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Review

The diversity of the DnaJ/Hsp40 family, the crucial partners for Hsp70 chaperones

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Abstract. DnaJ/Hsp40 (heat shock protein 40) proteins have been preserved throughout evolution and are important for protein translation, folding, unfolding, translocation, and degradation, primarily by stimulating the ATPase activity of chaperone proteins, Hsp70s. Because the ATP hydrolysis is essential for the activity of Hsp70s, DnaJ/Hsp40 proteins actually determine the activity of Hsp70s by stabilizing their interaction with substrate proteins. DnaJ/Hsp40 proteins all contain the J domain through which they bind to Hsp70s and can be categorized into three groups, depending on the presence of other domains. Six DnaJ homologs have been identified in *Escherichia coli* and 22 in *Saccharomyces cerevisiae*. Genome-wide analysis has revealed 41 DnaJ/Hsp40 family members (or putative members) in humans. While 34 contain the typical J domains, 7 bear partially conserved J-like domains, but are still suggested to function as DnaJ/Hsp40 proteins. DnaJA2b, DnaJB1b, DnaJC2, DnaJC20, and DnaJC21 are named for the first time in this review; all other human DnaJ proteins were dubbed according to their gene names, e.g. DnaJA1 is the human protein named after its gene DNAJA1. This review highlights the progress in studying the domains in DnaJ/Hsp40 proteins, introduces the mechanisms by which they interact with Hsp70s, and stresses their functional diversity.

Keywords. DnaJ, Hsp40, Hsp70, chaperone, heat shock.

Introduction

Molecular chaperones are involved in protein translation, folding, unfolding, translocation, and degradation. Heat shock protein 70s (Hsp70s) are key components of the cellular chaperone network. The expression of the Hsp70 family is either inducible by various stresses or constitutive. Some Hsp70s are differentially regulated at various stages of development. For example, Hsp70-2 and Hsc70t have been shown to play special roles in spermatogenesis [1, 2]. Hsp70s bind selectively to unfolded hydrophobic regions of substrate polypeptides, and their activity is controlled by the cycle of ATP binding, hydrolysis, and nucleotide exchange [3]. ATP hydrolysis converts Hsp70s from an open state with high association and dissociation rates for substrates to a closed state with low exchange rates [4]. This cycle is regulated by co-chaperones, such as members of the DnaJ family (also referred to as heat shock protein 40s, Hsp40s), which stimulate the ATP hydrolysis [5, 6]. Because the ATP hydrolysis is essential for the activity of Hsp70s, DnaJ/Hsp40 proteins actually determine the activity of Hsp70s by stabilizing their interaction with substrates. Numerous reports have demonstrated that Hsp70s and DnaJ/Hsp40s are implicated in various human diseases, such as neurodegenerative disorders [7–9].

DnaJ was first known to stimulate the ATPase activity of DnaK, the bacterial Hsp70 homolog, and to help replicate

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λ phage DNA in host cells [10, 11]. A large number of DnaJ homologs have been identified in both prokaryotes and eukaryotes. Six DnaJ homologs have been found in *Escherichia coli*, and 22 in *Saccharomyces cerevisiae* [12]. In mammals, more than 20 DnaJ homologs with diverse activities have been reported, but the exact numbers of such proteins in mammalian genomes remain unknown. Some of them may also regulate the activity of other chaperones, such as Hsp90. For example, the recycling of incompletely folded polypeptides from Hsp90 onto an Hsp70 protein might be regulated by the mammalian DnaJ protein TPR2/TTC2 /DJC7 [13]. Certain DnaJ proteins bind directly to unfolded protein substrates through their zinc fingers and C-terminal domains. *E. coli* DnaJ, the yeast cytosolic DnaJ protein Ydj1, and the yeast mitochondrial DnaJ protein Mdj1 can all bind to the substrates by themselves [14–16].

Structure and organization of domains in the DnaJ/Hsp40 family

All the members of the DnaJ/Hsp40 family contain the J domain through which they bind to their partner Hsp70s [17, 18]. With few exceptions, this domain is usually present at the N-terminal region of the proteins. The J domain is a 70-amino acid sequence consisting of four helices and a loop region between helices II and III that contains a highly conserved tripeptide of histidine, proline, and aspartic acid (the HPD motif) [19] (Fig. 1).

In addition to the J domain, many DnaJ/Hsp40 proteins contain other conserved regions, which are critical to their functions [20]. Based on the difference in these regions,

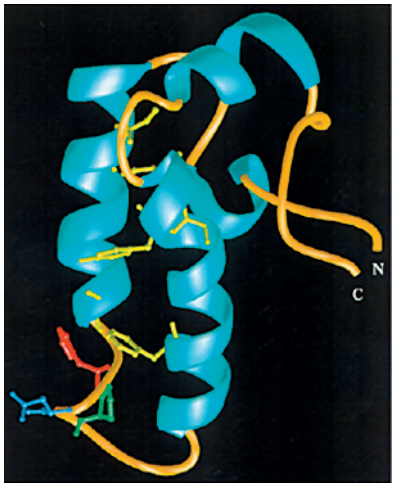

Figure 1. The structure of the HDJ-1 J domain (residues 1–76). The chain termini are labeled by the letters N and C. Helices I to IV (cyan), the loop and turns connecting the helices (orange), and the HPD tripeptide of His31 (red), Pro32 (green) and Asp33 (blue) are illustrated [19]. Six highly conserved residues at 19, 27, 44, 50, 51, and 54, which may stabilize the J domain, are shown in yellow.

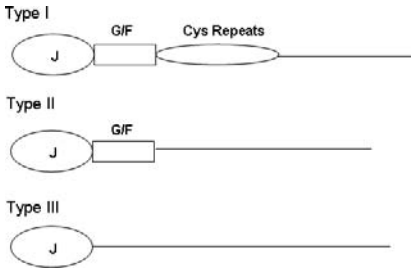

Figure 2. Classification of DnaJ/Hsp40 proteins. Depending on the presence of the Gly/Phe-rich region (G/F) and/or the cysteine repeats, a DnaJ/Hsp40 protein can be categorized as type I, II, or III.

DnaJ proteins can be categorized into three groups [21] (Fig. 2). Type I proteins are similar to *E. coli* DnaJ with the J domain, the Gly/Phe-rich region, and the cysteine repeats. Type II proteins possess the J domain and the Gly/Phe-rich region, but lack the cysteine repeats. Type III proteins do not have any of these conserved regions other than the J domain. Although type I and II proteins are different in their conserved regions, it seems that both types function similarly and bind to non-native substrates [22]. In contrast, the type III proteins may not bind to non-native polypeptides and thus should not function as molecular chaperones on their own.

Aside from the above conserved domains or regions, some DnaJ family members contain additional domains, which may determine the functional diversity of DnaJ proteins [23, 24]. For example, the mammalian DnaJ protein ERdj5/JPD1 promotes the formation of appropriate disulfide bonds of endoplasmic reticulum (ER) proteins, because of the presence of both the protein disulfide isomerase-like domain and the J domain; the latter sequesters the ER-associated Hsp70, BiP [25, 26]. Recently, a C-terminal region in DnaJ proteins was found to be essential for their dimerization and chaperone activity [27, 28].

Genome-wide analysis and classification of DnaJ/Hsp40 family members

DnaJ/Hsp40 proteins in an organism are present in much larger numbers than Hsp70 proteins. Six DnaJ homologs have been found in *E. coli.* In higher organisms, there are more such proteins. For example, there are at least 10 DnaJ-related proteins in *Plasmodium falciparum*. As indicated in a recent review [12], the genome of *S. cerevisiae* has 22 proteins that bear well-conserved J domains. Many mammalian DnaJ-like proteins have been reported, but the exact numbers are still unknown. In the database of the Human Genome Resource, 140 human protein entries are annotated as DnaJ-related. But many of them represent repeated entries (under different names) of the same genes, and some do not contain any DnaJ domains.

To determine the actual number of DnaJ-related proteins, we performed alignments for all these protein entries using the ClustalW program (Lasergene, DNASTAR Inc.). To ensure that our searches covered all the DnaJ/Hsp40 proteins in the human genome, we further carried out Blast searches for the HPD-containing proteins, and then looked for novel J domain-containing proteins. As a result, 41 J domain-containing proteins (or putative proteins), all of which contain the HPD motif, were identified in the human genome (Table 1). As shown in Figure 3, 34 of them bear typical J domains, one (DnaJC5G) has a J-like domain with a 16-residue insertion in helix II, and six (DnaJC6, 8, 13, 15, 19, and 20) contain J-like domains, whose helices III and IV are poorly conserved. Among these J-like domain-containing proteins, all but DnaJC5G and DnaJC8 have been suggested to function as DnaJ/Hsp40 proteins [29–33].

In addition, TSARG6 (DnaJB13) is highly homologous (45% identical) to the well-defined DnaJ protein, DnaJB5, over the 348-amino-acid region including the J domain, but it has the HPL sequence in place of the HPD motif. Since the HPD residues are critical to the function of the J domain, it is uncertain whether DnaJB13 can serve as a typical DnaJ protein. However, its sequence similarity with other DnaJ proteins suggests that it is probably functionally related to this family. There are most likely more such proteins in the human genome. In *S. cerevisiae*, Jip3/Pam16 encoded by the gene Yjl104w is 27% identical to the helices II and III region of the DnaJ protein Pam18, and also lacks the HPD residues. But its corresponding sequence DKE is predicted to form a HPD-like turn between the two helices [12]. It seems that Jip3 promotes Hsp70 recruitment without stimulating ATP hydrolysis [34].

The DnaJ/Hsp40 proteins in Table 1 were named based on the gene names in GenBank, where the letters D, N, and A in gene names are all in capitals, and A, B, and C represent type I, II, and III DnaJ domains, respectively. To distinguish the proteins from their genes and also to preserve the traditional nomenclatures of DnaJ proteins, we designated the proteins as DnaJ+type of domain (A, B, or C)+number. DnaJA2b, DnaJB1b, DnaJC2, DnaJC20, and DnaJC21 are identified and named for the first time in this review; all other proteins were dubbed according to their gene names, e.g. DnaJA1 is the human protein named after its gene DNAJA1.

Cellular localization and tissue distribution of DnaJ proteins

The function of DnaJ proteins is dependent on their localization. In a cell, they have been found in the cytosol, the nucleus, endosomes, mitochondria, the ER, and ribosomes (Table 1). In multicellular organisms, they can be expressed either specifically in a tissue or universally in all tissues. DnaJB2/HSJ1 has been shown to be preferentially expressed in brain [35], and rat rDJL is only detectable in liver and testis [36]. rDJL probably functions as a co-chaperone of Hsc70 and participates in vesicular trafficking during sperm acrosomogenesis [36]. In certain cases, the tissue-specific expression is achieved through alternative splicing of DnaJ transcripts. One of the two alternatively spliced isoforms of DnaJA1 is known to be highly expressed in testis and spermatogenic cells, whereas the other spliced form was highly abundant in brain and other tissues [37, 38].

The mechanisms by which DnaJ/Hsp40 proteins interact with Hsp70s

Hsp70 chaperones contain an amino-terminal ATPase domain and a carboxy-terminal polypeptide-binding domain. They recognize short hydrophobic polypeptide stretches and are regulated by the nucleotide-bound states. In the ATP-bound state, an Hsp70 protein exchanges polypeptide substrates rapidly, and in the ADP-bound state, it stably binds the substrates. In general, Hsp70s have very low basal ATPase activity. A co-chaperone DnaJ/Hsp40 protein can bind to substrate polypeptides by itself, and its J domain promotes ATP hydrolysis by the Hsp70 protein and the transfer of polypeptides to the Hsp70 protein. Nucleotide exchange returns the Hsp70 protein to the ATP-bound state [39, 40].

The nature of the interaction between DnaJ/Hsp40 proteins and Hsp70 proteins determines the function of these two families of proteins. In bacteria, the Hsp70 protein DnaK has a binding surface for DnaJ on its ATPase domain [41]. As analyzed in rat Hsc70, the C-terminal region of Hsp70s also contains a binding site for Hsp40 [42]. However, the ATPase domain is most likely the major binding site for DnaJ proteins. As for the sites on DnaJ proteins that bind to Hsp70 proteins, it is well established that the J domain, particularly the HPD motif, binds to Hsp70s [43]. Although the structure of the ATPase domain of Hsp70 proteins has been solved [44, 45], that of the intact Hsp70 proteins remains to be determined. Based on the published results from nuclear magnetic resonance (NMR) and mutant binding studies, a model structure of the complex with the Hsp70 protein, Hsc70, and the DnaJ/Hsp40 protein, auxilin (DnaJC6), has been constructed [46]. In this model (Fig. 4), H874 of the HPD loop of the auxilin J domain forms transient hydrogen bonds with A148, Y149, and N174 of the Hsc70 ATPase domain, whereas D876 of the HPD loop forms a salt bridge with R171 of the Hsc70 ATPase domain. D876 is also close to another ATPase arginine, R155. Both arginines are very highly conserved in Hsp70 family proteins [46].

Table 1. Forty-one DnaJ/Hsp40 proteins in the human genome.

Protein [1]	Amino acids	Other names	Homologs in *S. erevisiae* [2]	Localization	Function or predicted function
DnaJA1	397	DJ-2, DjA1, HDJ2, HSPF4	Ydj1	Cytosol	androgen signaling
DnaJA2	412	CPR3, DNJ3, HIRIP4	Ydj1	Cytosol	
DnaJA2b	415	AAB69313, cpr3	Ydj1		
DnaJA3	480	TID1, hTid-1	Mdj1	Mitochondria	
DnaJA4	426	dj4	Ydj1	Cytosol, nuclei	
DnaJA5	576		none		
DnaJB1	340	HSPF1, Hsp40	Sis1	Cytosol	protein degradation
DnaJB1b	339	caa44287	Sis1		
DnaJB2	351	HSJ1, HSPF3	Sis1		protein degradation
DnaJB4	337	DNAJW, Djb4, HLJ1	Sis1		
DnaJB5	386	Hsc40	Sis1		
DnaJB6	335	HHDj1, HSJ-2, MSJ-1	Sis1		protein folding
DnaJB7	309	HSC3	none		
DnaJB8	232	MGC33884	none		
DnaJB9	223	ERdj4, MDG1	none	ER, nuclei	
DnaJB11	358	ABBP-2, ERdj3, hDj9	Sis1	ER	protein folding, mRNA editing
DnaJB12	375	DJ10	none		
DnaJB14	379	EGNR9427	Hlj1		
DnaJC1	115	DNAJL1, ERdj1, HTJ1	none	ER, nuclei	translocation and radation
DnaJC2	760	SEC63L, ERdj2	Sec63	ER	protein translocation
DnaJC3	504	HP58, P58IPK, PRKRI	HRC558	ER	translation, viral pathogenesis
DnaJC4	311	HSPF2, MCG18	none		
DnaJC5	198	CSP	none		exocytosis
DnaJC5b	199	CSP-beta	none		
DnaJC5G	189	CSP-gamma	none		
DnaJC6	913	DJC6, auxilin	none		endocytosis
DnaJC7	484	TPR2, TTC2	STI1		protein folding
DnaJC8	264	SPF31	none		
DnaJC9	260	JDD1, SB73	none		
DnaJC10	793	ERdj5, JPDI	none	ER	
DnaJC11	559	FLJ10737	none		
DnaJC12	107	JDP1	none		estrogen signaling
DnaJC13	2232	RME-8	none	endosome	endocytosis
DnaJC14	702	DRiP78, HDJ3, LIP6	none	ER	protein transport
DnaJC15	150	DNAJD1, HSD18, MCJ	none		
DnaJC16	782	RP4–680D5.1	none		
DnaJC17	304	FLJ10634	none		
DnaJC18	358	MGC29463	none		
DnaJC19	116	TIM14, TIMM14	none	mitochondria	protein import
DnaJC20	235	HSC20, HscB	Jac1	mitochondria	protein assembly, energetics
DnaJC21	202	ZRF1, MPP11	Zuo1	ribosome	translation, folding

[1] DnaJA2b, DnaJB1b, DnaJC2, DnaJC20, and DnaJC21 are named for the first time in this review; all other proteins are dubbed according to their gene names, e.g. DnaJA1 represents the protein for the gene DNAJA1. All the proteins, which are encoded by distinct genes, contain typical J domains or J-like domains.

[2] The homologs in *S. cerevisiae* were obtained based on their significant homologies with the corresponding human proteins over the sequences beyond the J domains.

Different DnaJ proteins may interact with distinct Hsp70 proteins. In *E. coli*, there are three Hsp70 proteins, including DnaK, Hsc62/HscC, and Hsc66, and at least six DnaJ proteins, including DnaJ, CbpA, DjlA, Hsc20, DjlB, and DjlC/Hsc56. While Hsc66 mainly interacts with Hsc20, DnaK binds to all the DnaJ proteins, except DjlB, and Hsc62/HscC associates with all the DnaJ proteins, except DjlA. In the cytosol of budding yeast, there are also three Hsp70 proteins, Ssa, Ssb, and Ssz, and 14 distinct DnaJ proteins. As observed in *E. coli*, a specific Hsp70 protein in yeast can interact with either only one DnaJ protein or multiple such proteins [12].

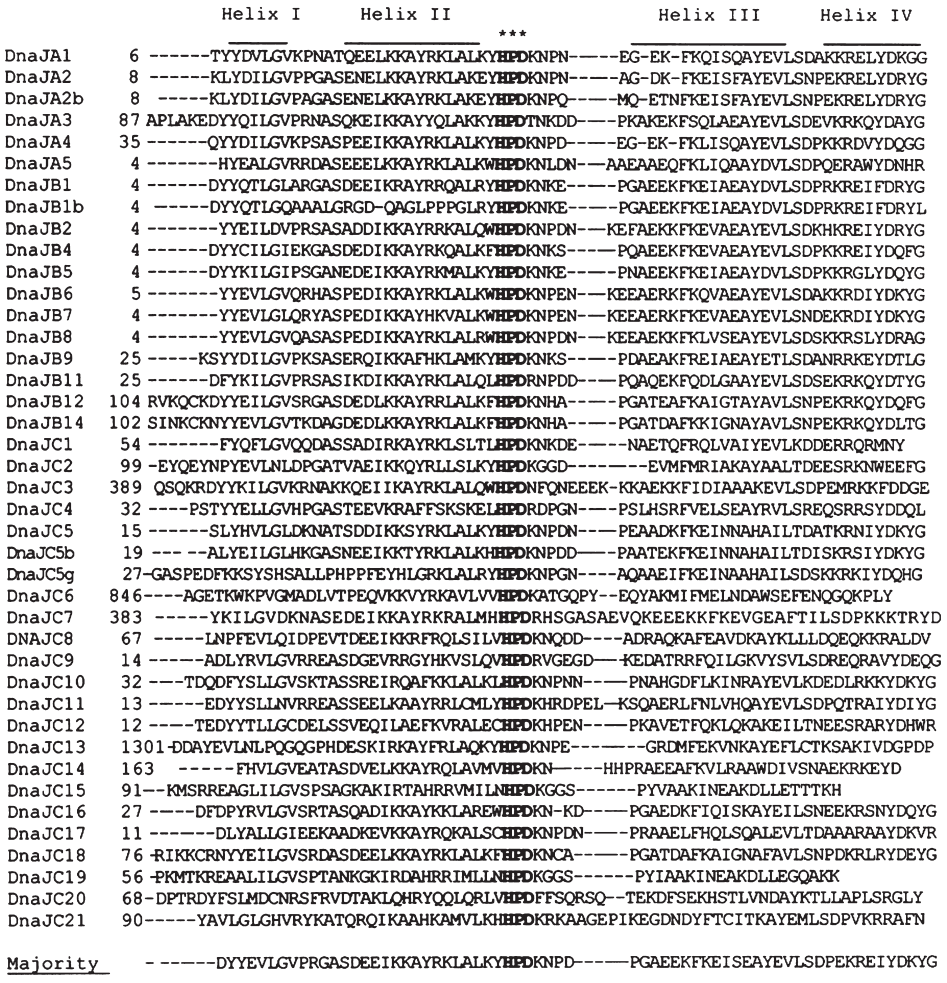

Figure 3. Alignment of J domains or J-like domains in 41 human DnaJ family members. These proteins mostly bear similar lengths of insertions among the four helices of their J domains as indicated. The HPD motif is marked with ***.

Functional diversity of DnaJ/Hsp40 proteins

It is well established that a DnaJ/Hsp40 protein acts as a co-chaperone for certain Hsp70 proteins. However, DnaJ in bacteria and certain other members of the DnaJ/Hsp40 family can be chaperones by themselves through binding to certain unfolded proteins and nascent chains [47]. DnaJ/Hsp40 proteins can also regulate other chaperones, such as the 90-kDa heat shock protein Hsp90. Hsp70 and Hsp90 cooperate in the folding of many substrates in the eukaryotic cytosol. The DnaJ/Hsp40 protein TPR2 (DnaJC7) has been suggested to mediate the retrograde transfer of substrates from Hsp90 onto Hsp70 [13].

Protein folding, refolding, and assembly

The folding of the newly translated polypeptides or the refolding of unfolded proteins into well-defined three-dimensional conformations is required for the formation or maintenance of functional proteins. DnaJ/Hsp40 proteins are crucial for the folding and refolding of proteins. Since there are some excellent recent reviews addressing the role of these proteins in protein folding in the cytosol and mitochondria [39, 48], this review focuses on the ER-related folding, refolding, and assembly of proteins.

Approximately one-third of newly synthesized proteins are transported into the lumen of the ER for folding and oligomerization and are then secreted for distal compartments. ER molecular chaperones, including DnaJ/Hsp40 proteins, can prevent the aggregation of these proteins and help them fold and assemble correctly [39, 49]. The Hsp70 homolog BiP and its co-factors are among the major chaperones in the mammalian ER and play a critical role in protein folding and assembly [47, 50]. BiP is up-regulated during ER stress [51]. Like other Hsp70 proteins, BiP switches between ATP and ADP-bound states to bind and release substrates. In the ATP-bound state, BiP binds and releases unfolded substrates rapidly. Hydrolysis of ATP stabilizes its association with unfolded proteins, and thus inhibits their aggregation. The release of ADP and rebinding of ATP lead to release and folding

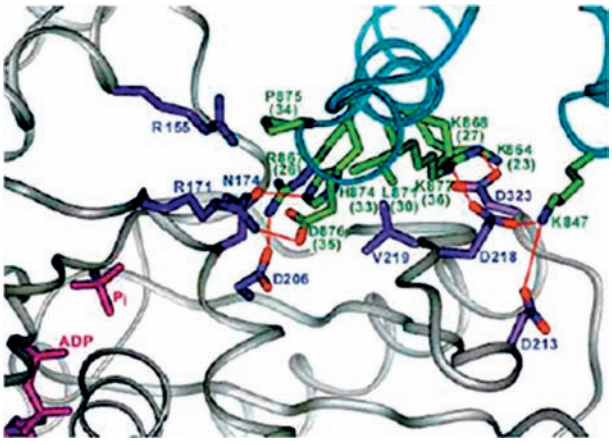

Figure 4. Potential contacts between the ATPase domain of Hsc70 and the J domain of auxilin. The auxilin J domain backbone (green-blue) and the ATPase backbone (gray) are shown [46]. Auxilin residues, green (DnaJ numbers in parentheses); ATPase residues, purple. Potential interprotein hydrogen bonds, dashed red lines.

of the nascent protein. DnaJ/Hsp40 proteins regulate the ATPase cycle of BiP by stimulating ATP hydrolysis [47]. Three ER-localized DnaJ homologs have been identified in yeast and at least five in mammals. The yeast ER DnaJ family members are each specific for regulating different functions of the yeast homolog of BiP, Kar2. Sec63, an essential transmembrane protein, assists Kar2 in translocating nascent proteins into the ER [52], and Scj1 helps Kar2 to fold and to assemble proteins in the ER lumen [53]. Jem1, by interacting with Kar2p, plays an important role in nuclear membrane fusion during mating [54]. All mammalian ER DnaJ homologs, such as ERdj1/Mtj1, ERdj2/hSec63, ERdj3/HEDJ/ERj3/ABBP-2, ERdj4/Mdg1, and ERdj5/JPDI, bind BiP *in vitro* and stimulate its ATPase activity [25, 26, 55–61]. ERdj3/ERj3, a soluble ER luminal protein [58], has been found to be a component of unassembled Ig heavy chain:BiP complexes and to bind directly to a number of nascent unfolded substrates, suggesting that it may be a co-factor for BiP functions in protein folding and assembly [60].

The critical role of P58^{IPK} in stress responses and the regulation of translation

Various cellular stresses may prevent protein folding, cause an accumulation of misfolded or malfolded proteins in the ER, and thus potentially induce cellular damage [62]. Phosphorylation of the eukaryotic translation initiation factor (eIF)2α is a key step for cells to respond to environmental stresses [63]. During virus infection and the unfolded-protein response (UPR) in the ER, protein kinase R (PKR, an interferon-induced, double-stranded RNA-activated kinase) and PKR-like ER kinase (PERK, an eIF2α kinase) are activated to reduce global protein synthesis (including the synthesis of viral proteins). PKR and PERK share common substrates, since they contain similar kinase domains (40% identity over the sequences of 189 residues). However, their stress signal-sensor domains and cellular localization (the cytosol and the ER, respectively) are different [64]. The mammalian DnaJ protein, DnaJC3/P58^{IPK}, is an important component of a negative feedback loop to inhibit eIF-2α signaling and to attenuate the UPR [65]. P58^{IPK} is induced through an ER stress response element in its promoter region, and inhibits PERK [66]. Upon influenza virus infection, P58^{IPK} can also downregulate the antiviral activities of PKR [67], thereby ensuring continual synthesis of viral proteins. The influenza virus therefore seems to find a way to escape from the host defense system. However, P58^{IPK} is unlikely to have evolved as a cellular gene to aid viral replication. The activation of P58^{IPK} in response to both the UPR and influenza virus infection suggests that P58^{IPK} might play a role in multiple stress responses [68]. Since the J domain of P58^{IPK} is required for the inhibition of PKR *in vivo* [69], P58^{IPK} most likely functions as a co-chaperone cooperating with Hsp70 to regulate PKR. P58^{IPK} has been demonstrated to stimulate the ATPase activity of Hsp70 [70], a process probably mediated by the C-terminal J domain of P58^{IPK}.

P58^{IPK} is universally expressed in the tissues of mice and humans, with especially high levels in liver and pancreas [71]. Mutant mice with P58^{IPK} deletion showed a gradual onset of glucosuria and hyperglycemia associated with increasing apoptosis of pancreatic islet cells [72], consistent with the critical role of P58^{IPK} in stress responses.

The role of Zuo1 in translation

Ribosomes play an essential role in converting genetic information into functional proteins by translating mRNA into linear polypeptides. However, ribosomes are also essential to fold the nascent polypeptides into correct three-dimensional conformations. The specialized molecular chaperones bind to nascent polypeptides and bridge translation with protein folding. In yeast, certain members of the Hsp70 and Hsp40 families are also found to associate with ribosomes. The yeast Hsp70 proteins, Ssb1 and Ssb2, are functionally interchangeable and stoichiometrically associated with ribosomes and can be cross-linked to short nascent chains that have just exited from the tunnel of ribosomes [73–75]. Therefore, Ssbs are thought to affect the translation process directly.

Little is known about the identity of the DnaJ protein partner of Ssbs. The essential DnaJ protein Sis1 binds to the small ribosomal subunit and is thought to be involved in translation initiation [76]. However, the DnaJ protein Zuo1 is considered a promising candidate, since it is also stoichiometrically associated with ribosomes [77]. Strains lacking Ssb or Zuo1 have the same phenotypes, with slowed growth and a hypersensitivity to cations [77–

79]. On the other hand, Zuo1 is found in a very stable complex, the ribosome-associated complex (RAC), together with another Hsp70, Ssz1 [80]. The phenotypes of the cells lacking Ssz1 are the same as those for Δssb or Δzuo1. Furthermore, Zuo1 can stimulate the ATPase activity of Ssb, but only when in complex with Ssz1. This stimulation is dependent on a functional J domain in Zuo1 [81]. The ribosome-associated molecular chaperones have been preserved throughout eukaryotic evolution. The human ortholog of Zuo1, Mpp11, when expressed in yeast, can partially substitute for Zuo1 by partnering with the multipurpose Hsp70 Ssa, the homolog of mammalian Hsc70, suggesting that Mpp11 can recruit the multifunctional soluble Hsc70 to nascent polypeptide chains as they exit the ribosome [82]. In short, certain DnaJ/Hsp40 proteins, together with specific Hsp70 proteins, can regulate protein translation by forming the RAC.

Protein translocation

Many earlier studies have demonstrated a role of DnaJ homologs in protein translocation [83, 84]. ERdj1/Mtj1 and ERdj2/hSec63 seem to be yeast Sec63p counterparts. ERdj1 possesses a ribosome-binding site [85], which should position it near translocating chains, and ERdj2 can be cross-linked to the Sec61 component of the translocon [86], suggesting that both may play roles in translocation. This review attempts to address the role of DnaJ/Hsp40 proteins in protein translocation by discussing their involvement in endocytosis.

The DnaJ proteins, auxilin (DnaJC6) and cyclin G-associated kinase (GAK/auxilin 2), have been shown to be important in the endocytic pathway [29]. Auxilin is specifically present in neuronal tissues, whereas GAK is expressed ubiquitously. Both of them can cooperate with the Hsc70 proteins to dissociate the endosomal protein, clathrin, from clathrin-coated vesicles (CCVs) and pits *in vitro* [87–89]. In contrast to other J domain-containing proteins, the J domain of auxilin is located at its C-terminal end and has an unusually long loop extending between helices I and II [90, 91]. In addition, auxilin has an N-terminal PTEN-like domain, which binds to PtdIns(4,5)P_2 [92], and a central clathrin-binding domain [93], which enables auxilin to induce polymerization of clathrin [94]. Furthermore, auxilin has been shown to bind to the clathrin adaptor protein AP2 [95, 96]. Unlike auxilin, GAK has an N-terminal kinase domain, which phosphorylates the μ subunits of AP2 [89, 97, 98].

Depletion experiments further support an important role for auxilins in endocytosis. In yeast, depletion of auxilin causes accumulation of CCVs with reduced cargo delivery to the vacuole and slows cell growth [99, 100]. Similarly, in *Caenorhabditis elegans*, RNA interference (RNAi) for auxilin markedly reduces receptor-mediated endocytosis of yolk protein in oocytes [101]. Depletion of GAK in HeLa cells also causes a marked reduction in the levels of perinuclear clathrin associated with the trans-Golgi network and in the number of clathrin-coated pits on the plasma membrane. In contrast to clathrin depletion, which does not prevent adaptors from assembling on the membrane, depletion of GAK causes a dramatic reduction in the levels of AP2. A similar phenotype is also observed for a dominant-negative form of Hsp70 [102].

Another example for a role of DnaJ proteins in endocytosis is from the DnaJ protein, receptor-mediated endocytosis 8 (RME-8). RME-8 was first discovered in a screen for endocytic defects in *C. elegans* [103], and was subsequently found to be important in fluid-phase endocytosis and receptor-mediated endocytosis of Boss in *Drosophila*. Genetic evidence suggests that RME-8 acts as a co-chaperone for Hsc70 in endocytosis [104]. In mammals, RME-8 has been shown to be critical for the trafficking of the cation-independent mannose 6-phosphate receptor (CI-MPR) between the trans-Golgi network and membranes of the endosomal system [30].

Protein degradation

Through a process called ER quality control, misfolded proteins are retained in the ER and eventually targeted for ER-associated degradation (ERAD) with the assistance of certain chaperones [105]. BiP plays an essential role in maintaining the permeability barrier of the ER translocon during early stages of protein translocation [106] and targeting misfolded proteins for proteasomal degradation [107, 108]. Yeast DnaJ proteins, Scj1p and Jem1p, may facilitate the retrotranslocation of ERAD substrates to the cytosol by preventing their aggregation in the ER [109].

Additional evidence for a role of Hsp70 in targeting proteins for proteasomal degradation comes from studies on the co-chaperone CHIP (C-terminus of heat-shock cognate 70 stress protein-interacting protein) [110, 111]. CHIP interacts with Hsp70 through a set of tetratricopeptide repeats and negatively regulates the ATPase and chaperone activity of Hsp70 [112]. CHIP contains the E3 ubiquitin ligase domain, the U box domain [113, 114], and can promote the ubiquitination of proteins [111]. The association of CHIP with Hsp70 promotes the ubiquitination of unfolded proteins, including the cystic fibrosis transmembrane conductance regulator (CFTR), glucocorticoid receptor, ErbB2/Her2 (a member of the epidermal growth factor receptor family), and Alzheimer's disease-related tau [110, 111, 115, 116]. It has been demonstrated that the membrane-localized DnaJ/Hsp40 protein, Hdj2, can assist Hsc70 and CHIP to promote ubiquitination of misfolded CFTR [117]. The DnaJ/Hsp40 proteins, human HSJ1 and yeast Ydj1, have also been shown to be involved in the proteasomal degradation of certain proteins [118, 119].

Conclusions and perspectives

This review has stressed the functional diversity of DnaJ/Hsp40 proteins, which are key partners for Hsp70 proteins. Genome-wide analysis in this review has revealed 41 DnaJ/Hsp40 family members (or putative members) in humans. Hsp70s are involved in protein translation, folding, unfolding, translocation, and degradation, whereas DnaJ/Hsp40 proteins are important for these processes primarily by promoting the ATP hydrolysis activity of Hsp70s. However, little is known about the mechanisms by which the activities of DnaJ/Hsp40 proteins are regulated [120]. For example, it has recently been shown that the cellular redox status can affect the co-chaperone activity of human DnaJ protein, Hdj2 [121], but the mechanism underlying this effect remains to be clarified. It is well established that degradation of certain membrane proteins, such as glucocorticoid receptor and ErbB2, requires Hsp70s. Whether or how DnaJ/Hsp40 proteins are involved in the degradation of such proteins still remains unknown. Degradation of these membrane proteins most likely requires specific E3 ubiquitin ligases. In addition to the E3 CHIP, there are several other membrane-associated E3s, such as RNF5/RMA1 [122] and Nrdp1/FLRF [123–125], which have been shown to promote the ubiquitination of specific membrane proteins. Furthermore, DnaJ/Hsp40 proteins may participate in other cellular activities, such as transcription and organelle biogenesis. Mrj has been shown to associate directly with the nuclear factor of activated T cells and to inhibit its transcriptional activity [126]. The yeast mitochondrial Hsp70, Ssq1, is implicated in the assembly of the mitochondrial Fe/S cluster, whereas the DnaJ protein, Jac1, might assist Ssq1 in this process [127]. Since a direct interaction between Ssq1 and Jac1 has not yet been observed, the importance of Jac1 in this process remains to be determined [48].

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