This is the Accepted Version of the Author's Manuscript.

Reis SD, Pinho BR, Oliveira JMA "Modulation of molecular chaperones in Huntington's disease and other polyglutamine disorders". Molecular Neurobiology. September 2016 DOI: 10.1007/s12035-016-0120-z

Link to Publisher:
https://link.springer.com/article/10.1007%2Fs12035-016-0120-z

Links to Full Text (RedCube, shared via Springer Nature) – View Only
http://rdcu.be/kwjE

Title page

Modulation of molecular chaperones in Huntington's disease and other polyglutamine disorders

Sara D. Reis, Brígida R. Pinho, Jorge M. A. Oliveira*

REQUIMTE/LAQV, Department of Drug Sciences, Pharmacology Lab, Faculty of Pharmacy, University of Porto, Porto, Portugal

*Corresponding author:
Jorge M. Ascenção Oliveira
Tel. +351 220428610
Email: jorgemao@ff.up.pt

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT): Strategic award UID/QUI/50006/2013, and by the research grant PTDC/NEU-NMC/0412/2014 (PI: JMAO), co-financed by the European Union (FEDER, QREN, COMPETE) – POCI-01-0145-FEDER-016577. SDR acknowledges FCT for her PhD Grant (PD/BD/113567/2015). BRP acknowledges FCT for her PostDoc Grant (SFRH/BPD/102259/2014). We thank Ana Isabel Duarte (PhD, U. Coimbra) and Maria Clara Quintas (PhD, U. Porto) for reading and commenting the initial manuscript draft.

Abstract

Polyglutamine expansion mutations in specific proteins underlie the pathogenesis of a group of progressive neurodegenerative disorders, including Huntington's disease, spinal and bulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, and several spinocerebellar ataxias. The different mutant proteins share ubiquitous expression and abnormal proteostasis, with misfolding and aggregation, but nevertheless evoke distinct patterns of neurodegeneration. This highlights the relevance of the full protein context where the polyglutamine expansion occurs, and suggests different interactions with the cellular proteostasis machinery. Molecular chaperones are key elements of the proteostasis machinery and therapeutic targets for neurodegeneration. Here we provide a focused review on Hsp90, Hsp70, and their co-chaperones, and how their genetic or pharmacological modulation affects the proteostasis and disease phenotypes in cellular and animal models of polyglutamine disorders. The emerging picture is that, in principle, Hsp70 modulation may be more amenable for long-term treatment by promoting a more selective clearance of mutant proteins than Hsp90 modulation, which may further decrease the necessary wild-type counterparts. It seems, nevertheless, unlikely that a single Hsp70 modulator will benefit all polyglutamine diseases. Indeed, available data, together with insights from effects on tau and alpha-synuclein in models of Alzheimer's and Parkinson's diseases, indicates that Hsp70 modulators may lead to different effects on the proteostasis of different mutant and wild-type client proteins. Future studies should include the further development of isoform selective inhibitors, namely to avoid off-target effects on Hsp in the mitochondria, and their characterization in distinct polyglutamine disease models to account for client protein-specific differences.

Keywords

Heat shock proteins; Hsp70; Huntington's disease; Neurodegeneration; Proteostasis; Mitochondria

Abbreviations

AR, androgen receptor; BAG1, bcl-2-associated athanogene 1; CACNA1A, α1A subunit of the voltage-dependent calcium channel Cav2.1; CHIP, C-terminal Hsc70-interacting protein; DRPLA, dentatorubral-pallidoluysian atrophy; ER, endoplasmic reticulum; gamitrinibs, geldanamycin mitochondrial matrix inhibitors; GGA, geranylgeranylacetone; Grp, glucose-regulated protein; HD, Huntington's disease; Hip, Hsp70-interacting protein; Hsc70, constitutive Hsp70; HSF1, heat shock factor 1; Hsp, heat shock protein; Htt, huntingtin; mHtt, mutant Htt; mtHsp70, mitochondrial Hsp70; NBD, nucleotide binding domain; NEF, nucleotide

exchange factor; polyQ, polyglutamine; SBD, substrate-binding domain; SBMA, spinal and bulbar muscular atrophy; SCA, spinocerebellar ataxia; STAGA, SPT3/TAF GCN5 complex; TBP, TATA-box binding protein; TFTC, TATA-binding protein-free TAF-containing complex; TIM23, translocase of the inner membrane; TRAP-1, tumor necrosis factor receptor associated protein-1; UPS, ubiquitin proteasome system;

1. Introduction

The successful folding and the conformational maintenance of newly synthesized proteins are essential for protein homeostasis (proteostasis). Additionally, cells must have mechanisms to regulate the localization, concentration, and the activity of different proteins in response to intrinsic and extrinsic stimuli [1,2]. Molecular chaperones are proteins that stabilize or assist the acquisition of the active conformation of other proteins, without being part of their final structure. Molecular chaperones are involved in multiple aspects of proteome maintenance, acting in protein trafficking, folding, aggregation, and degradation [3,4]. Although molecular chaperones were initially described as heat shock proteins (Hsp; as the first members were discovered to be upregulated under such stress conditions; [5]), it is currently known that most molecular chaperones are constitutively expressed and involved in maintaining proteostasis at any time [6].

Molecular chaperone families (Hsp40, Hsp60, Hsp70, Hsp90, Hsp100, and the small Hsp) were named according to the molecular weight of their members. Hsp100 has an important role in protein disaggregation in non-metazoans (e.g. yeast), but metazoans lack Hsp100 in the cytosol and nucleus [7,8]. Hsp60 chaperones are present in the cytosol and the mitochondria of eukaryotes, but seem to interact with a higher percentage of proteins in bacteria and archaea [3,9]. The small Hsp are ubiquitous chaperones that bind to non-native proteins, preventing their aggregation and facilitating further refolding or degradation [10-12]. Together, molecular chaperones are estimated to account for approximately 10% of the cellular mass, with Hsp90 and Hsp70 alone representing half of that [13]. Indeed, Hsp90 and Hsp70 are the main effectors of the mammalian protein homeostasis network, acting in a multiprotein complex that includes co-chaperones such as Hsp40 [14-16]. Hsp90 typically interacts with client proteins in their late stages of folding. Hsp90 stabilizes and mediates the final folding of client proteins, preserving their activity and inhibiting their degradation [6,17]. In contrast, Hsp70 typically interacts with proteins at an early stage of folding or with proteins with an abnormal folding, but not with their folded counterparts. The binding of Hsp70 to unfolded or misfolded proteins allows redirecting client proteins for either folding or degradation [6,18]. Hsp70 is also relevant in the context of protein aggregation, being able to bind protein aggregates and promote their disaggregation [19,20].

Defects in proteostasis are implicated in the process of aging and in the pathogenesis of several degenerative conditions, including the polyglutamine (polyQ) expansion disorders [21,22]. PolyQ disorders comprise Huntington's disease (HD), spinal and bulbar muscular atrophy (SBMA), six of the spinocerebellar ataxias (SCA 1-3, 6, 7, 17), and dentatorubral-pallidoluysian atrophy (DRPLA), and all stem from expanded CAG repeats in the coding regions of the affected genes, resulting in mutant proteins with expanded glutamine tracts [23].

These disorders further share an inverse correlation between the CAG repeat number and the age of onset, and are primarily neurodegenerative despite ubiquitous expression of the respective mutant protein that forms insoluble aggregates in affected neurons [24-26]. Additionally, nuclear localization of the expanded protein seems to be required for the induced toxicity in the majority of polyQ disorders [24,25]. Interestingly, although all these diseases share polyQ expansion in the affected protein, they affect different regions of the neural tissue [23]. This differential neurodegeneration highlights the relevance of the protein context where the polyQ is inserted, and strongly suggests that the different mutant polyQ proteins and their wild-type forms may interact differently with components of the molecular chaperone machinery, justifying a focused review.

The modulation of molecular chaperones has been mainly studied in the context of cancer therapy [27,28]. Evidence suggests, however, that molecular chaperones are also potential therapeutic targets for neurodegenerative diseases characterized by the accumulation and aggregation of misfolded proteins. Indeed, molecular chaperones co-localize with polyQ-containing insoluble aggregates and their expression is decreased in several polyQ disease models, suggesting that their modulation is worth investigating as a potential therapeutic approach [29-33]. This review focuses on the modulation of molecular chaperones in the context of polyQ disorders. The first section describes the roles and mechanisms of action of key members of the Hsp90 and Hsp70 families, together with their standard and novel small-molecule modulators. The second section integrates the available data on the genetic and pharmacological modulation of Hsp90/70 and associated chaperones in models of HD and other polyQ disorders, addressing how treatments affect mutant protein levels, aggregation, survival and other key disease phenotypes. This review ends with insights from other main neurodegenerative diseases, and a discussion including future directions in the field.

2. Molecular chaperones and the heat shock response

The heat shock response is a highly conserved cellular reaction to proteotoxic insults such as heat, oxidative stress and toxins. In mammals, the master regulator of this response is the constitutively expressed heat shock factor 1 (HSF1), which acts by inducing the transcription of Hsp genes. Proteotoxic insults activate HSF1, converting its inactive monomers into trimers with DNA binding activity and transactivation capacity [34,35]. Interaction with Hsp90 prevents the activation of monomeric or trimeric HSF1. Similarly, interaction with Hsp70 and Hsp40 inhibits HSF1 transactivation capacity (Fig. 1). These interactions seem to act as regulatory feedback mechanisms that coordinate HSF1 activity with the expression of its HSP targets, and with the state of the protein-folding environment [34-36].

2.1. The Hsp90 family

Hsp90 is a highly dynamic family of proteins, capable of adopting several distinct conformations, and being regulated by multiple interactors [37]. This chaperone family has multiple client proteins, including steroid receptors, kinases, and several other unrelated proteins, including intrinsically disordered proteins such as tau [38,39,15,40,41]. Clients are delivered to Hsp90 by Hsp70 and other co-factors [4]. Hsp90 typically interacts with client proteins in their late stages of folding by binding to hydrophobic residues scattered over a large surface area [6,18]. Hsp90 may stabilize these proteins, mediate their folding and bring about their activation, but Hsp90 is also involved in directing clients to proteasomal degradation [17].

The Hsp90 family belongs to the gyrase, histidine kinase, and MutL superfamily of ATPases [4]. Hsp90 is constituted by three domains: an N-terminal nucleotide binding domain (NBD) followed by a charged linker region, a middle domain – involved in client protein binding (although other domains may participate in client binding [39,42]), and a C-terminal domain [38] (Fig. 2a). Importantly, Hsp90 is active in the form of a dimer, assembled via interaction sites located in the C-terminal domain. This domain also mediates Hsp90 interactions with several co-chaperones [4].

The Hsp90 dimer undergoes an ATP-regulated cycle, similarly to other chaperones. Client protein binding affinity is regulated by the combined effects of ATP binding and hydrolysis, post-translational modifications, and interactions with co-chaperones [6,4,37] (Fig. 2b). Client proteins themselves also influence the conformational equilibrium in interplay with co-chaperones, thus acting as modulators of the Hsp90 machinery [39,40].

In eukaryotes, Hsp90 can be found in the cytosol, nucleus and organelles such as mitochondria and the endoplasmic reticulum. The nuclear localized Hsp90 represents only a small fraction of the cytosolic Hsp90, which translocates to the nucleus in response to stress and other stimuli [43,44]. In humans, Hsp90 exists as four isoforms: Hsp90α and Hsp90β represent the cytosolic forms, Grp (glucose-regulated protein) 94 is found in the endoplasmic reticulum (ER), and TRAP-1 (tumor necrosis factor receptor associated protein-1) is associated with mitochondria [45] (Table 1).

2.1.1. Grp94 – the endoplasmic reticulum Hsp90 isoform

Grp94 is found in the endoplasmic reticulum, and is the only Grp in the Hsp90 chaperone family (Table 1) [46,47]. In addition to Grp94, there are other Grp present in the endoplasmic

reticulum and also in the mitochondria. Collectively, Grp are chaperones induced upon ER stress, a state usually associated with ER calcium depletion and/or accumulation of misfolded proteins in the ER [46]. At least one third of the proteins in eukaryotic cells are synthesized at the ER membrane. After their synthesis, these proteins are translocated into the ER lumen where they acquire their functional structure. When proteins are properly folded, they undergo vesicular-mediated transport through the organelles of the secretory pathway [48-50]. When protein misfolding prevails, the unfolded protein response is activated, which is a mechanism that promotes ER protein-folding homeostasis. The unfolded protein response involves a transient reduction of protein synthesis, while enhancing protein folding, transport and ER-associated protein degradation and autophagy [49-51].

Unlike cytosolic Hsp90, Grp94 has no known co-chaperones [47]. Grp94 contributes to the ER protein quality control and calcium storage, being one of the major calcium binding proteins [52]. Free calcium levels regulate Grp94 chaperone activity via a calcium binding site, whose occupation enhances Grp94 association with client proteins [53]. The Grp94 molecular chaperone activity includes the direct folding and/or assembly of secreted and membrane proteins, and assists the targeting of misfolded proteins for degradation [47,52].

2.1.2. TRAP-1 – the mitochondrial Hsp90 isoform

TRAP-1 is a Hsp90 isoform that is predominantly found in the mitochondrial matrix, with a smaller fraction in the intermembrane space (Table 1) [54]. Like Grp94, TRAP-1 has no known co-chaperones [54]. TRAP-1 functions are incompletely understood but do not seem to overlap with the functions of other Hsp90 chaperones, and might be crucial for mitochondrial physiology [55,56]. The most extensively reported TRAP-1 function is cytoprotection, via an interaction with cyclophilin D that prevents mitochondrial permeability transition pore opening [55], and via decreasing reactive oxygen species by yet unknown mechanisms [57]. Additionally, TRAP-1 has been associated with mitochondrial morphology, through regulation of fission proteins [58]; with mitophagy, through improved molecular quality control that reduces the number of damaged mitochondria [59]; with the maintenance of a functional electron transport chain, by upregulation of complex I activity [59,60]; and with the ER stress response [61].

TRAP-1 was also found on the outer side of the ER and shown to regulate the ubiquitination of specific proteins destined to mitochondria through interaction with TBP7 (a component of the 19S proteasome subunit). Data support the hypothesis that TRAP-1 and TBP7 perform the quality control of proteins destined to mitochondria at the ER-mitochondria

interface: TRAP-1 avoids the mitochondrial import of damaged proteins, sequestering them to be refolded [62].

2.1.3. Hsp90 modulators

Hsp90 inhibitors are thought to decrease levels of client proteins by preventing their cycling with Hsp90, thereby diverting them to the UPS for degradation [63]. Non-selective Hsp90 inhibitors include naturally occurring compounds, such as geldanamycin and radicicol. Attempts to overcome pharmacological and toxicity issues sprouted derivatives of these drugs, and also purine analogues (e.g. BIIB021 and PU-H71) as improved Hsp90 inhibitors. Geldanamycin derivates include tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspimycin (IPI-504), and the primary active metabolite of tanespimycin (IPI-493/17-AG). Radicicol derivatives include ganetespib (STA-9090), AT-13387, KW-2478, NVP-AUY922, and the orally available NVP-HSP990. All the aforementioned drugs inhibit Hsp90 by competing with nucleotides for their binding site at the N-terminal, shifting the balance to client protein dissociation and thereby allowing its degradation [63-65]. Interestingly, it has been recently proposed that Hsp90 inhibitors that block the ATP binding pocket may impact on Hsp70 functions, given that the ATP cycles of Hsp90 and Hsp70 may be tightly coupled [15]. Other strategies being pursued to inhibit Hsp90 include the targeting of the Hsp90 C-terminal domain in an attempt to inhibit binding with co-chaperones [63-65]. In the context of neurodegenerative disorders, radicicol, geldanamycin, and the geldanamycin derivatives 17-AAG and 17-DMAG are the most frequently tested molecules, as addressed in section 3.

Strategies to identify isoform selective Hsp90 inhibitors are beginning to emerge. Most compounds are being targeted to regions outside the highly conserved nucleotide-binding site [65], but minor differences in the ATP-binding pockets are also being explored to develop Grp94 selective compounds [66]. Small-molecule Hsp90 inhibitors that selectively accumulate in mitochondria were also synthesized to preferentially target TRAP-1. In these compounds, designated as geldanamycin mitochondrial matrix inhibitors (gamitrinibs), the ATPase inhibitory component of 17-AAG is fused to a mitochondrial targeting moiety (triphenylphosphonium cation or 1 to 4 cyclic guanidinium repeats). Gamitrinibs were found to accumulate in mitochondria and inhibit the ATPase activity of TRAP-1 with in vitro and in vivo efficacy [67].

2.2. The Hsp70 family

The Hsp70 chaperone family is a ubiquitous class of proteins involved in several steps of the protein quality control, including protein folding, transport and degradation [37]. Hsp70 recognizes short and highly hydrophobic amino acid sequences, which often are integral components of the hydrophobic core of the proteins. Consequently, Hsp70 acts in unfolded and misfolded proteins that have these amino acids exposed and not in their native counterparts [18].

Hsp70 contain an NBD and a C-terminal substrate-binding domain (SBD) connected by a conserved hydrophobic linker region (Fig. 3a). Hsp70 activity depends on its dynamic interaction with Hsp40 co-chaperones (also called J proteins), the Hsp70-interacting protein (Hip), and nucleotide exchange factors (NEFs), which together regulate the nucleotide state of the Hsp70 ATPase domain. ATP binding or hydrolysis at the NBD alter the conformation of the SBD and, consequently, regulate Hsp70 interaction with client proteins. ATP binding opens the substrate-binding site, allowing interaction with client proteins. In contrast, ATP hydrolysis, which is promoted by Hsp40, closes the substrate-binding site, stabilizing the client protein binding [37,68]. Hip and NEFs interact with the NBD, in a mutually exclusive manner, to regulate client release: an Hip dimer locks ADP in the binding cleft, delaying client release [69], whereas a NEF releases ADP and, consequently, the client protein [37,68]. Proteins unable to fold properly may rebind to Hsp70 or be redirected to other chaperones or degradation pathways (Fig. 3b). In addition to its role in protein folding and degradation, Hsp70 is also involved in protein disaggregation, acting in cooperation with Hsp40 co-chaperones and the Hsp110 NEF in the formation of a chaperone complex with disaggregase activity [70,20] (Fig. 3c).

There are at least 14 Hsp70 isoforms in humans encoded by different genes (Table 2). Although the vast majority of Hsp70 proteins are mainly cytosolic and nuclear in localization, there are also Hsp70 members restricted to specific compartments, such as mitochondria and the ER. The four most studied Hsp70 isoforms are: the stress induced Hsp70 (Hsp72); the constitutive Hsp70 (Hsc70); the Grp78 or BiP, which is mainly located in the ER; and the mitochondrial Hsp70 (mtHsp70) [71].

2.2.1. The constitutive Hsc70 and the inducible Hsp72

The major constitutively active form of Hsp70 is Hsc70. Hsc70 is by far the most expressed Hsp70 in all tissues and is only mildly induced during stress conditions. Hsc70 and the inducible Hsp72 have high identity (87%; Table 2), and share major functions in protein folding, translocation, degradation and prevention of aggregation [72]. Still, differences in their C-terminal domain may account for some functional differences [73]. Indeed, while Hsp72 knockout mice survive, Hsc70 knockout is lethal, thus suggesting different survival roles for these isoforms [74]. Moreover, while under normal conditions Hsc70 regulates several

components of the synapse (e.g. N-type calcium channels, neurotransmitter synthesis and packaging, and vesicle recycling), Hsp72 only seems to function at the synapse under stress conditions [71]. Furthermore, Hsc70 is the only chaperone with a crucial role in recognizing substrates for chaperone-mediated autophagy [75].

2.2.2. Grp78 – the endoplasmic reticulum Hsp70 isoform

The most abundant ER chaperone is the Grp78, which shares 64% identity with Hsp72 [76] (Table 2). Similarly to the ER located Hsp90 isoform (Grp94), Grp78 acts as a calcium binding protein, functions in protein folding, and targets proteins for secretion or for the ER-associated degradation. The mechanisms involved in these processes are similar between Grp78 and the previously described Grp94 [48,49].

2.2.3. mtHsp70 – the mitochondrial Hsp70 isoform

mtHsp70 is constitutively expressed and has 52% sequence identity with Hsp72 (Table 2). It resides mainly in the mitochondria where it acts as the ATP-hydrolyzing subunit of TIM23 (translocase of the inner membrane; [77]), assisting the import and folding of nucleus-encoded mitochondrial precursor proteins [78,79]. Such precursor proteins contain targeting signals recognizable by receptors at the mitochondrial surface that target the precursors to different mitochondrial subcompartments [80]. Mitochondrial membrane potential per se is not enough to drive the complete translocation of proteins into the matrix. The additional energy required for this translocation via TIM23 comes from mtHsp70-dependent ATP hydrolysis [81]. mtHsp70 binds the unfolded polypeptide chain and drives its movement into the matrix [80], acting in the subsequent protein folding [82].

2.2.4. Hsp70 modulators

Different compounds have been identified and developed to target different aspects of Hsp70 function, but thus far all lack selectivity for individual Hsp70 isoforms. The first was 15-deoxyspergualin, which targets the Hsp70 SBD and allosterically stimulates Hsp70 ATPase activity [83,84]. Other compounds that interact with the Hsp70 SBD are 2-phenylethynesulfonamide, also known as pifithrin-μ [85], and geranylgeranylacetone (GGA) [86]. Pifithrin-μ inhibits Hsp70 folding activity by disrupting its association with co-chaperones,

such as Hsp40, and with client proteins [85]. The mechanism of action of GGA is still incompletely understood; being suggested that GGA binding to Hsp70 promotes its dissociation from HSF1, allowing HSF1 activation [86].

The majority of the developed Hsp70 modulators target the NBD, either stimulating or inhibiting Hsp70 ATPase activity. Those that stimulate Hsp70 ATPase activity include the dihydropyrimidines SW02 and 115-7c [87,88], whereas Hsp70 ATPase inhibitors include the dihydropyridine CE12 [89], VER155008 [90], myricetin [87,91], and apoptozole [92]. Compounds such as methylene blue and azure C were also reported to inhibit Hsp70 ATPase activity [87], possibly by oxidizing residues at the NBD [93].

The rhodocyanine MKT-077 targets the NBD inhibiting Hsp70 ATPase activity and stabilizing the ADP-bound conformation [94]. Researchers have been working to create MKT-077 analogs with reduced toxicity and increased potency and permeability. Improved MKT-077 analogs include YM-01 and the blood brain barrier permeable neutral analog YM-08 [95,96]. These compounds mimic the ADP-locking activity of the co-chaperone Hip at the NBD, increasing Hsp70 affinity for clients and consequently preventing their aggregation and facilitating transfer to downstream chaperones or degradation machineries [69,97].

2.3. The chaperone machinery and protein quality control

The Hsp70 and Hsp90 chaperone families play a major role in the proteostasis network, acting together with their co-chaperones as a multiprotein complex in protein quality control [3,14,16]. Hsp90 stabilizes the clients, inhibiting their ubiquitination and degradation, whereas Hsp70 promotes the ubiquitination and degradation of proteins that cannot be properly folded. Indeed, when Hsp90 cannot cycle with the client protein, ensuring the native folding state of the client protein, Hsp70 can redirect the misfolded protein to degradation pathways, such as chaperone-assisted proteasomal degradation or autophagy-associated pathways [98,99]. The latter pathways include chaperone-assisted selective autophagy and chaperone-mediated autophagy; both are associated with Hsc70 and may compensate for defects in the ubiquitin proteasome system (UPS), which is otherwise the main system responsible for the degradation of misfolded proteins [100,101].

The UPS relies on the coupling of ubiquitin chains to target proteins, signaling them for proteasomal degradation [102]. After protein recognition by chaperones, chaperone-dependent E3 ubiquitin ligases target E2 ubiquitin-conjugating enzymes to the protein, promoting its ubiquitination [14]. The co-chaperone CHIP is an E3 ligase that binds to both Hsp70 and Hsp90, and interacts with E2 ubiquitin-conjugating enzymes through a C-terminal U-box domain, thereby connecting chaperone complexes to the ubiquitination machinery [103]. The

co-chaperone BAG1 (bcl-2-associated athanogene 1) associates with Hsp70 and CHIP, promoting protein ubiquitination [4] and linking the chaperone system to the proteasome [98,104] (Fig. 4). Interestingly, although Hsp90 is considered to be primarily involved in client protein stabilization and inhibition of degradation, evidence suggests that Hsp90 may also promote the degradation of specific client proteins such as tau, a protein known to misfold and accumulate in Alzheimer's disease [105,106].

In the context of neurodegenerative disorders, the chaperone-assisted proteasomal degradation model presents two possible approaches to promote the degradation of aggregation-prone client proteins. The first is the inhibition of client protein stabilization by Hsp90, inhibiting the cycling of the not-yet-unfolded proteins with Hsp90 to promote its degradation. However, Hsp90 interacts with hundreds of proteins, therefore long-term treatment with Hsp90 inhibitors may lead to the unspecific degradation of multiple Hsp90 client proteins. Thus, the second possible pathway seems to be a better approach, which is the promotion of Hsp70-dependent degradation of already-unfolded or misfolded client proteins [14].

3. Modulation of molecular chaperones in Huntington's disease and other polyglutamine disorders

The expanded polyQ tract renders the affected protein highly prone to aggregation. Still, among polyQ disorders, aggregates may present different subcellular localizations, and different neuronal populations show the highest vulnerability [23,107,108]. Also, while the aggregates clearly indicate that proteostasis is altered, it remains uncertain if aggregates are the main mediators of neuronal dysfunction [109]. Indeed, aggregate formation may actually reduce the diffuse levels of the mutant protein and the associated risk of neuronal death [110]. Nevertheless, protein aggregates do sequester several proteins, including components of the transcriptional and protein quality control machineries, thus suggesting a role in transcriptional dysregulation and abnormal proteostasis that are common features to all polyQ disorders [107,111]. Either genetic or pharmacological modulation of molecular chaperones alters protein levels and aggregation, thus being potential therapeutic approaches for polyQ disorders [33,89,97].

3.1. Huntington's disease

In HD, the polyQ expansion occurs in the huntingtin (Htt) protein (Table 3). Htt interacts with multiple proteins involved in diverse cellular processes (e.g. gene transcription, energy

metabolism, cell signaling, and proteostasis), which are disrupted upon expression of mutant Htt; mHtt) [111,112]. Key components of HD pathology are mitochondrial dysfunction, transcriptional dysregulation and abnormal proteostasis [113].

Mitochondrial bioenergetics and dynamics are disturbed by expression of mHtt [114-116]. mHtt was shown to interact with mitochondria in both cellular and animal models, however, its exact sub-mitochondrial localization is still unclear [117,118]. mHtt was reported to disrupt mitochondrial protein import via direct interaction with the TIM23 complex [118], whose ATP-hydrolysing subunit is the chaperone mtHsp70 [77,81].

Transcriptional dysregulation in HD results from mHtt interaction with major components of the transcriptional machinery. In the context of chaperones, mHtt was found to interact with and sequester the transcription factor NF-Y in brains of HD mice. As this sequestration reduces the NF-Y dependent Hsp70 transcription, it may explain the reduced expression of Hsp70 in HD, and contribute for abnormal proteostasis [32].

Abnormal proteostasis in HD relates to the aggregation-prone features of mHtt, which seed nuclear and cytoplasmic aggregates that sequester transcription factors and quality control proteins, thereby disrupting normal transcription and protein clearance [111]. Indeed, the two major protein clearance pathways, UPS and autophagy, seem compromised in HD. Ubiquitin chains do accumulate in HD brains [119], however, this is not necessarily due to direct proteasome inhibition by mHtt. Instead, mHtt may overwhelm the proteostasis network, leading to increased levels of other misfolded proteins that are diverted to the UPS where they compete for the limited degradation capacity of the 26S proteasome [120]. Concerning autophagy, data suggest transcriptional dysregulation of autophagy-related genes in HD brains [121]. Also, mHtt may impair autophagosome trafficking [122] and cargo recognition [114,121-123]. Still, the hypothesis that wild-type Htt may function as a scaffold for selective autophagy cautions about the risk of decreasing both wild-type and mHtt with non-selective therapeutic strategies [124].

3.1.1. Genetic modulation of molecular chaperones in HD

Proteomic analysis of the mHtt interactome revealed that several members of chaperone families associate with mHtt. Moreover, levels of Hsp70 and Hsp40 are progressively reduced in brain tissues of HD animal models through a combination of sequestration and transcriptional dysregulation, suggesting that modulation of chaperones could be a therapeutic strategy in HD [111]. The genetic modulation of chaperone-mediated regulatory pathways in HD has been mainly studied by manipulating Hsp40 and Hsp70 levels and less frequently Hsp90, CHIP and HSF1 levels (Table 4). Overall, the overexpression or enhancement of chaperones reduced mHtt toxicity in HD cellular and animal models: Hsp40 and CHIP reduced mHtt aggregation and

improved HD phenotypes; Hsp90 modulation did not significantly alter HD phenotypes; and contradictory results have been obtained with modulation of HSF1 and Hsp70 (Table 4).

The chaperone-dependent E3 ubiquitin ligase CHIP was found to interact with mHtt and to induce its ubiquitination and degradation, in cells expressing N-terminal mHtt [125]. Consistently, CHIP overexpression reduced mHtt aggregation and toxicity in HD cellular and animal models [125-127].

HSF1 overexpression increased survival and reduced mHtt aggregation in skeletal muscle but not in brains of R6/2 HD mice, where HSF1 expression was not detected [128]. In contrast, overexpression of HSF1 in a bone-derived cell line increased mHtt aggregation and toxicity. The proposed explanation was that strategies that promote the heat shock response may increase proteotoxic stress, preferentially killing cells with high mHtt expression and this could lead in some cases to an underestimation of mHtt aggregation [129].

Overexpression of Hsp90 in cell lines showed no effect on mHtt aggregates [130]. Hsp90 silencing, however, reduced the levels of mutant Htt in both full-length and N-terminal forms, in cellular models, supporting the hypothesis that Htt is an Hsp90 client protein, and that preventing its cycling with Hsp90 promotes its degradation [131,132]. Further supporting this hypothesis is the fact that both wild-type and mutant N-terminal Htt, and full-length mutant Htt were found to interact with Hsp90 [131].

Hsp40 and Hsp70 overexpression have been studied alone or in combination in different HD models. While Hsp40 overexpression inhibited mHtt aggregation and/or toxicity in cells [130,133,134], primary neurons [135], *Xenopus laevis* [136], and mice [137], Hsp70 overexpression was less efficient in reducing mHtt aggregation than Hsp40. Indeed, HEK293 cells overexpressing Hsp40 exhibited fewer mHtt aggregates than those overexpressing Hsp70 [135]. Data suggest that Hsp70 overexpression reduces or does not alter mHtt aggregation, which may be related with the Hsp70 isoform that is modulated. For example, overexpression of Hsc70 decreased mHtt aggregation, while overexpression of inducible Hsp70 did not alter the amount of aggregates in Neuro2a cells expressing mHtt [133]. Most studies, however, do not specify which Hsp70 isoforms are being modulated. Co-expression of Hsp70 and Hsp40 synergistically reduced mHtt aggregation [130,134], highlighting the coordination between both Hsp70 and Hsp40 chaperones in mediating Htt protein degradation.

3.1.2. Pharmacological modulation of molecular chaperones in HD

Drugs that attempt to rectify the harmful consequences of mHtt (e.g. histone deacetylase inhibitors [138-140]) have shown promising results. Nevertheless, pharmacological strategies that focus on the causative agent itself, mHtt, via enhanced refolding, and/or increased

degradation are expected to provide upstream protection, and are being actively pursued, namely with drugs that modulate molecular chaperones.

Increased expression of molecular chaperones may be achieved pharmacologically by direct activators of HSF1 or by Hsp90 inhibitors. The latter dissociate HSF1 from its cytosolic complexes with Hsp90, allowing HSF1 nuclear translocation and increasing the expression of heat shock proteins [29,141]. By destabilizing the Hsp90 chaperone-client protein complexes, Hsp90 inhibitors also promote the degradation of client proteins [131]. Studies with Hsp90 inhibitors have found an inhibition of mHtt aggregation and/or a decrease of its toxicity (Table 5).

HSF1 activators include HSF1A, a benzyl pyrazole-based molecule [142] and F1, an unsaturated barbituric acid derivative [143]. Both HSF1A and F1 promoted Hsp70 expression and reduced mHtt aggregates in cells expressing mHtt [142,143]. In contrast, bone-derived cells treated with F1 showed increased mHtt aggregation, however, in this case F1 treatment was performed at least 48h after cellular transfection with Htt exon 1 [129], a sufficient time for mHtt aggregates to have already formed [120]. Together, these findings suggest that the chaperones induced by HSF1 treatment may act on mHtt oligomers but not on the larger aggregates.

Hsp70 has a crucial role in protein folding, transport and degradation [37] and its overexpression may reduce mHtt aggregation [130]. SW02, an Hsp70 ATPase stimulator, decreased mHtt aggregation in PC12 and yeast HD models, increasing soluble mHtt and toxicity in yeast, but unaffecting survival in PC12 cells [89]. Conversely, CE12, an Hsp70 ATPase inhibitor, increased mHtt soluble levels in yeast and increased mHtt aggregation and survival in yeast and PC12 cells, supporting the hypothesis that mHtt aggregates are less toxic than soluble mHtt [89].

3.2. Spinal and bulbar muscular atrophy

SBMA, also known as Kennedy's disease, is caused by polyQ expansion in the androgen receptor (AR) [26] (Table 3). The AR mediates the effects of androgens, being expressed in sexual and non-reproductive organs, such as the skeletal muscle, and the central nervous system [26]. AR-androgen complexes formed in the cytoplasm translocate to the nucleus where they activate gene transcription [144]. Mutant AR forms insoluble aggregates in the nucleus and cytoplasm [107], however, nuclear localization seems critical for toxicity since mutations in the nuclear localization signal or addition of nuclear export signals abolish mutant AR toxicity [145,146]. Still, ligand-dependent nuclear localization *per se* is insufficient for mutant AR-induced neurodegeneration, as DNA binding is also required [147].

As in HD, the pathogenesis of SBMA includes transcriptional dysregulation and mitochondrial dysfunction. Mutant AR decreases the expression of genes required for neuronal survival [148,149], and its N-terminal fragments were found to activate BAX-dependent apoptosis in neurons, stimulating cytochrome c release from mitochondria [150]. Further, mutant AR expression in mammalian cells was found to increase reactive oxygen species, depolarize mitochondria and to decrease transcription of genes associated with mitochondrial function, such as PGC-1β, TFAM, PPARγ, ND1, SOD2 [151].

3.2.1. Genetic modulation of molecular chaperones in SBMA

The AR is a classic Hsp90 client protein and the genetic modulation of chaperones in the context of SBMA has been performed by overexpression of CHIP, Hip, Hsp40 and Hsp70 in cell lines and less frequently in animal models. Available studies suggest that overexpression of chaperones decreases mutant AR levels, aggregates and toxicity (Table 6).

CHIP overexpression enhanced mutant AR ubiquitination and degradation, decreasing mutant AR levels in cells [152,153] and in SBMA transgenic mice, where it also decreased mutant AR aggregation and ameliorated motor symptoms [152]. Similarly, Hip overexpression decreased mutant AR aggregation by increasing its degradation in cells [97,154].

Hsp40 or Hsp70 overexpression decreased mutant AR aggregate formation and increased the survival of SBMA cell models (Table 5). Hsp70 overexpression in SBMA transgenic mice ameliorated motor function and decreased aggregation and levels of mutant AR. [155]. Overexpression of Hsp40 and Hsp70 likely decreases the aggregation of mutant AR by promoting its degradation via the UPS. Alternatively, both Hsp40 and Hsp70 also induce mutant AR solubilization, since their overexpression decreased mutant AR aggregation and increased its soluble form in presence of lactacystin, a proteasome inhibitor [156]. Co-expression of Hsp70 and Hsp40 presents synergic effects on reduction of mutant AR aggregate formation and on cell survival, reflecting that Hsp70 and Hsp40 act together in chaperoning misfolded AR [156,157].

3.2.2. Pharmacological modulation of molecular chaperones in SBMA

Several drugs targeting Hsp90 and Hsp70 chaperones have been tested in cellular and animal models of SBMA (Table 5). As with mHtt in HD models, Hsp90 inhibitors reduced aggregation and/or soluble levels of mutant AR, decreasing its toxicity in SBMA models [158-160]. The geldanamycin derivative 17-DMAG was 3-fold more potent than 17-AAG in reducing levels of

mutant AR in SH-SY5Y cells [159]. Proteasomal inhibition with MG-132 blocked the reduction of mutant AR levels induced by 17-AAG and 17-DMAG. In contrast, these Hsp90 inhibitors decreased mutant AR even when Hsp70 induction was blocked by protein synthesis inhibition with cycloheximide or by small interfering RNA. These results indicate that the effects of these Hsp90 inhibitors are highly dependent on the UPS, rather than on Hsp70 induction [159,160]. Autophagy also seems involved in the reduction of mutant AR after 17-AAG treatment. Indeed, 17-AAG may promote activation of the autophagy pathway, as indicated by the associated increases in LC3 mRNA and LC3-II protein levels in immortalized motor neurons. In addition, when autophagy was blocked with 3-methyladenine or with small-hairpin RNA against LC3, 17-AAG could not increase degradation of mutant AR [161].

GGA has been shown to induce the expression of molecular chaperones in various tissues, including the central nervous system, via disruption of the Hsp70-HSF1 complex, thus allowing HSF1 activation [86,162,163]. GGA did not alter the expression of the constitutive Hsc70, but up-regulated expression of Hsp70 and Hsp90 in cells expressing mutant AR [158]. GGA also decreased mutant AR levels and increased the survival of SBMA cellular and mouse models [158].

Methylene blue, which was identified in a high-throughput screen as an Hsp70 inhibitor [87], promoted the accumulation of mutant AR in HeLa cells. Since methylene blue may influence several cellular processes, the experiments were repeated in the presence of Hsp70 overexpression, and the associated loss of methylene blue effects on mutant AR indicates that they were mediated by Hsp70 inhibition [164].

YM-1 stabilizes Hsp70 in its ADP-bound state, mimicking the co-chaperone Hip and enhancing binding of Hsp70 to substrates. In a PC12 cell model of SBMA, YM-1 decreased the levels of mutant AR through increased degradation by the UPS. Importantly, the knockdown of Hsp70 reduced the effects of YM-1, implicating Hsp70 as its critical cellular target [97].

3.3. Spinocerebellar ataxias and dentatorubral-pallidoluysian atrophy

The SCA family comprises more than 35 genetically different types of progressive neurodegenerative disorders, six of which are polyQ expansion disorders [165]. SCA1, 2, 3 and 7 are caused by polyQ expansion in ataxin-1, 2, 3 and 7, respectively, whereas SCA6 and SCA17 result from polyQ expansions in the α1A subunit of the voltage-dependent calcium channel Cav2.1 (CACNA1A) and in the TATA-box binding protein (TBP), respectively [25] (Table 3). SCAs and DRPLA share many clinical and pathological features (Table 3), in fact, DRPLA patients with mildly expanded polyQ in the affected protein (atrophin-1; [166]) tend to exhibit pure cerebellar symptoms, such as ataxia [167]. Many of the ataxia-causing proteins are

reported to share interacting partners, suggesting that the disease phenotypes shared among these disorders may result from common molecular pathways [168]. Indeed, as will be described below, transcriptional dysregulation is a common feature to all of these disorders.

Ataxin-1 was reported to interact with RNA and several regulators of transcription, indicating its role in the regulation of gene expression [169-171]. Studies in transgenic animal models showed that expanded polyQ in ataxin-1 induced a dysregulation of the expression of genes critical for cerebellar development [172-174]. Importantly, nuclear localization of the mutant protein seems to be required for toxicity [175] and nuclear aggregates are common in SCA1 [108].

Ataxin-2 is thought to play a role in post-transcriptional and translation regulation [176]. Mice expressing ataxin-2 with a polyQ expansion showed deficits in the expression of Purkinje cells-specific genes [177,178], which were associated with translation dysregulation [177]. In contrast with SCA1, nuclear localization or aggregates formation of the expanded ataxin-2 are thought unnecessary for the pathogenesis of SCA2 [179].

Ataxin-3 has been associated with protein homeostasis and transcriptional regulation [180]. Ataxin-3 has ubiquitin-protease activity and up to three ubiquitin-binding motifs, being thought to directly regulate ubiquitination-dependent degradation pathways [181]. Additionally, ataxin-3 was shown to interact with the co-chaperone and E3 ligase CHIP and suggested to promote the turnover of CHIP substrates [182]. The expansion of the polyQ tract in ataxin-3 may alter the dynamics of ataxin-3 and CHIP interaction, targeting CHIP for degradation. In fact, expanded ataxin-3 presents an increased affinity for CHIP, and decreased levels of CHIP were found in a SCA3 mouse model [182]. Consistently with its function in transcription, expression of mutant ataxin-3 in SCA3 transgenic mice altered the expression of several genes including those involved in the heat shock response [31]. Although both cytoplasmic and nuclear aggregates have been described in SCA3 brains [180], the nucleus is thought to be the principal site of SCA3 pathogenesis [183].

CACNA1A, the protein mutated in SCA6, integrates the Cav2.1 calcium channel that is responsible for transmission initiation at fast synapses [25]. While there is lack of support for changes in Cav2.1 channel function in SCA6 [108,184], this disease has been associated with transcriptional dysregulation in Purkinje cells [185], being noteworthy that mutant CACNA1A aggregation preferentially occurs in the cytoplasm and rarely in the nucleus of Purkinje cells [186].

Ataxin-7 constitutes a subunit of the TFTC (TATA-binding protein-free TAF-containing complex) and the STAGA (SPT3/TAF GCN5 complex) complexes, both of which are crucial for gene transcription [108]. Not surprisingly, mutant ataxin-7 induced transcriptional dysregulation in SCA7 transgenic models [187-189], and the disease associates with nuclear aggregates of the polyQ-expanded protein [190].

TBP, the protein involved in SCA17, is an essential component of the transcriptional initiation complex [191], directing it to DNA via binding to the TATA-box [192]. Studies with SCA17 transgenic mice indicate that polyQ expansion in TBP alters its ability to bind DNA and transcriptional regulators, suggesting that transcriptional dysregulation contributes to SCA17 pathogenesis [193-196]. In SCA17, mutant TBP accumulates in intranuclear aggregates [190].

Atrophin-1, the protein involved in DRPLA, functions as a transcription regulator [166]. In *Drosophila* models [197] and brains of transgenic mice [198], polyQ expansion in atrophin-1 was shown to induce transcriptional dysregulation. Both patients and DRPLA animal models show nuclear accumulation of an N-terminal fragment of mutant atrophin-1, which forms aggregates and is associated with toxicity [190,199].

3.3.1. Genetic modulation of molecular chaperones in SCAs and DRPLA

Chaperone-associated genetic modulations in SCAs and DRPLA models include the overexpression of CHIP, Hsp40 and Hsp70, which were found protective by decreasing mutant proteins levels, aggregation, or toxicity (Table 7). Overexpression of the chaperone-dependent E3 ubiquitin ligase CHIP decreased mutant protein levels in cellular and *Drosophila* models of SCA1 and SCA3 [125,126,153,200,201], likely due to increased degradation via the UPS since the ubiquitination levels of mutant protein were increased [125,126]. As observed for mHtt aggregation [125,127], CHIP overexpression decreased mutant ataxin-3 aggregation in cells [125]. In contrast with these results, CHIP overexpression increased mutant ataxin-1 aggregation in cellular models, suggesting that CHIP may exert different effects depending on its client protein [200]. Additionally, CHIP modulation was found to alter the levels of unexpanded ataxin-1 and -3 proteins in cells (Table 7), indicating that CHIP can also regulate the degradation of wild-type forms of these proteins [33,200]. In agreement with the protective effects of CHIP overexpression, CHIP reduction increased mutant ataxin-3 aggregation and the severity of the phenotype in SCA3 transgenic mice [201].

Hsp40 overexpression decreased mutant protein aggregation in SCA1, SCA3 and DRPLA cellular models [33,128,136,202,203]. Hsp40 overexpression decreased mutant ataxin-3 aggregation while increasing its levels in cells, prompting the hypothesis that Hsp40 may bind mutant ataxin-3 and delay its degradation [33]. Hsp70 overexpression decreased mutant protein aggregation in SCA3, SCA7 and DRPLA cellular models [128,204,205]. In a SCA3 *Drosophila* model, overexpression of Hsp40 or Hsp70 reduced mutant ataxin-3 induced toxicity. Co-overexpression of Hsp40 and Hsp70 synergistically reduced toxicity while decreasing aggregates and increasing soluble ataxin-3 levels, thus associating reduced toxicity with altered solubility properties of mutant ataxin-3 [206]. As observed for CHIP, Hsp40 and Hsp70 were

shown to also regulate the levels of wild-type ataxin-3 in cells [33] (Table 7). While Hsp70 overexpression improved Purkinje neuron morphology and motor function in SCA1 mice [207], its overexpression had no effect on mutant ataxin-7 toxicity in SCA7 mice [204]. Concerning SCA17, overexpression of the constitutive Hsc70 ameliorated neuropathology in transgenic mice [208].

3.3.2. Pharmacological modulation of molecular chaperones in SCAs and DRPLA

Concerning SCAs and DRPLA, and as far as we could find in the literature, pharmacological modulation of chaperones has only been tested in SCA3 and SCA17 models, where Hsp90 inhibitors or inducers of chaperone expression (GGA, indole or NC001-8) were found protective by decreasing mutant protein aggregation and associated toxicity, in contrast with Hsp70 ATPase inhibition (azure C) (Table 5).

Hsp90 inhibition with geldanamycin, radicicol or 17-AAG in Drosophila SCA3 models decreased mutant ataxin-3 associated toxicity [141,142], and 17-AAG treatment also decreased mutant ataxin-3 aggregation and increased survival [141]. In SCA3 mice, Hsp90 inhibition with 17-DMAG improved motor function, while decreasing mutant ataxin-3 aggregation and levels, possibly via autophagy activation since 17-DMAG treatment increased both beclin-1 and LC3-II levels [209].

Treatment with GGA decreased mutant protein aggregation in SCA3 and SCA17 cells [205,210], but had no effect on mutant ataxin-3 toxicity in SCA3 Drosophila [141]. Indole and its derivate NC001-8 were recently tested in SCA models. Although the mechanisms by which these compounds induce chaperone expression remain uncertain, both decreased mutant protein aggregation in SCA3 and SCA17 cells [205,210], being reported to enhance autophagy in SCA3 cells [205].

The inhibition of Hsp70 ATPase activity with azure C increased levels of both normal and mutant ataxin-3 in cells, consistent with the hypothesis that Hsp70 can regulate the degradation of wild-type ataxin-3 [33].

3.4. Insights from other neurodegenerative disorders

In addition to polyQ diseases, Hsp70 modulators have been studied in the context of other neurodegenerative disorders. For instance, the Hsp70 ATPase activity stimulators SW02 and 115-7c were assessed for their effect on the proteostasis of tau and α-synuclein, which are proteins know to misfold and accumulate in Alzheimer's and Parkinson's diseases, respectively.

In HeLa cells, both compounds were found to increase tau levels [87]. While SW02 had no effect on α-synuclein levels in HeLa cells [87], 115-7c decreased α-synuclein aggregation in neuroglioma cells [211]. Inhibitors of the Hsp70 ATPase activity were also studied for their potential in the regulation of tau [87]. Contrasting with the observed effects in polyQ models, in which methylene blue and azure C increased protein levels of mutant AR and mutant ataxin-3, respectively, treatment with either of these compounds decreased both wild-type and mutant tau levels in HeLa cells and mice brain [87]. Additionally, neither methylene blue nor azure C altered α-synuclein levels in HeLa cells [87]. YM-1 and its blood brain barrier permeable neutral analog YM-8 were also found to reduce tau levels in HeLa cells and brain slices from tau transgenic mice [96,212]. As observed for SBMA models [97], the effect of YM-1 was blocked by proteasomal inhibition with epoximicin, suggesting that treatment with YM-1 promotes tau degradation via the UPS [212]. Taken together with data from polyQ disease models, the emerging picture is that chaperone modulators, and those of Hsp70 in particular, may lead to different effects on the proteostasis of different mutant and wild-type client proteins.

3.5. Pharmacological targeting of Hsp70: inhibition *versus* activation

Collectively, data from both genetic and pharmacological interventions generally support chaperone modulation as a potential therapeutic approach in polyQ disorders, while also evidencing that the chaperone system may exert different effects depending on the nature of the client protein. Importantly, although genetic interventions support Hsp70 activation as a promising approach for polyQ disorders, the pharmacological modulation of Hsp70 has proven to be more complex.

Two molecules known to inhibit Hsp70 ATPase activity presented different effects on mutant AR levels in SBMA cells (methylene blue increased whereas YM-1 decreased AR levels) [97,164]. One explanation for these distinct results may lay in the detailed mechanisms by which the compounds affect the biochemistry of Hsp70. In fact, YM-1 not only inhibits Hsp70 ATPase activity, but it also stabilizes the ADP-bound conformation of Hsp70. This is relevant because the binding affinity of the SBD is highly dependent on the state of the NBD: Hsp70 in an ATP-bound form has poor affinity for client proteins, whereas Hsp70 in an ADP-bound form has high affinity for client proteins [37]. Different Hsp70 modulators may thus evoke different effects on proteostasis depending on their Hsp70 binding site and the conformation state that they promote. As such, modulators that stabilize the ADP-bound conformation, such as YM-1, are expected to enhance Hsp70 affinity to client proteins, inhibiting protein aggregation and allowing Hsp70-dependent degradation. In contrast,

modulators that favor the ATP-bound conformation are expected to promote client release, and thus facilitate mutant protein aggregation.

The mechanisms that regulate the probability of the Hsp70 complex to allow degradation or refolding of a protein are incompletely understood. Several factors, such as the nature of a specific client, its conformational state, and the availability of downstream chaperones and degradation machineries are likely to contribute for that probability.

4. Concluding remarks

The modulation of molecular chaperones is emerging as a potential therapeutic approach in polyQ disorders. Although genetic approaches have been valuable tools to identify the pathways that modulate the toxicity of polyQ-expanded proteins, the translation of these findings to the clinical practice would benefit from pharmacological agents with the ability to modify disease progression. Hsp90 inhibitors include a variety of molecules that have been extensively studied in the context of polyQ disorders with promising results. However, when considering the long-term use of such inhibitors it is important to have in mind that Hsp90 interacts with not-yet-unfolded client proteins, preserving their stability and activity. Long-term treatment with Hsp90 inhibitors may thus lead to functional disruption and unspecific degradation of multiple Hsp90 client proteins. Taking this into account, the ideal approach might be to promote the degradation of not properly folded proteins that are no longer cycling with Hsp90. In principle, this may be achieved with drugs that modulate Hsp70, promoting Hsp70-dependent degradation. Current limitations are that all the available Hsp70 modulators lack selectivity for individual Hsp70 family members, increasing the chances of disrupting essential functions of multiple Hsp70 isoforms, including the Hsp70 present in the mitochondria. As such, in addition to the development of selective Hsp70 inhibitors, future studies in polyQ models should include the effects of Hsp70 modulators on mitochondrial Hsp70 functions, including mitochondrial protein import and folding. Moreover, given that the consequences of modulating Hsp70 activity seem to depend on the nature of the specific client protein, it currently seems unlikely that the same Hsp70 modulator will provide therapeutic benefits for all polyQ disorders. Furthermore, since Hsp70 may not only influence the levels of soluble mutant protein but also act upon aggregates, it will be important to clarify the relative pathological role of such protein species and how these are affected by different Hsp70 modulators.

Conflict of interest

The authors declare they have no conflict of interest

References cited only in Tables and Figure legends

[213-224]

References

1. Hipp MS, Park SH, Hartl FU (2014) Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends in cell biology 24 (9):506-514. doi:10.1016/j.tcb.2014.05.003

2. Balch WE, Morimoto RI, Dillin A, Kelly JW (2008) Adapting proteostasis for disease intervention. Science 319 (5865):916-919. doi:10.1126/science.1141448

3. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475 (7356):324-332. doi:10.1038/nature10317

4. Kim YE, Hipp MS, Bracher A, Hayer-Hartl M, Hartl FU (2013) Molecular chaperone functions in protein folding and proteostasis. Annual review of biochemistry 82:323-355. doi:10.1146/annurev-biochem-060208-092442

5. Lindquist S (1986) The heat-shock response. Annual review of biochemistry 55:1151-1191. doi:10.1146/annurev.bi.55.070186.005443

6. Karagoz GE, Rudiger SG (2015) Hsp90 interaction with clients. Trends in biochemical sciences 40 (2):117-125. doi:10.1016/j.tibs.2014.12.002

7. Doyle SM, Genest O, Wickner S (2013) Protein rescue from aggregates by powerful molecular chaperone machines. Nature reviews Molecular cell biology 14 (10):617-629. doi:10.1038/nrm3660

8. Kirstein J, Moliere N, Dougan DA, Turgay K (2009) Adapting the machine: adaptor proteins for Hsp100/Clp and AAA+ proteases. Nature reviews Microbiology 7 (8):589-599. doi:10.1038/nrmicro2185

9. Smith HL, Li W, Cheetham ME (2015) Molecular chaperones and neuronal proteostasis. Seminars in cell & developmental biology 40:142-152. doi:10.1016/j.semcdb.2015.03.003

10. Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and alpha-crystallins: dynamic proteins with flexible functions. Trends in biochemical sciences 37 (3):106-117. doi:10.1016/j.tibs.2011.11.005

11. Kampinga HH, Garrido C (2012) HSPBs: small proteins with big implications in human disease. The international journal of biochemistry & cell biology 44 (10):1706-1710. doi:10.1016/j.biocel.2012.06.005

12. Eyles SJ, Gierasch LM (2010) Nature's molecular sponges: small heat shock proteins grow into their chaperone roles. Proc Natl Acad Sci U S A 107 (7):2727-2728. doi:10.1073/pnas.0915160107

13. Shrestha L, Patel HJ, Chiosis G (2016) Chemical Tools to Investigate Mechanisms Associated with HSP90 and HSP70 in Disease. Cell chemical biology 23 (1):158-172. doi:10.1016/j.chembiol.2015.12.006

14. Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP (2015) Targeting Hsp90/Hsp70-based protein quality control for treatment of adult onset neurodegenerative diseases. Annu Rev Pharmacol Toxicol 55:353-371. doi:10.1146/annurev-pharmtox-010814-124332

15. Kirschke E, Goswami D, Southworth D, Griffin PR, Agard DA (2014) Glucocorticoid receptor function regulated by coordinated action of the Hsp90 and Hsp70 chaperone cycles. Cell 157 (7):1685-1697. doi:10.1016/j.cell.2014.04.038

16. Taipale M, Tucker G, Peng J, Krykbaeva I, Lin ZY, Larsen B, Choi H, Berger B, Gingras AC, Lindquist S (2014) A quantitative chaperone interaction network reveals the architecture of cellular protein homeostasis pathways. Cell 158 (2):434-448. doi:10.1016/j.cell.2014.05.039

17. Chiosis G, Dickey CA, Johnson JL (2013) A global view of Hsp90 functions. Nat Struct Mol Biol 20 (1):1-4. doi:10.1038/nsmb.2481

18. Mayer MP (2013) Hsp70 chaperone dynamics and molecular mechanism. Trends in biochemical sciences 38 (10):507-514. doi:10.1016/j.tibs.2013.08.001

19. Gao X, Carroni M, Nussbaum-Krammer C, Mogk A, Nillegoda NB, Szlachcic A, Guilbride DL, Saibil HR, Mayer MP, Bukau B (2015) Human Hsp70 Disaggregase Reverses Parkinson's-Linked alpha-Synuclein Amyloid Fibrils. Mol Cell 59 (5):781-793. doi:10.1016/j.molcel.2015.07.012

20. Nillegoda NB, Kirstein J, Szlachcic A, Berynskyy M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R, Guilbride DL, Wade RC, Morimoto RI, Mayer MP, Bukau B (2015) Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. Nature 524 (7564):247-251. doi:10.1038/nature14884

21. Labbadia J, Morimoto RI (2015) The biology of proteostasis in aging and disease. Annual review of biochemistry 84:435-464. doi:10.1146/annurev-biochem-060614-033955

22. Kaushik S, Cuervo AM (2015) Proteostasis and aging. Nature medicine 21 (12):1406-1415. doi:10.1038/nm.4001

23. Blum ES, Schwendeman AR, Shaham S (2013) PolyQ disease: misfiring of a developmental cell death program? Trends in cell biology 23 (4):168-174. doi:10.1016/j.tcb.2012.11.003

24. Orr HT, Zoghbi HY (2007) Trinucleotide repeat disorders. Annu Rev Neurosci 30:575-621. doi:10.1146/annurev.neuro.29.051605.113042

25. Orr HT (2012) Cell biology of spinocerebellar ataxia. J Cell Biol 197 (2):167-177. doi:10.1083/jcb.201105092

26. Katsuno M, Tanaka F, Adachi H, Banno H, Suzuki K, Watanabe H, Sobue G (2012) Pathogenesis and therapy of spinal and bulbar muscular atrophy (SBMA). Prog Neurobiol 99 (3):246-256. doi:10.1016/j.pneurobio.2012.05.007

27. Hong DS, Banerji U, Tavana B, George GC, Aaron J, Kurzrock R (2013) Targeting the molecular chaperone heat shock protein 90 (HSP90): lessons learned and future directions. Cancer treatment reviews 39 (4):375-387. doi:10.1016/j.ctrv.2012.10.001

28. Sherman MY, Gabai VL (2015) Hsp70 in cancer: back to the future. Oncogene 34 (32):4153-4161. doi:10.1038/onc.2014.349

29. Hay DG, Sathasivam K, Tobaben S, Stahl B, Marber M, Mestril R, Mahal A, Smith DL, Woodman B, Bates GP (2004) Progressive decrease in chaperone protein levels in a mouse model of Huntington's disease and induction of stress proteins as a therapeutic approach. Hum Mol Genet 13 (13):1389-1405. doi:10.1093/hmg/ddh144

30. Chafekar SM, Duennwald ML (2012) Impaired heat shock response in cells expressing full-length polyglutamine-expanded huntingtin. PLoS One 7 (5):e37929. doi:10.1371/journal.pone.0037929

31. Chou AH, Yeh TH, Ouyang P, Chen YL, Chen SY, Wang HL (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31 (1):89-101. doi:10.1016/j.nbd.2008.03.011

32. Yamanaka T, Miyazaki H, Oyama F, Kurosawa M, Washizu C, Doi H, Nukina N (2008) Mutant Huntingtin reduces HSP70 expression through the sequestration of NF-Y transcription factor. The EMBO journal 27 (6):827-839. doi:10.1038/emboj.2008.23

33. Gao XC, Zhou CJ, Zhou ZR, Zhang YH, Zheng XM, Song AX, Hu HY (2011) Co-chaperone HSJ1a dually regulates the proteasomal degradation of ataxin-3. PLoS One 6 (5):e19763. doi:10.1371/journal.pone.0019763

34. Akerfelt M, Morimoto RI, Sistonen L (2010) Heat shock factors: integrators of cell stress, development and lifespan. Nature reviews Molecular cell biology 11 (8):545-555. doi:10.1038/nrm2938

35. Anckar J, Sistonen L (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annual review of biochemistry 80:1089-1115. doi:10.1146/annurev-biochem-060809-095203

36. Neef DW, Jaeger AM, Thiele DJ (2011) Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. Nat Rev Drug Discov 10 (12):930-944. doi:10.1038/nrd3453

37. Saibil H (2013) Chaperone machines for protein folding, unfolding and disaggregation. Nature reviews Molecular cell biology 14 (10):630-642. doi:10.1038/nrm3658

38. Mayer MP, Prodromou C, Frydman J (2009) The Hsp90 mosaic: a picture emerges. Nat Struct Mol Biol 16 (1):2-6. doi:10.1038/nsmb0109-2

39. Karagoz GE, Duarte AM, Akoury E, Ippel H, Biernat J, Moran Luengo T, Radli M, Didenko T, Nordhues BA, Veprintsev DB, Dickey CA, Mandelkow E, Zweckstetter M, Boelens

R, Madl T, Rudiger SG (2014) Hsp90-Tau complex reveals molecular basis for specificity in chaperone action. Cell 156 (5):963-974. doi:10.1016/j.cell.2014.01.037

40. Lorenz OR, Freiburger L, Rutz DA, Krause M, Zierer BK, Alvira S, Cuellar J, Valpuesta JM, Madl T, Sattler M, Buchner J (2014) Modulation of the Hsp90 chaperone cycle by a stringent client protein. Mol Cell 53 (6):941-953. doi:10.1016/j.molcel.2014.02.003

41. Verba KA, Wang RY, Arakawa A, Liu Y, Shirouzu M, Yokoyama S, Agard DA (2016) Atomic structure of Hsp90-Cdc37-Cdk4 reveals that Hsp90 traps and stabilizes an unfolded kinase. Science 352 (6293):1542-1547. doi:10.1126/science.aaf5023

42. Rohl A, Rohrberg J, Buchner J (2013) The chaperone Hsp90: changing partners for demanding clients. Trends in biochemical sciences 38 (5):253-262. doi:10.1016/j.tibs.2013.02.003

43. Li J, Soroka J, Buchner J (2012) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta 1823 (3):624-635. doi:10.1016/j.bbamcr.2011.09.003

44. Taipale M, Jarosz DF, Lindquist S (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nature reviews Molecular cell biology 11 (7):515-528. doi:10.1038/nrm2918

45. Johnson JL (2012) Evolution and function of diverse Hsp90 homologs and cochaperone proteins. Biochim Biophys Acta 1823 (3):607-613. doi:10.1016/j.bbamcr.2011.09.020

46. Lee AS (2014) Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nature reviews Cancer 14 (4):263-276. doi:10.1038/nrc3701

47. Marzec M, Eletto D, Argon Y (2012) GRP94: An HSP90-like protein specialized for protein folding and quality control in the endoplasmic reticulum. Biochim Biophys Acta 1823 (3):774-787. doi:10.1016/j.bbamcr.2011.10.013

48. Behnke J, Feige MJ, Hendershot LM (2015) BiP and its nucleotide exchange factors Grp170 and Sil1: mechanisms of action and biological functions. J Mol Biol 427 (7):1589-1608. doi:10.1016/j.jmb.2015.02.011

49. Zhu G, Lee AS (2015) Role of the unfolded protein response, GRP78 and GRP94 in organ homeostasis. J Cell Physiol 230 (7):1413-1420. doi:10.1002/jcp.24923

50. Wang M, Kaufman RJ (2016) Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 529 (7586):326-335. doi:10.1038/nature17041

51. Melnyk A, Rieger H, Zimmermann R (2015) Co-chaperones of the mammalian endoplasmic reticulum. Sub-cellular biochemistry 78:179-200. doi:10.1007/978-3-319-11731-7_9

52. Eletto D, Dersh D, Argon Y (2010) GRP94 in ER quality control and stress responses. Seminars in cell & developmental biology 21 (5):479-485. doi:10.1016/j.semcdb.2010.03.004

53. Biswas C, Ostrovsky O, Makarewich CA, Wanderling S, Gidalevitz T, Argon Y (2007) The peptide-binding activity of GRP94 is regulated by calcium. Biochem J 405 (2):233-241. doi:10.1042/BJ20061867

54. Altieri DC (2013) Hsp90 regulation of mitochondrial protein folding: from organelle integrity to cellular homeostasis. Cell Mol Life Sci 70 (14):2463-2472. doi:10.1007/s00018-012-1177-0

55. Kang BH, Plescia J, Dohi T, Rosa J, Doxsey SJ, Altieri DC (2007) Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. Cell 131 (2):257-270. doi:10.1016/j.cell.2007.08.028

56. Rasola A, Neckers L, Picard D (2014) Mitochondrial oxidative phosphorylation TRAP(1)ped in tumor cells. Trends in cell biology 24 (8):455-463. doi:10.1016/j.tcb.2014.03.005

57. Rasola A, Bernardi P (2015) Reprint of "The mitochondrial permeability transition pore and its adaptive responses in tumor cells". Cell calcium 58 (1):18-26. doi:10.1016/j.ceca.2015.03.004

58. Takamura H, Koyama Y, Matsuzaki S, Yamada K, Hattori T, Miyata S, Takemoto K, Tohyama M, Katayama T (2012) TRAP1 controls mitochondrial fusion/fission balance through Drp1 and Mff expression. PLoS One 7 (12):e51912. doi:10.1371/journal.pone.0051912

59. Costa AC, Loh SH, Martins LM (2013) Drosophila Trap1 protects against mitochondrial dysfunction in a PINK1/parkin model of Parkinson's disease. Cell Death Dis 4:e467. doi:10.1038/cddis.2012.205

60. Zhang L, Karsten P, Hamm S, Pogson JH, Muller-Rischart AK, Exner N, Haass C, Whitworth AJ, Winklhofer KF, Schulz JB, Voigt A (2013) TRAP1 rescues PINK1 loss-of-function phenotypes. Hum Mol Genet 22 (14):2829-2841. doi:10.1093/hmg/ddt132

61. Takemoto K, Miyata S, Takamura H, Katayama T, Tohyama M (2011) Mitochondrial TRAP1 regulates the unfolded protein response in the endoplasmic reticulum. Neurochem Int 58 (8):880-887. doi:10.1016/j.neuint.2011.02.015

62. Amoroso MR, Matassa DS, Laudiero G, Egorova AV, Polishchuk RS, Maddalena F, Piscazzi A, Paladino S, Sarnataro D, Garbi C, Landriscina M, Esposito F (2012) TRAP1 and the proteasome regulatory particle TBP7/Rpt3 interact in the endoplasmic reticulum and control cellular ubiquitination of specific mitochondrial proteins. Cell Death Differ 19 (4):592-604. doi:10.1038/cdd.2011.128

63. Garcia-Carbonero R, Carnero A, Paz-Ares L (2013) Inhibition of HSP90 molecular chaperones: moving into the clinic. Lancet Oncol 14 (9):e358-369. doi:10.1016/S1470-2045(13)70169-4

64. Wang RE (2011) Targeting heat shock proteins 70/90 and proteasome for cancer therapy. Curr Med Chem 18 (27):4250-4264

65. Bhat R, Tummalapalli SR, Rotella DP (2014) Progress in the discovery and development of heat shock protein 90 (Hsp90) inhibitors. J Med Chem 57 (21):8718-8728. doi:10.1021/jm500823a

66. Duerfeldt AS, Peterson LB, Maynard JC, Ng CL, Eletto D, Ostrovsky O, Shinogle HE, Moore DS, Argon Y, Nicchitta CV, Blagg BS (2012) Development of a Grp94 inhibitor. Journal of the American Chemical Society 134 (23):9796-9804. doi:10.1021/ja303477g

67. Kang BH, Plescia J, Song HY, Meli M, Colombo G, Beebe K, Scroggins B, Neckers L, Altieri DC (2009) Combinatorial drug design targeting multiple cancer signaling networks controlled by mitochondrial Hsp90. J Clin Invest 119 (3):454-464. doi:10.1172/JCI37613

68. Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nature reviews Molecular cell biology 11 (8):579-592. doi:10.1038/nrm2941

69. Li Z, Hartl FU, Bracher A (2013) Structure and function of Hip, an attenuator of the Hsp70 chaperone cycle. Nat Struct Mol Biol 20 (8):929-935. doi:10.1038/nsmb.2608

70. Nillegoda NB, Bukau B (2015) Metazoan Hsp70-based protein disaggregases: emergence and mechanisms. Frontiers in molecular biosciences 2:57. doi:10.3389/fmolb.2015.00057

71. Lu TZ, Quan Y, Feng ZP (2010) Multifaceted role of heat shock protein 70 in neurons. Mol Neurobiol 42 (2):114-123. doi:10.1007/s12035-010-8116-6

72. Liu T, Daniels CK, Cao S (2012) Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. Pharmacol Ther 136 (3):354-374. doi:10.1016/j.pharmthera.2012.08.014

73. Ahn SG, Kim SA, Yoon JH, Vacratsis P (2005) Heat-shock cognate 70 is required for the activation of heat-shock factor 1 in mammalian cells. Biochem J 392 (Pt 1):145-152. doi:10.1042/BJ20050412

74. Daugaard M, Rohde M, Jaattela M (2007) The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. FEBS letters 581 (19):3702-3710. doi:10.1016/j.febslet.2007.05.039

75. Kaushik S, Cuervo AM (2012) Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends in cell biology 22 (8):407-417. doi:10.1016/j.tcb.2012.05.006

76. Maattanen P, Gehring K, Bergeron JJ, Thomas DY (2010) Protein quality control in the ER: the recognition of misfolded proteins. Seminars in cell & developmental biology 21 (5):500-511. doi:10.1016/j.semcdb.2010.03.006

77. Kang PJ, Ostermann J, Shilling J, Neupert W, Craig EA, Pfanner N (1990) Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature 348 (6297):137-143. doi:10.1038/348137a0

78. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N (2009) Importing mitochondrial proteins: machineries and mechanisms. Cell 138 (4):628-644. doi:10.1016/j.cell.2009.08.005

79. Stricher F, Macri C, Ruff M, Muller S (2013) HSPA8/HSC70 chaperone protein: structure, function, and chemical targeting. Autophagy 9 (12):1937-1954. doi:10.4161/auto.26448

80. Schmidt O, Pfanner N, Meisinger C (2010) Mitochondrial protein import: from proteomics to functional mechanisms. Nature reviews Molecular cell biology 11 (9):655-667. doi:10.1038/nrm2959

81. Dudek J, Rehling P, van der Laan M (2013) Mitochondrial protein import: common principles and physiological networks. Biochim Biophys Acta 1833 (2):274-285. doi:10.1016/j.bbamcr.2012.05.028

82. van der Laan M, Hutu DP, Rehling P (2010) On the mechanism of preprotein import by the mitochondrial presequence translocase. Biochim Biophys Acta 1803 (6):732-739. doi:10.1016/j.bbamcr.2010.01.013

83. Brodsky JL (1999) Selectivity of the molecular chaperone-specific immunosuppressive agent 15-deoxyspergualin: modulation of Hsc70 ATPase activity without compromising DnaJ chaperone interactions. Biochemical pharmacology 57 (8):877-880

84. Nadeau K, Nadler SG, Saulnier M, Tepper MA, Walsh CT (1994) Quantitation of the interaction of the immunosuppressant deoxyspergualin and analogs with Hsc70 and Hsp90. Biochemistry 33 (9):2561-2567

85. Leu JI, Pimkina J, Frank A, Murphy ME, George DL (2009) A small molecule inhibitor of inducible heat shock protein 70. Mol Cell 36 (1):15-27. doi:10.1016/j.molcel.2009.09.023

86. Otaka M, Yamamoto S, Ogasawara K, Takaoka Y, Noguchi S, Miyazaki T, Nakai A, Odashima M, Matsuhashi T, Watanabe S, Itoh H (2007) The induction mechanism of the molecular chaperone HSP70 in the gastric mucosa by Geranylgeranylacetone (HSP-inducer). Biochemical and biophysical research communications 353 (2):399-404. doi:10.1016/j.bbrc.2006.12.031

87. Jinwal UK, Miyata Y, Koren J, 3rd, Jones JR, Trotter JH, Chang L, O'Leary J, Morgan D, Lee DC, Shults CL, Rousaki A, Weeber EJ, Zuiderweg ER, Gestwicki JE, Dickey CA (2009) Chemical manipulation of hsp70 ATPase activity regulates tau stability. J Neurosci 29 (39):12079-12088. doi:10.1523/JNEUROSCI.3345-09.2009

88. Wisen S, Bertelsen EB, Thompson AD, Patury S, Ung P, Chang L, Evans CG, Walter GM, Wipf P, Carlson HA, Brodsky JL, Zuiderweg ER, Gestwicki JE (2010) Binding of a small molecule at a protein-protein interface regulates the chaperone activity of hsp70-hsp40. ACS Chem Biol 5 (6):611-622. doi:10.1021/cb1000422

89. Chafekar SM, Wisen S, Thompson AD, Echeverria A, Walter GM, Evans CG, Makley LN, Gestwicki JE, Duennwald ML (2012) Pharmacological tuning of heat shock protein 70

modulates polyglutamine toxicity and aggregation. ACS Chem Biol 7 (9):1556-1564. doi:10.1021/cb300166p

90. Massey AJ, Williamson DS, Browne H, Murray JB, Dokurno P, Shaw T, Macias AT, Daniels Z, Geoffroy S, Dopson M, Lavan P, Matassova N, Francis GL, Graham CJ, Parsons R, Wang Y, Padfield A, Comer M, Drysdale MJ, Wood M (2010) A novel, small molecule inhibitor of Hsc70/Hsp70 potentiates Hsp90 inhibitor induced apoptosis in HCT116 colon carcinoma cells. Cancer Chemother Pharmacol 66 (3):535-545. doi:10.1007/s00280-009-1194-3

91. Chang L, Miyata Y, Ung PM, Bertelsen EB, McQuade TJ, Carlson HA, Zuiderweg ER, Gestwicki JE (2011) Chemical screens against a reconstituted multiprotein complex: myricetin blocks DnaJ regulation of DnaK through an allosteric mechanism. Chem Biol 18 (2):210-221. doi:10.1016/j.chembiol.2010.12.010

92. Ko SK, Kim J, Na DC, Park S, Park SH, Hyun JY, Baek KH, Kim ND, Kim NK, Park YN, Song K, Shin I (2015) A small molecule inhibitor of ATPase activity of HSP70 induces apoptosis and has antitumor activities. Chem Biol 22 (3):391-403. doi:10.1016/j.chembiol.2015.02.004

93. Miyata Y, Rauch JN, Jinwal UK, Thompson AD, Srinivasan S, Dickey CA, Gestwicki JE (2012) Cysteine reactivity distinguishes redox sensing by the heat-inducible and constitutive forms of heat shock protein 70. Chem Biol 19 (11):1391-1399. doi:10.1016/j.chembiol.2012.07.026

94. Rousaki A, Miyata Y, Jinwal UK, Dickey CA, Gestwicki JE, Zuiderweg ER (2011) Allosteric drugs: the interaction of antitumor compound MKT-077 with human Hsp70 chaperones. J Mol Biol 411 (3):614-632. doi:10.1016/j.jmb.2011.06.003

95. Koren J, 3rd, Miyata Y, Kiray J, O'Leary JC, 3rd, Nguyen L, Guo J, Blair LJ, Li X, Jinwal UK, Cheng JQ, Gestwicki JE, Dickey CA (2012) Rhodacyanine derivative selectively targets cancer cells and overcomes tamoxifen resistance. PLoS One 7 (4):e35566. doi:10.1371/journal.pone.0035566

96. Miyata Y, Li X, Lee HF, Jinwal UK, Srinivasan SR, Seguin SP, Young ZT, Brodsky JL, Dickey CA, Sun D, Gestwicki JE (2013) Synthesis and initial evaluation of YM-08, a blood-brain barrier permeable derivative of the heat shock protein 70 (Hsp70) inhibitor MKT-077, which reduces tau levels. ACS Chem Neurosci 4 (6):930-939. doi:10.1021/cn300210g

97. Wang AM, Miyata Y, Klinedinst S, Peng HM, Chua JP, Komiyama T, Li X, Morishima Y, Merry DE, Pratt WB, Osawa Y, Collins CA, Gestwicki JE, Lieberman AP (2013) Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. Nat Chem Biol 9 (2):112-118. doi:10.1038/nchembio.1140

98. Kettern N, Dreiseidler M, Tawo R, Hohfeld J (2010) Chaperone-assisted degradation: multiple paths to destruction. Biological chemistry 391 (5):481-489. doi:10.1515/BC.2010.058

99. Pratt WB, Morishima Y, Gestwicki JE, Lieberman AP, Osawa Y (2014) A model in which heat shock protein 90 targets protein-folding clefts: rationale for a new approach to neuroprotective treatment of protein folding diseases. Experimental biology and medicine 239 (11):1405-1413. doi:10.1177/1535370214539444

100. Koga H, Martinez-Vicente M, Arias E, Kaushik S, Sulzer D, Cuervo AM (2011) Constitutive upregulation of chaperone-mediated autophagy in Huntington's disease. J Neurosci 31 (50):18492-18505. doi:10.1523/JNEUROSCI.3219-11.2011

101. Minoia M, Boncoraglio A, Vinet J, Morelli FF, Brunsting JF, Poletti A, Krom S, Reits E, Kampinga HH, Carra S (2014) BAG3 induces the sequestration of proteasomal clients into cytoplasmic puncta: implications for a proteasome-to-autophagy switch. Autophagy 10 (9):1603-1621. doi:10.4161/auto.29409

102. Dantuma NP, Bott LC (2014) The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. Front Mol Neurosci 7:70. doi:10.3389/fnmol.2014.00070

103. Edkins AL (2015) CHIP: a co-chaperone for degradation by the proteasome. Sub-cellular biochemistry 78:219-242. doi:10.1007/978-3-319-11731-7_11

104. Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C (2009) Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. The EMBO journal 28 (7):889-901. doi:10.1038/emboj.2009.29

105. Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, Ash P, Shoraka S, Zlatkovic J, Eckman CB, Patterson C, Dickson DW, Nahman NS, Jr., Hutton M, Burrows F, Petrucelli L (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest 117 (3):648-658. doi:10.1172/JCI29715

106. Thompson AD, Scaglione KM, Prensner J, Gillies AT, Chinnaiyan A, Paulson HL, Jinwal UK, Dickey CA, Gestwicki JE (2012) Analysis of the tau-associated proteome reveals that exchange of Hsp70 for Hsp90 is involved in tau degradation. ACS Chem Biol 7 (10):1677-1686. doi:10.1021/cb3002599

107. Gatchel JR, Zoghbi HY (2005) Diseases of unstable repeat expansion: mechanisms and common principles. Nature reviews Genetics 6 (10):743-755. doi:10.1038/nrg1691

108. Rub U, Schols L, Paulson H, Auburger G, Kermer P, Jen JC, Seidel K, Korf HW, Deller T (2013) Clinical features, neurogenetics and neuropathology of the polyglutamine spinocerebellar ataxias type 1, 2, 3, 6 and 7. Prog Neurobiol 104:38-66. doi:10.1016/j.pneurobio.2013.01.001

109. Walsh DM, Selkoe DJ (2016) A critical appraisal of the pathogenic protein spread hypothesis of neurodegeneration. Nature reviews Neuroscience 17 (4):251-260. doi:10.1038/nrn.2016.13

110. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431 (7010):805-810. doi:10.1038/nature02998

111. Labbadia J, Morimoto RI (2013) Huntington's disease: underlying molecular mechanisms and emerging concepts. Trends in biochemical sciences 38 (8):378-385. doi:10.1016/j.tibs.2013.05.003

112. Shirasaki DI, Greiner ER, Al-Ramahi I, Gray M, Boontheung P, Geschwind DH, Botas J, Coppola G, Horvath S, Loo JA, Yang XW (2012) Network organization of the huntingtin proteomic interactome in mammalian brain. Neuron 75 (1):41-57. doi:10.1016/j.neuron.2012.05.024

113. Schapira AH, Olanow CW, Greenamyre JT, Bezard E (2014) Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. Lancet 384 (9942):545-555. doi:10.1016/S0140-6736(14)61010-2

114. Guedes-Dias P, Pinho BR, Soares TR, de Proenca J, Duchen MR, Oliveira JM (2016) Mitochondrial dynamics and quality control in Huntington's disease. Neurobiol Dis 90:51-57. doi:10.1016/j.nbd.2015.09.008

115. Oliveira JM (2010) Mitochondrial bioenergetics and dynamics in Huntington's disease: tripartite synapses and selective striatal degeneration. Journal of bioenergetics and biomembranes 42 (3):227-234. doi:10.1007/s10863-010-9287-6

116. Oliveira JM, Lightowlers RN (2010) Could successful (mitochondrial) networking help prevent Huntington's disease? EMBO molecular medicine 2 (12):487-489. doi:10.1002/emmm.201000104

117. Oliveira JM (2010) Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. J Neurochem 114 (1):1-12. doi:10.1111/j.1471-4159.2010.06741.x

118. Yano H, Baranov SV, Baranova OV, Kim J, Pan Y, Yablonska S, Carlisle DL, Ferrante RJ, Kim AH, Friedlander RM (2014) Inhibition of mitochondrial protein import by mutant huntingtin. Nat Neurosci 17 (6):822-831. doi:10.1038/nn.3721

119. Bennett EJ, Shaler TA, Woodman B, Ryu KY, Zaitseva TS, Becker CH, Bates GP, Schulman H, Kopito RR (2007) Global changes to the ubiquitin system in Huntington's disease. Nature 448 (7154):704-708. doi:10.1038/nature06022

120. Hipp MS, Patel CN, Bersuker K, Riley BE, Kaiser SE, Shaler TA, Brandeis M, Kopito RR (2012) Indirect inhibition of 26S proteasome activity in a cellular model of Huntington's disease. J Cell Biol 196 (5):573-587. doi:10.1083/jcb.201110093

121. Hodges A, Strand AD, Aragaki AK, Kuhn A, Sengstag T, Hughes G, Elliston LA, Hartog C, Goldstein DR, Thu D, Hollingsworth ZR, Collin F, Synek B, Holmans PA, Young AB, Wexler NS, Delorenzi M, Kooperberg C, Augood SJ, Faull RL, Olson JM, Jones L, Luthi-

Carter R (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15 (6):965-977. doi:10.1093/hmg/ddl013

122. Wong YC, Holzbaur EL (2014) The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. J Neurosci 34 (4):1293-1305. doi:10.1523/JNEUROSCI.1870-13.2014

123. Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D, Cuervo AM (2010) Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 13 (5):567-576. doi:10.1038/nn.2528

124. Ochaba J, Lukacsovich T, Csikos G, Zheng S, Margulis J, Salazar L, Mao K, Lau AL, Yeung SY, Humbert S, Saudou F, Klionsky DJ, Finkbeiner S, Zeitlin SO, Marsh JL, Housman DE, Thompson LM, Steffan JS (2014) Potential function for the Huntingtin protein as a scaffold for selective autophagy. Proc Natl Acad Sci U S A 111 (47):16889-16894. doi:10.1073/pnas.1420103111

125. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 280 (12):11635-11640. doi:10.1074/jbc.M412042200

126. Al-Ramahi I, Lam YC, Chen HK, de Gouyon B, Zhang M, Perez AM, Branco J, de Haro M, Patterson C, Zoghbi HY, Botas J (2006) CHIP protects from the neurotoxicity of expanded and wild-type ataxin-1 and promotes their ubiquitination and degradation. J Biol Chem 281 (36):26714-26724. doi:10.1074/jbc.M601603200

127. Miller VM, Nelson RF, Gouvion CM, Williams A, Rodriguez-Lebron E, Harper SQ, Davidson BL, Rebagliati MR, Paulson HL (2005) CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. J Neurosci 25 (40):9152-9161. doi:10.1523/JNEUROSCI.3001-05.2005

128. Fujimoto M, Takaki E, Hayashi T, Kitaura Y, Tanaka Y, Inouye S, Nakai A (2005) Active HSF1 significantly suppresses polyglutamine aggregate formation in cellular and mouse models. J Biol Chem 280 (41):34908-34916. doi:10.1074/jbc.M506288200

129. Bersuker K, Hipp MS, Calamini B, Morimoto RI, Kopito RR (2013) Heat shock response activation exacerbates inclusion body formation in a cellular model of Huntington disease. J Biol Chem 288 (33):23633-23638. doi:10.1074/jbc.C113.481945

130. Sittler A, Lurz R, Lueder G, Priller J, Lehrach H, Hayer-Hartl MK, Hartl FU, Wanker EE (2001) Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. Hum Mol Genet 10 (12):1307-1315

131. Baldo B, Weiss A, Parker CN, Bibel M, Paganetti P, Kaupmann K (2012) A screen for enhancers of clearance identifies huntingtin as a heat shock protein 90 (Hsp90) client protein. J Biol Chem 287 (2):1406-1414. doi:10.1074/jbc.M111.294801

132. Ernst JT, Liu M, Zuccola H, Neubert T, Beaumont K, Turnbull A, Kallel A, Vought B, Stamos D (2014) Correlation between chemotype-dependent binding conformations of HSP90alpha/beta and isoform selectivity-Implications for the structure-based design of HSP90alpha/beta selective inhibitors for treating neurodegenerative diseases. Bioorganic & medicinal chemistry letters 24 (1):204-208. doi:10.1016/j.bmcl.2013.11.036

133. Jana NR, Tanaka M, Wang G, Nukina N (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum Mol Genet 9 (13):2009-2018

134. Rujano MA, Kampinga HH, Salomons FA (2007) Modulation of polyglutamine inclusion formation by the Hsp70 chaperone machine. Experimental cell research 313 (16):3568-3578. doi:10.1016/j.yexcr.2007.07.034

135. Zhou H, Li SH, Li XJ (2001) Chaperone suppression of cellular toxicity of huntingtin is independent of polyglutamine aggregation. J Biol Chem 276 (51):48417-48424. doi:10.1074/jbc.M104140200

136. Hageman J, Rujano MA, van Waarde MA, Kakkar V, Dirks RP, Govorukhina N, Oosterveld-Hut HM, Lubsen NH, Kampinga HH (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. Mol Cell 37 (3):355-369. doi:10.1016/j.molcel.2010.01.001

137. Labbadia J, Novoselov SS, Bett JS, Weiss A, Paganetti P, Bates GP, Cheetham ME (2012) Suppression of protein aggregation by chaperone modification of high molecular weight complexes. Brain : a journal of neurology 135 (Pt 4):1180-1196. doi:10.1093/brain/aws022

138. Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, Kurokawa R, Housman DE, Jackson GR, Marsh JL, Thompson LM (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. Nature 413 (6857):739-743. doi:10.1038/35099568

139. Guedes-Dias P, de Proenca J, Soares TR, Leitao-Rocha A, Pinho BR, Duchen MR, Oliveira JM (2015) HDAC6 inhibition induces mitochondrial fusion, autophagic flux and reduces diffuse mutant huntingtin in striatal neurons. Biochim Biophys Acta 1852 (11):2484-2493. doi:10.1016/j.bbadis.2015.08.012

140. Oliveira JM, Chen S, Almeida S, Riley R, Goncalves J, Oliveira CR, Hayden MR, Nicholls DG, Ellerby LM, Rego AC (2006) Mitochondrial-dependent Ca2+ handling in Huntington's disease striatal cells: effect of histone deacetylase inhibitors. J Neurosci 26 (43):11174-11186. doi:10.1523/JNEUROSCI.3004-06.2006

141. Fujikake N, Nagai Y, Popiel HA, Okamoto Y, Yamaguchi M, Toda T (2008) Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem 283 (38):26188-26197. doi:10.1074/jbc.M710521200

142. Neef DW, Turski ML, Thiele DJ (2010) Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. PLoS Biol 8 (1):e1000291. doi:10.1371/journal.pbio.1000291

143. Calamini B, Silva MC, Madoux F, Hutt DM, Khanna S, Chalfant MA, Saldanha SA, Hodder P, Tait BD, Garza D, Balch WE, Morimoto RI (2012) Small-molecule proteostasis regulators for protein conformational diseases. Nat Chem Biol 8 (2):185-196. doi:10.1038/nchembio.763

144. Orr HT (2012) Polyglutamine neurodegeneration: expanded glutamines enhance native functions. Curr Opin Genet Dev 22 (3):251-255. doi:10.1016/j.gde.2012.01.001

145. Takeyama K, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, Miura M, Tabata T, Kato S (2002) Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. Neuron 35 (5):855-864

146. Montie HL, Cho MS, Holder L, Liu Y, Tsvetkov AS, Finkbeiner S, Merry DE (2009) Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 18 (11):1937-1950. doi:10.1093/hmg/ddp115

147. Nedelsky NB, Pennuto M, Smith RB, Palazzolo I, Moore J, Nie Z, Neale G, Taylor JP (2010) Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. Neuron 67 (6):936-952. doi:10.1016/j.neuron.2010.08.034

148. Sopher BL, Thomas PS, Jr., LaFevre-Bernt MA, Holm IE, Wilke SA, Ware CB, Jin LW, Libby RT, Ellerby LM, La Spada AR (2004) Androgen receptor YAC transgenic mice recapitulate SBMA motor neuronopathy and implicate VEGF164 in the motor neuron degeneration. Neuron 41 (5):687-699

149. Katsuno M, Adachi H, Minamiyama M, Waza M, Doi H, Kondo N, Mizoguchi H, Nitta A, Yamada K, Banno H, Suzuki K, Tanaka F, Sobue G (2010) Disrupted transforming growth factor-beta signaling in spinal and bulbar muscular atrophy. J Neurosci 30 (16):5702-5712. doi:10.1523/JNEUROSCI.0388-10.2010

150. Young JE, Garden GA, Martinez RA, Tanaka F, Sandoval CM, Smith AC, Sopher BL, Lin A, Fischbeck KH, Ellerby LM, Morrison RS, Taylor JP, La Spada AR (2009) Polyglutamine-expanded androgen receptor truncation fragments activate a Bax-dependent apoptotic cascade mediated by DP5/Hrk. J Neurosci 29 (7):1987-1997. doi:10.1523/JNEUROSCI.4072-08.2009

151. Ranganathan S, Harmison GG, Meyertholen K, Pennuto M, Burnett BG, Fischbeck KH (2009) Mitochondrial abnormalities in spinal and bulbar muscular atrophy. Hum Mol Genet 18 (1):27-42. doi:10.1093/hmg/ddn310

152. Adachi H, Waza M, Tokui K, Katsuno M, Minamiyama M, Tanaka F, Doyu M, Sobue G (2007) CHIP overexpression reduces mutant androgen receptor protein and ameliorates

phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model. J Neurosci 27 (19):5115-5126. doi:10.1523/JNEUROSCI.1242-07.2007

153. Morishima Y, Wang AM, Yu Z, Pratt WB, Osawa Y, Lieberman AP (2008) CHIP deletion reveals functional redundancy of E3 ligases in promoting degradation of both signaling proteins and expanded glutamine proteins. Hum Mol Genet 17 (24):3942-3952. doi:10.1093/hmg/ddn296

154. Howarth JL, Glover CP, Uney JB (2009) HSP70 interacting protein prevents the accumulation of inclusions in polyglutamine disease. J Neurochem 108 (4):945-951. doi:10.1111/j.0022-3042.2008.05847.x

155. Adachi H, Katsuno M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, Kusakabe M, Yoshiki A, Kobayashi Y, Doyu M, Sobue G (2003) Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci 23 (6):2203-2211

156. Bailey CK, Andriola IF, Kampinga HH, Merry DE (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. Hum Mol Genet 11 (5):515-523

157. Kobayashi Y, Kume A, Li M, Doyu M, Hata M, Ohtsuka K, Sobue G (2000) Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. J Biol Chem 275 (12):8772-8778

158. Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, Doyu M, Sobue G (2005) Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci U S A 102 (46):16801-16806. doi:10.1073/pnas.0506249102

159. Tokui K, Adachi H, Waza M, Katsuno M, Minamiyama M, Doi H, Tanaka K, Hamazaki J, Murata S, Tanaka F, Sobue G (2009) 17-DMAG ameliorates polyglutamine-mediated motor neuron degeneration through well-preserved proteasome function in an SBMA model mouse. Hum Mol Genet 18 (5):898-910. doi:10.1093/hmg/ddn419

160. Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, Inukai A, Doyu M, Sobue G (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nature medicine 11 (10):1088-1095. doi:10.1038/nm1298

161. Rusmini P, Simonini F, Crippa V, Bolzoni E, Onesto E, Cagnin M, Sau D, Ferri N, Poletti A (2011) 17-AAG increases autophagic removal of mutant androgen receptor in spinal and bulbar muscular atrophy. Neurobiol Dis 41 (1):83-95. doi:10.1016/j.nbd.2010.08.023

162. Fujiki M, Kobayashi H, Abe T, Ishii K (2003) Astroglial activation accompanies heat shock protein upregulation in rat brain following single oral dose of geranylgeranylacetone. Brain Res 991 (1-2):254-257

163. Sinn DI, Chu K, Lee ST, Song EC, Jung KH, Kim EH, Park DK, Kang KM, Kim M, Roh JK (2007) Pharmacological induction of heat shock protein exerts neuroprotective effects in experimental intracerebral hemorrhage. Brain Res 1135 (1):167-176. doi:10.1016/j.brainres.2006.11.098

164. Wang AM, Morishima Y, Clapp KM, Peng HM, Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP (2010) Inhibition of hsp70 by methylene blue affects signaling protein function and ubiquitination and modulates polyglutamine protein degradation. J Biol Chem 285 (21):15714-15723. doi:10.1074/jbc.M109.098806

165. Jacobi H, du Montcel ST, Bauer P, Giunti P, Cook A, Labrum R, Parkinson MH, Durr A, Brice A, Charles P, Marelli C, Mariotti C, Nanetti L, Panzeri M, Rakowicz M, Sulek A, Sobanska A, Schmitz-Hubsch T, Schols L, Hengel H, Baliko L, Melegh B, Filla A, Antenora A, Infante J, Berciano J, van de Warrenburg BP, Timmann D, Szymanski S, Boesch S, Kang JS, Pandolfo M, Schulz JB, Molho S, Diallo A, Klockgether T (2015) Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. The Lancet Neurology 14 (11):1101-1108. doi:10.1016/S1474-4422(15)00202-1

166. Shen Y, Peterson AS (2009) Atrophins' emerging roles in development and neurodegenerative disease. Cell Mol Life Sci 66 (3):437-446. doi:10.1007/s00018-008-8403-9

167. Tsuji S (2012) Dentatorubral-pallidoluysian atrophy. Handbook of clinical neurology 103:587-594. doi:10.1016/B978-0-444-51892-7.00041-3

168. Lim J, Hao T, Shaw C, Patel AJ, Szabo G, Rual JF, Fisk CJ, Li N, Smolyar A, Hill DE, Barabasi AL, Vidal M, Zoghbi HY (2006) A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell 125 (4):801-814. doi:10.1016/j.cell.2006.03.032

169. de Chiara C, Menon RP, Strom M, Gibson TJ, Pastore A (2009) Phosphorylation of S776 and 14-3-3 binding modulate ataxin-1 interaction with splicing factors. PLoS One 4 (12):e8372. doi:10.1371/journal.pone.0008372

170. Lim J, Crespo-Barreto J, Jafar-Nejad P, Bowman AB, Richman R, Hill DE, Orr HT, Zoghbi HY (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 452 (7188):713-718. doi:10.1038/nature06731

171. Yue S, Serra HG, Zoghbi HY, Orr HT (2001) The spinocerebellar ataxia type 1 protein, ataxin-1, has RNA-binding activity that is inversely affected by the length of its polyglutamine tract. Hum Mol Genet 10 (1):25-30

172. Serra HG, Byam CE, Lande JD, Tousey SK, Zoghbi HY, Orr HT (2004) Gene profiling links SCA1 pathophysiology to glutamate signaling in Purkinje cells of transgenic mice. Hum Mol Genet 13 (20):2535-2543. doi:10.1093/hmg/ddh268

173. Serra HG, Duvick L, Zu T, Carlson K, Stevens S, Jorgensen N, Lysholm A, Burright E, Zoghbi HY, Clark HB, Andresen JM, Orr HT (2006) RORalpha-mediated Purkinje cell development determines disease severity in adult SCA1 mice. Cell 127 (4):697-708. doi:10.1016/j.cell.2006.09.036

174. Crespo-Barreto J, Fryer JD, Shaw CA, Orr HT, Zoghbi HY (2010) Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genet 6 (7):e1001021. doi:10.1371/journal.pgen.1001021

175. Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB, Zoghbi HY, Orr HT (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95 (1):41-53

176. Magana JJ, Velazquez-Perez L, Cisneros B (2013) Spinocerebellar ataxia type 2: clinical presentation, molecular mechanisms, and therapeutic perspectives. Mol Neurobiol 47 (1):90-104. doi:10.1007/s12035-012-8348-8

177. Dansithong W, Paul S, Figueroa KP, Rinehart MD, Wiest S, Pflieger LT, Scoles DR, Pulst SM (2015) Ataxin-2 regulates RGS8 translation in a new BAC-SCA2 transgenic mouse model. PLoS Genet 11 (4):e1005182. doi:10.1371/journal.pgen.1005182

178. Hansen ST, Meera P, Otis TS, Pulst SM (2013) Changes in Purkinje cell firing and gene expression precede behavioral pathology in a mouse model of SCA2. Hum Mol Genet 22 (2):271-283. doi:10.1093/hmg/dds427

179. Huynh DP, Figueroa K, Hoang N, Pulst SM (2000) Nuclear localization or inclusion body formation of ataxin-2 are not necessary for SCA2 pathogenesis in mouse or human. Nat Genet 26 (1):44-50. doi:10.1038/79162

180. Costa Mdo C, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97 (2):239-257. doi:10.1016/j.pneurobio.2011.11.006

181. Li X, Liu H, Fischhaber PL, Tang TS (2015) Toward therapeutic targets for SCA3: Insight into the role of Machado-Joseph disease protein ataxin-3 in misfolded proteins clearance. Prog Neurobiol 132:34-58. doi:10.1016/j.pneurobio.2015.06.004

182. Scaglione KM, Zavodszky E, Todi SV, Patury S, Xu P, Rodriguez-Lebron E, Fischer S, Konen J, Djarmati A, Peng J, Gestwicki JE, Paulson HL (2011) Ube2w and ataxin-3 coordinately regulate the ubiquitin ligase CHIP. Mol Cell 43 (4):599-612. doi:10.1016/j.molcel.2011.05.036

183. Bichelmeier U, Schmidt T, Hubener J, Boy J, Ruttiger L, Habig K, Poths S, Bonin M, Knipper M, Schmidt WJ, Wilbertz J, Wolburg H, Laccone F, Riess O (2007) Nuclear

localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. J Neurosci 27 (28):7418-7428. doi:10.1523/JNEUROSCI.4540-06.2007

184. Giunti P, Mantuano E, Frontali M, Veneziano L (2015) Molecular mechanism of Spinocerebellar Ataxia type 6: glutamine repeat disorder, channelopathy and transcriptional dysregulation. The multifaceted aspects of a single mutation. Front Cell Neurosci 9:36. doi:10.3389/fncel.2015.00036

185. Du X, Wang J, Zhu H, Rinaldo L, Lamar KM, Palmenberg AC, Hansel C, Gomez CM (2013) Second cistron in CACNA1A gene encodes a transcription factor mediating cerebellar development and SCA6. Cell 154 (1):118-133. doi:10.1016/j.cell.2013.05.059

186. Ishiguro T, Ishikawa K, Takahashi M, Obayashi M, Amino T, Sato N, Sakamoto M, Fujigasaki H, Tsuruta F, Dolmetsch R, Arai T, Sasaki H, Nagashima K, Kato T, Yamada M, Takahashi H, Hashizume Y, Mizusawa H (2010) The carboxy-terminal fragment of alpha(1A) calcium channel preferentially aggregates in the cytoplasm of human spinocerebellar ataxia type 6 Purkinje cells. Acta neuropathologica 119 (4):447-464. doi:10.1007/s00401-009-0630-0

187. Helmlinger D, Hardy S, Abou-Sleymane G, Eberlin A, Bowman AB, Gansmuller A, Picaud S, Zoghbi HY, Trottier Y, Tora L, Devys D (2006) Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. PLoS Biol 4 (3):e67. doi:10.1371/journal.pbio.0040067

188. Chou AH, Chen CY, Chen SY, Chen WJ, Chen YL, Weng YS, Wang HL (2010) Polyglutamine-expanded ataxin-7 causes cerebellar dysfunction by inducing transcriptional dysregulation. Neurochem Int 56 (2):329-339. doi:10.1016/j.neuint.2009.11.003

189. Yoo SY, Pennesi ME, Weeber EJ, Xu B, Atkinson R, Chen S, Armstrong DL, Wu SM, Sweatt JD, Zoghbi HY (2003) SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. Neuron 37 (3):383-401

190. Seidel K, Siswanto S, Brunt ER, den Dunnen W, Korf HW, Rub U (2012) Brain pathology of spinocerebellar ataxias. Acta neuropathologica 124 (1):1-21. doi:10.1007/s00401-012-1000-x

191. Vannini A, Cramer P (2012) Conservation between the RNA polymerase I, II, and III transcription initiation machineries. Mol Cell 45 (4):439-446. doi:10.1016/j.molcel.2012.01.023

192. Sainsbury S, Bernecky C, Cramer P (2015) Structural basis of transcription initiation by RNA polymerase II. Nature reviews Molecular cell biology 16 (3):129-143. doi:10.1038/nrm3952

193. Friedman MJ, Shah AG, Fang ZH, Ward EG, Warren ST, Li S, Li XJ (2007) Polyglutamine domain modulates the TBP-TFIIB interaction: implications for its normal function and neurodegeneration. Nat Neurosci 10 (12):1519-1528. doi:10.1038/nn2011

194. Friedman MJ, Wang CE, Li XJ, Li S (2008) Polyglutamine expansion reduces the association of TATA-binding protein with DNA and induces DNA binding-independent neurotoxicity. J Biol Chem 283 (13):8283-8290. doi:10.1074/jbc.M709674200

195. Shah AG, Friedman MJ, Huang S, Roberts M, Li XJ, Li S (2009) Transcriptional dysregulation of TrkA associates with neurodegeneration in spinocerebellar ataxia type 17. Hum Mol Genet 18 (21):4141-4152. doi:10.1093/hmg/ddp363

196. Huang S, Ling JJ, Yang S, Li XJ, Li S (2011) Neuronal expression of TATA box-binding protein containing expanded polyglutamine in knock-in mice reduces chaperone protein response by impairing the function of nuclear factor-Y transcription factor. Brain : a journal of neurology 134 (Pt 7):1943-1958. doi:10.1093/brain/awr146

197. Zhang S, Xu L, Lee J, Xu T (2002) Drosophila atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. Cell 108 (1):45-56

198. Suzuki K, Zhou J, Sato T, Takao K, Miyagawa T, Oyake M, Yamada M, Takahashi H, Takahashi Y, Goto J, Tsuji S (2012) DRPLA transgenic mouse substrains carrying single copy of full-length mutant human DRPLA gene with variable sizes of expanded CAG repeats exhibit CAG repeat length- and age-dependent changes in behavioral abnormalities and gene expression profiles. Neurobiol Dis 46 (2):336-350. doi:10.1016/j.nbd.2012.01.014

199. Nucifora FC, Jr., Ellerby LM, Wellington CL, Wood JD, Herring WJ, Sawa A, Hayden MR, Dawson VL, Dawson TM, Ross CA (2003) Nuclear localization of a non-caspase truncation product of atrophin-1, with an expanded polyglutamine repeat, increases cellular toxicity. J Biol Chem 278 (15):13047-13055. doi:10.1074/jbc.M211224200

200. Choi JY, Ryu JH, Kim HS, Park SG, Bae KH, Kang S, Myung PK, Cho S, Park BC, Lee do H (2007) Co-chaperone CHIP promotes aggregation of ataxin-1. Mol Cell Neurosci 34 (1):69-79. doi:10.1016/j.mcn.2006.10.002

201. Williams AJ, Knutson TM, Colomer Gould VF, Paulson HL (2009) In vivo suppression of polyglutamine neurotoxicity by C-terminus of Hsp70-interacting protein (CHIP) supports an aggregation model of pathogenesis. Neurobiol Dis 33 (3):342-353. doi:10.1016/j.nbd.2008.10.016

202. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19 (2):148-154. doi:10.1038/502

203. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19 (23):10338-10347

204. Helmlinger D, Bonnet J, Mandel JL, Trottier Y, Devys D (2004) Hsp70 and Hsp40 chaperones do not modulate retinal phenotype in SCA7 mice. J Biol Chem 279 (53):55969-55977. doi:10.1074/jbc.M409062200

205. Lin CH, Wu YR, Kung PJ, Chen WL, Lee LC, Lin TH, Chao CY, Chen CM, Chang KH, Janreddy D, Lee-Chen GJ, Yao CF (2014) The potential of indole and a synthetic derivative for polyQ aggregation reduction by enhancement of the chaperone and autophagy systems. ACS Chem Neurosci 5 (10):1063-1074. doi:10.1021/cn500075u

206. Chan HY, Warrick JM, Gray-Board GL, Paulson HL, Bonini NM (2000) Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in Drosophila. Hum Mol Genet 9 (19):2811-2820

207. Cummings CJ, Sun Y, Opal P, Antalffy B, Mestril R, Orr HT, Dillmann WH, Zoghbi HY (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum Mol Genet 10 (14):1511-1518

208. Yang S, Huang S, Gaertig MA, Li XJ, Li S (2014) Age-dependent decrease in chaperone activity impairs MANF expression, leading to Purkinje cell degeneration in inducible SCA17 mice. Neuron 81 (2):349-365. doi:10.1016/j.neuron.2013.12.002

209. Silva-Fernandes A, Duarte-Silva S, Neves-Carvalho A, Amorim M, Soares-Cunha C, Oliveira P, Thirstrup K, Teixeira-Castro A, Maciel P (2014) Chronic treatment with 17-DMAG improves balance and coordination in a new mouse model of Machado-Joseph disease. Neurotherapeutics 11 (2):433-449. doi:10.1007/s13311-013-0255-9

210. Kung PJ, Tao YC, Hsu HC, Chen WL, Lin TH, Janreddy D, Yao CF, Chang KH, Lin JY, Su MT, Wu CH, Lee-Chen GJ, Hsieh-Li HM (2014) Indole and synthetic derivative activate chaperone expression to reduce polyQ aggregation in SCA17 neuronal cell and slice culture models. Drug design, development and therapy 8:1929-1939. doi:10.2147/DDDT.S67376

211. Kilpatrick K, Novoa JA, Hancock T, Guerriero CJ, Wipf P, Brodsky JL, Segatori L (2013) Chemical induction of Hsp70 reduces alpha-synuclein aggregation in neuroglioma cells. ACS Chem Biol 8 (7):1460-1468. doi:10.1021/cb400017h

212. Abisambra J, Jinwal UK, Miyata Y, Rogers J, Blair L, Li X, Seguin SP, Wang L, Jin Y, Bacon J, Brady S, Cockman M, Guidi C, Zhang J, Koren J, Young ZT, Atkins CA, Zhang B, Lawson LY, Weeber EJ, Brodsky JL, Gestwicki JE, Dickey CA (2013) Allosteric heat shock protein 70 inhibitors rapidly rescue synaptic plasticity deficits by reducing aberrant tau. Biol Psychiatry 74 (5):367-374. doi:10.1016/j.biopsych.2013.02.027

213. Wyttenbach A, Carmichael J, Swartz J, Furlong RA, Narain Y, Rankin J, Rubinsztein DC (2000) Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington's disease. Proc Natl Acad Sci U S A 97 (6):2898-2903

214. Hansson O, Nylandsted J, Castilho RF, Leist M, Jaattela M, Brundin P (2003) Overexpression of heat shock protein 70 in R6/2 Huntington's disease mice has only modest effects on disease progression. Brain Res 970 (1-2):47-57

215. Tagawa K, Marubuchi S, Qi ML, Enokido Y, Tamura T, Inagaki R, Murata M, Kanazawa I, Wanker EE, Okazawa H (2007) The induction levels of heat shock protein 70 differentiate the vulnerabilities to mutant huntingtin among neuronal subtypes. J Neurosci 27 (4):868-880. doi:10.1523/JNEUROSCI.4522-06.2007

216. Guzhova IV, Lazarev VF, Kaznacheeva AV, Ippolitova MV, Muronetz VI, Kinev AV, Margulis BA (2011) Novel mechanism of Hsp70 chaperone-mediated prevention of polyglutamine aggregates in a cellular model of huntington disease. Hum Mol Genet 20 (20):3953-3963. doi:10.1093/hmg/ddr314

217. Wacker JL, Huang SY, Steele AD, Aron R, Lotz GP, Nguyen Q, Giorgini F, Roberson ED, Lindquist S, Masliah E, Muchowski PJ (2009) Loss of Hsp70 exacerbates pathogenesis but not levels of fibrillar aggregates in a mouse model of Huntington's disease. J Neurosci 29 (28):9104-9114. doi:10.1523/JNEUROSCI.2250-09.2009

218. Jiang Y, Lv H, Liao M, Xu X, Huang S, Tan H, Peng T, Zhang Y, Li H (2012) GRP78 counteracts cell death and protein aggregation caused by mutant huntingtin proteins. Neuroscience letters 516 (2):182-187. doi:10.1016/j.neulet.2012.03.074

219. Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, DeMartino GN, Marcelli M, Weigel NL, Mancini MA (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet 8 (5):731-741

220. Howarth JL, Kelly S, Keasey MP, Glover CP, Lee YB, Mitrophanous K, Chapple JP, Gallo JM, Cheetham ME, Uney JB (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Molecular therapy : the journal of the American Society of Gene Therapy 15 (6):1100-1105. doi:10.1038/sj.mt.6300163

221. Li L, Saegusa H, Tanabe T (2009) Deficit of heat shock transcription factor 1-heat shock 70 kDa protein 1A axis determines the cell death vulnerability in a model of spinocerebellar ataxia type 6. Genes to cells : devoted to molecular & cellular mechanisms 14 (11):1253-1269. doi:10.1111/j.1365-2443.2009.01348.x

222. Herbst M, Wanker EE (2007) Small molecule inducers of heat-shock response reduce polyQ-mediated huntingtin aggregation. A possible therapeutic strategy. Neuro-degenerative diseases 4 (2-3):254-260. doi:10.1159/000101849

223. Thomas M, Harrell JM, Morishima Y, Peng HM, Pratt WB, Lieberman AP (2006) Pharmacologic and genetic inhibition of hsp90-dependent trafficking reduces aggregation and promotes degradation of the expanded glutamine androgen receptor without stress protein induction. Hum Mol Genet 15 (11):1876-1883. doi:10.1093/hmg/ddl110

224. Labbadia J, Cunliffe H, Weiss A, Katsyuba E, Sathasivam K, Seredenina T, Woodman B, Moussaoui S, Frentzel S, Luthi-Carter R, Paganetti P, Bates GP (2011) Altered chromatin

architecture underlies progressive impairment of the heat shock response in mouse models of Huntington disease. J Clin Invest 121 (8):3306-3319. doi:10.1172/JCI57413
225. Mayer MP, Le Breton L (2015) Hsp90: breaking the symmetry. Mol Cell 58 (1):8-20. doi:10.1016/j.molcel.2015.02.022

Figure legends

Fig. 1. Regulation of Hsp transcription by heat shock factor 1 (HSF1). a Domain structure of human HSF1 (NCBI accession number: NP_005517.1). **b** Proteotoxic insults convert inactive monomers of HSF1 into trimers with DNA binding activity. HSF1 trimers bind heat shock elements (HSE) and induce transcription of HSP genes. The availability of heat shock proteins exerts a feedback regulation on HSF1 activity: Hsp90 can interact with either (i) monomeric or (ii) trimeric HSF1, preventing their activation; (iii) Hsp70 and Hsp40 can inhibit the transactivation capacity of HSF1 [34-36].

Fig. 2. The Hsp90 reaction cycle. a Domain structure of human Hsp90 family members (NCBI accession numbers: Hsp90α, AAI21063.1; Hsp90β, AAH68474.1; GRP94, AAH66656.1; TRAP-1, AAH23585.1). **b** The ATP-bound open state of the Hsp90 dimer interacts with inactive client proteins transferred from the Hsp70 system by the co-chaperone Hop (Hsp70-Hsp90 organizing protein). Hop and co-chaperones such as Cdc37 (cell division cycle 37 homolog) stabilize the open conformation of Hsp90, facilitating client binding and inhibiting ATP hydrolysis, whereas the activator of Hsp90 ATPase, Aha1, induces the transition to the closed conformation. The co-chaperone p23 stabilizes the Hsp90 closed conformation, trapping the client protein. After ATP hydrolysis, the client protein is released in an active state [4,225,42].

Fig. 3. Hsp70 reaction cycle. a Domain structure of human Hsp70 family members (NCBI accession numbers: Hsp72, AAH18740.1; Hsc70, AAH19816.1; GRP78, AAI12964.1; mtHsp70, AAH24034.1). **b** ATP binding promotes the opening of the substrate-binding site, allowing interaction of Hsp70 with unfolded proteins recruited by the co-chaperone Hsp40. Hsp40 stimulates ATP hydrolysis, inducing the shutting of the substrate-binding site and, consequently, the transition to the ADP-bound closed conformation. Hsp70-interacting protein (Hip) locks ADP in the nucleotide binding domain, delaying substrate release. In contrast, nucleotide exchange factors (NEFs), which have a high affinity for Hsp70 when bound to ADP, trigger ADP dissociation and induce protein release upon rebinding of ATP. After dissociation from Hsp70, proteins incapable of proper folding may rebind, or be transferred to downstream chaperones or to the degradation machinery [4,37,68]. **c** Simplified model of Hsp70-dependent protein disaggregation. Hsp40 co-chaperones (J proteins) target protein aggregates and recruit Hsp70. Hsp110 is also recruited to form a chaperone complex with Hsp70 and Hsp40, whose cooperative pulling forces (dashed red arrows) promote protein disaggregation. The NEF activity of Hsp110 triggers peptide release from the aggregate [70,20].

Fig. 4. Simplified model of Hsp90/Hsp70 chaperone machinery in client protein degradation. Dissociation from the Hsp90 machinery allows the unfolding of the client protein. Hsp70 binds the unfolded protein and recruits the chaperone-dependent E3 ubiquitin ligase CHIP. CHIP then targets E2 ubiquitin-conjugating enzymes to the unfolded protein promoting its ubiquitination and subsequent proteasomal degradation. This simplified model (adapted from [97]) does not include all possible pathways, being noteworthy that Hsp90 and CHIP may form a complex that is proposed to recognize and target for degradation clients such as phosphorylated tau [105].

Table 1. Proteins of the human Hsp90 family.

Gene	Protein designations [a]	Localization	Stress inducible	Identity (%) [b]
HSP90AA1	Heat shock protein 90kDa alpha class A member 1; **Hsp90α**; Hsp86; Hspc1	Cytoplasm, Nucleus	Yes	100
HSP90AB1	Heat shock protein 90kDa alpha class B member 1; **Hsp90β**; Hsp84; Hspc3	Cytoplasm, Nucleus	No	86
HSP90B1	Heat shock protein 90kDa beta member 1; **Grp94**; Gp96; endoplasmin; Hspc4	Endoplasmic reticulum	Yes	46
TRAP1	TNF receptor-associated protein 1; **TRAP1**; Hsp75; Hspc5	Mitochondria	-	30

[a] Protein designations used in this review are in **bold**.

[b] BLASTP results relative to the HSP90AA1 sequence on the NCBI database. Accession numbers: AAI21063.1; AAH68474.1; AAH66656.1; AAH23585.1.

Table 2. Proteins of the human Hsp70 family.

Gene	Protein designations [a]	Localization	Stress inducible	Identity (%) [b]
HSPA1A/B	Heat shock 70kDa protein 1A/B; Hsp70-1/-2; **Hsp72**	Cytoplasm, Nucleus	Yes	100
HSPA1L	Heat shock 70kDa protein 1-like; Hsp70-1l; Hsp70-HOM	Cytoplasm, Nucleus	No	90
HSPA2	Heat shock 70kDa protein 2	Cytoplasm, Nucleus	No	85
HSPA4	Heat shock 70kDa protein 4; Hsp70RY	Cytoplasm	Yes	33
HSPA4L	Heat shock 70kDa protein 4L; APG-1; Osp94	Cytoplasm	Yes	33
HSPA5	Heat shock 70kDa protein 5; **Grp78**; BiP	Endoplasmic reticulum	Yes	64
HSPA6	Heat shock 70kDa protein 6; HSP70B'	Cytoplasm, Nucleus	Yes	83
HSPA7	Heat shock 70kDa protein 7; HSP70B	Cytoplasm, Nucleus	Yes	84
HSPA8	Heat shock 70kDa protein 8; **Hsc70**; Hsp73	Cytoplasm, Nucleus	No	87
HSPA9	**mtHsp70**; Heat shock 70kDa protein 9; Grp75	Mitochondria	No	52
HSPA12A	Heat shock 70kDa protein 12A	Cytoplasm, Nucleus	-	26
HSPA12B	Heat shock 70kDa protein 12B	Cytoplasm, Nucleus	-	25
HSPA13	Heat shock 70kDa protein 13; STCH	Endoplasmic reticulum	No	39
HSPA14	Heat shock 70kDa protein 14; Hsp70L1	Cytoplasm	Yes	35

[a] Protein designations used in this review are in **bold**.

[b] BLASTP results relative to the HSPA1A sequence on the NCBI database. Accession numbers: AAH18740.1; NP_005518.3; AAH36107.1; NP_002145.3; NP_055093.2; AAI12964.1; NP_002146.2; P48741.2; AAH19816.1; AAH24034.1; NP_079291.2; AAI43933.1; P48723.1; Q0VDF9.1.

Adapted from [71, 79].

Table 3. Polyglutamine Disorders Summary

Disease (Prevalence [a])	Protein	Normal Q length	Pathogenic Q length	Neuropathology	Main clinical features
HD (5-10/100,000)	Huntingtin	6-34	36-121	Marked neuronal loss in the striatum and cerebral cortex	Chorea, dystonia, bradykinesia, rigidity, cognitive deficits, psychiatric problems
SBMA (1/30,000)	Androgen receptor	9-34	38-62	Degeneration of lower motor neurons in the anterior horn, bulbar region, and dorsal root ganglia	Motor weakness, gynecomastia, testicular atrophy, decreased fertility
SCA1 (1-2/100,000)	Ataxin-1	6-44[b]	39-91	Degeneration of Purkinje cells, cerebellar dentate, inferior olive and red nuclei	Ataxia, slurred speech, spasticity, cognitive impairments
SCA2 (1-2/100,000)	Ataxin-2	14-32	>32	Degeneration of Purkinje and granule neurons	Ataxia, decreased reflexes, retinopathy in infantile cases
SCA3 (1-2/100,000)	Ataxin-3	11-44	45-86	Degeneration of subthalamic nucleus, substantia nigra, dentate nucleus, pontine and cranial nerve nuclei	Ataxia, parkinsonism, spasticity
SCA6 (<1/100,000)	CACNA1A	4-18	19-33	Degeneration of Purkinje cells	Ataxia, dysarthria, nystagmus, tremors
SCA7 (<1/100,000)	Ataxin-7	4-35	>35	Degeneration of retina, cerebellar Purkinje and granule cells	Ataxia, blindness, cardiac failure in infantile cases
SCA17 (unknown)	TBP	25-40	42-66	Degeneration of small neurons in the caudate and putamen, Purkinje cells and frontal and temporal cortex	Ataxia, cognitive decline, seizures, and psychiatric problems
DRPLA (unknown)	Atrophin-1	6-35	49-93	Degeneration of Purkinje cells, cerebral cortex, globus palidus, striatum, dentate, subthalamic and red nuclei	Ataxia, seizures, choreoathetosis, dementia

[a] Worldwide prevalence values from http://www.orpha.net. Orpha numbers: ORPHA399; ORPHA481; ORPHA98755; ORPHA98756; ORPHA98757; ORPHA98758; ORPHA94147; ORPHA98759; ORPHA101.

[b] Normal SCA1 alleles are interrupted by 1-4 CAT sequences, whereas disease-causing alleles are uninterrupted.

Adapted from [23, 24].

Table 4. Genetic Hsp modulation in HD models

Protein	Modulation	Model	Mutant Protein		Survival	Other Outcomes	References
			Aggregation	Levels			
CHIP	Overexpression	**Zebrafish** embryos (Q82)	-	-	↑	improved morphology	[127]
		Drosophila (Q128)	-	-	-	↓ retinal degeneration	[126]
		Cells (Cos-7; Q82)	↓	↑	-	-	[127]
		Cells (Neuro2a; Q150)	↓	-	↑	↑ mHtt ubiquitination	[125]
		Cells (O23; Q74)	=	↓	-	-	[134]
	Reduction	**Mice** (N171-Q82)	↑	-	↓	↓ DARPP-32 levels in striatum; ↑ motor dysfunction	[127]
HSF1	Overexpression	**Mice** (R6/2 without HSF1 overexpression in brain)	↓ (skeletal muscle) = (brain)	-	↑	↓ skeletal muscle damage; = brain atrophy; = weight loss; = paw-clasping	[128]
		Cells (U2OS Tet-On; Q91)	↑	-	↓	-	[129]
Hsp90	Overexpression	**Cells** (COS-1; Q51)	=	-	-	-	[130]
	Reduction	**Cells** (HN10; Q72)	-	↓	-	-	[131]
		Cells (HEK293; Q73)	-	↓	-	-	[132]
Hsp40	Overexpresssion	**Mice** (R6/2)	↓	↑	-	↓ motor dysfunction; ↑ BDNF levels; = weight loss	[137]
		Xenopus laevis tadpole (Q119)	↓	-	-	-	[136]
		Primary neurons (striatum; Q120)	↓	-	-	↓ DNA fragmentation	[135]
		Cells (Neuro2a; Q150)	↓	-	↑	-	[133]
		Cells (COS-7; Q74)	↑	-	-	-	[213]
		Cells (PC12/SH-SY5Y; Q74)	=	-	-	-	
		Cells (COS-1; Q51)	↓	-	-	-	[130]
		Cells (HEK293; Q120)	↓	-	↑	↓ caspase activity	[135]
		Cells (O23/N2a; Q74)	↓	-	-	-	[134]
		Cells (Q119)	↓	-	↑	-	[136]
	Reduction	**Cells** (HEK293; Q74)	↓	-	-	-	[136]
Hsp70	Overexpression	**Mice** (R6/2)	=	-	=	↓ body weight loss; = clasping phenotype; = brain weight loss; = striatum size; = DARPP32 levels	[214]
		Mice (R6/2)	↓ (in early stages)	=	-	↑ weight loss; = motor dysfunction	[29]
		Primary neurons (striatum; Q120)	=	-	-	↓ DNA fragmentation	[135]
		Primary neurons (cortex; Q111)	-	-	↑	-	[215]
		Cells (Neuro2a: Q150)	↓ (Hsc70); = (Hsp72)	-	= (Hsc70)	-	[133]
		Cells (COS-7; Q74)	=	-	-	-	[213]
		Cells (HEK293; Q120)	=	-	↑	↓ caspase activity	[135]
		Cells (COS-1; Q51)	↓	-	-	-	[130]
		Cells (O23/N2a; Q74)	=	-	-	-	[134]
		Cells (Q119)	=	-	-	-	[136]
		Cells (SK-N-SH; Q103)	↓	↑	-	-	[216]
		Cells (Neuro2a; Q150)	↓(Grp78)	-	↑(Grp78)	↓ caspase activity (Grp78)	[217]
	Reduction	**Mice** (R6/2)	↑	-	↓	↑ motor dysfunction; worsened coat appearance; = weight loss	[215]
		Primary neurons (cerebellum; Q111)	-	-	↓	-	[218]

Table 5. Pharmacological Hsp modulation in polyglutamine disorders

Disease	Target	Pharmacodynamics	Drug	Model	Mutant Protein		Survival	Other outcomes	References
					Aggregation	Levels			
HD	HSF1	Activation	HSF1A	**Cells** (PC12; Q74)	↓	-	↑	-	[142]
			F1	**Cells** (PC12; Q74)	↓	=	-	-	[143]
				Cells (U2OS; Q91)	↑	-	-	-	[129]
	Hsp90	ATPase activity inhibition	Geldanamycin	***Drosophila*** (Q128)	-	-	-	↓ photoreceptor degeneration	[141]
				Slice (R6/2; hippocampus)	↓	=	-	-	[29]
				Cells (COS-1; Q72)	↓	-	-	-	[130]
			Radicicol	***Drosophila*** (Q128)	-	-	-	↓ photoreceptor degeneration	[141]
				Slice (R6/2; hippocampus)	↓	↑	-	-	[29]
			17-DMAG	**Cells** (COS-1; Q72)	↓	-	-	-	[222]
			17-AAG	***Drosophila*** (Q128)	-	-	-	↓ photoreceptor degeneration	[141]
				Cells (COS-1; Q72)	↓	-	-	-	[222]
			NVP-HSP990	**Mice** (R6/2)	↓	-	-	Improved rotarod performance; ↑ brain weight; = grip strength; = exploratory activity; = weight loss	[224]
			NVP-AUY922	**Cells** (HN10; Q72/ ES; Q150)	-	↓	-	-	[131]
	Hsp70	ATPase activity stimulation	SW02	**Yeast** (Q46)	↓	↑	↓	-	[89]
				Cells (PC12; Q103)	↓	-	=	-	
		ATPase activity inhibition	CE12	**Yeast** (Q46)	↑	↓	↑	-	
				Cells (PC12; Q103)	↑	-	↑	-	
SBMA	Hsp90	ATPase activity inhibition	Geldanamycin	**Cells** (MEFs; Q112)	↓	-	-	-	[223]
				Cells (MEFs/MN-1; Q112)	-	↓	-	-	[153]
			Radicicol	**Cells** (MEFs; Q112)	↓	-	-	-	[223]
			17-DMAG	**Mice** (Q97)	↓	↓	↑	↓ motor dysfunction; ↓ weight loss	[159]
				Cells (SH-SY5Y; Q97)	-	↓	-	-	
			17-AAG	**Mice** (Q97)	↓	↓	↑	↓ motor dysfunction; ↓ weight loss	[160]
				Cells (SH-SY5Y; Q97)	-	↓	-	-	
				Cells (SH-SY5Y; Q97)	-	↓	-	-	[159]
				Cells (NSC34; Q48)	↓	↓	-	-	[161]
	Hsp70	HSF-1 binding inhibition	GGA	**Mice** (Q97)	-	↓	↑	↓ motor dysfunction; ↓ weight loss	[158]
				Cells (SH-SY5Y; Q97)	=	↓	↑	-	
		ATPase activity inhibition	Methylene blue	**Cells** (HeLa; Q112)	-	↑	-	-	[164]
		ATPase activity inhibition / ADP-bound form stabilization	YM-1	***Drosophila*** (Q52)	-	-	-	↓ eye degeneration; ↑ eclosion	[97]
				Cells (PC12; Q112)	↓	↓	-	-	
SCA3	HSF1	Activation	HSF1A	***Drosophila*** (Q78)	-	-	-	↓ eye degeneration	[142]
	Hsp90	ATPase activity inhibition	Geldanamycin	***Drosophila*** (Q78)	-	-	-	↓ eye degeneration	[141]
				Cells (MN-1; Q78)	-	=	-	-	[153]
			Radicicol	***Drosophila*** (Q78)	-	-	-	↓ eye degeneration	[141]
			17-DMAG	**Mice** (Q135)	↓	↓	-	↓ motor dysfunction; = body weight; ↑ beclin-1 and LC3-II levels	[209]

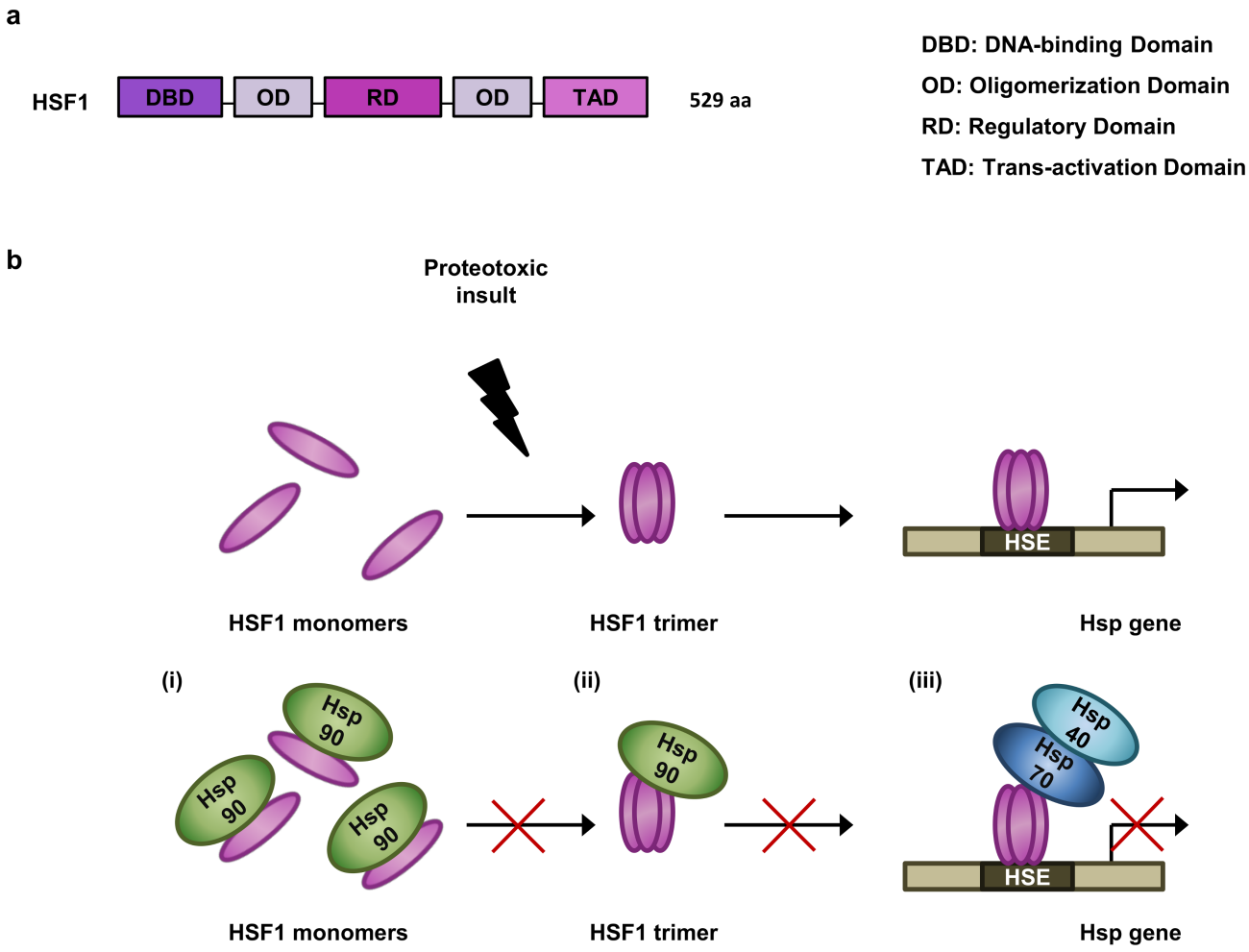
			17-AAG	***Drosophila*** (Q78)	↓	=	↑	↓ eye degeneration	[141]
				Drosophila (Q78)	-	-	-	↓ eye degeneration	[142]
	Hsp70	HSF1 binding inhibition	GGA	***Drosophila*** (Q78)	-	-	-	= eye degeneration	[141]
				Cells (HEK293; Q75)	↓	=	-	-	[205]
				Cells (SH-SY5Y; Q75)	↓	-	-	↑ neurite outgrowth	
		ATPase activity inhibition	Azure C	**Cells** (HEK293; Q22/71)	-	↑	-	↑ levels of wild type ataxin-3	[33]
	-	-	Indole/NC001-8	**Cells** (HEK293; Q75)	↓	=	-	↓ caspase activity; ↑ LC3-II levels; ↓ ROS production	[205]
				Cells (SH-SY5Y; Q75)	↓	-	-	↑ neurite outgrowth	
SCA17	**Hsp70**	HSF1 binding inhibition	GGA	**Cells** (SH-SY5Y; Q79)	↓	-	-	-	[210]
	-	-	Indole/NC001-8	**Slice** (cerebellum; Q109)	↓	-	-	-	
				Primary neurons (cerebellum; Q109)	↓	-	-	↑ neurite outgrowth	
				Cells (SH-SY5Y; Q79)	↓	-	-	-	

Table 6. Genetic Hsp modulation in SBMA models

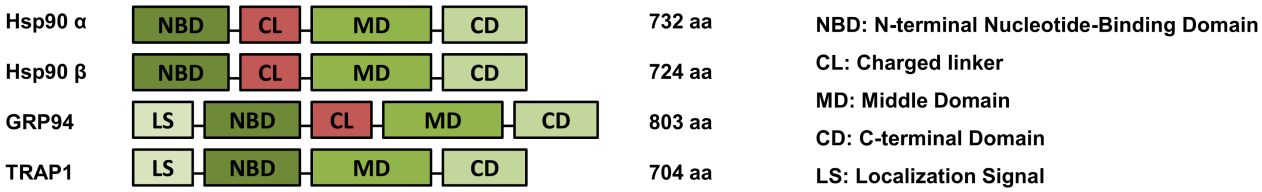
Protein	Modulation	Model	Mutant Protein		Survival	Other Outcomes	References
			Aggregation	Levels			
CHIP	Overexpression	**Mice** (Q97)	↓	↓	↑	↓ motor dysfunction; ↓ muscle atrophy; ↓ weight loss	[152]
		Cells (SH-SY5Y; Q65)	-	↓	-	-	
		Cells (MN-1; Q112)	-	↓	-	-	[153]
HIP	Overexpression	***Drosophila*** (Q52)	-	-	-	↑ eclosion	[97]
		Cells (N2a; Q51)	↓	-	-	-	[154]
		Cells (HeLa; Q112)	↓	↓	-	-	[97]
Hsp40	Overexpression	**Cells** (HeLa; Q48)	↓	-	-	-	[219]
		Cells (Neuro2a: Q97)	↓	-	↑	-	[157]
		Cells (MN hybrid cell; Q112)	↓	-	-	-	[156]
		Cells (N2a; Q51)	↓	-	-	-	[220]
		Cells (N2a; Q51)	↓	-	-	-	[154]
		Cells (Q72)	↓	-	-	-	[136]
Hsp70	Overexpression	**Mice** (Q97)	↓	↓	-	↓ motor dysfunction; ↓ weight loss	[155]
		Cells (Neuro2a: Q97)	↓	-	↑	-	[157]
		Cells (MN hybrid cell; Q112)	↓	-	-	-	[156]
		Cells (N2a; Q51)	↓	-	-	-	[220]
		Cells (N2a; Q51)	↓	-	-	-	[154]

Table 7. Genetic Hsp modulation in SCAs and DRPLA models

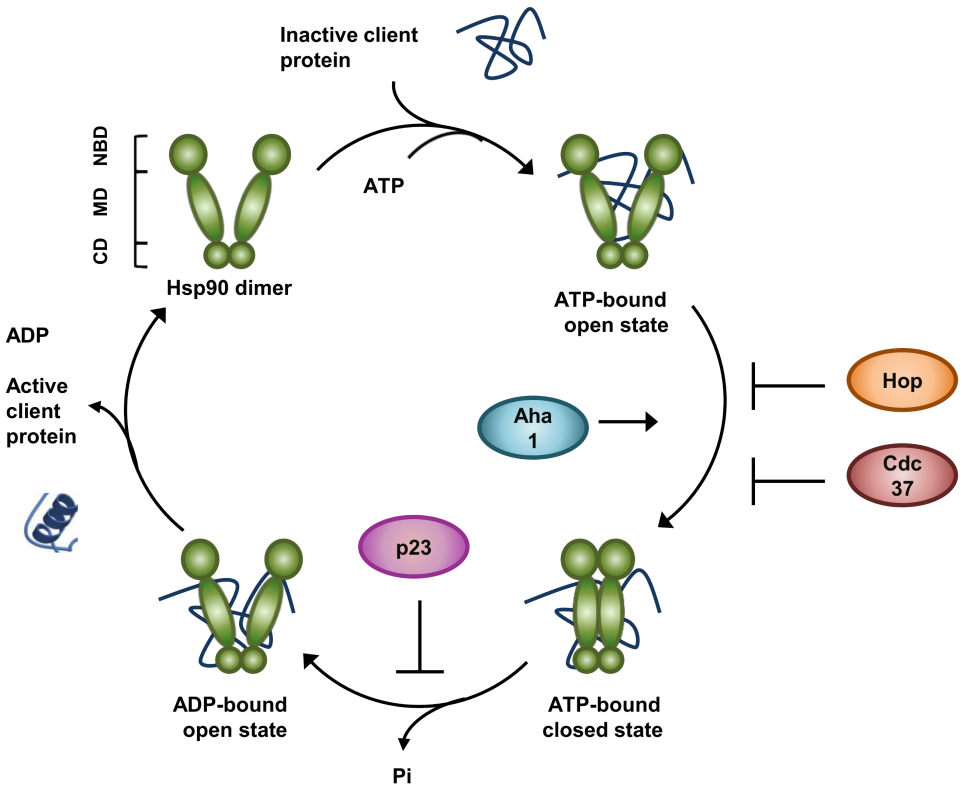
Disease	Protein	Modulation	Model	Mutant Protein		Survival	Other Outcomes	References
				Aggregation	Levels			
SCA1	CHIP	Overexpression	***Drosophila*** (Q2/30/82)	-	↓	-	↓ eye degeneration	[126]
			Cells (HeLa; Q82)	-	-	-	↑ mutant ataxin-1 ubiquitination	
			Cells (BOSC23; Q30/82)	↑	↓	-	↑ aggregation and ↓ levels of wild type ataxin-1; ↑ ubiquitination of both wild-type and mutant ataxin-1 in insoluble fraction	[200]
	Hsp40	Overexpression	**Cells** (HeLa; Q92)	↓	-	-	-	[202]
	Hsp70	Overexpression	**Mice** (B05)	=	-	-	improved motor function; improved purkinje cell morphology	[207]
SCA3	HSF1	Reduction	***Drosophila*** (Q78)	-	-	-	↑ eye degeneration	[141]
	CHIP	Overexpression	**Cells** (Neuro2a; Q80/130)	↓	↓	-	↑ mutant ataxin-3 ubiquitination;	[125]
			Cells (BOSC23; Q73)	-	↓	-	-	[200]
			Cells (HEK293/MN-1; Q78)	-	↓	-	-	[153]
			Cells (M17; Q80)	-	↓	-	-	[201]
			Cells (HEK293; Q80)	-	=	-	-	
			Cells (HEK293; Q22)	-	-	-	↑ ubiquitination and ↓ levels of wild type ataxin-3	[33]
		Reduction	**Mice** (Q71-B)	↑	-	-	↑ motor dysfunction; ↑ nuclear localization of mutant ataxin-3	[201]
			Cells (HEK293; Q22)	-	-	-	↑ levels of wild type ataxin-3	[33]
	Hsp40	Overexpression	***Drosophila*** (Q78)	-	-	↑	↓ eye degeneration	[206]
			Cells (Q82)	↓	-	-	-	[136]
			Cells (HEK293; Q22/71)	↓	↑	-	↑ ubiquitination and levels of wild type ataxin-3	[33]
			Cells (COS7/PC12; Q80)	↓	-	-	-	[203]
	Hsp70	Overexpression	***Drosophila*** (Q78)	-	-	↑	↓ eye degeneration	[206]
			Cells (COS7/PC12; Q80)	=	-	-	-	[203]
			Cells (HEK293; Q22)	-	-	-	↓ levels of wild type ataxin-3	[33]
			Cells (HEK293; Q75)	↓	-	-	-	[205]
SCA6	Hsp70	Reduction	**Cells** (HEK293; Q24)	-	-	=	-	[221]
SCA7	Hsp40	Overexpression	**Mice** (R7E; Hsp overexpression exclusively in rod photoreceptors)	-	-	-	= rod photoreceptors dysfunction	[204]
			Cells (HEK293; Q128)	=	-	-	-	
	Hsp70	Overexpression	**Mice** (R7E; Hsp overexpression exclusively in rod photoreceptors)	-	-	-	= rod photoreceptors dysfunction	[204]
			Cells (HEK293; Q128)	↓	-	-	-	
SCA17	Hsp70	Overexpression	**Mice** (Q105)	-	-	-	↓ degeneration of purkinje cells (Hsc70)	[208]
			Cells (PC12; Q105)	-	-	-	↓ neurite outgrowth deficit (Hsc70)	
DRPLA	HSF1	Overexpression	**Cells** (HeLa; Q81)	↓	-	-	-	[128]
	Hsp40	Overexpression	**Cells** (HeLa; Q81)	↓	-	-	-	
	Hsp70	Overexpression	**Cells** (HeLa; Q81)	↓	-	-	-	

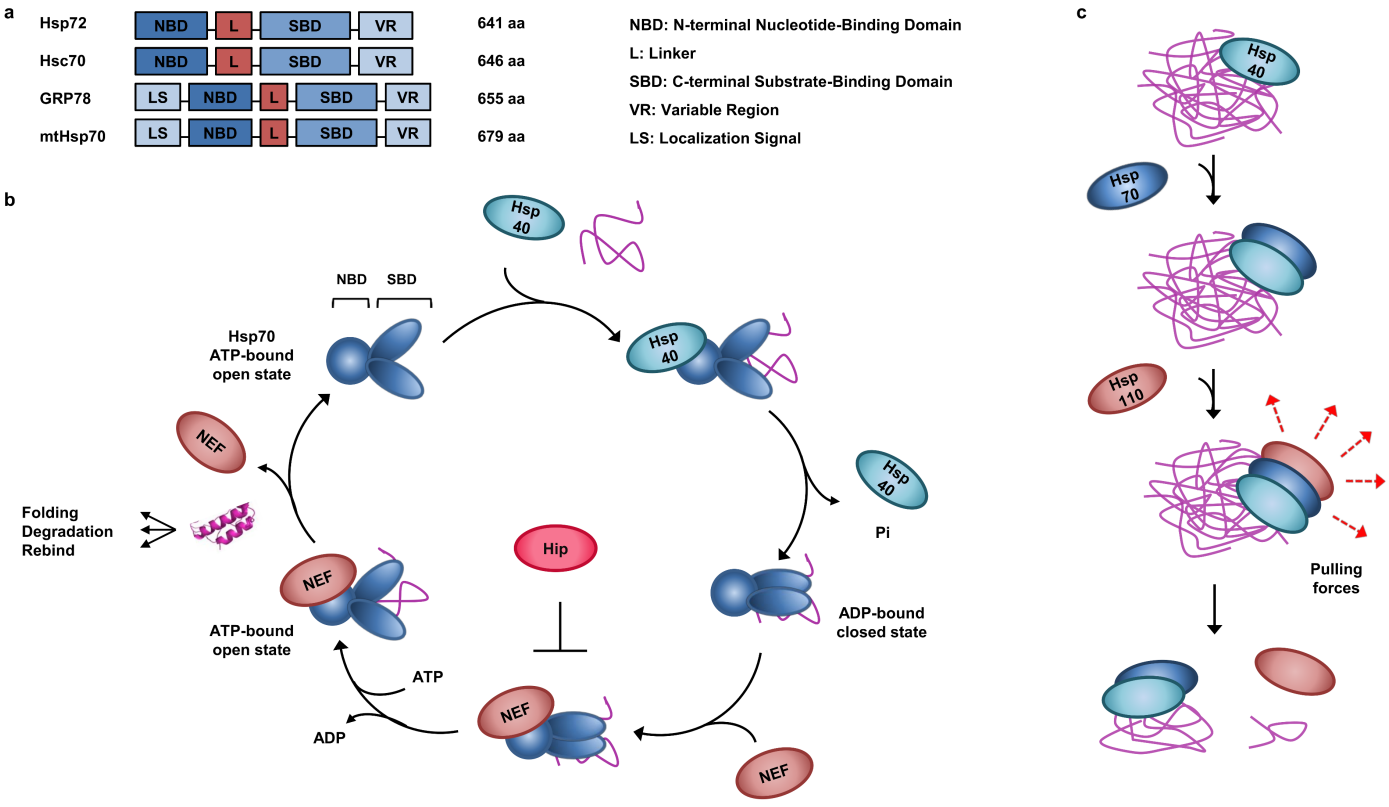

a
HSF1
DBD
OD
RD
OD
TAD
529 aa
DBD: DNA-binding Domain
OD: Oligomerization Domain
RD: Regulatory Domain
TAD: Trans-activation Domain
b
Proteotoxic insult
HSF1 monomers
HSF1 trimer
HSE
Hsp gene
(i)
(ii)
(iii)
Hsp 90
Hsp 40
Hsp 70
HSF1 monomers
HSF1 trimer
HSE
Hsp gene

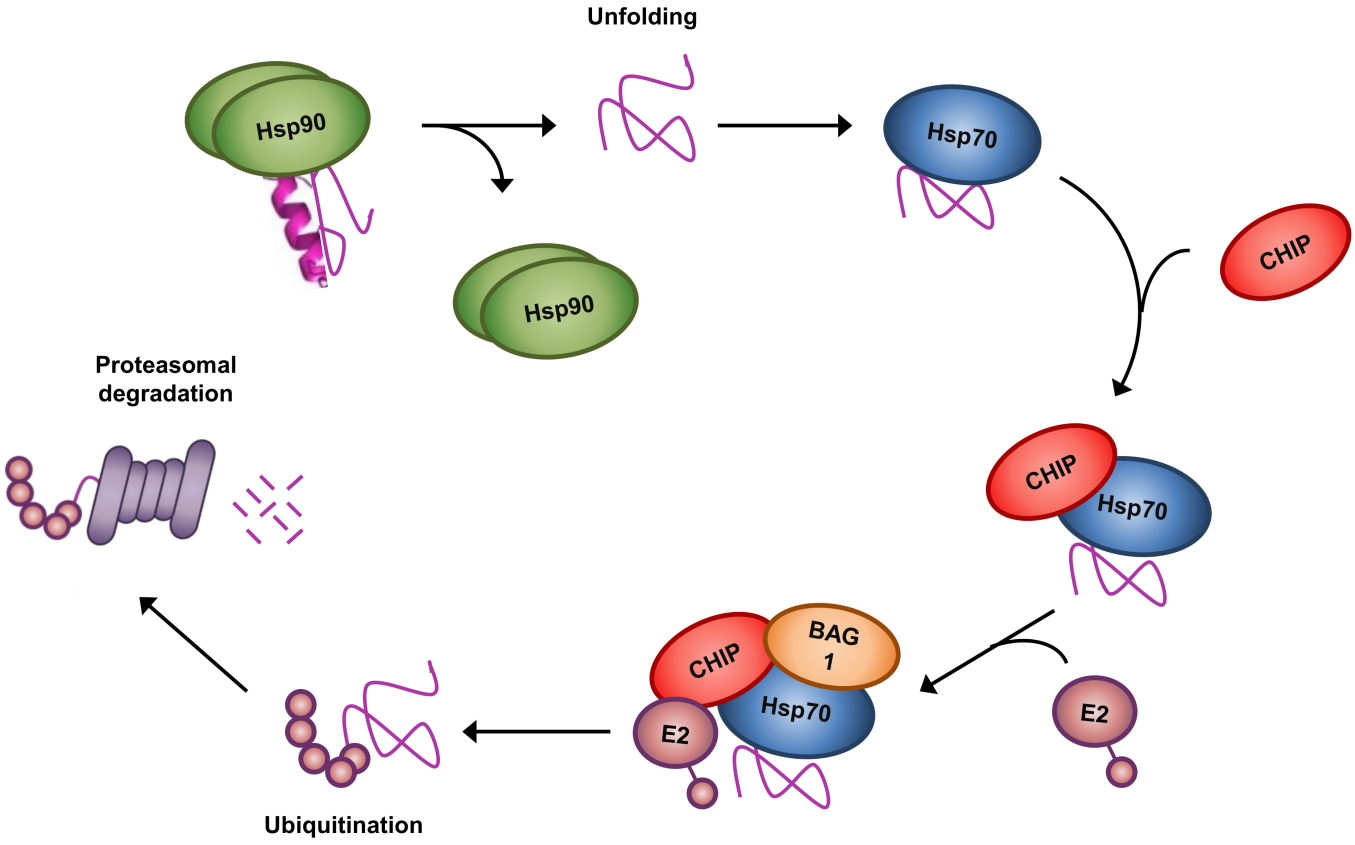
a


Hsp90 α
NBD
CL
MD
CD
732 aa
Hsp90 β
NBD
CL
MD
CD
724 aa
GRP94
LS
NBD
CL
MD
CD
803 aa
TRAP1
LS
NBD
MD
CD
704 aa
NBD: N-terminal Nucleotide-Binding Domain
CL: Charged linker
MD: Middle Domain
CD: C-terminal Domain
LS: Localization Signal

b


Inactive client protein
NBD
MD
CD
Hsp90 dimer
ATP
ATP-bound open state
Hop
Aha 1
Cdc 37
ADP
Active client protein
p23
ADP-bound open state
ATP-bound closed state
Pi


a
Hsp72
NBD
L
SBD
VR
641 aa
Hsc70
NBD
L
SBD
VR
646 aa
GRP78
LS
NBD
L
SBD
VR
655 aa
mtHsp70
LS
NBD
L
SBD
VR
679 aa
NBD: N-terminal Nucleotide-Binding Domain
L: Linker
SBD: C-terminal Substrate-Binding Domain
VR: Variable Region
LS: Localization Signal
b
Hsp 40
NBD
SBD
Hsp70
ATP-bound
open state
Hsp 40
Hsp 40
Pi
NEF
Folding
Degradation
Rebind
Hip
NEF
ATP-bound
open state
ADP-bound
closed state
ATP
NEF
ADP
NEF
c
Hsp 40
Hsp 70
Hsp 110
Pulling
forces


Unfolding
Hsp90
Hsp90
Hsp70
CHIP
CHIP
Hsp70
Proteasomal
degradation
CHIP
BAG
1
Hsp70
E2
E2
Ubiquitination