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Amyloid Precursor Protein and Alpha Synuclein Translation, Implications for Iron and Inflammation in Neurodegenerative diseases

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Summary

Recent studies that alleles in the hemochromatosis gene may accelerate the onset of Alzheimer's disease (AD) by five years have validated interest in the model in which metals (particularly iron) accelerate disease course. Biochemical and biophysical measurements demonstrated the presence of elevated levels of neurotoxic copper, zinc and iron in the brains of AD patients. Intracellular levels of amyloid precursor protein (APP) holoprotein were shown to be modulated via iron by a mechanism that is similar to the translation control of the ferritin L- and H mRNAs by Iron-responsive Element (IRE) RNA stem loops in their 5′untranslated regions (5′UTRs). Recently, we reported a putative IRE-like sequence to be present in the 5′UTR of the Parkinson's disease (PD) specific alpha synuclein (ASYN) transcript. ASYN encodes the non-Aβ component (NAC) of amyloid plaques. The demonstration of iron-dependent translation of APP mRNA, the involvement of metals in the plaque of AD patients and of increased iron in striatal neurons in the Substantia nigra (SN) of PD patients, have each encouraged the development of metal attenuating agents and iron chelators as a major new therapeutic strategy for the treatment of these neurodegenerative diseases. In the case of AD, metal based therapeutics may ultimately prove more cost effective than the use of an amyloid vaccine as the preferred anti-amyloid therapeutic strategy to ameliorate the cognitive decline of AD patients.

A. METALS AND ALZHEIMER'S DISEASE (AD) AND PARKINSON'S DISEASE (PD) PATHOLOGY

A.1. Introduction

Both extracellular amyloid plaques and intracellular neurofibrillary tangles are the predominant pathological features characterizing the clinical onset of AD (both early onset and late onset AD). A proximal pathological feature of AD is the formation of neurofibrillary tangles by the microtubule associated protein Tau (mutations in Tau cause hereditary frontotemporal dementia (1,2)). However, the amyloid precursor protein (APP) remains central to our understanding AD progression as a devastating neurodegenerative disease. We will discuss the relationship of metals to AD, referring to APP and amyloid

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formation (3). The chromosome 21 APP gene retains its status as being both the genetic cause of familial AD (FAD) (4) and production of the 40 -42 amino acid amyloid Aβ processing product of APP is the most significant therapeutic target and pathological marker of the AD brain causing amyloid plaque formation (5).

The marked increase in the steady-state levels of metals (Fe, Cu and Zn) in the AD (6) and PD brains (7) induce a gene expression pattern that is associated with deleterious consequences for neuronal survival (6). APP mRNA translational control by iron (8,9) and APP gene transcriptional control by copper (10) each provide new genetic support for the model that APP is a metalloprotein with an integral role in metal metabolism. Recently we identified and published that the alpha synuclein 5′UTR encodes an RNA sequences that closely resembles an active Iron-responsive Element (11).

Genetic and biochemical evidence has linked the biology of metals (Fe, Cu and Zn) to brain aging and major neurodegenerative diseases including AD (12,13). This phenomenon is also associated particularly with elevated iron in the midbrain of PD patients (7). Excess iron, as occurs after Stroke, closely regulates gene expression of the Alzheimer's APP gene at the level of message translation (8,14). This review describes the Fe-regulated control of intracellular levels of APP holoprotein which is modulated by a mechanism that is similar to the pathway by which iron controls the translation of the ferritin L- and H mRNAs by Iron-responsive Element (IRE) RNA stem loops in their 5′untranslated regions (5′UTRs) (8,15). More recently a putative IRE-like sequence was reported to be present in the 5′untranslated region of the Parkinson's disease alpha synuclein (ASYN) transcript (11). APP gene transcription was found to be responsive to copper deficit in a study in which the Cu depleted fibroblasts of a human fibroblasts over-expressing the Menkes protein (MNK) exhibited repressed transcription of APP through metal regulatory and copper regulatory sequences upstream of the 5′ cap site (16).

A.2. APP abundance and mis-processing are a genetic cause of familial Alzheimer's disease (FAD)

Approximately 10% of cases of AD are caused by familial genetic mutations. These cases are associated with autosomal dominant inheritance of APP (17,18) and presenilins PS-1 and PS-2 (19) gene mutations (20). Mutations within the APP gene on chromosome 21 cause altered cleavage of the Aβ-peptide from the APP holoprotein and have been genetically linked with early onset “Familial Alzheimer's Disease”(FAD) (17). APP and PS-1 gene mutations independently enhance cleavage of the precursor into the Aβ peptide [Aβ (1-40) and Aβ (1-42)] that accumulates in the brain as amyloid plaques (21). Trisomy of the APP gene is associated with Down's syndrome (DS) (22). Recently, as in DS, mounting evidence supports that simple APP abundance caused by duplication of the region of chromosome 21 harboring the APP gene, was 100% linked to familial AD in French pedigrees (23,24).

The abundant and ubiquitously expressed APP is a metalloprotein that spans membranes in the endoplasmic reticulum, trans-Golgi and on the cell membrane surface of most cell-types. Originally APP was shown to be a redox active copper-zinc binding protein of unknown function during normal health (25). Our unpublished data suggests that APP, itself, also binds iron. During normal physiology a group of metalloproteases known as the alpha-secretases (26) ADAM-10, ADAM-17 and TACE, cleave the APP to generate the neuroprotective 90 kDa ectodomain of APP (APP(s)) that is released from cells into the cortex, cerebrospinal fluid and the bloodstream; a cytoprotective event that occurs at an increased rate in activated astrocytes during the acute phase response (27).

During the course of AD, APP is the single substrate which is proteolyzed to produce the 40-42 amino acid Aβ peptide that aggregates to form the main component of amyloid

plaques (28). The pathogenic Aβ peptide encompasses amino acids 672-714 of APP-770 (longest alternatively spliced isoform of APP), wherein the amino terminus of Aβ includes 28 external amino acids and the remaining carboxyl-terminal half of Aβ is encoded by first residues of the trans-membrane region of nascent APP. A decade of research from multiple laboratories has shown that the γ-secretase multimer (Pen2, presenillin, APH1 and nicastrin) (29) and BACE (30) cleave APP to generate the pathogenic 40 to 42 amino acid Aβ peptide (31).

A.3. Alpha synuclein mutations and abundance are a cause of familial Parkinson's disease

Alpha-synuclein is the ∼15 kd cytosolic protein implicated in pathogenesis of neurodegenerative diseases referred to as α-synucleinopathies (32), including the movement disorders such as PD. The pathological hallmark of PD is the oligomerization of toxic ASYN commonly in Lewy bodies, but also in dystrophic neurites, and glial cytoplasmic inclusions (33). The mechanisms underlying PD have been associated with many cell-based lesions including ubiquitination and LRRK kinase leading to dopaminergic cell death (34). The discovery that mutations in ASYN can cause familial PD(11) (35), and that ASYN accumulates in Lewy bodies suggests that this protein participates in the pathophysiology of PD.

The straightforward protein abundance of ASYN was shown to be the cause of familial PD in families that expressed triplicate copies of the alpha synuclein gene (35), whereas missense mutations have been more frequently quoted in the scientific literature (34). Alpha synuclein knockout mice are fully viable (36), suggesting that the two homologous proteins γ and β synucleins perform a redundant and compensatory function most certainly associated with their its pre-synaptic localization (37). The role of alpha synuclein aggregation mediate neurotoxicity in the SN of Parkinson's disease models was supported by the finding that alpha synuclein knock out mice are resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurotoxicity (38).

A.4. Iron and spontaneous Alzheimer's and Parkinson's Disease

Metals (Fe, Cu, Zn) clearly provide one of the ultrastructural requirements needed for polymerization of Aβ peptide (39) in addition to the reported presence of pathological chaperones such as alpha-1 antichymotrypsin (ACT) or Apo-E (40). For example, elemental profiles (S, Fe, Cu, and Zn) were microscopically observed to be physically associated with amyloid plaques using Synchrotron scanning X-ray Fluorescence Microscopy (μ-XRF) (41) (Figure 1). Also Cu, Zn and Fe were demonstrated to be potent natural binding partners of Aβ with a dissociation constant at the attomolar affinity level (42,43). Iron and Cu, were demonstrated to be very important in mediating the neurotoxic action of Aβ protofibrils (44). At the molecular level, the histidine mediated zinc binding site was mapped to a contiguous sequence between positions 6 and 28 of the Aβ sequence (45). Amyloid Aβ is normally secreted from cells grown in culture as a monomer, but also as a covalently linked dimer, in the ratio 55: 30: 15 monomers: dimers: trimers (46). Human amyloid derived from oligomeric Aβ results from a tyrosine cross-lin ked oligomerization that is induced by metal catalyzed oxidation systems.

The current level of understanding of late onset AD has been advanced since the discovery that individuals homozygous for the E4 allele of apolipoprotein E (ApoE) are at increased risk for developing Alzheimer's Disease (47). Other genome scans have identified alpha 2 macroglobin involved in Aβ peptide clearance (48), the insulin degrading enzyme that degrades Aβ (49), alpha-1 antichymotrypsin as a pathological chaperone for profibril formation (40).

A.4.1. Iron and the AD brain—Recently the transferrin C2 allele, relevant to blood iron transport was characterized as a risk factor for AD (50) (51,52). Also several genetic studies demonstrated the clear involvement of the genotype of the hemochomatosis (HFE) gene, additional to the transferrin C2 allele, to increase the risk of AD (53) (54) (50), likely as a consequence of accelerated iron overload and compartmentalization in these individuals. The elegant work of Lee et al., (2006) confirmed the cell biological mechanisms of these AD genetic associations of HFE mutations to their cell based phenotypes in neuroblastoma cells consistent to oxidative stress events causing neurodegeneration (55). The original Italian study, for example, incorporated 107 patients with sporadic late-onset AD and 99 age-matched non-demented controls (56). These authors observed that patients carrying the mutant HFE-H63D allele had a mean age of onset at 71.7 +/- 6.0 years versus 76.6 +/- 5.8 years of those who were homozygous for the wild-type allele ($p = 0.001$). Further support came from a study using knockout mice deficient in the H-ferritin gene wherein Thompson et al., (2003) reported that null ferritin heterozygous mutants developed oxidative features in the cortex reminiscent of PD and AD (57).

The integral role iron plays in the etiology of AD pathology was directly supported from an MRI imaging study of the brains of Alzheimer's patients that revealed elevated levels of iron, particularly in the neurons of the basal ganglia (58). Confirming these results, elegant spectroscopic and microscopic studies demonstrated that amyloid plaques harbor increased burden of iron, copper and zinc (6) (41) (Figure 1). Iron and zinc levels were measured to be present at concentrations as high as the 1 mM level in the vicinity of amyloid plaques. There has been controversy as to whether the iron-Aβ peptide interaction was neurotrophic or neuroprotective. Aβ-peptide, itself, was found to be neurotrophic under some circumstances (59), and perhaps Aβ toxicity is only evident in the presence of metals. Certainly iron enhanced beta-amyloid action to accelerate Aβ-induced neuroblastoma cell death, providing one direct link between excessive iron and the known loss of neuronal function seen in AD patients (60). Bishop and Robinson (2003) reported that Aβ peptide reduced iron toxicity in rat cerebral cortex (61) although iron remains a candidate pathological mediator of AD (62).

In addition to iron, trace copper in the water supply dramatically accelerated amyloid plaque formation in APP transgenic mice (63). Thus our working model has to account for the fact that the Aβ amyloid precursor protein (APP) of AD is a copper binding metalloprotein (25). From *in vitro* experiments, copper, zinc and iron accelerated the aggregation the Aβ peptide and enhanced metal catalyzed oxidative stress associated with amyloid plaque formation (44).

Iron imbalance is evident in the AD brain as reflected by the altered expression of several iron proteins in the AD brain, including increased expression of P97 (an iron transporter (64) and potential biomarker (65,66). Ferritin expression is increased in the vicinity of amyloid plaques (67). These data are consistent with the involvement of hemachromatosis and transferrin genes as genetic factors that can increase the risk for late onset sporadic AD.

A.4.2. Iron and the PD brain—The role of iron to cause mis-aggregation has long been a feature of PD research (35), particularly since iron is found in increased abundance in the Substantia nigra, the region of maximal dopaminergic neuronal loss in PD (7). Consistent with this observation, ferritin over-expression, stored and safely sequestered excess iron and offset neuronal loss in mice subjected to the neurotoxic Parkinson's-inducing agent MPTP, suggesting that iron mediated events are important in PD (68). Iron, but also hydrogen peroxide, stimulates the production of intracellular aggregates that contain α-synuclein and ubiquitin as identified by immunocytochemistry, electron microscopy, or the histochemical staining with thioflavine S in striatal regions of the brain (35).

In summary, Metal catalyzed oxidative stress in the vicinity of amyloid remains a central pathological hallmark of AD in the brain cortex region, and metal catalyzed fibrilization of ASYN is proximal to neurotoxic events in the SN of Parkinson's disease patients (35).

B. FERRITIN TRANSLATIONAL CONTROL: LINKS TO APP AND ASYN *m*RNA EXPRESSION

B.1. Introduction

Iron imbalance, like inflammation, has been recognized genetically in the etiology of neurological disorders. Consistent with these observations, the iron storage protein ferritin is present at increased abundance in neuritic plaques (69) at the same time as the acute phase proteins alpha-1 antichymotrypsin (70). An elucidation of the mechanisms of brain iron homeostasis, as outlined in figure 2, will help our understanding of AD especially since iron binds to Aβ-peptide and enhances beta-amyloid toxicity (45,60,71,72). Excess iron accumulation is a consistent observation in the AD brain. Brain autopsy samples from AD patients have elevated levels of ferritin iron, particularly in the neurons of the basal ganglia (58) and most amyloid plaques contain iron and ferritin-rich cells (12).

This section will outline how iron enhances both the translation of APP mRNA and also cleavage of the Aβ-peptide domain of APP by the metaloprotease alpha secretase (73). Similar to ferritin, the protective effect of the major cleavage product of APP, APP(s), may derive from its capacity to detoxify iron and thus diminish iron/heme aggravated oxidative stress to neuronal cells (25,74). Clinically there is a reported decrease in the rate of decline in AD patients who were treated with the intramuscular iron chelator desferrioxamine (75), and part of this efficacy may be attributable to DFO repression of APP mRNA translation. We need to recognize the “UTRosome’ concept which in essence takes the 5′-UTR from an RNA entity to a RNA-protein complex consisting of proteins (Iron-regulatory proteins)(76) but also note that Smad proteins, and certain transcription factors, bind both proximal promoter region as well as the UTR (77).

Lessons from the genetic control of translation of ferritin mRNAs and transferrin receptor mRNA stability can be applied to the control of APP and also ASYN expression by iron. This information will be relevant to RNA directed drug discovery to offset AD and PD pathology by targeting and inhibiting translation of APP and ASYN gene expression without influencing general translation (78) (79).

B.2. Post-transcriptional Control of Ferritin and Transferrin Receptor (TfR) Expression

Ferritin is the intracellular iron storage protein composed of two subunits (L and H-subunits) that co-assemble to form the 240,000 dalton iron storage shell found in all tissues of the body (80) (Figure 2). Ferritin is cytoprotective (81) because the H-subunit oxidizes Fe^{2+}, the bioavailable form of iron that causes tissue damage via hydroxyl radicals (Fenton Reaction). Ferric iron then becomes stored as an ferric orthophosphate (Fe^{3+}-$P0_4$) crystal inside the internal cavity of the ferritin shell (80,82). Transferrin receptor (TfR) is present at the surface membrane of all cells and tissues. TfRs have a high affinity for binding iron-loaded transferrin (90,000 dalton protein), thereby facilitating the uptake of iron from the bloodstream into tissues (80,82). One of the major markers of any inflammation is lowered serum iron levels accompanied by a reduction in the steady-state levels of blood transferrin levels (83). There is also an increase in serum ferritin levels seen during inflammation (84). In the next sections, we will outline briefly how the iron-regulatory proteins IRP1and IRP2 control intracellular iron homeostasis by modulating ferritin mRNA translation and transferrin receptor mRNA stability (85), and will then compare this to the control of APP and ASYN expression.

B.2. 1. Iron-responsive Element (IRE) RNA stem loops and intracellular iron homeostasis—Transferrin receptors (TfRs) mediate iron uptake into most mammalian cell types wherein iron uptake decreases TfR mRNA stability via five distinct Iron-responsive Element RNA stem loops (IREs) in the 3′UTR of TfR mRNA. In fact, IREs modulate intracellular iron uptake by mediating both TfR mRNA and Divalent Metal Ion Transporter (DMT-1) mRNA stability. In a feedback regulatory circuit, during iron deficit the iron regulatory proteins, IRP1 and IRP2, increase the message stability of TfR mRNA and DMT-1 mRNA by binding to 3′UTR sequences and preventing their degradation (86).

Prior to assembling into the central iron storage protein in all cells, ferritin L- and H subunit translation is controlled by the iron regulatory proteins,IRP1 and IRP2, which bind at high affinity (K_d 40-100 pM) to highly conserved IREs RNA stem loops at the 5′ cap sites of the L- and H-ferritin mRNAs (86,87) (88) (Figures 2 and 3). The IRE/IRP interaction impedes the access of the small 40S ribosome subunit to the 5′ end of the ferritin mRNAs and thereby suppresses L- and H-ferritin mRNA translation (89-91). The potency of the IRE to regulate translation is position dependent (92). Iron influx relieves repression of ferritin translation by removing IRP1 from the ferritin IREs; IRP1 is simultaneously interconverted to a cytoplasmic cis-aconitase (93) (94) with an iron sulfur cluster, and IRP2 is degraded by iron influx (85,95). Removal of IRPs from the ferritin IREs restores recruitment of the 40S ribosome at the 5′ cap sites. Ferritin translation can take place after eIF4E promotes eIF4G binding to eIF3 and hence 40S ribosome recruitment (96) (Figure 3). The 40S ribosome then scans ferritin mRNAs before the eIF2-a dependent 60S ribosome subunit “joining step” occurs, after which protein synthesis begins at the optimal start codon (97). The ratio of IRP1 and IRP2 is tissue specific, but IRP2 is more abundant than IRP1 in pituitary cells (98).

Hormonal signaling via IRPs certainly modulates intracellular ferritin levels, and appears to be cell line specific. Interleukin-1 (IL-1) does not change steady-state levels of IRP1 and IRP2 but appears to enhance binding to IREs (99). As outlined in the next section, IL-1α and IL-1β also act through the downstream acute box domain in ferritin mRNA 5′UTR. Both Epidermal Growth Factor and Thyroid Releasing Hormone increased the phosphorylation of IRP1 and IRP2 and binding of IRPs to IREs, an event that was shown not always to be correlated with the direction of ferritin translation (98).

Oxidative stress modulates the IRE/IRP regulatory system as an additional control point of ferritin translation. For example, H_2O_2 as a model for oxidative stress was shown to rapidly activate IRP1 binding to ferritin IRE stem loops (100) (101). These data confirmed that rapid responses in the gene expression of ferritin (and TfR) to oxidative stress was mediated by IRPs (102). Toth et al., (1999) showed that hypoxia altered IRP1 binding to IREs and modulated cellular iron homeostasis in human hepatoma and erythroleukemia cells (103).

The absence of Iron Regulatory Protein-2 (IRP2), which controls iron homeostasis (Figure 1), was associated with a mis-regulated iron metabolism and ferritin translation and TfR mRNA stability in both the gut mucosa and the central nervous system (104). Iwai et al., (1998) previously showed that iron-mediated degradation of IRP2 requires the oxidation of key cysteines that reside within a 73-amino acid region (105) unique to IRP2 and not present in IRP1. However, a mutant IRP2 protein lacking this 73-amino acid region degraded at a rate similar to that of wild-type IRP2 (106). Dycke et al., (2007) showed this sequence is an active non-iron dependent cleavage domain in cultured breast cancer cells, and that it is unlikely that the iron-dependent degradation of IRP2 is mediated by haem binding to the intact 73 aa domain (107). These results are relevant to AD since oxidative stress is important during AD and the APP mRNA has an active IRE in its 5′UTR (Figure 4).

B. 2.2. Translation of Ferritin RNA by an Interleukin-1 responsive Acute Box Domain—To generate a molecular model for iron sequestration (as observed during anemia of chronic disease), we employed HepG2 cells grown in tissue culture (Figure 2). The anemia of the acute phase response was first observed during inflammation and disease progression by Cartwright in 1946 (108). Here, an enlargement of tissue ferritin pools was seen to be preceded by a marked increase of hepatic and spleen ferritin mRNA translation and subsequent ferritin gene transcription (109). Several groups then showed that enlarged ferritin pools could cause iron sequestration from the bloodstream into rat liver and spleen for tissue storage (84). Like tissue ferritin, serum ferritin may provide cytoprotection both to oxidized low density lipoprotein in vitro and to heme-aggravated damage (81).

For our model system of hepatic ferritin gene expression, interleukin-1 (IL-1) was found to significantly increase ferritin translation by signaling though a novel translation enhancer element, the acute box motif, downstream from the IREs at the 5′ cap site (110-112). This acute box motif is located immediately downstream from the IREs in the 5′UTRs of both the L- and H-ferritin transcripts, and was also found to be present in front of the start codon in the APP transcript (113). This observation was consistent with the pattern of both APP and ferritin expression in response to inflammation during both AD and the anemia associated with chronic disease.

We found that the acute box element in the 5′ leaders of the ferritin transcripts selectively interacted with the Poly C-binding Proteins (CP-1 and CP-2) (114). During erythropoeisis both CP-1 and CP-2 are known to be the mediators of globin mRNA stability (115) and Lipoxygenase mRNA translation (116). In hepatic cells, IL-1 increases ferritin translation at the 60S ribosome subunit “joining step” when protein synthesis begins at the optimal start codon (114,117) after the 40S ribosome has scanned the 5′UTR of ferritin mRNAs. We showed that IL-1 activated iron dependent translation of ferritin H-mRNA by removing the interaction between the CP-1, CP-2 and the H-mRNA acute box (114). Iron influx causes CP-1 to replace CP-2 from acute box residency (114).

The above observations have a biological correlate since serum ferritin levels are markedly increased in the bloodstream after the onset of an inflammation (118). Ferrtin in the serum was shown to be rich in the L-subunit and is known to be immunosuppressive (119). Clinically the average concentration of serum ferritin has been measured over a very broad dynamic range between 2 and 450 μg/liter. Serum ferritin levels are clinically used after trauma as an index of tissue damage and as a predictor of disease (i.e., hepatitis B virus infection was associated with increased serum ferritin levels as a disease biomarker (120). During progression of many chronic conditions the concentration of serum ferritin rises sharply, whereas this is to a level less than that observed during conditions of iron overload such as during hemochromatosis (1000 μg/ml). Inflammation reduces hepatic transferrin gene expression (121), reflecting the levels of transferrin bound iron versus free iron present in the bloodstream during inflammation (the anemia of chronic disease ((76,80)).

B.3. Post-Transcriptional Control of the Alzheimer's APP mRNA

B.3.1. Role an active APP IRE RNA stem-loop—Like ferritin, we characterized the presence of a fully functional iron responsive IRE stem loop in the APP 5′UTR. We originally reported that APP mRNA, but not APLP1 and APLP2 mRNAs, belongs to the family of transcripts that encodes a functional IRE RNA stem loop (8,15). Figure 4A shows the sequence alignment of APP 5′UTR sequences with the known IRE in H-ferritin mRNA. These alignments demonstrated an overall 67% sequence identity between APP 5′UTR sequences (+51 to +94) and the 44 nt IRE in H-ferritin mRNA (+12 to +59) (Figure 4A). Two clusters within this APP 5′UTR IRE-Type II domain showed >70% identity with the ferritin IRE sequences. First an 18 base sequence in APP mRNA (+51 to +66) was found to

be 72% similar to 5′ half of the H-mRNA IRE (+12 to +29). Second APP sequences (+82 to +94) (a 13 base cluster) were 76% identical to the loop domain of the H-ferritin IRE (+43 to +55). The IRE alignment shown in figure 4A demonstrated that the IRE-Type II sequence in the APP 5′UTR (+51 to +94) was sited immediately upstream of the IL-1 responsive acute box domain in the APP 5′UTR (+100 to -146). To ensure specificity, the 5′UTR sequences in the APLP-1 transcript only exhibited 25% percent homology with equivalent APP 5′UTR sequences.

This novel Iron-Responsive Element (Type II) in the 5′UTR of APP mRNA was fully functional as assessed by multiple separate transfection assays (8). RNA gel-shift experiments demonstrated that the mutant version of the APP 5′UTR cRNA probe no longer binds to IRP (Figure 4A) (8). Using RNA electrophoretic mobility shift assays (REMSA) we showed that IRP1 specifically binds to this APP IRE stem loop (8). The scientific consensus is that the canonical ferritin type of IRE preferentially binds IRP2 rather than IRP1 as the predominant pathway of IRE mediated control of iron homeostasis (122). Therefore it is of direct relevance to test which of IRP1 or IRP2 preferentially interacts with the APP 5′UTR to control iron dependent translation of APP expression in neural cells.

The fact that an IRP binds strongly to the APP 5′UTR suggests an integral involvement of APP holoprotein in iron metabolism. Comparison of the relative affinity measurement of IRP1/IRP2 as binding partners to the APP 5′UTR will provide useful information for screening novel lead compounds that limit APP translation, particularly if IRP1 and/or IRP2 expression can be functionally linked to APP expression.

Lahiri and his co-workers elegantly aligned the 5′UTR sequences of APP, APLP-1 and APLP-2 throughout the animal kingdom reporting, the presence of a “CAGA box” in the APP gene unique to amyloid plaque-forming species and absent in all APLP-1/2 genes with implications for AD (123). Here, the “CAGA” sequence was identified proximal to the “ATG” start codon and was unique to APP genes of amyloid plaque-forming genes surveyed. This CAGA box is immediately upstream of an interleukin-1-responsive element (the “acute box”, see next section). Interestingly, this proximal CAGA box is present in the 11 base apex of the CAGAGN stem-loop structure in both human and guinea pig APP mRNA IRE (see Figure 4A). We also note that the amyloid species specific CAGA box is indeed the same sequence responsible for binding to IRP1 in the core IRE sequence in the APP transcript (8,15). The convergence of these findings implies that IRP1 regulation of APP gene expression may yet be found to be associated with an intrinsic capacity of the encoded APP sequence to form brain amyloid and ultimately amyloid plaque *in vivo*.

The role of 5′-UTR can be considered in a broader context. We propose that APP 5′-UTR in concert with proximal promoter region (PPR) functions to both down-regulate and up-regulate APP gene expression (124). Notably, the proximal promoter (PPR) and the APP 5′UTR participate at both transcriptional and posttranscriptional levels. Based on our studies, multi-protein DNA- and RNA protein complexes in a gene's 5′-UTR and PPR may interact and cooperate as a unit that can regulate gene expression at both the transcriptional and post-transcriptional levels (82,124,125).

B.3.2. Translation of APP mRNA by an IL-1 Responsive Acute Box Domain—

As a Cu-Zn metalloprotein (25), we first reported that the APP gene is acutely regulated at the translational level by changes in cellular iron status in neuronal cells but also by IL-1 in astrocytes (113). Furthermore, IL-1-responsive sequences were characterized as active RNA regulatory elements in the 146 APP 5′UTR analogous to the IL-1 responsive acute-box RNA enhancer immediately in front of the start codons of the ferritin L- and H-chain mRNAs (113).

Since poly(C)-binding protein affects H-ferritin translation via its acute box domain, it is worth speculating that poly(C)-binding protein isoforms may also have a role in determining the expression of APP in response to IL-1 signaling. Mini-strokes associated with AD may generate an ischemic inflammation with the same genetic consequences (126). Zhu et al., (2002) used immunostaining to show that poly(C)-binding protein 1, but not poly(C)-binding protein 2, expression was increased in the ischemic boundary zone (penumbra) of the frontal cortex after 90 min of ischemia, and persisted for at least 72 h after reperfusion (127). These results demonstrated that poly(C)-binding protein 1 and poly(C)-binding protein 2 in cortical neurons are differentially affected by hypoxic/ischemic insults, suggesting that there are functional differences between poly(C)-binding protein isoforms. We are currently investigating the role of poly(C)-binding proteins relevant to the regulation of APP mRNA translational control.

B.3.3. Mechanisms for APP and Ferritin mRNA Translational Control—The 5′UTR specific translational control circuits that regulate ferritin L- and H-mRNAs in response to iron and IL-1 are summarized in the model shown in figure 3. The mRNA for FMR1 (Fragile X) presents as a typical eukaryotic mRNA in which RNA secondary structure suppresses protein synthesis (128). However, like the ferritin mRNAs, the 146 base APP 5′UTR is unique to the precursor transcript that encodes stable RNA secondary structure, the acute box, that regulates 40S ribosome scanning and facilitate the onset of APP synthesis (Figure 4A).

In sum, our data supports the hypothesis that IL-1 actively stimulates the translation of the APP transcript by a pathway that is similar to the translational regulation of the mRNAs encoding the L- and H-subunits of ferritin (112). We also reported that APP 5′UTR sequences encode functional sequences that are 75% similar to the iron-responsive element (IRE) in the mRNAs for the ferritin L- and H-subunits (Ferritin IREs) (Figure 3). There are common features of ferritin and APP translation by iron and Interleukin-1 (8,113). These two sets of mRNAs appear each to encode very different metalloproteins whose regulation is controlled by common RNA binding protein interactions but by radically different mechanisms.

B. 4. IREs in the Transcripts of Other Proteins of Iron Metabolism

A consistent finding is that IREs are involved in the post-transcriptional regulation of several genes that control intracellular iron homeostasis. The AD brain shows dysregulated binding of IRPs to IREs (129), which might have implications for the expression of APP and other iron-related proteins. Like ferritin, the other transcripts that encode active IREs in their 5′UTR include:- (i) the mRNA for erythroid aminoluvenyl synthase (eALAS). eALAS is the rate limiting enzyme that controls heme biosynthesis in the mitochondria of developing reticulocytes (130,131); (ii) transferrin (Tf) transports iron throughout the bloodstream to tissues, and translation of Tf is activated by binding of IRPs to the 5′UTR of the transferrin transcript (132); (iii) Thomson et al., (1999) reviewed the mechanism of action of 5′UTR specific IREs that control the translation of IREG-1 (ferroportin), which is responsible for iron efflux from the duodenum into the bloodstream and macrophage iron efflux; (iii) Like the transferrin receptor, the divalent metal ion transporter (DMT-1) is known to be regulated by an IRE in its 3′UTR as one of the alternatively spliced DMT-1 transcripts (86). DMT-1 is responsible for uptake of iron and other divalent cations from the gut into the bloodstream into (133,134).

B. 5. An IRE in the Parkinson's Disease ASYN Transcript

The biology of iron-regulatory proteins is highly relevant to that of movement disorders. IRP1 knockout mice, although viable, show an enhanced PD-like neurological phenotype in

a gene-dose manner. Here heterozygotes for the IRP1 gene knockout, that already have a double deletion in the IRP2 gene, showed an accelerated movement disorder with ataxia, bradykinesia and tremor (135,136) relative to counterparts with two allelic copies of the IRP1 gene.

ASYN mRNA translational control may be significantly de-regulated in Lewy Body Dementia brains (137,138). In this context, we reported sequences in the ASYN mRNA 5′untranslated region (5′UTR) to be homologous to the Iron-responsive Element (IRE) RNA stem-loops encoded in the 5′UTRs of transcripts for the L- and H-ferritin chains (iron-storage), erythroid amino-levulynyl synthase (eALAS) (heme-biosynthesis) (11), and the Alzheimer's APP (Cu/Zn-metalloprotein (8). Five 3′UTR IRE stem-loops control iron-dependent transferrin-receptor (TfR) mRNA stability and hence cellular iron transport (see the model for intracellular iron homeostasis in figure 2). The loop domain, CAGUG in the novel ASYN IRE is precisely juxtaposed to the splice junction of exons-1 and -2 (Fig. 4B) (RNA folding program of Zucker and colleagues (139)). The presence of this putative IRE in ASYN mRNA is of added significance since individual neurons in the Substantia nigral neurons of PD patients harbor twofold increased iron relative to aged matched controls (7). Therefore the ASYN IRE is a potential regulatory element through which iron influx may increase ASYN expression at the translational level. In neuronal cells this event would be predicted to contribute to disease pathology since ASYN over expression. [Trisomy of the chromosome 4 alpha synuclein locus has already been genetically linked to familial Parkinson's disease (140)]. It remains to be determined whether the 5′UTR specific IRE in ASYN mRNA is functional. Scherzer et al., (2008) demonstrated a GATA motif in intron-2 of the alpha synuclein gene (141). This study is consistent with possibility that the IRE in the ASYN 5′UTR may be fully functional and provides substantial further evidence for a role of alpha synuclein in iron metabolism.

B.6. APP mRNA Stability Coupled to Translation, Role of 5′UTR and 3′UTR sequences

Cytokines, which are physiologically relevant to AD, change the efficiency of APP mRNA translation and APP mRNA stability by signaling through 3′UTR RNA sequences (142) additional to the APP 5′UTR (113). By this route APP gene expression is increased at the post transcriptional level to generate more template for the deposition of more amyloid Aβ. Two cis-acting elements in the APP 3′UTR regulate the stability of the precursor transcript where TGFβ induces a 68 kDa protein to bind to an 81nt motif and thus stabilize the APP mRNA (143). Addition of serum growth factors overrides the action of another 3′UTR element, a 29-base sequence (2285-2313), that normally destabilizes APP mRNA in endothelial cells (and peripheral blood lymphocytes) (144).

In astrocytes, we found that APP gene expression was up-regulated by IL-1 at the translational level via 5′UTR sequences by a regulatory pattern similar to that for the universal iron storage protein, ferritin (112) (113). de Sauvage et al., (1991) reported that polyadenylation site selection in the APP mRNA 3′UTR was critical for its efficient translation in xenopus oocytes (145). The longer 3.3 Kb APP mRNA was translated 3-fold more efficiently than the shorter 3.042 Kb transcript. Mbella et al (2000) then observed that these 3′UTR sequences enhanced APP mRNA translation in mammalian (CHO) cells, and more closely mapped two guanosine residues that are crucial for this action (146). These authors used RNA gel-shift assays to determine that a translational repressor protein interacts with the shorter transcript (146). Thus in addition to the APP 5′UTR system, the presence of the 3′UTR specific 258 nucleotide poly(A) regulatory region (PAR) (nt +3042 to +3300) removes binding of this APP mRNA repressor and facilitates longer APP mRNA translation (146).

In sum, APP 3′ untranslated region sequences operate in conjunction with the APP 5′UTR to establish the post-transcriptional control of APP gene expression (and Aβ production) (146). Future studies will rigorously test how APP mRNA 5′UTR sequences and 3′UTR interact to effectively regulate the translation of the Aβ-precursor protein. We will continue to characterize how the anticholinesterase phenserine, as well as the trivalent iron chelator desferrioxamine, generate anti-amyloid efficacy *in vivo* perhaps by inhibition of APP mRNA translation via 3′UTR sequences acting in concert with APP 5′UTR sequences (147).

When mRNAs compete for ribosome binding, neither the cap structure nor the poly(A) tail alone are sufficient for optimal translation, but together they may synergize and direct ribosome entry to the 5′ end (148-150). However, the elegant studies of Chen et al., (1995) and others demonstrated that initiation of protein synthesis by the eukaryotic translational apparatus includes the formation of circular RNAs where the 3′ end interacts with the 5′ end of any given message (151). Using this model, an APP mRNA circularization step and internal ribosome entry may have to be invoked to explain the mechanism by which APP mRNA is translated. Consistent with an internal 40S ribosome entry mechanism to mediate APP translation, metal chelators inhibited precursor translation acting through an APP IRE stem loop that is positioned > 50 bases from the 5′ cap site of the APP transcript (15). The canonical IRE had previously been shown to no longer confer 5′ cap dependent translation when situated this distance from the 5′ cap site (92).

There is also notable RNA binding protein interaction between the coding region of the APP transcript and the fragile X mental retardation protein (FMRP), which is a cytoplasmic mRNA binding protein whose expression is absent in fragile X syndrome. Westmark and Malter (2007) showed that FMRP binds to the coding region of APP mRNA at a guanine-rich, G-quartet-like sequence (152). Stimulation of cortical synaptoneurosomes or primary neuronal cells with the metabotropic glutamate receptor agonist, DHPG, increased APP translation in wild-type but not fmr-1 knockout samples. This interaction may affect APP levels *in vivo*. Indeed, this mechanism may explain a recent human study by Sokol and colleagues who detected high levels of plasma APP in children with severely autistic behavior and aggression (153). Finally, a predicted IRE-like stem loop was found in the Aβ region of APP mRNA, and IRP1 and IRP2 may interact at this RNA sequence (154). However, mutations silent for familial AD disrupt this predicted IRE, thus down playing its pathogenic importance (155). We propose this coding sequence IRE-like stem loop present in Aβ may represent a binding site of an, as yet uncharacterized, disease associated RNA binding proteins other than IRP1 and IRP2.

B.7. Disease relevance of IRE dependent APP translational control

The brains of AD patients may exhibit disrupted iron distribution with a changed brain pattern of iron regulatory proteins (156). As discussed, ferritin is the universal iron storage protein, and the transferrin receptor (TfR) is the universal receptor responsible for transferrin-mediated iron transport. Ferritin and TfR are respectively regulated by modulated binding of IRPs to the 5′ cap site-and 3′UTR specific IRE RNA stem loops in each transcript (85). It has been demonstrated that 30% of AD brain samples displayed a stronger interaction between the IRP1 and IRP2 and the canonical IRE stem loop in the 5UTRs of the transcripts coding for H-ferritin and the 3′ untranslated region of the transferrin receptor mRNA (TfR-mRNA) (129). Since IRP binding to the IREs controls intracellular iron homeostasis, a change in this RNA binding affinity would be predicted to enhance iron transport into neuronal cells during AD, but decrease ferritin levels, and thus diminish the cellular iron storage capacity. In these circumstances, neuronal cells in the AD brain would harbor an enlarged cellular pool of dangerous unstored iron (129). This model of reduced ferritin synthesis may be accurate at earlier stages in AD progression, but the hypothesis has

to account for the fact that most amyloid plaques contain ferritin-rich cells. To account for this discrepancy, ferritin appears to be deposited into plaques at later stages in disease progression when iron homeostasis in neurons has been reset by the enhanced preceding increase in transferrin receptor activity. Certainly ferritin synthesis of microglial, rather than neuronal origin, may also account for most plaque associated ferritin in the AD brain (157).

Our report that the 5′ untranslated region of APP mRNA is related to the 5′UTRs of L- and H-ferritin mRNAs (113) provided a further direct link between iron metabolism and AD pathogenesis. IRP1/IRP2 appear to be the specific trans-activators of the APP 5′UTR translational control, and thereby central regulators of APP translation (8). The post-transcriptional regulatory domains in the APP transcript include the APP 5′ untranslated region comprising both IL-1 and iron dependent enhancers of APP translation. The presence of a functional IRE in the APP 5′UTR is a further indication for the essential requirements of metal metabolism for the regular hitherto unknown function of APP. This view is supported by the finding that the APP cytoplasmic tail can be interchanged with that of the transferrin receptor and maintain 50% endocytosis of transferrin bound iron (158).

C. CHELATION THERAPY FOR ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

C.1. Introduction (Overview of iron and copper chelators as therapeutic agents for AD treatment)

The presence of metals in the plaque of AD patients (Fig 1), and of increased iron in striatal neurons in the Substantia nigra (SN) of PD patients (7), together with the demonstration of iron dependent translation of APP mRNA (8), have each encouraged the development of metal attenuating agents and iron chelators as a major new therapeutic strategy for the treatment of these neurodegenerative diseases (159,160). In the case of AD, metal based therapeutics complements the use of anti Aβ passive immunity and the use of an amyloid vaccine (161) as part of the trend to combination therapeutic strategies to ameliorate the cognitive decline of AD patients. In keeping with a metal regulatory element (IRE-Type II) in the APP 5′untranslated region, we identified both iron and copper chelators to be a major class of APP 5′UTR-directed leads from our screen of a library of 1,200 FDA pre-approved drugs (162). Drug screens targeted to the APP 5′ untranslated region identified dimercaptopropanol (Hg and Pb chelators), and tetrathiolmobdylate (Cu chelator) as FDA pre-approved leads that limited APP holoprotein expression and (Aβ-peptide output) (163).

Iron and copper chelators appear to operate at two levels as therapeutic agents for the treatment of AD. A clinical trial that was run in Toronto (Canada) showed that desferrioxamine provided clear and effective therapeutic relief to Alzheimer's patients as registered by measurement of cognitive performance (75). Notably desferrioxamine, which is a highly specific iron chelator (Kd Fe^{3+} $=10^{-31}$ M), suppressed intracellular levels of APP holoprotein by inhibiting translation of APP mRNA from it's 5′ untranslated region 5′UTR) (164). An alternative mechanism for the anti-amyloid efficacy of chelators comes from the reported broad specificity of the copper-zinc-iron chelator, clioquinol, that dissolved extra cellular fibrillar Aβ and thereby diminished plaque burden (165).

Clioquinol has been tested in a Phase II clinical trial in Melbourne Australia, wherein the metal binding drug improved cognition in severe AD patients (166) but was withdrawn from trials because of impurities during preparation. More recently a replacement small molecule metal-protein attenuating compound, of the 8-hydroxyquinoline class PBT-2 has shown promise in later trials (13). PBT-2 was deemed more brain penetrable, and was designed to be safer and more efficacious than parent compound clioquinol.

C.2. Iron chelators (inhibitors of APP mRNA translation)

Although APP transcription is up-regulated by copper (167), Fe chelation with DFO consistently suppressed APP expression (and Aβ levels) without any change to APP mRNA levels in SH-SY5Y cells (8). A major mechanism for the observed anti-amyloid efficacy of Fe chelators is that these agents inhibit APP translation through the active iron responsive IRE RNA stem-loop in the APP 5′UTR (76). In support, EGCG in Chinese green tea exhibited potent iron-chelating activity comparable to that of the prototypic iron chelator desferrioxamine, and was found to dose dependently (1-10 μM) inhibit both the immature and full-length cellular holo-APP, as shown by two-dimensional gel electrophoresis, without altering APP mRNA levels. EGCG operated via the APP 5′UTR (9).

C.2.1. Intramuscular Injection of Desferrioxamine—Daily intramuscular injection of the intracellular Fe^{3+} chelator, desferrioxamine, was shown to decrease the cognitive decline in a large cohort of AD patients (75). Desferrioxamine (DFO) was at first thought to chelate aluminium, which was considered a risk factor for AD (168). However desferrioxamine is commonly used as an iron chelator in the treatment of Sickle Cell disease (169), and for treatment of patients suffering from poisoning during acute iron overload (170-172). Thus the mechanism of action of desferrioxamine in the treatment of AD is likely associated with iron chelation rather than aluminum chelation. Desferrioxamine appears to specifically suppresses APP mRNA translation (Figure 4) (164).

Desferrioxamine (Df) has a dissociation constant for binding to Fe^{3+} at 10^{-31}M which provides the very high specificity for the chelation of iron required for the treatment of patients with transfusion iron overload (170-172). As such DFO has been very closely monitored as clinical agent for 20 years (171). The main clinical drawback of desferrioxamine is that the chelator can only be administered by intramuscular injection (usually to Sickle Cell Disease patients in crisis). There has been an active program to introduce new orally active iron chelators (170-172).

C.2.2. Iron Chelators to Supercede desferioxamine (Deferiprone and Ferralex) —Deferiprone is the orally active iron chelator that has been most often used for the treatment of iron overload disorders (173). It will be of interest to test whether deferiprone provides efficacy for AD based on the experimental capacity of chelators to limit APP translation, as has been observed for both desferrioxamine (Fe^{3+} chelator) and dimercaptopropanol (Hg^{2+}, Cu^{2+} chelator)(174). There is controversy concerning the use of deferiprone as an agent to replace desferrioxamine since the chelator has caused hepatic fibrosis in cases of transfusion iron overload (172).

C.2.3. Iron Chelation and Tau Phosphorylation—Aside from the amyloid based model for AD, iron enhanced phosphorylation of Tau and the formation of the neurofibrillary tangles associated with the AD brain (160). In this report a novel trivalent cationic chelator, Feralex, effectively dissociated binding of aluminum and iron to hyperphosphorylated Tau (neurofibrillary tangle) pertinent to its use as a therapeutic agent for AD ((159)).

C.2.4. Future Directions for Iron Chelators—New oral iron chelators are rapidly being developed to combat disorders of iron overload such as sickle cell anemia, transfusional iron overload, including (a) the hexadentatephenolicaminocarboxylate HBED [n,N'bis(2-hydroxybenzyl)ethylenediamine-N,N-diacetic acid], (b) the tridentate desferrithiocin derivative 4′-OH-dadmDFT[4′dihydroxy-(S)-deszaddesmethyl-desferrithiocin, (S)-4,5-dihydro-2-(2,4-dihydroxylphenyl)-4-thiazolecarboxylic acid], (c) the tridentate triazole ICL670A[CGP72 670A; 4-[3,5-bis-(hydroxylphenyl)-1,2,4triazol-1-yl]-

benzoic acid], and (d), the bidentate hydroxypyridin-4-one deferoprone [L1,CP20: 1,2-dimethyl-3-hydroxypyridin-4-one] (170). The successful use of new oral iron chelators for blood-associated diseases will predict their therapeutic capacity for the treatment of AD. Their efficacy will be measured using the same both histochemical and nuclear magnetic resonance imaging techniques that originally identified that the brain cortex of Alzheimer's disease patients displayed an abnormal iron distribution of (58). New Fe chelators such as PBT2 (175) and Ferelex are under investigation (PBT2 in Phase II clinical trials for improvement in cognitive performance (159)). Some of their therapeutic impact may be mediated by mechanisms of translational repression of APP gene expression to subsequently generate anti-amyloid efficacy.

C.3. Antioxidants Retard Metal-dependent Amyloid Induced Oxidative Stress and Neurotoxicity

For a long time oxidative stress has been strongly associated with the neurotoxicity in Alzheimer's disease (176-178). It has been recently shown that over-expression of superoxide dismutase -1 protected neurons against Abeta-amyloid peptide toxicity (179). The antioxidant, Co-enzyme Q, has already been tested for efficacy in animal models receiving increased oxidative stress, and is currently being tested for efficacy to AD (180).

One important mechanism to link Aβ peptide induced neurotoxicity and oxidative damage came from the finding that Aβ itself, is a generator of metal-catalyzed oxidative stress (Aβ (1-42) > Aβ (1-40) (71,181). Electron spin trap measurements have shown that Aβ expressed in C-elegans does generate superoxides in solution (182). However, Fe, Cu and Zn bind strongly with Aβ peptide (42). Huang et al., (1999) demonstrated that Cu and Fe are reduced by Aβ peptides, and that this catalytic reaction transfers electrons to molecular oxygen thereby generating neurotoxic H_2O_2 and superoxide ions (44). Using in-vitro assays, the presence of iron or copper (100 nM) with Aβ (10 μM) was found to catalyze H_2O_2 production (71). Redox interactions between Aβ and Cu(II) and Fe(III) engender production of reduced metal ions, Cu(I) and Fe(II), and consequential generation of ROS- , H_2O_2 and OH^-. Like Cu(II), Zn(II) precipitates Aβ in-vitro (Aβ 1-42 > Aβ 1-40 > rat Aβ (1-40).

Amyloid induced neuronal loss in AD (44) can be explained by the binding of Aβ-peptides to copper and iron to catalyze the generation of toxic H_2O_2. Equation 1 shows the standard Fenton reaction by which iron or copper react with hydrogen peroxide and superoxides to generate the toxic and deadly hydroxyl radicals that mediate neuronal loss during AD (183). The presence of reduced copper (Cu^{1+}) and iron (Fe^{2+}) also implied that even more damaging hydroxyl radical would be formed by the Fenton and Haber-Weiss chemical reactions (39,184) (Equation 1).

The pathologically damaging production of hydrogen peroxide generated by Aβ may be the result of a corruption of a beneficial superoxide dismutase activity associated with Aβ. In fact, Aβ dimer may form a copper - zinc protein (8 kDa) protein that has intrinsic superoxide dismutase activity that is capable of converting O_2^- into H_2O_2. (185). This hydrogen peroxide can then be converted into two water molecules by catalase to complete an antioxidant function. Pathogenic corruption of Aβ function may occur when oxygen is consumed as a substrate instead of O_2^- radicals, thereby changing a potentially cytoprotective peptide into a pro-oxidant which has deleterious activity.

Trace amounts of copper in water induce Aβ-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease (63). At a therapeutic level, it is worth remembering that Zn^{2+} is redox-inert and appears to play an inhibitory role in H_2O_2-mediated Aβ toxicity by copper. After co-incubation of zinc with Cu(II), Zn(II) was found to rescue primary cortical and human embryonic kidney 293 cells that were exposed to Aβ 1-42, correlating

with the effect of Zn(II) in suppressing Cu(II)-dependent H_2O_2 formation from Aβ 1-42 (186). Other antioxidants will also prove beneficial to counteract the toxicity of the redox interaction between Fe and Cu and Aβ peptide. For example, coenzyme coenzyme Q10 is a well known nutraceutical that may serve this important antioxidant purpose for AD therapeutics (187).

C.4. Chelators (Cu Zn) that dissolve amyloid plaques and reduce oxidative burden

It is clear that the strategy of using chelators to eliminate both Aβ fibrilization and Aβ-dependent metal oxidative stress neurotoxicity could well provide a major new therapeutic impact on AD progression. Certainly the copper chelator, clioquinol, has been successfully used as an inhibitor of copper-dependent aggregation of Aβ peptide. Also clioquinol suppressed metal catalyzed H_2O_2 production by copper interacting with Aβ peptide (181). Thus clioquinol both chelated metals and dissolved amyloid plaques, and continuous diet with the chelator to transgenic mice over-expressing APP have improved amyloid burden, and slowed the rate of cognitive decline (165). Clioquinol underwent phase II clinical trails for its therapeutic impact to Alzheimer's Disease patients (166). As discussed, PBT-II, as developed by Prana, Ltd., is the current top replacement for clioquinol as an active agent that may dissolve amyloid plaques (188).

There remains considerable controversy as to whether copper actually accelerates the onset of AD. Two studies demonstrated that copper appeared to lower the amyloid burden in the AD brain of APP transgenic mice. In the first study Phinney et al., 2003 demonstrated an *in vivo* reduction of amyloid-Aβ by a mutant copper transporter (189). They used a well-known spontaneous mutation of a special strain of mutant toxic-milk mice that accumulate too much copper. Eventually those animals get a liver disease which is a facsimile of a human disorder called Wilson's disease. In direct contrast to the results of Sparks and Scheur (2003) (63), this study demonstrated that an excess of copper in the brain actually reduced of amyloid burden. Superoxide dismutase-1 activity was found to be stabilized by excess dietary copper and reduced amyloid Aβ production in APP23 transgenic mice (190). These data suggested that copper may not be is a causative risk factor in AD since excess of dietary copper can be beneficial. On the other hand copper deficiency associated with the Menkes disease protein (copper pump) reduced APP mRNA levels and APP proximal promoter activity in fibroblasts (10). This latter finding was consistent with the model that copper may enhance APP production, be amyloidogenic and act as a promoter of Alzheimer's Disease. To accommodate these controversies at the therapeutic level it may be possible to screen for drugs that alter copper transport and distribution in patients. These experimental lines will help us understand the interface between copper biology and APP biology. Interestingly, copper and iron metabolism are closely linked (133).

It is intriguing to speculate that iron-specific chelators (porphyrins) may be considered a viable therapeutic option in aging and AD (191). These findings are in keeping with an a earlier therapeutic strategy for AD that was designed to inhibit Aβ fibrilization, and thus block amyloid plaque formation in the pathogenesis of AD (192). To monitor drug efficacy in reducing the extent of Aβ fibrilization, scientists at Smith Kline and Beecham developed a monoclonal antibody-based assay (DELFIA) that detected fibril formation over the presence of monomer (dimer Aβ). Using this assay, hemin and related porphyrins were found to effectively inhibit Aβ -amyloid aggregation (191). The rank order of fibril inhibition in-vitro was hemin > hematin > zinc > protoporphyrin IX in the absence of cytotoxic action of the compounds (191).

Porphyrins and phthalocyannines also inhibited protease resistant prion protein formation relevant to Bovine Spongiform Encephalopathy (BSE) and the appearance of Creutzfeldt-Jakob disease in humans (193,194). Scrapie is transmitted by intra-peritoneal injection of

protease sensitive prion (PrP -sens), and porphyrin-specific suppression of PrP conversion to a pathogenic protease resistant PRP (PrP-res) occurs in the periphery before crossing the blood brain barrier to cause encephalopathy (193). By contrast, during the progression of AD amyloid plaques form in the brain cortex whereas heme and proteoporphyrin do not cross the blood-brain barrier, and thus it is uncertain that the use of porphyrines will have therapeutic impact in this case. Also shorter protofibrils of prefibrillar, diffusible assemblies of Aβ peptide are deleterious agents during the progression of Alzheimer's disease. These facts might argue against identifying drugs, like heme, targeted to inhibit the formation of larger fibrils as a treatment for AD (195).

Conclusions

In summary, brain levels of Zn, Cu, and Fe, and their binding proteins are dysregulated in the brain cortex of AD patients and dopaminergic neurons of PD patients (67,157). Levels of Zn and Fe are increased to concentrations as high as 1 mM in cerebral amyloid plaques (Cu is at 400 μM) (6). Normally Zn, Cu, and Fe are concentrated in the temporal cortex at relatively lower levels [eg: 7.94 μM Fe / mg protein, in the temporal cortex and 20 μM Fe / mg in the motor cortex (67)]. The distributions of both the brain iron storage proteins, transferrin and ferritin, are also altered in the white matter versus the gray matter in the brains of AD patients compared to age matched controls (69,196). Key to this review we discuss the implications of our discovery of fully functional IRE RNA stem loops in the 5′UTRs of the AD specific APP mRNA and the PD specific ASYN mRNA, which each encode two key proteins and causative agents for these neurodegenerative diseases. Iron also causes neural damage, behavioral changes and microgliosis in mouse behavioral models of each disease and we have not ruled out a role for the APP and ASYN IREs to contribute to the pathology of AD and PD. Thus, an alternative mechanism for the action of Fe chelators (such as DFO and EGGC) in their therapeutic efficacy against neurodegenerative diseases such as AD and PD, is their role as translation inhibitors of APP (or indeed alpha synuclein) transcripts. By contrast metal attenuating agents such as clioquinol and PBT-2 may well preferentially operate at the protein levels to dissolve amyloid plaques.

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Abbreviations

APP	Amyloid Precursor Protein
P97	mellanotransferrin
PS	Presenillin
ADAM	A Disintegrin and Metalloprotease Domain-10
ADAM-17	A Disintegrin and Metalloprotease Domain
TACE-1	Tumor Necrosis Factor alpha Converting Enzyme
ORF	Open Reading Frame
ASYN	Alpha synuclein
IRE	Iron-responsive Element
IRP	Iron-regulatory Protein
Cu	Copper
Fe	Iron
Zn	Zinc

Equation 1:

$$Fe^{+2}\ (Cu^{2+}) + H_2O_2 \longrightarrow Fe^{+3}\ (Cu^{1+}) + OH^- + OH^{\cdot}$$

Equation 1
Metal-catalyzed neuronal oxidative damage via hydroxyl radicals.

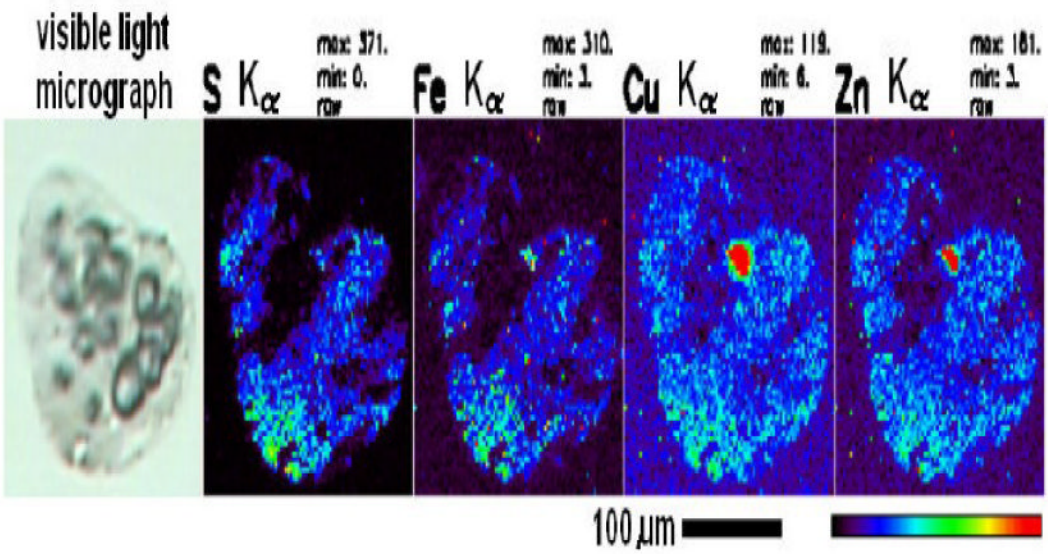


Figure 1. Elemental profiles (S, Fe, Cu, and Zn) in a typical Alzheimer's Aβ amyloid plaque

The cryo-sectioned (10 μm thichness) AD brain tissues were stained with 0.1% Thioflavin-T for amyloid plaques. The amyloid plaque-bearing human brain tissues were procured by LCM (Arcturus Pixcell IIE platform) and mounted on Si_3N_4 membrane grids (2.0 mm × 2.0 mm). Guided by the optical amyloid plaque images, the samples were excited with incident synchrotron X-ray of 10 keV for elemental Kα characteristic emission lines. Elemental profiles (S, Fe, Cu, and Zn) were obtained using Synchrotron scanning X-ray Fluorescence Microscopy (μ-XRF) at the Advanced Photon Source (APS) of the Argonne National Laboratory (ANL).

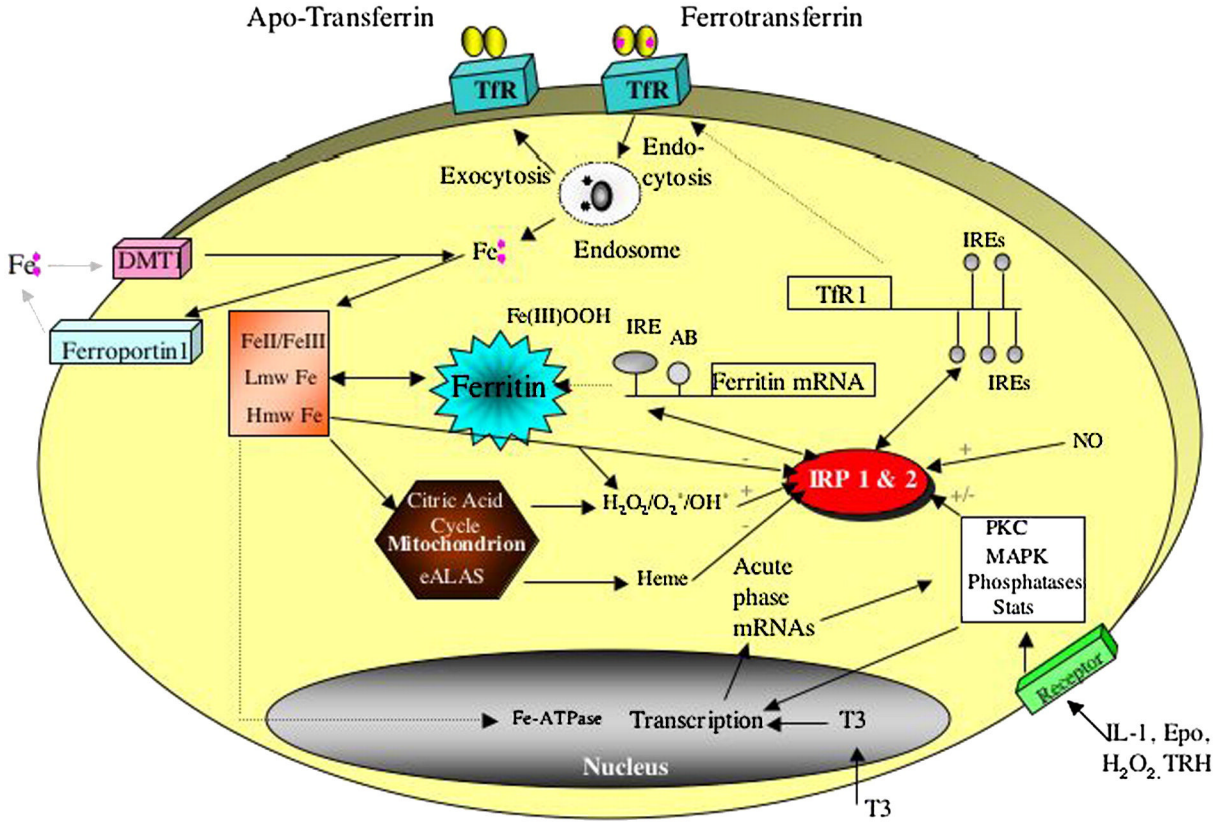


Figure 2. Intracellular Iron Homeostasis

Iron transit across the cell surface membrane is mediated by (i) ferrotransferrin internalization by the transferrin receptor (TfR), (ii) DMT-1, (iii) ferroportin mediated iron efflux from the duodenum into the blood. Ferritin mRNA translation is regulated by the modulated interaction between the IRPs and the IREs in the 5′UTR of ferritin mRNA. MAP kinase signaling events influence ferritin translation and transferrin receptor activity and expression.

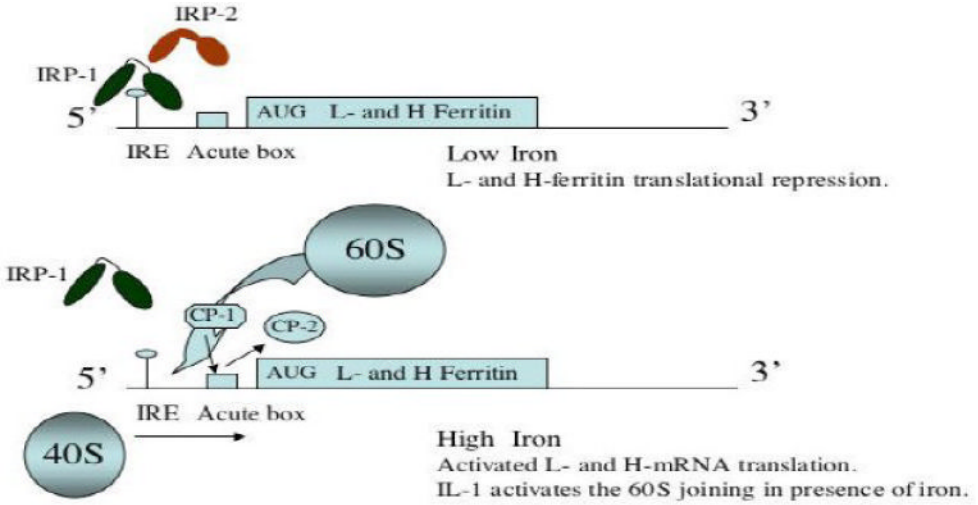
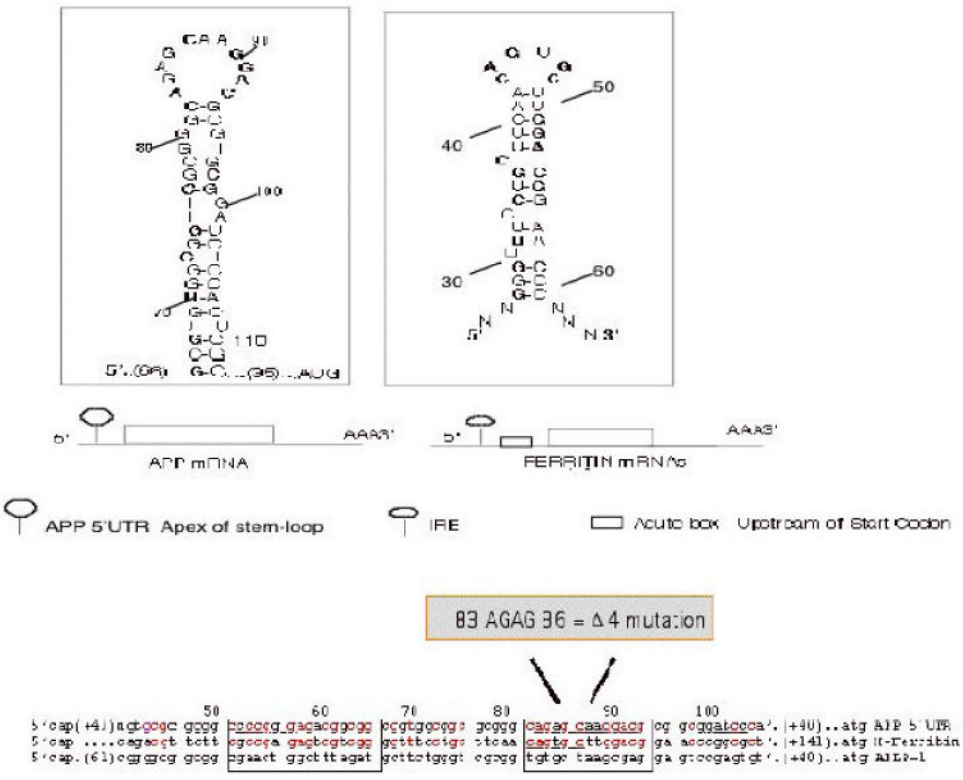


Figure 3. Model for Ferritin mRNA Translational Control

Iron releases IRP1/IRP2 from suppressing ferritin mRNA translation at the Iron responsive Element stem loops (IREs) specific to the Land H- mRNA 5′ cap sites. In the diagram, IRP1 and IRP2 are depicted as two domains separated by a hinge region (line). Our preliminary data suggests that the RNA binding protein (Poly C-binding proteins, CP-1 and CP-2) interact with the ferritin mRNA acute box (AB) domain (box) downstream from the IRE (114).

A



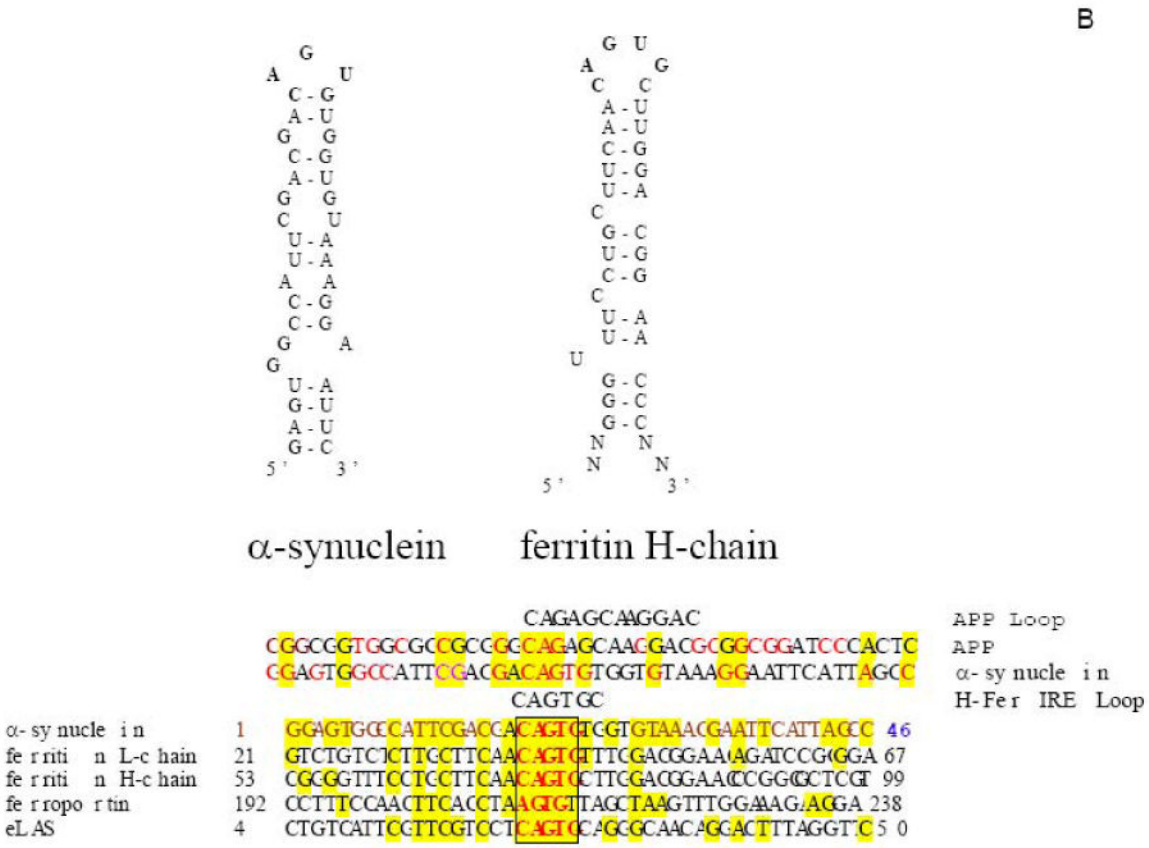


Figure 4.

Both APP and alpha synuclein (ASYN) 5′UTRs were predicted to fold into the stable RNA stem loops similar to the 5′UTR-specific IRE in H-ferritin transcript (“APP 5′UTR” DG = -54 kCal/mol) (Zuker et al., 2003 (139)). **Panel A** The APP mRNA IRE (Type II) was identified after alignment with the ferritin Iron-responsive Element (shown in two clusters of > 70% sequence similarity)(8). The APP IRE is a currently active drug target for translational repression of APP in AD therapeutics (197). **Panel B:** The ASYN 5′UTR encodes a CAGUGU motif at the exon-1/exon-2 splice junction. This ASYN 5′UTR stem loop (DG =53 kcal/mol) aligns with classical IRE (5′CAGUGN3′ loop motif) that controls L-& H-ferritin translation & transferrin receptor (TfR) mRNA stability. Boxed alignment of the ASYN 5′CAGUGU3′ motif against the IREs of ferritin H- and L- chains (iron storage), ferroportin (iron transport), erythroid eALAS (heme synthesis) mRNAs in addition to an alignment with IRE sequences in APP mRNA.