Molecular Neurobiology

ISSN0893-7648/00/21(1/2): 83–95/$13.25

The Cerebral Proteopathies

Neurodegenerative Disorders of Protein Conformation and Assembly

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Abstract

The abnormal assembly and deposition of specific proteins in the brain is the probable cause of most neurodegenerative disease afflicting the elderly. These "cerebral proteopathies" include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), prion diseases, and a variety of other disorders. Evidence is accumulating that the anomalous aggregation of the proteins, and not a loss of protein function, is central to the pathogenesis of these diseases. Thus, therapeutic strategies that reduce the production, accumulation, or polymerization of pathogenic proteins might be applicable to a wide range of some of the most devastating diseases of old age.

Index Entries: Aging; Alzheimer's disease; amyloid; amyotrophic lateral sclerosis; dementia; diabetes; Huntington's disease; Parkinson's disease; prion; proteopathy.

Proteopathology is Fundamental to Age-Related Neurodegenerative Disorders

By around 60 yr of age, the neuropathological harbingers of Alzheimer's disease (AD)—senile plaques and neurofibrillary tangles—begin to appear in the brain *(1,2).* Plaques and tangles originate in the abnormal assembly of specific proteins; the β-amyloid peptide (Aβ) forms the fibrillar cores of senile plaques, and the microtubule-associated protein *tau* fibrillizes into intraneuronal paired helical filaments, or neurofibrillary tangles. (Fig. 1). The prevalence of plaques and tangles increases with age *(1),* as does the risk of developing AD *(3).* Recent genetic and biochemical analyses have confirmed the long-held suspicion that the abnormal accumulation of Aβ and/or tau in the brain is central to AD and some related neurodegenerative disorders. Specific mutations in the gene encoding the precursor of Aβ (the β-amyloid precursor protein [βAPP]), and in the genes for the presenilins, produce autosomal dominant

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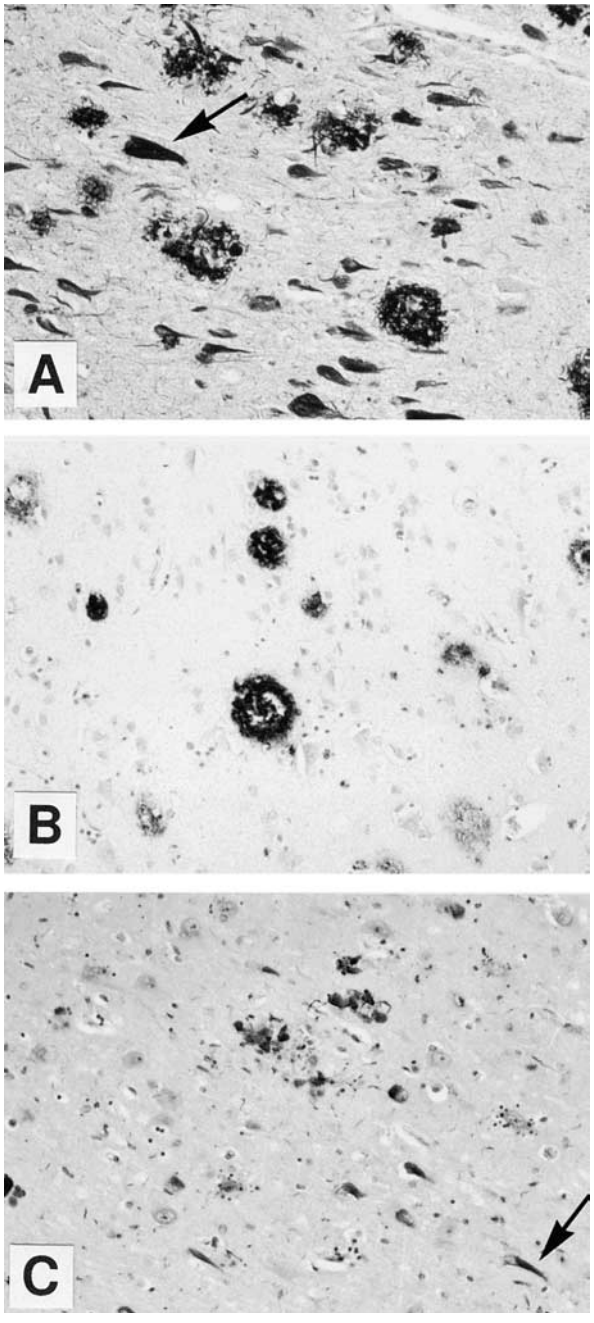

Fig. 1. The neuropathological hallmarks of AD. **(A)** Spherical accumulations of β-amyloid form the cores of senile plaques, and are distributed among neurofibrillary tangles (arrow) in this Gallyas silver-stained section of parahippocampal cortex in an Alzheimer's case; **(B)** Immunostaining with antibody 10D5 (courtesy of Dale Schenk, Elan Pharmaceuticals) detects only the β-amyloid deposits, some of which form the dense cores of classical senile plaques, and some of which are diffuse, lightly immunostained and noncongophilic. Neocortex; hematoxylin counterstain. **(C)** Ubiquitin immunoreactivity also is prominent in the Alzheimeric brain, particularly in structures exhibiting tau pathology (arrow shows a neurofibrillary tangle). Note also a senile plaque in the upper middle of panel **(C),** in which ubiquitin-positive abnormal neurites surround an unstained center. Parahippocampal cortex; hematoxylin counterstain.

AD *(4).* Persons carrying one or more apolipoprotein Eε4 alleles are at greater risk of developing AD than are noncarriers of the allele *(5,6).* Even in the nondemented elderly, senile plaques and neurofibrillary tangles are most abundant in people with one or more ε4 alleles *(1,2).* Indeed, all known genetic risk factors for AD increase the production and/or accumulation of Aβ in the brain *(7).* Although mutations in the gene for tau do not cause AD *per se,* they are linked to dementing disorders characterized by widespread neurofibrillary tangles, often in the absence of β-amyloid *(8–10).* Because <10% of AD cases are transmitted in an autosomal dominant, Mendelian fashion *(4),* the vast majority of cases have no identifiable genetic cause.

Aging is the major risk factor for AD *(3)* and other disorders of protein conformation and assembly. The steep rise in the occurrence of these diseases in the elderly appears to derive from posttranslational events, such as glycation, isomerization, phosphorylation, crosslinking, or partial denaturation of proteins *(11–15),* or possibly in the faulty transcription of DNA *(16).* The ensuing structural modifications can render proteins highly prone to self-aggregation. It is noteworthy that aggregation can be facilitated by transient, denaturing conditions such as increased temperature *(11)* and changes in pH *(17).* Once initiated, abnormal protein polymerization becomes self-perpetuating, eventually leading to disruption of cellular function and disease-specific clinical symptoms. We refer to diseases of abnormal protein conformation and assembly as the "proteopathies" (*18;* Table 1). Protein misfolding

Table 1
Cerbral Proteopathies

Disease	Major Protein
Alzheimer's Disease	β-peptide (Aβ) 4R, 3R tau
Cerebral Aβ Angiopathy	β-peptide (Aβ)
Multiple System Tauopathy (familial)	4 R tau
Progressive Supranuclear Palsy	4 R tau
Corticobasal Degeneration	4 R tau
Pick's Disease	3 R tau
Diffuse Lewy Body Disease	α-synuclein
Parkinson's Disease	α-synuclein
Multiple System Atrophy	α-synuclein
Amyotrophic Lateral Sclerosis (ALS)	α-synuclein
Familial ALS	SOD1 mutants
Triplet Repeat Disorders (HD, etc.)	polyglutamine inserts
Prion diseases (CJD, etc.)	prion protein
Familial British Dementia	ABri
Familial Danish Dementia	ADan
Familial Encephalopathy w/ Neuroserpin Inclusion Bodies (FENIB)	neuroserpin
Familial Cerebral Hemorrhage w/ Amyloidosis (Icelandic)	cystatin C
Familial Amyloidotic Neuropathy	transthyretin

and aggregation are integrally associated with virtually all known chronic human neurodegenerative diseases *(19–21),* and may contribute to neuronal death in acute brain injury as well *(22).* Unraveling the pathogenic mechanisms shared by the proteopathies may yield effective therapies for some of the most debilitating disorders of old age.

Amyloidoses are One Type of Proteopathy

Proteins usually fold rapidly into their normal, native conformations in vivo, but under pathogenic conditions, such as amino acid substitutions and denaturation, proteins can misfold and aggregate into insoluble clumps *(11,23).* Proteins vary in their disposition to self-aggregate, and those with the strongest inclination to do so are prime disease-causing suspects. Often, but not always, pathogenic proteins aggregate into conformations rich in intramolecular β-sheet *(24).* Fibrillar protein accumulations with a β-pleated sheet structure are birefringent after staining with the dye Congo Red, and are referred to generically as "amyloid" (Fig. 2), an adjective *cum* noun that originated from the early belief that the protein deposits might actually be starch *(see 25).* Amyloid can form in a number of organs from a variety of proteins that are unified mainly by their tendency to self aggregate into β-pleated sheets. Although amyloid is an indicator of various disease processes in humans, the β-sheet secondary structure is not inherently pathological, and even has utility elsewhere in nature, for example in the formation of silk fibers ["The amyloid fibril [is] the abortive effort of the vertebrate to rival the silkworm…" *(24)*].

Accumulations of amyloid are an obvious manifestation of certain proteopathies *(23–30),* and have instigated the determination of the amino acid and genetic sequences of most amyloid precursor proteins. Although β-amyloid and diffuse Aβ deposits are salient features of AD, the presence of amyloid plaques correlates rather poorly with dementia *(31).* This weak correlation has suggested to some that β-amyloid *per se* is not critical to AD. However, growing evidence indicates that the cytotoxic culprits in neurodegenerative diseases may be prefibrillar forms of the proteins, i.e., soluble species such as oligomers or protofibrils that are not readily detectable histologically *(20,32–36).* (A cytotoxic, nonfibrillar intermediate species of islet amyloid polypeptide may also contribute to the death of pancreatic β-cells in type II diabetes *[37–39]*). Amyloid (that is, the insoluble congophilic deposits) may simply be a terminal or ancillary stage of the proteopathic cascade that contributes only marginally to the clinical phenotype of neurodegenerative diseases. Moreover, a number of proteopathies never manifest the classical features of amyloid, for example the

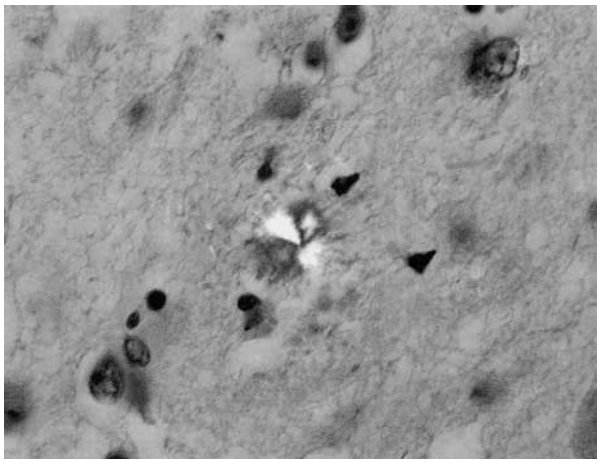

Fig. 2. A Congo Red-stained senile plaque in the center of the field exhibits characteristic Maltese-cruciform birefringence with crossed polarizing filters. This tinctorial/optical peculiarity is one of the defining characteristics of classical amyloid deposits. Hematoxylin counterstain.

triplet-repeat disorders, amyotrophic lateral sclerosis (ALS), and the synucleinopathies, and some maladies, such as the prion diseases, display amyloid inconsistently. What all of these diseases share, however, is abnormal protein assembly as a probable pathogenic process. The proteopathies therefore include all diseases of pathological protein misfolding and/or self-aggregation, regardless of whether classical amyloid is present. The proteopathy concept accommodates the entire cascade of molecular events that bring about the phenotype of the disorders, rather than singling out the amyloid deposits or inclusion bodies *per se* as putative causative lesions. As indicated earlier, the proteopathies are not restricted to the brain, but can include systemic disorders in which the pathogenic process is similar *(30,39–41).*

Protein Self-Aggregation, Not Loss of Function, Causes Disease

What is the evidence that the abnormal assembly of proteins is necessary and sufficient for brain disease? Some of the most compelling hints to date come from uncommon disorders which, though phenotypically heterogeneous, all are characterized by protein self-aggregation: familial British dementia, familial encephalopathy with neuroserpin inclusion bodies (FENIB), triplet-repeat disorders such as Huntington's disease (HD), and prion diseases.

Familial British Dementia

Familial British dementia is a very rare, autosomal dominant disease of motor function and cognition that was first described in the 1930s *(42).* The disorder usually becomes manifest around the fifth decade of life. Pathologically, the brain and spinal cord are beset by large numbers of neurofibrillary tangles, vascular amyloid deposits, and non-neuritic senile plaques. Significantly, the amyloid deposits do not contain Aβ but, rather, a previously unidentified, 4-kDa peptide termed "ABri" (*43;* a Danish variant of the peptide called "ADan" has recently been discovered *[44]*). ABri derives from a single, membrane-spanning precursor protein by an unusual mechanism: a single nucleotide change converts the stop codon to an arginine codon, extending the precursor protein from 266 to 277 residues. The errant cleavage product ABri consequently contains 23 amino acids from the normal precursor, the intrusive arginine, and 11 intron-derived amino acids. Because of its unusual origin, the peptide has no known homology in nature. Nevertheless, one salient feature that ABri shares with Aβ and other pathogenic proteins is a strong tendency to self-assemble (in this instance, ultimately forming amyloid). Both familial British dementia and AD can be linked to genetic mutations that enhance the amyloidogenicity of particular proteins. Pathologically, both diseases exhibit abundant amyloid deposits in the brain (as well as neurofibrillary tangles), but their distinct clinical phenotypes reflect disease-specific anatomical distributions of the lesions.

Familial Encephalopathy with Neuroserpin Inclusion Bodies

FENIB is another uncommon, autosomal dominant neurological disorder. The age of onset is between the second and fifth decades of life; afflicted patients undergo progressive, debilitating cognitive and behavioral decline and, in one family, epilepsy *(45).* The most notable neuropathological finding is eosinophilic inclusion bodies in neurons of the deep layers of the cerebral cortex, in the substantia nigra, and in other subcortical nuclei *(45,46).* These inclusions recently were found to contain loop-sheet polymers of a mutant form of the neuronal serine protease inhibitor neuroserpin *(45).* How this protein configuration causes cerebral disease is not yet known. However, compelling evidence that the abnormal polymerization of neuroserpin is key to the pathogenesis of FENIB comes from a liver disease involving the aggregation of a different serpin, α_1-antitrypsin. Loop-sheet polymerization of α_1-antitrypsin produces hepatocytic inclusions and cirrhosis *(47).* Significantly, liver disease does not develop in human "null" mutants with suppressed α_1-antitrypsin synthesis, indicating that hepatocytic pathology in α_1-antitrypsin-associated cirrhosis is owing to abnormal polymerization and deposition of the enzyme, and not to loss of function *(45).* What is more, neuroserpin inclusions are not "amyloid" by tinctorial or ultrastructural criteria. FENIB thus differs in many ways from familial British dementia, AD, and other proteopathies; however, in FENIB, as in virtually every age-related neurodegenerative disease, specific proteins precipitate as aggregates in the brain. Mutations that increase the concentration of the proteins and/or their inclination to self-aggregate raise the odds that the disease will appear, particularly with advancing age.

Triplet-Repeat Disorders

The triplet-repeat disorders are autosomal dominant, neurodegenerative illnesses that emerge in persons with abnormally expanded trinucleotide repeats. In the most common forms, the result is the production of proteins whose cleavage products are particularly inclined to self-aggregate (*see 19* for review). Many of the known triplet-repeat disorders, including HD, involve multiple, consecutive cytosine-adenine-guanine (CAG) codons that are translated into polyglutamine stretches in different proteins. The longer the pathogenic expansion of amino acids, the greater the proteins' propensity to self-aggregate, and the more aggressive the disease; transgenic animals and cultured cells expressing pathogenic polyglutamine expansions mimic the neuronal degeneration seen in the corresponding human diseases, and the cytotoxicity is directly related to the repeat expansion-length within the protein *(48).* An unusual pathologic feature of the polyglutamine diseases (and their transgenic animal models) is the presence of intranuclear inclusions that are immunoreactive for ubiquitin *(19,49).* Histologically detectable aggregates of mutant huntingtin generally precede the onset of behavioral symptoms and neuronal degeneration in a transgenic mouse model of HD *(50,51).* Intriguingly, in a transgenic model of Machado-Joseph triplet-repeat disease, cleaved MJD1a protein containing the expanded polyglutamines is more injurious to neurons than is the full-length protein, suggesting that brain region-specific cleavage events may determine the localized expression of pathology *(19,48).* Clinicopathological, genetic, and experimental evidence strongly suggests that the self-aggregation of the affected proteins, and not a loss of function, drives the pathogenesis of the polyglutamine disorders.

Prion Diseases

The prion diseases in humans include Creutzfeldt-Jakob disease (CJD), Kuru, Gerstmann-Straeussler-Scheinker disease, and fatal familial insomnia; there are also several nonhuman prion diseases, the best known being scrapie and bovine spongiform encephalopathy (BSE, the possible source of variant CJD in Britain and parts of continental Europe *[52]*). The key histopathological changes in prion

disease are cerebral spongiform change and plaque-like PrP immunoreactivity (Fig. 3), with neuronal loss and gliosis *(53),* although neither amyloid (i.e., congophilic) plaques nor spongiform change appears to be obligatory *(54).* It is now generally accepted that these spongiform encephalopathies are caused by the aggregation of misconformed prions, i.e., proteinaceous particles (PrP) that can, under the proper conditions, transmit disease from one individual to another *(54–56; see below).* Like other proteopathic agents, pathogenic PrP (PrP^{Sc}) has a strong proclivity for self-aggregation *(57).* The amplification of pathogenic prions in the nervous system apparently requires the conformational conversion of normal PrP (PrP^{C})—which is produced routinely in neurons and other mammalian cells—to PrP^{Sc} *(11,57).* Significantly, a number of PrP gene mutations have been discovered that cause autosomal dominant neurodegenerative disease *(53),* presumably by promoting the conversion of some PrP^{C} molecules to PrP^{Sc}. Although the molecular events involved may differ in the details, the abnormal assembly of prions as a general pathogenic mechanism *(57)* is similar to that postulated for other proteopathies *(18–21).*

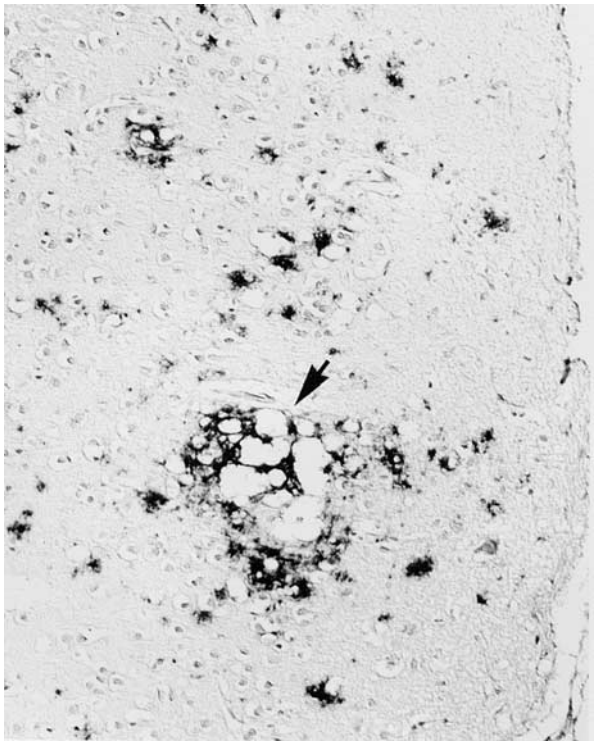

Fig. 3. Prion protein (PrP)-immunoreactivity and spongiform change (arrow) in the neocortex of a person who had died of Creutzfeldt-Jakob disease. Hematoxylin counterstain. Slide courtesy of Professor Rolf Warzok, University of Greifswald.

Seeding Accelerates Proteopathy

The formation of pathogenic protein assemblies can be impelled by seeding, or nucleation-dependent polymerization *(58).* Indeed, seeding may promote the pathogenesis of many, if not all, neurodegenerative proteopathies *(55,58,59),* as well as systemic amyloidoses *(60).* The seed is thought to be an abnormal structural variant of a protein (usually oligomeric or multimeric) that provides a template or assembly site for the organization of like molecules into ordered aggregates *(55,59,61).* Infectious prions appear to be PrP^{Sc} seeds that catalyze the transformation of PrP^{C} into the aggregation-prone PrP^{Sc} *(57);* the spontaneous (or sporadic) prion diseases arise in the rare instances when a chance seed is formed endogenously. "Transmissibility," then, may be largely a function of the facility with which exogenous seeds stimulate the conversion and/or aggregation of endogenous proteins, a role at which prions are especially adept. The species barriers in the transmission of prion diseases are thought to be owing to differences in the amino acid sequences between PrP^{Sc} and the host PrP^{C} *(54).*

There have been relatively few attempts to seed other cerebral proteopathies in vivo, and early attempts to transmit Alzheimeric pathology met with little success *(62–64).* Unlike prion diseases, which can be transmitted (within the limitations imposed by species differences) with a fair degree of fidelity, the full behavioral and pathological spectrum of AD's

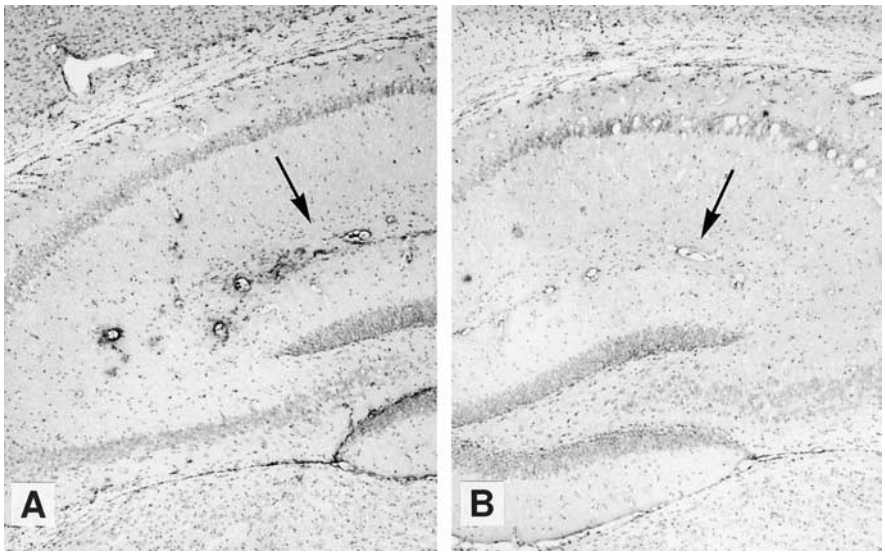

Fig. 4. Hippocampal Aβ-immunostaining in a Tg2576 mouse that had been unilaterally injected 5 mo earlier with dilute Alzheimeric neocortical extract. Panel **(A)** shows the injected hippocampus, and **(B)** shows the contralateral hippocampus in the same tissue section. Note the intense, mostly diffuse Aβ-immunoreactivity in the infused hippocampus, and the paucity of immunoreactivity in the noninjected hippocampus. The hippocampal fissure is indicated by arrows. Hematoxylin counterstain *(66).*

still has not been conveyed experimentally to any nonhuman species. However, there is growing evidence that β-amyloid pathology can be exogenously induced by diseased tissue. Marmosets inoculated intracerebrally with crude, 10% tissue homogenates from AD brains developed β-amyloid plaques and cerebrovascular amyloid after approx 6 yr *(65).* Recently, β-amyloid pathology was induced within a period of 1–5 mo in transgenic mice overexpressing human βAPP *(66).* In this latter study, Alzheimeric cortical homogenates were depleted of plaque cores, blood vessels, and tissue debris by centrifugation; the supernatant was diluted to 1% and injected unilaterally into the hippocampus and neocortex of 3-mo-old Tg2576 *(67)* βAPP-transgenic mice. Up until 4 wk post-injection, there was no Aβ-immunoreactivity in the brains of the transgenic mice; by 5 mo post-injection, however, the injected hemisphere was rife with Aβ deposits (Fig. 4). Immunoreactive Aβ was never found in injected, nontransgenic littermate control animals, and the uninjected hemisphere of transgenic mice contained only 10% of the immunoreactive Aβ seen in the injected hemisphere. The nature of the seed in the AD extracts remains unknown. Furthermore, the mice did not have other pathological or behavioral characteristics of AD at 8 mo of age—there was no evidence of behavioral dysfunction, neurofibrillary pathology or neuronal loss. However, the results show that Aβ deposition can be exogenously seeded by diseased human brain extracts, and indicate possible commonalities in the in vivo mechanisms by which proteopathology amplifies in diverse diseases.

Treatment of the Proteopathies

The concept that disease can be caused by the excessive accumulation of proteins is not new, but the realization that morbidity can result from components of the proteopathic cascade other than the manifest tissue deposits is gaining momentum. Indeed, proteopathic processes may lie at the root of a surprising

variety of chronic diseases. What are the prospects for effective therapies for the proteopathies? In fact, protein-targeting strategies are already being investigated. In 1994, a monoclonal antibody (MAb) to Aβ was first reported to bind selectively to β-amyloid deposits in the brains of living nonhuman primates, suggesting that Aβ antibodies might be useful in the diagnosis and treatment of AD (*68;* Fig. 5). However, to facilitate their entry into the brain, the antibodies were delivered directly into the CSF. Recently, Dale Schenk and colleagues took the antibody approach to therapy a significant step further, showing that immunization with the Aβ peptide can dramatically reduce the cerebral Aβ load in βAPP-transgenic mice *(69).* More recently, this group found that passive immunization with exogenous antibodies can stimulate the removal of cerebral amyloid deposits by macrophages *(70).* Obviously, similar strategies will be applied to other proteopathies. Whether immunization will be a practical and effective treatment/preventive remains an open question, but the results of clinical trials of Aβ-immunization in AD are expected within the next few years.

Fig. 5. Neocortical β-amyloid deposits that have been selectively labeled in vivo by MAb 10D5 infused into the cerebrospinal fluid of an aged rhesus monkey. Immunoreactivity was confined to the effective diffusion distance of the antibody into the brain parenchyma, and decreased in intensity with increasing distance from the cortical surface (upper left). This study provided the first evidence that MAbs might be used to target cerebral amyloid deposits in the living brain for diagnostic and therapeutic purposes *(68).*

Polymerization tends not to occur below a critical concentration of protein *(59);* once initiated, Aβ-fibril extension is linearly dependent on peptide concentration *(71).* The form of the protein that injures cells and tissues is not yet known with certainty, and may differ among the proteopathies. Furthermore, whether toxicity is owing to intracellular or extracellular polymerization remains unclear. However, reducing the production of the pathogenic polypeptide is an attractive strategy for treating some proteopathies. For example, it now appears that the long-sought proteases that liberate Aβ from its precursor protein have finally been identified *(72–74),* although the biology of these secretases is far from understood. Secretase inhibitors currently are leading candidates for preventive or disease-modifying AD therapy. One attraction of selectively inhibiting protein production is that the entire proteopathic cascade will be impeded (rather than a later step in the aggregation process), increasing the likelihood of averting the disease-causing state of the protein.

Another therapeutic option is to develop small molecules that block the aggregation of pathogenic proteins into toxic moieties *(75).* Studies with larger molecules suggest that this strategy is possible; for example, peptides can be designed that will inhibit amyloid fibrillogenesis *(76,77),* and existing aggregates can be dissociated in vitro by MAbs *(78).* Congo Red is a highly effective inhibitor of amyloid fibrillogenesis in vitro *(see 79),* and also inhibits huntingtin aggregation *(80).* In fact, Congo Red inhibits the aggregation of many different amyloidogenic proteins, regardless of their primary amino acid sequence. This finding is not entirely unexpected, inasmuch as: 1) amyloid is defined by staining and birefringence with Congo Red; and 2) X-ray diffraction analyses show that amyloid fibrils of heterogeneous origins have common

structural features, even in the absence of primary or tertiary structural homology among the amyloidogenic peptides *(17,81).* A possible advantage of aggregation inhibition, then, is that a single active compound with the appropriate pharmacokinetic profile might effectively treat multiple proteopathies.

The misfolding and aberrant polymerization of proteins in vivo does not occur in isolation; numerous auxiliary factors participate in the process *(60),* but their validity and practicality as therapeutic targets are not yet established. For example, osmotic, thermal, and oxidative stress all can disrupt the folding of proteins into their normal three-dimensional configurations *(82),* and pH is an important regulator of protein stability *(17).* An interesting array of substances are associated with protein deposits in vivo and appear to variously modulate their assembly and/or persistence. Serum amyloid P component and heparan sulfate proteoglycan, among other proteins, are bound to all types of amyloid *(39,60).* Serum amyloid A deposition is delayed in mice deficient in serum amyloid P *(83).* In addition, compounds that block the interaction of heparan sulfate proteoglycan with amyloid can inhibit amyloidogenesis *(60).* It should be noted that some molecular chaperones may inhibit the pathogenic process. Increased levels of the 70-kDa heat-shock protein (HSP70), a chaperone protein for Cu/Zn-superoxide dismutase, (SOD), diminish the formation of SOD aggregates and increase neuronal survival in vitro *(84).* Finally, tissue transglutaminase modifies proteins by transamidation of specific polypeptide-bound glutamines. The resulting bonds covalently cross-link and polymerize peptides into high molecular weight, more proteolysis-resistant protein aggregates, and may participate in the formation and/or stabilization of amyloid deposits and paired helical filaments *(14,15).* Inhibitors of transglutaminase reduce polyglutamine aggregate formation and associated apoptosis in cultured COS-7 cells *(85),* and could find broad use as anti-proteopathic agents.

Enhancing the cell's ability to subdue aggregation-prone proteins also would be expected to reduce pathology. The endoplasmic reticulum (ER) orchestrates the production and folding of myriad proteins; those that are not properly folded are eliminated by endoplasmic reticulum-associated degradation *(86).* If overwhelmed by unfolded proteins, as can happen with an overabundance of misfolded mutant proteins, the ER stress response is activated, which can be toxic to the cell. Enhancing the ability of the ER to deal with misfolded proteins, or inhibiting the endoplasmic reticulum stress response, could diminish proteopathic cytotoxicity. Similarly, manipulating the ability of proteasomes and/or aggresomes to cope with misfolded proteins *(82)* also is a theoretical therapeutic option, as is the augmentation of particular catabolic pathways *(87).*

Ubiquitination marks proteins that are destined for intracellular degradation, and is a regular component of proteopathic lesions of the nervous system (*21;* Fig 1C). In transfected, cultured striatal neurons, reduction of protein ubiquitination increased cellular toxicity even though it eliminated nuclear huntingtin aggregates *(88);* as with the amyloidoses, the results suggest that the aggregates themselves are relatively benign manifestations of a more insidious cytopathic process that is executed by pre-fibrillar structures. Interestingly, the familial Parkinson's disease (PD) gene product parkin turns out to be a ubiquitin protein ligase; disease-causing mutations in this enzyme reduce its activity, leading to a toxic increase in (as yet unidentified) cellular proteins *(89).*

Conclusions

Pathological, genetic, and biochemical data implicate the abnormal assembly of specific proteins in the pathophysiology of a remarkable array of neurodegenerative diseases. Precisely how the proteopathic cascade induces clinical disease, and why the disorders exhibit such remarkable phenotypic variability, remain to be clarified. Our growing understanding of the proteopathies suggests several therapeutic goals: 1) reduce the amount of pathogenic protein; 2) inhibit the binding of

proteins to themselves or to pathological chaperones; or 3) enhance cellular protein-clearance mechanisms. Recognition of the commonalities among the proteopathies should stimulate communication across seemingly disparate research areas, and thereby accelerate the discovery of preventives or treatments for a broad spectrum of neurodegenerative diseases.

Acknowledgments

We thank Dr. Margaret Walker for helpful comments and Prof. Rolf Warzok for excellent histological preparations. Special thanks also to Richard Rhodes, whose book *Deadly Feasts* awakened our dormant experimental plans to study the in vivo induction of cerebral β-amyloidosis.

References

1. Warzok R. W., Kessler C., Apel G., Schwarz A., Egensperger R., Schreiber D., et al. (1998) Apolipoprotein E4 promotes incipient Alzheimer pathology in the elderly. *Alzheimer Dis. Assoc. Disord.* **12,** 33–39.
2. Walker L. C., Pahnke J., Madauss M., Vogelgesang S., Pahnke A., Herbst E. W., et al. (2000) Apolipoprotein E4 promotes the early deposition of Aβ42 and then Aβ40 in the elderly. *Acta Neuropathol.* **100,** 36–42.
3. Kawas C. H. and Katzman R. (1999) Epidemiology of dementia and Alzheimer disease, in *Alzheimer Disease,* 2nd ed. (Terry R. D., Katzman R., Bick K. L., and Sisodia S. S., eds.), Lippincott Williams and Wilkins, Philadelphia, pp. 95–116.
4. St. George-Hyslop P. H. (1999) Molecular genetics of Alzheimer disease, in *Alzheimer Disease,* 2nd ed. (Terry R. D., Katzman R., Bick K. L., and Sisodia S. S., eds.), Lippincott Williams and Wilkins, Philadelphia, pp. 311–326.
5. Corder E. H., Saunders A. M., Strittmatter W. J., Schmechel D. E., Gaskell P. C., Small G. W., et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261,** 921–923.
6. Poirier J., Davignon J., Bouthillier D., Kogan S., Bertrand P., and Gauthier S. (1993) Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* **342,** 697–699.
7. Selkoe D. J. (1999) Biology of β-amyloid precursor protein and the mechanism of Alzheimer disease, in *Alzheimer Disease,* 2nd ed. (Terry R. D., Katzman R., Bick K. L. and Sisodia S. S., eds.) Lippincott Williams and Wilkins, Philadelphia, pp. 293–310.
8. Hutton M., Pérez-Tur J., and Hardy, J. (1998) Genetics of Alzheimer's disease. *Essays Biochem.* **33,** 117–131.
9. Tolnay M. and Probst A. (1999) Tau protein pathology in Alzheimer's disease and related disorders. *Neuropathol. Appl. Neurobiol.* **25,** 171–187.
10. Vogelsberg-Ragaglia V. V., Trojanowski J. Q., and Lee V. M.-Y. (1999) Cell biology of tau and cytoskeletal pathology in Alzheimer disease, in *Alzheimer Disease,* 2nd ed. (Terry R. D., Katzman R., Bick K. L., and Sisodia S. S., eds), Lippincott Williams and Wilkins, Philadelphia, pp. 359–372.
11. Carrell R. W. and Lomas D. A. (1997) Conformational disease. *Lancet* **350,** 134–138.
12. Münch G., Cunningham A. M., Riederer P., and Braak E. (1998) Advanced glycation endproducts are associated with Hirano bodies in Alzheimer's disease. *Brain Res.* **796,** 307–310.
13. Vincent I., Zheng J. -H., Dickson D. W., Kress Y., and Davies P. (1998) Mitotic phosphoepitopes precede paired helical filaments in Alzheimer's disease. *Neurobiol. Aging* **19,** 287–296.
14. Norlund M. A., Lee J. M., Zainelli G. M., and Muma N. A. (1999) Elevated transglutaminase-induced bonds in PHF tau in Alzheimer's disease. *Brain Res.* **851,** 154–163.
15. Lesort M., Tucholski J., Miller M. L., and Johnson G. V. (2000) Tissue transglutaminase: a possible role in neurodegenerative diseases. *Prog. Neurobiol.* **61,** 439–463.
16. van Leeuwen F. W., Burbach J. P. H., and Hol E. M. (1998) Mutations in RNA: a first example of molecular misreading in Alzheimer's disease. *Trends Neurosci.* **21,** 331–335.
17. Kelly J. W. (1998) The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. *Curr. Opin. Struct. Biol.* **8,** 101–106.
18. Walker L. C. and LeVine III H. (2000) The cerebral proteopathies. *Neurobiol. Aging* **21,** 559–561.
19. Kakizuka A. (1998) Protein precipitation: a common etiology in neurodegenerative disorders? *Trends Genet.* **14,** 396–402.
20. Koo E. H., Lansbury Jr. P. T., and Kelly J. W. (1999) Amyloid diseases: abnormal protein

aggregation in neurodegeneration. *Proc. Natl. Acad. Sci. USA* **96,** 9989,9990.
21. Tran P. B. and Miller R. J. (1999) Aggregates in neurodegenerative disease: crowds and power? *Trends Neurosci.* **22,** 194–197.
22. Hu B. R., Martone M. E., Jones Y. Z., and Liu C. L. (2000) Protein aggregation after transient cerebral ischemia. *J. Neurosci.* **20,** 3191–3199.
23. Dobson C. M. (1999) Protein misfolding, evolution and disease. *Trends Biol. Sci.* **24,** 329–332.
24. Glenner G. G. (1980) Amyloid deposits and amyloidosis: the beta-fibrilloses (Parts 1 and 2). *N. Engl. J. Med.* **302,** 1283–1292; 1333–1343.
25. Schwartz P. (1972) Ueber den Ursprung der Bezeichnung "Amyloidose". Ein Vorschlag zu ihrer Ersetzung. *Zentralbl. Algem. Pathol.* **115,** 453–462.
26. Cohen A. S. (1967) Amyloidosis. *N. Engl. J. Med.* **277,** 522–530.
27. Ghiso J., Wisniewski T., and Frangione B. (1994) Unifying features of systemic and cerebral amyloidosis. *Mol. Neurobiol.* **8,** 49–64.
28. Solomon A. and Weiss D. T. (1995) Protein and host factors implicated in the pathogenesis of light chain amyloidosis (AL amyloidosis). *Amyloid: Intl. J. Exp. Clin. Invest.* **2,** 269–279.
29. Kisilevsky R. and Fraser P. E. (1997) ABeta amyloidogenesis: unique, or variation on a systemic theme? *Crit. Rev. Biochem. Mol. Biol.* **32,** 361–404.
30. Kahn S. E., Andrikopoulos S., and Verchere C. B. (1999) Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes* **48,** 241–253.
31. Terry R. D. (1996) The pathogenesis of Alzheimer disease: an alternative to the amyloid hypothesis. *J. Neuropathol. Exp. Neurol.* **55,** 1023–1025.
32. Lambert M. P., Barlow A. K., Chromy B. A., Edwards C., Freed R., Liosatos M., et al. (1998) Diffusible, nonfibrillar ligands derived from Abetal-42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. USA* **95,** 6448–6453.
33. Lue L.-F., Kuo Y.-M., Roher A. E., Brachova L., Shen Y., Sue L., Beach T., Kurth J. H., Rydel R. E., and Rogers J. (1999) Soluble amyloid beta-peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am. J. Pathol.* **155,** 853–862.
34. McLean C. A., Cherny R. A., Fraser F. W., Fuller S. J., Smith M. J., Beyreuther K., et al. (1999) Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* **46,** 860–866.
35. Mucke L., Masliah E., Yu G. -Q., Mallory M., Rockenstein E. M., Tatsuno G., et al. (2000) High-level neuronal expression of $A\beta_{1-42}$ in wild-type human amyloid protein precursor transgenic mice: Synaptotoxicity without plaque formation. *J. Neurosci.* **20,** 4050–4058.
36. Ward R. V., Jennings K. H., Jepras R., Neville W., Owen D. E., Hawkins J., et al. (2000) Fractionation and characterization of oligomeric, protofibrillar and fibrillar forms of β-amyloid peptide. *Biochem. J.* **348,** 137–144.
37. Clark A., Morris J. F., Scott L. A., McLay A., Foulis A. K., Bodkin N. L., and Hansen B. C. (1991) Intracellular formation of amyloid fibrils in B-cells of human insulinoma and pre-diabetic monkey islets, in *Amyloid and Amyloidosis* (Natvig J. B., ed.) Kluwer, Amsterdam, pp. 453–456.
38. Janson J., Ashley R. H., Harrison D., McIntyre S., and Butler P. C. (1999) The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. *Diabetes* **48,** 491–498.
39. Hoeppener J. W. M., Ahren B., and Lips C. J. M. (2000) Islet amyloid and type 2 diabetes mellitus. *N. Engl. J. Med.* **343,** 411–419.
40. Eaton W. A. and Hofrichter J. (1995) The biophysics of sickle cell hydroxyurea therapy. *Science* **268,** 1142,1143.
41. Westermark P. (1997) Classification of amyloid fibril proteins and their precursors: An ongoing discussion. *Amyloid: Intl. J. Exp. Clin. Invest.* **4,** 216–218.
42. Worster-Drought C., Hill T. R., and McMenemey W. H. (1933) Familial presenile dementia with spastic paralysis. *J. Neurol. Psychopathol.* **14,** 27–34.
43. Vidal R., Frangione B., Rostagno A., Mead S., Revesz T., Plant G., and Ghiso J. (1999) A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature* **399,** 776–781.
44. Vidal R. G., Revesz T., Rostagno A., Bek T., Braendgaard H., Plant G., et al. (2000) A decamer duplication in the BRI gene originates a de-novo amyloid peptide that causes dementia in a Danish kindred. *Neurobiol. Aging* **21,** S58.
45. Davis R. L., Shrimpton A. E., Holohan P. D., Bradshaw C., Feiglin D., Collins G. H., et al. (1999) Familial dementia caused by polymerization of mutant neuroserpin. *Nature* **401,** 376–379.
46. Yerby M. S., Shaw C. -M., and Watson J. M. D. (1986) Progressive dementia and epilepsy in a young adult: unusual intraneuronal inclusions. *Neurology* **36,** 68–71.
47. Lomas D. A., Evans D. L., Finch J. T., and Carrell R. W. (1992) The mechanism of Z alpha1-antitrypsin accumulation in the liver. *Nature* **357,** 605–607.

48. Ikeda H., Yamaguchi M., Sugai S., Aze Y., Narumiya S., and Kakizuka A. (1996) Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. *Nature Genet.* **13,** 196–202.
49. Becher M. W., Kotzuk J. A., Sharp A. H., Davies S. W., Bates G. P., Price D. L., and Ross C. A. (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. *Neurobiol. Dis.* **4,** 387–397.
50. Bates G. P., Mangiarini L., and Davies S. W. (1998) Transgenic mice in the study of polyglutamine repeat expansion diseases. *Brain Pathol.* **8,** 699–714.
51. Turmaine M., Raza A., Mahal A., Mangiarini L., Bates G. P., and Davies S. W. (2000) Nonapoptotic neurodegeneration in a transgenic mouse model of Huntington's disease. *Proc. Natl. Acad. Sci. USA* **97,** 8093–8097.
52. Prusiner S. B. (1997) Prion diseases and the BSE crisis. *Science* **278,** 245–251.
53. Johnson R. T. and Gibbs C. J. (1998) Creutzfeldt-Jakob disease and related transmissible spongiform encephalopathies. *N. Engl. J. Med.* **339,** 1994–2004.
54. DeArmond S. J. and Prusiner S. B. (1995) Etiology and pathogenesis of prion diseases. *Am. J. Pathol.* **146,** 785–811.
55. Gajdusek D. C. (1994) Spontaneous generation of infectious nucleating amyloids in the transmissible and nontransmissible cerebral amyloidoses. *Mol. Neuriobiol.* **8,** 1–13.
56. Aguzzi A. and Weissmann C. (1997) Prion research: the next frontiers. *Nature* **389,** 795–798.
57. Prusiner S. B., Safar J., Cohen F. E., and DeArmond S. J. (1999) The prion diseases, in *Alzheimer Disease,* 2nd ed. (Terry R. D., Katzman R., Bick K. L., and Sisodia S. S., eds.) Lippincott Williams and Wilkins, Philadelphia, pp. 161–179.
58. Jarrett J. T. and Lansbury P. T. Jr. (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73,** 1055–1058.
59. Lansbury P. T. Jr. (1997) Structural neurology: Are seeds at the root of neuronal degeneration? *Neuron* **19,** 1151–1154.
60. Kisilevsky R. (1996) Anti-amyloid drugs: potential in the treatment of diseases associated with aging. *Drugs Aging* **8,** 75–83.
61. Serio T. R., Cashikar A. G., Kowal A. S., Sawicki G. J., Moslehi J. J., Serpell L., et al. (2000) Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* **289,** 1317–1321.
62. Goudsmit J., Morrow C. H., Asher D. M., Yanagihara R. T., Masters C. L., Gibbs C. J. Jr., and Gadjusek D. C. (1980) Evidence for and against the transmissibility of Alzheimer disease. *Neurology* **30,** 945–950.
63. Manuelidis E. E. and Manuelidis L. (1991) Search for a transmissible agent in Alzheimer's disease: studies of human buffy coat. *Curr. Top. Microbiol. Immun.* **172,** 275–280.
64. Godec M. S., Asher D. M., Kozachuk W. E., Masters C. L., Rubi J. U., Payne J. A., et al. (1994) Blood buffy coat from Alzheimer's disease patients and their relatives does not transmit spongiform encephalopathy to hamsters. *Neurology* **44,** 1111–1115.
65. Baker H. F., Ridley R. M., Duchen L. W., Crow T. J., and Bruton C. J. (1994) Induction of beta (A4)-amyloid in primates by injection of Alzheimer's disease brain homogenate. Comparison with transmission of spongiform encephalopathy. *Mol. Neurobiol.* **8,** 25–39.
66. Kane M. D., Lipinski W. J., Callahan M. J., Bian F., Durham R. A., Schwarz R. D., et al. (2000) Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. *J. Neurosci.* **20,** 3606–3611.
67. Hsiao K., Chapman P., Nilsen S., Eckman C., Harigaya Y., Younkin S., et al. (1996) Correlative memory deficits, A beta elevation, and amyloid plaques in transgenic mice. *Science* **274,** 99–102.
68. Walker L. C., Price D. L., Voytko M. L., and Schenk D. B. (1994) Labeling of cerebral amyloid in vivo with a monoclonal antibody. *J. Neuropathol. Exp. Neurol.* **53,** 377–383.
69. Schenk D., Barbour R., Dunn W., Gordon G., Grajeda H., Guido T., et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400,** 173–177.
70. Bard F., Cannon C., Barbour R., Burke R.-L., Games D., Grajeda H., et al. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature Med.* **6,** 916–919.
71. Esler W. P., Stimson E. R., Jennings J. M., Vinters H. V., Ghilardi J. R., Lee J. P., et al. (2000) Alzheimer's disease amyloid propagation by a template-dependent dock-lock mechanism. *Biochemistry* **39,** 6288–6295.

72. Vassar R., Bennett B. D., Babu-Khan S., Kahn S., Mendiaz E. A., Denis P., et al. (1999) Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286,** 735–741.
73. Farzan M., Schnitzler C. E., Vasilieva N., Leung D., and Choe H. (2000) BACE2, a beta-secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. *Proc. Natl. Acad. Sci. USA* **97,** 9712–9717.
74. Selkoe D. J. and Wolfe M. S. (2000) In search of gamma-secretase: presenilin at the cutting edge. *Proc. Natl. Acad. Sci. USA* **97,** 5690–5692.
75. LeVine III H. (in press, 2000) Protein mis-folding and neurodegenerative disease: therapeutic opportunities, in *Drug Discovery Strategy and Techniques* (Makriyannis A. and Biegel D., eds.), Marcel Dekker, New York.
76. Soto C., Sigurdsson E. M., Morelli L., Kumar R. A., Castano E. M., and Frangione B. (1998) Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nature Med.* **4,** 822–826.
77. Findeis M. A. (2000) Approaches to discovery and characterization of inhibitors of amyloid beta-peptide polymerization. *Biochim. Biophys. Acta* **1502,** 76–84.
78. Solomon B., Koppel R., Frankel D., and Hanan-Aharon E. (1997) Disaggregation of Alzheimer β-amyloid by site-directed mAb. *Proc. Natl. Acad. Sci. USA* **94,** 4109–4112.
79. LeVine III H. and Scholten J. D. (1999) Screening for pharmacologic inhibitors of amyloid fibril formation. *Meth. Enzymol.* **309,** 467–476.
80. Heiser V., Scherzinger E., Boeddrich A., Nordhoff E., Lurz R., Schugardt N., Lehrach H., and Wanker E. E. (2000) Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. *Proc. Natl. Acad. Sci.* **97,** 6739–6744.
81. Sunde M., Serpell L. C., Bartlam M., Fraser P. E., Pepys M. B., and Blake C. C. F. (1997) Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J. Mol. Biol.* **273,** 729–739.
82. Johnston J. A., Ward C. L., and Kopito, R. R. (1998) Aggresomes: a cellular response to misfolded proteins. *J. Cell Biol.* **143,** 1883–1898.
83. Botto M., Hawkins P. N., Bickerstaff M. C. M., Herbert J., Bygrave A. E., McBride A., et al. (1997) Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. *Nature Med.* **3,** 855–859.
84. Bruening W., Roy J., Giasson B., Figlewicz D. A., Mushynski W. E., and Durham H. D. (1999) Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. *J. Neurochem.* **72,** 693–699.
85. Igarashi S., Koide R., Shimohata T., Yamada M., Hayashi Y., Takano H., et al. (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. *Nature Genet.* **18,** 111–117.
86. Hampton R. Y. (2000) E. R. stress response: getting the UPR hand on misfolded proteins. *Curr. Biol.* **10,** R518–R521.
87. Iwata N., Tsubuki S., Takaki Y., Watanabe K., Sekiguchi M., Hosoki E., et al. (2000) Identification of the major Aβ1-42-degrading catabolic pathway in brain parenchyma: Suppression leads to biochemical and pathological deposition. *Nature Med.* **6,** 143–150.
88. Saudou F., Finkbeiner S., Devys D., and Greenberg M. E. (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* **95,** 55–66.
89. Shimura H., Hattori N., Kubo S., Mizuno Y., Asakawa S., Minoshima S., et al. (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nature Genet.* **25,** 302–305.