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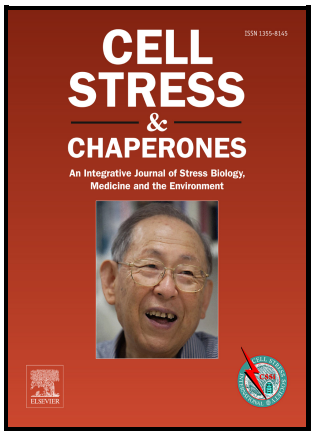

J-domain proteins: from molecular mechanisms to diseases

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J-domain proteins: from molecular mechanisms to diseases

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Abstract:

J-domain proteins (JDPs) are known to drive the functional specificity of Hsp70 chaperone machines. Here we report on the latest findings presented at the third international JDP workshop held in 2025 in Gdansk, Poland. Investigators from many different disciplines, including structural biology, genetics, chemical biology, translational research, computational sciences and biophysics took part in the meeting. This article includes short summaries of the seminars presented by many of the speakers, which provided exciting new insights into the chaperone-dependent and chaperone-independent functions of JDPs, some of which go beyond Hsp70-dependent functions. We also provide a revised classification of members of the (human) JDP family that emerged from open discussion at the meeting. This workshop continues to serve as the premier venue for discussions of JDP evolution, structure, function and roles in health, aging and disease.

Keywords: J-domain proteins (JDPs); functional classification; Hsp70

Note: The report does not include all details of what was shared and discussed at the meeting, either because some of these original data have not yet been accepted for publication elsewhere or because they concerned only preliminary observations.

Introduction to the meeting and keynote lecture by Elizabeth Craig

The third Cell Stress Society International-sponsored meeting on J-domain proteins (JDPs) began with an inspiring keynote lecture by ***Elizabeth Craig***, one of the pioneers in JDP-Hsp70 research. She reviewed the amazing scientific journey that led to the discovery of Hsp70 and its JDP cochaperones, from the initial cloning and sequencing of HSP genes to the discovery of their universal evolutionary conservation. She also demonstrated how yeast, which encodes multiple JDP-Hsp70 pairs, proved to be a wonderful model organism for establishing the fundamental principles that govern JDP-Hsp70 partner specificity, localization, and cellular functions under both stress and standard growth conditions.

The second part of the lecture covered recent work from her laboratory on two yeast JDPs: Zuo1 (see below) and Sis1. It has remained an enigma why Sis1 is the only essential cytosolic JDP in yeast. Using an elegant genetic screen for suppressors, she identified Tti1, a subunit of the heterotrimeric TTT chaperone complex, which is dedicated to the folding and/or maintenance of the phosphatidylinositol 3-kinase-related kinase (PIKK) proteins[1]. In line, Sis1 levels were found to be correlated to sensitivity to rapamycin, suggesting that Sis1 is essential because it is required for the folding and/or maintenance of PIKKs.

JDP nomenclature and classification

JDPs are known to diversify the Hsp70 chaperone systems to operate within a remarkable range of different processes. In many cases, JDPs bind to Hsp70s and stimulate their ATPase activity. However, it is becoming clear that this is not always the case. In this meeting summary, we provide short synopses of many of the seminars. Besides individual lectures, a general discussion was held about some inconsistencies in the current classification of the (human) JDPs into class A, B, and C members that have been revealed by detailed sequence analyses as well as structure and function insights[2]. Whereas it was decided not to rename these JDP members, the participants agreed to reorder some members to different classes that better reflect their structural and functional conservation. In addition, it was decided to subdivide the human class B JDPs into canonical (Sis1 like) and non-canonical members (see **Table I**)[3]. Another consensus conclusion of this meeting is that Hsp40 should no longer be used as the name for the family. Although some members of the human JDP family have a molecular weight close to 40 kDa, most members have entirely different molecular weights and are not heat shock regulated proteins. Thus, the term JDP family is considered to be a more accurate name defined by the characteristic that all of the family members contain a J-domain.

JDP Diversity and Evolution Across Species

As stated above, JDPs were categorized into three classes (A, B, and C) based on their sequence similarity to the *E. coli* DnaJ prototype. Class A JDPs contain a canonical N-terminal J-domain, a glycine-phenylalanine-rich (G/F) linker, two β-sandwich client-binding domains (CBD1 and CBD2), a zinc-finger β-hairpin motif within CBD1, and an extended C-terminal dimerization domain. Class B JDPs share the N-terminal J-domain and G/F region but lack the Zinc-finger β-hairpin domain (ZnF) protruding from CBD1. Class C JDPs are the most diverse, containing only the J-domain, which may itself appear anywhere in the sequence and is often embedded within various functional domains.

It is becoming increasingly clear, though, that this classification does not even begin to reflect the remarkable evolutionary plasticity of JDPs. ***Bartlomiej Tomiczek*** addressed this by performing an extensive phylogenetic analysis of class A and B JDPs across bacteria, archaea, and eukaryotes. His results showed, for example, that eukaryotic cytosolic class B JDPs are more closely related to eukaryotic class A JDPs than to bacterial class B proteins, suggesting that class B JDPs evolved independently from class A ancestors multiple times. To explore the functional consequences of this evolutionary divergence, the researchers reconstructed ancestral proteins, including a common AB ancestor and distinct early class A and class B prototypes. Functional analysis in yeast-deletion strains revealed that both the AB ancestor and the class A prototype could rescue the growth defect of Δydj1 (a class A yeast JDP), while only the class B ancestor complemented Δsis1 (a class B yeast JDP). In vitro, only the ancestral class B protein could disaggregate α-synuclein fibrils, functions also seen in its modern human homolog, DNAJB1 (see also below). Notably, the evolutionary loss of the zinc-finger domain in class B appears to have been a key adaptation enabling this JDP class to acquire disaggregation activity. The disaggregation activities, just like for DNAJB1 (see below), required interaction with the Hsp70 EEVD motif and the presence of the autoinhibitory G/F helix that seems to be present in all class B JDPs[4,5].

Further highlighting the diversity of JDP evolution, ***Gregory Blatch*** explored the rich chaperone network of *Plasmodium knowlesi*, a zoonotic malaria parasite of humans. Bioinformatic analysis revealed an unusually complex ensemble of at least 31 JDPs, five Hsp70s, and four Hsp90s. Moreover, one class B JDP appears to be exported into the host cell cytosol and involved in pathogenesis, suggesting evolved roles for JDPs in host-pathogen interaction and parasite virulence[6]. This complexity highlights the remarkable adaptability of the JDP-Hsp70 system in specialized cellular environments.

Reut Shalgi further expanded our understanding of JDP evolution by turning attention to isoform diversity, focusing on alternative splicing and heterocomplex formation in the JDP family. Mining transcriptomic databases, she identified hundreds of JDP isoforms, many of which lack the J-domain altogether[7]. These truncated variants may act independently of Hsp70, further diversifying the cellular chaperone landscape. Intriguingly, several of these isoforms formed Table heterodimers, broadening the range of JDP functional interactions and possibly tuning client specificity.

But is the canonical J-domain, that has a HPD motif to interact with Hsp70s, even required? ***Jason Gestwicki***, in collaboration with Jason Zhang and David Baker, set out to test this question using de novo protein design[8]. In their research, they created artificial J-domains, three-helix bundles capable of binding to Hsp70 and modulating its activity despite lacking the conserved HPD motif. Some designs activated the ATPase activity of Hsp70, while others only recruited Hsp70 without stimulating it. These synthetic modules not only call into question the evolutionary constraints on J-domain architecture but also open the door to building synthetic chaperone systems with customized activities.

Such functional engineering of JDPs was further explored by ***Pierre Genevaux***, who introduced a clever bacterial genetic assay to select for human JDP variants that counteract aggregation of human disease-associated proteins. By expressing libraries of mutant chaperones in bacteria sensitized to misfolded peptides, he could rapidly identify variants with enhanced protective activity. This platform now offers a powerful pipeline for evolving next-generation chaperones with therapeutic potential for neurodegenerative diseases (*unpublished*).

Finally, whereas JDPs have always been suggested as co-evolved with Hsp70s, ***Johannes Buchner*** showed that certain JDPs also directly connect to the Hsp90 chaperone system. Whereas it has been known for long that certain substrate of the JDP-Hsp70 system can be transferred to Hsp90, he showed how the nuclear migration protein NudC can interact with Ydj1 through an extended motif similar to the Hsp70 C-terminus and can directly transfer Ydj1-bound clients to Hsp90[9].

JDP in transcriptional regulation

Molecular chaperones, and especially JDP-Hsp70 chaperone pairs, play a key role in regulating gene expression. While the impact of the generic DnaK(Hsp70)/DnaJ(JDP)chaperones on transcription regulation in bacteria is well documented, it is currently not known whether specialized JDPs could be involved in this process too. ***Sébastien Dementin*** investigated the role of molecular chaperones in the stress-resistant marine bacterium *Shewanella oneidensis* and showed that it encodes a class C JDP, named AtcJ, that is present on the *atc J-A-B-C* operon and that it is essential for survival at low temperature[10]. Interestingly, the AtcB protein, under conditions of overproduction, was found to bind to and inhibit the RNA polymerase, and this effect was reversed by the combined action of AtcJ, AtcC, and DnaK. This suggests that the ability of *S. oneidensis* to grow in cold conditions possibly relies on DnaK-mediated regulation of transcription.

In yeast, the evolutionarily conserved heat shock response is controlled by the essential transcription factor Hsf1. Hsf1 is repressed by Hsp70, coupling substrate accumulation during stress to Hsf1 activation[11]. In his presentation, ***Axel Mogk*** demonstrated that the JDPs Ydj1, Sis1 and Apj1 together enable Hsp70 to control Hsf1 activity. In this case, the nuclear Apj1 protein specifically controls the attenuation phase of the heat shock response by assisting in the displacement of Hsf1 from heat shock elements on target DNA. Remarkably, the loss of Ydj1 and Apj1 causes almost complete Hsf1 activation and rescues the growth-sensitive phenotype of *ydj1* mutant cells. These exciting new discoveries highlight the fact that there are arrays of J-domain-containing proteins that provide synergistic control of the yeast Hsf1 at distinct phases of the heat shock response.

Ribosome-bound chaperones and co-translational protein folding

The efficient folding of newly synthesized proteins requires the coordination of various molecular chaperones on translating ribosomes[12]. In bacteria, this process is primarily carried out by generic chaperones such as trigger factor, DnaK/DnaJ and GroESL[13]. ***David Balchin*** presented his laboratory's research concerning the specific actions of such generic chaperones during co-translational folding in bacteria. Using β-galactosidase as a large multidomain nascent protein model, his study revealed key molecular determinants underlying the recognition of nascent polypeptides by both DnaJ and DnaK. He also presented evidence that molecular chaperones persistently bind to nascent chains without

antagonizing the folding process. Interestingly, hydrogen deuterium exchange – mass spectrometry data of ribosome nascent chain complexes in the presence of chaperones suggested that DnaJ binding to the nascent chains involves primarily its CBD1 and G/F region, and to a lesser extent its CBD2.

In eukaryotes, Hsp70 machines interact and cooperate with the two ribosome-associated chaperone complexes, the ribosome-associated complex (RAC) and the highly conserved nascent polypeptide-associated complex (NAC), to assist in the folding and maturation of newly synthesised proteins[14-16]. It has long been debated whether the RAC and NAC complexes can simultaneously bind to the ribosome exit tunnel. Using *in vivo* site-specific cross-linking approaches the group of ***Elizabeth Craig*** identified many sites of interaction between NAC and Zuo1 (the yeast homolog of the human DNAJC2), suggesting that indeed NAC and this JDP-Hsp70 chaperone systems can coexist at the ribosome tunnel exit *in vivo*[17], highlighting possible interplay between these systems. To identify the nascent chain interactome of RAC, ***Bernd Bukau*** and his colleagues conducted an extensive ribosome profiling study of the nascent chain interactome of RAC in yeast. Remarkably, the data showed that RAC can interact with nascent chains independently of the ribosome-targeted Hsp70 chaperone Ssb and interacts particularly with cysteine-rich sequences. Here, RAC may prevent oxidation of *e.g*. zinc-fingers and, in line, RAC deletion strains are hypersensitive to oxidative stress. Regarding the mechanism of RAC interaction with nascent chains, ***Sabine Rospert*** presented two solved cryo-EM structures of Ssb, revealing the Ssb ribosomal binding site and its interaction with a nascent model substrate. Alongside detailed biochemical and mutational analyses, these structures delineate the intricate cycle that positions the substrate binding domain of Ssb at the tunnel exit, ready to receive nascent chains in a RAC-dependent manner. This *unpublished* work provides key insights into the interplay between the components of the yeast ribosome-bound chaperone triad.

In addition to generic chaperones, dedicated chaperones at the ribosome can relieve the burden on the Hsp70 system by assisting essential components of the translation machinery. Indeed, the nascent eukaryotic elongation factor 1A (eEF1A) relies on a dedicated ribosome-associated chaperone (called Chp1) that binds NAC for its high-level expression[18,19]. ***Claes Andréasson*** presented new experimental data supporting the notion that the function of this dedicated chaperone is to unburden the Hsp70 system, and that Chp1 specifically delays the folding of the nascent G-domain of eEF1A during its biogenesis. This function is similar to that of Hsp70, which delays the folding of nascent domains at the ribosome. Together, these data suggest that the highly expressed nascent eEF1A has replaced a general Hsp70 with dedicated Chp1 to control the folding of the G-domain during its synthesis.

Regulation of organellar protein folding capacity by ER- and mitochondrial JDPs

Failure to maintain ER homeostasis can lead to deleterious conditions such as protein misfolding-related diseases and neurodegeneration. Misfolded ER membrane proteins are ubiquitinated and targeted for clearance by the ERAD pathway or autophagy. The ER transmembrane JDPs, DNAJB12 and DNAJB14, have been studied for their role in the degradation of misfolded membrane proteins. ***Doug Cyr*** showed that DNAJB12 recognizes and triages misfolded ER membrane proteins: upon Hsp70 recruitment, some of these misfolded proteins are then targeted for ERAD[20]. ERAD-resistant membrane proteins, however, can also be targeted to autophagic clearance by DNAJB12, in a manner that is inhibited by the DNAJB12^{HPD} -mutant, suggesting this action is Hsp70-dependent. Work by ***Jeff Brodsky*** had suggested that the distinction between ERAD and lysosomal degradation may also be

based on the extractability of the respective proteins from the lipid bilayer[21]. More recent data from his lab suggest that certain JDPs are required for the polyubiquitination of these ERAD substrates prior to targeting to the proteasome.

Ryo Ushioda and Ayano Kasai presented data suggesting that ER-resident JDPs can also regulate the autophagy pathway. ERdJ8 (DNAJC16) possesses a thioredoxin (Trx)-like domain and a transmembrane domain and not only the Trx-domain but also the J-domain of ERdJ8 is required to control the size of autophagosomes, the latter suggesting Hsp70 involvement. After transient ERdJ8 knockdown in cells, autophagosomes are smaller but the number of autophagosomal vesicles are elevated. Intriguingly, such cells are impaired in removing CCCP-damaged mitochondria (large cargo) but accumulate less polyglutamine-caused protein aggregates (smaller cargo).

The ER-localized Hsp70 chaperone, BiP, undergoes a rapid, reversible and inactivating post-translational modification, an AMPylation of BiP's threonine 518 by the AMP transferase, FICD[22]. ***David Ron*** showed that a monomeric form of FICD mediates AMPylation of BiP that then freezes BiP in a domain-docked state making it refractory to JPD stimulated ATP hydrolysis. A dimeric form of FICD can lead to the de-AMPylation and thereby re-activation of BiP. This PTM-mediated regulation of the responsiveness to JDPs adds a novel layer of fine-tuning of the activity of BiP that may serve to meet the protein folding demands of the ER. The importance of this regulation is highlighted by the findings that FICD mutations are associated with diabetes and neuronal dysfunction. David Ron also presented an update on his efforts to develop an assay to search for compounds that regulate FICD's activity.

Tool development was also pursued by several other participants of the meeting to gain mechanistic and physiological insights into the regulation and capacity of organellar chaperones. E.g., ***Dejana Mokranjac*** reported on her recent progress in developing FRET-based tools to analyse the conformation of Hsp70 in physiologically active mitochondria.

Christian Münch and Raymond Sutandy reported on their ongoing studies of the regulation of the human mitochondrial UPR (UPR^{mit}) via DNAJA1[23]. Upon mitochondrial misfolding stress, DNAJA1 is oxidized at two cysteines in its Zn-finger domain. This seems to be required for the activation of the UPR^{mit} as knockdown of DNAJA1 abrogates the UPR^{mit}. Yet, their data suggested that this is not sufficient and a second signal is required that involves the sequestration of Hsp70 to non-imported mitochondrial precursors that accumulate in the cytosol upon mitochondrial misfolding stress. The sequestration of Hsp70 in turn results in an activation of HSF-1. Indeed, HSF-1 knockout cells were found to not being able to induce a UPR^{mit}. Their research thus reveals how an integrated stress response involving multiple chaperones of different subcellular compartments ensures cellular integrity in response to proteotoxicity.

In the final presentation on organellar JDP-Hsp70 systems ***Jaroslaw Marszalek*** discussed how shifts in client binding preferences of Hsp70s have evolved – using as a model the yeast mitochondrial Hsp70 (Ssq1) that interacts with a single client, the scaffold protein on which FeS clusters are assembled. Ssq1 emerged via gene duplication from the mitochondrial Hsp70 that binds many clients. Using ancestral reconstruction of the substrate-binding domain and biochemical analysis of the 14 historical amino acid substitutions, they identified the subset responsible for the shift in binding specificity, demonstrating that effects are additive and independent of their order of addition. Structural analysis revealed that subtle rearrangements within the client/domain contact network were sufficient to

cause the specificity shift. These findings indicate that the binding preferences of Hsp70s are highly evolvable, supporting shifts in these preferences as key drivers of their functional diversification.

Structural and functional diversity of JDPs in protein aggregation prevention, (re)folding, disaggregation, and complex remodeling

A recurring theme throughout the meeting was the structural diversity of JDPs and how it underpins functional specialization. ***Matthias Mayer*** opened this discussion by exploring the role of dimerization in *E. coli* DnaJ. He presented data from Veronika Lashkul in his lab showing that while a dimeric architecture is dispensable for (re)folding of many substrates, a subset of client proteins does require it. Monomeric and heterodimeric DnaJ variants were active but differed quantitatively from the wild-type, indicating that while the J-domain remains functional in isolation, structural context fine-tunes efficiency *(unpublished).* The importance of context was also evident in the work of ***Chandan Sahi,*** which focused on Caj1, a highly specialized yeast JDP that localizes to the plasma membrane. Caj1 modulates the stability of amino acid permeases, impacting membrane integrity and sensitivity to amphotericin B. Intriguingly, Caj1-induced cytotoxicity could be compensated by other JDPs like Sis1 and Ydj1, suggesting a buffering capacity within the cytosolic JDP network. Whether Caj1 acts exclusively at the membrane or participates in more general proteostasis remains an open question[24]. In this context, work by ***Anita Manogaran*** showed that Sis1 and Ydj1 can also compensate for the loss of the Hsp70 proteins, Ssa1 and Ssa2, in limiting the formation of aberrant aggregates associated with stress responsive proteins[25]. These results suggest that the cytosolic JDP network provides flexibility in maintaining protein homeostasis, particularly when Hsp70 chaperones are compromised.

Pierre Goloubinoff expanded this theme by examining how different JDP-Hsp70 modules function at distinct stages of protein quality control. He showed that only Ydj1 (and to a lesser extent Ssa1) prevented heat-induced aggregation of luciferase in vitro, while Sis1 or Sse1 could not[26]. Interestingly, however, only Sis1, and not Ydj1, could support Ssa1-dependent disaggregation of pre-formed protein aggregates. Furthermore, this disaggregation activity was highly dependent on Sse-1 (i.e., the Hsp70 nucleotide exchange factor (NEF) that releases ADP from Hsp70 to allow binding of a new ATP) activity, which is increased in more solid protein aggregates.

The data of ***Krzysztof Liberek*** further reinforced this, showing that yeast Sis1 (and human DNAJB1) drive more abundant than yeast Ydj1 (and human DNAJA2) Hsp70 loading onto preformed aggregates, resulting in more effective disaggregation. Furthermore, his data outlined a multi-step model of disaggregation whereby Class B JDPs bind the EEVD motif of Hsp70 to relieve autoinhibition analogously as described for the human DNAJB1/Hsp70/Hsp110(NEF) disaggregation system[27]. Subsequently, the unlocked J-domain of JDP-Hsp70-aggregate complexes nucleate further Hsp70 recruitment. This layered regulation facilitates dense Hsp70 loading on aggregates and enhances disaggregation, emphasizing the architectural interplay between client, JDP, and chaperone[28,unpublished data]. The substrate and context dependence, as well as the timing of JDP action for handling protein aggregates in cells, were emphasized in ***Harm Kampinga's*** report. Whilst DNAJB1/Hsp70 modules (present during exposure to elevated temperatures in cells) are involved in disaggregation of protein aggregates early after an acute heat shock, HSF-1-induced DNAJA1 and DNAJB1 form complexes late after the heat shock to assist the Hsp70-mediated dismantling of persistent aggregates[29]. This is consistent with previously published *in vitro* data, showing that DnaJA1-DnaJB1-complexes form efficient Hsc70/Hsp110-mediated disaggregation modules to handle amorphous protein aggregates[30].

Besides handling protein aggregates induced by acute stresses like heat shock, Hsp70 machines have also been implicated in handling aggregates induced by pathological amyloids. In this context, ***Anne Wentink*** showed *in vitro* data on how preformed α-synuclein fibrils are disaggregated by the concerted action of DNAJB1, Hsp70 and Hsp110[31-33]. For α-synuclein, different polymorphs have been described, and the ability of the DNAJB1-Hsp70-Hsp110 complex to disassemble them declined with increasing polymorph stability and the ability of DNAJB1 binding to the different fibers. Alarmingly, at least *in vitro,* the disaggregation of preformed fibrils can generate seeding-competent intermediates that promote further aggregation, raising questions about the double-edged role of chaperones in neurodegenerative diseases. ***Janine Kirstein*** specifically investigated how the canonical class B JDP, DNAJB1, acts on aggregates of Huntingtin exon1 with a pathogenic polyQ expansion (HTTExon1Q48). Previous data from her lab had shown that DNAJB1 does not interact with the core of the polyQ stretch of HTTExon1Q48 but rather with its C-terminal flanking proline-rich stretch; DNAJB1 does so via the hinge region between its two C-terminal client binding domains[34].

The non-canonical class B JDPs, DNAJB6 and DNAJB8, act on amyloids in a manner distinct from DNAJB1. Whereas for polyQ they seem to directly interact with the amyloid core[35], they seem to have almost no activity on preformed fibrils. ***Sara Linse*** introduced a thermodynamic framework for understanding how the self-oligomerizing DNAJB6 (and likely also DNAJB8) interferes with amyloid formation. Her "Unhappy Chaperone Hypothesis"[36] posits that the incorporation of DNAJB6 into aggregates is driven by its high chemical potential in the soluble state. Her data across Aβ-42, α-synuclein, and tau aggregation strongly support co-aggregation as a key inhibitory mechanism, shifting how we view chaperone engagement with amyloid pathways. The cellular data from **Harm Kampinga's** group further support this hypothesis. Moreover, his group recently showed how such an early association of DNAJB6 during aggregate formation, supports an aggregation fragmentation mediated by Hsp70 and the proteasome's 19S cap, which is coupled to a piece-meal form of lysosomal degradation[37]. ***Lukasz Joachimiak*** dissected the differential roles of the closest paralog of DNAJB6, DNAJB8[38] and that of DNAJC7[39] in suppressing Tau aggregation. He showed that DNAJB8 engages with tau-oligomers (seeds) consistent with the mode of action suggested by Sara Linse. Even though DNAJB8 mainly exists as an oligomer, the monomers suffice to exert this activity. DNAJC7, on the other hand, prevents aggregation even at early stages by binding monomeric tau, in a reaction that is fully dependent on DNAJC7-Hsp70 interaction. Using novel fluorescent tagging strategies, Lukasz Joachimiak and colleagues further examined TDP-43 seeding and aggregation inside cells as a function of JDP chaperone expression levels and found that chaperones play differential roles depending on the cellular seeding load and associated stress machineries.

Kate Hyun Lee next showed that the JDP-Hsp70 system is also key to the resolution of heat-induced stress-granules (SG). Several cytosolic JDPs like DNAJA1, DNAJB1, and DNAJC7 (but not e.g. DNAJB6) accumulate in these stress-induced biomolecular condensates. Specific knockdown of each of these JDPs again pointed to DNAJB1 as being the most crucial member for promoting their ATP-dependent disassembly. *In vitro,* the SG-component G3BP1 forms condensates and while DNAJB1 can co-condensate with G3BP1, Hsp70 alone cannot unless recruited by DNAJB1, which then results in condensate remodeling *(unpublished).* ***Mike Cheetham*** also described the potential of some class B JDPs to modulate neuronal dysfunction and neurodegeneration in mouse, fly and stem cell-based models of ALS and FTD, further highlighting functional diversity and translational potential of JDP over-expression.

At the core of many of these observations lies the concept of "entropic pulling", proposed on theoretical grounds two decades ago. ***Paolo De Los Rios*** provided the first direct single-molecule validation of entropic pulling using nanopore-based force spectroscopy. Hsp70 was shown to exert pulling forces stronger than previously predicted, with strength scaling with the chaperone's size as predicted by the theory[40]. This confirms a long-standing theoretical prediction and places Hsp70-mediated unfolding within a physical, energetically grounded framework. ***Pierre Goloubinoff*** proposed that the yeast NEF, Hsp110, derived from an ancestral Hsp70 that relinquished substrate and JDP binding, enhances Hsp70-JDP activity through ATP-driven super-entropic strokes. These pulling forces, generated upon dimer association, would provide a non-canonical means to promote client unfolding and disaggregation[41]. Closing the loop between theory, simulations and experiment, ***Alessandro Barducci*** showed how Coarse-grained (CG) Molecular Dynamics simulations may act as an integrative framework for dissecting JDP dynamics at the molecular scale. Building on prior work combining CG simulations, smFRET and rate models to elucidate how Hsp70 converts ATP hydrolysis into mechanical work[42], it is now possible to leverage recent CG force-field advances to map the dynamical network of intermolecular JDP–Hsp70–substrate interactions during disaggregation.

Together, the presentations converged on a nuanced picture of the Hsp70 machinery in handling protein assemblies or aggregates: a modular, tunable force-generating system, modulated by the structural logic of JDPs, potentiated by Hsp110. Besides being involved in aggregate clearance, this force-generating system activity is also considered key to the remodeling of specific native protein oligomers, of organelles and for membrane homeostasis.

The diverse functions of Class C JDPs

While protection against protein aggregation is usually performed through protein repair (protein aggregation prevention, refolding, and / or disaggregation) it can also be done through protein removal, either by protein degradation or secretion. ***Janice Braun*** presented her results on DNAJC5 (CSPα-cysteine string protein) showing that, depending on its membrane localization, whether at the Golgi or at the lysosome, DNAJC5-mediated vesicle export into the extracellular space or endolysosomes eliminates a diverse set of misfolded proteins including α-synuclein, TDP-43, tau, SOD-1 and huntingtin. While the full range of DNAJC5-secreted substrates is still unknown, this class C JDP chaperone represents a previously undiscovered layer of protection against an array of neurodegenerative disorders. Indeed, mutations within DNAJC5 are known to lead to adult-onset neuronal ceroid lipofuscinosis, a rapidly developing neurodegenerative disease.

Class C JDPs anyways represent an exceptionally diverse and specialized group of co-chaperones, linking Hsp70 activity to a wide range of cellular processes. While class A and B JDPs and some class C JDPs function in core proteostasis pathways, many class C JDPs have evolved to mediate a wide array of unique biological roles, many of which are only now being uncovered.

Rina Rosenzweig presented a structural and functional characterization of one such specialized class C JDP, DNAJC12. Mutations in this chaperone cause hyperphenylalaninemia, a metabolic disorder also known as phenylketonuria and that is characterized by an accumulation of phenylalanine in the blood, typically due to malfunction of phenylalanine hydroxylase (PAH), the enzyme that converts phenylalanine into tyrosine. The Rosenzweig group discovered that DNAJC12 is a 700 kDa homo-oligomer that acts as a chaperone for PAH, preventing its misfolding and aggregation under conditions

of low phenylalanine. DNAJC12 binds misfolded PAH and sequesters it into large complexes that keep the enzyme inactive and prevent aggregation during stress. These complexes can subsequently be disassembled with the help of the Hsp70 chaperone system, potentially once the stress is resolved. Disease-causing mutations in DNAJC12 disrupt its ability to bind either PAH or Hsp70, thereby impairing its chaperone function (*unpublished*). ***Colin Hammond*** reported about another class C specialist JDP, DNAJC9, a histone chaperone involved in H3–H4 protein folding[43]. In a collaborative study with the laboratory of Munira Basrai, a global interactome analysis was performed showing that DNAJC9 depletion promotes the interaction of the centromeric histone variant CENP-A with the DNA-replication-associated histone chaperone MCM2[44]. Thus, DNAJC9 serves as a factor restricting CENP-A mislocalization which reduces chromosomal instability. Furthermore, ***Kausik Si*** identified a specific class C JDPs that regulates long-term memory in Drosophila. These chaperones appear to modulate the conformational state of Orb2, a memory-associated prion-like protein, positioning JDPs as developmental regulators that influence neural plasticity through highly selective substrate interactions (*unpublished*). Another intriguing example of class C JDPs found in the nervous system is Sacsin (DNAJC29), one of the largest human proteins and the longest class C JDP. Through structural modeling and knockout studies, ***Paul Chapple*** demonstrated that Sacsin ATP-dependent chaperone activity is crucial for the trafficking of synaptic adhesion proteins, directly linking its dysfunction to the neurodevelopmental disorder autosomal recessive spastic ataxia of Charlevoix-Saguenay. Paul also reported on homozygous missense variant in the ER-resident DNAJC3 that causes early onset diabetes and multisystemic neurodegeneration[45] and showed that depletion of DNAJC3 in cells greatly impairs their viability.

Together, these examples underscore the remarkable gamut of class C JDP functions. Far from their previously perceived role as peripheral components of the chaperone network, these chaperones now emerge as specialized regulators of critical cellular and developmental processes.

JDP mutants and disease

As already stated above, there are several diseases caused by mutations in JDPs. The above-mentioned mutations in class C JDPs are recessive and associated with loss of function. ***Conrad Weihl*** reported on myopathies caused by recessive loss of function mutations in the canonical class B protein DNAJB4 that cause respiratory weakness and that are associated with rimmed vacuoles and protein aggregates[46]. ***Mike Cheetham*** discussed the role of JDPs in motor neuropathies and ALS/FTD, with a particular focus on the LOF variants in DNAJB2 that cause Charcot Marie Tooth type 2 (CMT2) and distal Hereditary Motor Neuropathy (dHMN). ***Conrad Weihl*** also reported on his progress on dominant mutations in the non-canonical class B protein DNAJB6 that cause a subtype of limb-girdle muscular dystrophy (LGMDD1)[47,48]. LGMDD1 is also associated with rimmed vacuoles and TDP-43 positive aggregates. In some of the LGMD-associated DNAJB6 mutants, the autoinhibitory G/F helix is dysfunctional[49], whereby it is assumed to have dominant negative effects on the Hsp70 system. Whether some mutations in DNAJB6 may (also) cause a partial loss of function (LOF) directly is unclear, but data shown by ***Yajun Jiang*** suggested this might be the case (*unpublished*). In fact, he suggested that the type of mutation in DNAJB6 and whether they also display LOF phenotype may distinguish the distal from proximal forms of LGMD. Anyway, for the presumed dominant negative mutants, ***Conrad Weihl*** showed that Hsp70 gets trapped at the sarcomere when the disease mutants are expressed. This effect was reversed when interactions of the mutants with Hsp70 were genetically

(HPD mutant) or pharmacologically abrogated. The data not only highlight the importance of balanced and tightly regulated Hsp70- recruitment by class B JDPs via their auto-inhibitory helix to ensure a healthy cellular protein homeostasis but provides proof-of-concept for therapeutic approaches in these diseases.

Summary

It is becoming increasingly clear that JDPs play important roles across many aspects of health and disease. Yet, the function of these proteins is complex, and they are often challenging to study. The 2025 JDP workshop featured researchers across many career stages and using a wide variety of methods, including structural biology, imaging, genetics, cell biology, biophysics, computational sciences, mass spectrometry, biochemistry and chemical biology and many model systems (*e.g.* yeast, mice, cells). Indeed, there is great enthusiasm for the future of this field because it has embraced inter-disciplinary research. The consensus at the workshop is that the field is nearing a time when this knowledge can be translated into therapeutic opportunities.

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Table I: classification of the human JDPs (reclassified members indicated *in bold italics*)

Class	Main characteristics	Members (human)	Remarks
Class A	N-terminal J-domain, a short G/F-rich region, two β-sandwich client binding domains (CBD1 and CBD2) with a zinc-finger β-hairpin insertion in CBD1, and a C-terminal dimerization domain	DNAJA1, DNAJA2, DNAJA3, DNAJA4, ***DNAJB11***	DNAJB11 contains a β-hairpin that structurally resembles the ZnF- β-hairpin present in class A JDPs
Class B (canonical)	N-terminal J-domain, a long G/F-rich region, two β-sandwich client binding domains (CBD1 and CBD2), no zinc-finger β-hairpin, and a C-terminal dimerization domain	DNAJB1, DNAJB4, DNAJB5, DNAJB13	DNAJB3 is excluded as the human ortholog has a stop codon in the middle of its ORF; DNAJB13 lacks the HDP motif to interact with Hsp70

Class B' (non-canonical)*	N-terminal J-domain, a long G/F-rich region, unstructured S/T-rich region important for client binding and oligomerization , a C-terminal β-sheet domain(CTD) with putative substrate binding capacity, no zinc-finger domain, no C-terminal dimerization domain	***DNAJB2 (a,b), DNAJB6 (a,b), DNAJB7, DNAJB8***	Besides the unstructured S/T- rich stretch, the JDP members contain various other C-terminal domains
Class C	J-domain anywhere, no long G/F - rich region. Highly variable set of other domains, sometimes even lacking substrate binding domains	***DNAJB9, DNAJB12, DNAJB14,*** DNAJC1, DNAJC2, DNAJC3, DNAJC4, DNAJC5 (b,g), DNAJC7, DNAJC8, DNAJC9, DNAJC10, DNAJC11, DNAJC12, DNAJC13, DNAJC14, DNAJC15, DNAJC16, DNAJC17, DNAJC18, DNAJC19, DNAJC20, DNAJC21, DNAJC22, DNAJC23, DNAJC24, DNAJC25, DNAJC26, DNAJC27, DNAJC28, DNAJC29, DNAJC30	DNAJB9, DNAJB12, and DNAJB14 do not contain the long G/F rich region and lack the autoinhibitory helix of class B JDPs.

* note: Based on evolutionary relationships they must still be classified as class B (Malinverni, et al 2023)[2].

Declaration of interests

☐ 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.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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