Neurodegeneration: new clues on inclusions

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The rare neurological disorders frontotemporal dementia and British dementia have been linked to two mutant genes whose products constitute the fibrils that define the two disease pathologies. Two recent studies add to the mounting circumstantial case that protein fibrillization, inside (neurofibrillary tangles) or outside (amyloid plaques) of the neuron, may be pathogenic and suggest that either or both of these mechanisms could initiate Alzheimer's disease.

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Introduction

Modern criteria to diagnose Alzheimer's disease (AD)

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setting of dementia. As a deeper understanding of the composition and generation of these pathological entities emerged, a fierce debate developed concerning where these two very distinct entities are positioned in the AD pathogenic cascade. Further complicating the debate is a group of patients with neurodegenerative conditions that overlap clinically and pathologically with AD, but show a surprising variety of confounding lesions. Perhaps most common are those conditions with Lewy bodies that affect as many as 10–20% of patients who are clinically diagnosed with 'probable AD'. Lewy bodies are intracellular fibrillar deposits that typically contain the protein α-synuclein and often also contain additional abundant cytoplasmic proteins, including ubiquitin. They occur either in combination with extracellular amyloid plaques (Alzheimer's disease–Lewy body variant, AD–LBV; see Table 1) or alone (diffuse Lewy body disease; DLBD). To what extent these disorders represent variants of AD and at what point they diverge from the pathway that leads to classical AD can only be resolved by applying the tools of molecular genetics (to identify disease-linked mutations, determine disease-associated patterns of gene expression and construct gene-targeted animal models), cell biology (to determine the link between protein fibrillization and neuronal death) and protein chemistry (to characterize the responsible proteins and to find drug-like molecules to alter their behavior *in vivo*).

The earliest genetic information, now almost ten years old, strongly implicates the formation of extracellular amyloid plaque as a key process in the pathogenesis of AD. Although most cases of late-onset AD are sporadic, rare early-onset forms of AD (FAD) carry mutations that generally influence the production of Aβ, the major plaque protein [1]. This discovery focused attention on the fibrillization of Aβ as a key upstream event in AD pathogenesis. The ultimate test of this proposal will depend on studies of compounds that inhibit specific steps in the generation, assembly and deposition of Aβ. In order to test the effects of such compounds, it is critical to produce animal models with AD-like symptoms, in addition to AD-like pathology. Without such models we can only rely on the accumulation of circumstantial evidence regarding the role of the various fibrillar deposits.

Because other neurodegenerative diseases share many of the properties of AD [2], particularly with regard to the self-assembly of proteins into pathological aggregates, it is timely to discuss here two such rare diseases with recent insights that may apply to this entire category of diseases. These diseases are frontotemporal dementia (FTD) and

ning to emerge. FTD is characterized by intracellular accumulations of hyperphosphorylated tau isoforms that form a variety of inclusions, including, but not limited to, classical

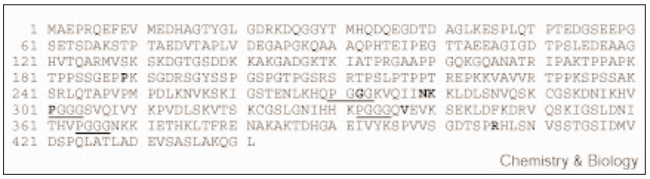
Table 1

Dementias characterized by fibrillar protein deposits.

Familial disease	Intracellular protein pathology	Extracellular protein pathology	Gene/protein
FAD	NFTs and Lewy bodies	Aβ amyloid plaques	APP/Aβ
FTD	NFTs and other tau inclusions	None	Tau/tau
FBD	NFTs	ABri amyloid plaques	BRI/ABri
Sporadic disease	**Intracellular protein pathology**	**Extracellular protein pathology**	**Fibrillar proteins**
AD	NFTs	Aβ amyloid plaques	Tau and Aβ
AD–LBV	Lewy bodies	Aβ amyloid plaques	α-Synuclein and Aβ
DLBD	Lewy bodies	None	α-Synuclein

FAD, familial Alzheimer's disease; FTD, frontotemporal dementia; FBD, British dementia.

Figure 1



Human tau sequence. One signature of the repeated sequences in the microtubule-binding domain is PGGG, which is underlined. Residues that are mutated in FTD are in bold.

AD NFTs. About 14 different mutations in the tau gene are responsible for about half of the cases clinically grouped under the term FTD (reviewed in [3]; see Figure 1). In contrast, FBD is characterized by both extracellular amyloid plaques (albeit of a different protein composition than those in AD) and NFTs. FBD is caused by a mutation in the gene encoding the major plaque protein [4].

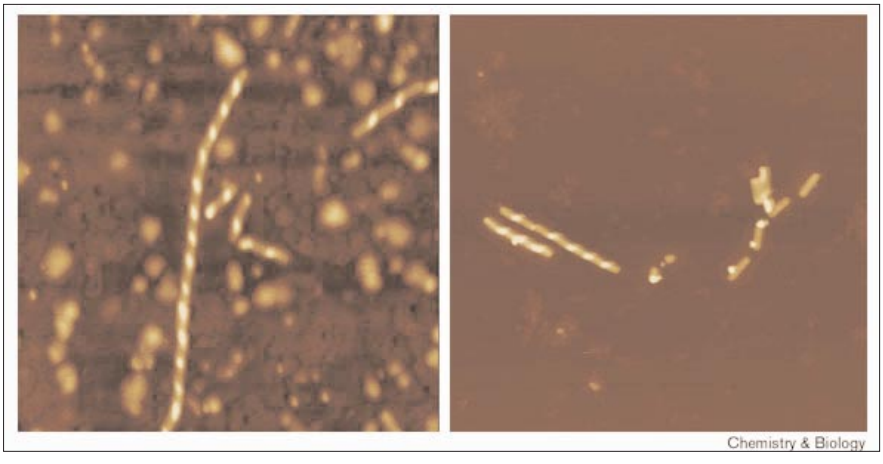
Frontotemporal dementia and mutations in the tau gene

The recent discovery of mutations in the tau gene that lead to the complex phenotypes collected under the clinical term fronto-temporal dementia (FTD) have pointed squarely at sites in tau that can lead to the intracellular accumulation of tau aggregates. Although these diseases are quite rare, the presence of intracellular tau aggregates, called neurofibrillary tangles, in AD suggests that a deeper understanding of how the tau protein self-assembles into abnormal filaments may have broader ramifications. In AD, tau assumes a characteristic ultrastructural appearance in which two filaments, each about 15 nm thick, helically wind around each other with a periodicity of 70–80 nm (Figure 2). This is not the only configuration found among tau aggregates in either AD or in FTD, however. Tau can form straight filaments or even diffuse intracellular free tau that is no longer found in its normal intracellular location, in association with microtubules. The one common denominator found in all tau inclusions is the presence of a hyperphosphorylated form of tau detectable with a variety of antibodies directed against specific phosphorylation sites. The origin of the hyperphosphorylated state of tau probably sits at or near the enigmatic center of the neurodegenerative disease process. One of the more intriguing ideas about the observed hyperphosphorylation of tau is that it represents the activation of a mitotic program in a cell (the neuron), which is irreversibly post-mitotic.

Tau is a substrate of the prolyl isomerase Pin1

Amide bonds involving the proline secondary amino group populate both *cis* and *trans* configurations and the interconversion between the two is often a slow step in protein folding. Consequently, prolyl isomerase enzymes exist that catalyze this reaction, possibly by a nucleophilic mechanism. The prolyl isomerase Pin1 isomerizes Ser–Pro or Thr–Pro bonds and therefore serves as a highly conserved and essential regulator of mitotic phosphoproteins [5–8]. The recent discovery that Pin1 may significantly contribute to the dysfunction of tau and the neurofibrillary tangle of AD provides an intriguing novel target [9]. The paper by Lu and colleagues [9] reports that Pin1 is depleted from the soluble fraction of brain and accumulates in the insoluble fraction, where it binds to the tau protein in the neurofibrillary tangles. Through its WW domain, Pin1 also showed specific and high-affinity binding to one tau peptide containing pThr231, one of the several sites on tau phosphorylated in AD brain. How the other tau phosphorylation sites in the Alzheimer brain might be affected by Pin1 activity is unknown. Interestingly, regarding the curious relationship to mitotic activation, this site is readily phosphorylated *in vitro* by cdc2 [10–12]. When tau is phosphorylated by cdc2 *in vitro*, its ability to bind and polymerize microtubules is reduced, an effect that can be reversed by incubation with Pin1. This key observation suggests that isomerization of a proline amide permits tau to bind to microtubules in the

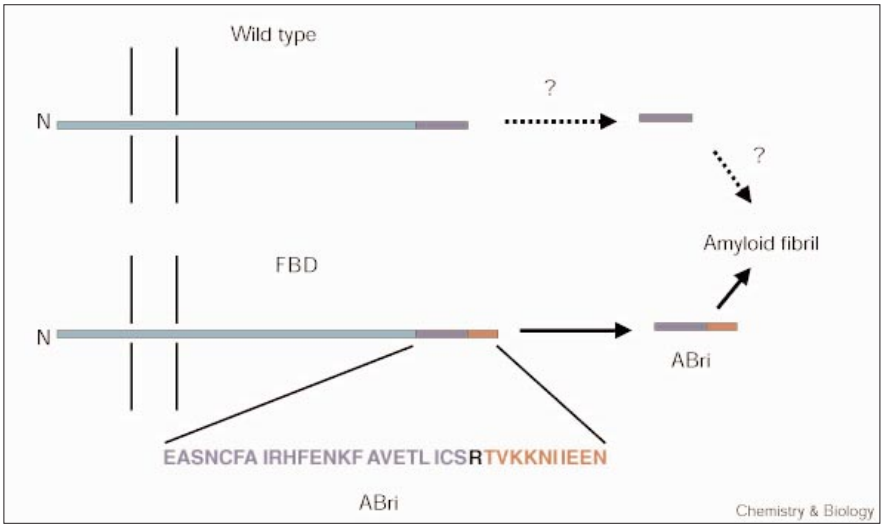
Figure 2



Atomic force microscopy images of brain-derived tau, in the form of paired helical filaments (PHFs). The helical periodicity of 70 nm is clearly visible in both images. These fibrils resemble amyloid fibrils produced *in vitro* from synthetic Aβ (helical periodicity of ~40nm). Images are 1.25 μm (left panel) and 1.5 μm (right panel) square. PHFs were isolated by Lisa Flanagan and imaged by James Harper.

Figure 3

A cartoon depiction of several possible scenarios to explain the pathogenicity of the FBD mutation. The known aspects are shown in color. These include the sequence of the ABri peptide and its relationship to the wild-type and mutant precursor sequences. (Note the carboxy-terminal extension of the mutant precursor in red.) It is also clear that the ABri peptide is cleaved from mutant precursor and that this peptide fibrillizes *in vivo* (solid arrows). In contrast, it is unknown whether the truncated version of ABri, derived from the wild-type precursor, is amyloidogenic or, in fact, whether it is released by proteolysis in the first place (see text). In addition, the putative transmembrane region has not been experimentally verified, nor has the orientation of the wild-type and mutant proteins [4]. See the note added in proof for a recent development.



presence of a phosphate group that is otherwise inhibitory towards binding. In attempting to relate the finding to the disease, the authors suggest that tau hyperphosphorylation may create a number of binding sites that exceeds the availability of Pin1. Alternatively, the sequestration of Pin1 in AD brain depletes soluble Pin1, which may lead to apoptosis [5].

Familial British dementia and mutations in the gene BRI

FBD is an autosomal dominant disorder of undetermined etiology. It appears to be a rare disease, but its clinical, genetic and pathological analysis has been facilitated by the existence of a large afflicted family, comprising more than 200 members and encompassing seven generations. FBD, like FAD and FTD, is a late-onset progressive dementia, but patients also exhibit other features associated with cerebellar involvement, such as spasticity and ataxia (balance problems). The FBD brain is characterized by amyloid plaques in the hippocampus and cerebellum, amyloid deposits in the vasculature, and, like FAD and FTD, cytoplasmic neurofibrillary tangles that resemble those characteristic of AD [13].

Although the FBD pathology resembles some types of FAD and prion disease, no staining of brain sections for Aβ or the prion protein could be demonstrated using immunohistochemical methods [14]. The identity of the FBD amyloid plaque protein was therefore unknown. A recent breakthrough paper describes the identification, using biochemical methods, of this protein and the subsequent discovery of the genetic basis for this rare disorder [4]. Using an approach similar to that which allowed George Glenner and coworkers [15] to identify Aβ as the major AD amyloid protein, the New York group [4] was able to purify an ~4 kDa peptide (designated ABri). Using partial sequence information from purified tryptic fragments, a previously uncharacterized gene on chromosome 13 was identified that encodes a 266 residue protein, designated the ABri precursor protein (Figure 3). Interestingly, the tryptic peptide derived from the ABri carboxyl terminus was perfectly homologous to a sequence to the 3′ side of the first stop codon and immediately before the next in-frame stop codon. This portion of the wild-type gene would not be expected to be translated.

Once the ABri precursor gene had been sequenced, the affected family members were analyzed in order to determine the molecular basis for FBD. Seven affected individuals had a single nucleotide transition in stop codon 267, which resulted in its replacement with an arginine residue. This change would allow translation to continue to the next stop codon, resulting in the addition of 11 residues at the protein carboxyl terminus (Figure 3). All the unaffected kindred retained the stop codon, as did 42 unrelated individuals with unrelated neurologic disorders and 72 normal individuals of comparable ethnicity (the stop codon was also conserved in the mouse sequence). Finally, antibodies generated against brain-derived FBD amyloid as well as synthetic fragments of ABri stained amyloid plaques in brain tissue sections from FBD patients, but not plaques from AD patients or patients with several other brain and systemic amyloidoses.

The ABri precursor protein is widely expressed in brain, as well as several peripheral tissues, such as the placenta, kidney and pancreas. Sequence analysis suggests that the ABri precursor has a single membrane-spanning segment (residues 52–74, Figure 3) and, furthermore, suggests that it is a 'type II' integral membrane protein, in which the short amino-terminal segment is located on the cytoplasmic face of the membrane. This topology has yet to be

confirmed (J. Ghiso, personal communication) and is suspect, because no signal sequence for insertion into the endoplasmic reticulum (ER) membrane was detected. The ABri sequence (Figure 3) is not homologous to any known amyloid proteins, which is not unexpected, because none of this group is homologous, despite the fact that they all form amyloid fibrils of similar morphology. The amino acid composition of ABri is unremarkable and offers no clues as to its amyloidogenicity. Several scenarios regarding the precursor-to-ABri conversion can be envisioned (Figure 3). First, the carboxy-terminal extension may create a cleavage site in the precursor that releases ABri into the extracellular space/ER/vesicle or into the cytoplasm, depending on the topology of the precursor. It seems unlikely, however, that the extension would create a new proteolytic site 24 residues upstream. Alternatively, both the wild-type and mutant precursors may be cleaved but the wild-type cleavage product may be soluble and easily degraded, in contrast to ABri, which is fibrillogenic. Finally, it is possible, but without precedent, that the carboxy-terminal extension could reverse the topology of the ABri precursor, leading to intracellular release of a fibrillogenic peptide (after cell death and lysis, plaques could end up extracellular). The observation that FBD amyloid contains fragments of a normally cytoplasmic protein, tubulin [16], may prove to be important in distinguishing between these scenarios and in determining the mechanism of ABri neurotoxicity.

Conclusions

FTD and FBD clearly constitute two more examples of neurodegenerative diseases that are characterized by fibrillar deposits and are autosomal dominantly inherited via point mutations in the genes encoding proteins that undergo fibrillization in these diseases. This list includes early-onset AD, early-onset Parkinson's disease and Huntington's disease [2]. It remains to be determined whether these diseases share an underlying neurotoxic mechanism and whether they may be treated using similar therapeutic strategies. It is clear that, as these diseases are more closely analyzed, disease-specific targets for therapeutic intervention, such as the PPI enzyme discussed above, will emerge.

Note added in proof

Some of the questions posed in Figure 3 are addressed in a recent study of the topology and metabolism of the products of the FBD gene (termed BRI-L by these authors, referred to as ABri precursor above) and its normal relative (BRI) (Kim, S.-H., *et al.*, & Sisodia, S.S. (1999). Furin mediates enhanced production of fibrillogenic ABri peptides in familial British dementia. *Nat. Neurosci.* **2**, 984-988). A variant of BRI in which both termini were ligated to epitope tags was expressed in mouse neuroblastoma cells. The predominant topology of the chimeric protein was demonstrated to be that predicted by Ghiso and coworkers [4] with the amino-terminal sequence in the cytoplasm. The mutant protein, BRI-L, was not analyzed by this method. However, the 'type II' topology of the mutant was supported by the fact that cultured cells transfected with either mutant or wild-type constructs secreted the expected carboxy-terminal cleavage products. Processing depended on the subtilisin-like protease furin. In the case of the mutant BRI construct, the amount of secreted peptide was increased relative to the wild-type construct. Finally, synthetic peptides corresponding to the carboxy-terminal sequences were shown to have different properties; under conditions where the peptide based on wild-type BRI was soluble, the ABri peptide formed small oligomeric structures that are not amyloid fibrils, but resemble the Aβ protofibril, a fibril precursor that has been extensively characterized by one of us (Harper, J.D., Wong, S.S., Lieber, C.M. & Lansbury, P.T. (1999). Assembly of an Aβ protofibril: an *in vitro* model for a possible early event in Alzheimer's disease. *Biochemistry* **38**, 8972-8890).

References

1. Selkoe, D. (1999). Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature Suppl.* **399**, A23-A31.
2. Lansbury, P.T. (1997). Structural neurology: are seeds at the root of neuronal degeneration? *Neuron* **19**, 1151-1154.
3. Wilhelmsen, K.C., Clark, L.N., Miller, B.L. & Geschwind, D.H. (1999). Tau mutations in frontotemporal dementia. *Dement. Geriatr. Cogn. Disord.* **10** (suppl 1), 88-92.
4. Vidal, R., *et al.,* & Ghiso, J. (1999). A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature* **399**, 776-781.
5. Lu, K.P., Hanes, S.D. & Hunter, T. (1996). A human peptidyl-prolyl isomerase essential for regulation of mitosis. *Nature* **380**, 544-547.
6. Ranganathan, R., Lu, K.P., Hunter, T. & Noel, J.P. (1997). Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* **89**, 875-886.
7. Yaffe, M.B., *et al.*, & Lu, K.P. (1997). Sequence-specific and phosphorylation-dependent proline isomerization: a potential mitotic regulatory mechanism. *Science* **278**, 1957-1960.
8. Shen, M., Stukenberg, P.T., Kirschner, M.W. & Lu, K.P. (1998). The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. *Genes Dev.* **12**, 706-20.
9. Lu, P.J., Wulf, G., Zhou, X.Z., Davies, P. & Lu, K.P. (1999). The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* **399**, 784-788.
10. Vincent, I., Rosado, M. & Davies, P. (1996). Mitotic mechanisms in Alzheimer's disease? *J. Cell Biol.* **132**, 413-425.
11. Vincent, I., Jicha, G., Rosado, M. & Dickson, D.W. (1997). Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain. *J. Neurosci.* **17**, 3588-3598.
12. Jicha, G.A., Lane, E., Vincent, I., Otvos, L., Jr., Hoffmann, R. & Davies, P. (1997). A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J. Neurochem.* **69**, 2087-2095.
13. Revesz, T., Holton, J.L., Doshi, B., Anderton, B.H., Scaravilli, F. & Plant, G.T. (1999). Cytoskeletal pathology in familial cerebral amyloid angiopathy (British type) with non-neuritic amyloid plaque formation. *Acta. Neuropathol. (Berl)* **97**, 170-176.
14. Ghiso, J., Plant, G.T., Revesz, T., Wisniewski, T. & Frangione, B. (1995). Familial cerebral amyloid angiopathy (British type) with nonneuritic amyloid plaque formation may be due to a novel amyloid protein. *J. Neurol. Sci.* **129**, 74-75.
15. Glenner, G.G. & Wong, C.W. (1984). Alzheimer's disease and Down's Syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* **122**, 1131-1135.
16. Baumann, M.H., Wisniewski, T., Levy, E., Plant, GT. & Ghiso, J. (1996). C-terminal fragments of alpha- and beta-tubulin form amyloid fibrils *in vitro* and associate with amyloid deposits of familial cerebral amyloid angiopathy British type. *Biochem. Biophys. Res. Commun.* **219**, 238-242.