Cotranslocation and Colocalization of hsp40 (DnaJ) with hsp70 (DnaK) in Mammalian Cells

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ABSTRACT. A novel 40-kDa heat-shock protein hsp 40 in mammalian cells has been recently identified to be a homolog of bacterial DnaJ protein. We have previously shown the colocalization of hsc70 (p73, constitutive form) with hsp40 in the nucleoli of heat-shocked HeLa cells. In this report we further investigated intracellular translocation and localization of hsp40 and hsp70 (both constitutive p73 and inducible p72) in several mammalian cells. Translocation kinetics of hsp40 during heating at mild temperature were almost the same as those of hsp70 in HeLa cells. Hsp40 colocalized not only with hsc70 (p73) but also with hsp70 (p72) in heat-shocked HeLa (human), HA-1 (Chinese hamster) and NRK (rat) cells. Direct interaction of hsp40 with hsp70 (p73 and/or p72) was observed in all cells tested by immunoprecipitation methods. Also, treatments of cells with cytoskeleton-acting drugs such as cytochalasin E, colchicine and taxol had no effect on the heat-induced translocation of hsc70 (p73) and hsp40 in NRK cells. These results strongly suggest that hsp40 and hsp70 (p73/p72) form a complex in the cytoplasm at normal temperature, translocate together and colocalize in the nuclei and nucleoli upon heat-shock, and that they may function cooperatively to repair (refold) denatured proteins under stress conditions.

In response to elevated temperature and other environmental stresses, living cells induce a set of proteins called heat-shock (stress) proteins (hsps) (45). Nearly twenty hsps have been identified in mammalian cells so far. Among them, hsp70 (70-kDa hsp) is one of the most abundant in cells, is highly conserved throughout evolution, and therefore has been extensively investigated in many laboratories (29, 51). The hsp70 family in mammalian cells is known to consist of at least four members, grp78 [localized in endoplasmic reticulum (ER)], grp75 (mitochondria), hsc70 (p73, cytoplasm and nucleus) and hsp70 (p72, cytoplasm and nucleus) (43, 51). While grp78, grp75 and hsc70 (p73) are constitutively expressed even in the absence of stress, hsp70 (p72) is barely detectable, if at all, at normal growth temperature and remarkably induced by heat shock or other stresses. In human cells, however, hsp70 (p72) is expressed constitutively and induced dramatically by stresses. In this paper we refer to both hsc70 (p73, constitutive form) and hsp70 (p72, inducible form) by the general term hsp70.

It has been shown that hsp70 has a molecular chaperone activity; it has an affinity for the hydrophobic region of partially denatured (unfolded) proteins induced by stresses and that of nascent (not completely folded) polypeptides emerging from translating ribosomes, and assist their correct folding using the energy of ATP (1, 8, 13, 16, 33, 41, 43, 49). Also, hsp70 is considered to maintain mitochondria- or ER-targeted proteins in a transport-competent (partially unfolded) state, so as to facilitate their entry into each organelle (7, 12).

Another notable feature of hsp70 in mammalian cells is the drastic changes in its localization upon heat shock, that is, the hsp70 usually present in cytoplasm translocates and accumulates in the nuclei, especially in the nucleoli during heat shock, and gradually returns again to the cytoplasm during the recovery period (36, 38, 42, 52). Mammalian hsp70 is known to have a nucleolar localization sequence (11, 32). It is considered that while the nucleolar localization sequence of hsp70 is buried inside the protein at normal temperature, the sequence is exposed outside at high temperature, and then hsp70 is translocated into the nucleoli. However, the precise mechanism of heat-induced nucleolar translocation of hsp70 is not clear at present.

Ohtsuka et al. (37) have recently found a novel 40-kDa heat-shock protein, hsp40, in mammalian and avian cells. Amino acid sequencing of N-terminal and
cDNA cloning of human hsp40 revealed that it is a mammalian homolog of bacterial DnaJ heat-shock protein (18, 34). Moreover, we have shown that hsp40 translocated into the nuclei and nucleoli upon heat shock as well as hsp70, colocalizes with hsc70 (p73) in heat-shocked HeLa cells, and that the kinetics of nucleolar accumulation and subsequent return to the cytoplasm of hsp40 are very similar to those of hsp70 (19). We had previously thought that hsp40 has a nucleolar localization sequence like hsp70. However, hsp40 does not seem to have the sequence judging from the amino acid sequence deduced from the nucleotide sequence of hsp40 cDNA (34). We therefore hypothesized that hsp40 and hsp70 form a complex in the cytoplasm and translocate together into the nucleoli upon heat shock.

In this report we obtained evidence of direct interaction between hsp40 and hsp70 (p73 and/or p72) and further investigated intracellular translocation and localization of hsp40 and hsp70 in several mammalian cells.

MATERIALS AND METHODS

Antibodies and chemicals. Five different antibodies were used in this study; rabbit anti-hsp40 polyclonal antibody (18), rat anti-hsc70 (p73) monoclonal antibody (1B5) (19, Ohtsuka et al., manuscript submitted for publication), mouse anti-hsp70 (p72) monoclonal antibody (RPN 1197, Amersham) and two different kinds of rabbit anti-hsp70 (p73 and p72) polyclonal antibodies (K01 and K04) (35). Horseradish peroxidase-conjugated goat anti-rabbit IgG, anti-rat IgG and ant.mouse IgG antibodies were purchased from Zymed (San Francisco, CA). FITC-conjugated goat anti-rabbit IgG, anti-rat IgG and anti-mouse IgG antibodies and rhodamine-conjugated goat anti-rabbit IgG antibody were from Cappel (West Chester, PA). Zymosan was from Zymed and apyrase from Sigma (St. Louis, MO). Cleavable cross linker, DSP [3,3’-dithiobis-(succinimidyl propionate)] and cytoskeleton-acting drugs (cytochalasin E, colchicine and taxol) were from Wako Pure Chemical (Osaka, Japan). Other reagents were from Wako and Nakalai Tesque (Kyoto, Japan), and were of the highest purity available from these companies.

Cells, cell culture, heating and chemical treatments. In this study, we used HeLa cells, HA-1 Chinese hamster fibroblasts (a gift of Dr. Andrei Laszlo, Washington University, St. Louis, MO), NRK (normal rat kidney) cells and 39-1 cells. NRK and 39-1 cells were gifts of Dr. Akira Masuda, Aichi Cancer Center Research Institute. The 39-1 cells were originally isolated as non-responder cells to growth factor-induced transformation by treating parent NRK cells with a chemical mutagen, MNNG (M-nitro-chloroacetamide (31). The 39-1 cells were found to be deficient in induction of hsp70 (p72) (44). These cells were grown in Dulbecco’s modified Eagle’s minimal essential medium (Nissui, Tokyo, Japan) supplemented with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS).

Cells at subconfluence in 100 mm or 35 mm culture dishes (Corning, NY) were heated by immersing the dishes sealed with Parafilm into a water bath, the temperature of which was controlled within ±0.1°C. Since the level of hsp70 (p72) and hsp40 expressed in non-heat-shocked control cells, especially in rodent cells, were too low to be detected by immunological techniques, cells were heated at 45°C for 10-15 min, then recovered at 37°C for 16 h in order to increase cellular amount of hsp40 as well as hsp70 (p72). We used these hsp-enriched cells in most of the present study. Cytochalasin E (final 1 μg/ml) and colchicine (final 20 μM) were added to the culture medium 2 h before, and taxol (final 1 μM) was added 16 h before test heating, and cells were heated in the presence of each drug.

Gel electrophoresis and immunoblotting. Cells were lysed in sodium dodecyl sulfate (SDS)-sample buffer (24) and boiled for 5 min. An aliquot (10 μl) of each sample was precipitated with 10% trichloroacetic acid and the precipitate was dissolved in 50 μl of 0.5 M NaOH followed by protein measurement with Pierce protein assay kit (Pierce, Rockford, IL). Equal amounts of each sample (3-5 μg/lane) were separated by one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% acrylamide gel. Proteins in the gel were then transferred to a nitrocellulose membrane (Schleicher & Schuell, Germany), which was 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 membrane was incubated first with the antibody described above and then with the second antibody (horseradish peroxidase-conjugated goat anti-rabbit IgG, anti-rat IgG or anti-mouse IgG). Peroxidase activity was detected using 4-chloro-1-naphthol as a substrate.

Immunofluorescence. For the analysis of the intracellular localization of hsp40 and hsp70, cells were grown on glass coverslips (9 mm × 9 mm) in 35 mm dishes. After various treatments, cells were washed with cold phosphate-buffered saline (PBS), and fixed in 100% methanol at −20°C for 10 min. The cells were incubated with 10% normal goat serum (MBL, Nagoya, Japan) in PBS for 1 h to inhibit nonspecific binding, and subjected to immunofluorescence staining. The anti-hsp40 antibody, anti-hsp70 antibody (KO4) and 1B5 anti-hsc70 (p73) monoclonal antibody were used as the first antibody, and FITC-conjugated goat anti-rabbit IgG or anti-rat IgG as the second antibody. For double immunofluorescence staining, the first antibody was a mixture of anti-hsp40 and 1B5 anti-hsc70 (p73) antibodies or a mixture of anti-hsp40 and anti-hsp70 (p72) antibodies. The second antibody was a mixture of rhodamine-conjugated goat-rabbit IgG and FITC-conjugated goat anti-rat IgG, or a mixture of rhodamine-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG. Cells were photographed through a Fluorophotomicroscope microscope (Nikon, Tokyo, Japan), using Tri-X Pan film (ASA 400, Kodak) for black-and-white prints and Kodacolor film (ASA 400, Kodak) for color prints.

Immunoprecipitation. For immunoprecipitation, cells at
subconfluences in 100 mm dishes were washed with cold PBS and harvested by treatment with 0.05% trypsin and 0.02% EDTA. The same number of cells was used in each experiment. After centrifugation, cells were resuspended in 1 ml of PBS containing 2 mM DSP [cleavable cross linker, diluted from 100 mM in dimethyl sulfoxide (DMSO)] and incubated for 30 min at room temperature with gentle shaking. The reaction of DSP was stopped by the addition of glycine (final 2 mM). Control cells were similarly treated with solvent alone (2% DMSO) without DSP. Cells were washed again with PBS and pelleted by centrifugation. They were then lysed with 500 µl of RIPA buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Triton X-100, 1% Na-deoxycholate) containing 1 µg/ml leupeptin, 1 µg/ml pepstatin and 1 mM PMSF. Also, 5 units of apyrase was added to the lysate to deplete endogenous ATP. The cell lysates were sonicated and clarified by centrifugation. The supernatants were preabsorbed with 50 µl of insoluble Protein A (Zysorbin, non-viable Staphylococcus aureus), which was previously boiled for 15 min in SDS-sample buffer and then washed with RIPA buffer. After 10 min incubation, the supernatants were clarified. Preabsorption was repeated once again. The supernatants were then incubated with 100 µl of anti-hsp70 antibody (KO4 for HeLa cells and KO1 for HA-1, NRK and 39-1 cells) or 100 µl of anti-hsp40 antibody for 60 min at 4°C. Zysorbin (100 µl) was then added and the incubation continued for 30 min. The immunoprecipitates were collected by centrifugation and washed five times with RIPA buffer. The precipitated proteins were released from the zysorbin by the addition of SDS-sample buffer followed by boiling for 5 min. The immunoprecipitates were then analyzed by SDS-PAGE followed by immunoblotting with anti-hsp70 antibody (KO4 for HeLa cells and KO1 for HA-1, NRK and 39-1 cells) or anti-hsp40 antibody.

RESULTS

Specificity of antibodies. We first examined the specificity of antibodies in four mammalian cells by immunoblotting. The anti-hsp40 antibody reacted specifically with HeLa, HA-1 and NRK cells; hsp40 was faintly detected in non-heat-shocked control cells and induced remarkably by heat shock (Fig. 1A). The anti-hsp70 (p73) monoclonal antibody (1B5) was highly specific for constitutive hsc70 (p73) in all cells tested, and the amount of hsc70 (p73) apparently did not change by heat shock (Fig. 1B). Mouse monoclonal antibody against hsp70 (p72) was also highly specific for inducible hsp70 (p72) (Fig. 1C). In HeLa cells, hsp70 (p72) was expressed in control cells and induced dramatically by heat shock (lanes 1 and 2 in Fig. 1C). On the other hand, hsp70 (p72) in rodent cells (HA-1 and NRK) was detected only after heat shock (lanes 4 and 6 in Fig. 1C). As previously reported, 39-1 cells were defective in hsp70 (p72)-induction (44). This was further supported in the present experiment (lanes 7 and 8 in Fig. 1C). Also, 39-1 cells seemed to be deficient in hsp40 induction (lanes 7 and 8 in Fig. 1A), because the level of hsp40 induction in 39-1 cells was lower than in parent NRK cells (compare lane 6 with lane 8 in Fig. 1A). Two anti-hsp70 polyclonal antibodies (KO1 and KO4) could recognize both hsc70 (p73) and hsp70 (p72), but their specificity differed in HeLa and rodent cells. KO1 antibody was more specific for hsp70 (p72) than for hsc70 (p73) in HeLa cells (lanes 1 and 2 in Fig. 1D), but similarly specific for both hsc70 (p73) and hsp70 (p72) in rodent cells (lanes 4 and 6 in Fig. 1D). While KO4 antibody was equally specific for both hsc70 (p73) and hsp70 (p72) in HeLa cells (lanes 1 and 2 in Fig. 1E), KO4 was more specific for hsc70 (p73) than for hsp70 (p72) in rodent cells (lanes 4 and 6 in Fig. 1E). Deficiency of hsp70 (p72) induction in 39-1 cells was again demonstrated by these polyclonal antibodies (lane 8 in Figs. 1D and 1E).

Since expression of hsp40 and hsp70 (p72) was very low or not detected in non-heat-shocked control cells, it was difficult to detect these hsps by immunofluorescence and immunoprecipitation. Therefore, the cells were heated at 45°C for 10-15 min and incubated at 37°C for

![Image of immunoblot analysis of the specificity of antibodies used in this study. Non-heat-shocked control cells (lanes 1, 3, 5 and 7) and cells heated at 45°C for 15 min, then recovered at 37°C for 16 h (lanes 2, 4, 6 and 8), were lysed in SDS-sample buffer and subjected to SDS-PAGE followed by immunoblotting with anti-hsp70 antibody (KO4 for HeLa cells and KO1 for HA-1, NRK and 39-1 cells) or anti-hsp40 antibody.](image-url)
16 h to increase the cellular amount of these hsp70s. In the following experiments, we used the hsp-enriched cells.

**Cotranslocation of hsp40 with hsp70.** As previously reported, when HeLa and NRK cells were continuously heated at 41°C, hsp70 once translocated into the nuclei and the nucleoli and returned again to the cytoplasm even during heating (20, 30). Also, in hsp-enriched cells, this entry and return of hsp70 was observed heated at 41°C, hsp70 once translocated into the nuclei and the nucleoli and returned again to the cytoplasm even during heating (20, 30). Also, in hsp-enriched cells, this entry and return of hsp70 was observed

![Image](https://via.placeholder.com/150)

**Fig. 2.** Translocation of hsp70 and hsp40 during continuous heating at 41.5°C in hsp-enriched HeLa cells. Hsp-enriched cells (previously heated at 45°C for 15 min then recovered at 37°C for 16 h) were heated at 41.5°C for 30 min (B and G), 1 h (C and H), 3 h (D and I) and 4 h (E and J), then fixed and processed for immunofluorescence staining with KO4 anti-hsp70 antibody (A–E) or anti-hsp40 antibody (F–J). Staining of hsp70 and hsp40 was performed independently. (A) and (F) indicate control cells heated at 45°C for 15 min then recovered at 37°C for 16 h. Cells with brightly stained nucleoli were counted as positive cells and the percentages of the positive cells were plotted against heating time at 41.5°C (lower panel). Each point represents at least 400 cells, a total of three independent experiments.
Colocalization of hsp40 with hsc70 (p73) and hsp70 (p72) in heat-shocked mammalian cells. Hsp-enriched cells were heated at 43°C for 30 min and recovered at 37°C for 3 h, then fixed and processed for double immunofluorescence staining as described under MATERIALS AND METHODS. (A–D) HeLa cells; (E–H) HA-1 cells; (I–L) NRK cells; (M–P) 39-1 cells. The hsc70 (p73)-specific fluorescence micrographs (FITC) are shown in (A), (E), (I) and (M), and the corresponding hsp40-specific fluorescence micrographs (rhodamine) in (B), (F), (J) and (N). The hsp70 (p72)-specific fluorescence micrographs are shown in (C), (G), (K) and (O), and the corresponding hsp40-specific fluorescence micrographs in (D), (H), (L) and (P).
during continuous heating at 42°C. We here examined whether the translocation pattern of hsp40 is the same as that of hsp70 during continuous heating. As shown in Fig. 2, not only hsp70 (probed with KO4 antibody) but also hsp40 once translocated into the nuclei and the nucleoli and returned to the cytoplasm during continuous heating, in the present case at 41.5°C, in hsp-enriched HeLa cells. Translocation kinetics of hsp70 and hsp40 were determined by counting cells with brightly stained nucleoli as positive cells, and the percentages of the positive cells were plotted against heating time. Translocation kinetics of hsp40 were very similar to those of hsp70 (Fig. 2, lower panel). In a previous study, we showed in HeLa cells that the kinetics of the accumulation in the nucleoli during heat shock (42°C for 2 h or 43°C for 30 min) and subsequent return to the cytoplasm during recovery period at 37°C of both hsp40 and hsp70 were very similar (19). These results strongly suggest that hsp40 and hsp70 translocate together upon heat shock.

Colocalization of hsp40 with both hsc70 (p73) and hsp70 (p72). We have previously reported the colocalization of hsp40 with hsc70 (p73) in heat-shocked HeLa cells (19). Here, we further examined whether hsp40 colocalizes not only with hsc70 (p73) but also with hsp70 (p72) in heat-shocked mammalian cells. Hsp-enriched cell were heated again at 43°C for 30 min to accumulate hsps in the nucleoli, then incubated at 37°C for 2–3 h. Under this treatment protocol, nucleoli of some cells were stained by both anti-hsp40 and anti-hsc70 (p73) antibodies, but nucleoli of other cells were not stained by these antibodies in HeLa (Figs. 3A and 3B), HA-1 (Figs. 3E and 3F) and NRK (Figs. 3I and 3J) cells, implying the colocalization of hsp40 with hsc70 (p73). Also, colocalization of hsp40 with hsp70 (p72) was observed in HeLa (Figs. 3C and 3D), HA-1 (Figs. 3G and 3H) and NRK (Figs. 3K and 3L) cells. Since induction of hsp40 and hsp70 (p72) was deficient in 39-1 cells (see Fig. 1), it was difficult to observe the colocalization of hsp40 with hsc70 (p73) and hsp70 (p72) (Figs. 3M, 3N, 3O and 3P).

Interaction of hsp40 with hsp70. In hsp-enriched cells (heated at 45°C for 15 min, then recovered at 37°C for 16 h), the majority (80–90%) of hsp40 and hsp70 (p73/p72) were localized in the cytoplasm as determined by cell fractionation methods (39). Physical interactions between hsp40 and hsp70 (p73/p72) in the hsp-enriched cells were examined by immunoprecipitation as described under MATERIALS AND METHODS. As shown in Fig. 4, anti-hsp70 antibody (KO4 for HeLa cells and KO1 for HA-1, NRK and 39-1 cells) could precipitate hsp40 (lane 2 in Fig. 4), and anti-hsp40 could precipitate mainly hsc70 (p73) (lane 3 in Fig. 4). Hsp70 (p72) in HeLa cells was also precipitated with anti-hsp40 antibody, but it was too faint to detect. However, when the peroxidase reaction was performed with an enhanced chemiluminescence system, hsp70 (p72) precipitated with anti-hsp40 antibody was detected in HeLa cells but not in other rodent cells (data not shown). Addition of ATP to the cell lysate resulted in the reduction of coimmunoprecipitation of hsp40 with hsp70 (data not shown). The coimmunoprecipitation of hsp40 with hsp70 (p73 and/or p72) was observed more prominently when cells were treated with cross linker DSP before
Fig. 5. Effects of cytoskeleton-acting drugs on heat-induced nucleolar translocation of hsc70 (p73) and hsp40 in NRK cells. Hsp-enriched NRK cells were incubated in the absence of drug (A–D) and in the presence of cytochalasin E for 2 h (E–H), colchicine for 2 h (I–L) and taxol for 16 h (M–P). Cells were then heated at 42°C for 2 h in the absence or presence of the drug, fixed and processed for immunofluorescence staining. The phase-contrast micrographs are shown in (A), (E), (I) and (M), and hsc70 (p73)-specific fluorescence micrographs (probed with 1B5 monoclonal antibody) of each corresponding field in (B), (F), (J) and (N). The phase-contrast micrographs are shown in (C), (G), (K) and (O), and hsp40-specific fluorescence micrographs (probed with anti-hsp40 antibody) of each corresponding field in (D), (H), (L) and (P). Bar, 10 μm.
We demonstrated in this report that hsp40 could physically interact with hsp70 in mammalian cells. We here investigated the translocation of hsp70 (p72) induced by heat shock in rat embryo fibroblasts. We have previously shown that disruption of cytoskeleton with cytochalasin E or colcemid had no effect on the translocation of hsp70 (p72) induced by heat shock in rat embryo fibroblasts. We here investigated the effect of cytoskeleton-acting drugs, such as cytochalasin E, colchicine and taxol, on the heat-induced translocation of hsp40 and hsc70 (p73) in NRK cells. Disruption of cytoskeletons (actin stress fiber with cytochalasin E or microtubules with colchicine) and stabilization of microtubules with taxol were checked by fluorescence staining of actin with rhodamine phalloidin and microtubules with anti-tubulin antibody (data not shown). As shown in Fig. 5, treatments with these drugs failed to inhibit the heat-induced translocation of both hsp40 and hsc70 (p73). These results are consistent with the idea of cotranslocation of hsp40 and hsp70 upon heat shock.

**DISCUSSION**

We demonstrated in this report that hsp40 could physically interact with hsp70 (p73 and/or p72) and colocalize not only with hsc70 (p73) but also with hsp70 (p72) in mammalian cells (Figs. 3 and 4). Also, we showed that the translocation kinetics of hsp40 during continuous heating at mild temperature were very similar to those of hsp70 (Fig. 2). These results suggest the cotranslocation of hsp40 and hsp70 upon heat shock.

Bacterial heat-shock protein DnaK (hsp70) is known to act together with DnaJ (hsp40) and GrpE in managing unfolded proteins such as nascent polypeptides being synthesized on the ribosomes and stress-damaged proteins (14, 15, 17, 25). Both DnaK and DnaJ bind to a target (unfolded) protein and mediate its correct folding using the energy of ATP, and GrpE facilitates release of the folded protein from DnaK and DnaJ (21, 27, 46, 55). Also, it has been shown that DnaJ and GrpE jointly stimulate ATPase activity of DnaK (28). Since affinity of DnaK for DnaJ is very weak, it is usually difficult to detect direct interaction of DnaK with DnaJ. Under some experimental conditions, however, DnaK-DnaJ complex has been observed in the in vitro system using purified proteins (22, 40). More recently, Frydman et al. (13) have shown that hsc70 (p73) in rabbit reticulocyte lysate is immunoprecipitated with anti-hsp40 antibody in the presence of translating ribosomes. We have recently shown that interaction of hsp70 with hsp40 in human oral squamous cell carcinoma cell line is dependent on the presence of ATP or unfolded protein (50). The results of coimmunoprecipitation of hsp40 with hsp70 in the present study suggest that at least a portion of these two hsps interacts directly. However, the possibility that hsp70 indirectly interacts with hsp40 through some target proteins cannot be excluded.

Exposure of HeLa cells to heat-shock is known to cause the translocation of more than ten protein, including hsp70, hsp40 and actin from the cytoplasm into the nuclei and nucleoli (23, 26, 54). Although hsp70 is known to have a nucleolar localization sequence, the mechanisms of heat-induced nuclear or nucleolar translocation of these proteins are not known at present (11, 32). Ohtsuka (34) recently isolated a human hsp40 cDNA and determined its nucleotide sequence. The amino acid sequence of hsp40 deduced from the nucleotide sequence does not seem to have a nucleolar localization consensus sequence such as found in hsp70. Therefore, the results of coimmunoprecipitation of hsp40 with hsp70 strongly suggest that these two hsps form a complex in the cytoplasm and translocate together into the nuclei and nucleoli upon heat shock. The similarity of the translocation kinetics of hsp40 and hsp70 (Fig. 2 and ref. 19) supports the idea of cotranslocation of these hsps.

Several DnaJ homologs (e.g., YDJ1, SEC63, SCJ1, SIS1 and MDJ1) have recently been identified in yeast by genetic screening (4, 10, 48). It has been shown that YDJ1p in cytoplasm and SEC63p in ER interact functionally with SSA1p (cytoplasmic hsp70) and BiP (hsp70 in ER), respectively (9, 47). YDJ1p is able to stimulate ATPase activity of SSA1p (9). Both YDJ1p and SSA1p are considered to be involved in protein import into cellular organelles (3, 12). Also both SEC63p and BiP have been shown to be necessary for protein import into ER (2). Another mammalian DnaJ homolog, HSJ1p, was also found to functionally interact with cytoplasmic hsp70 (5, 6). Both hsc70 (p73) and hsp40 in rabbit reticulocyte lysate as well as bacterial DnaK and DnaJ reportedly bind to the nascent polypeptide chains emerging from translating ribosomes and function as molecular chaperones mediating their correct folding (13). The overall findings indicate that the hsp70 (DnaK) / hsp40 (DnaJ) chaperone system (or chaperone machine) is ubiquitous in living cells.

Therefore, the data of colocalization of hsp40 with hsp70 suggest that these two hsps act cooperatively to repair (refold) denatured proteins under stress conditions. Purification of hsp40 and hsp70, which is now under way in our laboratory, is necessary for elucidation of the precise mechanisms of their molecular chaperone activity in the in vitro system.

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hsp40 (DnaJ) and hsp70 (DnaK) in Mammalian Cells

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M. Yamane et al.

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