

# Expression of the Murine Small Heat Shock Proteins hsp 25 and $\alpha$ B Crystallin in the Absence of Stress

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**Abstract.** Stress induces the synthesis of several large and small heat shock proteins (hsp's). Two related small hsp's, hsp25 and  $\alpha$ B crystallin exist in mice.  $\alpha$ B crystallin is an abundant protein in several tissues even in the absence of stress. Particularly high amounts accumulate in the eye lens. Here we show that hsp25 is likewise constitutively expressed in many normal adult tissues. In the absence of stress the protein is most abundant in the eye lens, heart, stomach,

colon, lung, and bladder. The stress-independent expression pattern of the two small hsp's is distinct. In several tissues the amount of hsp25 exceeds that accumulating in NIH 3T3 fibroblasts in response to heat stress. hsp25, like  $\alpha$ B crystallin, exists in a highly aggregated form in the eye lens. The expression of hsp25 and  $\alpha$ B crystallin in normal tissues suggests an essential, but distinct function of the two related proteins under standard physiological conditions.

ALL organisms respond to various forms of stress by synthesizing several heat shock proteins (hsp's)<sup>1</sup> which are believed to render the cells less vulnerable to the damaging potential of the stressful conditions (for reviews see Lindquist and Craig, 1988; Morimoto et al., 1990). However, most hsp genes are not only expressed in response to stress but are also induced by other physiological conditions. This stress-independent expression of hsp's is a widespread phenomenon and probably of vital importance. In yeast, interference with the basal expression of hsp's prevents cell growth (Sorgner and Palham, 1988; Wiederrecht et al., 1988). Stress-independent expression of the human hsp70 gene has been correlated both positively and negatively with cellular proliferation. The gene is induced in resting cells upon growth stimulation (Wu and Morimoto, 1985) and periodically in proliferating cells at the G1-S border of the cell cycle (Milarski and Morimoto, 1986). Others have demonstrated a correlation between the nonproliferative state and hsp70 expression (Kaczmarek et al., 1987; Iida and Yahara, 1984; Santoro et al., 1989; Holbrook et al., 1992; Feder et al., 1992). Moreover, there exist several hsp70 cognate genes which are expressed at all times and which are barely stress inducible (for a recent review see Phillips and Morimoto, 1991). The proteins of the hsp90 family are relatively abundant at the normal temperature and the basal level is augmented in response to stress. In addition, the *Drosophila* hsp90 gene is induced during oogenesis (Zimmerman et al., 1982). Likewise the two mouse hsp90 genes are subject

to developmental regulation (Lee, 1990). Some of the low molecular weight hsp's are developmentally regulated as well. In *Drosophila melanogaster*, a subset of them is induced during oogenesis while all of them are expressed in early pupae probably in response to the molting hormone ecdysone (for reviews see Pauli and Tissière, 1990; Arrigo and Tanguay, 1991). Two of the *Saccharomyces cerevisiae* hsp's, hsp26 and hsp84, strongly accumulate during sporulation (Kurtz et al., 1986).

Two small hsp's, hsp25 and  $\alpha$ B crystallin, have been detected in mice. Besides considerable sequence similarity and stress responsiveness they share the ability to form 17S cytoplasmic particles and to relocalize from the cytoplasm into or around the nucleus during a heat shock. Thus they are likely to serve similar functions.  $\alpha$ B crystallin is not only one of the most abundant eye lens proteins but is also present in several other tissues in substantial amounts (Iwaki et al., 1991; Dubin et al., 1989; Longoni et al., 1990; Bhat and Nagineni, 1989; Iwaki et al., 1990; Atomi et al., 1991; Kato et al., 1991). Its low basal level in fibroblasts under normal conditions has recently allowed its identification as a hsp (Klemenz et al., 1991b). However, little is known about stress-independent expression of hsp25. The hsp25 gene is estrogen-responsive (Fuqua et al., 1989) and has been found expressed in several estrogen sensitive human tissues and breast tumors (Ciocca et al., 1983; Seymour et al., 1990; Thor et al., 1991). However, a systematic search for stress-independent expression of hsp25 has not yet been reported.

The abundant expression of  $\alpha$ B crystallin in several normal tissues prompted us to investigate the stress-independent accumulation of the related protein hsp25. Here we demon-

1. Abbreviations used in this paper: HSF, heat shock transcription factors; hsp, heat shock protein; 2D, two dimensional.

strate that hsp25 is present in all analyzed organs. However, the level of accumulated hsp25 varies greatly between different organs. The highest level of hsp25 was found in the eye lens. Particularly high amounts were also found in heart, stomach, colon, lung, and bladder. The expression patterns of hsp25 and  $\alpha$ B crystallin are distinct. The constitutive level of hsp25 in several organs exceeds that obtained in heat-stressed fibroblasts.

## Materials and Methods

### Cell Culture Conditions

NIH 3T3 cells were grown in DME supplemented with 10% FCS and antibiotics. To induce the heat shock response, cells in 75 cm<sup>2</sup> culture flasks were submerged in a water bath at 42.5 or 44°C for 1 h.

### Western Blots

Cultured cells were washed twice with PBS, mechanically removed from the tissue culture plate with the help of a rubber policeman, and collected by centrifugation. Sample buffer for SDS-PAGE (Laemmli, 1970) or for two-dimensional (2D) gel electrophoresis (O'Farrell, 1975) was added directly to the cell pellets. Cell extracts in SDS sample buffer were immediately boiled for 5 min. Protein concentrations were determined by spotting aliquots on nitrocellulose membranes and staining with Amido black (Schaffner and Weissmann, 1973). Mouse organs were washed twice in PBS and frozen in liquid nitrogen. Cell extracts were prepared by crushing small pieces of tissues between two sheets of plastic foil in the presence of some dry ice with a hammer. The crushed material was transferred into an Eppendorf centrifugation tube containing gel electrophoresis sample buffer. Proteins were either separated on 12.5% SDS polyacrylamide gels (Laemmli, 1970) or by isoelectric focussing followed by SDS-PAGE (O'Farrell, 1975). The pH gradient was produced with a mixture of the ampholines (LKB) pH 3.5–10, pH 4–6, pH 6–8, and pH 9–11 at the ratio of 10:1:1:1. After electrophoretic separation, proteins were either silver stained (Morrissay, 1981) or electrophoretically transferred to nitrocellulose membranes. hsp's were detected using affinity-purified rabbit antibodies directed against pure human eye lens  $\alpha$ B crystallin or recombinant rat hsp25. The secondary antibodies were either coupled to alkaline phosphatase or HRP. The immunoreactive proteins were visualized by enzymatic staining or by chemiluminescence, respectively. Recombinant rat hsp25 was produced in *E. coli*

transfected with the expression plasmid pAKHSP25 (Gaestel et al., 1989). Hsp25 was purified on SDS polyacrylamide gels and used to immunize rabbits.

### Northern Blots

RNA was prepared from tissue culture cells according to Chomczynski and Sacchi (1987). 5  $\mu$ g of total RNA was separated on 1% agarose gels (MacMaster and Carmichael, 1977) and transferred to nylon membranes (Genescreen plus, New England Nuclear, Boston, MA). The DNA probe used to detect  $\alpha$ B crystallin mRNA was a 0.7-kb EcoRI-XbaI fragment from the plasmid pCry1 harboring a cDNA insert derived from mouse  $\alpha$ B crystallin mRNA (Klemenz et al., 1991a). The hsp25 and hsp70 mRNA were hybridized with a 0.9-kb XbaI-PstI fragment from plasmid pUC HSP25 (Gaestel et al., 1989) and a 1.7-kb HindIII-EcoRI fragment from the plasmid MHS 243 (Love and Moran, 1986), respectively. The DNA fragments were labeled by the oligonucleotide random priming method (Feinberg and Vogelstein, 1983, 1984).

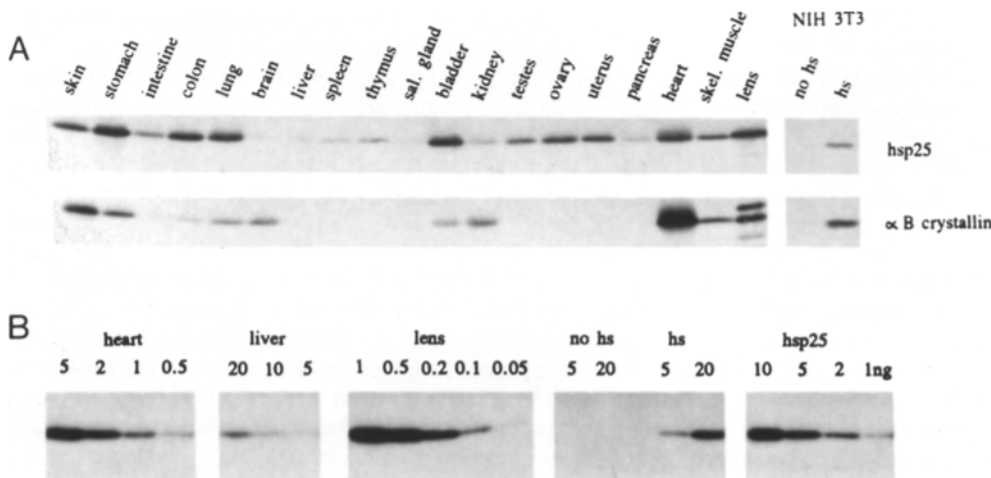
### Size Fractionation of Eye Lens Proteins

Dissected mouse eye lenses were shaken at 4°C for 16 h in 1 ml, 0.1 M Tris HCl, pH 7.3, 2 mM DTT. Insoluble material was removed by centrifugation at 12,000 rpm in a microcentrifuge for 30 min. Proteins were fractionated on a Bio Gel A-5 m gel filtration column (1.6  $\times$  85 cm) in the same buffer.

## Results

### Tissue-specific Expression of the Small hsp's

$\alpha$ B crystallin was originally found in the eye lens where it is one of the most abundant proteins. Later it was detected in many other tissues and it was undetectable in only very few cell types (Dubin et al., 1989; Bhat and Nagineni, 1989; Iwaki et al., 1990, 1991; Longoni et al., 1990; Atomi et al., 1991; Kato et al., 1991). To investigate whether the same holds true for hsp25 we prepared protein extracts from mouse organs and subjected them to Western blot analysis (Fig. 1 A). hsp25 could be detected in all analyzed tissues. The relative amount varied considerably between different tissues. The highest concentrations were found in the eye



**Figure 1.** Tissue-specific expression of small hsp's. (A) 5 and 30  $\mu$ g of protein extracts from all indicated tissues and NIH 3T3 fibroblasts were analyzed in the upper and lower rows, respectively, of A. Only 0.3  $\mu$ g eye lens extract was analyzed in the lower row. The Western blots were developed with antisera directed against hsp25 (upper row) and  $\alpha$ B crystallin (lower row). NIH 3T3 cells were heat shocked at 42.5°C for 1 h and further incubated at 37°C for 6 h (hs). The protein extracts from tissues and NIH 3T3 cells were analyzed on the same Western

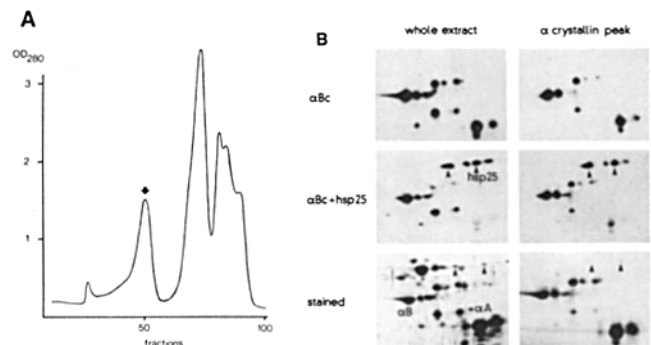
blot. hsp25 is detected more efficiently by its antiserum than  $\alpha$ B crystallin. Thus, the intensities of the hsp25 and  $\alpha$ B crystallin signals do not reflect the relative abundance of the two proteins within the same tissue. (B) Different amounts of extracts from tissues and NIH 3T3 cells, heat shocked (hs) or not (no hs) as well as purified recombinant hsp25 were analyzed on the same Western blot, developed with hsp25 antiserum. The amounts of proteins loaded (in  $\mu$ g for tissues and NIH 3T3 cells, in ng for purified hsp25) are indicated above the lanes.

lens. Particularly high amounts were also observed in heart, bladder, lung, colon, and stomach. The tissues with the lowest hsp25 concentration are liver, brain, and salivary gland. To estimate the absolute amounts of hsp25 in tissues we subjected various amounts of protein extracts from three selected organs as well as known quantities of pure hsp25 to Western blot analysis (Fig. 1 B). The concentration of the purified hsp25 was accurately determined by amino acid composition analysis. hsp25 is most abundant in the eye lens where it amounts to  $\sim 0.2$ –1% of total protein. This is at least 10 times less than  $\alpha B$  crystallin. In nonlenticular organs it amounts to 0.003–0.2%. The expression pattern of hsp25 was compared with that of  $\alpha B$  crystallin. We detected  $\alpha B$  crystallin in every analyzed organ. However, there was great variation in the amount of accumulated  $\alpha B$  crystallin in the different organs. The tissue distribution of the two small stress proteins is very different. Colon, ovary, and uterus are among the organs of particularly high hsp25 expression. However,  $\alpha B$  crystallin accumulates to only low levels in these organs. On the other hand,  $\alpha B$  crystallin is very abundant in the brain where the hsp25 level is very low.

### *hsp25 in the Eye Lens*

The  $\alpha$  crystallins ( $\alpha A$  and  $\alpha B$  crystallin) form high mol wt aggregates in the eye lens and can easily be separated from the other eye lens proteins by size fractionation. Evidence has been presented that  $\alpha B$  crystallin and hsp25 coaggregate (Zanema et al., 1989). To determine whether hsp25 is present in an aggregated state in the lens we subjected soluble eye lens proteins to size separation on a Bio Gel A-5m column (Fig. 2 A). The first protein peak which eluted immediately after the void volume contained the  $\alpha$  crystallins. An aliquot from this fraction and from a total eye lens extract each were analyzed on 2D gels. The proteins in these gels were either silver stained (Fig. 2 B, lower panel) or transferred to nitrocellulose membranes. Small hsp's were detected immunologically using the  $\alpha B$  crystallin antiserum (Fig. 2 B, upper panel) or a mixture of the  $\alpha B$  crystallin and the hsp25 antiserum (Fig. 2 B, middle panel). hsp25 cofractionates with the  $\alpha$  crystallins. The hsp25 antiserum detected four protein spots which correspond to unphosphorylated and various phosphorylated forms of hsp25 (see below). The arrowheads point to the two most abundant spots which were also detected on silver-stained gels.  $\alpha$  crystallin was present exclusively in the first protein peak of this column (not shown). Since the ratio of  $\alpha B$  crystallin to hsp25 is the same in the total protein extract and in the  $\alpha$  crystallin fraction, we conclude that most or all of the hsp25 in the lens is present in a highly aggregated form. Our  $\alpha B$  crystallin antibody detects multiple proteins on 2D Western blots. All of these proteins cofractionate with  $\alpha$  crystallin and are probably modified forms of  $\alpha B$  crystallin. Some cross-reactivity of the antiserum with  $\alpha A$  crystallin was also observed.

Silver staining of the 2D gels revealed that hsp25 is a minor eye lens protein. The efficiency of silver staining varies considerably for different proteins. Therefore we compared the staining efficiencies of the two highly purified small hsp's whose concentrations have been accurately determined by amino acid composition analysis and found them to be very similar (data not shown).



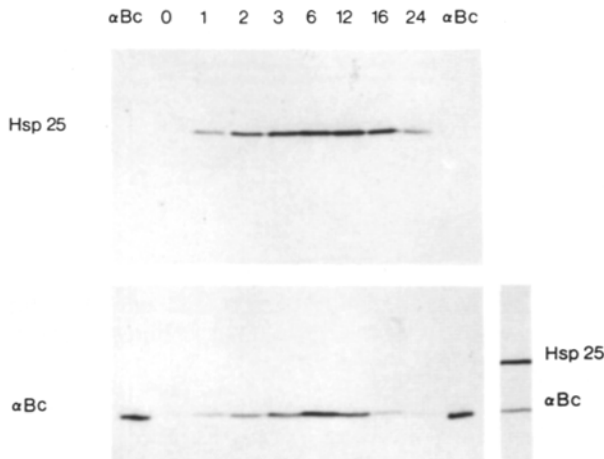
**Figure 2.** The two small hsp's exist in a highly aggregated form in the eye lens. (A) Soluble mouse eye lens proteins were size fractionated on a BioGel A-5 m column. The arrow indicates the protein peak which was analyzed in B. (B) Part of the same eye lens extract whose fractionation is shown in A as well as proteins in fraction number 51 of the BioGel A-5 m column ( $\alpha$  crystallin peak) were analyzed on 2D IEF gels. The proteins were either transferred to nitrocellulose (top and middle rows) or silver stained. The Western blots were developed with an anti  $\alpha B$  crystallin antiserum (top) or a mixture of antisera directed against the two small hsp's. The two major forms of hsp25 are indicated by arrowheads. The acidic end of the gels is on the right.

### *hsp25 and $\alpha B$ Crystallin Accumulate After a Heat Shock with Similar Kinetics*

We wished to compare the amount of small hsp's which accumulate under normal conditions in various tissues and in response to stress. The two small hsp's are barely detectable in unstressed NIH 3T3 fibroblasts, which allowed us to estimate their stress-mediated accumulation. Cells were exposed to a 1-h heat shock at 42.5°C. Cellular protein extracts were prepared at different times after the heat shock and were subjected to Western blot analysis (Fig. 3). After the heat stress  $\alpha B$  crystallin and hsp25 accumulated with the same kinetics. Peak levels of the two proteins were reached between 8 and 12 h. We were surprised to observe the relatively slow accumulation of the two hsp's in NIH 3T3 cells. hsp's are assumed to counteract deleterious effects of elevated temperature and other stresses. Therefore, their synthesis should occur as a rapid response to the insult. This is indeed true for hsp70 (Fig. 4). This gene is very rapidly induced. mRNA accumulation peaks shortly after the heat shock. In contrast, the kinetics of hsp25 and  $\alpha B$  crystallin mRNA accumulation was much delayed (Fig. 4). A more severe heat shock at 44°C delays the accumulation of all three tested hsp RNAs. However, the time gap between the appearance of hsp70 mRNA and the mRNAs encoding the small hsp's persists (Fig. 4). The small hsp genes, unlike the hsp70 gene, contain introns. The delayed accumulation of small hsp mRNA accumulation after a heat shock might be the consequence of impaired splicing at elevated temperature (for a review see Yost et al., 1990). If true, these two proteins should fail to accumulate during a continuous heat shock. We found, however, that the small hsp's accumulate with the same kinetics and to a similar extent after a heat pulse and during continuous heat shock at 42.5°C (data not shown).

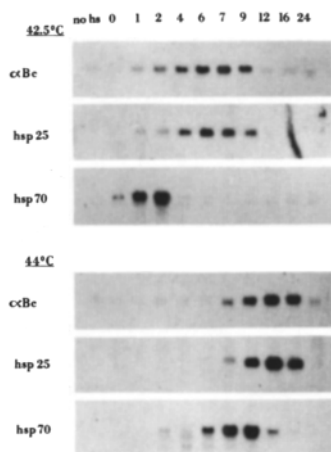
### *The Phosphorylation Pattern of hsp25 in Tissues and Heat-Shocked Fibroblasts*

Three isoforms of hsp25 representing unphosphorylated as

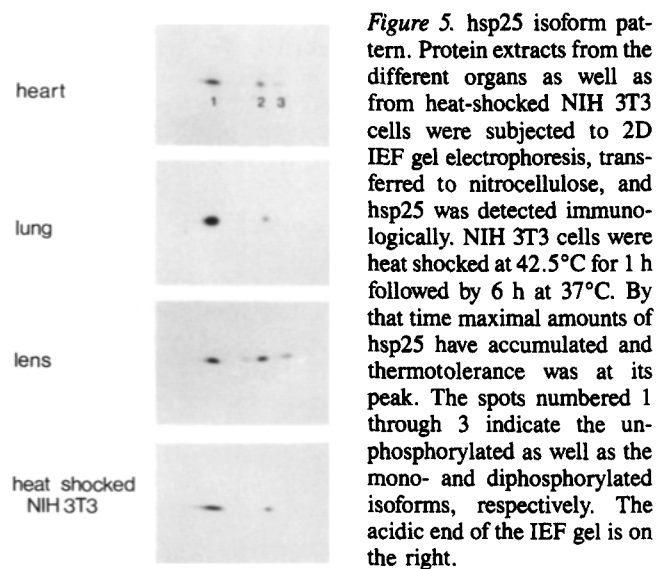


**Figure 3.** Heat shock-mediated expression of the two small hsp's in NIH 3T3 cells. Cells were heat shocked for 1 h at 42.5°C and subsequently incubated at 37°C for the time indicated (in hours). Western blots were developed with antisera directed against hsp25 (top),  $\alpha$ B crystallin (bottom, left) or a mixture of the two antisera. Purified  $\alpha$ B crystallin from a human eye lens was used as a marker. The human protein migrates slightly faster than the mouse protein.

well as mono- and diphosphorylated protein have been described. hsp25 is phosphorylated in response to heat shock (Welch, 1985) as well as to treatment with phorbol ester (Feuerstein and Cooper, 1983, 1984; Welch, 1985; Regazzi et al., 1988), serum (Welch, 1985), tumor necrosis factor  $\alpha$  (Darbon et al., 1990; Schütze et al., 1989; Hepburn et al., 1988; Robaye et al., 1989; Kaur and Saklatvala, 1988; Arigo, 1990), interleukin 1 (Kaur and Saklatvala, 1988), arsenite (Kim et al., 1984; Welch, 1985; Crete et al., 1990), and to platelet activation by thrombin (Mendelsohn et al., 1991). Two serine residues have been identified which are susceptible to phosphorylation by protein kinase C and cAMP-dependent protein kinase (Gaestel et al., 1991). Since phosphorylation of hsp25 is postulated to regulate hsp25 activity, it is of relevance to investigate the phosphorylation state of hsp25 in tissues under nonstressed conditions. Protein extracts from heart, lung, and lens as well as from heat-shocked NIH 3T3 cells were analyzed on 2D Western blots (Fig. 5). Unphosphorylated hsp25 predominated in all cell types. The proportion of phosphorylated hsp25 varied in



**Figure 4.** Stress-mediated accumulation of hsp mRNA. NIH 3T3 cells were heat shocked for 1 h at 42.5°C (upper half) or 44°C (lower half) and subsequently incubated at 37°C for the time period (in hours) indicated at the top of the figure. Northern blots were hybridized with probes which selectively recognized the  $\alpha$ B crystallin ( $\alpha$ Bc), the hsp25, and the hsp70 mRNA.



**Figure 5.** hsp25 isoform pattern. Protein extracts from the different organs as well as from heat-shocked NIH 3T3 cells were subjected to 2D IEF gel electrophoresis, transferred to nitrocellulose, and hsp25 was detected immunologically. NIH 3T3 cells were heat shocked at 42.5°C for 1 h followed by 6 h at 37°C. By that time maximal amounts of hsp25 have accumulated and thermotolerance was at its peak. The spots numbered 1 through 3 indicate the unphosphorylated as well as the mono- and diphosphorylated isoforms, respectively. The acidic end of the IEF gel is on the right.

different tissues and was the highest in lens. Thermotolerance slowly develops in NIH 3T3 fibroblasts in response to a heat shock (A. Aoyama and R. Klemenz, unpublished observation). 6 h after a heat shock when a thermotolerance reaches its maximal extent, cells were collected for hsp25 isoform analysis (Fig. 5, lower panel). Phosphorylation of hsp25 was not more extensive under these conditions than it was in unstressed heart, lens, kidney, and stomach (Fig. 5 and data not shown). To exclude the possibility that phosphatase and/or kinase activities in the tissue extracts distorted the phosphorylation pattern we have repeated the 2D gel analyses with extracts which have been incubated at room temperature for several hours and have obtained identical results.

## Discussion

Previous reports have revealed the widespread expression of the  $\alpha$ B crystallin gene (Dubin et al., 1989; Bhat and Nagineni, 1989; Iwaki et al., 1990, 1991; Longoni et al., 1990; Atomi et al., 1991; Kato et al., 1991). Here we demonstrate that the related protein hsp25 is expressed in all analyzed tissues as well. The extent of accumulated hsp25 and  $\alpha$ B crystallin in the various tissues varies enormously. However, this does not necessarily mean that their expression in individual cells varies correspondingly. hsp25 might be expressed in a subset of cells within an organ only as has been shown for  $\alpha$ B crystallin (Iwaki et al., 1990, 1991; Kato et al., 1991). Tissue-specific transcription has been described as the main regulatory mechanism underlying differential accumulation of  $\alpha$ B crystallin in tissues (Dubin et al., 1989, 1991). No such information is available concerning hsp25 gene expression. We have recently isolated the murine hsp25 gene (unpublished data) which will allow us to study its transcriptional expression pattern. Both small hsp genes are steroid hormone responsive. The hsp25 and  $\alpha$ B crystallin genes are regulated by estrogen (Fuqua et al., 1989) and glucocorticoids, respectively (our own unpublished observation). This differential hormone responsiveness might partly account for discordant expression.

The two small hsp's have been shown to establish stress

tolerance even in the absence of other hsp's (Landry et al., 1989; Berger and Woodward, 1983; A. Aoyama and R. Klemenz, unpublished observation). The phosphorylation of hsp25 in response to stress might modify its potency to protect against stress. Phosphorylated hsp25 has been observed in all analyzed tissues. Thus, given the high level of small hsp's in some cells and the presence of phosphorylated isoforms we expect these cells to be permanently stress tolerant. A great variability in thermosensitivity of different cell types has indeed been noted but a correlation between this property and the constitutive level of hsp's has not been described. Stress probably causes several kinds of defects to cells and the various hsp's are likely involved in different aspects of protection. It is unknown what injuries are prevented or repaired by small hsp's. Whatever the protective role of the small hsp is, it might be required by some cells at all times. Certain cells might need to be continuously protected because they are permanently exposed to stress or because they cannot tolerate the long lag between the onset of the insult and the establishment of the protected state. hsp25 and  $\alpha$ B crystallin might protect against different kinds of stress and their differential pattern of expression might indicate that different organs are exposed to different kinds of stress.

Many eye lens crystallins were identified as housekeeping enzymes (for reviews see Piatigorsky, 1989, 1992; Wistow and Piatigorsky, 1988; de Jong, 1989; Doolittle, 1988; Piatigorsky and Wistow, 1989, 1991). Their high abundance in the lens has been attributed to a proposed evolutionary mechanism termed gene sharing. According to that model, appropriate genes which encoded proteins suitable for the formation of a transparent lens became overexpressed in the evolving eye lens epithelium. The primordial  $\alpha$  crystallin/hsp25 gene probably encoded a small hsp before having been recruited as a crystallin gene. Subsequently, it must have undergone a duplication event which gave rise to the  $\alpha$  crystallin and hsp25 genes. The former duplicated again to yield the  $\alpha$ A and  $\alpha$ B crystallin genes. Thereafter, the  $\alpha$ A crystallin gene lost stress inducibility. The hsp25 gene has retained relatively high lenticular expressivity during the long time since the first gene duplication event, suggesting that it serves an important function in the lens. It is interesting to note that several other crystallins are either stress-inducible or related to stress proteins (Iida and Yahara, 1984, 1985; De Pomerai and Carr, 1987; Wistow, 1990). This may indicate that stress responsiveness was one of the selection forces operating during the evolution of the eye lens.

The delayed synthesis of small hsp's as compared with hsp70 in response to stress has previously been observed in *Drosophila melanogaster* cell lines (Di Domenico et al., 1982a,b), in HeLa cells (Hickey and Weber, 1982), and in rats (Blake, 1990). Our observation that  $\alpha$ B crystallin accumulates with the same delayed kinetics as hsp25 further demonstrates the relationship of these two hsp's and suggests a common mode of stress-mediated induction. The heat shock-induced accumulation of the chicken small hsp, hsp23, mainly results from an increased hsp23 half-life with little increase in mRNA synthesis and stability (Edington and Hightower, 1990). In contrast, the two murine small hsp's and their cognate mRNA accumulate in parallel. Thus, transcriptional activation of the small hsp gene seems to be the major regulatory mechanism as is the case for the *Dro-*

*sophila melanogaster* (Corces, 1980), *Caenorhabditis elegans* (Jones et al., 1989), *Saccharomyces cerevisiae* (Susek and Lindquist, 1990), and *Xenopus laevis* (Krone and Heikkila, 1989) small hsp's. Heat shock transcription factors (HSF) have been identified which bind to DNA sequence elements in the 5' flanking regions of hsp genes (for reviews see Lis et al. 1990; Wu et al., 1990; Sorger and Pelham, 1991). The kinetics of HSF activity as revealed by DNA binding assays parallels that of hsp70 mRNA synthesis (Morimoto and Milavski, 1990; Mosser et al., 1988). However, the strongest accumulation of hsp25 and  $\alpha$ B crystallin mRNA occurs at times when hsp70 transcription has already dropped to a very low level and HSF is expected to be inactivated. Therefore, it seems likely that the HSF which is instrumental for hsp70 gene expression is not required for stress-mediated induction of the small hsp genes. Heat-induced expression of a small hsp gene has indeed been observed under conditions where hsp70 gene induction had been inhibited by overexpressed hsp70 gene promoter sequences (Johnston and Kucey, 1988). A second human HSF with different HSE binding properties has recently been described (Schuetz et al., 1991). This protein or yet another related transcription factor might trigger heat-induced expression of the small hsp genes.

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*Note Added in Proof.* Stress-independent accumulation of the small hsp, hsp28, has recently been described in several normal human tissues (Kato, K., H. Shinohara, S. Goto, Y. Inaguma, R. Morishita, and T. Asano. 1992. *J. Biol. Chem.* 267:7718-7725).

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