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Intracellular Localization and Partial Amino Acid Sequence of a Stress-Inducible 40-kDa Protein in HeLa Cells

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ABSTRACT. **We earlier discovered a novel 40-kDa protein (hsp40) induced by heat shock and other stresses in mammalian and avian cells. In this report, we purified the hsp40 in HeLa cells, using modified two-dimensional gel electrophoresis, and determined the amino terminal amino acid sequence of this protein. The hsp40 is homologous to DnaJ, an *Escherichia coli* heat-shock protein, as well as to DnaJ-homologous proteins in yeast such as SCJ1, Sec63/Npl1, YDJ1 and SIS1. Indirect immunofluorescence staining using an anti-hsp40 polyclonal antibody demonstrated that hsp40 was localized faintly throughout the cell in non-heat-shocked cells and was accumulated in nuclei and nucleoli in heat-shocked cells. The intracellular localization of hsp40 was very similar to that of hsp70, suggesting that these two hsps colocalize in heat-shocked HeLa cells.**

When prokaryotic and eukaryotic cells are exposed to heat shock or other environmental stresses, they respond by synthesizing a family of proteins, the so-called stress or heat-shock proteins (hsps) (1, 28, 33). In mammalian cells, several hsps with molecular sizes of 110-, 100-, 90-, 78-, 75-, 73-, 72-, 60-, 47-, 32-, 28- and 8-kDa are known to be induced by heat shock or environmental stresses such as ethanol, amino acid analogues, heavy metals and viral infection (see ref. 38 for review). Recently, the functions of some hsps have been described. For example, hsp90 has been shown to associate with steroid hormone receptors or various protein kinases to regulate their activity and translocation through interaction with the cytoskeleton (19, 27, 31). Some members of the hsp70 family have been reported to be involved in the translocation of nascent proteins from the cytoplasm across the membrane of either the endoplasmic reticulum or mitochondria (3, 7, 9). Hsp47 is found to be a collagen-binding protein (20). Hsp32 and hsp8 are identified as heme oxygenase and ubiquitin, respectively (34, 5). In addition to these classical hsps, Ohtsuka *et al.* have recently found a novel heat-shock protein, hsp40, which is induced by heat shock, transient metals and azetidine carboxylic acid in mammalian and avian cells (26).

Here we report the purification of hsp40 in HeLa cells by modified two-dimensional gel electrophoresis, partial amino acid sequence of the amino terminal of hsp40, and intracellular localization of hsp40 by indirect immunofluorescence staining using an anti-hsp40 polyclonal antibody.

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Abbreviations used: hsp, heat-shock protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NEPHGE, nonequilibrium pH gradient electrophoresis; CBB, Coomassie brilliant blue; PBS, Dulbecco's phosphate-buffered saline.

MATERIALS AND METHODS

Cell culture, heating and metabolic labeling. HeLa cells were cultured in Dulbecco's modified Eagle's minimal essential medium (Nissui, Tokyo) supplemented with 10% fetal calf serum (Hazleton, U.S.A.) and maintained by passaging twice a week. Cells at subconfluence in 100-mm or 35-mm dishes (Corning, U.S.A.) were heated by immersing the dishes sealed with Parafilm into a water bath, the temperature of which was controlled within ±0.1°C (12). For metabolic labeling, cells were heated at 45°C for 15 min and incubated at 37°C for 1 h; the culture medium was then replaced with methionine-free culture medium supplemented with 10% dialyzed fetal calf serum, and 1.85 MBq (50 μCi) of [^{35}S]methionine was added to each dish. The cells were further incubated at 37°C for 5 h. Control cells were labeled at 37°C for 5 h. Cells were then rinsed with Dulbecco's phosphate-buffered saline (PBS), harvested with a rubber policeman and pelleted by centrifugation. They were lysed by sonication in O'Farrell's lysis buffer (21) (9.5 M Urea, 2% Nonidet P-40, 5% 2-mercaptoethanol, 2% Pharmalyte (pH 3–10)) containing 1 μg/ml of leu-

peptin, 1 μg/ml of pepstatin and 1 mM phenyl-methylsulfonyl fluoride as protease inhibitors. For one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting, cells were lysed in SDS-sample buffer (13) and boiled for 5 min. Protein amount was measured by a Pierce protein assay kit (Pierce, U.S.A.).

Gel electrophoresis. One-dimensional SDS-PAGE was performed by Laemmli's method (13) on a slab gel with a 10% acrylamide concentration. Two-dimensional (2D) gel electrophoresis using nonequilibrium pH gradient electrophoresis (NEPHGE) as the first dimension was performed by O'Farrell's methods (22) at 400 V for 3.5 h. The second dimension was done by Laemmli's method (SDS-PAGE). Approximately equal amounts of protein were applied to the first-dimension gels. After electrophoresis, the gels were stained with Coomassie brilliant blue R-250 (CBB) and photographed. For fluorography, the stained gels were soaked in 1 M sodium salicylate and shaken for 20 min, dried under a vacuum, and exposed to X-ray film (Kodak, X-Omat AR 5) at −80°C.

Purification of hsp40 and amino acid sequencing. We attempted to purify hsp40 by modified 2D gel electrophoresis because its purification by conventional column chromatography was difficult. HeLa cells were heated at 45°C for 15 min and recovered for 16 h; they were then lysed in O'Farrell's lysis buffer. The first-dimensional electrophoresis was performed using a slab gel instead of a rod gel. The position of hsp40 was determined with colored pI markers (Oriental Yeast, Osaka). The portion of hsp40 was cut out and applied to the second dimension. Consequently, the hsp40 was detected as a band. We call this method Slab-NEPHGE/SDS-PAGE 2D gel electrophoresis. It allowed us to apply 30–40 times the amount of proteins to the first-dimension gel as compared with ordinary 2D gel electrophoresis. Details of this method will be published separately. The purified hsp40 was blotted electrophoretically onto a PVDF membrane (BIO-RAD, U.S.A.) in a CAPS (cyclohexylaminopropane sulfonic acid) buffer (17), and sequenced directly by means of an automated gas-phase sequencer (Applied Biosystems, Model 470A).

Preparation of anti-hsp40 antibody. A band of hsp40 was cut from the Slab-NEPHGE/SDS-PAGE 2D gel, and hsp40 was eluted electrophoretically by Amicon Centrilutor (Amicon, U.S.A.). Typically, 10–20 μg of purified hsp40 was ob-

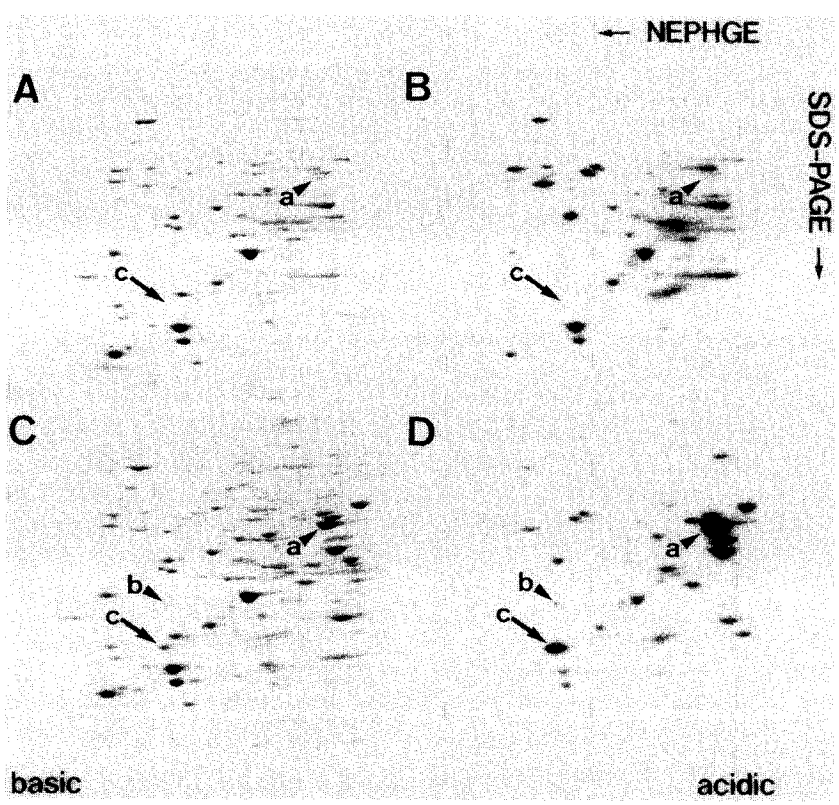

Fig. 1. Two-dimensional gel analysis of proteins in control (A and B) and heat-shocked (C and D) HeLa cells. A and B, Cells were grown at 37°C and labeled with [^{35}S]methionine for 5 h. C and D, Cells were heated at 45°C for 15 min then incubated at 37°C for 6 h and labeled with [^{35}S]methionine for the last 5 h. Cell lysate in O'Farrell's lysis buffer was analyzed by NEPHGE/SDS-PAGE 2D gel electrophoresis. Protein amounts applied to the first dimension were approximately 300 μg for both gels. Gels were stained with CBB (A and C) and processed for fluorography (B and D). a, hsp70 (p72, inducible form); b, hsp47; c, hsp40.

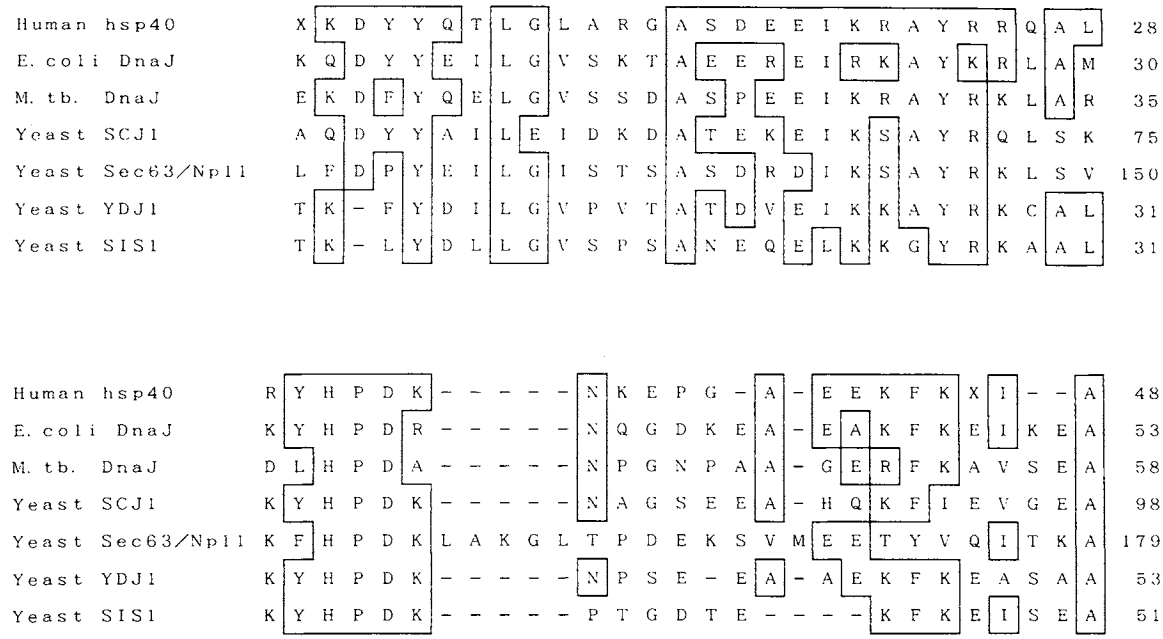

Fig. 2. Amino acid sequence of amino terminal of human hsp40 and comparison with amino acid sequence of *E. coli* DnaJ (2, 23), M. tuberculosis (M. tb.) DnaJ (14), yeast SCJ1 (4), yeast Sec63/Npl1 (32), yeast YDJ1 (6) and yeast SIS1 (16). Residues (one-letter code) shown in boxes are identical for hsp40 and other DnaJ-homologous proteins. Amino acid sequence of hsp40 was determined in three independent experiments, and the same results were obtained. Homology between hsp40 and DnaJ was identified by computer search of the protein sequence data base (NBRF/PIR). Dashes are introduced to optimize alignments. X denotes unidentified amino acid.

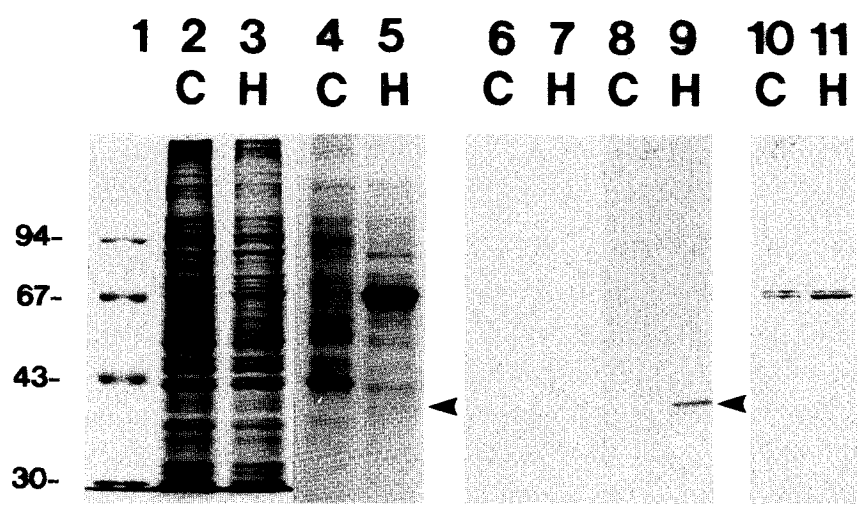

Fig. 3. Detection of hsp40 by one-dimensional SDS-PAGE and immunoblot analysis of proteins in control and heat-shocked HeLa cells with anti-hsp40 and anti-hsp70 antibodies. Lanes 2, 6, 8 and 10, control cells; lanes 3, 7, 9 and 11, cells were heated at 45°C for 15 min then incubated at 37°C for 16 h; lane 4, fluorography of control cells labeled with [^{35}S]methionine for 5 h at 37°C; lane 5, fluorography of cells heated at 45°C for 15 min, then incubated at 37°C for 6 h and labeled with [^{35}S]methionine for the last 5 h. Lane 1, molecular weight markers (in kilodaltons, Pharmacia, Sweden); lanes 2 and 3, gels stained with CBB; lanes 6 and 7, probed with pre-immune serum (1/1000 dilution); lanes 8 and 9, probed with anti-hsp40 antibody (1/1000 dilution); lanes 10 and 11, probed with anti-hsp70 antibody (1/400 dilution). This antibody recognizes both hsp70 (p72, inducible form, lower band) and hsc70 (p73, constitutive form, upper band). Arrowheads indicate hsp40.

tained from one Slab-NEPHGE/SDS-PAGE 2D gel. The purified hsp40 (approximately 70 μg) was mixed with an equal volume of Freund's complete adjuvant (Difco, U.S.A.) and injected subcutaneously into one rabbit. Two weeks later, additional hsp40 (25 μg in Freund's complete adjuvant) was injected again. Two weeks after the second injection, 25 μg of hsp40 in Freund's incomplete adjuvant was injected. Serum (25 ml) was collected one and two weeks after the last injection.

Immunoblotting and indirect immunofluorescence staining. The method of immunoblotting was used to examine the specificity of an anti-hsp40 antibody. The proteins of cell lysate in SDS sample buffer were separated by SDS-PAGE and transferred to a nitrocellulose membrane by the method of Towbin *et al.* (36). The membrane was then treated with 5% skim milk in TBS (50 mM Tris-HCl, pH 8.0, 154 mM NaCl) to inhibit nonspecific binding of first and second antibodies. The anti-hsp40 serum at a 1/1,000 dilution or rabbit anti-hsp70 serum (No. 4 rabbit as described in Refs. 12 and 24) at 1/400 dilution was used as the first antibody, and horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed, U.S.A.) at a 1/1,000 dilution as the second antibody.

For the analysis of the intracellular localization of hsp40 and hsp70, HeLa cells were grown on glass coverslips. After various treatments, cells were washed with cold PBS, and fixed in 100% methanol at -20°C for 10 min. The cells were treated with 10% normal goat serum in PBS to inhibit nonspecific binding. Then they were processed for immunofluorescence staining using rabbit anti-hsp40 serum (1/100 dilution) or rabbit anti-hsp70 serum (12, 24, 1/80 dilution) as the first antibody, and FITC-conjugated goat anti-rabbit IgG (H+L) (Zymed) as the second antibody (1/60 dilution). The cells on each coverslip were photographed through a Fluorophoto microscope (Nikon, Tokyo). Exposure time was exactly the same for every photograph in one series of experiments.

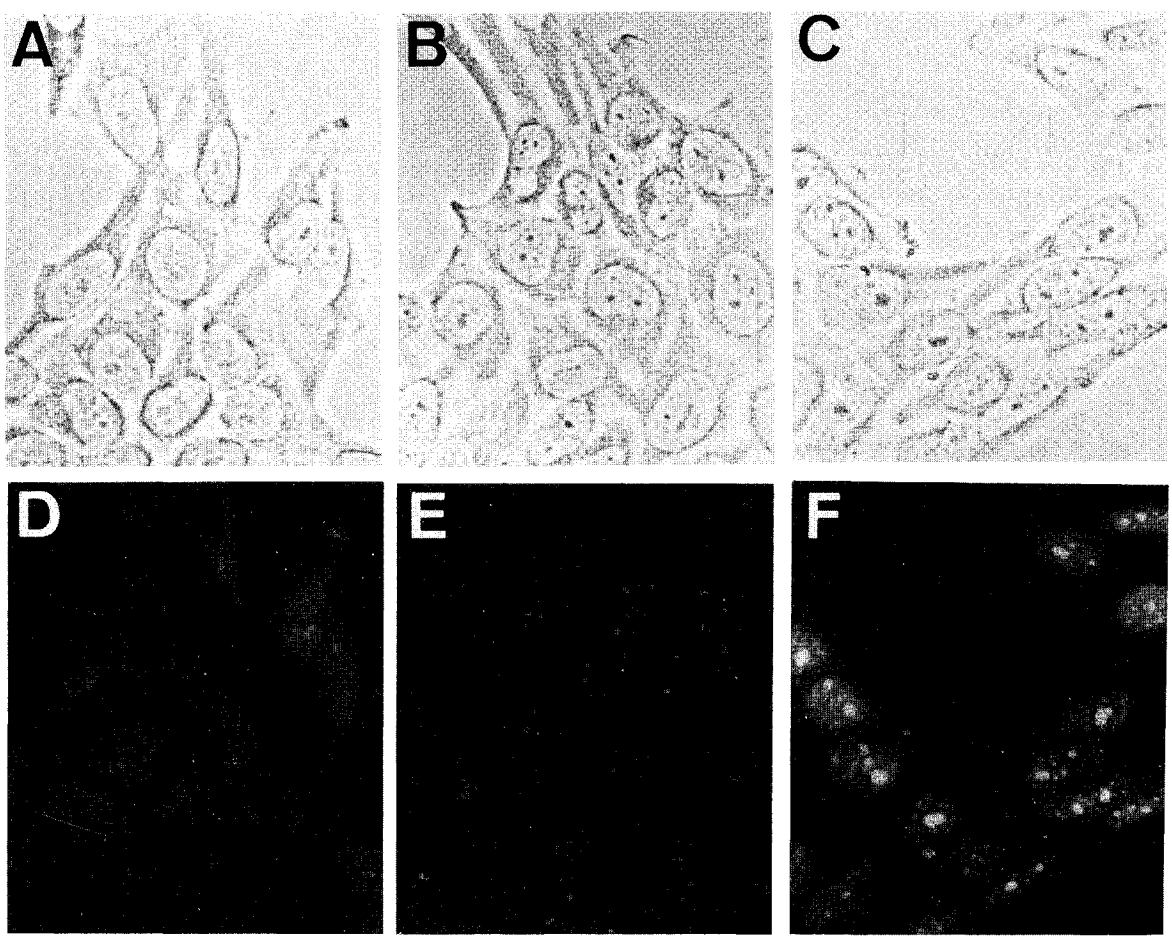

Fig. 4. Intracellular localization of hsp40 in HeLa cells. Cells growing at 37°C (A, B, D and E) or heated at 42°C for 2 h (C and F) were fixed and processed for immunofluorescence staining. A, B and C, phase contrast micrographs; D, E and F, fluorescence micrographs of each corresponding field. D and F, cells stained with an anti-hsp40 antibody (1/100 dilution); E, cells stained with a pre-immune serum (1/100 dilution). Exposure time was 1.5 sec for A, B and C and 30 sec for D, E and F. Note the brightly stained globular structures in F that correspond to the phase-dense nucleoli in C.

RESULTS

Induction of hsp40. We previously reported that hsp40, as well as other hsps (e.g., hsp110, hsp90, hsp70 and hsp47), was clearly induced by heat shock, sodium arsenite and azetidine carboxylic acid in HeLa cells, as demonstrated by NEPHGE/SDS-PAGE 2D gel electrophoresis (26). As shown in Figure 1, the hsp40 was barely detectable in non-heat-shocked control HeLa cells (Figs. 1A and 1B). However, it was detectable when the X-ray film was overexposed (data not shown), indicating that hsp40 is expressed constitutively at a very low level (26). In contrast, hsp40 was drastically induced by heat shock at 45°C for 15 min, and detected by Coomassie blue staining 6 h after the heat shock, as well as by metabolic labeling with [^{35}S]methionine (Figs. 1C and 1D). Hsp70 (p72, inducible form) and hsp47 were also induced under these experimental conditions (Figs. 1C and 1D).

Partial amino acid sequence of human hsp40. We have attempted to purify hsp40 by conventional column chromatography, but have not been successful thus far. In order to determine the partial amino acid sequence of hsp40, we sought to purify it by modified 2D gel electrophoresis (Slab-NEPHGE/SDS-PAGE), as described under MATERIALS AND METHODS. The purified hsp40 was blotted onto a PVDF membrane and sequenced directly by a protein sequencer. The amino terminal of human hsp40 is homologous to the *E. coli* heat-shock protein DnaJ (2, 23); 24 of the 48 amino acid residues are identical (50% identity) (Fig. 2). Also, the hsp40 is homologous to *M. tuberculosis* DnaJ (14) (54% identity), yeast SCJ1 (4) (44% identity), yeast Sec63/Npl1 (32) (42% identity), yeast YDJ1 (6) (54% identity) and yeast SIS1 (16) (44% identity). These DnaJ-related proteins are known to be highly homolo-

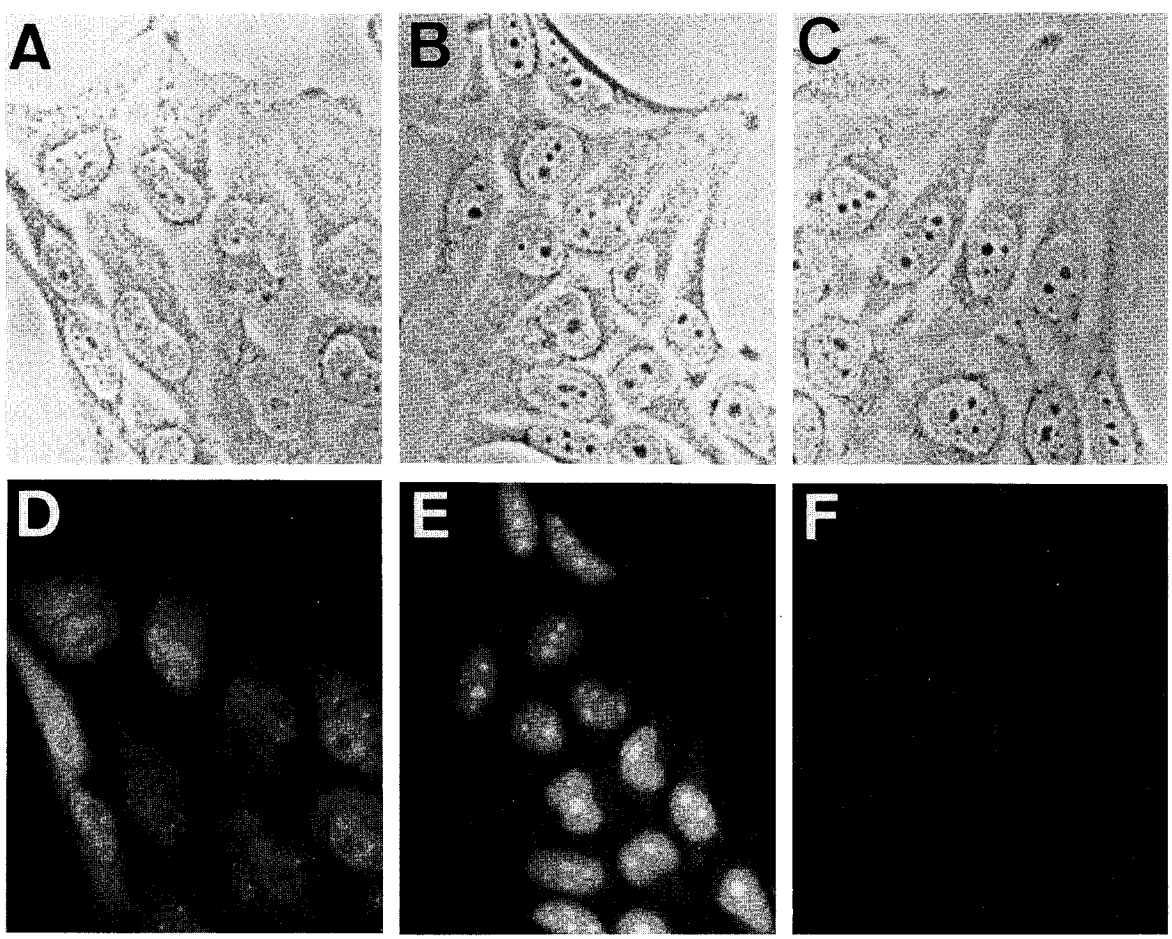

Fig. 5. Intracellular localization of hsp40 in HeLa cells. A and D, cells heated at 45°C for 15 min and incubated at 37°C for 16 h; B and E, cells heated at 45°C for 15 min and incubated at 37°C for 16 h, then heated again at 43°C for 30 mini; C and F, cells treated in the same mamner as in B and E. A, B and C, phase contrast micrographs; D, E and F, fluorescence micrographs of each corresponding field. D and E, cells stained with an anti-hsp40 antibody (1/100 dilution); F, cells stained with a pre-immune serum (1/100 dilution). Exposure time was 1.5 sec for A, B and C and 30 sec for D, E and F.

gous among one another in the amino terminal region. The bacterial DnaJ, as well as mammalian hsp40, is a basic protein with a molecular size of approximately 40-kDa. These results strongly suggest that hsp40 is a mammalian homologue of bacterial DnaJ protein. It is known that eukaryotic hsp90, hsp70 and hsp60 are homologous to bacterial heat-shock proteins HtpG, Dnak and GroEL, respectively (11). To our knowledge, this is the first report of a mammalian homologue to a bacterial DnaJ heat-shock protein.

Specificity of anti-hsp40 antibody. We prepared an anti-hsp40 antibody by immunizing a rabbit with electrophoretically purified human hsp40, as described under MATERIALS AND METHODS. We could not observe an increase of hsp40 in heat-shocked cells by one-dimensional SDS-PAGE gel stained with Coomassie blue (Fig. 3, lanes 2 and 3). In contrast, when cells were labeled with [^{35}S]methionine, a clear induction of hsp40 by heat shock was demonstrated by fluorography of SDS-PAGE gel (Fig. 3, lanes 4 and 5). It was also detected in heat-shocked cells by immunoblotting with an anti-hsp40 antibody (Fig. 3, lanes 8 and 9) that was highly specific for hsp40. Of course, no apparent signal was observed with a pre-immune serum (Fig. 3, lanes 6 and 7). The human hsp40 did not react with anti-SCJ1 antibody (4, a kind gift of Dr. P. Silver of Princeton) as determined by immunoblotting. As positive control, the same samples were probed with an anti-hsp70 antibody (Fig. 3, lanes 10 and 11). This antibody recognized both hsc70 (p73, constitutive form) and hsp70 (p72, inducible form), and an approximately fivefold increase in the amount of hsp70 (p72) was observed in heat-shocked cells as compared with control cells.

Intracellular localization of hsp40 in HeLa cells. The intracellular distribution of hsp40 was investigated by indirect immunofluorescence staining. In non-heat-shocked control cells, diffuse and very faint staining of the cytoplasm and faint staining of nucleoli were ob-

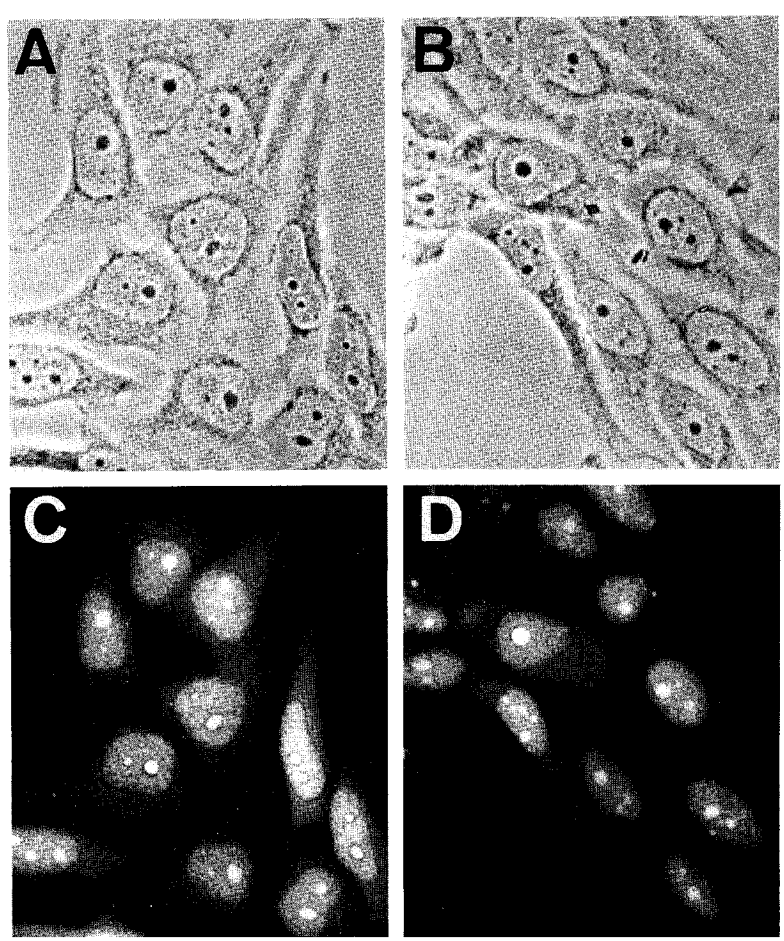

Fig. 6. Effect of cycloheximide on the translocation of hsp40 in HeLa cells. Cells were heated at 45°C for 15 min and incubated at 37°C for 16 h, then heated again at 43°C for 30 min in the absence (A and C) or presence (B and D) of cycloheximide (20 μg/ml). Cycloheximide was added to the culture dish 10 min before the second heating. A and B, phase contrast micrographs; C and D, fluorescence micrographs of each corresponding field. Cells were stained with anti-hsp40 antibody (1/100 dilution). Exposure times were 1.5 sec for A and B and 30 sec for C and D.

served (Figs. 4A and 4D). The faint staining of nucleoli, however, occurred with a pre-immune serum (Figs. 4B and 4E), indicating that faint staining of nucleoli with an anti-hsp40 was nonspecific. In contrast, when cells were heated at 42°C for 2 h, large and small globular structures in the nuclei, which corresponded to phase-dense nucleoli, were brightly stained (Figs. 4C and 4F). This indicates that hsp40 was induced during 42°C heating and translocated in the nuclei and nucleoli. However, the fluorescence signal was relatively weak. Since hsp40 was barely detectable in non-heat-shocked cells (Fig. 1 and Fig. 3), cells were heated at 45°C for 15 min and recovered at 37°C for 16 h in order to increase the amount of hsp40 in cells. The hsp40 was distributed throughout these cells (Figs. 5A and 5D). When the cells were heated again at 43°C for 30 min, the nuclei and especially the nucleoli were brightly stained (Figs. 5B and 5E). The pre-immune serum again showed very faint nucleoli staining in heat-shocked cells (Figs. 5C and 5F) which was similar to that in non-heat-shocked control cells (Figs. 4B and 4E). When cycloheximide (20 μg/ml) was added 10 min before the second heating at 43°C for 30 min, the rapid accumulation of hsp40 in nuclei and nucleoli in pre-heat-shocked cells was not inhibited (Fig. 6). This means that *de novo* synthesis of proteins is not necessary for the accumulation of hsp40 in the nuclei and nucleoli, and that heat shock *per se* induced the translocation of pre-existing hsp40 from cytoplasm into the nuclei.

We and other groups have previously reported that hsp70 in mammalian cells translocates from the cytoplasm into the nuclei, and especially into the nucleoli, by heat shock (25, 29, 37). Here, we reexamined the intracellular localization of hsp70 in HeLa cells using an anti-hsp70 antibody. As shown in Figure 3, both hsc70 (p73) and hsp70 (p72) were synthesized constitutively in

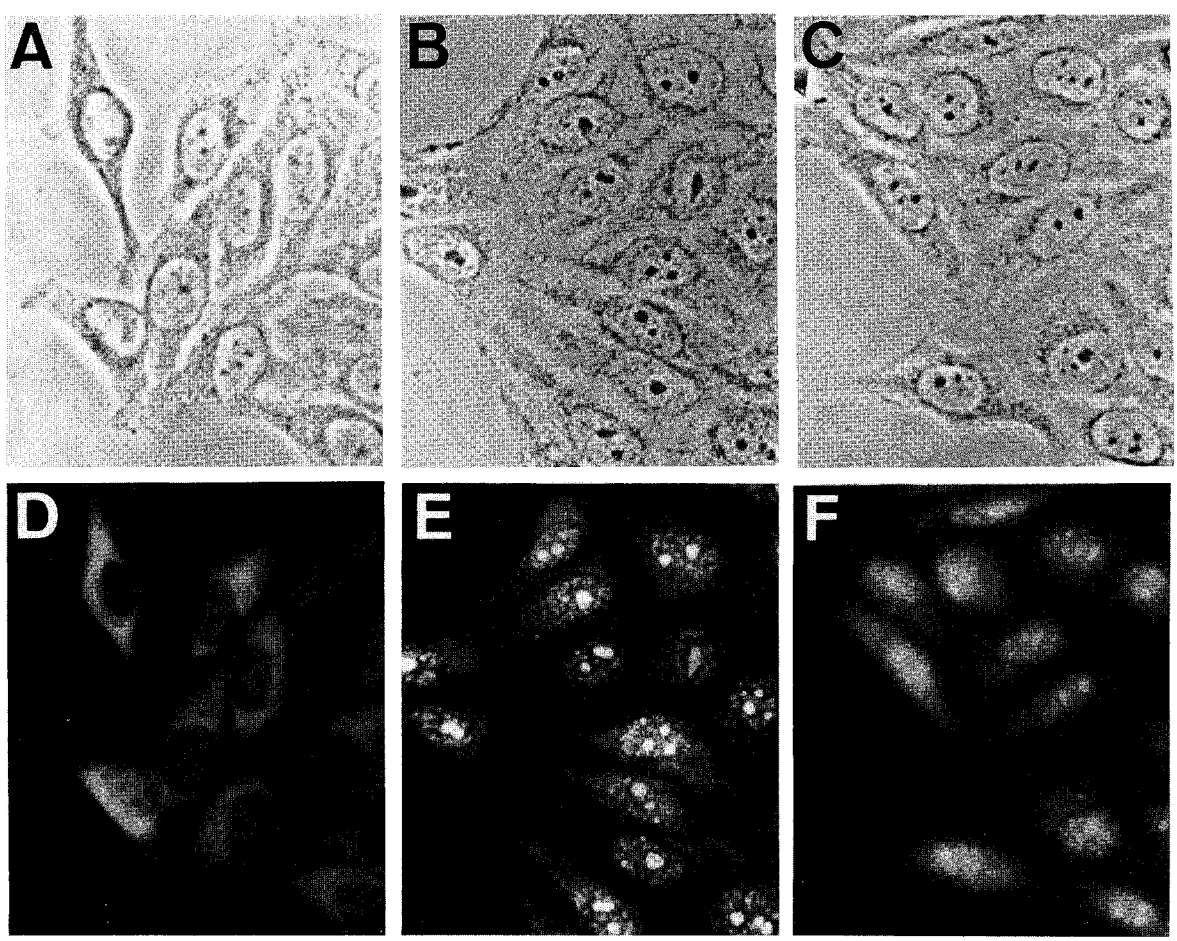

Fig. 7. Intracellular localization of hsp70 in HeLa cells. A and D, non-heat-shocked control cells; B and E, cells heated at 42°C for 2 h; C and F, cells heated at 43°C for 30 min. A, B and C, phase contrast micrographs; D, E and F, fluorescence micrographs of each corresponding field. After heating, cells were fixed and processed for immunofluorescence staining with an anti-hsp70 antibody (1/80 dilution). Exposure time was 1.5 sec for A, B and C and 20 sec for D, E and F. No appreciable fluorescent signal was observed with the pre-immune serum (12, 25, data not shown).

control cells, and the anti-hsp70 antibody recognized both hsc70 and hsp70. In non-heat-shocked control cells, hsc70/hsp70 were localized diffusely in cytoplasm (Figs. 7A and 7D). When cells were heated at 42°C for 2 h or at 43°C for 30 min, hsc70/hsp70 translocates rapidly from the cytoplasm into nuclei and nucleoli (Figs. 7B, 7E, 7C and 7E). The localization of hsc70/hsp70 was very similar to that of hsp40 in heat-shocked cells. These results suggest that hsp40 colocalizes with hsc70 and/or hsp70 in heat-shocked HeLa cells.

DISCUSSION

We here have determined the partial amino acid sequence of the amino terminal of a stress-inducible 40-kDa protein (hsp40) and have shown the intracellular localization of hsp40 in HeLa cells. The hsp40 has homology to the bacterial heat shock protein DnaJ and to the DnaJ-related proteins in yeast such as SCJ1, Sec63/Npl1, YDJ1 and SIS1 (Fig. 2). Both bacterial DnaJ protein and mammalian hsp40 are basic proteins with apparent molecular sizes of 40-kDa (2, 23, 26). These results strongly suggest that hsp40 is a mammalian homologue of bacterial DnaJ protein. Eukaryotic hsp90, hsp70 and hsp60 are known to be homologous to bacterial heat-shock proteins HtpG, DnaK and GroEL, respectively (see ref. 11 for review). To our knowledge, this is the first report of a mammalian homologue of a bacterial DnaJ protein. We are now in the process of cloning hsp40 cDNA in HeLa cells.

It has been shown that bacterial DnaJ protein as well as another heat-shock protein, DnaK (hsp70 homologue), acts together in the dissociation of a protein complex involved in replication of bacteriophage λ (11, 40), and in monomerization of Rep A dimer necessary for the replication of P1 plasmid in the *in vitro* system (39). Also, it has been reported that bacterial heat-shock proteins such as DnaK, DnaJ and GrpE play a role *in vivo* in renaturation of heat-denatured bacteriophage λ repressor protein (10) and that DnaK protein alone can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner (35). However, no direct evidence for the association of DnaK and DnaJ has been demonstrated so far. In eukaryotic cells, it has been proposed that hsp70 family members have a "molecular chaperoning activity" in the translocation of nascent protein from the cytoplasm across the membrane of either the endoplasmic reticulum or mitochondria (3, 7, 9). This implies that hsp70 has an affinity for unfolded proteins. It is also proposed that hsp70 associates with partially denatured (unfolded) proteins induced by heat shock and repairs (folds) them in the nuclei, which have very heat-sensitive structures, then dissociates from repaired proteins using the energy of ATP and returns to cytoplasm (15, 30).

We have demonstrated in the present study that hsp40 translocates rapidly from the cytoplasm to the nuclei, and especially to the nucleoli, upon brief heat shock in HeLa cells (Figs. 4 and 5). This is very similar to the localization of hsc70 (p73)/hsp70 (p72) (Fig. 7). These results suggest that hsp40 colocalizes with hsp70, and that they act together to repair heat-denatured proteins in heat-shocked nuclei and nucleoli by analogy with the function of bacterial DnaK and DnaJ proteins. Although hsp70 is known to have a nucleolar localization sequence (8, 18), the molecular mechanisms of translocation of hsp70 from cytoplasm to nuclei and nucleoli are not known at present. Also, it remains to be elucidated whether hsp40 translocates into nuclei and nucleoli together with hsp70 or not, and whether hsp40 has a nuclear or nucleolar localization sequence.

Recently, four yeast homologues of bacterial DnaJ protein have been identified: SCJ1 (4); Sec63/Npl1 (32); YDJ1 (6); and SIS1 (16). It has been reported that yeast SCJ1 protein is localized in the mitochondrial fraction (4), YDJ1 protein is concentrated in a perinuclear ring as well as in the cytoplasm (6), and that SIS1 protein is found throughout the cells but is concentrated in the nucleus (4). The authors of these studies, however, have not examined the localization of these proteins in heat-shocked yeast. It is suggested that SCJ1 and Sec63 proteins are involved in protein import into mitochondria and the endoplasmic reticulum, respectively (4, 32). The functions of YDJ1 and SIS1 are not known. In our preliminary experiments, most of the hsp40 was localized both in the microsome and cytosol fractions in pre-heat-shocked HeLa cells, as determined by the cell fractionation method. By analogy with the function of SCJ1 and Sec63, it is possible that mammalian hsp40 plays a role in protein import into subcellular organelles across the membrane in non-heat-shocked normal cells.

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REFERENCES

1. Atkinson, B.G., and Walden, D.B., editors (1985). *Chainges in Eukaryotic Gene Expression in Response to Environmental Stress*. Academic Press, New York, 379pp.
2. Bardwell, J.C.A., Tilly, K., Craig, E., King, J., Zylicz, M., and Georgopoulos, C. (1986). The nucleotide sequence of the *Escherichia coli* K12 *dnaJ*$^+$ gene. A gene that encodes a heat shock protein. *J. Biol. Chem.*, **261**: 1782–1785.
3. Beckman, R.P., Mizzen, L.A., and Welch, W.J. (1990). Interaction of hsp70 with newly synthesized proteins: Implications for protein folding and assembly. *Science*, **248**: 850–854.
4. Blumberg, H. and Silver, P.A. (1991). A homologue of the bacterial heat-shock gene *DnaJ* that alters protein sorting in

yeast. *Nature*, **349**: 627–630.
5. Bond, U. and Schlesinger, M.J. (1985). Ubiquitin is a heat shock protein in chicken embryo fibroblasts. *Mol. Cell. Biol.*, **5**: 949–956.
6. Caplan, A.J. and Douglas, M.G. (1991). Characterization of YDJ1: A yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.*, **114**: 609–621.
7. Chirico, W.J., Waters, M.G., and Blobel, G. (1988). 70K heat shock related proteins stimulate protein translocation into microsomes. *Nature*, **332**: 805–810.
8. Dang, C.V. and Lee, W.M.F. (1989). Nuclear and nucleolar targeting sequences of c-*erb*-A, c-*myb*, N-*myc*, p53, HSP70 and HIV *tat* proteins. *J. Biol. Chem.*, **264**: 18019–18023.
9. Deshaies, R.J., Koch, B.D., Werner-Washburne, M., Craig, E.A., and Schekman, R. (1988). A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptide. *Nature*, **332**: 800–805.
10. Gaitanaris, G.A., Papavassiliou, A.G., Rubock, P., Silverstein, S.J., and Gottesman, M.E. (1990). Renaturation of denatured λ repressor requires heat shock proteins. *Cell*, **61**: 1013–1020.
11. Georgopoulos, C., Ang, D., Liberek, K., and Zylicz, M. (1990). Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophage λ growth. In *Stress Proteins in Biology and Medicine* (R.I. Morimoto, A. Tissières, and C. Georgopoulos, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp191–221.
12. Hayashi, Y., Tohnai, I., Kaneda, T., Kobayashi, T., and Ohtsuka, K. (1991). Translocation of hsp-70 and protein synthesis during continuous heating at mild temperatures in HeLa cells. *Radiat. Res.*, **125**: 80–88.
13. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680–685.
14. Lathigra, R.B., Yound, D.B., Sweetser, D., and Young, R.A. (1988). A gene from *Mycobacterium tuberculosis* which is homologous to the DnaJ heat shock protein of *E. coli*. *Nucl. Acid Res.*, **16**: 1636.
15. Lewis, M.J. and Pelham, H.R.B. (1985). Involvement of ATP in the nuclear and nucleolar functions of the 70 kd heat shock protein. *EMBO J.*, **4**: 3137–3143.
16. Luke, M.M., Sutton, A., and Arndt, K.T. (1991). Characterization of SIS1, a *Saccharomyces cerevisiae* homologue of bacterial dnaJ protein. *J. Cell Biol.*, **114**: 623–638.
17. Matsudaira, P. (1987). Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. *J. Biol. Chem.*, **262**: 10035–10038.
18. Milarski, K.L. and Morimoto, R.I. (1989). Mutational analysis of the human hsp70 protein: Distinct domains for nucleolar localization and adenosine triphosphate binding. *J. Cell Biol.*, **109**: 1947–1962.
19. Miyata, Y. and Yahara, I. (1991). Cytoplasmic 8S glucocorticoid receptor binds to actin filaments through the 90-kDa heat shock protein moiety. *J. Biol. Chem.*, **266**: 8779–8783.
20. Nagata, K., Saga, S., and Yamada, K.M. (1986). A major collagen-binding protein of chick embryo fibroblasts is a novel heat shock protein. *J. Cell Biol.*, **103**: 223–229.
21. O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, **250**: 4007–4021.
22. O'Farrell, P.Z., Goodman, H.M., and O'Farrell, P.H. (1977). High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell*, **12**: 1133–1142.
23. Ohki, M., Tamura, F., Nishimura, S., and Uchida, H. (1986). Nucleotide sequence of the *Escherichia coli dnaJ* gene and purification of the gene product. *J. Biol. Chem.*, **261**: 1778–1781.
24. Ohtsuka, K., Tanabe, K., Nakamura, H., and Sato, C. (1986). Possible cytoskeletal association of 69,000- and 68,000-dalton heat shock proteins and structural relations among heat shock proteins in murine mastocytoma cells. *Radiat. Res.*, **108**: 34–42.
25. Ohtsuka, K., Nakamura, H., and Sato, C. (1986). Intracellular distribution of 73000 and 72000 dalton heat shock proteins in HeLa cells. *Int. J. Hyperthermia*, **2**: 267–275.
26. Ohtsuka, K., Masuda, A., Nakai, A., and Nagata, K. (1990). A novel 40-kDa protein induced by heat shock and other stresses in mammalian and avian cells. *Biochem. Biophys. Res. Commun.*, **166**: 642–647.
27. Opperman, H., Levinson, W., and Bishop, J.M. (1981). A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat shock protein. *Proc. Natl. Acad. Sci. USA*, **78**: 1067–1071.
28. Pardue, M.L., Feramisco, J.R., and Lindquist, S., editors (1989). *Stress-Induced Proteins*. Alan R. Liss, Inc., New York, 294pp.
29. Pelham, H.R.B. (1984). Hsp70 accelerate the recovery of nucleolar morphology after heat shock. *EMBO J.*, **3**: 3095–3100.
30. Pelham, H.R.B. (1986). Speculations on the functions of the major heat shock and glucose-regulated proteins. *Cell*, **46**: 959–961.
31. Pratt, W.B., Sanchez, E.R., Bresnik, E.H., Meshinchi, S., Scherrer, L.C., Dalman, F.C., and Welsh, M.J. (1989). Interaction of the glucocorticoid receptor with the *Mr* 90,000 heat shock protein: An evolving model of ligand mediated receptor transformation and translocation. *Cancer Res.*, **49**: 2222s–2229s.
32. Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J., and Silver, P. (1989). A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an *Escherichia coli* heat shock protein. *J. Cell Biol.*, **109**: 2665–2675.
33. Schlesinger, M.J., Ashburner, M., and Tissières, A., editors (1982). *Heat Shock from Bacteria to Man*. Cold Spring Harbor laboratory, Cold Spring Harbor, New York, 440pp.
34. Shibahara, S., Muller, R.M., and Taguchi, H. (1987). Transcriptional control of rat heme oxygenase by heat shock. *J. Biol. Chem.*, **262**: 12889–12892.
35. Skowyra, D., Georgopoulos, C., and Zylicz, M. (1990). The E. coli *dnaK* gene product, the hsp70 homolog, can reactivate heat-inactivated RNA polymerase in an ATP hydrolysis-dependent manner. *Cell*, **62**: 939–944.
36. Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedures and some applications. *Proc. Natl. Acad. Sci. USA*, **76**: 4350–4354.
37. Welch, W.J. and Feramisco, J.R. (1984). Nuclear and nucleolar localization of 72,000-dalton heat shock protein in heat shocked mammalian cells. *J. Biol. Chem.*, **259**: 4501–4513.
38. Welch, W.J. (1990). The mammalian stress response: Cell physiology and biochemistry of stress proteins. In *Stress Proteins in Biology and Medicine* (R.I. Morimoto, A. Tissières, and C. Georgopoulos, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp223–278.
39. Wickner, S., Hoskins, J., and McKenney, K. (1991). Monomerization of RepA dimers by heat shock proteins activates binding to DNA replication origin. *Proc. Natl. Acad. Sci.*

USA, **88**: 7903–7907.

40. Zylicz, M., Ang, D., Liberek, K., and Georgopoulos, C. (1989). Initiation of DNA replication with purified host- and bacteriophage-encoded proteins: the role of the dnaK, dnaJ and grpE heat shock proteins. *EMBO J.*, **8**: 1601–1608.

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