



Cell biology of protein misfolding: The examples of Alzheimer's and Parkinson's diseases

Dennis J. Selkoe

The salutary intersection of fundamental cell biology with the study of disease is well illustrated by the emerging elucidation of neurodegenerative disorders. Novel mechanisms in cell biology have been uncovered through disease-orientated research; for example, the discovery of presenilin as an intramembrane aspartyl protease that processes many diverse proteins within the lipid bilayer. A common theme has arisen in this field: normally-soluble proteins accumulate, misfold and oligomerize, inducing cytotoxic effects that are particularly devastating in the post-mitotic milieu of the neuron.

A blurring of the traditional distinction between basic and applied research is increasingly evident in the study of neurodegeneration. Complex and previously enigmatic diseases of post-mitotic neurons, such as Parkinson's, Huntington's and Alzheimer's diseases, seem to share elements of a common pathogenic process: the misfolding and progressive polymerization of otherwise soluble proteins. Although the molecular and cellular details vary greatly among these disorders, the tendency for highly soluble neuronal proteins to develop altered conformations as a function of time or genetic mutation and then aggregate inside cells — and in the case of Alzheimer's disease, also outside cells — precedes the earliest clinical signs of these diseases and is associated with profound neuronal dysfunction and death.

It is unclear whether protein misfolding and aggregation can explain the fundamental pathogenic basis for neurodegenerative diseases or rather represent an important but secondary step in the course of the disorders. Put another way, it remains to be determined whether inhibiting the oligomerization of the implicated proteins will actually prevent the clinical syndromes. A definitive answer will only come from human trials of compounds that target this mechanism. Perhaps the first disease in which such an answer may be obtained will be Alzheimer's disease.

The elucidation of many aspects of the Alzheimer riddle during the past two decades has relied heavily on the concepts and methods of cell biology. In turn, research focused squarely on Alzheimer's disease has sometimes helped to reveal new mechanisms and pathways in cell biology. One notable example is the discovery of presenilin and its strange ability to cleave diverse integral membrane proteins within the lipid bilayer. Many other examples of the mutually beneficial intersection of cell biology and research into Alzheimer's disease exist, including the study of the cytoskeleton, membrane trafficking, axoplasmic flow, intercellular signalling, synaptic function, and the biochemistry of kinases and proteases. Here, I will review our current understanding

of the cell biological mechanisms underlying Alzheimer's disease and then briefly compare the lessons learnt to those that are emerging from similar approaches to Parkinson's disease.

The Alzheimer phenotype

An enormous lead in the quest to decipher the biology of Alzheimer's disease (AD) has been provided by its robust cytopathological signature. Since 1907, the syndrome has been defined by the occurrence in brain regions serving memory and cognition of two types of lesions, neurofibrillary tangles and senile (amyloid) plaques (Fig. 1). Neurofibrillary tangles are masses of paired, helically wound protein filaments (PHF) lying in the cytoplasm of neuronal cell bodies and neuritic processes. Some of the PHF-bearing processes are clustered around the extracellular amyloid deposits, but many others are scattered widely in the neuropil of the limbic and association cortices. It seemed even in the early days of Alzheimer research that the PHF might be composed of a cytoskeletal protein gone awry — perhaps a microtubule or neurofilament subunit. Intensive research has established that PHF and the closely related straight filaments in tangles are insoluble, highly stable polymers of the microtubule-associated protein, tau^{1–6}. Tau constitutes a group of alternatively spliced cytoplasmic proteins that bear either three or four microtubule-binding domains and co-assemble with tubulin onto microtubules, where they stabilize these organelles and contribute to the formation of cross bridges between adjacent microtubules⁷.

The other major lesion is the amyloid plaque. These are extracellular deposits of insoluble, 8–10-nm amyloid fibrils that are polymers of the amyloid- β protein (A β)^{8,9}. One characteristic form of amyloid plaque is referred to as the neuritic plaque, namely, an extracellular core of amyloid fibrils intimately surrounded by dystrophic dendrites and axons — some of which contain bundles of PHF — as well as by activated microglia and reactive astrocytes¹⁰. Neuritic plaques occur in variable but generally high numbers in the molecular layer of the dentate gyrus of hippocampus, the amygdala, the association cortices of the frontal, temporal and parietal lobes, and within certain deep brain nuclei that

Dennis J. Selkoe is at the Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.
e-mail: dselkoe@rics.ewh.harvard.edu

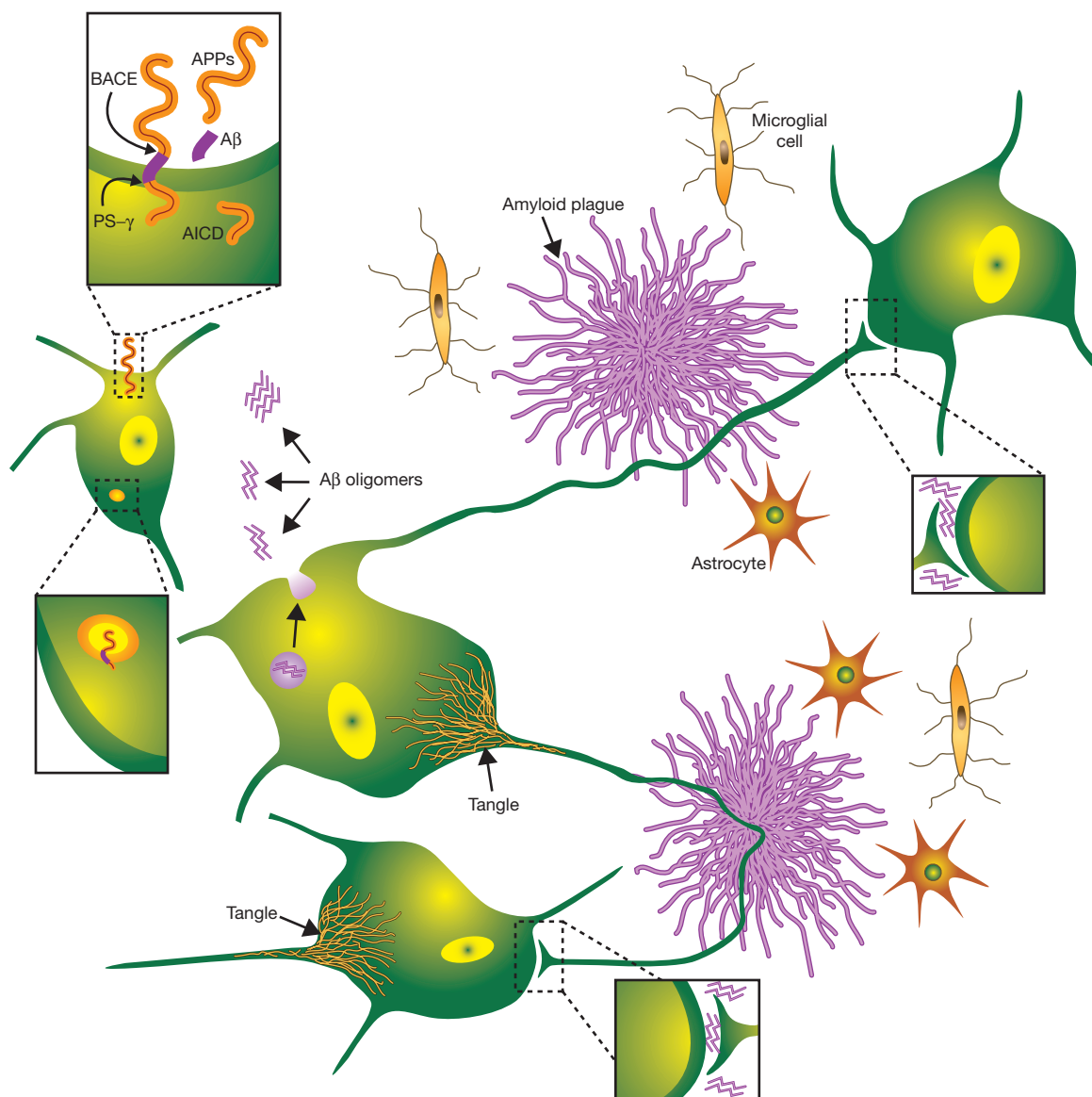


Figure 1 A model of key events underlying the pathogenesis of Alzheimer's disease, based on available evidence. APP molecules on the plasma membrane and in intracellular vesicles such as endosomes are cleaved by β -secretase (BACE) and the presenilin- γ -secretase complex (PS- γ) to liberate the A β region. A portion of A β peptides can oligomerize, initially intravesicularly, and be released into the interstitial fluid of brain, where soluble oligomers may diffuse into synaptic clefts and interfere with synaptic function by unknown mechanisms. A β oligomers can further

polymerize into insoluble amyloid fibrils that aggregate into spherical plaques, resulting in tortuosity and dysfunction of adjacent axons and dendrites. A major accompaniment of such events is the activation of kinases in the neuronal cytoplasm, leading to hyperphosphorylation of the microtubule-associated protein, tau, and its polymerization into insoluble filaments that aggregate as neurofibrillary tangles. Activated microglia and reactive astrocytes surrounding plaques participate in a local inflammatory response that may contribute to neurotoxicity.

project to these regions. The microscopic appearance of these 'mature' plaques suggests a focal deposit of amyloid protein that somehow incites a highly localized multicellular reaction.

In addition to neuritic plaques, an array of morphological variants of A β deposits is found in the Alzheimer brain. The advent of A β immunocytochemistry led to the recognition of innumerable 'diffuse plaques' in the limbic and association cortices and even in regions, such as striatum and cerebellum, that are not usually thought of as being targeted by the Alzheimer process. These diffuse deposits appear loose and granular by light microscopy and lack dystrophic neurites and altered astrocytes and microglia. The occurrence of

sometimes abundant diffuse plaques in the brains of some neurologically normal late middle-aged and elderly humans — and also in children with Down's syndrome (trisomy 21) as early as the second decade of life — has led to the hypothesis that they represent precursor lesions to the neuritic plaques, which bear fibrillar amyloid. Neuropathological analyses in humans, aged monkeys and mice transgenic for human APP have all supported this interpretation. Alzheimer brains typically exhibit a complex mixture of A β deposits that occur along a continuum from faint, wispy diffuse deposits to neurite- and glia-rich amyloid plaques, and even some dense amyloid cores devoid of surrounding cytopathology.

The elaborate processing of APP

A β is a 38–43-residue proteolytic fragment of APP¹¹ that is generated normally throughout life by virtually all mammalian cells^{12–14} (Fig. 2). APP is a ubiquitous Type 1 membrane glycoprotein that occurs as a heterogeneous group of polypeptides arising from alternative splicing, N- and O-linked glycosylation, phosphorylation, sulphation, glycosaminoglycan addition and complex proteolysis. A β is released from APP through sequential cleavages by β -secretase (also called BACE-1), a membrane-spanning aspartyl protease with its active site in the lumen¹⁵, and γ -secretase, an unusual intramembrane aspartyl protease containing presenilin at its catalytic site complexed with three other membrane proteins, nicastrin, Aph-1 and Pen-2 (refs 16–18). In addition, APP undergoes both constitutive and regulated secretory cleavages by certain metalloproteases referred to as α -secretases, principally believed to be ADAM 10 and ADAM 17 (refs 19, 20). The α -secretase cleavage occurs primarily between residues 16 and 17 of the A β region of APP, and any precursors so cleaved cannot yield A β peptides. Instead, processing by α -secretase followed by γ -secretase generates a smaller hydrophobic fragment referred to as p3 (refs 12, 21), whose normal function and role in Alzheimer's disease (if any) are unclear.

α -secretase processing releases the large APP ectodomain (APPs- α) from the cell (Fig. 2). Several biological activities have been ascribed to this secreted derivative *in vitro*, including as a trophic or neuroprotective factor, a serine protease inhibitor and a cell–substrate adhesion molecule. Sequential processing by α -secretase and γ -secretase also releases the APP intracellular domain (AICD), a portion of which can bind to the cytoplasmic adaptor protein, Fe65, and apparently help mediate aspects of nuclear signalling^{22–24}. However, there has been no consensus so far as to which transcripts may be regulated by complexes shown to contain AICD, Fe65 and the histone acetylase Tip60 (ref. 22). Genetic deletion of APP in mice produces a mild, non-lethal phenotype that includes relatively minor alterations in the adult CNS as well as defects in neurite development observed in cultured primary neurons^{25,26}. This lack of major consequence may be explained by an apparent functional redundancy with two APP homologues, APLP-1 (neuron-specific) and APLP-2 (ubiquitous). Whether transcriptional regulation is a *bona fide* function of APP and its two homologues remains to be seen.

Cellular and animal modelling provides insights into disease mechanisms

A recurring controversy in the study of proteins implicated in human neurodegenerative disorders is whether the polypeptide in question undergoes a loss or gain of function in the disease. But as the actual biochemical effects of mutations in such proteins are deciphered, attempting to answer this question seems to miss the mark. A compelling example is provided by the presenilins, missense mutations in which constitute the most common cause of dominantly inherited AD^{27,28}. The *Caenorhabditis elegans* homologue of presenilin, Sel-12, was identified as a critical mediator of Notch signalling, which is required for many cell-fate decisions in metazoans²⁹. It soon became apparent that loss of presenilin function prevents the proteolytic processing of Notch that releases its intracellular domain (NICD) to signal in the nucleus^{30–32} and likewise prevents the normal γ -secretase processing of APP to release A β ³³. The discovery that all presenilins contain two aspartate residues in transmembrane domains 6 and 7 of this putatively eight-spanning transmembrane polypeptide and that these represent the active site of γ -secretase revealed that presenilin functioned as an unprecedented intramembrane aspartyl protease^{34–36}. In this context, AD-causing missense mutations in human presenilin-1 or presenilin-2 alter the cleavage specificity of γ -secretase within the APP transmembrane domain, increasing the amount of a minor cleavage at the A β 42 residue while

slightly reducing the major cleavage at A β 40. This subtle perturbation does not notably alter the additional 'epsilon' cleavage (Fig. 2) of the Notch and APP transmembrane domains near their cytoplasmic faces, and therefore does not seem to interfere with NICD and AICD release. In short, the AD-causing presenilin mutations produce a subtle biochemical alteration of presenilin proteolytic function that cannot easily be construed as either loss or gain of function.

Over time, it has become clear that presenilin has many substrates, all of which have so far been shown to be single-spanning transmembrane proteins that must first undergo α -secretase-mediated shedding of their ectodomains before presenilin- γ -secretase can cleave within the membrane³⁷. It could be reasoned that presenilin missense mutations cause Alzheimer's disease by misprocessing one or more of these other substrates. However, when the effects of the other genes implicated so far in familial AD are considered — namely APP³⁸ and the ϵ 4 allele of apolipoprotein E³⁹ — it becomes clear that this is not the case. Most of the disease-causing missense mutations in APP are clustered at, or adjacent to, the β - or γ -secretase cleavage sites (Fig. 2). These increase A β 42 production roughly twofold or so, as has been shown in transfected cells, transgenic mice and the affected individuals themselves^{40–44}. The remaining APP mutations occur near the middle of the A β region and enhance the oligomerization and fibril formation of the resultant mutant A β peptides^{45–48}. Moreover, duplication of the APP gene in patients with Down's syndrome invariably leads to very early formation of diffuse A β deposits, followed by the age-dependent development of amyloid-rich plaques containing altered neurites, microglia and astrocytes, as well as neurofibrillary tangles⁴⁹. In the case of apoE4, inheritance of one or two alleles sharply increases the likelihood of developing AD⁵⁰ and enhances amyloid plaque levels in the brain⁵¹. The mechanism seems not to involve increased A β production but rather stabilization and decreased clearance of fibrillar A β deposits, as revealed by expression of human ApoE4 compared with ApoE3 in ApoE-knock-out mice⁵².

Common, 'idiopathic' cases of AD seem to be phenocopies of the inherited cases, both clinically and neuropathologically, except that the latter cases occur earlier in life. Thought of in this way, the genetically determined cases represent an acceleration of a disease process that occurs gradually with advancing age. Before inheritance of ApoE4 alleles was recognized as a specific risk factor for AD, these cases were considered to be part of the huge 'sporadic' AD population.

Cell biological consequences of A β accumulation

Taken together, the available information about genotype-to-phenotype conversions in familial AD supports the 'A β hypothesis': a chronic imbalance between the production and clearance of a small hydrophobic peptide with a tendency to misfold and aggregate leads gradually to synaptic and neuritic compromise and glial activation⁵³. But even if this general model is accepted, and it is not by some, the details of how neuronal dysfunction ensues remain unclear. Looking at the Alzheimer brain, there are many possible consequences. The dilated and tortuous dendrites and axons in the immediate vicinity of amyloid plaques suggest that altered intracellular transport and slowed nerve conduction may well contribute to compromised information processing in the limbic and association cortices⁵⁴. APP travels by fast axonal transport⁵⁵, and there is recent evidence that intact APP molecules may interact with kinesins, either directly or indirectly, and thus help to mediate the axonal transport of various molecules, including presenilin and other secretases⁵⁶. An attack by A β aggregates on local neuritic integrity and function could contribute to the development of cognitive failure.

But there is a potentially more subtle effect that could induce neuronal dysfunction early in the pre-clinical phase of the disease. The concept that subtle alterations of hippocampal synaptic efficacy may

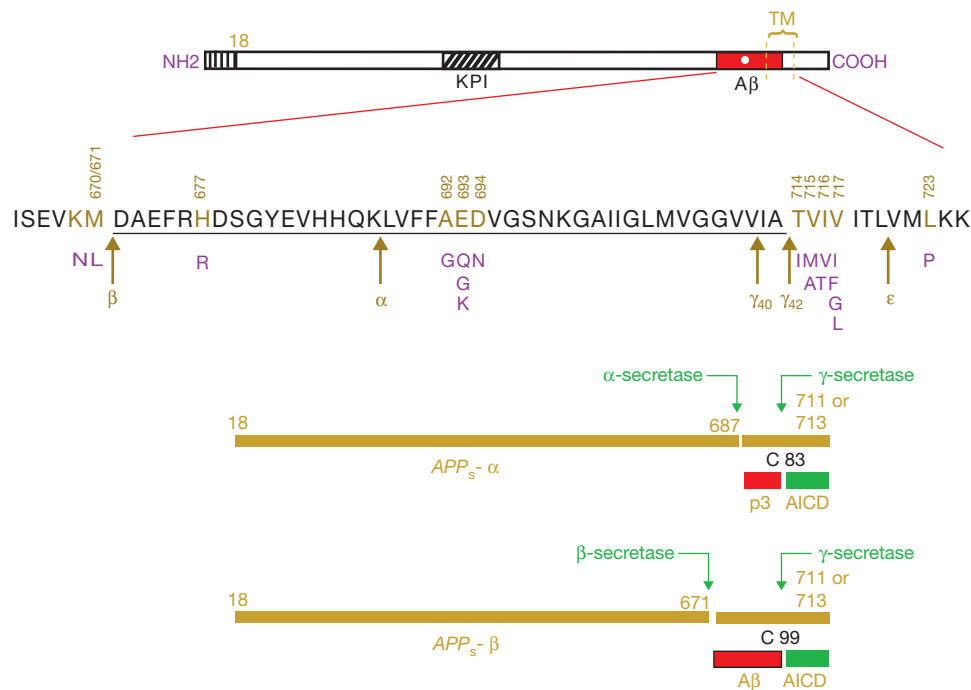


Figure 2 Schematic diagrams of the β -amyloid precursor protein (APP) and its principal proteolytic derivatives. The first line depicts the largest of the known APP alternative splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct linear positions. A 17-residue signal peptide occurs at the N-terminus. Two alternatively spliced exons of 56 and 19 amino acids are inserted at residue 289; the first contains a serine protease inhibitor domain of the Kunitz type (KPI). A single transmembrane domain (TM) at amino acids 700–723 is indicated. The amyloid β -peptide ($A\beta$) includes 28 residues just outside the membrane plus the first 12–14 residues of the TM. In the second line, the sequence within APP that contains the $A\beta$ and TM regions is expanded. The underlined residues represent the $A\beta$ 1–42 peptide. The purple letters below the wild-type sequence indicate the known missense mutations identified in certain families with Alzheimer's disease and/or hereditary cerebral haemorrhage

with amyloidosis. Three-digit numbers represent codon numbers (APP 770 isoform). In the third line, the first arrow indicates a site (after residue 687; same as white dot in $A\beta$ box in first line) of cleavage by α -secretase that enables secretion of the large, soluble ectodomain $APP_s-\alpha$ into the medium and retention of the 83-residue C-terminal fragment (C83) in the membrane. C83 can undergo cleavage by γ -secretase principally at residue 711 or residue 713 to release the p3 peptides. The fourth line depicts the alternative proteolytic cleavage after residue 671 by β -secretase that results in the secretion of the slightly truncated $APP_s-\beta$ molecule and the retention of a 99-residue C-terminal fragment in the membrane. C99 can also undergo cleavage by γ -secretase to release the $A\beta$ peptides. Cleavage of both C83 and C99 by γ -secretase at the epsilon (ϵ) site (line 2) releases the APP intracellular domain (AICD) into the cytoplasm. The order and interdependency of the γ - and ϵ cleavages have not been established

be caused by diffusible oligomeric assemblies of $A\beta$ has received support from the significant correlations between cortical levels of soluble $A\beta$ (which includes soluble oligomers) and the degree of cognitive impairment^{57–59}. Moreover, in certain APP transgenic mouse models, biochemical and electrophysiological evidence of synaptic alteration can be detected before visible plaque formation but after $A\beta$ levels start rising steadily^{60–63}. Mechanistically, cerebral microinjection of naturally secreted $A\beta$ oligomers can both inhibit hippocampal long-term potentiation⁶⁴ and interrupt memory of a learnt behaviour in living rats⁶⁵. Moreover, behavioural deficits in aged APP transgenic mice were reversed overnight by injection of an anti- $A\beta$ antibody, without any decrease in amyloid deposits⁶⁶. These and other findings suggest that soluble oligomers of $A\beta$ can directly compromise synaptic function and that amyloid plaques may function as reservoirs of fibrous polymers that are in equilibrium with these diffusible species.

A major adverse consequence of the cerebral accumulation of various $A\beta$ assembly forms is the induction of neurofibrillary changes in myriad cell bodies and processes. Precisely how this comes about is not yet clear. In general, a variety of distinct neuronal insults (including, for example, brain trauma, rare viral infections of the brain and lipid storage disorders) can lead to secondary alterations of tau that result

in NFT in diverse brain diseases. The genetics of AD suggest that $A\beta$ accumulation is the factor that ultimately triggers NFT formation in this disorder. Regardless of the specific precipitating factor, tau hyperphosphorylation can lead to the detachment of tau from microtubules and the consequent destabilization of these vital organelles^{7,67}. This tendency towards less stable microtubules represents a loss of tau function, but at the same time, a portion of the free tau molecules can now polymerize into insoluble cytoplasmic filaments (PHF). These can seemingly confer new, toxic effects that are likely to include disturbed axonal and dendritic transport, altered neuronal metabolism and structural changes in cell bodies and neurites. That this inexorable process of neurofibrillary degeneration is lethal to both the neuron and, ultimately, the patient, has been established by the discovery of missense and splicing mutations in tau that directly cause a group of less frequent dementing illnesses, which includes frontotemporal dementia with parkinsonism^{68–70}. In these disorders, the mutant tau molecules show decreased binding to microtubules, consequent loss of function, and florid NFT formation — all in the absence of $A\beta$ deposits. The lack of the latter lesions, coupled with the fact that inheritance of APP mutations invariably leads to NFT composed of wild-type tau, provides compelling genetic evidence that $A\beta$ build-up can induce tangles, but that the converse has not been established. This sequence is further

supported by studies in transgenic mouse models^{71–73}. Precisely how A β accumulation in AD leads to the hyperphosphorylation of wild-type tau molecules is under intensive study and may include changes in calcium homeostasis and consequent activation of certain kinases⁷⁴.

A further important consequence of progressive A β accumulation is the activation of local microglia and astrocytes in the vicinity of A β deposits, with attendant expression and release of various pro- and anti-inflammatory cytokines and acute-phase proteins⁷⁵. This process seems to represent a response of the brain's innate immune system to the build-up of A β . The epidemiological observation that prolonged usage of non-steroidal anti-inflammatory drugs such as ibuprofen offers partial protection against the development of AD could be explained by an anti-inflammatory effect, but such drugs have also been shown to function as direct modulators of γ -secretase that selectively lower A β 42 production⁷⁶.

Unresolved issues in the cell biology of Alzheimer's disease

Although progress in elucidating the biological mechanisms of AD has led to clinical trials of agents that might slow or even prevent the disease, numerous questions remain to be answered. Precisely where in the cell A β is generated and how much of it is then secreted continue to elicit experimental interest. APP traffics from the endoplasmic reticulum (ER) to the cell surface, and there is evidence for the production of A β peptides as early as in the ER but also in the Golgi and *trans*-Golgi network (TGN), at the plasma membrane and in recycling endosomes^{21,77–79}. It has been postulated that a portion of any A β peptides generated early in the secretory pathway (particularly A β 42) remains inside the cell, and these could contribute to intracellular A β accumulation, which has been documented in neurons in culture and in the Alzheimer brain^{79,80}. However, a substantial portion of A β peptides is secreted and thus found in extracellular fluids (including CSF and plasma¹³), and A β immunohistochemistry reveals extracellular deposits (that is, diffuse plaques of A β 42) very early in the development of AD-type neuropathology in Down's syndrome⁴⁹.

A closely related problem is determining where in the cell the presenilin- γ -secretase complex operates. This unique protease processes cell-surface Notch molecules after they make contact with ligands found on adjacent cells, but much of presenilin is found in the ER and Golgi, suggesting a 'spatial paradox'⁸¹. This issue has been clarified by detecting portions of presenilin⁸² and the other three γ -secretase components directly on the plasma membrane, where they complex with their cognate APP substrates (C83 and C99)⁸³. It is now important to understand where in the cell the γ -secretase complex first encounters its substrates, how APP substrates enter into the unusual active site of this channel-like protease, and how its many substrates manage to compete for processing in an orderly fashion. From a therapeutic perspective, it might be possible to inhibit β - or γ -secretase on the plasma membrane without requiring the inhibitor to enter the cell.

Although the gene products implicated in causing AD are expressed ubiquitously, the disorder targets the brain and, in particular, the limbic and association cortices. If it turns out that A β actually drives the disease, one explanation for this anatomical predilection could come from the realization that BACE-1 is expressed almost exclusively in brain and particularly in neurons. It is unclear to what extent the unusual asymmetry of neurons, with their very long axonal processes, is the key to understanding the development of the disease. The axonal transport of APP and its processing enzymes to synaptic terminals could be critical for the initiation of the disease, or else the generation of A β in neuronal cell bodies and dendrites (as well as in glia and vascular cells) could function to elevate the extracellular pool of A β 42 (including secreted oligomers), thus allowing its subsequent precipitation into innumerable plaques.

Finally, it may be possible to further unravel the multifaceted responses of neurons and glia to the insidious accumulation of A β during aging and in AD. Although transgenic mice may allow the dissection of elements of the reaction in a controlled temporal fashion⁷³, these models do not necessarily reproduce all aspects of the human disorder (for example, frank neuronal loss). Moreover, many cellular responses probably occur virtually simultaneously in humans and in transgenic mice. However, it may not be necessary to understand all the steps of pathogenesis in exquisite detail in order to intervene successfully with disease-modifying therapies.

A brief look at Parkinson's disease: parallels to and distinctions from AD

Although selective degeneration of dopaminergic and noradrenergic midbrain neurons was recognized as the essential pathological substrate of Parkinson's disease (PD) more than four decades ago, the molecular events causing this loss remained obscure in most patients. This situation has begun to change in the last few years with the discovery of no less than five genes that predispose individuals to rare, familial forms of the disease⁸⁴. Although the large majority of PD cases seem to occur sporadically, the portion caused by mutant genes is higher than once thought and is continuing to rise. As in the case of AD, infrequent familial forms of PD could turn out to be highly instructive as regards the pathogenesis of the common ('idiopathic') form of the disorder.

Dopaminergic neurons in the substantia nigra and noradrenergic neurons in the locus coeruleus develop characteristic filamentous inclusions in PD called 'Lewy bodies'. The identification of the first familial PD gene as α -synuclein⁸⁵ led to the recognition that the principal subunit protein of the filaments comprising Lewy bodies in idiopathic PD was wild-type α -synuclein^{86,87}. Although normally a 'natively unfolded' soluble protein, α -synuclein forms amyloid-like filaments under certain conditions that include mitochondrial complex I deficiency⁸⁸ and other forms of oxidative stress⁸⁹. Mice lacking α -synuclein are resistant to the neurotoxic effects of the complex I inhibitor, MPTP⁹⁰. α -Synuclein can also form small protofibrillar intermediates that may contribute to its cytotoxicity⁹¹. Very rare missense mutations in α -synuclein cause a dominant PD-like syndrome. Moreover, genomic triplication of the wild-type α -synuclein gene can cause PD⁹², a situation remarkably analogous to the duplication of APP in Down's syndrome that invariably induces the AD phenotype.

The second — and most common — mutant gene implicated in familial PD is parkin⁹³, and various loss-of-function mutations occurring in both alleles produce an aggressive, generally early form of PD in which dopaminergic midbrain neurons are lost, but the remaining cells usually lack Lewy bodies. Parkin is a cytoplasmic neuronal protein that contains two ring-finger domains, plus a ubiquitin-like domain at its amino terminus. This structure is consistent with parkin functioning as an E3 ubiquitin ligase, and such activity has been demonstrated^{94,95}. Several substrates for the ubiquitin-ligase function of Parkin have been suggested, but there is no agreement as to which of these are *bona fide* substrates *in vivo*, as homozygous deletion of Parkin in mice does not seem to elevate brain levels of these proteins, although it also does not produce dopaminergic neuronal loss⁹⁶. In the brains of such mice, the down-regulation of several proteins implicated in the oxidative stress process suggests that Parkin could also function in this pathway⁹⁷ and fits well with considerable epidemiological and animal modelling evidence that idiopathic PD may be triggered in some cases by chronic exposure to environmental toxins (for example, rotenone, paraquat or MPTP) that can inhibit mitochondrial complex I, promote free radical stress and induce Lewy-body-like inclusions containing α -synuclein in dopaminergic neurons^{98,99}.

Three other genes have been identified so far as being associated with very rare forms of familial parkinsonism: ubiquitin C-terminal hydrolase-L1 (UCH-L1)¹⁰⁰, DJ-1 (ref. 101) and Pink-1 (ref. 102). UCH-L1 also possesses enzymatic activity as a type of ubiquitin ligase¹⁰³, placing it in a pathway potentially related to parkin. DJ-1 is a cytoplasmic neuronal protein of unclear function, but structural homology considerations suggest it may normally mitigate against protein misfolding¹⁰⁴. Pink-1 is a mitochondrial protein kinase that seems to protect neurons from stress-induced mitochondrial dysfunction and apoptosis¹⁰².

At this early juncture in the study of genes that predispose to PD, it is unclear whether there will be a convergence of most or all of the implicated gene products in a common pathogenic pathway, analogous to the four confirmed AD genes that all lead to increased A β accumulation in the brain. Much work is now needed to determine whether the misfolding and oligomerization of α -synuclein has a central role in the pathogenesis of dopaminergic neuronal dysfunction analogous to that of A β in the Alzheimer cascade. There are several notable differences between the two syndromes: first, intraneuronal protein aggregates characterize PD, whereas both extra- and intra-neuronal aggregates accumulate abundantly in AD; second, the characteristic protein aggregates of PD are usually (but not always) absent in cases caused by the most commonly implicated gene (parkin); third, all genes implicated so far in familial AD are transmitted as autosomal-dominant traits, whereas familial PD can arise from both dominant and recessive inheritance; fourth, the Parkinson process targets primarily subcortical neurons, whereas the AD process principally compromises neurons in limbic and association cortices; and fifth, and perhaps most important, is the growing evidence that mitochondrial dysfunction, oxidative stress and the ubiquitin–proteasome pathway are centrally implicated in PD, whereas these may function in AD but seem to be farther downstream in the cascade. Despite these distinctions, it makes abundant sense to continue to look for opportunities to apply the lessons learnt from deciphering the role of misfolded proteins in AD to the effort to understand selective neuronal loss in the second most common neurodegenerative disease.

Factors that make otherwise soluble proteins prone to misfolding and aggregation

As already stated, a common feature of slowly progressive neurodegenerative disorders is the accumulation of protein aggregates rich in β -pleated sheet conformation that can confer toxic properties on cells. Clearly, a full answer to the question of why this misfolding occurs is not yet possible, just as a detailed understanding of the normal mechanisms of protein folding is not at hand. But factors that predispose certain proteins to misfolding have been recognized, based in part on emerging knowledge of the biochemistry of systemic amyloid diseases, such as primary (immunoglobulin) and secondary (serum amyloid A) amyloidosis. One such factor is the occurrence of a missense mutation in a polypeptide that enhances the likelihood that it will self-aggregate into dimers and higher-order assemblies. The mutation may directly foster homotypic protein interactions, as is the case for the intra-peptide mutations within the central hydrophobic core of A β . This process of folding into a β -sheet-rich conformation and aggregating helps bury otherwise exposed hydrophobic residues. Alternatively, a mutation may alter the normal properties of a protein, leading secondarily to its accumulation to levels that exceed the critical concentration required for self-association, as in the case of tau mutations that decrease the normal sequestration of tau on microtubules.

Proteolysis is another factor that can contribute to amyloidogenic potential. In the case of serum amyloid A protein, limited proteolysis creates a 76-residue fragment that is more prone to self-association than the parent molecule. In a different way, altered proteolysis of APP caused

by presenilin mutations increases the A β 42-to-A β 40 ratio, and the extra two hydrophobic residues (alanine and isoleucine) notably enhance the misfolding and aggregation of the peptide. In general, high local concentrations of a protein coupled with certain biochemical conditions (for example, low pH, exposure to hydrophobic surfaces on lipids and, in the example of α -synuclein, oxidative stress) may favour self-association. Deficiencies in protein chaperone systems may also contribute to a tendency for a polypeptide to lose its native structure and misfold. Of special importance is time, as achieving sufficiently high concentrations, overcoming energetic barriers to the loss of native structure and gain of β -sheet-rich structure, and forming a 'seed' for aggregation (for example, a dimer) all seem to require great time. The neurodegenerative diseases — and amyloidoses in general — are strongly age-dependent, even though in some cases a mutant protein has been highly expressed since before birth. Of course, a considerable part of this lag time represents the gradual accrual of large protein deposits that function as reservoirs of cytotoxic species and also the accrual of sufficient cell dysfunction and loss to cause clinically detectable symptoms.

All things considered, it is abundantly clear that disease-orientated research and the fundamental study of cell biology have found a highly salutary intersection in the effort to understand, and ultimately resolve, human neurodegeneration.

COMPETING FINANCIAL INTERESTS

The author declares competing financial interests: see *Nature Cell Biology* website for details.

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