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Review article

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Extracellular protein homeostasis in neurodegenerative diseases

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Abstract

The protein homeostasis (proteostasis) system encompasses the cellular processes that regulate protein synthesis, folding, concentration, trafficking and degradation. In the case of intracellular proteostasis, the identity and nature of these processes have been extensively studied and are relatively well known. By contrast, the mechanisms of extracellular proteostasis are yet to be fully elucidated, although evidence is accumulating that their age-related progressive impairment might contribute to neuronal death in neurodegenerative diseases. Constitutively secreted extracellular chaperones are emerging as key players in processes that operate to protect neurons and other brain cells by neutralizing the toxicity of extracellular protein aggregates and promoting their safe clearance and disposal. Growing evidence indicates that these extracellular chaperones exert multiple effects to promote cell viability and protect neurons against pathologies arising from the misfolding and aggregation of proteins in the synaptic space and interstitial fluid. In this Review, we outline the current knowledge of the mechanisms of extracellular proteostasis linked to neurodegenerative diseases, and we examine the latest understanding of key molecules and processes that protect the brain from the pathological consequences of extracellular protein aggregation and proteotoxicity. Finally, we contemplate possible therapeutic opportunities for neurodegenerative diseases on the basis of this emerging knowledge.

Sections

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Key points

- Evidence is accumulating that age-related impairment of extracellular proteostasis contributes to neuronal death in neurodegenerative diseases.
- Key elements implicated in the protective functioning of extracellular proteostasis are a growing family of constitutively secreted extracellular chaperones and extracellular proteases, including plasmin, which is regulated by the plasminogen activation system.
- Multiple studies have already demonstrated the benefits of administering exogenous extracellular chaperones or increasing their level of expression in animal models of neurodegenerative diseases.
- Strategies to manipulate elements of extracellular proteostasis have the potential to reduce excessive levels of misfolded proteins in the synaptic space and interstitial fluid and thereby to ameliorate associated disease pathologies.

Introduction

The human proteome comprises around 20,000 different proteins, many of which exist in a wide variety of cell-specific proteoforms[1]. In the face of this complexity, the proteostasis system, which regulates protein synthesis, folding, concentration, trafficking and degradation, has a crucial role in maintaining cell viability. While the proteostatic mechanisms that operate inside cells (intracellular proteostasis) have been investigated extensively[2–4], the mechanisms that operate in the extracellular space are yet to be fully elucidated. Considering that about 40% of human proteins are extracellular or membrane-associated[5], extracellular proteostasis is likely to be vital for defending cells, including brain cells, from the many pathological processes that underlie neurodegenerative diseases[6–9].

In the extracellular space, protein molecules are persistently exposed to an oxidizing environment and shear stresses associated with fluid circulation. These conditions lead to destabilization of the native states of proteins, which can result in misfolding[10,11]. A range of neurodegenerative diseases are associated with accumulation of specific misfolded proteins, in which hydrophobic regions that are buried in the native state become exposed, leading to the formation of potentially cytotoxic assemblies[12]. For example, in individuals with Alzheimer disease (AD), the amyloid-β (Aβ) peptide aggregates into small oligomers that damage brain cells and later form insoluble fibrillar aggregates that deposit into large amyloid plaques in the brain[13,14].

Among other classes of molecules that are important in extracellular proteostasis, constitutively secreted extracellular chaperones (ECs) are likely to be key players in protecting the brain from the pathological consequences of proteins that misfold and aggregate in extracellular fluids[8]. ECs protect cells by neutralizing the toxicity of extracellular protein aggregates and promoting their safe clearance and disposal. The first of the mammalian ECs to be identified was clusterin, which was originally described as an ATP-independent chaperone over 20 years ago[15]. Since then, around 20 ECs have been described[16–18] (Table 1). However, given that hundreds of intracellular chaperones and co-chaperones are known to exist, many more ECs could be identified in the future. Many independent lines of evidence point towards a direct involvement of ECs such as clusterin in protecting the brain from disease. Our current knowledge of the role of ECs in brain health and pathology has come from studies of genetics, disease-associated changes in EC expression and physical colocalization of ECs with disease-associated protein aggregates, as well as experimental studies of the effects of manipulating EC levels on disease pathologies[8].

Other important components of the extracellular proteostasis systems are proteases, protease inhibitors and endocytic cell surface receptors. In this Review, we outline current knowledge of the mechanisms of extracellular proteostasis, with an emphasis on the aspects linked to neurodegenerative diseases. We examine the latest understanding of key molecules and processes that protect the brain from the pathological consequences of extracellular protein aggregation and proteotoxicity. The Review includes brief outlines of the rapidly growing family of known ECs found in body fluids and a proposed involvement of the plasminogen activation system (PAS) in the clearance of extracellular protein deposits. Finally, we contemplate possible therapeutic opportunities for neurodegenerative diseases enabled by this emerging knowledge.

The proteostasis system

Intracellular proteostasis

The intracellular environment contains a wide variety of molecular chaperones and co-chaperones that help nascent proteins to fold correctly, facilitate protein translocation across lipid membranes, shield misfolded proteins from aberrant inter-molecular interactions, disassemble protein aggregates and direct misfolded proteins that cannot be refolded towards cellular degradation systems[2–4]. The folding and refolding of proteins inside cells is largely an ATP-dependent process, requiring the presence of at least micromolar levels of ATP. ATP is often found inside cells at millimolar concentrations and, at these high levels, has been suggested to act as a hydrotrope to help solubilize hydrophobic proteins[19]. The two primary systems that operate inside cells to degrade proteins are autophagy and the proteasome[2–4]; selective ubiquitination of misfolded proteins by a family of cytosolic ubiquitin ligases can direct the proteins to these systems for disposal. An outline of the main processes involved in intracellular proteostasis is presented in Fig. 1. For more detailed descriptions of the intracellular proteostasis system and its relevance to neurodegenerative diseases, we refer the reader to other sources[2,3,20].

Overview of extracellular proteostasis

Although the extracellular environment contains measurable amounts of both ATP and ATP-dependent foldase chaperones (in particular, heat shock protein 70 (HSP70)), the levels of these molecules are orders of magnitude lower than those found inside cells[21–23]. Consequently, ATP-dependent refolding of extracellular misfolded proteins is likely to play a minor part in the maintenance of extracellular proteostasis. However, locally increased extracellular concentrations of normally intracellular chaperones released from cells, such as HSP70 and HSP90, could enable ATP-independent binding to misfolded extracellular proteins to neutralize proteotoxicity and facilitate internalization and lysosomal degradation of the formed complexes. The proteasome is also present only at low levels in extracellular fluids[24], suggesting that other protease systems are likely to be involved in the proteolytic degradation of extracellular misfolded proteins in synaptic clefts, interstitial fluid (ISF) and cerebrospinal fluid (CSF).

Ample evidence indicates that a family of constitutively secreted molecular chaperones is present in the extracellular space and has key roles in binding to misfolded proteins to inhibit their toxicity

Table 1 | Extracellular proteins known to have chaperone activity

Protein	Molecular mass (kDa)	Plasma or serum concentration (μg ml^{-1})	Cerebrospinal fluid concentration (μg ml^{-1})	Types of protein aggregation inhibited	Refs.
α_2-Macroglobulin (A2M)	720	2,729	1.54	Amorphous and amyloid	67,140,141
Haptoglobin	94–116	1,250	0.6	Amorphous and amyloid	67,82
Vitronectin	75	610	0.025–0.072	Amorphous and amyloid	16,48
Transthyretin	55	260	15.5	Amyloid	73,142,143
Prothrombin	72	154	16.7	Amyloid	16,144,145
Clusterin	~60	125	2	Amorphous and amyloid	30,58
Complement component 1s (C1s)	28	93	–	Amyloid	16,146
Serum amyloid P (SAP)	~25	40	8.5	Amorphous and amyloid	147,148
Pregnancy zone protein (PZP)	720	10–30	–	Amorphous and amyloid	149,150
Plasminogen activator inhibitor 3 (PAI3)	46	6.3	0.05	Amorphous and amyloid	16,151
Complement component 1r (C1r)	83	5.5	–	Amyloid	16,146
Insulin-degrading enzyme (IDE)	110	2.0	0.006	Amyloid	152–154
Macrophage inhibitory factor (MIF)	37.5	0.0057	0.0024	Amorphous	155,156
Secreted protein acidic and rich in cysteine (SPARC)	35	0.0015	–	Amorphous	157,158
Neuroendocrine protein 7B2 (7B2)	27	0.000056	0.037	Amyloid	53,159–161
Neuroserpin	45	–	0.0007	Amyloid	73,142
Surfactant protein C (SP-C)	21	–	0.0005	Amorphous and amyloid	162,163
Integral membrane protein 2 (BRI2)	13.6	–	–	Amorphous and amyloid	163
Proprotein convertase subtilisin (proSAAS)	27	–	–	Amyloid	159,164
Caseins	~25	–	–	Amorphous and amyloid	165–167

The proteins in this table are arranged in the descending order of known plasma or serum concentration. These proteins have been demonstrated to inhibit the in vitro aggregation of various proteins to form amorphous (unstructured) or amyloid (fibrillar) aggregates.

and aggregation[17,25,26]. These ECs are also thought to form soluble complexes with misfolded proteins to mediate their uptake by receptor-mediated endocytosis and safe disposal through proteolytic degradation within lysosomes. In some cases, despite the operation of these protective mechanisms, large insoluble extracellular protein deposits can form. In these situations, extracellular proteolytic systems, such as the PAS, can play a part in clearing the deposits[27]. A summary of the main processes that operate to maintain extracellular proteostasis is presented in Fig. 2.

It should be noted that the extracellular and intracellular proteostasis systems work together. Proteins that misfold and aggregate inside brain cells can be extruded into the ISF, where they can be degraded by extracellular proteases, phagocytosed by microglia and astrocytes or cleared by the glymphatic system[28]. In turn, proteins that misfold and aggregate in the ISF can be redirected for cell uptake and subsequent intracellular degradation in lysosomes.

The expanding family of ECs

Since the discovery of clusterin[15], a range of other proteins with EC activity have been identified (Table 1). A study published in 2021 used a purpose-designed technique to recover and identify previously unknown ECs in human serum[16]. Among the currently known ECs, only serum amyloid P (SAP) protein has been reported to have some, albeit limited, ability to refold misfolded proteins: denatured lactate dehydrogenase was reactivated in the presence of a 10-fold molar excess of SAP[29]. Clusterin lacks any measurable independent refolding activity, although it was shown to support protein refolding in vitro when co-incubated with ATP and HSP70 (ref. [30]). The ability of other ECs to refold proteins has not been directly tested. However, given the low levels of extracellular ATP, most ECs seem likely to act as holdases, which bind to and stabilize misfolded proteins to inhibit their aggregation, but not as foldases, which would require ATP to perform protein refolding. For the majority of ECs, investigations into the nature of their chaperone activities in vivo are yet to be performed.

Endocytic cell receptors

A number of endocytic cell receptors have been reported to play an active part in extracellular proteostasis by mediating the uptake of EC complexes. Complexes formed between α_2-macroglobulin (A2M) and Aβ can be internalized by LDL receptor-related protein 1 (LRP1, also known as the A2M receptor) and degraded inside lysosomes[31]. Similarly, LRP2 (also known as megalin) mediates the uptake and degradation of clusterin and clusterin–Aβ complexes[32,33]. One study in mouse primary microglia and human monocyte-derived macrophages demonstrated that triggering receptor expressed on myeloid cells 2 (TREM2) binds and facilitates uptake of LDL-associated clusterin, and internalization of complexes of lipidated clusterin and Aβ was found to be TREM2-dependent[34]. Another clusterin receptor, plexin A4, is widely expressed in neurons in the brain and might also be involved in clusterin-mediated clearance of Aβ[35]. Mutations in the genes that encode TREM2 and plexin A4 are, like those in the clusterin (*CLU*) gene, associated with the risk of AD onset[35,36], strongly suggesting that receptor-mediated uptake and degradation of complexes formed between clusterin and Aβ can substantively affect AD pathology.

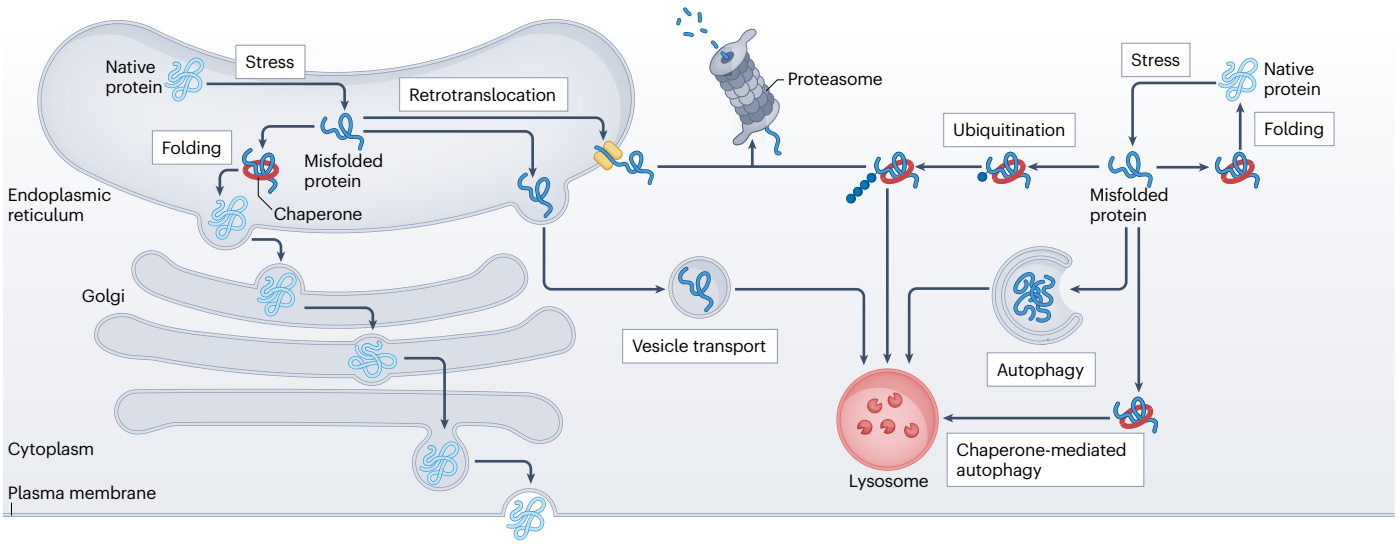

Fig. 1 | Overview of known intracellular proteostasis mechanisms. Inside cells, native proteins can fail to fold correctly or can change their state in response to various stressors to become misfolded proteins. Misfolded proteins can be refolded by ATP-dependent foldase chaperones[3]. If this process is unsuccessful, the misfolded proteins can become ubiquitinated and be degraded by proteasomes or within lysosomes[168]. Misfolded proteins inside the endoplasmic reticulum can be exported via vesicle transport or retrotranslocation for subsequent cytoplasmic degradation. Molecular chaperones assist in many of these processes[169]. For simplicity, cofactors are not shown.

Other cell receptors are also likely to be involved in mediating the uptake and safe disposal of complexes formed between ECs and misfolded proteins. The scavenger receptor inhibitor fucoidan was reported to inhibit the uptake of clusterin–misfolded protein complexes in an in vivo rat model, suggesting that widely expressed scavenger receptors play an important part in the clearance of these complexes[25]. Furthermore, in this model, the vast majority of radiolabelled complexes quickly became localized to the liver, suggesting that this organ has a major role in the safe disposal of EC–misfolded protein complexes formed in body fluids[25]. A 2020 report suggested that the heparin sulfate receptor mediates the uptake and degradation of clusterin–misfolded protein complexes[37]. However, the study lacked any direct confirmation of the involvement of the heparin sulfate receptor in the uptake of the complex. Furthermore, the researchers used clusterin bearing a large tandem fluorescent protein tag at the C terminus, which could affect the behaviour of the molecule as its mass approaches that of clusterin itself.

The evidence discussed in this section indicates that endocytic cell receptors are crucial elements of the extracellular proteostasis system and could be potential therapeutic targets for protein aggregation-related diseases, including those of the nervous system. Identification of additional key receptors that mediate the uptake of specific EC–misfolded protein complexes will be an important focus of future research.

Synergies between ECs and other extracellular proteostasis systems

The plasminogen activation system. The PAS is a group of plasma proteins that regulate the activation of the zymogen plasminogen to the active protease plasmin, which is best known for its role in digesting fibrin clots. However, studies in both in vitro and in vivo experimental systems have demonstrated that plasminogen and the tissue plasminogen activator (tPA) bind to many different protein aggregates to generate active plasmin, which then degrades the aggregates[38,39]. Later work showed that small protein fragments released by plasmin-mediated digestion of protein aggregates are bound by the ECs clusterin and A2M, giving rise to the proposal that the PAS and ECs work together to clear extracellular protein deposits from the body[27]. Collectively, these findings suggest that the PAS degrades large insoluble extracellular protein aggregates to release small soluble protein fragments that are bound by circulating ECs. Once bound, the protein fragments could be internalized by receptor-mediated endocytosis and safely proteolysed within lysosomes (Fig. 2). A role for the PAS in the clearance of insoluble pathological protein deposits is supported by multiple studies in mouse models. For example, following neurotrauma, the levels of extracellular misfolded tubulin aggregates were greater in a plasminogen knockout mouse model than in controls, and clearance of the aggregates was delayed[38].

In addition to clusterin and A2M, other ECs implicated in interactions with the PAS include haptoglobin, which is cleaved by urokinase plasminogen activator (uPA)[40]; prothrombin, which is cleaved by plasmin to produce thrombin (an enzyme that converts fibrinogen to fibrin to form blood clots); and PAI3, which is a uPA inhibitor. All of these ECs, and potentially others, might work together with the PAS in different biological contexts. A functional synergy between ECs and the PAS, and possibly other yet to be identified extracellular proteolytic systems, could be crucial for the systemic clearance of large extracellular protein deposits associated with neurodegenerative disease pathologies (Fig. 2).

Protease and protease inhibitor functions of ECs. Interestingly, all known ECs are multifunctional proteins and many also function as proteases or protease inhibitors. Insulin-degrading enzyme (IDE) is a constitutively active protease that cleaves Aβ and other peptides[41], and prothrombin, complement component 1s and complement component 1r are all zymogen proteases that are activated by proteolytic

cleavage or binding-induced conformational changes. By contrast, A2M and pregnancy zone protein both inhibit multiple proteases; clusterin inhibits several members of the metalloproteinase family[42]; haptoglobin inhibits cathepsin B[43]; and both PAI3 and neuroserpin inhibit serpin proteases. Clusterin, vitronectin and SAP show further functional diversity, as they all interact with complement proteins and might thereby modulate innate immune responses against tissue damage and pathological challenges[44,45]. In addition, clusterin, vitronectin and A2M can exert effects on cell adhesion and migration and on the recruitment of immune cells such as macrophages to sites of inflammation and/or infection[46–48]. During evolution, ECs might have acquired multifunctional roles to provide efficient responses against the challenges to proteostasis posed at sites of pathogen invasion, tissue damage and local inflammation, where toxic misfolded protein aggregates are likely to accumulate.

Extracellular proteostasis in neurodegenerative diseases

AD and cerebral amyloid angiopathy

Many ECs are found to be associated with Aβ plaques in vivo, indicating involvement in AD and other amyloid pathologies. These ECs include clusterin[49], A2M[50], haptoglobin[51], vitronectin[52], neuroendocrine protein 7B2 (7B2)[53], proSAAS[54], SAP[55] and integral membrane protein 2 (BRI2)[56]. The association of ECs with amyloid deposits could arise when their chaperone action is overwhelmed by an excess of aggregating protein, resulting in their physical entrapment within the deposit. In this section, we present evidence for the involvement of various ECs in amyloid pathology and discuss how these proteins might be harnessed as therapeutic targets.

Clusterin. Clusterin is one of the most potent inhibitors of in vitro Aβ aggregation known[57,58]. Genome-wide association studies have identified *CLU* as the third-largest genetic risk factor for late-onset AD[59], and changes in *CLU* expression are associated with the progression and severity of AD[60]. The single-nucleotide polymorphism (SNP) rs11136000T in the *CLU* gene has been proposed to increase *CLU* expression and delay the onset and progression of AD[61]. Furthermore, rare non-synonymous mutations and small insertion–deletion mutations in the *CLU* gene that are associated with AD result in reduced clusterin secretion, which in turn has been suggested to exacerbate the onset, progression and severity of AD[62]. When the amyloid precursor protein (APP) PS1 mouse model of Aβ amyloidosis was crossed with *CLU*$^{-/-}$ mice,

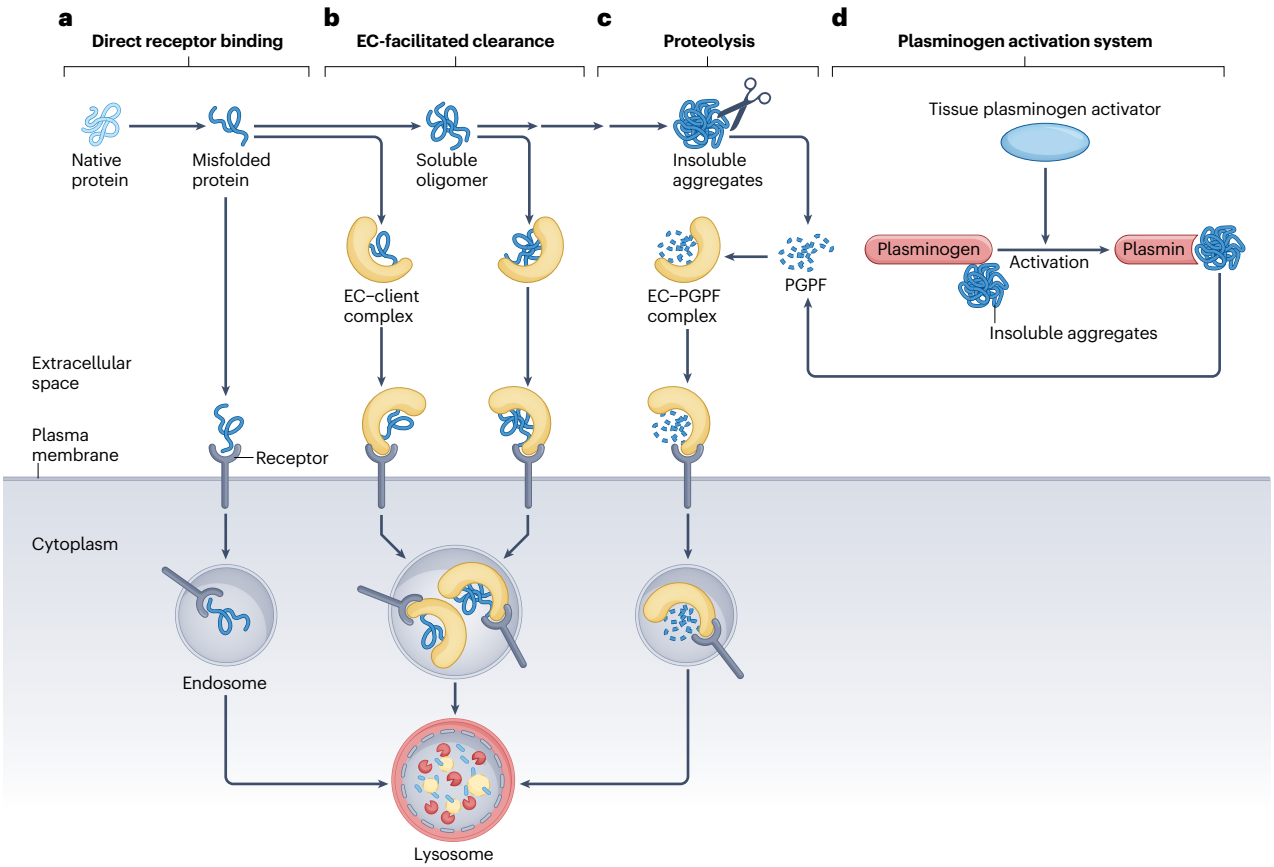

Fig. 2 | Overview of major known extracellular proteostasis mechanisms. **a**,**b**, Misfolded proteins in the interstitial fluid and cerebrospinal fluid can bind to cell surface receptors directly (part **a**) or form soluble complexes with extracellular chaperones (ECs) before binding to cell surface receptors (part **b**). **c**, Large insoluble protein aggregates can activate extracellular proteolytic systems, resulting in the digestion of the aggregates to form small protease-generated protein fragments (PGPFs), which in turn are bound by ECs. **d**, Plasminogen and tissue plasminogen activator bind to protein aggregates to generate active plasmin, which then degrades the aggregates to form PGPFs. In all situations, the misfolded proteins or resulting PGPFs are taken up by cells via receptor-mediated endocytosis and are directed to lysosomes for degradation.

suppression of *CLU* expression was associated with a marked decrease in plaque deposition in the brain parenchyma but an increase within the cerebrovasculature. These results suggest that in the absence of clusterin, Aβ clearance shifts from the brain to perivascular drainage pathways[63].

Multiple studies support the therapeutic potential of administering clusterin protein or peptides to ameliorate Aβ-related pathologies. The addition of purified clusterin increased the removal of $A\beta_{1-42}$ by macrophage-like U937 cells incubated in CSF from patients with AD[64]. Furthermore, exogenously added clusterin substantially reduced the binding of Aβ to blood vessels isolated from mice[63]. In vivo experiments have also demonstrated a benefit of clusterin treatment: peripheral administration of recombinant clusterin in the APP23 mouse model of AD reduced the levels of Aβ plaques and neuronal loss[65]. Similarly, intraventricular administration of a clusterin peptide (LVGRQLEEF, corresponding to clusterin residues 135–143) in the Tg6799 AD mouse model produced significant reductions in amyloid deposits and cognitive decline relative to placebo-treated mice[66].

α_2-Macroglobulin. A range of observations collectively suggest that the function of A2M is protective against AD. A2M inhibits the in vitro formation of amyloid by Aβ and other proteins[67], and A2M levels are elevated in the CSF of patients with AD[68]. Increased levels of A2M in the blood are associated with raised CSF concentrations of the neuronal injury markers tau and phosphorylated tau and with an almost threefold greater risk of progression to clinical symptoms of AD in humans[68]. The increased levels of A2M associated with AD could reflect one aspect of the attempt of the body to counter the accumulation of disease-associated toxic misfolded proteins. Further indicating the protective effects of A2M in AD, plasma A2M was found to be more oxidized in patients with AD than in neurologically healthy control individuals[69], and oxidation has been reported to enhance the chaperone activity of this protein[70]. Furthermore, functioning as a protease inhibitor, A2M can bind to serine proteases and the complexes formed are able to degrade Aβ: the V1000I SNP in A2M (which is located close to the thiolester active site involved in binding proteases) is associated with AD progression[71]. Finally, in an AD mouse model, drug-induced upregulation of A2M disrupted the production and aggregation of Aβ, induced Aβ efflux from the brain and ameliorated cognitive decline. The same drug also decreased the degradation of LRP1, which facilitated brain efflux of Aβ[72].

Transthyretin. In vitro, transthyretin is an effective inhibitor of amyloid formation by Aβ and other proteins but has little ability to inhibit amorphous protein aggregation[73]. Decreased CSF levels of transthyretin are observed in AD[74], and this protein has been shown to proteolytically cleave the Aβ peptide in vitro[75]. Overexpression of wild-type transthyretin in the APP23 mouse model of AD reduced cognitive and motor impairments, whereas silencing of the endogenous transthyretin gene promoted neuropathology[76].

Integral membrane protein 2. BRI2 binds to APP to inhibit Aβ production and inhibits Aβ aggregation via its BRICHOS domain. Patients with AD showed lower levels of BRI2–APP complexes in the hippocampus compared with controls, suggesting that depletion of BRI2 contributes to the development of AD[56]. In a 2021 in vitro study, hippocampal brain slices from wild-type mice were exposed to Aβ[77]. This exposure induced AD-like degradation of electrical activity, which was rescued by the addition of recombinant BRI2 BRICHOS domains. This study highlights the potential application of BRI2 as a treatment for AD and other amyloidogenic brain disorders.

Serum amyloid P protein. SAP is found at increased concentrations in the CSF of patients with AD[78]. In vitro, SAP was shown to bind directly to preformed amyloid fibrils and, consequently, to inhibit proteolysis of the fibrils[79]. Depletion of circulating SAP levels has, therefore, been pursued as a potential therapy for amyloidosis, although this strategy has yet to be trialled as a therapy for AD. Therapeutic approaches that have been investigated include chemical inhibitors of SAP binding to fibrils[80] and treatment with a humanized anti-SAP antibody, which was shown to reduce amyloid load in patients with systemic amyloidosis[81].

Haptoglobin. Haptoglobin is an effective inhibitor of both amorphous and amyloid protein aggregate formation in vitro, including Aβ aggregation[67,82]. Patients with AD have elevated serum levels of haptoglobin[83] and show significantly increased oxidation of plasma haptoglobin relative to controls. Oxidation of haptoglobin reduces its ability to inhibit the in vitro aggregation of Aβ to form amyloid[69], suggesting that the increase in haptoglobin oxidation in patients with AD might contribute to amyloid formation.

7B2 and proSAAS. Both 7B2 and proSAAS are specifically expressed by neuronal and endocrine cells[84,85]. These ECs dose-dependently inhibit the in vitro aggregation of Aβ at sub-stoichiometric levels and protect neuronal cells from Aβ toxicity[53,54], indicating therapeutic potential for AD.

The plasminogen activation system. In mouse models of AD, reduced levels of tPA, which activates plasminogen to plasmin, were associated with a greater load of Aβ plaques[86]. In addition, deletion of the tPA inhibitor PAI1 expression increased tPA-induced, plasmin-mediated cleavage of Aβ plaques[87]. Furthermore, in AD mouse models, elevated Aβ in the brain is associated with inhibition of the PAS, and intracerebral injection of Aβ in mice lacking either tPA or plasminogen, but not in wild-type mice, caused neuronal degeneration[88]. Additional results further implicating the PAS in AD include demonstrations that insoluble Aβ increases the expression of tPA mRNA in cerebral cortical neurons[89] and that the expression of PAI1 is increased in the CSF[90] and brains of patients with AD[87]. Aβ also increases the abundance of mRNA encoding uPA[91] – a protease that induces plasmin-mediated cleavage of insoluble Aβ-containing extracellular plaques[92]. Collectively, these observations support a model in which plasmin-mediated degradation of Aβ deposits, induced by tPA or uPA, is protective against Aβ pathology and, thus, increased PAI1 activity in the brains of patients with AD exacerbates pathology.

Transmissible spongiform encephalopathies

Transmissible spongiform encephalopathies (TSEs) constitute a family of rare progressive neurodegenerative diseases caused by an aggregation-prone prion protein that can propagate from one region of the nervous system to adjoining areas. According to the prion hypothesis[93], misfolded prion molecules can induce the misfolding of other prion molecules, thereby underpinning the propagation.

The prion neuropeptide 106–126 spontaneously aggregates to form fibrillar structures in vitro, and this aggregation is specifically inhibited by clusterin[94]. Clusterin binds directly to full-length prion protein[95] and has been found to be associated with prion plaques in several human TSEs, including Creutzfeldt–Jakob disease[96]. Clusterin

expression is upregulated in the cerebral cortex and cerebellum in Creutzfeldt–Jakob disease[96]. In mice transmitted with bovine spongiform encephalopathy infection, *Clu* knockout mice survived longer than *Clu*-expressing control mice. Although not quantified, the study reported that prion deposits were less aggregated (more diffuse) and greater astrocytosis was observed in the *Clu* knockout mice[97]. Assuming that the *Clu* knockout mice differed from control mice only in *Clu* expression, the authors suggested that clusterin alters the aggregation and extracellular deposition of prion protein and accelerates bovine spongiform encephalopathy pathogenesis. However, further work in animal models is needed to clarify the effects of clusterin in TSEs.

Interestingly, the recently identified EC vitronectin is reported to be a ligand of prion protein[98], although no study has yet examined this interaction in the context of TSEs. As vitronectin has a chaperone activity similar to clusterin[16], the influence of vitronectin and other ECs that affect prion aggregation on TSE pathologies is an area worthy of future research.

Other neurodegenerative diseases

In several other neurodegenerative diseases, evidence has emerged that propagation of pathology from affected neurons in one location to those in adjacent areas arises from the physical spread of protein aggregates. This hypothesis has been supported in studies of AD[99,100], Parkinson disease (PD)[101], Huntington disease[102] and amyotrophic lateral sclerosis[103]. If the spreading of pathology involves transit of the disease-relevant protein through the extracellular space, ECs and extracellular proteases are likely to influence disease pathologies. Few studies have examined the impact of extracellular proteostasis mechanisms in these diseases. However, as we outline in the text that follows, reports relating to α-synuclein, the propagating protein associated with PD, provide an enticing glimpse of potential new strategies to combat PD and other neurodegenerative diseases.

The neuropathological hallmarks of PD are the loss of dopamine-containing neurons in the substantia nigra and the presence of α-synuclein-containing intracellular inclusions[104]. α-Synuclein is thought to spread between neurons in the form of oligomers, although the mechanisms underlying this process are yet to be clearly established. *CLU* polymorphisms are associated with an increased risk of PD[105,106]. In addition, clusterin and A2M were both shown to bind directly to regions of exposed hydrophobicity on the surface of α-synuclein oligomers, substantially reducing the ability of the oligomers to permeabilize lipid membranes and stimulate the production of reactive oxygen species in a neuronal cell line[107]. Furthermore, one study found that extracellular clusterin potently inhibited the ability of extracellular α-synuclein to seed the formation of GFP–α-synuclein aggregates inside cells[108]. Another study reported that the translocation of extracellular α-synuclein into neighbouring cells was inhibited through cleavage by plasmin, and extracellular α-synuclein upregulated the expression of PAI1 (ref. [109]). Consequently, an excess of PAI1 in the brains of individuals with PD has been suggested to exacerbate pathology by inhibiting plasmin-mediated clearance of α-synuclein aggregates[110]. In line with this idea, increased levels of PAI1 have been linked to worse clinical prognosis in patients with PD[111].

Although the existing knowledge is fragmentary, it is consistent with the possibility that manipulation of key extracellular proteostasis elements, such as ECs and extracellular proteases, could provide powerful new avenues to combat the progression of PD and other neurodegenerative diseases. A better understanding of the mechanisms of pathological protein propagation will be important to support the development of such therapies.

Targeting extracellular proteostasis

In this section, we discuss opportunities for developing extracellular proteostasis regulators for therapeutic purposes. Many of the therapeutic approaches outlined in this section are based on earlier strategies developed to manipulate elements of intracellular proteostasis, which, unlike extracellular proteostasis, has been intensely studied for many decades[2,112].

EC-interacting compounds

Intracellular molecular chaperones can rescue misfolded intermediates or disrupt aberrant aggregates through various ATP-dependent mechanisms. These mechanisms, however, are not readily available to ECs, which are largely ATP-independent. As many ECs act as holdases by binding non-native states of proteins and presenting them for degradation, it could be possible to develop compounds that modulate the binding of ECs to their substrates (Fig. 3a). Indeed, chemical probes acting on protein–protein interactions have been described for intracellular chaperones[112] and are being investigated as possible treatments for cancer[113] and neurodegenerative diseases[114]. Another possibility is to develop chaperone-mimicking compounds that bind directly to non-native states of disease-related proteins and either act as holdases[115,116] (Fig. 3b) or prevent microscopic processes that depend on the non-native states, such as fibril-dependent secondary nucleation in protein aggregation[117,118] (Fig. 3c). Pharmacological upregulation of EC secretion might also be an option (Fig. 3d), although this approach could become problematic owing to the complexity of the protein aggregation process[108] and potential off-target effects[119].

Promoting endolysosomal protein degradation

Extracellular proteins can be targeted for intracellular degradation through the endolysosomal pathway (Fig. 3e). Proteolysis-targeting chimaeras (PROTACs)[120] use a targeting ligand attached to a protein-binding moiety to deliver bound protein cargo to a proteolytic system for degradation. Inspired by this mechanism, endogenous internalizing receptors, such as Fc receptors and lysosome-targeting receptors (LTRs), can be used to direct misfolded or damaged extracellular proteins to lysosomes. This targeted protein degradation approach has been implemented through lysosome-targeting chimaeras[121,122]. A lysosome-targeting chimaera consists of a ligand that is recognized by an LTR conjugated to a small molecule or antibody that binds to the extracellular or cell membrane protein that is being targeted for degradation. For example, the asialoglycoprotein receptor (ASGPR), an LTR specifically expressed on hepatocytes, was recruited to enable the degradation of extracellular proteins, and triantennary *N*-acetylgalactosamine, a ligand of the ASGPR, was conjugated to biotin or antibodies, leading to the internalization of extracellular protein targets into lysosomes for degradation in liver cell lines[123]. In a related approach, an antibody-based PROTAC was developed to degrade the PDL1 cell surface protein by directing it towards the lysosomal pathway through a transmembrane E3 ligase[124]. Similarly, molecular degraders of extracellular proteins acting through the ASGPR have been introduced as modular bifunctional molecules that mediate the formation of a ternary complex between an extracellular protein targeted for degradation (for example, a cytokine) and the ASGPR on hepatocytes[122].

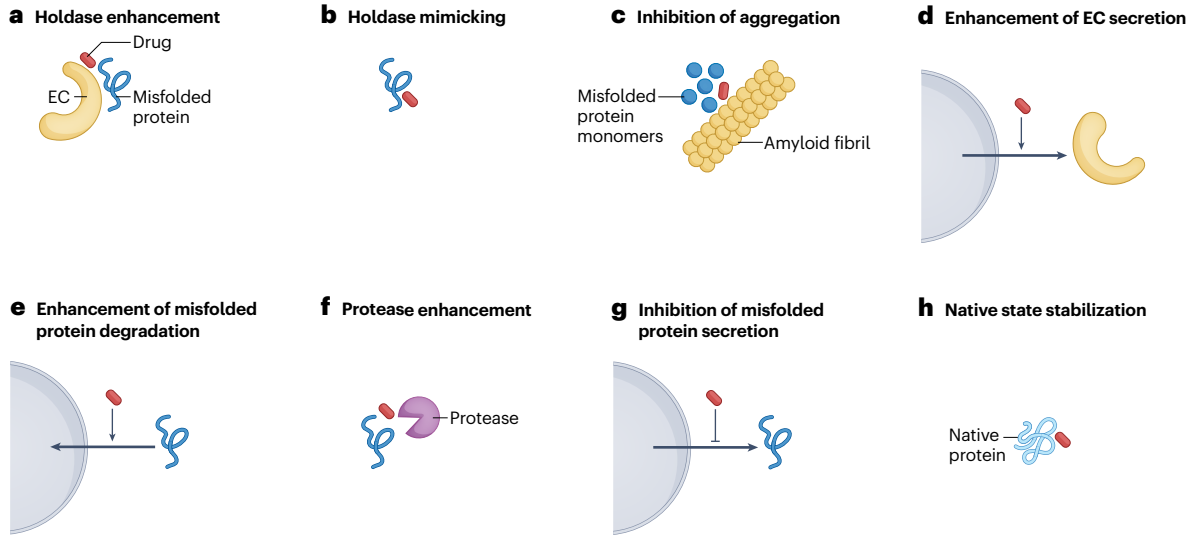

Fig. 3 | Small-molecule drugs to manipulate extracellular proteostasis. The figure depicts possible therapeutic strategies in which small-molecule drugs are used to manipulate aspects of extracellular proteostasis to reduce extracellular accumulation of misfolded proteins and ameliorate the resulting pathologies. Such drugs could enhance the efficiency of holdase chaperone action (part **a**); bind to and stabilize misfolded proteins (part **b**); directly inhibit protein aggregation (part **c**); increase the secretion of extracellular chaperones (ECs) (part **d**); promote the uptake and degradation of extracellular misfolded proteins (part **e**); enhance the ability of extracellular proteases to degrade misfolded proteins (part **f**); inhibit the secretion of misfolded proteins from cells (part **g**); or bind to and stabilize the native state of proteins (part **h**).

Activating extracellular proteases

A number of studies have investigated the potential of targeting extracellular proteases, including metalloproteases such as neprilysin and IDE, for drug development[125,126] (Fig. 3f). Epigallocatechin compounds have been shown to induce neprilysin and IDE by modulating the expression, secretion and catalytic activity of these proteases, leading to an increase in the degradation of Aβ peptides[41,127,128]. These small molecules can also modify the extracellular levels of disease-related proteins or their regulators directly by inhibiting the secretion pathway[124] (Fig. 3g).

Pharmacological chaperones

Pharmacological chaperones have been investigated as compounds that are capable of binding and stabilizing the native states of proteins that are prone to misfolding. An example is tafamidis, a drug currently used to treat familial amyloid polyneuropathy and familial and sporadic amyloid cardiomyopathy. This drug is thought to act by binding the active tetrameric form of transthyretin and preventing its dissociation, which is the first step in the aggregation pathway[129,130] (Fig. 3h). This approach might be extended to other disordered proteins, such as Aβ, to prevent their extracellular aggregation[131–133]. Similarly, chemical chaperones stabilize the native state of proteins by acting as osmolytes, which alter the physico-chemical properties of the environment of the protein. The human metabolite myoinositol, which acts as a chemical chaperone, was shown in a 2022 study to prevent the aggregation of lens crystallins, indicating its potential for the treatment of cataracts[134].

Conclusions and future directions

Given that our understanding of the processes that underpin extracellular proteostasis is still in its infancy, many questions concerning the role of this system in neurodegenerative diseases remain unanswered. Some of the arguably more important areas of focus are outlined below as suggestions for future research. We focus specifically on two areas in which advances can be expected to have the greatest impact — mapping of the entire extracellular proteostasis system, and the development of methods and models for systematic investigations of the identified components.

Our knowledge of the extracellular proteostasis system is much less advanced than that of its intracellular counterpart[3]. Therefore, generating a more complete list of the extracellular proteostasis components would be beneficial. For example, identification of major endocytic receptors that have a central role in the uptake and degradation of EC–misfolded protein complexes from extracellular fluids will strengthen our knowledge of this important branch of the extracellular proteostasis system. Early work suggests that the liver is a primary organ involved in the clearance of these complexes from the systemic circulation[25], so focusing on receptors expressed by hepatocytes and liver-resident macrophages could be a productive approach. Receptors expressed by microglia might be particularly important for the clearance of misfolded proteins from the ISF and CSF[135]. A better understanding of the cooperative actions of the PAS (and other extracellular proteolytic systems) and the EC network would advance us towards potentially harnessing this system to develop novel therapeutic strategies[27]. Studies over the past few years have revealed that IDE can proteolytically cleave Aβ and, independently of its peptidase activity, can also inhibit amyloid formation by Aβ and other amyloid-forming proteins[41]. These findings are of potential importance in the context of developing new treatments for AD.

A particularly important goal of future research is to develop methods and models to directly visualize the operation of extracellular proteostasis in vivo. This goal might be achieved in well-established transparent model organisms such as *Caenorhabditis elegans*[136], or by making use of brain cells, organoids and assembloids derived

from induced pluripotent stem cells[137,138]. In these model systems, ECs, misfolded proteins and other related components labelled with spectrally distinct fluorescent markers could be used for real-time monitoring of protein misfolding and aggregation, binding interactions with ECs, uptake of EC–misfolded protein complexes via cell surface receptors and trafficking of misfolded proteins to lysosomes for degradation. Also, the use of genetic screens[7] and optogenetics[139] in cellular and animal models of neurodegenerative diseases could enable the systematic study of the roles of ECs and other extracellular proteostasis components in regulating disease processes.

In the future, we anticipate that a more complete understanding of the main players in the extracellular proteostasis system and their various functions will enhance our ability to harness these players for therapeutic interventions and offer novel opportunities to combat neurodegenerative disorders.

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