REVIEW: BIOMEDICINE

Toxic Proteins in Neurodegenerative Disease

J. Paul Taylor,[1]* John Hardy,[2] Kenneth H. Fischbeck[1]

A broad range of neurodegenerative disorders is characterized by neuronal damage that may be caused by toxic, aggregation-prone proteins. As genes are identified for these disorders and cell culture and animal models are developed, it has become clear that a major effect of mutations in these genes is the abnormal processing and accumulation of misfolded protein in neuronal inclusions and plaques. Increased understanding of the cellular mechanisms for disposal of abnormal proteins and of the effects of toxic protein accumulation on neuronal survival may allow the development of rational, effective treatment for these disorders.

Neurodegenerative disorders as diverse as Alzheimer's disease, Parkinson's disease, prion diseases, Huntington's disease, frontotemporal dementia, and motor neuron disease all share a conspicuous common feature—aggregation and deposition of abnormal protein (Table 1). Expression of mutant proteins in transgenic animal models recapitulates features of these diseases (*1*). Neurons are particularly vulnerable to the toxic effects of mutant or misfolded protein. The common characteristics of these neurodegenerative disorders suggest parallel approaches to treatment, based on an understanding of the normal cellular mechanisms for disposing of unwanted and potentially noxious proteins.

Correct folding requires proteins to assume one particular structure from a constellation of possible but incorrect conformations. The failure of polypeptides to adopt their proper structure is a major threat to cell function and viability. Consequently, elaborate systems have evolved to protect cells from the deleterious effects of misfolded proteins. The first line of defense against misfolded protein is the molecular chaperones, which associate with nascent polypeptides as they emerge from the ribosome, promoting correct folding and preventing harmful interactions. A large fraction of newly translated proteins nonetheless fails to fold correctly, generating a substantial burden of defective polypeptide (*2*). These proteins are degraded primarily by the ubiquitin-proteasome system (UPS) (Fig. 1), a multicomponent system that identifies and degrades unwanted proteins (*3*). In addition to its role in clearing defective proteins, the UPS carries out selective degradation of many short-lived normal proteins, thereby contributing to the regulation of numerous cellular processes. Failure to detect and eliminate misfolded proteins may contribute to the pathogenesis of neurodegenerative disease. Conversely, it has been suggested that the UPS itself may be a target for toxic proteins (*4*).

Under some circumstances, misfolded proteins may evade the quality control systems designed to promote correct folding and eliminate faulty proteins. When they accumulate in sufficient quantity, misfolded proteins are prone to aggregation. Insoluble aggregates of disease-related proteins may be deposited in microscopically visible inclusions or plaques, the characteristics of which are often disease specific (Fig. 2). It has been widely assumed that the formation of intracellular inclusions is a passive process driven by mass-action chemistry, with self-assembly of misfolded monomers into a single growing aggregate. However, this assumption has been challenged by evidence that some intracellular inclusions are formed as part of a physiological response to excess misfolded protein. For example, some mutant proteins are delivered to inclusion bodies by dynein-dependent retrograde transport on microtubules (*5*). These actively formed inclusions have been designated "aggresomes." Mutant superoxide dismutase, found in some patients with familial amyotrophic lateral sclerosis, and polyglutamine-containing protein, such as generated in Huntington's disease, have been shown to form aggresomes in vitro (*4*, *6*). Inclusion formation by some mutant proteins is regulated by corticosteroids and by the activity of the stress kinase MEKK1, providing further evidence of an active process (*7*, *8*).

It has been suggested that in some circumstances inclusions may serve a protective role (*9*). This is supported by experimental models of polyglutamine disease, in which dissociation of inclusion formation from neuronal toxicity has been observed (*10*, *11*). Moreover, interruption of inclusion formation by mutant polyglutamine results in enhanced toxicity, suggesting that inclusion formation may be a mechanism to assist in the clearance of misfolded, noxious proteins (*12*). However, a protective role for inclusions does not preclude the possibility that the inherent aggregability of a mutant protein, i.e., the ten-

Table 1. Features of neurodegenerative disorders characterized by aggregation and deposition of abnormal protein.

Disease	Protein deposits	Toxic protein	Disease genes	Risk factor
Alzheimer's disease	Extracellular plaques	Aβ	APP* Presenilin 1† Presenilin 2†	apoE4 allele
	Intracellular tangles	tau		
Parkinson's disease	Lewy bodies	α-Synuclein	α-Synuclein* Parkin† UCHL1†	tau linkage
Prion disease	Prion plaque	PrP^{Sc}	PRNP*	Homozygosity at prion codon 129
Polyglutamine disease	Nuclear and cytoplasmic inclusions	Polyglutamine-containing proteins	9 different genes with CAG repeat expansion*	
Tauopathy	Cytoplasmic tangles	tau	tau*	tau linkage
Familial amyotrophic lateral sclerosis	Bunina bodies	SOD1	SOD1*	

*Pathogenic mutations are associated with a toxic gain of function. †Pathogenic mutations are associated with a loss of function.

[1]Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, [2]Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, USA.

*To whom correspondence should be addressed. E-mail: taylorjp@ninds.nih.gov

dency of the protein to aggregate, either with itself or with other proteins, is nonetheless important to its toxicity.

Evidence suggests that the neurotoxic species of β-amyloid in Alzheimer's disease are oligomers of the Aβ peptide, rather than of the mature, fibrillar form found in mature plaques (*13*). Oligomeric aggregates of a wide range of misfolded proteins may exhibit similar toxicity, perhaps caused by exposure of hydrophobic residues on their surface, permitting inappropriate interaction with a wide range of cellular targets (*14*).

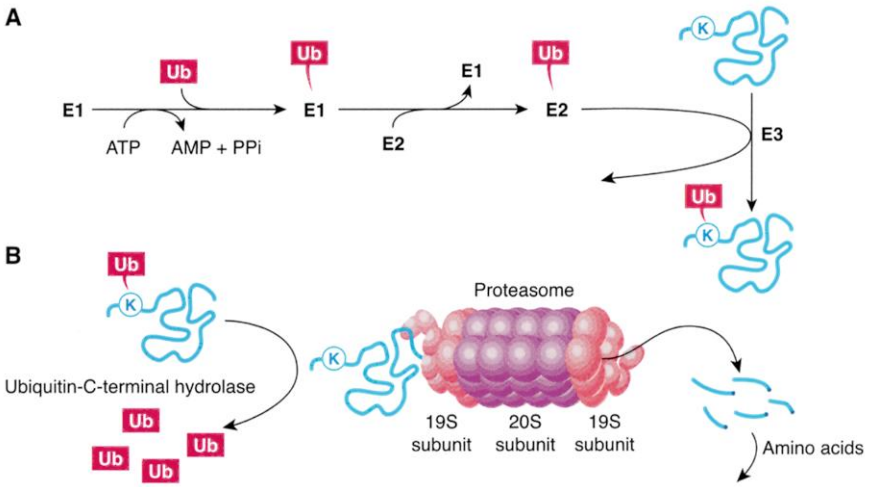

Fig. 1. The ubiquitin-proteasome system. (**A**) Proteins targeted for degradation are identified by covalent linkage to ubiquitin. Selective ubiquitination is accomplished by a series of enzymes (E1, E2, and E3) that constitute the ubiquitin ligase system. (**B**) Ubiquitinated substrates are recognized, unfolded, and degraded in an energy-dependent manner by the proteasome.

Alzheimer's Disease

Alzheimer's disease is the most common neurodegenerative disease, directly affecting about 2 million Americans. It is characterized by the presence of two lesions: the plaque, an extracellular lesion made up largely of the β-amyloid (Aβ) peptide, and the tangle, an intracellular lesion made up largely of the cytoskeletal protein tau. Although it is predominantly a disease of late life, there are families in which Alzheimer's disease is inherited as an autosomal dominant disorder of mid-life. Three genes have been implicated in this form of the disease: the amyloid precursor protein (APP) gene (*15*), which encodes the Aβ peptide; and the presenilin protein genes (PS1 and PS2), which encode transmembrane proteins (*16*, *17*).

Metabolism of APP generates a variety of Aβ species, predominantly a 40–amino acid peptide, $Aβ_{1-40}$, with a smaller amount of a 42–amino acid peptide, $Aβ_{1-42}$. This latter form of the peptide is more prone to forming amyloid deposits. Mutations in all three pathogenic genes alter the processing of APP such that a more amyloidogenic species of Aβ is produced (*18*). Although the precise function of the presenilins is still the subject of debate, it is clear from gene ablation experiments that presenilins are intimately involved in the COOH-terminal cleavage of Aβ (*19*), and the simplest explanation of the effects of presenilin mutations on APP processing is that they lead to an incomplete loss of function of the complex that processes APP (*20*, *21*).

The implication of these findings is that the process of Aβ deposition is intimately connected to the initiation of Alzheimer pathogenesis and that all the other features of the disease—the tangles and the cell and synapse loss—are secondary to this initiation; this is the amyloid cascade hypothesis for Alzheimer's disease (*22*). If this hypothesis is correct, then other genetic or environmental factors that promote Aβ deposition are likely to predispose to the disease, and seeking treatments that prevent this deposition is a rational route to therapy. The only gene confirmed to confer increased risk for typical, late-onset Alzheimer's disease is the apolipoprotein E4 allele (*23*), and apolipoprotein E gene knockouts have been shown to prevent Aβ deposition (*24*), consistent with the amyloid cascade hypothesis. Other genes predisposing to Alzheimer's disease are being sought, and it seems most likely that they too act by alteration of Aβ metabolism (*25*, *26*).

These findings suggest that Aβ metabolism is the key pathway to be targeted for therapy, and there has been much progress in this arena with transgenic mice that develop plaque pathology (*27*). Immunization of these transgenic mice with Aβ results in a reduction in pathology and better performance in behavioral tests, providing evidence that Aβ-directed therapy may be clinically relevant (*28*). Immunization may not turn out to be a practical approach to therapy, but the results of these animal studies have been an important proof of principle. It should be noted, however, that the APP transgenic mice used in these studies do not show tangles or cell loss, and it will be important to retest this strategy in newer, more complete models of the disease (*29*).

Parkinson's Disease

Parkinson's disease affects about half a million individuals in the United States and previously has been considered a nongenetic disorder. However, recent data increasingly implicate genetic factors in its etiology. Two genes are clearly associated with the disease: α-synuclein (PARK1) (*30*) and parkin (PARK2) (*31*). There is evidence implicating a third, ubiquitin COOH-terminal hydrolase (PARK5) (*32*, *33*), and there are at least five other linkage loci (PARK 3, 4, 6, 7, and 8), indicating additional contributing genes (*34*–*39*). The pathological hallmark of Parkinson's disease is the deposition within dopaminergic neurons of Lewy bodies, cytoplasmic inclusions composed largely of α-synuclein.

As the work on Alzheimer's disease has suggested, when multiple genes influence a single disorder, those genes may define a pathogenic biochemical pathway. It is not yet clear what this pathway might be in Parkinson's disease. The notion that it could be a pathway involved in protein degradation (*32*) has gained ground with the observations that parkin is a ubiquitin–protein ligase (*40*) and that parkin and α-synuclein may interact (*41*). In at least one patient, mutations in parkin led to Lewy body formation as seen in sporadic Parkinson's disease (*42*). The interaction of parkin with α-synuclein may be mediated by synphilin-1 (*43*). Another pathologically relevant substrate for parkin is the unfolded form of Pael, which is found to accumulate in the brains of patients with parkin mutations (*44*). If protein degradation is the key pathogenic pathway in Parkinson's disease, one may predict that additional Parkinson's disease loci encode other proteins in this same pathway. Dopaminergic neurons may be more sensitive to the disease process than other neurons because they sustain more protein damage through oxidative stress induced by dopamine metabolism. However, work on the molecular basis of Parkinson's disease is currently less advanced than work on other neurodegenerative diseases; as additional genes are found, other pathogenic mechanisms may emerge.

Prion Diseases

The most common human prion disease is sporadic Creutzfeldt-Jacob disease (CJD). Less common are the hereditary forms, including familial CJD, Gerstmann-Straussler-Scheinker disease, and fatal familial insomnia (*45*). Prion diseases are distinct from other neurodegenerative disorders by

virtue of their transmissibility. Although they share a common molecular etiology, the prion diseases vary greatly in their clinical manifestations, which may include dementia, psychiatric disturbance, disordered movement, ataxia, and insomnia. The pathology of prion diseases shows varying degrees of spongioform vacuolation, gliosis, and neuronal loss. The one consistent pathological feature of the prion diseases is the accumulation of amyloid material that is immunopositive for prion protein (PrP), which is encoded by a single gene on the short arm of chromosome 20.

Substantial evidence now supports the contention that prions consist of an abnormal isoform of PrP (*46*). Structural analysis indicates that normal cellular PrP (designated PrP^{C}) is a soluble protein rich in α helix with little β-pleated sheet content. In contrast, PrP extracted from the brains of affected individuals (designated PrP^{Sc}) is highly aggregated and detergent insoluble. PrP^{Sc} is less rich in α helix and has a greater content of β-pleated sheet. The polypeptide chains for PrP^{C} and PrP^{Sc} are identical in amino acid composition, differing only in their three-dimensional conformation.

It is suggested that the PrP fluctuates between a native state (PrP^{C}) and a series of additional conformations, one or a set of which may self-associate to produce a stable supramolecular structure composed of misfolded PrP monomers (*46*). Thus, PrP^{Sc} may serve as a template that promotes the conversion of PrP^{C} to PrP^{Sc}. Initiation of a pathogenic self-propagating conversion reaction may be induced by exposure to a "seed" of β-sheet–rich PrP after prion inoculation, thus accounting for transmissibility. The conversion reaction may also depend on an additional, species-specific factor termed "protein X" (*47*). Alternatively, aggregation and deposition of PrP^{Sc} may be a consequence of a rare, stochastic conformational change leading to sporadic cases. Hereditary prion disease is likely a consequence of a pathogenic mutation that predisposes PrP^{C} to the PrP^{Sc} structure.

The Polyglutamine Diseases

At least nine inherited neurological disorders are caused by trinucleotide (CAG) repeat expansion, including Huntington's disease, Kennedy's disease, dentatorubro-pallidoluysian atrophy, and six forms of spinocerebellar ataxia (*48*, *49*). These are all adult-onset diseases with progressive degeneration of the nervous system that is typically fatal. The genes responsible for these diseases appear to be functionally unrelated. The only known common feature is a CAG trinucleotide repeat in each gene's coding region, resulting in a polyglutamine tract in the disease protein. In the normal population, the length of the polyglutamine tract is polymorphic, generally ranging from about 10 to 36 consecutive glutamine residues. In each of these diseases, however, expansion of the polyglutamine tract beyond the normal range results in adult-onset, slowly progressive neurodegeneration. Longer expansions correlate with earlier onset, more severe disease.

These diseases likely share a common molecular pathogenesis resulting from toxicity associated with the expanded polyglutamine tract. It is now clear that expanded polyglutamine endows the disease proteins with a dominant gain of function that is toxic to neurons. Each of the polyglutamine diseases is characterized by a different pattern of neurodegeneration and thus different clinical manifestations. The selective vulnerability of different populations of neurons in these diseases is poorly understood but likely is related to the expression pattern of each disease gene and the normal function and interactions of the disease gene product. Partial loss of function of individual disease genes, although not sufficient to cause disease, may contribute to selective neuronal vulnerability (*50*, *51*).

Several years ago, it was recognized that expanded polyglutamine forms neuronal intranuclear inclusions in animal models of the polyglutamine diseases and the central nervous system of patients with these diseases (*52*). These inclusions consist of accumulations of insoluble aggregated polyglutamine-containing fragments in association with other proteins. It has been proposed that proteins with long polyglutamine tracts misfold and aggregate as antiparallel β strands termed "polar zippers" (*53*). The correlation between the threshold polyglutamine length for aggregation in experimental systems and the CAG repeat length that leads to human disease supports the argument that self-association or aggregation of expanded polyglutamine underlies the toxic gain of function. Although in some experimental systems the toxicity of expanded polyglutamine has been dissociated from the formation of visible inclusions, the formation of insoluble molecular aggregates appears to be a consistent feature of toxicity (*9*–*12*). The observed correlation between aggregation and toxicity in the polyglutamine diseases suggests a link with the other neurodegenerative diseases characterized by deposition of abnormal protein.

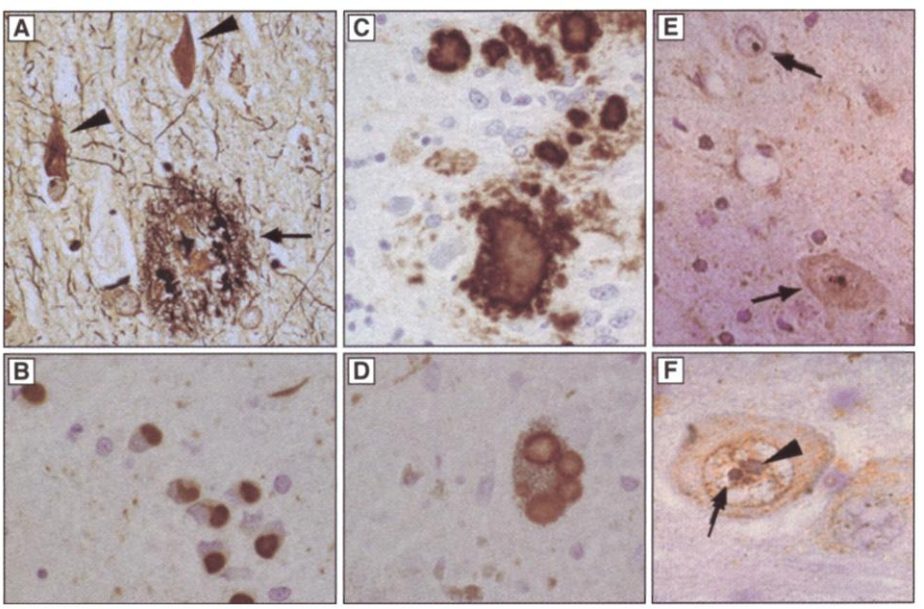

Fig. 2. Aggregation of misfolded proteins in microscopically visible inclusions or plaques in various neurodegenerative diseases. (**A**) Alzheimer's disease. Arrowhead, intracellular neurofibrillary tangles; arrow, extracellular amyloid plaque. (**B**) Fibrillar tau inclusions in Pick's disease. (**C**) PrP^{Sc} amyloid deposition in prion disease. (**D**) Multiple Lewy bodies in a nigral neuron in Parkinson's disease. (**E**) Neuronal intranuclear inclusions of mutant ataxin-3 in Machado-Joseph's disease. (**F**) Higher power micrograph of nuclear inclusion of mutant ataxin-3, demonstrating that it is distinct from the nucleolus. Magnification, ×40. [(A) to (D) courtesy of D. Dickson; (E) and (F) courtesy of H. Paulson.]

The Tauopathies

Tau has long been suspected of playing a causative role in human neurodegenerative disease, a view supported by the observation that filamentous tau inclusions are the predominant neuropathological feature of a broad range of sporadic disorders, including Pick's disease, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and the amyotrophic lateral sclerosis/parkinsonism-dementia complex. This group of disorders is collectively referred to as the "tauopathies"

(*54*). Filamentous tau deposition is also frequently observed in the brains of patients with Alzheimer's disease and prion diseases. The tau proteins are low molecular weight, microtubule-associated proteins that are abundant in axons of the central and peripheral nervous system. Encoded by a single gene on chromosome 17, multiple tau isoforms are generated by alternative splicing. The discovery that multiple mutations in the gene encoding tau are associated with frontotemporal dementia and parkinsonism (FTDP-17) provided strong evidence that abnormal forms of tau may contribute to neurodegenerative disease (*55*). Moreover, polymorphisms associated with the tau gene appear to be risk factors for sporadic CBD, PSP, and Parkinson's disease (*56*, *57*). Emerging evidence suggests that tau abnormalities associated with neurodegenerative disease impair tau splicing, favor fibrillization, and generally promote the deposition of tau aggregates.

Familial Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of upper and lower motor neurons. About 10% of ALS cases are inherited; the remainder are believed to be sporadic cases (*57*). Of the inherited cases, about 20% are caused by mutations in the gene encoding superoxide dismutase 1 (SOD1). More than 70 different pathogenic SOD1 mutations have been described; all are dominant except for the substitution of valine for alanine at position 90, which may be recessive or dominant. Neuropathologically, ALS is characterized by degeneration and loss of motor neurons and gliosis. Intracellular inclusions are found in degenerating neurons and glia (*58*). Familial ALS is characterized neuropathologically by neuronal Lewy body–like hyaline inclusions and astrocytic hyaline inclusions composed largely of mutant SOD1.

SOD1 is a copper-dependent enzyme that catalyzes the conversion of toxic superoxide radicals to hydrogen peroxide and oxygen. Mutations that impair the antioxidant function of SOD1 could lead to toxic accumulation of superoxide radicals. However, a loss-of-function mechanism for familial ALS is unlikely given that no motor neuron degeneration is seen in transgenic mice in which SOD1 expression has been eliminated. Moreover, overexpression of mutant SOD1 in transgenic mice causes motor neuron disease despite elevated SOD1 activity. This supports a role for a deleterious gain of function by the mutant protein, consistent with autosomal dominant inheritance. A pro-oxidant role for mutant SOD1 contributing to motor neuron degeneration has been proposed. This seems unlikely, however, given that ablation of the specific copper chaperone for SOD1, which deprives SOD1 of copper and eliminates enzymatic activity, has no effect on motor neuron degeneration in mutant SOD1 transgenic mice (*59*). More recently, attention has turned to the possible deleterious effects of accumulating aggregates of mutant SOD1. The notion that aggregation is related to pathogenesis is supported by the observation that murine models of mutant SOD1-mediated disease feature prominent intracellular inclusions in motor neurons, and in some cases within the astrocytes surrounding them as well (*60*). Although a variety of inclusions have been described in sporadic cases of ALS, there is scant evidence for deposition of SOD1 in these inclusions and no convincing evidence that aggregation contributes to the pathogenesis of sporadic ALS.

Therapeutic Implications

It remains unclear exactly how abnormal proteins could lead to neurodegenerative disease. Determining the mechanism of toxicity of mutant or misfolded, aggregation-prone protein remains the most important unresolved research problem for each of these diseases. Specific questions that need to be addressed include the following: (i) What is the toxic form of the protein: monomer, oligomer, or aggregate? (ii) Why are these particular proteins prone to aggregation, and what determines the particular cells affected? (iii) What are the primary targets of the toxic proteins? and (iv) Are targets shared across multiple diseases? Although the different diseases may ultimately involve different mechanisms, certain common themes have emerged, which could point the way to common therapeutic approaches.

Proposed mechanisms of toxicity include sequestration of critical factors by the abnormal protein (*61*–*63*), inhibition of the UPS (*4*), inappropriate induction of caspases and apoptosis (*64*), and inhibition by aggregates of neuron-specific functions such as axonal transport and maintenance of synaptic integrity (*65*, *66*). For example, mutant polyglutamine-containing proteins bind and deplete CREB-binding protein and other protein acetylases (*61*–*63*). That this may contribute to polyglutamine toxicity is supported by the finding that deacetylase inhibitors can mitigate the toxic effect (*67*, *68*). There is recent evidence that mutant polyglutamine can impede proteasome activity (*4*); the key role of proteasomes in maintaining cell viability indicates that this effect of the mutant protein could be important in mediating neuronal dysfunction and death. Caspase activation and apoptosis have been well demonstrated in cell culture models of polyglutamine disease, ALS, and Alzheimer's disease (*64*), and the role of apoptosis in polyglutamine disease and ALS is indicated by the mitigating effects of caspase inhibition in transgenic mouse models (*65*). Demonstration of apoptosis in patient autopsy samples is more difficult, perhaps because of the long time course and slow evolution of these disorders in humans or because different cell death pathways may be involved (*69*). Neurofilament changes and defects in axonal transport occur in ALS (*65*), and early synaptic pathology has been found in transgenic models of Alzheimer's disease (*66*). Other implicated mechanisms include excitotoxicity, mitochondrial dysfunction, oxidative stress, and the microglial inflammatory response. Indeed, downstream from the direct effects of mutant or misfolded protein in neurodegenerative diseases the mechanisms of toxicity likely diverge.

These insights into the role of toxic proteins in neurodegenerative disease suggest rational approaches to treatment. First, blocking the expression or accelerating the degradation of the toxic protein can be an effective therapy. Reducing expression of the mutant polyglutamine in transgenic mice can reverse the phenotype (*70*), and immune-mediated clearance of β-amyloid has a similar benefit in an animal model of Alzheimer's disease (*28*). Because fragments of the toxic proteins may be more pathogenic than the full-length protein and specific cellular localization may enhance toxicity, blocking proteolytic processing and intracellular transport are reasonable approaches to treatment. Other therapeutic strategies include inhibiting the tendency of the protein to aggregate (either with itself or with other proteins), up-regulating heat shock proteins that protect against the toxic effects of misfolded protein, and blocking downstream effects, such as triggers of neuronal apoptosis. Overexpression of heat shock protein can reduce the toxicity of both mutant polyglutamine and mutant α-synuclein (*71*, *72*), and caspase inhibition can reduce the toxicity of both polyglutamine and mutant SOD (*73*, *74*), indicating that therapeutic interventions of this type may apply across multiple neurodegenerative diseases. Pharmaceutical screens are now under way to identify agents that block the expression or alter the processing and aggregation of the toxic proteins responsible for neurodegenerative disease, or mitigate the harmful effects of these proteins on neuronal function and survival.

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