Quality control of protein folding in extracellular space

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The pathologies of many serious human diseases are thought to develop from the effects of intra- or extracellular aggregates of non-native proteins. Inside cells, chaperone and protease systems regulate protein folding; however, little is known about any corresponding mechanisms that operate extracellularly. The identification of these mechanisms is important for the development of new disease therapies. This review briefly discusses the consequences of protein misfolding, the intracellular mechanisms that control folding and the potential corresponding extracellular control processes. Finally, a new speculative model is described, which proposes that newly discovered extracellular chaperones bind to exposed regions of hydrophobicity on non-native, extracellular proteins to target them for receptor-mediated endocytosis and intracellular, lysosomal degradation.

Keywords: chaperones; extracellular space; protein folding; proteolysis; receptor-mediated endocytosis

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Introduction

It has been estimated that about 400 g of protein are synthesized and degraded each day in the human body. Individual proteins are degraded at extremely varied rates, with half-lives ranging from several minutes to many hours. Intracellularly, this variation in half-life has been attributed to differences in the intrinsic stability of proteins and the recognition of non-native structures by highly selective and precisely regulated protein quality-control systems.

Protein misfolding and disease

Correct protein folding is essential for the assembly of the functional machinery required by all living organisms. Mistakes in transcription or translation that result in errors as small as an amino-acid substitution can destabilize, and thus prevent, normal folding. In addition, environmental conditions such as macromolecular crowding, inappropriate ionic strength, oxidative stress and extremes of pH and temperature are known to promote the formation of misfolded states or slowly reacting intermediates that are trapped on the folding pathway. They also promote the partial unfolding of fully folded native proteins. Exposed hydrophobic regions on non-native proteins bind

to similar regions on nearby proteins by highly specific self-association (Rajan *et al*, 2001). The aggregating species can continue to bind exposed hydrophobic regions of neighbouring proteins and thus has the potential to form various stable structures, including soluble aggregates and insoluble deposits (Fig 1). All of these protein structures have been found to be associated with disease states. For example, soluble oligomeric protein aggregates have been implicated as cytotoxic species in various neurodegenerative disorders (Demuro *et al*, 2005). Insoluble protein deposits contribute to the pathology of a variety of serious human diseases (Table 1), including the amyloidoses that are characterized by insoluble, fibrillar protein aggregates with a β -sheet-rich structure.

Intracellular defences against non-native proteins

Prokaryotic and eukaryotic cells use post-translational quality-control systems, which include molecular chaperones and proteases and are designed to repair or remove damaged proteins. Consequently, misfolded intracellular proteins have three possible fates: rescue by chaperones, destruction by proteases and aggregation. Chaperones are a diverse group of proteins that selectively recognize and bind to exposed hydrophobic surfaces of non-native proteins in a stable non-covalent interaction. Several families of chaperones—defined by size, cellular compartment and function—work together to prevent protein aggregation and facilitate the correct folding of non-native proteins. Through regulated binding and release, chaperones can directly facilitate the folding of proteins by a mechanism that is ATP-dependent (as exemplified by heat-shock protein 90 (Hsp90), Hsp70 and Hsp60 in the cytosol) or independent (as exemplified by calnexin and calreticulin in the endoplasmic reticulum (ER); Fink, 1999; Hartl & Hayer-Hartl, 2002). Other types of chaperones—for example, the small Hsp25 and α -crystallin—bind reversibly to exposed hydrophobic regions on unfolded polypeptides but are generally unable to independently facilitate refolding. They are thought to protect non-native proteins from aggregation until “folding-helper” chaperones are available (Bross *et al*, 1999).

Misfolded proteins are degraded by a variety of proteases in different cell compartments. However, the main intracellular site of degradation is the proteasome, a barrel-shaped cytosolic protein complex that selectively degrades ubiquitin-tagged proteins (Hohfeld *et al*, 2001). Polyubiquitin chains can be attached to misfolded proteins in the ER lumen (Tsai *et al*, 2002), but ubiquitination predominantly occurs through the action of ubiquitin ligases and cofactors in the cytosol (Welchman *et al*, 2005). Proteins destined for secretion into the extracellular environment undergo a rigorous quality-control process within the ER lumen, which

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Fig 1 | Schematic diagram of protein aggregation pathways. For each protein, there is thought to be an ensemble of possible structures: native (N) and non-native (NN), the latter including partly unfolded intermediates (I₁ and I₂) and unfolded (U) structures. The non-native structures are prone to associate with each other to form aggregates, although native proteins may sometimes also aggregate to form fibres. Non-fibrillar (amorphous) aggregates result from non-specific interactions between many different conformations, whereas highly structured amyloid fibrils can be formed by β-sheet-rich intermediates. Soluble structures are shown in the internal blue rectangle, insoluble structures in the outer white region.

includes interaction with several ER-resident chaperones. Some of the ER chaperones, such as calnexin and calreticulin, are lectins that have a specialized role in the quality control of glycoprotein folding. Many quality-control substrates cycle between the ER and the Golgi during their maturation (Trombetta & Parodi, 2003). When misfolded proteins originally destined for secretion cannot be refolded, they are degraded by ER proteinases, directed to the lysosome or, more commonly, retro-translocated into the cytosol for proteasomal degradation (Fig 2). Under circumstances when the chaperone/protease machinery is overwhelmed, misfolded proteins may form insoluble aggregates inside the ER (Trombetta & Parodi, 2003) or in the cytosol (an aggresome). Once proteins are secreted, they escape these characterized intracellular quality-control processes and enter an environment in which the mechanisms of folding control—if they exist—have yet to be discovered.

Are extracellular proteins subject to quality control?

There is, on average, 15 litres of extracellular fluids in a 70 kg human, including five litres of blood. Compared with intracellular fluid, extracellular fluids have a lower protein concentration—6% in plasma and 2% in interstitial fluid, as opposed to 30% in cytosol—but are more oxidizing (Sitia & Braakman, 2003). Unlike intracellular

fluids, extracellular fluids are continuously subjected to shear stress—for example, through the pumping of plasma around the body—which is known to induce protein unfolding and aggregation (Ker & Chen, 1998). Intuitively, the relatively harsh extracellular conditions suggest that active protein quality-control mechanisms are required. Studies from past decades have provided substantive support for this hypothesis. For example, in the 1970s and 1980s it was shown that denatured plasma proteins were catabolized *in vivo* more rapidly than their native counterparts (Margineanu & Ghetie, 1981), and that the catabolic rates of plasma proteins correlate with their *in vitro* susceptibility to proteases (Dice & Goldberg, 1976). In addition, liposomes with greater surface-exposed hydrophobicity were cleared from the circulation more quickly than those coated with a hydrophilic polymer; it was proposed that clearance was enhanced by plasma factors that could bind to exposed hydrophobic surfaces on the liposomes (Senior *et al*, 1991). These data are consistent with the hypothesis that exposed hydrophobicity might be the structural change that identifies individual extracellular proteins as needing quality-control intervention, as occurs intracellularly. Further support for this hypothesis has come from the demonstration that a recently discovered extracellular chaperone, clusterin (see below), binds to its substrates by hydrophobic interactions (Poon *et al*, 2002).

What protein quality-control mechanism(s) actually operate in extracellular space? Substantial recent advances in understanding intracellular protein quality-control mechanisms highlight our near-complete lack of understanding of corresponding extracellular processes. While not excluding other possibilities, the potential involvement of chaperones and selective protease systems are clearly worth considering.

Extracellular chaperones and proteasome-like systems

Intracellular folding-helper chaperones—for example, Hsp70 & Hsp90—might be released from necrotic cells or during viral cell lysis. Indeed, they have been discovered in human plasma and associated with cell surfaces, in particular cancer cells. Several extracellular roles have been postulated for these chaperones, such as cancer cell invasiveness (Eustace *et al*, 2004) and immune presentation (Becker *et al*, 2002). If established, these specialized functions are obviously important; however, in the context of generic mechanisms for extracellular protein quality control, it is pertinent to note that these ‘normally intracellular’ chaperones are present extracellularly at very low (ng/ml) levels and require ATP to effect protein refolding. The abundance of extracellular ATP is at least 1,000 times lower than intracellular ATP (Farias *et al*, 2005). In the event of a large-scale presentation of extracellular non-native proteins—such as might occur during a chronic infection—the capacity of the low levels of Hsp70 and similar chaperones that are present extracellularly would be quickly exceeded. Thus, although a role for these chaperones in extracellular protein quality control cannot be excluded, it is far more likely that large-scale ‘handling’ of non-native proteins is dealt with by much more abundant extracellular chaperones (ECs). Two such ECs have recently been identified.

Clusterin, a ubiquitous and highly conserved secreted protein, is an efficient EC (Humphreys *et al*, 1999; Wilson & Easterbrook-Smith, 2000). Clusterin potently inhibits stress-induced protein aggregation by ATP-independent binding to non-native proteins to form soluble, high-molecular-weight complexes (Humphreys *et al*, 1999; Poon *et al*, 2000). More recently, it was established that haptoglobin (Hp; previously best known for its high-affinity binding to haemoglobin) is also an abundant EC with a chaperone action similar to clusterin (Yerbury *et al*, 2005). Both ECs are highly conserved, widely distributed glycoproteins that are present in most physiological fluids, including plasma and cerebrospinal fluid (Bowman & Kurosky, 1982; Jenne & Tschopp, 1992). They are composed of variably sized oligomers made up of different numbers of disulphide-linked α - and β -subunits, bind to a broad range of ligands, and have been detected in association with clinical amyloid deposits *in vivo* (Calero *et al*, 2000; Powers *et al*, 1981). In human plasma, clusterin is present at about 100 μ g/ml (Humphreys *et al*, 1999) and Hp at 0.3–1.9 mg/ml (Bowman & Kurosky, 1982). Immunoaffinity depletion of either clusterin or Hp from human serum renders proteins in whole serum more susceptible to aggregation and precipitation (Poon *et al*, 2000; Yerbury *et al*, 2005).

Neither clusterin nor Hp has the ability to independently refold heat-stressed, non-native enzymes (Poon *et al*, 2000; Yerbury *et al*, 2005). However, like the small heat-shock proteins, clusterin is able to preserve heat-inactivated enzymes in a state competent for subsequent ATP-dependent refolding by heat-shock chaperone 70 (Hsc70; Poon *et al*, 2000). Although it is possible that specifically extracellular folding-helper chaperones exist, they remain to be convincingly demonstrated. It has been reported that the plasma

Table 1 | Proteins forming insoluble deposits associated with disease

Protein involved	Disease(s)	Deposit structure
Amyloid- β	Alzheimer's disease	F, A
Tau	Alzheimer's disease	F
α -Synuclein	Parkinson's disease, Lewy body dementia	F, A F, A
Amylin*	Diabetes type 2	F, A
SOD1	Amyotrophic lateral sclerosis	F
β 2-Microglobulin*	Haemodialysis-related amyloidosis	F, A
Amyloid-A*	Reactive amyloidosis	F, A
Haemoglobin	Sickle cell anaemia	F
Huntingtin	Huntington's disease	F
PrP*	Creutzfeldt-Jakob disease	F, A
Androgen receptor	Spinobulbar muscular atrophy	F
Ataxins 1, 2 & 3	Spinocerebellar ataxias 1, 2 & 3	F
IgG*	Non-amyloid monoclonal IgG deposition disease	NF
Kerato-epithelin*	Certain corneal dystrophies	NF
Ten other proteins*	Systemic and cerebral hereditary amyloidosis	F, A

*Indicates the protein is deposited extracellularly. A, amyloid; F, fibrillar; NF, non-fibrillar. Data sourced from Korvatska *et al*, 1999; Lin *et al*, 2001; Sherman & Goldberg, 2001; Soto, 2001.

pentraxin serum amyloid P component (SAP) has some ATP-independent refolding activity *in vitro*. However, the physiological relevance of this is uncertain because, to effect significant refolding, SAP was required at a tenfold molar excess to the only substrate tested (lactate dehydrogenase; Coker *et al*, 2000). Further studies of the chaperone action of SAP using other substrates, and the investigation of potential interactions between SAP and other ECs, would be enlightening. SAP is a main plasma acute-phase protein in mice, but in humans, this role is fulfilled by the related pentraxin C-reactive protein (CRP; Mold *et al*, 2001); it is therefore tempting to speculate that CRP might also be an EC. Regardless, it is likely that other abundant ECs will be discovered in the future.

Previous studies have indicated that 50%–90% of plasma proteins are degraded in the liver and reticuloendothelial system (Bouma, 1982). This suggests that a principal component of any extracellular protein quality-control system comprises mechanisms for cellular uptake and intracellular degradation of aged/non-native proteins. However, this does not exclude the existence of selective mechanisms for the proteolysis of extracellular non-native proteins. Both ubiquitin and the proteasome are found in human plasma and their concentrations increase during disease, possibly reflecting their release from dead and dying cells. The plasma concentrations reported range from 7.7–200 ng/ml (Akarsu *et al*, 2001; Savas *et al*, 2003) and 2.1–2.4 μ g/ml (Lavabre-Bertrand *et al*, 2001; Stoebner *et al*, 2005), respectively. At these levels, ubiquitin and the proteasome are probably at least 300 times less abundant in human plasma than inside cells (Born *et al*, 1996; Lightcap *et al*, 2000). In addition, extracellular proteasome-mediated protein degradation would require extracellular ATP, which is scarce, as noted above. Therefore, current evidence does not support a major role for the mammalian ubiquitin/proteasome system in extracellular protein quality control. Nevertheless, the existence of other extracellular

Fig 2 | Outline of main intracellular controls of protein folding. In the cytosol, non-native proteins are recognized by chaperones and are targeted for refolding or proteolytic degradation by the proteasome or lysosome (Hohfeld *et al*, 2001). In the endoplasmic reticulum (ER), newly synthesized proteins or other non-native proteins are assisted to fold by chaperones. ER–Golgi cycling may occur at this stage. If a native conformation is achieved, the protein may be secreted from the cell. If a non-native conformation persists, it can be proteolytically degraded within the ER, retro-translocated to the cytosol and degraded by the proteasome, or transported to the lysosome (Trombetta & Parodi, 2003). Under some circumstances, aggregated non-native proteins can accumulate as insoluble deposits inside the ER or in the cytosol as an aggregosome. For simplicity, cofactors involved in many of these processes are not shown. See key for other details.

protease systems that might perform a similar function, perhaps independently of ATP, remains a possibility.

A control mechanism for extracellular protein folding

There are four, not mutually exclusive, theoretical models for extracellular quality control. Non-native extracellular proteins could be:

- Assisted to refold in the extracellular space. As discussed above, at present there is no evidence to substantiate this.
- Selectively endocytosed through receptors and degraded intracellularly. This seems feasible and will be discussed in more detail below.
- Randomly endocytosed and selected for degradation at the level of, for example, sorting endosomes, with native extracellular proteins recycled back to the extracellular space. This model cannot be excluded but at present there is no evidence to support it.
- Randomly endocytosed and non-selectively degraded intracellularly. This model can be excluded because it cannot account for the more rapid catabolism of denatured versus native plasma proteins observed *in vivo* (described above).

Although models (i) and (iii) cannot be excluded, recent advances support model (ii). In this model, a quality-control mechanism ‘senses’ increased hydrophobicity on non-native proteins to select them for ‘processing’. Obvious candidates for this sensing role are the recently identified ECs clusterin and Hp; other, yet to be identified ECs may also be involved. The formation of non-covalent complexes between ECs and non-native extracellular proteins would effectively label the latter for quality-control processing. The suggestion that ECs complex with misfolded proteins *in vivo* is strongly supported by the fact that *in vitro*, ECs inhibit the aggregation of purified proteins subjected to chemical and physical stresses by forming stable non-covalent complexes with them (Humphreys *et al*, 1999; Yerbury *et al*, 2005). In addition, the same (endogenous) ECs in unfractionated human serum inhibit heat-induced aggregation of serum proteins (Poon *et al*, 2000; Yerbury *et al*, 2005). It will be important to verify experimentally the formation of complexes between ECs and non-native proteins *in vivo*. There are precedents for the idea that by complexing with proteins, ECs can mediate the uptake of their protein ligands via cell-surface receptors and direct them towards intracellular degradation. For example, complexes formed *in vivo* between clusterin and the Alzheimer’s β -peptide

Fig 3 | Speculative model for extracellular chaperone-mediated clearance of non-native proteins. Non-native (NN) extracellular proteins are bound by extracellular chaperones (ECs) that mediate their uptake into cells by receptor-mediated endocytosis. EC–NN complexes are internalized and moved by vesicular transport to lysosomes, where they are degraded. Receptors are recycled back to the cell surface (not shown). The primary sites of action of this process are likely to be the liver and the reticuloendothelial system. See key for other details.

(amyloid- β , A β) are taken up by neural epithelial cells by way of megalin (LRP2)-mediated endocytosis and degraded in lysosomes (Hammad *et al*, 1997). Furthermore, Hp–haemoglobin complexes are quickly bound and internalized by CD163 expressed on human macrophages and are subsequently degraded (Kristiansen *et al*, 2001). We propose that by virtue of their interactions with both extracellular non-native proteins and cell surface receptors, ECs identify and deliver non-native proteins for cell uptake and degradation (Fig 3).

If this model is correct, then it would be expected that manipulation of EC expression may affect the clearance and/or aggregation of proteins in animal models. Recent work with clusterin-knockout mice supports the hypothesis that clusterin exerts important effects on protein clearance and aggregation *in vivo*. For example, a long-term study of clusterin-knockout mice showed that ageing mice developed a progressive glomerulopathy characterized by the accumulation of insoluble protein (containing immunoglobulins) in the kidney mesangium (Rosenberg *et al*, 2002). Another study of double-knockout mice with ablated expression of both clusterin and apolipoprotein E genes concluded that these two proteins worked together to inhibit the deposition of fibrillar A β in a model for Alzheimer's disease (DeMattos *et al*, 2004). Studies of Hp-knockout mice have not examined the effects of Hp on the aggregation/clearance of non-native extracellular proteins; it is, however, interesting to note that ablation of Hp expression caused a small but significant reduction in post-natal viability (Lim *et al*, 1998).

There are several cell-surface molecules that might be involved in receptor-mediated endocytosis of EC–(non-native) protein complexes. For example, clusterin interacts with several members of the low-density lipoprotein receptor (LDLR) family. Clusterin binds to chicken lipoprotein receptor 8 and an LDLR-related protein (Mahon *et al*, 1999), and uptake of clusterin–leptin complexes by apoER2 and very LDLR (VLDLR) has been proposed to facilitate leptin clearance (Bajari *et al*, 2003). Furthermore, clusterin and

LRP1/megalin have been implicated in the clearance of cellular debris by non-professional phagocytes (Bartl *et al*, 2001). Aside from CD163, another potential candidate receptor for Hp–(non-native) protein complexes is the CD11b/CD18 integrin (Mac-1/CR3), which binds to Hp, denatured proteins and the iC3b fragment of complement (Ross, 2000). SAP and CRP are also known to bind and be internalized by Fc receptors (Mold *et al*, 2001). These observations have provided leads to pursue in future studies of extracellular protein quality control.

Concluding remarks

If the hypothesis is correct that a quality-control system exists to oversee the folding of extracellular proteins, then it follows that the dysfunction of this system might contribute to the development of diseases associated with inappropriate extracellular protein aggregation and deposition. Therefore, elucidation of the mechanisms controlling extracellular protein folding could lead to the development of new treatments for serious human diseases.

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REFERENCES

- Akarsu E, Pirim I, Selcuk NY, Tombul HZ, Cetinkaya R (2001) Relation between serum ubiquitin levels and KT/V in chronic hemodialysis patients. *Nephron* **88**: 280–282
- Bajari TM, Strasser V, Nimpf J, Schneider WJ (2003) A model for modulation of leptin activity by association with clusterin. *FASEB J* **17**: 1505–1507
- Bartl MM, Luckenbach T, Bergner O, Ullrich O, Koch-Brandt C (2001) Multiple receptors mediate apoJ-dependent clearance of cellular debris into nonprofessional phagocytes. *Exp Cell Res* **271**: 130–141
- Becker T, Hartl FU, Wieland F (2002) CD40, an extracellular receptor for binding and uptake of Hsp70–peptide complexes. *J Cell Biol* **158**: 1277–1285

- Born LJ, Kharbanda KK, McVicker DL, Zetterman RK, Donohue TM Jr (1996) Effects of ethanol administration on components of the ubiquitin proteolytic pathway in rat liver. *Hepatology* **23**: 1556–1563
- Bourma JM (1982) Some aspects of plasma protein metabolism as compared with intracellular protein breakdown. *Acta Biol Med Germanica* **41**: 53–60
- Bowman BH, Kurosky A (1982) Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. *Adv Human Gen* **12**: 189–261
- Bross P, Corydon TJ, Andresen BS, Jorgensen MM, Bolund L, Gregersen N (1999) Protein misfolding and degradation in genetic diseases. *Human Mut* **14**: 186–198
- Calero M, Rostagno A, Matsubara E, Zlokovic B, Frangione B, Ghiso J (2000) Apolipoprotein J (clusterin) and Alzheimer's disease. *Microsc Res Tech* **50**: 305–315
- Coke, AR, Purvis A, Baker D, Pepys MB, Wood SP (2000) Molecular chaperone properties of serum amyloid P component. *FEBS Lett* **473**: 199–202
- DeMattos RB, Cirrito JR, Parsadanian M, May PC, Taylor JW, Harmony JAK, Aronow BJ, Bales KR, Paul SM, Holtzman DM (2004) ApoE and clusterin co-operatively suppress Ab levels and deposition: evidence that apoE regulates extracellular Ab metabolism *in vivo*. *Neuron* **41**: 193–202
- Demuro A, Mina E, Kayed R, Milston SC, Parker I, Glabe CG (2005) Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem* **280**: 17294–17300
- Dice JF, Goldberg AL (1976) Structural properties of rat serum proteins which correlate with their degradative rates *in vivo*. *Nature* **262**: 514–516
- Eustace BK *et al* (2004) Functional proteomic screens reveal an essential extracellular role for hsp90 α in cancer cell invasiveness. *Nat Cell Biol* **6**: 507–514
- Farias M 3rd, Gorman MW, Savage MV, Feigl EO (2005) Plasma ATP during exercise: possible role in regulation of coronary blood flow. *Am J Physiol – Heart Circ Physiol* **288**: H1586–H1590
- Fink, AL (1999) Chaperone-mediated protein folding. *Physiol Rev* **79**: 425–449
- Hamad SM, Ranganathan S, Loukinova E, Twal WO, Argraves WS (1997) Interaction of apolipoprotein J-amyloid β -peptide complex with low density lipoprotein receptor-related protein-2/megalin. A mechanism to prevent pathological accumulation of amyloid β -peptide. *J Biol Chem* **272**: 18644–18649
- Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**: 1852–1858
- Hohfeld J, Cyr DM, Patterson C (2001) From the cradle to the grave: molecular chaperones that may choose between folding and degradation. *EMBO Rep* **2**: 885–890
- Humphreys D, Carver JA, Easterbrook-Smith SB, Wilson MR (1999) Clusterin has chaperone-like activity similar to that of small heat-shock proteins. *J Biol Chem* **274**: 6875–6881
- Jenne DE, Tschopp J (1992) Clusterin: the intriguing guises of a widely expressed glycoprotein. *Trends Biochem Sci* **17**: 154–159
- Ker YC, Chen RH (1998) Shear-induced conformational changes and gelation of soy protein isolate suspensions. *Food Sci Technol-Lebensmittel-Wissenschaft Technol* **31**: 107–113
- Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* **10**: 524–530
- Korvatska E, Munier FL, Chaubert P, Wang MX, Mashima Y, Yamada M, Uffer S, Zografos L, Schorderet DF (1999) On the role of kerato-epithelin in the pathogenesis of 5q31-linked corneal dystrophies. *Invest Ophthalmol Vis Sci* **40**: 2213–2219
- Kristiansen M, Gravens JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. *Nature* **409**: 198–201
- Lavabre-Bertrand T, Henry L, Carillo S, Guiraud I, Ouali A, Dutaud D, Aubry L, Rossi JF, Bureau JP (2001) Plasma proteasome level is a potential marker in patients with solid tumors and hemopoietic malignancies. *Cancer* **92**: 2493–2500
- Lightcap ES, McCormack TA, Pien CS, Chau V, Adams J, Elliott PJ (2000) Proteasome inhibition measurements: clinical application. *Clin Chem* **46**: 673–683
- Lim SK, Kim H, bin Ali A, Lim YK, Wang Y, Chong SM, Costantini F, Baumann H (1998) Increased susceptibility in Hp knockout mice during acute hemolysis. *Blood* **92**: 1870–1877
- Lin J, Markowitz GS, Valeri AM, Kambham N, Sherman WH, Appel GB, D'Agati VD (2001) Renal monoclonal immunoglobulin deposition disease: the disease spectrum. *J Am Soc Nephrol* **12**: 1482–1492
- Mahon MG, Lindstedt KA, Hermann M, Nimpf J, Schneider WJ (1999) Multiple involvement of clusterin in chicken ovarian follicle development. Binding to two oocyte-specific members of the low density lipoprotein receptor gene family. *J Biol Chem* **274**: 4036–4044
- Margineanu I, Ghetie V (1981) A selective model of plasma protein catabolism. *J Theor Biol* **90**: 101–110
- Mold C, Gresham HD, Du Clos TW (2001) Serum amyloid P component and C-reactive protein mediate phagocytosis through murine Fc γ Rs. *J Immunol* **166**: 1200–1205
- Poon S, Rybchyn MS, Easterbrook-Smith SB, Carver JA, Wilson MR (2000) Clusterin is an ATP-independent chaperone with very broad substrate specificity that stabilizes stressed proteins in a folding-competent state. *Biochemistry* **39**: 15953–15960
- Poon S, Rybchyn MS, Easterbrook-Smith SB, Carver JA, Pankhurst GJ, Wilson MR (2002) Mildly acidic pH activates the extracellular molecular chaperone clusterin. *J Biol Chem* **277**: 39532–39540
- Powers JM, Schlaepfer WW, Willingham MC, Hall BJ (1981) An immunoperoxidase study of senile cerebral amyloidosis with pathogenetic considerations. *J Neuropath Exp Neurol* **40**: 592–612
- Rajan RS, Illing ME, Bence NF, Kopito RR (2001) Specificity in intracellular protein aggregation and inclusion body formation. *Proc Natl Acad Sci USA* **98**: 13060–13065
- Rosenberg ME, Girtan R, Finkel D, Chmielewski D, Barrie A, Witte DP, Zhu G, Bissler JJ, Harmony JAK, Aronow BJ (2002) Apolipoprotein J/clusterin prevents a progressive glomerulopathy of aging. *Mol Cell Biol* **22**: 1893–1902
- Ross GD (2000) Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/ α M β 2-integrin glycoprotein. *Crit Rev Immunol* **20**: 197–222
- Savas MC, Koruk M, Pirim I, Yilmaz O, Karakok M, Taysi S, Yilmaz A (2003) Serum ubiquitin levels in patients with nonalcoholic steatohepatitis. *Hepatogastroenterology* **50**: 738–741
- Senior J, Delgado C, Fisher D, Tilcock C, Gregoriadis G (1991) Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. *Biochim Biophys Acta* **1062**: 77–82
- Sherman MY, Goldberg AL (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* **29**: 15–32
- Sitia R, Braakman I (2003) Quality control in the endoplasmic reticulum protein factory. *Nature* **426**: 891–894
- Soto C (2001) Protein misfolding and disease: protein refolding and therapy. *FEBS Lett* **498**: 204–207
- Stoebner PE, Lavarbre-Bertrand T, Henry L, Guiraud I, Carillo S, Dandurand M, Joux JM, Bureau JP, Meunier L (2005) High plasma proteasome levels are detected in patients with metastatic malignant melanoma. *Br J Dermatol* **152**: 948–953
- Trombetta ES, Parodi AJ (2003) Quality control and protein folding in the secretory pathway. *Annu Rev Cell Dev Biol* **19**: 649–676
- Tsai B, Ye YH, Rapoport TA (2002) Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. *Nat Rev Mol Cell Biol* **3**: 246–255
- Welchman RL, Gordon C, Mayer RJ (2005) Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol* **6**: 599–609
- Wilson MR, Easterbrook-Smith SB (2000) Clusterin is a secreted mammalian chaperone. *Trends Biochem Sci* **25**: 95–98
- Yerbury J, Easterbrook-Smith SB, Rybchyn MS, Henriques C, Wilson MR (2005) The acute phase protein haptoglobin is a new member of a small family of mammalian extracellular chaperones. *Biochemistry* **44**: 10914–10925

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