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Molecular Chaperones and Co-Chaperones in Parkinson Disease

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Abstract

Parkinson disease, a progressive neurodegenerative disorder, is caused by the pathological accumulation of proteins, including the ubiquitous presynaptic protein α -synuclein. Alterations in the metabolism of α -synuclein have clearly been linked to neurodegeneration, and early steps in the pathological sequence of this protein include the formation of oligomers, fibrils, and small aggregates. Targeting these early steps of oligomerization is one of the main therapeutic approaches in the quest to develop disease-modifying agents. Molecular chaperones, molecules that can mediate the proper folding and refolding of client proteins, are vital to cell function and survival and thus have been explored as potential therapeutic agents. Important to Parkinson disease, chaperones are capable of preventing α -synuclein misfolding, oligomerization, and aggregate formation as shown in vitro and in Parkinson disease animal models. Furthermore, chaperones and associated co-chaperones are closely linked to pathways of protein degradation, like the ubiquitin-proteasome system and autophagy, and are thus able to remove irreversibly misfolded proteins. In this review, we summarize the role of molecular chaperones in Parkinson disease models and discuss the importance of preserving protein homeostasis to prevent neurodegeneration. We also review the growing number of exciting studies that have targeted molecular chaperone function as a novel therapeutic approach.

Keywords

neurodegeneration; Parkinson disease; α synuclein; molecular chaperone; heat shock protein (Hsp); co-chaperone

Molecular Chaperones in Health and Disease

Molecular chaperones are a class of proteins that play a key role in protein homeostasis in cells of all organisms. Molecular chaperones assist either in the folding of newly synthesized proteins or in the refolding of partially folded or misfolded proteins (Hartl and Hayer-Hartl 2002). In addition, molecular chaperones may assist in the decision making of whether to refold a misfolded protein or target it for degradation via one of two degradation pathways: the ubiquitin-proteasome system (UPS) or the autophagy-lysosomal pathway (ALP) (Wickner and others 1999). These normal cellular functions of molecular chaperones exist to sustain proper folding of proteins that otherwise may become misfolded under the crowded cellular milieu. Under normal cellular conditions, these roles are carried out by heat shock cognates (HSCs), which are constitutively expressed in the cells. However, under stress

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conditions, another set of molecular chaperones are expressed, heat shock proteins (HSPs), which are highly conserved and expressed in all living organisms. Heat shock proteins are expressed and activated under stress conditions including elevated temperatures and conditions such as oxidative stress (Kiffin and others 2004) and are a crucial group of proteins for the survival of cells. Hsp70, along with Hsp90, are the major families of chaperones induced in response to cellular stress. Other families of molecular chaperones include Hsp100, Hsp60, Hsp40, and Hsp27, all of which are classified according to their molecular weight.

The importance of this class of protein to proper cellular function is highlighted by the fact that the first major product of zygotic gene activity in mouse embryos is HSP 70 (Hsp70) (Bensaude and others 1983). Maintaining proteins at their normal functional conformation might prevent many types of diseases in which the cause of the disease is a protein loss/gain of function or aggregation. An example for the importance of molecular chaperones in preventing disease formation is given by protein conformational diseases, where the key pathological hallmarks are aggregated proteins. A strong link has been established between molecular chaperones and conformational diseases, as was first highlighted in trinucleotide repeat expansion diseases such as Huntington disease and spinocerebellar ataxias (Chai and others 1999) in which molecular chaperones were found to co-localize with pathological protein aggregates. Since then, HSPs have been found to play a role in several other neurodegenerative diseases including Parkinson disease (PD) (Ebrahimi-Fakhari and others 2011b; Muchowski and Wacker 2005).

The Link between Protein Aggregation and PD

Parkinson disease is the second most common neurodegenerative disorder after Alzheimer disease and the most common cause of parkinsonism. The disease afflicts approximately 1% to 10% of the population over 65 years old and is caused by the specific loss of dopaminergic neurons within the substantia nigra pars compacta (SNpc) (Wirdefeldt and others 2011). Degeneration of dopaminergic cells in this brain region results in a depletion of the neurotransmitter dopamine, which leads to the clinical manifestation of PD. The pathological hallmarks of the disease are intracellular protein inclusions termed Lewy bodies, which are found in surviving neurons in the brains of PD patients. Lewy bodies are comprised of different proteins including, but not limited to, α -synuclein (Irizarry and others 1998; Spillantini and others 1998), synphilin-1 (Wakabayashi and others 2000), and ubiquitin (Kuzuhara and others 1988). The presence of large amounts of ubiquitinated α -synuclein in Lewy bodies suggests a cellular attempt to sequester toxic α -synuclein aggregates and/or the failure of the cell to degrade protein inclusions. Lewy bodies are thought to represent the end point of the α -synuclein aggregation process, which begins with the formation of dimers and continues with the formation of small oligomers and protofibrils, leading to the end-stage fibrils. Although it still remains to be determined how α -synuclein induces cytotoxicity, there is striking evidence that α -synuclein plays a significant role in the formation and progression of PD. The first clue to α -synuclein's contribution to the disease process is the fact that aggregated α -synuclein is the main component of Lewy bodies, the pathological signature of PD (Irizarry and others 1998; Spillantini and others 1998). Another compelling link between α -synuclein aggregation and PD is suggested by the discovery of several familial forms of PD (FPD) in which a mutation in α -synuclein with an autosomal dominant inheritance causes early PD onset (Kruger and others 1998; Polymeropoulos and others 1997). Mutations in α -synuclein, as well as α -synuclein gene multiplication (Chartier-Harlin and others 2004), result in accelerated aggregation of α -synuclein compared to the wild-type protein, further linking its aggregation with disease onset and progression (Conway and others 1998). Other than linkage to FPD, the α -synuclein gene (SNCA) was also found to be linked to idiopathic PD. Genome-wide

association studies found that single nucleotide polymorphisms (SNPs) in the SNCA region have a significantly strong genetic association to increased PD risk (Edwards and others 2010). Although these studies point to the fact that α -synuclein aggregation is a key factor in PD etiology, the exact species of aggregated α -synuclein that cause cytotoxicity are still unknown. In recent years, the notion that Lewy bodies are the malicious form of α -synuclein aggregates has been replaced by an alternative hypothesis, suggesting that it is in fact the earlier steps of the aggregation process in which α -synuclein forms soluble oligomeric aggregates and protofibrils, which pose a toxic potential to cells (Danzer and others 2011; Tetzlaff and others 2008; Winner and others 2011). In fact, the formation of large cytoplasmic α -synuclein aggregates has been proposed to be a defense mechanism whereby the cell sequesters potentially toxic oligomers and protofibrils (Gispert-Sanchez and Auburger 2006). Supporting this notion, a postmortem study demonstrated that most SNpc neurons with apoptotic-like changes do not contain Lewy bodies, indicating that neuronal death through apoptosis may occur before the formation of Lewy bodies and that cells containing Lewy bodies are not predisposed for neuronal death (Tompkins and Hill 1997). A recent in vivo study demonstrated a more severe dopaminergic neuronal loss in rats injected with lentivirus-carrying α -synuclein gene variants that promote oligomer formation compared to gene variants that promote fibril formation, further establishing the role of soluble α -synuclein oligomers in disease etiology (Winner and others 2011). Similarly, a tight correlation between α -synuclein-soluble oligomers and cytotoxicity was demonstrated in PD animal models, and no such correlation was observed with mature fibrils (Karpinar and others 2009).

Taken together, these studies imply that soluble prefibrillar species of α -synuclein, rather than mature fibrils, are the potential cause of early disease onset in the familial PD cases. Although the mechanism of α -synuclein-induced toxicity remains unknown and the specific species causing cell loss is still controversial, there is an overall agreement within the scientific community that α -synuclein aggregation has a profound effect on PD development and progression. Thus, by interrupting this aggregation process by either preventing α -synuclein oligomerization or by modulating the size and amount of aggregates once formed, we will be able to address the cause of the disease and hopefully prevent the disease or at the very least slow down its progression.

Molecular Chaperones in PD

Molecular chaperones, being able to either prevent proteins from misfolding or target misfolded proteins for degradation, were found to play a key role in PD, as demonstrated by studies showing the presence of HSPs such as Hsp70, Hsp40, and TorsinA in Lewy bodies (Auluck and others 2002; McLean and others 2002; Shashidharan and others 2000; Outeiro and others 2006). The presence of HSPs in Lewy bodies may reflect a cellular attempt to regulate protein aggregation using molecular chaperones. This notion is supported by direct up-regulation of Hsp70, Hsp40, and Hsp27 in response to overexpression of α -synuclein demonstrated in a viral vector PD mouse model (St Martin and others 2007).

Ultimately, a normal age-related decrease in the ability of HSPs to regulate protein aggregation and degradation may contribute to the accumulation of toxic protein inclusions. The slow accumulation of proteins over time may, in turn, compromise the molecular chaperone's ability to maintain cellular proteins in the correct folding state, resulting in a vicious cycle that leads to the neurodegeneration.

By shedding light on the link between molecular chaperones and protein aggregation, we may be able to better understand disease mechanisms, identify novel therapeutic targets, and harness molecular chaperones' functions to inhibit or alter disease progression. In the

following sections, we will discuss the link between molecular chaperones and PD in further detail.

Hsp70

Hsp70 is the major protein up-regulated in response to cellular stress and as such is the most studied molecular chaperone. Many of these studies have linked Hsp70 to PD and to α -synuclein, demonstrating its beneficial effect both in vitro and in vivo and thus placing Hsp70 at the forefront of therapeutic approaches that are being investigated today.

Hsp70 contains two main domains, the N-terminal ATPase domain and the C-terminal ligand binding domain. Hsp70 is active in both ATP- and ADP-bound forms, although the ADP-bound form has enhanced chaperone activity (Hartl and Hayer-Hartl 2002). Hsp70 function involves several levels of regulation, ranging from regulation of its transcription to the regulation of its function once the protein is expressed. Heat shock transcription factor 1 (HSF1) is in a complex with HSPs in unstressed cells, rendering it inactive. When the cells are exposed to stress conditions, HSF1 is then released from the complex and enters the nucleus to bind heat shock elements in the promoter region of HSP genes, which leads to their transcription (Pirkkala and others 2001). Hsp70 function is also regulated at the protein level by co-chaperones that are critical for the activity of Hsp70. Molecular co-chaperones interact either with the N-terminus or C-terminus of Hsp70 and affect its chaperone activity. Co-chaperones that regulate the activity of Hsp70 through association with its N-terminus include the J domain containing the Hsp40 family of proteins, which increase the chaperones' function of Hsp70 by promoting the formation of the more active ADP-bound form of Hsp70 and increasing its ATPase activity (Hartl and Hayer-Hartl 2002). Other co-chaperones that are able to regulate the activity of Hsp70 through direct interaction with its N-terminal domain include ST13 (Hip), which also enhances the chaperone functions of Hsp70 via stabilization of its ADP-bound form (Hohfeld and others 1995), and BAG1, which negatively regulates the activity of Hsp70 either by destabilizing the protein through the dissociation of ADP or by competing with the co-factor Hip to positively regulate Hsp70 activity (Nollen and others 2001). Hsp70 function can also be regulated through its C-terminus, an example of which is the C-terminus Hsp70 interacting protein (CHIP). CHIP is an E3 ubiquitin ligase, which positively regulates Hsp70-dependent degradation using the UPS but negatively regulates the ATPase refolding activity of Hsp70 (Connell and others 2001). Interestingly, α -synuclein oligomers can inhibit Hsp70 chaperone function by interacting with the J domain co-chaperones (Hinault and others 2010).

Evidence that Hsp70 regulates the level of α -synuclein protein aggregates in PD has been demonstrated in several studies both in vitro and in vivo. Hsp70 can prevent, reduce, and clear α -synuclein oligomers and alleviate α -synuclein-induced toxicity in cells (Klucken and others 2004b; Luk and others 2008; McLean and others 2004). In yeast exposed to heat shock, the expression of Hsp70 was demonstrated to prevent α -synuclein-induced apoptosis (Flower and others 2005). A reduction in the toxicity of α -synuclein aggregates was also demonstrated by the complex formation between α -synuclein and Hsc70, an HSC that is constitutively expressed under normal cell conditions. In this work, Hsc70 was shown to preferably bind fibrillar over soluble α -synuclein and reduces its cellular toxicity (Pemberton and others 2011).

Moreover, a single amino acid substitution in the ATPase binding domain of Hsp70 (K71S), which results in loss of function, abolishes Hsp70's ability to protect against α -synuclein-induced toxicity (Klucken and others 2004a). In a study using fluorescence lifetime imaging, it was demonstrated that Hsp70 can affect α -synuclein conformation and interfere with the oligomerization of α -synuclein, thus directly linking Hsp70 chaperone function with α -synuclein aggregation (Klucken and others 2006b).

A connection between Hsp70 and α -synuclein aggregation and toxicity was demonstrated in vivo in rodent and fly models. Increased survival rate of dopaminergic cells in a *Drosophila melanogaster* model overexpressing α -synuclein was demonstrated following expression of Hsp70 (Auluck and others 2002). Similarly, in MPTP-treated rats, the direct targeting of Hsp70 into the SNpc was shown to prevent dopaminergic cell loss associated with the MPTP toxin (Dong and others 2005), and in a PD transgenic mouse model, the expression of Hsp70 was shown to reduce α -synuclein aggregation (Klucken and others 2004b).

Evidence that Hsp70 can also impact the levels of soluble α -synuclein oligomers was demonstrated in a postmortem analysis of brain tissue from patients with dementia with Lewy bodies (DLB) (Klucken and others 2006a), where a correlation between the expression of Hsp90, Hsp40, and Hsp27 and the amount of Triton X-100-soluble α -synuclein was observed. No correlation was observed in control cases as well as with the Triton-insoluble fraction, demonstrating a possible effect of HSPs only on soluble α -synuclein oligomers (Klucken and others 2006a).

Most recently, expression of Hsp70 was found to modify extracellular α -synuclein oligomers and rescue cells from extracellular α -synuclein-induced toxicity (Danzer and others 2011), further supporting a role for molecular chaperones in the cellular attempt to reduce and clear α -synuclein oligomers. As discussed above, many studies have demonstrated the profound effect that Hsp70 has on α -synuclein aggregation and toxicity, highlighting the prospective of Hsp70 as a disease-modifying agent.

Hsp90

Hsp90, like Hsp70, has an N-terminal ATPase domain, while the C-terminus of Hsp90 is responsible for protein dimerization. In PD patients, Hsp90 was found in filamentous aggregates within Lewy bodies and Lewy neurites (Uryu and others 2006). Also, in a large proteomic study, Hsp90 was found to associate with α -synuclein following oxidative stress (Zhou and others 2004). These studies demonstrate that Hsp90, like Hsp70, may play a role in the cellular attempt to modulate and clear protein inclusions. Hsp90 activity is also important for the stability of leucine-rich repeat kinase 2 (LRRK2). Hsp90 inhibition disrupts the LRRK2/Hsp90 complex, which results in increased degradation of LRRK2 by the proteasome (Wang and others 2008). Moreover, in a recent study, Hsp90 was demonstrated to bind to a region within α -synuclein and abolish its ability to bind vesicles (Falsone and others 2009). However, in the presence of ATP, Hsp90 was found to promote α -synuclein fibril formation (Falsone and others 2009). Hsp90 has also been found to suppress α -synuclein toxicity in a yeast model (Liang and others 2008). Recently, Hsp90, together with the recycling endosome regulator rab11a, was found to influence the recycling of α -synuclein (Liu and others 2009).

Small HSPs

Although less studied than Hsp70 and Hsp90, Hsp27 is a small molecular chaperone that is one of the main inducible HSPs, up-regulated in response to a variety of stresses including heat shock and oxidative conditions (Pauli and others 1990). In human DLB brains, up-regulation of Hsp27 mRNA was found (Outeiro and others 2006); Hsp27 was also found to be in Lewy bodies of DLB cases (McLean and others 2002). Hsp27 was shown to have a protective effect in tissue culture against α -synuclein-induced stress (Zourlidou and others 2004). In cultured dopaminergic neurons, Hsp27 was shown to prevent α -synuclein aggregation and have a neuroprotective effect against α -synuclein-induced toxicity (Outeiro and others 2006). Hsp27 was also shown to reduce apoptosis induced by 6-OHDA in PC12 cells by delaying cytochrome c release and caspase activation (Gorman and others 2005).

Another small molecular chaperone, α -crystallin B, was also found in Lewy bodies in cases of multiple system atrophy (Pountney and others 2005). α -synuclein was found to interact with α -crystallin B, and that interaction can diminish the chaperone function of α -crystallin B (Rekas and others 2004).

Co-Chaperones in PD

As described above, Hsp70 can either target a protein for degradation or refold a protein to prevent the exposure of hydrophobic regions that may result in subsequent aggregation. These two functions of Hsp70 are separate, and the likelihood that Hsp70 will perform either one of them depends on interactions with specific co-chaperones that act to regulate its function. Co-chaperones play a key role in the regulation of Hsp70 functions and can either promote the refolding function of Hsp70 or inhibit it and in doing so promote Hsp70-mediated degradation (Fig. 1). These regulatory attributes of co-chaperones on Hsp70 function are now investigated in the context of PD and α -synuclein and may prove to be an important therapeutic strategy.

CHIP

α -synuclein's tendency to aggregate is associated with PD initiation and progression; thus, targeting misfolded α -synuclein for degradation may be an important cellular response that enables the cell to cope with toxic α -synuclein aggregates. The importance of targeting α -synuclein for degradation is emphasized by familial parkinsonism with early disease onset found in people with a mutation in parkin, an E3 ubiquitin ligase (Kitada and others 1998). The decision to target a protein for degradation rather than refolding was shown to be partly dependent on CHIP, a co-chaperone of Hsp70 that interacts with its C-terminus. CHIP inhibits the ATPase activity of Hsp70, negatively regulates its refolding ability, and facilitates the chaperone-mediated protein degradation (Fig. 2) (Connell and others 2001). CHIP was found to be a chaperone-dependent U-box – containing E3 ubiquitin ligase, which enables it, together with Hsp70, to target proteins to the UPS (Meacham and others 2001). Linking CHIP to PD, several lines of evidence demonstrate a CHIP-mediated degradation of toxic α -synuclein oligomers, thus illustrating its therapeutic potential. First, CHIP has been identified in Lewy bodies using immunohistochemistry (Shin and others 2005). Furthermore, CHIP has been demonstrated to associate with α -synuclein and target it for degradation via proteosomal and lysosomal degradation pathways, resulting in reduced α -synuclein-induced toxicity (Shin and others 2005; Tetzlaff and others 2008). CHIP was also found to regulate the ubiquitination, degradation, and toxicity of LRRK2 (Ko and others 2009), mutations that are associated with familial PD with a recessive inheritance (Zimprich and others 2004), further linking CHIP and PD.

BAG5

Bcl-2 – associated athanogene 5 (BAG5) is a co-chaperone of Hsp70 and is part of the BAG protein family. Although BAG5 is the least characterized member of the BAG protein family, it has been linked to PD. Kalia and others (2004, 2011) have shown that BAG5 interacts with Hsp70 and parkin and that this interaction negatively regulates the refolding function of Hsp70. Furthermore, BAG5's interaction with parkin, independent of Hsp70, ameliorates the ligase activity of parkin by its sequestration within protein aggregates (Kalia and others 2004). In a recent article, BAG5 was also shown to bind CHIP, thereby negatively regulating its ability to target α -synuclein for degradation and consequently ameliorating its ability to reduce α -synuclein oligomerization (Kalia and others 2011). In rats injected with BAG5, an increase in dopaminergic cell loss was observed following exposure to MPTP, emphasizing the negative impact of BAG5 function to dopaminergic cell survival (Kalia and others 2004).

ST13

ST13, also known as Hsp70 interacting protein (Hip), is another co-chaperone of Hsp70. Hip stabilizes Hsp70/substrate complexes through interactions with the ATPase domain in its ADP-bound form (Fig. 2) (Hohfeld and others 1995). Hip was found to prevent the co-aggregation of Hsp70 and α -synuclein and in doing so prevent the depletion of Hsp70 (Roodveldt and others 2009). Another connection between PD and Hip is provided by an unbiased transcriptome analysis conducted on the blood of early-stage PD patients. In this study, a reduction in ST13 transcripts was observed in patient samples compared to healthy subjects (Scherzer and others 2007). Other than the discussed role of molecular chaperones in α -synuclein aggregation and degradation, this result also demonstrates the potential role of molecular chaperones as early PD biomarkers.

As discussed, the biological function of molecular chaperones and their ability to regulate cellular proteostasis, together with accumulating data linking molecular chaperones to PD etiology, highlight the important role that molecular chaperones have in PD initiation and progression. Better understanding of the control mechanisms that underlie molecular chaperone function may offer some answers to the puzzling questions surrounding PD and may provide us with potential therapeutic approaches to prevent and/or degrade the toxic protein aggregates that are associated with many neurodegenerative diseases including PD.

Molecular Chaperones as a Therapeutic Target in PD

While dopamine replacement strategies for PD can substantially improve motor symptoms in most patients, there is currently no therapeutic approach that can prevent or reverse the progression of neuronal degeneration in PD. There is therefore a growing and unmet need to identify novel therapeutic targets for neuroprotection and/or disease-modifying agents (Meissner and others 2011). By targeting well-characterized pathways that have been established by preclinical studies in disease models, rational drug design and testing will lead to promising new disease-modifying approaches. Translational approaches, to date, target mechanisms of oxidative stress (e.g., antioxidative agents such as green tea polyphenols), glutamate excitotoxicity (e.g., glutamate release inhibitors and glutamate receptor blockers), mitochondrial dysfunction (e.g., coenzyme Q10 and creatine), trophic factors (e.g., GDNF, neurturin and others), and altered protein metabolism. These targets are shared by many neurodegenerative conditions, and therefore, research efforts will not only benefit patients with PD but also patients who suffer from other major diseases such as Alzheimer disease or DLB.

Targets within protein metabolism include, but are not limited to, misfolding and aggregation (Conway and others 1998), posttranslational modification (Fujiwara and others 2002), and mechanisms of protein removal, recycling, and degradation (Ebrahimi-Fakhari and others 2011a; Ebrahimi-Fakhari and others 2012).

As detailed in the previous section, molecular chaperones are crucially involved in protein folding and refolding, a vital cellular function and an essential step in many protein conformational disorders. Thus, molecular chaperone function is a promising target that has the capacity to alter early pathological changes in PD, even before significant neurodegeneration has occurred (Fig. 3). The Hsp70 system in particular has been shown to be an important frontline defense mechanism employed by neurons to halt, prevent, or even reverse protein misfolding and toxicity.

A growing number of preclinical studies have investigated strategies to up-regulate or enhance chaperone function in neuronal populations at risk (Ebrahimi-Fakhari and others 2011b). In order to test the neuroprotective effects of chaperones, most studies have

employed pharmacological or protein transfer strategies, with promising results. Testing of compounds and therapeutic concepts in neurodegenerative diseases is again not limited to studies for PD and has been driven by research in many related areas, in particular the trinucleotide repeat expansion diseases (Hay and others 2004; Waza and others 2005). Based on similarities between disease models and the mechanisms at play, many of the compounds tested in other diseases might provide a rationale for studies in PD.

The main pharmacological agents aimed at Hsp70 can be classified into direct and indirect enhancers of Hsp70 function (summarized in Fig. 3 and Table 1). Indirect enhancers primarily inhibit Hsp90, which leads to the release of HSF1 from its complex with Hsp90 and thus to an increased expression of Hsp70 and other stress-induced chaperones (Zou and others 1998). Recruitment of Hsp70 to actively unfold stress-induced misfolded proteins can possibly prevent the formation of toxic protein species and aggregates. This cytoprotective ability is key to Hsp70- and Hsp90-targeting therapies.

An increasing number of small molecule inhibitors of Hsp90 have been studied in models of PD. The first compound that was studied extensively was geldanamycin, a naturally occurring antibiotic and the lead compound for a family of inhibitors termed ansamycin antibiotics (Whitesell and others 1994). Treatment with geldanamycin was found to effectively reduce α -synuclein oligomerization and aggregation while increasing α -synuclein clearance in cell culture models (Liu and others 2009; McLean and others 2004). With regard to neurodegeneration, geldanamycin has been shown to prevent α -synuclein-induced toxicity and cell death in cell culture (Liu and others 2009; McLean and others 2004), *Drosophila melanogaster* (Auluck and others 2002; Auluck and others 2005), and the MPTP mouse model of PD (Shen and others 2005). Although these findings have been very encouraging for the concept that chaperone function can be targeted and employed as a beneficial treatment in disease models, the use of geldanamycin has been limited for pharmacokinetic reasons, most importantly its poor solubility and blood-brain barrier penetration. Other members of the ansamycin group of inhibitors have been able to partly overcome these restrictions. 17-AAG (tanespimycin) and 17-DMAG (alvespimycin) are potent Hsp90 inhibitors with improved pharmacokinetic profiles (Schulte and Neckers 1998). Like geldanamycin, 17-AAG can attenuate α -synuclein toxicity, prevent oligomerization, and facilitate α -synuclein clearance in cultured cells (Danzon and others 2011; Putcha and others 2010). Recent studies have underscored the influence of 17-AAG on cellular degradation pathways. Notably, 17-AAG was found to promote proteasomal degradation of LRRK2 by affecting the interaction between LRRK2, the co-chaperone CHIP, and Hsp90, thus reducing LRRK2-mediated toxicity (Ding and Goldberg 2009; Ko and others 2009). Besides that, 17-AAG was also shown to effectively enhance macroautophagy and to accelerate α -synuclein clearance (Riedel and others 2010). Current phase I/II trials in various forms of cancer have demonstrated safety for the use of 17-AAG in patients, but its limited blood-brain barrier permeability remains an obstacle to use in neurodegenerative diseases. 17-DMAG displays improved aqueous solubility but unfortunately was found to cause unacceptable adverse events in cancer trials (Kim and others 2009). In summary, the clinical utility of all three compounds—geldanamycin, 17-AAG, and 17-DMAG—is uncertain, despite encouraging results from preclinical studies.

A novel group of synthetic small molecule inhibitors of Hsp90 was obtained through a compound library screen for scaffolds that selectively bind the ATP binding pocket of Hsp90, the same mechanism by which geldanamycin and analogs exert their function (Whitesell and others 1994). The lead compound has been termed SNX-2112. Although originally developed for antitumor effects, SNX-2112 and derivatives have important features for the use in neurodegenerative diseases: an excellent blood-brain barrier permeability with oral application and a very selective inhibition of Hsp90. Selectivity is

key to Hsp90 inhibitors because a global block of Hsp90 function is likely to result in a significant derangement of cellular circuits, making this an unfavorable approach. Pretreatment and co-treatment with SNX compounds in cell culture models of PD resulted in a reduction of both high molecular weight and monomeric α -synuclein as well as a significant reduction of α -synuclein oligomerization (Putcha and others 2010). The latter might be of particular importance given the implications of oligomeric species for α -synuclein-induced pathology and neurodegeneration (Danzer and others 2011; Winner and others 2011). In the study by Putcha and others (2010), a lead compound, SNX-0723, was identified and subjected to further in vivo pharmacokinetic and pharmacodynamic studies that revealed robust brain penetration and bioavailability, thus making this compound a promising candidate for future studies. Additional Hsp90 inhibitors that have been studied in other neurodegenerative diseases or cancer but not yet in PD models include radicicol, trichostatin A, retaspimycin, and novobiocin (Kim and others 2009).

Despite improved selectivity and pharmacodynamics of novel Hsp90 inhibitors and despite the promising results in disease models, the feasibility of using Hsp90 inhibitors for the treatment of neurodegenerative diseases is yet uncertain. The presence of potentially hundreds of Hsp90 client proteins involved in crucial cellular pathways and the strong toxic side effects observed with some of the current Hsp90 inhibitors pose significant conceptual challenges for this approach. A thorough understanding of the mechanisms by which Hsp90 regulates HSF1 activation will open new opportunities to the development of future Hsp90 inhibitors as therapeutics for neurodegenerative diseases associated with protein misfolding.

Direct enhancers of Hsp70 expression and/or chaperone function have shown promising results in models of a variety of neurodegenerative diseases. Arimoclomol, an enhancer of the Hsp70 system, has probably the greatest potential for use in PD given the successful application for amyotrophic lateral sclerosis, where this compound has even reached the level of clinical testing (Cudkowicz and others 2008). Arimoclomol acts by prolonging the binding of HSF1 to heat shock response elements in heat shock gene promoters and thus supports chaperone expression under stress conditions, an important characteristic that confers selectivity for cells at risk. Celastrol, a compound that helps to phosphorylate and activate HSF1, was found to significantly alleviate MPTP-induced neurodegeneration and to partly restore striatal dopamine levels in the MPTP mouse model of PD (Clerehugh and others 2005). Neuroprotective effects were also confirmed in a *Drosophila melanogaster* model (Faust and others 2009). Valproic acid, a classic anticonvulsant drug widely used in the treatment of epilepsy, was shown to induce Hsp70 through its action as a histone deacetylase (HDAC) inhibitor. Relevant to PD, valproic acid-induced Hsp70 activation was protective against rotenone-induced toxicity in treated cell culture and rats (Monti and others 2010; Pan and others 2005).

Achieving HSF1 activation and a neuroprotective chaperone response through inhibition of negative regulators such as chaperones (e.g., Hsp90 inhibitors) or protein kinases (e.g., celastrol) is of prospective therapeutic use in PD and other neurodegenerative diseases. However, the pleiotropic effects of such global approaches remain an important obstacle because derangement of the many cellular pathways and functions associated with these targets could have serious and detrimental consequences. Therefore, approaches that directly bind and act on HSF1 or compounds that even act outside of HSF1-dependent HSP expression might be a favorable approach.

A very interesting compound in this respect is the disaccharide trehalose. Trehalose is able to act as a chemical chaperone through direct interaction with client proteins and was also found to enhance protein clearance via the autophagy pathway (Sarkar and others 2007; Tanaka and others 2004). For example, mutant α -synuclein was shown to be subject to

trehalose-induced autophagy (Sarkar and others 2007). While these are encouraging results, further development of this compound for PD calls for a detailed characterization in mammalian animal models.

Gene therapy approaches have the potential to stably restore deficits in cellular pathways or to enhance protective cell functions. Viral-mediated enzyme replacement or expression of trophic factors in neurons has recently received considerable attention as a possible new approach to ameliorate or even cure PD (Bjorklund and Kordower 2010). With regards to chaperone-based therapies, targeted viral-mediated expression in brain regions and neuronal populations at risk for neurodegenerative changes can potentially be employed as a novel preventive or curative approach. A few *in vivo* studies have shed light on the efficacy of chaperone gene transfer in disease models (summarized in Fig. 3 and Table 2). As a classic model of neurodegeneration, the first experiments following this approach have been conducted in toxin-based models of PD. In the MPTP mouse model, adeno-associated virus (AAV)-mediated overexpression of Hsp70 in dopaminergic neurons was capable of preserving neuronal integrity and striatal dopamine levels (Dong and others 2005). Similarly, in a CDCrel-1 (a cytotoxic parkin substrate)-based rat model, AAV-based overexpression of H-BH, a constitutively active form of HSF1, or Hsp70, was able to ameliorate dopaminergic neurodegeneration (Jung and others 2008).

Viral-mediated delivery of chaperones in neurons has not only been limited to direct enhancement of Hsp70 but also has been used to express Hsp70 interacting proteins. Exogenous expression of Hsp104 in a co-expression model with A30P mutant α -synuclein in the SNpc of rats resulted in reduced α -synuclein-positive inclusions and an overall reduction of dopaminergic cell death (Lo Bianco and others 2008). AAV-mediated expression of BAG5 (DARA), a mutant and inactive form of the co-chaperone BAG5, which blocks the interaction between BAG5 and Hsp70, significantly improved the survival of dopaminergic neurons in the SNpc of MPTP-treated mice (Kalia and others 2004).

A novel methodology to deliver chaperones to neurons has emerged in the form of cell-penetrating peptide (CCP) technology. Even with systemic application, CCP as small carrier proteins can deliver cargo across the blood-brain barrier and cell membranes (Dietz and Bahr 2004). Fusion of Hsp70 to TAT (transactivator of transcription), one of the most widely used CCPs, can enhance delivery and cell penetration into neurons. In cultured primary neurons, for example, TAT-Hsp70 application significantly increased intracellular levels of Hsp70 and provided protection against insults like oxidative or glutamatergic cell stress (Lai and others 2005). TAT-Hsp70 was further found to protect against the toxic insult of MPTP application in different cell culture models (Nagel and others 2008). At the *in vivo* level, systemic application of TAT-Hsp70 through intraperitoneal injection in mice demonstrated robust delivery to dopaminergic neurons of the SNpc and again saved the target region from the insult created by MPTP application (Nagel and others 2008). Studies on the CCP-based enhancement of other chaperones, like Hsp40, or direct delivery of the transcription factor HSF1 are still limited, although promising with regards to Hsp70 up-regulation (Hou and Zou 2009; Kim and others 2008). Although delivery mechanisms for chaperones such as CCP are exciting new research avenues, safety and applicability clearly have to be further demonstrated in additional *in vivo* studies.

Conclusions

Molecular chaperone-based therapies are being developed for use in PD. Rationale for this approach is provided by strong evidence for the involvement of chaperones in crucial disease steps such as the formation and accumulation of proteins. As a vital cellular pathway, unbalanced and dysfunctional chaperone-mediated protein folding could result in

the onset or progression of protein conformational disorders. By enhancing chaperone function, early pathological changes can be counteracted to halt or even prevent neurodegeneration. Small molecule-based approaches have shown very promising results in proof-of-concept studies in disease models. Chronic and coordinated enhancement of molecular chaperone levels and function is therefore a promising new approach to target protein misfolding and accumulation in PD. However, unbalanced overexpression or the inhibition of targets with pleiotropic downstream functions or even functions outside the chaperone network might lead to significant cellular dysregulation or toxicity. With these and the specific limitations discussed above, the burden is now on future studies to identify the most capable approach to fight protein accumulation and toxicity in PD.

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Figure 1.

Figure 2.

Figure 3.

Table 1

Small Molecule Enhancer of Chaperone Function

Compound	Group	Target	References
Geldanamycin	Ansamycin antibiotics	Hsp90 inhibition	Auluck and others 2002; Auluck and others 2005; Liu and others 2009; McLean and others 2004; Shen and others 2005
17-AAG			Danzer and others 2011; Ding and Goldberg 2009; Ko and others 2009; Putcha and others 2010; Riedel and others 2010
SNX-0723 and derivatives	SNX compounds	Hsp70 inducer	Putcha and others 2010
Celastrol	Triterpene		Cleren and others 2005; Faust and others 2009
Valproic acid	HDAC inhibitor		Monti and others 2010; Pan and others 2005

Table 2

Gene Transfer Strategies to Up-Regulate Chaperone Function

Construct	Test model	References
AAV-Hsp70	MPTP mouse model	Dong and others 2005; Jung and others 2008
	AAV-CDCrel-1 rat model	
AAV-H-BH	AAV-CDCrel-1 rat model	Jung and others 2008
LV-Hsp104	LV-(h)A30P α -synuclein rat model	Lo Bianco and others 2008
AAV-BAG5 (DARA)	MPTP mouse model	Kalia and others 2004