA activity was inhibited by expressing *PKA-R1-BDK* and *CPT1* splicing was measured in both fed and starved flies and compared to *GFP*-expressing controls. Under fed conditions, both *GFP*-expressing and *PKA-R1-BDK* flies contain equal amounts of exon 6A-containing isoforms and exon 6B-containing isoforms of *CPT1* (Fig. 4A), suggesting that inhibiting PKA under fed conditions does not change *CPT1* splicing. Since starvation induces *CPT1* splicing to include exon 6A, and PKA activity is high during starvation, it was hypothesized that inhibiting PKA activity during

starvation would prevent the accumulation of *CPT1* isoforms that include exon 6A. Similar to the data in Figure 1B, starving *GFP*-expressing flies increased the amounts of exon 6A-containing isoforms (Fig. 4B). Consistent with the hypothesis, starving *PKA-R1-BDK* flies does not result in the accumulation of exon 6A-containing *CPT1* isoforms; in fact, the amount of exon 6B-containing isoforms increases in these flies (Fig. 4B). Together, these findings suggest that starvation activates PKA to regulate starvation-induced changes in *CPT1* splicing to produce isoforms that include exon 6A.

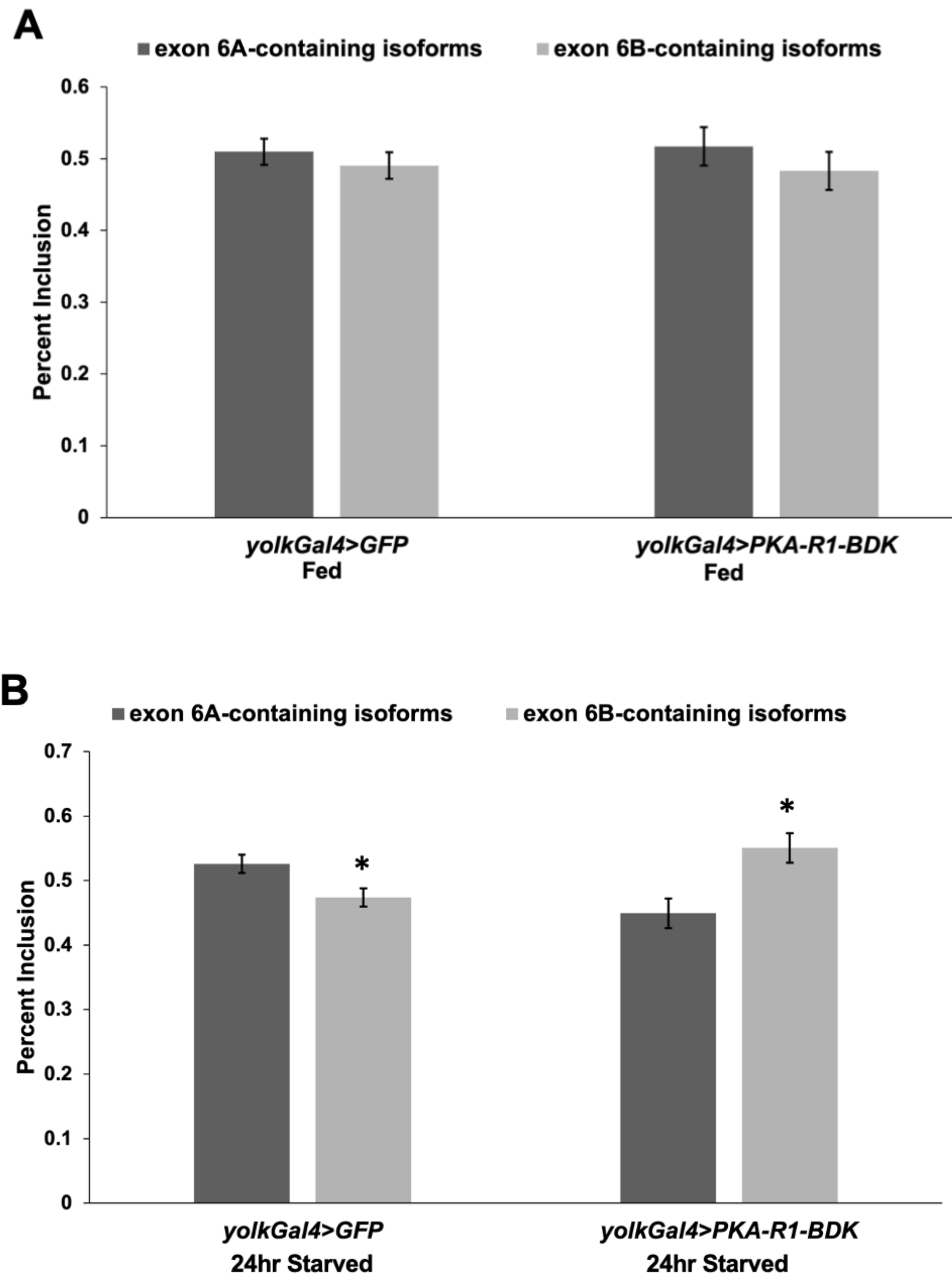



**Figure 6. PKA is necessary for the starvation-induced splicing of *CPT1*.**

**RNA was isolated fat bodies dissected from 1 week old female (A) fed and (B) 24 hour-starved *yolkGal4>GFP* and *yolkGal4>PKA-R1-BDK* flies (n=8). qPCR was performed for *CPT1* isoforms including either exon 6A or 6B and quantities were then normalized by *rp49,* and fractions of each transcript including either exon 6A or exon 6B that were expressed were calculated. Values are mean ± standard error. *, P < 0.05 by Student's *t* test comparing the percent inclusion of *CPT1* isoforms containing exon 6A to those containing exon 6B in each condition.**

# Chapter 4

# Discussion

In this study, the metabolic signals that regulate the splicing of *CPT1* in *Drosophila* fat tissue were investigated. It was found that a 24 hour starvation period induces the preferential inclusion of exon 6A in *CPT1* transcripts. Consistent with previous studies on *CPT1* splicing in *Drosophila* adipose tissue, our results strongly support that starvation is a contributing signal to the production of a more active CPT1 enzyme in order to increase the rate of fatty acid oxidation (Price et al 2010). After determining that starvation promoted the inclusion of exon 6A in *CPT1* transcripts, splicing factors were identified and characterized in *CPT1* splicing. The starvation-induced splicing of *CPT1* was found to be dependent on the nuclear protein transporter Tnpo-SR and the SR protein 9G8. Critically, the expression of both proteins during starvation promotes the generation of *CPT1* transcripts containing exon 6A, which produces a more enzymatically active CPT1 protein, suggesting that 9G8 and Tnpo-SR promote beta oxidation in *Drosophila* fat tissue when starved.

While it is unknown how *CPT1* splicing is regulated in humans, previous studies performed in mammalian systems have found other lipid metabolic enzymes whose splicing are regulated by nutrients. For example, *glucose-6-phosphate dehydrogenase* (*G6PD*), which encodes an enzyme of the pentose phosphate pathway that catalyzes the production nicotinamide adenine dinucleotide phosphate (NADPH) necessary for fatty acid synthesis, is regulated by starvation in mammals (Stincone et al 2014; Walsh et al 2013). Specifically, a starvation stimulus inhibits the removal of introns adjacent to exon 12 of mammalian *G6PD*, therefore reducing the production of the enzyme to decrease enzymatic efficiency and overall lipogenesis (Walsh et al 2013). Interestingly, another study identified *LPIN1*, which encodes the protein

lipin, to be alternatively spliced in cultured mammalian liver cells (Pihlajamäki et al., 2011). Lipins are phosphatases that remove phosphate groups from phosphatidic acid (Reue and Dwyer 2009), producing diacylglycerol that can be used as a substrate to make triacylglycerol (TAG). In humans, *LPIN1* splicing is mediated by the splicing factor SFRS10 (Siniossoglou et al 2013; Pihlajamäki et al., 2011). In cells with lower levels of *SFRS10*, *LPIN1* splicing is altered, resulting in increased lipogenesis (Pihlajamäki et al., 2011). However, whether nutrient availability affects *LPIN1* splicing is currently not known.

Despite the extensive understanding of the genes that encode these lipid enzymes in mammals, there is limited research on the alternative splicing of the *Drosophila G6PD* homolog, *Zwischenferment* (*Zw*), and the *Drosophila LPIN1* homolog, Lpin. *Zw* splicing has been shown to be regulated by 9G8, as decreasing *9G8* mRNA leads to altered *Zw* splicing that increases the RA and RB isoforms while reducing the RC transcripts (Weidman et al 2022). Similarly, *Lpin* is predicted to have many isoforms in *Drosophila* (Valente et al 2010), but whether *Lpin* splicing is regulated by 9G8 is unknown. This evidence links 9G8 to the alternative splicing of these metabolic genes, but whether starvation plays a role in altering the splicing of these genes requires further investigation. Nonetheless, these findings in *Drosophila* and mammalian cells all support a paradigm of starvation serving as a regulator of lipid metabolic enzymes and their activity.

The results have also identified the starvation activated kinase, PKA, as playing a role in *CPT1* splicing in *Drosophila* fat tissue. By overexpressing a mutant regulatory subunit of PKA (*R1-BDK*), this mutant was found to be effective in tissue-specific inhibition of the kinase, as TAG levels are increased (Figure 3C) as expected based on the well characterized role of PKA in promoting lipolysis (London et al 2020). This result is consistent with previous work

characterizing the *R1-BDK* mutant as an effective method to inhibit PKA signaling in other contexts such as wing development (Li et al., 1995; Kiger et al., 1999). Moreover, inhibiting PKA during starvation by expressing the *R1-BDK* mutant specifically in fat tissue blocks the inclusion of exon 6A in *CPT1* transcripts, suggesting that PKA relays a starvation signal to promote *CPT1* splicing to produce more active CPT1 enzymes. Together, these findings indicate that when presented with limited nutrients, PKA is activated to promote beta oxidation by promoting the preferential splicing of *CPT1* to include exon 6A, thus producing a more active beta oxidation enzyme.

Data from the lab have shown that SR protein 9G8 controls *CPT1* splicing under starvation conditions, but 9G8 regulation by other protein kinases to regulate lipid storage is poorly understood in *Drosophila* fat tissue. SR proteins, such as 9G8, contain regions of arginines and serines (RS domains) that can be phosphorylated by SR protein kinases (SRPK), which promote the interaction with Tnpo-SR leading to the translocation of these SR proteins into the nucleus (Gosh and Adams 2011; Lai et al 2000). Previous studies from the DiAngelo lab have identified the *Drosophila* SRPK homologs SRPK and SRPK79D to both promote exon 6B inclusion because knockdown of either *SRPK* or *SRPK79D* led to more *CPT1* transcripts containing exon 6A (Mercier et al., 2023). 9G8, on the other hand, promotes exon 6A inclusion because knocking down *9G8* results in more *CPT1* transcripts containing exon 6B (Gingras et al., 2014). These two results suggest that phosphorylation of 9G8 by SRPKs may prevent 9G8 from promoting the inclusion of exon 6A in *CPT1* transcripts (Figure 7). Moreover, PKA is known to promote lipid breakdown (Soeters et al 2012; Sakers et al., 2022; Schlaepfer and Joshi 2020) and alter *CPT1* splicing (Figure 6B), but whether PKA regulates SR proteins directly to control lipid homeostasis is unknown. Previous studies have shown that PKA phosphorylates

9G8 in human neuronal cells to alter the splicing of *tau*, which encodes a microtubule-associated protein (Gu et al 2012), but whether PKA phosphorylates 9G8 to control *CPT1* splicing and overall lipid storage in *Drosophila* requires further studies.

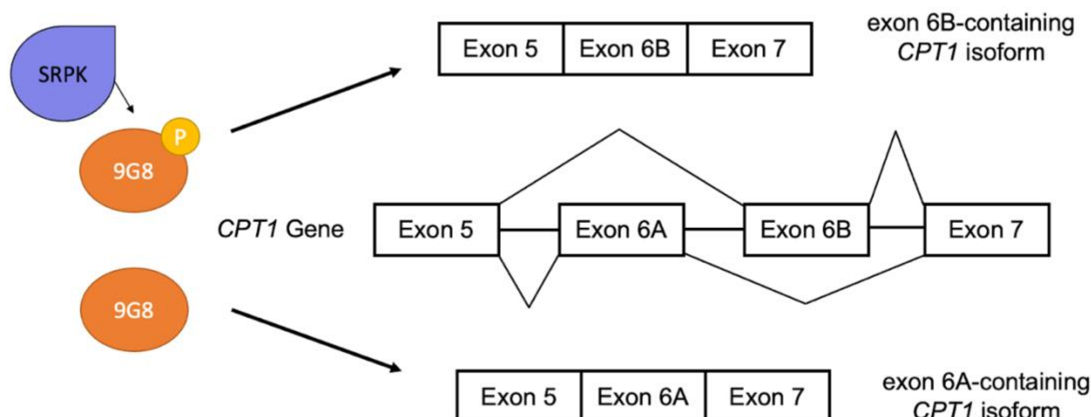



**Figure 7. Regulation of CPT1 splicing by SR proteins and SRPK.**

**9G8 promotes the usage of exon 6A in *CPT1* transcripts. In the presence of an SRPK, it can be speculated that 9G8 gets phosphorylated and loses the ability to promote exon 6A inclusion, leading to more *CPT1* transcripts including exon 6B.**

In addition to potentially phosphorylating 9G8 directly, it is possible that PKA could regulate 9G8 by phosphorylating SRPKs. SRPKs contain regions that can be phosphorylated by other protein kinases (Du et al 2021). In mammalian cells, the insulin-activated kinase Akt has been shown to phosphorylate and activate SRPKs (Zhou et al 2012). Activated SRPKs then phosphorylate SR proteins to regulate alternative splicing in response to the growth factor EGF (Zhou et al 2012). Whether this regulation is seen in *Drosophila* is unknown, but it is possible that insulin-like peptides could promote TAG storage and alter *CPT1* splicing by stimulating SRPKs to inhibit 9G8 in fly fat tissue. In contrast to insulin-like peptides, the adipokinetic hormone (AKH), a glucagon homolog in *Drosophila*, has been shown to effectively activate

PKA to regulate perilipin and stimulate lipolysis of lipid droplets (Beller et al 2010). PKA could be speculated to be activated by AKH during the fasted state results in more exon 6A-containing *CPT1* isoforms, which perhaps contributes to lower levels of TAG in starved flies. Although this process has yet to be studied in *Drosophila* fat tissues, PKA could also phosphorylate SRPKs, thereby inactivating its kinase domain and allowing 9G8 and other SR proteins to splice *CPT1* to include exon 6A (Figure 8). Future experimentation is required to identify and characterize phosphorylation levels of SR proteins by different protein kinases to control lipid homeostasis.

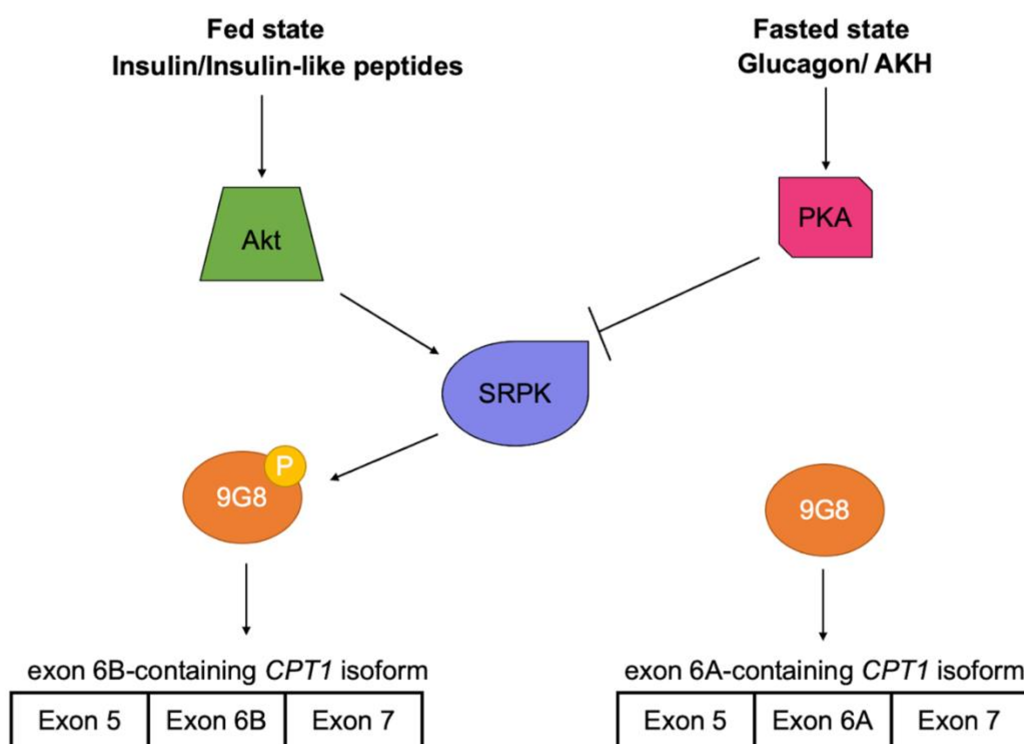



**Figure 8. Regulation of SRPK activity and CPT1 splicing by nutrients.**

**Under fed conditions, insulin/insulin-like peptides could activate Akt to promote SRPK activation, leading to 9G8 inactivation and CPT1-6B isoform production. During fasted conditions, glucagon/AKH could activate PKA to inhibit SRPKs, leading 9G8 to promote the inclusion of exon 6A in *CPT1* transcripts.**

In summary, the study has shown that starvation regulates *CPT1* splicing to produce *CPT1* transcripts containing exon 6A to promote lipid breakdown. The SR protein 9G8 and the

SR protein transporter Tnpo-SR are necessary to carry out starvation-induced *CPT1* splicing, and that PKA plays a role in modulating *CPT1* splicing during starvation. While only two major *CPT1* isoforms exist in *Drosophila* (Price et al., 2010), three *CPT1* isoforms (*CPT1A, CPT1B, CPT1C*) have been identified in mammals (Schreurs et al 2010); however, whether starvation regulates the splicing of these isoforms remains unknown. Furthermore, the *CPT1* splicing data described here increases our knowledge of how starvation regulates splicing of genes encoding for lipid metabolic enzymes such as G6PD and LPIN1 to regulate lipid storage in humans (Stincone et al 2014; Walsh et al 2013; Siniossoglou et al 2013; Pihlajamäki et al., 2011). Understanding the metabolic signals that regulate lipid storage allows us to better understand human metabolic diseases, such as obesity, perhaps better equipping us to generate new approaches to achieve metabolic homeostasis during these disease states.

# BIBLIOGRAPHY


Allemand, E., Dokudovskaya, S., Bordonné, R., & Tazi, J. (2002). A conserved Drosophila transportin-serine/arginine-rich (SR) protein permits nuclear import of Drosophila SR protein splicing factors and their antagonist repressor splicing factor 1. *Molecular Biology of the Cell*, *13*(7), 2436-2447.

Arking, D. E., & Chakravarti, A. (2009). Understanding cardiovascular disease through the lens of genome-wide association studies. *Trends in Genetics*, *25*(9), 387-394.

Beller, M., Bulankina, A. V., Hsiao, H. H., Urlaub, H., Jäckle, H., & Kühnlein, R. P. (2010). PERILIPIN-dependent control of lipid droplet structure and fat storage in Drosophila. *Cell Metabolism*, *12*(5), 521-532.

Beller, M., Sztalryd, C., Southall, N., Bell, M., Jäckle, H., Auld, D. S., & Oliver, B. (2008). COPI complex is a regulator of lipid homeostasis. *PLoS Biology*, *6*(11), e292.

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

Bhogal, J. K., Kanaskie, J. M., & DiAngelo, J. R. (2020). The role of the heterogeneous nuclear ribonucleoprotein (hnRNP) Hrb27C in regulating lipid storage in the Drosophila fat body. *Biochemical and Biophysical Research Communications*, *524*(1), 178–183. https://doi.org/10.1016/j.bbrc.2020.01.064

Cano-Gamez, E., & Trynka, G. (2020). From GWAS to function: using functional genomics to identify the mechanisms underlying complex diseases. *Frontiers in Genetics*, *11*, 424.

Chooi, Y. C., Ding, C., & Magkos, F. (2019). The epidemiology of obesity. *Metabolism*, *92*, 6-10.

Du, J. X., Zhu, G. Q., Cai, J. L., Wang, B., Luo, Y. H., Chen, C., ... & Dai, Z. (2021). Splicing factors: Insights into their regulatory network in alternative splicing in cancer. *Cancer Letters*, *501*, 83-104.

Fall, T., & Ingelsson, E. (2014). Genome-wide association studies of obesity and metabolic syndrome. *Molecular and Cellular Endocrinology*, *382*(1), 740-757.

Ghosh, G., Adams, J.A. Phosphorylation mechanism and structure of serine-arginine protein kinases. *FEBS Journal*, 278 (2011) 587-597. 10.1111/j.1742-4658.2010.07992.x.

Gingras, R. M., Warren, M. E., Nagengast, A. A., & Diangelo, J. R. (2014). The control of lipid metabolism by mRNA splicing in Drosophila. *Biochemical and Biophysical Research Communications*, *443*(2), 672–676. https://doi.org/10.1016/j.bbrc.2013.12.027

Gu, J., Shi, J., Wu, S., Jin, N., Qian, W., Zhou, J., ... & Liu, F. (2012). Cyclic AMP-dependent protein kinase regulates 9G8-mediated alternative splicing of tau exon 10. *FEBS Letters*, *586*(16), 2239-2244.

Guo, Y., Walther, T. C., Rao, M., Stuurman, N., Goshima, G., Terayama, K., Wong, J. S., Vale, R. D., Walter, P., & Farese, R. V. (2008). Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. *Nature*, *453*(7195), 657–661. https://doi.org/10.1038/nature06928

Jaworski, K., Sarkadi-Nagy, E., Duncan, R. E., Ahmadian, M., & Sul, H. S. (2007). Regulation of triglyceride metabolism. IV. Hormonal regulation of lipolysis in adipose tissue. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *293*(1), G1-G4.

Kelemen, O., Convertini, P., Zhang, Z., Wen, Y., Shen, M., Falaleeva, M., & Stamm, S. (2013). Function of alternative splicing. *Gene*, *514*(1), 1-30.

Keller, M., Hopp, L., Liu, X., Wohland, T., Rohde, K., Cancello, R., ... & Böttcher, Y. (2017). Genome-wide DNA promoter methylation and transcriptome analysis in human adipose tissue unravels novel candidate genes for obesity. *Molecular Metabolism*, *6*(1), 86-100.

Kiger, J. A., Eklund, J. L., Younger, S. H., & O'Kane, C. J. (1999). Transgenic inhibitors identify two roles for protein kinase A in *Drosophila* development. *Genetics*, 152(1), 281–290.

Lai, M. C., Lin, R. I., Huang, S. Y., Tsai, C. W., & Tarn, W. Y. (2000). A human importin-β family protein, transportin-SR2, interacts with the phosphorylated RS domain of SR proteins. *Journal of Biological Chemistry*, *275*(11), 7950-7957.

Lewis, G. F., Carpentier, A., Adeli, K., & Giacca, A. (2002). Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocrine Reviews*, *23*(2), 201-229.

Li, W., Ohlmeyer, J. T., Lane, M. E., & Kalderon, D. (1995). Function of protein kinase A in hedgehog signal transduction and Drosophila imaginal disc development. *Cell*, *80*(4), 553-562.

London, E., Bloyd, M., & Stratakis, C. A. (2020). PKA functions in metabolism and resistance to obesity: lessons from mouse and human studies. *The Journal of Endocrinology*, *246*(3), R51.

Loos, R. J., & Yeo, G. S. (2014). The bigger picture of FTO: the first GWAS-identified obesity gene. *Nature Reviews. Endocrinology*, *10*(1), 51–61. https://doi.org/10.1038/nrendo.2013.227

Majercak, J., Kalderon, D., & Edery, I. (1997). Drosophila melanogaster deficient in protein kinase A manifests behavior-specific arrhythmia but normal clock function. *Molecular and Cellular Biology*, *17*(10), 5915-5922.

Mercier, J., Nagengast, A. A., & DiAngelo, J. R. (2023). The role of SR protein kinases in regulating lipid storage in the Drosophila fat body. *Biochemical and Biophysical Research Communications*.

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

Nagle, C., Bhogal, J. K., Nagengast, A. A., & DiAngelo, J. R. (2022). Transportin-serine/arginine-rich (Tnpo-SR) proteins are necessary for proper lipid storage in the Drosophila fat body. *Biochemical and Biophysical Research Communications*, *596*, 1–5. https://doi.org/10.1016/j.bbrc.2022.01.087

Ohlmeyer, J. T., & Kalderon, D. (1997). Dual pathways for induction of wingless expression by protein kinase A and Hedgehog inDrosophila embryos. *Genes & Development*, *11*(17), 2250-2258.

Palou, M., Priego, T., Sánchez, J., Villegas, E., Rodríguez, A. M., Palou, A., & Picó, C. (2008). Sequential changes in the expression of genes involved in lipid metabolism in adipose tissue and liver in response to fasting. *Pflügers Archiv-European Journal of Physiology*, *456*(5), 825-836.

Pihlajamäki, J., Lerin, C., Itkonen, P., Boes, T., Floss, T., Schroeder, J., ... & Patti, M. E. (2011). Expression of the splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced lipogenesis. *Cell Metabolism*, *14*(2), 208-218.

Pi-Sunyer, F. X. (2002). The obesity epidemic: pathophysiology and consequences of obesity. *Obesity Research*, *10*(S12), 97S-104S.

Pospisilik, J. A., Schramek, D., Schnidar, H., Cronin, S. J., Nehme, N. T., Zhang, X., Knauf, C., Cani, P. D., Aumayr, K., Todoric, J., Bayer, M., Haschemi, A., Puviindran, V., Tar, K., Orthofer, M., Neely, G. G., Dietzl, G., Manoukian, A., Funovics, M., Prager, G., … Penninger, J. M. (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

Price, N. T., Jackson, V. N., Müller, J., Moffat, K., Matthews, K. L., Orton, T., & Zammit, V. A. (2010). Alternative exon usage in the single CPT1 gene of Drosophila generates functional diversity in the kinetic properties of the enzyme: differential expression of alternatively spliced variants in Drosophila tissues. *The Journal of Biological Chemistry*, *285*(11), 7857–7865. https://doi.org/10.1074/jbc.M109.072892

Reue, K., & Dwyer, J. R. (2009). Lipin proteins and metabolic homeostasis. *Journal of Lipid Research*, *50,* S109–S114. https://doi.org/10.1194/jlr.R800052-JLR200

Schlaepfer, I. R., & Joshi, M. (2020). CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential. *Endocrinology*, *161*(2), bqz046. https://doi.org/10.1210/endocr/bqz046

Schreurs, M., Kuipers, F., & Van Der Leij, F. R. (2010). Regulatory enzymes of mitochondrial β-oxidation as targets for treatment of the metabolic syndrome. *Obesity Reviews*, *11*(5), 380-388.

Sciabica, K. S., & Hertel, K. J. (2006). The splicing regulators Tra and Tra2 are unusually potent activators of pre-mRNA splicing. *Nucleic Acids Research*, *34*(22), 6612-6620.

Shabar, H., & DiAngelo, J. R. (2022). The regulation of lipid and carbohydrate storage by the splicing factor gene *snRNP-U1-70K* in the *Drosophila* fat body. *microPublication Biology*, *2022*, 10.17912/micropub.biology.000580. https://doi.org/10.17912/micropub.biology.000580

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

Siniossoglou, S. (2013). Phospholipid metabolism and nuclear function: roles of the lipin family of phosphatidic acid phosphatases. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1831*(3), 575-581.

Soeters, M. R., Soeters, P. B., Schooneman, M. G., Houten, S. M., & Romijn, J. A. (2012). Adaptive reciprocity of lipid and glucose metabolism in human short-term starvation. *American Journal of Physiology, Endocrinology and Metabolism*, *303*(12), E1397–E1407. https://doi.org/10.1152/ajpendo.00397.2012

Stincone, A., Prigione, A., Cramer, T., Wamelink, M. M., Campbell, K., Cheung, E., ... & Ralser, M. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biological Reviews*, *90*(3), 927-963.

Upadhyay, J., Farr, O., Perakakis, N., Ghaly, W., & Mantzoros, C. (2018). Obesity as a disease. *Medical Clinics*, *102*(1), 13-33.

Valente, V., Maia, R. M., Vianna, M. C., & Paçó-Larson, M. L. (2010). Drosophila melanogaster lipins are tissue-regulated and developmentally regulated and present specific subcellular distributions. *FEBS Journal*, *277*(22), 4775–4788. https://doi.org/10.1111/j.1742-4658.2010.07883.x

Walsh, C. M., Suchanek, A. L., Cyphert, T. J., Kohan, A. B., Szeszel-Fedorowicz, W., & Salati, L. M. (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. *Journal of Biological Chemistry*, *288*(4), 2816-2828.

Wang, G. S., & Cooper, T. A. (2007). Splicing in disease: disruption of the splicing code and the decoding machinery. *Nature Reviews. Genetics*, *8*(10), 749–761. https://doi.org/10.1038/nrg2164

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

Will, C. L., & Lührmann, R. (2011). Spliceosome structure and function. *Cold Spring Harbor Perspectives in Biology*, *3*(7), a003707.

Whiteman, E. L., Cho, H., & Birnbaum, M. J. (2002). Role of Akt/protein kinase B in metabolism. *Trends in Endocrinology & Metabolism*, *13*(10), 444-451.

Yamaguchi, M., & Yoshida, H. (2018). Drosophila as a model organism. *Advances in Experimental Medicine and Biology*, *1076*, 1–10. https://doi.org/10.1007/978-981-13-0529-0_1

Zheng, H., Yang, X., & Xi, Y. (2016). Fat body remodeling and homeostasis control in Drosophila. *Life Sciences*, *167*, 22-31.

Zhou, Z., Qiu, J., Liu, W., Zhou, Y., Plocinik, R. M., Li, H., ... & Fu, X. D. (2012). The Akt-SRPK-SR axis constitutes a major pathway in transducing EGF signaling to regulate alternative splicing in the nucleus. *Molecular Cell*, *47*(3), 422-433.

**ACADEMIC VITA**
**Huy Truong**

## EDUCATION

The Pennsylvania State University 2019-2023
B.S. Genetics and Developmental Biology
Honors Thesis: The Regulation of Carnitine Palmitoyltransferase 1 (CPT1) mRNA Splicing by Nutrient Availability in *Drosophila* Fat Tissue

## ACTIVITIES AND COMMUNITY INVOLVEMENT

Lion Ambassador—Penn State Berks 2020-2023
Provide tours to prospective students and assist with campus admission, as well as promoting and maintaining strong campus spirit and pride.

Orientation Leader—Penn State Berks 2020-2023
Participates in Welcome Weekend in helping students with move-in and acclimation. As a part of Lead Team, work with other Lead Team members to plan and coordinate Welcome Weekend for Fall 2022.

Honors Club—Penn State Berks 2019-2023
Participate in the Berks honors program in community and volunteer services. Honors participation also involves out-of-class educational trips to NYC and Broadway shows.

## PUBLICATION AND CONFERENCE

Published Essay 2021
Truong, H. G. (2021). Visual Rhetoric in Medical Illustrations and the Implications in Medical Practices. *Young Scholars in Writing*, *18*, 127-141.

Higher Education Council of Berks County (HECBC) Conference 2022
Presented my thesis-related research poster "Regulation of CPT1 Splicing by Protein Kinase A Under Starvation in Drosophila Fat Tissue" to faculty and students across many universities and higher institutions within Berks County at Penn State Berks.

## HONORS AND AWARDS

Student Marshal 2023
Schreyer Honors Scholar 2020-2023
Contribution to Science Award 2023
Evan Pugh Scholar Award, Junior 2022
President's Sparks Award, Penn State University Berks 2021
Excellence in Biomathematics 2022
President's Freshman Award, Penn State University Berks 2020

Chapman Family Trustee Scholarship 2020
Bunton Waller Scholarship 2019-2023
Jacquelyn G. Ciferri First Year English Award, Penn State University Berks 2020
Dean's List, Penn State University Berks 2019-2023

**RELEVANT EXPERIENCE**

Research Assistant—Pennsylvania State University Fall 2021-2023

Work alongside Dr. Justin DiAngelo to understand *Drosophila* genetics and metabolism, including homogenization protocols, assay procedures, and data analysis.

Teaching Assistant—Pennsylvania State University Spring 2021- Fall 2022

Assist professors in managing lecture sessions and student groups, grading, and work one-on-one with students outside class time. Assist students in biology, chemistry and mathematics in understanding concepts and preparation for exams, and work one-on-one with students on writing assignments and help develop good writing skills.

Learning and Writing Center Tutoring— Pennsylvania State University 2021-2023

Work one-on-one with students and writers from across all disciplines to help develop skills in science and writing-based classes. Assist students with understanding class assignments and writing prompts, and help students to grow as learner and writers in their respective fields and majors.