Title Page

Manuscript Title:

The Akt-mTOR pathway drives myelin sheath growth by regulating cap-dependent translation

Abbreviated Title:

mTOR-mediated translation drives myelination

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Number of pages: 77, including references and figures

Number of figures: 8

Acknowledgements:

We thank the Appel lab for helpful discussions, Drs. Caleb Doll and Alexandria Hughes for constructive comments on the manuscript, Dr. Katie Yergert for experimental guidance, and Rebecca O'Rourke for help with bioinformatics analysis. This work was supported by US National Institutes of Health (NIH) grant R01 NS095670 and a gift from the Gates Frontiers Fund to B.A. K.N.F-S. was supported by NIH F31 NS115261 and NIH T32 NS099042. The University of Colorado Anschutz Medical Campus Zebrafish Core Facility was supported by NIH grant P30 NS048154.

Conflict of Interest:

The authors declare no competing financial interests.

ABSTRACT

In the vertebrate central nervous system, oligodendrocytes produce myelin, a specialized proteolipid rich membrane, to insulate and support axons. Individual oligodendrocytes wrap multiple axons with myelin sheaths of variable lengths and thicknesses. Myelin grows at the distal ends of oligodendrocyte processes and multiple lines of work have provided evidence that mRNAs and RNA binding proteins localize to myelin, together supporting a model where local translation controls myelin sheath growth. What signal transduction mechanisms could control this? One strong candidate is the Akt-mTOR pathway, a major cellular signaling hub that coordinates transcription, translation, metabolism, and cytoskeletal organization. Here, using zebrafish as a model system, we found that Akt-mTOR signaling promotes myelin sheath growth and stability during development. Through cell-specific manipulations to oligodendrocytes, we show that the Akt-mTOR pathway drives cap-dependent translation to promote myelination and that restoration of cap-dependent translation is sufficient to rescue myelin deficits in mTOR loss-of-function animals. Moreover, an mTOR-dependent translational regulator co-localized with mRNA encoding a canonically myelin-translated protein in vivo and bioinformatic investigation revealed numerous putative translational targets in the myelin transcriptome. Together, these data raise the possibility that Akt-mTOR signaling in nascent myelin sheaths promotes sheath growth via translation of myelin-resident mRNAs during development.

SIGNIFICANCE STATEMENT

In the brain and spinal cord oligodendrocytes extend processes that tightly wrap axons with myelin, a protein and lipid rich membrane that increases electrical impulses and provides trophic support. Myelin membrane grows dramatically following initial axon

wrapping in a process that demands protein and lipid synthesis. How protein and lipid synthesis is coordinated with the need for myelin to be generated in certain locations remains unknown. Our study reveals that the Akt-mTOR signaling pathway promotes myelin sheath growth by regulating protein translation. Because we found translational regulators of the Akt-mTOR pathway in myelin, our data raise the possibility Akt-mTOR activity regulates translation in myelin sheaths to deliver myelin on demand to the places it is needed.

INTRODUCTION

During development, oligodendrocytes extend numerous processes that survey the environment and ensheath target axons with myelin. Notably, not all myelin sheaths produced by an individual oligodendrocyte are uniform in length and thickness (Murtie et al., 2007; Almeida et al., 2011; Chong et al., 2012) and changes in neural activity can influence the amount of myelin made on an axon (Hines et al., 2015; Koudelka et al., 2016; Mitew et al., 2018). Together, this suggests a mechanism whereby oligodendrocytes use localized cell signaling events to coordinate myelin production at distal locations with extracellular cues.

One strong candidate to mediate distal myelin production is the PI3K-Akt-mTOR signaling pathway, whose precise regulation is critical for proper myelin formation. When pathway activity was increased through either oligodendrocyte specific deletion of the antagonist phosphatase PTEN (Goebbels et al., 2010; Harrington et al., 2010) or overexpression of constitutively active Akt (caAkt) (Flores et al., 2008) oligodendrocytes made thicker myelin. Chronic treatment of mice overexpressing caAkt in oligodendrocytes with rapamycin, an mTOR inhibitor, reduced hypermyelination to wild type levels

(Narayanan et al., 2009), and decreased pathway activity resulting from oligodendrocyte-specific knockout of mTOR or Raptor, the defining protein of the mTORC1 complex, resulted in severe hypomyelination of the spinal cord (Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014). Additionally, animals harboring a mutation in the ubiquitin ligase Fbxw7, which targets mTOR for degradation (Mao et al., 2008), made longer myelin sheaths in an mTOR-dependent manner (Kearns et al., 2015).

Protein translation and myelin protein synthesis are among the numerous cell biological processes regulated by the Akt-mTOR pathway. One way that Akt-mTOR signaling regulates protein translation is through the eukaryotic translation initiation factor 4E binding protein (4E-BP) family of proteins. Unphosphorylated 4E-BPs inhibit cap-dependent translation initiation by binding to eIF4E on the 5' cap of mRNAs (Pause et al., 1994). Upon phosphorylation by the mTORC1 subcomplex, 4E-BP1 dissociates from its binding partner eIF4E, thereby allowing for recruitment of eIF4A and eIF4G to assemble the eIF4F translation initiation complex and promotion of translation (Pause et al., 1994). Dysregulation of Akt-mTOR signaling in oligodendrocytes results in aberrant levels of some myelin proteins (Flores et al., 2008; Narayanan et al., 2009; Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014; Zou et al., 2014). In vitro, myelin basic protein mRNA is packaged into granules and actively transported into some but not all myelin sheaths (Ainger et al., 1993; Ainger et al., 1997), where it is presumably translated. In support of this possibility, free ribosomes have been observed by electron microscopy in the distal ends of cultured oligodendrocytes (Lunn et al., 1997) and recently hundreds of other myelin resident mRNAs have been identified (Thakurela et al., 2016) and a subset validated in vivo (Yergert et al., 2021).

Here, using zebrafish as a model system, we directly tested the hypothesis that protein translation downstream of Akt-mTOR signaling promotes myelin development. We found that modulation of the Akt-mTOR signaling pathway controlled myelin sheath length and stability. Oligodendrocytes co-localized mRNA and translational regulators downstream of mTOR in myelin, and we identified hundreds of putative mTOR translational targets in myelin. Finally, we performed cell-specific manipulation of protein translation and found that Akt-mTOR signaling required cap-dependent translation to promote proper myelin development. Together, our data raise a model wherein mTOR signaling in myelin sheaths regulate sheath growth by promoting translation of myelin-resident mRNAs.

METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Bruce Appel (bruce.appel@cuanschutz.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Zebrafish lines and husbandry

All procedures were approved by the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Committee (IACUC) and performed to their standards. Wild-type non-transgenic embryos were generated by crosses of male and females of the AB strain. *Tg(sox10:mRFP)* embryos were generated by outcrossing *Tg(sox10:mRFP)^{vu234}* (Kucenas et al., 2008) carriers with wild-type AB fish. *mtor* mutant larvae were generated by incrosses of heterozygote *mtor^{xu015Gt}* (Ding et al., 2011) carriers. Embryos and larvae were raised at 28.5°C in E3 media (5mM NaCl, 0.17mM

KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄ at pH 7.4 with sodium bicarbonate) and staged according to hours and days postfertilization. Only larvae displaying good health and normal developmental patterns were used experimentally. Animals used were of indeterminate sex, as sexual differentiation is not apparent until later larval ages.

METHOD DETAILS

Genotyping

mtor mutant larvae were genotyped following imaging. Whole anesthetized larvae were lysed by incubation in 50mM NaOH at 95°C for 30 minutes followed by neutralization with 1/10th volume Tris pH 8.0. PCR amplification was performed using a small volume of lysis and the primers: forward primer: 5'-ATAAGAAAAGAAACCACATGTCATACC-3'; reverse primer: 5'-CTTACCACTCAGAGAGACCAAAG-3'; 5'LTR primer: 5'-CCCTAAGTACTTGTACTTTCACTTG-3'.

Plasmids and Expression Construct Generation

Expression constructs were generated by Multisite Gateway cloning using LR Clonase II Plus (Thermo Fisher Scientific) to recombine entry clones p5E, pME, and p3E into a destination vector, pDEST. All entry clones and expression constructs were screened by restriction digestion and sequence confirmed by Sanger sequencing. The following entry clones were used in this study:

p5E: *p5E-sox10*; *p5E-mbpa*

pME: *pME-mNeonCAAX*; *pME-myrAkt*; *pME-4EBP1*; *pME-4EBP1^{T37/46A}*; *pME-mScarletCAAX*; *pME-eIF4E*; *pME-mbpaCDS-24xMBS*; *pME-HA-NLS-tdMCP-tagRFP*

p3E: *p3E-P2A-mNeonCAAX*; *p3E-eGFP*; *p3E-P2A-myrAkt*; *p3E-polyA*; *p3E-mbpa-3'UTR*

pDEST: *pDEST-Tol2-pA*

pcDNA3-TORCAR (Addgene Plasmid 64927) (Zhou et al., 2015) and *pcDNA3-TORCAR(T/A)* (Addgene Plasmid 64928) (Zhou et al., 2015) were gifts from Jin Zhang and used to create *pME-4EBP1* and *pME-4EBP1^{T37/46A}*, respectively. *pHA-eIF4E* (Addgene Plasmid 17343) (Okumura et al., 2007) used to generate pME-eIF4E was a gift from Dong-Er Zhang. *pme-HA-NLS-tdMCP-tagRFP* (Addgene Plasmid 86244) (Campbell et al., 2015) was a gift from Florence Marlow.

Table of DNA oligonucleotides used for plasmid creation

<i>Construct</i>	<i>Template</i>	<i>Primers</i>
<i>pME-4EBP1</i>	Addgene Plasmid 64927	FWD: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCC GGGGGCAGCAG-3' REV: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTAAATGTC CATCTCAAACGTGTGACT-3'
<i>pME-4EBP1^{T37/46A}</i>	Addgene Plasmid 64928	FWD: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCC GGGGGCAGCAG-3' REV: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTAAATGTC CATCTCAAACGTGTGACT-3'
<i>pME-mScarletCAAX</i>	p3E-P2A-mScarlet CAAX	FWD: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGCCGC CACCATGGTGAGCAAG-3' REV: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTGGAGAG CACACA-3'
<i>pME-eIF4E</i>	Addgene Plasmid 17343	FWD: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGA GGCTTGGTACCG-3' REV: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTAAACAACA AACCTATTTTGTAGTGGTGGAGC-3'

<i>P3E-P2A-myrAkt</i>	pME-myrAkt	FWD: 5'-TAAGCAGGATCCGATGGGGAGCAGCAAG-3' REV: 5'-TAAGCAGAATTCATCGGCTGTGCCACTGG-3'
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Microinjections

Microinjections were performed into newly fertilized eggs generated by incrosses of wild-type AB or heterozygote *mtor^{xu015Gt}* carriers. Injection solutions contained 5 μ L 0.4M KCl, 250ng Tol2 mRNA, a total of 250ng plasmid DNA, and water to 10 μ L. In instances where multiple plasmids were injected, equal concentrations of each plasmid were used. Larvae were raised to the developmental timepoint indicated in individual experiments and selected for good health before inclusion in experiments.

Microscopy

For static imaging experiments, larvae were anesthetized with 0.06% tricaine and immobilized in 0.6% low-melt agarose with 0.4% tricaine. For dynamic live imaging experiments, larvae were immobilized in 0.3 mg/ml pancuronium bromide aided by a small tail slit and embedded in 1.2% low-melt agarose. For analysis of myelin sheath length and dynamics, we acquired images using a C-Apochromat 63x, 1.20 NA water immersion objective on a Zeiss CellObserver Spinning Disk confocal microscope equipped with a Photometrics Prime 95B camera. For analysis of subcellular localization, we acquired images using a Plan-Apochromat 63x, 1.4 NA oil immersion objective on a Zeiss LSM 880. Images were collected with Zen (Carl Zeiss), blinded, and processed/analyzed in ImageJ/Fiji (Schindelin et al., 2012).

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of myelin sheath length and number

Three dimensional images of single spinal cord oligodendrocytes were collected in Zen. We then used the Fiji plugin Simple Neurite Tracer (Longair et al., 2011) to quantify myelin sheath length and number. Briefly, blinded 8-bit z-stack images of single oligodendrocytes were opened in Simple Neurite Tracer. We traced each sheath and saved the path length (sheath length), path number (sheath number), and the sum of the path lengths (cumulative sheath length) in Excel for further analysis in R and Prism 9.

Quantification of myelin sheath dynamics

Three dimensional images of the spinal cord were collected every 15 minutes for 4 hours in Zen. Z-volume movies collected from individual larvae were opened in Fiji. We used the trace segmented line tool to trace individual myelin sheaths. Single myelin sheaths were measured in all frames and exported to Excel before continuing to the next. Myelin sheath dynamic data was then transferred to Prism 9 for visualization and analysis.

Colocalization quantification

Three dimensional images of spinal cord oligodendrocytes co-expressing *sox10:4E-BP1-eGFP* and *mbpa* MS2 reporter constructs were collected in Zen. We imported these images to Fiji and used JACoP (Just Another Colocalization Plugin) (Bolte and Cordelières, 2006) to quantify colocalization. Specifically, we isolated single optical planes of individual myelin sheaths and used JACoP to calculate Mander's Colocalization Coefficients, M1 and M2. M1 and M2 describe the proportion of protein 1 that colocalizes with protein 2 and vice versa, independent of fluorescent intensity. These data were saved in Excel and exported to Prism 9 for visualization and analysis.

Statistics

Plots were generated in R (version 3.6.3) with RStudio using ggplot2 (Wickham, 2016) or biomaRt (Durinck et al., 2009) or Prism 9. All statistical tests were performed in Prism 9. We first tested normality of data. In no instances were all datasets normal. For unpaired comparisons, we used Mann-Whitney test to compare ranks. For multiple comparisons, we first assessed overall significance with the Kruskal-Wallis test. If the Kruskal-Wallis test showed significance, we then performed pairwise Mann-Whitney tests with Bonferroni-Holm correction for multiple comparisons.

BIOINFORMATIC ANALYSIS

FIMO analysis

We used Find Individual Motif Occurrences (FIMO) (version 5.3.3), part of the MEME suite software, to identify transcripts containing TOP-like or CERT motifs in the myelin transcriptome. We downloaded the 5'UTR sequences from the myelin transcriptome and created position weight matrices corresponding to TOP-like or CERT motifs. We uploaded the 5'UTR sequences and position weight matrices to FIMO. We changed options to scan the given strand only and otherwise used default settings. We downloaded the resulting file and removed duplicates to generate the final list of unique genes containing TOP-like or CERT motifs.

Gene ontology analysis

We used DAVID software (version 6.8) to identify gene ontology terms associated with transcripts containing TOP-like or CERT motifs. We submitted Ensembl Gene IDs of transcripts identified as having these motifs and selected categories for biological process, cellular compartment, and up_keywords. We filtered for false discovery rate less

than 0.05 and removed any duplicate terms. We sorted terms from lowest to highest false discovery rate and selected the top 20 for analysis.

RESULTS

The Akt-mTOR pathway drives myelin sheath growth

We previously showed that homozygous *mtor* mutant zebrafish larvae express abnormally low levels of myelin genes (Kearns et al., 2015), consistent with studies from mice showing that conditional loss of mTOR function in oligodendrocytes results in a decreased expression of some myelin genes and proteins and decreased myelin sheath thickness (Wahl et al., 2014). For this study, we assessed the number and length of myelin sheaths formed by oligodendrocytes as a sensitive measure of how mTOR activity promotes myelin development. To label individual oligodendrocytes, we used Tol2-mediated transgenesis (Kwan et al., 2007) and *sox10* regulatory DNA to express the membrane localized fluorophore mNeonCAAX in oligodendrocyte lineage cells. We injected *sox10:mNeonCAAX* into newly fertilized eggs generated from incrosses of wild-type or *mtor* heterozygous zebrafish to generate homozygous *mtor* mutant loss-of-function animals. At 5 days post-fertilization (dpf) we imaged spinal cord oligodendrocytes (Figure 1B) and analyzed oligodendrocyte morphology. On average, myelin sheaths in *mtor* mutant larvae were ~35% shorter than those in wild-type larvae (Figure 1C). Additionally, oligodendrocytes in *mtor* mutant larvae produced fewer sheaths on average than those in wild-type larvae (Figure 1D). Together, this reduction of both myelin sheath length and number resulted in a nearly 50% reduction in the cumulative length of myelin produced by individual oligodendrocytes of *mtor* mutants when compared to wild-type

controls (Figure 1E). These data therefore show that mTOR function promotes the length and number of myelin sheaths formed by spinal cord oligodendrocytes.

In mice, oligodendrocytes programmed to express a constitutively active form of Akt expressed elevated levels of myelin mRNA and protein and produced significantly thicker myelin sheaths (Flores et al., 2008). To test whether elevated Akt signaling also increases myelin sheath length and number, we drove oligodendrocyte-specific expression of a constitutively active Akt1 allele. This allele, hereafter referred to as myrAkt, has an N-terminal myristoylation signal as well as threonine 308 and serine 473, which are the phosphorylation sites that result in full activation, mutated to aspartic acid (Aoki et al., 1998). To identify cells expressing this allele, we appended the viral P2A sequence followed by mNeonCAAX to generate the construct *sox10:myrAkt-P2A-mNeonCAAX*, which drives expression of both myrAkt and mNeonCAAX in oligodendrocyte lineage cells from the same construct. We injected *sox10:myrAkt-P2A-mNeonCAAX* into newly fertilized wild-type eggs and at 5 dpf imaged mNeonCAAX⁺ spinal cord oligodendrocytes for ensheathment analysis. On average, myelin sheaths generated by cells expressing myrAkt were ~30% longer than those made by wild-type controls (Figure 1C). Additionally, cells expressing myrAkt had slightly more sheaths than cells expressing a control, but the difference did not reach statistical significance (Figure 1D). Together, the increased individual myelin sheath length and number resulted in ~40% more myelin generated upon constitutive activation of Akt (Figure 1E). These data show that constitutive Akt activation in wild-type cells autonomously promotes myelin sheath elongation.

In mice, Akt and mTOR function in a common pathway to promote myelin gene and protein expression and myelin sheath thickness (Narayanan et al., 2009). Because Akt has numerous downstream targets, it was important to determine if the myelin sheath length-promoting activity of myrAkt requires mTOR function. To test this, we elevated Akt signaling in oligodendrocytes of *mtor* mutant larvae by injecting *sox10:myrAkt-P2A-mNeonCAAX* into newly fertilized eggs generated from incrosses of *mtor* mutant heterozygotes. If Akt and mTOR function in a common pathway to promote myelin sheath growth, we predicted that driving constitutively active Akt in oligodendrocytes of *mtor* mutant larvae would result in a myelin phenotype indistinguishable from *mtor* mutants alone. By contrast, if Akt does not require mTOR function to promote myelin sheath growth, we predicted driving constitutive Akt activation in oligodendrocytes of *mtor* mutant larvae would increase myelin sheath length. Strikingly, oligodendrocytes in *mtor* mutant larvae that expressed myrAkt produced shorter (Figure 1C) and fewer (Figure 1D) myelin sheaths than wild-type controls. These cells produced an average of ~50% less cumulative myelin sheath lengths than cells of wild-type larvae (Figure 1E). We then compared the myelin profiles of oligodendrocytes of *mtor* mutant larvae with those of *mtor* mutant larvae that also expressed constitutively active Akt and found the average length, number, and cumulative amount of myelin produced per cell were indistinguishable (Figure 1C-E). From this, we conclude that Akt requires mTOR function to promote myelin sheath length, a result that is comparable to the requirement in myelin sheath thickness (Narayanan et al., 2009).

Figure 1. Akt-mTOR pathway activity drives myelin sheath length

(A) Schematic representation of the Akt-mTOR pathway. (B) Representative images of single spinal cord oligodendrocytes of 5 dpf wild-type (WT) or *mtor* mutant larvae with or without expression of myrAkt. Dashed line highlights an individual myelin sheath. Scale bar, 10 μm. Quantification of average sheath length (C), sheath number per cell (D), and cumulative myelin sheath length (E). Violin plot solid lines represent median and dashed lines represent 25th and 75th percentiles. Overall significance was assessed using the Kruskal-Wallis test: sheath length, $p < 1 \times 10^{-12}$, $h = 362.3$; sheath number, $p = 3.98 \times 10^{-6}$, $h = 27.81$; cumulative sheath length, $p = 2 \times 10^{-12}$, $h = 57.40$. Individual data points are represented by orange dots. P values on figure are pairwise Mann-Whitney tests with Bonferroni-Holm correction for multiple comparisons. Sample sizes: WT - 16 fish, 16 cells, 161 sheaths; *mtor*^{-/-} - 23 fish, 23 cells, 206 sheaths; myrAkt - 18 fish, 18 cells, 197 sheaths; myrAkt + *mtor*^{-/-} - 22 fish, 22 cells, 184 sheaths.

mTOR pathway activity promotes myelin sheath stabilization

Changes in myelin sheath length could result from differences in growth rate, stability, or both. To understand which of these parameters was altered upon mTOR pathway loss-of-function, we performed time-lapse imaging of actively myelinating oligodendrocytes beginning at 72 hours post fertilization, when myelin sheath growth is highly dynamic. We collected three dimensional images of wild-type transgenic *sox10:mRFP* and *mtor* mutant larvae transiently expressing *sox10:mScarletCAAX* every 15 minutes for 4 hours (Figure 2A). Myelin sheath length was quantified at each timepoint during the imaging period. To understand the net change in myelin sheath length during the imaging period, we subtracted the length of a given sheath in the first frame from its length in the last frame. We found that on average myelin sheaths in wild-type larvae grew longer during the imaging period, whereas sheaths of *mtor* mutant larvae on average became shorter (Figure 2B). We calculated the mean change in sheath length at each time point by subtracting the length in the initial frame and analyzed those as a function of time. On average, sheaths in *mtor* mutant larvae displayed a steady net negative displacement during the imaging period compared to the net positive displacement of sheaths in wild-type larvae (Figure 2C). Together, these data show that mTOR signaling is required to drive myelin sheath growth during development.

Why did myelin sheaths in *mtor* mutant larvae not show a net growth during development? Did these sheaths not grow at all or did they grow but were not stabilized? To distinguish between these possibilities, we calculated the total amount of space an individual myelin sheath occupied during the imaging period. This measure, which we referred to as dynamic range, is defined by the maximum length of a given sheath

subtracted by the minimum length of that sheath. There was no difference in the average dynamic range of myelin sheaths in wild-type and *mtor* mutant larvae (Figure 2D), which shows that in the absence of mTOR signaling myelin sheaths are capable of growth but are not stabilized.

Figure 2. mTOR pathway activity promotes myelin sheath stabilization

(A) Images of myelin sheaths in the spinal cord of 72 hours post fertilization wild-type (WT) or *mtor* mutant larvae at 60 minute intervals during the imaging period. Inset, dashed line highlights an individual myelin sheath throughout the time course. Scale bar, 10 μ m. (B) Average net change (length at 240 minutes – length at 0 minutes) of sheaths in WT and *mtor* mutant larvae. (C) Mean change in sheath length from initial length as a function of time. Mann-Whitney, $p = 5.98 \times 10^{-5}$, $U = 258$. (D) Dynamic range (maximum length – minimum length) of sheaths in WT and *mtor* mutant larvae. Mann-Whitney, $p = 0.6746$, $U = 512$. N = WT - 8 animals, 42 sheaths; *mtor*^{-/-} - 7 animals, 28 sheaths.

Oligodendrocytes localize mTOR-regulated translational machinery to myelin sheaths

The localization of mRNAs (Ainger et al., 1993; Thakurela et al., 2016; Yergert et al., 2021), ribosomes (Lunn et al., 1997), and RNA binding proteins (Doll et al., 2020) in distal myelin sheaths support a model where local protein translation promotes myelination. We therefore investigated the possibility that translation factors that are regulated by mTOR signaling are localized to nascent myelin sheaths. To test this possibility, we generated a C-terminally tagged human 4E-BP1 fusion protein, an mTOR-dependent translational regulator, and expressed it specifically in oligodendrocytes using the Tol2-transgenesis system and *sox10* regulatory DNA. Imaging of oligodendrocytes at 4 dpf revealed that oligodendrocytes localized 4E-BP1-eGFP to myelin sheaths (Figure 3A). N-terminally tagged fusion proteins were similarly localized, indicating that sheath localization is not a consequence of the placement of the eGFP fusion. The localization of 4E-BP1-eGFP was similar to localization of F-actin that defines the leading edge of myelin sheaths (Figure 3A'). These observations therefore raise the possibility that translation regulated by 4E-BP1 downstream of Akt-mTOR signaling occurs at the leading edge of myelin sheaths.

For Akt-mTOR signaling to promote localized translation, we predicted that Akt-mTOR-dependent translational regulators would co-localize with myelin localized mRNAs. To investigate this possibility, we used fluorescent reporters to test if 4E-BP1 colocalized with *mbpa* mRNA, which encodes myelin basic protein, in vivo using the MS2-MCP RNA visualization system. The MS2-MCP system consists of an mRNA containing 24 MS2 binding sites (24xMBS), which form repetitive stem loops, and a fluorescently tagged MS2 coat protein (MCP), which specifically binds the 24xMBS stem loops to

visualize mRNA via fluorescent reporter (Bertrand et al., 1998). Our lab has previously used this system to reveal localization of RNA encoded by *mbpa*, a zebrafish orthologue of *Mbp*, to myelin sheaths (Yergert et al., 2021). We co-expressed 4E-BP1-eGFP and *mbpa* MS2-mScarlet in oligodendrocytes using the Tol2-transgenesis system and imaged eGFP/mScarlet⁺ spinal cord oligodendrocytes at 4 dpf (Figure 3B). Expression of 4E-BP1-eGFP and *mbpa* MS2 was highly colocalized in myelin sheaths (Figure 3B, inset). We performed colocalization analysis using JACoP (Just Another Colocalization Plugin) and calculated Mander's coefficient to quantify the amount of 4E-BP1-eGFP colocalized with *mbpa* mRNA and vice versa. We found that ~85% of *mbpa* mRNA colocalized with 4E-BP1-eGFP in myelin sheaths (Figure 3C), while 4E-BP1-eGFP colocalized with *mbpa* mRNA ~70% of the time (Figure 3C). Additionally, the percent of 4E-BP1-eGFP that colocalized with *mbpa* mRNA was highly variable, raising the possibility that 4E-BP1 regulates the translation of other myelin-resident mRNAs.

Figure 3. Oligodendrocytes colocalize translational regulators and mRNA in myelin. (A) Image of single 4 dpf spinal cord oligodendrocyte co-expressing *sox10:mScarletCAAX* to label membrane and *sox10:4EBP1-eGFP*. 4EBP1-eGFP signal observed in myelin sheaths and localized to the distal ends (inset, arrows). Scale bar, 10 μ m. (B) Colocalization of 4EBP1-eGFP and *mbpa* mRNA MS2 reporter in a spinal cord oligodendrocyte. 4EBP1-eGFP and *mbpa* colocalize to discrete puncta in the myelin sheath (inset, arrows). (C) Quantification of 4EBP1-eGFP and *mbpa* mRNA colocalization in individual myelin sheaths. Sample size: 7 animals, 7 cells, 43 sheaths.

Identification of putative translational targets in myelin

What myelin-resident mRNAs might be targets of mTOR-regulated translation? To address this question, we used bioinformatics to identify putative myelin-resident translational targets. Loss of 4E-BPs in cultured cells was sufficient to render translation of mRNAs containing terminal oligopyrimidine (TOP) or TOP-like motifs in their 5' untranslated region (5' UTR) resistant to inhibition by the mTOR inhibitor Torin 1 (Thoreen et al., 2012), indicating that mTOR signaling through 4E-BPs promotes the translation of 5' TOP or TOP-like motif containing transcripts. To identify putative 4E-BP1 translational targets, we used the function Find Individual Motif Occurrences (FIMO) in the package MEME Suite to scan the myelin transcriptome for TOP or TOP-like motifs (Figure 6A). We found that 324 of the 1195 annotated 5'UTRs in the myelin transcriptome contained a TOP-like motif (Figure 4B; Table 4-1), indicating that nearly 25% of mRNAs in the myelin transcriptome could be subject to translational regulation by mTOR-4E-BP1 signaling. Canonically, TOP-motif containing mRNAs encode proteins that are components of translational machinery (Yoshihama et al., 2002; Iadevaia et al., 2008). We identified such proteins in our dataset, including the 40S ribosomal proteins S2 and S18 (Rps2 and Rps18, respectively). Additionally, we identified other transcripts with known TOP motifs, such as vimentin (Meyuhas and Kahan, 2015), together indicating that our bioinformatic analysis accurately identified TOP and TOP-like motif containing mRNAs in the myelin transcriptome. To ask if myelin-resident mRNAs with TOP or TOP-like motifs encode specific functions, we performed gene ontology (GO) analysis. From this, we identified some processes indicative of developmental myelination, such as cell junction, membrane, nervous system development, and cytoplasm (Figure 4C). Similar to previous

analysis of myelin-resident transcripts (Yergert et al., 2021), we also identified terms associated with synapses and cell signaling (Figure 4C). These data support the possibility that 4E-BP1 regulated translation of specific myelin-resident mRNAs could promote myelination via synaptogenic-like mechanisms.

We next asked if any myelin-resident mRNAs were putative translational targets of the 4E-BP1 binding partner eIF4E. Studies from eIF4E haploinsufficient mice identified the shared *cis*-regulatory element Cytosine Enriched Regulator of Translation (CERT) present in ~70% of mRNAs that are translational targets of eIF4E (Truitt et al., 2015). To identify transcripts containing a CERT sequence in the myelin transcriptome, we used the FIMO function in MEME suite to search the 5'UTRs of myelin resident transcripts for CERT (Figure 5A). We found that nearly 50% (570/1195) of the annotated 5'UTRs in the myelin transcriptome contain the CERT sequence (Figure 5B; Table 5-1). Similar to 4E-BP1 targets CERT-containing transcripts were enriched for gene ontology terms associated with synapses and intracellular signaling, but also included different categories such as lipoprotein and ion channel (Figure 5C).

Because both 4E-BP1 and eIF4E regulate translation downstream of mTOR signaling, we defined the transcripts that contain both of these sequences. Of the 1195 annotated 5'UTRs in the myelin transcriptome, 245 possessed both a TOP-like motif and a CERT sequence (~20%) (Figure 6A). Approximately 75% of TOP-like motif containing also contained a CERT sequence (245/324) (Figure 6B; Table 6-1), whereas only ~40% of CERT-containing transcripts also contain a TOP-like motif (245/570) (Figure 6B). Myelin-resident transcripts containing both a TOP-like motif and CERT sequence represented GO terms associated with myelin development such as cell junction,

membrane, and nervous system development, but also represented distinct categories such as those associated with potassium and neurotransmitter transport (Figure 6C). Together, the similarities and differences among putative 4E-BP1 and eIF4E translational targets supports the possibility that translation regulated by these two proteins have both overlapping and distinct functions in myelin development.

Figure 4. Identification of putative myelin-resident 4E-BP1 translational targets
 (A) Schematic representation of the TOP-like motif position weight matrix used in bioinformatic identification of putative translational targets. (B) Percentage of 5'UTRs identified as containing a TOP or TOP-like motif. (C) Gene ontology of transcripts containing identified TOP-like motifs. Top 20 terms shown, ordered most to least significant by $-\log_2$ of false discovery rate. Counts correspond to number of genes associated with each term.

Figure 5. Identification of putative eIF4E myelin-resident translational targets

(A) Schematic representation of CERT position weight matrix used to identify transcripts of interest. (B) Percentage of myelin-resident mRNAs identified as having a CERT motif in the 5'UTR. (C) Gene ontology of transcriptions containing identified CERT motif. Top 20 terms shown, ordered most to least significant by $-\log_2$ of false discovery rate. Counts correspond to number of genes associated with each term.

Figure 6. Identification of putative 4E-BP1 and eIF4E shared targets.

(A) Percentage of myelin-resident mRNAs containing both a TOP-like motif and CERT sequence in their 5'UTRs. (B) Schematic representing the proportion of shared and unique translational targets between 4E-BP1 and eIF4E. (C) Gene ontology analysis of 4E-BP1 and eIF4E shared targets. Top 20 terms shown, ordered most to least significant by $-\log_2$ of false discovery rate. Counts correspond to number of genes associated with each term.

Cap-dependent translation regulated by Akt-mTOR signaling drives myelin sheath growth

Our data show that a downstream translational regulator of Akt-mTOR signaling localized to myelin and hundreds of myelin-localized transcripts contain *cis*-regulatory motifs that confer their translation sensitive to Akt-mTOR signaling. Does Akt-mTOR-dependent protein translation drive myelination? To assess this, we performed cell-specific manipulation of the downstream translational regulators 4E-BP1 and eIF4E. To first test if 4E-BP1 function is required for myelin development, we overexpressed a human allele of 4E-BP1 where threonines 37 and 46, which are obligate mTOR phosphorylation sites, were mutated to alanine (4EBP1^{T37/46A}). These mutations render the protein unable to be phosphorylated by mTORC1 and released from its translational repressor state (Gingras et al., 1999), thereby inhibiting 4E-BP1-mediated translation (Figure 7A). Using the Tol2-transgenesis system, we transiently expressed mNeonCAAX, a wild-type allele of human 4E-BP1 (4EBP1^{WT}) and mNeonCAAX, or 4EBP1^{T37/46A} and mNeonCAAX in oligodendrocyte lineage cells of wild-type animals using *sox10* regulatory DNA. Overexpression of 4EBP1^{WT} did not change myelin sheath length, number, or cumulative length of myelin made when compared to wild-type controls (Figure 7D-F). However, when mTOR-4E-BP1 signaling was perturbed by overexpression of the 4EBP1^{T37/46A} allele, oligodendrocytes generated myelin sheaths that were ~35% shorter than those of wild-type larvae or those expressing 4EBP1^{WT} (Figure 7D). Additionally, cells expressing 4EBP1^{T37/46A} produced fewer myelin sheaths than those of controls (Figure 7E). Together, the reduction in myelin sheath length and number resulted in a 50% reduction in the total

length of myelin produced by cells expressing 4EBP1^{T37/46A} (Figure 7F). From these data, we conclude myelin development requires 4EBP1-dependent translation.

As a direct test of whether the Akt-mTOR pathway requires 4E-BP1 function to drive myelination, we simultaneously manipulated Akt-mTOR signaling and 4E-BP1. To drive Akt-mTOR pathway activity in oligodendrocytes, we created the construct *sox10:mScarletCAAX-P2A-myrAkt*. Using the Tol2-transgenesis system, we expressed either *sox10:mScarletCAAX-P2A-myrAkt* alone or in combination with 4EBP1^{T37/46A} and mNeonCAAX in wild-type larvae. Simultaneous expression of myrAkt and 4EBP1^{T37/46A} resulted in a ~35% reduction in average myelin sheath length (Figure 7D). This was accompanied by a decrease in myelin sheath number (Figure 7E) and resulted in a nearly 50% reduction in the total length of myelin produced (Figure 7F). Individual and cumulative myelin sheath length as well as number produced by cells expressing both myrAkt and 4EBP1^{T37/46A} was not different than those of cells expressing 4EBP1^{T37/46A} alone (Figure 3D-F). To test if mTOR and 4EBP1 function in a common pathway to promote myelin development, we performed ensheathment analyses in *mtor* mutant larvae expressing either mNeonCAAX alone or 4EBP1^{T37/46A} and mNeonCAAX. Myelin sheath length, number, and cumulative length of myelin were unchanged in wild-type larvae expressing 4EBP1^{T37/46A} alone, *mtor* mutants, or *mtor* mutant larvae expressing 4EBP1^{T37/46A} (Figure 3D-F). From these data, we conclude that 4E-BP1-mediated translation downstream of Akt-mTOR signaling is required for proper myelin development.

Figure 7. 4E-BP1-dependent translation downstream of Akt-mTOR signaling promotes myelination

(A) Schematic illustrating the mechanism by which 4E-BPs promote translation initiation and the function disruption strategy. (B) Representative images of single 5 dpf spinal cord oligodendrocytes expressing controls or 4E-BP1 manipulation with or without simultaneous mTOR pathway manipulations. Scale bar, 10 μ m. Quantification of myelin sheath length (C), number (D), or cumulative myelin sheath length (E). Violin plot solid lines represent median and dashed lines represent 25th and 75th percentiles. Overall significance was assessed using the Kruskal-Wallis test: sheath length, $p < 1 \times 10^{-12}$, $h = 458.4$; sheath number, $p = 8.62 \times 10^{-6}$, $h = 33.44$; cumulative sheath length, $p < 1 \times 10^{-12}$, $h = 86.76$. Individual data points are represented by orange dots. Sample sizes: WT - 19 fish, 19 cells, 190 sheaths; 4EBP1^{wt} - 18 fish, 18 cells, 169 sheaths; 4EBP1^{T37/46A} - 22 fish, 22 cells, 179 sheaths; myrAkt - 16 fish, 16 cells, 155 sheaths; 4EBP1^{T37/46A} + myrAkt - 17 fish, 17 cells, 126 sheaths; *mtor*^{-/-} - 17 fish, 17 cells, 148 sheaths; 4EBP1^{T37/46A} + *mtor*^{-/-} - 20 fish, 20 cells, 154 sheaths. P values on figure are pairwise Mann-Whitney tests with Bonferroni-Holm correction for multiple comparisons.

Because our 4E-BP1^{T37/46A} data showed that dampening cap-dependent translation inhibits myelin development, we tested if increasing cap-dependent translation would drive myelin sheath elongation. Constitutive overexpression of eIF4E in the mouse brain exaggerates cap-dependent translation that results in alterations to synaptic morphology and physiology and animal behavior (Gkogkas et al., 2013; Santini et al., 2013). To test the role of eIF4E-mediated translation in our system, we expressed human eIF4E and mNeonCAAX in oligodendrocytes by Tol2-transgenesis. Oligodendrocytes expressing human eIF4E had, on average, a 10% increase in the average length of myelin sheaths (Figure 8B). This was accompanied by no change in the number of myelin sheaths produced (Figure 8C). Cumulatively, cells expressing human eIF4E produced a greater length of myelin sheaths (Figure 8D), but this did not reach statistical significance. Together, these data indicate that exaggerated protein translation alone is capable of driving myelin sheath elongation.

Our results showing 4E-BP1-dependent translation promotes myelination led us to conclude that translation initiation downstream of Akt-mTOR is required for myelin sheath elongation. As a direct test of this possibility, we exaggerated translation in oligodendrocytes of *mtor* mutant larvae by expressing human eIF4E and mNeonCAAX using Tol2 transgenesis and *sox10* regulatory elements. If translation downstream of mTOR signaling promotes myelination, we predicted *mtor* mutant myelin sheath length and number deficits would be rescued upon exaggerated translation achieved by eIF4E overexpression. Indeed, eIF4E overexpression in oligodendrocytes of *mtor* mutant larvae restored average myelin sheath length (Figure 8B), number (Figure 8C), and cumulative myelin sheath length (Figure 8D) to wild-type levels. These experiments provide strong

evidence that protein translation regulated by mTOR signaling is required for oligodendrocytes to produce proper length and number of myelin sheaths.

Figure 8. Upregulated eIF4E-mediated translation rescues mTOR loss-of-function

(A) Representative images of 5 dpf spinal cord oligodendrocytes in wild-type (WT) or *mtor* mutant larvae expressing human eIF4E or controls. Scale bar, 10 μm .

Quantification of myelin sheath length (B), number (C), and cumulative myelin sheath length (D). Violin plot solid lines represent median and dashed lines represent 25th and 75th percentiles. Overall significance was assessed by the Kruskal-Wallis test: sheath length, $p < 1 \times 10^{-12}$, $h = 120.3$; sheath number, $p = 0.0069$, $h = 12.15$; cumulative sheath length, $p = 3 \times 10^{-6}$, $h = 28.44$. Orange dots represent individual data points. Sample sizes: WT – 10 fish, 10 cells, 100 sheaths; eIF4E – 15 fish, 15 cells, 150 sheaths; *mtor*^{-/-} – 13 fish, 13 cells, 104 sheaths; *mtor*^{-/-} + eIF4E – 12 animals, 12 cells, 118 sheaths. P values on figure are pairwise Mann-Whitney tests with Bonferroni-Holm correction for multiple comparisons.

DISCUSSION

During development, oligodendrocytes ensheath axons with myelin to support nervous system function. The Akt-mTOR pathway is a key regulator of radial myelin sheath growth, but our understanding of how this pathway promotes myelin sheath elongation and the downstream signaling events it regulates to promote myelination remain limited.

By inhibiting 4E-BP1 function we found that protein translation initiated by Akt-mTOR signaling drives myelin sheath growth. Previous studies have shown that Akt-mTOR signaling in oligodendrocytes promotes both transcription and translation of some myelin genes and proteins (Flores et al., 2008; Narayanan et al., 2009; Tyler et al., 2009; Tyler et al., 2011; Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014; Zou et al., 2014; Kearns et al., 2015) and translation only of other myelin proteins (Tyler et al., 2009; Tyler et al., 2011; Bercury et al., 2014; Lebrun-Julien et al., 2014; Wahl et al., 2014). Consequently, the independent roles that transcription and translation downstream of Akt-mTOR signaling play in regulating myelin development are unclear, and investigations into how mTOR-dependent translation specifically promotes myelination have been limited. Ultrastructural analysis of myelin sheath thickness of 2-month-old 4E-BP1/2 knockout mice showed no change compared to wild-type siblings (Lebrun-Julien et al., 2014). From our data showing inhibited 4E-BP1-dependent translation results in shorter and fewer myelin sheaths, we would have predicted that increasing translation by removing 4E-BP1/2 function would lead to thicker myelin. However, it is possible that changes in myelin sheath thickness were apparent at younger ages not analyzed, consistent with the role of mTOR signaling in developmental myelination, or that mTOR

signaling regulates different mechanisms to promote radial versus lateral growth of myelin sheaths.

Consistent with the role of protein translation driving myelination downstream of Akt-mTOR signaling, we found that eIF4E overexpression cell autonomously rescued myelin sheath length and number deficits observed in mTOR loss-of-function mutants. eIF4E represents a point of convergence between Akt-mTOR signaling and the MAPK/ERK pathway. Phosphorylation of eIF4E at Ser201 by Mnk1 directly regulates eIF4E cap-binding activity downstream of ERK signaling (Waskiewicz et al., 1997). Pharmacological treatment of mice with the mTOR inhibitor rapamycin led to an upregulation of phospho-ERK1/2 in oligodendrocytes of the corpus callosum (Dai et al., 2014), indicating mTOR signaling may negatively regulate ERK signaling during development. To date crosstalk between mTOR and ERK signaling during myelination have proposed Insulin Receptor Substrate-1 (Dai et al., 2014) or p70S6K (Michel et al., 2015) as points of convergence. Our data raise the possibility that eIF4E represents yet another integration point of these pathways, and that upregulation ERK signaling during development could overcome myelin sheath elongation deficits seen upon mTOR loss-of-function.

Do 4E-BP1 and eIF4E promote myelin sheath growth by targeting translation of the same transcripts? By mining the myelin transcriptome for cis-regulatory elements that confer translational control by 4E-BP1 or eIF4E, we identified populations of mRNAs whose translation may be regulated by either 4E-BP1 or eIF4E alone or the two in tandem. In support of our identification of different subsets of genes, yeast genetic studies show that 4E-BP family members can regulate both eIF4E-dependent and independent

translation (Castelli et al., 2015). The myelin transcriptome is composed of hundreds of mRNAs with diverse functions (Thakurela et al., 2016). Gene ontology analysis revealed that the subsets of mRNAs we identified as potentially regulated by 4E-BP1 and/or eIF4E have distinct functions, raising the possibility that 4E-BP1 and eIF4E regulated transcripts have different functional consequences for myelin development. Another possibility is that 4E-BP1 and eIF4E function provide a level of regulation of selective translation based on extracellular signals. Additionally, interactions with RNA binding proteins such as FMRP, which localizes to and promotes myelination (Doll et al., 2020), could provide complex translational regulatory networks in myelin sheaths.

Our data provide preliminary support for a model wherein mTOR signaling in myelin sheaths promotes selective translation of myelin-resident mRNAs. Such a model has been tested in other cell types of the nervous system. In cultured cortical neurons, brain-derived neurotrophic factor (BDNF) stimulation promoted the phosphorylation of mTOR downstream targets 4E-BP and p70S6K in dendrites and drove neurite-localized translation of Arc and CaMKII in an mTOR-dependent manner (Takei et al., 2004). Our bioinformatic analyses revealed some CaMKII subunits as putative 4E-BP1 translational targets in myelin. Genetic studies in mice showed that both some CaMKII subunits (Waggener et al., 2013) and BDNF (Xiao et al., 2010; Lundgaard et al., 2013; Wong et al., 2013; Fletcher et al., 2018) promote central nervous system myelination, raising the possibility that BDNF coordinates new myelin synthesis by mTOR-dependent translation.

This model of mTOR signaling activating the translation of specific myelin-resident transcripts in a context-dependent manner is predicated on localized signaling and translation in myelin sheaths. In support of this possibility, we found a 4E-BP1 fusion

protein and *mbpa* mRNA localized to myelin during development. Additionally, a reporter for PIP₃ formation, the phospholipid that activates Akt, and phosphorylated Akt localized to the membrane of cultured oligodendrocytes (Snaidero et al., 2014). Moreover, in oligodendrocyte-dorsal root ganglion co-cultures, action potentials induced de novo translation of an *Mbp*-fluorescent reporter construct (Wake et al., 2011). In contrast to our proposed mechanism, these studies attributed *Mbp* translational regulation to Fyn kinase (Wake et al., 2011), however it is possible that differences in translational control could be dependent upon context or developmental age or even specific transcripts being regulated by different signaling mechanisms. Future work into this topic should aim to identify the spatial and temporal dynamics of signaling networks in nascent myelin sheaths as well as validate the local translation of myelin-resident mRNAs in vivo.

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Table 4-1. List of genes that are putative 4E-BP1 translational targets in myelin

Ensembl Gene ID	<i>Symbol</i>
ENSMUSG00000026077	Npas2
ENSMUSG00000026051	1500015O10Rik
ENSMUSG00000026185	Igfbp5
ENSMUSG00000026248	Mrpl44
ENSMUSG00000026163	Sphkap
ENSMUSG00000026463	Atp2b4
ENSMUSG00000006005	Tpr
ENSMUSG00000026482	Rgl1
ENSMUSG00000042671	Rgs8
ENSMUSG00000026584	Scyl3
ENSMUSG00000026576	Atp1b1
ENSMUSG00000070532	Ccdc190
ENSMUSG00000038370	Pcp4l1
ENSMUSG00000063659	Zbtb18
ENSMUSG00000015961	Adss
ENSMUSG00000016262	Sertad4
ENSMUSG00000026728	Vim
ENSMUSG00000048550	Thnsl1
ENSMUSG00000026959	Grin1
ENSMUSG00000058740	Kcnt1
ENSMUSG00000009216	Fam163b
ENSMUSG00000057738	Sptan1
ENSMUSG00000079484	Phyhd1
ENSMUSG00000035949	Fbxw2
ENSMUSG00000000247	Lhx2
ENSMUSG00000075334	Rprm
ENSMUSG00000026824	Kcnj3
ENSMUSG00000026826	Nr4a2
ENSMUSG00000027221	Chst1
ENSMUSG00000027168	Pax6
ENSMUSG00000027210	Meis2
ENSMUSG00000074923	Pak6
ENSMUSG00000027245	Hypk
ENSMUSG00000033256	Shf
ENSMUSG00000074872	Ctxn2
ENSMUSG00000027273	Snap25
ENSMUSG00000039033	Tasp1

ENSMUSG00000027419	Pcsk2
ENSMUSG00000060257	Scrt2
ENSMUSG00000027636	Sla2
ENSMUSG00000037771	Slc32a1
ENSMUSG00000074622	Mafb
ENSMUSG00000017943	Gdap1l1
ENSMUSG00000050373	Snx21
ENSMUSG00000017740	Slc12a5
ENSMUSG00000027560	Dok5
ENSMUSG00000090625	Gm20721
ENSMUSG00000039059	Hrh3
ENSMUSG00000027581	Stmn3
ENSMUSG00000027500	Stmn2
ENSMUSG00000027534	Snx16
ENSMUSG00000045031	Cetn4
ENSMUSG00000027722	Spata5
ENSMUSG00000036894	Rap2b
ENSMUSG00000027833	Shox2
ENSMUSG00000033900	Map9
ENSMUSG00000028086	Fbxw7
ENSMUSG00000028069	Gpatch4
ENSMUSG00000027935	Rab13
ENSMUSG00000028137	Celf3
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ENSMUSG00000053819	Camk2d
ENSMUSG00000028161	Ppp3ca
ENSMUSG00000028266	Lmo4
ENSMUSG00000028221	Pip4p2
ENSMUSG00000055373	Fut9
ENSMUSG00000044288	Cnr1
ENSMUSG00000073889	Il11ra1
ENSMUSG00000039137	Whrn
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ENSMUSG00000028484	Psip1
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ENSMUSG00000034610	Tut4
ENSMUSG00000032890	Rims3
ENSMUSG00000042616	Oscp1

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ENSMUSG000000051435	Fhad1
ENSMUSG000000066026	Dhrs3
ENSMUSG000000005045	Chd5
ENSMUSG000000029054	Gabrd
ENSMUSG000000038181	Chpf2
ENSMUSG000000044681	Cnpy1
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ENSMUSG000000000560	Gabra2
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ENSMUSG000000029516	Cit
ENSMUSG000000049550	Clip1
ENSMUSG000000029420	Rimbp2
ENSMUSG000000015944	Castor2
ENSMUSG000000037428	Vgf
ENSMUSG000000029712	Actl6b
ENSMUSG000000045348	Nyap1
ENSMUSG000000029634	Rnf6
ENSMUSG000000029757	Dync1i1
ENSMUSG000000029755	Dlx5
ENSMUSG000000062995	Ica1
ENSMUSG000000060882	Kcnd2
ENSMUSG000000017978	Cadps2
ENSMUSG000000068551	Zfp467
ENSMUSG000000037984	Neurod6
ENSMUSG000000002930	Ppp1r17
ENSMUSG000000062190	Lancl2
ENSMUSG000000035104	Eva1a

ENSMUSG00000047013	Fbxo41
ENSMUSG00000030067	Foxp1
ENSMUSG00000038390	Gpr162
ENSMUSG00000030122	Ptms
ENSMUSG00000000184	Ccnd2
ENSMUSG00000030350	Prmt8
ENSMUSG00000032652	Crebl2
ENSMUSG00000030218	Mgp
ENSMUSG00000030226	Lmo3
ENSMUSG00000068566	Myadm
ENSMUSG00000078816	Prkcg
ENSMUSG00000003549	Ercc1
ENSMUSG00000011751	Sptbn4
ENSMUSG00000009687	Fxyd5
ENSMUSG00000019194	Scn1b
ENSMUSG00000059824	Dbp
ENSMUSG00000058975	Kcnc1
ENSMUSG00000055409	Nell1
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ENSMUSG00000043831	Lysmd4
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ENSMUSG00000038646	Ramac
ENSMUSG00000070462	Tlnrd1
ENSMUSG00000042797	Aqp11
ENSMUSG00000030729	Pgm2l1
ENSMUSG00000037060	Cavin3
ENSMUSG00000036111	Lmo1
ENSMUSG00000038296	Galnt18
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ENSMUSG00000052889	Prkcb
ENSMUSG00000058966	Fam57b
ENSMUSG00000052301	Doc2a
ENSMUSG00000045114	Prrt2
ENSMUSG00000057176	Ccdc189
ENSMUSG00000030806	Stx1b
ENSMUSG00000030967	Zranb1
ENSMUSG00000045733	Sprn
ENSMUSG00000048583	Igf2
ENSMUSG00000037541	Shank2
ENSMUSG00000031543	Ank1

ENSMUSG00000031517	Gpm6a
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ENSMUSG00000033751	Gadd45gip1
ENSMUSG00000053693	Mast1
ENSMUSG00000031654	Cbln1
ENSMUSG00000046707	Csnk2a2
ENSMUSG00000036564	Ndr4
ENSMUSG00000031750	Il34
ENSMUSG00000031767	Nudt7
ENSMUSG00000032174	Icam5
ENSMUSG00000032177	Pde4a
ENSMUSG00000059974	Ntm
ENSMUSG00000070304	Scn2b
ENSMUSG00000046480	Scn4b
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ENSMUSG00000078307	AI593442
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ENSMUSG00000066607	Insyn1
ENSMUSG00000032297	Celf6
ENSMUSG00000032238	Rora
ENSMUSG00000032224	Fam81a
ENSMUSG00000032355	Mlip
ENSMUSG00000032368	Zic1
ENSMUSG00000046402	Rbp1
ENSMUSG00000032475	Nck1
ENSMUSG00000062296	Trank1
ENSMUSG00000039163	Cmc1
ENSMUSG00000032532	Cck
ENSMUSG00000019775	Rgs17
ENSMUSG00000015501	Hivep2
ENSMUSG00000078453	Abrac1
ENSMUSG00000059554	Ccdc28a
ENSMUSG00000004360	9330159F19Rik
ENSMUSG00000019785	Clvs2
ENSMUSG00000038332	Sesn1
ENSMUSG00000020098	Pcbd1
ENSMUSG00000020083	Fam241b
ENSMUSG00000049422	Chchd10

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ENSMUSG00000019960	Dusp6
ENSMUSG00000019966	Kitl
ENSMUSG00000053825	Ppfia2
ENSMUSG00000035864	Syt1
ENSMUSG00000052302	Tbc1d30
ENSMUSG00000044071	Fam19a2
ENSMUSG00000044005	Gls2
ENSMUSG00000044629	Cnrip1
ENSMUSG00000045671	Spred2
ENSMUSG00000044068	Zrsr1
ENSMUSG00000000861	Bcl11a
ENSMUSG00000032878	Ccdc85a
ENSMUSG00000053519	Kcnip1
ENSMUSG00000010803	Gabra1
ENSMUSG00000020333	Acsl6
ENSMUSG00000020524	Gria1
ENSMUSG00000049892	Rasd1
ENSMUSG00000018470	Kcnab3
ENSMUSG00000043419	Rnf227
ENSMUSG00000044122	Proca1
ENSMUSG00000048895	Cdk5r1
ENSMUSG00000018548	Trim37
ENSMUSG00000003949	Hlf
ENSMUSG00000056158	Car10
ENSMUSG00000017400	Stac2
ENSMUSG00000038255	Neurod2
ENSMUSG00000061718	Ppp1r1b
ENSMUSG00000038020	Rapgef1l
ENSMUSG00000046215	Rprml
ENSMUSG00000061086	Myl4
ENSMUSG00000020599	Rgs9
ENSMUSG00000020810	Cygb
ENSMUSG00000075410	Prcd
ENSMUSG00000020589	Fam49a
ENSMUSG00000057469	E2f6
ENSMUSG00000020580	Rock2
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ENSMUSG00000062563	Cys1

ENSMUSG00000044067	Gpr22
ENSMUSG00000020950	Foxg1
ENSMUSG00000035614	Togaram1
ENSMUSG00000021062	Rab15
ENSMUSG00000042700	Sipa1l1
ENSMUSG00000066392	Nrxn3
ENSMUSG00000007682	Dio2
ENSMUSG00000021007	Spata7
ENSMUSG00000021194	Chga
ENSMUSG00000037904	Ankrd9
ENSMUSG00000033499	Larp4b
ENSMUSG00000036006	Ripor2
ENSMUSG00000035910	Dcdc2a
ENSMUSG00000069255	Dusp22
ENSMUSG00000038546	Ranbp9
ENSMUSG00000021373	Cap2
ENSMUSG00000021379	Id4
ENSMUSG00000037989	Wnk2
ENSMUSG00000052087	Rgs14
ENSMUSG00000056222	Spock1
ENSMUSG00000005583	Mef2c
ENSMUSG00000021708	Rasgrf2
ENSMUSG00000045034	Ankrd34b
ENSMUSG00000021666	Gfm2
ENSMUSG00000021647	Cartpt
ENSMUSG00000021752	Kctd6
ENSMUSG00000021750	Fam107a
ENSMUSG00000021743	Fezf2
ENSMUSG00000054423	Cadps
ENSMUSG00000045201	Lrrc3b
ENSMUSG00000021820	Camk2g
ENSMUSG00000021773	Comtd1
ENSMUSG00000049532	Sall2
ENSMUSG00000022208	Jph4
ENSMUSG00000022212	Cpne6
ENSMUSG00000022216	Psme1
ENSMUSG00000021974	Fgf9
ENSMUSG00000022055	Nefl
ENSMUSG00000003469	Phyhip
ENSMUSG00000022099	Dmtn

ENSMUSG00000022103	Gfra2
ENSMUSG00000052584	Serp2
ENSMUSG00000033186	Mzt1
ENSMUSG00000033060	Lmo7
ENSMUSG00000061524	Zic2
ENSMUSG00000022269	March11
ENSMUSG00000022240	Ctnnd2
ENSMUSG00000022285	Ywhaz
ENSMUSG00000022296	Baalc
ENSMUSG00000059895	Ptp4a3
ENSMUSG00000048385	Scrt1
ENSMUSG00000033565	Rbfox2
ENSMUSG00000061740	Cyp2d22
ENSMUSG00000035805	Mlc1
ENSMUSG00000058441	Panx2
ENSMUSG00000052560	Cpne8
ENSMUSG00000045608	Dbx2
ENSMUSG00000029875	Ccdc184
ENSMUSG00000000552	Zfp385a
ENSMUSG00000008658	Rbfox1
ENSMUSG00000043811	Rtn4r
ENSMUSG00000038094	Atp13a4
ENSMUSG00000061751	Kalrn
ENSMUSG00000037196	Pacrg
ENSMUSG00000048826	Dact2
ENSMUSG00000044533	Rps2
ENSMUSG00000025739	Gng13
ENSMUSG00000063239	Grm4
ENSMUSG00000008668	Rps18
ENSMUSG00000007041	Clic1
ENSMUSG00000050705	2310061I04Rik
ENSMUSG00000003279	Dlgap1
ENSMUSG00000062691	Cebpz
ENSMUSG00000036533	Cdc42ep3
ENSMUSG00000045038	Prkce
ENSMUSG00000063889	Crem
ENSMUSG00000047879	Usp14
ENSMUSG00000036225	Kctd1
ENSMUSG00000056124	B4galt6
ENSMUSG00000057455	Rit2

ENSMUSG00000042834	Nrep
ENSMUSG00000043991	Pura
ENSMUSG00000044024	Rell2
ENSMUSG00000054477	Kcnn2
ENSMUSG00000005986	Ankrd13d
ENSMUSG000000032946	Rasgrp2
ENSMUSG00000006435	Neurl1a

Table 5-1. List of genes that are putative eIF4E translational targets in myelin

Ensembl Gene ID	<i>Symbol</i>
ENSMUSG000000057715	A830018L16Rik
ENSMUSG000000037509	Arhgef4
ENSMUSG000000026090	2010300C02Rik
ENSMUSG000000026083	Eif5b
ENSMUSG000000048814	Lonrf2
ENSMUSG000000026077	Npas2
ENSMUSG000000003135	Cnot11
ENSMUSG000000025981	Coq10b
ENSMUSG000000048495	Tyw5
ENSMUSG000000026185	Igfbp5
ENSMUSG000000026248	Mrpl44
ENSMUSG000000026241	Nppc
ENSMUSG000000049866	Arl4c
ENSMUSG000000026278	Bok
ENSMUSG000000064246	Chil1
ENSMUSG000000026452	Syt2
ENSMUSG000000006005	Tpr
ENSMUSG000000026482	Rgl1
ENSMUSG000000042671	Rgs8
ENSMUSG000000026584	Scyl3
ENSMUSG000000026576	Atp1b1
ENSMUSG000000015843	Rxrg
ENSMUSG000000038530	Rgs4
ENSMUSG000000070532	Ccdc190
ENSMUSG000000038370	Pcp4l1
ENSMUSG000000045259	Klhdc9
ENSMUSG000000026527	Rgs7
ENSMUSG000000053963	Stum
ENSMUSG000000026514	Cnih3
ENSMUSG000000026618	Iars2
ENSMUSG000000037624	Kcnk2
ENSMUSG000000016262	Sertad4
ENSMUSG000000016494	Cd34
ENSMUSG000000039145	Camk1d
ENSMUSG000000026728	Vim
ENSMUSG000000057914	Cacnb2
ENSMUSG000000017418	Arl5b

ENSMUSG00000043415	Otud1
ENSMUSG00000036617	Etl4
ENSMUSG00000026787	Gad2
ENSMUSG00000026959	Grin1
ENSMUSG00000058740	Kcnt1
ENSMUSG00000026925	Inpp5e
ENSMUSG00000036186	Fam69b
ENSMUSG00000009216	Fam163b
ENSMUSG00000057738	Sptan1
ENSMUSG00000015337	Endog
ENSMUSG00000000247	Lhx2
ENSMUSG00000026761	Orc4
ENSMUSG00000017144	Rnd3
ENSMUSG00000036202	Rif1
ENSMUSG00000075334	Rprm
ENSMUSG00000026824	Kcnj3
ENSMUSG00000026826	Nr4a2
ENSMUSG00000063145	Bbs5
ENSMUSG00000068882	Ssb
ENSMUSG00000051730	Mettl5
ENSMUSG00000070880	Gad1
ENSMUSG00000027016	Zfp385b
ENSMUSG00000034701	Neurod1
ENSMUSG00000027221	Chst1
ENSMUSG00000027168	Pax6
ENSMUSG00000027210	Meis2
ENSMUSG00000074923	Pak6
ENSMUSG00000027245	Hypk
ENSMUSG00000033256	Shf
ENSMUSG00000027378	Nphp1
ENSMUSG00000027394	Ttl
ENSMUSG00000027350	Chgb
ENSMUSG00000027270	Lamp5
ENSMUSG00000027273	Snap25
ENSMUSG00000039033	Tasp1
ENSMUSG00000027419	Pcsk2
ENSMUSG00000074736	Syndig1
ENSMUSG00000060257	Scrt2
ENSMUSG00000042745	Id1
ENSMUSG00000027612	Mmp24

ENSMUSG00000037843	Vstm2l
ENSMUSG00000037771	Slc32a1
ENSMUSG00000074622	Mafb
ENSMUSG00000050373	Snx21
ENSMUSG00000017754	Pltp
ENSMUSG00000017740	Slc12a5
ENSMUSG00000047907	Tshz2
ENSMUSG00000027560	Dok5
ENSMUSG00000027495	Fam210b
ENSMUSG00000070476	Fam217b
ENSMUSG00000039086	Ss18l1
ENSMUSG00000039059	Hrh3
ENSMUSG00000016346	Kcnq2
ENSMUSG00000027581	Stmn3
ENSMUSG00000002458	Rgs19
ENSMUSG00000037531	Mrpl47
ENSMUSG00000085007	Gm11549
ENSMUSG00000027714	Exosc9
ENSMUSG00000027716	Trpc3
ENSMUSG00000045031	Cetn4
ENSMUSG00000027722	Spata5
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ENSMUSG00000048332	Lhfp
ENSMUSG00000027797	Dclk1
ENSMUSG00000027799	Nbea
ENSMUSG00000036894	Rap2b
ENSMUSG00000027833	Shox2
ENSMUSG00000043300	B3galnt1
ENSMUSG00000028086	Fbxw7
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ENSMUSG00000044667	Plppr4
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ENSMUSG00000053819	Camk2d
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ENSMUSG00000028266	Lmo4
ENSMUSG00000036825	Ssx2ip
ENSMUSG00000039058	Ak5
ENSMUSG00000028207	Asph
ENSMUSG00000049225	Pdp1
ENSMUSG00000028221	Pip4p2
ENSMUSG00000028222	Calb1
ENSMUSG00000055373	Fut9
ENSMUSG00000058006	Mdn1
ENSMUSG00000044288	Cnr1
ENSMUSG00000028407	Smim27
ENSMUSG00000073889	Il11ra1
ENSMUSG00000028464	Tpm2
ENSMUSG00000045589	Frrs1l
ENSMUSG00000039137	Whrn
ENSMUSG00000048706	Lurap1l
ENSMUSG00000008575	Nfib
ENSMUSG00000028484	Psip1
ENSMUSG00000028488	Sh3gl2
ENSMUSG00000070923	Klhl9
ENSMUSG00000028556	Dock7
ENSMUSG00000028527	Ak4
ENSMUSG00000034610	Tut4
ENSMUSG00000028545	Bend5
ENSMUSG00000028634	Hivep3
ENSMUSG00000032890	Rims3
ENSMUSG00000046093	Hpcal4
ENSMUSG00000043872	Zmym1
ENSMUSG00000028789	Azin2
ENSMUSG00000028785	Hpca
ENSMUSG00000028909	Ptpru
ENSMUSG00000056596	Trnp1

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ENSMUSG000000078235	Fam43b
ENSMUSG000000046447	Camk2n1
ENSMUSG000000051435	Fhad1
ENSMUSG000000066026	Dhrs3
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ENSMUSG000000005045	Chd5
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ENSMUSG000000084845	Tmem240
ENSMUSG000000061601	Pclo
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ENSMUSG000000028949	Smarcd3
ENSMUSG000000056367	Actr3b
ENSMUSG000000039095	En2
ENSMUSG000000044681	Cnpy1
ENSMUSG000000063646	Jakmip1
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ENSMUSG000000090061	Nwd2
ENSMUSG000000037653	Kctd8
ENSMUSG000000000560	Gabra2
ENSMUSG000000029211	Gabra4
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ENSMUSG000000063430	Wscd2
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ENSMUSG000000029516	Cit
ENSMUSG000000029359	Tesc
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ENSMUSG000000049550	Clip1
ENSMUSG000000029406	Pitpnm2
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ENSMUSG000000029674	Limk1

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ENSMUSG00000043388	Tmem130
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ENSMUSG00000029757	Dync1i1
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ENSMUSG00000062995	Ica1
ENSMUSG00000029563	Foxp2
ENSMUSG00000060882	Kcnd2
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ENSMUSG00000030067	Foxp1
ENSMUSG00000030102	Itpr1
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ENSMUSG00000002265	Peg3
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ENSMUSG00000048481	Mypop

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ENSMUSG000000036578	Fxyd7
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ENSMUSG000000030731	Syt3
ENSMUSG000000070570	Slc17a7
ENSMUSG000000059824	Dbp
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ENSMUSG000000055409	Nell1
ENSMUSG000000030500	Slc17a6
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ENSMUSG000000078591	Hs3st4
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ENSMUSG000000052301	Doc2a
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ENSMUSG00000037316	Bag4
ENSMUSG00000052906	Ubxn8
ENSMUSG00000036306	Lzts1
ENSMUSG00000109523	Gdf1
ENSMUSG00000034799	Unc13a
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ENSMUSG00000053693	Mast1
ENSMUSG00000031654	Cbln1
ENSMUSG00000031760	Mt3
ENSMUSG00000031762	Mt2
ENSMUSG00000046707	Csnk2a2
ENSMUSG00000036564	Ndr4
ENSMUSG00000045538	Ddx28
ENSMUSG00000031906	Smpd3
ENSMUSG00000031731	Ap1g1
ENSMUSG00000033430	Terf2ip
ENSMUSG00000031767	Nudt7
ENSMUSG00000043687	1190005I06Rik
ENSMUSG00000031970	Dbn1
ENSMUSG00000050751	Pgbd5
ENSMUSG00000032174	Icam5
ENSMUSG00000032177	Pde4a
ENSMUSG00000053310	Nrgn
ENSMUSG00000046480	Scn4b
ENSMUSG00000059412	Fxyd2
ENSMUSG00000037493	Cib2
ENSMUSG00000032291	Crabp1
ENSMUSG00000032307	Ube2q2
ENSMUSG00000062309	Rpp25
ENSMUSG00000066607	Insyn1
ENSMUSG00000032297	Celf6
ENSMUSG00000032238	Rora

ENSMUSG00000062151	Unc13c
ENSMUSG00000032251	Irak1bp1
ENSMUSG00000034973	Dop1a
ENSMUSG00000032368	Zic1
ENSMUSG00000046402	Rbp1
ENSMUSG00000032475	Nck1
ENSMUSG00000070287	Slc35g2
ENSMUSG00000032560	Dnajc13
ENSMUSG00000032936	Camkv
ENSMUSG00000032589	Bsn
ENSMUSG00000062296	Trank1
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ENSMUSG00000032507	Fbxl2
ENSMUSG00000039163	Cmc1
ENSMUSG00000019775	Rgs17
ENSMUSG00000019796	Lrp11
ENSMUSG00000015501	Hivep2
ENSMUSG00000078453	Abrac1
ENSMUSG00000019986	Ahi1
ENSMUSG00000019997	Ccn2
ENSMUSG00000000296	Tpd52l1
ENSMUSG00000019785	Clvs2
ENSMUSG00000019777	Hdac2
ENSMUSG00000038332	Sesn1
ENSMUSG00000071324	Armc2
ENSMUSG00000047139	Cd24a
ENSMUSG00000050953	Gja1
ENSMUSG00000020098	Pcbd1
ENSMUSG00000020083	Fam241b
ENSMUSG00000020076	Ddx50
ENSMUSG00000071253	Slc25a16
ENSMUSG00000049422	Chchd10
ENSMUSG00000001120	Pcbp3
ENSMUSG00000020262	Adarb1
ENSMUSG00000055862	Izumo4
ENSMUSG00000004933	Matk
ENSMUSG00000034818	Celf5
ENSMUSG00000020248	Nfyb
ENSMUSG00000087651	1500009L16Rik
ENSMUSG00000054027	Nt5dc3

ENSMUSG00000019979	Apaf1
ENSMUSG00000020027	Socs2
ENSMUSG00000019943	Atp2b1
ENSMUSG00000019960	Dusp6
ENSMUSG00000019966	Kitl
ENSMUSG00000053825	Ppfia2
ENSMUSG00000035864	Syt1
ENSMUSG00000020186	Csrp2
ENSMUSG00000020189	Osbpl8
ENSMUSG00000052302	Tbc1d30
ENSMUSG00000025795	Rassf3
ENSMUSG00000020115	Tbk1
ENSMUSG00000044071	Fam19a2
ENSMUSG00000044005	Gls2
ENSMUSG00000020396	Nefh
ENSMUSG00000034209	Rasl10a
ENSMUSG00000057897	Camk2b
ENSMUSG00000020431	Adcy1
ENSMUSG00000044629	Cnrip1
ENSMUSG00000045671	Spred2
ENSMUSG00000044068	Zrsr1
ENSMUSG00000000861	Bcl11a
ENSMUSG00000032985	5730522E02Rik
ENSMUSG00000032878	Ccdc85a
ENSMUSG00000053519	Kcnip1
ENSMUSG00000051062	Fbl1
ENSMUSG00000018849	Wwc1
ENSMUSG00000010803	Gabra1
ENSMUSG00000049892	Rasd1
ENSMUSG00000033389	Arhgap44
ENSMUSG00000020900	Myh10
ENSMUSG00000018470	Kcnab3
ENSMUSG00000043419	Rnf227
ENSMUSG00000018451	6330403K07Rik
ENSMUSG00000020807	4933427D14Rik
ENSMUSG00000038807	Rap1gap2
ENSMUSG00000069814	Ccdc92b
ENSMUSG00000020848	Doc2b
ENSMUSG00000020846	Rflnb
ENSMUSG00000044122	Proca1

ENSMUSG00000002055	Spag5
ENSMUSG000000017344	Vtn
ENSMUSG000000048895	Cdk5r1
ENSMUSG000000020704	Asic2
ENSMUSG00000000805	Car4
ENSMUSG000000018548	Trim37
ENSMUSG000000046442	Ppm1e
ENSMUSG00000003948	Mmd
ENSMUSG000000056158	Car10
ENSMUSG000000017400	Stac2
ENSMUSG000000038255	Neurod2
ENSMUSG000000061718	Ppp1r1b
ENSMUSG000000038020	Rapgef1
ENSMUSG000000045007	Tubg2
ENSMUSG000000056938	Acbd4
ENSMUSG000000048878	Hexim1
ENSMUSG000000055805	Fmnl1
ENSMUSG000000046215	Rprml
ENSMUSG000000019590	Cyb561
ENSMUSG000000020599	Rgs9
ENSMUSG000000020810	Cygb
ENSMUSG000000075410	Prcd
ENSMUSG000000071454	Dtnb
ENSMUSG000000020657	Dnajc27
ENSMUSG000000020635	Fkbp1b
ENSMUSG000000020607	Fam84a
ENSMUSG000000057469	E2f6
ENSMUSG000000020580	Rock2
ENSMUSG000000062563	Cys1
ENSMUSG000000020644	Id2
ENSMUSG000000036188	Ankmy2
ENSMUSG000000021047	Nova1
ENSMUSG000000020950	Foxg1
ENSMUSG000000020993	Trappc6b
ENSMUSG000000049882	Vcpkmt
ENSMUSG000000021066	Atl1
ENSMUSG000000021071	Trim9
ENSMUSG000000044912	Syt16
ENSMUSG000000021062	Rab15
ENSMUSG000000015143	Actn1

ENSMUSG00000042700	Sipa1l1
ENSMUSG00000046791	Riox1
ENSMUSG00000071234	Syndig1l
ENSMUSG00000021244	Ylpm1
ENSMUSG00000066392	Nrxn3
ENSMUSG00000021007	Spata7
ENSMUSG00000021194	Chga
ENSMUSG00000037904	Ankrd9
ENSMUSG00000049792	Bag5
ENSMUSG00000021285	Ppp1r13b
ENSMUSG00000072825	Cep170b
ENSMUSG00000033499	Larp4b
ENSMUSG00000036006	Ripor2
ENSMUSG00000035910	Dcdc2a
ENSMUSG00000069255	Dusp22
ENSMUSG00000046573	Lym4
ENSMUSG00000038546	Ranbp9
ENSMUSG00000015396	Cd83
ENSMUSG00000000253	Gmpr
ENSMUSG00000021373	Cap2
ENSMUSG00000037989	Wnk2
ENSMUSG00000025867	Cplx2
ENSMUSG00000069227	Gprin1
ENSMUSG00000034891	Sncb
ENSMUSG00000034675	Dbn1
ENSMUSG00000021536	Adcy2
ENSMUSG00000052981	Ube2ql1
ENSMUSG00000001504	Irx2
ENSMUSG00000043190	Rfesd
ENSMUSG00000051098	Mblac2
ENSMUSG00000005583	Mef2c
ENSMUSG00000021548	Ccnh
ENSMUSG00000021708	Rasgrf2
ENSMUSG00000045034	Ankrd34b
ENSMUSG00000021666	Gfm2
ENSMUSG00000021647	Cartpt
ENSMUSG00000021719	Rgs7bp
ENSMUSG00000021743	Fezf2
ENSMUSG00000054423	Cadps
ENSMUSG00000045201	Lrrc3b

ENSMUSG00000021820	Camk2g
ENSMUSG00000021868	Ppif
ENSMUSG00000021895	Arhgef3
ENSMUSG00000021948	Prkcd
ENSMUSG00000058351	Smim4
ENSMUSG00000021943	Gdf10
ENSMUSG00000037536	Fbxo34
ENSMUSG00000022208	Jph4
ENSMUSG00000022212	Cpne6
ENSMUSG00000022216	Psme1
ENSMUSG00000022217	Emc9
ENSMUSG00000048281	Dleu7
ENSMUSG00000022040	Ephx2
ENSMUSG00000059456	Ptk2b
ENSMUSG00000003469	Phyhip
ENSMUSG00000022099	Dmtn
ENSMUSG00000022103	Gfra2
ENSMUSG00000052584	Serp2
ENSMUSG00000043881	Kbtbd7
ENSMUSG00000033186	Mzt1
ENSMUSG00000098557	Kctd12
ENSMUSG00000022132	Cldn10
ENSMUSG00000061524	Zic2
ENSMUSG00000044224	Dnajc21
ENSMUSG00000045763	Basp1
ENSMUSG00000022269	March11
ENSMUSG00000022240	Ctnnd2
ENSMUSG00000022285	Ywhaz
ENSMUSG00000037362	Ccn3
ENSMUSG00000022359	Wdyhv1
ENSMUSG00000022332	Khdrbs3
ENSMUSG00000059895	Ptp4a3
ENSMUSG00000048385	Scrt1
ENSMUSG00000033565	Rbfox2
ENSMUSG00000019146	Cacng2
ENSMUSG00000018865	Sult4a1
ENSMUSG00000058441	Panx2
ENSMUSG00000053137	Mapk11
ENSMUSG00000052560	Cpne8
ENSMUSG00000036273	Lrrk2

ENSMUSG00000045608	Dbx2
ENSMUSG00000029875	Ccdc184
ENSMUSG000000054855	Rnd1
ENSMUSG00000000552	Zfp385a
ENSMUSG00000014232	Cluap1
ENSMUSG00000008658	Rbfox1
ENSMUSG00000038055	Dexi
ENSMUSG00000043811	Rtn4r
ENSMUSG00000004366	Sst
ENSMUSG00000035790	Cep19
ENSMUSG00000061751	Kalrn
ENSMUSG000000087141	Plcxd2
ENSMUSG00000032965	Ift57
ENSMUSG00000052397	Ezr
ENSMUSG00000037196	Pacrg
ENSMUSG00000048826	Dact2
ENSMUSG00000079657	Rab26
ENSMUSG00000044533	Rps2
ENSMUSG00000025739	Gng13
ENSMUSG00000025738	Fbxl16
ENSMUSG00000073433	Arhgdig
ENSMUSG00000063239	Grm4
ENSMUSG00000034786	Gpsm3
ENSMUSG00000050705	2310061I04Rik
ENSMUSG00000038910	Plcl2
ENSMUSG00000003279	Dlgap1
ENSMUSG00000036533	Cdc42ep3
ENSMUSG00000059811	Atl2
ENSMUSG00000045038	Prkce
ENSMUSG00000063889	Crem
ENSMUSG00000047879	Usp14
ENSMUSG00000057766	Ankrd29
ENSMUSG00000036225	Kctd1
ENSMUSG00000056124	B4galt6
ENSMUSG00000042834	Nrep
ENSMUSG00000043991	Pura
ENSMUSG00000044024	Rell2
ENSMUSG00000036452	Arhgap26
ENSMUSG00000054477	Kcnn2
ENSMUSG00000032735	Ablim3

ENSMUSG00000005986	Ankrd13d
ENSMUSG000000049303	Syt12
ENSMUSG000000033760	Rbm4b
ENSMUSG000000032946	Rasgrp2
ENSMUSG000000090291	Lrrc10b
ENSMUSG000000058624	Gda
ENSMUSG000000067279	Ppp1r3c
ENSMUSG000000018821	Avpi1
ENSMUSG000000042532	Golga7b
ENSMUSG000000034336	Ina
ENSMUSG000000006435	Neurl1a
ENSMUSG000000034765	Dusp5
ENSMUSG000000084957	Bbip1

Table 6-1. List of genes that are putative shared 4E-BP1 and eIF4E translational targets in myelin

Ensembl Gene ID	<i>Symbol</i>
ENSMUSG00000026077	Npas2
ENSMUSG00000026185	Igfbp5
ENSMUSG00000026248	Mrpl44
ENSMUSG00000006005	Tpr
ENSMUSG00000026482	Rgl1
ENSMUSG00000042671	Rgs8
ENSMUSG00000026584	Scyl3
ENSMUSG00000026576	Atp1b1
ENSMUSG00000070532	Ccdc190
ENSMUSG00000038370	Pcp4l1
ENSMUSG00000016262	Sertad4
ENSMUSG00000026728	Vim
ENSMUSG00000026959	Grin1
ENSMUSG00000058740	Kcnt1
ENSMUSG00000009216	Fam163b
ENSMUSG00000057738	Sptan1
ENSMUSG00000000247	Lhx2
ENSMUSG00000075334	Rprm
ENSMUSG00000026824	Kcnj3
ENSMUSG00000026826	Nr4a2
ENSMUSG00000027221	Chst1
ENSMUSG00000027168	Pax6
ENSMUSG00000027210	Meis2
ENSMUSG00000074923	Pak6
ENSMUSG00000027245	Hypk
ENSMUSG00000033256	Shf
ENSMUSG00000027273	Snap25
ENSMUSG00000039033	Tasp1
ENSMUSG00000027419	Pcsk2
ENSMUSG00000060257	Scrt2
ENSMUSG00000037771	Slc32a1
ENSMUSG00000074622	Mafb
ENSMUSG00000050373	Snx21
ENSMUSG00000017740	Slc12a5
ENSMUSG00000027560	Dok5
ENSMUSG00000039059	Hrh3

ENSMUSG00000027581	Stmn3
ENSMUSG00000045031	Cetn4
ENSMUSG00000027722	Spata5
ENSMUSG00000036894	Rap2b
ENSMUSG00000027833	Shox2
ENSMUSG00000028086	Fbxw7
ENSMUSG00000028069	Gpatch4
ENSMUSG00000027935	Rab13
ENSMUSG00000028137	Celf3
ENSMUSG00000048458	Inka2
ENSMUSG00000027895	Kcnc4
ENSMUSG00000027894	Slc6a17
ENSMUSG00000053819	Camk2d
ENSMUSG00000028161	Ppp3ca
ENSMUSG00000028266	Lmo4
ENSMUSG00000028221	Pip4p2
ENSMUSG00000055373	Fut9
ENSMUSG00000044288	Cnr1
ENSMUSG00000073889	Il11ra1
ENSMUSG00000039137	Whrn
ENSMUSG00000008575	Nfib
ENSMUSG00000028484	Psip1
ENSMUSG00000034610	Tut4
ENSMUSG00000032890	Rims3
ENSMUSG00000078235	Fam43b
ENSMUSG00000046447	Camk2n1
ENSMUSG00000051435	Fhad1
ENSMUSG00000066026	Dhrs3
ENSMUSG00000005045	Chd5
ENSMUSG00000029054	Gabrd
ENSMUSG00000038181	Chpf2
ENSMUSG00000044681	Cnpy1
ENSMUSG00000000560	Gabra2
ENSMUSG00000029245	Epha5
ENSMUSG00000060961	Slc4a4
ENSMUSG00000029330	Cds1
ENSMUSG00000063430	Wscd2
ENSMUSG00000029544	Cabp1
ENSMUSG00000029516	Cit
ENSMUSG00000049550	Clip1

ENSMUSG00000029420	Rimbp2
ENSMUSG00000015944	Castor2
ENSMUSG000000037428	Vgf
ENSMUSG000000029712	Actl6b
ENSMUSG000000045348	Nyap1
ENSMUSG000000029634	Rnf6
ENSMUSG000000029757	Dync1i1
ENSMUSG000000029755	Dlx5
ENSMUSG000000062995	Ica1
ENSMUSG000000060882	Kcnd2
ENSMUSG000000017978	Cadps2
ENSMUSG000000068551	Zfp467
ENSMUSG000000062190	Lanc12
ENSMUSG000000047013	Fbxo41
ENSMUSG000000030067	Foxp1
ENSMUSG000000038390	Gpr162
ENSMUSG000000030122	Ptms
ENSMUSG000000030350	Prmt8
ENSMUSG000000032652	Crebl2
ENSMUSG000000078816	Prkcg
ENSMUSG000000003549	Ercc1
ENSMUSG000000009687	Fxyd5
ENSMUSG000000019194	Scn1b
ENSMUSG000000059824	Dbp
ENSMUSG000000058975	Kcnc1
ENSMUSG000000055409	Nell1
ENSMUSG000000030500	Slc17a6
ENSMUSG000000043831	Lysmd4
ENSMUSG000000070462	Tlnrd1
ENSMUSG000000042797	Aqp11
ENSMUSG000000030729	Pgm2l1
ENSMUSG000000038296	Galnt18
ENSMUSG000000058420	Syt17
ENSMUSG000000052889	Prkcb
ENSMUSG000000058966	Fam57b
ENSMUSG000000052301	Doc2a
ENSMUSG000000045114	Prrt2
ENSMUSG000000030806	Stx1b
ENSMUSG000000030967	Zranb1
ENSMUSG000000048583	Igf2

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ENSMUSG00000031543	Ank1
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ENSMUSG00000031654	Cbln1
ENSMUSG00000046707	Csnk2a2
ENSMUSG00000036564	Ndrp4
ENSMUSG00000031767	Nudt7
ENSMUSG00000032174	Icam5
ENSMUSG00000032177	Pde4a
ENSMUSG00000046480	Scn4b
ENSMUSG00000059412	Fxyd2
ENSMUSG00000032307	Ube2q2
ENSMUSG00000066607	Insyn1
ENSMUSG00000032297	Celf6
ENSMUSG00000032238	Rora
ENSMUSG00000032368	Zic1
ENSMUSG00000046402	Rbp1
ENSMUSG00000032475	Nck1
ENSMUSG00000062296	Trank1
ENSMUSG00000039163	Cmc1
ENSMUSG00000019775	Rgs17
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ENSMUSG00000078453	Abrac1
ENSMUSG00000019785	Clvs2
ENSMUSG00000038332	Sesn1
ENSMUSG00000020098	Pcbd1
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ENSMUSG00000049422	Chchd10
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ENSMUSG00000019960	Dusp6
ENSMUSG00000019966	Kitl
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ENSMUSG00000052302	Tbc1d30
ENSMUSG00000044071	Fam19a2
ENSMUSG00000044005	Gls2
ENSMUSG00000044629	Cnrip1
ENSMUSG00000045671	Spred2

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ENSMUSG000000049892	Rasd1
ENSMUSG000000018470	Kcnab3
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ENSMUSG000000048895	Cdk5r1
ENSMUSG000000018548	Trim37
ENSMUSG000000056158	Car10
ENSMUSG000000017400	Stac2
ENSMUSG000000038255	Neurod2
ENSMUSG000000061718	Ppp1r1b
ENSMUSG000000038020	Rapgef1
ENSMUSG000000046215	Rprml
ENSMUSG000000020599	Rgs9
ENSMUSG000000020810	Cygb
ENSMUSG000000075410	Prcd
ENSMUSG000000057469	E2f6
ENSMUSG000000020580	Rock2
ENSMUSG000000062563	Cys1
ENSMUSG000000020950	Foxg1
ENSMUSG000000021062	Rab15
ENSMUSG000000042700	Sipa1l1
ENSMUSG000000066392	Nrxn3
ENSMUSG000000021007	Spata7
ENSMUSG000000021194	Chga
ENSMUSG000000037904	Ankrd9
ENSMUSG000000033499	Larp4b
ENSMUSG000000036006	Ripor2
ENSMUSG000000035910	Dcdc2a
ENSMUSG000000069255	Dusp22
ENSMUSG000000038546	Ranbp9
ENSMUSG000000021373	Cap2
ENSMUSG000000037989	Wnk2
ENSMUSG000000005583	Mef2c
ENSMUSG000000021708	Rasgrf2
ENSMUSG000000045034	Ankrd34b

ENSMUSG00000021666	Gfm2
ENSMUSG00000021647	Cartpt
ENSMUSG00000021743	Fezf2
ENSMUSG00000054423	Cadps
ENSMUSG00000045201	Lrrc3b
ENSMUSG00000021820	Camk2g
ENSMUSG00000022208	Jph4
ENSMUSG00000022212	Cpne6
ENSMUSG00000022216	Psme1
ENSMUSG00000003469	Phyhip
ENSMUSG00000022099	Dmtn
ENSMUSG00000022103	Gfra2
ENSMUSG00000052584	Serp2
ENSMUSG00000033186	Mzt1
ENSMUSG00000061524	Zic2
ENSMUSG00000022269	March11
ENSMUSG00000022240	Ctnnd2
ENSMUSG00000022285	Ywhaz
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ENSMUSG00000033565	Rbfox2
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ENSMUSG00000052560	Cpne8
ENSMUSG00000045608	Dbx2
ENSMUSG00000029875	Ccdc184
ENSMUSG00000000552	Zfp385a
ENSMUSG00000008658	Rbfox1
ENSMUSG00000043811	Rtn4r
ENSMUSG00000061751	Kalrn
ENSMUSG00000037196	Pacrg
ENSMUSG00000048826	Dact2
ENSMUSG00000044533	Rps2
ENSMUSG00000025739	Gng13
ENSMUSG00000063239	Grm4
ENSMUSG00000050705	2310061I04Rik
ENSMUSG00000003279	Dlgap1
ENSMUSG00000036533	Cdc42ep3
ENSMUSG00000045038	Prkce
ENSMUSG00000063889	Crem
ENSMUSG00000047879	Usp14

ENSMUSG00000036225	Kctd1
ENSMUSG00000056124	B4galt6
ENSMUSG00000042834	Nrep
ENSMUSG00000043991	Pura
ENSMUSG00000044024	Rel2
ENSMUSG00000054477	Kcnn2
ENSMUSG00000005986	Ankrd13d
ENSMUSG00000032946	Rasgrp2
ENSMUSG00000006435	Neurl1a