A STUDY ON AN FMRP-MEDIATED TRANSLATIONAL SWITCH IN THE MGLUR-TRIGGERED TRANSLATION OF ARC AND SYNAPTIC PLASTICITY

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DEDICATION

I devotedly dedicate the first fruits of my labor to and thank my Rock of ages, the Ancient of Days, who changes not, whose compassion fails not, and from whom I draw comfort, protection, and strength on my journey.

To Dr. Kimberly Huber, I am beholden to your selfless and steadfast support, to your nobility to nurture and train students of science, and to your exemplary experiences and expertise that you have endowed me.

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To my parents, Romeo and Lorna, and brother, ‘Nong Jun, I delightedly dedicate this work to your amaranthine affection, boundless benevolence, and chivalric charity; and to the courageous company of family and friends, pedagogues and pupils alike, who graciously traveled alongside me, inspiring this philosophical pilgrimage with your “Tales of best sentence and moost solaas.”

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by

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FARR NIERE, Ph.D.

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The group 1 metabotropic glutamate receptor (mGluR)-stimulated protein synthesis and long-term synaptic depression (mGluR-LTD) are altered in a mouse model of Fragile X Syndrome, *Fmr1* knockout (KO) mouse. *Fmr1* encodes the Fragile X mental retardation protein (FMRP), a dendritic RNA-binding protein that functions, in part, as a translational suppressor. It is unknown if and how FMRP acutely regulates LTD and/or the rapid synthesis of new proteins required for LTD, such as the activity-regulated cytoskeletal-associated protein (Arc). The protein phosphatase PP2A dephosphorylates FMRP, which contributes to the translational activation of some target

mRNAs. Here, I report that PP2A and the dephosphorylation of FMRP at S500 are required for an mGluR-induced, rapid increase in dendritic Arc protein and LTD in rat and mouse hippocampal neurons. In the *Fmr1* KO neurons, basal, dendritic Arc protein levels and mGluR-LTD are enhanced, and the mGluR-triggered Arc synthesis is absent. A lentiviral-mediated expression of the wildtype FMRP in *Fmr1* KO neurons suppresses basal, dendritic Arc levels and mGluR-LTD, and restores the rapid mGluR-triggered Arc synthesis. A phosphomimic of FMRP (S500D) suppresses steady state dendritic Arc levels but does not rescue the mGluR-induced Arc synthesis. A dephosphomimic of FMRP (S500A) neither suppresses the basal, dendritic Arc levels nor supports the mGluR-induced Arc synthesis. Accordingly, expressing the S500D-FMRP in *Fmr1* KO neurons suppresses mGluR-LTD, whereas the S500A-FMRP has no effect. These data support a model whereby a phosphorylated FMRP at S500 functions to suppress the steady state and the mGluR-induced translation of Arc and mGluR-LTD. However, upon mGluR activation of PP2A, FMRP is rapidly dephosphorylated which contributes to the rapid, new synthesis of Arc and mGluR-LTD.

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Niere, F, Wilkerson JE, Huber KM. Evidence for a fragile X mental retardation protein-mediated translational switch in metabotropic glutamate receptor-triggered Arc translation and long-term depression. *J Neurosci* Apr 25; 32(17).

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4EBP – eIF4E-binding protein
aa – Amino acid
A2RE – A2 response element
Aβ – Amyloid β protein
AβPP – Amyloid β precursor protein
ABP/GRIP – AMPAR-binding protein/glutamate receptor-interacting protein
ACSF – Artificial cerebrospinal fluid
AF - AlexaFluor
AGO2 – Argonaute 2 protein
Akt – Protein kinase B
ALS – Amyotrophic lateral sclerosis
AMPAR – α-amino-3-hydroxy-5-methylisoxzaole-4-propionic acid-sensitive receptor
ANOVA – Analysis of variance
Arc/Arg3.1 – Activity-regulated cytoskeletal-associated/activity-regulated gene 3.1 protein homolog
ASD – Austism spectrum disorders
BamHI – *Bacillus amyloliquifaciens* restriction enzyme
bp – base-pair
CA1 – *Cornu Ammonis* region 1 of the hippocampus
CaMK-II – Calcium/calmodulin-dependent protein kinase
CaSR – Calcium-sensing receptor
CB1 – Cannabinoid receptor
CCD – Charge-coupled device
COS7 – Immortalized kidney cells of the African green monkey
CPEB – Cytoplasmic polyadenylation element-binding protein
CpG – Cytosine-phosphodiester bond- guanine
CyFIP – Cytoplasmic FMRP-interacting protein
DAG – 1,2-Diacylglycerol
dFXR – *Drosophila* fragile X-related
DHPG – 3,5-dihydroxyphenylglycine
DIC – Differential interference contrast
Dig - Digoxigenin
DIV – Days *in vitro*
DLGAP3 – discs, large (*Drosophila*) homolog-associated protein 3
DNA – Deoxyribonucleic acid
DSM-IV – Diagnostic and Statistical Manual for Mental Disorders IV
Dsp – Desmoplakin
DTE – Dendritic-targeting element
eCB – Endocannabinoid
EcoRI – *Escherichia coli* restriction enzyme
eEF2 – eukaryotic elongation factor 2
eEF2K – eukaryotic elongation factor 2 kinase
EGTA – Ethylene glycol tetraacetic acid

eIF2 – Eukaryotic initiation factor 2
eIF4E – Eukaryotic initiation factor 4E
EJC – Exon junction complex
EPSP – Excitatory postsynaptic potential
ER – Endoplasmic reticulum
ERK – Extracellular-signal-regulated kinase
EVH – Ena-Vasp homology
fEPSP – Field excitatory postsynaptic potential
FISH – Fluorescence *in situ* hybridization
FMR1 – Fragile X mental retardation 1 gene
FMRP – Fragile X mental retardation protein
FP – Extracellular field potentials
FXR – Fragile X-related
FXR1P – Fragile X-related protein 1
FXR2P – Fragile X-related protein 2
FXS – Fragile X syndrome
FUGW – Flap-ubiquitin promoter-GFP-woodchuck hepatitis virus posttranscriptional regulatory element
G_{α} – G-protein α subunit
GABA – γ-aminobutyric acid
GEF – Guanine nucleotide exchange factor
GDP – Guanosine diphosphate
GFP – Green fluorescent protein
GTP – Guanosine triphosphate
GluA2 – Glutamate AMPA receptor subunit 2
GluN2A – Glutamate NMDA receptor subunit 2A
GluN2B – Glutamate NMDA receptor subunit 2B
Gp 1 mGluR – Group 1 metabotropic glutamate receptor
GPCR – G-protein-coupled receptor
Grin2a – Glutamate receptor, ionotropic, N-methyl-D-aspartate 2a mRNA
HA – hemagglutinin
HCV – Hepatitis C virus
HEK – Human embryonic kidney cell
HeLa – Immortal human cervical cancer cell
HEPES – 4-(2-hydroxyethyl)-1-piperizineethanesulfonic acid
HITS-CLIP – High-throughput sequencing of mRNAs isolated by crosslinking immunoprecipitation
hnRNP – Heterogeneous nuclear ribonucleoprotein
hpr – Human promoter
Hz – Herz
kDa – kilodalton
KpnI – *Klebsiella pneumoniae* restriction enzyme
ID – Intellectual disability
IgG – Immunoglobulin G
iGluR – Ionotropic glutamate receptor

IP_3 – Inositol 1,4,5-trisphosphate
IR-DIC – Infrared illumination differential interference contrast
IVT – *in vitro* translation
KAR – Kainate receptor
kcRNA – kissing-complex ribonucleic acid
kD or kDa – kilodalton
KH – hnRNP K-homology domain
KIF – Kinesin superfamily of proteins
KLC – Kinesin light chain
KO – knock-out
LTD – Long-term depression
LFS – Low-frequency stimulation
LTP – Long-term potentiation
mAChR – muscarinic acetylcholine receptor
MAP1B – Microtubule-associated protein 1B
MAP2 – Microtubule-associated protein 2
MCPG – α-methyl-4-carboxyphenylglycine
MEF2 – Myocyte enhancer factor 2
mEPSC – miniature excitatory postsynaptic current
Met - Methinonine
mGluR – Metabotropic glutamate receptor
$mGlu_{1-5}$ – Metabotropic glutamate receptor subtype
miRNA – MicroRNA
mM – millimole
mOsm - Milliosmole
mRNA – Messenger ribonucleic acid
MPEP – 2-methyl-6-(phenylethynyl)pyridine
MPP – Medial perforant path
mTOR – Mammalian target of rapamycin
mV – Millivolt
NDF – N-terminal domain of FMRP
Ng - Neurogranin
Nlgn3 – Neuroligin 3
NLS – Nuclear localization signal
NMD – Nonsense-mediated RNA decay
NMDA – N-methyl-D-aspartate
NMDAR – N-methyl-D-aspartate-sensitive glutamate receptor
Nog – Noggin
nt – nucleotide
OA – Okadaic acid
PC – Purkinje cell
PEST – Proline (P), glutamate (E), serine (S), threonine (T)-rich region
PDZ – PSD-95/*Drosophila* disc large tumor suppressor/zonula occludens-1 protein
PF – Parallel fiber of the cerebellum
P-FMRP – Phospho-FMRP

PI3K – Phosphatidylinositol 3 kinase
PIP_2 – Phosphatidylinositol 4,5-bisphosphate
PKC – Protein kinase C
PLC – Phospholipase C
PML – Pro-myelocytic leukemia bodies
PP1 – Protein serine/threonine phosphatase type 1
PP2A – Protein serine/threonine phosphatase type 2A
PP2B – Protein serine/threonine protein phosphatase type 2B
PP-LFS – Paired-pulse low-frequency stimulation
PPxxFR – Proline rich motif
pre-miRNA – precursor microRNA
pri-miRNA – primary microRNA
pRSV – Rous sarcoma virus plasmid
PRMT – Protein arginine methyltransferase
PSD-95 – Post-synaptic density of 95 kD
PTP – Protein tyrosine phosphatase
Purα – Purine-rich single-stranded DNA or RNA-binding protein
RGG – Arginine-glycine-glycine-rich region
Rheb – Ras homologue enriched in the brain protein
RISC – RNA-induced silencing complex
RMS – Root mean square
RNA – Ribonucleic acid
rpS6 – Ribosomal protein S6
S6K1 – S6 kinase 1
SAPAP3 – SAP90/PSD-95-associated protein 3
SARE – Synaptic activity-responsive element
SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Shank – SH3 and multiple ankyrin repeat domains protein
SOD1 – Superoxide dismutase 1
SoSLIP – Sod1 stem-loop interacting with FMRP
STEP – Striatal-enriched protein tyrosine phosphatase (STEP)
SUMO – Small ubiquitin-like modifier
SV40 – Simian vacuolating virus 40
TIME-STAMP - time-specific tag for the age measurement of proteins
Tln2 – Talin 2
TMD – Transmembrane domain
TRBP – TAR RNA-binding protein
TSC – Tuberous sclerosis complex
t-RNA – Transfer ribonucleic acid
TTX - Tetrodotoxin
UpF – Up frameshift protein
UTR – Untranslated region
VFD – Venus flytrap domain
VGCC – Voltage-gated calcium channel
VSVG – Vesicular stomatitis virus G envelope

WT – Wildtype
ZBP – Zip-code binding protein

CHAPTER ONE

Introduction

The Fragile X Syndrome is the leading cause of autism and intellectual disability

The Fragile X Syndrome (FXS), though induced by changes in a single gene, *FMR1* (Fragile X Mental Retardation 1), presents a myriad of disorders. A national survey of 1,235 American children, who are over six years old with FXS, accounts that in boys, 96% have been diagnosed with developmental delay or intellectual disability, 46% with autism, and 70% with anxiety (Bailey et al., 2008). This survey also accounts that in girls, 64% exhibit developmental delay, 16% autism, and 56% anxiety. Autism or autism spectrum disorders (ASD), as defined in the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) (APA, 2000), is characterized by impairment in social interaction and communication, and repetitive patterns of behavior. ASD has a high prevalence, affecting one percent or one in every 110 children aged eight years (CDC, 2009). Defined in DSM-IV, intellectual disability (ID) or mental retardation consists of (1) significant below-average intellectual function and (2) significant limitations in adaptive performance all presenting before adulthood (APA, 2000). Another national survey of American children ages 3-17 documents that approximately one percent of this population suffers intellectual disability (Boyle et al., 2011).

Mutations in the *FMR1* gene are the leading cause of ASD and ID. Clinical studies within and without the United States estimate that approximately 2-5% of individuals diagnosed with autism have FXS (Chonchaiya et al., 2009; Estecio et al., 2002; Havlovicova et al., 2002; Reddy, 2005). A large scale study of individuals with

mental retardation in the Netherlands accounts one percent of this population has mutations in the *FMR1* (de Vries et al., 1997). FXS is also a leading cause of ID characterized by moderate ID in males and mild ID in females (Saul and Tarleton, 2011). Because individuals with ASD and ID grow old, the burden resulting from life-long nursing and productivity loss of affected individuals and their immediate caretakers is substantial. For a person suffering ASD, who lives to 65 years, the average cost to society is estimated at $3,000,000; while for an individual with ID, the average estimated cost is $1,000,000 (CDC, 2004; Ganz, 2007; Kogan et al., 2008). Because FXS negatively impacts so many, understanding and unraveling the molecular bases of neuronal disease in FXS is warranted.

FMR1 is the only gene involved in FXS

Research on FXS has made significant strides in characterizing the disease. One finding that has remarkably advanced the study of FXS is the identification of the *FMR1* gene at the previously observed cytogenetic "fragile" or constricted site of the X chromosome (Brown, 1990; Krawczun et al., 1985; Lubs, 1969; Silverman et al., 1983; Verkerk et al., 1991). *FMR1* is located at Xq27.3 and codes for the fragile X mental retardation protein (FMRP), which has been characterized as an RNA-binding protein that is ubiquitously expressed in many cell types (Ashley et al., 1993a; Verheij et al., 1993). Aside from neurological dysfunctions, persons with FXS often present other disorders such as dental crowding and malocclusion, congenital hip displacement, chronic otitis media, and hyperopia and astigmatism. Additional clinical phenotypes observed in individuals suffering FXS include long narrow faces and prominent jaws,

enlarged ears, and machroorchidism after puberty (Chonchaiya et al., 2009; Hersh and Saul, 2011).

The transcriptional silencing of *FMR1* is often the prime culprit in FXS. A variable trinucleotide (CpG) repeat, ranging from 5-44, lies within the 5' untranslated region (UTR) of *FMR1* (Saul and Tarleton, 2011). In FXS, the CpG repeat expands, between several hundred to thousand repeats, triggering methylation of the gene and consequently decreasing or preventing the transcription of *Fmr1* and synthesis of FMRP (Bell et al., 1991; Coffee et al., 1999; Heitz et al., 1991; Hornstra et al., 1993; Oberle et al., 1991; Pieretti et al., 1991; Sutcliffe et al., 1992). Aside from CpG expansion repeats, intragenic mutations in *FMR1* such as missense mutations or deletions that lead to the loss-of-function of FMRP also result in FXS (De Boulle et al., 1993; Gedeon et al., 1992; Hirst et al., 1995; Lugenbeel et al., 1995; Meijer et al., 1994; Wohrle et al., 1992). These findings clearly implicate the role of *FMR1* in FXS.

The Fragile X Mental Retardation Protein operates in RNA-associated biology of learning and memory

It is widely accepted that the absence of FMRP owing to the loss-of-function mutations in *FMR1* leads to FXS (Bassell and Warren, 2008). However, it is worth noting that mosaic individuals – persons having both premutations (~55-200 CpG repeats) and full mutations (>200 repeats) – and individuals having partially methylated full mutations can give rise to variable FMRP levels (Saul and Tarleton, 2011; Tassone et al., 1999). Interestingly, the levels of FMRP strongly correlate with ID and physical features – lower levels of FMRP are highly associated with more severe ID, larger ear-

width and prominence, and more extensible joints (Loesch et al., 2003a; Loesch et al., 2002; Loesch et al., 2003b; Tassone et al., 1999). These findings underscore the relevance of FMRP in FXS and cognitive function.

FMRP is an RNA-binding protein that is highly conserved throughout evolution, and the synthesis of its transcript is regulated by the transcription factor AP-2α (Lim et al., 2005a; Lim et al., 2005b). The *Fmr1* transcript has 17 exons, and as a result of exon skipping or the use of alternative splice sites, at least 12 *Fmr1* mRNA isoforms have been isolated so far (Eichler et al., 1993). Expression of the different spliced *Fmr1* mRNA isoforms varies over different developmental periods, tissues and microdomains, and generates different FMRP products ranging from 47-71 kDa that may or may not include domains such as the major phosphorylation site – Ser500 in humans, Ser499 in mouse, and Ser406 in *Drosophila* (Ashley et al., 1993b; Banerjee et al., 2010; Bassell and Warren, 2008; Didiot et al., 2008; Sittler et al., 1996; Xie et al., 2009). The longest isoform of FMRP contains 632 amino acids and several functional regions (Fig. 1). The N-terminal domain of FMRP (NDF, residues 1-200) contains an Agenet domain-like pair (residues 3-110), which bears sequence similarity to the Tudor family domain that can bind to methylated histones, and a nuclear localization signal (NLS, residues 115-150). The NDF can also form dimers and can serve as a binding site for RNA and FMRP-interacting proteins – nuclear FMRP-interacting protein (NUFIP), cytoplasmic FMRP-interacting protein (CYFIP), and fragile X related 1 and 2 proteins (FXR1P and FXR2P) (Adinolfi et al., 2003; Bardoni et al., 1997; Collins et al., 2010; Eberhart et al., 1996; Lacoux et al., 2012; Maurer-Stroh et al., 2003; Wu et al., 2011a). A subcellular fractionation study has demonstrated that FMRP is both present in the nuclear and

cytoplasmic sections, suggesting that FMRP may have nuclear and cytoplasmic functions (Sittler et al., 1996). The NLS of FMRP has moderate activity, and it is speculated that the NDF region interacts with a nuclear component, perhaps through NUFIP, that directs FMRP to the nucleus (Bardoni et al., 2003; Henderson and Eleftheriou, 2000; Sittler et al., 1996).

Like many RNA-binding proteins, FMRP has several regions that have been shown to be important in interacting with RNA and polyribosomes. FMRP contains two hnRNP K-homology domains (KH1 and 2, residues 280-400) and an arginine (R)-glycine (G)-rich region (RGG, residues 526-552) (Siomi et al., 1993). The KH domain, which exists in multiple copies, is a sequence of amino acids (~70 aa) that bind to single-stranded RNA or DNA (Valverde et al., 2008). A substitution mutation in the highly conserved isoleucine (Ile) to asparagine (Asp) at residue 367 (Ile367Asp) in the KH2 domain, which has been shown to associate with a kissing-complex motif in RNAs (kcRNAs), disrupts the binding of FMRP to its associated RNAs; and this disruption in the FMRP-RNA interaction may underlie the FXS pathology in a patient who bears this mutation (Darnell et al., 2005; De Boulle et al., 1993; Musco et al., 1996; Siomi et al., 1994). Unlike the KH2 domain, the RGG region interacts with a secondary structure that is composed of a G-quadruplex in the RNA stem, which is present in some mRNAs that code for synaptic proteins such as the microtubule associated protein 1B (MAP1B), the post-synaptic density of 95 kD (PSD-95), and the amyloid b precursor protein (AβPP) (Darnell et al., 2001; Menon et al., 2008; Todd et al., 2003; Westmark and Malter, 2007; Zalfa et al., 2007). The RGG domain is subject to posttranslational modifications that can modulate its binding to RNAs. The phosphorylation and the methylation of arginine

by the protein arginine methyltransferases (PRMT1, 3, and 4) can decrease the ability of FMRP to bind to the G-quadruplex structure and polyribosomes (Blackwell et al., 2010; Evans et al., 2012; Stetler et al., 2006).

The FXR family – FXR1P, FXR2P and FMRP – contains similar protein domains (NLS, KH1, KH2, NES, and RGG), giving rise to speculations that they have similar or redundant function; and there are several observations that support this idea (Siomi et al., 1995; Valverde et al., 2008; Zhang et al., 1995). Based on a comparative genomic analysis, FMRP, FXR1P, and FXR2P might have arisen from a common ancestral gene (Kirkpatrick et al., 2001). A comparative genomic sequence analysis also reveals that the human FXR1P and FXR2P, but not FMRP, contain a nucleolar targeting signal at the C-terminus. The *Drosophila* homolog, dFXR does not include the nucleolar targeting sequence (Kirkpatrick et al., 2001). While the KH2 domain of FMRP expresses exons 11 and 12, FXR1P, FXR2P, and dFXR do not, implying that FMRP may have a potential specialized function in binding RNAs at the KH2 region (Darnell et al., 2009; Kirkpatrick et al., 2001). FMRP is absent in yeast and *Caenorhabditis elegans* – the simpler eukaryotes – but FXR exists as a single gene, *dfxr*, in *Drosophila melanogaster* (Wan et al., 2000). In humans, *FXR1* and *FXR2* are located in chromosomes 3q.28 and 17p13.1 respectively. FXR1P and FXR2P are important proteins in vertebrates since a reduction or loss of *Fxr1* results in cardiac diseases in mice and zebrafish; and a mouse which lacks *Fmr1* and *Fxr2* displays exaggerated behavioral phenotypes (Mientjes et al., 2004; Spencer et al., 2006; Van't Padje et al., 2009). It is becoming apparent, however, that FMRP, FXR1P, and FXR2P have different roles. One study demonstrates that of the three members of the FXR family, only FMRP rescues synaptic connectivity deficits in

Drosophila, while all three rescue spermatogenesis defects (Coffee et al., 2010). Recent reports on FXR1P account that it regulates brain-specific microRNAs (miR-9 and miR-124) and mRNAs – desmoplakin (*Dsp*) and talin2 (*Tln2*) – that are important for cardiac muscle function (Whitman et al., 2011; Xu et al., 2011). FXR2P, through its interaction with the noggin (*Nog*) mRNA, regulates neurogenesis in the dentate gyrus but not in the subventricular zone (Guo et al., 2011). The redundancy of functional domains in the FXR family members suggests that one can compensate for the other, but it is now becoming evident from these studies that this is not the case as FMRP, FXR1P, and FXR2P function independently. A comparative study of FXR proteins has also determined that each member has different RNA and ribosome binding affinities (Darnell et al., 2009). Nonetheless, these studies establish that FMRP, FXR1P, and FXR2P are integral players in RNA-associated biology, and alterations in their function have severe physiological consequences. Considering that FMRP targets mRNAs that are involved in neuronal function, the disruption of FMRP activity predicates cognitive or learning dysfunction.

Long-term depression serves as a cellular model of learning and memory

The two most studied forms of synaptic plasticity that serve as cellular models of learning and memory, and experience-dependent plasticity – long-term potentiation (LTP) and long-term depression (LTD) – require long-lasting synaptic changes that rely on the synthesis of new proteins. LTP and LTD occur in many different brain regions and synaptic types, and it is now commonly accepted that the expression of LTP arises from the increased number or insertion of AMPARs (α-amino-3-hydroxy-5-

methylisoxazole-4-propionic acid-sensitive glutamate receptors) in the postsynaptic bouton, while LTD results from the removal of AMPARs (Malenka and Bear, 2004). However, changes in expression of other receptors such as NMDARs (N-methyl-D-aspartate-sensitive glutamate receptors), KARs (kainate receptors), and mGluRs (metabotropic glutamate receptors) can mediate LTD (Collingridge et al., 2010; Harney et al., 2006; Jin et al., 2007; Malenka and Bear, 2004; Park et al., 2006). LTD, unlike LTP, is characterized by a reduction in synaptic strength, such that a stimulation paradigm leads to a decrease in synaptic response as measured either by extracellular or whole-cell recording. Furthermore, LTD (also known as heterosynaptic LTD) can occur in the absence of presynaptic activation (Lynch et al., 1977).

There are several types of LTD that are characterized by the manner in which they are induced. Among these are: (1) NMDAR-LTD that requires activation of the NMDAR by a low-frequency stimulation (LFS, 0.5-5 Hz) or a brief application of NMDA; (2) mGluR-LTD that relies on the activation of mGluRs by a paired-pulse low-frequency stimulation (PP-LFS, 50 ms interstimulus interval pulses at 1 Hz for 15-20 minutes) or application of 3,5-dihydroxyphenylglycine (DHPG) or α-methyl-4-carboxyphenylglyice (MCPG); and (3) mAChR-LTD (muscarinic acetylcholine receptor-dependent LTD) that employs the activation of mAChRs by PP-LFS or the application of carbamoylcholine chloride (CCh) (Bashir et al., 1993; Bolshakov and Siegelbaum, 1994; Collingridge et al., 1983; Collingridge et al., 2010; Dudek and Bear, 1992; Huber et al., 2000; Ito et al., 1982; Kemp and Bashir, 1999; Lee et al., 1998; Malenka and Bear, 2004; Palmer et al., 1997; Volk et al., 2007). With the reproducibility of and the advent of selective inhibitors of LTD, the functional relevance of LTD in

learning and memory is becoming evident (Collingridge et al., 2010; Kemp and Manahan-Vaughan, 2007; Malenka and Bear, 2004; Massey and Bashir, 2007). Long-term depression truly serves as a cellular model of learning and memory as disruption of LTD, through manipulations of molecules (such as calcineurin, protein serine/threonine phosphatase 2A (PP2A), NMDA subunit (GluN2B) or Ca^{2+}/calmodulin-dependent protein kinase (CaMK-II and IV)) or pathways (such as the endocytosis of GluA2-containing AMPARs) that are involved in the expression of LTD, can upset learning-dependent behaviors that include working and episodic memory, reversal learning, fear extinction, vestibulo-ocular reflex, and spatial representation (Duffy et al., 2008; Hansel et al., 2006; Ho et al., 2000; Nicholls et al., 2008; Rochefort et al., 2011; Zeng et al., 2001).

The multifaceted mechanisms of the metabotropic glutamate receptor-mediated LTD underlie neuronal function and network

The metabotropic glutamate receptors are large proteins (~850-1200 aa) that contain an extracellular, hydrophilic N-terminal domain; a core of seven hydrophobic regions that represent the seven-transmembrane domain (TMD) and is characteristic of G-protein-coupled receptors (GPCRs); and a large hydrophilic C-terminus. Although mGluRs share very little homology to other GPCRs, members within the same group of mGluRs have high sequence homology (Hollmann and Heinemann, 1994). Class C GPCRs, to which the mGluRs, the Ca^{2+}-sensing receptor (CaSR) and the γ-aminobutyric acid type B ($GABA_B$) receptor belong, are characterized by a large, extracellular domain called the Venus Flytrap domain (VFD), which contains the ligand binding domain.

Generally, stimulation of a GPCR, which serves as a guanine nucleotide exchange factor (GEF), activates the intracellular heterotrimeric G-proteins through the exchange of a bound guanosine diphosphate (GDP) to a guanosine triphosphate (GTP). Upon GTP binding to a GPCR, the heterotrimeric G-protein dissociates to α and $\beta\gamma$ subunits that then can activate their respective effectors. The C-terminal domain is the least conserved region; it is highly variable and suspect to alternative splicing. The group 1 mGluR (Gp 1 mGluR), which is composed of $mGlu_1$ and $mGlu_5$, has several splice variants at the C-terminus – $mGlu_{1b}$ and $mGlu_{1d}$ contain 20 and 26 amino acids, respectively, instead of 318 amino acids that is expressed by $mGlu_{1a}$ (Gregory et al., 2011; Houamed et al., 1991; Mary et al., 1997; Masu et al., 1991; Pin et al., 2003). The C-terminal region of $mGlu_5$ serves as a target for many scaffolding proteins. The Homer protein, through its Ena-Vasp homology (EVH) domain, targets the proline-rich motif (PPxxFR) near the C-terminal tail of $mGlu_1$ and $mGlu_5$ (Pin et al., 2003; Shiraishi-Yamaguchi and Furuichi, 2007). The interaction of mGluRs and Homer proteins is crucial for proper mGluR signaling and for linking mGluRs to other scaffolding proteins, such as Shank, that are essential for normal calcium homeostasis (Mao et al., 2005b; Ronesi and Huber, 2008; Sala et al., 2005). It is well established that the activation of the Gp 1 mGluRs triggers a cascade of events that lead to LTD. However, a high-frequency stimulation (HFS) in the hippocampus promotes LTP in the dentate gyrus that is mediated by the $mGlu_1$ and $mGlu_5$ receptors (Anwyl, 1999, 2009; Bellone et al., 2008; Wu et al., 2008). The Gp 1 mGluR is associated with the activation of the $G_{\alpha q/11}$ family of heterotrimeric G proteins, while group 2 and group 3 mGluRs tend to be linked to the $G_{\alpha i/o}$ and inhibition of adenylyl cyclases (Gregory et al., 2011; Hollmann and Heinemann, 1994; Tanabe et al.,

1992). Canonically, stimulation of Gp 1 mGluRs activate the phospholipase C (PLC) and leads to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) resulting in the formation of inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylgycerol (DAG). In turn, the generation of IP_3 promotes the release of Ca^{2+} from the intracellular stores such as the endoplasmic reticulum (ER), while DAG activates protein kinase C (PKC). (Conn and Pin, 1997; De Blasi et al., 2001; Houamed et al., 1991; Masu et al., 1991; Nicoletti et al., 1986; Pin et al., 1994; Rose and Konnerth, 2001; Sladeczek et al., 1985; Sugiyama et al., 1987).

Although the activation of any mGluR can potentially trigger synaptic depression, the most common form of mGluR-induced LTD to date is mediated by the Gp 1 mGluR. In the CA1 (*Cornu Ammonis* 1) region of the hippocampus, mGluR-LTD can be generated by stimulating the Gp 1 mGluR alone as demonstrated by the application of DHPG, a Gp 1 mGluR agonist (Huber et al., 2000; Huber et al., 2001; Sacaan et al., 1998; Schoepp et al., 1994). A study has also defined that the activation of either $mGlu_1$ or $mGlu_5$ is sufficient to promote LTD (Volk et al., 2006). Stimulation of the CA3 Schaffer collateral axons to CA1 with paired-pulses of low-frequency stimulation (PP-LFS; 1 Hz, 50 ms interstimulus interval) results in synaptic depression that is commonly observed in adult rodents older than two weeks; however, PP-LFS-LTD relies on both the Gp 1 mGluR and mAChR (Huber et al., 2000; Huber et al., 2001; Kemp and Bashir, 1999; Moult et al., 2008; Nosyreva and Huber, 2006; Volk et al., 2006; Volk et al., 2007). In some brain regions, the generation of mGluR-dependent LTD also requires the activation of other receptors in addition to members of the Gp 1 mGluR. In the perirhinal cortex, a synergistic interaction among Gp 1 and Gp 2 mGluRs and

NMDARs is necessary for LTD; while in the cerebellum, a coincident synaptic activation of the parallel fibers (PF) and $mGlu_1$ activation of the Purkinje cells (PC) are necessary for mGluR-LTD (Cho et al., 2000; Collingridge et al., 2010; Ito et al., 1982; Linden et al., 1991; Luscher and Huber, 2010).

The molecular and signaling mechanisms of an mGluR-triggered endocytosis of the ionotropic glutamate receptor (iGluR), AMPAR, and its consequential synaptic depression are as varied as their respective mGluR-mediated induction and cell types (Fig. 2). In PCs that primarily express $mGlu_1$, synaptic depression relies on PKCα to phosphorylate Ser880 of GluA2 and trigger the internalization of a GluA2-containing AMPAR (Chung et al., 2003; Leitges et al., 2004; Steinberg et al., 2006; Xia et al., 2000). The phosphorylation of GluA2 at Ser880, which lies within the PDZ (PSD-95/*Drosophila* disc large tumor suppressor/zonula occludens-1 protein) binding domain, disrupts the interaction of GluA2 with the ABP/GRIP (AMPAR binding protein/glutamate receptor-interacting protein), which is a PDZ-containing scaffold protein (Chung et al., 2003; Takamiya et al., 2008). This disruption is believed to promote the exclusion or unfastening of phosphorylated GluA2-containing AMPARs from the PSD by translocating them through lateral diffusion to the extrasynaptic and clathrin-coat-rich endocytic zones where these receptors can be internalized (Blanpied et al., 2002; Lu et al., 2007). In the cerebellum, the expression of mGluR-LTD relies on an intracellular Ca^{2+} increase that is mediated through the voltage-gated Ca^{2+} channels (VGCC) (Collingridge et al., 2010; Gladding et al., 2009). Although the activation of the Gp 1 mGluR is coupled to the production of IP_3 that promotes Ca^{2+} release from the intracellular stores, mGluR-LTD in the hippocampus is independent of Ca^{2+} and PKC

(Fitzjohn et al., 2001; Schnabel et al., 1999). The removal of AMPARs from the surface that is triggered by DHPG application, however, relies on the dephosphorylation of GluA2 by the tyrosine phosphatase, striatal-enriched protein tyrosine phosphatase (STEP) (Moult et al., 2006; Zhang et al., 2008). The application of DHPG in hippocampal slices increases STEP, and the mGluR-induced translation of STEP is required for the endocytosis of AMPARs. Currently, it is unknown which tyrosine residue of GluA2 is the target of STEP. Aside from STEP, the microtubule-associated protein 1B (MAP1B), the activity-regulated cytoskeletal-associated protein/activity regulated gene 3.1 (Arc/Arg3.1), and the amyloid β protein (Aβ) have been implicated in mGluR-LTD. Similar to STEP, DHPG treatment of hippocampal slices increases MAP1B, a cytoskeleton protein that is present throughout neuronal development, Arc, which has been shown to associate with the endocytic machinery, and Aβ precursor protein (AβPP) (Chowdhury et al., 2006; Davidkova and Carroll, 2007; Park et al., 2008; Riederer, 2007; Waung et al., 2008; Westmark and Malter, 2007). The mGluR-induced increase in MAP1B is thought to support AMPAR internalization, whereby MAP1B sequesters GRIP and eventually hindering surface AMPAR expression. It is important to note that the application of NMDA also results in the increased expression of MAP1B; however, the stimulation-induced increase in MAP1B expression only seems to regulate in Gp 1 mGluR-dependent removal of surface AMPARs (Davidkova and Carroll, 2007). Arc, which is implicated in numerous neuronal functions, is likewise an appealing LTD-associated protein because of its interaction with dynamin and endophilin – proteins that are involved in the endocytosis of surface receptors (Chowdhury et al., 2006; Conner and Schmid, 2003; Praefcke and McMahon, 2004). An earlier study on Aβ has shown that

Aβ-induced synaptic depression occludes DHPG-induced LTD and relies on AMPAR endocytosis (Hsieh et al., 2006). A subsequent study has determined that Aβ induces LTD by attenuating glutamate reuptake, consequently desensitizing glutamate receptors, and ultimately leading to synaptic depression (Li et al., 2009). Taken together, these findings underscore the complexity of an mGluR-mediated synaptic depression. It is also worth noting that *STEP, MAP1B, Arc*, and *AβPP* are mRNA-targets of FMRP and that FMRP regulates their translation upon neuronal activation (Antar et al., 2005; Darnell et al., 2001; Darnell et al., 2011; Goebel-Goody et al., 2012; Park et al., 2006; Westmark and Malter, 2007; Zalfa et al., 2003).

The induction of mGluR-LTD is primarily regarded as a postsynaptic phenomenon, such that the signaling mechanisms induced by the stimulation of mGluRs converge on decreasing the surface expression AMPARs or altering the half-life of glutamate at the synaptic cleft. Substantial evidence, however, exists that an mGluR-induced depression can attenuate synaptic strength through presynaptic mechanisms by altering the release of glutamate, a neurotransmitter that acts on mGluRs, NMDARs, AMPARs, and KARs (Collingridge et al., 2010; Feinmark et al., 2003; Lovinger, 2008). One example of a Gp 1 mGluR-mediated synaptic depression that relies on presynaptic alteration is the endocannabinoid-mediated LTD (eCB-LTD). The activation of Gp 1 mGluRs and L-type calcium channels in the striatum results in a long-term depression that is mediated by the release of eCB from the postsynaptic compartment (Adermark and Lovinger, 2007; Gerdeman et al., 2002; Kreitzer and Malenka, 2005; Ronesi et al., 2004; Shen et al., 2008; Sung et al., 2001). The resultant eCB binds to presynaptic, GPCR($G_{\alpha i/o}$) cannabinoid receptors (CB1) causing a decrease in neurotransmitter release

(Lovinger, 2008). In the basal ganglia, the indirect pathway inhibits extraneous actions or movements (Mink, 1996; Nambu, 2004). Interestingly in a disease model of Parkinson's disease, eCB-LTD is absent in the indirect pathway, and restoring the eCB-LTD in the indirect pathway through inhibition of eCB degradation rescues muscle rigidity or catalepsy, a pronounced motor deficit observed in Parkinson's disease (Kreitzer and Malenka, 2007).

These discoveries collectively establish that the Gp 1 mGluR-mediated LTD occurs in many circuits and essentially participates in physiological, neuronal processes, because the dysregulation of mGluR-LTD results in pathological conditions. Studies on mGluR-LTD have likewise revealed that FXS presents many mGluR-associated dysfunctions. These physiologically relevant findings are discussed hereafter.

Metabotropic glutamate receptor-dependent events are altered in FXS

A mouse model of FXS (*Fmr1* KO), which was generated by inserting a neomycin cassette into exon 5 to prevent the synthesis of a functional FMRP, presents deficits in reversal learning, increased exploratory behavior, and macroorchidism (Bakker et al., 1994). Additionally, the *Fmr1* KO mouse displays disrupted mGluR signaling. One key finding that drew interest in deciphering the connection between Gp 1 mGluR and FMRP is the discovery that the *Fmr1* KO mouse expresses an exaggerated mGluR-LTD in the hippocampus and that the mGluR-LTD no longer relies on *de novo* protein synthesis (Huber et al., 2002; Nosyreva and Huber, 2006). In wildtype mice, mGluR-LTD that is induced synaptically or chemically by DHPG can be blocked by using translation inhibitors – anisomycin or cyclohexamide – or an mRNA cap analog

(m^7GpppG, where m is a methyl group) that competes with endogenous capped mRNAs for the eukaryotic initiation factor 4E (eIF4E), an essential component in cap-dependent translation (Huber et al., 2000). Recent findings have also established that the activity of Gp 1 mGluR in the *Fmr1* KO is enhanced causing altered neocortical activity or longer UP-states (Hays et al., 2011). Coincidentally, the mGluR-Homer interaction is disrupted in *Fmr1* KO mice, resulting in aberrant $mGlu_5$ activity that leads to elevated basal translation, neocortical hyperexcitability, and audiogenic seizures. Interestingly, by deleting Homer1a, which competes with Homer1 for the PPxxFR motif of $mGlu_5$, mGluR-Homer association is corrected as well as the aforementioned abnormal events (Ronesi et al., 2012).

FMRP functions in mGluR-associated translation

The regulation of mRNA translation can be exerted at the initiation or elongation stage. Summarily, translation initiation in eukaryotes requires the 43S ribosomal preinitiation complex formation, followed by the binding of the 43S ribosomal complex to the mRNA, and concluding with the addition of the 60S ribosomal subunit to form the 80S ribosomal complex (Costa-Mattioli et al., 2009). The 43S ribosomal preinitiation complex, which is composed of the eukaryotic initiation factor 2 (eIF2), GTP, a transfer RNA that is charged with methionine (Met-$tRNA_i^{Met}$), and a 40S ribosomal subunit, binds to the 5'-cap structure (m^7GpppX, where X is any nucleotide) of an mRNA. This cap-dependent translation also requires eIF4F, which consists the cap binding protein, eIF4E, eIF4A, and eIF4G1 or eIF4G2. Once the 43S is recruited to the 5'-cap, it travels along the 5'UTR until it reaches the initiation codon where the 60S subunit binds, forming the

translation machinery complex. After completion of the initiation complex, the elongation factors are mobilized to participate in the synthesis of proteins. The phosphorylation state of the eIF4E binding proteins (4E-BPs) regulates the translation initiation by competing with eIF4G for eIF4E binding. A hypophosphorylated 4E-BP binds to eIF4E with high affinity thereby repressing translation, while a hyperphosphorylated 4E-BP binds with less affinity, thereby allowing the eIF4F complex to form and subsequent translation to occur (Haghighat et al., 1995; Lin et al., 1994; Pause et al., 1994). The mammalian target of rapamycin (mTOR) is the major kinase that phosphorylates 4E-BP, and mTOR lies downstream of the phosphatidylinositol-3 kinase (PI3K) pathway (Hay and Sonenberg, 2004; Hou and Klann, 2004). Intriguingly, the activation of Gp 1 mGluR stimulates the PI3K pathway.

The influence of mTOR on translation-dependent synaptic plasticity is evident. Pretreatment of hippocampal slices with an mTOR inhibitor, rapamycin, blocks the DHPG-induced LTD (Hou and Klann, 2004). The tuberous sclerosis complex (TSC), which is composed of TSC1 (hamartin) and TSC2 (tuberin) and lies upstream of mTOR, inhibits mTOR activity through the small G protein, Ras homologue enriched in the brain protein (Rheb) (Kwiatkowski and Manning, 2005). In light of TSC as an mTOR inhibitor, a genetic reduction of *Tsc2* in the $Tsc2^{+/-}$ mouse displays elevated mTOR activity, attenuated mGluR-LTD, and surprisingly reduced basal protein synthesis (Auerbach et al., 2011). In the *Fmr1* KO mouse, mTOR activity is likewise elevated; however, basal protein synthesis is heightened and mGluR-LTD is enhanced (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006; Ronesi et al., 2012; Sharma et al., 2010). It is bewildering that an exaggerated mTOR activity causes opposing aberrant

phenotypes. This phenomenon can be explained if an effector of mTOR regulated FMRP function, which seems to be the case for the p70 ribosomal protein S6 kinase 1 (S6K1) that functions as the major kinase of FMRP (Costa-Mattioli et al., 2009; Narayanan et al., 2008b). FMRP is considered to be a translational regulator because it associates with translating or elongating polyribosomes in an mRNA dependent manner (Ceman et al., 2003; Darnell et al., 2011; Eberhart et al., 1996; Feng et al., 1997; Khandjian et al., 1996; Stefani et al., 2004). S6K1 also regulates the activity of the ribosomal protein S6 (rpS6), which is a vital component of the 40S ribosomal subunit (Meyuhas, 2008). Because numerous studies support that the phosphorylation of FMRP represses the translation of its associated mRNAs, mTOR, through S6K1, can regulate translation by modulating the phosphorylation of FMRP (Ceman et al., 2003; Coffee et al., 2011; Narayanan et al., 2007). These findings establish that mTOR is indeed a major modulator of protein synthesis as it can influence translation at the initiation stage and, through FMRP, modulate the elongation step as well.

Much like the initiation stage, the elongation phase of translation is subject to intricate regulation. The eukaryotic elongation factor 2 kinase (eEF2K) has gained attention as a key regulatory molecule of the elongation step as its activity is modulated by neuronal activity (Costa-Mattioli et al., 2009; Ryazanov et al., 1988; Sutton et al., 2007). The phosphorylation of eEF2, a substrate of eEF2K, has been empirically determined to attenuate global translational and promote local translation of "special" mRNAs (Chotiner et al., 2003; Davidkova and Carroll, 2007; Marin et al., 1997; Park et al., 2008). The observations that the mGluR-dependent synthesis of Arc and LTD are impaired in the *eEF2K* KO mice, and that a knock-down of *eEF2K* blocks the DHPG-

induced MAP1B translation all suggest that the eEF2K and its substrates are essential in mGluR-mediated processes (Davidkova and Carroll, 2007; Park et al., 2008). An interesting finding from the *eEF2K* KO study is that the application of cycloheximide, a translation inhibitor, at a low dose (50-100 nM) induces Arc translation and restores DHPG-LTD, hearkening to the idea that some form of translational inhibition may be necessary to promote the synthesis of certain proteins. These discoveries altogether posit that (1) the translation of activity-dependent synaptic proteins are generally suppressed during normal activity – perhaps through mRNA competition for initiation factors – only to be selectively upregulated upon synaptic stimulation; (2) the inhibition of general translation serves as a regulatory mechanism to ensure the synthesis of activity-dependent synaptic proteins upon synaptic activation; (3) the role of eEF2K in translation inhibition at the elongation step functions to provide a rapid and flexible response upon the commencement (within five minutes after activation) and termination of synaptic activation (Brendler et al., 1981; Park et al., 2008; Scheetz et al., 2000; Walden et al., 1981).

Because earlier studies demonstrated that FMRP associated with mRNAs and the translation machinery, work in understanding the role of FMRP as a translational regulator gained interest (Eberhart et al., 1996; Feng et al., 1997; Khandjian et al., 1996). Subsequent investigations on FMRP offered evidence that the protein primarily functions as a translational suppressor since animal models that lacked FMRP expressed elevated basal translation of its target-mRNAs such as *Map1b*, *αCamkII*, *Aβpp* – syntheses of their protein products are induced by mGluR activation – and the SH3 and multiple ankyrin repeat domains protein (*Shank 1*) (Hou et al., 2006; Lu et al., 2004; Schutt et al., 2009;

Westmark and Malter, 2007). Utilizing phosphorylation site mutants at Ser499 of the murine FMRP, which did not affect the association of FMRP with mRNAs, determined that the phosphomimetic form, S499D-FMRP (where serine was changed to aspartic acid), associated with stalled or non-translating polyribosomes, while the dephosphomimetic form, S499A-FMRP (where serine was changed to alanine), associated with translating polyribosomes (Ceman et al., 2003). This seminal finding established that phosphorylation at Ser499 affected the role of FMRP in regulating protein synthesis.

Recent work using a high-throughput sequencing of mRNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) technique gave further credence to the role of FMRP as an RNA-binding protein because FMRP was found to associate physically with over 800 mRNAs; and surprisingly, FMRP crosslinked to the coding sequence of more than 66% of its target mRNAs (Darnell et al., 2011). This study also strengthened the relevance of FMRP in ASD as several of the FMRP-target mRNAs such as *Tsc2*, *Shank3*, and *Nlgn3* (neuroligin 3) are believed to be autism candidate genes (Basu et al., 2009; Geschwind, 2011). In addition, 32 of the identified mRNA-targets are associated with the $mGlu_5$ receptor. By using an *in vitro* translation (IVT) system to perform run-off assays with puromycin, a drug that specifically targets actively translating polyribosomes, Darnell and colleagues determined that the FMRP-target mRNA, *Map1B*, had more residual ribosomes after run-off when FMRP was present compared to when a functional FMRP was eliminated. In other words, with FMRP in the IVT, *Map1B* and other target mRNAs contained more stalled ribosomes in comparison to conditions where FMRP was absent or knocked-down or when the KH2-mutant (I304N)

FMRP was present. The inhibitory effect of FMRP on *Map1b* could be relieved by competition using a kissing-complex RNA (kcRNA) ligand, to which FMRP bound with high affinity (Darnell et al., 2005). Furthermore, the synthesis of MAP1B increased in the presence of a kcRNA ligand compared to a mutated kcRNA – to which FMRP had little affinity – evincing that FMRP functions as a translational suppressor since sequestering or relieving FMRP from the ribosomal complex induces protein synthesis. Although it had been shown that the majority of FMRP is phosphorylated basally, Darnell and colleagues, however, did not examine whether a phosphorylated FMRP at Ser500 was involved in ribosomal stalling (Narayanan et al., 2007).

It is also worth noting that some of the FMRP-target mRNAs encode translation initiation factors such as eIF4G I and II (Darnell et al., 2011). These initiation factors are essential components for the formation of the preinitation complex that targets cap-dependent translation (Hinnebusch, 2011). It would be interesting to investigate whether the loss of FMRP results in elevated eIF4G and promotes increased cap-binding in cap-dependent translation. There is evidence that in the absence of FMRP cap-dependent translation of synaptic proteins such as MAP1B and αCaMK-II is elevated. Additional findings suggest that these elevated basal levels are a consequence of increased initiation complex formation through eIF4G or relief of ribosomal stalling (Ronesi et al., 2012). In *Drosophila*, dFXR interacts with eIF4G that is independent of the ribonucleoprotein complex (Papoulas et al., 2010). Although currently there is no evidence documenting the functional significance of dFXR-eIF4G interaction, the authors speculate that the interaction may be important in regulating translation initiation of specific transcripts in early embryos.

The phosphorylation of FMRP phosphorylation is a critical regulatory event in the translation of target-mRNAs

A landmark finding on FMRP function has determined that the phosphorylated and dephosphorylated forms of FMRP at Ser499 accompany contrasting translational states (Ceman et al., 2003). This work demonstrates that a dephosphorylated mimic of FMRP, whereby the serine residue at 499 is mutated to alanine (S499A), associates with translating ribosomes; and an aspartic acid-substituted FMRP (S499D), which mimics a phosphorylated FMRP at Ser499, cofractionates with stalled or non-translating ribosomes. In support of this observation, subsequent independent studies have revealed that a phosphorylated FMRP at Ser500 represses the translation of SAP90/PSD-95 associated protein 3 (SAPAP3, also known as discs, large (*Drosophila*) homolog-assoicated protein 3 (DLGAP3)) and Chickadee, a *Drosophila* homolog of Profilin (Coffee et al., 2011; Narayanan et al., 2007). *Sapap3* and *chickadee* are FMRP-target mRNAs. SAPAP3 is a postsynaptic scaffolding protein, and its absence is linked to obsessive-compulsive disorder, and Chickadee is involved in actin polymerization (Cooley et al., 1992; Welch et al., 2007; Welch et al., 2004).

The work by Narayanan and colleagues establishes that the dephosphorylation of FMRP is essential for a Gp 1 mGluR-induced translation of SAPAP3 (Narayanan et al., 2007). Summarily, the activation of Gp 1 mGluR by DHPG promotes a rapid dephosphorylation of FMRP, within one minute after stimulation, and relieves the translational repression on *Sapap3*; the removal of phosphate group(s) from FMRP is mediated by the protein serine/threonine phosphatase type 2 (PP2A). This work also

accounts that the catalytic subunit of PP2A (PP2Ac) does not only associate with $mGlu_5$, as previously demonstrated, but also interacts solely with FMRP and not with S499A-FMRP, a dephosphomimic form of FMRP (Mao et al., 2005a). Furthermore, by utilizing inhibitors against different neuronal phosphatases (PP1, PP2A, and PP2B), the investigators have determined that PP2A is the major phosphatase of FMRP. With the identification of PP2A as the major phosphatase of FMRP, the authors use okadaic acid, a pharmacological agent that inhibits PP2A at a low concentration (0.5 nM), to demonstrate that the inhibition of PP2A can prevent the dephosphorylation of FMRP and the DHPG-induced synthesis of SAPAP3. Based on these findings and other extant data, a model is proposed whereby the Gp 1 mGluR-triggered translation and resultant changes in neuronal activity such as LTD rely on a rapid dephosphorylation of FMRP by PP2A that then leads to the derepression of FMRP-target mRNAs. Upon removal of a phosphate group at Ser499 in mouse (Ser500 in humans or Ser406 in *Drosophila*) stalled ribosomes on target-mRNAs can proceed with the elongation process. This model is attractive because it accounts for the rapid, Gp 1 mGluR-dependent protein synthesis of FMRP-target mRNAs such as *Arc*, *Aβpp*, and *Map1b* that are all involved in LTD (Davidkova and Carroll, 2007; Park et al., 2008; Waung et al., 2008; Westmark and Malter, 2007). However, there is no direct evidence showing that the rapid translational regulation of FMRP or its dephosphorylation regulates mGluR-LTD. For my dissertation I have examined the influence of the phosphorylation of FMRP in modulating mGluR-LTD, and I have provided evidence that PP2A and the switch of FMRP from a phosphorylated to a dephosphorylated form serve as translational regulators of the mGluR-induced synthesis of Arc in dendrites and synaptic depression.

Although several studies consider FMRP as a repressor of translation because it has been observed that in the absence of FMRP there is elevated expression and FMRP-regulated protein synthesis (e.g. MAP1B, AβPP, and PSD-95) basally, evidence does exist that FMRP functions as a translational activator (Dolen et al., 2007; Hou et al., 2006; Muddashetty et al., 2007; Qin et al., 2011; Qin et al., 2005; Westmark and Malter, 2007). Unlike the aforementioned proteins, the synthesis of the superoxide dismutase 1 (SOD1) protein, which is an antioxidant metalloprotease that facilitates the conversion of toxic superoxides (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2), and implicated in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease, requires FMRP (Bechara et al., 2009; Fukai and Ushio-Fukai, 2011; Murakami et al., 2011; Siddique and Ajroud-Driss, 2011). One study has noted that in the absence of FMRP there is a decreased interaction between *Sod1* and translating ribosomes, and there is also a reduced expression of SOD1 in whole brain lysates, all suggesting that FMRP is essential for the synthesis of SOD1 (Bechara et al., 2009). The authors conclude that FMRP increases the translatability of *Sod1*. They have also discovered that the C-terminal region of FMRP, which houses the RGG domain, interacts with a novel triple stem-loop structure, why they termed SoSLIP (*Sod1* stem-loop interacting with FMRP), unlike the G-quadruplex structure found in other FMRP-target mRNAs (e.g. *Map1b, Aβpp*, and *Psd95*). Bechara and colleagues have determined that the interaction between FMRP and SoSLIP is necessary for SOD1 synthesis as mutations in any of the stem-loop structure attenuates the binding of FMRP to *Sod1* as measured by luciferase expression assays. With these evidences, the authors conclude that FMRP, through its interaction with the SoSLIP motif functions as a translational activator. The role of FMRP in enhancing translation may be

more common than previously thought. A recent report documents reduced expression of synaptic proteins such as GluN2A, MAP1B, and PSD-95 basally in the medial prefrontal and orbitofrontal cortices of the *Fmr1* KO mouse (Krueger et al., 2011). Because FMRP has differential effects on the translation of its target-mRNAs, it would be informative to investigate further whether FMRP influences the synthesis of proteins based on the FMRP-interacting domains of target mRNAs (G-quadruplex vs. SoSLIP motifs) or on the brain regions that the target-mRNAs are localized.

FMRP modulates translation through "RISCky" microRNAs

Posttranscriptional regulation by components of the RNA-induced silencing complex (RISC), such as the microRNAs (miRNAs), modulates protein synthesis through an miRNA-directed translational repression and mRNA destabilization (Bartel, 2009). There is evidence, however, that miRNAs do participate in active translation as miR-21 cosediments with polyribosomes in an mRNA-dependent manner; and in one study, FXR1P and Argonaute 2 (AGO2), a component of the miRNA pathway, are recruited by miR-369-3 to upregulate translation in serum-deprived HeLa cells (Maroney et al., 2006; Vasudevan et al., 2007). Aside from AGO2, a number of studies have also documented that FMRP interacts with components of the miRNA pathway to regulate translation. First discovered in *Caenorhabditis elegans*, miRNAs are small, genetically encoded, but non-coding RNAs of approximately 20-22 nucleotides (nt) long, that are processed from primary transcripts (pri-miRNAs) containing between several hundreds to thousands of nucleotides. After additional processing, pri-miRNAs are cleaved to become precursor miRNAs (pre-miRNAs) that contain approximately 80 nt. Dicer, an endonuclease,

together with the TAR RNA-binding protein (TRBP), a double-stranded RNA binding protein, further cut pre-miRNAs to single-stranded miRNAs. The mature miRNA or guide strand is then loaded into the RISC, which is composed of Dicer, TRBP, and AGO2, an endonuclease that cleaves RNA in mammals (Jin et al., 2004; Li and Jin, 2009; Vasudevan et al., 2007).

Pioneering studies in *Drosophila* have determined that dFXR physically interacts with dDicer, dAGO1, and dAGO2 (Caudy et al., 2002; Ishizuka et al., 2002). Subsequent work in vertebrates has likewise demonstrated that FMRP associates with Dicer, AGO1, and AGO2 (or mammalian eIF2C2) (Jin et al., 2004). Recent findings are now establishing the relevance of FMRP and the microRNA machinery and pathway in regulating translation of synaptic proteins, and consequentially synaptic plasticity. Working in concert, FMRP, miR-132, miR-125b, and AGO1 regulate the dendritic spine morphology and the synaptic strength of hippocampal neurons (Edbauer et al., 2010). Specifically, the overexpression of miR-132 induces the formation of mature, stubby spines, while miR-125b overexpression promotes the development or maintenance of immature, filopodia-like spines. Moreover, neurons that overexpress miR-132 have enhanced synaptic strength as measured by increases in the amplitude and frequency of the AMPAR-mediated miniature excitatory postsynaptic currents (mEPSC). Cells that overexpress miR-125b, however, display a reduction in mEPSC amplitude, cuntionally corroborating the observed immature spine morphology. It is important to note that the opposing effects of miR-132 and miR-125b rely on FMRP, because a knock-down of FMRP abolishes the spine phenotypes. Although the mechanism by which miR-132 strengthens the synapse is unknown, miR-125b exerts its effect by downregulating the

expression of GluN2A, an NMDAR subunit, whereby FMRP, miR-125b, and AGO1 synergistically target the 3'UTR of *Grin2a*, the GluN2A mRNA. In support of this finding, Darnell and colleagues have recently determined by using HITS-CLIP that *Grin2a*, is an FMRP-target mRNA (Darnell et al., 2011).

In addition to regulating the translation of GluN2A, FMRP and components of the miRNA pathway also affect the synthesis of PSD-95, another integral synaptic protein involved in defining the synaptic architecture and function (Bhattacharyya et al., 2009; Muddashetty et al., 2011; Xu et al., 2008). FMRP, miR-125a, and AGO2 cooperatively regulate Gp 1 mGluR-induced translation of PSD-95 (Muddashetty et al., 2011). The authors propose that during basal condition the phosphorylated form of FMRP recruits AGO2 and miR-125a to suppress the translation of PSD-95. Upon DHPG treatment, FMRP is dephosphorylated resulting in the destabilization of the AGO2-miR-125a complex with *Psd95* mRNA, and consequently promoting the synthesis of PSD-95. It is unclear, and it will be informative to examine, if the AGO2-miR125a complex with FMRP promotes scission or degradation of *PSD-95*.

The expression of several miRNAs is regulated by activity-dependent transcription factors, such as the myocyte enhancer factor 2 (MEF2) (Fiore et al., 2009). MEF2 has previously been shown to regulate activity-dependent synapse development by promoting the transcription of genes whose protein products inhibit synapse number (Flavell et al., 2006). In one study, the activity-dependent stimulation of MEF2 by potassium chloride (KCl), which depolarizes neurons, facilitates the expression of miR-134 (Fiore et al., 2009). Interestingly, miR-134 antagonizes the translation of Pumillo, a repressor of protein synthesis, thereby fostering dendritogenesis. The finding that MEF2

regulates a cluster of miRNAs is truly fascinating in light of a recent study, which demonstrates that a MEF2-induced synapse elimination requires FMRP (Pfeiffer et al., 2010). As FMRP interacts with miRNAs and other components of RISC, it is tantalizing to examine if MEF2 and FMRP work in conjuction through miRNAs to regulate synaptic plasticity. It will also be informative to test whether the process of synapse elimination is only mediated through a MEF2-FMRP interaction, since a MEF2-induced expression of miR-134 promotes the growth of dendrites

The phosphorylation state of FMRP, as previously demonstrated, influences the translation of its target-mRNAs (Ceman et al., 2003; Narayanan et al., 2007; Narayanan et al., 2008b). One study has observed that the phosphorylation of FMRP influences the association of FMRP with Dicer, a component of the microRNA machinery, inviting the speculation that the FMRP-Dicer interaction may serve as another regulatory mechanism in the translation of FMRP-associated mRNAs (Cheever and Ceman, 2009). Biochemical evidence demonstrates that the immunoprecipated, phosphorylated FMRP at Ser499 (P-FMRP) interacts with pre-miRNA and not with Dicer; however FMRP that is immunoprecipitated with an antibody that recognizes both the phospho- and dephosphorylated FMRP associates with Dicer but not with pre-miRNA. These findings are truly intriguing as they generate several questions regarding the role of FMRP, its phosphorylation, and Dicer in translation regulation. Does the apparent FMRP-Dicer interaction suggest that Dicer plays a role in the translation of FMRP-target mRNAs since Dicer does not associate with P-FMRP? Does P-FMRP sequester pre-microRNAs from Dicer? Because Dicer functions to reduce pre-miRNA to miRNA, does the phosphorylation of FMRP influence miRNA production, which can affect the translation

of miRNA-targeted mRNAs? Because Dicer facilitates the formation of RISC, is the FMRP-Dicer association a mechanism that ensures the scission of FMRP-target mRNAs after translation, thereby preventing runaway protein synthesis? Additional studies are indeed warranted to understand the function of this novel FMRP-Dicer interaction. Since components of RISC (e.g., Dicer, Ago2, and microRNA) are present at the synapse, dissecting the potential role of FMRP and its association with the microRNA pathway and machinery seems relevant in resolving synaptic disorders that are associated with FXS (Lugli et al., 2005; Lugli et al., 2008).

Deviant mGluR-dependent neuronal activity plagues FXS

A national survey of 1,394 (1090 males and 304 females) individuals with FXS has determined that epileptic seizures occurred in 14% males and 6% females and that seizures often happened in the developmental ages of 4-10 years (Berry-Kravis et al., 2010). Another survey of parents with children who express the full mutation accounts that 18% males (n = 976) and 7% females (n = 259) over six years old have had seizures (Bailey et al., 2008). These findings substantiate the relevance of FMRP in impaired neuronal activity as epileptic seizure is defined as "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain" (Fisher et al., 2005). One proposed reason for the occurrence of epileptic seizures in FXS is an impaired GABAergic system and circuit that promotes an imbalance between excitatory and inhibitory transmissions (Brooks-Kayal, 2011; Paluszkiewicz et al., 2011).

The activation of Gp 1 mGluR by DHPG in the hippocampus can induce an epileptogenic response that relies on translation, while synaptic stimulation does not

(Bianchi et al., 2009; Chuang et al., 2001; Chuang et al., 2005; Zhao et al., 2011). However in the *Fmr1* KO mouse, the synaptic release of glutamate is sufficient to generate an epileptogenic response, indicating that the absence of FMRP influences altered Gp 1 mGluR activity (Chuang et al., 2005). Because there are several mGluR-associated neuronal activities that are altered in the *Fmr1* KO, "the mGluR theory of fragile X mental retardation" has been proposed – overactive Gp 1 mGluR can drive its downstream signaling pathways that contribute to the symptoms observed in FXS (Bear et al., 2004). The collective work investigating the different neuronal activities in the *Fmr1* KO mouse has strengthened the pertinence of Gp 1 mGluR dysfunction in FXS. These studies have identified aberrant neuronal network events that can be linked to mGluRs. Among these are longer synchronous neuronal activity or UP states in layers four and five of the neocortex; enhanced and translation-independent mGluR-LTD; and higher incidence of audiogenic seizures (Hays et al., 2011; Huber et al., 2002; Nosyreva and Huber, 2006; Ronesi et al., 2012; Yan et al., 2005). Interestingly, dampening the activity of Gp 1 mGluR can rescue many of these disorders. Genetic reduction of $mGlu_5$ or a pharmacological inhibition of $mGlu_5$ activity with 2-methly-6-(phenylethynyl)pyridine (MPEP) can significantly reduce the duration of the UP-states and the incidence of audiogenic seizures (Dolen et al., 2007; Hays et al., 2011; Thomas et al., 2012; Yan et al., 2005). In the *Fmr1* KO, reduction of Homer1a, which disrupts the $mGlu_5$-Homer interactions necessary for proper $mGlu_5$-mediated signaling, can likewise diminish the anomalous UP-states and audiogenic seizures, pronouncing that an altered Gp 1 mGluR activity may lie at the heart of FXS (Ronesi et al., 2012).

It is worth noting that manipulations on Gp 1 mGluR and/or its effectors that lead to a reduction of the enhanced translation can rescue the exaggerated mGluR-LTD (Dolen et al., 2007). The enhanced mGluR-mediated LTD in the *Fmr1* KO can likewise be abrogated if the mGluR-LTD proteins, whose mRNAs are FMRP-targets, are reduced, FMRP itself is expressed, lithium is introduced or Gp 2 mGluR is inhibited (Choi et al., 2011; Park et al., 2008; Westmark et al., 2011). These studies strongly underscore that the exaggerated translation is at the core of this synaptic plasticity dysfunction since the reduction of LTD-associated proteins (e.g. AβPP and Arc), the attenuation of translation by lithium through the PI3K-Akt pathway or a decrease in IP3 production can mitigate the enhanced mGluR-LTD in the *Fmr1* KO animals (Choi et al., 2011; Liu et al., 2012). The effect of Gp 2 mGluR antagonism in translation-dependent mGluR-LTD is difficult to interpret since LY341495, a Gp 2 mGluR antagonist, enhances DHPG-LTD in the wildtype while attenuating it in the *Fmr1* KO mouse. Furthermore in the hippocampus, Gp 2 mGluR is predominantly expressed in the perisynaptic region of presynaptic terminals (Cosgrove et al., 2011). The authors have reasoned that the effect of Gp 2 mGluR antagonism may be mediated at the level of transcriptional regulation because the activation of Gp 2 mGluR represses cyclic AMP (cAMP) production through the Gp 2 mGluR-coupled $G_{i\alpha}$ subunit. As it is currently unknown the mechanism by which LY341495 mediates DHPG-LTD, It would there be elucidative to examine how LY341495 expresses LTD or whether the LY341495-induced attenuation of LTD is attained by reducing the endocytosis of AMPARs in the *Fmr1* KO

The activity-regulated cytoskeletal-associated protein arcs FMRP and mGluR-LTD

The activity-regulated cytoskeletal-associated protein/activity regulated gene 3.1 protein homolog (Arc/Arg3.1) has garnered heightened attention since its discovery in 1995. The interest stems from the participation of Arc in several functionally-relevant neuronal events such that alterations in Arc level adversely affect neuronal, cognitive, and behavioral performance (Greer et al., 2010; McCurry et al., 2010; Park et al., 2008; Plath et al., 2006; Waung et al., 2008). Because Arc is upregulated during neuronal processes linked to synaptic plasticity, dissecting the role of Arc in learning and memory-associated events, like mGluR-LTD, is profitable (Park et al., 2008; Rodriguez et al., 2005; Waung et al., 2008; Ying et al., 2002).

The attractiveness of Arc in mGluR-LTD, which is manifested as a consequence of surface AMPAR reduction, lies in the ability of Arc to interact with dynamin and endophilin 2 and 3, proteins that are involved in the endocytosis of AMPARs (Fig. 3) (Chowdhury et al., 2006; Collingridge et al., 2010; Gladding et al., 2009; Luscher and Huber, 2010). Arc is a critical molecule in AMPAR endocytosis because the expression of Arc alone, and not of endophilin 3 or an Arc construct lacking the endophilin binding domain, promotes the removal of AMPARs from the membrane, implying that Arc is essential in facilitating or initiating endocytosis (Chowdhury et al., 2006). Furthermore, Arc seemingly targets AMPARs preferentially since expressing Arc in hippocampal neurons only affects the miniature and evoked AMPAR-mediated synaptic currents and not the NMDAR-mediated currents or time decay constants (Rial Verde et al., 2006). Stimulation of Gp 1 mGluR by DHPG (50 or 100 μM for five minutes) increases Arc protein within five minutes following drug washout; and the rapid synthesis of Arc is

required for mGluR-LTD (Park et al., 2008; Waung et al., 2008). The reduction of Arc, through short-hairpin RNAs (shRNA) directed against *Arc*, prevents the DHPG-induced internalization of surface AMPARs and depression of mEPSC frequency. Moreover, the genetic deletion of Arc abolishes the DHPG-triggered removal of surface AMPARs and reduces both chemically- and synaptically-induced mGluR-LTD in the hippocampus. Collectively, these findings substantiate the pertinence of Arc in mGluR-LTD.

Intriguingly, high-frequency stimulation of the medial perforant path (MPP) to the dentate gyrus results in the accumulation of *Arc* along the site of where the stimulating electrode is located (Steward et al., 1998b). This ground-breaking finding displays and suggests that *Arc* is recruited and accumulates at the site of synaptic activity, thereby laying the groundwork of local translation. Additional work has recently established that *Arc* or another mRNA that contains the 3'UTR of *Arc* stably accumulates with polyribosomes at the base of dendritic spines, implying once again that Arc can be swiftly synthesized upon synaptic activity (Dynes and Steward, 2012). The evidence for necessity of Arc in hippocampal-dependent learning is substantial. Exposure of rats to a novel environment rapidly induces *Arc* transcription within five minutes after the exposure (Guzowski et al., 1999). Subsequent work has established the requirement for early Arc synthesis in long-term synaptic plasticity as the inhibition of Arc synthesis by oligonucleotides impairs the maintenance phase of LTP which was assessed by spatial bias during a hidden platform test of a Morris water task. The animals that received the oligonucleotides against *Arc* do not display a spatial bias towards the training location (Guzowski et al., 2000). These findings demonstrate that necessity of Arc translation

within a specified time window and the relevance of Arc in hippocampal-dependent learning.

The rapid translation of synaptic proteins like Arc, AβPP, αCaMK-II and MAP1B upon neuronal activation demands a process whereby these proteins can be swiftly delivered to synaptic sites that require changes (Davidkova and Carroll, 2007; Hou et al., 2006; Park et al., 2008; Waung et al., 2008; Westmark and Malter, 2007). Local translation is one mechanism ensuring a quick delivery of essential molecules that will effect synaptic plasticity. Although previously regarded to occur only in the soma, several lines of evidence substantiate the presence of local translation at active synapses (Sutton and Schuman, 2005). The discoveries that LTP and LTD require translation and that polyribosomes and mRNAs are in the postsynaptic site before and after stimulation all give credence to the idea that dendritically-localized protein synthesis is essential for synaptic plasticity (Frey et al., 1988; Frey and Morris, 1997; Huber et al., 2000; Kang and Schuman, 1996; Ostroff et al., 2002; Steward and Levy, 1982; Steward and Schuman, 2001). Because Gp 1 mGluR activation leads to a rapid increase of Arc in dendrites and internalization of surface AMPARs, it is likely that one mechanism for expressing DHPG-induced LTD relies on local translation as *Arc* mRNA is present in the dendrites and synaptoneurosomes (Bagni et al., 2000; Giorgi et al., 2007; Yin et al., 2002).

Arc, which significantly participates in synaptic plasticity, is highly regulated. Seizures, NMDAR activation, high-frequency stimulation and pharmacological application of BDNF, DHPG or forskolin, which activates the adenylate cyclase, rapidly induce *Arc* transcription, prompting *Arc* to be considered as an immediate-early gene (Bramham et al., 2010; Korb and Finkbeiner, 2011). Surprisingly, AMPAR activation in

hippocampal slices attenuates *Arc* transcription, proposing a negative feedback of *Arc* at the transcription level by heightened network activity (Rao et al., 2006). The *Arc* promoter contains two enhancer elements, namely a synaptic activity-responsive element (SARE) and a "Zeste-like" element, all of which are positively sensitive to synaptic activity (Kawashima et al., 2009; Pintchovski et al., 2009; Waltereit et al., 2001). One hour after transcription and traveling at a rate of 300 μm/hour, *Arc* mRNA can be located in the dendrites (Wallace et al., 1998). Another evidence that bespeaks of *Arc* as a dendritically-targeted mRNA is the presence of two dendritic-targeting elements (DTE) in the 3'UTR of the transcript and the requirement of the proximal DTE (within nt 2162-2513) for the translocation of *Arc* to distal dendrites (Kobayashi et al., 2005). *Arc* mRNA also contains an hnRNP A2 response element (A2RE), which directs RNAs to the dendrites via the A2 pathway (Gao et al., 2008). Interestingly, the A2 trafficking pathway of *Arc* utilizes the RNA granule structure, which contains several RNAs (e.g., *αCamkII* and *neurogranin* (*Ng*)), RNA-binding proteins (e.g., FMRP and Purα – a protein that binds to purine-rich single-stranded DNA or RNA), portions of the translational machinery (e.g., EF1α and ribosomal RNA), and kinesin (Barbarese et al., 1995; Carson et al., 1997; Gao et al., 2008; Kanai et al., 2004; Ma et al., 1995; Ohashi et al., 2002; Zalfa et al., 2003). The inclusion of FMRP and Purα in the RNA granule suggests that the mRNAs within this complex are translationally repressed (Ceman et al., 2003; Gallia et al., 2001; Zalfa et al., 2003). Although FMRP interacts with the kinesin light chain (KLC), which is a cargo-binding component of the KIF5 kinesin superfamily, thus serving as an adaptor protein, FMRP is not essential for the transport of *Arc* mRNA

(Dictenberg et al., 2008). Purα, however, is an essential molecule in the transport of RNA granules as loss of Purα suppresses the dispersion of RNA granules to the dendrites (Kanai et al., 2004). Intriguingly, FMRP is involved in the transport of *αCamkII* but not *Arc* upon DHPG treatment (Dictenberg et al., 2008). It is unknown, however, whether the dephosphorylation of FMRP plays a role in the activity-dependent transport of target-mRNAs. The C-terminal domain of FMRP interacts with KIF5 and is important for the mGluR-induced movement of RNA granules to the dendrites. Competition of the FMRP-KIF5 binding site results in increased dendritic length and number that are reminiscent of the filopodia phenotype of FXS (Dictenberg et al., 2008; Dolen et al., 2007; Grossman et al., 2006).

Perhaps due to the potency of Arc in mediating rapid changes in neuronal activity, *Arc* is subject to nonsense-mediated RNA decay (NMD) (Giorgi et al., 2007). A bioinformatic analysis reveals that *Arc* contains two introns in the 3'UTR, which allows the deposition of the exon junction complex (EJC), which can promote the degradation of the transcript. The presence of the two introns in the 3'UTR makes *Arc* a natural target for NMD. *Arc* strongly interacts with eIF4AIII, a component of EJC, and a reduction of eIF4AIII results in *Arc* enrichment and an increase in Arc expression. A knockdown of the up-frameshift 1 protein (UpF1), a regulator and component of NMD that binds to eIF4AIII, also leads to an increase in *Arc* transcript and protein. Collectively, these findings implicate that the NMD pathway negatively modulates the level of *Arc*. In the mammalian cells, NMD is generally restricted to newly synthesized mRNAs, and NMD preferentially targets CAP-dependent translation (Maquat, 2004). The presence of eIF4AIII on the transcript implies that *Arc* goes through a single pioneer round of protein

synthesis, since the recruitment of UpF1 by eIF4AIII to the EJC at the 3'UTR will destabilize the mRNA upon translation. However, it is unknown if one or more ribosomes are actively carrying-out the process of translation during the pioneer round. Giorgi and colleagues propose that the translation-dependent destabilization of *Arc* allows for a highly regulated synthesis of Arc spatially and/or temporally. Because *Arc* remains stable or is translationally suppressed during transport, *Arc* can accumulate in certain sites, such as the dendrites. Following activity, *Arc* mRNAs enriched in the dendrites can be translated simultaneously and rapidly. A recent finding has documented a specialized microdomain at the base of dendritic spines to which *Arc* docks (Dynes and Steward, 2012). It remains to be tested whether synaptic activity facilitates the movement of *Arc* to the spine or the translation of Arc at the base followed by the partitioning of the newly synthesized Arc to the spine. The nonsense-mediated RNA decay and translational silencing of *Arc* while in transport serve to restrict the synthesis of Arc in a time-dependent manner, which is defined by the pioneer round of translation – Arc synthesis is limited because *Arc* is quickly degraded upon the completion of translation. It is worth noting that only 59% of the *Arc* mRNA granules colocalize with eIF4AIII, indicating that *Arc*, which has a half-life of 47 minutes, may experience several rounds of translation. These NMD-uncoupled *Arc* mRNAs may be responsible for the observed increased Arc protein at 60 minutes after mGluR stimulation (Bramham et al., 2010; Giorgi et al., 2007; Park et al., 2008; Rao et al., 2006). It remains to be explored whether FMRP or its phosphorylation state influences the population of NMD-uncoupled *Arc*.

Arc is present in the dendrites and soma. However, the function of Arc in the soma remains elusive. In COS7 cells, Arc interacts with a novel, apoptosis-associated protein, Amida that is also expressed in the brain (Irie et al., 2000). Amida contains two NLS, and cotransfection of Arc and Amida results in the localization of Arc in the nucleus. Interestingly, the coexpression of Arc and Amida inhibits apoptosis that is induced by the expression of Amida alone. Arc, which contains a coiled-coil domain and a spectrin homology (SH) domain, also interacts with the pro-myelocytic leukemia bodies (PML) in the nucleus via the nuclear spectrin isoform (βSpIVΣ5) (Bloomer et al., 2007; Lyford et al., 1995). PMLs are covalently linked to small ubiquitin-like modifier 1 (SUMO-1) and are thought to be sites where transcriptional regulation occurs (Bloomer et al., 2007; Zhong et al., 1999). Within the C-terminus, Arc contains a PEST sequence – a region that is rich in proline (P), glutamate (E), serine (S), and threonine (T) – indicating that Arc may be targeted for proteasomal degradation (Rao et al., 2006). Arc also has two SUMOylation sites that influence its localization. LTP induction in the dentate gyrus of rats leads to the formation of SUMOylated Arc that fractionates with cytoskeletal components (Bramham et al., 2010). The different domains and protein interactions of Arc reveal the versatility and importance of Arc in cellular, neuronal network, and behavioral functions. With the apparent intimate relationship among Arc, FMRP, and Gp 1 mGluR, elucidating the mechanisms on how these molecular players synergistically modulate synaptic activity in physiological and disease states, like FXS, is worth the work.

MOTIVATION FOR STUDIES

Defects in synaptic plasticity, which underlies learning and memory, contribute to cognitive deficits as observed in individuals with autism and intellectual disability, such as those observed in fragile X syndrome (FXS) – a condition that is caused by loss of function mutations in the *Fmr1* gene. As an RNA-binding molecule, FMRP, the protein product of *Fmr1*, regulates the translation of dendritically localized mRNAs (Bassell and Warren, 2008; Zukin et al., 2009). Local or synaptic translation regulates long-term plasticity; and synaptic plasticity that relies on *de novo* dendritic protein synthesis is abnormal in the *Fmr1* KO mouse, which serves as an animal model for FXS.

The activation of the group 1 mGluR in hippocampal CA1 neurons results in a long-term depression of the excitatory synaptic transmission (mGluR-LTD) that requires a rapid synthesis of dendritic proteins that facilitate the reduction of AMPARs at the surface of the postsynaptic membrane (Waung and Huber, 2009). One proposed function of FMRP is to suppress the translation of its target-mRNAs (Darnell et al., 2011; Napoli et al., 2008); and in the *Fmr1* KO mice, mGluR-LTD is enhanced and independent of new protein synthesis (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006). Therefore, I import and have examined in this work the idea that the loss of FMRP-mediated translational suppression in the *Fmr1* KO mouse leads to an elevated steady state level of "LTD proteins," consequently generating LTD that occurs in the absence of new protein synthesis (Nosyreva and Huber, 2006).

A candidate "LTD protein" is the activity-regulated cytoskeletal-associated protein/activity-regulated gene 3.1 protein homolog (Arc/Arg3.1) (Park et al., 2008;

Waung et al., 2008). Arc is an immediate early gene that is induced in neurons in response to salient experiences (Link et al., 1995; Lyford et al., 1995). The translation of Arc likely contributes to the encoding of these experiences, because Arc is necessary for learning and experience-dependent synaptic and behavioral plasticity (Bramham et al., 2010). Following neuronal activity, *Arc* mRNA is rapidly transported to the dendrites where it is dendritically translated in response to Gp 1 mGluR stimulation (Park et al., 2008; Steward et al., 1998b; Waung et al., 2008). Arc is required for mGluR-LTD in both WT and *Fmr1* KO mice (Park et al., 2008; Waung et al., 2008). During the expression of mGluR-LTD, Arc functions to facilitate the endocytosis of AMPARs (Chowdhury et al., 2006). *Arc* mRNA interacts with FMRP (Iacoangeli et al., 2008b; Zalfa et al., 2003). However, it is unknown if or how FMRP regulates the synthesis of Arc in the dendrites under basal conditions or during plasticity-inducing events.

FMRP is generally considered to function as a translational suppressor; yet recent work demonstrates that the activation of Gp 1 mGluR can trigger a PP2A-mediated dephosphorylation of FMRP, which in turn reprieves the translational suppression of or activates the synthesis of FMRP-target mRNAs (Bassell and Warren, 2008). The phosphorylation of FMRP at a conserved serine (Ser500 in humans) is more associated with stalled polyribosomes. The phosphorylation of FMRP can stabilize a microRNA-Ago2 complex with the *Psd95* mRNA, whereby the phosphorylated FMRP complex suppresses the translation of PSD-95. In contrast, the dephosphorylation of FMRP at Ser500 is associated with translating polyribosomes (Ceman et al., 2003; Muddashetty et al., 2011). The activation of group 1 mGluR in neurons rapidly (< 1 minute) activates PP2A, which dephosphorylates FMRP at Ser500, and stimulates the translation of the

FMRP-target mRNAs, *Sapap3 and Psd95* (Muddashetty et al., 2011; Narayanan et al., 2007; Narayanan et al., 2008a). Numerous studies have been undertaken to elucidate and characterize FMRP and Arc and their function. However, little is known regarding the role of FMRP or its dephosphorylation in the rapid translational regulation of neuronal and synaptic function. In light of this, I have sought to satisfy the functional paucities in the Gp 1 mGluR-mediated synaptic depression. My efforts evince that PP2A and the dephosphorylation of FMRP intimately function as a translational switch in the mGluR-stimulated translation of Arc in the dendrites and LTD.

Figure 1. A schematic representation of FMRP and its domain architecture

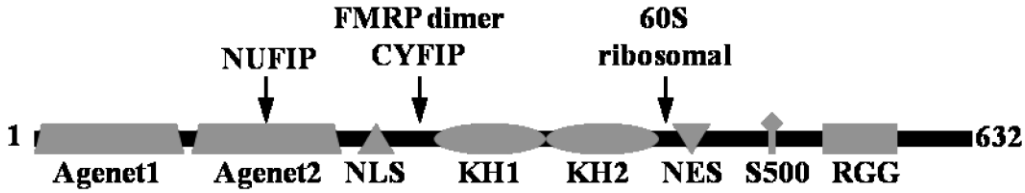

Figure 1. A schematic representation of FMRP and its domain architecture. The full-length human FMRP has approximately 632 amino acids. The top labels are the putative binding or interacting sites for the respective proteins: NUFIP (nuclear FMRP-interacting protein) and CYFIP (cytoplasmic FMRP-interacting protein). The bottom labels indicate the different domains: Tudor homology domain (aa ~3-110), Agenet; nuclear localization signal (~115-150), NLS; hnRNP K-homology binding domains (~280-400), KH1 and KH2; nuclear export signal (~425-441), NES; serine 500, S500; and arginine (R), glycine (G) glycine (G)-rich domains (~526-552), RGG. (Modified from Maurer-Stroh et al., 2003 and Adinolfi et al., 2003)

Figure 2. Mechanisms of translation-dependent mGluR-LTD

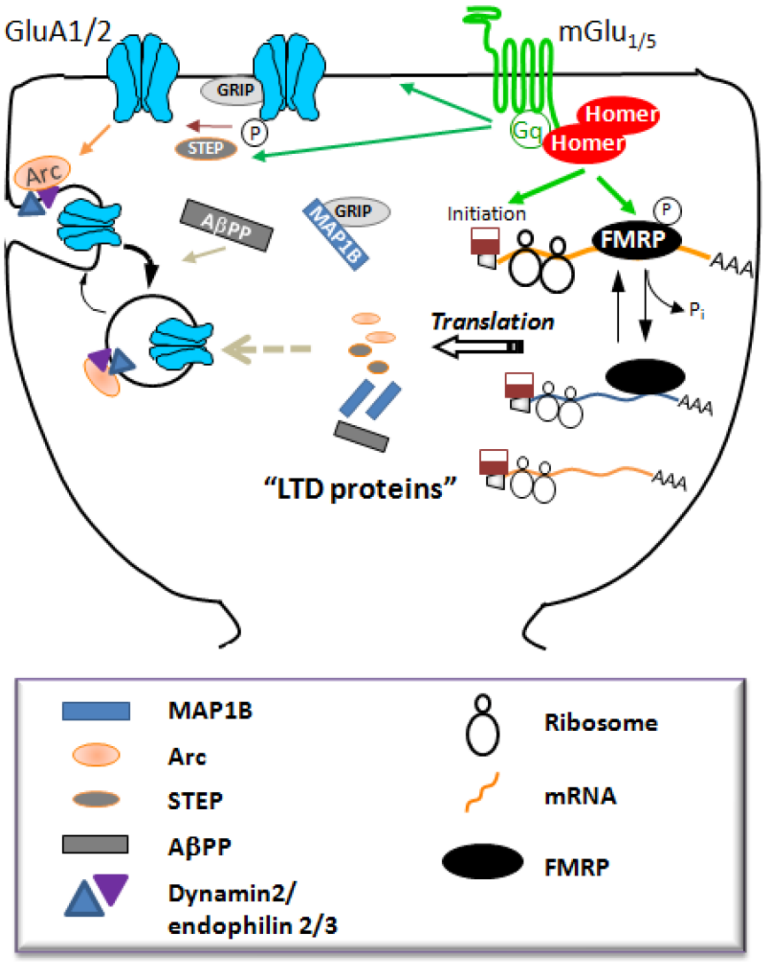

Figure 2. Mechanisms of translation-dependent mGluR-LTD. Stimulation of Gp 1 mGluR triggers the rapid endocytosis of AMPARs that requires the activity of the tyrosine phosphatase STEP (striatal-enriched protein tyrosine phosphatase) as well as existing Arc proteins. mGluRs also trigger the translation of proteins through the activation of translation initiation and dephosphorylation of FMRP, which is an RNA binding protein. Known proteins, whose synthesis is triggered by mGluRs and participate in mGluR-LTD, include MAP1B (microtubule-associated protein 1B) and AβPP (amyloid β precursor protein). (Modified from Luscher and Huber, 2010)

Figure 3. A schematic representation of the Arc protein and its different domains and binding regions

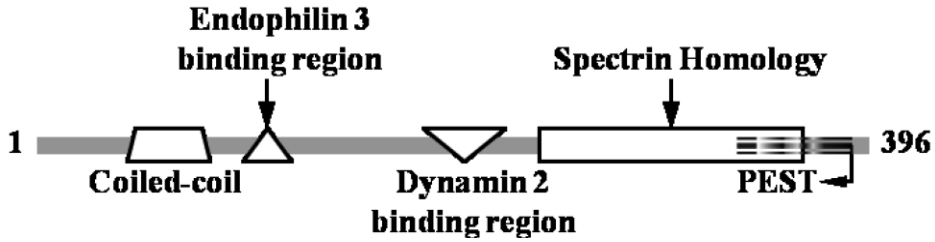

Figure 3. A schematic representation of the Arc protein and its different domains and binding regions. The coiled-coil (aa ~49-79) and spectrin homology (SH; ~228-375) domains can facilitate the interaction with other SH-containing proteins. The endophilin (~89-100) and dynamin 2 (~195-214) binding regions mediate the endocytosis of AMAPRs. The proline (P), glutamic acid (E), serine (S) and threonine (T)-rich regions (PEST, ~351-392, black and gray lines) may act to target Arc for proteosomal degradation. (Modified from Bramham et al., 2010)

CHAPTER TWO

Materials and Methods

Dissociated Hippocampal Neurons

Neuronal cultures were prepared from the CA1/CA3 hippocampal regions of P0-P2 male hooded Long-Evans rats, and male and female WT or *Fmr1* KO (C56Bl6CR strain) or Arc KO mice as described (Volk et al., 2007; Waung et al., 2008). The Arc KO mice were obtained from Dr. Kuan Hong Wang (National Institute of Mental Health / NIH) (Wang et al., 2006) and backcrossed to the C57Bl6CR mice. Neurons were plated in a Neurobasal A medium that is supplemented with B27, 0.5 μM glutamine and 1% fetal bovine serum at a density ~400 neurons/mm^2 onto glass coverslips that were coated overnight with 50 μg/ml poly-D-lysine and 25 μg/ml laminin. Cultures were fed at one day *in vitro* (DIV 1), and the medium was replaced once a week thereafter with a glial-conditioned medum from wildtype mouse glial cultures as described (Viviani, 2006). The experiments were performed at 18-21 DIV.

Immunocytochemistry

All experiments were performed on at least three independent cultures with 2-3 different coverslips per condition. For Arc, tubulin, and GFP immunofluorescence, the neurons were fixed and permeabilized with ice-cold methanol for 10 minutes, or fixed in 4% paraformaldehyde for 15 minutes at 37°C and permeabilized in 0.2% Triton-X for 10 minutes. Fixed cells were incubated in 1° anti-Arc (1:600, Synaptic Systems; 1:100, gift from Dr. Paul Worley (Lyford et al., 1995)), 1° anti-tubulin (1:600, Abcam), 1° anti-

MAP2 (1:1000, Millipore), and 1° anti-GFP (1:600, Aves Labs). Primary antibodies were detected with the subsequent application of the appropriate AlexaFluor555- (AF555), AF546-, AF488- or AF633-conjugated 2° antibody (Molecular Probes). To confirm the specificity of the two anti-Arc antibodies that were used in this study, immunocytochemistry was performed in dissociated hippocampal neuron cultures prepared from WT and *Arc* KO littermates (Wang et al., 2006). Figure 4 and Table 1 demonstrate the specificity of the Synaptic Systems anti-Arc antibody. Similar results were observed using the Arc antibody provided by Dr. Worley.

Fluorescence images were acquired on a Nikon TE2000 microscope with a cooled CCD camera (CoolSnap HQ; Roper Scientific). The images were subsequently quantified with a Metamorph Meta Imaging Series software (Molecular Devices). In any given experiment, images of cells across all conditions were collected on the same day using the same duration and intensity of the excitation light. For the acquisition of dendritic images, an oil immersion, 63X objective lens was used; and for the somatic images, a 20X objective lens was used. Healthy neurons were first identified by their smooth soma and multiple processes under DIC microscopy.

Using Metamorph, the images used for the somatic and dendritic analyses were set at a threshold value of at least thrice above background, which remained constant within an experiment. For the dendritic images, the background values for each image were obtained by measuring the average fluorescence intensity in a circular region of ~70 μm^2 adjacent to a neuron. The product of the total area and intensity above the threshold-value of the dendritic fluorescence, which was at least thrice the background, was quantified and normalized to the control (untreated or basal) conditions for each

experiment. The dendritic Arc immunofluorescence was analyzed in the most proximal 50 μm section of secondary dendrites. For each cell, three dendrites were analyzed. The fluorescence values of the three dendrites were then averaged and represented the value for a cell. A cell equals an n of one. For each condition 10-15 cells were analyzed per coverslip.

For somatic Arc measurement, the product of the total area and intensity, which was above the threshold-value, was quantified. A circular area of ~60 μm^2, which was placed in the center of a cell body, was used to designate the area to be measured. The threshold-value was at least thrice the background. To obtain background values for somatic imaging analysis, I measured the average fluorescence intensity outside of the soma using a circular area of ~60 μm^2. A threshold-value was acquired for each image. Five to ten regions from each coverslip were taken. Ten to thirty cell somas from each coverslip were analyzed, and 2-3 different coverslips were used for each condition. To aid the visualization of images in the figures, background fluorescence was manually subtracted using the "adjust levels" tool in Adobe Photoshop. For a given experiment, levels were adjusted in exactly the same manner as to maintain the same ratio of intensities across experimental conditions.

Lentivirus Production

$FMRP^{GFP}$ was cloned into the lentiviral vector FUGW (Lois et al., 2002) obtained from Dr. Thomas Sudhof (Stanford, University). The FUGW vector was digested with EcoRI and BamHI, and the 9136 base pair product (bp) was gel purified. The human wt-$FMRP^{GFP}$-hpr construct described previously (Darnell et al., 2005; Pfeiffer and Huber,

2007) was digested with EcoRI, and the 5411 bp product was gel purified and digested with BamHI. The 2593 bp product from the BamHI digestion of wt-FMRP^{GFP}-hpr was gel purified and ligated to the 9136 bp product of the FUGW digestion. The lentiviral constructs of S500D-FMRP^{GFP} and S500A-FMRP^{GFP} were made similarly as the FUGW-FMRP^{GFP}, using the FMRP-S500D-EGFP-hpr and FMRP-S500A-EGFP-hpr respectively as described (Pfeiffer and Huber, 2007). Briefly, S500A and S500D were created by introducing mutations into the wtFMRP-GFP using the QuikChange site-directed mutagenesis (Stratagene) of *KpnI*-fragment. The products were individually subcloned into pBluescript for mutagenesis then replaced in the WT construct. For production of the lentivirus, HEK293 cells were transfected with FMRP^{GFP} or FMRP phosphomutants and three helper plasmids (pRSV, pMDLg, and VSVG) using the FuGENE transfection reagent (Roche) according to the manufacturer's directions. After 48 hours, the media was collected, filtered through a 0.45 μm filter, aliquoted, and stored at -80°C. Hippocampal cultures were infected at 7-10 DIV at a titer (approximately 250-300 units/μl) to infect ~85% of neurons. Experiments were peprformed at 18-21 DIV.

Fluorescence in situ Hybridization

Anti-sense and sense oligonucleotide probes to *Arc* mRNA were obtained from Dr. Gary Bassell (Emory University) and labeled with digoxigenin (Dig; Roche). Fluorescent *in situ* hybridization (FISH) was performed as previously described (Antar et al., 2004; Dictenberg et al., 2008). Summarily, probes were detected by immunofluorescence with a Cy3-conjugated mouse anti-Dig antibody and a Cy3-conjugated anti-mouse IgG antibody (Jackson). Sense oligonucleotides against *Arc* were

used as negative controls. Experiments were performed on at least three separate cultures.

Electrophysiology

For extracellular recording in slices, acute hippocampal slices (400 μm) were prepared from postnatal day 21-55 hooded Long Evans rats as described (Fig. 5) (Volk et al., 2007). Dissected hippocampi were sliced in ice-cold dissection buffer containing (in mM): 2.6 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 5 $MgCl_2$, 212 sucrose, and 10 dextrose using a vibratome (Leica VT 1000S). The slices were transferred and allowed to recover for 1-5 hours in a chamber containing artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$ and 10 dextrose. To acquire extracellular recordings, slices were placed in a submerged recording chamber, maintained at 30°C, and perfused continuously with ACSF at a rate of 2-3 ml/min. Extracellular field potentials (FPs) were recorded using glass electrodes (1 MΩ) filled with ACSF and placed in the *stratum radiatum* of area CA1. FPs were evoked by monophasic stimulation (200 μs duration) of Schaffer collateral/commissural afferents with a concentric bipolar tungsten stimulating electrode. Stable baseline responses were collected every 30 seconds using a stimulation intensity (10-30 μA) yielding 50-60% of the maximal response. The initial slope of the FPs was used to measure the stability of synaptic responses and to quantify the magnitude of LTD. Chemically induced mGluR-LTD was elicited by the application of 100 μM DHPG for five minutes.

Whole cell voltage clamp recordings were performed on dissociated hippocampal neurons from WT, *Fmr1* KO, and infected *Fmr1* KO (GFP, wildtype $FMRP^{GFP}$, S500A-$FMRP^{GFP}$, S500D-$FMRP^{GFP}$). All experiments were performed on at least three separate cultures. Cells were visualized using a combination of infrared illumination differential interference contrast (IR-DIC) and GFP fluorescence to identify infected and non-infected neurons. Patch electrodes (3-7 MΩ) were filled with (in mM): 0.1 EGTA, 125 K-gluconate, 2.6 KCl, 1.3 NaCl, 10 HEPES, 4 ATP-Mg, 0.3 GTP-Na, 14 phosphocreatine-Tris; pH 7.2, adjusted to 285 mOsm with sucrose or H_2O. Cells were perfused at room temp at a rate of 0.5 ml/min in Tyrode solution that is composed of (in mM) 150 NaCl, 4 KCl, 2 $MgCl_2 \cdot 6H_2O$, 10 glucose, 10 HEPES, 2 $CaCl_2 \cdot 2H_2O$, 0.1 picrotoxin, 0.001 TTX; pH 7.4, adjusted to 310 mOsm. Cells were voltage-clamped at -60 mV. Series resistance and input resistance were measured in voltage clamp with 400 ms, -10 mV step from a -60 mV holding potential (Pfeiffer and Huber, 2007). Only cells with a holding current of less than 200 pA and a series resistance less than 40 MΩ were used for analysis. In addition, only cells that had a stable series resistance (< 25% change) after DHPG application were used. On average DHPG did not affect series resistance within any experimental group (Table 2). mEPSCs were detected off-line using an automatic detection program (MiniAnalysis; Synaptosoft Inc.) with a detection threshold set at a value of five-times the root mean square (RMS) noise.

Drug Application

For extracellular field potentials and western blotting in slices, the tissues were allowed to recover for one hour and then incubated for a minimum of three hours in

either ACSF/H_2O or ACSF/100 nM okadaic acid (OA). A brief application of 100 μM (RS)-3,5-dihydroxyphenylglycine (DHPG) for five minutes was used to induce LTD in all of the experiments. Dissociated rat hippocampal neurons were preincubated in either 10 or 100 nM OA, or 100 nM fostriecin for two hours. Cultured mouse neurons were preincubated in 10 nM OA for one hour because this was sufficient to enhance P-FMRP levels (Fig. 3C), and I found that 100 nM OA to be toxic to cultured mouse neurons. Similarly, incubation of acute mouse hippocampal slices for 1-3 hours in 100nM OA, as we used in rat slices, severely reduced their viability. This was evinced by small or nonexistent FP recordings, which precluded the investigation on the role of PP2A in mGluR-LTD in slices from WT or *Fmr1* KO mice. For experiments involving PP2A inhibitors, OA and fostriecin were present before, during, and after DHPG stimulation. For protein synthesis inhibition, cultures were incubated in 20 μM anisomycin 20 minutes before DHPG application and for the duration of the experiment. To inhibit Gp 1 mGluR activity and action potentials, dissociated *Fmr1*/GFP cultures were preincubated for 14-18h in 2-Methyl-6-(phenylethynyl)pyridine (MPEP, 10 mM) and LY367385 (100 mM), and TTX (1 mM) respectively.

Western Blot Analysis

For western blots on dissociated hippocampal neurons (P18-21), neurons were plated at a density of 4.5 – 5.0 X 10^4 cells, on 12 mm diameter coverslips. Cultured neurons were harvested in SDS sample buffer and boiled at 90°C for 10 minutes. A minimum of three coverslips were used for each condition. For western blots in hippocampal slices, slices were prepared from 21-28 days old WT and *Fmr1* KO mice or

21-35 days old hooded Long-Evans rats as described in acute hippocampal slice electrophysiology (Nosyreva and Huber, 2006). Slices were homogenized in RIPA buffer, and protein concentrations were measured with BCA Protein Assay (Pierce). Proteins were separated by SDS-PAGE and immunoblotted with 1° antibodies against Arc (1:20,000; Synaptic Systems), FMRP (2F5-1 antibody; 1:2000; (Gabel et al., 2004) provided by Dr. Jennifer Darnell; Rockefeller University), phospho-FMRP (1:400; Abcam), Akt (Cell Signaling; 1:5000), phospho-Akt (Cell Signaling; 1:5000), βIII-tubulin (1:5,000; Abcam), ERK1/2 (1:5,000; Cell Signaling), and actin (1:100,000; Millipore). Blots were washed and incubated in appropriate HRP-conjugated secondary antibody (1:5000; MP Biomedical, Aurora, OH). Bands were detected using enhanced chemiluminescence and densitometric images from scanned films were analyzed with Image J. For optical density quantification, Arc and P-FMRP were normalized to a loading control. Actin, total ERK, or tubulin was used as loading controls because the levels were unchanged with the experimental manipulations. For total ERK, bands at 42 and 44 kDa were quantified. All experiments were performed on at least three separate cultures or three separate pairs (WT/*Fmr1* KO) of animals.

Statistical Analysis

Student's t-test or two-way ANOVA, and posthoc Bonferroni or Newman-Keuls multiple comparison tests were used for statistical analysis where indicated * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure 4. The Synaptic System and Worley antibodies against Arc are specific in dissociated hippocampal neuron cultures

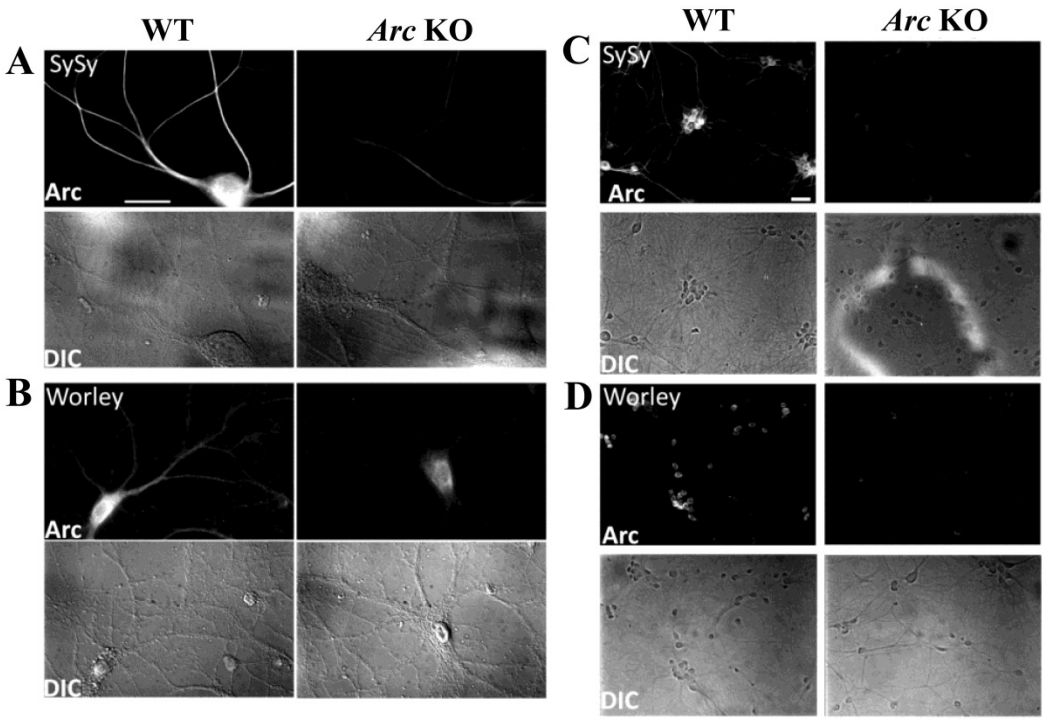

Figure 4. The synaptic System and Worley antibodies against Arc are specific in dissociated hippocampal neuron cultures. Representative images of immunocytochemistry with the Synaptic Systems (SySy) and Worley (Lyford et al., 1995) antibodies in cultures prepared from WT and Arc KO mouse littermates. **A, B**, Fluorescence and DIC images of WT and Arc KO neurons at high (60 X) magnification. Note: The images were taken focusing on the dendrites. The cell soma may or may not also be in focus in these images. Scale bar = 20 µm. **C, D,** Fluorescence and DIC images of WT and Arc KO neurons when focused on the cell bodies at (20 X) magnification. Scale bar = 50 µm. (Fig 4A from Niere et al, 2012; Figs 4B, C and D from Niere et al, unpublished observations)

Table 1. Quantification of the specificity of the Arc antibodies used in the study in the dendrites and soma of WT and *Arc* KO

	Synaptic Systems Arc Antibody		**Worley Arc Antibody**	
	WT	***Arc* KO**	**WT**	***Arc* KO**
Dendrite	1.00 ± 0.15 (n = 36)	0.20 ± 0.03*** (n = 31)	1.00 ± 0.14 (n = 20)	0.11 ± 0.04*** (n = 18)
Soma	1.00 ± 0.04 (n = 148)	0.28 ± 0.03*** (n = 95)	1.00 ± 0.05 (n = 191)	0.25 ± 0.03*** (n = 113)

Table 1. Quantification of the specificity of Arc antibodies used in the study immunofluorescence in the dendrites and soma of WT and *Arc* KO. Two rabbit polyclonal antibodies against Arc were tested – Synaptic Systems and a gift from Dr. Paul Worley. Dendritic images were taken at 60X, somatic images were at 20X. Dissociated hippocampal neurons were prepared from WT and Arc KO littermates. (From Niere et al, 2012)

Figure 5. A schematic diagram of an acute rodent hippocampal slice preparation for extracellular electrophysiology

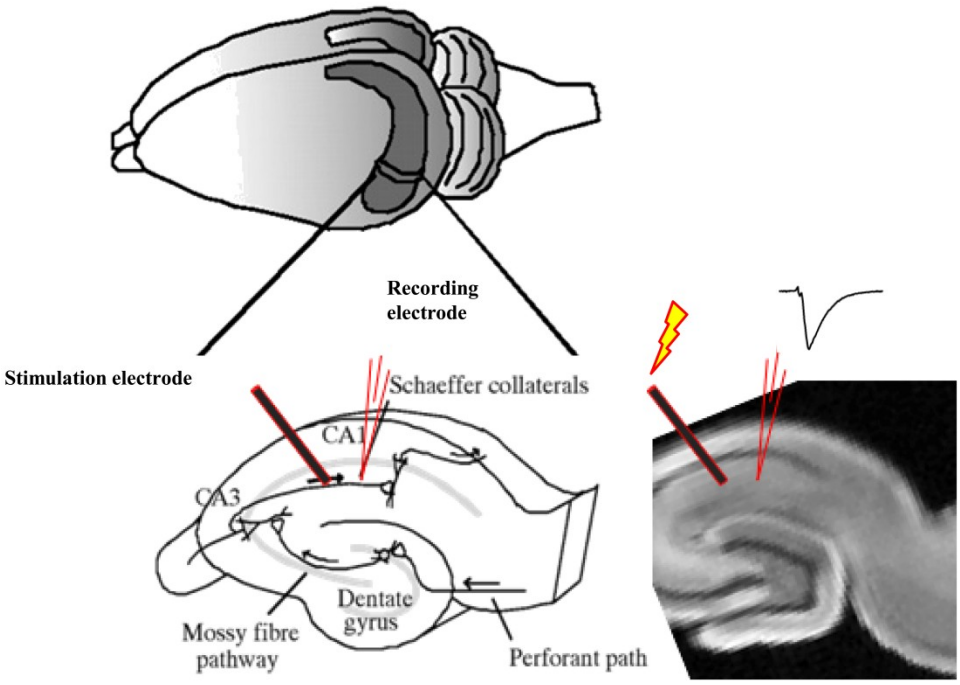

Figure 5. A schematic diagram of an acute rodent hippocampal slice preparation for extracellular electrophysiology. Hippocampi were removed and sliced are 400 mm. Following recovery, extracellular field potentials (FPs) are recorded using a glass electrode (red pointed tip; a sample response waveform is shown), which is filled with artificial cerebrospinal fluid (ACSF), placed in the *stratum radiatum* of area CA1. FPs are evoked using monophasic stimulation of the Schaffer collateral/commissural afferents with a concentric bipolar tungsten stimulation electrode (red and black bar). (Modified from Nguyen et al., 2006)

CHAPTER THREE

Results

PP2A is necessary for an early, immediate phase of mGluR-triggered LTD and increases in dendritic Arc protein

To determine whether PP2A and the dephosphorylation of FMRP played a role in mGluR-LTD, I incubated acute hippocampal slices prepared from mature Long-Evans rats in the PP2A inhibitor okadaic acid (OA). I used 100 nM OA, because OA is 100X more effective against PP2A in comparison to PP1 (Swingle et al., 2007), and a three-hour incubation of rat brain slices in 100 nM OA selectively inhibits PP2A over PP1 or PP2B (Gong et al., 2000). Furthermore, treating hippocampal slices with 100 nM OA for 1-3 hours was sufficient to increase the phosphorylation of FMRP at S499 and Akt, another PP2A substrate, as observed with western blotting using phosphospecific antibodies against these PP2A-target proteins (Fig. 6B) (Padmanabhan et al., 2009). Population or field excitatory postsynaptic potentials (fEPSP or FP) were measured in the *stratum radiatum* of area CA1 of the hippocampus in response to an extracellular stimulation of the CA3 Schaffer collateral axons as described (Huber et al., 2000). LTD was induced with the Gp 1 mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG; 100 μM; 5 min; (Huber et al., 2000)). The incubation of hippocampal slices in OA slightly reduced the LTD magnitude measured at 30-40 minutes after DHPG (86 ± 3% of baseline; n = 13) in comparison to interleaved vehicle-treated slices (vehicle (water); 76 ± 2% of baseline; n = 13 slices; $p < 0.05$; Fig. 6A). However, at one hour after washing out DHPG, the LTD magnitude of OA-treated slices returned to similar levels as the vehicle-

treated controls (OA: 80 ± 2%; vehicle: 76 ± 3%). These results suggested that PP2A is required for an early, but not a late phase of mGluR-LTD.

A brief mGluR stimulation results in a rapid (within five minutes after DHPG application) and persistent increase in Arc levels that last at least one hour after drug treatment. Previous work demonstrated that the rapid increases in Arc, in response to the Gp 1 mGluR agonist, DHPG, were due to the local translational activation of Arc from preexisting mRNAs in the dendrites (Park et al., 2008; Waung et al., 2008). Whereas, the later increases in Arc protein are likely due to the mGluR-induced transcription of *Arc* (Park et al., 2008; Taylor et al., 2010). Based on our FP data, we hypothesized that the role of PP2A in mGluR-LTD functions to regulate a rapid, FMRP-mediated translational control of LTD proteins, such as Arc, in the dendrites. To test this idea, I examined the effects of PP2A inhibitors on Gp 1 mGluR-induced increases of Arc protein in the dendrites of dissociated hippocampal neuron cultures using immunocytochemistry.

To confirm the specificity of the anti-Arc antibodies, I performed immunocytochemistry in dissociated hippocampal neuron cultures prepared from wildtype and *Arc* knockout littermates (Wang et al., 2006) (Fig. 4). My analysis determined that the two anti-Arc antibodies used in this study were selective for Arc (Table 1).

The pretreatment of cultures for one hour with 10 nM OA blocked the mGluR-induced increases in dendritic Arc levels that were observed at five minutes following DHPG treatment. However, the treatment with OA did not affect the basal Arc levels or the late increases of Arc in the dendrites Arc that were measured at 60 minutes post-DHPG (Fig. 7A). The application of 100 nM fostriecin, a PP2A inhibitor that is

structurally different from OA and 10,000-fold more selective for PP2A over PP1 (Swingle et al., 2007), also blocked the early, mGluR-induced increases in early dendritic Arc (Fig. 7B). Interestingly, the use of 100 nM fostriecin (Fig. 7B) or a higher concentration of OA (100 nM; basal: 100 ± 8%, 100 nM OA: 72 ± 6%) that causes the hyperphosphorylation of FMRP (Narayanan et al., 2007) suppressed the basal Arc levels in the dendrites of dissociated hippocampal neurons that were isolated from rats.

Dendritic Arc levels are elevated in Fmr1 KO neurons

To determine how FMRP and its phosphorylation state regulated Arc, I compared the levels of dendritic Arc in WT and *Fmr1* KO mice. *Arc* mRNA had been shown to interact with FMRP, either directly and/or indirectly, but it is unclear if or how FMRP regulates protein levels of Arc (Iacoangeli et al., 2008a; Park et al., 2008; Zalfa et al., 2003). Because Arc is deemed as an "LTD protein", I hypothesized that the levels of Arc are elevated in the hippocampal neurons of *Fmr1* KO mice and thus the heightened Arc levels contribute to the enhanced and protein synthesis-independent LTD that had been documented in these animals. However, I did not observe any difference in the total Arc levels between the hippocampi of WT and *Fmr1* KO mice (Fig. 8A). Surprisingly, I saw a trend towards a decreased Arc level in the hippocampus of the *Fmr1* KO mouse. FMRP had been suggested to function as a dendritic translational suppressor (Bassell and Warren, 2008). Therefore using immunocytochemistry, I examined the amount of dendritic Arc in dissociated neuronal cultures that were prepared from the hippocampi of WT and *Fmr1* KO mice. Consistent with the proposed function of FMRP as a translational suppressor, the Arc levels, as measured by immunofluorescence intensity,

were enhanced in the dendrites of *Fmr1* KO neurons (Fig. 8B). In contrast, the soma of the *Fmr1* KO neurons intriguingly expressed less Arc compared to WT neurons in sister cultures.

With these observations, I wanted to determine whether the enhanced level of Arc in the dendrites of the *Fmr1* KO cultures reflected a cell-autonomous effect of FMRP. To this end, I generated dissociated hippocampal neurons from the GFP/*Fmr1* mosaic mice that were obtained by crossing the *Fmr1* KO line with mice that carry a GFP vector on the X chromosome (Hadjantonakis et al., 1998; Hanson and Madison, 2007). As a result of X-inactivation, cells of heterozygous females are mosaic; that is, the GFP (+) cells express FMRP, which are therefore "wildtype", are intermingled with the GFP (-) cells that are "*Fmr1* KO". The co-expression of FMRP and GFP in the hippocampus of the GFP/*Fmr1* mosaic mice was confirmed by immunohistochemistry (Fig. 9A). In the dissociated neuron cultures of GFP/*Fmr1* mosaic mice, I observed similar results as that in the complete WT and *Fmr1* KO. The "*Fmr1* KO" (GFP-) cells had elevated Arc levels in dendrites in comparison to their neighboring "WT" (GFP+) cells on the same coverslip (Fig. $9B_2$). I also observed a similar trend in the soma of KO (GFP-) and WT (GFP) neurons, namely, the KO (GFP-) soma expressed less amount of Arc than the WT (GFP+). Furthermore, to verify my previous findings of elevated dendritic Arc levels in GFP- neurons, I performed a double-immunofluorescence staining for Arc and the microtubule-associated protein 2 (MAP2), which is a dendritic marker, in the GFP/*Fmr1* mosaic mice. Analysis of the double-immunofluorescence for Arc and MAP2, in which Arc was normalized to MAP2, I found that Arc remained elevated in *Fmr1* KO dendrites (Fig. $9B_2$).

I also examined the amount of somatic Arc in the GFP/*Fmr1* KO mice. Consistent with my previous observations in the complete WT and *Fmr1* KO neurons, I found a similar compartment specific regulation of Arc levels that was confirmed with two different rabbit polyclonal antibodies against Arc (from Synaptic Systems; Figs. 8B and $9B_2$; or from Dr. Paul Worley (Johns Hopkins University); Fig. $9B_3$) (Lyford et al., 1995).

Arc is an activity-dependent gene, and the elevated Arc levels could be a consequence of enhanced activity or excitability of the *Fmr1* KO neurons that can thereby drive *Arc* transcription (Link et al., 1995; Lyford et al., 1995). I tested this idea in two ways: first, by examining the level of *Arc* mRNA in the dendrites, and second by measuring the level of dendritic Arc protein levels by blocking neuronal activity. To measure dendritic *Arc* mRNA, I performed a fluorescent *in situ* hybridization (FISH) for *Arc* mRNA in the GFP/*Fmr1* mosaic mice as described (Dictenberg et al., 2008). Previous work has demonstrated that the *Fmr1* KO hippocampal neurons have normal steady state and mGluR-induced trafficking of *Arc* mRNA (Dictenberg et al., 2008; Steward et al., 1998a). Consistent with these reports, I did not detect a difference in the levels of dendritic *Arc* mRNA between WT (GFP+) cells and their neighboring *Fmr1* KO (GFP-) neurons in culture (Fig. 10; WT: 1.00 ± 0.08, n = 53; KO: 0.90 ± 0.08 (*Arc* FISH intensity; normalized to WT neurons) n = 52). To examine whether neuronal activity increased the expression of Arc in the dendrites, I treated the GFP/*Fmr1* mosaic cultures for 14-18 hours with one micromolar tetrodotoxin (TTX) to block action potentials. The blockade of activity by TTX reduced the dendritic Arc levels in both WT and *Fmr1* KO neurons. However, the application of TTX did not abolish the difference between

genotypes (Fig. 11). In other words, in the presence of TTX, the dendritic Arc levels in the *Fmr1* KO remained elevated in comparison to WT neurons. These results indicate that the elevated Arc in the *Fmr1* KO dendrites is not a consequence of an increased activity-dependent *Arc* transcription or dendritic mRNA levels. Collectively, these findings support a cell-autonomous role of FMRP in suppressing Arc translation in the dendrites.

FMRP and PP2A are necessary for a rapid, but not a late, mGluR-induced expression of dendritic Arc protein

Because I was interested in understanding the role of FMRP phosphorylation during the mGluR-triggered increase of Arc in the dendrites, I treated dissociated hippocampal cultures that were prepared from wildtype (WT) and *Fmr1* KO mice with 10 nM OA before stimulating the Gp 1 mGluR (Figs. 12 and 13). As I had earlier observed in the slices, treating the WT cultures with OA increased the levels of phosphorylated FMRP (Fig. 12C) (Narayanan et al., 2007). As in the dissociated rat neurons, the application of okadaic acid in the dissociated WT mouse neurons also blocked the rapid, mGluR-induced increases of Arc protein in the dendrites as determined by immunocytochemistry (Fig. $12A_2$) and total Arc protein by western blotting (Fig. 12B). As I had demonstrated in rat cultures, the late increases in dendritic Arc of WT mouse neurons, observed at one hour post-DHPG, were also resistant to OA (Fig. $12A_3$). In contrast to WT cultures, the stimulation of the Gp 1 mGluR by DHPG in the *Fmr1* KO neuron cultures failed to evoke a rapid expression of dendritic Arc (Fig. 13B). However at a late time point, DHPG elicited a small, but significant elevation of Arc in the

dendrites of *Fmr1* KO neurons (Fig. 13C) (Park et al., 2008). In the presence of OA, the *Fmr1* KO neurons that were treated with DHPG displayed a trend of increased Arc in the dendrites at one hour following Gp 1 mGluR stimulation; but this did not reach statistical significance despite the large number of cells that I analyzed ($p = 0.058$; OA; n =85; OA + DHPG n = 97; Fig. 13C). Therefore in the *Fmr1* KO, the role of PP2A in the late expression of dendritic Arc is unclear.

To determine whether FMRP plays a cell-autonomous role in the mGluR-triggered, rapid increases of Arc in the dendrites, I stimulated the Gp 1 mGluR of the GFP/*Fmr1* mosaic cultures with DHPG. Similar to what I had observed in the complete WT and *Fmr1* KO cultures, I found that DHPG induced a rapid increase of Arc in the dendrites of WT (GFP+) neurons, but not in neighboring *Fmr1* KO (GFP-) neurons (Fig. $9B_2$). Interestingly, the level of dendritic Arc in DHPG-treated WT neurons did not reach the level as observed basally in *Fmr1* KO neurons (Fig. $9B_2$). These results have led me to propose that the mGluR-triggered Arc translation comprises two phases, namely: (1) an early, immediate phase that is mediated by PP2A and FMRP, and (2) a late phase that is independent of these proteins and mediated by other translational or transcriptional control mechanisms, or both.

Numerous studies have demonstrated that the translation inhibitor anisomycin blocks mGluR-LTD in hippocampal slices prepared from WT, but not *Fmr1* KO mice (Hou et al., 2006; Nosyreva and Huber, 2006). Because a string of evidence implicates that Arc functions as an LTD protein (Park et al., 2008; Waung et al., 2008), I considered the possibility that the mGluR-triggered, late increases in dendritic Arc of *Fmr1* KO neurons may be, like LTD, independent of *de novo* translation. Therefore, I tested the

effects of anisomycin on the late increases of Arc in the dendrites of WT and *Fmr1* KO neurons. In the WT cultures, anisomycin completely abrogated the DHPG-induced Arc expression in the dendrites, and this finding is consistent with the influence of anisomycin in blocking mGluR-LTD (Fig. $12A_4$). Although DHPG evoked a small elevation of dendritic Arc in the anisomycin-treated *Fmr1* KO neurons, the increase was not statistically significant (Fig. $13A_4$). Therefore, these observations indicate that the late expression of Arc in the dendrites of *Fmr1* KO neurons also rely on translation (Fig. $13A_4$), which is likely from the mGluR-triggered generation of *Arc* mRNA (Park et al., 2008; Taylor et al., 2010). The fact that the *Fmr1* KO mice display a persistent mGluR-LTD in the presence of anisomycin suggests that mGluR-LTD does not utilize the late, mGluR-induced, expression of Arc; rather mGluR-LTD relies on preexisting Arc protein (Park et al., 2008).

Phosphomimic FMRP at S500 suppresses basal Arc, and the dephosphorylation of FMRP is necessary for mGluR-induced, early dendritic Arc expression

Because the phosphorylation of FMRP has been shown to stall translation, I examined whether the phosphorylation state of FMRP regulated the basal and mGluR-triggered Arc expression in the dendrites by conducting rescue experiments with an N-terminally-GFP tagged wildtype FMRP ($FMRP^{GFP}$) and phosphorylation site mutants of FMRP. My data from immunohistochemical analyses of dendritic Arc in WT and *Fmr1* KO mice that were presented earlier suggested that FMRP suppresses basal dendritic Arc levels and is necessary for the mGluR-induced, rapid synthesis of Arc in the dendrites. Consistent with this prediction, expressing $FMRP^{GFP}$ in dissociated *Fmr1* KO

hippocampal neurons reduced the basal dendritic Arc levels by 30% in comparison to sister *Fmr1* KO cultures that were infected with GFP only (Fig. $14A_2$). Furthermore, the expression of $FMRP^{GFP}$ restored the mGluR-induced, early dendritic Arc synthesis in these neurons (Fig. $14A_2$). The suppression of dendritic Arc levels by FMRP was not due to a general inhibition of dendritic size or protein expression, because $FMRP^{GFP}$ had no effect on the dendritic immunofluorescence of β3-tubulin (Fig. 14B). Intriguingly, the lentiviral-mediated transfection of $FMRP^{GFP}$ into *Fmr1* KO neurons did not affect the global level of Arc as assessed by western blotting (Fig. 15B), similar to what we had observed in WT cultures (see Fig. 8A). These results regarding the total levels of Arc in GFP- and $FMRP^{GFP}$-infected cells are tenable because $FMRP^{GFP}$ increased the basal amount of somatic Arc by 185 ±10% ($n = 191$; $p < 0.001$) in comparison to GFP-transfected neurons ($n = 127$) (Fig. $14A_3$). Nonetheless, the transfection of $FMRP^{GFP}$ into *Fmr1* KO cells rescued the DHPG-induced elevation of total Arc levels, which is in contrast to GFP-infected *Fmr1* KO cultures (Fig. 15C).

In light of my earlier findings that implicate the FMRP phosphatase, PP2A, in mGluR-triggered dendritic Arc expression, I investigated whether the dephosphorylation of FMRP at S500 regulated the mGluR-induced, early synthesis of Arc in the dendrites. Using the lentivirus system, I transfected dissociated hippocampal neurons that were prepared from *Fmr1* KO mice with GFP-tagged phosphorylation site mutants of $FMRP^{GFP}$, namely a dephosphomimic (S500A) and a phosphomimic (S500D). The S500A-$FMRP^{GFP}$ and S500D-$FMRP^{GFP}$ are expressed at similar levels to $FMRP^{GFP}$ in hippocampal neurons and are properly localized to the dendrites (Pfeiffer and Huber, 2007). As I have observed in wildtype $FMRP^{GFP}$-expressing cells, the S500D-$FMRP^{GFP}$-

infected *Fmr1* KO neurons had decreased basal dendritic Arc expression in comparison to GFP-infected sister cultures. However, S500D-FMRP^{GFP} did not rescue the DHPG-induced, rapid elevation of dendritic Arc levels (Fig. 16A). The expression of S500A-FMRP^{GFP} neither suppressed the Arc protein in dendrites, nor rescued the mGluR-stimulated increase in dendritic Arc (Fig. 16B). These results collectively promote a model whereby FMRP that is phosphorylated at S500 functions as a translational suppressor of *Arc* mRNA; and FMRP dephosphorylation at S500 by PP2A is necessary for the mGluR-triggered, rapid expression of Arc in the dendrites (Fig. 19).

The dephosphorylation of FMRP at S500 is required for mGluR-induced depression of mEPSCs

FMRP and its phosphorylation state at S500 differentially affected the basal and the mGluR-induced, rapid increases in dendritic Arc levels of cultured neurons. With these findings, I sought to determine in the dissociated mouse hippocampal cultures the effects of FMRP and its phosphorylation site mutants on the Gp 1 mGluR-stimulated depression of synaptic transmission. In the dissociated hippocampal neuron cultures prepared from rats, the DHPG-induced LTD is typically manifested as a decrease in the frequency of mEPSCs that requires postsynaptic tyrosine phosphatase (PTP) activity and Arc; DHPG-LTD also correlates with decreases in postsynaptic AMPAR surface expression (Moult et al., 2006; Sanderson et al., 2011; Snyder et al., 2001; Waung et al., 2008; Xiao et al., 2001). These results support a postsynaptic expression mechanism for DHPG-mediated LTD. In contrast to rat hippocampal cultures, the application of DHPG (100 μM; 5 min) in cultured WT mouse hippocampal neurons did not induce a depression

of mEPSC frequency (105 ± 8% of baseline; n = 11 cells) (Fig. 17, Table 2). However in *Fmr1* KO cultures, DHPG treatment resulted in a depression of mEPSC frequency (72 ± 5% of baseline; n = 8; measured at 5-15 minutes after DHPG washout; Fig. 17). These results are consistent with the enhanced, DHPG-induced LTD of evoked synaptic transmission that is observed in the *Fmr1* KO hippocampal slices (Huber et al., 2002).

To test whether the enhanced mGluR-induced depression of mEPSC frequency in the *Fmr1* KO neurons was due to an acute role of FMRP, as opposed to a developmental effect, I expressed GFP (control) or $FMRP^{GFP}$ in *Fmr1* KO neurons using a lentiviral-meidated system. Following the stimulation of the Gp 1 mGluR by DHPG application, *Fmr1* KO neurons that were infected with $FMRP^{GFP}$ failed to express a depression of mEPSC frequency (114 ± 6% of baseline; n = 11; Fig. 17), mimicking what I had observed in WT neurons. In contrast, the GFP-infected *Fmr1* KO neurons displayed a reduction in mEPSC frequency following DHPG application (76 ± 9% of baseline; n = 14) that was not different from the untransfected *Fmr1* KO neurons. I next examined whether the phosphorylation of FMRP at S500 affected the Gp 1 mGluR-induced depression of mEPSC frequency by expressing the S500A-$FMRP^{GFP}$ or the S500D-$FMRP^{GFP}$ in *Fmr1* KO cells. *Fmr1* KO neurons that were expressing the phosphomimic FMRP, S500D-$FMRP^{GFP}$, which suppressed both basal and mGluR-triggered dendritic Arc levels (see Fig. 16A), also failed to display a DHPG-induced depression of mEPSC frequency (125 ± 17% of baseline; n = 13; Fig. 6). However, cells that were infected with the dephosphomimic FMRP, S500A- $FMRP^{GFP}$, which neither suppressed basal dendritic Arc nor rescued the mGluR-stimulated Arc synthesis in the dendrites (see Fig. 16B), displayed a depression in mEPSC frequency following the activation of the Gp 1

mGluR (81 ± 6% of baseline; n = 16). Overall, I did not detect significant differences in mEPSC amplitude, series resistance, holding current or input resistance among the different genotypes or lentiviral-expressed proteins (Table 2). These findings collectively support the hypothesis that FMRP, when phosphorylated at S500, suppresses basal dendritic Arc levels and inhibits mGluR-LTD.

The inhibition of group 1 mGluR partially rescues the elevated basal dendritic Arc in Fmr1 KO

Recent work demonstrated that *Fmr1* KO neurons have enhanced overall protein synthesis rates, as measured by the incorporation of radiolabeled methionine (^{35}S Met), and that the elevated global protein synthesis can be equalized by the inhibitors of the group 1 mGluRs, $mGlu_1$ or $mGlu_5$ (Gross et al., 2010; Osterweil et al., 2010; Sharma et al., 2010). To determine whether the Gp 1 mGluR-enhanced translation rates led to an elevated steady state level of dendritic Arc, we treated GFP/*Fmr1* mosaic cultures for 14-18 hrs with the $mGlu_1$ and $mGlu_5$ antagonists, LY367385 (100 μm) and MPEP (10 μm), respectively. In contrast to TTX (see Fig. 11), blocking the activity of Gp 1 mGluR had no effect on the dendritic Arc levels of WT neurons. However, LY367385 and MPEP selectively reduced the Arc levels of *Fmr1* KO dendrites (Fig. 18). Interestingly, even in the presence of Gp 1 mGluR activity blockers, Arc levels in *Fmr1* KO dendrites remained significantly elevated. Prompted by these results, I hypothesize that there are two mechanisms or pathways that mediate the elevated Arc levels in the dendrites of *Fmr1* KO neurons: (1) enhanced Gp 1 mGluR-driven translation rates, and (2) abrogation

of translational suppression by the loss of interaction between FMRP and *Arc* mRNA, which physiologically occurs in WT neurons.

Figure 6. Okadaic acid, a PP2A inhibitor, reveals an early phase of mGluR-LTD

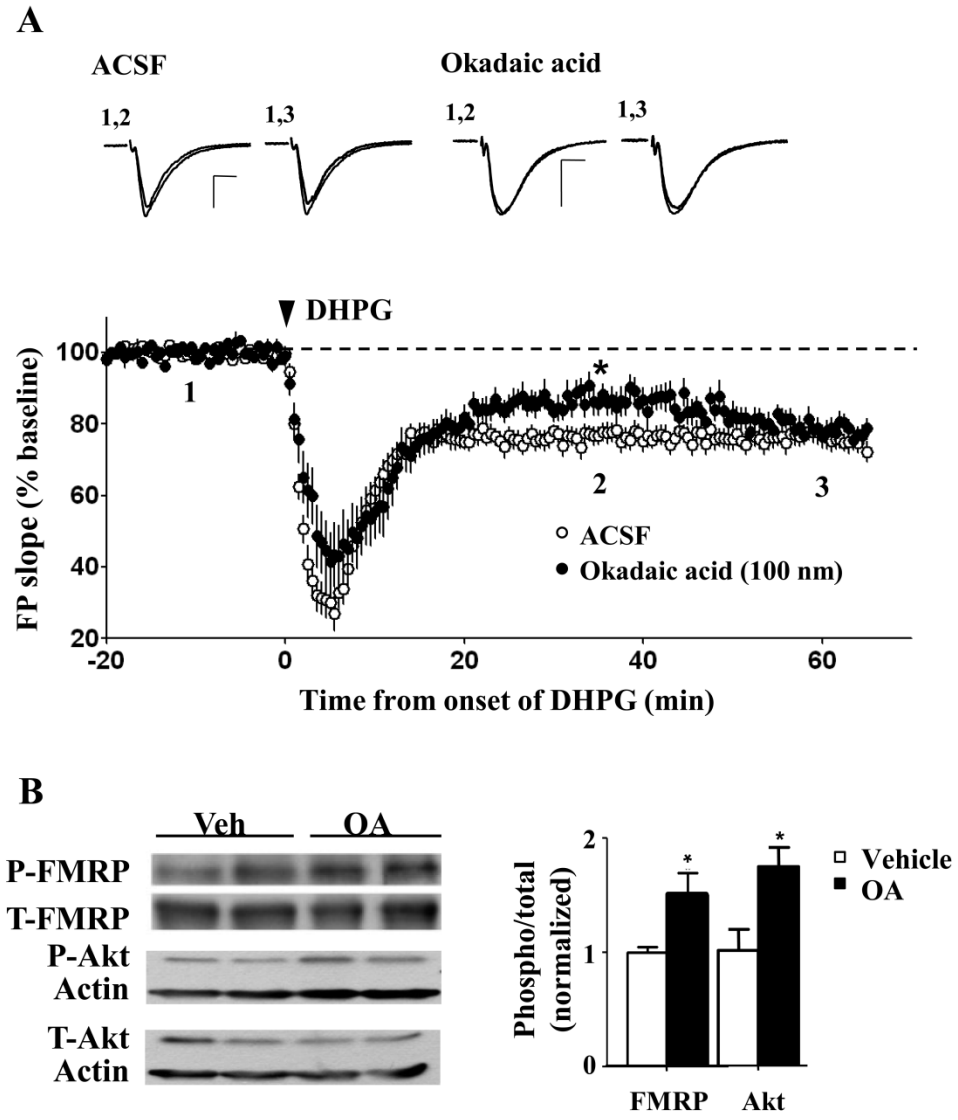

Figure 6. Okadaic acid, a PP2A inhibitor, reveals an early phase of mGluR-LTD. **A.** Incubation of acute rat hippocampal slices in the PP2A inhibitor, okadaic acid (OA, 100 nM), significantly reduces mGluR-LTD at 30-40 minutes (ACSF: 76 ± 2%, N = 13 slices; OA: 86 ± 3%, N = 13 slices; *p < 0.05) but not at 50-60 minutes (ACSF: 76 ± 3%; OA: 80 ± 2%) following DHPG application (100 μM, 5 minutes). Plotted are average (±SEM) initial slope values of FPs normalized to the pre-DHPG baseline. Inset: FP waveforms from a representative experiment are taken at the time points indicated on the

graph (1, 2 and 3). Calibration: 0.2 mV, 10 ms. **B.** Pretreatment of acute rat hippocampal slices with 100 nM OA (3 hours) increased the phosphorylated (S499) FMRP (P-FMRP) and phosphorylated Akt (P-Akt). Left panel: Representative western blot of P-FMRP and total FMRP (T-FMRP), and P-Akt and total Akt (T-Akt) from vehicle (H_2O)-treated and OA-treated slices. Right panel: Quantification of P-FMRP normalized to T-FMRP (Veh: 1.00 ± 0.05; OA: 1.51 ± 0.19; N = 3 rats; *p < 0.05), and P-Akt normalized to T-Akt (Veh: 1.00 ± 0.19; OA: 1.73 ± 0.17; N = 2 rats; *p < 0.05). (From Niere et al, 2012)

Figure 7. PP2A inhibitors define a rapid, early phase of mGluR-induced synthesis of dendritic Arc

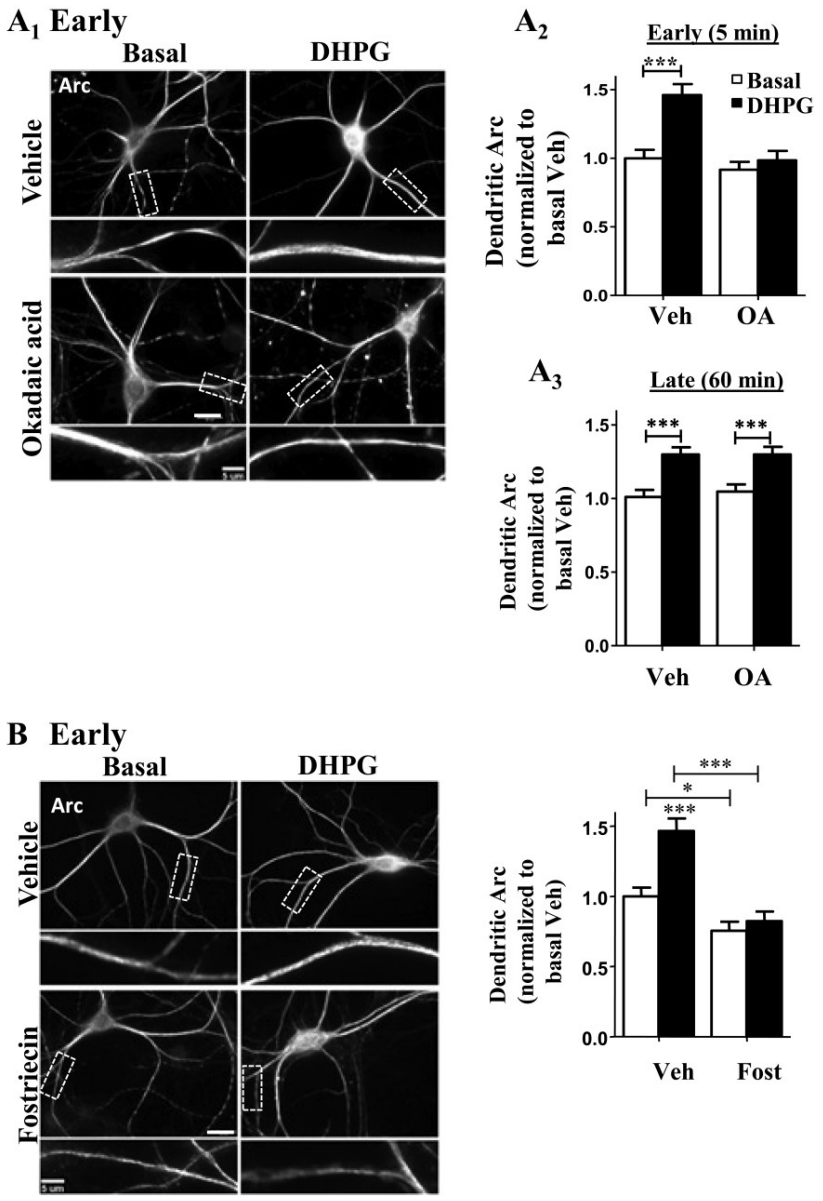

Figure 7. PP2A inhibitors define a rapid, early phase mGluR-induced synthesis of dendritic Arc. **A.** Application of OA (10 nM, 1 hour) prevents the rapid but not the late increase of Arc in the dendrites following the stimulation of Gp 1 mGluR by DHPG (100 mM, 5 minutes) in dissociated rat hippocampal neurons (DIV 18-21). **A_1**. Representative images of Arc immunofluorescence at 5 minutes after DHPG induction. Scale bar: 20

and 5 μm. **A_{2-3}**. Group averages (±SEM) of quantified dendritic Arc immunofluorescence intensity at 5 (**A_2**) and 60 (**A_3**) minutes after treatment with vehicle (H_2O) or DHPG for each condition. Arc levels are normalized to basal, vehicle-treated neurons of the same culture preparation. **A_2**. Vehicle = 1.00 ± 0.05; DHPG (normalized to basal vehicle) = 1.46 ± 0.08; OA = 0.92 ± 0.06; OA + DHPG = 0.99 ± 0.07; $p < 0.01$. **A_3**. Vehicle = 1.00 ± 0.05; DHPG = 1.30 ± 0.05; OA = 1.05 ± 0.05; OA + DHPG = 1.30 ± 0.05. **B.** Pretreatment of neuronal cultures with a structurally distinct PP2A inhibitor, fostriecin (100 nM, 1 hour, Fos) lowers basal Arc and prevents the rapid increase in dendritic Arc in response to DHPG. **B_1**. Representative images of Arc staining in dissociated hippocampal neurons (DIV 18-21) at 5 min after DHPG application. Scale bar: 20 and 5 μm. **B_2**. Quantification of dendritic Arc immunofluorescence at 5 minutes after treatment with vehicle (H_2O) or DHPG for each condition. Arc levels are normalized to basal, vehicle-treated cells. Vehicle = 1.00 ± 0.06; DHPG = 1.47 ± 0.09; Fos = 0.75 ± 0.07; Fos + DHPG = 0.82 ± 0.07. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis; two-way ANOVA, Bonferroni post-hoc comparison; *$p < 0.05$, ***$p < 0.001$. (From Niere et al, 2012)

Figure 8. FMRP suppresses basal Arc protein levels in the dendrites

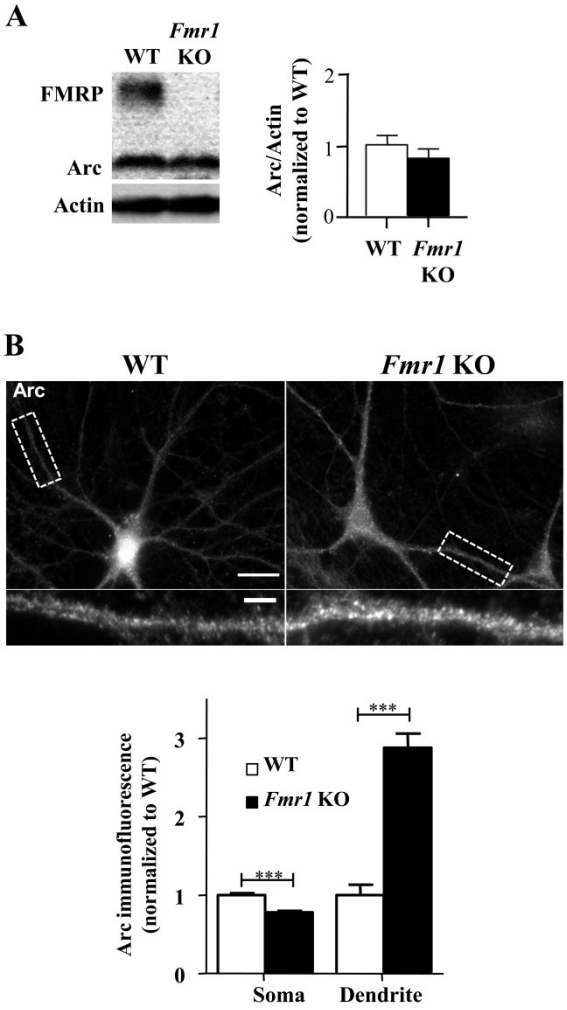

Figure 8. FMRP suppresses basal Arc protein levels in the dendrites. **A.** Total Arc levels in *Fmr1* KO are unchanged as assessed by western blotting of hippocampal homogenates from WT and *Fmr1* KO mice. Left panel: Representative western blot. Right panel: Group averages (±SEM) of Arc/actin levels normalized to WT. N = 12 mice per genotype. **B.** Dendritic Arc immunofluorescence is greater in *Fmr1* KO neurons. Top panel: Representative images of Arc immunofluorescence in dissociated hippocampal neurons (DIV 18-21) from WT and *Fmr1* KO. Scale bar: 20 and 5 μm. Bottom panel: Group averages (±SEM) of quantified somatic and dendritic Arc immunofluorescence levels. Soma: WT = 1.00 ± 0.02, *Fmr1* KO = 0.78 ± 0.02; Dendrite: WT = 1.00 ± 0.13, *Fmr1* KO = 2.88 ± 0.18. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by

two-way ANOVA, Bonferroni post-hoc comparison; ***$p < 0.001$. (From Niere et al, 2012 and unpublished observations)

Figure 9. Basal Arc levels are differentially expressed in the GFP/*Fmr1* mosaic mice

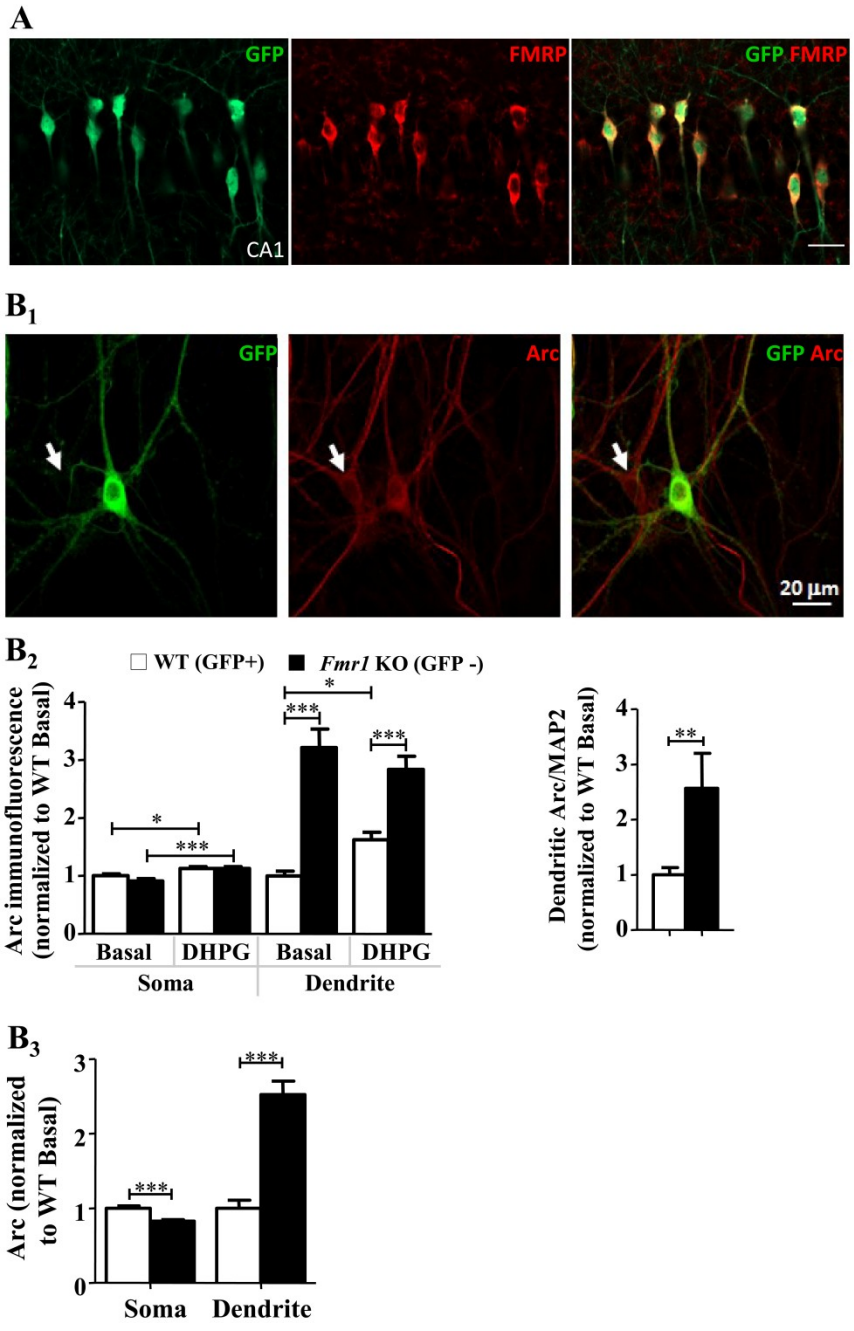

Figure 9. Basal dendritic Arc levels are differentially expressed in the GFP/*Fmr1* mosaic mice. **A.** Double immunofluorescence images of GFP (green) and FMRP (red) in

hippocampal CA1 tissue sections of GFP/*Fmr1* mosaic mice demonstrating the coexpression of GFP and FMRP. Scale bar: 20 μm. **B.** Basal dendritic Arc levels are elevated while somatic Arc levels are reduced in *Fmr1* KO neurons of GFP/*Fmr1* KO mosaic mice as quantified by two different antibodies against Arc: (**B_2**) Synaptic Systems rabbit polyclonal anti-Arc; (**B_3**) Anti-Arc antibody provided by Dr. Paul Worley (Lyford et al., 1995). **B_1**. Double immunofluorescence images of GFP (green) and Arc (red) in dissociated hippocampal neurons (DIV 18-21). White arrows indicate a *Fmr1* KO (GFP-) cell. **B_2**. Left panel: Quantification of somatic and dendritic Arc immunofluorescence levels from neighboring WT and *Fmr1* KO neurons using a rabbit polyclonal Arc antibody from Synaptic Systems in GFP/*Fmr1* KO mosaic cultures. Values are normalized to WT (GFP+) basal (vehicle (H_2O)-treated) neurons of the same culture. Soma: WT = 1.00 ± 0.03; KO = 0.91 ± 0.04; Dendrite: WT = 1.00 ± 0.08; KO = 3.21 ± 0.32. DHPG treatment of GFP/*Fmr1* mosaic cultures increases dendritic Arc levels in WT neurons, but not in neighboring *Fmr1* KO neurons where Arc levels are greatly elevated in basal conditions. Soma: WT (DHPG) = 1.09 ± 0.03 ; KO(DHPG) = 1.10 ± 0.03; Dendrite: WT (DHPG) = 1.62 ± 0.13; KO (DHPG) = 2.84 ± 0.23. Right panel: Quantification of the ratio of dendritic Arc/MAP2 immunofluorescence in neighboring WT and *Fmr1* KO neurons from GFP/*Fmr1* mosaic cultures. **B_3**. Soma: WT = 1.00 ± 0.03; KO = 0.83 ± 0.02. Dendrite: WT = 1.00 ± 0.11; KO = 2.5 ± 0.87. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (From Niere et al, 2012 and unpublished observations)

Figure 10. Dendritic *Arc* mRNA levels are similarly expressed in WT and *Fmr1* KO neurons of GFP/*Fmr1* mosaic mice

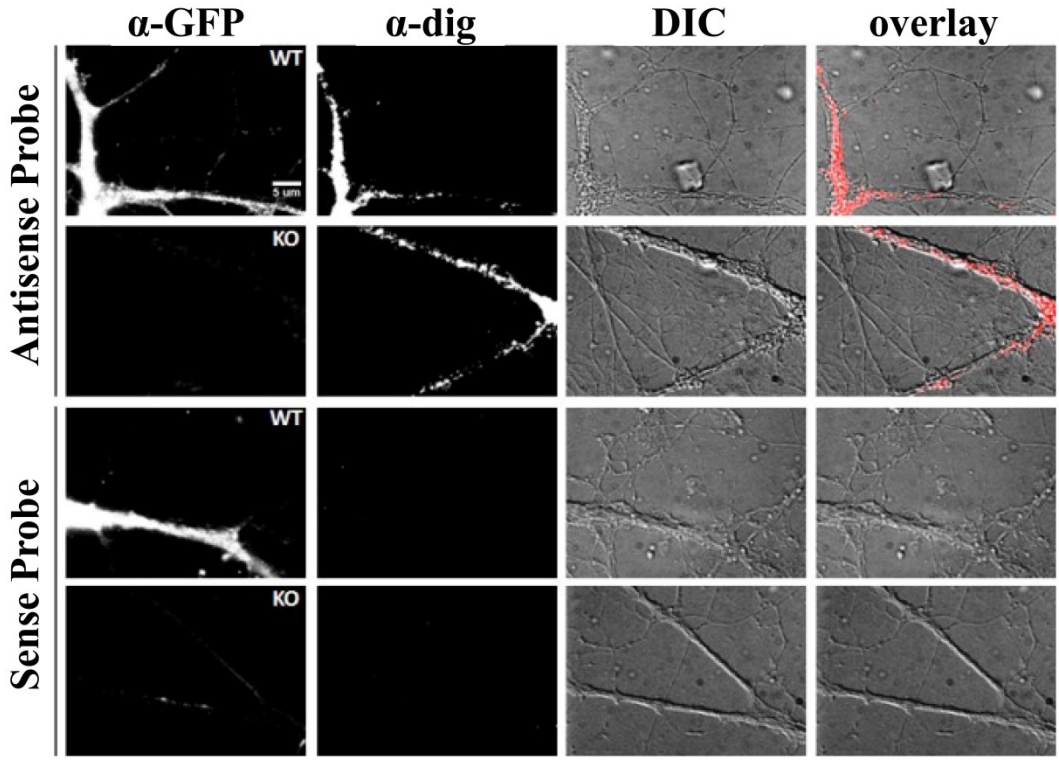

Figure 10. Dendritic *Arc* mRNA levels are similarly expressed in WT and Fmr1 KO neurons of GFP/Fmr1 mosaic mice. Fluorescence *in situ* hybridization (FISH) reveals comparable dendritic *Arc* mRNA levels in dissociated cultured WT and *Fmr1* KO neurons prepared from the hippocampi of GFP/*Fmr1* mosaic mice. α–GFP column: GFP staining identifies WT (GFP+) and *Fmr1* KO (GFP–) cells. WT: 1.00 ± 0.08, n = 53; KO = 0.90 ± 0.08 (*Arc* FISH intensity; normalized to WT neurons) α-dig column: digoxigenin staining identifies hybridized digoxigenin-labeled antisense and sense (control) oligo-probes. *Arc* sense probes are unstained. DIC column: DIC images of dendrites. Overlay column: overlay images of digoxigenin labeled probes (red) on DIC images of respective dendrites. Scale bar: 5 μm. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison. (From Niere et al, 2012)

Figure 11. Neuronal activity does not affect the difference in basal dendritic Arc levels between WT and *Fmr1* KO neurons of GFP/*Fmr1* mice

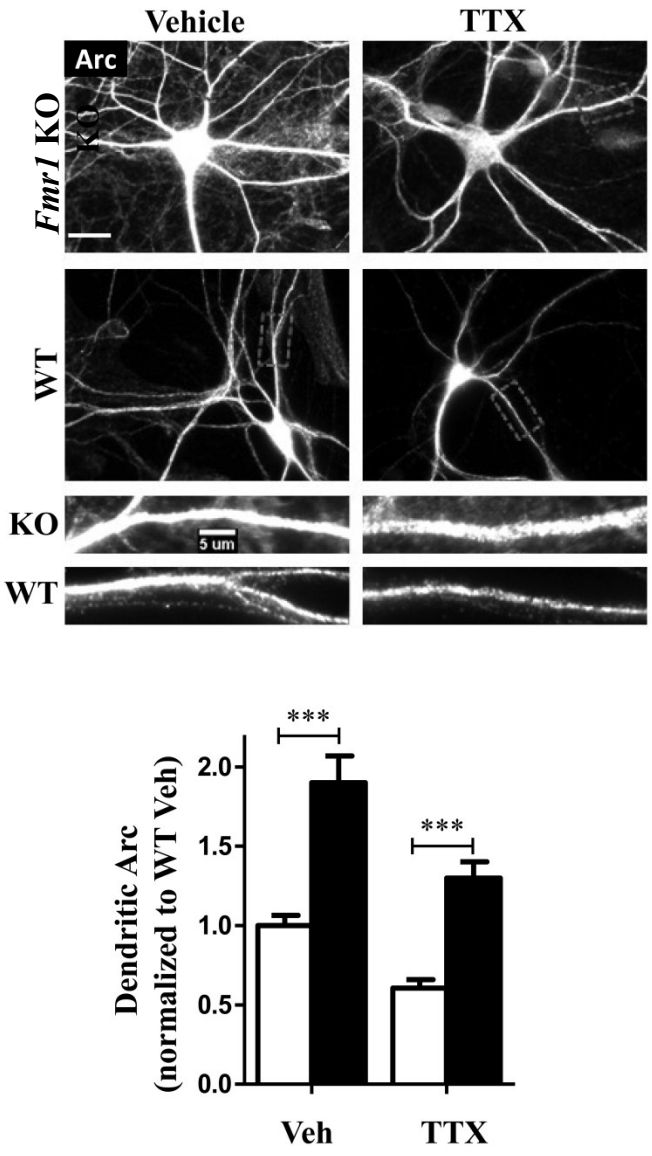

Figure 11. Neuronal activity does not affect the difference in basal dendritic Arc levels between WT and *Fmr1* KO neurons of GFP/*Fmr1* mice. Top panel: Representative images of Arc staining in WT and *Fmr1* KO neurons from GFP/*Fmr1* mosaic mice. Scale bar: 5 μm. Bottom panel: quantification of basal dendritic Arc levels in vehicle (H_2O) and TTX (1 μM, 14-18 hrs). Values are normalized to WT in vehicle. Veh: WT =

1.00 ± 0.06, *Fmr1* KO = 1.90 ± 0.18; TTX: WT = 0.61 ± 0.05, *Fmr1* KO = 1.30 ± 0.10. N = # cells per condition. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; ***$p < 0.001$. (From Niere et al, 2012)

Figure 12. PP2A is necessary for a rapid, mGluR-induced increase in dendritic Arc expression

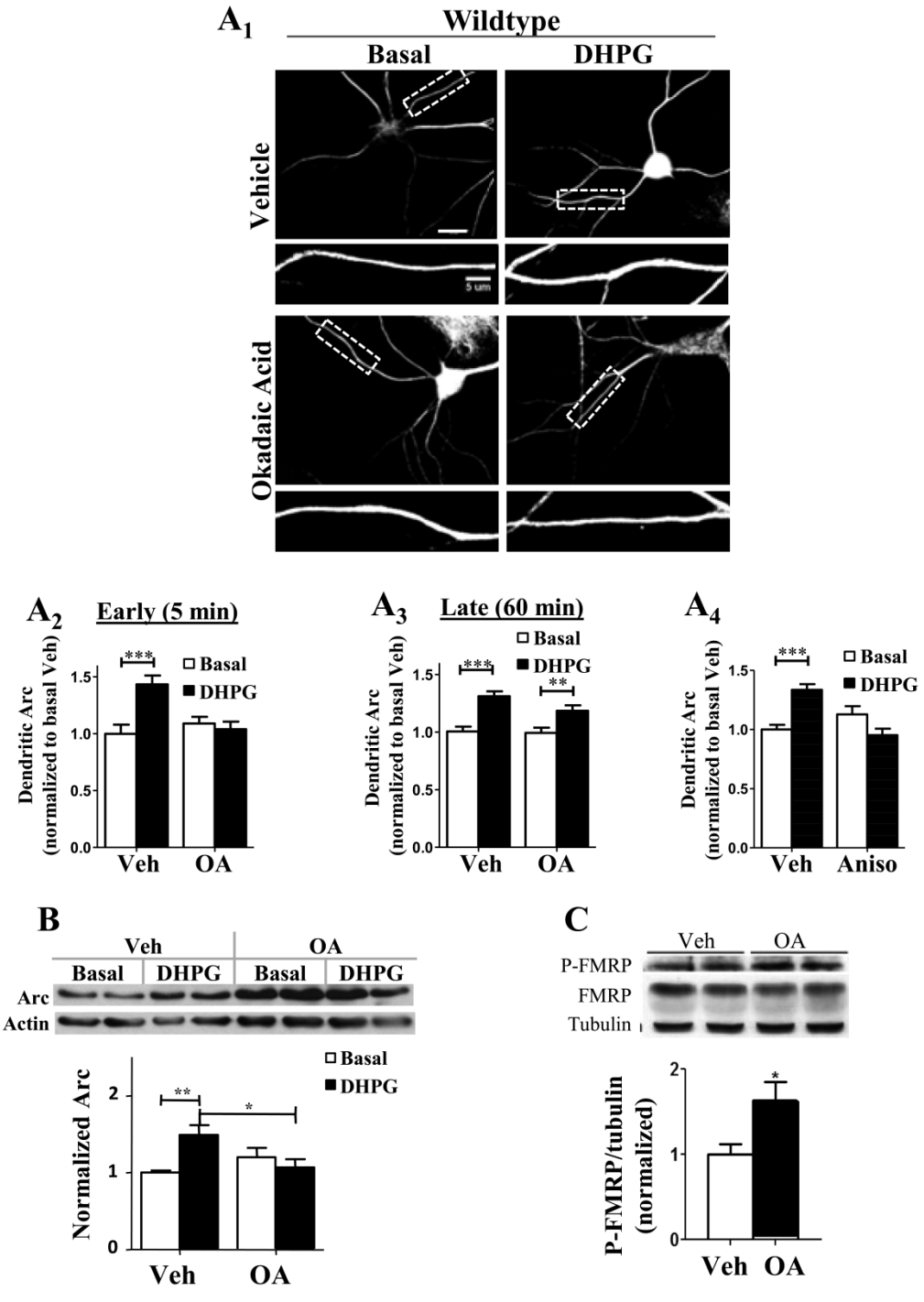

Figure 12. PP2A is necessary for a rapid, mGluR-induced increase in dendritic Arc expression. **A.** Application of OA (10 nM, 1 hr) blocks the rapid but not the late increase in dendritic Arc protein in response to mGluR activation by DHPG (100 μM, 5 minutes) in dissociated WT mouse hippocampal neurons. **A_1**. Representative images of Arc immunofluorescence at 5 minutes after DHPG or vehicle (H_2O) treatment. Scale bar: 20 and 5 μm. Quantification of dendritic Arc levels at 5 (**A_2**) and 60 minutes (**A_3**) after addition of DHPG. Values are normalized to basal, vehicle-treated cells. **A_2**. Vehicle = 1.00 ± 0.08; DHPG = 1.43 ± 0.08; OA = 1.09 ± 0.06; OA + DHPG = 1.04 ± 0.07. **A_3**. Vehicle = 1.00 ± 0.04; DHPG = 1.31 ± 0.04; OA = 0.99 ± 0.05; OA + DHPG = 1.19 ± 0.05. **A_4**. The translation inhibitor, anisomycin (20 μM, 20 minutes prior to DHPG application) blocks the mGluR-induced, late expression of dendritic Arc by DHPG. Values are normalized to basal, vehicle-treated cells. Vehicle = 1.00 ± 0.04; DHPG = 1.34 ± 0.05; Aniso = 1.13 ± 0.06; Aniso + DHPG = 0.95 ± 0.05. **B.** Pretreatment of OA (10 nM, 1 hour) prevents the DHPG-induced, rapid, total Arc synthesis in dissociated WT mouse hippocampal neurons. Top panel: Representative western blot of Arc and actin. Bottom panel: quantification of Arc levels normalized to basal, vehicle-treated cells. Vehicle = 1.00 ± 0.03; DHPG = 1.49 ± 0.13; OA = 1.20 ± 0.12; OA + DHPG = 1.07 ± 0.11; n = 6 cultures. Actin and total ERK were used as loading controls. There was no significant difference in the levels of actin or total ERK for each condition. **C**. P-FMRP level is elevated in OA-treated (10 nM, 1 hr), dissociated WT mouse hippocampal neurons. Top panel: Western blot of whole cell lysates from WT neurons in the presence of vehicle or OA. Lower panel: quantification of P-FMRP/tubulin normalized to vehicle-treated cells. Veh = 1.00 ± 0.12; OA = 1.62 ± 0.23; n = 3 mice. Statistical analysis by student t-test; $p<0.05$. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. For all four-group comparisons (A and B statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; *$p < 0.05$; **$p < 0.01$; ***$p<0.001$. (From Niere et al, 2012)

Figure 13. An mGluR-induced, rapid synthesis of dendritic Arc is absent in *Fmr1* KO neurons

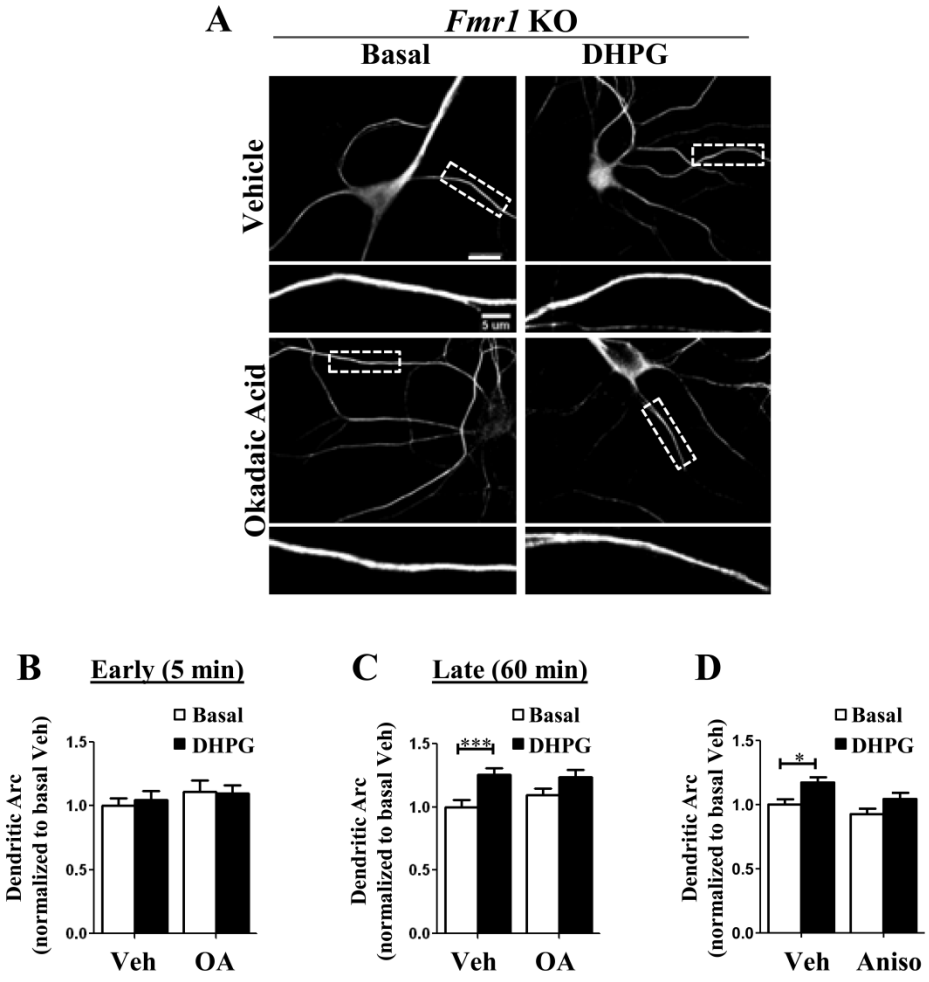

Figure 13. An mGluR-induced, rapid synthesis of dendritic Arc by DHPG (100 μM, 5 min) is absent in *Fmr1* KO neurons. **A**. Representative images of Arc immunofluorescence at 5 min after DHPG in vehicle or OA pretreated neurons. Scale bar: 20 and 5 μm. Quantification of dendritic Arc levels at 5 (**B**) and 60 minutes (**C**) after addition of DHPG. Values are normalized to basal, vehicle-treated cells. **B**. Vehicle = 1.00 ± 0.06; DHPG = 1.05 ± 0.07; OA = 1.11 ± 0.09; OA+DHPG = 1.10 ± 0.07. **C**. Effects of mGluR stimulation on dendritic Arc levels at 1 hour after DHPG in vehicle (Basal = 1.00 ± 0.06; DHPG = 1.26 ± 0.05) or OA treated cultures (OA = 1.09 ± 0.05; OA + DHPG = 1.24 ± 0.06; p = 0.058). **D.** Effects of mGluR stimulation on dendritic Arc levels at 1 hour after DHPG in vehicle (Basal = 1.00 ± 0.04; DHPG = 1.17 ± 0.04) or

anisomycin treated cultures (Aniso = 0.92 ± 0.04; Aniso + DHPG = 1.04 ± 0.05). Values are normalized to basal, vehicle-treated cells. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. For all four-group comparison statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. (From Niere et al, 2012)

Figure 14. Acute expression of wildtype FMRP in *Fmr1* KO neuron lowers basal dendritic Arc and rescues mGluR-induced, rapid expression of Arc

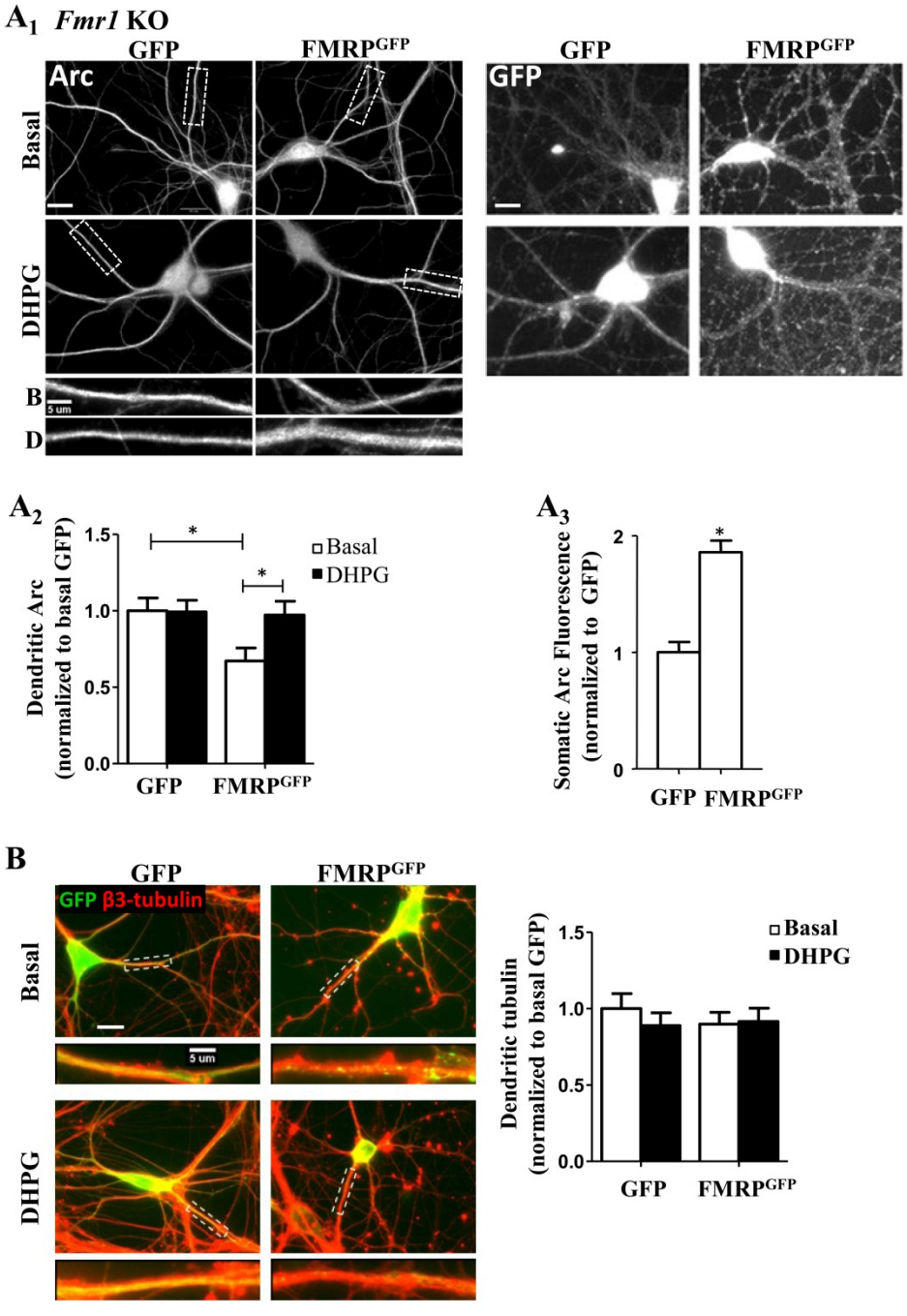

Figure. 14. Acute expression of wildtype FMRP in *Fmr1* KO neurons lowers basal dendritic Arc and rescues mGluR-induced, rapid expression of Arc. **A and C.** *Fmr1* KO neuronal cultures were transfected using lentivirus expressing GFP (control) or an N-terminally GFP tagged FMRP ($FMRP^{GFP}$). For quantification, values are normalized to GFP-infected, vehicle-treated (basal, H_2O) cells. $\mathbf{A_1}$. Representative images of Arc and GFP immunoflourescence of *Fmr1* KO neurons transfected with either GFP or $FMRP^{GFP}$ and then treated with vehicle or DHPG (100 μM, 5 minutes). Scale bar: 20 and 5 μm. $\mathbf{A_2}$. Quantification of dendritic Arc levels. Basal (GFP) = 1.00 ± 0.08; DHPG (GFP) = 0.99 ± 0.08; Basal ($FMRP^{GFP}$) = 0.67 ± 0.08; DHPG ($FMRP^{GFP}$) = 0.97 ± 0.09. **B.** Expression of GFP (control) or an N-terminally GFP tagged FMRP ($FMRP^{GFP}$) does not alter tubulin levels in the dendrites. Left panel: Representative images of double immunoflourescence for β3-tubulin (red) and GFP (green) of infected cells treated with vehicle or DHPG. Scale bar: 20 and 5 μm. Right panel: Tubulin levels are similar in GFP- and FMRP-infected cells. Basal (GFP) = 1.00 ±0.10; DHPG (GFP) = 0.89 ± 0.08; Basal ($FMRP^{GFP}$) = 0.90 ±0.08; DHPG ($FMRP^{GFP}$) = 0.92 ± 0.09. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. For all four-group comparisons statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. For B_2 a student's t-test was used. (From Niere et al, 2012)

Figure 15. Acute expression of wildtype FMRP in *Fmr1* KO neurons rescues total mGluR-induced, rapid expression of Arc

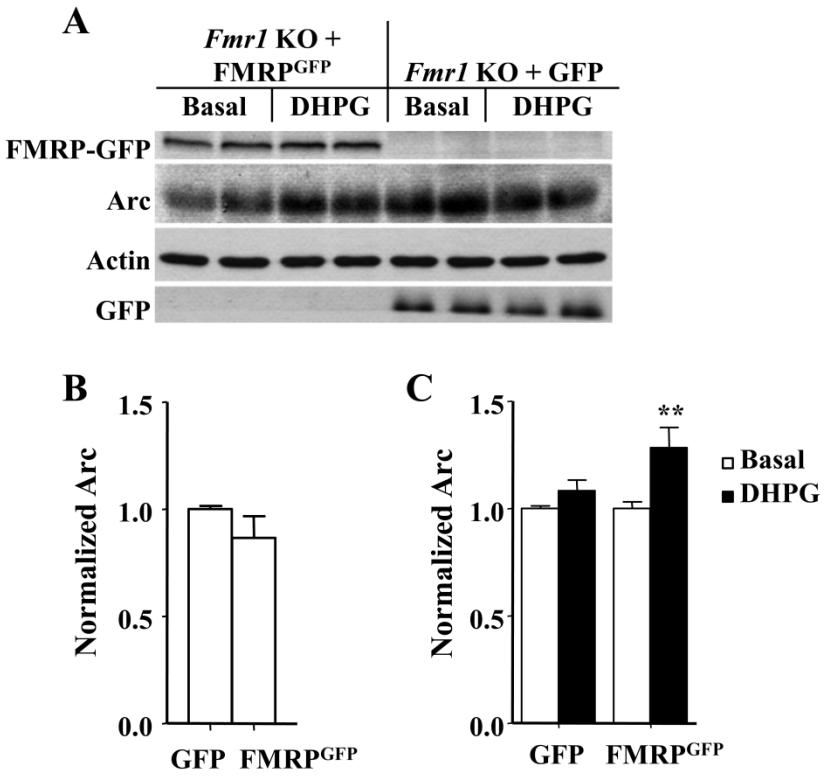

Figure 15. Acute expression of wildtype FMRP in *Fmr1* KO neurons rescues total mGluR-induced, rapid expression of Arc. **A**. Representative western blot of Arc and actin from *Fmr1* KO cultures transfected with either GFP or $FMRP^{GFP}$. **B**. GFP- and FMRP-transfected cells express similar levels of total Arc. GFP: 1.00 ± 0.02; FMRP = 0.92 ± 0.12. **C**. mGluR activation increases total Arc levels in $FMRP^{GFP}$-transfected *Fmr1* KO cells, but not GFP-transfected. Values are normalized to respective basal conditions. Basal (GFP) = 1.00 ± 0.02; DHPG (GFP) = 1.11 ± 0.06; Basal ($FMRP^{GFP}$) = 1.00 ± 0.03; DHPG ($FMRP^{GFP}$) = 1.29 ± 0.13; n = 5 independent cultures. For all four-group comparisons statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; **$p < 0.01$. For **B** a student's t-test was used. (From Niere et al, 2012)

Figure 16. Phosphomutant constructs of S500-FMRP differentially affect basal dendritic Arc and do not rescue the mGluR-induced, rapid dendritic Arc expression

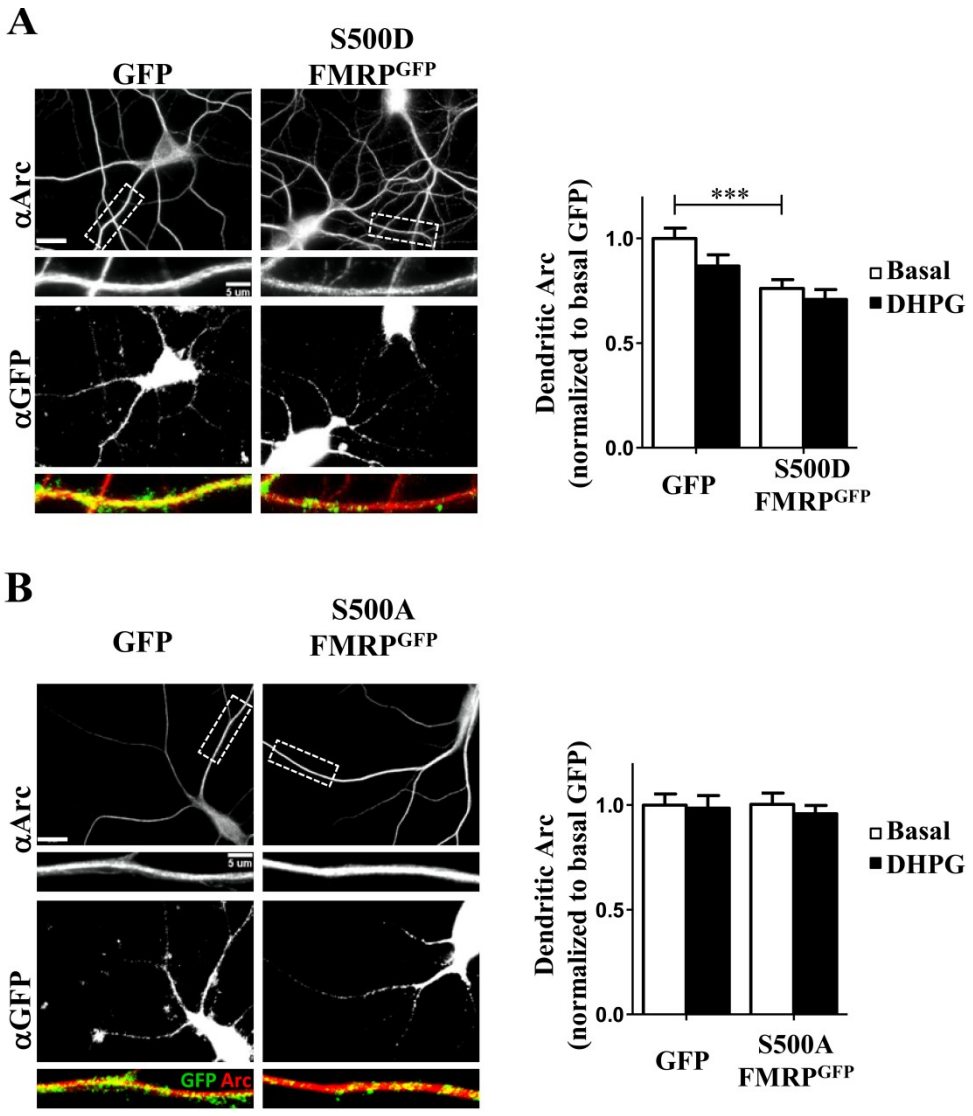

Figure 16. Phosphomutant constructs of S500-FMRP differentially affect basal dendritic Arc and do not rescue the mGluR-induced, rapid dendritic Arc expression. **A-B.** *Fmr1* KO neuron cultures were infected with lentivirus expressing either GFP (control) a phospho- or dephosphomimetic of $FMRP^{GFP}$ (S500D- or S500A-$FMRP^{GFP}$). For quantification of basal dendritic Arc, values are normalized to GFP-infected, vehicle-treated (basal, H_2O) cells. mGluR activation was induced by applying DHPG (100 μM, 5

minutes). Scale bar: 20 and 5 μm. **A.** S500D-FMRP^{GFP}, a phosphomimic form of FMRP, lowers basal dendritic Arc, but does not rescue mGluR-induced increase in dendritic Arc. Left panel: representative images of infected cells stained for stained for Arc (red) and GFP (green) under basal condition. Right panel: Basal (GFP) = 1.00 ± 0.05; DHPG (GFP) = 0.87 ± 0.05; Basal (S500D-FMRP^{GFP}) = 0.76 ± 0.04; DHPG (S500D-FMRP^{GFP}) = 0.71 ± 0.05. **B**. S500A, a dephosphomic form of FMRP, does not affect dendritic Arc levels under basal condition or upon mGluR stimulation with DHPG. Left panel: Representative images of infected cells stained for stained for Arc (red) and GFP (green) under basal conditions. Right panel: Basal (GFP) = 1.00 ± 0.05; DHPG (GFP) = 0.99 ± 0.06; Basal (S500A-FMRP^{GFP}) = 1.00 ± 0.05; DHPG (S500A-FMRP^{GFP}) = 0.96 ± 0.04. 10-15 cells/culture were obtained for each condition, and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; ***$p < 0.001$. (From Niere et al, 2012)

Figure 17. FMRP and its phosphorylation state at S500 affect mGluR-induced depression of mEPSCs in dissociated hippocampal neurons

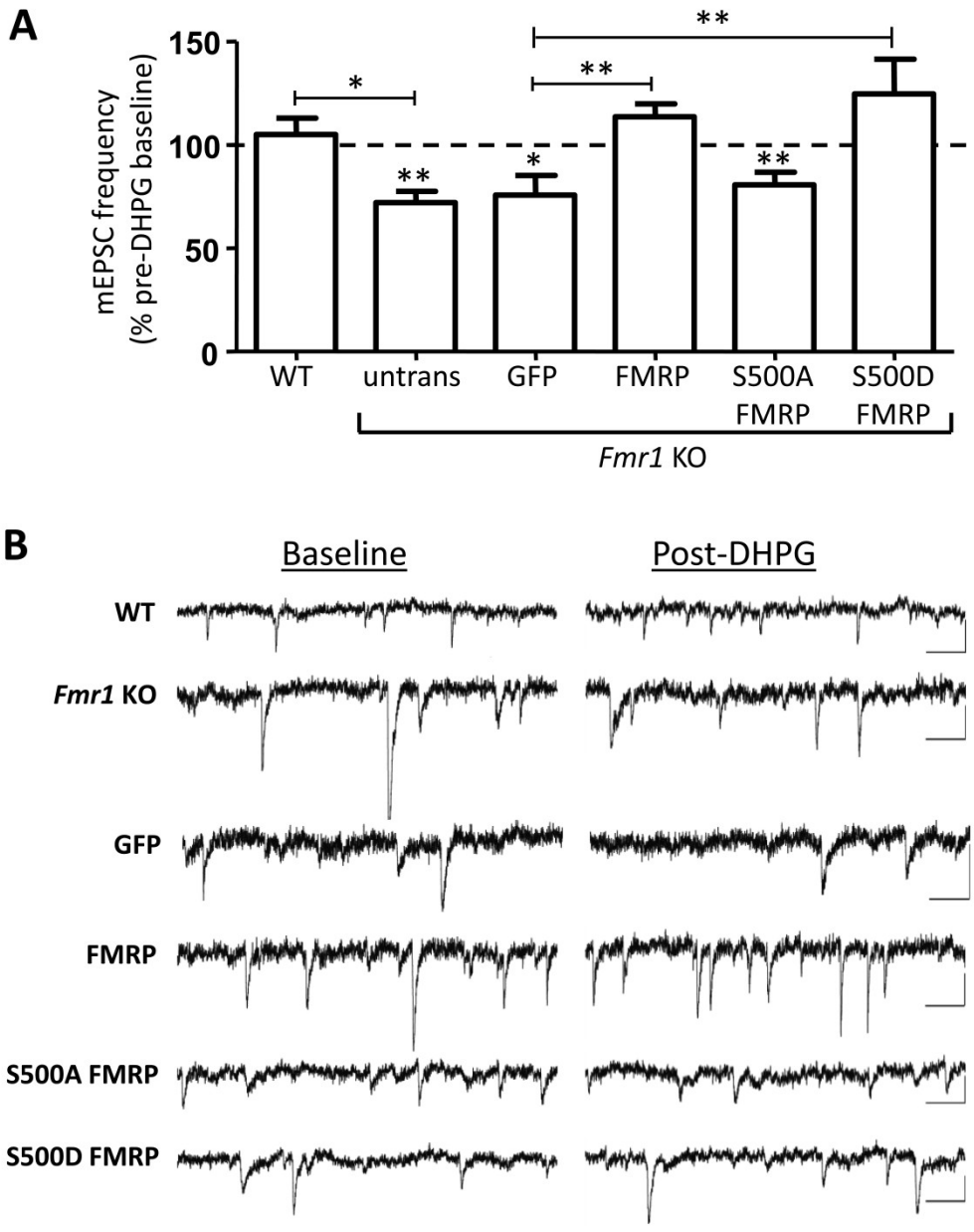

Figure 17. FMRP and its phosphorylation state at S500 affect mGluR-induced depression of mEPSCs in dissociated hippocampal neurons. **A.** Effects of brief mGluR activation

DHPG (100 μM, 5 min) on mEPSC frequency (measured at 5-15 minutes after DHPG onset) in cultured untransfected WT or *Fmr1* KO and *Fmr1* KO neurons with a lentiviral-mediated transfection of $FMRP^{GFP}$ S500A-$FMRP^{GFP}$, or S500D-$FMRP^{GFP}$. mEPSC frequency is normalized to a 5-minute pre-DHPG baseline. WT untransfected = 105 ± 8%; *Fmr1* KO untransfected = 72 ± 6%; *Fmr1* KO transfected with the following: GFP = 76 ± 9%; $FMRP^{GFP}$ = 114 ± 6%; S500A-$FMRP^{GFP}$ = 81 ± 6%; S500D-$FMRP^{GFP}$ = 125 ± 17%. Statistical analysis: Paired t-test; *$p < 0.05$, **$p < 0.01$. For comparison among groups, one-way ANOVA, Newman-Keuls post-hoc comparison was used; *$p < 0.05$, **$p < 0.01$. **B.** Representative mEPSC recordings during baseline and 10 min after DHPG application from WT, *Fmr1* KO, and *Fmr1* KO neurons transfected with GFP, $FMRP^{GFP}$ (FMRP), S500A-$FMRP^{GFP}$ (S500A FMRP) or S500D-$FMRP^{GFP}$ (S500D FMRP). Scale: 10 pA, 100 ms. (From Niere et al, 2012)

Table 2. Electrophysiological properties of *Fmr1* KO neurons with lentiviral-mediated expression of $FMRP^{GFP}$ and FMRP phosphorylation site mutants

		WT	*Fmr1 KO*				
		untrans	untrans	GFP	$FMRP^{GFP}$	S500A-FMRP	S500D-FMRP
SR (MΩ)	B	24 ± 2	19 ± 3	24 ± 2	28 ± 3	19 ± 1	19 ± 2
	D	26 ± 2	20 ± 3	25 ± 2	26 ± 3	19 ± 1	18 ± 1
R_n (MΩ)	B	209 ± 22	221 ± 26	163 ± 14	206 ± 24	166 ± 18	141 ± 21
	D	224 ± 22	215 ± 24	171 ± 15	204 ± 20	179 ± 24	149 ± 22
I_H (pA)	B	-36 ± 17	-62 ± 14	-75 ± 14	-54 ± 10	-55 ± 8	-91 ± 18
	D	-81 ± 17**	-114 ± 28**	-91 ± 13**	-66 ± 10*	-70 ± 9**	-105 ± 15*
mEPSC frequency (Hz)	B	1.4 ± 0.3	1.9 ± 0.3	1.3 ± 0.2	1.2 ± 0.3	1.6 ± 0.2	1.4 ± 0.2
	D	1.5 ± 0.3	1.4 ± 0.3**	1.0 ± 0.2*	1.5 ± 0.4	1.3 ± 0.2**	1.6 ± 0.3
mEPSC amplitude (pA)	B	14 ± 1	19 ± 2	14 ± 1	15 ± 1	16 ± 1	16 ± 1
	D	13 ± 1**	18 ± 2	14 ± 1	15 ± 1	16 ± 9	15 ± 1
V_m (mV)[$]	B	-53 ± 1	-54 ± 1	-50 ± 2	-53 ± 1	-53 ± 1	-52 ± 1
N (# of cells)		11	8	14	11	16	13

Table 2. Electrophysiological properties of *Fmr1*KO neurons with lentiviral-mediated expression of FMRPGFP and FMRP phosphorylation site mutants. Electrophysiological properties were measured before and after application of DHPG (100 mM, 5 minutes). On average no significant differences were observed in the series resistance within any experimental group. [$] not corrected for junction potential. Asterisks indicate that the DHPG treated condition (D) is different from baseline (B). *$p < 0.05$; **$p < 0.01$. (From Niere et al, 2012)

Figure 18. Inhibition of Gp 1 mGluR activity partially rescues the elevated basal dendritic expression in *Fmr1* KO neurons

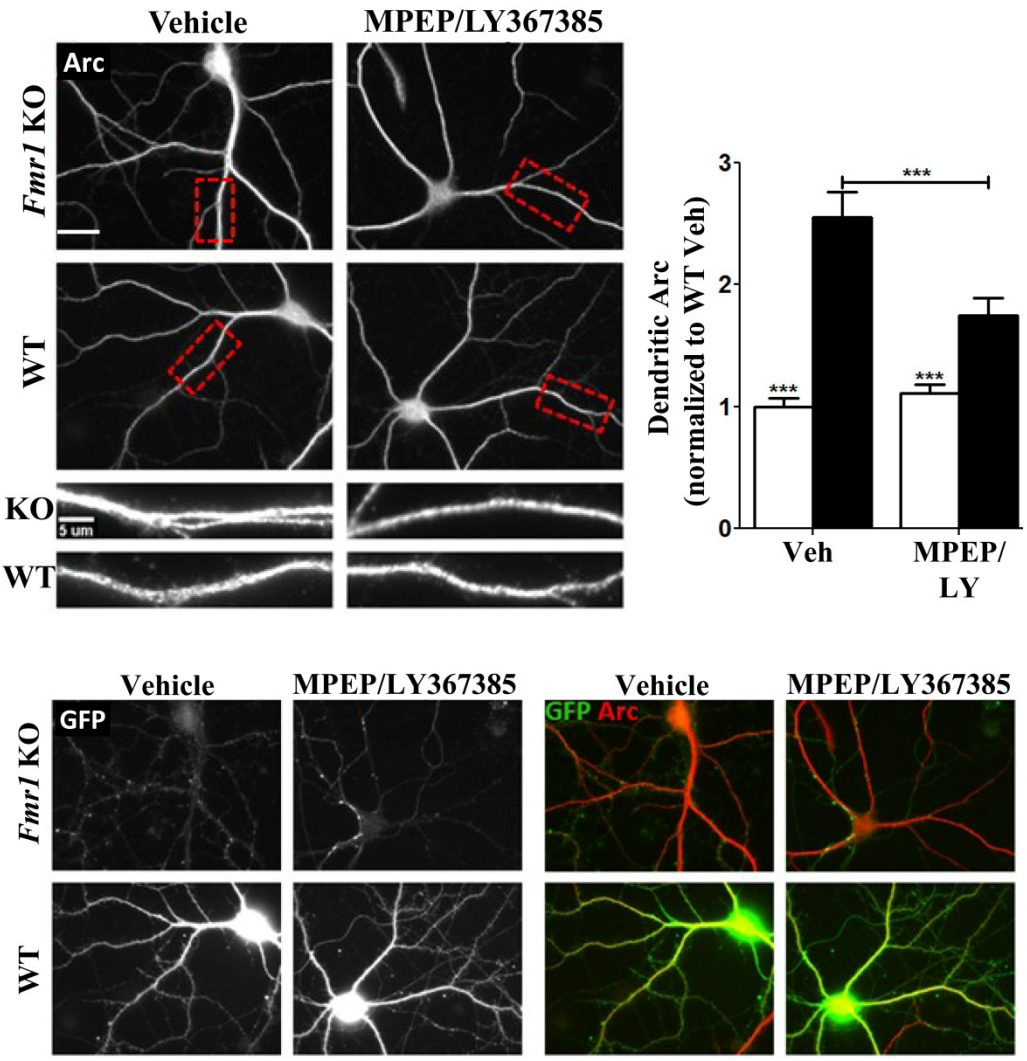

Figure 18. Inhibition of Gp 1 mGluR activity partially rescues the elevated basal dendritic Arc expression in Fmr1 KO neurons. Top left panel: Representative images of Arc staining in WT and *Fmr1* KO neurons from GFP/*Fmr1* mosaic mice. Scale bar: 5 μm. Bottom panel: Representative images of GFP staining to distinguish WT from *Fmr1* KO cells and merged images of Arc (red) and GFP (green). Top right panel: quantification of basal dendritic Arc levels in vehicle (H_2O) and MPEP (10 mM, 14-18 hours) and LY367385 (1mM, 14-18 hours). Values are normalized to WT in vehicle.

Veh: WT = 1.00 ± 0.07, *Fmr1* KO = 2.55 ± 0.21; MPEP/LY367385: WT = 1.11 ± 0.07, *Fmr1* KO = 1.75 ± 0.14. N = # cells per condition. 10-15 cells/culture were obtained for each condition and experiment was repeated in 3-4 independent culture preparations. Statistical analysis by two-way ANOVA, Bonferroni post-hoc comparison; ***$p < 0.001$. (From Niere et al, unpublished observations)

Figure 19. A working model of the acute role of FMRP regulation of Arc translation and mGluR-LTD

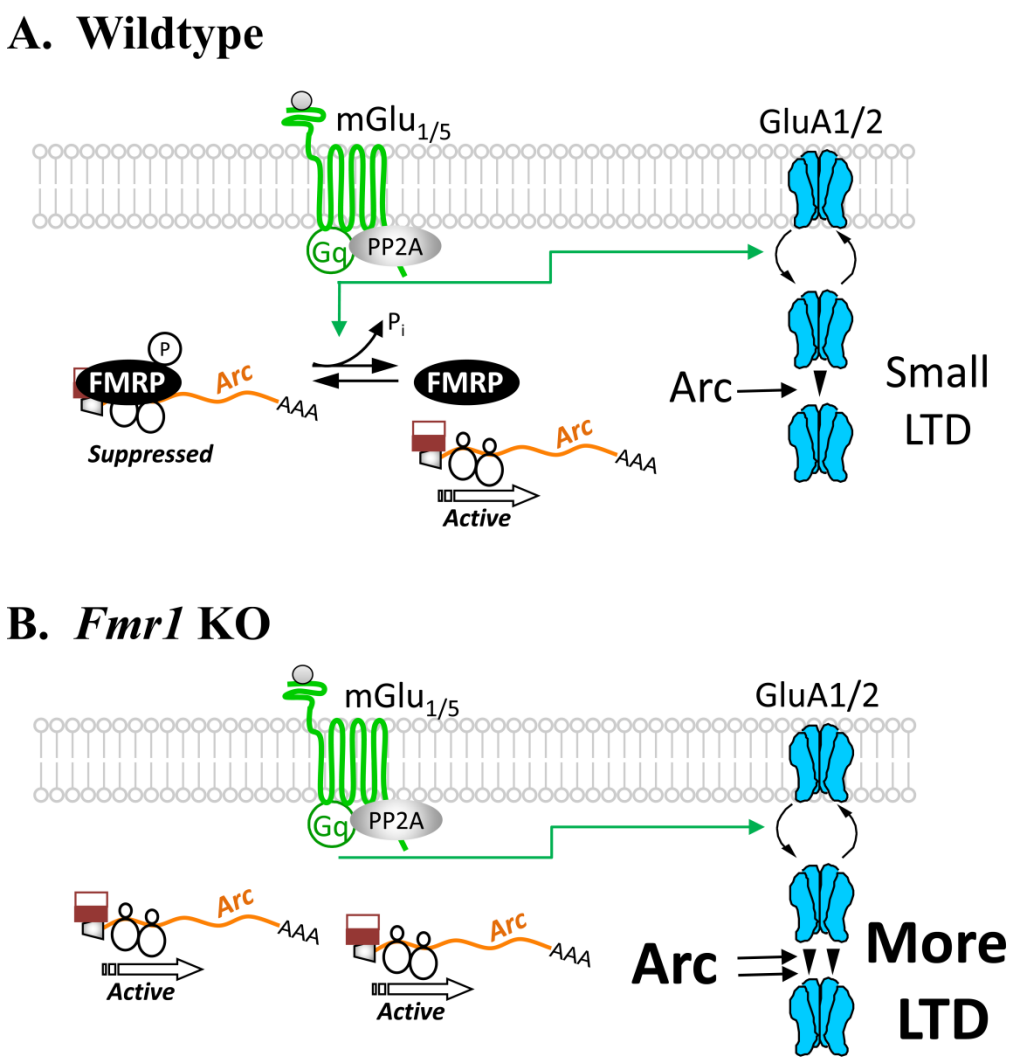

Figure 19. A working model of the acute role of FMRP regulation of Arc translation and mGluR-LTD. **A.** In wildtype mice, phosphorylated FMRP functions to suppress Arc translation in dendrites under basal or unstimulated conditions. As yet unknown for *Arc* mRNA, the translational suppression by FMRP may occur at the initiation or elongation step. Gp 1 mGluR agonism stimulates endocytosis of AMPA receptors (GluRA1 and 2) as well as PP2A mediated dephosphorylation of FMRP (Narayanan et al., 2007). FMRP dephosphorylation contributes to activation of new synthesis of Arc (Figs. 6, 7, 12, 13 and 16) which is required to maintain the persistent endocytosis of AMPARs that

underlies LTD (Figs. 6 and 7) (Park et al., 2008; Waung et al., 2008). **B.** In *Fmr1* KO mice the loss of FMRP mediated translational suppression of Arc leads to elevated steady state levels of dendritic Arc (Fig. 9). Gp 1 mGluR stimulation causes an endocytosis of AMPARs; but because of the elevated basal dendritic Arc levels in *Fmr1* KO dendrites (Fig. 2), no mGluR-stimulated synthesis of Arc is required to maintain LTD (Hou et al., 2006; Nosyreva and Huber, 2006). (From Niere et al, 2012)

CHAPTER FOUR

Discussion

FMRP serves an acute, cell-autonomous role in mGluR-dependent Arc translation and LTD

The group 1 mGluR-dependent LTD in the *Fmr1* KO mouse is enhanced and unreliant on protein synthesis (Hou et al., 2006; Huber et al., 2002; Nosyreva and Huber, 2006). However, it is unknown whether FMRP plays an acute role in mGluR-LTD or the nature of FMRP function in this form of synaptic plasticity. The work herein described provides evidence that FMRP functions as a translational switch to regulate the steady state dendritic Arc levels and the mGluR-triggered Arc synthesis during LTD. Furthermore, the collective findings from obtained the WT, *Fmr1* KO, and GFP/*Fmr1* mosaic mice reveal that FMRP functions cell-autonomously. In the hippocampal neurons of WT and *Fmr1* KO mice, I have found that Arc levels in the soma and dendrites and are disparate. The *Fmr1* KO neurons, in comparison to the cell soma of WT neurons, contain less Arc. However, the Arc levels in the dendrites of WT neurons are significantly lower compared to the *Fmr1* KO (Fig. 8B). Furthermore, I have observed that the activation of the Gp 1 mGluR swiftly increases the level of Arc in the dendrites of WT neurons but not of *Fmr1* KO (Figs. 8B and $12A_2$). These findings are also apparent in the dissociated hippocampal neurons of *GFP/Fmr1* mice, in which WT (GFP+) and *Fmr1* KO (GFP-) are intermingled (Fig. 9B). The similarity of the basal and the mGluR-stimulated Arc levels in the dendrites between WT and GFP+ cells and between *Fmr1* KO and GFP- cells reveals that FMRP regulates Arc in a cell-autonomous fashion.

I have also found that an acute, 10-day expression of FMRP in the *Fmr1* KO reduces the basal level of Arc in the dendrites and restores the Gp 1 mGluR-triggered synthesis of dendritic Arc (Fig. $14A_2$). Additionally, I have observed that FMRP, when phosphorylated at S500, functions to repress the steady state level of Arc in the dendrites and attenuate the mGluR-dependent synaptic depression, similar to what I have found in WT and FMRP-infected *Fmr1* KO cells (Fig. 16A). In response to Gp 1 mGluR stimulation by DHPG, FMRP is rapidly dephosphorylated by PP2A (Narayanan et al., 2007). This process is necessary for the mGluR-triggered, rapid increases in dendritic Arc and LTD, since *Fmr1* KO cells that are acutely expressing a phosphomutant form of FMRP (S500D-$FMRP^{GFP}$) fail to exhibit an increase in dendritic Arc following DHPG treatment (Fig. 16A). The phosphorylation of FMRP at S500 has been previously shown to associate with stalled or translationally-suppressed polyribosomes (Ceman et al., 2003). The dual translational control of FMRP also occurs with its other target mRNAs, such as SAPAP3, MAP1B, and PSD-95 (Coffee et al., 2011; Muddashetty et al., 2011; Narayanan et al., 2007), suggesting that the switch of FMRP from a phosphorylated to a dephosphorylated state may be a common mechanism, by which FMRP mediates translational control.

PP2A and FMRP dephosphorylation are necessary for an immediate, early phase of mGluR-triggered Arc translation and LTD

The role of the protein serine/threonine phosphatase, PP2A, in mGluR-LTD is ambiguous. Okadaic acid has been reported to enhance DHPG-LTD (Schnabel et al., 2001); however, the study uses OA at a greater concentration – 10X than my working

concentration of 100 nM – which can likely inhibit PP1 activity (Swingle et al., 2007). In contrast, another study accounts normal mGluR-LTD in a transgenic mouse that expresses the simian virus, SV40 small-t-antigen, which is a PP2A inhibitor (Nicholls et al., 2008). The level of PP2A activity in this transgenic mouse has not been demonstrated. The differences in species and slice preparation among studies may also contribute to these varying results. In my investigation on the role of PP2A in mGluR-LTD, I have used a low concentration of OA, which selectively inhibits PP2A (Gong et al., 2000). By using 100 nM OA, I have found that at this concentration an early, but not a late phase of DHPG-LTD is attenuated (Fig. 6A). Consistent with this finding, OA and a distinct PP2A inhibitor, fostriecin, block the immediate, mGluR-induced increase in dendritic Arc levels, but not those at one hour following DHPG treatment. Similar to what I have observed when PP2A is inhibited, the rapid, mGluR-induced elevation of dendritic Arc is absent in the hippocampal neurons of *Fmr1* KO; although, the late increase in dendritic Arc remains intact.

The expression of the wildtype FMRP, but not S500A- or S500D-FMRP, rescues the mGluR-induced, rapid synthesis of Arc (Figs. 14, 15 and 16). These findings implicate the necessity of the dephosphorylating step of FMRP in the mGluR-mediated translation. PP2A and $mGlu_5$ directly interact (Mao et al., 2005a); and upon activation of $mGlu_5$, PP2A quickly dephosphorylates FMRP within 1-2 minutes (Narayanan et al., 2007). If phosphorylated FMRPs are bound to mRNAs that are already initiated and FMRP functions to stall translating ribosomes as has been suggested (Ceman et al., 2003; Darnell et al., 2011), the swift dephosphorylation of FMRP by PP2A would provide a rapid mechanism to stimulate the synthesis of specific proteins within minutes of

activating $mGlu_5$. The data presented in this work and the published results from this lab support this proposed idea as the activation of the Gp 1 mGluR by DHPG induces – within five minutes following DHPG washout – a rapid synthesis of Arc in dendrites (Waung et al., 2008). The data that I have obtained from using the phosphomimic and dephosphomimic mutants of FMRP provide additional support for the relevance of rapid dephosphorylation in modulating translation, as other investigators and I have determined that the dephosphorylation of FMRP is vital in the mGluR-dependent translation of Arc. First, although it suppresses basal dendritic Arc, the expression of S500D-$FMRP^{GFP}$ in *Fmr1* KO neurons does not restore the Gp 1 mGluR-triggered elevation of Arc in the dendrites (Fig. 16A). Second, *Fmr1* KO neurons that have been infected with a dephosphomutant construct of FMRP (S500A-$FMRP^{GFP}$), which associates with translating polyribosomes, fail both in suppressing basal dendritic Arc and in expressing DHPG-induced increase in dendritic Arc (Fig. 16B) (Ceman et al., 2003). Third, the expression of FMRP in *Fmr1* KO restores the suppression of basal Arc and mGluR-dependent increase of Arc in the dendrites (Fig 14A). These three findings collectively implicate the necessity of shifting FMRP from a phosphorylated to a dephosphorylated form in the Gp 1 mGluR-mediated translation of Arc. It is solely the presence of a phospho-switchable FMRP and not of a permanently phosphorylated or dephosphorylated FMRP at S500 that promotes the Gp 1 mGluR-dependent synthesis of Arc in neuronal dendrites. The phosphorylation of FMRP suppresses basal dendritic Arc levels and mGluR-LTD in cultured mouse neurons. The activation of Gp 1 mGluR can also stimulate translation initiation and regulate elongation, which are required for mGluR-triggered translation of *Arc* and mGluR-LTD (Banko et al., 2006; Hou and

Klann, 2004; Park et al., 2008). These general translational control mechanisms likely work in concert with the dephosphorylation of FMRP to achieve the rapid and robust translational activation of specific mRNAs in response to the activation of $mGlu_1$ and $mGlu_5$.

Phosphorylated FMRP suppresses basal dendritic Arc levels and mGluR-LTD in cultured mouse neurons

I have found that the activation of the Gp 1 mGluR in cultured *Fmr1* KO hippocampal neurons induces a robust depression of synaptic function, which is reminiscent of the exaggerated mGluR-LTD that is observed in acute *Fmr1* KO hippocampal slices (Fig. 17) (Huber et al., 2002). In the complete WT and *Fmr1* KO cultures as well as in the GFP/*Fmr1* mosaic cultures, I have documented enhanced Arc levels in the dendrites, but not in the soma of *Fmr1* KO neurons (Figs. 8B, 9B and 14A). The compartment-dependent changes in Arc protein of *Fmr1* KO neurons may explain why differences in the total Arc protein between WT and *Fmr1* KO mice are not observed with western blots (Fig. 8A). As addressed earlier, the elevated dendritic Arc levels detected in *Fmr1* KO cells are due to a cell autonomous function of FMRP; and the enhanced basal Arc expression in the dendrites are not mediated by changes in dendritic *Arc* mRNA or neuronal activity (Figs. 10 and 11). Because basal dendritic Arc is decreased by a lentiviral-mediated expression of $FMRP^{GFP}$ and S500D-$FMRP^{GFP}$, but not S500A-$FMRP^{GFP}$, I infer that the phosphorylation of FMRP at S500 suppresses Arc in the dendrites. This conclusion is likewise supported by the finding that *Arc* mRNA in the dendrites is associated with the exon junction complex (EJC), suggesting it has not

undergone a pioneer round of translation (Giorgi et al., 2007). If there were increased activity, based on the interaction of *Arc* and the EJC, I would expect to have less *Arc* in the *Fmr1* KO compared to WT neurons as the translation of *Arc* coupled to the EJC promotes the degradation or destabilization of the transcript. Because 41% of *Arc* mRNA is not associated with the EJC, it is untenable to rule out the possibility that there is a higher translation rate of *Arc* that is not coupled with the EJC in *Fmr1* KO (Giorgi et al., 2007). FMRP and the EJC components eIF4III and UpF1 can be immunoprecipitated together. Therefore, it is plausible that FMRP may be necessary for the stabilization of *Arc* and the EJC; and the absence of FMRP may abrogate the nonsense-mediated RNA decay that couples *Arc* to a limited or pioneer round of translation. Additionally, it has not been determined how many ribosomes are involved in the pioneer round of translation. Although the phosphorylation state of FMRP does not alter its binding to mRNAs, FMRP-target mRNAs are more translatable, which may be due to a loss of ribosomal stalling, in the absence of FMRP, suggesting that the increased Arc expression in the dendrites may be due to an upregulation of the translation rate of *Arc* (Ceman et al., 2003; Darnell et al., 2011; Zalfa et al., 2006). Altogether, my findings implicate a cell-autonomous role for phosphorylated FMRP in mediating the dendritic translational suppression of *Arc* mRNA. However, the mechanism that promotes the exaggerated basal translation of Arc in the dendrites of *Fmr1* KO requires determination.

The differential effects of FMRP in the levels of somatic and dendritic Arc are unexpected. In agreement with previous results (Figs. 8B and 9B) (Dictenberg et al., 2008; Steward et al., 1998a), I have not observed a difference in the levels of dendritic *Arc* mRNA in the WT and *Fmr1* KO neurons (Fig. 10). It is plausible however that

FMRP does not regulate or interact with *Arc* mRNA in the cell soma either due to a change in the phosphorylation state *Arc* and/or FMRP or due to competitive interactions of FMRP with other proteins and RNA. Additional experiments are required to define the mechanisms of Arc regulation in the soma.

Similar to basal Arc levels, the acute lentiviral-mediated expression of $FMRP^{GFP}$ or S500D-$FMRP^{GFP}$, but not S500A-$FMRP^{GFP}$, suppresses the DHPG-induced LTD in *Fmr1* KO neurons (Fig. 17). These findings import that the enhanced mGluR-LTD in the *Fmr1* KO neurons stems from the exaggerated dendritic Arc levels and that a phosphorylated FMRP in WT neurons functions to suppress Arc levels and synaptic depression. To correlate the effects of mGluR activation on dendritic Arc levels and mGluR-LTD, we stimulated the Gp 1 mGluR by applying DHPG for five minutes to WT and $FMRP^{GFP}$-transfected *Fmr1* KO neurons to induce synaptic depression (Huber et al., 2002; Nosyreva and Huber, 2006; Waung et al., 2008). Using this protocol which elicits LTD in the acute hippocampal slices in rats, WT and *Fmr1* KO mice, and in cultured rat hippocampal neurons, I have observed an increase in dendritic Arc levels in cultured WT neurons and $FMRP^{GFP}$-infected *Fmr1* KO neurons (Figs. 6, 9B, 12A and 14A). Although the activation of the Gp 1 mGluR increases dendritic Arc in these neurons by 50%, a depression in mEPSCs is not apparent (Fig. 17). These results imply that the mGluR-induced Arc increases are insufficient to support mGluR-LTD in cultured WT and FMRP-expressing *Fmr1* KO hippocampal neurons.

FMRP may function as translational switch in mGluR-LTD

The dual regulation of translation by RNA-binding proteins is vital in mediating specific as well as localized protein expression in several types of polarized cells (Besse and Ephrussi, 2008). To attain site-specific synthesis of proteins, mRNA transcription must be restrained immediately after the production of the transcripts as well as their transport to the appropriate neuronal compartments. With the relevant cues, the translation of quiescent mRNAs is stimulated or reprieved. Because neurons are morphologically complex with thousands of distal synapses, local protein synthesis is vital for regulating individual synapses to ensure proper cellular function, thereby promoting normal development, plasticity, and cognition (Sutton et al., 2006). The cytoplasmic polyadenylation element-binding protein (CPEB) and the Zip code binding protein (ZBP) are examples of other RNA binding proteins that differentially regulate the translation or their mRNA-targets in response to extracellular signals and changes in their phosphorylation state (Huttelmaier et al., 2005; Richter, 2007). Importantly here, the phosphorylation of CPEB-1 by Aurora-A kinase is required for mGluR- and translation-dependent LTD in the Purkinje cells of the cerebellum and motor coordination (McEvoy et al., 2007). The work that I have described here implicates a similar repressor/activator function for the phospho/dephosphorylation switch of FMRP in regulating mGluR-LTD and the levels of Arc in the dendrites. Such dual regulation by RNA-binding proteins may serve as a common mechanism for translation-dependent synaptic plasticity.

The findings that the phospho/dephosphorylation states of FMRP have opposite effects on the translation of Arc suggest that FMRP possesses a dual repressor/activator function in protein synthesis. The phosphorylation of FMRP suppresses both basal and

mGluR-dependent Arc synthesis and mGluR-LTD, while the dephosphorylation of FMRP has the opposite effects. As with other FMRP-target mRNAs, the mGluR-triggered translation of Arc in the dendrites is absent in the *Fmr1* KO neurons (Hou et al., 2006; Muddashetty et al., 2007; Park et al., 2008; Westmark and Malter, 2007). This deficit may persist because basal Arc levels are at a "ceiling" or FMRP may be required for the mGluR-stimulated translation. In contrast to the wildtype $FMRP^{GFP}$, the S500D-$FMRP^{GFP}$ suppresses basal Arc, but does not rescue the mGluR-induced Arc synthesis. Therefore, these data implicate a requirement for FMRP dephosphorylation in the mGluR-dependent elevation of dendritic Arc. The early phase of mGluR-LTD in rat hippocampal slices relies in part on PP2A, the FMRP phosphatase, and Arc synthesis (Narayanan et al., 2007; Waung et al., 2008). Because the mGluR-triggered Arc synthesis requires PP2A and the dephosphorylation of FMRP at S500, my findings suggest that FMRP dephosphorylation regulates the early phase of mGluR-LTD in WT neurons. Interestingly, okadaic acid has a small effect on the magnitude of mGluR-LTD, but completely blocks the mGluR-triggered synthesis of Arc in cultured neurons. This observation could be due to the protein-synthesis independent component of mGluR-LTD (Hou et al., 2006; Huber et al., 2000; Nosyreva and Huber, 2006) that may be mediated by the existing Arc protein (Park et al., 2008; Waung et al., 2008) or posttranslational modifications (Moult et al., 2006). Additionally, FMRP may have other functions in translational control that are independent of its phosphorylation state (Napoli et al., 2008).

The influence of FMRP phosphorylation in controlling the translation of its mRNA-targets is evident in recent studies. The phosphorylation of FMRP at S499 in

mouse and S500 in humans suppresses the synthesis of PSD-95 by promoting a complex of the microRNA (miR-125a) and AGO2 with the 3'UTR of the *Psd95* mRNA. Upon mGluR stimulation, FMRP is dephosphorylated and releases AGO2 from the mRNA (Muddashetty et al., 2011). For the majority of its mRNA-targets, FMRP interacts with the coding sequence of the transcript where FMRP stalls polyribosomes and inhibits translation elongation (Darnell et al., 2011). It has been previously reported that the S500D-FMRP associates more with stalled polysomes while the S500A-FMRP associates more with translating polysomes (Ceman et al., 2003) suggesting that the phosphorylation of FMRP may contribute to ribosomal stalling.

An FMRP-mediated translational switch may function as a form of metaplasticity in response to Arc-inducing experience

Arc, an immediate early gene, is induced in hippocampal neurons in response to novelty and spatial exploration (Guzowski et al., 1999; Link et al., 1995; Lyford et al., 1995) and required for hippocampal-dependent learning (Guzowski et al., 2000; Plath et al., 2006). Upon induction, *Arc* mRNA is rapidly transported to the dendrites (Steward et al., 1998b), where evidence suggests it is translationally suppressed (Giorgi et al., 2007), likely by FMRP (this manuscript). Subsequent synaptic activation of the Gp 1 mGluR and PP2A may stimulate dendritic Arc translation and promote synaptic plasticity, but only in recently activated neurons that have suppressed dendritic *Arc* mRNA. An FMRP-mediated translational switch may function as a mechanism for metaplasticity (Abraham and Bear, 1996), in which the activation history of a neuron, as determined by the induction of *Arc*, affects subsequent synaptic plasticity of its inputs. Such metaplasticity

may contribute to the encoding of *Arc*-inducing experiences. In the absence of FMRP, as in Fragile X Syndrome patients, a deficit in this metaplastic mechanism may result in abnormal plasticity to sensory experiences or learning.

CHAPTER FIVE

Conclusion and Future Directions

The debilitating manifestations of FXS warrant a thorough examination of the FMRP-associated molecular changes that promote the pathology of neuronal dysfunctions. The results of my investigations have identified that FMRP and PP2A work synergistically to regulate Gp 1 mGluR-dependent translation of Arc and synaptic depression of excitatory transmission or LTD. By hindering the dephosphorylation of FMRP, either by manipulating the activity of PP2A or the phosphorylation site of FMRP at Ser500, the mGluR-induced expression of Arc in the dendrites is abrogated, corroborating earlier findings and predictions that FMRP in its phosphorylated form functions as a translational suppressor. Additional evidence that gives credence to the translationally suppressive nature of a phosphorylated FMRP is our finding that the S500D-FMRP reduces the basal level of dendritic Arc and prevents the mGluR-induced synthesis of Arc. Because a dephosphorylated FMRP does not affect the steady state or the mGluR-induced increase of Arc in the dendrites, these findings highlight the necessity of the phosphorylation of FMRP in regulating the steady state level of dendritic Arc, while the process of dephosphorylating FMRP is vital for the activity-induced translation of Arc in the dendrites. In the *Drosophila* model of FXS, *dfmr1*, FMRP and S500D-FMRP, but not S500A-FMRP, can rescue the elevated basal level of chickadee (Coffee et al., 2010). Together with the data herein, only a phosphorylated form of FMRP at Ser500 or a phosphorylatable FMRP can suppress protein synthesis. It is worth noting that 96% of FMRP is basally phosphorylated; and upon mGluR activation, all of the FMRP is dephosphorylated at five minutes while 40% remain dephosphorylated at 30

minutes following DHPG application (Narayanan et al., 2007). In the dendrites, I have observed that the dephosphorylation of FMRP, immediately after DHPG treatment, significantly increases the level of Arc by 30-60%. Although I do not detect elevated total Arc protein in the hippocampal homogenates of the *Fmr1* KO mice, the dendritic Arc level in the *Fmr1* KO CA1 and CA3 neurons is two-fold higher compared to WT neurons. The somatic analysis reveals that the *Fmr1* KO neurons have 20% less Arc protein than WT.

The findings described in this exhibit the importance of FMRP in the rapid synthesis of Arc following Gp 1 mGluR stimulation. However, the relevance of FMRP in regulating the mGluR-induced, immediate translation of Arc may only be relevant in the dendrites, since DHPG increases Arc in the soma of *Fmr1* KO and WT cells. The compartment-dependent difference between the levels of Arc in the dendrites and soma of *Fmr1* KO and WT cells suggest another layer of complexity in Arc expression and perhaps another role of FMRP in regulating Arc that may be pertinent in FXS. Do these compartment-dependent differences reflect a dysregulation of Arc at the level of translation, protein transport or protein degradation? Are these differences mediated by the loss of FMRP or by the phosphorylation state of FMRP?

Zalfa and colleagues have previously demonstrated that the *Arc* mRNA is more translatable in the *Fmr1* KO than WT as measured by the percent of messenger in polysomes (PMP) (Zalfa et al., 2003). In light of the data that I have presented, it may be worth considering whether the *Arc* mRNA is more permissive for translation in the *Fmr1* KO. One way to examine this is by measuring the synthesis rate of Arc using the time-specific tag for the age measurement of proteins (TIME-STAMP). Fusing Arc and

hemagglutinin (HA) with a hepatitis C virus (HCV) protease, which is sandwiched by the cognate protease recognition site, the Arc-HCV-HA fusion protein quickly undergoes a *cis*-regulated scission by the HCV protease. The cleavage results in the removal of the HA tag. Addition of a cell-permeable, small-molecule HCV protease inhibitor, BILN-2061, prevents the default cleavage, allowing the visualization and quantification of the newly synthesized Arc-HCV-HA protein. This method is useful because it provides a way to measure the basal or the activity-dependent translation of Arc-HCV-HA (Lin and Tsien, 2010).

The data which demonstrate that Arc is elevated in the dendrites but reduced in the soma of *Fmr1* KO neurons suggest that this compartment difference may arise from an aberrant transport of Arc from the soma to the dendrite or *vice versa*. Currently, there are no published reports regarding the trafficking of Arc protein. The general consensus is that a translationally-suppressed *Arc* is transported as part of an RNA granule to the dendrites, whereby neuronal activity can stimulate Arc synthesis. Since my work and that of others do not observe a difference in *Arc* between WT and *Fmr1* KO and do not detect elevated Arc in *Fmr1* KO, it is plausible that a deficiency in Arc transport develops in the absence of FMRP (Dictenberg et al., 2008; Krueger et al., 2011; Steward et al., 1998a). One possible scenario of increased somatic Arc in the WT is that FMRP or translation of an FMRP-target protein(s) that is involved in the transport of Arc is suppressed, thereby preventing the movement of Arc into the dendrites. In the absence of FMRP, Arc protein can easily travel to the dendrites as a result of losing the translational suppression exerted by FMRP. This hypothesis can be tested by expressing Arc that is tagged with a photoconvertible fluorescent protein in WT and *Fmr1* KO. Arc trafficking

can then be measured by performing live-cell imaging of with the aid of fluorescence photobleaching (Raab-Graham et al., 2006). As of now, Arc has been shown to interact with very few proteins in neurons – Endophilin, Dynamin, βIV spectrin splice variant βSpIVΣ5, and Presinilin (Bloomer et al., 2007; Chowdhury et al., 2006; Wu et al., 2011b). It is unknown if FMRP and Arc interact either directly or through some proteins; and it will be worth pursuing the mechanisms of Arc trafficking as Arc plays a vital role at the synapse and a novel role of Arc in the nucleus has recently been described (Bloomer et al., 2007; Chowdhury et al., 2006; Irie et al., 2000; Park et al., 2008; Waung et al., 2008). One enticing proposition regarding the influence of FMRP in Arc distribution throughout the neuron is through the regulation of FMRP in the expression of the catalytic subunit of PP2A (PP2Ac) as *Pp2ac* is an mRNA-target of FMRP (Castets et al., 2005). PP2A controls the dephosphorylation of Cofilin, hence affecting the arrangement of actin. Arc has been previously shown to regulate the phosphorylation of Cofilin, and through this interaction Arc effects the expression of LTP (Messaoudi et al., 2007).

One possible explanation for the elevated Arc expression in dendrites is that the absence of FMRP alters the degradation of *Arc*, perhaps by modulating the exon-junction complex. FMRP physically interacts with eIF4III, a vital EJC factor, and colocalizes with UpF1, a nonsense-mediated decay factor. Because EJC is instrumental in limiting the number of translation occurring on a particular transcript, disruption of EJC removes the translation brake beyond the pioneer round by preventing *Arc* decay. The phosphorylation state of FMRP has been demonstrated to influence the translation of its target mRNAs either through the association of FMRP with the translation machinery or

components of RISC. A current report documents that a phosphorylated FMRP, unlike its dephosphorylated form, is less susceptible to ubiquitination and degradation (Nalavadi et al., 2012). The dysregulated ubiquitination and degradation of Arc are implicated in a mouse model of the Angelman syndrome, a disease that presents intellectually disability. In this disease model, the loss of function of the ubiquitin protein E3 ligase, *UBE3A*, leads to excess global Arc, which is a substrate of Ube3A (Greer et al., 2010). Here, the authors observe that the absence of Ube3A results in an increase of total Arc protein by two-fold, a reduction of surface AMPAR expression, and a depression of mEPSC frequency. In light of these findings investigating whether the compartment-dependent difference of Arc expression relies on the ubiquitination and degradation of Arc or other proteins involved in its trafficking may be worthwhile.

The dysregulated expression of Arc and its contribution in altering normal electrophysiological functions are observed in several disease models of autism (Auerbach et al., 2011; Greer et al., 2010). However, the dysregulated Arc expression and its consequential effects on function that others and I see have not been documented in humans. Therefore, the question, how relevant is Arc expression is in human disease, still looms. It will be useful and informative to examine whether the observed molecular and electrophysiological changes in the *Fmr1* KO mouse neurons exist in human cells using neurons derived from induced pluripotent stem cells (iPSC) (Dolmetsch and Geschwind, 2011).

It is fascinating that the elevated Arc level observed in *Fmr1* KO neurons does not affect the basal synaptic transmission since overexpression of Arc greatly alters the synaptic landscape and function (Park et al., 2008; Waung et al., 2008). The stated

observations in this work together with previous findings bespeak that an exaggerated Arc level in the dendrites without a global increase in Arc protein may not be pathologically significant basally, but only upon LTD-inducing stimulus, which requires a rapid synthesis of proteins. Exposure to novelty induces *Arc* transcription and Arc translation; LTD is thought to encode novel objects in a spatial context (Kemp and Manahan-Vaughan, 2004, 2007; Kemp and Bashir, 1999). Because neurons with elevated dendritic Arc are more permissive to LTD, is an *Fmr1* KO mouse deficient in detecting or determining novelty? In addition, does the phosphorylation of FMRP alter novelty-related behaviors? Because the steady state expression of Arc in the dendrites affects a neuron's susceptibility to LTD, examining the degradation of Arc protein, whether it is compartment-dependent, may be worthwhile. As the rapid translation of Arc is implicated in the expression of LTP, it may be profitable to examine whether the elevated level of dendritic Arc in the *Fmr1* KO affects LTP (Messaoudi et al., 2007). Furthermore, it may be worth pursuing whether the phosphorylation state of FMRP regulates LTP, since the *Fmr1* KO mouse displays deficits in this form of synaptic plasticity (Desai et al., 2006; Hu et al., 2008; Shang et al., 2009; Yun and Trommer, 2011; Zhao et al., 2005).

The work entailed in this dissertation presents the necessity of FMRP in the mGluR-mediated translation of Arc and LTD. Particularly, findings from my studies describe the suppressive nature of a phosphorylated FMRP in the synthesis of Arc both basally and upon Gp 1 mGluR stimulation. The absence of FMRP is associated with several dysfunctions at the levels of translation to neuronal networks. I have provided evidence that FMRP is intricately linked to the expression of Arc and mGluR-dependent

synaptic plasticity. However, the extent of Arc function and its regulation, the nature of the compartment-dependent changes in FXS, and the functional role of these changes as well as the phosphorylation of FMRP at the behavioral level remain warranted.

BIBLIOGRAPHY

Abraham, W.C., and Bear, M.F. (1996). Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci *19*, 126-130.

Adermark, L., and Lovinger, D.M. (2007). Combined activation of L-type Ca2+ channels and synaptic transmission is sufficient to induce striatal long-term depression. J Neurosci *27*, 6781-6787.

Adinolfi, S., Ramos, A., Martin, S.R., Dal Piaz, F., Pucci, P., Bardoni, B., Mandel, J.L., and Pastore, A. (2003). The N-terminus of the fragile X mental retardation protein contains a novel domain involved in dimerization and RNA binding. Biochemistry *42*, 10437-10444.

Antar, L.N., Afroz, R., Dictenberg, J.B., Carroll, R.C., and Bassell, G.J. (2004). Metabotropic glutamate receptor activation regulates fragile x mental retardation protein and FMR1 mRNA localization differentially in dendrites and at synapses. J Neurosci *24*, 2648-2655.

Antar, L.N., Dictenberg, J.B., Plociniak, M., Afroz, R., and Bassell, G.J. (2005). Localization of FMRP-associated mRNA granules and requirement of microtubules for activity-dependent trafficking in hippocampal neurons. Genes, brain, and behavior *4*, 350-359.

Anwyl, R. (1999). Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res Brain Res Rev *29*, 83-120.

Anwyl, R. (2009). Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology *56*, 735-740.

APA (2000). Diagnostic and statistical manual of mental disorders: DSM-IV-TR, 4th, text revision edn (Washington, DC, American Psychiatric Association).

Ashley, C.T., Jr., Wilkinson, K.D., Reines, D., and Warren, S.T. (1993a). FMR1 protein: conserved RNP family domains and selective RNA binding. Science (New York, NY *262*, 563-566.

Ashley, C.T., Sutcliffe, J.S., Kunst, C.B., Leiner, H.A., Eichler, E.E., Nelson, D.L., and Warren, S.T. (1993b). Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nature genetics *4*, 244-251.

Auerbach, B.D., Osterweil, E.K., and Bear, M.F. (2011). Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature *480*, 63-68.

Bagni, C., Mannucci, L., Dotti, C.G., and Amaldi, F. (2000). Chemical stimulation of synaptosomes modulates alpha -Ca2+/calmodulin-dependent protein kinase II mRNA association to polysomes. J Neurosci *20*, RC76.

Bailey, D.B., Jr., Raspa, M., Olmsted, M., and Holiday, D.B. (2008). Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. Am J Med Genet A *146A*, 2060-2069.

Bakker, C.E., Verheij, C., Willemsen, R., Helm, R.v.d., Oerlemans, F., Vermey, M., Bygrave, A., Hoogeveen, A., Reyniers, E., Boule, K.D.*, et al.* (1994). Fmr1 knockout mice: a model to study fragile X mental retardation. The Dutch-Belgian Fragile X Consortium. Cell *78*, 23-33.

Banerjee, P., Schoenfeld, B.P., Bell, A.J., Choi, C.H., Bradley, M.P., Hinchey, P., Kollaros, M., Park, J.H., McBride, S.M., and Dockendorff, T.C. (2010). Short- and long-term memory are modulated by multiple isoforms of the fragile X mental retardation protein. J Neurosci *30*, 6782-6792.

Banko, J.L., Hou, L., Poulin, F., Sonenberg, N., and Klann, E. (2006). Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. J Neurosci *26*, 2167-2173.

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

Bardoni, B., Sittler, A., Shen, Y., and Mandel, J.L. (1997). Analysis of domains affecting intracellular localization of the FMRP protein. Neurobiol Dis *4*, 329-336.

Bardoni, B., Willemsen, R., Weiler, I.J., Schenck, A., Severijnen, L.A., Hindelang, C., Lalli, E., and Mandel, J.L. (2003). NUFIP1 (nuclear FMRP interacting protein 1) is a nucleocytoplasmic shuttling protein associated with active synaptoneurosomes. Exp Cell Res *289*, 95-107.

Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. Cell *136*, 215-233.

Bashir, Z.I., Jane, D.E., Sunter, D.C., Watkins, J.C., and Collingridge, G.L. (1993). Metabotropic glutamate receptors contribute to the induction of long-term depression in the CA1 region of the hippocampus. Eur J Pharmacol *239*, 265-266.

Bassell, G.J., and Warren, S.T. (2008). Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron *60*, 201-214.

Basu, S.N., Kollu, R., and Banerjee-Basu, S. (2009). AutDB: a gene reference resource for autism research. Nucleic Acids Res *37*, D832-836.

Bear, M.F., Huber, K.M., and Warren, S.T. (2004). The mGluR theory of fragile X mental retardation. Trends Neurosci *27*, 370-377.

Bechara, E.G., Didiot, M.C., Melko, M., Davidovic, L., Bensaid, M., Martin, P., Castets, M., Pognonec, P., Khandjian, E.W., Moine, H.*, et al.* (2009). A novel function for fragile X mental retardation protein in translational activation. PLoS Biol *7*, e16.

Bell, M.V., Hirst, M.C., Nakahori, Y., MacKinnon, R.N., Roche, A., Flint, T.J., Jacobs, P.A., Tommerup, N., Tranebjaerg, L., Froster-Iskenius, U.*, et al.* (1991). Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell *64*, 861-866.

Bellone, C., Luscher, C., and Mameli, M. (2008). Mechanisms of synaptic depression triggered by metabotropic glutamate receptors. Cell Mol Life Sci *65*, 2913-2923.

Berry-Kravis, E., Raspa, M., Loggin-Hester, L., Bishop, E., Holiday, D., and Bailey, D.B. (2010). Seizures in fragile X syndrome: characteristics and comorbid diagnoses. Am J Intellect Dev Disabil *115*, 461-472.

Besse, F., and Ephrussi, A. (2008). Translational control of localized mRNAs: restricting protein synthesis in space and time. Nat Rev Mol Cell Biol *9*, 971-980.

Bhattacharyya, S., Biou, V., Xu, W., Schluter, O., and Malenka, R.C. (2009). A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors. Nat Neurosci *12*, 172-181.

Bianchi, R., Chuang, S.C., Zhao, W., Young, S.R., and Wong, R.K. (2009). Cellular plasticity for group I mGluR-mediated epileptogenesis. J Neurosci *29*, 3497-3507.

Blackwell, E., Zhang, X., and Ceman, S. (2010). Arginines of the RGG box regulate FMRP association with polyribosomes and mRNA. Human molecular genetics *19*, 1314-1323.

Blanpied, T.A., Scott, D.B., and Ehlers, M.D. (2002). Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. Neuron *36*, 435-449.

Bloomer, W.A., VanDongen, H.M., and VanDongen, A.M. (2007). Activity-regulated cytoskeleton-associated protein Arc/Arg3.1 binds to spectrin and associates with nuclear promyelocytic leukemia (PML) bodies. Brain Res *1153*, 20-33.

Bolshakov, V.Y., and Siegelbaum, S.A. (1994). Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science (New York, NY *264*, 1148-1152.

Boyle, C.A., Boulet, S., Schieve, L.A., Cohen, R.A., Blumberg, S.J., Yeargin-Allsopp, M., Visser, S., and Kogan, M.D. (2011). Trends in the prevalence of developmental disabilities in US children, 1997-2008. Pediatrics *127*, 1034-1042.

Bramham, C.R., Alme, M.N., Bittins, M., Kuipers, S.D., Nair, R.R., Pai, B., Panja, D., Schubert, M., Soule, J., Tiron, A.*, et al.* (2010). The Arc of synaptic memory. Exp Brain Res *200*, 125-140.

Brendler, T., Godefroy-Colburn, T., Carlill, R.D., and Thach, R.E. (1981). The role of mRNA competition in regulating translation. II. Development of a quantitative in vitro assay. The Journal of biological chemistry *256*, 11747-11754.

Brooks-Kayal, A. (2011). Molecular mechanisms of cognitive and behavioral comorbidities of epilepsy in children. Epilepsia *52 Suppl 1*, 13-20.

Brown, W.T. (1990). The fragile X: progress toward solving the puzzle. Am J Hum Genet *47*, 175-180.

Carson, J.H., Worboys, K., Ainger, K., and Barbarese, E. (1997). Translocation of myelin basic protein mRNA in oligodendrocytes requires microtubules and kinesin. Cell Motil Cytoskeleton *38*, 318-328.

Castets, M., Schaeffer, C., Bechara, E., Schenck, A., Khandjian, E.W., Luche, S., Moine, H., Rabilloud, T., Mandel, J.L., and Bardoni, B. (2005). FMRP interferes with the Rac1 pathway and controls actin cytoskeleton dynamics in murine fibroblasts. Human molecular genetics *14*, 835-844.

Caudy, A.A., Myers, M., Hannon, G.J., and Hammond, S.M. (2002). Fragile X-related protein and VIG associate with the RNA interference machinery. Genes & development *16*, 2491-2496.

CDC (2004). Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment--United States, 2003. MMWR Morb Mortal Wkly Rep *53*, 57-59.

CDC (2009). Prevalence of autism spectrum disorders - Autism and Developmental Disabilities Monitoring Network, United States, 2006. MMWR Surveill Summ *58*, 1-20.

Ceman, S., O'Donnell, W.T., Reed, M., Patton, S., Pohl, J., and Warren, S.T. (2003). Phosphorylation influences the translation state of FMRP-associated polyribosomes. Hum Mol Genet *12*, 3295-3305.

Cheever, A., and Ceman, S. (2009). Phosphorylation of FMRP inhibits association with Dicer. RNA *15*, 362-366.

Cho, K., Kemp, N., Noel, J., Aggleton, J.P., Brown, M.W., and Bashir, Z.I. (2000). A new form of long-term depression in the perirhinal cortex. Nat Neurosci *3*, 150-156.

Choi, C.H., Schoenfeld, B.P., Bell, A.J., Hinchey, P., Kollaros, M., Gertner, M.J., Woo, N.H., Tranfaglia, M.R., Bear, M.F., Zukin, R.S.*, et al.* (2011). Pharmacological reversal of synaptic plasticity deficits in the mouse model of fragile X syndrome by group II mGluR antagonist or lithium treatment. Brain Res *1380*, 106-119.

Chonchaiya, W., Schneider, A., and Hagerman, R.J. (2009). Fragile X: a family of disorders. Adv Pediatr *56*, 165-186.

Chotiner, J.K., Khorasani, H., Nairn, A.C., O'Dell, T.J., and Watson, J.B. (2003). Adenylyl cyclase-dependent form of chemical long-term potentiation triggers translational regulation at the elongation step. Neuroscience *116*, 743-752.

Chowdhury, S., Shepherd, J.D., Okuno, H., Lyford, G., Petralia, R.S., Plath, N., Kuhl, D., Huganir, R.L., and Worley, P.F. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron *52*, 445-459.

Chuang, S.C., Bianchi, R., Kim, D., Shin, H.S., and Wong, R.K. (2001). Group I metabotropic glutamate receptors elicit epileptiform discharges in the hippocampus through PLCbeta1 signaling. J Neurosci *21*, 6387-6394.

Chuang, S.C., Zhao, W., Bauchwitz, R., Yan, Q., Bianchi, R., and Wong, R.K. (2005). Prolonged epileptiform discharges induced by altered group I metabotropic glutamate receptor-mediated synaptic responses in hippocampal slices of a fragile X mouse model. J Neurosci *25*, 8048-8055.

Chung, H.J., Steinberg, J.P., Huganir, R.L., and Linden, D.J. (2003). Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. Science (New York, NY *300*, 1751-1755.

Coffee, B., Zhang, F., Warren, S.T., and Reines, D. (1999). Acetylated histones are associated with FMR1 in normal but not fragile X-syndrome cells. Nature genetics *22*, 98-101.

Coffee, R.L., Jr., Tessier, C.R., Woodruff, E.A., 3rd, and Broadie, K. (2010). Fragile X mental retardation protein has a unique, evolutionarily conserved neuronal function not shared with FXR1P or FXR2P. Dis Model Mech *3*, 471-485.

Coffee, R.L., Jr., Williamson, A.J., Adkins, C.M., Gray, M.C., Page, T.L., and Broadie, K. (2011). In vivo neuronal function of the fragile X mental retardation protein is regulated by phosphorylation. Hum Mol Genet.

Collingridge, G.L., Kehl, S.J., Loo, R., and McLennan, H. (1983). Effects of kainic and other amino acids on synaptic excitation in rat hippocampal slices: 1. Extracellular analysis. Exp Brain Res *52*, 170-178.

Collingridge, G.L., Peineau, S., Howland, J.G., and Wang, Y.T. (2010). Long-term depression in the CNS. Nat Rev Neurosci *11*, 459-473.

Collins, S.C., Bray, S.M., Suhl, J.A., Cutler, D.J., Coffee, B., Zwick, M.E., and Warren, S.T. (2010). Identification of novel FMR1 variants by massively parallel sequencing in developmentally delayed males. Am J Med Genet A *152A*, 2512-2520.

Conn, P.J., and Pin, J.P. (1997). Pharmacology and functions of metabotropic glutamate receptors. Annual review of pharmacology and toxicology *37*, 205-237.

Conner, S.D., and Schmid, S.L. (2003). Regulated portals of entry into the cell. Nature *422*, 37-44.

Cooley, L., Verheyen, E., and Ayers, K. (1992). chickadee encodes a profilin required for intercellular cytoplasm transport during Drosophila oogenesis. Cell *69*, 173-184.

Cosgrove, K.E., Galvan, E.J., Barrionuevo, G., and Meriney, S.D. (2011). mGluRs modulate strength and timing of excitatory transmission in hippocampal area CA3. Mol Neurobiol *44*, 93-101.

Costa-Mattioli, M., Sossin, W.S., Klann, E., and Sonenberg, N. (2009). Translational control of long-lasting synaptic plasticity and memory. Neuron *61*, 10-26.

Darnell, J.C., Fraser, C.E., Mostovetsky, O., and Darnell, R.B. (2009). Discrimination of common and unique RNA-binding activities among Fragile X mental retardation protein paralogs. Human molecular genetics *18*, 3164-3177.

Darnell, J.C., Fraser, C.E., Mostovetsky, O., Stefani, G., Jones, T.A., Eddy, S.R., and Darnell, R.B. (2005). Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. Genes Dev *19*, 903-918.

Darnell, J.C., Jensen, K.B., Jin, P., Brown, V., Warren, S.T., and Darnell, R.B. (2001). Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. Cell *107*, 489-499.

Darnell, J.C., Van Driesche, S.J., Zhang, C., Hung, K.Y., Mele, A., Fraser, C.E., Stone, E.F., Chen, C., Fak, J.J., Chi, S.W.*, et al.* (2011). FMRP Stalls Ribosomal Translocation on mRNAs Linked to Synaptic Function and Autism. Cell *146*, 247-261.

Davidkova, G., and 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*, 13273-13278.

De Blasi, A., Conn, P.J., Pin, J., and Nicoletti, F. (2001). Molecular determinants of metabotropic glutamate receptor signaling. Trends Pharmacol Sci *22*, 114-120.

De Boulle, K., Verkerk, A.J., Reyniers, E., Vits, L., Hendrickx, J., Van Roy, B., Van den Bos, F., de Graaff, E., Oostra, B.A., and Willems, P.J. (1993). A point mutation in the FMR-1 gene associated with fragile X mental retardation. Nature genetics *3*, 31-35.

de Vries, B.B., van den Ouweland, A.M., Mohkamsing, S., Duivenvoorden, H.J., Mol, E., Gelsema, K., van Rijn, M., Halley, D.J., Sandkuijl, L.A., Oostra, B.A.*, et al.* (1997). Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group. Am J Hum Genet *61*, 660-667.

Desai, N.S., Casimiro, T.M., Gruber, S.M., and Vanderklish, P.W. (2006). Early postnatal plasticity in neocortex of Fmr1 knockout mice. Journal of neurophysiology *96*, 1734-1745.

Dictenberg, J.B., Swanger, S.A., Antar, L.N., Singer, R.H., and Bassell, G.J. (2008). A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. Dev Cell *14*, 926-939.

Didiot, M.C., Tian, Z., Schaeffer, C., Subramanian, M., Mandel, J.L., and Moine, H. (2008). The G-quartet containing FMRP binding site in FMR1 mRNA is a potent exonic splicing enhancer. Nucleic Acids Res *36*, 4902-4912.

Dolen, G., Osterweil, E., Rao, B.S., Smith, G.B., Auerbach, B.D., Chattarji, S., and Bear, M.F. (2007). Correction of fragile X syndrome in mice. Neuron *56*, 955-962.

Dolmetsch, R., and Geschwind, D.H. (2011). The human brain in a dish: the promise of iPSC-derived neurons. Cell *145*, 831-834.

Dudek, S.M., and Bear, M.F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences of the United States of America *89*, 4363-4367.

Duffy, S., Labrie, V., and Roder, J.C. (2008). D-serine augments NMDA-NR2B receptor-dependent hippocampal long-term depression and spatial reversal learning. Neuropsychopharmacology *33*, 1004-1018.

Dynes, J.L., and 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.

Eberhart, D.E., Malter, H.E., Feng, Y., and Warren, S.T. (1996). The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. Human molecular genetics *5*, 1083-1091.

Edbauer, D., Neilson, J.R., Foster, K.A., Wang, C.F., Seeburg, D.P., Batterton, M.N., Tada, T., Dolan, B.M., Sharp, P.A., and Sheng, M. (2010). Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron *65*, 373-384.

Eichler, E.E., Richards, S., Gibbs, R.A., and Nelson, D.L. (1993). Fine structure of the human FMR1 gene. Human molecular genetics *2*, 1147-1153.

Estecio, M., Fett-Conte, A.C., Varella-Garcia, M., Fridman, C., and Silva, A.E. (2002). Molecular and cytogenetic analyses on Brazilian youths with pervasive developmental disorders. J Autism Dev Disord *32*, 35-41.

Evans, T.L., Blice-Baum, A.C., and Mihailescu, M.R. (2012). Analysis of the Fragile X mental retardation protein isoforms 1, 2 and 3 interactions with the G-quadruplex forming semaphorin 3F mRNA. Mol Biosyst *8*, 642-649.

Feinmark, S.J., Begum, R., Tsvetkov, E., Goussakov, I., Funk, C.D., Siegelbaum, S.A., and Bolshakov, V.Y. (2003). 12-lipoxygenase metabolites of arachidonic acid mediate metabotropic glutamate receptor-dependent long-term depression at hippocampal CA3-CA1 synapses. J Neurosci *23*, 11427-11435.

Feng, Y., Absher, D., Eberhart, D.E., Brown, V., Malter, H.E., and Warren, S.T. (1997). FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. Mol Cell *1*, 109-118.

Fiore, R., Khudayberdiev, S., Christensen, M., Siegel, G., Flavell, S.W., Kim, T.K., Greenberg, M.E., and Schratt, G. (2009). Mef2-mediated transcription of the miR379-410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. The EMBO journal *28*, 697-710.

Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., and Engel, J., Jr. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia *46*, 470-472.

Fitzjohn, S.M., Palmer, M.J., May, J.E., Neeson, A., Morris, S.A., and Collingridge, G.L. (2001). A characterisation of long-term depression induced by metabotropic glutamate receptor activation in the rat hippocampus in vitro. J Physiol *537*, 421-430.

Flavell, S.W., Cowan, C.W., Kim, T.K., Greer, P.L., Lin, Y., Paradis, S., Griffith, E.C., Hu, L.S., Chen, C., and Greenberg, M.E. (2006). Activity-dependent regulation of MEF2

transcription factors suppresses excitatory synapse number. Science (New York, NY *311*, 1008-1012.

Frey, U., Krug, M., Reymann, K.G., and Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res *452*, 57-65.

Frey, U., and Morris, R.G. (1997). Synaptic tagging and long-term potentiation. Nature *385*, 533-536.

Fukai, T., and Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal *15*, 1583-1606.

Gabel, L.A., Won, S., Kawai, H., McKinney, M., Tartakoff, A.M., and Fallon, J.R. (2004). Visual experience regulates transient expression and dendritic localization of fragile X mental retardation protein. J Neurosci *24*, 10579-10583.

Gallia, G.L., Darbinian, N., Jaffe, N., and Khalili, K. (2001). Single-stranded nucleic acid-binding protein, Pur alpha, interacts with RNA homologous to 18S ribosomal RNA and inhibits translation in vitro. J Cell Biochem *83*, 355-363.

Ganz, M.L. (2007). The lifetime distribution of the incremental societal costs of autism. Arch Pediatr Adolesc Med *161*, 343-349.

Gao, Y., Tatavarty, V., Korza, G., Levin, M.K., and Carson, J.H. (2008). Multiplexed dendritic targeting of alpha calcium calmodulin-dependent protein kinase II, neurogranin, and activity-regulated cytoskeleton-associated protein RNAs by the A2 pathway. Mol Biol Cell *19*, 2311-2327.

Gedeon, A.K., Baker, E., Robinson, H., Partington, M.W., Gross, B., Manca, A., Korn, B., Poustka, A., Yu, S., Sutherland, G.R.*, et al.* (1992). Fragile X syndrome without CCG amplification has an FMR1 deletion. Nature genetics *1*, 341-344.

Gerdeman, G.L., Ronesi, J., and Lovinger, D.M. (2002). Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. Nat Neurosci *5*, 446-451.

Geschwind, D.H. (2011). Genetics of autism spectrum disorders. Trends Cogn Sci *15*, 409-416.

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

Gladding, C.M., Fitzjohn, S.M., and Molnar, E. (2009). Metabotropic glutamate receptor-mediated long-term depression: molecular mechanisms. Pharmacol Rev *61*, 395-412.

Goebel-Goody, S.M., Baum, M., Paspalas, C.D., Fernandez, S.M., Carty, N.C., Kurup, P., and Lombroso, P.J. (2012). Therapeutic implications for striatal-enriched protein tyrosine phosphatase (STEP) in neuropsychiatric disorders. Pharmacol Rev *64*, 65-87.

Gong, C.X., Lidsky, T., Wegiel, J., Zuck, L., Grundke-Iqbal, I., and Iqbal, K. (2000). Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease. J Biol Chem *275*, 5535-5544.

Greer, P.L., Hanayama, R., Bloodgood, B.L., Mardinly, A.R., Lipton, D.M., Flavell, S.W., Kim, T.K., Griffith, E.C., Waldon, Z., Maehr, R.*, et al.* (2010). The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc. Cell *140*, 704-716.

Gregory, K.J., Dong, E.N., Meiler, J., and Conn, P.J. (2011). Allosteric modulation of metabotropic glutamate receptors: structural insights and therapeutic potential. Neuropharmacology *60*, 66-81.

Gross, C., Nakamoto, M., Yao, X., Chan, C.B., Yim, S.Y., Ye, K., Warren, S.T., and Bassell, G.J. (2010). Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. J Neurosci *30*, 10624-10638.

Grossman, A.W., Aldridge, G.M., Weiler, I.J., and Greenough, W.T. (2006). Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. J Neurosci *26*, 7151-7155.

Guo, W., Zhang, L., Christopher, D.M., Teng, Z.Q., Fausett, S.R., Liu, C., George, O.L., Klingensmith, J., Jin, P., and Zhao, X. (2011). RNA-binding protein FXR2 regulates adult hippocampal neurogenesis by reducing Noggin expression. Neuron *70*, 924-938.

Guzowski, J.F., Lyford, G.L., Stevenson, G.D., Houston, F.P., McGaugh, J.L., Worley, P.F., and Barnes, C.A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. J Neurosci *20*, 3993-4001.

Guzowski, J.F., McNaughton, B.L., Barnes, C.A., and Worley, P.F. (1999). Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nat Neurosci *2*, 1120-1124.

Hadjantonakis, A.K., Gertsenstein, M., Ikawa, M., Okabe, M., and Nagy, A. (1998). Non-invasive sexing of preimplantation stage mammalian embryos. Nat Genet *19*, 220-222.

Haghighat, A., Mader, S., Pause, A., and Sonenberg, N. (1995). Repression of cap-dependent translation by 4E-binding protein 1: competition with p220 for binding to eukaryotic initiation factor-4E. The EMBO journal *14*, 5701-5709.

Hansel, C., de Jeu, M., Belmeguenai, A., Houtman, S.H., Buitendijk, G.H., Andreev, D., De Zeeuw, C.I., and Elgersma, Y. (2006). alphaCaMKII Is essential for cerebellar LTD and motor learning. Neuron *51*, 835-843.

Hanson, J.E., and Madison, D.V. (2007). Presynaptic FMR1 genotype influences the degree of synaptic connectivity in a mosaic mouse model of fragile X syndrome. J Neurosci *27*, 4014-4018.

Harney, S.C., Rowan, M., and Anwyl, R. (2006). Long-term depression of NMDA receptor-mediated synaptic transmission is dependent on activation of metabotropic glutamate receptors and is altered to long-term potentiation by low intracellular calcium buffering. J Neurosci *26*, 1128-1132.

Havlovicova, M., Propper, L., Novotna, D., Musova, Z., Hrdlicka, M., and Sedlacek, Z. (2002). [Genetic study of 20 patients with autism disorders]. Cas Lek Cesk *141*, 381-387.

Hay, N., and Sonenberg, N. (2004). Upstream and downstream of mTOR. Genes & development *18*, 1926-1945.

Hays, S.A., Huber, K.M., and Gibson, J.R. (2011). Altered neocortical rhythmic activity states in Fmr1 KO mice are due to enhanced mGluR5 signaling and involve changes in excitatory circuitry. J Neurosci *31*, 14223-14234.

Heitz, D., Rousseau, F., Devys, D., Saccone, S., Abderrahim, H., Le Paslier, D., Cohen, D., Vincent, A., Toniolo, D., Della Valle, G.*, et al.* (1991). Isolation of sequences that span the fragile X and identification of a fragile X-related CpG island. Science (New York, NY *251*, 1236-1239.

Henderson, B.R., and Eleftheriou, A. (2000). A comparison of the activity, sequence specificity, and CRM1-dependence of different nuclear export signals. Exp Cell Res *256*, 213-224.

Hersh, J.H., and Saul, R.A. (2011). Health supervision for children with fragile X syndrome. Pediatrics *127*, 994-1006.

Hinnebusch, A.G. (2011). Molecular mechanism of scanning and start codon selection in eukaryotes. Microbiol Mol Biol Rev *75*, 434-467, first page of table of contents.

Hirst, M., Grewal, P., Flannery, A., Slatter, R., Maher, E., Barton, D., Fryns, J.P., and Davies, K. (1995). Two new cases of FMR1 deletion associated with mental impairment. Am J Hum Genet *56*, 67-74.

Ho, N., Liauw, J.A., Blaeser, F., Wei, F., Hanissian, S., Muglia, L.M., Wozniak, D.F., Nardi, A., Arvin, K.L., Holtzman, D.M.*, et al.* (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. J Neurosci *20*, 6459-6472.

Hollmann, M., and Heinemann, S. (1994). Cloned glutamate receptors. Annual review of neuroscience *17*, 31-108.

Hornstra, I.K., Nelson, D.L., Warren, S.T., and Yang, T.P. (1993). High resolution methylation analysis of the FMR1 gene trinucleotide repeat region in fragile X syndrome. Human molecular genetics *2*, 1659-1665.

Hou, L., Antion, M.D., Hu, D., Spencer, C.M., Paylor, R., and Klann, E. (2006). Dynamic translational and proteasomal regulation of fragile X mental retardation protein controls mGluR-dependent long-term depression. Neuron *51*, 441-454.

Hou, L., and Klann, E. (2004). Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. J Neurosci *24*, 6352-6361.

Houamed, K.M., Kuijper, J.L., Gilbert, T.L., Haldeman, B.A., O'Hara, P.J., Mulvihill, E.R., Almers, W., and Hagen, F.S. (1991). Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. Science (New York, NY *252*, 1318-1321.

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., and Malinow, R. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. Neuron *52*, 831-843.

Hu, H., Qin, Y., Bochorishvili, G., Zhu, Y., van Aelst, L., and Zhu, J.J. (2008). Ras signaling mechanisms underlying impaired GluR1-dependent plasticity associated with fragile X syndrome. J Neurosci *28*, 7847-7862.

Huber, K.M., Gallagher, S.M., Warren, S.T., and Bear, M.F. (2002). Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A *99*, 7746-7750.

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

Huber, K.M., Roder, J.C., and Bear, M.F. (2001). Chemical induction of mGluR5- and protein synthesis--dependent long-term depression in hippocampal area CA1. Journal of neurophysiology *86*, 321-325.

Huttelmaier, S., Zenklusen, D., Lederer, M., Dictenberg, J., Lorenz, M., Meng, X., Bassell, G.J., Condeelis, J., and Singer, R.H. (2005). Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. Nature *438*, 512-515.

Iacoangeli, A., Rozhdestvensky, T.S., Dolzhanskaya, N., Tournier, B., Schutt, J., Brosius, J., Denman, R.B., Khandjian, E.W., Kindler, S., and Tiedge, H. (2008a). On BC1 RNA

and the fragile X mental retardation protein. Proceedings of the National Academy of Sciences of the United States of America *105*, 734-739.

Iacoangeli, A., Rozhdestvensky, T.S., Dolzhanskaya, N., Tournier, B., Schütt, J., Brosius, J., Denman, R.B., Khandjian, E.W., Kindler, S., and Tiedge, H. (2008b). On BC1 RNA and the fragile X mental retardation protein. Proc Natl Acad Sci U S A *105*, 734-739.

Irie, Y., Yamagata, K., Gan, Y., Miyamoto, K., Do, E., Kuo, C.H., Taira, E., and Miki, N. (2000). Molecular cloning and characterization of Amida, a novel protein which interacts with a neuron-specific immediate early gene product arc, contains novel nuclear localization signals, and causes cell death in cultured cells. The Journal of biological chemistry *275*, 2647-2653.

Ishizuka, A., Siomi, M.C., and Siomi, H. (2002). A Drosophila fragile X protein interacts with components of RNAi and ribosomal proteins. Genes & development *16*, 2497-2508.

Ito, M., Sakurai, M., and Tongroach, P. (1982). Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. J Physiol *324*, 113-134.

Jin, P., Zarnescu, D.C., Ceman, S., Nakamoto, M., Mowrey, J., Jongens, T.A., Nelson, D.L., Moses, K., and Warren, S.T. (2004). Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat Neurosci *7*, 113-117.

Jin, Y., Kim, S.J., Kim, J., Worley, P.F., and Linden, D.J. (2007). Long-term depression of mGluR1 signaling. Neuron *55*, 277-287.

Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuron *43*, 513-525.

Kang, H., and Schuman, E.M. (1996). A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science (New York, NY *273*, 1402-1406.

Kawashima, T., Okuno, H., Nonaka, M., Adachi-Morishima, A., Kyo, N., Okamura, M., Takemoto-Kimura, S., Worley, P.F., and Bito, H. (2009). Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons. Proceedings of the National Academy of Sciences of the United States of America *106*, 316-321.

Kemp, A., and Manahan-Vaughan, D. (2004). Hippocampal long-term depression and long-term potentiation encode different aspects of novelty acquisition. Proceedings of the National Academy of Sciences of the United States of America *101*, 8192-8197.

Kemp, A., and Manahan-Vaughan, D. (2007). Hippocampal long-term depression: master or minion in declarative memory processes? Trends Neurosci *30*, 111-118.

Kemp, N., and Bashir, Z.I. (1999). Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. Neuropharmacology *38*, 495-504.

Khandjian, E.W., Corbin, F., Woerly, S., and Rousseau, F. (1996). The fragile X mental retardation protein is associated with ribosomes. Nature genetics *12*, 91-93.

Kirkpatrick, L.L., McIlwain, K.A., and Nelson, D.L. (2001). Comparative genomic sequence analysis of the FXR gene family: FMR1, FXR1, and FXR2. Genomics *78*, 169-177.

Kobayashi, H., Yamamoto, S., Maruo, T., and Murakami, F. (2005). Identification of a cis-acting element required for dendritic targeting of activity-regulated cytoskeleton-associated protein mRNA. Eur J Neurosci *22*, 2977-2984.

Kogan, M.D., Strickland, B.B., Blumberg, S.J., Singh, G.K., Perrin, J.M., and van Dyck, P.C. (2008). A national profile of the health care experiences and family impact of autism spectrum disorder among children in the United States, 2005-2006. Pediatrics *122*, e1149-1158.

Korb, E., and Finkbeiner, S. (2011). Arc in synaptic plasticity: from gene to behavior. Trends Neurosci *34*, 591-598.

Krawczun, M.S., Jenkins, E.C., and Brown, W.T. (1985). Analysis of the fragile-X chromosome: localization and detection of the fragile site in high resolution preparations. Hum Genet *69*, 209-211.

Kreitzer, A.C., and Malenka, R.C. (2005). Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. J Neurosci *25*, 10537-10545.

Kreitzer, A.C., and Malenka, R.C. (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature *445*, 643-647.

Krueger, D.D., Osterweil, E.K., Chen, S.P., Tye, L.D., and Bear, M.F. (2011). Cognitive dysfunction and prefrontal synaptic abnormalities in a mouse model of fragile X syndrome. Proceedings of the National Academy of Sciences of the United States of America *108*, 2587-2592.

Kwiatkowski, D.J., and Manning, B.D. (2005). Tuberous sclerosis: a GAP at the crossroads of multiple signaling pathways. Human molecular genetics *14 Spec No. 2*, R251-258.

Lacoux, C., Di Marino, D., Pilo Boyl, P., Zalfa, F., Yan, B., Ciotti, M.T., Falconi, M., Urlaub, H., Achsel, T., Mougin, A.*, et al.* (2012). BC1-FMRP interaction is modulated by 2'-O-methylation: RNA-binding activity of the tudor domain and translational regulation at synapses. Nucleic Acids Res.

Lee, H.K., Kameyama, K., Huganir, R.L., and Bear, M.F. (1998). NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron *21*, 1151-1162.

Leitges, M., Kovac, J., Plomann, M., and Linden, D.J. (2004). A unique PDZ ligand in PKCalpha confers induction of cerebellar long-term synaptic depression. Neuron *44*, 585-594.

Li, S., Hong, S., Shepardson, N.E., Walsh, D.M., Shankar, G.M., and Selkoe, D. (2009). Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron *62*, 788-801.

Li, X., and Jin, P. (2009). Macro role(s) of microRNAs in fragile X syndrome? Neuromolecular Med *11*, 200-207.

Lim, J.H., Booker, A.B., Luo, T., Williams, T., Furuta, Y., Lagutin, O., Oliver, G., Sargent, T.D., and Fallon, J.R. (2005a). AP-2alpha selectively regulates fragile X mental retardation-1 gene transcription during embryonic development. Human molecular genetics *14*, 2027-2034.

Lim, J.H., Luo, T., Sargent, T.D., and Fallon, J.R. (2005b). Developmental expression of Xenopus fragile X mental retardation-1 gene. Int J Dev Biol *49*, 981-984.

Lin, M.Z., and Tsien, R.Y. (2010). TimeSTAMP tagging of newly synthesized proteins. Curr Protoc Protein Sci *Chapter 26*, Unit 26 25.

Lin, T.A., Kong, X., Haystead, T.A., Pause, A., Belsham, G., Sonenberg, N., and Lawrence, J.C., Jr. (1994). PHAS-I as a link between mitogen-activated protein kinase and translation initiation. Science (New York, NY *266*, 653-656.

Linden, D.J., Dickinson, M.H., Smeyne, M., and Connor, J.A. (1991). A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. Neuron *7*, 81-89.

Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., and Kuhl, D. (1995). Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc Natl Acad Sci U S A *92*, 5734-5738.

Liu, Z.H., Huang, T., and Smith, C.B. (2012). Lithium reverses increased rates of cerebral protein synthesis in a mouse model of fragile X syndrome. Neurobiol Dis *45*, 1145-1152.

Loesch, D.Z., Bui, Q.M., Grigsby, J., Butler, E., Epstein, J., Huggins, R.M., Taylor, A.K., and Hagerman, R.J. (2003a). Effect of the fragile X status categories and the fragile X mental retardation protein levels on executive functioning in males and females with fragile X. Neuropsychology *17*, 646-657.

Loesch, D.Z., Huggins, R.M., Bui, Q.M., Epstein, J.L., Taylor, A.K., and Hagerman, R.J. (2002). Effect of the deficits of fragile X mental retardation protein on cognitive status of fragile x males and females assessed by robust pedigree analysis. J Dev Behav Pediatr *23*, 416-423.

Loesch, D.Z., Huggins, R.M., Bui, Q.M., Taylor, A.K., and Hagerman, R.J. (2003b). Relationship of deficits of FMR1 gene specific protein with physical phenotype of fragile X males and females in pedigrees: a new perspective. Am J Med Genet A *118A*, 127-134.

Lois, C., Hong, E.J., Pease, S., Brown, E.J., and Baltimore, D. (2002). Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors. Science *295*, 868-872.

Lovinger, D.M. (2008). Presynaptic modulation by endocannabinoids. Handb Exp Pharmacol, 435-477.

Lu, J., Helton, T.D., Blanpied, T.A., Racz, B., Newpher, T.M., Weinberg, R.J., and Ehlers, M.D. (2007). Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. Neuron *55*, 874-889.

Lu, R., Wang, H., Liang, Z., Ku, L., O'Donnell W, T., Li, W., Warren, S.T., and Feng, Y. (2004). The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development. Proceedings of the National Academy of Sciences of the United States of America *101*, 15201-15206.

Lubs, H.A. (1969). A marker X chromosome. Am J Hum Genet *21*, 231-244.

Lugenbeel, K.A., Peier, A.M., Carson, N.L., Chudley, A.E., and Nelson, D.L. (1995). Intragenic loss of function mutations demonstrate the primary role of FMR1 in fragile X syndrome. Nature genetics *10*, 483-485.

Lugli, G., Larson, J., Martone, M.E., Jones, Y., and Smalheiser, N.R. (2005). Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. J Neurochem *94*, 896-905.

Lugli, G., Torvik, V.I., Larson, J., and Smalheiser, N.R. (2008). Expression of microRNAs and their precursors in synaptic fractions of adult mouse forebrain. J Neurochem *106*, 650-661.

Luscher, C., and Huber, K.M. (2010). Group 1 mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. Neuron *65*, 445-459.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., and Worley, P.F. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron *14*, 433-445.

Lynch, G.S., Dunwiddie, T., and Gribkoff, V. (1977). Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. Nature *266*, 737-739.

Ma, Z.W., Pejovic, T., Najfeld, V., Ward, D.C., and Johnson, E.M. (1995). Localization of PURA, the gene encoding the sequence-specific single-stranded-DNA-binding protein Pur alpha, to chromosome band 5q31. Cytogenet Cell Genet *71*, 64-67.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5-21.

Mao, L., Yang, L., Arora, A., Choe, E.S., Zhang, G., Liu, Z., Fibuch, E.E., and Wang, J.Q. (2005a). Role of protein phosphatase 2A in mGluR5-regulated MEK/ERK phosphorylation in neurons. The Journal of biological chemistry *280*, 12602-12610.

Mao, L., Yang, L., Tang, Q., Samdani, S., Zhang, G., and Wang, J.Q. (2005b). The scaffold protein Homer1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons. J Neurosci *25*, 2741-2752.

Maquat, L.E. (2004). Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol *5*, 89-99.

Marin, P., Nastiuk, K.L., Daniel, N., Girault, J.A., Czernik, A.J., Glowinski, J., Nairn, A.C., and Premont, J. (1997). Glutamate-dependent phosphorylation of elongation factor-2 and inhibition of protein synthesis in neurons. J Neurosci *17*, 3445-3454.

Maroney, P.A., Yu, Y., Fisher, J., and Nilsen, T.W. (2006). Evidence that microRNAs are associated with translating messenger RNAs in human cells. Nat Struct Mol Biol *13*, 1102-1107.

Mary, S., Stephan, D., Gomeza, J., Bockaert, J., Pruss, R.M., and Pin, J.P. (1997). The rat mGlu1d receptor splice variant shares functional properties with the other short isoforms of mGlu1 receptor. Eur J Pharmacol *335*, 65-72.

Massey, P.V., and Bashir, Z.I. (2007). Long-term depression: multiple forms and implications for brain function. Trends Neurosci *30*, 176-184.

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., and Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. Nature *349*, 760-765.

Maurer-Stroh, S., Dickens, N.J., Hughes-Davies, L., Kouzarides, T., Eisenhaber, F., and Ponting, C.P. (2003). The Tudor domain 'Royal Family': Tudor, plant Agenet, Chromo, PWWP and MBT domains. Trends Biochem Sci *28*, 69-74.

McCurry, C.L., Shepherd, J.D., Tropea, D., Wang, K.H., Bear, M.F., and Sur, M. (2010). Loss of Arc renders the visual cortex impervious to the effects of sensory experience or deprivation. Nat Neurosci *13*, 450-457.

McEvoy, M., Cao, G., Montero Llopis, P., Kundel, M., Jones, K., Hofler, C., Shin, C., and Wells, D.G. (2007). Cytoplasmic polyadenylation element binding protein 1-mediated mRNA translation in Purkinje neurons is required for cerebellar long-term depression and motor coordination. J Neurosci *27*, 6400-6411.

Meijer, H., de Graaff, E., Merckx, D.M., Jongbloed, R.J., de Die-Smulders, C.E., Engelen, J.J., Fryns, J.P., Curfs, P.M., and Oostra, B.A. (1994). A deletion of 1.6 kb proximal to the CGG repeat of the FMR1 gene causes the clinical phenotype of the fragile X syndrome. Human molecular genetics *3*, 615-620.

Menon, L., Mader, S.A., and Mihailescu, M.R. (2008). Fragile X mental retardation protein interactions with the microtubule associated protein 1B RNA. RNA *14*, 1644-1655.

Messaoudi, E., Kanhema, T., Soule, J., Tiron, A., Dagyte, G., da Silva, B., and Bramham, C.R. (2007). Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo. J Neurosci *27*, 10445-10455.

Meyuhas, O. (2008). Physiological roles of ribosomal protein S6: one of its kind. Int Rev Cell Mol Biol *268*, 1-37.

Mientjes, E.J., Willemsen, R., Kirkpatrick, L.L., Nieuwenhuizen, I.M., Hoogeveen-Westerveld, M., Verweij, M., Reis, S., Bardoni, B., Hoogeveen, A.T., Oostra, B.A*., et al.* (2004). Fxr1 knockout mice show a striated muscle phenotype: implications for Fxr1p function in vivo. Human molecular genetics *13*, 1291-1302.

Mink, J.W. (1996). The basal ganglia: focused selection and inhibition of competing motor programs. Prog Neurobiol *50*, 381-425.

Moult, P.R., Correa, S.A., Collingridge, G.L., Fitzjohn, S.M., and Bashir, Z.I. (2008). Co-activation of p38 mitogen-activated protein kinase and protein tyrosine phosphatase underlies metabotropic glutamate receptor-dependent long-term depression. J Physiol *586*, 2499-2510.

Moult, P.R., Gladding, C.M., Sanderson, T.M., Fitzjohn, S.M., Bashir, Z.I., Molnar, E., and Collingridge, G.L. (2006). Tyrosine phosphatases regulate AMPA receptor

trafficking during metabotropic glutamate receptor-mediated long-term depression. J Neurosci *26*, 2544-2554.

Muddashetty, R.S., Kelic, S., Gross, C., Xu, M., and Bassell, G.J. (2007). Dysregulated metabotropic glutamate receptor-dependent translation of AMPA receptor and postsynaptic density-95 mRNAs at synapses in a mouse model of fragile X syndrome. J Neurosci *27*, 5338-5348.

Muddashetty, R.S., Nalavadi, V.C., Gross, C., Yao, X., Xing, L., Laur, O., Warren, S.T., and Bassell, G.J. (2011). Reversible Inhibition of PSD-95 mRNA Translation by miR-125a, FMRP Phosphorylation, and mGluR Signaling. Mol Cell *42*, 673-688.

Murakami, K., Murata, N., Noda, Y., Tahara, S., Kaneko, T., Kinoshita, N., Hatsuta, H., Murayama, S., Barnham, K.J., Irie, K.*, et al.* (2011). SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid beta protein oligomerization and memory loss in mouse model of Alzheimer disease. The Journal of biological chemistry *286*, 44557-44568.

Musco, G., Stier, G., Joseph, C., Castiglione Morelli, M.A., Nilges, M., Gibson, T.J., and Pastore, A. (1996). Three-dimensional structure and stability of the KH domain: molecular insights into the fragile X syndrome. Cell *85*, 237-245.

Nalavadi, V.C., Muddashetty, R.S., Gross, C., and Bassell, G.J. (2012). Dephosphorylation-Induced Ubiquitination and Degradation of FMRP in Dendrites: A Role in Immediate Early mGluR-Stimulated Translation. J Neurosci *32*, 2582-2587.

Nambu, A. (2004). A new dynamic model of the cortico-basal ganglia loop. Prog Brain Res *143*, 461-466.

Napoli, I., Mercaldo, V., Boyl, P.P., Eleuteri, B., Zalfa, F., De Rubeis, S., Di Marino, D., Mohr, E., Massimi, M., Falconi, M.*, et al.* (2008). The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. Cell *134*, 1042-1054.

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

Narayanan, U., Nalavadi, V., Nakamoto, M., Thomas, G., Ceman, S., Bassell, G.J., and Warren, S.T. (2008a). S6K1 phosphorylates and regulates FMRP with the neuronal protein synthesis-dependent mTOR signaling cascade. J Biol Chem.

Narayanan, U., Nalavadi, V., Nakamoto, M., Thomas, G., Ceman, S., Bassell, G.J., and Warren, S.T. (2008b). S6K1 phosphorylates and regulates fragile X mental retardation protein (FMRP) with the neuronal protein synthesis-dependent mammalian target of

rapamycin (mTOR) signaling cascade. The Journal of biological chemistry *283*, 18478-18482.

Nicholls, R.E., Alarcon, J.M., Malleret, G., Carroll, R.C., Grody, M., Vronskaya, S., and Kandel, E.R. (2008). Transgenic mice lacking NMDAR-dependent LTD exhibit deficits in behavioral flexibility. Neuron *58*, 104-117.

Nicoletti, F., Wroblewski, J.T., Novelli, A., Alho, H., Guidotti, A., and Costa, E. (1986). The activation of inositol phospholipid metabolism as a signal-transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. J Neurosci *6*, 1905-1911.

Nosyreva, E.D., and Huber, K.M. (2006). Metabotropic receptor-dependent long-term depression persists in the absence of protein synthesis in the mouse model of fragile x syndrome. J Neurophysiol *95*, 3291-3295.

Oberle, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boue, J., Bertheas, M.F., and Mandel, J.L. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science (New York, NY *252*, 1097-1102.

Ohashi, S., Koike, K., Omori, A., Ichinose, S., Ohara, S., Kobayashi, S., Sato, T.A., and Anzai, K. (2002). Identification of mRNA/protein (mRNP) complexes containing Puralpha, mStaufen, fragile X protein, and myosin Va and their association with rough endoplasmic reticulum equipped with a kinesin motor. The Journal of biological chemistry *277*, 37804-37810.

Osterweil, E.K., Krueger, D.D., Reinhold, K., and Bear, M.F. (2010). Hypersensitivity to mGluR5 and ERK1/2 leads to excessive protein synthesis in the hippocampus of a mouse model of fragile X syndrome. J Neurosci *30*, 15616-15627.

Ostroff, L.E., Fiala, J.C., Allwardt, B., and Harris, K.M. (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. Neuron *35*, 535-545.

Padmanabhan, S., Mukhopadhyay, A., Narasimhan, S.D., Tesz, G., Czech, M.P., and Tissenbaum, H.A. (2009). A PP2A regulatory subunit regulates C. elegans insulin/IGF-1 signaling by modulating AKT-1 phosphorylation. Cell *136*, 939-951.

Palmer, M.J., Irving, A.J., Seabrook, G.R., Jane, D.E., and Collingridge, G.L. (1997). The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. Neuropharmacology *36*, 1517-1532.

Paluszkiewicz, S.M., Martin, B.S., and Huntsman, M.M. (2011). Fragile X syndrome: the GABAergic system and circuit dysfunction. Dev Neurosci *33*, 349-364.

Papoulas, O., Monzo, K.F., Cantin, G.T., Ruse, C., Yates, J.R., 3rd, Ryu, Y.H., and Sisson, J.C. (2010). dFMRP and Caprin, translational regulators of synaptic plasticity, control the cell cycle at the Drosophila mid-blastula transition. Development *137*, 4201-4209.

Park, S., Park, J.M., Kim, S., Kim, J.A., Shepherd, J.D., Smith-Hicks, C.L., Chowdhury, S., Kaufmann, W., Kuhl, D., Ryazanov, A.G.*, et al.* (2008). Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. Neuron *59*, 70-83.

Park, Y., Jo, J., Isaac, J.T., and Cho, K. (2006). Long-term depression of kainate receptor-mediated synaptic transmission. Neuron *49*, 95-106.

Pause, A., Belsham, G.J., Gingras, A.C., Donze, O., Lin, T.A., Lawrence, J.C., Jr., and Sonenberg, N. (1994). Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. Nature *371*, 762-767.

Pfeiffer, B.E., and Huber, K.M. (2007). Fragile X mental retardation protein induces synapse loss through acute postsynaptic translational regulation. J Neurosci *27*, 3120-3130.

Pfeiffer, B.E., Zang, T., Wilkerson, J.R., Taniguchi, M., Maksimova, M.A., Smith, L.N., Cowan, C.W., and Huber, K.M. (2010). Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. Neuron *66*, 191-197.

Pieretti, M., Zhang, F.P., Fu, Y.H., Warren, S.T., Oostra, B.A., Caskey, C.T., and Nelson, D.L. (1991). Absence of expression of the FMR-1 gene in fragile X syndrome. Cell *66*, 817-822.

Pin, J.P., Galvez, T., and Prezeau, L. (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther *98*, 325-354.

Pin, J.P., Joly, C., Heinemann, S.F., and Bockaert, J. (1994). Domains involved in the specificity of G protein activation in phospholipase C-coupled metabotropic glutamate receptors. The EMBO journal *13*, 342-348.

Pintchovski, S.A., Peebles, C.L., Kim, H.J., Verdin, E., and Finkbeiner, S. (2009). The serum response factor and a putative novel transcription factor regulate expression of the immediate-early gene Arc/Arg3.1 in neurons. J Neurosci *29*, 1525-1537.

Plath, N., Ohana, O., Dammermann, B., Errington, M.L., Schmitz, D., Gross, C., Mao, X., Engelsberg, A., Mahlke, C., Welzl, H.*, et al.* (2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. Neuron *52*, 437-444.

Praefcke, G.J., and McMahon, H.T. (2004). The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol *5*, 133-147.

Qin, M., Entezam, A., Usdin, K., Huang, T., Liu, Z.H., Hoffman, G.E., and Smith, C.B. (2011). A mouse model of the fragile X premutation: effects on behavior, dendrite morphology, and regional rates of cerebral protein synthesis. Neurobiol Dis *42*, 85-98.

Qin, M., Kang, J., Burlin, T.V., Jiang, C., and Smith, C.B. (2005). Postadolescent changes in regional cerebral protein synthesis: an in vivo study in the FMR1 null mouse. J Neurosci *25*, 5087-5095.

Raab-Graham, K.F., Haddick, P.C., Jan, Y.N., and Jan, L.Y. (2006). Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science (New York, NY *314*, 144-148.

Rao, V.R., Pintchovski, S.A., Chin, J., Peebles, C.L., Mitra, S., and Finkbeiner, S. (2006). AMPA receptors regulate transcription of the plasticity-related immediate-early gene Arc. Nat Neurosci *9*, 887-895.

Reddy, K.S. (2005). Cytogenetic abnormalities and fragile-X syndrome in Autism Spectrum Disorder. BMC Med Genet *6*, 3.

Rial Verde, E.M., Lee-Osbourne, J., Worley, P.F., Malinow, R., and Cline, H.T. (2006). Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron *52*, 461-474.

Richter, J.D. (2007). CPEB: a life in translation. Trends Biochem Sci *32*, 279-285.

Riederer, B.M. (2007). Microtubule-associated protein 1B, a growth-associated and phosphorylated scaffold protein. Brain Res Bull *71*, 541-558.

Rochefort, C., Arabo, A., Andre, M., Poucet, B., Save, E., and Rondi-Reig, L. (2011). Cerebellum shapes hippocampal spatial code. Science (New York, NY *334*, 385-389.

Rodriguez, J.J., Davies, H.A., Silva, A.T., De Souza, I.E., Peddie, C.J., Colyer, F.M., Lancashire, C.L., Fine, A., Errington, M.L., Bliss, T.V.*, et al.* (2005). Long-term potentiation in the rat dentate gyrus is associated with enhanced Arc/Arg3.1 protein expression in spines, dendrites and glia. Eur J Neurosci *21*, 2384-2396.

Ronesi, J., Gerdeman, G.L., and Lovinger, D.M. (2004). Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. J Neurosci *24*, 1673-1679.

Ronesi, J.A., Collins, K.A., Hays, S.A., Tsai, N.P., Guo, W., Birnbaum, S.G., Hu, J.H., Worley, P.F., Gibson, J.R., and Huber, K.M. (2012). Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome. Nat Neurosci.

Ronesi, J.A., and Huber, K.M. (2008). Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. J Neurosci *28*, 543-547.

Rose, C.R., and Konnerth, A. (2001). Stores not just for storage. intracellular calcium release and synaptic plasticity. Neuron *31*, 519-522.

Ryazanov, A.G., Shestakova, E.A., and Natapov, P.G. (1988). Phosphorylation of elongation factor 2 by EF-2 kinase affects rate of translation. Nature *334*, 170-173.

Sacaan, A.I., Santori, E.M., and Rao, T.S. (1998). (S)-4-carboxy-3-hydroxyphenylglycine activates phosphatidyl inositol linked metabotropic glutamate receptors in different brain regions of the neonatal rat. Neurochem Int *32*, 77-85.

Sala, C., Roussignol, G., Meldolesi, J., and Fagni, L. (2005). Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca2+ homeostasis at dendritic spines in hippocampal neurons. J Neurosci *25*, 4587-4592.

Sanderson, T.M., Collingridge, G.L., and Fitzjohn, S.M. (2011). Differential trafficking of AMPA receptors following activation of NMDA receptors and mGluRs. Mol Brain *4*, 30.

Saul, R.A., and Tarleton, J.C. (2011). FMR1-Related Disorders. In Gene Reviews [online], R. Pagon, T. Bird, C. Dolan, and S. K, eds. (Seattle, Washington, University of Washington).

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

Schnabel, R., Kilpatrick, I.C., and Collingridge, G.L. (1999). An investigation into signal transduction mechanisms involved in DHPG-induced LTD in the CA1 region of the hippocampus. Neuropharmacology *38*, 1585-1596.

Schnabel, R., Kilpatrick, I.C., and Collingridge, G.L. (2001). Protein phosphatase inhibitors facilitate DHPG-induced LTD in the CA1 region of the hippocampus. Br J Pharmacol *132*, 1095-1101.

Schoepp, D.D., Goldsworthy, J., Johnson, B.G., Salhoff, C.R., and Baker, S.R. (1994). 3,5-dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus. J Neurochem *63*, 769-772.

Schutt, J., Falley, K., Richter, D., Kreienkamp, H.J., and Kindler, S. (2009). Fragile X mental retardation protein regulates the levels of scaffold proteins and glutamate receptors in postsynaptic densities. The Journal of biological chemistry *284*, 25479-25487.

Shang, Y., Wang, H., Mercaldo, V., Li, X., Chen, T., and Zhuo, M. (2009). Fragile X mental retardation protein is required for chemically-induced long-term potentiation of the hippocampus in adult mice. J Neurochem *111*, 635-646.

Sharma, A., Hoeffer, C.A., Takayasu, Y., Miyawaki, T., McBride, S.M., Klann, E., and Zukin, R.S. (2010). Dysregulation of mTOR signaling in fragile X syndrome. J Neurosci *30*, 694-702.

Shen, W., Flajolet, M., Greengard, P., and Surmeier, D.J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. Science (New York, NY *321*, 848-851.

Shiraishi-Yamaguchi, Y., and Furuichi, T. (2007). The Homer family proteins. Genome Biol *8*, 206.

Siddique, T., and Ajroud-Driss, S. (2011). Familial amyotrophic lateral sclerosis, a historical perspective. Acta Myol *30*, 117-120.

Silverman, W., Lubin, R., Jenkins, E.C., and Brown, W.T. (1983). The strength of association between fragile (X) chromosome presence and mental retardation. Clin Genet *23*, 436-440.

Siomi, H., Choi, M., Siomi, M.C., Nussbaum, R.L., and Dreyfuss, G. (1994). Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. Cell *77*, 33-39.

Siomi, H., Siomi, M.C., Nussbaum, R.L., and Dreyfuss, G. (1993). The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. Cell *74*, 291-298.

Siomi, M.C., Siomi, H., Sauer, W.H., Srinivasan, S., Nussbaum, R.L., and Dreyfuss, G. (1995). FXR1, an autosomal homolog of the fragile X mental retardation gene. The EMBO journal *14*, 2401-2408.

Sittler, A., Devys, D., Weber, C., and Mandel, J.L. (1996). Alternative splicing of exon 14 determines nuclear or cytoplasmic localisation of fmr1 protein isoforms. Human molecular genetics *5*, 95-102.

Sladeczek, F., Pin, J.P., Recasens, M., Bockaert, J., and Weiss, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. Nature *317*, 717-719.

Snyder, E.M., Philpot, B.D., Huber, K.M., Dong, X., Fallon, J.R., and Bear, M.F. (2001). Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat Neurosci *4*, 1079-1085.

Spencer, C.M., Serysheva, E., Yuva-Paylor, L.A., Oostra, B.A., Nelson, D.L., and Paylor, R. (2006). Exaggerated behavioral phenotypes in Fmr1/Fxr2 double knockout mice reveal a functional genetic interaction between Fragile X-related proteins. Human molecular genetics *15*, 1984-1994.

Stefani, G., Fraser, C.E., Darnell, J.C., and Darnell, R.B. (2004). Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. J Neurosci *24*, 7272-7276.

Steinberg, J.P., Takamiya, K., Shen, Y., Xia, J., Rubio, M.E., Yu, S., Jin, W., Thomas, G.M., Linden, D.J., and Huganir, R.L. (2006). Targeted in vivo mutations of the AMPA receptor subunit GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. Neuron *49*, 845-860.

Stetler, A., Winograd, C., Sayegh, J., Cheever, A., Patton, E., Zhang, X., Clarke, S., and Ceman, S. (2006). Identification and characterization of the methyl arginines in the fragile X mental retardation protein Fmrp. Human molecular genetics *15*, 87-96.

Steward, O., Bakker, C.E., Willems, P.J., and Oostra, B.A. (1998a). No evidence for disruption of normal patterns of mRNA localization in dendrites or dendritic transport of recently synthesized mRNA in FMR1 knockout mice, a model for human fragile-X mental retardation syndrome. Neuroreport *9*, 477-481.

Steward, O., and Levy, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. J Neurosci *2*, 284-291.

Steward, O., and Schuman, E.M. (2001). Protein synthesis at synaptic sites on dendrites. Annual review of neuroscience *24*, 299-325.

Steward, O., Wallace, C.S., Lyford, G.L., and Worley, P.F. (1998b). Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. Neuron *21*, 741-751.

Sugiyama, H., Ito, I., and Hirono, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. Nature *325*, 531-533.

Sung, K.W., Choi, S., and Lovinger, D.M. (2001). Activation of group I mGluRs is necessary for induction of long-term depression at striatal synapses. Journal of neurophysiology *86*, 2405-2412.

Sutcliffe, J.S., Nelson, D.L., Zhang, F., Pieretti, M., Caskey, C.T., Saxe, D., and Warren, S.T. (1992). DNA methylation represses FMR-1 transcription in fragile X syndrome. Human molecular genetics *1*, 397-400.

Sutton, M.A., Ito, H.T., Cressy, P., Kempf, C., Woo, J.C., and Schuman, E.M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. Cell *125*, 785-799.

Sutton, M.A., and Schuman, E.M. (2005). Local translational control in dendrites and its role in long-term synaptic plasticity. Journal of neurobiology *64*, 116-131.

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

Swingle, M., Ni, L., and Honkanen, R.E. (2007). Small-molecule inhibitors of ser/thr protein phosphatases: specificity, use and common forms of abuse. Methods Mol Biol *365*, 23-38.

Takamiya, K., Mao, L., Huganir, R.L., and Linden, D.J. (2008). The glutamate receptor-interacting protein family of GluR2-binding proteins is required for long-term synaptic depression expression in cerebellar Purkinje cells. J Neurosci *28*, 5752-5755.

Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R., and Nakanishi, S. (1992). A family of metabotropic glutamate receptors. Neuron *8*, 169-179.

Tassone, F., Hagerman, R.J., Ikle, D.N., Dyer, P.N., Lampe, M., Willemsen, R., Oostra, B.A., and Taylor, A.K. (1999). FMRP expression as a potential prognostic indicator in fragile X syndrome. American journal of medical genetics *84*, 250-261.

Taylor, A.M., Dieterich, D.C., Ito, H.T., Kim, S.A., and Schuman, E.M. (2010). Microfluidic local perfusion chambers for the visualization and manipulation of synapses. Neuron *66*, 57-68.

Thomas, A.M., Bui, N., Perkins, J.R., Yuva-Paylor, L.A., and Paylor, R. (2012). Group I metabotropic glutamate receptor antagonists alter select behaviors in a mouse model for fragile X syndrome. Psychopharmacology (Berl) *219*, 47-58.

Todd, P.K., Mack, K.J., and Malter, J.S. (2003). The fragile X mental retardation protein is required for type-I metabotropic glutamate receptor-dependent translation of PSD-95. Proceedings of the National Academy of Sciences of the United States of America *100*, 14374-14378.

Valverde, R., Edwards, L., and Regan, L. (2008). Structure and function of KH domains. FEBS J *275*, 2712-2726.

Van't Padje, S., Chaudhry, B., Severijnen, L.A., van der Linde, H.C., Mientjes, E.J., Oostra, B.A., and Willemsen, R. (2009). Reduction in fragile X related 1 protein causes cardiomyopathy and muscular dystrophy in zebrafish. J Exp Biol *212*, 2564-2570.

Vasudevan, S., Tong, Y., and Steitz, J.A. (2007). Switching from repression to activation: microRNAs can up-regulate translation. Science (New York, NY *318*, 1931-1934.

Verheij, C., Bakker, C.E., de Graaff, E., Keulemans, J., Willemsen, R., Verkerk, A.J., Galjaard, H., Reuser, A.J., Hoogeveen, A.T., and Oostra, B.A. (1993). Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. Nature *363*, 722-724.

Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, F.P*., et al.* (1991). Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell *65*, 905-914.

Viviani, B. (2006). Preparation and coculture of neurons and glial cells. Curr Protoc Cell Biol *Chapter 2*, Unit 2 7.

Volk, L.J., Daly, C.A., and Huber, K.M. (2006). Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. Journal of neurophysiology *95*, 2427-2438.

Volk, L.J., Pfeiffer, B.E., Gibson, J.R., and Huber, K.M. (2007). Multiple Gq-coupled receptors converge on a common protein synthesis-dependent long-term depression that is affected in fragile X syndrome mental retardation. J Neurosci *27*, 11624-11634.

Walden, W.E., Godefroy-Colburn, T., and Thach, R.E. (1981). The role of mRNA competition in regulating translation. I. Demonstration of competition in vivo. The Journal of biological chemistry *256*, 11739-11746.

Wallace, C.S., Lyford, G.L., Worley, P.F., and Steward, O. (1998). Differential intracellular sorting of immediate early gene mRNAs depends on signals in the mRNA sequence. J Neurosci *18*, 26-35.

Waltereit, R., Dammermann, B., Wulff, P., Scafidi, J., Staubli, U., Kauselmann, G., Bundman, M., and Kuhl, D. (2001). Arg3.1/Arc mRNA induction by Ca2+ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. J Neurosci *21*, 5484-5493.

Wan, L., Dockendorff, T.C., Jongens, T.A., and Dreyfuss, G. (2000). Characterization of dFMR1, a Drosophila melanogaster homolog of the fragile X mental retardation protein. Mol Cell Biol *20*, 8536-8547.

Wang, K.H., Majewska, A., Schummers, J., Farley, B., Hu, C., Sur, M., and Tonegawa, S. (2006). In vivo two-photon imaging reveals a role of arc in enhancing orientation specificity in visual cortex. Cell *126*, 389-402.

Waung, M.W., and Huber, K.M. (2009). Protein translation in synaptic plasticity: mGluR-LTD, Fragile X. Curr Opin Neurobiol *19*, 319-326.

Waung, M.W., Pfeiffer, B.E., Nosyreva, E.D., Ronesi, J.A., and Huber, K.M. (2008). Rapid translation of Arc/Arg3.1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. Neuron *59*, 84-97.

Welch, J.M., Lu, J., Rodriguiz, R.M., Trotta, N.C., Peca, J., Ding, J.D., Feliciano, C., Chen, M., Adams, J.P., Luo, J.*, et al.* (2007). Cortico-striatal synaptic defects and OCD-like behaviours in Sapap3-mutant mice. Nature *448*, 894-900.

Welch, J.M., Wang, D., and Feng, G. (2004). Differential mRNA expression and protein localization of the SAP90/PSD-95-associated proteins (SAPAPs) in the nervous system of the mouse. J Comp Neurol *472*, 24-39.

Westmark, C.J., and Malter, J.S. (2007). FMRP mediates mGluR5-dependent translation of amyloid precursor protein. PLoS Biol *5*, e52.

Westmark, C.J., Westmark, P.R., O'Riordan, K.J., Ray, B.C., Hervey, C.M., Salamat, M.S., Abozeid, S.H., Stein, K.M., Stodola, L.A., Tranfaglia, M.*, et al.* (2011). Reversal of fragile X phenotypes by manipulation of AbetaPP/Abeta levels in Fmr1KO mice. PLoS One *6*, e26549.

Whitman, S.A., Cover, C., Yu, L., Nelson, D.L., Zarnescu, D.C., and Gregorio, C.C. (2011). Desmoplakin and talin2 are novel mRNA targets of fragile X-related protein-1 in cardiac muscle. Circ Res *109*, 262-271.

Wohrle, D., Kotzot, D., Hirst, M.C., Manca, A., Korn, B., Schmidt, A., Barbi, G., Rott, H.D., Poustka, A., Davies, K.E.*, et al.* (1992). A microdeletion of less than 250 kb, including the proximal part of the FMR-I gene and the fragile-X site, in a male with the clinical phenotype of fragile-X syndrome. Am J Hum Genet *51*, 299-306.

Wu, H., Zeng, H., Lam, R., Tempel, W., Amaya, M.F., Xu, C., Dombrovski, L., Qiu, W., Wang, Y., and Min, J. (2011a). Structural and histone binding ability characterizations of human PWWP domains. PLoS One *6*, e18919.

Wu, J., Harney, S., Rowan, M.J., and Anwyl, R. (2008). Involvement of group I mGluRs in LTP induced by strong high frequency stimulation in the dentate gyrus in vitro. Neurosci Lett *436*, 235-238.

Wu, J., Petralia, R.S., Kurushima, H., Patel, H., Jung, M.Y., Volk, L., Chowdhury, S., Shepherd, J.D., Dehoff, M., Li, Y.*, et al.* (2011b). Arc/Arg3.1 regulates an endosomal pathway essential for activity-dependent beta-amyloid generation. Cell *147*, 615-628.

Xia, J., Chung, H.J., Wihler, C., Huganir, R.L., and Linden, D.J. (2000). Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. Neuron *28*, 499-510.

Xiao, M.Y., Zhou, Q., and Nicoll, R.A. (2001). Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. Neuropharmacology *41*, 664-671.

Xie, W., Dolzhanskaya, N., LaFauci, G., Dobkin, C., and Denman, R.B. (2009). Tissue and developmental regulation of fragile X mental retardation 1 exon 12 and 15 isoforms. Neurobiol Dis *35*, 52-62.

Xu, W., Schluter, O.M., Steiner, P., Czervionke, B.L., Sabatini, B., and Malenka, R.C. (2008). Molecular dissociation of the role of PSD-95 in regulating synaptic strength and LTD. Neuron *57*, 248-262.

Xu, X.L., Zong, R., Li, Z., Biswas, M.H., Fang, Z., Nelson, D.L., and Gao, F.B. (2011). FXR1P but not FMRP regulates the levels of mammalian brain-specific microRNA-9 and microRNA-124. J Neurosci *31*, 13705-13709.

Yan, Q.J., Rammal, M., Tranfaglia, M., and Bauchwitz, R.P. (2005). Suppression of two major Fragile X Syndrome mouse model phenotypes by the mGluR5 antagonist MPEP. Neuropharmacology *49*, 1053-1066.

Yin, Y., Edelman, G.M., and Vanderklish, P.W. (2002). The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneurosomes. Proceedings of the National Academy of Sciences of the United States of America *99*, 2368-2373.

Ying, S.W., Futter, M., Rosenblum, K., Webber, M.J., Hunt, S.P., Bliss, T.V., and Bramham, C.R. (2002). Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. J Neurosci *22*, 1532-1540.

Yun, S.H., and Trommer, B.L. (2011). Fragile X mice: reduced long-term potentiation and N-Methyl-D-Aspartate receptor-mediated neurotransmission in dentate gyrus. J Neurosci Res *89*, 176-182.

Zalfa, F., Achsel, T., and Bagni, C. (2006). mRNPs, polysomes or granules: FMRP in neuronal protein synthesis. Curr Opin Neurobiol *16*, 265-269.

Zalfa, F., Eleuteri, B., Dickson, K.S., Mercaldo, V., De Rubeis, S., di Penta, A., Tabolacci, E., Chiurazzi, P., Neri, G., Grant, S.G.*, et al.* (2007). A new function for the fragile X mental retardation protein in regulation of PSD-95 mRNA stability. Nat Neurosci *10*, 578-587.

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

Zeng, H., Chattarji, S., Barbarosie, M., Rondi-Reig, L., Philpot, B.D., Miyakawa, T., Bear, M.F., and Tonegawa, S. (2001). Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. Cell *107*, 617-629.

Zhang, Y., O'Connor, J.P., Siomi, M.C., Srinivasan, S., Dutra, A., Nussbaum, R.L., and Dreyfuss, G. (1995). The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. The EMBO journal *14*, 5358-5366.

Zhang, Y., Venkitaramani, D.V., Gladding, C.M., Kurup, P., Molnar, E., Collingridge, G.L., and Lombroso, P.J. (2008). The tyrosine phosphatase STEP mediates AMPA receptor endocytosis after metabotropic glutamate receptor stimulation. J Neurosci *28*, 10561-10566.

Zhao, M.G., Toyoda, H., Ko, S.W., Ding, H.K., Wu, L.J., and Zhuo, M. (2005). Deficits in trace fear memory and long-term potentiation in a mouse model for fragile X syndrome. J Neurosci *25*, 7385-7392.

Zhao, W., Chuang, S.C., Bianchi, R., and Wong, R.K. (2011). Dual regulation of fragile X mental retardation protein by group I metabotropic glutamate receptors controls translation-dependent epileptogenesis in the hippocampus. J Neurosci *31*, 725-734.

Zhong, S., Hu, P., Ye, T.Z., Stan, R., Ellis, N.A., and Pandolfi, P.P. (1999). A role for PML and the nuclear body in genomic stability. Oncogene *18*, 7941-7947.

Zukin, R.S., Richter, J.D., and Bagni, C. (2009). Signals, synapses, and synthesis: how new proteins control plasticity. Front Neural Circuits *3*, 14.