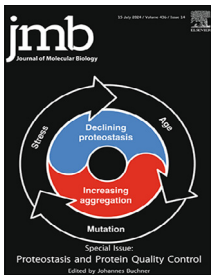
Review Article



Role of J-domain Proteins in Yeast Physiology and Protein Quality Control

Carmen Ruger-Herreros [1,2], Lucia Svoboda [1], Axel Mogk [1,*] and Bernd Bukau [1,*]

*1 - **Center for Molecular Biology of Heidelberg University (ZMBH),** DKFZ-ZMBH Alliance, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany*

*2 - **Instituto de Biomedicina de Sevilla (IBiS),** Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Avda. Manuel Siurot, s/n, E-41013 Sevilla, Spain*

***Correspondence to Axel Mogk and Bernd Bukau:** a.mogk@zmbh.uni-heidelberg.de (A. Mogk), bukau@zmbh.uni-heidelberg.de (B. Bukau)*

https://doi.org/10.1016/j.jmb.2024.168484

Edited by J. Buchner

Abstract

The Hsp70 chaperone system is a central component of cellular protein quality control (PQC) by acting in a multitude of protein folding processes ranging from the folding of newly synthesized proteins to the disassembly and refolding of protein aggregates. This multifunctionality of Hsp70 is governed by J-domain proteins (JDPs), which act as indispensable co-chaperones that target specific substrates to Hsp70. The number of distinct JDPs present in a species always outnumbers Hsp70, documenting JDP function in functional diversification of Hsp70. In this review, we describe the physiological roles of JDPs in the *Saccharomyces cerevisiae* PQC system, with a focus on the abundant JDP generalists, Zuo1, Ydj1 and Sis1, which function in fundamental cellular processes. Ribosome-bound Zuo1 cooperates with the Hsp70 chaperones Ssb1/2 in folding and assembly of nascent polypeptides. Ydj1 and Sis1 cooperate with the Hsp70 members Ssa1 to Ssa4 to exert overlapping functions in protein folding and targeting of newly synthesized proteins to organelles including mitochondria and facilitating the degradation of aberrant proteins by E3 ligases. Furthermore, they act in protein disaggregation reactions, though Ydj1 and Sis1 differ in their modes of Hsp70 cooperation and substrate specificities. This results in functional specialization as seen in prion propagation and the underlying dominant role of Sis1 in targeting Hsp70 for shearing of prion amyloid fibrils.

Introduction: The Hsp70 chaperone functional cycle and its control by J-domain proteins

Hsp70s represent a highly conserved but functionally versatile group of molecular chaperones. They have key functions in cellular protein quality control (PQC) by acting in a multitude of protein folding processes including *de novo* folding of newly synthesized proteins, membrane targeting of organellar proteins, prevention of aggregation of misfolded proteins, solubilization of aggregated proteins and control of regulatory circuits such as the heat shock response.[1] Hsp70 cooperates with other chaperones, especially the small Hsps, AAA+ disaggregases (e.g. Hsp104) and Hsp90, expanding Hsp70 activities in protein disaggregation and protein folding. This broad spectrum of Hsp70 functions within the PQC network protects cells from proteotoxic stressors such as heat shock, pathophysiological conditions and aging.

Hsp70s are composed of a nucleotide-binding domain (NBD) and a substrate-binding domain (SBD) which interacts with short, exposed

hydrophobic sequence stretches of substrates.[2] The nucleotide bound by the NBD dictates the substrate affinity of the SBD. In the ATP-bound state, Hsp70-substrate interactions are weak and transient, as substrates rapidly associate but also rapidly dissociate from the SBD. In contrast, closure of the SBD in the ADP-bound state leads to stable substrate trapping (Figure 1A). Spontaneous ATP hydrolysis by Hsp70 and the subsequent release of ADP are very slow, on the minute time scale, demanding factors that catalyze these rate-limiting steps. These tasks are accomplished by J-domain proteins (JDPs) and nucleotide exchange factors (NEFs), which together control affinities and kinetics of substrate interactions and allow for rapid cycles of substrate binding and release (Figure 1A).

JDPs are central and indispensable co-chaperones of Hsp70s. They confer multifunctionality of Hsp70 by targeting specific substrates to Hsp70, determining cellular localizations of Hsp70 and modulating its ATPase cycle. JDPs interact with the interface of the Hsp70 NBD and SBD[3] and stimulate ATP hydrolysis. This step is linked to the concurrent transfer of substrate from JDPs to Hsp70. The ability of JDPs to bind substrates is reflected in an autonomous chaperone activity. The ATPase and substrate binding cycle of Hsp70s is closed by NEFs which dissociate bound ADP from Hsp70. This ensures resetting of Hsp70 upon binding of ATP and displacement of bound substrates.

Overview on domain organization and classification of JDPs

All JDPs share the J-domain, which is composed of approx. 70 residues including the highly conserved HPD motif that is essential for Hsp70 interaction and ATPase stimulation (Figure 1B)[1]. Sequence alterations in J-domains dictate cooperation with specific Hsp70 partners[4] defining cooperating pairs of JDPs and Hsp70s as also summarized in[5]. While all JDPs share the J-domain, they differ in other regions considerably, thereby gaining functional specificities that are conferred to Hsp70 partners. Depending on the domains fused to the J-domain three distinct JDP classes have been defined (reviewed in.[6] Class A and B JDPs harbor two β-barrel domains (CTD I and CTD II) for substrate binding. Both also have an unstructured glycine/phenylalanine-rich region (GF) that links CTD I with the N-terminal J-domain, and a C-terminal dimerization domain (Figure 1B). Class A JDPs additionally harbor a zinc-finger-like region (ZFLR), which protrudes from CTD I and a C-terminal extension (CTE) (Figure 1B). The ZFLR is characterized by two zinc-binding sites, enclosing a β-hairpin. It has been implicated in transfer of bound substrate to Hsp70[7] and might additionally contribute to substrate interaction[8]. The CTE was recently shown to control self-assembly and chaperone activity of human DnaJA2[9]. Class C JDPs represent a highly heterogenous class with diverse domains, which determine functional specificities, fused to the J-domain. We will not cover these JDPs here and refer to other reviews.[5,6]

Class A and B JDPs can differ in substrate specificity due to sequence alterations in their CTD I and II domains. CTD I and CTD II independently recognize linear sequence motifs in substrate proteins enriched in aromatic and hydrophobic residues[10,11]. Differences in CTD binding specificities have been reported, which will expand the substrate spectrum of JDPs and contribute to functional diversification.[12] The dimerization of JDPs leads to the presence of at least four substrate binding sites within a protomer and opens the possibility that simultaneous occupancy of multiple sites increases substrate affinity through avidity effects. This might enable JDPs for both, rapid and transient screening of proteins, and distinction between native and misfolded or aggregated states.

Next to differences in substrate specificities, class A and class B JDPs additionally exhibit fundamental differences in their cooperation with the Hsp70 partner (see also[5] for more detailed summary). The J-domain of class B JDPs is not freely accessible but blocked through an autoinhibitory interaction with the GF region[13,14]. To overcome this autoinhibition, Class B JDPs require the presence of the C-terminal EEVD motif of eukaryotic Hsp70 for cooperation[13,14]. The Hsp70 EEVD extension binds to CTD I of class B JDPs, triggering the release of the J-domain through an allosteric change.[13] This two-step binding mode has functional implications on Hsp70 activities in disassembly reactions as detailed below.

Here, we describe the physiological functions of JDPs from the model organism *Saccharomyces cerevisiae*. We largely focus on the activities of cytosolic and nuclear JDPs in PQC. Functional and mechanistic principles established for yeast JDPs are at least partially transferable to human orthologs.

General physiology of JDPs in yeast

The number of distinct JDPs present in a species always outnumbers Hsp70s. A single Hsp70 can interact with various JDPs, rationalizing how a single Hsp70 machine can take over so many different functions. The copy number of JDPs differs substantially (Table 1) and their abundancies can dramatically change through induction of stress responses. As consequence, JDP copy numbers are dynamic and sensitive to growth conditions, allowing to modulate Hsp70 activity on demand.

Yeast encodes for 22 JDPs and 11 Hsp70s, including the cytosolic and nuclear Ssa1-4, the cytosolic Ssb1-2 and Ssz1, and the compartment-

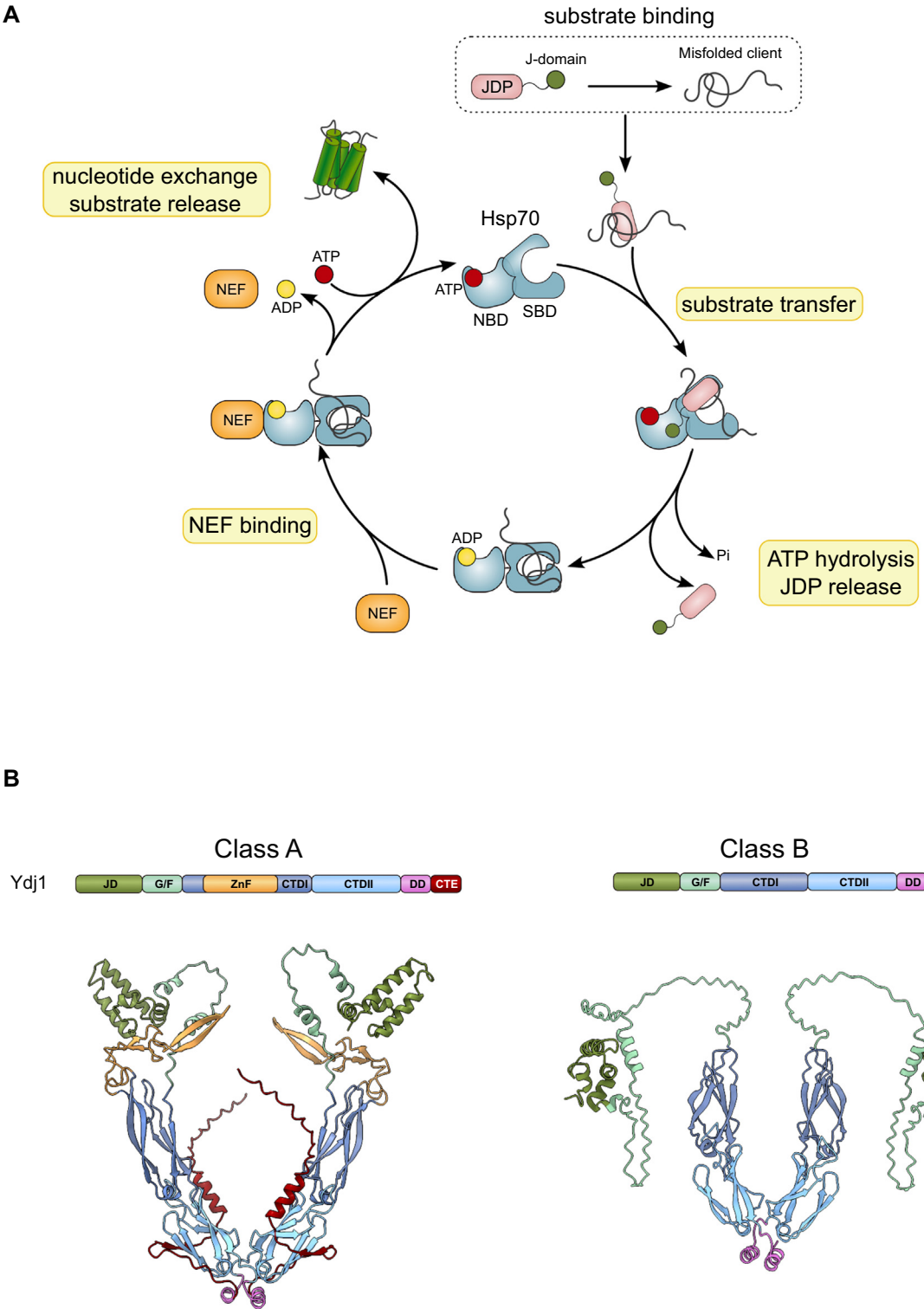


Figure 1. JDPs function as crucial co-chaperones of Hsp70. (A) The Hsp70 chaperone consists of a nucleotide binding domain (NBD) and a substrate binding domain (SBD). In the ATP-bound state substrate affinity is low due to an open SBD. J-domain proteins (JDPs) bind misfolded proteins and interact with Hsp70 via their J-domain. JDP binding and concurrent substrate transfer stimulates ATP hydrolysis by Hsp70 and leads to sequestration of substrate at the closed SBD. Nucleotide exchange factors (NEFs) bind to Hsp70 and trigger ADP release. ATP rebinding reopens the SBD and causes substrate release. (B) Domain organizations and structures of class A and class B JDPs with yeast Ydj1 and Sis1 as representatives. Both classes contain a J-domain, a disordered G/F region, two C-terminal substrate binding sites (CTDI, CTDII) and a dimerization domain (DD). Class A JDPs additionally harbor a zinc-finger-like region (ZFLR) and a C-terminal extension (CTE). Ydj1 and Sis1 structural models were created by AlphaFold2.

Table 1 Physiological functions and copy numbers of *Saccharomyces cerevisiae* JDPs.

JDP	Class	Localization	Copy numbers (molecules/cells)	Physiological function	growth phenotype
Ydj1	A	Cytosol/Nucleus	41,251 +/- 16,052	Folding and targeting (ER, mitochondria) of newly synthesized proteins; protein refolding and disaggregation, protein degradation	Non-essential, *ts* > 30 °C
Xdj1	A	Cytosol/ Mitochondria	1886 +/- 1303	Protein import into mitochondria	Non-essential
Apj1	A	Nucleus	1068 +/- 943	Protein disaggregation and degradation	Non-essential
Scj1	A	ER	3626 +/- 1693	Protein folding in ER	Non-essential, *ts*
Mdj1	C	Mitochondria	5987 +/- 1296	Protein folding in mitochondria	Non-essential, *ts*
Sis1	B	Cytosol/Nucleus	28,050 +/- 5749	Protein folding and disaggregation, prion propagation, targeting (mitochondria) of newly synthesized proteins, protein degradation	Essential
Djp1	B	ER membrane/ Mitochondria	5619 +/- 1781	Protein import to peroxisomes, re-targeting of mitochondrial precursors from ER	Non-essential
Caj1	B	Cytosol/Nucleus	12,469 +/- 1828	Unknown	Non-essential
Erj5	A	ER	2657 +/- 863	Protein folding at ER	Non-essential, *ts*
Jjj1	C	Cytosol/Nucleus	1973 +/- 558	Endocytosis, ribosome biogenesis	Non-essential, *ts*
Jjj3	C	Nucleus/Cytosol	2010 +/- 1269	Targeting of newly synthesized proteins to ER, diphthamide biosynthesis	Non-essential
Jac1	C	Mitochondria	2464 +/- 605	Iron-sulfur cluster assembly	Essential
Cwc23	C	Nucleus	1647 +/- 772	Spliceosomal complex disassembly	Essential
Swa2	C	Cytosol	2927 +/- 800	Clathrin coat disassembly (endocytosis)	Non-essential
Jem1	C	ER membrane	1663 +/- 430	ERAD pathway, kariogamy during conjugation	Non-essential
Jid1	C	ER membrane	414	ERAD pathway	Non-essential, *ts*
Jjj2	C	Cytosol/Nucleus	542 +/- 427	Unknown	Non-essential
Sec63	C	ER	9491 +/- 3050	Protein import into ER	Essential
Zuo1	C	Cytosol/Nucleus/ Mitochondria	46,597 +/- 15,022	Co-translational protein folding, ribosome biogenesis	Non-essential, *ts* < 20 °C
Mdj2	C	Mitochondria	802 +/- 620	Protein import into mitochondrial matrix	Non-essential
Pam18	C	Mitochondria	4907 +/- 2060	Protein import into mitochondrial matrix	Essential
Hlj1	B	ER	2774 +/- 915	ERAD pathway	Non-essential

JDP localizations and growth phenotypes were derived from from https://www.yeastgenome.org.[120] JDP copy numbers are derived from https://sites.google.com/view/yeastgenome-help/function-help/protein-information.

specific Kar2 (ER) and Ssc1 and, Ssq1, Ssc1 and Ssc3 (mitochondria) (Table 1). The presence of one functional gene copy of Ssa1-4 is essential for yeast viability, underlining the crucial function of the Ssa´s for cellular physiology and PQC. Similarly, the Hsp70 copies of the ER (Kar2) and mitochondria (Ssc1) are essential for yeast viability. Ssa1/2 are constitutively expressed and represent the most abundant Ssa proteins, whereas Ssa3/4 are induced upon stress conditions.[15,16] The cooperation of JDPs with Hsp70 largely follows their cellular co-localizations, however, specific cooperations also exist within the same compartment (Table 1). For instance, the ribosome-bound JDP Zuo1 only cooperates with Ssb1/2, whereas Jjj1, which also binds to the ribosome, only cooperates with Ssa1/2[17,18]. Concerning the roles of JDPs for yeast physiology, one can distinguish between generalists and specialists.[19] JDP generalists take part in fundamental PQC processes including protein folding, membrane targeting and degradation, whereas specialists function in specific cellular processes like ribosome biogenesis or pre-mRNA splicing.

Ydj1 is the central generalist among JDPs, which is reflected in its high copy number (approx. 41.000 molecules/cell, see Table 1). Its central role in yeast physiology and PQC is reflected in the poor growth of *ydj*1Δ mutant at 25 °C and a temperature-sensitive growth phenotype above 30 °C.[20] Overexpression of the J-domains of various JDPs including Ydj1, Sis1 and Apj1 is sufficient to rescue the severe growth defect of *ydj1* knockout at 30 °C.[20,21] This indicates that the very basic function of JDPs, the stimulation of ATP hydrolysis by Hsp70 and the resulting substate trapping, can be sufficient for JDP activity *in vivo*. However, J-domain overexpression does not fully substitute for absence of Ydj1, as cells remain temperature-sensitive. This documents crucial roles for additional domains of Ydj1 dictating substrate specificity.

Sis1 represents the second major and abundant (28.000 molecules/cell) JDP acting in PQC and is essential for yeast viability. Ydj1 and Sis1 have functional overlaps in PQC as documented by negative genetic interactions[22]. Deleting the substrate binding domains (CTDI and CTDII) of both Ydj1 and Sis1 is lethal, pointing to overlapping substrate pools of the JDPs[22]. However, differences in substrate specificity between Sis1 and Ydj1 also exist, which is indicated by the existence of distinct phenotypes of Sis1 and Ydj1 mutants. The analysis of hybrid Sis1/Ydj1 proteins revealed that these phenotypes can be rescued only if the authentic CTDs are expressed.[23]

Zuo1 is the third JDP generalist in PQC, though its function is clearly different as compared to Sis1 and Ydj1. Zuo1 binds to translating ribosomes, fulfilling a crucial function in the folding of newly synthesized proteins (see below). This general function of Zuo1 is reflected in a high copy number (approx. 47.000 molecules/cell) ensuring interaction with the majority of ribosomes.

JDP specialists act in diverse cellular pathways (Table 1) but will not be covered here. Notably, the boundaries between JDP generalists and specialists are sometimes blurry. For instance, the essential activity of Sis1 required for yeast viability is not linked to general PQC but to a specific class of substrates, phosphatidylinositol-3-kinase-related kinases (PIKK).[24] Overexpression of Tti1, a specialized chaperone for PIKK maturation, enables the otherwise lethal deletion of the *SIS1* gene, underlining that the most severe phenotype reported for Sis1 is not linked to its role as JDP generalist.[24]

Role of JDPs in folding and membrane targeting of newly synthesized proteins

Nascent polypeptides are immediately interacting with molecular chaperones once they leave the peptide exit tunnel of the translating ribosome. In yeast, the ribosome-bound Hsp70 members Ssb1/2 interact with newly synthesized proteins. Ssb1/2 undergo repeated cycles of binding and dissociation during nascent chain synthesis, interacting with short peptide stretches enriched for hydrophobic and basic residues once they appear close to the tunnel exit.[25,26] This Ssb1/2 activity is suggested to delay co-translational folding until all relevant information for folding of a domain becomes available through ongoing synthesis. Ssb1/2 thereby promote domain-wise folding, prevent nascent chain aggregation and coordinate folding events with downstream assembly reactions (Figure 2a)[27,28]. Ssb1/2 binding to nascent chains during translation is correlated to local alterations in translation speed[25,26], indicating a connection between co-translational action of chaperones and the rate of polypeptide synthesis.[25,29]

The functional recruitment of Ssb1/2 to ribosomes requires the ribosome-associated complex (RAC),[25] which is a stable heterodimer consisting of the JDP Zuo1 (Zuotin) and the non-canonical Hsp70 member Ssz1 (Figure 2a)[30]. Ssz1 harbors a truncated SBD and does not hydrolyze ATP, distinguishing it from generic Hsp70s[31]. The Zuo1 component of RAC has a binding site next to the peptide exit tunnel in the 60S ribosomal subunit, but via a long helical arm also reaches out to the 40S subunit[18,32,33]. Thereby Zuo1 is in proximity of the decoding center of the ribosome, perhaps accounting for the observation that Zuo1 can increase translational fidelity[18,34]. Zuo1 is the JDP partner of Ssb1/2 and stimulates ATP hydrolysis, thus ensuring the trapping of nascent chains by Ssb1/2. This classical JDP function of Zuo1 strictly depends on stable complex formation with Ssz1, Zuo1 is thus only functionally active in context of the RAC complex[31]. The dependence of Ssb1/2 on RAC is reflected in a shared set of phenotypes of respective mutant cells including cold-sensitive growth and sensitivity to cations and aminoglycosides[35–37], but potential roles of RAC in nascent chain folding that do not involve Ssb1/2 are not excluded. Furthermore, RAC and Ssb1/2 have a role in ribosome biogenesis.[38,39] Whether this function relies on the chaperoning of nascent ribosomal proteins is unclear.

Ssa1/2 have also been implicated in the co– and post-translational folding of newly synthesized proteins (Figure 2b)[40]. Ssa1/2 act in concert with Ssb1/2, as indicated by an enhancement of co-translational interactions of Ssa1 in the absence of Ssb1/2.[41] This might imply that Ssa1/2 can partially take over Ssb1/2 function in *de novo* protein folding, functioning as backup or repair system. It is unclear which JDPs function as recruiting factors of Ssa1/2 to newly synthesized proteins, but Sis1 and Ydj1 as most abundant cytosolic JDPs of Ssa1/2 are prime candidates.

JDPs with their organellar Hsp70 partner chaperones have crucial functions in targeting to

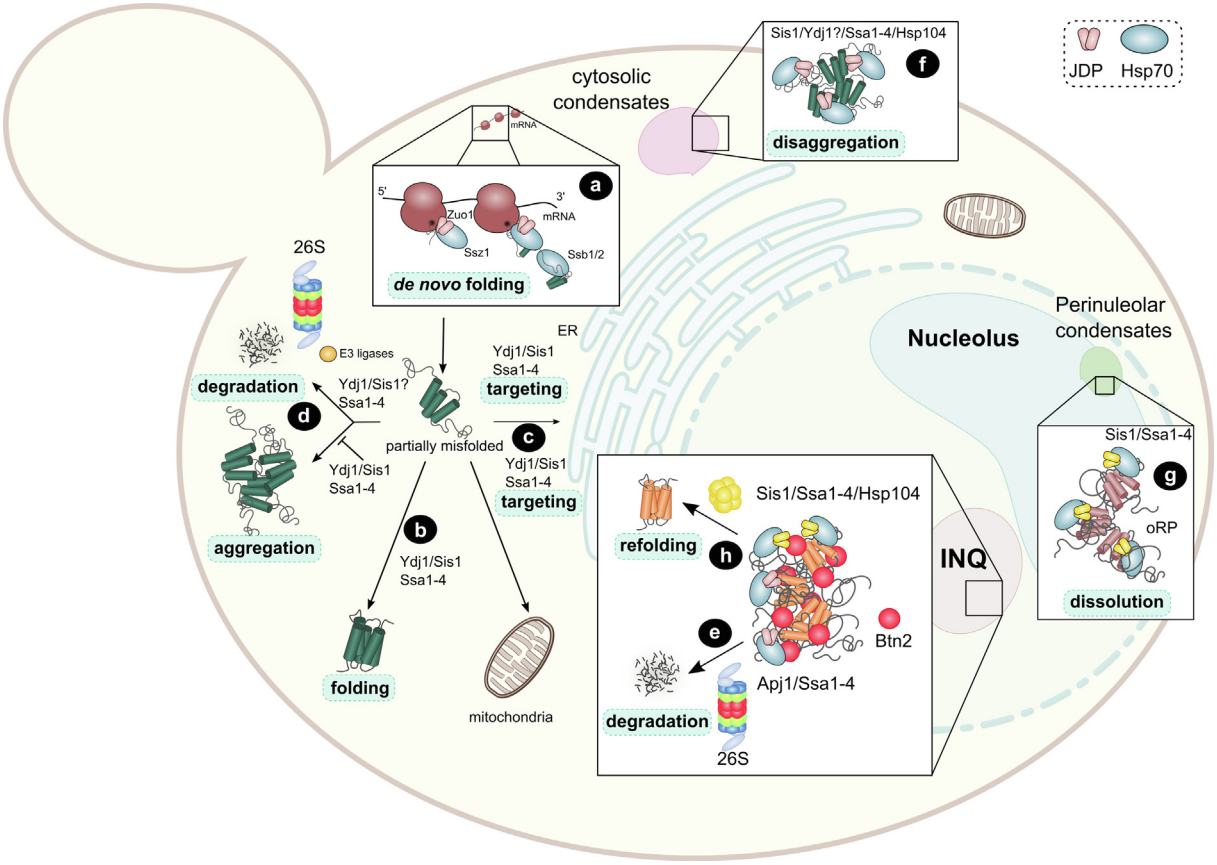


Figure 2. Functions of cytosolic and nuclear JDPs in yeast protein quality control. Ribosome-bound Zuo1 cooperates with Ssb1/2 in the folding of nascent polypeptides (a). Ydj1 and Sis1 are involved in post-translational folding or targeting of newly synthesized proteins (b/c). They can also support degradation of non-native proteins by either preventing protein aggregation or facilitating substrate ubiquitination by E3 ligases (d). JDPs together with Hsp70 also play crucial roles in disassembly/disaggregation reactions. They can act on stress granules or protein aggregates in the cytosol (f). Nuclear Sis1 releases orphan ribosomal proteins (oRPs) from stress-induced perinucleolar condensates (g). Sis1 also interacts with the sequestrase Btn2 that isolates misfolded proteins at INQ (intranuclear quality control compartment) and initiates disaggregation and refolding of sequestered substrates by Hsp70/Hsp104 (h). Alternatively, Apj1 cooperates with Hsp70 in INQ dissolution but targets substrates for proteasomal degradation (e).

and import of proteins into the ER and mitochondria. ER and mitochondria resident Hsp70/JDPs act inside the organelles (ER: JDPs Sec63/Scj1 and Hsp70 Kar2; mitochondria: JDPs Pam17/Xdj1/Mdj1 and Hsp70 Ssc1) by interacting with translocons and facilitating import and folding of the organellar proteins via ratcheting/entropic pulling[42]. Other specialized functions have been assigned to the JDPs Xdj1 and Djp1. Xdj1 interacts with the Tom22 import receptor at the outer membrane of mitochondria and promotes assembly of the TOM complex[43]. ER membrane localized Djp1 reroutes mislocalized mitochondrial inner membrane proteins from the ER back to mitochondria. This fail-safe mechanism was termed ER-SURF.[44]

Cytosolic Hsp70s and cooperating JDPs also support the targeting of newly synthesized proteins to the ER and mitochondria by keeping them in a transport-competent state. Ssb1/2 were shown to interact with a large variety of mitochondrial and ER-targeted proteins.[25,26] The aggregation of mitochondrial proteins in *Δssb1 Δssb2* knockouts[28] implies a functional relevance of this interaction. On the other hand mitochondrial function is rather enhanced in *Δssb1 Δssb2* cells due to release of glucose repression[45,46] indicating that loss of co-translational interactions between Ssb1/2 and mitochondrial precursors is not detrimental for mitochondrial activity.

A post-translational targeting route of newly synthesized proteins to the ER and mitochondria invokes cytosolic Ssa1/2 and their major JDP partners Ydj1 and Sis1 (Figure 2c). Mitochondrial proteins harboring a pre-sequence engage Ydj1 and Ssa1/2[47–49]. Sis1 and Ydj1 were shown to interact with hydrophobic transmembrane segments of newly synthesized signal-anchored (SA) outer membrane proteins of mitochondria, supporting their Ssa1/2-dependent targeting to the Tom70 receptor[50]. Overlapping functions of Ydj1 and Sis1

were also observed in the targeting of mitochondrial beta-barrel proteins, which are inserted into the outer membrane[51]. Similarly, a network of JDPs involving Sis1/Ydj1/Apj1/Jjj3 has been implicated in the targeting of tail-anchored (TA) proteins[52]. Here, the JDPs act upstream of the GET pathway by capturing substrates and facilitating their subsequent transfer to GET pathway components like Sgt2.[52]

JDPs have also been linked to nuclear import of misfolded proteins for proteasomal degradation[53]. This pathway is part of the cellular PQC system and was suggested to separate protein degradation in the nucleus from cytosolic protein synthesis, protecting nascent polypeptides from exhaustive degradation. Sis1 shuttles between nucleus and cytosol, enabling for the delivery of bound substrates to the nucleus. Restricting Sis1 localization to either cytosol or nucleus by fusing of NES and NLS sequences to Sis1 abolished substrate transfer to the nucleus[53]. Ydj1 was also implicated in targeting of misfolded proteins to nucleus,[54] however, its contribution might be indirect, by keeping substrates in a soluble state that is accessible for import factors.

JDP function in protein degradation: competitive versus cooperative activities

Besides chaperone-assisted (re)folding of proteins, the degradation of misfolded proteins by the ubiquitin proteasome system (UPS) constitutes a second branch of the PQC system. Misfolded proteins are selected by quality control E3 ligases, including Ubr1, San1, Rsp5 and Doa10, which poly-ubiquitinate substrates in cooperation with a cognate E2 conjugating enzyme and target the labeled substrates for degradation by the 26S proteasome.[55] How folding and degrading branches cooperate in the proteostasis network is an important yet largely unresolved question. Chaperones like Hsp70 might compete with E3 ligases for substrate binding and triage decisions will depend on kinetic partitioning: slow (re)folding kinetics will render a substrate more prone to interactions with E3 ligases and degradation. Alternatively, Hsp70 together with JDPs may directly cooperate with E3 ligases in protein degradation by presenting substrates for facilitated ubiquitination.

Positive genetic interactions between yeast *ssa*1Δ *ssa*2Δ and UPS mutants support the competition model: the temperature-sensitive growth of *ssa*Δ double mutants can be partially rescued by reducing ubiquitin-dependent protein degradation.[56,57] This implies that an imbalance between refolding and degrading activities represents one reason for the growth defects of *ssa*1Δ *ssa*2Δ cells. Thus, enhanced UPS-dependent degradation of Hsp70 substrates limits the growth of *ssa*1Δ *ssa*2Δ cells. Similarly, an imbalance between Hsp70-dependent (re)folding and proteolytic activities was noticed to be largely responsible for proteostasis and growth defects of mutants with decreased Hsp70 capacity.[58] Together these findings underline that a balance between refolding and degradation pathways is critical to cell viability. Still, various unstable PQC reporters are frequently stabilized in yeast Hsp70 mutants. This involves the degradation of abnormal proteins[59], ER proteins that are import-incompetent[60] and various misfolded reporters linked to ER-associated protein degradation (ERAD)[61–63]. The role of Hsp70 in protein degradation can be explained by keeping substrates in a soluble and thus accessible state for E3 ligase activity.[64,65] In this model Hsp70 acts passively, and kinetic partitioning is sufficient to explain ultimate substrate degradation.

Active roles of Hsp70 and cooperating JDPs in substrate presentation to E3 ligases have also been suggested (Figure 2d). Ydj1 was shown to play a role in ER-associated protein degradation (ERAD)[62]. Ydj1 is partially anchored to the ER membrane by farnesylation of the C-terminal CAAX motif,[66] supporting a scenario in which Ydj1 directly cooperates with an E3 ligase in ubiquitination of ERAD substrates at the ER membrane. Indeed, Ydj1 but also Sis1 are required for Doa10-mediated protein degradation, an E3 ligase that localizes to the ER and the inner nuclear membrane[9,57]. A physical interaction of Ydj1 with the E3 ligase Rsp5 has been reported upon heat shock, suggesting that Ydj1 transfers substrates to Rsp5 for ubiquitination and subsequent degradation[67]. Such JDP dependent ubiquitination was also reported for Sis1, which was shown to cooperate with Doa10[68] and Ubr1 in protein degradation.[69] However, it is usually difficult to distinguish between direct and indirect roles of JDPs and Hsp70 in protein degradation. Thus loss of Hsp70 and JDPs functions might affect substrate solubility as mentioned above or overwhelm the UPS system due to accumulation of non-native protein substrates. The molecular basis of how JDPs cooperate with E3 ligases remains unclear. Unravelling this mode of cooperation will help to understand how JDPs differentiate between refoldable and terminally misfolded conformers, which are transferred to E3 ligases.

An exclusive and direct connection to protein degradation has been shown for the nuclear JDP Apj1, which arose from gene duplication of *YDJ1*[70]. Apj1 exhibits strong negative genetic interactions with the SUMO-dependent E3-ligases Slx5/8 causing poor growth of double knockouts.[70] SUMOylated proteins strongly accumulate in *Δapj*1 Δ*slx5* double mutants, suggesting that the growth defects stem from reduced protein degradation. Apj1 has also been shown to direct terminally misfolded proteins and mistargeted mitochondrial

proteins for proteasomal degradation in the nucleus.[71] Here, Apj1 functions as a disaggregase that solubilizes substrates sequestered at INQ (intranuclear quality control compartment) for subsequent proteasomal degradation (Figure 2e). Notably, substrates sequestered at INQ can be SUMOylated,[72] suggesting a link between both reported Apj1 phenotypes.

Role of JDPs in the disassembly of diverse stress-induced protein assemblies

The disassembly of protein complexes is a core activity of Hsp70. Hsp70 can act on diverse physiological and pathological complexes and the broad substrate spectrum is again dictated by the diverse cooperating JDP proteins. JDPs either target specific regular protein complexes with physiological functions or recruit Hsp70 to stress-induced assemblies of aberrant proteins. The JDP Swa2 directs Ssa1/2 to vesicles, which are coated with a lattice composed of clathrin trimers that interact with one another to form a stable triskelion structure. Disassembly of the clathrin trimers by Hsp70 leads to vesicle uncoating, which is a crucial step during endocytosis[73]. Highly specific disassembly reactions during ribosome biogenesis and pre-mRNA-maturation are governed by the JDPs Jjj1 and Cwc23, which either displace the ribosome biogenesis factor Arx1 from pre60S particles[74], or act in the disassembly of the spliceosome,[75] respectively.

Heat shock conditions trigger the formation of diverse protein assemblies, whose dispersal not only requires JDP/Hsp70 activity but additionally the presence of the AAA+ disaggregase Hsp104[76–79]. Ssa1/2 recruit Hsp104 to the surface of assemblies through direct contacts[80] and activate the Hsp104 ATPase and threading activities,[81] leading to the extraction of sequestered protein. The kind of assemblies that form during heat shock depends on the severity of the stress regime. Moderate heat shock (up at 45 °C) triggers the formation of biomolecular condensates like cytosolic stress granules, which include a specific set of proteins typically linked to protein synthesis. The sequestered proteins are likely in a near-native state but not globally unfolded[78] and the assemblies are suggested to form via phase-separation and to exhibit a liquid-like character.[82] Poly(A)-binding protein, Pab1, is a central component of stress granules and an extremely rapid dispersal of Pab1 condensates by Hsp70 has been documented *in vitro*[83] (Figure 2f). Pab1 disaggregation by Hsp70 relies on the Hsp104 partner and the JDP Sis1[83], whereas Ydj1 is unable to assist Hsp70 in this disassembly reaction. Orphan ribosomal proteins (oRPs) form peri-nucleolar condensates upon heat shock[84]. Sis1 but not Ydj1 protects these condensates from solidification and mediates condensate dispersal during recovery phases for rapid regain of translation capacity[84] (Figure 2g). A Sis1-specific disassembly reaction was also noted for stress-induced assemblies in the yeast nucleus (INQ), which are formed via the sequestration activity of the heat shock protein Btn2[85]. Btn2 directly interacts with Sis1 and this interaction is crucial for Hsp70 and Hsp104 recruitment and subsequent INQ dissolution[86] (Figure 2h). All these findings raise the possibility that the class B JDP Sis1,but not the class A JDP Ydj1 has the unique ability to target stress-induced condensates. However, *in vivo* Ydj1 also localizes to cytosolic stress granules, implying that Ydj1 might act on a different pool of stress granule components. Such activity is awaiting reconstitution *in vitro*.

Severe heat stress (e.g. 50 °C) leads to the formation of amorphous, solid protein aggregates resulting from misfolded proteins. The disaggregation and reactivation of such assembly states by Hsp70/Hsp104 has been reconstituted *in vitro* using heat-labile model substrates (e.g. Luciferase)[87] and documented activity for both JDPs, Ydj1 and Sis1. Differences in the efficiency of Ydj1 or Sis1-mediated disaggregation reactions were noted and were dependent on the particular model substrate and the denaturation conditions applied. A mechanistic basis for these differences might stem from the number of Hsp70 molecules that is recruited by Ydj1 or Sis1 to the aggregate surface. While Sis1 binds more slowly to protein aggregates, it confers enhanced loading of Hsp70, which in turn increases recruitment of the Hsp100 disaggregase[88]. Enhanced loading of Hsp70 might also increase entropic pulling forces, leading to aggregate remodeling in absence of Hsp104.[88] Such activity has been documented for the human homolog of Sis1, DnaJB1, which enables disassembly of α-synuclein fibrils by human Hsc70 and the Hsp110 member Apg2[89]. Increased Hsp70 loading by Sis1 likely relies on the specific cooperation between the two chaperones, involving two distinct Hsp70 binding sites in Sis1 (J-domain and CTD I). The interaction between CTDI and the C-terminal EEVD motif of Hsp70 is likely more stable and additionally unaffected by the Hsp70 nucleotide state[88]. The increased number of JDP-Hsp70 interaction sites and the more robust interaction strength can explain enhanced Hsp70 recruitment. Yet, Sis1 regulatory mutants that enable for cooperation with Hsp70 lacking an EEVD motif, are still functional in protein disaggregation[14,88]. The lack of a strong disaggregation defect could be explained by the requirement for the Hsp104 disaggregase, which might blur differences in JDP-dependent Hsp70 loading. Of note, the reactivation of aggregated proteins is linked to thermotolerance, the ability of yeast cells to transiently survive a severe heat shock[90]. Thermotolerance development primarily relies on Ydj1[23,91] in conjunction with Ssa´s and

Hsp104, underlining that Ydj1 is operative in protein disaggregation *in vivo*.

JDP specificity in the propagation of prion amyloid fibrils

Yeast prions are amyloid fibers that form from alternative structural states of specific native proteins.[92] The prion state leads to loss of protein activity as exemplified by [PSI+] prions that form by sequestration of the translation termination factor Sup35 into amyloid fibrils.[93] As consequence, the readthrough frequency in [PSI+] cells is strongly increased as compared to [psi-] cells[94]. Prions are self-propagating units and prion replication requires a disaggregation reaction, leading to amyloid fiber breakage and the formation of smaller seeds that are passed to daughter cells[95]. The ends of the seeds allow for incorporation of novel prion subunits, leading to fiber growth and maintenance of the prion state. The fiber disaggregation reaction again involves cooperation of Hsp70 (Ssa1-4), JDPs and the Hsp104 disaggregase[80,96]. Extraction of a prion protein via Hsp104-mediated threading will cause fiber breakage. Loss of fiber breakage by disaggregase inactivation leads to prion curing, allowing to define roles of JDPs in prion propagation.[91] The contributions of JDPs in the fiber disassembly reaction are specific and dependent on prion identity. Sis1 represents the central JDP in prion propagation *in vivo* as multiple prion states including [PIN] (aggregation of Rnq), [PSI+] and [URE3] (aggregation of Ure2) crucially rely on this JDP.[97,98] Similarly, human DnaJB1 but not DnaJA1/2 (human homologs of Ydj1) activates Hsc70 clustering for disassembly of α-synuclein and Tau fibrils[99,100]. Sis1 depletion, similarly to Hsp104 inhibition, leads to an increase in prion aggregate size[101]. This underlines the crucial role of the Sis1-initiated fiber disaggregation reactions for prion propagation in cells and highlights the mechanistic similarities to disaggregation of stress-induced protein assemblies. Yeast prions can, however, also exhibit differing dependencies on JDPs. The prion variant [URE3-1] relies on Swa2 instead of Sis1[102]. The [SWI1] prion is affected by depletion of both, Ydj1 and Sis1.[103] This prion is potentially highly sensitive to alterations in Hsp70 capacity, which will be strongly affected by the loss of highly abundant Ydj1. Thus impacts of other JDPs on prion propagation might also be caused by indirect effects involving functional depletion of Sis1 and Hsp70.

Regulation of the Hsf1-mediated heat shock response: JDPs couple protein damage to chaperone gene expression

Yeast Hsf1 is the central transcription factor for adjusting the expression of PQC components to stress injuries thereby counteracting the deleterious effects of proteotoxic stress conditions[104]. Upon heat shock Hsf1 becomes transiently active and binds as a trimer to promoters harboring heat-shock elements (HSEs). Hsf1 is essential for yeast growth by ensuring basal levels of Hsp70 and Hsp90 chaperones, which both have housekeeping activities in maintaining protein homeostasis in non-stressed cells.[105]

Latent Hsf1 resides in the nucleus enabling for rapid activation and stress induction. In yeast cells the heat shock response involves an additional regulatory layer next to Hsf1 binding to HSEs: the stress-induced formation of Hsf1 and heat shock gene condensates (Figure 3). In these condensates heat shock genes that are separated by large distances or are even located on different chromosomes interact and form clusters[106]. This coalescence involves massive remodeling of chromatin and is dependent on the N-terminal domain of Hsf1.[107] Abolishing heat shock gene coalescence in ΔN-Hsf1 cells, however, does not substantially alter the magnitude and kinetics of the heat shock response, leaving the physiological relevance of heat shock gene clusters currently unanswered.[107]

Hsf1 activity is independently controlled by Hsp70 and phosphorylation, allowing to integrate signals from diverse pathways[108]. Phosphorylation transiently increases upon heat shock and correlates with increased transcriptional activity of Hsf1. However, phosphorylation is dispensable for general regulation of Hsf1, pointing to Hsp70 as central regulator.[108] Hsp70 represses Hsf1 activity by binding and sequestering the transcription factor (Figure 3).[108] Accordingly, Hsf1 is highly active in *Δssa1 Δssa2* cells.[24] The repressor function of Hsp70 is titratable allowing to adjust Hsf1 regulation to the accumulation of damaged proteins (e.g. INQ, oRP condensates) upon heat stress (Figure 3)[108,109]. Inhibiting protein synthesis diminishes the heat shock response, leading to the suggestion that newly synthesized proteins represent a main source of Hsp70 substrates upon heat shock.[110,111] This might also include proteins translocating to the ER or mitochondria, since hampering correct protein targeting to these organelles also triggers Hsf1 activation[112,113]. Stress granules, which readily form upon heat shock and are targets of Hsp70, might also contribute to Hsp70 depletion.[78,83,114]

Which JDP is targeting Hsp70 to Hsf1? Sis1 has been suggested to play a key role in Hsf1 activity control. A complex of Hsf1 and Ssa1 can be isolated from *E. coli* cells when co-expressed with Sis1. Although Sis1 is not part of the final complex, this observation implies that Sis1 can target Hsf1 as substrate to Hsp70.[110]

Sis1 localizes to the nucleoplasm in non-stressed cells and is thus ideally positioned to bind Hsf1. Upon stress Sis1 relocalizes to PQC centers at the ER surface and the nucleolus[115]. At the nucleo-

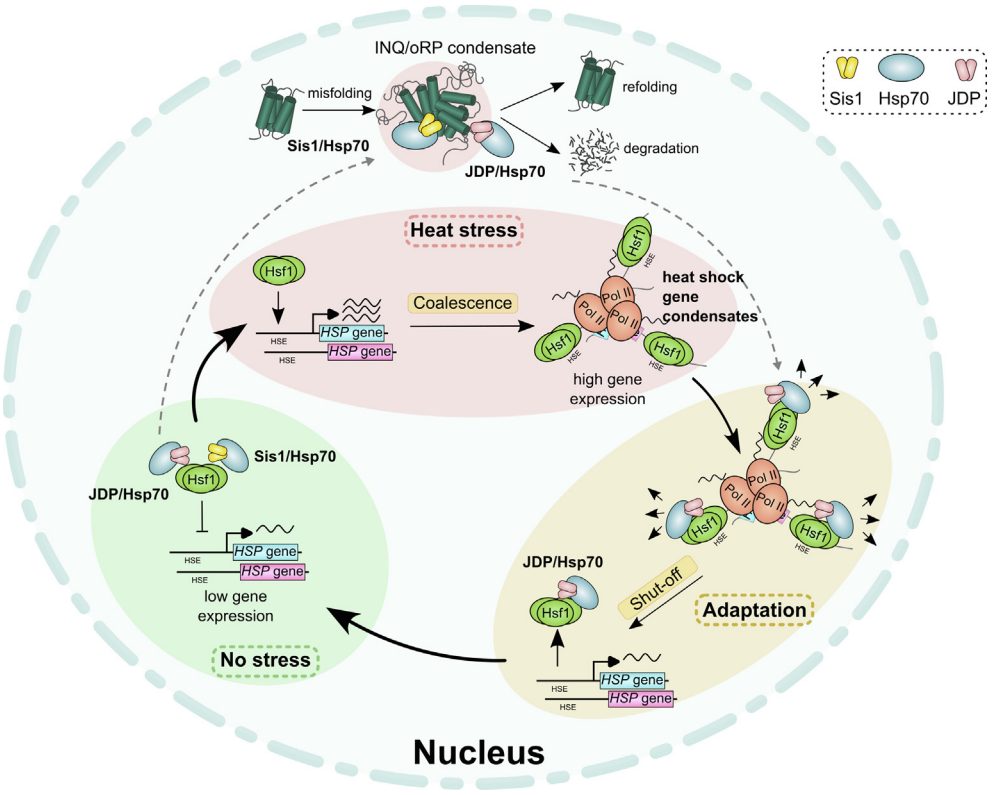


Figure 3. Role of yeast JDPs in regulating Hsf1 activity. In non-stressed cells Hsf1 is kept inactive through sequestration by Hsp70. Hsf1 targeting to Hsp70 involves Sis1 and other, so far unknown JDPs. Stress conditions lead to the accumulation of misfolded and aggregated proteins in the cytosol and nucleus and titrates Hsp70 and JDP capacity. This leads to Hsf1 activation and increased expression of genes encoding for heat shock proteins. Hsf1 activation is coupled to the formation of transcriptionally active condensates that form by Hsf1-dependent coalescence of heat shock gene loci located e.g. on different chromosomes. Stress adaptation leads to inactivation of Hsf1 (shut-off). This process depends on Hsp70 and so far unknown JDPs.

lus Sis1 was recently documented to bind aggregation-prone orphan ribosomal proteins (oRPs)[84]. oRPs form peri-nucleolar, dynamic condensates with liquid-like properties upon stress conditions and Sis1 protects oRP condensates from solidifying.[84] The accumulation of oRPs will therefore change Sis1 availability in the nuclear compartment and trigger rapid Hsf1 activation (Figure 3). In accordance with this model it was shown before that impairing ribosome biogenesis activates Hsf1[116,117]. Sis1 might additionally be depleted by misfolded proteins originating from the cytosol. Preferential targeting of cytosolic misfolded proteins into the nucleus[118] was reported and Sis1 has been implicated in this process.[53]

Surprisingly, Sis1 depletion and even a full gene knockout (rescued by Tti1 overproduction) only results in partial induction of the heat shock response, indicating that other JDPs also take part in downregulating Hsf1 activity[24]. Furthermore, Sis1 was recently shown to be dispensable for the shut-off phase of the heat shock response, representing a negative feedback loop to re-inactive HSE-bound Hsf1[119]. Hsf1-dependent induction of Hsp70 is required to re-repress Hsf1 activity,[109] indicating that Hsp70 also controls the shut-off phase and pointing to other JDPs being involved in this process (Figure 3). Activated Hsf1 differs from latent Hsf1 as (i) it is bound as a trimer to HSE and (ii) becomes part of heat shock gene condensates. These structural differences can rationalize the involvement of distinct JDPs in the induction and shut-off phases of the heat shock response. The identity of alternative JDPs participating in Hsf1 control is currently unknown. Overexpression of Ssa2 together with Ydj1 attenuates Hsf1 activity and *Δydj1* cells exhibit a partial induction of heat shock response.[108] These findings provide some evidence for a role of Ydj1 in Hsf1 control, however, it is difficult to differentiate direct from indirect effects, as Ydj1/Ssa2 overexpression might simply increase total Hsp70 capacity, while *Δydj1* suffer from severe growth defects and massive proteostasis defects.[108] In conclusion, current reports suggest that a network of JDPs controls the Hsf1-dependent heat shock response with specific contributions of distinct JDPs to Hsf1 activation and inactivation periods.

Concluding remarks

The analysis of yeast JDPs has led to seminal insights into their mechanistic cooperations with Hsp70 partners and their diverse roles in the cellular PQC network. The fundamental knowledge acquired for the yeast chaperones provided valuable information on JDP/Hsp70 functions in higher organisms including humans. Various central aspects of JDP functions, however, remain to be explored. How do the different modes of JDP-Hsp70 cooperation affect their physiological activities? How are overlapping functions of diverse JDPs in e.g. protein folding, protein disaggregation and the regulation of the heat shock response coordinated? The distinct substrate binding modes of major JDPs are still only vaguely defined. Furthermore, the roles and mechanisms of many JDP specialists still need to be explored. The analysis of yeast JDPs will presumably make again important contributions to these points.

CRediT authorship contribution statement

Carmen Ruger-Herreros: Writing – review & editing, Visualization, Conceptualization. **Lucia Svoboda:** Writing – review & editing, Visualization, Conceptualization. **Axel Mogk:** Writing – original draft, Supervision, Conceptualization. **Bernd Bukau:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

L.S. was supported by the Heidelberg Biosciences International Graduate School (HBIGS). This work was supported by a grant of the Deutsche Forschungsgemeinschaft (BU617/21-1 and KR3593/4-1) to B.B.

Received 20 October 2023;
Accepted 2 February 2024;
Available online 7 February 2024

Keywords:
Chaperone;
Hsp70;
protein folding;
protein degradation;
protein disaggregation;
prion

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