The HSP70 chaperone machinery: J proteins as drivers of functional specificity

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Abstract | Heat shock 70 kDa proteins (HSP70s) are ubiquitous molecular chaperones that function in a myriad of biological processes, modulating polypeptide folding, degradation and translocation across membranes, and protein–protein interactions. This multitude of roles is not easily reconciled with the universality of the activity of HSP70s in ATP-dependent client protein-binding and release cycles. Much of the functional diversity of the HSP70s is driven by a diverse class of cofactors: J proteins. Often, multiple J proteins function with a single HSP70. Some target HSP70 activity to clients at precise locations in cells and others bind client proteins directly, thereby delivering specific clients to HSP70 and directly determining their fate.

In their native cellular environment, polypeptides are constantly at risk of attaining conformations that prevent them from functioning properly and/or cause them to aggregate into large, potentially cytotoxic complexes. Molecular chaperones guide the conformation of proteins throughout their lifetime, preventing their aggregation by protecting interactive surfaces against non-productive interactions. Through such interactions, molecular chaperones aid in the folding of nascent proteins as they are synthesized by ribosomes, drive protein transport across membranes and modulate protein–protein interactions by controlling conformational changes[1,2]. In addition to these roles under optimal conditions, stresses can exacerbate protein conformational problems (for example, heat shock causes protein unfolding and oxygen radicals cause oxidation and nitrosylation). Although chaperones can facilitate folding or refolding, often such rejuvenation is not possible. In such cases, chaperones can facilitate degradation, either by simply preventing aggregation and thus keeping clients susceptible to proteolysis or by actively facilitating their transfer to proteolytic systems. These diverse functions of molecular chaperones typically involve iterative client binding and release cycles until the client has reached its final active conformation, or has entered the proteolytic system (FIG. 1).

Strikingly, the heat shock 70 kDa proteins (HSP70s), one of the most ubiquitous classes of chaperones, have been implicated in all of the biological processes mentioned above[2,3]. This Review focuses on the means by which the HSP70 molecular chaperone machinery participates in such diverse cellular functions. Their functional diversity is remarkable considering that within and across species, HSP70s have high sequence identity. They share a single biochemical activity: an ATP-dependent client-binding and release cycle combined with client protein recognition, which is typically rather promiscuous. This apparent conundrum is resolved by the fact that HSP70s do not work alone, but rather as 'HSP70 machines', collaborating with and being regulated by several cofactors. Here, using examples from yeast and humans, we discuss several such factors, particularly concentrating on how the array of J proteins (also known as HSP40s) orchestrates HSP70 functions.

The core HSP70 machinery

HSP70s never function alone. They invariably require a J protein and, almost always, a nucleotide exchange factor (NEF) as partners. These cofactors are key, as they regulate the binding of HSP70 to client proteins by affecting HSP70's interaction with nucleotides.

The HSP70 cycle. HSP70–client interaction is profoundly affected by the interaction with nucleotides. The 40 kDa amino-terminal adenine nucleotide-binding domain regulates the conformation of the 25 kDa carboxy-terminal peptide-binding domain (PBD), which binds to a five amino acid segment of clients that is enriched in hydrophobic residues[4,5] (FIG. 2). Through ATP hydrolysis and nucleotide exchange reactions, HSP70 cycles between

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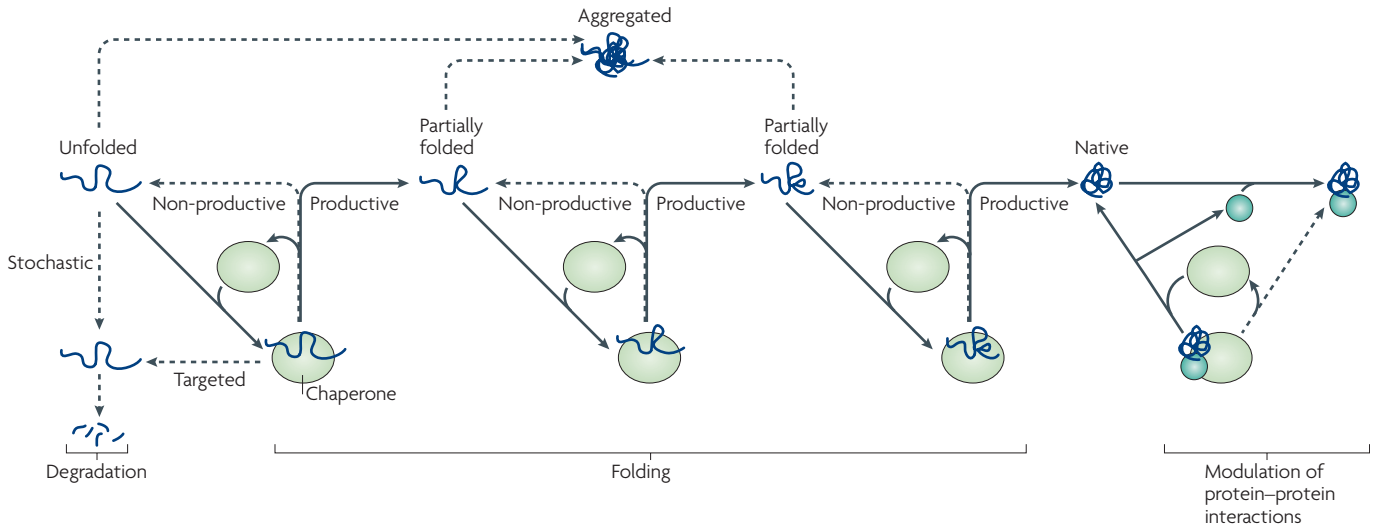


Figure 1 | **Protein folding and degradation through the client protein–chaperone binding and release cycle.** Chaperones were originally defined as "proteins that prevent improper interactions between potentially interactive surfaces and disrupt any improper liaisons that may occur"[134]. Chaperones are a group of structurally divergent proteins that interact with various non-native polypeptides, facilitating the acquisition of their native conformation without being associated with them when in their natively folded and functional structure (centre). However, recent evidence indicates that chaperone functions are not restricted to assisting protein folding and assembly, but also to facilitate client degradation through both proteasomal and autophagasomal pathways (left), as well as to stabilize or destabilize interactions between mature, folded proteins (right). In each of these processes, iterative cycles of client binding to and release from chaperones, which are often driven in an adenine nucleotide-dependent manner, prevent client aggregation. For example, productive folding occurs through a series of steps, and chaperones are recycled for client binding. If folding fails or a non-foldable client re-binds to the chaperone, the protein is degraded by the proteasome in a stochastic (passive) manner (left). Some chaperones can also actively direct clients towards degradation (targeted degradation). In addition, chaperones can bind folded proteins and induce conformational changes (right), thereby regulating protein–protein interactions and the functionality of protein complexes.

ATP- and ADP-bound states, which interact with client proteins in dramatically different ways. On and off rates for client binding are rapid in the ATP-bound state and slow in the ADP-bound state. Thus, engagement with clients is very fast in the ATP-bound state, but hydrolysis must occur to stabilize client interaction. However, the spontaneous transition between the two states is extremely slow, as HSP70's basal ATPase activity is low and nucleotides typically bind stably. Thus, HSP70 function requires cofactors: ATPase activity is stimulated by J proteins, facilitating client capture; dissociation of ADP is stimulated by NEFs, fostering client dissociation and, consequently, 'recycling' HSP70 molecules (FIG. 2a). We refer to this triad as the core HSP70 machinery.

Canonical model: J protein delivery and NEF-driven release. Much of the early biochemical work on HSP70 function focused on the ability of the core machinery to facilitate the folding of denatured proteins (such as luciferase) *in vitro*. This body of work established the 'canonical model' for the functioning of the HSP70 machinery[6–8] (FIG. 2a). This model has two key features in addition to those described above. First, the initial binding of an unfolded client protein by a J protein prevents its aggregation and 'delivers' it to HSP70, in addition to stimulating HSP70's ATPase activity. Second, dissociation of the client caused by NEF action gives the client the opportunity to fold into its active conformation. If folding is not yet complete, clients can re-bind such that reiterative cycles of client binding and release occur. In the simplest scenario, this cycling allows productive folding merely because aggregation is prevented during the time the client is bound. This canonical mechanism of function for the core HSP70 machinery has clearly been shown in some cases to be valid *in vivo*. Sometimes the mode of action of the machine is simpler, but usually it is more complicated and diverse, as we describe below.

The importance of regulation of the nucleotide cycle. It has become apparent that fine-tuning of the cycle of HSP70 interaction with client proteins is crucial. Overstimulation of ATPase may prevent the capture of clients and an excess of NEF activity may cause a premature release of captured clients. *In vivo*, the J protein and NEF cofactors are typically present in sub-stochiometric amounts relative to their partner HSP70. *In vitro*, a 10–20 times lower concentration of J protein than HSP70 is most efficient for the stimulation of client protein folding[9], and raising J protein levels in cells without a concomitant rise in HSP70s generally reduces refolding (J. Hageman and H.H.K, unpublished observations). Analogously, folding efficiency declines when NEF concentrations increase above optimal NEF/HSP70 ratios *in vitro*[10], and overexpression of a NEF in cells reduces the folding capacities of the HSP70 machinery[11].

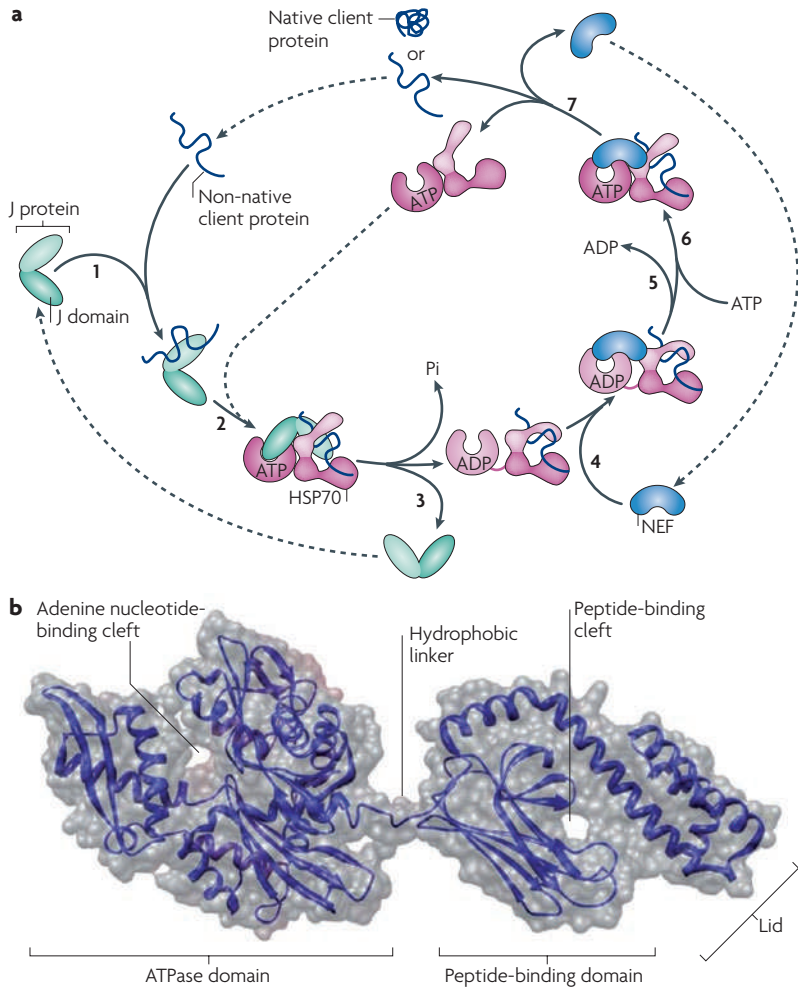


Figure 2 | **Canonical model of the core HSP70 machinery's mode of action in protein folding and HSP70 structure. a** | The mode of action of the heat shock 70 kDa protein (HSP70) core machinery, based on *in vitro* refolding studies of denatured proteins. J proteins bind to client proteins through their peptide-binding domain (1) and interact with HSP70–ATP through their J domain (2). The client rapidly, but transiently, interacts with the 'open' peptide-binding site of HSP70. ATP hydrolysis is stimulated by both the J domain and client, causing a conformational change in HSP70 that closes the helical lid over the cleft and stabilizes the client interaction, and the J protein then leaves the complex (3). A nucleotide exchange factor (NEF), which has a higher affinity for HSP70–ADP than HSP70–ATP, binds HSP70 (4). The ADP then dissociates through distortion of the ATP-binding domain (5), after which ATP binds to HSP70 (6). The client is released because of its low affinity for HSP70–ATP (7). ATP binding to HSP70 is favoured as cellular ATP concentrations are typically much higher than those of ADP. If the native state of the client is not attained on release, the J protein rebinds to its exposed hydrophobic regions and the cycle begins again. **b** | The structure of HSP70 with ADP bound to the nucleotide-binding domain[135] (protein data bank code 2KHO). The ATPase domain and peptide-binding domain are connected by a short, flexible, hydrophobic linker. These domains dock when in the ATP-bound state, which is also thought to displace the lid, allowing easy access and egress of the client protein from the cleft[17,136].

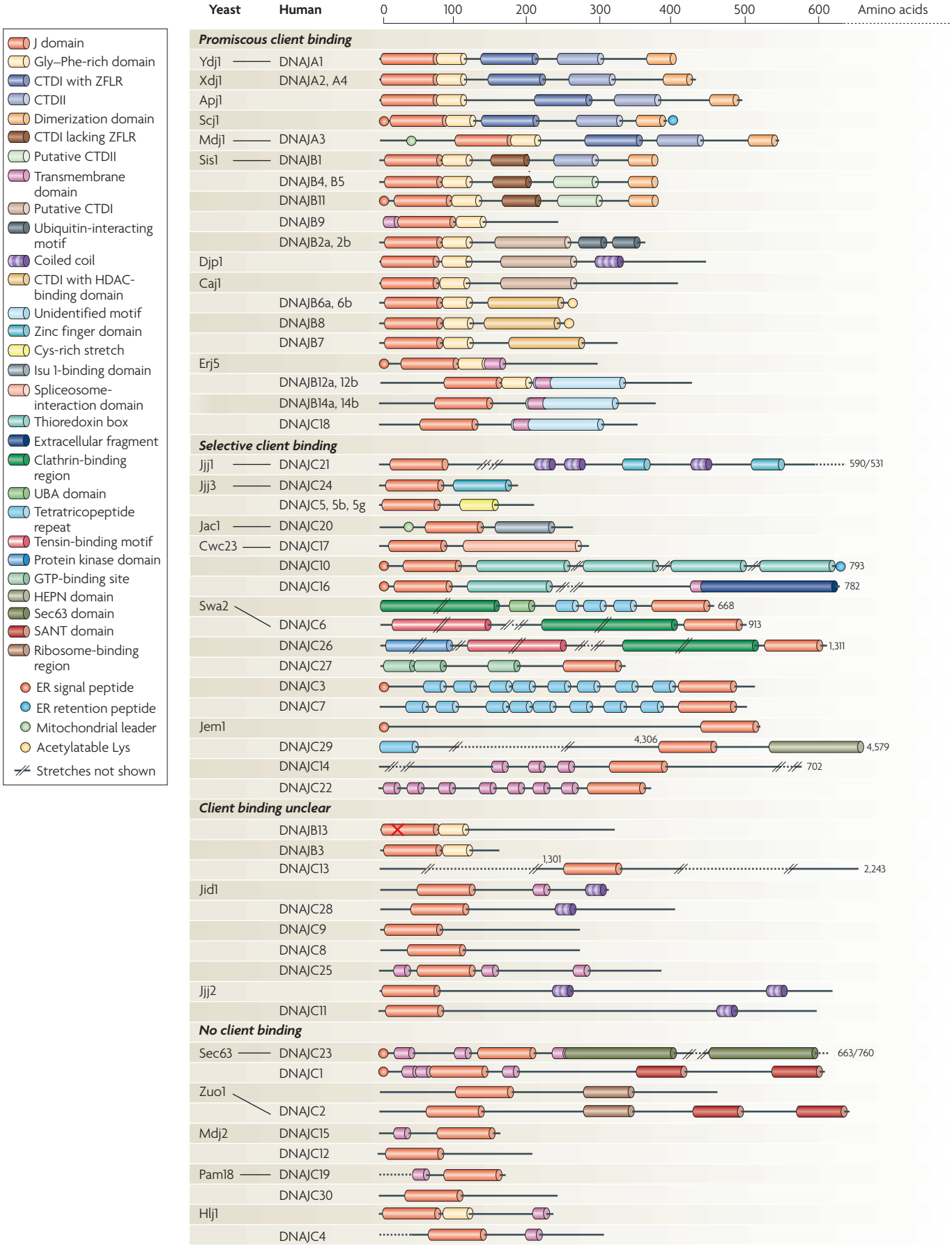
J proteins drive the multifunctionality of HSP70s. Although it is possible to imagine that the versatility of HSP70 function could be achieved primarily through the amplification and diversification of HSP70 genes during evolution[3], this does not seem to be the case. The number of HSP70s in each cell is rather limited. However, J proteins often far outnumber HSP70s and NEFs in a cellular compartment[12,13]. For example, in mammals there is one type of HSP70 in mitochondria and one type in the endoplasmic reticulum (ER), and four and six J proteins, respectively[12,13]. Overall, humans have only 11 HSP70s and 13 NEFs, but 41 J proteins (FIG. 3; see Supplementary information S1 (figure)). In addition, and in contrast to HSP70, J proteins show a large degree of sequence and structural divergence (FIG. 3), consistent with the idea that they play a major part in driving the multifunctionality of the HSP70 machinery.

Diversity of J protein structure

The J domain is both the defining domain and the key to a functional interaction with HSP70s as it stimulates their ATPase activity[12,13]. However, many J proteins bear little if any sequence or structural similarity outside this domain (FIG. 3; see Supplementary information S2 (figure)) and increasing evidence indicates that these non-homologous regions are primary determinants of HSP70 function.

J domain — the common denominator. J proteins, by definition, contain a highly conserved ~70 amino acid signature region, the J domain, which is named after the founding member, *Escherichia coli* DnaJ (FIG. 4a). Particularly conserved is a His, Pro and Asp tripeptide (HPD), which is in a loop between the two main helices (helix II and helix III). This HPD motif is crucial for J domain function; that is, stimulation of HSP70's ATPase activity. The exact mechanism of J domain-stimulated ATPase activity and the ensuing conformational changes resulting in stabilization of client interaction remain a matter of debate. However, it is established that exposed residues of the J domain form a surface for HSP70 interaction[14,15]. Crucial interactions occur with HSP70's ATPase domain and the adjacent flexible region, which links it to the client protein-binding domain (FIG. 2). These interactions are crucial for transmitting the conformation change necessary for closing the peptide-binding pocket[15–20].

J protein groups, structure and classification. The J protein family has often been referred to as the HSP40 family; but, as most members have a molecular weight that differs greatly from 40 kDa, we refer to them as J proteins throughout this Review. Despite the omnipresent J domain, J proteins, as a group, are strikingly dissimilar, with various additional domains (FIG. 3, see Supplementary information S2 (figure)). Historically, J proteins have been divided into three classes (class I, II and III, also known as class A, B and C, respectively)[13,21-23], with class I designation being based on the motifs and domains present in *E. coli* DnaJ. Thus, by definition, class I J proteins have an N-terminal J domain, followed by a Gly and Phe-rich region, four repeats of the CxxCxGxG type zinc finger motif and a C-terminal extension, which is now known to bind client proteins[24–26]. This type of C-terminal region is composed of two barrel topology domains, C-terminal domain I (CTD I) and CTD II. CTD I has a hydrophobic pocket in which client proteins are thought to bind, as well as a zinc-finger domain extruding from it, which may



◀ Figure 3 | **Diversity in domain architecture of yeast and human J proteins.** *Saccharomyces cerevisiae* and *Homo sapiens* J protein family members are clustered according to their known or presumed client-binding ability, and functional orthologues are connected by lines. For clarity, some domains and some differences between yeast and human orthologues are not shown. For more detailed information on the presumed localization and function of all J proteins, see Supplementary information S2 (figure). Class I and class II J proteins contain Gly and Phe-rich regions (of which the functional relevance is disputed; see main text). These are segments with more than 5 Gly and/or Phe residues in the first 25 amino acids carboxy-terminal to the J domain. In C terminal domain 1 (CTD I), canonical class I members have a zinc finger-like region (ZFLR) that class II members lack. However, class II members often have Cys-rich stretches and/or a binding site for histone deacetylases (HDACs). The dimerization domain has been firmly established for only a few class I and class II members (scYdj1, DnaJ subfamily A member 1 (*hs*DNAJA1), *sc*Scj1 and *sc*Sis1); for the other members this domain is presumed for simplicity. X in *hs*DNAJB13 indicates the lack of the canonical His, Pro and Asp (HPD) motif in the J domain. ER, endoplasmic reticulum; HEPN, higher eukaryotes and prokaryotes nucleotide-binding domain; SANT, Swi3, Ada2, N-CoR and TFIIIB domain; UBA, ubiquitin-associated domain.

also be involved in substrate binding[27,28] (FIG. 4b). The extreme C terminus is a dimerization domain and thus serves to increase the affinity for clients[29]. Proteins were classified as class II members if they had an N-terminal J domain with an adjacent Gly–Phe region, but lacked the zinc-finger domain. Any J proteins that did not have a structure that fitted the class I or class II classification were designated class III.

It must be emphasized that this historical classification does not relate to the biochemical function or mechanism of action of group members. In fact, both within and between classes II and III there are large structural and functional diversities (FIG. 3; see Supplementary information S2 (figure)). In particular, the importance of client binding, a crucial feature of many J proteins, is minimized by this classification system. Some class II J proteins, such as *Saccharomyces cerevisiae* Sis1, have client protein-binding domains that, despite having little sequence identity, are strikingly structurally similar to that of the class I yeast protein Ydj1 (REFS 26,30) (FIG. 4b). For clarity, we refer to such client protein-binding domains as 'DnaJ-like'. By contrast, some class II proteins seem to have no client-binding domain at all, whereas many, probably most, class III members do have domains that bind clients. However, data to date reveal no structural similarity of any class III J proteins to the DnaJ-like domain. Rather, many seem to bind one, or perhaps at most a handful, of clients[12] (FIG. 4c). Finally, the presence (type II) or absence (type III) of the Gly–Phe region has led to ambiguity, as the definition and function of this region are ill-defined. Besides serving as a linker between domains, the functional importance of the Gly-rich region is in question. In cases where it has been shown to be important, as in the function of *E. coli* DnaJ and yeast Sis1, the specific sequences found to be crucial were either peripheral to or insertions in the Gly-rich segment[31,32].

In sum, whereas these classifications may be helpful for nomenclature purposes[33], they should not be taken as informative regarding functionality. Indeed, the diversity of J proteins has led to complicated and often confusing nomenclature. Here, for human HSPs we use the recent NCBI accepted nomenclature[33], indicating where appropriate commonly used alternative names. The yeast protein nomenclature used here is based on names established in the *Saccharomyces* Genome Database. To underscore whether discussing proteins from *S. cerevisiae* or *Homo sapiens*, we use the prefixes '*sc*' and '*hs*', respectively.

J protein function without client binding

Despite the functional complexity of J proteins, it is important to note that the presence of only a J domain may be sufficient for some cellular functions (FIG. 5). Such is the case if the domain is localized to a particular site in a cellular compartment (FIG. 5b). This positioning maintains a high local J domain concentration, thus targeting HSP70 to particular client proteins at these sites, without the need for direct J protein interaction with the client itself (FIG. 5a,b).

In the simplest cases, a J protein consists of little besides the J domain and sequences required for localization. For example, the J domain of *sc*Hlj1 is positioned at the cytosolic face of the ER membrane by a single transmembrane domain, where it recruits soluble cytosolic HSP70s to assist in the degradation of proteins exiting the ER (that is, ER-associated degradation (ERAD))[34]. J proteins that are tethered near the polypeptide exit site of the ribosome[35] are another example of HSP70 recruitment to a site with a high concentration of clients (FIG. 6). In this case, positioning ensures the prevention of nascent chain aggregation and facilitates folding. Fungi have a specialized ribosome-associated HSP70, which is independently tethered to the ribosome[36]. However, higher eukaryotes rely on the ribosome-associated J protein DnaJ subfamily C member 2 (DNAJC2) for ribosomal recruitment of soluble *hs*HSPA8 (also known as HSC70), which itself has no intrinsic affinity for the ribosome[37,38] (FIG. 6).

A J domain positioned at the translocon of the inner mitochondrial membrane, which is responsible for translocating polypeptides from the cytosol into the matrix, is another example of a 'minimal J protein'. The HSP70 machinery of which this J protein (*sc*Pam18 or *hs*DNAJC19) is a part, is not involved in protein folding, rather it drives the movement of polypeptides through the translocon. However, the core HSP70 machinery (*sc*Pam18 or *hs*DNAJC19), the mitochondrial HSP70 (*sc*Ssc1 or *hs*HSPA9 (also known as GRP75)) and the NEF (*sc*Mge1 or *hs*BAP (also knowns as SIL1)) that form this 'import motor' follow the basic biochemical rules for HSP70 machines described above. In the process of driving polypeptide import[39], HSP70 binds to exposed hydrophobic sequences in unfolded translocating polypeptides and the J protein stimulates HSP70's ATPase activity to enhance its interaction with the client and the NEF, causing nucleotide release and, thus, dissociation of the client. In addition to the matrix-localized J domain, *sc*Pam18 (or *hs*DNAJC19) consists of a transmembrane domain, which localizes it to the mitochondrial inner membrane, and a short intermembrane space domain, which, in the case of the yeast protein, directly interacts with the translocon[40,41]. *sc*Ssc1 (and *hs*HSPA9)

Zinc finger
A small, functional, independently folded domain that requires the coordination of one or more zinc ions for structural stabilization. Zinc fingers vary widely in structure and can function in DNA and RNA binding, protein–protein interactions and membrane association.

ERAD
A pathway along which misfolded proteins are transported from the ER to the cytosol for proteasomal degradation.

Translocon
A complex of proteins that forms a channel in a membrane and is associated with the translocation of polypeptides from one cellular compartment to another.

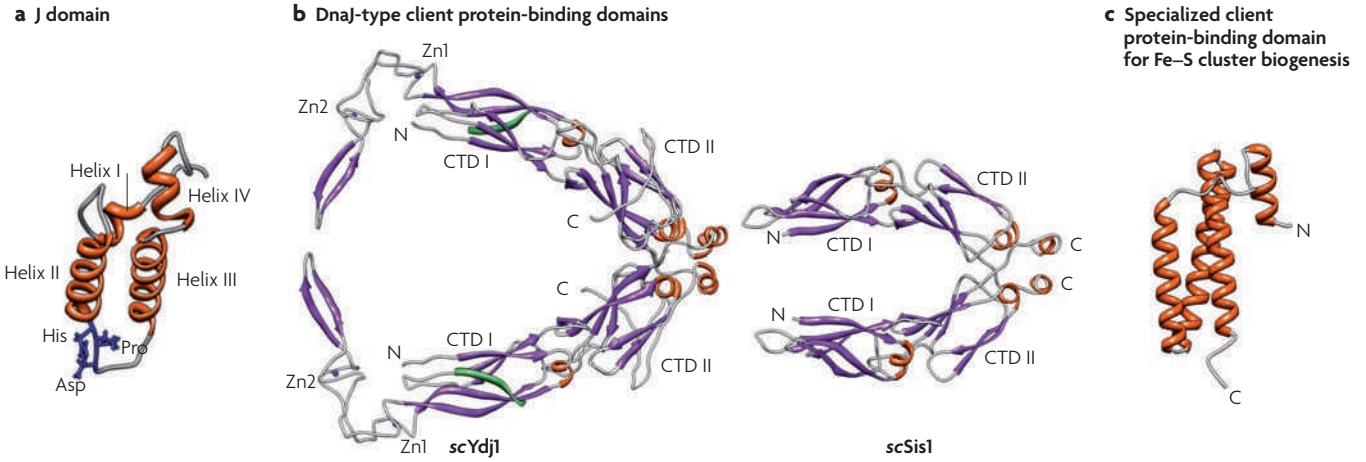


Figure 4 | **J domain and client protein-binding domain structures. a** | J domains contain four α-helices, with the central ones forming a coiled-coil motif around a hydrophobic core[137] (protein data bank (PDB) code 1XBL). The invariant His, Pro and Asp (HPD) tripeptide located in the loop between helix II and helix III is crucial for ATPase stimulation and *in vivo* function[12]. Residues in helix II and the neighbouring loop, including the HPD, form a heat shock 70 kDa protein (HSP70)-interaction face. **b** | Class I *sc*Ydj1 and class II *sc*Sis1 have similar client protein-binding domains, called DnaJ-type domains. The structure of amino acids 102–350 of *sc*Ydj1 (REF. 26; reconstruction of PDB codes 1NLT, IXAO (REF. 29) and 1C3G from Sis1) and 180–343 of *sc*Sis1 (REF. 30; PDB code 2B26) are shown. Both proteins have J domains at their amino termini, followed by a Gly and Phe-rich region. No full-length structure of a class I or class II J protein has been obtained owing to the flexibility of the Gly–Phe region. Both *sc*Ydj1 and *sc*Sis1 are dimers, with the dimerization domain at their carboxy termini. Each monomer of *sc*Ydj1 and *sc*Sis1 has two adjacent domains that are similar in structure, being predominantly composed of β-sheets. These are often referred to as C-terminal domain I (CTD I) and CTD II. *sc*Ydj1 (like DnaJ) has two zinc fingers (Zn1 and Zn2), which extend out from CTD I. In addition, *sc*Ydj1 has a CAAX motif at its extreme C terminus for farnesylation, a modification important for membrane localization and binding of some client proteins[138,139]. **c** | *sc*Jac1 (called HscB in *Escherichia coli*), which is important for Fe–S cluster biogenesis, has a specialized client protein-binding domain that has neither sequence nor structural similarities to the DnaJ-type domain[140]. Amino acids 63–171 of HscB are shown (PDB code 1FPO). The face pointing outwards interacts with an Fe–S cluster scaffold protein (Isu)[141].

is independently localized to the translocon[42,43]. Thus, when the HSP70 partner is localized (such as fungal ribosome-associated HSP70s), the tethered J domain is not necessary for recruitment of HSP70 *per se*. Beyond resulting in high local concentrations of both components, dual localization also precisely positions the J domain juxtaposition to HSP70 (FIG. 5b) for optimal ATPase stimulation. This precise positioning probably results in exquisite modulation of the interaction of HSP70 with client proteins (a translocating polypeptide in the case of the import motor).

Remarkably, for some cellular functions, the J domain (that is, the ability to stimulate HSP70's ATPase activity) may be sufficient, even without sub-compartment localization (FIG. 5a,c). This functional robustness is illustrated by the ability of the J domain of several cytosolic J proteins to rescue the severe defects caused by the absence of class I *sc*Ydj1, the most abundant J protein of the yeast cytosol[44]. Surprisingly, this rescue occurs even when a J domain is expressed at normal *sc*Ydj1 levels. This, and other observations, also underscores the idea that little if any specificity resides in J domains themselves[45]. Perhaps facilitation of folding of some newly synthesized proteins by cytosolic HSP70 needs a J protein only for stimulation of ATP hydrolysis and not for the direct binding, to prevent aggregation or increase the probability of its interaction with HSP70. However, it should be noted that this rescue of the effects of the absence of Ydj1 by the J domain is not complete. As discussed below, client protein binding by J proteins has many important functions.

J protein function in client binding

J protein client binding is crucial in many functions of the core HSP70 machinery, (FIG. 3). These client protein-binding functions are not restricted to preventing aggregation or supporting folding, but include protein degradation and remodelling of folded proteins. As described below, J proteins have a directive role in these cases.

Client protein binding for folding. Both *in vitro* and *in vivo* results strongly indicate an important role for J proteins with a DnaJ-like fold in *de novo* protein folding[25,46] (FIG. 2: FIG. 5c). However, some results seem to challenge the importance of client protein binding *per se* in the function of these proteins *in vivo*. A J domain alone can substantially substitute for *sc*Ydj1 as described above[44], and complete deletion of the client protein-binding domains of either *sc*Ydj1 or *sc*Sis1, the other abundant J protein of the yeast cytosol that has a DnaJ-like client protein-binding domain (FIG. 3; see Supplementary information S2 (figure)), had little phenotypic effect[47]. However, mutants lacking the C terminus of both *sc*Ydj1 and *sc*Sis1 are non-viable[47], implying that the client-binding ability of these

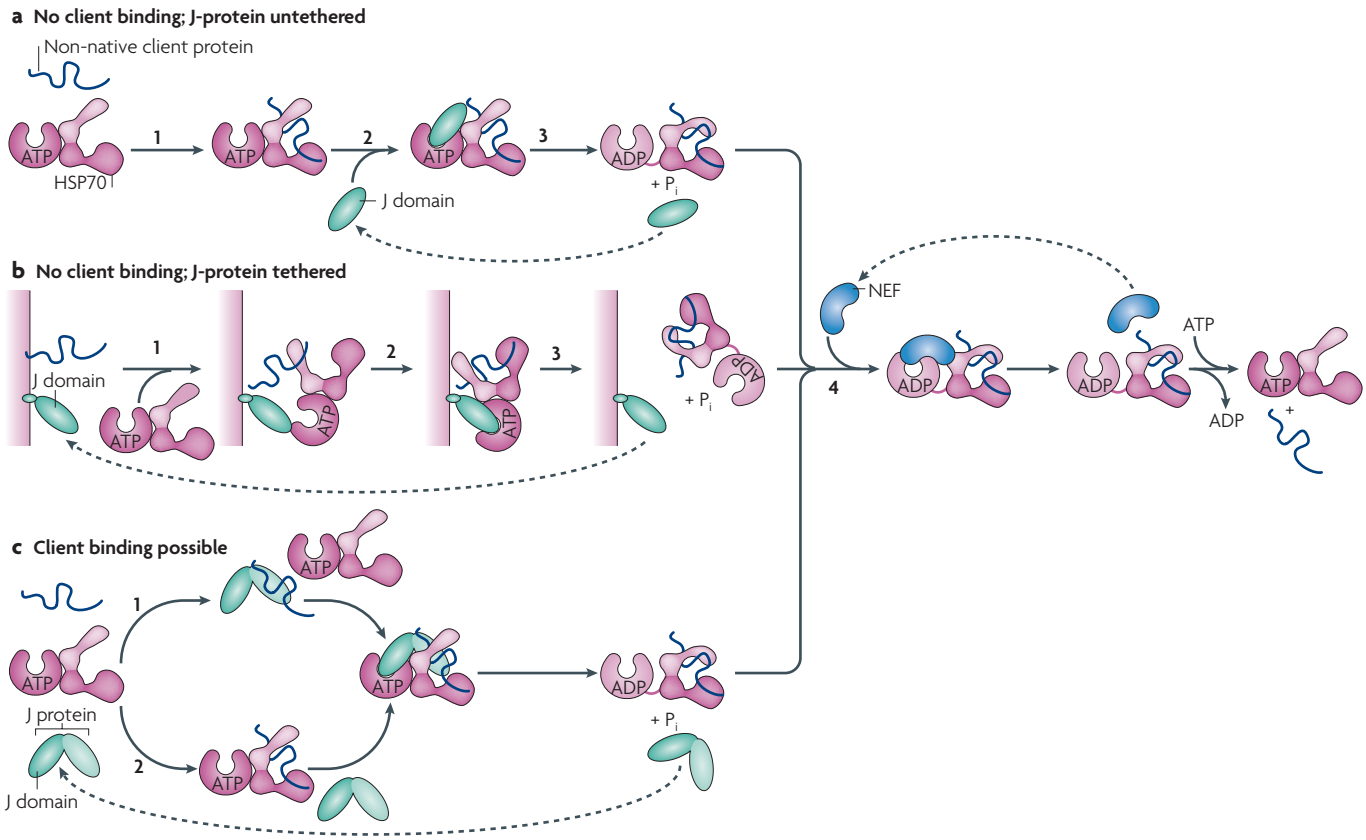


Figure 5 | **J protein function with or without client binding.** J proteins can act without binding to clients, either untethered (**a** and **c**) or tethered (**b**) to a particular site in the cell. **a** | The simplest J protein function is the action of a J domain in the absence of a client protein-binding domain, whereby it stimulates the ATPase activity of heat shock 70 kDa protein (HSP70), allowing HSP70–ATP to capture a client protein that has transiently entered its open peptide-binding cleft (1). The J domain then binds HSP70 (2) and stimulates ATP hydrolysis (3). In such cases, HSP70 is the driving force of client protein interaction, as there is no facilitation by the J protein, either through direct binding or by subcellular localization. **b** | If the J domain is tethered to a particular site in a cellular compartment, on initiation of client protein binding by HSP70–ATP (1), a high concentration of J domains is present (2), allowing efficient stimulation of ATP hydrolysis and thus client capturing by HSP70 (3). **c** | J proteins with client protein-binding domains can function in two modes: the J protein can bind the client first (as in the canonical model of J protein and HSP70 function; FIG. 2) and target it to HSP70 (1), or binding can occur directly to HSP70 (2; as in **a**). In the case of direct binding, J proteins serve only to stimulate HSP70 ATPase activity, even though a client-binding domain is present. Evidence for such an alternative pathway has been found in the mitochondrial Fe–S cluster biogenesis pathway in yeast with the specialized J protein *sc*Jac1 and its HSP70 partner *sc*Ssq1 (REF. 142). In all cases, release of the client is facilitated by nucleotide exchange factors (NEFs; 4).

cytosolic J proteins is an essential function *in vivo*. Thus, functional overlap between J proteins can mask the importance of client protein binding. This overlap is particularly remarkable as the peptide-binding domains have limited sequence homology (29% identity).

Another example of client binding being an essential feature of J protein function comes from studies on *hs*DNAJB11 (also known as ERdj3), an ER lumenal J protein with a DnaJ-like peptide-binding domain. Even in the absence of an active J domain, *hs*DNAJB11 can bind directly to several nascent, unfolded and mutant secretory pathway proteins, implying that this binding is HSP70-independent. As predicted from the canonical folding model, after client binding, HSP70 joins the complex, which leads to *hs*DNAJB11 client dissociation before protein folding is completed[48]. So, *hs*DNAJB11 seems to prevent client aggregation and 'presents' the client for HSP70-dependent folding. For its own release, *hs*DNAJB11 must recruit *hs*HSPA5 (also known as BiP) and stimulate its ATPase activity to convert HSPA5 into its high-affinity state for clients[49].

Client protein binding for degradation. Besides facilitating protein folding, several J proteins have specific functions in preventing aggregation and/or shunting clients towards degradative pathways. For example, the two closely related J proteins *hs*DNAJB6 and *hs*DNAJB8 were identified as potent inhibitors of aggregation and the associated toxicity of polyglutamine-containing proteins (polyQ proteins)[50–53]. Both client binding and prevention of aggregation were completely dependent on a Ser-rich stretch in the C terminus named the SSF-SST region[50]. This region shows no obvious sequence similarity to the canonical

PolyQ protein
A protein containing a tract of several Glu residues. In inheritable neurodegenerative disorders such as Huntington's disease, these Glu tracts are expanded, leading to disease-causing protein aggregation.

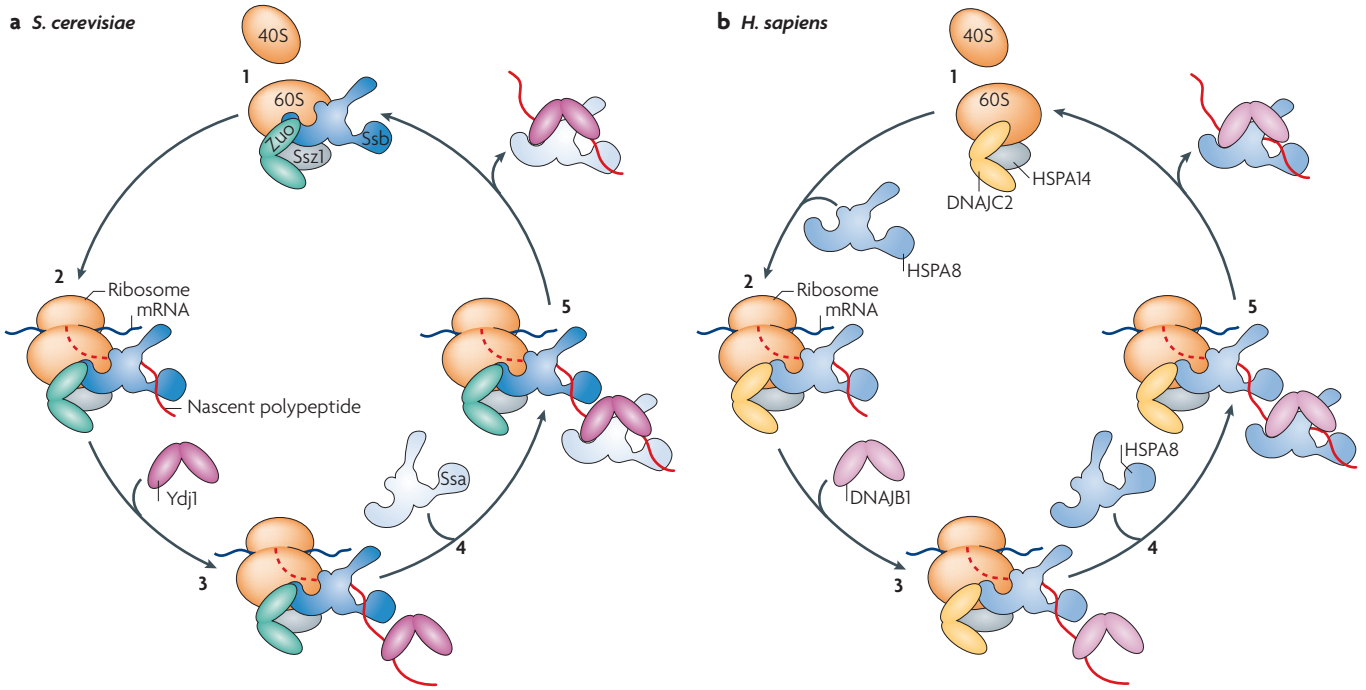


Figure 6 | **J protein tethering to the site of action.** Ribosome-associated chaperones are an example of J proteins tethering HSP70 to a site in a cellular compartment. All eukaryotes, illustrated here by *Saccharomyces cerevisisae* (**a**) and *Homo sapiens* (**b**), have a ribosome-associated J protein (*sc*Zuo1 and *hs*DNAJC2, respectively) that binds near the exit site of the 60S subunit, regardless of whether translation is occurring or not[37] (1). Fungi, but not other eukaryotes, also have a specialized ribosome-associated heat shock 70 kDa protein (HSP70), *sc*Ssb, that independently associates with the 60S subunit. *sc*Zuo1 and *sc*Ssb function as a J protein–HSP70 pair when a nascent polypeptide emerges from the ribosome[36] (**a**2). Later events in polypeptide folding include binding of soluble J proteins (3) and recruitment of soluble HSP70 before the completion of translation (4) and after nascent chain release (5). *S. cerevisiae* uses the abundant soluble J protein *sc*Ydj1 and the HSP70 *sc*Ssa. In humans, the ribosome-associated J protein DnaJ subfamily C member 2 (*hs*DNAJC2) recruits *hs*HSPA8 as a partner (**b**2), which also partners with the soluble *hs*DNAJB1 in downstream folding events (**b**3 and **b**4). Note that both *sc*Zuo1 and *hs*DNAJC2 form a stable heterodimer with an unusual HSP70 (*sc*Ssz1 or *hs*HSPA14). The function of this HSP70, which is not known to have client protein-binding activity, beyond being important for the ability of *sc*Zuo1 to stimulate the ATPase activity of its HSP70 partner *sc*Ssb[20], is not known.

DnaJ-type client protein-binding domain, and thus is probably a novel client-binding domain. The SSF-SST region has at least two additional functions. First, it is involved in forming higher-order structures. In contrast to J proteins with their canonical DnaJ-like client-binding domain, *hs*DNAJB6 and *hs*DNAJB8 do not form dimers, but rather exist *in vivo* as complexes of many different sizes, and their formation is dependent on the SSF-SST region. Second, the SSF-SST region is required for interaction with several histone deacetylases (HDACs). Intriguingly, the deacetylation of two C-terminal Lys residues in *hs*DNAJB8 is functionally important *in vivo*[50]. How clients bound to *hs*DNAJB6 or *hs*DNAJB8 are handled remains to be elucidated. Intriguingly, however, besides acting on polyQ proteins, both proteins are also able to inhibit aggregation of polyQ peptides (E. Reits, personal communication). Such peptides, which may arise from the proteasomal cleavage of full-length polyQ proteins, are highly aggregation-prone and have been implied as possible seeds in polyQ aggregation. Together, this suggests the possibility that *hs*DNAJB6 or *hs*DNAJB8 maintain aggregation-prone peptides in a form that is competent for peptidase degradation.

Some J proteins contain well-defined domains that specifically drive their clients towards degradation. *hs*DNAJB2 (also known as HSJ1) is closely related to *hs*DNAJB6 and *hs*DNAJB8 (REF. 50), but contains two ubiquitin-interacting motifs (UIMs) C-terminal to its putative client-binding domain that are not present in the other proteins[54] (FIG. 3). *hs*DNAJB2 not only efficiently targets misfolded targets, such as polyQ proteins, for degradation[55], but also antagonizes the refolding of heat denatured luciferase, which is mediated by *hs*HSPA1A (also known as the stress-inducible HSP70) and *hs*DNAJB1 (also know as HSP40)[56], by increasing luciferase ubiquitylation. Both the UIMs and the J domain are required for this action of *hs*DNAJB2, indicating an HSP70-dependent function[57] (FIG. 7a). This clearly illustrates that the fate of clients can be primarily determined by the J protein, rather than by the HSP70 component of the core machinery, as the same clients may either fold or be degraded, depending on whether they interact with *hs*DNAJB1 or *hs*DNAJB2. In these studies, *hs*DNAJB8 could also prevent aggregation of heat-unfolded luciferase but could not support refolding, unlike *hs*DNAJB2. However, refolding was not, or was only marginally, inhibited by *hs*DNAJB8, further

Histone deacetylase
An enzyme that removes acetyl groups from ε-N-acetyl lysines from histones and many other proteins. Acetylation (by histone acetyltransferases) and deacetylation is a common post-translational modification to regulate protein function.

Ubiquitin-interacting motif
A single α-helix motif oriented either parallel or antiparallel to the central β-strand that binds ubiquitin and can assist in protein degradation by the proteasome.

Ubiquitylation
The tagging of proteins with a small protein called ubiquitin by ubiquitin ligases. Tagging with multiple ubiquitin moieties leads to the binding of the tagged protein to the proteasome that will degrade it.

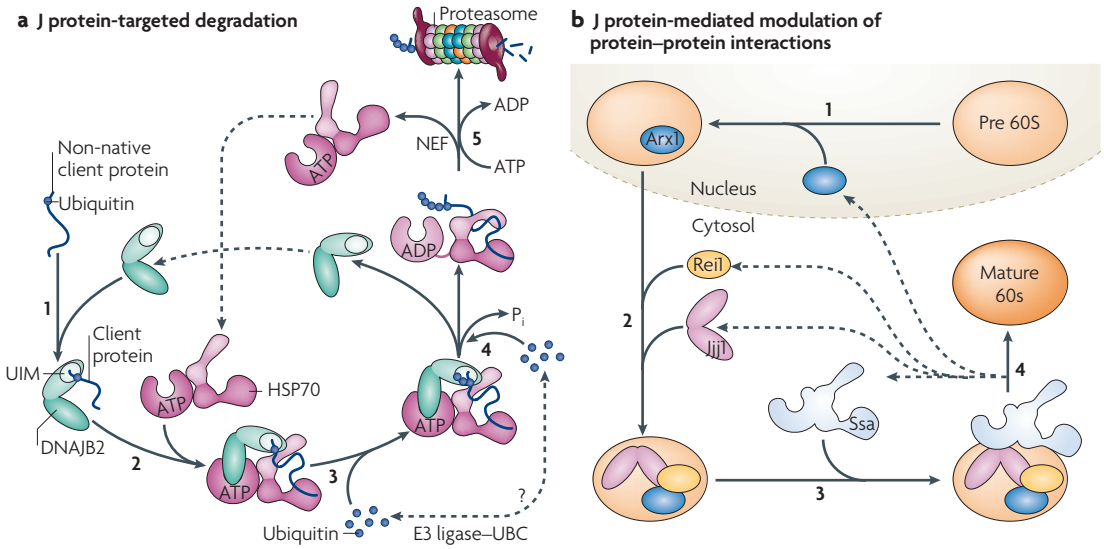


Figure 7 | **Examples of J protein function beyond protein refolding. a** | J protein-targeted degradation. The ubiquitin-interacting motifs (UIMs) in human DnaJ subfamily B member 2 (*hs*DNAJB2; also known as HSJ1) recognize clients that contain a monoubiquitin or polyubiquitin moiety (1). After transfer of the client to heat shock 70 kDa protein (HSP70) (2), E3 ligases (such as CHIP; also known as STUB1) and the ubiquitin conjugation machinery (UBC) can associate with the HSP70–*hs*DNAJB2 complex (the precise manner and specificity of this associations is unclear), leading to further ubiquitylation of the bound client (3,4). After the canonical ATP hydrolysis step (4) and nucleotide exchange factor (NEF)-mediated nucleotide exchange (5), the polyubiquitylated client released from HSP70 is transferred to the proteasome for degradation[54,55]. **b** | J protein-mediated modulation of protein–protein interactions. The alteration of interactions between mature, folded proteins is typically part of complex biological processes. Shown here is the role of the *Saccharomyces cerevisiae* J protein *sc*Jjj1 in the biogenesis of the 60S ribosome subunit; specifically, the destabilization of the biogenesis factor associated with ribosomal export complex protein 1 (*sc*Arx1) in the biogenesis of 60S[65–67]. *sc*Arx1 is loaded on the pre60S subunit in the nucleus (1). *sc*Jjj1 binds directly to the ribosome, as does *sc*Rei1, another cytosolic factor required for *sc*Arx1 destabilization and with which *sc*Jjj1 interacts (2). *sc*Jjj1 partners with soluble *sc*Ssa (3; another example of targeting of HSP70 by J protein localization (see FIG. 5c and FIG. 6). Once the *sc*Arx1–pre60S subunit interaction is destabilized, a step needed to generate the mature subunit (4), *sc*Arx1 is transported back into the nucleus where it engages in another cycle of subunit biogenesis.

suggesting that there is functional differentiation based on J protein diversity. However, it must be stressed that, besides directed targeting, protein fates also depend on the client itself. For example, in the case of non-foldable clients such as polyQ proteins, *hs*DNAJB1 can support client degradation[50,58,59]. However, in such cases, degradation is probably facilitated in a stochastic manner by perpetual cycles of client loading onto and release from HSP70 — that is, the client is simply kept competent for eventual degradation (FIG. 1).

More complex examples than simply the presence of UIMs also exist. *hs*DNAJC10 (also known as ERdj5) of the ER lumen not only has a putative peptide-binding domain but also has protein-disulphide isomerase and thioredoxin domains[60,61] (FIG. 3; see Supplementary information S2 (figure)). In addition, *hs*DNAJC10 is able to interact with ER degradation-enhancing α-mannosidase-like 1 (EDEM1). *hs*DNAJC10 cleaves aberrant disulphide bonds in EDEM1 clients in an HSP70-independent manner. Recruitment of the ER-resident HSP70 (*hs*HSPA5) by the J domain is needed for client release and transfer to components of the ER-associated degradation pathway, such as p97 (also known as VCP), for retrotranslocation to the cytosol and subsequent degradation by the proteasome[62].

Protein-disulphide isomerase with thioredoxin domain
A domain that can catalyse the formation and breakage of disulphide bonds between Cys residues in proteins as they fold. The typical thioredoxin fold refers to a canonical four-stranded antiparallel β-sheet sandwiched between two α-helices.

Working on folded client proteins. It is becoming apparent over time that mature, folded proteins are J protein clients more commonly than previously appreciated. Such functions often involve the 'remodelling' of large multiprotein complexes, affecting the stability of protein–protein interactions. Ironically, this was the first identified function for the *E. coli* DnaK–DnaJ machinery. For initiation of lambda phage DNA replication in *E. coli*, DnaK and DnaJ mediate the destabilization of the lambda P protein from the initiation complex, causing activation of the DNA helicase DnaB[63,64]. Whereas lambda replication involves the multifunctional DnaJ, in eukaryotes similar roles are often carried out by J proteins that specialize in a single function. *sc*Jjj1 (or *hs*DNAJC21) and *sc*Swa2 (or *hs*DNAJC6 (also known as auxilin)), are both conserved, structurally complex J proteins of the cytosol that destabilize protein–protein complexes. *sc*Jjj1 is required for one of the final steps in formation of the 60S ribosomal subunit[65,66]. In collaboration with the soluble constitutive HSP70 (*sc*Ssa or *hs*HSPA8) and *sc*Rei1, another cytosolic protein with which it interacts, *sc*Jjj1 facilitates the destabilization of two biogenesis factors, allowing them to recycle back to the nucleus[67] (FIG. 7b). *sc*Swa2 or *hs*DNAJC6, working with the same HSP70 family member, is required for

the uncoating of clathrin-coated vesicles. These vesicles are encased in an intricate lattice made up of clathrin trimers[68], which interdigitate with one another, forming a stable structure. Thus, this lattice must be destabilized; that is, uncoated, which entails the dissociation of trimers from the lattice, probably by rotation of one trimer relative to another[68]. Both *sc*Swa2 (or *hs*DNAJC6) and *sc*Ssa (or *hs*HSPA8) specifically bind clathrin, but at different sites[69–71], and, as a J protein–HSP70 pair, facilitate trimer release from the lattice[72].

Specialized J proteins with folded proteins as clients can also have roles other than the destabilization of multimeric complexes. In one case, chaperones seem to facilitate partial unfolding of the client. The ubiquitous mitochondrial J protein *sc*Jac1 (or *hs*DNAJC20) is required for the biogenesis of Fe–S clusters[73,74]. These clusters are assembled on a scaffold protein, *sc*Isu, and then transferred to recipient proteins[75]. *sc*Isu1 is the client protein of both *sc*Jac1 and the HSP70 with which it functions, with chaperone action being required for the transfer, but not the assembly, of the cluster[76–78]. In the simplest model, chaperone binding facilitates partial unfolding of *sc*Isu1, thus 'releasing' the cluster. Such a model is supported by recent observations that *E. coli* Isu attains several conformations *in vitro*, with one being markedly disordered[79]. Thus, binding of the J protein–HSP70 machinery may favour the disordered over the ordered conformation of *sc*Isu, and in so doing favour cluster transfer. This system also provides one of the few examples of the evolution of a specialized HSP70. A small subset of fungi have evolved a second, specialized mitochondrial HSP70 that functions with *sc*Jac1. Like *sc*Jac1, this specialized HSP70 (*sc*Ssq1) functions in cluster biogenesis only, with *sc*Isu being its only client protein[80]. Interestingly, most eukaryotes, including humans, use the multifunctional HSPA9 of the mitochondrial matrix in Fe–S cluster biogenesis[80]. In these organisms, the specificity for the system is driven by the J protein.

An HSP70-independent J protein function?

Besides functioning in the context of the core HSP70 machine, some J proteins have evolved functions that largely do not require their J domains and are thus independent of HSP70s. Such cases uncovered so far involve either the prevention of aggregation or modulation of the stability of protein complexes. For example, the ability of *hs*DNAJB6 and *hs*DNAJB8 to prevent accumulation of aggregated polyQ proteins, as described above, does not depend on their J domains — variants with alterations in or complete deletions of the J domain were only slightly impaired in preventing aggregation. This was due to a defect in supporting degradation of the unfolded, soluble polypeptides that required an interaction with HSP70, leading to the conclusion that aggregation prevention did not require HSP70 (REF. 50).

*sc*Cwc23 shows an extreme example of J domain dispensability in the modulation of protein–protein interactions. *sc*Cwc23, an essential protein with an N-terminal J domain (FIG. 3; see Supplementary information S2 (figure)), is required for disassembly of the spliceosome[81,82]. This is an essential process, as the spliceosome components must be reassembled for each pre-mRNA that is spliced. Whereas alterations in the extreme C terminus of *sc*Cwc23 cause a global defect in pre-mRNA splicing, complete deletion of the J domain has no effect. The J domain is functional, but its role is revealed only when the interaction between two other components required for spliceosome disassembly, *sc*Prp43 and *sc*Ntr1 (also known as Spp382), are affected. As *sc*Cwc23 interacts directly with *sc*Ntr1[83] through its C terminus[81], it is thought that this region is sufficient for its essential role in destabilization of the spliceosome. Probably, the J domain fine-tunes destabilization of spliceosomal components, but its absence is only noticeable when interaction among other proteins involved in the disassembly process are functionally compromised.

How did such J domain-independent, and thus HSP70-independent, functions of J proteins evolve? One can envision two extreme possibilities: J proteins have gained domains, making them more complex, and, in some cases, the added domains surpass the J domain in functional importance; and/or proteins that independently functioned in certain cellular processes gained a J domain, enabling the HSP70 system to fine-tune an existing function. In at least one instance, J domain function may have been lost during evolution. The J protein Rsp16 plays a part in flagellar stroke movement in *Chlamydomonas reinhardtii*[84], but a fragment lacking the J domain seems to function as well as the full-length protein in regulating flagellar beating[85]. Interestingly, although Rsp16 has the defining HPD motif, its orthologues from human (*hs*DNAJB13), zebrafish, mouse and mosquito do not, raising the possibility of an increase in functional importance of protein portions other than the J domain during evolution, with a concomitant loss or decrease in J domain function.

NEF and HSP70 multifunctionality

Although we argue above that J proteins are the prime drivers of HSP70 functional diversity, NEFs may play a part as well. Unlike J proteins, which have a common domain responsible for the effects on HSP70's nucleotide cycle, four different types of NEFs have been identified (see Supplementary information S1 (figure)) and no sequence similarity exists among them. Although they all interact with HSP70's ATPase domain, destabilization of nucleotide binding is accomplished in mechanistically distinct ways[86–88]. Some NEFs seem to function only in nucleotide release, but others have additional domains. Clear insight into how these other domains affect HSP70 function and relate to NEF activity is still lacking.

Acting only as NEFs. Two types of NEF, the GrpE-type and the HSP-binding protein 1 (HSPBP1)-type, seem to only have domains involved in nucleotide release. Bacteria and mitochondria have a single NEF, the GrpE-like type, which functions with resident HSP70s in functions from protein folding to translocation[89–92]. The cytosol and ER in eukaryotes contain the HSPBP-type NEFs *hs*HSPBPI (or *sc*Fes1) and *hs*BAP (or *sc*Sls1; also known as Sil1), respectively[93–96]. As with GrpE-types, the

Clathrin-coated vesicle
A vesicle surrounded by a polyhedral lattice of triskelion-shaped clathrin molecules that plays an important part in the selective sorting of cargo at the cell membrane, *trans*-Golgi network and endosomal compartments for multiple membrane traffic pathways.

Fe–S cluster
An ensemble of iron and sulphide centres found in various metalloproteins and crucial for the function of many proteins. They are best known for their role in oxidation-reduction reactions of mitochondrial electron transport, but they also have regulatory roles.

Spliceosome
A dynamic complex of specialized RNA and protein subunits that removes introns from a transcribed pre-mRNA segment (splicing).

HSPBP1-type NEFs seem to support 'classical' chaperone actions of HSP70 machines, from stress-related protein refolding reactions to ERAD[93,97].

The HSP70-like NEFs. The HSP110 proteins (*hs*HSPH1–*hs*HSPH4, *sc*Sse1, *sc*Sse2 and *sc*Lhs1) were initially grouped as HSP70 family members because of similarities in sequence. Like HSP70s, they consist of an N-terminal ATPase domain that is connected to a peptide-binding domain (in this case, a nine-stranded β-sandwich) by a flexible linker similar to that of HSP70 (REFS 98,99) (see Supplementary information S1 (figure)). Interestingly, at least some HSP110s, like HSP70s, can bind unfolded proteins and prevent their aggregation[99], raising the possibility that the interacting HSP70 and HSP110 chaperone pair act in concert, both binding client proteins. However, although HSP110s have ATPase activity, unlike the canonical HSP70s, they cannot employ a nucleotide-dependent, peptide-binding release cycle[86]. Therefore, in terms of their chaperone activity, their interaction with client proteins cannot be modulated; that is, HSP110s can only act as 'holdases'. Both *S. cerevisiae* and humans have stress-inducible HSP110 members (*sc*Sse2 and *hs*HSPH1, respectively), raising the possibility that they may store partially denatured clients under conditions of stress and then, using their NEF activity, help HSP70 facilitate their refolding[86,87,98,100–102].

The Bag family of NEFs. The Bag-type NEFs are the most complex NEF family, in terms of both the number of members and diversity of sequence. All contain a so-called Bag domain — an ~85 amino acid region that can interact with HSP70's ATPase domain[103]. Whereas *S. cerevisiae* has a single Bag-type NEF, *sc*Snl1, humans have six members — *hs*BAG1, *hs*BAG2, *hs*BAG3 (also known as CAIR1 and BIS), *hs*BAG4 (also known as SODD), *hs*BAG5 and *hs*BAG6 (also known as scythe and BAT3) (see Supplementary information S1 (figure)). All are found in the cytosol and/or nucleus. Specific interaction of *hs*BAG1, *hs*BAG2, *hs*BAG3 and *hs*BAG6 with HSP70 has been experimentally verified[103]. However, how the different members function in relation to HSP70 beyond their NEF activity remains unclarified. Intriguing hints exist, but no clear picture has emerged. The most obvious domain besides the defining Bag domain, found in *hs*BAG1 and *hs*BAG6, is a ubiquitin-like domain (UBL). The UBL may sort the Bag proteins to and associate them with the proteasome[104,105]. *hs*BAG3 has also been implicated in protein degradation but has no UBL and seems to support protein degradation through autophagy rather than the proteasome. This does not seem to be HSP70-specific. Rather, it is dependent on the small HSP family member *hs*HSPB8, with which it forms a stoichiometric complex[106,107]. Therefore, although *hs*BAG3 also associates with HSP70s *in vivo*, the precise role of *hs*BAG3 as a NEF for HSP70 is not yet understood.[107]. In summary, there is limited, if any, evidence that NEF activity or the mechanism by which nucleotides are released drive the specificity of HSP70 machines.

Autophagy
A catabolic process involving the engulfment of (usually damaged) organelles and long-lived proteins or protein aggregates by double-membrane vesicles (autophagosomes) that fuse with lysosomes, where their contents are degraded by acidic lysosomal hydrolases.

E3 ubiquitin ligase
A protein that catalyses the attachment of multiple ubiquitin moieties onto a target, an already monoubiquitylated protein. Polyubiquitylation marks proteins for degradation by the proteasome.

Beyond the core HSP70 machinery

It is important to keep in mind that the functional diversity of HSP70 machinery partly depends on partnerships with other chaperone systems and fine-tuning by a set of cofactors, as outlined below.

Chaperone partnerships. HSP70 machines do not often act alone, but rather act in concert with other chaperone machines (see Supplementary information S3 (figure)). Such partnering does not necessarily entail physical interactions between the different machineries. Instead, networks of chaperone activity exist that are driven by the affinity of partially folded proteins for particular chaperone systems. For example, in the folding of nascent polypeptides, HSP70s act upstream, binding the more extensively unfolded clients before their interaction with chaperonins[108,109]. However, adaptor proteins that interact directly with different chaperone machines and act as physical bridges between them also exist. Such is the case with the HSP70–HSP90 chaperone network, which is important for the final maturation steps of certain clients, for example hormone receptors and some transcription factors. The transfer of clients from HSP70 to HSP90 is facilitated by a tetratricopeptide repeat (TPR) protein — HSC70 and HSP90-organizing protein (*hs*HOP (also known as STIP1); or *sc*Sti) — that binds to both chaperones[110,111]. These and other partnerships, such as those with small HSPs[112,113], expand the repertoire of HSP70 function.

Nucleotide cycle regulation beyond the core HSP70 machinery. Several factors have been identified, which, although not universal and thus not part of the core HSP70 machinery, affect the HSP70 ATP–ADP hydrolysis and release cycle. The best studied factors, *hs*HIP (also known as p48) and *hs*CHIP (also known as STUB1), were identified over a decade ago. But, whether, or how, they functionally direct HSP70-dependent reactions *in vivo* remains elusive.

*hs*HIP was identified as a protein that preferentially binds to and stabilizes the ADP-bound state of HSP70 (REFS 114–116). *hs*HIP competes with the NEF *hs*BAG1 for binding to HSP70's ATPase domain[117], thereby slowing down the nucleotide cycle and extending the time during which clients are bound. Consistent with these biochemical properties, increasing *hs*HIP concentration generally enhances folding *in vitro*[114] and *in vivo*[118]. *hs*HIP also facilitates the assembly of HSP70s into multichaperone complexes with HSP90 (REF. 119). However, this effect seems to be independent of an interaction with HSP70, underscoring both the complexity and need for more information to understand *hs*HIP function.

*hs*CHIP has three distinct domains, an N-terminal TPR domain and an adjacent charged domain, both of which interact with HSP70, as well as a C-terminal U-box domain with E3 ubiquitin ligase activity[120,121]. This combination of domains lends itself to the hypothesis that the *hs*CHIP–HSP70 interaction shunts HSP70-bound clients to the proteolytic pathway. However, *hs*CHIP's biochemical properties and the results of *in vivo* experiments suggest a more specific role in the

targeted degradation of HSP90 clients[122–124] and perhaps a more antagonistic role regarding HSP70s. First, most clients (for example, steroid receptors, cystic fibrosis transmembrane conductance regulator (CFTR) and ERBB2) that show accelerated degradation in the presence of elevated *hs*CHIP expression are clients of HSP90. Second, *hs*CHIP has chaperone-like activity itself, as it can bind unfolded proteins and prevent their aggregation[125], which, depending on the client, may lead to either folding[126] or degradation[125]. Third, the binding of *hs*CHIP to ATP-bound HSP70 can inhibit the J protein-stimulated ATPase activity of HSP70 (REF. 120). This activity favours neither HSP70's binding to clients nor the stabilization of its client-bound form. So, rather than directing HSP70-bound clients towards degradation, these data suggest that *hs*CHIP, by inhibiting the ability of J proteins to stimulate HSP70's ATPase activity, may inhibit HSP70's binding to its own clients, thus promoting their degradation. Alternatively, *hs*CHIP's action on HSP70 could recruit and maintain HSP70 in a form that is able to rapidly interact with *hs*CHIP-bound clients once they have been ubiquitylated, to facilitate their transfer to the proteasome. Clearly, much more work is needed before *hs*CHIP function can be discussed with certainty.

Perspectives

Whereas HSP70 provides the 'horsepower' to the core machine, exquisite fine-tuning by cofactors, particularly J proteins, provides the machine with functional and client specificities. Despite the progress reported here, there is still much to learn at many levels. Understanding the means by which J proteins, NEFs and other proteins mechanistically alter the function of the core machinery requires insight that can only come from further structural and biochemical work. For example, determining whether individual J proteins bind client proteins, and with how much specificity, is paramount for a global understanding of this chaperone machinery. Also, more detailed biochemical, cell-free experiments using (unfolded) clients and the sequential addition of J proteins (with and without intact J domains), HSP70s and NEFs will be needed to gain better insights into the mode of action of the core machine and its dependence on its specific composition.

How to practically manipulate the machinery for clinical intervention is a future challenge. Because of their diversity, J proteins may be better, more specific targets than HSP70s. Indeed, manipulations of HSP70 in mice have had some desirable effects (such as cardioprotection[127,128] and delay in progression of neurodegenerative diseases[129–131]), but also some negative effects (such as carcinogenesis[132,133]). Screening for individual J proteins that specifically interact with disease-associated misfolded proteins or protein aggregates may be one possible approach to find candidates for more selective manipulation of the core machine in a specific disease. In cases where good cellular models of folding diseases are available, comparison of the effects of manipulating expression of the various J protein on the fate of the disease-associated clients could also lead to productive approaches. Clearly, in addition, more emphasis will need to be placed on generating *in vivo* transgenic and/or (conditional) knockout models targeting specific J proteins. In addition, basic information about many human J proteins is only rudimentary. Thus, there are many challenges and opportunities for insights into the fundamental specificities of J proteins that drive composition and function of the core machines, which hopefully will lead to practical, medical applications.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
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FURTHER INFORMATION
Harm H. Kampinga's homepage:
http://www.rug.nl/umcg/faculteit/disciplinegroepen/celbiologie/stralingenstresscelbiologie/index
Elizabeth A. Craig's homepage:
http://www.biochem.wisc.edu/faculty/craig/lab/default.aspx
***Saccharomyces* Genome Database:**
http://www.yeastgenome.org/

SUPPLEMENTARY INFORMATION
See online article: S1 (figure) | S2 (figure) | S3 (figure)
ALL LINKS ARE ACTIVE IN THE ONLINE PDF