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Molecular Chaperones as Rational Drug Targets for Parkinson's Disease Therapeutics

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Abstract

Parkinson's disease is a neurodegenerative movement disorder that is caused, in part, by the loss of dopaminergic neurons within the substantia nigra pars compacta of the basal ganglia. The presence of intracellular protein aggregates, known as Lewy bodies and Lewy neurites, within the surviving nigral neurons is the defining neuropathological feature of the disease. Accordingly, the identification of specific genes mutated in families with Parkinson's disease and of genetic susceptibility variants for idiopathic Parkinson's disease has implicated abnormalities in proteostasis, or the handling and elimination of misfolded proteins, in the pathogenesis of this neurodegenerative disorder. Protein folding and the refolding of misfolded proteins are regulated by a network of interactive molecules, known as the chaperone system, which is composed of molecular chaperones and co-chaperones. The chaperone system is intimately associated with the ubiquitin-proteasome system and the autophagy-lysosomal pathway which are responsible for elimination of misfolded proteins and protein quality control. In addition to their role in proteostasis, some chaperone molecules are involved in the regulation of cell death pathways. Here we review the role of the molecular chaperones Hsp70 and Hsp90, and the co-chaperones Hsp40, BAG family members such as BAG5, CHIP and Hip in modulating neuronal death with a focus on dopaminergic neurodegeneration in Parkinson's disease. We also review current progress in preclinical studies aimed at targeting the chaperone system to prevent neurodegeneration. Finally, we discuss potential future chaperone-based therapeutics for the symptomatic treatment and possible disease modification of Parkinson's disease.

Keywords

Bcl-2 associated athanogene (BAG) family; C-terminal Hsp70 interacting protein (CHIP); chaperones; co-chaperones; heat shock protein (Hsp); Hsp90 inhibitors; neurodegeneration; Parkinson's disease

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CONFLICT OF INTEREST

The authors have no conflict of interest.

INTRODUCTION

Parkinson's disease (PD) is a movement disorder affecting approximately 3% of the population over the age of 65 and is second only to Alzheimer's disease as the most common neurodegenerative disease [1]. The greatest known risk factor for PD is advancing age and, with the aging population, it is expected that the number of individuals seeking treatment for PD will dramatically increase over the next several decades. The motor symptoms which characterize PD include tremor, rigidity, bradykinesia, and postural instability. In addition, a number of non-motor symptoms, such as dementia, constipation, sleep disturbances, and mood symptoms, are increasingly recognized as also being common to the disease. This diversity of symptoms correlates with the neuropathologic changes that occur in widespread regions of the central and peripheral nervous systems affecting many different neurotransmitter systems.

The most important neuropathological hallmark of PD is considered to be the death of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). Nigral neurodegeneration results in a reduction of the neurotransmitter dopamine in the striatum, the main target of the SNpc, causing dysregulation within the basal ganglia. The presence of intracellular protein aggregates, known as Lewy bodies and Lewy neurites, within the surviving dopaminergic neurons of the SNpc is the defining neuropathological feature of the disease. Lewy bodies and Lewy neurites contain a number of proteins and are often highly ubiquitinated. 3-Synuclein is a major component of the filaments of Lewy bodies and Lewy neurites [2, 3]. Missense mutations (A53T, A30P, and E46K) in the 3-synuclein gene (*PARK1*), as well as duplications and triplications of the locus containing the 3-synuclein gene (initially *PARK4*) are associated with rare familial forms of PD. In addition, polymorphisms in the gene have been identified as risk factors for idiopathic, or sporadic, PD [4, 5].

Much of the current understanding of the cellular processes underlying the loss of dopaminergic neurons in the SNpc has emerged from investigations into animal models of the disease and from genetic studies of patients with PD. The etiology of PD in the majority of cases involves the interplay of both genetic and environmental factors. Research into the identification of environmental causes of PD has not yet revealed a definitive environmental agent but has led to the development of useful models of PD. These include models in which dopaminergic neurodegeneration is chemically induced using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine, or rotenone [6]. Investigation into these models has linked mitochondrial dysfunction, oxidative stress, and microglial activation to the pathogenesis of PD [6–10].

The identification of specific genes mutated in familial PD and of genetic susceptibility variants for idiopathic PD have implicated the abnormal handling and elimination of misfolded proteins in PD pathogenesis. Protein folding and the refolding of misfolded proteins are regulated by the chaperone system which is composed of molecular chaperones and co-chaperones. The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system are responsible for elimination of misfolded proteins and protein quality control. Mutations in genes that encode for components of these systems which are involved in protein homeostasis, or proteostasis, have been linked to familial PD. They include: 1) ubiquitin carboxy-terminal hydrolase L1 (*PARK5*), a deubiquitinating enzyme; 2) parkin (*PARK2*), a ubiquitin E3 ligase; 3) DJ-1 (*PARK7*), a protein that may have chaperone-like activity; and, 4) ATP13A2 (*PARK9*), a lysosomal P-type ATPase. More recently, heterozygous mutations in the 3-glucocerebrosidase (GBA) gene, which encodes the lysosomal enzyme affected in Gaucher disease, have been identified as susceptibility factors for idiopathic PD [11]. Furthermore, as discussed within this review, other genes which are

mutated in PD, such as PINK1 (*PARK7*) and LRRK2 (*PARK8*), may be regulated by chaperones and/or be linked to the UPS.

The majority of current pharmacological treatments for PD are aimed at increasing intracerebral dopamine levels and/or stimulating central dopamine receptors. These drugs are limited to the symptomatic management of some of the motor manifestations of PD. While there have been significant efforts to develop disease-modifying drugs for PD, neuroprotection trials to date have not yet clearly identified a drug that can delay or halt disease progression [12]. The targeting of well characterized molecular pathways involved in the dysfunctional cellular processes underlying the pathogenesis of PD may allow for rational drug design of disease-modifying drugs for PD. Here we will examine the emerging role of the chaperone system in PD and investigate molecular chaperones and co-chaperones as potential drug targets for PD therapeutics.

MOLECULAR CHAPERONES ARE REGULATORS OF PROTEOSTASIS

Molecular chaperones are defined as a group of molecules that mediate folding and/or unfolding of protein substrates, or the assembly and/or disassembly of larger protein aggregates [13]. The two major chaperone systems in mammals are Hsp70 and Hsp90 (Fig. 1A). Molecular co-chaperones may be defined as those molecules that regulate the ability of a chaperone to carry out its function. Co-chaperones can be classified according to the presence of different protein modules: the bcl-2 associated athanogene (BAG) domain, the tetratricopeptide repeat (TPR) domain, or the DnaJ or J domain (Fig. 1B). Chaperones and co-chaperones are intimately associated with the UPS [14] and autophagy-lysosomal pathway [15]. When unfolded or misfolded proteins cannot be refolded by chaperones, they are targeted to the proteasome or the lysosome for degradation. A major component of Lewy bodies in PD and other synucleinopathies, aside from 3-synuclein, are molecular chaperones and co-chaperones including Hsp70, Hsp90, Hsp27, Hsp40, Hsp60, BAG5, and CHIP [16–20], as well as components of the UPS. Molecular chaperones are also found as components of protein aggregates in other neurodegenerative diseases and models of neurodegeneration, such as polyglutamine (polyQ) disorders and prion diseases [21–27]. Given the central importance of chaperones in protein homeostasis, or proteostasis, they may serve as rational targets for the design of therapeutics in neurodegenerative diseases associated with aberrant proteins such as PD.

In response to potentially toxic conditions almost all cells, both prokaryotic and eukaryotic, possess inherent mechanisms to maximize the potential for their survival. A significant portion of this response is managed by a subclass of inducible chaperone molecules known as heat shock proteins (Hsp). The term “heat shock” refers to using increased temperature as a cellular stressor but importantly this class of proteins may be induced by any proteotoxic insult. The first heat shock responsive genes were discovered in 1962 by F. Ritossa and colleagues [28] when they noted a temperature increase induced puffing patterns in the polytene chromosomes of *Drosophila* salivary glands. In 1974, the first products of these genes were identified and the term “heat shock protein” was coined for two classes of protein products which were 70 kDa and 27 kDa in size: the Hsp70 and small Hsp families, respectively [28, 29].

Since then a large number of heat shock proteins have been identified. Constitutively expressed homologues of heat shock proteins, known as heat shock cognates (Hsc), have also been discovered and they are key players in cellular homeostasis involved in regulating a wide array of basic cellular processes. Chaperone functions which are important in the context of neurodegeneration include: the refolding and solubilization of misfolded proteins; the modulation of protein aggregation; the targeting of proteins which cannot be repaired by

the UPS or autophagy-lysosomal system; and, the suppression of cell death programs [14, 30, 31].

Hsp70, a Model Molecular Chaperone, and its Co-Chaperones

The Hsp70 chaperone family is the most well characterized group of molecular chaperones implicated in the regulation of dopaminergic neurodegeneration. Hsp70 chaperones are thought to be critical in the regulation of protein oligomerization and aggregation, which are believed to be central in the molecular pathogenesis of PD. The Hsp70 family of chaperones is large and complex with many members existing within eukaryotic cells. Each family member has a variable expression pattern and subcellular compartmentalization. Furthermore, some Hsp70 family members are expressed constitutively (e.g. Hsc70) whereas others are induced by cell stress (e.g. Hsp72) [32]. For the purposes of this review, the term “Hsp70” refers to any member of the Hsp70 family unless otherwise specified. Hsp70 chaperones promote protein refolding using a series of ATP-dependent “hold” and “fold” cycles. The N-terminal domain of Hsp70 contains an ATPase domain. The C-terminal region of Hsp70 has a peptide binding domain (PBD) which can bind unfolded or misfolded residues of a peptide substrate *via* the unfolded peptide substrate’s exposed hydrophobic residues [33–36]. The N-terminal ATPase domain mediates the hydrolysis of ATP to ADP, and this has conformational consequences on the C-terminal PBD. The ADP-Hsp70-substrate complex is considered stable, or in the “hold” conformation. The release of ADP in exchange for ATP is necessary for the release of the peptide substrate since the ATP-Hsp70-substrate complex conformation is less stable. The cycling between these two states is what is thought to ultimately allow for a protein to be refolded to its native state. A variety of mechanisms, including kinetic partitioning, Brownian ratchetting, and entropic pulling, have been proposed to explain how Hsp70 through its ATPase-dependent cycling may assist in both refolding of unfolded proteins and disassembly of protein aggregates [13].

A number of co-chaperones which interact with Hsp70 and regulate its ability to refold misfolded proteins have been identified. Hsp70 cooperates with DnaJ domain-containing proteins which are members of the Hsp40 family of co-chaperones. Most Hsp40 proteins are believed to recognize misfolded substrates and target them to Hsp70 through a direct interaction between Hsp40 and Hsp70. Hsp40 also enhances Hsp70 ATPase activity, thus promoting the formation of the more stable ADP-Hsp70-substrate complex [37, 38]. Co-chaperones that directly interact with the ATPase domain of Hsp70 also regulate the ability of Hsp70 to refold misfolded proteins. Hsp70 interacting protein (Hip, or ST13) binds to the ATPase domain of Hsp70 and stabilizes the ADP-Hsp70-substrate complex [39, 40]. In addition, BAG1 and other BAG family members interact with the ATPase domain of Hsp70. BAG1 is known to oppose the action of Hip, likely enhancing the release of ADP from Hsp70 through a conformational change [38, 41–45]. C-terminal Hsp70 interacting protein (CHIP) is another co-chaperone of Hsp70. CHIP is also an E3 ubiquitin ligase, thus serving as a link between the Hsp70 chaperone system and the UPS. CHIP and another co-chaperone, named Hsp70/Hsp90 organizing protein (Hop, or Sti1), regulate the association of Hsp70 with the Hsp90 chaperone complex. It is clear that a complex interplay between the various components of the Hsp70 chaperone system is necessary for protein refolding and protein aggregate disassembly. Their potential roles in the degeneration of dopaminergic neurons are discussed in subsequent sections.

MOLECULAR CHAPERONES AND CO-CHAPERONES IN NEURODEGENERATION

Hsp70 and Hsp40

The establishment of a role for the Hsp70 chaperone system in the molecular pathogenesis of PD follows work examining neurodegenerative diseases caused by trinucleotide repeat expansions, which include Huntington's disease and many of the spinocerebellar ataxias (SCAs). Initial work by Cummings and colleagues [25] established that molecular chaperones were present in the protein aggregates associated with SCA1 and demonstrated that overexpression of Hsp40 can reduce protein aggregate formation associated with ataxin-1, the mutant protein in SCA1, in cells. They later demonstrated that the expression of inducible Hsp70 *in vivo* suppresses neurotoxicity in a SCA1 mouse model [46]. Consistent with these findings, a screen for modifiers of neurodegeneration in a *Drosophila* model of polyQ expansion-mediated neurodegeneration identified a *Drosophila* Hsp40 homologue (dHJ1), in addition to a *Drosophila* TPR protein homologue (dTPR2), as suppressors of neurodegeneration [47]. Furthermore, the overexpression of molecular chaperones in *Drosophila* models of polyQ-mediated neurodegeneration has been shown to prevent toxicity associated with polyQ expansion whereas the suppression of chaperone activity enhances polyQ toxicity [48, 49].

A role for Hsp70 and Hsp40 in regulating the pathogenesis of PD is suggested by their prominence in Lewy bodies [16,19]. In addition, a screen of patients with PD has revealed a polymorphism in a promoter of Hsp70, which may affect the transcription of Hsp70, to be significantly more frequent in patients with PD [50]. Further evidence is provided by studies investigating cellular and animal models of PD. Overexpression of Hsp70 by heat shock has been found to prevent 3-synuclein-induced apoptosis in yeast [51] and to protect cultured PC12 cells treated with the active form of MPTP, 1-methyl, 4-phenylpyridinium ion (MPP+) [52]. Auluck and colleagues [16] demonstrated that Hsp70 can prevent dopaminergic neuron death in an 3-synuclein overexpression model of PD in *Drosophila*. In mouse models of PD, overexpression of Hsp70 has been shown to lead to a reduction of high molecular weight 3-synuclein species [18]. Furthermore, targeted overexpression of Hsp70 by adeno-associated virus (AAV) vector expression within the SNpc has been found to prevent dopaminergic neuron degeneration following the administration of MPTP [53] and to protect against toxicity mediated by the parkin substrate CDCrel-1 [54].

Protection by Hsp70 seems to be dependent, at least in part, on its refolding activity since the introduction of a mutation in the ATPase domain of Hsp70 (K71S) results in the inability of Hsp70 to refold proteins and the subsequent loss of its ability to mitigate 3-synuclein-mediated toxicity [50, 55]. Consistent with this, Hsp70 has been shown to change 3-synuclein conformation by fluorescence lifetime imaging [56], to prevent 3-synuclein oligomerization or fibril formation [57–59], and to mitigate the formation of 3-synuclein-containing protein aggregates [19, 60]. Hsp70 may also protect cells from 3-synuclein-mediated toxicity by sequestering 3-synuclein [61]. In contrast, Hsp70 prevents the sequestration of parkin in large deposits of protein aggregates, known as aggresomes [17], and synergizes with parkin to prevent cell death secondary to unfolded protein stress [62].

In addition to its role in refolding proteins and modulating protein aggregates, Hsp70 plays an important part in regulating protein degradation. For example, Hsp70 is a central molecule in the chaperone-mediated autophagy pathway, one of several pathways for the degradation of cytosolic proteins in lysosomes. 3-Synuclein may inhibit its ability to carry out this function [63]. Hsp70 may also prevent the inhibition of the UPS, which is detrimental to cell survival, by enhancing the degradation of proteotoxic substrates [17, 64–

67]. Similarly, co-chaperones of Hsp70, such as CHIP and BAG family members, seem to play a role in targeting misfolded mutant proteins to the UPS and thus modulate neurodegeneration [62, 65, 68–76].

Hsp70 has also been identified as a regulator of cell death pathways through its interaction with and inhibition of apoptosis-inducing proteins [77–80] and c-jun N-terminal kinase (JNK) [81–86]. Perhaps in addition to changing the solubility characteristics of misfolded proteins, oligomers, or aggregates, Hsp70 may prevent caspase and JNK activation and subsequent cell death through mechanisms that are possibly independent of its role as a chaperone molecule. Alternatively, Hsp70 has been shown to be a substrate of parkin in a degradation-independent manner [87] which may ultimately modulate signalling through the JNK cell death pathway [88].

Given the multiple roles of Hsp70, the exact mechanism (s) by which Hsp70 exerts its protective effects on the death of dopaminergic neurons in PD is likely to occur through its many seemingly divergent functions. However, it is clear that the Hsp70 chaperone system prevents neurodegeneration and thus the loss of chaperone function may then contribute to neurodegeneration. How this loss of chaperone function occurs still remains to be elucidated. It has been suggested that chaperone activity and the ability of a cell to handle proteotoxic insults decreases with age while the cell's proteotoxic load increases with age [14, 89]. The chaperone response may also be impaired with age [81, 90]. Furthermore, over the course of a neurodegenerative process, chaperones may become sequestered or trapped in protein aggregates. Although molecular chaperones and co-chaperones are found within inclusions in neurodegenerative diseases such as PD, the activity state of these molecules within the inclusions is difficult to assess. It is likely, since chaperones interact with components of protein aggregates, that chaperone depletion within the aggregates is detrimental to cellular homeostasis since the sequestration of the chaperone prevents it from performing its necessary functions within the cell. Differences in the ability to activate a significant chaperone response between regions in the nervous system may also contribute to the differential sensitivity observed in neurodegenerative disorders. Another possibility is that Hsp70 may become functionally sequestered by disease causing mutant proteins and the loss of chaperone-mediated inhibition of cell death leads to both protein aggregation and neurodegeneration [91].

Taken together, the data discussed above suggest that molecular chaperones, in particular the Hsp70 chaperone complex, can suppress the neurotoxicity associated with the expression of genes known to cause neurodegenerative diseases including PD. Strategies aimed at increasing endogenous Hsp70 expression or allowing for the delivery of exogenous Hsp70 may mitigate neurodegeneration in PD. However, Hsp70 family members are ubiquitously expressed and differentially localized within subcellular compartments. In addition, interactions between the constitutively active heat shock cognate family members and the inducible homologues are not yet clearly understood, especially in the context of neurodegeneration. Thus, care must be taken in the design and evaluation of potential therapeutics targeting this chaperone system in order to achieve maximal benefit with minimal disruption to a key system required for the maintenance of proteostasis.

BAG Family Proteins

The BAG family of proteins are considered to be co-chaperones of Hsp70 [92]. BAG1, the prototypic BAG family member, was cloned in 1995 based on its synergistic interaction with bcl-2 by Takayama and colleagues [42] and was also independently cloned as a steroid receptor associated protein (Rap46) based on its interaction with activated glucocorticoid receptor [93]. Subsequently, it was established that BAG1 interacts with the ATPase domain of the molecular chaperone Hsp70 [40, 94, 95]. The functional consequence of this

interaction was the inhibition of Hsp70-mediated refolding of misfolded reporter proteins *in vitro* [94, 95] and in cells [96, 97]. It has also been proposed that BAG1 competes for the same binding region of the positive co-chaperone of Hsp70, Hip [44, 97]. Taken together, it is likely that BAG1 is a negative co-chaperone of Hsp70 through its interaction with the ATPase domain of Hsp70 and by its ability to compete out the positive co-chaperone Hip.

The BAG domain was initially defined as approximately the last 50 amino acids in the C-terminus of BAG1 [98]. Based on homology with the BAG domain of BAG1, an entire family of BAG domain-containing proteins has been identified [92, 98] (Fig. 1B). BAG family homologues exist in plants, yeast, *C. elegans*, *Drosophila*, and mammals [92]. In humans, there are currently six known family members, designated BAG1 to BAG6, each containing a single BAG domain at its C-terminus with the exception of BAG5 which contains multiple BAG domains. BAG2 [98], BAG3 (also known as CAIR-1 or Bis) [99], BAG4 (also known as SODD) [100], BAG5 [17], and BAG6 (also known as BAT3 or Scythe) [101] have been shown to interact with Hsp70/Hsc70 and regulate its ability to refold misfolded substrates in a manner similar to that of BAG1. Aside from their formation of complexes with Hsp70, BAG family proteins functionally interact with a variety of other binding partners and coordinate diverse cellular processes such as stress signalling [102], cell division, cell differentiation, and cell death [95].

BAG family proteins also provide a link between the chaperone system and the UPS and autophagy protein degradation pathways. BAG1, like parkin, contains a ubiquitin-like domain (UBL) at its N-terminus. BAG1 has been shown to associate with the 26S proteasome in a manner that is likely dependent on its UBL and independent of BAG1 binding to Hsp70 [71]. Through this interaction, BAG1 may promote the formation of a trimeric proteasome-BAG1-Hsp70 complex and may serve as a bridge between the chaperone system and the UPS [71]. BAG1 has been shown to interact directly with siah-1, the human homologue of *Drosophila* seven in abstinence (SINA) [103]. Siah-1 is a p53-inducible RING finger-containing protein that has E3 ubiquitin ligase activity [104]. 3-Synuclein and synphilin-1, an 3-synuclein-interacting protein of unknown function [105], have both been identified as a substrate of siah-1 [106–108]. Demand and colleagues [73] have identified an interaction between BAG1 and CHIP and they suggest that BAG1 may shift the function of Hsp70 from refolding misfolded proteins to targeting proteins for degradation through its interactions with both CHIP and the UPS. Interestingly, BAG1 is also a substrate of CHIP and its ubiquitylation by CHIP, in cooperation with E2 ubiquitin-conjugating enzymes of the Ubc4/5 family, does not result in the degradation of BAG1. Rather, ubiquitylation of BAG1 by CHIP results in the formation of a non-canonical poly-ubiquitin chain which enhances the interaction between BAG1 and the UPS [76].

BAG2 has been shown to interact with CHIP [109, 110]. The BAG2-Hsp70-CHIP complex has recently been shown to be part of a degradation pathway for tau, a protein implicated in several neurodegenerative disorders including Alzheimer's disease [111]. This tau degradation pathway is regulated, in part, by the microRNA miR-128a which leads to decreased BAG2 levels and failure of the tau protein triage system. Doong and colleagues [112] have shown that BAG3 is associated with the UPS, possibly through an interplay with Hsp90. It is known that Hsp90, in a state of ATP depletion, will target client proteins for degradation through a complex recruiting Hsp70 and Hop. Overexpression of BAG3 was found to inhibit the degradation of client proteins, such as Akt, which are typically targeted to the UPS by Hsp90 in an ATP-depleted state. The BAG3-mediated inhibition of the targeting of these client proteins for degradation was not associated with decreased ubiquitylation of the proteins to be targeted to the UPS. Subsequently, BAG3 has been found to be a stimulator of macroautophagy in the degradation of huntingtin with polyQ

repeats [113, 114], the mutant protein in Huntington's disease, and in cellular models of aging [115].

BAG5 immunoreactivity has been identified within Lewy bodies and has been shown to interact with both Hsp70 and parkin [17]. BAG5 inhibits Hsp70 chaperone activity, mitigating the ability of Hsp70 to refold unfolded substrates and to prevent the sequestration of parkin within aggresomes. Furthermore, BAG5 binds directly with parkin independently of Hsp70 and negatively regulates its E3 ubiquitin ligase activity both *in vitro* and in cells. Adenoviral-mediated targeted overexpression of BAG5 in the SNpc has been found to result in enhanced dopaminergic neurodegeneration in the MPTP model of PD [17]. Through its inhibition of Hsp70 and parkin function, BAG5 serves as an important link between the chaperone system and the UPS. Thus, therapeutic strategies that decrease endogenous BAG5 levels or inhibit BAG5 activity may slow progression of neurodegeneration.

CHIP

CHIP, also known as STUB1, was cloned as a protein that interacts *via* its TPR domain with the C-terminus of Hsp70 [116] containing the EEVD motif. CHIP was initially found to inhibit Hsp70-mediated protein refolding *in vitro* through its opposing action against Hsp40. However in cells, CHIP has been shown to actually enhance Hsp70-mediated refolding of proteins, such as luciferase [117]. The importance of CHIP function within cells was realized over a series of studies that identified CHIP as a U-box-containing E3 ubiquitin ligase. The E3 activity of CHIP, together with its interaction with Hsp70, allows CHIP to serve as a co-chaperone which links the chaperone system to the UPS [68, 70, 74, 75, 118]. CHIP has also been found to interact with parkin and may be an E4-like protein that functions to enhance the E3 ubiquitin ligase activity of parkin [62]. BAG1 may facilitate the interaction between CHIP, Hsp70, and the UPS and thereby modulate protein quality control triage decisions by shifting the balance from protein refolding to protein degradation [73, 76]. CHIP immunoreactivity has been identified in Lewy bodies [119] and in tau positive inclusions [120] in disease tissue. CHIP associates with 3-synuclein leading to the prevention of toxic oligomer formation and increased degradation *via* both proteasomal and lysosomal pathways [119, 121]. More recently, CHIP has been shown to associate with and regulate LRRK2 toxicity by modulating LRRK2 stability through CHIP-mediated ubiquitin ligase activity [122, 123]. The molecular chaperone Hsp90 is present in the complex with CHIP and LRRK2, and inhibition of Hsp90 chaperone activity with geldanamycin or an analogue has been shown to enhance the degradation of LRRK2 [122, 123]. Thus, Hsp90 seems to mitigate CHIP-mediated degradation of LRRK2. CHIP may also play a role in inhibiting protein aggregation and cell death, potentially through the induction of Hsp70 expression [65, 124]. As a co-chaperone of Hsp70 that also serves as a link to the UPS through its E3 and E4 activities, CHIP acts as a dual purpose molecule. Therefore, CHIP may serve as a unique target for therapeutic intervention in the treatment of PD. By increasing the levels of CHIP or enhancing its co-chaperone and ubiquitin ligase activities, toxicity and subsequent cell death associated with 3-synuclein and LRRK2 may be reduced or prevented.

Hip

Hip, another co-chaperone of Hsp70, was only recently found to have a possible association with PD. In a transcriptome wide screen, the level of Hip mRNA expression was decreased in PD patients comparing individuals with early stage PD to healthy control subjects [125]. More recently, Roodveldt and colleagues [61] demonstrated that Hip can prevent the co-aggregation of Hsp70 with 3-synuclein resulting in an increase in available Hsp70 to carry out chaperone functions. The potential utility of chaperone mRNA expression profiles in the

diagnosis and prognosis of PD remains to be determined. If the decreased mRNA expression levels of Hip correlate with decreased or absent protein function, this may serve as another possible target to prevent disease progression by using strategies aimed at mitigating the loss of Hip expression or through the addition of exogenous Hip.

Hsp90

Hsp90 is a ubiquitously expressed molecular chaperone that has a central role in post-translational folding and stabilization of its client proteins. Its domain structure includes an N-terminal region with an ATP binding site and ATPase activity, a middle PBD, and a C-terminal region responsible for dimerization [126]. Hsp90 is regulated through dynamic interactions with a multitude of other chaperones including Hsp70 and Hsp40. Hop is thought to be responsible for mediating the transfer of a client protein from Hsp70 to Hsp90 by interacting with both proteins simultaneously [127]. If client proteins are not appropriately chaperoned by the Hsp90 complex, CHIP is recruited to the C-terminal TPR binding site of Hsp90 containing the EEVD motif and targets these client proteins for degradation by the UPS [74]. Inhibition of Hsp90 chaperone activity leads to the recruitment of heat shock transcriptional factor-1 (HSF-1) and subsequent induction of Hsp70 expression. Thus, Hsp90 inhibition is a potential strategy to increase endogenous Hsp70 protein levels.

Hsp90 has been shown to be a predominant heat shock protein within filamentous inclusions in synucleinopathies, such as Lewy bodies and Lewy neurites, most predominantly within brains from patients with PD [128]. PINK1 [129] and LRRK2 [122, 130] have recently been identified as Hsp90 client protein kinases. The L347P mutant of PINK1, which is associated with a recessive form of PD, is unable to bind the Hsp90 chaperone complex and has a decreased half-life relative to wild-type PINK1 which can bind to Hsp90. Consistent with this, cells treated with Hsp90 inhibitors demonstrate a significant reduction in wild-type PINK1 levels [131]. Similarly, Hsp90 maintains the stability of LRRK2 and the disruption of the Hsp90-LRRK2 interaction leads to degradation of LRRK2 by the UPS [132], possibly through the action of CHIP [122,130]. Indeed modulating these interactions by utilizing Hsp90 inhibitors, such as geldanamycin, has demonstrated a potentially beneficial therapeutic effect through the degradation of mutant pathogenic client proteins [122, 130–132] and may also prove to be beneficial in the treatment of PD [133, 134].

POTENTIAL CHAPERONE-BASED THERAPIES FOR PARKINSON'S DISEASE

Given that chaperones are regulators of diverse pathways involved in the protection of dopaminergic neurons and other neuronal subtypes, they serve as a rational target for the development of novel therapeutics for PD. Recent preclinical studies have investigated brain permeable small molecules that upregulate chaperone function in models of PD. Additional drugs that enhance the function of chaperones are currently being studied in other neurodegenerative disorders and various forms of cancer and, based on their mechanism of action, may be worthwhile studying in PD. Newer strategies to enhance chaperone activity in PD include viral-mediated gene therapy or TAT-based drug delivery to increase chaperone levels or to modulate chaperone activity. Pharmacological chaperones, or drugs with inherent chaperone activity, are also potential disease-modifying therapies for PD.

Drugs that Upregulate Molecular Chaperone Function

A number of small molecule inhibitors of Hsp90 have been studied in models of PD and other neurodegenerative diseases (Fig. 2). As described above, Hsp90 is part of a complex that negatively regulates the activity of the transcription factor HSF-1. Inhibition of Hsp90

chaperone function, by reducing its ATPase activity, results in activation of HSF-1 and subsequent increase in expression of protective stress-induced HSPs such as Hsp70 [135, 136]. The benzoquinone ansamycin antibiotic geldanamycin is a naturally occurring Hsp90 inhibitor which binds to an ATP site on Hsp90 and blocks its interaction with HSF-1, leading to enhanced Hsp70 expression. Geldanamycin has been found to prevent dopaminergic neuron cell death in 3-synuclein-induced toxicity in cell culture [60], in an 3-synuclein overexpression model of PD in *Drosophila* [133], and in the MPTP mouse model of PD [137]. However, geldanamycin has poor aqueous solubility, does not cross the blood brain barrier, and is associated with significant liver toxicity. To overcome these properties which limit its clinical use, numerous geldanamycin analogues have been designed including 17- (allylamino)-17-demethoxygeldanamycin (17-AAG, or tanespimycin) and 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG, or alvespimycin). These analogues are blood brain barrier permeable and are much less toxic than geldanamycin. They also have higher affinity for Hsp90 than geldanamycin. 17-AAG has been shown to upregulate Hsp70 expression while reducing α -synuclein protein levels and α -synuclein-mediated toxicity in a cell-based system [134]. 17-AAG has also been found to be neuroprotective in preclinical studies of Huntington's disease and SCAs [136, 138]. Currently, 17-AAG is in phase II trials as an anti-tumour compound [139, 140]. However, the clinical utility of 17-AAG and 17-DMAG may be limited because, while much less toxic than geldanamycin, they have caused varying degrees of hepatotoxicity in earlier cancer trials. In addition, they have limited oral availability and have been difficult to formulate [141, 142].

A novel class of synthetic small molecule Hsp90 inhibitors which are unrelated in structure and exhibit unique activities relative to geldanamycin and its derivatives is SNX-2112 (4-[6,6-dimethyl-4-oxo-3- (trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-1-yl]-2-[(trans-4-hydroxycyclohexyl)amino]benzamide) and its analogues [143, 144]. SNX-2112 was identified in a compound library screen for scaffolds that selectively bind to the ATP pocket of Hsp90. It is orally available and crosses the blood brain barrier. Recent *in vivo* pharmacokinetic and pharmacodynamic studies demonstrated that SNX-0723, another member of this drug class, also has good brain absorption and excellent oral bioavailability [134]. Furthermore, a group of these novel Hsp90 inhibitors can decrease α -synuclein oligomerization *in vitro* and rescue α -synuclein-induced toxicity [134], supporting further investigation *in vivo* to determine if Hsp90 inhibition can rescue 3-synuclein-mediated cell death in animal models. With the first phase I clinical trial of SNX-5422, the prodrug of SNX-2112 which is being developed for cancer therapy [145], the safety and tolerability of these drugs in human subjects is beginning to be assessed.

Other drugs that upregulate Hsp70 and, therefore, may be candidates for study in PD include radicicol, a naturally occurring antifungal that is structurally unrelated to geldanamycin, and its more stable oxime derivatives. Like geldanamycin, radicicol and its derivatives are Hsp90 inhibitors but they have up to 50-fold greater affinity for Hsp90 than geldanamycin. However, further drug development of radicicol would be required as it has limited oral bioavailability and blood brain barrier permeability [141, 146]. A number of other synthetic small molecule inhibitors of Hsp90 are currently being studied in clinical trials for cancer and may be potentially useful in PD including IPI-504 (or retaspimycin), which is a geldanamycin derivative [147], and STA-9090, which is structurally unrelated to geldanamycin [148].

Arimoclomol (Fig. 2) is an orally administered drug that upregulates expression of Hsp70 not by inhibiting Hsp90 but by activating HSF-1 [149, 150] (Fig. 2). It has been studied in a mouse model of amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative motor neuron disease, resulting in upregulation of Hsp70 with a decrease in the number of protein

aggregates in the spinal cord [151]. Also, there was a significant reduction in the degeneration of motor neurons [152]. In phase I and IIa clinical trials, arimoclomol has demonstrated adequate safety and tolerability [149, 153]. Currently, it is being tested in phase II clinical trials for ALS. Another HSF-1 activator, named HSF1A, was recently identified in a newly described high-throughput screen designed to identify novel small molecule activators of human HSF-1 [154]. HSF1A-mediated upregulation of Hsp70 protein expression has been confirmed in cell lines. Furthermore, HSF1-A has been shown to suppress aggregation of polyQ-expanded huntingtin and to decrease associated cell toxicity [154].

Viral-Mediated Strategies to Modulate Molecular Chaperones

In addition to small molecule-based therapies, there is potential for development of large therapeutic molecules for the treatment of PD. The use of proteins, peptides, and nucleic acid therapeutics has been limited by poor stability *in vivo* and lack of cellular uptake. However, new advances in recombinant viral vector technology have resulted in feasible gene therapy applications for delivery of large therapeutic molecules. Importantly, a recent phase I study of viral delivery of aromatic amino acid decarboxylase (AADC) in patients with moderately advanced PD provides proof-of-principle for the use of viral delivery systems in PD [155]. Gene therapy may provide strategies to upregulate the expression and/or activity of neuroprotective chaperones in neurons which are vulnerable to neurodegeneration in PD. Preclinical studies in animal models of PD have provided evidence for the potential of viral-mediated Hsp70 expression. One study using the MPTP mouse model demonstrated that Hsp70 gene transfer to striatal dopaminergic neurons by a recombinant AAV could protect against MPTP-induced dopaminergic cell death and the associated decline in striatal dopamine levels [53]. Another study using a rat model of PD showed that AAV vector-mediated overexpression of Hsp70, but not Hsp40, protected against dopaminergic neurodegeneration [54].

Recombinant viral vector technology has also been used to investigate neuroprotection by exogenous expression of proteins that can upregulate Hsp70 function. Hsp104 is a molecular chaperone in the AAA+ family of ATPases that can disaggregate large protein aggregates and rescue proteins trapped within these structures [156]. Hsp104 is expressed in fungi, plants, and bacteria but not in mammals. Nevertheless, Hsp104 can synergize with the mammalian Hsp70 chaperone system to act as a hybrid chaperone system which rescues trapped and aggregated proteins in human cells [157]. Expression of Hsp104 using a lentiviral vector in a rat model of PD reduced the formation of phosphorylated 3-synuclein inclusions and prevented nigrostriatal dopaminergic neurodegeneration induced by mutant A30P 3-synuclein [158].

Unlike the chaperone Hsp104 which synergizes with Hsp70, the co-chaperone BAG5 is a negative regulator of Hsp70. Thus, inhibition of BAG5 may be a feasible approach to upregulate Hsp70 function. As described above, BAG5 physically interacts with Hsp70 and inhibits Hsp70-mediated protein refolding [17]. BAG5-mediated Hsp70 inhibition is associated with increased dopaminergic cell death. The effect of BAG5 on the refolding activity of Hsp70 can be prevented by a mutant BAG5, named BAG5 (DARA). Unlike BAG5, BAG5 (DARA) does not physically interact with Hsp70 but can bind to wild-type BAG5 and may thereby mitigate the BAG5-Hsp70 interaction. To test whether DARA may protect against dopaminergic neurodegeneration by preventing the inhibition of Hsp70 by BAG5 *in vivo*, BAG5 (DARA) was expressed in the SNpc of MPTP-treated mice using targeted delivery of a recombinant adenoviral vector [17]. Adenovirus-mediated expression of BAG5 (DARA) in the SNpc resulted in an increase in dopaminergic neuronal survival, providing early preclinical evidence for its use as a potential neuroprotective strategy in PD.

Another strategy to enhance Hsp70 chaperone function is to use gene silencing to inhibit the expression of negative regulators of Hsp70. RNA interference (RNAi) has emerged as a possible method to reduce target gene expression in brain. Short RNA molecules, such as small interfering RNA (siRNA) and short hairpin RNA (shRNA) can be targeted to specific brain regions by stereotactic injection of recombinant viral vectors. Although RNAi has not yet been well studied in models of dopaminergic neurodegeneration, it can theoretically be applied to PD [159]. There is evidence for the use of RNAi to improve the motor and neuropathological findings in a mouse model of SCA1, supporting its possible utility for the treatment of neurodegenerative diseases.

Non-Viral Strategies to Modulate Molecular Chaperones

A non-viral strategy to deliver chaperones or peptides that upregulate chaperone function to neurons at risk of death is to use cell-penetrating peptides (CPPs). CPPs are short synthetic peptides with the most well characterized being CPPs derived from the transactivator of transcription (Tat) protein of human immunodeficiency virus [160]. When proteins, peptides, or nucleic acids are linked to CPPs, they are readily taken up by cells, or transduced. Transduction of Hsp70 in primary neuronal cultures can be achieved by linking a Tat-derived CPP to Hsp70 (Tat-Hsp70), resulting in increased intraneuronal levels of Hsp70 [161]. Systemic administration of Tat-Hsp70 in mice has been demonstrated to protect dopaminergic neurons of the SNpc against MPTP toxicity [162]. Preliminary studies suggest that intracellular Hsp70 chaperone function may also be increased using CPPs for transduction of proteins known to upregulate Hsp70 expression or activity, such as HSF-1 [163] and Hsp40 [164], respectively.

Drugs with Chaperone Activity

An interesting strategy in development for the treatment of neurological conditions is the use of pharmacological, or chemical, chaperones. Unlike the small molecule-based drugs described above which upregulate chaperone function of endogenous proteins, pharmacological chaperones are small molecules with inherent chaperone qualities; in particular, they act by promoting protein stability and/or folding. Trehalose, a naturally occurring disaccharide found in a variety of plant and animal species, has been identified as a pharmacological chaperone. Initial work in yeast cells has revealed that trehalose may stabilize proteins in their native conformation during heat shock [165]. Yeast cells rapidly degrade trehalose after heat shock at which point the molecular chaperone system becomes primarily responsible for the refolding of denatured proteins. Trehalose has been shown to prevent the aggregation of denatured proteins *in vitro* [165]. The ability of trehalose to prevent protein aggregation *in vivo* was tested in a transgenic mouse model of Huntington's disease [166]. Oral administration of trehalose was found to decrease formation of huntingtin aggregates in this mouse model. Furthermore, the transgenic mice treated with trehalose displayed improved motor function and extended lifespan. More recently, trehalose has been shown to enhance the clearance of the A30P and A53T mutants of 3-synuclein in dopaminergic cell lines [167] supporting further investigation into the use of pharmacological chaperones as potential therapeutic agents for PD.

Additional orally-administered pharmacological chaperones include 1-deoxygalactonojirimycin (AT1001, or migalastat hydrochloride) [168] and 1-deoxynojirimycin (AT2220) [169] which are being studied in clinical trials for Fabry disease and Pompe disease, respectively. Another pharmacological chaperone, AT2101, is being investigated for Gaucher disease. With an emerging genetic and possible pathophysiological link between Gaucher disease and PD [170, 171], preclinical studies of AT2101 and its derivatives for the treatment of PD are currently underway.

CONCLUSIONS

A growing body of research implicates abnormalities in proteostasis, or protein homeostasis, in the pathogenesis of a number of neurodegenerative conditions including PD. As described, the chaperone system is composed of molecular chaperones and co-chaperones and plays a critical role in protein folding and the refolding of misfolded proteins. Furthermore, chaperones are involved in the elimination of aberrant proteins through their close association with the UPS and lysosomal pathways for protein degradation. Evidence to date suggests that Hsp70 and its co-chaperones Hsp40, BAG1, CHIP, and Hip serve a protective function in the context of neurodegenerative diseases whereas the co-chaperone BAG5 enhances dopaminergic neuron death. The chaperone Hsp90 negatively regulates the activity of HSF-1, the transcription factor for Hsp70, and interacts with PINK1 and LRRK2 to regulate their protein levels. Through the utilization of drugs that enhance endogenous chaperone activity or by viral vector-mediated delivery of exogenous chaperones, it may be possible to modify disease progression. Indeed small molecule Hsp90 inhibitors plus viral and non-viral methods of modulating chaperone activity have already demonstrated promise in proof-of-concept pre-clinical work on PD. Such interventions may hold promise in the prevention of further neurodegeneration and may possibly play an augmenting role in strategies aimed for neurorestoration or replacement dopaminergic neurons. It has become increasingly evident that neurodegeneration in PD is not restricted to the dopaminergic neurons of the SNpc but rather involves the death of many different populations of neurons. Thus, similar approaches may be of significant benefit in mitigating the loss of these neurons and thus benefiting patients by slowing the progression of both the motor and non-motor symptoms of PD.

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ABBREVIATIONS

17-AAG	17- (allylamino)-17-demethoxygeldanamycin
17-DMAG	17-dimethylaminoethylamino-17-demethoxy-geldanamycin
AAV	Adeno-associated virus
BAG	Bcl-2 associated athanogene
CHIP	C-terminal Hsp70 interacting protein
CPP	Cell-penetrating peptide
EEVD	Glutamate-glutamate-valine-aspartate
Hip	Hsp70 interacting protein
Hop	Hsp70/Hsp90 organizing protein
HSF-1	Heat shock transcriptional factor-1
Hsp	Heat shock protein
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
PBD	Peptide binding domain
PD	Parkinson's disease
polyQ	Polyglutamine

SCA	Spinocerebellar ataxia
SNpc	Substantia nigra pars compacta
TPR	Tetratricopeptide repeat
UBL	Ubiquitin-like domain
UPS	Ubiquitin-proteasome system

References

1. Lang AE, Lozano AM. Parkinson's disease. First of two parts. *N Engl J Med*. 1998; 339(15):1044–1053. [PubMed: 9761807]
2. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997; 388(6645):839–840. [PubMed: 9278044]
3. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci USA*. 1998; 95(11):6469–6473. [PubMed: 9600990]
4. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG, Kruger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der BM, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. 2009; 41(12):1308–1312. [PubMed: 19915575]
5. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet*. 2009; 41(12):1303–1307. [PubMed: 19915576]
6. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003; 39(6):889–909. [PubMed: 12971891]
7. Beal MF. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann NY Acad Sci*. 2003; 991:120–131. [PubMed: 12846981]
8. Fiskum G, Starkov A, Polster BM, Chinopoulos C. Mitochondrial mechanisms of neural cell death and neuroprotective interventions in Parkinson's disease. *Ann NY Acad Sci*. 2003; 991:111–119. [PubMed: 12846980]
9. Ischiropoulos H, Beckman JS. Oxidative stress and nitration in neurodegeneration: cause, effect, or association? *J Clin Invest*. 2003; 111(2):163–169. [PubMed: 12531868]
10. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol*. 2003; 53(Suppl 3):26–36.
11. Aharon-Peretz J, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson's disease in Ashkenazi Jews. *N Engl J Med*. 2004; 351(19):1972–1977. [PubMed: 15525722]
12. Hung AY, Schwarzschild MA. Clinical trials for neuroprotection in Parkinson's disease: overcoming angst and futility? *Curr Opin Neurol*. 2007; 20(4):477–483. [PubMed: 17620885]
13. Goloubinoff P, De Los RP. The mechanism of Hsp70 chaperones:(entropic) pulling the models together. *Trends Biochem Sci*. 2007; 32(8):372–380. [PubMed: 17629485]
14. Sherman MY, Goldberg AL. Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron*. 2001; 29(1):15–32. [PubMed: 11182078]
15. Kon M, Cuervo AM. Chaperone-mediated autophagy in health and disease. *FEBS Lett*. 2010; 584(7):1399–1404. [PubMed: 20026330]

16. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science*. 2002; 295(5556):865–868. [PubMed: 11823645]
17. Kalia SK, Lee S, Smith PD, Liu L, Crocker SJ, Thorarinsdottir TE, Glover JR, Fon EA, Park DS, Lozano AM. BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. *Neuron*. 2004; 44(6):931–945. [PubMed: 15603737]
18. Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ. Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. *J Biol Chem*. 2004; 279(24):25497–25502. [PubMed: 15044495]
19. McLean PJ, Kawamata H, Shariff S, Hewett J, Sharma N, Ueda K, Breakefield XO, Hyman BT. TorsinA and heat shock proteins act as molecular chaperones: suppression of alpha-synuclein aggregation. *J Neurochem*. 2002; 83(4):846–854. [PubMed: 12421356]
20. Sharma N, Hewett J, Ozelius LJ, Ramesh V, McLean PJ, Breakefield XO, Hyman BT. A close association of torsinA and alpha-synuclein in Lewy bodies: a fluorescence resonance energy transfer study. *Am J Pathol*. 2001; 159(1):339–344. [PubMed: 11438481]
21. Yvert G, Lindenberg KS, Picaud S, Landwehrmeyer GB, Sahel JA, Mandel JL. Expanded polyglutamines induce neurodegeneration and trans-neuronal alterations in cerebellum and retina of SCA7 transgenic mice. *Hum Mol Genet*. 2000; 9(17):2491–2506. [PubMed: 11030754]
22. Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, Wanker EE. Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol Biol Cell*. 2001; 12(5):1393–1407. [PubMed: 11359930]
23. Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, DeMartino GN, Marcelli M, Weigel NL, Mancini MA. 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*. 1999; 8(5):731–741. [PubMed: 10196362]
24. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol*. 2000; 10(12):524–530. [PubMed: 11121744]
25. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat Genet*. 1998; 19(2):148–154. [PubMed: 9620770]
26. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL. Analysis of the role of heat shock protein(Hsp) molecular chaperones in polyglutamine disease. *J Neurosci*. 1999; 19(23):10338–10347. [PubMed: 10575031]
27. Berke SJ, Paulson HL. Protein aggregation and the ubiquitin proteasome pathway: gaining the UPPer hand on neurodegeneration. *Curr Opin Genet Dev*. 2003; 13(3):253–261. [PubMed: 12787787]
28. Ritossa F. A new puffing pattern induced by temperature shock and DNP in Drosophila. *Experientia*. 1962; 18:571–573.
29. Tissieres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. *J Mol Biol*. 1974; 84(3):389–398. [PubMed: 4219221]
30. Sakahira H, Breuer P, Hayer-Hartl MK, Hartl FU. Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. *Proc Natl Acad Sci USA*. 2002; 99(Suppl 4):16412–16418. [PubMed: 12189209]
31. Witt SN. Hsp70 molecular chaperones and Parkinson's disease. *Biopolymers*. 2010; 93(3):218–228. [PubMed: 19768775]
32. Fink AL. Chaperone-mediated protein folding. *Physiol Rev*. 1999; 79(2):425–449. [PubMed: 10221986]
33. Beissinger M, Buchner J. How chaperones fold proteins. *Biol Chem*. 1998; 379(3):245–259. [PubMed: 9563819]
34. Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. *Cell*. 1998; 92(3):351–366. [PubMed: 9476895]
35. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science*. 2002; 295(5561):1852–1858. [PubMed: 11884745]

36. Nollen EA, Morimoto RI. Chaperoning signaling pathways: molecular chaperones as stress-sensing 'heat shock' proteins. *J Cell Sci.* 2002; 115(Pt 14):2809–2816. [PubMed: 12082142]
37. Kelley WL. Molecular chaperones: How J domains turn on Hsp70s. *Curr Biol.* 1999; 9(8):305–308.
38. Hohfeld J. Regulation of the heat shock conjugate Hsc70 in the mammalian cell: the characterization of the anti-apoptotic protein BAG-1 provides novel insights. *Biol Chem.* 1998; 379(3):269–74. [PubMed: 9563821]
39. Hohfeld J, Minami Y, Hartl FU. Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle. *Cell.* 1995; 83(4):589–598. [PubMed: 7585962]
40. Hohfeld J, Jentsch S. GrpE-like regulation of the hsc70 chaperone by the anti-apoptotic protein BAG-1. *EMBO J.* 1997; 16(20):6209–6216. [PubMed: 9321400]
41. Zeiner M, Gebauer M, Gehring U. Mammalian protein RAP46: an interaction partner and modulator of 70 kDa heat shock proteins. *EMBO J.* 1997; 16(18):5483–5490. [PubMed: 9312007]
42. Takayama S, Sato T, Krajewski S, Kochel K, Irie S, Millan JA, Reed JC. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell.* 1995; 80(2):279–284. [PubMed: 7834747]
43. Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, Moarefi I. Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. *Science.* 2001; 291(5508):1553–1557. [PubMed: 11222862]
44. Gebauer M, Zeiner M, Gehring U. Proteins interacting with the molecular chaperone hsp70/hsc70: physical associations and effects on refolding activity. *FEBS Lett.* 1997; 417(1):109–113. [PubMed: 9395086]
45. Bimston D, Song J, Winchester D, Takayama S, Reed JC, Morimoto RI. BAG-1, a negative regulator of Hsp70 chaperone activity, uncouples nucleotide hydrolysis from substrate release. *EMBO J.* 1998; 17(23):6871–6878. [PubMed: 9843493]
46. Cummings CJ, Sun Y, Opal P, Antalffy B, Mestrl R, Orr HT, Dillmann WH, Zoghbi HY. Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. *Hum Mol Genet.* 2001; 10(14):1511–1518. [PubMed: 11448943]
47. Kazemi-Esfarjani P, Benzer S. Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science.* 2000; 287(5459):1837–1840. [PubMed: 10710314]
48. Chan HY, Warrick JM, Gray-Board GL, Paulson HL, Bonini NM. Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in *Drosophila*. *Hum Mol Genet.* 2000; 9(19):2811–2820. [PubMed: 11092757]
49. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. *Nat Genet.* 1999; 23(4):425–428. [PubMed: 10581028]
50. Wu YR, Wang CK, Chen CM, Hsu Y, Lin SJ, Lin YY, Fung HC, Chang KH, Lee-Chen GJ. Analysis of heat-shock protein 70 gene polymorphisms and the risk of Parkinson's disease. *Hum Genet.* 2004; 114(3):236–241. [PubMed: 14605873]
51. Flower TR, Chesnokova LS, Froelich CA, Dixon C, Witt SN. Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. *J Mol Biol.* 2005; 351(5):1081–1100. [PubMed: 16051265]
52. Quigley DJ, Gorman AM, Samali A. Heat shock protects PC12 cells against MPP+ toxicity. *Brain Res.* 2003; 993(1–2):133–139. [PubMed: 14642838]
53. Dong Z, Wolfer DP, Lipp HP, Bueler H. Hsp70 gene transfer by adeno-associated virus inhibits MPTP-induced nigrostriatal degeneration in the mouse model of Parkinson disease. *Mol Ther.* 2005; 11(1):80–88. [PubMed: 15585408]
54. Jung AE, Fitzsimons HL, Bland RJ, During MJ, Young D. HSP70 and constitutively active HSF1 mediate protection against CDCrel-1-mediated toxicity. *Mol Ther.* 2008; 16(6):1048–1055. [PubMed: 18398426]
55. Klucken J, Shin Y, Hyman BT, McLean PJ. A single amino acid substitution differentiates Hsp70-dependent effects on alpha-synuclein degradation and toxicity. *Biochem Biophys Res Commun.* 2004; 325(1):367–373. [PubMed: 15522241]

56. Klucken J, Outeiro TF, Nguyen P, McLean PJ, Hyman BT. Detection of novel intracellular alpha-synuclein oligomeric species by fluorescence lifetime imaging. *FASEB J*. 2006; 20(12):2050–2057. [PubMed: 17012257]
57. Luk KC, Mills IP, Trojanowski JQ, Lee VM. Interactions between Hsp70 and the hydrophobic core of alpha-synuclein inhibit fibril assembly. *Biochemistry*. 2008; 47(47):12614–12625. [PubMed: 18975920]
58. Huang C, Cheng H, Hao S, Zhou H, Zhang X, Gao J, Sun QH, Hu H, Wang CC. Heat shock protein 70 inhibits alpha-synuclein fibril formation *via* interactions with diverse intermediates. *J Mol Biol*. 2006; 364(3):323–336. [PubMed: 17010992]
59. Outeiro TF, Putcha P, Tetzlaff JE, Spoelgen R, Koker M, Carvalho F, Hyman BT, McLean PJ. Formation of toxic oligomeric alpha-synuclein species in living cells. *PLoS One*. 2008; 3(4):e1867. [PubMed: 18382657]
60. McLean PJ, Klucken J, Shin Y, Hyman BT. Geldanamycin induces Hsp70 and prevents alpha-synuclein aggregation and toxicity *in vitro*. *Biochem Biophys Res Commun*. 2004; 321(3):665–669. [PubMed: 15358157]
61. Roodveldt C, Bertoni CW, Andersson A, van der Goot AT, Hsu ST, Fernandez-Montesinos R, de JJ, van Ham TJ, Nollen EA, Pozo D, Christodoulou J, Dobson CM. Chaperone proteostasis in Parkinson's disease: stabilization of the Hsp70/alpha-synuclein complex by Hip. *EMBO J*. 2009; 28(23):3758–3770. [PubMed: 19875982]
62. Imai Y, Soda M, Hatakeyama S, Akagi T, Hashikawa T, Nakayama KI, Takahashi R. CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity. *Mol Cell*. 2002; 10(1):55–67. [PubMed: 12150907]
63. Yang F, Yang YP, Mao CJ, Cao BY, Cai ZL, Shi JJ, Huang JZ, Zhang P, Liu CF. Role of autophagy and proteasome degradation pathways in apoptosis of PC12 cells overexpressing human alpha-synuclein. *Neurosci Lett*. 2009; 454(3):203–208. [PubMed: 19429084]
64. Tsai YC, Fishman PS, Thakor NV, Oyler GA. Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. *J Biol Chem*. 2003; 278(24):22044–22055. [PubMed: 12676955]
65. Chavez Zobel AT, Loranger A, Marceau N, Theriault JR, Lambert H, Landry J. Distinct chaperone mechanisms can delay the formation of aggregates by the myopathy-causing R120G alphaB-crystallin mutant. *Hum Mol Genet*. 2003; 12(13):1609–1620. [PubMed: 12812987]
66. Bailey CK, Andriola IF, Kampinga HH, Merry DE. Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. *Hum Mol Genet*. 2002; 11(5):515–523. [PubMed: 11875046]
67. Adachi H, Katsuno M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, Kusakabe M, Yoshiki A, Kobayashi Y, Doyu M, Sobue G. 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*. 2003; 23(6):2203–2211. [PubMed: 12657679]
68. Murata S, Minami Y, Minami M, Chiba T, Tanaka K. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep*. 2001; 2(12):1133–1138. [PubMed: 11743028]
69. Murata S, Chiba T, Tanaka K. CHIP: a quality-control E3 ligase collaborating with molecular chaperones. *Int J Biochem Cell Biol*. 2003; 35(5):572–578. [PubMed: 12672450]
70. Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM. The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat Cell Biol*. 2001; 3(1):100–105. [PubMed: 11146634]
71. Luders J, Demand J, Hohfeld J. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. *J Biol Chem*. 2000; 275(7):4613–4617. [PubMed: 10671488]
72. Hung WJ, Roberson RS, Taft J, Wu DY. Human BAG-1 proteins bind to the cellular stress response protein GADD34 and interfere with GADD34 functions. *Mol Cell Biol*. 2003; 23(10):3477–3486. [PubMed: 12724406]

73. Demand J, Alberti S, Patterson C, Hohfeld J. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr Biol*. 2001; 11(20):1569–1577. [PubMed: 11676916]
74. Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J, Patterson C. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat Cell Biol*. 2001; 3(1):93–96. [PubMed: 11146632]
75. Cardozo CP, Michaud C, Ost MC, Fliss AE, Yang E, Patterson C, Hall SJ, Caplan AJ. C-terminal Hsp-interacting protein slows androgen receptor synthesis reduces its rate of degradation. *Arch Biochem Biophys*. 2003; 410(1):134–140. [PubMed: 12559985]
76. Alberti S, Demand J, Esser C, Emmerich N, Schild H, Hohfeld J. Ubiquitylation of BAG-1 Suggests a Novel Regulatory Mechanism during the Sorting of Chaperone Substrates to the Proteasome. *J Biol Chem*. 2002; 277(48):45920–45927. [PubMed: 12297498]
77. Saleh A, Srinivasula SM, Balkir L, Robbins PD, Alnemri ES. Negative regulation of the Apaf-1 apoptosome by Hsp70. *Nat Cell Biol*. 2000; 2(8):476–483. [PubMed: 10934467]
78. Ravagnan L, Gurbuxani S, Susin SA, Maise C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger JM, Garrido C, Kroemer G. Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nat Cell Biol*. 2001; 3(9):839–843. [PubMed: 11533664]
79. Jaattela M, Wissing D, Kokholm K, Kallunki T, Egeblad M. Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases. *EMBO J*. 1998; 17(21):6124–6134. [PubMed: 9799222]
80. Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Taylor P, Morimoto RI, Cohen GM, Green DR. Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol*. 2000; 2(8):469–475. [PubMed: 10934466]
81. Volloch V, Mosser DD, Massie B, Sherman MY. Reduced thermotolerance in aged cells results from a loss of an hsp72-mediated control of JNK signaling pathway. *Cell Stress Chaperones*. 1998; 3(4):265–271. [PubMed: 9880239]
82. Park HS, Lee JS, Huh SH, Seo JS, Choi EJ. Hsp72 functions as a natural inhibitory protein of c-Jun N-terminal kinase. *EMBO J*. 2001; 20(3):446–456. [PubMed: 11157751]
83. Mosser DD, Caron AW, Bourget L, Denis-Larose C, Massie B. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Mol Cell Biol*. 1997; 17(9):5317–5327. [PubMed: 9271409]
84. Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. *Mol Cell Biol*. 2000; 20(19):7146–7159. [PubMed: 10982831]
85. Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, Sherman MY. Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. *J Biol Chem*. 1997; 272(29):18033–18037. [PubMed: 9218432]
86. Gabai VL, Meriin AB, Yaglom JA, Volloch VZ, Sherman MY. Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. *FEBS Lett*. 1998; 438(1–2):1–4. [PubMed: 9821948]
87. Moore DJ, West AB, Dikeman DA, Dawson VL, Dawson TM. Parkin mediates the degradation-independent ubiquitination of Hsp70. *J Neurochem*. 2008; 105(5):1806–1819. [PubMed: 18248624]
88. Liu M, Aneja R, Sun X, Xie S, Wang H, Wu X, Dong JT, Li M, Joshi HC, Zhou J. Parkin regulates Eg5 expression by Hsp70 ubiquitination-dependent inactivation of c-Jun NH2-terminal kinase. *J Biol Chem*. 2008; 283(51):35783–35788. [PubMed: 18845538]
89. Nardai G, Csermely P, Soti C. Chaperone function and chaperone overload in the aged. A preliminary analysis. *Exp Gerontol*. 2002; 37(10–11):1257–1262. [PubMed: 12470839]
90. Gutschmann-Conrad A, Heydari AR, You S, Richardson A. The expression of heat shock protein 70 decreases with cellular senescence *in vitro* and in cells derived from young and old human subjects. *Exp Cell Res*. 1998; 241(2):404–413. [PubMed: 9637782]
91. Okado-Matsumoto A, Fridovich I. Amyotrophic lateral sclerosis: a proposed mechanism. *Proc Natl Acad Sci USA*. 2002; 99(13):9010–9014. [PubMed: 12060716]

92. Takayama S, Reed JC. Molecular chaperone targeting and regulation by BAG family proteins. *Nat Cell Biol.* 2001; 3(10):237–241.
93. Zeiner M, Gehring U. A protein that interacts with members of the nuclear hormone receptor family: identification and cDNA cloning. *Proc Natl Acad Sci USA.* 1995; 92(25):11465–11469. [PubMed: 8524784]
94. Zeiner M, Niyaz Y, Gehring U. The hsp70-associating protein Hap46 binds to DNA and stimulates transcription. *Proc Natl Acad Sci USA.* 1999; 96(18):10194–10199. [PubMed: 10468585]
95. Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, Morimoto RI, Reed JC. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. *EMBO J.* 1997; 16(16):4887–4896. [PubMed: 9305631]
96. Nollen EA, Brunsting JF, Song J, Kampinga HH, Morimoto RI. Bag1 functions *in vivo* as a negative regulator of Hsp70 chaperone activity. *Mol Cell Biol.* 2000; 20(3):1083–1088. [PubMed: 10629065]
97. Nollen EA, Kabakov AE, Brunsting JF, Kanon B, Hohfeld J, Kampinga HH. Modulation of *in vivo* HSP70 chaperone activity by Hip and Bag-1. *J Biol Chem.* 2001; 276(7):4677–4682. [PubMed: 11076956]
98. Takayama S, Xie Z, Reed JC. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J Biol Chem.* 1999; 274(2):781–786. [PubMed: 9873016]
99. Doong H, Price J, Kim YS, Gasbarre C, Probst J, Liotta LA, Blanchette J, Rizzo K, Kohn E. CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. *Oncogene.* 2000; 19(38):4385–4395. [PubMed: 10980614]
100. Briknarova K, Takayama S, Homma S, Baker K, Cabezas E, Hoyt DW, Li Z, Satterthwait AC, Ely KR. BAG4/SODD protein contains a short BAG domain. *J Biol Chem.* 2002; 277(34):31172–31178. [PubMed: 12058034]
101. Thress K, Song J, Morimoto RI, Kornbluth S. Reversible inhibition of Hsp70 chaperone function by Scythe and Reaper. *EMBO J.* 2001; 20(5):1033–1041. [PubMed: 11230127]
102. Song J, Takeda M, Morimoto RI. Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. *Nat Cell Biol.* 2001; 3(3):276–282. [PubMed: 11231577]
103. Matsuzawa S, Takayama S, Froesch BA, Zapata JM, Reed JC. p53-inducible human homologue of Drosophila seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. *EMBO J.* 1998; 17(10):2736–2747. [PubMed: 9582267]
104. Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. RING fingers mediate ubiquitin-conjugating enzyme(E2)-dependent ubiquitination. *Proc Natl Acad Sci USA.* 1999; 96(20):11364–11369. [PubMed: 10500182]
105. Engelender S, Kaminsky Z, Guo X, Sharp AH, Amaravi RK, Kleiderlein JJ, Margolis RL, Troncoso JC, Lanahan AA, Worley PF, Dawson VL, Dawson TM, Ross CA. Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat Genet.* 1999; 22(1):110–114. [PubMed: 10319874]
106. Szargel R, Rott R, Eyal A, Haskin J, Shani V, Balan L, Wolosker H, Engelender S. Synphilin-1A inhibits seven in absentia homolog(SIAH) and modulates alpha-synuclein monoubiquitylation and inclusion formation. *J Biol Chem.* 2009; 284(17):11706–11716. [PubMed: 19224863]
107. Rott R, Szargel R, Haskin J, Shani V, Shainskaya A, Manov I, Liani E, Avraham E, Engelender S. Monoubiquitylation of alpha-synuclein by seven in absentia homolog(SIAH) promotes its aggregation in dopaminergic cells. *J Biol Chem.* 2008; 283(6):3316–3328. [PubMed: 18070888]
108. Nagano Y, Yamashita H, Takahashi T, Kishida S, Nakamura T, Iseki E, Hattori N, Mizuno Y, Kikuchi A, Matsumoto M. Siah-1 facilitates ubiquitination and degradation of synphilin-1. *J Biol Chem.* 2003; 278(1):23–33.
109. Arndt V, Daniel C, Nastainczyk W, Alberti S, Hohfeld J. BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. *Mol Biol Cell.* 2005; 16(12):5891–5900. [PubMed: 16207813]
110. Dai Q, Qian SB, Li HH, McDonough H, Borchers C, Huang D, Takayama S, Younger JM, Ren HY, Cyr DM, Patterson C. Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. *J Biol Chem.* 2005; 280(46):38673–38681. [PubMed: 16169850]

111. Carrettiero DC, Hernandez I, Neveu P, Papagiannakopoulos T, Kosik KS. The cochaperone BAG2 sweeps paired helical filament- insoluble tau from the microtubule. *J Neurosci.* 2009; 29(7):2151–2161. [PubMed: 19228967]
112. Doong H, Rizzo K, Fang S, Kulpa V, Weissman AM, Kohn EC. CAIR-1/BAG-3 abrogates heat shock protein-70 chaperone complex-mediated protein degradation: accumulation of poly-ubiquitinated Hsp90 client proteins. *J Biol Chem.* 2003; 278(31):28490–28500. [PubMed: 12750378]
113. Carra S, Seguin SJ, Landry J. HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. *Autophagy.* 2008; 4(2):237–239. [PubMed: 18094623]
114. Carra S, Seguin SJ, Lambert H, Landry J. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. *J Biol Chem.* 2008; 283(3):1437–1444. [PubMed: 18006506]
115. Gamerding M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* 2009; 28(7):889–901. [PubMed: 19229298]
116. Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY, Patterson C. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol.* 1999; 19(6):4535–4545. [PubMed: 10330192]
117. Kampinga HH, Kanon B, Salomons FA, Kabakov AE, Patterson C. Overexpression of the cochaperone CHIP enhances Hsp70-dependent folding activity in mammalian cells. *Mol Cell Biol.* 2003; 23(14):4948–4958. [PubMed: 12832480]
118. Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C. CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation. *J Biol Chem.* 2001; 276(46):42938–42944. [PubMed: 11557750]
119. Shin Y, Klucken J, Patterson C, Hyman BT, McLean PJ. The co-chaperone carboxyl terminus of Hsp70-interacting protein(CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. *J Biol Chem.* 2005; 280(25):23727–23734. [PubMed: 15845543]
120. Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, De LM, McGowan E, Lewis J, Prihar G, Kim J, Dillmann WH, Browne SE, Hall A, Voellmy R, Tsuboi Y, Dawson TM, Wolozin B, Hardy J, Hutton M. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Hum Mol Genet.* 2004; 13(7):703–714. [PubMed: 14962978]
121. Tetzlaff JE, Putcha P, Outeiro TF, Ivanov A, Berezovska O, Hyman BT, McLean PJ. CHIP targets toxic alpha-Synuclein oligomers for degradation. *J Biol Chem.* 2008; 283(26):17962–17968. [PubMed: 18436529]
122. Ko HS, Bailey R, Smith WW, Liu Z, Shin JH, Lee YI, Zhang YJ, Jiang H, Ross CA, Moore DJ, Patterson C, Petrucelli L, Dawson TM, Dawson VL. CHIP regulates leucine-rich repeat kinase-2 ubiquitination, degradation, and toxicity. *Proc Natl Acad Sci USA.* 2009; 106(8):2897–2902. [PubMed: 19196961]
123. Ding Q, Lewis JJ, Strum KM, Dimayuga E, Bruce-Keller AJ, Dunn JC, Keller JN. Polyglutamine expansion, protein aggregation, proteasome activity, and neural survival. *J Biol Chem.* 2002; 277(16):13935–13942. [PubMed: 11782460]
124. Dai Q, Zhang C, Wu Y, McDonough H, Whaley RA, Godfrey V, Li HH, Madamanchi N, Xu W, Neckers L, Cyr D, Patterson C. CHIP activates HSF1 and confers protection against apoptosis and cellular stress. *EMBO J.* 2003; 22(20):5446–5458. [PubMed: 14532117]
125. Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, Fefer D, Schwarzschild MA, Schlossmacher MG, Hauser MA, Vance JM, Sudarsky LR, Standaert DG, Growdon JH, Jensen RV, Gullans SR. Molecular markers of early Parkinson's disease based on gene expression in blood. *Proc Natl Acad Sci USA.* 2007; 104(3):955–960. [PubMed: 17215369]
126. Hahn JS. The Hsp90 chaperone machinery: from structure to drug development. *BMB Rep.* 2009; 42(10):623–630. [PubMed: 19874705]
127. Johnson BD, Schumacher RJ, Ross ED, Toft DO. Hop modulates Hsp70/Hsp90 interactions in protein folding. *J Biol Chem.* 1998; 273(6):3679–3686. [PubMed: 9452498]

128. Uryu K, Richter-Landsberg C, Welch W, Sun E, Goldbaum O, Norris EH, Pham CT, Yazawa I, Hilburger K, Micsenyi M, Giasson BI, Bonini NM, Lee VM, Trojanowski JQ. Convergence of heat shock protein 90 with ubiquitin in filamentous alpha-synuclein inclusions of alpha-synucleinopathies. *Am J Pathol.* 2006; 168(3):947–961. [PubMed: 16507910]
129. Weihofen A, Ostaszewski B, Minami Y, Selkoe DJ. Pink1 Parkinson mutations, the Cdc37/Hsp90 chaperones and Parkin all influence the maturation or subcellular distribution of Pink1. *Hum Mol Genet.* 2008; 17(4):602–616. [PubMed: 18003639]
130. Ding X, Goldberg MS. Regulation of LRRK2 stability by the E3 ubiquitin ligase CHIP. *PLoS One.* 2009; 4(6):e5949. [PubMed: 19536328]
131. Moriwaki Y, Kim YJ, Ido Y, Misawa H, Kawashima K, Endo S, Takahashi R. L347P PINK1 mutant that fails to bind to Hsp90/Cdc37 chaperones is rapidly degraded in a proteasome-dependent manner. *Neurosci Res.* 2008; 61(1):43–48. [PubMed: 18359116]
132. Wang L, Xie C, Greggio E, Parisiadou L, Shim H, Sun L, Chandran J, Lin X, Lai C, Yang WJ, Moore DJ, Dawson TM, Dawson VL, Chiosis G, Cookson MR, Cai H. The chaperone activity of heat shock protein 90 is critical for maintaining the stability of leucine-rich repeat kinase 2. *J Neurosci.* 2008; 28(13):3384–3391. [PubMed: 18367605]
133. Auluck PK, Bonini NM. Pharmacological prevention of Parkinson disease in *Drosophila*. *Nat Med.* 2002; 8(11):1185–1186. [PubMed: 12411925]
134. Putcha P, Danzer KM, Kranich L, Scott A, Silinski M, Mabbett S, Hicks CD, Veal JM, Steed PM, Hyman BT, McLean PJ. Brain permeable small molecule inhibitors of Hsp90 prevent alpha-synuclein oligomer formation and rescue alpha synuclein-induced toxicity. *J Pharmacol Exp Ther.* 2010; 332(3):849–857. [PubMed: 19934398]
135. Dickey CA, Eriksen J, Kamal A, Burrows F, Kasibhatla S, Eckman CB, Hutton M, Petrucelli L. Development of a high throughput drug screening assay for the detection of changes in tau levels -- proof of concept with HSP90 inhibitors. *Curr Alzheimer Res.* 2005; 2(2):231–238. [PubMed: 15974923]
136. Fujikake N, Nagai Y, Popiel HA, Okamoto Y, Yamaguchi M, Toda T. Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. *J Biol Chem.* 2008; 283(38):26188–26197. [PubMed: 18632670]
137. Shen HY, He JC, Wang Y, Huang QY, Chen JF. Geldanamycin induces heat shock protein 70 and protects against MPTP-induced dopaminergic neurotoxicity in mice. *J Biol Chem.* 2005; 280(48):39962–39969. [PubMed: 16210323]
138. Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, Inukai A, Doyu M, Sobue G. 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. *Nat Med.* 2005; 11(10):1088–1095. [PubMed: 16155577]
139. Heath EI, Hillman DW, Vaishampayan U, Sheng S, Sarkar F, Harper F, Gaskins M, Pitot HC, Tan W, Ivy SP, Pili R, Carducci MA, Erlichman C, Liu G. A phase II trial of 17-allylamino-17-demethoxygeldanamycin in patients with hormone-refractory metastatic prostate cancer. *Clin Cancer Res.* 2008; 14(23):7940–7946. [PubMed: 19047126]
140. Ramanathan RK, Trump DL, Eiseman JL, Belani CP, Agarwala SS, Zuhowski EG, Lan J, Potter DM, Ivy SP, Ramalingam S, Brufsky AM, Wong MK, Tutchko S, Egorin MJ. Phase I pharmacokinetic-pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin(17AAG, NSC 330507): a novel inhibitor of heat shock protein 90, in patients with refractory advanced cancers. *Clin Cancer Res.* 2005; 11(9):3385–3391. [PubMed: 15867239]
141. Chiosis G, Tao H. Purine-scaffold Hsp90 inhibitors. *IDrugs.* 2006; 9(11):778–782. [PubMed: 17096299]
142. Cysyk RL, Parker RJ, Barchi JJ Jr, Steeg PS, Hartman NR, Strong JM. Reaction of geldanamycin C17-substituted analogues with glutathione: product identifications pharmacological implications. *Chem Res Toxicol.* 2006; 19(3):376–381. [PubMed: 16544941]
143. Chandarlapaty S, Sawai A, Ye Q, Scott A, Silinski M, Huang K, Fadden P, Partdrige J, Hall S, Steed P, Norton L, Rosen N, Solit DB. SNX2112; a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers. *Clin Cancer Res.* 2008; 14(1):240–248. [PubMed: 18172276]

144. Okawa Y, Hideshima T, Steed P, Vallet S, Hall S, Huang K, Rice J, Barabasz A, Foley B, Ikeda H, Raje N, Kiziltepe T, Yasui H, Enatsu S, Anderson KC. SNX-2112; a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling *via* Akt and ERK. *Blood*. 2009; 113(4):846–855. [PubMed: 18948577]
145. Taldone T, Gozman A, Maharaj R, Chiosis G. Targeting Hsp90: small-molecule inhibitors and their clinical development. *Curr Opin Pharmacol*. 2008; 8(4):370–374. [PubMed: 18644253]
146. Taldone T, Sun W, Chiosis G. Discovery and development of heat shock protein 90 inhibitors. *Bioorg Med Chem*. 2009; 17(6):2225–2235. [PubMed: 19017562]
147. Hanson BE, Vesole DH. Retaspimycin hydrochloride (IPI-504): a novel heat shock protein inhibitor as an anticancer agent. *Expert Opin Investig Drugs*. 2009; 18(9):1375–1383.
148. Lin TY, Bear M, Du Z, Foley KP, Ying W, Barsoum J, London C. The novel HSP90 inhibitor STA-9090 exhibits activity against Kit-dependent and -independent malignant mast cell tumors. *Exp Hematol*. 2008; 36(10):1266–1277. [PubMed: 18657349]
149. Lanka V, Wieland S, Barber J, Cudkowicz M. Arimoclomol: a potential therapy under development for ALS. *Expert Opin Investig Drugs*. 2009; 18(12):1907–1918.
150. Kalmar B, Greensmith L. Activation of the heat shock response in a primary cellular model of motoneuron neurodegeneration-evidence for neuroprotective and neurotoxic effects. *Cell Mol Biol Lett*. 2009; 14(2):319–335. [PubMed: 19183864]
151. Kalmar B, Novoselov S, Gray A, Cheetham ME, Margulis B, Greensmith L. Late stage treatment with arimoclomol delays disease progression and prevents protein aggregation in the SOD1 mouse model of ALS. *J Neurochem*. 2008; 107(2):339–350. [PubMed: 18673445]
152. Kieran D, Kalmar B, Dick JR, Riddoch-Contreras J, Burnstock G, Greensmith L. Treatment with arimoclomol, a coinducer of heat shock proteins, delays disease progression in ALS mice. *Nat Med*. 2004; 10(4):402–405. [PubMed: 15034571]
153. Cudkowicz ME, Shefner JM, Simpson E, Grasso D, Yu H, Zhang H, Shui A, Schoenfeld D, Brown RH, Wieland S, Barber JR. Arimoclomol at dosages up to 300 mg/day is well tolerated and safe in amyotrophic lateral sclerosis. *Muscle Nerve*. 2008; 38(1):837–844. [PubMed: 18551622]
154. Neef DW, Turski ML, Thiele DJ. Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. *PLoS Biol*. 2010; 8(1):e1000291. [PubMed: 20098725]
155. Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, VanBrocklin HF, Wright JF, Bankiewicz KS, Aminoff MJ. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology*. 2009; 73(20):1662–1669. [PubMed: 19828868]
156. Glover JR, Lindquist S. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell*. 1998; 94(1):73–82. [PubMed: 9674429]
157. Mosser DD, Ho S, Glover JR. *Saccharomyces cerevisiae* Hsp104 enhances the chaperone capacity of human cells and inhibits heat stress-induced proapoptotic signaling. *Biochemistry*. 2004; 43(25):8107–8115. [PubMed: 15209506]
158. Lo BC, Shorter J, Regulier E, Lashuel H, Iwatsubo T, Lindquist S, Aebischer P. Hsp104 antagonizes alpha-synuclein aggregation and reduces dopaminergic degeneration in a rat model of Parkinson disease. *J Clin Invest*. 2008; 118(9):3087–3097. [PubMed: 18704197]
159. Manfredsson FP, Lewin AS, Mandel RJ. RNA knockdown as a potential therapeutic strategy in Parkinson's disease. *Gene Ther*. 2006; 13(6):517–524. [PubMed: 16267570]
160. Heitz F, Morris MC, Divita G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. *Br J Pharmacol*. 2009; 157(2):195–206. [PubMed: 19309362]
161. Lai Y, Du L, Dunsmore KE, Jenkins LW, Wong HR, Clark RS. Selectively increasing inducible heat shock protein 70 *via* TAT-protein transduction protects neurons from nitrosative stress and excitotoxicity. *J Neurochem*. 2005; 94(2):360–366. [PubMed: 15998287]
162. Nagel F, Falkenburger BH, Tonges L, Kowsky S, Poppelmeier C, Schulz JB, Bahr M, Dietz GP. Tat-Hsp70 protects dopaminergic neurons in midbrain cultures and in the substantia nigra in models of Parkinson's disease. *J Neurochem*. 2008; 105(3):853–864. [PubMed: 18182047]

163. Hou Y, Zou J. Delivery of HSF1(+) protein using HIV-1 TAT protein transduction domain. *Mol Biol Rep.* 2009; 36(8):2271–2277. [PubMed: 19190998]
164. Kim SA, Chang S, Yoon JH, Ahn SG. TAT-Hsp40 inhibits oxidative stress-mediated cytotoxicity *via* the inhibition of Hsp70 ubiquitination. *FEBS Lett.* 2008; 582(5):734–740. [PubMed: 18258197]
165. Singer MA, Lindquist S. Multiple effects of trehalose on protein folding *in vitro* and *in vivo*. *Mol Cell.* 1998; 1(5):639–648. [PubMed: 9660948]
166. Tanaka M, Machida Y, Niu S, Ikeda T, Jana NR, Doi H, Kurosawa M, Nekooki M, Nukina N. Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat Med.* 2004; 10(2):148–154. [PubMed: 14730359]
167. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J Biol Chem.* 2007; 282(8):5641–5652. [PubMed: 17182613]
168. Flanagan JJ, Rossi B, Tang K, Wu X, Mascioli K, Donaudo F, Tuzzi MR, Fontana F, Cubellis MV, Porto C, Benjamin E, Lockhart DJ, Valenzano KJ, Andria G, Parenti G, Do HV. The pharmacological chaperone 1-deoxyojirimycin increases the activity and lysosomal trafficking of multiple mutant forms of acid alpha-glucosidase. *Hum Mutat.* 2009; 30(12):1683–1692. [PubMed: 19862843]
169. Khanna R, Soska R, Lun Y, Feng J, Frascella M, Young B, Brignol N, Pellegrino L, Sitaraman SA, Desnick RJ, Benjamin ER, Lockhart DJ, Valenzano KJ. The pharmacological chaperone 1-deoxygalactonojirimycin reduces tissue globotriaosylceramide levels in a mouse model of Fabry disease. *Mol Ther.* 2010; 18(1):23–33. [PubMed: 19773742]
170. Hruska KS, Goker-Alpan O, Sidransky E. Gaucher disease and the synucleinopathies. *J Biomed Biotechnol.* 2006; 2006(3):78549. [PubMed: 17047314]
171. Neumann J, Bras J, Deas E, O’Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R, Segarane B, Singleton A, Lees A, Hardy J, Houlden H, Revesz T, Wood NW. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson’s disease. *Brain.* 2009; 132(Pt 7):1783–1794. [PubMed: 19286695]

Fig. 1.

(A) Domain structure of molecular chaperones Hsp70 and Hsp90. (B) Domain structure of selected co-chaperones: BAG domain-containing family members BAG1 to BAG6; TPR domain-containing co-chaperones CHIP, Hip, and Hop; and, DnaJ domain-containing co-chaperone Hsp40.

Geldanamycin and derivatives

Novel Hsp90 inhibitors

R=H

SNX-5422

R=C(O)CH₂NH₂

Radicalol

Arimoclomol

Fig. 2.

Chemical structures of Hsp90 inhibitors: geldanamycin and its derivatives 17-AAG, 17-DMAG, and IPI-504; SNX-2112 and its prodrug SNX-5422; and, radicalol. Chemical structure of arimoclomol, which activates HSF-1.