Molecular Neurobiology

ISSN0893-7648/94/8(2–3): 79–87/$5.80

The "Brave New World" of Transmissible Spongiform Encephalopathy (Infectious Cerebral Amyloidosis)

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Abstract

The story of transmissible human spongiform encephalopathy, from its origins to the present time, enjoys the commentary of a cast of characters from Shakespeare's imaginary island in *The Tempest*, with a brief visit to the real island of Tasmania for a bird's eye view of the prion, and some concluding thoughts about the current state of research in the netherworlds of molecular biology and physical chemistry.

Index Entries: Creutzfeldt-Jakob disease; Gerstmann-Sträussler-Scheinker disease; kuru; amyloidosis; prion; spongiform encephalopathy.

Origins

> *"Two of these fellows you Must know and own; this thing of darkness I Acknowledge mine." (V. i. 274–276)*[1]

In 1920, while working in Alzheimer's Neuropsychiatric Clinic in Breslau, Hans Creutzfeldt reported the case of a "new and unusual type of neurological disease" in a young woman he had seen several years before *(1,2)*. The patient's mother had died of unknown causes at age 56, and two siblings were mentally defective. She presented at age 22 with tremors, spasticity, and pyramidal signs, and shortly thereafter became ataxic and progressively demented. Her later symptoms included nystagmus, rigidity, myoclonus, and mutism, and she died in status epilepticus 12 mo after the onset of illness. Examination of the brain revealed moderate cerebral atrophy with a patchy, diffuse neuronal loss, pronounced astroglial hypertrophy, and bilateral degeneration of the corticospinal tracts. Neuronal vacuolation was not mentioned.

A year later, in 1921, Alfons Jakob at the University of Hamburg published four cases that he thought resembled Creutzfeldt's case, grouping them together as examples of "spastic pseudo-

[1]Quotations with line citations are from *The Complete Works of Shakespeare*, edited by H. Craig (published by Scott, Foreman and Company, Chicago, 1951), which follows the text of the 1864 "Globe" edition of W. G. Clark and W. A. Wright.

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sclerosis" *(3–5)*. On review, it appears that three of the cases may have been the result of other causes *(6)*, but the remaining case was that of a 42-yr-old man whose illness began with aching legs, vertigo, and abdominal pain, followed by leg weakness, ataxia, diplopia, and progressive mental deterioration. He later developed dysarthria and facial twitching, before dying in a totally demented stuporous state some 9 mo after the onset of his illness. Microscopic examination of the brain showed changes that were in almost all essentials similar to those of Creutzfeldt's case, but without the corticospinal tract degeneration. Again, the presence of neuronal vacuolation was not mentioned.

"What seest thou else
In the dark backward and abysm of time?" (I. ii. 49–50)

Microscopic slides from Creutzfeldt's case have never been reviewed, but reexamination of slides from Jakob's case revealed, in addition to the changes he described, a diffuse vacuolation of the neuropile involving all areas of the cerebral cortex as well as the molecular layer of the cerebellum *(6)*. Although we shall never know whether Jakob was correct in thinking that Creutzfeldt had reported the first case of the disease that now bears their names, and although neither he nor Creutzfeldt commented on the spongiform change in the brains of their patients, Jakob can at least be credited with having reported the first retrospectively verified case of human spongiform encephalopathy. Over the next several years, Jakob and his students gradually acquired a fuller appreciation of spongiform encephalopathy as a pathologic entity, including the first case of familial Creutzfeldt-Jakob disease (CJD) *(7,8)*.

"They
say there's but five upon this isle. We are three of them; if th' other two be brained like us, the state totters." (III. ii. 4–7)

Meanwhile, in Vienna, Josef Gerstmann, who was later to be joined by Ernst Sträussler and Isaak Scheinker, described the first family afflicted with what we now call Gerstmann-Sträussler-Scheinker disease (GSS) *(9,10)*. A young woman of 26 presented herself to the neurological clinic with the story that 1 yr before she experienced a sudden loss of equilibrium, and had since become progressively clumsy, with slurred speech, uncontrollable mood swings, and failing memory. When examined, she was severely ataxic, with marked intention tremors and generalized hypotonicity, emotional lability, and moderate intellectual impairment. During the next several years, she developed pyramidal signs, nystagmus, Parinaud's syndrome, pseudobulbar swallowing difficulties, and an increasingly severe cerebellar syndrome, all associated with progressive dementia. She died 6 yr after the onset of an illness resembling that of her father and several of his relatives. At autopsy, the brain was atrophic, with a diffuse neuronal degeneration, glial proliferation, and vacuolation that was most marked in the cerebellum, which also contained many multicentric argentophilic plaques.

Yesterday and Today

Despite this recognition of both familial and sporadic forms of human spongiform encephalopathy before the end of the 1920s, the next three decades witnessed few cases and little interest in these exotic diseases, which remained threaded within a heterogeneous fabric of other neurodegenerative disorders. One reason for this confusion was the enduring failure of neuropathologists to credit spongiform change as the lynchpin of diagnosis, because of an excusable reluctance to attribute significance to holes. As late as 1968, cases of nonspongiform disease still contaminated three comprehensive reviews of CJD, of which the best known included the statement that "neuronal losses and astrogliosis are invariable findings in J-C disease, but status spongiosis is incidental and exists in addition to the basic pathology" *(11)*.

"Poor worm, thou art infected!" (III. i. 32)

This was the setting in which a remarkable series of discoveries burst on the scene in the 1960s that included:

1. The discovery of kuru, an epidemic neurodegenerative disorder limited to a single isolated people in the Eastern Highlands of New Guinea *(12)*;
2. The realization that kuru shared pathologic similarities with scrapie *(13)*, a disease of sheep that had been shown 30 yr earlier to be transmissible *(14)*;
3. The subsequent experimental transmission of kuru to primates *(15)*; and
4. Recognition of the neuropathological similarity of kuru to CJD and GSS, which were in turn successfully transmitted to primates *(16–18)*.

The connection between human and animal disease was of particular importance, in that the con-

siderable body of experimental work that had already been conducted on scrapie could now be applied to CJD and GSS, in return for which the financial cornucopia that often attends medical discoveries was opened to the comparatively indigent world of veterinary research. Among other benefits, the development of convenient laboratory rodent models led to an immense amplification of research on all forms of spongiform encephalopathy.

> *"Sir, I am vexed;*
> *Bear with my weakness; my old brain is troubled:*
> *Be not disturb'd with my infirmity . . ." (IV. i. 158–160)*

The many facets of this research bonanza included both clinical and basic areas of interest. The confusion over what was and was not CJD gave way to the bedrock criterion of experimental transmissibility, permitting its precise definition as a multisystem disorder characterized in the majority of cases by the subacute evolution of dementia, cerebellar and other neurologic signs, myoclonus, and a distinctive pattern of periodic EEG activity; and pathologically by the triad of spongiform vacuolation, gliosis, and neuronal loss (amyloid plaques occur in 5–10% of cases) *(19–22)*.

In the laboratory, most studies were directed at understanding the virus-like pathogenesis of disease and in characterizing the nature of the infectious agent (reviewed in chapters 11–14 in ref. *[23]*), but researchers were much embarrassed by the agent's stubborn refusal to reveal itself in any morphologically visible form. At length, in 1981, a fibrillar structure was consistently identified by electron microscopy in brain extracts from scrapie-infected, but not normal mice *(24)*, and was soon found to be present as well in humans with spongiform encephalopathy *(25)*. There followed an elegant series of experiments in which (1) highly purified infectious preparations of these fibrils were shown to consist of a 27–30-kDa proteinase-resistant protein fragment *(26)*, and (2) N-terminus sequencing of the fragment permitted its corresponding cDNA to be used to probe a scrapie-infected brain DNA library, and identify a gene encoding the full-length 33–35-kDa precursor protein *(27)*. The most surprising discovery in all of this work was that the protein was not encoded by a foreign DNA molecule, but by a host gene.

> *"This is as strange a maze as e'er men trod;*
> *And there is in this business more than nature*
> *Was ever conduct of . . ." (V. i. 244–246)*

What was a normal host protein doing as the major component of infectious brain extracts? The possibility of a molecule other than nucleic acid having replicative properties had been foreseen 20 yr earlier, when the implications of a variety of inactivation studies became impossible to ignore. In particular, the demonstration that UV and ionizing radiation were comparatively ineffective in doses far exceeding those needed to inactivate the nucleic acid in all conventional viruses *(28)* compelled a consideration of alternative replicative mechanisms. Several papers seriously addressed the possibility that the replicating agent was a piece of cell membrane, a ligand, a complex glycolipid, or a protein *(29–37)*. No great attention was paid to these highly speculative ideas, but now, with a real protein in hand that copurified with infectivity, it became possible to examine the problem experimentally.

Thus was born, or rather reborn, the prion, a name originally given to a group of southern hemisphere seabirds (Fig. 1), but later proposed as an eponym for this "proteinaceous infectious particle" *(38)*. The earlier ornithologic usage proved to be remarkably prescient, given its eponymic capacity to stretch or contract its wings to accommodate evolving knowledge about its nature.

> *"And ye that on the sands with printless foot*
> *Do chase the ebbing Neptune and do fly him*
> *When he comes back . . ." (V. i. 34–36)*

A recent traveler to Tasmania provides the following picturesque account of his first visit to Prion Bay:

> As I left the heights and began my descent toward the long beach of Prion Bay, everything changed. The bare, wind-tossed tops gave way to some of the thickest, most tangled and tortured rain forest it has ever been my misfortune to encounter. Out of the bright light and into a gray-green gloom of a nefarious netherworld, I attempted to follow a trail that had the remarkable ability of vanishing in the difficult places, leaving me scrambling through mud and slime and decaying mossbeds without any sense of direction—except down *(39)*.

The full-length protein was found to have 253 amino acids and a number of interesting physicochemical features (Fig. 2), including a region of glycine-rich octapeptide repeats near its N-terminus, two large and complex asparagine-linked sugar

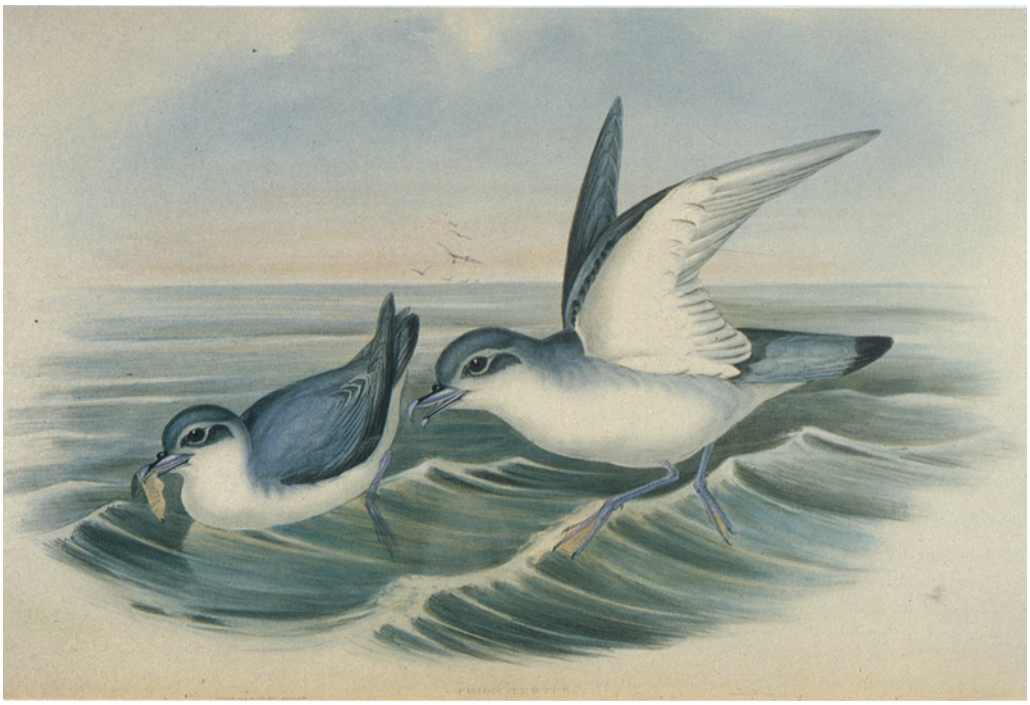

Fig. 1. The "original" prion (genus *Pachyptila*), a family of southern hemisphere seabirds. Reproduced from John Gould, *The Birds of Australia*, vol. 7, 1848, published by the author, printed by Richard and John E. Taylor, London, unpaginated, plate No. 54 (Prion *turtur*). Courtesy of Smithsonian Institution Libraries.

moities, a disulfide bond, a glycophosphatidylinositol membrane anchor, and a predicted secondary structure of comparatively high β-sheet potential *(27,40–45)*. Surprisingly, the protein isolated from normal individuals did not differ in any of these features from the protein isolated from affected individuals, and so left unexplained the question of its replicative potential that, by inference, was judged to reside in some other posttranslational alteration of its normal configuration *(46–50)*.

> *"Their understanding*
> *Begins to swell, and the approaching tide*
> *Will shortly fill the reasonable shore,*
> *That now lies foul and muddy." (V. i. 87–90)*

At this point, molecular genetics entered on the scene. From classical genetic studies, it had long been known that certain characteristics of both natural and experimental scrapie were genetically influenced *(51–53)*; the responsible gene was now shown to be closely linked, and probably identical to, the gene encoding this newly discovered protein *(54–60)*. Coincidentally, a search began for mutations in the corresponding human gene among families with spongiform encephalopathy, yielding more than a dozen different point and insert mutations in over a hundred studied families: in fact, no family is known to exist that does not carry one or another mutation, with new ones being discovered every year *(61)*.

Each of these mutations, of which the most common occur at codons 102, 178, and 200, is associated with a more or less distinct clinical syndrome: the mutation at codon 102 is associated with the classical features of GSS (indeed, the original family has been shown to carry this mutation) *(62)*; families with the codon 178 mutation may show either a CJD-like or thalamic syndrome, and families with the codon 200 mutation, apart from a somewhat earlier age at onset, have an illness indistinguishable from sporadic CJD. However, there is substantial phenotypic blurring of the lines between different genotypes: Similar illnesses can occur among individuals with different mutations, and different illnesses can occur among individuals with the same mutation, even within the same family *(63)*.

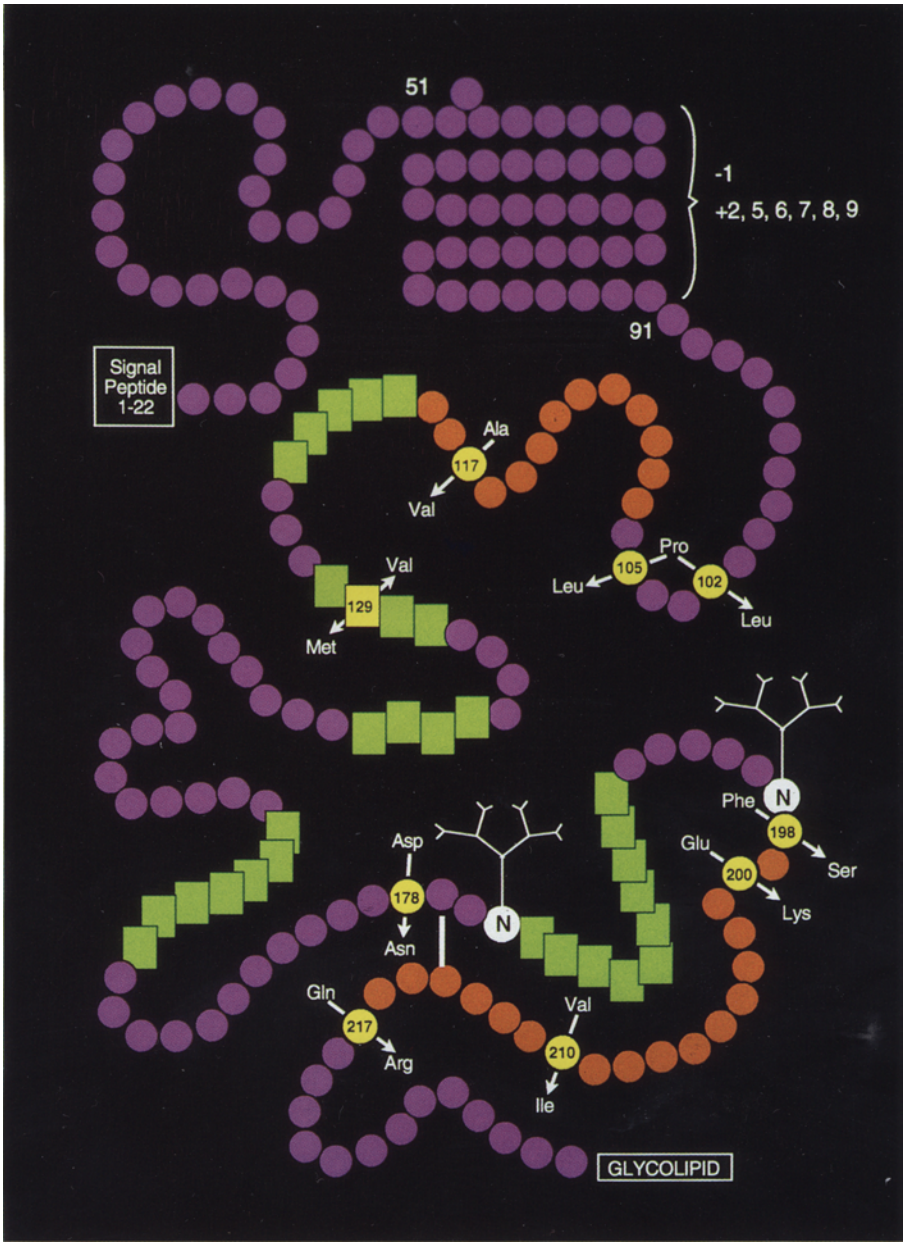

Fig. 2. The "modern" prion, an acronym for "proteinaceous infectious particle," the presumed replicating agent of the spongiform encephalopathies. Although more colorful, it is distinctly less graceful than its ancestor. The 230 amino acid backbone is shown as having a predicted secondary structure that has a random turn and coil (pink circles), α helix (orange circles), or β (green rectangles) configuration. A disulfide bond links two cysteine residues at positions 179 and 214, and two complex sugar groups are attached to asparagine residues at positions 181 and 197. Other posttranslational modifications involve cleavage of an N-terminus 22 residue signal peptide, and replacement of a C-terminus 23 residue sequence by a glycophosphatidyl-inositol membrane anchor. Disease-specific point mutations are indicated by single arrows through their encoded amino acids (yellow), and a polymorphism at codon 129 is shown by a double arrow through its corresponding amino acid. In addition, the region of tandem octapeptide repeats between positions 51 and 91 is the site of several disease-specific inserts containing between two and nine (extra) repeats, and at least one polymorphic octapeptide deletion.

"I find not
Myself disposed to sleep." (II. i. 201–202)

One of the more remarkable observations from molecular genetic analysis has been the discovery that a polymorphic codon that is not disease-specific is responsible for each of two distinctive syndromes associated with the pathogenic mutation at codon 178. When the mutated allele carries the codon 129 valine coding triplet, the disease has an early age at onset and comparatively long duration, but except for an absence of periodic EEG activity, shows the typical symptomatic and neuropathological spectrum of sporadic CJD. When the mutated allele carries the codon 129 methionine coding triplet, the disease takes a quite different aspect, with early and important "vegetative" disorders, in particular the occurrence of intractable insomnia (from which it derived its name of "fatal familial insomnia"), and a neuropathological picture that chiefly involves neuronal loss and gliosis of thalamic nuclei, without spongiform degeneration *(64)*.

In addition, homozygosity of codon 129 for either valine or methionine appears to influence the tempo of disease in families with pathogenic mutations in codons other than 178 *(65–67)* and to predispose to infection in environmentally acquired disease unrelated to mutations, most notably in patients with iatrogenic CJD resulting from contaminated human growth hormone therapy *(68–70)*.

"How camest thou in this pickle?" (V. i. 281)

In spite of the great temptations of molecular genetics, the heyday of mutation hunters has probably come and gone: It is unlikely that the next dozen mutations will add very much to what we have come to appreciate from the first dozen, which is that mutations are at the very least predisposing, and quite possibly causative. Although there is no evident clustering of mutations in any one region of the gene, all code for predicted non-β domains, and we know that synthetic peptides duplicating these regions have the capacity of undergoing spontaneous in vitro transformation into β-sheet amyloid fibrils *(71–73)*. We also know that mixtures of the alternative normal and mutant peptides from these regions are more fibrillogenic than either component peptide *(73)*, that at least one peptide is neurotoxic in vitro *(74)*, and that both normal and mutant protein species coexist in vivo within amyloid deposits *(75)*.

Taken together, these observations suggest that mutationally altered amino acids may function in vivo to transform their surrounding peptide sequences from a non-β to β configuration, pushing the entire protein across a solubility threshold to form amyloid fibrils. This altered molecule might then function as a physical constraint to newly formed proteins in a template-induced auto-patterning mechanism, leading to the accumulation of ever-increasing amounts of an amyloid protein that is toxic to neurons.

The idea is not new. Premolecular biologists had long since imagined this possibility, and even made reference to studies of cytoplasmic inheritance in lower organisms, from which it had been concluded that

> newly formed molecules do not enter a vacuum, but a structured cell, the molecules of which are an essential part of the determinism for locating, orienting, and patterning new molecular formations . . . A modified structure might, by itself constituting a change in the milieu, no longer permit the formation of the normally patterned structure, but permit reproduction of only the modified form. Such interactions at the molecular level might then result in hereditary extragenic variations *(76)*.

As satisfying as this theory appears, it addresses only the pathogenetic issue of amyloid formation, and not the more interesting problem of how the first protein molecule is triggered to undergo its transformation into an abnormal, replication-inducing isoform of the normal protein.

Tomorrow

"O, wonder!
How many goodly creatures are there here!
How beauteous mankind is! O brave new world,
That has such people in't!" (V. i. 181–184)

What are the most promising future approaches to these issues? Many of us think that we should put behind us the question of the participation of a nucleic acid that has not been identified, take the protein that has been identified, and run with it as far as our imagination and experimental techniques will carry us. The answers we are seeking may appear along the way, and if they do not, we can always start again in another direction. The most fruitful current research can be broadly categorized as belonging to the worlds of molecular biology and genetics, biochemistry, and biophysics.

From the world of molecular biology and genetics, we have approaches that involve both humans and animals. Familial forms of human disease with demonstrated mutations still do not account for the approx 90% of patients with sporadic disease, in whom germ-line mutations do not occur, at least not in the translated portion of the gene. Sporadic disease could nevertheless in theory result from such mutations in untranslated parts of the gene, from somatic mutations, or from random errors in transcription or translation. In this regard, it is interesting to recall that familial patients with the codon 200 mutation are phenotypically indistinguishable from sporadic patients, which might be a clue to an abnormality in the same region of the protein in sporadic cases.

In animals, genetic engineering studies are already well under way. Transgenic mice experiments have demonstrated the influence of introduced genes on disease phenotypes *(77,78)*, and have produced animals carrying a construct of the human codon 102 mutation that spontaneously develop a spongiform encephalopathy in the absence of extraneous infection *(79)*. "Knockout" mice, in which the gene has been rendered nonfunctional, have so far proved resistant to extraneous infection, proving that the encoded host protein is a necessary component of the disease process, and implying (but not proving) that it is itself the infectious agent *(80)*. Current experiments combining transgenic and "knock-out" animals involve a dizzying range of crossed and back-crossed mouse strains and gene copy numbers that threaten to become almost as difficult to interpret as the classical scrapie agent-mouse strain experiments of an earlier era, but that in the fullness of time should yield a coherent explanation of what is presently a very complicated story.

From the world of biochemistry, the eventual conclusion of efforts to purify the normal and altered precursor proteins should answer the crucial question of their conformational differences. Parallel studies of uninfected and infected cell cultures could identify the physiologic effects and metabolic fate of the normal protein, and discern the different metabolic pathways between the normal and altered protein.

From the world of biophysics, we can expect more in vitro studies on the mechanism of amyloid formation. We have seen that synthetic peptides corresponding to predicted α-helix domains may undergo in vitro transformation into β-sheet fibrils, especially pronounced when normal and mutant sequence peptides are mixed together, and that such peptides can be neurotoxic. The next logical step will be to study the dynamics and conditions of fibril formation to determine if in fact mutant peptides are capable of inducing normal peptides to make amyloid fibrils, and to try to duplicate these conditions, first in cell cultures and then in experimental animals.

From each of these disciplines, alone or in concert, may come information of therapeutic interest. Molecular geneticists may develop a practical method to obliterate the gene in humans, possibly by using antisense RNA packaged in a harmless viral vector. Biochemists and molecular biologists may be able to devise pharmacological strategies to prevent the abnormal transformation of the protein or to interrupt its aberrant metabolic pathway. Biophysicists may discover how to inhibit the initial pathogenetic nucleation, perhaps by the technique of crystal structure mismatches, similar in action to that of detergents on immiscible solutions *(81)*. We are indeed at the threshold of a "brave new world" for infectious amyloidosis, where, even in our present state of imperfect knowledge, we can now begin to envision ways to prevent or cure these rare but devastating diseases.

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