Human DnaJ Homologs dj2 and dj3, and bag-1 Are Positive Cochaperones of hsc70*

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Received for publication, March 10, 2000, and in revised form April 27, 2000 Published, JBC Papers in Press, May 16, 2000, DOI 10.1074/jbc.M002021200

DnaJ is an essential cochaperone of mammalian heat shock cognate 70 (hsc70) protein. We previously found that dj2 (HSDJ/hdj-2/rdj1), rather than dj1 (hsp40/hdj-1), is a partner DnaJ for the hsc70-based chaperone system. Here, we compared the distribution of dj1, dj2, and the newly found dj3 (cpr3/DNJ3/HIRIP4/rdj2) in cultured cells. Both dj2 as well as dj3 were farnesylated and were ubiquitously expressed. In immunocytochemical and subfractionation studies, these two proteins colocalized with hsc70 under normal conditions. However, dj1 and hsc70 apparently colocalized in the nucleioli after heat shock. Simultaneous depletion of dj2 and dj3 from rabbit reticulocyte lysate markedly reduced mitochondrial import of pre-ornithine transcarbamylase and refolding of guanidine-denatured luciferase. Re-association of either dj2 or dj3 led to recovery of these reactions. In a reconstituted system, both hsc70-dj2 and hsc70-dj3 were effective in protein refolding. Anti-apoptotic protein bag-1 further stimulated ATP hydrolysis and protein refolding by both pairs. Thus, dj2 and dj3 are the partner DnaJs of hsc70 within the cell, functionally similar and much more efficient than dj1, and bag-1 is a positive cochaperone of the hsc70-dj2 and hsc70-dj3 systems.

The heat-shock cognate protein 70 (hsc70)1 is a major molecular chaperone present in the mammalian cytosol and mediates various cellular processes, including protein folding and traffic. Among numerous cochaperones for hsc70 (1), an essential group is the DnaJ family (2). The mammalian dj2 (HSDJ/hdj-2/rdj1) (3–6) and dj3 (cpr3/DNJ3/HIRIP4/rdj2) (5, 7) contain all the domains found in bacterial DnaJ. These orthodox members of the DnaJ subfamily have J-, G/F-, and zinc-finger domains. The zinc-finger domain of bacterial DnaJ coordinates two zinc atoms and is important for binding to denatured protein substrate (8). dj2 and dj3 also have the “CAAX” prenylation motif at their COOH termini, and we showed that dj2 is farnesylated (9). The protein sequence of dj3 is 55% identical to the dj2 sequence. dj3, dj2, and hsc70 are all highly conserved among mammals (99% identity between human and rat proteins).

On the other hand, dj1 (hsp40/hdj-1) (10) is a noncanonical member of DnaJ, which lacks the zinc-finger domain. Because dj1 was identified earlier among mammalian Dnas, many studies of the hsc70 chaperone system were done on dj1. One proposed function for the hsc70-dj1 system is its involvement in folding of nascent polypeptides (11). The *in vitro* protein refolding mediated by the hsc70-dj1 system was also reported (12).

We previously developed a system of depletion of hsc70, dj1, and dj2 from reticulocyte lysates and of their re-addition, to assess the roles of these chaperones (13). We found that the hsc70-dj2 chaperone pair is required for mitochondrial import of pre-ornithine transcarbamylase (pOTC) and refolding of chemically denatured luciferase. Unexpectedly, dj1 depletion had little effect on luciferase refolding. A reconstituted system using purified hsc70 and dj2 at physiological concentrations, but not the hsc70-dj1 pair, facilitated luciferase refolding.

In the present study, we investigated properties and roles of the newly found dj3 in comparison with those of dj1 and dj2. dj3 as well as dj2 was farnesylated. The pattern of dj1, dj2, and dj3 expression in various tissues was tested at the protein and mRNA levels, as well as the inducibility upon heat shock in cultured cells. Immunofluorescence and organelle fractionation were used to examine the intracellular localization of these Dnas before and after heat-shock. All these data indicate that dj2 and dj3 are functional partners of hsc70. In chaperone depletion experiments from reticulocyte lysates, dj2 and dj3 were equally effective in facilitating protein import into mitochondria and in luciferase refolding. In reconstitution experiments with purified chaperones, hsc70-dj2 and hsc70-dj3 pairs, but not the hsc70-dj1 pair, were effective in protein refolding. Refolding was further accelerated by bag-1, an anti-apoptotic and grpE-like protein (14–16). Effects of dj1–3 and bag-1 proteins on ATPase activity of hsc70 were also tested. The status of the hsc70-based chaperone cycle on combinations of these cochaperones was discussed. Taken together, hsc70-dj2 and hsc70-dj3 pairs possess much stronger chaperone activities than do the hsc70-dj1 pair in mitochondrial protein import and protein folding under normal conditions.

**EXPERIMENTAL PROCEDURES**

Chaperone Proteins—Human dj3 cDNA was amplified by polymerase chain reaction (PCR) from human liver cDNA and cloned into pGEM-T (Promega) to generate pGEMT-hdj3. The PCR oligonucleotides were 5'-GCCGCGCCGCGCATGCTAAGCGTCG-3' (sense) and 5'-TCCCATCGTGCAATTTGTTTGCGAGA-3' (antisense), giving a fragment of 1353 base pairs. The nucleotide sequence was verified by sequencing. Nucleotide sequence coding for the hexahistidine tag was inserted into the Apol-I-NcoI site of pGEMT-hdj3, which is located just prior to the initial methionine of dj3, using a pair of oligonucleotides, 5'-CGGATCCATCAGCATCATCATCATCATC-3' and 5'-CATGGAATCAGTAT-3'. A mutated DNA fragment was cut by *Pst*I and *Bam*HII digestion, and inserted into the pVL1393...
baculovirus transfer vector. Human H$_2$dj and H$_3$dj3 were expressed by the baculovirus system in partially farnesylated forms and purified as described (13). Human dj3 was also expressed as Histag and Stag fusion protein in Escherichia coli. The coding sequence for dj3 was inserted into pET30a (Novagen) to generate pET30-hdj3. BL21(DE3) cells were transformed with pET30-hdj3 plasmid, and Histagged- and Stagged-dj3 protein was induced with 1 mm isopropyl-1-thio-β-D-galactopyranoside. The fusion dj3 protein was purified using a Ni$^{2+}$-NTA-Sepharose column. The fraction containing Histagged- and Stagged-dj3 protein was further purified by preparative SDS-polyacrylamide gel electrophoresis (PAGE). The purified protein was injected into rabbits to obtain antiserum. Human H$_3$dj and rat hsc70 were prepared as described (13). Human baculovirus-infected insect cells were transformed with pET30-hdj3 plasmid, and Histagged- and Stagged-dj3 protein was induced with 1 mm isopropyl-1-thio-β-D-galactopyranoside. The fusion dj3 protein was purified using a Ni$^{2+}$-NTA-Sepharose column. The fraction containing Histagged- and Stagged-dj3 protein was further purified by preparative SDS-polyacrylamide gel electrophoresis (PAGE). The purified protein was injected into rabbits to obtain antiserum. Human H$_3$dj and rat hsc70 were prepared as described (13).

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RESULTS

Intracellular Concentration of dj3 Protein—To analyze the nature of dj3, we expressed and purified histidine-tagged hu-

FIG. 1. Intracellular concentrations of DnaJs. A, purified chaperones (0.5 μg each) were analyzed using SDS-PAGE followed by Coomassie Brilliant Blue staining. Lane 1, rat hsc70; lane 2, E. coli-expressed human H$_3$dj1; lane 3, baculovirus-expressed human H$_3$dj2; lane 4, baculovirus-expressed human H$_3$dj3; lane 5, E. coli-expressed human H$_3$bag-1, B, immunoblot analysis of dj1 (a), dj2 (b and c), and dj3 (d) in rabbit reticulocyte lysate (lane 1, 20 μg of protein), HeLa-S3 cell extract (lane 2, 2 μg), CHO-K1 cell extract (lane 3, 2 μg of protein), and rat liver extract (lane 4, 2 μg of protein). Antibodies used were affinity-purified rabbit polyclonal anti-dj1 (a), -dj2 (b), and -dj3(d); and mouse monoclonal anti-dj2 (c). Purified chaperones were used as standards (lanes 5–7; a, H$_3$dj1 (0.25, 0.5, and 1 ng); b and c, H$_3$dj2 (0.5, 1, and 2 ng); d, H$_3$dj3 (5, 10, and 20 ng). For detecting cross reactivity, H$_3$dj3 migrated as polypeptides of 49,000 and 47,000 proteins represented the unfarnesylated and farnesylated mature forms, respectively (see below). We have shown that H$_3$man dj2 is farnesylated at Cys-394 and that the unfarnesylated form migrates faster than the farnesylated form (9). dj3 as well as dj2 contains a “CAAX box.” The M$_r$ 49,000 and 48,000 proteins represented the unfarnesylated precursor form and farnesylated mature form, respectively (see below).

Fig. 1B shows immunoblot analyses of three DnaJs in rabbit
reticulocytes, HeLa-S3 cells, CHO-K1 cells, and rat liver. Endogenous dj1 was detected as a Mr 40,000 band and represented 0.02–0.15% of the total protein (Fig. 1B, a). dj2 was detected as a Mr 46,000 band and represented about 0.07–0.2% of the total protein (Fig. 1B, b and c) (13). dj3 was detected as a Mr 47,000 band, being close to the migration of dj2. Content of dj3 was estimated to be 0.1–0.8% of total protein, this value being a little more abundant than that of dj2 (panel d). dj2 and dj3 proteins are similar (55% identical) and the affinity-purified dj2 polyclonal antibody cross-reacted with dj3 with an about 15-fold lower affinity (panel b). In contrast, the monoclonal antibody against dj2 did not cross-react with dj3 (panel c).

Farnesylation of dj3 (5) as well as dj2 (5, 9, 20) was confirmed in vitro. The farnesyl group was incorporated into the monoclonal antibody against dj2 did not cross-react with dj3 (1, 2, or 4 µg of protein) of total extract (lanes 1) and nuclear (lane 2), mitochondria (lane 3), microsomal (lane 4), and cytosolic (lane 5) fractions were loaded and analyzed by immunoblot analysis. Total amount of proteins obtained from 3.3 g of the tissue (660 mg of protein) was 5.2 mg of protein for nuclear fraction, 53 mg for mitochondrial fraction, 110 mg for microsomal fraction, and 120 mg for cytosolic fraction, respectively.

**Subcellular Distribution of the Three DnaJs in Rat**—We examined subcellular localization of the three DnaJs in rat liver, where the subcellular fractionation procedure is established (Fig. 2). The fractionation was assessed by distribution of the marker proteins. hsc70 was recovered mostly in the cytosolic fraction and partly in the microsomal and nuclear fractions as described (21). Most of dj1 was recovered in the cytosolic fraction, but a significant amount was also recovered in the nuclear fraction. Little dj1 was found in the microsomal fraction where a significant amount of hsc70 was present. dj2 and dj3 distributed similarly. These two DnaJs were mostly present in the microsomal and cytosolic fractions.

Expression and Heat Shock Induction of the DnaJ mRNAs—Distribution of mRNAs for hsc70 and DnaJs in human tissues was studied. hsc70 mRNA was uniformly detected in various tissues (Fig. 3A). dj1 mRNA was detected most strongly in the lung, much less in the heart and placenta, and very low in other tissues. However, different batches of the blot revealed much less marked expression in the lung (data not shown). dj1 mRNA may differ between individuals or be induced under certain conditions. Indeed, dj1 mRNA is markedly induced by heat shock (see below). Two dj2 mRNA species were detected in all tissues but with some variations. A major dj3 mRNA species of about 2.2 kilobases (kb) and a minor one of about 3.0 kb were also detected in all tissues, but were relatively abundant in the heart and skeletal muscle, and distribution differed from that of dj2 mRNA. We also examined the tissue distribution of bag-1 mRNA. bag-1 mRNA of about 1.4 kb was detected in all tissues, but was abundant in the heart and skeletal muscle, like dj3 mRNA.

We next examined effects of heat shock on expression of chaperone genes in cultured cells (Fig. 3B). A slight induction of hsc70 mRNA by heat shock was observed in these cell lines. 1.4- to 1.6-fold increases, on average, were seen in two independent experiments. Increases of more than 7-fold of dj1 mRNA were observed as described (10). The two dj2 mRNA species were also induced 3- to 4-fold. In contrast to dj1 and dj2 mRNAs, dj3 mRNA showed little increase with heat treatment (1.0- to 1.2-fold). bag-1 mRNA was little changed by the heat treatment.
Effect of Heat Shock on Intracellular Distribution of DnaJs—Localization of the three DnaJ proteins in HeLa-S3 cells was compared with that of hsc70, using indirect immunofluorescence microscopy. Under normal conditions, hsc70 localized almost exclusively in the cytoplasm (Fig. 4A). When cells were heat-shocked, hsc70 migrated from the cytoplasm and became condensed in the nucleoli (Fig. 4B), in agreement with reported data (21–23).

dj1 showed different localization patterns from that of hsc70, under normal conditions (Fig. 4A). Both cytoplasm and nuclear matrix were stained, in accord with published results (24). Neither the nucleolus nor the nuclear envelope was stained. When the cells were heat-shocked, dj1 accumulated in the nucleoli and became condensed in the nucleoli (Fig. 4B), in agreement with reported data (21–23).

Under normal conditions, the staining pattern of dj2 mostly overlapped with that of hsc70 (Fig. 4A: c and d; e and f). However, dj2 behaved differently from hsc70 under heat-shocked conditions. dj2 showed a concentrated pattern in a specific perinuclear region (Fig. 4B: c and d; e and f). Localization of dj3 was similar to that of dj2, in both normal and heat-shocked conditions (Fig. 4A and B): d, f, and h). Indeed, double staining experiment showed colocalization of dj2 and dj3 proteins (data not shown). Like dj2, a small portion of dj3 appeared to be translocated into the nucleus after heat treatment, but accumulation to the nucleolus was never apparent. Davis et al. (20) reported that dj2 accumulates to the nucleolus after heat shock. This discrepancy may be due to the longer heat shock.

Effect of dj3 Depletion on pOTC Import into Mitochondria—Effect of dj3 on import of rat pOTC into isolated mitochondria was assessed, using the precursor protein synthesized in dj3-depleted lysate. Depletion of the chaperones from the lysate was monitored by immunodetection (Fig. 5A). When the lysate was treated with the anti-dj3-coupled resin, over 85% of the dj3 was removed. The dj3 depletion had little effect on the amount of remaining dj2 and hsc70. Depletion of dj2 from the lysate was efficient and did not affect the level of remaining hsc70 and dj1 (13). However, not only dj2 but also dj3 was removed from the anti-dj2-treated lysate.

We reported that dj2 depletion led to impairment of rat pOTC import into mitochondria in vitro (13). Because dj3 was simultaneously removed from the dj2-depleted lysate, we tested the effect of H6dj3 re-addition to the dj2-dj3-depleted lysate on pOTC import (Fig. 5B). No decrease in pOTC synthesis was observed in dj2-dj3-depleted lysate. Import of pOTC synthesized in the dj2-dj3-depleted lysate was about 40% that of control.
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Fig. 5. Effect of dnaJ depletion of and re-addition to rabbit reticulocyte lysate on import of rat pOTC into rat liver mitochondria. A, immuno depletion was done using antibody-coupled Sepharose resin. The extent of the depletion for the endogenous chaperones was assessed by immunoblot analysis. Protein molecular mass markers are serum albumin (Mr 69,000) and ovalbumin (Mr 46,000). B, rat pOTC was synthesized in the mock-depleted lysate (Mock-Depleted, 2.8 kBq) or in the dj2-dj3-depleted lysate without re-addition (dj2-dj3-Depleted, 3.0 kBq) or with re-addition of 0.4 μM H6dj2 (Depleted + H6dj2, 3.8 kBq) or with re-addition of 0.4 μM H6dj3 (Depleted + H6dj3, 3.8 kBq) before translation, was subjected to import assay. C, rat pOTC synthesized in the mock-depleted lysate (Mock-Depleted, 2.8 kBq) or in the dj3-depleted lysate without re-addition (dj3-Depleted, 1.0 kBq), with or without re-addition of 0.4 μM H6dj3 (Depleted + H6dj3, 1.0 kBq) before translation, was subjected to import assay. The results were visualized and quantitated by imaging plate analysis, using a BAS2000 analyzer.

of the precursor synthesized in the control lysate. Import of pOTC was completely restored by the re-addition of purified H6dj2 prior to translation. When H6dj3 was added again to the dj2-dj3-depleted lysate, the import was mostly recovered. The concentration of H6dj3 (0.4 μM) used was similar to that in the lysate. This finding indicates that either dj2 or dj3 is required for pOTC import into mitochondria.

When the dj3-depleted lysate was tested for pOTC import, a moderate decrease was observed (Fig. 5C). Import of pOTC was reduced by about 20%, and the reduction was almost completely overcome by adding either H6dj2 or H6dj3. Because more than 80% of dj2 remained in the dj3-depleted lysate, dj2 is likely to support pOTC import. Thus, dj2 and dj3 were functionally equivalent in rat pOTC import into mitochondria.

Requirement of dj2 and dj3 for Import of Various Precursor Proteins—In previous studies, we found that the hsc70 required for mitochondrial import varied among precursor proteins (26). We then asked whether dj2 and dj3 are also required for other precursor proteins where the requirement for hsc70 varies by 0–60% for import. When import of human pOTC, pre-aspartate aminotransferase, pre-serine:pyruvate aminotransferase, and an artificial fusion precursor protein (27) in which the presequence of human pOTC was fused to green fluorescence protein (pOTC-GFP) was tested, dependence on hsc70 roughly paralleled that on dj2-dj3 among the precursor proteins (data not shown).

Effect of dj3 Depletion on Luciferase Refolding—We next examined the roles of dj3 in folding of chemically denatured firefly luciferase, as a model substrate. We proposed that the hsc70-dj2 chaperone system is the major system for refolding of denatured luciferase (13). When chemically denatured luciferase was incubated in the mock-depleted reticulocyte lysate, luciferase was efficiently refolded within 60 min at 25°C (Fig. 6). However, refolding of luciferase was markedly decreased in the dj2-dj3-depleted lysate. When 0.4 μM H6dj2 was added to the dj2-dj3-depleted lysate prior to the refolding assay, refolding recovered almost completely. When 0.4 μM H6dj3 was added, a more efficient refolding was achieved. Again, either dj2 or dj3 protein is required for luciferase refolding.

When dj3-depleted lysate was tested for luciferase refolding, a moderate decrease was observed. Refolding in the dj3-depleted lysate at 90 min decreased by 20%, compared with refolding in the control lysate. When 0.4 μM H6dj3 was added to the dj3-depleted lysate, refolding was fully restored. Similarly, the re-addition of 0.4 μM H6dj2 recovered the refolding. These results indicate that dj2 and dj3 are functionally equivalent in luciferase refolding.

Refolding of Luciferase by Purified Chaperones—Refolding of luciferase by dj3 was further studied using purified components. bag-1/RAP46 is an anti-apoptotic protein regulating hsc70 (28, 29), and was shown to be a grpE homolog in mam-
FIG. 7. Effect of bag-1 on refolding of luciferase by hsc70-dj1 (A), hsc70-dj2 (B), hsc70-dj3 (C), and hsc70-dj2,dj3 (D) system. Chemically denatured luciferase (5.0 nM) was renatured at 25 °C in the presence of various combinations of hsc70 (1.8 μM), H6dj1 (0.5 μM), H6dj2 (0.4 μM), H6dj3 (0.4 μM), and H6bag-1 (0.4 μM). All refolding mixtures contained 16 μM bovine serum albumin. Luciferase refolding was nil in the absence of chaperones and serum albumin. H6bag-1 alone had no apparent effect on luciferase refolding.

A combination of hsc70 and H6dj2 efficiently refolded the denatured luciferase (Fig. 7B). However, kinetics of the refolding differed from that in the lysate, as we noted earlier (13). Refolding in the reconstituted system was slow for the initial 10 min and then proceeded almost linearly up to 60 min. This initial slow refolding was improved when 0.4 μM H6bag-1 protein was included. No time lag was observed, and more than 50% of luciferase was refolded within 20 min, similar to refolding kinetics for the reticulocyte lysate. The maximum yield of the refolded luciferase exceeded 60% with this tertiary chaperone system.

H6dj3 together with hsc70 led to a moderate refolding of luciferase (Fig. 7C). This binary chaperone system also showed slow refolding kinetics for the initial 10 min. When H6bag-1 was included to the hsc70-H6dj3 system, refolding was further improved, with no initial lag.

Finally, we asked whether H6dj2 and H6dj3 can synergistically function in luciferase refolding (Fig. 7D). The kinetics of luciferase refolding was little affected by simultaneous addition of H6dj2 and H6dj3 to hsc70, compared with H6dj2 or H6dj3 alone. Further addition of H6bag-1 enhanced luciferase refolding. These results indicate that a substoichiometric amount of either dj2 or dj3 is sufficient for activation of hsc70-based refolding activity, whereas bag-1 further accelerates refolding kinetics.

FIG. 8. Effect of bag-1 and DnaJs on ATPase activity of hsc70. A, representative time course of ATPase assay is shown. ATPase activity of hsc70 was linear up to 120 min. B, effect of combinations of DnaJ and bag-1 proteins on ATPase activity of hsc70 is shown. The results are presented as the mean ± S.D. (n = 3). Concentrations of chaperones added were the same as in Fig. 7. Bovine serum albumin (16 μM) was also added.

Thus hsc70-dj2 and hsc70-dj3 chaperone pairs are much more efficient in luciferase refolding than is the hsc70-dj1 pair at physiological concentrations of respective chaperones. Although bag-1 was reported to suppress chaperone activity of the hsc70-dj1 pair (14, 15), bag-1 did accelerate chaperone function of hsc70-dj2 and hsc70-dj3 pairs.

Effects of DnaJs and bag-1 on ATPase Activity of hsc70—Effects of three DnaJs on ATPase activity of hsc70 were studied. As shown in Fig. 8A, hydrolysis of ATP by hsc70 was almost linear up to 120 min. The ATPase activity of hsc70 was 0.78 pmol/min/μg of hsc70 protein or 0.056 mol/min/mol of hsc70. Similar values for ATPase activity of mammalian hsc70 have been documented previously (14, 30).

In the presence of H6dj1, ATPase activity increased by 3.1-fold (Fig. 8B), in accord with published data (30). Either H6dj2 or H6dj3 enhanced ATPase activity of hsc70 by 3.7- to 3.8-fold. These results indicate that all three DnaJs are equally effective in enhancing ATPase activity of hsc70. H6bag-1, on the other hand, did not enhance the ATPase activity, which means a function of bag-1 is different from those of DnaJs. When H6bag-1 was included to the hsc70-DnaJ pairs, 1.8- to 3.0-fold ATPase activities were observed. Whether or not bag-1 is the sole chaperone with grpE-like functions in the mammalian cytosol remains to be determined.

DISCUSSION

In the present study, we analyzed the roles of recently found dj3 in mitochondrial protein import and protein folding, in comparison with dj1 and dj2. Unexpectedly, we found that our depletion system for dj2 (13) also depleted dj3 simultaneously. Re-addition experiments revealed that dj3 could replace dj2 in

malian cytosol (14). Thus we tested the effect of H6bag-1 on our hsc70-DnaJ chaperone pairs.

As shown in Fig. 7A, hsc70 alone did not facilitate luciferase refolding. Low refolding activity was apparently not specific to the chaperone activity of hsc70, because similar refolding was observed in the presence of serum albumin (13). When 0.4 μM H6dj1 together with 1.8 μM hsc70 were added, luciferase refolding was inefficient. Under the substoichiometric ratio of dj1 to hsc70 similar to that in the lysate, luciferase refolding was reproducibly low. Further addition of H6bag-1 protein improved luciferase refolding, albeit the yield of refolded luciferase was much lower than that obtained in the case of reticulocyte lysate.

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facilitating mitochondrial pOTC import and luciferase refolding. Single depletion for dj3 only resulted in a moderate inhibition, in both assays. dj2 and dj3 enhanced ATPase activity of hsc70 protein. Intracellular localization of these two DnaJs was similar, under normal and heat-shocked conditions. These findings also suggest a functional overlap between dj2 and dj3 in living cells. However, tissue distribution of dj3 mRNA differs from that of dj2 mRNA, although these mRNAs are expressed ubiquitously. Furthermore, dj2 mRNA but not dj3 mRNA is heat-induced. Thus, dj2 and dj3 apparently have similar functions also suggest a functional overlap between dj2 and dj3 in living cells. However, tissue distribution of dj3 mRNA differs from that of dj2 mRNA, although these mRNAs are expressed ubiquitously. Furthermore, dj2 mRNA but not dj3 mRNA is heat-induced. Thus, dj2 and dj3 apparently have similar functions. Their mRNA is ubiquitously expressed. The intracellular localization of these two DnaJs was observed. While dj2 and dj3 localized in a limited perinuclear region, localization of hsc70 was not limited to this region. These results reflect distinct roles of these DnaJs on hsc70, under different conditions. Under normal conditions, dj2 and dj3 mainly modulate chaperoning activities of hsc70 in the cytoplasm. On the other hand, when hsc70 accumulates in the nucleoli under heat-shocked conditions, hsc70 may be regulated mainly by dj1. Hot shock induction of dj1 mRNA may support this view.

The importance of ubiquitously expressed bag-1 on mitochondrial protein import remains to be elucidated. Anti-mouse bag-1 antibody could not detect bag-1 in rabbit reticulocyte lysates, and thus could not assess the roles of bag-1. Interestingly, multiple bag-1-related proteins are present in euakaryotes (32). Bag-1 accelerated refolding of denatured luciferase together with either hsc70-dj2 or hsc70-dj3 pair (Fig. 7). In contrast, the hsc70-dj1 pair could not support luciferase refolding even in the presence of bag-1. Thus it is clear that hsc70-dj2 and hsc70-dj3 pairs possess stronger chaperone activities than do the hsc70-dj1 pair. The zinc-finger domain of orthodox DnaJ proteins may be necessary for the strength of these activities.

We found that dj2 is the most efficient partner of hsc70 with regard to mitochondrial protein import and protein refolding. Recently, dj2 was shown to facilitate early steps of assembly of the cystic fibrosis transmembrane conductance regulator in the endoplasmic reticulum (24). Involvement of the hsc70-dj2 chaperone pair in polyglutamine repeat disorders (34, 35) and in defects of a mutant glucocorticoid receptor (36) has also been documented. It remains to be tested whether dj3 can replace proposed functions of dj2. On the other hand, dj3 was found to be a farnesylated protein in a rat cDNA library (5) and in a human gene, which restored cell cycle progression in yeast (7). It also remains to be tested whether dj2 can suppress cell cycle defect in yeast. We are now disrupting genes for these DnaJs to better understand the precise role(s) of respective DnaJs in vivo.

Acknowledgments—We thank M. Takiguchi for the gift of human liver cDNA, colleagues of our laboratory for suggestions and technical advice, and M. Ohara for comments on the manuscript.

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