Chaperone Activity of α B-crystallin Suppresses Tubulin Aggregation through Complex Formation

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ABSTRACT. α B-Crystallin, one of the small heat shock proteins, is constitutively expressed in lens as well as in nonlenticular tissues. It can function as a molecular chaperone for other lens crystallins and some other proteins. Its nonocular function is unknown although some reported one of them is related to cytoskeletal networks and/or components. In the present study, we demonstrate the association of α B-crystallin with tubulin. Immunoprecipitation experiments using L6 myoblast cell lysate with anti- α B-crystallin antibody resulted in the coprecipitation of α -tubulin, which was apparently temperature-dependent. Further, purified α B-crystallin prevented the turbidity development of purified tubulin molecule at 37°C in vitro. Sucrose gradient centrifugation revealed that this chaperone activity was accompanied by the formation of large complex of α B-crystallin and tubulin dimer. These results indicate that one of the nonlenticular functions of α B-crystallin may be the protection of tubulin subunits of microtubules.

Living organisms are continuously exposed to various stresses. At cellular level, cells respond to these insults by expressing and synthesizing a group of proteins collectively known as heat shock proteins (HSPs) (21, 22). Many of this class of proteins are now known to have the ability to function as molecular chaperones. Molecular chaperones are involved in such diverse fundamental protein regulation as prevention of irreversible protein aggregation, renaturation of denatured proteins, correct folding of newly synthesized proteins, protein membrane transport, and establishment of signal transduction pathways. Among the HSP family, HSP70 and HSP60 are relatively well characterized regarding its mode of action (9, 10). However, low molecular weight HSPs (small HSPs; sHSPs) are poorly characterized both in their mode of action and substrate specificity (1).

In 1982, α -crystallin, a major lens protein, was reported to have striking homology to other sHSPs (12) and indeed to be induced by heat shock (19). Horwitz

(11) demonstrated that α -crystallin can function as molecular chaperone preventing other lens crystallins from heat-induced aggregation, suggesting that α -crystallin provides lens transparency. The non-lenticular expression of α -crystallin, especially B chain (α B-crystallin), has been reported in many tissues including brain, heart, kidney, and skeletal muscle (4, 5, 8, 14, 17, 18, 29). The function of this protein outside the lens is poorly understood, although some research reported it is related to cytoskeletal networks. For example, Iwaki *et al.* (13) demonstrated that there was disorganization in actin microfilament network in glial cell line C6 when antisense strategy was used. Nicholl and Quinlan demonstrated using lens lysate that α -crystallin can function as molecular chaperone in assembly of intermediate filament vimentin (24). Atomi *et al.* demonstrated that α B-crystallin decreased in atrophying soleus muscle using hind-limb suspension method, an animal model for muscle atrophy. They also showed that α B-crystallin localized in a striated pattern indicative of association with structural components and that its expression was highly dependent on physiological activity and/or cellular morphology in the skeletal muscle (2, 3). These evidences strongly suggested that the function of α B-crystallin outside the lens is related to cytoskeletal networks as well as cytoskeletal components.

Microtubules (MTs) are involved in many important biological processes such as transport of cellular components, maintenance of cell polarity, and formation of mitotic apparatus. The component of MTs, tubulin mol-

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Abbreviations: BCA, bicinchoninic acid; BSA, bovine serum albumin; EGTA, ethylene glycolbis (b-aminoethyl ether) N,N,N',N'-tetraacetic acid; GTP, guanosine 5'-triphosphate; HSP, heat shock protein; MAP, microtubule associated protein; MT, microtubule; PIPES, piperazine-N,N'-bis (2-ethanesulfonic acid); SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; sHSP, small heat shock protein; TRiC, TCP-1 ring complex.

ecule is well known to be a labile protein and to lose its activity rapidly even in relatively mild conditions (26).

In this report, we investigated the possible biochemical association between α B-crystallin and tubulin by immunoprecipitation, using specific antibody produced against C-terminal peptide of α B-crystallin. We found that α B-crystallin is associated with tubulin in L6 cell lysate. Furthermore, we found that α B-crystallin has chaperone activity for tubulin molecules to prevent aggregation through formation of binary complex. From these results, we suggest that one of the functions of α B-crystallin outside the lens is the protection of tubulin molecules.

MATERIALS AND METHODS

Materials. Bovine α -crystallin, BSA (bovine serum albumin), EGTA (ethylene glycol-bis (b-aminoethyl ether) N,N,N',N'-tetraacetic acid), PIPES (piperazine-N,N'-bis (2-ethanesulfonic acid)) were purchased from Sigma Chemical Co. (St. Louis, MO). GTP (guanosine 5'-triphosphate) was from Seikagaku Corp. (Tokyo, Japan). Porcine brain was from local slaughterhouse. BCA (bicinchoninic acid) protein assay kit was from Pierce Chemical Co. (Rockford, IL). All other chemicals were from Wako Pure Chemical Industries, Ltd (Osaka, Japan). All reagents were of the purest grade available.

Preparation of Antibodies. Antibodies C1 and N1 were raised against C-((SH)KPAVTAAPKK) and N-(Ac-MDIAIH HPWIC(SH)) terminal peptides of rat α B-crystallin (kindly synthesized and purified by Dr. S. Aimoto, Research Center for Protein Engineering, Institute for Protein Research, Osaka University, Japan) conjugated to BSA with m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) (25). The immunoglobulins, precipitated by 50% NH_4SO_4 and dialyzed against PBS, were purified using the C- and N-terminal peptide conjugated affinity chromatography using MBS linker (Pierce) and EAH Sepharose-4B (Pharmacia Biotech KK, Shinagawa, Japan).

Cell Culture. L6E9 cells (a generous gift from Dr. T. Endo, Chiba University, Japan) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum.

Immunoprecipitation. Semiconfluent L6 cells were lysed with lysis buffer (20 mM PIPES (pH 6.8), 1 mM MgCl_2 , 1 mM EGTA, 1% NP-40, 1 mM PMSF, 10 $\mu\text{g}/\text{ml}$ leupeptin), left on ice for 10 min. The lysate was centrifuged at 4°C at 18,500 g for 10 min. Protein G-Sepharose (Pharmacia Biotech) was added to the supernatant and the mixture was rocked for 30 min at 37°C, followed by centrifugation at 4°C at 18,500 g for 5 min. Anti- α B-crystallin C-terminal peptide together with Protein G-Sepharose was added to the supernatant and the mixture was rocked for 2 hours at 37°C. As controls, polyclonal anti-catalase (raised with rabbit) (Calbiochem-Novabiochem International, 219010) and rabbit control IgG (Inter-

Cell Technologies) were used. Protein G-Sepharose alone was also used as a control. In some experiments, the temperature was maintained at 4°C or room temperature (24°C) throughout incubation time. The immune complex was pelleted by centrifugation at 4°C at 2,000 g for 3 min, followed by extensive wash with lysis buffer 5 times. Precipitated proteins were boiled in SDS-PAGE sample buffer for 5 min, followed by SDS-PAGE and immunoblotting using monoclonal anti- α -tubulin antibody (Sigma, T9026) as primary antibody and horseradish peroxidase-conjugated anti-mouse antibody as secondary antibody. Immunoblot was visualized with enhanced chemiluminescence (ECL) western blotting kit (Amersham).

Protein Purification. α B-Crystallin was purified from bovine α -crystallin (Sigma) using ion-exchange chromatography in the presence of urea according to de Jong *et al.* (7). Tubulin was purified according to Shelanski *et al.* (31) from porcine brain. Microtubule protein obtained by two cycles of assembly-disassembly was passed through phosphocellulose column and concentrated using Amicon Centricon 30 (Amicon, Beverly, MA). PC-tubulin (phosphocellulose-purified tubulin) was stored frozen at -80°C until use. Protein concentration of α B-crystallin and PC-tubulin was determined with BCA Protein Assay kit using bovine serum albumin (BSA) as standards.

Chaperone Activity Assay. PC-tubulin solution was mixed with various concentrations of purified α B-crystallin. The final PC-tubulin concentration was fixed at 1.5 mg/ml in PME buffer (0.1 mM PIPES (pH 6.8), 1 mM MgCl_2 , 1 mM EGTA) supplemented with 1 mM GTP. The mixture was then subjected to incubation at 37°C for 6 hours. The aggregation was monitored by measuring turbidity of the solution using spectrophotometer (Beckman DU-65) thermostated with water bath. Monitoring wavelength was 350 nm. Absorbance was recorded every 4 minutes.

Sucrose Gradient Centrifugation. PC-Tubulin solution (1.5 mg/ml, reaction volume 100 μl) in the absence and presence of α B-crystallin (0.1 mg/ml) was incubated at 37°C for various hours, then subjected to 2.1 ml 10–40% (w/w) sucrose gradient centrifugation in PME supplemented with 0.1 mM GTP. Samples were centrifuged at 55,000 rpm in Beckman TLS-55 rotor at 5°C for 90 min, followed by fractionation into 22 fractions (10 μl) from the top manually. S value was calculated from the rotor specification and experimental conditions. The fractions were mixed with SDS sample buffer and subjected to SDS-PAGE, followed by quantification using NIH-IMAGE 1.60.

RESULTS

Characterization of α B-Crystallin Antibody. Polyclonal antibody against C-terminal 10 amino acid peptides of rat α B-crystallin was produced and purified by peptide affinity chromatography. This antibody reacted specifically with α B-crystallin in L6 myoblast cell lysate

(Fig. 1A, lane 4) and with purified bovine α B-crystallin (lane 5), but not with α - nor β -tubulin (lane 6).

Complex Formation of α B-Crystallin and Tubulin in the L6 Cell Lysate as Revealed by Immunoprecipitation. Since some papers reported the association of α B-crystallin with cytoskeletal networks or elements, we examined the possibility of interaction with cytoskeletal components by immunoprecipitation. As α B-crystallin was reported to have chaperone activity for some proteins, it was possible that α B-crystallin chaperones for cytoskeletal components. Tubulin is well known to be a labile protein, which prompted us to examine the interaction between α B-crystallin and tubulin. Immunoprecipitation using α B-crystallin antibody with L6 myoblast cell lysate resulted in coprecipitation of α -tubulin (Fig. 1B). When control experiments using anti-catalase antibody or control rabbit IgG were carried out, copre-

cipitation of tubulins was not observed (Fig. 1B). Therefore, the coprecipitation with α B-crystallin mentioned above was not the result of binding of tubulin to rabbit IgG or Protein G-Sepharose, or the results of nonspecific tubulin aggregation or microtubule assembly. However, when immunoprecipitation was conducted at 4°C, we did not observe the coprecipitation of tubulins with the immune complex. The complex formation was apparently temperature-dependent (Fig. 1C).

α B-Crystallin has a Chaperone Activity for Suppression of Tubulin Aggregation. Complex formation of α B-crystallin and tubulin in L6 cells as revealed by immunoprecipitation suggested the function of α B-crystallin as molecular chaperone for tubulin molecule. Tubulin molecule is known to be a highly labile protein and to lose its activity very rapidly (26, 27). Chaperone activity of α -crystallin has been reported for other lens crystallins (11). Thus we examined whether α B-crystallin can function as molecular chaperone for this labile protein. Tubulin can be easily denatured even in mild solution conditions, i.e., neutral in pH and incubated at 37°C. As a result, tubulin undergoes aggregate formation and the turbidity of the solution increases as shown in curve 3 of Fig. 2A. This increase in turbidity does not reflect tubulin self-assembly because 1) in the solution conditions that lack MAPs and assembly-promoting reagent such as dimethylsulfoxide (DMSO), purified tubulin cannot self-assemble, 2) cooling the turbid solution to 4°C, which would have depolymerized assembled MTs, did not result in decrease of turbidity (data not shown), and 3) microtubule assembly is a much more rapid reaction which reaches its plateau 15–30 minutes after warming the solution. Therefore, this turbidity increase was most likely due to nonspecific irreversible aggregate formation. In contrast, when purified α B-crystallin was present in the solution, the turbidity increase was considerably suppressed (curves 1 and 2). This suppression was dependent on the concentration of α B-crystallin as shown in Fig. 2B. This effect was evident at very low concentrations of α B-crystallin (0.05 mg/ml). However, at higher concentrations of α B-crystallin, we observed slightly enhanced turbidity development, which cannot be explained at present (Fig. 2B).

Complex formation between α B-crystallin and tubulin as revealed by sucrose gradient centrifugation. The results obtained with immunoprecipitation and turbidity measurement experiments suggest that α B-crystallin prevented tubulin aggregation by binding directly since turbidity experiment was carried out with purified components. To confirm this, samples incubated for various hours in the absence or presence of α B-crystallin were subjected to 10–40% (w/w) sucrose gradient centrifugation. When incubated for 5 minutes at 37°C, tubulin remained in upper fractions, indicating that it still was native (Fig. 2C, a). As expected from its labile na-

Fig. 1. Coprecipitation of α -tubulin with α B-crystallin as revealed by immunoprecipitation. A. Specificity of anti- α B-crystallin C-terminal peptide antibody. Myoblast L6 cell lysate (lanes 1 and 4), purified α B-crystallin (lanes 2 and 5), and PC-tubulin (lanes 3 and 6) were subjected to SDS-PAGE, followed by staining with CBB (lanes 1–3) or immunoblotted with anti- α B-crystallin C-terminal peptide antibody (lanes 4–6). Proteins loaded were 40 μ g, 1 μ g, 2 μ g, 5 μ g, 20 ng, 1 μ g, from lane 1 to 6, respectively. Molecular weight markers (lane M) are phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), and soybean trypsin inhibitor (20 kD). B. Coprecipitation of α -tubulin by immunoprecipitation with anti- α B-crystallin antibody. L6 cell lysate (lane 1), immunoprecipitate with anti- α B-crystallin antibody (lane 2), immunoprecipitate with anti-catalase antibody (lane 3), immunoprecipitate with control rabbit IgG (lane 4), and pellet fraction incubated only with Protein G-Sepharose (lane 5) were subjected to SDS-PAGE, followed by immunoblotting with monoclonal anti- α -tubulin antibody. Faintly reacting bands moving slightly fast in lanes 2–4 may be the result of cross-reaction of secondary anti-mouse IgG antibody to rabbit IgG heavy chain. Incubation with antibody in immunoprecipitation was conducted at 37°C. C. Temperature-dependence of complex formation of α B-crystallin and α -tubulin. L6 cell lysate (lane 1), immunoprecipitate with anti- α B-crystallin antibody at 37°C (lane 2), at room temperature (24°C, lane 3), and at 4°C (lane 4) were subjected to SDS-PAGE, followed by immunoblotting with monoclonal anti- α -tubulin antibody. At lower temperature, α -tubulin was not contained in the immune complex.

Fig. 2. A. Prevention of aggregation of tubulin by α B-crystallin. Purified PC-tubulin (1.5 mg/ml) was incubated at 37°C in the absence (curve 1) and in the presence of 0.05 mg/ml (curve 3) or 0.1 mg/ml (curve 2) of α B-crystallin, and the turbidity was measured. B. Concentration-dependence of aggregation prevention by α B-crystallin. The turbidity at 6 hours of incubation was plotted as a function of α B-crystallin concentration. C. Prevention of formation of large aggregate of tubulin by α B-crystallin as revealed by sucrose gradient centrifugation. After 5 min (a), 3 hours (b), and 6 hours (c) of incubation, the samples without (α B-C(-)) or with 0.1 mg/ml of α B-crystallin (α B-C(+)) were subjected to 10%–40% sucrose gradient centrifugation. Tubulin concentration of each fraction was calculated. Particles of intermediate sizes were formed in the presence of α B-crystallin, while in its absence tubulin became very large aggregate. Centrifugation was from left to right. D. Complex formation between α B-crystallin and tubulin as revealed by sucrose gradient centrifugation. The same gels as in C (incubation time was 5 min, 3 hours, and 6 hours for a, b, and c, respectively) were quantified for both tubulin and α B-crystallin. Note that the y axis for tubulin is not the same as in C to show the intermediate fractions clearly. Tubulin and α B-crystallin were recovered from the same fractions.

ture, tubulin underwent formation of large aggregate when the solution was incubated for 3 hours (Fig. 2C, b) and a substantial amount of tubulin was recovered from the bottom of the gradient ($>300S$), which was more evident at 6 hours of incubation (Fig. 2C, c). In the presence of α B-crystallin, tubulin was recovered from upper fractions as in the absence at 5 minutes of incubation (Fig. 2C, a). However, when α B-crystallin was present in the solution, the formation of large tubulin aggregate was considerably suppressed at a longer time of incubation (Fig. 2C, b, c). Instead, tubulin was recovered from intermediate fractions. When the same fractions were quantified for α B-crystallin, α B-crystallin was recovered from upper fractions ($\sim 20S$) at 5 minutes of incubation. At longer incubation, it was recovered from intermediate fractions together with tubulin (Fig. 2D, a-c). It is likely that α B-crystallin and tubulin form the complex directly. It should be noted that both the ratio of tubulin to α B-crystallin and size of the complex seem to increase when the incubation was prolonged.

DISCUSSION

We show here that α B-crystallin can function as molecular chaperone to prevent aggregation of tubulin. Chaperone activity of α -crystallin was demonstrated by Horwitz (11) and other researchers (15, 23, 28, 32). They reported that α -crystallin prevents aggregation of other lens crystallins, some enzymes, and test proteins. In many cases, molecular chaperones recognize hydrophobic surfaces and patches which become exposed either in nascent folding or in unfolding by denaturation. Besides the general tendency to bind to hydrophobic areas of other proteins, it seems that there exists class-specificity in preference for the substrates among HSP/chaperone family. Das *et al.* (6) showed that α A-crystallin recognizes its substrates with relatively native-like structure, in contrast to HSP70s. They suggested that α -crystallin and other sHSPs recognize 'aggregation-prone' proteins. Taking general features of chaperones and this class-specificity into consideration, tubulin may be a preferred substrate for α B-crystallin chaperoning. Prakash and Timasheff (26) showed that tubulin molecule shows conformational changes in long-term incubation in the absence of stabilizing agents. Prasad *et al.* (27) demonstrated using fluorescent apolar probe that the decay of tubulin molecule involves the appearance of hydrophobic areas and that at this time the secondary structure is still maintained. Taken together, we consider that α B-crystallin recognizes denatured tubulin in its very early step(s) of denaturation and by binding to this denaturation intermediate, α B-crystallin suppresses further aggregation.

We show here that association of tubulin with α B-

crystallin in cell lysate is apparently temperature-dependent in immunoprecipitation experiment. This temperature dependency was also reported by Raman *et al.* (28), demonstrating that this activity appears only above $30^{\circ}C$ and steeply increases with temperature, reaching its maximum above $50^{\circ}C$. Thus it may be that chaperone activity of α B-crystallin was not fully activated in the condition of the present study, *i.e.*, at $37^{\circ}C$. Our finding that α B-crystallin associates with tubulin in physiological temperature may be attributable partly to lability of tubulin and its abundance in cell lysate.

In our study, the complex formed between α B-crystallin and tubulin was very large. It is not likely that there exists a chaperone-substrate complex much larger than ribosomes *in vivo*. We consider that *in vivo*, α B-crystallin transiently traps denaturation intermediate in a soluble form and hands it to and/or presents it to other chaperones/proteases. One candidate is TCP-1 Ring Complex (TRiC), which is reported to be involved in nascent folding of tubulins and actin (20, 33). It is also possible that HSP70 or HSP90 is sufficient for the refolding of mature tubulins. Further studies regarding coordination with other chaperones are required for the reasonable evaluation of functions of α B-crystallin/sHSPs for cytoskeletal components and other substrates.

Even in the absence of apparent stresses, α B-crystallin and sHSP are constitutively expressed in many tissues and cell types (17). α B-crystallin is also expressed in many neurodegenerative diseases (14, 29, 30). The aggregation-prone property of tubulin together with its abundance may be a serious problem to the cells. It is possible that those tissues and cells that are rich in intrinsically unstable proteins such as tubulin, especially in its depolymerized state, require a chaperone system like α B-crystallin/sHSPs and/or other HSPs for maintaining cellular functions. This is consistent with recent finding by Kato *et al.* (16) that α B-crystallin was induced by the treatment with a microtubule-depolymerizing drugs in C6 cells. Abnormal modifications of MAPs as seen in brains in some pathological conditions may cause destabilization of MTs and tubulin subunit and in turn enhance the requirement of chaperones.

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REFERENCES

1. ARRIGO, A.-P. and LANDRY, J. 1994. Expression and function

- of low-molecular-weight heat shock proteins. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R.I., Tissieres, A., and Georgopoulos, C., eds.). Cold Spring Harbor Laboratory Press, New York, pp.335–373.
2. ATOMI, Y., YAMADA, S., and HONG, Y.-M. 1990. Dynamic expression of α B-crystallin in skeletal muscle Effects of unweighting, passive stretch and denervation. *Proc. Japan Acad.*, **66 Ser B**: 203–208.
  3. ATOMI, Y., YAMADA, S., and NISHIDA, T. 1991. Early changes of α B-crystallin mRNA in rat skeletal muscle to mechanical tension and denervation. *Biochem. Biophys. Res. Commun.*, **181**: 1323–1330.
  4. ATOMI, Y., YAMADA, S., STROHMAN, R., and NONOMURA, Y. 1991. α B-crystallin in skeletal muscle: purification and localization. *J. Biochem.*, **110**: 812–822.
  5. BHAT, S.P. and NAGINENI, C.N. 1989. α B subunit of lens-specific protein alpha crystallin is present in other ocular and non-ocular tissues. *Biochem. Biophys. Res. Commun.*, **158**: 319–325.
  6. DAS, K.P., PETRASH, J.M., and SUREWICZ, W.K. 1996. Conformational properties of substrate proteins bound to a molecular chaperone α -crystallin. *J. Biol. Chem.*, **271**: 10449–10452.
  7. DEJONG, W.W., ZWEERS, A., VERSTEEG, M., and NUYTERWINDT, E.C. 1984. Primary structures of the alpha-crystallin A chains of twenty-eight mammalian species, chicken and frog. *Eur. J. Biochem.*, **141**: 131–140.
  8. DUBIN, R.A., WAWROUSEK, E.F., and PIATIGORSKY, J. 1989. Expression of the murine alpha B crystallin gene is not restricted to the lens. *Mol. Cell. Biol.*, **9**: 1083–1091.
  9. FRYDMAN, J. and HARTL, F.U. 1994. Molecular chaperone functions of hsp70 and hsp60 in protein folding. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R.I., Tissieres, A., and Georgopoulos, C., eds.). Cold Spring Harbor Laboratory Press, New York, pp.251–284.
  10. HIGHTOWER, L.E., SADIS, S.E., and TAKENAKA, I.M. 1994. Interactions of vertebrate hsc70 and hsp70 with unfolded proteins and peptides. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R.I., Tissieres, A., and Georgopoulos, C., eds.). Cold Spring Harbor Laboratory Press, New York, pp.179–208.
  11. HORWITZ, J. 1992. α -Crystallin can function as a molecular chaperone. *Proc. Natl. Acad. Sci. USA*, **89**: 10449–10453.
  12. INGOLIA, T.D. and CRAIG, E.A. 1982. Four small *Drosophila* heat shock proteins are related to each other and to mammalian α -crystallin. *Proc. Natl. Acad. Sci. USA*, **79**: 2360–2364.
  13. IWAKI, T., IWAKI, A., TATEISHI, J., and GOLDMAN, J.E. 1994. Sense and antisense modification of glial α B-crystallin production results in alterations of stress fiber formation and thermoresistance. *J. Cell Biol.*, **125**: 1385–1393.
  14. IWAKI, T., KUME-IWAKI, A., LIEM, R.K.H., and GOLDMAN, J.E. 1989. α B-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. *Cell*, **57**: 71–78.
  15. JAKOB, U., GAESTEL, M., ENGEL, K., and BUCHNER, J. 1993. Small heat shock proteins are molecular chaperones. *J. Biol. Chem.*, **268**: 1517–1520.
  16. KATO, K., ITO, H., INAGUMA, Y., OKAMOTO, K., and SAGA, S. 1996. Synthesis and accumulation of α B crystallin in C6 glioma cells is induced by agents that promote the disassembly of microtubules. *J. Biol. Chem.*, **271**: 26989–26994.
  17. KLEMENZ, R., ANDRES, A.-C., FROHLI, E., SCHAFER, R., and AOYAMA, A. 1993. Expression of the murine small heat shock proteins hsp25 and alpha B crystallin in the absence of stress. *J. Cell Biol.*, **120**: 639–645.
  18. KLEMENZ, R., FROHLI, E., AOYMAA, A., HOFFMANN, S., SIMPSON, R.J., MORITZ, R.L., and SCHAFER, R. 1991. Alpha B crystallin accumulation is a specific response to Ha-ras and v-mos oncogene expression in mouse NIH 3T3 fibroblasts. *Mol. Cell. Biol.*, **11**: 803–812.
  19. KLEMENZ, R., FROHLI, E., STEIGER, R.H., SCHAFER, R., and AOYAMA, A. 1991. α B-Crystallin is a small heat shock protein. *Proc. Natl. Acad. Sci. USA*, **88**: 3652–3656.
  20. LEWIS, V.A., HYNES, G.M., ZHENG, D., SAIBIL, H., and WILLISON, K. 1992. T-complex polypeptide-1 is a subunit of a heteromeric particle in the eukaryotic cytosol. *Nature*, **358**: 249–252.
  21. LINDQUIST, S. 1981. Regulation of protein synthesis during heat shock. *Nature*, **293**: 311–314.
  22. LINDQUIST, S. 1986. The heat-shock response. *Annu. Rev. Biochem.*, **55**: 1151–1191.
  23. MERCK, K.B., GROENEN, P.J.T.A., VOORTER, C.E.M., DE HAARD-HOEKMAN, W.A., HORWITZ, J., BLOEMENDAL, H., and DE JONG, W.W. 1993. Structural and functional similarities of bovine α -crystallin and mouse small heat-shock protein. *J. Biol. Chem.*, **268**: 1046–1052.
  24. NICHOLL, I.D. and QUINLAN, R.A. 1994. Chaperone activity of α -crystallins modulates intermediate filaments assembly. *EMBO J.*, **13**: 945–953.
  25. PATERSON, Y. 1987. In *Current Protocol in Molecular Biology* (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K., eds.). Green Publishing Associates Inc. and John Wiley and Sons, Inc., pp.11.15.1–4.
  26. PRAKASH, V. and TIMASHEFF, S.N. 1982. Aging of tubulin at neutral pH. *J. Mol. Biol.*, **160**: 499–515.
  27. PRASAD, A.R.S., LUDUENA, R.F., and HOROWITZ, P.M. 1986. Bis(8-anilino-naphthalene-1-sulfonate) as a probe for tubulin decay. *Biochemistry*, **25**: 739–742.
  28. RAMAN, B. and RAO, C.M. 1994. Chaperone-like activity and quaternary structure of α -crystallin. *J. Biol. Chem.*, **269**: 27264–27268.
  29. RENKAWEK, K., DE JONG, W.W., MERCK, K.B., FRENKEN, C.W.G.M., and VAN WORKUM, F.P.A. 1992. α B-crystallin is present in reactive glia in Creutzfeldt-Jakob disease. *Acta Neuropathologica*, **83**: 324–327.
  30. RENKAWEK, K., VOORTER, C.E., BOSMAN, G.J., VAN WORKUM, F.P., and DE JONG, W.W. 1994. Expression of α B-crystallin in Alzheimer's disease. *Acta Neuropathologica*, **87**: 155–160.
  31. SHELANSKI, M.L., GASKIN, F., and CANTOR, C.R. 1973. Microtubule assembly in the absence of added nucleotides. *Proc. Natl. Acad. Sci. USA*, **70**: 765–768.
  32. WANG, K. and SPECTOR, A. 1994. The chaperone activity of bovine α crystallin Interaction with other lens crystallins in native and denatured states. *J. Biol. Chem.*, **269**: 13601–13608.
  33. YAFFE, M.B., FARR, G.W., MIKLOS, D., HORWICH, A.L., STERNLICHT, M.L., and STERNLICHT, H. 1992. TCP1 complex is a molecular chaperone in tubulin biogenesis. *Nature*, **358**: 245–248.

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