JPDI, a Novel Endoplasmic Reticulum-resident Protein Containing Both a BiP-interacting J-domain and Thioredoxin-like Motifs*

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Several endoplasmic reticulum (ER)-resident luminal proteins have a characteristic ER retrieval signal, KDEL, or its variants at their C terminus. Our previous work searching EST databases for proteins containing the C-terminal KDEL motif predicted some novel murine proteins, one of which designated JPDI (J-domain-containing protein disulfide isomerase-like protein) is characterized in this study. The primary structure of JPDI is unique, because in addition to a J-domain motif adjacent to the N-terminal translocation signal sequence, four thioredoxin-like motifs were found in a single polypeptide. As examined by Northern blotting, the expression of JPDI was essentially ubiquitous in tissues and almost independent of ER stress. A computational prediction that JPDI is an ER-resident luminal protein was experimentally supported by immunofluorescent staining of epitope-tagged JPDI-expressing cells together with glycosylation and protease protection studies of this protein. JPDI probably acts as a DnaJ-like partner of BiP, because a recombinant protein carrying the J-domain of JPDI associated with BiP in an ATP-dependent manner and enhanced its ATPase activity. We speculate that for the folding of some proteins in the ER, chaperoning by BiP and formation of proper disulfide bonds may synchronously occur in a JPDI-dependent manner.

Most eukaryotic membrane or secretory proteins are thought to be folded in the endoplasmic reticulum (ER). Glycosylation and disulfide bond formation are involved in the correct folding of these proteins because inhibition of such post-translational modifications causes accumulation of unfolded proteins in the ER. As described below, various molecular chaperones and folding enzymes located in the ER play crucial roles in these cellular events (1).

BiP, also known as GRP78, is an ER-resident member of the Hsp70 chaperone family. As reviewed by Gething (2), it is apparent that in the ER, many unfolded proteins are chaperoned by BiP. Through the maintenance of solubility by BiP, unfolded proteins are occasionally subjected to ER-associated degradation (3–5). Furthermore, translocation of newly synthesized proteins into the ER requires BiP, and it seems that BiP plays multiple roles in the protein translocation machinery (6–8). In addition, Ire1 and PERK, sensors for accumulation of unfolded proteins in the ER, are negatively regulated by BiP (9, 10). Similar to other Hsp70 family chaperones, BiP carries an N-terminal ATPase domain and an adjacent substrate-binding domain. The biochemical properties of these domains in Hsp70 family chaperones are well documented (11). ATP binding to the ATPase domain causes low affinity but rapid binding of chaperone substrates to the substrate-binding domain, whereas subsequent ATP hydrolysis stabilizes substrate binding.

ATPase activity of Hsp70 family chaperones is regulated by various co-chaperones, including J-proteins (11, 12). The defining feature of J-proteins is an ~70-amino acid-residue signature termed the J-domain. Through this domain, J-proteins interact with their Hsp70 chaperone partners to stimulate ATP hydrolysis. Up until now, several types of ER-resident J-proteins have been identified. Over the full-length of the molecules, yeast Saccharomyces cerevisiae Scj1 and mammalian HEDJ (13) share common structural features with bacterial DnaJ, which has an intrinsic ability to bind some unfolded proteins and assists in chaperoning by its Hsp70 partner DnaK (11, 14). It has been demonstrated experimentally that Scj1 and another J-protein, Jem1, act to chaperone unfolded proteins in the yeast ER (5, 15). In contrast, yeast Sec63 (16), its putative mammalian orthologue hSec63 (17), and mammalian MTJ1 (18, 19), all of which are transmembrane J-proteins, seem to be directly involved in protein translocation across the ER membrane. Their similarity to bacterial DnaJ is restricted to the J-domain. In addition, mammalian cells contain a fourth ER-resident J-protein termed ERdj4 (20).

Another distinct family of ER-resident proteins, of which protein disulfide isomerase (PDI) is best documented (21, 22), is defined by the presence of thioredoxin-like motifs in their primary structure. PDI promotes oxidative folding of secretory proteins through catalyzing thiol-disulfide exchange reactions including disulfide formation, disulfide reduction, and disulfide isomerization. In this case, the thioredoxin-like motifs act as the catalytic active centers of PDI (23). Furthermore, it is widely accepted that PDI acts not only as an enzyme but also as a chaperone, and in this case, Tsai et al. (24) proposed that the thioredoxin-like motifs play a regulatory role.

BiP, PDI, and several other soluble proteins resident in the
Fig. 1. Amino acid sequence and computational analysis of JPDI. A, deduced amino acid sequence of murine JPDI. The dotted underline and arrow, respectively, indicate the predicted translocation signal sequence and its cleavage site. The underline and double underlines, respectively, indicate the J-domain and thioredoxin-like motifs. A conserved HPD sequence in the J-domain motif is shadowed, and the His residue replaced by Gln in the H63Q mutation is indicated in boldface. A putative N-linked glycosylation site is indicated by an open circle, and the KDEL motif is boxed. HA-tagged JPDI (JPDI-HA) carries a HA tag at the position indicated by an asterisk. B, schematic representation of murine JPDI. C, comparison of JPDI amino acid sequences in mouse, human (GenBank™ accession number AK027647), and C. elegans (GenBank™ accession number AL032657). Identical amino acids are shadowed. The J-domain and thioredoxin-like motifs are underlined and double-underlined, respectively.
ER carry a conserved tetrapeptide sequence, KDEL, at their C terminus (25). This KDEL motif and its variants are termed the ER retrieval signal, and it is commonly accepted that proteins carrying this signal are bound by a receptor in the Golgi apparatus and that the receptor-ligand complex returns to the ER (26). We previously searched EST databases for proteins carrying this C-terminal KDEL motif (27). This data base search predicted some novel proteins, one of which was named EP58 and is characterized in our previous report (27). Here we describe studies on another protein, JPDI (J-domain-containing PDI-like protein), which is a new type of ER-resident J-protein carrying thioredoxin-like motifs.

METHODS

Plasmids—As described previously (27), murine total RNA was subjected to reverse transcriptase-PCR using an oligo(dT) primer for the reverse transcription. For the PCR, the primer set 5′-CCGGAAATTC-CAACATGGGAGTCTGGTAAAGATGAC-3′ and 5′-CCGGAAATTC-GATCTTCTGATGCCGTTCTCC-3′ was employed. Both primers are specific to the predicted sequence of JPDI with the exception of additional EcoRI sites (italics) plus CCG and a Kozak sequence (underlined) (28). The resulting reverse transcriptase-PCR product carrying the JPDI cDNA was digested by EcoRI and inserted into the EcoRI site of pBluescript II KS+. Two plasmids were obtained by digesting one of the obtained plasmids with BamHI and EcoRI, and the other by digesting with BamHI and HindIII: the JPDI cDNA fragment inserted into the EcoRI site of pBluescript II KS+ was sequenced, and it was found to be identical to the corresponding sequence of the isolated cDNA fragment (data not shown).

Cells—Mammalian cells were cultured in a 5% CO2 atmosphere, and serum-free medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.5% transferrin. Murine NIH3T3 cells were used for the Northern blot analysis, and human HeLa cells were used for the experiments with HA epitope-tagged JPDI. Both were cultured in a medium containing 10% fetal bovine serum. Human fibroblasts were cultured in a medium containing 10% fetal bovine serum and 1% nonessential amino acids. All cell cultures were performed in a humidified atmosphere containing 5% CO2 at 37°C. The medium was changed twice a week, and the cells were grown to confluence before use. Transient transfections were performed with LipofectAMINE (Gibco BRL) as described previously (27).

Northern Blot Analysis—Northern blots were prepared from total RNA isolated from cultured cells as described previously (27). The blots were hybridized with a JPDI cDNA probe (top) and a β-actin probe (bottom), respectively. The mRNA level of JPDI was quantified using a BAS imaging plate (Fuji film, Tokyo, Japan).

RESULTS

Identification of a J-domain Containing PDI-like Protein—A mouse multi-tissue Northern blot was purchased from Clontech. Expression of the J-domain-encoding sequence was re-oxidized from the resulting plasmid by BamHI and EcoRI digestion and inserted into the corresponding sites of pBluescript II KS+. The J-domain-encoding fragment was quantified and normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal standard.

Cell Culturing and Transfection—All of the mammalian cells were cultured in α-minimum Eagle’s medium (ICN) supplemented with 10% fetal bovine serum at 37°C and 5% CO2. Transient transfection of plasmids was performed as described previously (30).

Northern Blot Analysis—As probes for Northern blot analysis, the following cDNA fragments were random primer-labeled with α-[32P]dCTP: murine JPDI and murine BiP (entire coding region); human glyceraldehyde-3-phosphate dehydrogenase (+75 to +1019); and human β-actin. Mouse multi-tissue Northern blot was purchased from Clontech. Extraction of total RNA from cultured cells and Northern blotting were performed as described previously (30). After hybridization, blots were washed in 2x SSC at 65°C and exposed to BAS imaging plate (Fuji film, Tokyo, Japan).

Analyses of HA-tagged JPDI Expressed in Mammalian Cultured Cells—36 h post-transfection of pCAG-JPDI-HA, HeLa cells were lysed with 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1% Nonidet P-40, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM phenylmethylsulfonfyl fluoride. After denaturing of proteins by incubation in 0.5% SDS and 1% 2-mercaptoethanol at 100°C for 10 min, the lysates were treated with 50 mM sodium citrate (pH 5.5), endo-β-N-acetylglu-
An ER-resident J-protein Carrying Thioredoxin-like Motifs

**Fig. 3.** Subcellular localization of JPDI-HA. A, NIH3T3 cells were transiently transfected with pCAG-JPDI-HA and incubated for 36 h. Cells then were fixed and co-stained with mouse 12CA5 anti-HA and rabbit anti-PDI antibodies. Fluorescein isothiocyanate- and rhodamine-conjugated secondary antibodies were used. B, 36 h post-transfection of pCAG-JPDI-HA, HeLa cells were lysed and treated with EndoH for the indicated times, and JPDI-HA was detected by anti-HA Western blot analysis. g and d indicate glycosylated and deglycosylated forms, respectively. A nonspecific protein band is indicated by an asterisk. C, 36 h post-transfection of pCAG-JPDI-HA, HeLa cells were homogenized for preparation of microsomes. The resulting microsomes were incubated with or without proteinase K (ProK) and Triton X-100 (1% Trx-100) and subsequently subjected to anti-HA and anti-BiP Western blot analysis. A nonspecific protein band is indicated by an asterisk.

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cosaminidase H (EndoH) (a MBP-tagged version commercially called EndoHEx New England BioLabs), and 5 units/1 μg of lysate protein at 37 °C.

Isolation of microsomes and protease protection assays were performed as described previously (27) with the exception that proteinase K was used at 1 μg/ml. In both EndoH treatment and protease protection assays, the resulting samples were subjected to Western blot analysis using the following materials: 12CA5 anti-HA monoclonal antibody (Nippon Boehringer Ingelheim, Tokyo, Japan) and rabbit anti-human BiP antibody as primary antibodies; horseradish peroxidase-conjugated donkey anti-mouse IgGs (Dako) and anti-rabbit IgGs (Amersham Biosciences) as secondary antibodies; and ECL Western blotting detection system (Amersham Biosciences). Immunofluorescent staining of cultured cells was performed as described previously (30).

**Preparation of Recombinant Proteins**—N-terminal GST and C-terminal (His)₆ double-tagged JPDI J-domain and its His6Q mutant version (designated GST-J and GST-J(H63Q), respectively) were expressed from pGEX-J and pGEX-J(H63Q), respectively. *Escherichia coli* BL21-CodonPlus (DE3)-RIL (Stratagene) cells transformed with these plasmids were cultured in LB broth containing 50 μg/ml ampicillin at 37 °C to A₆₀₀ 1.2. The cultures were then shifted to 20 °C, and isopropyl-1-thio-β-D-galactopyranoside was added (final concentration, 0.1 mM). After further culture for 80 min, cells were harvested and resuspended in lysis buffer (phosphate-buffered saline containing 2 mM EDTA, 2 mM 2-mercaptoethanol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride). The cells were then lysed by the addition of Triton X-100 (Trx-100, final concentration of 1%) and sonication. The lysates were clarified by centrifugation twice (9,500 × g for 20 min and 100,000 × g for 30 min) and then loaded into a 1-ml bed of glutathione-Sepharose 4B (Amersham Biosciences) column pre-equilibrated with lysis buffer. The column was washed with 5 ml of lysis buffer, 10 ml of wash buffer A (lysis buffer plus 1 M KCl and 0.1% Trx-100), and 5 ml of wash buffer B (50 mM Tris-HCl (pH 7.5), 10 mM ATP, 10 mM Mg(OAc)₂, 200 mM KOAc used to remove contaminating DnaK). Bound proteins were eluted with 8 ml of elution buffer A (50 mM Tris-HCl, 20 mM reduced glutathione (pH 8.0)) and loaded into a 0.5-ml bed of nickel-nitrilotriacetic acid Superflow (Qiagen) column pre-equilibrated with binding buffer A (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole (pH 8.0)). The column was washed with wash buffer C (50 mM NaH₂PO₄, 1 mM NaCl, 20 mM imidazole (pH 8.0)), and bound proteins were eluted with lysis buffer B (50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole (pH 8.0)). Peak fractions were then pooled and dialyzed against buffer 88lK (20 mM HEPES (pH 6.8), 75 mM KOAc, 250 mM sorbitol, 5 mM Mg(OAc)₂, 10% glycerol).

Unfused GST was obtained from pGEX-5X-2 in *E. coli* BL21-CodonPlus (DE3)-RIL by culturing cells in the presence of 1 mM isopropyl-1-thio-β-D-galactopyranoside at 37 °C for 3 h and purified using the above-mentioned glutathione-Sepharose column chromatography and MonoQ (Amersham Biosciences) column chromatography, subsequently. The peak fractions were then pooled and dialyzed against buffer 88IK (88 mM HEPES (pH 6.8), 75 mM KOAc, 250 mM sorbitol, 5 mM Mg(OAc)₂, 10% glycerol).

An *E. coli* strain expressing (His)₆-tagged hamster BiP protein was purified as described previously (31) and dialyzed against buffer B88 (the same composition as buffer 88IK with the exception of 150 mM KOAc).

**GST Pull-down Assay**—Purified proteins (GST-J, GST-J(H63Q), and unfused GST) were adjusted to the same concentrations with buffer 88IK. 3 μg of each protein then was diluted to 80 μl with binding buffer B (20 mM HEPES, 100 mM KCl, 5 mM MgCl₂, 0.1% Nonidet P-40, 2%
sequence as the query, a subsequent BLAST search against the GenBank™ data base predicted that it is part of a cDNA (GenBank™ accession number AK004617). This cDNA, which was generated and identified in the mouse full-length cDNA encyclopedia project (33), encodes a previously uncharacterized 793-amino acid-residue protein termed here as JPDI.

Computational analyses using PSORT II (psort.nibb.ac.jp/form2.html) and Searching Motif (www.motif.genome.ad.jp) programs indicated as shown in Fig. 1, A and B, that JPDI contains several significant sequence features together with the C-terminal KDEL motif. First, JPDI putatively bears an N-terminal translocation signal sequence that is cleaved between Gly-31 and Thr-32. The predicted molecular mass of signal sequence-cleaved JPDI is calculated as ~87 kDa, which well coincides with endoglycosidase-treated HA-tagged JPDI from cell lysate (Fig. 3B) as described below. Second, a 66-amino acid-residue J-domain motif was found adjacent to the translocation signal sequence. Finally, similar to PDI, JPDI contains multiple thioredoxin-like motifs (-WCXXC-).

As shown in Fig. 1C, translating the BLAST search against the GenBank™ data base using the JPDI amino acid sequence as the query predicted similar uncharacterized proteins in humans (90% identical) and in Caenorhabditis elegans (37% identical). These putative orthologues of murine JPDI commonly carry all of the above mentioned sequence features. No homologue was found in S. cerevisiae.

Expression of JPDI—To determine the tissue distribution of JPDI expression, Northern blot detection of JPDI mRNA was performed using multi-tissue Northern blot, a commercial Northern blot membrane on which mouse poly(A)+ RNA preparations from various tissues were blotted. The JPDI probe almost ubiquitously detected duplicate bands (4.0 and 3.3 kilobases) in all of the tissues examined (Fig. 2A). The 4.0-kilobase transcript was major in most of the tissues with the exception that the 3.3-kilobase transcript was highly expressed in the testis. Relatively speaking, JPDI expression was high in heart, liver, kidney, and testis and low in spleen and skeletal muscle.

Several mammalian ER-resident chaperones and folding enzymes including BiP and PDI are transcriptionally induced by ER stress through intracellular signaling pathways as described previously (34). To investigate whether the expression of JPDI is induced under ER stress conditions, NIH3T3 cells were treated with the ER stress inducer tunicamycin, an N-glycosylation inhibitor, or thapsigargin, a calcium pump inhibitor. Thereafter, total RNA preparations were analyzed by Northern blotting, which showed that JPDI mRNA was only slightly induced (~1.3-fold) under conditions in which BiP mRNA was induced ~10-fold (Fig. 2, B and C). Similar results were obtained using Ltk− cells (data not shown). Unlike the result in multi-tissue Northern blot, only the 3.3-kilobase transcript was detected in these cultured cells.

JPDI Is a ER-resident Luminal Protein—JPDI carries only one significant hydrophobic segment, which is positioned at the N terminus and predicted to be a cleavable translocation signal, on the basis of its amino acid sequence. Because of this sequence feature and the C-terminal KDEL motif, JPDI seemed to be an ER-resident luminal protein. Therefore, we undertook the task of confirming this idea experimentally by using cultured cells transiently expressing JPDI tagged with a HA tag at the position just N-terminally adjacent to the C-terminal KDEL motif (hereafter called JPDI-HA) (see Fig. 1A).

First, JPDI-HA-expressing NIH3T3 cells were doubly immunostained with anti-HA antibody and anti-PDI antibody. As detected by fluorescence microscopy, both JPDI-HA staining and PDI staining yielded classical ER patterns (Fig. 3A, upper
and middle panels). The yellow/orange color in the overlay image indicates co-localization of JPDI-HA and PDI (Fig. 3A, lower panel). Similar images were obtained from JPDI-HA-expressing HeLa and COS7 cells.

Lysates of JPDI-HA-expressing HeLa cells were treated next with EndoH and subjected to SDS-PAGE followed by Western blot detection of JPDI-HA. As shown in Fig. 3B, EndoH treatment increased the mobility of JPDI in SDS-PAGE. This result indicates that JPDI is modified by N-linked glycosylation that is cleavable by EndoH.

Finally, microsomes prepared from JPDI-HA-expressing HeLa cells were treated with proteinase K, and proteins protected from protease digestion were detected by Western blot analysis. As shown in Fig. 3C, neither JPDI-HA nor BiP was digested unless the microsomal membrane was lysed by detergent Trx-100.

The J-domain of JPDI Binds to BiP—The localization of JPDI to the ER implies that JPDI may act as a DnaJ-like partner of BiP. This possibility was tested by experiments designed to observe in vitro interaction of the JPDI J-domain with BiP. At the beginning of in vitro analyses, a fusion of GST-JPDI J-domain (His)_6 tag named GST-J was expressed in bacteria and purified by glutathione affinity and subsequent nickel-chelating chromatography. As monitored by CBB staining after SDS-PAGE separation, contaminating protein was virtually undetectable in the purified preparation of GST-J (Fig. 4A).

A tripeptide motif, HPD, in the J-domain is quite highly conserved among J-proteins. As initially demonstrated using E. coli DnaJ (35), an amino acid replacement of the HPD motif to QPD causes a complete loss of J-domain functions. In murine JPDI, the amino acid residues positioned at 63–65 correspond to this motif (see Fig. 1A). Thus, as a negative control in the following experiments, we generated GST-J(H63Q), a mutant version of GST-J where the HPD motif is replaced by QPD (see Fig. 1A). An unfused version of GST (hereafter called GST) was also employed as another type of negative control. Similar to GST-J, CBB staining after SDS-PAGE separation confirmed that GST-J, GST, and BiP were purified to homogeneity (Fig. 4A).

For a pull-down experiment to test J-domain binding to BiP, glutathione-Sepharose beads carrying GST-J, GST-J(H63Q), or GST were incubated with BiP in the absence or presence of ATP. The subsequent analysis of pulled-down proteins showed that BiP does not bind with GST-J(H63Q) or GST but it does with GST-J and that this binding requires ATP (Fig. 4B).

The J-domain of JPDI Stimulates ATP Hydrolysis by BiP—It is well known that the ATPase activity of Hsp70 family chaperones is enhanced by the J-domain of their specific J-protein partners. Thus, we next asked whether the J-domain of JPDI stimulates in vitro ATP hydrolysis by BiP. First, 1 μg of BiP was incubated without additional protein or with 0.5 μg each of GST, GST-J, or GST-J(H63Q) in ATPase assay buffer (the molar ratio of BiP to GST-J or GST-J(H63Q) was 1.4). The results shown in Fig. 5, A and B, indicate that GST and GST-J(H63Q) had little or no ability to stimulate ATP hydrolysis by BiP and that, on the contrary, ATPase activity of BiP was enhanced ~2-fold by the addition of GST-J. This ATP hydrolysis assay was performed next using various concentrations of GST, GST-J, or GST-J(H63Q), which confirmed that only GST-J stimulates ATP hydrolysis by BiP (Fig. 5C).

DISCUSSION

Our computational search for proteins bearing a C-terminal ER retrieval signal predicted a novel murine protein, JPDI. Remarkably, JPDI is the first identified protein carrying both a J-domain and thioredoxin-like motifs in a single polypeptide chain (Fig. 1). Because the similarity of JPDI to other J-pro-
teins is restricted to the J-domain, we suspect that JPDI plays a role(s) quite different from that of other J-proteins.

According to a further computational data base search, humans and C. elegans carry putative orthologues of murine JPDI. The expression of JPDI mRNA was nearly ubiquitous in all the tissues examined, and ER stressors induced JPDI mRNA expression only very slightly (Fig. 2). These observations imply that JPDI may have stress-independent and constitutive function(s) commonly required in metazoan cells. As shown in Fig. 2A, two different sizes of JPDI mRNA were detected in various murine tissues. We suspect that these transcripts were produced by alternative termination or by alternative splicing at the 3′-untranslated region, because a 3′-RACE analysis predicted multiple 3′-untranslated region lengths of JPDI mRNA (data not shown).

We next experimentally confirmed cellular localization of JPDI to the ER lumen using mammalian cultured cells transiently expressing an HA epitope-tagged version of JPDI designated here as JPDI-HA. To avoid dysfunction of the N-terminal translocation signal sequence and the C-terminal KDEL (see Fig. 1A). A SDS-PAGE mobility shift of JPDI-HA by EndoH digestion indicates the N-linked glycosylation and thus luminal localization of this protein (Fig. 3B). We suspect that JPDI is glycosylated at the N-glycosylation motif NX(S/T) (see Fig. 1A). Moreover, JPDI-HA in a microsomal fraction was protected from protease digestion (Fig. 3C). This result strongly suggests luminal localization of the entire region of JPDI. Localization of JPDI to the ER was demonstrated by anti-HA immunofluorescent staining, which yielded an ER-like pattern almost overlapping that of anti-PDI staining (Fig. 3A). Moreover, the above mentioned susceptibility of JPDI-HA to EndoH digestion also implies the ER localization of JPDI, because, in general, N-linked glycosyl chains are modified such that they are not susceptible to EndoH digestion in the mammalian Golgi apparatus.

Here we also presented two lines of in vitro evidence that JPDI can interact with BiP as its DnaJ-like partner via the J-domain. First, a GST pull-down assay indicated ATP-dependent binding of BiP to the JPDI J-domain (Fig. 4). A requirement for hydrolyzable ATP for the J-protein-Hsp70 chaperone interaction is well documented (32, 36). The ATPase activity of BiP next was significantly enhanced by the JPDI J-domain (Fig. 5). The following observations indicate that our preparation of JPDI J-domain interacts with BiP as its functional partner rather than as an unfolded protein substrate, which also binds to Hsp70 chaperones to stimulate their ATPase activity (11).

First, unlike the case for J-protein partners, the binding of unfolded protein substrates to Hsp70 chaperones is inhibited by ATP (36). Second, amino acid replacement of the highly conserved HDP motif with QPD in the J-domain, which commonly causes dysfunction of various J-proteins (35, 37), significantly decreased the ability of the JPDI J-domain to bind to BiP and to stimulate its ATPase activity (Figs. 4 and 5). Considering the high degree of specificity of a J-domain for its Hsp70 chaperone partner (38), it is likely that BiP is the natural Hsp70 chaperone partner of JPDI. However, we cannot exclude the possibility that other ER-resident Hsp70 family proteins including GRP170 (39) interact with JPDI.

Thus far, we have failed to demonstrate any PDI-like activities for JPDI, although the multiple thioredoxin-like motifs of JPDI imply its activity in disulfide formation. Heterologous expression of murine JPDI was not able to rescue the lethal phenotype of a PDI gene disruption in S. cerevisiae (data not shown). In our in vitro assay, oxidative refolding of denatured ribonuclease A was not promoted by JPDI, and we have not shown so far thiol-dependent reductase activity of recombinant JPDI, which catalyzes the reduction of insulin disulfides by dithiothreitol (data not shown). Nevertheless, if we suppose a disulfide isomerization activity of JPDI, it is possible to speculate that this reaction occurs synergistically with chaperoning by BiP. In this scenario, BiP may be recruited by the JPDI J-domain and bind unfolded protein substrates to maintain their conformation, thus making them accessible to the JPDI thioredoxin-like catalytic centers for disulfide isomerization. Cooperation between BiP and PDI was demonstrated in an in vitro refolding reaction of a denatured and reduced model protein (40). Moreover, another thioredoxin-like motif-containing ER-resident protein, ERP57, was proposed to function together with the lectin-like chaperones calnexin and calreticulin to promote glycoprotein folding (41, 42). The above mentioned hypothesized function of JPDI would be a novel example of synergy between a chaperone and a folding enzyme. An alternative hypothesis for JPDI function, which is also a speculation derived from the knowledge of PDI, is that JPDI acts to bind with unfolded proteins. If this idea is correct, it is possible that like bacterial DnaJ JPDI functions to bring unfolded proteins to its Hsp70 chaperone partner. In this case, the thioredoxin-like motifs may play a regulatory role in retrotranslocation from the ER to cytosol as hypothesized (24). Otherwise, JPDI may somehow help the function of BiP in the protein translocation machinery. We anticipate that our further studies including the generation of JPDI-deficient cells will clarify the biochemical and physiological function(s) of JPDI.

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REFERENCES

1. Stevens, F. J., and Argon, Y. (1999) Semin. Cell Dev. Biol. 10, 443–454
2. Gething, M.-J. (1999) Semin. Cell Dev. Biol. 10, 465–472
3. Plummer, R. K., Bohmner, S., Bordallo, J., Sommer, T., and Wolf, D. H. (1997) Nature 388, 891–895
4. Brodsky, J. L., Werner, E. D., Dubas, M. E., Goeckeler, J. L., Kruse, K. B., and McCracken, A. A. (1999) J. Biol. Chem. 274, 3453–3460
5. Nishikawa, S. I., Saw, T.-W., Kato, Y., Brodsky, J. L., and Endo, T. (2001) J. Cell Biol. 153, 1061–1070
6. Vogel, J. P., Mira, L. M., and Rose, M. D. (1990) J. Cell Biol. 110, 1885–1895
7. Hamman, B. D., Hendershot, L. M., and Johnson, A. E. (1998) Cell 92, 747–758
8. Matlack, K. E., Mieselwitz, B., Plath, K., and Rapoport, T. A. (1999) Cell 97, 533–564
9. Bertolotti, A., Yang, Z., Hendershot, L. M., Harding, H. P., and Ron, D. (2000) Nat. Cell Biol. 2, 326–332
10. Ozakura, K., Kimata, Y., Higashio, H., Tsuru, A., and Kohno, K. (2000) Biochem. Biophys. Res. Commun. 279, 445–450
11. Bukau, B., and Horwich, A. L. (1998) Cell 92, 351–366
12. Kelley, W. L. (1998) Trends Biochem. Sci. 23, 223–227
13. Yu, M., Haslam, R. H., and Haslam, D. B. (2000) J. Biol. Chem. 275, 24984–24992
14. Wawrynow, A., and Zylstra, M. (1997) in Molecular Chaperones and Protein Folding Catalysis (Gething, M.-J., ed) pp. 88–98, Oxford University Press, Oxford, United Kingdom
15. Silverstein, S., Schlenstedt, G., Silver, P. A., and Gilmore, R. (1998) J. Cell Biol. 143, 921–933
16. Brodsky, J. L. (1997) in Molecular Chaperones and Protein Folding Catalysts (Gething, M.-J., ed) pp. 98–108, Oxford University Press, Oxford, United Kingdom
17. Skowronek, M. H., Rotter, M., and Haas, I. G. (1999) Biol. Chem. Hoppe-Seyler 380, 1133–1138
18. Chevalier, M., Rhee, H., Elguindi, E. C., and Blond, S. Y. (2000) J. Biol. Chem. 275, 19620–19627
19. Dudek, J., Yolken, J., Bies, C., Geth, S., Muller, A., Lerner, M., Feick, P., Schafer, K. H., Morgenstern, E., Hennessy, F., Bluth, G. L., Janochek, K., Hein, N., Scholes, F., Frien, M., Nastazinszky, W., and Zimmermann, R. (2002) EMBO J. 21, 9258–9267
20. Shen, Y., Meunier, L., and Hendershot, L. M. (2002) J. Biol. Chem. 277, 19547–19556
21. Novoa, R. (1999) Semin. Cell Dev. Biol. 10, 481–483
22. Freedman, R. B., Klappa, P., and Ruddock, L. W. (2002) EMBO Rep. 3, 1138–1140
23. Darby, N. J., and Creighton, T. E. (1995) Biochemistry 34, 16770–16780
24. Tsai, B., Rodighiero, C., Lencer, W. I., and Rapoport, T. A. (2001) Cell 104, 937–948
25. Munro, S., and Pelham, H. R. (1987) Cell 48, 899–907
26. Pelham, H. R. (1996) Cell Struct. Funct. 21, 413–419
27. Kimata, Y., Ochoki, K., Nomura-Furuwatari, C., Hosoda, A., Tsuru, A., and
An ER-resident J-protein Carrying Thioredoxin-like Motifs

Kohno, K. (2000) *Gene* (Amst.) **261**, 321–327
28. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872
29. Niwa, H., Yamamura, K., and Miyazaki, J. (1991) *Gene* (Amst.) **108**, 193–199
30. Iwawaki, T., Hosoda, A., Okuda, T., Kamigori, Y., Nomura-Furuwatari, C., Kimata, Y., Tsuru, A., and Kohno, K. (2001) *Nat. Cell Biol.* **3**, 158–164
31. Wei, J., and Hendershot, L. M. (1995) *J. Biol. Chem.* **270**, 26670–26676
32. Corsi, A. K., and Schekman, R. (1997) *J. Cell Biol.* **137**, 1483–1493
33. Kawai, J., Shinagawa, A., Shibata, K., Yoshine, M., Itoh, M., Ishii, Y., Arakawa, T., Haru, A., Fukunishi, Y., Konno, H., Adachi, J., Fukuda, S., Aizawa, K., Izawa, M., Nishi, K., Kiyosawa, H., Kondo, S., Yamanaka, I., Saito, T., Okazaki, Y., Gojobori, T., Iono, H., Kasukawa, T., Saito, R., Kadota, K., Matsuda, H., Ashburner, M., Batalov, S., Casavant, T., Fleischmann, W., Gaasterland, T., Gissi, C., King, B., Kochwa, H., Kuehl, P., Lewis, S., Matsuo, Y., Nikaido, I., Poso, G., Quackenbush, J., Schriml, L. M., Staubli, F., Suzuki, R., Tomita, M., Wagner, L., Washio, T., Sakai, K., Okido, T., Furuno, M., Aono, H., Baldarelli, R., Barsh, G., Blake, J., Boffelli, D., Bojunga, N., Carninci, P., de Bonaldo, M. F., Brenwald, M. J., Bult, C., Fletcher, C., Fujita, M., Garbolino, M., Gustincich, S., Hill, D., Hofmann, M., Hume, D. A., Kamiya, M., Lee, N. H., Lyons, P., Marchionni, L., Mashima, J., Mazzarelli, J., Mombaerts, P., Nordone, P., Ring, B., Ringwald, M., Rodriguez, I., Sakamoto, N., Sasaki, H., Sato, K., Schonbach, C., Seya, T., Shibata, Y., Storch, K. F., Suzuki, H., Teyo-ska, K., Wang, K. H., Weitz, C., Whittaker, C., Wilming, L., Wynshaw-Boris, A., Yoshida, K., Hasegawa, Y., Kawaji, H., Kohsukue, S., and Hayashizaki, Y. (2001) *Nature* **409**, 685–690
34. Lee, K., Tirasophon, W., Shen, X., Michalak, M., Prywes, R., Okada, T., Yoshida, H., Mori, K., and Kaufman, R. J. (2002) *Genes Dev.* **16**, 452–466
35. Wall, D., Zylicz, M., and Georgopoulos, C. (1994) *J. Biol. Chem.* **269**, 5446–5451
36. Wawrzynow, A., and Zylicz, M. (1995) *J. Biol. Chem.* **270**, 19300–19306
37. Tsai, J., and Douglas, M. G. (1996) *J. Biol. Chem.* **271**, 9347–9354
38. Schlenstedt, G., Harris, S., Rase, B., Lill, E., and Silver, P. A. (1995) *J. Cell Biol.* **129**, 979–988
39. Lin, H. Y., Masso-Welch, P., Di, Y. P., Cai, J. W., Shen, J. W., and Subjeck, J. R. (1993) *Mol. Biol. Cell* **4**, 1109–1119
40. Mayer, M., Kies, U., Kammermeier, R., and Buchner, J. (2000) *J. Biol. Chem.* **275**, 29421–29425
41. Oliver, J. D., van der Wal, F. J., Bulleid, N. J., and High, S. (1997) *Science* **275**, 86–88
42. Oliver, J. D., Roderick, H. L., Llewellyn, D. H., and High, S. (1999) *Mol. Biol. Cell* **10**, 2573–2582
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