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There is a family of currently untreatable serious human diseases that arise from the inappropriate misfolding and aggregation of extracellular proteins. At present our understanding of mechanisms that operate to maintain proteostasis in extracellular body fluids is limited but has significantly advanced with the discovery of a small but growing family of constitutively secreted extracellular chaperones (ECs). The available evidence strongly suggests that these chaperones act as both sensors and disposal-mediators of misfolded proteins in extracellular fluids, thereby normally protecting us from disease pathologies. It is critically important to further increase our understanding of the mechanisms that operate to effect extracellular proteostasis, as this will be essential knowledge upon which to base the development of effective therapies for some of the world's most debilitating, costly and intractable diseases.

Keywords
proteostasis, chaperones, extracellular, CMMB

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Extracellular Chaperones and Proteostasis

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Summary

- Processes acting to sense and control protein misfolding in extracellular fluids have previously been poorly studied.
- Recent work has identified a small but growing family of secreted chaperones that are abundant in extracellular fluids.
- These extracellular chaperones (ECs) stabilize misfolded proteins and are implicated in mediating their systemic clearance via receptor-mediated endocytosis.
- This action operates to normally protect the human body from disease pathologies arising from the inappropriate misfolding and aggregation of extracellular proteins.
- A better understanding of the processes that maintain extracellular proteostasis will open up new therapeutic opportunities for currently untreatable diseases.

Future issues to be resolved for the topic

- What are the specific receptors involved in clearing EC-misfolded protein complexes from extracellular fluids?
- Which protease systems act to help clear extracellular protein deposits and do these synergise with ECs to safely accomplish this task?
- Is it possible to treat disease pathologies arising from inappropriate extracellular protein misfolding by pharmacologically manipulating the in vivo expression levels of ECs (or their chaperone activities)?

Mini-glossary

- Extracellular chaperones: secreted proteins generally having a sHSP-like chaperone action (i.e. ATP-independent ability to stabilize misfolded proteins, preventing their aggregation and precipitation).
- Proteostasis: all those processes that act to maintain the steady state levels, distribution and native fold of the proteome.

Important acronyms

EC extracellular chaperone
α_2M **α_2**-macroglobulin
HSP heat shock protein
sHSP small heat shock protein
Aβ amyloid beta peptide
AD Alzheimer's disease
TTR transthyretin
LRP low density lipoprotein receptor-related protein
Apo apolipoprotein
TLR toll-like receptor

Abstract
There is a family of currently untreatable serious human diseases that arise from the inappropriate misfolding and aggregation of extracellular proteins. At present our understanding of mechanisms that operate to maintain proteostasis in extracellular body fluids is limited but has significantly advanced with the discovery of a small but growing family of constitutively secreted extracellular chaperones (ECs). The available evidence strongly suggests that these chaperones act as both sensors and disposal-mediators of misfolded proteins in extracellular fluids, thereby normally protecting us from disease pathologies. It is critically important to further increase our understanding of the mechanisms that operate to effect extracellular proteostasis, as this will be essential knowledge upon which to base the development of effective therapies for some of the world's most debilitating, costly and intractable diseases.

Keywords
Extracellular chaperones; proteostasis; protein folding; protein deposition diseases; clearance

Running title: Extracellular chaperones and proteostasis

1.0 Introduction

The term *proteostasis* refers to the maintenance of the proteome as a set of individual proteins in a conformation, concentration and location that is required for their correct function (1). Proteostasis is critical for the maintenance of organismal viability and operates in both the intracellular and extracellular environments. By far the better characterized systems relate to the intracellular environment, which has been the focus of decades of research, leading to the identification of many important components and processes (see 2.0 below).

The pathologies of many serious human diseases (the so-called Protein Deposition Diseases) are associated with the aggregation and deposition of misfolded proteins (Table 1). Generally speaking, protein aggregates form when protein concentration exceeds solubility (2). Despite this, many proteins normally function at the upper edge of their solubilities (3). This means that any small changes in protein concentration or solubility (due to mutations or a change in the environment) may tip the delicate balance leading to aggregation and deposition. Chaperones have emerged as ubiquitous and critical players in proteostasis systems, where they perform a variety of roles including inhibiting protein aggregation, maintaining the solubility of and refolding misfolded proteins, and protein trafficking. As is true for proteostasis in general, knowledge of extracellular chaperones (ECs) has lagged well behind that of their intracellular counterparts. Nevertheless, in recent years it has become clear that there is a growing family of abundant proteins in the extracellular fluids of metazoans that share functional characteristics with the intracellular small heat shock proteins (sHSPs). These abundant ECs are able to bind to, and keep soluble, proteins that are misfolded as a result of mutations or stresses and inhibit their aggregation. Furthermore, the ECs are strongly implicated in clearing these aggregating proteins from extracellular spaces and facilitating their degradation, thereby playing a pivotal role in maintaining extracellular proteostasis.

This review will provide a critical overview of the current understanding of the processes that operate in extracellular proteostasis with a particular focus on emerging knowledge of the ECs. A brief outline of intracellular proteostasis systems follows (2.0) because this provides background for the ensuing consideration of corresponding processes in the extracellular context.

2.0 Intracellular proteostasis

In order to produce properly functioning proteins the processes of transcription, RNA processing and transport, translation, protein folding, protein transport and ultimately protein degradation must be tightly regulated (1). Arguably the most important elements of the proteostasis machinery are the chaperones which have been defined by some as proteins that interact with other proteins to stabilize them or to help them acquire their native conformation (4). These broad functional characteristics mean that chaperones play a role in many cellular functions including protein folding, assembly of complexes, protein trafficking, protein degradation, and controlling protein aggregation and disaggregation. There are over a hundred chaperone genes in mammalian genomes, therefore no single chaperone performs all the roles identified above. Several families of chaperones reside inside mammalian cells and have previously been categorized on the

basis of their molecular weights, including the sHSPs, and the HSP40, HSP60, HSP70, HSP90, and HSP100 families. The various chaperones have differing actions and distinct functional roles in protein quality control. For example, HSP70 is known to play a role early in the protein folding process, interacting with ribosomes, growing peptide chains and newly synthesized polypeptides (5). In contrast, HSP60 and HSP90 members act further downstream to provide an enclosed environment with hydrophobic surfaces to assist in the folding of specific protein clients (5, 6). Once folded, a range of physiological stresses can cause a protein to partially unfold or misfold. Chaperones such as HSP100 and the sHSPs can recognize misfolded proteins and, in co-operation with folding chaperones such as HSP70, allow them to refold (7).

When maintenance of correct protein folding is no longer possible, cells contain a number of systems to remove damaged or misfolded proteins. The ubiquitin-proteasome system recognizes, labels and degrades stubbornly misfolded proteins. There are many hundreds of ubiquitin ligases (8) that through a series of highly regulated events covalently attach polyubiquitin chains to misfolded proteins; ubiquitinated proteins are subsequently transferred to the proteasome as substrates for degradation (9). In addition, damaged cytosolic proteins can be degraded by lysosomes via three distinct mechanisms, macroautophagy, microautophagy and chaperone-mediated autophagy (10). Chaperones are involved in controlling the movement of intractably misfolded proteins towards degradation machinery. For example HSP70 can, depending on the co-factors involved, promote folding (5), degradation through the ubiquitin-proteasome system (11), chaperone-mediated autophagy (12) or even actively partition misfolded proteins into inclusions such as the aggresome (13).

Most of the current information on the function of chaperones relates to those found inside cells. However, chaperones are also found in other compartments, both within and outside cells. De novo folding of proteins destined for secretion occurs in the endoplasmic reticulum (ER), where a network of chaperones and other protein quality control mechanisms act to ensure that proteins are correctly folded before they are released from the cell. Synthesis of proteins destined for secretion begins on ER-associated ribosomes. The microenvironment to which polypeptides are exposed in the ER is similar to that of the extracellular space; both environments contain a relatively high concentration of calcium ions and are oxidizing (14). Consequently, there are specialized chaperones and enzymes that assist in the maturation of secreted proteins. The ER contains members of the classical chaperone families HSP70 (BiP), HSP40, HSP90 and a member of the HSP100 family. Notably, there is an absence of HSP60 family members in the ER which means that secreted proteins rely for folding exclusively on binding and release from folding chaperones such as BiP and the HSP90 family member GRP94. In the case of glycoproteins, further maturation of protein folding is achieved with assistance from lectin chaperones such as calnexin and calreticulin. These lectin chaperones work downstream of classic chaperones such as BiP through a cycle of binding and release which controls de-glucosylation and re-glucosylation via specific glucosyltransferase enzymes. The addition of glucose to the folding protein signals another round of binding and release, and only when the protein is fully folded will it exit this cycle and be released to the secretory pathway (15). Oxidoreductases of the protein disulfide isomerase (PDI) family also contribute to protein folding within the ER. PDIs

catalyze the oxidation reaction required to form disulfide bonds by acting as electron acceptors (14); PDIs can also isomerize disulfide bonds, rearranging inappropriate disulfide linkages to attain native structures.

The ER quality control network strives to ensure that only fully folded native proteins are secreted and is able to retain most misfolded proteins within the ER (16). One mechanism of retention is thought to involve chaperones that contain C-terminal ER retention sequences, such as BiP (17), physically directing bound misfolded proteins back to the ER. However, only structures that are recognized by ER chaperones are able to be held back. As a result, some proteins with "native-like" folds (e.g. some mutated forms of transthyretin) are able to evade the quality control system and exit to the extracellular space (18). Once in the extracellular space, the proteome is out of reach of the well-described intracellular proteostasis systems and must be "maintained" by other mechanisms.

3.0 Extracellular proteostasis

Once in the extracellular space, secreted proteins will be bathed in large volumes of extracellular fluids (approximately 5 liters of blood and 10 liters of interstitial and other fluids in an average human). As flagged above, this environment is oxidizing and in the case of blood plasma especially, is subjected to ongoing shear stress during its enforced circulation around the body. These stresses will ensure that with time, dependent on the stability of individual proteins, extracellular proteins will misfold and need replacement. Studies from 30-40 years ago showed that misfolded forms of plasma proteins were more rapidly degraded than their natively folded precursors (19), hinting that a system operated in vivo to recognize and dispose of "damaged" extracellular proteins. Knowing what we do now about intracellular proteostasis, it would in fact be absolutely remarkable if there were not corresponding systems to deal with the potentially pathological consequences of extracellular protein misfolding. The known list of serious human diseases arising from excessive inappropriate extracellular protein misfolding and aggregation (Table 1) draws a line under this imperative. Importantly, however, owing to the major physical differences between the intracellular and extracellular environments, exactly the same systems cannot operate in both locations. For example, the concentration of nucleotide phosphates such as ATP, used by intracellular chaperones to energize protein refolding, is several orders of magnitude lower in extracellular fluids than inside cells (20). Thus, chaperone-mediated protein refolding appears a much more difficult proposition in the extracellular context. Similarly, although very low levels of proteasome (which also requires ATP) and even normally intracellular chaperones have been found in extracellular fluids, their concentrations are orders of magnitude lower than inside cells (21-23) indicating that they are unlikely to have the capacity to play any substantive role in protecting the organism from the challenges posed by misfolding extracellular proteins present at much higher levels.

Then what mechanisms do operate extracellularly to protect metazoans from "aged", misfolded and aggregating proteins? Theoretical options include refolding (unlikely in light of the above), extracellular proteolysis, and physical clearance from extracellular

fluids for subsequent intracellular degradation (Figure 1). There is some evidence that the plasmin/plasminogen system may have the ability to proteolyse pre-formed extracellular protein deposits (24-26), although a lot more work is required here to better understand how major a role this might play *in vivo*. In addition, misfolded or aggregated proteins may themselves be recognized by specific receptors on the surfaces of some cells, however, in many cases this has been demonstrated to have subsequent pro-inflammatory effects (see 5.2). A body of work in recent years has identified a series of ECs that, in most cases, share functional similarities with the sHSPs in that they lack ATPase activity and cannot refold proteins (see 4.0). They are able to stabilize misfolded proteins, however, and keep them soluble, which not only inhibits their aggregation and toxicity but also facilitates their efficient delivery to receptors which may be the key to safely clearing these potentially dangerous species from extracellular spaces (see 5.0 & 5.3).

4.0 ECs

4.1 Clusterin

Clusterin was originally named for its propensity to cause cell clustering *in vitro* (27), however, owing to its multifunctional nature it is also known by many alternative names including apolipoprotein J (ApoJ), SP-40,40, sulfated glycoprotein 2 and complement lysis inhibitor. The clusterin gene encodes a precursor polypeptide that is extensively glycosylated and internally cleaved to form the α and β subunits, which are linked by five disulphide bridges in the mature protein (28). The structure of clusterin is yet to be fully resolved, however, by sequence analysis it is predicted that clusterin contains three amphipathic α-helices and two coiled-coil α-helices (29, 30). It has also been proposed that the binding site on clusterin for a diverse range of hydrophobic ligands is a molten-globule-like pocket formed by intrinsically disordered regions and amphipathic α-helices (31). The gamut of functions (other than chaperone) proposed for clusterin includes, but is not limited to, regulation of complement (32) and apoptosis (33, 34), protease inhibition (35), and lipid transport (30). This diversity of putative functions most likely reflects the ability of clusterin to bind to an extremely broad range of structurally diverse ligands. The concentrations of clusterin in blood plasma and cerebrospinal fluid (CSF) are 35-105 μg/ml and 1.2-3.6 μg/ml, respectively (36, 37). However, clusterin expression is up-regulated in response to many different stresses including tissue injury (38), aging (39), and in diseases including Alzheimer's disease (AD) (40, 41), atherosclerosis (42), diabetes (42) and cancer (43). While the clusterin gene is known to encode a secretory signal, in some instances is appears that clusterin is retained within cells. It has been suggested that this is the result of the translation of a form of clusterin lacking the secretory signal (44). Conversely, it has been shown that full-length clusterin can be retrotranslocated into the cytosol in response to ER stress (45). Another form of clusterin directed to the nucleus is reportedly the result of alternative splicing of the clusterin gene (46, 47). The mechanisms by which different isoforms of clusterin may be generated is still highly controversial and further studies are necessary to clarify this.

Clusterin is a potent sHSP-like chaperone that has been shown to inhibit stress-induced amorphous protein aggregation and the fibrillar aggregation of many amyloidogenic proteins and peptides (48-56). The structural elements responsible for the chaperone activity of clusterin are not yet known, however, its ability to bind to misfolded proteins

is thought to be related to its surface hydrophobicity which is enhanced by acidic pH (49). The chaperone activity of clusterin is ATP-independent and in the case of amorphously aggregating clients, results in the formation of soluble, high molecular mass complexes ≥ 40 000 kDa (57). Immunodepletion of clusterin from human blood plasma renders plasma proteins susceptible to stress-induced precipitation (49). The near ubiquitous expression of clusterin, and its constitutive presence in many biological fluids, suggests that it performs a fundamentally important protective role in vivo. Supporting this, clusterin knockout mice have increased tissue damage after heat-shock (58), myosin-induced auto-immune myocarditis (59) or post-ischemic brain injury (60). Moreover, it has been demonstrated that ageing clusterin knockout mice develop glomerular neuropathy, directly implicating clusterin in the clearance of pathological protein deposits (61). Additionally, clusterin is found colocalized with misfolded protein deposits in many diseases (Table 1).

Two recent independent genome-wide association studies identified polymorphisms in clusterin as a strong genetic risk factor for AD (62, 63). Clusterin has been shown to influence amyloid formation by binding to prefibrillar aggregates rather than binding to the monomeric protein/peptide or mature amyloid fibrils (50-52). Depending on the ratio of clusterin to the fibril forming client, clusterin may either prevent further growth or promote elongation (51), and may either prevent or exacerbate the cytotoxicity of amyloidogenic peptides *in vitro* (51, 56, 64, 65) (see also 5.1). It has been shown that clusterin markedly enhances the clearance of $A\beta_{1-42}$ at the blood-brain barrier (66), presumably via the receptor known as meglain/LRP-2 (67). However, in a mouse model of AD, clusterin knockout has been shown to reduce fibrillar Aβ amyloid deposition and neurotoxicity (68). A similar result was shown for ApoE knockout mice, however, double knockout of clusterin and ApoE resulted in early disease onset and a marked increase in Aβ peptide levels and amyloid formation (69). Thus, while the available data shows that clusterin can influence amyloid fibril formation and facilitate the clearance of Aβ, the role of clusterin in AD remains unresolved.

4.2 Haptoglobin

Haptoglobin is well known for its role as a haemoglobin-binding protein and also as an acute phase reactant. In humans there are three major haptoglobin phenotypes (Hp1-1, Hp1-2 and Hp2-2) depending on the presence of two principal alleles Hp1 and Hp2, which encode the α^1 and α^2 subunits, respectively. The simplest form of haptoglobin is type Hp1-1, which consists of a disulfide-linked $\alpha^1\beta$ dimer (70). An additional cysteine residue in the α^2 chain allows for the formation of large complex disulfide-linked polymers in Hp2-1 and Hp2-2 which can form species up to 900 kDa in mass (71, 72). Homology with complement receptor 1 has been used to predict structural elements including the location of complement control protein domains, a CD163-binding region and the hemoglobin-binding site (73). Additionally, it has been proposed that a large hydrophobic region adjacent to the hemoglobin-binding site is responsible for the chaperone activity of haptoglobin (73, 74).

Haptoglobin is found in most extracellular fluids, with concentrations of 0.3-2.0 mg/ml (75) and 0.5-2 μg/ml (76) in human plasma and CSF, respectively. The hepatic

expression of haptoglobin is strongly upregulated by inflammatory mediators such as IL-6, oncostatin M, and leukemia inhibitory factor (77). Sequestration of hemoglobin by haptoglobin is an important protective mechanism that reduces the amount of free hemoglobin and iron available to catalyze oxidative reactions (78). Other proposed roles for haptoglobin include, but are not limited to, regulation of cathepsin B activity (79), angiogenesis (80) and the immune system (81). In support of the latter, haptoglobin knockout mice have lower counts of mature T and B cells and display reduced adaptive immune responses (82). Haptoglobin phenotype has been implicated in several diseases including atherosclerosis where it is appears that the Hp2-2 phenotype is associated with increased risk and poor prognosis (reviewed in (83)). Unfortunately, studies have not yet examined the relationship between haptoglobin phenotype and the many protein deposition diseases.

Like clusterin, all three haptoglobin phenotypes have been shown to inhibit stress-induced amorphous protein aggregation of a wide range of client proteins *in vitro* (84, 85), and immunodepletion of haptoglobin from human blood plasma has been shown to render plasma proteins susceptible to precipitation (85). Complexation with hemoglobin reduces but does not abolish the chaperone activity of haptoglobin, which supports that the binding sites on haptoglobin for hemoglobin and misfolded client proteins are discrete (74, 86). By size exclusion chromatography it appears that complexes formed between haptoglobin and misfolded proteins are comparable in mass to those involving clusterin ($\geq$ 40 000 kDa; (85)), however, little else is known about their physical characteristics. In contrast to clusterin, decreased pH reduces both the hydrophobicity and chaperone activity of hapoglobin (85). Hp2-1 has been shown to inhibit amyloid formation by a number of amyloidogenic proteins/peptides, however, this is currently limited to a single study and the effect of other haptoglobin phenotypes has not yet been investigated (86). Nevertheless, it appears that at substoichiometric levels Hp2-1 inhibits amyloid formation by forming stable complexes with client protein and preventing their elongation (86).

4.3 α_2-Macroglobulin (α_2M)

α_2M is a multifunctional protein that is best known for its role as a broad spectrum protease inhibitor. X-ray crystallography data and homology modeling against complement component C3 have been used to predict that α_2M is formed by numerous macroglobulin domains, an alpha helical TED (thiol ester-containing) domain, and a CUB (complement protein subcomponents C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein 1) (87). The quaternary structure of α_2M involves four identical 180 kDa chains, which covalently pair by disulfide bonds and then non-covalently associate to form a 720 kDa tetramer (88). The ability of α_2M to act as a protease inhibitor is due to "bait regions" which are not present in the related complement proteins. Upon cleavage of one or more of the bait regions by a protease, native α_2M transitions to a more compact "activated" conformation, which migrates further than native α_2M during native gel electrophoresis (89). During this transition, each disulfide-bonded dimer can covalently trap a protease within a steric "cage", tethered via an intramolecular thiol ester bond (90). Small nucleophiles including methylamine or ammonium ions can also activate α_2M by directly attacking the thiol ester bond (89). The activation of α_2M exposes a cryptic receptor recognition site for low-density lipoprotein receptor-related protein (LRP; also known as the α_2M receptor)(90). In addition to acting

as a protease inhibitor, it has been demonstrated that binding to α_2M can enhance antigen presentation (91) and α_2M is widely reported to act as a carrier of cytokines, growth factors and hormones, particularly in its activated form (92, 93).

α_2M is expressed by many tissues and is highly abundant in extracellular fluids. The concentrations of α_2M in human plasma and CSF are 1.5–2 mg/ml and 1.0–3.6 mg/ml, respectively (90, 94). In humans, plasma levels of α_2M are known to decline with age (95). While α_2M expression is upregulated during the acute phase in rats (96), plasma concentrations of α_2M do not increase during the acute phase in humans (97). Consistent with it interacting with misfolded proteins in vivo, α_2M is found colocalized with misfolded protein deposits in many diseases (Table 1). In particular, α_2M is topical in the field of AD owing to its ability to bind to and facilitate the clearance of Aβ via LRP (98-100). Several independent studies have reported that polymorphism in α_2M is a genetic risk factor for AD (101-105), however, several other studies have failed to show this association (106, 107). There is also vigorous debate about whether mutations in LRP are linked with AD (106, 108, 109).

Similar to clusterin and haptoglobin, α_2M has been shown to have a "holdase"-type chaperone activity which inhibits amorphous and fibrillar protein aggregation in vitro (86, 110). At present the structural elements responsible for the chaperone activity of α_2M are not known. Investigation of the chaperone activity of α_2M against stress-induced amorphous protein aggregation is currently limited to a single study which suggested that this activity was abolished by protease activation, however, α_2M retained the ability to trap proteases after binding to misfolded proteins and α_2M-protease-misfolded protein complexes were recognized by LRP (111). α_2M has been shown to inhibit amyloid formation by a large number of substrates (86) and to protect cells against Aβ toxicity in vitro (112, 113). As for the other ECs, α_2M appears to suppress amyloid formation by interacting with prefibrillar species that occur early in the aggregation process (86). A recent study showed that mildly acidic pH or 0.5 mM sodium dodecyl sulphate (which induce dissociation α_2M tetramers into dimers), increased the binding of α_2M to β_2-microglobulin (114). This report proposed that dimeric α_2M may be more chaperone-active than the tetramer, however, currently it is unknown whether α_2M dimers are generated in humans in vivo.

4.4 Caseins

The caseins are a heterogeneous mixture of four (unrelated) phosphoproteins that include α_{S1}-, α_{S2}-, β- and κ-casein, and are the primary components of milk micelles. All of the caseins lack a well-defined tertiary structure, existing as 'natively unfolded' proteins that self-associate into casein micelles, which serves as the transport vesicle for calcium to mammalian neonates. Both α_S- (made up of α_{S1}- and α_{S2}- subunits) and β-casein act as chaperones to inhibit the stress-induced amorphous aggregation of client proteins (115), as well the fibrillar aggregation of Aβ (116). Their chaperone activity is pH (117) and phosphorylation (118) dependent with activity being highest at the pH range typical of milk (i.e. 6.8 - 7.0). As for clusterin, the caseins act as 'holdase' chaperones by forming high molecular mass complexes with client proteins but do not have refolding activity (117, 119). Evidence of the physiological relevance of this chaperone action comes from findings reporting the presence of calcified amyloid-like deposits (known as *corpora*

amylacea) in bovine, rat and canine mammary tissue (120-123). Moreover, when isolated from the other casein proteins, α_{S2}- and κ-casein readily aggregate into amyloid fibrils under conditions of physiological pH and temperature (124-126). Thus, the ability of α_{S1}- and β-casein to function as chaperones and associate with other proteins (including the other caseins) is essential for the formation and stability of casein micelles and may also play a role in the prevention of mammary *corpora amylacea.*

4.5 Other ECs

In addition to clusterin, α_2M, haptoglobin and caseins, several other secreted proteins have been reported to have chaperone activity (summarized below). In several cases, analysis of the chaperone activity of these proteins is limited to a single study, thus, further characterization of their interactions with misfolded proteins is needed before they can be recognized as genuine ECs.

The ε4 allele of apolipoprotein E (ApoE) is a firmly established genetic risk factor for late-onset AD (127). It has been demonstrated that ApoE binds to Aβ and fragments of amyloidogenic gelsolin and prion protein (128, 129). Binding to ApoE reportedly increases the β-sheet content of these peptides (129), and promotes amyloid formation (130). However, similar to clusterin, it appears that depending on the conditions tested, ApoE can also inhibit amyloid formation by influencing either the nucleation or elongation phases (131, 132). In mice, ApoE genotype differentially regulates the clearance of Aβ from the brain; complexes formed between ApoE ε2 or ApoE ε3 and Aβ are cleared faster than those formed between ApoE ε4 and Aβ (133, 134). Although not proven, this may be the critical activity that promotes AD in carriers of the ApoE4 genotype. ApoE is found colocalized with misfolded proteins in a large number of diseases including with Aβ in AD (135) and Down's Syndrome (135), with prion protein in spongiform encephalopathies (135), with human islet amyloid peptide in diabetes (136), with drusen in macular degeneration (137), and in atherosclerotic plaques (138). In addition to clusterin (ApoJ) and ApoE, a third apolipoprotein, ApoAI, has been reported to influence Aβ aggregation and toxicity in vitro (139).

Albumin is by far the most abundant plasma protein, and is known to be an important carrier of many different molecules including Aβ (140). Several studies have reported that albumin inhibits stress-induced amorphous protein aggregation and amyloid formation *in vitro* (141-144). Compared to most recognized chaperones, on a molar basis albumin is considerably less efficient at preventing protein aggregation (57, 145, 146), however, given its abundance this activity may be physiologically relevant. A recent report suggested that the "chaperone" activity of albumin involves the formation of high molecular mass complexes, however, the data showed that proportionally only a very small amount of protein formed high molecular mass species when stressed in the presence of albumin (143). Further work is needed in order to determine whether albumin can preferential bind to misfolded proteins, or whether the chaperone-like activity of albumin at high concentrations is the result of weak non-specific interactions.

The “secreted protein acidic and rich in cysteine” (SPARC) is a multifunctional protein that promotes extracellular matrix remodelling by inhibiting collagen fibrillogenesis (147), and acts as an intracellular chaperone for procollagen (148). Aging SPARC knockout mice develop cataract and abnormal collagen deposition, supporting that the ability of SPARC to act as a collagen chaperone is important in vivo (149, 150). In human patients with cataract SPARC is upregulated (151), possibly in response to stress (152). SPARC has been demonstrated to, at substoichiometric concentrations, prevent the aggregation of heat denatured alcohol dehydrogenase (153). Little is known about the mechanism of this activity and whether it applies to misfolded proteins more broadly, thus further studies are warranted.

Serum amyloid P (SAP: a member of the pentraxin family) is known to bind to a diverse array of ligands (154-156), however, no clear biological function for this protein has yet been established. It has been reported that SAP has ATP-independent refolding chaperone activity, however, this was achieved using a very high molar excess of SAP and even then the recovery of heat-denatured lactate dehydrogenase activity was only 25% (157). Nevertheless, SAP is universally found colocalized with amyloid deposits in disease (158), which supports that it preferentially binds to amyloidogenic proteins in vivo. In vitro, SAP has been shown to inhibit amyloid fibril formation and increase the solubility of Aβ (159), however, the association of SAP with amyloid also protects the fibrils from proteolytic degradation (160). Knockout of SAP expression has been shown to delay amyloid deposition in a mouse model of reactive amyloidosis, suggesting that it plays a pro-amyloidogenic role (161).

Fibrinogen, a major blood protein that plays an important role in clotting, was reported as inhibiting stress-induced amorphous protein aggregation and amyloid formation (162). A later report from the same group, however, suggested that the chaperone activity is mediated exclusively by the α_EC domain which is only present in a minor isoform of fibrinogen known as fibrinogen-420 (163). When present at equimolar concentrations fibrinogen-420 reduced the heat-induced precipitation of citrate synthase by around 50% (163). In comparison, purified α_EC was a more potent chaperone, however, while free α_EC can be liberated from fibrinogen-420 as a result of proteolysis, its concentration in vivo is likely to be only a small fraction of that of fibrinogen-420 which is normally around 35 μg/ml in human plasma (164).

Two secreted lipocalin-type proteins, α_1-acid glycoprotein and lipocalin-type prostaglandin D synthase (L-PGDS)/β-trace are both reported to have chaperone-like activity (165, 166). For L-PGDS/β-trace this has only been addressed by a single study, which found that (i) L-PGDS/β-trace binds to monomeric and fibrillar Aβ and is found colocalized with Aβ plaques in vivo, (ii) L-PGDS/β-trace inhibits Aβ fibril formation, and (iii) Aβ deposition is enhanced in L-PGDS/β-trace deficient mice and decreased in L-PGDS/β-trace overexpressing mice compared to wild-type control mice (165). For α_1-acid glycoprotein, again the available data is limited to a single study which reported that α_1-acid glycoprotein inhibited the in vitro aggregation of a range of proteins (166). The same researcher also reported in a similar one-off study that α_1-antitrypsin has chaperone-like activity (167). These latter two studies, however, lacked suitable non-chaperone control proteins with which to compare the effects of α_1-acid glycoprotein and α_1-

antitrypsin on protein aggregation. Moreover, preferential binding of α_1-acid glycoprotein and α_1-antitrypsin to misfolded proteins has not been demonstrated.

5.0 Physiological roles of ECs

ECs are proposed to patrol extracellular spaces for misfolded and aggregated proteins. This function has implications for the clearance of aged or damaged proteins and, importantly, the protection of cells and tissues from the toxic or physically disruptive effects of protein aggregates. Cellular contact with misfolded or aggregated proteins can result in direct toxic effects (see 5.1), inflammatory signaling (see 5.2) or indeed endocytosis and degradation (see 5.3). The outcome depends on the cell types and specific receptors involved, and on the actions of the ECs.

5.1 Direct effects on ECs on the toxicity of protein aggregates

Although all aggregate species on the amyloid forming pathway may be toxic, it has become apparent that smaller soluble aggregates, commonly known as oligomers, are the most toxic species. These oligomers, even those generated from proteins not associated with disease, have been shown to be more toxic than both the precursor protein/peptide from which they are made and the fibrils generated from them (168). The mechanism(s) of oligomer toxicity remain unclear, however, common structural epitopes and exposed hydrophobicity have been correlated with aggregate toxicity in vitro (169, 170). Very hydrophobic protein aggregates may interact with cell surface receptors leading to changes in intracellular signal transduction cascades, potentially leading to cell death (171) or, alternatively, insert into and then interfere directly with membrane integrity resulting in toxicity (172). In the context of amyloid formation, ECs interact most strongly with oligomers formed early in the aggregation pathway (50, 51, 86), probably via the exposed hydrophobic residues thought to responsible for cellular toxicity. Indeed, it is likely that this is a common mechanism by which a range of ECs, such as clusterin, α_2M, haptoglobin and ApoE protect cells from misfolded or aggregated proteins (51, 173, 174). Recent insights into the mechanism of these interactions have come from studies exploiting advanced microscopy techniques. Single molecule fluorescence analyses were used to show for the first time that clusterin forms stable, soluble complexes with a broad range of Aβ oligomers (ranging from dimers to 50-mers) and by doing so can inhibit fibrillogenesis and enhance the concentration of soluble Aβ species following disaggregation of pre-formed fibrils (50). Furthermore, atomic force and confocal microscopy was used to show that clusterin and α_2M physically associate with HypF-N protein oligomers to induce them to form larger assemblies; this inhibited binding of the oligomers to cell membranes and consequently their cytotoxicity (173).

However, it is important to note that the effects of ECs on the toxicity of protein oligomers are context-dependent. For example, clusterin and α_2M were shown to enhance the cytotoxicity of Aβ to PC12 cells and LAN5 cells, respectively (56, 175). In contrast, other work has shown that clusterin and α_2M can protect cells from Aβ toxicity in primary rat mixed neuronal cultures (64, 100). Furthermore, when Aβ was aggregated in the presence of clusterin at a ratio of clusterin:Aβ of 1:10, it was less toxic than Aβ alone

to SH-SY5Y cells. However, when this same experiment was performed using a ratio of clusterin:Aβ of 1:500, the species formed were more toxic (51). These apparently opposing outcomes probably arise as a result of stoichiometry-dependent differential effects of ECs on oligomer structure. When present at relatively high ratios of chaperone:client, the ECs may be able to effectively mask most of the hydrophobicity exposed on the oligomers (thereby reducing their toxicity). In contrast, when present at lower ratios of chaperone:client, the ECs may structurally stabilize the oligomers, leading to the generation of more oligomers, but be present at insufficient levels to shield all the hydrophobic regions exposed on the oligomers.

5.2 Anti-inflammatory effects of ECs There are many reports describing the effects of clusterin, haptoglobin and α_2M on the immune system (59, 176-180). Some of these, such as the ability of α_2M to enhance antigen presentation (91) and haptoglobin-facilitated clearance of hemoglobin (180), clearly fall outside of the scope of this review and as such, will not be discussed here. With direct relevance to their function as ECs, a large number of recent studies have now shown that amyloidogenic peptides and aggregates of misfolded proteins are potently immunostimulatory (reviewed in (181)). Moreover, it has been suggested that hydrophobicity is universally recognized as a damage-associated pattern by the innate immune system (182). The rationale for this hypothesis comes in part from the fact that innate immune systems receptors such as toll-like receptors (TLRs) and scavenger receptors are highly promiscuous and bind to a very large number of ligands. These ligands are structurally diverse, however, most share the trait of normally being either hydrophobic or prone to exposing large areas of hydrophobicity when they are damaged or modified (e.g. bacterial lipopolysacharride (183)). Furthermore, it has recently been shown that when exposed to gold nanoparticles the expression of pro-inflammatory cytokines by splenocytes correlates with the surface hydrophobicity of these particles (184).

Protein misfolding is accompanied by chronic inflammatory pathology in many diseases including AD, prion disease, arthritis, macular degeneration and atherosclerosis. Reports describing the in vitro activation of microglia and astrocytes via stimulation of scavenger receptors and TLRs by amyloidogenic peptides are too numerous to address individually here, therefore, just a few examples will be discussed (reviewed in (181)). Fibrillar Aβ reportedly interacts with an ensemble of innate immune receptors including SR-AI, CD36, CD14, TLR-2, TLR-4 and formyl peptide receptor 2 (185-187), the net effect being upregulation of pro-inflammatory genes such as iNOS, COX2 and TNFα, and the initiation of respiratory burst (187, 188). A role for amyloids in platelet activation has also been suggested and CD36 and von Willebrand factor receptor glycoprotein Ibα were implicated in this process (189). Direct comparison of Aβ oligomers and Aβ fibrils suggests that small oligomers of Aβ are more potent stimulators of microglia and astrocytes (187, 190, 191), and supports the hypothesis that the hydrophobicity of the agonist is important. The ability of misfolded proteins to stimulate pro-inflammatory responses does not appear to be limited to amyloid, for example amorphous aggregates formed by the denaturation of large globular proteins have been shown to stimulate nitric oxide and superoxide production in macrophages (192). This activity was attributed to interaction of the aggregates with $\beta_1\beta_2$ integrins, MAC-1 and receptor for advanced glycation end products (RAGE) (192, 193).

Taken together the findings of the aforementioned studies strongly support that misfolded proteins are inherently immunostimulatory and this may be an important mechanism by which they contribute to the pathology of disease. Considering that inflammation is a state in which numerous stresses including heat and the concentration of free radicals are increased, it is possible that misfolded proteins and inflammation together generate a self-perpetuating cycle. Although the anti-inflammatory actions of the ECs may involve several mechanisms, close examination of their biological activities supports that at least some of their immunomodulatory effects are linked to their inherent property to bind and mask areas of exposed hydrophobicity on molecules. For example, the binding of α_2M to cytokines, which is currently considered a major mechanism by which it exerts immunomodulatory effects, is driven by hydrophobic interactions in many cases (92, 93, 194). Similarly, hydrophobic interactions are central to the interactions of clusterin with the complement system (32). Thus, it is tempting to speculate that an additional, yet to be characterized immunomodulatory activity of ECs, may be the direct result of their ability to mask regions of exposed hydrophobicity on misfolded proteins and other ligands, thereby reducing their ability to participate in pro-inflammatory signaling.

5.3 EC-mediated clearance of protein aggregates

In addition to directly shielding cells from hydrophobic protein aggregates, ECs may also protect cells by playing an important role in physically clearing misfolded proteins from extracellular fluids. Although receptors that can recognize and directly bind to misfolded or aggregated proteins have been identified, continued aggregation will result in the formation of insoluble deposits which have restricted access to cell surface receptors and that may persist in the body for extended periods. Additionally, there is evidence that the recognition of misfolded proteins by receptors may in fact contribute to their pathological effects (see above). The formation of complexes between ECs and misfolded proteins inhibits further aggregation of the latter, maintains them in solution and enhances the efficiency with which they are delivered to cell surface receptors for clearance. For example, SH-SY5Y cells expressing the α_2M receptor (LRP) are more resistant to Aβ toxicity in the presence of α_2M than cells that do not (175). In this context, the protection afforded by LRP expression could be inhibited with receptor-associated protein (a ligand that inhibits binding of species to LRP), further supporting the notion that internalization of α_2M-Aβ complexes is cytoprotective. Along similar lines, in the presence of α_2M, Aβ was cytotoxic to LRP-negative LAN5 cells but not when the LAN5 cells were transfected with LRP (175). When Aβ is added into AD patient CSF, it is more toxic to SH-SY5Y cells than Aβ added into control CSF; adding ECs (clusterin, α_2M and haptoglobin) suppresses this toxicity and this effect coincides with a more efficient cellular uptake of Aβ (112). Furthermore, it has been demonstrated that clusterin-Aβ complexes bind to the receptor megalin on the surface of mouse teratocarcinoma F9 cells, and are subsequently internalized, via receptor mediated endocytosis, transported to lysosomes and degraded (195). Likewise, complexes formed between protease-activated α_2M and Aβ bind LRP and are internalized in U87 cells and subsequently degraded (99). Importantly, in vivo studies also strongly support that clusterin and α_2M facilitate the clearance of Aβ via interactions with lipoprotein receptors (66, 196). In a rat model, complexes formed between clusterin and misfolded client proteins are quickly and specifically taken up by liver hepatocytes and degraded within lysosomes (197). This uptake can be delayed by in

vivo injection of fucoidin, an inhibitor of scavenger receptors, implicating these in receptor-mediated endocytosis of the chaperone-client complexes. This may reflect a process in which protein aggregates are maintained in solution in complex with ECs until cell surface pattern recognition receptors bind to hydrophobic or misfolded protein epitopes exposed on the complexes and mediate their cellular uptake. Scavenger receptors also reportedly facilitate the uptake of methylamine-activated α_2M by liver endothelial and kupffer cells (198), however, previous studies have not addressed whether this is also a pathway by which α_2M facilitates the clearance of misfolded client proteins. It is well known that human macrophages use the CD163 receptor to bind and internalize haptoglobin-hemoglobin complexes for subsequent degradation (199); however, the identity of receptor(s) that may function in clearing haptoglobin-misfolded protein complexes is not yet known. Taken together, the available evidence suggests that ECs protect cells from toxic and pro-inflammatory protein aggregates both by masking regions of exposed hydrophobicity on them and by promoting their receptor-mediated cellular uptake and degradation (Figure 2). An intriguing question which requires further investigation is whether extracellular proteolysis systems (e.g. plasminogen/plasmin) might synergize with ECs to digest and clear insoluble extracellular protein deposits. Although much remains to be done to identify all the relevant cell surface receptors involved in the systemic clearance of EC-misfolded protein complexes, scavenger and lipoprotein receptors are strongly implicated in clearing complexes incorporating clusterin and α_2M.

6.0 Therapeutic opportunities

Available treatments for extracellular protein deposition diseases are currently limited to reducing their symptoms. Without effective prophylactics or cures, the already heavy burden of diseases such as AD, macular degeneration and arthritis will continue to grow within our aging society. Thus, there is an urgent need to better understand the fundamental biological systems that normally protect the body from accumulating misfolded proteins in extracellular spaces. Many amyloidoses result from the accumulation of a single protein. To stem the production of this protein would provide a first line of defense against its accumulation. In cases where the disease-relevant protein is primarily synthesized by the liver, organ transplantation is a drastic but effective means by which to control the disease. Currently, liver transplant is most common for the treatment of transthyretin (TTR) related familial amyloidosis (200), and has been successfully used to treat other forms of amyloidosis including those resulting from mutation in fibrinogen α-chain or lysozyme (201, 202). Nevertheless, surgery of this kind carries serious risk and unless taken as a preemptive measure, damage to other organs may already be severe at the time of transplantation. Moreover, the disease will continue to progress if the amyloidogenic protein is expressed by other tissues. Suppression of the expression of amyloid forming proteins using antisense oligonucleotides or small interfering RNA are promising new therapeutic strategies (203, 204). Treatment of this kind, however, is suitable only if knockout of the target does not negatively impact upon overall organismal health, such as is the case for TTR (205), but not other examples such as the amyloid precursor protein (APP) (206). Rather than target the expression of APP, an alternative strategy to prevent/treat AD is to reduce the expression of the enzymes

responsible for the production of $A\beta_{1-42}$ (207), since is well known that $A\beta_{1-42}$ has a higher tendency to aggregate compared to $A\beta_{1-40}$ peptide.

A variety of small molecules are known to inhibit the aggregation/fibrillogenesis of disease-relevant proteins in vitro (reviewed in (208)). Unfortunately, clinical use of these compounds is not possible due to their lack of specificity, the high concentrations required to elicit an effect and their low tolerability in vivo. Therefore, current research is focused on identifying molecules that specifically target amyloidogenic proteins and disrupt their aggregation. A successful example of this is the drug Tafamadis, which has recently been approved by the European Medicines Agency (209). Tafamadis inhibits amyloid formation by stabilizing the tetrameric form of TTR, the dissociation of which into monomers is the rate-limiting step in TTR amyloid formation (210). Promising novel peptide-based strategies are also currently under development, including "β-sheet breakers", which have been shown to reduce amyloid deposition in mouse models of AD (211). A major limitation of anti-aggregation strategies is the lack of knowledge surrounding precisely which of the aggregated species are responsible for disease (reviewed in (212)). This limitation may be overcome by the development of therapeutics that not only influence the aggregation of misfolded proteins, but also efficiently target them for disposal. Importantly, a recent study comparing AD patients with normal controls showed that the levels of Aβ (1-40 and 1-42) production were the same in both groups but that the clearance of Aβ was significantly decreased in AD patients, strongly implicating impaired Aβ clearance in AD pathogenesis (213). Immunotherapy has been investigated as a means to increase the clearance of disease-relevant proteins/peptides. Active immunization using $A\beta_{1-42}$ peptide and passive immunization using antibodies raised against $A\beta_{1-42}$ have both been demonstrated to reduce Aβ deposition and cognitive decline in mice (214, 215). An early clinical trial using full length $A\beta_{1-42}$ as the immunogen in humans was halted due to the incidence of meningoencephalitis in a small proportion of the patients (216), nevertheless, long-term follow up of the patients from this trial showed significantly less cognitive decline and brain volume loss in those patients who had generated an antibody response during the trial compared to controls (217). The results of a more recent clinical trial suggest that side effects such as meningoencephalitis may be avoided by using a shorter fragment of $A\beta_{1-42}$ as the immunogen, rather than the full length peptide (218).

The discovery of ECs is an important landmark in our developing understanding of the mechanisms comprising extracellular proteostasis. Further characterization of the activities of the ECs, in particular their ability to facilitate the clearance of misfolded extracellular proteins (see section 5.3), will open up new avenues for the development of novel therapies. Exogenous administration of the normally intracellular sHsp αB-crystallin is protective in animal models of acute ischemic and autoimmune disease (219-222). The effect of αB-crystallin is potently anti-inflammatory and it has been suggested that this is directly related to its ability to sequester misfolded proteins (223). Considering that the activity of the ECs is similar to that of αB-crystallin, it is tempting to speculate that increasing their extracellular concentrations may have a similar therapeutic effect. Given that they are normally secreted it may be possible to increase the concentrations of ECs by administration directly in to the blood stream, however entry to the nervous system would be problematic due to the blood brain barrier. Alternatively, increases in

EC concentration could be achieved by targeting regulatory elements in the promoters responsible for their expression (224-227). However, the overexpression of clusterin been implicated in cancer pathogenesis and protection from chemotherapy drugs (43), thus, the possible side effects of the upregulation of ECs needs to be carefully evaluated. As outlined above (see section 5.1 and 5.2) there is strong evidence indicating that ECs reduce the toxicity of misfolded proteins, depending on the ratio of EC to amyloid forming protein, and the presence (or not) of specific receptors to promote clearance of complexes formed between the two molecules (51). Therefore, when targeting EC expression as a therapeutic strategy, it is important to consider the pathways by which chaperone-misfolded client protein complexes are cleared. For instance, in AD, downregulation of LRP at the blood brain barrier is coupled with increased expression of several LRP ligands (228-231), suggesting that accumulation of Aβ may in part be the result of overwhelming of LRP. In this scenario, increasing the concentration of α_2M may not have any therapeutic benefit unless the expression of LRP is also increased. A final intriguing possibility yet to be explored is to pharmacologically manipulate the in vivo chaperone activity of endogenous ECs.

7.0 Conclusions

Proteostasis is critical to maintain organismal viability, and logically must operate in all body spaces. Knowledge of those processes that achieve this in extracellular body spaces are only now being identified but are likely to depend heavily upon the involvement of recently discovered, constitutively secreted ECs. The available evidence strongly suggests that these chaperones act as both sensors and disposal-mediators of misfolded proteins in extracellular fluids. Their actions are likely to normally defend the human body from a range of serious diseases arising from inappropriate extracellular protein aggregation and deposition. It is therefore critically important to advance knowledge of ECs and how they integrate with various molecular and cellular mechanisms to effect extracellular proteostasis. This is essential if we are to one day identify effective therapies for what are currently some of the world's most debilitating, costly and intractable diseases.

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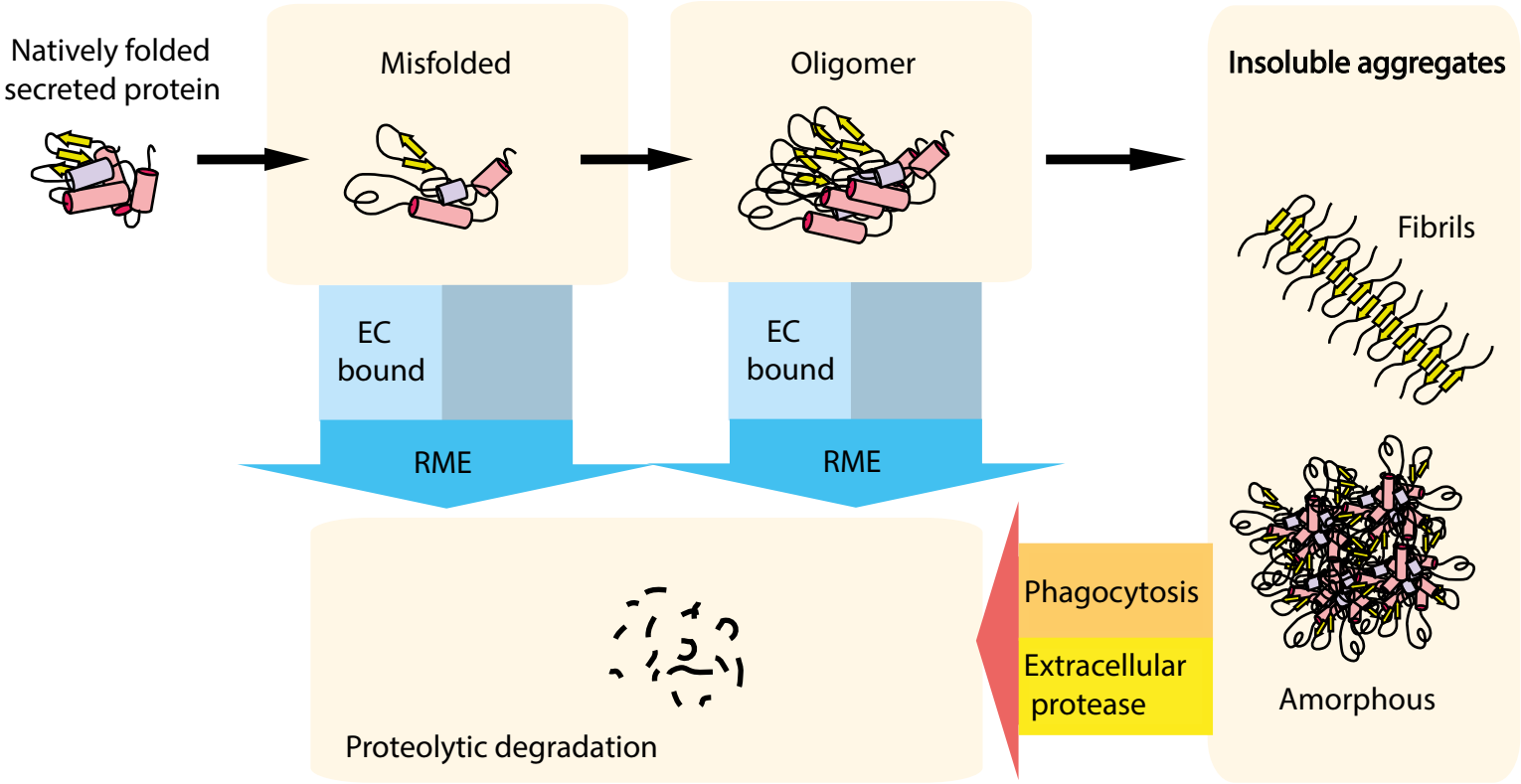
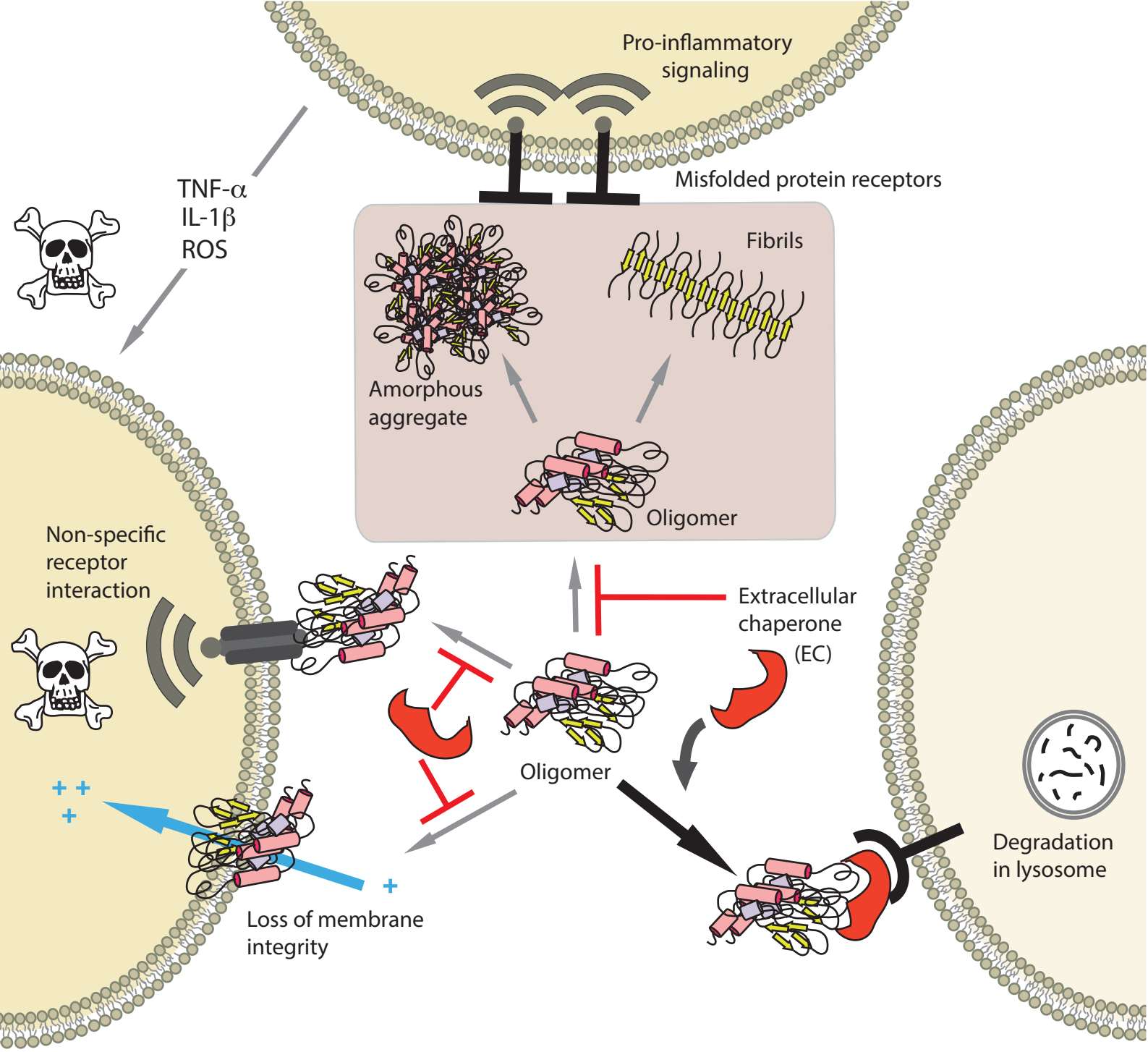
Table 1. Some examples of extracellular protein deposition disease, the protein/peptide implicated in their pathology and the ECs found co-localized with these deposits.

Disease	Aggregating protein/peptide	Co-localized chaperones
Alzheimer's disease	Aβ	Clusterin (232) α_2M (175) Haptoglobin (233)
Spongiform encephalopathies	Prion Protein	Clusterin (234) α_2M (235)
Macular Degeneration	Major contribution by vitronectin and complement components.	Clusterin (236)
Atherosclerosis	ApoB-100	Clusterin (237) α_2M (238)
Familial British dementia	ABri	Clusterin (239)
Familial Danish Dementia	ADan	Clusterin (240)
Down's syndrome	Aβ	Clusterin (241)
Type II Diabetes	Human Islet Amyloid Peptide	Clusterin (242)
Hemodialysis-related amyloidosis	β_2-Microglobulin	α_2M (243)
Amyloidotic cardiomyopathy	Transthyretin	Clusterin (244)
Systemic Amyloidosis	Immunoglobulin light chain	Clusterin (245)
Corneal Dystrophies	Keratoepithelin	Clusterin (246)
Glomerulonephritis	IgA	Haptoglobin (247)
Corpora amylacea	β-lactoglobulin, α-lactalbumin and other undetermined proteins	αS_2-casein and β-casein (248)

Figure 1. Major elements of extracellular proteostasis. Proteins have undergone rigorous quality control before they are secreted, generally in a natively folded state. Once in the extracellular environment, they encounter a variety of stresses which can cause them to partially unfold and populate misfolded states. Misfolded proteins can aggregate into soluble oligomers and subsequently into insoluble fibrillar or amorphous aggregates. Extracellular chaperones (ECs) are known to form stable complexes with misfolded protein species, including misfolded monomers and oligomers. These complexes maintain misfolded proteins in solution and facilitate their clearance from extracellular fluids via receptor mediated endocytosis (RME) and degradation in lysosomes. In some cases misfolded, modified or aggregated proteins can also be cleared via RME without the involvement of ECs; large insoluble aggregates must be phagocytosed. Furthermore, extracellular proteases such as plasmin may be activated by protein aggregates and subsequently degrade them.

Figure 2. Model for the effects of ECs on toxicity and inflammation driven by misfolded extracellular proteins. Misfolded proteins and aggregates can be toxic to cells by a variety of mechanisms including disruption of membrane integrity, inducing changes in intracellular signal transduction cascades, and indirectly by eliciting pro-inflammatory signaling in immune cells Extracellular chaperones (ECs) are likely to be cytoprotective because of their ability to shield hydrophobic residues on the surfaces of these species that can mediate interactions with cell membranes and receptors. The actions of the ECs also inhibit the formation of larger aggregates and facilitate their efficient clearance, further reducing potential pathology.


Natively folded secreted protein
Misfolded
Oligomer
Insoluble aggregates
Fibrils
EC bound
EC bound
RME
RME
Phagocytosis
Extracellular protease
Amorphous
Proteolytic degradation


Pro-inflammatory
signaling
Misfolded protein receptors
TNF-α
IL-1β
ROS
Fibrils
Amorphous
aggregate
Oligomer
Non-specific
receptor
interaction
Extracellular
chaperone
(EC)
Oligomer
Degradation
in lysosome
Loss of membrane
integrity