Characterizing RNA Granule-Associated Stalled Polysomes

Mina Naz Anadolu

Neurology and Neurosurgery
McGill University, Montreal, Quebec

November 2021

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

For my father, Dr. Rauf Yücel Anadolu

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

"Set your life on fire. Seek those who fan your flames."
-Rumi

I would not be here today if it wasn't for the generous contributions and unwavering support of the following individuals:

I am eternally grateful to my supervisor Dr. Wayne Sossin, for recognizing the spark in me, nurturing my curiosity, and showing me how to be an example scientist and do great science. I learned a great deal from Wayne: how to foster the collaborative spirit, how to be patient with yourself and others, how to be a sceptic, how to argue well and write persuasively, how to be pragmatic and most importantly, how not to fuse with a hypothesis. I would also like to thank Wayne for his patience and encouragement during the darkest times of my life; we made it through.

I would also like to thank my mentor Dr. Heidi McBride, and my advisory committee, Dr. Keith Murai, and Dr. Ed Ruthazer, for empowering me and for inspiring me to pursue my crazy ideas. I would also like to thank past and present Sossin Lab members: John Dyer, Tyson Graber, Robyn McAdam, Carole Abi Farah and Xiaotang Fan, for sharing their wisdom, for being my support mechanism in the lab and for helping me become a fierce scientist. I owe my sharp wit to the delicious pork buns Xiaotang fed me. I would also like to thank our outstanding undergraduate researchers Irene Shin and Teodora Markova for their contributions to my publications.

I would also like to recognize the efforts of my extraordinary collaborators Jingyu Sun, Senthilkumar Kailasam, Konstanze Simbriger, Seyed Mehdi Jafarnejad, Mehdi Amiri, Sarah Herbert-Seropian, Erika Freemantle and Dushyant Jahagirdar, who supported my research under the leadership of Dr. Joaquin Ortega, Dr. Christos Gkogkas, Dr. François Lefebvre, Dr. Jean-

Claude Lacaille, and Dr. Nahum Sonenberg. I would like to thank Dr. Ronan da Silva and Dr. Vincent Soubannier for helping translate my abstract. I'd also like to acknowledge the kind support of the members of the Montreal Neurological Institute's Center of Neurological Disease Models (CNDM), Cathy Hunt, Lisa Volume, Jennifer Henri, Madeline Arkilanian.

I must admit, I would not have made it without my pillar of strength, my dad Prof. Dr. Rauf Yucel Anadolu, MD, who taught me to persevere, and my grandparents Prof. Dr. Sema Yavuzer, MD and Prof. Dr. Sinasi Yavuzer, MD, who encouraged me to follow my dreams and be versatile. I also thank the strong female academics in my life who are my role models: my mother, Prof. Dr. Rana Anadolu Dizioglu, MD, Dr. Gulgun Koyuncuoglu, Prof. Ulku Bozkurt Nilsen, Dr. Josephine Nalbantoglu, and Tumay Krugman. Your graceful intensity inspires me.

Lastly, I would like to thank my multicultural Canadian family who watched over me, who picked me up when I fell down, and who gave me the best advice when I needed it the most: Melissa and Jim Costigan, Dr. Ronan da Silva, Kirsten Joe, Nazli Bozoglu, Dr. Brian Foo, Dr. Vincent Soubannier, Jeanne Madranges, Dr. Marie-Pier Girouard, Jaminie Vas, Nathalie Marcal, Allison Bloom, Nataly Urena, Yasemin Erdogan, Cigdem Erdogan, Dr. Valerio Piscopo and Michael Oliver. Thank you, friends, for the many cups of coffee and tea, and for lending an ear.

This work was supported by Canadian Institute of Health Research (CIHR) grant 374967 to Wayne Sossin, an award from the Azrieli foundation to Dr. Wayne Sossin and Dr. Joaquin Ortega that I helped prepare the application for, by a Fondation Santé grant and a FORTH-IMBB-BR start-up grant to Christos Gkogkas. I would also like to thank staff at the Facility for Electron Microscopy Research (FEMR) at McGill University; FEMR is supported by the Canadian Foundation for Innovation, the Quebec Government and McGill University.

CONTRIBUTION OF AUTHORS

It takes a village to make great science happen. I'd like to give credit to and thank the following individuals for their contributions to the two scholarly publications in this thesis:

Chapter Two: This chapter was co-authored with Dr. Wayne Sossin and recently published as Chapter 11 of the Oxford Handbook of Neuronal Protein Synthesis, Oxford University Press, New York, NY, USA. The Oxford Handbook of Neuronal Protein Synthesis was edited by Dr. Wayne Sossin. I made the 3 figures in the chapter using biorender.com. I also contributed the cover image for the book from my original painting titled "Granules of life", acrylic on canvas.

Chapter Three: This chapter consists of a manuscript prepared and submitted to Nucleic Acids Research, titled "*Ribosomes in RNA granules are stalled on mRNA sequences that are consensus sites for FMRP association"*, by Mina N. Anadolu, Senthilkumar Kailasam, Konstanze Simbriger, Jingyu Sun, Tedora Markova, Seyed Mehdi Jafarnejad, Francois Lefebvre, Joaquin Ortega, Christos G. Gkogkas, Wayne S. Sossin. I prepared the RNA Granule and polysome enriched fractions using a protocol adapted from the Khandjian group (El Fatimy et al., 2016). I did the characterization of sucrose gradient fractions using Coomassie and Western Blot. I also identified RNA Granule proteins using western blot and quantified the results. Jingyu Sun prepared the EM grids and took EM images of negative stained stalled polysomes I purified. I did the nuclease digestion of the enriched fractions and purified monosomes for footprinting. I also did RNA extraction from whole brain lysate for sequencing. Konstanze Simbriger made the footprint libraries from the enriched monosomal fractions I prepared. Senthilkumar Kailasam performed the bioinformatic analysis of the footprints, including footprint mapping onto identified mRNAs, calculation of periodicity, plotting of 3' and 5' overhangs, correlation analysis between our biological and experimental replicates. Dr. Wayne Sossin generated the list of highly enriched and

abundant mRNAs from our sequencing data, and ran the GO Analysis as well as Homer motif analysis; I prepared the figures. Teodora Markova did the correlation analysis comparing our mRNAs to data sets published by other groups and prepared the whisker-box plots. Dr. Wayne Sossin provided me with a short list of RNA binding proteins with consensus sites that match the motifs identified by Homer. I prepared the figures for the motif analysis, showing the consensus sequences and their matching RBPs, overlapping consensus sites. Dr. Mehdi Jafarnejad did the RNA extraction and preliminary bioanalysis of stalled footprints, not included in this version of the manuscript. Similarly, Mehdi Amiri did the preliminary UV analysis of sucrose gradients I prepared, also not shown in this version of the manuscript. I prepared all the figures and illustrations using biorender.com. I wrote the paper with help from Dr. Wayne Sossin, Dr. Joaquin Ortega and Dr. Christos Gkogkas. This research study was designed by Dr. Wayne Sossin, Dr. Christos Gkogkas, Dr. Joaquin Ortega and Dr. Francois Lefebvre.

ABSTRACT

Neurons have numerous mechanisms to regulate the transcription, transport and translation of mRNAs important for neurodevelopment and learning-related synaptic plasticity. When these regulatory mechanisms fail, aberrant mRNAs and dysregulated proteins can accumulate, disrupting homeostasis, and leading to the miswiring of the nervous system and intellectual disability. One example is a severe type of Autism Spectrum Disorder called Fragile X Syndrome, in which patients lack the RNA binding fragile X mental retardation protein (FMRP) that regulates the translation of many mRNAs important for synaptic function. The mechanisms of translational control that are disrupted in neurodevelopmental disorders, including Fragile X Syndrome, are not clear. Our lab has suggested that the mRNAs that are regulated by FMRP in the healthy brain, including the mRNA for the Microtubule associated protein 1b (Map1b), utilize stalled polysomes as a means of transport and regulated translation, and that this is the critical form of translational control that is disrupted during development. FMRP, as well as several other proteins associated with RNA granules are found to be enriched in a sedimentation-based purification of RNA granules from healthy post-natal rat brains. We used nucleases to digest the purified polysomes from RNA granules into monosomes and characterized the stalling profiles using Ribosome Profiling.

Analysis of ribosome footprints with RNASeq revealed that polysomes recruit a large quantity of cytoskeletal mRNAs involved in neuronal development including those regulated by translation elongation through eEF2 phosphorylation. In contrast, proteins regulated by translation initiation, such as those regulated by TOR activation or through the phosphorylation of eIF4E, were not enriched. The footprints showed an enrichment for mRNAs previously predicted to be regulated by stalled polysomes, as well as many mRNAs implicated in Autism Spectrum Disorders, as identified by the Simons Foundation Autism Research Initiative (SFARI). Most importantly, we

observed a significant enrichment of footprints on mRNAs previously identified by HITS-CLIP to be associated with FMRP, suggesting that a large proportion of these CLIPs were generated from RNA granule-associated mRNAs. In our footprints we saw different sized reads, which we divided into small (<25 nt), medium (26-32 nt) and large (>32 nt) reads. We detected an abundance of larger size ribosomal footprints with an average size of 35 nt in the RNA granules, which cluster in the coding region of mRNAs, indicating putative ribosome stalling sites. The larger footprints showed reproducible consensus peaks in the open reading frame that were enriched in purine-rich sequences, which correspond to consensus sequences for FMRP HIT-CLIPS as well as interaction motifs for m6A modifications. The evidence supports the stalling of elongating ribosomes on specific mRNA sequences. These sequences may be directly targeted by FMRP and other RBPs that are implicated in the regulation of stalled mRNAs and their recruitment to RNA Granules for transport. Alternatively, these sequences may stall elongating ribosomes through an independent mechanism, and techniques such as CLIP identify these sequences as consensus sequences for RBPs because the RBPs associate with the ribosomes that are stalled on these sequences.

RÉSUMÉ

Les neurones possèdent de nombreux mécanismes pour réguler la transcription, le transport et la traduction des ARNm qui sont importants pour le développement neurologique et la plasticité synaptique liée à l'apprentissage. Lorsque ces mécanismes de régulation échouent, des ARNm aberrants et des protéines dérégulées peuvent s'accumuler, perturbant l'homéostasie et conduisant à un mauvais câblage du système nerveux et à une déficience intellectuelle. Un exemple est un type sévère de trouble du spectre autistique, appelé syndrome du X fragile, dans lequel les patients n'ont pas la protéine de retard mental X fragile (protéine appelée FMRP) qui est une protéine de liaison à l'ARN et qui régule la traduction de nombreux ARNm importants pour la fonction synaptique. Les mécanismes de contrôle traductionnels qui sont perturbés dans les troubles neurodéveloppementaux, y compris le syndrome du X fragile, ne sont pas tout à fait clairs. Notre laboratoire a suggéré que les ARNm régulés par la FMRP dans le cerveau sain, y compris l'ARNm de la protéine associée aux microtubules 1b (Map1b), utilisent des polysomes bloqués comme moyen de transport et de régulation de la traduction, et que c'est ceci la forme déterminante de contrôle de la traduction interrompue pendant le développement. La FMRP, ainsi que plusieurs autres protéines associées aux granules d'ARN, se sont révélées enrichies dans une purification par sédimentation de granules d'ARN provenant de cerveaux postnataux de rats sains. Nous avons utilisé des nucléases pour digérer les polysomes purifiés à partir de granules d'ARN en monosomes et nous avons caractérisé les profils de blocage à l'aide du profilage de ribosomes.

L'analyse des empreintes de ribosomes avec la technique RNASeq a révélé que les polysomes recrutent une grande quantité d'ARNm du cytosquelette qui sont impliqués dans le développement neuronal, y compris ceux régulés par l'élongation de la traduction au travers de la phosphorylation de eEF2. En revanche, les protéines régulées par l'initiation de la traduction,

telles que celles régulées par l'activation de TOR ou par la phosphorylation de eIF4E, n'étaient pas enrichies. Les empreintes ont montré un enrichissement pour les ARNm précédemment prédits pour être régulés par des polysomes bloqués, ainsi que de nombreux ARNm impliqués dans les troubles du spectre autistique, comme identifiés par la Simons Foundation Autism Research Initiative (SFARI). Plus important encore, nous avons observé un enrichissement significatif des empreintes sur les ARNm précédemment identifiés par HITS-CLIP comme étant associés à la FMRP, ce qui suggère qu'une grande proportion de ces CLIP ont été générées à partir d'ARNm associés aux granules d'ARN. Dans nos empreintes, nous avons observé des lectures de tailles différentes, que nous avons divisées en lectures petites (<25 nt), moyennes (26-32 nt) et grandes (> 32 nt). Nous avons détecté une abondance d'empreintes ribosomales de plus grande taille ; avec une taille moyenne de 35 nt ; dans les granules d'ARN, qui se regroupent dans la région codante des ARNm, indiquant des sites putatifs de blocage des ribosomes. Les empreintes plus grandes ont montré des pics consensus reproductibles dans le cadre de lecture ouverte qui étaient enrichis en séquences riches en purine, ce qui correspond à des séquences consensus des HIT-CLIPS de la FMRP ainsi qu'à des motifs d'interaction pour les modifications de m6A. Ces preuves soutiennent le blocage des ribosomes allongeant sur des séquences d'ARNm spécifiques. Ces séquences peuvent être directement ciblées par la FMRP et d'autres protéines de liaison à l'ARN qui sont impliquées dans la régulation des ARNm bloqués et leur recrutement en granules d'ARN pour leur transport. Dans une hypothèse alternative, ces séquences pourraient bloquer les ribosomes allongeant par un mécanisme indépendant, et des techniques telles que la CLIP identifie ces séquences comme des séquences consensus pour les protéines liant l'ARN parce que celles-ci s'associent aux ribosomes qui sont bloqués sur ces séquences.

RATIONALE AND CONTRIBUTION TO ORIGINAL KNOWLEDGE

RNA Granules play an important role in neurons as they deliver precious mRNA cargo from the cell body to local synaptic sites. Many mRNAs stored and transported in **RNA Granules** are important for neurodevelopment. When the proteins that manage this precious cargo are mutated, it can lead to severe neurodevelopmental disorders and autism. Some examples are FMRP in Fragile X Syndrome (FXS), DDX3 in DDX3X-related Neurodevelopmental Disorder (DDX3X-NDD), PUR-Alpha in PURA Syndrome. In the case of Fragile X Syndrome, a mutation in the X chromosome results in the loss of FMRP, which, in the normal brain, regulates the translation of mRNAs like Map1b that are important for synaptic plasticity and the endocytosis of AMPA receptors during mGluR-LTD. Without the regulatory control of FMRP mRNAs like Map1b are excessively translated, disrupting the homeostasis of the local proteome and the excitatory-inhibitory balance of neuronal circuits during neurodevelopment. As a result, patients with Fragile X have severe intellectual and learning disability among other debilitating physical and behavioral symptoms that significantly affect their quality of life.

If we were to find a way to reconstitute regulation of Map1b and other mRNAs in the absence of FMRP, we can possibly bring the system back to homeostasis. For this we needed to understand how Map1b and other mRNAs regulated by FMRP are translated in the healthy brain. Understanding the mechanisms that govern mRNA transport and translation during neurodevelopment is the first step towards understanding how these mechanisms can be manipulated to develop therapies that benefit patients.

The scholarly work in this thesis establishes this foundation, by reviewing new and existing literature on mRNA transport and translation, and by bringing new perspectives to understanding the role of FMRP in regulating neurodevelopmental mRNAs. This work challenges our dogmatic view of translation and provides compelling evidence to cement the idea that reversible stalling of elongating ribosomes is a cellular strategy specialized to neurons for the regulation of neurodevelopmental mRNAs in a fast and efficient way, independent of translation initiation. This work also casts a new light on ribosomes as RNA binding proteins, and how they may contribute to the regulation of stalled mRNAs through interaction with proteins such as FMRP. I present to you a new point of view on neuronal translation that reconciles existing findings in the field and reveals new avenues to be explored. The work in its entirety by no means provides a direct and complete clinical therapy to Fragile X, however, it offers a launchpad for future translational work that has the potential to change lives.

LIST OF SYMBOLS AND ABBREVIATIONS

3’UTR – 3-prime Untranslated Region

40S – Small Ribosomal Subunit (eukaryotic)

43S – Preinitiation Complex with 40S ribosome

4EBP – 4E Binding Protein

5'UTR – 5-prime Untranslated Region

60S – Large Ribosomal subunit (eukaryotic)

80S – Complete Ribosome (eukaryotic)

aaRS – aminoacyl-tRNA Synthetase

ABCE1 – ATP Binding Cassette Subfamily E Member 1

Akt – Ak strain transforming kinase (also known as Protein Kinase B, PKB)

AMPA – L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR – AMPA Receptor

ASD – Autism Spectrum Disorder

ATP – Adenosine Tri-Phosphate

Bbs2 – Bardet-Biedl Syndrome 2 Protein

BDNF – Brain Derived Neurotrophic Factor

bp – base pair

cAMP – cyclic-Adenosine Monophosphate

Cdc42 – Cell division control protein 42

CK2 – Casein Kinase 2 (also known as Protein Kinase CK2)

CRMP2 – Collapsin Response Mediator Protein 2

Cryo-EM – Cryo-Electron Microscopy

CYFIP1 – Cytoplasmic FMR1 Interacting Protein 1

DCC – Deleted in Colorectal Cancer

DDX3 – Deadbox Protein 3

DNA – Deoxyribonucleic Acid

eEF – eukaryotic Elongation Factor

eEF1 – eukaryotic Elongation Factor 1

eEF1 – eukaryotic Elongation Factor 1

eEF2 – eukaryotic Elongation Factor 2

eIF – eukaryotic Initiation Factor

eIF1 – eukaryotic Initiation Factor 1

eIF1A – eukaryotic Initiation Factor 1A

eIF2 – eukaryotic Initiation Factor 2

eIF2α – eukaryotic Initiation Factor 2 alpha subunit

eIF3 – eukaryotic Initiation Factor 3

eIF4A – eikaryotic Initiation Factor 4A

eIF4E – eukaryotic Initiation Factor 4E

eIF4F – eukaryotic Initiation Factor 4F

eIF4G – eukaryotic Initiation Factor 4G

eIF5 – eukaryotic Initiation Factor 5

EJC – Exon Junction Complex

EJC – Exon Junction Complex

Elav – Embryonic Lethal Abnormal Vision

eRF1 – eukaryotic release factor 1

eRF3 – eukaryotic translation termination factor 3

ERK – Extracellular signal-regulated Kinase

FMRP – Fragile-X Mental Retardation Protein

Fxr1 – FMR1 Autosomal Homolog 1

FXS – Fragile X Syndrome

FXS – Fragile X Syndrome

GDP – Guanosine Di-Phosphate

GLUA1 – glutamate ionotropic receptor AMPA type subunit 1

GSK3β – Glycogen synthase kinase 3 beta

GTP – Guanosine Tri-Phosphate

HITS-CLIP – High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation

hnRNPA2B1 – heterogenous nuclear Ribonucleoprotein A2B1

iPSC – induced Pluripotent Stem Cell

KD – Knockdown

KO – Knockout

LKB1 – Liver Kinase B1 (also known as Serine/Threonine kinase 11, STK11)

LTD – Long Term Depression

LTP – Long Term Potentiation

m6A – N’6 methyladenosine
MAPK – Mitogen-activated Protein Kinase
mGluR – metabotropic Glutamate Receptor
mGluR-LTD – metabotropic Glutamate Receptor dependent Long Term Depression
MMP9 – Matrix Metallopeptidase 9
MNK1/2 – (MAPK)-interacting kinases 1 and 2
mRNA – messenger RNA
mTOR – mammalian Target of Rapamycin Kinase (also known as mechanistic target of rapamycin)
mTORC1 – mechanistic target of rapamycin complex 1
mTORC2 – mechanistic target of rapamycin complex 2
Myc – MYC Proto-Oncogene
NGD – No Go Decay
NMD – Nonsense Mediated Decay
NMDA – N-methyl-D-aspartate
NMDAR – NMDA Receptor
NPC – Neural Precursor Cell (also known as Neural Progenitor Cell)
Nrp1 – Neuropilin-1 precursor
nt – nucleotide
NT3 – Neurotrophin-3
PABP – Poly-A Binding Protein
PI3K – Phosphatidlyinositol-3-kinase
PIP3 – Phosphatidylinositol-3,4,5-triphosphate
PKA – Protein Kinase A
PKC – Protein Kinase C
Prox1 – Prospero Homeobox 1
PTC – Premature Termination Codon
PTEN – Phosphate and Tensin Homolog phosphatase
PurA – Pur-alpha
Rab1B – Ras-related protein 1B
Rac – Ras-related C3 botulinum toxin substrate GTPase
Raptor – Regulatory-associated protein of mTORC1
RBP – RNA Binding Protein

RGC – Radial Glial Cell

RhoA – Ras homolog family member A GTPase

Rictor – Rapamycin-insensitive companion of mTORC2

RNA – Ribonucleic Acid

S6K – Ribosomal Protein S6 Kinase

SFARI – Simons Foundation Autism Research Initiative

SGK – Serine/Threonine-Protein Kinase

SMD – Staufen Mediated Decay

Stau – Staufen

TDP43 – TAR DNA binding protein 43

TIA1 – T-Cell-Restricted Intracellular Antigen-1

Trk – Tropomyosin receptor kinase

tRNA – transporter RNA

TSC1/2 – Tuberous Sclerosis Proteins 1 and 2 (also known as Hamartin and Tuberin)

TTX – Tetrodotoxin

ULK1 – Unc-51 Like Autophagy Activating Kinase 1

UPF1 – Up frame shift 1 (also known as RENT1)

VZ - ventricular zone

ZBP1 – Zipcode Binding Protein 1

τ1 – Tau 1 (also known as Microtubule associated protein Tau1)

1 CHAPTER ONE: Introduction

1.1 Preface

Nucleotides are the building blocks of life and the key to organisms' diversity on earth. A single nucleotide difference can set us apart from each other, and even break our existence. On its own, a nucleotide is nothing but mere chemical bonds of carbon and hydrogen but, when a string of 3 nucleotides come together, to make a codon, all of a sudden it means something. Just like this, 3 letters at a time, the manual for life can be translated into proteins that make up a living, breathing work of art that can walk, talk, and write its own horror blockbusters. In this chapter we will briefly overview how mRNAs are translated into proteins one codon at a time, and how this process is regulated. We will then explore the role of translational control in different stages of neurodevelopment and discuss how dysregulated translation can lead to neurodevelopmental disorders. This chapter ends with a concise exposé on initiation-dependent forms of translation and neurodevelopmental disease, setting the stage for the next chapter where we will dive deeper into initiation-independent forms of translation.

1.2 How translation works

It all begins when DNA is copied into a messenger RNA. However, transcription is just the first step in unraveling the genetic code. Translation is the process by which messenger RNA is read and transformed into a chain of amino acids, which can be folded in a particular manner to produce a protein that has a specific function inside the cell. Essentially, translation is a way for the cell to produce and regulate its own machinery. Translation occurs in 3 main steps: initiation, elongation and termination and is carried out by a complicated RNA/protein machine called the ribosome in concert with a number of important protein factors, which are often named eukaryotic initiation factors (eIFs) and eukaryotic elongation factors (eEFs).

During initiation, the ribosome is assembled at the initiation codon (usually methionine) and this step is usually the rate-limiting step of translation(Aitken & Lorsch, 2012; Jackson, Hellen, & Pestova, 2010). Initiation begins with the formation of the ternary complex, which is comprised of a methionine-charged initiator tRNA and eIF2-GTP. The ternary complex then associates with the 40S ribosomal subunit (small subunit, S refers to the "Svedberg" unit based on speed of sedimentation in a centrifuge) through association with eIF1, eIF1A, eIF3 and eIF5, forming what is called the "preinitiation complex", also referred to as the 43S(Aitken & Lorsch, 2012; Jackson et al., 2010). This preinitiation complex is then recruited to the 5' cap (7-methylguanosine) structure of an mRNA through interactions with the eIF4F complex consisting of eIF4E (the protein that recognizes the 5'cap), eIF4G (a scaffold that links eIF4E and proteins in the preinitiation complex to associate the mRNA to the 43S complex), and the RNA helicase eIF4A. eIF4G also binds to the poly-A binding protein (PABP) which is bound to the polyadenylated 3' end of the mRNA (Jackson et al., 2010). The looping of the mRNA brings the PABP at the 3' end of the mRNA to come into close proximity with the 5' cap structure, which promotes translation and re-

initiation when translation is ended (Jackson et al., 2010; Vicens, Kieft, & Rissland, 2018). The recruited preinitiation complex then scans the mRNA in a 5' to 3' direction to find the AUG start codon; the portion of the mRNA that remains 5' of the initiation codon is thus referred to as the 5' untranslated region (5'UTR) (Aitken & Lorsch, 2012; Jackson et al., 2010). Upon recognition of the start codon, the eIF2-GTP and eIF5-GTP are hydrolyzed facilitating the dissociation of other initiation factors, allowing 60S subunit (large subunit) joining (Aitken & Lorsch, 2012; Jackson et al., 2010). The formation of this 80S ribosomal complex with a P-site peptidyl tRNA marks the end of translation initiation.

The 80S ribosomal complex can now read the mRNA, 3 nucleotides at a time, and assemble a chain of amino acids based on the codons it reads through a process called elongation. To better understand how elongation occurs, it is important to note that the 80S ribosome has 3 inner channels where incoming tRNAs will pass through: the Aminoacyl tRNA site (A-site) where tRNAs charged with amino acids can enter the ribosome, the Peptidyl tRNA site (P-site) which is also where the polypeptide exit channel is located, and the Exit site (E-site) where the empty deacylated tRNA can exit the ribosome from (Lareau, Hite, Hogan, & Brown, 2014). The first round of elongation begins when eEF1 brings in an aminoacyl tRNA to the A-site. The anti-codon of the tRNA carrying the amino acid must match the 3 nt codon on the mRNA for the amino acid to the incorporated into the peptide chain, and the availability of aminoacyl tRNAs can help or hinder the rate of translation elongation (Brackley, Romano, & Thiel, 2011). If the tRNA anti-codon matches the mRNA codon, then the ribosome enzymatically catalyzes the joining of the two amino acids via an internalized peptidyl transferase (the amino acid attached to the P-site tRNA, and the second amino acid that is attached to the new A-site tRNA in the A-channel) through GTP hydrolysis and de-acylation of the P-site tRNA so it is no longer attached to the growing peptide chain with an ester bond (Cooper,

2000). This GTP powered peptide joining executed by the ribosome is not well understood, however hypotheses attributing this phenomenon to quantum mechanics and energy carry-over from an elongation factor (GTPase) like eEF1 have emerged (Gindulyte et al., 2006; Świderek, Marti, Tuñón, Moliner, & Bertran, 2015; Yonath, 2017). Peptide joining results in the shifting of the ribosomal confirmation into what is known as the Hybrid state, in which the inner two tRNAs are tilted and are slightly shifted into A/P and P/E positions (Agirrezabala et al., 2008)**.** The peptide chain is now attached to the A/P site tRNA, that will soon be shifted fully into the P-channel. eEF2 is the elongation factor that facilitates this shift by binding to the hybrid ribosome, hydrolyzing its bound GTP and causing the ribosome to ratchet and translocate on the mRNA in a 5' to 3' direction (Dever & Green, 2012; Fritz & Boris-Lawrie, 2015). This ratcheting motion not only moves the ribosome on the mRNA by 3 nucleotides, but also ejects the E-site tRNA and moves the A/P tRNA to the P-site so that a new A-site aminoacyl tRNA can come in and the steps will repeat again. The ejected tRNAs no longer associated with an amino acid are then re-charged by an aminoacyl-tRNA Synthethase (aaRS) through a process called aminoacylation and are recycled (McClain, 1993).

The ribosome continues elongating on the mRNA in a 5' to 3' direction until it reaches a Stop Codon (UAG, UAA, UGA) where termination occurs. The stop codon is not recognized by any tRNA, but instead recruits a complex of termination factors eRF1 and eRF3-GTP (Fritz & Boris-Lawrie, 2015) . The close proximity of the GGQ motif of the eRF1 to the peptidyl transferase center of the ribosome results in the hydrolysis of the eRF3-GTP, which facilitates a conformational change in the large ribosomal subunit and the release of the polypeptide chain from the ribosomal complex (Fritz & Boris-Lawrie, 2015). After this, ABCE1 mediated ATP hydrolysis causes the dissociation of the ribosomal complex and the completion of mRNA translation (Dever & Green, 2012; Fritz & Boris-Lawrie, 2015). At termination, an important quality control

mechanism exists for removal of mRNAs with premature stop sites (either due to genetic mutations, incorrect splicing, or mRNA cleavage). This is important as truncated proteins often have dominant negative effects and are deleterious for cell health. This quality control program, called nonsense-mediated decay (NMD), is organized by a protein, UPF1, which is recruited to the stop site through interactions with the release factors and, in the case of a premature termination codon, interacts with proteins on the Exon Junction Complex (EJC) downstream of the elongating ribosome, leading to the disassembly of the ribosomal complex and destruction of the polypeptide (Brogna & Wen, 2009; Ghosh, Ganesan, Amrani, & Jacobson, 2010; Ivanov, Gehring, Kunz, Hentze, & Kulozik, 2008)**.** An additional quality control mechanism called No-Go decay resolves stalled ribosomes through recognition of ribosome collisions and will be important in our discussion of stalled polysomes in chapter 3 and 4 (Harigaya & Parker, 2010; Simms, Yan, & Zaher, 2017).

1.3 How translation is regulated

Apart from quality control mechanisms that safeguard translation, there is a plethora of control mechanism that regulate translation itself. The regulation of translation is essential to ensure that the correct proteins are produced at the right place and at the right time and in the correct quantity. There are both mRNA specific mechanisms of translational regulation, largely mediated by specific RNA binding proteins (RBPs) binding to specific sites in an mRNA, and more general mechanisms (although these are still affected by the composition of the mRNA). Below we review the most important general mechanisms of translational regulation: the availability of eIF4E to bind to the cap, and the phosphorylation of the key translation factors eIF2α and eEF2.

A major way to regulate translation initiation is through eIF4E binding to proteins that prevent eIF4E from binding to eIF4G (Richter & Sonenberg, 2005). This inhibits translation

initiation by blocking the eIF4E bound mRNA from finding the 40S ribosome. eIF4E is also phosphorylated, and while this phosphorylation has important physiological effects, how it regulates translation is not well understood and will not be discussed further in this section (I. S. Amorim et al., 2018; Shveygert, Kaiser, Bradrick, & Gromeier, 2010). While there are many proteins that can bind eIF4E in the cell, often linked to specific RNA binding proteins to regulate specific mRNAs, the general proteins involved in this process are known as the eIF4E binding proteins (4EBPs) (Richter & Sonenberg, 2005). 4EBPs themselves are regulated by phosphorylation by the mechanistic target of rapamycin complex 1 (mTORC1), where phosphorylation prevents 4EBPs from binding 4E and thus mTORC1 activity is linked to translational activation (Amorim, Lach, & Gkogkas, 2018).

mTORC1 is identified by its component Raptor, and there is a separate complex, mTORC2 identified by Rictor. Both mTORC1 and mTORC2 are essential for cell growth and survival, as they are responsible for distinct functions in the cell. In addition to 4EBP, mTORC1 controls cell growth through phosphorylation of S6 kinase and cellular autophagy via ULK1 (Inês S. Amorim et al., 2018; Foster & Fingar, 2010; Limon & Fruman, 2012). mTORC2 major targets are downstream kinases Akt, PKC, SGK and Rho family GTPases Cdc42, Rac and RhoA and these also regulate cell survival and cytoskeletal dynamics in response to growth factors (Foster & Fingar, 2010; Urbanska, Gozdz, Swiech, & Jaworski, 2012). The activation of these two mTOR complexes is highly regulated by the availability of energy and the presence of appropriate growth factors (Bond, 2016). While the two pathways have different tasks, there is significant cross talk allowing one cascade to modulate the activity of the other. For example, the activation of Akt downstream of mTORC2 strongly regulates activation of mTORC1. Alternatively, S6K substrates regulate activation of mTORC2 (Bond, 2016).

Another major regulator of translation initiation is the phosphorylation of eIF2α, which has a dominant negative effect on translation initiation. eIF2-GTP is part of the ternary complex together with a methionine charged tRNA, which following the recognition of the start codon is hydrolyzed into eIF2-GDP and dissociates from the ribosomal complex. eIF2-GDP is then recycled into eIF2-GTP by a multi-subunit GEF called eIF2B, allowing eIF2-GTP to associate with a new Methionine-charged tRNA (Jackson et al., 2010). The phosphorylated eIF2α subunit binds to and inhibits eIF2B, resulting in the inhibition of general protein synthesis (Bogorad, Lin, & Marintchev, 2017). eIF2α phosphorylation is the key step in the integrated stress response and distinct stresses activate one of four dedicated (only one target) eIF2α kinases involved in integrated stress response (Boye & Grallert, 2020; Clemens, 2001). While phosphorylation of eIF2α strongly inhibits overall translation by lowering levels of the ternary complex, certain mRNAs important for the cellular response to stress have ben programmed to be activated by eIF2α phosphorylation (Bellato & Hajj, 2016). These mRNAs have methionines in the mRNA that are before (upstream) of the methionine that initiates the protein encoded by this mRNA. Under normal conditions this 'upstream' methionine leads to translation of short open reading frames followed by termination, but when levels of ternary complex are low, these methionines are bypassed and the methionine that translates the stress related protein is used instead (Barbosa, Peixeiro, & Romão, 2013; Bellato & Hajj, 2016).

Similar to the phosphorylation of eIF2, the phosphorylation of eEF2 can also inhibit translation but in a different way, through blocking the binding of eEF2 to the ribosomal complex during translation elongation. This is not a dominant negative effect and phosphorylation acts to reduce the levels of active eEF2, slowing translation elongation. eEF2 is phosphorylated by a dedicated kinase eEF2K that is a regulatory hub as well, activated by stresses such as loss of ATP

and calcium influxes, but inhibited by mTOR (Proud, 2015). Similar to eIF2a, there appears to be a subset of mRNAs that are translated more efficiently after phosphorylation of eEF2, but unlike eIF2α where the mechanism for this is well characterized (bypassing upstream open reading frames) how these mRNAs are translationally activated by eEF2 phosphorylation is still a mystery (discussed in chapter 2) (Kenney et al., 2016; S. Park et al., 2008; Sossin & Costa-Mattioli, 2019).

1.4 Role of Translation control in Neurodevelopment:

While translation is important for many neuronal processes including synaptic plasticity and the response to aging, this thesis mainly is concerned with the role of translational control in neuronal development, and readers can refer to reviews for summaries of other important roles for translational regulation in neurons (Buffington, Huang, & Costa-Mattioli, 2014; Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009; Klann & Dever, 2004; Skariah & Todd, 2021; Sossin & Costa-Mattioli, 2019; Michael A. Sutton & Schuman, 2005). Neuronal development consists of many steps including neurogenesis, neuronal differentiation, axonal and dendritic growth, axon guidance, synapse formation and pruning, homeostasis and synaptic plasticity. The synthesis of proteins that are critical for these processes is tightly regulated and neurons have evolved a plethora of control mechanisms to ensure that proteins are made at the right place and at the right time. A growing body of evidence points to dysregulated translation of mRNAs involved in these key functions as an underlying cause for many genetic causes of neurodevelopmental disorders (Y. C. Chen, Chang, & Huang, 2019; C. G. Gkogkas et al., 2013; Gkogkas & Sonenberg, 2013; Kelleher & Bear, 2008; Emanuela Santini & Borgkvist; E. Santini et al., 2013; Yang, Smibert, Kaplan, & Miller, 2014; Zahr et al., 2018). Below we discuss each of these functions, their role in normal neurodevelopment and examine the mechanisms of translational control that are important for these processes and the evidence linking translational dysregulation to neurodevelopmental disorders.

1.4.1 Neurogenesis and Neuronal Differentiation

The organization of the brain and its regions, each of which will have specialized functions, occurs through the birth, differentiation and migration of progenitor cells called stem cells. Neuronal stem cells have the capability to differentiate into many different types of neurons/glia cells depending on the intrinsic and extrinsic cues that affect their fate. These cues often dictate the expression of proteins within the developing progenitor cells through a combination of transcriptional and translational control mechanisms. Thus, the presence of a wide variety of neuronal cells in the brain relies on post-transcriptional and translational control mechanisms such as sub-cellular mRNA localization, RNA decay, post-transcriptional modifications, and binding of RBPs that repress or activate translation.

In the developing cerebral cortex, neuroepithelial cells in the Ventricular Zone divide symmetrically to generate a pool of neural progenitor cells (NPCs), that either continue to replicate to make more NPCs or are transformed into Radial Glial Cells (RGCs) as they mature. The RGCs can either continue to proliferate or undergo an asymmetric division to leave an RGC and a neuronal daughter cell, or later in development, a glial cell. These decisions occur at least in part through the spatiotemporally regulated expression of mRNAs that allow them to remain pluripotent while also maintaining the capability to differentiate into non-proliferative cells. Indeed, it appears that many mRNAs required for neuronal/glial differentiation are already transcribed in the RGCs and progenitors, but their translation is repressed until differentiation is induced (Hoye & Silver, 2021)

Early on in neurodevelopment, soon after Neuroepithelial cells in the ventricular zone give rise to RGCs, which sprout neurites in two directions: one basal and one apical process. The apical process generates end feet that anchor the RGC body to the ventricular zone, while the basal process

extends to reach the basal lamina. The basal process acts as a scaffold for newly born excitatory neurons to climb as they migrate and reach different layers of the developing cortex. As neurodevelopment progresses and the neuronal layers in the cortical plate become thicker, the basal process undergoes significant morphological change to elongate. Both the polarization of the basal process and its elongation is facilitated by mRNA transport and local translation (Pilaz, Lennox, Rouanet, & Silver, 2016).

In many cases, cell fate during neurogenesis is controlled by localizing some mRNAs specifically to one cell after an asymmetric division. This has been particularly well studied in Drosophila, in which there are many examples of mRNAs that are asymmetrically expressed such as the mRNAs for oskar, nanos, bicoid, and gurken, which are necessary to determine cell polarization and body axis during oogenesis (Lasko, 2012). For neuronal differentiation, Drosophila NPCs regulate the localization of miranda, which is restricted to the apical side of neuronal progenitor cells and is important for determining the fate of dividing progenitors in entering neuronal differentiation (Hoye & Silver, 2021). In Drosophila, the localization of Miranda depends on Staufen (Broadus, Fuerstenberg, & Doe, 1998) as does the asymmetric segregation of prospero mRNA in developing neuroblasts, which facilitates their asymmetric division (Vessey et al., 2012). In vertebrates, as well, many neurogenic mRNAs depend on Staufen for correct localization in NPCs, such as the orthologue of prospero (Prox1) and Bardet-Biedl Syndrome 2 (Bbs2). Staufens, which bind to double stranded RNA in the 3'UTRs of these transcripts, are also spatiotemporally regulated, segregating into basal ganglion cells during progenitor cell division and thus promoting the differentiation of neuroblasts (Kusek et al., 2012). In fact, Staufen 2 has been shown to target 1500 mRNAs in the developing mouse cortex (Kusek et al., 2012). Additional RNA binding proteins such as PUMILIO and SMAUG have also been implicated in regulating the

segregation of mRNAs encoding proteins important for RGC cell fate (Amadei et al., 2015; Goldstrohm, Hall, & McKenney, 2018)

Thus, the asymmetric division of RGCs allows the asymmetric inheritance of mRNAs and proteins that favor one cell fate over another. The asymmetric localization of the mRNA is also tied to the translational repression of neurogenic factors until after cell division, where translation is activated to drive neuronal differentiation of the daughter cell (Hoye & Silver, 2021).

Another trans-acting factor that affects mRNA localization and expression during neurogenesis is the fragile X mental retardation protein (FMRP), whose loss causes the neurodevelopmental disorder, Fragile X syndrome. FMRP has been shown to be associated with hundreds of mRNAs that are expressed preferentially in the basal RGC in a granule located to the basal end feet of the RGCs (Hoye & Silver, 2021; Pilaz et al., 2016). Loss of FMRP perturbs neuronal differentiation in human IPSC-derived neurons (Boland et al., 2017; Sunamura, Iwashita, Enomoto, Kadoshima, & Isono, 2018).

Another important mechanism for repression of mRNAs during neurogenesis is through a protein called eIF4E-Transporter. This protein, similar to 4EBPs, binds eIF4E and prevents it from binding to eIF4G (Ferraiuolo et al., 2005). It is recruited to mRNA through its binding to the RBP, Pumilio and is important for repression of Pumilio bound mRNAs. These mRNAs are required for neuronal differentiation and removal of this repression is required for neuronal differentiation (Hoye & Silver, 2021; Yang et al., 2014).

While localized transport and translational control of neurogenic factors via RBP binding are efficient methods of regulating the spatiotemporal expression of mRNAs important for cell fate, neuronal differentiation is also tied to the regulation of ribosome biogenesis and composition, which play an important role in early stages of differentiation and may contribute to regulating

different mRNAs in different cell types. The rates of ribosome biogenesis are significantly higher in early neuroepithelial cells in the VZ, which is later inhibited by Myc signalling and coupled to the increased proliferation of neuronal progenitors (Chau et al., 2018; Hoye & Silver, 2021). The regulation of the production of ribosomal subunits and their availability may dictate the rates of translation in different cellular compartments at different developmental stages.

1.4.2 Axonal Polarization

Neurons are highly polarized cells, meaning they have a sub-cellular organization that allows them to process information efficiently. For this reason, neuronal cells grow processes that differentiate into dendrites that receive information, or an axon that relays information to the next cell in the network. Initially, growing neurons sprout premature neurites, one of which will be selected and polarized to generate a pioneering axon. The remaining neurites need to be suppressed in a way that permits only one neurite to become specialized as an axon to ensure that information is relayed in one direction. The selection and polarization of the pioneering axon relies heavily on cues from the environment that regulate the segregation of mRNAs and proteins in the developing neuron to promote local cytoskeletal changes (Mili & Macara, 2009). While some cues promote the growth and polarization of an axon, other cues may suppress this mechanism in other neurites not selected to become the axon but rather the dendrites (Arimura & Kaibuchi, 2007). Axon growth and polarization is a very complex process that requires the competition and delicate balance of positive and negative signals and crosstalk of the molecular cascades that govern the factors that modify the actin and microtubule network. In particular, a series of positive feedback loops ensure that the decision of one neurite to become an axon becomes an all or none decision (Yoshimura, Arimura, & Kaibuchi, 2006).

Axon polarization requires significant modification of the actin and microtubule cytoskeleton (Yoshimura et al., 2006). These modifications are facilitated by intracellular signal transduction cascades that are often activated by extracellular cues (Cioni, Koppers, & Holt, 2018). The leading edge of neurites express receptor proteins that can recognize positive and negative cues that are either secreted, membrane bound or expressed in the extracellular matrix (Jung, Yoon, & Holt, 2012). When a cell surface receptor recognizes its corresponding ligand in the extracellular environment, it activates an intracellular cascade which, in turn, activates downstream kinases or small G proteins such as rac, cdc-42 and rho that promote or inhibit the expression and activity of proteins that modify the cytoskeleton, promoting or inhibiting the decision of the neurite to become an axon (Barnes & Polleux, 2009). The convergence of all of these pathways governs axon polarization.

Secreted factors are soluble ligands that are released into the extracellular matrix that can directly bind cell surface receptors. Neurotrophins are some of the most powerful secreted factors that can promote the growth and polarization of a new axon. Brain Derived Neurotrophic Factor (BDNF) and Neurotrophin 3 are two examples that have been implicated in initiating axon polarization (Arikkath, 2020). The neurotrophins bind distinct Trk receptors and promote different but complementary pathways of axon polarization. BDNF binding to TrkB receptors on the surface activates a downstream cascade that activates PI3-kinase, which in turn promotes the production of PIP3, an activator of protein kinase AKT (Arikkath, 2020). One important role of AKT is to activate the TOR pathway to increase translation of important factors important for axonal determination. Inhibition of TORC1 reduces axon formation. Proteins translated downstream of local TORC1 activation critical for axon determination are Rap1B (That activates CDC-42 and downstream actin cytoskeletal changes) and CRMP2 and Tau that are involved in microtubule

polymerization. Microtubule modifications regulated partly through microtubule binding proteins such as these are critical for axon determination (Arikkath, 2020). AKT also acts in protein-synthesis independent pathways to promote axon determination through inhibition of GSK3beta, which removes the phosphorylation of sites on microtubule associated proteins (such as Tau1 or CRMP2) and leads to increased polymerization of tubulin, a critical factor in axon determination. BDNF can also promote axon polarization through the activation of PKA, which in turn phosphorylates LKB1, which activates downstream kinases that also increase microtubule polymerization (Arikkath, 2020). BDNF can also amplify its own signal through a cAMP second messenger cascade, leading to the recruitment of more TrkB receptors creating a positive feedback loop (Schelski & Bradke, 2017). Feedback loops are, in general, an important component of the axon determination pathway, which is programmed to be an all or nothing decision. Thus, translation of Rap1B leads to Rap1B activation of AKT leading to TOR activation and further translation of Rab1B (Schwamborn & Püschel, 2004). NT3, on the other hand, acts through TrkC receptors to both increase and inhibit axon determination. It's signalling leads to an influx of Calcium eventually leading to the activation of RhoA, which results in the collapse of the actin cytoskeleton, blocking the polarization of more than one axon (Takano, Xu, Funahashi, Namba, & Kaibuchi, 2015). Thus, the negative and positive pathways overlap to ensure that only one axon undergoes growth and polarization.

1.4.3 Axonal and Dendritic Outgrowth

Among the mRNAs localized to the growing axon are transcripts that encode ribosomal proteins (Cajigas et al., 2012; Shigeoka et al., 2019). As neurons grow out processes that differentiate into axon and dendrites, and become increasingly compartmentalized, it is crucial for neurons to be able to continue the synthesis of key proteins in regions far away from the cell body

and respond to guidance cues from the environment to find their way in the developing neural circuit. While ribosome levels are important for outgrowth, ribosomes cannot be assembled locally. They are made only in the nucleolus in a highly regulated process that requires hundreds of genes acting in a coordinated pathway (Kumar, 2021; Leary & Huang, 2001). However, transport of ribosomal mRNAs to the growing axon may lead to ribosomal heterogeneity through the regulated replacement of ribosomal subunits and this may assert an extra level of regulation in a cell-type specific or subcellular manner, to optimize the production of proteins crucial for neurite outgrowth, branching and sub-cellular organization or compartmentalization (Genuth & Barna, 2018).

For example, the local production of the S4 ribosomal protein has been shown to be necessary for axonal growth (Shigeoka et al., 2019). Moreover, guidance cues such as Netrin 1 stimulate the translation of S4 and other ribosomal and axonal mRNAs located in the axon growth cone (Koppers et al., 2019; Shigeoka et al., 2019). Interestingly, disrupting the local synthesis of S4 affects the local population of ribosomes and inhibits translation, which is required for axon growth and branching.

While local synthesis of ribosomal mRNAs in dendrites has not yet to be shown to be critical for dendritic growth, overall ribosomal levels controlled by synthesis of ribosomal proteins has been shown to be important (Slomnicki et al., 2016). Disrupting ribosomal protein synthesis also disrupts BDNF-mediated dendritic branching, eventually leading to dendritic degeneration (Slomnicki et al., 2016). It is important to note that ribosomal proteins also play key roles in the mTOR molecular signalling cascades and their basal response to BDNF, which should be differentiated from their requirement for protein-synthesis dependent dendritic growth and branching (Slomnicki et al 2016).

The mTOR cascades that regulate the growth and maintenance of the dendritic tree have distinct functions: mTORC1 has been implicated in the translation of mRNAs important for microtubule dynamics and mTOR-mediated dendritic growth. Whereas, mTORC2 has been implicated in regulating the activity of kinases such as Akt and Rho family GTPases Cdc42, Rac and RhoA which regulate actin dynamics. Both pathways have been shown to be valuable for dendritic growth as there is some cross-talk between the two cascades. mTORC2's effects on dendritic growth can be partially attributed to it's effect on mTORC1 through Akt mediated regulation of S6K phosphorylation and the inhibition of TSC1/2 upstream of Rheb (Urbanska et al., 2012). mTORC1 activation downstream of Rheb has been implicated in axonal growth (Gong et al., 2015). The combination of two parallel signalling cascades that can regulate each other's activity downstream of growth and/or guidance molecules, controls the basal and stimulus-dependent responses of growing axons and dendrites. In addition to this, the regulation of the translation of microtubule associated and ribosomal mRNAs that play a role in reorganizing the cytoskeleton creates an extra layer of complexity and control over neurite outgrowth in response to external cues.

1.4.4 Axon Guidance

Axon guidance is a crucial step in neurodevelopment in which growing axons respond to chemical and physical cues in their environment to find their way in the developing brain. Growing axons express a variety of receptor proteins that are tuned to different guidance cues, some of which attract and some of which repel them (A. B. Huber, Kolodkin, Ginty, & Cloutier, 2003). Similar to axon polarization, the recognition of a guidance cue by a membrane bound receptor activates a downstream cascade that affects actin and microtubule cytoskeleton

formation, allowing the growing cone to turn and change directions. Guidance cues also assert regulation over the local proteome through these signalling cascades (Koppers et al., 2019).

The region of high growth at the end of a growing axon is called the growth cone. The growth cone is organized into 3 main regions: 1) The C-Domain, which consists of microtubule bundles from the body of the axon that extend towards the leading edge of the growing axon, 2) The T-Zone where the microtubules end and the actin-myosin mesh provide structural support for the growing actin bundles, and 3) The P-domain where rapid polymerization and de-polymerization of actin lamellopodia and filopodia drives the leading edge of the growth cone to advance and turn (Lowery & Vactor, 2009; Suter & Forscher, 1998).The remodelling of these actin and microtubule networks in the axonal growth cone rely on the recognition of guidance ques by cell surface receptors. These cell surface receptors and the guidance cues that activate them have been studied extensively, and are classified into ligand-receptor pairs that either attract or repel the growing axons (O'Donnell, Chance, & Bashaw, 2009). What is notable about these ligand-receptor pairs is that some guidance cues can activate more than one receptor and the response of a growing axon depends on the combination of receptors that it expresses on its surface (Garbe & Bashaw, 2004; A. B. Huber et al., 2003). For this reason, regulation of guidance receptors is critical to correct axon guidance. While this is mainly regulated by the trafficking these receptors to and from the cell surface and the proteolysis of these receptors (O'Donnell et al., 2009), in one case, regulated local synthesis of a guidance receptor determines when an axon can respond to a cue (Brittis, Lu, & Flanagan, 2002)

There are many mRNAs that are localized to the growing axon and growth cone (Zivraj et al., 2010). Ligand-receptor pairs are often coupled to downstream translational machinery that can regulate the local translation of mRNAs important for the guidance of the growing axon (Koppers

et al., 2019). The interaction of some external guidance ques with membrane bound receptors results in rapid changes in the axon cytoskeleton that dependent on local protein synthesis (Campbell & Holt, 2001; Jung et al., 2012). Attractive guidance cues like Netrin-1 and BDNF activates the asymmetric translation of mRNAs important for actin cytoskeleton assembly such as B-actin mRNA (localized by the Zipcode binding protein ZBP1) on the P-domain of the growth cone that is adjacent to the source of the guidance cue, whereas repulsive ques such as SEMA3A and SLIT2 activates translation of mRNAs of proteins that facilitate the disassembly of actin such as the translation of RhoA and Cofilin, leading to cue-specific turning. Local translation of transported mRNAs downstream of Netrin 1 and Sema3A is sufficient to induce axon turning even when the cell somas have been severed from growing axons (Campbell & Holt, 2001; Jung et al., 2012). Interestingly, both of these attractive and repulsive cues converge onto the mTOR pathway and the direction of the turning response relies on which mRNAs are translated locally (Campbell & Holt, 2001; Shigeoka et al., 2019)

Global translation in developing neurons is regulated by mTOR in a translation-initiation dependent manner (Hay & Sonenberg, 2004; Sengupta, Peterson, & Sabatini, 2010). This pathway can also be activated locally to translate mRNAs in an asymmetrical way to induce growth cone turning, such as in B-actin translation in response to the chemoattractant cue Netrin 1 (Campbell & Holt, 2001; K.-M. Leung et al., 2006; Yao, Sasaki, Wen, Bassell, & Zheng, 2006). Netrin 1 binding to DCC activates the PI3K-AKT-mTOR pathway, resulting in phosphorylated S6K mediated translational activation of B-actin (Leung et al., 2018; K.-M. Leung et al., 2006; Welshhans & Bassell, 2011), as well as through the inhibition of 4E-BP1. Netrin binding to DCC can also result in the ubiquitin-mediated degradation of PTEN, further promoting PIP3 production, and the activation of ERK1/2 (MAPK) which removes the TSC1/2 inhibition on mTOR, resulting

in more activation of mTOR downstream effectors (Jung et al., 2012). Ephrin A, a chemorepellent guidance cue, can utilize the same pathway to suppress local translation to induce growth cone repulsion, by binding surface Eph B receptors and leading to the inhibition of ERK1/2 and the activation of the mTOR inhibitor TSC1/2, inhibiting local translation of actin-cytoskeleton promoting proteins (Nie et al., 2010). Hence chemo-attractive and chemorepellent cues can converge onto the same pathway to regulate the local proteome of the growth cone. Moreover, cues that activate the local translation of some mRNAs may also inhibit the global translation of other mRNAs (Byung C. Yoon et al., 2012).

The involvement of cis and trans acting factors that can regulate the transport, stability and translation of mRNAs adds to the complexity of spatiotemporal regulation of guidance cue-dependent local protein synthesis. For example, axon growth cone turning induced by local B-actin synthesis in response to BDNF requires the phosphorylation of the RNA binding protein Zbp1, which can not only bind mRNAs and facilitate their asymmetric translocation (hence the name Zipcode binding protein), but also repress their translation to prevent premature expression in the cytoplasm (Huttelmaier et al., 2005). FMRP is another RBP that regulates the translation of transported mRNAs in both axons and dendrites. FMRP acts downstream of guidance cues such as Semaphorin 3A, leading to protein synthesis-dependent growth cone collapse (Li, Bassell, & Sasaki, 2009). mRNAs, such as the one encoding microtubule binding protein Map1b, that are regulated by FMRP accumulate in the growing axon following Sema3A activation (Li et al., 2009). FMRP represses mRNAs when it is in a phosphorylated state, and it's dephosphorylation and re-phosphorylation may be regulated by the crosstalk between different signalling cascades. While FMRP was initially thought to be phosphorylated downstream of TORC1 by S6K, more recent evidence shows that FMRP is phosphorylated by CK2 , a constitutively active kinase, independent

of the mTORC1-S6K pathway (Bartley et al., 2016; Bartley, O'Keefe, & Bordey, 2014). Upon phosphorylation of FMRP by CK2, FMRP undergoes secondary phosphorylation on other residues that are regulated downstream of PP2A and mGluR1 activation (Bartley et al., 2016). FMRP is briefly/transiently dephosphorylated in an activity dependent way, which likely results in brief bursts of local translation of FMRP-repressed mRNAs (Bartley et al., 2016; Narayanan et al., 2007; Niere, Wilkerson, & Huber, 2012).

Another factor that contributes to the regulation of localized translational control downstream of guidance cues is the sequestering and release of the local population of ribosomes, which allows the tight coupling of the site of protein synthesis to the area near receptor activation (Tcherkezian, Brittis, Thomas, Roux, & Flanagan, 2010). Netrin 1 receptor DCC is able to capture and sequester ribosomes through binding to L5 and Netrin 1 binding to DCC can promote both translation initiation and the release of ribosome-mRNA complexes sequestered by DCC to allow the formation of local polysomes (Koenig, Martin, Titmus, & Sotelo-Silveira, 2000; Tcherkezian et al., 2010). Other guidance receptors such as Neuropilin 1 and Robo 2 also interact with ribosomes in an mRNA-dependent way and their activation by guidance cues triggers local translation (Koppers et al., 2019), whereas others such as EphB receptors are not coupled to ribosomes and do not require local protein synthesis for EphB-mediated growth cone collapse. Interestingly, each guidance receptor binds different RNA binding proteins (DCC binds hnRNPA2B1, Nrp1 binds Staufen1), and they each regulate a different subset of mRNAs (almost half of which that are regulated by Staufen1, hRNPA2B1, Elav1 and Fxr1), asserting a higher level of selectivity on guidance cue mediated translational control. Upon binding of a guidance to cue to its associated receptor, the interacting ribosomes are released resulting in fast and local translation of associated mRNAs (Koppers et al., 2019). The co-activation of more than one guidance receptor adds another

level of complexity to regulating the axon growth cone dynamics in response to the environment (Koppers et al., 2019). It is unclear whether these receptors can bind and sequester translationally active ribosomes already associated with mRNAs to inhibit their translation. The regulation of ribosomal mRNAs may also play a role in generating ribosomal heterogeneity and may assert further spatiotemporal control on the selection of other local mRNAs to be translated (Kondrashov et al., 2011)

1.4.5 Synapse Formation, Pruning and Homeostasis

Once growing axons and dendrites find their way to their posts where they will become a part of the neural network, they need to make synaptic connections with surrounding neurons and become electrically coupled. This process is governed by transmembrane and transsynaptic cell adhesion molecules that can orchestrate synapse formation in a bidirectional way (Südhof, 2018). Cell adhesion molecules link the distinct pre-synaptic active zone to the post-synaptic scaffold, forming a synaptic junction that facilitates the chemical signal transduction of electrical signals from the pre-synaptic neuron to the post-synaptic neuron. Most importantly, the different cell adhesion molecules involved can determine whether the formed synapse will be inhibitory or excitatory. While the synthesis of pre- and post- synaptic components is important, local translation is not a requirement for synapse formation. Presynaptically, these steps appear to be downstream of fusion of vesicles containing active zone components (Garner, Waites, & Ziv, 2006)

In contrast, activation of adhesion proteins in the post-synaptic cell leads to signaling to attract proteins in the post-synaptic density. In particular activation of DCC, the netrin receptor, leads to accumulation of post-synaptic density proteins and this is blocked by protein synthesis inhibitors (Goldman et al., 2013; X. Zhou et al., 2020). While netrin-induced increases in synapse formation are protein-synthesis dependent, whether other induced synapse forming steps require

protein synthesis is less clear. Moreover, since synapse stabilization, pruning and synapse size are under control of protein synthesis, it may be difficult to differentiate a role of protein synthesis in the initial steps of synapse formation or in synapse stabilization. For example, when recreating synapses in cultures of Aplysia, the initial steps of synapse formation occur in a few hours and do not require protein synthesis (Coulson & Klein, 1997). However, if EPSPs are measured 12 hours later, synapse maintenance at this time point shows an absolute requirement for protein synthesis (Lyles, Zhao, & Martin, 2006).This presumably reflects a requirement for protein synthesis in synapse stabilization, not in synapse formation. Similar studies have not yet been done in vertebrate systems, but results are likely to be similar. Most spines formed using live imaging have life times of less than 24 hours suggesting the rate-limiting step for synapse formation may be synapse stabilization as opposed to synapse formation (Holtmaat & Svoboda, 2009).

1.4.6 Homeostatic Scaling

Perhaps one of the most impressive feats of neurons is their ability to adapt to synaptic changes and still maintain an internal status quo, an excitable state, a delicate balance of inhibition and excitation. Following network connecting synaptic events, the neuron is able to maintain that connection and adjust its level of excitability when there is not enough or too much activation, while also maintaining the ability to make new connections (Turrigiano & Nelson, 2000). All thanks to local homeostatic scaling linking activity to internal protein synthesis machinery. Both action potentials and miniature synaptic events are able to exert control over the scaling ability of synaptic sites by regulating local protein synthesis in a bidirectional way. (Megumi Mori, Penney, & Haghighi, 2021; M. A. Sutton, Taylor, Ito, Pham, & Schuman, 2007; M. A. Sutton, Wall, Aakalu, & Schuman, 2004). Responses to both LTP and LTD inducing stimuli are tightly regulated while

homeostatic plasticity is also maintained by modulating excitability. This modulation can be done either presynaptically or postsynaptically.

Spontaneous synaptic activity is coupled to local protein synthesis and plasticity. When miniature excitatory post synaptic potentials are blocked with TTX, leading to blockade of NMDA receptors, the protein synthesis program is kicked into high gear and there is an increase in synaptic strength through increased insertion of GLUA1 homotetramers at the synapse (M. A. Sutton et al., 2006; M. A. Sutton et al., 2004).This is a protein synthesis dependent phenomenon (M. A. Sutton et al., 2004). Activity causes this program to be inhibited, however if a pre-synapse goes silent, the post synapse would upregulate its surface receptors to increase its sensitivity to the signal. Consequently, spontaneous synaptic activity through NMDAR causes the downregulation of translation at these synapses. This effect contributes to the local homeostasis, the maintenance of status quo, at the dendritic synapse. (M. A. Sutton et al., 2006)

Activity dependent regulation of protein synthesis has been shown to be downstream of the translation elongation factor eEF2 (M. A. Sutton et al., 2007). Miniature postsynaptic transmission induces the phosphorylation of eEF2, which inhibits it and reduces global protein synthesis levels. Synaptic activation and firing of action potentials promotes the dephosphorylation of eEF2 and increases protein synthesis. Inhibiting the phosphorylation of eEF2 results in upregulation of translation (M. A. Sutton et al., 2007). We had previously demonstrated the bidirectional regulation of eEF2 phosphorylation in synaptic plasticity in *Aplysia californica* neurons (McCamphill, Farah, Anadolu, Hoque, & Sossin, 2015).

Of course, as postsynaptic changes occur, presynaptic changes are also occurring. Factors such as BDNF can act as retrograde signals to affects the presynaptic cell/compartment (Jakawich et al., 2010). For example, the activation of TOR is required for the upregulation of presynaptic

neurotransmitter release, whereas when postsynaptic TOR is inhibited, presynaptic homeostatic scaling is also blocked (Henry et al., 2018; Megumi Mori et al., 2021; Penney et al., 2012). Interestingly, inhibiting TOR also blocks BDNF secretion and retrograde presynaptic scaling (Henry et al., 2012).

1.5 Initiation Dependent Translation in Neurodevelopmental Diseases

Studies in the last decade have uncovered the importance of the excitation-inhibition balance in the brain for neurodevelopment, learning and memory, and how disrupting this balance can result in many neurological disorders including epilepsy, autism spectrum disorders, depression and even psychiatric disorders such as Schizophrenia. Moreover, these imbalances are often caused by mutations or manipulations that affect neuronal translational control during development (I. S. Amorim et al., 2018; Inês S. Amorim et al., 2018). In attempting to make the genotype/phenotype explanation, it is often unclear which of the many developmental steps regulated by translation discussed above is most critical (i.e. neurogenesis vs axon guidance vs homeostatic changes) for the phenotypic effect. It is also difficult to characterize because most studies on neuronal development are conducted in rodents and may have different rate-limiting steps than in human neuronal development, where diseases are found. Disruptive intervention at any level of the cellular cascade can result in similar yet different neurological pathologies, and their experimental perturbation can uncover distinct regulatory mechanisms governing different subsets of mRNAs. In the section below, we will focus on disorders linked to deficits in translation initiation. Disorders linked to translational elongation will be discussed in chapter 2.

One of the hubs for translational regulation in neurodevelopment is the step of initiation that, when disrupted, can lead to numerous neurodevelopmental, psychiatric, and

neurodegenerative disorders. One of the pathways that activates translation initiation is the PI3K/TOR pathway through phosphorylation of 4EBP controlling the availability of eIF4E (Christos G. Gkogkas et al., 2013). Mutations in humans that activate TORC1 (PTEN, FRMP, etc) lead to neurodevelopmental disorders in humans and these are phenocopied in mice (Zhou & Parada, 2012). Similarly, more specific manipulations that just regulate 4E availability (4E overexpression in rodents and humans, 4EBP KO in mice) also lead to neurodevelopmental disorders, which have been implicated in Autism spectrum disorders (Auerbach, Osterweil, & Bear, 2011; Christos G. Gkogkas et al., 2013; Jeste, Sahin, Bolton, Ploubidis, & Humphrey, 2008; Kwon et al., 2006; O'Roak et al., 2012; Zhou & Parada, 2012).

One of the targets of this pathways is eIF4E, the cap-binding translation initiation factor that is required for ribosome recruitment to the eIF4F complex, is an important translational regulator that has been implicated in ASD, Anixety and Depression (I. S. Amorim et al., 2018; Christos G. Gkogkas et al., 2013). eIF4E is phosphorylated downstream of the MAPK/ERK pathway (via MNK1/2 phosphorylation of 4E to encourage initiation (Bramham, Jensen, & Proud, 2016; Panja et al., 2014), as well as activated by the PI3K/TORC pathway (via phosphorylation by mTORC1 through dissociation of 4E-BP to free up 4E for initiation;(Richter & Sonenberg, 2005)) and is influenced by a plethora of growth factors and extracellular stimuli, including BDNF and glutamate, to increase translation initiation.

Notably, eIF4E is implicated in ASD such that it's overactivation downstream of mTOR leads to the production of many postsynaptic proteins, including cell adhesion molecules such as neuroligins, and significantly alters the excitatory-inhibitory balance at the synapse in favour of increased excitation, which can be mimicked by either the overexpression of 4E or knockdown of

4EBP (Christos G. Gkogkas et al., 2013; Rubenstein & Merzenich, 2003; E. Santini et al., 2013; Carson C. Thoreen et al., 2012)

4E has also been linked to the initiation of FMRP regulated mRNAs, through interaction with FMRP and CFIP1 (Napoli et al., 2008; Nowicki et al., 2007). One of the effects of increased protein synthesis in Fragile X is the increase in mTOR activation, although the effects could be indirect. The enhanced mTOR pathway signalling in Fragile X could be linked to the lack of FMRP to repress 4E-mediated initiation of FMRP-linked mRNAs (Hoeffer et al., 2012).

It is important to mention that perturbation of upstream messengers of the mTOR pathway, including PTEN and TSC1/2, leads to similar neurodevelopmental pathologies to 4E overactivation, highlighting the importance of mTOR in regulating the initiation of many mRNAs important for development, synaptic plasticity and establishing the excitatory-inhibitory balance in the brain.

1.6 Initiation Independent Translation

So far, we have reviewed the role of translational control in different steps of neurodevelopment, and how dysregulated translation initiation can lead to neurodevelopmental disorders. In the next chapter, we will focus on localized protein synthesis and the evidence supporting the role of initiation-independent forms of translation in neurodevelopment. It is essential that we first understand why initiation-independent forms of translation are necessary for fast and local synaptic plasticity to be able to appreciate the multi-layered regulatory power of RNA Granules, and the stalled polysomes contained within, in the transport, storage and translation of select mRNAs. To avoid redundancy in topics, I invite you to dive into the next chapter to learn more about initiation independent translation.

1.7 References

Agirrezabala, X., Lei, J., Brunelle, J. L., Ortiz-Meoz, R. F., Green, R., & Frank, J. (2008). Visualization of the Hybrid State of tRNA Binding Promoted by Spontaneous Ratcheting of the Ribosome. *Molecular Cell, 32*(2), 190-197. doi:10.1016/j.molcel.2008.10.001

Aitken, C. E., & Lorsch, J. R. (2012). A mechanistic overview of translation initiation in eukaryotes. *Nature Structural & Molecular Biology, 19*(6), 568-576. doi:10.1038/nsmb.2303

Amadei, G., Zander, M., Yang, G., Dumelie, J., Vessey, J., Lipshitz, H., . . . Miller, F. (2015). A Smaug2-Based Translational Repression Complex Determines the Balance between Precursor Maintenance versus Differentiation during Mammalian Neurogenesis. *Journal of Neuroscience, 35*, 15666-15681. doi:10.1523/JNEUROSCI.2172-15.2015

Amorim, I. S., Kedia, S., Kouloulia, S., Simbriger, K., Gantois, I., Jafarnejad, S. M., . . . Gkogkas, C. G. (2018). Loss of eIF4E Phosphorylation Engenders Depression-like Behaviors via Selective mRNA Translation. *J Neurosci, 38*(8), 2118-2133. doi:10.1523/JNEUROSCI.2673-17.2018

Amorim, I. S., Lach, G., & Gkogkas, C. G. (2018). The Role of the Eukaryotic Translation Initiation Factor 4E (eIF4E) in Neuropsychiatric Disorders. *Frontiers in Genetics, 9*(561). doi:10.3389/fgene.2018.00561

Arikkath, J. (2020). Mechanisms of axon polarization in pyramidal neurons. *Molecular and Cellular Neuroscience, 107*, 103522. doi:https://doi.org/10.1016/j.mcn.2020.103522

Arimura, N., & Kaibuchi, K. (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nature Reviews Neuroscience, 8*(3), 194-205. doi:10.1038/nrn2056

Auerbach, B. D., Osterweil, E. K., & Bear, M. F. (2011). Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature, 480*(7375), 63-68. doi:10.1038/nature10658

Barbosa, C., Peixeiro, I., & Romão, L. (2013). Gene expression regulation by upstream open reading frames and human disease. *PLoS Genet, 9*(8), e1003529. doi:10.1371/journal.pgen.1003529

Barnes, A. P., & Polleux, F. (2009). Establishment of axon-dendrite polarity in developing neurons. *Annual review of neuroscience, 32*, 347-381. doi:10.1146/annurev.neuro.31.060407.125536

Bartley, C. M., O'Keefe, R. A., Blice-Baum, A., Mihailescu, M. R., Gong, X., Miyares, L., . . . Bordey, A. (2016). Mammalian FMRP S499 Is Phosphorylated by CK2 and Promotes Secondary Phosphorylation of FMRP. *eNeuro, 3*(6). doi:10.1523/ENEURO.0092-16.2016

Bartley, C. M., O'Keefe, R. A., & Bordey, A. (2014). FMRP S499 is phosphorylated independent of mTORC1-S6K1 activity. *PLOS ONE, 9*(5), e96956. doi:10.1371/journal.pone.0096956

Bellato, H. M., & Hajj, G. N. (2016). Translational control by eIF2α in neurons: Beyond the stress response. *Cytoskeleton (Hoboken), 73*(10), 551-565. doi:10.1002/cm.21294

Bogorad, A. M., Lin, K. Y., & Marintchev, A. (2017). Novel mechanisms of eIF2B action and regulation by eIF2α phosphorylation. *Nucleic Acids Res, 45*(20), 11962-11979. doi:10.1093/nar/gkx845

Boland, M. J., Nazor, K. L., Tran, H. T., Szücs, A., Lynch, C. L., Paredes, R., . . . Loring, J. F. (2017). Molecular analyses of neurogenic defects in a human pluripotent stem cell model of fragile X syndrome. *Brain, 140*(3), 582-598. doi:10.1093/brain/aww357

Bond, P. (2016). Regulation of mTORC1 by growth factors, energy status, amino acids and mechanical stimuli at a glance. *Journal of the International Society of Sports Nutrition, 13*(1), 8. doi:10.1186/s12970-016-0118-y

Boye, E., & Grallert, B. (2020). eIF2α phosphorylation and the regulation of translation. *Current Genetics, 66*(2), 293-297. doi:10.1007/s00294-019-01026-1

Brackley, C. A., Romano, M. C., & Thiel, M. (2011). The Dynamics of Supply and Demand in mRNA Translation. *PLOS Computational Biology, 7*(10), e1002203. doi:10.1371/journal.pcbi.1002203

Bramham, C. R., Jensen, K. B., & Proud, C. G. (2016). Tuning Specific Translation in Cancer Metastasis and Synaptic Memory: Control at the MNK–eIF4E Axis. *Trends in Biochemical Sciences, 41*(10), 847-858. doi:10.1016/j.tibs.2016.07.008

Brittis, P. A., Lu, Q., & Flanagan, J. G. (2002). Axonal Protein Synthesis Provides a Mechanism for Localized Regulation at an Intermediate Target. *Cell, 110*(2), 223-235. doi:10.1016/S0092-8674(02)00813-9

Broadus, J., Fuerstenberg, S., & Doe, C. Q. (1998). Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate. *Nature, 391*(6669), 792-795. doi:10.1038/35861

Brogna, S., & Wen, J. (2009). Nonsense-mediated mRNA decay (NMD) mechanisms. *Nature Structural & Molecular Biology, 16*(2), 107-113. doi:10.1038/nsmb.1550

Buffington, S. A., Huang, W., & Costa-Mattioli, M. (2014). Translational control in synaptic plasticity and cognitive dysfunction. *Annu Rev Neurosci, 37*, 17-38. doi:10.1146/annurev-neuro-071013-014100

Cajigas, I. J., Tushev, G., Will, T. J., tom Dieck, S., Fuerst, N., & Schuman, E. M. (2012). The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron, 74*(3), 453-466. doi:10.1016/j.neuron.2012.02.036

Campbell, D. S., & Holt, C. E. (2001). Chemotropic Responses of Retinal Growth Cones Mediated by Rapid Local Protein Synthesis and Degradation. *Neuron, 32*(6), 1013-1026. doi:10.1016/S0896-6273(01)00551-7

Chau, K. F., Shannon, M. L., Fame, R. M., Fonseca, E., Mullan, H., Johnson, M. B., . . . Lehtinen, M. K. (2018). Downregulation of ribosome biogenesis during early forebrain development. *Elife, 7*, e36998. doi:10.7554/eLife.36998

Chen, Y. C., Chang, Y. W., & Huang, Y. S. (2019). Dysregulated Translation in Neurodevelopmental Disorders: An Overview of Autism-Risk Genes Involved in Translation. *Dev Neurobiol, 79*(1), 60-74. doi:10.1002/dneu.22653

Cioni, J. M., Koppers, M., & Holt, C. E. (2018). Molecular control of local translation in axon development and maintenance. *Curr Opin Neurobiol, 51*, 86-94. doi:10.1016/j.conb.2018.02.025

Clemens, M. J. (2001). Initiation factor eIF2 alpha phosphorylation in stress responses and apoptosis. *Prog Mol Subcell Biol, 27*, 57-89. doi:10.1007/978-3-662-09889-9_3

Cooper, G. M. (2000). *The cell : a molecular approach* (2nd ed. ed.). Washington, D.C.

Sunderland, Mass.: ASM Press ;

Sinauer Associates.

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational Control of Long-Lasting Synaptic Plasticity and Memory. *Neuron, 61*(1), 10-26. doi:10.1016/j.neuron.2008.10.055

Coulson, R. L., & Klein, M. (1997). Rapid Development of Synaptic Connections and Plasticity Between Sensory Neurons and Motor Neurons of Aplysia in Cell Culture: Implications for Learning and Regulation of Synaptic Strength. *Journal of Neurophysiology, 77*(5), 2316-2327. doi:10.1152/jn.1997.77.5.2316

Dever, T. E., & Green, R. (2012). The Elongation, Termination, and Recycling Phases of Translation in Eukaryotes. *Cold Spring Harbor Perspectives in Biology, 4*(7). doi:10.1101/cshperspect.a013706

Ferraiuolo, M. A., Basak, S., Dostie, J., Murray, E. L., Schoenberg, D. R., & Sonenberg, N. (2005). A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay. *J Cell Biol, 170*(6), 913-924. doi:10.1083/jcb.200504039

Foster, K. G., & Fingar, D. C. (2010). Mammalian Target of Rapamycin (mTOR): Conducting the Cellular Signaling Symphony*. *Journal of Biological Chemistry, 285*(19), 14071-14077. doi:https://doi.org/10.1074/jbc.R109.094003

Fritz, S., & Boris-Lawrie, K. (2015). The RNPs of eukaryotic translation control. *Trends in Cell and Molecular Biology, 10*, 105-130.

Garbe, D. S., & Bashaw, G. J. (2004). Axon guidance at the midline: from mutants to mechanisms. *Crit Rev Biochem Mol Biol, 39*(5-6), 319-341. doi:10.1080/10409230490906797

Garner, C. C., Waites, C. L., & Ziv, N. E. (2006). Synapse development: still looking for the forest, still lost in the trees. *Cell Tissue Res, 326*(2), 249-262. doi:10.1007/s00441-006-0278-1

Genuth, N. R., & Barna, M. (2018). Heterogeneity and specialized functions of translation machinery: from genes to organisms. *Nat Rev Genet, 19*(7), 431-452. doi:10.1038/s41576-018-0008-z

Ghosh, S., Ganesan, R., Amrani, N., & Jacobson, A. (2010). Translational competence of ribosomes released from a premature termination codon is modulated by NMD factors. *Rna, 16*(9), 1832-1847. doi:10.1261/rna.1987710

Gindulyte, A., Bashan, A., Agmon, I., Massa, L., Yonath, A., & Karle, J. (2006). The Transition State for Formation of the Peptide Bond in the Ribosome. *Proc Natl Acad Sci U S A, 103*(36), 13327-13332.

Gkogkas, C. G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D. B., . . . Sonenberg, N. (2013). Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature, 493*(7432), 371-377. doi:10.1038/nature11628

Gkogkas, C. G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D. B., . . . Sonenberg, N. (2013). Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature, 493*(7432), 371-377. doi:10.1038/nature11628

Gkogkas, C. G., & Sonenberg, N. (2013). Translational control and autism-like behaviors. *Cell Logist, 3*(1), e24551. doi:10.4161/cl.24551

Goldman, J. S., Ashour, M. A., Magdesian, M. H., Tritsch, N. X., Harris, S. N., Christofi, N., . . . Kennedy, T. E. (2013). Netrin-1 promotes excitatory synaptogenesis between cortical neurons by initiating synapse assembly. *J Neurosci, 33*(44), 17278-17289. doi:10.1523/JNEUROSCI.1085-13.2013

Goldstrohm, A. C., Hall, T. M. T., & McKenney, K. M. (2018). Post-transcriptional Regulatory Functions of Mammalian Pumilio Proteins. *Trends Genet, 34*(12), 972-990. doi:10.1016/j.tig.2018.09.006

Gong, X., Zhang, L., Huang, T., Lin, T. V., Miyares, L., Wen, J., . . . Bordey, A. (2015). Activating the translational repressor 4E-BP or reducing S6K-GSK3β activity prevents accelerated axon growth induced by hyperactive mTOR in vivo. *Hum Mol Genet, 24*(20), 5746-5758. doi:10.1093/hmg/ddv295

Harigaya, Y., & Parker, R. (2010). No-go decay: a quality control mechanism for RNA in translation. *Wiley Interdiscip Rev RNA, 1*(1), 132-141. doi:10.1002/wrna.17

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes Dev, 18*(16), 1926-1945. doi:10.1101/gad.1212704

Henry, F. E., McCartney, A. J., Neely, R., Perez, A. S., Carruthers, C. J., Stuenkel, E. L., . . . Sutton, M. A. (2012). Retrograde changes in presynaptic function driven by dendritic mTORC1. *J Neurosci, 32*(48), 17128-17142. doi:10.1523/jneurosci.2149-12.2012

Henry, F. E., Wang, X., Serrano, D., Perez, A. S., Carruthers, C. J. L., Stuenkel, E. L., & Sutton, M. A. (2018). A Unique Homeostatic Signaling Pathway Links Synaptic Inactivity to Postsynaptic mTORC1. *J Neurosci, 38*(9), 2207-2225. doi:10.1523/jneurosci.1843-17.2017

Hoeffer, C. A., Sanchez, E., Hagerman, R. J., Mu, Y., Nguyen, D. V., Wong, H., . . . Tassone, F. (2012). Altered mTOR signaling and enhanced CYFIP2 expression levels in subjects with fragile X syndrome. *Genes Brain Behav, 11*(3), 332-341. doi:10.1111/j.1601-183X.2012.00768.x

Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience, 10*(9), 647-658. doi:10.1038/nrn2699

Hoye, M. L., & Silver, D. L. (2021). Decoding mixed messages in the developing cortex: translational regulation of neural progenitor fate. *Curr Opin Neurobiol, 66*, 93-102. doi:10.1016/j.conb.2020.10.001

Huber, A. B., Kolodkin, A. L., Ginty, D. D., & Cloutier, J. F. (2003). Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annu Rev Neurosci, 26*, 509-563. doi:10.1146/annurev.neuro.26.010302.081139

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., . . . Singer, R. H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature, 438*(7067), 512-515. doi:10.1038/nature04115

Ivanov, P. V., Gehring, N. H., Kunz, J. B., Hentze, M. W., & Kulozik, A. E. (2008). Interactions between UPF1, eRFs, PABP and the exon junction complex suggest an integrated model for mammalian NMD pathways. *EMBO J, 27*(5), 736-747. doi:10.1038/emboj.2008.17

Jackson, R. J., Hellen, C. U. T., & Pestova, T. V. (2010). The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology, 11*(2), 113-127. doi:10.1038/nrm2838

Jakawich, S. K., Nasser, H. B., Strong, M. J., McCartney, A. J., Perez, A. S., Rakesh, N., . . . Sutton, M. A. (2010). Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron, 68*(6), 1143-1158. doi:10.1016/j.neuron.2010.11.034

Jeste, S. S., Sahin, M., Bolton, P., Ploubidis, G. B., & Humphrey, A. (2008). Characterization of autism in young children with tuberous sclerosis complex. *J Child Neurol, 23*(5), 520-525. doi:10.1177/0883073807309788

Jung, H., Yoon, B. C., & Holt, C. E. (2012). Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. *Nature Reviews Neuroscience, 13*, 308. doi:10.1038/nrn3210

Kelleher, R. J., 3rd, & Bear, M. F. (2008). The autistic neuron: troubled translation? *Cell, 135*(3), 401-406. doi:10.1016/j.cell.2008.10.017

Kenney, J. W., Genheden, M., Moon, K. M., Wang, X., Foster, L. J., & Proud, C. G. (2016). Eukaryotic elongation factor 2 kinase regulates the synthesis of microtubule-related proteins in neurons. *J Neurochem, 136*(2), 276-284. doi:10.1111/jnc.13407

Klann, E., & Dever, T. E. (2004). Biochemical mechanisms for translational regulation in synaptic plasticity. *Nature Reviews Neuroscience, 5*(12), 931-942. doi:10.1038/nrn1557

Koenig, E., Martin, R., Titmus, M., & Sotelo-Silveira, J. R. (2000). Cryptic Peripheral Ribosomal Domains Distributed Intermittently along Mammalian Myelinated Axons. *The Journal of Neuroscience, 20*(22), 8390. doi:10.1523/JNEUROSCI.20-22-08390.2000

Kondrashov, N., Pusic, A., Stumpf, C. R., Shimizu, K., Hsieh, A. C., Ishijima, J., . . . Barna, M. (2011). Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell, 145*(3), 383-397. doi:10.1016/j.cell.2011.03.028

Koppers, M., Cagnetta, R., Shigeoka, T., Wunderlich, L. C., Vallejo-Ramirez, P., Qiaojin Lin, J., . . . Holt, C. E. (2019). Receptor-specific interactome as a hub for rapid cue-induced selective translation in axons. *Elife, 8*. doi:10.7554/eLife.48718

Kumar, V. (2021). Chapter 7 - Ribosomal biogenesis in eukaryotes. In V. Kumar (Ed.), *Emerging Concepts in Ribosome Structure, Biogenesis, and Function* (pp. 129-150): Academic Press.

Kusek, G., Campbell, M., Doyle, F., Tenenbaum, S. A., Kiebler, M., & Temple, S. (2012). Asymmetric segregation of the double-stranded RNA binding protein Staufen2 during mammalian neural stem cell divisions promotes lineage progression. *Cell Stem Cell, 11*(4), 505-516. doi:10.1016/j.stem.2012.06.006

Kwon, C. H., Luikart, B. W., Powell, C. M., Zhou, J., Matheny, S. A., Zhang, W., . . . Parada, L. F. (2006). Pten regulates neuronal arborization and social interaction in mice. *Neuron, 50*(3), 377-388. doi:10.1016/j.neuron.2006.03.023

Lareau, L. F., Hite, D. H., Hogan, G. J., & Brown, P. O. (2014). Distinct stages of the translation elongation cycle revealed by sequencing ribosome-protected mRNA fragments. *Elife, 3*, e01257. doi:10.7554/eLife.01257

Lasko, P. (2012). mRNA localization and translational control in Drosophila oogenesis. *Cold Spring Harbor Perspectives in Biology, 4*(10), a012294. doi:10.1101/cshperspect.a012294

Leary, D. J., & Huang, S. (2001). Regulation of ribosome biogenesis within the nucleolus. *FEBS Lett, 509*(2), 145-150. doi:10.1016/s0014-5793(01)03143-x

Leung, K.-M., Lu, B., Wong, H. H.-W., Lin, J. Q., Turner-Bridger, B., & Holt, C. E. (2018). Cue-Polarized Transport of β-actin mRNA Depends on 3′UTR and Microtubules in Live Growth Cones. *Frontiers in Cellular Neuroscience, 12*(300). doi:10.3389/fncel.2018.00300

Leung, K.-M., van Horck, F. P. G., Lin, A. C., Allison, R., Standart, N., & Holt, C. E. (2006). Asymmetrical β-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nature Neuroscience, 9*(10), 1247-1256. doi:10.1038/nn1775

Li, C., Bassell, G., & Sasaki, Y. (2009). Fragile X mental retardation protein is involved in protein synthesis-dependent collapse of growth cones induced by Semaphorin-3A. *Frontiers in Neural Circuits, 3*(11). doi:10.3389/neuro.04.011.2009

Limon, J., & Fruman, D. (2012). Akt and mTOR in B Cell Activation and Differentiation. *Frontiers in immunology, 3*, 228. doi:10.3389/fimmu.2012.00228

Lowery, L. A., & Vactor, D. V. (2009). The trip of the tip: understanding the growth cone machinery. *Nature Reviews Molecular Cell Biology, 10*(5), 332-343. doi:10.1038/nrm2679

Lyles, V., Zhao, Y., & Martin, K. C. (2006). Synapse Formation and mRNA Localization in Cultured Aplysia Neurons. *Neuron, 49*(3), 349-356. doi:https://doi.org/10.1016/j.neuron.2005.12.029

McCamphill, P. K., Farah, C. A., Anadolu, M. N., Hoque, S., & Sossin, W. S. (2015). Bidirectional regulation of eEF2 phosphorylation controls synaptic plasticity by decoding neuronal activity patterns. *J Neurosci, 35*(10), 4403-4417. doi:10.1523/JNEUROSCI.2376-14.2015

McClain, W. H. (1993). Rules that Govern tRNA Identity in Protein Synthesis. *Journal of Molecular Biology, 234*(2), 257-280. doi:https://doi.org/10.1006/jmbi.1993.1582

Mili, S., & Macara, I. G. (2009). RNA localization and polarity: from A(PC) to Z(BP). *Trends in cell biology, 19*(4), 156-164. doi:10.1016/j.tcb.2009.02.001

Mori, M., Penney, J., & Haghighi, P. (2021). Regulation of synaptic homeostasis by translational mechanisms. *The Oxford Handbook of Neuronal Protein Synthesis*, 297.

Napoli, I., Mercaldo, V., Boyl, P. P., Eleuteri, B., Zalfa, F., De Rubeis, S., . . . Bagni, C. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell, 134*(6), 1042-1054. doi:10.1016/j.cell.2008.07.031

Narayanan, U., Nalavadi, V., Nakamoto, M., Pallas, D. C., Ceman, S., Bassell, G. J., & Warren, S. T. (2007). FMRP phosphorylation reveals an immediate-early signaling pathway triggered by group I mGluR and mediated by PP2A. *J Neurosci, 27*(52), 14349-14357. doi:10.1523/jneurosci.2969-07.2007

Nie, D., Di Nardo, A., Han, J. M., Baharanyi, H., Kramvis, I., Huynh, T., . . . Sahin, M. (2010). Tsc2-Rheb signaling regulates EphA-mediated axon guidance. *Nature Neuroscience, 13*(2), 163-172. doi:10.1038/nn.2477

Niere, F., Wilkerson, J. R., & Huber, K. M. (2012). Evidence for a fragile X mental retardation protein-mediated translational switch in metabotropic glutamate receptor-triggered Arc translation and long-term depression. *J Neurosci, 32*(17), 5924-5936. doi:10.1523/JNEUROSCI.4650-11.2012

Nowicki, S. T., Tassone, F., Ono, M. Y., Ferranti, J., Croquette, M. F., Goodlin-Jones, B., & Hagerman, R. J. (2007). The Prader-Willi phenotype of fragile X syndrome. *Journal of Developmental & Behavioral Pediatrics, 28*(2), 133-138.

O'Donnell, M., Chance, R. K., & Bashaw, G. J. (2009). Axon growth and guidance: receptor regulation and signal transduction. *Annual review of neuroscience, 32*, 383-412. doi:10.1146/annurev.neuro.051508.135614

O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P., . . . Eichler, E. E. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature, 485*(7397), 246-250. doi:10.1038/nature10989

Panja, D., Kenney, Justin W., D'Andrea, L., Zalfa, F., Vedeler, A., Wibrand, K., . . . Bramham, Clive R. (2014). Two-Stage Translational Control of Dentate Gyrus LTP Consolidation Is Mediated by Sustained BDNF-TrkB Signaling to MNK. *Cell reports, 9*(4), 1430-1445. doi:10.1016/j.celrep.2014.10.016

Park, S., Park, J. M., Kim, S., Kim, J. A., Shepherd, J. D., Smith-Hicks, C. L., . . . Worley, P. F. (2008). Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron, 59*(1), 70-83. doi:10.1016/j.neuron.2008.05.023

Penney, J., Tsurudome, K., Liao, E. H., Elazzouzi, F., Livingstone, M., Gonzalez, M., . . . Haghighi, A. P. (2012). TOR is required for the retrograde regulation of synaptic homeostasis at the Drosophila neuromuscular junction. *Neuron, 74*(1), 166-178. doi:10.1016/j.neuron.2012.01.030

Pilaz, L.-J., Lennox, A. L., Rouanet, J. P., & Silver, D. L. (2016). Dynamic mRNA Transport and Local Translation in Radial Glial Progenitors of the Developing Brain. *Current Biology, 26*(24), 3383-3392. doi:https://doi.org/10.1016/j.cub.2016.10.040

Proud, C. G. (2015). Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans, 43*(3), 328-332. doi:10.1042/BST20140323

Richter, J. D., & Sonenberg, N. (2005). Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature, 433*(7025), 477-480. doi:10.1038/nature03205

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav, 2*(5), 255-267. doi:10.1034/j.1601-183x.2003.00037.x

Santini, E., & Borgkvist, A. (2021). Dysregulated Translation in Autism Spectrum Disorder. In *The Oxford Handbook of Neuronal Protein Synthesis*.

Santini, E., Huynh, T. N., MacAskill, A. F., Carter, A. G., Pierre, P., Ruggero, D., . . . Klann, E. (2013). Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature, 493*(7432), 411-415. doi:10.1038/nature11782

Schelski, M., & Bradke, F. (2017). Neuronal polarization: From spatiotemporal signaling to cytoskeletal dynamics. *Molecular and Cellular Neuroscience, 84*, 11-28. doi:https://doi.org/10.1016/j.mcn.2017.03.008

Schwamborn, J. C., & Püschel, A. W. (2004). The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. *Nat Neurosci, 7*(9), 923-929. doi:10.1038/nn1295

Sengupta, S., Peterson, T. R., & Sabatini, D. M. (2010). Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress. *Molecular Cell, 40*(2), 310-322. doi:10.1016/j.molcel.2010.09.026

Shigeoka, T., Koppers, M., Wong, H. H., Lin, J. Q., Cagnetta, R., Dwivedy, A., . . . Holt, C. E. (2019). On-Site Ribosome Remodeling by Locally Synthesized Ribosomal Proteins in Axons. *Cell reports, 29*(11), 3605-3619 e3610. doi:10.1016/j.celrep.2019.11.025

Shveygert, M., Kaiser, C., Bradrick, S. S., & Gromeier, M. (2010). Regulation of eukaryotic initiation factor 4E (eIF4E) phosphorylation by mitogen-activated protein kinase occurs through modulation of Mnk1-eIF4G interaction. *Molecular and Cellular Biology, 30*(21), 5160-5167. doi:10.1128/MCB.00448-10

Simms, C. L., Yan, L. L., & Zaher, H. S. (2017). Ribosome Collision Is Critical for Quality Control during No-Go Decay. *Mol Cell, 68*(2), 361-373.e365. doi:10.1016/j.molcel.2017.08.019

Skariah, G., & Todd, P. K. (2021). Translational control in aging and neurodegeneration. *Wiley Interdiscip Rev RNA, 12*(4), e1628. doi:10.1002/wrna.1628

Slomnicki, L. P., Pietrzak, M., Vashishta, A., Jones, J., Lynch, N., Elliot, S., . . . Hetman, M. (2016). Requirement of Neuronal Ribosome Synthesis for Growth and Maintenance of the Dendritic Tree. *J Biol Chem, 291*(11), 5721-5739. doi:10.1074/jbc.M115.682161

Sossin, W. S., & Costa-Mattioli, M. (2019). Translational Control in the Brain in Health and Disease. *Cold Spring Harb Perspect Biol, 11*(8). doi:10.1101/cshperspect.a032912

Südhof, T. C. (2018). Towards an Understanding of Synapse Formation. *Neuron, 100*(2), 276-293. doi:10.1016/j.neuron.2018.09.040

Sunamura, N., Iwashita, S., Enomoto, K., Kadoshima, T., & Isono, F. (2018). Loss of the fragile X mental retardation protein causes aberrant differentiation in human neural progenitor cells. *Sci Rep, 8*(1), 11585. doi:10.1038/s41598-018-30025-4

Suter, D. M., & Forscher, P. (1998). An emerging link between cytoskeletal dynamics and cell adhesion molecules in growth cone guidance. *Curr Opin Neurobiol, 8*(1), 106-116. doi:https://doi.org/10.1016/S0959-4388(98)80014-7

Sutton, M. A., Ito, H. T., Cressy, P., Kempf, C., Woo, J. C., & Schuman, E. M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell, 125*(4), 785-799. doi:10.1016/j.cell.2006.03.040

Sutton, M. A., & Schuman, E. M. (2005). Local translational control in dendrites and its role in long-term synaptic plasticity. *Journal of Neurobiology, 64*(1), 116-131. doi:https://doi.org/10.1002/neu.20152

Sutton, M. A., Taylor, A. M., Ito, H. T., Pham, A., & Schuman, E. M. (2007). Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron, 55*(4), 648-661. doi:10.1016/j.neuron.2007.07.030

Sutton, M. A., Wall, N. R., Aakalu, G. N., & Schuman, E. M. (2004). Regulation of dendritic protein synthesis by miniature synaptic events. *Science, 304*(5679), 1979-1983. doi:10.1126/science.1096202

Świderek, K., Marti, S., Tuñón, I., Moliner, V., & Bertran, J. (2015). Peptide Bond Formation Mechanism Catalyzed by Ribosome. *Journal of the American Chemical Society, 137*(37), 12024-12034. doi:10.1021/jacs.5b05916

Takano, T., Xu, C., Funahashi, Y., Namba, T., & Kaibuchi, K. (2015). Neuronal polarization. *Development, 142*(12), 2088-2093. doi:10.1242/dev.114454

Tcherkezian, J., Brittis, P. A., Thomas, F., Roux, P. P., & Flanagan, J. G. (2010). Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell, 141*(4), 632-644. doi:10.1016/j.cell.2010.04.008

Thoreen, C. C., Chantranupong, L., Keys, H. R., Wang, T., Gray, N. S., & Sabatini, D. M. (2012). A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature, 485*(7396), 109-113. doi:10.1038/nature11083

Turrigiano, G. G., & Nelson, S. B. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol, 10*(3), 358-364. doi:10.1016/s0959-4388(00)00091-x

Urbanska, M., Gozdz, A., Swiech, L. J., & Jaworski, J. (2012). Mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2) control the dendritic arbor morphology of hippocampal neurons. *J Biol Chem, 287*(36), 30240-30256. doi:10.1074/jbc.M112.374405

Vessey, J. P., Amadei, G., Burns, S. E., Kiebler, M. A., Kaplan, D. R., & Miller, F. D. (2012). An asymmetrically localized Staufen2-dependent RNA complex regulates maintenance of mammalian neural stem cells. *Cell Stem Cell, 11*(4), 517-528. doi:10.1016/j.stem.2012.06.010

Vicens, Q., Kieft, J. S., & Rissland, O. S. (2018). Revisiting the Closed-Loop Model and the Nature of mRNA 5'-3' Communication. *Molecular Cell, 72*(5), 805-812. doi:10.1016/j.molcel.2018.10.047

Welshhans, K., & Bassell, G. J. (2011). Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J Neurosci, 31*(27), 9800-9813. doi:10.1523/JNEUROSCI.0166-11.2011

Yang, G., Smibert, C. A., Kaplan, D. R., & Miller, F. D. (2014). An eIF4E1/4E-T complex determines the genesis of neurons from precursors by translationally repressing a proneurogenic transcription program. *Neuron, 84*(4), 723-739. doi:10.1016/j.neuron.2014.10.022

Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J., & Zheng, J. Q. (2006). An essential role for β-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nature Neuroscience, 9*(10), 1265-1273. doi:10.1038/nn1773

Yonath, A. (2017). Quantum mechanic glimpse into peptide bond formation within the ribosome shed light on origin of life. *Structural Chemistry, 28*(5), 1285-1291. doi:10.1007/s11224-017-0980-5

Yoon, Byung C., Jung, H., Dwivedy, A., O'Hare, Catherine M., Zivraj, Krishna H., & Holt, Christine E. (2012). Local Translation of Extranuclear Lamin B Promotes Axon Maintenance. *Cell, 148*(4), 752-764. doi:10.1016/j.cell.2011.11.064

Yoshimura, T., Arimura, N., & Kaibuchi, K. (2006). Signaling Networks in Neuronal Polarization. *The Journal of Neuroscience, 26*(42), 10626-10630. doi:10.1523/jneurosci.3824-06.2006

Zahr, S. K., Yang, G., Kazan, H., Borrett, M. J., Yuzwa, S. A., Voronova, A., . . . Miller, F. D. (2018). A Translational Repression Complex in Developing Mammalian Neural Stem Cells that Regulates Neuronal Specification. *Neuron, 97*(3), 520-537.e526. doi:10.1016/j.neuron.2017.12.045

Zhou, J., & Parada, L. F. (2012). PTEN signaling in autism spectrum disorders. *Curr Opin Neurobiol, 22*(5), 873-879. doi:10.1016/j.conb.2012.05.004

Zhou, X., Gueydan, M., Jospin, M., Ji, T., Valfort, A., Pinan-Lucarré, B., & Bessereau, J.-L. (2020). The netrin receptor UNC-40/DCC assembles a postsynaptic scaffold and sets the synaptic content of GABAA receptors. *Nat Commun, 11*(1), 2674. doi:10.1038/s41467-020-16473-5

Zivraj, K. H., Tung, Y. C., Piper, M., Gumy, L., Fawcett, J. W., Yeo, G. S., & Holt, C. E. (2010). Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. *J Neurosci, 30*(46), 15464-15478. doi:10.1523/jneurosci.1800-10.2010

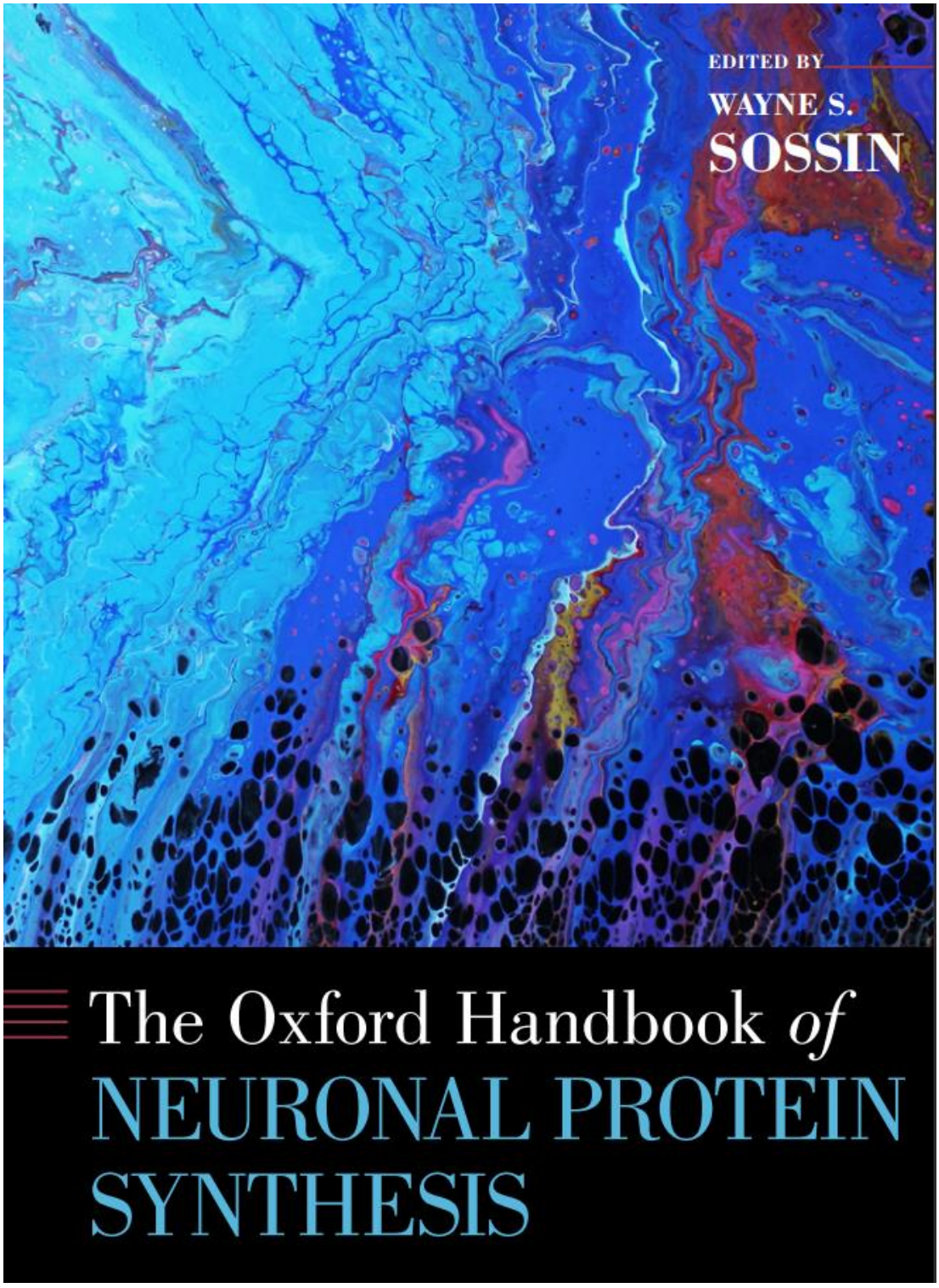
2 CHAPTER TWO: Focusing on mRNA Granules and Stalled Polysomes Amidst Diverse Mechanisms Underlying mRNA Transport, mRNA Storage, and Local Translation

2.1 Preface

This chapter contains the first of two scholarly works presented in this manuscript-based thesis. Published recently by Oxford University Press in the "Oxford Handbook of Neuronal Protein Synthesis", this chapter consists of a thorough review of the literature on mRNA transport, mRNA storage and local translation, and introduces advances in the field that expand our understanding of ribonucleoparticles. The purpose of this chapter is to unpack neuronal RNA Granules and discuss the complex ways in which neuronal mRNAs important for neurodevelopment are regulated. We also discuss the evidence supporting the role of Stalled Polysomes as a reversible cellular strategy utilized by neurons to regulate the transport and translation of neuronal mRNAs important for neurodevelopment and synaptic plasticity, independent of translation initiation. This chapter demonstrates my deep understanding of the field and contributes to original knowledge by connecting the dots between existing literature and emerging hypotheses and promotes the acceptance of polysome stalling as a neuron-specific translational control mechanism. The topics discussed in this chapter establish a foundation upon which the following chapter, and second scholarly work in this thesis, rests upon.

.

Cover Image: “Granules of life” by Mina N. Anadolu, acrylic on canvas. This painting is my romantic rendition of what I imagine the inside of a neuron would look like; the intricate and interwoven gradients of proteins and mRNA, the delicate balance of excitation and inhibition, EPSPs, and the phase separated granules that have become an inseparable part of my life.



Focusing on mRNA granules and stalled polysomes amidst diverse mechanisms underlying mRNA transport, mRNA storage and local translation.

Mina N. Anadolu[1] and Wayne S. Sossin[1]

[1]Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4

Published in Oxford Handbook of Neuronal Protein Synthesis
Oxford University Press, 2021

2.2 Abstract

In neurons, mRNAs are transported to distal sites to allow for localized protein synthesis. There are many diverse mechanisms underlying this transport. For example, an individual mRNA can be transported in an RNA transport particle that is tailored to the individual mRNA and its associated binding proteins. In contrast, some mRNAs are transported in liquid-liquid phase separated structures called neuronal RNA Granules that are made up of multiple Stalled Polysomes, allowing for rapid initiation-independent production of proteins required for synaptic plasticity. Moreover, neurons have additional types of liquid-liquid phase separated structures containing mRNA, such as Stress Granules and P bodies. We will discuss the relationships between all of these structures, what proteins distinguish them, and the possible roles they play in the complex control of mRNA translation at distal sites that allow neurons to use protein synthesis to refine their local proteome in many different ways.

2.3 Introduction

The control of local mRNA translation in distal dendrites and axons is an important feature of neuronal signaling. Local translation plays multiple roles in neuronal function including: (i) determining the proteome of distal sites (Glock, Heumüller, & Schuman, 2017; S. Miller et al., 2002), (ii) allowing local homeostasis in the face of local hyper- or hypo-excitability (M. Mori, Penney, & Haghighi, 2019; M. A. Sutton & Schuman, 2006) (iii) responding to guidance cues important for growth-cone dynamics during neuronal development (Cioni et al., 2018), and (iv) providing the plasticity-related proteins required for translation-dependent forms of synaptic plasticity (Costa-Mattioli et al., 2009; Sossin & Costa-Mattioli, 2019). These distinct roles for local translation are likely to be implemented by different mechanisms of translational control. Of

particular interest, since multiple forms of plasticity require local translation, it is likely that distinct control mechanisms exist for each distinct type of synaptic plasticity.

Local translation is a common feature in many cellular settings and general principles of local translation have been largely elucidated (Huang & Richter, 2004; Palacios & St Johnston, 2001; Wilhelm & Vale, 1993). These include: (i) a mechanism for repression of mRNA translation during transport, (ii) a mechanism for active transport of mRNAs to local sites using actin or microtubule tracks, and (iii) a mechanism for de-repression of the mRNA either when reaching the appropriate target or when appropriate signals are received. In particular, detailed mechanisms for all of these features have been elucidated for particularly well-studied examples, such as Ash1 in yeast daughter cells (Paquin & Chartrand, 2008), localized translation of morphology-determining factors during Drosophila development (Becalska & Gavis, 2009; Palacios & St Johnston, 2001) and the local translation of beta-actin mRNA in neurons (Eliscovich, Buxbaum, Katz, & Singer, 2013; Huttelmaier et al., 2005; Wong et al., 2017).

In the above examples, transport, repression and activation are understood at the level of the individual mRNAs that are regulated. However, mRNAs are also transported to distal sites of neurons in bulk within specialized RNA Granules (Kiebler & Bassell, 2006). This chapter will focus on these granules, their components, their mechanisms of repression and their mechanisms of reactivation, and how these granules are critical for both synaptic plasticity and neurodevelopment. There has been growing interest in the macro-organization of RNAs in liquid-liquid-phase separated structures in cells, such as P bodies and Stress Granules (P. Anderson & Kedersha, 2006; Protter & Parker, 2016). These structures, particularly Stress Granules, have been implicated in multiple neurological disorders (Wolozin & Ivanov, 2019). This chapter will also

address the relationship of the RNA Granules important for transport and other types of RNA structures present in neurons.

2.4 Evidence for mRNA transport and local translation at distal sites in the nervous system

The first evidence for localized translation in the central nervous system came from a study by Colman and colleagues in 1982, in which they studied the incorporation of Myelin Basic Protein (MBP) into newly myelinated membranes in the developing rat brain. They observed that MBP was synthesized in ribosomes highly enriched in the processes of myelinating oligodendrocytes where the MBP was being incorporated into the myelin sheath (Colman et al., 1982). Next, the observation of clusters of ribosomes localized within dendritic spines of granule cells, identified by electron microscopy (EM) in the rat dentate gyrus, suggested that dendritic spines could be hot spots for localized translation of mRNAs (Steward and Levy, 1982). The first mRNA to be localized to dendrites was Microtubule-associated Protein 2 (MAP-2), while other mRNAs were localized exclusively to the soma (Garner et al., 1988). Since this discovery, thousands of mRNAs have been shown to be present in dendrites (Cajigas et al., 2012) although some mRNAs are much more abundant than others. The most quantitative analysis of mRNA enrichment using nanostring technology showed that the top 10 most abundant mRNAs, including CAMKII, dendrin, and beta-actin make up over 40% of the total mRNA in neuronal processes (Cajigas et al., 2012). Many of these mRNAs encode proteins of particular abundance in dendrites, suggesting that a major role of their transport is to keep the levels of these proteins high in distal processes (Glock et al., 2017). In addition, distinct subsets of mRNAs have also been found in axons (Ostroff et al., 2019; Shigeoka et al., 2016; Taylor et al., 2009; Willis et al., 2005).

The large number of mRNAs that can be found in neuronal processes raises the question of whether all these mRNAs have dendritic sorting signals or whether the sorting mechanism is not precise and some of the mRNAs found in dendrites represent this low fidelity sorting process. Indeed, the absence of some mRNAs in dendrites may be due to active retention mechanisms that prevent some mRNAs from being transported (Martinez et al., 2019; Y. Mori, Imaizumi, Katayama, Yoneda, & Tohyama, 2000; Vicario et al., 2015), as opposed to lacking a distal targeting signal. Moreover, many studies using artificial constructs, designed to produce mRNAs with no known targeting sequences, find that these mRNAs are found in dendrites at similar levels to targeted mRNAs, although cryptic signals in the vectors cannot be ruled out (Bauer et al., 2019; Langille, Ginzberg, & Sossin, 2019; Lebeau et al., 2011). While the localization signal for some mRNAs (e.g beta-actin zip code; hNRPA2 binding sites), have been elucidated (Kislauskis, Zhu, & Singer, 1997; Shan, Munro, Barbarese, Carson, & Smith, 2003) there appears to be many distinct signals that allow for sorting of mRNAs to neuronal processes, often with redundant and dispersed signals present in the mRNA (Y. Mori et al., 2000). The mechanism by which most mRNAs are selected for targeting, and what determines the percentage of a transcript that is to be transported to local sites in neurons is still largely unknown.

It is widely believed that mRNA localization is driven by recognition of specific sequences in the mRNA by RNA Binding Proteins (RBPs). Protein-protein interactions between these RBPs allows for combinatorial interactions and the formation of complexes that then interact with active transport motors (Jansen & Niessing, 2012), translational activators and translational repressors, creating a framework for a hierarchical organization for mRNA localization and abundance (Mayya & Duchaine, 2019). Multiple RBPs can bind to the same or similar sequences on the mRNA, often competing for binding, and this competition can determine the fate of the mRNA. For example,

the embryonic lethal abnormal visual system (ELAV) family of RBPs bind to AU Rich Elements (often termed AREs) and stabilize mRNAs by competing with other RBPs that bind to the same ARE and lead to degradation (Simone & Keene, 2013). Similar competitions can determine transport versus retention (Gardiner, Twiss, & Perrone-Bizzozero, 2015). Thus, the competition between RBPs is important for post-transcriptional RNA control. Each type of neuron expresses a distinct cohort of RBPs suggesting that the mRNAs transported in each neuron could be distinct. Indeed, mRNAs enriched in the dendrites of Purkinje neurons are quite different from those found in hippocampal neurons (Bian, Chu, Schilling, & Oberdick, 1996).

Even with the multiplicity of RBPs and their interactions, there remain a large number of other factors that determine the fate of mRNAs. At the level of the transcript, post-transcriptional modifications of mRNAs, such as alternative 3'ends (Fontes et al., 2017) and RNA modifications (methylation, etc.) (Zhang et al., 2018) can alter the complement of RBPs that bind to mRNAs. All these moving parts change during development and in response to recent neuronal activity. Thus, there is great room for complexity in post-transcriptional regulation of mRNA stability, localization and translational activation (Figure 1).

2.1 Evidence for Stimulus Dependent Translation of Localized mRNAs and Synaptic Plasticity

Following the evidence supporting the transport and translation of specific mRNAs in neuronal processes and sub-compartments, the first direct evidence linking stimulus-dependent local translation of dendritic mRNAs to synaptic plasticity came from Kang and Schuman (Kang & Schuman, 1996) who showed that increases in synaptic strength mediated by BDNF requires local translation.

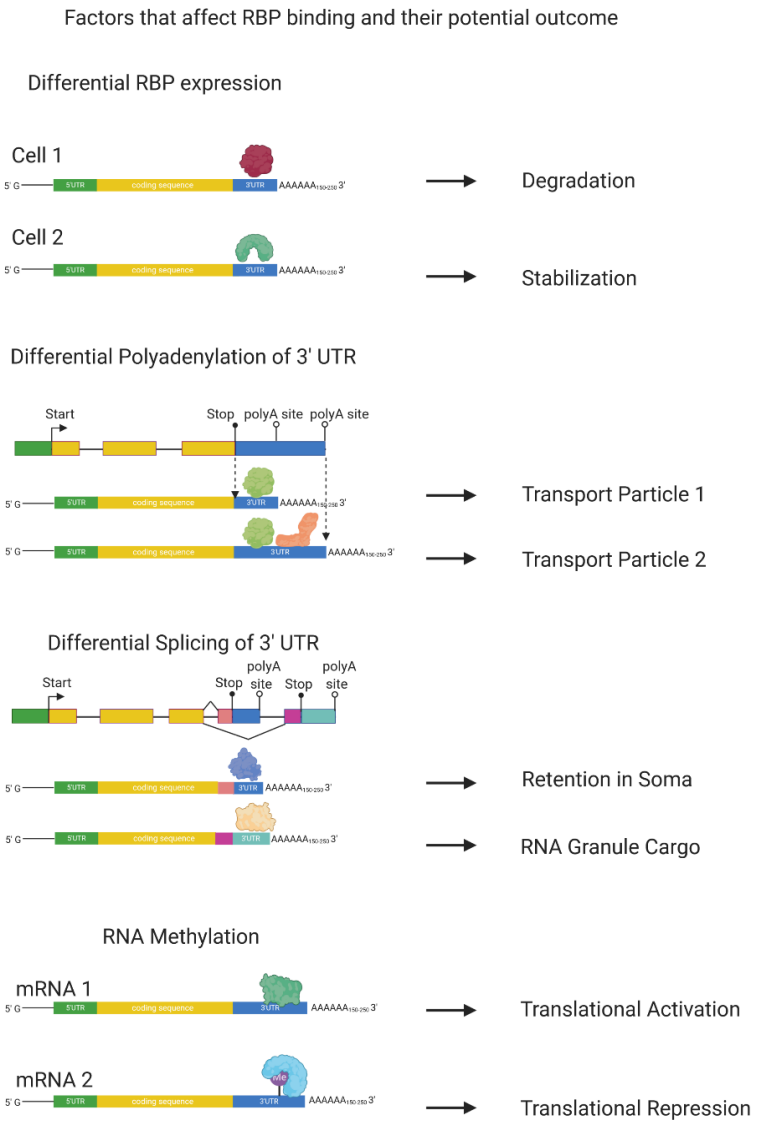


Figure 1. Factors that affect RBP binding to mRNA and their potential outcome.
RBP binding to mRNAs plays an important role in the localization and fate of mRNAs. It should be noted that we do not mean to imply that the specific differences in RBPs or the mRNA on the left necessarily lead to the different regulation on the right. These are just examples; and any of the different regulations could be linked to the differences in RBP binding A) Differential RBP expression in different cells can determine the outcome for the same mRNA, for example, the binding of one RBP in Cell 1 may lead to degradation while the binding of another RBP in Cell 2 may lead to stabilization of that mRNA. B) Differential polyadenylation of an mRNA based on different polyA sites in the 3'UTR can result in longer 3'UTRs, as well as one or more RBP binding sites to be retained in the final mRNA. Based on this, one or more RBPs can bind to the transcript to determine which transport particle the mRNA will be recruited into. It is worth noting that the interaction of two RBPs bound to the same transcript may be important for the outcome. C) Differential splicing of the 3' UTR of transcripts may also affect the binding of different RBPs. One splice variant may lead to interaction with an RBP leading to the retention of the mRNA in the soma, while another splice variant may allow the binding of an RBP that will recruit the mRNA to RNA Granules as cargo to be shipped to distal sites. D) Post-transcriptional modifications such as the Methylation of a transcript may facilitate the interaction of different RBPs with the Methylated versus non-methylated mRNA. The non-methylated mRNA may be translationally active, whereas the methylated mRNA may be translationally repressed by the RBP that is bound to it.

Another pioneering study in *Aplysia californica* sensory neurons showed that Serotonin could stimulate local translation in neurites and that this played multiple roles in the increase in synaptic strength induced by Serotonin, including activation of transcription back in the cell soma (K. C. Martin et al., 1997). This was also one of the first systems in which specific mechanisms of translational control in neurons was examined, as the increase in translation induced by Serotonin was blocked by Rapamycin, implicating the mechanistic Target of Rapamycin Complex 1 (mTORC1) pathway in this process (Casadio et al., 1999; Yanow, Manseau, Hislop, Castellucci, & Sossin, 1998). Another influential finding was that long-term depression induced by activation of metabotropic Glutamate receptors (mGLUR-LTD) in rat hippocampal neurons, required local protein synthesis and could be achieved even after dendrites were separated from the cell soma (K. M. Huber, Kayser, & Bear, 2000). Soon afterwards, examples of local translation of specific mRNAs during synaptic plasticity were found, including Calcium-calmodulin-dependent protein kinase II (CAMKII) alpha (Ouyang, Rosenstein, Kreiman, Schuman, & Kennedy, 1999; Scheetz, Nairn, & Constantine-Paton, 2000), S6 (Khan, Pepio, & Sossin, 2001) and Activity-related cytoskeleton-associated protein (Arc) (Zalfa et al., 2003). The use of fluorescent protein synthesis reporters firmly established that synaptic plasticity-inducing stimuli were associated with increases in local translation (Aakalu, Smith, Nguyen, Jiang, & Schuman, 2001; Job & Eberwine, 2001; D. O. Wang et al., 2009).

The role of local translation in homeostatic plasticity has also been strongly established by a number of studies (M. Mori et al., 2019). Pioneering work by the Holt group showed the importance of local translation in the growth cone for responding to guidance cues (Cioni et al., 2018; B. C. Yoon, Zivraj, & Holt, 2009). A number of recent studies have firmly established that local axonal translation is present at the basal state (Hafner, Donlin-Asp, Leitch, Herzog, &

Schuman, 2019) and is important for response to injury (Hanz et al., 2003) and presynaptic forms of plasticity (Younts et al., 2016). Thus, the requirement of local translation for many forms of synaptic plasticity in both axons and dendrites is strongly supported by recent and past findings.

2.2 Neuronal Ribosome-Containing RNA Granules

Similar to localization of mRNAs, the first evidence for the granular transport of mRNAs in the nervous system came from oligodendrocytes and studies of MBP mRNA, where MBP mRNA and translational machinery (elongation factor 1 a (EF1a) and rRNA) were found to be colocalized to large supramolecular structures in the oligodendrocyte periphery (Barbarese et al., 1995). This was soon followed by similar findings in neurons where Kosik and colleagues (Knowles et al., 1996), who first defined the term neuronal RNA Granule for these granules containing both mRNA and ribosomes, demonstrated their microtubule dependent transport in dendrites. Large ribosome-containing granules could be purified by sucrose gradient sedimentation (Krichevsky & Kosik, 2001) as they were denser than both monosomes and polysomes, thus passing through the gradients to form a pellet. This highlights a potential technical issue when examining neuronal translation, since many studies use sucrose sedimentation to determine which mRNAs are on polysomes, but mostly ignore the pellet fraction, despite numerous studies showing the presence of RNA Granules containing collections of polysomes in the dense pellet (Aschrafi, Cunningham, Edelman, & Vanderklish, 2005; El Fatimy et al., 2016; Elvira et al., 2006; Krichevsky & Kosik, 2001). Importantly, despite the abundance of ribosomes in the pellet, this fraction was translationally repressed (Krichevsky & Kosik, 2001). However, neuronal activity was able to dissociate these granules, and lead to an increase in ribosomes now being found in the polysome fraction (Aschrafi et al., 2005; Krichevsky & Kosik, 2001). Another indication that these

RNA Granule associated polysomes were repressed is that the ribosomes in these granules were aggregated and compacted compared to normal polysomes (El Fatimy et al., 2016; Elvira et al., 2006). This was observed even when the granules were formed in an in-vitro translational assay from neuronal extracts (Darnell et al., 2011). These granules were also shown to be resistant to nuclease treatment suggesting that their compact structure can protect the contents from degradation (Darnell et al., 2011). One important unresolved issue is that the large, aggregated compacted ribosomes are not easily seen in electron microscopy (EM) of dendrites that have been used to characterize polysomes in dendrites. It may be that it is difficult to distinguish a circular translating polysome rosette from a compacted RNA granule in EM. Interestingly, polysomes found in dendrites are somewhat insensitive to Puromycin (Dynes & Steward, 2012) consistent with these polysomes either representing ribosomes not involved in translation or representing Stalled Polysomes (discussed below). However, the large collections of polysomes as seen in some studies (Krichevsky & Kosik, 2001) are not seen in EMs. These aggregates could form after homogenization perhaps because the granules have a higher propensity for aggregation than normal polysomes. Another possibility is that the aggregates are masked in neurons and are not identifiable in EM. The best evidence for masking comes from the Singer lab with the observation that many transported mRNAs were resistant to probes before protease treatment (Buxbaum, Wu, & Singer, 2014). Interestingly, neuronal stimulation opened up these granules to probes, consistent with the idea that RNA Granules dissociate to free mRNAs for translation (Buxbaum et al., 2014).

2.3 Components of Neuronal RNA Granules

A number of proteomics studies have identified a large number of proteins found in these large aggregates of ribosomes isolated from neurons. More specifically, two proteomic studies were done based on purification by sedimentation (El Fatimy et al., 2016; Elvira et al., 2006) and

one based on a pull-down by tail of kinesin5a subunit of Kinesin1 (KIF5a) (Kanai, Dohmae, & Hirokawa, 2004). While the KIF5a study also purified a densely sedimenting particle (1000S), it should be noted that unlike the other studies granules identified by KIF5a pulldown only identified one ribosomal protein. This is likely due to technical aspects of the proteomics (bands were isolated from 2-D gels where the extremely positively charged ribosomal proteins had likely run off, and only one of the most negatively charged ribosomal proteins, L3, was found at the very edge of the gel) (Kanai et al., 2004). Other than the ribosomal proteins, the shared proteomics results of this study as well as the high density of the purified particle suggests that the same structure was identified. The major protein components (other than ribosomal proteins) detected in these proteomic studies are RBPs and some RBPs were shared between the three studies (Fragile X mental retardation protein (FMRP), Pur-alpha and beta, Staufen, Synaptotagmin Binding Cytoplasmic RNA Interacting Protein (Syncrip, also known as heterogeneous ribonucleoprotein particle (hnRNP-Q, hnRNP-U), despite the different developmental stages of the tissues used in the three studies (E18 vs. P5 vs. adult). Many more proteins were found in at least two studies including cytoplasmic activation/proliferation-associated protein 1 (Caprin-1, also known as RNA granule protein 105), Ras-GTPase-activating protein-binding proteins (G3BPs), ELAVs, Interleukin-enhancer binding protein, Insulin-like growth factor mRNA binding proteins (IMPs; also known as Zip-code binding protein (ZBP)) and other hnRNPs. There are some additional proteins, not necessarily RBPs that have been implicated in RNA biology, such as dead box RNA helicases (DDX)1 and DDX 3, RNA transcription, translation and transport factor (RTRAF, also known as CGI-99)- a protein in a complex with DDX1-, UP Frameshift mutation 1 (UPF1), plasminogen activator inhibitor 1 RNA binding protein (PAI1 RBP, also known as SERPINE1 mRNA binding protein (SERBP1) that were found in at least two of the studies. While some of

these proteins can also be found in generic proteomic studies of polysomes (Reschke et al., 2013) their relative enrichment in these preparations suggests specific roles in these granules, either in selecting mRNA cargo, forming the compact ribosome bundles, connecting to motors, or in the actual stalling process itself. Another striking finding in all these studies is the absence of most translation factors implicated in translation initiation, including any component of the initiating complex, eIF4G, consistent with the status of these structures as translationally repressed.

2.4 RNA Granules and Liquid-Liquid Phase Separation

Many RNA structures (nucleolus, P bodies, Stress Granules) are known to retain their structure through liquid-liquid phase separation (Langdon & Gladfelter, 2018; Ryan & Fawzi, 2019). This phase separation of RNA structures is achieved in part by RBPs with low complexity disordered domains that are found abundantly in these structures (Harrison & Shorter, 2017; Mittag & Parker, 2018). Indeed, many of the proteins with disordered domains important for Stress Granules (Harrison & Shorter, 2017) are also present in RNA Granules based on the proteomic studies outlined above (Figure 2). Live imaging of RNA granules demonstrated that they were visible in differential interference contrast microscopy (DIC), indicating that a phase separation had occurred (L. C. Miller et al., 2009). It is not clear if the phase separation seen in these structures is also important for the repression of translation seen in RNA Granules, perhaps if several factors important for translation are segregated out of these structures. Also, how liquid-liquid phase separated structures can be tethered to and transported on microtubules is an issue that has not yet been addressed. Interestingly, relationships between Stress Granules and the Endoplasmic Reticulum (ER) have generated a model for how membraneless organelles can interact with other structures through protein-protein interactions (Lee, Cathey, Wu, Parker, & Voeltz, 2020). It is likely that a true understanding of the transport of these granules and their repression, and removal

of repression, may require a better understanding of how liquid-liquid phase interactions are regulated.

2.5 RNA Transport Particles

There have been a number of proteomic studies examining the mRNA transport particles that contain mRNAs but lack ribosomes (Sossin & DesGroseillers, 2006) often purified through a specific RBP that marks this particle. Two distinct particles and associated proteins were isolated using affinity purification with antibodies against either Staufen 2 (Stau2) or Barentsz (Btz) (Fritzsche et al., 2013). It was observed that less than 50% of the 150 proteins identified in this study were present in both particles, supporting the idea of heterogeneity in these transport particles based on the RBPs that are present. The mRNAs contained within the two isolated particles were also distinct. The Barentsz particle contained components of the Exon Junction Complex (EJC), suggesting that the mRNA contained within was not previously translated since translation is known to displace the EJC from the message. It is also possible that this particle may be specific to mRNAs that contain EJC in their 3' untranslated region, such as the mRNA for Arc that would be the target of Nonsense-Mediated Decay if fully translated (Giorgi et al., 2007). The Stau2 particle shared a number of features with Stalled Polysomes (that also contain Stau2), including UPF1, FMRP, Pur-alpha (Figure 2). One complication of this study is the exclusion of many proteins that were immunoprecipitated as they were also present in control immunoprecipitations; this included ribosomal proteins and many other proteins that were previously identified in the Stalled Polysome purifications (Fritzsche et al., 2013). Since the RNA Granules are very dense, it is possible that they would pellet in the control precipitations, making it difficult to distinguish the relationship between the Stau2 particle isolated here and the RNA Granules described previously containing Stau2 (Elvira et al., 2006).

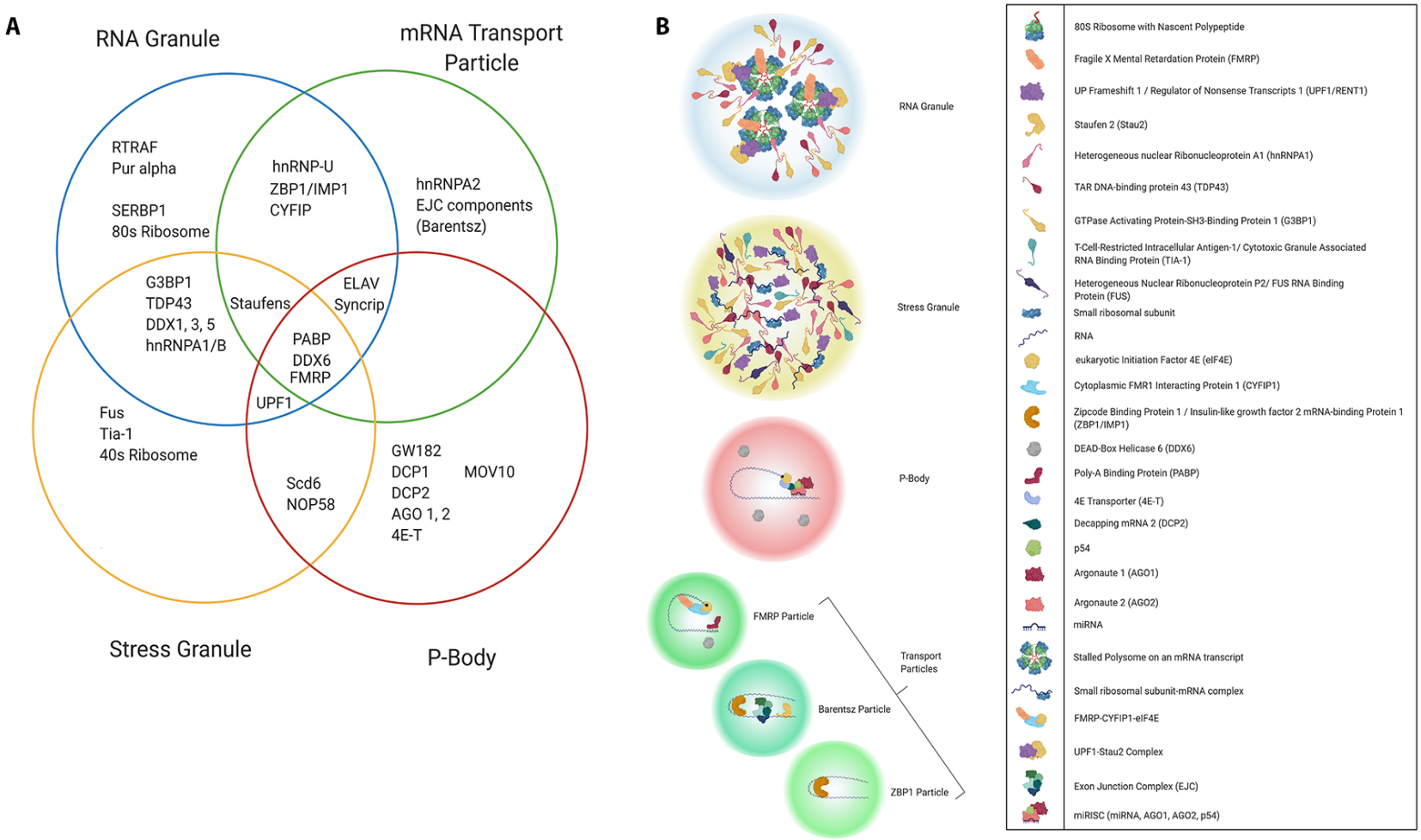


Figure 2. RNA containing Ribonucleoparticles

There are a number of particles in neurons that contain RNA, including the RNA Granule, Stress Granule, mRNA transport particles and P-bodies. Each particle is equipped with a different set of RBPs and other proteins that allows them to have a specialized purpose in the transport and regulation of mRNAs. A) Although each particle has a distinct purpose, there is an overlap of some proteins that are shared among these particles. B) Key for the important RBPs/proteins that define each particle and its purpose. C) Structure and relative organization of each ribonucleoparticle.

HnRNPA2 is another RBP that appears to be critical for mRNA transport to distal sites and many transported mRNAs have a binding site for hnRNPA2 (Muslimov, Patel, Rose, & Tiedge, 2011; Shan et al., 2003) however, the composition of these transport particles have not been well characterized. FMRP binds to cytoplasmic FMR1 interacting protein family (CYFIP) that represses specific mRNAs by repressing translation initiation through eIF4E (Napoli et al., 2008) (Figure 2). Another RBP implicated in transport in both axons and dendrites is ZBP1/IMP1. It has been most closely associated with the transport and translational regulation of beta-actin mRNA in dendrites (Huttelmaier et al., 2005) and axons (Welshhans & Bassell, 2011). Purification of ZBP1/IMP1 granules from cell lines using antibodies to this RBP revealed a structure containing small ribosomal subunits, EJC, and a number of RBPs (Jonson et al., 2007). Although these granules resembled Stress Granules (see below) in containing small, but not large ribosomal subunits, they did not colocalize with Stress Granules in these cells as marked by G3BP1 and FMRP (Jonson et al., 2007). Their relationship to the Barentsz particle that also contains EJC is not clear, perhaps due to the different cell types (neurons vs HEK293 cells) from which the particles were isolated. In summary, there are a wide variety of RNA transport particles that have been identified, mainly based on immunoprecipitation with a particular RBP. It is not clear, however, whether specific RBPs can define a particular transport particle and how distinct these identifications are, as well as how many distinct transport particles exist. It is conceivable that every transported mRNA, based on their binding to individual set of RBPs, defines its own transport particle.

2.6 Comparison to other mRNA containing granules

2.6.1 Stress Granules

Stress Granules are formed as part of the integrated stress response which can be induced by multiple types of stress (proteostatic stress, oxidative stress, starvation, etc) that converge on

the phosphorylation of initiation factor eIF2 alpha, and store mRNAs stalled at initiation until the stress is resolved. As a consequence of eIF2 alpha phosphorylation, initiation is blocked before subunit joining, and thus stress granules contain mRNAs bound to the small ribosomal subunit but lacking the large ribosomal subunit (P. Anderson & Kedersha, 2006). The lack of the large ribosomal subunit is the clearest distinction between neuronal RNA Granules and Stress Granules. Stress Granules are organized by specific RBPs, such as G3BP and TIA1, with low complexity disordered domains that are important for the liquid-liquid phase separation (Protter & Parker, 2016). Notably, some of these proteins such as G3BPs are shared with neuronal RNA Granules (Figure 2), perhaps due to both structures requiring the liquid-liquid phase separation. It thus brings up a concern that some characterization of Stress Granules using co-localization with shared proteins like G3BP in neurons (S. Martin & Tazi, 2014; Sahoo, Smith, Perrone-Bizzozero, & Twiss, 2018) may confuse Stress Granules and neuronal RNA Granules. It would be preferable that co-localization with a large ribosomal subunit protein should be used to distinguish the two. Since transported mRNAs can also be blocked at initiation after association with the small ribosomal subunit, some transport particles may also contain small but not large ribosomal subunits. The composition of these transport granules may be quite similar to stress granules, although in one case an RNA transport particle containing the small subunit did not contain G3BP1 (Jonson et al., 2007). In contrast, if mRNA transport particles contain mRNAs for which initiation was blocked before 43 S joining (eg. if eIF4E is prevented from binding to eIF4G), these would not be expected to be associated with small subunits during transport. Thus, not all RNA transport particles would be expected to contain small ribosomal subunits.

2.6.2 P bodies and RISC particles

Two other RNA-related structures in neurons are P bodies and RNA-induced silencing complexes (RISC particles). P bodies are sites of RNA degradation, and indeed are defined by the presence of decapping enzymes (Franks & Lykke-Andersen, 2008) and ribonucleases important for RNA degradation (Siwaszek, Ukleja, & Dziembowski, 2014). However, they may act as storage granules as well (Luo, Na, & Slavoff, 2018; Parker & Sheth, 2007). RISC particles represent complexes in which miRNAs repress translation. While miRNA binding usually leads to degradation of the mRNA (Fabian & Sonenberg, 2012), there are a number of examples in neurons for the reversibility of miRNA-mediated repression, particularly at distal sites in neurons, suggesting local regulation by miRNAs (Banerjee, Neveu, & Kosik, 2009; Kenny et al., 2014; Muddashetty et al., 2011). This could be due to either transport of RISC particles, or local production of miRNAs. Interestingly, markers of the RISC complex, including Argonaute (AGO) and GW (glycine-tryptophan repeat) protein of 182 kD (GW182), are also found in P bodies (J. Liu et al., 2005), making it difficult to differentiate putative P bodies and RISC complexes. A granule with similarity to P bodies has been implicated in mRNA transport and translational control at distal sites in Drosophila neurons (Barbee et al., 2006; Hillebrand et al., 2010). While most of the P body/RISC proteins are not found in neuronal RNA granules, they do share some components, such as DDX6 and FMRP (Figure 2). One study examined the co-localization of RNA Granule markers and P body markers in neuronal processes, which also showed some overlap (L. C. Miller et al., 2009). There are a number of possibilities concerning the relationship of these structures. It is possible that mRNA granules containing mRNAs and ribosomes also contain mRNAs with RISC complexes on their target mRNAs that would regulate the mRNAs once they are released from the granule. It is also possible that there is a distinct mRNA transport particle in

neurons where mRNAs are repressed by miRNA-mediated mechanisms in a P body-like transport particle that shares components with neuronal RNA granules. Further studies are required to differentiate these possibilities.

2.7 Evidence for Stalled Polysomes in RNA granules

Large RNA Granules in neurons are made up of a mixture of transported mRNAs and ribosomes (Krichevsky & Kosik, 2001). While it is possible that this is due to co-transport of mRNAs stalled before elongation and ribosomal subunits not engaged in translation, multiple lines of evidence below suggest that these granules are made up of Stalled Polysomes, where translation has been stalled during elongation. Thus, some local translation can be mediated by reactivation of Stalled Polysomes as opposed to activating translation initiation pathways (Graber et al., 2013). What is the purpose for using stalled ribosomes for local translation in neurons? Neurons are highly dynamic cells that exhibit fast synaptic responses to incoming stimuli in order to process information at a physiologically relevant rate. This process often requires the production of new proteins at local synaptic sites in a fast and efficient manner, which then alters synaptic strength. One mechanism for translating proteins quickly is to bypass initiation, the rate limiting step for translation, by assembling multiple ribosomes on an mRNA and then stalling at elongation or termination, allowing for fast production of proteins after this stall is removed.

Because the critical distinction of RNA Granule-mediated translation is the lack of requirement for initiation, specific initiation inhibitors have been key to providing evidence for these structures. These compounds only block the initial formation of polysomes, not their elongation. One class of inhibitors, including Pateamine A and Hippuristanol, are dominant negative inhibitors of eIF4A that trap the mRNA scanning complex, preventing initiation before subunit joining (Shen & Pelletier, 2019). Another class, exemplified by Homoharringtonine and

Bruceantin, block only the very first step of elongation, allowing ribosomes that have already passed the first step of translation elongation to continue elongating (Robert et al., 2009). These compounds allow for identification of stalled polysomes in-situ as they allow run-off of translating polysomes but prevent formation of new polysomes. Thus, polysomes that remain after prolonged treatment with initiation inhibitors are, by definition, stalled. Tools are then required to distinguish Stalled Polysomes from ribosomes not in the process of translation. A major distinguishing feature is that Stalled Polysomes contain nascent polypeptide chains, but ribosomes not involved in translation do not. There are two methods to identify nascent chains: Nascent Chain Ribopuromycylation (RPM) and SunTagging, which are described below.

Puromycin covalently attaches to the nascent polypeptide chain on translating ribosomes and eventually leads to the dissociation of the polypeptide and ribosome. However, if Puromycin is added in conjunction with an elongation inhibitor such as Emetine, the nascent chain becomes trapped on the ribosome, thus one can label ribosomes with nascent chains using this Ribopuromycylation (RPM) technique (David, Bennink, & Yewdell, 2013). By inducing ribosomal run-off with initiation inhibitors prior to the addition of Puromycin, RPM will only label Stalled Polysomes. Indeed, in hippocampal neurites, large puncta of polysomes resistant to run-off were observed. The number of these puncta decreased after activation of mGLURs, consistent with the reactivation of Stalled Polysomes and their subsequent disappearance (Graber et al., 2013). Interestingly, Emetine is not necessary to prevent the dissociation of the nascent chain and stalled ribosomes in the initiation-inhibitor resistant RPM puncta, presumably since these ribosomes are already stalled and Puromycin dissociation requires translocation (Langille et al., 2019).

Another way to image nascent peptides is the SunTag method, in which multiple copies of an antigen for a fluorescently-tagged nanobody are incorporated into a message along with a degradation signal, allowing for the imaging of the translated nascent chains, only before translation is complete (Yan, Hoek, Vale, & Tanenbaum, 2016). The co-localization of Sun-Tag and RPM puncta after run-off of translating polysomes firmly established the presence of Stalled Polysomes in neurites (Langille et al., 2019).

One can also distinguish translation that comes from Stalled Polysomes by examining translation in the presence of initiation inhibitors in conjunction with the nascent protein synthesis reporter Click-it Azidohomoalanine (AHA). Using this technique, it was shown that in processes of cultured hippocampal neurons, activation of mGLURs leads to a strong initiation-independent increase in new protein synthesis consistent with reactivation of stalled polysomes (Graber et al., 2013). If Stalled Polysomes are important for production of proteins and plasticity, then these proteins and the types of synaptic plasticity they are associated with should be distinguished by their dependence of elongation inhibitors (such as Cycloheximide or Anisomycin), and their independence of initiation inhibitors. Indeed, production of Map-1b (Graber et al., 2013), and Arc (Na et al., 2016), as well as mGLUR-LTD (Graber et al., 2013) have all been shown to depend on elongation, but not initiation. A form of synaptic plasticity called Intermediate Term Facilitation (ITF) in the invertebrate *Aplysia californica* is also blocked by elongation, but not by initiation inhibitors (McCamphill et al., 2015) suggesting that this method of fast and local translation is evolutionarily conserved.

2.8 One mRNA vs multiple mRNAs in transport

Imaging of mRNAs in dendrites using sensitive in-situ hybridization has strongly indicated that most mRNAs are present individually and do not co-localize with other mRNAs or even with separate mRNAs encoding the same protein (Batish, van den Bogaard, Kramer, & Tyagi, 2012; Mikl, Vendra, & Kiebler, 2011). This is in contrast to the evidence that large RNA Granules appear to consist of multiple polysomes. Imaging of Stalled Polysomes appears to show aggregates of large structures (L. C. Miller et al., 2009). When imaging tagged FMRP, there appears to be coalescence and dissociation of smaller particles from these larger structures, indicating units or individual arrays of stalled polysomes, coming on or off (El Fatimy et al., 2016). RPM puncta of Stalled Polysomes also appears more consistent with the presence of multiple Stalled Polysomes in one structure, particularly when using super resolution STORM microscopy the number of Puromycin associated with an individual granule appears larger than what would be expected from a single polysome (Graber et al., 2017). If one compares the density of in-situ hybridization derived puncta with the density of RPM puncta, it is clear that there are many more individual molecules of mRNA than RPM puncta in dendrites (at least 10-fold higher numbers of a single mRNA than total number of RPM puncta in Graber et al, 2017). Thus, RNA Granules containing Stalled Polysomes are sparse compared to transport particles containing individual mRNAs. This is consistent with some puzzling findings. The first is that initiation inhibitors did not significantly decrease the number of initiation inhibitor resistant RPM puncta seen in dendrites, despite the ability of these inhibitors to strongly decrease basal translation in dendrites (Graber et al., 2013). This could be due to translation in processes coming mainly from mRNAs with one or only a few ribosomes attached (Biever et al., 2020) that are under the detection limit for RPM puncta. Second, when expressing an mRNA containing SunTag epitopes, there was a high percentage of RPM

puncta that overlapped with the nascent peptides (Langille et al., 2019). If polysomes were transported individually, then one would expect only the polysomes containing the mRNA encoding the SunTag epitope to show colocalization of RPM and SunTag signal, while all other polysomes containing other messages to label only for RPM. In contrast, virtually all RPM puncta were also positive for Sun-Tag suggesting that either under these conditions this mRNA competed out all others for generation of granules, or that there were many polysomes in each granule (Langille et al., 2019). Thus, these data suggest that in dendrites while most mRNAs for a particular message are either in transport particles or individual translating ribosomes, a small subset of mRNAs may be found in larger aggregates of Stalled Polysomes, available for quick release upon appropriate stimuli.

2.9 How are Stalled Polysomes stalled?

If stalled polysomes represent a source of mRNAs underlying local translation, it is important to understand how they are stalled and unstalled. There are several signals that appear to be important for this process (Figure 3) and these are detailed below. However, despite some clues from proteins that appear to be required for stalling and unstalling, the fundamental mechanisms involved in these processes are still unknown.

2.9.1 FMRP

FMRP is enriched in neuronal RNA Granules (El Fatimy et al., 2016; Elvira et al., 2006; Kanai et al., 2004). This protein is encoded for by an X-linked gene subject to amplification of a CGG repeat in the 5’ untranslated region of the Fragile-X gene that leads to (i) hypermethylation, (ii) a lack of production of the mRNA encoding FMRP, and (iii) the absence of functional FMRP

protein (Garber, Visootsak, & Warren, 2008). Loss of FMRP leads to the Fragile-X Syndrome, including severe intellectual disability, epilepsy and often Autism Spectrum Disorder symptoms (Garber et al., 2008). One common finding in animal models of Fragile-X is an increase in overall translation, suggesting a normal role for FMRP in repressing translation (Richter, Bassell, & Klann, 2015). The FMRP protein was found to segregate with polysomes in sucrose gradients, suggesting a role in elongation (Khandjian, Corbin, Woerly, & Rousseau, 1996). Run-off of translating polysomes left a fraction of polysomes still associated with FMRP, suggesting that FMRP was specifically associated with stalled polysomes (Ceman et al., 2003). This form of FMRP was phosphorylated at a specific residue (serine 499) (Ceman et al., 2003), initially thought to be due to S6 kinase, but now shown to be partly due to CK2 and other kinases (Bartley et al., 2016; Bartley et al., 2014). Dephosphorylation of this residue is thought to be important for mGLUR-LTD (Niere et al., 2012) and this is consistent with the phosphorylation playing a role in the reactivation of Stalled Polysomes and the production of proteins that are also required for mGLUR-LTD (Graber et al., 2013) (Figure 3). Perhaps, the most direct association between FMRP and Stalled Polysomes are studies where FMRP was required for stalling of transcripts shown separately to be associated with FMRP (Darnell et al., 2011). Unlike most RBPs that associate with the 3' untranslated regions of their mRNA targets, the majority of sites for FMRP association were located in the coding region (Darnell et al., 2011), consistent with FMRP's association with the ribosome as opposed to with specific sites on its target mRNAs (E. Chen, Sharma, Shi, Agrawal, & Joseph, 2014). Loss of FMRP was also associated with increased elongation rates for a number of messages, consistent with a role for FMRP in the regulation of elongation (Udagawa et al., 2013).

2.9.2 UPF1-Staufen 2 interactions

UPF1 is an RNA helicase that plays a major role in Nonsense-Mediated Decay (Kim & Maquat, 2019). It is recruited when the mRNA reaches the stop codon through an interaction with eukaryotic release factors 1 and 3 and stalls termination. If an EJC containing UPF2/3 exists downstream of the stop codon, indicating that the stop codon is premature, then interactions between UPF1 and UPF2/3 leads to the phosphorylation of UPF1 and subsequent decay of the message (Kim & Maquat, 2019). Staufen binding to UPF1 can also lead to its phosphorylation and can result in Staufen-Mediated Decay for some messages that harbor Stau-binding sites in their 3' untranslated region (E. Park & Maquat, 2013). Since both UPF1 and Stau2 have been found in proteomics of RNA Granules (El Fatimy et al., 2016; Elvira et al., 2006), it suggests that the UPF1/Stau2 interaction may be repurposed to generate Stalled Polysomes. Indeed, removal of UPF1 or Stau2 leads to a decrease in the number of Stalled Polysomes, initiation-independent translation and mGLUR-LTD (Graber et al., 2017). Moreover, both initiation-independent translation and mGLUR-LTD can be rescued with re-expression of Stau2, but not with a form of Stau2 that does not bind UPF1, demonstrating the importance of their interaction for the regulation of Stalled Polysomes (Graber et al., 2017). UPF1 dephosphorylation was also induced by mGLUR-LTD, similar to FMRP (Graber et al., 2017) (Figure. 3). In this study, the role of UPF1 and Stau2 were also examined for mRNA transport and production of a specific protein, Map1b, which is required for mGLUR-LTD (Davidkova & Carroll, 2007). Both UPF1 and Staufen were required for the transport of the mRNA encoding Map1b, as well as the induced translation of the protein following unstalling (Graber et al., 2017). Importantly Map1b is also a transcript highly regulated by FMRP (Lu et al., 2004), linking the two distinct regulators of Stalled Polysomes.

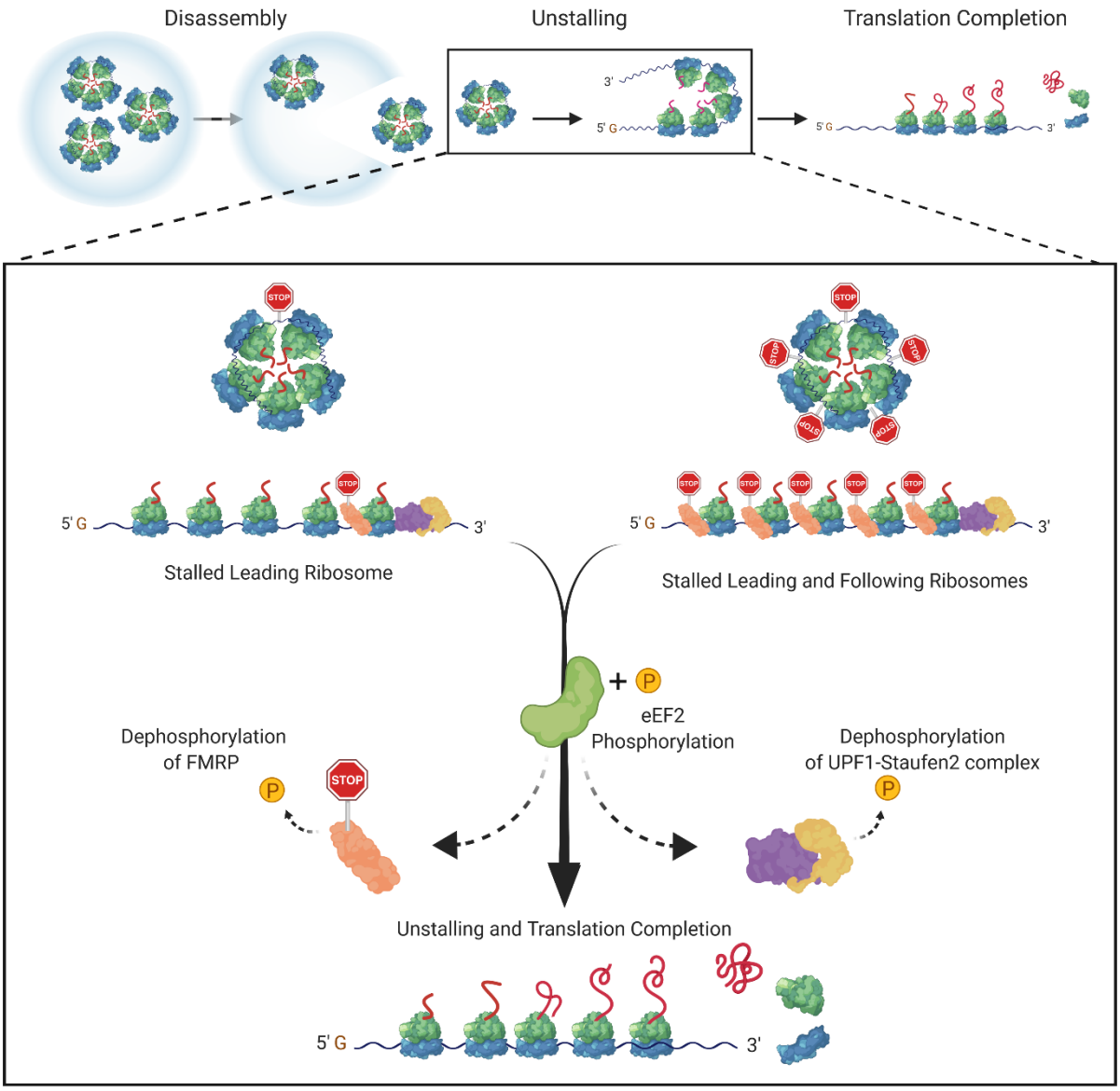


Figure 3. Release of RNA-Granule Associated Stalled Polysomes
RNA Granule associated Stalled Polysomes are a specialized mechanism for neurons to regulate the expression of mRNAs important for synaptic plasticity during development. Once RNA Granules arrive at their designated local sites, appropriate cues induce the disassembly of one or more stalled polysomes to dissociate from the liquid-liquid phase separated granule structure and unravel to expose the contents to translation factors. Unstalling of polysomes on the mRNA occurs when the repression induced by RBPs such as FMRP and UPF1-Staufen2 complex, is removed and this is facilitated by signals such as the activation of mGluRs. It has been established that the phosphorylation of eEF2 is required for the release of stalled polysomes, however the exact mechanism is unclear. In terms of stalling, there are two possibilities: A) Only the leading ribosome is stalled, causing a pile up of the trailing ribosomes on the mRNA, and B) Both leading and trailing ribosomes are stalled. The activation of mGluRs leads to the phosphorylation of eEF2 and the dephosphorylation of FMRP and the UPF1-Staufen2 complex, resulting in unstalling and completion of translation.

2.9.3 eEF2 phosphorylation

There is a correlation between synaptic plasticity events that appear to require reactivation of stalled polysomes (i.e. elongation-dependent, initiation-independent) and plasticity events that require phosphorylation of the elongation factor eEF2, reviewed in (Sossin & Costa-Mattioli, 2019) (Figure 3). eEF2 is the elongation factor important for ratcheting the mRNA forward through the ribosome and phosphorylation inactivates eEF2 through removing its ability to bind the ribosome (Proud, 2015). What underlies this correlation is not clear but may suggest some relationship between the structure of the stalled polysome and the role of eEF2 (See Chapter Four: Discussion - *The eEF2 conundrum).*

2.9.4 Prevention of Nonsense-Mediated and No-go Decay

No-Go Decay is a dedicated pathway in cells for the recovery of stalled polysomes and degradation of their partially translated nascent chains (Buskirk & Green, 2017). The stability of Stalled Polysomes in neuronal processes would require a mechanism to prevent this form of decay. Indeed, none of the proteins specific to No-Go Decay process are present in the proteomics of Stalled Polysomes. Similarly, UPF1 phosphorylation normally leads to Nonsense Mediated Decay, as does the association of Staufen with an mRNA (Schoenberg & Maquat, 2012). Thus, Stalled Polysomes in neurons containing phosphorylated UPF1 would need a mechanism to prevent this form of decay as well. Similar to No-Go Decay, none of the proteins (other than UPF1) normally associated with Nonsense-Mediated Decay are present in the proteomics of RNA Granules.

2.10 Stalled Polysomes and neurodevelopmental disorders

The loss of FMRP is the leading genetic cause of intellectual deficiency and Autism (Garber et al., 2008). Recently, a number of other proteins implicated in Stalled Polysomes have been found to cause intellectual deficiency when mutated. This includes the RBPs Pur-alpha (Reijnders et al., 2018), Syncrip (Lelieveld et al., 2016), hnRNP U (Bramswig et al., 2017), and the most abundant RNA helicase in the polysomes DDX3 (Snijders Blok et al., 2015; X. Wang et al., 2018). While most of these RBPs have also been implicated in other granules or other translational regulatory steps, it is still striking that so many of the proteins central to the proteome of the Stalled Polysome, when altered, result in neurodevelopmental disorders. mRNAs associated with FMRP are highly enriched for those encoding proteins whose mutation causes intellectual deficiency and Autism (Darnell et al., 2011). If this represents the mRNAs mostly regulated through Stalled Polysomes, it would be consistent with the dysregulation of many of the proteins implicated in these granules causing neurodevelopmental disorders. Moreover, Stalled Polysomes have mostly been characterized in developmental settings, using neuronal cultures or purified from tissues of embryonic or developing brains. While a quantitative developmental profile has not been accomplished, it is possible that this form of mRNA transport is much more important in developmental stages than in the mature brain.

2.11 Conclusions

There are many structures implicated in the transport of mRNAs to distal sites in neurons and their local translational regulation. One particular neuronal RNA granule consisting of stalled polysomes appears to play a unique role in the control of mRNAs produced for certain forms of plasticity such as mGLUR-LTD, and loss of this regulation is associated with neurodevelopmental

disorders. Since many of the proteins are shared between this granule and other structures such as mRNA transport particles, stress granules and P bodies, it is still difficult to ascertain particular roles for any one RNA-containing structure. Nevertheless, it is clear that a better understanding of these different modes of transport will be required for a deeper understanding of local mRNA translation and its role in the healthy and diseased brain. In the case of Fragile-X Syndrome, the restoration of homeostasis through the regulation of stalled polysome dependent translation even in the absence of FMRP, holds great promise.

2.12 Acknowledgements

This work was funded by a CIHR grant to WSS and WSS is a James McGill Professor. We thank Arkady Khotoursky and Keith Murai for their helpful comments. The figures were created with biorender.com

2.13 References

Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C., & Schuman, E. M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron, 30*(2), 489-502. doi:10.1016/s0896-6273(01)00295-1

Anderson, P., & Kedersha, N. (2006). RNA granules. *J Cell Biol, 172*(6), 803-808. doi:10.1083/jcb.200512082

Aschrafi, A., Cunningham, B. A., Edelman, G. M., & Vanderklish, P. W. (2005). The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci U S A, 102*(6), 2180-2185. doi:10.1073/pnas.0409803102

Banerjee, S., Neveu, P., & Kosik, K. S. (2009). A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron, 64*(6), 871-884. doi:10.1016/j.neuron.2009.11.023

Barbarese, E., Koppel, D. E., Deutscher, M. P., Smith, C. L., Ainger, K., Morgan, F., & Carson, J. H. (1995). Protein translation components are colocalized in granules in oligodendrocytes. *J Cell Sci, 108*(8), 2781-2790.

Barbee, S. A., Estes, P. S., Cziko, A. M., Hillebrand, J., Luedeman, R. A., Coller, J. M., . . . Ramaswami, M. (2006). Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron, 52*(6), 997-1009. doi:10.1016/j.neuron.2006.10.028

Bartley, C. M., O'Keefe, R. A., Blice-Baum, A., Mihailescu, M. R., Gong, X., Miyares, L., . . . Bordey, A. (2016). Mammalian FMRP S499 Is Phosphorylated by CK2 and Promotes Secondary Phosphorylation of FMRP. *eNeuro, 3*(6). doi:10.1523/ENEURO.0092-16.2016

Bartley, C. M., O'Keefe, R. A., & Bordey, A. (2014). FMRP S499 is phosphorylated independent of mTORC1-S6K1 activity. *PLoS One, 9*(5), e96956. doi:10.1371/journal.pone.0096956

Batish, M., van den Bogaard, P., Kramer, F. R., & Tyagi, S. (2012). Neuronal mRNAs travel singly into dendrites. *Proc Natl Acad Sci U S A, 109*(12), 4645-4650. doi:10.1073/pnas.1111226109

Bauer, K. E., Segura, I., Gaspar, I., Scheuss, V., Illig, C., Ammer, G., . . . Kiebler, M. A. (2019). Live cell imaging reveals 3'-UTR dependent mRNA sorting to synapses. *Nat Commun, 10*(1), 3178. doi:10.1038/s41467-019-11123-x

Becalska, A. N., & Gavis, E. R. (2009). Lighting up mRNA localization in Drosophila oogenesis. *Development, 136*(15), 2493-2503. doi:10.1242/dev.032391

Bian, F., Chu, T., Schilling, K., & Oberdick, J. (1996). Differential mRNA transport and the regulation of protein synthesis: selective sensitivity of Purkinje cell dendritic mRNAs to translational inhibition. *Mol Cell Neurosci, 7*(2), 116-133. doi:10.1006/mcne.1996.0009

Biever, A., Glock, C., Tushev, G., Ciirdaeva, E., Dalmay, T., Langer, J. D., & Schuman, E. M. (2020). Monosomes actively translate synaptic mRNAs in neuronal processes. *Science, 367*(6477). doi:10.1126/science.aay4991

Bramswig, N. C., Ludecke, H. J., Hamdan, F. F., Altmuller, J., Beleggia, F., Elcioglu, N. H., . . . Wieczorek, D. (2017). Heterozygous HNRNPU variants cause early onset epilepsy and severe intellectual disability. *Hum Genet, 136*(7), 821-834. doi:10.1007/s00439-017-1795-6

Buskirk, A. R., & Green, R. (2017). Ribosome pausing, arrest and rescue in bacteria and eukaryotes. *Philos Trans R Soc Lond B Biol Sci, 372*(1716). doi:10.1098/rstb.2016.0183

Buxbaum, A. R., Wu, B., & Singer, R. H. (2014). Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science, 343*(6169), 419-422. doi:10.1126/science.1242939

Cajigas, I. J., Tushev, G., Will, T. J., tom Dieck, S., Fuerst, N., & Schuman, E. M. (2012). The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron, 74*(3), 453-466. doi:10.1016/j.neuron.2012.02.036

Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., . . . Kandel, E. R. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell, 99*(2), 221-237. doi:10.1016/s0092-8674(00)81653-0

Ceman, S., O'Donnell, W. T., Reed, M., Patton, S., Pohl, J., & Warren, S. T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet, 12*(24), 3295-3305. doi:10.1093/hmg/ddg350

Chen, E., Sharma, M. R., Shi, X., Agrawal, R. K., & Joseph, S. (2014). Fragile X mental retardation protein regulates translation by binding directly to the ribosome. *Mol Cell, 54*(3), 407-417. doi:10.1016/j.molcel.2014.03.023

Cioni, J. M., Koppers, M., & Holt, C. E. (2018). Molecular control of local translation in axon development and maintenance. *Curr Opin Neurobiol, 51*, 86-94. doi:10.1016/j.conb.2018.02.025

Colman, D. R., Kreibich, G., Frey, A. B. , & Sabatinie, D. D. (1982) Synthesis and incorporation of myelin polypeptides into CNS myelin. *J Cell Biol*, *95*(2), 598-608. DOI:10.1083/jcb.95.2.598

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*(1), 10-26. doi:10.1016/j.neuron.2008.10.055

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., . . . Darnell, R. B. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell, 146*(2), 247-261. doi:10.1016/j.cell.2011.06.013

David, A., Bennink, J. R., & Yewdell, J. W. (2013). Emetine optimally facilitates nascent chain puromycylation and potentiates the ribopuromycylation method (RPM) applied to inert cells. *Histochem Cell Biol, 139*(3), 501-504. doi:10.1007/s00418-012-1063-8

Davidkova, G., & Carroll, R. C. (2007). Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors in hippocampus. *J Neurosci, 27*(48), 13273-13278. doi:10.1523/JNEUROSCI.3334-07.2007

Dynes, J. L., & Steward, O. (2012). Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation. *J Comp Neurol, 520*(14), 3105-3119. doi:10.1002/cne.23073

El Fatimy, R., Davidovic, L., Tremblay, S., Jaglin, X., Dury, A., Robert, C., . . . Khandjian, E. W. (2016). Tracking the Fragile X Mental Retardation Protein in a Highly Ordered Neuronal RiboNucleoParticles Population: A Link between Stalled Polyribosomes and RNA Granules. *PLoS Genet, 12*(7), e1006192. doi:10.1371/journal.pgen.1006192

Eliscovich, C., Buxbaum, A. R., Katz, Z. B., & Singer, R. H. (2013). mRNA on the move: the road to its biological destiny. *J Biol Chem, 288*(28), 20361-20368. doi:10.1074/jbc.R113.452094

Elvira, G., Wasiak, S., Blandford, V., Tong, X. K., Serrano, A., Fan, X., . . . Sossin, W. S. (2006). Characterization of an RNA granule from developing brain. *Mol Cell Proteomics, 5*(4), 635-651. doi:10.1074/mcp.M500255-MCP200

Fabian, M. R., & Sonenberg, N. (2012). The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol, 19*(6), 586-593. doi:10.1038/nsmb.2296

Fontes, M. M., Guvenek, A., Kawaguchi, R., Zheng, D., Huang, A., Ho, V. M., . . . Martin, K. C. (2017). Activity-Dependent Regulation of Alternative Cleavage and Polyadenylation During Hippocampal Long-Term Potentiation. *Sci Rep, 7*(1), 17377. doi:10.1038/s41598-017-17407-w

Franks, T. M., & Lykke-Andersen, J. (2008). The control of mRNA decapping and P-body formation. *Mol Cell, 32*(5), 605-615. doi:10.1016/j.molcel.2008.11.001

Fritzsche, R., Karra, D., Bennett, K. L., Ang, F. Y., Heraud-Farlow, J. E., Tolino, M., . . . Kiebler, M. A. (2013). Interactome of two diverse RNA granules links mRNA localization to translational repression in neurons. *Cell Rep, 5*(6), 1749-1762. doi:10.1016/j.celrep.2013.11.023

Garber, K. B., Visootsak, J., & Warren, S. T. (2008). Fragile X syndrome. *Eur J Hum Genet, 16*(6), 666-672. doi:10.1038/ejhg.2008.61

Gardiner, A. S., Twiss, J. L., & Perrone-Bizzozero, N. I. (2015). Competing Interactions of RNA-Binding Proteins, MicroRNAs, and Their Targets Control Neuronal Development and Function. *Biomolecules, 5*(4), 2903-2918. doi:10.3390/biom5042903

Garner, C. C., Tucker, R. P., & Matus, A. (1988) Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. *Nature 336*(6200), 674-677. doi:10.1038/336674a0

Giorgi, C., Yeo, G. W., Stone, M. E., Katz, D. B., Burge, C., Turrigiano, G., & Moore, M. J. (2007). The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. *Cell, 130*(1), 179-191. doi:10.1016/j.cell.2007.05.028

Glock, C., Heumüller, M., & Schuman, E. M. (2017). mRNA transport & local translation in neurons. *Curr Opin Neurobiol, 45*, 169-177. doi:https://doi.org/10.1016/j.conb.2017.05.005

Graber, T. E., Freemantle, E., Anadolu, M. N., Hebert-Seropian, S., MacAdam, R. L., Shin, U., . . . Sossin, W. S. (2017). UPF1 Governs Synaptic Plasticity through Association with a

STAU2 RNA Granule. *J Neurosci, 37*(38), 9116-9131. doi:10.1523/jneurosci.0088-17.2017

Graber, T. E., Hebert-Seropian, S., Khoutorsky, A., David, A., Yewdell, J. W., Lacaille, J. C., & Sossin, W. S. (2013). Reactivation of stalled polyribosomes in synaptic plasticity. *Proc Natl Acad Sci U S A, 110*(40), 16205-16210. doi:10.1073/pnas.1307747110

Hafner, A. S., Donlin-Asp, P. G., Leitch, B., Herzog, E., & Schuman, E. M. (2019). Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. *Science, 364*(6441). doi:10.1126/science.aau3644

Hanz, S., Perlson, E., Willis, D., Zheng, J. Q., Massarwa, R., Huerta, J. J., . . . Fainzilber, M. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron, 40*(6), 1095-1104. doi:10.1016/s0896-6273(03)00770-0

Harrison, A. F., & Shorter, J. (2017). RNA-binding proteins with prion-like domains in health and disease. *Biochem J, 474*(8), 1417-1438. doi:10.1042/BCJ20160499

Hillebrand, J., Pan, K., Kokaram, A., Barbee, S., Parker, R., & Ramaswami, M. (2010). The Me31B DEAD-Box Helicase Localizes to Postsynaptic Foci and Regulates Expression of a CaMKII Reporter mRNA in Dendrites of Drosophila Olfactory Projection Neurons. *Front Neural Circuits, 4*, 121. doi:10.3389/fncir.2010.00121

Huang, Y. S., & Richter, J. D. (2004). Regulation of local mRNA translation. *Curr Opin Cell Biol, 16*(3), 308-313. doi:10.1016/j.ceb.2004.03.002

Huber, K. M., Kayser, M. S., & Bear, M. F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science, 288*(5469), 1254-1257.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., . . . Singer, R. H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature, 438*(7067), 512-515. doi:10.1038/nature04115

Jansen, R. P., & Niessing, D. (2012). Assembly of mRNA-protein complexes for directional mRNA transport in eukaryotes--an overview. *Curr Protein Pept Sci, 13*(4), 284-293. doi:10.2174/138920312801619493

Job, C., & Eberwine, J. (2001). Identification of sites for exponential translation in living dendrites. *Proc Natl Acad Sci U S A, 98*(23), 13037-13042. doi:10.1073/pnas.231485698

Jonson, L., Vikesaa, J., Krogh, A., Nielsen, L. K., Hansen, T., Borup, R., . . . Nielsen, F. C. (2007). Molecular composition of IMP1 ribonucleoprotein granules. *Mol Cell Proteomics, 6*(5), 798-811. doi:10.1074/mcp.M600346-MCP200

Kanai, Y., Dohmae, N., & Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron, 43*(4), 513-525. doi:10.1016/j.neuron.2004.07.022

Kang, H., & Schuman, E. M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science, 273*(5280), 1402-1406. doi:10.1126/science.273.5280.1402

Kenny, P. J., Zhou, H., Kim, M., Skariah, G., Khetani, R. S., Drnevich, J., . . . Ceman, S. (2014). MOV10 and FMRP regulate AGO2 association with microRNA recognition elements. *Cell Rep, 9*(5), 1729-1741. doi:10.1016/j.celrep.2014.10.054

Khan, A., Pepio, A. M., & Sossin, W. S. (2001). Serotonin activates S6 kinase in a rapamycin-sensitive manner in Aplysia synaptosomes. *J Neurosci, 21*(2), 382-391.

Khandjian, E. W., Corbin, F., Woerly, S., & Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. *Nat Genet, 12*(1), 91-93. doi:10.1038/ng0196-91

Kiebler, M. A., & Bassell, G. J. (2006). Neuronal RNA Granules: Movers and Makers. *Neuron, 51*(6), 685-690. doi:https://doi.org/10.1016/j.neuron.2006.08.021

Kim, Y. K., & Maquat, L. E. (2019). UPFront and center in RNA decay: UPF1 in nonsense-mediated mRNA decay and beyond. *Rna, 25*(4), 407-422. doi:10.1261/rna.070136.118

Kislauskis, E. H., Zhu, X., & Singer, R. H. (1997). beta-Actin messenger RNA localization and protein synthesis augment cell motility. *J Cell Biol, 136*(6), 1263-1270. doi:10.1083/jcb.136.6.1263

Knowles, R. B., Sabry, J. H., Martone, M. E., Deerinck, T. J., Ellisman, M. H., Bassell, G. J., & Kosik, K. S. (1996). Translocation of RNA Granules in Living Neurons. *The Journal of Neuroscience, 16*(24), 7812.

Krichevsky, A. M., & Kosik, K. S. (2001). Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron, 32*(4), 683-696.

Langdon, E. M., & Gladfelter, A. S. (2018). A New Lens for RNA Localization: Liquid-Liquid Phase Separation. *Annu Rev Microbiol, 72*, 255-271. doi:10.1146/annurev-micro-090817-062814

Langille, J. J., Ginzberg, K., & Sossin, W. S. (2019). Polysomes identified by live imaging of nascent peptides are stalled in hippocampal and cortical neurites. *Learn Mem, 26*(9), 351-362. doi:10.1101/lm.049965.119

Lebeau, G., Miller, L. C., Tartas, M., McAdam, R., Laplante, I., Badeaux, F., . . . Lacaille, J. C. (2011). Staufen 2 regulates mGluR long-term depression and Map1b mRNA distribution in hippocampal neurons. *Learn Mem, 18*(5), 314-326. doi:10.1101/lm.2100611

Lee, J. E., Cathey, P. I., Wu, H., Parker, R., & Voeltz, G. K. (2020). Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science, 367*(6477). doi:10.1126/science.aay7108

Lelieveld, S. H., Reijnders, M. R., Pfundt, R., Yntema, H. G., Kamsteeg, E. J., de Vries, P., . . . Gilissen, C. (2016). Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nat Neurosci, 19*(9), 1194-1196. doi:10.1038/nn.4352

Liu, J., Rivas, F. V., Wohlschlegel, J., Yates, J. R., 3rd, Parker, R., & Hannon, G. J. (2005). A role for the P-body component GW182 in microRNA function. *Nat Cell Biol, 7*(12), 1261-1266. doi:10.1038/ncb1333

Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell W, T., Li, W., . . . Feng, Y. (2004). The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in

brain neuron development. *Proc Natl Acad Sci U S A, 101*(42), 15201-15206. doi:10.1073/pnas.0404995101

Luo, Y., Na, Z., & Slavoff, S. A. (2018). P-Bodies: Composition, Properties, and Functions. *Biochemistry, 57*(17), 2424-2431. doi:10.1021/acs.biochem.7b01162

Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., . . . Kandel, E. R. (1997). Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell, 91*(7), 927-938. doi:10.1016/s0092-8674(00)80484-5

Martin, S., & Tazi, J. (2014). Visualization of G3BP stress granules dynamics in live primary cells. *J Vis Exp*(87). doi:10.3791/51197

Martinez, J. C., Randolph, L. K., Iascone, D. M., Pernice, H. F., Polleux, F., & Hengst, U. (2019). Pum2 Shapes the Transcriptome in Developing Axons through Retention of Target mRNAs in the Cell Body. *Neuron, 104*(5), 931-946 e935. doi:10.1016/j.neuron.2019.08.035

Mayya, V. K., & Duchaine, T. F. (2019). Ciphers and Executioners: How 3'-Untranslated Regions Determine the Fate of Messenger RNAs. *Front Genet, 10*, 6. doi:10.3389/fgene.2019.00006

McCamphill, P. K., Farah, C. A., Anadolu, M. N., Hoque, S., & Sossin, W. S. (2015). Bidirectional regulation of eEF2 phosphorylation controls synaptic plasticity by decoding neuronal activity patterns. *J Neurosci, 35*(10), 4403-4417. doi:10.1523/JNEUROSCI.2376-14.2015

Mikl, M., Vendra, G., & Kiebler, M. A. (2011). Independent localization of MAP2, CaMKIIalpha and beta-actin RNAs in low copy numbers. *EMBO Rep, 12*(10), 1077-1084. doi:10.1038/embor.2011.149

Miller, L. C., Blandford, V., McAdam, R., Sanchez-Carbente, M. R., Badeaux, F., DesGroseillers, L., & Sossin, W. S. (2009). Combinations of DEAD box proteins distinguish distinct types of RNA: protein complexes in neurons. *Mol Cell Neurosci, 40*(4), 485-495.

Miller, S., Yasuda, M., Coats, J. K., Jones, Y., Martone, M. E., & Mayford, M. (2002). Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron, 36*(3), 507-519. doi:10.1016/s0896-6273(02)00978-9

Mittag, T., & Parker, R. (2018). Multiple Modes of Protein-Protein Interactions Promote RNP Granule Assembly. *J Mol Biol, 430*(23), 4636-4649. doi:10.1016/j.jmb.2018.08.005

Mori, M., Penney, J., & Haghighi, P. (2019). Regulation of synaptic homeostasis by translational mechanisms. In W. Sossin (Ed.), *The Oxford Handbook of Neuronal Protein Synthesis*: Oxford Press.

Mori, Y., Imaizumi, K., Katayama, T., Yoneda, T., & Tohyama, M. (2000). Two cis-acting elements in the 3' untranslated region of alpha-CaMKII regulate its dendritic targeting. *Nat Neurosci, 3*(11), 1079-1084. doi:10.1038/80591

Muddashetty, R. S., Nalavadi, V. C., Gross, C., Yao, X., Xing, L., Laur, O., . . . Bassell, G. J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol Cell, 42*(5), 673-688. doi:10.1016/j.molcel.2011.05.006

Muslimov, I. A., Patel, M. V., Rose, A., & Tiedge, H. (2011). Spatial code recognition in neuronal RNA targeting: role of RNA-hnRNP A2 interactions. *J Cell Biol, 194*(3), 441-457. doi:10.1083/jcb.201010027

Na, Y., Park, S., Lee, C., Kim, D. K., Park, J. M., Sockanathan, S., . . . Worley, P. F. (2016). Real-Time Imaging Reveals Properties of Glutamate-Induced Arc/Arg 3.1 Translation in Neuronal Dendrites. *Neuron, 91*(3), 561-573. doi:10.1016/j.neuron.2016.06.017

Napoli, I., Mercaldo, V., Boyl, P. P., Eleuteri, B., Zalfa, F., De Rubeis, S., . . . Bagni, C. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell, 134*(6), 1042-1054. doi:10.1016/j.cell.2008.07.031

Niere, F., Wilkerson, J. R., & Huber, K. M. (2012). Evidence for a fragile X mental retardation protein-mediated translational switch in metabotropic glutamate receptor-triggered Arc translation and long-term depression. *J Neurosci, 32*(17), 5924-5936. doi:10.1523/JNEUROSCI.4650-11.2012

Ostroff, L. E., Santini, E., Sears, R., Deane, Z., Kanadia, R. N., LeDoux, J. E., . . . Klann, E. (2019). Axon TRAP reveals learning-associated alterations in cortical axonal mRNAs in the lateral amgydala. *Elife, 8*. doi:10.7554/eLife.51607

Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M., & Kennedy, M. B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J Neurosci, 19*(18), 7823-7833.

Palacios, I. M., & St Johnston, D. (2001). Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu Rev Cell Dev Biol, 17*, 569-614. doi:10.1146/annurev.cellbio.17.1.569

Paquin, N., & Chartrand, P. (2008). Local regulation of mRNA translation: new insights from the bud. *Trends Cell Biol, 18*(3), 105-111. doi:10.1016/j.tcb.2007.12.004

Park, E., & Maquat, L. E. (2013). Staufen-mediated mRNA decay. *Wiley Interdiscip Rev RNA, 4*(4), 423-435. doi:10.1002/wrna.1168

Parker, R., & Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Mol Cell, 25*(5), 635-646. doi:10.1016/j.molcel.2007.02.011

Protter, D. S. W., & Parker, R. (2016). Principles and Properties of Stress Granules. *Trends Cell Biol, 26*(9), 668-679. doi:10.1016/j.tcb.2016.05.004

Proud, C. G. (2015). Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans, 43*(3), 328-332. doi:10.1042/BST20140323

Reijnders, M. R. F., Janowski, R., Alvi, M., Self, J. E., van Essen, T. J., Vreeburg, M., . . . Baralle, D. (2018). PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature. *J Med Genet, 55*(2), 104-113. doi:10.1136/jmedgenet-2017-104946

Reschke, M., Clohessy, J. G., Seitzer, N., Goldstein, D. P., Breitkopf, S. B., Schmolze, D. B., . . . Pandolfi, P. P. (2013). Characterization and analysis of the composition and dynamics of the mammalian riboproteome. *Cell Rep, 4*(6), 1276-1287. doi:10.1016/j.celrep.2013.08.014

Richter, J. D., Bassell, G. J., & Klann, E. (2015). Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nat Rev Neurosci, 16*(10), 595-605. doi:10.1038/nrn4001

Robert, F., Carrier, M., Rawe, S., Chen, S., Lowe, S., & Pelletier, J. (2009). Altering chemosensitivity by modulating translation elongation. *PLoS One, 4*(5), e5428. doi:10.1371/journal.pone.0005428

Ryan, V. H., & Fawzi, N. L. (2019). Physiological, Pathological, and Targetable Membraneless Organelles in Neurons. *Trends Neurosci, 42*(10), 693-708. doi:10.1016/j.tins.2019.08.005

Sahoo, P. K., Smith, D. S., Perrone-Bizzozero, N., & Twiss, J. L. (2018). Axonal mRNA transport and translation at a glance. *J Cell Sci, 131*(8). doi:10.1242/jcs.196808

Scheetz, A. J., Nairn, A. C., & Constantine-Paton, M. (2000). NMDA receptor-mediated control of protein synthesis at developing synapses. *Nat Neurosci, 3*(3), 211-216. doi:10.1038/72915

Schoenberg, D. R., & Maquat, L. E. (2012). Regulation of cytoplasmic mRNA decay. *Nat Rev Genet, 13*(4), 246-259. doi:10.1038/nrg3160

Shan, J., Munro, T. P., Barbarese, E., Carson, J. H., & Smith, R. (2003). A molecular mechanism for mRNA trafficking in neuronal dendrites. *J Neurosci, 23*(26), 8859-8866.

Shen, L., & Pelletier, J. (2019). Selective targeting of the DEAD-box RNA helicase eukaryotic initiation factor (eIF) 4A by natural products. *Nat Prod Rep*. doi:10.1039/c9np00052f

Shigeoka, T., Jung, H., Jung, J., Turner-Bridger, B., Ohk, J., Lin, J. Q., . . . Holt, C. E. (2016). Dynamic Axonal Translation in Developing and Mature Visual Circuits. *Cell, 166*(1), 181-192. doi:10.1016/j.cell.2016.05.029

Simone, L. E., & Keene, J. D. (2013). Mechanisms coordinating ELAV/Hu mRNA regulons. *Curr Opin Genet Dev, 23*(1), 35-43. doi:10.1016/j.gde.2012.12.006

Siwaszek, A., Ukleja, M., & Dziembowski, A. (2014). Proteins involved in the degradation of cytoplasmic mRNA in the major eukaryotic model systems. *RNA Biol, 11*(9), 1122-1136. doi:10.4161/rna.34406

Snijders Blok, L., Madsen, E., Juusola, J., Gilissen, C., Baralle, D., Reijnders, M. R., . . . Kleefstra, T. (2015). Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Effects on Wnt Signaling. *Am J Hum Genet, 97*(2), 343-352. doi:10.1016/j.ajhg.2015.07.004

Sossin, W. S., & Costa-Mattioli, M. (2019). Translational Control in the Brain in Health and Disease. *Cold Spring Harb Perspect Biol, 11*(8). doi:10.1101/cshperspect.a032912

Sossin, W. S., & DesGroseillers, L. (2006). Intracellular trafficking of RNA in neurons. *Traffic, 7*(12), 1581-1589. doi:10.1111/j.1600-0854.2006.00500.x

Steward, O., & Levy, W. B. (1982) Preferential localization of polyribosomes under dendritic spines in granule cells of the dentate gyrus. *J. Neurosci*, *2*(3) 284-291, doi10.1523/JNEUROSCI.02-03-00284

Sutton, M. A., & Schuman, E. M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. *Cell, 127*(1), 49-58. doi:10.1016/j.cell.2006.09.014

Taylor, A. M., Berchtold, N. C., Perreau, V. M., Tu, C. H., Li Jeon, N., & Cotman, C. W. (2009). Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J Neurosci, 29*(15), 4697-4707. doi:10.1523/JNEUROSCI.6130-08.2009

Udagawa, T., Farny, N. G., Jakovcevski, M., Kaphzan, H., Alarcon, J. M., Anilkumar, S., . . . Richter, J. D. (2013). Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology. *Nat Med, 19*(11), 1473-1477. doi:10.1038/nm.3353

Vicario, A., Colliva, A., Ratti, A., Davidovic, L., Baj, G., Gricman, L., . . . Tongiorgi, E. (2015). Dendritic targeting of short and long 3' UTR BDNF mRNA is regulated by BDNF or NT-3 and distinct sets of RNA-binding proteins. *Front Mol Neurosci, 8*, 62. doi:10.3389/fnmol.2015.00062

Wang, D. O., Kim, S. M., Zhao, Y., Hwang, H., Miura, S. K., Sossin, W. S., & Martin, K. C. (2009). Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science, 324*(5934), 1536-1540. doi:10.1126/science.1173205

Wang, X., Posey, J. E., Rosenfeld, J. A., Bacino, C. A., Scaglia, F., Immken, L., . . . Moretti, P. (2018). Phenotypic expansion in DDX3X - a common cause of intellectual disability in females. *Ann Clin Transl Neurol, 5*(10), 1277-1285. doi:10.1002/acn3.622

Welshhans, K., & Bassell, G. J. (2011). Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J Neurosci, 31*(27), 9800-9813. doi:10.1523/JNEUROSCI.0166-11.2011

Wilhelm, J. E., & Vale, R. D. (1993). RNA on the move: the mRNA localization pathway. *J Cell Biol, 123*(2), 269-274. doi:10.1083/jcb.123.2.269

Willis, D., Li, K. W., Zheng, J. Q., Chang, J. H., Smit, A. B., Kelly, T., . . . Twiss, J. L. (2005). Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. *J Neurosci, 25*(4), 778-791. doi:10.1523/JNEUROSCI.4235-04.2005

Wolozin, B., & Ivanov, P. (2019). Stress granules and neurodegeneration. *Nat Rev Neurosci, 20*(11), 649-666. doi:10.1038/s41583-019-0222-5

Wong, H. H., Lin, J. Q., Strohl, F., Roque, C. G., Cioni, J. M., Cagnetta, R., . . . Holt, C. E. (2017). RNA Docking and Local Translation Regulate Site-Specific Axon Remodeling In Vivo. *Neuron, 95*(4), 852-868 e858. doi:10.1016/j.neuron.2017.07.016

Yan, X., Hoek, T. A., Vale, R. D., & Tanenbaum, M. E. (2016). Dynamics of Translation of Single mRNA Molecules In Vivo. *Cell, 165*(4), 976-989. doi:10.1016/j.cell.2016.04.034

Yanow, S. K., Manseau, F., Hislop, J., Castellucci, V. F., & Sossin, W. S. (1998). Biochemical pathways by which serotonin regulates translation in the nervous system of Aplysia. *J Neurochem, 70*(2), 572-583. doi:10.1046/j.1471-4159.1998.70020572.x

Yoon, B. C., Zivraj, K. H., & Holt, C. E. (2009). Local translation and mRNA trafficking in axon pathfinding. *Results Probl Cell Differ, 48*, 269-288. doi:10.1007/400_2009_5

Younts, T. J., Monday, H. R., Dudok, B., Klein, M. E., Jordan, B. A., Katona, I., & Castillo, P. E. (2016). Presynaptic Protein Synthesis Is Required for Long-Term Plasticity of GABA Release. *Neuron, 92*(2), 479-492. doi:10.1016/j.neuron.2016.09.040

Zalfa, F., Giorgi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., . . . Bagni, C. (2003). The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell, 112*(3), 317-327. doi:10.1016/s0092-8674(03)00079-5

Zhang, F., Kang, Y., Wang, M., Li, Y., Xu, T., Yang, W., . . . Jin, P. (2018). Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. *Hum Mol Genet, 27*(22), 3936-3950. doi:10.1093/hmg/ddy292

3 CHAPTER THREE: Ribosomes in RNA granules are stalled on mRNA sequences that are consensus sites for FMRP association

3.1 Preface

This chapter contains the second of two scholarly works presented in this manuscript-based thesis. In this chapter, we present our latest scientific research on the topic of Stalled Polysomes that we described in detail in the previous chapter. Submitted to Nucleic Acids Research, this work provides much needed evidence linking FMRP to stalled polysomes by examining the ribosomal footprints of stalled mRNAs contained within RNA Granules. This work opens new avenues to better understand how ribosomes may be reversibly stalled on neuronal mRNAs and creates a foundation for future Cryo-EM work looking more closely at stalled ribosomes and their interactions with putative stalling factors. This work also highlights the complexity of the many regulatory pathways that converge to solve the complex problem of regulating mRNAs important for neurodevelopment and synaptic plasticity. This chapter demonstrates my research potential and ability to apply my knowledge in a practical way. This manuscript contributes to original knowledge by providing new information on neuronal protein synthesis that necessitates a revision of our collective (and often dogmatic) knowledge of translational control and ribosomal stalling.

Ribosomes in RNA granules are stalled on mRNA sequences that are consensus sites for FMRP association.

Mina N. Anadolu[1], Senthilkumar Kailasam[2], Konstanze Simbriger[3], Jingyu Sun[4,5], Teodora Markova[1], Seyed Mehdi Jafarnejad[6], Francois Lefebvre[2], Joaquin Ortega[4,5], Christos G. Gkogkas[7], Wayne S. Sossin[1,4,*]

[1]Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, Canada. [2]Canadian Centre for Computational Genomics, McGill University, Montreal, Quebec, H3A 0G1, Canada. [3]Department of Pharmacology, Medical University Innsbruck, Innsbruck Austria Division of Biomedical Research, A-6020 Innsbruck, Austria [4]Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec H3A 0C7, Canada, [5]Centre for Structural Biology, McGill University, Montreal, Quebec, H3G 0B1, Canada. [6]Patrick G. Johnston Centre for Cancer Research, The Queen's University of Belfast, BT9 7AE, Belfast, Northern Ireland, UK [7] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, University Campus, 45110 Ioannina, Greece

Author contributions: Performed Research (MNA, KS, JS, SMJ), Analyzed Data (MNA, SK, TM, WSS), Designed research (FL, JO, CGG, WSS), Wrote paper (MNA, WS, JO, CGG)

Competing Interest Statement. There are no competing interests

Keywords: Ribosome Footprinting, FMRP, Stalled polysome, Neuronal RNA granule. Translational control

3.2 Abstract

Local translation in neurons is mediated in part by the reactivation of stalled polysomes. However, the mechanism for stalling of the polysomes is not understood. Stalled polysomes may be enriched within neuronal RNA granules defined by dense collections of compacted ribosomes found in the pellet of sucrose gradients used to separate polysomes from monosomes. We find that this fraction, isolated from P5 rat brains of both sexes, is enriched in proteins implicated in stalled polysome function, such as the fragile X mental retardation protein (FMRP) and Up-frameshift mutation 1 homolog (UPF1). Ribosome profiling of this fraction showed an abundance of footprint reads derived from mRNAs of cytoskeletal proteins implicated in neuronal development and an enrichment of footprint reads on RNA binding proteins. Compared to those usually found in ribosome profiling studies, the footprint reads were more extended on their 3'end and were found in reproducible peaks in the mRNAs. These footprint reads were enriched in motifs previously associated with mRNAs cross-linked to FMRP in vivo, independently linking the ribosomes in the sedimented pellet to the ribosomes associated with FMRP in the cell. The data supports a model in which specific sequences in mRNAs act to stall translation elongation in neurons, attracting FMRP and beginning a process where stalled ribosomes are packaged and transported in RNA granules.

3.3 Significance Statement

This work finds that neuronal ribosomes in RNA granules are concentrated at consensus sites previously identified through cross-linking FMRP to mRNAs in the brain. This strongly links the compacted ribosomes found in the pellet of sucrose gradients from brain extracts to stalled ribosomes regulated by FMRP and provides important insights into how stalling is accomplished. Many mRNAs important for neurodevelopment are enriched in these ribosomes. These results suggest that many studies on translation in the brain may need to be revised. The larger size of the ribosomal footprints on stalled polysomes and their sedimentation in the pellet of sucrose gradients suggests mRNAs found in these structures have not been assessed in many studies of neuronal translation.

3.4 Introduction

In neurons, the local translation of mRNAs at distal synaptic sites is essential for maintaining the local proteome (Glock et al., 2017), developmental processes (Cioni et al., 2018), homeostasis of excitability (M. Mori et al., 2019), and synaptic plasticity (Sossin & Costa-Mattioli, 2019). Local translation requires the transport of mRNAs from the soma to distal sites in a repressed state, followed by their reactivation, either when the mRNA reaches its correct location or after an appropriate stimulus (M. N. Anadolu & Sossin, 2020). Two major forms of mRNA transport have been defined in neurons: the transport of mRNAs that are repressed prior to the start of translational elongation, and the transport of mRNAs for which elongation has begun but then stalled (Sossin & DesGroseillers, 2006). The mRNAs repressed before elongation lack large ribosomal subunits and are often transported in dedicated mRNA transport particles. In contrast, the mRNAs repressed at elongation are transported as stalled polysomes (Krichevsky & Kosik, 2001; Sossin & DesGroseillers, 2006). The local reactivation of translation from mRNAs transported in stalled polysomes can be distinguished from mRNAs blocked at initiation using drugs such as homoharringtonine (HHT) that specifically block the first step of translation elongation, thus blocking translation from mRNA transport particles but not RNA granules (M. N. Anadolu & Sossin, 2020). Using this tool, several physiological processes have been shown to be supported by initiation-independent protein synthesis, such as the local production of microtubule associated protein 1B (Map1B) and the induction of a form of long-term depression stimulated by the activation of metabotropic glutamate receptors (mGluR-LTD) in vertebrates, as well as the induction of a type of intermediate term synaptic plasticity in the invertebrate model system *Aplysia californica* (Graber et al., 2017; Graber et al., 2013; McCamphill et al., 2015).

Stalled polysomes may be transported in large liquid-liquid phase separated structures termed neuronal RNA granules (M. N. Anadolu & Sossin, 2020). These were first described in oligodendrocytes where they transported myelin basic protein mRNA to myelin synthesis sites (Barbarese et al., 1995). The term neuronal RNA granule was first used to describe a sedimented fraction containing ribosomes and repressed mRNAs lacking initiation factors (Krichevsky & Kosik, 2001). These large collections of ribosomes can be separated from normal polysomes based on their high density (Aschrafi et al., 2005; El Fatimy et al., 2016; Elvira et al., 2006; Kanai et al., 2004; Krichevsky & Kosik, 2001). The proteomic characterization of these structures is consistent with the possibility that they are stalled polysomes (El Fatimy et al., 2016; Elvira et al., 2006; Kanai et al., 2004). Indeed, they are enriched in mRNAs such as Map1B that undergo initiation-independent protein synthesis (El Fatimy et al., 2016).

Stalled polysomes may also be necessary for neuronal development and the regulation of developmentally expressed mRNAs through association with the fragile X mental retardation protein (FMRP), a protein that when lost, results in the neurodevelopmental disorder Fragile X syndrome (Garber et al., 2008). UV cross-linking of FMRP to mRNA in neurons showed that FMRP was mainly associated with the coding region of mRNAs (Darnell et al., 2011), consistent with the possibility of FMRP associating with ribosomes. Indeed, FMRP has been shown to be associated with stalled ribosomes in several studies (Aschrafi et al., 2005; Ceman et al., 2003; Darnell et al., 2011; El Fatimy et al., 2016; Shah et al., 2020). A number of mRNA sequences enriched in the regions of mRNAs cross-linked with FMRP have been identified (B. R. Anderson, Chopra, Suhl, Warren, & Bassell, 2016; M. Ascano, Jr. et al., 2012) suggesting that specific mRNA sequences may be important for determining which mRNAs are recruited to FMRP-containing stalled polysomes. FMRP was also shown to be enriched in RNA granules isolated by sucrose

granule sedimentation (El Fatimy et al., 2016), consistent with the idea that this dense RNA granule fraction contains stalled polysomes.

The mechanism for stalling polysomes during elongation in neurons is not known. Recently, the nonsense-mediated decay factor UPF1 was implicated in this process. Decreasing levels of UPF1 reduced the levels of initiation-independent translation, the local production of Map1B and disrupted the induction of mGluR-LTD (Graber et al., 2017). Since UPF1 is known to be attracted to ribosomes when they reach the stop codon through association with eukaryotic release factors (Kim & Maquat, 2019), this suggests that stalled mRNAs within RNA granules are blocked at the release step of translation termination. To test this, we used ribosome profiling, also known as ribosome footprinting, to elucidate the position of ribosomes on mRNAs through sequencing the ribosome protected fragments (footprint reads) after nuclease treatment (Ingolia, 2014). We took advantage of the presumed enrichment of stalled polysomes in heavy fractions of sucrose gradients to create an enriched fraction and identify the sites occupied by ribosomes in these structures. The footprint reads identified by ribosome profiling of the sedimented RNA granules were larger than expected and produced reproducible peaks, which were highly enriched in motifs previously associated with FMRP target mRNAs (B. R. Anderson et al., 2016; M. Ascano, Jr. et al., 2012) and consensus sites for m6A modifications in the brain (Zhang et al., 2018). Contrary to our prediction, the footprint reads were not enriched at the stop codon, but instead were slightly biased towards the first half of the open reading frame of transcripts. We propose a stochastic model in which stalling of ribosomes at specific motifs in mRNAs in neurons attracts FMRP, starting a process of ribosome compaction and RNA granule assembly that allows transport of stalled ribosomes to distal sites in neurons for local translation.

3.5 Results

3.5.1 Isolation of the pellet enriched in compacted ribosomes

To enrich for RNA granules and presumed stalled ribosomes from P5 rat whole brain homogenate, we adapted a previous protocol (El Fatimy et al., 2016) for sucrose gradient sedimentation that involves a high-velocity 2-hour spin over a 60% sucrose pad to enrich for all polysomes, followed by an additional 45-minute short spin on a 15-60% sucrose gradient to further sediment heavier particles (Fig. 1A). From this second centrifugation, we collected fractions from the top and separated the proteins on an SDS gel followed by staining with Coomassie blue to visualize the contents of each fraction (Fig. 1B). We performed immunoblotting with an antibody against the ribosomal protein S6. We observed peaks of S6 both in fractions 5-6 and in the pellet (hereafter called granule fraction) consistent with the separation of polysomes from the denser compacted ribosomes of the RNA granule (Fig. 1B). Indeed, the granule fraction contained compacted ribosomes as observed under negative staining electron microscopy (Fig. 1C), consistent with previous descriptions of stalled polysomes (Darnell et al., 2011) and RNA granules (El Fatimy et al., 2016; Elvira et al., 2006; Krichevsky & Kosik, 2001).

To further validate our enriched preparation, we performed western blot analysis on the starting material, fraction 5-6 (polysomes) and granule fraction, examining RNA binding proteins (RBPs) known to be markers for stalled polysomes and RNA granules. For quantification, we standardized the levels of the RBPs to the levels of the ribosomal protein S6 to normalize for the number of ribosomes in each fraction. FMRP has been linked to stalled polysomes in several studies (Ceman et al., 2003; E. Chen et al., 2014; Darnell et al., 2011; El Fatimy et al., 2016; Graber et al., 2013) and was highly enriched in the granule fraction (Fig. 1D, E).

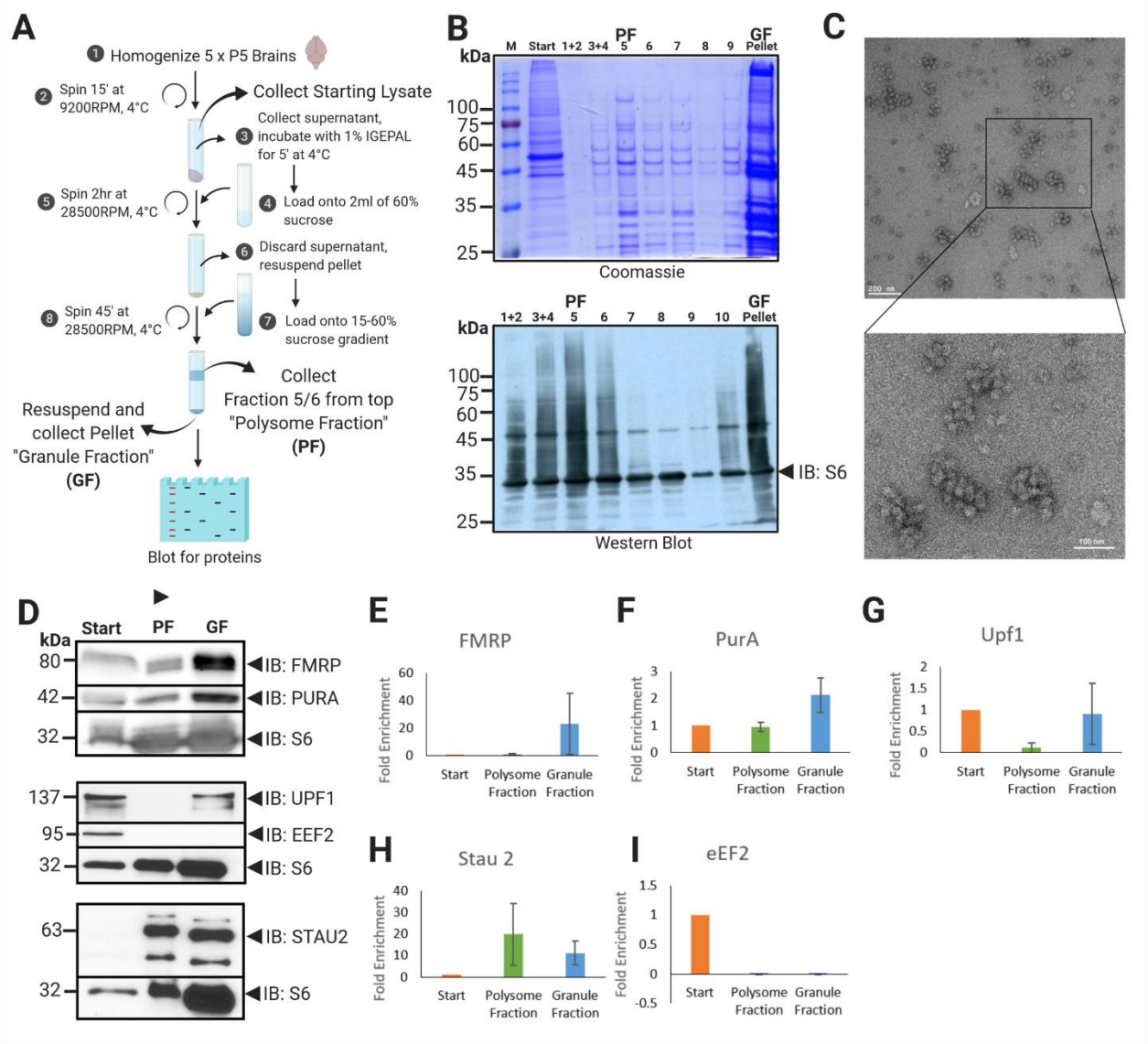


Figure 1. Isolation of a pellet enriched in compacted ribosomes.
(A) Summary of protocol for isolating the pellet from P5 rat whole brain homogenate using sucrose gradient fractionation. (B) Top: SDS-page stained with Coomassie brilliant blue showing enrichment of the characteristic distribution of ribosomal proteins in Fraction 5 (Polysome Fraction; PF) and Pellet (Granule Fraction; GF); Bottom: Immunoblot analysis demonstrating strong enrichment of S6 ribosomal protein in Fraction 5 (PF) and Pellet (GF); Lanes are described on top (M for molecular weight marker). P, pellet. (C) Negative stained electron micrograph of the Pellet fraction shows compacted ribosomes; inset showing close up of compacted ribosome structure (D) Immunoblot analysis of starting material, Polysome Fraction (PF; Fraction 5/6) and Granule Fraction (GF; Pellet) stained for RBPs and other factors implicated in RNA granules and stalled polysomes: FMRP, PURA, Upf1, Stau2 and eEF2. (E-I) Quantification of Western Blots. The fold enrichment compared to starting material normalized to levels of S6 (see methods) is shown for each RBP: FMRP (N=3), PURA (N=3), Upf1 (N=2), Stau2 (N=2), eEF2 (N=3). Error bars represent SEM.

Similarly Pur-alpha (PURA), a protein that marks RNA granules in neurons (El Fatimy et al., 2016; Kanai et al., 2004) whose loss leads to neurodevelopmental disorders (Reijnders et al., 2018), was significantly enriched in the granule fraction compared to polysomes (Fig. 1D, F). UPF1, the key component of the nonsense-mediated decay pathway (Kim & Maquat, 2019), has been shown to play an independent role in the formation of stalled polysomes in neurons (Graber et al., 2017) and is enriched in the granule fraction, compared to polysomes (Fig. 1D, G). UPF1 interacts with Staufen 2 (STAU2), and this interaction is important for the formation of stalled polysomes (Graber et al., 2017). However, while STAU2 was present in the granule fraction, it was more enriched in polysomes (Fig. 1D, H). This may be due to an important role of STAU2 in regulating other forms of translation (Heraud-Farlow & Kiebler, 2014). Interestingly, we were unable to detect the presence of the eukaryotic elongation factor 2 (eEF2) in the polysome or the granule fraction (Fig. 1D, I). Given the proposed role for eEF2 phosphorylation in the reactivation of stalled polysomes (McCamphill et al., 2015; Sossin & Costa-Mattioli, 2019), the absence of eEF2 suggests that this may be due to a requirement for eEF2 release from normal translating polysome to restart stalled polysomes. Alternatively, the lack of cycloheximide in our purification may have allowed eEF2 to diffuse away, since cycloheximide blocks the elongation step of translation potentially trapping eEF2 on polysomes. Overall, the protein composition of the granule fraction is consistent with an enrichment of RNA granules containing stalled ribosomes.

3.5.2 Cleavage of compacted ribosomes into monosomes

Ribosome profiling is based on nuclease digestion of mRNA that is not protected by ribosomes, isolation of monosomes containing the protected mRNA, followed by library construction and sequencing. Compacted ribosomes are resistant to cleavage by nucleases (Darnell et al., 2011). Based on the previous observation that high-salt conditions cause the unpacking of

the compacted ribosomes (El Fatimy et al., 2016), we treated the granule fraction with 400 mM of sodium chloride for 10 minutes before dilution to reduce the salt concentration back to physiological levels (150 mM) followed by incubation with RNase I. After RNase digestion we resedimented the fractions on a sucrose gradient to evaluate the digestion. We found that this treatment was able to cleave the compacted ribosome clusters to monosomes, measured based on the movement of S6 ribosomal protein immunoreactivity from the granule fraction to fraction 2 of the sucrose gradient (Fig. 2A-D). We observed that the high-salt treatment improved the digestion by nuclease at low nuclease concentrations, although some lots of nuclease led to complete cleavage even without the need for salt (Supp Fig. 1). At the higher concentration of nuclease (1 µl: 100 U), there was no difference in digestion in the presence or absence of salt (Fig 2A-D; Supp Fig. 1).

The effect of the high-salt and nuclease treatments was also visualized using negative staining electron microscopy (EM) (Fig 2E, F). Samples either untreated or treated with the high-salt buffer, nuclease (low concentration; 0.5 µl: 50 U) or both were deposited on the EM grids and negatively stained. Consistent with the sedimentation results, we observed that the high-salt and nuclease treatments, when applied separately, induced partial unpacking of the ribosome clusters. Only when both treatments were applied to the sample, the EM images showed that the ribosome clusters had dissociated into monosomes.

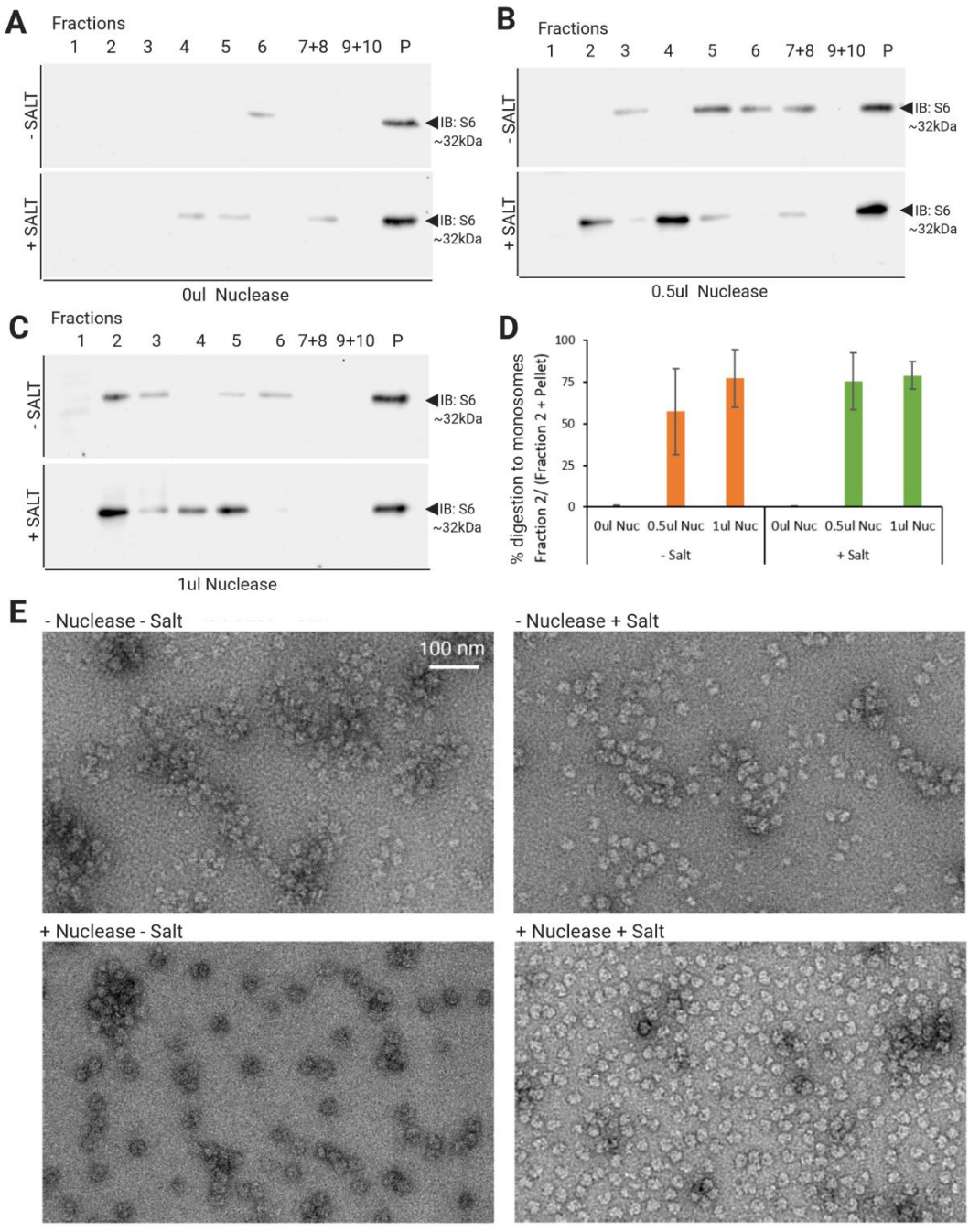


Figure 2. Cleavage of compacted stalled polysomes into monosomes
(A-C) Western Blot analysis for S6 ribosomal protein of fractions collected after first treating the pellet from the first sucrose gradient with 0ul (A), 0.5ul (B) and 1ul (C) RNase I, with or without pre-treatment of 400 mM NaCl (-SALT: TOP; +SALT: BOTTOM), followed by a second sucrose gradient (see Methods). (D) Generation of monosomes by digestion of pelleted granule fraction with RNase I. Quantification of digestion represented as the ratio of Fraction 2/(Fraction 2 + Pellet), N=4 biological replicates, error bars represent SEM. (E) Electron micrographs of negatively stained pellet fraction following treatment with RNase I, with and without pre-treatment with Salt (-Nuclease -Salt: TOP LEFT; -Nuclease +Salt: TOP RIGHT; +Nuclease – Salt: BOTTOM LEFT; +Nuclease +Salt: BOTTOM LEFT). Scale bar represents 100 nm. Note that EM images represent the pellet fraction treated with Nuclease and Salt prior to the second sucrose gradient, and not the purified monosomes in Fraction 2 that are quantified in the above Western Blots.

3.5.3 Ribosome Footprints of the mRNAs protected by ribosomes in the pellet fraction

After treatment with RNase I, the cleaved ribosomes from the granule fraction were loaded onto a second 15-60% sucrose gradient and centrifuged to separate the monosomes before RNA extraction, library preparation and sequencing of the footprint reads (Fig. 3A). As we are interested in stalled polysomes, no attempt was made to prevent ribosome run-off, and cycloheximide was not present during the tissue homogenization. We tested whether the presence or absence of cycloheximide has an impact on ribosome footprints (Supp. Fig 2). There was little effect of the omission of cycloheximide on the mRNAs detected by footprint reads as seen by a high Pearson's correlation and clustering by principal components analysis (PCA) between relative numbers of footprint reads in the presence or absence of cycloheximide (Supp. Fig 2). Similarly, nuclease digestion was performed after a brief treatment with high salt; while this treatment slightly improved the digestion (Fig. 2), the mRNAs detected by footprint reads were very similar to the salt untreated sample, again determined by a high Pearson's correlation and clustering by PCA (Supp Fig 2). For consistency, only the samples prepared in the absence of cycloheximide and the presence of salt during digestion were used for the results reported below. Overall, we generated five biological replicates of libraries of footprint reads from the cleaved compacted ribosomes in the pellet fraction and the results below are from averages of the five replicates after normalizing for transcript length and library size (Ritchie et al., 2015).

Ribosome profiling reads are usually generated from canonical fragment sizes between 28 and 34 nucleotides (Ingolia, 2014). Surprisingly, the peak read size (36-37 nucleotides) from the ribosome protected reads was larger than this canonical ribosome protected fragment (Fig. 3B). It has been previously reported that classical ribosomes produce medium sized 27-29 nucleotide

footprints and small 20-22 nucleotide footprints should the ribosome have an open A site (C. C. Wu, Zinshteyn, Wehner, & Green, 2019). The larger reads we observed may be due to (i) an altered state of the stalled polysome, (ii) increased protection due to associated RNA binding proteins, or (iii) incomplete digestion. It is also possible that even larger reads are present since the libraries were generated after running a gel to size select the ribosome protected RNAs (see Methods). The longer reads map better to the coding sequence (CDS) than shorter reads (Fig. 3C). Alignment at the start and stop codons showed that the excess length of the reads was due to extension at the 3'end of the footprint reads. While approximately 14 nt was protected at the 5' end, regardless of the length of the read, the extension at the 3'end increased with the read length giving a diagonal line on a plot of read length vs. distance from start or stop codon (Fig. 3D). Ribosome footprint reads should show periodicity due to the three nucleotide code in the mRNA and reads over 32 nt showed higher periodicity than the shorter reads (Fig. 3E). We had predicted that footprint reads from stalled polysomes would be most enriched at the stop codon and the 3' end of the message due to the requirement of UPF1 for the formation of stalled polysomes and the recruitment of UPF1 at the stop codon. However, we did not observe a bias for footprint reads near the stop codon. Instead, there was some bias in the large footprint reads for the first part of the message (Fig. 3F). Taken together, ribosome profiling of dissociated monosomes derived from compacted ribosomes in the RNA granule pellet from P5 rat brain reveals enrichment in large ribosomal footprints (>32 nt), which carry a prominent 3'end extension, display a preference for the first part of the mRNA CDS, and do not display a bias for the stop codon.

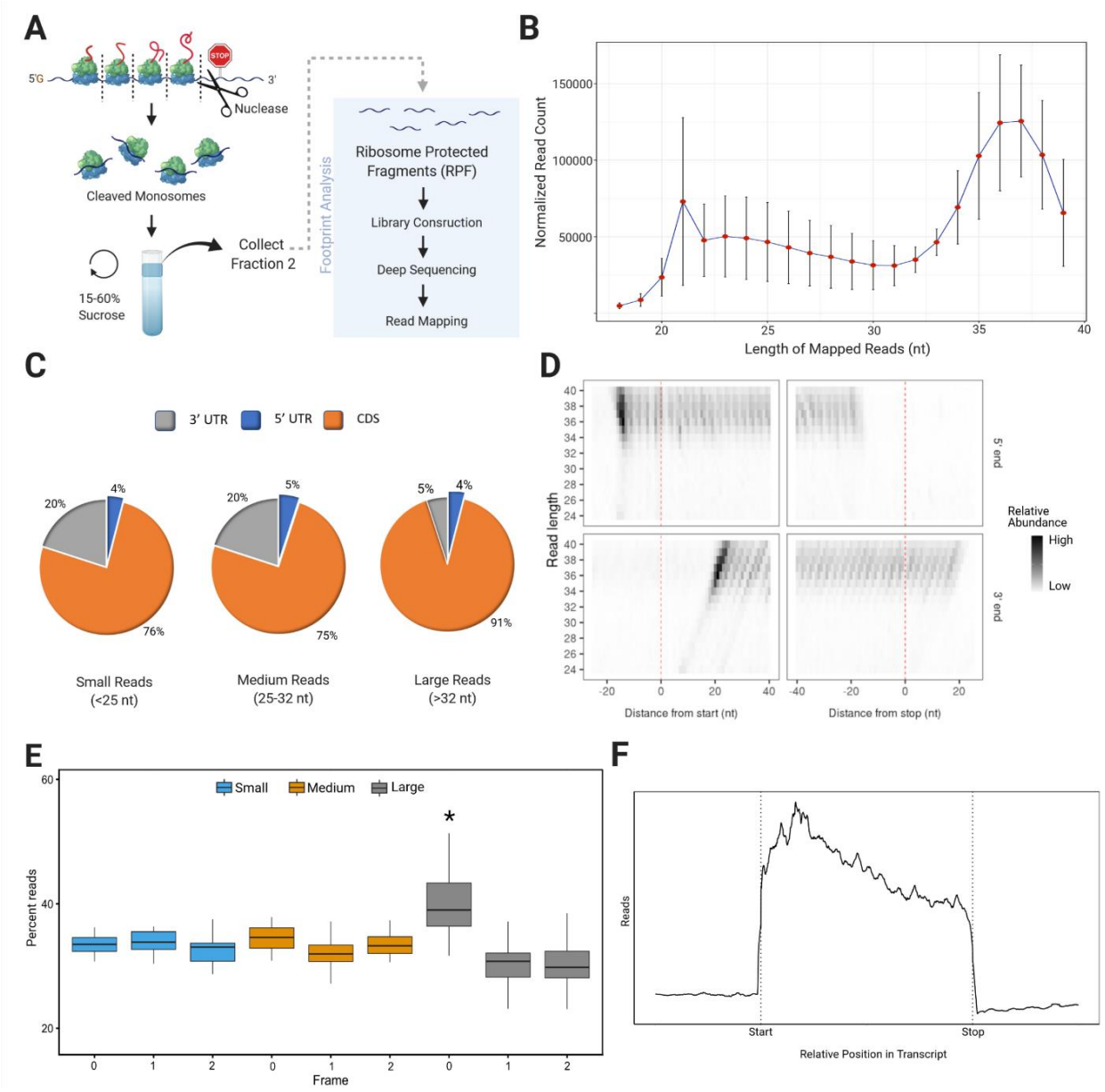


Figure 3. Ribosome footprinting of the pellet fraction

(A) Diagram summarizing Footprinting procedure. (B) Size distribution of normalized footprint reads, n=5 biological replicates, SEM. (C) Read coverage of different sized footprints (small <25 nt, medium 25-32 nt, large >32 nt) to 3'UTR, 5'UTR and CDS. (D) The number of read extremities (shading) for each read length (Y-axis) based on distance from start (left) and stop (right) with the beginning of the read (5') on top and the end of the read (3') on bottom. Data are shown for one biological replicate, but results are similar for all replicates. (E) Periodicity statistics indicate that long reads (33-40) in frame 0 have significantly more periodicity than frame 1 and frame 2 for long reads, or any frame for short (21-24) and medium reads (25-32) (ANOVA F (261,8) =13.9, $p<0.001$; Post-Hoc Tukey HSD test, Long reads in 0 frame*, $p<0.001$ against long reads in frame 1 and frame 2 and other reads in frame 0) SD, n=39 long, 40 medium, 11 short (N is based on each read length in each biological replicate; not all read lengths are present in all biological replicates); Errors are SD. (F). Distribution of large reads with the CDS of all transcripts normalized to the same length shows that reads are biased to the first half of the transcript. Data are shown for one biological replicate, but results are similar for all replicates.

3.5.4 Analysis of mRNAs that are abundant and enriched in footprint reads from compacted ribosomes

We were interested in identifying what mRNAs represent the most abundant constituent of compacted ribosomes and what mRNAs are enriched in compacted ribosomes compared to total mRNA levels. From the five biological replicates, we calculated the average for Abundance (reads per kb of mRNA; RPKM) of footprint reads to determine the most abundant constituents of the compacted ribosomes. To determine what mRNAs are enriched in these ribosomes, we calculated what is characteristically called the translation efficiency (abundance of ribosome footprints/abundance of total mRNA as determined by conventional RNA seq of the starting fraction) for each mRNA (Table 1). However, in the context of presumed stalled ribosomes, translation efficiency may be a misleading term, so we renamed this measure as enrichment for the present study. Gene Ontology (GO) analysis of the 100 mRNAs with the largest abundance showed significant over-representation of mRNAs encoding cytoskeletal proteins that are expressed developmentally in neuronal projection and synaptic compartments (Fig. 4A), including Map1b (Table 1), an mRNA we had previously shown to be translated in dendrites through reactivation of stalled polysomes (Graber et al., 2017; Graber et al., 2013). While cytoskeletal mRNAs dominate the most abundant GO category and are also enriched (Table 1), they are also relatively abundant in the total mRNA population. The GO analysis for enriched mRNAs showed a significant over-representation of mRNAs encoding RNA binding proteins or proteins involved in RNA metabolism (Fig. 4B), including the gene mutated in ALS; FUS (Table 2). These results were similar regardless of whether the 50, 200 or 500 most abundant or enriched mRNAs were selected for the analysis (Supp. Table 2,3)

Top 20 most enriched mRNAs

Gene Name	logFC	P-value	FDR	Total RNA RPKM	Footprints RPKM
FUS	3.16434772	6.49E-10	9.56E-08	69.7831423	337.199324
FAM168A	2.94304308	7.81E-07	1.60E-05	52.1322914	130.2335745
PRRT1	2.89433174	1.85E-06	3.16E-05	26.2478904	55.2638004
NCL	2.87065155	4.50E-13	4.21E-10	63.0689626	366.6690471
RBM25L1	2.67673894	5.59E-08	2.27E-06	4.69685407	22.57140369
HNRNPD	2.63687085	5.04E-10	8.14E-08	135.136829	590.9433885
GATAD2B	2.55905455	1.39E-08	8.75E-07	34.7720695	118.9687961
SOX9	2.45296112	1.01E-05	0.00012227	25.4556107	59.89774259
UBQLN2	2.43529701	4.11E-11	1.25E-08	101.635634	385.068685
HNRNPA2B1	2.43013679	5.74E-10	8.95E-08	158.368037	668.3078643
PRR36	2.41730546	1.44E-06	2.58E-05	23.624002	46.51721335
ZMIZ1	2.37502673	7.30E-07	1.53E-05	38.2509104	89.47685878
LOC103694210	2.3707282	2.29E-07	6.30E-06	16.8011259	76.08347195
TAF15	2.36234981	2.89E-07	7.61E-06	10.1106776	38.79032835
MNT	2.36190606	3.14E-08	1.55E-06	10.7221613	28.72871691
AHCYL1	2.36154326	3.68E-08	1.70E-06	59.2481984	150.2720528
HNRNPA3	2.35579919	1.63E-10	3.46E-08	53.2766435	197.1313486
LOC108348151	2.35324232	1.93E-05	0.00020574	11.6881118	46.27995067
VAMP2	2.30892138	4.34E-07	1.00E-05	473.446629	912.2714551
SF3A2	2.29351137	3.99E-07	9.54E-06	20.2483045	41.1681424

Table 1. Top 20 most enriched mRNAs
Top 20 enriched mRNAs sorted on the fold change (FC) in global footprint reads per kilobase per million reads (Footprint RPKM) compared to Total RNA reads per kilobase per million reads (Total RNA RPKM). mRNAs were excluded if the RPKM was predominantly due to reads less than 32 bp (>2 fold) or if the total RPKM was less than 5. Also shown are two evaluations of the significance of the fold change, the P value, and the false discovery rate (FDR). mRNAs highlighted in red match the GO term mRNA or RNA binding.

Top 20 most abundant mRNAs

Gene Name	logFC	P-value	FDR	Total RNA RPKM	Footprints RPKM
ACTB	1.940985547	1.53E-08	9.23E-07	3311.57669	9408.327001
TUBB5	1.033645345	1.37E-05	1.55E-04	3698.919325	7404.359382
TUBB2B	1.63257495	4.98E-07	1.13E-05	1913.684313	5570.961651
YWHAE	1.711033554	7.60E-09	5.65E-07	1688.064086	4751.885604
TUBB3	1.244833653	2.40E-07	6.48E-06	2052.960915	4594.904687
YWHAZ	1.05722464	1.71E-06	2.96E-05	2004.358037	4216.85062
STMN2	1.400937863	2.81E-06	4.44E-05	1692.26231	3719.643634
CALM1	0.154694995	4.87E-01	6.19E-01	3093.888941	3625.962055
DPYSL2	1.24010485	2.46E-09	2.57E-07	1568.286012	3524.363783
YWHAG	1.82197446	1.01E-09	1.32E-07	1166.611045	3340.758587
CALM3	-0.309008441	7.89E-02	1.58E-01	3403.092834	2994.609636
CALM2	0.862970381	7.06E-04	3.78E-03	1549.807564	2953.038226
UCHL1	1.003234991	4.34E-05	3.93E-04	1439.792879	2647.37674
DPYSL3	2.223810027	1.16E-10	2.71E-08	698.2863694	2518.842074
ZWINT	0.887865185	8.91E-04	4.56E-03	1426.858532	2492.284205
MAP1B	1.865404024	1.12E-10	2.71E-08	755.4364087	2259.892443
STMN1	0.273922938	2.08E-01	3.31E-01	1722.323579	2160.340529
GAP43	0.569199253	1.27E-03	6.09E-03	1409.864902	2067.364803
CRMP1	1.496025361	3.20E-09	3.00E-07	882.8247474	1959.479377
YWHAB	1.791487719	8.44E-09	6.04E-07	578.6707707	1808.787351

Table 2. Top 20 most abundant mRNAs
Top 20 most abundant mRNAs sorted on the Footprint reads per kilobase per million reads (RPKM) compared to Total RNA reads per kilobase per million reads (Total RNA RPKM). Also shown are the log fold change (Footprint RPKM compared to total RNA RPKM). Also shown are evaluations of the significance of the fold change (the P value and the False Discovery Rate (FDR). mRNAs highlighted in blue match the GO term for cytoskeleton constituent of cytoskeletal binding.

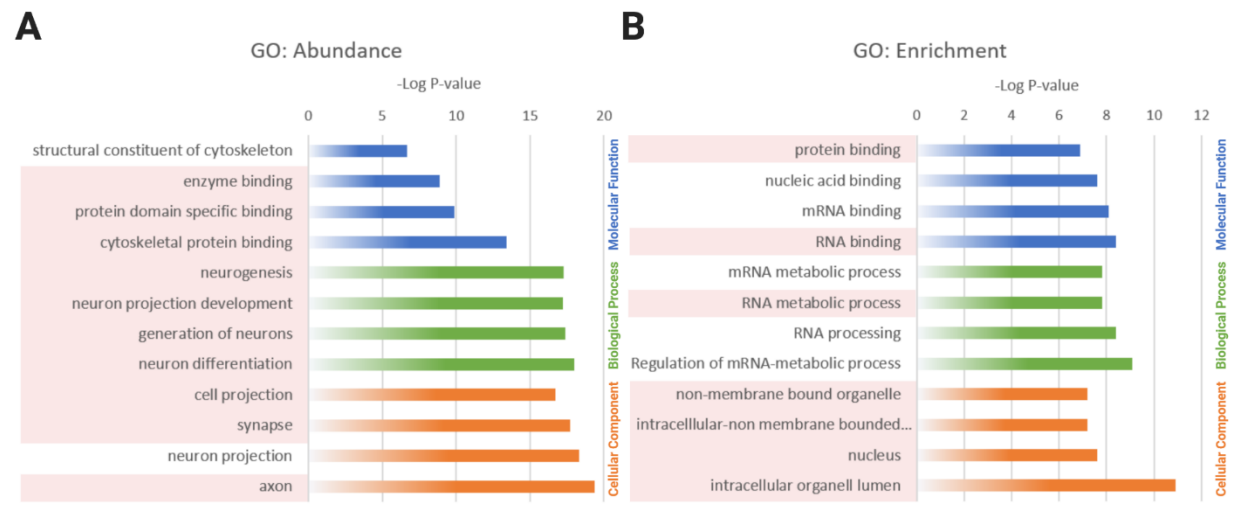


Figure 4. GO Analysis of footprint mRNAs
(A-B) Gene Ontology (GO) terms of selected comparisons for most abundant (A) and most enriched (B) mRNAs found in reads based on -Log P-values. Terms highlighted in red represent terms involving RNA binding for enrichment and cytoskeletal related terms for abundance. Complete table with all significant findings in Supp Table 2.

We next determined if mRNAs regulated by elongation or initiation in the nervous system were over-represented in our samples either through abundance or enrichment. Strikingly, mRNAs whose translation is regulated by elongation through eEF2 phosphorylation (Kenney et al., 2016) were significantly abundant and enriched in the footprint reads (Fig. 5A, B). In contrast, mRNAs regulated by initiation, through eIF4E phosphorylation (I. S. Amorim et al., 2018), TOR activation (including TOP mRNAs) (C. C. Thoreen et al., 2012) or through upstream ORFs after eIF2 alpha phosphorylation was increased by the stimulation of metabotropic glutamate receptors (Di Prisco et al., 2014), were not enriched in the preparation (Fig. 5A, B). While TOP mRNAs, mainly encoding ribosomal subunits are relatively abundant and known to be transported in neurons, we observed a significant de-enrichment of footprint reads from TOP mRNAs in footprint libraries of the compacted ribosomes (Fig. 5A), consistent with the notion that transported mRNAs blocked at initiation are not enriched in this preparation.

We next determined whether mRNAs that are predicted to be enriched in stalled polysomes are particularly enriched/abundant in the footprint reads of compacted ribosomes. A recent publication identified mRNAs protected by ribosomes resistant to ribosomal run-off in neuronal slices (Shah et al., 2020). While this publication focused on the proportion of each mRNA for which ribosomes ran-off, their data also identified the mRNAs with the most protected fragments remaining after a long period of run-off (60 min). The 200 runoff-resistant mRNAs with the most reads remaining at 60 minutes were significantly enriched and abundant in our preparation (Fig. 5C, D). FMRP, an RBP highly enriched in the sedimented pellet, has been associated with stalled polysomes (Ceman et al., 2003; Darnell et al., 2011). The mRNAs associated with FMRP in neurons, identified through cross-linking immunoprecipitation (CLIP), are also more resistant to ribosome run-off (Darnell et al., 2011).

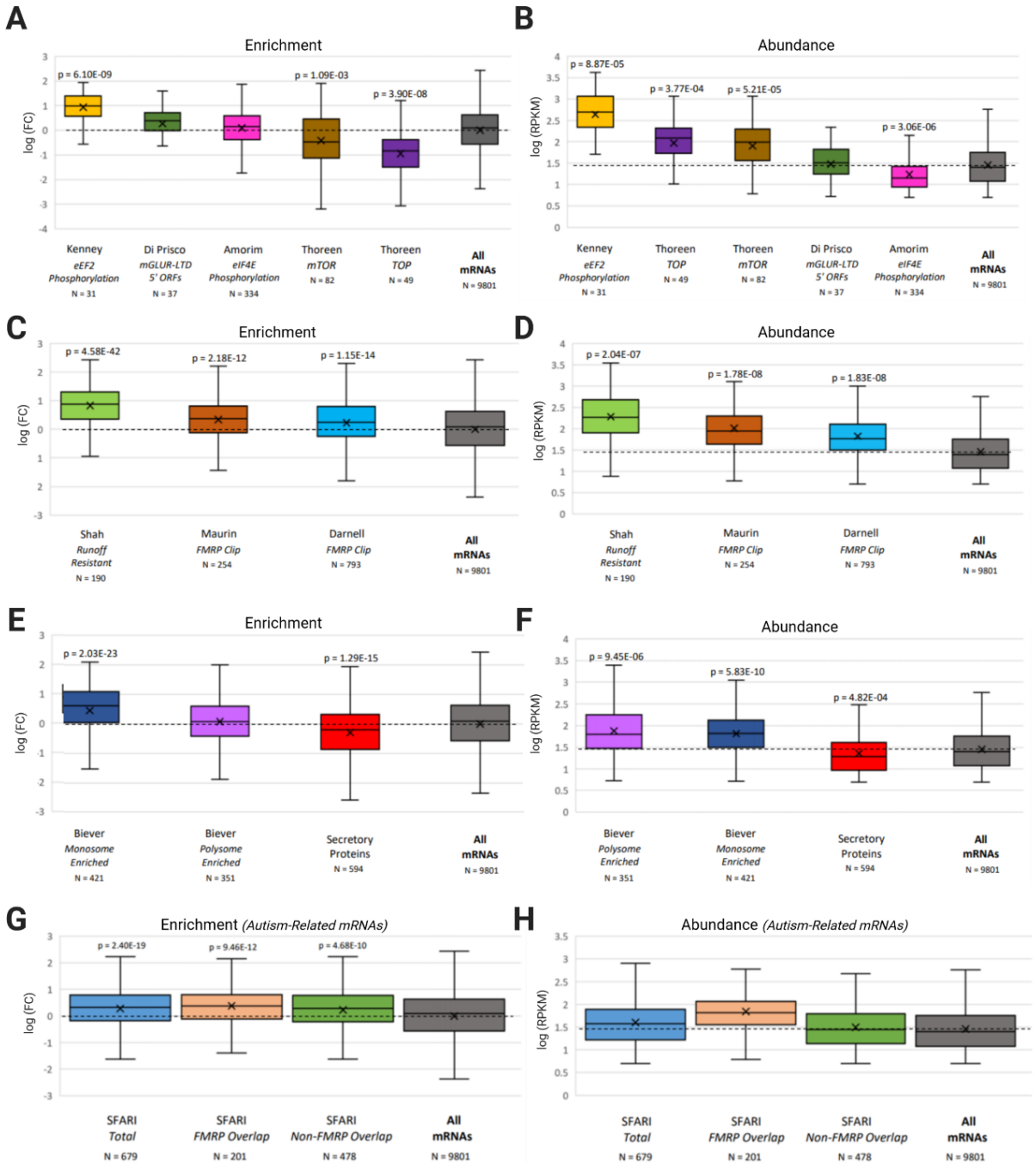


Figure 5. Correlation analysis of mRNAs abundant and enriched in ribosome footprints
(A) Enrichment and (B) Abundance comparison of footprint reads to mRNAs regulated by translation elongation (30) eIF4E phosphorylation (31) mTOR (32) TOP mRNAs (32) and mRNAs upregulated by mGluR with upstream open reading frames (33). (C) Enrichment and (D) Abundance comparison of runoff-resistant mRNAs (19) and mRNAs that are CLIPped by FMRP (17, 34). (E) Enrichment and (F) Abundance comparison of mRNAs translated preferentially by monosomal and polysomal mRNAs in the neuropil (35) and secretory mRNAs (secretory proteins with reviewed annotation from UNIPROT), compared to all mRNAs. (G-H) Enrichment and Abundance comparison of autism-related mRNAs from the SFARI database (syndromic and levels 1-3). The total SFARI group was also divided into ones that are also in the FMRP CLIP group (17,34). and ones that are not. For all groups there was a cut-off of 5 RPKM to avoid mRNAs not expressed in the nervous system. P values from comparison to all mRNAs (Students t test with Bonferroni correction for multiple tests (n=14 for all comparisons in figure). Only Significant P values ($p<0.01$ after correction are shown). The N for each comparison group is shown under the group.

We examined the abundance/enrichment of two separate FMRP CLIP studies from brain tissue and both were significantly enriched/abundant in our preparation (Darnell et al., 2011; Maurin et al., 2018) (Fig. 5C, D).

We also examined several other datasets to better evaluate the mRNAs with footprint reads in the pelleted compact ribosomes. Secreted proteins (and their associated mRNAs) are stalled by their signal peptide and then co-translationally inserted into the endoplasmic reticulum (ER). The transport of secreted mRNAs stalled at elongation would also involve the transport of ER in the RNA granules; while transportation of ER is possible, we suspected that secretory mRNAs would be de-enriched in the RNA granules and this was indeed the case (Fig. 5E). Recently, mRNAs that are preferentially translated from monosomes in neuronal processes were identified (Biever et al., 2020). While we predicted that these mRNAs would also be depleted from our preparation, they were significantly enriched (Fig. 5E), while mRNAs preferentially transported in polysomes were not significantly enriched (Fig. 5E). However, total mRNA levels for the preferentially polysomal transported mRNAs were significantly higher than the total mRNA levels for the preferentially monosomal translated mRNAs at this developmental timepoint (158 ± 17 polysome RPKM (n=327) vs. 72 ± 4 monosome RPKM (n=458), S.E.M, $p<0.001$ Student's t-test).

Since translation from stalled polysomes is implicated in neurodevelopmental disorders, we examined if protected reads from Autism-related genes from the Simons Foundation Autism Research Initiative (SFARI) database were enriched and abundant in the protected reads. These mRNAs were significantly enriched in our data compared to all mRNAs (Fig. 5G, H). There is a significant overlap with FMRP CLIPped mRNAs in this dataset, but both FMRP CLIPped SFARI mRNAs and non-CLIPped SFARI mRNAs were equally enriched in the protected reads (Fig. 5 G, H).

Finally, some of the enrichments we observed may be due to our preparation being specifically from the nervous system. To account for this bias, we repeated this analysis using only mRNAs known to be transported in neuronal processes (Biever et al., 2020). While the mRNAs known to be transported in neuronal processes were significantly enriched and abundant in our preparation (Supp Fig 3) when we restricted the total set of mRNAs to only include this set, all of the results above were replicated (Supp Fig 4) suggesting our results are not biased due to enrichment of neuronally transported mRNAs.

3.5.5 The ribosome protected reads are enriched in sequences matching FMRP CLIPs

If stalled polysomes are indeed enriched in the compacted ribosome-containing pellet, the footprint reads should help identify where on the mRNA ribosomes are stalled. Examination of the distribution of the large reads (>32 nt) on individual messages revealed highly non-uniform distribution of reads on mRNAs (Fig. 6A). As peaks in ribosome protected fragments are often non-reproducible (B. Liu et al., 2019), we used stringent criteria to identify peaks. First, peaks were defined for each mRNA in each library based on a maximum value higher than the average RPKM for the mRNA and a minimum width of 18 nucleotides at the half maximum height. Second, the peak had to be present in at least 3 of the 5 biological replicates (Fig. 6B; Supp Fig. 5). Using these stringent criteria, we identified 766 peaks in 524 mRNAs (Supp Table 4). While 90% of the mRNAs had only one or two peaks, Map1B, the mRNA most associated with stalled polysomes, had the largest number of identified peaks (10; Fig. 6A).

Contrary to our initial hypothesis that peaks representing stalled footprint reads would be clustered around the stop codon, only 6 of the 766 total peaks were at the stop codon, and, similar to the overall coverage of footprint reads, the peaks were biased to the first half of the message

with an average position of 0.37 ± 0.26 (SD) where 0 is the start codon, and 1 is the stop codon. The average length of the sequence within a peak was 36 nt ± 6 nt, similar to the most common footprint read size (Fig. 3B), and there was no clear evidence for the presence of collided ribosomes (peaks of twice the size of a ribosome protected fragments). We also identified peaks in the smaller reads, but only 65% of these peaks were in the CDS, and there was little overlap with the peaks in the long reads. In contrast, 94% of the peaks from large reads were in the CDS.

We next examined whether there were any consensus sequences in these peaks of footprint reads. We used an unbiased sequence motif search approach with the HOMER program (Heinz et al., 2010). HOMER uses relative enrichment and requires a background sequence. To remove usage bias for mRNA sequences, we used similarly sized fragments from mRNAs with no peaks as our background selection. The Homer program identified three highly significant consensus sequences in the peaks above the false discovery rate determined by the program (Fig. 6C). Notably, the most significant consensus sequence (p=1e-67) matched a consensus sequence (WGGA) previously derived from analyzing FMRP CLIP sequences (B. R. Anderson et al., 2016; M. Ascano, Jr. et al., 2012), which also overlapped with the consensus sites for m6A methylation (RGACH or RRACT) in the nervous system (Zhang et al., 2018) (Fig. 6D-F). The motif was not biased to the start or end of the protected reads (average position of motif in reads was 19 ± 9 bases) and thus does not represent sequences selected because they were difficult for nucleases to digest, since sequences selected due to resistance to digestion would be at the end or beginning of the read. Analysis of 36 nt in front of the peaks or 36 nt behind the peaks did not result in a HOMER consensus sequence above the false discovery rate determined by the program. Similarly, peaks identified using small or medium-sized reads also did not result in a HOMER consensus sequence above this cut-off.

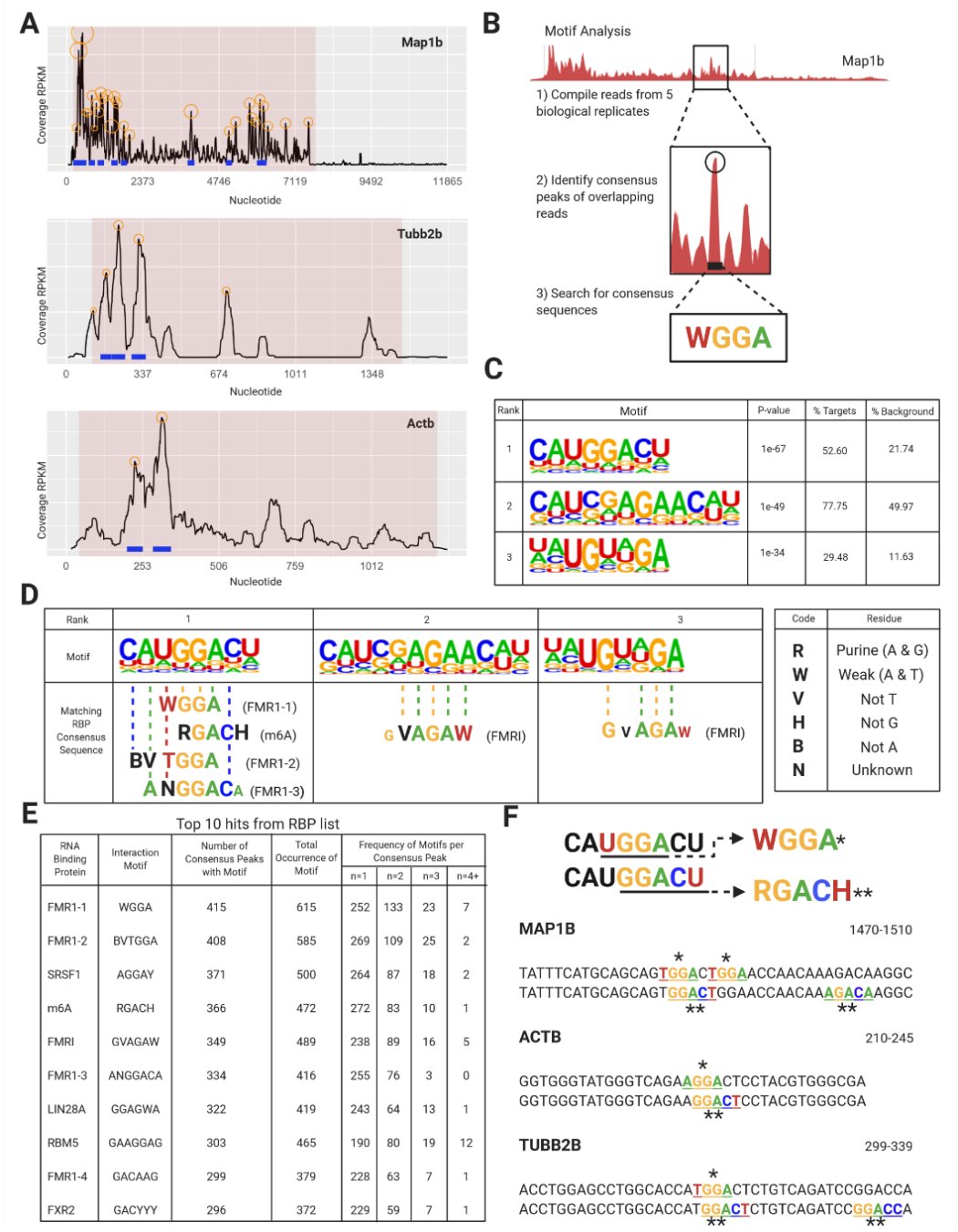


Figure 6. Sequences underlying ribosome protected fragments are enriched in sequences matching FMRP CliPs.
(A) mRNA profiles of Map1b, Beta-actin and Tubulin 2b showing reproducible consensus peaks in the CDS; circles represent consensus peaks of footprint reads mapping to the same sequence, blue lines represent reproducible consensus peaks across biological replicates, red shading represents CDS. All replicates are shown in Supp. Fig 5. (B) Diagram summarizing how motif analysis is done. (C) Results from the HOMER program showing the only 3 consensus sequences above the cut-off provided by HOMER. (D) Homer identified motifs overlapped with matching interaction motifs for RBPs listed in brackets. Residues that do not match are given in smaller font. RIGHT: Code key for residue annotation. (E) Table of top 10 RBPs with RNA interaction motifs present in consensus peaks. The number of peaks with multiple hits are also shown as Frequency of Motifs per Consensus Peak; n=x represents the number of motifs per consensus peak. All RBPs motifs examined are shown in in Supp. Table 4. (F) Top ranked consensus sequence from HOMER showing overlapping sites for interaction motifs WGGA and RGACH, and their corresponding residues on a single peak from Map1b, Beta-actin and Tubulin 2b. Both sequences given for each protein are identical, but they have been annotated to show clusters of motifs that map to the same consensus sequence. Numbers on the right indicate the location of the sequence in each mRNA that correspond to a consensus peak (blue) on the mRNA profiles shown in (A).

We also performed a directed search of the peaks for sequences matching consensus binding sites of RBPs (Van Nostrand et al., 2020) using FIMO (Grant, Bailey, & Noble, 2011). Consistent with the non-biased search, FMRP CLIP consensus sites had the most matches (Fig. 6D, E; Supp Table 5). Strikingly, these motifs mainly recognized purine-rich sequences (Fig. 6E; 76% of nucleotides in the top 10 RBP motifs are purines). However, the overall peak nucleotides are not enriched in purines compared to the background sequences (both at 57% purines). The abundance of purines in the consensus peaks suggests a possible role for the RBP PURA, a protein highly enriched in RNA Granules, but without a known consensus binding site. Of the 766 peaks, there were 415 peaks with a WGGA site, and also many peaks with multiple WGGA sites in the peak making a total of 615 WGGA sites in consensus peaks (Fig. 6E; Supp Table 4). The number of multiple matches (39% of peaks with a WGGA have multiple WGGA sites) was far in excess of those found in the corresponding background sequences (11 ± 1 %, from 10 separate selections of 750 background sequences with a WGGA peak). Of the 615 WGGA sites found in the peaks, 182 WGGA sites overlapped with an m6A site (examples shown in Fig. 6F). This percentage of WGGA sites overlapping with an RGACH site (30%) was significantly more than the number of overlaps seen in WGGA sites in our background samples (19 + 1.3%; 10 separate selections of 1300 WGGA sites from background sequences). Moreover, there were also many examples where m6A sites and WGGA sites were in the same peak but did not overlap. Of the 415 peaks with a WGGA site, 315 (76%) had an m6A consensus site in the same peak significantly more than were found in the background sample (39 ± 4%, 10 separate selections of 750 background sequences with a WGGA peak).

There were also many matches to other FMRP CLIP consensus sites (Fig. 6D). After including GVAGAW and GACAAG over 80% of the 766 consensus peaks contained an FMRP

CLIP sequences or m6A consensus sites suggesting a strong sequence bias for these sites in the regions of the mRNA enriched in footprint reads (Supp Table 4).

3.6 Discussion

3.6.1 The granule fraction containing compacted ribosomes is an enriched preparation for stalled ribosomes

Previous reports showed that increased density could be used to segregate compacted ribosomes from normal polysomes (Aschrafi et al., 2005; El Fatimy et al., 2016; Elvira et al., 2006; Krichevsky & Kosik, 2001). However, these studies did not show that these compacted ribosomes are stalled. An association between stalled polysomes and compacted ribosomes was first made based on FMRP association (Darnell et al., 2011), however in that study the RNA granules were not isolated by sedimentation. Here, we show that the sedimented pellet (granule fraction) containing compacted ribosomes is not only enriched for FMRP (as had been previously shown (El Fatimy et al., 2016)), but also for UPF1, an additional protein functionally implicated in stalled polysomes (Graber et al., 2017). Moreover, mRNAs that were previously shown to resist ribosomal run-off by initiation inhibitors (Shah et al., 2020), or to be regulated at the elongation stage (Kenney et al., 2016) were over-represented in the footprint reads generated from this fraction. All of these findings are consistent with the granule fraction enriching for ribosomes that were stalled in neurons.

It should be noted that this preparation represents a snapshot of P5 brains. This sedimentation protocol is complicated by the presence of myelin at later stages of development (El Fatimy et al., 2016). Still, if feasible, it is likely that the mRNAs found in the granule fraction would be different at the adult stage since many of mRNAs with the most abundant footprint reads

that we found in this fraction are developmentally implicated in neuronal outgrowth. A related protocol developed for E18 rat brains showed fewer ribosomes when performed in adults (Elvira et al., 2006). However, stalled polysomes and some of the mRNAs isolated here (notably Map1b) are implicated in mGluR-LTD, a plasticity that is present mainly in mature brains (Nosyreva & Huber, 2005). Thus, it is plausible that the role of stalled polysomes may change as the brain develops.

3.6.2 Cytoskeletal and RNA binding proteins represent abundant and enriched mRNAs, respectively

Axon and dendrite outgrowth, coupled with synapse formation, occur at high rates in P5 rat brains (Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013). Indeed, the most abundant footprint reads are on cytoskeletal mRNAs and mRNAs encoding proteins that are highly enriched in growth cones (such as 14-3-3 proteins) (Kent et al., 2010). Indeed, the most abundant number of footprint reads is found on beta-actin, whose local translation in both axons and dendrites is important for neuronal outgrowth (Eom, Antar, Singer, & Bassell, 2003; K. M. Leung et al., 2006). Somewhat more surprising was the high enrichment for RNA binding proteins in the footprint reads (Fig. 4). This suggests an important homeostatic aspect for translation from stalled polysomes, where the increased translation of RBPs will have critical effects on the translation of other messages not necessarily present in stalled polysomes.

3.6.3 Identification of conserved motifs enriched in footprint read peaks from compacted ribosomes.

The finding that the footprint reads derived from the compacted ribosome granule fraction are distributed mainly in large peaks is consistent with the enrichment of stalled ribosomes in this fraction. These peaks are strikingly enhanced in sequences previously defined as enriched in

FMRP CLIPs (Fig. 6). This is consistent with the strong enrichment of FMRP in the granule fraction and the abundance and enrichment of mRNAs previously identified as associated with FMRP using CLIP experiments (Fig. 5). While most of the FMRP CLIP consensus sequences have not been directly shown to bind to FMRP, the WGGA sequence can be directly bound by FMRP (M. Ascano, Jr. et al., 2012). However, the major FMRP binding sites (G quartets and Kissing sequence) are not enriched in FMRP CLIP consensus sites (B. R. Anderson et al., 2016). Moreover, if ribosomes protect this sequence, it is unclear how FMRP would gain access to these sequences. However, it is possible that FMRP initially binds this sequence and this is followed by ribosome occupation and stalling. It is also possible that these sequences are enriched in FMRP CLIPs because FMRP is specifically associated with stalled ribosomes and these sequences specify where ribosomes would be stalled independently of FMRP. In this scenario, the sequences would not be directly bound by FMRP. Instead, FMRP would be crosslinked to sequences near to, but not protected by the ribosome. Since the CLIP sequences are approximately 100 bp, this is entirely consistent with both the CLIP and ribosome footprint read data.

The sites are also enriched in a consensus site for m6A modification. Interestingly, mRNAs with m6A sites are selectively transported in neurons (Merkurjev et al., 2018) and this methylation plays an important role in neurodevelopment (Widagdo & Anggono, 2018). Moreover, mRNAs that are associated with FMRP by CLIP experiments have previously been shown to be highly enriched for m6A modifications in neurons (Zhang et al., 2018) and this has been proposed to play a role in FMRP-mediated nuclear export (Hsu et al., 2019; Westmark, Maloney, Alisch, Sokol, & Lahiri, 2020). However, whether FMRP directly binds to m6A, interacts with m6A readers, or is associated with m6A through some other indirect interaction is not clear. There has been some indication that m6A directly leads to the stalling of ribosomes (Choi et al., 2016) or it may be that

some m6A reader in neurons is important for the stall. Again, it is unclear how the reader would get access to the mRNA sequences protected by the ribosome, but similar to FMRP the sequence may be recognized first and then later occupied by the ribosome. It will be interesting in the future to determine the specific relationship between m6A methylation and stalled polysomes and whether initial findings of specific roles for m6A methylation in the developing brain are linked to their possible role in stalling translation in RNA granules.

3.6.4 Stalled Polysomes or Stalled ribosomes?

Stalled polysomes would be predicted to show a series of protected reads following the initial stalled peak. These peaks upstream of the stall site, corresponding to ribosomes stalled behind the leading ribosome, would not be predicted to show any specificity (consensus sequences), as the leading ribosome dictates the stall. If, for example, four ribosomes were stalled behind the leading ribosome, this would mean only 20% of the ribosomes would be stalled on a stalling sequence. This model does not fit the data we observe. The majority of consensus peaks contain sites previously observed for FMRP Clips or m6A consensus sites, and there is little periodicity in the placement of peaks.

We did not search for disome or higher periodicity ribosome footprints (Han et al., 2020), as we performed ribosome footprints on purified monosomes. However, we did not observe other signs of collided ribosomes, such as periodic peaks or a size of peaks corresponding to two or more ribosomes. Indeed, ribosomes that are stalled due to collision with a downstream ribosome recruit a collection of specific factors to resolve the stall and rescue the ribosomes (Buskirk & Green, 2017). These factors are not present in proteomics of RNA granules (El Fatimy et al., 2016; Elvira et al., 2006; Kanai et al., 2004) and indeed, if these stalled ribosomes are meant to be transported and reactivated, they need to be protected from the surveillance mechanisms normally used for

prematurely stopped or collided ribosomes, which lead to unstalling and/or degradation of the proteins (Buskirk & Green, 2017). Our data is consistent with a model in which a controlled form of stalling attracts specific factors in neurons, such as FMRP, that likely play a role in the compaction of the stalled ribosome/polysome and package the structure into a granule for transport before collisions occur. Thus, the compacted ribosomes may not represent collided ribosomes on a polysome, but collections of ribosomes from distinct mRNAs that are packaged together. Indeed, the mRNAs that are preferentially translated by monosomes in dendrites are enriched in this compacted ribosome pellet. The packaging of many distinct mRNAs in the same mRNA granule is consistent with the finding that *in situ* RNA granules containing stalled polysomes probably contain many distinct mRNAs (Langille et al., 2019).

3.7 Conclusions

While there have been assumed links between the compacted ribosomes that sediment in sucrose gradients, stalled polysomes identified by resistance to ribosomal run-off in neuronal dendrites and FMRP's association with stalled polysomes, there were previously no direct connections between these various lines of research. Identifying an enrichment for FMRP CLIP consensus sequences in protected reads in compacted ribosomes from the pellet of sucrose gradients establishes these links. Notably, most investigations of translation regulation in neuronal tissues do not consider the pellet fraction after separation of polysomes using sucrose gradients. Thus, they are not accounting for this pool of translationally repressed mRNAs. For ribosome profiling from the initial polysome pellet, the use of translation efficiency needs to be re-evaluated, because many of the ribosomes in this initial pellet are presumably stalled. Moreover, some studies only include footprints of the canonical size and may exclude the larger size footprints that we

observed in this preparation. Thus, these results have large implications for the interpretation of many studies of neuronal translation.

3.8 Materials and Methods

3.8.1 Purification of RNA Granules.

RNA Granules were purified, from whole brain homogenate harvested from five-day-old (P5) Sprague Dawley rats, using a protocol adapted from a previous purification (El Fatimy et al., 2016). Five P5 rat brains were homogenized in RNA Granule Buffer (20 mM TRIS-HCl pH 7.4 [Fisher; BP152-1], 150 mM NaCl [Fisher; BP358-212], 2.5 mM $MgCl_2$ [Fisher; M33-500]) supplemented with 1 mM DTT [Sigma; D9163], 1 mM EGTA [Sigma; E8145], EDTA-free protease inhibitor [Roche; 04693132001]. Note that cycloheximide [Abcam; ab120093] was not added to the homogenization buffer unless explicitly stated. Homogenate was centrifuged 15 minutes in a Thermo Scientific T865 fixed angle rotor at 9200 rpm (6117 RCF) at 4˚C to spin down cellular debris. The supernatant was treated with 1% IGEPAL CA-630 [Sigma; I8896] for 5 minutes at 4˚C on a rocker. The sample was then loaded onto a 2 ml 60% sucrose [Calbiochem; 8550] cushion (dissolved in supplemented RNA Granule Buffer) in a Sorvall 36 ml tube [Kendro; 3141, Thermo Scientific], filled to top with additional RNA Granule Buffer and centrifuged for 2 hours in a Thermo Scientific AH-629 swing-bucket rotor at 28500 rpm (56660 RCF) at 4˚C to achieve the polysome pellet. The pellet was re-suspended in RNA Granule Buffer, gently dounced and loaded over a 15-60% linear sucrose gradient (gradient was made with RNA Granule Buffer) that was prepared in advance using a gradient maker [Biocomp Gradient Master], and centrifuged for 45 minutes at 28500 rpm (56660 RCF) at 4˚C in an AH-629 swing bucket rotor. Fractions of 3.5 ml were then collected from the top, and the remaining pellet was rinsed once and then

resuspended using RNA Granule Buffer. For some experiments, such as Electron Microscopy, the resuspended pellet was used directly, or treated with salt and nuclease to break up the ribosome clusters into monosomes (see below). For some experiments, such as Ribosome Footprinting, the fractions were precipitated overnight at -20 ˚C by adding 7 ml of chilled 100% ethanol. The precipitated samples were then centrifuged for 45 minutes at 3900 RPM (2177 RCF) at 4˚C in an Eppendorf 5810/5810 swing bucket rotor before collection using RNA Granule Buffer.

3.8.2 Nuclease and Salt Treatments.

To break up the stalled polysomes compacted in the RNA Granule into monosomes for ribosome footprint analysis, we treated the pellet fraction with salt and nuclease. The pellet was incubated with RNA granule buffer containing 400 mM NaCl for 10 minutes at 4˚C on a rocker(El Fatimy et al., 2016). Before nuclease treatment, the NaCl concentration was reduced back to 150 mM by diluting the sample with a NaCl-free RNA Granule Buffer. The sample was then treated with 100 U of RNase I [100 U/µl; Ambion AM2294, Thermo Fisher] for 30 minutes at 4˚C on a rocker. The nuclease was quenched with 100 U of SuperaseIN [20 U/µl; Invitrogen #AM2696, Thermo Fisher], and the samples were re-run on a fresh 15-60% sucrose gradient to separate mononsomes. Fraction 2 was precipitated overnight at -20 ˚C by adding 7 ml of chilled 100% ethanol. The precipitated samples were then centrifuged for 45 minutes at 3900 RPM (2177 RCF) at 4˚C in an Eppendorf 5810/5810 swing bucket rotor before collection using RNA Granule Buffer.

3.8.3 Electron microscopy

The untreated pellet fraction, or after treatment with either high-salt or nuclease or both treatments were deposited on the EM grids. The ribosome concentration of each sample was adjusted to ~80 ng/ml (~25 nM) using RNA Granule Buffer before applying them to the grids. In the case of the sample treated with both nuclease and high salt the concentration of sample applied

to the grid was 9.2ng/ml (2.9 nM). We used 400-mesh copper grids freshly coated with a continuous layer of thin carbon for these experiments. Grids were glow-discharged at 5 mA for 15 seconds and then floated on a 5-µl drop of the diluted sample for 2 min. Excess of sample was blotted away with filter paper (Whatman #1), and to stain them, they were subsequently floated in a 5-µl drop of a 1% uranyl acetate solution for 1 min. Excess of stain was blotted away, and the grids were dried on air and store in regular grid boxes. The EM images were acquired on a Tecnai F20 electron microscope operated at 200 kV using a room temperature side entry holder. Images were collected in a Gatan Ultrascan 4000 4 k × 4 k CCD Camera System Model 895 at a nominal magnification of 60,000x. Images produced by this camera had a calibrated pixel size of 1.8 Å/pixel. The total electron dose per image was ~50 e-/Å2. Images were collected using a defocus of approximately −2.7 µm. Images were prepared for figures using the Adobe Photoshop program.

3.8.4 Immunoblotting and Quantification of Enrichment.

For immunoblotting, SDS sample buffer was added to each sample before loading onto a 10% acrylamide gel. The resolved proteins were either stained with Coomassie brilliant blue [Fisher; 821616] or transferred onto a 0.45 µm nitrocellulose membrane (Bio Rad; 1620115) for immunoblotting. The membranes were blocked with 5% BSA (Sigma; 9647) in Tris Buffered Saline with Tween (TBS-T [Tris: Fisher, BP152-1; NaCl: Fisher, BP358-212; Tween: Fisher, BP337]) before incubation with the following primary antibodies in 1:1000 dilution: rabbit anti-S6 [Cell Signaling #2217], rabbit anti-FMRP [Cell Signaling; #4317], rabbit anti-eEF2 [Cell Signaling; #2332S], rabbit anti-Upf1 [Abcam; ab133564], mouse anti-Stau2 [Medimabs; MM0037-P], rabbit anti-PurA [Abcam; ab79936]. Membranes were washed with TBS-T after incubation. Detection was done using HRP-conjugated secondary antibodies [Invitrogen] before ECL [Perkin Elmer; NEL105001EA] reaction and imaging using the Bio Rad ChemiDoc digital

imager. For quantification of RBP enrichment, membranes were stripped with a Western Blot Stripping Buffer [ZmTech Scientific; S208070] and re-probed with rabbit anti-S6 [Cell Signaling #2217] antibody, followed by detection with HRP-conjugated secondary antibodies [Invitrogen].

Quantification of signal intensity was done using ImageJ software. We selected full lane ROIs and quantified single bands corresponding to the observed kDa size of each protein. We then used the corresponding anti-S6 signal intensity to quantify the amount of examined protein per S6 ribosomal protein for each fraction. For each experiment the protein/S6 value was normalized to the protein/S6 value from the starting material. Following this normalization, biological replicates were averaged. For salt and nuclease experiments, we calculated the proportion of Granule Fraction Polysomes (Pellet) digested into monosomes (Fraction 2) by doing the following calculation: Fraction 2/(Fraction 2 + Pellet). In one experiment we added Fraction 1 and 2 together because we detected some residual monosomes in Fraction 1, likely caused by spillover of Fraction 2 from pipetting.

3.8.5 Footprint sequencing library construction and sequencing.

Fraction 2 from the monosome purification was centrifuged at 4,444 x g at 4 °C for 60 min, the isopropanol taken off, the pellet air-dried, and re-suspended in 10 mM Tris-HCl pH 7. We then proceeded with a standard hot phenol-acid extraction (Acid Phenol:Chloroform mix 125:24:1, Invitrogen, AM9722). The samples were resuspended in nuclease-free water and quantified on a Spectrophotometer.

All samples were depleted of ribosomal RNA using the Ribo-Zero Gold (Human/Mouse/Rat) Kit (Illumina) (replicates 1-3 and cycloheximide treated) or the NEBNext® rRNA Depletion Kit (Human/Mouse/Rat)(E6350, NEB) (replicates 4-5 and no salt treatment) The footprint samples were size-selected using the 17 and 34 nt markers as a guide on a 15% TBE-Urea polyacrylamide

gel (Thermo Scientific), while total RNA samples were randomly heat fragmented by incubating at 95 °C in an alkaline fragmentation solution (50 mM $NaCO_3$ pH 9.2, 2 mM EDTA) for 40 min, yielding comparably sized fragments to footprints. All samples were de-phosphorylated using PNK (T4, NEB). The quality and concentration of samples was assessed by running an Agilent Small RNA chip (Agilent Technologies), and sequencing libraries were generated using the NEXTflex™ Small RNA Sequencing Kit v3 (PerkinElmer, NOVA-5132-06), according to the manufacturer's instructions. Samples were balanced and pooled for sequencing with Edinburgh Genomics on NovaSeq S1/2 flow cells yielding 50 bp paired-end reads.

3.8.6 Riboseq and RNAseq data analysis

Adaptor sequences and low-quality score containing bases (*Phred score < 30*) were trimmed from reads using Cutadapt v2.8 (*-j 8 -u 4 -u -4 -Z -o*) (M. Martin, 2011). Noncoding RNAs were removed by custom scripts following mapping these contaminant reads using Bowtie2 v2.3.5 (*--phred33 --very-sensitive*) (Langmead & Salzberg, 2012). The unmapped reads were aligned to reference rat genome (Rnor_6.0.94) using *STAR v2.7.3a (Dobin et al., 2013)*with options previously described (Biever et al., 2020) (*--twopassMode Basic --twopass1readsN -1 --seedSearchStartLmax 15 --outSJfilterOverhangMin 15 8 8 8 -- outFilterMismatchNoverReadLmax 0.1*). QuantMode with STAR was used to obtain genomic and transcript coordinates (Dobin et al., 2013). Assigning RPF reads to genomic features (CDS, UTRs) was based on Genome annotation (Rnor_6.0.94). Only a single transcript isoform, with the highest APPRIS score (Rodriguez et al., 2013), was considered per gene. All raw reads from these experiments have been deposited at the GEO dataset at NCBI (number to be confirmed).

Raw counts obtained using *featurecounts v2.2.0* (Liao, Smyth, & Shi, 2014) were analyzed using the R package *limma* (Ritchie et al., 2015). From this package, transcript abundance was

obtained for riboseq and RNAseq data in reads per kb of mRNA (RPKM). Enrichment was determined for each transcript by dividing riboseq RPKM by RNAseq RPKM. False discovery rates (FDR) and p values were determined for enrichment in the package, and nominal p-values were corrected for multiple testing using the Benjamini-Hochberg method.

Gene Ontology (GO) enrichment analysis was performed with *gProfileR* (Reimand et al., 2016). Enrichment p-values were based on a hypergeometric test using the set of known Rat genes as background. Sample correlation based on normalised read count was obtained using the R package *limma* (Ritchie et al., 2015). We used Ribowaltz (Lauria et al., 2018) to assign length dependant P-site correction and periodicity assignment to each reads.

3.8.7 Peak identification

Sites enriched with RPF were identified using normalized riboseq profiles using peak identification function within *IDPmisc*, a R package (Harrell Jr & Dupont, 2019). A peak width of minimum 18 nt and peak height above the mean peak height within the transcript was used as the criteria to define a region with significant enrichment of RPFs. Only peaks region that overlap (at least 90% nt overlap) in at least 3 samples were considered using BEDtools v2.29.2 (Quinlan & Hall, 2010)

3.8.8 Motif analysis

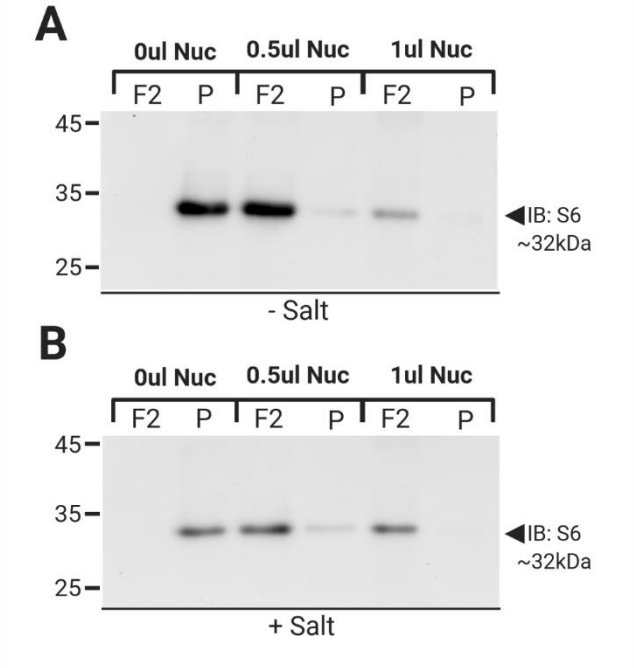
The peak regions were scanned for known human RNA-binding protein motifs using the *FIMO* program (Grant et al., 2011) which is part of MEME Suite (Bailey et al., 2009). Only search results with a p-value less than the threshold of 0.5 were considered. De novo motif finding was performed using peak from RPFs. *HOMER* tool (Heinz et al., 2010) was used for this analysis (*findMotifs.pl -rna)*. Background sequences were randomly selected from transcripts with no peaks.

3.9 Acknowledgements

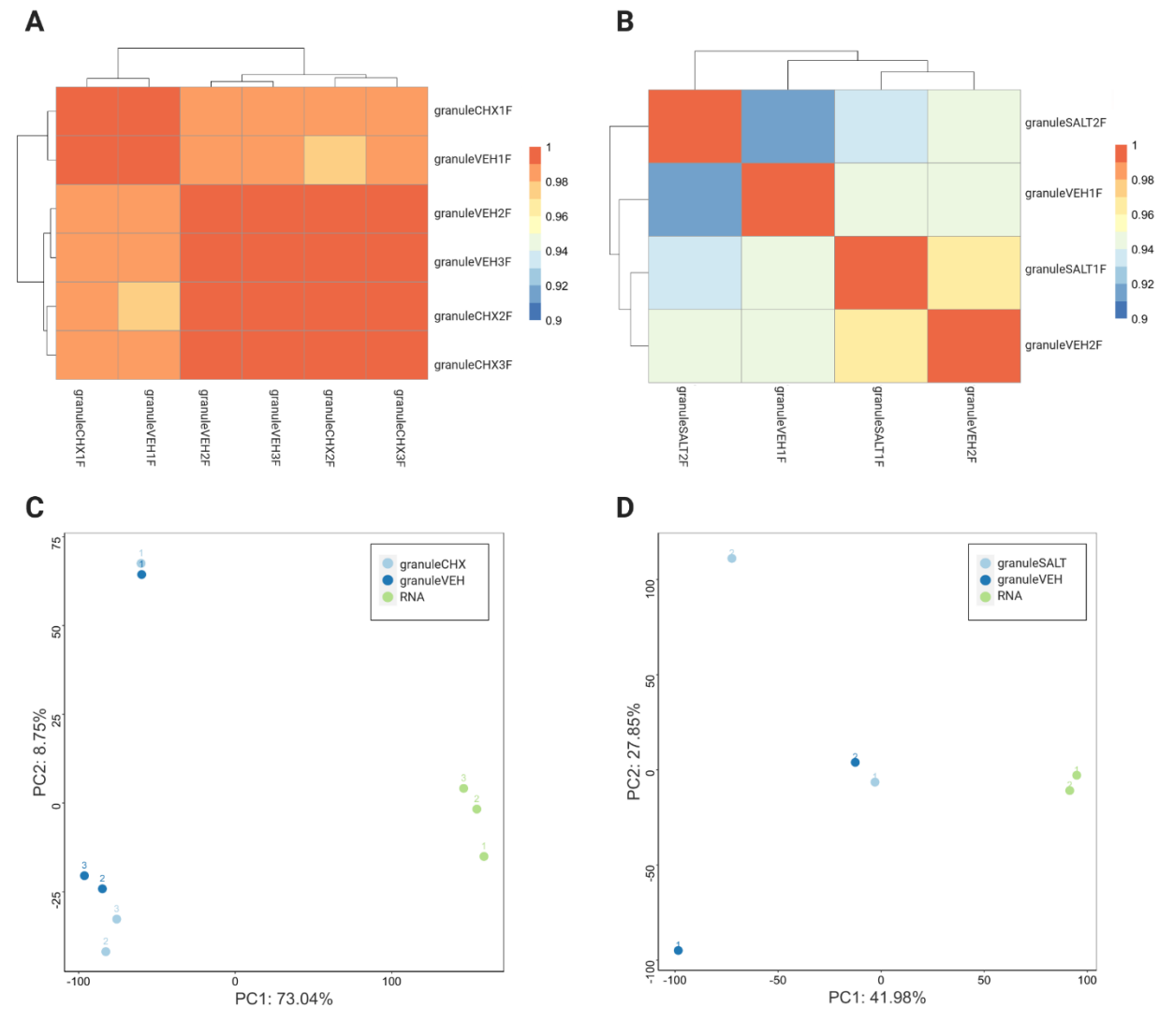
This work was supported by Canadian Institute of Health Research (CIHR) project grant 374967 to W.S.S, an award from the Azrieili foundation to WSS and JO, and by a Fondation Santé grant and a FORTH-IMBB-BR start-up grant to CGG. W.S.S. is a James McGill Professor and SMJ is supported by a Queen's University Belfast Patrick Johnston Research Fellowship. We thank staff at the Facility for Electron Microscopy Research (FEMR) at McGill University. FEMR is supported by the Canadian Foundation for Innovation, the Quebec government, and McGill University. Figures were created using Biorender.com

3.10 Supplemental Figures

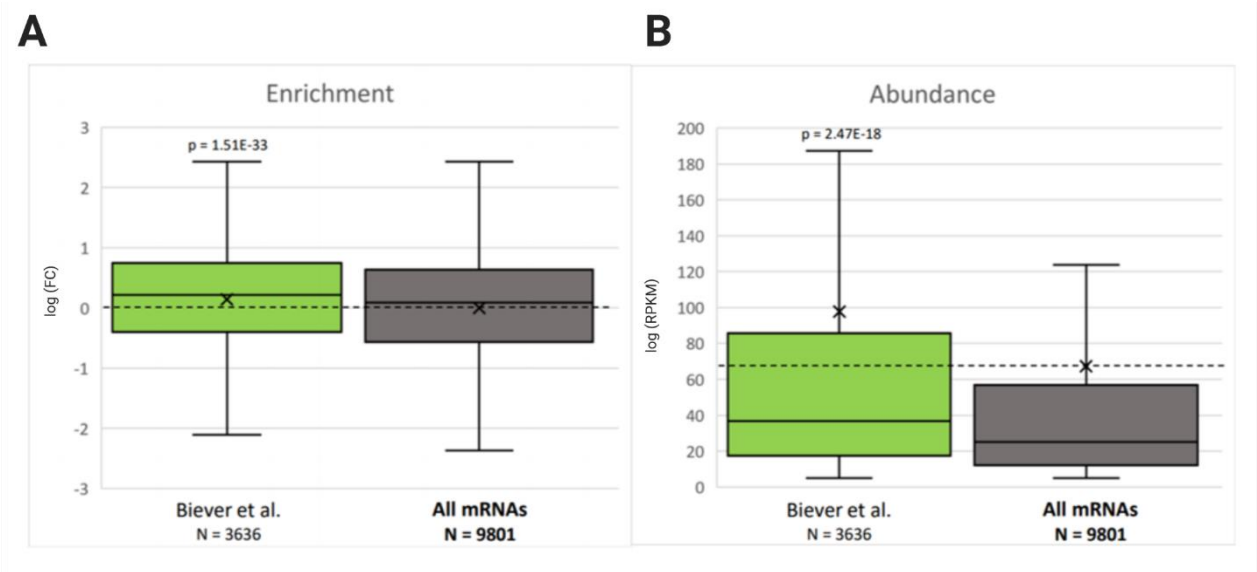
The complete sequencing data of footprint reads from this study can be found in Excel file format on biorxiv.org at https://doi.org/10.1101/2021.02.22.432349. This document was not appended directly to this Thesis because of the large size of the file.



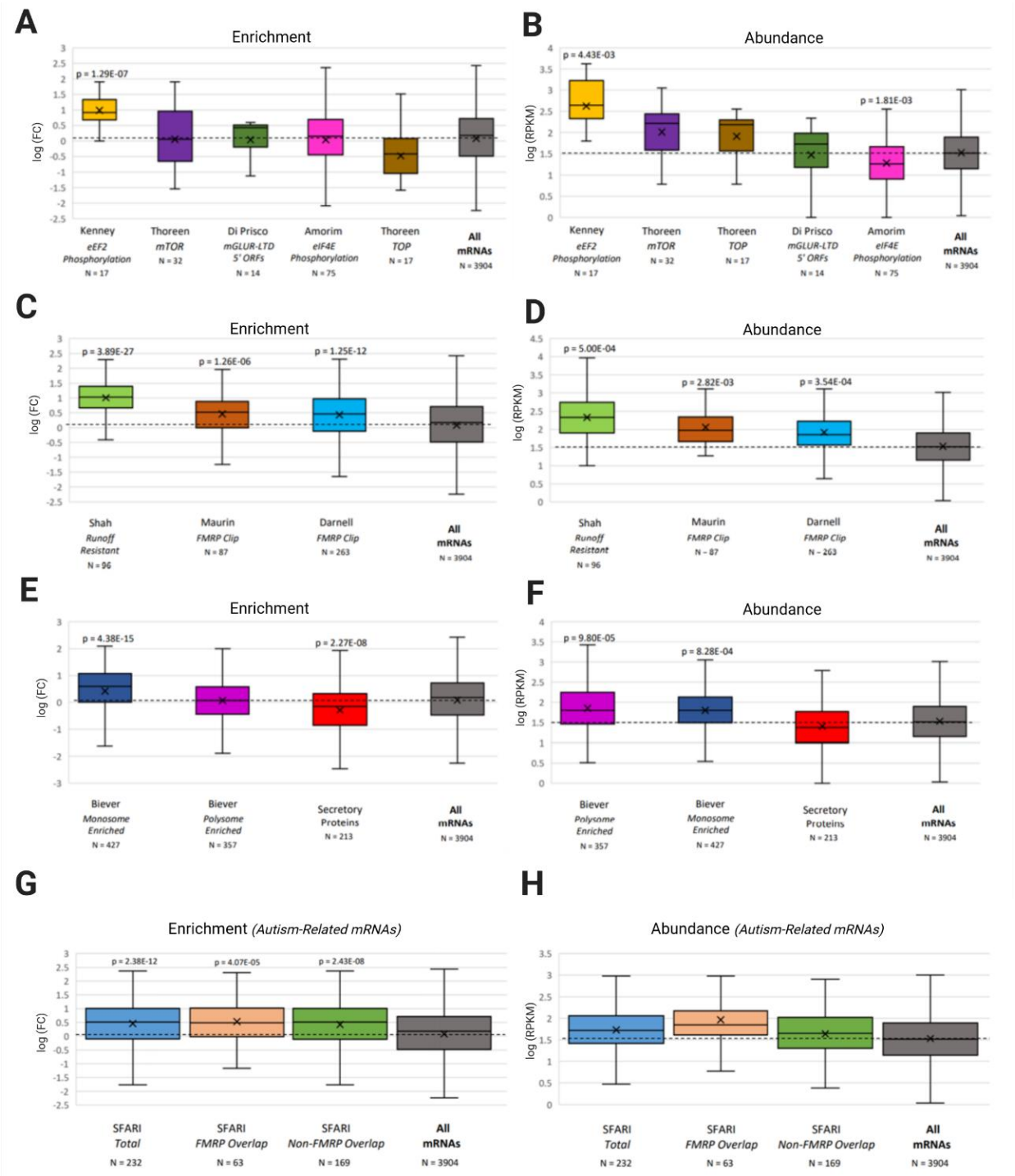
Supplemental Figure 1. Complete digestion of pellet fraction.
Western Blot analysis for S6 ribosomal protein of purified fractions collected after first treating the pellet from the first sucrose gradient with 0ul (A), 0.5ul (B) and 1ul (C) RNase I, with or without pre-treatment of 400 mM NaCl (-SALT: TOP; +SALT: BOTTOM), followed by a second sucrose gradient (see Methods). Only fraction 2 was loaded in this experiment. In this example, almost complete digestion even with lower concentrations of nuclease and no salt was observed. Increased nuclease digestion appeared to be related to different batches of nuclease and different amounts of time nuclease was stored, but this was not systematically analyzed.



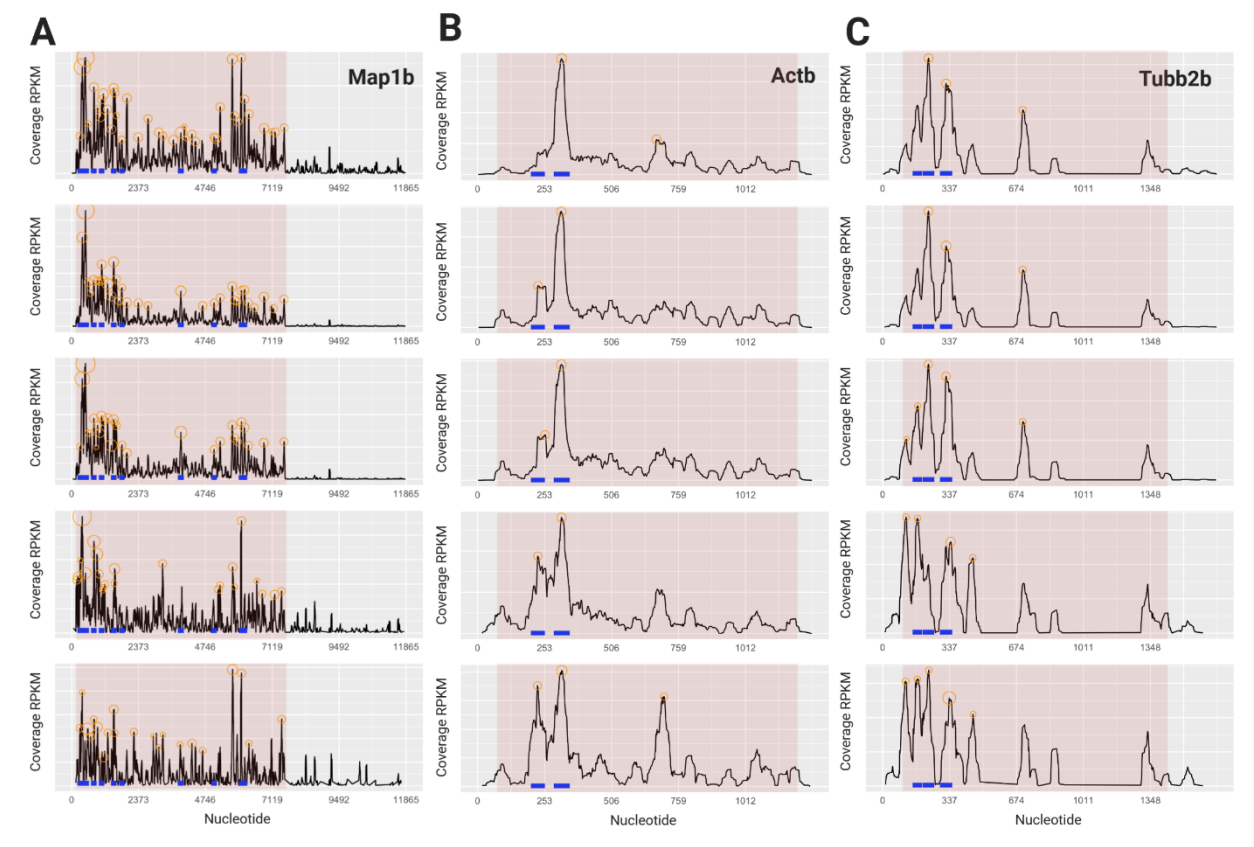
Supplemental Figure 2. No effect of Salt or Chx treatment on RNA reads
(A-B) Heat map for the comparison of biological replicates accomplished in the presence or absence of cycloheximide (Chx) (A) or for the comparison of biological replicates accomplished in the presence or absence of salt (B). Warmer colors indicating higher correlation between groups. Differences between biological samples was equal to or higher than the differences seen with treatment (C-D) Principal component analysis for the comparison of biological replicates accomplished in the presence or absence of cycloheximide (Chx) including RNA-SEQ of starting material (C) or for the comparison of biological replicates accomplished in the presence or absence of salt including RNA-SEQ of starting material (D). Differences between biological samples was equal to or higher than the differences seen with treatment. The footprint reads were clustered separately from the RNA-SEQ of total mRNA. All calculations were done with the Limma program (28).



Supplemental Figure 3. Enrichment and Abundance compared to transported mRNAs
(A) Enrichment and (B) Abundance comparison of footprint reads to mRNAs classified as transported to distal sites as determined (35). For all groups there was a cut-off of 5 RPKM to avoid mRNAs not expressed in the nervous system. P values from comparison to all mRNAs (Students t test with Bonferroni correction for multiple tests (n=2 for all comparisons in figure). Only Significant P values ($p<0.01$ after correction are shown). The N for each comparison group is shown under the group.



Supplemental Figure 4. Comparisons only using neuronal transported mRNAs
(A) Enrichment and (B) Abundance comparison of footprint reads to mRNAs regulated by translation elongation (30) eIF4E phosphorylation (31) mTOR (32) TOP mRNAs (32) and mRNAs upregulated by mGluR with upstream open reading frames (33). (C) Enrichment and (D) Abundance comparison of runoff-resistant mRNAs (19) and mRNAs that are CLIPped by FMRP (17, 34). (E) Enrichment and (F) Abundance comparison of mRNAs translated preferentially by monosomal and polysomal mRNAs in the neuropil (35) and secretory mRNAs (secretory proteins with reviewed annotation from UNIPROT), compared to all mRNAs. (G-H) Enrichment and Abundance comparison of autism-related mRNAs from the SFARI database (syndromic and levels 1-3). The total SFARI group was also divided into ones that are also in the FMRP CLIP group (17,34). and ones that are not. For all groups there was a cut-off of 1RPKM to avoid mRNAs not expressed at this point in development. P values from comparison to all mRNAs (Students t test with Bonferroni correction for multiple tests (n=14 for all comparisons in figure). Only Significant P values ($p<0.01$ after correction are shown). The N for each comparison group is shown under the group.



Supplemental Figure 5. Read Maps of all 5 biological replicates.
(A-C) Footprint read maps for Map1b (A), Beta-actin (B) and Tubulin 2b (C) for 5 biological replicates. Circles represent consensus peaks of footprint reads mapping to the same sequence, blue lines represent reproducible consensus sequences across biological replicates. Criteria for identifying consensus sequences (blue lines) is that at least 3 of 5 biological replicates have a consensus peak at this site. Red shading represents coding sequence (CDS).

3.11 References

Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C., & Schuman, E. M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron, 30*(2), 489-502. doi:10.1016/s0896-6273(01)00295-1

Amorim, I. S., Kedia, S., Kouloulia, S., Simbriger, K., Gantois, I., Jafarnejad, S. M., . . . Gkogkas, C. G. (2018). Loss of eIF4E Phosphorylation Engenders Depression-like Behaviors via Selective mRNA Translation. *J Neurosci, 38*(8), 2118-2133. doi:10.1523/JNEUROSCI.2673-17.2018

Anadolu, M. N., Kailasam, S., Simbriger, K., Sun, J., Markova, T., Jafarnejad, S. M., . . . Sossin, W. S. (2021). Ribosomes in RNA granules are stalled on mRNA sequences that are consensus sites for FMRP association. *bioRxiv*, 2021.2002.2022.432349. doi:10.1101/2021.02.22.432349

Anadolu, M. N., & Sossin, W. S. (2020). Focusing on mRNA granules and stalled polysomes amidst diverse mechanisms underlying mRNA transport, mRNA storage and local translation. In W. Sossin (Ed.), *Oxford Hanbook of Neuronal Protein Synthesis*. Oxford Press.

Anderson, B. R., Chopra, P., Suhl, J. A., Warren, S. T., & Bassell, G. J. (2016). Identification of consensus binding sites clarifies FMRP binding determinants. *Nucleic Acids Res, 44*(14), 6649-6659. doi:10.1093/nar/gkw593

Anderson, P., & Kedersha, N. (2006). RNA granules. *The Journal of Cell Biology, 172*(6), 803-808. doi:10.1083/jcb.200512082

Arpat, A. B., Liechti, A., De Matos, M., Dreos, R., Janich, P., & Gatfield, D. (2020). Transcriptome-wide sites of collided ribosomes reveal principles of translational pausing. *Genome Res, 30*(7), 985-999. doi:10.1101/gr.257741.119

Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J. B., Nusbaum, J. D., Corcoran, D. L., . . . Tuschl, T. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature, 492*(7429), 382-386. doi:10.1038/nature11737

Ascano, M., Mukherjee, N., Bandaru, P., Miller, J. B., Nusbaum, J. D., Corcoran, D. L., . . . Tuschl, T. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature, 492*(7429), 382-386. doi:10.1038/nature11737

Aschrafi, A., Cunningham, B. A., Edelman, G. M., & Vanderklish, P. W. (2005). The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci U S A, 102*(6), 2180-2185. doi:10.1073/pnas.0409803102

Athar, Y. M., & Joseph, S. (2020). The Human Fragile X Mental Retardation Protein Inhibits the Elongation Step of Translation through Its RGG and C-Terminal Domains. *Biochemistry, 59*(40), 3813-3822. doi:10.1021/acs.biochem.0c00534

Atkin, A. L., Altamura, N., Leeds, P., & Culbertson, M. R. (1995). The majority of yeast UPF1 co-localizes with polyribosomes in the cytoplasm. *Mol Biol Cell, 6*(5), 611-625. doi:10.1091/mbc.6.5.611

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., . . . Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res, 37*(Web Server issue), W202-208. doi:10.1093/nar/gkp335

Banerjee, S., Neveu, P., & Kosik, K. S. (2009). A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron, 64*(6), 871-884. doi:10.1016/j.neuron.2009.11.023

Barbarese, E., Koppel, D. E., Deutscher, M. P., Smith, C. L., Ainger, K., Morgan, F., & Carson, J. H. (1995). Protein translation components are colocalized in granules in oligodendrocytes. *Journal of Cell Science, 108*(8), 2781-2790.

Barbee, S. A., Estes, P. S., Cziko, A. M., Hillebrand, J., Luedeman, R. A., Coller, J. M., . . . Ramaswami, M. (2006). Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron, 52*(6), 997-1009. doi:10.1016/j.neuron.2006.10.028

Bartley, C. M., O'Keefe, R. A., Blice-Baum, A., Mihailescu, M. R., Gong, X., Miyares, L., . . . Bordey, A. (2016). Mammalian FMRP S499 Is Phosphorylated by CK2 and Promotes Secondary Phosphorylation of FMRP. *eNeuro, 3*(6). doi:10.1523/ENEURO.0092-16.2016

Bartley, C. M., O'Keefe, R. A., & Bordey, A. (2014). FMRP S499 is phosphorylated independent of mTORC1-S6K1 activity. *PLOS ONE, 9*(5), e96956. doi:10.1371/journal.pone.0096956

Batish, M., van den Bogaard, P., Kramer, F. R., & Tyagi, S. (2012). Neuronal mRNAs travel singly into dendrites. *Proc Natl Acad Sci U S A, 109*(12), 4645-4650. doi:10.1073/pnas.1111226109

Bauer, K. E., Segura, I., Gaspar, I., Scheuss, V., Illig, C., Ammer, G., . . . Kiebler, M. A. (2019). Live cell imaging reveals 3'-UTR dependent mRNA sorting to synapses. *Nat Commun, 10*(1), 3178. doi:10.1038/s41467-019-11123-x

Becalska, A. N., & Gavis, E. R. (2009). Lighting up mRNA localization in Drosophila oogenesis. *Development, 136*(15), 2493-2503. doi:10.1242/dev.032391

Bian, F., Chu, T., Schilling, K., & Oberdick, J. (1996). Differential mRNA transport and the regulation of protein synthesis: selective sensitivity of Purkinje cell dendritic mRNAs to translational inhibition. *Mol Cell Neurosci, 7*(2), 116-133. doi:10.1006/mcne.1996.0009

Biever, A., Glock, C., Tushev, G., Ciirdaeva, E., Dalmay, T., Langer, J. D., & Schuman, E. M. (2020). Monosomes actively translate synaptic mRNAs in neuronal processes. *Science, 367*(6477). doi:10.1126/science.aay4991

Bogorad, A. M., Lin, K. Y., & Marintchev, A. (2017). Novel mechanisms of eIF2B action and regulation by eIF2α phosphorylation. *Nucleic Acids Res, 45*(20), 11962-11979. doi:10.1093/nar/gkx845

Bramswig, N. C., Ludecke, H. J., Hamdan, F. F., Altmuller, J., Beleggia, F., Elcioglu, N. H., . . . Wieczorek, D. (2017). Heterozygous HNRNPU variants cause early onset epilepsy and severe intellectual disability. *Hum Genet, 136*(7), 821-834. doi:10.1007/s00439-017-1795-6

Buskirk, A. R., & Green, R. (2017). Ribosome pausing, arrest and rescue in bacteria and eukaryotes. *Philos Trans R Soc Lond B Biol Sci, 372*(1716). doi:10.1098/rstb.2016.0183

Buxbaum, A. R., Wu, B., & Singer, R. H. (2014). Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science, 343*(6169), 419-422. doi:10.1126/science.1242939

Cajigas, I. J., Tushev, G., Will, T. J., tom Dieck, S., Fuerst, N., & Schuman, E. M. (2012). The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron, 74*(3), 453-466. doi:10.1016/j.neuron.2012.02.036

Campbell, D. S., & Holt, C. E. (2001). Chemotropic Responses of Retinal Growth Cones Mediated by Rapid Local Protein Synthesis and Degradation. *Neuron, 32*(6), 1013-1026. doi:10.1016/S0896-6273(01)00551-7

Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., . . . Kandel, E. R. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell, 99*(2), 221-237. doi:10.1016/s0092-8674(00)81653-0

Ceman, S., O'Donnell, W. T., Reed, M., Patton, S., Pohl, J., & Warren, S. T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet, 12*(24), 3295-3305. doi:10.1093/hmg/ddg350

Chen, E., Sharma, M. R., Shi, X., Agrawal, R. K., & Joseph, S. (2014). Fragile X mental retardation protein regulates translation by binding directly to the ribosome. *Mol Cell, 54*(3), 407-417. doi:10.1016/j.molcel.2014.03.023

Choi, J., Ieong, K. W., Demirci, H., Chen, J., Petrov, A., Prabhakar, A., . . . Puglisi, J. D. (2016). N(6)-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. *Nat Struct Mol Biol, 23*(2), 110-115. doi:10.1038/nsmb.3148

Cioni, J. M., Koppers, M., & Holt, C. E. (2018). Molecular control of local translation in axon development and maintenance. *Curr Opin Neurobiol, 51*, 86-94. doi:10.1016/j.conb.2018.02.025

Cook, D., Sanchez-Carbente Mdel, R., Lachance, C., Radzioch, D., Tremblay, S., Khandjian, E. W., . . . Murai, K. K. (2011). Fragile X related protein 1 clusters with ribosomes and messenger RNAs at a subset of dendritic spines in the mouse hippocampus. *PLOS ONE, 6*(10), e26120. doi:10.1371/journal.pone.0026120

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. *Neuron, 61*(1), 10-26. doi:10.1016/j.neuron.2008.10.055

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., . . . Darnell, R. B. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell, 146*(2), 247-261. doi:10.1016/j.cell.2011.06.013

David, A., Bennink, J. R., & Yewdell, J. W. (2013). Emetine optimally facilitates nascent chain puromycylation and potentiates the ribopuromycylation method (RPM) applied to inert cells. *Histochem Cell Biol, 139*(3), 501-504. doi:10.1007/s00418-012-1063-8

Davidkova, G., & Carroll, R. C. (2007). Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors

in hippocampus. *J Neurosci, 27*(48), 13273-13278. doi:10.1523/JNEUROSCI.3334-07.2007

Di Prisco, G. V., Huang, W., Buffington, S. A., Hsu, C. C., Bonnen, P. E., Placzek, A. N., . . . Costa-Mattioli, M. (2014). Translational control of mGluR-dependent long-term depression and object-place learning by eIF2alpha. *Nat Neurosci, 17*(8), 1073-1082. doi:10.1038/nn.3754

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., . . . Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics, 29*(1), 15-21. doi:10.1093/bioinformatics/bts635

Dynes, J. L., & Steward, O. (2012). Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation. *J Comp Neurol, 520*(14), 3105-3119. doi:10.1002/cne.23073

El Fatimy, R., Davidovic, L., Tremblay, S., Jaglin, X., Dury, A., Robert, C., . . . Khandjian, E. W. (2016). Tracking the Fragile X Mental Retardation Protein in a Highly Ordered Neuronal RiboNucleoParticles Population: A Link between Stalled Polyribosomes and RNA Granules. *PLoS Genet, 12*(7), e1006192. doi:10.1371/journal.pgen.1006192

Eliscovich, C., Buxbaum, A. R., Katz, Z. B., & Singer, R. H. (2013). mRNA on the move: the road to its biological destiny. *J Biol Chem, 288*(28), 20361-20368. doi:10.1074/jbc.R113.452094

Elvira, G., Wasiak, S., Blandford, V., Tong, X. K., Serrano, A., Fan, X., . . . Sossin, W. S. (2006). Characterization of an RNA granule from developing brain. *Mol Cell Proteomics, 5*(4), 635-651. doi:10.1074/mcp.M500255-MCP200

Enam, S. U., Zinshteyn, B., Goldman, D. H., Cassani, M., Livingston, N. M., Seydoux, G., & Green, R. (2020). Puromycin reactivity does not accurately localize translation at the subcellular level. *Elife, 9*, e60303. doi:10.7554/eLife.60303

Eom, T., Antar, L. N., Singer, R. H., & Bassell, G. J. (2003). Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J Neurosci, 23*(32), 10433-10444.

Fabian, M. R., & Sonenberg, N. (2012). The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol, 19*(6), 586-593. doi:10.1038/nsmb.2296

Fontes, M. M., Guvenek, A., Kawaguchi, R., Zheng, D., Huang, A., Ho, V. M., . . . Martin, K. C. (2017). Activity-Dependent Regulation of Alternative Cleavage and Polyadenylation During Hippocampal Long-Term Potentiation. *Sci Rep, 7*(1), 17377. doi:10.1038/s41598-017-17407-w

Foster, K. G., & Fingar, D. C. (2010). Mammalian Target of Rapamycin (mTOR): Conducting the Cellular Signaling Symphony*. *Journal of Biological Chemistry, 285*(19), 14071-14077. doi:https://doi.org/10.1074/jbc.R109.094003

Franks, T. M., & Lykke-Andersen, J. (2008). The control of mRNA decapping and P-body formation. *Mol Cell, 32*(5), 605-615. doi:10.1016/j.molcel.2008.11.001

Fritz, S., & Boris-Lawrie, K. (2015). The RNPs of eukaryotic translation control. *Trends in Cell and Molecular Biology, 10*, 105-130.

Fritzsche, R., Karra, D., Bennett, K. L., Ang, F. Y., Heraud-Farlow, J. E., Tolino, M., . . . Kiebler, M. A. (2013). Interactome of two diverse RNA granules links mRNA localization to translational repression in neurons. *Cell reports, 5*(6), 1749-1762. doi:10.1016/j.celrep.2013.11.023

Garber, K. B., Visootsak, J., & Warren, S. T. (2008). Fragile X syndrome. *Eur J Hum Genet, 16*(6), 666-672. doi:10.1038/ejhg.2008.61

Gardiner, A. S., Twiss, J. L., & Perrone-Bizzozero, N. I. (2015). Competing Interactions of RNA-Binding Proteins, MicroRNAs, and Their Targets Control Neuronal Development and Function. *Biomolecules, 5*(4), 2903-2918. doi:10.3390/biom5042903

Genuth, N. R., & Barna, M. (2018). Heterogeneity and specialized functions of translation machinery: from genes to organisms. *Nat Rev Genet, 19*(7), 431-452. doi:10.1038/s41576-018-0008-z

Giorgi, C., Yeo, G. W., Stone, M. E., Katz, D. B., Burge, C., Turrigiano, G., & Moore, M. J. (2007). The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. *Cell, 130*(1), 179-191. doi:10.1016/j.cell.2007.05.028

Glock, C., Heumüller, M., & Schuman, E. M. (2017). mRNA transport & local translation in neurons. *Curr Opin Neurobiol, 45*, 169-177. doi:https://doi.org/10.1016/j.conb.2017.05.005

Gong, X., Zhang, L., Huang, T., Lin, T. V., Miyares, L., Wen, J., . . . Bordey, A. (2015). Activating the translational repressor 4E-BP or reducing S6K-GSK3β activity prevents accelerated axon growth induced by hyperactive mTOR in vivo. *Hum Mol Genet, 24*(20), 5746-5758. doi:10.1093/hmg/ddv295

Graber, T. E., Freemantle, E., Anadolu, M. N., Hebert-Seropian, S., MacAdam, R. L., Shin, U., . . . Sossin, W. S. (2017). UPF1 Governs Synaptic Plasticity through Association with a STAU2 RNA Granule. *J Neurosci, 37*(38), 9116-9131. doi:10.1523/jneurosci.0088-17.2017

Graber, T. E., Hebert-Seropian, S., Khoutorsky, A., David, A., Yewdell, J. W., Lacaille, J. C., & Sossin, W. S. (2013). Reactivation of stalled polyribosomes in synaptic plasticity. *Proc Natl Acad Sci U S A, 110*(40), 16205-16210. doi:10.1073/pnas.1307747110

Grant, C. E., Bailey, T. L., & Noble, W. S. (2011). FIMO: scanning for occurrences of a given motif. *Bioinformatics, 27*(7), 1017-1018. doi:10.1093/bioinformatics/btr064

Hafner, A. S., Donlin-Asp, P. G., Leitch, B., Herzog, E., & Schuman, E. M. (2019). Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. *Science, 364*(6441). doi:10.1126/science.aau3644

Han, P., Shichino, Y., Schneider-Poetsch, T., Mito, M., Hashimoto, S., Udagawa, T., . . . Iwasaki, S. (2020). Genome-wide Survey of Ribosome Collision. *Cell Rep, 31*(5), 107610. doi:10.1016/j.celrep.2020.107610

Hanson, G., & Coller, J. (2018). Codon optimality, bias and usage in translation and mRNA decay. *Nature Reviews Molecular Cell Biology, 19*(1), 20-30. doi:10.1038/nrm.2017.91

Hanz, S., Perlson, E., Willis, D., Zheng, J. Q., Massarwa, R., Huerta, J. J., . . . Fainzilber, M. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron, 40*(6), 1095-1104. doi:10.1016/s0896-6273(03)00770-0

Harrell Jr, F. E., & Dupont, M. C. (2019). Package Hmisc. Vienna, Austria.

Harrison, A. F., & Shorter, J. (2017). RNA-binding proteins with prion-like domains in health and disease. *Biochem J, 474*(8), 1417-1438. doi:10.1042/BCJ20160499

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes Dev, 18*(16), 1926-1945. doi:10.1101/gad.1212704

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., . . . Glass, C. K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell, 38*(4), 576-589. doi:10.1016/j.molcel.2010.05.004

Heraud-Farlow, J. E., & Kiebler, M. A. (2014). The multifunctional Staufen proteins: conserved roles from neurogenesis to synaptic plasticity. *Trends Neurosci, 37*(9), 470-479. doi:10.1016/j.tins.2014.05.009

Hillebrand, J., Pan, K., Kokaram, A., Barbee, S., Parker, R., & Ramaswami, M. (2010). The Me31B DEAD-Box Helicase Localizes to Postsynaptic Foci and Regulates Expression of a CaMKII Reporter mRNA in Dendrites of Drosophila Olfactory Projection Neurons. *Front Neural Circuits, 4*, 121. doi:10.3389/fncir.2010.00121

Hobson, B. D., Kong, L., Hartwick, E. W., Gonzalez, R. L., & Sims, P. A. (2020). Elongation inhibitors do not prevent the release of puromycylated nascent polypeptide chains from ribosomes. *Elife, 9*, e60048. doi:10.7554/eLife.60048

Holt, C. E., Martin, K. C., & Schuman, E. M. (2019). Local translation in neurons: visualization and function. *Nat Struct Mol Biol, 26*(7), 557-566. doi:10.1038/s41594-019-0263-5

Hsu, P. J., Shi, H., Zhu, A. C., Lu, Z., Miller, N., Edens, B. M., . . . He, C. (2019). The RNA-binding protein FMRP facilitates the nuclear export of N (6)-methyladenosine-containing mRNAs. *J Biol Chem, 294*(52), 19889-19895. doi:10.1074/jbc.AC119.010078

Huang, Y. S., & Richter, J. D. (2004). Regulation of local mRNA translation. *Curr Opin Cell Biol, 16*(3), 308-313. doi:10.1016/j.ceb.2004.03.002

Huber, K. M., Kayser, M. S., & Bear, M. F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science, 288*(5469), 1254-1257.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., . . . Singer, R. H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature, 438*(7067), 512-515. doi:10.1038/nature04115

Ikeuchi, K., Izawa, T., & Inada, T. (2019). Recent Progress on the Molecular Mechanism of Quality Controls Induced by Ribosome Stalling. *Frontiers in Genetics, 9*(743). doi:10.3389/fgene.2018.00743

Ingolia, N. T. (2014). Ribosome profiling: new views of translation, from single codons to genome scale. *Nat Rev Genet, 15*(3), 205-213. doi:10.1038/nrg3645

Jansen, R. P., & Niessing, D. (2012). Assembly of mRNA-protein complexes for directional mRNA transport in eukaryotes--an overview. *Curr Protein Pept Sci, 13*(4), 284-293. doi:10.2174/138920312801619493

Job, C., & Eberwine, J. (2001). Identification of sites for exponential translation in living dendrites. *Proc Natl Acad Sci U S A, 98*(23), 13037-13042. doi:10.1073/pnas.231485698

Jonson, L., Vikesaa, J., Krogh, A., Nielsen, L. K., Hansen, T., Borup, R., . . . Nielsen, F. C. (2007). Molecular composition of IMP1 ribonucleoprotein granules. *Mol Cell Proteomics, 6*(5), 798-811. doi:10.1074/mcp.M600346-MCP200

Jung, H., Yoon, B. C., & Holt, C. E. (2012). Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. *Nature Reviews Neuroscience, 13*, 308. doi:10.1038/nrn3210

Kanai, Y., Dohmae, N., & Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron, 43*(4), 513-525. doi:10.1016/j.neuron.2004.07.022

Kang, H., & Schuman, E. M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science, 273*(5280), 1402-1406. doi:10.1126/science.273.5280.1402

Keenan, R. J., Freymann, D. M., Stroud, R. M., & Walter, P. (2001). The signal recognition particle. *Annu Rev Biochem, 70*, 755-775. doi:10.1146/annurev.biochem.70.1.755

Kenney, J. W., Genheden, M., Moon, K. M., Wang, X., Foster, L. J., & Proud, C. G. (2016). Eukaryotic elongation factor 2 kinase regulates the synthesis of microtubule-related proteins in neurons. *J Neurochem, 136*(2), 276-284. doi:10.1111/jnc.13407

Kenny, P. J., Zhou, H., Kim, M., Skariah, G., Khetani, R. S., Drnevich, J., . . . Ceman, S. (2014). MOV10 and FMRP regulate AGO2 association with microRNA recognition elements. *Cell reports, 9*(5), 1729-1741. doi:10.1016/j.celrep.2014.10.054

Kent, C. B., Shimada, T., Ferraro, G. B., Ritter, B., Yam, P. T., McPherson, P. S., . . . Fournier, A. E. (2010). 14-3-3 proteins regulate protein kinase a activity to modulate growth cone turning responses. *J Neurosci, 30*(42), 14059-14067. doi:10.1523/JNEUROSCI.3883-10.2010

Khan, A., Pepio, A. M., & Sossin, W. S. (2001). Serotonin activates S6 kinase in a rapamycin-sensitive manner in Aplysia synaptosomes. *J Neurosci, 21*(2), 382-391.

Khandjian, E. W., Corbin, F., Woerly, S., & Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. *Nat Genet, 12*(1), 91-93. doi:10.1038/ng0196-91

Kiebler, M. A., & Bassell, G. J. (2006). Neuronal RNA Granules: Movers and Makers. *Neuron, 51*(6), 685-690. doi:https://doi.org/10.1016/j.neuron.2006.08.021

Kim, Y. K., & Maquat, L. E. (2019). UPFront and center in RNA decay: UPF1 in nonsense-mediated mRNA decay and beyond. *Rna, 25*(4), 407-422. doi:10.1261/rna.070136.118

Kislauskis, E. H., Zhu, X., & Singer, R. H. (1997). beta-Actin messenger RNA localization and protein synthesis augment cell motility. *J Cell Biol, 136*(6), 1263-1270. doi:10.1083/jcb.136.6.1263

Knowles, R. B., Sabry, J. H., Martone, M. E., Deerinck, T. J., Ellisman, M. H., Bassell, G. J., & Kosik, K. S. (1996). Translocation of RNA Granules in Living Neurons. *The Journal of Neuroscience, 16*(24), 7812.

Koppers, M., Cagnetta, R., Shigeoka, T., Wunderlich, L. C., Vallejo-Ramirez, P., Qiaojin Lin, J., . . . Holt, C. E. (2019). Receptor-specific interactome as a hub for rapid cue-induced selective translation in axons. *Elife, 8*. doi:10.7554/eLife.48718

Krichevsky, A. M., & Kosik, K. S. (2001). Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron, 32*(4), 683-696.

Langdon, E. M., & Gladfelter, A. S. (2018). A New Lens for RNA Localization: Liquid-Liquid Phase Separation. *Annu Rev Microbiol, 72*, 255-271. doi:10.1146/annurev-micro-090817-062814

Langille, J. J., Ginzberg, K., & Sossin, W. S. (2019). Polysomes identified by live imaging of nascent peptides are stalled in hippocampal and cortical neurites. *Learn Mem, 26*(9), 351-362. doi:10.1101/lm.049965.119

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods, 9*(4), 357-359. doi:10.1038/nmeth.1923

Lauria, F., Tebaldi, T., Bernabo, P., Groen, E. J. N., Gillingwater, T. H., & Viero, G. (2018). riboWaltz: Optimization of ribosome P-site positioning in ribosome profiling data. *PLoS Comput Biol, 14*(8), e1006169. doi:10.1371/journal.pcbi.1006169

Lebeau, G., Miller, L. C., Tartas, M., McAdam, R., Laplante, I., Badeaux, F., . . . Lacaille, J. C. (2011). Staufen 2 regulates mGluR long-term depression and Map1b mRNA distribution in hippocampal neurons. *Learn Mem, 18*(5), 314-326. doi:10.1101/lm.2100611

Lee, J. E., Cathey, P. I., Wu, H., Parker, R., & Voeltz, G. K. (2020). Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science, 367*(6477). doi:10.1126/science.aay7108

Lelieveld, S. H., Reijnders, M. R., Pfundt, R., Yntema, H. G., Kamsteeg, E. J., de Vries, P., . . . Gilissen, C. (2016). Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nat Neurosci, 19*(9), 1194-1196. doi:10.1038/nn.4352

Leung, K.-M., van Horck, F. P. G., Lin, A. C., Allison, R., Standart, N., & Holt, C. E. (2006). Asymmetrical β-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nature Neuroscience, 9*(10), 1247-1256. doi:10.1038/nn1775

Leung, K. M., van Horck, F. P., Lin, A. C., Allison, R., Standart, N., & Holt, C. E. (2006). Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci, 9*(10), 1247-1256. doi:10.1038/nn1775

Li, C., Bassell, G., & Sasaki, Y. (2009). Fragile X mental retardation protein is involved in protein synthesis-dependent collapse of growth cones induced by Semaphorin-3A. *Frontiers in Neural Circuits, 3*(11). doi:10.3389/neuro.04.011.2009

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics, 30*(7), 923-930. doi:10.1093/bioinformatics/btt656

Limon, J., & Fruman, D. (2012). Akt and mTOR in B Cell Activation and Differentiation. *Frontiers in immunology, 3*, 228. doi:10.3389/fimmu.2012.00228

Liu, B., Molinaro, G., Shu, H., Stackpole, E. E., Huber, K. M., & Richter, J. D. (2019). Optimization of ribosome profiling using low-input brain tissue from fragile X syndrome model mice. *Nucleic Acids Res, 47*(5), e25. doi:10.1093/nar/gky1292

Liu, J., Rivas, F. V., Wohlschlegel, J., Yates, J. R., 3rd, Parker, R., & Hannon, G. J. (2005). A role for the P-body component GW182 in microRNA function. *Nat Cell Biol, 7*(12), 1261-1266. doi:10.1038/ncb1333

Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell W, T., Li, W., . . . Feng, Y. (2004). The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development. *Proc Natl Acad Sci U S A, 101*(42), 15201-15206. doi:10.1073/pnas.0404995101

Lucas, B. A., Lavi, E., Shiue, L., Cho, H., Katzman, S., Miyoshi, K., . . . Maquat, L. E. (2018). Evidence for convergent evolution of SINE-directed Staufen-mediated mRNA decay. *Proceedings of the National Academy of Sciences, 115*(5), 968-973. doi:10.1073/pnas.1715531115

Luo, Y., Na, Z., & Slavoff, S. A. (2018). P-Bodies: Composition, Properties, and Functions. *Biochemistry, 57*(17), 2424-2431. doi:10.1021/acs.biochem.7b01162

Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., . . . Kandel, E. R. (1997). Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell, 91*(7), 927-938. doi:10.1016/s0092-8674(00)80484-5

Martin, M. (2011). Cutadapt removes adapter sequences from high-trhoughput sequencing reads. *EMBnet.journal, 17*, 10-12. doi:doi:https://doi.org/10.14806/ej.17.1.200.

Martin, S., & Tazi, J. (2014). Visualization of G3BP stress granules dynamics in live primary cells. *J Vis Exp*(87). doi:10.3791/51197

Martinez, J. C., Randolph, L. K., Iascone, D. M., Pernice, H. F., Polleux, F., & Hengst, U. (2019). Pum2 Shapes the Transcriptome in Developing Axons through Retention of Target mRNAs in the Cell Body. *Neuron, 104*(5), 931-946 e935. doi:10.1016/j.neuron.2019.08.035

Maurin, T., Lebrigand, K., Castagnola, S., Paquet, A., Jarjat, M., Popa, A., . . . Bardoni, B. (2018). HITS-CLIP in various brain areas reveals new targets and new modalities of RNA binding by fragile X mental retardation protein. *Nucleic Acids Res, 46*(12), 6344-6355. doi:10.1093/nar/gky267

Mayya, V. K., & Duchaine, T. F. (2019). Ciphers and Executioners: How 3'-Untranslated Regions Determine the Fate of Messenger RNAs. *Front Genet, 10*, 6. doi:10.3389/fgene.2019.00006

McCamphill, P. K., Farah, C. A., Anadolu, M. N., Hoque, S., & Sossin, W. S. (2015). Bidirectional regulation of eEF2 phosphorylation controls synaptic plasticity by decoding neuronal activity patterns. *J Neurosci, 35*(10), 4403-4417. doi:10.1523/JNEUROSCI.2376-14.2015

Merkurjev, D., Hong, W. T., Iida, K., Oomoto, I., Goldie, B. J., Yamaguti, H., . . . Wang, D. O. (2018). Synaptic N(6)-methyladenosine (m(6)A) epitranscriptome reveals functional

partitioning of localized transcripts. *Nat Neurosci, 21*(7), 1004-1014. doi:10.1038/s41593-018-0173-6

Mikl, M., Vendra, G., & Kiebler, M. A. (2011). Independent localization of MAP2, CaMKIIalpha and beta-actin RNAs in low copy numbers. *EMBO Rep, 12*(10), 1077-1084. doi:10.1038/embor.2011.149

Miller, L. C., Blandford, V., McAdam, R., Sanchez-Carbente, M. R., Badeaux, F., DesGroseillers, L., & Sossin, W. S. (2009). Combinations of DEAD box proteins distinguish distinct types of RNA: protein complexes in neurons. *Mol Cell Neurosci, 40*(4), 485-495.

Miller, S., Yasuda, M., Coats, J. K., Jones, Y., Martone, M. E., & Mayford, M. (2002). Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron, 36*(3), 507-519. doi:10.1016/s0896-6273(02)00978-9

Min, E. E., Roy, B., Amrani, N., He, F., & Jacobson, A. (2013). Yeast Upf1 CH domain interacts with Rps26 of the 40S ribosomal subunit. *Rna, 19*(8), 1105-1115. doi:10.1261/rna.039396.113

Mittag, T., & Parker, R. (2018). Multiple Modes of Protein-Protein Interactions Promote RNP Granule Assembly. *J Mol Biol, 430*(23), 4636-4649. doi:10.1016/j.jmb.2018.08.005

Molitor, L., Bacher, S., Burczyk, S., & Niessing, D. (2021). The Molecular Function of PURA and Its Implications in Neurological Diseases. *Frontiers in Genetics, 12*(267). doi:10.3389/fgene.2021.638217

Mori, M., Penney, J., & Haghighi, P. (2019). Regulation of synaptic homeostasis by translational mechanisms. In W. Sossin (Ed.), *The Oxford Handbook of Neuronal Protein Synthesis*: Oxford Press.

Mori, Y., Imaizumi, K., Katayama, T., Yoneda, T., & Tohyama, M. (2000). Two cis-acting elements in the 3' untranslated region of alpha-CaMKII regulate its dendritic targeting. *Nat Neurosci, 3*(11), 1079-1084. doi:10.1038/80591

Muddashetty, R. S., Nalavadi, V. C., Gross, C., Yao, X., Xing, L., Laur, O., . . . Bassell, G. J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol Cell, 42*(5), 673-688. doi:10.1016/j.molcel.2011.05.006

Muslimov, I. A., Patel, M. V., Rose, A., & Tiedge, H. (2011). Spatial code recognition in neuronal RNA targeting: role of RNA-hnRNP A2 interactions. *J Cell Biol, 194*(3), 441-457. doi:10.1083/jcb.201010027

Na, Y., Park, S., Lee, C., Kim, D. K., Park, J. M., Sockanathan, S., . . . Worley, P. F. (2016). Real-Time Imaging Reveals Properties of Glutamate-Induced Arc/Arg 3.1 Translation in Neuronal Dendrites. *Neuron, 91*(3), 561-573. doi:10.1016/j.neuron.2016.06.017

Napoli, I., Mercaldo, V., Boyl, P. P., Eleuteri, B., Zalfa, F., De Rubeis, S., . . . Bagni, C. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell, 134*(6), 1042-1054. doi:10.1016/j.cell.2008.07.031

Narayanan, U., Nalavadi, V., Nakamoto, M., Pallas, D. C., Ceman, S., Bassell, G. J., & Warren, S. T. (2007). FMRP phosphorylation reveals an immediate-early signaling pathway triggered

by group I mGluR and mediated by PP2A. *J Neurosci, 27*(52), 14349-14357. doi:10.1523/jneurosci.2969-07.2007

Nie, D., Di Nardo, A., Han, J. M., Baharanyi, H., Kramvis, I., Huynh, T., . . . Sahin, M. (2010). Tsc2-Rheb signaling regulates EphA-mediated axon guidance. *Nature Neuroscience, 13*(2), 163-172. doi:10.1038/nn.2477

Niere, F., Wilkerson, J. R., & Huber, K. M. (2012). Evidence for a fragile X mental retardation protein-mediated translational switch in metabotropic glutamate receptor-triggered Arc translation and long-term depression. *J Neurosci, 32*(17), 5924-5936. doi:10.1523/JNEUROSCI.4650-11.2012

Nosyreva, E. D., & Huber, K. M. (2005). Developmental switch in synaptic mechanisms of hippocampal metabotropic glutamate receptor-dependent long-term depression. *J Neurosci, 25*(11), 2992-3001. doi:10.1523/JNEUROSCI.3652-04.2005

Ostroff, L. E., Santini, E., Sears, R., Deane, Z., Kanadia, R. N., LeDoux, J. E., . . . Klann, E. (2019). Axon TRAP reveals learning-associated alterations in cortical axonal mRNAs in the lateral amgydala. *Elife, 8*. doi:10.7554/eLife.51607

Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M., & Kennedy, M. B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J Neurosci, 19*(18), 7823-7833.

Palacios, I. M., & St Johnston, D. (2001). Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu Rev Cell Dev Biol, 17*, 569-614. doi:10.1146/annurev.cellbio.17.1.569

Paquin, N., & Chartrand, P. (2008). Local regulation of mRNA translation: new insights from the bud. *Trends Cell Biol, 18*(3), 105-111. doi:10.1016/j.tcb.2007.12.004

Park, E., & Maquat, L. E. (2013). Staufen-mediated mRNA decay. *Wiley Interdiscip Rev RNA, 4*(4), 423-435. doi:10.1002/wrna.1168

Parker, R., & Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Mol Cell, 25*(5), 635-646. doi:10.1016/j.molcel.2007.02.011

Penney, J., Tsurudome, K., Liao, E. H., Elazzouzi, F., Livingstone, M., Gonzalez, M., . . . Haghighi, A. P. (2012). TOR is required for the retrograde regulation of synaptic homeostasis at the Drosophila neuromuscular junction. *Neuron, 74*(1), 166-178. doi:10.1016/j.neuron.2012.01.030

Pochopien, A. A., Beckert, B., Kasvandik, S., Berninghausen, O., Beckmann, R., Tenson, T., & Wilson, D. N. (2021). Structure of Gcn1 bound to stalled and colliding 80S ribosomes. *Proceedings of the National Academy of Sciences, 118*(14), e2022756118. doi:10.1073/pnas.2022756118

Poulopoulos, A., Murphy, A. J., Ozkan, A., Davis, P., Hatch, J., Kirchner, R., & Macklis, J. D. (2019). Subcellular transcriptomes and proteomes of developing axon projections in the cerebral cortex. *Nature, 565*(7739), 356-360. doi:10.1038/s41586-018-0847-y

Protter, D. S. W., & Parker, R. (2016). Principles and Properties of Stress Granules. *Trends Cell Biol, 26*(9), 668-679. doi:10.1016/j.tcb.2016.05.004

Proud, C. G. (2015). Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans, 43*(3), 328-332. doi:10.1042/BST20140323

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics, 26*(6), 841-842. doi:10.1093/bioinformatics/btq033

Reijnders, M. R. F., Janowski, R., Alvi, M., Self, J. E., van Essen, T. J., Vreeburg, M., . . . Baralle, D. (2018). PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature. *J Med Genet, 55*(2), 104-113. doi:10.1136/jmedgenet-2017-104946

Reimand, J., Arak, T., Adler, P., Kolberg, L., Reisberg, S., Peterson, H., & Vilo, J. (2016). g:Profiler-a web server for functional interpretation of gene lists (2016 update). *Nucleic Acids Res, 44*(W1), W83-89. doi:10.1093/nar/gkw199

Reschke, M., Clohessy, J. G., Seitzer, N., Goldstein, D. P., Breitkopf, S. B., Schmolze, D. B., . . . Pandolfi, P. P. (2013). Characterization and analysis of the composition and dynamics of the mammalian riboproteome. *Cell reports, 4*(6), 1276-1287. doi:10.1016/j.celrep.2013.08.014

Richter, J. D., Bassell, G. J., & Klann, E. (2015). Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nat Rev Neurosci, 16*(10), 595-605. doi:10.1038/nrn4001

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res, 43*(7), e47. doi:10.1093/nar/gkv007

Robert, F., Carrier, M., Rawe, S., Chen, S., Lowe, S., & Pelletier, J. (2009). Altering chemosensitivity by modulating translation elongation. *PLOS ONE, 4*(5), e5428. doi:10.1371/journal.pone.0005428

Rodriguez, J. M., Maietta, P., Ezkurdia, I., Pietrelli, A., Wesselink, J. J., Lopez, G., . . . Tress, M. L. (2013). APPRIS: annotation of principal and alternative splice isoforms. *Nucleic Acids Res, 41*(Database issue), D110-117. doi:10.1093/nar/gks1058

Ryan, V. H., & Fawzi, N. L. (2019). Physiological, Pathological, and Targetable Membraneless Organelles in Neurons. *Trends Neurosci, 42*(10), 693-708. doi:10.1016/j.tins.2019.08.005

Sahoo, P. K., Smith, D. S., Perrone-Bizzozero, N., & Twiss, J. L. (2018). Axonal mRNA transport and translation at a glance. *J Cell Sci, 131*(8). doi:10.1242/jcs.196808

Scheetz, A. J., Nairn, A. C., & Constantine-Paton, M. (2000). NMDA receptor-mediated control of protein synthesis at developing synapses. *Nat Neurosci, 3*(3), 211-216. doi:10.1038/72915

Schoenberg, D. R., & Maquat, L. E. (2012). Regulation of cytoplasmic mRNA decay. *Nat Rev Genet, 13*(4), 246-259. doi:10.1038/nrg3160

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol, 106-107*, 1-16. doi:10.1016/j.pneurobio.2013.04.001

Sengupta, S., Peterson, T. R., & Sabatini, D. M. (2010). Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress. *Molecular Cell, 40*(2), 310-322. doi:10.1016/j.molcel.2010.09.026

Shah, S., Molinaro, G., Liu, B., Wang, R., Huber, K. M., & Richter, J. D. (2020). FMRP Control of Ribosome Translocation Promotes Chromatin Modifications and Alternative Splicing of Neuronal Genes Linked to Autism. *Cell Rep, 30*(13), 4459-4472 e4456. doi:10.1016/j.celrep.2020.02.076

Shan, J., Munro, T. P., Barbarese, E., Carson, J. H., & Smith, R. (2003). A molecular mechanism for mRNA trafficking in neuronal dendrites. *J Neurosci, 23*(26), 8859-8866.

Shen, L., & Pelletier, J. (2019). Selective targeting of the DEAD-box RNA helicase eukaryotic initiation factor (eIF) 4A by natural products. *Nat Prod Rep*. doi:10.1039/c9np00052f

Shi, Z., Fujii, K., Kovary, K. M., Genuth, N. R., Röst, H. L., Teruel, M. N., & Barna, M. (2017). Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. *Mol Cell, 67*(1), 71-83.e77. doi:10.1016/j.molcel.2017.05.021

Shigeoka, T., Jung, H., Jung, J., Turner-Bridger, B., Ohk, J., Lin, J. Q., . . . Holt, C. E. (2016). Dynamic Axonal Translation in Developing and Mature Visual Circuits. *Cell, 166*(1), 181-192. doi:10.1016/j.cell.2016.05.029

Shigeoka, T., Koppers, M., Wong, H. H., Lin, J. Q., Cagnetta, R., Dwivedy, A., . . . Holt, C. E. (2019). On-Site Ribosome Remodeling by Locally Synthesized Ribosomal Proteins in Axons. *Cell reports, 29*(11), 3605-3619 e3610. doi:10.1016/j.celrep.2019.11.025

Shu, H., Donnard, E., Liu, B., Jung, S., Wang, R., & Richter, J. D. (2020). FMRP links optimal codons to mRNA stability in neurons. *Proc Natl Acad Sci U S A, 117*(48), 30400-30411. doi:10.1073/pnas.2009161117

Simone, L. E., & Keene, J. D. (2013). Mechanisms coordinating ELAV/Hu mRNA regulons. *Curr Opin Genet Dev, 23*(1), 35-43. doi:10.1016/j.gde.2012.12.006

Siwaszek, A., Ukleja, M., & Dziembowski, A. (2014). Proteins involved in the degradation of cytoplasmic mRNA in the major eukaryotic model systems. *RNA Biol, 11*(9), 1122-1136. doi:10.4161/rna.34406

Slomnicki, L. P., Pietrzak, M., Vashishta, A., Jones, J., Lynch, N., Elliot, S., . . . Hetman, M. (2016). Requirement of Neuronal Ribosome Synthesis for Growth and Maintenance of the Dendritic Tree. *J Biol Chem, 291*(11), 5721-5739. doi:10.1074/jbc.M115.682161

Snijders Blok, L., Madsen, E., Juusola, J., Gilissen, C., Baralle, D., Reijnders, M. R., . . . Kleefstra, T. (2015). Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Effects on Wnt Signaling. *Am J Hum Genet, 97*(2), 343-352. doi:10.1016/j.ajhg.2015.07.004

Sossin, W. S., & Costa-Mattioli, M. (2019). Translational Control in the Brain in Health and Disease. *Cold Spring Harb Perspect Biol, 11*(8). doi:10.1101/cshperspect.a032912

Sossin, W. S., & DesGroseillers, L. (2006). Intracellular trafficking of RNA in neurons. *Traffic, 7*(12), 1581-1589. doi:10.1111/j.1600-0854.2006.00500.x

Spencer, C. M., Serysheva, E., Yuva-Paylor, L. A., Oostra, B. A., Nelson, D. L., & Paylor, R. (2006). Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. *Hum Mol Genet, 15*(12), 1984-1994. doi:10.1093/hmg/ddl121

Südhof, T. C. (2018). Towards an Understanding of Synapse Formation. *Neuron, 100*(2), 276-293. doi:10.1016/j.neuron.2018.09.040

Sutton, M. A., Ito, H. T., Cressy, P., Kempf, C., Woo, J. C., & Schuman, E. M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell, 125*(4), 785-799. doi:10.1016/j.cell.2006.03.040

Sutton, M. A., & Schuman, E. M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. *Cell, 127*(1), 49-58. doi:10.1016/j.cell.2006.09.014

Sutton, M. A., Taylor, A. M., Ito, H. T., Pham, A., & Schuman, E. M. (2007). Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron, 55*(4), 648-661. doi:10.1016/j.neuron.2007.07.030

Sutton, M. A., Wall, N. R., Aakalu, G. N., & Schuman, E. M. (2004). Regulation of dendritic protein synthesis by miniature synaptic events. *Science, 304*(5679), 1979-1983. doi:10.1126/science.1096202

Taylor, A. M., Berchtold, N. C., Perreau, V. M., Tu, C. H., Li Jeon, N., & Cotman, C. W. (2009). Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J Neurosci, 29*(15), 4697-4707. doi:10.1523/JNEUROSCI.6130-08.2009

Tcherkezian, J., Brittis, P. A., Thomas, F., Roux, P. P., & Flanagan, J. G. (2010). Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell, 141*(4), 632-644. doi:10.1016/j.cell.2010.04.008

Thoreen, C. C., Chantranupong, L., Keys, H. R., Wang, T., Gray, N. S., & Sabatini, D. M. (2012). A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature, 485*(7396), 109-113. doi:10.1038/nature11083

Turrigiano, G. G., & Nelson, S. B. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol, 10*(3), 358-364. doi:10.1016/s0959-4388(00)00091-x

Udagawa, T., Farny, N. G., Jakovcevski, M., Kaphzan, H., Alarcon, J. M., Anilkumar, S., . . . Richter, J. D. (2013). Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology. *Nat Med, 19*(11), 1473-1477. doi:10.1038/nm.3353

Ule, J., Hwang, H. W., & Darnell, R. B. (2018). The Future of Cross-Linking and Immunoprecipitation (CLIP). *Cold Spring Harb Perspect Biol, 10*(8). doi:10.1101/cshperspect.a032243

Urbanska, M., Gozdz, A., Swiech, L. J., & Jaworski, J. (2012). Mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2) control the dendritic arbor morphology of hippocampal neurons. *J Biol Chem, 287*(36), 30240-30256. doi:10.1074/jbc.M112.374405

Van Nostrand, E. L., Freese, P., Pratt, G. A., Wang, X., Wei, X., Xiao, R., . . . Yeo, G. W. (2020). A large-scale binding and functional map of human RNA-binding proteins. *Nature, 583*(7818), 711-719. doi:10.1038/s41586-020-2077-3

Vicario, A., Colliva, A., Ratti, A., Davidovic, L., Baj, G., Gricman, L., . . . Tongiorgi, E. (2015). Dendritic targeting of short and long 3' UTR BDNF mRNA is regulated by BDNF or NT-3 and distinct sets of RNA-binding proteins. *Front Mol Neurosci, 8*, 62. doi:10.3389/fnmol.2015.00062

Wang, D. O., Kim, S. M., Zhao, Y., Hwang, H., Miura, S. K., Sossin, W. S., & Martin, K. C. (2009). Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science, 324*(5934), 1536-1540. doi:10.1126/science.1173205

Wang, X., Posey, J. E., Rosenfeld, J. A., Bacino, C. A., Scaglia, F., Immken, L., . . . Moretti, P. (2018). Phenotypic expansion in DDX3X - a common cause of intellectual disability in females. *Ann Clin Transl Neurol, 5*(10), 1277-1285. doi:10.1002/acn3.622

Welshhans, K., & Bassell, G. J. (2011). Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J Neurosci, 31*(27), 9800-9813. doi:10.1523/JNEUROSCI.0166-11.2011

Westmark, C. J., Maloney, B., Alisch, R. S., Sokol, D. K., & Lahiri, D. K. (2020). FMRP Regulates the Nuclear Export of Adam9 and Psen1 mRNAs: Secondary Analysis of an N(6)-Methyladenosine Dataset. *Sci Rep, 10*(1), 10781. doi:10.1038/s41598-020-66394-y

Widagdo, J., & Anggono, V. (2018). The m6A-epitranscriptomic signature in neurobiology: from neurodevelopment to brain plasticity. *J Neurochem, 147*(2), 137-152. doi:10.1111/jnc.14481

Wilhelm, J. E., & Vale, R. D. (1993). RNA on the move: the mRNA localization pathway. *J Cell Biol, 123*(2), 269-274. doi:10.1083/jcb.123.2.269

Willis, D., Li, K. W., Zheng, J. Q., Chang, J. H., Smit, A. B., Kelly, T., . . . Twiss, J. L. (2005). Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. *J Neurosci, 25*(4), 778-791. doi:10.1523/JNEUROSCI.4235-04.2005

Wolozin, B., & Ivanov, P. (2019). Stress granules and neurodegeneration. *Nat Rev Neurosci, 20*(11), 649-666. doi:10.1038/s41583-019-0222-5

Wong, H. H., Lin, J. Q., Strohl, F., Roque, C. G., Cioni, J. M., Cagnetta, R., . . . Holt, C. E. (2017). RNA Docking and Local Translation Regulate Site-Specific Axon Remodeling In Vivo. *Neuron, 95*(4), 852-868 e858. doi:10.1016/j.neuron.2017.07.016

Wu, B., Zhang, H., Sun, R., Peng, S., Cooperman, B. S., Goldman, Y. E., & Chen, C. (2018). Translocation kinetics and structural dynamics of ribosomes are modulated by the conformational plasticity of downstream pseudoknots. *Nucleic Acids Res, 46*(18), 9736-9748. doi:10.1093/nar/gky636

Wu, C. C., Zinshteyn, B., Wehner, K. A., & Green, R. (2019). High-Resolution Ribosome Profiling Defines Discrete Ribosome Elongation States and Translational Regulation during Cellular Stress. *Mol Cell, 73*(5), 959-970 e955. doi:10.1016/j.molcel.2018.12.009

Xu, W. B., & Roufa, D. J. (1996). The gene encoding human ribosomal protein S24 and tissue-specific expression of differentially spliced mRNAs. *Gene, 169*(2), 257-262. doi:10.1016/0378-1119(96)88652-5

Yamashita, A. (2013). Role of SMG-1-mediated Upf1 phosphorylation in mammalian nonsense-mediated mRNA decay. *Genes to Cells, 18*(3), 161-175. doi:https://doi.org/10.1111/gtc.12033

Yan, X., Hoek, T. A., Vale, R. D., & Tanenbaum, M. E. (2016). Dynamics of Translation of Single mRNA Molecules In Vivo. *Cell, 165*(4), 976-989. doi:10.1016/j.cell.2016.04.034

Yanow, S. K., Manseau, F., Hislop, J., Castellucci, V. F., & Sossin, W. S. (1998). Biochemical pathways by which serotonin regulates translation in the nervous system of Aplysia. *J Neurochem, 70*(2), 572-583. doi:10.1046/j.1471-4159.1998.70020572.x

Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J., & Zheng, J. Q. (2006). An essential role for β-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nature Neuroscience, 9*(10), 1265-1273. doi:10.1038/nn1773

Yoon, Byung C., Jung, H., Dwivedy, A., O'Hare, Catherine M., Zivraj, Krishna H., & Holt, Christine E. (2012). Local Translation of Extranuclear Lamin B Promotes Axon Maintenance. *Cell, 148*(4), 752-764. doi:10.1016/j.cell.2011.11.064

Yoon, B. C., Zivraj, K. H., & Holt, C. E. (2009). Local translation and mRNA trafficking in axon pathfinding. *Results Probl Cell Differ, 48*, 269-288. doi:10.1007/400_2009_5

Younts, T. J., Monday, H. R., Dudok, B., Klein, M. E., Jordan, B. A., Katona, I., & Castillo, P. E. (2016). Presynaptic Protein Synthesis Is Required for Long-Term Plasticity of GABA Release. *Neuron, 92*(2), 479-492. doi:10.1016/j.neuron.2016.09.040

Zalfa, F., Giorgi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., . . . Bagni, C. (2003). The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell, 112*(3), 317-327. doi:10.1016/s0092-8674(03)00079-5

Zhang, F., Kang, Y., Wang, M., Li, Y., Xu, T., Yang, W., . . . Jin, P. (2018). Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. *Hum Mol Genet, 27*(22), 3936-3950. doi:10.1093/hmg/ddy292

Zhao, T., Chen, Y.-M., Li, Y., Wang, J., Chen, S., Gao, N., & Qian, W. (2021). Disome-seq reveals widespread ribosome collisions that promote cotranslational protein folding. *Genome Biology, 22*(1), 16. doi:10.1186/s13059-020-02256-0

Zivraj, K. H., Tung, Y. C., Piper, M., Gumy, L., Fawcett, J. W., Yeo, G. S., & Holt, C. E. (2010). Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. *J Neurosci, 30*(46), 15464-15478. doi:10.1523/jneurosci.1800-10.2010

4 CHAPTER FOUR: Discussion and Conclusions

4.1 Preface:

In the following sections I will discuss the possible mechanisms for stalling and maintenance of RNA Granule associated stalled polysomes, with a focus on mRNA, protein, and ribosome interactions. Because of the complexity of the mRNA life cycle and the diversity of cellular mechanisms that regulate mRNAs from transcription to translation, many processes such as splicing, mRNA-editing, and how mRNAs are selected for this pathway (for example, by export/targeting sequences etc.) will not be discussed in much detail. The purpose of the following debate is to weigh the potential impact and interplay of dynamic mechanisms surrounding translation elongation and how these may be adapted by neurons to make reversible stalling a robust translational control method. I will propose new hypotheses based on my interpretations of the data presented in the previous chapter, within the context of the existing knowledge in the field and attempt to reconcile differences observed by other groups. Here, I will also briefly discuss caveats and shortcomings of the techniques used and propose future directions.

4.2 Mechanism for Stalling

Our finding that there are large peaks where ribosomes stall on mRNAs in the compacted ribosomes of the pellet fraction (Chapter 3) supports the idea that a percentage of these structures represent stalled ribosomes, and that there are specific motifs on the mRNA where ribosomes are preferentially stalled. While these stall sites overlap with consensus sequences for FMRP previously identified by HIT-CLIPS, it is uncertain whether there is direct binding by FMRP or other RBPs to these stall site sequences. At this point, we do not have any evidence demonstrating direct binding of FMRP to an identified site on stalled mRNAs. Because the HIT-CLIP method utilizes cross-linking of FMRP to its associated mRNA prior to immunoprecipitation, it is not possible to ascertain whether FMRP is associated with the mRNA or the ribosome occupying that space on the mRNA. There is evidence for both models. Previous studies have shown that FMRP can interact directly with the ribosome (Athar & Joseph, 2020; Ceman et al., 2003; E. Chen et al., 2014). There is also some evidence for FMRP directly binding to the WGGA motif, although usually when this motif is repeated and is likely to form a G quartet (M. Ascano et al., 2012). High resolution single particle cryo-EM analysis could answer this question; if FMRP is bound to stalled ribosomes, it should be detectable. Repeating this experiment with animals lacking FMRP should also shed light on this issue. If FMRP is bound to the ribosome and stabilizes the stalled state, the lack of FMRP should reduce the number of stalled ribosomes, but the WGGA motif may still be observed. In contrast, if FMRP directly binds to the WGGA state to stall ribosomes, this motif should no longer be found in stalled ribosomes in the absence of FMRP. One issue however, is the presence of the FMRP-related proteins FMR1 and FMR2, that also likely play similar roles in the nervous system (Cook et al., 2011; Spencer et al., 2006). These may reduce the changes observed but given the strong phenotype due to the loss of FMRP, a change should still be detected.

Our lack of a mechanism for the type of ribosome stalling observed in Chapter 3 suggests we should be open to multiple mechanisms by which stalling could occur. Reversible ribosome stalling may be mediated by three main methods discussed below: 1) RBP-mediated stalling, 2) mRNA-mediated stalling, 3) Ribosome-mediated stalling.

4.3 RBP-mediated stalling

The presence of consensus peaks in the ribosome footprints that map to specific sequences on the stalled mRNAs infers that these sequences may be targeted by RNA binding proteins that slow down or stall elongating ribosomes. There is a plethora of RNA binding proteins and translation factors identified in the proteomics of the RNA granule that could potentially interact with the motifs we've identified (Mina N. Anadolu et al., 2021; Elvira et al., 2006). While we've succeeded in creating a short list of proteins whose consensus sequences overlap with the putative stalling motifs, the mechanism by which these proteins contribute to stalling is still unclear. Our most recent findings provided us with a fresh perspective on our previous hypothesis and created new avenues to develop our understanding of how stalling may occur. In light of the new evidence, we will review these hypotheses and the supporting evidence below.

4.3.1 The Termination Hypothesis, Stau 2 and UPF-1:

Our initial hypothesis emerged from the concept that the simplest explanation to the following question is likely the correct one: Where could ribosomes naturally stall during elongation that could determine their fate? The simplest answer was prior to termination. Prior to translation termination and the disassembly of the ribosomal-peptidyl complex, the elongating ribosome reaches the stop codon and is temporarily stalled. During this time, the Nonsense Mediated Decay (NMD) protein Upf1 inspects the stalled ribosome to determine whether the stop codon is premature (PTC), in which case there is a downstream Exon Junction Complex that can

interact with and facilitate the phosphorylation of Upf1 to activate RNA decay, or whether the ribosome has encountered a legitimate stop codon and translation termination can occur (Yamashita, 2013). We had hypothesized that this Upf1-mediated mechanism could be adapted to stall the first elongating ribosome at or close to the termination site and facilitate the recruitment of the stalled mRNA-ribosome complex to RNA granules. This hypothesis was also in line with the idea that stalling the first ribosome in the polysome would be sufficient to induce stalling of the consequent trailing ribosomes, similar to a traffic stop, and that releasing the first ribosome would also be sufficient to release the polysome and result in fast and local protein expression.

Indeed, we had previously found that Upf1 and Stau 2 are required for the formation of stalled polysomes in hippocampal cultures (Graber et al., 2017). We had observed that knocking down Upf1 and Stau 2 independently of each other, not only disrupted the formation of stalled polysomes but also their maintenance during transport, marked by a significant reduction of initiation inhibitor-resistant ribopuromycylation and initiation-independent translation downstream of mGLUR activation, in both Upf1 and Stau2 KD conditions (Graber et al., 2017). Moreover, UPF1 was required for the transport of stalled polysome associated mRNAs such as Map1b, as well as the initiation-independent translation downstream of mGLUR activation and induction of mGluR-LTD(Graber et al., 2017).. When the interaction of these two proteins were uncoupled using lentiviral knockdown of Stau 2 and re-constitution with a mutated construct that is lacking the Upf1 binding TBD domain (Stau2ΔTBD), the same phenotype as the above knockdowns were seen, suggesting that the interaction between the two proteins was also important for the formation and release of stalled polysomes (Graber et al., 2017).. As a negative control, we also knocked down the Nonsense Mediated Decay factor downstream of Upf1, PNRC1, and did not see an effect,

eliminating the possibility of decay pathway contribution to this phenomenon, and decoupling the role of Upf1 in the regulation of stalled transcripts from its role in NMD (Graber et al., 2017).

Based on these findings, we had initially hypothesized that Stau 2 binding to determinants in the 3'UTR would interact with UPF1 recruited to the stop codon, but instead of mediating Staufen-Mediated Decay as it does in other tissues (E. Park & Maquat, 2013), this would be the first step in the formation of a stalled polysome that will be packaged into RNA granules in neurons. A strong prediction of this model is that stalled ribosomes would preferentially occupy the region before and around the stop codon. Below we will unpack the evidence against this initial hypothesis and propose alternative mechanisms that may govern stalling.

4.3.2 Evidence against the Termination Hypothesis:

Contrary to our predictions, the majority of footprint reads from the compacted ribosomes in the pellet fraction mapped to peaks in the first half of messages, closer to the 5' end, with an apparent lack of consensus peaks near the termination site (Chapter 3). The lack of stalled ribosomes near the stop codon thus rules out our initial hypothesis; the compacted ribosomes found in the pellet are likely not generated by a stall mediated by UPF1 at the stop codon. It is possible that the model was correct, but that ribosomes stalled in an UPF1 dependent manner are not found in the heavy pelleting fraction of the sucrose gradient. However, we do observe a large enrichment of UPF1 in this fraction, consistent with UPF1 regulation of stalled ribosomes. Given the importance of the Upf1-Stau2 interaction for stalling and the new information we have gathered from ribosome footprints, a new interpretation must be made.

The most readily available interpretation is that Upf1-Stau2 are more important for the packaging or targeting of mRNAs to RNA Granules rather than inducing stalling. Since Stau2 selectively binds mRNAs with double-stranded 3'UTR elements, it could be involved in selecting

mRNAs with special 3'UTRs for stalling. Through direct interaction, Stau2 could then recruit Upf1 to the 3'UTR, as it does for Staufen Mediated Decay (SMD). This implies that the trigger that activates the decay pathway is somehow bypassed, likely through the inhibition of Upf1 phosphorylation. The two proteins may then work together to facilitate the assembly of the compact cluster structure that will be packaged into RNA granules prior to transport. Depleting the two proteins independently of each other, or disrupting their interaction, would also disrupt the transport and expression of stalled polysome-associated mRNAs such as Map1b (consistent with Graber et al., 2017), most likely by disrupting their recruitment to this pathway.

Alternatively, UPF1 may directly bind to the ribosome and participate in stalling (Atkin, Altamura, Leeds, & Culbertson, 1995; Min, Roy, Amrani, He, & Jacobson, 2013). UPF1 recruitment of STAU2 would then be a mechanism involved in compacting the ribosomes and perhaps for selecting the mRNAs that are stalled, but in this case UPF1 would not be recruited by a stop codon, or by STAU2, but by protein-protein interactions with other proteins important for stalling.

4.3.3 The Roadblock Hypothesis:

The roadblock hypothesis is a simple solution to the problem of stalling and can explain how the binding of RBPs to the mRNA downstream of the elongating ribosome could initiate stalling and facilitate the Upf1-Stau2 dependent compaction or packaging. For this to occur, elongating ribosomes moving in the 5' to 3' direction must encounter a roadblock, an RBP downstream of the ribosome, which is recognized by the ribosome and causes it to stall or slow down. Even if new tRNAs are available at the A site of the ribosome, a physical hindrance blocking the translocation of the ribosome might just do the trick. This might explain the large protected footprints we observed: the stalled ribosome and downstream RBP that is in close proximity to it

could occupy a larger portion of the mRNA than a ribosome alone would, leaving footprints that are 35 nt and larger. Considering that the size of a classical ribosomal footprint is 26-28 nt, and 21 nt when the A-site is empty, this hypothesis could explain the observation of 35 nt footprints containing consensus sites, and potentially rule out the contribution of collided ribosomes or disomes, which would be 58 nt in size (Arpat et al., 2020; Zhao et al., 2021).

This model, however, makes 2 key assumptions: 1) Any binding motif for a downstream RBP would be expected to be selectively at one end of the footprint, flanking the 3' end downstream of the ribosome; 2) There would not be any ribosomes downstream of the leading ribosome that gets stopped behind the roadblock. Indeed, if the ribosome is protecting motifs in the center of the protected fragment, it is difficult to understand how any RBP could gain access to this site to induce stalling. We observed that these large protected fragments align at the 5' end with overhangs of various sizes on the 3' end, suggesting that the blockage is not downstream of the ribosome. This brings up the idea that elongating ribosomes are, perhaps, not stalled by RBPs blocking forward movement of the ribosomes, but rather held back, stabilized and locked into place like an emergency break on a car would, and "protected" by RBP interactions. It must also be noted that because of the size selection step of ribosome footprinting, we have not observed fragments larger than ~39 nt. Hence it would be premature to rule out the contribution of collided ribosomes without repeating the ribosome footprinting with larger sized fragments (like the 58nt footprints of disomes) included in the read mapping.

4.3.4 m6A Readers as stalling mechanism.

The motif identified under ribosome peaks in the compacted ribosome pellet also contained a consensus site for m6A modification. Thus, post-transcriptional modifications of the mRNA by adding m6A could also play a role in the stalling mechanism. There are a large number of RBPs

known to bind to m6A sites that confer regulatory control on mRNAs (Dan Ohtan Wang, 2021). FMRP has been identified as an m6A reader and its ability to interact with ribosomes and regulate the transport and translation of mRNAs supports the idea of its role as a stalling factor(Hsu et al., 2019). Interestingly, we have observed the presence of one or more m6A and FMRP consensus sequences (identified by HIT-CLIPs) on a single mRNA, often times clustering under the footprint consensus peaks. This by no means should be a surprise given the popular knowledge in the field that FMRP binds and represses mRNAs. However, the means by which FMRP interacts with the RNA could be mediated by m6A markers in combination with ribosomes that are paused on consensus sequences, rather than a direct recognition of an FMRP binding motif on the mRNA.

The evidence supporting direct binding of FMRP to a dedicated motif coding region of mRNAs is weak. Most studies conducted on FMRP regulation on mRNAs have utilized the HIT-CLIP method of cross-linking FMRP to the mRNAs and then immunoprecipitating the cross-linked complex (Ule, Hwang, & Darnell, 2018), which yields a small number of consensus sequences that studies have claimed could be the binding sequence for FMRP. However, direct binding of FMRP to the coding region of regulated mRNAs has not been demonstrated. It is possible that FMRP is found to be cross-linked to some consensus sequences simply because it is there, interacting with a ribosome that is stalled on the consensus sequence. While FMRP is an m6A reader, there is a possibility that the stalling could be due to the m6A modification stalling the polysomes and FMRP binding to the stalled polysomes to stabilize them. However, the m6A sites are not restricted to the 3’overhang, so an m6A reader protein binding and then stalling an elongating ribosome has the same issues as raised above in the roadblock hypothesis.

4.3.5 Role for PurA

A major RBP found in the proteomics of RNA granules, is the purine rich mRNA binding protein PurA. The deletion of PurA leads to neurodevelopmental disorders, autism and what is referred to as PurA syndrome (Molitor, Bacher, Burczyk, & Niessing, 2021).

We observed an enrichment for PurA in our pellet fraction, consistent with the idea that PurA is associated with mRNAs regulated by stalled polysomes. Moreover, the motifs we found are, in general, quite purine rich, suggesting a role for PurA binding to the motif in regulating stalling. However, similar to the arguments above, these motifs are protected by the ribosome and not specifically located in the 3' overhang, thus how PurA would induce stalling is not clear. Then again, it is still possible that PurA is associated with the ribosome in such close proximity that the large size of the footprints could be attributed to this ribosome-protein complex sitting on the mRNA.

4.4 mRNA-mediated stalling

Much emphasis has been given to RBPs for interacting with an mRNA and determining its fate. However, mRNAs can also regulate their own translation based on their codon composition, secondary structure and though the many post-translational modifications that it can undergo.

4.4.1 Codon optimality:

Ribosomes can slow down or pause on non-optimal or GA/Purine rich sequences on the mRNA. Usually mRNAs with low codon optimality have low translational efficiency (Hanson & Coller, 2018), perhaps due to the collisions that occur while the mRNA is stalled waiting for the rare tRNA and the induction of NOGO decay (Ikeuchi, Izawa, & Inada, 2019). This may be advantageous for the packaging of mRNAs, as ribosomes that slow down or stall on specific

sequences would allow the recruitment of factors that affect the resolution of the stall (decay vs packaging vs termination). Hence, the role of FMRP could simply be to associate with ribosomes that are slowed or paused on a strip of non-optimal codons to stabilize the pause into a controlled stall. This could be either through binding to stalled ribosomes and clamp or tether them down onto the mRNA, therefore creating a straight 5' anchor with a 3' overhang as we observed, or through binding directly to mRNAs that have the motif sequences that are labelled with m6A modifications. In this case, FMRP could bind upstream of ribosomes giving us a clean 5' read alignment, while consequently occupying a consensus sequence marked by m6A markers and interacting with the ribosome, giving us a large 35 nt footprint. The loss of FMRP would remove this upstream "clamp" and cause stalled polysomes to be leaky. Hence, codon optimality could contribute to the stalling mechanism by slowing down elongating ribosomes at certain locations in the coding region for regulatory factors to come in and repress elongation, which would also allow de-repressed ribosomes to continue elongating normally once repression is removed.

4.4.2 mRNA secondary structures:

mRNA secondary structures play an important role in how mRNAs are translated. mRNAs can form closed loop structures when the 3' Poly-A-tail comes into close proximity with the 5' Cap structure. This closed loop structure can repress mRNAs at the stage of translation initiation such as when a 3'UTR binding protein binds to eIF4E and thus prevents initiation by inhibiting eIF4E-eIF4G binding. This is the model for FMRP to block translational initiation through FMRP binding to CYFIP which binds to eIF4E (Napoli et al., 2008; B. Wu et al., 2018). Other types of mRNA secondary structures in the coding region, such as stem-loops or pseduoknots, could also act as regulatory hubs, both for the interaction of RBPs and to stall elongating ribosomes(Bao et al., 2020). Staufens are known to bind stem-loop structures in the 3'UTR of mRNAs to regulate their

entry to the SMD pathway. Hence, Staufen 2 can perhaps bind the 3'UTRs of specific mRNAs and interact with UPF1 to select mRNAs that will be stalled and recruited to RNA granules.

While we did not see any evidence for double stranded mRNAs in our footprints, the size selection done during the footprinting process could have eliminated reads with palindromic sequences that are larger than 39 nt and can form secondary structures such as stem loops. Bioinformatic predictions of double stranded mRNA is not simple since there can be long distances between the two strands. Indeed, Staufen sites in the 3'UTR often consist of two stretches of matching RNA sequences far apart (Lucas et al., 2018), and yet Staufens are certainly implicated in the formation of the stalled ribosomes. The nuclease we use to do the footprinting, RNase I, does not cut double stranded RNA, hence I would predict that a second round of Footprinting analysis excluding the size selection step could yield surprising results. The 3' overhang of the large reads in our current data set could also be attributed to incomplete cleavage of the interface between the ribosome and double stranded mRNA.

Indeed, there may be two independent events occurring, one at the consensus site in the coding region, and one at a double stranded site and the two may be quite the distance apart, only being brought together by protein-protein interactions that form a closed loop structure, resembling the structure formed by the FMRP-CYFIP interaction that regulates initiation. New hypotheses can emerge from this explanation, on how a 3' UTR bound Staufen 2 that binds a nearby Upf1 could come into close proximity with an FMRP-ribosome complex sitting on a WGGA consensus sequence.

4.5 Argument for Ribosome-mediated stalling

A major RBP not discussed so far is the ribosome itself. Cells have developed strategies to deal with unexpected pauses in translation to avoid erroneous transcripts and incomplete proteins

from accumulating in the cell. However, reversible stalling for the transport and regulation of mRNAs appears to be specific to the nervous system (See Chapter 2). If the ribosome is what recognizes the motifs we have identified and causes the stalling, then neuronal ribosomes must be distinct. Neuronal ribosomes may be structurally different in a way that allows them to stall on consensus sequences. Different compartments of a neuron may express different types of ribosomes with different subunits or rRNA components, distinct ribosomal subunits, or post-transcriptional modifications of rRNA or post-translational modification of ribosomal proteins.

4.5.1 Ribosomal Heterogeneity

While direct evidence that ribosomes are heterogeneous in neuronal processes is lacking, there is indirect evidence that this may occur. First, mRNAs encoding ribosomal subunits are some of the major transported mRNAs (Holt, Martin, & Schuman, 2019; Poulopoulos et al., 2019), suggesting that local translation of subunits may lead to distinct composition of ribosomes, either due to removal/replacement of ribosomal proteins or distinct processing of ribosomal proteins in the periphery (Shi et al., 2017); L10a and S25 have been proposed to be important heterogenous components of ribosomes in other studies (reviewed in (Genuth & Barna, 2018)). Indeed, axonal synthesis requires local production of ribosomal protein S4 (Shigeoka et al., 2019). There may also be specific ribosomal proteins expressed only in neurons; for example there is a specific splice isoform of S24 that is nervous system specific (Xu & Roufa, 1996). Finally, some post-transcriptional modifications of rRNA appear enriched in the nervous system (Dan Ohtan Wang, 2021). Thus, there are many possibilities for nervous system specific ribosomes that could be programmed for stalling.

4.6 Maintenance of Stalled Ribosomes

Stalled Polysomes are formed in the soma where translation of target mRNAs is initiated and then quickly paused. This mRNA-ribosome and peptidyl complex can be easily destroyed and must be protected from the intracellular environment during its transport from the soma to local synaptic sites. For the precious cargo to arrive safely at its intended location, there must be different orders of magnitude of protection that need to occur to maintain the stalled complex and divert it away from RNA surveillance and decay pathways. As a first layer of protection, the integrity of the stalled polysomes packaged into RNA granules need to first be masked from the cytoplasmic environment, nucleases/proteases and surveillance mechanisms. Second, the mRNA and the loaded ribosomal complex on the mRNA need to remain intact so they can resume translation at their target local sites. Third, the emerging polypeptide chain attached to the ribosomes that are stalled on the mRNA in the stage of translation elongation need to be protected. Hence, the proper maintenance of stalled polysomes is as important and likely as complex as their formation. In this section, we explore the evidence and theories of how stalled polysomes are maintained.

4.6.1 Phase separation as a means of protection

One might argue that the liquid-liquid phase separated structure of the RNA granule (see Chapter 2) would be sufficient to protect the inner components during active transport. This oil-like separation of the granule components from the remainder of the intracellular environment could be established with the help of low complexity disordered domain containing proteins that can create a scaffold to coat and protect the contents from the cytoplasm. Examples of this have been well studied in Stress Granules, in which proteins such as TDP43 and G3BP assist the formation of low complexity disordered protein aggregates that helps separate stress granule

contents from the surroundings (Protter & Parker, 2016). RNA Granules share some proteins with Stress Granules, including TDP43 and G3BP, suggesting that the mechanism by which the two particles are formed are similar (Chapter 2). While Stress Granules and RNA Granules resemble each other, they have completely different functions. However, both ribonucleoparticles regulate the expression of mRNAs and proteins in a temporospatial manner that is dependent on some form of stimulus, whether it be stress, growth factors or synaptic activation. Thus, it is not far fetched to imagine that these two could utilize similar mechanisms for the formation and maintenance of their liquid-liquid phase separated structures.

4.6.2 Role for FMRP as a stabilizer of stalled polysomes

FMRP is a major protein component of the compacted ribosomes and has been associated with stalled polysomes in a number of studies described previously. FMRP is also implicated in the stabilization of the mRNA in the case of non-optimal codons (Shu et al., 2020). Since non-optimal codons causes ribosomal stalling, this suggests a general role for FMRP in protection of stalled polysomes. How FMRP interacts with the surveillance machinery for the NoGo decay pathway will be an interesting line of research to investigate in the future.

4.6.3 Evading collision-induced mRNA decay (mRNA decay in general)

No-Go Decay is induced by ribosomal collision and a number of proteins that activate this pathway, specifically recognize the collided ribosomes including the ubiquitin ligase Hel2/ZNF598 and GCN1 (Ikeuchi et al., 2019; Pochopien et al., 2021). One way to stabilize stalled polysomes in neurons is to prevent this collision. Is there a collision-detection system like in a car that regulates the rate at which the ribosome is moving based on the one in front of it? Is there a specific intra-ribosomal distance such that the second ribosome in the array is only allowed to initiate at specific intervals? Is it possible that once the polysome is stalled, then initiation of subsequent ribosomes

is also inhibited to avoid pile-up of ribosomes that may lead to a collided ribosome? Perhaps this is the role of FMRP in stabilizing stalled polysomes?

Another possible explanation is the “bumper hypothesis”, which proposes that there are RNA- or ribosome- binding proteins that act as a “bumper” between elongating ribosomes to block possible collisions. One strong piece of evidence in support of this hypothesis is the size of the footprint fragments which are unusually large; the footprints could represent an RNA or ribosome binding protein in complex with the footprinted ribosomes hence resulting in a larger footprint. While we did not detect a pattern of peaks separated by a small distance, further analysis of the spacing of the peak sequences from the identified motifs could reveal more about this possibility.

One finding that challenges this hypothesis is the alignment of the large footprints on the 5’ end with a growing 3’ end. If there was a leading ribosome that was stalled on the mRNA, causing a pile-up of ribosomes behind it, then the footprint should align on the 3’ end with varying lengths of “following ribosomes” tailing back towards the 5’ end? Hence, we would propose the hypothesis that stalled ribosomes are “held back” by an RBP on the 5’ end rather than be blocked on the 3’ end from moving forward or bumping into the leading ribosome. Upon reaching a target sequence, elongating ribosomes may perhaps become “tethered” or “clamped down” onto the mRNA, which could reduce the risk of ribosome collision.

4.6.4 Maintaining the growing polypeptide

As the ribosomes elongate on the mRNA, the growing polypeptide emerges from the peptide exit channel. In normal translation, this peptide is guided and folded with the help of many chaperones to ensure that the peptide chain is correctly folded into a functional protein. In the case of stalled polysomes, it is expected that ribosomes that are frozen at different locations on the mRNA will also have a growing polypeptide chain associated with them at the time of

stalling. Is it the way stalling occurs that blocks the nascent polypeptide chain from dissociating or is it the way that stalled polysomes are packaged into a liquid-liquid phase separated structure that protects all the contents including the polypeptide? In my opinion it is a combination of both. It is a delicate negotiation of RNA and protein where the peptide must remain attached to the tightly packaged ribosomes and be protected from proteases. Our lab has previously established the presence of chaperones in the RNA granule proteomics, supporting the hypothesis that stabilization occurs at the nascent peptide level (Elvira et al., 2006).

4.7 Nascent peptides as a stalling mechanism

Stalling of ribosomal translocation mediated by the nascent peptide is well established concept through the signal sequence paradigm where appearance of the peptide attracts the signal recognition complex that then stalls further translation (Keenan, Freymann, Stroud, & Walter, 2001). Hence, blocking the exit of the peptide from the exit channel, may also contribute to the stalling mechanism since blocking peptide exit would also be expected to hinder the tRNA movement through the ribosome.

Evidence suggesting a role for nascent peptides comes from experiments using puromycin. Puromycin is a small molecule that can enter the A channel of an 80S ribosome and conjugate to the last amino acid added. Because of this, Puromycin causes premature peptide chain termination, causing the puromycylated polypeptide chain to dissociate from the tRNA and the peptide to diffuse away (Enam et al., 2020; Hobson, Kong, Hartwick, Gonzalez, & Sims, 2020). However, the ribopurmycylation assay we have used to identify stalled polysomes in situ depends on the puromycylated peptide remaining on the ribosome (Graber et al., 2017; Graber et al., 2013). This was confirmed using SunTag, a distinct technique to identify nascent peptides on a ribosome where neuronal nascent peptides at distal sites were resistant to dissociation by puromycin (Langille et

al., 2019). This is distinct for neuronal ribosomes as puromyclyated peptides diffuse from other ribosomes, even in the presence of elongation inhibitors (Enam et al., 2020; Hobson et al., 2020). This suggests that in the stalled neuronal ribosomes, the peptide does not have the ability to diffuse away, even after dissociation from the tRNA by puromycin. This is consistent with stalling through prevention of peptide movement through the exit channel. Future work, perhaps with examination of the structure of stalled polysomes at the atomic level through cryo-electron microscopy may address this question.

4.8 Future Directions

With the new evidence we uncovered, we also revealed caveats of existing methods as well as new questions that need to be answered. More work is needed to understand the precise mechanism of stalling and unstalling, and to overcome the limitations of the techniques used. Some burning questions that remain are as follows:

4.8.1 Can the footprint reads be larger?

After RNA extraction from the digested pellet monosomes, there is a size selection step that requires the cutting of a gel on which the footprints are run. This is done manually, taking the 17mer and 34mer size markers into account. Depending on how close the gel is cut to the markers, there could be a discrepancy in sequencing results, causing some larger fragments to be lost in the process. Based on the size markers we used, it is possible that we missed out on the larger polysomal footprints that run above the 35mer marker. For the revised version of the manuscript, size selection should be done up to 50mer and with the guide of a standardized excision tool, to ensure that large stretches of polysomes that may leave larger footprints (with periodicity) can be identified. To be able to ascertain whether there are large polysomes on the pellet mRNAs, and to

identify interaction sites for ribosome-RBP-mRNA complexes, this is a simple solution that may yield great rewards.

4.8.2 FMRP-Ribosome interaction

FMRP has been shown to interact with the 80S ribosome (E. Chen et al., 2014). Hence, it must be clarified whether the consensus sequences associated with FMRP HITS-CLIP targets is the result of a direct interaction of FMRP with the mRNA or with the ribosome that is occupying that stretch of mRNA. Because the HITS-CLIP technique requires cross-inking of the proteins to the mRNA, it is possible that an adjacent or associated ribosome be covalently crosslinked and co-precipitated with FMRP. The direct interaction between FMRP and Ribosome could be inducing the stall, or FMRP may simply tether and stabilize the ribosome that gets stalled on the mRNA. To address this, high resolution Cryo-EM techniques will provide powerful insight. 3D reconstruction and single particle analysis of digested pellet monosomes may reveal interaction partners on or inside the ribosomal complex. In vitro re-constitution of FMRP to a preparation of neuronal ribosomes, or even in situ reconstitution to Fragile X patient derived cells, and the consequent Cryo-EM or TEM imaging of ribosomes is an ambitious yet promising route that can be followed. Cryo-Electron Tomography, a spectacular technique that provides wide-field view and atomic resolution of samples, could reveal the structural morphology of RNA Granules and stalled polysomes and perhaps show where FMRP and other RBPs could be bound.

4.8.3 Ribosomal Heterogeneity and Ribosome as a stalling factor

Ribosomes are truly remarkable machines that read and translate mRNA. Hence, it is possible that the ribosome is contributing to stalling, through subunit heterogeneity or modification, conformational changes and even rRNA-mRNA interaction. Single particle Cryo-EM analysis of ribosomes that run in different fractions of the sucrose gradient may reveal interesting differences

between stalled and actively translating ribosomes. Also, analyzing the intricate interaction of ribosomal RNA and proteins with the mRNA in the 60S-40S mRNA interface of the ribosome may yield some answers.

4.8.4 Leaky RNA Granules in Fragile X

Ultimately, experiments comparing RNA granules of FXS patients or FMR1 KO animals with healthy controls is necessary to ascertain FMRP's direct role in regulating stalled ribosomes. This endeavor will be taken on by the new student I am training in the lab who will begin with FMR1 KO mice lines; I am excited to see the results. Do KO animals have less stalled polysomes? What is different about their activity dependent translation? What is different about the ribosomal footprints?

4.8.5 The eEF2 conundrum

One protein that has haunted me since the beginning of my graduate degree is the elusive elongation factor eEF2. eEF2 is not only required for the translocation of the ribosome on the mRNA during translation, but the phosphorylation of eEF2 has been shown time and time again to be required for the release of stalled polysomes, production of Map1b and the induction of mGluR-LTD. As a protein that transiently interacts with the ribosomal complex, eEF2 is hard to catch. I was unable to detect eEF2 in my western blots, however occasionally I would see a faint band of 15kDa size with the C-terminal eEF2 antibody. For long, we hypothesized that eEF2 may be stuck or sequestered on the ribosomal complex and released through phosphorylation, and our lab's previous proteomics analysis of RNA Granules revealed a short C-terminal fragment approximately the same size. We are not as confident about this hypothesis, as it is highly likely that phosphorylation of eEF2 could be freeing up translation factors that are needed for a small burst of translation in a partially phase separated compartment of a disassembled RNA granule.

However, there is some evidence in the field indicating that eEF2 or the eEF2 binding pocket of the ribosome may be modified to facilitate this. eEF2 has a diphthamide modification that gets ADP-ribosylated when energy levels are low in the cell; his modification allows eEF2 to be inhibited yet remain on the ribosomal complex (S. Liu et al., 2012; Mateus-Seidl et al., 2019). This is the same residue that gets ADP ribosylated as a result of the deadly diphtheria toxin (Mateyak & Kinzy, 2013). There are also E3 ligases that modify the eEF2 binding pocket and affect the affinity of eEF2 to the ribosomal complex (Y. Zhou et al., 2020). These modifications must be explored to better understand how eEF2 may be involved in this process.

4.8.6 mRNA structures

mRNAs are not straight strips of nucleotides that ribosomes can race through. mRNAs also have an intrinsic structure based on the residues that can sterically interact with each other, and form double-stranded stretches of mRNA like hairpin structures. mRNAs can also be formed into closed loop structures through interaction of RNA binding proteins. A more thorough analysis of codon optimality of footprint mRNAs, a search for palindromic sequences and location where mRNA strands are brought to close proximity to each other need to be examined. While the evidence supporting the involvement of RNA binding proteins in ribosome stalling is striking, it is important not to overlook the secrets embedded in the text that is being translated; one must read between the lines, per se!

4.9 References

Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C., & Schuman, E. M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron, 30*(2), 489-502. doi:10.1016/s0896-6273(01)00295-1

Agirrezabala, X., Lei, J., Brunelle, J. L., Ortiz-Meoz, R. F., Green, R., & Frank, J. (2008). Visualization of the Hybrid State of tRNA Binding Promoted by Spontaneous Ratcheting of the Ribosome. *Molecular Cell, 32*(2), 190-197. doi:10.1016/j.molcel.2008.10.001

Aitken, C. E., & Lorsch, J. R. (2012). A mechanistic overview of translation initiation in eukaryotes. *Nature Structural & Molecular Biology, 19*(6), 568-576. doi:10.1038/nsmb.2303

Amadei, G., Zander, M., Yang, G., Dumelie, J., Vessey, J., Lipshitz, H., . . . Miller, F. (2015). A Smaug2-Based Translational Repression Complex Determines the Balance between Precursor Maintenance versus Differentiation during Mammalian Neurogenesis. *Journal of Neuroscience, 35*, 15666-15681. doi:10.1523/JNEUROSCI.2172-15.2015

Amorim, I. S., Kedia, S., Kouloulia, S., Simbriger, K., Gantois, I., Jafarnejad, S. M., . . . Gkogkas, C. G. (2018). Loss of eIF4E Phosphorylation Engenders Depression-like Behaviors via Selective mRNA Translation. *J Neurosci, 38*(8), 2118-2133. doi:10.1523/JNEUROSCI.2673-17.2018

Amorim, I. S., Lach, G., & Gkogkas, C. G. (2018). The Role of the Eukaryotic Translation Initiation Factor 4E (eIF4E) in Neuropsychiatric Disorders. *Frontiers in Genetics, 9*(561). doi:10.3389/fgene.2018.00561

Anadolu, M. N., Kailasam, S., Simbriger, K., Sun, J., Markova, T., Jafarnejad, S. M., . . . Sossin, W. S. (2021). Ribosomes in RNA granules are stalled on mRNA sequences that are consensus sites for FMRP association. *bioRxiv*, 2021.2002.2022.432349. doi:10.1101/2021.02.22.432349

Anadolu, M. N., & Sossin, W. S. (2020). Focusing on mRNA granules and stalled polysomes amidst diverse mechanisms underlying mRNA transport, mRNA storage and local translation. In W. Sossin (Ed.), *Oxford Hanbook of Neuronal Protein Synthesis*. Oxford Press.

Anderson, B. R., Chopra, P., Suhl, J. A., Warren, S. T., & Bassell, G. J. (2016). Identification of consensus binding sites clarifies FMRP binding determinants. *Nucleic Acids Res, 44*(14), 6649-6659. doi:10.1093/nar/gkw593

Anderson, P., & Kedersha, N. (2006). RNA granules. *The Journal of Cell Biology, 172*(6), 803-808. doi:10.1083/jcb.200512082

Arikkath, J. (2020). Mechanisms of axon polarization in pyramidal neurons. *Molecular and Cellular Neuroscience, 107*, 103522. doi:https://doi.org/10.1016/j.mcn.2020.103522

Arimura, N., & Kaibuchi, K. (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nature Reviews Neuroscience, 8*(3), 194-205. doi:10.1038/nrn2056

Arpat, A. B., Liechti, A., De Matos, M., Dreos, R., Janich, P., & Gatfield, D. (2020). Transcriptome-wide sites of collided ribosomes reveal principles of translational pausing. *Genome Res, 30*(7), 985-999. doi:10.1101/gr.257741.119

Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J. B., Nusbaum, J. D., Corcoran, D. L., . . . Tuschl, T. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature, 492*(7429), 382-386. doi:10.1038/nature11737

Ascano, M., Mukherjee, N., Bandaru, P., Miller, J. B., Nusbaum, J. D., Corcoran, D. L., . . . Tuschl, T. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature, 492*(7429), 382-386. doi:10.1038/nature11737

Aschrafi, A., Cunningham, B. A., Edelman, G. M., & Vanderklish, P. W. (2005). The fragile X mental retardation protein and group I metabotropic glutamate receptors regulate levels of mRNA granules in brain. *Proc Natl Acad Sci U S A, 102*(6), 2180-2185. doi:10.1073/pnas.0409803102

Athar, Y. M., & Joseph, S. (2020). The Human Fragile X Mental Retardation Protein Inhibits the Elongation Step of Translation through Its RGG and C-Terminal Domains. *Biochemistry, 59*(40), 3813-3822. doi:10.1021/acs.biochem.0c00534

Atkin, A. L., Altamura, N., Leeds, P., & Culbertson, M. R. (1995). The majority of yeast UPF1 co-localizes with polyribosomes in the cytoplasm. *Mol Biol Cell, 6*(5), 611-625. doi:10.1091/mbc.6.5.611

Auerbach, B. D., Osterweil, E. K., & Bear, M. F. (2011). Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature, 480*(7375), 63-68. doi:10.1038/nature10658

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., . . . Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res, 37*(Web Server issue), W202-208. doi:10.1093/nar/gkp335

Banerjee, S., Neveu, P., & Kosik, K. S. (2009). A coordinated local translational control point at the synapse involving relief from silencing and MOV10 degradation. *Neuron, 64*(6), 871-884. doi:10.1016/j.neuron.2009.11.023

Bao, C., Loerch, S., Ling, C., Korostelev, A. A., Grigorieff, N., & Ermolenko, D. N. (2020). mRNA stem-loops can pause the ribosome by hindering A-site tRNA binding. *Elife, 9*, e55799. doi:10.7554/eLife.55799

Barbarese, E., Koppel, D. E., Deutscher, M. P., Smith, C. L., Ainger, K., Morgan, F., & Carson, J. H. (1995). Protein translation components are colocalized in granules in oligodendrocytes. *Journal of Cell Science, 108*(8), 2781-2790.

Barbee, S. A., Estes, P. S., Cziko, A. M., Hillebrand, J., Luedeman, R. A., Coller, J. M., . . . Ramaswami, M. (2006). Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron, 52*(6), 997-1009. doi:10.1016/j.neuron.2006.10.028

Barbosa, C., Peixeiro, I., & Romão, L. (2013). Gene expression regulation by upstream open reading frames and human disease. *PLoS Genet, 9*(8), e1003529. doi:10.1371/journal.pgen.1003529

Barnes, A. P., & Polleux, F. (2009). Establishment of axon-dendrite polarity in developing neurons. *Annual review of neuroscience, 32*, 347-381. doi:10.1146/annurev.neuro.31.060407.125536

Bartley, C. M., O'Keefe, R. A., Blice-Baum, A., Mihailescu, M. R., Gong, X., Miyares, L., . . . Bordey, A. (2016). Mammalian FMRP S499 Is Phosphorylated by CK2 and Promotes Secondary Phosphorylation of FMRP. *eNeuro, 3*(6). doi:10.1523/ENEURO.0092-16.2016

Bartley, C. M., O'Keefe, R. A., & Bordey, A. (2014). FMRP S499 is phosphorylated independent of mTORC1-S6K1 activity. *PLOS ONE, 9*(5), e96956. doi:10.1371/journal.pone.0096956

Batish, M., van den Bogaard, P., Kramer, F. R., & Tyagi, S. (2012). Neuronal mRNAs travel singly into dendrites. *Proc Natl Acad Sci U S A, 109*(12), 4645-4650. doi:10.1073/pnas.1111226109

Bauer, K. E., Segura, I., Gaspar, I., Scheuss, V., Illig, C., Ammer, G., . . . Kiebler, M. A. (2019). Live cell imaging reveals 3'-UTR dependent mRNA sorting to synapses. *Nat Commun, 10*(1), 3178. doi:10.1038/s41467-019-11123-x

Becalska, A. N., & Gavis, E. R. (2009). Lighting up mRNA localization in Drosophila oogenesis. *Development, 136*(15), 2493-2503. doi:10.1242/dev.032391

Bellato, H. M., & Hajj, G. N. (2016). Translational control by eIF2α in neurons: Beyond the stress response. *Cytoskeleton (Hoboken), 73*(10), 551-565. doi:10.1002/cm.21294

Bian, F., Chu, T., Schilling, K., & Oberdick, J. (1996). Differential mRNA transport and the regulation of protein synthesis: selective sensitivity of Purkinje cell dendritic mRNAs to translational inhibition. *Mol Cell Neurosci, 7*(2), 116-133. doi:10.1006/mcne.1996.0009

Biever, A., Glock, C., Tushev, G., Ciirdaeva, E., Dalmay, T., Langer, J. D., & Schuman, E. M. (2020). Monosomes actively translate synaptic mRNAs in neuronal processes. *Science, 367*(6477). doi:10.1126/science.aay4991

Bogorad, A. M., Lin, K. Y., & Marintchev, A. (2017). Novel mechanisms of eIF2B action and regulation by eIF2α phosphorylation. *Nucleic Acids Res, 45*(20), 11962-11979. doi:10.1093/nar/gkx845

Boland, M. J., Nazor, K. L., Tran, H. T., Szücs, A., Lynch, C. L., Paredes, R., . . . Loring, J. F. (2017). Molecular analyses of neurogenic defects in a human pluripotent stem cell model of fragile X syndrome. *Brain, 140*(3), 582-598. doi:10.1093/brain/aww357

Bond, P. (2016). Regulation of mTORC1 by growth factors, energy status, amino acids and mechanical stimuli at a glance. *Journal of the International Society of Sports Nutrition, 13*(1), 8. doi:10.1186/s12970-016-0118-y

Boye, E., & Grallert, B. (2020). eIF2α phosphorylation and the regulation of translation. *Current Genetics, 66*(2), 293-297. doi:10.1007/s00294-019-01026-1

Brackley, C. A., Romano, M. C., & Thiel, M. (2011). The Dynamics of Supply and Demand in mRNA Translation. *PLOS Computational Biology, 7*(10), e1002203. doi:10.1371/journal.pcbi.1002203

Bramham, C. R., Jensen, K. B., & Proud, C. G. (2016). Tuning Specific Translation in Cancer Metastasis and Synaptic Memory: Control at the MNK–eIF4E Axis. *Trends in Biochemical Sciences, 41*(10), 847-858. doi:10.1016/j.tibs.2016.07.008

Bramswig, N. C., Ludecke, H. J., Hamdan, F. F., Altmuller, J., Beleggia, F., Elcioglu, N. H., . . . Wieczorek, D. (2017). Heterozygous HNRNPU variants cause early onset epilepsy and severe intellectual disability. *Hum Genet, 136*(7), 821-834. doi:10.1007/s00439-017-1795-6

Brittis, P. A., Lu, Q., & Flanagan, J. G. (2002). Axonal Protein Synthesis Provides a Mechanism for Localized Regulation at an Intermediate Target. *Cell, 110*(2), 223-235. doi:10.1016/S0092-8674(02)00813-9

Broadus, J., Fuerstenberg, S., & Doe, C. Q. (1998). Staufen-dependent localization of prospero mRNA contributes to neuroblast daughter-cell fate. *Nature, 391*(6669), 792-795. doi:10.1038/35861

Brogna, S., & Wen, J. (2009). Nonsense-mediated mRNA decay (NMD) mechanisms. *Nature Structural & Molecular Biology, 16*(2), 107-113. doi:10.1038/nsmb.1550

Buffington, S. A., Huang, W., & Costa-Mattioli, M. (2014). Translational control in synaptic plasticity and cognitive dysfunction. *Annu Rev Neurosci, 37*, 17-38. doi:10.1146/annurev-neuro-071013-014100

Buskirk, A. R., & Green, R. (2017). Ribosome pausing, arrest and rescue in bacteria and eukaryotes. *Philos Trans R Soc Lond B Biol Sci, 372*(1716). doi:10.1098/rstb.2016.0183

Buxbaum, A. R., Wu, B., & Singer, R. H. (2014). Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science, 343*(6169), 419-422. doi:10.1126/science.1242939

Cajigas, I. J., Tushev, G., Will, T. J., tom Dieck, S., Fuerst, N., & Schuman, E. M. (2012). The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron, 74*(3), 453-466. doi:10.1016/j.neuron.2012.02.036

Campbell, D. S., & Holt, C. E. (2001). Chemotropic Responses of Retinal Growth Cones Mediated by Rapid Local Protein Synthesis and Degradation. *Neuron, 32*(6), 1013-1026. doi:10.1016/S0896-6273(01)00551-7

Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., . . . Kandel, E. R. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell, 99*(2), 221-237. doi:10.1016/s0092-8674(00)81653-0

Ceman, S., O'Donnell, W. T., Reed, M., Patton, S., Pohl, J., & Warren, S. T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. *Hum Mol Genet, 12*(24), 3295-3305. doi:10.1093/hmg/ddg350

Chau, K. F., Shannon, M. L., Fame, R. M., Fonseca, E., Mullan, H., Johnson, M. B., . . . Lehtinen, M. K. (2018). Downregulation of ribosome biogenesis during early forebrain development. *Elife, 7*, e36998. doi:10.7554/eLife.36998

Chen, E., Sharma, M. R., Shi, X., Agrawal, R. K., & Joseph, S. (2014). Fragile X mental retardation protein regulates translation by binding directly to the ribosome. *Mol Cell, 54*(3), 407-417. doi:10.1016/j.molcel.2014.03.023

Chen, Y. C., Chang, Y. W., & Huang, Y. S. (2019). Dysregulated Translation in Neurodevelopmental Disorders: An Overview of Autism-Risk Genes Involved in Translation. *Dev Neurobiol, 79*(1), 60-74. doi:10.1002/dneu.22653

Choi, J., Ieong, K. W., Demirci, H., Chen, J., Petrov, A., Prabhakar, A., . . . Puglisi, J. D. (2016). N(6)-methyladenosine in mRNA disrupts tRNA selection and translation-elongation dynamics. *Nat Struct Mol Biol, 23*(2), 110-115. doi:10.1038/nsmb.3148

Cioni, J. M., Koppers, M., & Holt, C. E. (2018). Molecular control of local translation in axon development and maintenance. *Curr Opin Neurobiol, 51*, 86-94. doi:10.1016/j.conb.2018.02.025

Clemens, M. J. (2001). Initiation factor eIF2 alpha phosphorylation in stress responses and apoptosis. *Prog Mol Subcell Biol, 27*, 57-89. doi:10.1007/978-3-662-09889-9_3

Cook, D., Sanchez-Carbente Mdel, R., Lachance, C., Radzioch, D., Tremblay, S., Khandjian, E. W., . . . Murai, K. K. (2011). Fragile X related protein 1 clusters with ribosomes and messenger RNAs at a subset of dendritic spines in the mouse hippocampus. *PLOS ONE, 6*(10), e26120. doi:10.1371/journal.pone.0026120

Cooper, G. M. (2000). *The cell : a molecular approach* (2nd ed. ed.). Washington, D.C.

Sunderland, Mass.: ASM Press ;

Sinauer Associates.

Costa-Mattioli, M., Sossin, W. S., Klann, E., & Sonenberg, N. (2009). Translational Control of Long-Lasting Synaptic Plasticity and Memory. *Neuron, 61*(1), 10-26. doi:10.1016/j.neuron.2008.10.055

Coulson, R. L., & Klein, M. (1997). Rapid Development of Synaptic Connections and Plasticity Between Sensory Neurons and Motor Neurons of Aplysia in Cell Culture: Implications for Learning and Regulation of Synaptic Strength. *Journal of Neurophysiology, 77*(5), 2316-2327. doi:10.1152/jn.1997.77.5.2316

Darnell, J. C., Van Driesche, S. J., Zhang, C., Hung, K. Y., Mele, A., Fraser, C. E., . . . Darnell, R. B. (2011). FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell, 146*(2), 247-261. doi:10.1016/j.cell.2011.06.013

David, A., Bennink, J. R., & Yewdell, J. W. (2013). Emetine optimally facilitates nascent chain puromycylation and potentiates the ribopuromycylation method (RPM) applied to inert cells. *Histochem Cell Biol, 139*(3), 501-504. doi:10.1007/s00418-012-1063-8

Davidkova, G., & Carroll, R. C. (2007). Characterization of the role of microtubule-associated protein 1B in metabotropic glutamate receptor-mediated endocytosis of AMPA receptors in hippocampus. *J Neurosci, 27*(48), 13273-13278. doi:10.1523/JNEUROSCI.3334-07.2007

Dever, T. E., & Green, R. (2012). The Elongation, Termination, and Recycling Phases of Translation in Eukaryotes. *Cold Spring Harbor Perspectives in Biology, 4*(7). doi:10.1101/cshperspect.a013706

Di Prisco, G. V., Huang, W., Buffington, S. A., Hsu, C. C., Bonnen, P. E., Placzek, A. N., . . . Costa-Mattioli, M. (2014). Translational control of mGluR-dependent long-term depression and object-place learning by eIF2alpha. *Nat Neurosci, 17*(8), 1073-1082. doi:10.1038/nn.3754

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., . . . Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics, 29*(1), 15-21. doi:10.1093/bioinformatics/bts635

Dynes, J. L., & Steward, O. (2012). Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation. *J Comp Neurol, 520*(14), 3105-3119. doi:10.1002/cne.23073

El Fatimy, R., Davidovic, L., Tremblay, S., Jaglin, X., Dury, A., Robert, C., . . . Khandjian, E. W. (2016). Tracking the Fragile X Mental Retardation Protein in a Highly Ordered Neuronal RiboNucleoParticles Population: A Link between Stalled Polyribosomes and RNA Granules. *PLoS Genet, 12*(7), e1006192. doi:10.1371/journal.pgen.1006192

Eliscovich, C., Buxbaum, A. R., Katz, Z. B., & Singer, R. H. (2013). mRNA on the move: the road to its biological destiny. *J Biol Chem, 288*(28), 20361-20368. doi:10.1074/jbc.R113.452094

Elvira, G., Wasiak, S., Blandford, V., Tong, X. K., Serrano, A., Fan, X., . . . Sossin, W. S. (2006). Characterization of an RNA granule from developing brain. *Mol Cell Proteomics, 5*(4), 635-651. doi:10.1074/mcp.M500255-MCP200

Enam, S. U., Zinshteyn, B., Goldman, D. H., Cassani, M., Livingston, N. M., Seydoux, G., & Green, R. (2020). Puromycin reactivity does not accurately localize translation at the subcellular level. *Elife, 9*, e60303. doi:10.7554/eLife.60303

Eom, T., Antar, L. N., Singer, R. H., & Bassell, G. J. (2003). Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J Neurosci, 23*(32), 10433-10444.

Fabian, M. R., & Sonenberg, N. (2012). The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat Struct Mol Biol, 19*(6), 586-593. doi:10.1038/nsmb.2296

Ferraiuolo, M. A., Basak, S., Dostie, J., Murray, E. L., Schoenberg, D. R., & Sonenberg, N. (2005). A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay. *J Cell Biol, 170*(6), 913-924. doi:10.1083/jcb.200504039

Fontes, M. M., Guvenek, A., Kawaguchi, R., Zheng, D., Huang, A., Ho, V. M., . . . Martin, K. C. (2017). Activity-Dependent Regulation of Alternative Cleavage and Polyadenylation During Hippocampal Long-Term Potentiation. *Sci Rep, 7*(1), 17377. doi:10.1038/s41598-017-17407-w

Foster, K. G., & Fingar, D. C. (2010). Mammalian Target of Rapamycin (mTOR): Conducting the Cellular Signaling Symphony*. *Journal of Biological Chemistry, 285*(19), 14071-14077. doi:https://doi.org/10.1074/jbc.R109.094003

Franks, T. M., & Lykke-Andersen, J. (2008). The control of mRNA decapping and P-body formation. *Mol Cell, 32*(5), 605-615. doi:10.1016/j.molcel.2008.11.001

Fritz, S., & Boris-Lawrie, K. (2015). The RNPs of eukaryotic translation control. *Trends in Cell and Molecular Biology, 10*, 105-130.

Fritzsche, R., Karra, D., Bennett, K. L., Ang, F. Y., Heraud-Farlow, J. E., Tolino, M., . . . Kiebler, M. A. (2013). Interactome of two diverse RNA granules links mRNA localization to translational repression in neurons. *Cell reports, 5*(6), 1749-1762. doi:10.1016/j.celrep.2013.11.023

Garbe, D. S., & Bashaw, G. J. (2004). Axon guidance at the midline: from mutants to mechanisms. *Crit Rev Biochem Mol Biol, 39*(5-6), 319-341. doi:10.1080/10409230490906797

Garber, K. B., Visootsak, J., & Warren, S. T. (2008). Fragile X syndrome. *Eur J Hum Genet, 16*(6), 666-672. doi:10.1038/ejhg.2008.61

Gardiner, A. S., Twiss, J. L., & Perrone-Bizzozero, N. I. (2015). Competing Interactions of RNA-Binding Proteins, MicroRNAs, and Their Targets Control Neuronal Development and Function. *Biomolecules, 5*(4), 2903-2918. doi:10.3390/biom5042903

Garner, C. C., Waites, C. L., & Ziv, N. E. (2006). Synapse development: still looking for the forest, still lost in the trees. *Cell Tissue Res, 326*(2), 249-262. doi:10.1007/s00441-006-0278-1

Genuth, N. R., & Barna, M. (2018). Heterogeneity and specialized functions of translation machinery: from genes to organisms. *Nat Rev Genet, 19*(7), 431-452. doi:10.1038/s41576-018-0008-z

Ghosh, S., Ganesan, R., Amrani, N., & Jacobson, A. (2010). Translational competence of ribosomes released from a premature termination codon is modulated by NMD factors. *Rna, 16*(9), 1832-1847. doi:10.1261/rna.1987710

Gindulyte, A., Bashan, A., Agmon, I., Massa, L., Yonath, A., & Karle, J. (2006). The Transition State for Formation of the Peptide Bond in the Ribosome. *Proc Natl Acad Sci U S A, 103*(36), 13327-13332.

Giorgi, C., Yeo, G. W., Stone, M. E., Katz, D. B., Burge, C., Turrigiano, G., & Moore, M. J. (2007). The EJC factor eIF4AIII modulates synaptic strength and neuronal protein expression. *Cell, 130*(1), 179-191. doi:10.1016/j.cell.2007.05.028

Gkogkas, C. G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D. B., . . . Sonenberg, N. (2013). Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature, 493*(7432), 371-377. doi:10.1038/nature11628

Gkogkas, C. G., Khoutorsky, A., Ran, I., Rampakakis, E., Nevarko, T., Weatherill, D. B., . . . Sonenberg, N. (2013). Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature, 493*(7432), 371-377. doi:10.1038/nature11628

Gkogkas, C. G., & Sonenberg, N. (2013). Translational control and autism-like behaviors. *Cell Logist, 3*(1), e24551. doi:10.4161/cl.24551

Glock, C., Heumüller, M., & Schuman, E. M. (2017). mRNA transport & local translation in neurons. *Curr Opin Neurobiol, 45*, 169-177. doi:https://doi.org/10.1016/j.conb.2017.05.005

Goldman, J. S., Ashour, M. A., Magdesian, M. H., Tritsch, N. X., Harris, S. N., Christofi, N., . . . Kennedy, T. E. (2013). Netrin-1 promotes excitatory synaptogenesis between cortical neurons by initiating synapse assembly. *J Neurosci, 33*(44), 17278-17289. doi:10.1523/JNEUROSCI.1085-13.2013

Goldstrohm, A. C., Hall, T. M. T., & McKenney, K. M. (2018). Post-transcriptional Regulatory Functions of Mammalian Pumilio Proteins. *Trends Genet, 34*(12), 972-990. doi:10.1016/j.tig.2018.09.006

Gong, X., Zhang, L., Huang, T., Lin, T. V., Miyares, L., Wen, J., . . . Bordey, A. (2015). Activating the translational repressor 4E-BP or reducing S6K-GSK3β activity prevents accelerated axon growth induced by hyperactive mTOR in vivo. *Hum Mol Genet, 24*(20), 5746-5758. doi:10.1093/hmg/ddv295

Graber, T. E., Freemantle, E., Anadolu, M. N., Hebert-Seropian, S., MacAdam, R. L., Shin, U., . . . Sossin, W. S. (2017). UPF1 Governs Synaptic Plasticity through Association with a STAU2 RNA Granule. *J Neurosci, 37*(38), 9116-9131. doi:10.1523/jneurosci.0088-17.2017

Graber, T. E., Hebert-Seropian, S., Khoutorsky, A., David, A., Yewdell, J. W., Lacaille, J. C., & Sossin, W. S. (2013). Reactivation of stalled polyribosomes in synaptic plasticity. *Proc Natl Acad Sci U S A, 110*(40), 16205-16210. doi:10.1073/pnas.1307747110

Grant, C. E., Bailey, T. L., & Noble, W. S. (2011). FIMO: scanning for occurrences of a given motif. *Bioinformatics, 27*(7), 1017-1018. doi:10.1093/bioinformatics/btr064

Hafner, A. S., Donlin-Asp, P. G., Leitch, B., Herzog, E., & Schuman, E. M. (2019). Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. *Science, 364*(6441). doi:10.1126/science.aau3644

Han, P., Shichino, Y., Schneider-Poetsch, T., Mito, M., Hashimoto, S., Udagawa, T., . . . Iwasaki, S. (2020). Genome-wide Survey of Ribosome Collision. *Cell Rep, 31*(5), 107610. doi:10.1016/j.celrep.2020.107610

Hanson, G., & Coller, J. (2018). Codon optimality, bias and usage in translation and mRNA decay. *Nature Reviews Molecular Cell Biology, 19*(1), 20-30. doi:10.1038/nrm.2017.91

Hanz, S., Perlson, E., Willis, D., Zheng, J. Q., Massarwa, R., Huerta, J. J., . . . Fainzilber, M. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron, 40*(6), 1095-1104. doi:10.1016/s0896-6273(03)00770-0

Harigaya, Y., & Parker, R. (2010). No-go decay: a quality control mechanism for RNA in translation. *Wiley Interdiscip Rev RNA, 1*(1), 132-141. doi:10.1002/wrna.17

Harrell Jr, F. E., & Dupont, M. C. (2019). Package Hmisc. Vienna, Austria.

Harrison, A. F., & Shorter, J. (2017). RNA-binding proteins with prion-like domains in health and disease. *Biochem J, 474*(8), 1417-1438. doi:10.1042/BCJ20160499

Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes Dev, 18*(16), 1926-1945. doi:10.1101/gad.1212704

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., . . . Glass, C. K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory

elements required for macrophage and B cell identities. *Mol Cell, 38*(4), 576-589. doi:10.1016/j.molcel.2010.05.004

Henry, F. E., McCartney, A. J., Neely, R., Perez, A. S., Carruthers, C. J., Stuenkel, E. L., . . . Sutton, M. A. (2012). Retrograde changes in presynaptic function driven by dendritic mTORC1. *J Neurosci, 32*(48), 17128-17142. doi:10.1523/jneurosci.2149-12.2012

Henry, F. E., Wang, X., Serrano, D., Perez, A. S., Carruthers, C. J. L., Stuenkel, E. L., & Sutton, M. A. (2018). A Unique Homeostatic Signaling Pathway Links Synaptic Inactivity to Postsynaptic mTORC1. *J Neurosci, 38*(9), 2207-2225. doi:10.1523/jneurosci.1843-17.2017

Heraud-Farlow, J. E., & Kiebler, M. A. (2014). The multifunctional Staufen proteins: conserved roles from neurogenesis to synaptic plasticity. *Trends Neurosci, 37*(9), 470-479. doi:10.1016/j.tins.2014.05.009

Hillebrand, J., Pan, K., Kokaram, A., Barbee, S., Parker, R., & Ramaswami, M. (2010). The Me31B DEAD-Box Helicase Localizes to Postsynaptic Foci and Regulates Expression of a CaMKII Reporter mRNA in Dendrites of Drosophila Olfactory Projection Neurons. *Front Neural Circuits, 4*, 121. doi:10.3389/fncir.2010.00121

Hobson, B. D., Kong, L., Hartwick, E. W., Gonzalez, R. L., & Sims, P. A. (2020). Elongation inhibitors do not prevent the release of puromycylated nascent polypeptide chains from ribosomes. *Elife, 9*, e60048. doi:10.7554/eLife.60048

Hoeffer, C. A., Sanchez, E., Hagerman, R. J., Mu, Y., Nguyen, D. V., Wong, H., . . . Tassone, F. (2012). Altered mTOR signaling and enhanced CYFIP2 expression levels in subjects with fragile X syndrome. *Genes Brain Behav, 11*(3), 332-341. doi:10.1111/j.1601-183X.2012.00768.x

Holt, C. E., Martin, K. C., & Schuman, E. M. (2019). Local translation in neurons: visualization and function. *Nat Struct Mol Biol, 26*(7), 557-566. doi:10.1038/s41594-019-0263-5

Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience, 10*(9), 647-658. doi:10.1038/nrn2699

Hoye, M. L., & Silver, D. L. (2021). Decoding mixed messages in the developing cortex: translational regulation of neural progenitor fate. *Curr Opin Neurobiol, 66*, 93-102. doi:10.1016/j.conb.2020.10.001

Hsu, P. J., Shi, H., Zhu, A. C., Lu, Z., Miller, N., Edens, B. M., . . . He, C. (2019). The RNA-binding protein FMRP facilitates the nuclear export of N (6)-methyladenosine-containing mRNAs. *J Biol Chem, 294*(52), 19889-19895. doi:10.1074/jbc.AC119.010078

Huang, Y. S., & Richter, J. D. (2004). Regulation of local mRNA translation. *Curr Opin Cell Biol, 16*(3), 308-313. doi:10.1016/j.ceb.2004.03.002

Huber, A. B., Kolodkin, A. L., Ginty, D. D., & Cloutier, J. F. (2003). Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annu Rev Neurosci, 26*, 509-563. doi:10.1146/annurev.neuro.26.010302.081139

Huber, K. M., Kayser, M. S., & Bear, M. F. (2000). Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science, 288*(5469), 1254-1257.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., . . . Singer, R. H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature, 438*(7067), 512-515. doi:10.1038/nature04115

Ikeuchi, K., Izawa, T., & Inada, T. (2019). Recent Progress on the Molecular Mechanism of Quality Controls Induced by Ribosome Stalling. *Frontiers in Genetics, 9*(743). doi:10.3389/fgene.2018.00743

Ingolia, N. T. (2014). Ribosome profiling: new views of translation, from single codons to genome scale. *Nat Rev Genet, 15*(3), 205-213. doi:10.1038/nrg3645

Ivanov, P. V., Gehring, N. H., Kunz, J. B., Hentze, M. W., & Kulozik, A. E. (2008). Interactions between UPF1, eRFs, PABP and the exon junction complex suggest an integrated model for mammalian NMD pathways. *EMBO J, 27*(5), 736-747. doi:10.1038/emboj.2008.17

Jackson, R. J., Hellen, C. U. T., & Pestova, T. V. (2010). The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature Reviews Molecular Cell Biology, 11*(2), 113-127. doi:10.1038/nrm2838

Jakawich, S. K., Nasser, H. B., Strong, M. J., McCartney, A. J., Perez, A. S., Rakesh, N., . . . Sutton, M. A. (2010). Local presynaptic activity gates homeostatic changes in presynaptic function driven by dendritic BDNF synthesis. *Neuron, 68*(6), 1143-1158. doi:10.1016/j.neuron.2010.11.034

Jansen, R. P., & Niessing, D. (2012). Assembly of mRNA-protein complexes for directional mRNA transport in eukaryotes--an overview. *Curr Protein Pept Sci, 13*(4), 284-293. doi:10.2174/138920312801619493

Jeste, S. S., Sahin, M., Bolton, P., Ploubidis, G. B., & Humphrey, A. (2008). Characterization of autism in young children with tuberous sclerosis complex. *J Child Neurol, 23*(5), 520-525. doi:10.1177/0883073807309788

Job, C., & Eberwine, J. (2001). Identification of sites for exponential translation in living dendrites. *Proc Natl Acad Sci U S A, 98*(23), 13037-13042. doi:10.1073/pnas.231485698

Jonson, L., Vikesaa, J., Krogh, A., Nielsen, L. K., Hansen, T., Borup, R., . . . Nielsen, F. C. (2007). Molecular composition of IMP1 ribonucleoprotein granules. *Mol Cell Proteomics, 6*(5), 798-811. doi:10.1074/mcp.M600346-MCP200

Jung, H., Yoon, B. C., & Holt, C. E. (2012). Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. *Nature Reviews Neuroscience, 13*, 308. doi:10.1038/nrn3210

Kanai, Y., Dohmae, N., & Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron, 43*(4), 513-525. doi:10.1016/j.neuron.2004.07.022

Kang, H., & Schuman, E. M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science, 273*(5280), 1402-1406. doi:10.1126/science.273.5280.1402

Keenan, R. J., Freymann, D. M., Stroud, R. M., & Walter, P. (2001). The signal recognition particle. *Annu Rev Biochem, 70,* 755-775. doi:10.1146/annurev.biochem.70.1.755

Kelleher, R. J., 3rd, & Bear, M. F. (2008). The autistic neuron: troubled translation? *Cell, 135*(3), 401-406. doi:10.1016/j.cell.2008.10.017

Kenney, J. W., Genheden, M., Moon, K. M., Wang, X., Foster, L. J., & Proud, C. G. (2016). Eukaryotic elongation factor 2 kinase regulates the synthesis of microtubule-related proteins in neurons. *J Neurochem, 136*(2), 276-284. doi:10.1111/jnc.13407

Kenny, P. J., Zhou, H., Kim, M., Skariah, G., Khetani, R. S., Drnevich, J., . . . Ceman, S. (2014). MOV10 and FMRP regulate AGO2 association with microRNA recognition elements. *Cell reports, 9*(5), 1729-1741. doi:10.1016/j.celrep.2014.10.054

Kent, C. B., Shimada, T., Ferraro, G. B., Ritter, B., Yam, P. T., McPherson, P. S., . . . Fournier, A. E. (2010). 14-3-3 proteins regulate protein kinase a activity to modulate growth cone turning responses. *J Neurosci, 30*(42), 14059-14067. doi:10.1523/JNEUROSCI.3883-10.2010

Khan, A., Pepio, A. M., & Sossin, W. S. (2001). Serotonin activates S6 kinase in a rapamycin-sensitive manner in Aplysia synaptosomes. *J Neurosci, 21*(2), 382-391.

Khandjian, E. W., Corbin, F., Woerly, S., & Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. *Nat Genet, 12*(1), 91-93. doi:10.1038/ng0196-91

Kiebler, M. A., & Bassell, G. J. (2006). Neuronal RNA Granules: Movers and Makers. *Neuron, 51*(6), 685-690. doi:https://doi.org/10.1016/j.neuron.2006.08.021

Kim, Y. K., & Maquat, L. E. (2019). UPFront and center in RNA decay: UPF1 in nonsense-mediated mRNA decay and beyond. *Rna, 25*(4), 407-422. doi:10.1261/rna.070136.118

Kislauskis, E. H., Zhu, X., & Singer, R. H. (1997). beta-Actin messenger RNA localization and protein synthesis augment cell motility. *J Cell Biol, 136*(6), 1263-1270. doi:10.1083/jcb.136.6.1263

Klann, E., & Dever, T. E. (2004). Biochemical mechanisms for translational regulation in synaptic plasticity. *Nature Reviews Neuroscience, 5*(12), 931-942. doi:10.1038/nrn1557

Knowles, R. B., Sabry, J. H., Martone, M. E., Deerinck, T. J., Ellisman, M. H., Bassell, G. J., & Kosik, K. S. (1996). Translocation of RNA Granules in Living Neurons. *The Journal of Neuroscience, 16*(24), 7812.

Koenig, E., Martin, R., Titmus, M., & Sotelo-Silveira, J. R. (2000). Cryptic Peripheral Ribosomal Domains Distributed Intermittently along Mammalian Myelinated Axons. *The Journal of Neuroscience, 20*(22), 8390. doi:10.1523/JNEUROSCI.20-22-08390.2000

Kondrashov, N., Pusic, A., Stumpf, C. R., Shimizu, K., Hsieh, A. C., Ishijima, J., . . . Barna, M. (2011). Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell, 145*(3), 383-397. doi:10.1016/j.cell.2011.03.028

Koppers, M., Cagnetta, R., Shigeoka, T., Wunderlich, L. C., Vallejo-Ramirez, P., Qiaojin Lin, J., . . . Holt, C. E. (2019). Receptor-specific interactome as a hub for rapid cue-induced selective translation in axons. *Elife, 8*. doi:10.7554/eLife.48718

Krichevsky, A. M., & Kosik, K. S. (2001). Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron, 32*(4), 683-696.

Kumar, V. (2021). Chapter 7 - Ribosomal biogenesis in eukaryotes. In V. Kumar (Ed.), *Emerging Concepts in Ribosome Structure, Biogenesis, and Function* (pp. 129-150): Academic Press.

Kusek, G., Campbell, M., Doyle, F., Tenenbaum, S. A., Kiebler, M., & Temple, S. (2012). Asymmetric segregation of the double-stranded RNA binding protein Staufen2 during mammalian neural stem cell divisions promotes lineage progression. *Cell Stem Cell, 11*(4), 505-516. doi:10.1016/j.stem.2012.06.006

Kwon, C. H., Luikart, B. W., Powell, C. M., Zhou, J., Matheny, S. A., Zhang, W., . . . Parada, L. F. (2006). Pten regulates neuronal arborization and social interaction in mice. *Neuron, 50*(3), 377-388. doi:10.1016/j.neuron.2006.03.023

Langdon, E. M., & Gladfelter, A. S. (2018). A New Lens for RNA Localization: Liquid-Liquid Phase Separation. *Annu Rev Microbiol, 72*, 255-271. doi:10.1146/annurev-micro-090817-062814

Langille, J. J., Ginzberg, K., & Sossin, W. S. (2019). Polysomes identified by live imaging of nascent peptides are stalled in hippocampal and cortical neurites. *Learn Mem, 26*(9), 351-362. doi:10.1101/lm.049965.119

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat Methods, 9*(4), 357-359. doi:10.1038/nmeth.1923

Lareau, L. F., Hite, D. H., Hogan, G. J., & Brown, P. O. (2014). Distinct stages of the translation elongation cycle revealed by sequencing ribosome-protected mRNA fragments. *Elife, 3*, e01257. doi:10.7554/eLife.01257

Lasko, P. (2012). mRNA localization and translational control in Drosophila oogenesis. *Cold Spring Harbor Perspectives in Biology, 4*(10), a012294. doi:10.1101/cshperspect.a012294

Lauria, F., Tebaldi, T., Bernabo, P., Groen, E. J. N., Gillingwater, T. H., & Viero, G. (2018). riboWaltz: Optimization of ribosome P-site positioning in ribosome profiling data. *PLoS Comput Biol, 14*(8), e1006169. doi:10.1371/journal.pcbi.1006169

Leary, D. J., & Huang, S. (2001). Regulation of ribosome biogenesis within the nucleolus. *FEBS Lett, 509*(2), 145-150. doi:10.1016/s0014-5793(01)03143-x

Lebeau, G., Miller, L. C., Tartas, M., McAdam, R., Laplante, I., Badeaux, F., . . . Lacaille, J. C. (2011). Staufen 2 regulates mGluR long-term depression and Map1b mRNA distribution in hippocampal neurons. *Learn Mem, 18*(5), 314-326. doi:10.1101/lm.2100611

Lee, J. E., Cathey, P. I., Wu, H., Parker, R., & Voeltz, G. K. (2020). Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science, 367*(6477). doi:10.1126/science.aay7108

Lelieveld, S. H., Reijnders, M. R., Pfundt, R., Yntema, H. G., Kamsteeg, E. J., de Vries, P., . . . Gilissen, C. (2016). Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nat Neurosci, 19*(9), 1194-1196. doi:10.1038/nn.4352

Leung, K.-M., Lu, B., Wong, H. H.-W., Lin, J. Q., Turner-Bridger, B., & Holt, C. E. (2018). Cue-Polarized Transport of β-actin mRNA Depends on 3′UTR and Microtubules in Live Growth Cones. *Frontiers in Cellular Neuroscience, 12*(300). doi:10.3389/fncel.2018.00300

Leung, K.-M., van Horck, F. P. G., Lin, A. C., Allison, R., Standart, N., & Holt, C. E. (2006). Asymmetrical β-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nature Neuroscience, 9*(10), 1247-1256. doi:10.1038/nn1775

Leung, K. M., van Horck, F. P., Lin, A. C., Allison, R., Standart, N., & Holt, C. E. (2006). Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci, 9*(10), 1247-1256. doi:10.1038/nn1775

Li, C., Bassell, G., & Sasaki, Y. (2009). Fragile X mental retardation protein is involved in protein synthesis-dependent collapse of growth cones induced by Semaphorin-3A. *Frontiers in Neural Circuits, 3*(11). doi:10.3389/neuro.04.011.2009

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics, 30*(7), 923-930. doi:10.1093/bioinformatics/btt656

Limon, J., & Fruman, D. (2012). Akt and mTOR in B Cell Activation and Differentiation. *Frontiers in immunology, 3*, 228. doi:10.3389/fimmu.2012.00228

Liu, B., Molinaro, G., Shu, H., Stackpole, E. E., Huber, K. M., & Richter, J. D. (2019). Optimization of ribosome profiling using low-input brain tissue from fragile X syndrome model mice. *Nucleic Acids Res, 47*(5), e25. doi:10.1093/nar/gky1292

Liu, J., Rivas, F. V., Wohlschlegel, J., Yates, J. R., 3rd, Parker, R., & Hannon, G. J. (2005). A role for the P-body component GW182 in microRNA function. *Nat Cell Biol, 7*(12), 1261-1266. doi:10.1038/ncb1333

Liu, S., Bachran, C., Gupta, P., Miller-Randolph, S., Wang, H., Crown, D., . . . Leppla, S. H. (2012). Diphthamide modification on eukaryotic elongation factor 2 is needed to assure fidelity of mRNA translation and mouse development. *Proceedings of the National Academy of Sciences, 109*(34), 13817-13822. doi:10.1073/pnas.1206933109

Lowery, L. A., & Vactor, D. V. (2009). The trip of the tip: understanding the growth cone machinery. *Nature Reviews Molecular Cell Biology, 10*(5), 332-343. doi:10.1038/nrm2679

Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell W, T., Li, W., . . . Feng, Y. (2004). The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development. *Proc Natl Acad Sci U S A, 101*(42), 15201-15206. doi:10.1073/pnas.0404995101

Lucas, B. A., Lavi, E., Shiue, L., Cho, H., Katzman, S., Miyoshi, K., . . . Maquat, L. E. (2018). Evidence for convergent evolution of SINE-directed Staufen-mediated mRNA decay. *Proceedings of the National Academy of Sciences, 115*(5), 968-973. doi:10.1073/pnas.1715531115

Luo, Y., Na, Z., & Slavoff, S. A. (2018). P-Bodies: Composition, Properties, and Functions. *Biochemistry, 57*(17), 2424-2431. doi:10.1021/acs.biochem.7b01162

Lyles, V., Zhao, Y., & Martin, K. C. (2006). Synapse Formation and mRNA Localization in Cultured Aplysia Neurons. *Neuron, 49*(3), 349-356. doi:https://doi.org/10.1016/j.neuron.2005.12.029

Martin, K. C., Casadio, A., Zhu, H., Yaping, E., Rose, J. C., Chen, M., . . . Kandel, E. R. (1997). Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function

for local protein synthesis in memory storage. *Cell, 91*(7), 927-938. doi:10.1016/s0092-8674(00)80484-5

Martin, M. (2011). Cutadapt removes adapter sequences from high-trhoughput sequencing reads. *EMBnet.journal, 17*, 10-12. doi:doi:https://doi.org/10.14806/ej.17.1.200.

Martin, S., & Tazi, J. (2014). Visualization of G3BP stress granules dynamics in live primary cells. *J Vis Exp*(87). doi:10.3791/51197

Martinez, J. C., Randolph, L. K., Iascone, D. M., Pernice, H. F., Polleux, F., & Hengst, U. (2019). Pum2 Shapes the Transcriptome in Developing Axons through Retention of Target mRNAs in the Cell Body. *Neuron, 104*(5), 931-946 e935. doi:10.1016/j.neuron.2019.08.035

Mateus-Seidl, R., Stahl, S., Dengl, S., Birzele, F., Herrmuth, H., Mayer, K., . . . Brinkmann, U. (2019). Interplay between reversible phosphorylation and irreversible ADP-ribosylation of eukaryotic translation elongation factor 2. *Biological Chemistry, 400*(4), 501-512. doi:doi:10.1515/hsz-2018-0280

Mateyak, M. K., & Kinzy, T. G. (2013). ADP-ribosylation of translation elongation factor 2 by diphtheria toxin in yeast inhibits translation and cell separation. *The Journal of biological chemistry, 288*(34), 24647-24655. doi:10.1074/jbc.M113.488783

Maurin, T., Lebrigand, K., Castagnola, S., Paquet, A., Jarjat, M., Popa, A., . . . Bardoni, B. (2018). HITS-CLIP in various brain areas reveals new targets and new modalities of RNA binding by fragile X mental retardation protein. *Nucleic Acids Res, 46*(12), 6344-6355. doi:10.1093/nar/gky267

Mayya, V. K., & Duchaine, T. F. (2019). Ciphers and Executioners: How 3'-Untranslated Regions Determine the Fate of Messenger RNAs. *Front Genet, 10*, 6. doi:10.3389/fgene.2019.00006

McCamphill, P. K., Farah, C. A., Anadolu, M. N., Hoque, S., & Sossin, W. S. (2015). Bidirectional regulation of eEF2 phosphorylation controls synaptic plasticity by decoding neuronal activity patterns. *J Neurosci, 35*(10), 4403-4417. doi:10.1523/JNEUROSCI.2376-14.2015

McClain, W. H. (1993). Rules that Govern tRNA Identity in Protein Synthesis. *Journal of Molecular Biology, 234*(2), 257-280. doi:https://doi.org/10.1006/jmbi.1993.1582

Merkurjev, D., Hong, W. T., Iida, K., Oomoto, I., Goldie, B. J., Yamaguti, H., . . . Wang, D. O. (2018). Synaptic N(6)-methyladenosine (m(6)A) epitranscriptome reveals functional partitioning of localized transcripts. *Nat Neurosci, 21*(7), 1004-1014. doi:10.1038/s41593-018-0173-6

Mikl, M., Vendra, G., & Kiebler, M. A. (2011). Independent localization of MAP2, CaMKIIalpha and beta-actin RNAs in low copy numbers. *EMBO Rep, 12*(10), 1077-1084. doi:10.1038/embor.2011.149

Mili, S., & Macara, I. G. (2009). RNA localization and polarity: from A(PC) to Z(BP). *Trends in cell biology, 19*(4), 156-164. doi:10.1016/j.tcb.2009.02.001

Miller, L. C., Blandford, V., McAdam, R., Sanchez-Carbente, M. R., Badeaux, F., DesGroseillers, L., & Sossin, W. S. (2009). Combinations of DEAD box proteins distinguish distinct types of RNA: protein complexes in neurons. *Mol Cell Neurosci, 40*(4), 485-495.

Miller, S., Yasuda, M., Coats, J. K., Jones, Y., Martone, M. E., & Mayford, M. (2002). Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron, 36*(3), 507-519. doi:10.1016/s0896-6273(02)00978-9

Min, E. E., Roy, B., Amrani, N., He, F., & Jacobson, A. (2013). Yeast Upf1 CH domain interacts with Rps26 of the 40S ribosomal subunit. *Rna, 19*(8), 1105-1115. doi:10.1261/rna.039396.113

Mittag, T., & Parker, R. (2018). Multiple Modes of Protein-Protein Interactions Promote RNP Granule Assembly. *J Mol Biol, 430*(23), 4636-4649. doi:10.1016/j.jmb.2018.08.005

Molitor, L., Bacher, S., Burczyk, S., & Niessing, D. (2021). The Molecular Function of PURA and Its Implications in Neurological Diseases. *Frontiers in Genetics, 12*(267). doi:10.3389/fgene.2021.638217

Mori, M., Penney, J., & Haghighi, P. (2019). Regulation of synaptic homeostasis by translational mechanisms. In W. Sossin (Ed.), *The Oxford Handbook of Neuronal Protein Synthesis*: Oxford Press.

Mori, M., Penney, J., & Haghighi, P. (2021). Regulation of synaptic homeostasis by translational mechanisms. *The Oxford Handbook of Neuronal Protein Synthesis*, 297.

Mori, Y., Imaizumi, K., Katayama, T., Yoneda, T., & Tohyama, M. (2000). Two cis-acting elements in the 3' untranslated region of alpha-CaMKII regulate its dendritic targeting. *Nat Neurosci, 3*(11), 1079-1084. doi:10.1038/80591

Muddashetty, R. S., Nalavadi, V. C., Gross, C., Yao, X., Xing, L., Laur, O., . . . Bassell, G. J. (2011). Reversible inhibition of PSD-95 mRNA translation by miR-125a, FMRP phosphorylation, and mGluR signaling. *Mol Cell, 42*(5), 673-688. doi:10.1016/j.molcel.2011.05.006

Muslimov, I. A., Patel, M. V., Rose, A., & Tiedge, H. (2011). Spatial code recognition in neuronal RNA targeting: role of RNA-hnRNP A2 interactions. *J Cell Biol, 194*(3), 441-457. doi:10.1083/jcb.201010027

Na, Y., Park, S., Lee, C., Kim, D. K., Park, J. M., Sockanathan, S., . . . Worley, P. F. (2016). Real-Time Imaging Reveals Properties of Glutamate-Induced Arc/Arg 3.1 Translation in Neuronal Dendrites. *Neuron, 91*(3), 561-573. doi:10.1016/j.neuron.2016.06.017

Napoli, I., Mercaldo, V., Boyl, P. P., Eleuteri, B., Zalfa, F., De Rubeis, S., . . . Bagni, C. (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. *Cell, 134*(6), 1042-1054. doi:10.1016/j.cell.2008.07.031

Narayanan, U., Nalavadi, V., Nakamoto, M., Pallas, D. C., Ceman, S., Bassell, G. J., & Warren, S. T. (2007). FMRP phosphorylation reveals an immediate-early signaling pathway triggered by group I mGluR and mediated by PP2A. *J Neurosci, 27*(52), 14349-14357. doi:10.1523/jneurosci.2969-07.2007

Nie, D., Di Nardo, A., Han, J. M., Baharanyi, H., Kramvis, I., Huynh, T., . . . Sahin, M. (2010). Tsc2-Rheb signaling regulates EphA-mediated axon guidance. *Nature Neuroscience, 13*(2), 163-172. doi:10.1038/nn.2477

Niere, F., Wilkerson, J. R., & Huber, K. M. (2012). Evidence for a fragile X mental retardation protein-mediated translational switch in metabotropic glutamate receptor-triggered Arc

translation and long-term depression. *J Neurosci, 32*(17), 5924-5936. doi:10.1523/JNEUROSCI.4650-11.2012

Nosyreva, E. D., & Huber, K. M. (2005). Developmental switch in synaptic mechanisms of hippocampal metabotropic glutamate receptor-dependent long-term depression. *J Neurosci, 25*(11), 2992-3001. doi:10.1523/JNEUROSCI.3652-04.2005

Nowicki, S. T., Tassone, F., Ono, M. Y., Ferranti, J., Croquette, M. F., Goodlin-Jones, B., & Hagerman, R. J. (2007). The Prader-Willi phenotype of fragile X syndrome. *Journal of Developmental & Behavioral Pediatrics, 28*(2), 133-138.

O'Donnell, M., Chance, R. K., & Bashaw, G. J. (2009). Axon growth and guidance: receptor regulation and signal transduction. *Annual review of neuroscience, 32*, 383-412. doi:10.1146/annurev.neuro.051508.135614

O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P., . . . Eichler, E. E. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature, 485*(7397), 246-250. doi:10.1038/nature10989

Ostroff, L. E., Santini, E., Sears, R., Deane, Z., Kanadia, R. N., LeDoux, J. E., . . . Klann, E. (2019). Axon TRAP reveals learning-associated alterations in cortical axonal mRNAs in the lateral amgydala. *Elife, 8*. doi:10.7554/eLife.51607

Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M., & Kennedy, M. B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J Neurosci, 19*(18), 7823-7833.

Palacios, I. M., & St Johnston, D. (2001). Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu Rev Cell Dev Biol, 17*, 569-614. doi:10.1146/annurev.cellbio.17.1.569

Panja, D., Kenney, Justin W., D'Andrea, L., Zalfa, F., Vedeler, A., Wibrand, K., . . . Bramham, Clive R. (2014). Two-Stage Translational Control of Dentate Gyrus LTP Consolidation Is Mediated by Sustained BDNF-TrkB Signaling to MNK. *Cell reports, 9*(4), 1430-1445. doi:10.1016/j.celrep.2014.10.016

Paquin, N., & Chartrand, P. (2008). Local regulation of mRNA translation: new insights from the bud. *Trends Cell Biol, 18*(3), 105-111. doi:10.1016/j.tcb.2007.12.004

Park, E., & Maquat, L. E. (2013). Staufen-mediated mRNA decay. *Wiley Interdiscip Rev RNA, 4*(4), 423-435. doi:10.1002/wrna.1168

Park, S., Park, J. M., Kim, S., Kim, J. A., Shepherd, J. D., Smith-Hicks, C. L., . . . Worley, P. F. (2008). Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron, 59*(1), 70-83. doi:10.1016/j.neuron.2008.05.023

Parker, R., & Sheth, U. (2007). P bodies and the control of mRNA translation and degradation. *Mol Cell, 25*(5), 635-646. doi:10.1016/j.molcel.2007.02.011

Penney, J., Tsurudome, K., Liao, E. H., Elazzouzi, F., Livingstone, M., Gonzalez, M., . . . Haghighi, A. P. (2012). TOR is required for the retrograde regulation of synaptic homeostasis at the

Drosophila neuromuscular junction. *Neuron, 74*(1), 166-178. doi:10.1016/j.neuron.2012.01.030

Pilaz, L.-J., Lennox, A. L., Rouanet, J. P., & Silver, D. L. (2016). Dynamic mRNA Transport and Local Translation in Radial Glial Progenitors of the Developing Brain. *Current Biology, 26*(24), 3383-3392. doi:https://doi.org/10.1016/j.cub.2016.10.040

Pochopien, A. A., Beckert, B., Kasvandik, S., Berninghausen, O., Beckmann, R., Tenson, T., & Wilson, D. N. (2021). Structure of Gcn1 bound to stalled and colliding 80S ribosomes. *Proceedings of the National Academy of Sciences, 118*(14), e2022756118. doi:10.1073/pnas.2022756118

Poulopoulos, A., Murphy, A. J., Ozkan, A., Davis, P., Hatch, J., Kirchner, R., & Macklis, J. D. (2019). Subcellular transcriptomes and proteomes of developing axon projections in the cerebral cortex. *Nature, 565*(7739), 356-360. doi:10.1038/s41586-018-0847-y

Protter, D. S. W., & Parker, R. (2016). Principles and Properties of Stress Granules. *Trends Cell Biol, 26*(9), 668-679. doi:10.1016/j.tcb.2016.05.004

Proud, C. G. (2015). Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans, 43*(3), 328-332. doi:10.1042/BST20140323

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics, 26*(6), 841-842. doi:10.1093/bioinformatics/btq033

Reijnders, M. R. F., Janowski, R., Alvi, M., Self, J. E., van Essen, T. J., Vreeburg, M., . . . Baralle, D. (2018). PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature. *J Med Genet, 55*(2), 104-113. doi:10.1136/jmedgenet-2017-104946

Reimand, J., Arak, T., Adler, P., Kolberg, L., Reisberg, S., Peterson, H., & Vilo, J. (2016). g:Profiler-a web server for functional interpretation of gene lists (2016 update). *Nucleic Acids Res, 44*(W1), W83-89. doi:10.1093/nar/gkw199

Reschke, M., Clohessy, J. G., Seitzer, N., Goldstein, D. P., Breitkopf, S. B., Schmolze, D. B., . . . Pandolfi, P. P. (2013). Characterization and analysis of the composition and dynamics of the mammalian riboproteome. *Cell reports, 4*(6), 1276-1287. doi:10.1016/j.celrep.2013.08.014

Richter, J. D., Bassell, G. J., & Klann, E. (2015). Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nat Rev Neurosci, 16*(10), 595-605. doi:10.1038/nrn4001

Richter, J. D., & Sonenberg, N. (2005). Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature, 433*(7025), 477-480. doi:10.1038/nature03205

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res, 43*(7), e47. doi:10.1093/nar/gkv007

Robert, F., Carrier, M., Rawe, S., Chen, S., Lowe, S., & Pelletier, J. (2009). Altering chemosensitivity by modulating translation elongation. *PLOS ONE, 4*(5), e5428. doi:10.1371/journal.pone.0005428

Rodriguez, J. M., Maietta, P., Ezkurdia, I., Pietrelli, A., Wesselink, J. J., Lopez, G., . . . Tress, M. L. (2013). APPRIS: annotation of principal and alternative splice isoforms. *Nucleic Acids Res, 41*(Database issue), D110-117. doi:10.1093/nar/gks1058

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav, 2*(5), 255-267. doi:10.1034/j.1601-183x.2003.00037.x

Ryan, V. H., & Fawzi, N. L. (2019). Physiological, Pathological, and Targetable Membraneless Organelles in Neurons. *Trends Neurosci, 42*(10), 693-708. doi:10.1016/j.tins.2019.08.005

Sahoo, P. K., Smith, D. S., Perrone-Bizzozero, N., & Twiss, J. L. (2018). Axonal mRNA transport and translation at a glance. *J Cell Sci, 131*(8). doi:10.1242/jcs.196808

Santini, E., & Borgkvist, A. (2021). Dysregulated Translation in Autism Spectrum Disorder. In *The Oxford Handbook of Neuronal Protein Synthesis*.

Santini, E., Huynh, T. N., MacAskill, A. F., Carter, A. G., Pierre, P., Ruggero, D., . . . Klann, E. (2013). Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature, 493*(7432), 411-415. doi:10.1038/nature11782

Scheetz, A. J., Nairn, A. C., & Constantine-Paton, M. (2000). NMDA receptor-mediated control of protein synthesis at developing synapses. *Nat Neurosci, 3*(3), 211-216. doi:10.1038/72915

Schelski, M., & Bradke, F. (2017). Neuronal polarization: From spatiotemporal signaling to cytoskeletal dynamics. *Molecular and Cellular Neuroscience, 84*, 11-28. doi:https://doi.org/10.1016/j.mcn.2017.03.008

Schoenberg, D. R., & Maquat, L. E. (2012). Regulation of cytoplasmic mRNA decay. *Nat Rev Genet, 13*(4), 246-259. doi:10.1038/nrg3160

Schwamborn, J. C., & Püschel, A. W. (2004). The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity. *Nat Neurosci, 7*(9), 923-929. doi:10.1038/nn1295

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol, 106-107*, 1-16. doi:10.1016/j.pneurobio.2013.04.001

Sengupta, S., Peterson, T. R., & Sabatini, D. M. (2010). Regulation of the mTOR Complex 1 Pathway by Nutrients, Growth Factors, and Stress. *Molecular Cell, 40*(2), 310-322. doi:10.1016/j.molcel.2010.09.026

Shah, S., Molinaro, G., Liu, B., Wang, R., Huber, K. M., & Richter, J. D. (2020). FMRP Control of Ribosome Translocation Promotes Chromatin Modifications and Alternative Splicing of Neuronal Genes Linked to Autism. *Cell Rep, 30*(13), 4459-4472 e4456. doi:10.1016/j.celrep.2020.02.076

Shan, J., Munro, T. P., Barbarese, E., Carson, J. H., & Smith, R. (2003). A molecular mechanism for mRNA trafficking in neuronal dendrites. *J Neurosci, 23*(26), 8859-8866.

Shen, L., & Pelletier, J. (2019). Selective targeting of the DEAD-box RNA helicase eukaryotic initiation factor (eIF) 4A by natural products. *Nat Prod Rep*. doi:10.1039/c9np00052f

Shi, Z., Fujii, K., Kovary, K. M., Genuth, N. R., Röst, H. L., Teruel, M. N., & Barna, M. (2017). Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. *Mol Cell, 67*(1), 71-83.e77. doi:10.1016/j.molcel.2017.05.021

Shigeoka, T., Jung, H., Jung, J., Turner-Bridger, B., Ohk, J., Lin, J. Q., . . . Holt, C. E. (2016). Dynamic Axonal Translation in Developing and Mature Visual Circuits. *Cell, 166*(1), 181-192. doi:10.1016/j.cell.2016.05.029

Shigeoka, T., Koppers, M., Wong, H. H., Lin, J. Q., Cagnetta, R., Dwivedy, A., . . . Holt, C. E. (2019). On-Site Ribosome Remodeling by Locally Synthesized Ribosomal Proteins in Axons. *Cell reports, 29*(11), 3605-3619 e3610. doi:10.1016/j.celrep.2019.11.025

Shu, H., Donnard, E., Liu, B., Jung, S., Wang, R., & Richter, J. D. (2020). FMRP links optimal codons to mRNA stability in neurons. *Proc Natl Acad Sci U S A, 117*(48), 30400-30411. doi:10.1073/pnas.2009161117

Shveygert, M., Kaiser, C., Bradrick, S. S., & Gromeier, M. (2010). Regulation of eukaryotic initiation factor 4E (eIF4E) phosphorylation by mitogen-activated protein kinase occurs through modulation of Mnk1-eIF4G interaction. *Molecular and Cellular Biology, 30*(21), 5160-5167. doi:10.1128/MCB.00448-10

Simms, C. L., Yan, L. L., & Zaher, H. S. (2017). Ribosome Collision Is Critical for Quality Control during No-Go Decay. *Mol Cell, 68*(2), 361-373.e365. doi:10.1016/j.molcel.2017.08.019

Simone, L. E., & Keene, J. D. (2013). Mechanisms coordinating ELAV/Hu mRNA regulons. *Curr Opin Genet Dev, 23*(1), 35-43. doi:10.1016/j.gde.2012.12.006

Siwaszek, A., Ukleja, M., & Dziembowski, A. (2014). Proteins involved in the degradation of cytoplasmic mRNA in the major eukaryotic model systems. *RNA Biol, 11*(9), 1122-1136. doi:10.4161/rna.34406

Skariah, G., & Todd, P. K. (2021). Translational control in aging and neurodegeneration. *Wiley Interdiscip Rev RNA, 12*(4), e1628. doi:10.1002/wrna.1628

Slomnicki, L. P., Pietrzak, M., Vashishta, A., Jones, J., Lynch, N., Elliot, S., . . . Hetman, M. (2016). Requirement of Neuronal Ribosome Synthesis for Growth and Maintenance of the Dendritic Tree. *J Biol Chem, 291*(11), 5721-5739. doi:10.1074/jbc.M115.682161

Snijders Blok, L., Madsen, E., Juusola, J., Gilissen, C., Baralle, D., Reijnders, M. R., . . . Kleefstra, T. (2015). Mutations in DDX3X Are a Common Cause of Unexplained Intellectual Disability with Gender-Specific Effects on Wnt Signaling. *Am J Hum Genet, 97*(2), 343-352. doi:10.1016/j.ajhg.2015.07.004

Sossin, W. S., & Costa-Mattioli, M. (2019). Translational Control in the Brain in Health and Disease. *Cold Spring Harb Perspect Biol, 11*(8). doi:10.1101/cshperspect.a032912

Sossin, W. S., & DesGroseillers, L. (2006). Intracellular trafficking of RNA in neurons. *Traffic, 7*(12), 1581-1589. doi:10.1111/j.1600-0854.2006.00500.x

Spencer, C. M., Serysheva, E., Yuva-Paylor, L. A., Oostra, B. A., Nelson, D. L., & Paylor, R. (2006). Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. *Hum Mol Genet, 15*(12), 1984-1994. doi:10.1093/hmg/ddl121

Südhof, T. C. (2018). Towards an Understanding of Synapse Formation. *Neuron, 100*(2), 276-293. doi:10.1016/j.neuron.2018.09.040

Sunamura, N., Iwashita, S., Enomoto, K., Kadoshima, T., & Isono, F. (2018). Loss of the fragile X mental retardation protein causes aberrant differentiation in human neural progenitor cells. *Sci Rep, 8*(1), 11585. doi:10.1038/s41598-018-30025-4

Suter, D. M., & Forscher, P. (1998). An emerging link between cytoskeletal dynamics and cell adhesion molecules in growth cone guidance. *Curr Opin Neurobiol, 8*(1), 106-116. doi:https://doi.org/10.1016/S0959-4388(98)80014-7

Sutton, M. A., Ito, H. T., Cressy, P., Kempf, C., Woo, J. C., & Schuman, E. M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell, 125*(4), 785-799. doi:10.1016/j.cell.2006.03.040

Sutton, M. A., & Schuman, E. M. (2005). Local translational control in dendrites and its role in long-term synaptic plasticity. *Journal of Neurobiology, 64*(1), 116-131. doi:https://doi.org/10.1002/neu.20152

Sutton, M. A., & Schuman, E. M. (2006). Dendritic protein synthesis, synaptic plasticity, and memory. *Cell, 127*(1), 49-58. doi:10.1016/j.cell.2006.09.014

Sutton, M. A., Taylor, A. M., Ito, H. T., Pham, A., & Schuman, E. M. (2007). Postsynaptic decoding of neural activity: eEF2 as a biochemical sensor coupling miniature synaptic transmission to local protein synthesis. *Neuron, 55*(4), 648-661. doi:10.1016/j.neuron.2007.07.030

Sutton, M. A., Wall, N. R., Aakalu, G. N., & Schuman, E. M. (2004). Regulation of dendritic protein synthesis by miniature synaptic events. *Science, 304*(5679), 1979-1983. doi:10.1126/science.1096202

Świderek, K., Marti, S., Tuñón, I., Moliner, V., & Bertran, J. (2015). Peptide Bond Formation Mechanism Catalyzed by Ribosome. *Journal of the American Chemical Society, 137*(37), 12024-12034. doi:10.1021/jacs.5b05916

Takano, T., Xu, C., Funahashi, Y., Namba, T., & Kaibuchi, K. (2015). Neuronal polarization. *Development, 142*(12), 2088-2093. doi:10.1242/dev.114454

Taylor, A. M., Berchtold, N. C., Perreau, V. M., Tu, C. H., Li Jeon, N., & Cotman, C. W. (2009). Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J Neurosci, 29*(15), 4697-4707. doi:10.1523/JNEUROSCI.6130-08.2009

Tcherkezian, J., Brittis, P. A., Thomas, F., Roux, P. P., & Flanagan, J. G. (2010). Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell, 141*(4), 632-644. doi:10.1016/j.cell.2010.04.008

Thoreen, C. C., Chantranupong, L., Keys, H. R., Wang, T., Gray, N. S., & Sabatini, D. M. (2012). A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature, 485*(7396), 109-113. doi:10.1038/nature11083

Thoreen, C. C., Chantranupong, L., Keys, H. R., Wang, T., Gray, N. S., & Sabatini, D. M. (2012). A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature, 485*(7396), 109-113. doi:10.1038/nature11083

Turrigiano, G. G., & Nelson, S. B. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol, 10*(3), 358-364. doi:10.1016/s0959-4388(00)00091-x

Udagawa, T., Farny, N. G., Jakovcevski, M., Kaphzan, H., Alarcon, J. M., Anilkumar, S., . . . Richter, J. D. (2013). Genetic and acute CPEB1 depletion ameliorate fragile X pathophysiology. *Nat Med, 19*(11), 1473-1477. doi:10.1038/nm.3353

Ule, J., Hwang, H. W., & Darnell, R. B. (2018). The Future of Cross-Linking and Immunoprecipitation (CLIP). *Cold Spring Harb Perspect Biol, 10*(8). doi:10.1101/cshperspect.a032243

Urbanska, M., Gozdz, A., Swiech, L. J., & Jaworski, J. (2012). Mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2) control the dendritic arbor morphology of hippocampal neurons. *J Biol Chem, 287*(36), 30240-30256. doi:10.1074/jbc.M112.374405

Van Nostrand, E. L., Freese, P., Pratt, G. A., Wang, X., Wei, X., Xiao, R., . . . Yeo, G. W. (2020). A large-scale binding and functional map of human RNA-binding proteins. *Nature, 583*(7818), 711-719. doi:10.1038/s41586-020-2077-3

Vessey, J. P., Amadei, G., Burns, S. E., Kiebler, M. A., Kaplan, D. R., & Miller, F. D. (2012). An asymmetrically localized Staufen2-dependent RNA complex regulates maintenance of mammalian neural stem cells. *Cell Stem Cell, 11*(4), 517-528. doi:10.1016/j.stem.2012.06.010

Vicario, A., Colliva, A., Ratti, A., Davidovic, L., Baj, G., Gricman, L., . . . Tongiorgi, E. (2015). Dendritic targeting of short and long 3' UTR BDNF mRNA is regulated by BDNF or NT-3 and distinct sets of RNA-binding proteins. *Front Mol Neurosci, 8*, 62. doi:10.3389/fnmol.2015.00062

Vicens, Q., Kieft, J. S., & Rissland, O. S. (2018). Revisiting the Closed-Loop Model and the Nature of mRNA 5'-3' Communication. *Molecular Cell, 72*(5), 805-812. doi:10.1016/j.molcel.2018.10.047

Wang, D. O. (2021). RNA Modifications in the Central Nervous System. *The Oxford Handbook of Neuronal Protein Synthesis*, 153.

Wang, D. O., Kim, S. M., Zhao, Y., Hwang, H., Miura, S. K., Sossin, W. S., & Martin, K. C. (2009). Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science, 324*(5934), 1536-1540. doi:10.1126/science.1173205

Wang, X., Posey, J. E., Rosenfeld, J. A., Bacino, C. A., Scaglia, F., Immken, L., . . . Moretti, P. (2018). Phenotypic expansion in DDX3X - a common cause of intellectual disability in females. *Ann Clin Transl Neurol, 5*(10), 1277-1285. doi:10.1002/acn3.622

Welshhans, K., & Bassell, G. J. (2011). Netrin-1-induced local beta-actin synthesis and growth cone guidance requires zipcode binding protein 1. *J Neurosci, 31*(27), 9800-9813. doi:10.1523/JNEUROSCI.0166-11.2011

Westmark, C. J., Maloney, B., Alisch, R. S., Sokol, D. K., & Lahiri, D. K. (2020). FMRP Regulates the Nuclear Export of Adam9 and Psen1 mRNAs: Secondary Analysis of an N(6)-Methyladenosine Dataset. *Sci Rep, 10*(1), 10781. doi:10.1038/s41598-020-66394-y

Widagdo, J., & Anggono, V. (2018). The m6A-epitranscriptomic signature in neurobiology: from neurodevelopment to brain plasticity. *J Neurochem, 147*(2), 137-152. doi:10.1111/jnc.14481

Wilhelm, J. E., & Vale, R. D. (1993). RNA on the move: the mRNA localization pathway. *J Cell Biol, 123*(2), 269-274. doi:10.1083/jcb.123.2.269

Willis, D., Li, K. W., Zheng, J. Q., Chang, J. H., Smit, A. B., Kelly, T., . . . Twiss, J. L. (2005). Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. *J Neurosci, 25*(4), 778-791. doi:10.1523/JNEUROSCI.4235-04.2005

Wolozin, B., & Ivanov, P. (2019). Stress granules and neurodegeneration. *Nat Rev Neurosci, 20*(11), 649-666. doi:10.1038/s41583-019-0222-5

Wong, H. H., Lin, J. Q., Strohl, F., Roque, C. G., Cioni, J. M., Cagnetta, R., . . . Holt, C. E. (2017). RNA Docking and Local Translation Regulate Site-Specific Axon Remodeling In Vivo. *Neuron, 95*(4), 852-868 e858. doi:10.1016/j.neuron.2017.07.016

Wu, B., Zhang, H., Sun, R., Peng, S., Cooperman, B. S., Goldman, Y. E., & Chen, C. (2018). Translocation kinetics and structural dynamics of ribosomes are modulated by the conformational plasticity of downstream pseudoknots. *Nucleic Acids Res, 46*(18), 9736-9748. doi:10.1093/nar/gky636

Wu, C. C., Zinshteyn, B., Wehner, K. A., & Green, R. (2019). High-Resolution Ribosome Profiling Defines Discrete Ribosome Elongation States and Translational Regulation during Cellular Stress. *Mol Cell, 73*(5), 959-970 e955. doi:10.1016/j.molcel.2018.12.009

Xu, W. B., & Roufa, D. J. (1996). The gene encoding human ribosomal protein S24 and tissue-specific expression of differentially spliced mRNAs. *Gene, 169*(2), 257-262. doi:10.1016/0378-1119(96)88652-5

Yamashita, A. (2013). Role of SMG-1-mediated Upf1 phosphorylation in mammalian nonsense-mediated mRNA decay. *Genes to Cells, 18*(3), 161-175. doi:https://doi.org/10.1111/gtc.12033

Yan, X., Hoek, T. A., Vale, R. D., & Tanenbaum, M. E. (2016). Dynamics of Translation of Single mRNA Molecules In Vivo. *Cell, 165*(4), 976-989. doi:10.1016/j.cell.2016.04.034

Yang, G., Smibert, C. A., Kaplan, D. R., & Miller, F. D. (2014). An eIF4E1/4E-T complex determines the genesis of neurons from precursors by translationally repressing a proneurogenic transcription program. *Neuron, 84*(4), 723-739. doi:10.1016/j.neuron.2014.10.022

Yanow, S. K., Manseau, F., Hislop, J., Castellucci, V. F., & Sossin, W. S. (1998). Biochemical pathways by which serotonin regulates translation in the nervous system of Aplysia. *J Neurochem, 70*(2), 572-583. doi:10.1046/j.1471-4159.1998.70020572.x

Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J., & Zheng, J. Q. (2006). An essential role for β-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nature Neuroscience, 9*(10), 1265-1273. doi:10.1038/nn1773

Yonath, A. (2017). Quantum mechanic glimpse into peptide bond formation within the ribosome shed light on origin of life. *Structural Chemistry, 28*(5), 1285-1291. doi:10.1007/s11224-017-0980-5

Yoon, Byung C., Jung, H., Dwivedy, A., O'Hare, Catherine M., Zivraj, Krishna H., & Holt, Christine E. (2012). Local Translation of Extranuclear Lamin B Promotes Axon Maintenance. *Cell, 148*(4), 752-764. doi:10.1016/j.cell.2011.11.064

Yoon, B. C., Zivraj, K. H., & Holt, C. E. (2009). Local translation and mRNA trafficking in axon pathfinding. *Results Probl Cell Differ, 48*, 269-288. doi:10.1007/400_2009_5

Yoshimura, T., Arimura, N., & Kaibuchi, K. (2006). Signaling Networks in Neuronal Polarization. *The Journal of Neuroscience, 26*(42), 10626-10630. doi:10.1523/jneurosci.3824-06.2006

Younts, T. J., Monday, H. R., Dudok, B., Klein, M. E., Jordan, B. A., Katona, I., & Castillo, P. E. (2016). Presynaptic Protein Synthesis Is Required for Long-Term Plasticity of GABA Release. *Neuron, 92*(2), 479-492. doi:10.1016/j.neuron.2016.09.040

Zahr, S. K., Yang, G., Kazan, H., Borrett, M. J., Yuzwa, S. A., Voronova, A., . . . Miller, F. D. (2018). A Translational Repression Complex in Developing Mammalian Neural Stem Cells that Regulates Neuronal Specification. *Neuron, 97*(3), 520-537.e526. doi:10.1016/j.neuron.2017.12.045

Zalfa, F., Giorgi, M., Primerano, B., Moro, A., Di Penta, A., Reis, S., . . . Bagni, C. (2003). The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell, 112*(3), 317-327. doi:10.1016/s0092-8674(03)00079-5

Zhang, F., Kang, Y., Wang, M., Li, Y., Xu, T., Yang, W., . . . Jin, P. (2018). Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. *Hum Mol Genet, 27*(22), 3936-3950. doi:10.1093/hmg/ddy292

Zhao, T., Chen, Y.-M., Li, Y., Wang, J., Chen, S., Gao, N., & Qian, W. (2021). Disome-seq reveals widespread ribosome collisions that promote cotranslational protein folding. *Genome Biology, 22*(1), 16. doi:10.1186/s13059-020-02256-0

Zhou, J., & Parada, L. F. (2012). PTEN signaling in autism spectrum disorders. *Curr Opin Neurobiol, 22*(5), 873-879. doi:10.1016/j.conb.2012.05.004

Zhou, X., Gueydan, M., Jospin, M., Ji, T., Valfort, A., Pinan-Lucarré, B., & Bessereau, J.-L. (2020). The netrin receptor UNC-40/DCC assembles a postsynaptic scaffold and sets the synaptic content of GABAA receptors. *Nat Commun, 11*(1), 2674. doi:10.1038/s41467-020-16473-5

Zhou, Y., Kastritis, P. L., Dougherty, S. E., Bouvette, J., Hsu, A. L., Burbaum, L., . . . Silva, G. M. (2020). Structural impact of K63 ubiquitin on yeast translocating ribosomes under oxidative stress. *Proc Natl Acad Sci U S A, 117*(36), 22157-22166. doi:10.1073/pnas.2005301117

Zivraj, K. H., Tung, Y. C., Piper, M., Gumy, L., Fawcett, J. W., Yeo, G. S., & Holt, C. E. (2010). Subcellular profiling reveals distinct and developmentally regulated repertoire of growth cone mRNAs. *J Neurosci, 30*(46), 15464-15478. doi:10.1523/jneurosci.1800-10.2010

5 APPENDIX

McGill

March 16, 2015

Animal Certificate

This is to certify that **Dr. Wayne S Sossin, Department of Neurology & Neurosurgery, Montreal Neurological Institute,** currently holds an approved Animal Use Protocol # **2012-7285** with McGill University and its Affiliated Hospitals' Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation

Start date: March 1, 2015

Expiration date: March 1, 2016

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Claude Lalande
Assistant Director, Animal Compliance Office
Office of Vice-Principal (Research and International Relations)
Room 429, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4

McGill

February 23, 2016

Animal Certificate

This is to certify that **Dr. Wayne Sossin**, MNI, currently holds an approved **Animal Use Protocol # 2012-7285** with McGill University and its Affiliated Hospital's Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation.

Start date: March 1, 2016

Expiration date: February 28, 2017

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Suzanne Smith
Director, Animal Compliance Office
Office of Vice-Principal (Research and International Relations)
Room 429, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4

McGill

March 3, 2017

Animal Certificate

This is to certify that **Dr. Wayne Sossin**, Montreal Neurological Institute, currently holds an approved **Animal Use Protocol # 2012-7285** with McGill University and its Affiliated Hospital's Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation.

Start date: March 1, 2017

Expiration date: February 28, 2018

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Suzanne Smith
Director, Animal Compliance Office
Office of Vice-Principal (Research and Innovation)
Suite 325, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4
suzanne.smith@mcgill.ca

McGill

March 15, 2018

Animal Certificate

This is to certify that **Dr. Wayne S Sossin, Department of Neurology & Neurosurgery, Montreal Neurological Institute,** currently holds an approved Animal Use Protocol # **2012-7285** with McGill University and its Affiliated Hospitals' Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation

Start date: March 1, 2018

Expiration date: March 1, 2019

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Claude Lalande
Assistant Director, Animal Compliance Office
Office of Vice-Principal (Research and Innovation)
Suite 325, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4
claude.lalande@mcgill.ca

McGill

February 25, 2019

Animal Certificate

This is to certify that **Dr. Wayne S. Sossin, Department of Neurology and Neurosurgery, Montreal Neurological Institute,** currently holds an approved Animal Use Protocol # **2012-7285** with McGill University and its Affiliated Hospitals' Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation

Start date: March 1, 2019

Expiration date: March 1, 2020

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Melanie Tremblay, Ph.D
Animal Ethics and Compliance Admin | Animal Compliance Office
Office of Vice-Principal (Research and Innovation)
Suite 325, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4
animal.approvals@mcgill.ca

McGill

February 4, 2020

Animal Certificate

This is to certify that **Dr. Wayne Steven Sossin, Department of Neurology and Neurosurgery,** currently holds an approved Animal Use Protocol # **2012-7285** with McGill University and its Affiliated Hospitals' Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation

Start date: March 1, 2020

Expiration date: March 1, 2021

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Melanie Tremblay, Ph.D
Animal Ethics and Compliance Admin | Animal Compliance Office
Office of Vice-Principal (Research and Innovation)
Suite 325, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4
animal.approvals@mcgill.ca

McGill

February 1, 2021

Animal Certificate

This is to certify that **Dr. Wayne Steven Sossin, Department of Neurology and Neurosurgery,** currently holds an approved **Animal Use Protocol # 2012-7285** with McGill University and its Affiliated Hospital's Research Institutes for the following project:

Animal Use Protocol Title: Identifying translational regulons underlying memory formation

Start date: March 1, 2021

Expiration date: February 28, 2022

McGill University and Affiliated Hospitals Research Institutes recognize the importance of animal research in our efforts to further our knowledge of natural processes, diseases and conservation. Research, educational and testing projects are conducted with full commitment to the wellbeing of the animal subjects. In order to limit animal use to meritorious research or educational projects, the institution relies on stringent peer review processes, along with assessment of ethical issues by the Animal Care Committee. McGill University recognizes that the use of animals in research, teaching and testing carries significant responsibilities. The institution will continue to develop and maintain guidelines and regulations, following the high standards established by the Canadian Council on Animal Care. It is committed to conducting the highest-quality research and to providing animals with the best care.

Cynthia Lavoie

Cynthia Lavoie
Animal Ethics and Compliance Administrator
Animal Compliance Office
Office of Vice-Principal (Research and Innovation)
Suite 325, James Administration Building, McGill University
845 Sherbrooke Street West, Montreal, Quebec, Canada H3A 0G4
animal.approvals@mcgill.ca

McGill | Graduate and Postdoctoral Studies

Doctoral Thesis Nomination of Examiners Form

Submit this completed form to doctoralthesissubmission.gps@mcgill.ca from your official McGill email address with a single PDF version of your thesis. Please verify that all signatures are included on page 3 of this form.

Name: ANADOLU (Last) MINA (First) **McGill ID:** 260352337

Email Address: mina.anadolu@mail.mcgill.ca **Degree:** PhD

Unit*: Integrated Program in Neuroscience (IPN)

**Unit refers to a department, a division, a school, an institute, or a Faculty/University-wide graduate program*

Exact Thesis Title: Capitalize proper nouns only (E.g., The politics and economics of the Free Trade deal) and use words rather than symbols (e.g., carbon dioxide instead of CO2).

Note: The convocation booklet will display the thesis title in lower case except for proper nouns.

Characterizing RNA Granule-Associated Stalled Polysomes

Supervisory Committee (list all members)

Thesis Supervisor

Full Name/Title
Dr. Wayne Sossin (Supervisor)

Tel: (514) 398 – 1486 / _____ (extension)

E-mail: wayne.sossin@mcgill.ca

Mailing Address (Full campus address where applicable)
BT110 - 3801 rue University (Montreal Neurological Institute, The Neuro)
H3A 2B4 Montreal, QC

Thesis Co-Supervisor (if applicable)

Full Name/Title

Tel: (_____) _____ – _____ / _____ (extension)

E-mail:

Mailing Address (Full campus address where applicable)

Other Members

Full Name/Title	Unit*
Dr. Ed Ruthazer (Advisory Committee Member)	IPN-MNI
Dr. Keith Murai (Advisory Committee Member)	IPN-MGH

McGill | Graduate and Postdoctoral Studies

Names and addresses in the following two sections **must be complete** with full names (no initials), and complete Unit* and non-McGill addresses, including building and room number, street address, city, province, postal code, e-mail address, as well as telephone number(s).

Internal Examiner

The Unit* has established that the internal examiner has agreed to evaluate the thesis. GPS is responsible for sending the internal examiner a copy of the thesis. The internal examiner must not be in conflict of interest according to McGill's policy (see University conflict of interest regulations).

Full Name/Title
Dr. Timothy Kennedy

Tel: (514) 398 – 7136 / _____ (extension)

E-mail: timothy.kennedy@mcgill.ca

Mailing Address (Full campus address where applicable)
BT105 - 3801 rue University (Montreal Neurological Institute, The Neuro)
H3A 2B4, Montreal, QC

External Examiner

The Unit* has established that the external examiner has agreed to evaluate the thesis. GPS is responsible for sending the external examiner a copy of the thesis. The external examiner must be able to examine the student and the thesis at arm's length, free of conflict of interest from any source. The test of whether a conflict of interest might exist is whether it could appear to a reasonable outside person that evaluation of the thesis may be affected by anything other than the merits of the thesis document. The candidate's Unit* must take reasonable steps to avoid recommending an examiner whose relationship with the candidate, the supervisor, or their research could be seen as jeopardizing an impartial judgment on the thesis. Any individual asked to examine a thesis must declare possible sources of conflict (see checklist).

Full Name/Title
Dr. Paul De Koninck

Tel: (418) 663 – 5747 / 4721 (extension)

E-mail: paul.dekoninck@neurosciences.ulaval.ca

Mailing Address (Full campus address where applicable)
F-5581 - 2601 Chemin de la Canardière (Universite Laval, Cervo)
G1J 2G3, Québec, QC

Certifications and Signatures

The Unit* head or delegate certifies that

- ☑ All other degree requirements have been met
- ☑ The nominated internal examiner is not in conflict of interest according to McGill's policy (see University conflict of interest regulations)
- ☑ There is no conflict of interest with nominated external examiner (see checklist)

McGill | Graduate and Postdoctoral Studies

Supervisor(s) certifies that:

- ☑ The thesis meets GPS guidelines for preparation and initial submission
- ☑ The thesis meets scholarly standards for partial fulfillment of the degree
- ☑ If relevant, all ethics and compliance certificates required have been properly obtained and copies are on file with the appropriate offices
- ☑ There is no conflict of interest with nominated internal examiner according to McGill's policy (see University see University conflict of interest regulations)
- ☑ There is no conflict of interest with nominated external examiner (see checklist)

The student certifies that:

- ☑ Thesis meets GPS guidelines for preparation and submission
- ☑ If relevant, all ethics and compliance certificates required have been properly obtained and copies are on file with the appropriate offices
- ☑ If relevant, appropriate permissions have been obtained to include copyrighted material in the thesis
- ☑ If relevant, appropriate permissions have been obtained from co-authors of manuscripts included in the thesis for such inclusion
- ☑ In the Preface, contributions of student to each chapter are explicitly stated
- ☑ In the Preface, contribution of any co-authors to each chapter have been explicitly stated

The supervisor and student acknowledge that there will be no further contact with the examiners after submission of this form. Any contact with examiners by the supervisor or student after submission of this form constitutes a conflict of interest and the examination process **will be cancelled.**

Date	Student's Signature	Print Name
25-02-2021	Mina N. Anadolu Digitally signed by Mina N. Anadolu Date: 2021.02.25 17:09:22 -05'00'	MINA ANADOLU
26-02-2021	Wayne Sossin Digitally signed by Wayne Sossin DN: cn=Wayne Sossin, o=Mcgill University, ou, email=wayne.sossin@mcgill.ca, c=CA Date: 2021.02.26 09:47:28 -05'00'	WAYNE SOSSIN
March 1, 2021	Reza Farivar-Mohseni Digitally signed by Reza Farivar-Mohseni Date: 2021.03.01 16:01:43 -05'00'	Reza Farivar-Mohseni
Date	Unit* Head's or Delegate** Signature	Print Name
	Supervisor's Signature	
	Co-Supervisor's Signature	

**Unit refers to a department, a division, a school, an institute, or a Faculty/University-wide graduate program*
*** Please attach a copy of approval granting signing authority if signed by delegate other than the Graduate Program Director*

Revised March 2018

RE: Chapter

BRUNSTEIN, Ada <Ada.Brunstein@oup.com>
Fri 2021-02-26 10:27 AM

To: Mina Anadolu <mina.anadolu@mail.mcgill.ca>
Cc: Wayne Sossin, Dr. <wayne.sossin@mcgill.ca>

Hi Mina,

Yes, please consider this email as confirmation that you may use your chapter and cover image in your PhD thesis for non-commercial use.

Thank you again for your wonderful work for the handbook!
Ada

From: Mina Anadolu <mina.anadolu@mail.mcgill.ca>
Sent: Friday, February 26, 2021 10:19 AM
To: BRUNSTEIN, Ada <Ada.Brunstein@oup.com>
Cc: Wayne Sossin, Dr. <wayne.sossin@mcgill.ca>
Subject: Re: Chapter

Dear Ada,

I hope all is well with you and that you've had a pleasant week! I'm completing my PhD studies this year and I'd like to include the chapter in my manuscript-based PhD thesis. For this I would like to ask OUP for permission to use the content of my chapter, as well as the cover image, in my thesis for non-commercial use. The doctorate thesis is the only document for which the content will be used. It would be fantastic if I could get a permission letter from you.

Thank you for your help!

Kind regards,

Mina

neuro

Mina N. Anadolu
PhD Candidate, Neuroscience
Sossin Lab, McGill University
mina.anadolu@mail.mcgill.ca
514-649-2561
The Neuro | **#OpenScience**
3801 University Street, Montreal, Quebec H3A 2B4
theneuro.ca