Scilight

Biomolecular Mechanisms and Innovations
https://www.sciltp.com/journals/bmi

Mini Review

The Central Role and Therapeutic Potential of Cochaperone Networking of Chaperones in the Regulation of Biocondensates

Gregory L. Blatch [1,2,*] and Adrienne L. Edkins [1,*]

[1] Biomedical Biotechnology Research Unit, Department of Biochemistry, Microbiology and Bioinformatics, Rhodes University, Makhanda 6140, South Africa
[2] The Vice Chancellery, The University of Notre Dame Australia, Fremantle, WA 6959, Australia
* Correspondence: g.blatch@ru.ac.za or greg.blatch@nd.edu.au (G.L.B.); a.edkins@ru.ac.za (A.L.E.)

How To Cite: Blatch, G.L.; Edkins, A.L. The Central Role and Therapeutic Potential of Cochaperone Networking of Chaperones in the Regulation of Biocondensates. *Biomolecular Mechanisms and Innovations* **2026**, *1*(1), 3.

Received: 16 November 2025
Revised: 20 December 2025
Accepted: 23 December 2025
Published: 1 January 2026

Abstract: Many heat shock proteins are essential molecular chaperones that safeguard proteome integrity (proteostasis) under both normal and stress conditions. These chaperones, especially heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90), and their associated cochaperones, play central roles in cellular protein quality control (PQC) pathways, assisting in the folding of nascent polypeptides, refolding of stress-denatured proteins, and prevention of toxic aggregation. Dysregulation of this proteostasis network is implicated in numerous diseases, from neurodegenerative proteinopathies to cancer. Biocondensates are dynamic, membraneless phase-separated compartments that are emerging as major components of the PQC system. In this review, we discuss how the regulatory networking of chaperones by cochaperones, particularly J domain proteins (JDPs; also called Hsp40 or DNAJ proteins), contributes to biocondensate formation and protein disaggregation to maintain proteostasis. Furthermore, we highlight new mechanistic insights into phase separation and aggregate clearance with therapeutic potential.

Keywords: heat shock protein; Hsp70; Hsp90; molecular chaperone; cochaperone; J domain protein; cellular stress; biocondensate; phase separation

1. Introduction

Stress responses are fundamental to life, spanning effects from the cellular level to whole-organism health. A key element of the cellular stress response is the maintenance of protein homeostasis (proteostasis) by molecular chaperones, notably the heat shock protein families. The major chaperones heat shock protein 70 (Hsp70) and heat shock protein 90 (Hsp90) facilitate the proper folding, assembly, trafficking, and degradation of other proteins, thereby preserving the integrity of the proteome under normal and stress conditions [1,2]. When cells are exposed to physiological stress (e.g., heat, oxidative stress, infection), the resulting protein unfolding and misfolding can overwhelm the protein quality control (PQC) system. If mismanaged, this leads to toxic protein aggregates and loss of proteostasis, contributing to diseases often termed “proteinopathies”, including neurodegenerative disorders like Alzheimer’s disease and Parkinson’s disease [3].

Molecular chaperones function as multiprotein complexes composed of proteins with defined functions. Hsp70 and Hsp90 are ATP-regulated molecular machines that undergo substantial conformational changes to function as holdases that prevent protein aggregation, and foldases that catalyse *de novo* or stress-related protein (re)folding [4]. Chaperones are regulated by a cohort of diverse proteins known as cochaperones that modify their function by regulating ATP hydrolysis, nucleotide exchange or mediating client protein delivery [5]. Cochaperones outnumber chaperones, and play a pivotal role in diversifying and tailoring chaperone function to a specific context or subcellular location [6]. Hsp70 has the largest class of cochaperones in the J-domain containing proteins (JDPs;

also known as Hsp40 or DNAJ proteins), which regulate the rate of ATP hydrolysis and hence the chaperone activity of Hsp70s [7,8]. Multiple JDPs are distributed across all cellular compartments and provide an extensive network by which to regulate Hsp70 [8]. In addition, Hsp70s are regulated by nucleotide exchange factors (NEFs) that function after ATP hydrolysis to permit the exchange of ADP for ATP necessary for the Hsp70 folding cycle to progress [7]. Therefore, chaperone function involves the formation of multiple complexes involving protein-protein interactions between chaperones, cochaperones and client proteins. This higher order networking is critical for maintenance of proteostasis, including in the context of biomolecular condensates [9].

Biomolecular condensates (to be referred to as biocondensates), dynamic, phase-separated compartments that concentrate proteins and RNA without a delimiting membrane, are emerging as major components of the cellular PQC system. These biocondensates, formed via liquid-liquid phase separation (LLPS), act as transient organelles where specific biochemical reactions are organized and accelerated [10]. Physiological biocondensates play important roles in a range of cellular processes, including regulation of splicing, ribosome assembly and pathogen responses [11–26] (Table 1; Figure 1). Physiological biocondensates are responsive, often associated with cellular stress, and represent an important cellular reaction to maintain protein homeostasis. Pathological biocondensates develop from physiological biocondensates due to aging, mutations or perturbation of the cellular environment. In contrast to the dynamic, responsive and reversible nature of physiological biocondensates, pathological biocondensates are associated with protein aggregation and become static and irreversible, culminating in disease if not resolved [27] (Figure 1). In this review, we discuss mechanistic aspects of cochaperone networking of molecular chaperones in the formation of physiological biocondensates, and their roles in disaggregating proteins within pathological biocondensates to maintain proteostasis.

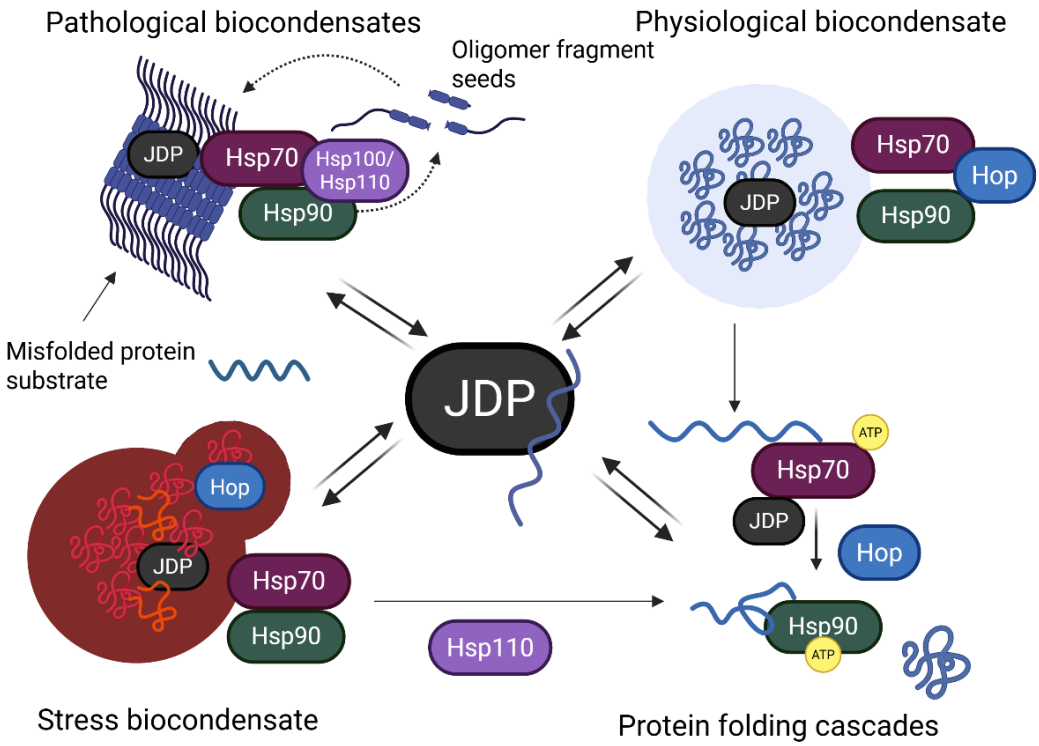

Figure 1. Cochaperone-networked chaperones regulate cellular biocondensates. Chaperone networks including the major chaperones, Hsp70 and Hsp90, and associated cochaperones, Hsp70/Hsp90 organising protein (Hop), J-domain containing proteins (JDPs) and Hsp110 nucleotide exchange factors (NEFs) regulate biocondensates. JDPs are central to physiological biocondensates, functioning to network biocondensates to chaperone folding cascades through their primary association with client proteins (i.e., unfolded proteins; or misfolded proteins formed through various stress or disease states). JDPs and Hop undergo liquid-liquid phase separation into stress-induced condensates (stress biocondensates), where they function as holdases until chaperones can be recruited to triage clients towards folding pathways. JDPs are able to bind stably to pathological biocondensates consisting of highly ordered large protein aggregates (e.g., amyloid fibres), and then recruit chaperone systems to promote disaggregation and disassembly (Hsp100 and Hsp110). However, production of smaller oligomers from such pathological biocondensates can act as seeds to promote further large aggregates. Image created in Biorender.com.

Table 1. Examples of physiological biocondensates.

Name	Cellular Location	Function	Reference
Stress granule (SG)	Nucleus and Cytoplasm	mRNA storage and translational repression (in cytoplasm); transcription and splicing regulation during stress (in nucleus)	[11,12]
U bodies	Cytoplasm	Storage and assembly of small nuclear ribonucleoproteins (snRNPs)	[13]
Nucleolus	Nucleus	Ribosome assembly	[14]
P bodies	Nucleus and Cytoplasm	Post-transcriptional regulation, translational repression and mRNA decay	[11,12,15]
Cajal body	Nucleus	snRNPs and small nucleolar ribonucleoproteins (snoRNPs) assembly and processing; required for splicing, ribosome biogenesis and telomere maintenance	[16,17]
Gems	Nucleus	Enriched in SMN; participate in snRNP assembly with Cajal bodies	[18,19]
PML bodies	Nucleus	Protein SUMOylation; transcriptional regulation, genome integrity, antiviral responses	[20]
Nuclear speckles	Nucleus	Splicing and mRNA processing	[16,21,22]
Paraspeckles	Nucleus	Regulation of gene expression by selective RNA sequestration; cellular responses to viral infection	[16,22–24]
Purinosomes	Cytoplasm	*De novo* purine biosynthesis	[25,26]

2. Molecular Chaperones and Physiological Biocondensates

Recent studies suggest that biocondensates serve as "central clearing houses" for proteostasis, sequestering misfolded proteins and coordinating their refolding or degradation [28]. Molecular chaperones are prominently involved in both the formation and function of such biocondensates. For example, Hsp90 and Hsp70, together with their cochaperones, can partition into stress-induced biocondensates (e.g., stress granules, SGs) that store and manage misfolded proteins [11,12,29,30] (Table 1; Figure 1). Important Hsp90 cochaperones include Aha1, responsible for stimulation of Hsp90 ATPase activity required for client protein release [31], Cdc37, a kinase specific cochaperone for Hsp90, and Hsp70/Hsp90-organizing protein, Hop (also called stress-inducible protein 1, Sti1; or stress-inducible phosphoprotein 1, Stip1), that acts as a scaffold for transfer of client proteins between the Hsp70 and Hsp90 chaperones [32]. The pro-folding Hsp70 and Hsp90 complexes can be diverted to target proteins for degradation via the action of cochaperones like C-terminal Hsp70-binding protein (CHIP/STUB1) [33]. The JDP cochaperones of Hsp70 all contain the signature J domain, and in addition, type I/class A JDPs include a glycine/phenylalanine (G/F) rich region, a C-terminal substrate-binding domain I (CTD-I) with an embedded zinc-finger-like region (with 4 repeats of the cysteine repeat motif CXXCXGXG), and a C-terminal substrate-binding domain II (CTD-II). Type II/Class B JDPs lack the zinc-finger-like region, while type III/class C JDPs only contain the J domain in addition to other specialised domains [7,34]. JDP isoforms all use the J domain to stimulate the basal ATP hydrolysis activity of Hsp70 that is required for protein folding, in addition to other functions that are dictated by the combination of domains in the JDP isoform

Mechanistically, chaperones can drive biocondensate formation by multivalent interactions. Hsp90 can undergo LLPS under certain conditions, and a recent study found that Hsp90α can form biocondensates through interactions with client proteins containing repetitive RG-rich motifs [35]. This condensation of Hsp90 may concentrate specific client proteins and chaperones together, creating a localized environment for efficient folding or refolding [35,36]. Hsp70 can undergo LLPS *in vitro*, while studies in cells have revealed that Hsp70 is involved in the formation and dynamics of certain biocondensates, such as nuclear bodies and SGs [37]. The LLPS property of Hsp70 may drive its association with biocondensates, a process that appears to involve the interaction of its C-terminal substrate-binding domain with certain clients (e.g., fused in sarcoma, FUS) [37]. Furthermore, there is increasing evidence that cochaperones of Hsp70 and Hsp90 are also capable of sequestering misfolded proteins under stressful conditions, independently from their chaperone partners, and potentially as the primary components in the genesis of biocondensates. Hop was recently found to phase-separate and sequester misfolded proteins during stress, effectively nucleating protective biocondensates independently of chaperone machinery, with Hsp90 being recruited later in the process to aid in protein refolding [30] (Figure 1). Indeed, Hop has been identified as a

component of SGs and P granules and has been listed as a core component in the RNA granule database [38]. Hop has also been reported to be a major component of purinosomes, liquid-like biocondensates implicated in *de novo* purine biosynthesis [39,40]. Interestingly, Hop is predicted to have a high propensity for phase-separation (so-called droplet forming capability), and this was recently experimentally confirmed using a novel intracellular assay [41]. Furthermore, the N-terminal tetratricopeptide repeat (TPR)-aspartic acid-proline (DP) domains (the TPR1-DP1 domains) were critical for droplet formation, suggesting that this region could adopt a suitably disordered conformation, enabling multiple multivalent weak interactions necessary for phase separation.

Biocondensates appear and dissolve in a tightly regulated spatiotemporal manner. Under proteotoxic stress (e.g., heat shock), cells rapidly assemble SGs enriched in mRNAs, translation factors, and chaperones, pausing translation and preventing aggregation of unfolded proteins [29]. The JDP family of Hsp70 cochaperones appear to act early and potentially drive the formation of biocondensates through their ability to bind unfolding proteins and sequester them into biocondensates, while Hsp70 and Hsp90 are upregulated to handle downstream refolding tasks [42] (Figure 1). These biocondensates function as hold-in-place repositories; they are not mere deposits of damaged proteins, but rather are active sites where misfolded proteins await triage (refolding by chaperones, or degradation via autophagy/proteasomes). Indeed, experimental disruption of biocondensate assembly has pathological consequences. For example, when key scaffold proteins or chaperones are mutated such that biocondensates cannot form properly, cells show heightened sensitivity to stress and may develop features of neurodegenerative disease [10]. Such "biocondensatopathies" have been proposed as a category of disease where aberrant phase transitions (either failure to form beneficial biocondensates, or formation of toxic, irreversible aggregates) underlie pathology [43]. Hence, cochaperones and their associated chaperones involved in biocondensate formation and dynamics could be targets for intervention; either to enhance their function (in diseases of protein aggregation) or to inhibit their hijacking by disease processes (such as cancer and viral infections).

There is increasing evidence that JDPs, together with their partner Hsp70s, not only suppress protein aggregation through the formation of biocondensates but also play an important role in the dispersal of biocondensates when the stress is removed [44–47] (Table 2; Figure 1). For example, cellular genetic studies on the yeast chaperone machinery, showed that an Hsp70 (Ssa1), a JDP (Sis1) and an Hsp100 (Hsp104) were able to suppress the aggregation of poly(A) binding protein (Pab1) and promote the disassembly of Pab1-containing biocondensates and aggregates [44]. In addition, biochemical studies on the yeast system showed that an Hsp70 (Ssa2), a JDP (Sis1, but not Ydj1) and an Hsp100 (Hsp104), were able to rapidly dissolve heat-induced Pab1-containing biocondensates [45]. Indeed, a considerable body of research has shown that JDPs (especially class B JDPs, the DNAJBs) are key components of biocondensates, playing an important role in the sequestration of specific client proteins into biocondensates during stressed and disease states, and then working with Hsp70 and other chaperone machinery (Hsp90, Hsp100 and Hsp110) to dissolve the biocondensates and promote the (re)folding (or degradation) of such proteins [44–60] (Table 2; Figure 1). This disassembly activity of chaperones is ATP-dependent and crucial for rebooting normal cellular organization after stress. Consequently, chaperones have two major roles in biocondensate biology: they not only assist in the genesis of protective biocondensates during stress but also help dismantle them when they are no longer needed, thus resetting cellular proteostasis. Interfering with either function can be detrimental; prolonged biocondensate existence might sequester needed proteins and stall cell growth, whereas inhibition of biocondensate formation could leave cells unprotected during acute stress [27].

The ability of this network of cochaperone-regulated chaperones to modulate phase-separated compartments also opens new possibilities for drug discovery. Some researchers speculate that tuning biocondensate behavior (for example, using small molecules to enhance or inhibit specific biocondensate-associated chaperones) could combat diseases characterized by aberrant protein aggregation [43,61]. For instance, promoting the formation of chaperone-rich biocondensates might help cells cope with toxic misfolded proteins in neurodegeneration, whereas preventing cancer cells from forming pro-survival biocondensates (sometimes called "stress granule addiction" in tumor cells) could make them more vulnerable to therapy. Indeed, targeting the biocondensate pathway has been suggested as a novel therapeutic approach [61]. Overall, the interplay between chaperones and biocondensates is a new frontier in cellular stress biology, where further research into the mechanistic details will undoubtably lead to the development of therapeutic interventions against so-called biocondensatopathies.

Table 2. J-domain proteins (JDP) associated with biocondensates.

Name	Cell Type	Biocondensate Type *	Function *	Disease Context *	Reference
DNAJA1/Hdj2	HeLa	SGs	Phase separates with FUS, stabilizing it against amyloid aggregation	ALS and FTLD; *in vitro* model of amyloid aggregation	[48]
	U2OS	SGs	Identified in SGs by proteomics	-	[49]
	HeLa	Nuclear paraspeckles	Colocalizes to FUS-containing nuclear paraspeckles	ALS	[46]
DNAJB1/Hdj1	HeLa	SGs	Phase separates with FUS, stabilizing it against amyloid aggregation	ALS and FTLD; *in vitro* model of amyloid aggregation	[48]
	QBI-293, Neuro2A, NSC-34, skeletal muscle	Pathological condensates	Prevents TDP-43 aggregation	ALS and FTLD	[50]
DNAJB2a/Hsj1a	HEK293T	Pathological condensates	Prevents TDP-43 aggregation; involves J-domain and Hsp70	ALS	[51]
DNAJB5	HEK293T and neurons	Pathological condensates	Protects against TDP-43 aggregation	ALS and FTLD; mouse model	[52]
DNAJB6	HEK293T	Cytosolic	Suppresses poly-Q aggregation (huntingtin-derived)	Huntington's disease	[53]
	HeLa, 293T	Nuclear bodies	Suppresses TDP-43 aggregation during heat shock; involves J-domain and Hsp70/HSPA1A	ALS and FTLD	[54]
	Fly muscle and S2 cells, human brain	Pathological condensates	Suppresses aggregation of hnRNPA2B1	hIMB and LGMD	[55]
DNAJB6b	HEK293	Pathological condensates	Suppresses poly-GA aggregation (C9orf72-derived)	ALS and FTD	[56]
DNAJB12	HEK293T and neurons	Pathological condensates	Protects against FUS aggregation	ALS	[57]
DNAJB14	HEK293T and neurons	Pathological condensates	Protects against FUS aggregation and restores proteostasis	ALS	[57]
DNAJC7	HEK293T	SGs	Identified in SGs by proteomics	ALS and FTD	[58]
	HeLa	Nuclear paraspeckles	Colocalizes to FUS-containing nuclear paraspeckles; promotes disassembly	ALS	[46]
	HEK293A	Pathological condensates	Colocalizes to pathological TDP-43 condensates	ALS	[59]
Ydj1	Yeast	Osmotic shock foci	Forms cytoplasmic foci in response to hyperosmosis	-	[60]
		SGs	Identified in SGs by proteomics	-	[49]
			SG disassembly to promote recovery of translation	-	[47]
Sis1	Yeast	Osmotic shock foci	Forms cytoplasmic foci in response to hyperosmosis	-	[60]
		SGs	SG disassembly to promote clearance by autophagy	-	[47]
		Pab1 condensates	Dispersal of Pab1 condensates	-	[45]
		Pab1 condensates	Suppression of Pab1 aggregation; promotes disassembly	-	[44]
		Cytosolic	Suppresses poly-Q aggregation (huntingtin-derived)	Huntington's disease	[53]

* Abbreviations: ALS, amyotrophic lateral sclerosis; C9orf72, chromosome 9 open reading frame 72; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; FUS, fused in sarcoma; hIBM, Hereditary inclusion body myopathy; hnRNPA2B1, heterogeneous nuclear ribonucleoproteins A2B1; LGMD, limb-girdle muscular dystrophy; Pab1, poly(A) binding protein; SG, stress granules; TDP-43, transactive response DNA-binding protein of 43 kDa (TDP-43).

3. Molecular Chaperones and Pathological Biocondensates

A critical function of these cochaperone-chaperone networks is to prevent proteins from aggregating or to rescue proteins that have fallen into aggregated states. Stressful conditions, including disease states, can readily cause unprotected non-sequestered proteins to aggregate. While proteins sequestered into biocondensates are protected, certain stress conditions, especially chronic disease states, can promote conversion of condensates from a dynamic liquid-like droplet state into a pathological gel-to-solid-like aggregated state which are recognised as pathological condensates (Table 2; Figure 1). These protein aggregates come in broadly two forms: amorphous aggregates, which are relatively disordered clumps of misfolded proteins, and amyloid fibrils, which are highly ordered, beta-sheet-rich aggregates [62]. Both types can be toxic to cells, but they pose different challenges for disaggregation. Amorphous aggregates often result from acute stress (heat shock, oxidative damage) and can sometimes be resolubilized by chaperones. Amyloid fibrils, by contrast, are associated with chronic diseases (e.g., amyloid-β in Alzheimer's disease, and α-synuclein in Parkinson's disease) and are notoriously stable and resistant to unfolding [63].

Cells have evolved dedicated disaggregase machines to resolve and clear aggregates. In bacteria and yeast, this role is performed by a two-component system: an Hsp70 chaperone (with its JDP cochaperones) recognizes and binds aggregates, and a powerful AAA+ ATPase (Hsp100 family, e.g., ClpB in bacteria or Hsp104 in yeast) applies mechanical force to pull apart aggregated polypeptides [64,65]. Early landmark studies demonstrated that *Escherichia coli* ClpB, working in concert with DnaK (Hsp70) and DnaJ (JDP), could reverse protein aggregation and reactivate proteins, effectively "unboiling an egg" at a molecular level [66,67]. Likewise, seminal studies in yeast showed that Hsp104 was required for thermotolerance by disassembling protein aggregates [68]. Furthermore, recent evidence suggests that in yeast the class B JDP, Sis1, is highly effective at recognizing aggregates and recruiting Hsp70 (Ssa1/2) and Hsp104 to promote disaggregation [69,70]. The Hsp100 disaggregase hexamers thread polypeptides through a central pore, using ATP-driven conformational changes to exert pulling forces, while Hsp70/JDP hold onto the aggregate and feed it to the Hsp100, like a rope into a winch [64,67,71] (Figure 2). Using this mechanism, a number of different types of aggregates can be disassembled in yeast, including: phase-transitioned gels; pre-amyloid oligomers; amyloids; and prions [71,72]. Unfolded polypeptides released by Hsp100 can then refold spontaneously or with the assistance of molecular chaperones, most likely a class A JDP and Hsp70 (e.g., Ydj1 and Ssa1/2 in yeast) [73] (Figure 2). However, the fate of the fragmented aggregate remains controversial, particularly in the case of yeast prion amyloids. Paradoxically, while Hsp104 has been reported under certain conditions to cure cells of prions when overexpressed, at normal expression levels it has been shown to fragment prion amyloids into multiple smaller pieces that seed prion propagation and accelerate prion conversion [72] (Figure 2). Interestingly, unlike the propagation activity that requires Hsp70 and a JDP, the curing activity requires Hsp90 and the co-chaperone Hop (Sti1 in yeast) [70,74].

Intriguingly, the metazoan cytosol and nucleus lack an Hsp100 homolog; animals do not encode an Hsp104/ClpB equivalent for these compartments [75]. Yet, metazoan cells can still clear aggregates, implying that Hsp70-based systems have evolved an augmented capability. Indeed, in higher eukaryotes, the disaggregation machinery consists of Hsp70 (particularly inducible Hsp72 and constitutive Hsc70), working with certain JDPs and NEFs (Figure 2). Key NEFs in mammals include Hsp110 family members (e.g., human HSPH family; [76]) which not only recharge Hsp70's ATP cycle but also act as holdases that bind unfolded polypeptides. The cooperation of Hsp70, JDP, and Hsp110 can dissolve amorphous aggregates and has some activity even against amyloids [75]. Metazoan cells evolved an Hsp110-dependent disaggregase complex, repurposing the Hsp70 system for disaggregation in the absence of Hsp100. In essence, JDPs can recognize and bind specific aggregates leading to recruitment of multiple Hsp70s, and then with the assistance of NEFs that help recycle Hsp70s, this JDP-Hsp70-NEF machinery collaboratively pulls apart the aggregated proteins (Figure 2). The JDPs from class B (such as DNAJB1 or DNAJB4) play a central role by binding first to amyloid fibres with nanomolar affinity and high density [77,78]. Biophysical studies have determined that the amyloid binding site is the CTD-II domain within DNAJB1 [79–81]. The JDPs then recruit ATP-bound Hsp70s to the amyloid fibres using a bidentate mechanism involving their J domain and the CTD-I domain, with the C-terminal EEVD motif of the Hsp70 releasing the auto-inhibition of the J domain by CTD-I [78,80,82,83]. Once bound to the JDP and amyloid, Hsp70 is stimulated to hydrolyse its ATP, resulting in even tighter binding to the complex [77,78,84]. The resultant cluster of tightly bound Hsp70/JDP molecules at the core of the amyloid is proposed to generate significant entropy loss due to excluded volume effects (and a more ordered state). However, the tight clusters will be prone to increased random collisions of the Hsp70s against one another and the amyloid fibre, triggering an increase in entropy and net movement away from the aggregate. This generates a pulling force on the amyloid fibre, gradually freeing it from the aggregate, with the resulting further increase in entropy (freedom of movement of all moving parts) [78,82,84,85]

(Figure 2). The formation of the Hsp70/JDP cluster is necessary but not sufficient for optimal disaggregase activity, and Hsp110 is also required to enable efficient disaggregation (Figure 2). Hsp110 appears to function not as a general NEF, but rather as a selective NEF that uses its bulk to boost the entropically-driven disaggregase activity of the aggregate-bound Hsp70/JDP cluster, and two theories have been proposed in this regard: (i) selective association of Hsp110 with loosely-bound inactive Hsp70s promotes disaggregase activity [86]; and (ii) selective association of Hsp110 with the tightly-bound active Hsp70/JDP clusters promotes disaggregase activity [87]. Theory (i) assumes that bulky Hsp110 would selectively target isolated loosely-bound inactive Hsp70s due to reduced steric hinderance compared to the tightly-bound active Hsp70-JDP clusters [86]. In this model, the unproductive Hsp70s would be recycled for further cluster formation, or to create clearings around clusters, promoting their growth and associated enhanced disaggregase activity. On the other hand, theory (ii) makes the counter assumption that the Hsp70/JDP clusters form where there is reduced steric hindrance, making such clusters more favorable to Hsp110 binding [87]. Hence, in this model the binding of Hsp110 directly to Hsp70/JDP clusters would increase their effective size, which would enhance the entropic pulling strokes and associated disaggregase activity. Whatever the precise mechanism, it is clear that class B JDPs enable targeting of specific aggregates and the recruitment of a partner Hsp70 that is the engine room of entropic force generation, and that the bulky NEF, Hsp110, is the regulatory component ensuring that the disaggregation machinery operates efficiently. Both models are compatible with subsequent spontaneous or chaperone-assisted folding (e.g., Hsp70/JDP) of unfolded proteins [53] (Figure 2). However, Hsp110-dependent disaggregases have also been reported to fragment amyloid fibres (e.g., α-synuclein and amyloid-β) into small seeding-competent oligomers that are capable of spreading pathology, as outlined in the following sections (Figure 2).

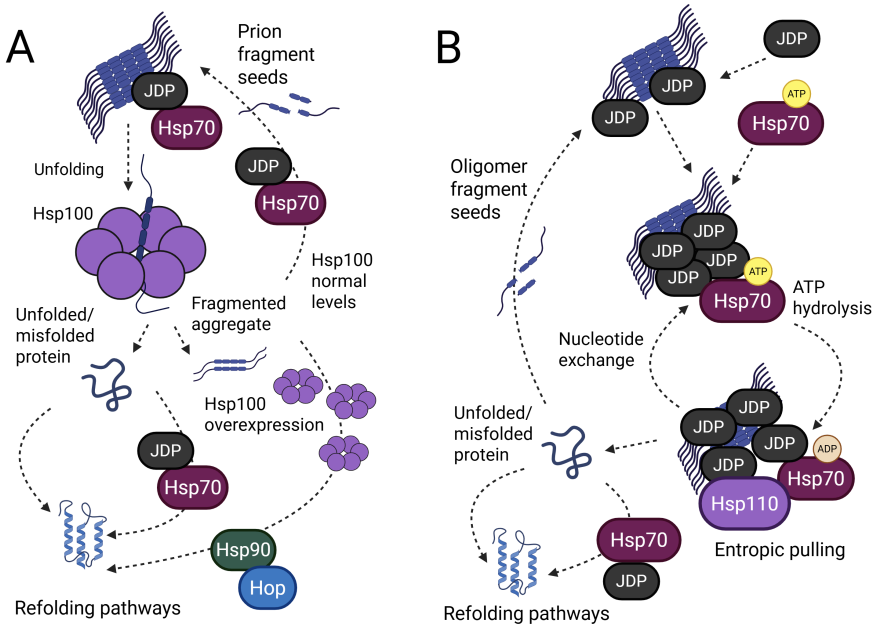

Figure 2. Comparison of the mechanisms of Hsp100-dependent and Hsp110-dependent disaggregases in biocondensate processing. A. In bacteria and yeast, Hsp100 chaperones collaborate with Hsp70 and JDP to unfold aggregated proteins. The Hsp70/JDP complex delivers the aggregate to the Hsp100 disaggregase, where the protein is unfolded via threading through the central channel of the Hsp100 hexamer. Thereafter, the unfolded protein can refold spontaneously or via chaperone-mediated pathways (e.g., via the Hsp70/JDP pathway). However, in the context of specific aggregates (e.g., prions in yeast), two different fates of the fragmented aggregates have been reported: elevated levels of Hsp100 can result in aggregate removal (via the Hsp90-Hop pathway); while normal levels of Hsp100 can generate small fragments that seed further aggregation (via the Hsp70/JDP pathway). B. In metazoans, Hsp110s function as selective nucleotide exchange factors (NEFs) for disaggregation catalysed by Hsp70/JDP complexes. In this case, JDP proteins, particularly the class B type (DNAJB), accumulate within the aggregates and recruit ATP-bound Hsp70. Stimulation of Hsp70 ATP hydrolysis results in ADP-bound Hsp70 that has high affinity for aggregates and results in entropic pulling required for disaggregation. Hsp110 fulfils a specific NEF function that is critical to promote entropic pulling and permit disaggregation. Unfolded proteins emerging from the aggregate can refold spontaneously or via chaperone-mediated pathways (e.g., via Hsp70/JDP pathways). Alternatively, it has been reported that Hsp110-dependent disaggregases can fragment amyloid fibres (e.g., α-synuclein and amyloid-β) into small seeding-competent oligomers that are capable of spreading pathology. Image created in Biorender.com.

Effectively measuring disaggregation activity in complex cellular environments is non-trivial, and outcomes can depend on the context. For example, in actively dividing cells, the "success" of disaggregation might be reflected in restored cell proliferation or prevention of cell death. In non-dividing, long-lived cells like neurons, other readouts, such as recovery of synaptic function or neurite outgrowth, are more relevant, since neuronal death may not occur until very late even if aggregates cause dysfunction. Studies in neurodegenerative disease models have shown that if expression of an aggregation-prone protein is halted early enough, cells (and even whole organisms) can often clear existing aggregates over time and recover function [88,89]. Using a Huntington's disease mouse model, it was shown that turning off the mutant huntingtin gene led to the gradual disappearance of aggregates and reversal of neurological symptoms [89]. Similar results have been observed in models of spinocerebellar ataxia and tauopathies; early intervention allowing endogenous chaperones and proteolytic systems to catch up and clear the aggregates to restore near-normal cellular function [90–92]. These findings underscore that timing is critical; once aggregates inflict irreversible damage (such as neuron loss or widespread synapse destruction), merely disaggregating proteins may not bring back lost cells or connections. Thus, any therapeutic strategy aiming to enhance disaggregation must be deployed sufficiently early in the disease course, before the "point of no return" where functional deficits become permanent.

Another challenge in the condensate disaggregation field is identifying the truly toxic species of aggregates. It is now well appreciated that large, insoluble inclusion bodies (visible aggregates) may actually be less toxic than smaller, soluble oligomers of misfolded proteins [93,94]. In Alzheimer's disease, for example, diffusible oligomers of amyloid-β correlate better with neurotoxicity than the final amyloid plaques [94,95]. The same is true for α-synuclein in Parkinson's disease. These small aggregates can evade quality control and travel between cells, spreading pathology. From a therapeutic standpoint, we must ask: can chaperone systems disaggregate these oligomeric species effectively, and if so, do they neutralize or worsen the threat? Disaggregation of amyloid fibrils or oligomers might inadvertently generate more toxic, seeding-competent species if conducted in an unregulated manner. The Hsp70/Hsp110 disaggregase can fragment amyloid fibres of α-synuclein, producing smaller oligomers that are capable of propagating pathology in cell and animal models [96] (Figure 2). It was demonstrated that while Hsp70/Hsp110 can break down α-synuclein fibrils, the resulting species were more toxic and could spread between cells, effectively exacerbating the proteopathic seed load. Hence, the chaperone disaggregase system is "a double-edged sword"; essential for clearing aggregates, but also potentially involved in generating harmful oligomers as by-products [96]. This also implies that sequestration of aggregates into stable condensates is, at least to some extent, cytoprotective by confinement that prevents dissemination of these pathological species.

Therefore, any therapeutic approach to enhance disaggregation must be coupled with strategies to safely dispose of the disaggregated proteins. Ideally, disaggregation of pathogenic amyloids should be followed immediately by proteolytic degradation or neutralization of the released monomers/oligomers. Cells do have chaperone-mediated mechanisms for the removal of disaggregated proteins: the ubiquitin-proteasome system and autophagy [97–102]. For instance, Hsp70 and its cochaperones can target protein aggregates for proteasomal degradation by facilitating ubiquitin ligase-mediated recruitment of the 26S proteasomal machinery. Alternatively, enhancing autophagy might be used in tandem with chaperone upregulation to ensure that once aggregates are disassembled, the proteins are promptly cleared. Recent studies have revealed a specialized type of autophagy, termed aggrephagy, which appears to involve the selective autophagic degradation of protein aggregates [103]. Interestingly, aggrephagy requires fragmentation of aggregates followed by compaction, before their autophagic disposal, with DNAJB6, Hsp70 and Hsp110 being crucial for aggregate fragmentation, but also requiring the activity of a component of the 26S proteasomal machinery (the 19S regulatory particle) [103].

In summary, protein disaggregases (whether Hsp70/JDP/Hsp100-based in yeast/bacteria or Hsp70/JDP/Hsp110-based in metazoans) are crucial for proteostasis, especially under stress and in the context of pathological condensates. These systems have proven benefits, as seen in models where boosting chaperone expression alleviates proteotoxicity. But their operation in the context of amyloid disease requires a nuanced approach to avoid collateral damage. Ongoing research is dissecting how disaggregase activity is regulated in cells. For example, cells might suppress disaggregation of certain amyloids to avoid generating seeds, opting instead to sequester them in inclusion bodies. A deeper understanding of these regulatory circuits will inform how disaggregases can be safely harnessed in therapy. With careful tuning, it might become possible to coax cells to dismantle toxic aggregates in diseases like Alzheimer's disease, while simultaneously preventing the rebound of toxicity by promoting downstream clearance. This balance of timing, targeting, and tandem clearance will define the success of future interventions aimed at toxic protein aggregates.

4. Conclusions

Stress response pathways at the molecular level offer a compelling yet complex arena for therapeutic intervention. Here, we examined how cochaperone-regulated molecular chaperones, the central players in proteostasis, present both opportunities and challenges for the therapeutic modulation of biocondensates. Cochaperones such as Hop, JDPs and NEFs in concert with their chaperone partners Hsp70 and Hsp90, are integral to maintaining protein homeostasis in biocondensates through the regulation of physiological biocondensate cycling, and the disaggregation-based resolution of pathological condensates. These cochaperone-chaperone-driven processes enable cells to survive various stresses and disease states by isolating and resolving misfolded proteins, with finely tuned regulation required to avoid pathological outcomes. Understanding these regulatory mechanisms in greater detail will enable the development of therapeutic strategies to enhance beneficial aspects (e.g., boosting adaptive SG responses in neurodegeneration) or inhibit detrimental ones (e.g., SG overuse by cancer cells to resist chemotherapy). Moreover, our expanding knowledge of cellular stress networks, for instance, how cochaperones and chaperones interface with proteolysis, immune signaling, and cell death pathways, will lead to more holistic therapeutic strategies. Indeed, any therapeutic intervention targeting toxic aggregates should be tightly coupled to enhancement of aggregate clearance processes (e.g., the ubiquitin-proteasome system and autophagy). In some cases, combining a cochaperone-chaperone inhibitor with another agent (like an autophagy inducer or an immune checkpoint inhibitor) could yield synergistic outcomes, attacking the disease on multiple fronts. In conclusion, cochaperone-chaperone networks sit at a crossroads of pathways critical for cell survival under duress, and hence by deepening our understanding of their role in biocondensate dynamics, novel intervention points will be revealed for sustainable therapeutic solutions to biocondensatopathies.

Author Contributions

G.L.B.: Conceptualization; G.L.B. and A.L.E.: writing—original draft preparation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding

Research in the laboratory of ALE is supported by grants from the Department of Science, Technology and Innovation and National Research Foundation (DSTI/NRF) South African Research Chair (SARChI) grant (grant number: 98566), Rhodes University, the Academy of Medical Sciences Newton Advanced Fellowship and by the MRC Africa Research Leaders award (MR/V030701/1) which is jointly funded by the UK Medical Research Council (MRC) and the UK Foreign, Commonwealth & Development Office (FCDO) under the MRC/FCDO Concordat agreement and is carried out in the frame of the Global Health EDCTP3 Joint Undertaking (UKRI MRC). Research conducted by GLB is supported by the Incentive Funding for Rated Researchers Programme of the NRF, South Africa (grant number: RA22111973834).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

All the data associated with this article can be found within the body of the article.

Conflicts of Interest

Given the role as an Editorial Board Member, Gregory L. Blatch had no involvement in the peer review of this paper and had no access to information regarding its peer-review process. Full responsibility for the editorial process of this paper was delegated to another editor of the journal.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Rosenzweig, R.; Nillegoda, N.B.; Mayer, M.P.; et al. The Hsp70 chaperone network. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 665–680. https://doi.org/10.1038/s41580-019-0133-3.
2. Schopf, F.H.; Biebl, M.M.; Buchner, J. The HSP90 chaperone machinery. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 345–360. https://doi.org/10.1038/nrm.2017.20.
3. Prodromou, C.; Aran-Guiu, X.; Oberoi, J.; et al. HSP70–HSP90 Chaperone Networking in Protein-Misfolding Disease. In *The Networking of Chaperones by Co-Chaperones*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 389–425. https://doi.org/10.1007/978-3-031-14740-1_13.
4. Edkins, A.L.; Boshoff, A. General Structural and Functional Features of Molecular Chaperones. *Adv. Exp. Med. Biol.* **2021**, *1340*, 11–73. https://doi.org/10.1007/978-3-030-78397-6_2.
5. Dean, M.E.; Johnson, J.L. Human Hsp90 cochaperones: Perspectives on tissue-specific expression and identification of cochaperones with similar in vivo functions. *Cell Stress Chaperones* **2021**, *26*, 3–13. https://doi.org/10.1007/s12192-020-01167-0.
6. Johnson, J.L. Mutations in Hsp90 cochaperones result in a wide variety of human disorders. *Front. Mol. Biosci.* **2021**, *8*, 787260. https://doi.org/10.3389/fmolb.2021.787260.
7. Kampinga, H.H.; Craig, E.A. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 579–592. https://doi.org/10.1038/nrm2941.
8. Marszalek, J.; De Los Rios, P.; Cyr, D.; et al. J-domain proteins: From molecular mechanisms to diseases. *Cell Stress Chaperones* **2024**, *29*, 21–33. https://doi.org/10.1016/j.cstres.2023.12.002.
9. Mayer, M.P.; Blair, L.; Blatch, G.L.; et al. Stress biology: Complexity and multifariousness in health and disease. *Cell Stress Chaperones* **2024**, *29*, 143–157. https://doi.org/10.1016/j.cstres.2024.01.006.
10. Alberti, S.; Hyman, A.A. Biomolecular condensates at the nexus of cellular stress, protein aggregation disease and ageing. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 196–213. https://doi.org/10.1038/s41580-020-00326-6.
11. Garg, M.; Lamba, S.; Rajyaguru, P.I. Emerging trends in the disassembly of stress granules and P-bodies. *Cell Rep.* **2025**, *44*, 116340. https://doi.org/10.1016/j.celrep.2025.116340.
12. Riggs, C.L.; Kedersha, N.; Ivanov, P.; et al. Mammalian stress granules and P bodies at a glance. *J. Cell Sci.* **2020**, *133*, jcs242487. https://doi.org/10.1242/jcs.242487.
13. Liu, J.L.; Gall, J.G. U bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P bodies. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11655–11659. https://doi.org/10.1073/pnas.0704977104.
14. Dogra, P.; Kriwacki, R.W. Phase separation via protein-protein and protein-RNA networks coordinates ribosome assembly in the nucleolus. *Biochim. Biophys. Acta Gen. Subj.* **2025**, *1869*, 130835. https://doi.org/10.1016/j.bbagen.2025.130835.
15. Luo, Y.; Na, Z.; Slavoff, S.A. P-Bodies: Composition, properties, and functions. *Biochemistry* **2018**, *57*, 2424–2431. https://doi.org/10.1021/acs.biochem.7b01162.
16. Choi, S.; Kim, K.K. Nuclear ribonucleoprotein condensates as platforms for gene expression regulation. *Genes Genomics* **2025**, *47*, 935–951. https://doi.org/10.1007/s13258-025-01661-8.
17. Staněk, D. Coilin and Cajal bodies. *Nucleus* **2023**, *14*, 2256036. https://doi.org/10.1080/19491034.2023.2256036.
18. Malatesta, M.; Scassellati, C.; Meister, G.; et al. Ultrastructural characterisation of a nuclear domain highly enriched in survival of motor neuron (SMN) protein. *Exp. Cell Res.* **2004**, *292*, 312–321. https://doi.org/10.1016/j.yexcr.2003.08.022.
19. Wang, I.F.; Reddy, N.M.; Shen, C.K. Higher order arrangement of the eukaryotic nuclear bodies. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 13583–13588. https://doi.org/10.1073/pnas.212483099.
20. Dorosz, K.; Majewska, L.; Kijowski, J. Structure and function of PML nuclear bodies: A brief overview of key cellular roles. *Biomolecules* **2025**, *15*, 291. https://doi.org/10.3390/biom15091291.
21. Giudice, J.; Jiang, H. Splicing regulation through biomolecular condensates and membraneless organelles. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 683–700. https://doi.org/10.1038/s41580-024-00739-7.
22. Ilık, İ.A.; Aktaş, T. Nuclear speckles: Dynamic hubs of gene expression regulation. *FEBS J.* **2022**, *289*, 7234–7245. https://doi.org/10.1111/febs.16117.
23. Milcamps, R.; Michiels, T. Involvement of paraspeckle components in viral infections. *Nucleus* **2024**, *15*, 2350178. https://doi.org/10.1080/19491034.2024.2350178.
24. Wang, Y.; Chen, L.L. Organization and function of paraspeckles. *Essays Biochem.* **2020**, *64*, 875–882. https://doi.org/10.1042/ebc20200010.
25. Pedley, A.M.; Benkovic, S.J. A new view into the regulation of purine metabolism: The purinosome. *Trends Biochem. Sci.* **2017**, *42*, 141–154. https://doi.org/10.1016/j.tibs.2016.09.009.
26. Pedley, A.M.; Pareek, V.; Benkovic, S.J. The purinosome: A case study for a mammalian metabolon. *Annu. Rev. Biochem.* **2022**, *91*, 89–106. https://doi.org/10.1146/annurev-biochem-032620-105728.

27. Akaree, N.; Secco, V.; Levy-Adam, F.; et al. Regulation of physiological and pathological condensates by molecular chaperones. *FEBS J.* **2025**, *292*, 3271–3297. https://doi.org/10.1111/febs.17390.
28. Kohler, V.; Andréasson, C. Reversible protein assemblies in the proteostasis network in health and disease. *Front. Mol. Biosci.* **2023**, *10*, 1155521. https://doi.org/10.3389/fmolb.2023.1155521.
29. Glauninger, H.; Wong Hickernell, C.J.; Bard, J.A.M.; et al. Stressful steps: Progress and challenges in understanding stress-induced mRNA condensation and accumulation in stress granules. *Mol. Cell* **2022**, *82*, 2544–2556. https://doi.org/10.1016/j.molcel.2022.05.014.
30. Rutledge, B.S.; Kim, Y.J.; McDonald, D.W.; et al. Stress-inducible phosphoprotein 1 (Sti1/Stip1/Hop) sequesters misfolded proteins during stress. *FEBS J.* **2025**, *292*, 3634–3658. https://doi.org/10.1111/febs.17389.
31. Amoah, D.P.; Mercier, R.; Alyousef, G.; et al. Collaboration between two conserved sequence motifs drives ATPase stimulation of Hsp90 by Aha1. *Protein Sci.* **2025**, *34*, e70359. https://doi.org/10.1002/pro.70359.
32. Schwarz, K.; Baindur-Hudson, S.; Blatch, G.L.; et al. Hsp70/Hsp90 organising protein (Hop): Coordinating much more than chaperones. In *The Networking of Chaperones by Co-Chaperones*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 81–125. https://doi.org/10.1007/978-3-031-14740-1_3.
33. Chakraborty, A.; Edkins, A.L. CHIP: A co-chaperone for degradation by the proteasome and lysosome. In *The Networking of Chaperones by Co-Chaperones*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 351–387. https://doi.org/10.1007/978-3-031-14740-1_12.
34. Cheetham, M.E.; Caplan, A.J. Structure, function and evolution of DnaJ: Conservation and adaptation of chaperone function. *Cell Stress Chaperones* **1998**, *3*, 28–36. https://doi.org/10.1379/1466-1268(1998)003<0028:sfaeod>2.3.co;2.
35. Hu, J.; Dong, H.; Li, Y.; et al. Hsp90α forms condensate engaging client proteins with RG motif repeats. *Chem. Sci.* **2024**, *15*, 10508–10518. https://doi.org/10.1039/d4sc00267a.
36. Zhang, Y.; Pyo, A.G.T.; Kliegman, R.; et al. The exchange dynamics of biomolecular condensates. *Elife* **2024**, *12*, RP91680. https://doi.org/10.7554/eLife.91680.
37. Li, Y.; Gu, J.; Wang, C.; et al. Hsp70 exhibits a liquid-liquid phase separation ability and chaperones condensed FUS against amyloid aggregation. *iScience* **2022**, *25*, 104356. https://doi.org/10.1016/j.isci.2022.104356.
38. Millar, S.R.; Huang, J.Q.; Schreiber, K.J.; et al. A new phase of networking: The molecular composition and regulatory dynamics of mammalian stress granules. *Chem. Rev.* **2023**, *123*, 9036–9064. https://doi.org/10.1021/acs.chemrev.2c00608.
39. An, S.; Kumar, R.; Sheets, E.D.; et al. Reversible compartmentalization of de novo purine biosynthetic complexes in living cells. *Science* **2008**, *320*, 103–106. https://doi.org/10.1126/science.1152241.
40. French, J.B.; Zhao, H.; An, S.; et al. Hsp70/Hsp90 chaperone machinery is involved in the assembly of the purinosome. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 2528–2533. https://doi.org/10.1073/pnas.1300173110.
41. Miki, T.; Hashimoto, M.; Shimizu, M.; et al. Intracellular evaluation of protein droplet-forming capability using self-assembling peptide tags. *Chem. Sci.* **2025**, *16*, 14384–14391. https://doi.org/10.1039/d5sc00871a.
42. Moritz, M.N.O.; Silva, N.S.M.; Rocha, A.M.F.; et al. Supramolecular assemblies and condensate aggregation: The role of human chaperones during stress. *Trans. R. Soc. S. Afr.* **2025**, *80*, 5–25. https://doi.org/10.1080/0035919X.2025.2519206.
43. Patel, A.; Mitrea, D.; Namasivayam, V.; et al. Principles and functions of condensate modifying drugs. *Front. Mol. Biosci.* **2022**, *9*, 1007744. https://doi.org/10.3389/fmolb.2022.1007744.
44. Buchholz, H.E.; Martin, S.A.; Dorweiler, J.E.; et al. Hsp70 chaperones, Ssa1 and Ssa2, limit poly(A) binding protein aggregation. *Mol. Biol. Cell* **2025**, *36*, ar66. https://doi.org/10.1091/mbc.E25-01-0027.
45. Yoo, H.; Bard, J.A.M.; Pilipenko, E.V.; et al. Chaperones directly and efficiently disperse stress-triggered biomolecular condensates. *Mol. Cell* **2022**, *82*, 741–755.e11. https://doi.org/10.1016/j.molcel.2022.01.005.
46. Gao, C.; Gu, J.; Zhang, H.; et al. Hyperosmotic-stress-induced liquid-liquid phase separation of ALS-related proteins in the nucleus. *Cell Rep.* **2022**, *40*, 111086. https://doi.org/10.1016/j.celrep.2022.111086.
47. Walters, R.W.; Muhlrad, D.; Garcia, J.; et al. Differential effects of Ydj1 and Sis1 on Hsp70-mediated clearance of stress granules in *Saccharomyces cerevisiae*. *RNA* **2015**, *21*, 1660–1671. https://doi.org/10.1261/rna.053116.115.
48. Gu, J.; Liu, Z.; Zhang, S.; et al. Hsp40 proteins phase separate to chaperone the assembly and maintenance of membraneless organelles. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 31123–31133. https://doi.org/10.1073/pnas.2002437117.
49. Jain, S.; Wheeler, J.R.; Walters, R.W.; et al. ATPase-modulated stress granules contain a diverse proteome and substructure. *Cell* **2016**, *164*, 487–498. https://doi.org/10.1016/j.cell.2015.12.038.
50. Wang, P.; Wander, C.M.; Yuan, C.X.; et al. Acetylation-induced TDP-43 pathology is suppressed by an HSF1-dependent chaperone program. *Nat. Commun.* **2017**, *8*, 82. https://doi.org/10.1038/s41467-017-00088-4.
51. Chen, H.J.; Mitchell, J.C.; Novoselov, S.; et al. The heat shock response plays an important role in TDP-43 clearance: Evidence for dysfunction in amyotrophic lateral sclerosis. *Brain* **2016**, *139*, 1417–1432. https://doi.org/10.1093/brain/aww028.
52. San Gil, R.; Pascovici, D.; Venturato, J.; et al. A transient protein folding response targets aggregation in the early phase of TDP-43-mediated neurodegeneration. *Nat. Commun.* **2024**, *15*, 1508. https://doi.org/10.1038/s41467-024-45646-9.

53. Klaips, C.L.; Gropp, M.H.M.; Hipp, M.S.; et al. Sis1 potentiates the stress response to protein aggregation and elevated temperature. *Nat. Commun.* **2020**, *11*, 6271. https://doi.org/10.1038/s41467-020-20000-x.
54. Udan-Johns, M.; Bengoechea, R.; Bell, S.; et al. Prion-like nuclear aggregation of TDP-43 during heat shock is regulated by HSP40/70 chaperones. *Hum. Mol. Genet.* **2014**, *23*, 157–170. https://doi.org/10.1093/hmg/ddt408.
55. Li, S.; Zhang, P.; Freibaum, B.D.; et al. Genetic interaction of hnRNPA2B1 and DNAJB6 in a *Drosophila* model of multisystem proteinopathy. *Hum. Mol. Genet.* **2016**, *25*, 936–950. https://doi.org/10.1093/hmg/ddv627.
56. Liu, F.; Morderer, D.; Wren, M.C.; et al. Proximity proteomics of C9orf72 dipeptide repeat proteins identifies molecular chaperones as modifiers of poly-GA aggregation. *Acta Neuropathol. Commun.* **2022**, *10*, 22. https://doi.org/10.1186/s40478-022-01322-x.
57. Rozales, K.; Younis, A.; Saida, N.; et al. Differential roles for DNAJ isoforms in HTT-polyQ and FUS aggregation modulation revealed by chaperone screens. *Nat. Commun.* **2022**, *13*, 516. https://doi.org/10.1038/s41467-022-27982-w.
58. Markmiller, S.; Soltanieh, S.; Server, K.L.; et al. Context-dependent and disease-specific diversity in protein interactions within stress granules. *Cell* **2018**, *172*, 590–604.e13. https://doi.org/10.1016/j.cell.2017.12.032.
59. Evangelista, B.A.; Cahalan, S.R.; Ragusa, J.V.; et al. Tandem detergent-extraction and immunoprecipitation of proteinopathy: Scalable enrichment of ALS-associated TDP-43 aggregates. *iScience* **2023**, *26*, 106645. https://doi.org/10.1016/j.isci.2023.106645.
60. Alexandrov, A.I.; Grosfeld, E.V.; Dergalev, A.A.; et al. Analysis of novel hyperosmotic shock response suggests 'beads in liquid' cytosol structure. *Biol. Open* **2019**, *8*, bio044529. https://doi.org/10.1242/bio.044529.
61. Mitrea, D.M.; Mittasch, M.; Gomes, B.F.; et al. Modulating biomolecular condensates: A novel approach to drug discovery. *Nat. Rev. Drug Discov.* **2022**, *21*, 841–862. https://doi.org/10.1038/s41573-022-00505-4.
62. Hipp, M.S.; Park, S.H.; Hartl, F.U. Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol.* **2014**, *24*, 506–514. https://doi.org/10.1016/j.tcb.2014.05.003.
63. Chiti, F.; Dobson, C.M. Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. *Annu. Rev. Biochem.* **2017**, *86*, 27–68. https://doi.org/10.1146/annurev-biochem-061516-045115.
64. Doyle, S.M.; Hoskins, J.R.; Wickner, S. Collaboration between the ClpB AAA+ remodeling protein and the DnaK chaperone system. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11138–11144. https://doi.org/10.1073/pnas.0703980104.
65. Haslberger, T.; Weibezahn, J.; Zahn, R.; et al. M domains couple the ClpB threading motor with the DnaK chaperone activity. *Mol. Cell* **2007**, *25*, 247–260. https://doi.org/10.1016/j.molcel.2006.11.008.
66. Mogk, A.; Tomoyasu, T.; Goloubinoff, P.; et al. Identification of thermolabile *Escherichia coli* proteins: Prevention and reversion of aggregation by DnaK and ClpB. *EMBO J.* **1999**, *18*, 6934–6949. https://doi.org/10.1093/emboj/18.24.6934.
67. Zietkiewicz, S.; Krzewska, J.; Liberek, K. Successive and synergistic action of the Hsp70 and Hsp100 chaperones in protein disaggregation. *J. Biol. Chem.* **2004**, *279*, 44376–44383. https://doi.org/10.1074/jbc.M402405200.
68. Parsell, D.A.; Kowal, A.S.; Singer, M.A.; et al. Protein disaggregation mediated by heat-shock protein Hsp104. *Nature* **1994**, *372*, 475–478. https://doi.org/10.1038/372475a0.
69. Masison, D.C.; Reidy, M.; Kumar, J. J Proteins counteract amyloid propagation and toxicity in yeast. *Biology* **2022**, *11*, 1292. https://doi.org/10.3390/biology11091292.
70. Wickner, R.B.; Hayashi, Y.; Edskes, H.K. Anti-prion systems in *Saccharomyces cerevisiae*. *J. Neurochem.* **2025**, *169*, e70045. https://doi.org/10.1111/jnc.70045.
71. Shorter, J.; Southworth, D.R. Spiraling in control: Structures and mechanisms of the Hsp104 disaggregase. *Cold Spring Harb. Perspect. Biol.* **2019**, *11*, a034033. https://doi.org/10.1101/cshperspect.a034033.
72. Kushnirov, V.V.; Dergalev, A.A.; Alexandrov, A.I. Amyloid fragmentation and disaggregation in yeast and animals. *Biomolecules* **2021**, *11*, 1884. https://doi.org/10.3390/biom11121884.
73. Wyszkowski, H.; Janta, A.; Sztangierska, W.; et al. Class-specific interactions between Sis1 J-domain protein and Hsp70 chaperone potentiate disaggregation of misfolded proteins. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2108163118. https://doi.org/10.1073/pnas.2108163118.
74. Stanford, K.E.; Zhao, X.; Kim, N.; et al. Overexpression of Hsp104 by causing dissolution of the prion seeds cures the yeast [*PSI*$^{+}$] prion. *Int. J. Mol. Sci.* **2023**, *24*, 10833. https://doi.org/10.3390/ijms241310833.
75. Nillegoda, N.B.; Bukau, B. Metazoan Hsp70-based protein disaggregases: Emergence and mechanisms. *Front. Mol. Biosci.* **2015**, *2*, 57. https://doi.org/10.3389/fmolb.2015.00057.
76. Kampinga, H.H.; Hageman, J.; Vos, M.J.; et al. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* **2009**, *14*, 105–111. https://doi.org/10.1007/s12192-008-0068-7.
77. Gao, X.; Carroni, M.; Nussbaum-Krammer, C.; et al. Human Hsp70 disaggregase reverses Parkinson's-linked α-synuclein amyloid fibrils. *Mol. Cell* **2015**, *59*, 781–793. https://doi.org/10.1016/j.molcel.2015.07.012.
78. Wentink, A.S.; Nillegoda, N.B.; Feufel, J.; et al. Molecular dissection of amyloid disaggregation by human HSP70. *Nature* **2020**, *587*, 483–488. https://doi.org/10.1038/s41586-020-2904-6.

79. Ayala Mariscal, S.M.; Pigazzini, M.L.; Richter, Y.; et al. Identification of a HTT-specific binding motif in DNAJB1 essential for suppression and disaggregation of HTT. *Nat. Commun.* **2022**, *13*, 4692. https://doi.org/10.1038/s41467-022-32370-5.
80. Faust, O.; Abayev-Avraham, M.; Wentink, A.S.; et al. HSP40 proteins use class-specific regulation to drive HSP70 functional diversity. *Nature* **2020**, *587*, 489–494. https://doi.org/10.1038/s41586-020-2906-4.
81. Irwin, R.; Faust, O.; Petrovic, I.; et al. Hsp40s play complementary roles in the prevention of tau amyloid formation. *Elife* **2021**, *10*, e69601. https://doi.org/10.7554/eLife.69601.
82. De Los Rios, P.; Ben-Zvi, A.; Slutsky, O.; et al. Hsp70 chaperones accelerate protein translocation and the unfolding of stable protein aggregates by entropic pulling. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 6166–6171. https://doi.org/10.1073/pnas.0510496103.
83. Goloubinoff, P.; De Los Rios, P. The mechanism of Hsp70 chaperones: (entropic) Pulling the models together. *Trends Biochem. Sci.* **2007**, *32*, 372–380. https://doi.org/10.1016/j.tibs.2007.06.008.
84. Schneider, M.M.; Gautam, S.; Herling, T.W.; et al. The Hsc70 disaggregation machinery removes monomer units directly from α-synuclein fibril ends. *Nat. Commun.* **2021**, *12*, 5999. https://doi.org/10.1038/s41467-021-25966-w.
85. Vilar, J.M.G.; Rubi, J.M.; Saiz, L. Chaperone-driven entropic separation of amyloid nanofilament bundles. *Phys. Rev. X* **2025**, *15*, 011041. https://doi.org/10.1103/PhysRevX.15.011041.
86. Wentink, A.; Rosenzweig, R. Protein disaggregation machineries in the human cytosol. *Curr. Opin. Struct. Biol.* **2023**, *83*, 102735. https://doi.org/10.1016/j.sbi.2023.102735.
87. Rebeaud, M.E.; Fauvet, B.; De Los Rios, P.; et al. Hsp110 nucleotide exchange factors may amplify Hsp70-disaggregation by enhanced entropic pulling. *J. Biol. Chem.* **2025**, *301*, 110450. https://doi.org/10.1016/j.jbc.2025.110450.
88. Martín-Aparicio, E.; Yamamoto, A.; Hernández, F.; et al. Proteasomal-dependent aggregate reversal and absence of cell death in a conditional mouse model of Huntington's disease. *J. Neurosci.* **2001**, *21*, 8772–8781. https://doi.org/10.1523/jneurosci.21-22-08772.2001.
89. Yamamoto, A.; Lucas, J.J.; Hen, R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* **2000**, *101*, 57–66. https://doi.org/10.1016/s0092-8674(00)80623-6.
90. DeVos, S.L.; Miller, R.L.; Schoch, K.M.; et al. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. *Sci. Transl. Med.* **2017**, *9*, eaag0481. https://doi.org/10.1126/scitranslmed.aag0481.
91. Sydow, A.; Van der Jeugd, A.; Zheng, F.; et al. Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic Tau mutant. *J. Neurosci.* **2011**, *31*, 2511–2525. https://doi.org/10.1523/jneurosci.5245-10.2011.
92. Van der Jeugd, A.; Hochgräfe, K.; Ahmed, T.; et al. Cognitive defects are reversible in inducible mice expressing pro-aggregant full-length human Tau. *Acta. Neuropathol.* **2012**, *123*, 787–805. https://doi.org/10.1007/s00401-012-0987-3.
93. Alam, P.; Bousset, L.; Melki, R.; et al. α-Synuclein oligomers and fibrils: A spectrum of species, a spectrum of toxicities. *J. Neurochem* **2019**, *150*, 522–534. https://doi.org/10.1111/jnc.14808.
94. Selkoe, D.J.; Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* **2016**, *8*, 595–608. https://doi.org/10.15252/emmm.201606210.
95. Haass, C.; Selkoe, D.J. Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid β-peptide. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101–112. https://doi.org/10.1038/nrm2101.
96. Tittelmeier, J.; Sandhof, C.A.; Ries, H.M.; et al. The HSP110/HSP70 disaggregation system generates spreading-competent toxic α-synuclein species. *EMBO J.* **2020**, *39*, e103954. https://doi.org/10.15252/embj.2019103954.
97. Bard, J.A.M.; Goodall, E.A.; Greene, E.R.; et al. Structure and Function of the 26S Proteasome. *Annu. Rev. Biochem.* **2018**, *87*, 697–724. https://doi.org/10.1146/annurev-biochem-062917-011931.
98. Hjerpe, R.; Bett, J.S.; Keuss, M.J.; et al. UBQLN2 mediates autophagy-independent protein aggregate clearance by the proteasome. *Cell* **2016**, *166*, 935–949. https://doi.org/10.1016/j.cell.2016.07.001.
99. Gatica, D.; Lahiri, V.; Klionsky, D.J. Cargo recognition and degradation by selective autophagy. *Nat. Cell Biol.* **2018**, *20*, 233–242. https://doi.org/10.1038/s41556-018-0037-z.
100. Lamark, T.; Johansen, T. Mechanisms of selective autophagy. *Annu. Rev. Cell Dev. Biol.* **2021**, *37*, 143–169. https://doi.org/10.1146/annurev-cellbio-120219-035530.
101. Ma, X.; Lu, C.; Chen, Y.; et al. CCT2 is an aggrephagy receptor for clearance of solid protein aggregates. *Cell* **2022**, *185*, 1325–1345.e22. https://doi.org/10.1016/j.cell.2022.03.005.
102. Mauthe, M.; Kampinga, H.H.; Hipp, M.S.; et al. Digest it all: The lysosomal turnover of cytoplasmic aggregates. *Trends Biochem. Sci.* **2023**, *48*, 216–228. https://doi.org/10.1016/j.tibs.2022.09.012.
103. Mauthe, M.; van de Beek, N.; Mari, M.; et al. A chaperone-proteasome-based fragmentation machinery is essential for aggrephagy. *Nat. Cell Biol.* **2025**, *27*, 1448–1464. https://doi.org/10.1038/s41556-025-01747-1.