

# Protein Misfolding, Amyloid Formation, and Neurodegeneration: A Critical Role for Molecular Chaperones?

Minireview

Paul J. Muchowski<sup>1</sup>

Department of Pharmacology  
University of Washington  
Seattle, Washington 98195

The most conspicuous feature of many neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease, is the occurrence of protein aggregates in ordered fibrillar structures known as amyloid found inside and outside of brain cells. The appearance of aggregates in diseased brains implies an underlying incapacity in the cellular machinery of molecular chaperones that normally functions to prevent the accumulation of misfolded proteins. Here we review recent studies that have revealed a critical role for molecular chaperones in several neurodegenerative disorders.

## *Neurodegenerative Diseases, Brain Lesions, Amyloid, and Molecular Chaperones*

Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative disorders, affecting a combined ~6 million people in the United States alone. AD is the most common senile dementia, where it is estimated 10% of people over the age of 65 are afflicted, whereas PD is the most common movement disorder, affecting 1%–2% of this same population group. In AD and PD, the vast majority of cases are idiopathic. In contrast, Huntington's disease (HD) is a less common neurodegenerative disorder that affects at least 1 in 10,000 people in the general population. The mutation that causes HD is an expansion of CAG repeats (encoding polyglutamine) in the gene huntingtin. The mutation is inherited in an autosomal dominant manner, and the same mutational mechanism is responsible for a growing number of less common neurodegenerative disorders that include the spinocerebellar ataxias (SCAs). AD, PD, and HD each display characteristic patterns of neuronal cell loss and behavioral phenotypes. In AD, cell loss is most prominent in the neocortex and hippocampus, and is accompanied by memory loss, dementia, and impairment in other forms of cognition and behavior. PD is characterized by a loss of dopaminergic neurons in the substantia nigra and is accompanied by muscle rigidity, bradykinesia, and resting tremor. In HD, cells in the striatum and cortex are most affected, resulting in progressive chorea, rigidity, and dementia.

What then do these apparently disparate neurodegenerative diseases have in common? One histological feature they share is the occurrence of lesions in brains, consisting of an intra- or extracellular accumulation of misfolded, aggregated, and ubiquitinated proteins that are intimately associated with neurodegeneration. The lesions observed in AD are intracellular neurofibrillary tangles that contain the protein Tau and extracellular plaques that contain  $\beta$ -amyloid (A $\beta$ ) peptides (reviewed

by Selkoe, 2001). In PD, the lesions are called Lewy bodies, are found in the cytoplasm, and are composed primarily of the protein  $\alpha$ -synuclein (reviewed by Goedert, 2001). In HD, the lesions are observed as intranuclear and cytoplasmic inclusion bodies that contain the protein huntingtin (reviewed by Zoghbi and Orr, 2000). All of the proteins found in the above-mentioned lesions share a propensity to form an ordered fibrillar structure called amyloid (reviewed by Rochet and Lansbury, 2000). Amyloid formation is thought to arise from partial unfolding and exposure of hydrophobic surfaces that are normally buried in the core of a folded protein, thus increasing attractive forces among protein molecules. Mutations that increase the production of amyloid cause rare, early-onset forms of AD and PD (reviewed by Goedert, 2001; Selkoe, 2001). In HD, the length of the polyglutamine (polyQ) expansion in huntingtin correlates directly with kinetics of amyloid formation and indirectly with age of onset and severity of the disease (reviewed by Zoghbi and Orr, 2000). For AD, PD, and HD, the precise relationship between deposition of amyloid and initiation of pathogenesis has been the subject of intense scrutiny.

An additional feature that AD, PD, and HD share in common is the presence of molecular chaperones and components of the ubiquitin-proteasome degradation system in the brain lesions characteristic of each disease (reviewed by Clark and Muchowski, 2000; Sherman and Goldberg, 2001). All cells and organelles possess a machinery of molecular chaperones whose function is to mediate the proper folding of other proteins and to insure that these proteins maintain their native conformations during conditions of stress (reviewed by Hartl and Hayer-Hartl, 2002). In addition to their functions in co- and posttranslational folding, chaperones are required for the translocation of many proteins across cellular membranes, are involved in macromolecular assembly and disassembly, and facilitate the transfer of misfolded proteins to the proteasome for degradation. Many chaperones are also components of signal transduction cascades that mediate transcriptional responses to stress, including those that lead to the suppression of apoptosis. Chaperones function by binding transiently to exposed hydrophobic surfaces in target proteins in a manner that is regulated by ATP-induced conformational changes that occur in their substrate binding domains. Chaperones function to insure that exposed hydrophobic surfaces are shielded from aberrant interactions with other nearby proteins and folding intermediates in the concentrated milieu of the cell. A defining feature of all chaperones is their predilection for binding to folding and misfolding intermediates.

## *Molecular Chaperones Suppress Neurodegeneration in Fly and Mouse Models of Polyglutamine Diseases*

Since 1998, approximately 15 publications have reported the effects of overexpression of chaperones in cellular models of polyQ aggregation and toxicity. Despite the various polyQ proteins and cell types used in these studies, in general these papers reported similar

<sup>1</sup>Correspondence: mucho@u.washington.edu

results, showing that members of the Hsp70 and Hsp40 families of chaperones suppress the aggregation and toxicity of polyQ-containing proteins (reviewed by Sherman and Goldberg, 2001).

The most compelling data that supports a critical role for chaperones in protein misfolding diseases comes from recent *in vivo* studies in fruit fly and mouse models of neurodegenerative disorders (Auluck et al., 2002; Cummings et al., 2001; Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000; Warrick et al., 1999). In the first study, a *Drosophila melanogaster* model of Machado-Joseph disease (also known as SCA3) was used to characterize the effects of overexpression of human Hsp70. Expression of the expanded polyQ protein was driven by an eye-specific promoter, causing severe degeneration of external eye structures and eventual loss of the retina (Warrick et al., 1999). Overexpression of human Hsp70 completely suppressed the external eye defects mediated by the expression of expanded polyQ in these flies, and partially restored retinal structure. In a second set of experiments, expression of the expanded polyQ protein under the control of a neuron-specific promoter caused widespread neurodegeneration and also decreased fly viability. Overexpression of human Hsp70 in these flies suppressed neurodegeneration and prolonged lifespan by 2-fold. Perhaps most intriguing, expression of the expanded polyQ protein in a fly line bearing a dominant-negative *Drosophila* Hsp70 augmented the severity and kinetics of neurodegeneration, suggesting that under normal conditions the endogenous fly Hsp70 may partially mitigate the toxic effects of the expanded polyQ protein (Warrick et al., 1999). Paradoxically, at the level of light microscopy, there appeared to be no effect on aggregate formation by the polyQ protein.

Two subsequent studies took advantage of genetic tools available in *Drosophila* to perform screens for modifiers of polyQ-induced toxicity. In the first study, two genes were isolated that dramatically reduced polyQ toxicity. The first gene encoded a *Drosophila* homolog of human Hsp40/HDJ1 (dHDJ1), a co-chaperone for Hsp70 *in vivo*, and the second gene encoded a *Drosophila* homolog of human tetratricopeptide repeat protein 2 (Kazemi-Esfarjani and Benzer, 2000). Interestingly, both proteins contain a conserved J domain that is known to stimulate the ATPase activity of Hsp70 and may function *in vivo* to help target folding intermediates to Hsp70. In an independent study, dHDJ1 was also isolated as a suppressor of polyQ-mediated toxicity induced by the expression of full-length human SCA1 in fruit flies (Fernandez-Funez et al., 2000).

The remarkable finding that overexpression of chaperones can suppress neurodegeneration *in vivo* has been extended to mammals, where it was shown that increasing levels of Hsp70 in a mouse model of SCA1 mitigates neurodegeneration (Cummings et al., 2001). Importantly, overexpression of Hsp70 not only reduced pathological changes in SCA1 mice, but also behavioral phenotypes. However, as in the fly models, there appeared to be no changes in aggregate formation by SCA1 as judged by light microscopy.

#### **Molecular Chaperones Suppress Neurodegeneration in a Fly Model for Parkinson's Disease**

While accumulating evidence indicates a neuroprotective role for chaperones in HD and other polyQ-mediated

disorders, whether or not their overexpression could have a beneficial effect in a major disorder of the nervous system (i.e., AD or PD) was an unanswered question, until recently. Bonini and colleagues asked if overexpression of Hsp70 could affect neuropathology in a *Drosophila* model of PD. In this model, overexpression of  $\alpha$ -synuclein driven by the 3,4-dihydroxyphenylalanine (DOPA) decarboxylase gene promoter leads to a selective and progressive loss of dopaminergic neurons. Indeed, not only could Hsp70 overexpression suppress the loss of dopaminergic neurons, but expression of a dominant-negative Hsp70 exacerbated the neurodegenerative phenotype significantly (Auluck et al., 2002). Interestingly, expression of the dominant-negative Hsp70 caused some dopaminergic neuron loss even in the absence of  $\alpha$ -synuclein, suggesting a role for endogenous Hsp70 in neuronal survival. The authors also showed that a small percentage of Lewy Bodies in postmortem PD brain tissue react with antibodies raised against Hsp70 and Hsp40, suggesting that these chaperones may play a role in the human disease. These results expand the protective role of chaperones to a major neurodegenerative disease, and are intriguing because PD may have a completely different molecular mechanism than HD and the polyQ diseases.

#### **Mechanisms of Chaperone Protection in Models of Neurodegenerative Diseases**

If chaperones suppress neurodegenerative phenotypes in models of protein misfolding disorders *in vivo* but have no discernible effect on aggregation of the disease-causing proteins, what then is the mechanism of protection? One clue may come from *in vitro* studies that examined the physical and biochemical effects of chaperones on fibril formation by a truncated version of the huntingtin protein (Muchowski et al., 2000). In our studies, the Hsp70/Hsp40 chaperone system inhibited in an ATP-dependent manner the self-assembly of polyQ proteins into amyloid-like fibrils. Electron microscopy demonstrated that the chaperones caused the formation of amorphous (as opposed to protofibrillar or fibrillar), detergent-soluble aggregates. The chaperones were most active in preventing fibrillization when added during the lag phase of the polymerization reaction, indicating that the molecular species with which they preferentially interact exists in an intermediate, pre-fibrillar state. Hsp70 alone had only a modest effect on inhibiting fibril formation, but in the presence of its co-chaperone Hsp40, complete suppression of fibril formation was observed. Consistent with *in vitro* results, overexpression of Hsp70 or Hsp40 in yeast and mammalian cell models of polyQ aggregation inhibited the formation of large, detergent-insoluble inclusion bodies, resulting instead in the accumulation of detergent-soluble inclusions. Not surprisingly, fibrillar aggregates could not be detected at the ultrastructural level by electron microscopy under these cellular conditions (Sittler et al., 2001). The synergistic effects of chaperones to increase polyQ solubility were confirmed in an *in vivo* model of polyQ-induced neurodegeneration in *Drosophila* (Chan et al., 2000). In this model, overexpression of Hsp70 and/or Hsp40 enhanced the solubility of polyQ proteins concomitant with protection against neurodegeneration, but without having any effect on the morphology of polyQ aggregates as judged by light microscopy. These studies collectively underscore the importance of analyzing aggregation in

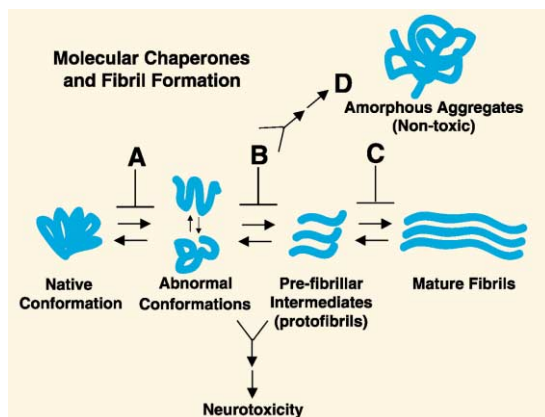


Figure 1. A Model for Molecular Chaperone Suppression of Neurotoxicity

Molecular chaperones may suppress neurotoxicity of amyloid-forming proteins by preventing their conversion between native conformations and toxic conformations (A), by preventing the formation of pre-fibrillar intermediates (B), by preventing the conversion between pre-fibrillar intermediates and mature fibrils (C), and/or by facilitating the conversion of toxic intermediates into nontoxic amorphous aggregates (D). Irrespective of the neuroprotective mechanisms they invoke, an excess of chaperones in cells leads to the formation of detergent-soluble and amorphous aggregates (D) that are degraded more readily by the proteolytic machinery, thus preventing aberrant protein interactions that would otherwise lead to a cascade of events that culminate in neurodegeneration.

disease models by methods other than conventional light microscopy, which poorly detects morphological changes in protein aggregates and is unable to detect underlying physical and biochemical changes in the disease-causing proteins that are likely to be more pertinent to neurotoxicity. This situation is reminiscent of studies in AD which have shown that levels of soluble forms of A $\beta$  more closely correlate with pathology than levels of insoluble, fibrillar forms present in amyloid plaques (Walsh et al., 2002; reviewed by Selkoe, 2001). These toxic soluble forms (see below) are presumably distinct from the “amorphous,” detergent-soluble forms that one would predict might be increased by chaperone treatment in models of AD.

Chaperones are one of the best examples of multifunctional proteins, and their protection against neurodegeneration may result from one or more of their activities in cells, perhaps in addition to their ability to inhibit fibril formation directly. In one recent study, the Hsp70/Hsp40 chaperone system was shown not only to enhance the solubility of expanded polyQ proteins, but to also increase the degradation of these proteins through the 26S proteasome (Bailey et al., 2002). The increase in polyQ solubility was accompanied by a 40% decrease in the half-life of the expanded polyQ proteins. This important result indicates that an excess of chaperone capacity in the cell can shift the equilibrium between amorphous (detergent-soluble) and fibrillar (detergent-insoluble) aggregates such that the cell’s proteolytic machinery can more efficiently turn over the toxic polyQ proteins. Nevertheless, the mechanism chaperones use to facilitate clearance of misfolded proteins by the proteasome remains to be elucidated, and may indicate a useful target for pharmacological intervention. A role for

chaperones in other cellular pathways such as in the suppression of signaling cascades that lead to apoptosis, or in protection against defects in synaptic transmission, are some examples of areas that are being investigated actively that may help explain chaperone suppression of neurotoxicity. Clearly, the effects of chaperones in multiple cellular pathways will have to be deciphered in order to understand which of these effects are primary and which are secondary in protection against neurodegeneration.

#### **Pre-Fibrillar Intermediates (Protofibrils) and Neurotoxicity**

Recent studies support the hypothesis that pre-fibrillar intermediates (protofibrils), and not mature amyloid fibrils, may be the key toxic species in AD, PD, and HD. In vitro studies with two mutants of  $\alpha$ -synuclein associated with inherited forms of PD showed that both mutations cause an acceleration of oligomerization, and not fibrillization (Conway et al., 2000). In a related study that supports an important role for protofibrils, oxidation-induced adduct formation between  $\alpha$ -synuclein and catecholamines, including dopamine, caused an accumulation of protofibrils, and this process was reversed by addition of anti-oxidants (Conway et al., 2001). Consistent with a toxic role for protofibrils in AD, small oligomers of A $\beta$  potently inhibited hippocampal long-term potentiation in vivo, while fibrillar and monomeric forms had no effect (Walsh et al., 2002). In an independent study, when two proteins not known to be involved in disease were incubated under conditions where they formed amyloid fibrils, only the pre-fibrillar intermediates and not the mature fibrils caused cytotoxicity (Buciantini et al., 2002). Evidence for a toxic role for pre-fibrillar intermediates in HD comes from our study which showed that disruption of the microtubule cytoskeleton in cell models for huntingtin aggregation unmasks a glutamine length-dependent toxicity under conditions where the huntingtin protein exists in a completely nonfibrillar state (Muchowski et al., 2002). The results from this study are consistent with an accumulation of data that dissociates aggregation of polyQ-containing proteins (judged at the level of light microscopy) from toxicity (reviewed by Zoghbi and Orr, 2000). In light of results that highlight the toxic nature of protofibrils, and based on the known affinity of chaperones for folding intermediates, it is tempting to speculate that chaperones protect against neurodegeneration in animal models by acting at the level of protofibrils. It might be argued that by binding to protofibrils, chaperones induce a conformational change that leads to the generation of amorphous, nontoxic aggregates, which at the same time also decreases the cellular concentration of mature amyloid fibrils.

#### **Future Directions**

While it is becoming clear that chaperones can have a profound influence on solubility, aggregation, fibril formation, and toxicity of proteins that misfold and cause neurodegenerative disorders, many issues remain to be examined. The disease-causing proteins associated with AD, PD, and HD can apparently misfold into multiple toxic conformations and pre-fibrillar intermediates with different morphologies. However, the structural nature and, just as importantly, downstream targets of these intermediates remain uncharacterized and will need to be elucidated if they are to be pursued as drug targets.

Chaperones may protect against neurodegenerative disorders in animal models by acting at one or more steps in the pathway of fibril formation (Figure 1). Chaperones may convert toxic conformations of misfolded proteins to nontoxic forms that can be tolerated by cells, although direct biophysical evidence for this hypothesis is lacking. Chaperones may also prevent the formation of toxic pre-fibrillar intermediates, or accelerate their conversion to nontoxic amorphous aggregates that can be turned over more easily by the proteolytic machinery. Although evidence indicates that chaperones act on pre-fibrillar intermediates and not mature fibrils (Muchowski et al., 2000), the molecular basis of these interactions is not known and should be resolved.

The functions and targets of chaperones in neurons and glia during normal aging and in other neurodegenerative disorders including AD are largely unknown. Since overexpression of a dominant-negative Hsp70 mutant causes a neurodegenerative phenotype in an otherwise wild-type fruit fly (Auluck et al., 2002), it is likely that the overall levels of Hsp70 and other endogenous chaperones may be intimately related to the age of onset, kinetics of progression, and severity of pathological and behavioral phenotypes in animal models of neurodegenerative disease. Chaperones play key roles in numerous cellular pathways, and interference with their normal functions due to altered protein levels or activities would be expected to have enormous consequences that would be manifested in a multitude of pathological and behavioral phenotypes. This may explain why unbiased genetic screens have implicated numerous and diverse cellular pathways as having physiological importance in the pathogenesis of neurodegenerative disorders. To help resolve this issue, it will be crucial to determine the temporal pattern of chaperone expression and capacity in brain cells during normal aging and in various neurodegenerative diseases. It is possible that chaperone capacity in brain cells diminishes as a function of aging as damaged proteins accumulate due to various forms of stress, and that this process is merely accelerated in neurodegenerative disorders. It is important to note that the expression and degradation of chaperones and disease-causing proteins in cells are in a constant state of equilibrium, thus even a modest increase in cellular chaperone capacity may be sufficient to delay disease onset or severity. Consistent with this hypothesis, in a conditional transgenic mouse model of HD, blockade of polyQ expression in symptomatic mice leads to the disappearance of neuronal inclusions and reversal of motor dysfunction (Yamamoto et al., 2000). While the mechanism which underlies this reversal has not been elucidated, the results suggest that shifting the equilibrium in favor of polyQ degradation (for example, by overexpressing chaperones) will have beneficial therapeutic effects even after behavioral symptoms and pathology have been exhibited. However, the effects of overexpression of chaperones will have to be evaluated carefully in animal models, because adverse cellular consequences to high levels of chaperones have been reported. Finally, since chaperones are known to function in a cooperative manner in vivo, it seems likely that increasing the expression of multiple chaperones may be required to obtain a beneficial impact on disease

progression and severity in patients with neurodegenerative disorders.

In summary, recent studies suggest that chaperones may play a critical role in neurodegeneration and aging, and this class of proteins holds much promise as a therapeutic target for the treatment of neurodegenerative disorders. While the identification of small molecules that can enhance chaperone expression or activities in brain cells may seem like a daunting task, it is worthy to note that a small molecule antagonist of the chaperone Hsp90 is currently in clinical trials for the treatment of recurrent, refractory breast cancer (Bagatell et al., 2001).

#### Selected Reading

- Auluck, P.K., Chan, H.Y., Trojanowski, J.Q., Lee, V.M., and Bonini, N.M. (2002). *Science* 295, 865–868.
- Bagatell, R., Khan, O., Paine-Murrieta, G., Taylor, C.W., Akinaga, S., and Whitesell, L. (2001). *Clin. Cancer Res.* 7, 2076–2084.
- Bailey, C.K., Andriola, I.F., Kampinga, H.H., and Merry, D.E. (2002). *Hum. Mol. Genet.* 11, 515–523.
- Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C.M., and Stefani, M. (2002). *Nature* 416, 507–511.
- Chan, H.Y., Warrick, J.M., Gray-Board, G.L., Paulson, H.L., and Bonini, N.M. (2000). *Hum. Mol. Genet.* 9, 2811–2820.
- Clark, J.I., and Muchowski, P.J. (2000). *Curr. Opin. Struct. Biol.* 10, 52–59.
- Conway, K.A., Lee, S.J., Rochet, J.C., Ding, T.T., Williamson, R.E., and Lansbury, P.T., Jr. (2000). *Proc. Natl. Acad. Sci. USA* 97, 571–576.
- Conway, K.A., Rochet, J.C., Bieganski, R.M., and Lansbury, P.T., Jr. (2001). *Science* 294, 1346–1349.
- Cummings, C.J., Sun, Y., Opal, P., Antalfy, B., Mestrl, R., Orr, H.T., Dillmann, W.H., and Zoghbi, H.Y. (2001). *Hum. Mol. Genet.* 10, 1511–1518.
- Fernandez-Funez, P., Nino-Rosales, M.L., de Gouyon, B., She, W.C., Luchak, J.M., Martinez, P., Turiegano, E., Benito, J., Capovilla, M., Skinner, P.J., et al. (2000). *Nature* 408, 101–106.
- Goedert, M. (2001). *Nat. Rev. Neurosci.* 2, 492–501.
- Hartl, F.U., and Hayer-Hartl, M. (2002). *Science* 295, 1852–1858.
- Kazemi-Esfarjani, P., and Benzer, S. (2000). *Science* 287, 1837–1840.
- Muchowski, P.J., Schaffar, G., Sittler, A., Wanker, E.E., Hayer-Hartl, M.K., and Hartl, F.U. (2000). *Proc. Natl. Acad. Sci. USA* 97, 7841–7846.
- Muchowski, P.J., Ning, K., D'Souza-Schorey, C., and Fields, S. (2002). *Proc. Natl. Acad. Sci. USA* 99, 727–732.
- Rochet, J.C., and Lansbury, P.T., Jr. (2000). *Curr. Opin. Struct. Biol.* 10, 60–68.
- Selkoe, D.J. (2001). *Physiol. Rev.* 81, 741–766.
- Sherman, M.Y., and Goldberg, A.L. (2001). *Neuron* 29, 15–32.
- Sittler, A., Lurz, R., Lueder, G., Priller, J., Lehrach, H., Hayer-Hartl, M.K., Hartl, F.U., and Wanker, E.E. (2001). *Hum. Mol. Genet.* 10, 1307–1315.
- Walsh, D.M., Klyubin, I., Fadeeva, J.V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., and Selkoe, D.J. (2002). *Nature* 416, 535–539.
- Warrick, J.M., Chan, H.Y., Gray-Board, G.L., Chai, Y., Paulson, H.L., and Bonini, N.M. (1999). *Nat. Genet.* 23, 425–428.
- Yamamoto, A., Lucas, J.J., and Hen, R. (2000). *Cell* 101, 57–66.
- Zoghbi, H.Y., and Orr, H.T. (2000). *Annu. Rev. Neurosci.* 23, 217–247.