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### Abstract

Small heat-shock proteins (sHsps) and clusterin are molecular chaperones that share many functional similarities despite their lack of significant sequence similarity. These functional similarities, and some differences, are discussed. sHsps are ubiquitous intracellular proteins whereas clusterin is generally found extracellularly. Both chaperones potently prevent the amorphous aggregation and precipitation of target proteins under stress conditions such as elevated temperature, reduction and oxidation. In doing so, they act on the slow off-folding protein pathway. The conformational dynamism and aggregated state of both proteins may be crucial for their chaperone function. Subunit exchange is likely to be important in regulating chaperone action; the dissociated form of the protein is probably the chaperone-active species rather than the aggregated state. They both exert their chaperone action without the need for hydrolysis of ATP and have little ability to refold target proteins. Increased expression of sHsp and clusterin accompanies a range of diseases, e.g. Alzheimer's, Creutzfeldt-Jakob and Parkinson's diseases, that arise from protein misfolding and deposition of highly structured protein aggregates known as amyloid fibrils. The interaction of sHsps and clusterin with fibril-forming species is discussed along with their ability to prevent fibril formation, probably via utilization of their chaperone ability.

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## Small heat-shock proteins and clusterin: intra- and extracellular molecular chaperones with a common mechanism of action and function?

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### **Abstract**

Small heat-shock proteins (sHsps) and clusterin are molecular chaperones that share many functional similarities despite their lack of significant sequence similarity. These functional similarities, and some differences, are discussed. sHsps are ubiquitous intracellular proteins whereas clusterin is generally found extracellularly. Both chaperones potently prevent the amorphous aggregation and precipitation of target proteins under stress conditions such as elevated temperature, reduction and oxidation. In doing so, they act on the slow off-folding protein pathway. The conformational dynamism and aggregated state of both proteins may be crucial for their chaperone function. Subunit exchange is likely to be important in regulating chaperone action; the dissociated form of the protein is probably the chaperone-active species rather than the aggregated state. They both exert their chaperone action without the need for hydrolysis of ATP and have little ability to refold target proteins. Increased expression of sHsp and clusterin accompanies a range of diseases, e.g. Alzheimer's, Creutzfeldt-Jakob and Parkinson's diseases, that arise from protein misfolding and deposition of highly structured protein aggregates known as amyloid fibrils. The interaction of sHsps and clusterin with fibril-forming species is discussed along with their ability to prevent fibril formation, probably via utilization of their chaperone ability.

## 1. Introduction

The conventional view of a protein, e.g. derived from X-ray crystallography, is that of a static entity comprised of a series of  $\alpha$ -helices,  $\beta$ -sheets etc. folded into a well-defined tertiary, globular structure. Of course, this is misleading as proteins are highly dynamic species that sample conformational space about a mean structure. This conformational pliability is important for protein function, for example, during interactions between proteins and binding partners. NMR spectroscopy is particularly useful for determining regions of proteins that have conformational mobility and are correspondingly relatively unstructured in solution. Often these regions become structured when interacting with their target [1,2]. Furthermore, many proteins exist as essentially unstructured species in their native state [1-3].

For globular proteins, there are occasions when they exist as partially folded and even unfolded species. For example, after synthesis, the polypeptide chain is unfolded and must fold to its correct functional conformation. To cross a biological membrane or move into a different cellular compartment, a protein must unfold from its native state. Furthermore, if a protein is subjected to stress, e.g. elevated temperature, shear, low pH, oxidation, it may partially unfold. Intermediately folded or unfolded proteins expose greater hydrophobicity to solution which encourages mutual association (aggregation), misfolding and possibly precipitation. The cell has various mechanisms available to counteract this. Arguably the most important of these mechanisms is the production of molecular chaperones, a large group of structurally unrelated proteins whose function is to interact with destabilized proteins to prevent their aggregation and encourage correct protein folding. Probably the most well studied groups of molecular chaperones are the Hsp70 and Hsp60 families, which operate in a sequential and synergistic manner to stabilize unfolded and partially folded proteins following their synthesis on the ribosome [4]. The folding pathway for a protein is relatively rapid: typically a protein will fold in a few milliseconds from its linear, unfolded state. As a result, the intermediately folded states of the folding protein are only transient species. In fact, many proteins do not require the intervention of molecular chaperones to fold correctly [4]. However, if the intermediately folded states linger for too long, e.g. because of difficulty in folding due to mutation or because of cellular stress, these states may mutually associate and enter the slow, off-folding pathway which leads to amorphous protein aggregation and potentially precipitation (Fig. 1).

In many cases, aggregation and precipitation of proteins are highly deleterious to cell viability and are hallmarks of diseases generally classified as protein misfolding or conformational diseases, e.g. Alzheimer's, Parkinson's, Huntington's and Creutzfeldt-Jakob diseases, cataract and type II diabetes. In all these diseases, with the possible exception of cataract, the precipitated state of the proteins is highly ordered, existing in a cross  $\beta$ -sheet array arranged into long amyloid fibrils. Like the off-folding pathway, the pathway to amyloid fibril formation occurs via one or more intermediately folded states which lead on to soluble, small protofibril aggregates that precede the insoluble amyloid fibril (Fig. 1). During physiological stress conditions, a unique class of molecular chaperones is often utilized to prevent large-scale protein aggregation. These types of chaperones are the focus of this review article. In particular, this discussion will focus on intracellular small heat-shock proteins (sHsps) and the extracellular protein, clusterin.

Although these two types of proteins share minimal structural similarity at the primary (and probably higher) levels, they function in a very similar manner and have strong parallels in their pattern of expression and reactivity in diseases related to protein misfolding.

## **2. The structure, function and distribution of sHsps and clusterin.**

### Small heat-shock proteins (sHsps)

sHsps are a diverse family of intracellular molecular chaperones that are found in all organisms [5-9]. In humans, they are found in many tissues at varying levels depending on the stage of development and the level of physiological stress. From examination of the human genome, ten sHsps have been identified [10,11]. The reasons for such diversity are currently unclear. The role of sHsps in cells seems to be multi-faceted with their overall theme of action being that they interact to stabilize partially folded states of other proteins. sHsps are expressed constitutively and regulate processes as diverse as actin polymerization and caspase protein inactivation [7-9]. However, sHsp expression is also significantly increased during conditions of cellular stress that cause protein unfolding. The chaperone action of sHsps was described over ten years ago; *in vitro*, sHsps prevented the stress-induced aggregation of a variety of unrelated target proteins at a stoichiometry of up to one subunit of sHsp to one subunit of the target protein [12,13].

The principal lens protein,  $\alpha$ -crystallin, is a sHsp and is comprised of two closely related subunits, A and B.  $\alpha$ B-crystallin, in particular, is found in significant levels in many extra-lenticular organs including the heart, kidney and brain. The lens is composed of a very high concentration of crystallin proteins ( $\alpha$ ,  $\beta$  and  $\gamma$ ) arranged in a close-range, supra-molecular order that allows for proper refraction of light. Apart from the outer (epithelial) layer of cells, there is almost no protein turnover in the lens. Furthermore, the lens continues to grow throughout life. As a result, the crystallin proteins have to be very stable and long-lived.  $\alpha$ -Crystallin helps maintain lens transparency by dual actions: it contributes to structural order and it functions as a molecular chaperone to prevent the aggregation and precipitation of lens crystallins [5-7].

sHsps have subunit masses in the range of 16 to 43 kDa but, in the main, exist as large heterogeneous aggregates of 300 to 1000 kDa in mass. sHsps are predominantly  $\beta$ -sheet in secondary structure. Crystal structures are available for Hsp16.9 (from wheat) and Hsp16.5 (from the archaeon *Methanococcus jannaschii*) [8,9]. Unlike the mammalian sHsps, which predominantly form heterogeneous aggregates, these two sHsps adopt well-defined quaternary arrangements of 24- and 12-mers, respectively. Both protein aggregates are characterized by having a large central cavity which is also detected in low-resolution cryoEM images of  $\alpha$ B-crystallin aggregates [7-9]. No crystal structure is available for any mammalian sHsp, however, the position of the  $\beta$ -sheets within  $\alpha$ A-crystallin, as determined by site-specific spin labeling studies of  $\alpha$ A-crystallin mutants, is very similar to those in the two sHsp crystal structures [14]. Therefore, the mammalian sHsps probably have a similar tertiary structure to other sHsps. At the level of quaternary structure, subtle differences in the interfaces between the subunits may explain why mammalian sHsps form heterogeneous aggregates while wheat and *M. jannaschii* sHsps form aggregates of well-defined stoichiometry. sHsps contain three structural regions: a variable N-terminal domain, the C-terminal (or  $\alpha$ -crystallin) domain of approximately 80 amino acids in length which is conserved between sHsps, and a C-terminal extension of variable length

and sequence [8,9]. NMR spectroscopy indicates that the heterogeneous mammalian sHsps have a highly flexible and unstructured region at their extreme C-terminus [9]; this conclusion is supported by sequence analysis using PONDR, a computer program used to predict regions of disorder within proteins (Fig. 2a) [3,15]. In contrast, the well-ordered sHsps, Hsp16.9 and Hsp16.5, lack this feature and have only a short C-terminal extension [9]. In mammalian sHsps, the flexible C-terminal extension is very important in chaperone action; its function seems to be as a solubilizing region for the relatively hydrophobic sHsp and the complex it makes with the destabilized target protein during chaperone action [9].

### Clusterin

Clusterin is constitutively expressed in mammals as a secreted (extracellular) protein and is found at relatively high concentrations in a variety of biological fluids, e.g. in plasma and semen it is present at about 100  $\mu\text{g/ml}$  and 400  $\mu\text{g/ml}$ , respectively [16]. Clusterin was first described, and named, because of its ability to cause clustering of a variety of cell types [17]. Since then, it has been found in a wide range of cellular environments associated with many disparate proteins and has been ascribed a bewildering array of functions, ranging from being a regulator of apoptosis, through to a lipid transporter and a regulator of complement [17]. In 1999, we reported that clusterin prevented the *in vitro* aggregation and precipitation of a variety of unrelated proteins that had been subjected to elevated temperature or reduction [18]; this established the sHsp-like chaperone action of clusterin. Clusterin was shown to be much more efficient than  $\alpha$ -crystallin in preventing the precipitation from solution of stressed target proteins. The chaperone ability of clusterin is consistent with its propensity to interact with so many different proteins *in vivo* and its enhanced expression under conditions of cellular stress.

Clusterin is expressed as a 75-80 kDa heterodimeric protein that is post-translationally internally cleaved to give two chains,  $\alpha$  and  $\beta$ , that remain joined by five disulfide bonds. The protein is heavily glycosylated such that up to 30% of its mass is comprised of sugar [17]. Clusterin exists in a range of aggregated states. Like the mammalian sHsps, there is no crystal structure available for clusterin and there is a paucity of information about its structural features. Structure prediction suggests that the protein has significant contiguous regions of disordered (possibly molten globule) conformation that separate other regions of well-defined secondary structure, such as amphipathic  $\alpha$ -helical regions and coiled-coil  $\alpha$ -helices [3,17]. Our NMR spectroscopic investigations have shown that clusterin has extensive regions of little ordered conformation and significant flexibility [Campbell, Rekas et al., unpublished results]. The putative assignment of resonances in the NMR spectrum and hence their location in the amino acid sequence coincides well with those regions predicted by Dunker et al. [3] (Fig. 2b). On this basis, clusterin has been categorized as an intrinsically disordered protein [3,15]. Such inherent disorder and flexibility would hinder the crystallization of clusterin and are potentially crucially important factors in its chaperone action (discussed in detail below). Interestingly, there is limited sequence similarity (around 25%) between the  $\beta$ -chain of clusterin and the C-terminal ( $\alpha$ -crystallin) domain of  $\alpha\text{B}$ -crystallin; the latter includes the putative chaperone active site of  $\alpha\text{B}$ -crystallin [17].

### 3. Parallels between the structure and mechanism of chaperone action of sHsps and clusterin

From the above, it is apparent that there are significant similarities between sHsps and clusterin. They are dynamic proteins, containing stretches of amino acids that have conformational flexibility and disordered structure. Clusterin is predicted to have a greater degree of disorder than sHsps. In clusterin, the disorder is localized over a variety of regions in the molecule, as opposed to  $\alpha$ -crystallin, where disorder and flexibility are predominantly found in the C-terminal extension (Fig. 2). Mammalian sHsps and clusterin are both found as heterogeneous aggregates. They are highly promiscuous in their chaperone interactions and prevent the stress-induced precipitation of a broad range of target proteins. The available evidence strongly suggests that (i) sHsps and clusterin recognize and bind to partially unfolded target proteins that expose significant hydrophobicity to solution, and (ii) these interactions are mainly hydrophobic in nature [19,20].

Clusterin and sHsps undertake their chaperone action independently of ATP hydrolysis. However,  $\alpha$ -crystallin does bind ATP [21]. The functional consequences of this property are not understood although there have been claims that ATP binding and hydrolysis enhance the chaperone ability of  $\alpha$ -crystallin [22]. Nevertheless, clusterin and sHsps have little or no ability to refold destabilized target proteins to which they have complexed; they prevent large-scale protein aggregation and precipitation by forming non-covalent complexes with the stressed proteins but they can not release them in a folded state. Therefore, they are unlike ATP-dependent chaperones such as Hsp70 and Hsp60 which are intimately involved in folding nascent proteins [4]. However, when complexes formed between stress-inactivated enzymes and either sHsps or clusterin are incubated together with Hsp70 and ATP, it is possible to recover folded and functionally active enzyme molecules [8,9,23].

It is clear that from our studies that sHsps and clusterin prefer to interact with slowly aggregating target proteins [9,24]. Thus, these chaperones do not recognize intermediately folded states of target proteins that are present only transiently on the rapid protein unfolding/folding pathway (Fig. 1) or as a result of rapid aggregation and precipitation [9,24]; in contrast, Hsp70 binds to these species. The implication is that kinetic factors are important determinants in regulating the action of molecular chaperones. Nature may have devised a simple mechanism for chaperones to distinguish between short-lived versus longer-lived intermediately folded states of target proteins. As a result, the unfolding/folding pathway of proteins is the domain of chaperones such as Hsp70 and Hsp60 whereas the off-folding pathway is where sHsps and clusterin act. How is this selectivity obtained? A clue to answering this question comes from a consideration of the importance of subunit exchange in the chaperone actions of sHsps and possibly clusterin. In an elegant series of studies, Bova et al. [e.g. 25] measured the rate of subunit exchange for different sHsps under varying conditions of pH, temperature, cations and the presence of bound target proteins. For  $\alpha$ A-crystallin, elevated temperature led to an increase in subunit exchange rate and a concomitant enhancement of chaperone ability [25]. The corollary is that the dissociated, probably dimeric sHsp [8], is the chaperone-active species and that the dynamic association and dissociation of subunits is important in regulating sHsp chaperone action. Possibly, the dissociated dimeric species has its chaperone binding site exposed and available for interaction with the destabilized target protein whereas this region is buried in the chaperone-inactive

aggregated species [9]. Indeed, ESR studies of spin-labeled  $\alpha$ A-crystallin mutants indicate that the putative chaperone binding site is buried in the aggregate [14].

For clusterin, the subunit exchange rate has not been measured. Unlike sHsps, the chaperone ability of clusterin is not significantly affected by temperature. The chaperone ability of  $\alpha$ -crystallin is reduced significantly at lower pH whereas that of clusterin is enhanced [20]. For clusterin, this suggests that it exerts its chaperone action extracellularly at sites of local acidosis. The effect of pH on the chaperone action is not accompanied by any gross structural change in clusterin but correlates with a shift to smaller aggregated species, particularly to the heterodimeric form [20]. Thus, like sHsps, an enhancement of the chaperone ability of clusterin is accompanied by a shift to smaller aggregates. The implication is that the chaperone binding site(s) in clusterin are buried in the aggregates and become available to bind to target proteins upon dissociation. Thus, a similar mechanism of chaperone action can be proposed for sHsps and clusterin which combines the features of the involvement of subunit exchange, dissociation and interaction with long-lived, potentially aggregating, intermediately folded target proteins (Fig. 3).

As sHsps and clusterin are likely to be intimately involved in preventing target protein aggregation *in vivo*, it is understandable that their expression is elevated in a variety of diseases that are associated with protein misfolding, aggregation and precipitation. An obvious example of this is the high concentration of  $\alpha$ -crystallin in the eye lens, which minimizes crystallin protein aggregation and hence cataract formation. In diseases characterized by the formation of amyloid fibrils, sHsps (e.g.  $\alpha$ B-crystallin) and clusterin are often associated with these deposits. The presence of these chaperones may arise from a response of the cell to the presence of the aggregating and precipitating fibril species. As the majority of fibril deposits occur extracellularly, clusterin may be the more relevant chaperone *in vivo* for regulating fibril formation. *In vitro*,  $\alpha$ B-crystallin and clusterin have both been found to inhibit amyloid fibril formation, e.g. by the amyloid  $\beta$ -peptide (A $\beta$ ), the putative causative agent in Alzheimer's disease [26-28]. However, in cell culture experiments,  $\alpha$ A-crystallin enhanced A $\beta$ -induced neurotoxicity, possibly by stabilizing the small soluble protofibril species (Fig. 1) [26] which is neurotoxic. Recent evidence strongly suggests that soluble protofibril species are the pathogenic agents in amyloid diseases [29,30]. In cell culture experiments in the presence of clusterin, conflicting results were obtained with clusterin enhancing oxidative stress caused by A $\beta$  in [27] but being protective under the conditions used in [28]. Our *in vitro* studies have shown that fibril formation by apolipoprotein C-II (apoC-II) is potently inhibited by clusterin and  $\alpha$ -crystallin [16,31]. When exerting this effect, neither chaperone forms a complex with apoC-II. Instead, they interact with an early fibrillogenic intermediate of apoC-II and cycle it back to its monomeric state. Our recent experiments have also shown that  $\alpha$ -crystallin and clusterin inhibit fibril formation by a variety of fibril-forming peptides and proteins including  $\alpha$ -synuclein, the putative causative agent in Parkinson's disease [Rekas, Thorn, Yerbury et al., unpublished results].

Finally, both proteins may have roles in regulating apoptosis. For sHsps, this arises from the ability of  $\alpha$ B-crystallin and Hsp27 to interact with caspases [32,33]. It is known that overexpression of clusterin can protect cells from a variety of agents that otherwise induce apoptosis. However, the mechanism(s) by which clusterin exerts these effects are currently unknown.



#### 4. Unanswered questions and directions for sHsp and clusterin research

It is apparent from the above discussion that many advances have been made over the past ten years in understanding structure/function aspects of sHsps and clusterin. The major catalyst for this progress has been the seminal observations of their chaperone action [12,18]. However, there are many fundamental aspects about their structure and chaperone action that are not yet understood.

The major drawback to research into both chaperones is the dearth of detail about their structure. For clusterin, there is no specific structural information available above the primary level. As discussed above, the two available sHsp crystal structures are for well-ordered non-mammalian aggregates. As mammalian sHsps exist predominantly as heterogeneous aggregates, and this heterogeneity is important in their chaperone action, it will be important to define the structural features of mammalian sHsps. When this information is available for sHsps and clusterin, it should provide significant insight into the mechanistic details of their chaperone actions, and the differences between the two. Both chaperones are dynamic and heterogeneous species and clusterin is heavily glycosylated; thus, it is likely that they will be problematic to crystallize, limiting the utility of X-ray crystallography as a means of elucidating their structures. Recent advances in NMR spectroscopy, such as the development of Transverse Relaxation Optimized Spectroscopy (TROSY) methods, may provide a route to determine the structures of these large chaperone aggregates [34]. It would also be valuable to determine the chaperone binding site(s) in clusterin; this might be accomplished via cross-linking studies with bound target peptides and proteins, as has been done for sHsps [8,35]. Furthermore, the hypothesis that subunit exchange is a crucial factor in the activation of clusterin chaperone action could be tested by directly measuring the rate of clusterin subunit exchange and its variation with pH, temperature and the presence of target protein.

The observation, *in vitro*, that partially folded target proteins complexed to sHsps and clusterin can be refolded by the action of Hsp70 coupled with ATP hydrolysis implies that similar mechanism(s) may exist *in vivo*, e.g. when intracellular conditions return to normal after stress, Hsp70 may function to refold the target proteins bound to sHsps (Fig. 3). Obviously, Hsp70 is an intracellular protein, so the *in vitro* experiments with it and clusterin are not physiologically relevant [23]. Whatever, there may be extracellular chaperones that function in an Hsp70 plus ATP manner to refold target proteins complexed to clusterin. However, arguing against this is that extracellular ATP levels are low. Instead, other extracellular chaperones may function to tag target proteins and facilitate their degradation. Two other extracellular molecular chaperones have been identified, serum amyloid protein [36] and haptoglobin [37]. Like clusterin, both have sHsp-like chaperone ability, i.e. a propensity to interact with destabilized target proteins to prevent their precipitation with little ability to refold them. Their individual efficiency of chaperone action, however, varies significantly. Understanding the interactions between these three extracellular chaperones (and potentially other chaperones), e.g. their possible synergistic chaperone interactions and their efficiency of interactions with target proteins, may shed light on the relative roles of these chaperones *in vivo*. For example, each chaperone may have different efficiency in interacting with target proteins that are aggregating at varying rates, i.e. one chaperone may be specific for rapidly aggregating proteins on their off-folding, amorphous

aggregation pathway whereas another may have preference for slowly aggregating proteins on the amyloid fibril-forming pathway (Fig. 1). There may even be the potential for one chaperone to direct a target protein from the amyloid to amorphous aggregation pathway. Similar behavior may be present intracellularly for different chaperones, e.g. Hsp70 may force amyloidogenic proteins on to the amorphous aggregation pathway [38] where they could be sequestered by the action of sHsps. To determine these various possibilities will require the implementation of a variety of *in vitro*, cellular and *in vivo* experiments.

Stemming from these experiments will be a much greater appreciation of the role that sHsps and clusterin play in protein misfolding or conformational diseases, particularly ones in which amyloid fibril formation is involved. Thus, under fibril-forming conditions it will be ascertained whether clusterin and sHsps over-expression is deleterious to cell viability due to their potentiation of neurotoxicity via stabilization of intermediately-folded protofibril species, as has been implied from *in vitro* and cell culture studies [26,27]. The role of individual chaperones, and their possible synergistic interactions, in the modulation of fibril formation will also become much clearer.

In addition to their role in regulating protein aggregation, sHsps and clusterin levels are elevated under a range of other diseases associated with stress. For example, sHsp expression is increased in a variety of cancers, multiple sclerosis and under ischemic conditions [9,32,39]. In some cancers, this may be detrimental because it enhances the tumor-forming propensity of the cells due to the increased protection from apoptosis. Investigation of sHsp and clusterin chaperone ability will enable a greater appreciation of their role in these diseases and potentially as therapeutics in their treatment, e.g. as agents to improve recovery in patients with cardiac infarction.

Many of the diseases associated with protein misfolding occur in old age. Thus, cataract is a major burden on the health care budget in the developed world and causes blindness in an estimated 20 million people world-wide. A greater understanding of the  $\alpha$ -crystallin/sHsp chaperone action will have implications for the development of therapeutics to treat and prevent cataract. In amyloid diseases, fibril formation arises from a partial breakdown in the mechanisms for removal of toxic, partially folded proteins. With the aging population, protein misfolding diseases will increase in their prevalence. Furthermore, since fibril formation is a generic characteristic of proteins [40], it is feasible that other diseases will be characterized as arising from protein misfolding. There is therefore a requirement to understand the fundamental chemistry of these diseases and there will be a major need for the development of therapeutics for their treatment. The involvement of molecular chaperones in these processes provides a possible avenue for therapeutic intervention, e.g. as agents to modulate amyloid fibril formation [38].

## References

1. Dyson, H.J. and Wright, P.E. (2002) Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* **12**, 54-60.
2. Uversky, V.N. (2002) What does it mean to be natively unfolded? *Eur. J. Biochem.* **269**, 2-12.

3. Dunker, A.K., Lawson, J.D., Brown, C.J., Williams, R.M., Romero, P., Oh, J.S., Oldfield, C.J., Campen, A.M., Ratliff, C.M., Hipps, K.W., Ausio, J., Nissen, M.S., Reeves, R., Kang, C., Kissinger, C.R., Bailey, R.W., Griswold, M.D., Chiu, W., Garner, E.C., and Obradovic, Z. (2001) Intrinsically disordered protein. *J. Mol. Graph. Model.* **19**, 26-59.
4. Hartl, F.U. and Hayer-Hartl, M. (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852-1858.
5. Horwitz, J. (2000) The function of alpha-crystallin in vision. *Cell Dev. Biol.* **11**, 53-60.
6. Horwitz, J. (2003) Alpha-crystallin. *Exp. Eye Res.* **76**, 145-153.
7. Derham, B.K. and Harding, J.J. (1999)  $\alpha$ -Crystallin as a molecular chaperone. *Prog. Ret. Eye Res.* **18**, 463-509.
8. van Montfort, R., Slingsby, C. and Vierling, E. (2002) Structure and function of the small heat shock protein/ $\alpha$ -crystallin family of molecular chaperones. *Adv. Prot. Chem.* **59**, 105-156.
9. Treweek, T.M., Morris, A.M. and Carver, J.A. (2003) Intracellular protein unfolding and aggregation: the role of small heat-shock chaperone proteins. *Aust. J. Chem.* **56**, 357-367.
10. Kappé, G., Franck, E., Verschuure, P., Boelens, W.C., Leunissen, J.A.M. and de Jong, W.W. (2003) The human genome encodes 10  $\alpha$ -crystallin-related small heat shock proteins: HspB1-10. *Cell Stress Chap.* **8**, 53-61.
11. Fontaine, J.M., Rest, J.S., Welsh, M.J. and Benndorf, R. (2003) The sperm outer dense fiber protein is the 10<sup>th</sup> member of the superfamily of mammalian small stress proteins. *Cell Stress Chap.* **8**, 62-69.
12. Horwitz, J. (1992)  $\alpha$ -Crystallin can function as a molecular chaperone. *Proc. Natl. Acad. Sci. USA* **89**, 10449-10453.
13. Jakob, U., Gaestel, M., Engel, K. and Buchner, J. (1993) Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* **268**, 1517-1520.
14. Koteiche, H.A. and Mchaourab, H.S. (1999) Folding pattern of the  $\alpha$ -crystallin domain in  $\alpha$ A-crystallin determined by site-directed spin labeling. *J. Mol. Biol.* **294**, 561-577.
15. Dunker, A.K., Brown, C.J., Lawson, J.D., Iakoucheva, L.M. and Obradovic, Z. (2002) Intrinsic disorder and protein function. *Biochemistry* **41**, 6573-6582.
16. Hatters, D.M., Wilson M.R., Easterbrook-Smith, S.B., and Howlett, G.J. (2002) Suppression of apolipoprotein C-II amyloid formation by the extracellular chaperone, clusterin. *Eur. J. Biochem.* **269**, 2789-2794.

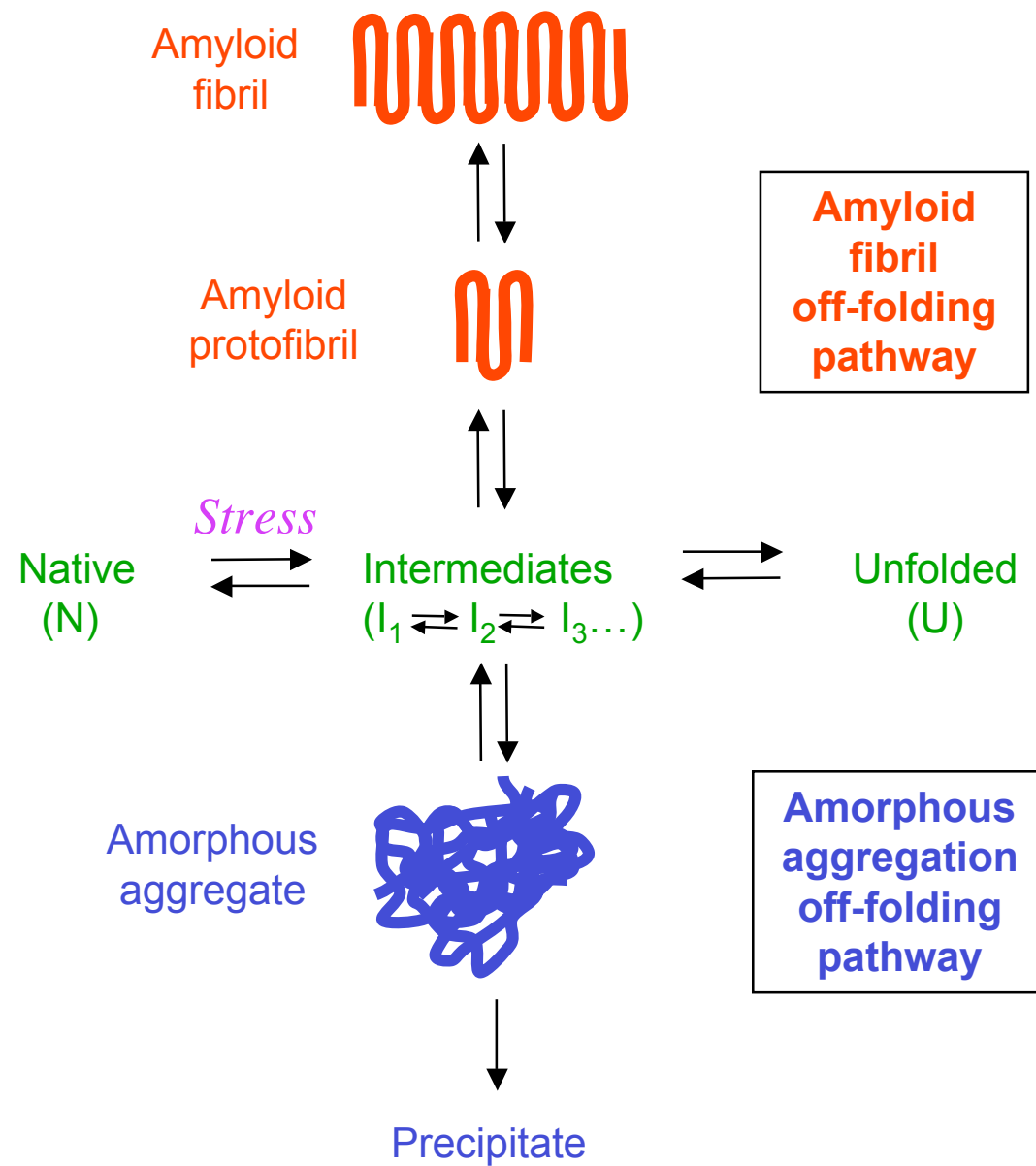
17. Wilson, M.R. and Easterbrook-Smith, S.B. (2000) Clusterin is a secreted mammalian chaperone. *Trends Biochem. Sci.* **25**, 95-98.
18. Humphreys, D.T., Carver, J.A., Easterbrook-Smith, S.B. and Wilson, M.R. (1999) Clusterin has chaperone-like activity similar to that of small heat-shock proteins. *J. Biol. Chem.* **274**, 6875-6881.
19. Das, K.P. and Surewicz, W.K. (1995) Temperature-induced exposure of hydrophobic surfaces and its effect on the chaperone activity of  $\alpha$ -crystallin. *FEBS Lett.* **369**, 321-325.
20. Poon, S., Rybchyn, M.S., Easterbrook-Smith, S.B., Carver, J.A., Pankhurst, G.J. and Wilson, M.R. (2002) Mildly acidic pH activates the extracellular chaperone clusterin. *J. Biol. Chem.* **277**, 39532-39840.
21. Palmisano, D.V., Groth-Vasselli, B., Farnsworth, P.N. and Reddy, M.C. (1995) Interaction of ATP and lens alpha-crystallin characterized by equilibrium binding studies and intrinsic fluorescence spectroscopy. *Biochim. Biophys. Acta* **1246**, 91-97.
22. Muchowski, P.J. and Clark, J.I. (1998) ATP-enhanced molecular chaperone functions of the small heat shock protein human  $\alpha$ B-crystallin. *Proc. Natl. Acad. Sci. USA* **95**, 1004-1009.
23. Poon, S., Easterbrook-Smith, S.B., Rybchyn, M.S., Carver, J.A. and Wilson, M.R. (2000) Clusterin is an ATP-independent chaperone with broad specificity that stabilizes stressed proteins in a folding-competent state. *Biochemistry* **39**, 15953-15960.
24. Poon, S., Treweek, T.M., Wilson, M.R., Easterbrook-Smith, S.B. and Carver, J.A. (2002) Clusterin is an extracellular chaperone that specifically interacts with slowly aggregating proteins on their off-folding pathway. *FEBS Lett.* **513**, 259-266.
25. Bova, M.P., Ding, L.-L., Horwitz, J. and Fung, B. K.-K. (1997) Subunit exchange of  $\alpha$ A-crystallin. *J. Biol. Chem.* **272**, 29511-29517.
26. Stege, G.J., Renkawek, K., Overkamp, P.S., Verschuure, P., van Rijk, A.F., Reijnen-Aalbers, A., Boelens, W.C., Bosman, G.J., de Jong, W.W. (1999) The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity. *Biochem. Biophys. Res. Commun.* **262**, 152-156.
27. Oda T., Wals P., Osterburg H.H., Johnson S.A., Pasinetti G.M., Morgan T.E., Rozovsky I., Stine W.B., Snyder S.W., Holzman T.F., *et al.* (1995) Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1-42) and forms slowly sedimenting A beta complexes that cause oxidative stress.
28. Other clusterin/A beta reference
29. Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M., Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* **416**, 507-11.

30. Kaye, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W. and Glabe, C.G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486-489.
31. Hatters, D.M., Lindner, R.A., Carver, J.A. and Howlett, G.J. (2001) The molecular chaperone,  $\alpha$ -crystallin, inhibits amyloid formation by apolipoprotein C-II. *J. Biol. Chem.* **276**, 33755-33761.
32. Concannon, C.G., Gorman, A.M. and Samali, A. (2003) On the role of Hsp27 in regulating apoptosis. *Apoptosis* **8**, 61-70.
33. Kamradt, M.C., Chen, F., Sam, S. and Cryns, V.L. (2002) The small heat shock protein  $\alpha$ B-crystallin negatively regulates apoptosis during mitogenic differentiation by inhibiting caspase-3 activation. *J. Biol. Chem.* **277**, 38731-38736.
34. Fiaux, J., Bertelsen, E.B., Horwich, A.L. and Wüthrich, K. (2002) NMR analysis of a 900K GroEL-GroES complex. *Nature* **418**, 207-210.
35. Sharma, K.K., Kumar, R.S., Kumar, G.S. and Quinn, P.T. (2000) Synthesis and characterization of a peptide identified as a functional element in  $\alpha$ A-crystallin. *J. Biol. Chem.* **275**, 3767-3771.
36. SAP ref.
37. Haptoglobin ref.
38. Muchowski, P.J. (2002) Protein misfolding, amyloid formation and neurodegeneration: a critical role for molecular chaperones? *Neuron* **35**, 9-12.
39. Clark, J.I. and Muchowski, P.J. (2000) Small heat-shock proteins and their potential role in human disease. *Curr. Opin. Struct. Biol.* **10**, 52-59.
40. Dobson, C.M. (2001) The structural basis of protein folding and its links with human disease. *Phil. Trans. Roy. Soc. Lond. B* **356**, 133-145.

### Figure captions

**Figure 1.** A schematic diagram of the folding/unfolding and off-folding pathways of a protein. A native protein (N) unfolds via a variety of intermediate states ( $I_1$ ,  $I_2$ ,  $I_3$ , ...) which can progress to the unfolded state (U). If the intermediate states linger for long enough, they can enter the off-folding pathways. These comprise two possible routes: (i) the amorphous aggregation pathway which arises from large-scale interaction, amorphous aggregation and, ultimately, irreversibly precipitated species and (ii) the slower amyloid fibril-forming pathway which leads to insoluble, highly ordered cross  $\beta$ -sheet fibril arrays via the formation of small, soluble protofibril species.

**Figure 1**



**Figure 2.** Intrinsic disorder in mammalian sHsps and clusterin as predicted by the computer program, PONDR [3,15]. (a) Human  $\alpha$ A-crystallin exhibits significant disorder at its C-terminus, a region that corresponds well to the predicted C-terminal extension of  $\alpha$ A-crystallin and the flexible region determined by NMR spectroscopy [9]. (b) Human clusterin has multiple regions of disorder that correspond well with those accessible to proteases [3]. The residue numbers for clusterin include its N-terminal signal peptide (22 amino acids in length) which is leaved off following export from the cytoplasm.

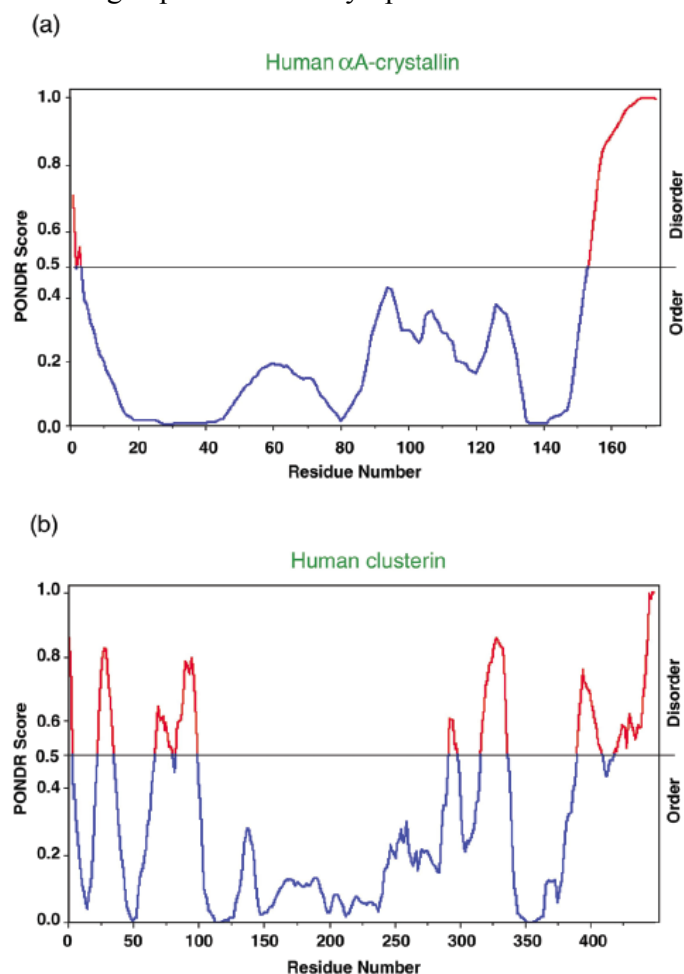


Figure 3. A schematic mechanism of chaperone action of sHsps and clusterin [9,20,24]. Both chaperones selectively interact with a slowly aggregating, relatively disordered, intermediately folded protein ( $I_2$ ) on its off-folding pathway. In doing so, this species is stabilized. The dynamic nature of the equilibrium between the monomeric and aggregated  $I_2$  species facilitates interaction with the similarly dynamic chaperones. It is hypothesized that dissociated forms of the chaperones, i.e. the sHsp dimer and the clusterin heterodimer, are the chaperone-active species. They interact with  $I_2$  which is subsequently sequestered into a high-mass complex containing both proteins. It is possible to recover natively folded target protein (N) via the action of another chaperone, e.g. Hsp70 in the case of intracellular sHsps, coupled to ATP hydrolysis. It is not known whether such a mechanism operates extracellularly with clusterin-stabilized  $I_2$  proteins. The diagram represents the interaction of sHsps and clusterin with amorphously aggregating target proteins. When interacting with amyloid fibril-forming target proteins (under slowly aggregating conditions), complexation may not occur; instead, the partially folded protofibril species is stabilized and large-scale aggregation is prevented [16,26-28,31].



**Figure 3**

Folding/unfolding pathway (reversible and fast)

