Iron-responsive-like elements and neurodegenerative ferroptosis

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A set of common-acting iron-responsive 5′untranslated region (5′UTR) motifs can fold into RNA stem loops that appear significant to the biology of cognitive declines of Parkinson's disease dementia (PDD), Lewy body dementia (LDD), and Alzheimer's disease (AD). Neurodegenerative diseases exhibit perturbations of iron homeostasis in defined brain subregions over characteristic time intervals of progression. While misfolding of Aβ from the amyloid-precursor-protein (APP), alpha-synuclein, prion protein (PrP) each cause neuropathic protein inclusions in the brain subregions, iron-responsive-like element (IRE-like) RNA stem–loops reside in their transcripts. APP and αsyn have a role in iron transport while gene duplications elevate the expression of their products to cause rare familial cases of AD and PDD. Of note, IRE-like sequences are responsive to excesses of brain iron in a potential feedback loop to accelerate neuronal ferroptosis and cognitive declines as well as amyloidosis. This pathogenic feedback is consistent with the translational control of the iron storage protein ferritin. We discuss how the IRE-like RNA motifs in the 5′UTRs of APP, alpha-synuclein and PrP mRNAs represent uniquely folded drug targets for therapies to prevent perturbed iron homeostasis that accelerates AD, PD, PD dementia (PDD) and Lewy body dementia, thus preventing cognitive deficits. Inhibition of alpha-synuclein translation is an option to block manganese toxicity associated with early childhood cognitive problems and manganism while Pb toxicity is epigenetically associated with attention deficit and later-stage AD. Pathologies of heavy metal toxicity centered on an embargo of iron export may be treated with activators of APP and ferritin and inhibitors of alpha-synuclein translation.

Iron balance influences healthy cognition throughout the human lifespan

Levels of dietary heavy metal exposure, especially from iron, have long been linked to cognitive problems in neonates and children as much as in neurodegenerative diseases of the aging population (Carlson et al. 2009; Brunette et al. 2010; Chiou et al. 2020). Michael Georgieff and his colleagues reported that neuronal iron uptake by the divalent metal ion transporter DMT-1 (Slc11a2) was essential for normal neuronal development of the hippocampus while Slc11a2 expression was induced by spatial memory training. Deletion of Slc11a2 was shown to disrupt neuronal development in the hippocampus and to alter spatial memory behavior in rodent models while remedies are sought to prevent childhood a dietary anemia that can limit learning and cognitive well-being of children (Auerbach and Georgieff 2020; Geng et al. 2020). Supporting the consensus that anemia thwarts cognitive skills, genetic manipulation of the dietary iron uptake protein DMT-1 in the hippocampus reflected early stage forgetfulness reminiscent of Alzheimer's disease (AD) phenotypes, including changes to the AD specific amyloid precursor protein (APP) and APP binding proteins (Carlson et al. 2008).

We review the impact of iron in the posttranscriptional regulation of the genes products of neurodegenerative cognitive decline, namely, AD as well as the major alpha-synucleinopathies of Parkinson's disease (PD), PD with dementia (PDD), and Lewy body dementia (LBD). Maintenance of cognitive health and memory retention lasts over a lifetime (Smolen et al. 2019) and may involve breakdowns of iron homeostasis linked to the loss of normal function of APP, alpha-synuclein (αsyn) and Prion protein (PrP) when accelerating neurogenerative processes (Lumsden et al.

2018; Zhang et al. 2020a). Neurodegenerative dementia can be modeled not only via plaque and Lewy body pathologies, but also through the path leading to accelerated perturbations of iron homeostasis and ferroptosis as causes of neural death (Bartzokis et al. 2010, 2011; Zhang et al. 2020a).

A balance exits such that too little iron is associated with anemia in newborn babies and has frequently been linked to cognitive liabilities in young children (Geng et al. 2020). Too much iron underpins hemochromatosis, a pathology that was evaluated by James Connor and colleagues as a fundamental factor in neurodegeneration in amyotrophic lateral sclerosis patients as well as a means to explain oxidative and cognitive decline during aging (Nandar and Connor 2011; Nandar et al. 2014; Meadowcroft et al. 2018; Song et al. 2020). Systemic conditions of iron overload include genetic mutations in the genes that cause hemochromatosis (HFE, HFE2), hepcidin (HAMP) and transferrin receptor-2 (TFR2) (Piperno et al. 2020). Reduced hepcidin synthesis leads to increased intestinal iron absorption and an increase of release of Fe from macrophages. This results in tissue iron overload (Piperno et al. 2020). Excess brain Fe and changed energy production may accelerate AD and also PD as well as be a driver of oxidative stress and ferroptosis in vascular dementias resulting from mini strokes and microhemorrhages (Maynard et al. 2002; Barnham and Bush 2008; Bush and Tanzi 2008; Ayton et al. 2015a; Corriveau et al. 2016; Jiang et al. 2017; Stockwell et al. 2017; Newman et al. 2019; Piperno et al. 2020; Zhang et al. 2020a)

Ferroptosis is actually an iron regulated necrosis, which differs from apoptosis by exhibiting shrunken/condensed mitochondria

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caused by excess intracellular REDOX active iron and the absence of the antioxidant glutathione (Liddell 2015; Abdalkader et al. 2018). Associated with this ferroptosis, neurons and pancreatic islet cells exhibit increases in REDOX-active lipids in diseases such as in diabetes, AD, PD (Abdalkader et al. 2018; Bruni et al. 2018; Zhang et al. 2020a). Ferrostatin-1 is an antioxidant that traps peroxyl radicals, and thus serves as a potent inhibitor of ferroptosis while rescuing nerve cells as a neuroprotectant against Huntington's disease and preventing neurotoxicity from excess glutamate in cell models (Skouta et al. 2014). Brain iron loading was reported to impair DNA methylation and alter GABAergic function in mice, again reflecting the impact of iron load as an effector of ferroptosis to undermine behavioral with cognitive outcomes (Ye et al. 2019). Small molecules have been suggested as useful agents to rescue afflicted iron uptake deficits in defined patient sets (Grillo et al. 2017).

The classic paradigm of cellular iron sensing is the canonical interaction between iron regulatory Proteins (IRP1 and IRP2) and iron responsive element (IRE) RNA stem loops (IRP/IRE interaction) that controls iron homeostasis (as shown in Figs. 1, 2; Thomson et al. 1999; Goforth et al. 2010). IRP1 and IRP2 binding to IREs is under tight control responding to changes in intracellular iron levels in a coordinate manner by differentially regulating ferritin mRNA translation rates and transferrin receptor (TfR) mRNA stability (Anderson et al. 2012). Iron sensing is governed by an IRE/IRP interaction that increase TfR mRNA mRNA stability in

the absence of iron to stimulate the cell's requirement for Fe-import. In a negative feedback loop, iron influx causes release of IRPs from the 5 IRE stem loops in the 3′UTR of TfR mRNA to enable ribonuclease digestion of TfR mRNAs to reduce excessive Fe. import (Thomson et al. 1999). There are many activators of iron homeostasis, including thyroid hormone linked to energy metabolism, that operates via IRP1 and IRP2 interactions with 5′UTR specific IRE stem loops in ferritin L and H as well as and Hif-2-alpha mRNAs (Figs. 1, 2; Leedman et al. 1996; Rogers 1996; Goforth et al. 2010; Sanchez et al. 2011; Anderson et al. 2012, 2013; Ghosh et al. 2013, 2018; Anderson and Leibold 2014). Ferritin expresses a cytoprotective ferroxidase activity in its H-chain, accounting for an added Fe storage capacity in mice that prevents MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxicity (Kaur et al. 2003). In fact, ferritin replenishment prevents ferroptosis as a target for protection against cardiomyopathy (Fang et al. 2019, 2020; Hernandez-Gallardo and Missirlis 2020).

The endocrinal peptide hepcidin can be regulated to control iron overload by modulating FPN sequestration and export of iron from tissues into the blood stream (Yin et al. 2018). On the other hand, loss of IRP1 is linked to polycythemia as well as having a role in the pulmonary and cardiovascular systems, essential for erythropoiesis by regulating the IRE in the 5′UTR Hif-2-alpha mRNA (Ghosh et al. 2015). IRP2(−/−) mice develop microcytic anemia, erythropoietic protoporphyria and a progressive neurological disorder while both IRP2 and IRP1 have a key role for securing
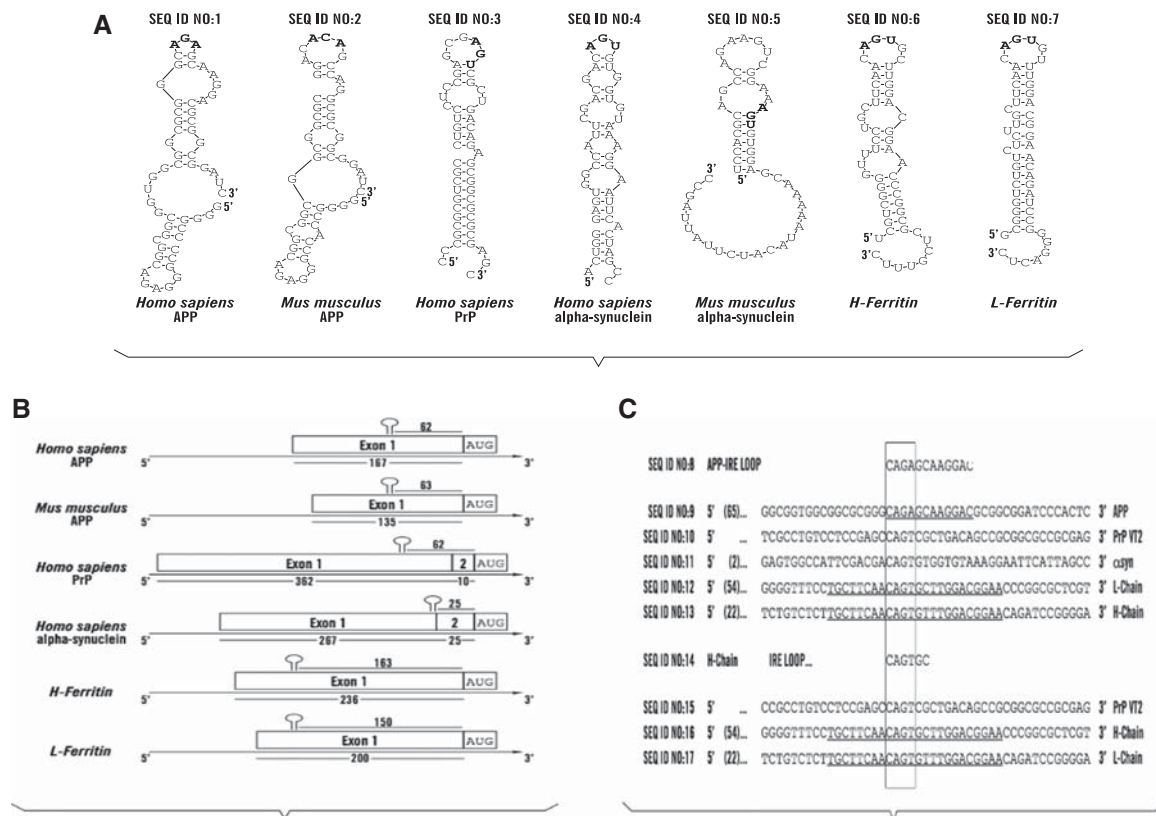

Figure 1. RNA stem loops common to the 5′UTRs in the mRNAs for L- and H-ferritin and the key neurodegenerative transcripts for the amyloid precursor protein, alpha synuclein and Prion Protein. (A) IRE-like AGU/AGA tri-loops and potential iron-regulatory protein binding motifs in key neurodegenerative disease transcripts for human and mouse APP and *SNCA* mRNAs and in the human PrP and ferritin- L and H transcripts; further links between iron and AD and PD. (B) Organization of the positions of the IRE-like RNA stem loops in the 5′untranslated regions of human and mouse APP mRNAs and in the human PrP, *SNCA* and ferritin- L and H transcripts. (C) Alignment of the central AGU and AGA motifs in the 5′UTRs of human APP, αsyn and PrP transcripts relative to those for H- and H-ferritin.

mitochondrial iron sufficiency (LaVaute et al. 2001; Galy et al. 2010; Zumbrennen-Bullough et al. 2014). Of note, the regulatory action of the downstream IL-1 responsive "acute box" motif extends the contribution of this classic paradigm of RNA mediated control of iron homeostasis by ferritin causing iron sequestration to also include the poly C binding proteins (PCBPs) (Thomson et al. 2005). Iron sequestration is a feature of the anemia of chronic disease. The cytosolic 5′UTR specific RNA binding proteins IRP1 as well as PCBPs not only control ferritin translation in the cytosol, these RNA binding proteins can enter the nucleus and regulate transcriptional events in adaptive iron homeostasis (Makayev and Liebhaber 2002; Thomson et al. 2005; Hernandez-Gallardo and Missirlis 2020).

We will discuss how environmental Mn and Pb accumulation in the body can be toxic to neurons potentially via ferroptosis (Kim et al. 2013). See the section Iron regulatory paths of ferritin, APP translation to mitigate metal neurotoxicity by manganese and lead exposures. Mn/Pb exposure to cells induces IRP1 dependent repression of translation of APP and the ferritin-H chain mRNAs (Fig. 4). Loss of APP translation limits the cell's capacity to form Fe-export complexes comprising of APP(s) and ferroportin (FPN) to increase intracellular toxic REDOX active iron (Venkataramani et al. 2018). Mn and Pb also inhibited IRP1 dependent translation of the heavy subunit of iron storage in multimer ferritin (Rogers et al. 2016; Khan et al. 2017).

Common role of iron in the translational expression and function of neurodegenerative disease proteins

Iron related function for APP, PrP, and αsyn central to Alzheimer's disease, Creutzfeldt-Jakob disease (CJD), and Parkinson's disease

There has been considerable support that the neurodegenerative proteins APP and alpha-synuclein (αsyn) as well as the prion protein (PrP) have a role to maintain cellular iron homeostasis in healthy individuals (Singh et al. 2009, 2013, 2014; Duce et al. 2010; Baksi et al. 2016; Baksi and Singh 2017; Bailey and Kosman 2019; Lahiri et al. 2019) (see model in Fig. 2). The last decade has introduced discoveries that APP and its secreted APP(s) ectodomain can facilitate FPN dependent cellular iron export to provide a novel central antioxidant defense to prevent iron overload in neuronal and endothelial cells (Duce et al. 2010; McCarthy et al. 2014; Rogers et al. 2016; Dlouhy et al. 2019; Tsatsanis et al. 2019, 2020). In these studies, appraisal of cellular iron status was reflected by complementary changes in cellular ferritin and transferrin receptor levels, thus supporting the function of APP695α to facilitate the export of iron from neurons. APP was originally shown to bind to FPN and then stimulate

FPN-dependent iron release from neurons (Duce et al. 2010, 2013). This work also showed, unlike normal mice, app−/− mice were more vulnerable to dietary iron exposure, which caused Fe^{2+} accumulation and oxidative stress in cortical neurons.

Mounting evidence suggests that, like APP, the key neurodegenerative proteins αsyn of PD and PrP of CJD also harbor a key physiological role in iron homeostasis. First, alpha-synuclein is a cytosolic ferrireductase (Davies et al. 2011), which facilitates the uptake of transferrin-bound iron in retinal cells with implications for visual manifestations of PD in some patients (Baksi et al. 2016). Excess αsyn fibrilization impairs ferritinophagy in the outer retina in vivo and in retinal-pigment-epithelial (RPE) cells, action that results in the accumulation of iron-rich ferritin (Baksi and Singh 2017). Second, PrP was modleled to have a role in iron homeostasis since PrP knockout mice exhibited reduced iron uptake and transport (Singh et al. 2009, 2013, 2014). Supporting a common link between PrP, APP, and αsyn in iron metabolism, we identified a significant homology in the 5′UTRs of each of their transcripts (see Fig. 1; Singh et al. 2009). A molecular crosstalk
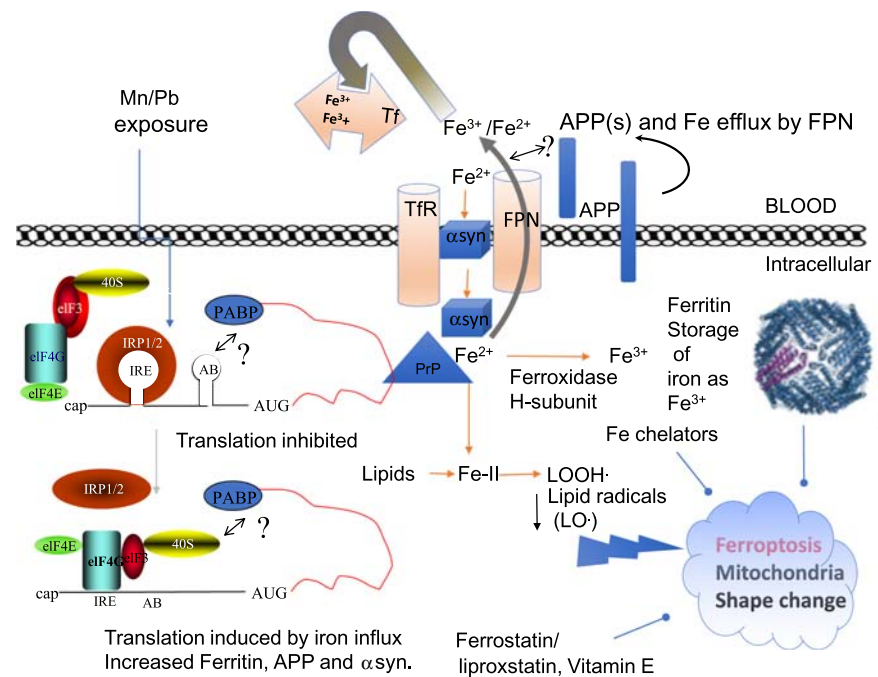

Figure 2. A Model that merges the canonical IRE/IRP dependent pathways of translation of ferritin and APP to generate balanced iron homeostasis with a depiction of the reported roles of APP, αsyn, and PrP in iron transport (Singh et al. 2014; Baksi et al. 2016; Venkataramani et al. 2018; Tsatsanis et al. 2020). IRP1 and IRP2 are translation repressors in the absence of cellular iron and under conditions of iron chelation (Rogers et al. 2008). Iron influx releases IRPs from the IRE RNA stem loops in the 5′UTRs of L- and H-ferritin, Hif-2α and APP mRNAs to enhance the translation of these neuroprotective proteins (Cho et al. 2010; Goforth et al. 2010; Anderson et al. 2013; Chung et al. 2014). IRP2 in degraded from cells in conditions of iron influx while its deficiency has been associated with neurodegeneration (Zumbrennen-Bullough et al. 2014). Neurotoxic exposures to Mn and Pb repress IRP dependent translation of APP and ferritin H-chain (Venkataramani et al. 2018), a pathological path that we propose can enhance Fe levels to cause ferroptosis. Amyloidosis and α-synucleinopathy advance plaque and Lewy body formations while ferroptosis and neuronal viability appear to accelerate neurodegeneration with cognitive declines. There are testable links between by Nrf2 dependent gene expression of heme oxygenase and H-ferritin and glutathione production as a means to limit ferroptosis (Abdalkader et al. 2018; Song et al. 2020). Mn and Pb inhibition of APP can be predicted to promote ferroptosis and Fe buildup by interfering with APP/FPN complexes for Fe efflux while also toxically blocking ferritin translation, as associated with the production of cytotoxic reactive oxygen species (Rogers et al. 2016; Venkataramani et al. 2018; Fang et al. 2020), iron overload impairs epigenetic DNA methylation patterns to disrupt GABA neurotransmission (Ye et al. 2019). Ultimately the generation of highly dangerous lipid radicals resulting from iron overload can be scavenged by ferrostatin, the standard diagnostic inhibitor of ferroptosis, as well as by ferritin (Balla et al. 1992), activators of hepcidin (Yin et al. 2018), and by the application of nanochelators (Kang et al. 2019).

does exist between misfolded proteins in AD and prion diseases (Morales et al. 2012). Finally, the importance of iron homeostasis extends to amyotrophic lateral sclerosis (ALS) since the familial ALS transcript "C9ORF72" encodes an IRE-like RNA stem loop in its 5′untranslated region that resembles the iron-responsive element-like RNA stem loop in the human cis-aconitase mRNA (Lu et al. 2016).

The overall role of αsyn in neurons has yet to be established although this protein is critical for synaptic dopamine neurotransmission (Oaks and Sidhu 2011) and represents1% of all proteins while15% of αsyn is associated with mitochondrial respiration (Ellis et al. 2005; Bendor et al. 2013). αsyn was found to promote dilation of the exocytotic fusion pore with implications for the synapse and neurotransmission (Logan et al. 2017; Pathak et al. 2017); this process may well yet depend on iron as scribed by the findings of N. Singh and her colleagues (Baksi et al. 2016; Baksi and Singh 2017). Of note, GATA transcription factors directly regulate the Parkinson's disease-linked SNCA gene while the heme metabolism genes including erythroid aminolevulinic acid synthase, ferrochelatase, and biliverdin reductase form a block of correlated gene expression in 113 samples of human blood, where αsyn naturally abounds (Scherzer et al. 2007). Supporting current *SNCA* 5′UTR directed therapeutic strategies for the treatment of PD, lowering the steady state levels of αsyn caused no defects to mitochondrial bioenergetics or loss of viability to neurons, typical of ferroptosis (Figs. 2, 3; Dixon et al. 2012, 2014; Pathak et al. 2017; Stockwell et al. 2017).

Like APP mRNA, The 5′UTR of the alpha-synuclein transcript was predicted to encode an iron responsive-like element (IRE-like) RNA stem loop (Fig. 1; Rogers et al. 2002a,b, 2011; Friedlich et al. 2007; Cho et al. 2010). This finding and subsequent confirmations were consistent with fact that αsyn itself facilitates the uptake of transferrin-bound iron in retinal cells (Baksi et al. 2016; Baksi and Singh 2017). In models of PD, alpha-synuclein associated iron buildup has been linked to ferroptosis and morphological changes to mitochondria as an early preapoptotic event in the neurodegeneration of dopaminergic neurons (Fig. 2; Zhang et al. 2020a). In addition, induction of αsyn infectivity and spread was reported to result from excess Mn exposures that increases cellular Fe driven oxidative stresses and can induce Parkinsonism phenotypes (Rogers et al. 2016; Harischandra et al. 2018, 2019a; Venkataramani et al. 2018; Zhang et al. 2020b). Indeed we will discuss how translation activators of APP mRNA can be therapeutically used to prevent Mn toxic consequences to generate ferroptosis as much as αsyn translation inhibitors can be advanced to prevent Lewy body formation and thus PD/DLB pathology (Zhang et al. 2020b). See the section Therapies to limit αsyn expression. Specific iron-associated damage can be offset by the use of novel chelation treatment options for PD with small molecules or transferrin based iron chelation (Ayton et al. 2015b; Finkelstein et al. 2017).

APP cleavage by α-, β-, and γ-secretases, provides a hypothesis to describe how these proteases may contribute to AD pathogenesis and how rare missense mutations in APP and γ- secretase may cause familial AD (De Strooper and Karran 2016; Walsh and Selkoe 2020). However, the common role of iron homeostasis linked to the biology of APP αsyn and PrP may yet yield therapies that address pathologies related to perturbed iron homeostasis in the neurodegenerative diseases previously associated with this altered processing misfolding (Jin et al. 2018). Increases in the genetic copy numbers of APP and αsyn genes generate familial AD and PD, respectively (Huang et al. 2017, 2019) while genetic variations in the APP promoter correlate with AD risk (Lahiri et al. 2005b; Maloney et al. 2010), and APP gene duplications cause early-onset AD (Rovelet-Lecrux et al. 2006) These findings show that APP dose and levels of expression are indeed important for AD pathogenesis. The finding that Apo-E increases APP transcription 4–sixfold in human neurons by activation of cFos-containing AP-1 transcription factors suggests that it may be possible to influence APP transcription pharmacologically as an approach to AD prevention (Huang et al. 2017, 2019). It has already been possible to target APP mRNA translation in order to diminish APP and limit amyloid production as a therapeutic option for AD. Some of these latter studies include the use of re-purposed iron chelators and anticholinesterases, some of which were tested in clinicals of efficacy trials for AD (Crapper McLachlan et al. 1991; Shaw et al. 2001; Bush and Tanzi 2008; Winblad et al. 2010; Rogers et al. 2019)

Iron-regulatory proteins (IRP) control of translation of APP, alpha-synuclein (αsyn), and prion protein (PrP) transcripts in conditions of iron influx

The iron-regulatory proteins (IRP-1 and -2) are the RNA-binding proteins that iron-dependently interact with IRE RNA stem loops that control the 5′UTR driven translation of the light and heavy subunits of ferritin, a genetic activity to enhance the iron storage capacity of all cell types, inclusive of promoting viability of neurons undergoing oxidative stress (Cho et al. 2010; Venkataramani et al. 2018). IRP1 and IRP2 determine rates of cellular iron acquisition by controlling the mRNA stability of the transferrin receptor (TfR) via 3′UTR specific RNA protein interactions. (Rogers et al. 2008; Cho et al. 2010; Sanchez et al. 2011)

Collaborative work demonstrated that the 5′-UTR of APP mRNA, like ferritin, controls APP expression at the level of translation in response to both iron and interleukin-1 (Thomson et al. 1999; Rogers et al. 1999, 2002a). This applies to modulate β-amyloid production in astrocytes (Rogers et al. 1999) while APP itself facilitates the central iron exporter FPN to efflux excess iron from neurons and endothelial cell (Duce et al. 2010; Kosman 2019; Rogers et al. 2019; Tsatsanis et al. 2020). The fact that IRP1 controls rates of APP translation in neuronal cell lines and rodent models is consistent with the major physiological function of APP to facilitate FPN dependent export of iron from Fe burdened neurons (Cho et al. 2010; Duce et al. 2010; Ayton et al. 2015c; Venkataramani et al. 2018). Debomoy Lahiri and his colleagues collaboratively reported microRNA-346 and iron are positive regulators of APP translation via the IRE RNA stem loops in 5′UTR of the mRNA (Long et al. 2019).

Excess brain iron confers suboptimal lethality to neurons by common pathways that toxically accelerate IRE/IRP disruption of ferritin translation and intracellular iron homeostasis to cause ferroptosis while glutathione acts as opposing antioxidant (Faucheux et al. 2002; Lee et al. 2009; Zhou and Tan 2017). We discuss the use of APP and alpha-synuclein inhibitors of translation when targeted to iron responsive-like elements in each of their transcripts to prevent αsyn buildup and its fibrilization into Lewy bodies. However, αsyn and PrP modulators can be modeled also to provide medicinal agents to prevent ferroptosis in vulnerable neurons, i.e., from iron and heavy metal oxidative death (Zhang et al. 2020a). The model in Figure 2 merges the IRE/IRP dependent ferritin regulatory pathways of translation with iron transport roles of αsyn, APP, and PrP. These pathways combine to generate ferroptosis in the event of neurotoxic exposures of cells to excess Fe, Mn, and Pb (Figs. 2, 4). Certainly, ferroptosis undermines neuronal viability and is associated with neurodegenerative disease of cognitive decline as much as by amyloidogenesis in AD and Lewy body formations via α-synucleinopathy in PD (Zhang et al. 2020a). Related efficacies exist between the synthesis antioxidant glutathione by Nrf2 inducible gene products as much as by the uses of modulators of APP and αsyn translation to adjust cellular Fe transport. Each of these

paths can be tested to prevent ferroptosis early in the onset of neurodegeneration (Abdalkader et al. 2018; Song et al. 2020).

Increases of intracellular iron are associated with the dopaminergic neurons of the substantia nigra (SN) in the midbrain of PD patients (Jellinger et al. 1990; Oakley et al. 2007). This elevation of the SN iron pool has the potential to release IRP1/IRP2 from repressing IRE stem loop dependent translation of ferritin. The lack of IRP2 in knockout mice does accelerate ferritin expression in defined neurons (LaVaute et al. 2001). Elevated iron also can induce misexpression of the markers such as tyrosine hydroxylase and dopamine, which are hallmarks of the PD brain (Salvatore et al. 2005). Iron acting through IRE/IRP systems certainly induces αsyn production as well as that of APP in the PD brains (Rogers et al. 2011; Ayton et al. 2015c). The IRE-like AGU sequences are on the apex of the 5′UTR specific stem loop of αsyn mRNA, as is shown in Figures 1 and 3. This *SNCA* mRNA stem loop is uniquely folded to offer a new therapeutic RNA target to control αsyn translation (Rogers et al. 2011; Zhang et al. 2020b).

Small molecules targeted to the APP, αsyn, and PrP 5′UTRs to improve iron homeostasis

APP gene expression is regulated at the level of translation in astrocytes and some neuronal lines by the inflammatory cytokine IL-1 and by iron influx while transcriptional induction is clear in endothelial and some neuronal lines (Goldgaber et al. 1989; Rogers et al. 1999, 2002a; Lahiri et al. 2005a). This regulatory circuit contributes to known acute phase events in AD that compliment apo-lipoprotein-E and alpha-anti-chymotrypsin pathogenic chaperoning of amyloidogenic progression in an inflammatory cascade mounted by astrocytes and microglia (Nilsson et al. 2004; Potter and Chial 2019). APP mRNA translation is controlled by the same iron-dependent 5′UTR specific IRP/IRE RNA protein interactions that set rates of ferritin and FPN mRNA translation and the mRNA stabilization of DMT-1 and transferrin receptor (Qureshi et al. 2008; Rogers et al. 2008; Olivares et al. 2009; Cho et al. 2010; Duce et al. 2010; Venkataramani et al. 2018). To maintain homeostasis, iron influx into neurons releases binding of IRP1 from the IRE folded from 5′UTR sequences in APP mRNA, activity that relieves the precursor from translational repression while IL-1 coinduces alpha-secretase and APP(s) secretion to enhance Fe efflux (Tsatsanis et al. 2020).

Mechanistically, the fact that iron controls rates of APP translation is consistent with gene knockout studies by Sh-RNA in neural cell lines where a 20-fold reduction of intracellular IRP1 caused a fivefold increase of APP expression in neural cells (Cho et al. 2010). These findings are consistent with the model that neurodegeneration in AD is not only the result of protein fibrilization, however perturbations iron homeostasis run parallel to amyloidosis. In fact, the physiological role of APP in iron homeostasis centers on its contribution to FPN dependent export of excess iron from Fe burdened neurons, as yet to be fully characterized in terms of REDOX activity (Dlouhy et al. 2019). Both amyloid and Mn exposures certainly caused a reduction of intracellular APP (APPs) in cells that reflected a blockade of iron export and loss of viability (Venkataramani et al. 2018; Tsatsanis et al. 2020). Using transfection based studies, this embargo Fe export was shown to be lifted when translation of the human APP was uncoupled from its repression in the absence of IREs (Venkataramani et al. 2018).

The consequences of overexpression of the APP in the onset of AD, includes that of duplications of the APP gene (Sleegers et al. 2006) and transcriptional activations (Geller and Potter 1999; Theuns et al. 2006; Potter et al. 2016). These events cause familial AD via amyloidosis and congophilic amyloid angiopathy (CAA) in families, the so called pathology in "Dup-APP" forms of familial AD (Rovelet-Lecrux et al. 2006; Buss et al. 2016; Rogers et al.

2019). Chromosome 21 nondisjunction with its APP gene has been elegantly modeled in cases of spontaneous AD (Geller and Potter 1999). By analogy, gene duplication and triplication of alpha-synuclein also causes a rare familial PD with later onset dementia (PDD) (Singleton and Gwinn-Hardy 2004; Nishioka et al. 2006). Alpha-synuclein fibrilization is a feature of sporadic PD as well as Lewy body dementia (LBD), the second most common cognitive liability after AD and vascular dementia (Cantuti-Castelvetri et al. 2005).

We described the practicable means by which inhibition of APP expression at the level of translation of its mRNA can be used as an anti-amyloid strategy to treat Down syndrome with amyloidosis as well as during the pathology in "Dup-APP" (Buss et al. 2016; Rogers et al. 2019). Simple abundance of the APP and αsyn genes, and therefore their transcripts and protein levels, can cause neurodegeneration. Therefore, novel agents under investigation include 5′untranslated region inhibitors of both APP and alpha-synuclein translation. In the first case these provide anti amyloid efficacy for older AD patients (Bandyopadhyay et al. 2013) and, second, prevent α-synucleinopathy in PD and LBD patients (Ross et al. 2010b, Kuo et al. 2019). The existence and uses of highly selective 5′untranslated region inhibitors and activators that had been high-throughput screened (HTS) to modulate APP, αsyn, and PrP translation are summarized as follows:

(a) Since APP mRNA translation is up-regulated by iron and Interleukin-1 via 5′UTR sequences (Rogers et al. 1999, 2002a; Long et al. 2019), it is highly feasible that APP 5′UTR directed translation blockers provide anti-amyloid efficacy for AD patients (Bandyopadhyay et al. 2013). Indeed, the anti-cholinesterase phenserine was shown to target APP 5′UTR sequences (Shaw et al. 2001) and then underwent clinical trials for AD efficacy (Greig et al. 2005; Yu et al. 2013). Its enantiomer posiphen is currently undergoing clinical trials as a therapeutic agent to prevent AD (Teich et al. 2018). The compound JTR-009 is a low molecular weight benzimidazole modeled to intercalate into the IRE-type-II RNA stem loop in the 5′UTR of APP mRNA, an action that irreversibly replaces IRP1 to chemically block APP mRNA translation (Rogers et al. 2002a; Bandyopadhyay et al. 2013; Teich et al. 2018). Table 1 summarizes data from these papers showing JTR-009 has a planar aromatic structure capable of reducing Aβ levels in neural cell lines (*IC-50 ∼ 50 nM) and may also generate a capacity to limit amyloid plaques in mouse models of AD and in aging AD patients similar to the clinically tested anti-amyloid associated APP mRNA translation blocker posiphen (Teich et al. 2018).

(b) Manganese and lead exposure to children and working adults increases toxicity to neurons and the risk of cognitive problems in defined brain subregions during development and after aging, for example, Lowered IQ (Vollet et al. 2016) (iron regulatory paths of ferritin, APP translation to mitigate metal neurotoxicity by manganese and lead exposures section). To thwart the toxicity of these metals in the absence of long-term accumulation of amyloid, both APP and ferritin 5′UTR activators can be predicted to be trophic to such metal exposed neurons. The identity of a high-throughput screen to identify such APP 5′UTR translation activators is outlined in PUBCHEM-AID-1276. In addition, the known M1 selective muscarinic agonist AF102B is an existing FDA approved agent with the property to induce APP 5′UTR driven translation (Rogers et al. 2019). Induced levels of APP(s) may drive increased formation and distribution of FPN into intracellular and membrane-associated complexes with APP (Tsatsanis et al. 2019). A FPN-targeting sequence, (K/R)EWEE, present in APP and APLP2, but not APLP1, may indeed help modulate FPN-dependent iron efflux in the presence of an intrinsic or

alternate active multicopper ferroxidase such as in haephestin and/or ceruloplasmin (Dlouhy et al. 2019). These molecular events can be modeled to increase Fe efflux sufficient to relieve cells of iron surfeit and prevent Fenton conversion of ROS into lethal hydroxyl radicals (Figs. 2, 4; Rogers et al. 2005; Fisher 2007; Fisher et al. 2016; Rogers et al. 2019; Mou et al. 2020) (iron regulatory paths of ferritin, APP translation to mitigate metal neurotoxicity by manganese and lead exposures section).

(c) The prion specific PrP 5′UTR translation blocker - BL-1- was described in the high-throughput screen (HTS) described in PubChem AID: 488862. This benzimidazole was selected as an inhibitor of intracellular expression of prion protein after HTS conducted in cultured SHSY5Y neurons. In fact, the use of BL-1 to prevent prion accumulation can be used for treating amyloid mediated damage in AD since PrP is known to be an Aβ receptor while prion antibodies are anti-amyloid in their profile of efficacy in rodent models (Lauren et al. 2009; Barry et al. 2011). Additional to this anti-prion therapeutic property of BL-1, the capacity of this agent to reduce PrP translation may also remediate perturbed iron uptake into the brain. PrP has a role in iron uptake (Fig. 2; Singh et al. 2009); Inhibitors of PrP translation, such as demonstrated by BL-1, can activate translation of H-ferritin via its canonical IRE while increases of ferritin. This action is to be tested to rescue cells from iron overload, ferroptosis, and thereby improve neuronal viability from oxidative assault.

(d) Several agents have been screened via 5′UTR dependent transfection-based assays to limit *SNCA* mRNA translation to treat PD, PDD, and LBD. The clinical trial drug posiphen was reported to inhibit translation of both brain and intestinal αsyn (Kuo et al. 2019). Similar to pharmacological increases translation of APP and ferritin as a therapy to prevent ferroptosis-associated damage to cultured neurons, *SNCA* 5′UTR blockers as enlisted in PUBCHEM-AID 1827, are to be used to limit alpha-synuclein expression to prevent ferroptosis in the onset of PD (Fig. 2; Zhang et al. 2020a). These SNCA 5′UTR blockers will have the added benefit of preventing Mn accelerated spread of αsyn fibrils (Fig. 4; Harischandra et al. 2018, 2019a).

Therapies to limit αsyn expression

The biology and expression of αsyn: strategies to limit α-synucleinopathy to prevent PD and Lewy body dementia

Alpha-synuclein (αsyn) and PD pathology: αsyn is the ~15 kDa protein implicated in pathogenesis of α-synucleinopathies, especially in the substantia nigra (SN) in the mid brain of patients with Parkinson's disease, the most prevalent movement disorder in humans (Ueda et al. 1993; Burre et al. 2018). Prodromal symptoms exist early in the onset of PD, including constipation and rapid eye movement sleep behavior disorder (RBD) (Stocchi and Torti 2017; Taguchi et al. 2020a,b). In the case of Dementia with Lewy bodies (DLB), Lewy body variant of Alzheimer's disease. and multiple system atrophy, αsyn undergoes a conformational change and oligomerization to cause a toxic gain of function and neurodegenerative deposition of aggregates commonly in Lewy bodies, also in dystrophic neurites and glial cytoplasmic inclusions (Lee et al. 2004) (LBD). Defined α-synucleinopathies are a central cause of dopaminergic cell death and PD pathology (Fanning et al. 2019; Kuo et al. 2019). Other familial PD associated genes include LRRK2 (Bakshi et al. 2019). Neurodegenerative REDOX active iron and αsyn fibrils are a coordinate feature of the SN in PD progression (Halliday et al. 2005; Jiang et al. 2017; Stockwell et al. 2017).

The intertwined links between iron and αsyn is supported by several lines of evidence: (i) the iron-binding pigment in neuromelanin is an MRI imaging target that accompanies neuronal depletion in the PD brain (Double et al. 2002; Halliday et al. 2005; Oakley et al. 2007; Chen et al. 2017). (ii) αsyn Itself, in health, has an integrative role in cellular iron transport (Baksi et al. 2016; Baksi and Singh 2017; Haining and Achat-Mendes 2017; Sulzer et al. 2018). As noted, increased expression of αsyn impairs ferritinophagy in the retinal pigment epithelium with Implications for retinal iron dys-homeostasis in PD and in dopaminergic neurotransmission (Baksi and Singh 2017). (iii) The presence of iron colocalized with αsyn in Lewy bodies, the increases of iron in the SN and the correlation between polymorphisms of the several genes implicated in iron metabolism and PD support a role for iron in the neurodegeneration (Oakley et al. 2007; Jiang et al. 2017). (iv) there is a uniquely folded version of an IRE-like RNA stem loop in the 5′UTR of *SNCA* mRNA (Figs. 1, 3; Mikkilineni et al. 2012). This *SNCA* 5′UTR potentially regulates brain αsyn by iron while the *SNCA* 5′UTR binds to IRP1 in a different manner than for IREs that control ferritin translation (Friedlich et al. 2007). The Ferritin L- and H-mRNAs encode a classic IRE RNA stem loop in their 5′UTRs that interacts with both IRP1 and IRP2 translational repressors in low iron conditions while the operational range of the *SNCA* 5′UTR in response to iron has yet to be recorded (Thomson et al. 2005; Wang et al. 2007a,b; Rogers et al. 2011).

The Braak stages of PD include later stage cognitive impairment that overlaps with AD and is present in PD with dementia (PDD) (Jellinger 2000). Alpha-synuclein and amyloid Aβ pathomechanisms converge via interaction of these two amyloidogenic proteins; coprecipitating into β-pleated oligomers and insoluble fibrils (Han et al. 1995; Jensen et al. 1997; Conway et al. 1998) although Aβ and αsyn rarely colocalize in brain amyloid deposits (Trojanowski and Lee 2001). Lewy body dementia (LBD) brains exhibit lowered *SNCA* mRNA but higher insoluble αsyn protein, suggesting misregulated translation additional to its clearance by chaperones (Cantuti-Castelvetri et al. 2005; Rogers et al. 2011). Alpha-synuclein and its mRNA were discordantly expressed relative to each other in LBD patients compared to controls (Cantuti-Castelvetri et al. 2005). In this case, the spread of αsyn is a feature of memory impairment and neuronal death (Adamowicz et al. 2017). Certainly alpha-synuclein mRNA is recruited to polysomes in response to exposures of HEK293 cells to ferric ammonium citrate, indicating translational control circuits at work (Febbraro et al. 2012). Published data on the reduction of αsyn levels shows that limiting levels of this protein does not adversely affect mitochondrial bioenergetics in rodent neurons (Logan et al. 2017; Pathak et al. 2017). In support, the phenotype of mice expressing the *SNCA* gene knockout are viable and this fact further justifies the therapeutic benefit to limit αsyn expression in the brain as much as to accelerate and target its clearance.

Clinicians currently have little choice other than to treat PD patients with standard disease symptomatic therapies such as rasagaline, a monoamine oxygenase inhibitor (Youdim and Lavie 1994) that prevents L-dopamine degradation (Youdim and Weinstock 2001; Trudler et al. 2014). For this reason, PD, PDD, and LBD are all α-synucleinopathies that provide an excellent justification for the use of *SNCA* 5′UTR directed translation blockers as a rationale for our strategy to limit αsyn translation in both PD and manganism patients. We will describe the therapeutic action of selective translation blockers of the *SNCA* transcript via its uniquely folded IRE in its 5′UTR (Rogers et al. 2011; Mikkilineni et al. 2012; Kuo et al. 2019; Zhang et al. 2020b). The model in Figure 3 shows that αsyn mRNA encodes 5′UTR specific stem loop with a CAGUGU loop motif in the apex of the IRE-like stem loop which

can be regulated by iron via IRP1 and IRP2. This is a relevant translational regulatory circuit at play during the etiology of PD. The *SNCA* mRNA encodes this RNA motif as a drug target to prevent PD and PDD either dependently or independently of IRP1 and IRP2 (Zhang et al. 2008).

Parkinson's disease and PD with dementia (PDD)

As stated in the Common role of iron in the translational expression and function of neurodegenerative disease proteins section, duplication or triplication of the *SNCA* gene can lead to familial PD where increased dose of this pathogenic protein is correlated to severity of symptoms (i.e., "triple repeat *SNCA* patients" show dementia) (Singleton et al. 2003; Chartier-Harlin et al. 2004; Singleton and Gwinn-Hardy 2004; Ahn et al. 2008; Ikeuchi et al. 2008). Polymorphic variability in the promoter of the *SNCA* gene was also linked to its increased expression as a risk factor for PD (Chiba-Falek et al. 2006). Since increased expression of αsyn can genetically cause and progress PD, high-throughput screens were conducted to advance efficacies with selective lead 5′UTR directed translation blockers of *SNCA* mRNA (Table 1). As described in the RNA based therapies used as α-syn translation inhibitors for PD, PDD, LBD section, the αsyn translation blocker probe "Syn-516" had been published from this screening campaign to provide proof-of concept for this strategy (Fig. 3; Ross et al. 2010b).

Dementia with Lewy bodies (DLB)

Consistent with strategies to pharmacologically control αsyn translation rates and prevent its overexpression neuropathologically, DLB is characterized by the accumulation of aggregated alpha-synuclein protein in Lewy bodies and Lewy neurites similar to Parkinson's disease. Extrapyramidal motor features characteristic of PD are common in DLB patients, but are not essential for the clinical diagnosis of DLB (Outeiro et al. 2019). In fact, αsyn fibrilization in several parts of the brain generates the third most common dementia as contributed by LBD patients in the population (Cantuti-Castelvetri et al. 2005, 2007). LBD is often preceded by prodromal "REM (rapid eye movement) sleep behavior disorder (RBD)" (Posturna and Berg 2019). The exact cause of DLB is unknown it but involves widespread deposits of abnormal deposits of αsyn that form Lewy bodies or Lewy neurites of the diseased brain stem (Walker et al. 2015). DLB and PDD, jointly known as Lewy body dementias, are subject to clinical trials and meta-analyses that provide the basis for the treatment of cognitive, neuropsychiatric, and motor symptoms in patients with LBD. There is expert support for the application of treatments for related conditions, such as PD, for the management of common symptoms (e.g., autonomic dysfunction) in patients with Lewy body dementia. However future clinical trials need to focus on the treatment of symptoms specific to patients with LBD (McKeith et al. 2020; Taylor et al. 2020). Association between αsyn blood transcripts and neuroimaging indicated a presence of PD/DLB at work in the brains of those to be affected with both PD and DLB (Locascio et al. 2015). All of these clinical features remain consistent with RNA based therapeutic approaches that target and inhibit the RNA secondary structure of *SNCA* 5′UTR outlined in the RNA based therapies used as α-syn translation inhibitors for PD, PDD, LBD section and in Figure 3.

RNA based therapies used as α-syn translation inhibitors for PD, PDD, LBD

Introduction

There is compelling support to pharmacologically limit expression of αsyn with translation blockers of the *SNCA* transcript (Fig. 1). This is supported by the use of immunotherapeutic strategies in the brains to limit αsyn in PD patients (Spencer et al. 2016, 2017; Kuo et al. 2019). As noted above, duplication or triplication of the *SNCA* gene leads to familial PD and dementia (PDD) (Chartier-Harlin et al. 2004; Singleton and Gwinn-Hardy 2004; Ahn et al. 2008; Ikeuchi et al. 2008). Experimental siRNA infusions demonstrated it is possible to limit αsyn in animal models (Lewis et al. 2008) although small molecules are more amenable to medicinal chemical optimizations and development for an oral drug therapies. Dopaminergic toxicity is diminished in mice with genetic deletion of brain αsyn levels (Dauer et al. 2002; Song et al. 2004; Alvarez-Fischer et al. 2008; Chen et al. 2013, 2017). We reported the use of the 5′UTR of *SNCA* mRNA as a uniquely structured RNA target to identify novel high-throughput screened potent and selective αsyn translation inhibitors for future therapeutic development (Table 1; Ross et al. 2010b). We will discuss the proof of concept that the drug posiphen was shown to limit αsyn expression in the cortical neurons expressed in PAC transgenic mice that express the complete 145 kb human *SNCA* gene (Friedlich et al. 2007; Kuo et al. 2010, 2019; Rogers et al. 2011). The abundant αsyn is subject to Mn induced toxic spread and αsyn fibrilization from exosomes to cause neighboring inflammation as a factor on the etiology of PD (Venkataramani et al. 2018; Harischandra et al. 2019b). This consideration further supports our small molecule-oriented therapies to suppress αsyn translation as a therapy for PD.

In comparison to the L- and H ferritin mRNAs, which encode a classic IRE RNA stem loops that interact with both the IRP1 and IRP2 translation repressors, the IRE-like stem loop of *SNCA* mRNAs is formed at the splice junction of the first two exons in *SNCA* gene (Figs. 1, 3; Thomson et al. 2005; Wang et al. 2007a,b, Zhang et al. 2008, Olivares et al. 2009, Rogers et al. 2011). The 5′UTR of *SNCA* mRNA is a uniquely folded RNA target to identify relatively safe drugs, such as posiphen, that selectively and therapeutically limit αsyn expression in midbrain and cortical neurons, as expressed in PAC transgenic mice with the complete 145 kb *SNCA* gene (Rogers et al. 2011; Mikkilineni et al. 2012; Kuo et al. 2019; Taguchi et al. 2020a) This *SNCA* 5′UTR RNA target has been used to identify potential intercalators that bind and interact

Table 1. *SNCA* 5′UTR inhibitors, incl. Syn-516, B1, A3, A6 are available to be medicinally advanced

SNCA 5′UTR Translation modulator	IC-50/AC-50 SNCA 5′UTR	Selectivity. PGL3	Selectivity PrP 5′UTR
Syn-517 (activator). Ross et al. MLPCN Probe report 2010a.	2.036 µM	20-fold	100-fold
Posiphen. (Inhibitor.) Rogers et al. JNT. 2011	5 µM	80-fold	29-fold
Syn-516 (Inhibitor.) Ross et al. MLPCN Probe report 2010b.	1.8 µM	111-fold	111-fold
A3. inhibitor and PET imaging agent SNCA mRNA.	5.8 µM	21-fold	18-fold
B1. Alkaloid related to vindoline SNCA 5′UTR inhibitor, PET image agent.	0.134 µM	31-fold	17-fold
A6. piperazine potent SNCA 5′UTR inhibitor	0.1 µM	58-fold	126-fold

Shown are their IC-50 values and fold-selectivity over control 5′UTRs in transfected with pGL3 and PrP 5′UTR encoding reporter transgenes. The Syn-516 probe is the top αsyn translation blocker and Syn-517 is an activator probe, as was outlined in the MLPCN probe report (Ross et al. 2010b).

with the specificity into the predicted RNA stem loop to interfere with *SNCA* mRNA translation relative to ferritin mRNA (Ross et al. 2010b; Zhang et al. 2020b). *SNCA* 5′UTR modulators are modeled to leave the cellular Fe storage capacity unchanged, thus maintaining iron homeostasis and viability of dopaminergic neurons (Table 1; Figs. 1, 3; Jiang et al. 2017).

FDA-approved glycosides provide proof that the SNCA 5′UTR is a valid target for PD

We stably transfected H4 neural cells with a human *SNCA* 5′UTR–luciferase construct, and then screened 780 natural products to identify top inhibitors of αsyn translation (Rogers et al. 2011). Of 17 hit SNCA 5′UTR directed natural product (NP) translation inhibitors*, four natural products, the cardiac glycosides strophanthidine, digoxigenin, sarmentogenin plus mycophenolic-acetate, exhibited IC-50 sec at <5 µM in dose-response. Western blotting confirmed strophanthidine reduced αsyn expression in SK-N-SN and SHSY5Y neural cell lines (IC-50 < 1 µM). Strophanthidine maintained cellular β-actin levels as well as levels of H-ferritin (IRE encoding mRNA), ensuring specificity toward *SNCA* mRNA; gitoxigenin exerted no αsyn inhibition. In the future It will be possible to advance such *SNCA* 5′UTR inhibitors, for example, by establishing efficient adeno-associated viral (AAV) vector constructs, which include the *SNCA* 5′UTR element, and use their neuron-specific (e.g., synapsin-1) promoters to drive the expression of human wild-type αsyn and mutant missense versions of associated with αsyn fibrilization (Cai et al. 2018).

Posiphen inhibits the SNCA 5′UTR as a valid in vivo therapeutic drug target for PD

The clinically tested anti-cholinesterase (AChe) phenserine is a physostigamine analog that reached phase III clinical assessment for AD and had been characterized to inhibit APP mRNA translation though its 5′UTR (Shaw et al. 2001; Venti et al. 2004; Utsuki et al. 2006). Posiphen (PS) is its enantiomer and is a well-tolerated drug and has been administered at doses of 75 mg versus 20 mg/kg/day in mice to suppress APP (Aβ) translation in vivo (Farlow 2004; Lahiri et al. 2007). Posiphen does not itself exhibit AChe activity and has been approved for clinical trials for AD while it also exhibited anti-αsyn efficacy in murine models of PD (Teich et al. 2018; Kuo et al. 2019). Because there was noted to be a 50% sequence similarity between the APP and *SNCA* 5′UTRs (Friedlich et al. 2007), we had originally predicted overlap in the spectrum of drugs that would suppress APP mRNA translation through its 5′UTR with those that might also suppress steady state levels of neuronal αsyn (Rogers et al. 2011).

Posiphen was first shown to have potential as a PD therapy when observed to repress the translation of αsyn (IC-50–5 µM) in primary neurons explanted for the brains of the *SNCA* 5′UTR competent PAC-Tg (SNCA) mouse model of PD (Rogers et al. 2011; Mikkilineni et al. 2012). An in vivo study extended posiphen's potential as a PD therapy since the drug reduced αsyn levels in both the brains and gut neurons of these mice (Kuo et al. 2019). In this study, posiphen normalized colonic motility reflecting prodromal gastrointestinal dysfunction in early PD before the onset of motor disturbances. Three-month PS treatment restored stool motility in PAC-Tg-SNCA mice overexpressing double familial PD mutations while biochemical determination for αsyn/β-actin was measured in the brain cortices postsacrifice. Indeed α-syn levels were lowered in the brain and guts of this mouse model of PD (Kuo et al. 2019). Untreated mice were compared to counterparts treated at doses of 10, 25, 50, 75 mg/kg/day PS for 3 wk (Kuo et al. 2019). Consistent was the fact that PS therapeutically limited APP and *SNCA* 5′UTRs ex vivo in neurons explanted from PAC/Tg(SNCA)

SNCA-1 mRNA and START CODON.

CGACAGTGTGGT   AUG

5′ — Exon-1 | Exon-2 — 3′

GGAGTGGCCATTCGACGACAGTGTGGTGTAAAGGAATTCATTAGCC   α-synuclein
GTCTGTCTCTTGCTTCAACAGTGTTTGGACGGAACAGATCCGGGGA   L ferritin
CGGGGTTTCCTGCTTCAACAGTGCTTGGACGGAACCCGGCGCTCGT   H ferritin
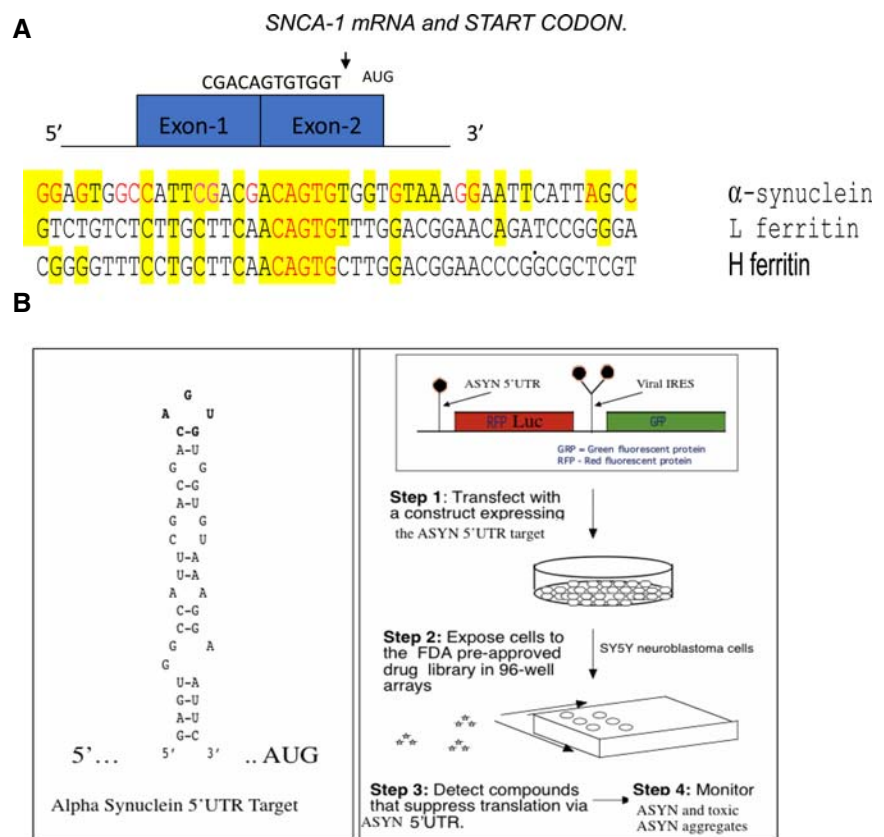
B

Alpha Synuclein 5′UTR Target

Figure 3. The 5′Untranslated region of alpha synuclein mRNA was the screening target for identifying αsyn translation blockers to address the need for Parkinson's disease and PDD and LBD therapies. The *SNCA* 5′UTR can be tested also to alleviate conditions of excess environmentally induced α-synucleinopathy (Friedlich et al. 2007; Rogers et al. 2011; Mikkilineni et al. 2012; Harischandra et al. 2019b). (*A*) The unique RNA target in the 5′UTR of the *SNCA* transcript. Depicted is the *SNCA* 5′UTR with its two exons immediately in front of the *SNCA* gene start codon (Olivares et al. 2009). Shown is the linear alignment of the *SNCA* 5′UTR with those for L- and H-ferritin mRNAs in the vicinity of their IRE RNA stem loops. (*B*) High-throughput screen for small molecule inhibitors of the RNA stem loop folded from the major neuronal neurodegenerative *SNCA* mRNA (MULTIFOLD, as described in Cho et al. 2010). Shown is a version of the transfection-based screen to identify *SNCA* 5′UTR directed translation inhibitors of a downstream luciferase reporter gene. During typical screens, inhibitors selectively maintained translation of a control GFP gene driven from an internal ribosome entry site (IRES) RNA structure. This RNA targeting technology was used to identify translation inhibitors of APP mRNA for AD therapy (Rogers et al. 2002a,b). The list of key HTS inhibitors of the *SNCA* 5′UTR are shown in PUBCHEM website as AID 1813. (Collaboration between Neurochemistry Laboratory at MGH and the Broad Inst. Cambridge, MA, https://pubchem.ncbi.nlm.nih.gov/bioassay/1813#section=Description

mice (Mikkilineni et al. 2012). A cautionary note is that the metabolic byproducts of posiphen from the liver could include derivatives that cause cholinergic excitability (Mikkilineni et al. 2012). This attractive AD drug may therefore present as somewhat of challenge for usage in PD patients where a gait problems already exist (Mikkilineni et al. 2012).

The high-throughput screened (HTS) small molecules Syn-516 and Syn-517 as proof of selectivity of SNCA 5′untranslated region as a drug target for PD, PDD, and LBD

Because of its unique RNA stem loop structure, the *SNCA* 5′UTR provides an excellent drug target to screen and identify suppressors of αsyn translation via an IRE-like stem loop not found in β and γ-syn mRNAs (Figs. 1, 3; Rogers et al. 2011; Mikkilineni et al. 2012; Zhang et al. 2020b). RNA targeted intercalators screened to bind the *SNCA* 5′UTR may well therapeutically interfere with the translation of *SNCA* mRNAs to a greater extent than for L- and H-ferritin mRNAs. This criterion should maintain normal iron homeostasis and viability of dopaminergic neurons while limiting α-synucleinopathy (Jiang et al. 2017). We will discuss the properties of the αsyn translation blocker probe "Syn-516" that was been published from a HTS to provide proof-of concept for our strategy (Ross et al. 2010b).

Based on the HTS screening campaign for αsyn translation blockers, 35 novel 5′-untranslated directed agents were identified after an exhaustive screen/counter-screen campaign from the Broad Inst., Cambridge, MA (Table 1). The compound "Syn-516" was published in 2012 as a highly selective and potent agent in an official probe report of the Molecular Libraries Probe Production Centers Network (MLPCN) of the NIH and designated as "ML-150" (Ross et al. 2010a,b). A representative four further top *SNCA* 5′UTR modulators from this MLPCN screen are shown in Table 1 with their listed IC_{50} and AC_{50} values. These AC_{50}s were found on PUBCHEM to be even lower and more selective than Syn-516. It is feasible to test medicinal analogs of the best *SNCA* 5′UTR blocker from this list and determine its IC-50 profile for αsyn inhibition in induced pluripotent stem cells (ipSCs) differentiated to dopaminergic neurons from a patient with familial PD for triple copies of the *SNCA* gene (Bakshi et al. 2018, 2019). Useful information about the MLPCN probe and αsyn inhibitor Syn-516 is listed in PUBCHEM-AID 1827. During this same screen, the agent Syn-517 was cherry-picked as the most potent/selective translation activator of the 5′UTR in SNCA mRNA (bioassay AID 1814) (Ross et al. 2010a,b).

During screen/counter-screening, the lead αsyn translation blocker Syn-516 exhibited a "hit" cutoff of ≥62% inhibition at 7.5 μM where 2498 compounds were originally identified as inhibitors of SNCA 5′UTR driven luciferase expression. Of these, 2193 compounds had been retested at eight-point conc. series (20–0.16 μM) in the primary screening cell line. Compounds that had IC-50 values <1 μM were successfully retested (1381/2193, 63% rate). Syn-516 was advanced after two counter-screens (Table 1). First, the H4-2a neural cell line with the *SNCA* 5′-UTR stem–loop removed (H4-C) was screened (Z′ = 0.65) while precluding cytotoxic agents to cause no global reductions in transcription/ translation. Second, a similar exclusion of PrP 5′UTR inhibitors was undertaken (Ross et al. 2010b). Cultured H4 neuronal cells were transfected with a construct containing the PrP 5′UTR upstream of luciferase (H4-PRP) and were assayed to confirm the compound Syn-516 was specific for the *SNCA* 5′UTR RNA stem–loop (Z′ = 0.75). Syn-516 inhibited 5′UTR driven translation of αsyn with an IC-50 of 1.8 μM and was initially found >34-fold selective over transfectants lacking this 5′UTR (H4-C) and from the 5′UTR of PrP mRNA (H4 H4-PrP) (counter-screens). N.B. inhibi-

tion commenced in the 50 nM range while the IC-50 value is shown Table 1.

Syn-516 was advanced as an SNCA 5′UTR directed inhibitor probe over the other *SNCA* 5′UTR directed inhibitors listed in Table 1 since this agent was the first compound to be assayed that displayed a metabolic tolerance and predicted suitability as required by the MLPCN for future in vivo studies. The chemical structure of the Syn-516 probe is N,N-Dimethyl-6-({[1-(1-naphthyl)-1H-tetrazol-5-yl]thio}methyl)-1,3,5-triazine-2,4-diamine and it is commercially available and bears a prominent di-amino thiazine structure similar to that present in lamotrigine (a drug used by bipolar patients). Lamotrigine is rapidly absorbed after oral administration with bioavailability at 98% with a plasma Cmax from 1.4 to 4.8 h. Similarly, western blotting and ELISAs showed the MLPCN probe Syn-517 dose responsively increased neural αsyn expression in SHSY5Y cells without change to steady state levels of β-actin (AID 2627).

To monitor the capacity of Syn-516 to increase IRE/IRP binding in a therapeutic mode, RNA protein binding measurements will be required. Scatchard analysis has been successfully used for these kinds of studies when establishing the dissociation (K_d) for the APP–IRE interaction with IRP1 (30 pM compared to $K_d = 40$ pM for the H-ferritin IRE and IRP1) (Allerson et al. 1999; Cho et al. 2010). In the future, efficacies to limit αsyn will be assessed by AAV-αsyn constructs expressing wt. and mutant alpha-synuclein from cDNA inserts with a bonafide *SNCA* 5′UTR cassette in front of the αsyn start codon (Cai et al. 2018). It will be necessary to determine which of the four focused *SNCA* 5′UTR inhibitors shown in Table 1 is most effective at an IC-50 in the 10–100 nM range compared to posiphen. This level of potency will be required to medicinally establish the in vivo efficacy for Syn-516 compared to posiphen since αsyn has a 48–72 h. half-life. αsyn exists in an equilibrium between being an in situ tetramer while it is the monomer form that favors fibrilization (Bartels et al. 2011; Dettmer et al. 2013, 2015a,b; Selkoe et al. 2014; Luth et al. 2015; Nuber et al. 2018; Fanning et al. 2019). For favorable blood brain barrier determinations, the use of in vitro membrane permeability assays can be conducted with endothelial cells (Lok et al. 2009). Optimal formulations of Syn-516 will be used for in vivo assays so as to correlate its capacity to increase the RNA binding of IRP1 to the *SNCA* 5′UTR with repression of *SNCA* mRNA translation.

Table 1 illustrates the point that there are currently no PET tracers available for imaging α-synuclein fibrils or *SNCA* mRNA in the human brain. This table shows the first generation of PET radiotracers targeting SNCA mRNA. There are in the pipeline probes pre-identified to target the *SNCA* 5′UTR that will provide a useful tool for PET imaging and prediction of α-synucleinopathies in PD and PD-related dementias. Using radiolabeled derivatives of SNCA 5′UTR directed small molecules, we plan to define the localization of *SNCA* mRNA in anatomic human brain during the etiology of PD and conduct in vivo PET imaging of how *SNCA* mRNA accumulates in the brains of animal models and in human patients at the Martinos Center (MGH). As an example, the agent B1 (analog of vindoline) demonstrated excellent labeling and ADME properties when used as a PET imaging agent to monitor the brain distribution of *SNCA* mRNA in mice (C. Wang, personal communications; Gilbert et al. 2019).

Mn was shown to increase the infective spread of alpha-synuclein pathology from the gut to the brain (Harischandra et al. 2018). In fact, alpha-synuclein is neuroprotective at low doses of Mn exposure to neurons (Harischandra et al. 2015). This progression of Mn pathology supports the therapeutic strategy of Figures 2–4 for RNA targeting with APP and ferritin translation activators compared with the possibility of utilizing *SNCA* 5′UTR directed inhibitors. Certainly, pharmacological conditioning with αsyn inhibitors may generate therapeutic

efficacy as a novel means to mitigate cognitive deficits of Mn associated α-synucleinopathy.

Iron regulatory paths of ferritin, APP translation to mitigate metal neurotoxicity by manganese and lead exposures

Manganese and lead (Pb) as neurotoxins

Manganese (Mn) and lead (Pb) exposure is associated with a range of brain developmental defects leading to lowered IQ (Vollet et al. 2016) and to the onset of memory loss as people age (Zawia and Basha 2005; Rogers et al. 2016; Venkataramani et al. 2018). Later-life risk for AD/PD after childhood exposures to lead are discussed (Basha et al. 2005b). Excess iron causes hemochromatosis whereas manganese and manganism are a behavioral/ movement crisis during working lives (Rogers et al. 2019). Mn in children leads to neuropsychological underscoring (Carpenter 2001; Rodan et al. 2018; Sarkar et al. 2018). Pb exposure from paint in the western world, and Pb in the industrial settings in the third world, leads to ADHD and then to later risks for ferroptosis and associated neural death from iron oxidation of lipid radicals, resulting in neurodegenerative disease such as AD (Bolin et al. 2006; Ashok et al. 2015; Rogers et al. 2016; Venkataramani et al. 2018). Several metals, including Pb, can trigger fibrilization of methionine-oxidized alpha-synuclein specific to an increased risk for PD (Yamin et al. 2003) while adult Mn toxicity is already a form of Parkinsonism (Peres et al. 2016). There are seminal works on early-life exposure of environmental factors such as Pb on late life disorders as per the LEARn (latent early-life associated regulation) pathway (Lahiri et al. 2009). This work provides a perspective for a disease particularly as complex as AD. Dementiacan be viw4ed as a transformation from many environmental effectors rather than only being considered as being a genetic a state (Maloney and Lahiri 2016).

Manganism

Biology and clinical pathological features of Mn toxic exposure

Mn exposure from welding settings affects all age groups to cause psychiatric, cognitive, and motor disturbances (Chia et al. 1993) including, bradykinesia, rigidity, tremor, and gait problems. Elevated environmental exposure of Mn causes the neurological disorder of manganism with disrupted movement features resembling idiopathic PD. Mn treated rats display motor deficits and striatal oxidative stress (Cordova et al. 2013) with iron flow from the blood to cerebral spinal fluid (Zheng et al. 1999; Tong et al. 2014). Motor disturbances in PD respond to Levadopa therapy while manganism patients treated with levodopa do not exhibit clinical benefit (Lu et al. 1994). In PD, motor disturbances are caused by a loss of neurons in the SN and to dopaminergic nerve terminals while Mn overexposure additionally incudes the loss of other monoaminergic neurons in the striatal globus pallidus (Cordova et al. 2013; Chen et al. 2014, 2015). Depending on exposure, Mn^{2+} and Mn^{3+} induce gait disturbances and toxic reactive oxygen species in rats neuronal Fe burden (Malecki et al. 1999).

Manganism has been documented for ~150 yr while Mn is an essential dietary element and a key cofactor in many enzymatic reactions, including the antioxidant enzyme superoxide dismutase (Keen and Hurley 1987). These devastating gait disorders extend to young adult workers while environmental Mn exposure enhances prion like αsyn spread and synthesis (Venkataramani et al. 2018; Harischandra et al. 2019b). Excess Mn promotes the aggregation and prion-like cell-to-cell exosomal transmission of αsyn that can coalesce into the fibrils that generate Lewy bodies

(Harischandra et al. 2019a,b). One familial form of PD is caused by recessive mutations to "Park-9," a lysosomal Type-5 ATPase cation pump that regulates Mn efflux from neurons while also serving in the same molecular pathway of αsyn in Mn homeostasis (Gitler et al. 2009; Chesi et al. 2012; Guilarte 2013; Kong et al. 2015; Medici et al. 2015).

Mn and iron transport and the potential for neuronal death by ferroptosis

Mutations in the Mn exporter SLC39A14 causes Mn-induced toxicity in the liver and a brain Parkinsonian dysfunction (Zheng et al. 1999; Tuschl et al. 2016; Hutchens et al. 2017). However, while SLC39A14 (ZIP14) has a critical role in maintaining Mn homeostasis in mice, this transporter has a role in iron and zinc transport and ferroptosis when misregulated (Xin et al. 2017). Certainly, Mn exposure and toxicity to neurons appears operate via ferroptosis by interfering with IRE/IRP regulation of translation of APP where APP(s) directly can interact with FPN to accelerate iron efflux (Venkataramani et al. 2018; Tsatsanis et al. 2020). This loss of APP generates enhanced intracellular REDOX active Fe^{2+} in the event of reduced levels of APP/PPN Fe-export complexes as well as resulting from reductions of the iron storage protein ferritin (Khan et al. 2017; Venkataramani et al. 2018). Mn exposure to cells certainly blocks IRE-driven translation of not only APP but also the H-subunit of ferritin, the cell's central neuroprotective iron storage multimer (see Fig. 4; Lumsden et al. 2018).

Iron-transporter proteins are linked to the maintenance of physiologic Mn levels and balance, including that of divalent metal ion transporter-1 (DMT1) for iron import (Garrick et al. 2006; Salazar et al. 2008) and FPN for iron export (Mitchell et al. 2014). In fact, FPN plays a central role in regulating both iron and Mn efflux from neurons (Madejczyk and Ballatori 2012). Mice expressing dominant negative mutations to FPN encoding the flatiron phenotype exhibited tissue iron-overload similar to hemochromatosis in humans (Johnson and Wessling-Resnick 2007) and these were shown also deficient in Mn export (Seo and Wessling-Resnick 2015). Transfection based overexpression of the wild-type FPN transgene eliminated excess toxic Mn burden and protected neural cells from oxidative overload while the flatiron mutated FPN failed to protect Mn treated neurons (Seo and Wessling-Resnick 2015). As noted above, APP belongs to a family of proteins that physically interact by FPN, as initiated in pathway by IRP1 dependent translation sufficient to promote APP/FPN dependent neuronal efflux of excess iron in conditions of Mn exposure (Duce et al. 2010; Hahl et al. 2013; Ayton et al. 2015c; Venkataramani et al. 2018; Tsatsanis et al. 2020). Lei et al. (2012) found that a microtubule-associated tau deficiency induced Parkinsonism with dementia by impairing APP-mediated iron export by FPN (Lei et al. 2012).

APP is a copper-zinc metalloprotein (Multhaup et al. 1996; White et al. 1999) whose expression, and that of ferritin, are eliminated as an early event immediately before Mn is most toxic to neurons (Venkataramani et al. 2018). Figure 4 incorporates the published model that, while iron increased both APP and ferritin-H protein levels in SH-SY5Y cells, Mn exposure dose-responsively inhibited expression of these key iron-associated protective proteins. Toxicity only became evident after 24 h when levels of APP and ferritin has been reduced via 5′UTR directed translation inhibition (Venkataramani et al. 2018). As shown in Figure 4, Mn interfered with IRE/IRP repression pathways to regulate APP and ferritin so as to deprive primary neural cells of two key proteins that contribute to healthy Fe metabolism (Balla et al. 1992; Rogers et al. 2002a,b; Bandyopadhyay et al. 2006, 2007; Cho et al. 2010; Duce et al. 2010). Clearly, Mn exposure can activate IRP1 to ablate APP and ferritin in cells, a property that causes an increase in the accumulation of iron-II as a REDOX active and neuro-

destructive agent (Fig. 4). In sum, increases of the APP(s) ectodomain sufficient to bind and facilitate FPN dependent cellular iron export may thereby provide an antiferroptotic therapy to mitigate the consequences of heavy metal exposure (Rogers et al. 2019; Tsatsanis et al. 2020). Alpha-synuclein blockers also offer potential neurotherapeutic agents that serve to protect neurons from ferroptosis. Certainly, oleic acid accumulation accelerates neurotoxicity when αsyn is overexpressed (Gitler et al. 2009; Fanning et al. 2019). Oleic acid peroxidation is toxic apparently as a consequence of αsyn dependent iron accumulation (Singh et al. 2014; Baksi et al. 2016).

For the purpose of therapies, perturbations of Mn transport and load are physiologically complex while being directly linked to movement and cognitive disorders such as for PDD (Lin et al. 2017; Xia et al. 2017; Jiang et al. 2020). Under the age of 40, pharmacological increases of IRE independent APP expression and APP (s) secretion offers a novel strategy to prevent Mn interference of translation of the APP and ferritin, suggesting the possible means to prevent cognitive problems for younger adult (Fig. 4; Nitsch et al. 2000; Rogers et al. 2019). For example, these authors showed an intervention with the known FDA drug AF120B was shown to activate APP 5′UTR directed translation and cleavage to APP(s) to potentially enhance FPN dependent excess iron and Mn export from prone neurons. AF102B is an alpha secretase activator that limits amyloid production (Beach et al. 2001). As a second example, yohimbine is a safe supplement as an alpha-2 adrenergic antagonist that limited cortico-amygdala circuit abnormalities in a genetic mouse strain with impaired fear extinction behavior (Hefner et al. 2008). Since yohimbine is a H-ferritin translation activator (Tibodeau et al. 2006; Rogers et al. 2016, 2019), this agent can be modeled to prevent Mn induced cell death via these ferroptosis pathways (Fig. 4; Morse et al. 2004; Stockwell et al. 2017).

Lead neurotoxicity

Lead poisoning is a problem to children in the third world as well as remaining a significant risk in the water supply and in preexisting lead paint and water supplies in the USA (Flint, Michigan) (Hanna-Attisha et al. 2016; Hanna-Attisha and Kuehn 2016). Acute environmental lead exposure generates nerve cell death with inflammatory encephalopathy and hemorrhage (Struzynska et al. 2001; Hossain et al. 2004). Early acute Pb exposure may well promote epigenetic changes that cause AD later in life (Basha et al. 2005a,b; Bakulski et al. 2012; Bihaqi et al. 2012; Eid et al. 2016). In the US and Canada, endangered populations would appreciate the relevance of the proposed "4R's" (remediation, renovation, reallocation, and research) against the Pb crisis (Maloney et al. 2018).

Pb is toxic to neurons, potentially via ferroptosis, by interfering with IRE/IRP driven translation of APP and the ferritin-H chain (Fig. 4; Rogers et al. 2016). By this pathway Pb, like Mn, has the capacity to cause heightened intracellular REDOX active Fe^{2+} is caused by reduced levels of APP/PPN Fe-export complexes, also loss of iron storage in ferritin (Khan et al. 2017). Neurotoxicity depends on Apo E3/ ApoE4 genotypes in rodents with a more pronounced effect in females (Engstrom et al. 2017). Early-life exposure to Pb activates microRNAs to target AD proteins (Masoud et al. 2016) and human tau phosphorylation (Dash et al. 2016). Pb exerts neurotoxicity when transported into cells by the central iron import protein *DMT-1* (Zhu et al. 2012; An et al. 2015). Redox-active ferrous iron accumulates in neurons of the brains of Pb-mice exposed (Zhu et al. 2013; Zhou et al. 2014) As is the case for manganese neurotoxicity, the presence of Pb exposure at chronic sublethal doses to neurons can be directly assessed for its capacity to embargo iron export leading to its toxic excess (Zhu et al. 2012; An et al. 2015; Rogers et al. 2016).

Pb caused IRP1 translationally repressed the L- and H-chains of the assembled multimer of ferritin via IRE RNA stem loops in the 5′UTRs of their mRNAs, thus disabling the cell's Fe storage capacity (Figs. 1, 4; Rogers et al. 2016). At the same time, Pb repressed IRP-1 dependent translation of the APP, the key facilitator of the
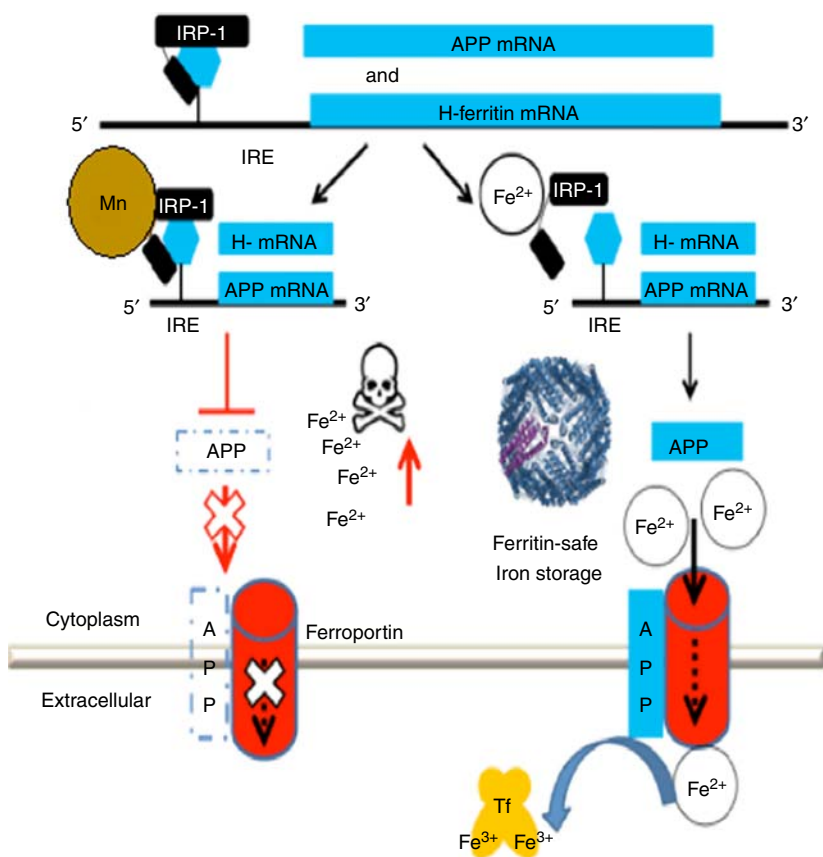

Figure 4. Model for manganese neurotoxicity invoking activation of APP and ferritin translation by iron-regulatory Proteins. (*Right*) In healthy individuals, APP facilitates FPN dependent iron export from neurons. (*Left*) Mn, like Pb exposure, interferes with APP and ferritin by blocking the translation of their mRNAs when causing super-repression by IRP1. Reduced FPN driven iron export by loss of APP (s) and the loss of Fe storage (ferritin) was shown to induce an embargo of iron inside the cell causing reactive oxygen species and REDOX active cell death/ferroptosis (Rogers et al. 2016; Venkataramani et al. 2018). Excess iron forms in magnetic nanoparticles throughout the amyloid plaque during AD (Plascencia-Villa et al. 2016) while ferritin was increased in brains and cerebrospinal fluid of AD patients (Ayton et al. 2015a; Ayton et al. 2016). Of note, amyloid form APP is protective in rodents burdened from acute bacterial infection/oxidative neurotoxicity (Kumar et al. 2016).

export of excess iron by FPN (Duce et al. 2010; Ayton et al. 2015c). In fact, epigenetic changes induced by early Pb exposure caused long-term induction APP transcription while the precursor is cleaved by β- and γ-secretase to generate the 40–42 residue amyloid peptide which increases the risk of AD at a later stage in life (Walsh and Selkoe 2016).

High dose Pb poisoning is clinically treated with the metal chelator succimer though this treatment presents side-effects (Horowitz 2001; Stangle et al. 2007; Rahman et al. 2014; Wirbisky et al. 2014). The testable model shown in Figure 4 proposes 5′UTR dependent activators of ferritin/APP translational axis of iron homeostasis indeed will mitigate Pb neurotoxicity at sublethal chronic dose exposure range (Rogers et al. 2016; Long et al. 2019). It is possible to characterize the druggable biological and therapeutic properties of small molecule αsyn blockers versus ferritin/APP activators in each case to prevent toxic metal induced ferroptosis and neurodegeneration (Duce et al. 2010; Abdalkader et al. 2018; Venkataramani et al. 2018; Tsatsanis et al. 2020). To treat Pb-based neurotoxicity, FDA preapproved and high affinity small-molecule modulators of IRE/IRP dependent translational induction might induce APP translation and APP(s) sufficient to form enough adaptive APP/FPN iron efflux complexes to efflux toxic overload of Fe. This is also the case for Mn exposures and L- and H ferritin subunits translation activators are also a desirable therapeutic route to provide protection from toxicity from both heavy metals (Tibodeau et al. 2006).

Conclusions

Mn and Pb toxicity to cultured neurons is associated with disrupted translation of APP whose loss causes disruption of neuronal iron export by FPN to generate conditions pf embargoed REDOX active intracellular iron (Rogers et al. 2016; Venkataramani et al. 2018). The model shown in Figure 4 depicts that APP mRNA is translationally controlled by IRP1 while APP and APP(s) binds to FPN to accelerate Fe and Mn efflux from cells (Duce et al. 2010; Rogers et al. 2019; Tsatsanis et al. 2020). IRP2 as well as IRP1 are the RNA binding proteins that set a healthy physiological balance for iron homeostasis when interacting with IREs in the untranslated regions of ferritin and transferrin receptors transcripts to control their intracellular steady state levels (Thomson et al. 1999, 2005; Cho et al. 2010). We originally showed that the influx of excess iron into neurons increased APP translation via 5′UTR specific IRE/IRP interactions (Rogers et al. 2002a). Subsequent alpha-secretase cleaved APP(s) favored conditions for a neuroprotective efflux of excess iron after its interaction with FPN, particularly in conditions of heightened brain iron overload (Cho et al. 2010; Duce et al. 2010; McCarthy et al. 2014).

The alpha-synuclein and APP 5′UTRs have been useful mRNA targets for identifying and advancing translation inhibitors such as the anti-cholinesterase phenserine and its enantiomer posiphen. Clinical trials have tested their action as bonafide anti-amyloid and anti-alpha synuclein agents respectively for treatment of AD and PD (Teich et al. 2018; Kuo et al. 2019). Extending from these agents, AF102B is a neuroprotective m-1 muscarinic agonist used to treat Sjogren's syndrome and can also induce APP(s) secretion resulting from alpha-secretase activation of the nonamyloidogenic pathway of APP expression (Fisher 2000; Fisher et al. 2000). AF102B, similar to high through put screened APP 5′UTR dependent translation activators, is a starting point for medicinal treatments to mitigate acute Pb and Mn toxicity. This drug can be tested for its capacity to enhance APP/FPN complexes to efflux toxically embargoed iron and prevent ROS accumulation (Figs. 2, 4). That is, this therapy my well apply especially in the younger population suffering from environmental/industrial heavy metal

exposures at an age long before such Mn or Pb exposed subjects are at risk from the onset of amyloidogenic cascade as observed in older AD subjects.

Since Mn induces the spread of alpha-synuclein (Harischandra et al. 2015, 2018, 2019a), we propose that αsyn inhibitors represent another potential set of therapeutic agents for offsetting Mn toxicity as well as in treating neuronal death in the course of dementia-associated diseases such as and PDD and LBD. APP 5′UTR activators might be used to pharmacologically restore APP translation even in the event of Pb and/or Mn interference via APP 5′UTR sequences (Rogers et al. 2016; Long et al. 2019). The clinical the use of alpha-synuclein inhibitors, in contrast, might promote iron homeostasis when preventing alpha-synucleinopathies in addition to a promoting efficacy from excess iron load in conditions of αsyn overexpression (Ross et al. 2010b; Mikkilineni et al. 2012; Zhang et al. 2020b). In future, translation activators of ferritin include the drugs yohimbine and the novel agent BL-1 as candidates for neuroprotection against heavy metal stresses (Tibodeau et al. 2006)

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