Heat Shock Response of the Rat Lens

Wilfried W. de Jong, Willeke A. Hoekman, John W. M. Mulders, and Hans Bloemendal
Department of Biochemistry, University of Nijmegen, 6500 HB Nijmegen, The Netherlands

Abstract. The sequence relationship between the small heat shock proteins and the eye lens protein α-crystallin (Ingolia, T. D., and E. E. Craig, 1982, *Proc. Natl. Acad. Sci. USA,* 79:2360–2364) prompted us to subject rat lenses in organ culture to heat shock and other forms of stress. The effects on protein synthesis were followed by labeling with [^{35}S]methionine and analysis by one- and two-dimensional gel electrophoresis and fluorography. Heat shock gave a pronounced induction of a protein that could be characterized as the stress protein SP71. This protein probably corresponds to the major mammalian heat shock protein hsp70. Also two minor proteins of 16 and 85 kD were induced, while the synthesis of a constitutive heat shock–related protein, P73, was considerably increased. The synthesis of SP71 started between 30 and 60 min after heat shock, reached its highest level after 3 h, and had stopped again after 8 h. In rat lenses that were preconditioned by an initial mild heat shock, a subsequent shock did not cause renewed synthesis of SP71. This effect resembles the thermotolerance phenomenon observed in cultured cells. The proline analogue azetidine-2-carboxylic acid, zinc chloride, ethanol, and calcium chloride did not, under the conditions used, induce stress proteins in the rat lens. Sodium arsenite, however, had very much the same effects as heat shock. Calcium ionophore A23187 specifically and effectively induced the synthesis of the glucose-regulated protein GRP78. No special response to stress on crystallin synthesis was noticed.

THE heat shock response of eukaryotic cells is characterized by the induction of a specific set of heat shock or stress proteins, and the concurrent development of thermotolerance, i.e., increased resistance to subsequent stress (34, 42). It has been shown that also in vivo and in organ culture several mammalian tissues respond to heat shock or other forms of stress by the synthesis of one or more heat shock proteins (5–7, 9, 10, 28).

A study of the effects of stress on the vertebrate eye lens is of interest for a number of reasons. The eye lens might be expected to need a very efficient protection or resistance against chemical and physical insults, because the differentiated lens fiber cells are never broken down or replaced (3, 11), and hence have to maintain a reasonable degree of structural and functional integrity throughout life. On the other hand the lens is quite likely to become exposed to stressful conditions because of its superficial location in combination with the absence of direct blood supply and innervation which hampers an efficient and rapid homeostatic regulation of the intracellular environment. Moreover, the older lens fiber cells loose their nuclei and ribosomes (3) and hence the ability to respond to stress by the synthesis of heat shock proteins. Chronic exposure of the lens to elevated temperatures induces cortical cataracts (39).

Also the intriguing structural relationships between the heat shock proteins and the lens-specific crystallins make a study of the stress response of the eye lens of special relevance. The major lens protein α-crystallin shows considerable sequence similarity with the small heat shock proteins of *Drosophila* (17, 37), the nematode *Caenorhabditis* (32), soybean (35), and *Xenopus* (2). A more limited sequence similarity has also been reported between a frog γ-crystallin and *Drosophila* hsp70 (43), as well as an apparent immunological relationship between a chicken fibroblast heat shock protein and a β-crystallin polypeptide (33).

We therefore have analyzed the response of rat lenses in organ culture to heat shock and other stressful conditions. Because also in situ the lens obtains its nutrients by perifusion from the aqueous humor, the results obtained in organ culture should well reflect the in vivo stress response of the eye lens.

Materials and Methods

Eye Lenses and Other Tissues

All rat and mouse lenses and other tissues were immediately removed after death by decapitation or cervical dislocation, and directly placed in incubation medium of 37°C. Eye lenses were dissected from Wistar rats of different ages and from 1-mo-old Swiss mice. The lenses were not decapsulated. Liver, lung, brain, and thigh muscle were used from 10-d-old Wistar rats and cut in pieces of ~1 mm^3 for incubation.

Heat Shock Conditions

All stress experiments were started within 15 min after death of the animal. Each lens or three to four pieces of tissue were incubated in 0.5 ml of medium. Eagle's minimum essential medium (modified with Earle's salts and 2 g/liter $NaHCO_3$, but without glutamine and methionine) (No. 16-222-49, Flow Laboratories, Inc., McLean, VA) was supplemented per 100 ml with 10 ml dialyzed

newborn calf serum (Boehringer Mannheim Diagnostics, Inc., Houston, TX), 1 ml glutamine (200 mM), 0.05 ml penicillin (10^6 U/5 ml), and 0.05 ml streptomycin (1 g/5 ml). Heat shock was carried out in this medium for different times and at different temperatures, as specified. Thereafter the lenses, or other tissues, were labeled for 6 h at 37°C in 0.5 ml minimum essential medium containing 10 or 25 μCi [^{35}S]methionine (Amersham Corp., Arlington Heights, IL). Incubations were stopped by rinsing twice with cold phosphate-buffered saline (PBS). To detect possible heat shock proteins containing no methionine, labeling was carried out by incubation for 6 h at 37°C in Eagle's minimum essential medium supplemented with 100 μCi/0.5 ml ^{3}H-amino acid mixture (TRK-440, Amersham Corp.).

Other Stress Conditions

Lenses of 10-d-old rats were incubated in 0.5 ml complete minimum essential medium supplemented with either L-azetidine-2-carboxylic acid (5 mM; Serva Fine Biochemicals Inc., Garden City Park, NY; 12 h incubation at 37°C) (47), sodium arsenite (150 μM; 1 h at 37°C) (19), zinc chloride (500 μM; 1 h at 37°C) (12), ethanol (6%; 1 h at 37°C) (22), or calcium chloride (6 mM, 24 h at 4°C) (14). Incubation with calcium ionophore A23187 (7 μM; Eli Lilly Co., Indianapolis, IN; 10 h at 37°C) (21) was carried out in 0.5 ml minimum essential medium containing 10 μCi [^{35}S]methionine. The other lenses were labeled, after washing with complete medium at 37°C, by incubation for 6 h at 37°C in 0.5 ml minimum essential medium containing 25 μCi [^{35}S]methionine.

Electrophoretic Analysis

Lenses and other tissues were homogenized in 0.5 ml SDS sample buffer. Proteins were electrophoretically separated on 13% polyacrylamide gels, containing 0.1% SDS (20), or on two-dimensional gels according to O'Farrell (30). The isofocusing gels contained ampholines (LKB Instruments, Inc., Gaithersburg, MD) in the pH ranges 3.5–10 and 6–8, mixed in a ratio of 3:5. Gels were stained with Coomasssie Brilliant Blue (45). For detection of labeled proteins the gels were processed for fluorography (4), dried, and exposed to Kodak X-Omat AR film.

Results

Temperature-dependence of Heat Shock Protein Induction

Lenses of 32-d-old Wistar rats were incubated in complete medium for 30 min at temperatures between 37° and 47°C, and subsequently labeled for 6 h at 37°C in a medium containing [^{35}S]methionine. Analysis of the synhesized proteins by SDS PAGE and fluorography (Fig. 1 *a*) revealed the appearance at 43° and 45°C of a major newly synthesized protein of apparent M_r 70,000 with a concomitant decrease of normal protein synthesis. After incubation for 30 min at 47°C, all protein synthesis had ceased. Comparison of samples containing equal amounts of incorporated radioactivity (Fig. 1 *b*) showed also a relative increase in synthesis of proteins with apparent masses of 71 and 85 kD at 43°C.

The optimum temperature for heat shock protein induction seemed to be age-dependent, since lenses of 6-d-old rats did not synthesize heat shock proteins after a 15-min incubation at 45°C (Fig. 1 *c*). Interestingly the synthesis of the lens crystallins still continued at a low level at 45°C, like in the 32-d-old lenses. The heat shock response was otherwise qualitatively similar in lenses of 6- and 32-d-old rats.

Characterization of the Heat-induced Rat Lens Proteins

A more detailed analysis of the heat shock response in the rat lens was obtained by two-dimensional gel electrophoresis and fluorography of the proteins synthesized in the control and heat-shocked lens. Comparison of parts *a* and *b* of Fig. 2 reveals that a 1 h incubation at 43°C indeed gave a pronounced induction of the 70-kD protein, which is only detectable in minute amounts in the control lens. Two minor proteins of ~16 and 85 kD were also induced by the heat

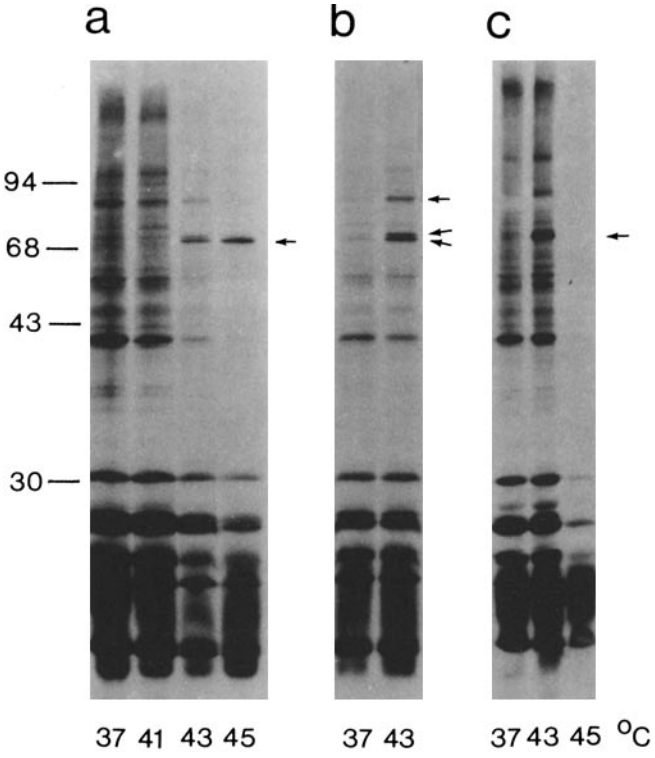

Figure 1. Effect of elevated temperature on protein synthesis in rat lenses in organ culture. Fluorographs are shown of SDS gel electrophoretic patterns of labeled proteins. (*a*) Lenses of 32-d-old rats were incubated for 30 min at the indicated temperatures, and then labeled for 6 h with 10 μCi of [^{35}S]methionine. Equal amounts of lens protein were applied in each lane. (*b*) Comparison of control lens (37°C) and lens incubated at 43°C, with equal amounts of radioactivity applied in each lane (same samples as in lanes 37° and 43°C, respectively, in *a*). (*c*) Labeled lens proteins of 6-d-old rats after incubation for 15 min at 37°, 43°, and 45°C. In contrast to the lenses of 32-d-old rats, the 6-d-old lenses do not synthesize heat shock proteins after incubation at 45°C. The positions of the heat-induced proteins (right) and the molecular weight markers (left) are indicated. These markers are: carbonic anhydrase, 30 kD; ovalbumin, 43 kD; bovine serum albumin, 68 kD; and phosphorylase a, 94 kD.

shock, while a protein of ~71 kD occurred in considerably greater amounts as compared with the control pattern. The 70-kD protein occurred as a major charge species of pI 5.9, as estimated by comparison with the known isoelectric points of αA_2-crystallin (pI 5.92; 11), lens γ actin (pI 5.45; 29), and vimentin (pI 5.35; 38). The molecular weight and isoelectric point of this rat lens stress protein are in good agreement with published values for the major mammalian heat shock–induced proteins (hsp70), which range between M_r 66,000 and 72,000, and pI 5.6 and 6.3 (26, 34, 44, 48). Also the position after two-dimensional gel electrophoresis of the rat lens 70-kD protein relative to actin, tubulin, and vimentin corresponds very well with published patterns for the major heat shock– or stress-induced protein in other cell types, in culture, or in vivo (6, 15, 19, 26, 44).

The same major 70-kD stress protein as in lens was also induced by heat shock treatment of other tissues of 10-d-old rats (Fig. 3). The extent of induction of this stress protein showed considerable differences among the various tissues. Currie and White (6) observed the induction of a similar protein in many different rat tissues after hyperthermia in

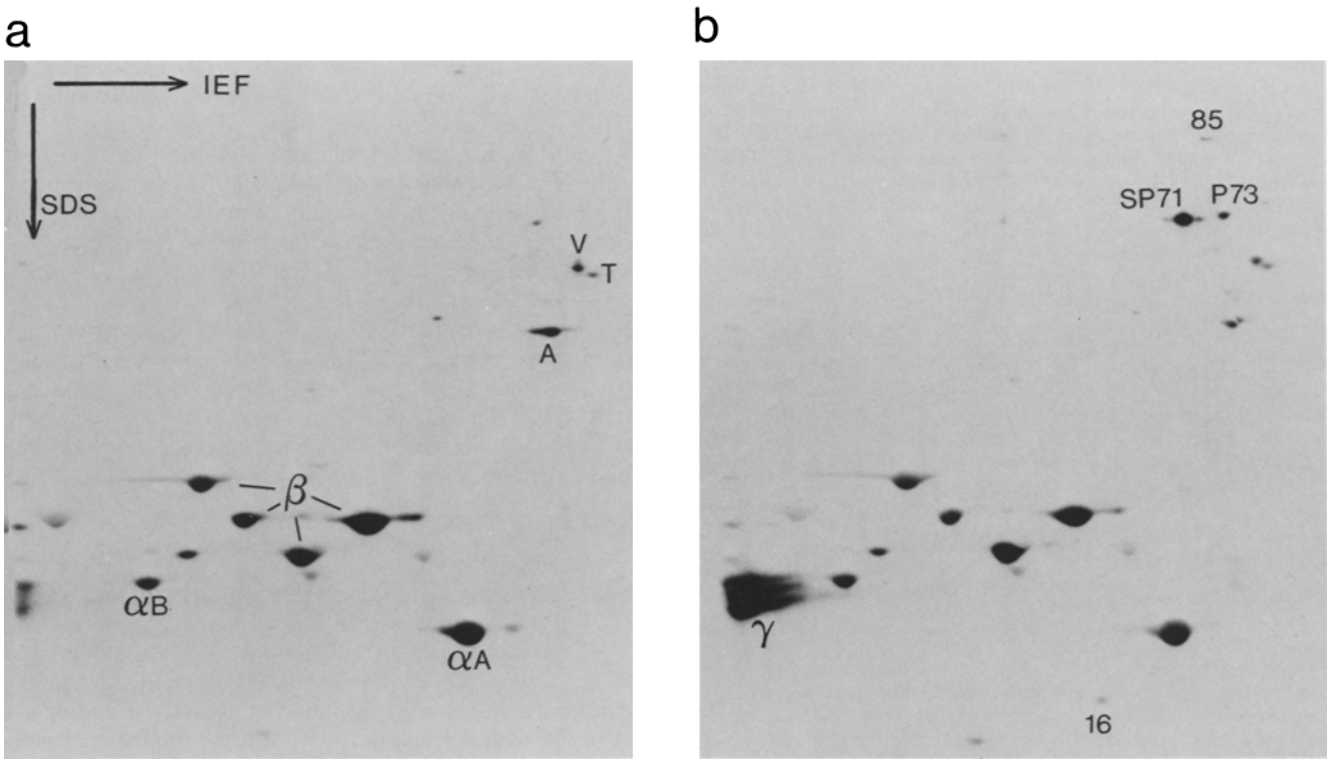

Figure 2. Two-dimensional electrophoretic analysis of heat-induced proteins in the rat lens. Fluorographs are shown of labeled proteins of 10-d-old rat lenses after incubation for 1 h at 37°C (*a*) and at 43°C (*b*), and subsequent labeling for 6 h at 37°C with 25 μCi of [^{35}S]methionine. The heat-induced proteins are indicated in *b*, and some of the crystallin subunits and cytoskeletal proteins in *a*. The heat-induced lens proteins of 70 and 71 kD are identified as SP71 and P73, respectively. *A*, actin; *V*, vimentin; T, β-tubulin (cf. reference 15). In *a* the γ-crystallins have largely run off the gel.

vivo, as well as in tissue slices. The identity of the major rat lens heat shock protein and this protein, called SP71 (15, 49), could convincingly be demonstrated by the fact that the corresponding mouse lens heat shock protein, like mouse SP71 (49), is slightly more acidic than the rat protein (Fig. 4). We therefore will further designate the rat lens 70-kD heat shock protein as SP71.

The lens protein of 71 kD, of which the synthesis increased upon heat shock, can reasonably be identified as P73 (15, 26, 49), a constitutive protein present at considerable levels in many investigated normal cell types, and of which the synthesis is indeed stimulated by heat shock. It has been shown that P73 is closely related to SP71 (15, 26). These proteins correspond indeed to the two primary members of the 70-kD family of heat shock proteins; the former representing the constitutive form, and the latter the highly inducible form. The minor heat-induced 85kD lens protein might well correspond to the common mammalian heat shock protein hsp90 (34, 47, 48). Finally, the minor 16 kD heat-induced lens protein does not seem to correspond with any heat shock protein described so far in other mammalian cells or tissues. The smallest heat shock proteins reported in mammalian cells have apparent molecular masses between 25,000 and 30,000 (13, 19, 34, 44, 47), and have moreover been reported not to become labeled by [^{35}S]methionine (13, 19).

In relation with the latter observation we assessed the possible induction of heat shock proteins that do not contain methionine, by labeling 6-d-old rat lenses, after a 30-min heat shock at 43°C, with ^{3}H-amino acid mixture. Fluorography after two-dimensional gel electrophoresis of these ^{3}H-labeled lens proteins did not reveal any additional heat-induced polypeptides, neither in the 25–30-kD range, nor of any other size (data not shown). The 25–30-kD mammalian heat shock proteins have indeed not been reported to be induced upon stress of intact organs or tissues, in vivo or in vitro (5–7, 9, 10, 28), nor are they induced in certain cultured cell types (19).

Kinetics of SP71 Induction in the Lens

Mammalian cells in culture show a characteristic pattern of synthesis of heat shock proteins after induction (13, 23, 36, 40). At first the synthesis of heat shock proteins rapidly increases and generally reaches a maximum between 2 and 6 h. The synthesis then decreases to normal levels within 8–24 h after heat shock. We have followed in the rat lens culture the levels of SP71 synthesis at different times after a heat shock of 30 min at 43°C (Fig. 5). It appeared that the synthesis of SP71 started between 30 and 60 min after heat shock, reached its highest levels after ~3 h, and leveled off to negligible amounts again after 8 h. Normal protein synthesis in the lens cells had apparently completely stopped immediately after the heat shock, started again at very low level within 30 min, even before SP71, and still increased when the synthesis of SP71 was already on its return.

Thermotolerance of the Rat Lens

One of the key features associated with heat shock protein synthesis in cultured cells is the phenomenon of thermotolerance (23, 40, 42, 47). After an initial heat shock and concomitant induction of heat shock protein synthesis, it

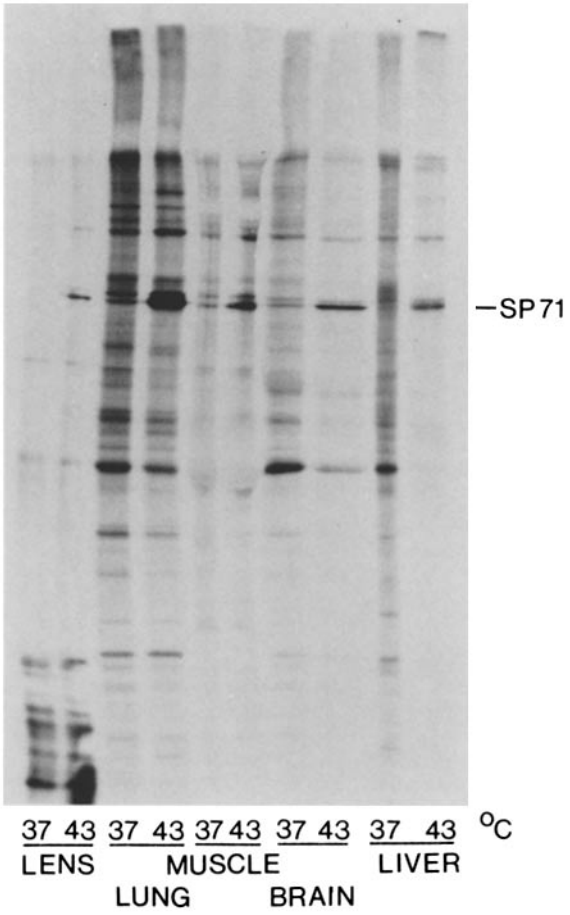

Figure 3. Induction of heat shock protein synthesis in different rat tissues. The lenses and pieces of lung, muscle, brain, and liver of a 10-d-old rat were incubated for 0.5 h at 37°C (control) or 43°C, and labeled for 6 h at 37°C with 25 μCi of [^{35}S]methionine. The labeled proteins are compared by SDS PAGE and fluorography.

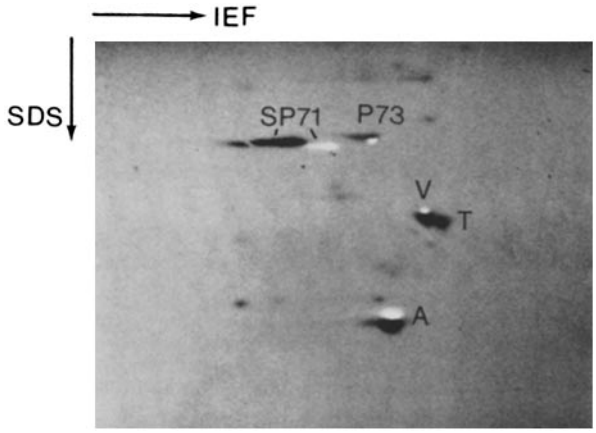

Figure 4. Comparison of the major heat-induced protein in rat and mouse lenses. Lenses of a 20-d-old rat and a 32-d-old mouse were incubated for 15 min at 43°C, and labeled for 4 h at 37°C with 25 μCi of [^{35}S]methionine. The labeled proteins were separated by two-dimensional electrophoresis. A contact negative of the fluorograph of the mouse lens proteins was placed over the fluorograph of the rat lens proteins. The mouse proteins appear as white spots and the rat proteins as black spots. Actin, vimentin, and tubulin are indicated as *A, V,* and T, respectively.

appears that the cells have become more resistant to a subsequent, more severe heat treatment, as evidenced by a better maintenance of normal levels of protein synthesis and a higher survival rate of the cells. Moreover, the second heat shock does not, or to a much lesser extent, induce the renewed synthesis of heat shock proteins. Since the development of thermotolerance might be one of the major biological functions of the heat shock proteins, it was of interest to establish the occurrence of this phenomenon in the cultured rat lens. We used the extent of induction of SP71 and the overall resistance of normal lens protein synthesis to a second heat treatment as criteria for the development of thermotolerance. Fig. 6 reveals that such a second heat shock of preconditioned lenses indeed did not lead to a renewed stimulation of SP71 synthesis, but the basal level of protein synthesis was not detectably protected by the initial heat shock.

Response to Other Forms of Stress

The induction of heat shock protein synthesis in cell culture is also triggered by a variety of other stressful conditions (34, 42). The types of induced heat shock proteins and the extent and patterns of induction are, however, quite variable. We examined the effects on the rat lens in organ culture of some of the commonly used stress-inducing agents. the fluorography in Fig. 7 shows that in this system the proline analogue L-azetidine-2-carboxylic acid (lane *3*) had no apparent influ-

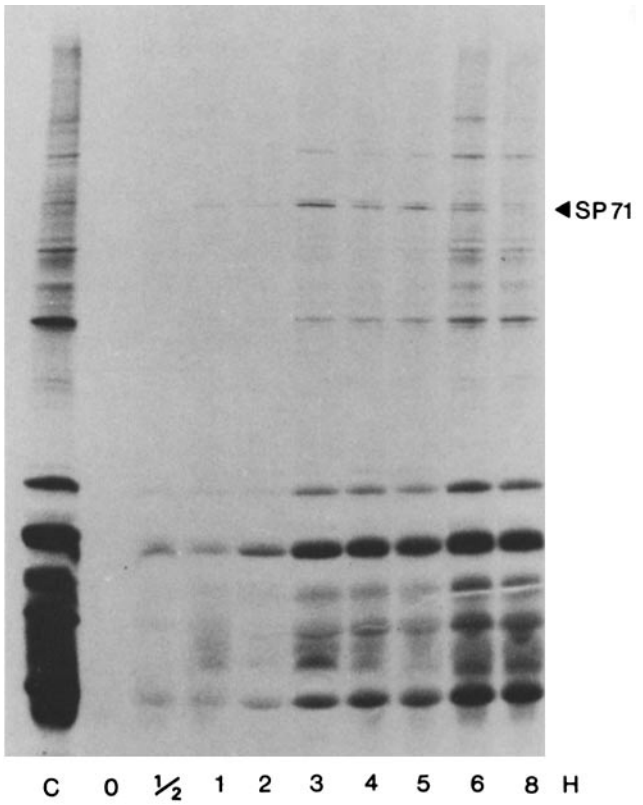

Figure 5. Kinetics of SP71 induction in rat lens. Lenses of 16-d-old rats were triggered by incubation for 30 min at 43°C, followed by different development times (0–8 h) at 37°C, and a final labeling with [^{35}S]methionine (25 μCi in 250 μl medium) for 30 min at 37°C. Labeled proteins are analyzed by SDS PAGE and fluorography. Labeled proteins from a control lens (incubated for 30 min at 37°C, followed by a 30 min labeling) are shown in the first lane.

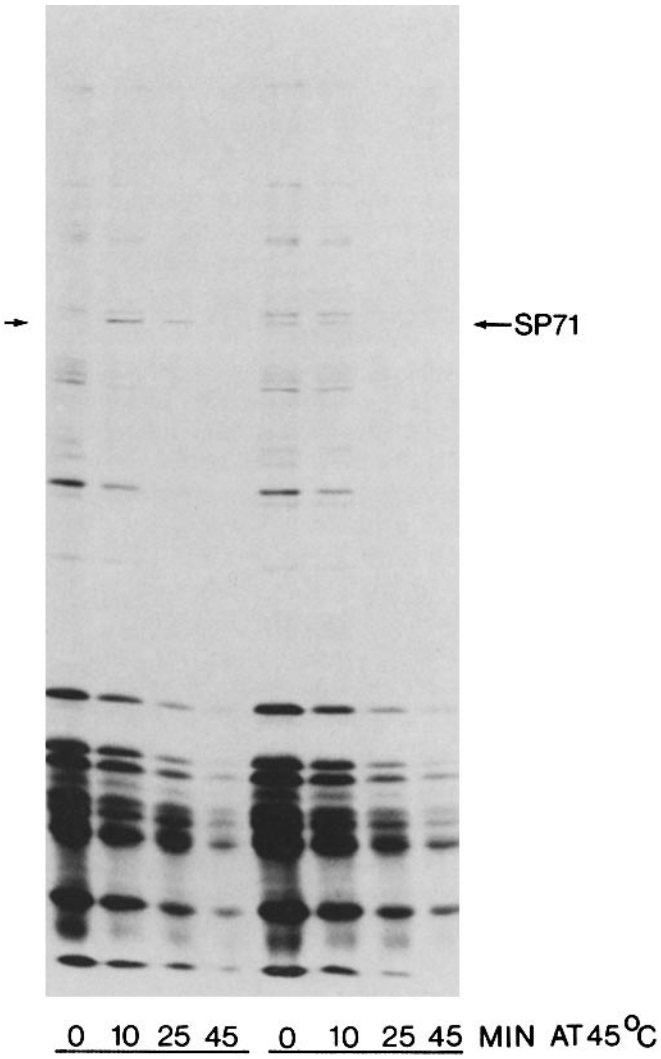

Figure 6. Thermotolerance of rat lens. Lenses of 19-d-old rats were triggered by preincubation for 30 min at 43°C, developed for 4 h at 37°C, and either directly labeled or exposed to a second heat shock at 45°C during 10 min, 25 min, or 45 min. Thereafter the lenses were labeled for 6 h at 37°C with 10 μCi of [^{35}S]methionine. Control lenses were preincubated for 30 min at 37°C, and otherwise treated in a similar way. Labeled proteins are analyzed by SDS PAGE and fluorography; equal amounts of protein were applied. The times of heat shock at 45°C, after the preincubation at 37°C (left) or 43°C (right) and development, are shown.

ence on lens protein synthesis, and did not induce SP71. Arsenite (lane *4*) induced the synthesis of both SP71 and P73, as well as that of a 32-kD protein, and enhanced also the synthesis of the 85-kD heat shock protein. Zinc (lane *5*) again had little effect, like ethanol (lane *6*). High levels of Ca^{2+} in the medium led to a dramatic decrease in overall protein synthesis, but did not induce heat shock protein synthesis, whereas the calcium ionophore A23187 induced the pronounced synthesis of a 74-kD stress protein.

A more detailed analysis by two-dimensional gel electrophoresis and fluorography revealed that the effect of arsenite (Fig. 8*a*) was very similar to that of heat shock, albeit that SP71 is less induced than P73. Arsenite also induced the 16-kD stress protein. The 32-kD protein, visible in one-dimensional PAGE (Fig. 7), is not found back on the two-dimensional gel, possibly because it might correspond to the very acidic stress protein induced by arsenate and arsenite in cultured rat myoblasts (19). Ca^{2+} turned out to equally decrease the synthesis of all lens proteins (Fig. 8*b*) as compared with the control (Fig. 2*a*), although the synthesis of P73 showed a slight relative increase. Finally the most conspicious effect of the calcium ionophore A23187 is the specific induction of the 74-kD protein, which thus is likely to correspond to GRP78, the major of the two calcium ionophore- and glucose-regulated proteins in mammalian cells in culture (25, 48, 51). In fact the position of the rat lens 74-kD stress protein on the two-dimensional gel corresponds exactly with that reported for GRP78 (15).

Discussion

The heat shock and stress response has mostly been studied in cultured cells. This approach has provided a wealth of information about this phenomenon, which however is not necessarily applicable to the processes as they occur in the

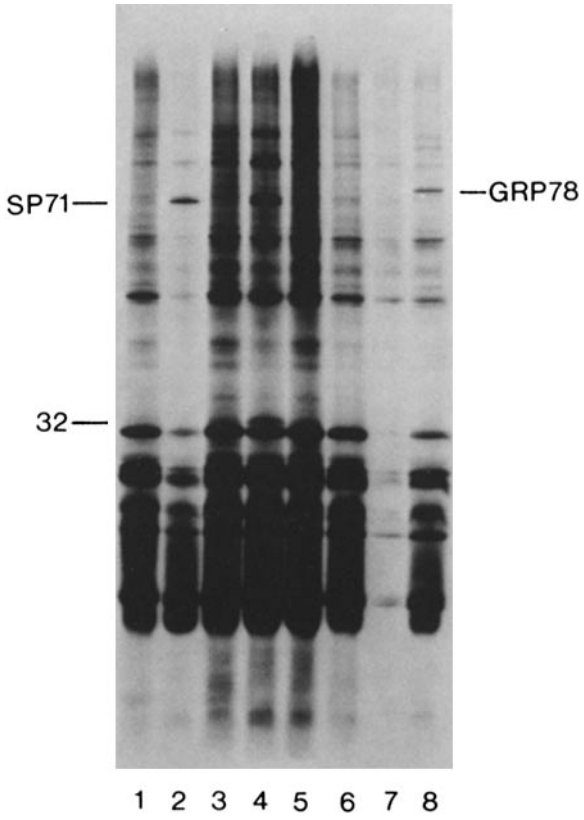

Figure 7. Response of rat lens to other forms of stress. Fluorography of SDS PAGE of total labeled proteins from 10-d-old rat lenses after different forms of stress. Lane *1*, control lens; lane *2*, heat shock for 1 h at 43°C; lane *3*, 5 mM azetidine-carboxylic acid; lane *4*, 150 μM sodium arsenite; lane *5*, 500 μM zinc chloride; lane *6*, 6% ethanol; lane *7*, 6 mM calcium chloride; lane *8*, 7 μM calcium ionophore A23187. The control and all stressed lenses were labeled, after the stress period, for 6 h at 37°C with 25 μCi [^{35}S]methionine, apart from the lenses treated with calcium ionophore, which were labeled during the stress for 10 h with 10 μCi [^{35}S]methionine. Equal amounts of protein were applied. Because individual lenses of rats of the same age show considerable variation in biosynthetic activity, some of the stressed lenses can have incorporated more radioactivity than the control.

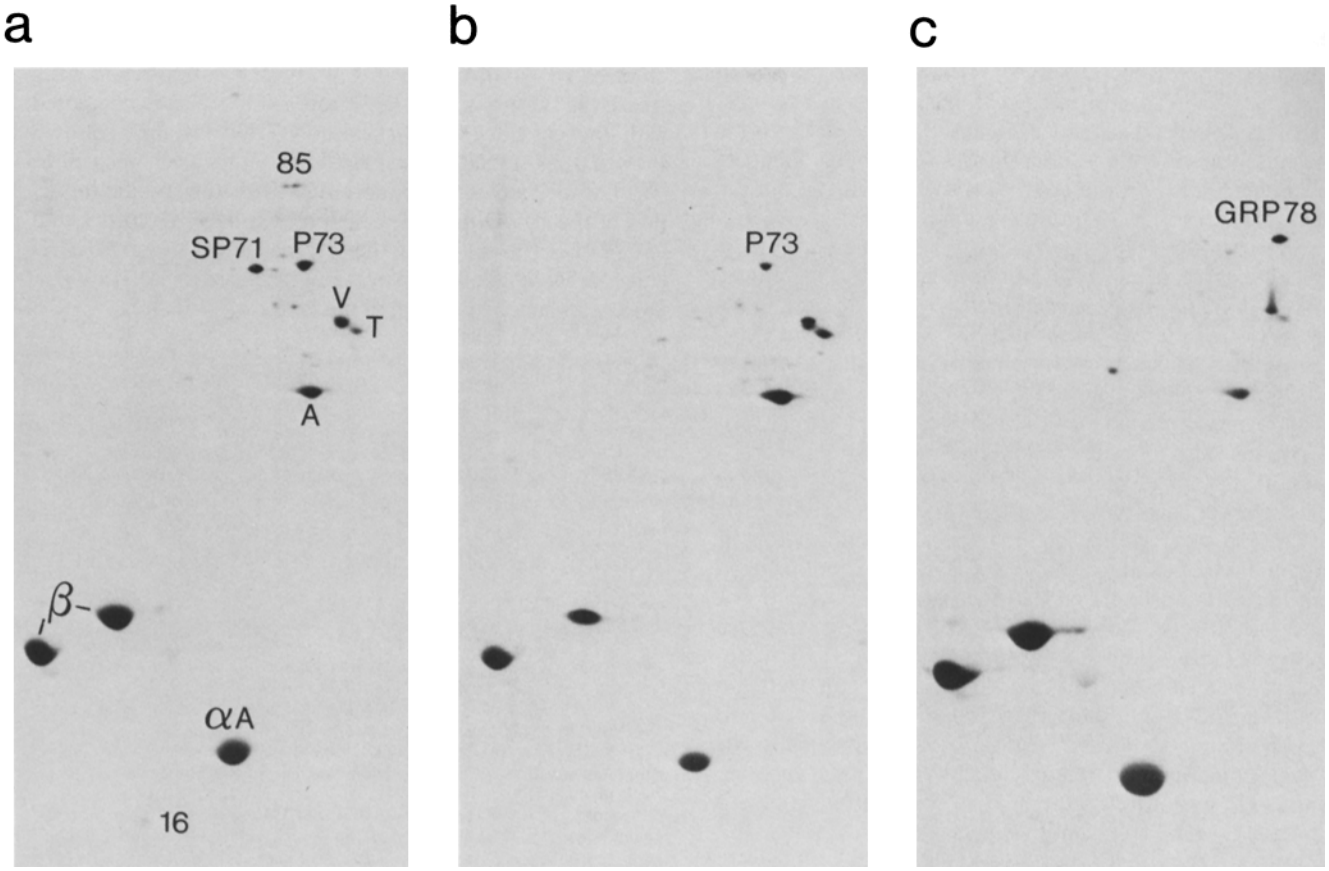

Figure 8. Comparison of the effects of arsenite (*a*), calcium (*b*), and calcium ionophore (*c*) on protein synthesis in rat lenses. Fluorographs are shown of the two-dimensional gels of the samples in lanes *4*, *7*, and *8* of Fig. 7. See Fig. 2 *a* and *b* for comparison with normal and heat-shocked lenses.

living organism. It has been shown that increased temperature and other forms of stress and trauma in living mammals or excised tissues mainly induce the synthesis of a 71–74-kD stress protein (5–7, 9, 10). The other size classes of heat shock proteins, which are induced to varying extents in different mammalian cell types in culture, are not observed in intact mammalian organs, or only at low levels. It has been demonstrated in mouse (41) and pig (27) that also in vivo heat shock results in thermotolerance, as evidenced by an increased resistance to subsequent heating. The study of acquired thermotolerance in living organisms is of relevance for the treatment of tumors by hyperthermia, based on the fact that transformed cells are hypersensitive to heat.

The eye lens in organ culture is a suitable system to follow the effects of stress on an intact organ. In fact the conditions of the lens in culture fairly well correspond with the in vivo situation, where the lens also receives its nutrients from the perifusing aqueous humor. The lens, therefore, can be maintained for periods of over 9 d without deterioration of its cellular metabolism or loss of functional and structural integrity (31, 39). The stress responses that we observed in the rat lens in organ culture are thus likely to reflect the processes that will occur in vivo under comparable conditions. It has previously been shown that incubation of rat lenses at elevated temperatures (40°C and upward) or at elevated glucose levels induces cataractous morphological changes (39). The morphological and biochemical changes associated with cataract formation also occur in vitro by incubation of rat or mouse lenses in glucose-free medium, as well as in media containing ouabain or calcium ionophore A23187 (16).

The major effect of heat shock on the lens is the induction of a 70-kD protein that can be identified as the stress protein SP71 observed upon stress in other mammalian tissues and organs (5–7, 9, 10). Since the lens only consists of an anterior single layer of epithelial cells and the very specific differentiated lens fiber cells, this further extends the number of cell types in which SP71 induction can occur (6). Our results contradict the suggestion that SP71 is synthesized by injury of microvasculature (49), because this is absent from the lens.

There exists considerable confusion concerning the nomenclature of the 70-kD heat shock proteins, as for the other size classes of heat shock proteins. This is due to the considerable variation in the reported numbers and characteristics of the 70-kD heat shock proteins and related constitutive and stress proteins in different organisms and in different cell types of the same species. The problem is aggravated by the use of different gel systems in different laboratories, resulting in varying estimates of apparent molecular masses and isoelectric points, and by the use of a great variety of stress conditions. However, the evidence indicates that SP71 is identical to the major heat shock protein hsp70, which has been demonstrated to be immunologically related in a great variety of eukaryotic organisms (18). Also the gene encoding hsp70 has been highly conserved during evolution (26). Even in *E. coli* a heat-inducible gene is present encoding a protein that is 48% homologous to *Drosophila* hsp70 (1). The identity of

rat lens SP71 and the major rodent hsp70 can be deduced from the following observations. SP71 and hsp70 are the only heat shock or stress proteins of M_r ~70,000 which are not, or hardly, detectable in unstressed cells, in contrast to a number of related, so-called heat shock cognate proteins of which the synthesis also increases under heat shock, but which are already constitutively present at lower levels in the normal unstressed cell (6, 26, 46). On two-dimensional gel electrophoresis both SP71 and hsp70 are the most basic as well as the lowest molecular weight polypeptides within the cluster of 68–74-kD heat shock and heat shock cognate proteins in rodents (6, 26). In many cell types SP71 and hsp70 do occur in several isoelectric point variants, in contrast to the more acidic unique polypeptides of the constitutively expressed heat shock–related proteins (5, 10, 26, 46).

In rat lens also a protein corresponding to the heat shock–related P73 (15, 26) can be identified. It is already present in the unstressed lens, but its synthesis increases considerably after heat shock (Fig. 2*b*). In that respect, and considering its electrophoretic position relative to SP71, it can probably be identified as the product of the gene corresponding with the mouse hsc70 gene (26). The rat lens P73 also migrates in exactly the same position on two-dimensional gels as a microtubule-associated heat shock–related 68-kD protein described in rat brain (24) and baby hamster kidney and HeLa cells (44). The minor 16,000-kD heat-induced polypeptide in the rat lens could not be related to any previously reported mammalian heat shock protein. Heat shock proteins in this size class have thus far only been described in plants and nematodes. Interestingly, the15–18-kD heat shock proteins in soybean (35) and the 16-kD heat shock proteins of the nematode *Caenorhabditis elegans* (32) do in fact show, like the 22–27-kD heat shock proteins of *Drosophila* (17, 37) and the hsp30 in *Xenopus* (2), considerable sequence homology with vertebrate α-crystallin.

As in other cell types, arsenite induces in rat lens the same proteins as heat shock, and in addition, a specific 32-kD protein which has also been reported in rat myoblasts (19). Interesting is the strikingly increased synthesis of a 74-kD stress protein under the influence of the calcium ionophore A23187. This protein is constitutively present at a very low level in control and heat-shocked lenses. It is known that calcium ionophore A23187 specifically induces in mammalian cells the synthesis of two stress proteins that are also induced by glucose starvation, and hence designated as the glucose-regulated proteins GRP78 and GRP94 (21, 25, 48, 51). The rat lens 74-kD stress protein has indeed a position on two-dimensional gel electrophoresis, relative to SP71, P73, actin, vimentin, and tubulin, which corresponds exactly with the glucose-regulated protein GRP78 in rat aorta (15). GRP78 is, like the rat lens 74-kD stress protein, slightly heat-inducible (25). GRP78 has, like hsp70 and hsp90, a highly acidic amino terminus (21), which can possibly interact with basic chromosomal proteins, but may also form a suitable environment to bind Ca^{2+} (21). The eye lens in vitro readily develops cataract by increased Ca^{2+} influx and by glucose starvation, two processes that are intimately associated in the lens (8, 14). GRP78 may thus have an important function in minimizing the irreversible damaging effects of such conditions.

The presence of heat shock protein-like sequences in α-crystallin, and possibly also in β- and γ-crystallin, does not influence their level of synthesis under heat shock. Their synthesis is not stimulated by heat shock, nor is it notably more resistant as compared with, for instance, actin and vimentin. It may be that the hsp-like sequences provide a thermodynamically very stable conformation, which might be advantageous for the long-living crystallins (50). Another possibility that might be suggested is that the presence of hsp-like sequences in the crystallins provides them with hsp-like properties, which may be favorable by providing protection against stressful conditions in the deeper layers of the lens, where synthesis of heat shock proteins no longer can occur.

This investigation was carried out under the auspices of the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Received for publication 3 July 1985, and in revised form 31 July 1985.

References

1. Bardwell, J. C. A., and E. A. Craig. 1984. Major heat shock gene of *Drosophila* and the *Escherichia coli* heat-inducible *dnaK* gene are homologous. *Proc. Natl. Acad. Sci. USA.* 81:848–852.

2. Bienz, M. 1984. Developmental control of the heat shock response in *Xenopus*. *Proc. Natl. Acad. Sci. USA.* 81:3138–3142.

3. Bloemendal, H., editor. 1982. Molecular and Cellular Biology of the Eye Lens. John Wiley & Sons, Inc., New York. 469.

4. Bonner, W. W., and A. Laskey. 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83–88.

5. Cosgrove, J. W., and I. R. Brown. 1983. Heat shock protein in mammalian brain and other organs after a physiologically relevant increase in body temperature induced by D-lysergic acid diethylamide. *Proc. Natl. Acad. Sci. USA.* 80:569–573.

6. Currie, R. W., and F. P. White. 1981. Trauma-induced protein in rat tissues: a physiological role for a "heat shock" protein? *Science (Wash. DC).* 214:72–73.

7. Currie, R. W., and F. P. White. 1983. Characterization of the synthesis and accumulation of a 71-kilodalton protein induced in rat tissues after hyperthermia. *Can. J. Biochem. Cell Biol.* 61:438–446.

8. Duncan, G., and T. J. C. Jacob. 1984. Influence of external calcium and glucose on internal total and ionized calcium in the rat lens. *J. Physiol.* 357:485–493.

9. Freedman, M. S., B. D. Clark, T. F. Cruz, J. W. Gurd, and I. R. Brown. 1981. Selective effects of LSD and hyperthermia on the synthesis of synaptic proteins and glycoproteins. *Brain Res.* 207:129–145.

10. Hammond, G. L., Y.-K. Lai, and C. L. Markert. 1982. Diverse forms of stress lead to new patterns of gene expression through a common and essential metabolic pathway. *Proc. Natl. Acad. Sci. USA.* 79:3485–3488.

11. Harding, J. J., and M. J. C. Crabbe. 1984. The lens: development, proteins, metabolism and cataract. *In* The Eye. 3rd edition. H. Davson, editor. Academic Press, Inc., New York. 207–492.

12. Heikkila, J. J., G. A. Schultz, I. Kostas, and L. Gedamu. 1982. Expression of a set of fish genes following heat or metal ion exposure. *J. Biol. Chem.* 257:12000–12005.

13. Hickey, E. D., and L. A. Weber. 1982. Modulation of heat-shock polypeptide synthesis in HeLa cells during hyperthermia and recovery. *Biochemistry.* 21:1513–1521.

14. Hightower, K. R., and V. N. Reddy. 1982. Ca^{++}-induced cataract. *Invest. Ophthalmol. Vis. Sci.* 22:263–267.

15. Hightower, L. E., and F. P. White. 1982. Preferential synthesis of rat heat-shock and glucose-regulated proteins in stressed cardiovascular cells. *In* Heat Shock: From Bacteria to Man. M. Schlesinger, A. Ashburner, and A. Tissières, editors. Cold Spring Harbor Laboratory, New York, 369–377.

16. Hu, T.-S., P. Russell, and J. H. Kinoshita. 1982. *In vitro* incubation parallelling changes occurring during mouse cataract formation. *Exp. Eye Res.* 35:521–533.

17. Ingolia, T. D., and E. A. Craig. 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.

18. Kelley, P. M., and M. J. Schlesinger. 1982. Antibodies to two major chicken heat shock proteins cross-react with similar proteins in widely divergent species. *Mol. Cell. Biol.* 2:267–274.

19. Kim, Y.-J., J. Shuman, M. Sette, and A. Przybyla. 1983. Arsenate induces stress proteins in cultured rat myoblasts. *J. Cell Biol.* 96:393–400.

20. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

21. Lee, A. S., J. Bell, and J. Ting. 1984. Biochemical characterization of the 94- and 78-kilodalton glucose-regulated proteins in hamster fibroblasts. *J. Biol. Chem.* 259:4616–4621.

22. Li, G. C., and G. M. Hahn. 1978. Ethanol-induced tolerance to heat and to adriamycin. *Nature (Lond.).* 274:699–701.

23. Li, G. C., D. C. Shrieve, and Z. Werb. 1982. Correlations between synthesis of heat-shock proteins and development of tolerance to heat and to adriamycin in Chinese hamster fibroblasts. *In* Heat Shock: From Bacteria to Man. M. Schlesinger, A. Ashburner, and A. Tissières, editors. Cold Spring Harbor Laboratory, New York. 395–404.

24. Lim, L., C. Hall, T. Leung, and S. Whatley. 1984. The relationship of the rat brain 68 kDa microtubule-associated protein with synaptosomal plasma membranes and with the *Drosophila* 70 kDa heat-shock protein. *Biochem. J.* 224:677–680.

25. Lin, A. Y., and A. S. Lee. 1984. Induction of two genes by glucose starvation in hamster fibroblasts. *Proc. Natl. Acad. Sci. USA.* 81:988–992.

26. Lowe, D. G., and L. A. Moran. 1984. Proteins related to the mouse L-cell major heat shock protein are synthesized in the absence of heat shock gene expression. *Proc. Natl. Acad. Sci. USA.* 81:2317–2321.

27. Martinez, A. A., A. Meshorer, J. L. Meyer, G. M. Hahn, L. F. Fajardo, and S. D. Prionas. 1983. Thermal sensitivity and thermotolerance in normal porcine tissues. *Cancer Res.* 43:2072–2075.

28. Morimoto, R. I., and E. J. B. Fodor. 1984. Cell-specific expression of heat shock proteins in chicken reticulocytes and lymphocytes. *J. Cell Biol.* 99:1316–1323.

29. Mousa, G. Y., and J. R. Trevithick. 1979. Actin in the lens: changes in actin during differentiation of lens epithelial cells *in vivo*. *Exp. Eye Res.* 29:71–81.

30. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007–4021.

31. Owers, J., and G. Duncan. 1979. The viability of the bovine lens in organ culture. *Exp. Eye Res.* 28:739–742.

32. Russnak, R. H., D. Jones, and E. P. M. Candido. 1983. Cloning and analysis of cDNA sequences coding for two 16 kilodalton heat shock proteins (hsps) in *Caenorhabditis elegans*: homology with the small hsps of *Drosophila*. *Nucleic Acids Res.* 11:3187–3205.

33. Schlesinger, M. J. 1985. Stress response in avian cells. *In* Changes in Gene Expression in Response to Environmental Stress. B. G. Atkinson and D. B. Walden, editors. Adademic Press, Inc., New York. 183–195.

34. Schlesinger, M. J., M. Ashburner, and A. Tissières, editors. 1982. Heat Shock: From Bacteria to Man. Cold Spring Harbor Laboratory, New York. 440.

35. Schöffl, F., E. Raschke, and R. T. Nagao. 1984. The DNA sequence analysis of soybean heat-shock genes and identification of possible regulatory promotor elements. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2491–2497.

36. Slater, A., A. C. B. Cato, G. M. Sillar, J. Kioussis, and R. H. Burdon. 1981. The pattern of protein synthesis induced by heat shock of HeLa cells. *Eur. J Biochem.* 117:341–346.

37. Southgate, R., A. Ayme, and R. Voellmy. 1983. Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster at locus 67B. *J. Mol. Biol.* 165:35–57.

38. Sredy, S., D. Roy, and A. Spector. 1984. Identification of two of the major phosphorylated polypeptides of the bovine lens utilizing a lens cAMP-dependent protein kinase system. *Curr. Eye Res.* 3:1423–1431.

39. Stewart-De Haan, P. J., M. O. Creighton, M. Sanwal, W. M. Ross, and J. R. Trevithick. 1981. Effects of vitamin E on cortical cataractogenesis induced by elevated temperature in intact rat lenses in medium 199. *Exp. Eye Res.* 32:51–60.

40. Subjeck, J. R., and J. J. Sciandra. 1982. Coexpression of thermotolerance and heat-shock proteins in mammalian cells. *In* Heat Shock: From Bacteria to Man. M. Schlesinger, A. Ashburner, and A. Tissières, editors. Cold Spring Harbor Laboratory, New York. 405–411.

41. Suit, H. D., and P. H. Blitzer. 1980. Thermally induced resistance to thermal damage. *Ann. NY Acad. Sci.* 335:379–382.

42. Tanguay, R. M. 1983. Genetic regulation during heat shock and function of heat shock proteins: a review. *Can. J. Biochem. Cell Biol.* 61:387–394.

43. Tomarev, S. I., A. S. Krayev, K. G. Skryabin, A. A. Bayev, and G. G. Gause Jr. 1982. The nucleotide sequence of a cloned cDNA corresponding to one of the γ-crystallins from the eye lens of the frog *Rana temporaria*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 146:315–318.

44. Wang, C., R. H. Gomer, and E. Lazarides. 1981. Heat shock proteins are methylated in avian and mammalian cells. *Proc. Natl. Acad. Sci. USA.* 78:3531–3535.

45. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406–4412.

46. Welch, W. J., and J. R. Feramisco. 1984. Nuclear and nucleolar localization of the 72,000-dalton heat shock protein in heat-shocked mammalian cells. *J. Biol. Chem.* 259:4501–4513.

47. Welch, W. J., J. I. Garrels, and J. R. Feramisco. 1982. The mammalian stress proteins. *In* Heat Shock: From Bacteria to Man. M. Schlesinger, A. Ashburner, and A. Tissières, editors. Cold Spring Harbor Laboratory, New York. 257–266.

48. Welch, W. J., J. I. Garrels, G. P. Thomas, J. J.-C. Lin, and J. R. Feramisco. 1983. Biochemical characterization of the mammalian stress proteins and identification of two stress proteins as glucose- and Ca^{2+}-ionophore-regulated proteins. *J. Biol. Chem.* 258:7102–7111.

49. White, F. P., and R. W. Currie. 1982. A mammalian response to trauma: the synthesis of a 71-kD protein. *In* Heat Shock: From Bacteria to Man. M. Schlesinger, A. Ashburner, and A. Tissières, editors. Cold Spring Harbor Laboratory, New York. 379–386.

50. Wistow, G. 1985. Domain structure and evolution in α-crystallins and small heat-shock proteins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 181:1–6.

51. Wu, F. S., Y.-C. Park, D. Roufa, and A. Martonosi. 1981. Selective stimulation of the synthesis of an 80,000-dalton protein by calcium ionophores. *J. Biol. Chem.* 256:5309–5312.