Brain Pathology ISSN 1015-6305

MINI-SYMPOSIUM: "Prion-Like" Templated Misfolding in Neurodegenerative Disorders

From Soluble Aβ to Progressive Aβ Aggregation: Could Prion-Like Templated Misfolding Play a Role?

Yvonne S. Eisele[1,2]

[1] DZNE, German Center for Neurodegenerative Diseases, Tübingen, Germany.
[2] Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany.

Keywords
Aβ, Alzheimer's disease, cerebral β-amyloidosis, cerebral beta-amyloid angiopathy, prion-like aggregation, seeding, templated misfolding.

Corresponding author:
Yvonne S. Eisele, PhD, German Center for Neurodegenerative Diseases (DZNE), c/o Hertie-Institute for Clinical Brain Research, Otfried-Müller-Straße 27, D-72076 Tübingen, Germany (E-mail: *yvonne.eisele@uni-tuebingen.de*)

Received 4 February 2013
Accepted 4 February 2013

doi:10.1111/bpa.12049

Abstract

Accumulation, aggregation and deposition of Aβ peptides are pathological hallmarks in the brains of individuals affected by Alzheimer's disease (AD) or by cerebral β-amyloid angiopathy (Aβ-CAA). While Aβ is a peptide of yet largely unknown function, it is constantly produced in the human brain where it normally remains in a soluble state. However, Aβ peptides are aggregation prone by their intrinsic ability to adopt alternative conformations rich in β-sheet structure that aggregate into oligomeric as well as fibrillar formations. This transition from soluble to aggregated state has been hypothesized to initiate the pathological cascade and is therefore subject to intensive research. Mounting evidence suggests prion-like templated misfolding as the biochemical phenomenon responsible for promoting progressive Aβ aggregation. Here, we review studies *in vitro* and *in vivo* that suggest that cerebral Aβ aggregation may indeed progress via prion-like templated misfolding. The implications of these findings are discussed with respect to understanding initiation and progression of the disease and to developing therapeutics.

INTRODUCTION

More than 100 years ago, neuropathologist, Alois Alzheimer, discovered peculiar changes in the brains of individuals who had died from dementia: amyloid plaques, neurofibrillary tangles and, to varying extent, vascular amyloidosis (3, 31, 53, 58). Today, we know that extracellular amyloid plaques and vascular amyloid are proteinaceous deposits mainly composed of Aβ peptides (51). In contrast, neurofibrillary tangles are intracellular aggregates of hyperphosphorylated tau proteins (50). Normally retaining a soluble state, Aβ peptides generated through proteolytic cleavage from the amyloid precursor protein (APP), as well as tau proteins, are integrated into cellular physiology (33, 50). However, in Alzheimer's disease (AD), Aβ peptides and tau proteins adopt alternative, misfolded conformations rich in β-sheet structure that renders them aggregation prone and promotes association into oligomeric as well as fibrillar structures. The abundance of these pathological deposits increases with disease severity and compromises synaptic and neuronal function. In advanced stages of AD, vast areas of the brain are affected (13), particularly in the so-called default mode network (78), and significant synaptic and neuronal loss causes progressive dementia (79).

The amyloid cascade hypothesis of AD proposes that Aβ misfolding and aggregation are the central and earliest events in the disease and trigger a sequence of pathological changes that result in tau hyperphosphorylation and aggregation, brain inflammation and ultimately synapse as well as neuronal loss, and dementia (35). Conclusive evidence is derived largely from rare forms of familial AD (<3%) caused by mutations in APP or in the catalytic subunits of the γ-secretase complex, that is, presenilin 1 (PS1) and presenilin 2 (PS2), responsible for releasing Aβ peptides from APP (33). These mutations lead to subtle changes in APP metabolism with increased production of Aβ peptides and/or to generation of more aggregation prone variants (7, 12, 16, 19, 20, 52, 76). Similarly, an increase in aggregation prone Aβ peptides caused by increased production and/or reduced degradation and clearance is hypothesized to initiate the more common sporadic cases of AD. Aβ aggregates, especially small units termed oligomers, have been shown to be neurotoxic and to alter synaptic function (57), although by themselves they may not be sufficient to lead to dementia, correlating only insufficiently with cognitive decline (14, 47, 54, 59, 91). Current state of knowledge suggests that Aβ toxicity in AD is at least partially mediated via tau hyperphosphorylation and aggregation that promote neuronal dysfunction (38, 72, 88). This part of the mini-symposium focuses on the mechanisms and consequences of Aβ aggregation. Initiation and spreading of tau aggregation and concomitant pathological changes are reviewed in Clavaguera *et al* (this issue).

AD is not the only disorder with signature Aβ aggregation. In cerebral β-amyloid angiopathy (Aβ-CAA), a sporadic or rare hereditary pathological condition in the elderly, characteristic Aβ deposits are found almost exclusively in the walls of small- to medium-sized blood vessels in the leptomeninges and in the cortex (9). These vascular Aβ deposits can weaken vessel walls

and compromise vascular function. Abundant vascular Aβ deposits can cause intracerebral microbleeds or hemorrhages as well as neurodegeneration and cognitive impairment. Pathological accumulation of Aβ peptides has also been described in sporadic inclusion body myositis (sIBM), an inflammatory and degenerative muscle disease (5, 60, 87); however, the contribution of Aβ aggregation to sIBM is controversial (32).

While it seems evident that the transition from soluble Aβ peptides to misfolded and aggregated versions plays an important role in these diseases, many questions still remain open and urgently await answers in order to develop efficacious treatments. These unanswered questions include: How are misfolding and aggregation of normally soluble Aβ peptides initiated? With disease progression, Aβ aggregation affects vast areas and neuronal networks of the central nervous system (CNS)—what is the relationship of these aggregates? Do they form independently at the different sites? Or are they related?

Interestingly, new insights arise from similarities between Aβ aggregation and protein aggregation observed in prion disorders, similarities with regard to a biochemical phenomenon termed templated misfolding.

NUCLEATION-DEPENDENT POLYMERIZATION, SEEDING AND PRION-LIKE TEMPLATED MISFOLDING

It has been proposed that Aβ aggregation as well as the aggregation of other amyloidogenic proteins follows a nucleation-dependent polymerization process similar to crystallization (36, 40). Such polymerization reactions consist of two phases: the initial nucleation phase ("lag phase") and the growth phase (Figure 1A). During the nucleation phase, the native monomer undergoes a series of kinetically unfavorable conformational changes and misfolded or oligomeric species can be formed. These are unstable because the intra- and intermolecular interactions do not outweigh the entropic costs of misfolding and aggregation. However, under certain conditions an ordered, putatively multimeric nucleus can be formed. Once the nucleus is present, more

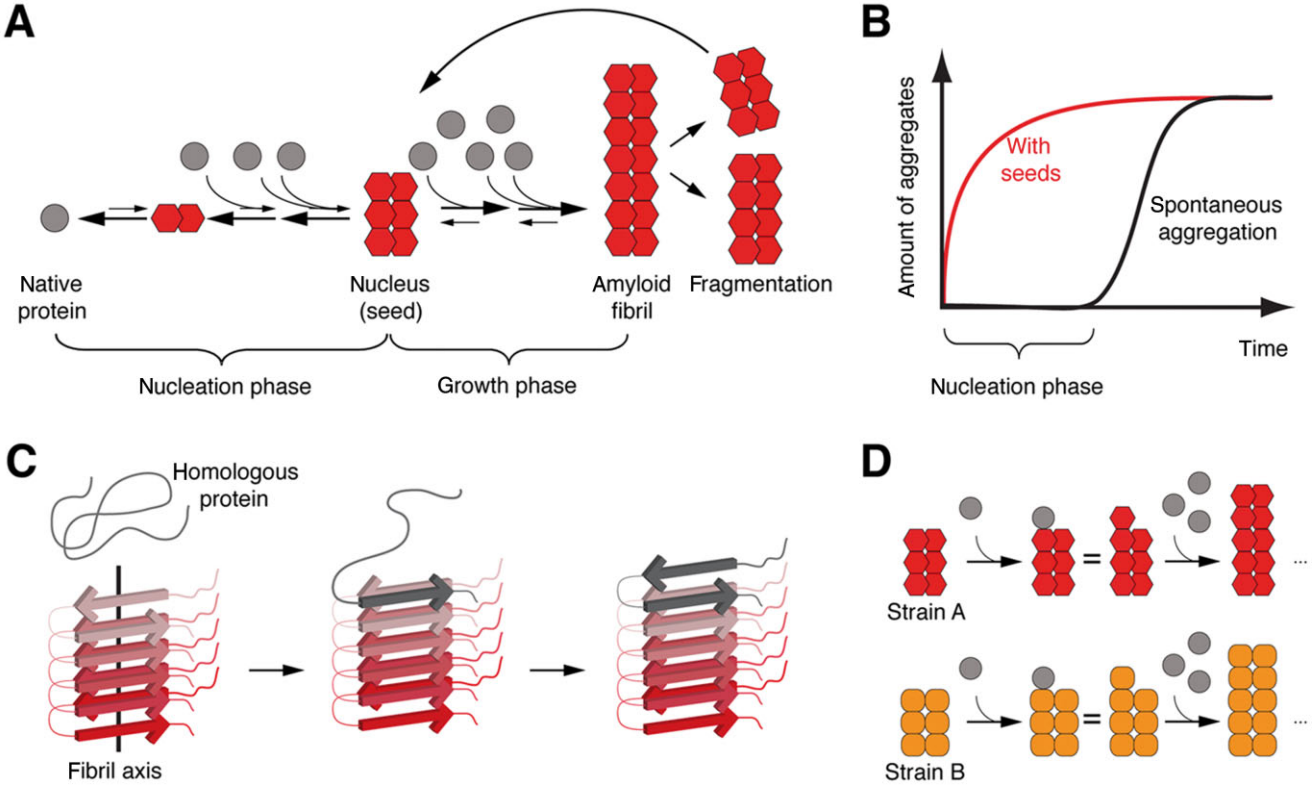

Figure 1. *Nucleation-dependent polymerization, seeding and templated misfolding.* **A.** Under certain circumstances, some proteins can adopt an alternative, misfolded conformation that allows for multimerization. The initial conformational changes are kinetically unfavorable and may not occur under physiological conditions. However, once a nucleus ("seed," putatively multimeric) is formed, the properties of the nucleus allow for relatively rapid incorporation of homologous proteins into amyloid fibrils. Fragmentation of fibrils generates more nuclei resulting in a vicious cycle of progressive protein misfolding and aggregation. **B.** Schematic representation of protein aggregation kinetics. Spontaneous aggregation is delayed because of the thermodynamically unfavorable nucleus formation. Addition of preformed seeds leads to immediate aggregate formation and circumvents the nucleation phase. **C.** Structure analysis of amyloid fibrils reveals typical sets of in-register β-sheets arranged parallel to the fibril axis. This structure has been suggested to serve as a template for the congruent incorporation of homologous, native or partially unfolded proteins that orient their peptide chains accordingly, hence termed templated misfolding. **D.** Some proteins can adopt diverse misfolded conformations that can be associated with different pathogenic properties. These conformational differences are referred to as "strains" and can be propagated by templated misfolding.

cognate proteins can be incorporated relatively rapidly into the aggregate ("growth phase"). In this model, aggregation is concentration- and time-dependent, and the rate-limiting step of fibrillization is the formation of the nucleus. Moreover, fragmentation of fibrils generates new seeds and boosts the polymerization cascade (25). Seeding, which is the addition of a preformed nucleus, circumvents the lag phase and initiates immediate aggregate formation (Figure 1B). Support for nucleation-dependent polymerization of Aβ peptides is derived from *in vitro* aggregation of synthetic or recombinant Aβ peptides in solution (48, 61, 64, 77). With regard to AD, this model takes into account that Aβ fibrillization in the human brain is normally a slow process: initial amyloid plaque formation may take decades in individuals who develop AD and may never even occur in histologically significant amounts at very old age (14). However, once Aβ aggregation has been initiated, the misfolding and deposition seems to inevitably progress.

Interestingly, structure analysis of fibrils formed by amyloidogenic proteins or fragments thereof provides a plausible and attractive explanation for the efficient incorporation of homologous molecules into the preformed nucleus. Amyloid fibrils were found to typically consist of sets of β-sheets arranged parallel to the fibril axis, with β-sheets either parallel or antiparallel and usually in-register (24). The side chains within and in between the sheets are tightly interdigitated and form a dry steric zipper devoid of water in the interface. Such structures allow for compact stacking of molecules and are stabilized by intermolecular interaction. Homologous molecules can be incorporated accordingly and this leads to progressive growth of the amyloid fibril (Figure 1C). For Aβ fibrils, such structures were proposed from analyses of synthetic or recombinant Aβ peptides aggregated *in vitro* by several groups (8, 18, 48, 63, 77, 84, 85). Although detailed structural information of Aβ fibrils in brain tissue is not available yet, it is conceivable that similar structures are formed *in vivo* (44, 61). Such fibrils can grow from one or both ends, depending on the polarity of the fibril. The growth rate depends (i) on the availability of soluble, homologous molecules for incorporation; (ii) on the efficiency of incorporation; and (iii) on the rate of fragmentation that generates more molecular interfaces for interaction of nucleus and homologous molecules. So, essentially proteins in their abnormal multimeric conformation seem to directly interact with native or partially unfolded homologous proteins and template them into the abnormal, misfolded form. Therefore, this phenomenon has also been termed "templated misfolding." In case a given protein can adopt two or more differently organized aggregate formations that are reliably sustained by templated misfolding, these distinct aggregates are known as different "strains" of aggregates (2, 24) (Figure 1D). Different strains of aggregates can have different pathological properties and may differ in their growth and fragmentation rates (2).

The principle of templated misfolding was first proposed and is best characterized for the misfolding cascade of prion proteins (17, 66) (Kretzschmar and Tatzelt, this issue). Only recently similar mechanisms of misfolding are discussed for other proteins associated with neurodegenerative diseases, for example, Aβ, tau (Clavaguera *et al*, this issue), α-synuclein (George *et al* this issue) as well as several others (65, 67). With respect to proteins for which this principle of misfolding is still under debate, it is often referred to as "prion-like" misfolding. However, an important difference exists between the prion diseases and other disorders with putative "prion-like" protein misfolding: only prion diseases have been shown to be "infectious" in a sense that the disease-causing agent can be acquired from the environment and "infect" a new organism. There is no evidence that any of the other disorders with putative "prion-like" protein aggregation are "infectious" or "transmissible" in a similar sense. This is likely because of the absence of an "infectious cycle" (1). AD is a disorder that only occurs in humans and the signature protein aggregates are confined to the individual's brain (21, 83). Moreover, clinical characteristics of AD do not suggest an infectious nature (1). Even in patients treated with cadaver-derived human growth hormone, no evidence for iatrogenic transmissibility of AD or other protein aggregation disorders was found, except prion diseases (37). However, within the brain, templated misfolding of Aβ peptides may explain disease progression.

EVIDENCE FOR PRION-LIKE TEMPLATED MISFOLDING OF Aβ

In the human brain, Aβ peptides are constantly generated from APP by proteolytic cleavage by β-secretase (BACE-1) generating the N-terminus and by the γ-secretase complex generating the C-terminus (33). γ-Secretase cleavage generates Aβ peptides of various lengths ranging from 37 to 43 residues (68). The 40-residue peptide Aβ (1–40) is the most abundant species in normal brain, followed by Aβ (1–42) species (94). In general, longer Aβ peptides are more aggregation prone than shorter ones (15, 68).

In vitro synthetic or recombinant Aβ peptides readily form aggregates of different kinds: low to high *n* oligomers have been described as well as protofibrils, annular aggregates and mature fibrillar structures (90). Aggregation *in vitro* is concentration-dependent and the precise conformation depends on the Aβ species studied (eg, Aβ40 or Aβ42) as well as on agitation, temperature, pH and peptide concentration. A detailed comparison of the structures of Aβ oligomers and fibrils is beyond the scope of this review and has been described elsewhere (24, 26, 86). Interestingly, Aβ aggregation *in vitro* can be initiated by the addition of preformed Aβ aggregates ("seeds") (36, 40, 74, 93). This circumvents the lag phase and supports the concept of nucleation-dependent aggregation. When different polymorphs of Aβ fibrils were used for *in vitro* seeding, the structural properties of the seeds were reliably propagated to the seeded aggregates indicating templated misfolding (64).

However, most evidence for prion-like templated misfolding of Aβ comes from inoculation studies in transgenic (tg) rodents. The full spectrum of AD is a human-specific disorder and only partially similar pathological changes including cerebral Aβ deposition were found in other mammals. Although the reasons for this are not fully understood, it is likely a combination of differences in APP and Aβ sequence as well as shorter lifespan of other mammals in comparison to the aging human society. Therefore, numerous tg mice and a few tg rats overexpressing human APP and generating human Aβ peptides were made and are often used to study cerebral β-amyloidosis (4, 41, 70). Most of these tg rodents recapitulate age-related onset of Aβ aggregation in the brain in the form of parenchymal plaques and vascular β-amyloid very similar to the lesions characteristic for AD. The age-of-onset varies between the different tg lines and depends on the levels of APP

overexpression and related Aβ levels. Insertion of APP mutations associated with familial AD (FAD) or additional overexpression of human presenilin variants with AD-causing mutations promotes earlier Aβ deposition.

Several laboratories have studied "seeded" induction of cerebral Aβ aggregation in tg rodents (43, 55, 56, 92). The experimental paradigm typically uses dilute homogenates from brains of AD patients or of aged APP tg mice with abundant cerebral β-amyloidosis. Minute amounts of these homogenates are injected into the brains of young, predepositing APP tg rodents and the development of cerebral β-amyloidosis is monitored over time.

The essential findings are (see also Figure 2) as follows: Brain extracts from AD patients or from aged APP tg mice containing *in vivo* generated Aβ aggregates are capable of inducing premature cerebral β-amyloidosis in young APP tg rodents (22, 34, 43, 45, 55, 56, 73, 80, 92). Interestingly, extracts derived from aged APP tg mice with abundant cerebral β-amyloidosis were similarly effective as extracts from AD cases ruling out that factors exclusive to the human brain are essential (55). In contrast, control extracts from healthy individuals or aged wild-type mice without Aβ aggregates fail to induce cerebral β-amyloidosis (22, 45, 55). Likewise, induction of cerebral β-amyloidosis is prevented when Aβ peptides are immunodepleted from the extract or when extracts are treated with formic acid resolving any tertiary protein structure. This indicates that misfolded Aβ peptides are essential components of the "seeding extract" (55). In addition, seeding is significantly reduced when Aβ peptides are neutralized by anti-Aβ antibodies (55). Induced cerebral β-amyloidosis was found to be time-dependent and occurs in histologically detectable amounts with a delay of several months (22, 34, 45, 55, 92). Induction of cerebral β-amyloidosis was also found to depend on the concentration of seeds applied (55). This supports the concept that more seeds, that is, more interfaces for templated misfolding can more efficiently recruit soluble Aβ peptides for templated misfolding.

The lag time until induced Aβ deposits are immunohistochemically detectable is not only dependent on the amount of seeds applied but also on the level of APP overexpression in the tg line used. Lower APP overexpression and thus lower availability of soluble Aβ peptides for misfolding goes along with an extended lag phase (34, 55, 56). In the brains of non-tg mice, no induced Aβ deposition was found with the same kind and amount of extracts and same incubation time (43, 55). Murine Aβ sequence differs from human Aβ sequence by three amino acids and can possibly not easily accommodate the misfolded amyloid state of human Aβ peptides. This again would indicate specificity of templated misfolding. However, it should not be overlooked that, all other rodent lines inoculated generate higher amounts of Aβ peptides because of tg overexpression. Therefore, it is conceivable that lowest availability of soluble Aβ peptides in non-tg mice is not sufficient for seeded induction of histopathologically detectable Aβ aggregation within the lifespan of these mice although templated misfolding may occur at low levels.

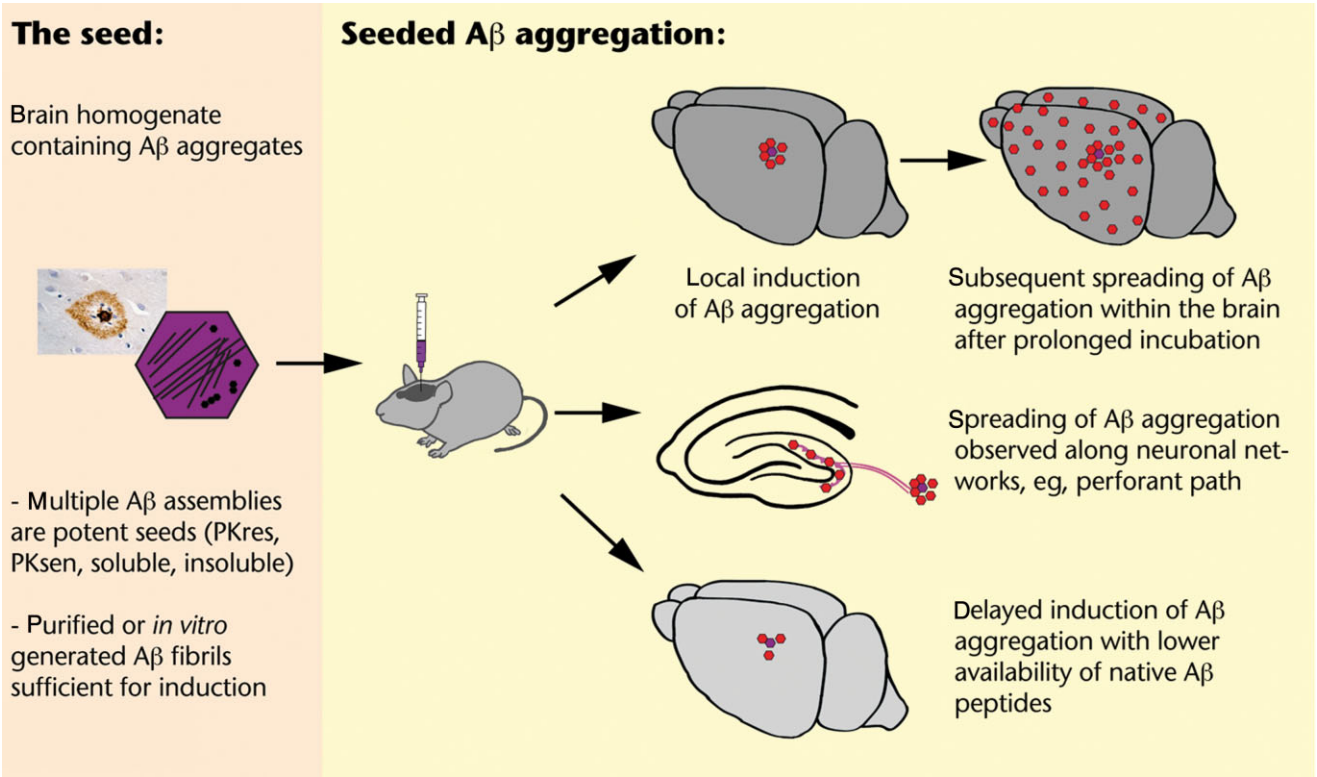

Figure 2. *Schematic overview on induction and spreading of Aβ deposits in brains of young APP transgenic mice following intracerebral seeding with brain homogenates containing aggregated Aβ peptides (22, 23, 34, 45, 55, 80).* [PKres = proteinase K resistant; PKsen = proteinase K sensitive under standard conditions (50 μg PK/mL homogenate, 30 min, 37°C)].

Interestingly, multiple Aβ assemblies seem capable of seeding Aβ aggregation, that is, Aβ aggregates resistant or sensitive to standard proteinase K treatment, soluble or insoluble aggregates when differentiated by 100 000 × g ultracentrifugation, and may thus represent a continuum of seeding-capable Aβ aggregates (45). Soluble seeds seemed to exert high activity, probably because of the presence of many molecular interfaces for templated misfolding (45). This interpretation is also supported by the observation that fragmentation increases the seeding capacity of a given extract (45).

Interestingly, induced deposits are first apparent locally at the site of injection (22, 34, 55). With prolonged incubation times, induced Aβ aggregates increase locally but also appear in neuroanatomically connected brain regions: inoculations into the entorhinal cortex induced Aβ deposits in the outer molecular layer of the dentate gyrus, that is, the terminal zone of projections of the perforant path which connects the entorhinal cortex to the hippocampus (22, 42) and vice versa (89). It was observed that local application of Aβ seeds can induce widespread Aβ deposition throughout cortex and hippocampus in APP tg mice that would otherwise not have any histochemically detectable Aβ deposition at this age (34). This suggests that Aβ aggregates may actively or passively traffic to neighboring and interconnected brain areas. Remarkably, peripheral application of Aβ seeds can also induce cerebral β-amyloidosis in young APP tg mice (23). However, it is unclear how this finding would relate to the human situation.

Recently, Aβ fibrils purified from β-amyloid-laden mouse brains as well as fibrils generated *in vitro* from synthetic Aβ were shown to be capable of inducing cerebral β-amyloidosis in young APP tg mice and are discussed as the final proof that misfolded Aβ by itself promotes prion-like templated misfolding and induces associated pathology (80).

Perhaps the most convincing studies suggesting prion-like templated misfolding of Aβ peptides are observations of reliable propagation of conformational strains. It is well known that Aβ deposits in the AD brain are heterogeneous in histopathological appearance both within and among brain regions and patients. Amyloid plaques are diffuse or compact, smaller or larger in appearance and this is also reflected in the biochemical composition (49, 82, 83). When different extracts were used for the inoculation studies, derived from either APPPS1 tg mice with small and compact, more punctate plaques (69) or from APP23 tg mice with larger and more filamentous and diffuse plaques (81), the characteristic plaque morphology of the donor was replicated in the host (Figure 3) (55). This indicates that the type of Aβ aggregates present in the seeding extract has a major influence in shaping the induced deposits. This could be conveyed by prion-like templated misfolding. At present, it is not well known if polymorphic Aβ

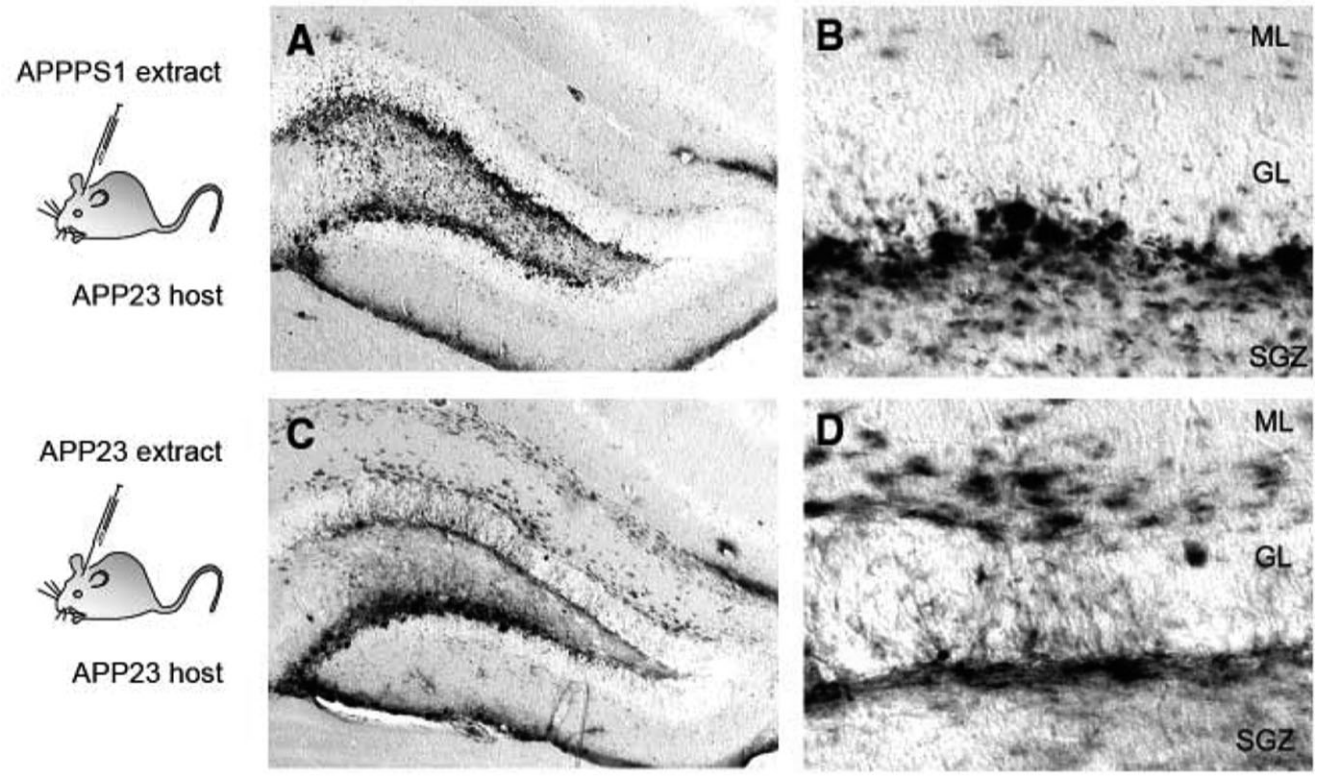

Figure 3. *Seeding in young APP23 transgenic hosts induced different morphotypes of Aβ deposits when different extracts were used for intracerebral inoculations.* Note the more punctate appearance of induced deposits with APPPS1 extract, especially in the subgranular cell layer (SGZ) (**A, B**) in contrast to more filamentous and diffuse Aβ deposits induced with APP23 extract (**C, D**). The morphotypes of the induced Aβ deposits resemble the Aβ deposits found in the brains of the donors of the extracts [from Meyer-Luehmann *et al* (55). Reprinted with permission from AAAS]. ML = molecular layer; GL = granular cell layer.

"strains" are also polyfunctional and are linked to differences in toxicity or the clinical spectrum of AD.

Taken together, all these studies *in vitro* and *in vivo* support the concept of prion-like templated misfolding of Aβ peptides as defined earlier. Although tg rodents predisposed to developing cerebral β-amyloidosis were used, the observation of induced Aβ deposits in tg lines without Aβ deposits within the normal lifespan suggests true seeded conversion of normally soluble Aβ peptides and not simply acceleration of an ongoing process (56, 73). However, other possible explanations should not be neglected and will largely have to take into account that inoculation of Aβ aggregates may cause secondary effects that indirectly promote Aβ aggregation, especially in environments with unnaturally high concentrations of Aβ peptides. Regarding the observed spreading of Aβ deposits within neuronal networks, indirect effects of compromised neuronal functions that may affect APP processing or Aβ levels are conceivable. Moreover, these experiments in tg rodents focused on Aβ aggregation, and therefore do not allow a conclusion if induction of cerebral Aβ deposition would provoke the whole spectrum of clinical AD. However, it was repeatedly observed that the induced deposits resemble typical AD-like Aβ deposits and can be accompanied by dystrophic neurites with typical hyperphosphorylation of tau proteins, as well as micro- and astrogliosis (22, 23, 34, 45, 55, 92). While induced deposits may be largely diffuse initially, a more compact and congophilic appearance was noted with longer incubation times, indicating maturation toward more pathological lesions (45). In addition, region-specific differences in induced deposits may point to the importance of additional cellular factors shaping Aβ aggregation, for example, induced deposits in the striatum of APP23 tg mice were largely diffuse within the observation time whereas cortical or hippocampal inoculation resulted in more compact deposits (22). Last but not least, minute amounts of Aβ aggregates may be sufficient to trigger progressive tau aggregation and pathology (11, 29).

Naturally, no such inoculation studies can be undertaken in humans. Attempts in nonhuman primates were initially inconclusive (30), but subsequent work has shown that intracerebral inoculation of crude homogenates from AD brain into marmosets can induce β-amyloid deposition after incubation times of at least 5–6 years (6, 71). However, because of the high prevalence of AD and Aβ-CAA and the suggestive spreading within brain networks (13, 78), it will be important to further probe this concept for understanding the human disorders and for developing therapeutics.

IMPLICATIONS OF PRION-LIKE TEMPLATED MISFOLDING OF Aβ PEPTIDES

These findings have multiple implications with regard to future research and to the development of therapeutics for the treatment and the prevention of Aβ-misfolding disorders. Disease-specific Aβ aggregation in the brains of individuals in prodromal AD stages has been detected many years to decades before clinical symptoms manifest (39, 62). Aβ seeds if present in body fluids (eg, CSF) could serve as an early and disease-specific biomarker, complementing currently used measurements of total CSF Aβ and tau levels (10). However, it is at present unknown if any kind of Aβ seed exists outside the brain, and if so, how efficient its capability to transmit the misfolded state via templated misfolding would be. Evidently, if such seeds would exist, accidental transmission between individuals must be avoided, particularly as the seeds may be chemically stable and resistant to some common inactivation procedures.

Our current understanding of the kinetics of Aβ aggregation and the indications for prion-like templated misfolding highlights several possibilities for therapeutic intervention: First, by keeping Aβ levels below the critical (local) concentration for aggregation. This could be achieved by inhibition or modulation of the enzymes releasing Aβ from its precursor (28, 95), by increasing Aβ degradation (75) or by promoting its clearance. Second, by removing existing aggregates. Some enzymes have been shown to be capable of degrading or dissociating small Aβ aggregates (75). In addition, immunotherapy was shown to be efficient in removing Aβ deposits (46). Third, by capping templated misfolding or by promoting the formation of less toxic aggregates. In principle, the cascade of progressive templated misfolding would be stopped if the molecular interfaces for templated misfolding are not available (24). Similarly, if differentially toxic Aβ aggregate versions exist, a shift toward less toxic and more biologically inert forms could be beneficial. Fourth, preventing the spreading of Aβ aggregation to neighboring or interconnected tissue may limit the detrimental effects and concomitant progressive cognitive deficits. Fifth, and maybe most importantly, compounds that preserve neuronal and synaptic integrity and function could keep dementia at bay. The prediction would be that all disease-modifying strategies are most effective as prevention or in the early stages of the disease because severe pathology and massive neuron loss in late stages are likely irreversible. Such early or preventive therapeutics may have to be taken for many years or decades, and therefore, side effects should be avoided and well-tolerated yet effective medication would be desirable. This is especially challenging as we yet lack a good understanding of the physiological functions of APP, its diverse fragments including Aβ and the enzymes involved in APP processing. In the end, likely not one but several of the possible therapeutic approaches outlined above may be needed for preventing cognitive decline. There is a silver lining for causative or preventive AD treatment as several compounds targeting Aβ-generating enzymes, anti-Aβ immunotherapy and others are already being tested in clinical trials and some are under consideration for preventive trials (27). While Aβ aggregation may be the most relevant trigger for Aβ-CAA, it may only be the starting point for AD. Changes in tau phosphorylation and eventually tau aggregation may be the effectors leading to AD neurodegeneration (38, 72, 88). As tau aggregation likewise seems to propagate by prion-like templated misfolding (Clavaguera *et al*, this issue), it will be important for any Aβ-targeting AD therapeutics to stop Aβ aggregation before tau aggregation proceeds beyond the point of no return.

CONCLUSIONS

Prion-like templated misfolding of Aβ peptides provides a plausible framework to explain the progressive accumulation and spreading of Aβ aggregation within the AD brain. This concept has far-reaching implications for possible therapeutic interventions. In particular, the need for early intervention before a fatal cascade of Aβ misfolding leads to neuropathological

damage beyond repair. However, more work is needed to fully understand the characteristics of this process and its contribution to AD and other Aβ-misfolding disorders. At present, prion-like templated misfolding is discussed as a driving force in several neurodegenerative disorders. This could unify experimental and translational approaches to these increasingly prevalent and devastating disorders.

FUNDING

The work in our laboratory was supported by grants from the Competence Network on Degenerative Dementias (BMBF-01G10705) and the Alzheimer Forschung Initiative e.V. (AFI).

CONFLICT OF INTEREST

The author declares that she has no conflicting interest related to this article.

ACKNOWLEDGMENTS

The author would like to thank Mathias Jucker, Lary Walker, Matthias Staufenbiel and all members of the laboratory for the support and helpful discussion.

REFERENCES

1. Aguzzi A (2009) Cell biology: beyond the prion principle. *Nature* **459**:924–925.
2. Aguzzi A, Heikenwalder M, Polymenidou M (2007) Insights into prion strains and neurotoxicity. *Nat Rev Mol Cell Biol* **8**:552–561.
3. Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. *Allg Zeitschr Psychiatr* **64**:146–148.
4. Ashe KH, Zahs KR (2010) Probing the biology of Alzheimer's disease in mice. *Neuron* **66**:631–645.
5. Askanas V, Engel WK (2006) Inclusion-body myositis: a myodegenerative conformational disorder associated with Abeta, protein misfolding, and proteasome inhibition. *Neurology* **66** (Suppl. 1):S39–S48.
6. Baker HF, Ridley RM, Duchen LW, Crow TJ, Bruton CJ (1994) Induction of beta (A4)-amyloid in primates by injection of Alzheimer's disease brain homogenate. Comparison with transmission of spongiform encephalopathy. *Mol Neurobiol* **8**:25–39.
7. Bentahir M, Nyabi O, Verhamme J, Tolia A, Horre K, Wiltfang J *et al* (2006) Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. *J Neurochem* **96**:732–742.
8. Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, Botto RE, Meredith SC (1998) Propagating structure of Alzheimer's beta-amyloid(10-35) is parallel beta-sheet with residues in exact register. *Proc Natl Acad Sci U S A* **95**:13407–13412.
9. Biffi A, Greenberg SM (2011) Cerebral amyloid angiopathy: a systematic review. *J Clin Neurol* **7**:1–9.
10. Blennow K, Zetterberg H, Fagan AM (2012) Fluid biomarkers in Alzheimer disease. *Cold Spring Harb Perspect Med* **2**:a006221.
11. Bolmont T, Clavaguera F, Meyer-Luehmann M, Herzig MC, Radde R, Staufenbiel M *et al* (2007) Induction of tau pathology by intracerebral infusion of amyloid-beta-containing brain extract and by amyloid-beta deposition in APPxTau transgenic mice. *Am J Pathol* **171**(6):2012–2020.
12. Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T *et al* (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron* **17**:1005–1013.
13. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**:239–259.
14. Braak H, Braak E (1997) Frequency of stages of Alzheimer-related lesions in different age categories. *Neurobiol Aging* **18**:351–357.
15. Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A *et al* (1992) Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J Biol Chem* **267**:546–554.
16. Citron M, Westaway D, Xia W, Carlson G, Diehl T, Levesque G *et al* (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat Med* **3**:67–72.
17. Colby DW, Prusiner SB (2011) Prions. *Cold Spring Harb Perspect Biol* **3**:a006833.
18. Colletier JP, Laganowsky A, Landau M, Zhao M, Soriaga AB, Goldschmidt L *et al* (2011) Molecular basis for amyloid-beta polymorphism. *Proc Natl Acad Sci U S A* **108**:16938–16943.
19. De Jonghe C, Esselens C, Kumar-Singh S, Craessaerts K, Serneels S, Checler F *et al* (2001) Pathogenic APP mutations near the gamma-secretase cleavage site differentially affect Abeta secretion and APP C-terminal fragment stability. *Hum Mol Gen* **10**:1665–1671.
20. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J *et al* (1996) Increased amyloid-beta42 (43) in brains of mice expressing mutant presenilin 1. *Nature* **383**:710–713.
21. Duyckaerts C, Delatour B, Potier MC (2009) Classification and basic pathology of Alzheimer disease. *Acta Neuropathol* **118**:5–36.
22. Eisele YS, Bolmont T, Heikenwalder M, Langer F, Jacobson LH, Yan ZX *et al* (2009) Induction of cerebral beta-amyloidosis: intracerebral versus systemic Abeta inoculation. *Proc Natl Acad Sci U S A* **106**:12926–12931.
23. Eisele YS, Obermuller U, Heilbronner G, Baumann F, Kaeser SA, Wolburg H *et al* (2010) Peripherally applied Abeta-containing inoculates induce cerebral beta-amyloidosis. *Science* **330**: 980–982.
24. Eisenberg D, Jucker M (2012) The amyloid state of proteins in human diseases. *Cell* **148**:1188–1203.
25. Falsig J, Nilsson KP, Knowles TP, Aguzzi A (2008) Chemical and biophysical insights into the propagation of prion strains. *HFSP J* **2**:332–341.
26. Fandrich M (2012) Oligomeric intermediates in amyloid formation: structure determination and mechanisms of toxicity. *J Mol Biol* **421**:427–440.
27. Gerald Z, Ockert W (2012) Alzheimer's disease market: hope deferred. *Nat Rev Drug Discov* **12**:19–20.
28. Ghosh AK, Brindisi M, Tang J (2012) Developing β-secretase inhibitors for treatment of Alzheimer's disease. *J Neurochem* **120**(Suppl. 1):71–83.
29. Gotz J, Chen F, van Dorpe J, Nitsch RM (2001) Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. *Science* **293**:1491–1495.
30. Goudsmit J, Morrow CH, Asher DM, Yanagihara RT, Masters CL, Gibbs CJ Jr, Gajdusek DC (1980) Evidence for and against the transmissibility of Alzheimer disease. *Neurology* **30**:945–950.
31. Graeber MB, Mehraein P (1999) Reanalysis of the first case of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* **249** (Suppl. 3):10–13.
32. Greenberg SA (2009) Inclusion body myositis: review of recent literature. *Curr Neurol Neurosci Rep* **9**:83–89.
33. Haass C, Kaether C, Thinakaran G, Sisodia S (2012) Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med* **2**:a006270.

34. Hamaguchi T, Eisele YS, Varvel NH, Lamb BT, Walker LC, Jucker M (2012) The presence of Aβ seeds, and not age per se, is critical to the initiation of Aβ deposition in the brain. *Acta Neuropathol* **123**:31–37.
35. Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**:353–356.
36. Harper JD, Lansbury PT Jr (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. *Annu Rev Biochem* **66**:385–407.
37. Irwin DJ, Abrams JY, Schonberger LB, Leschek EW, Mills JL, Lee VM, Trojanowski JQ (2013) Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. *JAMA Neurol* **4**:1–7.
38. Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J *et al* (2010) Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell* **142**:387–397.
39. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW *et al* (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**:119–128.
40. Jarrett JT, Lansbury PT Jr (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73**:1055–1058.
41. Jucker M (2010) The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nat Med* **16**:1210–1214.
42. Jucker M, Walker LC (2011) Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann Neurol* **70**:532–540.
43. Kane MD, Lipinski WJ, Callahan MJ, Bian F, Durham RA, Schwarz RD *et al* (2000) Evidence for seeding of beta-amyloid by intracerebral infusion of Alzheimer brain extracts in beta-amyloid precursor protein-transgenic mice. *J Neurosci* **20**:3606–3611.
44. Kuo YM, Kokjohn TA, Beach TG, Sue LI, Brune D, Lopez JC *et al* (2001) Comparative analysis of amyloid-beta chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. *J Biol Chem* **276**:12991–12998.
45. Langer F, Eisele YS, Fritschi SK, Staufenbiel M, Walker LC, Jucker M (2011) Soluble Aβ seeds are potent inducers of cerebral β-amyloid deposition. *J Neurosci* **31**:14488–14495.
46. Liu YH, Giunta B, Zhou HD, Tan J, Wang YJ (2012) Immunotherapy for Alzheimer disease: the challenge of adverse effects. *Nat Rev Neurol* **8**:465–469.
47. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L *et al* (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol* **15**(3):853–862.
48. Luhrs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H *et al* (2005) 3D structure of Alzheimer's amyloid-beta(1-42) fibrils. *Proc Natl Acad Sci U S A* **102**:17342–17347.
49. Maarouf CL, Daugs ID, Spina S, Vidal R, Kokjohn TA, Patton RL *et al* (2008) Histopathological and molecular heterogeneity among individuals with dementia associated with Presenilin mutations. *Mol Neurodegener* **3**:20.
50. Mandelkow EM, Mandelkow E (2012) Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med* **2**:a006247.
51. Masters CL, Selkoe DJ (2012) Biochemistry of amyloid β-protein and amyloid deposits in Alzheimer disease. *Cold Spring Harb Perspect Med* **2**:a006262.
52. Mastrangelo P, Mathews PM, Chishti MA, Schmidt SD, Gu Y, Yang J *et al* (2005) Dissociated phenotypes in presenilin transgenic mice define functionally distinct gamma-secretases. *Proc Natl Acad Sci U S A* **102**:8972–8977.
53. Maurer K, Volk S, Gerbaldo H (1997) Auguste D and Alzheimer's disease. *Lancet* **349**(9064):1546–1549.
54. McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K *et al* (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* **46**(6):860–866.
55. Meyer-Luehmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, Kilger E *et al* (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science* **313**:1781–1784.
56. Morales R, Duran-Aniotz C, Castilla J, Estrada LD, Soto C (2012) De novo induction of amyloid-β deposition *in vivo*. *Mol Psychiatry* **17**:1347–1353.
57. Mucke L, Selkoe DJ (2012) Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med* **2**:a006338.
58. Muller U, Winter P, Graeber MB (2013) A presenilin 1 mutation in the first case of Alzheimer's disease. *Lancet Neurol* **12**(2):129–130.
59. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000) Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* **283**:1571–1577.
60. Nogalska A, D'Agostino C, Engel WK, Klein WL, Askanas V (2010) Novel demonstration of amyloid-β oligomers in sporadic inclusion-body myositis muscle fibers. *Acta Neuropathol* **120**:661–666.
61. Paravastu AK, Qahwash I, Leapman RD, Meredith SC, Tycko R (2009) Seeded growth of β-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. *Proc Natl Acad Sci U S A* **106**:7443–7448.
62. Perrin RJ, Fagan AM, Holtzman DM (2009) Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature* **461**:916–922.
63. Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R (2002) A structural model for Alzheimer's beta-amyloid fibrils based on experimental constraints from solid state NMR. *Proc Natl Acad Sci U S A* **99**:16742–16747.
64. Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP, Tycko R (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* **307**: 262–265.
65. Polymenidou M, Cleveland DW (2012) Prion-like spread of protein aggregates in neurodegeneration. *J Exp Med* **209**:889–893.
66. Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. *Science* **216**:136–144.
67. Prusiner SB (2012) Cell biology. A unifying role for prions in neurodegenerative diseases. *Science* **336**:1511–1513.
68. Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y, Dolios G, Hirotani N, Horikoshi Y *et al* (2005) Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase. *J Neurosci* **25**:436–445.
69. Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L *et al* (2006) Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep* **7**:940–946.
70. Radde R, Duma C, Goedert M, Jucker M (2008) The value of incomplete mouse models of Alzheimer's disease. *Eur J Nucl Med Mol Imaging* **35**(Suppl. 1):S70–S74.
71. Ridley RM, Baker HF, Windle CP, Cummings RM (2006) Very long term studies of the seeding of beta-amyloidosis in primates. *J Neural Transm* **113**:1243–1251.

72. Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T *et al* (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* **316**:750–754.
73. Rosen RF, Fritz JJ, Dooyema J, Cintron AF, Hamaguchi T, Lah JJ *et al* (2012) Exogenous seeding of cerebral β-amyloid deposition in βAPP-transgenic rats. *J Neurochem* **120**:660–666.
74. Roychaudhuri R, Yang M, Hoshi MM, Teplow DB (2009) Amyloid beta-protein assembly and Alzheimer disease. *J Biol Chem* **284**:4749–4753.
75. Saido T, Leissring MA (2012) Proteolytic degradation of amyloid β-protein. *Cold Spring Harb Perspect Med* **2**:a006379.
76. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N *et al* (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* **2**:864–870.
77. Schmidt M, Sachse C, Richter W, Xu C, Fandrich M, Grigorieff N (2009) Comparison of Alzheimer Abeta(1-40) and Abeta(1-42) amyloid fibrils reveals similar protofilament structures. *Proc Natl Acad Sci U S A* **106**:19813–19818.
78. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD (2009) Neurodegenerative diseases target large-scale human brain networks. *Neuron* **62**:42–52.
79. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT (2011) Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* **1**:a006189.
80. Stohr J, Watts JC, Mensinger ZL, Oehler A, Grillo SK, DeArmond SJ *et al* (2012) Purified and synthetic Alzheimer's amyloid beta (Aβ) prions. *Proc Natl Acad Sci U S A* **109**:11025–11030.
81. Sturchler-Pierrat C, Abramowski D, Duke M, Wiederhold KH, Mistl C, Rothacher S *et al* (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc Natl Acad Sci U S A* **94**:13287–13292.
82. Tekirian TL, Saido TC, Markesbery WR, Russell MJ, Wekstein DR, Patel E, Geddes JW (1998) N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. *J Neuropathol Exp Neurol* **57**:76–94.
83. Thal DR, Capetillo-Zarate E, Del Tredici K, Braak H (2006) The development of amyloid beta protein deposits in the aged brain. *Sci Aging Knowledge Environ* **2006**:re1.
84. Torok M, Milton S, Kayed R, Wu P, McIntire T, Glabe CG, Langen R (2002) Structural and dynamic features of Alzheimer's Abeta peptide in amyloid fibrils studied by site-directed spin labeling. *J Biol Chem* **277**:40810–40815.
85. Tycko R (2011) Solid-state NMR studies of amyloid fibril structure. *Ann Rev Phys Chem* **62**:279–299.
86. Tycko R, Wickner RB (2013) Molecular structures of amyloid and prion fibrils: consensus versus controversy. *Acc Chem Res*. doi: 10.1021/ar300282r.
87. Vattemi G, Nogalska A, King Engel W, D'Agostino C, Checler F, Askanas V (2009) Amyloid-beta42 is preferentially accumulated in muscle fibers of patients with sporadic inclusion-body myositis. *Acta Neuropathol* **117**:569–574.
88. Vossel KA, Zhang K, Brodbeck J, Daub AC, Sharma P, Finkbeiner S *et al* (2010) Tau reduction prevents Abeta-induced defects in axonal transport. *Science* **330**:198.
89. Walker LC, Callahan MJ, Bian F, Durham RA, Roher AE, Lipinski WJ (2002) Exogenous induction of cerebral beta-amyloidosis in betaAPP-transgenic mice. *Peptides* **23**:1241–1247.
90. Walsh DM, Selkoe DJ (2007) A beta oligomers—a decade of discovery. *J Neurochem* **101**:1172–1184.
91. Wang J, Dickson DW, Trojanowski JQ, Lee VM (1999) The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathological aging. *Exp Neurol* **158**(2):328–337.
92. Watts JC, Giles K, Grillo SK, Lemus A, DeArmond SJ, Prusiner SB (2011) Bioluminescence imaging of Abeta deposition in bigenic mouse models of Alzheimer's disease. *Proc Natl Acad Sci U S A* **108**:2528–2533.
93. Wetzel R (2006) Kinetics and thermodynamics of amyloid fibril assembly. *Acc Chem Res* **39**:671–679.
94. Wolfe MS (2007) When loss is gain: reduced presenilin proteolytic function leads to increased Abeta42/Abeta40. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep* **8**:136–140.
95. Wolfe MS (2012) γ-Secretase as a target for Alzheimer's disease. *Adv Pharmacol* **64**:127–153.