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αB-Crystallin is a small heat shock protein

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ABSTRACT **Sequence similarity between αB-crystallin and small heat shock proteins (HSPs) has prompted us to investigate whether αB-crystallin expression is induced by heat shock. Indeed, accumulation of αB-crystallin was detected immunologically in NIH 3T3 cells after incubation at elevated temperatures and after addition of Cd^{2+} or sodium arsenite to these cells. Two-dimensional gel electrophoresis revealed identity between αB-crystallin from eye lenses and from heat-treated fibroblasts. The promoter of the αB-crystallin gene was fused to the bacterial chloramphenicol acetyltransferase gene and was shown to confer heat inducibility on this reporter gene in transient transfection assays. A perfect heat shock element within the promoter region is likely to mediate this response. Small HSPs and αB-crystallin were shown to share the following two physical properties: (*i*) they form supramolecular structures with sedimentation values around 17 S and (*ii*) they are associated with the nucleus at high temperatures and are localized in the cytoplasm under normal conditions. We conclude that αB-crystallin has to be considered a member of the class of small HSPs.**

Heat shock and numerous other stress conditions lead to the rapid induction of several genes whose protein products are collectively called heat shock proteins (HSPs) (for recent reviews see refs. 1–3). The HSPs have been grouped into several classes on the basis of their size and sequence homology. Members of the class of small HSPs have molecular masses in the range 15–40 kDa. All analyzed organisms possess at least one small HSP gene. In mammals, birds, and yeast this class of HSPs is represented by a single member (4–9), whereas in *Drosophila melanogaster* and plants there appear to be multiple small HSPs (10, 11). Small HSPs aggregate to form characteristic ring-shaped structures called heat shock granules (4, 12–15). These structures resemble prosomes or proteosomes but are distinct entities (16). Their biochemical function is unknown. Under heat shock conditions the small HSPs associate with the nucleus. Following a heat shock they slowly relocalize to the cytoplasm (17–19). It is still a matter of debate whether the small HSPs are actually transported into the nucleus at high temperatures or whether they are entrapped by the intermediate filaments, which, under heat shock conditions, collapse onto the nucleus (for a review see ref. 3). The amino acid sequences of the small HSPs from different organisms are only poorly conserved. However, striking sequence similarities exist between vertebrate α-crystallins and small HSPs (5, 20–23). α-Crystallins were originally found in eye lenses, where they are among the most abundant proteins (24, 25). There exist two forms of α-crystallins, αA and αB, which are closely related (26). Considerable amounts of αB-crystallin, but not αA-crystallin, are present in many nonlenticular tissues (27–31). Moreover, αB-crystallin gene expression has been observed in various diseased cells, including astrocytes of patients suffering from Alexander disease (32), scrapie-infected hamster brain cells (33), and NIH 3T3 mouse fibroblasts expressing Ha-*ras* and v-*mos* oncogenes (34). The frequent observation of this protein under pathological conditions in conjunction with the striking sequence similarity between αB-crystallin and the small HSPs prompted us to investigate whether the αB-crystallin gene is subject to stress regulation.

Here we present evidence indicating that αB-crystallin is indeed a HSP. The protein accumulates under stress conditions, aggregates into supramolecular structures with the same sedimentation coefficient as heat shock granules, and is found associated with the nucleus under heat shock conditions.

MATERIALS AND METHODS

Cells. We used exponentially growing NIH 3T3 and NIH[LTR-*mos*] cells. The latter cell line had been derived from NIH 3T3 cells by the stable integration of a mouse mammary tumor virus long terminal repeat (LTR)-driven v-*mos* gene (35). The v-*mos* oncogene was induced by the addition of dexamethasone (1 μM). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml). For heat treatment the culture dishes were sealed in plastic bags and submerged in a water bath.

Protein Extraction and Gel Electrophoresis. Cells were lysed either by hypotonic shock or with detergent. For hypotonic lysis, cells were washed twice with phosphate-buffered isotonic saline (PBS, Dulbecco–Vogt formulation, without Ca^{2+} and Mg^{2+}) and then allowed to swell for 5 min in 10 mM Tris·HCl, pH 8/0.1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride (5 ml per 15-cm dish) on ice. Cells were disrupted in a Dounce homogenizer and large cell debris and nuclei were removed by centrifugation at 500 × *g* for 10 min. Particulate material was pelleted by centrifugation at 100,000 × *g* for 30 min. The proteins in the S100 supernatant were concentrated by precipitation with ammonium sulfate (50% saturation). For detergent lysis, cells from a 15-cm dish were first washed twice with PBS and then collected by centrifugation in the same buffer. One milliliter of lysis buffer [10 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/10 mM NaCl/0.1 mM phenylmethylsulfonyl fluoride/0.5% (vol/vol) Triton X-100] was added and detergent-soluble and -insoluble fractions were separated by centrifugation in an Eppendorf centrifuge for 5 min. SDS sample buffer and 5× SDS sample buffer were added to the pellets and the supernatants, respectively. Proteins were fractionated in SDS/12.5% polyacrylamide gels (36). Protein concentrations were determined by spotting aliquots of the extracts in SDS sample buffer onto nitrocellulose filters and staining with amido black (34).

Western Blots. Proteins were transferred electrophoretically onto nitrocellulose membranes. The primary antiserum was raised in rabbits against purified human αB crystallin and was purified by affinity chromatography (unpublished work). Protein blots were incubated with this antibody at room temper-

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Abbreviations: HSP, heat shock protein; HSE, heat shock element; HSF, heat shock factor; CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat.

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ature for 16 hr. The secondary antibody was anti-rabbit IgG coupled to alkaline phosphatase (Promega). The immunocomplexes were visualized enzymatically by immersing the blots in 100 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$ containing nitro blue tetrazolium (33 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (16 μg/ml).

Size Fractionation of Proteins on Sucrose Gradients. NIH[LTR-*mos*] cells, untreated or incubated with 1 μM dexamethasone for 24 hr, were lysed in hypotonic buffer and S100 extracts were prepared as described above. The extracts were further centrifuged at 100,000 × *g* for 18 hr. The resulting pellets were dissolved in 1.5 ml of buffer A (10 mM Tris·HCl, pH 8/150 mM NaCl/0.1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride). Insoluble material was removed by brief centrifugation in an Eppendorf centrifuge, and the protein solutions were layered onto 5–30% sucrose gradients in buffer A. After centrifugation for 16 hr at 35,000 rpm in a Beckman SW40 rotor at 4°C, 1-ml fractions were collected from the bottom. Proteins were concentrated from an aliquot of each fraction by trichloroacetic acid precipitation and subjected to SDS/PAGE.

Construction of pKSαB-CAT. A 711-base-pair (bp) DNA fragment containing nucleotides +45 to −666 of the αB-crystallin gene (28) was amplified by polymerase chain reaction using total DNA isolated from NIH[LTR-*mos*] cells as template. The oligodeoxynucleotides 5′-CTGCAGGAGGGCAAGGAGAGG-3′ (−666 to −646) and 5′-TGTGGCTAGATGAATGCAGAGTCGG-3′ (+21 to +45) were used as primers. The synthesized αB-crystallin promoter region was purified from an agarose gel and cloned into the *Sma* I site of the chloramphenicol acetyltransferase (CAT) vector $KS^+SV0CAT$ (37). The plasmid with the αB-crystallin promoter region in the same orientation as the CAT gene is designated pKSαB-CAT.

Transient Transfection of NIH 3T3 Cells. NIH 3T3 cells were plated at 2×10^6 per 15-cm dish 24 hr before the addition of DNA/calcium phosphate precipitates. Thirty micrograms of pKSαB-CAT DNA and 10 μg of pSVA2PAP DNA (38) were added to each culture and kept at 37°C for 6 hr. The cells were washed three times with PBS, and DMEM containing 10% fetal bovine serum was added. These cells were subjected directly to heat treatment by transferring the dishes into a water bath at 39°C or 44°C. After heat treatment, the cells were incubated at 37°C for 12 hr before being harvested.

CAT Assay. CAT assays were performed by the method of Gorman *et al.* (39) with modifications. Cells were washed three times with PBS. Extract was prepared by sonicating the cells in 200 μl of 40 mM Tris·HCl, pH 7.8/1 mM EDTA/150 mM NaCl. To determine the efficiency of transfection, half the extract was incubated at 65°C for 30 min and used to assay alkaline phosphatase activity (38). The remaining half was centrifuged for 30 min in an Eppendorf centrifuge at 4°C. The supernatant was removed and incubated at 65°C for 15 min. The resulting precipitate was removed by centrifugation for 15 min at 4°C. The supernatant was used for the CAT assay. The assay mixture (130 μl), containing 250 mM Tris·HCl (pH 7.5), 0.5 mM EDTA; [^{14}C]chloramphenicol (0.1 μCi, 57 mCi/mmol; 1 Ci = 37 GBq), and 8 μg of protein, was preincubated at 37°C for 10 min. The CAT reaction was started by the addition of 20 μl of 4 mM acetyl-CoA (0.53 mM final concentration) to the reaction mixture and stopped after 10 min by the addition of 1 ml of ethyl acetate. The products were extracted into the ethyl acetate phase, concentrated, and analyzed by thin-layer chromatography.

RESULTS

αB-Crystallin Accumulation Under Stress Conditions. To study the possible heat inducibility of αB-crystallin synthesis, we subjected NIH 3T3 cells to a thermal shock. After 1 hr at 42.5°C the cells were returned to 37°C. Total cell extracts were prepared at various times thereafter by lysis in SDS sample buffer (36). Immunoblots were prepared and αB-crystallin was detected with an affinity-purified monospecific antiserum raised in rabbits against pure human αB-crystallin (unpublished work). No αB-crystallin was detected in nonshocked cells (Fig. 1, lane 1). However, heat shock was followed by a strong, transient accumulation of αB-crystallin with maximal amounts found between 8 and 12 hr (lanes 2–6). Purified αB-crystallin from mouse eye lenses was used as a marker (lanes 11–13). It consisted of ≈70% αA-crystallin and ≈30% αB-crystallin. Using this standard, we estimated that the accumulation of αB-crystallin following a heat shock amounts to 0.1–0.2% of total cellular protein. Besides hyperthermia, numerous other stress conditions have been described that lead to HSP synthesis. Among them are the incubation of cells with Cd^{2+} or with sodium arsenite. We found that treatment of NIH 3T3 cells with these compounds caused substantial accumulation of αB-crystallin (Fig. 1, lanes 7, 8, and 10). These results clearly indicate that αB-crystallin is a HSP.

αB-Crystallins from Eye Lens and Heat-Shocked Fibroblasts Comigrate in Two-Dimensional Gels. The small murine HSP (HSP27) and αB-crystallin share extensive sequence similarity (5, 20–23). It was quite possible that our polyclonal antibody also detected HSP27. To ensure that the immunoreactive protein in heat-shocked cells was indeed αB-crystallin and not crossreactive HSP27, we subjected cell extracts to two-dimensional Western blot analysis. A single immunoreactive spot was detected among the proteins of heat-shocked NIH 3T3 cells. This protein was not detectable in nonshocked cells (Fig. 2*A*, compare uppermost and second panels). In the eye lens extract we detected one prominent spot, which was unphosphorylated αB-crystallin (Fig. 2*A*, third panel). The several minor spots probably represent phosphorylated αB-crystallin (41, 42) and degradation products. A mixture of an eye lens extract and the proteins from heat-shocked NIH 3T3 cells was also analyzed (Fig. 2*A*, bottom panel). Comigration of the immunoreactive protein of heat-shocked cells and the major form of eye lens αB-crystallin suggests identity between these proteins and clearly indicates that the HSP we detected was indeed αB-crystallin and not HSP27.

Cofractionation of αB-Crystallin and Nuclei Under Heat Shock Conditions. Cell fractionation studies from various laboratories have invariably localized the small HSP of *D. melanogaster* and mammalian cells inside or firmly associated with the nucleus at heat shock temperatures (17–19). They relocalize to the cytoplasm upon return of the system

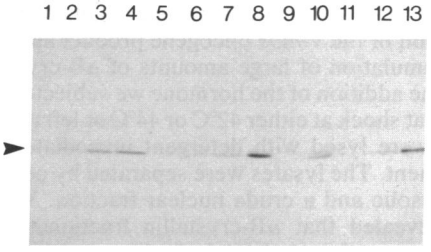

FIG. 1. Accumulation of αB-crystallin under stress conditions. NIH 3T3 cells were incubated for 1 hr at 42.5°C and returned to 37°C for 4 hr (lane 2), 8 hr (lane 3), 12 hr (lane 4), 16 hr (lane 5), or 24 hr (lane 6). NIH 3T3 cells were exposed to 100 μM sodium arsenite for 4 hr (lane 7) and 8 hr (lane 8) or to 100 μM $CdCl_2$ for 6.5 hr (lane 10). Untreated cells are represented in lanes 1 and 9. Total cellular extracts were prepared by lysis in SDS sample buffer. Samples (25 μg of protein) were electrophoresed in an SDS/12.5% polyacrylamide gel and a Western blot was prepared. αB-Crystallin was prepared from mouse eye lenses and 10 ng (lane 11), 20 ng (lane 12), or 40 ng (lane 13) was applied to the gel. Approximately 30% of the α-crystallin protein is αB-crystallin (arrowhead).

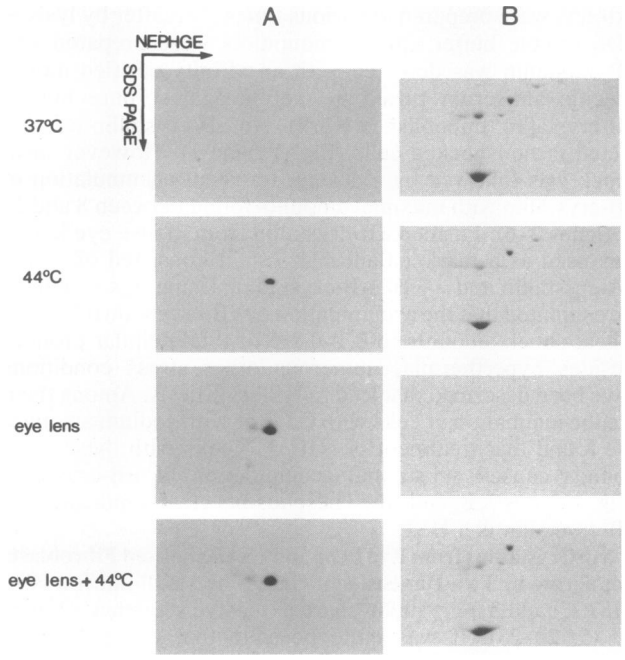

FIG. 2. Heat-induced αB-crystallin comigrates in two-dimensional gels with eye lens αB-crystallin. NIH 3T3 cells were subjected to the following heat treatment: successive incubations for 1 hr each at 39°C, 37°C, and 44°C, followed by a 2-hr incubation at 37°C. Heat-treated and untreated cells were lysed and S100 supernatants were prepared. Twenty micrograms of protein from NIH 3T3 cells, 1 μg of mouse eye lens proteins, and a mixture of the two were subjected to nonequilibrium pH-gradient gel electrophoresis (NEPHGE) followed by SDS/PAGE (40). The lower half of the gel was transferred to nitrocellulose and αB-crystallin was detected immunologically (*A*). The upper half of the gel was stained with Coomassie brilliant blue to verify equal loading of proteins (*B*). Only the relevant portions of the Western blots and only a section of the stained gels are shown. The acidic side of the first-dimension gel is to the left.

to normal temperatures. Since the temperature dependence of intracellular localization is a characteristic feature of the small HSPs, we investigated whether αB-crystallin behaves similarly. We previously showed (34) that high amounts of αB-crystallin accumulated in NIH 3T3 cells in response to the expression of the v-*mos* and Ha-*ras* oncogenes. The NIH 3T3-derived cell line NIH[LTR-*mos*] contains a v-*mos* gene driven by the glucocorticoid hormone-inducible promoter of mouse mammary tumor virus. Treatment of these cells with the synthetic hormone dexamethasone results in the transient accumulation of the v-*mos* oncogene product and the subsequent accumulation of large amounts of αB-crystallin. One day after the addition of the hormone we subjected such cells to a 1-hr heat shock at either 42°C or 44°C or left them at 37°C. The cells were lysed with detergent immediately after the heat treatment. The lysates were separated by centrifugation into a cytosolic and a crude nuclear fraction. Western blot analysis revealed that αB-crystallin fractionates with the non-nuclear proteins at physiological temperatures and with crude nuclei at heat shock temperatures (Fig. 3). Thus αB crystallin and the small HSP exhibit the same temperature-dependent fractionation behavior.

αB-Crystallin Forms 17S Particles in Fibroblasts. The small HSPs of various organisms aggregate into globular structures with a sedimentation coefficient of ≈17 S. We investigated whether αB-crystallin in fibroblasts is present in an aggregated form reminiscent of such heat shock granules. For this purpose we treated NIH[LTR-*mos*] cells with dexamethasone for 1 day and analyzed the sedimentation behavior of the αB-crystallin that accumulated under these conditions. Dexamethasone-treated cells and untreated cells were hypotonically lysed. The soluble proteins were fractionated by sucrose gradient centrifugation and the fractions were analyzed by SDS/PAGE. The analysis revealed the exclusive presence of a 23-kDa protein in dexamethasone-treated cells (Fig. 4 *Upper*). Western blotting of a similar gel run in parallel detected αB-crystallin in the same fractions in which the 23-kDa protein was observed. Again, this protein was present only among the polypeptides from dexamethasone-treated cells (Fig. 4 *Lower*). It is very likely that the 23-kDa protein and αB-crystallin are identical. The immunoreactive protein sedimented on a sucrose gradient with the same or a very similar sedimentation coefficient as the heat shock granules. The results from this and the previous experiment lead us to conclude that the classical HSPs and αB-crystallin must have very similar physical properties.

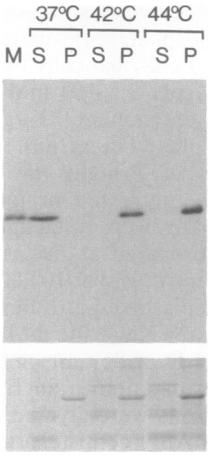

FIG. 3. Cofractionation of αB-crystallin with nuclei at high temperature. NIH[LTR-*mos*] cells were treated with 1 μM dexamethasone for 1 day to induce the oncogene-mediated accumulation of αB-crystallin. Thereafter they were incubated for 1 hr at the temperature indicated above the lanes. Cells were detergent-lysed and soluble (lanes S) and particulate (lanes P) fractions were obtained by centrifugation of the lysates. Samples (10 μg of protein) were fractionated by SDS/12.5% PAGE. The lower half of the gel was subjected to Western blot analysis (*Upper*) and the upper half was stained with Coomassie brilliant blue. A section of the stained gel is shown (*Lower*). Mouse eye lens extract (1 μg) served as marker for αB-crystallin (lane M).

The αB-Crystallin Promoter Is Heat-Inducible. The accumulation of HSPs under stress conditions is most often the consequence of induced transcriptional activation of their cognate genes (43). To assay transcriptional activation of the αB-crystallin gene during heat shock, we fused a 711-bp DNA fragment of its promoter region (28) to the bacterial CAT gene. This chimeric DNA was introduced into NIH 3T3 cells and CAT activity was determined in cells that had been kept at 37°C or were heat shocked. Heat treatment led to a very strong increase in CAT activity (Fig. 5). This increase was more pronounced in cells that were subjected to two heat treatments rather than one (Fig. 5, lane 3). Quantitation of the CAT activity revealed a 9-fold and a 12-fold induction in the cells that underwent one and two successive heat treatments, respectively. Hence, the αB-crystallin gene promoter is subject to stress-mediated transcriptional control.

DISCUSSION

We have shown that αB-crystallin accumulation in mouse NIH 3T3 cells is a response to cellular stress. By definition, this criterion classifies αB-crystallin as a HSP. There are several reasons to consider αB-crystallin a member of the family of small HSPs. (*i*) Its molecular weight falls within the size range of small HSPs. (*ii*) Strong sequence similarity

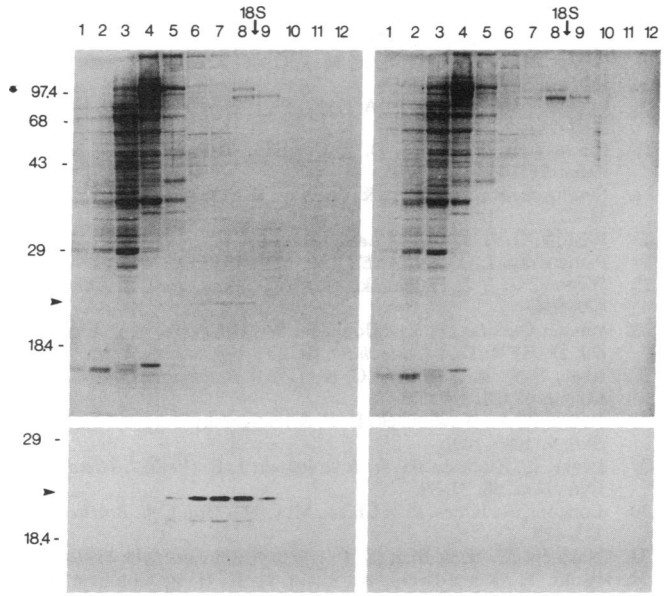

FIG. 4. Fractionation of αB-crystallin on a sucrose gradient. NIH[LTR-*mos*] cells were treated for 24 hr with 1 μM dexamethasone or were left untreated. Concentrated proteins from the S100 supernatants were applied to 5–30% sucrose gradients and centrifuged at 35,000 rpm in an SW40 rotor for 16 hr. Fractions (1 ml) were collected from the bottom of the tubes and aliquots of each fraction were analyzed in parallel SDS/polyacrylamide gels (36). One gel was stained with Coomassie brilliant blue (*Upper*), and the proteins from the relevant part of the other gel were transferred to nitrocellulose for immunological detection of αB-crystallin (*Lower*). Gels were loaded with proteins from hormone-treated cells (*Left*) or untreated cells (*Right*). Fraction 1 is from the top of the gradient. Position of αB-crystallin is indicated by the arrowhead. Molecular mass markers (kDa) are given at left. Total mouse RNA was fractionated on a parallel sucrose gradient and 0.5-ml fractions were collected. The position of 18S rRNA is indicated between fractions 8 and 9.

between αB-crystallin and most small HSPs is evident (27–31). (*iii*) Like the small HSPs, αB-crystallin is found in the detergent-insoluble fraction during a heat shock and in the soluble fraction thereafter. (*iv*) αB-Crystallin aggregates into supramolecular structures with the same sedimentation characteristics as heat shock granules (4, 12, 15).

The αB-crystallin promoter is subject to a heat-regulated transcriptional control mechanism. Transcriptional regulation of many HSP genes at elevated temperatures is mediated by the heat shock factor (HSF), which binds to the heat shock element (HSE) in the promoter region of these genes (for recent reviews see refs. 1, 43, and 44). Functional HSEs consist of at least three contiguous 5-bp units with alternating NGAAN and NTTCN sequence motifs (44, 45). The HSF binds as a trimer to these HSEs, probably with each monomer contacting one 5-bp unit (46). Sequence analysis of the available promoter region of the αB-crystallin gene revealed one perfect HSE between positions −39 and −53 from the start site (Fig. 6). Two contiguous 5-bp units are found further upstream, at −376 to −385. This distal DNA sequence is expected to bind HSF *in vitro* but is unlikely to mediate heat shock-dependent transcription on its own (45). Nevertheless it may enhance transcription in conjunction with the proximal HSE.

The induced expression of the Ha-*ras* and v-*mos* oncogenes in NIH 3T3 cells triggers the transient induction of the αB-crystallin gene. The same conditions did not lead to HSP70 gene expression, indicating that these oncogenes do not elicit a general stress response (47). Activation of small HSP genes in the absence of stress has been observed by several investigators. Particularly well studied is the expression of small

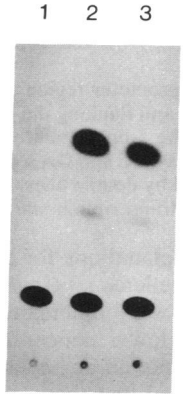

FIG. 5. Heat-mediated transcriptional activation of the αB-crystallin promoter as detected by CAT assay. A DNA fragment of 711 bp spanning 666 bp from the promoter and 45 bp from the nontranslated leader region of the αB-crystallin gene (28) was fused to the CAT gene. NIH 3T3 cells were cotransfected with DNA of this construct together with pSVA2PAP, an expression plasmid encoding a human placental alkaline phosphatase gene driven by the simian virus 40 early promoter (38). Heat treatment was performed 8 hr after transfection. Cells either were incubated at 44°C for 1 hr and collected 12 hr later (lane 3) or were first treated at 39°C for 1 hr, and, after a recovery period of 1 hr at 37°C, were subjected to a second heat shock at 44°C for 1 hr before being collected 12 hr later (lane 2). Control cells were left at 37°C (lane 1). Alkaline phosphatase activity was measured in parallel with the CAT activity from a portion of the transfected cells and served as a control for transfection efficiency. The alkaline phosphatase activities under the hyperthermic conditions were inhibited 14- to 31-fold. This reduction is probably a consequence of the selected expression of HSPs under hyperthermic conditions. Therefore, the CAT activities could not reliably be compared to an internal standard and were compared based only on the amount of protein used in the assay.

HSP genes under various developmental conditions in *D. melanogaster* (48). Likewise, selective expression of the HSP22 gene has been observed in the indirect flight muscle cells of flightless *D. melanogaster* mutants (49, 50). That single small HSP genes or various combinations thereof can be activated selectively under specific conditions suggests complex arrays of controlling sequence elements in their promoter regions. The expression of several HSP genes during development and heat shock is indeed regulated by completely different promoter elements (for a review see ref. 48).

The well-known sequence similarity between αB-crystallin and the small HSPs has led two groups to study the heat inducibility of the α-crystallin genes in eye lens epithelial cells (51, 52). No increased synthesis of α-crystallins was noticed in those experiments, suggesting that α-crystallins are not bona fide HSPs. αB-Crystallin is one of the major eye lens proteins and we assume that its gene is constitutively induced at the maximal level in this tissue. Therefore αB-crystallin gene expression in the lens epithelial cells might be refractory to further stimulation by a heat shock.

The physiological function of small HSPs is not known, but there is good evidence for their involvement in the establishment of heat tolerance (53, 54). It is quite possible that αB-crystallin participates in this task as well. Deletion of the only known small HSP gene of *Saccharomyces cerevisiae* was without any effects on several parameters tested, including heat tolerance (55, 56). The buildup of heat tolerance could be of such foremost importance for cell survival that redundant mechanisms might have evolved to establish the required conditions. A second, related HSP gene might exist in yeast, whose product fulfills the same or a similar task as HSP27. Such a putative gene could be the equivalent of αB-crystallin.

FIG. 6. HSEs in the promoter region of the αB-crystallin gene. The sequences including and flanking the two and three contiguous 5-bp units are shown. The 5-bp units are underlined and the conserved central nucleotides TTC and GAA are indicated by dots. The TATA box is emphasized by double lines. The numbers indicate the distance from the major transcription start site (28, 34).

We propose two explanations for the high abundance of αB-crystallin in the eye lens. (*i*) *Gene sharing*. Many of the crystallins, which are very abundant in the lens, are also present, but at much lower concentrations, in most other tissues, where they function as housekeeping enzymes (24, 25, 57–59). It is assumed that they serve a structural role in the lens rather than their normal enzymatic role. Their physical properties, which allow their association into a highly transparent structure, might have led to their recruitment as components of the lens fibers during the evolution of the eye. αB-Crystallin might have originally evolved as a HSP. Its ability for ordered aggregation might have determined its involvement in the formation of the eye lens. (*ii*) *Heat tolerance*. A large fraction of the lens consists of fiber cells that have lost their nuclei and translational capacity. Therefore, they can no longer respond to stress by synthesizing HSPs. However, these cells are particularly vulnerable because of their proximity to environmental stress sources. In addition, the lens is devoid of blood vessels, which prevents a rapid homeostatic regulation. The constitutive high level of αB-crystallin might render the lens permanently stress-tolerant. If this explanation is correct, it implies that high concentrations of αB-crystallin can confer stress tolerance in the absence of the other HSPs. Since these cells no longer synthesize RNA or proteins, their requirements for stress protection might be relaxed.

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