

Protein Aggregation in the Brain: The Molecular Basis for Alzheimer's and Parkinson's Diseases

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Developing effective treatments for neurodegenerative diseases is one of the greatest medical challenges of the 21st century. Although many of these clinical entities have been recognized for more than a hundred years, it is only during the past twenty years that the molecular events that precipitate disease have begun to be understood. Protein aggregation is a common feature of many neurodegenerative diseases, and it is assumed that the aggregation process plays a central role in pathogenesis. In this process, one molecule (monomer) of a soluble protein interacts with other monomers of the same protein to form dimers, oligomers, and polymers. Conformation changes in three-dimensional structure of the protein, especially the formation of β -strands, often accompany the process. Eventually, as the size of the aggregates increases, they may precipitate as insoluble amyloid fibrils, in which the structure is stabilized by the β -strands interacting within a β -sheet. In this review, we discuss this theme as it relates to the two most common neurodegenerative conditions—Alzheimer's and Parkinson's diseases.

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INTRODUCTION

Extracellular fibrous amyloid deposits or intracellular inclusions containing abnormal protein fibrils characterize many neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis, frontal temporal dementia, and the human prion diseases (1). Burgeoning evidence suggests that accumulation of proteins capable of forming amyloid deposits may represent a common pathological mechanism for these diverse illnesses (2,3). In each of these diseases,

misfolding of a particular protein can lead to its aggregation, involving a process in which monomers interact to form dimers, oligomers, and eventually insoluble fibrillar deposits. Alzheimer's and Parkinson's diseases, representative examples that account for the majority of cases of neurodegenerative diseases, are reviewed here with an emphasis on the fundamental importance of aggregation as the pathological trigger. For each disease, we first outline the major characteristics and then present the evidence for the crucial role of soluble oligomers

(rather than the end-stage insoluble fibrils) of the aggregating protein— β -amyloid protein (A β) in Alzheimer's disease and α -synuclein in Parkinson's disease. Finally, we discuss future therapeutic and diagnostic approaches, based on current understanding of A β and α -synuclein pathobiology.

ALZHEIMER'S DISEASE: INCIDENCE AND SYMPTOMS

First identified by Alois Alzheimer in 1906, Alzheimer's disease is an irreversible, progressive brain disease that slowly destroys memory and cognitive skills (4). It is the most common cause of dementia, accounting for more than half of all such cases, and currently affects more than 24 million people worldwide, with 4.6 million new cases each year (5). Age is the single biggest known risk factor, with the incidence of the disease increasing from one in ten of those over 65 to almost half of those over 85 (6,7). There is no strong sex or race effect, but because women tend to live longer than men there are more

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women with Alzheimer's disease. The average duration of the disease is about 8 years, but it can last in excess of 20 years.

The disease is divided into two categories based on the age of onset. Early-onset Alzheimer's disease is extremely rare, accounting for only ~2% of all cases. It develops between the ages of ~30 and 60 years, and more than half of all such cases are genetic, with a strong Mendelian inheritance pattern (8). Late-onset Alzheimer's disease is by far the most common form of the disease. It too has a genetic predisposition but appears to involve several gene polymorphisms, some yet to be identified, that individually or in combination increase one's risk for developing Alzheimer's disease (9). A genetic component for late-onset disease is supported by the finding that the age at onset of Alzheimer's disease is significantly more variable for concordant nonidentical twins than concordant identical twins, providing evidence that genetic background strongly influences the timing of the disease (10). Genetic factors that predispose to Alzheimer's disease are difficult to identify because their inheritance does not cause the disease phenotype, but rather modulates the age of onset.

The disease progression is similar for both early- and late-onset Alzheimer's disease and is arbitrarily split into three overlapping stages: early/mild, moderate, and severe. The initial onset of Alzheimer's disease is insidious, with memory loss the earliest and most frequently cited symptom. In a living person, there is no precise diagnostic test that confirms Alzheimer's disease. A diagnosis of probable Alzheimer's disease is achieved by excluding other conditions that might explain the observed symptoms. Currently the most important diagnostic tool for clinicians is neuropsychological and mental status testing. The Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for diagnosing dementia requires loss of two or more domains, including

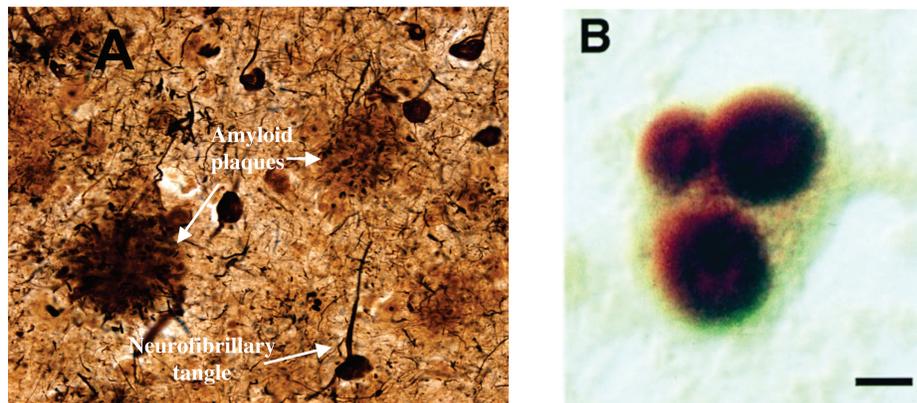


Figure 1. Pathological hallmarks of Alzheimer's and Parkinson's diseases. (A) Tangles and plaques in Alzheimer's disease. Neurofibrillary tangles are intraneuronal and consist of paired helical filaments, the subunit of which is a microtubule-associated protein called tau that has been phosphorylated at multiple sites (dark staining structures). Amyloid plaques are extracellular and are largely composed of a ~4-kDa protein called the amyloid β -protein ($A\beta$) (round diffuse structures). See Acknowledgements for source information on panel A. (B) Lewy bodies in Parkinson's disease. Nerve cell with 3 Lewy bodies that are double-stained for α -synuclein (brown) and ubiquitin (blue). Where only α -synuclein is stained, the color appears as pale reddish-brown, but where ubiquitin also is stained, the superposition of color gives a dark black and brown appearance. The blue staining is not seen on its own, because all the ubiquitin-immunoreactive structures are also positive for α -synuclein. The halo of each Lewy body is strongly immunoreactive for ubiquitin, whereas both the core and the halo of each Lewy body are immunoreactive for α -synuclein. Bar, 10 μ m. Panel B has been reproduced from Figure 3 of Spillantini *et al.* (96), © 1993–2005 by the National Academy of Sciences of the USA, all rights reserved.

memory, language, calculation, orientation, and judgment (11). Neuropsychological tests, such as the Mini-Mental State Examination (MMSE), can provide clinical insight into the patient's cognitive changes. Patients are considered to have mild cognitive impairment, not Alzheimer's disease, if they present with memory loss but have only minimal impairment in other cognitive domains and are not functionally impaired at work or home. Thereafter, computed tomography or magnetic resonance imaging (MRI) can be used to discriminate between other forms of dementia. An experienced physician can diagnose Alzheimer's disease with up to 90% accuracy. However, a definitive diagnosis of Alzheimer's disease requires not only the presence of severe dementia but also postmortem confirmation of two histopathological features, tangles and plaques (Figure 1A) (12).

EMERGING DIAGNOSTIC TOOLS

Techniques for imaging the brain are progressing, and both the sensitivity and specificity for diagnosis of Alzheimer's disease are constantly improving. For instance, MRI of the hippocampal formation can be used to identify atrophy found in patients with mild cognitive impairment and Alzheimer's disease, as such atrophy is absent in the normal elderly. Similarly, changes in the volume of the fusiform gyrus can be used to distinguish between mild cognitive impairment and Alzheimer's disease, whereas common cerebral alterations such as enlarged ventricular spaces are used to support the diagnosis of Alzheimer's disease (13). Positron emission tomography (PET) allows for visualization of metabolism in various regions of the brain by using ^{18}F -2-deoxy-2-fluoro-D-glucose (FDG) as a surrogate marker of glucose metabolism. In patients with

early Alzheimer's disease, decreased metabolism in parieto-temporal association cortex and cingulate gyrus and more marked changes in the medial temporal region and parieto-temporal association cortex are detected. As the condition progresses, abnormal PET-FDG is also obvious in the frontal association cortex. In addition, changes detected in regional cerebral perfusion studies by single photon emission computed tomography (SPECT) can distinguish between mild Alzheimer's disease and forms of vascular dementia (14).

As will be discussed below, accumulation of A β in the brain, manifesting as β -sheet rich plaques, is a hallmark of Alzheimer's disease. Recently, researchers at the University of Pittsburgh developed a thioflavin T analog, Pittsburgh compound B (PIB), which binds β -sheet-rich fibrils (15). This compound crosses the blood-brain barrier and binds amyloid deposits in the brain parenchyma, where binding of PIB labeled with carbon-11 can be detected by PET imaging. As one would expect, an inverse correlation exists between FDG-PET imaging of glucose metabolism in the parietal cortex and PIB binding (16). This novel *in vivo* imaging technique provides promise for more definitive diagnosis of Alzheimer's disease by detecting the pathognomonic A β accumulation; following the progression of Alzheimer's disease in individual patients; and, tracking changes in plaque burden in response to amyloid-lowering therapeutics (see below).

NEUROPATHOLOGICAL HALLMARKS OF ALZHEIMER'S DISEASE

Microscopically, the Alzheimer brain is characterized by the presence of extracellular amyloid plaques and intraneuronal neurofibrillary tangles. Amyloid plaques display a broad range of morphologic and biochemical characteristics and contain numerous proteins, the principal of which is A β (17,18). A β is a ~4-kDa protein with a common core sequence but heterogeneous N- and C-termini. The most common form of A β is 40 amino acids long and is called A β_{40} . A β_{42} , a less

abundant form of this protein that differs only by having two additional amino acid residues at the C-terminus, is particularly associated with disease (19). Compact, neuritic amyloid plaques contain thioflavin S and Congo red-positive fibrillar deposits with both A β_{40} and A β_{42} present (20). Diffuse plaques, on the other hand, are not fibrillar and consist almost exclusively of A β_{42} . These immature deposits may be detected in the brains of young patients with Down's syndrome before the manifestation of Alzheimer's disease-type dementia or in brain regions that do not display the complete extent of Alzheimer's disease pathology described above (21). As a result, diffuse plaques are considered precursors to mature, neuritic plaques. Dilated, dystrophic neurites, activated microglia, and reactive astrocytes can be found within and immediately surrounding neuritic plaques (22). The processes of neurons found herein display abnormal signs of enlarged lysosomes and numerous mitochondria.

Neurons bearing neurofibrillary tangles, composed of hyperphosphorylated forms of the microtubule-associated protein, tau, are also frequently found proximate to amyloid deposits (23,24), and their temporal and spatial appearance more closely reflects disease severity than does the appearance of amyloid plaques (25,26). Neurofibrillary tangles are not specific to Alzheimer's disease, however, and are found in other disorders (for example, subacute sclerosing panencephalitis and progressive supranuclear palsy) not associated with the cognitive dysfunction and memory impairment that characterize Alzheimer's disease (27). Indeed a growing body of genetic and biochemical evidence suggests that neurofibrillary tangles are downstream of A β . Specifically, experimental evidence suggests that abnormal A β accumulation triggers tau pathology (28,29), and tau has been proposed as an essential mediator of A β -induced neurotoxicity (30); however, the steps connecting A β to tau remain undefined. A β has been shown to induce the calpain-mediated cleavage of tau, leading to the generation of a toxic 17-kDa frag-

ment (31), and to induce abnormal tau phosphorylation at disease-relevant sites (32,33); a recent study even suggested that tau phosphorylation is the limiting factor in A β -induced neurotoxicity (34). Similarly, tau appears to play a central role in the memory deficits apparent in certain transgenic mouse models of Alzheimer's disease (35). Together, these results suggest that A β plays an initiating role in a pathogenic cascade that requires altered metabolism of tau to result in disease.

A MOLECULAR EXPLANATION OF ALZHEIMER'S DISEASE: THE A β HYPOTHESIS

Considerable genetic, animal-modeling, and biochemical data have emerged to suggest that A β plays a central role in initiating Alzheimer's disease. A β is derived from the amyloid precursor protein (APP) by the action of two aspartyl proteases called β - and γ -secretases (Figure 2) (36-38). APP is first cleaved by β -secretase shedding its large ectodomain and leaving a membrane-bound C-terminal stub (39,40). This 99-amino acid stub is subsequently cleaved by γ -secretase and A β is released (41) (Figure 2). Depending on the exact point of cleavage by γ -secretase, two main forms of A β , comprising either 40 or 42 amino acid residues, are produced. The proportion of A β_{42} to A β_{40} formed is particularly noteworthy, because the longer form of A β is far more prone to oligomerize and form fibrils than the more abundantly produced A β_{40} peptide. Production of A β is a normal process, but in a small number of individuals the overproduction of A β , or an increased proportion of the 42-amino acid form, appears sufficient to cause early-onset Alzheimer's disease (see Table 1).

The evidence in support of a causative role for A β in Alzheimer's disease is as follows:

1. Localization of the *APP* gene to chromosome 21 and the observation that Alzheimer's disease-like neuropathology is invariably seen in Down's syndrome (trisomy 21) (Table 1) (42,43). This point is further supported by de-

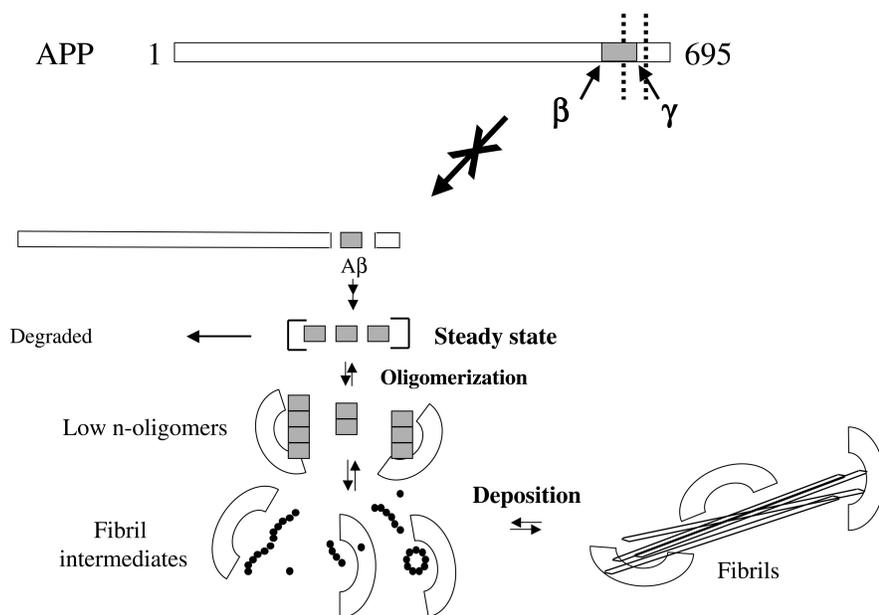


Figure 2. Production of Aβ by proteolytic cleavage from APP followed by association of Aβ to form oligomers and fibrils, showing potential targets for anti-amyloid therapies. Aβ, the gray shaded box, is cleaved from APP by sequential action of 2 proteases; β-secretase carries out the initial cleavage to form the N-terminus of Aβ; γ-secretase then cleaves the C99 stub to produce the C-terminus of Aβ. The parallel dotted lines represent a membrane bilayer in which part of the C-terminal region of APP is anchored. Hence γ-secretase activity is a protease that cleaves a substrate within a membrane. Production of Aβ by secretase action leads to Aβ monomer, the concentration of which in the steady state is a balance between formation and degradation. Monomers can associate to form small oligomers that increase in size and eventually lead to fibril formation. One anti-amyloid strategy is to inhibit the enzymatic action of either secretase (shown by a black cross). A second strategy is to remove soluble and deposited Aβ using antibodies (shown as semicircles).

tection of a rare case of Down’s syndrome in which the distal location of the chromosome 21q breakpoint left the patient diploid for the APP gene (44). This individual showed no signs of dementia, and amyloid deposition was essentially absent from the brain upon death at age 78. In addition, duplication of APP is also associated with early-onset Alzheimer’s disease (42).

2. Synthetic Aβ peptides are toxic to hippocampal and cortical neurons, both in culture and *in vivo* (45–47).
3. Inherited mutations in the APP gene that immediately flank or localize within the Aβ region and increase the amount or aggregation properties of Aβ are sufficient to precipitate early-onset Alzheimer’s disease. Mutations lying outside the Aβ domain are prox-

imate to the β- and γ-cleavage sites and elevate Aβ production or increase the Aβ₄₂/Aβ₄₀ ratio (48–51). The five point mutations that lie within the Aβ sequence are clustered around the central hydrophobic core of Aβ and cause an increase in steady-state levels of Aβ and/or an increased propensity of the resultant Aβ to aggregate.

4. Inherited mutations within the *presenilin 1* and 2 genes increase the Aβ₄₂/Aβ₄₀ ratio throughout life and cause very early and aggressive forms of Alzheimer’s disease. In this regard, presenilin has been found to contribute the active site of the protease (γ-secretase) that generates the C-terminus of Aβ (Figure 2) (19,52).
5. In humans, *Apo E*, which codes for apolipoprotein E, has three common alleles, ε2, ε3, and ε4, and genetic epidemiological studies show that the ε4 allele is a major risk factor for developing late-onset Alzheimer’s disease, whereas the ε2 allele appears to be protective. Importantly, ε4 is associated with more extensive and fulminant Aβ deposition than is ε2 (53,54).
6. Mice transgenic for mutant human APP show a time-dependent increase in extracellular Aβ and develop certain neuropathological and behavioral changes similar to those seen in Alzheimer’s disease (55).
7. Finally, injection of synthetic Aβ into the brains of tau transgenic mice accelerates tau hyperphosphorylation and leads to tangle formation reminiscent of the other hallmark that characterizes Alzheimer’s disease (Figure 1A), whereas reducing endogenous expression of tau ameliorates behavioral deficits in APP transgenic mice (35).

Aβ TOXICITY: THE IMPORTANCE OF STRUCTURE

Aβ is a natural product present in the brains and cerebrospinal fluid (CSF) of normal subjects (36,56,57). The presence of Aβ itself does not lead to neurodegeneration, but neuronal injury ensues as a result of the ordered self-association of Aβ molecules. Within the amyloid

Table 1. Genetics of early-onset Alzheimer’s disease.

Gene	Age of onset, years	Aβ phenotype
APP trisomy 21	50s	Aβ _{total} production increased
APP mutations	50s	Aβ _{total} production increased Aβ ₄₂ /Aβ ₄₀ ratio increased
APP triplication of APP gene	50s	Aβ _{total} production increased
Presenilin 1	40s and 50s	Aβ ₄₂ /Aβ ₄₀ ratio increased
Presenilin 2	50s	Aβ ₄₂ /Aβ ₄₀ ratio increased

plaques that characterize Alzheimer's disease, A β is organized into fibrils 6–10 nm in diameter, whereas *in vitro*, A β readily assembles into very similar amyloid fibrils. Many studies have demonstrated that when synthetic A β is pre-incubated to form amyloid fibrils, such preparations are directly toxic to neurons (46,58).

One important caveat when considering the activity of A β assemblies is the dynamic nature of the aggregation process. Initial studies clearly demonstrated that aggregation of A β was essential for toxicity, but characterization of the assemblies used was limited and it was assumed that, because amyloid fibrils were detectable, it was fibrils that mediated the observed toxicity. Yet this ignored the fact that in patients dying with Alzheimer's disease there is a relatively weak correlation between the severity of dementia and the density of fibrillar amyloid (59).

In contrast, robust correlations between the levels of soluble A β and the extent of synaptic loss and severity of cognitive impairment have been demonstrated (60,61); the term soluble A β refers to all forms of A β that remain in aqueous solution following high speed centrifugation of brain extracts. To date, most studies of soluble A β brain levels have employed assays that cannot identify the aggregation state of the species detected (62). Thus, although one cannot attribute the effects to a specific assembly form of A β , the solubility of the species in aqueous buffer following ultracentrifugation (typically >100,000g for >1 h) would indicate that the preparations used are free of fibrillar assemblies. Furthermore, in very recent studies, antibodies reported to be specific for oligomeric, but not monomeric or fibrillar, A β revealed abundant anti-oligomer reactivity in soluble extracts of Alzheimer's disease brain, but none in age-matched controls (63).

IDENTIFICATION OF NEUROTOXIC, NONFIBRILLAR A β AGGREGATES

SDS-stable dimers and trimers (so-called low-n oligomers) of A β have been

detected in the buffer-soluble fraction of human cerebral cortex and in human CSF (57,64). Similar oligomers are also formed by a fibroblast cell line genetically manipulated to express mutant human APP (7PA2 cells). These cells produce and secrete significant amounts of SDS-stable low-n oligomers of A β that migrate in denaturing SDS-polyacrylamide gels with molecular weights consistent with dimers, trimers, and occasionally tetramers (65,66). Because of the easy maintenance and fast growth rate of these cells, 7PA2 culture medium has provided a convenient tool to investigate the biological activities of low-n A β oligomers. This led to the discovery that A β oligomers can inhibit hippocampal long-term potentiation (an electrophysiological measure of synaptic plasticity) (67,68), impair complex learned behavior in the live rat (69), and reduce the density of dendritic spines in cultured hippocampal neurons (70,71).

Additional support for a role for pre-fibrillar A β assemblies in Alzheimer's disease pathogenesis comes from studies using synthetic A β peptides. The first nonfibrillar assemblies identified were protofibrils; these heterogeneous structures range from spherical assemblies of ~5 nm diameter to short, flexible rods of up to 200 nm in length (72,73). The principal difference between protofibrils and mature fibrils is size and relative solubility; fibrils are frequently several microns long and are often associated with other fibrils, whereas protofibrils tend not to be associated with other protofibrils and seldom exceed 150 nm in length. Consequently, unlike fibrils, they do not sediment upon low-speed centrifugation (73,74). Protofibrils can be generated under a variety of biochemical conditions and appear to behave as true fibril intermediates in that they can both form fibrils and dissociate to lower-molecular-weight species. Acute application of protofibrils *in vivo* rapidly alters synaptic physiology, whereas chronic application causes cell death (75). A second soluble, nonfibrillar assembly of synthetic A β called A β -derived diffusible ligands

(ADDLs), appear as spheres with a diameter ~5 nm and migrate in polyacrylamide gels at ~4, 8, 16, and 18 kDa. ADDLs are formed only under certain specific *in vitro* conditions but can cause neuronal death and block long-term potentiation in *ex vivo* preparations (76,77). A recent study reported that synthetic ADDL preparations can bind excitatory synapses (78) and cause a reduction in spine density (79) similar to the findings observed with soluble A β oligomers secreted in cell culture.

Together these results provide compelling evidence that soluble nonfibrillar forms of A β are potent neurotoxins. Indeed, in the human brain it is likely that multiple A β assemblies that are in dynamic equilibrium simultaneously alter neuronal, astrocytic, and microglial function, and that different toxic effects may occur virtually concurrently in various regions of the cerebral cortex. Thus removal or neutralization of such toxic species is an attractive therapeutic strategy.

CANDIDATE A β -BASED THERAPIES AND DIAGNOSTICS

To date, there is no effective treatment that can prevent progression of Alzheimer's disease; available drugs can only delay worsening of symptoms. Therefore there is urgent need for therapies that alter the progression of Alzheimer's disease. The A β hypothesis posits that increased steady-state levels and consequent A β assembly is the primary event driving Alzheimer's disease pathogenesis (Figure 3). The rest of the disease process is believed to result from this aberrant assembly. A number of different anti-amyloid therapies are under development; two examples are discussed and illustrated in Figure 2: decreasing the production of soluble A β monomer and removing soluble and deposited A β .

Reduction of A β levels is particularly attractive because it may be possible to titrate A β down to concentrations that will not support oligomerization. It would be anticipated that cell-penetrant

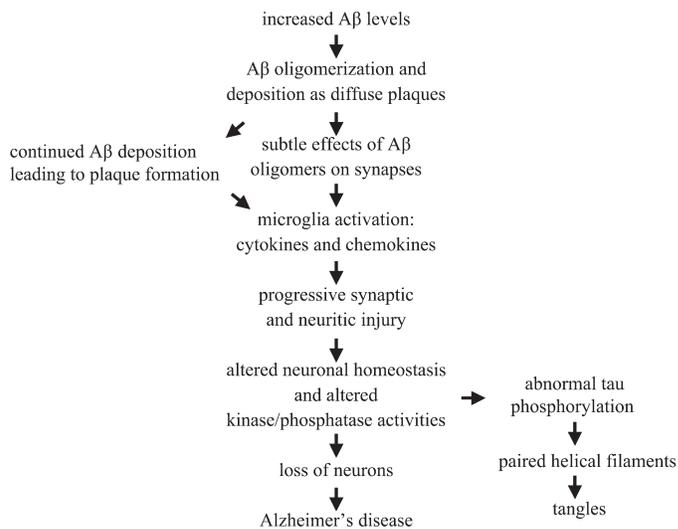


Figure 3. The A β hypothesis: an increase in the concentration of A β , especially the 42-amino acid form, is the underlying cause for the pathological features of Alzheimer's disease.

agents that could reduce intracellular and/or extracellular monomer levels below the critical concentration needed for oligomerization would thus prevent A β from assembling into toxic structures. The development of potent highly selective inhibitors of β - and γ -secretases that can readily enter the brain and lower A β production (Figure 2) is being actively pursued. Similarly, efforts are also ongoing to develop small molecules that can upregulate the enzymes that control A β degradation and thus lower A β levels by increasing A β catabolism.

Anti-A β immunotherapy employs antibodies that recognize multiple different toxic A β assemblies by both directly neutralizing them and preventing their toxic effect, by promoting microglial clearance, and/or by redistributing A β from the brain to the systemic circulation. This approach has already been shown to reduce cerebral A β levels, decrease amyloid-associated gliosis and neuritic dystrophy, and alleviate memory impairment in transgenic mouse models of Alzheimer's disease (80). More importantly, Alzheimer's disease patients that were immunized with aggregated A β showed diminished cognitive decline and slowed disease progression compared with pa-

tients that received placebo (81). Unfortunately, this phase IIa trial had to be stopped prematurely because 18 of the 298 patients who had been immunized developed meningoencephalitis. Notably, in four cases that have since come to autopsy (two affected with encephalitis and two not), all showed evidence of clearance of amyloid deposits. Thus in the first clinical test of the A β hypothesis it appears that (as in preclinical studies of mouse models) targeted removal of cortical A β beneficially modifies Alzheimer's disease progression. Efforts are ongoing to develop an equally effective immunization protocol that avoids induction of encephalitis. Thus there is good reason to believe that therapies directed at preventing the generation of toxic A β assemblies will soon come to the clinic and that, unlike current therapies, they will actually halt further deterioration and offer the potential of restoring normal cognitive function.

With the advancement of potentially disease-modifying therapies, there is an urgent need to develop methods for use in early *ante mortem* diagnosis. This is required, not only from a clinical standpoint, but also because it affects the integrity of clinical trials and epidemiolog-

ical research. Currently there are at least four methods that have evolved from our better understanding of the disease process: analysis of A β species in CSF (82); visualization of amyloid plaques by PET as discussed above (15,83); measurement of A β in peripheral blood (84); and measurement of total tau and/or phospho-tau in CSF (85,86).

Given the genetic evidence supporting a prominent role for A β_{42} in disease, many studies have investigated the diagnostic utility of measuring A β_{42} in CSF. For Alzheimer's disease patients, A β_{42} levels in CSF are typically reduced to around 50% of the level found in controls. The mean sensitivity and specificity to discriminate between Alzheimer's disease and normal aging are both >85% (87). However, decreased CSF A β_{42} is found in certain patients with frontotemporal dementia and vascular dementia, and measurement of CSF A β_{42} alone is insufficient to discriminate between Alzheimer's disease and these dementias (88). CSF A β_{40} is unchanged or slightly increased in Alzheimer's disease (89); consequently a decrease in the ratio of A β_{42} /A β_{40} in CSF has been found in Alzheimer's disease, and this decrease seems more pronounced than the reduction of CSF A β_{42} alone (90). Alzheimer's disease is also associated with a significant increase in CSF tau and phospho-tau levels (85,86), and combining measurement of total tau, A β_{42} , and phospho-tau identifies incipient Alzheimer's disease in patients with mild cognitive impairment with very high accuracy (90,91).

The reduced level of CSF A β_{42} in Alzheimer's disease is believed to be caused by deposition of A β_{42} in senile plaques, hence leaving lower levels of A β_{42} to diffuse into CSF. Accordingly, studies have found a strong correlation between low A β_{42} in CSF and high retention of PIB (83). Factors that may contribute to reduced A β_{42} levels, in addition to deposition in senile plaques, include formation of A β_{42} oligomers that escape ELISA detection and binding of A β_{42} to other proteins that block the antibody recognition

of A β . For instance, ELISA measurements of plasma A β levels in Alzheimer's disease have yielded conflicting data and this could, in part, reflect an inability to measure A β oligomers. This possible confounder might differ for antibodies used in different ELISA protocols and could explain some of the contradictory results. Development of anti-A β dimer/oligomer-specific antibodies should obviate concerns about epitope masking due to A β self-association and may provide a useful system to measure A β dimer/oligomer levels in both CSF and plasma. Indeed, a small number of preliminary studies suggests that measurement of A β oligomers will be of benefit (63,92). If this holds true in larger studies, one would anticipate that combining measurement of disease-linked assembly forms (oligomers) of A β together with measurement of tau in CSF and PIB binding in brain will provide a highly specific and sensitive means of measuring both early and incipient Alzheimer's disease.

PARKINSON'S DISEASE: INCIDENCE, SYMPTOMS, AND DIAGNOSIS

Named after the English physician who first described the clinical syndrome in "An Essay on the Shaking Palsy" published in 1817 [reprinted in (93)], Parkinson's disease is an irreversible, progressive neurodegenerative disease that impairs movement. Incidence increases markedly with age; hence young-onset Parkinson's disease, defined as occurrence before age 40, accounts for just 5% of newly diagnosed cases (Parkinson's Disease Society). Upon reaching the 65–69 age range, 0.6% of the population are affected, increasing to 2.6% of those aged 85–89 (94), making it the second most common neurodegenerative illness after Alzheimer's disease. The majority of cases of Parkinson's disease are idiopathic and sporadic. However, studies within the past few years indicate that a substantial number of cases do have a genetic component. The three principal signs of parkinsonism, the clinical phenotype that defines the disease, are rest-

ing tremor, often beginning in one finger, bradykinesia, and rigidity. Whereas dementia is the defining feature of Alzheimer's disease, cognitive ability remains intact in most Parkinson's disease sufferers, at least in the early stages of the disease.

PATHOLOGY AND MECHANISM OF DISEASE

Pathologically, the most obvious brain structure affected by Parkinson's disease is a part of the substantia nigra called the pars compacta. Because patients suffering from other neurological disorders can display parkinsonian features, a definitive diagnosis of Parkinson's disease can be confirmed only by *post mortem* histopathological examination of the substantia nigra for loss of pigmented neurons and presence of Lewy bodies in remaining neurons. Identification of Lewy bodies has been facilitated by immunostaining for particular proteins, initially for ubiquitin and more recently for α -synuclein, now regarded as the major protein constituent (95). Such staining reveals filaments that, when purified and examined by immunoelectron microscopy, can be seen to contain α -synuclein (96) (Figure 1B) and resemble filaments formed when purified recombinant α -synuclein is allowed to aggregate *in vitro* (97).

In the mid-twentieth century, Arvid Carlsson showed that dopamine acted as the neurotransmitter in neurons of the substantia nigra pars compacta, work for which he shared the Nobel Prize in 2000. These neurons operate in a pathway that controls voluntary movement. This involves signals being relayed from the cerebral cortex through the basal ganglia back to the cortex and then on to muscles. Neurons from the substantia nigra pars compacta project axons that release dopamine in synapses on interneurons in the striatum. As the dopamine-containing neurons die, failure to complete this circuit results in inability to coordinate movement. Neuromelanin, the black pigment that gives the substantia nigra its name, is a byproduct from the metabolic

pathway for dopamine synthesis. When the symptoms of Parkinson's disease first become apparent more than 70% of the dopamine-containing neurons have already been lost, releasing their neuromelanin and hence turning the tissue less black. It is now apparent, however, that many regions of the brain are affected in Parkinson's disease, and indeed in the early stages it may affect only a lower region of the brain stem called the medulla oblongata, spreading gradually upward through the basal ganglia into the cortical areas (98).

One of the most obvious questions when considering the etiology of Parkinson's disease is why dopaminergic neurons are particularly vulnerable. Until recently no clear genetic links were apparent, and one hypothesis was that the metabolic pathways for dopamine synthesis might be at the root of the problem (99). There is evidence that dopamine metabolites can increase levels of reactive oxygen species that damage cells, especially mitochondria; for example, neuromelanin binds heavy metals that can lead to free radical production (100). Another hypothesis, that postulated exposure to environmental toxins as the cause, was given impetus by two apparently diverse findings that were both subsequently linked to mitochondrial dysfunction. First, during the 1970s and 80s, some users of illegal recreational drugs developed parkinsonism that was traced to the ingestion of a contaminant called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (101). Itself relatively innocuous, this compound is lipid-soluble and so crosses the blood-brain barrier, where it is oxidized by monoamine oxidase-B, present in astrocytes, to the toxic 1-methyl-4-phenylpyridinium (MPP⁺) ion. The unique susceptibility of dopaminergic neurons to this toxin is due to it being taken up by the dopamine transporter. Once inside the neuron, it is concentrated in the mitochondria, where it binds NADH dehydrogenase, inhibiting complex I of the electron transport chain and leading to mitochondrial dysfunction (102). Second,

epidemiological studies had suggested a link between long-term exposure to agricultural pesticides, such as rotenone and paraquat. Rotenone, a plant derivative used as an insecticide, is known to be an inhibitor of NADH dehydrogenase. Chronic infusion of either rotenone or MPTP in rodents results in parkinsonism-like behavior and pathology, including the formation of inclusion bodies, and indeed these are among the best animal models for the human disease (103). It has also been suggested that exposure to paraquat may alter the expression and aggregation of α -synuclein (see below).

The view that Parkinson's disease was almost entirely a sporadic disease due to metabolic dysfunction caused by environmental toxins has altered dramatically during the past 10 years. The first hereditary connection was a link to the protein α -synuclein, and so its genetic locus on chromosome 4 became known as *PARK1*. Nevertheless, mutations in the gene for α -synuclein account for only a tiny percentage of Parkinson's disease cases. Several additional loci, named *PARK2* through *PARK12*, have since been implicated (Online Mendelian Inheritance in Man; Johns Hopkins University, Baltimore; MIM no. 168600, 2007). The most promising candidates are listed in Table 2.

α -SYNUCLEIN

In 1997, a mutation in the α -synuclein gene leading to a single amino acid substitution (Ala53Thr) was detected in a family with an early-onset autosomal dominant form of Parkinson's disease with high penetrance (104). Spurred by this report, researchers searched for and detected α -synuclein in Lewy bodies (Figure 1B) and in so doing further implicated α -synuclein as a major player in Parkinson's disease pathogenesis (105). Subsequently, two additional disease-causing mutations were identified in the α -synuclein gene, each causing a different single amino acid substitution (106,107).

α -Synuclein belongs to a family of three closely related proteins (the others

Table 2. Genetic mutations linked to hereditary forms of Parkinson's disease.

Locus	Protein	Function
<i>PARK1</i>	α -synuclein	Putative chaperone
<i>PARK2</i>	parkin	Ubiquitin E3 ligase
<i>PARK6</i>	PINK1	Putative serine/threonine kinase
<i>PARK7</i>	DJ-1	Putative chaperone
<i>PARK8</i>	LRRK2	Putative serine/threonine and tyrosine kinase

being β - and γ -synucleins) that are encoded by three distinct genes. In humans, minor spliced variants of α -synuclein occur, but the predominant form in the brain is a small, soluble protein of 140 residues that accounts for about 1% of total protein in neurons, where it is localized in presynaptic nerve terminals. The primary structure of α -synuclein is characterized by three distinct regions (108):

- the N-terminal region (1–60) contains several degenerative repeats of an 11-amino acid sequence related to an α -helical lipid-binding motif of apolipoproteins that mediates protein binding to phospholipid vesicles
- the central region (61–95) is composed of extremely hydrophobic amino acid residues, and part of this region has been implicated in association of monomers into larger aggregates that ultimately form amyloid fibrils (109)
- the C-terminal region (96–140) is hydrophilic and rich in the amino acid residue proline and the acidic residues glutamate and aspartate; its size and sequence vary markedly between species, and it has been suggested to confer chaperone activity to α -synuclein (110)

Until recently, the function of α -synuclein remained unclear, and indeed mice deficient in α - or β -synuclein, or both, survive with no very obvious brain defects (111). α -Synuclein lacks secondary or tertiary structure, so it belongs to the family of natively unfolded proteins, many of which act as chaperones. It is known to interact with several other proteins, whereas exposure to lipid micelles induces a structural change to α -helix in the N-terminal region (112).

In the presynaptic nerve terminals, α -synuclein associates with membranes of synaptic vesicles and may have a regulatory role in inhibiting neurotransmitter release (113). Support for a chaperone function for α -synuclein has come from genetically manipulated mice in which cysteine-string protein- α (CSP α) was deleted (114). CSP α is a synaptic vesicle protein that acts as a cochaperone for SNARE proteins. α -Synuclein appears to be able to act as an auxiliary chaperone, complementing the chaperone activity of CSP α .

A MOLECULAR EXPLANATION OF PARKINSON'S DISEASE: THE α -SYNUCLEIN HYPOTHESIS

In an analogous manner to the involvement of A β in Alzheimer's disease, considerable genetic, animal-modeling, and biochemical data have emerged to suggest that α -synuclein plays a central role in initiating Parkinson's disease.

1. Solutions of α -synuclein, or synthetic peptide fragments derived from it, associate into oligomers that eventually aggregate to produce amyloid fibrils whose morphology resembles that of fibrils purified from Lewy bodies (97,115).
2. The three mutant forms described above, each involving a single amino acid substitution, are associated with autosomal dominant Parkinson's disease. The mutations increase the aggregation rate of the resultant α -synuclein (115,116).
3. Duplication or triplication of the α -synuclein gene on one chromosome, giving 50% or 100% increase in expression of protein, causes parkinsonism (117–119). Age of onset of disease

and its severity are proportional to gene copy number.

4. α -Synuclein is the principal constituent of Lewy bodies. Much of this α -synuclein has been posttranslationally modified by sequence truncation at the C-terminus, phosphorylation at Ser129 (120,121), or nitration (122); these modifications have been shown to increase the rate at which oligomers are formed (121,123). Dopamine and related catecholamines have been shown to interact with α -synuclein and stabilize the protofibril stage of aggregation (124,125), providing a possible explanation for the increased susceptibility of dopaminergic neurons. Levels of soluble oligomers of α -synuclein are also affected by fatty acids, being upregulated by polyunsaturated fatty acids (126).
5. α -Synuclein aggregation is also increased in the presence of metals and pesticides, which may be environmental risk factors (127). For example, exposure to paraquat induced increased expression and aggregation of α -synuclein in mice. The authors speculate that these increased levels of α -synuclein may form part of the neuronal response to toxic insult, but that the increasing protein concentration or its interaction with paraquat may lead to the production of deleterious aggregates (128). Iron levels are elevated in substantia nigra from Parkinson's disease patients (129), and *in vitro* it has been demonstrated that, in the presence of iron, solutions of α -synuclein give rise to reactive oxygen species that could be toxic to cells (100).
6. Oligomers of α -synuclein are toxic to some cell cultures, including the dopaminergic SH-SY5Y human neuroblastoma cell line (130). As is the case for Alzheimer's disease, there is mounting evidence that the toxic species is not the final fibril, but early aggregates, which are soluble oligomers on the pathway to fibril formation (131). A recent study demonstrated that the affinity of α -synuclein for a phospholipid mem-

brane is a function of its degree of aggregation, with tightest binding by an intermediate formed during the conversion from monomeric to fibrillar state (132).

7. Increased human α -synuclein expression in transgenic flies (133) and mice (134) is accompanied by neuronal dysfunction and loss of synaptic terminals and/or neurons, the formation of lesions similar to those found in Parkinson's disease brain, and the development of motor abnormalities.
8. Expression of mutant forms of α -synuclein in cells promotes mitochondrial defects and cell death and enhances susceptibility to oxidative stress. On the other hand, mice deficient in synuclein are resistant to toxicity induced by MPTP and other mitochondrial toxins (135).

Taken together, all these studies provide strong evidence for a central role for α -synuclein in the pathogenesis of Parkinson's disease and, as is the case for Alzheimer's disease, recent evidence implicates small aggregates rather than insoluble fibrils as the toxic species, though the exact mechanism of toxicity remains unclear (131,136).

Genetic evidence also implicates several other proteins, listed in Table 2, some of which have been found to be associated with mitochondria. Because these subcellular organelles are also the initial site of damage by environmental toxins, sporadic and hereditary Parkinson's disease may converge in that both types of disease have at their root mitochondrial dysfunction. The *PARK2* locus codes for the enzyme parkin, which is a ubiquitin E3-ligase. Parkin can protect cells against mutant α -synuclein, and an obvious explanation might be that α -synuclein is a parkin substrate. This has not been convincingly demonstrated, however; rather, a connection with mitochondrial function may be involved, because parkin-null mutants in both fly (137) and mouse (138) models had compromised mitochondrial function and increased oxidative damage.

PINK1 and DJ-1 have both been shown to be required for normal mitochondrial function, and mutations in either of these proteins can decrease mitochondrial viability. Loss of PINK1 kinase activity adversely affects mitochondria under stressful conditions (139), whereas DJ-1 may protect against intracellular oxidative conditions (140) and also prevent α -synuclein aggregation (141). Mitochondrial deficiency will ultimately result in decreased ATP production, reducing proteasome activity and allowing excessive amounts of cellular proteins, including α -synuclein, to build up. Indeed, one of the other major protein constituents of Lewy bodies is ubiquitin, suggesting a general malfunction of the ubiquitin-proteasome system. LRRK2 is a kinase (142) whose connection with Parkinson's disease pathology remains unclear. The proteins mentioned above and their potential interactions in the pathways leading to Parkinson's disease are illustrated in Figure 4. Whether any particular individual succumbs to Parkinson's pathology may depend on a complex set of circumstances that include genetic factors, environmental exposures, and loss of cellular protective mechanisms. As suggested by Sulzer (143), development of pathology may require "multiple hits" that involve some combination of these factors; this might explain the difficulties encountered in explaining low penetrant inheritance.

EMERGING DIAGNOSTIC TOOLS

Despite progress in understanding the underlying disease mechanism, there remains an urgent need to develop methods for use in diagnosis of Parkinson's disease. To date, there is no serological or urine test that can confirm the diagnosis of Parkinson's disease. Routine blood, CSF, and urine tests yield normal results. Neither CT nor MRI scans of the head show abnormalities in idiopathic Parkinson's disease, but may sometimes help to diagnose a secondary parkinsonism due to tumor, infarction, etc. Deoxyglucose PET scans of the brain reveal an abnormal pattern of increased glucose

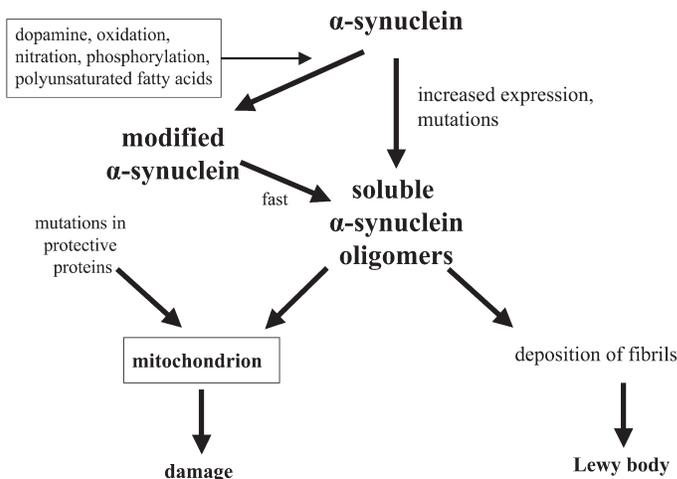


Figure 4. Summary of α -synuclein's role in Parkinson's disease pathology. Cellular α -synuclein levels or chemical structure may be altered by overexpression, mutations, or chemical modifications (such as phosphorylation, nitration, oxidation, exposure to metal ions or toxins, or adduct formation with dopamine quinone). The increased oxidative environment of dopaminergic neurons is likely to exacerbate some of these processes, making these cells especially vulnerable. The end result is increased oligomer formation that may damage mitochondrial membranes. The amyloid-containing Lewy body is likely to be much less toxic than this precursor, and it may be the case that cells that rapidly produce Lewy bodies survive best. Other mutations in parkin, PINK1, and DJ-1 are likely to compromise the ability of mitochondria to resist stress.

metabolism in the globus pallidus, and fluorodopa (F-dopa) PET shows loss of striatal dopamine characteristic of Parkinson's disease (144). The PET/F-dopa measures dopamine function and SPECT/B-CIT tags the dopamine transporter. It has to be kept in mind, however, that PET/F-dopa is an indirect measure of striatal dopamine levels, and dopa decarboxylase activity only poorly correlates with nigral cell counts. The need for an accurate diagnostic method is amply demonstrated by studies indicating that clinical diagnosis during life is correct only about 75% of the time, even in specialist research centers (145). This is serious not only from a clinical diagnostic standpoint, but also because it affects the efficacy of clinical trials and epidemiological research. At present, the most advanced imaging biomarker for Parkinson's disease is F-dopa. The amyloid-binding ligand PIB (see above) has been shown to bind filaments of α -synuclein generated *in vitro* but does not interact with sections from Parkin-

son's brain that contain Lewy bodies, suggesting that the amyloid in these *in vivo* lesions is less accessible (146). Beyond ensuring optimal use of existing imaging biomarkers, it is critical to foster the development of new biomarkers. Given the likely multiple etiologies of Parkinson's disease and clear heterogeneity in expression of the clinical manifestations and progression, several biomarkers will probably be necessary to fully understand the disorder. To this end, two recent approaches have yielded some positive indicators, though neither is yet at the stage of providing an established diagnostic protocol. In the first approach, microarrays were used to detect changes in mRNA expression in blood cells, and a set of 22 genes was found to have differential expression in patient samples compared with control samples (147). The second approach was based on high-performance liquid chromatographic assays for a large number (many hundreds) of low-molecular-weight analytes in plasma, so-called

metabolomic profiling, that identified several potential biomarkers (148).

CURRENT AND FUTURE THERAPIES

The realization that Parkinson's disease was related to a deficit in dopamine production soon led to the first effective treatment, because dopamine levels can be supplemented pharmacologically. Dopamine is synthesized in neurons from L-tyrosine by a two-step process. Tyrosine hydroxylase catalyses the synthesis of 3,4-dihydroxy-L-phenylalanine (L-dopa or levodopa); this then is decarboxylated by dopa decarboxylase. Dopamine cannot cross the blood-brain barrier, but its metabolic precursor, L-dopa, can. This is usually given in conjunction with carbidopa, an inhibitor of peripheral decarboxylase that cannot cross the blood-brain barrier but prevents metabolism of L-dopa before it reaches its target tissue, where it is converted to dopamine by neuronal decarboxylase. Other current therapies include drugs, such as monoamine oxidase B inhibitors that reduce dopamine metabolism or dopamine receptor agonists, and physical interventions, such as deep brain stimulation with electrical pulses or surgical ablation of regions of the basal ganglia. However, L-dopa remains the standard treatment for Parkinson's disease, although it has undesirable side effects and efficacy wanes after treatment for several years.

In the longer term, transplantation of stem cells may eventually allow regeneration of neurons, and this might be the ultimate goal for treatment of most neurodegenerative diseases, though in the more immediate future other approaches are required. Because amyloidogenic proteins display toxic properties only when they form oligomers, one strategy is to prevent them from forming aggregates. Recently, simvastatin has been shown to reduce α -synuclein aggregation in cell cultures, providing a possible clue to a reported epidemiological link between simvastatin and a reduced incidence of Parkinson's disease (149). Molecules that can

bind and disrupt fibril formation have been discovered for several amyloidogenic proteins. Their mode of action seems to be insertion into the newly forming protein assemblies, which are held together by interactions between β -strands in an incipient β -sheet, preventing additional β -strands from being added. Several peptides derived from the central hydrophobic region (residues 68–72) of α -synuclein have been shown to prevent aggregation of the full-length protein (150). A cell-permeable version of one of these inhibitors was able to reduce toxicity in cells transfected with mutant A53T α -synuclein (151). The flavinoid compound, baicalein, has also been shown to inhibit α -synuclein aggregation and indeed to disaggregate existing fibrils (152). However, a potential danger in this approach is that although fibril formation might be prevented, smaller assemblies might be stabilized, as seems to be the case with baicalein. Because there is now good evidence that even low-n oligomers are toxic (see above regarding A β oligomers in Alzheimer's disease), this strategy could well be counterproductive, as it might shift the equilibrium of aggregation away from fibrils to more hazardous oligomeric species. Another approach would be to remove synuclein using antibodies in a manner analogous to the Alzheimer's disease therapy discussed above. Immunization of a human α -synuclein transgenic mouse to generate anti- α -synuclein antibodies reduced the deposits of α -synuclein in intracellular neuronal cell bodies (153). However, this approach is at least 5 years behind the analogous putative Alzheimer's therapy, which itself has encountered severe technical difficulties due to development of encephalitis.

CONCLUSIONS

During the past few years there has been mounting evidence that the underlying pathology in several neurodegenerative diseases arises from the production of soluble oligomeric assemblies of a protein characteristic of each disease. In the

case of Alzheimer's and Parkinson's diseases, these proteins are A β and α -synuclein, respectively. It is clear that such oligomers are deleterious to neurons in their vicinity, though the molecular mechanisms by which damage occurs remain to be established. Emerging therapies are likely to be based on preventing such assemblies from forming and/or persisting. When oligomeric assemblies grow in size, they can form insoluble deposits of amyloid fibrils. Emerging diagnostic tools are likely to include the early detection of these fibrils.

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