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MEETING REVIEW

Function, evolution, and structure of J-domain proteins

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Abstract

Hsp70 chaperone systems are very versatile machines present in nearly all living organisms and in nearly all intracellular compartments. They function in many fundamental processes through their facilitation of protein (re)folding, trafficking, remodeling, disaggregation, and degradation. Hsp70 machines are regulated by co-chaperones. J-domain containing proteins (JDPs) are the largest family of Hsp70 co-chaperones and play a determining role functionally specifying and directing Hsp70 functions. Many features of JDPs are not understood; however, a number of JDP experts gathered at a recent CSSI-sponsored workshop in Gdansk (Poland) to discuss various aspects of J-domain protein function, evolution, and structure. In this report, we present the main findings and the consensus reached to help direct future developments in the field of Hsp70 research.

Keywords Heat shock protein 70 (Hsp70) · J-domain proteins (JDPs) · 8-stranded β-sandwich domain (SBDβ)

Introduction

In April 2018, researchers gathered for the first time to attend a workshop centered on the function of J-domain proteins (JDPs), essential co-chaperones of heat shock protein 70 (Hsp70). Hsp70 chaperone machines, which are present in all cellular compartments, play active roles in protein homeostasis by transiently binding to many different polypeptide substrates, via an adenine nucleotide–dependent cycle. Invariably, a J-domain protein co-chaperone is required for this process. Through their defining J-domain, JDPs stimulate the hydrolysis of Hsp70-bound ATP and stabilize` the interaction with substrate polypeptides. This interaction cycle is completed by substrate and ADP release, a process facilitated by interaction of Hsp70 with another critical co-chaperone—a nucleotide exchange factor (Cheetham and Caplan 1998; Craig and Marszalek 2017; Cyr et al. 1994; Kampinga and Craig 2010; Liberek et al. 1991).

At a structural level, Hsp70 alternates between an open, ATP-bound state, with fast substrate association/dissociation rates and a closed, ADP-bound state, with much slower substrate association/dissociation rates. Nucleotide hydrolysis and exchange trigger critical, global conformational changes in all three structural units of Hsp70: an N-terminal nucleotide binding domain (NBD), an 8-stranded β-sandwich domain (SBDβ), and a C-terminal α-helical lid domain (SBDα). The NBD and SBDβ are connected via a highly conserved linker, which is essential for allostery and ATP hydrolysis (Mayer 2018; Swain and Gierasch 2006; Zuiderweg et al. 2013). The J-domain interacts with the open ATP-bound state, in which the SBDα and SBDβ, as well as the linker, are docked onto the NBD.

J-domain proteins differ in expression, regulation, and sequence. Many organisms express multiple members of this family; the domains outside their J-domain are the ones that drive specificity of the system by delivering specific substrate polypeptides or by attracting Hsp70 partners to their site of

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actions. Most of the J-domain proteins initially identified (e.g., DnaJ of *Escherichia coli*, Ydj1 and Sis1 of fungi, and DNAJB1/Hsp40 of mammalian cells) were both constitutively expressed and heat inducible. It is now known that most members are in fact constitutively expressed in cells and that many are not regulated by stress. In addition, in multi-cellular organisms, their expression is heterogeneous among cells and tissues (Hageman and Kampinga 2009). In the next sections, we provide a summary of the workshop discussions, highlighting the latest research in the field, and attempt to align the nomenclature and classification of J-domain proteins with these findings.

Diversity, classification, and nomenclature of J-domain proteins

An Hsp70 usually has more than one J-domain protein (JDP) partner. Yet, quantitative proteomics discussed by Pierre Goloubinoff demonstrates that the sum of concentrations of all JDPs in a given cellular compartment (cytosol, ER, or mitochondria) is about 10 times lower than that of all Hsp70s. In other words, JDP co-chaperones act as sub-stoichiometric catalysts of Hsp70s in vivo, consistent with the results of many in vitro experiments (e.g., Sharma et al. 2010). As reported by Chandan Sahi and Pablo Pulido, plant genomes are especially rich in JDP coding genes. Over hundred members are present in *Arabidopsis thaliana* and other plant species (Finka et al. 2011), with the functionality of many conserved across long evolutionary time scales—from plants to the yeast *Saccharomyces cerevisiae*. However, the emergence of highly complex J-domain protein: Hsp70 networks cannot be explained just by the increased number of J-domain proteins. Besides increasing number, regulatory differences, and sub-functionalization, as well as heterodimeric J-domain protein interactions are driving evolution of highly complex, robust chaperone networks with unprecedented functional diversity. Together, this complexity implies that modulation of J-domain protein concentration and functions could be a feasible way to specifically manipulate the function of Hsp70 machines.

The classification and nomenclature of J-domain proteins, and even the name of this family of proteins, have been a matter of debate for a long time. The workshop participants agreed that the nomenclature of J-domain proteins requires revision, as many alternative names are currently used in the literature such as DNAJ proteins, Hsp40 proteins, and J-proteins. Here, we propose to use J-domain proteins according to their defining J-domain. Further, we propose that the abbreviation of JDP is a convenient and suitably differentiating shorthand. This name implies that related proteins lacking a functional J-domain (see, e.g., Pulido and Leister 2018) are not considered members of the family, although such proteins may provide useful information about evolutionary history and functional diversification.

The original classification scheme subdivided JDPs into three major classes (A, B, C) based on the presence or absence of structural features of bacterial DnaJ—the first member of the family described in the literature. Class A and class B members have their J-domain at the N-terminus, similar to DnaJ, whereas in the class C members, this domain can be anywhere in the protein. In class A JDPs, as in DnaJ, the J-domain is followed by a glycine/phenylalanine (G/F)-rich region of ~ 30 residues, two homologous β-barrel domains, the first containing a zinc-binding motif, and a C-terminal dimerization domain. By definition, class B JDPs contain a G/F-rich region adjacent to the N-terminal J-domain. In more C-terminal regions, they may contain the double β-barrel without the zinc-binding motif (in humans DNAJB1, − 4, − 5, and − 11; in fungi Sis1) or another substrate interaction domain or no substrate binding domain at all. Class C JDPs encompass all JDPs that do not fall into either class A or B, but do contain a functional J-domain. They may have a wide variety of other domains or functional motifs either N- or C-terminal of the J-domain (Craig and Marszalek 2017; Kampinga and Craig 2010). An original class C requirement was the absence of a G/F region next to the J-domain. The participants of the meeting, including Mike Cheetham, one of the originators of the concept behind this classification, agreed that this DnaJ-centered classification is outdated as more JDPs have been identified, as exemplified by the fact that most currently known JDPs belong to class C. Furthermore, as reported by Pierre Genevaux, based on his extensive phylogenetic analysis of prokaryotic JDPs, a huge reservoir of unexplored class C JDPs exist in bacteria and phages (Perrody et al. 2012).

Jarek Marszalek and Chandan Sahi emphasized that a new JDP classification should be based on evolutionary relatedness, not on function. Even within the most conserved prototypical class A JPDs, functional divergence has evolved following their emergence via gene duplication, as evidenced by the closely related proteins Xdj1, Apj1, and Ydj1 in yeast (Sahi et al. 2013). Moreover, as reported by Paolo De los Rios, a division between class A and B JDPs has deep evolutionary roots. By using machine learning techniques, he showed remarkably that members of classes A and B can be distinguished from one another with high accuracy, based solely on the sequences of their J-domains.

The participants of the meeting committed themselves to work toward a new more biologically meaningful JDP classification based on a combination of sequence/structure data, extensive survey of genomic data, and high-quality phylogenetic analyses. This collaborative effort will involve Jaime Huerta-Cepas who reported on his in silico studies on how to identify homologs, paralogs, and orthologs.

Functions of JDP domains

The J-domain

The definition above requires that for a protein to be called a J-domain protein, it must be able to functionally interact with an Hsp70. To date, all functionally defined J-domains have a His-Pro-Asp (HPD) motif between helices II and III that is essential for the stimulation of the ATPase activity of Hsp70s. However, we still know relatively little about the precise structural requirements and allostery involved in the functional interactions between JPDs and Hsp70s.

Based on molecular simulations using both coarse-grained and atomistic models combined with co-evolutionary sequence analysis, Alessandro Barducci reported on an evolutionarily conserved interaction surface formed by helix II of the J-domain and a structurally contiguous region of Hsp70, involving lobe IIA of the nucleotide binding domain, the interdomain linker, and the β-sandwich of the substrate binding domain (SBD) (Malinverni et al. 2017). This mode of interaction was confirmed by recent X-ray structures of a complex between the J-domain of *Escherichia coli* DnaJ and its Hsp70 partner, DnaK, presented by Matthias Mayer (Kityk et al. 2018). The J-domain binds on top of the linker contacting NBD and SBDβ via polar and electrostatic interactions. In particular, the HPD motif accesses two pathways of polar and hydrophobic interactions that converge in the catalytic site for ATP hydrolysis. In addition, contacts between the J-domain and the SBDβ connect the J-domain with an intramolecular signaling pathway that transduces the signal from a bound substrate to the NBD. Mutant analysis demonstrated that these contacts couple the substrate signal to the J-domain action, explaining the synergistic action of substrates and the J-domain in stimulating ATP hydrolysis.

Despite these important advances, how a J-domain recognizes a particular Hsp70 partner is still largely an open question. This question was addressed by Bartlomiej Tomiczek from Jarek Marszalek's group using the class C JDP, Hsc20, which specializes in the biogenesis of iron-sulfur clusters. Hsc20 has changed Hsp70 partners twice during evolution (Delewski et al. 2016). Molecular dynamic simulations combined with evolutionary analyses demonstrated that partner recognition involves evolutionary variable J-domain residues that co-evolved with complementary residues of its Hsp70 partner. Meanwhile, the triggering of Hsp70's allosteric transition involves interaction of the invariant HPD motif of the J-domain and evolutionary conserved residues of Hsp70, as described above for the DnaJ/DnaK interaction.

The G/F-rich region

In the original class typing, the flexible glycine/phenylalanine (G/F)-rich region was used to distinguish JDP class B from class C proteins. Even though class A and B typically make up the vast majority of JDP molecules in a cellular compartment, there is very limited insight into the function of the G/F-rich region. Early work provided hints that the G/F-rich region could be involved in modulating Hsp70's substrate binding activity (Wall et al. 1995) or in substrate binding itself (Perales-Calvo et al. 2010). That autosomal dominant mutations in the G/F-rich region of the human DNAJB6 are associated with a heritable muscle disease (Ruggieri et al. 2016) underscores the functional importance of this region. Also, early work on class A Ydj1 and class B Sis1 of *S. cerevisiae* provided hints that the G/F-rich regions of the two classes differ functionally (Yan and Craig 1999). Consistent with this idea, Carlos Ramos presented results of a solution structure analysis of Sis1 and Ydj1 that revealed features within the N-terminus of the G/F region and J-domain of Sis1 that are not present in Ydj1. Clearly, more experiments are required to understand the G/F-rich region's functional relevance.

Beyond the J-domain and G/F-rich region

JDP diversity beyond the J-domain and G/F-rich region is enormous. Other domains serve to regulate intracellular localization, associations with/in membranes, and, most particularly, substrate binding. A theme of the meeting was the ability of JDPs to triage, through binding, the same client, depending on their folding state, for productive folding or degradation in a reaction catalyzed by the same Hsp70.

Douglas Cyr and Jason Young reported on the biosynthetic folding and trafficking of the chloride channel, cystic fibrosis transmembrane regulator (CFTR). DNAJA1 and HSPA8 (Hsc70) co-translationally bind to the cytosolic facing, CFTR nucleotide binding domain 1 (NBD1) for assisted folding. Using synthetic peptide and competition assays, Jason Young showed that DNAJA1 was even more selective in binding to CFTR, i.e., at sites that mapped to regions critical for the folding of CFTR; DNAJA2 was less selective, and generally matched with Hsc70 sites, suggesting that HSPA8 and the DNAJAs act coordinately on these structurally labile regions. Interestingly, the main other class A JDP, DNAJA2, assists in the maintenance of mature misfolded CFTR. Douglas Cyr showed that another class B JDP, DNAJB12 is involved in monitoring the quality of CFTR folding and triages ERAD-sensitive and ERAD-resistant forms of misfolded membrane proteins between pathways for proteasomal degradation or ERQC autophagy (Grove et al. 2011).

Together, this highlights how different JDPs can bind to different sites in the same substrate, depending on their surface exposure, i.e., folding state. Harm Kampinga brought up comparable data of Linda Hendershot, who examined binding of ER-JDPs: ERdj3, ERdj4, and ERdj5 to two secretory pathway proteins—immunoglobulin γ1 heavy chain and NS-1 κ light chain (Behnke et al. 2016). Binding sites for the pro-folding

ERdj3 (and BiP) were frequent and dispersed throughout the substrate proteins, whereas pro-degradation, ERAD-associated ERdj4 and ERdj5 specifically recognized a distinct type of rarer sequence with a high-predicted aggregation potential.

JDP/Hsp70 and higher order chaperone networks

Many JDPs, especially the class A and some class B members, have been known to function as homodimers for some time. Nevertheless, the surprising complexity of physical and functional interaction networks was a general theme of the meeting. Mikko Taipale presented his collaborative work with Anne-Claude Gingras's laboratory using affinity purification coupled to mass spectrometry, proximity biotinylation (BioID), and pairwise interaction assay LUMIER to systematically characterize the interactomes of all human JDPs and Hsp70s. Preliminary analysis of the network shows that JDPs have very distinct subcellular localizations in human cells and show large differences in substrate interaction.

Consistent with the results of Taipale's unbiased screen, experimental evidence reported by Nadinath Nillegoda, Cecilia Emanuelsson, and Harm Kampinga, points to the idea that higher order complexes formed among JDPs can further fine-tune substrate selection, play a role in fate determination of client proteins, and sometimes be obligatory for productive chaperone action. For example, oligomerization of DNAJB11 and DNAJB12 lead to efficient binding, folding, and assembly of proteins in the ER lumen (Chen et al. 2017) and membrane (Li et al. 2017), respectively. Self-oligomerization presumably provides additional substrate interaction sites allowing for increased avidity toward unfolded/misfolded proteins over natively folded proteins, as exemplified by the efficient recognition and suppression of protein aggregates of amyloidogenic proteins by DNAJB6 (Hageman et al. 2010; Månsson et al. 2018).

Nadinath Nillegoda discussed how transient hetero-oligomer formation by class A and class B JDPs is required for Hsp70-based disaggregases (e.g., between DNAJA2 and DNAJB1) to target a range of protein aggregates (Nillegoda et al. 2015, 2018). Such hetero-complexing, regulated by naturally occurring reversion of electrostatic potentials at the complex forming interfaces (Nillegoda et al. 2017), could presumably bring together an assortment of distinct substrate binding modules to efficiently recognize and bind to, for example, heterogeneous surfaces exposed on amorphous protein aggregates. As discussed by Janine Kirstein, such a cooperative network between JDPs appears to be vital for proteotoxic stress recovery at an organismal level (Kirstein et al. 2017).

JDPs and functional diversification and specialization of Hsp70 machines

Several reviews have been written describing the different functions to which JDPs steer Hsp70 machines (Cheetham and Caplan 1998; Craig and Marszalek 2017; Cyr et al. 1994; Kampinga and Craig 2010). Below, we highlight two examples on the extremes of functional specialization and diversity reported at the meeting: complex JDP-Hsp70 machinery at exits of channels through which unfolded polypeptides pass and the diversity of JDP structure and function in the ER.

JDPs at channel exits

Sabine Rospert reviewed recent advances in understanding of the eukaryotic ribosome–associated complex (RAC)—a heterodimer between class C JDP Zuo1 and atypical Hsp70 Ssz1 (Zhang et al. 2017). In fungi, this JDP complex partners with the ribosome-bound Hsp70 homolog Ssb and in metazoans with the soluble Hsp70/Hsc70, facilitating folding of nascent polypeptide chains. Zuo1 has a remarkable structure and precise association with the ribosome. One interaction positions the J-domain on the 60S subunit at the exit of the channel through which the nascent chain passes, called the ribosome tunnel; the other interaction is with a RNA helix of the 40S subunit that extends from the mRNA decoding site. Through these interactions, the RAC-Ssb system not only facilitates de novo protein folding, but also ensures fidelity of protein translation.

JDPs are also crucial for assisting Hsp70 machines in protein import into mitochondria and the ER (Craig 2018). In both cases, the channel through which the protein passes must be gated, increasing the challenge. In the case of the ER, an open channel endangers the steep ER to cytosol Ca^{2+} gradient. Richard Zimmermann discussed BiP (HSPA5) action in regulating the closed/open state of the Sec61 channel by ER JDPs. Membrane embedded class C Sec63 drives opening of the channel. The two JDPs, ERj3 (DNAJB11) and ERj6 (DNAJC3), partner with BiP as modulators for channel closing.

Protein homeostasis in the ER

Protein folding and maturation in the ER is a multistep process that involves several JDPs at each step (Melnyk et al. 2015). Ryo Ushioda highlighted the function of one highly specialized, multifunctional class C JDP, ERdj5 (DNAJC10). This JDP has two thioredoxin-like domains that provide it with reductase activity by which it facilitate ER-associated degradation of misfolded proteins, via transfer of the substrates to BiP. Intriguingly, ERdj5 works in parallel, with BiP, as a regulator of SERCA2b, a Ca^{2+}

pump on ER membrane. This regulation is via ERdj5 oligomerization (Ushioda et al. 2016), reminiscent of the type of complex remodeling by JPD/Hsp70 actions found in early studies on lambda phage replication, and clathrin uncoating.

Nonetheless, many JDP proteins exist of which no clear function has been described so far. One of them is class C JDP, ERdj8 (DNAJC16). Kaz Nagata showed that ERdj8 is an ER-membrane protein with both the J and thioredoxin domains facing the luminal side of the ER. ERdj8 localizes at the ER-mitochondria contact sites. Levels of ERdj8 control the size of autophagosomes in mammalian cells as well as in *C. elegans*. This control is lost in an HPD mutant of ERdj8, implying that its action requires ER-resident Hsp70 activity. Interestingly, ERdj8 is upregulated under proteotoxic stress.

JPDs in protein disaggregation and refolding

One of the most discussed aspects of JPDs was their ability to promote refolding of stress unfolded or aggregated proteins, and functional differences among structurally related JDPs in this regard. Krzysztof Liberek presented data on the role of yeast *Saccharomyces cerevisiae* cytosolic JDPs, Ydj1 (class A), and Sis1 (class B) in Hsp104-Hsp70-dependent refolding of proteins from aggregates. JDPs are required for Hsp70 binding to aggregates which in turn allows Hsp104 disaggregase to interact with Hsp70 bound to aggregates to initiate polypeptide disentangling. Using a biolayer interferometry technique, it was shown that Sis1 more efficiently attracts Hsp70 to aggregates than Ydj1. In agreement, Sis1 is more efficient in disaggregation and refolding of substrates from aggregates in vitro. These results differentiate the functions of Sis1 and Ydj1 in disaggregation and suggest that JDP-dependent binding of Hsp70 to aggregates is critical in the disaggregation process.

Work of Jason Young and Nadinath Nillegoda revealed that actions of different JDPs also seem to highly depend on the folding and aggregation status of the client. For example, Jason Young reported that DNAJA2, but not DNAJA1, promoted refolding of heat denatured, aggregated luciferase (Baaklini et al. 2012), while DNAJB1 interfered with refolding. However, when luciferase aggregates were formed in the presence of one of the members of the small Hsp family HSPB1, both DNAJB1 and DNAJA2 were effective in reactions that required Hsp70 and the NEF, Hsp110. In the studies of Nadinath Nillegoda, it was evident that reaction kinetics and disaggregation efficiencies were highly dependent on the aggregate status of the luciferase substrate and the absence or presence of small HSP during aggregate formation (Nillegoda et al. 2015, 2017).

JDPs and disease

Dysregulated expression, JDP mutations, and disease

Faulty or elevated levels of otherwise functional chaperones can contribute to or be causal for disease (so-called chaperonopathies). Richard Zimmermann, Mike Cheetham, and Harm Kampinga addressed how mutations in JDP proteins such as Sec63 (Linxweiler et al. 2017), DNAJB2 (Zarouchlioti et al. 2018) or DNAJB6, and other chaperones (Kakkar et al. 2014) can lead to inherited neuro-, cardiac-, or motor-neuropathies. In fact, of all known chaperonopathies, over 50% are due to mutations in JDP genes and each cause a specific type of disease again underscoring that these proteins are functionally distinct.

Chaperone network(s) seem often to be upregulated in several types of cancer cells, facilitating tumor cell survival, supporting proliferation and metastasis, and development of chemoresistance. Maciej Zylicz reported the association of poor survival of breast cancer patients with mutated p53 and simultaneous elevated levels of MDM2 protein with high expression levels of DNAJB1, DnaJB6, and low expression levels of DNAJB4 and DNAJB12. This rewiring of the pattern of JDP expression may affect complex formation between mutated p53, MDM2, and TAp73 tumor suppressor and their formation into amyloid-like, nuclear aggregates (Wawrzynow et al. 2018).

Protein aggregation diseases

There was much discussion about the relationship between JDPs and protein aggregation diseases, particularly age-related, neuro-, and muscular degenerative diseases. Work over the past decade has revealed that these aggregates are likely not all the same. Intriguingly, aggregation of different disease-specific polypeptides can be attenuated by overexpression of different (single) class A or B JDPs, further underscoring the idea that JDPs evolved to bind to different exposed, interactive surfaces (Kakkar et al. 2014). Mike Cheetham described results consistent with the idea that members of the "non-canonical" class B, which do not share the CTD domain with the class A or—B JDPs, e.g., DNAJB2, DNAJB6, and DNAJB8, are particularly efficient in suppressing aggregation of some disease-specific polypeptides and can even delay disease onset in mouse models of these diseases (Zarouchlioti et al. 2018). Interestingly, these JDPs share a region of high similarity that links the C-terminal to the so-called S/T domain. This region corresponds to the recently modeled beta-sandwich in DNAJB6 by Cecilia Emanuelsson (Soderberg et al. 2018). The S/T region is essential for the anti-aggregation function of DNAJB6 (Kakkar et al. 2016; Månsson et al. 2018) and may be important for client protein binding in this sub-class of JDPs. Mike Cheetham reported on

the additional importance of two UIM domains in DNAJB2. These can bind polyubiquitin chains that are important for the anti-aggregation action of DNAJB2 with some substrate proteins (e.g., polyQ expanded huntingtin (Htt) and mutant SOD1), but not others (e.g., mutant Parkin and TDP-43), emphasizing how the same JDP can have different interactions with client proteins (Labbadia et al. 2012; Zarouchlioti et al. 2018). Further underscoring that many of the diseases-causing proteins are highly different, even though they all seem to form non-native, non-functional, and likely toxic aggregates are recent findings in the lab of Jason Gestwicki related to the aggregation of tau. His data revealed that class A type JDPs (DNAJA2 in particular), rather than class B type JDPs, are the most rate-limiting components of the Hsp70 machine in dealing with tau aggregation (Mok et al. 2018), also further underlining the substrate selectivity of JDPs.

Evidence is accumulating that Hsp70 chaperone complexes also can break up pre-existing amyloid aggregates. Janine Kirstein reported on how a complex of Hsc70, Hsp110, and selected JDP can also resolubilize fibrils made of polyglutamine fragments, revealing how JDPs, in particular class B JDPs like DNAJB1, can be rate-limiting factors in cells to profoundly reduce polyglutamine aggregation (Scior et al. 2018). A functionally similar complex exists in nematodes (HSP-1, DNJ-13, and HSP-110), raising the important question of how J-domain proteins functionally compare across species.

All these data also argue that drugs targeting JDP-Hsp70 interactions could be a powerful way not only to study their functions, but also to specifically target them for disease intervention. Jason Gestwicki discussed his group's efforts to identify such molecules by high-throughput screening (HTS). He provided an update on emerging HTS methodology that can be used to detect the increase in ATPase activity caused by binding of JDPs to Hsp70s (Taylor et al. 2018). Using this approach, they reported molecules that promote interactions between Hsp70 and the conserved HPD motif of the JDPs.

HSP70-independent functions of JDPs

While the ability to functionally interact with Hsp70 is defined as an essential feature, JDPs can have Hsp70-independent functions as well. For example, Richard Zimmermann described experiments showing that the JDP Sec63 of the ER membrane is capable of facilitating opening of the Sec61 channel for translocation of some, but not all, polypeptides without involvement of the ER-resident Hsp70 protein BiP (Hassdenteufel et al. 2018).

How common such Hsp70-independent action is in the cell was a point of discussion. It was noted that caution is required in interpreting results, particularly for in vivo experiments. For example, HPD mutants have often been used to demonstrate such Hsp70 independent functions. But given that DNAJs often form dimers or multimers, misinterpretation can result, especially in the case of overexpression studies and when the endogenous JDP is still being expressed. It is clear that JDPs can bind to/hold substrates without the help of Hsp70, but generally for full, efficient client processing interaction with Hsp70s is required. Aggregation suppressive capacities of certain JDPs may be so potent that Hsp70 dependency is not always revealed even though it is required for full activity.

Hsp70 substrate binding cycle: beyond JDPs

Understanding JDP function is not possible without understanding the entire Hsp70 substrate binding cycle. NEFs, obligatory Hsp70 co-chaperones like JDPs, determine the lifetime of the substrate-chaperone complex. The eukaryotic cytosol and endoplasmic reticulum have at least two structurally distinct NEFs (Hsp110/Grp170 and armadillo-type NEFs), while animal cells have diversified BAG-type NEFs in contrast to the single GrpE type NEF found in bacteria and mitochondria (Bracher and Verghese 2015). But, some NEFs are more than nucleotide exchange factors. Claes Andréasson highlighted how armadillo-type NEFs in both the cytosol and endoplasmic reticulum employ a substrate-mimicking release domain to prevent rebinding of substrates to Hsp70, subsequent to exchange-accelerated substrate release (Gowda et al. 2018; Rosam et al. 2018). Interestingly, BAG domain NEFs carry similar unstructured domains required for efficient substrate release (Rauch et al. 2016).

Beyond the role of co-chaperones, the effect of Hsp70 binding on the conformation of substrate is a central question. Rina Rosenzweig reported her findings using nuclear magnetic resonance (NMR) spectroscopy to structurally characterize a small, folding-competent protein domain, TRF1, in complex with Hsp70 (Rosenzweig et al. 2017). Hsp70 binding results in a globally unfolded conformation of TRF1. However, it is able to form local secondary structure, with a significant amount of heterogeneity even within each bound ensemble. But, Hsp70 binding prevents formation of non-native, long-range interactions that would otherwise be present in the unbound, unfolded substrate and would result in protein misfolding and aggregation.

Outstanding questions

There are still many gaps in our knowledge about JDPs: how they evolved, how they precisely work, and how they collaborate with other JDPs within or outside the context of the Hsp70 machines. Whereas evidence is emerging that different JDPs serve to direct certain clients to Hsp70, we still lack substantial information regarding JDP client specificity. The

findings that different JDPs can bind to and act on the same substrate (in part related to its folded state) also still needs to be better understood. Is this activity related to the different fate decisions, such as folding versus degradation? Do some proteins or folded states require multiple JDPs (e.g. class A and B) for processing? Do certain JDPs act in concert with specific NEFs or other Hsp70 co-factors, E3 ligases such as CHIP, or even the proteasome or autophagosomal machinery? What is the role of post-translational modifications to steer (control) JDP localization (e.g., many JDPs are prenylated) or to fine-tune interactions with Hsp70 or substrates? How are partnerships between JDPs and HSP70s balanced at the molecular level allowing multiple members of JDP and Hsp70 families to co-exist without interference?

Members of the workshop noted that the detailed insight into the structure-function-evolution relationships of JDPs across different species is also lacking. Such knowledge is important because it could facilitate the use of different model systems to address specific disease–relevant questions. However, for many proteins annotated as JDPs, no or limited functional information is yet available. For several proteins annotated as JDPs, it is not even known whether they can functionally interact with Hsp70 machines (which, as stated, should be a criterion for being called a JDP).

These and likely many more questions stimulated the attendees of this JDP-meeting to agree to organize another meeting in 2020 in which we also hope to attract some Hsp70 and NEF specialists to further unravel the magic of HSP70 chaperone machines that (should) keep us healthy and busy.

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