

Heat Shock Proteins and Amateur Chaperones in Amyloid-Beta Accumulation and Clearance in Alzheimer's Disease

Micha M. M. Wilhelmus · Robert M. W. de Waal ·
Marcel M. Verbeek

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Abstract The pathologic lesions of Alzheimer's disease (AD) are characterized by accumulation of protein aggregates consisting of intracellular or extracellular misfolded proteins. The amyloid- β ($A\beta$) protein accumulates extracellularly in senile plaques and cerebral amyloid angiopathy, whereas the hyperphosphorylated tau protein accumulates intracellularly as neurofibrillary tangles. "Professional chaperones", such as the heat shock protein family, have a function in the prevention of protein misfolding and subsequent aggregation. "Amateur" chaperones, such as apolipoproteins and heparan sulfate proteoglycans, bind amyloidogenic proteins and may affect their aggregation process. Professional and amateur chaperones not only colocalize with the pathological lesions of AD, but may also be involved in conformational changes of $A\beta$, and in the clearance of $A\beta$ from the brain via phagocytosis or active transport across the blood-brain barrier. Thus, both professional and amateur chaperones may be involved in the aggregation, accumulation, persistence, and clearance of $A\beta$ and tau and in other $A\beta$ -associated reactions such as inflammation associated with AD lesions, and may, therefore, serve as potential targets for therapeutic intervention.

Keywords Alzheimer's disease · Amyloid · Heat shock proteins · Heparan sulfate proteoglycans · Apolipoproteins · Amyloid beta-protein · Chaperones

Introduction

Pathological lesions consisting of intra- and/or extracellular accumulations of misfolded proteins are characteristic for neurodegenerative diseases such as Alzheimer's disease (AD). AD is characterized by three distinct pathological lesions: senile plaques (SPs), neurofibrillary tangles (NFTs), and cerebrovascular amyloid angiopathy (CAA) [1]. Both SPs and CAA are formed by extracellular deposition of aggregated amyloid-beta protein ($A\beta$), whereas NFTs consist of intracellular aggregates of hyperphosphorylated tau protein in the cytoplasm of neurons [2, 3]. The $A\beta$ protein is a 4-kDa proteolytic cleavage product [2] of the transmembrane amyloid- β precursor protein (APP). The two major forms of $A\beta$ in human brain are $A\beta_{1-40}$ and $A\beta_{1-42}$, differing from each other only by two amino acids. Cerebral production of $A\beta$ is balanced by clearance from the brain either via active transport across the blood-brain barrier (BBB) or via uptake and degradation of $A\beta$ by microglial cells and astrocytes [4–6]. Active transport of $A\beta$ is mediated by $A\beta$ receptors that are capable of transporting $A\beta$, or $A\beta$ in complex with other proteins, across the BBB [7]. In contrast to normal brain, the cerebral $A\beta$ balance is disturbed in AD brains, resulting in accumulation and aggregation of $A\beta$.

$A\beta$ aggregation includes the formation of $A\beta$ oligomers, protofibrils, and eventually, mature fibrils. Both $A\beta$ oligomers and protofibrils are considered the most toxic forms of $A\beta$ that initiate degeneration of neurons and of cells within the vasculature, such as smooth muscle cells and pericytes [8, 9]. $A\beta$ aggregates do not clear from the brain as efficiently as soluble $A\beta$, and thus, directly lead to increased levels of $A\beta$ in the brain [10]. Furthermore, deposition of $A\beta$ in SPs is accompanied by attraction and activation of both microglial cells and astrocytes [11–13]. Activation of these

M. M. M. Wilhelmus · M. M. Verbeek (✉)
Department of Neurology and Alzheimer Centre,
Radboud University Nijmegen Medical Centre,
830 LKN, P.O. Box 9101, 6500 HB Nijmegen, Netherlands
e-mail: m.verbeek@cukz.umcn.nl

M. M. M. Wilhelmus · R. M. W. de Waal
Department of Pathology,
Radboud University Nijmegen Medical Centre,
Nijmegen, Netherlands

cell types results in increased secretion of pro-inflammatory cytokines as part of a neuro-inflammatory reaction.

Chaperones can be defined as proteins that: (1) have a role in the intracellular handling of misfolded proteins, (2) induce conformational changes of proteins, (3) act as transporter of proteins. “Professional” chaperones, such as the heat shock protein family (Hsp), are defined as proteins that have a specific function in facilitating normal folding of proteins and intracellular handling of misfolded proteins. Members of the Hsp family recognize misfolded proteins and transport them to the proteasome for degradation. Therefore, this protein family acts as the first line of defense against toxicity induced by misfolded proteins such as A β and tau. In contrast to professional chaperones, “amateur” chaperones can be defined as proteins that bind to other proteins and induce conformational changes or, alternatively, serve as transporter proteins. Examples of putative amateur chaperones are apolipoprotein E (ApoE), heparan sulfate proteoglycans (HSPGs), and complement factors such as C1q. They have, in contrast to the professional chaperones, primarily an extracellular function. In this paper, we will review the role of both amateur and professional chaperones in the pathogenesis of AD.

A β -Binding Proteins in Extracellular Interaction with A β

Apolipoproteins

The apolipoprotein family consists of proteins that conjugate with lipids to form different classes of lipoprotein particles. In human brain, several members of this protein family are expressed, such as apolipoprotein E (ApoE), apolipoprotein J (ApoJ), and apolipoprotein D (apoD).

ApoE is a major determinant of lipid transport and metabolism and is expressed in brain by astrocytes, microglia, pericytes, and smooth muscle cells [14–18]. In human, three common isoforms are expressed: apoE2, apoE3, and apoE4 which are all products of alleles at a single gene locus [19, 20].

The ϵ 4 allele of ApoE is the major genetic risk factor for AD, whereas the ϵ 2 allele appears to be protective against AD [21–24]. As ApoE immunoreactivity was found in extracellular amyloid deposits in subjects with AD, it has been suggested that it affects amyloidogenesis [25, 26]. In vitro studies provided evidence for a direct interaction of ApoE with A β and the formation of stable complexes [27, 28]. Binding of ApoE to A β is, however, ApoE isoform-dependent (ϵ 2> ϵ 3> ϵ 4) [29, 30] and depends on the degree of lipidation [29]. Lipidation of ApoE also seems a major factor in its effect on A β -mediated cellular toxicity [18]. In addition, ApoE4 promotes the conversion of soluble A β into β -sheet-rich amyloid more than ApoE3 [31–33].

In contrast to its effect on A β in vitro where a consistent accelerating effect of ApoE on A β aggregation is observed, the effect of ApoE on A β deposition in transgenic (Tg) mice studies is less equivocal. In early studies, both A β immunoreactivity and amyloid formation were reduced in ApoE knockout mice [31, 32]. In addition, CAA and associated microhemorrhages were also suppressed in ApoE knockout mice [34]. This effect might be due to the absence of ApoE/A β complexes [35]. In contrast to the effects of murine ApoE in the early studies, both human ApoE3 and ApoE4 suppressed A β deposition in Tg mice [36]. In addition, when these mice aged, ApoE4 induced a tenfold higher deposition of fibrillar A β than ApoE3 [37]. Consistent with these latter results, human ApoE4 accelerated A β deposition in APPSwe Tg mice relative to human ApoE3 [38]. In addition, when human ApoE3 or ApoE4 were knocked in in Tg mice, A β deposition was reduced compared to mice carrying endogenous ApoE at 9 months, and at 15 months, substantial CAA was observed in mice with human ApoE4, but not with human ApoE3, and, in either case, parenchymal A β was sparse [39]. Thus isoform- and species-specific differences in ApoE direct the aggregation or clearance of A β . Furthermore, it is suggested that the presence of ApoE facilitates internalization and degradation of A β from brain parenchyma by astrocytes [40] and human ApoE may reduce A β deposition in mouse brain by facilitating A β transport across the BBB [36]. Although these Tg mice studies seem contradictory, ApoE clearly affects conformational changes of A β and functions as an A β -transporter protein.

Besides colocalization of ApoE with A β in AD brains, ApoE is also found within neurons containing NFTs [25] where it is able to interact directly with tau protein [41]. Furthermore, ApoE has an isoform-dependent effect on tau phosphorylation, as ApoE3 binds to tau in vitro, whereas ApoE4 fails to bind tau [42]. In addition, an ApoE4-dependent increase in phosphorylated tau has been observed [43–45].

Neuroinflammation in AD comprises both activation of microglial cells and astrocytes and activation of the complement system. A β deposits in brain are associated with activated microglia and astrocytes, but also with elevated levels of complement [5, 6, 46]. ApoE may have an anti-inflammatory effect by suppressing microglial and astrocytic activation [47–50]. ApoE-deficient mice demonstrate increased levels of IL-6 and TNF α after LPS stimulation, suggesting a role of ApoE in inflammatory gene regulation [51]. In addition, ApoE isoform-dependent (ϵ 2< ϵ 3< ϵ 4) differences in nitric oxide (NO) levels have been observed in microglia cells [52]. Transgenic mice expressing the ApoE4 protein isoform show a greater NO production than mice expressing the ApoE3 protein isoform. These data indicate that ApoE4 has a less efficient

anti-inflammatory affect, and thus, may accelerate the development of AD.

Apolipoprotein J, also known as clusterin or SP-40/40, is a highly conserved heterodimeric secreted glycoprotein expressed in brain by epithelial and neuronal cells [53]. ApoJ colocalizes with fibrillar A β deposits, and it is suggested that it prevents misfolding and aggregation of soluble A β [54–56]. ApoD is a glycoprotein associated with high-density lipoproteins in human plasma and also has a high expression level in human brain [57], but neither its physiological role nor its ligand has been identified. ApoD levels are increased in the hippocampus of AD patients and in ApoE-deficient mice [58, 59].

In conclusion, ApoE and ApoJ can be regarded as amateur chaperones that regulate A β aggregation in vitro. By accelerating the A β aggregation process towards mature fibril formation, (human) ApoE prevents formation of toxic A β intermediates such as oligomers and protofibrils, and thus, may have a protective function towards development of AD. Moreover, ApoE protects against the development of AD by suppressing the inflammatory reactions associated with AD lesions. Besides its role in inducing conformational changes in A β , ApoE facilitates A β clearance from brain by serving as a transporter molecule of A β , which will be discussed in paragraph 4.

Heparan Sulfate Proteoglycans

Proteoglycans are members of a large family of macromolecules with a wide variety of functions ranging from simple physical support to various effects on cell adhesion, motility, proliferation, differentiation, and even tissue morphogenesis. They are composed of linear sulfated polysaccharides (glycosaminoglycans, GAGs), consisting of disaccharide units, covalently bound to a core protein. One of the members of this superfamily is the heparan sulfate proteoglycan (HSPG) family characterized by polymers of repeating disaccharides, *N*-acetylglucosamine and glucuronic acid, which can be subsequently modified by sulfatation [60, 61]. HSPGs can be subdivided into a family of extracellular matrix proteins, including perlecan, agrin, and collagen XVIII, and a family of cell surface proteins, including syndecans and glypicans [60, 62].

Ever since GAGs were demonstrated in amyloid deposits, the proteoglycans became of interest in amyloidogenesis. The presence of HSPGs in SPs, CAA, and NFTs in AD brains was already demonstrated in the late 1980s [63–65]. Only when antibodies became available that could identify the various individual HSPG species was it described that perlecan colocalized with all three lesions characteristic of AD brains [65–67]. However, we were not able to confirm these findings [68, 69]. Furthermore, it was shown that in both diffuse and classic SPs, several other HSPGs were found, such as agrin,

glypican 1, and syndecan 1-3, whereas collagen XVIII is only present in classic SPs and CAA [69–72].

These data suggest that HSPGs interact with A β , thereby contributing to development or persistence of SPs or CAA. HSPGs isolated from Engelbreth–Holm–Swarm tumor prevented proteolytic breakdown of aggregated A β [73]. In addition, both agrin and perlecan directly interacted with A β and promoted conversion of non-fibrillar A β into fibrillar A β [70, 74–76]. Although the interaction between HSPGs and A β is likely mediated predominantly by the sulfate moieties of the GAGs, a role for the protein backbone in A β aggregation could not be excluded [77, 78]. As sulfated GAGs were also demonstrated in NFTs in AD brains [79], these macromolecules may also play a role in tangle development. Indeed, sulfated GAGs may induce the formation of paired helical filaments by stimulating tau phosphorylation [80].

As heparan sulfates bind to A β and interfere with its fibrillogenesis, they are interesting candidates for therapeutic intervention [81]. GAG mimetics are able to inhibit this binding and may block the formation of β -pleated sheets and adherence of A β to the cell surface [82]. The use of GAG mimetics has already been explored in mouse models where they reduced progression of inflammation-associated amyloidosis [83]. The efficacy of one of these compounds is currently being tested in a human phase III trial.

As exemplified by ApoE, A β -binding proteins may play a role in the inflammatory reactions in AD brains. Recently, it was demonstrated that the semi-synthetic proteoglycan analogue dextran sulfate blocks activation of the complement cascade [84]. In addition, chondroitin sulfate proteoglycans are also known to bind to C1q and prevent the formation of the C1 complex in vitro [85]. By doing so, chondroitin sulfate proteoglycans inhibit normal complement function. Furthermore, heparin has long been regarded as a potential complement inhibitor [86].

In conclusion, HSPGs do not only colocalize with A β and tau, but they also contribute to the development of these lesions. The role of HSPGs in A β aggregation might even be a protective one. HSPGs prevent the persistence of toxic A β forms, e.g., oligomers or protofibrils, and transform them into more harmless aggregates, i.e., the classic senile plaques containing mature A β fibrils that are less toxic than the intermediate aggregates. In addition, HSPGs might play a role in the development of AD lesions by inhibiting complement activation. According to the definitions, HSPGs can therefore be regarded as amateur chaperones. Their ability to recognize a variety of proteins may originate from the heterogeneous structure of the heparan sulfate chains. The negatively charged HS chains are structurally heterogeneous and bind a diverse repertoire of proteins, such as amyloid A, protease-resistant prion protein, α -synuclein, and tau, providing HSPGs with the

ability to interact with a wide range of intracellular and extracellular amyloidogenic proteins [61].

Complement Factors

The complement system is an ancient host defense mechanism which is involved in boosting antibody activity. The system consists of a group of soluble serum proteins C1–C9 and is activated either by immunoglobulin M or G bound to a foreign particle or directly by microorganisms. Proteins such as Hageman factor, C4 binding protein, CDS46, CD59, and C1 inhibitor regulate the complement system. In AD, the complement system is overexpressed and activated [46]. The A β protein itself activates this system, and complement factor concentrations are increased in AD brains [87–89]. A β induces C3 and C4 in AD, and elevated levels of the membrane attack complex (MAC) composed of C5–C9 have been observed [90, 91]. In addition, factors such as Hageman factor, C1q, C3, and C5–9 are commonly found in SPs and NFTs [87, 92, 93]. C1q is associated with A β deposits and directly binds fibrillar A β which activates the complement cascade [94]. In contrast, the complement inhibitor (C1 INH) is downregulated in AD [95, 96]. Thus, an activated complement system is a general feature observed in AD. However, the contribution of complement to the pathogenesis of AD is controversial.

On the one hand, it is suggested that complement activation protects against A β -induced toxicity and even contributes to reducing the accumulation of A β in SPs [97]. Transgenic mice expressing complement inhibitors develop increased AD-pathology, whereas increased complement C3 production was associated with a reduction of A β deposition [97]. Thus, the complement activation in the brain may be beneficial in AD and possibly also other neurodegenerative diseases [98–100].

However, complement activation may lead to accelerated neurodegeneration as well. Activation of complement in an antibody-independent fashion is achieved by binding of aggregated, but not soluble, A β to C1q [12, 90, 101, 102]. This latter finding suggests that in AD, aggregated A β induces chronic complement activation. Thus, C1 binding to fibrillar A β deposits may precede microglial activation. Both A β and pro-inflammatory stimuli are able to activate microglia, which results in increased A β and cytokine production [103]. Furthermore, cultured human microglial cells show an increase in cytokine production after co-stimulation of A β with C1q and serum amyloid P (SAP) [104]. This suggests that microglia may get triggered by both A β - and SP-associated factors such as C1q, which results in the secretion of pro-inflammatory cytokines and A β , both accelerating neurodegeneration.

Although none of the complement factors directly regulate conformational changes of A β , complement

activation as a whole plays a role in the A β aggregation *in vivo*. Therefore, complement factors might act as amateur chaperones, although their exact role in A β aggregation remains to be elucidated.

Professional Chaperones

Heat shock proteins (Hsp) are professional chaperones. They are highly conserved proteins constitutively expressed in most cells under normal conditions where they play a role in cellular metabolism and help normal folding processes [105]. In addition, during cell stress, they bind unfolded proteins to keep them in their native state [105]. Heat shock proteins can be divided into two different families based on size and function: classic Hsps such as Hsp100, Hsp90, Hsp70, Hsp60, and the small heat shock proteins (sHsps). Hsps with a molecular weight of 60 kD or more possess an ATP-binding site and are actively involved in the process of refolding of misfolded proteins [106]. Small Hsps, with a molecular weight of 40 kD or less, lack this ATP-binding site and assist the Hsps in their refolding function [107]. The role of Hsps in misfolded protein recognition and refolding is illustrated in Fig. 1.

Small Heat Shock Proteins

Small Hsps function as molecular chaperones that can prevent proteins from adopting an incorrect conformation [108]. The sHsp family is characterized by the presence of an α -crystallin domain, a stretch of 80–100 amino acids in the C terminal half of the proteins [109]. So far, the sHsp family comprises ten sHsps, including α B-crystallin, Hsp27, Hsp20, HspB8, and HspB2/B3 [110]. Although sHsps are

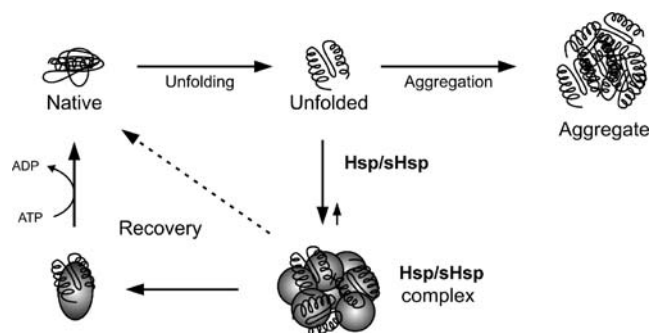


Fig. 1 The role of heat shock proteins (*Hsp*) and small heat shock proteins (*sHsps*) in recognition and refolding of unfolded and misfolded proteins. Unfolded or misfolded proteins are recognized by Hsps and sHsps. Together with these unfolded or misfolded proteins, Hsps and sHsps form a complex. In addition, Hsps recover unfolded or misfolded proteins back to their native form using ATP. If unfolded or misfolded protein are not recognized by the Hsp/sHsps, these unfolded or misfolded proteins are capable of forming aggregates

predominantly expressed in muscle cells, several family members are also found in human brain.

In AD, α B-crystallin and Hsp27 are upregulated and expressed by astrocytes surrounding SPs and NFTs [111–114], whereas Hsp20, HspB2, and HspB8 colocalize with A β in SPs and CAA [115, 116]. Although α B-crystallin or Hsp27 do not colocalize with A β in SPs, direct interaction between A β and these sHsps in addition to Hsp20 and HspB8 has been demonstrated [111, 117–119]. In addition, high-affinity binding of α B-crystallin and A β has been observed in eye lenses from AD patients [120]. Furthermore, α B-crystallin is able to prevent mature A β fibril formation, retaining it in a non-fibrillar, but likely a protofibrillar state, which is highly toxic to neurons [121]. Recently, we demonstrated that α B-crystallin, Hsp20, and HspB8 inhibit A β -mediated toxicity towards cerebrovascular cells probably by preventing aggregation of A β at the cell surface [116, 117]. Others showed that Hsp27 directly binds to hyperphosphorylated tau, thereby protecting against cell death [122].

Hsps are involved in the formation and persistence of misfolded protein aggregates. They are upregulated in several neurodegenerative diseases, such as AD, Creutzfeldt–Jakob disease, and Parkinson’s disease probably as a reaction to the formation of misfolded proteins [113, 123–126]. However, despite of increased intracellular levels, they are unable to prevent accumulation of A β in AD possibly because of decreased chaperone activity. In aged rats, this was illustrated by a significant decrease of Hsp90 function [127], resulting in diminished hepatic chaperone capacity. Furthermore, the increasing amount of damaged or misfolded proteins as a result of defects in protein degradation might lead to a total decrease in chaperone activity in aged cells [128]. Thus, the state of misfolded protein recognition and repair systems, such as the (s)Hsp system, might be of great importance in the development of neurodegenerative diseases.

Miscellaneous Proteins

Apart from the above-described proteins, several other molecules are also associated with the pathological lesions of AD, and some of these can be regarded as amateur chaperones. Acute phase proteins, such as α_1 -antichymotrypsin (ACT), α_2 -macroglobulin (α 2M), and SAP, are all associated with A β deposition [129–132]. ACT is a serine protease inhibitor of the serpin family, and in AD, ACT levels are upregulated, and binding of ACT with A β induces A β fibrillogenesis [133–135]. Furthermore, when ACT is overexpressed in transgenic mice, an increased plaque load in the brains of these mice and impaired spatial learning is observed [134, 135]. α 2M also binds A β , although in contrast to ACT, this binding prevents A β fibril

formation and fibril-associated neurotoxicity [136, 137]. α 2M promotes the protease-mediated degradation of α 2M/A β complexes and contributes to clearance of A β from the brain (discussed in paragraph 4) [138, 139]. The glycoprotein SAP belongs to the pentraxin family and is a common component of all known types of amyloid fibrils. SAP is upregulated in AD and protects amyloid fibrils from proteolysis *in vitro* [140, 141]. SAP not only colocalizes with SPs and interacts with aggregated A β ; SAP oligomers also bind and activate C1 [142]. Both C1 and SAP may bind to fibrillar A β deposits *in vivo* and induce microglial activation, as cultured human microglial cells show an increase in cytokine production after co-stimulation of A β with C1q and SAP [104]. These observations further strengthen the above-noted suggestion that not only A β , but also several A β -binding proteins, are capable of activating the complement system, and thus, contribute to neuroinflammation in AD. In addition, both α 2M and ACT, in contrast to SAP, can be regarded as amateur chaperones, as they regulate conformational changes of A β .

Tissue-type plasminogen activator (tPA) regulates activation of plasminogen into plasmin and is expressed in various regions of the brain especially in the hippocampus [143]. Several reports suggested an important role for tPA in AD, as the tPA system is involved in A β turnover [144, 145]. Fibrillar forms of A β stimulate tPA activity *in vitro*, whereas in AD patients, a reduction of tPA activity is observed in the affected areas [144, 146]. Although tPA has no effect on conformational changes of A β , it might play a role in the clearance of A β from the brain (paragraph 4).

The actin-regulatory protein gelsolin is found both intracellularly and in plasma [147, 148]. Plasma gelsolin can be considered an amateur chaperone, as it binds A β and not only inhibits its A β fibrillization but is also capable of degrading preformed A β fibrils [149, 150]. Furthermore, gelsolin inhibits A β -mediated neurotoxicity [151].

One of the major gangliosides in the brain is GM1. Soluble A β binds GM1 and the formed complexes accelerate A β fibrillogenesis by acting as a seed for A β [152]. In the presence of GM1, A β is more neurotoxic than A β alone, and cholesterol-rich membranes demonstrate accelerated A β binding due to the formation of GM1 clusters [153, 154]. As GM1 is a major component of lipid rafts and recent studies suggest that A β accumulation in these lipid rafts is an early event in AD development, GM1 might play an important role in the early steps in AD pathogenesis [155, 156].

In summary, several proteins are associated with A β aggregates in the AD brain and contribute to the aggregation of A β and should, therefore, be considered as amateur chaperones. In addition, they might play a role in triggering inflammation.

A β -Binding Proteins and Intracellular Interactions with A β

Intracellular accumulation of A β already starts in the ER or in the Golgi apparatus of the cell [157–159]. Intracellular A β is associated with neuronal damage [160, 161], and intraneuronal accumulation of A β in transgenic mice was correlated with impairments in synaptic plasticity [162]. Intraneuronal accumulation of A β in those brain areas affected earliest in AD suggests a possible relation between intracellular A β and development of AD [160].

A few proteins that interact with intracellular A β and affect its intracellular fate have been identified. The endoplasmic reticulum amyloid beta-peptide-binding protein binds intracellular A β and mediates neurotoxicity in neuronal cells by forming an intracellular target for A β [163]. In addition, the mitochondrial enzyme amyloid- β alcohol dehydrogenase also binds A β inside neurons, resulting in the production of free radicals [164]. However, whether these intracellular A β -binding proteins affect aggregation of A β within the cells remains unknown. Therefore, both these proteins cannot, for the time being, be defined as amateur chaperones of A β .

The first lines of defense against misfolded and aggregated proteins are the professional chaperones, which counteract these processes and are able to stimulate clearance of misfolded proteins by proteosomal degradation. Newly synthesized proteins are folded by several other proteins, such as immunoglobulin-binding protein (BiP)/glucose-regulated protein (GRP78), and calnexin. GRP78 is a member of the Hsp70 protein family and interacts with intracellular APP. GRP78 regulates APP and A β secretion by intervening between APP and β -/ γ -secretases within the cell [165].

It is not surprising that in AD, where misfolded protein molecules accumulate, both Hsp90 and Hsp70 synthesis is increased. Several members of the Hsp family directly interact with intracellular A β , but only recently, Hsp70 was identified as a protector against intracellular A β accumulation [166, 167]. Besides, immunoreactivity of both Hsp90, 70, and Hsp60 is found in SPs [132], which suggests that these professional chaperones may not only interact with misfolded protein in the cell interior [168–171]. In addition, it has also been postulated that up-regulation of Hsp90 and Hsp70 may suppress the formation of NFTs by partitioning tau into a productive folding pathway and thereby preventing its aggregation [172]. Recently, it was demonstrated that the chaperone CHIP–Hsc70 complex conjugates ubiquitin to hyperphosphorylated tau, which enhances cell survival by elimination of soluble hyperphosphorylated tau [173]. These data suggest that the cell increases production of the Hsps to cope with the presence of misfolded proteins such as hyperphos-

phorylated tau and accumulating A β . At some point, this protective mechanism seems to fail, however. In line with this hypothesis, it was shown that the actin and tubulin specific chaperone Hsp60 is decreased in AD, resulting in a decrease of cytoskeletal proteins in AD-affected neurons [174]. Thus, both production and function of Hsps seems to be disturbed in AD, which might result in the accumulation of misfolded proteins. The role of other Hsps in regulating intracellular A β or tau folding remains to be investigated (Table 1).

A β -Binding Proteins and A β Clearance

A β -binding proteins, amateur chaperones, play a role in the clearance of A β from brain by functioning as a transporter molecule. Two major pathways govern A β clearance. By the first pathway, A β is removed from brain to blood via active transport across the BBB. This active transport is performed by specialized transporters, so-called “A β -receptors”, expressed by endothelial cells. Second, A β is removed from brain via phagocytosis by both microglial cells and astrocytes. In both pathways, interaction of A β with cell surface A β -receptors is crucial; therefore, the expression levels of A β -binding proteins might contribute to A β clearance by regulating its binding with A β receptors.

The low-density lipoprotein receptor-related protein-1 (LRP-1) binds A β in a complex with ApoE at the abluminal side of the endothelium and internalizes these ApoE/A β complexes followed by degradation in lysosomes or transport into the plasma [4, 175]. However, LRP-1 also mediates transport of free A β across the BBB [10]. In contrast to LRP-1, the receptor for advanced glycation end products (RAGE) transports A β from the circulation into the central nervous system [176]. Similar to RAGE, the A β receptor megalin is also involved in the transport of A β from blood to brain, although megalin probably plays only a minor role in A β transport. Furthermore, megalin binds A β /ApoE complexes rather than free A β [177, 178]. Clearance of A β /ApoE complexes from brain might be ApoE isoform-dependent. ApoE4 forms less stable complexes with A β than ApoE3 or ApoE2; therefore, ApoE4 reduces A β transport efficiency across the BBB. Additionally, as described above (paragraph 2), ApoE4 enhances A β aggregation more efficiently than ApoE3, which also inhibits clearance. On the other hand, the LDL receptor shows a marked preference for the ApoE3 and ApoE4 isoforms and binds the ApoE2 isoform poorly [179]. Given the similarity between the LDL receptor family, other LDL receptors, such as the LRP-1 receptor, might display similar specificities towards the ApoE isoforms, but this has not been reported yet. Moreover, lipidation of ApoE also affects clearance of ApoE and ApoE/A β complexes from brain, as LRP

Table 1 Summary of the expression of chaperones in AD brains and their interaction and effects on A β and tau

| | SP/CAA | NFT | Direct interaction | Effects on A β or tau in general |
|--------------------------------|--------|-----|--------------------|--|
| Apolipoproteins | | | | |
| ApoE | + | + | A β /tau | ↑ Fibrillar A β /↓ hyperph. Tau |
| ApoJ | + | ? | A β | ↓ A β aggregation |
| HSPGs | | | | |
| Perlecan | ± | ± | A β | HSPGs: |
| Agrin | + | – | A β | ↓ Proteolytic breakdown A β |
| Glypican 1 | + | – | ? | ↑ Non-fibrillar → fibrillar A β |
| Syndecan 1–3 | + | – | ? | ↑ Phosphorylation tau |
| Collagen XVIII | + | – | ? | |
| GAGs | + | + | A β /tau | |
| Complement factors | | | | |
| Hageman Factor | + | + | ? | A β activates complement in AD |
| C1q | + | + | A β | C3 ↓ A β deposition |
| C3/C4 | + | + | A β | |
| C5-9 | + | + | ? | |
| Heat shock proteins | | | | |
| Hsp90 | + | ? | Tau | ↓ Tau aggregation |
| Hsp70 | + | ? | A β /tau | ↓ Tau aggregation |
| Small Hsps | | | | |
| α B-crystallin | – | – | A β | ↓ A β fibril formation |
| Hsp27 | – | ± | A β /tau | ↓ A β fibril formation |
| Hsp20 | + | – | A β | ↓ A β fibril formation |
| HspB2/B3 | + | + | – | No effect |
| HspB8 | + | – | A β | ↓ A β fibril formation |
| Acute phase proteins | | | | |
| α_1 -antichymotrypsin | + | – | A β | ↑ A β fibrillization |
| α_2 -macroglobulin | + | – | A β | |
| serum amyloid P | + | + | A β | ↑ A β fibrillization |
| Miscellaneous compounds | | | | |
| tPA | – | – | A β | ↓ A β fibril formation |
| Gelsolin | – | – | A β | ↑ A β fibrillization |

Expression of chaperones in a specific lesion is illustrated as follows: present (+), by conflicting reports (±), absence (–), and unknown (?); ↓ = inhibition or down-regulation, ↑ = induction or up-regulation

SP Senile plaques, CAA cerebral amyloid angiopathy, NFT neurofibrillary tangles, HSPGs heparan sulphate proteoglycans, A β amyloid-beta, Hsp heat shock proteins, Apo apolipoproteins, SAP serum amyloid P, tPA tissue-type plasminogen activator, GAGs glycosaminoglycans, LDLR low-density lipoprotein receptor, LRP-1 LDL receptor protein-1, BBB blood–brain barrier

preferentially binds lipid-rich forms of ApoE [179]. These data indicate that A β -binding proteins, especially ApoE, and possibly, ApoJ, play an important role in transport of A β across the BBB and that both the ApoE isoform and the ApoE lipidation state affect A β clearance. In addition to ApoE and ApoJ, the A β -binding protein α 2M also forms complexes with A β . As α 2M is a ligand for LRP-1, these α 2M/A β complexes might undergo LRP-1-mediated endocytosis and degradation or translocation into the plasma [7, 139].

Stimulation of the transport of A β across the BBB demonstrated to be an effective therapeutic approach in AD, as several studies demonstrated elevated levels of A β in the plasma of mice after passive immunization with anti-A β antibodies or Fab fragments [180–182], and decline in cognitive performance was arrested in patients that received vaccination [183]. However, the occurrence of severe meningoencephalitis in human patients after active immu-

nization with A β hampered widespread application of this type of therapy. Administration of A β -binding proteins that demonstrate similar positive effects, but possibly, without the severe immune reactions associated with antibody therapy, might provide an alternative strategy. An interesting example of such an A β -binding protein is gelsolin. This protein has high affinity for A β and reduces A β levels in a transgenic mouse model of AD [184]. Furthermore, administration of gelsolin or GM1 in PS/APP mice resulted in decreased A β aggregation in the brains [184]. Both gelsolin and GM1 act as a “peripheral sink” for A β . Although both compounds did not enter the brain, they lowered soluble A β concentrations in the blood, shifted the balance between blood and cerebral A β concentrations, and accordingly, stimulated A β transport over the BBB. Therefore, other A β -binding proteins administered in the circulation might also act as “peripheral sinks” [181, 184].

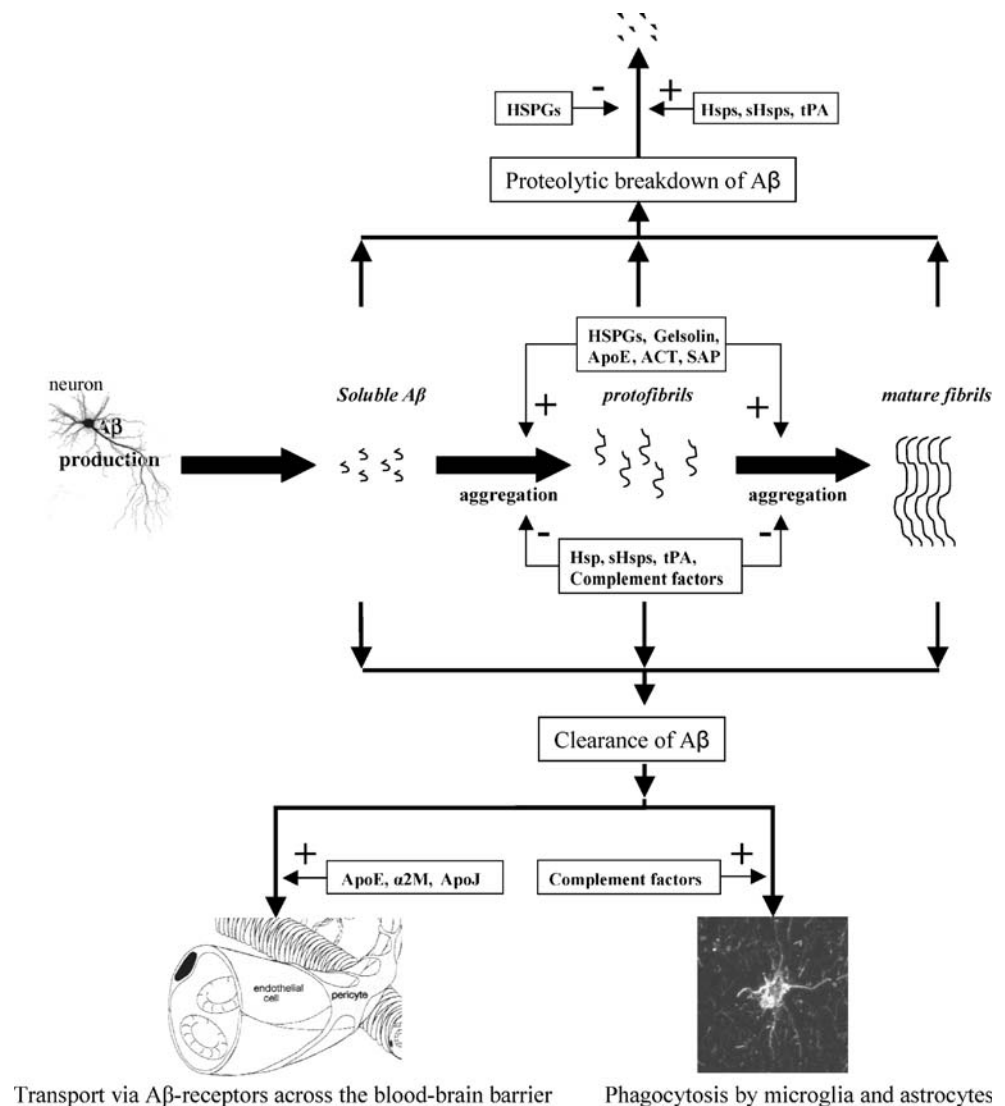
Both activated microglial cells and activated astrocytes are associated with A β deposition and may internalize A β fragments via phagocytosis [185–187]. Activation of the complement system is, among others, achieved by Hsps such as Hsp60 and Hsp70, which are able to induce phagocytosis by microglia, and thus, clearance of A β [132, 188, 189]. In addition, the absence of ApoE reduces internalization and degradation of A β by astrocytes in the brain, demonstrating that ApoE is directly involved in the clearance of A β from brain via phagocytosis by microglial cells and astrocytes [40]. tPA might also contribute to clearance of A β , as it accelerates A β clearance from transgenic mouse brains [146]. Thus, as A β -chaperones contribute to activation of the complement system or activation of microglial cells and astrocytes, these proteins might contribute to the clearance of A β from the brain via phagocytosis.

Concluding Remarks

Professional chaperones, such as the heat shock protein family, and amateur chaperones, such as apolipoproteins and HSPGs and several other proteins, have a role in the intracellular handling of misfolded proteins, induce conformational changes of proteins, or act as transporter of proteins (Fig. 2). This suggests that these chaperones form interesting therapeutic targets in the prevention and treatment of neurodegenerative diseases.

In the process of clearance of A β from the brain, A β -binding partners might play important roles by acting as A β transporter proteins in both the receptor-mediated clearance of A β across the BBB but also as a “peripheral sink” for A β . Both ApoE isotype and local concentrations in the brain might regulate A β transport across the BBB, but as this transport is receptor-mediated, other A β -binding

Fig. 2 The putative role of chaperones in amyloid- β (A β) fibril formation, proteolytic breakdown, and clearance from the brain. In Alzheimer’s disease, soluble A β , predominantly produced in neurons, is converted into β -sheet rich protofibrils and eventually forms mature A β fibrils. The conversion from soluble A β to protofibrils and fibrils, which accumulate in senile plaques and cerebral amyloid angiopathy, is enhanced by chaperones as apolipoprotein E (ApoE), Gelsolin, α_1 -antichymotrypsin (ACT) and several heparan sulphate proteoglycans (HSPGs), which function as catalysts. In contrast, the heat shock protein family, tissue-type plasminogen activator (tPA) and complement factors prevent the transition of soluble A β into protofibrils and mature fibrils. Furthermore, heat shock proteins and tPA stimulate the proteolytic breakdown of (proto) fibrils, whereas HSPGs prevent this breakdown. Finally, the clearance of A β from the brain across the blood–brain barrier is stimulated by ApoE, ApoJ, and α_2 -macroglobulin (α_2 M), whereas complement factors stimulate phagocytosis-mediated clearance of A β by activated microglia and astrocytes



proteins might also fulfill such a role. In addition, transport of aggregated A β across the BBB is less efficient than soluble A β . Thus, by preventing self-aggregation of A β , A β -binding proteins contribute to the clearance of A β from the brain. As a therapeutic strategy, A β -binding proteins serving as a “peripheral sink”, such as gelsolin, seem promising [184].

Overexpression of professional chaperones, such as the Hsps, to prevent aggregation of misfolded proteins will have to be evaluated carefully, as they also interact with other chaperones and are dependent on this interaction to fulfill some of their functions. This strategy may therefore result in instability of the cell-stress mechanism, which may cause the system to collapse. A solution may be found in the overexpression of several chaperones, which may be required to achieve an impact on the progression of the disease.

Another pitfall in the use of professional chaperones as therapeutic agents is their ability to bind misfolded proteins and keep them in an intermediate conformation. This type of conformation might even be more toxic than the aggregated state. As an example, co-incubations of α B-cystallin with A β are more toxic to neurons than A β alone [121]. Furthermore, Hsps are most likely to be involved in early development of neurodegenerative diseases, given their natural function. Yet, the role of this protein family in maturation of the neurodegenerative lesions remains to be elucidated.

In conclusion, studying the role of chaperones, both professional and amateur, in the pathophysiology of AD will provide us with a better understanding of the mechanisms underlying the formation and accumulation of toxic aggregates in AD, which, eventually, will lead to the design of more effective therapeutic strategies.

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