Cell. Mol. Life Sci. (2015) 72:4127–4137
DOI 10.1007/s00018-015-1996-x

Cellular and Molecular Life Sciences

REVIEW

Regulation of αA- and αB-crystallins via phosphorylation in cellular homeostasis

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Received: 14 May 2015 / Revised: 10 July 2015 / Accepted: 16 July 2015 / Published online: 26 July 2015
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Abstract αA-Crystallin (αA) and αB-crystallin (αB) are small heat shock proteins responsible for the maintenance of transparency in the lens. In non-lenticular tissues, αB is involved in both maintenance of the cytoskeleton and suppression of neurodegeneration amongst other roles. Despite their importance in maintaining cellular health, modifications and mutations to αA and αB appear to play a role in disease states such as cataract and myopathies. The list of modifications that have been reported is extensive and include oxidation, disulphide bond formation, C- and N-terminal truncation, acetylation, carboxymethylation, carboxyethylation, carbamylation, deamidation, phosphorylation and methylation. Such modifications, notably phosphorylation, are alleged to cause changes to chaperone activity by inducing substructural changes and altering subunit exchange dynamics. Although the effect modification has on the activities of αA and αB is contentious, it has been proposed that these changes are responsible for the induction of hyperactivity and are thereby indirectly responsible for protein deposition characteristic of many diseases associated with αA and αB. This review compiles all reported sites of αA and αB modifications, and investigates the role phosphorylation, in particular, plays in cellular processes.

Keywords Chaperones · PTMs · Structure · Proteostasis

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Introduction

The human lens proteome is dominated by the crystallins, a long-lived family of proteins that encompasses the α-, β- and γ-crystallins. In the lenticular environment, the crystallins are susceptible to the accumulation of post-translational modifications, due largely to their extended life spans. Such modifications, notably phosphorylation, have also been reported in extralenticular αB, often being implicated in the regulation of the protein's activity [3, 36–50]. Although such modifications have been extensively reported in the lens in recent years [23–25, 27–31], the implications, particularly for αA and αB functions, have yet to be characterised.

The crystallins

The two subunits of α-crystallin, αA and αB, also known as HSPB4 and HSPB5, respectively, both have an approximate molecular mass of 20 kDa [51], share 60 % sequence similarity [52], and exist in the cytoplasm of the lens fibres at a ratio of three A subunits to one B subunit [53] where they form a dynamic polydisperse population of hetero-oligomers with an average mass of ~800 kDa [51]. α-Crystallin oligomers exist in a state of dynamic equilibrium, undergoing subunit exchange where αA and αB subunits are readily interconverted [54]. As a member of the sHsp family [55–57], each subunit contains a highly conserved α-crystallin domain containing 5 β-strands labelled β2- β5, β6/7, β8 and β9 [58]. The α-crystallin domain is flanked by the C- and N-terminal domains [59]. The N-terminal domain contains an antiparallel β-sheet involving the residues 44–65 [60], while the C-terminal domain is highly variable and is capped with a flexible

C-terminal tail [61]. Dimers, the building block of the oligomer [60, 62], are formed via interactions between the β6/7 strands of the α-crystallin domain of two monomers [60, 63, 64]. They form an antiparallel β-sheet at the interface [63, 64], while the β4 and β8 strands form a groove lined with charged amino acids [60, 65] that participate in ionic bonds that stabilise the complex [62–64]. Hydrophobic binding sites within the groove bind the C-terminal I-X-I motif of neighbouring dimers to form hexamers [60, 62–66]. Due to difficulty in achieving protein crystallisation, the structure of larger oligomers is based largely on conjecture with multiple models being proposed [60, 64–66]. However, it is generally believed that the hexamers interact to form larger structures that feature a roughly spherical shape with binding sites located either as pockets on the surface [60], or within the hollow interior accessible through openings in the shell [52, 64, 67], as depicted in Fig. 1. It has been reported that αB shows a bias towards a 24meric structure [64], that allows the formation of differently sized constructs via either the association of extra dimers to the external pockets [60] or the addition and subtraction of subunits to the shell itself [64, 66]. In the latter, the subtraction of subunits would lead to the exposure of hydrophobic regions found on the inner walls [64] and extensions [65], and the production of higher activity smaller oligomers [34] and, eventually, monomers [32, 68], allowing for the regulation of activity mediated by the dissociation of subunits. The molecular changes to dimers required for these dynamic interconversions, which involve the breakage of two C-terminal interactions, are reportedly minor, with only a slight change to the angle of interactions of the C-terminal regions and the groove observed [66]. Polydispersity is thought to be achieved as a result of the reverse and forward binding of the palindromic I-X-I motif to the β4–β8 groove, and the presence of 3 different registration shifts at the interface [58]. Considering the homogeneity of the oligomers, it is likely that no currently proposed model fully characterises the true quaternary structure of α-crystallin, especially as many focus on the 24meric oligomer [60, 64], potentially overstating the prevalence or importance of the hexameric substructure. Instead, it is more likely that the true structure resembles an amalgamation of various proposed models [69].

Chaperone activity of α-crystallin

α-Crystallin oligomers have been reported to exist in two substructural formations: a high-affinity monomeric and a low-affinity dimeric substructures [68]. The major substrates of αA and αB in the lens fibre cell are λ- and β-crystallin [21], although interactions with 'house-keeping' enzymes such as enolase [70], cytoskeletal elements [71] and membrane-associated proteins also occur [72, 73]. It appears that the quaternary structure of the protein with a hydrophilic exterior and a hydrophobic interior, is vital to chaperone activity, as it has been observed that the structure is conserved [74] and resistant to changes in the amino acid sequence [61]. Furthermore, the addition of a negative charge to the protein surface such as through citraconylation has been seen to increase activity [75]. This is significant considering that various PTMs, which can alter surface charge, have also been found to affect the chaperone activities of αA and αB [57, 76–78].

α-Crystallins in health and disease

While αA is found almost exclusively in the lens [52] with trace amounts being detected in the thymus, spleen, retina [79], liver, kidney and pancreas [80], αB is also found in relatively high amounts in non-lenticular tissue, such as the brain, heart, muscle tissue [52], nervous system, retina, iris, thyroid, colon, squamous epithelia, placenta, spermatocytes and tissues subjected to high oxidative stress such as in the kidneys [79]. Expression of αB has been found to be induced by infection [81], oncogene expression [82], osmotic stress [83], heat shock [56], dexamethasone, cadmium, sodium arsenite [84] and muscle stretch [79, 85].

As αB is expressed ubiquitously throughout body tissues, the functions it serves in maintaining health are highly diverse. In the lens, the crystallins are vital in lens transparency by maintaining a medium that is optimal for light transmission [86]. In non-lenticular tissue, however, αB has often been associated with neurodegenerative disorders, for example being overexpressed and co-deposited in protein inclusion bodies such as Rosenthal fibres associated with Alexander's disease [87], where it is believed to serve a neuroprotective role by preventing the aggregation of GFAP [6]. It has also been shown that αB has anti-apoptotic properties, preventing cell death in response to disease such as stroke [88] or infection [81], and preventing damage caused by the inflammation response itself [81]. αB is also known to be involved in maintenance of the cytoskeleton, binding to and stabilising components of the cytoskeleton in response to stress such as ischaemia [1]. Considering αA's comparatively low abundance in non-lenticular tissues, its role outside the lens has not been as extensively studied. However, it has been implicated with cases of pancreatic cancer, where it has been proposed that it inhibits carcinogenesis by retarding cell migration, as well as activating protein-1 (AP-1) and upregulating components of the TGFβ signalling pathway [80]. These roles are summarised in Table 1.

Despite its overall role in maintaining the health of cells, overexpression or mutations in αB have been associated with particular disease states (Table 2) [87, 91]. For

example, it is believed that the accumulation of αB may cause the deterioration of the microenvironment surrounding neurons associated with Alzheimer's Disease [87]. αB has also been identified as an oncoprotein, where it promotes neoplastic changes and invasiveness and prevents apoptosis in basal-like breast carcinomas [93]. αB has also been implicated in the increased risk of recurrence in head and neck cancers, [94] as well as the inhibition of TRAIL-induced apoptosis in various cancer cell lines [95]. Mutations in αA and αB have been associated with cataract [7–16] and various myopathies [10, 17–20].

Central to all of these functions is the ability of αA and αB to act as a molecular chaperones, which in the lens is also essential for the maintenance of lens transparency.

Post-translational modification of α-Crystallin

Owing to the protein longevity and the negligible protein turnover of the lens, lenticular αA and αB are vulnerable to the accumulation of PTMs with age. Such modifications are first detectable in the foetal lens [96] with the modification profile changing little after 17 years of age [97]. The levels of intact αA and αB itself have been shown to decrease with age, with little full-length isoforms remaining after 75 years of age [53]. However, αA is lost at a greater rate, suggesting αB has a higher level of stability [53].

Modifications that have been reported are summarised in Table 3.

When considering the effect such modifications have on the structure and function of αA and αB, it has become apparent that a holistic approach is required, treating it as a complex system of interacting modifications rather than standalone events. For example, in vitro experiments performed by Chaves et al. [107] and Asomugha et al. [108] showed that deamidation of αA and αB at N123 and N146, respectively, and truncation alter structure and function, leading to a decrease in chaperone function [107] and an increase in β-sheet formation and oligomeric size [108]. However, when present on the same protein, an ameliorating effect was seen with the loss of activity being limited [108]. Furthermore, while deamidation, C-terminal truncation and a combination of the two produced a compact tertiary structure, N-terminal truncation and a combination of deamidation and N-terminal truncation resulted in a relaxed tertiary structure [108].

In the context of the lens, N- and C-terminal truncation, oxidation of methionine residues, deamidation [21, 22], cleavage at deamidated residues [22], phosphorylation and intramolecular disulphide bond formation [21] have all been associated with the insoluble protein fraction. This has led to the implication of such modifications in protein insolubilisation events. One mechanism proposed involves deamidation-induced conformational changes that lead to disulphide bond formation between oxidised cysteine residues [21, 22] and exposure of the hydrophobic interior [21]. Insoluble complexes form as a consequence and are held together by strong hydrogen bonds [21]. Although various modifications have been associated with this process, this paper focuses on phosphorylation, as it is one of the most abundant and most widely researched modifications to both αA and αB.

Table 1 Suggested roles of αA- and αB-crystallins in non-lenticular tissue

Functions	References
αA	
Inhibition of carcinogenesis	[80]
αB	
Anti-apoptosis	[4, 42, 81, 89, 90]
Protection against oxidative damage	[4, 89]
Maintenance of cytoskeletal integrity	[1–4]
Suppression of neurodegeneration	[5, 6]
Formation of inclusion bodies in protein conformation diseases	[4, 10, 87, 91, 92]
Promotion of neoplastic changes and invasiveness of cancerous cells	[93, 94]
Anti-inflammation	[88, 90]

Table 2 Pathologically significant mutations of αA and αB in humans

Disease	Species	Mutation	References
Autosomal dominant congenital cataract	αB	D140N	[9]
		450delA	[8]
		P20S	[11]
		R120G	[10]
	αA	R116C	[7, 12]
		R49C	[13]
		R116H	[14]
		R54P	[15]
Autosomal recessive congenital cataract	αA	W9X	[16]
Desmin-related myopathy	αB	R120G	[10]
Dilated cardiomyopathy	αB	R157H	[17]
		G157S	[18]
Myofibrillar myopathy	αB	Q151X	[20]
		464delCT	[20]
Late-onset distal vacuolar myopathy	αB	G154S	[19]

Phosphorylation of αA and αB

33–50 % of lenticular αA and αB has been reported to undergo phosphorylation [74, 86], with phosphorylated

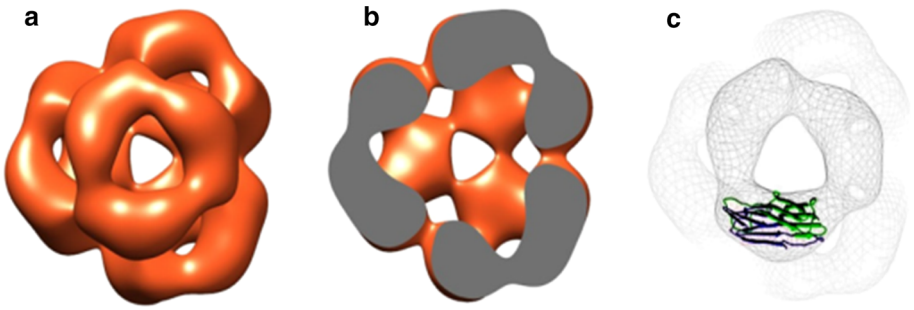

Fig. 1 Model of recombinant human αB quaternary structure. Depicted as surface representation (**a**), density cross section (**b**) and with dimeric α-crystallin domain of Hsp16.5 from *M. jannaschii* overlay (**c**). Adapted from Peschek et al. [52]

products being present from birth [96]. The phosphorylation status of the crystallins is in dynamic equilibrium under physiological conditions [109] and in dividing, differentiating or newly differentiated cells, like that of the lenticular epithelium [96]. Phosphorylation of αA and αB can also be induced by oxidative agents such as Fenton reagent [32, 110, 111], and by stress conditions such as ischaemia [35] and heat stress [109].

In vivo, the majority of αA and αB is phosphorylated via the cAMP-dependent pathway in a reversible serine-specific manner [79]. It has been a long-held belief that the dominant sites of phosphorylation in α-crystallin are Ser19, Ser45 and Ser59 for αB, and Ser122 for αA. The enzymes believed to be responsible for enzymatic phosphorylation at these sites are p44/42 MAP kinase for the Ser19 and Ser45 residues of αB and MAPKAP kinase 2 for the Ser59 residue of αB and Ser122 residue of αA [112]. Despite the dominance of these sites in the literature, a multitude of other sites of phosphorylation, including at threonine and tyrosine residues, for both subunits have been recently reported (Table 4).

Although much less prominent in the literature, non-enzymatic autophosphorylation has also been observed in lenticular αA and αB [79]. A little is known about the mechanism of autophosphorylation in the lens, but it has been shown in vitro that both αA and αB are able to undergo non-enzymatic phosphorylation in the presence of either Mn^{+} [113] or Mg^{+} [114] and ATP. Notably, it has been shown that under conditions of limited cAMP-dependent kinase concentration, αB experiences 10 × higher rates of phosphorylation than αA, while incubation with 1 % deoxycholate causes the disaggregation of αA into tetramers and a 10× higher rate of autophosphorylation compared to αB [115], suggesting preferences for different phosphorylation pathways. Recently, a mechanism of non-enzymatic dephosphorylation has also been proposed that involves the spontaneous β-elimination of phosphate groups from phosphoserine and phosphothreonine residues and the subsequent glutathionylation of the resultant dehydroalanine [116] intermediate [28]. The presence of such non-enzymatic pathways in the lens is significant as they provide a mechanism of reversible phosphorylation in a tissue that is generally believed to be largely metabolically and enzymatically inactive. Although it is as yet unclear what proportion of the phosphorylation observed in the lens is residual from early enzymatic phosphorylation events, and how much results from subsequent non-enzymatic events, it is likely that αA and αB enzymatically phosphorylated in the metabolically active cells of the outer cortex or gradually dephosphorylated non-enzymatically as it forms part of the metabolically inactive nucleus, where a dynamic balance of non-enzymatic phosphorylation and dephosphorylation events occur.

The effect phosphorylation has on the chaperone activity of αA and αB, if any, has met with contention in the literature. Earlier studies by Ito et al. [120] reported a decrease in chaperone activity when thermal aggregation assays were performed utilising phosphomimics. They attributed this to an observed decrease in oligomeric size. These findings were supported by Kamei et al. [117] who reported a 30 % decrease in the ability of bovine phosphoextracts to prevent the heat-induced aggregation of β_L-crystallin. A complimentary study by Augusteyn et al. [118], however, reported no effect on chaperone activity. More recently, studies utilising phosphomimics have found that the addition of one or more negative charges onto αB acts to increase target protein affinity [33, 119], increase oligomeric polydispersity [33], increases the rate of subunit exchange, decreases stability [35] and decrease average oligomeric size [33, 120]. It has been proposed that such modifications to the N-terminal domain region of αA and αB induce flexibility and structural changes that lead to increased exposure of substrate binding sites [34]. Phosphorylation in this region has also been shown to interrupt intersubunit interactions, leading to the dissociation of larger oligomers to form smaller oligomers with higher activity [33, 34]. It has been suggested that this hyperactivity induces aggregation rather than prevents it, as αA and αB reach saturation and codeposit with the target proteins at an earlier stage [33]. This is supported by a study by Aquilina et al. [32] that reported both a change in oligomeric substructure i.e. a preference towards high-affinity monomeric substructure and away from low-

Table 3 Modification sites identified in human lens αA and αB

Subunit	Modification	Residue	References
αA	Oxidation	Met1	[21–23, 30, 98, 99]
		Trp9	[30, 98, 99]
		Met138	[21–24]
		Tyr18	[24]
		Tyr34	[24]
		His154	[99]
	Disulphide bond	Cys131–Cys142	[21, 22]
	Deamidation	Gln6	[21–23, 30]
		Gln50	[22, 23]
		Gln90	[21, 22, 26, 30]
		Asn101	[22, 23]
		Gln104	[21, 26]
		Asn123	[23, 26, 30]
		Gln126	[23, 26]
		Gln147	[21, 22, 26, 30]
	Truncation	Met1–Gln50	[22]
		Glu102–Ser173	[22]
		Ser173	[22]
		Met1–Arg65	[21]
		Met1–Phe80	[21]
		Ala152–Ser173	[22]
		Ser169–Ser173	[22]
	Acetylation	Met1	[23, 99]
		Lys70	[24, 100, 101]
		Lys78	[24]
		Lys88	[24]
		Lys145	[24]
	Carboxymethylation	Lys11	[23]
	Carboxyethylation	Lys11	[23]
	Carbamylation	Lys99	[23]
	Methylation	His79	[99]
		His154	[23]
		Arg21	[24]
		Lys88	[24]
		Val89	[99]
		Gln90	[99]
		Ile146	[99]
		Gln147	[99]
		His154	[99]
		Arg157	[99]
	Ethylation	Thr13	[99]
		His79	[99]
		Val89	[99]
		Ile146	[99]
	Formylation	His79	[98]
	Conversion to DHA	Ser59	[98]
	dkpD formation	Asp67	[102]

Table 3 continued

Subunit	Modification	Residue	References
αB	Oxidation	Met1	[21–23, 25, 99, 103]
		Trp9	[23, 99]
		Trp60	[24, 99]
		Met68	[21–25, 99, 103]
		Tyr48	[24]
	Deamidation	Gln26	[23, 26]
		Asn78	[21, 26]
		Gln108	[21, 22, 26, 104]
		Asn146	[23, 26, 30, 99]
	Carboxymethylation	Lys82	[23]
		Lys90	[23]
		Lys92	[23]
		Lys103	[23]
		Lys174	[23]
	Carboxyethylation	Lys92	[23]
		Lys175	[23]
	Acetylation	Met1	[23, 99, 103, 105]
		Lys92	[24, 106]
	Methylation	His83	[23, 99]
		Arg22	[24]
		Arg50	[24]
	Ethylation	His8	[99]
		Val93	[99]
		Gln108	[99]
	Carbamylation	Met1	[99]
		Glu164	[99]
		Lys166	[23]
		Lys92	[99, 106]
		Lys175	[101]
	Truncation	Met1–Pro46	[105]
		Gln151–Lys175	[103]
		Val152–Lys175	[103]
		Ser153–Lys175	[103]
		Gly154–Lys175	[103]
		Pro155–Lys175	[103]
		Arg163–Lys175	[103]
		Glu164–Lys175	[103]
		Ala171–Lys175	[103]
		Ala172–Lys175	[25]
		Lys175	[22, 103, 105]
		Pro130–Lys175	[104]
		Met1–His7	[22]

affinity dimeric substructure, coupled with an earlier onset of the reduction-induced aggregation of α-lactalbumin. Taken together, it appears that the hyperactivity observed recently upon phosphorylation of αA and αB is a result of

Table 4 Phosphorylation sites of human lens αA and αB

Subunit	Site	References
αA	S13	[28]
	S20	[27, 28]
	S45	[24, 27]
	S51	[27]
	S59	[27, 28]
	S62	[27, 28]
	S66	[27, 29]
	S81	[27, 29]
	S122	[23, 24, 27, 30, 31]
	S127	[27]
	S130	[27]
	S134	Thornell, unpublished
	S140	[24]
	S162	[27, 28]
	S172	[27]
	S173	[27]
	T13	[24, 27]
	T43	[27]
	T55	[27]
	T86	[27]
	T140	[24]
	T148	[27–29]
	T168/S169	[27]
	T153	[27, 29]
	Y47	[27]
	Y118	[27]
αB	S19	[23, 24, 27, 29, 31, 103]
	S21	[24, 27, 29]
	S43	[24, 27]
	S45	[24, 27, 31]
	S53	[24, 27, 28]
	S59	[23–25, 27–31]
	S66	[27]
	S76	[23, 24, 27, 29]
	S85	[27]
	S136	[27]
	S138	[27, 28]
	S139	[27–29]
	S153	[27]
	T63	[27]
	T170	[28]
	T132	[27]
	T134	[27]
	T158	[27]

the increased exposure of substrate binding sites [34] brought about by the dissociation of dimeric oligomer substructures [32]. This process also provides a mechanism to regulate the activity of αA and αB in non-lenticular tissues.

Roles of phosphorylated αA and αB

In the context of the lens, a little is known of the significance of αA or αB phosphorylation. However, reports of the involvement of phosphorylated αB in various pathways including stress response [39, 40, 42, 44–46, 48, 49, 88], the regulation of apoptosis [38, 50], actin dynamics [3, 39, 40] and protein quality control [36, 41] suggest that it is pivotal in many cellular processes in non-lenticular tissues.

It has been widely reported that phosphorylation of αB is induced in response to various stresses via activation of the p38/MAPK pathway [3, 36, 45, 49]. Although this appears to often be an intrinsic step in the cytoprotection of cells by αB, the protective pathways that follow are wide and varied. In cardiomyocytes subjected to induced ischemic stress, phosphorylation of αB at the Ser59 residue [42, 44] and Ser45 [44] was accompanied by concurrent translocation of 15–20 % of the total αB pool to the z-lines of sarcomeres [37, 42–44]. Although it was reported that the translocation was not due exclusively to the phosphorylation event [44], inhibiting phosphorylation at Ser59 leads to cell death [42]. It was proposed that the cytoprotective properties of associated phosphorylated αB were due to the stabilisation of myofibrils [42] and the prevention of osmotic stress and associated swelling [44]. αB has also been reported to inhibit caspase-3 activation in both cardiomyocytes in response to hyperosmotic and hypoxic stress [46] and in differentiating myoblasts [50]. In the case of stressed cardiomyocytes, cell death was prevented by phosphorylation at Ser59 [46]. However, the cytoprotective properties of nonphosphorylated αB in preventing differentiation-induced apoptosis in myoblasts were lost following multiple phosphorylation [50], indicating that the phosphorylation is involved in both positive and negative regulation of apoptotic processes under different conditions. Phosphorylation at Ser59 has also been implicated in the expression and regulation of the cytoprotectant protein, Bcl2 [38, 45]. Exposure of myoblasts to TNF-α has been seen to induce phosphorylation of αB, leading to a cascade of events resulting in the nuclear uptake and increased expression of Bcl2 [45]. In conjunction with this, exposure to vinblastine, a microtubule-depolymerisation agent, has been shown to induce αB phosphorylation in breast epithelial carcinoma cell line MCF7, inducing association with Bcl2 [38]. It is believed that this prevents translocation of Bcl2 into the mitochondria, repressing Bcl2 anti-apoptotic ability [38], thereby limiting cell immortality and cancer pathogenesis. Dual phosphorylation at Ser59 in conjunction with Ser45 induced by PAR-2 activation has also been implicated in the protection of astrocytes against

Table 5 Role of phosphorylated αA and αB in lenticular and non-lenticular tissues

Role	Mechanism	Residues	References
Cytoprotection (inhibition of apoptosis)	Localisation to Z-bands of myocytes following ischaemia.	Ser59	[37, 42, 43]
		Ser45 and Ser59	[44]
	Induces Bcl2 expression in myoblasts in presence of TNF-α.	Ser59	[45]
	Inhibits caspase-3 activation in myocytes following hyperosmotic or hypoxic stress.	Ser59	[46]
	Nuclear speckle formation following heat shock.	Ser45 and Ser59	[47, 48]
	Activated by PAR-2 in astrocytes in presence of C2-ceramide and staurosporine.	Ser45 and Ser59	[49]
Regulation of apoptosis (promotes apoptosis)	Reduces anti-apoptotic activity of αB in differentiation-induced cell death of myoblasts.	Ser19, Ser45 and Ser59	[50]
	Associates with and inhibits anti-apoptotic activity of Bcl2 in vinblastine treated breast epithelial carcinoma cells.	Ser59	[38]
Modulation of actin dynamics	Associates with and stabilises cytoskeletal components at focal adhesions in myoblasts treated with vinblastine and cytochalasin-D.	Ser59	[3]
	Associates with proteins of the actin nucleation complex involved with membrane protrusion and cell migration in migratory lenticular epithelial cells.	Ser59	[39]
	Negatively modulates actin depolymerisation in vitro.		[40]
Protein quality control	Participates in the ubiquitin–proteasome system to degrade excess protein in disused rat soleus muscle.	Ser59	[36]
	Accumulates in and potentially facilitates formation of aggresomes in MG-132-treated glioma cells.	Ser59	[41]

cytotoxic stress induced with C2-ceramide and staurosporine [49], and in the formation of nuclear speckles in HeLa cells [47, 48]. It was reported that αB phosphorylated at Ser59 interacts with Gemin-3, part of the Survival Motor Neuron complex [48], and is translocated into the nucleoplasm where phosphorylation at Ser45 is involved in speckle formation [47]. Nuclear speckles act as depots to ensure a readily available source of chaperone in the event of cellular stress, upon which αB is then released from the speckles to prevent cell death [48]. Interestingly, the hyperphosphorylated R120G αB mutant is unable to be translocated into the nucleus due to the formation of cytoplasmic inclusions [47]. It has been reported that αB which preferentially phosphorylated at Ser59 accumulates in brains from Alexander's [121, 122] and Alzheimer's disease patients [121]. However, as similar accumulation was also seen in aged control brains [121, 122], it remains unclear whether phosphorylation state is important for neuroprotective properties of αB in neurodegenerative disorders.

Observations of increased phosphorylation at the Ser59, and to a lesser extent the Ser45 residue and concurrent translocation of αB to the insoluble fraction of rat soleus muscle lead to the suggestion that phosphorylated αB is involved in the ubiquitin–proteasome system [36], which is involved in the degradation of proteins. This was supported by the observation that treatment of U373 MG human glioma cells with the proteasome inhibitor MG-132 induced phosphorylation of αB at Ser59 which was accompanied by translocation to aggresomes [41]. It was proposed that αB alters the structure of intermediate filaments, facilitating aggresome formation, in conjunction with targeting and degrading ubiquitinated proteins [41]. However, although phosphorylation was induced by MG-132 treatment, it was not seen to be required for aggresome association [41], implying a different role for the phosphorylated form.

It has been shown that any perturbation to the cytoskeletal structure i.e. actin, microfilaments, microtubules or intermediate filaments, induces the p38/MAPKAP-2 pathway and thereby phosphorylation of αB at Ser59 [3]. Phosphorylated αB is believed to associate with and stabilise cytoskeletal components [3], but in murine myoblasts treated with vinblastine and cytochalasin-D, known disrupters of the cytoskeleton, phosphorylated αB was also seen to relocate to cytoplasmic focal adhesions where actin stress fibres attach [3]. In the lens, both αA and αB have been seen to localise at the leading membrane of migrating epithelial cells, suggesting that they are involved in actin dynamics including those associated with membrane protrusion and cell adhesion in cell mobility [39].Unlike αA, however, the association of αB appears to be mediated by phosphorylation at Ser59, leading to interactions with the proteins Arp3, a component of the actin nucleation complex, WAVE-1, Abi

and β-catenin, which is involved in cell adhesion, and the actin meshwork [39]. Actin polymerisation is also believed to be modulated by both αA and αB [40]. Unphosphorylated αA and αB have both been shown to inhibit the cytochalasin-D-induced polymerisation of actin, an ability that is lost upon phosphorylation [40]. This suggests that the dynamic phosphorylation state of both subunits is involved in the regulation of actin polymerisation and depolymerisation events during cellular remodelling processes such as those associated with epithelial cell differentiation in the lens [40]. Information on the significance of αA phosphorylation in non-lenticular tissue is sparse due to its relatively low abundance. However, if αA phosphorylation is of more import in the lens than in other tissues due to its higher abundance, it may also help explain why αA is relatively more susceptible to autophosphorylation compared to αB [115], as the enzymatic activity in the lens is somewhat suppressed.

These roles are summarised in Table 5.

It has become clear that modifications to αA and αB such as phosphorylation not only are responsible for the regulation of chaperone activity of the proteins [32–34, 120], but it is greatly influential in the roles they take on in cellular processes [3, 36–50]. Although phosphorylation, particularly of αB, appears to be important in maintaining cellular health, it may also be involved in the disruption of proteostasis that can result in protein deposition diseases such as Alexander's disease [121, 122]. Further understanding of the processes that contribute to these conditions is therefore vital, particularly when considering the longevity of the proteins involved and their propensity to be modified in such a way.

Acknowledgments The help of Heath Ecroyd with the editing of this paper is gratefully acknowledged.

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