Chapter 14
Link Between Metal Homeostasis and Neurodegenerative Diseases: Crosstalk of Metals and Amyloidogenic Proteins at the Synapse

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Abstract Increasing evidence suggests that disruption of metal homeostasis contributes to the pathogenesis of various neurodegenerative diseases, including Alzheimer's disease, Lewy body diseases, vascular dementia, and prion diseases. Conformational changes of disease-related proteins such as ß-amyloid protein, α-synuclein, and prion proteins are well-established contributors to the synaptotoxicity, neurotoxicity, and pathogenesis of these diseases. Recent studies have revealed that these proteins are metalloproteins that coexist in synapses and play significant roles in the maintenance of metal homeostasis in synapses. Trace elements such as zinc (Zn), iron (Fe), copper (Cu), and aluminum (Al) bind to these proteins, thereby influencing their conformations and functions. Additionally, these metals have common binding sites; binding of metals to proteins is nonspecific. Therefore, metal-metal interactions at synapses contribute to the neurodegenerative processes. We present a current review of the role of trace elements in the functions and toxicity of disease-related proteins, as well as in the pathogenesis of neurodegenerative diseases. Possible therapeutic approaches related to metal homeostasis are discussed.

Keywords Calcium homeostasis • Iron homeostasis • Neurotoxicity • Alzheimer's disease • Prion disease

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14.1 Introduction

The brain is a unique organ, representing only 2 % of the typical human body weight, but consuming 20 % of the total oxygen used by the body. The disproportionate oxygen metabolism of the brain contributes to increased susceptibility to oxidative stress. The brain is primary composed of two cell types: neurons and glial cells. Neurons generally do not regenerate and are maintained for the individual's entire life span. Therefore, neurons are susceptible to accumulation of toxic substances such as heavy metals. The blood-brain barrier (BBB), which is composed of tight junctions between endothelial cells, is one mechanism to prevent brain exposure to toxic substances. However, this barrier system is not perfect, and when the brain experiences long durations of toxic substance exposure, the substances may accumulate and result in adverse effects such as Minamata disease.

Essential trace elements including iron (Fe), zinc (Zn), and copper (Cu) exist in the brain and have crucial roles in normal brain functions, such as myelination, neurotransmitter synthesis, and neural information processing. Deficiency of these metals produces severe adverse effects on central nervous system functions, especially learning and memory. Moreover, growing evidence suggests that dyshomeostasis of metals, either excess or deficiency, is implicated in the pathogenesis of various neurodegenerative diseases including Alzheimer's disease (AD), prion diseases, Lewy body diseases (Parkinson's disease, dementia with Lewy bodies (PD/DLB), etc.), and vascular dementia [1, 2]. All of these diseases, except for vascular dementia, include abnormal deposition of disease-related proteins in the brain. The disease-associated proteins, termed amyloidogenic proteins, are ß-amyloid protein (AßP) in AD, prion protein in prion diseases, and α-synuclein in Lewy body diseases. Although the primary sequences are identical to typical proteins, the disease-associated proteins form fibril-like structures of oligomers with the ß-pleated sheet structures (amyloid fibrils) and are associated with neurotoxicity. Since the conformational changes to these amyloidogenic proteins are central to the pathogenesis, a new category, termed "conformational diseases" (protein-misfolding diseases), has proposed [3]. Metals are of particular interest as factors that influence conformational changes to the amyloidogenic proteins [4]. Recent studies have demonstrated that these amyloidogenic proteins or their precursor proteins (such as amyloid precursor protein; APP) possess the binding ability to metals and thereby participate in regulating metal homeostasis. Furthermore, metals and most of these amyloidogenic proteins are co-localized at the synapses which are the crucial node of neural networks. Table 14.1 summarizes the structure and properties of theses amyloidogenic proteins.

In the current neurometallomics study, we investigated the molecular mechanisms of neurodegenerative diseases, focusing on metal-protein interactions and metal-metal interactions at the synapse. Here, we describe the characteristics of three essential elements (Fe, Zn, Cu) and one toxic element (Al) in the brain, and thereafter, we review the functions and neurotoxicity of metals as well as the

Table 14.1 Characteristics of amyloidogenic proteins and the related peptides

Disease	The primary sequence of amyloidogenic protein or its fragment peptide	Binding metal	ß-sheet formation	Cytotoxicity	Channel formation	Functions
Alzheimer's disease	*AβP(1-42)*	Al	+	+	+	(Functions of APP)
	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA	Zn				Neuronal proliferation and development
		Cu				Neurite outgrowth
		Fe				Fe homeostasis
Prion disease	Prion protein: *PrP106-126*	Zn	+	+	+	SOD activity
	MANLGCWMLVLFVATWSDLGLCKKRPKPGG WNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPH GGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKP *KTNMK HMAGAAAAGAVVGGLG*GYVL GSAMSRPIIH FGSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDC VNITIKQHTVT TTTKGENFTE TDVKMMERVVEQMCITQY ERESQAYYQRGS SMVLFSSPPVILLISFLIFL IVG	Cu				SOD activity
		Fe				Cu homeostasis
		Mn				Zn homeostasis
						Ferrireductase activity
Lewy body disease	α-synuclein; *NAC* (a *fragment of α-synuclein)*	Cu	+	+	+	Dopamine release
	MDVFMKGLSKAKEGVVAAAEKTKQGVAEAA GKTKEGVLYVGSKTKEGVVHGVTTVAEKTK *EQVSNVGGAVVTGVTAVAHKTVEGAGNFAA ATGLV*KKDQKNESGFGPEGTMENSENMPVNPNNETY EMPPEEEYQDYDPEA	Fe				Ferrireductase activity
		Al				

The sequence of fragment peptide of each amyloidogenic protein (PrP106-126, NAC) is indicated in italic form
In "functions" of Alzheimer's disease, possible functions of APP are noted

implications for neurodegenerative disease pathogenesis, based on recent studies from our lab and those by other authors.

14.2 Properties of Metals in the Brain

14.2.1 Iron (Fe) in the Brain

Considerable amounts of trace elements such as Fe, Zn, and Cu exist in the brain, in addition to ubiquitous elements such as sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg). The distribution of each metal differs across brain regions [5]. Among these trace elements, Fe is the most abundant in the brain and in the whole body. Fe is essential for numerous biological functions as an enzyme cofactor for metabolic processes such as the oxygen transport, oxidative phosphorylation, and energy transfer. Fe has critical roles in specialized brain functions such as the synthesis of dopaminergic neurotransmitters and the myelination. Therefore, Fe deficiency impairs learning abilities, especially in children or infants, and it produces impaired working ability or learning ability also in adults [6]. However, excess Fe can generate reactive oxygen species (ROS) that damage DNA, proteins, and lipids and can therefore be toxic to neurons [7]. Fe exists in two different forms: ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}). In general, oxidized Fe^{3+} is insoluble and exists extracellularly, whereas reduced Fe^{2+} is soluble and intracellularly located. Orally administrated Fe is primary absorbed from the gastrointestinal pathway by divalent metal transporter-1 (DMT-1) as Fe^{2+} [8]. Once it enters the circulation, Fe^{2+} ions are oxidized to Fe^{3+} by ferroxidases such as ferritin or ceruloplasmin. It binds with transferrin, an iron binding protein that binds two Fe^{3+}ions. Transferrin-bound iron (Fe^{3+}) crosses BBB *via* transferrin receptors and enters into the cells. Finally, Fe^{3+} is reduced to Fe^{2+} by ferrireductase and function as a cofactor for neuronal enzymes such as tyrosine hydroxylase, which is necessary for the dopamine synthesis. Thus, Fe levels as well as the ratio between Fe^{2+} and Fe^{3+} are strictly regulated in normal brains. Two amyloidogenic proteins, prion protein and α-synuclein, reportedly possess ferrireductase activity [9, 10]. Ferroportin, a transmembrane protein, controls Fe export from intracellular to extracellular compartments. Recent findings suggest that APP, a precursor protein of Alzheimer's AßP, binds to ferroportin and regulates Fe efflux [11].

The iron-responsive element/iron-regulatory protein (IRE/IRP) network also regulates Fe levels [12]. The mRNA encoding Fe-binding proteins such as ferritin or transferrin possess IRE domains in their mRNA. This system regulates production of Fe-binding proteins and prevents the formation of free Fe^{2+} and toxic free radicals. In Fe-deficient conditions, IRP binds to the IREs and inhibits their expression. As the concentration of free Fe^{2+} increases, binding of Fe to IRP and the expression of transferrin is downregulated, whereas that of ferritin is upregulated, thereby decreasing the amount of free Fe^{2+}. The expression of two

amyloid-related proteins, APP and α-synuclein, is also controlled by Fe [13] (*see* Sects. 14.3, 14.4, and 14.5).

14.2.2 Zinc (Zn) in the Brain

Zn is the second most abundant element in the brain. Zn is essential for most organisms and has important roles in various physiological functions such as mitotic cell division, immune system functioning, synthesis of proteins and DNA, and as a cofactor to more than 300 enzymes or metalloproteins. Recent studies have revealed that Zn signaling plays crucial roles as a second messenger in various human biological systems [14]. Zn deficiency in children results in dwarfism, delayed mental and physical development, immune dysfunction, and learning disabilities [15]. Zn deficiency also produces learning disorders, taste disorders, and odor disorders also in adults [16].

The human body contains approximately 2g of Zn, primary located in the testes, muscle, liver, and brain tissues. In the brain, Zn occurs at the highest concentrations in the hippocampus, amygdala, cerebral cortex, thalamus, and olfactory cortex [17]. The total Zn content of the hippocampus is estimated to be 70–90 mg/kg (dry weight). Although some Zn in the brain 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 is stored in the presynaptic vesicles of excitatory glutamatergic neurons and is secreted from these vesicles into synaptic clefts, together with glutamate, during neuronal excitation. The concentration of this secreted Zn is estimated to be 1–100 μM [18], although this value is still controversial. Secreted Zn modulates overall brain excitability by binding with *N*-methyl-D-aspartate (NMDA)-type glutamate receptors, GABA receptors, and glycine receptors. Recent studies have suggested that the secreted Zn^{2+} is critical to information processing, synaptic plasticity, learning, and memory [19, 20]. Indeed, Zn^{2+} in the hippocampus is essential for the induction of long-term potentiation (LTP), a form of synaptic information storage that has become a well-known paradigm for the mechanisms underlying memory formation [21].

There are two factors in the maintenance of Zn homeostasis: metallothioneins and Zn transporters. Metallothioneins are ubiquitous metal-binding proteins with 68 amino acids, which bind seven metal atoms (such as Zn, Cu, Cd, etc.) via its 20 cysteine residues. There are three types of metallothioneins: MT-1, MT-2, and MT-3. MT-1 and MT-2 are ubiquitously expressed throughout the whole body, whereas MT-3 is primary localized in the central nervous system [22]. Uchida et al. found that neuronal growth inhibitory factor (GIF) which inhibits neurite extensions and prevents neuronal death was decreased in the brains of AD patients and determined that GIF is equivalent to MT-3 [23]. Therefore, MT-3 (GIF) is implicated in AD-associated neuronal death [24].

Zn transporters also control Zn homeostasis by facilitating Zn influx when it is deficient and efflux when it is present in excess [25]. There are two types of mammalian Zn transporters: ZnT transporters and Zrt-, Irt-like protein (ZIP) transporters. ZnT transporters are involved with the gene family for solute carrier (*SLC30*) and decrease intracellular Zn via facilitation of Zn efflux from cells. There are 14 types of ZnT transporters in mammals, including ZnT-1 and ZnT-3, which are co-localized with chelatable Zn in the brain. ZnT-1 is a membrane protein with six transmembrane domains and is widely distributed in mammalian cells. ZnT-1 has a pivotal role in Zn efflux and in protection from excess Zn. The expression of ZnT-1 is induced after transient global ischemia and decreases following dietary Zn deficiency [26]. ZnT-3 is localized to the membranes of presynaptic vesicles, transports Zn into synaptic vesicles, and maintains high Zn concentrations in the vesicles. Although the physiological roles of ZnT-3 have not fully elucidated, ZnT-3 knockout mice have depleted synaptic Zn and impaired memory formation [27].

ZIP transporters are another type of Zn transporter encoded by *SLC39* genes. ZIP transporters increase cytosolic Zn by promoting transport from extracellular to intracellular compartments. Fourteen ZIP genes have been identified in mammal, and the ZIP transporters are localized to cell membranes or in the membranes of the Golgi apparatus or endoplasmic reticulum (ER). These transporters control Zn influx into subcellular organs, and Zn transporter mutations produce severe diseases such as Ehlers-Danlos syndrome [28].

14.2.3 Cu (Copper) in the Brain

Cu is essential for brain functions and is a cofactor for numerous enzymes, such as cytochrome C, superoxide dismutase, lysyl oxidase, and tyrosinase. Cu is involved in Fe homeostasis as a component of ceruloplasmin and has neuroprotective activity as a component of Cu/Zn superoxide dismutase, an endogenous antioxidant. Cu deficiency results in adverse effects on myelination. Cu is a redox-active metal and exists both as oxidized Cu^{2+} and reduced Cu^{+}. Thus, excess free Cu is toxic because it produces ROS and binds with the thiol groups of functional proteins. The Cu transporters, ATP7A and ATP7B, transport Cu in ATP dependently. Another Cu transporter, Ctr1, is also involved in neuronal Cu uptake. Mutations of these transporters are linked with neurodegenerative diseases such as Wilson disease and Menkes disease [29]. Recent studies suggest that Cu has modulatory effects on neuronal information processes. Intracellular Cu accumulates in synaptic vesicles and is then released into the synaptic clefts during neuronal excitation, similarly to Zn [30]. Its concentration is estimated to be approximately 15 μM. Although the physiological roles of the released Cu are still controversial, Cu reportedly blocks glutamate receptors and modulates neuronal spontaneous activity [31].

14.2.4 Aluminum (Al) in the Brain

Given that Al is implicated in many neurodegenerative diseases due to its peculiar chemical characteristics, it can be considered a neurotoxic metal. Al is the third most abundant element in the earth's crust. Despite its widespread distribution throughout the environment, Al is not essential to living organisms. In contrast, Al reportedly inhibits more than 300 essential enzymatic functions. Al is suspected to contribute to various neurodegenerative diseases including Alzheimer's disease (AD) [1, 32], amyotrophic lateral sclerosis (ALS) and Parkinsonism dementia (PD) in the Kii Peninsula and Guam [33], dialysis encephalopathy [34], and Gulf War syndrome [35].

Al exhibits only one oxidation state, Al^{3+}. In acidic solutions with pH < 4, Al^{3+} exists as a soluble octahedral hexahydrate $Al(H_2O)_6{}^{3+}$ (Al^{3+}). As the pH increases, its solubility decreases and $Al(OH)_3$ precipitates at neutral pH. Al^{3+} has affinity for negatively charged, oxygen-donor ligands. Inorganic and organic phosphates, carboxylate, and deprotonated hydroxyl groups form strong bonds with Al^{3+}. Owing to these chemical characteristics, Al^{3+} binds to the phosphate groups of DNA and RNA, affecting DNA topology and influencing the expression of various genes essential for brain functions. Al^{3+} also binds to the phosphate groups of nucleoside di- and triphosphates, such as ATP, and can thus influence energy metabolism. Al also inhibits the functions of various protein kinases and phosphatases. Furthermore, Al^{3+} has a very low ligand-exchange rate compared to other metals. For example, the ligand-exchange rate of Mg^{2+} is 10^5 times faster than that of Al^{3+}, and therefore, Al^{3+} inhibits enzymes with Mg^{2+} cofactors. Al^{3+} also inhibits biological processes involving rapid Ca^{2+} exchange: the exchange rate for Al^{3+} is 10^8 times slower than that of Ca^{2+}. Therefore, Al cannot participate in Ca^{2+}- or Mg^{2+}-related enzymatic reactions and has an extended half-life in the body.

The strong positive charges and a relatively small ionic radius of Al^{3+} in comparison to other metal ions such as Ca^{2+}, Zn^{2+}, and Na^{+} facilitate Al^{3+} binding firmly to metal-binding amino acids (histidine, His; tyrosine, Tyr; arginine, Arg; etc.) or phosphorylated amino acids and thereby influences their conformations. Al is a well-known protein cross-linker and is therefore used as a leather-tanning agent.

Additionally, Al^{3+} has characteristics similar to Fe and binds to Fe-binding proteins such as transferrin and ferritin. Al^{3+} also stimulates Fe-induced membrane lipid peroxidation and causes oxidative damage in vitro and in vivo, although Al^{3+} does not directly affect peroxidation [36].

Overall, Al influences various processes induced by Ca, Mg, Fe, and other elements. Metal-metal interaction by Al will be discussed later in this chapter.

14.3 Alzheimer' s Disease (AD) and Metals

14.3.1 Amyloid Hypothesis

AD is a severe senile type of dementia that affects a large proportion of the elderly population worldwide. In Japan, there were more than 4,000,000 patients with senile dementia in 2013, and the number is increasing annually. The number of patients with mild cognitive incidence (MCI), which is the precursor stage of senile dementia, is also estimated to be more than 4,000,000. AD accounts for approximately half of patients with senile dementia.

AD is characterized by profound memory loss and the inability to form new memories. The pathological hallmarks of AD are the presence of numerous extracellular deposits, including senile plaques and neurofibrillary tangles (NFTs), and the selective loss of synapses and neurons in the hippocampal and cerebral regions [37]. Indeed, there is a strong correlation between the decrease in the number of synapses and the severity of memory impairment [38]. The major components of NFTs and senile plaques are phosphorylated tau protein and AßP, respectively.

Numerous biochemical, cell biological, and genetic studies support the idea termed "amyloid cascade hypothesis," which proposes that AßP accumulation and the consequent neurodegeneration are central to AD, although this hypothesis requires further investigation [39, 40]. Recent studies of the identified AßP species have indicated that the oligomerization of AßP and its conformational changes are critical to the neurodegeneration process.

AßP is a small peptide of 39–43 amino acid residues, which results from cleavage of a large precursor protein (APP; amyloid precursor protein) at the N-terminus by the ß-APP cleaving enzyme (BACE) and the intramembrane cleavage of its C-terminus by γ-secretase (Fig. 14.1). Genetic studies of early-onset cases of familial AD have indicated that APP mutations and AßP metabolism are associated with AD [41]. Presenilins are considered to be one of γ -secretases, and their mutations also account for the majority of cases of early-onset familial AD [42].

Yankner et al. reported that the first 40 amino acid residues of AßP (AßP (1–40)) causes the death of cultured rat hippocampal neurons or neurodegeneration in the brains of experimental animals [43]. AßP is a hydrophobic peptide with an intrinsic tendency to self-assemble and form SDS-stable oligomers in aqueous solutions. The monomeric form of AßP has a randomly coiled structure and is also less toxic. Oligomeric AßPs have ß-pleated sheet structures and form insoluble aggregates, termed amyloid fibrils. The neurotoxicity of AßP (1–40) peptides is enhanced by the process of "aging" (aggregation under incubation at 37 °C for several days), compared to freshly prepared AßP(1–40), and correlates with its ß-sheet contents [44]. Jarrett and Lansbury demonstrated that AßP formed oligomers via a nucleation-dependent process and that AßP (1-42) forms seeds for the aggregation of AßP (1-40) [45]. These results suggest that the ratio of AßP (1-40) and AßP (1-42) is critical to the pathogenesis. The point mutations of APP are located near

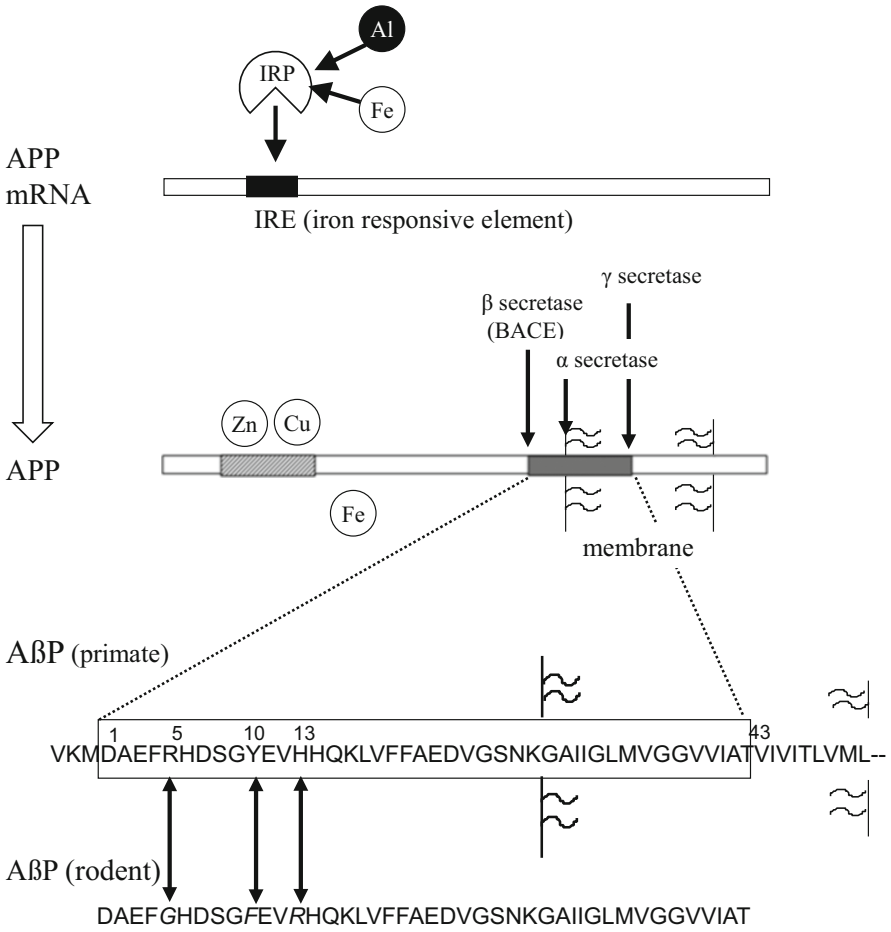

Fig. 14.1 Structure of AßP and its secretion from APP
AßP is secreted by the cleavage of the APP N-terminus by ß-secretase (BACE), followed by intramembrane cleavage of the C-terminus by γ-secretase. APP also binds to Cu or Zn. Human AßP and rodent AßP differ by three amino acids (Arg^5, Tyr^{10}, and His^{13}). The expression of APP is regulated by Fe and Al

the γ-secretase cleavage site of AßP (1-42) and influence the ratio of AßP (1-40) and AßP (1-42). Other mutations in presenilin genes influence the ratio of AßP (1-40) and AßP (1-42) by increasing production of AßP (1-42) in transfected cell lines.

14.3.2 Interaction Between Metals and AßP

AßP is secreted in the cerebrospinal fluid (CSF) of young individuals as well as in that of elderly individuals and non-dementia individuals [46]. Therefore, factors that accelerate or inhibit oligomerization may essentially contribute to the pathogenesis of AD. Several factors, such as the concentration of peptides, pH, composition of solvents, and temperature, influence the oligomerization processes. Oxidations, mutations, and racemization of AßP enhance its oligomerization. Additionally, substances including cholesterol or its oxidation products, apolipoprotein E, transthyretin, rifampicin, curcumin, aspirin, and ß-sheet breaker peptide, inhibit AßP oligomerization in vitro (Fig. 14.2) [47].

It is well established that rodent AßP exhibits a reduced tendency for oligomerization compared to human AßP in vitro, and AßP accumulation is rarely observed in the brains of rodents (rats or mice) compared to primates (humans or monkeys) [48]. As shown in Fig. 14.1, the amino acid sequences of human and rodent AßP are similar, but rodent AßP differs at three amino acids (Arg^5, Tyr^{10}, and His^{13}) compared to primate AßP. Considering that all of these amino acids are able to bind metals and metals are important determinants for the conformation of proteins, it is possible that trace elements such as Al, Zn, Cu, and Fe may play central roles in the accumulation of AßP in the human brain. Al is of particular interest since epidemiological Al is considered to be a risk factor of AD [49]. Al^{3+} has strong positive charges and a relatively small ionic radius in comparison with the other metal ions. Thus, Al^{3+} firmly binds to metal-binding amino acids and induces conformational changes to proteins. Exley et al. first demonstrated that Al induces a conformational change in AßP (1-40), using circular dichroism

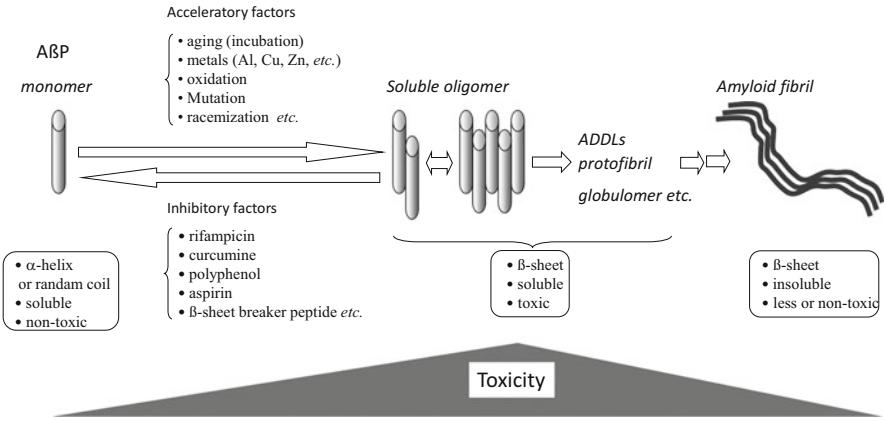

Fig. 14.2 Oligomerization of AßP. AßP monomers exhibit random or α-helix structures. However, under aging conditions or in the presence of acceleratory factors, AßP self-aggregates and forms several types of oligomers (SDS-soluble oligomers, ADDLS, globulomers, protofibrils, etc.) before finally forming insoluble aggregates (amyloid fibrils). Oligomeric soluble Aßs are toxic, although the monomeric and fibril aggregates are relatively nontoxic

(CD) spectroscopy [50]. Al also promotes aggregation of ^{125}I-labeled AßP (1-40), with similar findings as for Fe and Zn [51]. We have developed a system for investigating AßP polymerization that involves immunoblotting and precipitation. Using this system, we have demonstrated that Al enhances oligomerization of AßP (1-40) and forms SDS-stable oligomers in vitro [52, 53]. The aggregated AßP (1-40) is redissolved by adding an Al chelator, deferoxamine (DFO). The oligomerization induced by Al is more marked than that induced by other metals, including Zn, Fe, Cu, and Cd. Furthermore, we have demonstrated that Al-aggregated AßPs bind tightly to the surface of cultured neurons and form fibrillar deposits, meanwhile, Zn-aggregated AßPs are rarely observed on the surface of cultured neurons. Furthermore, Al inhibits AßP degradation resulting from conformational changes and enhances AßP accumulation.

Other trace metals such as Zn and Cu enhance AßP oligomerization. Bush et al. have demonstrated that Zn^{2+}, at a concentration similar to that in the CSF, produced AßP aggregation [54]. They reported that Cu^{2+} also binds to AßP, induces its aggregation, and increases ROS levels [55].

Despite all oligomers possess ß-pleated sheet structure, not all oligomers are equally neurotoxic. Recent studies using size-exclusion chromatography, gel electrophoresis, and atomic force microscopy revealed that several stable types of oligomers have been reported: naturally occurring soluble oligomers (dimmers or trimers), AßP-derived diffusible ligands (ADDLs), AßP globulomers, and protofibrils [4]. Hartley et al. separated aggregated AßP(1–40) into low-molecular-weight (mainly monomers), protofibrillar, and fibril fractions using size-exclusion chromatography and found that the protofibrillar fraction produced robust changes in the electrical activity of cultured neurons and was neurotoxic, but fibrils did not [56]. Walsh et al. found that SDS-stable oligomers exist in conditioned medium with cultured cells exogenously expressing the human APP gene [57]. The natural AßP oligomers obtained from the CSF of AD patients induced the loss of dendritic spines and synapses and blocked LTP [58].

The characteristics (size or shape) of AßP oligomers formed in the presence of Al, Zn Cu, and Fe are revealed to be identical by morphological analysis using atomic force microscopy [59]. Additionally, Sharma et al. revealed that Zn-aggregated AßPs are less toxic than Cu-aggregated AßP [60]. Meanwhile, Al-aggregated AßPs reportedly caused cytoskeletal changes, mitochondrial dysfunction, and increased production of ROS [61]. Bolognin et al. investigated aggregation and toxicity of AßP induced by Al, Cu, Fe, and Zn, finding that Al-aggregated AßPs induce overproduction of APP and tau, but AßP oligomers formed in the presence of other metals did not [62]. Everett et al. reported that AßP has the catalytic activity to reduce Fe^{3+} to redox-active Fe^{2+} and found that Al enhanced the reductive activity of AßP [63].

Metals can also participate in AßP-induced neurodegeneration pathways. Our previous studies, as well as numerous other studies, have demonstrated that AßPs are directly incorporated onto the surface of the cellular membrane and create unregulated pore-like channels that have cytotoxic effects [64–66]. These “amyloid channels” are giant multilevel pores that can facilitate the transport of large

amounts of Ca^{2+} [67]. Zn binds to His residues, which are exposed to the internal site of the amyloid pore and inhibit Ca^{2+} influx [68, 69]. We found that AßP induced abnormal increases in $[Ca^{2+}]_i$ using a high-resolution multi-site video imaging system in conjunction with a Ca^{2+}-sensitive fluorescent dye (fura-2) [70]. Numerous studies, including those from our lab, have revealed that prion protein fragment peptide or α-synuclein also form amyloid channels on membranes [65, 66]. Zn can regulate Ca influx via these amyloid channels.

14.3.3 Interactions Between Metals and APP or Presenilin

Despite the wide distribution of APP in the brain, its physiological roles have not been elucidated. APP has distinct binding domains for Cu, Zn, and Fe [71]. Wong et al. found that APP does not possess ferroxidase activity, but binds to the Fe transporter ferroportin and enhances Fe efflux [11]. Furthermore, APP mRNA contains an IRE domain as well as ferritin, and its expression is regulated by Fe, as described in Fig. 14.1 [72]. Therefore, APP regulates Fe homeostasis, and Fe controls APP expression. There are other important findings implicating Fe homeostasis in the AD pathogenesis. Fe-related genes, such as transferrin C2 or the hemochromatosis gene, are risk factors for AD [73]. Imagawa et al. reported that Fe supplementation is effective for recovery of cognitive functions in patients with AD [74].

APP has two Cu-binding domains in its N-terminal and possesses the ability to reduce Cu^{2+} to Cu^{+} [75]. Cu induces the dimerization of APP, AßP production, and trafficking of APP from the ER to neurites [76]. Cu also influences APP processing and the expression of APP. These results suggest that APP has crucial roles in the maintenance of metal homeostasis, particularly Fe homeostasis. When this homeostasis is disrupted, increased Fe causes ROS production or increased APP causes AßP production, both initiate the degenerative processes. Indeed, APP knockout mice exhibited increased Cu levels in the brain [77]. Cicotosto et al. reported that knockout of APP or its analogue APLP2 in mice resulted in changes in the distribution of Cu, Zn, Fe, and Ca [78]. Decreased levels of ferroportin and accumulation of Fe in the brains of AD patients have been reported [79].

It is very possible that Al influences Fe homeostasis. As noted previously, Al has characteristics similar to Fe and binds to Fe-binding proteins, including ferritin and transferrin, or to Fe chelators such as DFO. Al^{3+} also binds to IRP [80, 81] and thus affects the expression of various Fe-regulated genes containing IREs, thereby causing elevation in the Fe concentration. Additionally, Al reportedly enhances the expression of APP [82–85]. Furthermore, Al influences the uptake of Fe into cultured neurons or glial cells [86].

Presenilins, one of the γ-secretases, and their mutations account for most cases of early-onset AD. Presenilins mainly exist in the ER and they are implicated in Ca homeostasis [87, 88]. Recent studies demonstrate that presenilins are also metal-binding proteins and participate in the neuronal uptake of Zn and Cu [89]. Presenilins also promote the uptake of dietary Cu [90].

14.4 Prion Disease and Metals

14.4.1 Overview

Prion diseases are fatal neurodegenerative diseases that include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, as well as Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and kuru disease in humans [91]. The common pathological hallmarks are the spongiform degeneration of neurons and glial cells, and the accumulation of amyloidogenic prion protein (PrP) in the brain of the infected animals or person. Prion diseases are also called transmissible spongiform encephalopathies, since their characteristic infections can be initiated by the administration of pathogenic tissue. Although the molecular pathogenesis and transmission mechanisms of prion diseases are still controversial, it is widely accepted that the conformational conversion of normal cellular prion protein (PrP^{C}) to an abnormal scrapie-type isoform (PrP^{Sc}) is related to the transmissible characteristics of prion diseases. PrP^{C} is a 30–35 kDa cell surface glycoprotein anchored at 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^{C} differs from PrP^{Sc} in that PrP^{Sc} is resistant to protease digestion, has high ß-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, the protease-resistant PrP^{Sc} can then aggregate. Next, fibril formation induces other PrP^{C} molecules in the brain to misfold and aggregate.

14.4.2 Interactions Between Metals and PrP

The physiological roles of PrP^{C} are still controversial; however, increasing evidence suggests that PrP^{C} is a metalloprotein involved in metal homeostasis [92]. PrP^{C} contains 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-terminal (Fig. 14.3). In 1997, Brown et al. reported decreased levels of Cu in the brains of PrP-knockout mice compared to wild-type mice. The activity of Cu-dependent enzymes was also reduced in PrP-null mice [93]. Jackson et al. reported that PrP^{C} binds four Cu atoms at its octa-repeat domain and binds two additional Cu atoms to two other histidine (His) residues, His^{96} and His^{111} [94]. 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+}. PrP^{C} reportedly transports Cu from the extracellular space to the intracellular space via endocytosis and regulates the intracellular concentration of Cu. PrP^{C} possesses or modulates Cu/Zn superoxide dismutase (Cu/Zn SOD) activity in the brain and has protective

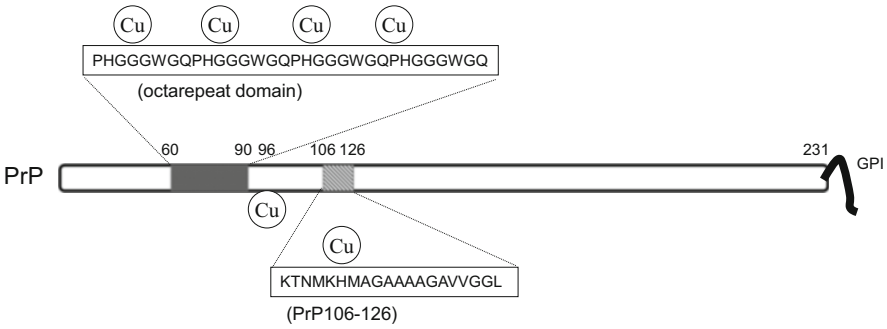

Fig. 14.3 The structure of the prion protein and its metal -binding properties

roles against oxidative stress [95]. Recent studies have suggested that PrP^C regulates the function of the NMDA-type glutamate receptor in a Cu-dependent manner [96]. In contrast, Cu^{2+} influences the gene expression and cellular trafficking of PrP [97]. It is also possible that the depletion of PrP^C and a subsequent increase in Cu could cause oxidative damage to neurons [98]. Furthermore, PrP-deficient neurons exhibit lower glutathione reductase activity and increased susceptibility to damage by hydrogen peroxide [99].

Zn^{2+} has the next highest affinity for binding with PrP, after Cu^{2+}. As discussed previously, Zn accumulates in synaptic vesicles, and a considerable amount of Zn (~100 μM) is released with glutamate during neuronal excitation. Since the concentration of Zn in the brain is much higher than the concentration of Cu (~15 μM), it is possible that Zn^{2+} influences PrP^C binding to Cu [100]. Moreover, recent bioinformatics analysis has revealed the evolutionary similarities between prion genes and genes encoding the ZIP transporters [101]. Of 14 ZIP transporters, sequence similarities have been reported between PrP^C and N-terminal ectodomains of ZIP5, ZIP6, and ZIP10. Furthermore, PrP^C co-localizes with ZIP5 in neuroblastoma cells [102]. Watt et al. found that PrP^C facilitates the uptake of Zn into neuronal cells and that the effect is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors [103]. These authors hypothesized that PrP^C functions as a Zn sensor, detecting extracellular levels up to a particular threshold. Indeed, PrP^C reportedly attenuates Zn-induced neurotoxicity.

In addition to Cu and Zn, other metals such as Fe and Mn are associated with PrP. Singh et al. suggested that PrP^C functions as a ferrireductase that is responsible for reducing Fe^{3+} to bioavailable Fe^{2+} and modulating the cellular uptake of Fe [104]. PrP-knockout mice exhibit altered Fe metabolism [105]. Decreased levels of ferroxidase activity and transferrin have been observed in the cerebrospinal fluid of patients with CJD [106].

Furthermore, several studies have suggested that Mn may facilitate prion diseases. Johnson et al. investigated the levels of trace elements in prion-infected hamster brains using X-ray photoelectron emission microscopy with synchrotron radiation and found both reduced Cu and increased Mn in plaques composed of prion proteins [107]. Thackray et al. reported that PrP^C loses its SOD-like activity

when Cu is replaced with Mn [108]. Furthermore, Mn enhances the stability of PrP in soils and increases its infectivity [109]. The risk of contracting prion disease in elk, termed "chronic wasting disease," has been associated with Mg deficiency and increased levels of Mn [110]. A recent epidemiological survey also suggested a relationship between the pathogenesis of CJD and Mn imbalance [111].

14.4.3 Toxicity of PrP Fragments and Metals

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. PrP^{C} is thought to regulate Cu homeostasis and to have antioxidant and cytoprotective effects against neurotoxicity induced by Cu^{2+} or free radicals. We have demonstrated that PrP fragment with octa-repeat domain attenuated Cu-induced neurotoxicity [112]. Thus, PrP^{C} depletion may initiate various neurodegenerative processes.

Meanwhile, 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 lab) have used synthetic fragment peptides of PrP (PrP106–126), because methodological difficulties occur when attempting to use a whole prion protein due to its strongly infectious characteristics. PrP106–126 has been used as a model peptide of PrP^{Sc}, as it coincides with the proposed sequence for ß-sheet structures, forms aggregates of ß-sheet structures to produce amyloid fibrils that share several characteristics with PrP^{Sc}, and causes the apoptotic death of cultured neurons or glial cells [113]. Additionally, PrP106–126 has the ability to bind to metals including Cu^{2+} and Zn^{2+} [114].

We observed that PrP106–126 forms ß-sheet structures during the "aging" process (incubation at 37 °C for several days) using a thioflavin T (ThT) fluorescence assay, far-UV circular dichroism (CD) spectroscopy, and atomic force microscopy (AFM) imaging [112]. Furthermore, aged PrP106–126 produced significant neurotoxicity in primary cultured rat hippocampal neurons. These characteristics are quite similar to that of AßP. We have added various trace elements to solutions of PrP106–126 during the aging processes and evaluated the resulting conformational changes and neurotoxicity. The presence of either Zn^{2+} or Cu^{2+} during the aging process significantly attenuated PrP106–126 neurotoxicity, whereas the presence of Al^{3+}, Fe^{2+}, and Fe^{3+} did not produce significant changes. Additionally, we investigated the effects of these metal ions on the ß-sheet formation of PrP106–126. We have utilized the changes in fluorescence of ThT, which binds with pleated ß-sheet structures, to observe the oligomerization of PrP106–126. The ThT fluorescence for solutions of aged PrP106–126 increased compared with freshly dissolved PrP106–126 solutions. The ThT fluorescence of PrP106–126 incubated with Zn^{2+}, Fe^{2+}, or Fe^{3+} was significantly decreased compared with aged PrP106–126 alone. In particular, the addition of Cu^{2+} dramatically decreased ThT fluorescence levels similar to fresh PrP106–126. Aged PrP106–126

forms amyloid fibrils with a distinctively straight and long morphology on mica plates as observed by AFM, although no fiber-like structures could be observed in freshly prepared PrP106–126. However, PrP106–126 aged with Cu or Zn exhibited different morphological features compared to aged PrP106–126 alone. Although the CD spectra of aged PrP106–126 exhibited ß-sheet structures, the CD spectra of PrP106–126 aged with Cu^{2+} exhibited random coil-like structures. Our results suggest that Cu and Zn influenced the ß-sheet formation of PrP106–126 and thereafter attenuated its neurotoxicity. These findings are consistent with other studies that Cu inhibits ß-sheet formation by PrP111–126 [115]. Thakur et al. investigated the conformational changes of full-length PrP by NMR and reported that Cu did not produce oligomerization of PrP at physiological temperatures and that Cu may act as an attenuator in prion diseases [116]. Although it is widely accepted that Cu enhances the aggregation of other amyloidogenic proteins such as AßP and α-synuclein, the effects of Cu on the conformational changes to amyloidogenic proteins may be complex and may depend on peptide structures [117].

14.5 Lewy Body Diseases and Metals

Lewy body disease is a category that includes Parkinson's disease (PD), dementia with Lewy body (DLB), and multiple system atrophy (MSA). These diseases commonly exhibit abnormal cellular inclusions, termed Lewy bodies, which is the accumulation of α-synuclein, and therefore are termed "synucleopathy" [118]. DLB includes approximately 25 % senile dementia and has common pathological changes with AD, such as the deposition of senile plaques and tau protein. Moreover, the fragment peptide of α-synuclein, non-amyloid component (NAC), is co-accumulated with AßP in Alzheimer's senile plaques. The oligomerization and fibrillation of α-synuclein have been implicated in the formation of Lewy bodies and the etiology of Lewy body diseases. It is demonstrated that α-synuclein is a 140-amino acid protein, abundantly present in the brain and particularly located in the nucleus and in the presynaptic terminals.

Involvement of metals in Lewy body diseases and α-synuclein has been extensively researched, since Mn toxicity exhibits Parkinson-like symptoms [119] and Fe-rich regions such as the substantia nigra are particularly vulnerable in Parkinson's diseases [104]. Accumulation of Fe and Al has been reported in Lewy bodies of Parkinson patients. Furthermore, α-synuclein aggregation is accelerated by Cu and Al in vitro. Uversky et al. found the metals, such as Al and Mn, enhance the α-synuclein oligomerization [120]. Cu binds to α-synuclein in its N-terminal domain and C-terminal domain [121].

Increasing evidence suggests that α-synuclein primary exists in the presynaptic terminals of dopaminergic neurons and controls dopamine release, synaptic functions, and synaptic plasticity. Additionally, α-synuclein reportedly binds Fe and functions as a ferrireductase, which reduces Fe^{3+} to bioavailable Fe^{2+}

[9]. Furthermore, α-synuclein mRNA has an IRE domain as well as APP or ferritin, and Fe regulates its expression [122]. In the postmortem brains of patients with Parkinson patient, Fe levels as well as the ratio of Fe^{2+} to Fe^{3+} were changed [123].

14.6 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 ischemia [124]. Following transient global ischemia or stroke, the interruption of blood flow and the resulting oxygen-glucose deprivation induce long-lasting membrane depolarization and result in excessive glutamate release into synaptic clefts. The excess glutamate then overstimulates receptors including NMDA-type receptors, AMPA-type receptors, and kainate-type receptors. Finally, Ca^{2+} dyshomeostasis (the entry of large quantities of Ca^{2+} to glutamate-responsive neurons) triggers delayed death of vulnerable populations of neurons, such as pyramidal neurons in the hippocampus, which is associated with learning and memory. Development of an infarct and the subsequent cognitive dysfunction produce the pathogenesis of VD. Approximately 30 % of patients with stroke experience show symptoms of dementia within 3 months of the initial stroke.

Increasing evidence suggests that Zn is central to ischemia-induced neuronal death and the pathogenesis of VD [125]. In ischemic conditions, as much as 300 μM Zn is co-released with glutamate into the synaptic clefts following membrane depolarization [126] and exhibits apoptotic cell death. Furthermore, the chelatable Zn moved from presynaptic terminals into cell bodies of degenerated neurons [127]. The increase in intracellular Zn^{2+} levels ($[Zn^{2+}]_i$), namely, Zn translocation, occurs in vulnerable neurons in the CA1 or CA3 regions of the hippocampus after transient global ischemia. Administration of calcium EDTA (Ca EDTA), a membrane-impermeable Zn chelator, blocked Zn translocation, protected hippocampal neurons after transient global ischemia, and reduced the infarct volume [128]. At least three major routes of Zn^{2+} entry were identified: voltage-gated Ca^{2+} channels (VGLC), NMDA-type glutamate receptors, and AMPA/kainate-type glutamate receptors [129]. Although NMDA-type glutamate receptors are present in most neurons, the permeability of Zn^{2+} and Ca^{2+} through AMPA/kainate channels is greater for NMDA receptor.

In the normal physiological condition, hippocampal neurons typically express AMPA receptors with GluR2 subunits, which are poorly permeable to divalent cations including Ca^{2+} and Zn^{2+}. However, following ischemia, there is an acute reduction in GluR2 subunit expression, and neurons possess specific types of AMPA receptors with channels that are directly Ca^{2+}-permeable (Ca-AMPA/kainate channels; Ca-A/K-R). The appearance of Ca-AMPA/kainate channels result in increased permeability of Ca^{2+}, thereby enhancing toxicity. Therefore, the

expression of Zn^{2+}-permeable Ca-AMPA/kainate channels and the entry of Ca^{2+} and/or Zn^{2+} through the channels are mediators of the delayed neuronal death that follows ischemia. Considering that Ca EDTA, a Zn chelator, attenuates ischemia-induced downregulation of the GluR2 gene [128], Zn is also implicated in the transcriptional regulation of Ca-AMPA/kainate channels.

We investigated the molecular mechanism of Zn-induced neuronal death using GT1-7 cells (immortalized hypothalamic neurons), which are much more sensitive to Zn than other neuronal cells [130, 131]. GT1-7 cells possess neuronal characteristics such as neurite extensions, secretion of gonadotropin-releasing hormone (GnRH), and expression of neuron-specific proteins or receptors including microtubule-associated protein 2 (MAP2), tau protein, neurofilament, synaptophysin, GABAA receptors, dopamine receptors, and L-type Ca^{2+} channels [132]. We demonstrated that the ER stress pathway, the mitochondrial energy pathway, and the disruption of Ca homeostasis contribute to Zn-induced apoptosis. Screening for substances that are protective against Zn neurotoxicity yielded findings that carnosine (ß-alanyl histidine) attenuates Zn-induced neuronal death [133].

14.7 Hypothesis: Crosstalk of Metals and Amyloidogenic Proteins at the Synapse

The evidence indicates that the amyloidogenic proteins (or their fragment peptides), including APP (AβP), prion protein (PrP106-126), and α-synuclein (NAC), share similarities such as oligomerization with β-pleated sheet structures, neurotoxicity, and the ability to binding metals, as shown in Table 14.1. Furthermore, recent findings demonstrate that these proteins are co-localized at the synapse. APP occurs primarily in the presynaptic membrane, PrP^{C} occurs in postsynaptic membranes, and α-synuclein occurs primarily in the presynaptic cytosol and to a lesser extent in membranes. Additionally, presenilins are predominantly located in the ER and also occur in the presynaptic and postsynaptic membranes.

The synapse is a local site of communications between neurons. It is small, but is the crucial node of brain neural networks. Neurotransmitters and metals (Zn or Cu) are co-released from synaptic vesicles in the presynaptic terminals to the synaptic clefts and bind to receptors in the postsynaptic domains (PSDs). Synapses are vulnerable regions for these neurodegenerative diseases, since synaptic plasticity is essential to memory formation. The synaptic cleft is conceptualized as a cylinder with 20 nm height and 200 nm radius [134]. Thus, the distance between presynaptic terminals and the postsynaptic membranes (~20 nm) may be small enough for proteins at the presynaptic terminal to interact with proteins in the postsynaptic terminal. Considering the small volume of synaptic clefts, it is plausible that neurotransmitters or metals are concentrated at synaptic clefts and their levels may be much higher than the level in the extracellular fluid. The concentration of

glutamate in the synaptic cleft is estimated to reach a few millimolar range after 1 ms of neuronal depolarization, and the Zn concentration after depolarization is estimated to be 1–100 μM. Thus, amyloidogenic proteins must certainly interact with other proteins in a considerable amount of Zn and Cu.

Considering the combined evidence, we hypothesize that metal imbalance at the synapse contributes to the pathogenesis of neurodegenerative diseases. Under typical physiological conditions (Fig. 14.4), Zn can be released with glutamate and can bind with NMDA-type glutamate receptors or other receptors, inhibiting overall brain excitability. Secreted Zn can diffuse across the synaptic cleft, spill over to neighboring synapses, and influence the activity of neighboring synapses

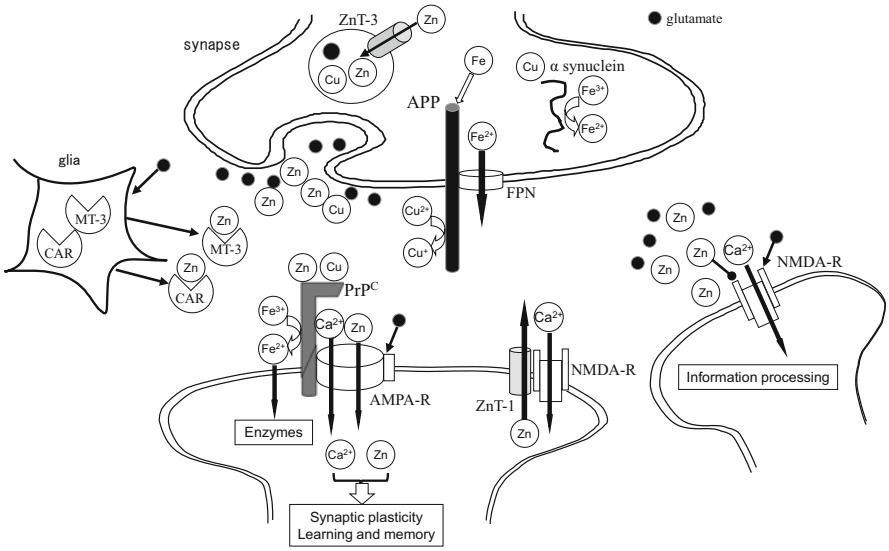

Fig. 14.4 Crosstalk between trace elements and amyloidogenic proteins at the synapse under normal condition. Zn and glutamate accumulate in synaptic vesicles and are released into synaptic clefts during neuronal excitation. Zn^{2+} regulates Ca^{2+} influx through glutamate receptors (NMDA-R, Ca-A/K), modulates neuronal information, and is implicated in the maintenance of synaptic plasticity and memory formation, similarly to Ca^{2+}. Zn has important roles in information processing in the targeted neuron and also in neighboring neurons. APP and α-synuclein exist in the presynaptic domain, and PrP^C is localized to the postsynaptic domain. These proteins are closely associated with the synaptic cleft and Zn and have cytoprotective roles via the regulation of metal homeostasis and protection from free radicals. PrP^C binds to AMPA-type glutamate receptors and regulates Zn^{2+} levels similarly to ZIP Zn transporters. Additionally, the ZnT-1 Zn transporter is localized to 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. 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. MT-3 and carnosine are released from glial cells, into synaptic clefts, and are also implicated in regulation of excess Zn. *ZnT-1* zinc transporter 1, *AMPA-R* AMPA-type glutamate receptor, *NMDA-R* NMDA-type glutamate receptor, *MT-3* metallothionein 3

dose-dependently. Since glutamate produces excitation and Zn produced inhibition, differing concentrations of glutamate and Zn in the adjacent synapses create precise modulation of neuronal activity. Therefore, it is possible that Zn may have neuromodulator roles, transmitting the spatiotemporal information of neuronal activity. This may enable "lateral inhibition," based on signaling contrast, and may be based on synaptic plasticity [135]. Synaptic Zn enters the postsynaptic neurons through Ca^{2+} channels and NMDA channels and regulates functions of various channels and receptors. Recent evidence suggests that the ZnT-1 transporter, which enhances Zn efflux to the extracellular compartment, is localized in postsynaptic membranes [136]. The ZnT-1 transporter binds with NMDA-type glutamate receptors and regulates the activity. In comparison, PrP^C, an analogue of ZIP zinc transporters, localizes in postsynaptic membranes binding with AMPA-type glutamate receptor, which facilitate Zn influx. Thus, it is likely that synaptic Zn levels are controlled by both ZnT-1 and PrP^C. MT-3 (GIF) secreted from neurons or glia may also regulate Zn homeostasis at synapses. Another contributor to Zn homeostasis is carnosine (CAR), an endogenous antioxidant and anti-cross-linking peptide, which is synthesized in glial cells [137].

Cu is also secreted at synaptic clefts following neuronal excitation. PrP^C binds to Cu at its N-terminal domain and regulates synaptic Cu levels. It is also possible that PrP^C provides Cu to APP or to NMDA-type glutamate receptor, thereby influencing the production of AβP or the neuronal excitability. APP also regulates Cu levels by reducing Cu^{2+} to Cu^{+}, and both APP and PrP^C reportedly attenuate Cu-induced toxicity.

Furthermore, APP controls Fe homeostasis by binding with ferroportin and promotes Fe efflux. In contrast, both PrP^C and α-synuclein possess ferrireductase activity, regulate the Fe^{2+}/Fe^{3+} ratio in synapses, and thereby control neurotransmitter synthesis.

We have demonstrated the complex and subtle interactions between metals and amyloidogenic proteins at the synapse. When the homeostasis of metals is disrupted, synapses and neuron degradations occur, which contribute to the pathogenesis of neurodegenerative diseases (Fig. 14.5).

Interactions between one metal and other metals may disrupt metal homeostasis. Varying types of metal ions usually share binding sites, although their binding constants differ. In particular, Cu^{2+} and Zn^{2+}share similar chemical characteristics and interact with each other. Similarly, Al^{3+} and Fe^{3+} share similar characteristics and interact with each other. Thus, the disruption of metal homeostasis may occur in the presence of toxic metals such as Al or the excess of essential metals such as Zn, Cu, or Fe.

In case of the pathogenesis of AD, Al enters into the brain. Al binds to Fe-binding proteins, inhibits IRP/IRE pathway as described previously, and influences the expression of Fe-binding proteins in the brain, including APP, α-synuclein, and ferritin, thereby increasing Fe levels. Overexpression of APP induced by Al results in accumulation of AβP. Al and other metals also accelerate AβP oligomerization and enhance the neurotoxicity. AβP oligomers may form amyloid channels on synaptic membranes, produce Ca dyshomeostasis, initiating

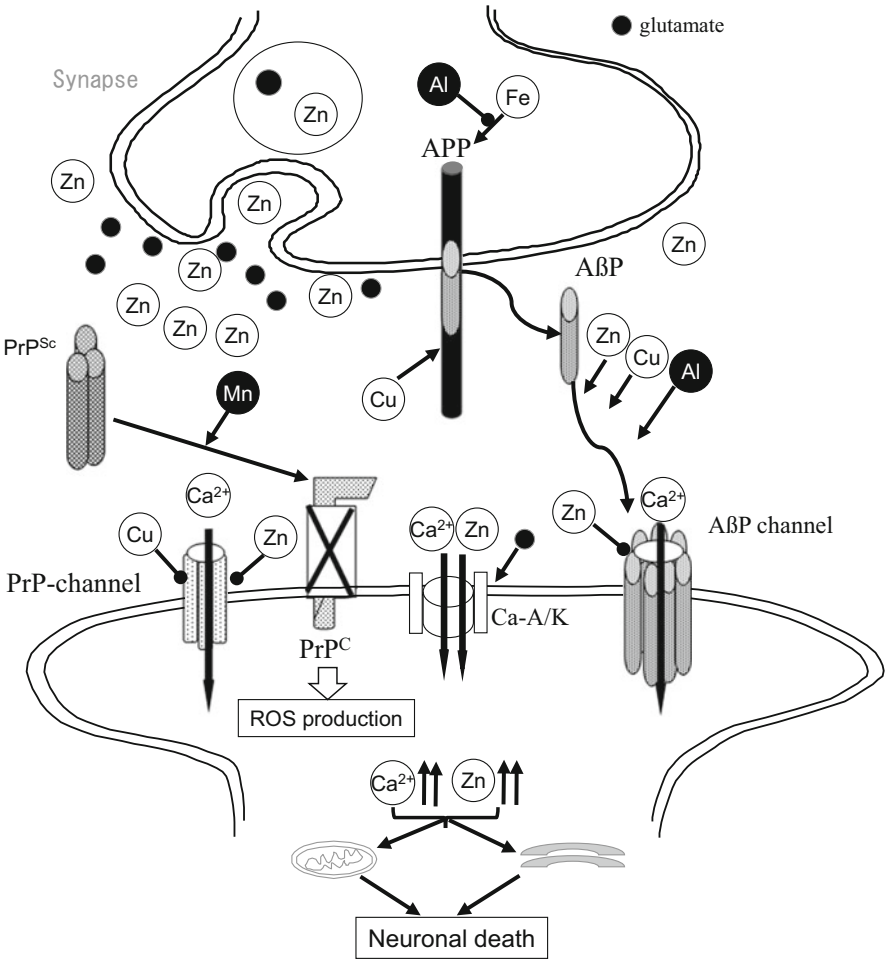

Fig. 14.5 Disruption of metal homeostasis and the pathogenesis of neurodegenerative diseases. When toxic metals such as Al cross the blood-brain barrier, Al binds various Fe-binding proteins and influences Fe homeostasis, which initiates formation of free radicals and upregulation of APP and results in overproduction of AßP. Al also causes the oligomerization and accumulation of AßP. These adverse effects may enhance the synaptotoxicity and neurotoxicity of AβP and produce AD pathogenesis. Oligomerized AβPs form pore-like structures (AβP channels) in membranes, cause Ca dyshomeostasis, and initiate apoptotic neuronal death. Conversely, Zn inhibits amyloid channels and has neuroprotective properties. However, excess Zn resulting from overexcitation during ischemia also disrupts metal homeostasis at the synapse, initiates neurodegeneration, and contributes to the pathogenesis of vascular dementia. When exogenous PrP^{Sc} enters into the brain, it stimulates conversion of endogenous PrP^{C} and results in PrP^{C} depletion and PrP^{Sc} accumulation. The loss of neuroprotective functions by PrP^{C} produces oxidative stress, enhances AßP neurotoxicity, and results in neuronal death. Accumulated PrP^{Sc} forms Ca^{2+}-permeable channels (PrP channel) in membranes and disrupts Ca homeostasis

synaptotoxicity and neurotoxicity, and produce the pathogenesis of AD. Zn inhibits AβP amyloid channel formations and neurotoxicity [69]. Zn also infounces Al-induced neurotoxicity [138]. In this context, Zn may have two faces in the pathogenesis of AD.

In case of the pathogenesis of prion diseases, pathogenetic PrP^{Sc} enters the brain primary by food contamination, triggers conformational changes to PrP^{C}, and causes conversion of PrP^{C} to PrP^{Sc}. PrP^{C} is neuroprotective, since it has antioxidant activity and attenuates AßP neurotoxicity. Loss of neuroprotective PrP^{C} may disrupt metal homeostasis. Increased Cu at the synapse produces the oxidative stress and influences the APP expression and processing. Thus, the depletion of normal PrP^{C} results in oxidative stress, enhances the neurotoxicity of AßP, and results in apoptotic death of neurons and glia. Additionally, accumulated PrP^{Sc} may form channels (PrP channel) in the membrane and thereby disrupt Ca homeostasis as well as AßP. Cu^{2+} and Zn^{2+} inhibit the conformational conversion from PrP^{C} to PrP^{Sc} and also attenuate PrP^{Sc}-induced Ca dyshomeostasis and neurotoxicity. However, Mn may enhance PrP^{Sc} toxicity by influencing the conversion of PrP^{C}.

In the case of total ischemia, excess Zn is released with glutamate, which produces disruption of Ca homeostasis, triggers mitochondrial energy depletion and ER stress, and contributes to neurodegeneration. Zn also enhances the expressions of Ca-permeable AMPA-type glutamate receptors and enhances Ca dyshomeostasis. Considering that vascular degeneration may be linked with the early-stage AD, Zn neurotoxicity may also be associated with AD.

14.8 Metal-Related Treatments for Neurodegenerative Diseases

Several studies have investigated metal chelators as potential treatment for neurodegenerative diseases. Clioquinol (5-chloro-7-iodo-8-quinolinol) and its derivatives have been assessed in therapeutic trials for AD. Clioquinol (quinoform), a chelator of Cu^{2+} or Zn^{2+}, inhibits oligomerization of AßP and attenuates the accumulation of amyloid in the brains of experimental animals [139]. Clinical trials using its analogue, PBT2, are under investigation [140]. Deferoxamine (DFO), a chelator of Al and Fe, attenuates the decline of daily living skills in AD patients [141]. Intranasal application of DFO reportedly attenuates memory loss and amyloid deposition [142]. Silicates, which couple with Al and reduce its toxicity, are also candidates for chelation therapy in AD [143].

Clioquinol treatment also had beneficial effects on scrapie-induced memory impairment [144]. It was also revealed that D-(-)-penicillamine, a Cu^{2+}-specific chelator, attenuated the pathogenesis of prion diseases in vivo [145].

Our screen for substances that protect against PrP106–126-induced neurotoxicity revealed that carnosine may be a candidate treatment modality for prion diseases [112]. Carnosine is a water-soluble dipeptide contained in mammalian muscles and

in the brain, particularly in the olfactory bulbs [137]. Carnosine has antioxidant and anti-glycation properties as well as the ability to bind to metals. Additionally, carnosine has anti-cross-linking properties, inhibits the oligomerization of AßP, and attenuates neurodegeneration in a mouse model of AD [146]. We previously reported that carnosine inhibited Zn^{2+}-induced neuronal death after ischemia [2, 133]. Considering these beneficial characteristics, carnosine may have neuroprotective functions in the brain. Based on these findings, we have published a patent for carnosine as a possible basis for drugs designed to treat vascular dementia [147].

14.9 Conclusions

We have demonstrated the complex and subtle interactions between metals and amyloidogenic proteins at the synapse. This crosstalk 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 neurodegenerative diseases. Our findings suggest that metal dyshomeostasis may be one of the common mechanisms for this pathogenesis and may elucidate the enigmatic roles of trace elements in neurodegenerative diseases and preventive drug development. In conclusion, our working hypothesis may contribute to enhanced understanding of the role of metals in the neurodegenerative diseases. Further research regarding "neurometallomics" is necessary, particularly in relation to the molecular mechanisms of synaptic degeneration and the quantitative analysis of neurometals.

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