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To cite this article: Jay Rasmussen, Mathias Jucker & Lary C. Walker (2017) Aβ seeds and prions: How close the fit?, Prion, 11:4, 215-225, DOI: 10.1080/19336896.2017.1334029

To link to this article: http://dx.doi.org/10.1080/19336896.2017.1334029
Aβ seeds and prions: How close the fit?

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\textbf{ABSTRACT.} The prion paradigm is increasingly invoked to explain the molecular pathogenesis of neurodegenerative diseases involving the misfolding and aggregation of proteins other than the prion protein (PrP). Extensive evidence from \textit{in vitro} and \textit{in vivo} studies indicates that misfolded and aggregated Aβ peptide, which is the probable molecular trigger for Alzheimer’s disease, manifests all of the key characteristics of canonical mammalian prions. These features include a β-sheet rich architecture, tendency to polymerize into amyloid, templated corruption of like protein molecules, ability to form structurally and functionally variant strains, systematic spread by neuronal transport, and resistance to inactivation by heat and formaldehyde. In addition to Aβ, a growing body of research supports the view that the prion-like molecular transformation of specific proteins drives the onset and course of a remarkable variety of clinicopathologically diverse diseases. As such, the expanded prion paradigm could conceptually unify fundamental and translational investigations of these disorders.

\textbf{KEYWORDS.} Abeta, Alzheimer, amyloid, aging, dementia, neurodegeneration, prion, proteopathy, seeding, tau

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Received April 24, 2017; Revised May 15, 2017; Accepted May 16, 2017.


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Since the initial clinicopathologic characterization of Kuru and the discovery that human spongiform encephalopathies are transmissible, researchers have hypothesized that these extraordinary maladies might share etiologic features with other neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease.1,2 A key mechanistic commonality is the pathologic accumulation of specific misshapen proteins within the brain.3 In the prion diseases, this process can be initiated by infection, i.e., the invasion of the body by exogenous seeds of misfolded, aggregated prion protein (PrPSc), which proliferate by the templated corruption of naive PrP molecules and ultimately impair brain function. However, most human prion diseases are not instigated by infection, but rather begin with the endogenous generation of PrPSc, which then multiplies by the same molecular mechanism as that induced by exogenous PrP-prions.4

Emerging data support the hypothesis that other neurodegenerative disorders similarly involve the endogenous misfolding, aggregation, and systematic spread of disease-specific proteins within the brain, reminiscent of PrP-prions.3,5,6 Unlike PrP-prions, however, these diseases are not infectious in the sense of transmissibility from one person to another under ordinary conditions.3,7 Hence, despite important molecular similarities, the fact that prions are defined as infectious agents has generated debate in the scientific community as to whether the term ‘prion’ should be expanded to include pathogenic proteins that are not infectious by conventional definitions.3

Comparing Aβ Seeds to PrP-Prions

The defining property of prions is the self-propagation of alternatively folded protein conformations by the templated corruption of like proteins.5 By this molecular definition alone, the seeds of Aβ and many other proteins can be defined as prions. However, other traits have been invoked to characterize infectious PrP-prions, including the tendency to form amyloid, folding into heterogeneous strains, resistance to inactivation, and spread by cellular transport mechanisms. These features define the broad limits of prion pathobiology within the context of the core definition – ‘proteins that acquire alternative conformations that become self-propagating’5 - and as we and others have found, many of the ancillary qualities associated with prions also are not unique to the prion protein. Here we consider, point by point, how key attributes of Aβ seeds compare to those of PrP-prions.

Aβ seeds and PrP-prions are rich in β-sheet and prone to forming amyloid

PrPSc has an enhanced tendency to form amyloid,6,14 a general state in which a β-sheet-rich misfolded protein polymerizes into fibrils that yield a characteristic X-ray diffraction pattern, and that further coalesce into masses with
distinctive histologic staining patterns.\textsuperscript{14,15} Although the misfolded state imparts important functional capacities to PrP-prions, the formation of amyloid is not mandatory for the induction of disease.\textsuperscript{16-18} Indeed, PrP-prions are heterogeneous entities; PrP can assume alternative conformations with varying properties (‘strains’; see below), and PrP\textsubscript{Sc} assembles into a spectrum of bioactive multimers that range from small oligomers to large amyloid fibrils.\textsuperscript{19}

Using the dye Congo red, Divry first reported the amyloid nature of senile plaques in AD nearly a century ago.\textsuperscript{20} In the 1980s, the amyloidogenic protein in Alzheimer plaques and cerebral amyloid angiopathy (CAA) was identified as what is now known as A\beta,\textsuperscript{21,22} and around the same time the amyloid deposits that are present to varying degrees in prion diseases were found to consist of PrP.\textsuperscript{23} In addition to Congo red, newer amyloid-specific dyes such as the luminescent conjugated oligothiophenes (LCOs) have been used to characterize deposits of A\beta and PrP in tissue sections.\textsuperscript{24-26} These dyes derive their selectivity for amyloid from their interaction with the \(\beta\)-sheet-rich regions of misfolded protein complexes, and as such they have been helpful in defining polymorphic amyloid strains.\textsuperscript{26,27} Although misfolded A\beta frequently forms amyloid in AD, as in prion disease, amyloid per se is not obligatory for the manifestation of Alzheimer’s disease (more on this below).

\textbf{A\beta seeds and PrP-prions are resistant to inactivation}

Owing to the stability conferred by the amyloid state, PrP-prions can be remarkably resistant to physicochemical degradation, a quality that contributes to their persistence and infectivity, and therefore has important public health implications.\textsuperscript{28} Both PrP-prions and A\beta seeds are resistant to inactivation by heat and formaldehyde.\textsuperscript{28-31} Furthermore, both agents are extraordinarily long-lasting in the living brain; when A\beta seeds were infused into the brains of APP-knockout mice (which are incapable of replicating the seeds), some seeding-competent A\beta remained even after 6 months in the recipients.\textsuperscript{32} Similarly, PrP-prions have been reported to retain their infectivity after nearly 20 months in the brains of hosts incapable of replicating prions.\textsuperscript{33} Additionally, aggregated A\beta is resistant to degradation by proteases,\textsuperscript{34} similar to PrP-prions (for which this property is exploited in the diagnostic detection of disease).\textsuperscript{35} However, analysis of the A\beta-inducing species revealed that small, soluble seeds harbor considerable amyloid-inducing activity, and that these small seeds are readily inactivated by proteinase-K,\textsuperscript{34,36} comparable to the high specific activity,\textsuperscript{19} and proteinase K-sensitivity of oligomeric forms of PrP.\textsuperscript{37}

\textbf{A\beta Seeds and PrP-prions Propagate by Molecular Templating}

Earlier \textit{in vitro} studies demonstrated that preformed A\beta seeds are able to convert monomeric A\beta into extended fibrils by molecular conformational templating (i.e., seeding).\textsuperscript{38} A series of experiments in transgenic mouse models expressing human-sequence APP showed that infusion of small amounts of brain extracts containing aggregated A\beta can induce the formation of plaques and CAA \textit{in vivo}, and that aggregated A\beta is essential for the seeding effect (see ref.\textsuperscript{39} for review). Similar to PrP-prion seeding, the A\beta-seeding effect can be achieved \textit{de novo} in rodent models in which such pathology is not normally seen within the average lifespans of the animals.\textsuperscript{40,41}

\textbf{Misfolded A\beta and PrP Aggregate into Variant, Inducible Strains}

The identification of structurally and functionally distinct prion strains has helped to explain how the pathobiology of these molecules is influenced by their architecture, beyond the linear amino acid sequence. PrP-prions can misfold and assemble into seeds with varying structural and functional features that can be passed on to subsequent generations of prions.\textsuperscript{42} Different prion strains also spur specific spatiotemporal patterns of PrP-deposition, while at the same time producing distinct
neurodegenerative phenotypes that are encoded by their biochemical fingerprint.\textsuperscript{43} Analogously, \(A\beta\) can misfold and assemble into strain-like structural variants both \textit{in vitro} and \textit{in vivo}.\textsuperscript{29,44-52} To determine whether the structural properties of \(A\beta\) can be seeded in new hosts, cross-seeding experiments were undertaken in two transgenic mouse lines that develop phenotypically dissimilar patterns of \(A\beta\) distribution and plaque size as they age.\textsuperscript{29} Intracerebral inoculation of brain extract from one mouse line into the other engendered seeded plaques that were morphological hybrids of the predominant plaques generated by the donor and the host.\textsuperscript{29} Subsequent experiments using conformation-sensitive LCOs showed that the fluorescence spectra emitted by cross-seeded plaques differed from the spectra emitted by endogenous, unseeded plaques in the same transgenic mouse lines.\textsuperscript{51}

These findings were confirmed and extended in an \textit{ex vivo} hippocampal slice culture model in which the morphology and LCO-spectral signatures of seeded plaques were influenced by the mouse line that furnished the initial seed as well as the isotype of \(A\beta\) added to the medium.\textsuperscript{53} The strain-like differences in seeded plaques on the hippocampal slices were associated with different ratios of the two commonly generated \(A\beta\) isoforms of 40 and 42 amino acids (\(A\beta\)40 and \(A\beta\)42), as had been seen in the \textit{in vivo} cross-seeding experiments.\textsuperscript{51} Other studies have found that \(A\beta\)40 and \(A\beta\)42 differentially influence the strain type, and that distinct strain-like features of synthetic \(A\beta\) seeds created \textit{in vitro} are retained after \textit{in vivo} passage.\textsuperscript{48}

\(A\beta\) deposits in humans with AD can differ in terms of morphology,\textsuperscript{54} ligand binding,\textsuperscript{55} solid-state nuclear magnetic resonance signature,\textsuperscript{56} and biophysical attributes such as conformational stability.\textsuperscript{52} In an initial study, brain samples from two AD patients with dissimilar clinical histories were used to seed the aggregation of synthetic \(A\beta\)40 \textit{in vitro}, and the resulting \(A\beta\) fibrils showed distinct nuclear magnetic resonance and electron-microscopic characteristics.\textsuperscript{50} The authors proposed that a single, dominant \(A\beta\) structure is propagated by seeded nucleation in each brain, and that this molecular structure influences clinical progression. In a larger cohort of patients, these findings were extended to show different molecular configurations of \(A\beta\) assemblies in rapidly progressing AD cases compared to normally progressing cases.\textsuperscript{56} Similarly, different rates of cognitive decline have been correlated with distinct conformations of \(A\beta\)42.\textsuperscript{57} These experiments suggest that rapidly progressing AD may be associated with a particularly virulent strain of misfolded \(A\beta\).

An alternative \(A\beta\) strain also has been implicated in a hereditary type of AD linked to a mutation within the \(A\beta\) sequence (E22G; the “arctic” mutation). These patients harbor \(A\beta\) plaques with a core largely devoid of amyloid; when brain extracts from arctic AD patients were injected into APP23 transgenic mice (which express the non-mutant sequence of human \(A\beta\)), cerebral blood vessels were surrounded by unusual fuzzy \(A\beta\) deposits, unlike the CAA in mice seeded with extracts from non-arctic AD cases.\textsuperscript{49,58} In addition to reinforcing the \(A\beta\)-strain hypothesis, studies of arctic-mutant \(A\beta\) underscore the important (and often unrecognized) point that, while \(A\beta\) aggregation is central to AD, classical amyloid deposits are not obligatory for the clinical expression of the disease. Collectively, these experiments show that, like PrP-prions, particular strains of aggregated \(A\beta\) can be propagated \textit{in vitro} and \textit{in vivo} by molecular templating. While the existing evidence strongly indicates that \(A\beta\) structure is associated with disease phenotype, the critical link between the pathophysiological traits and molecular architecture of \(A\beta\) have not yet been fully defined.

\textbf{\(A\beta\) Seeds and PrP-prions Translocate within and to the Brain}

Intraocular injection of PrP-prions has shown that the agent can be conveyed from one part of the nervous system to another by neuronal transport mechanisms.\textsuperscript{59-62} Similarly, \(A\beta\) seeds introduced focally into the brain induce \(A\beta\) aggregation that propagates systematically to interconnected regions.\textsuperscript{63,64} \textit{In vitro} studies indicate that the trafficking of \(A\beta\) seeds is
mediated by neuronal uptake, transport and release mechanisms.\textsuperscript{65,66} Within cells, soluble Aβ is concentrated in the acidic environment of endosomes/lysosomes, where it assembles into higher molecular weight seeds.\textsuperscript{67} There is also evidence that membrane-associated, non-fibrillar Aβ in mitochondria has robust seeding capacity.\textsuperscript{68} Notably, placement of Aβ seeds into the peritoneal cavity is capable of inducing Aβ deposition in the brain,\textsuperscript{69,70} though the mechanisms involved in the translocation of the seeds from periphery to brain are uncertain. These findings demonstrate the extensive mobility of Aβ within and outside of the CNS, with implications for the iatrogenic induction of Aβ aggregation in humans (below).

**Aβ Seeds can Induce Aβ Deposition in Humans**

Despite longstanding experimental evidence for the prion-like seeding of Aβ in vitro and in vivo, the relevance of the seeding paradigm to humans has been uncertain. Recent studies aimed at bridging this gap have involved the opportunistic evaluation of Aβ pathology in tissues from patients who had been treated with human cadaver-derived growth hormone or dura mater implants.\textsuperscript{71-75}

In the first such investigations, the patients who were analyzed had died of iatrogenic Creutzfeldt-Jakob disease (iCJD) years after having received cadaveric growth hormone or dura mater; hypothesizing that the treatment materials were also likely to have harbored Aβ seeds from donors who had died with AD (or incipient AD), the researchers demonstrated that recipients who contracted iCJD also had significantly greater cerebral Aβ deposition than did control patients.\textsuperscript{71-74} The link between cadaveric growth hormone treatment and Aβ deposition was subsequently confirmed in a larger cohort of iCJD patients; significantly, increased Aβ deposition also occurred in hormone recipients who died of causes other than CJD, indicating that the Aβ deposition is not caused by the prionopathy.\textsuperscript{75}

A noteworthy caveat in these analyses is that the patients with Aβ pathology did not have AD-like tauopathy at the time of death, and thus they did not fulfill the pathologic criteria for fully developed AD. This is surprising for two reasons; first, experimental work has demonstrated that aggregated Aβ is capable of inciting tauopathy in experimental animals,\textsuperscript{76} and that Aβ can cross-seed tau assembly to produce potent, in vivo-active tau seeds.\textsuperscript{77} Second, pituitaries that contained Aβ seeds are likely to have contained tau seeds as well,\textsuperscript{78} and tau itself can be induced to misfold and polymerize in a prion-like fashion;\textsuperscript{79} hence, if tau seeds survived the purification process and entered the brain (neither of which is known), a direct tau seeding effect would have been expected. Further scrutiny of the cadaveric growth hormone and dura mater recipients will be needed to determine whether tauopathy might also be inducible in humans.

The implications of these findings for the risk of AD in surviving recipients of human-derived growth hormone and dura mater transplants are ambiguous. A study of growth hormone recipients in the US found no evidence of an increased incidence of AD as of 2008,\textsuperscript{78} but the growth hormone preparations in the US included a stringent purification step after 1977 that likely eliminated most PrP-prions.\textsuperscript{80} Since no cases of iCJD have occurred in US patients treated after 1977, it is possible that these recipients also would be less likely to have been exposed to Aβ (or tau) seeds. In addition, because the analyzed recipients of tainted biologics had died of CJD or other causes at relatively young ages,\textsuperscript{71-75} it is not possible to know whether they would have developed AD after a longer incubation period, especially given that Aβ deposition begins in the AD brain decades before the emergence of cognitive decline.\textsuperscript{81} It is worth noting that the kinetics of protein misfolding and aggregation are likely to be important for the effective transmission of proteopathies, and differences in kinetics could explain the potency of exogenous PrP-prions at eliciting disease compared to Aβ in similar exposure scenarios.

Improvements in the production and preparation of biologic agents have essentially eliminated the risk of iatrogenic CJD.\textsuperscript{80} However, longer-term follow-up of patients who received...
cadaveric growth hormone and dura mater transplants during this problematic period is needed to establish the risk of AD with certainty. In light of evidence for the seeded induction of Aβ deposition and the cerebral invasion of peripheral Aβ seeds in animal models,69,70 there is also a need for more extensive surveillance of Aβ pathology that might be linked to other sources such as blood and blood products.

The Most Effective Aβ Seeds and PrP-prions are Generated in Vivo

Finally, it is worth highlighting the puzzling observation that both Aβ seeds and PrP-prions are most potent when generated in living tissues. Initial investigations of Aβ seeding in mouse models demonstrated that brain extracts containing aggregated Aβ strongly induce Aβ deposition, whereas synthetic Aβ that was pre-aggregated in vitro was ineffective within the same incubation timeframe.29 Subsequent studies employing a longer incubation period showed that synthetic Aβ seeds can induce deposition in APP-transgenic mice, albeit with low potency relative to brain-derived seeds.82 Generating efficacious PrP seeds in vitro also has been a persistent challenge, but aggregation of recombinant PrP in the presence of specific co-factors can markedly increase their infectivity.83 Likewise, when synthetic Aβ is aggregated on living tissue slices in culture, the resulting Aβ seeds induce robust Aβ deposition in vivo.53 Determining why proteinaceous seeds that develop in living tissues are more potent than seeds aggregated in vitro is an important objective for future research.

CONCLUSIONS AND OPEN QUESTIONS

The original definition of prions as proteinaceous infectious particles84 has hindered the expansion of the prion concept to other proteopathies, in part because of concern that these diseases might be perceived as contagious under everyday circumstances.3 For this reason, and because prion-like mechanisms are increasingly recognized in many realms of biology,11-13,85-89 we have argued that ‘proteinaceous nucleating particles’ would serve as a more inclusive (and less alarming) definition of prions.3 Aβ seeds undoubtedly meet the key criteria to qualify as prions, and evidence is growing that the prion paradigm includes several other proteopathies as well.3,5,6,79 However, many issues require resolution; among these: What is the connection between seeding capacity and toxicity? How do the conformation and size of aggregating proteins influence their pathobiology? How is Aβ linked to tauopathy in the AD cascade? Do different Aβ strains explain the resistance of non-human species (and perhaps some humans) to AD?90 What factors influence the conversion of pathogenic proteins to a prion-like state? How are the proteins processed and transferred by cells? And given growing evidence for prion-like processes in a wide spectrum of diseases, how can the expanded prion principle help to unify the search for new therapeutic objectives?

ABBREVIATIONS

Aβ       amyloid-β  
AD       Alzheimer’s disease  
APP      Aβ-precursor protein  
CAA      cerebral amyloid angiopathy  
CJD      Creutzfeldt-Jakob disease  
iCJD     iatrogenic Creutzfeldt-Jakob disease  
LCO      luminescent conjugated oligothiophene  
PrP      prion protein  
PrPSc    prion protein scrapie, PrP in a pathogenic conformation

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

ACKNOWLEDGMENTS

We gratefully acknowledge enlightening conversations with Harry LeVine, Yury Chernoff, David Lynn, Anil Mehta, as well as the members of the Walker and Jucker laboratories for their many contributions.
FUNDING

This work was supported by the NIH under Grants P50 AG025688, RR00165, OD11132 (LCW); the Alexander von Humboldt Foundation (LCW); and the EC Joint Program on Neurodegenerative Diseases under Grant JPND-NeuTARGETs (MJ).

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