The Small Heat-shock Protein αB-Crystallin Promotes FBX4-dependent Ubiquitination*

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αB-Crystallin is a small heat-shock protein in which three serine residues (positions 19, 45, and 59) can be phosphorylated under various conditions. We describe here the interaction of αB-crystallin with FBX4, an F-box-containing protein that is a component of the ubiquitin-protein isopeptide ligase SCF (SKP1/CUL1/F-box). The interaction with FBX4 was enhanced by mimicking phosphorylation of αB-crystallin at both Ser-19 and Ser-45 (S19D/S45D), but not at other combinations. Ser-19 and Ser-45 are preferentially phosphorylated during the mitotic phase of the cell cycle. Also αB-crystallin R120G, a mutant found to co-segregate with a desmin-related myopathy, displayed increased interaction with FBX4. Both αB-crystallin S19D/S45D and R120G specifically translocated FBX4 to the detergent-insoluble fraction and stimulated the ubiquitination of one or a few yet unknown proteins. These findings implicate the involvement of αB-crystallin in the ubiquitin/proteasome pathway in a phosphorylation-and cell cycle-dependent manner and may provide new insights into the αB-crystallin-induced aggregation in desmin-related myopathy.

αB-Crystallin is a 20-kDa protein that is highly expressed in eye lens and muscle tissues and to a lesser extent in many other tissues such as brain, skin, and kidney (1). It is a member of the family of small heat-shock proteins (HSPs),1 which are characterized by the presence of a conserved α-crystallin domain (2). Ten different small HSPs are expressed in human (3), mostly in muscle tissues (1). An important property of αB-crystallin is its ability to bind unfolding proteins (4). This chaperone-like activity might help to prevent protein aggregation during stress conditions (5) and thus increase the stress resistance of the cell (6, 7). More specific functions of αB-crystallin are its apparent inhibition of apoptosis, possibly by preventing the activation of procaspase-3 (8), and its association with cytoskeletal components under normal conditions (9), which is more pronounced during stress (10–12). The interaction with type III intermediate filaments might modulate the assembly of these proteins in the cell and prevent inappropriate interactions between bundled intermediate filaments (13). αB-crystallin can be phosphorylated at three different positions: Ser-19 and Ser-45 are mainly phosphorylated during mitosis, and Ser-59 under various stress conditions (14, 15). Although the phosphorylation of αB-crystallin is thus clearly regulated by the cellular conditions, it is not clear what role phosphorylation plays in the functioning of αB-crystallin.

A missense mutation in αB-crystallin (R120G) has been shown to co-segregate with desmin-related myopathy in a French family (16). Desmin-related myopathies are usually adult-onset neuromuscular diseases characterized by the accumulation of aggregates of cytoplasmic desmin in conjunction with other proteins. In this French family, these inclusion bodies were found to contain large amounts of αB-crystallin R120G. Interestingly, αB-crystallin is also found in cytoplasmic inclusions in various neurological disorders such as Alzheimer’s, Parkinson’s, Huntington’s, Alexander’s, and diffuse Lewy body disease (5, 17, 18), but its role in these diseases remains elusive.

The ubiquitin/proteasome pathway plays a prominent role in the pathogenesis of most of these neurodegenerative disorders (19). Ubiquitin-positive proteins are consistently found in the inclusion bodies that are characteristic of these diseases (20, 21). This suggests that the proteins responsible for aggregate formation may have a role in ubiquitin-dependent degradation (21, 22). Rapid protein degradation via the ubiquitin/proteasome pathway is dependent on the ubiquitination of the substrate protein by a ubiquitin ligase (23, 24). There are many different ubiquitin ligases, which can be classified into two families, those containing either RING finger domain proteins or HECT domain proteins (25). The ubiquitin-protein isopeptide ligase SCF (SKP1/CUL1/F-box) complex, which also contains the RING finger protein RBX1, captures the target protein via the F-box protein (26–30). The F-box is a conserved domain of ~40 residues that is responsible for recruiting bound target proteins to the SCF complex by interacting with SKP1 (27). Ubiquitin moieties are then transferred through the SCF complex to the target protein, ultimately resulting in the attachment of a polyubiquitin chain (31). This tag is readily recognized by the 26 S proteasome and leads to the degradation of the target protein (32). F-box proteins capture their targets often in a phospho-dependent manner (27, 29) and generally have obvious domains for target protein interactions such as leucine-rich repeats or WD40 repeats (26, 27, 29).

In this study, we identify the F-box protein FBX4 as an interactor of αB-crystallin. The interaction with FBX4 seems to depend on the phosphorylation status of αB-crystallin, but is also enhanced by the mutation R120G. Binding of FBX4 to αB-crystallin stimulates the ubiquitination of a detergent-insoluble protein, which probably destined this protein for ubiquitin-dependent degradation.

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1 The abbreviations used are: HSPs, heat-shock proteins; HA, hemagglutinin; CHAPS, [3-[3-cholamidopropyl]dimethylammonio]1-propane-sulfonic acid.
**Experimental Procedures**

**Plasmids, Cell Culture, Transfections, and Antibodies**—The coding regions of wild-type human α-crystallin, α-crystallin S19A/S45A (kindly provided by Jack Liang, Harvard Medical School, Boston), and human α-crystallin R116C cDNAs were cloned into the polylinker region of the expression vector pIRES (Clontech) and the yeast two-hybrid vectors pEG202 and pJG4-5. Mutations were introduced site-directed mutagenesis (Stratagene). The complete coding region of FBX4 cDNA was obtained by reverse transcription-PCR from total HeLa RNA and cloned into the pIRES, pEG202, and pJG4-5 vectors. SKP1 cDNA was obtained by PCR using the HeLa library as a DNA source and cloned into the pEG202 and pJG4-5 vectors. The cDNAs coding for vimentin and desmin were cloned into the yeast two-hybrid vectors pEG202 and pJG4-5 vectors. DNA fragments encoding the sequence of HA-ubiquitin were cloned in the pBSK II- vector (kindly provided by Dirk Bohmann, European Molecular Biology Laboratory, Heidelberg, Germany).

HeLa cells were grown at 37 °C in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum (PAA Laboratories), 100 units penicillin, and 200 μg/ml streptomycin in the presence of 5% CO2. Transfections of plasmids into HeLa cells were performed by lipofection using the FuGENE™ 6 system (Roche Molecular Biochemicals) as described by the manufacturer. Immunoblotting was performed with mouse monoclonal anti-α-crystallin antibody (Riken Cell Bank), monoclonal anti-HA antibody (Roche Molecular Biochemicals), monoclonal anti-HSP70 antibody (Stressgen Biotech Corp.), monoclonal anti-β-actin antibody (Sigma), and rabbit polyclonal anti-FBX4 serum (obtained from a rabbit immunized with purified recombinant FBX4 protein).

**Two-hybrid Screening**—For the interaction screening, yeast strain EGY48 (ura3 trp1 his3 leu2-3,112; Clontech), containing bait plasmid pEGα-crystallin and LacZ reporter plasmid pK103, was transformed with the pBG-α-crystallin (kindly provided by Roger Brent, The Molecular Sciences Institute, Berkeley, CA). Transformants were selected on plates lacking histidine, uracil, and tryptophan. A total of ~1 × 106 transformants were obtained. After induction of prey expression, ~3 × 106 colony-forming units were plated on galactose plates lacking leucine to select for clones coding for proteins able to interact with the α-crystallin fusion protein.

**Northern Blot Analysis**—The tissue distribution of FBX4 and α-crystallin mRNAs was analyzed using human multiple-tissue Northern blots (Clontech). A 710-bp BglII-Ncol cDNA fragment of FBX4 and a 342-bp BamHI cDNA fragment of human α-crystallin were labeled with [α-32P]dCTP using a random primer kit (Invitrogen). Hybridization was performed at 65 °C in 0.25 M Na2HPO4, 7% SDS, 1% bovine serum albumin, 1 mM EDTA, and 100 μg herring sperm DNA. Following hybridization, filters were washed under high stringency conditions (0.025 M Na2HPO4, 1% SDS, and 1 mM EDTA at 65 °C) and visualized by autoradiography. The blots were first hybridized with the FBX4 probe, stripped, and subsequently reprobed for α-crystallin.

**Detergent Solubility**—Cells were harvested by trypsinization and solubilized by sonication. Protein concentrations were determined using the 2-D Quant kit (Amersham Biosciences), and the samples were further diluted to appropriate concentrations in 2 mM thioura, 6 mM urea, 0.8% Immobilized pH gradient buffer 3–10 (Amersham Biosciences), 2% CHAPS, 10 mM diethiothreitol, and bromphenol blue. Four micrograms of protein was analyzed on pH 3–10 gradient strips (7 cm; Amersham Biosciences) by rehydration loading. After isoelectric focusing, the strips were equilibrated two times for 10 min in 50 mM Tris-HCl (pH 8.8), 6% urea, 30% glycerol, 2% SDS, and bromphenol blue containing 10 mg/ml diethiothreitol and 25 mg/ml iodoacetamide, respectively. The strips were placed on small conventional 12% SDS-polyacrylamide gels. These gels were subsequently blotted and stained for ubiquitin conjugates and α-crystallin.

**RESULTS**

**Yeast Two-hybrid Screening for α-Crystallin-interacting Proteins**—Yeast two-hybrid screening was performed with an α-crystallin-LexA fusion protein (bait) to select proteins (preys) that are able to interact with α-crystallin. We have shown before that the α-crystallin bait is not hampered by its chaperone-like activity and can interact specifically with well known interactors such as α-crystallin and HSP27 (33). The cDNA library used for screening was derived from HeLa cells. HeLa cells have a low expression of α-crystallin and thus may contain α-crystallin-binding proteins. Most of the selected clones contained the cDNA coding for the α-type proteasomal subunit a7/C8, which has previously been shown to be a specific interactor of α-crystallin (33). One of the selected clones contained a cDNA coding for the C-terminal part (residues 179–387) of the F-box protein FBX4. This protein belongs to a large family of F-box-containing proteins, which are components of the SCF ubiquitin-protein isopeptide ligases (26, 28) and function in phosphorylation-dependent ubiquitination of specific substrate proteins. The interaction between α-crystallin and the truncated FBX4 protein seemed to be specific and thus not due to improper folding of the prey because FBX4(179–387) did not interact with the α-crystallin homolog aA-crystallin or with desmin (Table I). However, no interaction with α-crystallin could be detected when full-length FBX4 was used (Table I). Full-length FBX4 seemed to be properly expressed in the two-hybrid system because it was able to bind SKP1 (Table I), which is the F-box-binding component of the SCF complex. Thus, the N-terminal region of FBX4 strongly reduces the affinity of its C-terminal region for α-crystallin.

**Mimicking Phosphorylation of α-Crystallin at Ser-19 and Ser-45 Stimulates Interaction with FBX4**—F-box proteins bind their substrate proteins often in a phosphorylation-dependent manner. Because α-crystallin can be phosphorylated at Ser-19, Ser-45, and Ser-59 (14), it is possible that phosphorylation...
of αB-crystallin is needed to allow interaction with full-length FBX4. These serines were therefore replaced, in different combinations, with negatively charged aspartic acid residues, which mimic phosphorylation (34). As controls, non-phosphorylatable mutants were made in which the serines were replaced with alanines. Interestingly, αB-crystallin S19D/S45D showed a weak but specific interaction with full-length FBX4 compared with wild-type αB-crystallin and S19A/S45A (Table 1). αB-Crystallin mutants in which a single serine or a combination of two other serine residues was replaced did not show any detectable interaction. Furthermore, the S19D/S45D/S59D mutant had a very similar affinity compared with the S19D/S45D mutant. These results indicate that the interaction with FBX4 might be regulated by phosphorylation of αB-crystallin at Ser-19 and Ser-45. It is of interest that phosphorylation of αB-crystallin at these two serine residues is specifically enhanced during the mitotic phase of the cell cycle, whereas phosphorylation of Ser-59 is reduced (15).

αB-Crystallin R120G Stimulates FBX4 Interaction—Aggregate formation might well be affected by the ubiquitination process. For this reason, we were interested whether αB-crystallin R120G, which causes intracellular aggregates, enhances the interaction with FBX4. Interestingly, αB-crystallin R120G was able to bind FBX4 with a similar affinity compared with the S19D/S45D mutant (Table 1). A corresponding missense mutation in αA-crystallin (R116C) that causes congenital cataracts (35) did not stimulate the interaction of αA-crystallin with FBX4 (Table 1). This result suggests that FBX4 might play a role in the aggregate formation caused by the R120G mutation of αB-crystallin.

αB-Crystallin S19D/S45D and R120G Interact with FBX4 in HeLa Cell Extracts—We next wanted to confirm the association of full-length FBX4 with αB-crystallin S19D/S45D and R120G in another protein-protein interaction assay. To this end, co-immunoprecipitation experiments were performed with lysates of HeLa cells cotransfected with different eukaryotic expression constructs (see “Experimental Procedures”). FBX4 was immunoprecipitated from the cell lysates with anti-FBX4 antibodies coupled to protein A-Sepharose beads, and the immunoprecipitates were analyzed on Western blots stained with a monoclonal antibody directed against αB-crystallin. In the lower panel (Input), each lane contained 1% of the HeLa extract used for the immunoprecipitation.

Expression of FBX4—A physiologically meaningful interaction between αB-crystallin and FBX4 requires that both proteins are expressed in similar tissue types. We therefore compared the tissue distribution of FBX4 and αB-crystallin by Northern blotting (Fig. 2). The transcript of FBX4 was found to be ∼1.8 kb, which is in good agreement with the full-length 1.5-kb FBX4 cDNA without a poly(A) tail (GenBank™/EBI accession number NM_012176), and showed a tissue expression profile similar to that presented by Cenciarelli et al. (28). Importantly, most tissues expressing αB-crystallin transcripts also expressed FBX4, although the ratio varied between the different tissues.

FBX4 Cotranslocates with αB-Crystallin to the Detergent-insoluble Fraction—Under stress conditions, αB-crystallin translocates from the detergent-soluble to the detergent-insoluble fraction in a phosphorylation-independent manner (12, 36, 37). However, there are indications that, under non-stress conditions, phosphorylation of αB-crystallin, at least at Ser-59,
might have an effect on detergent solubility (38). For this reason, we were interested in the detergent solubility of aB-crystallin S19D/S45D in HeLa cells under regular culture conditions. We found about twice as much aB-crystallin S19D/S45D in the detergent-insoluble fraction compared with wild-type aB-crystallin or S19A/S45A as determined by quantification (Fig. 3, lower left panel; and data not shown). This suggests that, upon phosphorylation at Ser-19 and Ser-45, aB-crystallin might have an increased affinity for a detergent-insoluble structure. In good agreement with the fact that the R120G substitution stimulates the formation of aB-crystallin-containing aggregates, the pathological hallmark of the disease it causes, we found this aB-crystallin mutant predominantly in the detergent-insoluble fraction (Fig. 3, lower left panel).

We wanted next to analyze the effect of aB-crystallin on the solubility of FBX4. For this purpose, we cotransfected FBX4 with wild-type aB-crystallin or its mutants in HeLa cells (Fig. 3, right panels). When FBX4 was transfected alone, it was completely soluble, and it remained soluble when cotransfected with wild-type aB-crystallin or S19A/S45A. However, when FBX4 was cotransfected with either aB-crystallin S19D/S45D or R120G, a proportional fraction of FBX4 also became detergent-insoluble (Fig. 3, upper right panel). Thus, aB-crystallin S19D/S45D and R120G seem to recruit FBX4 to a detergent-insoluble structure.

Coexpression of FBX4 with aB-Crystallin S19D/S45D or R120G Induces Ubiquitination of Detergent-insoluble Proteins—FBX4, like other F-box proteins, can function as an adaptor molecule to induce the ubiquitination of a bound protein (28). It might thus well be that FBX4 induces the ubiquitination of aB-crystallin S19D/S45D and R120G or of proteins bound to these isoforms of aB-crystallin. To test this, HeLa cells were transfected with a combination of three expression constructs coding for one of the isoforms of aB-crystallin, for FBX4, and for HA-tagged ubiquitin (see “Experimental Procedures”). The HA tag enabled us to determine the total amount of ubiquitinated proteins present in the transfected HeLa cells by Western blotting (39). The distribution of the different aB-crystallin isoforms and FBX4 between detergent-soluble and -insoluble fractions was similar to that found without coexpression of HA-tagged ubiquitin (cf. Fig. 3, right panels; and data not shown). Interestingly, upon coexpression of FBX4 with aB-crystallin S19D/S45D or R120G (upper panels) range in size from 34 kDa to much larger than 98 kDa. The asterisk indicates an unidentified endogenous HA-immunopositive soluble protein.

**Fig. 4.** Ubiquitination is stimulated in the presence of FBX4 and aB-crystallin S19D/S45D or R120G. HeLa cells were cotransfected with pBSSK–HA-ubiquitin (HA-Ub) and either a pIRES construct coding for wild-type aB-crystallin (WT), S19D/S45D (SD), S19A/S45A (SA), or R120G (RG) alone (lanes 3–10) or additionally with pIRES-FBX4 (lanes 13–20). After 2 days, the cells were lysed, fractionated into detergent-insoluble and -soluble fractions (i and s lanes, respectively), subjected to SDS-PAGE and Western blotting, and subsequently stained for the HA epitope (upper panel) and FBX4 (lower panel). The ubiquitinated proteins (X-Ub(n)) range in size from 34 kDa to much larger than 98 kDa. The asterisk indicates an unidentified endogenous HA-immunopositive soluble protein.

**Fig. 5.** HSP70 is not induced upon cotransfection of the different isoforms of aB-crystallin together with FBX4 and HA-ubiquitin. A, HeLa cells were cotransfected with pBSSK–HA-ubiquitin and either a pIRES construct coding for wild-type aB-crystallin (WT), S19D/S45D (SD), S19A/S45A (SA), or R120G (RG) alone or additionally with pIRES-FBX4. Total cell extracts were prepared after 2 days and analyzed by SDS-PAGE and subsequent immunoblotting with anti-β-actin (lower panel), anti-HSP70 (middle panel), and anti-HA (upper panel) antibodies. The ubiquitinated proteins are indicated by X-Ub(n). The sizes of the protein markers are shown on the left in kilodaltons. The asterisk indicates an unidentified endogenous HA-immunopositive protein. B, HeLa cells were subjected to a heat shock at 45 °C for 30 min and analyzed for HSP70 induction: no heat shock (0), 1-h recovery (1), 3-h recovery (3), 6-h recovery (6), and 18-h recovery (18). β-Actin was used as a loading control.
\(\text{aB-crystallin S19D/S45D or R120G, a strong signal of ubiquitinated proteins could be detected in the detergent-insoluble fraction, but not in the detergent-soluble fraction (Fig. 4, compare lanes 15 and 16 and lanes 19 and 20). These ubiquitinated proteins were not detected when FBX4 was coexpressed with wild-type aB-crystallin or S19A/S45A (Fig. 4, lanes 13, 14, 17, and 18). Also other control proteins such as \(\beta_\text{B}-\text{crystallin and HSP27 were tested and found not to stimulate ubiquitination by FBX4 (data not shown), underlining the specificity of the ubiquitination reaction.}

Importantly, the ubiquitination was dependent on the presence of FBX4 because coexpression of aB-crystallin S19D/S45D or R120G with HA-tagged ubiquitin alone gave no or very little ubiquitinated protein in the detergent-insoluble fraction (Fig. 4, compare lanes 5 and 15 and lanes 9 and 19). No higher molecular mass species of aB-crystallin or FBX4 could be detected upon Western blotting with their respective antisera (data not shown), making it unlikely that one of these proteins itself becomes ubiquitinated. These results indicate that aB-crystallin S19D/S45D and R120G are able to ubiquitinate one or more proteins concomitantly with the recruitment of FBX4 to the detergent-insoluble fraction. Similar ubiquitinated products were seen in a mouse C2 cell line (data not shown); thus, the target protein is present in different cell lines.

**HSP70 Is Not Induced upon Overexpression of aB-Crystallin and FBX4**—One could imagine that overexpression of aB-crystallin S19D/S45D or R120G with FBX4 and HA-ubiquitin somehow induces in itself a stress response and that this stress reaction is responsible for the observed ubiquitination. To exclude this possibility, we estimated the levels of the endogenous stress-inducible HSP70 in the transfected cells by Western blotting (Fig. 5). No significant increase in HSP70 levels could be detected upon coexpression with FBX4 and aB-crystallin S19D/S45D or R120G compared with the different control transfected HeLa cells (Fig. 5A, middle panel), whereas upon heat stress, HSP70 was induced (Fig. 5B). A similar negative result was obtained by cotransfecting a stress-inducible reporter construct (data not shown). Thus, overexpression of aB-crystallin S19D/S45D or R120G together with FBX4 induced the ubiquitination of one or more proteins in non-stressed cells (Fig. 5A, upper panel).

**Analysis of Ubiquitination by Two-dimensional Electrophoresis**—To determine the number and sizes of proteins that are ubiquitinated by FBX4 and aB-crystallin S19D/S45D or R120G, the ubiquitinated proteins were analyzed by two-dimensional gel electrophoresis. Each target protein to which an additional amount of ubiquitin was added was detected upon Western blotting with their respective antisera. The shape of the curve is dependent on the charge of the protein. Detergent-insoluble fractions, in which the ubiquitinated proteins were enriched, were isolated from HeLa cells transfected with HA ubiquitin, pIRES-FBX4, and a pIRES construct coding for wild-type aB-crystallin (A), S19D/S45D (B), S19A/S45A (C), or R120G (D). After 2 days, the cells were lysed and fraction into detergent-soluble and -insoluble fractions. The detergent-insoluble fraction was dissolved by ultrasonication in buffer containing 6 M urea, 2 M thiourea, and 2% CHAPS and separated by isoelectric focusing and in the second dimension by 12% SDS-PAGE. After blotting onto nitrocellulose membranes, immunostaining was successively performed for aB-crystallin and the HA epitope. Proteins staining for aB-crystallin are indicated, as well as the region in which ubiquitin staining of unknown protein(s) was observed (X-Ub\(_{\text{n}}\)). aB-Crystallin displays charge heterogeneity, which may be due to modifications such as phosphorylation or deamidination (44). The arrowheads indicate the smallest ubiquitinated protein (~35 kDa).

![Image](image.png)

**Fig. 6. Ubiquitin-protein conjugates resolved by two-dimensional electrophoresis.** HeLa cells were cotransfected with pBSSK - HA ubiquitin, pIRES-FBX4, and a pIRES construct coding for wild-type aB-crystallin (A), S19D/S45D (B), S19A/S45A (C), or R120G (D). After 2 days, the cells were lysed and fractionated into detergent-soluble and -insoluble fractions. The detergent-insoluble fraction was dissolved by ultrasonication in buffer containing 6 M urea, 2 M thiourea, and 2% CHAPS and separated by isoelectric focusing and in the second dimension by 12% SDS-PAGE. After blotting onto nitrocellulose membranes, immunostaining was successively performed for aB-crystallin and the HA epitope. Proteins staining for aB-crystallin are indicated, as well as the region in which ubiquitin staining of unknown protein(s) was observed (X-Ub\(_{\text{n}}\)). aB-Crystallin displays charge heterogeneity, which may be due to modifications such as phosphorylation or deamidination (44). The arrowheads indicate the smallest ubiquitinated protein (~35 kDa).

**DISCUSSION**

We have characterized a new interactor of aB-crystallin, the F-box protein FBX4. The interaction between FBX4 and aB-crystallin is most likely regulated by phosphorylation at Ser-19 and Ser-45. This combination is of particular interest because these serines are mainly phosphorylated during mitosis (15), suggesting that the interaction with FBX4 might play a role during cell division. In our experiments, phosphorylation at Ser-19 and Ser-45 is mimicked by replacing these residues with negatively charged aspartic acids (Fig. 1 and Table I). It is possible that phosphorylated serines give a stronger interaction than aspartic acid residues because a phosphate group has two negative charges. For this reason, it cannot be excluded that phosphorylation of just one of the two serines is already sufficient to stimulate the interaction with FBX4. Because Ser-19 and Ser-45 are phosphorylated by different kinases (15), it would be interesting to determine whether both phosphorylation-mimicking mutations are indeed necessary for FBX4 interaction.

We made the interesting observation that not only the phosphorylation-mimicking mutations stimulate the interaction of aB-crystallin with FBX4, but also the mutation R120G, which causes a desmin-related myopathy. Ser-19 and Ser-45 are localized in the N-terminal domain, and R120G in the a-crystallin domain. The interaction of aB-crystallin R120G with FBX4...
could be the result of a conformational change induced by the mutation and resembling the phosphorylated form. Because the structure of αB-crystallin is not known, it cannot be excluded that these residues are actually close to each other in the tertiary structure. In wheat HSP16.9, the crystal structure of which is known, Arg-108 is located at a position equivalent to Arg-120 in αB-crystallin and forms a salt bridge with a negatively charged residue (41). Thus, due to the loss of Arg-120, a negative charge might become available, and if at the right position, can mimic phosphorylation.

We found that N-terminally truncated FBX4, FBX4-(179–387), interacted efficiently not only with αB-crystallin S19D/S45D and R120G, but also with wild-type αB-crystallin (Table I). This is remarkable because full-length FBX4 did not interact with wild-type αB-crystallin during mitosis (15). Both phosphorylation of αB-crystallin might be an important step in understanding the role of phosphorylation of which is known, Arg-108 is located at a position equivalent to Arg-120, a negative charge might become available and, if at the right position, can mimic phosphorylation. It would be interesting to find out if this protein is the target protein ubiquitinated by αB-crystallin S19D/S45D and R120G during mitosis (15). Both phosphorylation of αB-crystallin and R120G also for some other proteins, it has been shown that the truncated version is a stronger interactor than the full-length protein (42). It should be emphasized that, in the case of FBX4-(179–387), this is not due to improper folding because the translocation of the mutants to the detergent-insoluble fraction by a yet unknown protein. It may thus well be that the ubiquitination induced by the regulated interaction between FBX4 and αB-crystallin is a generally occurring process.

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