

Aberrant translation of proteins implicated in Alzheimer's disease pathology

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Abstract

Introduction

Control of protein manufacture at the point of translation is a crucial step in the regulation of gene expression and has shown to be important to many neurological processes, for example, synaptic plasticity and memory formation. The aim of this review was to discuss aberrant translation of proteins implicated in Alzheimer's disease pathology.

Discussion

Aberrant translation has been linked with neurodegenerative conditions such as Alzheimer's disease; however, it is not fully understood how this aberrant protein synthesis occurs or how this may be sustained in Alzheimer's disease. Cell stressors such as oxidative stress may enhance the translation of Alzheimer's disease-associated proteins (e.g. amyloid precursor protein), and new research suggests that the cell survival response (e.g. eIF2-alpha phosphorylation) may inadvertently up-regulate the translation of proteins such as BACE1; a process mediated in this instance by an upstream open reading frame located within the BACE1 5'UTR.

Conclusion

The research discussed in this review article has identified that in addition to regulation at the point of transcription and post-translational protein processing, the levels of proteins

which negatively associate with Alzheimer's disease pathology may also be controlled at the point of translation. Stressors such as oxidative stress may drive the transcription of amyloid precursor protein and the cleavage of amyloid precursor protein and may also enhance the translational efficiency of both amyloid precursor protein and the secretase responsible for cleaving amyloid precursor protein into its cytotoxic Abeta42 fragment. We suggest that selectively inhibiting the translation machinery in combination with reducing the levels of oxidative stress may represent a new therapeutic avenue for the treatment of Alzheimer's disease.

Introduction

Translation

Gene expression comprises a series of complex and tightly regulated events, starting with transcription and ending with protein synthesis. In a simple model, translation initiation of messenger RNA begins when the cap-binding protein complex is recruited to the cap complex. This complex then scans the 5'UTR of the messenger RNA in a 5' to 3' direction until a start codon is recognised and synthesis is initiated (reviewed in depth by Jackson et al.¹); the process of translation then progresses through three stages: initiation, elongation, and termination. The cap-binding complex that forms at the initiation step is an assembly of a multitude of translation initiation factors, while the 5'-untranslated region (5'UTR) may differ in length, possess features such as tertiary structures, upstream open reading frames (uORFs) and RNA protein-binding sites²⁻⁴. Importantly, each

feature may act as a regulatory element independently enhancing or repressing the rate of protein synthesis (see Jackson et al.¹).

Transcription and mRNA processing can be a lengthy process; therefore regulation of gene expression at the point of transcription may be insufficiently rapid to function as a responder to a stimuli or stress. One hypothesis suggests that the cell can overcome this constraint by transcribing reservoirs of inactive messages that remain dormant awaiting translational activation only when protein is required—an idea supported by data showing that mRNA levels only partly correlate with protein levels⁵. This model allows for transcripts to be 'activated', then proteins rapidly synthesised from stored templates. It is unsurprising that most detailed investigations into the regulation of translation have focussed on the area of stress responses, where the rate of protein manufacture may directly determine cell survival. However, recent research has also identified this step in the gene expression pathway to be a key stage of control in the manufacture of proteins linked with the pathology of progressive neurodegenerative conditions such as Alzheimer's disease (AD). The aim of this review was to discuss new and exciting research that identifies putative mechanisms of regulation of proteins associated with AD.

Discussion

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

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Function of translation within neurons and neurodegenerative disease

Translation is a key step in the regulation of cellular gene expression in the central nervous system (CNS). CNS cells may have a relatively large distance between the nucleus and subcellular domains; therefore, localised control of protein synthesis provides a ready means of changing the proteome in distal regions of the cell in response to stimuli, signal or stress (reviewed by Liu-Yesucevitz et al.⁶)⁷. Recent research has identified that aberrations in translation are not trivial and may profoundly affect a range of neurological functions within the brain⁶. Deregulation of the translation initiation machinery (e.g. proteins such as eIF4e) has been linked to the development of autism spectrum disorder, aberrant memory formation and CNS disease such as amyotrophic lateral sclerosis and fragile X syndrome^{8,9} (see Liu-Yesucevitz et al.⁶). Given the proven links between the machinery that regulates protein synthesis and a diverse range of complex diverse neurological processes such as memory consolidation, it is unsurprising that aberrant control of translation has been implicated in a number of different complex polygenic neurodegenerative diseases.

Potent regulators of translation are now known to be active within the brain. Small-molecule inhibitors such as the prostaglandin 15d-PGJ2 and short non-coding RNAs (e.g. BC200 or the protein PDCD4¹⁰⁻¹²), all function to regulate the proteome via a selective inhibition of translation. Importantly, new research has identified that the levels of a number of these endogenous inhibitors link with the pathology of some CNS diseases such as AD (e.g. PDCD4 and BC200^{13,14}), and although it is tempting to hypothesise that this link is a direct consequence of the deregulation of the synthesis of individual proteins associated with AD such as amyloid, as yet there is no definitive

model to describe exactly how regulators such as BC200 may affect disease pathology.

New research is starting to explore the function of these endogenous inhibitors of translation initiation within CNS cells and, by extension, may explain their role in diseases such as AD. Mice knockout for the snRNA inhibitor BC1 (BC200 orthologue) exhibits hyper-excitability, whereas mice overexpressing eIF4e show abnormal synaptic plasticity in the hippocampus, a region implicated in the early stages of AD¹⁵. It is interesting to note that neuronal hyper-excitability amplifies the synaptic release of A β (see Noebels¹⁶) and low levels of amyloid enhance synaptic plasticity¹⁷. It could be speculated that given the role of amyloid in synaptic connectivity, the cell faced with the challenge of toxic amyloid and increased neuronal cell death chooses to increase neuronal outgrowth to form new connections and by doing so stimulates the amyloid pathway, thereby creating a pathogenic feedback loop. It is less speculative, however, to suggest that translation is a key step in the regulation of dendritic protein synthesis^{7,18}.

Intriguingly, research has identified elevated levels of PDCD4 in post-mortem AD brain tissue¹³, although it is unclear from the wider data currently available if this is an attempt by AD-afflicted cells to assert additional control over the proteome via inhibition of the translation initiation factor eIF4a or if this is an aberrant overexpression of PDCD4 that exacerbates or worsens disease symptoms. The microRNA (miR21) that regulates PDCD4 is also deregulated in response to AD amyloid-beta¹⁹ and may either directly or indirectly promote neurite outgrowth²⁰. In experiments conducted using primary mouse microglial cells, the prostaglandin 15d-PGJ2 blocked the elevation of amyloid-beta-induced proinflammatory cytokines²¹ and protected enteric glial cells against

oxidative stress²²—however it is not evident how much, if any, of these cytoprotective properties are due to the selective inhibition of translation or other functional properties of each molecule, for example acting as an agonist of the PPAR-gamma pathway.

Other proteins function to stimulate or modify translation initiation indirectly. The neuronal specific RNA-binding protein HuD has been demonstrated to act as a modifier of translation and may directly enhance the activity of the translation initiation factor eIF4A²³. Importantly, it has been identified that among those specific mRNA transcripts bound and regulated by HuD, a surprising number of messages that associate with AD pathology may be positively or negatively regulated by this protein (e.g. tau and acetylcholinesterase^{24,25}). 4EB-PB inhibits translation by binding to eIF4E. This protein has also been shown to suppress global translation under stress and that the levels of this molecule may direct the levels of tau protein²⁶. This hints at a complex web of protein/RNA interactions that underpin the progression of AD via multiple interacting pathways. It is interesting to note that we were able to demonstrate that the synthesis of a number of proteins that drive the progression of AD can be suppressed by small-molecule inhibitors of translation²⁷ and where tested that this class of inhibitor is well tolerated in mice²⁸.

Translation—regulation in the amyloid pathway

The levels of key proteins linked with AD pathology may be regulated post-transcriptionally. Transcripts of the amyloid pathway genes such as amyloid precursor protein (APP), beta secretase (BACE1), and presenilin-1 (PS1) possess 5'UTR regulatory features that include, but are not limited to, uORFs, iron response

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elements, translational enhancers and RNA-binding sites (see Chatterjee and Pal²⁹). These sequences have a profound effect on the ultimate levels of these proteins and may be exploited by the cell as a means of co-ordinating the expression of the amyloid pathway.

It is now widely accepted that the transcription, synthesis and processing of APP into the toxic amyloid-beta 42 (abeta42) protein can all be directly and indirectly influenced by different cell stressors; new research however identifies a previously unreported stimulatory effect of oxidative stress on the synthesis of APP. Oxidative stress can induce APP promoter demethylation, elevating transcription of APP mRNA³⁰. This same stress may indirectly promote the cleavage of APP into the toxic amyloid pathway³¹, and it is logical that oxidative stress also drives the translation of APP. Although this suggests that APP transcription, translation and processing are a tightly co-ordinated process, it has yet to be established precisely how APP translation is up-regulated. It is, however, known that the translation of BACE1, an enzyme that functions to process amyloid beta into toxic abeta 42, is also enhanced by oxidative stress³², a process mediated by an uORF located within the 5'UTR of the BACE1 mRNA. This uORF feature leads to the up-regulation of BACE1 translation upon the phosphorylation of the translation factor eIF2-alpha (eIF2a)³². For most mRNAs, the phosphorylation of eIF2a is a stress-induced signal intended to reduce protein synthesis (reviewed by Yamasaki and Anderson³³); however, unusually in this instance, phosphorylation of eIF2a stimulates beta secretase synthesis. Also, as abeta42 causes oxidative stress within the cell, it is possible to envisage a feedback loop where oxidative stress drives not only the transcription and cleavage of APP but also the translation of APP and the translation of BACE1, since oxidative DNA damage

increases the level of eIF2-alpha phosphorylation during the cellular response DNA damage³¹. This new research identifies an exciting new link between oxidative stress and the regulation of amyloid processing, which requires further investigation.

Translation as a point of control in DNA damage pathway

Proteins negatively associated with AD pathology elicit many damaging intracellular effects within neuronal cells. Exposure to toxic amyloid beta induces DNA damage and mitochondrial dysfunction (reviewed by Mao and Reddy³⁴), while facets of the DNA damage response pathway such as Chk1 and Chk2 may drive the aberrant phosphorylation of tau³⁵. Like other stress responses, the global regulation of translation following a DNA-damaging stress has been demonstrated to be critical to cell survival^{31,36}. In order to maintain the expression of DNA repair genes, translation is reprogrammed to allow for the selective manufacture of DNA damage-specific proteins which allows specific mRNAs to be translated while other messages become translationally inactive (reviewed by Kondrashov et al.³⁷).

Cells respond to DNA damage through the activation of a multitude of pathways that directly and indirectly control the regulation of post-transcriptional gene expression (see Kondrashov et al.³⁷); however, new research suggests that this mechanism may inadvertently impact on the synthesis of AD-associated proteins and synaptic plasticity. In order to promote cell survival, kinases such as DNA-PK regulate protein synthesis during UV-induced DNA damage³⁶. Another 'master' kinase ATM can directly phosphorylate e4E-BP³⁸ interfering with translational initiation, while ATR kinase has been shown to be involved in regulation of mRNA splicing and polyadenylation³⁹. In response to DNA-damaging stresses such as UV, the kinase GCN2

(general control non-derepressible-2) phosphorylates eIF2a, thereby modifying global translation as a stress response to promote cell survival⁴⁰. Importantly, this links with the recent work discussed above by Mouton-Liger et al.³², which suggests that eIF2-alpha phosphorylation may drive the translation of BACE1, the beta secretase responsible for cleaving the APP into the toxic amyloid pathway. DNA damage may therefore drive a negative feedback loop in which aberrant phosphorylation resulting from DNA damage drives toxic amyloid processing, which in turn results in DNA damage via oxidative stress induced by toxic amyloid. Also, the phosphorylation of e4E-BP and the action of the kinase ATR both impact on synaptic plasticity and may also contribute to AD pathology, although further research will be required to establish how these pathways intercede.

Conclusion

This new research discussed in this article identifies both a stimulant and a putative mechanism for the enhanced translation of AD-associated proteins. This research hints at a complex interplay between translation regulation, cell stress and various cell stress and DNA damage response pathways. We previously identified that selectively inhibition of translation at the point of translation initiation may provide a viable strategy for reducing the levels of proteins negatively associated with AD while also providing some protection against oxidative stress; this new research further supports the idea that a small molecule that can selectively control protein manufacture via modulation of translation may represent a new and dynamic therapeutic avenue for the treatment of diseases such as AD. Although there is growing research to support the rationale for this strategy for treatment, there is as yet no ready supply of a non-toxic selective

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small-molecule inhibitor of translation with which to test this hypothesis.

Abbreviations list

AD, Alzheimer's disease; APP, amyloid precursor protein; CNS, central nervous system; uORF, upstream open reading frame; UTR, untranslated region

References

- Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol.* 2010 Feb;11(2):113–27.
- Appasamy SD, Ramlan EI, Firdaus-Raih M. Comparative sequence and structure analysis reveals the conservation and diversity of nucleotide positions and their associated tertiary interactions in the riboswitches. *PLoS One.* 2013 Sep;8(9):e73984.
- Barbosa C, Peixeiro I, Romão L. Gene expression regulation by upstream open reading frames and human disease. *PLoS Genet.* 2013;9(8):e1003529.
- Amadio M, Pascale A, Wang J, Ho L, Quattrone A, Gandy S, et al. nELAV proteins alteration in Alzheimer's disease brain: a novel putative target for amyloid-beta reverberating on AbetaPP processing. *J Alzheimers Dis.* 2009;16(2):409–19.
- Brockmann R, Beyer A, Heinisch JJ, Wilhelm T. Posttranscriptional expression regulation: what determines translation rates? *PLoS Comput Biol.* 2007 Mar;3(3):e57.
- Liu-Yesucevitz L, Bassell GJ, Gitler AD, Hart AC, Klann E, Richter JD, et al. Local RNA translation at the synapse and in disease. *J Neurosci.* 2011 Nov;31(45):16086–93.
- Martin KC, Zukin RS. RNA trafficking and local protein synthesis in dendrites: an overview. *J Neurosci.* 2006 Jul;26(27):7131–4.
- Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, et al. Autism-related deficits via dysregulated eIF4E-dependent translational control. *Nature.* 2013 Jan;493(7432):371–7.
- Hoeffler CA, Cowansage KK, Arnold EC, Banko JL, Moerke NJ, Rodriguez R, et al. Inhibition of the interactions between eukaryotic initiation factors 4E and 4G impairs long-term associative memory consolidation but not reconsolidation. *Proc Natl Acad Sci U S A.* 2011 Feb;108(8):3383–8.
- Kim WJ, Kim J, Jang SK. Anti-inflammatory lipid mediator 15d-PGJ2 inhibits translation through inactivation of eIF4A. *EMBO J.* 2007 Dec;26(24):5020–32.
- Kondrashov AV, Kiefmann M, Ebnet K, Khanam T, Muddashetty RS, Brosius J. Inhibitory effect of naked neural BC1 RNA or BC200 RNA on eukaryotic in vitro translation systems is reversed by poly(A)-binding protein (PABP). *J Mol Biol.* 2005 Oct;353(1):88–103.
- Suzuki C, Garces RG, Edmonds KA, Hiller S, Hyberts SG, Marintchev A, et al. PDCD4 inhibits translation initiation by binding to eIF4A using both its MA3 domains. *Proc Natl Acad Sci U S A.* 2008 Mar;105(9):3274–9.
- Soler-López M, Zanzoni A, Lluís R, Stelzl U, Aloy P. Interactome mapping suggests new mechanistic details underlying Alzheimer's disease. *Genome Res.* 2011 Mar;21(3):364–76.
- Mus E, Hof PR, Tiedge H. Dendritic BC200 RNA in aging and in Alzheimer's disease. *Proc Natl Acad Sci U S A.* 2007 Jun;104(25):10679–84.
- Santini E, Huynh TN, MacAskill AF, Carter AG, Pierre P, Ruggero D, et al. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. *Nature.* 2013 Jan;493(7432):411–5.
- Noebels J. A perfect storm: Converging paths of epilepsy and Alzheimer's dementia intersect in the hippocampal formation. *Epilepsia.* 2011 Jan;52 (Suppl 1):39–46.
- Puzzo D, Privitera L, Leznik E, Fà M, Staniszewski A, Palmeri A, et al. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci.* 2008 Dec;28(53):14537–45.
- Schuman EM, Dynes J, Steward O. Synaptic regulation of translation of dendritic mRNAs. *J Neurosci.* 2006 Jul;26(27):7143–6.
- Schonrock N, Ke YD, Humphreys D, Staufienbiel M, Ittner LM, Preiss T, et al. Neuronal microRNA deregulation in response to Alzheimer's disease amyloid-beta. *PLoS One.* 2010 Jun;5(6):e11070.
- Sayed D, Rane S, Lypowy J, He M, Chen IY, Vashistha H, et al. MicroRNA-21 targets Sprouty2 and promotes cellular outgrowths. *Mol Biol Cell.* 2008 Aug;19(8):3272–82.
- Xu J, Barger SW, Drew PD. The PPAR-gamma Agonist 15-Deoxy-Delta-prostaglandin J(2) Attenuates Microglial Production of IL-12 Family Cytokines: Potential Relevance to Alzheimer's Disease. *PPAR Res.* 2008;349185.
- Abdo H, Mahé MM, Derkinderen P, Bach-Ngohou K, Neunlist M, Lardeux B. The omega-6 fatty acid derivative 15-deoxy- $\Delta^{12,14}$ -prostaglandin J2 is involved in neuroprotection by enteric glial cells against oxidative stress. *J Physiol.* 2012 Jun;590(Pt 11):2739–50.
- Fukao A, Sasano Y, Imataka H, Inoue K, Sakamoto H, Sonenberg N, et al. The ELAV protein HuD stimulates cap-dependent translation in a Poly(A)- and eIF4A-dependent manner. *Mol Cell.* 2009 Dec;36(6):1007–17.
- Atlas R, Behar L, Sapoznik S, Ginzburg I. Dynamic association with polysomes during P19 neuronal differentiation and an untranslated-region-dependent translation regulation of the tau mRNA by the tau mRNA-associated proteins IMP1, HuD, and G3BP1. *J Neurosci Res.* 2007 Jan;85(1):173–83.
- Deschênes-Furry J, Mousavi K, Bolognani F, Neve RL, Parks RJ, Perrone-Bizzozero NI, et al. The RNA-binding protein HuD binds acetylcholinesterase mRNA in neurons and regulates its expression after axotomy. *J Neurosci.* 2007 Jan;27(3):665–75.
- Li X, Alafuzoff I, Soininen H, Winblad B, Pei JJ. Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. *FEBS J.* 2005 Aug;272(16):4211–20.
- Bottley A, Phillips NM, Webb TE, Willis AE, Spriggs KA. eIF4A inhibition allows translational regulation of mRNAs encoding proteins involved in Alzheimer's disease. *PLoS One.* 2010 Sep;5(9).
- Ishikawa C, Tanaka J, Katano H, Senba M, Mori N. Hippuristanol reduces the viability of primary effusion lymphoma cells both in vitro and in vivo. *Mar Drugs.* 2013 Sep;11(9):3410–24.
- Chatterjee S, Pal JK. Role of 5'- and 3'-untranslated regions of mRNAs in human diseases. *Biol Cell.* 2009 May;101(5):251–62.
- Gu X, Sun J, Li S, Wu X, Li L. Oxidative stress induces DNA demethylation and histone acetylation in SH-SY5Y cells:

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potential epigenetic mechanisms in gene transcription in A β production. *Neurobiol Aging*. 2013 Apr;34(4):1069–79.

31. Tan JL, Li QX, Ciccotosto GD, Crouch PJ, Culvenor JG, White AR, et al. Mild oxidative stress induces redistribution of BACE1 in non-apoptotic conditions and promotes the amyloidogenic processing of Alzheimer's disease amyloid precursor protein. *PLoS One*. 2013 Apr;8(4):e61246.

32. Mouton-Liger F, Paquet C, Dumurgier J, Bouras C, Pradier L, Gray F, et al. Oxidative stress increases BACE1 protein levels through activation of the PKR-eIF2 α pathway. *Biochim Biophys Acta*. 2012 Jun;1822(6):885–96.

33. Yamasaki S, Anderson P. Reprogramming mRNA translation during stress. *Curr Opin Cell Biol*. 2008 Apr;20(2):222–6.

34. Mao P, Reddy PH. Aging and amyloid beta-induced oxidative DNA damage and mitochondrial dysfunction in Alzheimer's disease: implications for early intervention and therapeutics. *Biochim Biophys Acta*. 2011 Nov;1812(11):1359–70.

35. Iijima-Ando K, Zhao L, Gatt A, Shenton C, Iijima K. A DNA damage-activated checkpoint kinase phosphorylates tau and enhances tau-induced neurodegeneration. *Hum Mol Genet*. 2010 May;19(10):1930–8.

36. Powley IR, Kondrashov A, Young LA, Dobbyn HC, Hill K, Cannell IG, et al. Translational reprogramming following UVB irradiation is mediated by DNA-PKcs and allows selective recruitment to the polysomes of mRNAs encoding DNA repair enzymes. *Genes Dev*. 2009 May;23(10):1207–20.

37. Kondrashov AV, Spriggs KA, Bushell M, Willis AE. Co-ordinated regulation of translation following DNA damage. *Cell Cycle*. 2009 Oct;8(19):3067–8.

38. Yang DQ, Kastan MB. Participation of ATM in insulin signalling through phosphorylation of eIF-4E-binding protein 1. *Nat Cell Biol*. 2000 Dec;2(12):893–8.

39. Chandris P, Giannouli CC, Panayotou G, Kletsas D. Compromise in mRNA processing machinery in senescent human fibroblasts: implications for a novel potential role of Phospho-ATR (ser428). *Biogerontology*. 2010 Aug;11(4):421–36.

40. Jiang HY, Wek RC. GCN2 phosphorylation of eIF2 α activates NF- κ B in response to UV irradiation. *Biochem J*. 2005 Jan;385(Pt 2):371–80.