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Heat Shock Protein 27 Stimulates Recovery of RNA and Protein Synthesis Following a Heat Shock

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Abstract Constitutive expression of human hsp27 resulted in a 100-fold increase in survival to a single lethal heat shock in CHO cells without effecting the development of thermotolerance. A possible mechanism for the thermoprotective function of hsp27 may be increased recovery of protein synthesis and RNA synthesis following a heat shock. A lethal heat shock (44°C, 30 min) results in a 90% reduction in the rate of protein synthesis in non-tolerant cells. Control transfected cells recovered protein synthesis to a pre-heat shock rate 10 h after the heat shock; while cell lines that constitutively express human hsp27 recovered 6 h after the heat shock. Thermotolerant cells had a 50% reduction in protein synthesis, which recovered within 7 h following the heat shock. The same lethal heat shock (44°C, 30 min) reduced RNA synthesis by 60% in the transfected cell lines, with the controls recovering in 7 h; while the hsp27 expressing cell lines recovered within 5 h. Thermotolerant cells had a 40% reduction in RNA synthesis and were able to recover within 4 h. The enhanced ability of hsp27 to facilitate recovery of protein synthesis and RNA synthesis following a heat shock may provide the cell with a survival advantage. J. Cell. Biochem. 66:153–164, 1997.

Key words: thermotolerance; molecular chaperone; breast cancer and CHO cells

All cells have the ability to survive and subsequently adapt to environmental stresses. The best-characterized adaptive response is the expression of the heat shock proteins (hsps) following exposure to elevated temperatures. In mammalian cell lines there are four major classes of hsp expressed: hsp27, hsp70, hsp90, and hsp110 [Carper et al., 1987; Welch, 1992; Craig et al., 1994]. During the same time that cells express hsps, they also develop thermotolerance, an increased ability to survive a second heat shock. It has been assumed that the hsps play a crucial role in the development of thermotolerance since they are expressed simultaneously. However, several comprehensive reviews have concluded that there is a lack of strong evidence demonstrating a critical role for hsp expression in the development of thermotolerance [Carper et al., 1987; Lindquist and Craig, 1988; Tomasovic, 1989).

Most eukaryotic cell lines that express hsps express at least one small heat shock protein in the MW range of 20 to 30 kDa [Ciocca et al., 1993]. In human cell lines hsp27 is expressed as four isoforms, all but one of which are phosphorylated. Following a heat shock, hsp27 is rapidly phosphorylated, forming large heat shock granules which can migrate into the nucleus [Arrigo and Welch, 1987]. Recently, it has been reported that expression of the human hsp27 gene product can protect rodent cell lines from heat induced cytotoxicity [Landry et al., 1989]. Several functions for hsp27 have been reported including the ability to stabilize actin filaments following a heat shock [Lavoie et al., 1993, 1995], drug resistance [Oesterreich et al., 1993], as well as being a molecular chaperone [Jakob et al., 1993].

Experiments described here were designed to investigate the function of hsp27. We constructed CHO cell lines that express the human hsp27 from the full-length cDNA [Carper et al., 1990]. We found a significant survival advantage; however, the development of thermotoler-

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ance was not affected by the expression of hsp27. Hsp27 did not provide any protection to complex cellular processes such as RNA or protein synthesis. It was found that hsp27 did greatly stimulate the recovery of these processes following a single heat shock. We propose that it is this increased rate of recovery that enables cells that constitutively express hsp27 to have an enhanced survival advantage.

METHODS

Cell Lines and Culture Conditions

Cultures of Chinese hamster ovary (CHO) cells were maintained in exponential growth in McCoy's 5A (modified) medium containing 10% fetal bovine serum supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml). Stable transfected cell lines were maintained under the above conditions with the addition of 400 μg/ml G418 sulfate (Gibco, Grand Island, NY). All cultures were maintained at 37°C in humidified incubators with 5% CO_2:95% air. All experiments were initiated with exponentially growing cultures in the absence of G418.

Vector Construction

The constitutive expression vector (pβ27) utilizing the human full-length hsp27 cDNA [Carper et al., 1990] was constructed by cloning the EcoRI cDNA fragment into the Sal I site of pHβAPr-1-neo [Gunning et al., 1987] by a blunt end ligation after filling in the fragments. This vector, shown in Figure 1, contains the β actin promoter (PRM), 5′ untranslated region (5′UTR) and an intron splice site (ss) followed by a multiple cloning site (MCS) and an SV40 polyadenylation signal (SV40 poly A).

Transfection

A calcium phosphate method was used for transfection (Bethesda Research Labs, Gaithersburg, MD). Cells (1×10^6) were fed with fresh serum-containing media 2 h prior to the addition of 20 to 40 μg of DNA. Twenty-four hours following the addition of DNA, cells were washed and serum-containing media was added to the cells. Twenty-four hours after washing, cells were put under selection pressure by the addition of serum containing media supplemented with 400 μg/ml of G-418 sulfate. Individual colonies were isolated 2 weeks later and continuously cultured in the presence of 400 μg/ml of G418 sulfate.

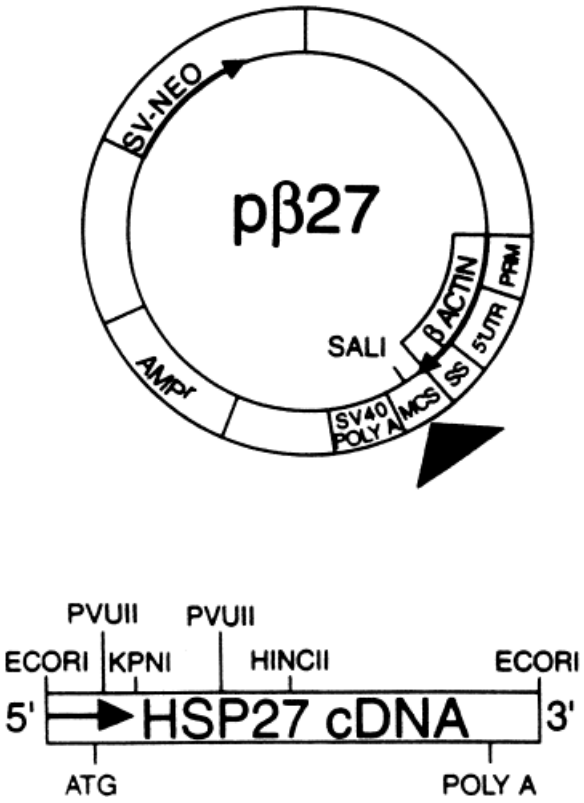

Fig. 1. Plasmid pβ27 used for the constitutive expression of hsp27 under control of the β-actin promoter. The gene for resistance to neomycin is under control of the simian virus early promoter.

Western Analysis

Equal amounts of total cellular proteins were separated by one-dimensional SDS/PAGE on a 12% acrylamide gel [Laemmli, 1970]. Immediately after electrophoresis, the proteins were electroluted onto a sheet of nitrocellulose in a 20% methanol transfer buffer. The nitrocellulose was blocked with 2% bovine serum albumin prior to the addition of the primary monoclonal antibody to hsp27 [Fuqua et al., 1989] at a final concentration of 1.4 μg/ml. Following three washes in PBS containing 0.5% Tween-20 and two washes in phosphate buffered saline (PBS), the secondary antibody (goat-anti-mouse IgG Fc specific alkaline-phosphate conjugate [Sigma A14181, Sigma, St. Louis, MO]) was added and gently shaken for 1 h at room temperature. After three washes in PBS containing 0.5% Tween-20 and two washes in PBS, the secondary antibody was detected by the alkaline phosphatase method [Harlow and Lane, 1988].

Two-Dimensional Electrophoresis

CHO cells were grown to a density of 40,000 cells per 10 × 75 mm culture tube. Protein was labeled by the incorporation of [^{3}H] leucine (800 Ci/mmol, New England Nuclear, Boston, MA) at a concentration of 0.1 µCi/µl in a final volume of 50 µl of McCoy's 5A media containing 10% fetal bovine serum. Cells were incubated under standard growth conditions following the heat shock. Two-dimensional electrophoresis was performed according to the method of O'Farrell [O'Farrell, 1975] as follows: Isoelectric focusing was carried out in glass tubes of inner diameter 2.0 mm, using 2.0% certified Resolytes pH 4–8 ampholites for 9,600 volthours. The final tube gel pH gradient extended from about pH 4.0 to 8.5 as measured by a surface pH electrode and colored acetylated cytochrome pI markers (Calbiochem, La Jolla, CA). After equilibration for 10 min in SDS sample buffer (10% glycerol, 50 mM dithiothreitol, 2.3% SDS, and 0.0625 M Tris, pH 6.9) the tube gels were sealed to the top of 10% acrylamide slab gels (0.75 mm thick) and SDS gel electrophoresis carried out for about 4 h at 12.5 mA/gel. The slab gels were run until the bromophenol blue dye front had reached the bottom of the gel. Radioactive molecular weight markers were also run on the gel. The slab gels were fixed in 50% methanol, 10% acetic acid. The gels were then impregnated with En3hance (New England Nuclear) dried at 60°C, and exposed to Kodak (Rochester, NY) X-Omat X-ray film at −80°C for fluorography.

Clonogenic Survival

Colony-forming assays were performed as previously described [Carper et al., 1991]. Briefly, following a heat shock, all treated cells were harvested, counted, and then incubated at various cell densities in complete media lacking G418 sulfate medium for 9–12 days to allow for colony growth. Colonies that grew from the surviving cells were stained with crystal violet and counted (>50 cells/colony). The percentage survival was calculated as 100× (number of colonies formed/number of experimental cells plated)/(number of control colonies formed/number of control cells plated).

HSP27 Determination

Cells were labeled as described above for two-dimensional electrophoresis. The total cellular protein was solubilized by the addition of SDS sample buffer (0.1 M sodium phosphate buffer [pH 7.2], 1% SDS, 1% 2-mercaptoethanol, 0.25 M sucrose) and the samples were then heated in a boiling water bath for 1 min. SDS/PAGE was performed with 12% acrylamide gels [Laemmli, 1970]. Equal amounts of radioactivity were added to each lane. Apparent molecular weights were determined by using molecular markers (Bethesda Research Labs). After electrophoresis, gels were fixed in: 10% (v/v) isopropanol; 5% (v/v) glacial acetic acid; 85% (v/v) water. The gels were then impregnated with En3hance (New England Nuclear), dried at 60°C, and exposed to Kodak X-Omat XAR5 X-ray film at −80°C for fluorography. The level of constitutive expression of the human hsp27 was quantitated using a densitometer (Bio-Rad, Richmond, CA, model 620), operated in the linear response range of the X-ray film.

Rate of Protein Synthesis

Cells were grown to a density of 40,000 cells per 10 × 75 mm culture tube in complete media lacking G418 sulfate. Protein was labeled by the incorporation of [^{3}H] leucine (800 Ci/mmol; New England Nuclear) at a concentration of 0.1 µCi/ul in a final volume of 50 µl of McCoy's 5A media containing 10% fetal bovine serum for 1 h. The labeling solution was removed and the culture tubes washed three times with 1.0 ml of PBS, then 100 µl of SDS sample buffer was added and the samples were boiled for 1 min. Aliquots of 5 µl were removed and added to 500 µl of 10% trichloroacetic acid (TCA) to precipitate proteins, which were filtered onto Whatman (Clifton, NJ) GC/A filters, washed in ice cold 5% TCA, dried and the filters counted (Packard, Meriden, CT, 1900 CA liquid scintillation counter). The protein content in each sample was determined in triplicate using the Bicinchoninic acid method [Smith et al., 1985]. All time point samples were taken in triplicate.

Rate of RNA Synthesis

Cells were plated at a density of 250,000 in a 60 mm tissue culture dish and fed with 5.0 ml of complete media lacking G418 sulfate 24 h prior to labeling. Following a 44°C, 30 min heat shock, the media was removed, the cells washed one time with 5.0 ml of phosphate buffered saline prior to the addition of 1.0 ml of complete media

containing [^{3}H-5,6] Uridine at a specific activity of 10 μCi/ml. After a 1 h incubation under normal growth conditions, the labeling solution was removed and the cells washed three times with 5 ml of phosphate buffered saline prior to the addition of 1.0 ml of 5% trichloroacetic acid (TCA). Cells were harvested by scraping, collected into a microfuge tube, washed two times with 1 ml of 5% TCA, and resuspended overnight in 600 μl of 0.2% NaOH. Radioactivity in each sample was determined in triplicate using a Packard 1900 CA liquid scintillation counter. The protein content in each sample was determined in triplicate using the Bicinchoninic acid procedure [Smith et al., 1985]. All time point samples were taken in triplicate.

Denaturation/Renaturation of α-Glucosidase

Denaturation and renaturation were carried out following the method of Jakob [Jakob et al., 1993]. Briefly, α-glucosidase (Boehringer Mannheim, Indianapolis, IN) was denatured at a concentration of 12.9 μM in 8 M urea, 0.1 M potassium phosphate, 1 mM EDTA, 20 mM dithioerythritol, pH 7.0, for 1.5 h. Renaturation was initiated by a 100-fold dilution in 40 mM HEPES-KOH, pH 7.5, with vigorous stirring in glass vessels. Denaturation and renaturation reactions were carried out at 20°C. Alpha-glucosidase activity was monitored according to Kopetzky [Kopetzki et al., 1989]. Human hsp27 (Stress-Gen, Victoria, B.C. Canada), ovalbumin (Polyscience, Warrington, PA), and aldolase (Polyscience) were resuspended and stored at −70°C prior to use.

RESULTS

Control (pHβAPr-1-neo) or the constitutive expression vector (pβ27) was transfected into Chinese hamster ovary (CHO) cells and single colonies were isolated following selection in G418 sulfate. The cell lines were maintained in the presence of G418 sulfate. These cell lines were characterized for expression of hsp27 by one-dimensional SDS/PAGE. All cell lines showed the expression of an endogenous hsp27 following a heat shock. The pβ27 transfected cell lines also showed the constitutive expression of a protein at 27 kD. This is the expected result from the pβ27 plasmid containing the hsp27 cDNA. One control transfected cell line (B2-6) and one pβ27 cell line (B19-5), demonstrating a significant increase in survival following a heat shock, were selected for further study.

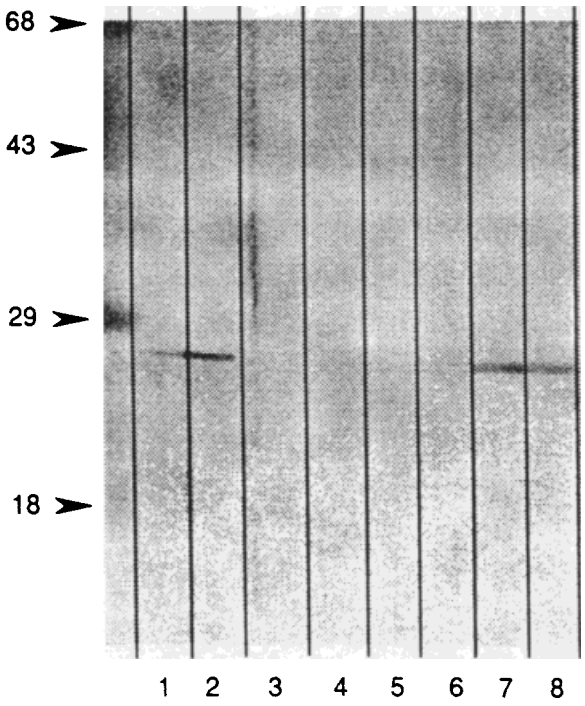

Fig. 2. Western analysis of human hsp27 expression. Odd-numbered lanes contain proteins expressed prior to heating while even-numbered lanes contain proteins expressed 8 h after a 45°C, 10 min heat shock. All lanes have been loaded with protein from 0.5×10^6 cells. Molecular mass markers are indicated by arrowheads. **Lanes 1** and **2** correspond to the human cell line A549; **lanes 3** and **4** to the parental CHO cell line; **lanes 5** and **6** to B2-6 (a control transfected cell line), and **lanes 7** and **8** correspond to B19-5 (a cell line transfected with plasmid pβ27).

To demonstrate that the 27 kD protein expressed from pβ27 plasmid was indeed the human form of hsp27, Western analysis was employed using a monoclonal antibody that specifically recognizes the human form of hsp27 (Fig. 2). Lanes 1 and 2 in Figure 2 demonstrate that the human cell line that was used as the source of the hsp27 cDNA has a low level of constitutive expression of hsp27 and that the expression is dramatically increased following a heat shock. Lanes 3 and 4 (Fig. 2) show that the CHO parental cell line does not express a human hsp27 protein. The control transfected cell line, B2-6, also failed to express any human hsp27 protein (Fig. 2, lanes 5 and 6). A cell line transfected with pβ27, B19-5, showed constitutive expression of the human hsp27 (Fig. 2, lane 7), which slightly decreased following a heat shock (Fig. 2, lane 8). Heat shock failed to increase the expression of the human hsp27 in any pβ27 transfected cell line due to the fact that hsp27 expression is under control of a constitutive promoter.

To demonstrate conclusively that the pβ27 plasmid was responsible for the constitutive expression of a human hsp27, two-dimensional gel electrophoresis was performed (Fig. 3). Figure 3A contains proteins that are expressed prior to a heat shock while Figure 3B contains proteins that are expressed following a heat shock in the control transfected cell line. An identical pattern of protein expression was seen in the parental cell line (data not shown). The arrows indicate the position of the three major isoforms of the rodent hsp27 which are labeled a, b, and c. These different isoforms are thought to represent different levels of phosphorylation of hsp27. In a pβ27 transfected cell line (B19-5), two isoforms of the human hsp27 protein which are expressed constitutively are indicated by triangles labeled A and B (Fig. 3C). These isoforms migrate at unique isoelectric points from that of the endogenous hamster hsp27 isoforms. Following a heat shock the pβ27 transfected cell line showed a normal endogenous hsp27 heat shock response (i.e., expression of the a, b, and c isoforms of the rodent hsp27) and a decrease in the constitutive expression of the human isoforms of hsp27 (Fig. 3D).

The ability of hsp27 to protect CHO cells from a single lethal heat dose was investigated (Fig. 4). A 2.5 log increase in survival is seen in cells that constitutively express hsp27 vs. either parental or control transfected cell line. The amount of constitutively expressed hsp27 correlated with an increase in survival in the pβ27 transfected cell lines (Fig. 5).

The ability of hsp27 to affect the development of thermotolerance was also investigated. Figure 6 contains the survival data to a single 90 min 44°C heat shock (hatched bars) as well as the survival to a split dose of 90 min at 44°C (solid bars) in the parental cell line; control transfected cell line (B2-6) and a pβ27 transfected cell line (B19-5). The split dose heating of 30 min at 44°C followed by recovery at 37°C for 8 h before the final heating of 44°C for 60 min allows for the development of thermotolerance. Both the parental and control transfected cell lines had similar responses and developed thermotolerance to the same extent. The pβ27 transfected cell line was better able to survive a single heat shock but developed thermotolerance to the same extent as the control transfected cell line.

Since protein synthesis is affected by heating, we examined the effect of constitutive hsp27 synthesis on this process. Figure 7 shows the rate of protein synthesis in the control transfected cell lines (B2-6 and B18-13), the thermotolerant cell line (B2-6TT), and hsp27 expressing cell lines (B19-5 and B18-16). Protein synthesis was dramatically reduced (90%) in non-thermotolerant cell lines, while the thermotolerant cell line showed a 50% reduction following a heat shock. Within 7 h the thermotolerant cell line recovered protein synthesis to pre-heat shock rates, while the control transfected cell lines took almost 10 h to recover protein synthesis. Hsp27 expressing cell lines were able to recover protein synthesis in about 6 h following the 44°C, 30 min heat shock.

Because there was a difference in the rate of protein synthesis, we also investigated the rate of total RNA synthesis following a heat shock. Figure 8 shows the rate of RNA synthesis in control transfected cell lines (B2-6 and B18-13), a thermotolerant cell line (B2-6TT), and hsp27 expressing cell lines (B19-5 and B18-16). RNA synthesis was decreased by 40% in the thermotolerant cell line and by 60% in the non-thermotolerant cell lines following a heat shock. Within 5 h the thermotolerant and hsp27 expressing cell lines recovered RNA synthesis to pre-heat shock rates, while it took the control transfected cell lines 7 h to recover RNA synthesis to the pre-heat shock rate.

To determine if hsp27 has the ability to act as a molecular chaperone, we investigated the refolding of α-glucosidase in the presence or absence of hsp27. Following denaturation in 8 M urea, α-glucosidase will spontaneously refold and regain activity (up to 30% of the control rate) upon lowering the concentration of urea (Fig. 9A,B). The addition of human hsp27 during the renaturation period stimulated the recovery of activity up to 45%. To determine if this increased recovery was due to a specific molecular chaperone activity of hsp27, α-glucosidase was allowed to refold in the presence of a different protein, ovalbumin. Surprisingly, ovalbumin was able to stimulate recovery of enzymatic activity just as effectively as hsp27 (Fig. 9A). Aldolase was more effective than hsp27 in stimulating recovery of enzymatic activity (Fig. 9B).

DISCUSSION

In this study, we constructed CHO cell lines that constitutively express human hsp27. Expression of hsp27 did not affect the development of thermotolerance but did affect thermal sensitivity. Both protein synthesis and total RNA synthesis decreased dramatically follow-

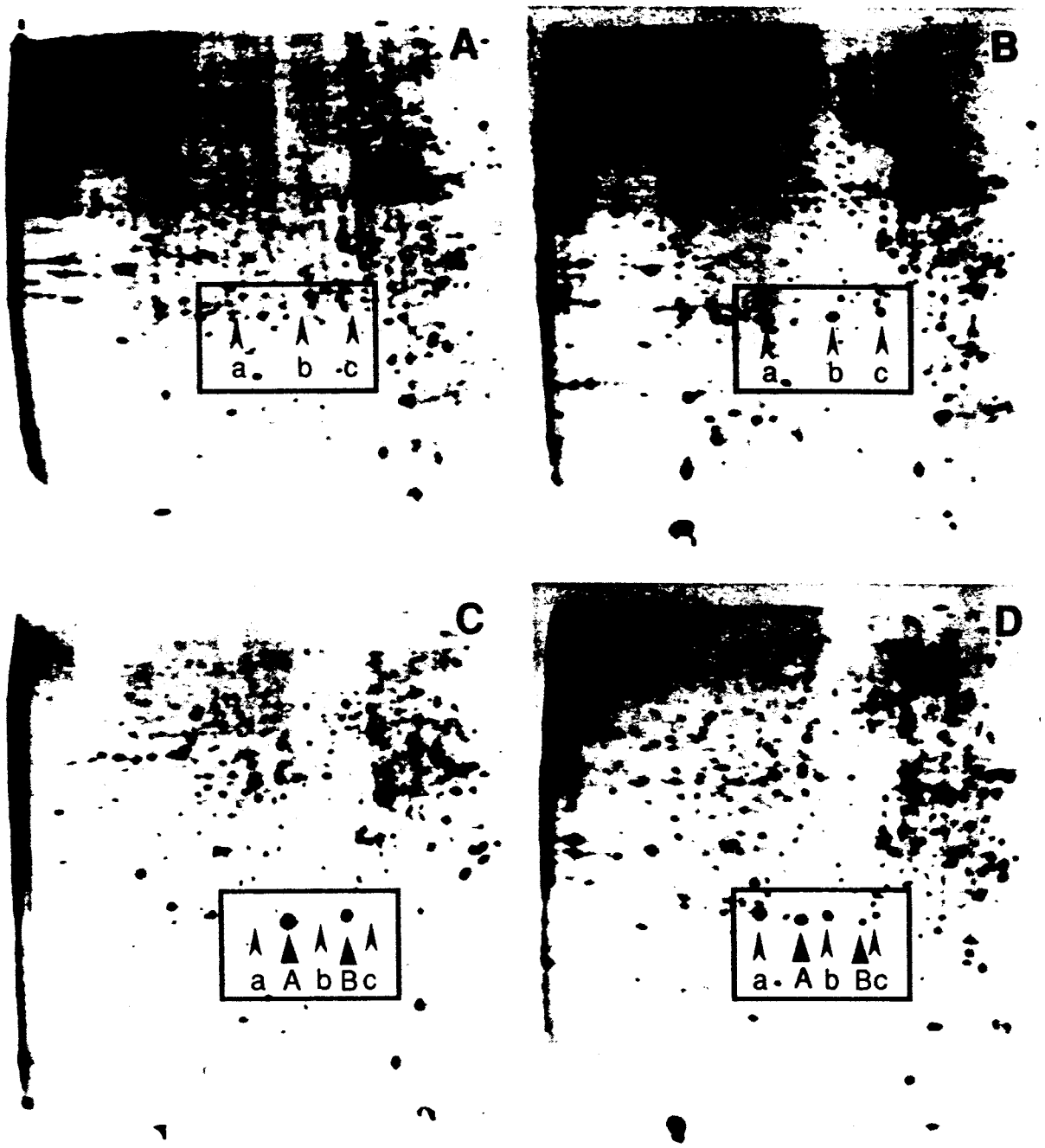

Fig. 3. Two-dimensional gel electrophoresis of transfected cell lines. **A** and **B** contain proteins expressed prior to (A) or following (B) a 45°C, 10 min heat shock in a control transfected cell line (B2-6). **C** and **D** contain proteins expressed prior to (C) or following (D) a 45°C, 10 min heat shock in cell line B19-5 transfected with plasmid pβ27. All gels contain protein from 75,000 cells. The position of the endogenous rodent hsp27 isoforms (a, b, and c) are indicated by arrowheads while the human hsp27 isoforms (A, B) are indicated by triangles.

ing a heat shock. In CHO cell lines that express human hsp27, there was a more rapid recovery of both cellular processes. The more rapid recovery of these processes could afford the cells more time to repair heat-induced damage prior to cell division.

The process whereby cells develop thermotolerance has been actively investigated for the past 20 years. No model explaining this phenomenon has found wide acceptance. Since cells express heat shock proteins at about the same time they develop thermotolerance, it has been

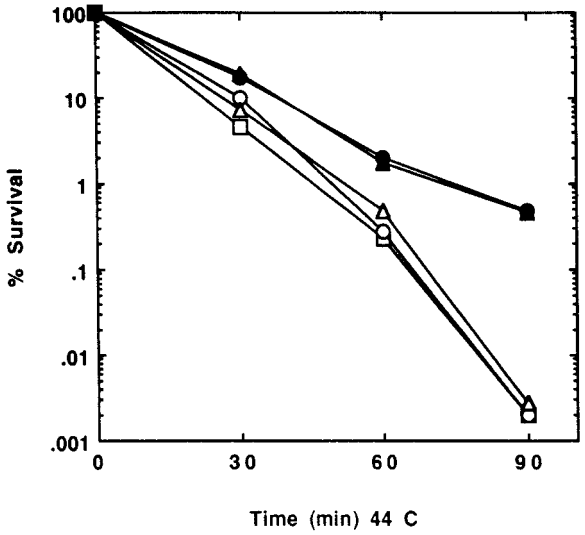

Fig. 4. Effect of hsp27 expression on survival to a single heat shock. The open squares represent the parental CHO cell line. The control transfected cell lines are represented by open circles (B2-6) and open triangles (B18-13). The pβ27 transfected cell lines are represented by filled circles (B19-5) and filled triangles (B18-16). Error bars are smaller than the symbols used.

proposed that hsps are essential in the thermotolerant response. To test this hypothesis, a variety of cell lines have been engineered to constitutively or conditionally express specific hsps in order to determine what effects they have on thermotolerance. In yeast cell, overexpression of hsp27 or hsp70 (SSA1, SSA2, and SSA4) had no effect on thermal sensitivity (the ability to survive a single heat shock) or thermotolerance (the ability to survive two or more heat shocks) [Weitzel and Li, 1993]. In contrast, rat fibroblasts that constitutively express human hsp70 show an increased ability to survive a single heat dose, while the development of thermotolerance was unaffected [Li et al., 1991]. In mammalian cell lines engineered to constitutively express hsp27 (COS cells, Chinese hamster lung fibroblasts, Ehrlich ascites tumor cells), there is an increase in survival to a single heat dose [Landry et al., 1989; Knauf et al., 1992; Mehlen et al., 1993]. These observations are consistent with our findings. It is interesting to note that the constitutive expression of a Drosophila hsp27 protein was also able to increase survival to a single heat dose in Chinese hamster lung fibroblasts cell line [Rollet et al., 1992].

Our results show that constitutive expression of the human hsp27 in CHO cells results in an enhanced ability to survive a single lethal heat shock. Normally, following a heat shock the human hsp27 is phosphorylated on serine residues at positions 15, 78, and 82 [Landry et al., 1992]. In the CHO cell lines used in our study, it appears that one of the phosphorylation sites is primarily being used (i.e., only two isoforms of the human hsp27 are seen in Fig. 3).

Similar results have been reported. When Chinese hamster cells were transfected with the human hsp27 gene and proteins were labeled with [^{3}H]-leucine only two isoforms were observed; however, when proteins were labeled with $H_3{}^{32}PO_4$ following a heat shock the three phosphorylated forms were observed [Landry et al., 1989]. The increased ability of our hsp27 expressing cell lines to survive a lethal heating is very similar to that reported for other Chinese hamster cell lines expressing the human hsp27 [Landry et al., 1989]. Interestingly, when a mutant form of hsp27 was constructed such that the three phosphorylation sites were mutated to glycine residues, the ability of the human hsp27 to repair actin microfilaments and protect against thermal cytotoxicity in Chinese hamster cells was completely obliterated [Lavoie et al., 1995]. Without a doubt the phosphorylation of hsp27 is an important factor in the function of the protein. We believe that Chinese hamster cells can properly phosphorylate the human hsp27, which results in an increased ability to survive a heat shock.

Our results (Fig. 6) clearly demonstrate that even though the initial survival to a single heat shock can vary greatly depending upon the amount of hsp27 expressed, all cell lines measured developed thermotolerance to the same extent. Therefore, the overexpression of hsp27 is not sufficient for the development of thermotolerance. Similar results have been reported [Landry et al., 1989]. The overexpression of hsp70 has also resulted in an increased survival to a single heat dose but had no effect on the development of thermotolerance [Li et al., 1991]. It would appear the overexpression of single heat shock protein is not sufficient for the development of thermotolerance. Since the normal response to a heat shock is an upregulation of many hsps (at least four major families) it is not surprising that any single hsp is insufficient for the development of thermotolerance. Currently, experiments are underway to determine if constitutive expression of multiple heat shock proteins will result in the development of thermotolerance.

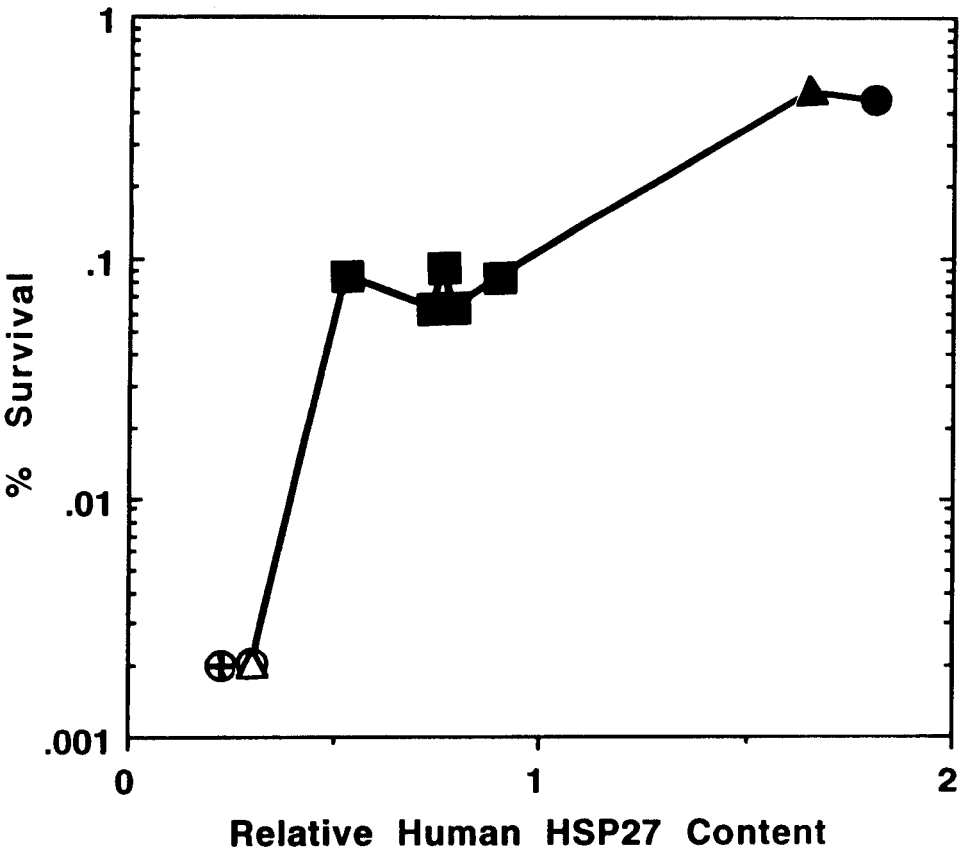

Fig. 5. Correlation between levels of human hsp27 and survival. Survival was determined by colony formation following exposure to 44°C for 90 min. Levels of hsp27 and actin were determined using densitometry. Hsp27 values were normalized relative to the signal obtained for actin. The circle containing the cross represents the level of hamster hsp27 in parental CHO cells. The control transfected cell lines are represented by the open circle (B2-6) and the open triangle (B18-13). The filled squares represent various cell lines transfected with pβ27. The filled circle represents the level of human hsp27 in the B19-5 cell line and the filled triangle represents the levels of human hsp27 in the B18-16 cell line.

Almost every cellular process is affected following a heat shock [Tomasovic, 1989]. We chose to examine both protein synthesis and total RNA synthesis. Following a heat shock, there is a dramatic decrease (90%) in protein synthesis in CHO cell lines that are not thermotolerant. Thermotolerant cells only displayed about a 50% reduction in the rate of protein synthesis following a heat shock and were able to recover to pre-heat shock levels in about 7 h.

In control transfected cell lines protein synthesis took about 10 h to recover, while in the hsp27 expressing cell line protein synthesis recovered in only 6 h. These results are different than those reported for a Chinese hamster lung fibroblasts cell line that constitutively expresses human hsp27 [Laszlo et al., 1993]. In that study, constitutive expression of hsp27 had no effect on the recovery of protein synthesis following a heat shock [Laszlo et al., 1993].

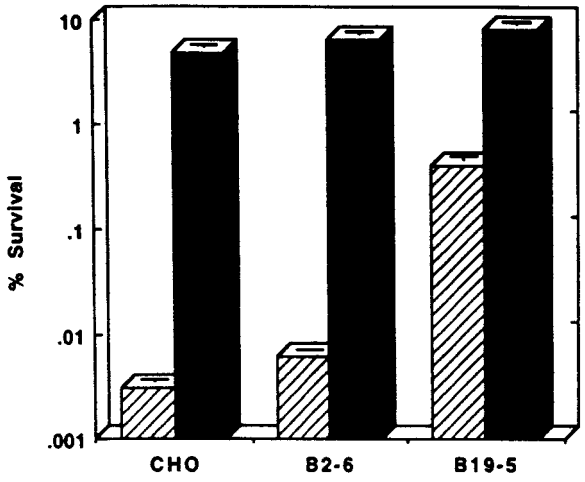

Fig. 6. Effect of human hsp27 expression on the development of thermotolerance. The survival response of cell lines to either a continuous 90 min, 44°C heat shock (hatched bars) or a 30 min, 44°C heating followed by 8 h at 37°C (to allow thermotolerance to develop), then heat for 60 min at 44°C (solid bars), is shown.

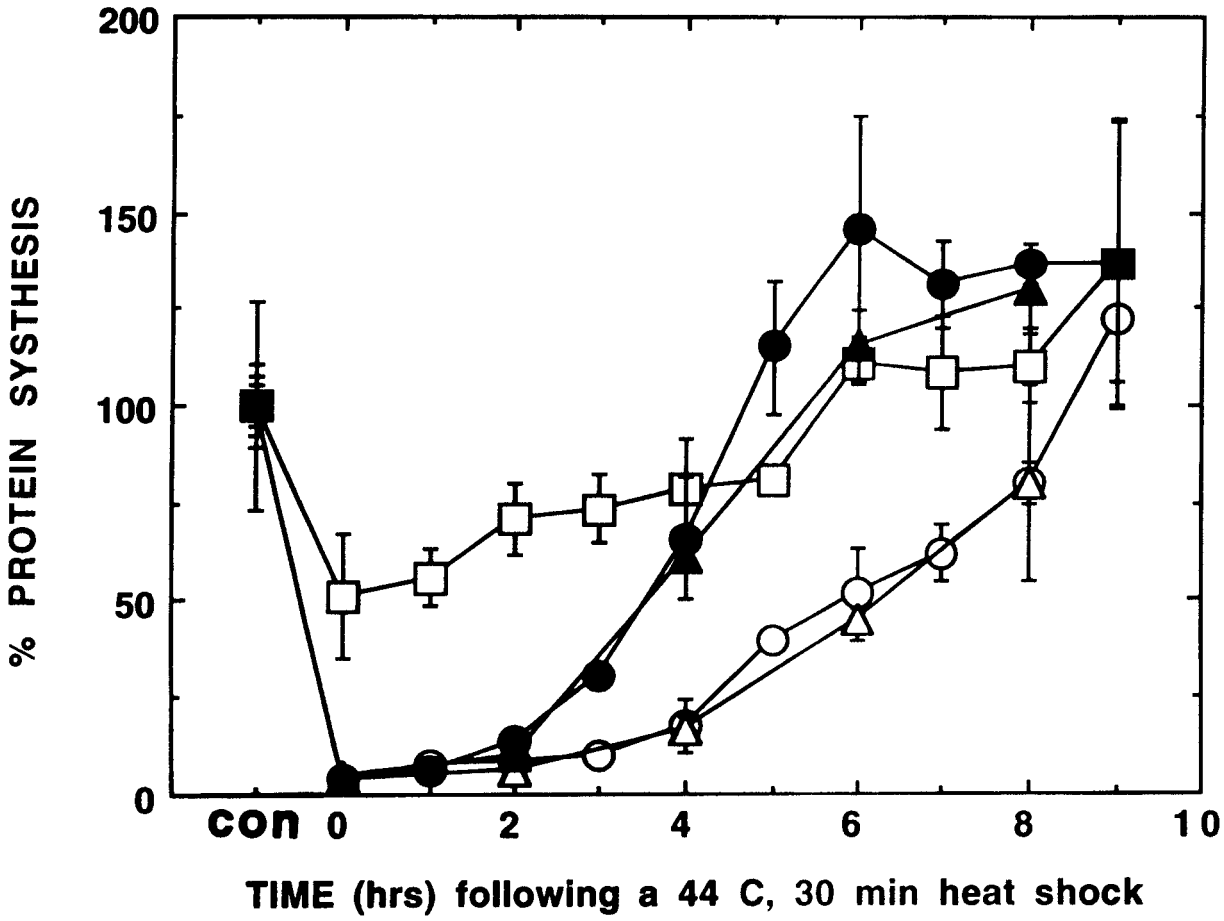

Fig. 7. Recovery of protein synthesis following heat shock. The rate of protein synthesis in the control transfected cell lines B2-6 (open circles) and B18-13 (open triangles), the thermotolerant control transfected cell line B2-6TT (open squares), and hsp27 constitutively expressing cell lines B19-5 (solid circles) and B18-16 (solid triangles) is shown by hour intervals prior to (CON) or following a 44°C, 30 min heat shock. Protein synthesis was measured as the number of TCA perceptible counts divided by the amount of protein in the sample. Each time point represents the average of three culture tubes, each of which had three samples removed for analysis.

These differences could be due to cell line variations, or to the fivefold higher level of expression of hsp27 in the cell line used in this study (a relative human hsp27 content of 0.4 [Laszlo et al., 1993] vs. 2.0 [this study]). Our data clearly indicate that hsp27 is responsible for a more rapid recovery of protein synthesis following a heat shock.

RNA synthesis rates also are reduced following a heat shock. Under the experimental conditions used, there was a 60% reduction in non-thermotolerant cells and only a 40% reduction in thermotolerant cells. The rate of RNA synthesis in the thermotolerant cells recovered within 5 h. The rate of RNA synthesis in the control transfected cell lines recovered after 7 h, while in the hsp27 expressing cell lines, RNA synthesis recovered within 5 h. A faster recovery of RNA synthesis following a heat shock in Chinese hamster lung fibroblasts that constitutively express hsp27 has recently been reported [Laszlo et al., 1993] and is in agreement with our observations. Our data indicate that hsp27 is also responsible for a more rapid recovery of RNA synthesis following a heat shock.

Thermotolerance is a complex cellular response to an increase in normal growth temperature. In trying to delineate the physiological events involved in this response, we have constructed CHO cell lines that constitutively express a human hsp27. Hsp27 does not appear to protect the cell from heat induced damage in regard to the rate of protein and RNA synthesis. Hsp27 appears to play a role in the recovery of these processes after the damage has occurred. The constitutive expression of hsp27

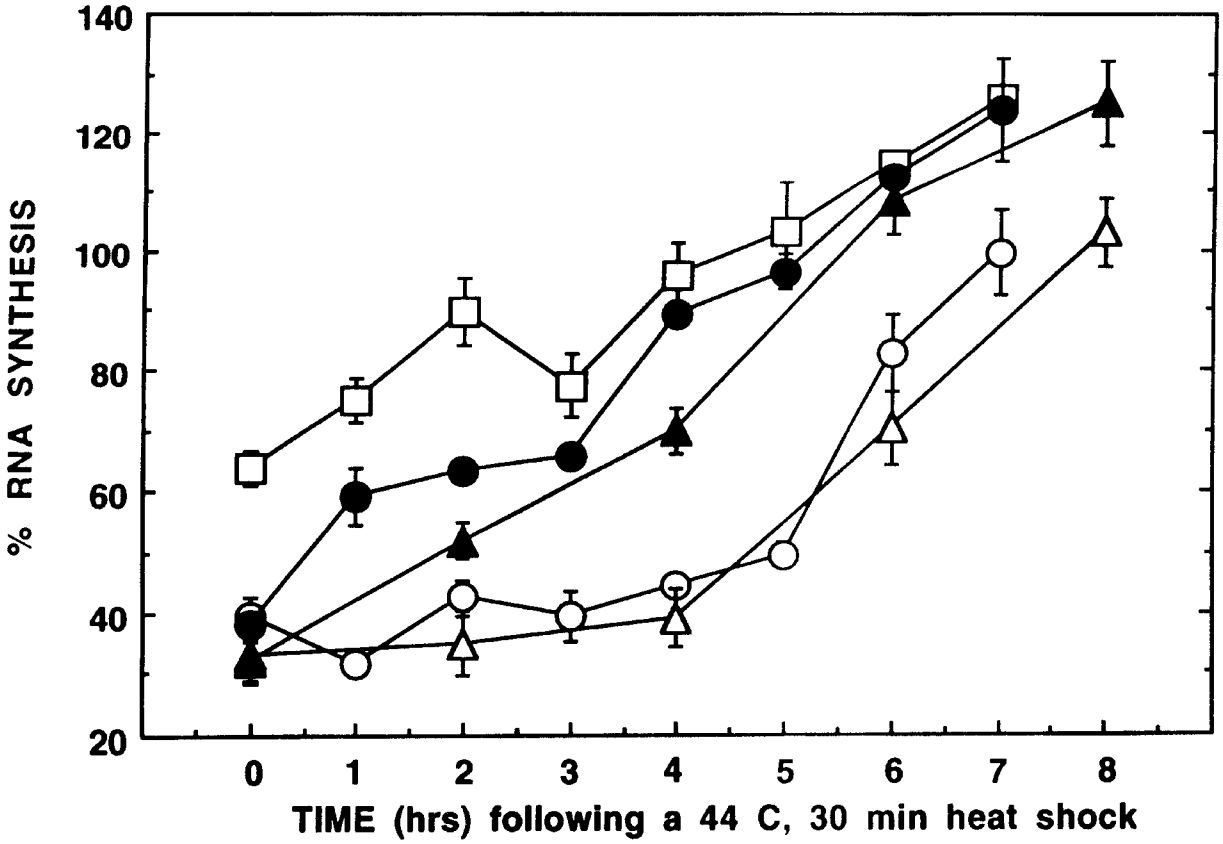

Fig. 8. Recovery of total RNA synthesis following heat shock. The rate of RNA synthesis in control transfected cell lines B2-6 (open circles) and B18-13 (open triangles), the thermotolerant control transfected cell line B2-6TT (open squares), and hsp27 constitutively expressing cell lines B19-5 (solid circles) and B18-16 (solid triangles) is shown by hour intervals following a 44°C, 30 min heat shock. The rate of RNA synthesis prior to heating was defined as 100%. RNA synthesis was measured as the amount of uridine incorporated divided by the amount of protein in the sample. Each time point represents the average of three flasks, each of which had three samples removed for analysis.

can result in a thousandfold increase in survival to a single heat shock. The exact mechanism whereby hsp27 results in such a dramatic increase in survival is still open to debate. We and others have shown that it affects the recovery of complex physiological processes. The rate of recovery of these processes may be of critical importance in repairing cellular damage prior to cell division. The ability of hsp27 to help stabilize the actin filaments following a heat shock may also be important in increasing survival to a lethal stress [Lavoie et al., 1993, 1995]. Hsp27 clearly affects the two processes that we have investigated but these may not be the critical target in cell survival. Perhaps the ability to develop thermotolerance involves upregulation of hsp27 or phosphorylation of hsp27. Our results show that the human hsp27 does greatly affect the recovery rate of both protein and RNA synthesis as well as thermal sensitivity. This increased recovery may well be responsible for the thermal sensitivity. It is also clear that even though the human hsp27 has these dramatic effects, the development of thermotolerance is unaffected. Therefore, the presence of a large amount of hsp27 prior to heating is not sufficient for thermotolerance. Other proteins, factors, or physical events must occur for the development of thermotolerance.

Hsp27 as well as α-crystallin (a protein with sequence homology to hsp27 [Lindquist and Craig, 1988]) have been proposed to have a molecular chaperone activity [Horwitz, 1992; Jakob et al., 1993]. However, when we investigated the ability of hsp27 to help an enzyme refold following urea denaturation, we found that the assay is not capable of measuring a molecular chaperone activity. The ability of ovalbumin (45,000 MW) to work as well as hsp27 in helping α-glucosidase to refold, indicates that

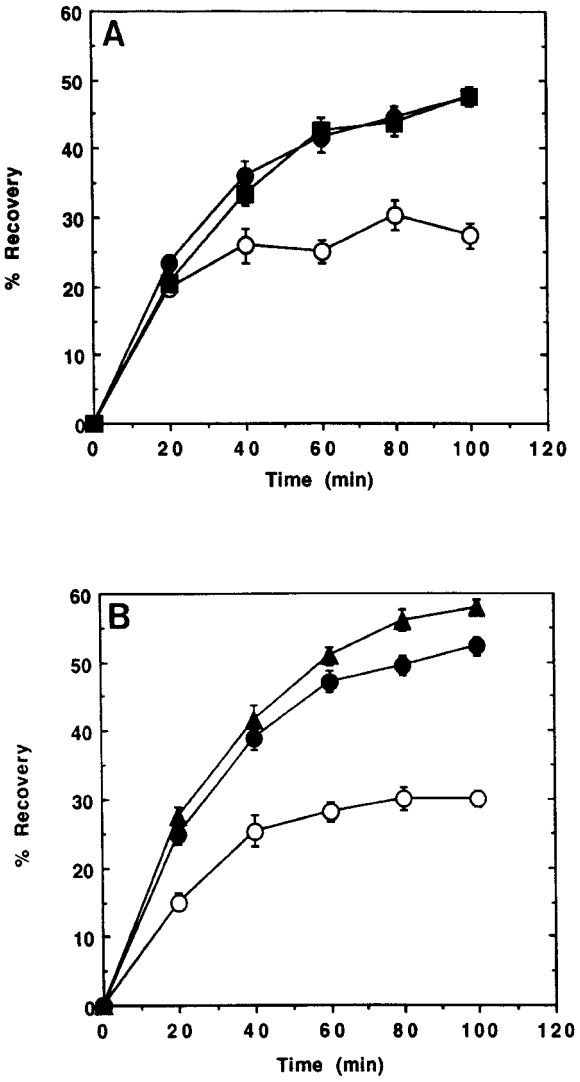

Fig. 9. Recovery of α-glucosidase activity following denaturation in urea. **A:** The kinetics of reactivation of α-glucosidase in the absence of additional proteins (open circles), in the presence of 140 nM Hsp27 (filled circles), and in the presence of 140 nM ovalbumin (filled squares). **B:** The kinetics of reactivation of α-glucosidase in the absence of additional proteins (open circles), in the presence of 140 nM Hsp27 (filled circles), and in the presence of 140 nM aldolase (filled triangles).

hsp27 has a non-specific effect in protein refolding. Alpha-glucosidase refolds spontaneously following dilution of urea, but it can recover activity to a greater extent if another protein is present, presumably due to the other protein's ability to bind urea and thus lower the effective urea concentration. This hypothesis is supported by our data that a large protein, aldolase (158,000 MW), is more effective in helping α-glucosidase refold than smaller proteins. Our results indicate that if hsp27 has a molecular chaperone activity of helping proteins refold, this assay is incapable of detecting it due to the problem of urea partitioning. The ability of hsp27 to prevent protein unfolding in vitro as measured by thermal aggregation of α-glucosidase appears to be specific since lysozyme and IgG had no effect [Jakob et al., 1993]. However, expression of hsp27 in vivo had no protective effect on the physiological reactions involving RNA synthesis and protein synthesis. If hsp27 can act as a molecular chaperone in vivo, it appears to have its most dramatic effects following a heat shock. Further studies are needed to determine if hsp27 can actually aid in protein refolding following thermal denaturation.

The physiological role of hsp27 overexpression may be very profound. Breast cancer patients whose tumors express high levels of hsp27 have greater incidence of tumor recurrence in comparison to patients whose tumors have a low level of hsp27 expression [Thor et al., 1991; Love and King, 1994]. Chinese hamster cells that constitutively express hsp27 are better able to survive certain types of chemotherapeutic agents [Huot et al., 1991]. Our studies show that CHO cells that constitutively express hsp27 are better able to survive cellular stresses such as heat and Adrimycin (data not shown) but have no increased survival to ionizing radiation (data not shown). It is interesting to speculate that breast cancer patients who demonstrate elevated levels of hsp27 may benefit more from adjuvant treatments of ionizing radiation than from chemotherapeutic treatments. Recently we have shown that hsp27 in an ER positive human breast cancer cell line results in making the transfected cells better targets for lymphocyte mediated killing by gamma delta T-cells [Mahvi et al., 1993]. However, hsp27 overexpression in an ER negative human breast cell line failed to stimulate lymphocyte mediated killing (Mahvi et al. manuscript submitted). Therefore, even though overexpression of hsp27 can result in an increased ability to survive certain types of cellular stress it may render the cell more susceptible to immune detection. We are far from understanding all of the physiological implications of overexpression of hsp27.

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