The multifaceted nature of α B-crystallin

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Abstract

In vivo, small heat-shock proteins (sHsps) are key players in maintaining a healthy proteome. α B-crystallin (α B-c) or HspB5 is one of the most widespread and populous of the ten human sHsps. Intracellularly, α B-c acts via its molecular chaperone action as the first line of defence in preventing target protein unfolding and aggregation under conditions of cellular stress. In this review, we explore how the structure of α B-c confers its function and interactions within its oligomeric self, with other sHsps, and with aggregation-prone target proteins. Firstly, the interaction between the two highly conserved regions of α B-c, the central α -crystallin domain and the C-terminal IXI motif and how this regulates α B-c chaperone activity are explored. Secondly, subunit exchange is rationalised as an integral structural and functional feature of α B-c. Thirdly, it is argued that monomeric α B-c may be its most chaperone-species active, but at the cost of increased hydrophobicity and instability. Fourthly, the reasons why hetero-oligomerisation of α B-c with other sHsps is important in regulating cellular proteostasis are examined. Finally, the interaction of α B-c with aggregation-prone, partially folded target proteins is discussed. Overall, this paper highlights the remarkably diverse capabilities of α B-c as a caretaker of the cell.

Keywords Small heat-shock proteins · α B-crystallin · Molecular chaperone · Oligomerisation · Protein unfolding · Protein aggregation · Proteostasis

Abbreviations

α A-c	α A-crystallin
α B-c	α B-crystallin
ACD	α -Crystallin domain
A β	Amyloid beta
DLS	Dynamic light scattering
FRET	Förster resonance energy transfer
HMW	High-molecular weight
IXI	IXI motif
K_d	Dissociation constant
MS	Mass spectrometry
PDB	Protein Data Bank
RCM	Reduced and carboxymethylated
R_g	Radius of gyration
R_h	Hydrodynamic radius

SANS	Small-angle nuclear scattering
SAXS	Small-angle X-ray scattering
sHsp	Small heat-shock protein
UPP	Ubiquitin-proteasome pathway
WT	Wild type
γ S-c	γ S-crystallin

Proteostasis and small heat-shock proteins

For a protein to function in the cell, it must be expressed at its correct concentration and location, while maintaining its native, functional conformation. Proteins drive nearly all cellular processes. In human cells, a complex and extensive network of thousands of proteins interacts cooperatively to ensure that the proteome functions correctly and efficiently. Collectively, this process is termed proteostasis, a portmanteau of the words protein and homeostasis (Hipp et al. 2019).

A reduction in proteostasis efficiency (for example as occurs with age) can promote non-native protein conformations due to partial protein unfolding, potentially leading to protein misfolding and aggregation. Undesirable consequences such as a loss of protein function and/or the generation of toxic intermediate species can then occur (Janson et al. 1999;

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Winner et al. 2011). Broadly, protein aggregation is classified into two types. Fibrillar aggregation refers to an ordered association of monomers to form amyloid fibrils, which have a characteristic cross- β -sheet arrangement, and a rod-like morphology (Chiti and Dobson 2017). Due to the cellular toxicity of intermediate, oligomeric and end-stage protein species formed during the aggregation process, amyloid fibril formation is implicated in diseases such as Parkinson's, Alzheimer's and type II diabetes (Janson et al. 1999). Amorphous aggregation is a disordered association of monomeric proteins, lacking a well-defined structure. Amorphous aggregates are generally not considered to be cytotoxic. Both aggregated states are more thermodynamically stable than their native folded counterpart, highlighting the importance of the proteostatic network to maintain the protein's native, functional fold (Hipp et al. 2019).

A major component of the proteostatic machinery is molecular chaperones. They are a diverse set of proteins that assist in the folding of the nascent polypeptide chain. Chaperones prevent proteins from unfolding and aggregating and can also refold partially unfolded proteins that are prone to, or have, aggregated. During cellular stress conditions, ATP-independent chaperones are upregulated, interact with and bind to unfolding proteins to prevent their further aggregation. Subsequently, ATP-dependent chaperones refold them into their correct native state. If the protein cannot be refolded, it is targeted for degradation via the ubiquitin-proteasome or the autophagosomal-lysosomal systems. Together with the protein synthesis machinery, the degradation systems keep proteins expressed at the correct level within the cell. An inappropriate increase in protein expression leads to protein aggregation and perturbs proteostasis. An excellent review by Hipp et al. is recommended for further discussion of cellular proteostasis (Hipp et al. 2019).

Inside the cell, small heat-shock proteins (sHsps) are constitutively expressed at a basal level but their expression is upregulated significantly during stress conditions such as elevated temperature (Treweek et al. 2015). They are considered to be the cell's first line of defence against the deleterious effects of protein unfolding. Certain regions of sHsps are highly conserved across all phyla, highlighting the importance of sHsps as key proteins of life (Kriehuber et al. 2010). In a *C. elegans* mutant with an ageing phenotype, a 13–90-fold increase in sHsp levels occurred compared to the wild-type organism (in contrast to the levels of other molecular chaperones), illustrating the crucial role of sHsps in protecting against age-related degradation of the proteome (Walther et al. 2015). We have recently reviewed the structure, interactions and function of sHsps (Treweek et al. 2015; Carver et al. 2018; Cox et al. 2014).

sHsps are ATP-independent molecular chaperones. There are ten human sHsps (Table 1), of which α B-crystallin (α B-c) or HspB5 is a widespread and populous sHsp. It is also one of

Table 1 Agreed nomenclature and common name, monomeric mass, amino acid length, and tissue location of human small heat-shock proteins

Agreed nomenclature	Common name(s)	Monomeric mass (kDa)	Amino acid length	Localisation
HSPB1	Hsp27	22.3	205	Ubiquitous
HSPB2	MKBP	20.2	182	Heart and skeletal muscle
HSPB3	HSPL27	17.0	150	Heart and skeletal muscle
HSPB4/CRYAA	α A-crystallin	19.9	173	Eye lens
HSPB5/CRYAB	α B-crystallin	20.2	175	Ubiquitous
HSPB6	Hsp20	17.1	160	Ubiquitous
HSPB7	HspB7, cardiovascular heat shock protein (cvHsp)	18.6	170	Heart and skeletal muscle
HSPB8	HspB8, α -crystallin C chain, E2-induced gene 1 protein, protein kinase H11	21.6	196	Ubiquitous
HSPB9	Cancer/testis antigen 51 (CT51)	17.5	159	Testes
HSPB10	ODF1, outer dense fibre protein 1	28.4	250	Testes

Modified and adapted from Mymrikov et al. 2017

the major lens proteins where it coexists with its closely related sHsp, α A-crystallin (α A-c). Human α B-c is 175 amino acids in length and is organised in a tripartite arrangement (Fig. 1). The N-terminal region (residues 1–65) is mostly unstructured and plays an important role in target protein interaction and binding and oligomerisation (Carver et al. 2017). The central, conserved α -crystallin domain (ACD) (residues 66–148) is predominantly β -sheet in conformation and adopts an immunoglobulin fold (Clark et al. 2011). The C-terminal region (residues 149–175) is dynamic and unstructured; it contains the conserved IXI motif (I159, P160 and I161) and the highly flexible C-terminal extension (residues 164–175) (Carver et al. 2017).

Similar to most other mammalian sHsps, α B-c exists as a heterogenous oligomer. Its monomeric mass is approximately 20 kDa, with an average oligomeric mass of 650 kDa, spanning a 420 to 980 kDa range. The subunits have a continuous probability distribution, ranging from 10 to 40 subunits (Aquilina et al. 2003). The size and oligomeric distribution

Fig. 1 Above: Schematic of the three structural regions within the primary amino acid sequence of human α B-c: the N-terminal region, the α -crystallin domain (ACD) and the C-terminal region. The C-terminal region contains the IXI motif (residues 159–161), and the flexible C-terminal extension (Ext.). Below: Structures of α B-c and its mutants deposited in the Protein Data Bank (PDB) to date. **a, e** Models of the α B-c oligomer reconstructed using solid state NMR (Jehle et al. 2011), and a triple hybrid approach of cryo-EM, NMR and structural modelling

(Braun et al. 2011), respectively. **b–d, f–i** Monomeric or dimeric ACD of WT α B-c solved by NMR or X-ray diffraction. **j, k** Dimeric ACD of R120G α B-c solved by solution NMR (Rajagopal et al. 2015) and X-ray diffraction (Clark et al. 2011), respectively. **l** Two dimeric ACDs of E117C α B-c solved by X-ray diffraction (Hochberg et al. 2014). The yellow spheres represent the inter-subunit disulphide bond at position 117. All structures are described in Table S3 with their PDB code, name, deposited date and method for structural elucidation

of α B-c varies with environment (i.e. pH, temperature etc.), and post-translational modifications such as phosphorylation in its N-terminal region (Ecroyd et al. 2007). The subunits spontaneously associate to form a dimer (Fig. 1). Its large size, dynamism and heterogeneity have made α B-c a challenging protein to study structurally.

Dysfunction of α B-c is associated with a plethora of human diseases (Table 2). Due to its ubiquitous intracellular expression, α B-c interacts with a wide range of target proteins (more than 115 proteins (Oughtred et al. 2019)), and during chaperone action, α B-c inhibits both amorphous and fibrillar target protein aggregation (Treweek et al. 2015).

In this review, we focus on the structure–function relationships of α B-c, and its interactions with itself, unfolding proteins and other sHsps.

What is the chaperone-active species of α B-crystallin?

Alpha-crystallin domain and the IXI motif interaction: autoregulation of chaperone function

The ACD and the IXI sequence are the most highly conserved regions across the sHsp family. What are the functional roles

of these regions, and what do the interactions between them confer? Despite the isolated ACD having chaperone ability by itself (Hochberg et al. 2014), the interaction between the ACD and the IXI appears to modulate α B-c's chaperone activity. Under normal physiological intracellular conditions, the interactions between these two regions may maintain α B-c in a less active form, for example via oligomerisation.

Structurally, interest in the interaction of the ACD with the IXI sequence was heightened upon the crystal structure determination of the ACDs of α A-c and α B-c (with their IXI sequences attached (Fig. 2b)), in which the IXI motif was observed to associate with the ACD to promote its higher order oligomerisation (Laganowsky et al. 2010). However, this is a reversible interaction, which led to implication that the (de)attachment of the IXI to the ACD was involved in chaperone action. The literature provides conflicting evidence whether this interaction enhances or diminishes the chaperone ability of α B-c. For example, the ACD with the IXI (ACD+IXI) completely suppressed the amorphous aggregation of destabilised alcohol dehydrogenase (ADH) with comparable efficiency to full length α B-c, whereas the ACD without the IXI (ACD-IXI) was inefficient as a molecular chaperone (Laganowsky et al. 2010). Similarly, ACD+IXI species was better at preventing the amyloid fibrillar aggregation of α -synuclein compared to ACD-IXI (Liu et al. 2018). In a

Table 2 Association with disease and localisation of human α B-crystallin

Disease association	Localised tissue	Ref
Alexander disease	Brain; astrocytes, Rosenthal fibres	Iwaki et al. 1989; Heaven et al. 2016
Alzheimer's disease	Brain	Renkawek et al. 1994
Amyotrophic lateral sclerosis	Brain (mice)	Bergemalm et al. 2010
Breast cancer	Breast	Kim et al. 2015a
Cardiomyopathy	Heart	Inagaki et al. 2006
Cataract	Eye	Berry et al. 2001
Colorectal cancer	Large intestine	Li et al. 2017; Shi et al. 2017
Creutzfeldt-Jakob disease, fatal familial insomnia	Brain; parietal lobe and thalamus	Wang et al. 2013; Renkawek et al. 1992
Desmin-related myopathy	Skeletal muscle	Vicart et al. 1998
Diabetic retinopathy	Eye	Chen et al. 2017
Endometriosis	Uterus	Moberg et al. 2015
Familial amyloidotic polyneuropathy	Skin	Magalhães et al. 2010
Guillain-Barré syndrome	Cerebrospinal fluid	Wanschitz et al. 2008
Head, neck and mouth squamous cell carcinoma	Head, neck, mouth	Yilmaz et al. 2015; Annertz et al. 2014
Multiple sclerosis	Brain; astrocytes and oligodendrocytes	Bsibsi et al. 2014; van Noort et al. 1995
Multiple system atrophy	Brain; glial cytoplasmic inclusions	Pountney et al. 2005
Parkinson's disease	Brain; substantia nigra	Liu et al. 2015
Renal cancer	Kidney	Kim et al. 2015b
Thyroid cancer	Thyroid	Mineva et al. 2005

separate assay, the ACD-IXI was more potent at suppressing the aggregation of α -synuclein compared to full length wild-type α B-c (WT- α B-c) (Liu et al. 2018), suggesting that the ACD-IXI had enhanced chaperone ability. These results differ from that with other fibrillar aggregating target proteins such as reduced and carboxymethylated (RCM) κ -casein and amyloid- β ($A\beta$)₁₋₄₂, which required a higher or equal concentration of the ACD-IXI to match the level of suppression achieved by WT- α B-c (Hochberg et al. 2014).

Mutations of the isoleucine residues 159 and 161 of the IXI sequence to glycine in α B-c (GXG- α B-c) prevented binding of the IXI sequence to the ACD (Delbecq et al. 2012) (see Table S1 for a list of other non-disease-related mutations of α B-c). GXG- α B-c had enhanced chaperone ability against amorphously aggregating reduced insulin (Pasta et al. 2004) and α -lactalbumin (Pasta et al. 2004). Previously, we defined the disordered molten globule-like intermediate of α -lactalbumin as the species which interacts with α B-c (Lindner et al. 1997; Carver et al. 2002). Interestingly, GXG- α B-c possessed a different mechanism to inhibit α -lactalbumin aggregation than WT- α B-c. α -Lactalbumin was allowed to aggregate until the end of its lag phase, at which point α -lactalbumin nuclei, pre-formed aggregates and some monomers are present, but the protein had not yet commenced large-scale aggregation. Upon

addition of GXG- α B-c at the end of the α -lactalbumin lag phase, GXG- α B-c suppressed its aggregation to a comparable degree compared to when GXG- α B-c was present at the start of the lag phase, i.e. when the reducing agent was added and α -lactalbumin is in its native globular monomeric state. By contrast, when WT- α B-c was co-incubated with α -lactalbumin at the end of its lag phase, it was unable to inhibit its aggregation, despite WT- α B-c preventing aggregation when present initially. The difference in chaperone ability between WT- and GXG- α B-c for aggregating α -lactalbumin suggests the absence of the ACD and IXI interaction allows α B-c to interact with a wider variety of aggregating species, by inter-converting into a more effective chaperone (Delbecq and Klevit 2018). Our previous results are consistent with this, in which the destabilised, disease-causing mutant R120G α B-c interacts very well with reduced α -lactalbumin, but results in complexation and precipitation of both proteins (Treweek et al. 2005). Overall, these observations highlight that access to the chaperone binding site(s) of α B-c is crucial to regulate controlled α B-c chaperone action (Treweek et al. 2005).

It is important to note that the chaperone effectiveness of α B-c (and all sHsps) is dependent upon many factors such as the rate of aggregation of the target protein, the nature of the aggregation (amorphous or fibrillar) and the

Fig. 2 ACD structures of various types of α B-c. Top. Amino acid sequence from residues 70 to 150 of WT α B-c ACD, with the β -stands numbered. **a** WT-ACD dimer solved by solution state NMR (PDB:2N0K). The positions of mutations are indicated by the green (H104), blue (R120) and purple (S135) spheres. **b** X-ray crystal structure of the WT-ACD monomer with a peptide from the C-terminal region interacting with the ACD (PDB: 4M5S). The C-terminal peptide is

coloured cyan, with the IXI motif (residues 159–161) in black. The extended yellow segment represents the C-terminal region. **c** R120G-ACD dimer solved by X-ray diffraction (PDB:2Y1Z). **d** X-ray crystal structure of the E117C-ACD dimer (PDB: 4M5T). The inter-subunit disulphide bond between E117C, i.e. across the β 6+7 dimer interface is indicated by the yellow spheres. Extended yellow segments represent the C-terminal region

type of stress involved (for example in amorphous aggregation whether it is elevated temperature, reduction etc.). For further discussion regarding these factors, see Cox et al. (2016), Carver et al. (2002) and Kulig and Ecroyd (2012). Thus, comparisons between the chaperone ability of α B-c (and other sHsps) is difficult, and should be undertaken with standardised in vitro conditions, i.e. the same target protein(s), type of stress and under physiological conditions, as has been done for eight of the human sHsps with well-characterised amorphously aggregating target proteins (Mymrikov et al. 2017). Most likely in solution, the ACD of α B-c with its IXI sequence bound and unbound are in equilibrium, which together with the other properties of α B-c, such as its dynamism and subunit exchange, regulate access to the chaperone binding site(s) of α B-c and hence its promiscuous chaperone effectiveness. Finally, although there are most likely various chaperone interacting sites on α B-c (which would go some way to explain its promiscuous chaperone ability), there is some

evidence that α B-c interacts with amorphously aggregating target proteins via its N-terminal region and amyloid fibril-forming proteins via its ACD (Mainz et al. 2015).

Alpha-crystallin domain and IXI interaction: effect on the structure of α B-crystallin

How does the interaction between the ACD and the IXI affect the structure of α B-c? Dynamic light scattering (DLS) and glycerol density gradient centrifugation showed that GXG- α B-c had a slightly larger hydrodynamic radius, polydispersity and molecular weight compared to WT- α B-c (Pasta et al. 2004). Jehle et al. studied the structure of α B-c at pH 6.5, a pH reflective of cellular stress such as hypoxia, ischemic stroke and the normal environment of the eye lens (Jehle et al. 2010). From small-angle X-ray scattering (SAXS) experiments, WT- α B-c is 24 to 30% larger in size at a pH of 6.5 compared to physiological pH (7.5), with radius of gyration (R_g) values of 6.7 nm and 5.4 nm, and $D_{\max}$ values of 30 and

19 nm, respectively. Additionally, NMR studies showed chemical shift perturbations of the isoleucine and threonine residues in the β 8 strand in the ACD and the C-terminal region of α B-c in comparing the two α B-c species (Jehle et al. 2010). Taken together, the authors attributed this to release of the IXI motif from the ACD. Hence, the ACD to IXI interaction may be a regulatory mechanism for chaperone action, in which ACD+IXI is capable of suppressing protein aggregation at a basal (constitutive) level, but release of the IXI motif enhances α B-c chaperone ability enabling interaction with, and binding to, unfolding target proteins under conditions of cellular stress.

Alpha-crystallin domain and IXI interaction: initiator of subunit exchange

The IXI sequence binding to the ACD also regulates subunit exchange within and between the α B-c oligomers. Subunit exchange of sHsps was first observed by native PAGE; upon mixing α A-c and α B-c in a 1:1 ratio at 37 °C, an additional band appeared which had an isoelectric point exactly between the two subunits (van den Oetelaar et al. 1990). Using mutants which block the ACD and IXI interaction (S135Q), and one that prevents subunit exchange at the dimer interface (E117C) by disulphide cross-linking between subunits (Hochberg et al. 2014) (Fig. 2d), Dulbecq et al. (2012) showed that subunit exchange cannot occur unless the IXI sequence of one α B-c population interacts with the ACD of the second population (Delbecq et al. 2015). Hence, the ACD and IXI interaction is involved in regulating subunit exchange and the recruiting of subunits into the α B-c oligomer.

Subunit exchange and the structure of α B-crystallin

Why subunit exchange occurs between α B-c oligomers remains contentious, particularly in relation to its role in chaperone action. Inoue et al. (2016) used small-angle neutron scattering (SANS) to investigate the structural changes of α B-c during subunit exchange of a mixture of deuterated and non-deuterated α B-c. Upon mixing, the time-dependent change in absolute scattering intensity ($I(Q)$) of deuterated α B-c was measured. At 37 °C, it took approximately 22 h for the intensity to reach $\sim 0.00 I(Q) \text{ (cm}^{-1}\text{)}$, reflective of complete and equal mixing of the two populations. The exchange rate was greatest from 0 to ~ 4 h, then gradually reached a plateau at 10 h. These data are in good agreement with Förster resonance energy transfer (FRET) measurements of subunit exchange of other sHsps (Bova et al. 1997). The overall size of the α B-c oligomer during subunit exchange was also determined. The R_g value remained stable for the first ~ 2 h, but then gradually decreased (Inoue et al. 2016). The error associated with the R_g value increased as time progressed, which was attributed to the dynamic nature of

α B-c. Taken together, it was concluded that subunit exchange promotes structural reorganisation within the homo-oligomers of α B-c.

Subunit exchange and the oligomeric size of α B-c are temperature dependent

The rate of subunit exchange of α B-c (and other sHsps) increases at higher temperature (Inoue et al. 2016; Bova et al. 1997; Ahmad et al. 2008). The size of α B-c as quantified by SAXS (R_g), the hydrodynamic radius as measured by dynamic light scattering (R_h) and the number of monomeric subunits was constant at around 53 Å, 81 Å and 26 subunits at 10, 25 and 37 °C, respectively. At 48 °C, however, these values increased to approximately 61 Å, 84 Å and 40 subunits, respectively (Inoue et al. 2016). The increase in size of α B-c above physiological temperatures may reflect a more active rearrangement of the protein customised for the stress associated with elevated temperature. Reddy et al. (2000) showed that an increase in temperature from 20 to 65 °C decreased the clustered surface hydrophobicity of α B-c, as monitored by the fluorescent dye, bis-ANS, which binds to exposed, clustered hydrophobic protein regions (Reddy et al. 2000). However, when the chaperone ability of α B-c over this temperature range was monitored, its ability to inhibit the amorphous aggregation of reduced insulin and α -lactalbumin remained the same but it was a slightly worse chaperone at higher temperatures against temperature-induced aggregation of target proteins (Reddy et al. 2000). α A-c behaved in an opposite manner to α B-c in these chaperone assays. Similar to WT- α B-c, the phosphorylation-mimicking mutant of α B-c, in which negatively charged aspartic acid residues replaced the *in vivo* phosphorylation sites at Ser19, 45 and 59 (i.e. 3D- α B-c) (Ahmad et al. 2008), completely inhibited the aggregation of reduced insulin over the temperature range from 20 to 45 °C. Similar to WT- α B-c, 3D- α B-c better suppressed the aggregation of reduced insulin as the temperature increased (i.e. $<40\%$ protection at ~ 20 °C compared to $\sim 100\%$ protection at 45 °C) (Ahmad et al. 2008). Hence, 3D- α B-c behaved better than WT- α B-c as a chaperone.

Overall, assessing the effect of temperature on α B-c chaperone effectiveness is not easy because of the variables described above, i.e. factors that vary with temperature such as the rate and extent of target protein aggregation along with structural changes in both α B-c and the native and intermediately folded forms of the target proteins.

Next, the relationship is explored between subunit exchange and the chaperone function of α B-c, excluding temperature as a contributing factor. The relationship is ambiguous. In disagreement with Ahmad et al. (2008), a triple serine to glutamate mutant (3E- α B-c) mimicking *in vivo* phosphorylation at the three N-terminal sites mentioned above had a higher rate of subunit exchange compared to WT- α B-c

(Pescehek et al. 2013). 3E- α B-c and WT- α B-c suppressed and enhanced the aggregation of p53, respectively. However, both proteins were equally effective at suppressing the aggregation of malate dehydrogenase (Pescehek et al. 2013). Mass spectrometry studies (Ecroyd et al. 2007) showed that the 3D mutant of α B-c has a bias towards even numbered oligomers in its distribution between 24 and 32 subunits, an observation that may be directly relevant to the difference in the chaperone ability of 3D- and WT- α B-c. Bova et al. (1997) studied the subunit exchange of α A-c using FRET and found that the binding of amorously aggregating target proteins slowed down subunit exchange significantly (Bova et al. 1997). In other words, subunit exchange is reduced upon binding to the target protein. However, whether subunit exchange is affected differently in the presence of an amorphous or fibrillar aggregating unfolding target protein remains unclear.

Cross-linking of α -crystallin immobilises subunit exchange. Dimethylsuberimidate cross-linked α -crystallin suppressed the aggregation of heat-denatured β L-crystallin, but required a two- to five-fold higher concentration than its non-cross-linked counterpart (Sharma and Ortwerth 1995). However, when α -crystallin was cross-linked with dimethyl 3,3'-dithiobispropionimidate, it was ineffective as a chaperone (Sharma and Ortwerth 1995). Glutaraldehyde cross-linked α -crystallin had enhanced chaperone activity (Augusteyn 2004). Cross-linking specifically at the dimer interface by introducing a cysteine at position 117 locks the ACD into a dimer and prevents subunit exchange (Hochberg et al. 2014) (Fig. 2d). After mixing ^{13}C labelled and unlabelled E117C-ACD, nanoelectrospray mass spectrometry (MS) revealed no subunit exchange between the two species under oxidising conditions. WT-ACD suppressed amyloid fibrillar aggregation of $\text{A}\beta_{1-42}$, but dimeric E117C-ACD was more potent. Moreover, the E117C-ACD was better than WT-ACD at rescuing cells from $\text{A}\beta_{1-42}$ induced toxicity (Hochberg et al. 2014). Furthermore, immobilisation of α B-c onto a solid-phase support, with concomitant prevention of subunit exchange, led to greatly enhanced α B-c chaperone activity against a range of target proteins that aggregated amorously and in an amyloid fibrillar manner (Garvey et al. 2011).

In the main, these results imply that inhibition of α B-c subunit exchange enhances its chaperone action. Similar to the effect of temperature, it is difficult to assess how subunit exchange affects the chaperone ability of α B-c because of the variables described above. However, it may be an important aspect, along with other structural and functional characteristics of α B-c, to exert an orchestrated response to prevent protein aggregation.

Subunit exchange and the active monomer

Many studies have concluded or presumed that the dimer is the fundamental building block of α B-c (and all sHsps), and

hence is the minimal species required for its chaperone action. Evidence to support this includes the two crystal structures of the α B-c ACD (amino acids 67–157 (Bagnris et al. 2009) (Fig. 2i) and 68–162 (Laganowsky et al. 2010) (Fig. 2h), respectively) which spontaneously form a dimer. The dimer is stabilised by electrostatic and other non-covalent (hydrogen bonds and hydrophobic) interactions by an anti-parallel arrangement of the β 6+7 strand from each monomer at the dimer interface, which are present in different registers (Laganowsky et al. 2010). Furthermore, Hsp26, a sHsp from baker's yeast, dissociates into a dimer from a 24-mer under heat shock conditions (Haslbeck et al. 1999; Benesch et al. 2010). Overall, these observations support the dimer as the fundamental and functional building block of the α B-c oligomer. However, there is literature consistent with the monomer being the oligomer building block and the most active species against unfolding proteins during chaperone action. Aquilina et al. (2003) used tandem MS/MS coupled with collision-induced dissociation to selectively remove subunits held together by non-covalent interactions within the α B-c oligomer. They found the presence of both even and odd number of subunits within the oligomer, suggesting that the monomer rather than the dimer is the building block of the α B-c oligomer (Aquilina et al. 2003). The dimer also dissociates into monomers at low pH upon protonation of a histidine, and monomers are present to a small extent at physiological pH (Rajagopal et al. 2015).

The naturally occurring R120G mutation in α B-c is responsible for cataract and desmin-related myopathy (Fig. 2c). The mutation leads to large-scale oligomerisation of the protein and poor chaperone effectiveness *in vitro* (Vicart et al. 1998; Delbecq and Klevit 2018; Hussein et al. 2015). Treweek et al. (2005) showed that this mutant was inherently unstable, since it is aggregated, unfolded and degraded with time (Treweek et al. 2005). Moreover, its poor chaperone ability was explained by rapid interaction with the target protein (reduced α -lactalbumin), leading to precipitation of the complex (Treweek et al. 2005). In α B-c, the R120G mutation is located on the β 6+7 strand of the ACD, which forms the dimer interface (Fig. 2a and c). At pH 6.0, size exclusion chromatography revealed a smaller species of WT-ACD compared to that at pH 7.2 (Bagnris et al. 2009) which may reflect dissociation of the dimer into monomers, as independently observed in NMR studies (Jehle et al. 2009). However, the R120G-ACD mutant was the same size at both pH values, pointing to its inability to dissociate under acidic conditions which mimic stress conditions. The ineffectiveness of R120G- α B-c as a molecular chaperone could be explained by its inability to dissociate into monomers, and in turn, the importance of the monomeric species to prevent protein aggregation.

In addition, disruption of the dimer interface by altering the charge at the interface by replacing the highly conserved H104

in the loop between strands 5 and 6 with a positively charged lysine (H104K- α B-c) led to dissociation of the α B-c dimer to a monomer (Rajagopal et al. 2015) (Fig. 2a). An α B-c species with substantially enhanced chaperone ability against reduced α -lactalbumin resulted from this mutation. Finally, native MS revealed an increase in the number of α B-c monomers with increased temperature, which was accompanied by a concomitant increase in the rate of subunit exchange. Interestingly, the same oligomeric α B-c structure detected at 25 °C remained intact at 47 °C (Inoue et al. 2016), suggesting that the increase in monomer dissociation and an intact oligomeric structure are key characteristics dictating chaperone function. These conclusions may explain why α B-c has an enhanced ability to prevent protein aggregation at higher temperature (Reddy et al. 2000).

Why are monomers efficient at preventing protein aggregation? They are not required in large amounts at basal levels to maintain proteostasis but under conditions of elevated temperature, an increase in the rate of subunit exchange can release more monomers, allowing for a greater number of molecules to interact with unfolding target proteins. Moreover, monomers as the active species may explain why α B-c can prevent the aggregation of proteins at a very low stoichiometric ratio compared to the target protein. This efficient stoichiometry is appropriate in the cell, as the destabilised target proteins are often present at low levels and aggregate slowly (for example within the eye lens). Hence, a high concentration of the active monomeric species is not required to prevent the unfolding and aggregation of the target protein. However, as the presence of the monomer would increase the hydrophobicity of α B-c and decrease its solubility, a balance between the monomer and oligomer is crucial for optimal chaperone action. Consistent with these arguments, recently Alderson et al. (2019) showed via sophisticated NMR techniques that the transient Hsp27 (HspB1) monomer was highly chaperone-active, but was unstable and aggregated readily, highlighting the importance of the monomer-dimer-oligomer equilibrium for the overall structure, stability and chaperone function of sHsps such as α B-c. Hsp27 is a closely related sHsp to α B-c with both proteins being present intracellularly and co-associating (more below).

Subunit exchange and hetero-oligomerisation

In vitro and in vivo subunit exchange leads to hetero-oligomerisation of sHsps with other sHsps. In the eye lens, hetero-oligomerisation between α B-c and α A-c via subunit exchange has been well recognised for many years, the reasons for which are probably twofold: (i) to stabilise one or both proteins and (ii) to contribute to the correct refractive index of the lens and its transparency. Outside the lens,

hetero-oligomerisation occurs between α B-c and Hsp27 (Katos et al. 1992) and Hsp20 (Kato et al. 1994).

Brady et al. (2001) highlighted the importance of the α A-c and α B-c interaction. Using knock-out mice of α A-c (α A-c $-/-$), they observed that α A-c $-/-$ formed large inclusions in the lens predominantly containing α B-c. The α A-c $-/-$ mice developed mild cataract as early as 7 weeks of age with α B-c in the insoluble fraction of their lenses, and no α B-c in the soluble fraction. By contrast, age-matched wild-type mice (α A-c $+/+$) had α B-c in the soluble fraction of their lenses, with no α B-c detected in the insoluble fraction (Brady et al. 1997). Thus, these results suggest that the interaction between α A-c and α B-c is crucial to maintain lens transparency, and in turn, to prevent aggregation of crystallin proteins, mainly in this case α B-c, implying that intra-ocularly, α A-c acts as a chaperone for α B-c to maintain the latter's solubility and stability. A corollary of this is that α A-c is intrinsically a more stable protein than α B-c, as has been confirmed in a variety of in vitro studies comparing the stabilities of the two proteins (Reddy et al. 2000). In the reverse set of in vivo studies, α B-c knock-out mice developed myopathy but not cataract, a result that should be interpreted with caution, as HspB2 was also knocked out with α B-c, although HspB2 does not reside in the lens (Brady et al. 2001). However, knock-in mice harbouring a homozygous R120G mutation in α B-c (R120G- α B-c $+/+$) developed lens opacity within 3 to 8 weeks, compared to more than 40 weeks for WT- α B-c mice (Andley et al. 2011). Biochemical characterisation of the lens proteins from these mice revealed a larger oligomeric mass of R120G- α B-c compared to WT- α B-c, with the R120G- α B-c becoming even larger and scattering more light as the mice aged. From these experiments, it is concluded that both α A-c and α B-c and their interaction are crucial for lens transparency and also to maintain the other lens crystallins (β and γ) as functional proteins by keeping them in solution.

Functionally, the hetero-oligomer is a better chaperone at suppressing the aggregation of β _L-crystallin compared to α A-c or α B-c (Srinivas et al. 2008). The suppression of β _L-crystallin is optimal when α A-c and α B-c oligomerise at a 3:1 ratio, as found in the young eye lens. The α A-c/ α B-c hetero-oligomers are better chaperones for native substrates such as γ -crystallin at temperatures exceeding 50 °C. However, the hetero-oligomers have a decreased chaperone ability compared to the homo-oligomer of α B-c for non-native target proteins like citrate synthase at temperatures below 45 °C (Srinivas et al. 2008). Hence, the synergistic association of α A-c and α B-c and their respective homo-oligomerisation provide chaperone activity potentially for a wide range of proteins in and outside the lens.

Fundamentally, it would seem that α B-c is not a very stable protein. For example, it is less thermally stable and more prone to aggregation than α A-c (Fu and Liang 2003). Hence, α A-c may provide thermal stability to α B-c through chaperone interaction and co-oligomerisation/complexation; a similar phenomenon occurs with Hsp27 and α B-c with elevated temperature (Fu and Liang 2003). Srinivas et al. (2008) found that the 3:1 complex of α A-c with α B-c did not precipitate above 100 °C, but α B-c on its own precipitated at around 70 °C. Complexation rescued α B-c from temperature-induced unfolding and the complex was more resistant to denaturation by guanidine hydrochloride. Finally, the 3:1 complex and α A-c were less susceptible to non-enzymatic glycation by methyl glyoxal, a post-translational modification in aged lenses, compared to α B-c (Srinivas et al. 2010). Taken together, hetero-oligomerisation of α A-c and α B-c enhances α B-c stability and facilitates chaperone activity against a wide range of destabilised target proteins. It is concluded that the α A-c and α B-c complex is a key player in the maintenance of proteostasis in the lens, an organ in which there is no protein turnover in all but the peripheral epithelial cell layer. A similar situation may prevail outside the lens where the hetero-oligomerisation of α B-c with Hsp27 stabilises the former.

sHsps prevent other sHsps from misfolding and aggregating via subunit exchange

Subunit exchange between different sHsps can regulate the size of sHsp oligomers. sHsps are robust proteins, but are susceptible to misfolding and aggregation under certain conditions. Hussein et al. (2015) expressed three α B-c mutants underlying human disease (R120G, 450 Δ A and 464 Δ CT), which each formed large, insoluble aggregates (see Table S2 for a full list of disease related-mutants of α B-c). Co-expression with α A-c and Hsp27 led to complexation with the α B-c mutants, significantly reducing their aggregation and consistent with α A-c and Hsp27 acting in a chaperone role to stabilise these mutant α B-c proteins (Hussein et al. 2015). Similarly, Chavez Zobel et al. (2003) observed that R120G α B-c formed small aggregates in the cell's cytoplasm, then formed large perinuclear aggregates after a few days. Co-expression of WT- α B-c, HspB8 and Hsp27 with R120G α B-c prevented the formation of large aggregates, although the smaller aggregates were still present. Co-immunoprecipitation confirmed the complexation and subunit exchange of WT α B-c and Hsp27 with R120G α B-c (Chavez Zobel et al. 2003). Additionally, the rate of subunit exchange with various α A-c mutants and WT α B-c is slower compared to WT α A-c and α B-c (Raju et al. 2012), implying that the subunit exchange is favoured between functional/non-mutant sHsps compared to their mutant/disease counterparts.

Hetero-oligomers also form between α B-c and severely C-terminally truncated α A-c such as α A-c₁₋₆₂ (the N-terminal domain of α A-c), a modification that occurs in aged and/or diabetic lenses (Thampi et al. 2002). Proteolytic and ubiquitinating enzymes sourced from human lymphatic endothelial cell lysate, reflective of the ubiquitin-proteasome pathway (UPP), were co-incubated with hetero-oligomers formed between α A-c₁₋₆₂ and full length α A-c or α B-c. Hetero-oligomerisation of WT- α A-c or WT- α B-c with α A-c₁₋₆₂ made the latter more resistant to degradation compared to α A-c₁₋₆₂ alone. However, hetero-oligomerisation with α A-c₁₋₆₂ also changed the resistance to degradation by the UPP of WT- α A-c and WT- α B-c. WT- α A-c became more susceptible to degradation, whereas WT- α B-c became more resistant (Wu et al. 2012). Similarly, the interaction of these α A-c mutants with Hsp27 stimulated the UPP, a response mediated by Hsp27 (Zhang et al. 2010). Considering that the cell rapidly degraded α A-c₁₋₆₂, its accumulation may cause undesirable effects on the cell. Thus, the hetero-oligomerisation of α A-c₁₋₆₂ with WT α A-c or α B-c may prevent α A-c₁₋₆₂ from degradation but have the adverse consequences of promoting cellular toxicity and thereby contribute to a decrease in proteostasis.

How does α B-crystallin recognise and interact with unfolding target proteins?

The ubiquitous expression of α B-c and its association with more than 19 human diseases (Table 2) highlight the importance of α B-c intracellularly. However, how α B-c exerts its chaperone functionality of protein stabilisation, in particular its recognition of unfolding proteins to prevent them from aggregating, is not understood in detail. In this section, the means by which α B-c interacts with fibrillar and amorphously aggregating proteins are discussed.

α B-crystallin and amyloid fibrillar aggregation

The initial discovery and subsequent investigations of α B-c (and sHsps) in vitro chaperone ability concentrated on their ability to prevent the aggregation of amorphously aggregating target proteins under various stress conditions. The first determination that sHsps could prevent the aggregation of amyloid fibril-forming proteins under physiological conditions was made with the observation that lens α -crystallin potently prevented the aggregation of apolipoprotein C-II, an unstructured extracellular protein that forms well-defined amyloid fibrils, at neutral pH (Hatters et al. 2001). Subsequently, it has been determined that α B-c can suppress the aggregation under physiological conditions of a plethora of fibril-forming proteins such as α -synuclein, amyloid beta (A β),

transferrin, κ -casein, ataxin-3 and β 2-microglobulin (Rekas et al. 2004, 2007; Dehle et al. 2010; Robertson et al. 2010). Co-incubation of α B-c upon the commencement of fibrillar aggregation led to elongation of the lag phase, the period during which oligomeric, prefibrillar nuclei are formed, and resulted in a decrease in fibril elongation (Kulig and Ecroyd 2012; Rekas et al. 2004; Esposito et al. 2013). Hence, it has been proposed that α B-c interacts with fibril-forming proteins early along their aggregation pathway, for example with monomeric and oligomeric species to prevent their aggregation. One example is κ -casein. κ -Casein forms fibrils via a novel mechanism that involves dissociation from an oligomer and lacks a significant lag phase (Ecroyd et al. 2008, 2010). The resultant monomeric form is highly amyloidogenic. When compared with the chaperone activity of α B-c at equal stoichiometric ratios against the aggregation of $A\beta_{1-40}$, which undergoes standard nucleation-dependent polymerisation with a significant lag phase, α B-c was 33% less effective at inhibiting the aggregation of κ -casein (Dehle et al. 2010). Thus, α B-c interacts well with long-lived, destabilised monomeric forms of target proteins to suppress their fibrillar aggregation (Dehle et al. 2010), i.e. ones that are slowly aggregating.

β 2-Microglobulin forms amyloid fibrils and its aggregation and deposition in tissues is associated with dialysis-related amyloidosis. Esposito et al. (2013) observed using MS the lack of higher order species (i.e. dimers, trimers, tetramers) of β 2-microglobulin and the presence of β 2-microglobulin monomers after 20 days of incubation with α B-c at a 60:1 molar ratio of β 2-microglobulin: α B-c. It was concluded that in vitro, α B-c interacts with and dissociates β 2-microglobulin oligomers into monomers (Esposito et al. 2013). The latter ability of α B-c demonstrates an important aspect of α B-c as a chaperone, i.e. it can prevent the formation of oligomeric β 2-microglobulin species, which are generally regarded as the most cytotoxic entities (Winner et al. 2011). In agreement with these observations, Rekas et al. (2007) used dual polarisation interferometry to demonstrate that α B-c interacts with monomeric α -synuclein, i.e. very early along its fibril-forming pathway, to prevent the formation of oligomeric nuclei or protofibrils (Rekas et al. 2007). Similarly, α B-c inhibits the amyloid fibril formation of apolipoprotein C-II by dissociating mature amyloid fibrils into monomers (Binger et al. 2013). Moreover, α B-c caused large-scale association of apolipoprotein C-II fibrils, in addition to transient interactions with the monomeric forms (Binger et al. 2013). In addition, Tkachenko et al. (2018) observed that α B-c redirects pre-formed nuclei of reduced α -lactalbumin into its monomeric form (Tkachenko et al. 2018). WT- α B-c binds and dissociates nuclei of α -lactalbumin, while the isolated central domain (WT-ACD) bound free and monomeric α -lactalbumin. Amyloid fibril aggregation can occur through two steps: primary nucleation, an association of monomers only, in which

the nucleus elongates faster in comparison to its dissociation to a monomer, and secondary nucleation, in which a pre-existing aggregate promotes monomers to form nuclei and/or bind to the fibril (Linse 2017). We have shown that α B-c inhibits amyloid fibril formation by preventing secondary nucleation events, for example by α B-c binding to and stabilising fibrils (Binger et al. 2013; Waudby et al. 2010; Shamma et al. 2011). Hence, α B-c possesses multiple means to suppress amyloid fibrillar aggregation, deeming it a highly effective chaperone under conditions of cellular stress that lead to protein unfolding.

As described above, WT-ACD has chaperone activity and preferentially interacts with fibrillar aggregating proteins compared to those which aggregate amorously (Mainz et al. 2015). It is generally believed that α B-c interacts with partially unfolded target proteins via predominantly hydrophobic interactions. NMR experiments revealed transient interactions between the hydrophobic β -sheet region in $A\beta_{1-40}$ with the hydrophobic groove of the β 4- β 8 strands of the ACD in α B-c (Mainz et al. 2015). Ghosh et al. (2008) identified peptide regions of α B-c which were the most effective at inhibiting amyloid fibril formation. Out of six sequences, the following two (residues 73–85 and 101–110) were located in the ACD, and were the most chaperone-active regions to prevent the aggregation of α -synuclein, β 2-microglobulin and transthyretin (Ghosh et al. 2008). For α -synuclein and the isolated K19 repeat region of Tau protein, WT-ACD was better at preventing their aggregation compared to full length WT- α B-c (Liu et al. 2018). Overall for fibril-forming target proteins, WT-ACD has comparable or enhanced chaperone ability to WT- α B-c, which suggests preferential interaction of the ACD with fibrillar aggregating proteins (Mainz et al. 2015).

The ability of α B-c to mask hydrophobic residues is important to mitigate the cytotoxicity of these species. For example, although the overexpression of α B-c did not reduce the number of α -synuclein inclusions in a brain neuroglioma cell line, it substantially decreased α -synuclein-induced cytotoxicity via reducing its exposed hydrophobicity (Outeiro et al. 2006). This study also indicated the α B-c can bind to mature amyloid fibrils. Specifically, Waudby et al. (2010) showed through in vitro immunogold labelling and electron microscopy that α B-c uniformly binds along the length of mature α -synuclein fibrils, which stabilised the fibrils and prevented their elongation (Waudby et al. 2010). They proposed that binding of α B-c to the hydrophobic regions of α -synuclein fibrils reduces their toxicity, especially by blocking sites for fragmentation and secondary nucleation (Waudby et al. 2010). Hence, hydrophobic interactions involving α B-c play a key role to mitigate protein aggregation, and α B-c interacts with fibrillar aggregating species at all stages of its aggregation pathway, deeming it an efficient molecular chaperone to inhibit amyloid aggregation (Trewick et al. 2015) (Fig. 3).

Fig. 3 Mechanisms of α B-c to prevent amyloid fibril formation. α B-c interacts with amyloid fibril-forming proteins at all stages of their aggregation. (1) α B-c interacts with monomers to prevent their aggregation. α B-c also dissociates oligomers and pre-formed nuclei into monomers (Rekas et al. 2007; Esposito et al. 2013; Binger et al. 2013; Tkachenko et al. 2018). (2) α B-c prevents secondary nucleation of amyloidogenic proteins (Binger et al. 2013; Waudby et al. 2010; Shammas et al. 2011).

(3) α B-c prevents fibril fragmentation by (4) binding along the length of mature amyloid fibrils (Waudby et al. 2010) and thereby stabilising them. All steps prevent the formation of amyloid fibrils. Therefore, α B-c is multifaceted in its ability to prevent amyloid fibril formation. The X-ray crystal structure of α B-c in the schematic is PDB:3L1G (Laganowky et al. 2010). The amyloid fibril in the schematic is a cryo-EM structure of a rod polymorph of α -synuclein (PDB: 6CU7, Li et al. 2018)

α B-c prevents the formation of amyloid fibrils through transient interactions. Liu et al. (2018) determined a dissociation constant (K_d) of $275 \pm 105 \mu\text{M}$ with the $\beta 4/\beta 8$ strands of α B-c interacting with α -synuclein, consistent with weak binding affinity between the two proteins (Liu et al. 2018). Similarly, amyloid β fibrils had a K_d of $2.1 \pm 0.4 \mu\text{M}$ when interacting with α B-c (Shammas et al. 2011). Transient interactions between α B-c and RCM- α -lactalbumin (Kulig and Ecroyd 2012), κ -casein (Rekas et al. 2007) and $\beta 2$ -microglobulin (Esposito et al. 2013) have also been reported. These weak and transient interactions ensure that large-scale depletion of available α B-c does not occur intracellularly, for example via high molecular weight (HMW) complex formation with target proteins. As a result, viable levels of α B-c are maintained to act as a molecular chaperone to prevent protein aggregation, which is particularly important in the lens where minimal protein turnover occurs.

α B-crystallin and amorphous aggregation

In a different manner to fibrillar aggregation, many studies have shown that α B-c suppresses the amorphous protein aggregation of a wide range of target proteins by forming a HMW complex between both proteins, particularly under conditions of significant cellular stress. For example, when α B-c was co-incubated with α -lactalbumin under reducing conditions which favour amorphous aggregation, size exclusion chromatography revealed the formation of a non-covalent, HMW complex that was held together by hydrophobic interactions in the main (Kulig and Ecroyd 2012). Mainz et al. (2015) observed an increase in the size of the complex

between α B-c and amorphously aggregating lysozyme upon increasing the concentration of lysozyme (Mainz et al. 2015). Additionally, co-incubation of α B-c and lysozyme resulted in the presence of new cross-peaks in the solid state NMR spectrum of α B-c which were attributed to complex formation between the N-terminal region of α B-c and lysozyme, implying that this region of α B-c is involved in binding to amorphously aggregating target proteins. In contrast, the ACD of α B-c inhibited the amorphous aggregation of α -lactalbumin at 37°C , even at a 1:80 α B-c-ACD: reduced α -lactalbumin molar ratio (Hochberg et al. 2014).

α B-crystallin and aggregation of lens proteins

Ninety percent of the water-soluble proteins expressed in the human eye lens are crystallins (Andley 2007), consisting of α (47%), β (44%) and γ (9%) (Delaye and Tardieu 1983). All three crystallins can form amyloid fibrils or amorphous aggregates with the appropriate combination of temperature, denaturant or acidic pH (Meehan et al. 2004; Wu et al. 2014). Indeed, mutations (Table S2), age-related truncations and post-translational modifications in crystallins lead to their destabilisation, aggregation, precipitation and the development of opacity and eventually cataract. A variety of single-point mutations in α B-c cause congenital cataract due to amyloid fibril formation of the mutated crystallin (Table S2). For example, the G18V mutation in γ S-crystallin (G18V- γ S-c) is responsible for hereditary, childhood-onset cataract (Sun et al. 2005) and is thermodynamically less stable than WT- γ S-c (Ma et al. 2009). The in vitro ability of α B-c and its lens partner α A-c to suppress the aggregation of β - and γ -

crystallins has been extensively explored. When co-incubated at elevated temperature with α B-c, α B-c had a stronger and preferential interaction with the G18V- γ S-c, whilst forming substantially larger complexes than with WT- γ S-c. ^{15}N - ^1H HSQC solution NMR spectra of mixtures of the two crystallins also revealed a broadening of $\sim 50\%$ of the cross-peaks of G18V- γ S-c despite the overall fold of G18V- γ S-c and WT- γ S-c being very similar (Kingsley et al. 2013), implying non-specific interactions of α B-c with γ S-c, consistent with other studies on the interaction of α B-c with destabilised target proteins that aggregate to form amyloid fibrils (Rekas et al. 2007; Robertson et al. 2010; Esposito et al. 2013). Thus, α B-c preferentially interacts with destabilised proteins over those in their native state, demonstrating its ability to maintain the folded proteome and contribute to proteostasis.

Finally, the chaperone activity of α B-c is highly robust. Bovine α -crystallin enhances its chaperone ability against various amorphous and fibrillar target proteins when it is covalently immobilised onto a fluorinated ethylene propylene copolymer in aqueous solution (Garvey et al. 2011). Moreover, α B-c forms amyloid fibrils upon prolonged exposure to elevated temperature, and at pH 2 (Meehan et al. 2004). Both types of α B-c amyloid fibrils prevented amorphous and fibrillar protein aggregation, demonstrating that α B-c is still functional under a variety of conditions (Garvey et al. 2017).

In the aged lens, taking into account the extensive post-translational modification of α B-c and the other crystallins, the absence of protein clearance pathways, a significant reduction in glutathione levels and no protein turnover, HMW complex formation may decrease the limited amount of intact α B-c and α A-c in the lens, increasing the propensity of crystallins to aggregate and cataract to form (Kingsley et al. 2013). Moreover, although the complex of the amorphously misfolded crystallin and α B-c remains largely soluble, increasing the concentration of the target crystallin protein can make the complex insoluble (Carver et al. 2018; Mainz et al. 2015). Extra-lenticularly, although α B-c is an excellent chaperone to halt aggregation, it must be accompanied by a functional proteostatic system to prevent unfavourable consequences from the accumulation of misfolded and aggregated proteins in the cell.

Future directions and conclusions

Future directions

Despite recent advances providing great insight into the structure of α B-c and how it exerts its chaperone function in vitro, a major question is how relevant these conclusions are to the situation in vivo. The following experiments may provide answers to this question. Firstly, recombinant protein purification using standard expression in *E. coli* does not give rise to

post-translational modifications. As briefly discussed above, post-translational modifications, for example phosphorylation, affect the structure and chaperone ability of sHsps. A plethora of modifications such as deamidation (Hains and Truscott 2010), glycation (Abraham et al. 2008), oxidation (Shum et al. 2005) and truncation (Thampi et al. 2002) of α B-c have been reported, particularly with lens-derived α B-c. Hains and Truscott determined the extent of deamidation of asparagine and glutamine residues of crystallins in normal, cataract and foetal human lens (Hains and Truscott 2010). The location and extent of deamidation differed depending on the age and type of human lens and the particular crystallin protein (Hains and Truscott 2010). Although this study shed light on a major post-translational modification, a comprehensive study to map all post-translational crystallin modifications and their respective extent has not yet been completed. Current technologies, including high resolution mass spectrometry coupled with powerful deconvolution algorithms which allow extended modification searching, could assist with this investigation. Identifying these post-translational modifications and their subsequent effect on the structure and chaperone action of α B-c (and other sHsps) will lead to a deeper understanding of how α B-c (i) exerts its chaperone action, (ii) whether in vitro studies are relevant to in vivo conditions and (iii) how lens α B-c retains its chaperone function for many years. They may reveal that different post-translational modifications occur at various stages of life and thereby provide targets for anti-cataract therapeutics.

Secondly, trapping various conformations of α B-c in vivo, especially to determine the chaperone-active conformation of α B-c, is a challenge due to its ever-changing and dynamic nature, as well as maintaining its structure upon lysis of the cell. However, understanding which conformation is the most populated in disease and non-disease states would provide a better understanding on how α B-c exerts its chaperone ability. Current techniques such as single-molecule fluorescence, the ability to capture transient interactions using engineered fusion proteins and cryo-EM provide a promising approach to trap the various species of α B-c in the cell.

Thirdly, how one molecule of α B-c responds when faced with a combination of unfolding fibrillar and amorphously aggregating protein(s) is of interest, as in vitro studies almost invariably have investigated the interaction of α B-c with a single target protein that is aggregating either amorphously or in a fibrillar manner. Intracellularly, it is likely that α B-c interacts with more than one protein on a very short timeframe. Despite the efforts of inert polymeric crowding agents to mimic macromolecular crowding, the conclusions drawn are limited, as the cellular environment is highly dynamic and complex. Similarly, how α B-c recognises destabilised, unfolding proteins, how it can differentiate between different aggregating species (monomers, transiently misfolding species, oligomers etc.) and how the structure

and dynamics of α B-c sample target protein conformations are not fully understood.

Finally, intracellularly, there is limited information on how α B-c interacts with ATP-dependent proteins such as Hsp70 to which α B-c HMW complexes off-load partially folded, intermediate states of target proteins for refolding, despite recent light having been shed on this interaction between Hsp27 and fibrillar aggregating target proteins (Freilich et al. 2018). To what extent a decline in proteostasis and the accumulation of misfolded proteins affect this transfer, and whether this process is inherently error-prone, would provide insight into the cooperative and synergistic interactions of molecular chaperones.

Conclusions

α B-c is arguably the most important member of the sHsp family. It has a diversity of functions, based around its chaperone ability to stabilise other proteins against unfolding, misfolding and possible aggregation. α B-c, along with other sHsps, forms the first line of defence against cellular stress. The dysregulation of α B-c function underlies a plethora of human diseases. In this paper, we have reviewed how the structure of α B-c relates to its chaperone action. Specifically, we explored the interactions between the ACD and the IXI, which may act as an on-off switch during basal proteome regulation and stress conditions. Secondly, we discussed how subunit exchange affects the chaperone ability and structure of α B-c. Thirdly, we argued that monomeric α B-c is the most active species to prevent target protein aggregation, which in doing so, requires a trade-off between enhanced chaperone activity and self-association due to the greater hydrophobicity of this form of the protein. Fourthly, we examined the role of hetero-oligomerisation between α B-c and other sHsps, and how this affects its chaperone ability and may act as a proteostatic modulator against aggregation-prone sHsps. Finally, we discussed how α B-c interacts with target proteins and prevents their amorphous or fibrillar aggregation.

A detailed understanding of the mechanism of α B-c chaperone action may provide avenues towards the treatment and prevention of protein aggregation diseases, which are intimately linked to proteostatic dysregulation.

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