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REVIEW ARTICLE

Conformational Dynamics of Hsp90 and Hsp70 Chaperones in Treating Neurodegenerative Diseases: Insights from the *Drosophila* Model

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Abstract
Protein aggregates of misfolded proteins are a pathological hallmark of nearly all neurological disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and various polyglutamine diseases such as Huntington's disease. Selective distribution in different cellular compartments highlights their core functions in cellular homeostasis. Investigating the cellular protein quality control system has become a significant strategy for counteracting protein aggregates and their toxic consequences. Heat shock proteins (Hsps) are crucial in regulating protein quality control, contributing to both protein aggregation and disaggregation. Beyond their well-known role in oncogenesis, several studies have identified Hsp90 as a key regulator of the functional stability of neuronal proteins. Similarly, Hsp70 is believed to promote cell survival by interacting with components of apoptotic and pro-survival pathways in neurodegeneration. Thus, targeting Hsp90 and Hsp70 represents a promising therapeutic strategy for treating neurodegenerative disorders. This review provides a comprehensive overview of the structure, mode of action, and roles of Hsp90 and Hsp70. Additionally, *Drosophila melanogaster* is highlighted as an effective model system for studying the roles of Hsp70 and Hsp90 in the proteinopathies associated with neurodegenerative diseases.

Keywords Co-chaperones, *Drosophila* · Heat shock proteins · Hsp70, neurodegenerative disease · Hsp90

Introduction

Protein homeostasis (proteostasis)—the equilibrium of protein synthesis, folding, trafficking, assembly, and degradation—is critical for proper cellular function and cells have devised various strategies to maintain it during times of stress. Proteostasis dysfunction develops with age and is linked to several human diseases. Proteostasis relies heavily on molecular chaperones. Hsp70 proteins are key components of the molecular chaperon. Heat shock protein 70 (Hsp70), in particular, plays a vital role in protein folding, disaggregation, and degradation (Zuiderweg et al. 2017). The Hsp70 family is the most well-known and conservative group of HSPs. The prokaryotic system has a single Hsp70 (the archetypal DnaK), whereas eukaryotes have a greater number of genes that code for different Hsp70 isoforms (Fernández-Fernández et al. 2017).

They are found in different structure forms, Isoform stages are managed in reaction to cell needs (for example, boom and tissue-precise needs). Organisms express Hsp70 isoforms in a variety of ways, with expression and localization varying greatly. Humans have a constitutive expression of heat shock cognate (Hsc70), whereas stress causes Hsp70 levels to rise. Hsc70 folds newly synthesized proteins, which is an important cell function, which explains the differences in expression (Evans et al. 2010). Hsp70s are distinguished by three primary features: first, variations in substrate reputation and allosteric regulation; second, variations imposed by way of target site localization, which includes Hsp70s binding at ribosomal exit tunnels and membrane translocons wherein nascent polypeptides emerge; and third, the type of cooperation with the large community of J-protein co-chaperones (Bracher and Verghese 2015). Cooperation with different cell chaperone systems, consisting of small warmth surprise proteins, chaperonins (bacterial and mitochondrial

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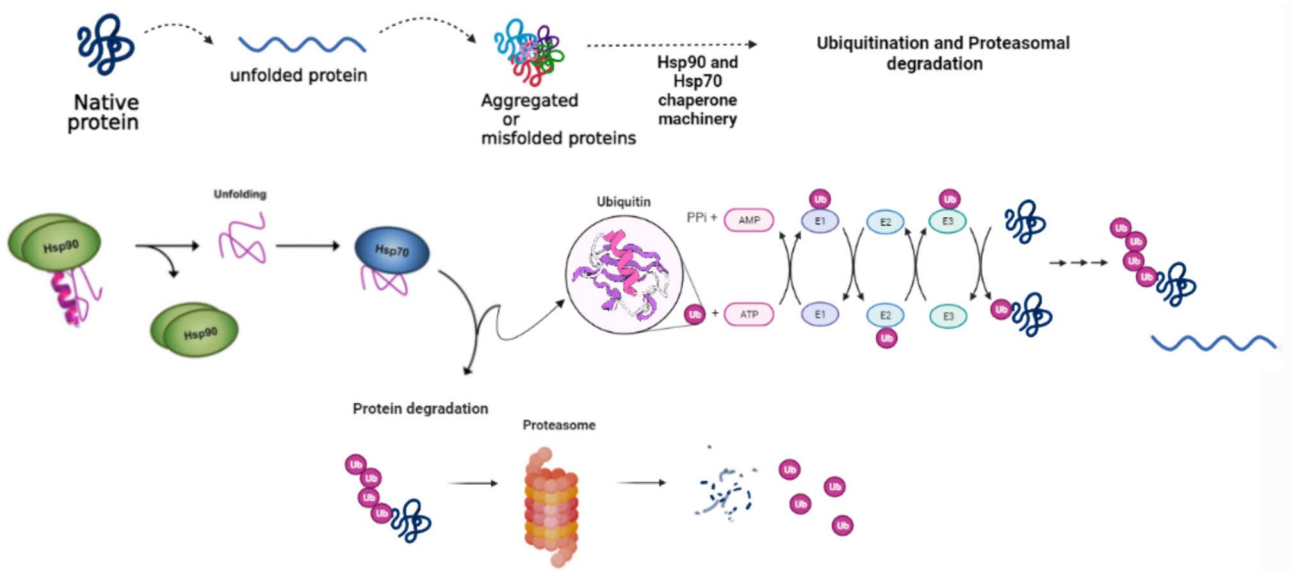

Fig. 1 Schematic diagram showing the Hsp90/ Hsp70 chaperone machinery in the proteasome degradation of misfolded and aggregated proteins

GroEL– GroES, eukaryotic Hsp90, and Hsp100, and protein degradation systems), will increase the useful range of Hsp70 participants.

The shape and allosteric pathway of the Hsp70 chaperones, their mechanism for interacting with substrates, and their affiliation with the mechanical elements of the Hsp70 community were described in this review. The Hsp70's structural traits as (J-domain Proteins) JDPs and Nucleotide Exchange Factors (NEFs) were spotlighted, in addition, to describing new concepts of Hsp70 community law via way of means of those co-chaperones and different molecular partners.

In eukaryotic cells, Hsp90 is widely abundant, contributes to 1 to 2% of total cellular proteins in the body, and is known to be responsible for their appropriate maturation, activation, and degradation (Taipale et al. 2010). Hsp90 interacts with different substrate proteins in the cell, generally termed clients. In various cellular pathways, many of these proteins are involved the regulations of protein folding and degradation, cell growth, chromatin remodeling, cellular trafficking, and differentiation (Vasilaki and Jackson 2013). Three domains are structurally included in Hsp90, namely the N-terminal ATP-binding domain, the middle domain as well as the C-terminal dimerization domain. The Hsp90 dimer forms conformational modifications, and interplay with co-chaperones and ATP stabilizing different conformations which is necessary for the client proteins to be properly folded (Schopf et al. 2017). On the other hand, Hsp90 and its associated secreted forms lead to the growth and progression of severe pathologies such as cancer and neurodegenerative diseases.

The onset of neurodegenerative disorders, characterized by protein aggregation, often involves the inhibition of Hsp90, which activates heat shock factor-1 (HSF-1). This activation, in turn, boosts the activity of Hsp70, Hsp40, and other chaperones, facilitating the disaggregation and degradation of proteins using proteasome process (Pratt et al., 2015; Rai 2023)(Fig.1). However, recent research suggests a dual role for Hsp90 in neurodegeneration: while it aids in maintaining the functional integrity of aberrant neuronal proteins, it also contributes to the aggregation of toxic aggregates. *Drosophila melanogaster* has served as a pivotal genetic model system for over a century due to its rapid reproduction and minimal maintenance requirements. Its evolutionary homology with primates makes it an invaluable tool for studying the cellular and molecular processes underlying development and disease. The dynamic nervous system of *Drosophila*, mirroring human systems, coupled with its vast collection of mutants affecting neural development, further enhances its utility as a model organism.

The pioneering investigation of the heat shock response in *Drosophila* has solidified its status as an ideal model for such studies, free from ethical concerns. This response triggers transcriptional activity and the production of new proteins in various tissues following heat shock. The transcription of Heat shock proteins (Hsps) is predominantly regulated by HSF, binding to the promoter of downstream target genes during stress conditions. Inhibition of the heat shock response leads to the accumulation of misfolded proteins, a primary driver of neurological disorders. Through both in vivo and in vitro studies, researchers aim to unravel the mechanisms underlying protein misfolding and develop therapeutic strategies to combat disease progression. This

review provides a comprehensive overview of the structure, mode of action, and roles of Hsps in neurodegenerative diseases, along with potential interventions to mitigate their effects (Fig. 1 (adapted from Pratt et al., 2015)

Hsp70 domain organization and structure

All members of the Hsp70 family have strongly conserved sequences and structures. An N-terminal nucleotide-binding domain (NBD) and its average relative molecular mass is 45kDa, followed by another 15-kDa substrate-binding domain (SBD), a helical lid domain, and a C terminal have disorder sequences. The interdomain linker may be a short liner that connects these two functional domains. The NBD is formed from two significant lobes, I and II. There is a deep cleft between the 2 lobes where the nucleotide binds. Each lobe is further subdivided into A and B subdomains. ATP coordinates all four subdomains within the hydrolysis center at the rock bottom of this deep cleft, regulating lobe movements (Evans et al. 2010).

SBD features a distinct fold, consisting of a twisted-sandwich domain (SBD), a -helical lid domain, and a C-terminal unstructured region of an unknown feature. The polypeptide-binding cleft with assets designed for one hydrophobic amino alkanoic acid side chain is found within the SBD. Two pairs of -strands surround a scissors-like opening within the cleft, and two concentric sets of upward protruding loops enclose the peptide backbone (Wang et al. 2022; Rosenzweig et al. 2019; Kityk et al. 2012, 2018).

The high affinity to the peptide substrate and low rate of association and dissociation of the substrate are mirrored in the structure of the isolated SBD. This domain has properties in the absence of nucleotides and when bound to ADP. NMR studies have revealed that in the ADP-bound state, NBD and SBD oscillate profoundly independently of one another, with only highly conservative flexible joints connecting them (Kityk et al., 2012; Alvira et al., 2014) (Fig 2).

The binding of ATP and NBD, on the other hand, causes a major rearrangement of the entire structure, increasing the substrate's association and dissociation rates by 100 and 1000 times, respectively, and decreasing its affinity for peptides ten to fifty times These dramatic structural rearrangements can be seen in the ATP-linked open conformation of *E. coli* Hsp70 DnaK. SBD and SBD are linked on both sides of NBD and are isolated from each other (Evans et al. 2010; Parkhitko et al. 2019).

Conformation changes of Hsp70

It was difficult to get full-length Hsp70 in an ATP-bound state. (a) The ATP-bound state is short-lived due to constant ATP hydrolysis; (b) the interaction between the two domains is too transient to be captured by structural analysis; and (c) purified Hsp70 proteins tend to form low-ordered, flexible oligomers, which is incompatible with structural analysis (Hirth 2010; Gupta et al. 2020; Zuehlke and Johnson 2010). Furthermore, none of the nonhydrolyzable ATP analogs that have been tested so far have been able to induce allosteric coupling in Hsp70s in the same way that ATP does. The key is to maintain a functional ATP-bound state that is compatible with structural analysis.

The NBD of Hsp70 has vulnerable ATPase activity and the binding/hydrolysis of ATP to ADP lets for allosteric law of the interdomain conformation and substrate binding of the SBD. Hsp70 has a low affinity for substrate when bound to ATP, but it binds and releases quickly. After ATP hydrolysis to ADP, the substrate affinity will dramatically increase, and the affiliation and dissociation prices additionally decrease. Cochaperones namely DnaJ (HSP40) and GrpE, where DnaJ contains a J domain at its N terminal which is represented by the HPD (Histidine-proline-aspartate) motif and form J domain proteins and nucleotide exchange factors (NEFs) help Hsp70 for their functions. The binding of Jdomain proteins induces ATP hydrolysis, which allows the exposed hydrophobic region of substrates to be trapped and the SBD to be closed. NEFs participate in an ATP– ADP exchange through the catalyst of Hsp70 releasing ADP, which results in the conversion of Hsp70 open conformation and release of substrates (Gupta et al. 2020; Zuehlke and Johnson 2010).

Hsp90 domain organization and structure

The identification of heat shock proteins culminated in the heat shock reaction being unraveled which is also termed a cellular stress response. This reaction is a typical physiological mechanism in nearly all living organisms and involves improvements in the profile of gene expression through the upregulation of Hsp (Lindquist 1986). To facilitate cell survival and shield cellular proteins from the possibility of disruption or aggregation, the over-expression of HSPs has been identified. A host of mechanisms raise the expression of Hsps, namely environmental and chemical causes, physiological causes of non-stress and disease states, concerning heat shock (Zhang et al. 2015; Pirkkala et al. 2001).

Three key retained domains form the overall molecular configuration of Hsp90 homologs namely, the N-terminal domain (NTD), the C-terminal domain (CTD), and the

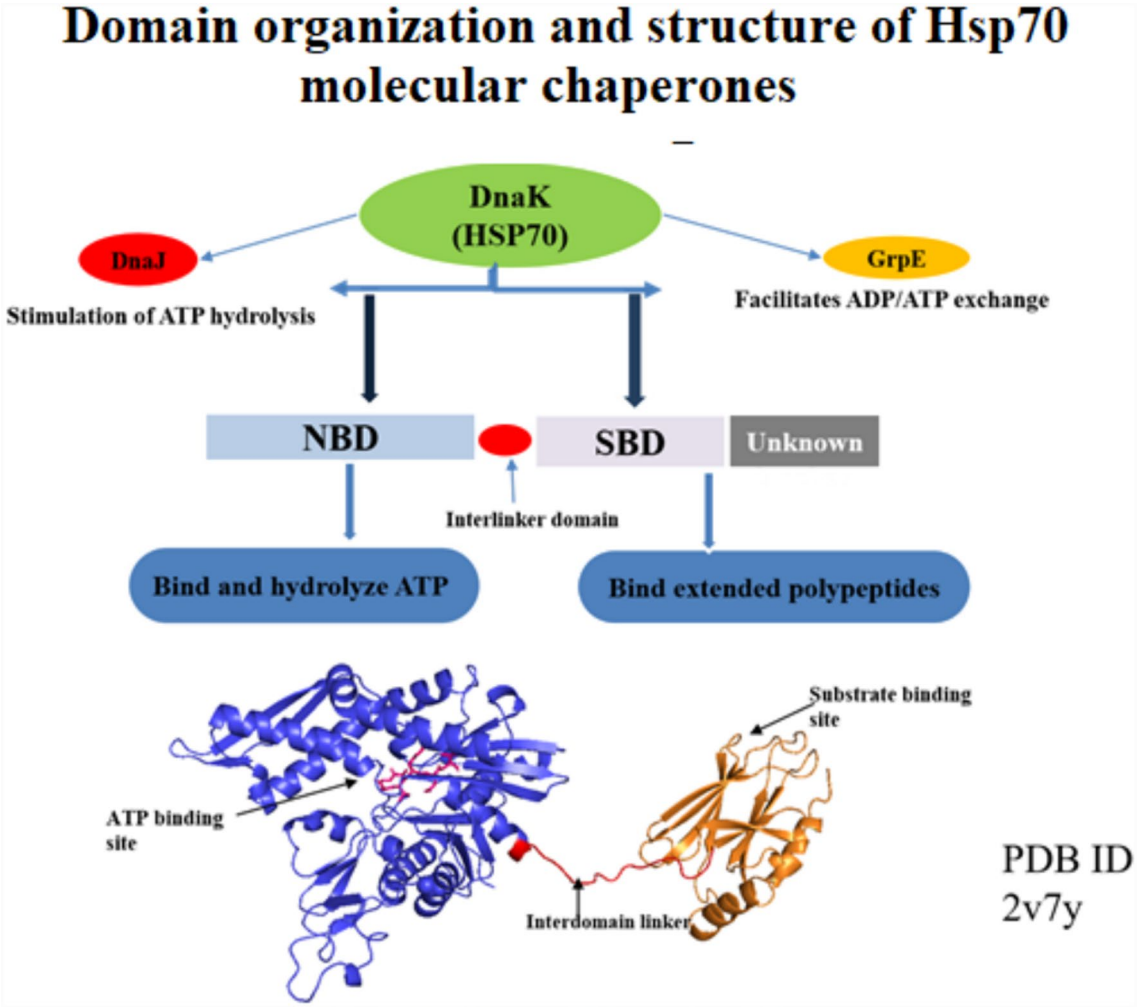

Fig. 2 Represents the structural and domain organization of Hsp70 molecular chaperone: shows the ATP binding site of Hsp70 to its substrates through the interdomain linker (Data Sources: PDB ID 2v7y)

middle domain (MD) (Sreedhar et al. 2004). There is a vector charger linker domain for eukaryotes that connects the NTD to the MD (Yamagishi et al. 2012). A particular role is performed by each domain inside the Hsp90 structure. The NTD binds to the ATP, hence it is termed a nucleotide-binding site. Based on the Hsp90 isoform and its cellular site either in the cytoplasm or the ER, the CTD is accountable for protein dimerization (illustrated in Table 1).

The involvement of ATPase activity and the switch between the closed and open states is the core concept by which Hsp90 acts (Urban et al. 2012). HSP90 appears as a flexible homodimer and its monomers comprise three structural domains, namely NTD, MD, and CTD. ATP binding takes place in the ATP binding site in the Hsp90 NTD region, culminating in a sequence of confirmative occurrences. A shorter portion of the N-domain is termed an ATP lid, which facilitates the transport over the binding pocket and binds to the corresponding N-domain of the other homodimer, eventually ending in a twisted and compacted dimer (Aghdassi et al. 2007). As a result, N- and M-domains become closer, progressing to the completion of the "split ATPase" site. The N domains of the Hsp90 homodimers disentangle with ADP and Pi release following hydrolysis of ATP, while the Hsp90 recovers to its initial open conformation state. Involving a dynamic mechanism termed the Hsp90 chaperone loop, members of the Hsp90 family execute their folding role of client proteins.

Earlier reviews have shown how Hsp90 mediates the folding of its client proteins, but there is still no full explanation of the protein folding cycle. The Hsp90 chaperone framework involves the coordination of various molecules, including co-chaperones, partner proteins, and immunophilins, which function precisely and dynamically to support the Hsp90 fold efficiently.

Table 1 Key features of Hsp90 domains

Domain Name	Characteristics	References
N-Terminal domain (NTD)	• Contains an ATP binding motif that possesses similarity with the GHKL (Gyrase, Hsp90, histidine kinase, and MutL) superfamily and also differentiates from the corresponding motifs of Hsp70 or protein kinases • Employed as a major target for Hsp90 inhibitors such as geldanamycin and radicicol	(Panaretou et al. 1998)
Charged linker region (CR)	• Links the N = terminal domain to the middle domain (MD) • CR domain is highly charged and has variable length as well as amino acid composition • Charged linker region has been known to enhance the resilience and dynamicity to deal with the eukaryotic cells' populated setting	(Noritake et al. 2009)
Middle domain (MD)	• By binding the γ-phosphate of the ATP identified for the NTD, it activates the Hsp90 function, thus altering its ATPase activity • In addition, multiple experiments have shown that this domain is involved in binding co-chaperones such as Aha1 and engaging with protein molecules	(Diao et al. 2005)
C-Terminal domain (CTD)	• Two major sites include one for calmodulin binding and the other for homodimerization with Hsp90. Additionally, it includes a nucleotide-binding site that works after N-terminal site occupation and acts as the N-terminal ATPase activity allosteric regulator	(Rajagopalan et al. 1996)

Conformational changes in the Hsp70 and Hsp90 with the interaction of client proteins

The NBD of Hsp70 controls the interaction with the client protein and its SBD identifies the hydrophobic regions in the client during the initial stages of its folding. These two domains are connected by a flexible linker which exhibits low ATPase activity when it is not bound to a client protein (Gupta et al. 2020). Thus, to stimulate its ATPase activity, a co-chaperon, J domain protein family passages client protein to Hsp70. After the leaving of J protein from this complex, Hsp70 is taken to its apo-form by a nucleotide-exchange factor releasing ADP from it. This conformational change makes the NBD free to engross ATP, leading the α-helical lid to "open" and releasing client protein in a mature form. This cycle continues until the client attains the native conformation.

Hsp40, belongs to a main co-chaperone of J domain protein family that works with the Hsp70 and helps in the recruitment of client protein to Hsp70 followed by transfer of client proteins to Hsp90 with the association of another co-chaperone STI1 (also called HOP or HSP-organizing protein in humans). Thereby, Hsp90 reconfigures abnormally folded proteins to their normal states through its ATP hydrolysis and structural rearrangement (Gupta et al. 2020; Zuehlke and Johnson 2010).

The conformational changes in Hsp70 and Hsp90 upon interaction with client proteins are crucial for their chaperone activity. Here's how these changes mechanistically occur:

Hsp70 Conformational Changes:

a. ADP-Bound State: In the resting state, Hsp70 binds ADP and adopts a low-affinity conformation, with its SBD in an open, accessible configuration.
b. Client Binding: Upon interaction with a client protein, Hsp70 undergoes a conformational change triggered by ATP hydrolysis. ATP binding leads to closure of the SBD, trapping the client protein in a hydrophobic pocket.
c. ATP Hydrolysis**:** ATP hydrolysis to ADP induces further conformational changes, weakening the affinity of Hsp70 for the client protein.
d. Nucleotide Exchange: The exchange of ADP for ATP, facilitated by factors NEFs, promotes the release of the client protein.

Hsp90 Conformational Changes:

a. N-Terminal ATP-Binding Domain: Hsp90 consists of an N-terminal ATP-binding domain (NBD), a middle domain, and a C-terminal dimerization domain.
b. ATP Binding: ATP binding to the NBD induces dimerization of Hsp90, resulting in closed conformation.
c. Client Binding: Client proteins interact with the closed conformation of Hsp90, stabilizing the ATP-bound state.
d. ATP Hydrolysis: ATP hydrolysis leads to opening of the NBD, destabilizing the client protein, and promoting its maturation or degradation.
e. C-Terminal Domain Interaction: The C-terminal domain of Hsp90 modulates the ATPase activity and client protein interactions of the NBD, influencing the chaperone cycle.

f. Co-chaperone Regulation: Co-chaperones, such as J-proteins and immunophilins, regulate the ATPase activity and conformational dynamics of Hsp70 and Hsp90.

These co-chaperones modulate the chaperone cycle by promoting ATP hydrolysis, assisting in client protein binding and release, and coordinating the action of Hsp70 and Hsp90. In summary, the conformational changes in Hsp70 and Hsp90, driven by nucleotide binding and hydrolysis, are essential for their interaction with client proteins. These changes regulate client protein binding, folding, and release, ultimately determining the fate of the client protein in terms of maturation, degradation, or refolding.

Protein–Protein interaction of Hsp90 and Hsp70 in *Drosophila*

In *Drosophila melanogaster*, the Hsp90 and Hsp70 machinery plays a crucial role in refolding misfolded proteins. Here's how it generally works under mechanism:

Recognition of Misfolded Proteins: When proteins misfold due to various stressors or genetic mutations, they expose hydrophobic regions that are typically buried in their native structure. Chaperones like Hsp70 recognize these exposed hydrophobic patches and bind to the misfolded proteins, preventing them from aggregating further.

Hsp70-Mediated Folding Assistance: Once bound to misfolded proteins, Hsp70 uses ATP hydrolysis to undergo conformational changes, resulting in the transient opening of its substrate-binding domain. This allows the misfolded protein to be captured and guided along a folding trajectory.

Hsp90 Stabilization and Maturation: The Hsp70-bound misfolded proteins may then interact with Hsp90. Hsp90 stabilizes partially folded intermediates, preventing their aggregation and allowing them to reach their native state. Hsp90 also facilitates the maturation of client proteins by providing a scaffold for the assembly of multiprotein complexes involved in various cellular processes.

Cooperation with Co-chaperones: Throughout the refolding process, Hsp70 and Hsp90 cooperate with a variety of co-chaperones, including J-proteins and nucleotide exchange factors (NEFs). These co-chaperones regulate the ATPase activity of Hsp70 and Hsp90, modulate their substrate specificity, and assist in the transfer of client proteins between different chaperone systems.

Protein Disaggregation and Degradation: In cases where misfolded proteins cannot be refolded, the Hsp70/Hsp90 machinery may facilitate their disaggregation, allowing them to re-enter the folding pathway or be targeted for degradation by the proteasome or autophagy pathways. Overall, the concerted action of Hsp70 and Hsp90, along with their co-chaperones, enables the efficient refolding of misfolded proteins in *Drosophila melanogaster*, thereby maintaining proteostasis and cellular homeostasis.

Two chaperones Hsp90 and Hsp70 were discovered to interact directly (Kravats et al., 2017) (Fig. 3). Studies looking into the mechanism of collaboration between Hsp90, and Hsp70 Pulldown assays and BioLayer Interferometry are two examples. Hsp90 binds to the DnaK NBD protein. Using molecular docking, the complex of Hsp90 and Hsp70 was discovered. Swiss model was used to create the atomic resolution model of *Drosophila melanogaster* Hsp70 using the best template protein sequence of Bovin (80.51%) sequence identity, PDB ID 2qw9, and 3-D structure model quality was validated by Ramachandran (RC) plot. RCSB tool was used to download the structure of Hsp90 (PDB I is 3) and performed docking of Hsp70 and Hsp90 using Patchdock and for the visualization and rotation of the model, PyMol was used.

Role of Hsps in neurodegenerative diseases of *Drosophila*

Neurodegenerative diseases are progressive disorders that affect specific cellular populations in the central and peripheral nervous systems. The genes associated with sporadic and familial cases have been identified and thus empowering the development of animal models. Invertebrates such as *Drosophila* acts as an excellent in vivo system to understand the cellular mechanisms of neurodegeneration using the genetic studies and for the testing of therapeutic drugs in the treatment of devastating degenerative diseases. In addition, the internal cellular architecture of the *Drosophila* tissue has been extremely well characterized, and many imaging techniques and antibody reagents are accessible to probe the underlying defects associated with a particular degenerative phenotype (Bonini and Fortini 2003). The aggregations of misfolded proteins and dysfunctional organelles such as mitochondria, Endoplasmic reticulum which exacerbates the major pathological feature of various neurological disorder including Parkinson's disease (PD), Huntington's disease (HD), Amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD). The dysfunctional mitochondria constitute a major cytotoxic feature of PD caused the loss of dopaminergic neuron, wherein the aggregations of Hsp70 expression observed in the larval brain of $park^{13/+}$mutant flies (Rai and Roy 2022). A Geldanamycin (GM) derivative, 17-AAG was also selective in other poly-Q diseases against neurodegeneration. Notably, in a *Drosophila* model of spinocerebellar ataxia (SCA), it resisted compound eye degeneration and body inclusion development as well as rescued lethality. Suppression of neurodegeneration in the HD fly model was also noted. In the *Drosophila* models, the knockdown of

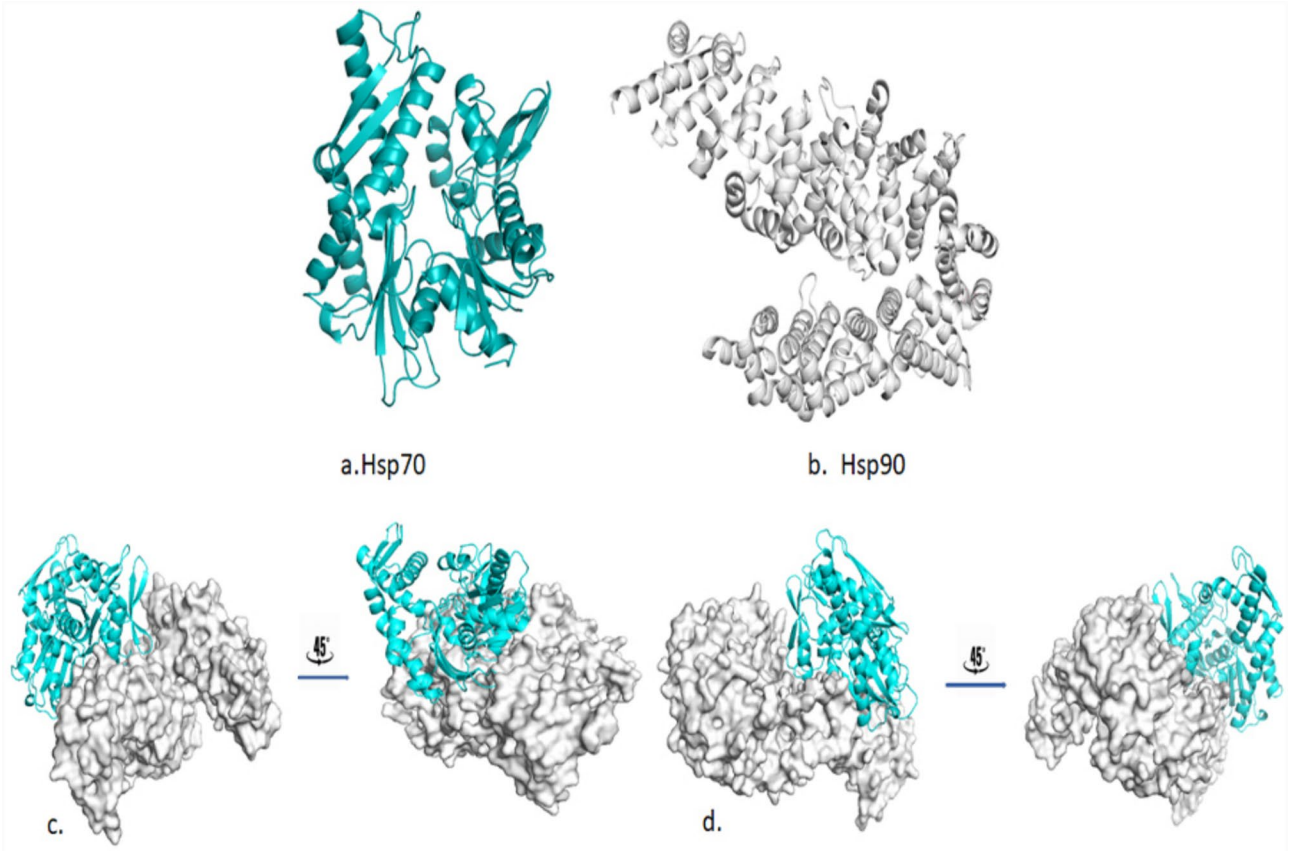

Fig. 3 **a** and **b** The model of the Hsp90 full-length dimer in the apo conformation was built from PDB code 3 now and HSP70 PDB code 2qw9. **c**. and **d**. showing the docked surface-rendered model of the apo structure of Hsp90 and a cartoon model of DnaK

HSF-1 prevented the activation of molecular chaperones and the clinical efficacy of 17-AAG on polyQ-induced neurodegeneration, implying that the therapeutic efficacy of 17-AAG was predominantly mediated by HSF1 (Fujikake et al. 2008).

In majority of the disease case, an RNA binding protein called transactive DNA binding protein-43 (TDP-43) normally being found in the nuclear region, speciously localize in the cytoplasm of neurons and glial cells forming aggregates and caused the reason of neuronal cell death. In AD, the most common type of dementia, mainly afflicting aging population and this disease is also characterized by the accumulation of misfolded and truncated proteins, Amyloid β and Tau in the brain. As foresaid accumulation of misfolded proteins is a leading cause of neurodegeneration. Herein, Hsps mediated the clearance and refolding of these misfolded proteins and can restore normal cellular functions using the Hsp90/Hsp70 chaperone machinery system (Jarrett and Lansbury 1993; Soto et al. 2006; Minoia et al. 2014).

The explanation for neurodegenerative diseases correlated with protein aggregation is that Hsp90 inhibition stimulates HSF-1 to stimulate the development of Hsp70 and Hsp40, and chaperones, which in turn facilitate disaggregation and degradation of associated proteins (Lu et al. 2007). In addition, Hsp90 retains the functional integrity of anomalous capacity neuronal proteins, thereby facilitating and preserving the aggregation of toxic concretes (Zou et al. 1998). A list of co-chaperones of Hsp90 involved in various neurodegenerative diseases is enumerated in Table 2.

Subjected to stress circumstances, cells typically adapt with heat shock response (HSR) activation, followed by intensified synthesis of a variety of cytoprotective Hsps that alleviate cytotoxicity, such as those induced by misfolded and denatured proteins. HSF-1's role is controlled by Hsp90 (Zou et al. 1998). Namely, Hsp90 binds to HSF-1 under non-stressed conditions and retains the transcription factor in a monomeric state. HSF-1 is released from the Hsp90 complex by stress, heat shock, or inhibition of Hsp90, resulting in its trimerization, activation, and translocation to the nucleus where it activates a heat shock response, manifesting in the development of Hsps such as Hsp70 and Hsp40 (Muchowski and Wacker 2005). It has been reported elsewhere that Hsp90 inhibition releases HSF-1 from the Hsp90 complex culminating in resulting Hsps generation and the use of Hsp70 by Hsp90 inhibitors is well known for neurodegenerative disease models (Neckers et al. 1999). An inhibitor of Hsp90 referred to as

Table 2 Co-chaperones of HSP90 involved in various neurodegenerative diseases

Co-chaperone name	Functions	Neurodegenerative disease
USP19	Cytoplasmic Ubiquitin-Specific Protease 19 (USP19) modulates aggregation of Polyglutamine-Expanded Ataxin-3 and Huntingtin through the HSP90 Chaperone (He et al. 2016)	HD
Aha1	The Hsp90 co-chaperone, ATPase homolog 1 (Aha1), causes the aggregations of tau reported in mouse model (Shelton et al. 2017)	AD
Cdc37	CDC37 is a molecular co-chaperone that functions with Hsp90 to promote folding of kinases including Pink1, a mitochondrial associated protein in case of PD (Rakovic et al. 2011) and Cdc37 destabilizes tau and leads to its clearance in case of AD (Bohush et al. 2019a)	AD PD
CHP-1	CHP-1 (CHORD containing protein-1) as a regulator of centrosome duplication and as a protein involved in the maintenance of genomic stability with the involvement of Hsp90 reported in *Drosophila* and mouse (Bohush et al. 2019b; Ferretti et al. 2010)	PD
CacyBP/SIP	Calcyclin binding protein and Siah-1 interacting protein have an important role in microtubules organization and interacts with the Tau protein, a pathological biomarker of AD (Wasik et al. 2013)	AD

GM has been known to induce a dose-dependent rise of Hsp70 in the AD cellular model and the primary cortical neurons of rats, and decreased the quantity of insoluble tau and the baseline amounts of tau phosphorylation stimulated by okadaic acid. Treatment with the purine-scaffold Hsp90 inhibitor called PU24FCl in primary cortical neurons also resulted in a dose-dependent rise in Hsp70 (Luo et al. 2010).

Neurodegenerative disorders are often represented by misfolded proteins acquiring toxic functions. In these cases, toxicity can arise from a disparity between the usual ability of chaperones and the development of harmful protein species. An elevated chaperone expression, indicating potential therapeutic interventions, can counteract the neurotoxicity of these molecules (Lu et al. 2007). Increased Hsp70 levels have been demonstrated to facilitate tau solubility and tau binding to microtubules in multiple model systems of Alzheimer's disease (AD) (Dou et al. 2003). The tendency of Aβ to aggregate was also blocked by Hsp70 and thereby resulted in decreasing the toxicity of Aβ in neuronal cultures (Evans et al. 2010). It has been reported elsewhere that inhibition of Hsp90 leads to the further expression of Hsps such as Hsp70. For neurodegenerative disease models, inhibition of Hsp90 results in the release of HSF-1 from the Hsp90 complex culminating in resultant Hsps generation and thereby leading to activation of Hsp70. An inhibitor of Hsp90 termed GM, triggered a dose-dependent raise of Hsp70 in the AD cell model and the primary cortical neurons of rats, and decreased the amount of insoluble tau and the baseline amounts of tau phosphorylation triggered by okadaic acid (Neckers et al. 1999). Another group of researchers reported that KU32, an Hsp90 inhibitor of a diverse chemical type, mediated and protected Hsp70 from Aβ-driven toxicity in SH-SY5Y neuroblastoma cultures (Luo et al. 2008). In multiple in vitro and in vivo models of neurodegenerative disease, HSF-1 stimulation by Hsp90 inhibitors was documented, indicating Hsp90 inhibition as a way of modulating Hsp levels in the diseased brain, to defend against the toxic proteins that occur during the neurodegenerative process.

Recent data indicates an appropriate role for Hsp90 in preserving the functional integrity of anomalous capability neuronal proteins, in addition to the regulation of HSF-1. The most frequent neurodegenerative disorder, PD, is marked by the magnitude of pathogenic episodes (Westerlund et al. 2010), many of which have recently been related to Hsp90. An earlier report suggested that Hsp90 affects the binding of alpha-synuclein vesicles and the development of amyloid fibrils, two pathways that are closely related to alpha-synuclein folding (Falsone et al. 2009). In PD, a significant change in the level of HSp70 expression was seen in the larval brain of *park*^{13} heterozygous mutant in comparison to control individual (Rai and Roy 2022). Earlier literature also suggested that Hsp90 also encouraged fibril development through oligomeric complexes in an ATP-dependent fashion. Luo et al. demonstrated that the stability of p35 and p25, neuronal proteins that cause CDK5 by complexation progressing to anomalous tau phosphorylation and mutant but not wild tau protein, was sustained by Hsp90 in tauopathy. Inhibition of Hsp90 yielded a decline in the pathogenic function of these proteins, resulting in the dose- and time-dependent removal of aggregate tau (Xu et al. 2007). For the myriad aberrant processes that promote the development of the neurodegenerative phenotype, Hsp90 tends to act as a biochemical buffer. Hsp90 inhibition by small molecules ends up in the disintegration by a proteasome-mediated cascade of the Hsp90/aberrant protein complexes, contributing mainly to the degradation of the dysfunctional proteins. These studies indicate that inhibition of Hsp90 in neurodegenerative diseases can provide a dual treatment strategy. On one hand, its positive impacts may originate from the activation of Hsp70 and other chaperones worthy of rerouting the development of neuronal aggregates and suitable for protecting against protein toxicity, indicating Hsp90 inhibition as a pharmacological treatment modality to clinically improve the expression of molecular

chaperone proteins for the treatment of neurodegenerative diseases where the aggregation is crucial to the pathway. Inhibition of Hsp90, on the other hand, may enhance protein hyperphosphorylation and subsequent aggregation by reducing anomalous neuronal protein activity.

Conclusion and future prospective

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43538-024-00325-7.

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Data availability Not applicable.

Declarations

Competing interest The author declares no conflict of interest.

Ethical standards This article does not contain any studies with human participants.

Informed consent statement Not applicable.

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