Post-transcriptional regulation of amyloid precursor protein by microRNAs and RNA binding proteins

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Abbreviations: APP, amyloid precursor protein; Aβ, amyloid beta; AD, Alzheimer’s disease; miRNAs, microRNAs; IL-1, interleukin-1; TGFβ, transforming growth factor β; 5'UTR, 5' untranslated region; 3'UTR, 3' untranslated region; FMRP, fragile-X mental retardation protein; SN, synaptoneurosomes; mGluR, type 1 metabotropic glutamate receptor; DHPG, 3,5-dihydroxyphenylglycine; hnRNPC, heterogeneous nuclear ribonucleoprotein C; IRES, internal ribosome entry site; CAT, chloramphenicol acetyltransferase; REMSA, RNA electrophoretic mobility shift assay; IRE, iron response element; Res, responsive elements; RISC, RNA-induced silencing complex

Amyloid Precursor Protein (APP) and its proteolytic product amyloid beta (Aβ) are critical in the pathogenesis of Alzheimer’s Disease (AD). APP gene duplication and transcriptional upregulation are linked to AD. In addition, normal levels of APP appear to be required for some physiological functions in the developing brain. Several studies in mammalian cell lines and primary neuron cultures indicate that RNA binding proteins and microRNAs interacting with regulatory regions of the APP mRNA modulate expression of APP post-transcriptionally. However, when the various mechanisms of APP post-transcriptional regulation are recruited and which of them are acting in a synergistic fashion to balance APP protein levels, is unclear. Recent studies suggest that further investigation of the molecules and pathways involved in APP post-transcriptional regulation are warranted.

Introduction

APP is ubiquitously expressed and its biological functions are not completely understood, although the study of APP deficient mice and, more recently, the use of APP siRNAs has begun to clarify APP functions. Neuronal APP modulates neurites outgrowth1 and neuronal migration2 and APP gene dosage seems to be relevant for proper migration of neuronal cells in the developing cortex.3 APP expression is developmentally regulated and reaches its highest levels during postnatal brain maturation and synaptogenesis.3,4 APP is targeted to the presynaptic terminal as well as dendrites5 where it has been found to potentiate the formation and maintenance of synapses.6 Genetic ablation of APP impairs memory in mice.7 APP is a type I integral membrane protein with a single transmembrane domain, a large extracellular N-terminal region and a shorter C-terminal tail. APP is processed through two main proteolytic processing pathways: amyloidogenic processing, which leads to generation of Aβ and non-amyloidogenic processing, which is prominent in most cell types. The extracellular domain is released by either α- or β-secretase and the remaining transmembrane domain is further cleaved by γ-secretase. The cleavage of APP by α-secretase within the Aβ sequence precludes the formation of Aβ peptides. Among the Aβ peptides produced by amyloidogenic processing, Aβ42 is more prone to form fibrillar aggregates.8

In the brain, APP and Aβ are critical in the pathogenesis of AD,9 which is the most common form of dementia in the elderly. The pathology, which affects primarily the hippocampus and the cortical areas, is characterized by early synaptic defects, neuronal dysfunction, Aβ plaques which contain Aβ aggregates and neurofibrillary tangles which consist primarily of aggregated forms of the microtubule-associated protein tau.

While familial cases of AD shed some light on the causes of the disease, more than 90% of AD cases arise sporadically and the aetiology of AD is still unknown, although neuropathological and cognitive abnormalities are similar in familial and sporadic AD.

Genetic studies have identified autosomal-dominant mutations in APP or presenilin (the key component of γ-secretase) in familial AD cases. The majority of APP mutations appear to enhance Aβ aggregation and APP cleavage by presenilin variants results in higher levels of the more amyloidogenic Aβ42 peptide, therefore Aβ load in the brain has been suggested to trigger neuronal dysfunction.9 In the last decade, abnormal
factor for AD21 and increased APP expression has been observed in ischemic conditions in the brain are also proposed to be a causal factor for AD21. The APP gene has also been detected. In addition, individuals with trisomy 21 (the APP gene is located on chromosome 21) are at increased risk of developing AD. Cytokines, such as IL-1 (Interleukin-1) and TGFβ, which are increased in the brain of AD patients, 13,14 are involved in the transcriptional and post-transcriptional regulation of APP. 15-18 Moreover, elevated levels of iron have been detected in the AD brain9 and intracellular levels of APP are modulated by a mechanism similar to the post-transcriptional regulation of ferritin mRNA, by iron response element stem loops in the 5’ untranslated region. Perturbations which induce ischemic conditions in the brain are also proposed to be a causal factor for AD21 and increased APP expression has been observed in ischemic brain tissue.22

This review summarizes the current knowledge of APP post-transcriptional regulation and examines the role post-transcriptional regulation plays in AD.

APP expression is extensively regulated at the post-transcriptional level by a number of different RNA-binding proteins that control both APP mRNA stability and translation (Fig. 1). Western and Malter23 demonstrated that the Fragile-X Mental Retardation Protein, FMRP, binds APP mRNA and regulates its translation. FMRP is highly expressed in the brain and its reduction causes Fragile X Syndrome, which is characterized by cognitive impairment, suggesting that FMRP plays an important role in memory formation and normal cognition. FMRP harbors multiple RNA-binding motifs, including two tandem KH motifs and an RGG box, although the exact roles played by the different RNA-binding domains in target recognition are unclear. Two RNA structures have been suggested to mediate the interaction between FMRP and its target mRNAs, the G-quartet RNA motif and the RNA kissing complex. APP mRNA has been purified from subcellular preparations enriched in presynaptic and postsynaptic structures (synaptoneurosomes, SN), suggesting that APP protein, which has been previously localized to dendrites and post-synaptic densities, may be produced through local translation. Indeed, FMRP associates with APP mRNA in SN preparations, as demonstrated by immunoprecipitation and RT-PCR analysis. Moreover, FMRP is required for type 1 metabotropic glutamate receptor (mGluR)-dependent translation of APP mRNA. Stimulation with the mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) induces rapid dissociation of FMRP from APP mRNA, correlated with an increase in APP translation. These data indicate that the binding of FMRP to the APP mRNA represses its translation.

In vitro RNase protection assays revealed that FMRP binds to the highly conserved G-rich region somewhat upstream of the predicted G-quartet motif. The relevance of this G-rich region in FMRP translational repression of APP mRNA was confirmed by a subsequent study.24 Interestingly, Gorospe and co-workers found that FMRP association with APP mRNA is modulated by competitive binding of another RNA-binding protein, heterogeneous nuclear ribonucleoprotein (hnRNP) C, at the same G-rich motif. FMRP and hnRNP C appear to have opposite effects on APP mRNA translation: the binding of hnRNP C enhances translation, whereas FMRP represses it. FMRP may repress translation via different mechanisms,25 the fact that APP mRNA co-localizes with P bodies when bound to FMRP, suggests that FMRP may block translation initiation of APP mRNA by modulating the assembly of the cap dependent initiation complex.26

hnRNP C promotes translation of Unr mRNA by stimulating IRES (internal ribosome entry site) dependent translation.27 In the case of APP mRNA, however, it is not clear whether hnRNP C stimulation of translation is dependent on the IRES present in the APP 5’UTR or is simply the result of alleviating FMRP mediated repression. Whether hnRNP C binding of
APP mRNA is linked to mGluR stimulation is also unknown. It appears that hnRNP C not only regulates the translation of APP mRNA, but also increases its stability by binding to a highly conserved 29 nt element within the 3'UTR, approximately 200 nt downstream of the stop codon. The same 29 nt sequence is also the target of the RNA-binding protein nucleolin. However, the role of nucleolin in regulating APP mRNA translation and/or stability is presently unknown, although nucleolin has been detected in a ribonucleoprotein complex with FMRP. While the 29 nt sequence appears to de-stabilize the APP mRNA, another sequence located in the first 52 bases downstream of the stop codon seems to have the opposite effect and increases mRNA stability. Six cytoplasmic proteins have been shown to interact with the 52 nt element: nucleolin, Rck/p54, plasminogen activator inhibitor-RNA binding protein 1 (PAI/RBP1), Y-box binding protein 1 (YB1), autoantigen La/Sjogren syndrome antigen B (La/SS-B) and elongation factor 1α (EF1α). Whether these proteins are present in a single complex or bind APP mRNA independently, is unknown. The identified proteins may interact with each other within a complex through both RNA dependent and independent interactions. However, the role of the individual proteins in APP regulation remains to be defined. Only Rck/p54 has been studied further: overexpression of Rck/p54 increases both APP mRNA and protein levels. Rck/p54 is a member of the DEAD-box family of RNA helicases that modulate mRNA secondary structure, which has been implicated in translation and mRNA degradation. Although the precise role of Rck/p54 in the regulation of APP mRNA stability remains to be elucidated, Rck/p54 helicase activity could maintain the APP mRNA in a favorable conformation for interacting with other elements of the multi-protein complex to stabilize the APP mRNA.

Another 81 nt sequence within the APP 3'UTR, at approximately 600 nt from the stop codon, may be involved in post-transcriptional regulation of APP. Insertion of this 81 nt cis-regulatory element into the chloramphenicol acetyltransferase (CAT) mRNA conferred TGFβ mediated mRNA stability in transfected human astrocytes. Although a TGFβ responsive protein forming a 68 kDa RNA-protein complex was proposed to mediate stabilization of the APP mRNA, further characterization of the protein has not yet been conducted. Interestingly, two polyadenylation sites are present in the APP 3'UTR and they give rise to a short and a long transcript. Selection of the last polyadenylation site, generating the long transcript, increased the efficiency of translation in Xenopus oocytes. Mbella et al. observed that the last 258 nucleotides of the 3'UTR enhanced APP mRNA translation in mammalian cells and mapped two guanine residues that are crucial for this action. Using an RNA electrophoretic mobility shift assay (REMSA), Mbella et al. determined that the shorter transcript interacts with a translational repressor and the presence of the last 258 nucleotides blocks the binding of this repressor. The molecular characterization of this RNA binding protein remains to be conducted.

More recently, using in vivo UV crosslinking with Argonaute immunoprecipitation in HEK293 cells, a fragment containing nt 1,106–1,146, near the end of the APP 3'UTR, was isolated. Identification of the microRNAs involved in this interaction will be of considerable interest.

In addition to the regulatory elements in the 3'UTR and the coding region, the 5'UTR also contains several elements that control the translation of APP mRNA, including an IRES, an IL-1 translation enhancer element and an iron responsive element (IRE). Rogers and colleagues found that the levels of APP are regulated by an RNA stem loop element within the 5'UTR similar to the originally identified IRE that regulates the translation of the L- and H-ferritin mRNAs in response to intracellular iron levels. IRP1, one of two iron regulatory proteins (IRP), binds the APP IRE in human neuroblastoma cells in response to intracellular iron chelation with desferrioxamine. Moreover, the increased binding of IRP1 to the APP IRE as a function of iron chelation was correlated with a rapid decrease in APP protein levels. These observations support a model previously proposed for the regulation of ferritin translation, in which IRP1, in the absence of iron, binds tightly to the ferritin mRNA IREs and prevents recruitment of the 40S ribosome at the 5' cap sites. Increased intracellular iron also appears to affect APP mRNA translation via an IRES located within the first 50 nt of the 5'UTR. In glioma cells, endogenous APP is translated in a cap-independent manner, as demonstrated both by treatment with rapamycin (an inhibitor of cap-dependent translation) and by knocking down the cap-binding protein elongation factor 4E (eEF-4E). The actual mechanism by which the levels of iron affect APP IRES activity is unknown, since it is still unclear whether cap-dependent and cap-independent translation of APP mRNA represent two alternative mechanisms by which intracellular iron concentration controls APP levels, perhaps in different cell types or in combination with other signals. Interestingly, several studies suggest that elevated intracellular iron may be linked to neuronal loss in the AD brain, where there is an enrichment of iron without a corresponding increase of ferritin. Iron may have multiple detrimental effects in the aetiology of AD. First, by enhancing APP synthesis and increasing Aβ peptide accumulation; second, by physically associating with amyloid plaques and potentially favouring the polymerization of Aβ. Inflammatory mechanisms have also been implicated in AD. Increased levels of IL-1 have been associated with increased APP immunoreactivity in the AD brain. In this regard, a 90 nt element in the 5'UTR upstream of the cap site conferred translational regulation by IL-1α and IL-1β to a CAT reporter gene. The IL-1 responsive 90 nt element did not affect steady-state mRNA levels of the reporter gene, but did increase its translation.

**Regulation of APP Translation by microRNAs**

Recently, several studies have reported that APP expression is post-transcriptionally regulated by microRNAs (Fig. 1), which are small, 21-nucleotides, double stranded non-coding RNAs that modulate gene expression at the post-transcriptional level in many species, including mammals. One strand of an miRNA is incorporated into the Argonaute-containing RNA-induced silencing complex (RISC) and drives the RISC to bind target mRNAs, leading to translational repression and/or mRNA destabilization. The miRNAs can modulate the expression of...
multiple mRNA targets and are candidates for temporally and spatially regulating several processes in neurons.

The 3'UTR of APP mRNA is a direct target for several microRNAs: miR-106a and miR-520c, miR-20a family members (including miR-20a, miR-106b and miR-17-5p), and miR-101. In transfected human HEK-293 cells, miR-520c and miR-106a negatively regulate expression of reporter genes containing the predicted APP target sequence in their 3'UTR. When overexpressed in human cell lines, both miR-106a and miR-520c reduced endogenous APP levels by 50% compared to cells transfected with a non-targeting miRNA. However, only miR-106a is expressed in a neuronal context. Overexpression of miR-20a (and miR-17-5p/106b) post-transcriptionally reduced endogenous APP expression in both non-neuronal and neuronal cells. Moreover, reduction of miR-20a, miR-17-5p and miR-106b levels was correlated with an increase in APP protein levels in developing mouse brain and in primary cultured neurons. Although two putative miR-20 family responsive elements (REs) are predicted within the APP 3'UTR (nt 461–467 and nt 709–715) only the nt 461–467 sequence functionally interacted with miR-20 family miRNAs. Interestingly, the nt 709–715 sequence, which did not interact with miRNAs, is part of the 81 nt cis-regulatory element which associated with the 68 kDa complex that stabilizes the APP mRNA (see above). When miRNA expression levels in human AD brains were analyzed, miR-106b was significantly reduced with respect to control samples. However, since APP levels varied among the different samples, a tight correlation between miR-106b and APP levels in AD tissues was not obtained. More recently, the regulation of APP expression by the RISC/miRNA pathway was examined in primary rat hippocampal neurons. First, silencing of Ago2 in hippocampal neurons increased APP protein levels, suggesting that APP translation may be regulated by an miRNA pathway. Among miRNAs potentially targeting the APP 3'UTR, miR-101 was selected for further study because two putative miR-101 target sites (nt 242–248 and nt 531–537), which are conserved among the human, rat and murine APP genes, are present in the 3'UTR. miR-101 is a brain-enriched miRNA and its expression is inversely correlated with APP in developing rat hippocampal neurons and tissues. Using site directed mutagenesis, a functional interaction between miR-101 and the RE at nt 242–248 was demonstrated. The inhibition of endogenous miR-101 increased APP levels, whereas lentiviral-mediated miR-101 overexpression significantly reduced APP and Aβ load in hippocampal neurons. These data support the hypothesis that miR-101-1 is a repressor of APP expression. Moreover, as described for other miRNAs, miR-101 is downregulated in the human AD cerebral cortex, suggesting that miR-101 downregulation may play a role in the development of AD.

Conclusions and Perspectives

Multiple cis-acting elements present within the 5’UTR, the coding sequence or the 3’UTR of the APP mRNA and several trans-acting molecules including RNA binding proteins and microRNAs, have been shown to regulate APP expression (Fig. 1). Many of these studies have been performed in vitro using cell lines rather than in post-mitotic neuronal cells or in vivo in the brain. However, APP regulation by FRMP has been studied both in vitro and in vivo and these studies revealed that APP post-transcriptional regulation may occur in specific neuronal compartments such as synapses.

The studies summarized above highlight the fact that APP translation may be either activated or reduced by RNA binding proteins and may also be repressed by an miRNA/RISC pathway. Are these systems working in synergistic or competitive fashion? In this regard, it will be interesting to explore whether repression of APP mediated by microRNA-3’UTR associations influences the interaction of FMRP with the coding region of the APP transcript. Indeed, in mouse brain, a functional interaction between an miRNA, Ago1, EMRF and the 3’UTR of target mRNAs has recently been described. In addition, the possibility that microRNAs regulating APP expression through a functional interaction with the APP 3’UTR also target other molecules which affect FMRP binding to APP mRNA cannot be excluded.

Which post-transcriptional mechanisms regulating APP are present and functional in brain regions susceptible to AD remains to be determined. Indeed, preliminary investigations have indicated that IRP1 binding to the APP 5’UTR occurs in human brain tissue. Expression profiles of miRNAs obtained from temporal cortices of sporadic AD brains have shown that miR-101 and miR-106 are downregulated compared to normal human brain tissue. The identification of molecules and pathways actively involved in APP post-transcriptional regulation in the AD brain may contribute to understanding the molecular basis of AD and lead to novel therapeutic targets.

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References

1. Hoe HS, Lee KJ, Carney RS, Lee J, Markova A, Lee JY, et al. Interaction of nelin with amyloid precursor protein promotes neurite outgrowth. J Neurosci 2009; 29:7459-73.
2. Young-Pearse TL, Bai J, Chang R, Zheng JB, LoTurco JJ, Selkoe DJ. A critical function for β-amyloid precursor protein in neuronal migration revealed by in utero electroporation. Development; 2004; 131:699-710.
3. Loffler J, Huber G. Beta-amyloid precursor protein isoforms in various rat brain regions and during brain development. J Neurochem 1992; 59:1316-24.
4. Moya KL, Benowitz LI, Schneider GE, Allinquant B. The amyloid precursor protein is developmentally regulated and correlated with synaptogenesis. Dev Biol 1994; 161:597-603.
5. Sahu SL, Ilkin AE, Buchbaum JD, Greengard P. The amyloid precursor protein and its regulatory protein, FE65, in growth cones and synapses in vivo and in vitro. J Neurosci 2005; 23:5407-541.
6. Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herrn J. Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci 2006; 26:7212-21.
10. Rovelt-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerriere A, Vital A, et al. APP locus duplications cause autosomal dominantly-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet 2006; 38:450-3.

11. Podlisny MB, Lee G, Selkoe DJ. Gene dosage of the amyloid beta precursor protein in Alzheimer's disease. Science 1987; 238:669-71.

12. Theuns J, Brouwers N, Engelborghs S, Seegers K, Bogaerts V, Corset E, et al. Promoter mutations that increase amyloid precursor-protein expression are associated with Alzheimer disease. Am J Hum Genet 2006; 78:936-46.

13. Griffin WS, Stanley LC, Ling C, White L, MacLeod V, Perron L, et al. Brain interleukin 1 and 5-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Proc Natl Acad Sci USA 1989; 86:7611-5.

14. Flanders C, Lippa CF, Smith T, Pollen DAW, Sporn MB. Altered expression of transforming growth factor-beta in Alzheimer's disease. Neurology 1995; 45:1561-9.

15. Connor RD, Scahill R, Knott J, Rossor MN, Laquerriere A, Vital A, et al. APP locus duplication of amyloid beta-protein precursor mRNA is upregulated by interleukin-45:1561-9. Neurology 1999; 53:1561-9.

16. Rubinsztein DC, Verheyen G, De Deyn PP, et al. Regulation of amyloid beta-protein precursor mRNA expression in a normal human astrocyte cell line: evidence for a role of nuclear factor kappa B. J Biol Chem 2002; 277:45518-28.

17. Hentze MW, Caugham SW, Roussad TA, Bariccalo JG, Dancis A, Harford JB, et al. Identification of the iron-responsive element for the translational regulation of human ferritin mRNA. Science 1987; 238:1570-3.

18. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009; 10:126-39.

19. Patel N, Houng D, Miller N, Ansaloni L, Huang Q, Rogers JT, et al. MicroRNAs can regulate human APP levels. Mol. Neurodegener 2008; 3:10.

20. Hebert SS, Horre K, Nicolai L, Bergmans B, Papadopoulos AL, Delacourte A, et al. MicroRNA regulation of Alzheimer's Amyloid precursor protein expression. Neurobiol Dis 2009; 33:422-8.

21. Kalaria RN. The role of cerebral ischemia in Alzheimer's disease. Neurobiol Aging 2000; 21:321-30.