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Synthetic prions and other human neurodegenerative proteinopathies

Q1 Nhat Tran Thanh Le[a], Joanna Narkiewicz[a], Suzana Aulić[a], Giulia Salzano[a], Hoa Thanh Tran[a], Denis Scaini[b], Fabio Moda[c], Gabriele Giachin[a,*], Giuseppe Legname[a,d,**]

[a] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy
[b] Life Science Department, University of Trieste, Trieste, Italy
[c] Carlo Besta Neurological Institute, Department of Neuropathology and Neurology 5, Milan, Italy
[d] Elettra-Sincrotrone Trieste S.C.p.A., Area Science Park, Basovizza, Trieste, Italy

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ABSTRACT

Transmissible spongiform encephalopathies (TSE) are a heterogeneous group of neurodegenerative disorders. The common feature of these diseases is the pathological conversion of the normal cellular prion protein (PrP^C) into a β-structure-rich conformer-termed PrP^{Sc}. The latter can induce a self-perpetuating process leading to amplification and spreading of pathological protein assemblies. Much evidence suggests that PrP^{Sc} itself is able to recruit and misfold PrP^C into the pathological conformation. Recent data have shown that recombinant PrP^C can be misfolded *in vitro* and the resulting synthetic conformers are able to induce the conversion of PrP^C into PrP^{Sc} *in vivo*. In this review we describe the state-of-the-art of the body of literature in this field. In addition, we describe a cell-based assay to test synthetic prions in cells, providing further evidence that synthetic amyloids are able to template conversion of PrP into prion inclusions. Studying prions might help to understand the pathological mechanisms governing other neurodegenerative diseases. Aggregation and deposition of misfolded proteins is a common feature of several neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and other disorders. Although the proteins implicated in each of these diseases differ, they share a common prion mechanism. Recombinant proteins are able to aggregate *in vitro* into β-rich amyloid fibrils, sharing some features of the aggregates found in the brain. Several studies have reported that intracerebral inoculation of synthetic aggregates lead to unique pathology, which spread progressively to distal brain regions and reduced survival time in animals. Here, we review the prion-like features of different proteins involved in neurodegenerative disorders, such as α-synuclein, superoxide dismutase-1, amyloid-β and tau.

1. Introducing prions and prion diseases

The physiological cellular form of the prion protein (PrP^C) is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein localized on the outer leaflet of the cellular membrane with highest expression levels at the presynaptic membrane of neurons (Herms et al., 1999; Horiuchi et al., 1995; Prusiner, 1998). The mature human PrP^C (HuPrP) is composed of 209 residues including a largely unstructured N-terminal region and a globular α-helix rich C-terminal domain (Zahn et al., 2000). Despite being highly conserved among mammals, its physiological function has not been established with certainty. Proposed PrP^C functions range from neuronal growth and differentiation (Steele et al., 2006), synaptic plasticity (Caiati et al., 2013; Maglio et al., 2004), and cell signaling (Mouillet-Richard et al., 2000; Santuccione et al., 2005), to NMDA receptor modulator (Khosravani et al., 2008) and brain

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; AFM, atomic force microscopy; ALS, amyotrophic lateral sclerosis; dpi, days post injection; HuPrP, human PrP^C; HX-MS, hydrogen/deuterium exchange measured using mass spectrometry; LBs, Lewy bodies; PD, Parkinson's disease; PMCA, protein misfolding cyclic amplification; PrP, prion protein; PrP^C, physiological, α-helical form of the cellular prion protein; PrP^{Sc}, prion, the pathological isoform of PrP^C; PK, proteinase-K; rec, recombinant; SOD1, superoxide dismutase-1; Tg, transgenic; TSE, transmissible spongiform encephalopathies.

* Corresponding author at: Scuola Internazionale Superiore di Studi Avanzati (SISSA), Via Bonomea 265, 34136 Trieste, Italy. Tel.: +39 040 3787 760; fax: +39 040 3787 702.
** Corresponding author at: Scuola Internazionale Superiore di Studi Avanzati (SISSA), Via Bonomea 265, 34136 Trieste, Italy. Tel.: +39 040 3787 715; fax: +39 040 3787 702.
E-mail addresses: giachin@sissa.it (G. Giachin), legname@sissa.it (G. Legname).

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metal homeostasis (Pushie et al., 2011). Defining PrP^C function(s) remains an absolute requirement for understanding transmissible spongiform encephalopathies (TSE). These neurodegenerative diseases are caused by the posttranslational conversion of PrP^C into a β-sheet enriched, partially proteinase-K (PK) resistant isoform denoted PrP^{Sc} or prion (Prusiner, 1982).

In humans, TSE include idiopathic forms as sporadic Creutzfeldt-Jakob disease (sCJD), sporadic fatal insomnia (sFI) and the variably proteinase sensitive prionopathies (VPSPr). Familial CJD (fCJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI) and prion protein cerebral amyloid angiopathy (PrP-CAA) are genetic forms of human TSE. The acquired forms are transmitted from human to human, as iatrogenic CJD (iCJD) and Kuru; or from cattle to human, as variant CJD (vCJD) (Head and Ironside, 2012). In animals, relevant TSE are scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease (CWD) in cervids (Imran and Mahmood, 2011).

The central molecular event during prion diseases is the self-propagating conformational conversion of PrP^C to PrP^{Sc}. This postulate is known as the "protein-only" hypothesis (Prusiner, 1998). Two different mechanisms of prion replication have been proposed. In the *template assistance model* PrP^{Sc} exists as a monomer that is thermodynamically more stable than PrP^C (Prusiner, 1991). In the rare event that a PrP^{Sc} molecule is formed spontaneously (or provided exogenously) it can template the misfolding of PrP^C by direct interaction. In this model, the critical step in the conversion is the formation of a dimer between PrP^{Sc} and PrP^C, or a partially destabilized folding intermediate of PrP^C denoted as PrP^*. PrP^{Sc} acts as a template able to catalyze the refolding of PrP^C to a thermodynamically more stable PrP^{Sc} conformation. The *nucleation-polymerization model* proposes that the conversion between PrP^C and PrP^{Sc} is reversible, but the PrP^{Sc} monomer is much less stable than PrP^C (*i.e.* the equilibrium strongly favors PrP^C). Stabilization of PrP^{Sc} occurs only upon formation of a stable oligomeric nucleus. Once the nucleus has formed, monomeric PrP^C could efficiently add to it by adopting the conformation of PrP^{Sc}. The rate-limiting step in this mechanism is not the conformational conversion itself but the nucleation step. This step, responsible for the lag phase in the spontaneous conversion, can be by-passed and accelerated by addition of preformed PrP^{Sc} seeds (Jarrett and Lansbury, 1993).

One of the most important recent advancements in prion biology has been the discovery of the *de novo* generation of prion infectivity from recombinant (rec) protein sources. Amyloid fibrils prepared *in vitro* from bacterially expressed rec PrP^C (recPrP) have confirmed that PrP^{Sc} is the principal causative agent of TSE, providing the definitive proof for the prion hypothesis. These recPrP amyloid fibrils can be used as a synthetic surrogate of PrP^{Sc} to obtain a model for understanding the structural and molecular basis of prion conversion.

In the next section, we present an overview of considerable progress that has been made in our understanding of prion diseases through the development of several protocols for producing amyloid fibrils made by recPrP.

1.1. Discovering the first synthetic prion strains

The earliest efforts to define the process of prion conversion have been described in the 1990s by Caughey and collaborators. Purified PrP^C was incubated with different PrP^{Sc} strains derived from scrapie-diseased animals. The interaction with PrP^{Sc} resulted in the formation of a PK-resistant form (Kocisko et al., 1994). The incubation of PrP^C with two different strains of PrP^{Sc}, the hyper and drowsy strains of hamster transmissible mink encephalopathy, generated two distinct sets of PK-resistant forms (Bessen et al., 1995). Additionally, the mouse/hamster chimeric PrP^C, termed MH2M, extracted from cell culture has been converted into a PK-resistant form after incubation with the Syrian hamster (SHa) 263 K scrapie strain. However, no infectivity was detected when the converted material was inoculated into wild-type mice (Hill et al., 1999). These pioneering studies recapitulate many features associated with prion transmission *in vitro*, demonstrating that the direct interaction between PrP^{Sc} and PrP^C is one of the key events during the conformational transition.

Another largely explored strategy consisted of using several physico-chemical approaches to induce misfolding of the recPrP into β-strand rich states. Such studies are relevant because they address the question whether PrP^C alone is sufficient for the spontaneous formation of prions without the presence of any exogenous agent. A plethora of studies have attempted to provide an answer to this question, but these experiments have largely failed in producing infectivity *in vivo* or the infectivity potential has not been tested in animal model yet [reviewed in Benetti and Legname, 2009; Legname et al., 2012].

In 2004 the production of synthetic prions *via* the *in vitro* conversion of recPrP was reported (Legname et al., 2004). In an earlier study (Baskakov et al., 2002) the same authors analyzed in detail the misfolding pathways of the truncated fragment of recPrP leading to β-sheet rich conformers. Depending on the reaction conditions, two misfolded forms were adopted: at acidic pH values and in the presence of partially denaturing urea a PrP^{Sc}-like oligomer was observed; whereas under neutral or slightly acidic pH values and at low concentration of urea recPrP aggregated in fibrillar structures which developed into amyloids. Importantly, in this work authors discovered that the addition of a seed of pre-folded amyloid to the reaction substantially reduced the time of the fibrillization (called lag phase) process, demonstrating that recPrP fibrils can be induced by seeding. Starting from these findings, Legname and collaborators addressed the question of whether these synthetic fibrils were infectious when inoculated into mice. The pre-folded amyloid fibrils (denoted as "unseeded") and the seeded fibrils composed of recMoPrP(89–230) were intracerebrally injected into transgenic (Tg) mice which overexpress MoPrP(89–230). Seeded amyloid fibrils exhibited shorter incubation time (382 days) and PK-resistance than unseeded (473 days and PK-sensitivity). Interestingly, the neuropathological features associated with seeded and unseeded amyloids were different in terms of vacuolation, gray matter PrP^{Sc} deposition and conformational stability as measured by the GndHCl concentration required to denature half of the sample (Legname et al., 2004, 2005). Subsequent serial passages of these strains led to shortened incubation periods and a decreased conformational stability of the resulting prions. Combining these data with those available for naturally occurring prion strains, it was shown that the length of the incubation time in mice is directly proportional to the conformational stability of the prion strain (Colby et al., 2007; Legname et al., 2006).

1.2. New synthetic prions confirm the protein-only hypothesis

As follow-up of the first synthetic prion experiment (Legname et al., 2004), a series of recPrP amyloid fibers were produced from the same laboratory and intracerebrally injected in Tg mice overexpressing full-length PrP^C at 4–8 times normal levels (Tg4053). Interestingly, different inocula were able to propagate *in vivo* and induce the formation of different prion strains (Colby et al., 2009). In another study from Prusiner's group, authors performed serial inoculation passages of biochemically different synthetic prions showing that synthetic prions may reach a common, adapted and convergent state with similar physico-chemical features (Ghaemmaghami et al., 2013). Additionally, Colby and

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