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## Molecular Chaperones in Parkinson's Disease – Present and Future

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### Abstract

Parkinson's disease, like many other neurodegenerative disorders, is characterized by the progressive accumulation of pathogenic protein species and the formation of intracellular inclusion bodies. The cascade by which the small synaptic protein  $\alpha$ -synuclein misfolds to form distinctive protein aggregates, termed Lewy bodies and Lewy neurites, has been the subject of intensive research for more than a decade. Genetic and pathological studies in Parkinson's disease patients as well as experimental studies in disease models have clearly established altered protein metabolism as a key element in the pathogenesis of Parkinson's disease. Alterations in protein metabolism include misfolding and aggregation, post-translational modification and dysfunctional degradation of cytotoxic protein species.

Protein folding and re-folding are both mediated by a highly conserved network of molecules, called molecular chaperones and co-chaperones. In addition to the regulatory role in protein folding, molecular chaperone function is intimately associated with pathways of protein degradation, such as the ubiquitin-proteasome system and the autophagy-lysosomal pathway, to effectively remove irreversibly misfolded proteins. Because of the central role of molecular chaperones in maintaining protein homeostasis, we herein review our current knowledge on the involvement of molecular chaperones and co-chaperones in Parkinson's disease. We further discuss the capacity of molecular chaperones to prevent or modulate neurodegeneration, an important concept for future neuroprotective strategies and summarize the current progress in preclinical studies in models of Parkinson's disease and other neurodegenerative disorders. Finally we include a discussion on the future potential of using molecular chaperones as a disease modifying therapy.

### Keywords

neurodegeneration; Parkinson's disease; alpha-synuclein; Lewy body; molecular chaperone; proteasome; autophagy; lysosome; heat shock protein (Hsp); Hsp90 inhibitor

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## PART I: THE ROLE OF MOLECULAR CHAPERONES IN PARKINSON'S DISEASE

### INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in industrialized countries, currently affecting around 1% of the population over the age of 60 years [1]. It is predicted that the incidence of PD will rise dramatically in the coming decades as populations around the globe increasingly age. Clinically, PD is characterized by four cardinal features, namely bradykinesia, rigor, tremor and postural instability, which are often accompanied by other motor- and non-motor symptoms including cognitive impairment [2]. Pathologically, PD is defined by progressive neurodegeneration in defined regions of the brain and the presence of distinct intracellular inclusion bodies, termed Lewy bodies and Lewy neurites [3]. These aggregates are proteinaceous in nature and contain abundant ubiquitinated and phosphorylated variants of the small, pre-synaptic protein  $\alpha$ -synuclein [4]. Lewy bodies and Lewy neurites are not the only defining pathological hallmarks of PD and are also characteristic of Dementia with Lewy bodies (DLB) [4–6]. Moreover  $\alpha$ -synuclein positive inclusions are found in glia cells in multiple system atrophy [7] and Lewy body pathology has been identified in a variety of other neurodegenerative diseases such as sporadic and familial Alzheimer's disease (AD) [8], Down's syndrome [9] and neurodegeneration with brain iron accumulation type 1 [10]. Besides being a major component of Lewy bodies, the central role of  $\alpha$ -synuclein in PD has been supported by genetic studies in familial and sporadic forms of the disease. Missense mutations in the gene for  $\alpha$ -synuclein (A53T, A30P and E46K) [11–13] as well as gene duplications [14–16] can lead to rare familial cases of PD. In addition, polymorphisms in the  $\alpha$ -synuclein gene, as identified by several genome-wide association studies, have been confirmed as a major risk factor for sporadic PD [17–19].

A common theme shared by PD and many other neurodegenerative disorders is the abnormal folding or clearance of potentially cytotoxic protein species. The cascade of  $\alpha$ -synuclein related pathology is believed to progress from the misfolding of  $\alpha$ -synuclein, to the formation of oligomers, the maturation of protofibrils, and on to heavily insoluble fibrils and finally full-blown aggregates. This process has been recapitulated *in vitro*, where recombinant wild-type  $\alpha$ -synuclein has been shown to aggregate and mutations have been found to accelerate the formation of oligomers and protofibrils [20]. Alpha-synuclein has traditionally been described as a natively unfolded monomer of about 14 kDa that acquires an  $\alpha$ -helical secondary structure upon binding to lipid membranes [21, 22]. A recent study, however, has challenged this notion by demonstrating that  $\alpha$ -synuclein is present as an  $\alpha$ -helically folded tetramer when isolated under non-denaturing conditions [23]. Moreover the authors of this study found that cell-derived native  $\alpha$ -synuclein has a greater lipid-binding capacity compared to the recombinant protein and importantly adopts a helical conformation even in the absence of lipids. Interestingly, the native tetramer also displays a lower propensity to aggregate into fibrils. These compelling results have the potential to significantly impact future research by adding a novel stage to the pathological sequence.

Destabilization of the native  $\alpha$ -helical tetramer conformation may precede  $\alpha$ -synuclein misfolding and aggregation and thus compounds that stabilize the native tetramers may emerge as novel therapeutic strategies. Looking downstream of this step, a growing body of recent evidence suggests that oligomeric intermediates are indeed the species toxic to neurons [24–27]. Hence, preventing the early steps of oligomerization and aggregation holds the promise to halt the degenerative process associated with protein misfolding and accumulation. Although the exact mechanism by which oligomeric  $\alpha$ -synuclein contributes to neuronal degeneration is not yet fully understood, one prominent hypothesis suggests that

$\alpha$ -synuclein oligomers might expose promiscuous hydrophobic domains on the protein, which interact with other proteins or lipid membranes in an aberrant way. Interrupting  $\alpha$ -synuclein oligomerization is therefore recognized as a potential therapeutic approach. Protein folding and re-folding is mediated by a network of highly conserved molecules termed chaperones and co-chaperones. In order to maintain intracellular protein homeostasis chaperones interact with pathways of protein degradation that regulate the turnover of irreversibly damaged or misfolded proteins. The two major protein degradation pathways for  $\alpha$ -synuclein *in vivo* are the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway (ALP) [28]. The latter includes three distinctive subtypes: microautophagy, macroautophagy and chaperone-mediated autophagy. Here, we review the role of molecular chaperones in PD and discuss future therapeutic strategies related to protein folding and degradation.

## MOLECULAR CHAPERONES IN THE PATHOGENESIS OF PARKINSON'S DISEASE

Molecular chaperones or heat shock proteins (HSPs) comprise a heterogeneous group of highly conserved molecules that are critical for maintaining protein homeostasis [29]. Based on their molecular weight they can be classified into different families including HSP40, HSP60, HSP70, HSP90, HSP100 and the small HSPs. While chaperones are molecules that directly mediate the folding of nascent proteins or the renaturation of misfolded proteins, co-chaperones are defined by their assistive role to this process. Important co-chaperones include the BAG-domain containing family (Bag1–6), the TPR-domain containing family (CHIP, Hip, Hop) and the DnaJ-domain containing co-chaperone Hsp40. Cells constitutively express specific molecular chaperones to guarantee adequate folding and refolding of client proteins and such homologues of heat shock proteins, known as heat shock cognates (Hsc), are key regulators of basic cell functions. However, most chaperones are induced after chemical or physical cell stress, for example as a consequence of hyperthermia, hypoxia, oxidative stress or exposure to toxins [30]. Accumulation of unfolded proteins during cellular stress effectively provokes chaperone expression by a signaling cascade that involves the transcription factor heat shock factor 1 (HSF-1). This regulatory element is part of a feedback loop by which chaperone expression is adjusted to optimize cell survival. In its inactive monomeric state HSF-1 is constitutively present in the cytosol. This resting state is preserved by the association with Hsp90 [31]. Exposure to proteotoxic stress enables HSF-1 to dissociate from Hsp90 to undergo phosphorylation, trimerization, and nuclear translocation. In the nucleus, HSF-1 induces the coordinated expression of Hsp70 and other heat shock proteins via binding to heat shock response elements in the promoter of the respective genes [32]. Once adequate levels of molecular chaperones have reached the cytosol, Hsp90 again stabilizes and inactivates HSF-1, therefore creating a dynamic equilibrium that allows the cell to adjust to endogenous or exogenous stress stimuli [33, 34]. Chaperones are not only cytoprotective because of their ability to limit protein misfolding and aggregation but also by a wide array of other functions such as stabilization of cytoskeleton elements and anti-apoptotic effects, for example by blocking Apaf1 (Apoptotic protease activating factor 1) signaling [35]. A summary of the many functions of chaperones is provided in Figure 1. The exact molecular mechanisms of chaperone-mediated protein folding is outside the scope of this review but has been the subject of other recent reviews [29, 36]. With focus on disease mechanisms, molecular chaperone function and malfunction has been implicated in a wide range of diseases [37–39] and their role in neurodegenerative diseases in particular has received considerable attention in recent years [40–43].

**Chaperones in Parkinson's disease**—Research exploring the role of molecular chaperones in PD follows seminal studies in other neurodegenerative diseases. Polyglutamine (PolyQ) repeat diseases, such as Huntington's disease (HD) and spinocerebellar ataxia (SCA), have been among the first to be studied in this respect [44–

47]. In PD, the first evidence for an involvement of molecular chaperones was provided by pathological studies that identified Hsp90, Hsp70, Hsp60, Hsp40 and Hsp27 as components of Lewy bodies, although to a varying extend [48–51]. Importantly, in one of these initial studies, Auluck et al. [50] demonstrated that Hsp70 co-expression could prevent dopaminergic cell death in a *Drosophila melanogaster* model of  $\alpha$ -synuclein toxicity. In the opposite direction, interference with the endogenous chaperone system by introducing a mutation in the ATPase domain of Hsp70 (K71S) exacerbated the pathological phenotype. Despite the dramatic protective effects on cell survival, no change in the number, size or distribution of  $\alpha$ -synuclein positive perinuclear inclusions was discernible. However, aggregates were found to contain Hsp70 just like Lewy bodies in PD cases. The authors of this seminal report concluded that I) Hsp70 may be a critical part of the neuronal arsenal that mitigates  $\alpha$ -synuclein toxicity and II) the presence of chaperones in aggregates could result in their cellular depletion, due to sequestration, and thus subsequent loss of chaperone function may lead to degeneration [50] (also see the accompanying editorials [52, 53]). Both hypotheses have been confirmed and extended in subsequent studies (Figure 1).

### **Hypothesis I. Molecular chaperones protect neurons against $\alpha$ -synuclein induced toxicity**

—As detailed above, molecular chaperones are induced in response to cellular stress. Experiments using heat shock induced expression of chaperones demonstrated that induction of Hsp70 can prevent  $\alpha$ -synuclein induced cell death in a yeast model of PD [54], can mitigate neurotoxicity induced by the mitochondrial toxin rotenone in acute rat brain slices [55] and can significantly ameliorate MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) toxicity in cultured SK-N-SH [56] or PC12 cells [57]. Like heat shock treatment, the insult created by toxins like MPTP, rotenone or lactacystin generated an endogenous although transient heat shock response leading to increased expression of Hsp90, Hsp70 and other chaperones in cultured dopaminergic MES cells [58] and mouse models of PD [49, 59]. Likewise, targeted overexpression of  $\alpha$ -synuclein in the substantia nigra pars compacta (SNpc) of mice resulted in increased mRNA levels of Hsp70, Hsp40 and Hsp27 as assayed by quantitative PCR (polymerase chain reaction) in dopaminergic cells recovered by laser capture microscopy [60]. Collectively these studies support the general principle that a cellular attempt to counteract proteotoxic stress in disease models involves recruiting molecular chaperones.

Exogenous overexpression of Hsp70 and other chaperones has proven beneficial in various PD models (Figure 1). In cell culture models of  $\alpha$ -synuclein aggregation and inclusion formation, co-expression of torsinA (a homologue of yeast Hsp104), Hsp40 [48], Hsp27 [61], or Hsp70 [62, 63] led to reduced aggregate formation [48, 61–63] and lowered total and detergent-insoluble fractions of misfolded  $\alpha$ -synuclein [62, 63]. Using time-lapse imaging of fluorescently labeled  $\alpha$ -synuclein, Opazo et al. nicely demonstrated that aggregate formation and toxicity induced by a C-terminally truncated form of  $\alpha$ -synuclein, could be significantly reduced by co-expressing Hsp70 in living cells [64]. Likewise, Hsp27 was found to be particularly effective in preventing cell death induced by mutant A30P and A53T  $\alpha$ -synuclein in cells [65]. Interestingly, Hsp70 has also been shown to bind  $\alpha$ -synuclein filaments and mitigate their inhibitory effect on proteasome function, a potentially key pathway for neurodegeneration [66].

*In vivo*, Klucken et al. confirmed the anti-aggregation effect of Hsp70 overexpression by crossing human  $\alpha$ -synuclein transgenic mice with transgenic mice overexpressing rat Hsp70 and finding a significant reduction in TritonX-insoluble aggregated  $\alpha$ -synuclein [63]. By contrast, a recent study found no effect in human A53T mutant  $\alpha$ -synuclein transgenic mice after crossing with human Hsp70 transgenic mice [67]. This double transgenic mouse line displayed a worse motor phenotype and no change in  $\alpha$ -synuclein, neither total levels nor aggregation, was discernible, arguing that, at least in this model, frank overexpression of

Hsp70 alone does not affect  $\alpha$ -synuclein pathology or toxicity [67]. The lack of an effect on  $\alpha$ -synuclein aggregation could be possibly due to a partial dysfunction of overexpressed human Hsp70 in transgenic mice, an explanation that is difficult to prove or disprove *in vivo*. Supporting this notion, human Hsp70 transgenic mice did not show any change in levels of endogenous  $\alpha$ -synuclein [67]. Furthermore it seems reasonable to assume that an exclusive overexpression of Hsp70 without the concerted action of other chaperones and co-chaperones is not fully effective *in vivo*, a lesson that has implications for therapeutics that stably restore or enhance Hsp70.

How do molecular chaperones interfere with  $\alpha$ -synuclein misfolding and aggregation? A number of studies suggest that molecular chaperones can directly bind  $\alpha$ -synuclein and inhibit fibrillization (Figure 1). Zhou et al. [58] found that endogenous Hsp70 is associated with  $\alpha$ -synuclein aggregates in cultured MES cells after rotenone treatment. Furthermore, the formation of  $\alpha$ -synuclein aggregates as well as rotenone-mediated mitochondrial inhibition, oxidative stress and cell death were all ameliorated by exogenous overexpression of Hsp70. As for a mechanism, Hsp70 was shown to effectively bind prefibrillar species and in doing so prevent key steps of  $\alpha$ -synuclein aggregation [68]. These data favor smaller, more soluble species, that are potentially more accessible to mechanisms of protein removal, although no reduction in the net cytotoxic effect of  $\alpha$ -synuclein aggregation was observed in this *in vitro* study [68]. Hsc70 was recently found to effectively bind  $\alpha$ -synuclein fibrils and to sequester  $\alpha$ -synuclein in an assembly incompetent state but this occurred only in the absence of ATP and in a co-chaperone (Hdj1 and 2) dependent manner [69]. When added to a murine cell line (H-END) the Hsc70-coated  $\alpha$ -synuclein fibrils were significantly less toxic, suggesting a detoxifying effect of chaperone binding to  $\alpha$ -synuclein aggregates [69]. Looking closer at the molecular interaction of Hsp70 and  $\alpha$ -synuclein, inhibition of  $\alpha$ -synuclein fibrillization was found to require a transient and reversible interaction of Hsp70's substrate-binding domain and the core hydrophobic region of soluble  $\alpha$ -synuclein [70, 71]. Using a fluorescence lifetime imaging (FLIM) based assay Hsp70 has been shown to alter  $\alpha$ -synuclein conformation by inducing it to adopt an open conformational state that discourages the formation of  $\alpha$ -synuclein- $\alpha$ -synuclein intermediates [72]. Thus Hsp70 can reduce  $\alpha$ -synuclein oligomer formation and toxicity in living cells by specifically preventing or destabilizing  $\alpha$ -synuclein- $\alpha$ -synuclein interactions or by enhancing clearance of oligomeric products [27]. Interestingly, oligomer formation of secreted extracellular  $\alpha$ -synuclein was found to be dramatically reduced when Hsp70 was co-expressed [73]. This modulatory effect on extracellular  $\alpha$ -synuclein oligomers was independent of the total level of  $\alpha$ -synuclein in the extracellular environment and occurred concomitant with an increase in extracellular Hsp70, potentially through simultaneous secretion of  $\alpha$ -synuclein and the chaperone Hsp70 [73].

Another molecular chaperone that has been shown to affect the process of  $\alpha$ -synuclein assembly is Hsp90. Hsp90 can bind to  $\alpha$ -synuclein and abolish its ability to associate with vesicles [74]. Hsp90 can also enhance fibril formation via an intermediate oligomeric pathway, which contrasts findings with Hsp70 and other chaperones and which remains to be evaluated *in vivo* [74]. The authors of this report suggest that Hsp90, by stabilizing fibrils, may act as a scavenger protein that shifts the equilibrium towards mature fibrils rather than smaller and potentially toxic oligomers [74].

**Hypothesis II: Depletion of molecular chaperones exacerbates protein toxicity and neurodegeneration**—The idea that sequestration of chaperones in protein aggregates could result in a general depletion of chaperones has been explored in post-mortem studies in PD cases and in disease models. Integral to this concept seems the finding that chaperone activity and the resistance to proteotoxic insults declines with aging while proteotoxic stress load increases over the lifetime of a cell. This is particularly true for post-

mitotic and high-metabolizing cells like neurons (reviewed in [75, 76]). In PD patients, a polymorphism in the Hsp70-1 gene was reported that is linked to transcriptional dysregulation and therefore perhaps increases susceptibility to PD [77]. The first post-mortem pathological studies that explored chaperones in PD noted the occurrence of  $\alpha$ B-crystallin, a small HSP, or Hsp27 positive neurons in PD patients but not in matched controls [78, 79]. The distribution of  $\alpha$ B-crystallin positive neurons followed a distinct pattern and overlapped with the severity and regional spread of Lewy body pathology, although  $\alpha$ B-crystallin was not only restricted to Lewy body bearing neurons [79]. As discussed in the previous section, studies have demonstrated the association of several chaperones with PD pathology and promoted the concept that chaperones and other components of protein metabolism might be critical players in PD [48–51]. Following these initial reports, several studies have measured levels of chaperones in different brain regions in PD, DLB and other synucleinopathies and revealed a correlation between levels of chaperones and detergent soluble  $\alpha$ -synuclein [49, 61, 80–83] suggesting an interaction mainly between chaperones and the bio-available fraction of  $\alpha$ -synuclein. Expression and protein levels of Hsp90, Hsp70, Hsp40 and Hsp27 were found to be either elevated or unchanged depending on the fraction and tissue used, allowing for no definite conclusions. Interestingly, however, the mitochondrial variant of Hsp70, known as mtHsp70 or mortalin, was found to be decreased in PD [84]. Given the many implications of this protein as an interacting chaperone with DJ-1 and its role in oxidative stress, this could have broader significance for mitochondrial homeostasis and neurodegeneration. Recent findings also suggest that Hsc70, together with other proteins involved in lysosomal targeting and degradation, is significantly reduced in PD cases [82, 85]. This may indicate impaired chaperone-mediated autophagy as an underlying cause or consequence of PD pathology. Supporting the concept that chaperones are functionally impaired due to sequestration into  $\alpha$ -synuclein aggregates and mature Lewy bodies, Hinault and colleagues reported that  $\alpha$ -synuclein oligomers are capable of effectively inhibiting the Hsp70/Hsp40 system by interacting with the J-domain co-chaperones [86].

**Chaperones and other key proteins in PD**—The relationship of molecular chaperones to other genetically-linked PD proteins has also been explored. Although limited in comparison to the broad investigations of  $\alpha$ -synuclein, important information can be garnered from these studies. In brief, Hsp70 was found to co-localize with and refold misfolded *parkin* (*PARK2*), allowing it to adopt its native conformation [87]. The Hsp70/Hsp40 complex was also shown to prevent sequestration of parkin into large, stress-induced protein aggregates called aggresomes [88, 89]. Interestingly, a recent study also showed that parkin might interact with mortalin [90] by rescuing the deleterious effects of mortalin knockdown, suggesting that the interaction of parkin and mortalin affects the role of mortalin in mitochondrial homeostasis [90]. *DJ-1* (*PARK7*) expression was shown to lead to increased levels of Hsp70 [91] and wild-type as well as mutant DJ-1 were found to associate with Hsp70, mortalin, and the co-chaperone CHIP (C-terminus of Hsp70 Interacting Protein) following proteasome inhibition [92]. The same study also suggested that translocation of DJ-1 into mitochondria following oxidative stress is carried out in a chaperone dependent manner [92]. In addition, L166P mutant DJ-1 was shown to form a large complex involving interactions with parkin, Hsp70 and CHIP [93]. Although mutations in the small ubiquitin hydrolase, *UCH-L1* (*PARK5*), are not unequivocally a cause of familial PD, it was found to physically interfere with Hsp90, Hsp70, and the lysosomal adapter protein Lamp2a, a known receptor for chaperone-mediated autophagy [94, 95]. The mitochondrial kinase *PINK1* (*PARK6*) appears to be a client protein of Hsp90 and is stabilized, cleaved, distributed, and degraded in an Hsp90 dependent manner [96, 97]. Studies addressing the interaction between *LRKK2* (*PARK8*), Hsp90 and CHIP are discussed below.

**Co-chaperones**—The activity of molecular chaperones is controlled by the dynamic association of various co-chaperones. Co-chaperones can modulate the activity of their chaperones in diverse ways: They can affect the recruitment of client proteins, mediate the binding to other chaperones or regulate their ATPase activity. Co-chaperones are also grouped into families. The most important for PD are the BAG-domain containing family (Bag1-6), the DnaJ-domain containing family (HSP40) and the TPR-domain containing family (CHIP, Hip, Hop).

BAG-domain containing chaperones are a heterogeneous family of multidomain proteins. All homologs contain one BAG-domain at their C-terminus except for Bag-5, which contains five. All BAG proteins have been shown to physically interact with Hsp70 but serve different functions [42]. Bag-1 was shown to regulate Hsp70's ATPase domain in a negative fashion [98, 99], potentially by competing with the positive co-chaperone of Hsp70, Hip [100]. Bag-2 was found to interact with CHIP, a TPR-domain co-chaperone of Hsp70 that is linked to the UPS through its E3 ubiquitin ligase activity. Bag-3 provides another link to protein degradation pathways because it is associated with both the UPS and autophagy pathways [101, 102]. Bag-5 has been identified as a component of Lewy bodies and has been shown to cooperate with Hsp70 and parkin [88]. It was found to mitigate the refolding capacity of Hsp70 and to inhibit the E3 ubiquitin ligase activity of parkin, therefore providing another important link between the chaperone system, PD and protein degradation [88]. Recently, Bag-5 was found to exist in a complex with both Hsp70 and CHIP [103] in which it inhibits CHIP's E3 ligase activity and thus mitigates CHIP's ability to ubiquitinate  $\alpha$ -synuclein and to inhibit oligomer formation [103].

TPR-domain containing co-chaperones can bind the C-terminus of Hsp70 or Hsp90 to regulate the association with client proteins and the assembly of the chaperone machinery. The co-chaperone CHIP has particular relevance to PD because of its dual role as an enhancer of Hsp70-mediated protein folding [104] and as an E3 ubiquitin ligase [103, 105, 106]. CHIP can therefore act as a co-chaperone that links the chaperone network to protein degradation pathways and the ubiquitin-proteasome system in particular [107, 108]. CHIP was also found to associate with parkin and to possibly act as an E4 ubiquitin ligase-like protein that enhances the E3 ligase activity of parkin [109]. CHIP is a component of Lewy bodies in DLB [110] and tau aggregates in AD [111]. Perhaps most interesting, CHIP ubiquitinates and associates with  $\alpha$ -synuclein and can prevent the formation of toxic  $\alpha$ -synuclein oligomers by facilitating removal via both the proteasome and lysosomal pathways [103, 110, 112]. In two recent studies, CHIP was found to associate with LRRK2 and Hsp90, forming a complex that regulates LRRK2-induced toxicity through modulation of LRRK2 stability via the action of CHIP's ligase activity [113, 114]. In this complex Hsp90 was found to interact with LRRK2 and to mitigate CHIP-mediated degradation of LRRK2 through the proteasome [113–116]. Hip, another TPR-domain containing co-chaperone of Hsp70, was recently identified as a possible player in PD by a transcriptome wide screen that revealed significantly decreased levels of Hip mRNA in PD patients compared to controls [117]. In addition, it recently became evident that Hip can prevent the co-aggregation of Hsp70 and  $\alpha$ -synuclein, therefore recovering levels of biologically active Hsp70 [118]. Hence, Hip can be viewed as an interesting target for therapeutic strategies that aim to prevent chaperone sequestration in  $\alpha$ -synuclein aggregates.

Taken together, the studies reviewed above strongly implicate a role for molecular chaperones in the pathogenesis of PD (Figure 1). Evidence is derived from pathological and genetic studies in PD patients and pathophysiological studies in a variety of disease models. The data discussed suggest that chaperones and the Hsp70 system in particular can suppress toxicity associated with  $\alpha$ -synuclein or the other proteins implicated in PD. This growing

body of investigations sets the stage for approaches that target molecular chaperones as a disease modifying therapy in PD, as reviewed in the following section.

## **PART II: MOLECULAR CHAPERONES AS A NOVEL THERAPEUTIC TARGET IN PARKINSON'S DISEASE**

### **MOLECULAR CHAPERONES AS A THERAPEUTIC TARGET**

Treatment of neurodegenerative disorders is a challenge to clinicians because of the slow progressive nature of the disease, the profound neurodegeneration prior to the onset of clinical symptoms and the lack of early diagnostic biomarkers. To date, therapeutics for PD are aimed at increasing cerebral dopamine levels or stimulating central dopamine receptors, respectively. Levodopa, in combination with a peripheral dopa-decarboxylase inhibitor, remains the mainstay for initial therapy in most cases. Surgical measures include deep-brain-stimulation, which has been recognized as an effective therapy for selected cases. While these treatment options substantially improve quality of life and functional capacity for a variable period of time, no current treatment halts or reverses neuronal degeneration in PD [119, 120]. As the disease progresses, first and second-line options for symptomatic treatment of motor and non-motor manifestations, including cognitive impairment, eventually fail, leaving patients severely disabled. The cause of death in many patients with PD remains obscure but pneumonia is the most common explanation given the problems of disease management in the terminal phase of the illness. Because current clinical trials have been unable to establish neuroprotective therapies for clinical use, significant effort now focuses on the identification of novel disease modifying agents [119, 121]. Targeting well-characterized molecular pathways in the pathogenesis of neurodegenerative disorders will lead to rational drug design and testing of novel disease modifying agents. Emerging pathways for potential therapeutic targets include mechanisms of oxidative stress, mitochondrial dysfunction, glutamate excitotoxicity, apoptosis and protein metabolism (Figure 2). Targets in protein metabolism pathways include misfolding and aggregation, post-translational modifications, and protein degradation pathways such as the UPS and the ALP. Pathologically misfolded proteins may cause cellular dysregulation and damage by a variety of interrelated mechanisms, including aggregation and misfolding of other proteins, inhibition of chaperones by direct interaction and sequestration into aggregates, impairment of protein degradation pathways, membrane destabilization and synaptic dysfunction with these pathological events all converging at the same endpoint - cell death and inflammation.

As discussed in the previous section, protein folding and refolding involves the orchestrated action of a network of interacting molecules. The highly conserved machinery of molecular chaperones acts in conjunction with degradation pathways to effectively eliminate misfolded or damaged proteins. It is noteworthy that in the context of neurodegeneration, these mechanisms of protein quality control significantly change in the aging brain, eventually paving the way for protein conformational disorders associated with aging [75, 76]. Although the molecular cascade leading to pathological accumulation and aggregation of  $\alpha$ -synuclein and other proteins in PD is yet not fully understood, protein homeostasis appears to be a key element. Therefore molecular chaperones hold the promise to alter early pathological changes in proteinopathies such as PD [40, 42]. It is widely recognized that the Hsp70 chaperone system is an important cellular defense element that can halt, prevent and eventually even reverse protein misfolding and neurodegeneration.

A growing number of preclinical studies have investigated pharmacological and gene therapy strategies to upregulate chaperone function with promising results. While a few recent studies have tested brain permeable small molecule enhancers of chaperone function in animal models of PD, a lot of the ideas for our current concepts stem from studies in other



neurodegenerative disorders and various forms of cancer. Based on their mechanism of action many of the agents tested in other diseases may be suitable for studies in PD.

## PHARMACOLOGIC UPREGULATION OF MOLECULAR CHAPERONES

Pharmacological agents targeting molecular chaperones have mainly focused on the Hsp70 system and can be classified in three groups: 1) Hsp90 inhibitors, 2) modulators of HSF-1 and 3) compounds with direct chaperone activity (Table 1).

**HSP90 inhibitors**—Hsp90 inhibitors increase the activity of the transcription factor HSF-1 and thus lead to increased expression of stress-induced proteins such as Hsp70 [31, 122]. As detailed above, the interplay between Hsp90 and HSF-1 can be regarded as a molecular switch that can activate a cytoprotective stress-response that will counteract the pathogenic aggregation of proteins [123]. A wealth of information about Hsp90 inhibitors has been generated in cancer studies (reviewed in [37, 39, 124, 125]). In cancer cells, Hsp90 protects a range of mutated or overexpressed oncoproteins from misfolding and degradation. Hence, Hsp90 can be regarded as a crucial line of support for cancer cell survival. This knowledge has led to considerable progress in the development of small molecule inhibitors of Hsp90 with more than 60 completed or ongoing clinical trials for various forms of cancer. The first inhibitor to enter clinical trials was 17-AAG (tanespimycin) in 1999 and since then numerous other inhibitors have undergone clinical testing [124, 125]. Parallels with the cancer studies can be drawn to neurodegenerative disorders where the detrimental consequences of an inefficient degradation of pathogenic proteins are equally dramatic.

**Geldanamycin and analogues**—Geldanamycin (GA) is a naturally occurring Hsp90 inhibitor and the lead compound for a group of agents termed ansamycin antibiotics [126]. This class of inhibitors acts by replacing ATP in the ATP binding pocket in the N-terminal domain of the Hsp90 chaperone [126]. GA has been well characterized in different PD models. Several studies in cultured cells have demonstrated that GA can reduce  $\alpha$ -synuclein oligomerization, aggregation and increase  $\alpha$ -synuclein clearance [127–129]. Interestingly, inhibition of Hsp90 with GA also leads to reduced re-secretion of extracellularly applied  $\alpha$ -synuclein via exocytosis and attenuates extracellular  $\alpha$ -synuclein toxicity induced by the neurotoxins rotenone and MPTP [129]. Similarly, upregulation of Hsp70 in  $\alpha$ -synuclein transfected cells by Hsp90 inhibition resulted in a reduction in secreted, extracellular  $\alpha$ -synuclein oligomers and attenuated their toxic effects [73]. Secretion of  $\alpha$ -synuclein is essential to the proposed mechanism of prion-like cellular transmission and spreading of  $\alpha$ -synuclein pathology, a very exciting new avenue in PD research [130]. The influence of molecular chaperone function on secretion and uptake of  $\alpha$ -synuclein might therefore be of great interest for future studies. A second noteworthy discovery is that GA regulates AMPA (2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid) receptor trafficking and therefore may be able to reduce glutamatergic excitotoxicity [131, 132]. With regards to PD pathogenesis, GA was shown to prevent dopaminergic cell death and  $\alpha$ -synuclein-induced pathology in cell culture [127], *Drosophila melanogaster* [50, 133, 134] and the MPTP mouse model of PD [135]. Besides PD, GA has also shown beneficial effects in models of several other important neurodegenerative disorders, including models of HD [136–138], AD [111] and amyotrophic lateral sclerosis (ALS) [139, 140]. Despite these very encouraging findings, the use of GA has been limited by a variety of reasons but mainly because of its poor aqueous solubility, poor blood-brain barrier permeability and significant liver toxicity [141]. Analogues of GA have been able to partly overcome these limitations and also show higher affinity for Hsp90 [142]. **17-AAG** (17-allylamino-17-demethoxygeldanamycin, or tanespimycin) and **17-DMAG** (17-dimethylaminoethylamino-17-demethoxygeldanamycin, or alvespimycin) are potent Hsp90 inhibitors with an improved side-effect profile [143]. 17-AAG ameliorates  $\alpha$ -synuclein

toxicity, decreases intracellular  $\alpha$ -synuclein levels in cultured cells [144] and prevents extracellular  $\alpha$ -synuclein oligomerization [73]. Recent studies have shown that 17-AAG influences the interaction between LRRK2, CHIP and Hsp90 and can reduce LRRK2 toxicity by promoting degradation through the proteasome [113, 114]. Importantly, 17-AAG was also found to effectively induce macroautophagy and hence facilitate clearance of  $\alpha$ -synuclein [145]. Like GA, treatment with 17-AAG has proven beneficial in several models of neurodegeneration, e.g. 17-AAG suppressed polyglutamine-induced neurodegeneration in an HSF-1 dependent manner in *Drosophila melanogaster* models of HD and SCA [146]. An elegant study by Waza et al. [147] demonstrated the therapeutic potential of 17-AAG in a mouse model of spinal bulbar muscular atrophy (SBMA). In this disease, Hsp90 is required to stabilize mutant androgen receptors. By blocking Hsp90, 17-AAG treatment destabilizes the pathogenic receptor and Hsp70 upregulation accelerates its degradation, leading to a significantly ameliorated phenotype in this model. As well as these promising findings for neurodegeneration, 17-AAG is currently being evaluated in several phase II/III trials for various forms of cancer [39, 125]. Although it has been shown to be safe for humans, poor blood brain barrier permeability remains a limiting factor for its clinical use for neurodegenerative disorders. 17-DMAG is an advanced formulation of 17-AAG with improved aqueous solubility but unfortunately was found to be associated with unacceptable toxicity in cancer trails [125]. Taken together the clinical utility of GA, 17-AAG and 17-DMAG is questionable, despite very encouraging results from studies in various disease models.

**Novel Hsp90 inhibitors: SNX compounds**—An interesting novel group of synthetic small molecule inhibitors of Hsp90 are derived from a compound termed **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). Developed for their potent Hsp90 inhibition and excellent antitumor activity [148, 149], novel derivatives of SNX-2112 (see below) have important advantages: they selectively inhibit Hsp90, are orally available and display excellent blood brain barrier permeability. In a recent study, a panel of SNX compounds, selected for their chemical properties to favor brain penetration, was assessed for effects on  $\alpha$ -synuclein oligomerization and toxicity in cell culture models [144]. In the study by Putcha et al., a significant reduction of  $\alpha$ -synuclein oligomerization was achieved in transfected cells [144]. Additionally, a subset of SNX compounds effectively reduced both high-molecular weight and monomeric species of  $\alpha$ -synuclein. These findings suggest that SNX compounds promote refolding and degradation of  $\alpha$ -synuclein through the efficient induction of Hsp70 by Hsp90 inhibition. A lead compound, **SNX-0723**, was identified based on its strong preventive effect on  $\alpha$ -synuclein oligomerization and *in vivo* pharmacokinetic and pharmacodynamic studies demonstrated robust brain absorption and excellent bioavailability, making this compound a promising candidate for further evaluation.

**Modulators of HSF-1**—Modulation of HSF-1 activity has received considerable attention as a therapeutic strategy because, by promoting the expression of Hsp70 and other chaperones, modulators of HSF-1 tackle intracellular chaperone function at the transcriptional level. Recruitment of Hsp70 and other chaperones to actively unfold and disassemble stress-induced protein aggregates can prevent the accumulation of toxic protein species.

**Arimoclomol**—One modulator of HSF-1, Arimoclomol, has received considerable attention as a potential ALS therapy [150–155] and thus may be a good candidate for study in PD. Arimoclomol, a derivate of bimeclocholol [156, 157], is an orally administered drug that acts through co-induction of HSF-1 under stress conditions [158]. After providing evidence for improved motor function, motor neuron survival and increased life span in

SOD1 mutant mice [151], Arimoclocholol is now in clinical testing for ALS [152]. It was found to have adequate tolerability and safety in a phase I/IIa clinical trial [152]. Now in a phase II/III clinical trial, results are awaited eagerly. It is important to understand that Arimoclocholol co-induces HSP expression by prolonging the binding of HSF-1 to the heat shock response element found in heat shock gene promoters [151, 158]. Therefore it acts only under conditions of cellular stress and ongoing heat shock response [151], an important feature that may convey selectivity for stressed neurons, e.g. dopaminergic cells in PD.

**HSF1A**—Given the importance of HSF-1 as the master regulator of chaperone gene transcription and the limitations of global Hsp90 inhibition, small molecules that directly modulate this transcription factor are of great interest. A recently published study [159], used a sophisticated high-throughput screen in yeast to identify a novel activator of HSF-1, coined HSF1A. This novel small molecule increases chaperone protein expression by promoting key steps of HSF-1 activation without binding or affecting Hsp90. HSF1A was found to enhance HSF-1 trimerization, translocation into the nucleus and activation through phosphorylation. HSF1A-mediated Hsp70 induction reduced the de-novo formation of protein aggregates and ameliorated polyglutamine-induced cytotoxicity in both a cell-culture and *Drosophila melanogaster* model of HD [159].

**Celastrol**—Celastrol is an effective Hsp70 inducer, although its mechanisms of action are pleiotropic and include anti-inflammatory and anti-oxidant properties [160]. Celastrol not only induces the hyperphosphorylation of HSF-1 and triggers its binding to chaperone gene promoters, it also regulates Hsp90's binding to co-chaperones [161]. Important to PD, celastrol treatment significantly attenuated MPTP-induced cell death and partly restored striatal dopamine levels in the MPTP mouse model [162]. In addition, it was also neuroprotective in a *Drosophila melanogaster* model of PD [163]. In the mutant SOD1 mouse model of ALS, orally administered celastrol resulted in delayed disease onset, improved motor function, and attenuated cell loss in the lumbar spinal column [164]. Likewise celastrol prevented polyglutamine aggregation and toxicity in transfected cells [165, 166].

**Geranylgeranylacetone**—Another interesting compound is geranylgeranylacetone (GGA). Originally developed as an antiulcer drug, GGA has been shown to be neuroprotective in animal models of transient cerebral ischemia [167–169] and cerebral hemorrhage [170] through effective induction of Hsp70 potentially via protein kinase C mediated phosphorylation of HSF-1 [167–169]. Furthermore, GGA suppressed polyglutamine-induced toxicity in cell culture and a mouse models of SBMA [171].

**Valproic acid**—Valproic acid is a classic anticonvulsive drug used in treating epilepsy and as a mood-stabilizing agent prescribed for bipolar disorder. Laboratory studies have identified valproic acid as a histone deacetylase (HDAC) inhibitor and inducer of Hsp70 [172]. It is cytoprotective under conditions of oxidative stress [173–175] and thus relevant to PD, against rotenone toxicity in cultured cells [176] and rats [177].

**Statins**—Other widely used drugs that potentially exert protective effects through modulation of molecular chaperone activity are statins. Statins or HMG-CoA (3-hydroxy-3-methyl-glutaryl coenzyme A) reductase inhibitors are important and frequently prescribed therapeutics for lowering cholesterol levels as a preventive measure for cardiovascular disease. Besides the known effects on cholesterol synthesis, statins may have pleiotropic actions including immunomodulatory, anti-oxidative and anti-apoptotic effects [178]. The impact of statin therapy on PD has been explored in laboratory and epidemiological studies but to date it remains an open question whether long-term treatment with statins will

influence the risk and progression of PD in a favorable or unfavorable way [178]. With regards to neuroprotection, it is noteworthy that statins have been shown to target molecular chaperones, e.g. by effecting the phosphorylation of Hsp90 [179] or by stimulating the expression of Hsp27 [180, 181], although the relevance of these findings to PD pathology still has to be evaluated in detail.

**Compounds with direct chaperone activity**—Another attempt to promote protein homeostasis under cellular stress conditions involves the addition of so-called chemical chaperones. These molecules minimize unproductive protein-protein interaction and stabilize partly folded intermediates during refolding or aggregation. Chemical chaperones can act synergistically with molecular chaperones and additionally may interact with protein degradation pathways.

**Trehalose**—Direct chaperone activity has been ascribed to trehalose, a stable disaccharide with unique physico-chemical properties [182]. Trehalose acts as a chemical chaperone through direct protein-trehalose interaction and protects cells against various stress conditions. For example, trehalose binds to PolyQ expanded mutant huntingtin, which leads to reduced disease pathology and toxicity *in vitro* and *in vivo* due to stabilization of the partly unfolded mutant protein [183]. In multiple cell lines it was demonstrated that trehalose can induce autophagy and thus facilitate clearance of aggregate-prone proteins including mutant  $\alpha$ -synuclein [184, 185]. In addition to not being toxic to cells, trehalose was found to have anti-apoptotic properties through enhanced removal of pro-apoptotic proteins via autophagy [184]. This myriad of protective actions, covering both direct chaperone function and enhancement of degradation mechanisms, together with its lack of toxicity, give trehalose qualities that might be useful for studies in many neurodegenerative disorders including PD.

## VIRAL VECTOR-MEDIATED UPREGULATION OF MOLECULAR CHAPERONES

Gene therapy approaches promise important advantages over pharmacological strategies with small-molecules. With the goal being a stable, targeted restoration of cellular deficits and the prevention of disease progression, gene therapy has, in theory, the potential to ameliorate or even cure PD. Besides the transplantation of fetal grafts, viral vector-mediated expression of trophic factors or transmitter enzymes has emerged as a promising new avenue for curative approaches (reviewed in [186, 187]). After a series of proof-of principle phase I studies for viral vector-based strategies, randomized controlled double-blind clinical trials are underway [188, 189]. Based on their properties, such as stable gene expression, specificity and a favorable safety profile, only adeno-associated virus (AAV) and lentiviral vectors are currently in clinical testing [190]. Viral vector-based strategies may provide novel ways to upregulate the expression and activity of neuroprotective chaperone systems in vulnerable neuron populations at risk. Preclinical studies in animal models of PD have provided evidence for the potential of **viral-mediated Hsp70 expression** (Table 1). In the MPTP mouse model, Hsp70 gene transfer to striatal dopaminergic neurons by a recombinant AAV vector protected against MPTP-induced dopaminergic cell death and the associated decline in striatal dopamine levels [191]. Another study using a rat model of PD, based on AAV-mediated overexpression of CDCrel-1, a parkin substrate toxic to dopaminergic cells, showed that AAV-mediated overexpression of Hsp70 or mutant H-BH [192], a constitutively active form of HSF-1, can prevent dopaminergic neurodegeneration [193]. Although only a moderate improvement (approximately 20%) in survival of dopaminergic neurons could be achieved in this model, the study supports viral-mediated upregulation of Hsp70 as a novel target for neuroprotection. In contrast to the encouraging data for Hsp70 overexpression or HSF-1 enhancement, targeted overexpression of Hsp40 did not prevent

but exacerbated neurodegeneration in the same study, a controversy that remains to be explained [193].

Recombinant viral vectors have also been used to investigate neuroprotection by exogenous expression of Hsp70 interacting proteins. Co-injection of **Hsp104** and A30P mutated  $\alpha$ -synuclein into the substantia nigra of rats using lentiviral vectors reduced the formation of phosphorylated  $\alpha$ -synuclein inclusions and prevented nigrostriatal dopaminergic neurodegeneration induced by mutant A30P  $\alpha$ -synuclein [194].

The co-chaperone BAG5 interacts with Hsp70 and serves as a negative regulator for Hsp70-mediated refolding of client proteins [88]. A mutant form of BAG5, termed **BAG5 (DARA)**, however, binds to wild-type BAG5 and therefore compromises BAG5-HSP70 interaction [88, 103]. AAV-mediated expression of BAG5 (DARA) in the SNpc of MPTP-treated mice resulted in an increased survival of dopaminergic neurons while targeted expression of BAG5 enhanced degeneration [88].

### CELL-PENETRATING PEPTIDE TECHNOLOGY-MEDIATED UPREGULATION OF MOLECULAR CHAPERONES

Apart from viral-mediated expression, **cell-penetrating peptides (CCP)** have emerged as an alternative strategy to deliver or enhance chaperones in neurons [195, 196]. CCPs are small, basic protein domains that can deliver compounds across cell membranes and the blood-brain-barrier. The most commonly used CCP is the basic domain of TAT (trans-activator of transcription) derived from the human immunodeficiency virus (HIV) [195, 196]. Delivery and cell-penetration of Hsp70 can be enhanced by fusion to TAT. Administration of **TAT-Hsp70** increases levels of Hsp70 in cultured primary neurons and efficiently protects against peroxynitrite and glutamate-induced cell stress [197]. In models of PD, TAT-Hsp70 protected primary neurons and cultured mesencephalic neurons against the insult of MPTP [198]. When administered systemically in mice through intraperitoneal injection, penetration through the blood-brain-barrier and transduction of dopaminergic neurons of the SNpc was observed [198]. Again, TAT-Hsp70 protected against MPTP toxicity, leading to increased survival and integrity of dopaminergic neurons in this model. Preliminary studies also suggest that CCP-mediated delivery of Hsp40 or HSF-1 can increase Hsp70 expression and function, thus potentially exerting similar protective effects [199, 200]. Although clearly advantageous as described above, the clinical usefulness of CCP-mediated delivery of molecular chaperones or CCP technology in general remains to be determined in future studies.

### CONCLUDING REMARKS AND FUTURE CHALLENGES

A number of exciting therapeutic interventions targeting molecular chaperone function are under development for use in PD. Promising candidates include small molecule inhibitors of Hsp90 and gene therapy strategies to upregulate Hsp70. Important lessons can be learned from studies in other neurodegenerative conditions, where compounds have already entered clinical trials. Parallels can also be drawn to studies in cancer where drug development and evaluation has pushed forward at a miraculous pace. Despite encouraging results in disease models and the existence of exciting new compounds waiting to be tested for PD, the same limitations that apply to all neuroprotective therapies on trial will challenge testing of chaperone-based therapeutics.

Neuroprotection has been defined as an intervention “that produces enduring benefits by favorably influencing the underlying etiology or pathogenesis and thereby forestalling onset of illness or clinical decline” [201]. The challenges for testing neuroprotective therapies today include, but are not limited to, the following issues:

1. Evaluation of beneficial effects in currently available animal models is limited because no model convincingly recapitulates all features of PD pathology [202]. Toxin-based models are adequate to study effects of dopamine-replacement therapies but may not appropriately mimic the slow and progressive nature of neurodegeneration in PD patients. Genetic models also have their caveats [203–205]. The level of nigrastratial pathology is relatively modest in most models and a strong disease phenotype is clearly missing. An approach to combine genetic models with the application of neurotoxins may provide a novel concept, more suitable for preclinical testing of neuroprotective strategies [206].
2. Clinical trial design has also been a challenge for testing neuroprotective effects in PD patients. The range of difficulties faced in current trials is outside the scope of this discussion but has been the subject of numerous excellent reviews and editorials [119, 120]. Importantly, population selection, timing of the intervention, appropriate endpoints, and readouts such as novel biomarkers are areas of uncertainty and call for improvement.
3. Clinical parkinsonism is heterogeneous in terms of underlying pathology with regards to cause, severity and progression. Enhancement of chaperone function holds the promise to prevent protein misfolding, accumulation and aggregation and hence cell death. It remains a conceptual question, however, if a single agent targeted at a single pathway, among numerous other established disease pathways, will have an enduring neuroprotective effect. This challenge obviously applies to therapeutics that aim at improving molecular chaperone function. The idea of using a combination of agents for the same pathway in conjunction with agents that target different pathways seems only natural to overcome this problem. In this respect, much can be learned from drug development in other diseases such as cancer or atherosclerosis. As an example, a combination of chaperone inducing agents with agents that facilitate protein clearance via the UPS [207] or ALP [208, 209] seems to be a potential testable approach.
4. Biomarkers are tools that assist in finding the right diagnosis, tracking disease progression and that help in identifying new therapeutic targets. Despite significant efforts, reliable biomarkers for the pre-clinical phase of PD have been notoriously difficult to establish. Neuroprotective therapies, aimed at preventing neurodegeneration, however, will have their greatest impact during the pre-symptomatic phase of the disease, before significant pathology has accumulated. The translation of novel techniques in genomics, proteomics and metabolomics into reliable, sensitive and specific biomarkers will therefore be a crucial step for testing of disease modifying agents.

In summary, targeting molecular chaperones as a therapeutic strategy in PD is a viable approach to prevent or modify disease progression. Small molecules and gene therapy strategies have already shown very encouraging results in proof-of-concept pre-clinical studies. With the limitations discussed above, the development of novel compounds applicable to clinical testing is anticipated and future studies will determine the best approach for using molecular chaperones to tackle protein toxicity in PD.

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## Glossary

<b>AAV</b>	adeno-associated virus
<b>AD</b>	Alzheimer's disease
<b>ALP</b>	autophagy-lysosomal pathway
<b>ALS</b>	amyotrophic lateral sclerosis
<b>CCP</b>	cell-penetrating peptides
<b>DLB</b>	Dementia with Lewy bodies
<b>GA</b>	Geldanamycin
<b>GGA</b>	geranylgeranylacetone
<b>HD</b>	Huntington's disease
<b>Hsp</b>	heat shock protein
<b>LV</b>	lentivirus
<b>MPTP</b>	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
<b>PD</b>	Parkinson's disease
<b>PolyQ</b>	polyglutamine
<b>SCA</b>	spinocerebellar ataxia
<b>SnPC</b>	substantia nigra pars compacta
<b>SBMA</b>	spinal bulbar muscular atrophy
<b>TAT</b>	trans-activator of transcription
<b>UPS</b>	ubiquitin-proteasome system

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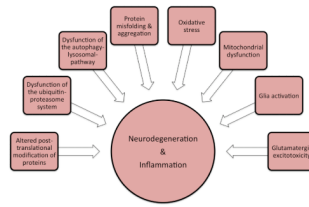
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**Figure 1.** Synopsis. The role of molecular chaperones in Parkinson’s disease can be summarized by two leading hypotheses: I) Molecular chaperones protect neurons against  $\alpha$ -synuclein induced toxicity and II) Depletion of molecular chaperones exacerbates protein toxicity and neurodegeneration. Molecular chaperones are neuroprotective because of a myriad of actions that mitigate protein misfolding and aggregation. UPS (ubiquitin-proteasome system); ALP (autophagy-lysosomal pathway)



**Figure 2.** Synopsis of the pathological cascades proposed to be involved in Parkinson's disease. Mechanisms at play include altered protein metabolism and mitochondrial function as well as cellular stress induced by reactive-oxygen species or glutamatergic excitotoxicity. All pathways are interrelated and converge into the final steps of neurodegeneration and inflammation.

Table 1

## Molecular chaperones as a therapeutic target

<b>A) Pharmacologic upregulation of molecular chaperones</b>		
<b>Compound</b>	<b>Target</b>	<b>Investigated for:</b>
Geldanamycin	Hsp90 Inhibitor	PD [54, 127–129, 133–135] AD [111] ALS [139, 140] PolyQ disease [136–138, 210]
17-AAG	Hsp90 Inhibitor	PD [73, 113, 114, 144, 145] ALS [211] PolyQ disease [146, 147, 210, 211]
17-DMAG	Hsp90 Inhibitor	PolyQ disease [212]
SNX-0723 and others	Hsp90 Inhibitor	PD [144]
Arimoclomol	HSF-1 enhancer	ALS [150–152]
HSF1A	HSF-1 enhancer	PolyQ disease [159]
Celastrol	Hsp70 induction	PD [162, 163] AD [213] ALS [164] PolyQ disease [165, 166]
Geranylgeranylacetone (GGA)	Hsp70 induction	PolyQ disease [171]
Valproate	Hsp70 induction	PD [176, 177] AD [214] ALS [215] PolyQ disease [216]
Trehalose	Direct chaperone function and mTOR independent autophagy induction	PD [184, 185] AD [185, 217, 218] ALS [219] PolyQ disease [183, 184, 220]
<b>B) Viral-mediated upregulation of chaperone function</b>		
AAV-Hsp70		MPTP mouse model of PD [191] AAV-CDCrel-1 rat model of PD [193]
AAV-H-BH (constitutively active form of HSF-1)		AAV-CDCrel-1 rat model of PD [193]
LV-Hsp104		LV-(h)A30P $\alpha$ -synuclein rat model of PD [194]
AAV-BAG5 (DARA)		MPTP mouse model of PD [88]
<b>C) Cell-penetrating peptide technology-mediated upregulation of chaperone function</b>		
TAT-Hsp70		MPTP in cell culture [198] MPTP mouse model of PD [198]

AAV (adeno-associated virus); AD (Alzheimer's disease); ALS (amyotrophic lateral sclerosis); LV (lentivirus); PD (Parkinson's disease); PolyQ disease (polyglutamine repeat expansion disease); TAT (trans-activator of transcription)