Review: prion protein and amyloid beta interactions

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Abstract

In 2009 it was discovered that prion protein (PrP) binds amyloid beta oligomers (Aβo), potentially acting as a receptor on the surface of neurons. PrP was additionally suggested to mediate at least some of the toxic effects of Aβo in Alzheimer's disease (AD), a proposition which has proven enormously controversial. In the past three years, several studies have produced apparently strong evidence both for and against the notion that PrP mediates Aβo toxicity and AD pathology. Though no consensus has been reached on the overall importance of PrP in AD pathology, recent efforts to elucidate the signaling pathways that are activated by Aβo binding to PrP have provided consistent evidence that this binding event causes activation of the Src family kinase Fyn, leading to downstream phosphorylation events with potential relevance to AD.

Introduction

Background on PrP

Prion protein (PrP) was discovered, and gained notoriety, for its capacity to convert into an infectious agent that can propagate and cause disease in the absence of nucleic acids [Prusiner 1982]. Human PrP is a 208 amino acid GPI-anchored glycoprotein of unknown native function encoded by the gene *PRNP*, ubiquitously expressed and abundant in the central nervous system. It is ordinarily found in a healthy 'cellular' conformation (PrP^{C}) but its conversion to an infectious 'scrapie' conformation (PrP^{Sc}) is responsible for the class of rapidly fatal, untreatable diseases

known as transmissible spongiform encephalopathies (TSEs) or prion diseases. These include Creutzfeldt-Jakob Disease, Fatal Familial Insomnia, Gerstmann-Straussler-Scheinker syndrome, and kuru in humans, and scrapie, bovine spongiform encephalopathy ('mad cow') and chronic wasting disease in other mammals. The PrP amino acid sequence is highly conserved among mammals, yet knockout mice, cows and goats are healthy, fertile and viable [Bueler 1992, Richt 2007, Benestad 2012]. Certain knockout phenotypes have been reported under stress or late in life [Steele 2007, Bremer 2010].

Although PrP's native function is unknown, its participation in certain interactions is well established. Over a decade ago it was shown that cross-linking of PrP on mature neurons leads to activation of the tyrosine kinase Fyn [Mouillet-Richard 2000], but the significance of this signal transduction pathway has not been understood. More recently, it has been shown that PrP inhibits the activity of BACE1, reducing the formation of Aβ [Parkin 2007]. The importance of these two roles will be explored further below.

Background on Aβ

Alzheimer's disease (AD) is the most common cause of dementia. It is characterized by two main neuropathological features: the accumulation of amyloid beta (Aβ) plaques and the formation of neurofibrillary tangles (NFTs) of hyperphosphorylated Tau protein. 95 - 99% of AD cases are late onset and idiopathic (of unknown molecular origin); the remainder are early onset familial forms caused by mutations in amyloid precursor protein (APP) or presenilins 1 or 2 (PSEN1 and PSEN2) [Bekris 2010]. APP is a Type I transmembrane protein which undergoes sequential cleavage by β-secretase (BACE1) and γ-secretase (a complex which includes both PSEN1 and PSEN2) to produce 40- or 42-residue N-terminal fragments known as $A\beta_{40}$ and $A\beta_{42}$ [Vassar 2009]. Mutations in APP, PSEN1 and PSEN2 that cause familial AD have been reported to increase the ratio of $A\beta_{42}$ to $A\beta_{40}$.

These peptides are present in the AD brain as monomers, as oligomers and as larger plaques. This review will use the term "Aβ" to refer generally to these peptides, regardless of aggregation state, and "Aβo" to refer specifically to oligomers. Oligomers of $A\beta_{42}$ are suspected to comprise the toxic species in AD [Bekris 2010]. The relationship between Aβ accumulation and Tau pathology in AD is not yet clear. Mutations in Tau (MAPT) in humans cause frontotemporal dementia (FTD), not AD, and some transgenic mouse models with only APP or PSEN mutations fail to form NFTs, leading some researchers to employ mice with both APP and MAPT mutations in order to recapitulate a more full set of human neuropathological changes associated with AD [Bryan 2009].

Though the toxic role of Aβ in the Alzheimer's brain is still poorly understood, certain species of Aβ are known to acutely impair neurons. Injected Aβ inhibits long-term potentiation (LTP) in the rat hippocampus [Walsh 2002] and cause memory and behavioral changes in rats [Lesne 2006]. However, the exact size (monomer, dimer, trimer, etc.) and structure (globular, spherical, fibrillar, etc.) of the toxic Aβ species is highly controversial [Benilova 2012].

Interest in the mechanism of this acute neuronal impairment by Aβ led to a cDNA library screen to identify proteins that physically interact with synthetic Aβo, which revealed a single strong hit: PrP [Lauren 2009]. PrP bound to synthetic Aβo with an order of magnitude stronger affinity than any other Aβo binding partners and appeared to account for about 50% of total and Aβo binding to cells. Moreover, Aβo was found to inhibit LTP in wild-type mouse hippocampal slices but not *Prnp*$^{-/-}$ slices, implicating PrP as a receptor necessary for Aβo-induced neuronal impairment. This phenotype could be rescued by 6D11, a monoclonal antibody against mouse PrP residues 93-109, approximately covering the putative Aβo binding site at residues 95-110. For clarity, note that all mice and cultures used in these experiments were uninfected with scrapie and therefore all

findings are assumed to refer to PrP^{C} and therefore to describe a native function of PrP. Aβo binding to PrP^{Sc} has not been shown.

The finding that PrP binds Aβo has been replicated several times and never disputed [Biasini 2012]. However, the finding that PrP is required for Aβo impairment of neurons and, by implication, for Alzheimer's pathogenesis, has proven enormously controversial. Though no clear answers have emerged yet, this paper will review recent findings with a view to understanding the current status of three questions:

1. Does PrP mediate acute neuronal impairment by Aβo?
2. Does PrP mediate Alzheimer's pathology in transgenic animal models?
3. What are the molecular consequences of PrP/Aβo interaction?

1. Does PrP mediate acute neuronal impairment by Aβo?

The claim that PrP is required for Aβo-induced neuronal impairment was quickly challenged in a battery of experiments [Kessels 2010]. Most importantly, Kessels reproduced the exact same system that Lauren had studied: long-term potentiation in the CA1 region of the mouse hippocampus using brain slices from wild-type and *Prnp*$^{-/-}$ mice. Kessels' synthetic $A\beta_{42}$ oligomers impaired neuronal LTP equally in both genotypes, implying that PrP expression is not necessary for the Aβo blockade of LTP. Kessels further examined two other established neuronal phenotypes of Aβo exposure: dendritic spine loss in neurons overexpressing APP or exposed to $A\beta_{42}$ oligomers, and synaptic depression in neurons expressing APPct100, a truncated form of APP that results in high Aβ production. Both of these phenotypes were equally observed in wild-type and *Prnp*$^{-/-}$ brain slices, confirming the dispensibility of PrP for Aβo's effects.

In agreement with Kessels' findings in brain slices, another group reported that intraventricular injections of synthetic $A\beta_{42}$ oligomers caused behavioral changes equally in wild-type and *Prnp*$^{-/-}$ mice [Balducci 2010]. The behavioral measurements involved exposing mice to a collection of novel and familiar objects and measuring the time that the mice spent with each. By default, mice prefer novel objects over familiar ones, and Balducci found that this held true for wild-type mice after injection with vehicle, injection with synthetic Aβ in its 'initial state' (presumably monomers), or injection with Aβ fibrils, confirming previous findings that Aβ monomers and fibrils are not acutely toxic. The preference for novel objects was diminished, however, following injection with synthetic $A\beta_{42}$ oligomers. The authors interpreted this as an inability to *discriminate* between objects, thus implying memory impairment. This reduction in discrimination was found in wild-type and *Prnp*$^{-/-}$ mice, thus supporting the conclusion that Aβo's effects on memory are independent of PrP. This finding has been questioned: Balducci's data show $A\beta_{42}$ oligomer-injected mice spending *more* time with familiar objects than novel ones, which other authors have taken to imply a change in *preference*, not *discrimination* [Gimbel & Nygaard 2010]. Indeed, Balducci's data suggest that this change in preference is actually stronger in *Prnp*$^{-/-}$ mice than in wild-type mice, which could suggest some PrP-dependent mechanism, albeit with an unexpected direction of effect.

While the best interpretation of the mouse behavioral data can be debated, Balducci's and Kessel's results uniformly failed to support the notion that the acutely deleterious effects of $A\beta_{42}$ oligomers on the brain are mediated by binding to PrP. However, it is increasingly recognized that preparations of Aβo and/or of Aβ aggregates can differ dramatically between laboratories [No Authors Listed 2011]. It is therefore of great importance that a different team not only replicated Lauren's original result with synthetic $A\beta_{42}$ oligomers but also extended it to authentic materials, using Aβ species derived from human AD brain tissue [Freir 2011].

Noting that the size, structure, and concentration of Aβo could explain the discrepancies between Kessel's and Lauren's results, Freir was careful to provide an exact description of the procedures used to synthesize biotinylated and unbiotinylated $A\beta_{42}$ oligomers *in vitro*. Freir's synthetic oligomers proved to inhibit LTP in hippocampal slices from wild-type mice but not from *Prnp*$^{-/-}$ mice. Further noting that synthetic Aβo may not represent a toxic species found *in vivo*, Freir also exposed the hippocampal slices to soluble extracts isolated from postmortem brain tissue from AD patients and from control, non-demented subjects. As expected, the control extracts had no effect. The AD brain extracts were found to inhibit LTP in wild-type brain slices but not in *Prnp*$^{-/-}$ brain slices.

Freir next considered whether the PrP/Aβo interaction could represent a therapeutic target in AD. In the wild-type brain slices, Freir discovered that LTP impairment by Aβo could be blocked by either of two well-characterized monoclonal antibodies against PrP. One of these antibodies, ICSM-35, binds to PrP residues 95-110, identical to the putative binding site of Aβo. The other, ICSM-18, binds at PrP alpha-helix 1 - residues 145-154 [Riek 1996] - and thus its mechanism of action with regards to Aβo is not immediately obvious. Based on the known epitope of ISCM-18 and a crystal structure of the antibody bound to PrP^C [Antonyuk 2009] Freir dismisses the possibility of steric hindrance as a mechanism, instead proposing that the antibody may block hypothetical PrP:PrP interactions which could be required for Aβo binding. ISCM-18 and ICSM-35 are slated for a clinical trial as therapeutic agents in prion diseases [MRC Prion Unit, accessed April 22, 2013], raising the possibility that if successful they may also be investigated as AD therapeutics. Another team also investigated the feasibility of blocking the Aβo-PrP interaction using antibodies against PrP, and found that Fab D13, which targets PrP residues 96-104, prevented Aβo-induced LTP impairment in rat hippocampal slices, while Fab R1, which binds to PrP at residues 225-231, the most extreme C-

terminus of the protein, had no effect [Barry 2011]. This is in agreement with Freir's finding that the PrP/Aβo interaction can be disrupted immunologically, at least through binding at certain PrP epitopes if not others.

By using extracts from AD postmortem brain tissue, Freir and Barry both confirmed that authentically derived species of Aβo induce LTP impairment in a PrP-dependent manner. This may be reconciled with Kessel's and Balducci's findings by assuming that the latter two groups' synthetic Aβo preparations differed from the synthetic preparations of both Lauren and Freir in containing an oligomeric Aβ species that (1) induces neuronal impairment by a PrP-independent mechanism, and (2) is not found *in vivo* in the AD brain. Alternately, point (1) may be accepted but point (2) may be substituted for an assumption that said species *does* exist in the AD brain but was not successfully extracted by Freir's or Barry's protocols.

In either case, though, hippocampal LTP impairment is merely one system in which to model the deleterious effects of Aβo in the brain. At the same time as the above studies were being carried out, experiments were underway to assess the importance of PrP for the appearance of AD pathology *in vivo* in transgenic mouse models of AD.

2. Does PrP mediate Alzheimer's pathology in transgenic animal models?

Many transgenic mouse models of AD use familial early onset AD-causing mutations in either APP, PSEN1, or both [Bryan 2009]. Two separate groups crossed mice with both APP and PSEN1 mutations (hereafter "AD" mice) with *Prnp*$^{-/-}$ mice in order to assess the relevance of PrP expression to AD pathology *in vivo*, with completely conflicting results [Gimbel & Nygaard 2010, Calella 2010].

The first group used already-characterized APPswe/PSen1ΔE9 mice, which form Aβ plaques but have not been reported to form NFTs [Jankowsky 2004]. These AD mice have also been reported to show significantly reduced lifespans compared to wild-type mice, though the investigators reporting this offered several caveats to their study, including the use of non-littermates, mixed genetic background and possible viral infections [Halford & Russell 2009]. This first group reported that AD/*Prnp*$^{-/-}$ mice were indistinguishable from controls in Morris water maze performance (a test of spatial learning and memory), synapse loss, and survival, even while AD/*Prnp*$^{+/+}$ mice deteriorated severely on all of these measures. The AD/*Prnp*$^{-/-}$ mice produced APP and Aβ, and deposited Aβ plaques, in equal quantities as AD/*Prnp*$^{+/+}$ mice, yet did not appear to be impaired by the presence of this Aβ [Gimbel & Nygaard 2010].

The second group used a separate, but similar, already-characterized AD mouse model known as APPPS1^{+} [Radde 2006]. Compared to APPswe/PSen1ΔE9, the APPPS1 model carries the same 'Swedish' APP mutation but a different PSEN1 mutation (L166P instead of ΔE9). These mice were crossed to *Prnp*$^{-/-}$ mice and evaluated for a set of phenotypes different than used in the above-described study [Calella 2010]. Calella evaluated the mice primarily by electrophysiological measurements of LTP at four months of age, and reported no difference between AD/*Prnp*$^{+/+}$, AD/*Prnp*$^{+/-}$, and AD/*Prnp*$^{-/-}$ mice. Calella reported no reduced survival in any genotype, precluding any comparison with Gimbel & Nygaard's results. A potential confounder was impure genetic background: the mouse crosses performed to generate these mice were insufficient to recombine mouse chromosome *Mmu2*, location of both *Prnp* and the AD transgenes. To rule out these confounding effects, Calella also crossed the AD mice to transgenics overexpressing PrP or overexpressing a mutant form of PrP lacking the GPI anchor. These transgenes did not exacerbate the LTP impairment phenotype in AD mice; to the contrary, the overexpression of PrP trended toward rescue, and the overexpression of unanchored PrP significantly rescued the phenotype.

Because these studies used similar but not identical AD mouse models and measured different phenotypes, a direct comparison is nearly impossible. Gimbel & Nygaard's behavioral phenotypes are more variable and difficult to measure than Calella's electrophysiological measurement; whether either represent an informative model of human AD pathology is debatable. Calella notes that Gimbel & Nygaard fail to address the problem of residual genetic linkage on *Mmu2*. Further, Calella's finding that overexpression of PrP does not exacerbate LTP impairment does appear to rule out a dose-dependent effect of PrP, at least on LTP.

A third mouse study, using yet a third mouse model, successfully used passive immunization with a monoclonal antibody against PrP to rescue AD phenotypes [Chung 2010]. This study used APP/PS1 transgenic mice [Holcomb 1998], which express the same APP 'Swedish' mutation as the two mouse models mentioned above, along with yet a third PSEN1 mutation, M146L. Treated mice received peripheral injections of the 6D11 antibody, which recognizes PrP amino acids 93-109 (covering the critical region for Aβo binding), while controls received IgG or vehicle. After two weeks of treatment starting at 8 months of age, the mice were subjected to a maze test and a blinded observer counted the number of 'errors' (entries into an arm of the maze where the reward had already been consumed) the mice made. The APP/PS1 mice treated with vehicle or IgG consistently made 8-10 errors before successfully consuming all 8 rewards, while the mice treated with 6D11 made only ~4 errors, similar to wild-type controls. Aβ and PrP levels in the 6D11-treated mice were indistinguishable from vehicle- or IgG-treated, implying a mechanism whereby 6D11 occludes PrP, blocking binding to Aβo, rather than accelerating degradation of either protein.

The results of this study are fairly surprising for several reasons. First, this is the earliest evidence of *peripheral* antibody injections significantly affecting PrP activity in the CNS. Peripheral injections

of anti-PrP antibodies have been shown to abolish peripheral prion infections but have so far proven unable to occlude a large enough fraction of PrP in the brain to detectably slow the course of established CNS prion infections [White 2003]. To achieve this, the authors report having used 'large' (1 mg/day) doses of antibodies, and cite evidence that ~0.1% of passively administered antibodies enter the CNS [Bard 2000]. Second, APP/PS1 mice are reported to have significant Aβ plaque burden by eight months of age, and so an apparently complete reversal of one behavioral symptom after only 2 weeks of treatment is contrary to the growing body of evidence that AD immunotherapy is most effective if administered early in the disease course [Lemere & Masilah 2010]. Third, if cognitive ability as measured in the radial arm maze is presumed to reflect LTP-based memory and learning, then this result is contrary to that of Calella. For all of these reasons, Chung's study represents a potentially very high-impact finding, limited by the authors' decision to rely on only one behavioral test and no other phenotypic measurements. Although Chung's study was published after Calella's, it does not address possible reasons for the discrepancy between their results.

In sum, the three AD mouse model studies provide no more clear agreement than the brain slice and Aβo-injected mouse studies discussed earlier. All authors agreed, however, that Aβo does bind to PrP. Calella's conclusions are phrased mildly, arguing that PrP mediation of Aβo toxicity is not 'universal' and allowing the possibility that further research may clarify whether PrP is a potential therapeutic target in AD. Accordingly, the molecular consequences of the PrP/Aβo interaction have been intensively studied over the past two years. The next section of this review will summarize current hypotheses.

3. What are the molecular consequences of PrP/Aβo interaction?

Because PrP is known to transduce signals through Fyn [Mouillet-Richard 2000], and overexpression of Fyn has been reported to exacerbate AD phenotypes [Chin 2005], Fyn is a natural candidate for mediating Aβo/PrP signaling. Recently, antibodies specific to pY416-Fyn detected increased Fyn activation after incubation with human AD brain-derived Aβo in wild-type neurons but not in *Prnp*$^{-/-}$ neurons, demonstrating that PrP acts as an Aβo receptor leading to Fyn activation [Um 2012]. Fyn signaling through other receptors was preserved in *Prnp*$^{-/-}$ neurons but no Aβo-induced Fyn activation was detected, implicating PrP as an indispensible intermediate in all Aβo/Fyn signaling. In PrP-expressing neurons, exposure to Aβo caused a five-fold increase in the amount of phosphorylated NR2B, a subunit of the NMDA receptor. The effect was absent in cells lacking PrP or Fyn, and reduced proportionally in heterozygous knockouts of either gene, suggesting a dose-dependent mechanism. Phosphorylated NMDARs were shown to endocytose less frequently, with a peak threefold increase in the ratio of cell surface to internalized NMDARs. The resultant changes in calcium signaling caused excitotoxicity detectable by cellular release of LDH, and also caused dendritic spine loss. This dendritic spine loss could be prevented by PrP ablation or by antibodies against NMDARs, and was proposed to possibly explain the seizures observed in AD/WT mice but not in AD/*Prnp*$^{-/-}$ mice. Taken together, Um's findings imply that the Aβo-, PrP- and Fyn-dependent phosphorylation of the NMDA receptor has dramatic consequences, potentially explaining the epileptic activity, excitotoxicity and dendritic spine loss associated with AD.

Shortly thereafter, another group provided evidence for an equally compelling piece of the AD puzzle, demonstrating that Aβo/ PrP-induced Fyn activation leads to Tau phosphorylation, potentially tying together the two major histopathological phenotypes of AD [Larson 2012]. First, Larson answered a basic question which Um had left untouched: because PrP is anchored to the exoplasmic leaflet of the plasma membrane while Fyn is intracellular, the two are likely to need an

additional interacting partner. Co-immunoprecipiation of PrP and Fyn from AD brain extracts showed caveolin-1 as the third party, confirming earlier findings from cell culture [Mouillet-Richard 2000]. Larson next used a battery of antibodies to ask which species of Aβ was co-precipitating with PrP, and identified $A\beta_{42}$ dimers as the sole species binding PrP. Cortical neurons derived from primary mouse tissues were shown to exhibit elevated Fyn activation after incubation with human AD brain-derived Aβo, and this effect could be abrogated by antibodies against certain epitopes of PrP. Moreover, this activation of Fyn more than doubled the amount of Tau protein phosphorylated at Y18, and changed the intracellular distribution of Tau; this effect could be abolished by anti-PrP antibodies. In vivo experiments on AD mice showed Fyn activation and Tau pathology to be somewhat reduced in heterozygous PrP knockout mice, dramatically reduced in homozygous knockout mice, and exacerbated in PrP overexpressers.

Together, Um's and Larson's work tell an important story about how PrP may mediate AD pathology. The two studies agree that Aβo binds PrP, activating Fyn and causing downstream pathology. Although the studies point to two different effects, on NMDA receptors versus Tau, the findings are non-conflicting. Importantly, Um's and Larson's colleagues were on opposite sides of the original debate, suggesting that a pathological activation of Fyn may be, for now at least, the first non-controversial result of Aβo/PrP binding.

Current work seems to support Um's and Larson's conclusions. Another study recently confirmed Fyn activation upon Aβo binding to PrP, and detected membrane disruption as a cytotoxic marker when this binding event occurred [Rushworth 2013]. Interestingly, that study also found that the binding event induced endocytosis and prevented PrP's inhibition of BACE1, leading to increased production of Aβ, pointing to a possible vicious cycle mechanism.

Discussion

After a few years of controversy, the field may finally be heading toward consensus on at least a subset of conclusions about Aβo and PrP. It is widely agreed that Aβo binds PrP with high affinity and it now appears clear that this binding event induces activation of Fyn via caveolin-1, and that Fyn's phosphorylation of downstream targets could explain at least some of AD pathology. The downstream targets proposed here - NR2B and Tau - have the potential to finally explain the role of excitotoxicity in AD and the molecular pathway connecting Aβ accumulation with Tau tangles in the AD brain.

Meanwhile, although the reasons for discrepant results in earlier experiments have still not been fully elucidated, investigators seem to have accepted the importance of using authentically derived Aβ from human brains and, after some nudging [No Authors Listed 2011], recent work shows a trend toward explaining oligomer preparations and methodologies in greater detail, which may help to make results more reproducible in the future.

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