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Essay

Eukaryotic Homologues of *Escherichia coli* dnaJ: A Diverse Protein Family That Functions with HSP70 Stress Proteins

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INTRODUCTION

The 70-kD heat shock proteins (hsp70) have been identified in all free living organisms so far examined. They were originally identified because of their highly inducible expression on heat shock, but are now known to be constitutively expressed as well, with specific family members targeted to different cellular organelles. They function as molecular chaperones by binding to other polypeptides. Such interactions, which appear to have only limited specificity (Flynn *et al.*, 1991), have been correlated with various aspects of protein dynamics such as protein folding, assembly, dissasembly, membrane transport, and protection from the effects of heat and other stresses. The dissociation of hsp70:polypeptide complexes is dependent on ATP hydrolysis (see Gething and Sambrook, 1992 and Hartl *et al.*, 1992 for reviews). In *Escherichia coli*, this event is regulated by the dnaJ and grpE gene products, which interact with dnaK (the *E. coli* hsp70), stimulate its ATPase, and cause complex dissociation. DnaJ also appears to function independently of dnaK as a molecular chaperone (Liberek *et al.*, 1990; Wickner *et al.*, 1991, Gamer *et al.*, 1992; Langer *et al.*, 1992; Ohki *et al.*, 1992). Langer *et al.* (1992) have proposed that dnaJ functions with dnaK as a molecular chaperone in a polypeptide folding pathway also involving the groEL and groES chaperonin proteins.

E. coli dnaJ was discovered as an essential host component for phage λ replication, where it functions with dnaK in the assembly of a functional preprimosomal complex. Both genes form an operon (in the order dnaK, J) in several eubacterial species including *Bacillus subtilis* (Wetzstein *et al.*, 1992), *Borrelia burgdorferi* (Anzola *et al.*, 1992) *Brucella ovis* (Cellier *et al.*, 1992), *Caulobacter crescentus* (Marczynski and Shapiro, 1992), *Clostridium acetobutylicum* (Narberhaus *et al.*, 1992), and *E. coli* (Bardwell *et al.*, 1986; Ohki *et al.*, 1986). Both are coordinately induced by heat stress, but at steady state dnaK is present at a tenfold greater concentration in the cell (5000 molecules of dnaK per cell vs. 500 molecules per cell of dnaJ; Bardwell *et al.*, 1986). Mutation in dnaK or dnaJ results in similar phenotypes, but neither gene is essential. These mutant phenotypes include inviability at temperatures over 42°C, cessation of DNA and RNA synthesis at the restrictive temperature, and defects in protein degradation and cell division (leading to pronounced filamentation). *E. coli* strains deficient in either dnaK or dnaJ overexpress other heat shock inducible proteins (such as the chaperonin groEL and groES) and are incompetent for phage replication at any temperature (for reviews see Georgopoulos *et al.*, 1990, Ang *et al.*, 1991). Recent reports indicate that dnaK and dnaJ function in secretion (Wild *et al.*, 1992) and in preventing protein aggregation (Gragerov *et al.*, 1992). Both proteins can bind independently to $\sigma 32$, which regulates hsp gene expression in *E. coli* (Gamer *et al.*, 1992).

In the last two years, a number of reports have emerged from several laboratories characterizing eukaryotic homologues of *E. coli* dnaJ. Compared with eukaryotic hsp70 proteins, which share ≥50% sequence identity, however, eukaryotic dnaJ homologues are rather diverse. This essay presents these new data and discusses the possible functions of these proteins.

STRUCTURE OF *E. coli* dnaJ AND ITS EUKARYOTIC HOMOLOGUES

E. coli dnaJ is a 43-kD protein that behaves as a dimer in solution (Zylicz *et al.*, 1985; Bardwell *et al.*, 1986; Ohki *et al.*, 1986). A 70-amino acid sequence at the *N* terminus, termed the "J" domain, is conserved in all proteins sharing sequence identity with dnaJ (see Figure 1). It is the presence of this domain in a protein that defines it as dnaJ-like, because this is the domain that is thought to interact with hsp70 proteins (see below).

Distal to the J domain is a sequence rich in glycine and phenylalanine (the "G/F" domain in Figure 1), which is thought to act as a "hinge" region, lacking in secondary structure and separating the J domain from a cysteine rich region in the middle of the protein. The spacing of cysteine residues in this domain is CxxCxGxG (single letter code, where x is usually charged or polar) repeated four times. This distribution of cysteines is similar to that found in some zinc binding proteins (see Berg, 1990, for review) and might be expected to form

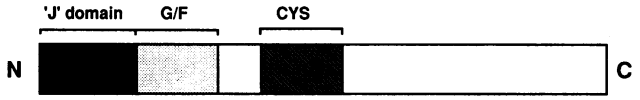

Figure 1. Organization of domains in *E. coli* dnaJ. "J" domain includes the first 70 amino acids. G/F, glycine and phenylalanine rich region; Cys, cysteine rich repeats.

two sub-domains that are each coordinated at their base by zinc ions as in a zinc "finger." In contrast to the N terminal half of dnaJ, the C terminal half is not organized into any immediately obvious domain structure. However, there is considerable sequence similarity between the C terminal half of dnaJ and some eukaryotic homologues (see below). For sequence comparisons see Blumberg and Silver, 1991; Caplan and Douglas, 1991; Luke *et al.*, 1991; Cheetham *et al.*, 1992; Kurihara and Silver, 1992; Zhu *et al.*, 1993.

Eukaryotic dnaJ-like proteins (see Table 1) have been characterized in both unicellular and metazoan eukaryotes. They may be roughly divided into two groups based on their sequence similarity with the *E. coli* prototype. First are proteins that share a similar domain structure with dnaJ, as described above, and are probably functional homologues. That is, they each contain an N terminal J domain, a G/F region, a central cysteine rich domain, and sequence similarity throughout the C terminal half with dnaJ. These include the yeast proteins SCJ1, YDJ1, and plant proteins ANJ1 and LDJ1. They average 35% identical amino acids with dnaJ when full length protein sequences are compared, but average 51% identity in the J domain. ANJ1 and LDJ1 are 88% identical and share a C terminal CaaX box sequence similar to that found in YDJ1 but not dnaJ. The CaaX box sequence (where C is cysteine, "aa" stands for two, usually aliphatic, amino acids and X is either cys, met, gln, or ser) is a substrate for prenyltransferase enzymes that catalyse the addition of a farnesyl lipid moiety to the C terminus (see Cox and Der, 1992 for review). The addition of this 15 carbon branched lipid moiety to the C terminus of YDJ1 has been demonstrated (Caplan *et al.*, 1992b) and shown to affect the ability of this protein to function at high (>37°C) growth temperatures. SCJ1 also contains a specialized sequence, a putative N-terminal mitochondrial targeting pre-sequence (Blumberg and Silver, 1991).

The second group of eukaryotic dnaJ-like proteins share more limited sequence similarity. These include some that are fairly similar to dnaJ, having the N terminal J domain and G/F region (as well as similarity in

Table 1. Eukaryotic dnaJ-like proteins

Protein	Organism	Localization	Domains[a]			Reference
			J	G/F	CYS	
SCJ1	*S. cerevisiae*	Mitochondria	+	+	+	Blumberg and Silver, 1991
SEC63	*S. cerevisiae*	ER membrane	+	−	−	Sadler *et al.*, 1989
SIS1	*S. cerevisiae*	Cytosol/nucleus	+	+	−	Luke *et al.*, 1991
YDJ1(MAS5)	*S. cerevisiae*	Cytosol	+	+	+	Caplan and Douglas 1991, Atencio and Yaffe 1992
Zuotin	*S. cerevisiae*	Nucleus	+	−	−	Zhang *et al.*, 1992
dnaJ[b]	*S. pombe*	?	+	?	?	Kurihara and Silver, 1992
YDJ1[c]	*C. elegans*	?	?	?	+	McCombie *et al.*, 1992
SCJ1[d]	*C. elegans*	?	+	+	?	Waterston *et al.*, 1992
CSP	*D. melanogaster*	Brain	+	−	−	Zinsmaier *et al.*, 1990
LDJ1	*A. porrum* (leek)	?[e]	+	+	+	J. Bessouie, 1993 (submitted)
ANJ1	*A. nummularia*	Roots/shoots	+	+	+	J.-K. Zhu *et al.*, 1993
HSJ1	Human	Brain	+	+	−	Cheetham *et al.*, 1992
HDJ1	Human	HeLa	+	−	−	Raabe and Manley, 1991
HSP40	Human	Cytosol/nucleus	+	?[f]	?	Hattori *et al.*, 1992
HLADRB10401[g]	Human	Cell surface	†	−	−	Albani *et al.*, 1992
RESA	*P. falciparum*	Red cell surface	+	−	−	Bork *et al.*, 1992

[a] The domains: J, conserved N terminal J domain of *dnaJ*; G/F, glycine phenylalanine region; cys, cysteine rich region.

[b] Unpublished sequence in Genbank, first noted by Kurihara and Silver, 1992.

[c] Discovered as expressed sequence tag (est) as part of the *C. elegans* sequencing project. Complete sequences unknown, and domain assignments are presumptive except for cysteine rich domain.

[d] Discovered as expressed sequence tag (est) as part of the *C. elegans* sequencing project. Complete sequences unknown, and domain assignments are presumptive except for partial J and G/F domains (assuming one frame shift in est).

[e] LDJ1 and ANJ1 are 88% identical and are probably functional homologues.

[f] Only the N terminal portion of hsp40 has been sequenced.

[g] HLADRB10401 shares 6 identical amino acids at the C terminal end of the J domain. † symbol denotes this is not a complete J domain.

the C terminus), yet lack the central cysteine rich region (eg., yeast SIS1; human HSJ1, and HDJ1), and some that contain only the conserved J and sometimes the G/F domain in common with dnaJ. The sequence identity between dnaJ and the latter group averages 44% over the 70 amino acid J domain, but there is otherwise no other sequence similarity. Members of this group include SEC63 and Zuotin; cysteine string protein (CSP) and the ring-infected erythrocyte surface antigen (RESA) protein of *Plasmodium falciparum*, which displays the least sequence identity of the group with dnaJs J domain (30%, Bork *et al.*, 1992). This group may have their J domains either at the N terminus of the protein where it is in dnaJ (eg., SIS1, HSJ1, and HDJ1) or elsewhere (eg., Zuotin, SEC63 and RESA).

Perhaps the most intriguing eukaryotic protein to contain sequence similarity with dnaJ is a human MHC class II allele HLA DRB10401 (Albani *et al.*, 1992). The sequence similarity is limited to a small amino acid stretch containing a seven out of nine identical match. In dnaJ, this region corresponds to amino acids 61–70, at the distal end of the conserved J domain. In HLA DRB10401, this stretch is between amino acids 70–79 that would place it in the vicinity of the putative peptide binding domain, which is thought to share structural similarity with the polypeptide binding pocket of hsp70 proteins (Rippmann *et al.*, 1991). Even though the sequence similarity is over a very small region, these proteins are antigenically related. Antibodies raised against a peptide of this MHC class II allele sequence will bind dnaJ protein. Furthermore, antibodies raised against dnaJ in rabbits will bind to the HLA DRB10401 protein. These results are especially interesting in view of the genetic linkage between this particular allele and patients with rheumatoid arthritis, an autoimmune disease (Albani *et al.*, 1992). Indeed, the etiology of rheumatoid arthritis has been discussed in terms of shared epitopes between bacterial heat shock proteins and self antigens that may be localized to the cell surface. (see Winfield and Jarjour, 1991 for review).

Some viral proteins also share limited sequence similarity with dnaJ. As first noted by Cheetham *et al.* (1992), the N-terminal region of large and small T-antigens of the Budgerigar Fledgling Disease Virus (BFVD, Rott *et al.*, 1988) are 64.3% identical over 28 amino acids to a conserved region of dnaJ's J domain. BFVD is a papovavirus that infects. *Melopsittacus undulatus.* Similar sequences are conserved in another papovavirus, SV40 (see Rott *et al.*, 1988 for sequence comparison). Because dnaJ is essential for phage replication in *E. coli*, the presence of such sequences in T antigens is suggestive of dnaJ function being conserved for papovavirus replication.

Although not a eukaryote, the nitrogen fixing bacterium *Rhizobium fredii* contains a dnaJ-like protein (nolC) that has diverged from dnaJ (Krishnan and Pueppke, 1991). The first 80 amino acids of nolC (which functions in host range specificity) are 68% identical with *E. coli* dnaJ. The overall similarity, however, is 30%, and nolc has neither a distinct G/F domain nor the cysteine rich repeats found in dnaJ.

In general, eukaryotic dnaJ homologues sharing the more limited sequence similarity have probably become specialized. This has been demonstrated directly in yeast since phenotypes resulting from mutations in YDJ1/MAS5 are partially suppressed by *E. coli* dnaJ and Saccharomyces cerevisiae SIS1 (Caplan *et al.*, 1992a), and fully suppressed by *Atriplex nummularia* ANJ1 (Zhu *et al.*, 1993). Overexpression of YDJ1, however, cannot suppress phenotypes resulting from SIS1 gene deletion, suggesting that the latter has a more specialized function (Luke *et al.*, 1991). The SIS1 gene is essential, whereas the YDJ1 gene is not (Caplan and Douglas, 1991; Luke *et al.*, 1991).

INTRACELLULAR LOCALIZATION AND TISSUE SPECIFIC EXPRESSION

The intracellular localization of S. cerevisiae dnaJ homologues has been characterized. SCJ1, discovered by Blumberg and Silver (1991), cofractionates with mitochondria consistant with this protein having a mitochondrial targeting presequence. Both SIS1 and YDJ1 proteins are cytoplasmic but also partially associated with yeast nuclei, with SIS1 showing intranuclear and YDJ1 partial perinuclear localization by indirect immunofluorescence experiments. Further characterization using subcellular fractions revealed SIS1 to be partially released from the nuclei with ribonuclease (RNase) treatment. SIS1 protein also stably interacts with another protein termed p40 in a large macromolecular complex (Luke *et al.*, 1991). The perinuclear association of YDJ1 was correlated with enrichment at the cytosolic side of ER membranes (Caplan and Douglas, 1991). Enrichment of YDJ1 at membranes also correlates with the presence of the farnesyl lipid moiety, since a mutant YDJ1 protein that could not be farnesylated was found to have a reduced membrane binding capacity (Caplan *et al.*, 1992a).

SEC63 has been localized to the membrane of the ER where this integral membrane protein associates with several other proteins in the "translocon," a translocation apparatus that spans the membrane (Deshaies *et al.*, 1991). The J domain of SEC63 has recently been shown by Feldheim *et al.*, (1992) to protrude into the ER lumen where, genetic studies suggest, it interacts with the yeast Bip protein (see below). The most recently discovered yeast dnaJ-like protein, Zuotin, was purified from nuclear extracts and binds to oligonucleotides that adopt a putative Z-DNA conformation. Zuotin also has a sequence motif similar to histone H1 (Zhang *et al.*, 1992). Disruption of ZUO1 (Zuotin) results in a slow growth phenotype.

RESA protein of *P. falciparum* is synthesized as a soluble protein in the parasite. After invasion of human

erythrocytes, however, the protein is released and becomes localized to the cytoplasmic side of the plasma membrane, where it interacts with spectrin (Brown *et al.*, 1985; Foley *et al.*, 1991).

Two dnaJ-like proteins found in metazoans have been localized specifically to brain tissue; *Drosophila melanoganster* CSP (which also localizes to retina) and human HSJ1. Although these two proteins are unrelated, apart from their similarity to dnaJ, both are found in two forms that probably result from alternative splicing (Zinsmaier *et al.*, 1990; Cheetham *et al.*, 1992). Other human dnaJ homologues, HDJ1 and hsp40 have only been characterized in HeLa cells, and their tissue distribution is unknown. However, Hattori *et al.* (1992) have demonstrated that hsp40 localizes throughout the cell except under heat shock conditions, when it relocalizes to the nucleus (see below). In *A. nummularia*, Zhu *et al.* have localized ANJ1 mRNA to all cell types examined but detect it in higher concentration in stems and shoot tips, both proliferating tissues (Zhu and Hasegawa, unpublished data).

REGULATION OF HSP70 ATPASE ACTIVITY

The cellular role of the hsp70 family of proteins as molecular chaperones is well documented (Gething and Sambrook, 1992). Analysis of regulation of hsp70 chaperone activity has focused on the paradigm defined by studies in the prokaryote. Hsp70 proteins function by reversibly binding to nascent or partially denatured polypeptides to prevent nonproductive interactions either with themselves or other cellular components (Pelham, 1986; Beckmann *et al.*, 1990). The first step in this process is the recognition and binding of polypeptides that occurs at a putative polypeptide binding groove located in the C terminus of hsp70 proteins (Chappell *et al.*, 1987, Rippmann *et al.*, 1991). The C terminus is the least conserved domain in hsp70 proteins and variations in the amino acid composition of this region are thought to dictate specificity of substrate binding (Craig and Gross, 1991).

Dissociation of hsp70:polypeptide complexes requires ATP hydrolysis (Lewis and Pelham, 1985; Munro and Pelham, 1986; Flynn *et al.*, 1989; Liberek *et al.*, 1991; Cyr *et al.*, 1992). The ATPase domain is highly conserved and is located in the N terminal two thirds of hsp70 (Chappell *et al.*, 1987; Flaherty *et al.*, 1990). Because total intracellular ATP concentrations are in the millimolar range, hsp70 activity is not regulated by ATP availability. Instead, the binding of polypeptide substrates (Flynn *et al.*, 1989) or interaction with regulatory proteins (Liberek *et al.*, 1991; Cyr *et al.*, 1992) appear to modulate chaperone activity by stimulating the ATPase reaction.

Details of the mechanism of polypeptide stimulation of the ATPase activity of hsc70 and its regulation have recently been considered (Flynn *et al.*, 1989; Palleros *et al.*, 1991; Sedis and Hightower, 1992). After ATP hydrolysis in hsc70 ADP remains bound with a sixfold higher affinity than ATP. Thus ADP/ATP exchange must take place in order for cycles of polypeptide binding and release by hsc70 to occur (Palleros *et al.*, 1991). Polypeptide binding to hsc70-ADP increases these exchange rates which correlate with the magnitude of polypeptide stimulated ATP hydrolysis (Sedis and Hightower, 1992). Thus the ADP/ATP exchange rate limits ATP hydrolysis by hsc70 and polypeptide binding to hsc70 stimulates ATP hydrolysis by accelerating rates of ADP/ATP exchange (Sedis and Hightower, 1992).

The ATPase activity of dnaK also appears to be regulated by the rate of ADP/ATP exchange. The interaction of dnaK with grpE alone stimulates ATPase activity twofold (Liberek *et al.*, 1991), which is the same extent as that observed for polypeptide stimulation of hsc70 (Sedis and Hightower, 1992). However, the large increase in dnaK ATPase activity by the combination dnaJ and grpE (see above; Liberek *et al.*, 1991) suggests that other events in addition to stimulation of ADP/ATP exchange participate to regulate the ATPase activity of dnaK. The result of regulating this exchange on dnaK's chaperoning activity was demonstrated, since grpE stimulates the dnaJ and ATP dependent dissociation of dnaK-rhodanese complexes in in vitro protein folding assays (Langer *et al.*, 1992).

Study of the nature of hsp70 and dnaJ homologue interactions are just beginning. Deletion analysis indicates that the amino terminal 106 amino acids of dnaJ interact with dnaK to stimulate ATP hydrolysis. This region contains the J domain and the G/F region of dnaJ (Karazi and McMacken, personal communication). At present the dnaJ recognition site on dnaK has not been described although several possibilities are being considered. One is that dnaJ homologues interact with hsp70 at the polypeptide binding site to stimulate ATP hydrolysis. If this were the case then dnaJ family members should interfere with substrate binding activities of hsp70 proteins. A second possibility, which has received some experimental support, is that dnaJ cooperates to stabilize substrate binding to dnaK (Langer *et al.*, 1992). Also, a large molar excess of YDJ1 does not block binding of unfolded protein to hsp70^{SSA1} (Cyr and Douglas, unpublished data). Thus dnaJ homologues appear to interact at a distinct regulatory site on hsp70 protein and not directly at the polypeptide binding pocket. DnaJ homologue binding to this regulatory site could stimulate ATP hydrolysis by altering the conformation of the ATPase domain to increase rates of ADP/ATP exchange. Alternatively, the dnaJ homolog binding could stimulate hsp70 ATPase activity by altering the conformation of the ATPase domain in a manner analogous to the mechanism for the stimulation of RAS GTPase activity by GTPase Activating Proteins (reviewed in Lowy *et al.*, 1991).

If polypeptide binding serves to stimulate ATP hydrolysis by hsp70 and this stimulation is sufficient to dissociate bound polypeptides, why then does the cell require dnaJ homologues to carry out similar functions? This question is answered in part by observation that some hsp70-polypeptide complexes are stable in the presence of ATP (Pelham, 1990; Chirico, 1992; Cyr *et al.*, 1992). Thus dnaJ homologues are important for regulated dissociation of polypeptides from hsp70 that would be otherwise stable. Indeed, ATP alone has little effect on $hsp70^{SSA1}$:carboxy methylated lactalbumin (CMLA) complex stability, but in combination with YDJ1 stimulates dissociation (Cyr *et al.*, 1992).

ROLE IN INTRACELLULAR PROTEIN TRANSPORT AND ORGANELLE BIOGENESIS

Although dnaJ-like proteins have been discovered in several different eukaryotes, a more complete picture of their varied functions is documented from studies in yeast, where the complementary approaches of genetics and biochemistry have been exploited. Of the five known *S. cerevisiae* dnaJ-like proteins (Table 1), four have been found to function in transport of other polypeptides into organelles.

Both YDJ1 and SEC63 have been shown to function in two separate transport pathways. Mutants in SEC63 were found through different genetic screens to affect transport of other polypeptides across the ER membrane and into the nucleus (Rothblatt *et al.*, 1989 and Sadler *et al.*, 1989). YDJ1 functions in the cytosol to transport polypeptides targeted to both mitochondria and ER (Atencio and Yaffe, 1992; Caplan *et al.*, 1992a). This common function in two separate pathways suggests a role for chaperone proteins at an early step in the targeting and sorting of polypeptides following their synthesis. Both YDJ1 and SEC63 appear to function with hsp70 proteins. YDJ1 probably interacts with the cytosolic $hsp70^{SSA1}$ and $hsp70^{SSA2}$ proteins, whereas SEC63 appears to interact with KAR2, the yeast Bip in the lumen of the ER (see below). Cytosolic Hsp70 proteins are thought to maintain polypeptides in a transport competent conformation before their import into mitochondria or translocation across the ER membrane (see Gething and Sambrook, 1992 and Hartl *et al.*, 1992 for reviews). The precise nature of the transport competent state is unknown, but peptides bound to dnaK have been shown to be in an extended conformation (Landry *et al.*, 1992), which is also necessary for polypeptide translocation into mitochondria or the ER.

Using pulse labeling and immunoprecipitation techniques, Atencio and Yaffe (1992) and Caplan *et al.* (1992a) have demonstrated that efficient import of precursor proteins into mitochondria depends on YDJ1. Like hsp70 proteins, YDJ1 appears to have a posttranslational function in mitochondrial import. This was demonstrated by monitoring the import of mitochondrial precursor proteins under conditions when translation and import had become uncoupled. Under these conditions import of precursors was impaired in a YDJ1 mutant strain compared with the wild type (Caplan *et al.*, 1992a). Smith and Yaffe (1991) also observed posttranslational import of mitochondrial precursors to be impaired in a MAS3 mutant strain. The MAS3 gene encodes the heat shock transcription factor that regulates the synthesis of heat inducible genes, which includes YDJ1 (Atencio and Yaffe, 1992). Interestingly, overexpression of YDJ1 (MAS5) by itself, from a non-heat shock promoter is insufficient to reverse the import defect in the MAS3 mutant strain, and co-expression with $hsp70^{SSA1}$ results in only a partial reversal (Atencio and Yaffe, personal communication). A role for SCJ1 in transport was invoked when its overexpression caused the missorting to mitochondria of a polypeptide that would otherwise be nuclear localized (Blumberg and Silver, 1991).

YDJ1 protein facilitates translocation of α-factor but not KAR2, carboxypeptidase Y (CPY) nor diaminopeptidylpeptidase B across the ER membrane (Caplan *et al.*, 1992a). HSP70, however, appears to facilitate the translocation of several proteins including CPY (Deshaies *et al.*, 1988). These data may reflect differences between the function of YDJ1 and hsp70 in posttranslational transport processes. Alternatively, this variance may result from differences in experimental approach, since Deshaies *et al.* (1988) monitored the effects of hsp70 depletion over several hours, while Caplan *et al.* (1992a) observed the mutant phenotypes within 30 min at the restrictive temperature.

The role of YDJ1 appears to be evolutionarily conserved, since *E. coli* dnaJ can suppress the transport defects when expressed in a YDJ1 mutant strain (Caplan *et al.*, 1992a). Secretion in *E. coli* is facilitated by both dnaJ and dnaK (Wild *et al.*, 1992). The mechanism by which hsp70 and YDJ1 proteins function together in these transport processes is uncertain, but two possibilities may be proposed. First, YDJ1p may function by stimulating hsp70s ATPase resulting in dissociation of hsp70:polypeptide complexes (as discussed above), freeing the polypeptide for interaction with a putative receptor or other component of the translocation apparatus. This idea is supported by the failure of a purified mutant YDJ1 protein to stimulate $hsp70^{SSA1}$ ATPase in vitro (Caplan *et al.*, 1992a). Furthermore, the enhanced localization of YDJ1 at cellular membranes due to farnesylation may regulate dissociation of hsp70-polypeptide complexes at the point of import/translocation. However, the precise function of YDJ1 farnesylation appears to be complex. Cell fractionation studies have localized YDJ1 to microsomes but not mitochondria. Furthermore, high intracellular concentrations of both *E. coli* dnaJ and *S. cerevisiae* SIS1 proteins can fully substitute for YDJ1 function in intracellular transport, yet neither is prenylated (Caplan *et al.*, 1992a).

A second possible role for YDJ1 in intracellular protein transport may be that of a molecular chaperone rather than as a regulator of hsp70. This function would involve YDJ1p itself binding to "substrate" proteins independent of or in conjunction with hsp70 and stabilizing them in the transport competent conformation. While there is mounting evidence that eukaryotic dnaJ homologues interact with hsp70 proteins, their putative chaperoning functions remain relatively uncharacterized. In *E. coli*, dnaJ stably interacts with several proteins including λP protein (Alfano and McMacken, 1989; Liberek *et al.*, 1990), bacteriophage P1 repA protein (Wickner *et al.*, 1991) and *E. coli* σ32 (Gamer *et al.*, 1992). dnaK also interacts with these proteins suggesting that dnaJ and dnaK function together but may bind independently to their target proteins. This is consistent with the results of Langer *et al.* (1992), who demonstrated that dnaK and dnaJ synergistically prevent aggregation of rhodanese as it refolds.

While YDJ1 may have a general role in polypeptide transport events in the cytosol, SEC63 functions as part of a translocation apparatus in the membrane of the ER (Sadler *et al.*, 1989; Deshaies *et al.*, 1991; Sanders *et al.*, 1992). Different mutations in the SEC63 gene cause defects in translocation of pre-proteins into the ER (Toyn *et al.*, 1988; Rothblatt *et al.*, 1989) and also the nucleus (Sadler *et al.*, 1989). However, the J domain of SEC63, which protrudes into the lumen, appears to function solely in the ER transport pathway (Rothblatt *et al.*, 1989; Sadler *et al.*, 1989; Feldheim *et al.*, 1992). Genetic evidence indicates SEC63 interacts with KAR2. The mutation in SEC63 that blocks ER translocation is located in the conserved J domain (Feldheim *et al.*, 1992), and allele specific suppressors of this mutation have been mapped to the KAR2 gene product (Scidmore and Rose, personal communication). The region of SEC63 that functions in nuclear protein localization, appears to be restricted to the cytoplasmic C terminal half of the protein (Nelson *et al.*, 1993).

Although the details of SEC63 and KAR2 interaction are unclear, both proteins are required together for preprotein translocation. It has been suggested that Bip may function in the assembly of SEC63 with other translocon components and also in later stages of translocation (Rappaport, 1992; Sanders *et al.*, 1992). Insights from mitochondrial import studies would suggest that KAR2 (and also perhaps SEC63) is required to power the nascent protein through the channel. The requirement for KAR2 in translocation is quite specific, because a normally cytosolic hsp70 (SSA1) cannot functionally substitute for it in an in vitro reconstitution assay (Brodsky *et al.*, 1993). Also, YDJ1 does not stimulate the ATPase activity of (mammalian) BiP but does stimulate hsp70^{SSA1} (Cyr and Douglas, unpublished data). The SEC63 and KAR2 proteins copurify (Brodsky and Schekman personal communication), indicating that they can stably interact, whereas the YDJ1 and hsp70^{SSA1} proteins do not (Cyr *et al.*, 1992).

ROLE IN THE STRESS RESPONSE

Because synthesis of *E. coli* dnaJ is upregulated in response to heat stress (Bardwell *et al.*, 1986), one might expect similar regulation of eukaryotic dnaJ homologues. Indeed, Hattori *et al.*, 1992 have characterized hsp40 to be a highly inducible dnaJ-like protein. Hsp40 is synthesized at very low levels at the normal growth temperature of 37°C and, as stated above, is localized throughout the cell. Upon heat shock, however, synthesis of the protein is increased and hsp40 relocalizes to the nucleus and becomes concentrated in the nucleoli. Heat shock induced relocalization to the nucleus is also a property of hsp70 (see Pelham, 1990 and references therein) and hsp26 (Rossi and Lindquist, 1989) proteins. On recovery from heat shock, hsp70 can be released from the nucleus in a manner requiring ATP hydrolysis (Lewis and Pelham, 1985). This requirement suggests a possible role for a dnaJ-like protein, since it could function by stimulating hsp70 dissociation from nuclear components. Another possible role for a dnaJ like protein may also be that of molecular chaperone, binding to nuclear components and stabilizing them against denaturation or aggregation. Recent work by Gragerov *et al.*, (1992) suggests that dnaK and dnaJ function together in preventing protein aggregation in *E. coli*.

A functional role for a dnaJ homologue in the stress response that is hsp70 independent has been suggested by Zhu *et al.* (1993). These investigators observed transcriptional upregulation of ANJ1 upon salinity stress in tissue culture, under conditions when transcriptional levels of hsp70 were unchanged. Since both ANJ1 and hsp70 are upregulated co-ordinately upon heat stress, this result suggests a separate function for ANJ1 when osmolarity changes.

In *S. cerevisiae*, both SIS1 and YDJ1 transcriptional levels are increased twofold upon heat shock (Luke *et al.*, 1991; Atencio and Yaffe, 1992), suggesting that these proteins play a role in the stress response. Also, raising the incubation temperature of wild-type *S. cerevisiae* to 37°C for 1 h leads to a redistribution of YDJ1 from 20 to 50% membrane bound. This relocalization requires farnesylation of YDJ1, as does cell viability at 37°C (Caplan *et al.*, 1992b).

CONCLUDING REMARKS

The discovery of eukaryotic dnaJ homologues supports the view that hsp70 function is regulated, or at least assisted by other proteins. While this may have been predicted based on previous studies in prokaryotes, the diversity of eukaryotic dnaJ-like proteins is surprising. It seems that hsp70 structure has remained well conserved, while the apparently modular nature of dnaJ

has been exploited by evolution. It is, therefore, the J domain itself that bears the dnaJ signature and not necessarily the cysteine rich or G/F domains shown in Figure 1. Whether this domain in proteins like zuotin or csp is sufficient for interaction with hsp70, as it appears to be for SEC63, remains to be determined. If it is, then this domain in these proteins probably catalyzes specific protein assembly/dissasembly events.

The mechanism of how dnaJ and hsp70 function together is still to be resolved, and the role of dnaJ's stimulation of hsp70's ATPase is central to this question. It seems unlikely that dnaJ proteins exclusively catalyze dissociation of hsp70:polypeptide complexes since prokaryotic dnaJ appears to have chaperoning functions of its own. Indeed, activation of phage RepA protein appears to require dnaJ as the chaperone and dnaK for catalyzing complex dissociation (Wickner *et al.*, 1991). The function of prokaryotic dnaK and dnaJ also requires grpE, for which no eukaryotic homologue has been found. However, the recent discovery of eukaryotic chaperonins localized to the cytosol (Lewis *et al.*, 1992; Yaffe *et al.*, 1992) indicates that protein folding pathways involving hsp70 and dnaJ homologues may be conserved.

Zhu *et al.* (1993) has observed antigenically related proteins of SEC63 and SCJ1 in a higher plant. We suspect, therefore, that the five currently known dnaJ-like proteins found in yeast may be common to all eukaryotic cells for protein folding and assembly in different organelles. The discovery of tissue specific homologues of dnaJ further leads to the notion that particular protein folding events in vivo are managed by specific chaperone proteins. Extrapolation of this idea leads to the prospect of many dnaJ-like proteins being expressed, not only in restricted cell types, but also at specific stages of development in complex organisms. This may be tested in the near future as the DNA sequences of whole genomes becomes available.

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