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Review

Phosphorylation of αB-crystallin: Role in stress, aging and patho-physiological conditions☆

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ABSTRACT

Background: αB-crystallin, once thought to be a lenticular protein, is ubiquitous and has critical roles in several cellular processes that are modulated by phosphorylation. Serine residues 19, 45 and 59 of αB-crystallin undergo phosphorylation. Phosphorylation of S45 is mediated by p44/42 MAP kinase, whereas S59 phosphorylation is mediated by MAPKAP kinase-2. Pathway involved in S19 phosphorylation is not known.

Scope of review: The review highlights the role of phosphorylation in (i) oligomeric structure, stability and chaperone activity, (ii) cellular processes such as apoptosis, myogenic differentiation, cell cycle regulation and angiogenesis, and (iii) aging, stress, cardiomyopathy-causing αB-crystallin mutants, and in other diseases.

Major conclusions: Depending on the context and extent of phosphorylation, αB-crystallin seems to confer beneficial or deleterious effects. Phosphorylation alters structure, stability, size distribution and dynamics of the oligomeric assembly, thus modulating chaperone activity and various cellular processes. Phosphorylated αB-crystallin has a tendency to partition to the cytoskeleton and hence to the insoluble fraction. Low levels of phosphorylation appear to be protective, while hyperphosphorylation has negative implications. Mutations in αB-crystallin, such as R120G, Q151X and 464delCT, associated with inherited myofibrillar myopathy lead to hyperphosphorylation and intracellular inclusions. An ongoing study in our laboratory with phosphorylation-mimicking mutants indicates that phosphorylation of R120GαB-crystallin increases its propensity to aggregate.

General significance: Phosphorylation of αB-crystallin has dual role that manifests either beneficial or deleterious consequences depending on the extent of phosphorylation and interaction with cytoskeleton. Considering that disease-causing mutants of αB-crystallin are hyperphosphorylated, moderation of phosphorylation may be a useful strategy in disease management. This article is part of a Special Issue entitled Crystallin Biochemistry in Health and Disease.

1. Introduction: αB-crystallin

αB-crystallin, over the last two decades, gained prominence due to its association with multiple cellular functions. In addition to its well studied "chaperone-like" activity in preventing aggregation of several proteins, it has been shown to play a role in a variety of cellular functions such as cell cycle, differentiation, apoptosis, gene expression and associated with several disease conditions. Manifestation of such a multiplicity of functions requires structural and/or functional regulation, induced by post translational events. Several years ago we have shown that a structural perturbation induced at about heat shock temperatures, leads to significant increase its activity. This phenomenon, in subsequent studies, was observed with a few other small heat shock proteins. Phosphorylation, yet another post-translational event, appears to play a significant role in modulating structure and function of αB-crystallin. Charge repulsions, due to phosphorylation of oligomeric proteins, can lead to alterations in quaternary structure and consequently, interacting surfaces and interacting partners. We focus on the phosphorylation of αB-crystallin in relation to its oligomeric structural change, role in cellular functions, aging, stress and diseases.

αB-crystallin, a ubiquitous sHsp [1–3], has a highly conserved stretch that adopts a β-sandwich, immunoglobulin-like fold called the "α-crystallin domain (ACD)", which is a characteristic "hallmark" of the sHsp family [4–6]. The ACD is flanked by the less conserved and flexible N-terminal domain (NTD) and the C-terminal extension (CTE) that is rich in charged residues and includes a semi-conserved "IXI/V" motif [4–6]. αB-crystallin is particularly abundant in the eye lens along with another sHsp, αA-crystallin, and is important for the formation of highly refractive lens and maintenance of its transparency [see reviews [7 & 8]. It is also present at high levels in cardiac and skeletal muscles [3,9–11], where it localizes to the sarcomeric region [11] and protects cardiomyocytes from ischemic stress [12]. Immunostaining and subcellular fractionation

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show redistribution of αB-crystallin from cytosol to intercalated disks and Z lines of the myofibrils upon ischemia [13]. It exhibits ATP-independent molecular chaperone property in preventing aggregation of proteins [14, reviews 15–17]. It exhibits pleotropic roles in several cellular processes such as protein folding, protein degradation, differentiation and apoptosis [see reviews 17–19]. It is also found in extracellular fluids [20,21], possibly through exosome-mediated secretion [22, 23], and exhibits anti-inflammatory property [24–28]. It seems to have several cellular and extracellular target proteins [27, review 29]. The mechanism(s) of the pleotropic functions and promiscuous interactions of sHsps in general, and αB-crystallin in particular, has not yet been completely understood. We earlier proposed "dynamic partitioning" hypothesis for the same [17]. αB-crystallin has three phosphorylation sites (S19, S45 & S59) at its NTD which seem to play a critical role in its functions. As emerging from the following sections, phosphorylation of αB-crystallin has both beneficial and deleterious outcomes. Thus, spatio-temporally controlling phosphoryaltion of αB-crystallin might emerge as a strategy to manage stress and age-related complications.

2. Phosphorylation of αB-crystallin

Earlier studies on phosphorylation of αB-crystallin dealt with mainly rat and bovine lenses. For example, treating rat eye lens with H_2O_2 stimulates the phosphorylation of αB-crystallin [30]. Phosphorylation of αB-crystallin in cultured bovine articular chondrocytes is induced by phorbol 12-myristate 13-acetate, a potent tumor promoter which activates protein kinase C [31]. αB-crystallin phosphorylated at S19 or 21, S45 and S59 residues has been found in bovine eye lens [32–34]. cAMP-dependent phosphorylation of αB-crystallin from bovine lens extract has been reported [35,36]. Eye lens α-crystallin, composed of αA- and αB-crystallin subunits, is believed to be phosphorylated on serine residues by cAMP-dependent and cAMP-independent mechanisms (see review [37]). The cAMP-independent pathway was believed to be an autophosphorylation that has been demonstrated in vitro [37]. Disaggregation of αA-, but not αB-crystallin, into tetramers results in an appreciable increase in autophosphorylation activity [37]. It was suggested that cAMP-dependent phosphorylation occurs preferentially to αB-crystallin than αA-crystallin [37]. αB-crystallin exhibits autokinase activity in vitro [38].

Serine residues at 19, 45, and 59 of αB-crystallin are phosphorylated upon exposure of human glioma (U373 MG) cells to various stimuli such as heat, arsenite, phorbol 12-myristate 13-acetate, okadaic acid, H_2O_2, anisomycin, and high concentrations of NaCl or sorbitol [39]. The study also indicated that cascade of events involving p38 MAP kinase and p44 MAP kinase are responsible for the phosphorylation of αB-crystallin [39]. Unlike in the case of αB-crystallin from bovine lens extracts [35,36], the activators of cAMP-dependent protein kinase such as forskolin, cholera toxin, and dibutyryl cAMP, did not stimulate the phosphorylation of αB-crystallin significantly in U373 MG cells [39].

Phosphorylation at S45 of αB-crystallin is enhanced during mitosis of cells and its presence is also detected in the tissue sections of mouse embryos [40]. Phosphorylation at each site in αB-crystallin is regulated differently/differentially during mitosis [40]. Increased levels of S45 and S19 phosphorylation of αB-crystallin, but decreased levels of S59 phosphorylation, compared to control cells, have been found in the extracts of cells from the mitotic phase [40]. Purified mitogen-activated protein kinase, p44/42 MAP kinase selectively phosphorylates S45 while the substrate of the p38 MAP kinase, mitogen-activated protein kinase-activated protein-2 (MAPKAP kinase-2) selectively phosphorylates S59 of αB-crystallin [40]. The kinase responsible for S19 phosphorylation of αB-crystallin is yet to be identified.

2.1. Phosphorylation of αB-crystallin upon aging, stress and diseases

Phosphorylation of αB-crystallin is generally found to be elevated upon aging, stress or diseases. Investigations on the role of αB-crystallin (and Hsp27) and their phosphorylation status in muscle wasting either due to disuse of muscle or aging show that the levels of αB-crystallin and Hsp27 are increased both in the muscles of aged compared to young patients and in disused muscles [41,42]. The levels of αB-crystallin phosphorylated at S59 and to some extent at S45 are increased in the insoluble fraction in aged muscles as well as in disused muscles [42,43], along with enhanced levels of phosphorylated p38 and p44/42 MAP kinases [43]. Increased phosphorylated αB-crystallin was also observed in aged or cataractous eye lens [44,45].

Heat stress increases not only the expression level of αB-crystallin but also its phosphorylation and increases its level in the insoluble fraction [46]. Treating cells with certain drugs/reagents that can disrupt the cytoskeleton [47], cause oxidative stress [48] or inhibit the proteosomal machinery [49] also lead to increased phosphorylation of αB-crystallin and accumulation in the insoluble fraction. For example, inhibition of proteasomal activity by MG-132 in U373 MG cells leads to activation of p38 and p44/42 MAP kinases, increased phosphorylation of αB-crystallin (and Hsp27), and localization in aggresomes [49]. Oxidative stress is shown to increase the phosphorylation of αB-crystallin, leading to co-localization with tubulin [48]. Heat stress elevates the expression of αB-crystallin in cultured hippocampal neurons [50]. While the non-phosphorylated forms of αB-crystallin (and Hsp25) were found in the perikaryon and nucleus, the S19 and S45 phosphorylated forms of αB-crystallin localized to axons and dendrites, the S59 phosphorylated form localized to the dendrites along the plasma membrane [51].

Accumulation of αB-crystallin in the Rosenthal fibers that consist of granular deposits associated with intermediate filaments occurs in the brains of Alexander disease patients [52–54]. Phosphorylated αB-crystallin (at S59 and S45) was found predominantly in the insoluble fraction of brain homogenates of Alexander and Alzheimer disease patients [55]. Increased levels of phosphorylated αB-crystallin (S45 &S59) were also found in the brain tissue of Down syndrome people [56]. The expression of αB-crystallin is increased in brain samples from Alzheimer's disease and correlates with phosphorylated tau and neurofilaments [57]. Increased expression of αB-crystallin and its S59 phosphorylated form in a sub-population of glial cells with or without the deposition of hyperphosphorylated tau has been observed in several tauopathies such as Alzheimer's, Pick's disease, corticobasal degeneration, and argyrophilic grain disease, and phosphorylation of αB-crystallin appears to be a protective factor [58].

Thus, during aging, stress and disease conditions, the expression of αB-crystallin is not only elevated but its phosphorylation is also enhanced and is represented in the insoluble fractions in many instances.

3. Phosphorylation destabilizes αB-crystallin, increases dynamics of subunit exchange and alters the distribution of the oligomeric populations

Most of the studies addressing the effect of phosphorylation on the structure and dynamics of αB-crystallin use phosphorylation-mimicking mutants where the phosphorylable serine residues are replaced with either aspartate or glutamate to impart negative charges. A study from our laboratory has shown that the pseudo-phosphorylation mimic (3D-αB-crystallin or S19D/S45D/S59D-αB-crystallin) exhibits decreased mean hydrodynamic radius (6.4 nm) compared to that of αB-crystallin (8.0 nm) and is more susceptible to urea-induced denaturation [59] (Fig. 1). Various biophysical studies have shown that the mean oligomeric size of the pseudo-phosphorylation mimics of αB-crystallin is lower than that of the wild-type αB-crystallin [59–61]. αB-crystallin phosphorylated in vitro using purified MAPKAP kinase 2 also showed a tendency to form lower oligomeric species compared to wild type αB-crystallin [61].

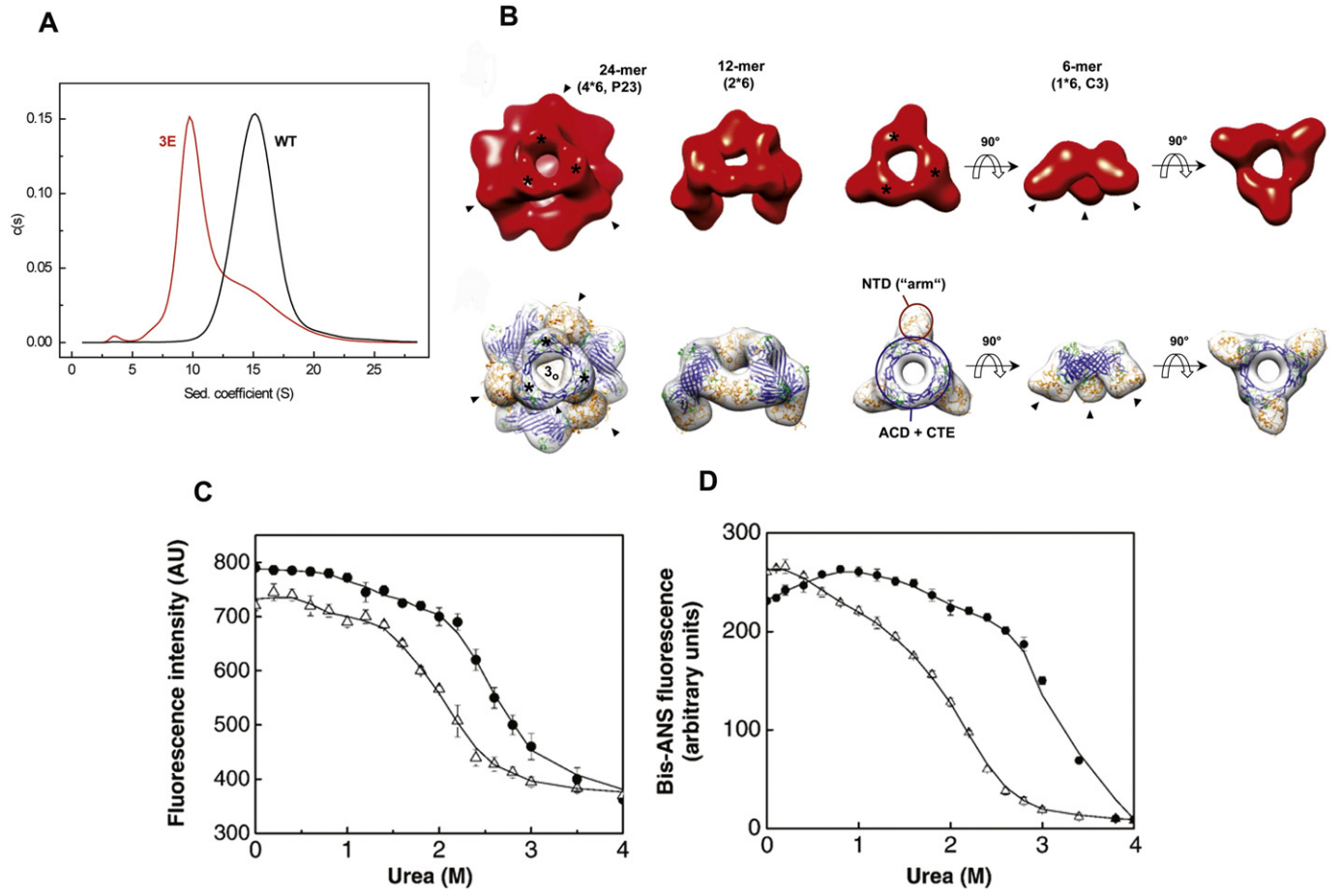

Fig. 1. Phosphorylation mimic of αB-crystallin exhibits altered distribution of ensembles, populating lower oligomeric species and decreased stability. (A) Altered distribution of phosphorylation mimic (3E, i.e., S19E/S45E/S59E mutant) towards lower sedimentation coefficients compared to that of the wild type (WT) αB-crystallin shown by Peschek et al. [61]. (B) 3Dimensional reconstructed model of 3E αB-oligomers (24-,12-,6-mer) observed in cryo-EM which could be superimposable with the corresponding oligomers of the docked pseudoatomic models proposed by Braun et al. [67], indicating the hierarchical assembly principle of the wild type αB-crystallin oligomers is preserved in the phosphomimic 3E-αB-crystallin shown by Peschek et al. [61]. Decreased stability of phosphorylation mimic (3D-αB-crystallin which exhibits decreased mean hydrodynamic radius of ~6.4 nm) towards urea-induced unfolding compared to the wild type αB-crystallin (exhibiting mean hydrodynamic radius of ~8.0 nm) shown by a study from our laboratory (Ahmad et al. [59]) by monitoring intrinsic tryptophan fluorescence (C) and bis-ANS binding (D). Figures shown in panels A & B are adapted from Peschek et al. [61] with permission from PNAS, USA. Figures shown in panels C & D are adapted from Ahmad et al. [59] with permission from Elsevier (through RightsLink).

The rapid subunit exchange process (rate constants in range of min^{-1}) is a unique feature of polydisperse vertebrate sHsps as well as monodisperse plant sHsps in contrast to a very slow subunit exchange rate (in the range of day^{-1}) of other representative multimeric proteins such as tetrameric transthyretin or hepatitis B capsid protein [see review 62]. Subunit exchange studies have shown that the pseudo-phosphorylation mimics of αB-crystallin exhibit significantly higher rate of subunit exchange than the wild type αB-crystallin [59,61]. Thus, αB-crystallin, through its inherent subunit exchange process, exists in a spectrum of inter-converting oligomeric ensembles, and phosphorylation increases the rate of conversion among the spectrum of oligomeric species with preponderance to the lower oligomers. Though the rapid subunit exchange process among the oligomers of αB-crystallin would limit the precise determination of the molecular mass by various techniques used under equilibrium conditions [63], it is evident that phosphorylation or its mimicking mutations have a tendency to alter the distribution of the oligomers towards lower oligomeric populations. In addition to various biophysical methods such as gel-filtration and sedimentation velocity measurements, a recent cryo-EM imaging study reveals the preponderance of lower oligomeric populations (6-mer and 12-mers) in the pseudo-phosphorylation mimic of αB-crystallin [61]; see Fig. 1. From the 3-dimensional-reconstructed cryo-EM images of αB-crystallin and its phosphorylation mimics, it was observed that while the symmetric 24-mer constitutes ~42% of the αB-crystallin ensemble, it constitutes only ~6% of the pseudo-phosphorylation mimic (3E-αB-crystallin) [61]. The species (6- and 12-mers) present at low abundance in αB-crystallin, contribute up to ~65% of the ensembles of 3E-αB-crystallin [61].

The reconstructed models of the cryo-EM images also show that the pseudo-phosphorylation mimics retain the gross structural features of αB-crystallin as described below. Though no high resolution oligomeric structure of full-length αB-crystallin (as well as other mammalian sHsps) is available as yet, there is a considerable advancement in the understanding of the structural aspects of αB-crystallin in recent years. Studies involving analytical ultra centrifugation, small angle X-ray scattering, NMR, negative-stained TEM, cryo-EM from different laboratories show that the oligomerization of αB-crystallin follows hierarchical interactions involving the ACD, the CTE and the NTD in the assembly of subunits with dimer as the stable building block [64–67]. ACD of αB-crystallin shows β-sheet-rich immunoglobulin-like fold which can assemble as dimer [64,68–70]. The ACD forms the primary inter-subunit interaction surfaces through anti-parallel pair-wise interactions between extended β6–β7 strand from the two subunits [64,69,70]. A hexameric unit is formed by three dimers through the conserved IXI motif from the C-terminus of one dimer binding to hydrophobic pockets formed by the β4 and β8 strands on the edge of another dimer [65–67]. Interactions involving the NTD form higher-order multimers [65–67]. Four triangular hexamers arranged with tetrahedral

symmetry form a 24mer assembly with a central cavity [65–67]. Interactions of dimeric building blocks with the existing openings in the shell of the 24-mer make further higher-order multimeric assembly [66]. Similarly, models of polydispersed species of αB-crystallin ranging from 12-mer to 48-mer including the odd numbered multimeric species of 23-mer are also formed [67].

However, the models of oligomeric assembly proposed by different studies [66,67] differ with respect to finer details of accommodating the N-terminus. Both the models of a 24mer assembly show the C-termini facing towards the surface as well as towards the inner cavity (see [66,67] for details). However, the N-terminal region is arranged inward to the central cavity in the model proposed by Jehle et al. [66], while it is arranged towards the surface in the model proposed by Braun et al. [67]. While the aspect with respect to spatial organization of the N-terminal region within the oligomeric assembly is still to be clearly understood, the proximity of the N-terminal regions from different subunits due to the oligomeric assembly is experimentally demonstrated. The cysteine mutants of αB-crystallin at the phosphorylable serine residues (19, 45 &59) could form disulfide cross-links [61]. Charge repulsion upon phosphorylation (at the NTD), thus, would have destabilizing effects on the oligomeric assembly of αB-crystallin [61,67] and therefore could shift the mean populations of αB-crystallin to hexamer and 12-mer in phosphorylation-mimicking mutants. The 3-dimensional-reconstruction of the cryo-EM images of the phosphorylation-mimicking mutant of αB-crystallin has been shown to retain the gross structural features of the hexameric assembly of the wild type protein [61] (Fig. 1B). Mass spectrometric studies have indicated that phosphorylation could disrupt dimeric substructure within the polydispersed oligomeric assembly of αB-crystallin [71].

Thus, phosphorylation of αB-crystallin has destabilizing effect, shifts the distribution of oligomeric species towards lower assemblies (fewer subunits) and increases the dynamic property of subunit exchange. As discussed in our earlier study [59], phosphorylation-induced destabilization of structure and assembly could be a general phenomenon by which phosphorylation-mediated functional regulations are achieved by the cellular system. Other examples of this effect include (i) phosphorylation-induced destabilization as a regulatory mechanism of the DNA-binding helix of the leucine zipper-type transcription factors such as Fos and Jun. [72], (ii) phosphorylation of histone 1 causing destabilization of chromatin structure [73] and (iii) phosphorylation of microtubule-associated proteins leading to destabilization of microtubule assembly [74]. It also appears that phosphorylation-induced destabilization can promote protein/peptide aggregation. For example, phosphorylation at a single serine residue of Trp-cage miniprotein destabilizes the native fold and the resultant highly dynamic structures form amyloid-like ordered aggregates with high intermolecular β-sheet formation [75]. Interestingly, our preliminary investigation (described in Section 9) indicates that phosphorylation-mimicking mutation of the myopathy-causing R120GαB-crystallin promotes its aggregation.

4. Phosphorylation modulates the function of αB-crystallin

Either as homo-oligomers or as hetero-oligomers with αA-crystallin (as isolated from the eye lens), αB-crystallin exhibits molecular chaperone activity in preventing amorphous as well as ordered fibrillar aggregation of a variety of target proteins (see reviews [15–17]). It also helps in refolding some enzymes either alone or along with other with ATP-dependent molecular chaperones by various mechanisms (see reviews [15–17]). In order to understand the role of phosphorylation in the molecular chaperone function of αB-crystallin, various phosphorylation-mimicking mutants have been studied.

with many target protein aggregation systems as described in Table 1. There seems to be both decreased and increased chaperone activities of the phosphorylation-mimicking mutants compared to wild type αB-crystallin with respect to different targets tested.

Varied results have also been reported on the chaperone activity of phosphorylated form(s) of αB-crystallin. Eye lens α-crystallin (composed of both αA- and αB-crystallin) binds to intermediate filaments and plays a role in its assembly [78]. The isolated forms of both the unphosphorylated and phosphorylated forms of αB-crystallin from bovine lens were reported to be equally effective in preventing in vitro assembly of glial fibrillary acidic protein (GFAP) and vimentin in an ATP-independent manner [78]. They also increase the soluble pool of these proteins upon incubation with the preformed filaments [78]. Another study has reported that the isolated mono-phosphorylated αB-crystallin exhibits ~30% less chaperone activity compared to the unphosphorylated form towards heat-induced aggregation of β-crystallin [79]. These results should be taken with caution as the isolated forms of unphosphorylated and phosphorylated αB-crystallin were treated with denaturants during the isolation procedure; these isolated forms may or may not be identical to the respective native proteins. However, human αB-crystallin phosphorylated in vitro has been shown to exhibit increased chaperone activity against heat-induced aggregation of malate dehydrogenase and p53 [61].

The varied results reported on the effect of phosphorylation of αB-crystallin on its chaperone activity can be considered as contradictory. However, it is possible that these variations might indeed indicate modulation of the activity upon phosphorylation, depending on the target

Table 1
Modulation of the chaperone activity of αB-crystallin upon phosphorylation-mimicking mutations.

Phosphorylation mimicking mutation in αB-crystallin	Assay model	Temperature (°C)	Chaperone activity with respect to αB-crystallin	Reference
S19D/S45D/S59D	Heat-induced aggregation of lactate dehydrogenase	50	Decreased	[60]
S19D/S45D/S59D	Refolding of luciferase	30	Decreased	[60]
S19D, S45D, S59D, S45D/S59D and S19D/S45D/S59D	Binding of destabilized T4 lysozyme mutants	23 and 37	Increased	[76]
S19D, S19D/S45D, S19D/S45D/S59D	Heat induced aggregation of β-crystallin and catalase	60	Increased	[77]
S19D, S19D/S45D, S19D/S45D/S59D	Fibril formation of ccβ-Trp	37	Decreased	[77]
S19D/S45D/S59D	Fibril formation of RCMκ-casein	37	Increased	[77]
S19D/S45D/S59D	DTT-induced aggregation of insulin	25 to 45	Increased	[59]
S19D/S45D/S59D	Heat-induced aggregation of citrate synthase	43	Increased	[59]
S19D/S45D/S59D	SDS-induced amyloid fibril formation of α-synuclein	25,37 &43	Increased	[59]
S19E,S45E,S59D, S19E/S45E, S19E/S59E, S45E/S59E S19E/S45E/S59E	Heat-induced aggregation of malate dehydrogenase	45	Increased	[61]
S19E,S45E,S59D, S19E/S45E, S19E/S59E, S45E/S59E S19E/S45E/S59E	Heat-induced aggregation of p53	42	Increased	[61]

protein and its interactions. As seen from the Table 1, the chaperone activity of phosphorylation-mimicking mutants of αB-crystallin increases in relatively more number of cases, indicating that phosphorylation, in general, has an augmentative effect. It is interesting to note that at the target protein to chaperone ratio used in the study, wild type αB-crystallin did not prevent the aggregation of p53 at 42 °C [61]. However, at the same target protein to chaperone ratio, 3EαB-crystallin completely prevented the aggregation of p53 [61]. As described in our earlier study [80], though αB-crystallin is a general chaperone and prevents the aggregation of a variety of target proteins tested, its efficiency in terms of ratio at which it can prevent the aggregation completely varies with the target proteins. The factors that could be involved in the observed target-protein-dependent efficiency may include the nature of the intermediates/destabilized states, the kinetics of their formation and the avidity of the interaction between the chaperone and the aggregation-prone species. It has been observed that two types/modes of interactions are involved in the molecular chaperone activities of sHsps and the relative involvement may depend on the nature of the intermediates of the target protein [76,80]. Given the in vivo scenario where multiple clients exist for αB-crystallin, the relative target-protein-dependent chaperone efficiency of αB-crystallin may give rise to apparent target protein discrimination upon phosphorylation in some cases — i.e., decreased interactions with some clients and increased interactions with other clients leading to different physiological consequences. Target protein discrimination effect upon phosphorylation has been demonstrated for another sHsp, Hsp27 [see reviews 29, 81]. Thus, phosphorylation of αB-crystallin (and probably other sHsps) may have both augmentative and apparent target-discriminatory effects.

5. "Dynamic partitioning": apparent target discrimination effect of phosphorylation of αB-crystallin

Considering a large number of clients/targets known so far for αB-crystallin (and Hsp27) [29] and different target protein interacting sites spanning NTD, ACD and CTE of αB-crystallin depending on the substrates [70,82–88], the mechanism by which sHsps, including αB-crystallin, function in vivo appears to involve pseudo-specific/broad specific interactions (see review [17]). Therefore, we have proposed recently a "Dynamic partitioning" hypothesis to understand the observed pleotropic functions and promiscuous interactions of sHsps (for details see review [17]). Briefly, the dynamic ensembles of sHsp species dynamically partition to substrates mediated by two types of interactions. Type 1 interaction with various targets depends on the nature of their equilibrium species populated among native or native-like species to early unfolding intermediates and is reversible/transient. Some pseudo-specific regions (for example, IPV motif of Bag 3 interacts with the β4–β8 groove of ACD of αB-crystallin/Hsp20 [89]) on the targets become temporally accessible to sHsps (determined by phosphorylation of targets, stress conditions or loss or gain of interaction with other partners of complexes). This leads to distribution of populations of reversible complexes. This distribution (dynamic fractional populations) of reversible complexes is subject to temporal variations due to stimuli that can cause alteration in both number and accessibility of interacting sites on the target proteins. Binding-induced stabilization or steric constraints upon their interaction with sHsps would determine the apparent selectivity towards target proteins exhibited by sHsps in some cases. Type I interactions help maintain cellular homeostasis under permissible and early stress conditions, giving the cellular system kinetic advantage to cope up with perturbations and help elicit further protective mechanisms [17].

Type 2 interactions which are relatively stronger and less reversible occur with aggregation-prone unfolding intermediates and/or highly destabilized target proteins or assemblies. Temperature-dependent perturbations of sHsps (see review [17]), flexibility of N- and C-terminal regions [62,66] and the increased dynamic exchange-mediated change in the distribution of quaternary structures [61,63] would enable the sHsps to wrap around/mask the exposed hydrophobic surfaces and stabilize the complex of some target proteins. Such relatively more stable complex formed under conditions of stress could either be refolded, failing which it could be targeted for degradation.

By virtue of shifting the distribution of oligomeric population towards lower sized particles [59–61], phosphorylation theoretically increases the number of species of αB-crystallin which has potential to mediate type 1 and/or type 2 interactions with targets. As we have shown earlier, though 3D-αB-crystallin exhibits increased chaperone activity compared to wild type αB-crystallin, it still exhibits temperature-dependent increase (further) in its chaperone activity and also exhibits relatively less stability [59]. Thus, phosphorylation, in general, is expected to increase the capacity (augmentation) of αB-crystallin towards both type 1 and type 2 interactions. However, in the in vivo situation where multiple targets co-exist, the interaction of phosphorylated species of αB-crystallin could decrease with respect to one or a set of targets, while increasing with respect to other/sets of targets, leading to increased partitioning effect and apparent target discrimination.

6. Effect of phosphorylation on functions of αB-crystallin in some cellular processes which have both beneficial and deleterious implications

αB-crystallin participates in several cellular processes such as stress-induced apoptosis, differentiation, cell cycle and angiogenesis where phosphorylation of αB-crystallin is indicated to play an important role. While its protective role in stress-induced apoptosis, differentiation and cell-cycle has beneficial implications, its role as a pro-angiogenic factor and in anti-apoptotic functions (depending on the context) may have deleterious implications (especially in cancer, age-related macular degeneration etc.).

6.1. Phosphorylation of αB-crystallin in its anti-apoptotic function: role in stress tolerance and myogenic differentiation

αB-crystallin has been shown to prevent apoptosis induced by various stresses including oxidative stress, drugs such as staurosporin and doxorubicin and cytokines such as TNFα [91–99; reviews 17,90]. αB-crystallin is known to be involved at various stages in mitochondrial-mediated apoptotic pathway as well as in NFκB-activated up-regulation of the anti-apoptotic protein, Bcl-2 [see reviews 17, 90]. Various stages at which αB-crystallin exhibits anti-apoptotic function in the mitochondrial-mediated apoptotic pathway include (i) interaction with Bax, the pro-apoptotic molecule, inhibiting its translocation into mitochondria and the subsequent release of cytochrome c [91], (ii) translocation of itself to mitochondria upon oxidative stress and protecting it [98], (iii) interacting with cytochrome-c and preventing its oxidation [98], which may prevent cytochrome-c-mediated interaction of Apaf-1 with procaspase-9 to form apoptosome and (iv) interacting with procaspase-3, preventing its maturation to active caspase-3 [92,93,95]. The role of phosphorylation of αB-crystallin in anti-apoptotic activity is not completely understood. However, there are some reports indicating importance of its phosphorylation in its anti-apoptotic function as discussed below.

Phosphorylation of αB-crystallin on serine residues 19, 45 and 59 plays an important role in preventing methylglyoxal-induced apoptosis in ARPE-19 retinal pigment epithelial cells [99]. Mimicking the phosphorylation of αB-crystallin on S59 was found to be necessary and sufficient to inhibit caspase-3 and to protect cardiac myocytes against hyperosmotic or hypoxic stress [94]. However, though αB-crystallin confers cytoprotection through its anti-apoptotic function by inhibiting the proteolytic activation of caspase 3 during myogenic differentiation of C2C12 myoblasts, the 3E pseudo phosphorylation mimic of αB-crystallin (mimicking hyperphosphorylation) was reported to be

completely devoid of anti-apoptotic function [93]. Interestingly, phosphorylation also seems to antagonize its anti-apoptotic function — preferential interaction of S59 phosphorylated αB-crystallin with anti-apoptotic protein, Bcl-2, leads to its sequestration and hence down-regulation of the anti-apoptotic activity of αB-crystallin [96]. Whether this phenomenon is part of any feed-back mechanism operating in phosphorylation-regulated anti-apoptotic function of αB-crystallin needs investigation. A recent study showed that S59 phosphorylated αB-crystallin binds to the mitochondrial voltage-dependent anion channel 1 and adenine nucleotide translocater, inhibiting the release of cytochrome c upon myocardial infarction [100].

αB-crystallin is important in muscle tissue homeostasis as the knockout mice for αB-crystallin die prematurely with extensive muscle wastage [101]. The level of αB-crystallin is increased (up to ~10-fold) during muscle cells differentiation and it plays an anti-apoptotic role during the differentiation process [93,102]. S59 phosphorylation of αB-crystallin is also increased during the differentiation of C2C12 myoblasts to myotubes, as shown by a study from our laboratory [103]. αB-crystallin inhibits maturation/activation of caspase-3 and -8 in response to TNF-α treatment [92]. TNFα is an inflammatory cytokine produced by immune cells and muscle cells during development, exercise or injury [104,105]. TNFα acts as a mitogen in skeletal muscle [105,106] and is essential for muscle tissue homeostasis [104]. TNFα modulates the activity of NFκB, a ubiquitous transcription factor that regulates the expression of many genes mediating pathways either related to cytoprotection or cell death [107,108]. Expression of αB-crystallin as well as TNFα increases in response to stress and differentiation [102,103]. Owing to its inflammatory effects, TNFα may trigger cytotoxic effects upon prolonged exposure but is required during the differentiation process. The elevated expression of αB-crystallin could be the balancing protective process. In order to understand the relation between the elevated expression of αB-crystallin and TNFα, a study from our laboratory [97] investigated the effect of over expression of αB-crystallin in C2C12 mouse myoblasts on TNFα-induced differentiation and cell death. The study showed that αB-crystallin promotes NFκB activation in a phosphorylation-dependent manner and protects myoblasts against TNFα-induced cytotoxicity [97]. αB-crystallin interacts with IKKβ and enhances its kinase activity, which leads to phosphorylation and subsequent degradation of IκBα, a negative regulator of NFκB, facilitating the nuclear translocation of the transcription factor [97]. One of the targets of NFκB is the transcription of Bcl-2, an anti-apoptotic protein which was found to be up-regulated, hence protecting the cells from TNFα-induced cytotoxicity [97]. Phosphorylation of αB-crystallin at S59 residue is essential for the activation of NFκB [97]. Incidentally, TNFα also activates p38-MAP kinase [109]. The study also showed that S59 phosphorylation is not required for association of αB-crystallin with the IKK complex but is essential for enhancing the kinase activity of IKKβ [97].

6.2. Possible role of phosphorylation of αB-crystallin in cell cycle regulation

SCF E3 ubiquitin ligase, which is primarily responsible for cyclin D1 degradation, consists of the F-box protein, Fbx4, and αB-crystallin, as the substrate adaptors [110,111]. D-type (1, 2 and 3) cyclins bind cyclin-dependent kinases 4 or 6 (cdk4/6), which catalyze the phosphorylation of Rb proteins and promote G1/S phase transition [111]. αB-crystallin is phosphorylated at S19 and S45 during the mitotic phase of the cell cycle [112]. The interaction of αB-crystallin with FBX4 was found to be increased by mimicking phosphorylation of αB-crystallin at both S19 and S45 [112]. Cyclin D1 is seen to be elevated frequently in human cancer [111]. Inhibition of ubiquitin-dependent proteolysis of cyclin D1 is believed to be a primary mechanism of elevated levels of cyclin D1 in human tumors [111]. Thus, as the FBX4-αB-crystallin complex is involved in the substrate recognition of SCF E3 ligase, phosphorylation of αB-crystallin, particularly at S19 and S45, might play a critical role in tumor suppression [111].

6.3. Phosphorylation of αB-crystallin and angiogenesis

Angiogenesis (neovascularisation) is an important phenomenon that promotes tumor growth [113,114] and also responsible for compromised vision in retinopathy of prematurity, diabetic retinopathy and age-related macular degeneration [115]. Angiogenesis occurs as an adaptive response to tissue hypoxia and is dependent on the accumulation of the hypoxia inducible factors (HIFs) [116]. Hypoxia increases the expression of αB-crystallin in piglet heart [117] and in retina [118]. The importance of αB-crystallin in retina during hypoxia is evident from the finding that αB-crystallin knock-out mouse shows accelerated retinal degeneration in chemically induced hypoxia compared to the wild type mouse [119].

Proteomic analysis of the bovine capillary endothelial cells showed elevated levels of αB-crystallin and S59 phosphorylated αB-crystallin during tubular morphogenesis [120]. αB-crystallin facilitates angiogenesis by chaperoning the vascular endothelial growth factor (VEGF) [118,121,122]. VEGF-A promotes vascular permeability and angiogenesis and is particularly considered as a potent angiogenic stimulator in pathologic neovascularisation [123]. αB-crystallin expression is associated with distant metastases formation in head and neck squamous cell carcinoma patients, and siRNA-mediated knockdown of αB-crystallin results in decrease in VEGF-A secretion and decreased cell motility [121]. αB-crystallin is significantly increased and colocalized with tumor vessels in a breast cancer xenograft [122]. It has been shown that αB-crystallin is an important chaperone of the unfolded protein response pathway and is a key component in the activation of the intracellular autocrine (intracrine) VEGF pathway by protecting VEGF from intracellular degradation in the tumor endothelial cells [122]. Both αB-crystallin and VEGF-A were colocalized in cytoplasm and in endoplasmic reticulum and their interaction was demonstrated by immunoprecipitation experiment [118]. S59 phosphorylated αB-crystallin is up-regulated during retinal hyperoxia and is prominently localized to neovascular tufts [118]. The S59 phosphorylated form binds to VEGF-A, protects it from degradation in the endoplasmic reticulum and results in increased VEGF-A protein secretion [118]. All these studies provide ample evidence that αB-crystallin, especially the S59 phosphorylated form, is important in intracrine and autocrine VEGF signaling pathways, involved in folding and secretion of VEGF-A as well as protecting it from proteolytic degradation and helps in angiogenesis.

7. Phosphorylated αB-crystallin partitions to cytoskeleton and nucleoskeleton

As described in the earlier sections, increased levels of phosphorylation of αB-crystallin as well as their partitioning into the insoluble fraction are observed upon aging and stress (also see Figs. 2 & 3). αB-crystallin has been shown to interact with microtubules, microfilaments and intermediate filaments of cytoskeleton networks. Many studies have shown increased localization of phosphorylated αB-crystallin to the cytoskeleton. One of the early effects of stresses such as ischemia and hyperthermia is the disruption of the cytoskeletal assembly, which leads to cell death. Chronic perturbation of the intermediate filament network or the severe disorganization of other cytoskeletal networks, results in the activation of p38 MAP kinase which leads to S59 phosphorylation of αB-crystallin and its colocalization with cytoskeletal elements [47]. Depending on the cytoskeletal network that is disorganized, activation of kinases RhoK, PKC or PKA upstream of p38 MAP kinase selectively activate it, resulting in phosphorylation of αB-crystallin [47].

αB-crystallin localizes to the cytoplasm in confluent lens epithelial cell cultures, and to the lamellipodia (leading edges of cell membranes) in migrating cells [124]. This localization of αB-crystallin is S59 phosphorylation-dependent; inhibition of its phosphorylation decreased its localization to the lamellipodia. αB-crystallin colocalized with the actin meshwork, β-catenin, WAVE-1, Abi-2 and Arp3,

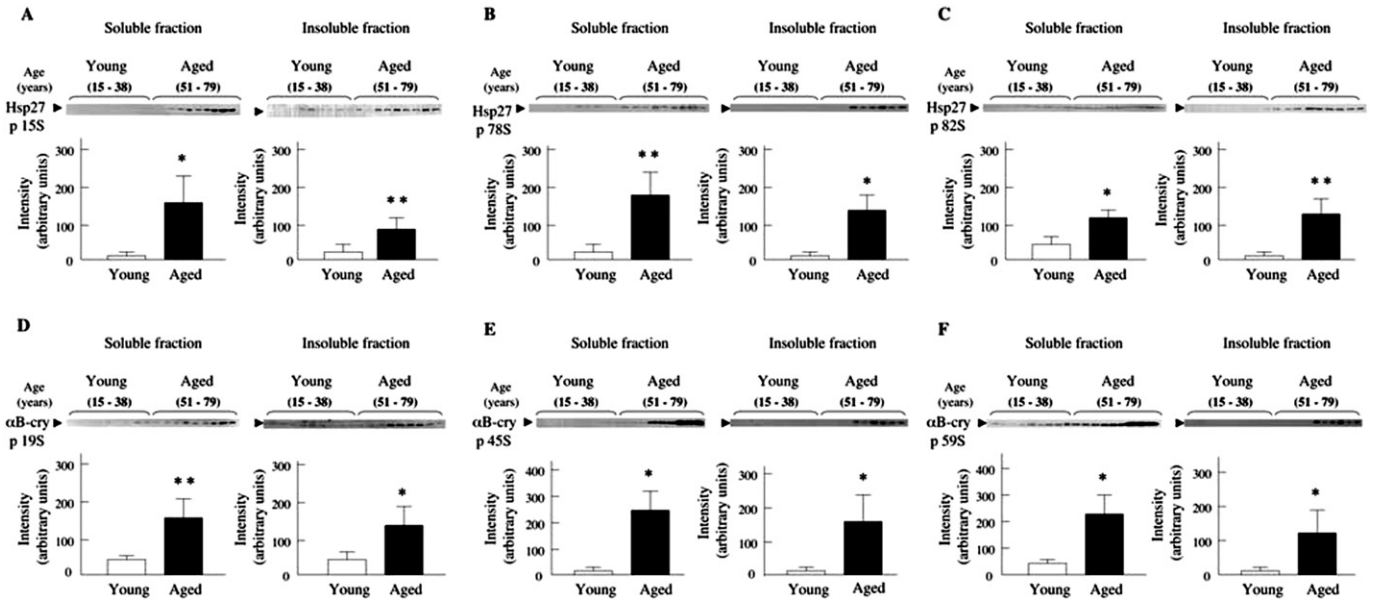

Fig. 2. Age-dependent partitioning of phosphorylated Hsp27 (A to C) and αB-crystallin (D to F) in to insoluble fraction shown by Yamaguchi et al. [42]. The figure and the following figure legend are reproduced from Yamaguchi et al. [42] with permission from Oxford university press (through RightsLink). Phosphorylation states of Hsp27 and αB-crystallin were determined from vastus lateralis muscle samples using Western blot analysis with phosphorylation site antibodies against S15, S78, or S82 peptide in Hsp27 (A–C) and with phosphorylation site antibodies against S19, S45, or S59 peptide in αB-crystallin (D–F) [42]. Study groups consisted of 9 young (15–38 years) and 9 aged (51–79 years) patients [42]. Data are expressed as means of 6, standard error of the mean. *p, 0.01, **p, 0.001 for young versus aged patients [42].

indicating a role for αB-crystallin in actin dynamics during cell migration [124]. An earlier study from our laboratory addressed the interaction of αB-crystallin with actin in vivo using H9C2 rat cardiomyoblast cell line [46]. αB-crystallin, which is uniformly distributed in the cytosol, translocates/binds to actin filaments upon heat stress [46]. αB-crystallin binding to actin filament upon mild heat stress also inhibits the

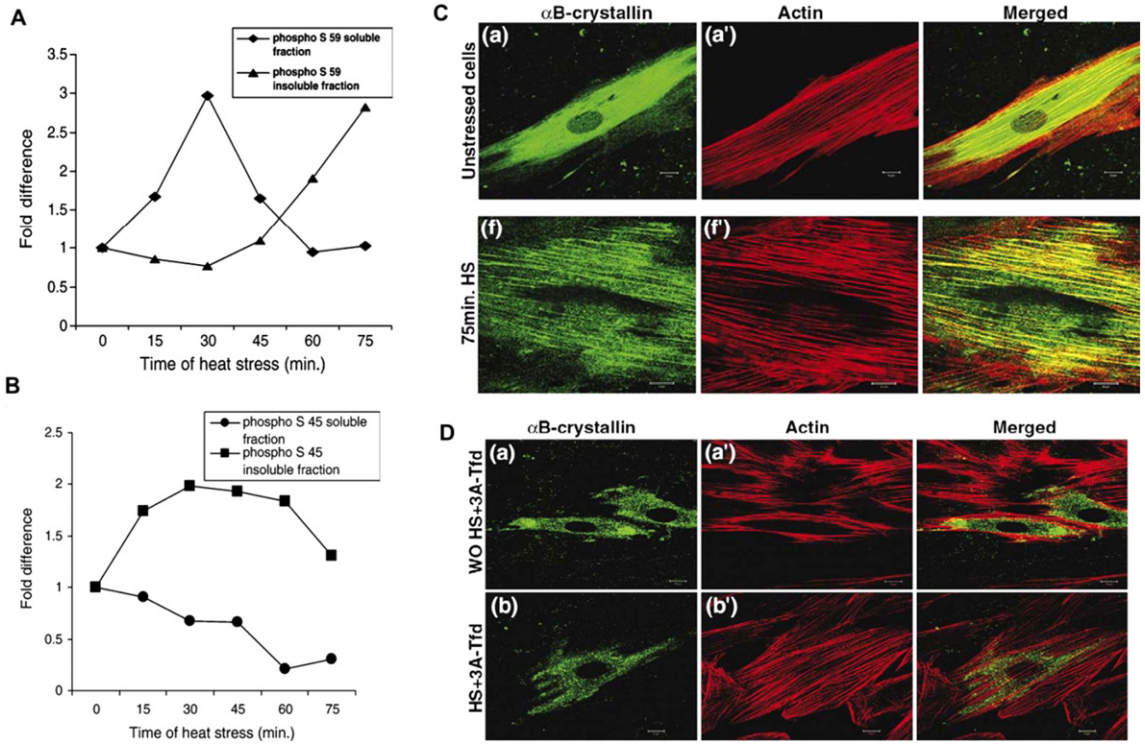

Fig. 3. Phosphorylation promotes the partitioning of αB-crystallin in to insoluble fraction and destabilized actin cytoskeleton upon heat stress shown by a study from our laboratory, Singh et al. [46]. Analysis of time-dependent changes in the soluble and insoluble fractions of αB-crystallin upon heat stress probed by Western blotting using S59 (A) and S45 (B) phospho specific antibodies. (C) Immunostaining and confocal imaging shows the increased co-localization of αB-crystallin with actin filaments in H9C2 cardiomyocytes upon heat stress. (D) Immunostaining and confocal images of the cells transfected with non-phosphorylable αB-crystallin (3A or S19A/S45A/S59A-αB-crystallin) does not show significant interaction with actin filaments either without (WO HS + 3A-Tfd) or with (HS + 3A-Tfd) heat stress, indicating importance of phosphorylation in partitioning into cytoskeleton elements. Figures shown in panels C & D are adapted from Singh et al. [46] with permission from Elsevier (through RightsLink). Note: the panel-labels inside the figures (as component) are reproduced from the original articles which are not referred to here.

cytochalasin D-induced deformation of actin filaments in vivo [46]. Time-dependent partitioning of S45- and S59-phosphorylated αB-crystallin in to insoluble fractions during stress has been demonstrated [46]. Thus, αB-crystallin is differentially phosphorylated at different serine residues and may influence its association with actin stress fibers. Transiently transfected H9C2 cells with an HA-tagged non-phosphorylable mutant of αB-crystallin (3AαB-crystallin) showed that the distribution of 3AαB-crystallin is similar to that seen for wild-type αB-crystallin in unstressed cells. However, 3AαB-crystallin fails to co-localize with actin stress fibers upon heat stress, indicating that phosphorylation of αB-crystallin is important for its association with actin stress fibers (see Fig. 3). Moreover, treating the cells with specific inhibitors of the p44 (PD98059) and p38 (SB202190) MAP kinases (which are involved in the phosphorylation of the S45 and S59 residues, respectively) almost completely inhibits the association of αB-crystallin with actin stress fibers. Thus, the study [46] showed that the association of αB-crystallin with actin filaments is phosphorylation-dependent and phosphorylation of both S45 and S59 is involved in the association. However, an earlier in vitro study reported that phosphorylated bovine eye lens αB-crystallin has comparable or slightly decreased interaction with actin [124]. αB-crystallin or its phosphorylated forms increase the rate of actin polymerization, and prevent the heat-induced aggregation of filamentous actin in vitro [125]. αB-crystallin also inhibits the cytochalasin D-induced depolymerization of actin filaments, whereas phosphorylated αB-crystallin exhibits marked decrease in such an activity in vitro [125]. However, it may be noted that the phosphorylated forms of αA- and αB-crystallin used in the in vitro study were obtained by refolding from 6 M urea. αB-crystallin has also been shown to affect microtubule assembly and prevent heat-induced aggregation of tubulin [126]. αB-crystallin can interact with tubulin subunits to regulate the equilibrium between tubulin and microtubules [127]. Low molar ratios of αB-crystallin: tubulin were found to be favorable, while high molar ratios were found to be unfavorable for microtubule assembly [127]. Regions of αB-crystallin interacting with tubulin with both increasing and inhibiting potentials towards tubulin assembly have been reported [128]. This suggests that the levels of interactable αB-crystallin pool can determine the microtubule dynamics. Immunostaining and subcellular fractionation showed redistribution of αB-crystallin from a cytosolic pool to intercalated disks and Z lines of the myofibrils, with increased fraction of phosphorylated form, upon rapid ischemia [13]. Over-expression of αB-crystallin in cardiac myocytes protects the cells from ischemia-induced cell death and stabilizes microtubules [129]. αB-crystallin, but not Hsp27, was found to protect the microtubule integrity after simulation of ischemia [129]. S59 phosphorylated αB-crystallin was localized at the microtubule organizing centers as well as was co-localized with γ-tubulin in centrosomes [130].

αB-crystallin has been shown to be involved in early cellular responses of rat and porcine myocardium to ischemia [13]. Ischemia-induced redistribution of αB-crystallin from a cytosolic pool to intercalated disks and Z lines of the myofibrils has been shown to be accompanied by its phosphorylation [13]. The time course of translocation of αB-crystallin to myofibrils during ischemia has been found to be correlated with phosphorylation (to the extent of ~20%) of αB-crystallin [131]. During initiation period of ischemia, αB-crystallin translocates to the Z-line, while prolonged ischemia (irreversibly damaging) leads to extreme stretching of myofibrils and concomitant extension of αB-crystallin localization from the Z-line area to I-bands [131]. During reperfusion after short period of ischemia (reversible ischemia), the localization of αB-crystallin is reversed from Z-line, whereas upon reperfusion after irreversibly damaging ischemia αB-crystallin remains bound to the overstretched, damaged myofibrils which are no longer capable of contraction [131]. αB-crystallin has been found to translocate to the N(2)-line area of myofibrillar I-bands of rat cardiomyocytes, where it interacts with titin [132]. Titin extracted from ischemic pig myocardium was shown to copurify with αB-crystallin [132]. Titin in I-band extends as muscles are stretched. It is made up of immunoglobulin-like modules (having β-sheet secondary structure) and two extensible regions (called N2B and PEVK) with no defined secondary structure: αB-crystallin (recombinant protein) binds to the N2B region of titin, but not to the PEVK region [133]. Atomic force microscopic study showed that higher stretching forces were needed to unfold the immunoglobulin-like domains (eight-domain fragment of titin) in the presence of αB-crystallin, indicating that αB-crystallin stabilizes this domain [133]. Thus, association with αB-crystallin protects I-band titin from stress liable to cause domain unfolding [133]. It is possible that compared to the unphosphorylated form, phosphorylated αB-crystallin partitions more and/or binds strongly to the N2B region of titin, which might contribute to the observed [131] increased, irreversible association especially with damaged myofibrils (that cannot contract). Increased phosphorylation of αB-crystallin at S59 and partitioning of the phosphorylated αB-crystallin to the insoluble fractions have been reported in the retina [134], heart [135] and skeletal muscle [136] of experimentally induced diabetic rats. Phosphorylated αB-crystallin was found to be associated with desmin of the skeletal muscles of diabetic rats [136].

αB-crystallin (and other sHsps) binds to intermediate filaments [137] and it has been proposed that specific combination of desmin and R120G mutation in αB-crystallin compromises cell viability causing desmin-related myopathy [137]. Hyperphosphorylation of the mutant is discussed later in Section 9. Co-localization of S59 phosphorylated αB-crystallin with aggregates of mutant desmin has been observed [47].

αB-Crystallin colocalizes with the splicing factor SC35 in the nucleus [138]. An earlier study from our laboratory has shown that αB-crystallin translocates to nucleus upon heat stress with a speckled appearance, colocalizing with the Lamin A/C nucleoskeleton assembly as well as with SC35, probably protecting the nucleoskeleton which also harbors the splicing machinery [139]. Phosphorylation of αB-crystallin at S59 has been shown to be important for its nuclear import through the survival motor neuron (SMN) complex [140,141]. Thus, it is likely that the phosphorylated form of αB-crystallin imported to the nucleus partitions to the nucleoskeleton assembly, protecting it from stress-induced destabilization.

8. αB-crystallin in cardioprotection: dual roles of phosphorylation

The amount of αB-crystallin in heart is as high as up to 3% of the total protein [11]. Many studies point out the cardioprotective role of αB-crystallin. Two aspects that emerge regarding the mechanism of cardioprotection offered by αB-crystallin are: (i) anti-apoptotic activity of αB-crystallin, and (ii) its binding to and stabilizing the cytoskeleton elements. Both these aspects are interrelated to some extent. As discussed in previous sections, phosphorylation of αB-crystallin is important for its anti-apoptotic function as well as binding or partitioning to the cytoskeleton. However, there are reports of poor outcome upon increased phosphorylation of αB-crystallin.

One of the causes of myocardial cell death upon ischemia and reperfusion is the disruption of the plasma membrane, caused by the disintegration of the plasma-membrane bound cytoskeleton and destabilization of the plasmalemmal lipid bilayer [142,143]. A brief period of hyperthermia has been shown to improve post-ischemic ventricular recovery in rats [144,145]. Transient ischemia followed by reperfusion is known to induce tissue damage. Though the knockout of αB-crystallin and HspB2 genes in mice had no effect on the myofibrillar structure or on most of the hemodynamic properties except for lower left ventricular end-systolic dimension, the knockout mice hearts were found to exhibit greater apoptotic and necrotic tissue damage following ischemia and reperfusion compared to wild type mice hearts. They also exhibited poorer post-ischemic contractile recovery and a significantly reduced GSH to GSSG ratio, which could be partly responsible for the oxidative damage [146]. Transgenic over-expression of αB-crystallin in mice has been shown to improve myocardial contractile performance of mouse hearts subjected to 20 min ischemia followed

by a 3 h recovery period compared to wild type mouse hearts. The transgenic mice were also shown to exhibit decreased oxidative stress, distinctly reduced infarct size and attenuated apoptotic cell death [147]. The level of platelet derived growth factor (PDGF-BB) increases, which in turn induces S59 phosphorylation of αB-crystallin by p38 MAP kinase during myocardial infarction in a mouse model [148]. In mouse hearts subjected to ex vivo ischemia, a 3-fold increase in the translocation of S59 phosphorylated αB-crystallin to mitochondria was observed and inhibition of S59 phosphorylation increased tissue damage and decreased functional recovery upon reperfusion [149]. S59 phosphorylated αB-crystallin interacts with mitochondria, thereby protecting the myocardium at least partly by modulating the mitochondrial permeability and reducing ischemia/reperfusion-induced injury [149]. As activated p38 MAP kinase translocated to the mitochondria soon after ischemia, it was suggested that during ischemia, phosphorylation of αB-crystallin occurred in the mitochondria at least to some extent, where it modulates mitochondrial damage during reperfusion [150]. As mentioned earlier, phosphorylated αB-crystallin interacts with voltage dependent anion channel-1 and adenine nucleotide transporter during myocardial infarction, inhibiting the release of cytochrome c into the cytosol, thus inhibiting apoptosis [100]. αB-crystallin is known to be up-regulated in hearts subjected to biomechanical stress and suppress cardiac hypertrophic response [151]. The role of phosphorylation in its protective response to cardiac hypertrophy is not clear.

Over-expression of αB-crystallin in transgenic mice also offers protection to heart against apoptosis and necrosis during myocardial ischemia and reperfusion [147]. Ischemia or oxidative stress can lead to the activation of p38 and MAPKAPK-2 in the heart [152,153]. Exposure of cardiac myocytes to sorbitol (hyperosmotic stressor) stimulated MKK6, p38, and MAPKAP-K2 and increased S59 phosphorylation of αB-crystallin, leading to its cytoprotective effect [154]. Blocking p38-mediated MAPKAPK-2 activation, and thus inhibiting phosphorylation of αB-crystallin, enhances apoptosis of myocytes [154]. Interestingly, exogenous administration of αB-crystallin significantly improves murine cardiac function after ischemia-reperfusion injury [155]. Cultured cardiac myocyte cells that expressed non-phosphorylable mutant of αB-crystallin (serine residues at 19, 45, 59 are replaced by alanine, 3AαB-crystallin), exhibited more stress-induced apoptosis compared to control cells [94]. Cells expressing phosphorylation-mimicking mutants [either S19A-S45A-S59EαB-crystallin or S19E-S45E-S59EαB-crystallin (3EαB-crystallin)] exhibited less activation of caspase 3 and apoptosis (3-fold) than cells expressing the non-phosphorylable mutant, 3AαB-crystallin [94].

Ischemic preconditioning in rat heart increases phosphorylation (~27%) and increased translocation of αB-crystallin to detergent insoluble fraction [156]. As mentioned earlier, during severe but brief ischemia in which the damage could be reversed, phosphorylated αB-crystallin is shown to translocate from the soluble pool to the myofibrillar Z-lines. However, in the case of severe chronic ischemia that causes irreversible damage, phosphorylated αB-crystallin localizes to the I-bands from the Z-lines [131]. This translocation of αB-crystallin correlates with the extent of damage to cardiac myofibrils during ischemia and reperfusion [131]. Therefore, it appears that stress-induced translocation of αB-crystallin (in correlation with its phosphorylation) to insoluble fraction (cytoskeleton, nucleoskeleton) would be reversible at initial stages of stress which may provide beneficial outcome, but becomes irreversible upon prolonged stress which may lead to deleterious outcome.

Another example where phosphorylation of αB-crystallin (and Hsp27) may have deleterious outcome is in the procedure of cardioplegia. Cardioplegia and cardiopulmonary bypass, increase the phosphorylation of αB-crystallin and Hsp27, and shift them to the detergent insoluble fraction, to the I-bands of the myofilament in the human myocardium [157]. αB-crystallin is phosphorylated at the S45 and S59 residues, whereas Hsp27 is phosphorylated at S15, S27 and S82 residues. The increased phosphorylation of αB-crystallin and Hsp27 is negatively correlated with cardiac function after surgery [157]. Inhibition of p38 MAP kinase, and thus the phosphorylation of αB-crystallin and Hsp27, or the over-expression of non-phosphorylable Hsp27 (all the three phosphorylable serines are replaced by alanines, 3A-Hsp27) were shown to significantly improve cardiac function after cardioplegic arrest [158]. Experiments with myocytes that over-express 3A-Hsp27 improved contractile function even when the phosphorylation of Hsp27 was not inhibited. These observations may be interpreted to suggest that unphosphorylated Hsp27 is important for contractile recovery; phosphorylation per se does not appear to affect contractile recovery, but it could deplete the pool of unphosphorylated Hsp27 [158], thereby negatively affecting cardiac function. While such details with respect to αB-crystallin are not available, similar interpretation might be applicable to αB-crystallin as well. As discussed in a later section, the extent of phosphorylation and the formation of mixed oligomers of phosphorylated forms of these sHsps with their unphosphorylated forms or with subunits of other sHsps may determine the outcome, either beneficial or deleterious. The phosphorylated forms which have increased partitioning to insoluble fractions would also take along the unphosphorylated subunits as mixed oligomers in to the insoluble fractions.

Simulating sarcopenia by muscle disuse (rat hind limb muscles) results in increased levels of phosphorylation of αB-crystallin (and Hsp27) and increased partitioning to the insoluble fractions which is returned to normal level upon recovery [43]. Age-related changes and disease/stress conditions also reflect increased phosphorylation and partitioning into insoluble fraction [41,42,44,45,53–58,131]. Therefore, it is likely that phosphorylation of αB-crystallin (also Hsp27) may display protective effect at initial stages (reversible state of phenomenon), while its increased accumulation during prolonged (irreversible stage of phenomenon) stress, diseases or aging would have deleterious outcome. The mechanism of the manifestation of the deleterious outcome may include depletion of unphosphorylated form due to partitioning of the mixed oligomers to the insoluble fractions (cytoskeleton or nucleoskeleton) which may be required for other targets and/or for the cytoskeleton/nucleoskeleton dynamics, impairment of which leads to degeneration [159–162]. Thus, hyperphosphorylation of αB-crystallin may have detrimental effect. There are considerable indications that hyperphosphorylation would also occur upon mutations in αB-crystallin which lead to familial cardiomyopathy as discussed below.

9. Hyper phosphorylation of αB-crystallin upon myofibrillar myopathy-causing mutations: the case of R120GαB-crystallin

The point mutation, R120G, in αB-crystallin leads to desmin-related myopathy as well as congenital cataract [163]. Intracellular aggregates containing both the mutant αB-crystallin and desmin have been observed upon expressing the mutant R120GαB-crystallin in muscle cell lines [163,164]. Even modest expression levels of R120GαB-crystallin in a transgenic mouse model recapitulate dominant negative phenotype like altered desmin filaments in the cardiomyocytes, impaired myofibril alignment and cardiac hypertrophy similar to that observed for the desmin-related cardiomyopathies [164]. Cardiac-specific expression of R120GαB-crystallin in a transgenic mouse model showed altered cytoskeletal network, alterations in mitochondrial-sarcomere architecture, leading to a reduction in the maximal rate of oxygen consumption, alterations in the permeability and compromised inner membrane potential, ultimately triggering apoptotic pathways, cardiomyocyte death, dilation, and heart failure [165]. Adenovirus-mediated transfection of cardiomyocytes with R120GαB-crystallin expression resulted in altered contractile mechanics [165]. While expression of wild type αB-crystallin leads to decreased aggregation of myopathy-causing desmin mutant in HEK cells, a double transgenic mouse model expressing both the myopathy-causing desmin mutant and R120GαB-crystallin

exhibits stronger cardiac hypertrophic response and dies of congestive heart failure before 7 weeks [166].

R120GαB-crystallin expression leads to formation of aggresomes with high amyloid oligomers and is proposed to be the primary toxic species affecting the viability of cardiomyocytes [167]. The levels of aggresome (containing R120GαB-crystallin) did not correlate with disease, as blocking aggresome formation led to increased levels of toxic amyloid oligomer and decreased cardiomyocyte viability [168]. Expression of Hsp22 or Hsp25 could prevent the formation of R120GαB-crystallin amyloid oligomers [169]. While wild-type αB-crystallin has a beneficial role in the formation of desmin filament networks, R120GαB-crystallin promotes desmin filament aggregation due to its increased binding to desmin [137,170]. Proteosomal inhibition and altered autophagy have also been seen upon expression of R120GαB-crystallin in cells [171,172]. While all these studies highlight the dominant negative role of R120GαB-crystallin, the role of phosphorylation of the mutant protein has not been specifically addressed. However, there are a few studies suggesting the importance of the phosphorylation of the mutant protein in its intracellular aggregate/inclusion formation.

Fig. 4 shows examples of hyperphosphorylation of R120GαB-crystallin partitioning into insoluble inclusions demonstrated by several earlier studies. Comparative Western blot analysis of protein extracts of the hearts of wild type and R120GαB-crystallin transgenic mice showed hyperphosphorylation of R120GαB-crystallin at all three S19, S45 and S59 residues [140]. The inclusions formed upon expressing R120GαB-crystallin in HeLa cells also showed strong staining against anti-S45P and S59P αB-crystallin antibodies [140]. Phosphorylation of αB-

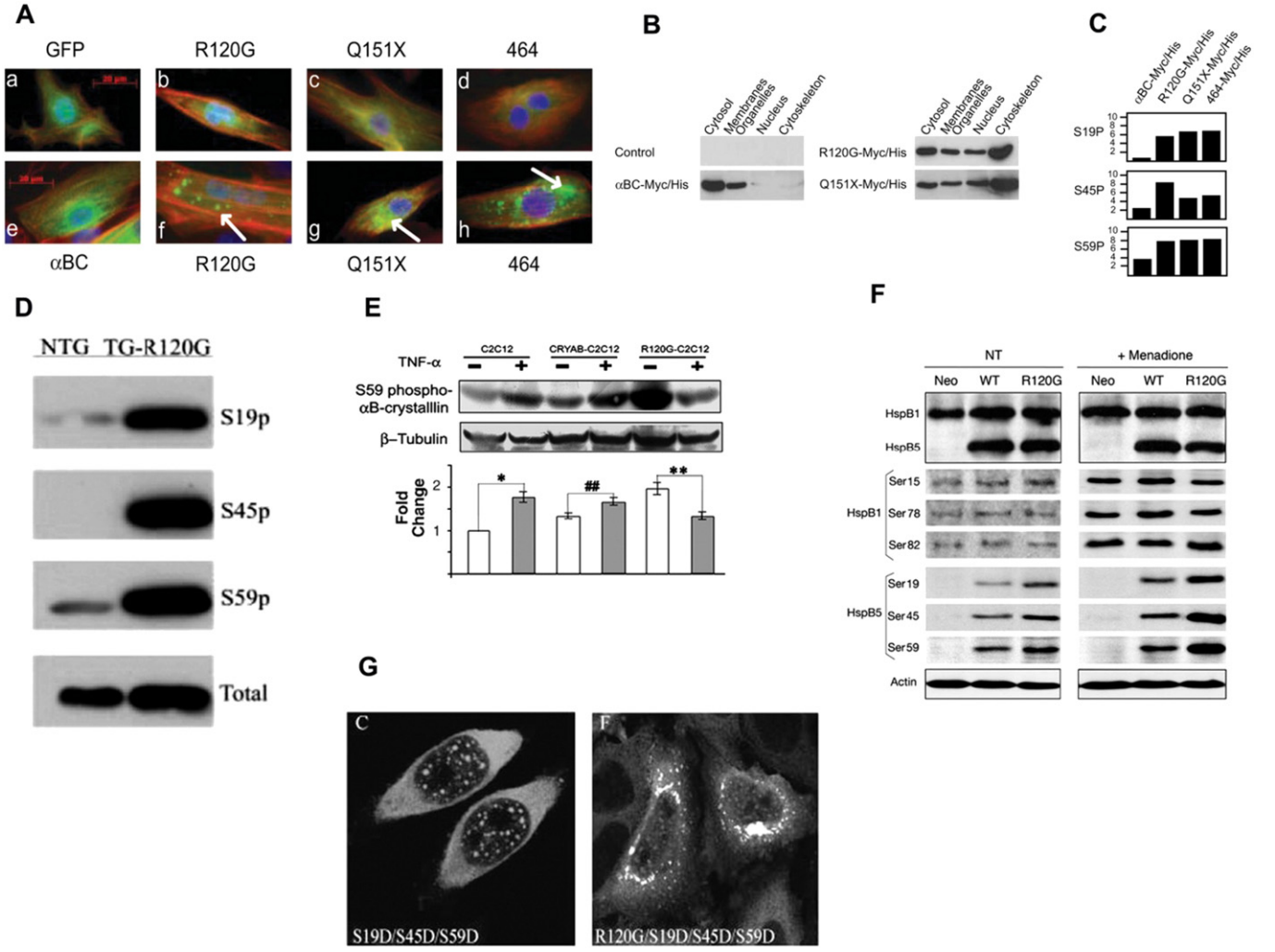

Fig. 4. Hyperphosphorylation and partitioning into insoluble inclusions of myopathy causing mutants of αB-crystallin from the literature. (A) Formation of intracellular inclusions of indicated myopathy causing mutants of αB-crystallin shown by Simon et al. [174]. Fluorescence images of neonatal cardiomyocytes cells expressing CFP-tagged indicated mutants of αB-crystallin (achieved by adenovirus mediated transfection). Images of cells without (a–e) and with protein aggregates (f–h) are shown. (B) Partitioning of the wild type and the indicated myopathy-causing mutants of αB-crystallin (Myc or His-tagged) in to the indicated sub-cellular fraction of COS cells shown by Simon et al., [174] by Western-blotting. Note the increased partitioning of the mutants to the cytoskeleton fraction. (C) Increased levels of phosphorylation (at S19, S45 and S59) of the expressed Myc/His tagged mutant αB-crystallin (as indicated) compared to the wild type in COS cells shown by Simon et al. [174] by Western blotting analysis using phospho-specific antibodies. (D) Hyperphosphorylation of R120Gα-crystallin expressed in transgenic mice probed by Western blotting using phosphor-specific antibodies shown by den Engelsman et al. [140]. (E) Hyperphosphorylation (probed at S59) of R120GαB-crystallin expressed upon stably transfected in C2C12 mouse myoblast cells which is negatively modulated by treating with the cytokine, TNFα shown by a study from our laboratory (Adhikari et al. [97]) by Western blot analysis using S59P-specific antibodies. (F) Increased phosphorylation of R120G mutant compared to the wild type (WT) αB-crystallin (HspB5) upon expressing in HeLa cells which is further augmented upon oxidative stress induced by menadione shown by Simon et al. [175] by Western blot using various phospho-specific antibodies. The figure also shows the phosphorylation of another sHsp, Hsp27 (HspB1) (G) Phosphorylation-mimicking mutant of R120GαB-crystallin, R120G/S19D/S45D/S59D, forms perinuclear inclusions when expressed in HeLa cells as shown by den Engelsman et al. [140]. The figure also shows intra nuclear staining of the phosphorylation mimicking mutant of the wild type αB-crystallin (S19D/S45D/S59D) which is not prominently seen in the case of R120Gmutant. Figures shown in panels A–C are adapted from Simon et al. [174]; figure shown in panels D and G are adapted from den Engelsman et al. [140]; the research [174,140] were originally published in the Journal Biological Chemistry© the American Society for Biochemistry and Molecular Biology. Figure shown in panel E is adapted from Adhikari et al. [97] with permission from Elsevier (through RightsLink). Figure shown in panel F is adapted from Simon et al. [175] (open access material). Note: the panel-labels inside the figures (as component) reproduced from the original articles which are not referred to here.

crystallin at S59 is required for its nuclear import through its interaction with the survival motor neuron (SMN) protein, which is affected in the case of the R120G αB-crystallin [140].

Expression of the myofibrillar myopathy causing mutants R120GαB-crystallin and other mutants (with truncated C-terminal region, Q151X, and 464delCT [173]) in COS-7 cells showed hyperphosphorylation at all the three serine residues [174]). Intracellular inclusions of all the three mutants were found in some cells, and subcellular fractionation analysis indicates partitioning of the mutant proteins into cytoskeleton [174]. Thus, the hyperphosphorylation-tendency is not just restricted to R120GαB-crystallin but also seems to occur with other mutants of αB-crystallin.

R120GαB-crystallin is phosphorylated at S19, S45 and S59 at higher level than the wild type αB-crystallin when expressed in HeLa cells [175]. The level of phosphorylated R120GαB-crystallin further increased upon oxidative stress induced by treating with menadione [175]. Upon oxidative stress, both the wild type and the mutant proteins partition into insoluble fractions [175]. A study from our laboratory also showed that the mouse C2C12 myoblasts stably expressing R120GαB-crystallin showed high phosphorylation (as probed at S59) [97]. Interestingly, treating the cells with TNFα led to decrease in the observed phosphorylation levels of the mutant protein [97]; the reason for such decrease in the phosphorylation is not yet understood. Thus, based on the above described studies, it is reasonable to conclude that R120GαB-crystallin is inherently hyperphosphorylated regardless of cell type, and external agents could modulate its hyperphosphorylation. Other mutants (Q151X, and 464delCT) also seem to get hyperphosphorylated and form insoluble intracellular aggregates [174]. However, the reason for such hyperphosphorylation is not understood and needs further investigations. The possibilities include: (i) the mutant proteins trigger stress response which leads to sustained activation of p38 MAP kinase pathway, and/or (ii) the mutation-induced conformational change, and changes in the oligomeric assembly/distribution make them susceptible/amenable for phosphorylation.

The interaction of phosphorylated wild type αB-crystallin with SMN protein leads to nuclear import of the protein [140]. Though hyperphosphorylated R120GαB-crystallin strongly interacts with SMN, both the SMN protein and phosphorylated R120GαB-crystallin colocalize in the cytoplasmic and perinuclear aggregates [140]. It was also observed that R120GαB-crystallin and desmin also colocalize in the intracellular inclusions [163,164]. The formation of insoluble inclusions may be due to increased affinity of the mutant protein for its targets and formation of insoluble complexes leading to co-aggregation. Increased aggregation propensity of mutant protein may also contribute to formation of insoluble inclusions. We are currently investigating these aspects. Fig. 5A shows that the pseudo phosphorylation mutant of R120GαB-crystallin forms inclusion bodies upon expressing it in *Escherichia coli*. It is to be noted that wild type αB-crystallin, the phosphorylation mimic of αB-crystallin and R120GαB-crystallin are expressed in soluble forms in *E. coli* as reported earlier [59,176]. When the purified inclusion body of 3D-R120GαB-crystallin was dissolved in 6 M urea and subjected to refolding, it yielded a clear solution which was further purified to homogeneity by gel-filtration and ion-exchange chromatography. In order to investigate its thermal stability, we have performed temperature-dependent turbidity measurement as optical density at 360 nm of a 0.2 mg/ml sample of 3D-R120GαB-crystallin in sodium phosphate buffer and compared it with those of the wild type αB-crystallin, 3D-αB-crystallin and R120GαB-crystallin (Fig. 5B). Interestingly, the turbidity of the sample of 3D-R120GαB-crystallin sharply increases at around 45 °C and reaches maximum at ~55 °C and then decreases with further increase in temperature. The sharp fall of turbidity value at higher temperature is due to flocculation and consequent removal of the aggregated sample from the optical path. Wild type αB-crystallin shows a relatively minor gradual increase in turbidity value beyond 65 °C which further increases above 80 °C. 3D-αB-crystallin shows a relatively sharp increase in turbidity value above

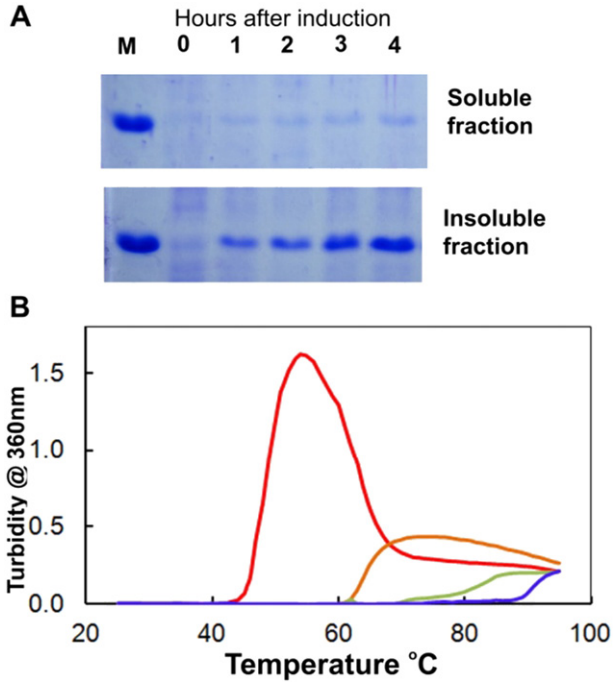

Fig. 5. Phosphorylation-mimicking mutations in R120GαB-crystallin promote aggregation. The mutations were introduced in the cDNA clone of R120GαB-crystallin by site-directed mutagenesis. (A) The expression of the protein (S19D/S45D/S59D-R120GαB-crystallin (3D-R120GαB-crystallin)) in *E. coli* (BL21 DE3) was induced by 1 mM IPTG and samples were withdrawn at the indicated time points and analyzed for soluble and insoluble fraction by SDS-PAGE. The figure shows the Coomassie brilliant blue stained gel, indicating that expressed 3D-R120GαB-crystallin partitions predominantly in the insoluble inclusion bodies. M represents a sample of purified αB-crystallin used as marker. (B) Temperature-dependent turbidity (measured as optical density at 360 nm) of the 0.2 mg/ml protein sample in 20 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl. 3D-R120GαB-crystallin (red line); wild type αB-crystallin (green line), R120GαB-crystallin (violet line) and the phosphorylation mimic of αB-crystallin, 3D-αB-crystallin (orange line). Turbidity measurements were performed using a Shimadzu UV–Vis −2600 spectrophotometer equipped with a Peltier (model S-1700) temperature controller. A temperature ramp of 2 °C/min was used.

62 °C. Surprisingly, R120GαB-crystallin shows increase in turbidity only above 85 °C. From these results it is evident that phosphorylation-mimicking mutations have general tendency to destabilize either wild type or the R120G αB-crystallin. However, the extent of destabilization effect in the case of 3D-R120GαB-crystallin is the highest. Further detailed study on these aspects of aggregation propensities, effect of mixed oligomer or hetero-oligomer formation and interaction with desmin are currently underway. Our finding presented here indicates that phosphorylation increases the aggregation propensity of R120GαB-crystallin which, at least in part, is involved in the molecular basis of pathological manifestation in the cardiomyopathy-causing mutation.

10. Conclusions and perspectives

It is emerging from several studies from different laboratories that (i) aging and various stresses lead to accumulation/increased phosphorylation of αB-crystallin in several tissues such as eye lens, retina, heart and skeletal muscle, (ii) phosphorylated αB-crystallin partitions into insoluble fractions (cytoskeleton) and (iii) phosphorylation of αB-crystallin has dual role that manifests both in beneficial and deleterious outcomes depending on the extent and duration of stress and phosphorylation; initial stage (reversible) seems to be beneficial while advanced stage (irreversible) is deleterious. Though complete understanding on the subject of role of phosphorylation of αB-crystallin in its function needs further investigations with respect to

given cellular context, at least one possibility of beneficial and deleterious outcome depending on the extent of phosphorylation and/or stress seems to be supported by various studies from different laboratories as discussed in the review.

Only a fraction of αB-crystallin is phosphorylated in vivo, depending on the various stimuli or stress or their duration. For example only 10% of αB-crystallin is phosphorylated in cells exposed to heat and NaCl and 15–20% in cells treated with reagents such as arsenite, PMA, anisomycin, H_2O_2, and sorbitol [39]. Ischemic preconditioning in rat heart increases phosphorylation of αB-crystallin ~27% [156]. We can presume with confidence that mixed oligomers of phosphorylated subunits of αB-crystallin exist with unphosphorylated subunits and/or as hetero-oligomers with the subunits of other sHsps in vivo due to dynamic subunit exchange process as well as possible phosphorylation of a few subunits of the existing oligomers. Therefore, understanding the structural and functional aspects of the mixed oligomer forms (with varying degree of phosphorylation) is important. An earlier study from our laboratory has shown that the phosphorylation mimic, 3DαB-crystallin exhibits enhanced rate of subunit exchange [59]. Importantly, our earlier study [59] as well as a recent study from another laboratory [61] show that mixed oligomer formation could in fact modulate the resultant chaperone activity, indicating that the degree of phosphorylation would have functional influence on the oligomeric species of αB-crystallin. However, the finer details of structural and functional outcome of mixed oligomer formation are not completely understood. Dominant effects of R120GαB-crystallin on oligomeric size distribution and phosphorylation levels of Hsp27 have been demonstrated in HeLa cells [175]. Phosphorylation may have destabilizing effects, the extent of destabilization varying with the degree of phosphorylation in the mixed oligomers. As discussed earlier, phosphorylation may modulate the "dynamic partitioning", giving rise to apparent discriminative effects. The extent of such modulation depends of the extent of phosphorylation in the mixed oligomeric species.

The dynamics of cytoskeleton are crucial for cell survival [159–161]. Given the knowledge from the literature that phosphorylated αB-crystallin increasingly binds to or partitions into the cytoskeleton, the interaction would be critical for cytoskeleton stability and dynamics, and therefore for cell survival (schematically illustrated in Fig. 6).

The cytoskeleton is a highly dynamic network of protein filaments that spans through the cytosol of eukaryotic cells [161]. Actin filaments and microtubuli provide structure, points of attachments and are determinants of the shape of cells. They are important for intracellular transport, cell movement and cell adhesion [161]. They are also important for mitochondrial organization and function in the cells [177,178]. Therefore, a critical balance of stabilization and dynamics of cytoskeleton is important for cell survival and cellular functions. For example, anticancer drugs have been developed that either destabilize or over stabilize the microtubules triggering cell-death and are in use clinically [179]. Therefore, lower degree of phosphorylation of αB-crystallin and hence its interaction with cytoskeleton may be necessary for optimal stabilization and the protective outcome, while too much stabilization of cytoskeleton by excessively phosphorylated αB-crystallin might disturb the cytoskeleton dynamics and trigger deleterious consequences. In addition, excessive partitioning to the cytoskeleton (or nucleoskeleton) would also deplete both the unphosphorylated and phosphorylated subunits (due to mixed oligomer formation) in the soluble fractions, thereby preventing its interactions with other targets (for example those involved in apoptotic pathways). Thus, phosphorylation of αB-crystallin may have dual role in eliciting either beneficial or deleterious outcomes depending on the extent of phosphorylation and its increased interaction with cytoskeleton. While involvement of other conditional, cellular context-dependent mechanism(s) for the dual role is also possible, it is still to be understood. As mentioned earlier, reports of phosphorylation of αB-crystallin both increasing and decreasing its anti-apoptotic activity are known. Therefore, understanding the relation between extent of phosphorylation, mixed-oligomer formation, relative partitioning/interactions of phosphorylated αB-crystallin/mixed

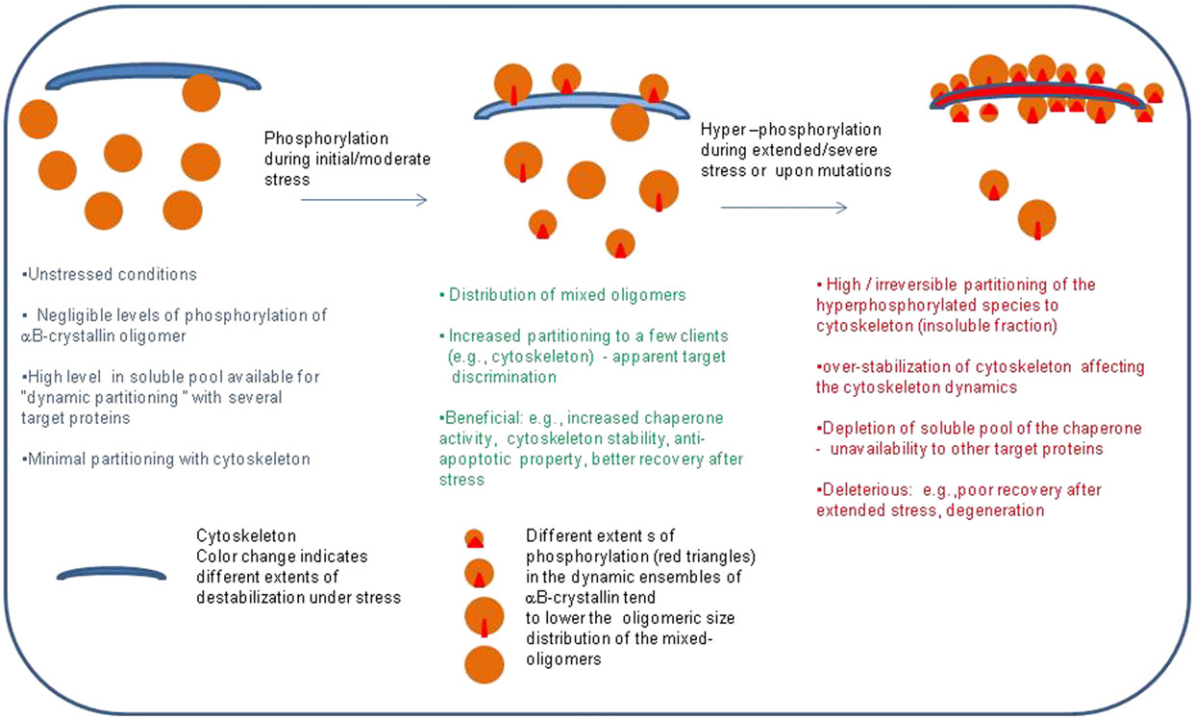

Fig. 6. Schematic representation of "Dynamic Partitioning" of αB-crystallin to one of its targets, the cytoskeleton, depending on the extent of phosphorylation. Stresses (caused by heat, reactive oxygen species, proteosomal inhibition or drugs) and chronic or acute inflammation lead to sustained activation of pathways (especially p38 MAPK), which result in progressive phosphorylation of αB-crystallin. Thus, phosphorylated αB-crystallin accumulates with aging, stresses or diseases and partitions into insoluble fractions (cytoskeletal). This phenomenon could also lead to the depletion of unphosphorylated subunits in the soluble fractions due to their existence as mixed oligomers in vivo, resulting in beneficial or deleterious outcomes, depending on the degree of accumulation of phosphorylated subunits.

oligomers to cytoskeleton and other factors involved in the apoptotic pathway is important. It is possible that the populations of αB-crystallin species available for interactions with factors of apoptotic pathways would still exist at the low levels of phosphorylation. However, hyperphosphorylation leads to increased partitioning of phosphorylated/mixed oligomers of αB-crystallin to the cytoskeleton and hence depletion of αB-crystallin species available for interactions with factors of apoptotic pathways and hence decreased anti-apoptotic activity. Mutations (e.g., R120G) in αB-crystallin also lead to hyperphosphorylation and formation of insoluble inclusions. Thus, all the above mentioned aspects lead us to propose "good-in-moderation and bad-in-excess" hypothesis for phosphorylation of αB-crystallin.

As discussed earlier, hyperphosphorylation of mutants of αB-crystallin which is involved in some cardiomyopathies has been demonstrated. Various mechanisms proposed for the basis of desmin-related cardiomyopathy caused by the R120G mutation in αB-crystallin include (i) altered conformation and oligomeric size with decreased chaperone property [176,180], (ii) improper interactions between the mutant protein and desmin [137,163,164] and intermediate filaments affecting mitochondria-sarcomere architecture [165], (iii) formation of cytotoxic amyloid oligomers [167] and (iv) proteosomal inhibition and modulation of autophagy [171,172]. Though which is the primary cause and which are consequences is debatable, interfering with these improper associations and aggregation seems to have better outcome. For example geranylgeranylacetone-induced expression of Hsp22 or Hsp25 and cardiac-specific expression of Hsp22 in transgenic R120αB-crystallin mice resulted in decreased amyloid oligomer and aggregates, improved cardiac function and survival [181]. Disrupting the interactions of R120GαB-crystallin with Hsp27 by a peptide aptamer has been shown to abolish the dominant negative effect of the mutant protein [182]. Interestingly, exercise in the transgenic mice with cardiac-specific expression of R120GαB-crystallin improves the survival (100%, while all unexercised mice had died) [183]. The pre-amyloid oligomer level was found to be decreased by 47% compared with the unexercised mice [183]. Other independent studies have shown that exercise can increase the levels of heat shock proteins including sHsps (see review [184,185]). Exercise might also decrease phosphorylation of sHsps since about ~60% decrease in the oxidative stress-induced phosphorylation (at Thr180 &Tyr182) of p38 MAPK (activation) is shown in exercised rats [186].

Considering the inherent hyperphosphorylation of the mutant protein, independent of the cell type in which it is expressed (see Fig. 4), it is likely that phosphorylation is a key aspect in several observations made with mutation-associated pathology. Therefore, inhibition or lowering the extent of phosphorylation should have therapeutic potential. p38 MAP kinase pathway and p44 kinase pathways are obvious targets moderating phosphorylation. As S45 phosphorylation of αB-crystallin is mostly involved in cell cycle and S59 phosphorylation seems to be prominent under stress conditions, targeting one of the prominent stress- or inflammation-related kinase pathways, i.e., p38 MAP kinase pathway could be a choice for moderation of the phosphorylation of αB-crystallin. So far there seems to be no inhibitor known for MAPKAP kinase-2 (substrate of the p38 MAPK) which phosphorylates αB-crystallin (and Hsp27). However, inhibition of either p38 MAPK or its activation (upstream to p38MAPK) would be useful. Inhibitors of p38 MAPK have been developed to target the kinase for some human diseases such as arthritis, cancer involving inflammation [187,188]. Transient/temporary inhibition of p38 MAP kinase pathway could be preferred as low level of phosphorylation of αB-crystallin would have protective effects. This also minimizes deleterious consequences, if any, of completely inhibiting the kinase. Experimental verification of this possibility would be very useful for developing strategies for managing several pathological conditions involving the role of αB-crystallin or other sHsps. Thus, we conclude that phosphorylation of αB-crystallin seems to have both beneficial and deleterious outcomes depending on the extent of phosphorylation. Moderation of hyperphosphorylation of αB-crystallin and/or its disease-causing mutants might form one of the future strategies for disease-management.

Conflict of interest

Authors claim no conflict of interest

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References

[1] S.P. Bhat, C.N. Nagineni, Alpha B subunit of lens-specific protein alpha-crystallin is present in other ocular and non-ocular tissues, Biochem. Biophys. Res. Commun. 158 (1989) 319–325.
[2] R.P. Taylor, I.J. Benjamin, Small heat shock proteins: a new classification scheme in mammals, J. Mol. Cell. Cardiol. 38 (2005) 433–444.
[3] K. Kato, H. Shinohara, N. Kurobe, Y. Inaguma, K. Shimizu, K. Ohshima, Tissue distribution and developmental profiles of immunoreactive alpha B crystallin in the rat determined with a sensitive immunoassay system, Biochim. Biophys. Acta 1074 (1991) 201–208.
[4] G. Kappé, E. Franck, P. Verschuure, W.C. Boelens, J.A. Leunissen, W.W. de Jong, The human genome encodes 10 alpha-crystallin-related small heat shock proteins: HspB1-10, Cell Stress Chaperones 8 (2003) 53–61.
[5] W.W. de Jong, G.J. Caspers, J.A. Leunissen, Genealogy of the α-crystallin-small heat shock protein superfamily, Int. J. Biol. Macromol. 22 (1998) 151–162.
[6] T. Kriehuber, T. Rattei, T. Weinmaier, A. Bepperling, M. Haslbeck, J. Buchner, Independent evolution of the core domain and its flanking sequences in small heat shock proteins, FASEB J. 24 (2010) 3633–3642.
[7] A.R. Clark, N.H. Lubsen, C. Slingsby, sHSP in the eye lens: crystallin mutations, cataract and proteostasis, Int. J. Biochem. Cell Biol. 44 (2012) 1687–1697.
[8] J. Horwitz, Alpha-crystallin, Exp. Eye Res. 76 (2003) 145–153.
[9] T. Iwaki, A. Kume-Iwaki, J.E. Goldman, Cellular distribution of alpha B-crystallin in non-lenticular tissues, J. Histochem. Cytochem. 38 (1990) 31–39.
[10] T. Iwaki, A. Iwaki, J.E. Goldman, Alpha B-crystallin in oxidative muscle fibers and its accumulation in ragged-red fibers: a comparative immunohistochemical and histochemical study in human skeletal muscle, Acta Neuropathol. 85 (1993) 475–480.
[11] G. Lutsch, R. Vetter, U. Offhauss, M. Wieske, H.J. Gröne, R. Klemenz, I. Schimke, J. Stahl, R. Benndorf, Abundance and location of the small heat shock proteins HSP25 and alphaB-crystallin in rat and human heart, Circulation 96 (1997) 3466–3476.
[12] J.L. Martin, R. Mestril, R. Hilal-Dandan, L.L. Brunton, W.H. Dillmann, Small heat shock proteins and protection against ischemic injury in cardiac myocytes, Circulation 96 (1997) 4343–4348.
[13] N. Golenhofen, W. Ness, R. Koob, P. Htun, W. Schaper, D. Drenckhahn, Ischemia-induced phosphorylation and translocation of stress protein alpha B-crystallin to Z lines of myocardium, Am. J. Physiol. 274 (1998) H1457–H1464.
[14] J. Horwitz, Alpha-crystallin can function as a molecular chaperone, Proce. Natl. Acad. Sci. U.S.A. 89 (1992) 10449–10453.
[15] E. Ganea, Chaperone-like activity of alpha-crystallin and other small heat shock proteins, Curr. Protein Pept. Sci. 2 (2001) 205–225.
[16] M. Haslbeck, T. Franzmann, D. Weinfurtner, J. Buchner, Some like it hot: the structure and function of small heat-shock proteins, Nature Struct. Mol. Biol. 12 (2005) 842–846.
[17] R. Bakthisaran, R. Tangirala, C.M. Rao, Small heat shock proteins: role in cellular functions and pathology, Biochim. Biophys. Acta 1854 (2015) 291–319.
[18] W.C. Boelens, Cell biological roles of αB-crystallin, Prog. Biophys. Mol. Biol. 115 (2014) 3–10.
[19] A. Parcellier, E. Schmitt, M. Brunet, A. Hammann, E. Solary, C. Garrido, Small heat shock proteins HSP27 and alphaB-crystallin: cytoprotective and oncogenic functions, Antioxid. Redox Signal. 7 (2005) 404–413.
[20] B. Stoevring, O. Vang, M. Christiansen, AlphaB-crystallin in cerebrospinal fluid of patients with multiple sclerosis, Clin. Chim. Acta 356 (2005) 95–101.
[21] O. Kozawa, H. Matsuno, M. Niwa, D. Hatakeyama, K. Kato, T. Uematsu, AlphaB-crystallin, a low-molecular-weight heat shock protein, acts as a regulator of platelet function, Cell Stress Chaperones 6 (2001) 21–28.
[22] R.K. Gangalum, I.C. Atanasov, Z.H. Zhou, S.P. Bhat, AlphaB-crystallin is found in detergent-resistant membrane microdomains and is secreted via exosomes from human retinal pigment epithelial cells, J. Biol. Chem. 286 (2011) 3261–3269.
[23] A. Clayton, A. Turkes, H. Navabi, M.D. Mason, Z. Tabi, Induction of heat shock proteins in B-cell exosomes, J. Cell Sci. 118 (2005) 3631–3638.
[24] J.G. Masilamoni, E.P. Jesudason, B. Baben, C.E. Jebaraj, S. Dhandayuthapani, R. Jayakumar, Molecular chaperone alpha-crystallin prevents detrimental effects of neuroinflammation, Biochim. Biophys. Acta 1762 (2006) 284–293.

[25] S.S. Ousman, B.H. Tomooka, J.M. van Noort, E.F. Wawrousek, K.C. O'Connor, D.A. Hafler, R.A. Sobel, W.H. Robinson, L. Steinman, Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination, Nature 448 (2007) 474–479.
[26] A. Arac, S.E. Brownell, J.B. Rothbard, C. Chen, R.M. Ko, M.P. Pereira, G.W. Albers, L. Steinman, G.K. Steinberg, Systemic augmentation of αB-crystallin provides therapeutic benefit 12 h poststroke onset via immune modulation, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13287–13292.
[27] J.B. Rothbard, M.P. Kurnellas, S. Brownell, C.M. Adams, L. Su, R.C. Axtell, R. Chen, C.G. Fathman, W.H. Robinson, L. Steinman, Therapeutic effects of systemic administration of chaperone αB-crystallin associated with binding proinflammatory plasma proteins, J. Biol. Chem. 287 (2012) 9708–9721.
[28] A. Klopstein, E. Santos-Nogueira, I. Francos-Quijorna, A. Redensek, S. David, X. Navarro, R. López-Vales, Beneficial effects of αB-crystallin in spinal cord contusion injury, J. Neurosci. 32 (2012) 14478–14488.
[29] A.P. Arrigo, B. Gibert, Protein interactomes of three stress inducible small heat shock proteins: HspB1, HspB5 and HspB8, Int. J. Hyperth. 29 (2013) 409–422.
[30] K. Wang, W. Ma, A. Spector, Phosphorylation of alpha-crystallin in rat lenses is stimulated by H2O2 but phosphorylation has no effect on chaperone activity, Exp. Eye Res. 61 (1995) 115–124.
[31] F. Bennardini, C. Juliano, D. Benetti, M. Mian, M. Chiesi, A. Mattana, F. Franconi, Alpha B crystallin is constitutively expressed in cultures of bovine articular chondrocytes, Biochem. Biophys. Res. Commun. 208 (1995) 742–747.
[32] R. Chiesa, M.A. Gawinowicz-Kolks, N.J. Kleiman, A. Spector, Definition and comparison of the phosphorylation sites of the A and B chains of bovine alpha-crystallin, Exp. Eye Res. 46 (1988) 199–208.
[33] C.E.M. Voorter, W.A. de Haard-Hoekman, E.C. Roersma, H.E. Meyer, H. Bloemendal, W.W. de Jong, The in vivo phosphorylation sites of bovine alpha B-crystallin, FEBS Lett. 259 (1989) 50–52.
[34] J.B. Smith, Y. Sun, D.L. Smith, B. Green, Identification of the posttranslational modifications of bovine lens alpha B-crystallins by mass spectrometry, Protein Sci. 1 (1992) 601–608.
[35] A. Spector, R. Chiesa, J. Sredy, W. Garner, cAMP-dependent phosphorylation of bovine lens alpha-crystallin, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 4712–4716.
[36] R. Chiesa, M.A. Gawinowicz-Kolks, N.J. Kleiman, A. Spector, The phosphorylation sites of the B2 chain of bovine alpha-crystallin, Biochem. Biophys. Res. Commun. 144 (1987) 1340–1347.
[37] M. Kantorow, J. Piatigorsky, Phosphorylations of alpha A- and alpha B-crystallin, Int. J. Biol. Macromol. 22 (1998) 307–314.
[38] M. Kantorow, J. Piatigorsky, Alpha-crystallin/small heat shock protein has autokinase activity, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3112–3116.
[39] H. Ito, K. Okamoto, H. Nakayama, T. Isobe, K. Kato, Phosphorylation of alphaB-crystallin in response to various types of stress, J. Biol. Chem. 272 (1997) 29934–29941.
[40] K. Kato, H. Ito, K. Kamei, Y. Inaguma, I. Iwamoto, S. Saga, Phosphorylation of alphaB-crystallin in mitotic cells and identification of enzymatic activities responsible for phosphorylation, J. Biol. Chem. 273 (1998) 28346–28354.
[41] P. Doran, J. Gannon, K. O'Connell, K. Ohlendieck, Aging skeletal muscle shows a drastic increase in the small heat shock proteins alphaB-crystallin/HspB5 and cvHsp/HspB7, Eur. J. Cell Biol. 86 (2007) 629–640.
[42] T. Yamaguchi, H. Arai, N. Katayama, T. Ishikawa, K. Kikumoto, Y. Atomi, Age-related increase of insoluble, phosphorylated small heat shock proteins in human skeletal muscle, J. Gerontol.A Biol. Sci. Med. Sci. 62 (2007) 481–489.
[43] K. Kato, H. Ito, K. Kamei, I. Iwamoto, Y. Inaguma, Innervation-dependent phosphorylation and accumulation of alphaB-crystallin and Hsp27 as insoluble complexes in disused muscle, FASEB J. 16 (2002) 1432–1434.
[44] C.-H. Huang, Y.-T. Wang, C.-F. Tsai, Y.-J. Chen, J.-S. Lee, S.-H. Chiou, Phosphoproteomics characterization of novel phosphorylated sites of lens proteins from normal and cataractous human eye lenses, Mol. Vis. 17 (2011) 186–198.
[45] Y. Ueda, M.K. Duncan, L.L. David, Lens proteomics: the accumulation of crystallin modifications in the mouse lens with age, Invest. Ophthalmol. Vis. Sci. 43 (2002) 205–215.
[46] B.N. Singh, K.S. Rao, T. Ramakrishna, N. Rangaraj, C.M. Rao, Association of alphaB-crystallin, a small heat shock protein, with actin: role in modulating actin filament dynamics in vivo, J. Mol. Biol. 366 (2007) 756–767.
[47] N. Launay, B. Goudeau, K. Kato, P. Vicart, A. Lilienbaum, Cell signaling pathways to alphaB-crystallin following stresses of the cytoskeleton, Exp. Cell Res. 312 (2006) 3570–3584.
[48] I.K. Aggeli, I. Beis, C. Gaitanaki, Oxidative stress and calpain inhibition induce alpha B-crystallin phosphorylation via p38-MAPK and calcium signalling pathways in H9c2 cells, Cell. Signal. 20 (2008) 1292–1302.
[49] H. Ito, K. Kamei, I. Iwamoto, Y. Inaguma, R. García-Mata, E. Sztul, K. Kato, Inhibition of proteasomes induces accumulation, phosphorylation, and recruitment of HSP27 and alphaB-crystallin to aggresomes, J. Biochem. 131 (2002) 593–603.
[50] B.B. Kirbach, N. Golenhofen, Differential expression and induction of small heat shock proteins in rat brain and cultured hippocampal neurons, J. Neurosci. Res. 89 (2011) 162–175.
[51] T. Schmidt, B. Bartelt-Kirbach, N. Golenhofen, Phosphorylation-dependent subcellular localization of the small heat shock proteins HspB1/Hsp25 and HspB5/αB-crystallin in cultured hippocampal neurons, Histochem. Cell Biol. 138 (2012) 407–418.
[52] J.E. Goldman, E. Corbin, Isolation of a major protein component of Rosenthal fibers, Am. J. Pathol. 130 (1988) 569–578.
[53] N. Tomokane, T. Iwaki, J. Tateishi, A. Iwaki, J. Goldman, Rosenthal fibers share epitopes with alpha B-crystallin, glial fibrillary acidic protein, and ubiquitin, but not with vimentin. Immunoelectron microscopy with colloidal gold, Am. J. Pathol. 138 (1991) 875–885.
[54] T. Iwaki, A. Kume-Iwaki, R.K.H. Liem, J.E. Goldman, Alpha B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain, Cell 57 (1989) 71–78.
[55] K. Kato, Y. Inaguma, H. Ito, K. Iida, I. Iwamoto, K. Kamei, N. Ochi, H. Ohta, M. Kishikawa, Ser-59 is the major phosphorylation site in alphaB-crystallin accumulated in the brains of patients with alexander's disease, J. Neurochem. 76 (2001) 730–736.
[56] S. Palminiello, K. Jarzabek, K. Kaur, M. Walus, A. Rabe, G. Albertini, A.A. Golabek, E. Kida, Upregulation of phosphorylated alphaB-crystallin in the brain of children and young adults with Down syndrome, Brain Res. 1268 (2009) 162–173.
[57] C. Björkdahl, M.J. Sjögren, X. Zhou, H. Concha, J. Avila, B. Winblad, J.J. Pei, Small heat shock proteins Hsp27 or alphaB-crystallin and the protein components of neurofibrillary tangles: tau and neurofilaments, J. Neurosci. Res. 86 (2008) 1343–1352.
[58] I. López-González, M. Carmona, L. Arregui, G.G. Kovacs, I. Ferrer, αB-crystallin and HSP27 in glial cells in tauopathies, Neuropathology 34 (2014) 517–526.
[59] M.F. Ahmad, B. Raman, T. Ramakrishna, C.M. Rao, Effect of phosphorylation on alpha B-crystallin: differences in stability, subunit exchange and chaperone activity of homo and mixed oligomers of alpha B-crystallin and its phosphorylation-mimicking mutant, J. Mol. Biol. 375 (2008) 1040–1051.
[60] H. Ito, K. Kamei, I. Iwamoto, Y. Inaguma, D. Nohara, K. Kato, Phosphorylation-induced change of the oligomerization state of alpha B-crystallin, J. Biol. Chem. 276 (2001) 5346–5352.
[61] J. Peschek, N. Braun, J. Rohrberg, K.C. Back, T. Kriehuber, A. Kastenmüller, S. Weinkauf, J. Buchner, Regulated structural transitions unleash the chaperone activity of αB-crystallin, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) E3780–E3789.
[62] E. Basha, H. O'Neill, E. Vierling, Small heat shock proteins and α-crystallins: dynamic proteins with flexible functions, Trends Biochem. Sci. 37 (2012) 106–117.
[63] G.K. Hochberg, J.L. Benesch, Dynamical structure of αB-crystallin, Prog. Biophys. Mol. Biol. 115 (2014) 11–20.
[64] S. Jehle, P. Rajagopal, B. Bardiaux, S. Markovic, R. Kuhne, J.R. Stout, V.A. Higman, R.E. Klevit, B.J. van Rossum, H. Oschkinat, Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers, Nat. Struct. Mol. Biol. 17 (2010) 1037–1042.
[65] J. Peschek, N. Braun, T.M. Franzmann, Y. Georgalis, M. Haslbeck, S. Weinkauf, J. Buchner, The eye lens chaperone alpha-crystallin forms defined globular assemblies, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 13272–13277.
[66] S. Jehle, B.S. Vollmar, B. Bardiaux, K.K. Dove, P. Rajagopal, T. Gonen, H. Oschkinat, R.E. Klevit, N-terminal domain of alphaB-crystallin provides a conformational switch for multimerization and structural heterogeneity, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6409–6414.
[67] N. Braun, M. Zacharias, J. Peschek, A. Kastenmüller, J. Zou, M. Hanzlik, M. Haslbeck, J. Rappsilber, J. Buchner, S. Weinkauf, Multiple molecular architectures of the eye lens chaperone αB-crystallin elucidated by a triple hybrid approach, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 20491–20496.
[68] J.P. Mornon, D. Halaby, M. Malfois, P. Durand, I. Callebaut, A. Tardieu, Alpha-crystallin C-terminal domain: on the track of an Ig fold, Int. J. Biol. Macromol. 22 (1998) 219–227.
[69] C. Bagnéris, O.A. Bateman, C.E. Naylor, N. Cronin, W.C. Boelens, N.H. Keep, C. Slingsby, Crystal structures of α-crystallin domain dimers of αB-crystallin and Hsp20, J. Mol. Biol. 392 (2009) 1242–1252.
[70] G.K. Hochberg, H. Ecroyd, C. Liu, D. Cox, D. Cascio, M.R. Sawaya, M.P. Collier, J. Stroud, J.A. Carver, A.J. Baldwin, C.V. Robinson, D.S. Eisenberg, J.L. Benesch, A. Laganowsky, The structured core domain of αB-crystallin can prevent amyloid fibrillation and associated toxicity, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E1562–E1570.
[71] J.A. Aquilina, J.L. Benesch, L.L. Ding, O. Yaron, J. Horwitz, C.V. Robinson, Phosphorylation of alphaB-crystallin alters chaperone function through loss of dimeric substructure, J. Biol. Chem. 279 (2004) 28675–28680.
[72] L. Szilak, J. Moitra, D. Krylov, C. Vinson, Phosphorylation destabilizes alpha-helices, Nat. Struct. Biol. 4 (1997) 112–114.
[73] L.J. Kaplan, R. Bauer, E. Morrison, T.A. Langan, G.D. Fasman, The structure of chromatin reconstituted with phosphorylated H1. Circular dichroism and thermal denaturation studies, J. Biol. Chem. 259 (1984) 8777–8785.
[74] A. Ebneth, G. Drewes, E.M. Mandelkow, E. Mandelkow, Phosphorylation of MAP2c and MAP4 by MARK kinases leads to the destabilization of microtubules in cells, Cell Motil. Cytoskel. 44 (1999) 209–224.
[75] J. Kardos, B. Kiss, A. Micsonai, P. Rovó, D.K. Menyhárd, J. Kovács, G. Váradi, G.K. Tóth, A. Perczel, Phosphorylation as conformational switch from the native to amyloid state: trp-cage as a protein aggregation model, J. Phys. Chem. B 119 (2015) 2946–2955.
[76] H.A. Koteiche, H.S. McHaourab, Mechanism of chaperone function in small heat-shock proteins. Phosphorylation-induced activation of two mode binding in alphaB-crystallin, J. Biol. Chem. 278 (2003) 10361–10367.
[77] H. Ecroyd, S. Meehan, J. Horwitz, J.A. Aquilina, J.L. Benesch, C.V. Robinson, C.E. Macphee, J.A. Carver, Mimicking phosphorylation of alphaB-crystallin affects its chaperone activity, Biochem. J. 401 (2007) 129–141.
[78] I.D. Nicholl, R.A. Quinlan, Chaperone activity of alpha-crystallins modulates intermediate filament assembly, EMBO J. 13 (1994) 945–953.
[79] A. Kamei, T. Hamaguchi, N. Matsuura, K. Masuda, Does post-translational modification influence chaperone-like activity of alpha-crystallin? I. Study on phosphorylation, Biol. Pharm. Bull. 24 (2001) 96–99.
[80] K. Rajaraman, B. Raman, T. Ramakrishna, C.M. Rao, Interaction of human recombinant alphaA- and alphaB-crystallins with early and late unfolding intermediates of citrate synthase on its thermal denaturation, FEBS Lett. 497 (2001) 118–123.
[81] A.P. Arrigo, B. Gibert, HspB1 dynamic phospho-oligomeric structure dependent interactome as cancer therapeutic target, Curr. Mol. Med. 12 (2012) 1151–1163.

[82] J.G. Ghosh, A.K. Shenoy Jr., J.I. Clark, Interactions between important regulatory proteins and human alphaB crystallin, Biochemistry 46 (2007) 6308–6317.
[83] K.K. Sharma, H. Kaur, K. Kester, Functional elements in molecular chaperone alpha-crystallin: identification of binding sites in alpha B-crystallin, Biochem. Biophys. Res. Commun. 239 (1997) 217–222.
[84] K.K. Sharma, R.S. Kumar, G.S. Kumar, P.T. Quinn, Synthesis and characterization of a peptide identified as a functional element in alphaA-crystallin, J. Biol. Chem. 275 (2000) 3767–3771.
[85] J.A. Aquilina, S.J. Watt, The N-terminal domain of alphaB-crystallin is protected from proteolysis by bound substrate, Biochem. Biophys. Res. Commun. 353 (2007) 1115–1120.
[86] J. Bhattacharyya, E.G. Padmanabha Udupa, J. Wang, K.K. Sharma, Mini-alphaBcrystallin: a functional element of alphaB-crystallin with chaperone-like activity, Biochemistry 45 (2006) 3069–3076.
[87] J.G. Ghosh, M.R. Estrada, J.I. Clark, Interactive domains for chaperone activity in the small heat shock protein, human alphaB crystallin, Biochemistry 44 (2005) 14854–14869.
[88] S. Narayanan, B. Kamps, W.C. Boelens, B. Reif, AlphaB-crystallin competes with Alzheimer's disease beta-amyloid peptide for peptide-peptide interactions and induces oxidation of Abeta-Met35, FEBS Lett. 580 (2006) 5941–5946.
[89] M. Fuchs, D.J. Poirier, S.J. Seguin, H. Lambert, S. Carra, S.J. Charette, J. Landry, Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction, Biochem. J. 425 (2009) 245–255.
[90] J. Acunzo, M. Katsogiannou, P. Rocchi, Small heat shock proteins HSP27 (HspB1), αB-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death, Int. J. Biochem. Cell Biol. 44 (2012) 1622–1631.
[91] Y.W. Mao, J.P. Liu, H. Xiang, D.W. Li, Human alphaA- and alphaB-crystallins bind to Bax and Bcl X(S) to sequester their translocation during staurosporine-induced apoptosis, Cell Death Differ. 11 (2004) 512–526.
[92] M.C. Kamradt, F. Chen, V.L. Cryns, The small heat shock protein αB-crystallin negatively regulates cytochrome c- and caspase-8-dependent activation of caspase-3 by inhibiting its autoproteolytic maturation, J. Biol. Chem. 276 (2001) 16059–16063.
[93] M.C. Kamradt, F. Chen, S. Sam, V.L. Cryns, The small heat shock protein AlphaBcrystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation, J. Biol. Chem. 277 (2002) 38731–38736.
[94] L.E. Morrison, H.E. Hoover, D.J. Thuerauf, C.C. Glembotski, Mimicking phosphorylation of alphaB-crystallin on serine-59 is necessary and sufficient to provide maximal protection of cardiac myocytes from apoptosis, Circ. Res. 92 (2003) 203–211.
[95] M.C. Kamradt, M. Lu, M.E. Werner, T. Kwan, F. Chen, A. Strohecker, S. Oshita, J.C. Wilkinson, V. Yu, P.G. Oliver, C.S. Dudkett, D.J. Buchsbaum, A.F. LoBuglio, V.C. Jordan, V.L. Cryns, The small heat shock protein AlphaB-crystallin is a novel inhibitor of TRAIL-induced apoptosis that suppresses the activation of caspase-3, J. Biol. Chem. 280 (2005) 11059–11066.
[96] N. Launay, A. Tarze, P. Vicart, A. Lilienbaum, Serine 59 phosphorylation of {alpha}B-crystallin down-regulates its anti-apoptotic function by binding and sequestering Bcl-2 in breast cancer cells, J. Biol. Chem. 285 (2010) 37324–37332.
[97] A.S. Adhikari, B.N. Singh, K.S. Rao, C.M. Rao, αB-crystallin, a small heat shock protein, modulates NF-κB activity in a phosphorylation-dependent manner and protects muscle myoblasts from TNF-α induced cytotoxicity, Biochim. Biophys. Acta 1813 (2011) 1532–1542.
[98] R.S. McGreal, W.L. Kantorow, D.C. Chauss, J. Wei, L.A. Brennan, M. Kantorow, αBcrystallin/sHSP protects cytochrome c and mitochondrial function against oxidative stress in lens and retinal cells, Biochim. Biophys. Acta 1820 (2012) 921–930.
[99] W.J. Jeong, J.H. Rho, Y.G. Yoon, S.H. Yoo, N.Y. Jeong, W.Y. Ryu, H.B. Ahn, W.C. Park, S.H. Rho, H.S. Yoon, Y.H. Choi, Y.H. Yoo, Cytoplasmic and nuclear anti-apoptotic roles of αB-crystallin in retinal pigment epithelial cells, PLoS One 7 (2012) e45754.
[100] A. Mitra, T. Basak, K. Datta, S. Naskar, S. Sengupta, S. Sarkar, Role of α-crystallin B as a regulatory switch in modulating cardiomyocyte apoptosis by mitochondria or endoplasmic reticulum during cardiac hypertrophy and myocardial infarction, Cell Death Dis. 4 (2013), e582.
[101] J.P. Brady, D.L. Garland, D.E. Green, E.R. Tamm, F.J. Giblin, E.F. Wawrousek, AlphaBcrystallin in lens development and muscle integrity: a gene knockout approach, Invest. Ophthalmol. Vis. Sci. 42 (2001) 2924–2934.
[102] H. Ito, K. Kamei, I. Iwamoto, Y. Inaguma, K. Kato, Regulation of the levels of small heat-shock proteins during differentiation of C2C12 cells, Expt. Cell Res. 266 (2001) 213–221.
[103] B.N. Singh, K.S. Rao, C.M. Rao, Ubiquitin–proteasome-mediated degradation and synthesis of MyoD is modulatedby αB-crystallin, a small heat shock protein, during muscle differentiation, Biochim. Biophys. Acta 1803 (2010) 288–299.
[104] R.A. Collins, M.D. Grounds, The role of tumor necrosis factor-alpha (TNF-alpha) in skeletal muscle regeneration. Studies in TNF-alpha (−/−) and TNF-alpha (−/−) / LT-alpha (−/−) mice, J. Histochem. Cytochem. 49 (2001) 989–1001.
[105] Y.P. Li, R.J. Schwartz, TNF-alpha regulates early differentiation of C2C12 myoblasts in an autocrine fashion, FASEB J. 8 (2001) 1413–1415.
[106] Y.P. Li, TNF-alpha is a mitogen in skeletal muscle, Am. J. Physiol. Cell Physiol. 285 (2003) c370–c376.
[107] H.L. Pahl, Activators and target genes of Rel/NF-KB transcription factors, Oncogene 18 (1999) 6853–6866.
[108] S. Shishodia, B.B. Aggarwal, Nuclear factor-κB activation: a question of life or death, J. Biochem. Mol. Biol. 35 (2002) 28–40.
[109] J. Raingeaud, S. Gupta, J.S. Rogers, M. Dickens, J. Han, R.J. Ulevitch, R.J. Davis, Proinflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine, J. Biol. Chem. 270 (1995) 7420–7426.
[110] D.I. Lin, O. Barbash, K.G. Kumar, J.D. Weber, J.W. Harper, A.J. Klein-Szanto, A. Rustgi, S.Y. Fuchs, J.A. Diehl, Phosphorylation-dependent ubiquitination of cyclin D1 by the SCF(FBX4-alphaB crystallin) complex, Mol. Cell 24 (2006) 355–366.
[111] O. Barbash, J.A. Diehl, SCFFbx4/αB-crystallin E3 ligase—when one is not enough, Cell Cycle 7 (2008) 2983–2986.
[112] J. den Engelsman, V. Keijsers, W.W. de Jong, W.C. Boelens, The small heat-shock protein αB-crystallin promotes FBX4-dependent ubiquitination, J. Biol. Chem. 278 (2003) 4699–4704.
[113] G. Bergers, L.E. Benjamin, Tumorigenesis and the angiogenic switch, Nat. Rev. Cancer 3 (2003) 401–410.
[114] B.R. Zetter, Angiogenesis and tumor metastasis, Annu. Rev. Med. 49 (1998) 407–424.
[115] J.S. Penn, A. Madan, R.B. Caldwell, M. Bartoli, R.W. Caldwell, M.E. Hartnett, Vascular endothelial growth factor in eye disease, Prog. Retin. Eye Res. 27 (2008) 331–371.
[116] G.H. Fong, Mechanisms of adaptive angiogenesis to tissue hypoxia, Angiogenesis 11 (2008) 121–140.
[117] P. Louapre, J.F. Grongnet, R.M. Tanguay, J.C. David, Effects of hypoxia on stress proteins in the piglet heart at birth, Cell Stress Chaperones 10 (2005) 17–23.
[118] S. Kase, S. He, S. Sonoda, M. Kitamura, C. Spee, E. Wawrousek, S.J. Ryan, R. Kannan, D.R. Hinton, αB-crystallin regulation of angiogenesis by modulation of VEGF, Blood 115 (2010) 3398–3406.
[119] J. Yaung, R. Kannan, E.F. Wawrousek, C. Spee, P.G. Sreekumar, D.R. Hinton, Exacerbation of retinal degeneration in the absence of alpha crystallins in an in vivo model of chemically induced hypoxia, Exp. Eye Res. 86 (2008) 355–365.
[120] A. Dimberg, S. Rylova, L.C. Dieterich, A.K. Olsson, P. Schiller, C. Wikner, S. Bohman, J. Botling, A. Lukinius, E.F. Wawrousek, L. Claesson-Welsh, AlphaBcrystallin promotes tumor angiogenesis by increasing vascular survival during tube morphogenesis, Blood 111 (2008) 2015–2023.
[121] C. van de Schootbrugge, J. Bussink, P.N. Span, F.C. Sweep, R. Grénman, H. Stegeman, G.J. Pruijn, J.H. Kaanders, W.C. Boelens, αB-crystallin stimulates VEGF secretion and tumor cell migration and correlates with enhanced distant metastasis in head and neck squamous cell carcinoma, BMC Cancer 13 (2013) 128–135.
[122] Q. Ruan, S. Han, W.G. Jiang, M.E. Boulton, Z.J. Chen, B.K. Law, J. Cai, αB-crystallin, an effector of unfolded protein response, confers anti-VEGF resistance to breast cancer via maintenance of intracrine VEGF in endothelial cells, Mol. Cancer Res. 9 (2011) 1632–1643.
[123] N. Ferrara, H.P. Gerber, J. LeCouter, The biology of VEGF and its receptors, Nat. Med. 9 (2003) 669–676.
[124] R. Maddala, P.V. Rao, α-Crystallin localizes to the leading edges of migrating lens epithelial cells, Exp. Cell Res. 306 (2005) 203–215.
[125] K. Wang, A. Spector, Alpha-crystallin stabilizes actin filaments and prevents cytochalasin-induced depolymerization in a phosphorylation-dependent manner, Eur. J. Biochem. 242 (1996) 56–66.
[126] J.H. Xi, F. Bai, R. McGaha, U.P. Andley, Alpha-crystallin expression affects microtubule assembly and prevents their aggregation, FASEB J. 20 (2006) 846–857.
[127] S.A. Houck, J.I. Clark, Dynamic subunit exchange and the regulation of microtubule assembly by the stress response protein human alphaB crystallin, PLoS One 5 (2010) e11795.
[128] J.G. Ghosh, S.A. Houck, J.I. Clark, Interactive domains in the molecular chaperone human alphaB crystallin modulate microtubule assembly and disassembly, PLoS One 2 (2007) e498.
[129] W.F. Bluhm, J.L. Martin, R. Mestril, W.H. Dillmann, Specific heat shock proteins protect microtubules during simulated ischemia in cardiac myocytes, Am. J. Physiol. 275 (1998) H2243–H2249.
[130] Y. Inaguma, H. Ito, I. Iwamoto, S. Saga, K. Kato, AlphaB-crystallin phosphorylated at Ser-59 is localized in centrosomes and midbodies during mitosis, Eur. J. Cell Biol. 80 (2001) 741–748.
[131] N. Golenhofen, P. Htun, W. Ness, R. Koob, W. Schaper, D. Drenckhahn, Binding of the stress protein alpha B-crystallin to cardiac myofibrils correlates with the degree of myocardial damage during ischemia/reperfusion in vivo, J. Mol. Cell. Cardiol. 31 (1999) 569–580.
[132] N. Golenhofen, A. Arbeiter, R. Koob, D. Drenckhahn, Ischemia-induced association of the stress protein alpha B-crystallin with I-band portion of cardiac titin, J. Mol. Cell. Cardiol. 34 (2002) 309–319.
[133] B. Bullard, C. Ferguson, A. Minajeva, M.C. Leake, M. Gautel, D. Labeit, L. Ding, S. Labeit, J. Horwitz, K.R. Leonard, W.A. Linke, Association of the chaperone alphaB-crystallin with titin in heart muscle, J. Biol. Chem. 279 (2004) 7917–7924.
[134] V.S. Reddy, G. Raghu, S.S. Reddy, A.K. Pasupulati, P. Suryanarayana, G.B. Reddy, Response of small heat shock proteins in diabetic rat retina, Invest. Ophthalmol. Vis. Sci. 54 (2013) 7674–7982.
[135] V.S. Reddy, C.U. Kumar, G. Raghu, G.B. Reddy, Expression and induction of small heat shock proteins in rat heart under chronic hyperglycemic conditions, Arch. Biochem. Biophys. 558 (2014) 1–9.
[136] V.S. Reddy, S. Jakhotia, P.Y. Reddy, G.B. Reddy, Hyperglycemia induced expression, phosphorylation, and translocation of αB-crystallin in rat skeletal muscle, IUBMB Life 67 (2015) 291–299.
[137] J.L. Elliott, M.D. Perng, A.R. Prescott, K.A. Jansen, G.H. Koenderink, R.A. Quinlan, The specificity of the interaction between αB-crystallin and desmin filaments and its impact on filament aggregation and cell viability, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 368 (2013) 20120375.
[138] A.E. van Rijk, G.J. Stege, E.J. Bennink, A. May, H. Bloemendal, Nuclear staining for the small heat shock protein alphaB-crystallin colocalizes with splicing factor SC35, Eur. J. Cell Biol. 82 (2003) 361–368.
[139] A.S. Adhikari, K.S. Rao, N. Rangaraj, V.K. Parnaik, C.M. Rao, Heat stress-induced localization of small heat shock proteins in mouse myoblasts: intranuclear lamin

A/C speckles as target for alphaB-crystallin and Hsp25, Exp. Cell Res. 299 (2004) 393–403.
[140] J. den Engelsman, D. Gerrits, W.W. de Jong, J. Robbins, K. Kato, W.C. Boelens, Nuclear import of {alpha}B-crystallin is phosphorylation-dependent and hampered by hyperphosphorylation of the myopathy-related mutant R120G, J. Biol. Chem. 280 (2005) 37139–37148.
[141] J. den Engelsman, C. van de Schootbrugge, J. Yong, G.J. Pruijn, W.C. Boelens, Pseudophosphorylated αB-crystallin is a nuclear chaperone imported into the nucleus with help of the SMN complex, PLoS ONE 8 (2013), e73489.
[142] C. Ganote, S. Armstrong, Ischaemia and the myocyte cytoskeleton: review and speculation, Cardiovasc. Res. 27 (1993) 1387–1403.
[143] M.D. Sage, R.B. Jennings, Cytoskeletal injury and subsarcolemmal bleb formation in dog heart during in vitro total ischemia, Am. J. Pathol. 133 (1988) 327–337.
[144] R.W. Currie, M. Karmazyn, M. Kloc, K. Mailer, Heat-shock response is associated with enhanced postischemic ventricular recovery, Circ. Res. 63 (1988) 543–549.
[145] M. Karmazyn, K. Mailer, R.W. Currie, Acquisition and decay of heat-shock-enhanced postischemic ventricular recovery, Am. J. Physiol. 259 (1990) H424–H431.
[146] L.E. Morrison, R.J. Whittaker, R.E. Klepper, E.F. Wawrousek, C.C. Glembotski, Roles for αB-crystallin and HSPB2 in protecting the myocardium from ischemia-reperfusion-induced damage in a KO mouse model, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H847–H855.
[147] P.S. Ray, J.L. Martin, E.A. Swanson, H. Otani, W.H. Dillmann, D.K. Das, Transgene overexpression of B crystalline confers simultaneous protection against cardiomyocyte apoptosis and necrosis during myocardial ischemia and reperfusion, FASEB J. 15 (2001) 393–402.
[148] E. Shu, H. Matsuno, S. Akamastu, Y. Kanno, H. Suga, K. Nakajima, A. Ishisaki, S. Takai, K. Kato, Y. Kitajima, O. Kozawa, AlphaB-crystallin is phosphorylated during myocardial infarction: involvement of platelet-derived growth factor-BB, Arch. Biochem. Biophys. 438 (2005) 111–118.
[149] J.K. Jin, R. Whittaker, M.S. Glassy, S.B. Barlow, R.A. Gottlieb, C.C. Glembotski, Localization of phosphorylated alphaB-crystallin to heart mitochondria during ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 294 (2008) H337–H344.
[150] R. Whittaker, M.S. Glassy, N. Gude, M.A. Sussman, R.A. Gottlieb, C.C. Glembotski, Kinetics of the translocation and phosphorylation of alphaB-crystallin in mouse heart mitochondria during ex vivo ischemia, Am. J. Physiol. Heart Circ. Physiol. 296 (2009) H1633–H1642.
[151] A.R. Kumarapeli, H. Su, W. Huang, M. Tang, H. Zheng, K.M. Horak, M. Li, X. Wang, Alpha B-crystallin suppresses pressure overload cardiac hypertrophy, Circ. Res. 103 (2008) 1473–1482.
[152] N. Maulik, T. Yoshida, Y.L. Zu, M. Sato, A. Bannerjee, D.K. Das, Ischemic preconditioning triggers tyrosine kinase signaling: a potential role for MAPKAP kinase 2, Am. J. Physiol. 275 (1998) H1857–H1864.
[153] A. Nakano, C.P. Baines, S.O. Kim, S.L. Pelech, J.M. Downey, M.V. Cohen, S.D. Critz, Ischemic preconditioning activates MAPKAPK2 in the isolated rabbit heart: evidence for involvement of p38 MAPK, Circ. Res. 86 (2000) 144–151.
[154] H.E. Hoover, D.J. Thuerauf, J.J. Martindale, C.C. Glembotski, αB-crystallin gene induction and phosphorylation by MKK6-activated p38, J. Biol. Chem. 275 (2000) 23825–23833.
[155] J.B. Velotta, N. Kimura, S.H. Chang, J. Chung, S. Itoh, J. Rothbard, P.C. Yang, L. Steinman, R.C. Robbins, M.P. Fischbein, αB-crystallin improves murine cardiac function and attenuates apoptosis in human endothelial cells exposed to ischemia-reperfusion, Ann. Thorac. Surg. 91 (2011) (2011) 1907–1913.
[156] P. Eaton, W. Fuller, J.R. Bell, M.J. Shattock, AlphaB crystallin translocation and phosphorylation: signal transduction pathways and preconditioning in the isolated rat heart, J. Mol. Cell. Cardiol. 33 (2001) 1659–1671.
[157] R.T. Clements, N.R. Sodha, J. Feng, S. Mieno, M. Boodhwani, B. Ramlawi, C. Bianchi, F.W. Sellke, Phosphorylation and translocation of heat shock protein 27 and alphaB-crystallin in human myocardium after cardioplegia and cardiopulmonary bypass, J. Thorac. Cardiovasc. Surg. 134 (2007) 1461–1470.
[158] R.T. Clements, J. Feng, B. Cordeiro, C. Bianchi, F.W. Sellke, p38 MAPK-dependent small HSP27 and αB-crystallin phosphorylation in regulation of myocardial function following cardioplegic arrest, Am. J. Physiol. Heart Circ Physiol. 300 (2011) H1669–H1677.
[159] P.P. Gan, J.A. McCarroll, S.T. Po'uha, K. Kamath, M.A. Jordan, M. Kavallaris, Microtubule dynamics, mitotic arrest, and apoptosis: drug-induced differential effects of betaIII-tubulin, Mol. Cancer Ther. 9 (2010) 1339–1348.
[160] V.E. Franklin-Tong, C.W. Gourlay, A role for actin in regulating apoptosis/programmed cell death: evidence spanning yeast, plants and animals, Biochem. J. 413 (2008) 389–404.
[161] M. Gellert, E.M. Hanschmann, K. Lepka, C. Berndt, C.H. Lillig, Redox regulation of cytoskeletal dynamics during differentiation and de-differentiation, Biochim. Biophys. Acta 1850 (2015) 1575–1587.
[162] P. Meinke, A.A. Makarov, P.L. Thành, D. Sadurska, E.C. Schirmer, Nucleoskeleton dynamics and functions in health and disease, Cell Health Cytoskelet. 7 (2015) 57–69.
[163] P. Vicart, A. Caron, P. Guicheney, Z. Li, M.C. Prévost, A. Faure, D. Chateau, F. Chapon, F. Tomé, J.M. Dupret, D. Paulin, M. Fardeau, A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy, Nat. Genet. 20 (1998) 92–95.
[164] X. Wang, H. Osinska, R. Klevitsky, A.M. Gerdes, M. Nieman, J. Lorenz, T. Hewett, J. Robbins, Expression of R120G-alphaB-crystallin causes aberrant desmin and alphaB-crystallin aggregation and cardiomyopathy in mice, Circ. Res. 89 (2001) 84–91.
[165] A. Maloyan, A. Sanbe, H. Osinska, M. Westfall, D. Robinson, K. Imahashi, E. Murphy, J. Robbins, Mitochondrial dysfunction and apoptosis underlie the pathogenic process in alpha-B-crystallin desmin-related cardiomyopathy, Circulation 112 (2005) 3451–3461.
[166] X. Wang, R. Klevitsky, W. Huang, J. Glasford, F. Li, J. Robbins, AlphaB-crystallin modulates protein aggregation of abnormal desmin, Circ. Res. 93 (2003) 998–1005.
[167] A. Sanbe, H. Osinska, J.E. Saffitz, C.G. Glabe, R. Kayed, A. Maloyan, J. Robbins, Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10132–10136.
[168] A. Sanbe, H. Osinska, C. Villa, J. Gulick, R. Klevitsky, C.G. Glabe, R. Kayed, J. Robbins, Reversal of amyloid-induced heart disease in desmin-related cardiomyopathy, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13592–13597.
[169] A. Sanbe, J. Yamauchi, Y. Miyamoto, Y. Fujiwara, M. Murabe, A. Tanoue, Interruption of CryAB-amyloid oligomer formation by HSP22, J. Biol. Chem. 282 (2007) 555–563.
[170] M.D. Perng, S.F. Wen, P. van den IJssel, A.R. Prescott, R.A. Quinlan, Desmin aggregate formation by R120G alphaB-crystallin is caused by altered filament interactions and is dependent upon network status in cells, Mol. Biol. Cell 15 (2004) 2335–2346.
[171] J. Liu, Q. Chen, W. Huang, K.M. Horak, H. Zheng, R. Mestril, X. Wang, Impairment of the ubiquitin–proteasome system in desminopathy mouse hearts, FASEB J. 20 (2006) 362–364.
[172] Q. Zheng, H. Su, M.J. Ranek, X. Wang, Autophagy and p62 in cardiac proteinopathy, Circ. Res. 109 (2011) 296–308.
[173] D. Selcen, A.G. Engel, Myofibrillar myopathy caused by novel dominant negative alpha B-crystallin mutations, Ann. Neurol. 54 (2003) 804–810.
[174] S. Simon, J.M. Fontaine, J.L. Martin, X. Sun, A.D. Hoppe, M.J. Welsh, R. Benndorf, P. Vicart, Myopathy-associated alphaB-crystallin mutants: abnormal phosphorylation, intracellular location, and interactions with other small heat shock proteins, J. Biol. Chem. 282 (2007) 34276–34287.
[175] S. Simon, V. Dimitrova, B. Gibert, S. Virot, N. Mounier, M. Nivon, C. Kretz-Remy, V. Corset, P. Mehlen, A.P. Arrigo, Analysis of the dominant effects mediated by wild type or R120G mutant of αB-crystallin (HspB5) towards Hsp27 (HspB1), PLoS One 8 (2013), e70545.
[176] L.V. Kumar, T. Ramakrishna, C.M. Rao, Structural and functional consequences of the mutation of a conserved arginine residue in alphaA and alphaB crystallins, J. Biol. Chem. 274 (1999) 24137–24141.
[177] H.M. Ni, J.A. Williams, W.X. Ding, Mitochondrial dynamics and mitochondrial quality control, Redox Biol. 4C (2014) 6–13.
[178] Y. Ikeda, A. Shirakabe, C. Brady, D. Zablocki, M. Ohishi, J. Sadoshima, Molecular mechanisms mediating mitochondrial dynamics and mitophagy and their functional roles in the cardiovascular system, J. Mol. Cell. Cardiol. 78 (2015) 116–122.
[179] R.O. Carlson, New tubulin targeting agents currently in clinical development, Expert Opin. Investig. Drugs 17 (2008) 707–722.
[180] M.P. Bova, O. Yaron, Q. Huang, L. Ding, D.A. Haley, P.L. Stewart, J. Horwitz, Mutation R120G in alphaB-crystallin, which is linked to a desmin-related myopathy, results in an irregular structure and defective chaperone-like function, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6137–6142.
[181] A. Sanbe, T. Daicho, R. Mizutani, T. Endo, N. Miyauchi, J. Yamauchi, K. Tanonaka, C. Glabe, A. Tanoue, Protective effect of geranylgeranylacetone via enhancement of HSPB8 induction in desmin-related cardiomyopathy, PLoS One 4 (2009), e5351.
[182] B. Gibert, S. Simon, V. Dimitrova, C. Diaz-Latoud, A.P. Arrigo, Peptide aptamers: tools to negatively or positively modulate HSPB1(27) function, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 368 (2013) 20120075.
[183] A. Maloyan, J. Gulick, C.G. Glabe, R. Kayed, J. Robbins, Exercise reverses preamyloid oligomer and prolongs survival in alphaB-crystallin-based desmin-related cardiomyopathy, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 5995–6000.
[184] J.P. Morton, A.C. Kayani, A. McArdle, B. Drust, The exercise-induced stress response of skeletal muscle, with specific emphasis on humans, Sports Med. 39 (2009) 643–662.
[185] A. McArdle, M.J. Jackson, Exercise, oxidative stress and ageing, J. Anat. 197 (2000) 539–541.
[186] K. Vichaiwong, E.J. Henriksen, C. Toskulkao, M. Prasannarong, T. Bupha-Intr, V. Saengsirisuwan, Attenuation of oxidant-induced muscle insulin resistance and p38 MAPK by exercise training, Free Radic. Biol. Med. 47 (2009) 593–599.
[187] V.C. Kyttaris, Kinase inhibitors: a new class of antirheumatic drugs, Drug Des. Dev. Ther. 6 (2012) 245–250.
[188] S. Kumar, J. Boehm, J.C. Lee, p38 MAP kinases: key signalling molecules as therapeutic targets for inflammatory diseases, Nat. Rev. Drug Discov. 2 (2003) 717–726.