Coilin Expression During Nucleolar Stress in the Drosophila Brain

Kathryn Rose DeLeo

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Biochemistry Commons, and the Molecular Biology Commons

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_theses/5321

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
COILIN EXPRESSION DURING NUCLEOLAR STRESS IN THE
DROSOPHILA BRAIN

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Biological Sciences

by
Kathryn Rose DeLeo
B.S. Louisiana State University, 2018
May 2021
ACKNOWLEDGEMENTS

I would like to thank my committee members Dr. SeYeon Chung and Dr. Anne Grove for your direction and assistance in graduate school. To my lab mates, past and present, Shova Pandey, Dr. Sonu Baral Shrestha, Gracie Hubacek, Dr. Himanshu Raje, Matthew Tran, Anh Nguyen, Erica Huang, and Phelan Sewell, thank you for your assistance, support, and friendship; you have made the lab such a loving home. I am eternally grateful to my family, especially the members of the Core Four, Shannon, Wayne, and Eileen DeLeo, who have been there for me since the start. Thank you to my friends Celine Richard, Scott Grimmell, Sam Lanjewar, Marisa Myers, and Phoebe Clark who have helped me throughout my life and in graduate school; your friendship has been invaluable. Thank you to Carrie and Karen for always being there for me and encouraging me to do better.

Most of all, thank you to my advisor Dr. Patrick DiMario. Your enthusiasm for research inspires and motivates everyone you meet. You have made research such an enjoyable experience for me and cultivated an incredibly supportive lab environment. It is the reason I chose to come to graduate school. Thank you for your dedication to mentoring and teaching me, first as an undergraduate and now as a graduate student. Your advice and wisdom have been a comfort during the low points. I could not have done this without your unfailing support. You’re pretty fly as a PI.
# TABLE OF CONTENTS

ABSTRACT ...............................................................................................................................iv

CHAPTER 1. LITERATURE REVIEW .................................................................................. 1
  1.1. Ribosome Biogenesis .............................................................................................. 1
  1.2. Treacher Collins Syndrome ..................................................................................... 1
  1.3. Nopp140 .................................................................................................................. 4
  1.4. The Cajal Body ......................................................................................................... 6
  1.5. Coilin ....................................................................................................................... 11
  1.6. Scope and Aims of Research ................................................................................. 12

CHAPTER 2. MUSHROOM BODY NEUROBLASTS IN THE DROSOPHILA BRAIN MANTAIN COILIN EXPRESSION DURING NUCLEOLAR STRESS .................................. 14
  2.1. Introduction ............................................................................................................ 14
  2.2. Results .................................................................................................................... 18
  2.3. Discussion .............................................................................................................. 21
  2.4. Materials and Methods .......................................................................................... 22

CHAPTER 3. CONFLICTING LOCALIZATION OF THE COILIN NULL TRUNCATED PROTEIN .................................................................................................. 25
  3.1. Introduction ............................................................................................................ 25
  3.2. Results .................................................................................................................... 28
  3.3. Discussion .............................................................................................................. 28
  3.4. Materials and Methods .......................................................................................... 32

CHAPTER 4. CONCLUSIONS AND FUTURE STUDIES .................................................... 34

APPENDIX. GFP FUSION TO COILIN’S CARBOXYL TERMINUS ALTERS COILIN EXPRESSION PATTERNS .................................................................................. 36

REFERENCES .................................................................................................................. 37

VITA ..................................................................................................................................... 51
ABSTRACT

Impairment of ribosome biogenesis disrupts cellular homeostasis and, in the process, causes a state of nucleolar stress in a cell. This triggers human syndromes collectively known as ribosomopathies. One such ribosomopathy is the Treacher Collins syndrome (TCS) which causes congenital craniofacial birth defects and hearing loss in patients. In TCS migration of neural crest cells during development is hindered by the loss of the ribosome assembly factor, treacle, and consequent apoptosis of these progenitor cells. Modeling this disorder in Drosophila was previously accomplished by disruption of Nopp140, treacle’s closest equivalent in Drosophila. During nucleolar stress, the Mushroom Body neuroblast population demonstrates resilience to the loss of Nopp140 with continued proliferation. These fruit flies also show an increase in total protein quantity of coilin, an integral component of the nuclear ribonucleoprotein assembly site, the Cajal body. We examined the variation of affected cell populations in TCS and other ribosomopathies by looking coilin expression at the neuroblasts with and without Nopp140. Neuroblasts in wild type brains already showed higher coilin levels relative to neurons. Coilin also showed a substantial distribution across the nucleoplasm of neuroblasts instead of appearing in bright Cajal body foci. In the absence of Nopp140, the Mushroom Body neuroblasts had increased coilin levels compared to other neuroblasts and neurons. We suspect that coilin plays a role not only in the selective resilience of Mushroom Body neuroblasts but also in cellular nucleolar response pathways.
CHAPTER 1. LITERATURE REVIEW

1.1. Ribosome Biogenesis

Ribosome production is a critical process for the cell because ribosomes are responsible for the synthesis of proteins by translation of messenger RNA (mRNA). Ribosome biogenesis involves transcription of pre-ribosomal RNA (pre-rRNA) mainly by RNA polymerase I, cleavage of nascent transcripts, modifications of the pre-rRNA, and synthesis of associated ribosomal proteins and ribosome assembly factors (RAFs) (Bleichert and Baserga, 2011). The ribosome biogenesis process primarily occurs in the nucleolus where small nucleolar ribonucleoprotein complexes (snoRNPs) add modifications to the pre-rRNA, most commonly 2’-O-methylation by C/D box snoRNPs and pseudouridylation by H/ACA box snoRNPs (Sloan et al., 2017). RAFs primarily serve as chaperones for RNA or RNA containing complexes such as snoRNPs throughout ribosome biogenesis (Talkish et al., 2014). Over half of RNA polymerase II’s mRNA output generates ribosomal proteins and RAFs, and pre-rRNA makes up 60% of a cell’s total RNA yield (Woolford and Baserga, 2013). Since such a large portion of cellular energy is dedicated to the production of ribosomes, disruption of any process in ribosome biogenesis can be extremely detrimental to cellular homeostasis (Warner, 1999).

1.2. Treacher Collins Syndrome

In the case of homeostasis disruption, the cell enters a state of nucleolar stress, and diseases called ribosomopathies can occur (He and DiMario, 2011). Clinical
manifestations of the different ribosomopathies depend on the specific part of ribosome biogenesis being disrupted, but many share conditions such as hematologic diseases, disruption of regular development resulting in structural malformations, and increased risk of cancer (Gérus et al., 2011). Although ribosomes are ubiquitous, any subset of defects present only appears in specific cell populations rather than globally (Narla and Ebert, 2010). Ribosomopathies primarily affect stem cell and some progenitor cell populations. Due to their high metabolic activity, defects in ribosome production would be more acutely felt in these cell populations (Dixon et al., 2006).

The ribosomopathy Treacher Collins syndrome (TCS) occurs in 1 out of 50,000 births, the highest incidence of any craniofacial disorder (Wise et al., 1997). In this autosomal dominant syndrome, individuals can have an array of craniofacial defects. They have hearing loss due to deformities of the external ear, narrowing of ear canals, and misshapen middle ear bones (Dixon et al., 2006; Phelps et al., 1981). Patients also experience a slanted drooping eye opening shape with some missing eyelid tissue and fewer eyelashes. Cleft palate and malformation of the cheek and jaw bones are also common in TCS (Fazen et al., 1967; Rovin et al., 1964). Perinatal death can occur if the patient has a severely underdeveloped airway (Edwards et al., 1996). The TCS phenotype presents with varying penetrance and expressivity of these clinical symptoms (Dixon and Dixon, 2004; Dixon et al., 2006; Teber et al., 2004). TCS can be inherited, but the majority of cases are due to new in utero mutations rather than inherited mutations of the TCOF1 gene (Jones et al., 1975). The many polymorphisms of TCOF1 have been suggested as the cause for this disorder’s varying expressivity, while the varying penetrance in TCS has been attributed to the genetic background of
the affected individual. Currently, there is no conclusive evidence for this theory (Dixon and Dixon, 2004; He and DiMario, 2011; Teber et al., 2004).

The craniofacial defects of TCS result from embryonic apoptosis of select neural crest cells preventing the typical migration of these cells into the pharyngeal arches. TCS specifically affects the neural crest cells that differentiate into craniofacial tissues (Dixon et al., 2006; He and DiMario, 2011; Jones et al., 2008), but it is uncertain why only this cell population is affected, and no other stem cell and progenitor cell populations are affected. p53-dependent apoptosis of the cephalic neural crest cells occurs due to a mutation in the human TCOF1 gene that encodes treacle (He et al., 2015; Sakai and Trainor, 2009). The murine homologue of TCOF1 is widely expressed during embryogenesis and adulthood, but during embryogenesis, elevated expression was documented in the pharyngeal arches and the cranial neural folds (Dixon et al., 1997b). A greater demand for treacle in the cephalic neural crest cells could explain the specificity of the affected cell population in TCS and potentially similar circumstances in other ribosomopathies (He and DiMario, 2011).

TCOF1 haploinsufficient mice displayed reduced viability as well as a significant loss of mature ribosomes in the neural crest cells (Dixon et al., 1997b; Sakai and Trainor, 2009). The resulting nucleolar stress triggered p53-induced cell cycle arrest and subsequent apoptosis (Dixon et al., 2006; Sakai and Trainor, 2009). p53 does inhibit ribosome production, but the initial decrease in mature ribosomes is likely due to the spatiotemporal haploinsufficiency of the RAF, treacle, which facilitates rDNA transcription and snoRNP function within the dense fibrillar component (DFC) and the fibrillar center (FC) of the nucleolus (He et al., 2015; Sakai and Trainor, 2009; Zhai and
Comai, 2000). Treacle promotes transcription of ribosomal DNA genes by binding of their promoter and 5’ external transcribed spacer (5’ ETS) regions; recruitment of the upstream binding factor (UBF), an activator of Pol I transcription, via interactions of treacle’s carboxyl terminus with UBF; and anchoring the Pol I complex in the nucleolus via interaction of its central acidic and basic repeat domain with Pol I (Lin and Yeh, 2009; Valdez et al., 2004). Interestingly, Drosophila does not express UBF or treacle. Vertebrate treacle promotes 2’-O-methylation of pre-rRNA by its interaction with NOP56, a core component of the C/D box snoRNP methylation complex, during telophase when rRNA production suppression is relieved (Gonzales et al., 2005; Hayano et al., 2003; He and DiMario, 2011).

1.3. Nopp140

The Drosophila melanogaster protein most related to treacle in structure as well as in function is the RAF Nopp140. Both Nopp140 and treacle are present in vertebrates, but there is no treacle in invertebrates (He and DiMario, 2011). Nopp140 and treacle both share an amino terminal LisH domain which is thought to be a potential homo-dimerization site (He and DiMario, 2011; Kim et al., 2004) as well as ten central acidic and basic repeat sequences (Dixon et al., 1997a; Meier and Blobel, 1992). However, their carboxyl terminal domains differ with Drosophila Nopp140 expressing two isoforms by alternative splicing: Nopp140-RGG and Nopp140-True. The Nopp140-True isoform resembles rat Nopp140 with around 60% identical sequence, but the Nopp140-RGG isoform has a distinctly different carboxyl end rich in RGG tripeptide motifs (Waggener and DiMario, 2002). As phosphoproteins Nopp140 and treacle are
both heavily phosphorylated which is necessary for interacting with nuclear localization signal binding proteins (Isaac et al., 2000; Meier and Blobel, 1992). Chen et al. (1999) indicate Nopp140’s participation in rRNA synthesis by showing an interaction between Nopp140 and the subunit RPA194 of Pol I (Chen et al., 1999). This has raised speculations about a potential role for Nopp140 in bringing pre-rRNA processing machinery and pre-rRNA transcription machinery together similar to the CTD of Pol II (Chen et al., 1999; He and DiMario, 2011; Yang et al., 2000). In addition to Nopp140 and treacle’s shared function as a participant in Pol I transcription, Nopp140 has also been identified as an activator of Pol II transcription by interactions with TFIIB (Miau et al., 1997).

Unlike treacle’s involvement with only the C/D box snoRNP complex, Nopp140 is associated with 2’-O-methylation as well as with pseudouridylation of pre-rRNA through interactions with NAP57 and GAR1, core components of the H/ACA box snoRNP pseudouridylation complex. However, Nopp140 exhibits a much higher protein-protein binding affinity for NAP57 than for the core proteins of the C/D box snoRNP complex suggesting a greater involvement in the H/ACA box snoRNP pathway as reported by Yang et al. (2000). The Nopp140 and snoRNP interaction is mediated by the phosphorylation status of Nopp140. However, a snoRNP’s function does not depend on its interactions with Nopp140, so Nopp140 only interacts with the snoRNP complexes as a chaperone (Wang et al., 2002). While treacle is only found in the nucleolus, Nopp140 concentrates in both the nucleolus and the Cajal body (CB) in addition to traveling through the cytoplasm (Isaac et al., 1998; Meier and Blobel, 1992). Nopp140 colocalizes with snoRNPs in the CB, supporting the role of Nopp140 as a chaperone
that transports snoRNPs between the nucleolus and the CB where snoRNPs are modified and temporarily stored post-transcription (Darzacq et al., 2002; Wang et al., 2002; Yang and Meier, 2003).

1.4. The Cajal Body

1.4.1. Cajal Body Assembly and Disassembly

The Cajal body (CB) was first identified in 1903 by Ramón y Cajal as a small accessory body near the nucleolus of neurons (Cajal, 1903). However, it was later described as coiled bodies by Monneron and Bernhard due to the sphere of fibrous coils they saw under the electron microscope (Monneron and Bernhard, 1969). Multiple names arose in the literature such as spheres and Binnenkörper, but the nuclear body was finally dubbed the Cajal body by Joe Gall in honor of its original discoverer (Bier et al., 1967; Callan and Lloyd, 1960; Gall et al., 1999). CBs have been found in vertebrates, invertebrates, and plants, but they are not found in every cell type of an organism (Gall, 2003). They are most prevalent in cells with increased metabolic activity (Morris, 2008; Ogg and Lamond, 2002). CBs normally form within the nucleoplasm. However, they have been found in the nucleolus in human breast cancer cells, but they are not formed in the cytoplasm. CBs diffuse through the interchromatin space, but their mobility decreases during active transcription when they are most strongly associated with chromatin (Platani et al., 2002).

These nuclear structures form when transcription is active, typically during G1 phase (Andrade et al., 1993; Ferreira et al., 1994; Hebert and Matera, 2000; Rebelo et al., 1996). The protein coilin self-associates at its amino terminus to make a homodimer
scaffold to which the components of the Cajal body can attach (Hebert and Matera, 2000; Liu et al., 2009; Wu et al., 1994). Factors regulating the self-association of coilin have been hypothesized to dwell in the nucleus due to CBs’ inability to form in the cytoplasm (Ochs et al., 1994; Shpargel et al., 2003). This homodimer structure seems to be a prevailing configuration amongst nuclear bodies, where a key protein self-associates, and the body assembles around the homodimer such as Sam68 in Sam68 bodies, PML in PML bodies, and the survival motor neuron protein (SMN) in gems (Chen et al., 1997; Hebert and Matera, 2000; Lorson et al., 1998; Misteli, 2001; Perez et al., 1993; Shpargel et al., 2003).

The degree of phosphorylation of coilin’s carboxyl terminus regulates its own amino terminal self-association domain. Without the correct phosphorylation status for that species and coilin’s self-association domain, the CB cannot form. In mice Shpargel et al. (2003) identified two serine residues that must be constitutively phosphorylated for CB formation. The necessity of coilin for CB formation established coilin as the marker protein for the CB (Hearst et al., 2009; Hebert and Matera, 2000; Liu et al., 2009; Shpargel et al., 2003). Similar to its assembly mechanism, the CB breaks down during mitosis due to coilin hyperphosphorylation which prevents the self-association of coilin. During interphase coilin has about twelve to fifteen phosphorylated serines with half in the carboxyl terminus of the protein. Two additional serine residues have been identified as mitotic phosphorylation sites; however, additional sites that prompt mitotic disassembly of CBs are unknown but predicted (Andrade et al., 1993; Carmo-Fonseca et al., 1993; Hearst et al., 2009; Hebert and Matera, 2000).
Coilin also regulates the number of CBs that form in the nucleus by phosphorylation of serine residues in its carboxyl terminal domain. Overexpression of coilin does not necessarily result in increased CB formation. The response seems to be species-dependent due to differences in the carboxyl domain. Shpargel et al. (2003) found the human carboxyl domain had an inhibitory effect on formation while the murine carboxyl domain promoted CB formation. Overexpression of gemin4, a CB component, dose-dependently caused a decrease in CB number by disruption of CB formation (Meier et al., 2018). In zebrafish embryogenesis, CB number is temporally regulated due to developmental gene expression requirements. As many as 30 CBs were observed during the first day of embryonic development (Strzelecka et al., 2010a).

1.4.2. Cajal Body Functions

CBs are the site for the biogenesis, modification, and assembly of many RNP complexes. They have been shown to contain snRNAs and snoRNAs as well as many of the modifiers of these RNAs and components of their mature RNP complexes. Once the RNPs have acquired their mature configuration, they are transported from the CB to their targets throughout the cell (Isaac et al., 1998; Makarov et al., 2013). snoRNPs are abundant in the CB, but snoRNA modification within the CB is not well defined (Enwerem et al., 2014). However, hypermethylation of its 5'-cap was ascribed to that location (Meier, 2017). After all processing, they are targeted to the nucleolus where they methylate and/or pseudouridylate pre-rRNA (Sloan et al., 2017).

Final modifications and assembly of snRNAs into individual snRNP complexes (U1, U2, etc.) also occurs in the CB before they are released for pre-mRNA splicing by
the fully assembled spliceosome (Isaac et al., 1998). snRNP biogenesis begins in the nucleus where the snRNAs (U1, U2, U4, and U5) are transcribed, capped with an m7G cap, and extended at their 3’-end (Gerard et al., 2010; Strzelecka et al., 2010b). The transcripts exit the nucleus where the SMN complex facilitates the addition of Sm proteins to the snRNA (Bizarro et al., 2015; Hyjek et al., 2015). After trimethylation of the m7G cap in the cytoplasm, the snRNPs return to the nucleus and locate within the CB (Gerard et al., 2010; Hyjek et al., 2015; Strzelecka et al., 2010b). In the CB, small Cajal body-specific RNPs (scaRNPs) are responsible for the modification of snRNAs. The scaRNA U85 oversees their processing and assembly into mature snRNPs (Darzacq et al., 2002; Enwerem et al., 2014; Strzelecka et al., 2010b). Box C/D and box H/ACA scaRNP complexes contain the methyltransferase fibrillarin and the pseudouridylase dyskerin, respectively. The enzymatic action of these two scaRNP complex components stabilizes the snRNA conformation within the snRNP complex. These last modifications are crucial for proper functioning of the functional spliceosome (Cao et al., 2018).

Cajal bodies are responsible for telomerase RNA biogenesis as well. Telomerase reverse transcribes telomere DNA to maintain chromosome integrity. The telomerase holoenzyme is actually an RNP with a H/ACA scaRNA on its 3’ end that serves as a reference for telomere sequence extension. The telomerase RNA component (TERC) also participates in the enzyme’s catalytic activity (Chartrand, 2016; Freund et al., 2014; Zhang et al., 2011). TERC is transported to the CB by Telomerase Cajal body protein 1 (TCAB1, also called WDR79) which interacts with TERC at its CB localization signal, the CAB box sequence (Broome and Hebert, 2013; Machyna et al., 2015; Zhang et al.,
Bizarro et al. (2019) suggested that modifications like hypermethylation and pseudouridylation are used to regulate telomerase activity (Kim et al., 2010; Verheggen et al., 2002). Key CB components like coilin also associate with TERC in the CB and with active telomerase; however, coilin’s importance is debatable (Enwerem et al., 2014; Poole et al., 2016). In coilin knockout cells, telomerase activity is unaffected, but with a partial coilin reduction, telomerase and telomeres cannot coalesce (Stern et al., 2012; Zhong et al., 2012). Studies in cancer cells indicate that modification of TERC and its assembly with component proteins into the telomerase holoenzyme occurs in the CBs of cells with a high cell division rate (Chen and Greider, 2004; Handwerger and Gall, 2006; Jady et al., 2003; Zhu et al., 2004).

The CB stores telomerase until DNA replication, upon which telomerase leaves for telomere maintenance (Artandi and DePinho, 2010; Jady et al., 2004; Jady et al., 2006; Machyna et al., 2015). When TCAB1 or the CAB box sequence is missing, telomerase does not return to the CB. This reduces its activity, and telomeres shorten (Chen et al., 2018; Cristofari et al., 2007; Vogan et al., 2016; Zhong et al., 2011). Yet, telomeres lengthen due to mislocalization during knockdown of Nopp140. Nopp140 traps scaRNPs like telomerase in the CB, but without it scaRNPs are not sequestered there leaving them free to lengthen telomeres without regulation (Bizarro et al., 2019). Interestingly, Drosophila melanogaster does not use telomerase to maintain chromosomal integrity. Instead, it uses retrotransposons to add to the ends of its chromosomes (Mason et al., 2008).
1.5. Coilin

Coilin is an intrinsically disordered protein with many post-translational modification sites (Frege and Uversky, 2015). Most coilin homologues contain conserved, slightly structured amino and carboxyl domains along with a non-conserved, disordered central domain. A Tudor-like domain was found in the carboxyl terminus of Arabidopsis coilin; however, its function is not yet established (Bellini, 2000; Machyna et al., 2015; Makarov et al., 2013; Shanbhag et al., 2010). Coilin is also known as p80-coilin due to its molecular weight (MW) of 80 kDa in humans, mice, and Xenopus (Bellini, 2000; Liu et al., 2009). In multiple species, the predicted MW and the MW discerned from gel electrophoresis do not match with the predicted MW being much smaller. The intrinsically disordered nature of the protein is thought to be the cause of the inconsistency (Makarov et al., 2013).

Coilin is not only present in CBs but also diffusely in the nucleoplasm of Xenopus germinal vesicles, the large nucleus of the oocyte. While it is much more concentrated in the CBs, the majority of coilin was found to be soluble in the nucleoplasm with some of it interacting with the U7 small nuclear ribonucleoprotein (snRNP), a histone pre-mRNA 3′-end processing snRNP. Bellini and Gall speculated that coilin forms a weak interaction with the U7 snRNP for transport to the CB which is in close proximity to the histone genes’ transcription site (Bellini and Gall, 1998; Cioce and Lamond, 2005). However, in Drosophila, U7 snRNP and coilin locate to a different nuclear body, the histone locus body (HLB) (Liu et al., 2009).

In addition to processing by scaRNPs at the CB, snRNA also undergoes modifications from coilin’s RNase capabilities in the CU region of U2 snRNA. During
coilin knockdown, U1 and U2 snRNA transcripts aggregate and cannot progress to later stages of spliceosome assembly (Broome and Hebert, 2012). Coilin also acts as an anchor to keep snRNPs in the CB; mutant coilin redistributes snRNPs within the nucleus (Bizarro et al., 2019; Bohmann et al., 1995). Coilin even contributes to the cell’s DNA damage response. Coilin congregates in the nucleolus when cisplatin, γ-irradiation, and UVA induced DNA damage occur. Coilin can then help halt rRNA synthesis by inhibiting Pol I through interactions with its largest subunit, RPA-194, and the regulator UBF (Bartova et al., 2014; Gilder et al., 2011).

1.6. Scope and Aims of Research

Here, we show that neuroblasts express more widespread nucleoplasmic coilin than surrounding neurons similar to Liu et al. (2009)’s male and female germline cells’ coilin expression. We also describe a feature of a resilient population of NBs in the Drosophila brain. These Mushroom body neuroblasts (MBNBs) expressed more coilin relative to other neuroblasts and neurons during Nopp140 disruption induced nucleolar stress. These flies experience nucleolar stress due to inadequate biogenesis of functional ribosomes. As expected, this deficiency affects the protein synthesis capacity for the cell, reducing it by half (He et al., 2015). However, increased coilin expression while the cell is deficient in functional ribosomes suggests an important role for coilin during the nucleolar stress experienced when Nopp140 is deleted. A coilin mediated mechanism might attempt to compensate for the loss of Nopp140, or it could just participate in a general nucleolar stress pathway. Even if the coilin is merely maternal stores, the reason for storage of the protein would be an exciting insight into coilin’s
function. Also the increased coilin expression is specific to the MBNBs emphasizing the need to expand on previous inquiries into their resilience (Baral et al., 2020). Coilin’s role in their durability could provide insight into the mechanisms of ribosomopathies’ selectivity in affected stem cell and progenitor cell populations. We also present our observations of a coilin null truncated protein’s contradictory localization patterns. The resolution of this problem could lead to insights about coilin’s structure and functional domains.
2.1. Introduction

Ribosomes play a critical role in the production of proteins for the entire cell. Even more critical is the successful production of these ribosomes. This process begins with transcription of pre-ribosomal RNA (pre-rRNA) which will be incorporated into the mature ribosome (Bleichert and Baserga, 2011). Concurrent with its incorporation, the pre-rRNA transcript is modified by snoRNPs (Sloan et al., 2017). RAFs assist with the assembly, transport, and function of snoRNPs (Talkish et al., 2014). Ribosome biogenesis requires high energy input and consumes over half of cellular transcription efforts, and therefore, it has a significant impact on cellular homeostasis (Woolford and Baserga, 2013). When the process is disrupted, the cell enters a state of nucleolar stress resulting in diseases called ribosomopathies (He and DiMario, 2011; Narla and Ebert, 2010; Warner, 1999).

In the ribosomopathy Treacher Collins Syndrome (TCS), mutation of the human TCOF1 gene encoding treacle, a ribosome assembly factor, prevents proper migration of embryonic neural crest cells presenting as craniofacial defects in affected individuals (He and DiMario, 2011; Sakai and Trainor, 2009). In the absence of treacle, ribosome biogenesis is impaired and the neural crest cells have a significant reduction in ribosome number (Sakai and Trainor, 2009; Zhai and Comai, 2000). Without enough ribosomes, a nucleolar stress response initiates p53-dependent apoptosis of neural crest cells (Dixon et al., 2006; Sakai and Trainor, 2009). These cephalic neural crest
cells never migrate into the pharyngeal arches, so craniofacial tissues are malformed and underdeveloped. Even though other stem cell and progenitor cell populations also have an increased demand for ribosomes to sustain the cells’ metabolic activity and high proliferation rate, only the cephalic neural crest cells are affected in TCS (Dixon et al., 2006). A higher threshold for sufficient embryonic levels of treacle in these cells has been suggested as a possibility for their sensitivity to treacle’s loss compared to other stem cell and progenitor cell populations, but there is no definitive study on this yet (He and DiMario, 2011).

To model TCS and nucleolar stress, Baral et al. (2020) disrupted treacle’s closest counterpart in the fruit fly Drosophila melanogaster, Nopp140, using CRISPR and balanced the disruption (J11) with the third chromosome balancer TM3. The heterozygous J11/TM3 larvae progressed to adulthood, but with some embryonic lethality demonstrating a haploinsufficiency for Nopp140 similar to the haploinsufficiency observed in TCS. However, the homozygous J11 larvae did not progress past the second instar larval stage with the majority of neural stem cells called neuroblasts (NBs) failing to proliferate at this point. Fibrillarin, the box C/D scaRNP methyltransferase, was also expelled from the nucleolus. As an essential enactor of pre-rRNA 2’-O-methylation, the loss of fibrillarin demonstrates the severity of nucleolar stress experienced by these neuroblasts. However, in the homozygous J11 larval brain, one subset of NBs, the Mushroom Body NBs (MBNBs), displayed resilience to nucleolar stress by continued proliferation and differentiation of their derived cell lineages as well as preservation of fibrillarin in the nucleolus compared to other NBs during nucleolar stress. This difference
in affected NBs is similar to the variation in affected stem cell populations in ribosomopathies like TCS (Baral et al., 2020; Cao et al., 2018).

Neuroblasts are the neural stem cells that form the larval CNS: the ventral nerve cord and the two central brain lobes (Birkholz et al., 2015; Homem and Knoblich, 2012). There are four classes of NBs: Type I, Type II, Mushroom Body, and Optic lobe (Baral et al., 2020). In the Drosophila life cycle, the insect progresses from an embryo to a larva, then a pupa, and finally an adult fly. During the larval stage, larvae develop through three substages: first, second, and third instar larval stages (Fernandez-Moreno et al., 2007). During embryonic and larval stages, neuroblasts undergo asymmetric division to produce another NB and a smaller ganglion mother cell (GMC). GMCs then differentiate into two neurons (Pearson et al., 2009). Embryonic neuroblasts are determined by Notch and Delta lateral inhibition in the neuroepithelium (Homem and Knoblich, 2012). During late embryogenesis, most NBs enter a quiescence stage until late first instar larval stage when they reenter the cell cycle if adequate nutrients are available. However, MBNBs do not enter the quiescence stage and continue the cell cycle without any dependence on nutrient availability (Ito and Hotta, 1992; Kunz et al., 2012; Prokop and Technau, 1994; Sipe and Siegrist, 2017). During the first and second instar larval stages NBs increase in number to about 60-85 NBs. The NBs stop dividing about one day after pupa formation, but the four MBNBs continue dividing for double the time of the other NB types (Ito and Hotta, 1992; Pearson et al., 2009).

MBNBs are the precursors to a structure in each central brain lobe responsible for olfactory sensory information, memory, and learning (Kunz et al., 2012; Kurusu et al., 2002). This structure is called the mushroom body, named for its mushroom-shaped
arrangement of neurons. Over 2,000 neurons called Kenyon cells constituting the mushroom body are all derived from the four MBNBs (Aso et al., 2009; Ito et al., 1997). In larval development, the lineages of the four MBNBs are identical, and one MBNB alone is enough to produce the entire mushroom body (Ito et al., 1997; Kunz et al., 2012; Zhu et al., 2003). The redundancy of the four MBNBs, the ability to continue cell division regardless of nutrient availability, and extended proliferation time all underscore the importance of the MBNBs during larval neurogenesis.

One observation made in our Nopp140 deficient larvae, was an overall upregulation of coilin expression (DeLeo and DiMario, 2018). Coilin is the marker protein for the CB, the site of snoRNP, snRNA, and spliceosome modification and assembly, and coilin regulates the CB’s formation and structure in the nucleus (Handwerger and Gall, 2006; Hebert and Matera, 2000; Shpargel et al., 2003). Nopp140 associates with the CB when chaperoning snoRNPs’ travel between the nucleolus and CBs in the nucleoplasm (Darzacq et al., 2002; Wang et al., 2002; Yang and Meier, 2003). Nopp140 and coilin have also been shown to interact, so we theorized that the observed nucleolar stress induced coilin upregulation would be most prevalent in Drosophila’s closest cell type to neural crest cells, the neuroblasts, and more specifically in the particularly robust MBNBs (Isaac et al., 1998). In this study, we show coilin expression in NBs resembles its diffuse nucleoplasmic expression in germline stem cells, and that during nucleolar stress in the larval brain, neural coilin expression is greatest in the MBNBs.
2.2. Results

2.2.1. Coilin Expression Variability within the Larval Brain

A variety of cell types contain coilin concentrated in Cajal bodies, but some stem cell populations have coilin expression throughout the nucleoplasm. Liu et al. (2009) demonstrated widespread nucleoplasmic coilin expression instead of exclusive localization to CB foci in both male and female germline stem cells in *Drosophila*. Here, we report more coilin expression in the NBs of *Drosophila* brains compared to the neurons which only had singular CB foci (Figure 2.1, white arrows for neuronal CB foci). The enlarged nuclei of the still diploid NBs can be seen as darker holes in the DAPI staining due to the large size of the NBs (Figure 2.1, panel b). The coilin labeling spans the entire nucleus apart from the nucleoli which can be identified by dark holes in the middle of the coilin staining (Figure 2.1, panel a, yellow arrows). The NBs had a wide distribution of nucleoplasmic coilin staining; however, coilin was still not present in the nucleoli of the NBs which is consistent with coilin localization patterns.

2.2.2. Mushroom Body Neuroblasts Maintain Coilin Expression under Nucleolar Stress

To model and study nucleolar stress in *Drosophila*, a CRISPR-derived *Nopp140* disruption line called *J11/TM3* was made in our lab by Sonu Baral Shrestha. Baral et al. (2020) demonstrated a null phenotype in the *J11* line with haploinsufficiency in the heterozygotes and second instar larval lethality in the homozygotes. The MBNBs were the only class of NBs able to withstand the loss of Nopp140 prior to larval lethality. We wanted to see if the classes of NBs also differed in their coilin expression during nucleolar stress. To investigate this, we did EdU studies in *J11* homozygous and
Figure 2.1. Increased Coilin Expression in Wild Type Larval Neuroblasts. Coilin was stained with GP3 anti-coilin (1:3,000) antibody in \( w^{1118} \) wild type larval central brain lobes (\( n=21 \)). Larvae were dissected on day 3 after larval hatching (ALH). Yellow arrows point to nucleoli of neuroblasts, and white arrows point to Cajal bodies of neurons. DNA is stained with DAPI. All scale bars represent 20 \( \mu \)m.

heterozygous second instar larvae. The \( TM3 \) balancer chromosome expresses GFP just in the gut of larvae which allowed us to differentiate between J11 homozygotes and heterozygotes. We selected for J11 homozygotes by collecting the GFP negative larvae and selected for J11 heterozygotes by collecting the GFP positive larvae. Second instar larvae were identified by mouth hook teeth number (Alpatov, 1929; Bodenstein, 1950), and EdU labeling was used to identify the four MBNBs as they are the only NB lineages still actively dividing during nucleolar stress in homozygous J11 larvae (Baral et al., 2020).

With EdU labeling, we saw a decrease in the number of EdU positive or actively dividing cells per \( \mu \)m\(^2\) in J11 heterozygotes as compared to wild type cells (Figure 2.2, panels e and h, respectively) reminiscent of the haploinsufficiency observed for the TCOF1 gene in TCS (Dixon et al., 1997b). However, these quantifications were not statistically significantly. Previous experiments indicate that the \( J11 \) heterozygotes exhibit an intermediate phenotype in survivability, so more repetitions of the EdU
experiment might be helpful to substantiate this. The number of EdU-positive cells further decreased to the characteristic four MBNBs per central brain lobe for the homozygous J11 (Figure 2.2, panel b). The smaller EdU-positive cells adjacent to the MBNBs are GMCs of that MBNB lineage. Coilin was expressed in the nucleoplasm of NBs and in the CBs of neurons in the J11 heterozygotes as seen in wild type brains. However, in the J11 homozygotes, each MBNB had a greater coilin signal intensity relative to other NBs and neurons in the rest of the central brain lobes.

Figure 2.2. Mushroom Body Neuroblasts Maintain Coilin Expression under Nucleolar Stress. The EdU labeling assay shows actively dividing cells along with GP3 anti-coilin (1:4,000) labeling in J11 homozygotes (a-c; n=8), J11 heterozygotes (d-f; n=10), and w1118 wild type (g-i; n=12) second instar larval central brain lobes. Panels a-c show two overlapping brain lobes. DNA is stained with DAPI. All scale bars represent 20 µm.
2.3. Discussion

Ribosome biogenesis constitutes a large but necessary resource sink for a cell and an even greater sink for cells with high metabolic demands like stem cells (Dixon et al., 2006; Warner, 1999). Neuroblasts in wild type larvae were shown to have coilin expression characteristic of other stem cell populations (Liu et al., 2009). Although all of coilin’s functions have not yet been definitively determined, we believe the expression pattern is due to coilin’s general involvement in the ribosome biogenesis pathway by its role in the CB at the least. Other proteins involved in ribosome biogenesis such as RAFs also have elevated expression levels in stem cells (Baral et al., 2020; Brombin et al., 2015) supporting this theory.

The diffuse coilin expression throughout the nucleoplasm of NBs is curious. This could represent multiple CBs or excess “free” coilin not concentrated within CBs perhaps due to extra expression or some inhibition of CB formation as a result of other factors that are in limiting amounts even though coilin is present. Coilin has been shown to interact with the U7 snRNP, a histone pre-mRNA processing snRNP, in HLBs in the nucleoplasm, so histone pre-mRNA 3’-end formation could potentially be more important in stem cells (Bellini and Gall, 1998; Liu et al., 2009). Coilin could also have additional functions outside of the CB not yet explored. Not only is the purpose of increased coilin unknown but also its source. An increase in coilin expression at the transcriptional or translational level would be expected in NBs, but the increased coilin signal could also be due to stockpiling of maternal coilin instead of nascent zygotic protein expression.
Our lab recently documented increased ribosome number in MBNBs in the J11 Nopp140 disruption larvae and is currently investigating their source. The cells being in a state of nucleolar stress suggests that ribosome production is not likely to have increased, so we predict that these ribosomes within MBNBs are retained maternal ribosomes. Maternal coilin could also be stored for zygotic use in the same way as these ribosomes. This could involve a mechanism specific to stem cells even without the impetus of nucleolar stress. Maternal stores could also be applied to the observation of increased coilin signal in MBNBs in homozygous J11 larvae. Stem cells are highly metabolically active cells and are, therefore, especially affected in ribosomopathies (Dixon et al., 2006). In the ribosomopathy Treacher Collins Syndrome, select neural crest cells are the affected progenitor cell population, and when modeling this disease in fruit flies, the neuroblasts are the equivalent affected stem cells (Baral et al., 2020). The resilience of MBNBs could be in part due to maternal coilin retention. However, much more work needs to be done to support either theory.

2.4. Materials and Methods

2.4.1. Fly Lines/Stocks

The w^{1118} line was used as a wild type control (Bloomington stock #3605). The A7.6-J11^{non-DeRed}/TM3, GFP, Ser, a heterozygous Nopp140 knockout line, was made in the DiMario lab by Sonu Shrestha Baral and is referred to as J11/TM3 (Baral et al., 2020). The third chromosome balancer w^{+}: Sb[1]/TM3, P[w[+mC]=ActGFP]JMR2, Ser[1] simply referred to as Sb/TM3, GFP, Ser (Bloomington stock #4534) was used to
make J11/TM3, GFP, Ser. All fly stocks were maintained at room temperature on standard fly food cornmeal medium.

2.4.2. Immunostaining and Fluorescence Microscopy

Larvae and flies were dissected and fixed in buffer B (2.18 mM KH$_2$PO$_4$, 14.54 mM K$_2$HPO$_4$, 75 mM KCl, 25 mM NaCl, 3.3 mM MgCl$_2$) (de Cuevas and Spradling, 1998) with 2% paraformaldehyde. Fixation proceeded for 30 minutes. The tissue was washed in PBST (PBS with 0.1% TX-100) for 30 minutes. The tissue was blocked in 3% BSA in PBST for 30 minutes. The primary antibody was Guinea Pig 3 (GP3) against the carboxyl-terminus of *D. melanogaster* coilin, a gift from the Gall lab (Liu et al., 2009), diluted with 3% BSA in PBST to either a 1:4,000 or 1:3,000 dilution. The tissue was incubated in primary antibody overnight at 4°C with gentle but visible shaking. The tissue was washed with 1.5% BSA in PBST for two hours and then incubated in 3% BSA in PBST for one hour. The tissue was incubated for four hours at 4°C in the secondary antibody Goat anti-Guinea Pig Alexa Flour 546 (A-11074, Invitrogen) to either a 1:600 or 1:400 dilution in 3% BSA in PBST. The tissue was washed with PBST overnight at 4°C, counterstained with DAPI (Polysciences) at 1:1,000 in PBST for ten minutes, and mounted in PBST for imaging. The tissues were imaged with either the Leica SP8 Confocal Microscope with a White Light Laser system at the LSU Shared Instrumentation Facility or a conventional fluorescence microscope using a Zeiss Axioskop with a SPOT RTSE digital camera. All PBST solutions included azide to prevent bacterial growth. Unless specified all steps were performed at room temperature.
2.4.3. EdU labeling

Within ten minutes of larval dissection in PBS in DEPC water, the tissue was exposed to 20 µM EdU in PBS for either 30 minutes or two hours. The tissue was fixed for 30 minutes in buffer B as described above (de Cuevas and Spradling, 1998) with 2% paraformaldehyde and blocked with 3% BSA in PBST (PBS with 0.1% TX-100) for 25 minutes. The EdU was labeled with Alexa Fluor 488 using the Click-iT reaction cocktail from an Invitrogen Click-iT EdU Imaging Kit (C10337) for 30 minutes. Tissues were then immunostained, counterstained with DAPI, and imaged as previously described. All EdU labeling steps were performed at room temperature.

2.4.4. Image Analysis and Assembly

Image J software and Spot 5.6 Advanced software were used for microscopy image analysis and editing. Adobe Photoshop was used to assemble all microscopy images.
CHAPTER 3. CONFLICTING LOCALIZATION OF THE COILIN NULL TRUNCATED PROTEIN

3.1. Introduction

The protein coilin is an essential component of the Cajal body, a nuclear suborganelle. As in all biology, form follows function for coilin. The protein is largely unstructured but has conserved terminal domains with slight structure (Bellini, 2000; Frege and Uversky, 2015). Discrepancies in predicted and observed molecular weight have been attributed to the disordered nature of coilin. In several species, the molecular weight observed by gel electrophoresis is smaller than predicted (Makarov et al., 2013). Coilin’s amino terminal self-association domain forms a homodimer, and this serves as the foundations for the CB (Hebert and Matera, 2000). The other structured region in coilin is a Tudor-like domain in the carboxyl terminus of coilin, but it has no known functions (Makarov et al., 2013; Shanbhag et al., 2010). The phosphorylation of serine residues in the carboxyl terminus of coilin dictates both the abundance of CBs and cellular localization (Shpargel et al., 2003). Phosphorylation by VRK1 also prevents proteasomal degradation of coilin (Cantarero et al., 2015). Nuclear kinases CDK2-cyclin E, casein kinase II, VRK1, and VRK2 can all phosphorylate coilin, but phosphatases that act specifically on coilin have not been confirmed (Hebert and Matera, 2000; Liu et al., 2000; Sanz-Garcia et al., 2011; Shpargel et al., 2003).

Phosphorylation is an important regulatory mechanism for coilin, and several neurodegenerative disorders have been tentatively attributed to its faulty regulation (Sanz-Garcia et al., 2011). Although its role in spinocerebellar ataxia type 1 (SCA1) is unknown, human coilin has been shown to interact with SCA1’s culpable protein,
ataxin-1 (Hong et al., 2003). Spinal muscular atrophy (SMA) is another neurodegenerative disease potentially reliant on accurate coilin regulation. SMA is caused by inaccurate localization of the SMN complex. Often this occurs due to mutation of the survival of motor neurons protein (SMN), but localization of the SMN complex relies not only on the SMN protein but also on its interaction with coilin’s carboxyl terminal RG box at the CB during snRNP biogenesis (Hebert et al., 2001). The affinity of this interaction is regulated by symmetrical dimethylarginines (sDMAs) in coilin’s RG box, but when arginine methylation is inhibited SMN locates to Gemini bodies or gems. Gems and CBs are very similar, but gems do not contain coilin or snRNPs. Additionally, sDMAs are essential for coilin homodimerization, so lack of sDMAs can affect CB formation as well (Boisvert et al., 2002; Hebert et al., 2002; Hebert et al., 2001). The absence of sDMA in coilin has also been observed in the neurological disorder Huntington’s (Ratovitski et al., 2015).

Although coilin is clearly involved in crucial cellular pathways, it’s necessity for survival of an organism varies. In Arabidopsis and Drosophila, viability is not affected by loss of functional coilin. Drosophila’s syncytial blastoderm allows sharing of snRNPs during embryogenesis which might prevent coilin loss from becoming too detrimental. Without coilin, nuclear bodies containing subsets of the CB components have been found around the nucleus, supporting coilin’s role as a structural binder for the CB. However, vertebrates like Danio rerio and Mus musculus have more severe phenotypes. In mice there is reduced fertility and fewer homozygous coilin loss-of-function progeny, but coilin loss is not lethal. In zebrafish, lack of functional coilin
reduces snRNPs, spliced mRNAs, and CB size and number causing lethality within one day (Cao et al., 2018; Liu et al., 2009; Machyna et al., 2015; Tucker et al., 2001).

Abnormal localization of coilin does indicate key structural information. Fusion of a GFP tag to the carboxyl terminus of coilin increases number of CB, but fusion of a GFP tag to the amino terminus of coilin does not affect its localization (Hebert and Matera, 2000; Liu et al., 2009; Shpargel et al., 2003). Nucleolar accumulation has also been observed during artificial inhibition of transcription, in human breast cancer cells, during serine/threonine phosphatase inhibition, and during mutation of serine residue S202 to aspartic acid (Carmo-Fonseca et al., 1992; Lyon et al., 1997; Ochs et al., 1994; Sleeman et al., 1998). Mutation of coilin serine 202 to alanine (S202A) does not affect localization of the protein, but the mutation S202D causes nucleolar accumulation. This mutation to aspartic acid mimics a constitutively phosphorylated site while S202A mimics a constitutively unphosphorylated site. Therefore, phosphorylation does seem to be key in coilin’s localization (Lyon et al., 1997). Removal of other phosphorylation sites at coilin’s carboxyl terminus increases CB number. These localization observations have led to a theory of coilin’s structure (Shpargel et al., 2003).

Hebert and Matera (2000) stated that a nucleolar localization sequence (NoLS) was hidden by interaction of acidic serine patches in the carboxyl domain with nuclear localization sequences (NLSs) just after the amino terminal self-interaction domain. They identified a potential NoLS sequence between the two NLSs of human coilin with two acidic serine patches located before the RG box. Here we describe similar observations of a truncated Drosophila melanogaster coilin protein and the similar potential structure to Hebert and Matera (2000). However, our speculations for
Drosophila coiling structure are questionable because we also present conflicting results. We have not found a satisfactory explanation for these contradictory observations.

### 3.2. Results

The coiling null fly line *coil 199* expresses a truncated coiling protein without its amino terminus, amino acids 1-144 (Beumer et al., 2008). Here, we report labeling of the truncated coiling protein in the nucleolus of coiling null larvae (Figure 3.1, part A, yellow arrows). Using only the secondary antibody, background labeling was seen in the cytoplasm, but there was no nucleolar staining as seen in Figure 3.1, part C with yellow arrows pointing to the unlabeled nucleoli of cells. Wild type flies showed normal CB foci and nucleoplasmic labeling when using the R2T anti-coiling primary antibody (Figure 3.1, part B). This nucleolar localization of the truncated coiling lacking its amino terminal self-interacting domain parallels similar results found with human coilin in Hebert and Matera (2000). However, when repeating this experiment with another primary antibody, GP3 anti-coiling, we detected no truncated coiling protein in *coil 199* larvae (Figure 3.2). Yellow arrows in Figure 3.2, panels a, e, and i all point to empty nuclei of coiling null larvae’s brain, imaginal disc, and proventriculus, respectively. However, typical CBs were seen in wild type neurons, the imaginal disc, and the proventriculus (Figure 3.2, panels c, g, and k, respectively).

### 3.3. Discussion

While using a coiling null fly line, *coil 199*, as a negative control for other experiments, we stumbled upon the alarming observation of coiling labeling in these fly
lines. Upon further investigation, we realized this was a truncated protein produced in this coilin null line, and its localization was due to the truncation. Hebert and Matera (2000) demonstrated nucleoplasmic localization of truncated human coilin missing its amino terminal domain. The coilin was not in CBs because it was missing its self-

---

Figure 3.1. Nucleolar Truncated Coilin in the Coilin Null Fly Line. R2T anti-coilin (1:2,000) with goat anti-rabbit AF 546 (1:300) was used to detect the truncated coilin protein (A,C) in the brain and gastric caecum (a,e), the fat bodies (b,f), the Malpighian tubules (c,g), and the proventriculus of coil 199 flies (d,h,k,l) and to detect the endogenous coilin protein in the brain (B) of w^1118 flies. The yellow arrows point to the nucleoli. DNA is stained with DAPI. All scale bars represent 20 μm. The coilin null fly line and coilin antibodies were gifts from the Gall lab (Beumer et al., 2008; Liu et al., 2009).
interacting domain. It was present in the nucleoplasm because it still had its NLSs, acidic serine patches, and NoLS, but it was not in the nucleolus because the NoLS was still concealed by interaction of the NLSs and acidic serine patches. Hebert and Matera (2000) also demonstrated human coilin with only amino acids 1-248 in the nucleolus and CBs. This particular truncation was present in the nucleolus because it was missing at least one acidic serine patch which exposed the NoLS, but it was also present in CBs because it still had its self-interacting domain and NLSs. If *Drosophila melanogaster* coilin shares a similar pattern of functional domains with human coilin, then the

![Figure 3.2. No Detection of Truncated Coilin in the Coilin Null Fly Line.](image)

Figure 3.2. No Detection of Truncated Coilin in the *Coilin Null* Fly Line. GP3 anti-coilin (1:3,000) with goat anti-guinea pig AF 546 (1:400) was used to detect the truncated coilin protein or endogenous coilin protein in the brain (a-d), imaginal disc (e-h), and proventriculus (i-l) of *coil 199* and *w*1118 flies. The yellow arrows point to nuclei of *coil 199* cells (a,b,e,f,i,j) and the nuclear Cajal bodies of *w*1118 cells (c,d,g,h,k,l). DNA is stained with DAPI. All scale bars represent 20 µm. The *coilin null* fly line and coilin antibodies were gifts from the Gall lab (Beumer et al., 2008; Liu et al., 2009).
nucleolar localization of the truncated protein is reasonable. The coil 199 truncated coilin protein only has amino acids 145-633, therefore, it is missing its self-interacting domain and cannot self-associate which prevents CB formation. It is also missing its NLS from the truncation, so it is not present in the nucleoplasm. However, the truncated protein is found in the nucleolus, so it must have another method of entry into the nucleus before going to the nucleolus. It might be able to enter by diffusion if small enough, or an unidentified NLS could still be present in the truncated protein. In this case, it would still be found in the nucleolus because the NoLS was exposed by removal of some fraction of the NLSs in coilin. This type of phosphorylative control of NoLS exposure has been demonstrated with a retinoblastoma protein by (Harbour et al., 1999). When analyzing the sequence of Drosophila coilin we found a potential NoLS sequence: KNKSKNKK, based on the work of Fang et al. (2017). It is in the correct location between potential monopartite and bipartite NLSs and the carboxyl phosphorylation sites of coilin.

After this interpretation, we attempted to replicate the results but found no nucleolar coilin in the same coilin null line. Different coilin antibodies were used in the two experiments, but they make the results even more confusing. The coilin antibodies were made using full length Drosophila melanogaster coilin. A set of antibodies against the amino terminus of coilin, amino acids 1-146, were made (R2T) and a set of antibodies against the carboxyl terminus of coilin, amino acids 147-634, were made (GP3). R2T should not be able to recognize the truncated coilin protein, but GP3 should because of which part of the coilin protein they recognize (Beumer et al., 2008; Liu et al., 2009). However, we found that GP3 did not label truncated coilin, but R2T did.
Beumer et al. (2008) showed no labeling of protein from coilin null ovaries using GP3 which is consistent with what we found. We believe R2T was labeling truncated coilin because it is a polyclonal serum, but we still cannot explain why GP3 would then be unable to label the truncated coilin protein.

3.4. Materials and Methods

3.4.1. Fly Lines/Stocks

The \textit{w}^{1118} line was used as a wild type control (Bloomington stock #3605). The coilin null line \textit{coil}^{199} was a gift from the Gall lab (Beumer et al., 2008). All fly stocks were maintained at room temperature on standard fly food cornmeal medium.

3.4.2. Immunostaining and Fluorescence Microscopy

Larvae and flies were dissected within 5 minutes and fixed in buffer B (2.18 mM KH$_2$PO$_4$, 14.54 mM K$_2$HPO$_4$, 75 mM KCl, 25 mM NaCl, 3.3 mM MgCl$_2$) (de Cuevas and Spradling, 1998) with 4% paraformaldehyde for 10 minutes. The tissue was washed in PBST (PBS with 0.1% TX-100) for 30 minutes. The tissue was blocked in 3% BSA in PBST for 30 minutes. Three primary antibodies against the amino-terminus of \textit{D. melanogaster} coilin were used: Guinea Pig 1 and 2 (GP1 and GP2) and Rabbit 2 (R2T), and one primary antibody against the carboxyl-terminus of \textit{D. melanogaster} coilin, Guinea Pig 3 (GP3), was used. All coilin antibodies were gifts from the Gall lab (Liu et al., 2009). Guinea pig antibodies were diluted with 3% BSA in PBST to a 1:3,000 dilution, and the rabbit antibody was diluted with 3% BSA in PBST to a 1:2000. The tissue was incubated in primary antibody overnight at 4°C. The tissue was washed with
1.5% BSA in PBST for two hours and then incubated in 3% BSA in PBST for one hour. The tissue was incubated for four hours at 4°C in the secondary antibodies Goat anti-Guinea Pig Alexa Flour 546 (A-11074, Invitrogen) and Goat anti-Rabbit Alexa Flour 546 (A-11035, Invitrogen) to either a 1:400 (anti-guinea pig) or 1:300 (ani-rabbit) dilution in 3% BSA in PBST. The tissue was washed with PBST overnight at 4°C, counterstained with DAPI (Polysciences) at 1:1,000 in PBST for ten minutes, and mounted in PBST for imaging. The tissues were imaged with either the Leica SP8 Confocal Microscope with a White Light Laser system at the LSU Shared Instrumentation Facility or a conventional fluorescence microscope using a Zeiss Axioskop with a SPOT RTSE digital camera. All PBST solutions included azide to prevent bacterial growth. Unless specified all steps were performed at room temperature.

3.4.3. Image Analysis and Assembly

Image J software and Spot 5.6 Advanced software were used for microscopy image analysis and editing. Adobe Photoshop was used to assemble all microscopy images.
CHAPTER 4. CONCLUSIONS AND FUTURE STUDIES

We have shown an upregulation of coilin in Nopp140 deficient flies. We suspect that in the absence of Nopp140, coilin compensates for the RAF’s loss in a nucleolar stress response pathway. It is not likely that increased coilin levels would be the cell’s attempt to make more CBs because it is the phosphorylation status of its carboxyl terminal domain that controls CB number, not its total amount (Shpargel et al., 2003). Coilin might take over the role of a chaperone for snRNPs, but the mechanism by which coilin compensates for loss of Nopp140 is not an answer we expect soon. We also hope to study if this response is exclusive to the loss of Nopp140. Other stressors such as other RAFs that associate with the CB and coilin could potentially induce coilin overexpression as well as other RAFs not associated with the CB or even just an overall loss of nucleolar function. If coilin has a direct role in compensating for the loss of Nopp140, then we do not expect an upregulation in coilin expression with these other means of inducing nucleolar stress. However, if the role of coilin in nucleolar stress is more extensive, then we could potentially see a change in expression for one of these factors. This could guide the focus of our future studies into the specific mechanism of coilin’s upregulation and role in nucleolar stress.

To study the role of coilin in nucleolar stress, we propose the following aims: 1) determine if coilin upregulation is triggered by Nopp140 loss specifically, loss of other RAFs, or general nucleolar stress due to inhibition of rDNA transcription, 2) characterize coilin’s expression pattern among different tissues and with respect to cellular location in the absence of Nopp140, and 3) determine if the source of increased coilin is transcription, translation, or maternal stores. Completion of these aims will help us
understand how coilin may compensate for the loss of Nopp140 during nucleolar stress. In turn, we will gain insight about the nucleolar stress pathways that occur in TCS and other ribosomopathies.

We also have unresolved questions from our research. We hope to clarify the quantification of actively dividing cells in wild type and J11 heterozygous brains. This could improve our understanding of the variation in phenotype of differentially affected cell populations in TCS, and it would verify the degree to which Nopp140 deficient NBs exhibit haploinsufficiency. To establish *Drosophila melanogaster* coilin’s functional domains, the conundrum of the truncated coilin protein’s localization will also be addressed. Confirming coilin’s structure and its similarity to human coilin’s structure could improve understanding of the neurological disorders in which coilin has been implicated.
APPENDIX. GFP FUSION TO COILIN’S CARBOXYL TERMINUS ALTERS COILIN EXPRESSION PATTERNS

Transgenic Expression of eGFP Tagged Coilin. (A) eGFP amino terminally tagged coilin transgene is expressed under the endogenous promoter at both the attP18(X)6C12 site on the X chromosome referred to as P18 and the P2(3L)68A4 site on the third chromosome referred to as P2 in adult female flies. The GFP exposure time for w^{1118} and P18 GFP-Coilin nurse cells differed with times of 2.7 sec (e) and 1.1 sec (g), respectively. Typical CB foci were observed for eGFP amino terminal tagged coilin. (B) eGFP carboxyl terminally tagged coilin transgene is expressed under the endogenous promoter at the P18 site in adult male flies. Abnormal nuclear aggregates of coilin were observed. This has previously been documented for human coilin with GFP by Hebert and Matera (2000) and for Drosophila melanogaster coilin with YFP by Liu et al. (2009).
REFERENCES


processing is directed by the encoding region. Nucleic Acids Research 38, 370-381.


the pseudogene-encoded coilp1 in telomerase and box C/D scaRNP biogenesis. RNA Biol 13, 955-972.


VITA

Kathryn Rose DeLeo attended Louisiana State University and graduated magna cum laude with a B.S. in Biochemistry and with College Honors in May 2018. She joined Dr. Patrick DiMario’s lab in her sophomore year of undergraduate studies and completed an undergraduate honors thesis. She continued her research in the lab as a graduate student in August 2018. Kathryn is a candidate for graduation with a Master of Science degree in Biochemistry in May 2021.