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The Regulation of A β PP Expression by RNA-Binding Proteins

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Abstract

Amyloid β -protein precursor (A β PP) is cleaved by β - and γ -secretases to liberate amyloid beta (A β), the predominant protein found in the senile plaques associated with Alzheimer's disease (AD) and Down syndrome (Masters et al., 1985). Intense investigation by the scientific community has centered on understanding the molecular pathways that underlie the production and accumulation of A β . Therapeutics that reduce the levels of this tenacious, plaque-promoting peptide may reduce the ongoing neural dysfunction and neuronal degeneration that occurs so profoundly in AD. A β PP and A β production are highly complex and involve still to be elucidated combinations of transcriptional, post-transcriptional, translational and post-translational events that mediate the production, processing and clearance of these proteins. Research in our laboratory for the past two decades has focused on the role of RNA binding proteins (RBPs) in mediating the post-transcriptional as well as translational regulation of *APP* messenger RNA (mRNA). This review article summarizes our findings, as well as those from other laboratories, describing the identification of regulatory RBPs, where and under what conditions they interact with *APP* mRNA and how those interactions control A β PP and A β synthesis.

Keywords

A β PP; A β ; FMRP; hnRNP C; nucleolin; post-transcriptional gene regulation

1. Introduction

The *APP* gene is located on human chromosome 21 and codes for A β PP, a ubiquitously expressed, transmembrane protein that localizes to post-synaptic densities, axons, dendrites and neuromuscular junctions (NMJ) (Akaaboune et al., 2000; Shigematsu et al., 1992), consistent with its multiple roles in cell adhesion (Soba et al., 2005), synapse formation (Torroja et al., 1999; Yang et al., 2005) and synapse maturation (Akaaboune et al., 2000). Differential processing of A β PP by non-amyloidogenic (α - and γ -) or amyloidogenic (β - and γ -) secretases produces soluble A β PP (sA β PP α and sA β PP β), truncated or full-length amyloid-beta (A β) and variable length carboxy-terminal fragments (CTFs). The over-production and ultimate aggregation of A β is associated with AD and Down syndrome pathology with current evidence suggesting a more pathogenic role for soluble, pre-

aggregated A β . Despite decades of investigation, much remains to be learned about the normal physiological function of A β and other A β PP catabolites as well as the cellular and molecular pathways that regulate their generation. Clearly, a better understanding of A β PP biology and gene expression will provide new avenues for therapeutic intervention in a number of highly prevalent and currently untreatable neurological disorders.

Our laboratory has studied the mechanisms that underlie the stability and translation of *APP* mRNA. These studies were triggered by the realization that the 3' untranslated regions (3' UTR) of mouse, rat, human and other species of *APP* mRNA contained islands of substantial homology, consistent with the presence of *cis-acting* elements. We hypothesized that *APP* mRNA was subject to activation-dependent changes in stability and/or translation. Such events could account for both increased mRNA under steady state levels as well as increased translation. Indeed, we and other groups have successfully defined multiple *cis*-regulatory elements within the 5'-UTR, coding region (CR) and 3'-UTR of *APP* mRNA that mediate the post-transcriptional stability and/or translation of *APP* mRNA (Zaidi and Malter, 1994; Amara et al., 1999; Rogers et al., 2002; Westmark et al., 2006; Westmark and Malter, 2007) as well as identified a number of RBPs that bind to these *cis*-elements (Zaidi et al., 1994; Zaidi and Malter, 1995; Westmark and Malter, 2007; Brody et al., 2008; Lee et al., 2010; Cho et al., 2010). These mRNA/RBP complexes control the degradation, stability and/or translation of *APP* mRNA, which directly determines how much A β PP is synthesized and thus available for processing towards or away from amyloidogenic endpoints.

This review article summarizes our findings, as well as those from the Rogers, Amara and Gorospe laboratories, describing the mRNA/RBP complexes and signaling pathways that regulate A β PP expression. A detailed understanding of the molecular interactions that regulate A β PP synthesis may provide novel avenues for therapeutic intervention in the treatment of AD and other A β -related disorders.

2. *Cis*-regulatory elements in *APP* mRNA

mRNA stability is an important control point in gene expression. The mRNAs of certain classes of regulatory proteins, such as oncogenes, cytokines, lymphokines and transcriptional activators are extremely labile (Brewer, 1991; Shaw and Kamen, 1986). Many of these mRNAs contain a common AUUUA pentamer in their 3'-UTR, which confers message instability through interactions with RBPs, such as AU-rich binding factor (AUBF) (Malter, 1989; Gillis and Malter, 1991), AUF1 (Wang et al., 1998) or TTP (Lai et al., 1999). In the early 1990s, we were interested in defining the contribution of mRNA stability to steady-state levels of *APP* mRNA in neurons. A large number of AU-rich mRNAs are expressed in the central nervous system and we hypothesized that *APP* would also be a labile mRNA due to the presence of multiple 3'-UTR AUUUA motifs in its 3'-UTR. The first of its four AUUUA elements in the context of an AU-rich region is located approximately 65 bases downstream from the stop codon in both human and murine *APP* mRNA. The AUUUA motif is frequently identified in a similar location in other labile mRNAs (Caput et al., 1986; Shaw and Kamen, 1986). Somewhat unexpectedly, we found that *APP* mRNA has a half-life of 4–5 hr in primary cells, which is intermediate between labile and stable housekeeping genes (Zaidi and Malter, 1994; Westmark and Malter, 2001a). These data suggested, and were later confirmed, that the aggregate content of AUUUA motifs did not correlate well with mRNA stability. The AUUUA motifs in *APP* mRNA are individually arranged and separated by approximately 50 bases whereas these motifs are tandemly stacked (AUUUUAUUUA etc.) in labile mRNAs such as IL-2 or GM-CSF (Lagnado et al., 1994; Zubiaga et al., 1995). Thus, the moderate instability of *APP*

mRNA in resting cells is consistent with an alternative *cis*-element presumably distinct from the AUUUA motifs.

Indeed, deletion mapping of the 3'-UTR indicated that a pyrimidine-rich 29-base element (29-be) approximately 200 bases downstream from the stop codon (bases 2285–2313) mediated *APP* mRNA decay (Zaidi et al., 1994). This element is highly conserved in human and murine sequences and is 69% AU-rich although it lacks the canonical AUUUA motifs (Zaidi et al., 1994). Mutant *APP* mRNA containing a randomized 29-be was far more stable than WT with a half-life >10 hr (Zaidi and Malter, 1994). Later, we showed that heterologous mRNAs containing the 29-be in their 3'-UTR showed similar destabilization (Westmark et al., 2006). Interestingly, when repositioned into the CR by deletion of the stop codon, the 29-be failed to function (Westmark et al., 2006). These results suggested a topologic constraint on the element. We hypothesized that the transiting ribosome might displace a protein required for function and/or the tertiary conformation of the mRNA. Thus, the 29-be clearly functions in *cis* to destabilize *APP* mRNA and must be located within the 3'-UTR, not the CR.

The majority of studies regarding *APP* mRNA stability have focused on the 3'-UTR. As *APP* mRNAs are produced by alternative splicing of a single copy gene and all share an identical 3'-UTR, all resultant *APP* mRNAs would be co-regulated. However, whether this is actually true has not been rigorously tested in cells. In addition to the 29-be, two additional, and opposing *cis*-regulatory elements in the 3'-UTR of *APP* mRNA have been identified. Amara and colleagues found an 81-be (positions 2730–2810) that destabilized *APP* mRNA (Amara et al., 1999), and our laboratory identified a 52-be immediately downstream from the translation stop codon that stabilized *APP* mRNA (Westmark et al., 2006). The proximal 200 bases of the 3'-UTR of *APP* mRNA thus contain both the 29-be and 52-be. Sequence conservation over the entirety of this region suggested maintenance of an important regulatory element (Westmark et al., 2006). Computer modeling of this 250 base segment predicts a highly stable structure with the terminal 29-be and 52-be in apposition to each other (Westmark et al., 2006). To test if the aggregate structure and/or the individual elements controlled *APP* mRNA decay, we deleted the entire 200 base intervening sequence between the stop codon and the 29-be. These mRNAs showed similar half-lives to those simply lacking the 52-be (Westmark et al., 2006). To ensure this was due to the loss of specific regulatory sequence rather than spacing and/or changes in conformation, we deleted the 54 bases immediately upstream from the 29-be. These mutant mRNAs had near normal stability (Westmark et al., 2006). Thus, the 52-be is most likely a bona fide stabilizer of *APP* mRNA that acts independently but in opposition to the 29-be.

In addition to the 3'-UTR elements, there are also *cis*-regulatory elements in the 5'-UTR and CR of *APP* mRNA. As for other mRNAs, element location often (but not always) has functional repercussions. Thus not surprisingly, CR and 5'-UTR locations have been associated with translational control rather than mRNA stability. For example, Rogers and coworkers identified a type II iron response element (IRE) in the 5'-UTR of *APP* mRNA (+51 to +144 from the 5' cap site) (Rogers et al., 2002). This stem loop RNA IRE was highly homologous to translational control elements in the 5'-UTR of the light (L) and heavy (H) ferritin genes and was located immediately upstream from an interleukin-1 (IL1) responsive acute box domain (+101 to +146) (Rogers et al., 1999; Rogers et al., 2002). Recently, we identified a guanine-rich area in the CR of *App* mRNA that bound to fragile X mental retardation protein (FMRP) [*APP* refers to the human and *App* to the mouse transcript]. Binding was maintained under basal conditions but was lost within minutes of metabotropic glutamate receptor 5 (mGluR₅)-mediated signaling. As *App* mRNA translation was increased in the absence of FMRP binding (after mGluR₅ agonists), these data suggest the CR element mediates translational repression (Westmark and Malter, 2007). These

results were later confirmed by the Gorospe laboratory that further demonstrated that this element differentially interacts with FMRP and hnRNP C to modulate translation (Lee et al., 2010).

3. RNA binding proteins that interact with *APP* mRNA

Given the numerous *cis*-elements described above, it is hardly surprising that multiple RBPs have been identified that interact with *APP* mRNA. Many of these proteins are known to bind to multiple mRNAs and have diverse functions including nuclear/cytoplasmic shuttling, RNA helicase activity, iron homeostasis and translational repression. Interestingly, with the exception of the 5'-UTR IRE-like domain, the *cis*-elements in *APP* mRNA do not show obvious or substantial homology to other mRNAs. As it is unlikely that RBPs have evolved to specifically regulate *APP* mRNA, these results suggest that the tertiary structure assumed by the element likely plays an important role in RBP recognition and binding.

3.1 hnRNP C and Nucleolin

The search for RBPs began by probing whole cell lysates from neuronal tumor lines with radioactive fragments of the 29-be. After relatively short incubations under conditions of physiologic salt and high concentrations of irrelevant, competitor RNA (typically tRNAs), reactions were treated with RNase to digest nonspecific interactions prior to nondenaturing gel electrophoresis. RBP/RNA interactions were identified by the presence of a slowly migrating band above that of free radioactive RNA in an electrophoretic gel mobility shift assay (EMSA). Specificity was established by the competition of the slowly migrating complex with increasing amounts of unlabeled probe RNA but not with similar concentrations of irrelevant RNA. The molecular mass of the complex was determined by treating reactions with UV light prior to SDS-PAGE. These methods detected multiple RBPs in cytosolic lysates prepared from normal and transformed human cells (Zaidi et al., 1994).

Obviously, identification of these RBPs was a critical next step prior to characterization of their role in *APP* mRNA stability. Through classical, biochemical, chromatographic methods, we fractionated cytosolic lysates prepared from human leukemia T-cells and followed binding activity by RNA EMSAs. Ultimately, the 29-be interacted *in vitro* with five cytoplasmic RBPs of 70, 48, 47, 39 and 38 kDa (Zaidi et al., 1994). Northwestern blotting with radiolabeled RNA confirmed that the purified proteins interacted specifically with the 29-be. After SDS-PAGE isolation and mass spectrometry analysis, the p47, p48 and p70 RBPs were identified as fragments of 110-kDa nucleolin and the p38 and p39 were identified as hnRNP C1 and hnRNP C2, respectively (Zaidi and Malter, 1995). The 70 kDa fragment of nucleolin and the hnRNP C proteins bound to the 5'-half of the 29-be while the 47 and 48 kD fragments of nucleolin likely bound to the 3'-end (Zaidi and Malter, 1995).

hnRNP C1 and C2 are members of a family of RBPs involved in the packaging of heterogeneous nuclear RNA (hnRNA) and its conversion to mRNA in the nucleus (Choi and Dreyfuss, 1984), and they showed some specificity for uridine-rich sequences (Wilusz and Shenk, 1990). hnRNP A1 and C were previously identified as RBPs that shuttle between the nucleus and cytoplasm and were capable of specifically binding to reiterated AUUUA sequences found in the 3'-UTRs of labile mRNAs (Hamilton et al., 1993). The hnRNP A and C families have been implicated in chromatin remodeling (Mahajan et al., 2005), replicator-binding protein (Huang et al., 2011), mammalian telomerase (Ford et al., 2000) and internal ribosome entry site complexes (Holcik et al., 2003). They have established roles in the prevention of transcriptional silencing (Huang et al., 2011), splicing (Choi et al., 1986) and translation (Millard et al., 2000; Holcik et al., 2003).

Nucleolin is a 110 kDa multifunctional phosphoprotein that plays roles in chromatin condensation (Erard et al., 1988), the transcription and processing of rRNA (Jordan, 1987; Egyhazi et al., 1988; Belenguer et al., 1989; Bouvet et al., 1998; Ginisty et al., 1998), transcriptional regulation (Yang et al., 1994), cell proliferation (Derenzini et al., 1995), differentiation and maintenance of neural tissue (Kibbey et al., 1995), apoptosis (Brockstedt et al., 1998), nuclear/cytoplasmic shuttling (Borer et al., 1989), mRNA stability (Zaidi et al., 1994) and mRNP assembly and masking (Yurkova and Murray, 1997). The carboxy-terminus of nucleolin has intrinsic RNA helicase activity capable of unwinding mRNA and thereby increasing its susceptibility to RNase attack (Tuteja et al., 1995). Nucleolin has been implicated in the post-transcriptional regulation of several mRNAs (Sengupta et al., 2004; Singh et al., 2004; Fahling et al., 2005). We discuss the opposing stabilizing (hnRNP C) and destabilizing (nucleolin) activities of these proteins in regard to *APP* mRNA post-transcriptional stability in the next section.

3.2 RBPs that bind to the 52-base element

The 52-be adjacent to the stop codon in *APP* mRNA bound to several RBPs based on EMSAs when interrogated with whole cell lysates from K562 cells (Broytman et al., 2008). We purified interacting proteins by RNA affinity chromatography using a biotinylated 52-be tethered to a streptavidin affinity matrix. Protein fractions were analyzed by northwestern blotting and active bands were excised and identified by mass spectrometry. We identified 6 proteins including RCK/p54, nucleolin, plasminogen activator inhibitor-RNA binding protein 1 (PAI-RBP1), autoantigen La, elongation factor 1 α (EF1 α) and Y box binding protein (YB1). We confirmed these RBPs as true 52-be targets by performing EMSA with K562 lysates and radiolabeled 52-be RNA followed by excising the protein/RNA complexes, running them on SDS-PAGE and immunoblotting against the putative targets (Broytman et al., 2008). With the exception of nucleolin, which binds to the 29-be, these RBPs were not known to bind to *APP* mRNA. EMSA with radiolabeled RNA of the first 250 bases of the *APP* 3'-UTR produced 3 complexes. All six 52-be binding proteins were found in the heaviest migrating complex. The intermediate migrating complex contained nucleolin, autoantigen La and YB1, and the fastest migrating complex only contained YB1 and La (Broytman et al., 2008).

Co-immunoprecipitation studies indicated that nucleolin and EF1 α both co-immunoprecipitated with all of the other 5 proteins (Broytman et al., 2008). However, the co-immunoprecipitations of nucleolin with La or YB1 was RNA-dependent, whereas its co-immunoprecipitation with RCK/p54, PAI-RBP1 and EF1 α was RNA-independent. Likewise, EF1 α co-immunoprecipitations with La and YB1 were RNA-dependent and co-immunoprecipitations with nucleolin, RCK/p54 and PAI-RBP1 were RNA independent (Broytman et al., 2008). These data suggest that the RBP complex at the 52-be in the 3'-UTR of *APP* mRNA contains both protein/protein interactions (nucleolin, EF1 α , RCK/p54 and PAI-RBP1) and protein/mRNA interactions (YB1, La) (Broytman et al., 2008).

To confirm that these *in vitro* interactions mirror those seen in intact cells, we immunoprecipitated all six RBPs from the cytosol fraction of SH-SY5Y human neuroblastoma cell line and demonstrated by RT/qPCR analysis that *APP* mRNA was specifically present in the co-immunoprecipitate (Broytman et al., 2008). The RBPs involved in protein/52-be RNA interactions are YB1 and La. As there is little sequence homology between their known mRNA targets and *APP* mRNA, YB1 and La binding at the 52-be are likely mediated by the secondary or tertiary structure of the RNA. Nucleolin, on the other hand, while a well-established RBP, is tethered to the 52-be/protein complex via protein/protein interactions. YB1 is a member of the highly conserved cold shock domain family of proteins and is a multifunctional nucleic acid binding protein implicated in transcriptional activation and repression, cellular and viral mRNA binding, mRNA

stabilization and translational control (Ansari et al., 1999; Bouvet et al., 1995; Capowski et al., 2001; Chen et al., 2000; Didier et al., 1988; Ranjan et al., 1993; Stenina et al., 2000). The autoantigen La is a well-characterized RBP that has been shown to stabilize histone mRNA (McLaren et al., 1997).

The other RBPs that form protein/protein interactions at the 52-be are EF1 α , RCK/p54 and PAI-RBP1. EF1 α regulates the transfer of aminoacyl-tRNAs to the ribosome and is involved in the nuclear export of proteins (Khacho et al., 2008). Broytman and colleagues were the first to show that it bound to an mRNA and possibly functioned in mRNA stabilization (Broytman et al., 2008). Its role in the posttranscriptional regulation of *APP* mRNA could involve utilization of energy from GTP hydrolysis to facilitate the binding of cofactors, to catalyze a change in secondary/tertiary structure of the 52-be or the energy-dependent transport of the RBP/*APP* mRNA complexes out of the nucleus (Broytman et al., 2008). RCK/p54 is a member of the DEAD-box helicase family of proteins, which have known roles in splicing, ribosome biogenesis, RNA transport, degradation and translation (Akao et al., 1995). RCK/p54 is a component of both processing (P)-body and cytoplasmic polyadenylation element-binding protein (CPEB) translation repressor complexes (Minshall et al., 2007; Minshall et al., 2009). PAI-RBP1 is an RBP that interacts with the adenosine-rich region of the cyclic nucleotide-responsive sequence in 3'-UTR of type-1 plasminogen activator inhibitor mRNA (Heaton et al., 2001) to regulate post-transcriptional stability (Heaton et al., 2003). The 52-be does not contain an adenosine-rich stretch consistent with its RNA independent binding to PAI-RBP1.

3.3 IRP1

Iron homeostasis is regulated post-transcriptionally in mammalian cells through iron regulatory proteins (IRP). IRP1 and IRP2 bind with high affinity to iron response element (IRE) stem-loop structures in the transcripts of iron storage proteins such as ferritin (Kim et al., 1995; Thomson et al., 2000). IRP are released from L- and H-ferritin transcripts during iron influx resulting in the latter's increased translation (Cox et al., 1991; Clarke et al., 2006). IRP1 modulates A β PP translation by binding to an IRE stem-loop structure in the 5'-UTR of the *APP* mRNA (Cho et al., 2010). Intracellular iron chelation increases the binding of IRP1 to the IRE thereby decreasing A β PP expression. Conversely, shRNA knockdown of IRP1 increases A β PP translation (Cho et al., 2010). Interestingly, the light and heavy ferritin messages bind to both IRP1 and IRP2 whereas only IRP1 binds to the IRE of *APP* mRNA (Cho et al., 2010).

3.4 FMRP

FMRP is a multi-functional mRNA binding protein involved in the transport, localization and translational regulation of a subset of dendritic mRNAs and is required for normal dendrite development (Santoro et al., 2011). The protein has 2 hnRNP K homology domains and 1 RGG box as well as nuclear localization and export signals. The RGG box of FMRP binds to intramolecular G-quartet sequences in target mRNAs (Darnell et al., 2001) while the KH2 domain has been proposed to bind to kissing complex RNAs based on *in vitro* selection assays (Darnell et al., 2005). In dendrites, FMRP is predominantly found in the post-synaptic density (PSD) associated with polysomes or nontranslating ribonucleoprotein (RNP) particles. The RNPs contain the FMRP homologs FXR1 and 2, YB1, nucleolin, the guide RNA BC1 and mRNA targets (Bagni and Greenough, 2005). Hundreds of mRNA ligands have been identified (Brown et al., 2001; Chen et al., 2003; Dolzhanskaya et al., 2003; Miyashiro et al., 2003) with many having the potential to influence synapse formation and synaptic plasticity. FMRP has been implicated in translational repression (Brown et al., 2001; Lagerbauer et al., 2001; Li et al., 2001; Mazroui et al., 2002; Miyashiro et al., 2003; Zalfa et al., 2003), and in brain, co-sediments with both translating polyribosomes (Stefani

et al., 2004) and with mRNPs (Zalfa et al., 2003). In addition to G-quartets and kissing complex structures, FMRP binds to uridine-rich mRNAs (Chen et al., 2003; Dolzhanskaya et al., 2003). It has been proposed that FMRP is an “immediate early protein” at the synapse orchestrating synaptic development and plasticity (Irwin et al., 2000). FMRP binds to a guanine-rich sequence in the CR of *App* mRNA (Westmark and Malter, 2007) and competes for binding to this sequence with hnRNP C (Lee et al., 2010). When FMRP is bound, translation is repressed. When hnRNP C is bound, translation is active. FMRP also protects the 29-be in the 3'-UTR of *App* mRNA from ribonuclease digestion (Westmark and Malter, 2007), but it is not known at this time if FMRP binds directly to the 3'-UTR or if protection occurs through protein/protein interactions.

4. Post-transcriptional gene regulation of *APP* mRNA

Post-transcriptional gene regulation is a complex process involving interaction of *cis*-regulatory elements in the 3'-UTR of mRNAs with *trans* factors or RBPs. These interactions are regulated by cell signaling events and likely result in alterations in higher order structures of the mRNAs. A summary of the identified *cis*-regulatory elements and *trans* factors involved in the pos-transcriptional gene regulation of *APP* mRNA is depicted in Figure 1. The role of microRNAs (miRNAs) is also evolving as an important control mechanism in post-transcriptional gene regulation. The effects of these *cis*-elements and *trans*-factors on message stability and translation can be studied by supplementation of a rabbit reticulocyte lysates (RRL) translation system with *in vitro* synthesized mRNAs harboring mutated *cis*-elements, transduction of bacterially expressed TAT-conjugated RBPs or transfection of cDNA constructs into cells of choice.

4.1 The 3'-UTR

All *APP* mRNA isoforms share a common 1,134 base 3'-UTR, suggesting that the above described elements and their cognate RBPs can coordinately regulate all forms simultaneously. We directly examined the role of these *trans* factors in the turnover and translation of *APP* mRNA in RRL. A mutant *APP* mRNA containing a randomized 29-be (MT) was 3–4-fold more stable and synthesized 2–4-fold more A β PP than WT *APP* mRNA in RRL (Rajagopalan et al., 1998). Therefore, the 29-be functioned as an *APP* mRNA destabilizer in this system. RNA EMSAs with the RRL suggested the presence of endogenous nucleolin, but failed to show hnRNP C binding activity. However, WT *APP* mRNA was stabilized and coded for 6-fold more A β PP when translated in RRL system supplemented with exogenous hnRNP C. Translation of the MT *APP* mRNA was not affected by supplementation of the RRL with hnRNP C (Rajagopalan et al., 1998). Therefore, interactions with hnRNP C stabilized *APP* mRNA when those events occurred in the 3'-UTR. As described later, when hnRNP C bound to the guanine-rich CR domain, the resulting phenotype showed enhanced translation without a change in stability (Lee et al., 2010). Thus, depending on the site of interaction, RBPs can affect distinct components of the post-transcriptional regulatory system.

The 29-be also functions as a destabilizer in intact, proliferating human umbilical vein endothelial cells (HUVEC) (Rajagopalan and Malter, 2000). Optimal proliferation of HUVEC requires medium containing epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF-1) and vascular endothelial growth factor (VEGF). We transfected HUVECs with *in vitro* transcribed chimeric mRNAs containing the 5'-UTR and CR of beta-globin attached to the entire WT or MT 3'-UTR of *APP* mRNA. The WT and MT *globin-APP* mRNAs decayed with identical half-lives. Removal of all supplemental growth factors from the culture medium significantly accelerated the decay of the transfected WT *globin-APP* mRNA, but only elicited a moderate decrease in the half-life of the MT mRNA (Rajagopalan and Malter, 2000). Thus,

the destabilizing function of the 29-be in the 3'-UTR of *APP* mRNA can be modulated through alterations in growth factor-mediated signaling.

The situation with the 52-be is more complex. As described above, nucleolin, PAI-RBP1, RCK/p54, autoantigen La, EF1 α and YB1 complex with this domain (Westmark et al., 2006; Broytman et al., 2008). Since RCK/p54 belongs to a well-conserved family of RNA helicases with known roles in RNA degradation (Akao et al., 1995; Collier et al., 2001; Tanner and Linder, 2001; Chu and Rana, 2006; Weston and Sommerville, 2006), we first investigated the effects of altering its levels on *APP* mRNA and protein levels in human cells (Broytman et al., 2008). Augmentation of intracellular RCK/p54 protein levels, either through TAT-protein transduction or through cDNA transfection of RCK/54 constructs resulted in increased *APP* mRNA stability and A β PP production (Broytman et al., 2008).

In addition to the 29-be and 52-be, there is a more 3' *cis*-element that also mediates mRNA decay. The half-life of *APP* mRNA is increased by binding of an unidentified protein to this 81 nucleotide fragment in the 3'-UTR to form a 68 kDa RNA-protein complex, which is responsive to TGF β (Amara et al., 1999). The 3'-UTR of *App* mRNA also contains a cytoplasmic polyadenylation element (CPE) at nucleotide 2853 and 2-AAUAAA elements at bases 3098 and 3107 (mouse sequence, accession #NM_007471). Both polyadenylation sites in *App* mRNA are utilized with the longer mRNA showing more efficient ribosomal mobilization and translation than the shorter (de Sauvage et al., 1992).

4.2 Higher Order Structure Interactions

The first 247 bases of the 3'-UTR of *APP* mRNA, which include the 29-be and 52-be, are highly conserved among species and computer modeling suggests that these two *cis*-elements could associate in apposition to each other to form a stable structure (Westmark et al., 2006). Nucleolin and/or RCK/p54, which have RNA helicase activity (Akao et al., 1995; Tuteja et al., 1995), could mediate this RNA/RNA interaction. The hypothesis that the 2 elements interact suggests functional consequences. Consistent with this, chimeric mRNAs composed of the globin 5'-UTR and CR fused to the 3'-UTR of *APP* mRNA were unstable when the 52-be was removed (Westmark et al., 2006). Transfection of SH-SY5Y cells with chimeric mRNAs containing the GFP CR and *APP* 3'-UTR resulted in down-regulation of endogenous A β PP, but only when both the 52-be and 29-be were present in the chimera (Westmark et al., 2006). These data suggest that the 52-be acts in opposition to the 29-be to control *APP* mRNA stability. Since mRNA produced from transfected DNA containing the 3'-UTR of *APP* reduced endogenous A β PP levels (Westmark et al., 2006), our data suggest that the overexpression of exogenous *APP* 3'-UTR sequestered RBPs that normally functioned to stabilize endogenous *APP* mRNA. With reduced *APP* mRNA stability, less A β PP was translated. These data may also explain the observation that A β PP levels are commonly reduced in transgenic animals expressing human *APP* mRNAs (Rockenstein et al., 1995). From a therapeutic perspective, decoy *APP* 3'-UTR DNA constructs could be targeted to neuronal cells to reduce endogenous *APP* mRNA and protein expression in diseases such as AD and Down syndrome (Westmark et al., 2006).

4.3 Signaling

Growth factors activate a number of cell surface receptors and downstream signaling pathways. We assessed the effect of agonists and antagonists of several well-studied receptors and second messenger systems on *APP* mRNA levels (Westmark and Malter, 2001a). Drugs that affected protein kinase A, protein kinase C and phospholipase C signal transduction pathways did not alter *APP* mRNA levels significantly; however, activation of extracellular-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family, as well as calcium signaling modulated *APP* mRNA levels. We found a

rapid 35–40% reduction in steady state levels of *APP* mRNA upon stimulation of peripheral blood mononuclear cells (PBMC) with phorbol ester or calcium ionophore, which caused a rapid activation of ERK that could be blocked with the specific inhibitor U0126. *APP* mRNA underwent biphasic decay upon ERK activation with a rapid, initial decrease in message quantity followed by prolonged stability. We have proposed that the initial unstable phase was due to nucleolin, and the later stable phase was a consequence of hnRNP C binding to the 29-be. Thus, depending on which RBP occupied the 29-be, *APP* mRNA levels were up- or down-regulated.

Conversely, ERK activation up-regulated *nucleolin* mRNA levels in PBMC after stimulation with phorbol ester, which was accompanied by accumulation and decreased processing of nucleolin protein (Westmark and Malter, 2001b). Higher levels of nucleolin were accompanied by increased binding of the 70-kDa-nucleolin fragment to the 29-be. In RRL, supplementation with nucleolin decreased *APP* mRNA stability and protein production. The initial drop in the biphasic decay of *APP* mRNA corresponds temporally with increased nucleolin production. Thus, our data suggests that nucleolin is upregulated in response to ERK activation and then unwinds *APP* mRNA allowing RNase attack. The destabilizing effect of nucleolin on *App* mRNA appears independent of the 29-be *cis*-element as the MT *APP* mRNA also decays rapidly.

Other signaling molecules affect the post-transcriptional stability and translation of *APP* mRNA through the 81 nucleotide instability element in the 3'-UTR, the IRE in the 5'-UTR or the guanine-rich sequence in the CR. TGF β increases the half-life and steady state levels of *APP* mRNA through interaction of an unidentified protein with the 81 nucleotide instability element in the 3'-UTR (Amara et al., 1999). IL-1 α and IL- β up-regulate A β PP synthesis through the 5'-UTR IRE without changing steady state levels of *APP* mRNA (Rogers et al., 1999). Activation of group 1 mGluR signaling increases the translation of A β PP by modulating the binding of FMRP to a guanine-rich sequence in the CR of *APP* mRNA (Westmark and Malter, 2007).

4.4 microRNAs

In addition to RBPs, miRNAs can also bind to *cis*-regulatory elements in mRNAs to post-transcriptionally alter protein production. Several miRNAs including hsa-miR-106a, hsa-miR-520c, miR-101 and miR-20 bind to the 3'-UTR of *APP* mRNA and negatively regulate reporter gene expression (Patel et al., 2008; Ruberti et al., 2010; Long and Lahiri, 2011).

5. Translational regulation of *APP* mRNA

App mRNAs are highly associated with polyribosomes in rat brain (Denman et al., 1991) suggesting that translational regulation could play an important role in A β PP production. At least two identified *cis*-elements regulate translation of *APP* mRNA including an IRE in the 5'-UTR (Rogers et al., 2002) and a guanine-rich element in the CR (Westmark and Malter, 2007; Lee et al., 2010). RBPs, which modulate ribosomal access and initiation, often mediate translational control at the synapse. Two of the best-known examples are IRP1 and FMRP.

5.1 Translational Regulation via the IRE in the 5'-UTR of *APP* mRNA

The 5'-UTR of *APP* mRNA contains an IRE that binds to IRP1, which modulates A β PP translation in response to intracellular iron levels (Cho et al., 2010). Upon intracellular iron chelation, IRP1 binding to the IRE is increased and translation is decreased.

5.2 Translational Regulation via the Guanine-Rich *Cis*-Element in the CR of *APP* mRNA

FMRP represses the translation of a subset of dendritic mRNAs containing G-quartets. We have demonstrated through crosslinking immunoprecipitation (CLIP) assays that FMRP binds directly to a guanine-rich *cis*-element in the CR of *App* mRNA (Westmark and Malter, 2007). Specific activation of group 1 mGluRs (mGluR₁ and mGluR₅) with dihydroxyphenylglycine (DHPG) releases FMRP from *App* mRNA resulting in elevated translation, which can be blocked with the mGluR₅-specific inhibitor MPEP. In *Fmr1*^{KO} neurons, A β PP expression is constitutively elevated and non-responsive to DHPG treatment (Westmark and Malter, 2007).

The Gorospe laboratory confirmed our finding that FMRP binds to the guanine-rich region in the CR of *App* mRNA and represses translation (Lee et al., 2010). They were working with a human neuroblastoma cell line, BE2-M17, and we were using brain lysates from mice. Thus, FMRP is a bona fide target for the guanine-rich *cis*-element in both human and rodent mRNAs as well as in both established and primary cells. The Gorospe laboratory further demonstrated through CLIP analyses that hnRNP C interacts directly with the guanine-rich CR *cis*-element of *APP* mRNA and, through a series of elegant siRNA silencing and RBP transfection experiments, that FMRP and hnRNP C modulate translation in opposing directions (Lee et al., 2010). Their data suggest that FMRP and hnRNP C competitively bind to *APP* mRNA to modulate protein synthesis. When hnRNP C is bound, translation is enhanced, and when FMRP is bound, translation is repressed. The interaction of hnRNP C with *App* mRNA was significantly more abundant in *Fmr1*^{KO} mice, which lack FMRP and exhibit elevated protein translation (Lee et al., 2010).

5.3 Other RBPs that Affect A β PP Translation

Interestingly, the Gorospe laboratory also demonstrated that *APP* mRNA associates with Ago1 and Ago2 proteins through RNA-independent protein/protein interactions (Lee et al., 2010). Ago1, Ago2 and RCK/p54 reside in P-bodies, which are sites where nontranslating mRNAs accumulate before sorting for transient storage or degradation (Barbee et al., 2006; Eulalio et al., 2007; Parker and Sheth, 2007; Balagopal and Parker, 2009). They further demonstrated that FMRP interacts with RCK/p54 and does not repress translation in the absence of RCK/p54, Ago1 or Ago2 (Lee et al., 2010). When FMRP is overexpressed, *APP* mRNA is more abundant in RCK/p54 immunoprecipitates suggesting that FMRP represses translation by enhancing the association of *APP* mRNA with P-bodies. Co-transfection of a pMS2-APP reporter construct expressing a chimeric RNA with 24 tandem MS2 RNA hairpins and the *APPCR cis*-element with plasmid pMS2-YFP, which has a NLS and expresses the chimeric fluorescent protein MS2-YFP, allowed tracking of the subcellular localization of the *MS2-APP* RNA by confocal microscopy. Some RNA colocalized in the cytoplasm with RCK/p54, but was lost upon overexpression of hnRNP C or silencing of FMRP. Thus, FMRP appears to repress the translation of *APP* mRNA by recruiting the transcript to P-bodies, and hnRNP C promotes translation by competing with FMRP for interaction with the CR *cis*-element thus preventing localization of *APP* mRNA to P-bodies (Lee et al., 2010).

Thus, there appears to be competition between stabilizing and destabilizing RBPs at multiple *cis*-regulatory elements of *APP* mRNA. At the 29-be, hnRNP C (stabilizing) and nucleolin (destabilizing) differentially modulate message stability. At the guanine-rich area in the CR, FMRP represses and hnRNP C activates protein synthesis. When we were characterizing the FMRP binding site in *App* mRNA, we found that the 29-be in the 3'-UTR was also protected from T1 ribonuclease digestion of anti-FMRP immunoprecipitates (Westmark and Malter, 2007). As FMRP is a known binding partner of both nucleolin and YB1 (Ceman et al., 1999), this data suggests that a large ribonucleoprotein complex forms to bring multiple

cis-regulatory elements of *APP* mRNA in close proximity. Overall, *APP* mRNA contains several identified *cis*-elements that bind to numerous RBPs, either directly or through protein/protein interactions, to mediate mRNA stability, decay, polyadenylation and translation.

5.4 mGluR-mediated signaling and *App* mRNA

Fragile X Syndrome (FXS) is characterized by excessive mGluR signaling (Bear et al., 2004). Slices from *Fmr1^{KO}* mice reveal excess LTD in response to mGluR₅ agonists (Huber et al., 2002). At a molecular level, basal mGluR₅ signaling is normally attenuated by FMRP through the latter's interactions with and translational suppression of dendritic mRNAs. Upon mGluR₅ activation, translation of post-synaptic mRNAs is transiently enhanced through loss of FMRP-mediated suppression (Bear et al., 2004). We have demonstrated that activation of group 1 mGluR signaling increases translation of A β PP, which can be blocked with the specific mGluR₅ antagonist MPEP (Westmark and Malter, 2007). Therefore, mGluR₅ and FMRP appear to play important roles in the translation and hence accumulation of A β PP at synapses. Consistent with these data, *Fmr1^{KO}* mice have constitutively elevated A β PP levels and older mice exhibit increased A β ₁₋₄₀ and A β ₁₋₄₂ cortical loads compared to WT controls (Westmark and Malter, 2007). Thus, it is very tempting to speculate that mGluR₅ antagonists could be used to reduce *APP* mRNA translation in Alzheimer's disease as well as FXS. As discussed below, we have recently provided evidence that the pathobiology of FXS may indeed reflect altered A β PP and A β levels.

As A β is overexpressed in the brain of *Fmr1^{KO}* mice, we explored the possibility that genetic reduction of A β PP and A β in these mice would rescue characteristic FXS phenotypes (Westmark et al., 2011). We found partial or complete rescue of audiogenic seizures, anxiety, the ratio of mature versus immature dendritic spines and mGluR-LTD after removal of one *App* allele (*Fmr1^{KO}/App^{HET}* mice) (Westmark et al., 2011). Furthermore, plasma A β ₁₋₄₂ levels were significantly reduced in full-mutation FXS males compared to age-matched controls suggesting that A β is sequestered in the brain and could serve as a plasma-based biomarker to facilitate disease diagnosis or assess therapeutic efficacy (Westmark et al., 2011).

The molecular basis for defective synapse formation in FXS is unknown, but likely reflects the loss of FMRP-regulated translation of synaptic mRNA. Dendrites contain all of the translational machinery (mRNAs, ribosomes, tRNA and translation initiation, elongation and release factors) necessary for protein biosynthesis (Tiedge and Brosius, 1996; Inamura et al., 2003). Our research demonstrates that *App* mRNA is a previously unappreciated target for FMRP-mediated translational repression at the synapse.

6. The need for new therapeutic approaches

The ultimate reason to understand the cellular and molecular pathways underlying A β PP and A β production is to facilitate the rationale development of reliable and safe therapeutics. All of the currently approved drugs for the treatment of AD act on healthy neurons to (1) compensate for reduced acetylcholine in the case of cholinesterase inhibitors, or (2) modulate NMDA receptor activity in the case of memantine. They improve cognitive ability for a year or less, but do not reduce amyloid plaque or neurofibrillary tangle accumulation, neuronal degeneration or subsequent disease progression.

A β immunotherapy has proved very effective in reducing soluble A β , amyloid plaque and soluble tau as well as associated cognitive decline; however, there are questions about its safety (Broytman and Malter, 2004; Morgan, 2005). Human clinical trials testing active immunization against A β were suspended because 6% of the patients developed

meningoencephalitis (Check, 2002). In AD mouse models characterized by prominent vascular A β deposition (APP23 and PDAPP mice), immunization resulted in a two-fold increase in hemorrhages (Pfeifer et al., 2002; Racke et al., 2005). The alternative, passive immunization (anti-A β administration), may prove just as effective in reducing A β without the side effects. In AD mouse models, passive immunization with an antibody directed against A β induced clearance of preexisting amyloid in the brain (Bard et al., 2000) and reverted A β -associated memory deficits (Kotilinek et al., 2002). There are many on-going trials with such agents at present.

β - and γ -secretase inhibitors could provide viable therapeutics that reduce A β generation. Specificity remains an issue with the use of γ -secretase inhibitors as these drugs also inhibit proteolytic processing of other proteins such as Notch that are critical for cellular function. Targeting other components of the γ -secretase complex may avoid these issues (Panza et al., 2011).

Studies described herein suggest several possible alternative avenues for therapeutic intervention. Drugs that target the metallobiology of AD, such as desferrioxamine, clioquinol, tetrathiomolybdate, dimercaptopropanol and VK-28 as well as natural antioxidants, such as curcumin and ginkgo biloba, could modulate IRE/IRP1 *cis/trans* interactions in the 5'-UTR and thereby regulate translation of A β PP (Bandyopadhyay et al., 2010). Decoy RNAs or miRNAs that target the 3'-UTR *cis*-regulatory elements could modulate RBP binding and thereby regulate *APP* message stability. Drugs influencing mGluR₅/FMRP signaling could repress translation of A β PP and reduce amyloidogenic processing. Chronic dosing with the mGluR₅ inhibitor fenobam reduced A β levels in mouse models of AD and FXS (Malter et al., 2010). This drug has never been tested as an AD therapeutic, but it has been approved for phase I & II clinical trials (Pecknold et al., 1980; Friedmann et al., 1980; Lapierre and Oyewumi, 1982; Pecknold et al., 1982). It was also designated as an orphan drug for the treatment of FXS in November of 2006. Despite these advances and on-going studies, continued research in the field of *APP*mRNA/RBP interactions will likely provide novel therapeutic insights to target A β production and plaque formation.

In conclusion, post-transcriptional gene regulation of *APP*mRNA is mediated by several *trans* factors (RBPs) that bind to *cis*-regulatory elements in the UTRs or CR in response to cell signaling events. Our laboratory has identified two *cis*-regulatory elements in the proximal 3'-UTR and one in the CR of *APP*mRNA while other laboratories have identified a destabilizing element in the 3'-UTR and a translational element in the 5'-UTR. Numerous RBPs interact with these *cis*-elements to modulate post-transcriptional message stability and translation. It is highly probable that the sophistication of the RBP complexes that regulate the stability and translation of *APP*mRNA resembles the complexity of the better-studied transcriptional and translational machinery. Remaining components of the *APP*mRNA/RBP complex(es) remain to be identified as do the post-translational modifications and signaling pathways that mediate the mRNA/protein interactions. Our work the past two decades as well as that of our colleagues has just scraped the surface of the *APP*mRNA/RBPs iceberg.

Highlights

1. Multiple RBPs interact with distinct *cis*-elements in *APP*mRNA.
2. hnRNP C and nucleolin bind to 3'-UTR *cis*-elements and mediate message stability.
3. IRP1 binds to an IRE in the 5'-UTR of *APP*mRNA to regulate translation.

4. FMRP and hnRNP C compete for binding to a CR G-rich element to modulate translation.
5. *APP* mRNA is subject to diverse post-transcriptional regulatory processes.

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References

- Akaaboune M, Allinquant B, Farza H, Roy K, Magoul R, Fisman M, Festoff BW, Hantai D. Developmental regulation of amyloid precursor protein at the neuromuscular junction in mouse skeletal muscle. *Mol Cell Neurosci*. 2000; 15:355–367. [PubMed: 10845772]
- Akao Y, Marukawa O, Morikawa H, Nakao K, Kamei M, Hachiya T, Tsujimoto Y. The rck/p54 candidate proto-oncogene product is a 54-kilodalton D-E-A-D box protein differentially expressed in human and mouse tissues. *Cancer Res*. 1995; 55:3444–3449. [PubMed: 7614484]
- Amara FM, Junaid A, Clough RR, Liang B. TGF-beta(1), regulation of Alzheimer amyloid precursor protein mRNA expression in a normal human astrocyte cell line: mRNA stabilization. *Brain Res Mol Brain Res*. 1999; 71:42–49. [PubMed: 10407185]
- Ansari SA, Safak M, Gallia GL, Sawaya BE, Amini S, Khalili K. Interaction of YB1 with human immunodeficiency virus type 1 tat and TAR RNA modulates viral promoter activity. *J Gen Virol*. 1999; 80:2629–2638. [PubMed: 10573156]
- Bagni C, Greenough WT. From mRNP trafficking to spine dysmorphogenesis: The roots of fragile X syndrome. *Nat Rev Neurosci*. 2005; 6:376–387. [PubMed: 15861180]
- Balagopal V, Parker R. Polysomes, P bodies and stress granules: States and fates of eukaryotic mRNAs. *Curr Opin Cell Biol*. 2009; 21:403–408. [PubMed: 19394210]
- Bandyopadhyay S, Huang X, Lahiri DK, Rogers JT. Novel drug targets based on metallobiology of Alzheimer's disease. *Expert Opin Ther Targets*. 2010; 14:1177–1197. [PubMed: 20942746]
- Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luedeman RA, Collier JM, Johnson N, Howlett IC, Geng C, Ueda R, Brand AH, Newbury SF, Wilhelm JE, Levine RB, Nakamura A, Parker R, Ramaswami M. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron*. 2006; 52:997–1009. [PubMed: 17178403]
- Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock T. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med*. 2000; 6:916–919. [PubMed: 10932230]
- Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004; 27:370–377. [PubMed: 15219735]
- Belenguer P, Baldin V, Mathieu C, Prats H, Bensaid M, Bouche G, Amalric F. Protein kinase NII and the regulation of rDNA transcription in mammalian cells. *Nucleic Acids Res*. 1989; 17:6625–6636. [PubMed: 2780290]
- Borer RA, Lehner CF, Eppenberger HM, Nigg EA. Major nucleolar proteins shuttle between nucleus and cytoplasm. *Cell*. 1989; 56:379–390. [PubMed: 2914325]
- Bouvet P, Diaz JJ, Kindbeiter K, Madjar JJ, Amalric F. Nucleolin interacts with several ribosomal proteins through its RGG domain. *J Biol Chem*. 1998; 273:19025–19029. [PubMed: 9668083]
- Bouvet P, Matsumoto K, Wolffe AP. Sequence-specific RNA recognition by the *Xenopus* Y-box proteins. an essential role for the cold shock domain. *J Biol Chem*. 1995; 270:28297–28303. [PubMed: 7499328]

- Brewer G. An A + U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro. *Mol Cell Biol.* 1991; 11:2460–2466. [PubMed: 1901943]
- Brockstedt E, Rickers A, Kostka S, Laubersheimer A, Dorken B, Wittmann-Liebold B, Bommert K, Otto A. Identification of apoptosis-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3. *J Biol Chem.* 1998; 273:28057–28064. [PubMed: 9774422]
- Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, Darnell RB, Warren ST. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell.* 2001; 107:477–487. [PubMed: 11719188]
- Broytman O, Westmark PR, Gurel Z, Malter JS. Rck/p54 interacts with APP mRNA as part of a multi-protein complex and enhances APP mRNA and protein expression in neuronal cell lines. *Neurobiol Aging.* 2008; 30:1962–1974. [PubMed: 18378046]
- Broytman O, Malter JS. Anti- β : The good, the bad, the unforeseen. *J Neurosci Res.* 2004; 75:301–306. [PubMed: 14743443]
- Capowski EE, Esnault S, Bhattacharya S, Malter JS. Y box-binding factor promotes eosinophil survival by stabilizing granulocyte-macrophage colony-stimulating factor mRNA. *J Immunol.* 2001; 167:5970–5976. [PubMed: 11698476]
- Caput D, Beutler B, Hartog K, Thayer R, Brown-Shimer S, Cerami A. Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators. *Proc Natl Acad Sci U S A.* 1986; 83:1670–1674. [PubMed: 2419912]
- Ceman S, Brown V, Warren ST. Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex. *Mol Cell Biol.* 1999; 19:7925–7932. [PubMed: 10567518]
- Check E. Nerve inflammation halts trial for Alzheimer's drug. *Nature.* 2002; 415:462. [PubMed: 11823817]
- Chen CY, Gherzi R, Andersen JS, Gaietta G, Jurchott K, Royer HD, Mann M, Karin M. Nucleolin and YB1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. *Genes Dev.* 2000; 14:1236–1248. [PubMed: 10817758]
- Chen L, Yun SW, Seto J, Liu W, Toth M. The fragile X mental retardation protein binds and regulates a novel class of mRNAs containing U rich target sequences. *Neuroscience.* 2003; 120:1005–1017. [PubMed: 12927206]
- Cho HH, Cahill CM, Vanderburg CR, Scherzer CR, Wang B, Huang X, Rogers JT. Selective translational control of the Alzheimer amyloid precursor protein transcript by iron regulatory protein-1. *J Biol Chem.* 2010; 285:31217–31232. [PubMed: 20558735]
- Choi YD, Grabowski PJ, Sharp PA, Dreyfuss G. Heterogeneous nuclear ribonucleoproteins: Role in RNA splicing. *Science.* 1986; 231:1534–1539. [PubMed: 3952495]
- Choi YD, Dreyfuss G. Isolation of the heterogeneous nuclear RNA-ribonucleoprotein complex (hnRNP): A unique supramolecular assembly. *Proc Natl Acad Sci U S A.* 1984; 81:7471–7475. [PubMed: 6594697]
- Chu CY, Rana TM. Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54. *PLoS Biol.* 2006; 4:e210. [PubMed: 16756390]
- Clarke SL, Vasanthakumar A, Anderson SA, Pondarre C, Koh CM, Deck KM, Pitula JS, Epstein CJ, Fleming MD, Eisenstein RS. Iron-responsive degradation of iron-regulatory protein 1 does not require the fe-S cluster. *EMBO J.* 2006; 25:544–553. [PubMed: 16424901]
- Coller JM, Tucker M, Sheth U, Valencia-Sanchez MA, Parker R. The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. *RNA.* 2001; 7:1717–1727. [PubMed: 11780629]
- Cox TC, Bawden MJ, Martin A, May BK. Human erythroid 5-aminolevulinate synthase: Promoter analysis and identification of an iron-responsive element in the mRNA. *EMBO J.* 1991; 10:1891–1902. [PubMed: 2050125]
- Darnell JC, Fraser CE, Mostovetsky O, Stefani G, Jones TA, Eddy SR, Darnell RB. Kissing complex RNAs mediate interaction between the fragile-X mental retardation protein KH2 domain and brain polyribosomes. *Genes Dev.* 2005; 19:903–918. [PubMed: 15805463]

- Darnell JC, Jensen KB, Jin P, Brown V, Warren ST, Darnell RB. Fragile X mental retardation protein targets G quartet mRNAs important for neuronal function. *Cell*. 2001; 107:489–499. [PubMed: 11719189]
- de Sauvage F, Kruys V, Marinx O, Huez G, Octave JN. Alternative polyadenylation of the amyloid protein precursor mRNA regulates translation. *EMBO J*. 1992; 11:3099–3103. [PubMed: 1353447]
- Denman R, Potempska A, Wolfe G, Ramakrishna N, Miller DL. Distribution and activity of alternatively spliced Alzheimer amyloid peptide precursor and scrapie PrP mRNAs on rat brain polysomes. *Arch Biochem Biophys*. 1991; 288:29–38. [PubMed: 1680310]
- Derenzini M, Sirri V, Tere D, Ochs RL. The quantity of nucleolar proteins nucleolin and protein B23 is related to cell doubling time in human cancer cells. *Lab Invest*. 1995; 73:497–502. [PubMed: 7474921]
- Didier DK, Schiffenbauer J, Woulfe SL, Zacheis M, Schwartz BD. Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. *Proc Natl Acad Sci U S A*. 1988; 85:7322–7326. [PubMed: 3174636]
- Dolzanskaya N, Sung YJ, Conti J, Currie JR, Denman RB. The fragile X mental retardation protein interacts with U-rich RNAs in a yeast three-hybrid system. *Biochem Biophys Res Commun*. 2003; 305:434–441. [PubMed: 12745094]
- Egyhazi E, Pigon A, Chang JH, Ghaffari SH, Dreesen TD, Wellman SE, Case ST, Olson MO. Effects of anti-C23 (nucleolin) antibody on transcription of ribosomal DNA in chironomus salivary gland cells. *Exp Cell Res*. 1988; 178:264–272. [PubMed: 3169130]
- Erard MS, Belenguer P, Caizergues-Ferrer M, Pantaloni A, Amalric F. A major nucleolar protein, nucleolin, induces chromatin decondensation by binding to histone H1. *Eur J Biochem*. 1988; 175:525–530. [PubMed: 3409881]
- Eulalio A, Behm-Ansmant I, Izauralde E. P bodies: At the crossroads of posttranscriptional pathways. *Nat Rev Mol Cell Biol*. 2007; 8:9–22. [PubMed: 17183357]
- Fahling M, Steege A, Perlewitz A, Nafz B, Mrowka R, Persson PB, Thiele BJ. Role of nucleolin in posttranscriptional control of MMP-9 expression. *Biochim Biophys Acta*. 2005; 1731:32–40. [PubMed: 16153722]
- Ford LP, Suh JM, Wright WE, Shay JW. Heterogeneous nuclear ribonucleoproteins C1 and C2 associate with the RNA component of human telomerase. *Mol Cell Biol*. 2000; 20:9084–9091. [PubMed: 11074006]
- Friedmann C, Davis L, Ciccone P, Rubin R. Phase II double blind controlled study of a new anxiolytic, fenobam (McN-3377) vs placebo. *Curr Ther Res*. 1980; 274 144-144-151.
- Gillis P, Malter JS. The adenosine-uridine binding factor recognizes the AU-rich elements of cytokine, lymphokine, and oncogene mRNAs. *J Biol Chem*. 1991; 266:3172–3177. [PubMed: 1993689]
- Ginisty H, Amalric F, Bouvet P. Nucleolin functions in the first step of ribosomal RNA processing. *EMBO J*. 1998; 17:1476–1486. [PubMed: 9482744]
- Hamilton BJ, Nagy E, Malter JS, Arrick BA, Rigby WF. Association of heterogeneous nuclear ribonucleoprotein A1 and C proteins with reiterated AUUUA sequences. *J Biol Chem*. 1993; 268:8881–8887. [PubMed: 8473331]
- Heaton JH, Dlakic WM, Gelehrter TD. Posttranscriptional regulation of PAI-1 gene expression. *Thromb Haemost*. 2003; 89:959–966. [PubMed: 12783107]
- Heaton JH, Dlakic WM, Dlakic M, Gelehrter TD. Identification and cDNA cloning of a novel RNA-binding protein that interacts with the cyclic nucleotide-responsive sequence in the type-1 plasminogen activator inhibitor mRNA. *J Biol Chem*. 2001; 276:3341–3347. [PubMed: 11001948]
- Holcik M, Gordon BW, Korneluk RG. The internal ribosome entry site-mediated translation of antiapoptotic protein XIAP is modulated by the heterogeneous nuclear ribonucleoproteins C1 and C2. *Mol Cell Biol*. 2003; 23:280–288. [PubMed: 12482981]
- Huang L, Fu H, Lin CM, Conner AL, Zhang Y, Aladjem MI. Prevention of transcriptional silencing by a replicator-binding complex consisting of SWI/SNF, MeCP1, and hnRNP C1/C2. *Mol Cell Biol*. 2011; 31:3472–3484. [PubMed: 21690294]

- Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A*. 2002; 99:7746–7750. [PubMed: 12032354]
- Inamura N, Hoshino S, Uchiumi T, Nawa H, Takei N. Cellular and subcellular distributions of translation initiation, elongation and release factors in rat hippocampus. *Brain Res Mol Brain Res*. 2003; 111:165–174. [PubMed: 12654516]
- Irwin SA, Galvez R, Greenough WT. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb Cortex*. 2000; 10:1038–1044. [PubMed: 11007554]
- Jordan G. At the heart of the nucleolus. *Nature*. 1987; 329:489–490. [PubMed: 3309675]
- Khacho M, Mekhail K, Pilon-Larose K, Pause A, Cote J, Lee S. eEF1A is a novel component of the mammalian nuclear protein export machinery. *Mol Biol Cell*. 2008; 19:5296–5308. [PubMed: 18799616]
- Kibbey MC, Johnson B, Petryshyn R, Jucker M, Kleinman HK. A 110-kD nuclear shuttling protein, nucleolin, binds to the neurite-promoting IKVAV site of laminin-1. *J Neurosci Res*. 1995; 42:314–322. [PubMed: 8583499]
- Kim HY, Klausner RD, Rouault TA. Translational repressor activity is equivalent and is quantitatively predicted by in vitro RNA binding for two iron-responsive element-binding proteins, IRP1 and IRP2. *J Biol Chem*. 1995; 270:4983–4986. [PubMed: 7890603]
- Kotilinek LA, Bacskai B, Westerman M, Kawarabayashi T, Younkin L, Hyman BT, Younkin S, Ashe KH. Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J Neurosci*. 2002; 22:6331–6335. [PubMed: 12151510]
- Lagerbauer B, Ostareck D, Keidel EM, Ostareck-Lederer A, Fischer U. Evidence that fragile X mental retardation protein is a negative regulator of translation. *Hum Mol Genet*. 2001; 10:329–338. [PubMed: 11157796]
- Lagnado CA, Brown CY, Goodall GJ. AUUUA is not sufficient to promote poly(A) shortening and degradation of an mRNA: The functional sequence within AU-rich elements may be UUAUUUA(U/A)(U/A). *Mol Cell Biol*. 1994; 14:7984–7995. [PubMed: 7969138]
- Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS, Blackshear PJ. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol Cell Biol*. 1999; 19:4311–4323. [PubMed: 10330172]
- Lapierre Y, Oyewumi L. Fenobam: Another anxiolytic? *Curr Ther Res*. 1982; 31 95-95-101.
- Lee EK, Kim HH, Kuwano Y, Abdelmohsen K, Srikantan S, Subaran SS, Gleichmann M, Mughal MR, Martindale JL, Yang X, Worley PF, Mattson MP, Gorospe M. hnRNP C promotes APP translation by competing with FMRP for APP mRNA recruitment to P bodies. *Nat Struct Mol Biol*. 2010; 17:732–739. [PubMed: 20473314]
- Li Z, Zhang Y, Ku L, Wilkinson KD, Warren ST, Feng Y. The fragile X mental retardation protein inhibits translation via interacting with mRNA. *Nucleic Acids Res*. 2001; 29:2276–2283. [PubMed: 11376146]
- Long JM, Lahiri DK. MicroRNA-101 downregulates Alzheimer's amyloid-beta precursor protein levels in human cell cultures and is differentially expressed. *Biochem Biophys Res Commun*. 2011; 404:889–895. [PubMed: 21172309]
- Mahajan MC, Narlikar GJ, Boyapaty G, Kingston RE, Weissman SM. Heterogeneous nuclear ribonucleoprotein C1/C2, MeCP1, and SWI/SNF form a chromatin remodeling complex at the beta-globin locus control region. *Proc Natl Acad Sci U S A*. 2005; 102:15012–15017. [PubMed: 16217013]
- Malter JS, Ray BC, Westmark PR, Westmark CJ. Fragile X syndrome and Alzheimer's disease: Another story about APP and beta-amyloid. *Curr Alzheimer Res*. 2010; 7:200–206. [PubMed: 20088809]
- Malter JS. Identification of an AUUUA-specific messenger RNA binding protein. *Science*. 1989; 246:664–666. [PubMed: 2814487]
- Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A*. 1985; 82:4245–4249. [PubMed: 3159021]

- Mazroui R, Huot ME, Tremblay S, Filion C, Labelle Y, Khandjian EW. Trapping of messenger RNA by fragile X mental retardation protein into cytoplasmic granules induces translation repression. *Hum Mol Genet.* 2002; 11:3007–3017. [PubMed: 12417522]
- McLaren RS, Caruccio N, Ross J. Human La protein: A stabilizer of histone mRNA. *Mol Cell Biol.* 1997; 17:3028–3036. [PubMed: 9154801]
- Millard SS, Vidal A, Markus M, Koff A. A U-rich element in the 5'-untranslated region is necessary for the translation of p27 mRNA. *Mol Cell Biol.* 2000; 20:5947–5959. [PubMed: 10913178]
- Minshall N, Kress M, Weil D, Standart N. Role of p54 RNA helicase activity and its C-terminal domain in translational repression, P-body localization and assembly. *Mol Biol Cell.* 2009; 20:2464–2472. [PubMed: 19297524]
- Minshall N, Reiter MH, Weil D, Standart N. CPEB interacts with an ovary-specific eIF4E and 4E-T in early *Xenopus* oocytes. *J Biol Chem.* 2007; 282:37389–37401. [PubMed: 17942399]
- Miyashiro KY, Beckel-Mitchener A, Purk TP, Becker KG, Barret T, Liu L, Carbonetto S, Weiler II, Greenough WT, Eberwine J. RNA cargoes associating with FMRP reveal deficits in cellular functioning in *Fmr1* null mice. *Neuron.* 2003; 37:417–431. [PubMed: 12575950]
- Morgan D. Mechanisms of A-beta plaque clearance following passive A-beta immunization. *Neurodegener Dis.* 2005; 2:261–266. [PubMed: 16909007]
- Panza F, Frisardi V, Solfrizzi V, Imbimbo BP, Logroscino G, Santamato A, Greco A, Seripa D, Pilotto A. Interacting with gamma-secretase for treating Alzheimer's disease: From inhibition to modulation. *Curr Med Chem.* 2011 [Epub ahead of print].
- Parker R, Sheth U. P bodies and the control of mRNA translation and degradation. *Mol Cell.* 2007; 25:635–646. [PubMed: 17349952]
- Patel N, Hoang D, Miller N, Ansaloni S, Huang Q, Rogers JT, Lee JC, Saunders AJ. MicroRNAs can regulate human APP levels. *Mol Neurodegener.* 2008; 3:10. [PubMed: 18684319]
- Pecknold J, McClure D, Appeltauer L. Fenobam in anxious outpatients. *Curr Ther Res.* 1980; 274:119–123.
- Pecknold JC, McClure DJ, Appeltauer L, Wrzesinski L, Allan T. Treatment of anxiety using fenobam (a nonbenzodiazepine) in a double-blind standard (diazepam) placebo-controlled study. *J Clin Psychopharmacol.* 1982; 2:129–133. [PubMed: 7042771]
- Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, Mathews PM, Jucker M. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science.* 2002; 298:1379. [PubMed: 12434053]
- Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, Pirooz KS, Jordan WH, Brown DD, Hoffman WP, Holtzman DM, Bales KR, Gitter BD, May PC, Paul SM, DeMattos RB. Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. *J Neurosci.* 2005; 25:629–636. [PubMed: 15659599]
- Rajagopalan LE, Malter JS. Growth factor-mediated stabilization of amyloid precursor protein mRNA is mediated by a conserved 29-nucleotide sequence in the 3'-untranslated region. *J Neurochem.* 2000; 74:52–59. [PubMed: 10617105]
- Rajagopalan LE, Westmark CJ, Jarzembowski JA, Malter JS. hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. *Nucleic Acids Res.* 1998; 26:3418–3423. [PubMed: 9649628]
- Ranjan M, Tafuri SR, Wolffe AP. Masking mRNA from translation in somatic cells. *Genes Dev.* 1993; 7:1725–1736. [PubMed: 8370522]
- Rockenstein EM, McConlogue L, Tan H, Power M, Masliah E, Mucke L. Levels and alternative splicing of amyloid beta protein precursor (APP) transcripts in brains of APP transgenic mice and humans with Alzheimer's disease. *J Biol Chem.* 1995; 270:28257–28267. [PubMed: 7499323]
- Rogers JT, Randall JD, Cahill CM, Eder PS, Huang X, Gunshin H, Leiter L, McPhee J, Sarang SS, Utsuki T, Greig NH, Lahiri DK, Tanzi RE, Bush AI, Giordano T, Gullans SR. An iron-responsive element type II in the 5'-untranslated region of the Alzheimer's amyloid precursor protein transcript. *J Biol Chem.* 2002; 277:45518–45528. [PubMed: 12198135]

- Rogers JT, Leiter LM, McPhee J, Cahill CM, Zhan SS, Potter H, Nilsson LN. Translation of the Alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5'-untranslated region sequences. *J Biol Chem.* 1999; 274:6421–6431. [PubMed: 10037734]
- Ruberti F, Barbato C, Cogoni C. Post-transcriptional regulation of amyloid precursor protein by microRNAs and RNA binding proteins. *Commun Integr Biol.* 2010; 3:499–503. [PubMed: 21331224]
- Santoro MR, Bray SM, Warren ST. Molecular mechanisms of fragile X syndrome: A twenty-year perspective. *Annu Rev Pathol.* 2011 [Epub ahead of print].
- Sengupta TK, Bandyopadhyay S, Fernandes DJ, Spicer EK. Identification of nucleolin as an AU-rich element binding protein involved in bcl-2 mRNA stabilization. *J Biol Chem.* 2004; 279:10855–10863. [PubMed: 14679209]
- Shaw G, Kamen R. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell.* 1986; 46:659–667. [PubMed: 3488815]
- Shigematsu K, McGeer PL, McGeer EG. Localization of amyloid precursor protein in selective postsynaptic densities of rat cortical neurons. *Brain Res.* 1992; 592:353–357. [PubMed: 1280522]
- Singh K, Laughlin J, Kosinski PA, Covey LR. Nucleolin is a second component of the CD154 mRNA stability complex that regulates mRNA turnover in activated T cells. *J Immunol.* 2004; 173:976–985. [PubMed: 15240685]
- Soba P, Eggert S, Wagner K, Zentgraf H, Siehl K, Kreger S, Lower A, Langer A, Merdes G, Paro R, Masters CL, Muller U, Kins S, Beyreuther K. Homo- and heterodimerization of APP family members promotes intercellular adhesion. *EMBO J.* 2005; 24:3624–3634. [PubMed: 16193067]
- Stefani G, Fraser CE, Darnell JC, Darnell RB. Fragile X mental retardation protein is associated with translating polyribosomes in neuronal cells. *J Neurosci.* 2004; 24:7272–7276. [PubMed: 15317853]
- Stenina OI, Poptic EJ, DiCorleto PE. Thrombin activates a Y box-binding protein (DNA-binding protein B) in endothelial cells. *J Clin Invest.* 2000; 106:579–587. [PubMed: 10953033]
- Tanner NK, Linder P. DExD/H box RNA helicases: From generic motors to specific dissociation functions. *Mol Cell.* 2001; 8:251–262. [PubMed: 11545728]
- Thomson AM, Rogers JT, Leedman PJ. Thyrotropin-releasing hormone and epidermal growth factor regulate iron-regulatory protein binding in pituitary cells via protein kinase C-dependent and -independent signaling pathways. *J Biol Chem.* 2000; 275:31609–31615. [PubMed: 10889193]
- Tiedge H, Brosius J. Translational machinery in dendrites of hippocampal neurons in culture. *J Neurosci.* 1996; 16:7171–7181. [PubMed: 8929426]
- Torroja L, Packard M, Gorczyca M, White K, Budnik V. The drosophila beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction. *J Neurosci.* 1999; 19:7793–7803. [PubMed: 10479682]
- Tuteja N, Huang NW, Skopac D, Tuteja R, Hrvatic S, Zhang J, Pongor S, Joseph G, Faucher C, Amalric F. Human DNA helicase IV is nucleolin, an RNA helicase modulated by phosphorylation. *Gene.* 1995; 160:143–148. [PubMed: 7642087]
- Wang XY, Hoyle PE, McCubrey JA. Characterization of proteins binding the 3' regulatory region of the IL-3 gene in IL-3-dependent and autocrine-transformed hematopoietic cells. *Leukemia.* 1998; 12:520–531. [PubMed: 9557611]
- Westmark CJ, Westmark PR, O'Riordan KJ, Ray BC, Hervey CM, Salamat MS, Abozeid SH, Stein KM, Stodola LA, Tranfaglia M, Burger C, Berry-Kravis EM, Malter JS. Reversal of fragile X phenotypes by manipulation of APP/Abeta levels in Fmr1 mice. *PLoS One.* 2011; 6:e26549. [PubMed: 22046307]
- Westmark CJ, Malter JS. FMRP mediates mGluR5-dependent translation of amyloid precursor protein. *PLoS Biol.* 2007; 5:e52. [PubMed: 17298186]
- Westmark CJ, Malter JS. Extracellular-regulated kinase controls betaamyloid precursor protein mRNA decay. *Brain Res Mol Brain Res.* 2001a; 90:193–201. [PubMed: 11406297]
- Westmark CJ, Malter JS. Up-regulation of nucleolin mRNA and protein in peripheral blood mononuclear cells by extracellular-regulated kinase. *J Biol Chem.* 2001b; 276:1119–1126. [PubMed: 11042220]

- Westmark PR, Shin HC, Westmark CJ, Soltaninassab SR, Reinke EK, Malter JS. Decoy mRNAs reduce beta-amyloid precursor protein mRNA in neuronal cells. *Neurobiol Aging*. 2006; 27:787–796. [PubMed: 16672170]
- Weston A, Sommerville J. Xp54 and related (DDX6-like) RNA helicases: Roles in messenger RNP assembly, translation regulation and RNA degradation. *Nucleic Acids Res*. 2006; 34:3082–3094. [PubMed: 16769775]
- Wilusz J, Shenk T. A uridylyate tract mediates efficient heterogeneous nuclear ribonucleoprotein C protein-RNA cross-linking and functionally substitutes for the downstream element of the polyadenylation signal. *Mol Cell Biol*. 1990; 10:6397–6407. [PubMed: 1701018]
- Yang G, Gong YD, Gong K, Jiang WL, Kwon E, Wang P, Zheng H, Zhang XF, Gan WB, Zhao NM. Reduced synaptic vesicle density and active zone size in mice lacking amyloid precursor protein (APP) and APP-like protein 2. *Neurosci Lett*. 2005; 384:66–71. [PubMed: 15919150]
- Yang TH, Tsai WH, Lee YM, Lei HY, Lai MY, Chen DS, Yeh NH, Lee SC. Purification and characterization of nucleolin and its identification as a transcription repressor. *Mol Cell Biol*. 1994; 14:6068–6074. [PubMed: 8065340]
- Yurkova MS, Murray MT. A translation regulatory particle containing the *Xenopus* oocyte Y box protein mRNP3+4. *J Biol Chem*. 1997; 272:10870–10876. [PubMed: 9099743]
- Zaidi SH, Malter JS. Nucleolin and heterogeneous nuclear ribonucleoprotein C proteins specifically interact with the 3'-untranslated region of amyloid protein precursor mRNA. *J Biol Chem*. 1995; 270:17292–17298. [PubMed: 7615529]
- Zaidi SH, Denman R, Malter JS. Multiple proteins interact at a unique cis-element in the 3'-untranslated region of amyloid precursor protein mRNA. *J Biol Chem*. 1994; 269:24000–24006. [PubMed: 7929050]
- Zaidi SH, Malter JS. Amyloid precursor protein mRNA stability is controlled by a 29-base element in the 3'-untranslated region. *J Biol Chem*. 1994; 269:24007–24013. [PubMed: 7929051]
- Zalfa F, Giorgi M, Primerano B, Moro A, Di Penta A, Reis S, Oostra B, Bagni C. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. *Cell*. 2003; 112:317–327. [PubMed: 12581522]
- Zubiaga AM, Belasco JG, Greenberg ME. The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol Cell Biol*. 1995; 15:2219–2230. [PubMed: 7891716]

APP mRNA *cis*-Elements

- ① iron response element (translational)
- ② G-rich region (translational)
- ③ 29-base element (destabilizing)
- ④ 52-base element (stabilizing)
- ⑤ 81-base element (destabilizing)
- ⑥⑦ CPEs (translational)

RNA Binding Proteins

	Ago1		hnRNP C
	Ago2		La
	CPEB		nucleolin
	EF1α		RCK/p54
	FMRP		PAI-RBP1
	IRP1		YB-1

Figure 1.

Post-transcriptional gene regulation and *APP* mRNA. *APP* mRNA contains two translational (IRE and guanine-rich), one stabilizing (52-base), two destabilizing (29- and 81-base) and two cytoplasmic polyadenylation elements. ①An IRE is located in the 5'-UTR of *APP* mRNA and translation is repressed when IRP1 is bound. ②FMRP and hnRNP C compete for binding to the guanine-rich region in the CR of *APP* mRNA. Translation is repressed when FMRP is bound and active when hnRNP C is bound. The remaining, identified *cis*-elements in *APP* mRNA are located in the 3'-UTR. ③Several proteins (nucleolin, YB1, La, PAI-RBP1, EF1α and RCK/p54) form a complex at a 52-be stabilizing element adjacent to the stop codon. YB1 and La interact directly with the message. RCK/p54 is a component of P-bodies along with Ago1 and Ago2. FMRP interacts with RCK/p54 suggesting that FMRP represses translation by recruiting *APP* mRNA to P-bodies. ④hnRNP C and nucleolin bind to a 29-be element approximately 200 bases downstream from the stop codon. When hnRNP C is bound, *APP* mRNA is stable whereas nucleolin increases decay. As FMRP is a known binding partner of both nucleolin and YB1, these data suggest that a large ribonucleoprotein complex forms to bring multiple *cis*-regulatory elements of *APP* mRNA in close proximity to mediate post-transcriptional events. ⑤An 81-nt destabilizing element is located further

downstream in the 3'-UTR. The identity of the RBPs that binds to this *cis*-element has yet to be determined. ⑥,⑦ Two CPEs are located near the poly(A) tail in *APP* mRNA and interact with the polyadenylation machinery. Both sites are utilized, but the longer mRNA exhibits more efficient ribosome mobilization and translation. RBPs that interact directly with *APP* mRNA are depicted as oval-shaped whereas those that bind through protein/protein interactions are rectangular in shape.