Volume 9 | Number 6 | June 2017 | Pages 587–804

Metallomics

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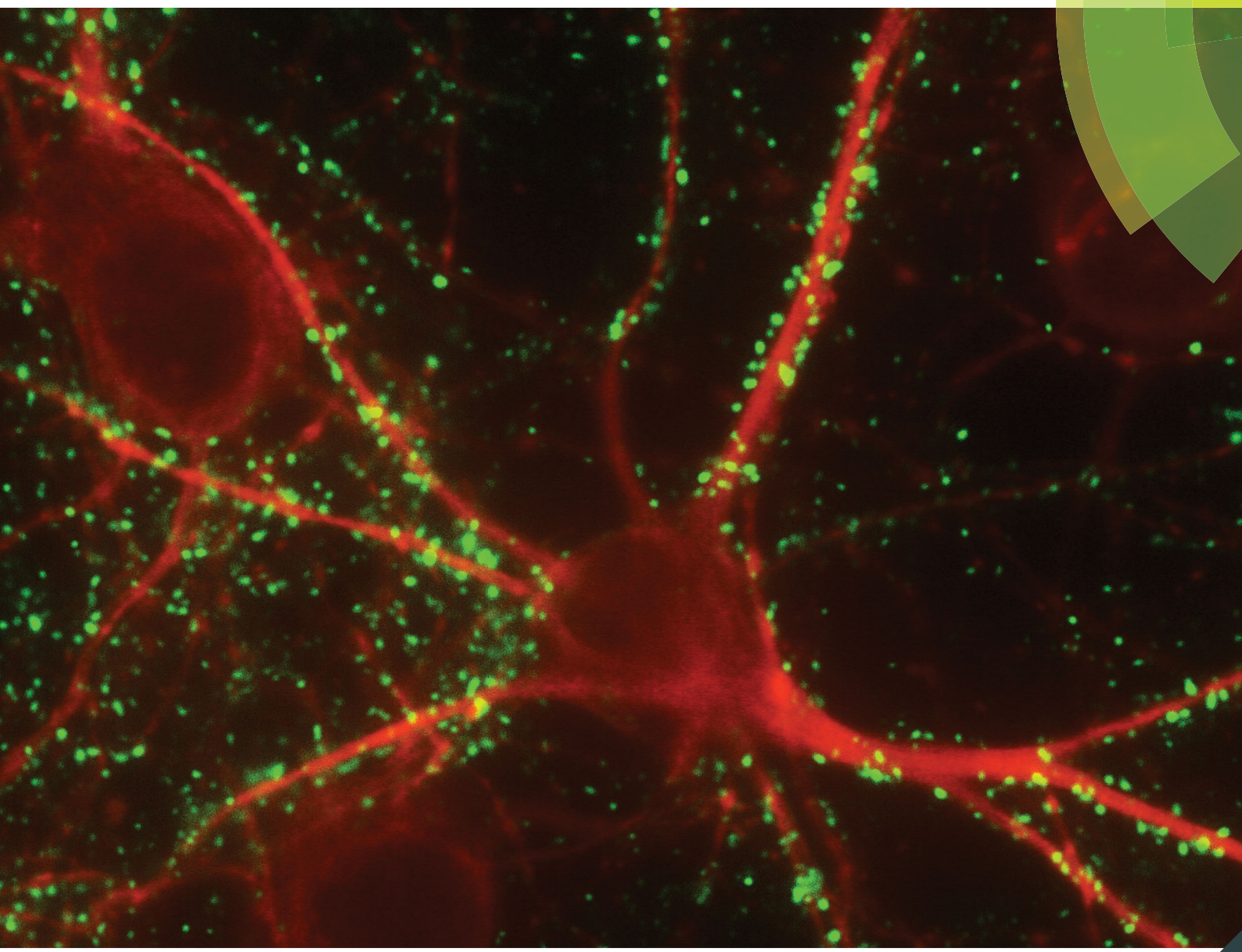

ISSN 1756-591X

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

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Cite this: *Metallomics*, 2017, 9, 619

Cross talk between neurometals and amyloidogenic proteins at the synapse and the pathogenesis of neurodegenerative diseases

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Received 19th February 2017,
Accepted 24th April 2017

DOI: 10.1039/c7mt00046d

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Increasing evidence suggests that disruption of metal homeostasis contributes to the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, prion diseases, Lewy body diseases, and vascular dementia. Conformational changes of disease-related proteins (amyloidogenic proteins), such as β-amyloid protein, prion proteins, and α-synuclein, are well-established contributors to neurotoxicity and to the pathogenesis of these diseases. Recent studies have demonstrated that these amyloidogenic proteins are metalloproteins that bind trace elements, including zinc, iron, copper, and manganese, and play significant roles in the maintenance of metal homeostasis. We present a current review of the role of trace elements in the functions and toxicity of amyloidogenic proteins, and propose a hypothesis integrating metal homeostasis and the pathogenesis of neurodegenerative diseases that is focused on the interactions among metals and between metals and amyloidogenic proteins at the synapse, considering that these amyloidogenic proteins and metals are co-localized at the synapse.

Introduction

Considerable amounts of trace elements, including iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn), exist in the brain at different levels and distributions.[1] These elements, termed 'neurometals', are essential for life and play crucial roles in normal brain functions, such as neurotransmitter synthesis, neural information processing, and neuronal myelination.[2] Therefore, deficiency of these neurometals produces severe adverse effects on central nervous system functions, especially learning and memory. Additionally, increasing evidence suggests that dyshomeostasis of neurometals, either through their excess or deficiency, is involved in the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease (AD), prion diseases, Lewy body diseases, and vascular dementia (VD).[3–6] It is widely accepted that conformational changes of disease-related proteins are central to the pathogenesis of these neurodegenerative diseases, except for VD. The disease-related proteins, termed amyloidogenic proteins, including β-amyloid protein (AβP) in AD, prion protein (PrP) in prion diseases, and α-synuclein in Lewy body diseases, exhibit similarities in their formation of fibril-like structures of oligomers with β-pleated sheet structures (amyloid fibrils), their accumulation in patients' brains, and their induction of neurotoxicity.[7] Metals are important determinants of protein conformation. These amyloidogenic proteins or their precursor proteins reportedly possess the ability to bind metals and thereby participate in regulating metal homeostasis. Moreover, these amyloidogenic proteins are co-localized at the synapse, which is a small but essential compartment for numerous functions including learning and memory, and is vulnerable in these neurodegenerative diseases. Additionally, Zn^{2+} and Cu^{2+} can be secreted into synaptic clefts,[8,9] and an excess of these metals contributes to ischemia-induced neuronal death and the pathogenesis of VD.[10] In the current review, we first present a brief overview describing the characteristics of neurometals and then provide evidence based on our recent studies and those of others linking metals and these neurodegenerative diseases. We propose an integrative hypothesis associating the interactions between neurometals and amyloidogenic proteins at the synapse with the pathogenesis of some neurodegenerative diseases.

Properties of neurometals

Metallomics studies have shown the brain to possess several distinct characteristics. Despite its small volume (typically only 2% of the human body weight), the brain uses approximately 20% of the body's total oxygen. Because of its disproportionate oxygen and blood flow, the brain is susceptible to oxidative

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stress and reactive oxygen species (ROS) caused by the presence of Fe. The brain requires several trace elements for intrinsic functions, such as myelination and neurotransmitter synthesis. However, neurons in the brain are susceptible to the accumulation of toxic heavy metals, because these neurons generally do not regenerate but are maintained for the individual's entire life span. The blood–brain barrier, which is composed of tight junctions between endothelial cells, prevents brain exposure to toxic substances, but this barrier system is imperfect, as shown in Minamata disease.

Among these neurometals, Fe is the most abundant in the brain as well as in the whole body. Iron is essential for numerous biological functions as an enzyme cofactor for metabolic processes, such as oxygen transport, oxidative phosphorylation, and energy transfer.[11] Iron has critical roles in specialized brain functions, including synthesis of the neurotransmitter dopamine and myelination. Therefore, Fe deficiency in children or infants impairs learning, and in adults, it impairs working memory and learning.[12] However, excess Fe generates ROS that damages DNA, proteins, and lipids and can therefore be toxic to neurons. Iron exists in two different forms: ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}). Orally administered Fe is primarily absorbed from the gastrointestinal pathway through the divalent metal transporter-1 (DMT1) as Fe^{2+}. Once it enters the circulation, Fe^{2+} ions are oxidized to Fe^{3+} by ferroxidases, such as hephaestin or ceruloplasmin. The Fe^{3+} binds with transferrin, an iron-binding protein that binds two Fe^{3+} ions, crosses the blood–brain barrier *via* transferrin receptors, and enters neurons or glial cells. The Fe^{3+} is reduced to bioactive Fe^{2+} by ferrireductase and transferred to neuronal enzymes, which require Fe^{2+} as a cofactor. Therefore, Fe levels as well as the ratio between Fe^{2+} and Fe^{3+} are strictly regulated in normal brains.

Zinc is the second most abundant element in the brain, occurring at the highest concentrations in the hippocampus, amygdala, cerebral cortex, thalamus, and olfactory cortex in the brain.[13] Although some Zn binds firmly to metalloproteins or enzymes, a substantial fraction (approximately 10% or more) either forms free Zn ions (Zn^{2+}) or is loosely bound and is histochemically detectable by staining using chelating reagents. This chelatable Zn^{2+} is stored in the presynaptic vesicles of excitatory glutamatergic neurons and is secreted into synaptic clefts with glutamate during neuronal excitation. The secreted Zn^{2+} modulates overall brain excitability by binding *N*-methyl-D-aspartate (NMDA) type glutamate receptors, γ-aminobutyric acid (GABA) receptors, and glycine receptors.[14] The secreted Zn^{2+} is critical to information processing, synaptic plasticity, learning, and memory.[15,16] Zinc deficiency in children results in dwarfism, delayed mental and physical development, immune dysfunction, and learning disabilities, whereas in adults Zn deficiency produces learning, taste, and odor disorders.[17]

Two factors come into play in the maintenance of Zn homeostasis: metallothioneins (MTs)[18] and Zn transporters.[19] There are three types of metallothioneins, MT1, MT2, and MT3. MT1 and MT2 are ubiquitously expressed throughout the whole body, whereas MT3 is primary localized in the central nervous system. Two types of mammalian Zn transporters exist: ZnT transporters and Zrt-, Irt-like protein (ZIP) transporters. ZnT transporters facilitate Zn^{2+} efflux when it is in excess, and ZIP transporters facilitate Zn^{2+} influx when it is deficient. Among the 14 types of ZnT transporters in mammals, ZnT-1 has a pivotal role in Zn^{2+} efflux and in protection from excess Zn^{2+} in the brain. ZnT-3, localized in the membranes of presynaptic vesicles, transports Zn^{2+} into synaptic vesicles and maintains high Zn^{2+} concentrations in these vesicles. ZIP transporters are localized in cell membranes or in the membranes of the Golgi apparatus or endoplasmic reticulum (ER), and control Zn^{2+} influx into subcellular organs.

Copper, the third most abundant element in the brain, is a cofactor for numerous enzymes, such as cytochrome *C*, superoxide dismutase, lysyl oxidase, and tyrosinase, and is essential for brain functions.[20] Copper is involved in Fe homeostasis as a component of ceruloplasmin, a ferroxidase enzyme, and has neuroprotective activity as a component of Cu/Zn superoxide dismutase (Cu/Zn-SOD), an endogenous antioxidant. Copper deficiency results in adverse effects on myelination. Copper is a redox-active metal and exists as both oxidized Cu^{2+} and reduced Cu^{+}. Excess free Cu is toxic because it produces ROS and binds with the thiol groups of functional proteins. Copper excess or deficiency caused by mutations of Cu transporters has been linked to neurodegenerative diseases, including Wilson disease and Menkes disease.[21] Recent studies suggest that intracellular Cu^{2+} accumulates in synaptic vesicles and is then released into synaptic clefts during neuronal excitation, similar to Zn^{2+}.[9] The released Cu^{2+} has modulatory effects on neuronal information processes, reportedly binds to NMDA-type glutamate receptors and other receptors, and modulates neuronal excitability.[22]

Manganese is essential for the function of various enzymes, including hydrolases, lyases, and ligases, and is involved in metabolic processes, such as protein glycosylation and lipid synthesis, as well as in immune function.[23] Manganese also participates as a cofactor of Mn-SOD, which facilitates the detoxification of free radicals. However, excess Mn is toxic and causes Parkinsonism-like syndromes.[24]

Because different levels of these neurometals coexist in various brain regions, the interaction of one metal ion with another is important. Different metal ions usually share binding sites but have different binding constants. Neurometals commonly bind to several amino acid residues, including His, Arg, and Tyr, or to phosphorylated amino acids in proteins. For example, it is widely known that Cu and Zn interact with one another and bind the same metal-binding proteins, such as metallothioneins. Al shares similar characteristics with Fe and can bind to iron-binding proteins, such as transferrin and ferritin. Manganese has characteristics similar to Fe and is transported by binding with DMT1 as Mn^{2+} and exported by binding with transferrin as Mn^{3+}. Several types of Zn transporters (ZIP8 and ZIP14) also transport Fe, cadmium (Cd), and Mn in addition to Zn.[25] Therefore, metal–metal interactions are extremely important in exerting functions.

Alzheimer's disease and metals

Interactions between metals and AβP

AD is a severe type of senile dementia that affects a large proportion of the elderly population worldwide. In Japan, more

than four million patients with senile dementia were reported in 2013, and the number is increasing annually. The disease is responsible for approximately half of all senile dementia and is characterized by profound memory loss and inability to form new memories. The pathological hallmarks of AD are the presence of numerous extracellular deposits termed senile plaques and intracellular neurofibrillary tangles, and the selective loss of synapses and neurons in the hippocampal and cerebral regions.[26] The major components of neurofibrillary tangles and senile plaques are phosphorylated tau protein and AβP, respectively.

Numerous biochemical, cell biological, and genetic studies support the amyloid cascade hypothesis, which proposes that AβP accumulation and the consequent neurodegeneration are central to AD pathogenesis.[27,28] Cleavage of a large precursor protein (APP; amyloid precursor protein) at the N-terminus by the β-APP cleaving enzyme (BACE) and the intramembrane cleavage of its C-terminus by γ-secretase results in AβP, a small peptide of 39–43 amino acid residues (Fig. 1A). Genetic studies of early-onset cases of familial AD have indicated that APP mutations and AβP metabolism are associated with AD.[29] Mutations of presenilins, which are the components of γ-secretases,[30] also account for most cases of early-onset familial AD.[31]

Yankner *et al.* reported that the first 40 amino acid residues of AβP, AβP(1–40), cause the death of cultured rat hippocampal neurons or neurodegeneration in the brains of experimental animals.[32] The oligomerization of AβP and its conformational changes are critical to the AβP-induced neurodegeneration process. AβP is a hydrophobic peptide and has an intrinsic tendency to self-assemble and form oligomers in aqueous solutions. The monomeric form of AβP has a randomly coiled structure and is less toxic than oligomeric AβPs, which have β-pleated sheet structures and form insoluble aggregates termed amyloid fibrils. Compared with that of freshly prepared AβP(1–40), the neurotoxicity of AβP(1–40) peptides is enhanced by the process of ''aging'' (aggregation under incubation at 37 °C for several days) and correlates with its β-sheet content.[33]

In young individuals as well as in elderly individuals with or without dementia, AβP is secreted in the cerebrospinal fluid (CSF).[34] Therefore, factors that accelerate or inhibit oligomerization may essentially alter the pathogenesis of AD.[35] Several factors, such as the concentration of peptides, composition and pH of solvents, and temperature, influence oligomerization. Oxidation, mutation, and racemization of AβP enhance its oligomerization, whereas other substances, including cholesterol or its oxidation products, apolipoprotein E, transthyretin, rifampicin, curcumin, aspirin, and β-sheet breaker peptide, inhibit AβP oligomerization *in vitro* (Fig. 1B).

Trace elements, including Al, Zn, Cu, and Fe, are accelerating factors, because metals are important determinants of protein conformation. It is well established that AβP accumulation is rarely observed in the brains of rodents (rats or mice), as compared with the brains of primates (humans or monkeys), and that rodent AβP exhibits a reduced tendency for oligomerization compared with human AβP *in vitro*.[36] As shown in Fig. 1C, although the amino acid sequences of human and rodent AβP are similar, they differ at three amino acids, with the human AβP having Arg5, Tyr10, and His13. Considering that these three amino acids can bind metals, it is plausible that the metal binding of these amino acid residues contributes to the amyloidogenicity of human AβP. Indeed, His13 is reportedly critical for the binding of Zn and Cu.[37]

Among these metals, Al^{3+} is of particular interest because the Al^{3+} in drinking water is considered to be an environmental risk factor for AD.[38] Compared with other metal ions, Al^{3+} has strong positive charges and a relatively small ionic radius.[7] Thus, Al^{3+} firmly binds to metal-binding amino acids and induces conformational changes in proteins. After developing a system for investigating AβP oligomerization that involves immunoblotting and precipitation, we demonstrated that Al enhances oligomerization of AβP(1–40) and forms sodium dodecyl sulfate (SDS)-stable oligomers *in vitro*.[39,40] The oligomerization induced by Al is more marked than that induced by other metals, including Zn, Fe, Cu, and Cd. Furthermore, Al-aggregated AβPs exhibit tight binding to the surface of cultured neurons and form fibrillar deposits. By contrast, Zn-aggregated AβPs are rarely observed on the surface of cultured neurons.

Other trace metals, for example Zn and Cu, also enhance AβP oligomerization. Bush *et al.* have demonstrated that Zn^{2+}, at a concentration similar to that in the CSF, produces AβP aggregation.[41] They also reported that Cu^{2+} binds to AβP, induces its aggregation, and increases ROS levels.[42] Faller *et al.* investigated the detailed characteristics and dynamics of AβP binding to Zn or Cu using mass spectrometry, UV-visible spectroscopy and fluorescence spectroscopy.[43] They found that Cu-AβP causes the production of ROS,[44] and their thermodynamic analysis revealed that the binding constants for AβP are much smaller compared to those of other metal-binding proteins such as metallothionein and SOD.[45] However, the role of these metal ions in AβP oligomerization is still controversial. The aggregation profiles are influenced by several experimental conditions such as pH, buffer contents, and peptide sequence.[46] Eury *et al.* investigated the coordination of Cu^{2+} to human or murine AβP using CD spectroscopy and NMR spectroscopy, and found that the coordination is pH-dependently and sequence-dependently different.[47] Moreover, recent studies demonstrate that metal-induced oligomerization of AβP is complex and not all oligomers are equally neurotoxic, despite all oligomers possessing β-pleated sheet structure. The characteristics (size or shape) of AβP oligomers formed in the presence of Al, Zn Cu, and Fe are identical, as assessed by morphological analysis using atomic force microscopy.[48] Additionally, Sharma *et al.* revealed that Zn-aggregated AβPs are less toxic than Cu-aggregated AβPs.[49] By contrast, Al-aggregated AβPs reportedly cause cytoskeletal changes, mitochondrial dysfunction, and increased ROS production compared to Zn- or Cu-aggregated AβPs.[50]

Although several studies have investigated the use of metal chelators as potential treatments for neurodegenerative diseases, the effects of these chelators are controversial since their precise target is not yet determined. For example, clioquinol (quinoform), a chelator of Cu^{2+} or Zn^{2+}, reportedly inhibits

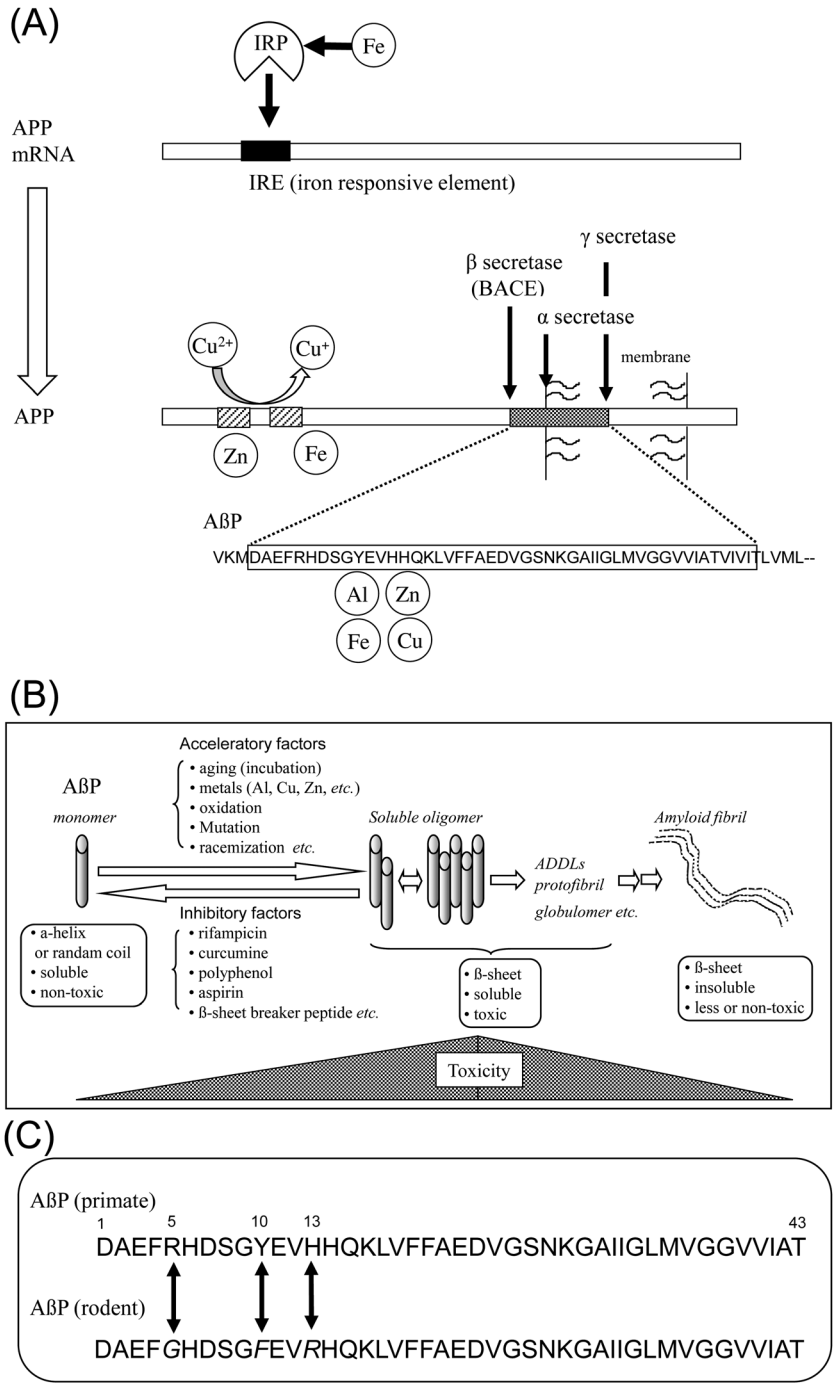

Fig. 1 Implications of metals in APP metabolism and AβP neurotoxicity. (A) Structure of AβP and its secretion from APP. AβP is secreted by the cleavage of the N-terminus of APP by β-secretase (BACE), followed by intra-membrane cleavage of the C-terminus by γ-secretase. APP also binds to Cu or Zn. The expression of APP is regulated by Fe and Al. (B) Oligomerization of AβP. AβP monomers exhibit random or α-helix structures. However, under aging conditions or in the presence of acceleratory factors, AβP self-aggregates and forms several types of oligomers (SDS-soluble oligomers, ADDLs, globulomers, protofibrils, *etc.*) before finally forming insoluble aggregates (amyloid fibrils). Oligomeric soluble Aβs are toxic, whereas the monomeric and fibril aggregates are relatively nontoxic. (C) Sequences of primate AβP and rodent AβP. Sequences of primate AβP and rodent AβP differ by three amino acids (Arg[5], Tyr[10], and His[13]).

oligomerization of AβP and attenuates the accumulation of amyloid in the brains of experimental animals.[51] Clinical trials using the clioquinol analogue PBT2 are reported to be effective.[52] However, Sampson *et al.* analyzed the results of several clinical trials and concluded that these chelators did not have any significant effect on cognition.[53] Other chelators such as deferoxamine,[54,55] a chelator of Al and Fe, or silicates,[56] which couple with Al and reduce its toxicity, are also candidates for chelation therapy in AD. However, there is still not enough evidence for the establishment of the new treatment.

Considering that these neurometals are essential for life and their deprivation by chelation is sometimes harmful, prudent trials are necessary for its evaluation. Indeed, it is widely known that clioquinol causes subacute myelo-optico-neuropathy (SMON).[57]

Interactions between metals and APP or presenilin

Despite the wide distribution of APP in the brain, its physiological roles are not well understood. APP has two Cu and/or Zn binding domains at its N-terminus and possesses the ability to reduce Cu^{2+} to Cu^{+}.[58] Copper induces the dimerization of APP and trafficking of APP from the ER to neurites.[59] Cu and Zn influence the expression and processing of APP and enhance AβP production.[60]

One functional role of APP is to regulate Fe homeostasis. Wong *et al.* found that APP binds to ferroportin, an iron transporter that regulates Fe^{2+} efflux.[61] Decreased levels of ferroportin and accumulation of Fe have been reported in the brains of AD patients.[62]

In general, the expression of Fe-binding proteins is regulated by the iron-responsive element/iron regulatory protein (IRE/IRP) network.[63] This system regulates production of Fe-binding proteins and prevents the formation of free Fe^{2+} and toxic free radicals. The mRNA encoding Fe-binding proteins, such as ferritin or transferrin, possesses IRE domains. In Fe-deficient conditions, IRP binds to the IREs and inhibits their expression. As the concentration of free Fe^{2+} increases, Fe binds to IRP and alters the expression of Fe-binding proteins (the expression of transferrin is downregulated, whereas that of ferritin is upregulated), thereby decreasing the amount of free Fe^{2+}. Rogers *et al.* found that APP mRNA contains an IRE domain as well as ferritin, and its expression is regulated by Fe.[64] Therefore, APP regulates Fe homeostasis, and conversely, Fe controls APP expression. Other important findings implicate Fe homeostasis in AD pathogenesis. Fe-related genes, such as transferrin C2 or the hemochromatosis gene, are risk factors for AD.[65] Imagawa *et al.* reported that Fe supplementation is effective for recovery of cognitive functions in patients with AD.[66]

These results suggest that APP plays crucial roles in the maintenance of metal homeostasis. When this homeostasis is disrupted, increased Fe or Cu causes ROS production or increased APP expression causes AβP production, and both initiate degenerative processes. Indeed, knockout of APP or its analogue APLP2 in mice results in increased Cu levels in the brain[67] and altered distribution of Cu, Zn, Fe, and Ca.[68] Ayton *et al.* demonstrated that APP-knockout mice exhibit iron-induced death of nigral neurons.[69] As discussed previously, Al has characteristics similar to Fe and binds to Fe-binding proteins, such as transferrin or ferritin. Al^{3+} reportedly binds to IRP[70] and thus may affect the expression of various Fe-regulated genes containing IREs, thereby causing elevation in Fe concentration. Indeed, Al reportedly enhances the expression of APP.[71,72] Pratico *et al.* found that mice transfected with the human APP gene (Tg 2576 mice), a mouse model of AD, and fed Al exhibit pathological changes similar to the human AD brain, including a marked increase in the amount of secreted and accumulated AβP as well as increased deposition of senile plaques.[73]

Presenilins, which are the components of γ-secretases, exist mainly in the ER and are implicated in Ca homeostasis maintenance, protein trafficking, and protein degradation.[74] Recent studies have demonstrated that presenilins are metal binding proteins and are implicated in the neuronal uptake of Zn and Cu,[75] which reportedly influence presenilin-induced trafficking pathways, γ-secretase activity, and APP processing.[60] Meanwhile, Zn influences the production of presenilins.[76]

However, in spite of this evidence, the link between AD and metals is complex and still enigmatic. Recent meta-analyses of metal levels in AD brains revealed that the results were highly heterogeneous, and that Zn was not significantly changed and Cu was depleted in AD patients. They also reported the lower level of ceruloplasmin and Cu in the CSF of AD patients.[77] Several studies about Zn supplementation suggested the protective roles of Zn.[78,79] Thus, further research about the bioavailability of metals and their neurotoxicity linked with protein conformational changes is necessary.

Prion diseases and metals

Interactions between metals and prion protein

Prion diseases are fatal neurodegenerative diseases that include scrapie in sheep and bovine spongiform encephalopathy in cattle as well as Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome, and Kuru disease in humans.[80] The common pathological hallmarks are the spongiform degeneration of neurons and glial cells and the accumulation of amyloidogenic prion protein (PrP) in the brain. Because prion diseases have characteristic infections that can be initiated by the administration of pathogenic tissue, they are also called transmissible spongiform encephalopathies. It is widely accepted that the conformational conversion of normal cellular prion protein (PrP^{C}) to an abnormal scrapie-type isoform (PrP^{Sc}) in pathogenic tissue is related to the transmissible characteristics of prion diseases. PrP^{C} is a 30–35 kDa cell surface glycoprotein anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) domain, and is ubiquitously expressed throughout the entire body, particularly in the brain. Both PrP^{C} and PrP^{Sc} have the same characteristics in terms of chemical modifications as well as the same primary sequence. However, PrP^{Sc} is resistant to protease digestion, has high β-sheet secondary structure, and has a propensity to form insoluble amyloid fibrils. When the misfolded PrP^{Sc} enters the body, for example by the administration of contaminated food or by iatrogenic contamination, the protease-resistant PrP^{Sc} can then aggregate and induce misfolding of other PrP^{C} molecules in the brain, which aggregate to become PrP^{Sc}.

Although the physiological roles of PrP^{C} are still controversial, increasing evidence suggests that PrP^{C} is a metalloprotein involved in metal homeostasis and possesses antioxidant and cytoprotective effects against the neurotoxicity induced by Cu^{2+}

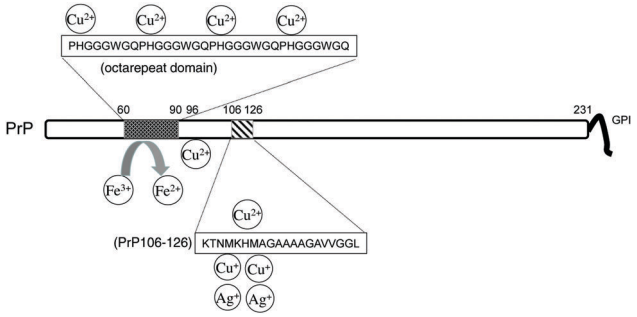

Fig. 2 Prion protein structure and metal-binding sites.

or free radicals.[81] PrP^C comprises 208 amino acid residues, and possesses a highly conserved octa-repeat domain composed of multiple tandem copies of the eight-residue sequence PHGGGWGQ in its N-terminus (Fig. 2). In 1997, Brown *et al.* reported decreased levels of Cu in the brains of PrP-knockout mice compared with that of wild-type mice.[82] The activity of Cu-dependent enzymes was also reduced in PrP-null mice. Jackson *et al.* reported that PrP^C binds four Cu atoms at its octa-repeat domain and binds two additional Cu atoms through two other His residues, His96 and His111. They also demonstrated that other metal ions including Zn^{2+}, Mn^{2+}, and Ni^{2+} can bind to these sites, although with lower affinity than Cu^{2+}.[83] Moreover, Cu^+ and Ag^+ reportedly bind to Met109 and Met112 of PrP.[84] Reportedly, PrP^C transports Cu^{2+} from the extracellular space to the intracellular space *via* endocytosis and regulates the intracellular concentration of Cu^{2+}, possesses or modulates Cu/Zn SOD activity in the brain, and has protective roles against oxidative stress.[85] This normal cellular prion protein also regulates the function of the NMDA-type glutamate receptor in a Cu-dependent manner.[86] By contrast, Cu^{2+} influences the gene expression and cellular trafficking of PrP^C.[87]

The ion with the next highest affinity, after Cu^{2+}, for binding PrP^C is Zn^{2+}. Bioinformatics analysis has revealed the evolutionary similarities between prion genes and genes encoding ZIP-type Zn transporters.[88] Among the 14 ZIP transporters, sequence similarities have been reported between PrP^C and the N-terminal ectodomains of ZIP5, ZIP6, and ZIP10. Watt *et al.* found that PrP^C facilitates the uptake of Zn^{2+} into neuronal cells and that the effect is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors.[89] These authors hypothesized that PrP^C functions as a Zn^{2+} sensor, detecting extracellular levels of Zn^{2+} up to a particular threshold. Indeed, PrP^C reportedly attenuates Zn^{2+}-induced neurotoxicity. Recent studies demonstrate that the PrP-like domain of ZIP6/ZIP10 is critical in the regulation of embryonic development, cell migration, and NCAM1 phosphorylation.[90]

In addition to Cu and Zn, other metals such as Fe and Mn are also associated with PrP. Singh *et al.* demonstrated that PrP^C functions as a ferrireductase that is responsible for reducing Fe^{3+} to Fe^{2+}.[91] The Fe^{2+} is translocated into cells by DMT1 or ZIP14 and is transferred to neuronal enzymes.[92] PrP-knockout mice reportedly exhibit altered Fe metabolism.[93] Decreased levels of ferroxidase activity and transferrin have been observed in the CSF of patients with CJD.[94]

Several studies have suggested that Mn facilitates prion diseases.[95] Thackray *et al.* reported that PrP^C loses its SOD-like activity when Cu is replaced with Mn.[96] Furthermore, Mn enhances the stability of PrP in soils and increases its infectivity.[97] The risk of contracting prion disease in elk, termed chronic wasting disease, has been associated with Mg deficiency and increased levels of Mn.[98] An epidemiological survey also suggests a relationship between the pathogenesis of CJD and Mn imbalance.[99]

The neurodegenerative mechanisms of prion diseases include three possibilities: the loss of normal protective functions of PrP^C, the gain of toxic functions of PrP^{Sc}, or a combination of both. The depletion of PrP^C may initiate various neurodegenerative processes, such as the oxidative damages caused by increased Cu and Fe, the increased susceptibility to damage by hydrogen peroxide with lower glutathione reductase activity, and the depletion of neurotransmitters.[100]

Interactions between PrP^{Sc} and metals

The abnormal scrapie-type isoform of prion protein PrP^{Sc} produces synaptic impairment and apoptosis in neurons and astrocytes *in vitro* and *in vivo*. To investigate the mechanisms of PrP^{Sc} neurotoxicity, researchers (including our laboratory) have used a synthetic fragment peptide of PrP (PrP106–126), because methodological difficulties occur when attempting to use a whole prion protein due to its strong infectious characteristics.[101] The PrP106–126 fragment has been used as a peptide model of PrP^{Sc}, as it coincides with the proposed sequence for β-sheet structures, forms aggregates of β-sheet structures to produce amyloid fibrils that share several characteristics with PrP^{Sc}, and causes the apoptotic death of cultured neurons or glial cells. Additionally, PrP106–126 has the ability to bind metals, including Cu^{2+} and Zn^{2+}.[102] We found that PrP106–126 forms β-sheet structures during the "aging" process (which involves incubation at 37 °C for several days) and that aged PrP106–126 produces significant neurotoxicity in primary cultured rat hippocampal neurons.[103] These characteristics are quite similar to those of AβP. We added various trace elements to solutions of PrP106–126 during the aging process and evaluated the resulting conformational changes and neurotoxicity. The presence of either Zn^{2+} or Cu^{2+} during the aging process significantly attenuates PrP106–126 neurotoxicity and significantly decreases the conformational changes of PrP106–126. Our results suggest that Cu and Zn influence the β-sheet formation of PrP106–126 and thereafter attenuate its neurotoxicity. Therefore, PrP^C regulates the homeostasis of Cu, Zn and Fe, and conversely, these metals regulate the neurotoxicity of PrP^{Sc} by affecting its conformation.

However, there is controversy about the effects of Cu on the conformational changes of other amyloidogenic proteins. Cu reportedly enhances the aggregation of various amyloidogenic proteins including AβP and α-synuclein,[43,104] but inhibits the oligomerization of human islet amyloid peptide (amylin).[105] The inconsistency may depend on the experimental conditions such as pH, sequence, *etc*. Valensin *et al.* investigated the binding of Cu^{2+} and Ni^{2+} to human or chicken PrP using NMR spectrometry and CD spectroscopy, and found that pH

influences the conformation in a sequence-dependent manner.[106] Therefore, the effects of trace elements on the conformational changes of amyloidogenic proteins are complex and need further investigation.

Lewy body diseases and metals

Lewy body diseases include Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy.[107] These diseases commonly exhibit abnormal cellular inclusions called Lewy bodies, which are the accumulation of α-synuclein, and are therefore known as synucleinopathies. DLB includes approximately 25% of all senile dementia cases and shares some pathological changes with AD, such as the deposition of senile plaques and tau protein. Moreover, the α-synuclein fragment peptide called non-amyloid component (NAC) co-accumulates with AβP in the senile plaques of patients with AD. The oligomerization and fibrillation of α-synuclein have been implicated in the formation of Lewy bodies and the etiology of Lewy body diseases, similar to AβP and PrP. Existing as a 140-amino acid protein, α-synuclein is abundantly present in the brain (Fig. 3), primarily in presynaptic terminals, and is thought to play a role in maintaining a supply of synaptic vesicles at the presynaptic terminals and in regulating the release of the neurotransmitter dopamine as well as in synaptic functions and plasticity.[108]

The association of metals with Lewy body diseases and α-synuclein has been extensively researched,[109] as patients with Mn toxicity exhibit Parkinson-like symptoms and because Fe-rich regions, such as the substantia nigra, are particularly vulnerable in Parkinson's diseases. Accumulation of Fe and Al has been reported in Lewy bodies of patients with Parkinson's disease. Uversky *et al.* found that metals, including Al and Mn, enhance α-synuclein oligomerization.[110] Cu^{2+} binds to α-synuclein at its N-terminal and C-terminal domains and promotes oligomerization of α-synuclein and formation of toxic oligomers (annular protofibrils).[111] α-Synuclein reportedly protects against Mn toxicity and regulates Mn and Ca levels.[112] Furthermore, α-synuclein binds Fe and functions as a ferrireductase, reducing Fe^{3+} to bioavailable Fe^{2+}, similar to PrP^{C}.[113] By contrast, Fe regulates the expression of α-synuclein, as its mRNA has an IRE domain as well as APP or ferritin.[114] In postmortem brains derived from patients with Parkinson's disease, Fe levels and the ratio of Fe^{2+} to Fe^{3+} were reportedly changed compared with those in control postmortem brains.[115]

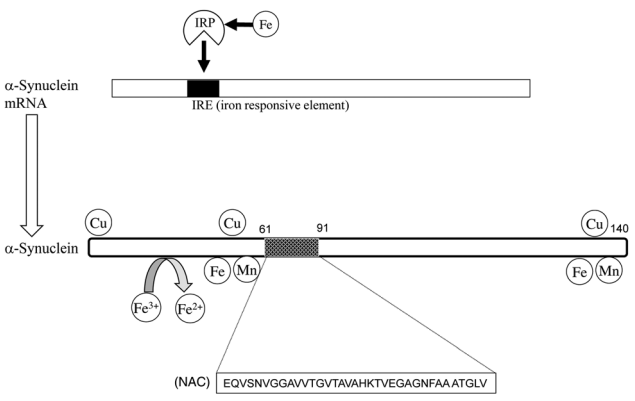

Fig. 3 α-Synuclein structure and metal-binding sites.

Vascular dementia and metals

Vascular dementia (VD) is a degenerative cerebrovascular disease that accounts for approximately one-third of senile dementia cases. Risk factors include aging, sex (male), diabetes, and high blood pressure. The most common type of VD is caused by a series of small strokes or transient global ischemia.[116] The interruption of blood flow and the resulting oxygen–glucose deprivation induce long-lasting membrane depolarization after transient global ischemia or stroke, and thereafter cause excessive glutamate release into synaptic clefts. The excess glutamate then overstimulates its receptors, and the entry of large quantities of Ca^{2+} triggers the delayed death of vulnerable populations of neurons, such as pyramidal neurons in the hippocampus, which is a region associated with learning and memory.

Increasing evidence suggests that Zn is central to ischemia-induced neuronal death and the pathogenesis of VD.[6,117] In ischemic conditions, excess Zn^{2+} is co-released with glutamate into synaptic clefts following membrane depolarization, and the neurons exhibit apoptotic cell death. Furthermore, an increase in intracellular Zn^{2+} ($[Zn^{2+}]_i$) levels, called Zn translocation, occurs in vulnerable neurons in the CA1 or CA3 region of the hippocampus prior to the onset of the delayed neuronal death that follows transient global ischemia.[118] Administration of calcium ethylenediamine tetraacetic acid (Ca EDTA), a membrane-impermeable Zn chelator, blocks Zn translocation, protects hippocampal neurons after transient global ischemia, and reduces infarct volume.[119] However, the role of Zn in ischemia-induced neurodegeneration is still controversial. Several studies reported the beneficial characteristics of Zn supplementation in heat ischemia and testis ischemia;[120,121] meanwhile, chronic Zn supplementation increased neuroinflammation in hypoxia-ischemia.[122] These inconsistent results may depend on the complex roles of Zn since Zn plays various important functions in various regions. Thus, the neurotoxicity of Zn as well as its entry into the brain (*i.e.* bioavailability) should be more precisely investigated.

We have investigated the molecular mechanisms of Zn^{2+}-induced neuronal death by using immortalized hypothalamic neurons called GT1-7 cells, which possess neuronal characteristics[123] and are much more sensitive to Zn^{2+} than other neuronal cells.[124] We demonstrated that the ER stress pathway, the mitochondrial energy pathway, and the disruption of Ca^{2+} homeostasis all contribute to Zn-induced apoptosis.[125–128]

Furthermore, we recently found that sublethal concentrations of Cu^{2+} strongly enhance Zn^{2+}-induced neurotoxicity and the expression of ER stress-related genes.[129] Fig. 4A exhibits that the cell viability exposed to Zn^{2+} is significantly decreased in concentration dependent manner of Cu^{2+}. GT1-7 cells exposed

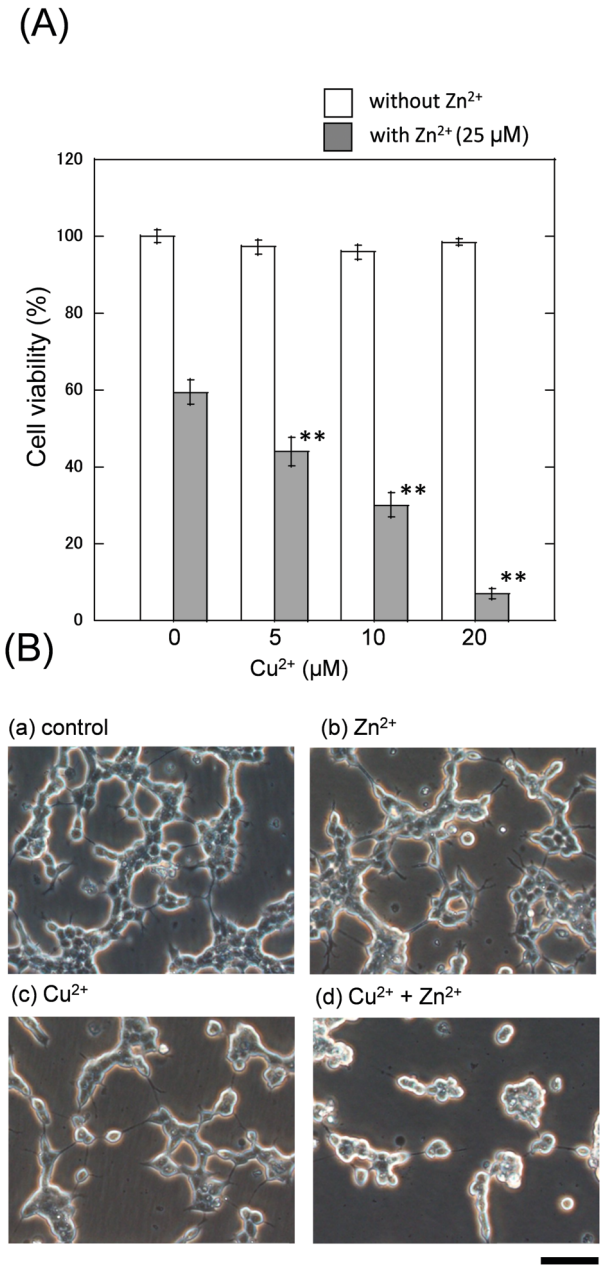

Fig. 4 Cu^{2+}-enhanced Zn^{2+}-induced neurotoxicity. (A) Various concentrations of $CuCl_2$ (0–20 μM) without $ZnCl_2$ or with 25 μM $ZnCl_2$ were administered to GT1-7 cells. After 24 h, cell viability was determined using the WST-8 method. Data are presented as means ± S.E.M. *$p < 0.05$, **$p < 0.01$ *versus* 25 μM $ZnCl_2$ alone (Tukey's test). (B) Phase-contrast images of GT1-7 cells after 24 h of exposure to 30 μM Zn^{2+} (b), 20 μM Cu^{2+} (c), and 30 μM Zn^{2+} with 20 μM Cu^{2+} (d). The scale bar represents 100 μm. GT1-7 cells cultured in serum-free conditions display neuron-like characteristics with neurite extension (a, control). After 24 h of exposure to Zn^{2+}, some cells exhibit round cell bodies with retracted neurites (b). Although Cu^{2+} applied alone does not lead to significant changes (c), exposure to a combination of Cu and Zn enhances neurodegeneration (d).

to the combination of Zn^{2+} and Cu^{2+} exhibited marked degenerative properties compared to treatment with either Zn^{2+} or Cu^{2+} alone (Fig. 4B). Considering that Cu^{2+} is released in synaptic clefts, similar to Zn^{2+}, the synergistic interactions between Zn^{2+} and Cu^{2+} may play critical roles in the pathogenesis of VD. These findings may explain the region-specific vulnerability of neurons after ischemia. We previously reported that carnosine (β-alanyl histidine) attenuates Zn^{2+}-induced neuronal death and prevents Zn^{2+}-induced ER stress. Carnosine is a water-soluble dipeptide present in mammalian muscles and in the brain, particularly in the olfactory bulbs.[130] Carnosine has antioxidant and antiglycation properties as well as the ability to bind metals. Additionally, carnosine possesses anti-cross-linking properties, inhibits the oligomerization of AβP, and attenuates neurodegeneration in a mouse model of AD.[131] Our chemical screen for substances that protect against PrP106–126-induced neurotoxicity indicated that carnosine may be a candidate treatment for prion diseases.[103] Considering these beneficial characteristics, carnosine may have neuroprotective functions in the brain. Based on these findings, we published a patent for carnosine to be used as a basis for drugs designed to treat vascular dementia.[132,133]

Neurotoxic mechanisms common to both amyloidogenic proteins and metals

Based on the aforementioned findings, a relatively new concept, termed 'conformational diseases', was proposed, suggesting that the conformational structure of the protein is an important determinant of its toxicity and, consequently, the development of the related disease.[134] Moreover, these amyloidogenic proteins share common characteristics in their neurotoxic mechanisms.[135]

Although exposure to AβP adversely affects neuronal survival, as it leads to the production of ROS, induction of cytokines and ER stress, and an abnormal increase in intracellular calcium ($[Ca^{2+}]_i$) levels, disrupted Ca^{2+} homeostasis is considered to be an important determinant, given that it occurs upstream of the other effects. In 1993, Arispe *et al.* first demonstrated that AβP(1–40) directly incorporates into artificial lipid bilayer membranes and forms cation-selective ion channels.[136] The channels, termed amyloid channels, were revealed to be giant multilevel pores that allow a large amount of Ca^{2+} to pass through. Durell *et al.* proposed a 3-D structural model of the amyloid channels obtained from a computer simulation of the secondary structure of AβP(1–40) in membranes that showed 5- to 8-mers aggregating to form pore-like structures on the membranes.[137] The multimeric (tetramer to hexamer) pore-like structures of the AβPs on liposomes were observed by using atomic force microscopy. These results strongly support the amyloid channel hypothesis, which suggests that the direct incorporation of AβPs and the subsequent imbalance of Ca^{2+} that occurs through amyloid channels are the primary events in AβP neurotoxicity. In this respect, AβP may share a toxicity mechanism with some antimicrobial or antifungal peptides that also exhibit channel-forming activity and cell toxicity. Electrophysiological and morphological studies have demonstrated that PrP and α-synuclein also exhibit similarities with AβP in their formation of pores (amyloid channels).[138,139] Lal *et al.* used gel electrophoresis and atomic force microscopy imaging to investigate the oligomerization and conformational

changes of AβP, α-synuclein, amylin, and other amyloidogenic proteins, and demonstrated that these amyloidogenic proteins form annular channel-like structures on bilayer membranes.[140] Using high-resolution multisite video imaging with fura-2 as a fluorescent cytosolic free calcium reporter probe, we have shown that the amyloidogenic peptides AβP, PrP106–126, and NAC increase $[Ca^{2+}]_i$ levels.[141] Considering these results together (shown in Table 1), it is suggested that the oligomerization of disease-related amyloidogenic proteins and the introduction of apoptotic degeneration by disruption of calcium homeostasis *via* unregulated amyloid channels may be the molecular basis of neurotoxicity in these diseases.

Metals also participate in the formation and toxicity of amyloid channels. The activity of amyloid channels is blocked by Zn^{2+} ions, which bind His residues on the inner side of the pore.[142] We have also demonstrated that channel activity on neuronal membranes is inhibited by the addition of Zn^{2+} and restored by a zinc chelator, *o*-phenanthroline.[143] Kourie *et al.* investigated the characteristics of channels formed by PrP106–126, concluding that Cu^{2+} modulates channel activity.[144] Additionally, Cu^{2+} reportedly enhances pore formation by α-synuclein.[145]

Hypothesis: cross talk among metals and amyloidogenic proteins in the synapse

As shown in Table 1, amyloidogenic proteins share common characteristics, including oligomerization with β-pleated sheet structures, neurotoxicity, pore formation, Ca^{2+} dyshomeostasis, and the ability to bind metals. Additionally, all amyloidogenic proteins are reportedly co-localized at the synapse, small but crucial nodes of neural networks. APP is found primarily in the presynaptic membrane,[146] PrPC in postsynaptic membranes,[147] and α-synuclein in the presynaptic cytosol and to a lesser extent in membranes.[107] Presenilins are predominantly located in the ER, but also occur in presynaptic and postsynaptic membranes.[148]

Furthermore, the synapse is the primary target of neurodegeneration.[149] The vulnerability of synapses in these neurodegenerative diseases is especially problematic because synaptic plasticity is essential for memory formation. Neurotransmitters and metals (Zn^{2+} or Cu^{2+}) are co-released from synaptic vesicles located at presynaptic terminals into synaptic clefts, where they bind to receptors at postsynaptic densities (Fig. 5A). The synaptic cleft can be conceptualized as a cylinder with a height of 20 nm and a radius of 200 nm.[150] It is plausible that neurotransmitters or metals are concentrated in synaptic clefts, and their levels may be much higher than those in the extracellular fluid. Indeed, the concentration of glutamate in the synaptic cleft is estimated to reach the mM range after 1 ms of neuronal depolarization. The level of Zn^{2+} in the synapse is estimated to be 1–100 μM, although still controversial,[151,152] whereas its level in the CSF is less than 1 μM. The level of Cu^{2+} in the synapse is estimated to be approximately 15 μM.[153] Secreted Zn^{2+} binds with NMDA-type glutamate receptors or other receptors and controls the excitability of the target neuron. It is possible that secreted Zn^{2+} can diffuse across the synaptic cleft, spill over to neighboring synapses, and modulate the activity of

Table 1 Characteristics of amyloidogenic proteins

Disease	The primary sequence of amyloidogenic protein or its fragment peptide	Metal	β-Sheet formation	Cytotoxicity	Pore formation	Ca dyshomeostasis
Alzheimer's disease	AβP(1–42) *DAEFRHDSGYEVHHQKLVFFAEDVG* *SNKGAIIGLMVGGVVIA*	Al, Zn, Cu, Fe	+	+	+	+
Prion disease	Prion protein: PrP106–126[a] MANLGCWMLVLFVATWSDL GLCKKRPKPGGWNTGGS RYPGQGSPGGNRY PPQGGGGWGQPHGGGW GQPHGGGWGQPHGGGWG QPHGGGWGQGGG THSQWNKPSKP*KTNMKHMAGAAAA* *GAVVGGLG*GYVL GSAMSRPIIHF GSDYEDRYYRENMHR YPNQVYYRPMDEY SNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKM MERVVEQMCITQYERESQA YYQRGSSMVLFSSPP VILLISFLIFL IVG	Zn, Cu, Mn, Fe	+	+	+	+
Lewy body diseases	α-Synuclein; NAC (a fragment of α-synuclsin)[a] MDVFMKGLSKAKEGVVA AAEKTKQGVAEAAGKTKE GVLYVGSKTKEGVVHGVTT VAEKTK*EQVSNVGGAVVTGVTAVA* *HKTVEGAGNFAAATGLV*KKDQKNESG FGPEGTMENSENMPVNPNNETYEM PPEEEYQDYDPEA	Cu, Fe, Mn, Al	+	+	+	+

[a] The sequence of fragment peptide of each amyloidogenic protein (PrP106–126, NAC) is indicated in italic form.

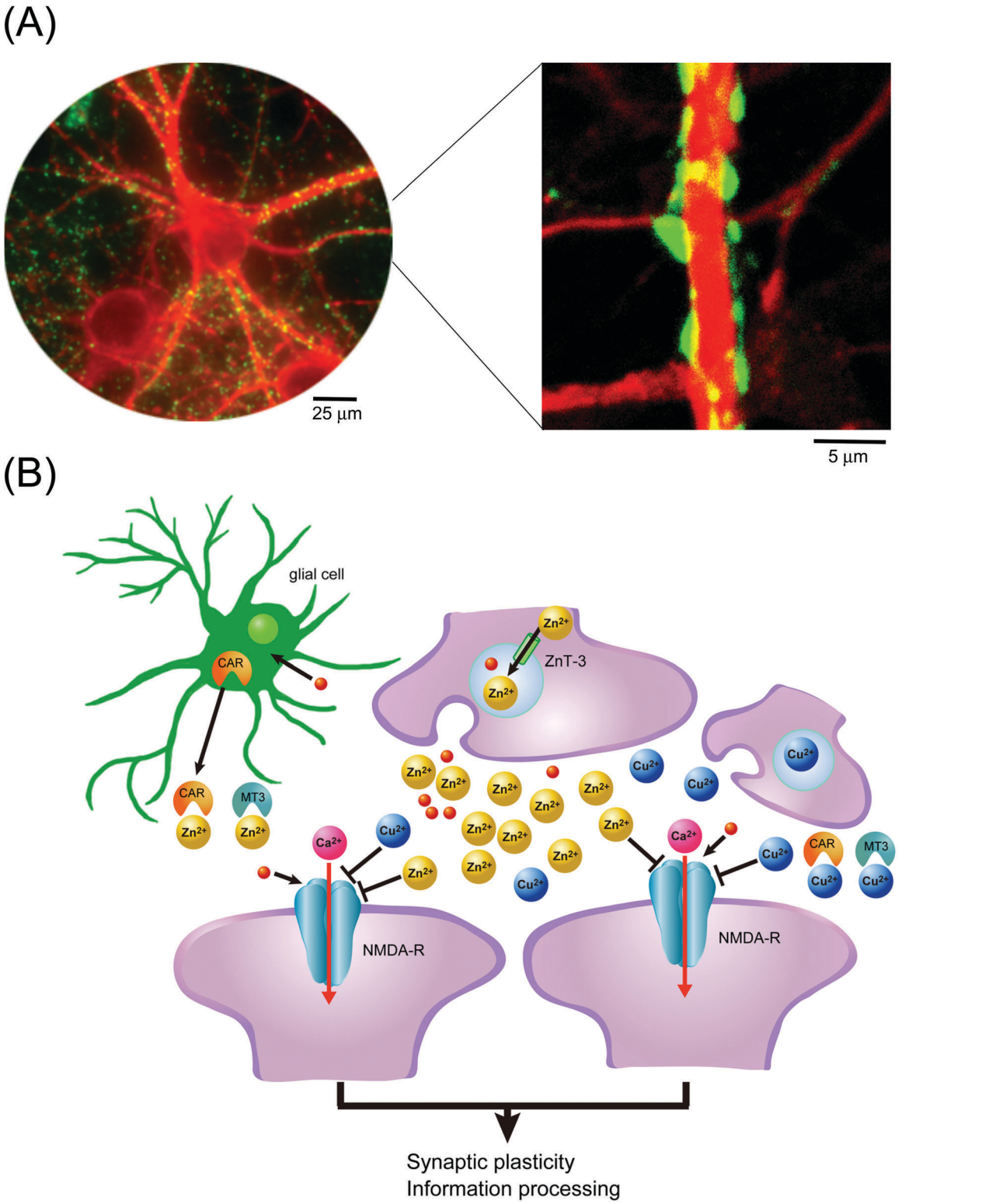

Fig. 5 Zn^{2+} and Cu^{2+} roles at synapses. (A) Laser confocal images of synapses at lower (left) and higher magnification (right). Primary cultured rat hippocampal neurons double-immunostained with antibodies to MAP2 (red) and synapsin1 (green). (B) Under physiological conditions, Zn^{2+} is stored in presynaptic vesicles, released with glutamate, and inhibits NMDA-type glutamate receptors (NMDA-R). The Zn^{2+} spilled over to the neighboring synapse modulates excitability and is implicated in the maintenance of synaptic plasticity and memory formation. The Cu^{2+} also secreted during neuronal excitation acts similar to Zn^{2+}. ZnT-1, zinc transporter 1; AMPA-R, AMPA-type glutamate receptor; NMDA-R, NMDA-type glutamate receptor; MT-3, metallothionein 3.

these neighboring synapses, as shown in Fig. 5B. Because glutamate produces excitation and Zn^{2+} produces inhibition, it is plausible that differing concentrations of glutamate and Zn^{2+} in adjacent synapses transmit the spatiotemporal information of neuronal activity and create precise modulation of neuronal activity. This modulation of neuronal activity at adjacent synapses generates contrasting signals that may enable lateral inhibition and be the basis for synaptic plasticity.[154] The released Cu^{2+} also spills over to neighboring synapses and modulates brain excitability by binding to NMDA-type glutamate receptors and other receptors.

Based on the aforementioned findings, we developed a hypothetical scheme describing the interactions among metals and amyloidogenic proteins at the synapse (Fig. 6). In normal physiological conditions, considerable amounts of metals exist in the synaptic cleft, and amyloidogenic proteins are thought to

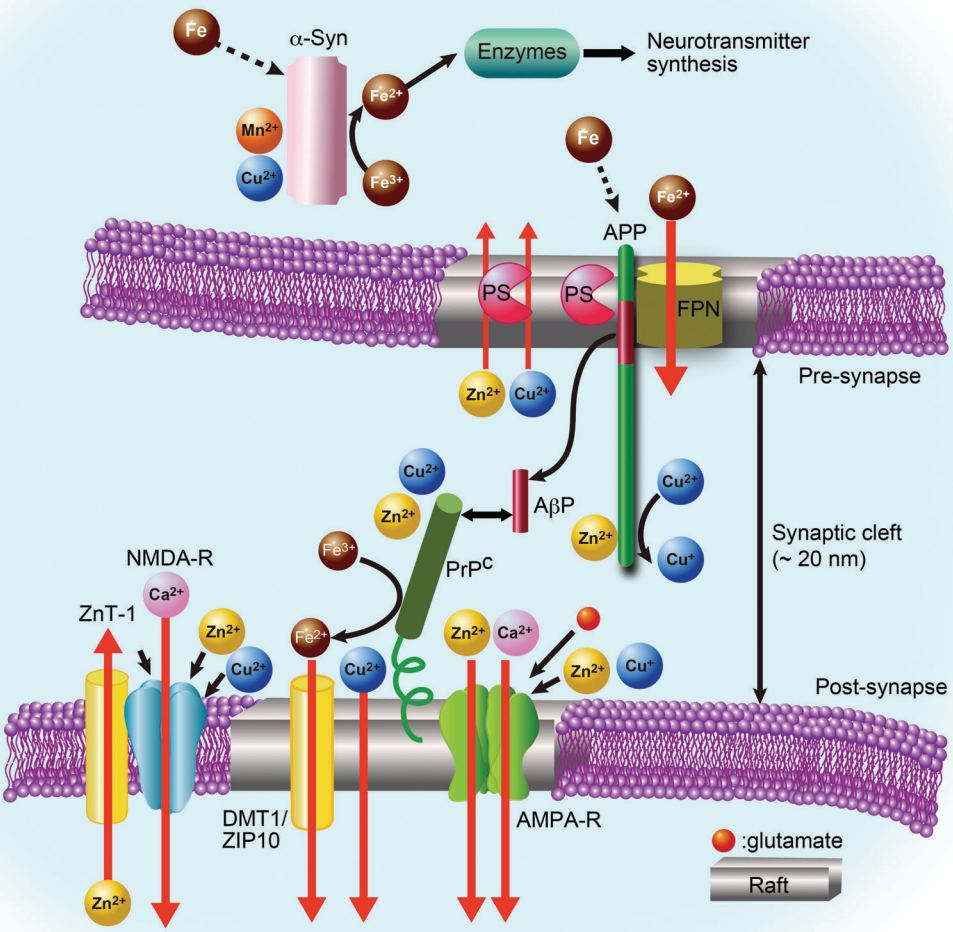

Fig. 6 Hypothesis: cross talk between trace elements and amyloidogenic proteins at the synapse. APP and α-synuclein exist in the presynaptic domain, whereas PrP^C is localized in the postsynaptic domain. These proteins are closely associated with both the synaptic cleft and Zn^{2+} and Cu^{2+}, and they have cytoprotective roles *via* the regulation of metal homeostasis and protection from free radicals. APP and PrP^C are localized on lipid rafts with their binding proteins. PrP^C binds to AMPA-type glutamate receptors and regulates Zn^{2+} levels, similar to ZIP Zn transporters. Additionally, the ZnT-1 Zn transporter is localized in postsynaptic membranes that express NMDA-type glutamate receptors and regulates Zn homeostasis. Moreover, PrP^C has SOD activity and also regulates Cu^{2+} levels, which influence APP sequencing. Presenilins also affect uptake of Zn^{2+} and Cu^{2+}. APP converts Cu^{2+} to Cu^{+} and regulates Cu at the synapse. PrP^C and α-synuclein have ferrireductase activity, which converts Fe^{3+} to Fe^{2+}. APP binds to ferroportin and thereby regulates Fe^{2+} efflux. MT3 and carnosine are released from glial cells, into synaptic clefts and are also implicated in the regulation of excess Zn. Details are provided in the text. FPN, ferroportin; PS, presenilins; α-Syn, α-synuclein.

play neuroprotective roles in the maintenance of metal homeostasis, especially in regulating the metal level at the synapse. The distance across the synaptic cleft (approximately 20 nm) may be sufficiently short for proteins at the presynaptic terminal to interact with proteins at the postsynaptic terminal. Therefore, the amyloidogenic proteins must certainly interact with other proteins while surrounded by a considerable amount of Zn^{2+} and Cu^{2+}. Indeed, PrP^{C} reportedly binds to AβP oligomers and attenuates their neurotoxicity.[155] α-Synuclein also influences the processing of APP.[156] Furthermore, both APP and PrP^{C} are located in lipid rafts, microdomains that are enriched in gangliosides, glycosphingolipids, and cholesterol.[157,158] Considering the negative charges of lipid rafts owing to the sialic acid of gangliosides, lipid rafts may serve as working platforms for the amyloidogenic proteins and metals.

Furthermore, isoform PrP^{C} controls Zn^{2+} influx into the cells as an analogue of ZIP transporters, by binding with AMPA-type glutamate receptors to regulate synaptic Zn^{2+} levels. The ZnT-1 transporter, which enhances Zn efflux to the extracellular compartment, is also localized in postsynaptic membranes and regulates the activity of NMDA-type glutamate receptors.[159]

PrP^{C} binds to Cu *via* its N-terminal domain and regulates synaptic Cu levels. It is possible that PrP^{C} provides Cu to APP or to NMDA-type glutamate receptors, thereby influencing the production of AβP or neuronal excitability. APP binds Cu and regulates Cu levels by reducing Cu^{2+} to Cu^{+}. Both APP and PrP^{C} reportedly attenuate Cu-induced toxicity. α-Synuclein also binds to intracellular Cu or Mn and regulates their levels in the presynaptic domains, and presenilins also control the influx of Cu^{2+} and Zn^{2+}.

APP binds ferroportin and regulates Fe^{2+} efflux. By contrast, both PrP^{C} and α-synuclein act as ferrireductases to regulate the Fe^{2+}/Fe^{3+} ratio in synapses. The Fe^{2+} ions are translocated into cells by DMT1 or ZIP14 and transferred to enzymes, including neurotransmitter synthetases. In contrast, Fe levels regulate the expression of APP and α-synuclein.

Other regulatory factors for metal homeostasis operate at the synapse. The metallothionein MT3 secreted from neurons or glia may also regulate Zn homeostasis at synapses. In addition, MT3 has been implicated in AD-associated neuronal death, since the neuronal growth inhibitory factor (GIF) that is decreased in the brains of patients with AD was determined to be MT3.[160] Considering that the binding constant of MTs and Cu or Zn is much smaller compared to that of AβP,[45] it is possible that MT3 plays a role in buffering of metals in normal synapses.

Another contributor to metal homeostasis is carnosine, an endogenous antioxidant and anti-cross-linking peptide synthesized and secreted from glial cells.[161] Carnosine is reportedly decreased during aging. MT3 is reportedly downregulated in AD patients, and carnosine is age-dependently decreased.[162]

These complex and subtle interactions between metals and amyloidogenic proteins maintain synaptic functions and protect neurons from ROS and excess metals. PrP^{C} functions as a Cu/Zn-SOD and protects neurons from ROS induced by excess Cu or Fe. When these homeostatic mechanisms are disrupted, various adverse effects trigger degenerative processes in synapses and neurons, which contribute to the pathogenesis of neurodegenerative diseases. For example, accumulated Al influences Fe homeostasis by binding to Fe-binding proteins, including APP, α-synuclein, and ferritin, thereby increasing Fe levels. Overexpression of APP induced by Al results in accumulation of AβP. Aluminum and other metals also accelerate AβP oligomerization and enhance neurotoxicity. The AβP oligomers may form amyloid channels on synaptic membranes, produce Ca^{2+} dyshomeostasis, initiating synaptotoxicity and neurotoxicity, and produce the pathogenesis observed in AD.

Pathogenetic PrP^{Sc} in contaminated food enters the brain and triggers conformational changes in PrP^{C}, which causes conversion of PrP^{C} to PrP^{Sc}. Loss of the neuroprotective PrP^{C} results in oxidative stress, enhances the neurotoxicity of AβP, and results in the apoptotic death of neurons and glia. Additionally, accumulated PrP^{Sc} may form amyloid channels in the membrane and thereby disrupt Ca^{2+} as well as AβP homeostasis. Manganese may enhance PrP^{Sc} toxicity by influencing the conversion of PrP^{C}. The oligomerization of α-synuclein caused by toxic metals accelerates the formation of amyloid channels and induces neurotoxicity. In the case of global brain ischemia, excess Zn^{2+} as well as Cu^{2+} is released into the synaptic cleft. Thereafter, excess Zn^{2+} triggers various neurodegenerative pathways including disruption of Ca^{2+} homeostasis, mitochondrial energy depletion, and ER stress. The different ratio between Zn^{2+} and Cu^{2+} may influence the vulnerability of neurons in the brain regions. Considering that vascular degeneration may be linked to the early stage of AD, Zn^{2+} neurotoxicity may also be associated with AD.

Conclusions

We presented the complex and subtle interactions between metals and amyloidogenic proteins at the synapse. This cross talk is essential for normal brain functions, and, therefore, the disruption of metal homeostasis may contribute to the conformational changes of amyloidogenic proteins and to the pathogenesis of the related neurodegenerative diseases. Our findings suggest that metal dyshomeostasis may be a common mechanism for the pathogenesis of these diseases, may elucidate the enigmatic roles of trace elements in neurodegenerative diseases, and may be important in preventive drug development. In conclusion, our working hypothesis may shed light on the role of metals in neurodegenerative diseases. However, the precise roles of neurometals in neurodegenerative diseases are still under investigation. Further research on neurometallomics will be necessary, particularly in relation to the molecular mechanisms of synaptic degeneration and the quantitative analysis of neurometals.

Acknowledgements

This work was partially supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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