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Prions, prionoid complexes and amyloids: the bad, the good and something in between

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Summary

Prions are infectious agents causing transmissible spongiform encephalopathies in humans and animals. These protein-based particles template conformational changes in a host-encoded prion protein to an insoluble self-like conformation. Prions are also present in yeast, where they support protein-based epigenetic inheritance. There is emerging evidence that prion-like (prionoid) particles can support a variety of pathological and beneficial functions. The recent data on the prionoid spread of other pathological amyloids are discussed in light of differences between prions and prion-like aggregates. On the other hand, prion-like action has also been found to support important functions such as memory, and amyloids were shown to have a variety of physiological roles from storage to scaffolding in simple organisms and in humans. Higher-order protein complexes play important roles in signalling. Many death-fold domains can polymerise upon nucleation to enhance sensitivity and induce a robust response. Although these polymers are structurally different from amyloids, some of them are characterised by prionoid activities, such as intercellular spread. The initial activation of these complexes is vital for organismal health, whereas prolonged activation leading to unresolved inflammation underlies autoinflammatory and other diseases. Prionoid complexes play important roles far beyond prion diseases and neurodegeneration.

Key words: *prion; amyloid; prion-like, prionoid; functional amyloid; inflammasome; signalosome; ASC (apoptosis-associated speck-like protein containing a CARD); MAVS; nucleated polymerisation*

AIM2	cytosolic DNA sensor absent in melanoma 2
ALS	amyotrophic lateral sclerosis
ASC	apoptosis-associated speck-like protein containing a CARD
CARD	caspase activation and recruitment domain
CJD	Creutzfeldt-Jakob disease
CPEB	cytoplasmic polyadenylation element-binding protein
Csg	curli-specific genes
DAMP	danger-associated molecular pattern
Hsp	heat shock protein
MAVS	mitochondrial antiviral signalling protein
MDA-5	melanoma differentiation-associated protein 5
NAIP	nucleotide-binding domain-containing inhibitor of apoptosis protein
NBD	nucleotide binding domain
NLR	nucleotide-binding domain- and leucine-rich repeat domain-containing receptor
NLRC4	NLR family CARD domain-containing protein 4
PAMP	pathogen-associated molecular pattern
PLD	prion-like domain
PrP	prion protein
PrP^{c}	cellular prion protein
PrP^{Sc}	prion protein scrapie
PYD	pyrin domain
RIG-I	retinoic acid-inducible gene I
RIP	receptor-interacting protein kinase
TSE	transmissible spongiform encephalopathy

Introduction

Organisms and cells depend on the correct folding of proteins into their native structure. Some proteins can adopt alternative, non-native conformations, which can obstruct normal cell function. Pathological conditions that arise from protein misfolding into insoluble amyloid deposits are called amyloidoses [1]. *In vivo* amyloids are characterised as extracellular fibrillary deposits and show green birefringence upon Congo red binding [2]. Although there are fewer than 25 proteins and peptides that form pathological amyloid *in vivo* [3], many proteins and peptides can form amyloid fibrils under appropriate conditions *in vitro* [4]. Isolated amyloid fibrils are monitored by transmission electron microscopy (fig. 1A) and have a cross-β-sheet core, which displays a characteristic diffraction pattern [8]. Amyloid fibrils are also characterised by a predominantly β-sheet secondary structure and bind specific dyes (fig. 1A). Enormous effort has been put into determining the atomistic details of the cross-β spine, which was challenging even for short amyloid-forming peptides [3, 9–13]. An amyloid spine is composed of in-register parallel or antiparallel β-sheets (fig. 1B), stabilised by hydrogen bonds and hydrophobic interactions, making amyloid assembly static and irreversible [14]. Amyloid formation follows typical sigmoidal growth kinetics, which is a characteristic of nucleated self-assembly reactions [15, 16] (fig. 1C).

The microscopic events defining amyloid fibrillisation are primary nucleation, elongation (growth of (proto)fibrils by the addition of monomers), and the formation of new nuclei, which can occur through the fragmentation of existing polymers or through monomer-dependent secondary nucleation [15] (fig. 1D). In fact, these processes are already present in the lag phase [16], which can be shortened by the addition of preformed amyloid seeds (fig. 1C).

In the past, amyloids had a fairly poor reputation in connection with neurodegenerative diseases and systemic amyloidoses. A special place among amyloids is reserved for prions, which are protein-based infectious particles, either causing prion diseases or being connected to epigenetic inheritance in unicellular organisms. Several amyloids were shown to have a prion-like (prionoid) phenotype, and to spread among cells and seed aggregates when injected into animals, but transmissibility was not demonstrated. However, knowledge on functional amyloids is also emerging.

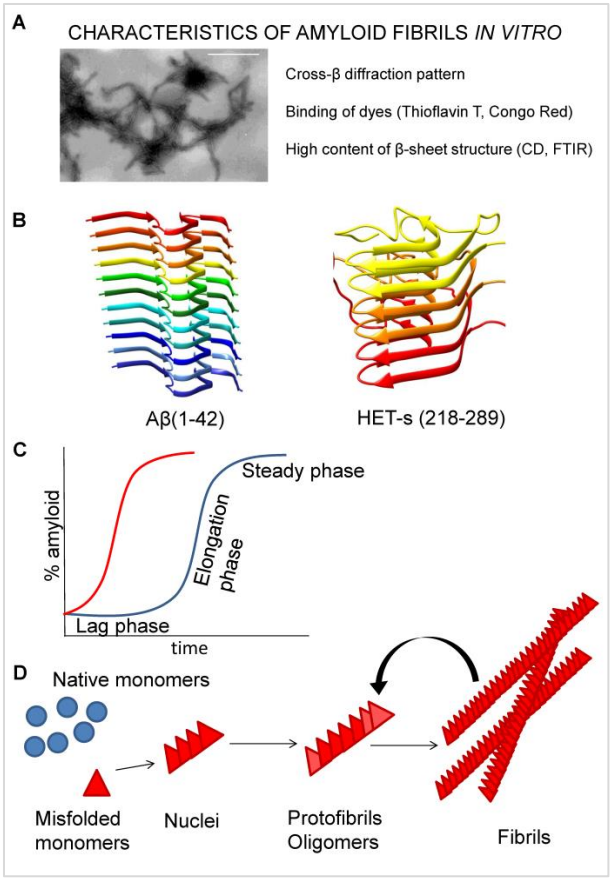

Figure 1: Characteristics of amyloid structure and polymerisation. (A) Amyloid fibrils (fibrils prepared from recombinant prion protein are shown, bar represents 500 nm) can be identified by transmission electron microscopy. Amyloids also have a characteristic X-ray diffraction pattern, bind amyloid-selective dyes and have a high content of β-sheet secondary structure, as determined by circular dichroism (CD) or Fourier transform infrared spectroscopy (FTIR). (B) Examples of amyloid structure (Aβ(1-42), fibril structure (PDB: 2MXU [5],), and HET-s (218-289) structure (PDB: 2KJ3 [6],) determined by solid-state nuclear magnetic resonance. Structural images were generated by use of UCSF Chimera [7]. (C) The kinetics of amyloid formation follow a typical sigmoidal curve (blue). The addition of preformed seeds shortens the lag phase (red curve). (D) A schematic representation of amyloid fibril formation. Misfolded proteins form a nucleus, which is able to capture and transform monomers. In the process of elongation, intermediate structures such as oligomers and protofibrils are formed, which further grow to mature fibrils. Mature fibrils are either spontaneously or enzymatically disintegrated, providing further perpetuation of amyloid formation.

In recent years, many prionoid amyloid and non-amyloid assemblies were observed to be important for vital processes throughout the tree of life. In this review, prions and disease-related amyloids will briefly be summarised in order to introduce the concept of prions and amyloids, with focus on nonpathological prionoid assemblies and amyloids (table 1). I will conclude by introducing the higher-order protein assemblies that are vital for signalling and innate immune responses. It seems likely that protein aggregation represents an ancient mechanism primarily facilitating beneficial functions, which only rarely leads to pathological conditions.

Prions and disease-related amyloids

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are infectious neurological disorders that affect humans and animals [91, 92]. Prion diseases can have sporadic, transmissible (less than 1%), or genetic occurrence (5–15%) [93]. The latter type arises from mutations in the *PRNP* gene that encodes the full-length prion protein (PrP), whose recently discovered physiological role is to promote myelin homeostasis by stimulating G protein-coupled receptor Adgrg6 [94]. The transmissibility of prion diseases is well documented. Bovine spongiform encephalopathy, or mad cow disease, affected more than 280 000 cattle. In addition, there is a wealth of evidence that the ingestion of contaminated beef triggered a variant form of Creutzfeldt-Jakob disease (CJD) in humans (more than 200 patients, predominantly in the UK) [93]. There are also almost 500 cases of iatrogenic CJD, in which prion disease was acquired through medical procedures, such as dura mater grafting or treatment with growth hormone extracted from pituitary glands [95]. Carleton Gajdusek earned a Nobel Prize for his investigations of kuru [96], a prion disease that was transmitted through ritualistic cannibalism among the Fore people in Papua New Guinea.

TSEs have all the characteristics of infectious diseases, such as transmissibility, species barriers and the presence of strains, yet the infectious particle was long unidentified. In the 1960s, Griffith proposed a theory that the infectious agent of scrapie (prion disease in sheep) is, in fact, a protein [97]. To isolate scrapie's infectious agent, the infectivity of brain homogenates was rigorously characterised by the Nobel laureate Stanley Prusiner, who coined the term "prion" to describe infectious agents causing transmissible spongiform encephalopathies as "a small proteinaceous infectious particle that is resistant to inactivation by most procedures that modify nucleic acids" [17]. The laborious work to prove the protein-only hypothesis, however, had only started, and it took almost 30 years for the hypothesis proposing that a protein can be the causative agent of a transmissible disease to be widely accepted. The protein-only hypothesis proposes that the main component of the infectious agent causing TSE is an aberrant conformation of prion protein, PrP^{Sc}. PrP^{Sc} differs from normal PrP^{c} in the secondary structure, as it is enriched in β-sheets and forms insoluble amyloid aggregates (or oligomers). PrP-deficient mice are resistant to infection [98], demonstrating that host-encoded PrP is exploited as a substrate during a pathological process in which PrP^{Sc} imposes conformational changes to a self-like conformation on the PrP^{c} [92, 99].

The structure of PrP^{Sc} and the mechanism of PrP^{c} pathological conversion remain elusive, despite many efforts [100]. Many animal models for studying prion disease have been established, in which prion characteristics, such as strains and species barriers, are stably reproduced. Prion strains possibly arise from various architectures of PrP^{Sc} [101]. Although the transmissibility of prions has been long established, the final proof for the protein-only hypothesis was much delayed. Legname et al. were the first to show that bacterial misfolded prion protein can induce neurological disease in transgenic mice overexpressing a truncated version of prion protein [102, 103]. Recombinant amyloid fibrils can also induce prion diseases in wild-type animals, although at low efficiency [104]. A technique called protein misfolding cyclic amplification brought a new boost to prion research, as it enabled the amplification of literary undetectable prion seeds using PrP^{c} or recombinant prion protein as a substrate [105–107]. This technique enabled the preparation of recombinant bacterially expressed misfolded protease-resistant prion protein that caused transmissible disease in wild-type mice [18]. The first study used RNA isolated from mouse liver as a cofactor, which was replaced later by totally synthetic components, and finally a *de novo* recombinant prion was produced in a novel facility, never exposed to native prions [108]. We can all appreciate the enormous efforts needed to provide evidence that prion diseases are transmitted through proteinaceous infectious particles. The reader is encouraged to learn more about the history of prion investigations in other reviews [91–93].

Are other amyloidoses also transmissible?

The deposition of β-sheet-rich amyloid aggregates is a common hallmark of many amyloidoses. Among these diseases, neurodegenerative diseases are the most prevalent. Above, I briefly introduced the development of the protein-only hypothesis and its verification. The legitimate question arising is whether amyloids in diseases other than TSEs are also prions. There are many features that are shared among prions and prionoid proteins (for a review, see [109–112]): amyloid polymerisation and seeding *in vitro* (fig. 1C) was demonstrated for α-synuclein, amyloid β (Aβ), tau and some other proteins connected to neurodegenerative diseases. Cell-to-cell transmission is also well documented for amyloid or oligomeric forms of those proteins. The most fascinating feature, although not unexpected, is the *in vivo* seeding and spreading of aggregates upon intracerebral inoculation. Aβ plaques, but not neurofibrillary tangles, were induced in primates inoculated with homogenates of Alzheimer patients' brains [19–21].

Table 1: A summary of reviewed prions, prionoids and functional amyloids. Note that the review only focuses on the functions of aggregated forms, and some proteins have functions related to their monomeric form. A limited number of references is listed, thus the reader should refer to the text for more information.

		Protein	Type; function	References
Harmful	Neurodegeneration	PrP^{Sc}	Prion; the causative agent of prion disease; prion strains and species barrier; transmissibility	[17, 18]
		Amyloid β	Prionoid; the hallmark of Alzheimer's disease; cell-to-cell and *in vivo* spread upon inoculation documented; indications for the presence of the species barrier and strains	[19–27]
		Tau	Prionoid; tau deposits are the hallmark of Alzheimer's disease and other taupathies; cell-to-cell and *in vivo* spread upon inoculation documented; indications for the presence of the species barrier and strains	[28–31]
		α-synuclein	Prionoid; α-synuclein deposits are the hallmarks of Parkinson's disease, Lewy-body dementia, etc.; cell-to-cell and *in vivo* spread upon inoculation documented; indications for the presence of the species barrier and strains	[32–36]
	Systemic amyloidosis	Amyloid A	Prionoid; serum amyloid A is the hallmark of systemic amyloidosis; potential transmission through ingestion	[37–39]
Beneficial	Memory	CPEB	Prionoid; mediates the persistence of memory in *Aplysia*; acts as prion when heterologously expressed in yeast	[40–42]
		Orb2	Prionoid; mediates the maintenance of long-term courtship memory in *Drosophila*	[43, 44]
		CPEB3	Prionoid; involved in the persistence of hippocampal-based explicit memory in mice; acts as prion when heterologously expressed in yeast	[45–47]
		Luminidependens	Prionoid; prion-like domain acts as prion when heterologously expressed in yeast	[48]
	Structure/storage	Curli (CsgA and CsgB)	Functional amyloid; *Escherichia coli* biofilm formation	[49–55]
		TasA	Functional amyloid; *Bacillus subtilis* biofilm formation	[56–60]
		Silkmoth chorion	Functional amyloid; eggshell formation	[61, 62]
		Spider silk	Functional amyloid; spider silk formation	[63–65]
		Peptide hormones	Functional amyloid; storage of peptide hormones in high concentration in membrane-enclosed secretory granules; functional peptide monomers are released in extracellular environment	[66]
		Xvelo	Functional amyloid; forms Balbiani bodies in dormant oocytes from *Xenopus laevis*	[67]
	DNA replication control	RepA	Functional amyloid oligomer; prevents plasmid replication by handcuffing	[68–71]
	Catalytic	Pmel17	Binding of highly reactive melanogenic precursors and accelerated synthesis of melanin	[72]
"In between"	Non-Mendelian inheritance	Yeast prions	Prions; usually loss-of-function phenotypes, transmitted through mating, cell division or cytoduction; strains widely reported	[73–78]
	Signalling	MAVS	Non-amyloid helical polymer; acts as prion in yeast; crucial for defence against RNA viruses; linked to autoinflammatory syndromes	[79, 80] [80–83]
		NLRC4	Non-amyloid wheel-like structure; crucial in innate immune response against intracellular bacteria; linked to autoinflammatory syndromes	[84–86]
		ASC	Non-amyloid helical polymer; acts as prion in yeast; prionoid transfer among cells demonstrated; crucial in the innate immune response; linked to autoinflammatory syndromes	[79, 87–89]
		RIP1/RIP3 necroptosome	Functional amyloid; drives necroptosis in virus infected cells	[90]

The prion-like behaviour of exogenous aggregates of Aβ [22–25] or tau [28–30] was also demonstrated after the inoculation of transgenic mice with recombinant *in vitro* prepared amyloid fibrils. Transgenic and wild-type mice also supported the prion-like spread and seeding of brain or recombinant α-synuclein aggregates [32–35]. The information regarding *in vivo* seeding and the spreading of recombinant amyloids from proteins connected to amyotrophic lateral sclerosis (ALS) is scarce, present only for superoxide dismutase-1 protein [113], but due to emerging developments in the field of ALS, new studies on the transmission of protein aggregates are expected soon (for a review, see [114]).

Prion diseases are characterised by the existence of prion strains and species barriers. Similarly, there are indications for the existence of a variety of Aβ aggregates that manifest in differences in seeded aggregation [25] and neurotoxicity [115]. Inoculation of brain homogenates from different genetic Alzheimer's disease cases into a mouse model results in distinguishable forms of pathology [26]. Different synthetic Aβ preparations induce distinct changes in the mouse model of Alzheimer's disease [27]. There is also some evidence for the existence of tau [31] and α-synuclein [36] strains, and evidence for ALS-related strains (reviewed in [110]) is emerging.

Whereas prionoid spread and seeding have been observed in animal models, and the evidence for strains and species barrier is emerging for non-TSE neurodegeneration-related amyloid proteins, rigorous evidence for natural donor/host transmission resulting in characteristic diseases is missing. There are, however, some indications for possible infectivity. Two recently published studies reported the presence of Aβ pathology (grey matter or vascular) in 50% of studied growth hormone-originated iatrogenic CJD cases [116] or in 71% of dura mater transplant-originated iatrogenic CJD [117]. On the other hand, the presence of Aβ pathology in sporadic CJD cases was very low, demonstrating that Aβ pathology does not usually co-exist with TSEs. The age of iatrogenic CJD patients was also unusually low for Aβ deposition, and genetic factors predisposing individuals to Aβ pathology-related diseases were excluded. Further analyses of larger numbers of cases are nevertheless needed to draw conclusive assumptions on potential iatrogenically derived Aβ pathology. Interestingly, more evidence for the potential transmission of systemic amyloidosis is already present. Amyloid A seeds were transmitted to mice orally [37] and via the ingestion of foie gras [38], or through the ingestion of the faeces of affected cheetahs [39], suggesting that infectivity leads to a high incidence of AA amyloidosis in captive populations.

Evidence of the experimental transmission of protein aggregates on the cellular and organismal levels is emerging. The major obstacle to recognising other amyloidoses as potentially transmissible is the lack of evidence for natural transmission, despite the fact that non-prion amyloidoses are much more common than prion diseases. Although there are some indications for potential iatrogenic or natural amyloid infection, further studies will estimate whether there is a real threat, or whether prions are unique in their capability to transmit diseases in natural environments.

Yeast prions

For mammalian prions, the term defines proteinaceous infectious agents that transfer infectivity through the promotion of conformational

changes in the host-encoded substrate to a self-like pathological conformation. In 1994, Wickner proposed that a similar mechanism might also exist in yeast, where a specific epigenetic inheritance was observed [73]. Prion forms of yeast prion proteins are self-perpetuating amyloid oligomers/aggregates, which are transmitted through mating, cytoduction or cell division. Protein-only infection was also demonstrated by adding amyloid fibrils to prion-free cells. Today, more than 10 different yeast prions are known. Several of them have multiple strains/variants, depend on the fractionation by the heat shock protein Hsp104, and at least some yeast prions are harmful (for a review, see [118, 119]). In most characterised yeast prions, a specific region is the determinant of prion activity. This region is characterised by an enrichment in asparagine or glutamine [120–122] and has a low propensity for any particular secondary structure, as determined by various computational algorithms. This domain can exist as a soluble monomer or an ordered aggregate and is probably unnecessary for the normal function of associated domains [123].

Yeast prions are usually recognised via the loss-of-function phenotype, which is dominant and transmitted through mating or to progeny [74–76]. The loss of function is achieved through prion-induced conformational changes and the aggregation of particular yeast proteins. One of the most investigated yeast prions is [*PSI*$^+$], which is the prion form of translational termination factor Sup35. The presence of [*PSI*$^+$] causes the recruitment and loss of function of Sup35, so translational termination is less efficient at nonsense codons. Because of the premature stop codon in the ade1-14 genes (adenine biosynthesis pathway), non-prion [*PSI*$^-$] cells are not able to grow without supplemented adenine, and accumulate a red intermediate when grown on complex growth medium. The presence of [*PSI*$^+$] induces the loss of function of Sup35, causing premature stop codons to be read through; such cells are able to grow without supplemented adenine and are white on complex growth medium (reviewed in [118]). Such tests are also used in Sup35 complementation assays, in which heterologous prion-like domains (PLDs) are placed instead of the Sup35 prion domain [124]. Such assays are particularly important for the screening and identification of new prion-like proteins and also enable different prion strains to be distinguished, for example via different colours of colonies on complex growth medium, where the level of inherently different ratios of prion/non-prion Sup35 results in different shades of pink. Interestingly, the mating of cells propagating different prion variants results in the domination of the more aggregated variant [77, 78].

It is fascinating that yeast prions survived selective pressure for so long, suggesting that they may prove beneficial in certain situations or environmental niches [125]. Whereas most newly identified prion-like proteins are tested for their prion-like activity in yeast, Sup35 or its prion domain also have prionoid behaviour when overexpressed in mammalian cells [126, 127], demonstrating the conservation of this phenomenon throughout kingdoms and evolution.

The prions to remember

How memories are formed and how they are retained for a lifetime, particularly since they are facilitated at a molecular level by relatively short-lived molecules, has been a very challenging problem to address. Studies of the sea slug *Aplysia* lead by Nobel laureate Eric Kandel and Kausik Si demonstrated that cytoplasmic polyadenylation element-binding protein (CPEB) mediates the persistence of memory [40, 41]. CPEB is an RNA-binding protein, and similar proteins can act as translational repressors or activators of target mRNAs [128]. CPEB is up-regulated by serotonin and provides the continuous local protein synthesis at activated synapses [129], which is facilitated by CPEB in non-native conformation. CPEB has a glutamine/asparagine-enriched prion-like domain, similar to yeast prions. In yeast, heterologously expressed CPEB exists in two distinct conformations, of which the multimeric form is capable of self-perpetuation and prionoid transmission [41]. Later it was shown that in naïve synapses, CPEB is monomeric. Synaptic activation triggers the conversion of CPEB to a self-sustaining oligomeric state, which marks activated synapses and enables the prolonged translation of target messenger RNAs at affected synapses [42]. CPEB overexpression is controlled by serotonin, inducing the down-regulation of miR-22, which has multiple binding sites on CPEB messenger RNA [130].

From *Aplysia* studies, we now understand that the prion-like self-perpetuation of CPEB marks activated synapses to store long-term memories. It seems that the controlled expression of CPEB and possible other mechanisms enable only the marking and maintenance of triggered synapses; how this is achieved on a molecular level remains to be solved.

More knowledge on how neurones switch on the programme for the persistence of memory and how memory is stabilised has been gained from studies on another model organism, the fruit fly. Orb2 is a *Drosophila* homologue of CPEB, critical for the maintenance of the long-term courtship memory through a prion-like mechanism [43, 44]. Before the memory is formed, an isoform Orb2B is abundant in neurones, but isoform Orb2A is very unstable, although the isoform Orb2A was shown to be critical for the persistence of memory. Orb2A can be stabilised by the protein Tob (transducer of Erb2). The stability of this complex is regulated by phosphorylation by LIM kinase and dephosphorylation by protein phosphatase 2A. The activation of LIM kinase and protein phosphatase 2A can be regulated in a synapse-specific manner, restricting Orb2 multimerisation to activated synapses [131].

There is substantial evidence that similar mechanisms are employed for long-term memory persistence in mammals. A mouse orthologue CPEB3 was demonstrated to be "a key mediator of the consolidation and persistence of hippocampal-based explicit memory" [45]. Although there are several mammalian CPEB homologues, CPEB3 contains a prion-like domain and exhibits prion-like behaviour in yeast; it forms amyloid and detergent-resistant oligomers and is transmitted to the progeny [46]. Fioritti and colleagues [45] show that CPEB3 is a translational regulator that acts as a repressor in the basal state. Upon stimulation of the hippocampal neurones, CPEB3 is de-SUMOylated (SUMO: small ubiquitin-like modifier), which allows it to switch to an oligomeric state, acting as a regulator of translation in response to learning-related activity [47]. In contrast to invertebrate orthologues, CPEB3 aggregates are not long-lived, but the aggregation is increased upon restimulation of animals [45]. Interestingly, while Kandel's group studied CPEB3 conditional knockout mice, transgenic mice completely lacking CPEB3 have an improved spatial memory compared with controls [132], suggesting that there are unknown mechanisms that could compensate for the total lack of CPEB3.

Unlike mammalian prions and amyloids connected to neurodegenerative diseases, *Aplysia* CPEB, *Drosophila* Orb2, and murine CPEB3 self-perpetuating multimeric forms do not kill cells, but instead harbour important biological functions and may serve as a persistent form of information, which is later discussed also in the context of prionoid assemblies mediating signalling.

Plant prion-like proteins revealed by a bioinformatics screen

As described above, prion-like proteins mediate molecular memories in sea slugs, fruit flies, and mice. Although many prions have been found in yeast, no prion has yet been identified in plants. Plants also form epigenetically founded memories when exposed to stressors such as cold, drought and pathogens [133].

The whole proteome of *Arabidopsis* was screened with an algorithm detecting prion-like domains [134], revealing almost 500 proteins with PLDs [48]. Half of all proteins involved in the autonomous flowering pathway contain PLDs. These prion-like domains were expressed in yeast to determine whether they can form prion phenotypes. Three domains formed prion-like sodium dodecyl sulphate-resistant oligomers, with the prion-like domain of the luminidependens protein providing a faithful phenotypic switch, and could propagate a spectrum of prion strains, which were maintained through generations. These prions were not dependent on Hsp104, but rather on other heat shock proteins such as Hsp70 and Hsp90. Whether luminidependens exhibits prionoid properties in *Arabidopsis* and whether this is involved in flowering decisions remains to be elucidated.

Functional amyloids from biofilm formation to securing genetic material

In the previous section, nonpathological amyloids involved in memory demonstrated that prionoid activity can be of vital importance. Amy-

loids can also perform a variety of functions, from scaffolding and storage to replication control. Such amyloids have been observed in bacteria as well as in humans, demonstrating that amyloid fold is beneficial in certain circumstances.

Microbial amyloids paved the way to recognition of functional amyloid

One of the first pieces of evidence for functional amyloids came from bacteria. In the last decade, microbial amyloids were shown to function in biofilm formation, changing the properties of microbial surfaces and contributing to interaction with host tissue and immune system evasion [135]. Functional microbial amyloids have been found in Gram-positive and Gram-negative bacteria and fungi, and are reviewed elsewhere [135, 136]. To demonstrate the major differences between functional and pathological amyloid, two examples of extracellular microbial amyloid will be presented here.

Chapman et al. [49] demonstrated that curli pili, which are present on the surface of uropathogenic *Escherichia coli*, have characteristics of amyloid. In contrast to pathological amyloids, the assembly of curli is not the result of protein misfolding, but rather the orchestrated effort of products encoded on two divergently transcribed operons (seven curli-specific genes, Csgs). The main components of curli amyloid are CsgA and CsgB. CsgA, which is secreted as unstructured protein [50], interacts with cell-surface-associated CsgB to form amyloid [51]. CsgD regulates the expression of *csgBAC* operon; CsgG makes pores in the outer membrane. This process is facilitated by CsgC. CsgA and CsgB are translocated from the periplasm to the extracellular milieu through CsgG pores [52]. CsgF helps to expose CsgB to the surface and CsgE facilitates the access of CsgA to CsgG pores. Both CsgF and CsgE seem to have chaperone-like functions in the curli assembly [53, 54]. Interestingly, CsgA orthologues from different bacterial species can cross-seed in *in vitro* fibrillation assay, suggesting that these heterogeneous curli fibres might participate in biofilms produced by various species co-existing in nature [55].

Another well-defined example of a bacterial amyloid is TasA in *Bacillus subtilis*. TasA has been shown to be involved in biofilm formation [56]. As in the case of *E. coli* curli amyloids, the formation of *B. subtilis* biofilm is tightly regulated. Proteins needed for the formation of *B. subtilis* amyloids are encoded in one operon containing three genes [57]. TasA forms amyloid fibres that are tethered to the peptidoglycan layer of the cell wall by TapA [58]. TapA also promotes TasA polymerisation and must be produced by the same cell for extracellular amyloids to be formed. The third protein encoded in the operon is SipW, a signal peptidase cleaving TasA and TapA into mature forms [59]. Additional regulation comes outside this operon. SinR represses the expression of *tasA* when biofilm formation is not favourable [60].

Another phenomenon connected to functional amyloids involves RepA. RepA (plasmid-encoded initiator protein) is a plasmid-encoded protein responsible for triggering and controlling the plasmid replication of *Pseudomonas* pPS10. The RepA-WH1 domain fibrillises *in vitro*, which is accelerated by specific DNA [68] and can be transmitted from mother to daughter cells [69, 70]. RepA controls and inhibits plasmid replication via the mechanism called handcuffing. The replication origins of two DNA molecules are handcuffed via a bridge of RepA amyloid oligomers, which has been detected by the use of amyloid-specific antibodies [71]. The prevention of plasmid over-replication by amyloid RepA represents a novel function in which amyloid complexes are involved and is vital for cells.

The heterologous expression of proteins in bacteria frequently leads to the formation of inclusion bodies that also have the characteristics of amyloid [137], from which it is possible to extract functional proteins; they are not only regarded as products of a safety mechanism avoiding toxicity, but also as potential drug-release systems [138].

Spider silk and silkmoth chorion

Silkmoth chorion is the major component of eggshell and its properties protect the oocyte and developing embryo from the environment. It is composed of low molecular weight proteins, which are able to form amyloid [61]. During amyloid formation, chorion peptides first form nuclei of a liquid crystalline nature, which collapse to form amyloid fibrils. On the molecular level, chorion peptides transform from a left-handed parallel β-helix to an antiparallel β-pleated sheet [62]. Amyloid-like nanofibrils have also been found in the gland of the spider *Nephila edulis* [63]. Spider silk proteins have been characterised *in vitro* [64, 65], which opened a perspective field of research and the design of recombinant spider silk [139–142] in addition to other amyloid-based materials [143].

Pmel17 amyloid within melanosomes accelerates the covalent polymerisation of reactive small molecules into melanin

The study by Fowler et al. [72] was the first to demonstrate functional amyloid formation in mammals. Pmel17 is trafficked to early melanosomes as a transmembrane protein. Within this compartment, the non-aggregating full-length protein is proteolytically processed, releasing an amyloid-prone fragment Mα, which rapidly polymerises into fibres confined within the melanosome. Mα fibres bind highly reactive melanogenic precursors, accelerate their polymerisation into melanin and inhibit the diffusion of these cytotoxic intermediates across the melanosome membrane. In addition to facilitating melanin biogenesis, Mα fibres also prevent cytotoxicity, which is associated with melanogenic precursors. Fowler and co-workers demonstrated that Mα fibres are amyloid in nature and that melanin synthesis can be facilitated by recombinant Mα fragment amyloids. They also suggested an important difference between functional and pathological amyloid. Recombinant Mα fibrillates at least four orders of magnitude faster than Aβ or α-synuclein in identical conditions [72], suggesting that Mα fibrillisation bypasses the formation of toxic intermediates [144, 145]. Mutant gelsolin is similarly proteolytically cleaved during secretion, leading to the slow deposition of extracellular gelsolin amyloid and gelsolin amyloid disease. Pmel17 amyloid is thus formed quickly to avoid more toxic oligomeric species and confined to a membrane-enclosed container, preventing cell damage that could be caused by mature amyloid.

Peptide hormones stored in amyloid conformation

Hormone-producing cells can store peptide hormones for an extended period of time in membrane-enclosed secretory granules. Maji et al. [66] tested 42 peptide hormones and demonstrated that almost three quarters formed amyloids upon *in vitro* incubation with glucosaminoglycans. Adrenocorticotrophic hormones, which are derived from the same prohormone and secreted together, formed amyloid only when incubated together and lacked the ability to cross-seed the other partner, suggesting that they formed mixed fibrils [66]. Co-aggregation was also demonstrated for ghrelin and obestatin. This study also showed that functional peptide monomers are released from amyloid aggregates, demonstrating that in this case amyloid formation is not an irreversible process. Although some of the peptides exhibited moderate neurotoxicity in the amyloid state, the authors argue that toxicity could be substantially lower in cells where amyloids are enclosed by membrane. They also demonstrated amyloid-specific staining and characteristics of isolated secretory granules of a mouse pituitary tumour neuroendocrine cell line and from rat pituitary tissue. This study introduced a different view of functional amyloid, showing that cells can pack hormones in high concentration in the form of amyloid fold and wrap amyloid aggregates with membrane to protect against cytotoxicity. Upon the appropriate trigger, these granules are released from cells and functional monomers are released from amyloid.

Balbiani bodies are protected by the amyloid network of Xvelo

The Balbiani body is a non-membrane-surrounded compartment consisting of mitochondria, RNA, endoplasmic reticulum and Golgi, present in early oocytes. It specifies the germline identity by forming germ plasm in frogs and fish, but its function in mammals is not known [146]. Buckyball protein is the main organiser of zebrafish Balbiani bodies, as the loss of the functional buckyball led to the absence of Balbiani bodies and disrupted oocyte polarity [147, 148]. Recently, Boke et al. [67] isolated Balbiani bodies from *Xenopus laevis* and analysed their content with quantitative mass spectrometry. They showed that the buckyball orthologue named Xvelo is highly enriched in Balbiani bodies. Balbiani bodies were very stable upon isolation and resisted high ionic strength

and temperature disintegration. Moreover, they stained with an amyloid-specific dye, thioflavin T. Xvelo is driven to Balbiani bodies by its N-terminal prion-like domain and makes a structural matrix in which organelles are incorporated. Recombinant Xvelo forms microscale amyloid-like networks *in vitro*. Boke et al. tested several proteins that have prion-like domains and RNA-binding domains, but only Xvelo and the buckyball targeted Balbiani bodies. The prion-like domains of these two proteins could be exchanged while retaining Balbiani body targeting, further demonstrating the specificity and homology of these two proteins. Full-length Xvelo could form networks and cluster mitochondria in egg extracts, demonstrating the sufficiency of Xvelo for Balbiani body-like assembly. Xvelo did not co-aggregate with other proteins with prion-like domains. Balbiani bodies are present in premature oocytes, whereas they either disappear or disperse into germ plasm in mature oocytes, suggesting that the amyloid network protects RNA and organelles, but can be disintegrated when appropriate. Although for now only buckyball and Xvelo have been demonstrated to be the main structural organisers of Balbiani bodies, they indicate that the amyloid network of a specific natively disordered protein with a prion-like domain could be an evolutionarily conserved mechanism for the protection of germline components in dormant cells.

Prionoid complexes spread (to) signalling and immunity

Prion-like complexes are not rare in processes necessary for normal cell or organism function, such as signalling [149]. Many signalling molecules are modular, where at least one of the domains belongs to the death fold. The death-fold domains are the death domain, death-effector domain, caspase activation and recruitment domain (CARD) and pyrin domain (PYD) [150]. This fold is composed of a six-helix bundle and has the tendency to form homotypic interactions and filament-like structures *in vitro* and *in vivo*. Those filaments are rich in α-helical secondary structure and do not express amyloid characteristics, yet they have some characteristics of prionoid behaviour. Cai et al. [79] tested domains belonging to different classes of the death fold for their ability to compensate for the yeast prion $Sup35^{NM}$ domain in the white/red screening assay, and showed that several were able to cluster the Sup35 translation termination domain to cause a loss of function. They extensively characterised the prion-like behaviour of the CARD domain of mitochondrial antiviral signalling protein ($MAVS^{CARD}$) and PYD domain of apoptosis-associated speck-like protein containing a CARD (ASC^{PYD}), which are described in detail below.

MAVS aggregates promote antiviral signalling

Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs, e.g., RIG-I and MDA5) detect very low amounts of viral RNA molecules to induce a robust antiviral signalling [151]. RIG-I, upon sensing viral RNA, oligomerises with the N-terminal domain, which is composed of tandem CARDs (2CARD). Oligomerised RIG-I recruits protein MAVS via CARD-CARD interaction. Activated MAVS signals to kinases, which activate the transcription factors, nuclear factor-κB and interferon regulatory factor 3 to induce antiviral defence.

Before activation with RIG-I-like receptors, there are multiple interactions between adjacent domains to keep MAVS in the dormant conformation [152]. Viral RNA induces RIG-I 2CARDs to form short filaments [153] or to form a tetramer upon covalent and noncovalent interactions with ubiquitin [151, 154]. RIG-I^{2CARD} tetramer has a helical geometry, where the second CARD of 2CARD continues the helical turn made by the first CARD [154]. The $MAVS^{CARD}$ domains extend the filament formation nucleated by the helical tetramer of RIG-I^{2CARD} [155] (fig. 2A). Upon activation, MAVS (which is tethered to the mitochondrial membrane), forms large rod-like aggregates at the mitochondrial surface [80–83] (fig. 2A). Aggregated MAVS is, in fact, the active signalling complex. Mutations that abrogate filament formation also inactivate signalling activity in cells [83, 153, 154]. *In vitro*, prepared MAVS fibrils are able to induce endogenous MAVS polymerisation in cells, demonstrating their prionoid activity [80]. The $MAVS^{CARD}$ domain has been shown to have the prionoid phenotype in yeast [79].

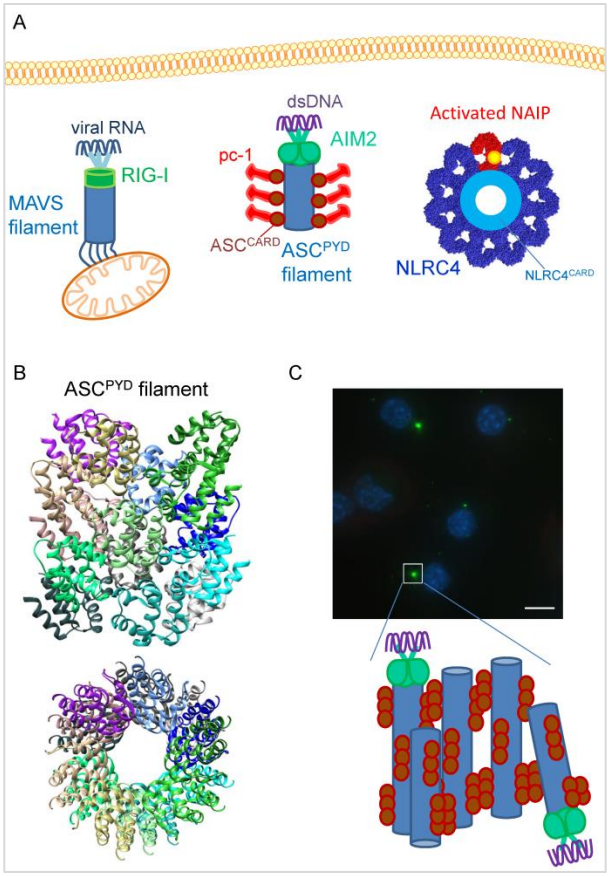

Figure 2: Innate immunity signalling platforms with a prionoid character. (A) The schematic representation of the assembly of RIG-I-nucleated MAVS filament (left), AIM2-nucleated ASC filament (middle), and NAIP-nucleated NLRC4 oligomer (right). In each case, nucleators are first activated by respective PAMP binding. The scheme of NLRC4 is based on the NLRC4 oligomer structure (PDB: 3JBL [85]). (B) The structure of ASC^{PYD} filament as determined by electron microscopy (PDB: 3J63 [87]) reveals a hollow three-start helical assembly. (C) Upon activation of nucleators, ASC is recruited to perinuclear region to form ASC specks (immunofluorescence-labelled endogenous ASC specks are shown in green and nuclei are depicted in blue, bar represents 10 μm). Upon activation of PYD-containing receptors such as NLRP3 or AIM2, ASC specks are formed by the polymerisation of ASC^{PYD} domains and crosslinking of ASC^{PYD} filaments by ASC^{CARD} domains [156]. Structural images were generated by use of UCSF Chimera [7].

AIM2 = cytosolic DNA sensor absent in melanoma 2; ASC = apoptosis-associated speck-like protein containing a CARD; CARD = caspase activation and recruitment domain; MAVS = mitochondrial antiviral signalling protein; NAIP = nucleotide-binding domain-containing inhibitor of apoptosis protein; NLRC4 = NLR family CARD domain-containing protein 4; PAMP = pathogen-associated molecular pattern; PYD = pyrin domain; RIG-I = retinoic acid-inducible gene I

There are indications that melanoma differentiation-associated protein 5 (MDA5) uses oligomer or filament formation slightly different from RIG-I to nucleate MAVS filaments, the mechanisms of which are currently under investigation [157–159]. MDA5 gain-of-function mutations are linked to autoinflammatory syndromes [160, 161]. As with other aggregates with substantial chemical stability, there is always a question of the resolution of such complexes and, in this case, antiviral signalling silencing. A recent study reported that MAVS aggregates are tagged for elimination with mitochondrial resident E3 ligase membrane associated ring-CH-type finger 5 (MARCH5), which transfers ubiquitin chains to specific residues of MAVS and labels them for proteasome-mediated degradation [162].

NLRC4 upon activation with NAIP recruits more NLRC4 molecules to support maximal multiplication of the input signal

The nucleotide binding domain- (NBD) and leucine rich repeat domain-containing proteins (NLRs) are a family of cytosolic proteins. Upon sensing either pathogen-associated (PAMPs) or danger-associated molecular patterns (DAMPs), some of these cytosolic receptors form caspase-activating platforms called inflammasomes. The major function of the canonical inflammasomes is the activation of procaspase-1, which induces pro-interleukin-1β and pro-interleukin-18 cytokine maturation and cleaves gasdermin D [163, 164]. The N-terminal part of gasdermin D forms pores in the membranes [165–168], thus inducing pyroptosis, a specific type of inflammatory cell death.

NLR family CARD domain-containing protein 4 (NLRC4) contains a CARD domain that is able to recruit procaspase-1 via its CARD domain directly. NLRC4 responds to cytosolic flagellin and to proteins of the type 3 secretion system (reviewed in [169, 170]). In the absence of triggers, the NLRC4 molecule is present in autoinhibited form, stabilised via multiple interactions between its domains and adenosine diphosphate [171]. In fact, NLRC4 is not the real sensor of PAMPs, which are detected by a family of NBD-domain-containing inhibitor of apoptosis proteins (NAIPs). NLRC4 activation is initiated by respective NAIPs recognising bacterial ligands [172] and structural rearrangement exposing the NAIP nucleating surface. Activated NAIP binds to the NLRC4 receptor surface, which is already exposed in the inactive form. The binding of NAIP to NLRC4 induces structural rearrangement of NLRC4, exposing the nucleating surface of NLRC4, which is capable of recruiting additional NLRC4 molecules, consequently forming wheel-like [84, 85] or spiral [86] structures, in which NAIP is present only at the start (fig. 2A). It is possible that NAIP-induced NLRC4 prion-like oligomerisation into 10- to 12-oligomeric wheel-like structures provides a seed for the oligomerisation of procaspase-1 via the CARD domain, which has also been shown to form filaments *in vitro* [85, 173]. $NLRC4^{CARD}$ is also able to recruit ASC^{CARD} and induce ASC^{PYD} filament formation [156, 174]. Mutations in NLRC4 cause autoinflammatory syndromes [175, 176].

As in the case of MAVS polymerisation, NLRC4 oligomerisation enables the detection of small amounts of PAMPs present and the amplification of the signal to induce a strong inflammatory response.

ASC specks continue to promote inflammation upon secretion

NLRC4 is not the only NLR that forms inflammasomes. NLRP3, the NLR family protein containing a pyrin domain 3, is able to sense a plethora of DAMPs from adenosine triphosphate [177] and monosodium urate crystals [178] to Aβ aggregates [179] and prion protein fibrils [180]. NLRP3 and other pyrin domain-containing receptors, such as cytosolic DNA sensor absent in melanoma 2 (AIM2) [181], recruit adaptor ASC via PYD-PYD interaction, and ASC further uses its CARD domain to catch pro-caspase-1 (fig. 2A). ASC^{PYD} has been identified as a prion domain in the yeast assay [79] and formed filaments *in vitro* and in mammalian cells upon overexpression [87]. ASC prion-like aggregation has been initiated by the addition of respective receptors NLRP3 and AIM2, which serve as nuclei from which the ASC^{PYD} filament grows [87] (fig. 2A). ASC^{CARD} domains are clustered and initiate the formation of pro-caspase-1^{CARD} short filaments growing out of ASC^{PYD} filament in a star-like pattern *in vitro* [87]. The cryo-electron microscopy structure of human [87] and mouse [167] ASC^{PYD} filament has been determined, revealing a three-start helical filament (fig. 2B). As in the case of $MAVS^{CARD}$ filament, in the ASC filament the secondary structure is also retained and filaments do not bind amyloid-specific dyes. Mutations that inhibit filament formation also abrogate inflammasome signalling [87]. Upon activation *in vivo*, ASC is recruited to the perinuclear region into a structure called a speck [182] (fig. 2C). Interestingly, ASC specks also appear upon activation of NLRC4, where NLRC4-mediated pyroptosis is ASC independent [183–185], but interleukin-1β maturation is strongly enhanced in the presence of ASC [156, 183]. Yeast-based assay [79], extensive mutagenesis [156] and the use of the ASC^{CARD}-specific single domain antibody fragment [174] have revealed that CARD domains of ASC enable the crosslinking of ASC^{PYD} filaments into ASC specks (fig. 2C), which is necessary for the caspase-1 maturation of cytokines. Yeast and *in vitro* studies have demonstrated the prion-like characteristics of ASC, but, even more importantly, ASC specks are released from cells and are active in the extracellular space. Released ASC aggregates re-enter phagocytes and are able to induce NLRP3 activation as classical particulate triggers via phagocytosis and lysosomal destabilisation [186], but they are also able to seed and recruit endogenous ASC molecules in the absence of upstream receptors [88, 89]. Moreover, extracellular ASC specks have been found in both mouse models and patients with cryopyrin-associated autoinflammatory syndromes, demonstrating that prionoid behaviour supports overstimulation and the progression of pathology [88, 89]. ASC specks have been found in the cerebrospinal fluid of patients with traumatic brain injury [187]. Whereas a neutralising antibody against ASC reduced inflammation in a mouse model of traumatic brain injury [188], anti-ASC antibody opsonised ASC specks, increased engulfment and boosted the proinflammatory response in crystal-induced peritonitis [89], which opens new strategies for intervention with such protein-based particles, for example via neutralising antibodies lacking an Fc region, such as single-domain antibody fragments [174].

RIP1/RIP3 necroptosome is an amyloid-signalling complex

Some viral infections induce programmed necrosis (necroptosis). Li et al. showed that receptor-interacting protein kinases 1 and 3 (RIP-1, RIP-3) interact through their RIP homotypic interaction motifs (RHIMs) and form amyloid fibrils *in vitro* [90]. RIP-1/RIP-3 fibrils are extensively characterised by the binding of amyloid-selective dyes, circular dichroism spectroscopy, X-ray diffraction and solid-state nuclear magnetic resonance. Although isolated RIP-1 or RIP-3 form fibrils, they are irregular and short and the formation is slow. On the other hand, preformed seeds accelerate RIP-1 fibrillation, and selected mutations in either of the complex partners decrease fibril formation. Upon necroptosis induction in cells, RIP-1 and RIP-3 are clustered into punctate-like structures, which are stained with thioflavin T. No thioflavin T labelling has been observed in untreated cells. Mutations that inhibit fibril formation also abolish punctate complexing and kinase activation. Li et al. demonstrated that RIP-1 and RIP-3 form a functional cross-β amyloid signalling complex, which by proximity-induced activation mediates necroptosis [90].

Of the signalling platforms described, only RIP1/RIP3 present with characteristics of amyloids; others are nucleated polymers of molecules that conserve their death fold, but nevertheless exhibit some prionoid characteristics, as discussed below. In fact, there are more examples of such signalling platforms, also called supramolecular organising centres [189], such as CARMA1/Bcl10/MALT1 [190]. These signalling platforms are assembled via nucleated polymerisation, where the nucleator induces the polymerisation of another protein. The major function of the supramolecular organising centres is to amplify a signal that reaches a certain threshold to maximum, which enables an efficient immune response and pathogen clearance [189]. Some of these signalosomes destine the cell to die, which is effective in the case of intracellular pathogens and promotes further induction of the immune system. In humans, these pathways are controlled on transcriptional and post-transcriptional levels to avoid unwanted activation [191]. Mutations in the nucleator proteins, such as NLRP3, NLRC4 or MDA5, cause autoinflammatory syndromes [160, 161, 175, 176, 192]. The fact that functional ASC specks are released from activated cells and induce further signalling in nascent cells, which is a prionoid behaviour, opens questions on how these complexes are disassembled or inactivated when infection has been cleared out. An efficient innate immune response is needed to fight against pathogens, but can exacerbate underlying disease when it responds to DAMPs or when inflammation is perpetuated in a prionoid way.

Conclusion

Higher-order protein assemblies, either with pathological functions or supporting vital functions, are being discovered in a wide variety of organisms from unicellular organisms, such as bacteria and yeast, to humans. The aim of this review was to give an overview of this versatile group of proteins, which expose their either detrimental or beneficial functions through monomer polymerisation/aggregation (table 1). First, the hard work leading to the recognition of mammalian prions as infectious agents was presented. Other disease-related amyloids resemble prions in their self-templating propagation, cell-to-cell transmissibility,

possible existence of species barrier and strains. The difference between prions and disease-related prionoids is that conclusive evidence of transmission in non-prion amyloidoses has not been presented so far. Amyloids, in addition to being the hallmark of various neurodegenerative diseases, have important structural, storage, information transfer and protective functions (table 1), which is not surprising if one takes the stability of amyloid structures into account. Even pathological amyloid fibrils, which are not the most toxic or infectious protein species [144], have a damage control function by sequestering more toxic oligomers. There are, however, some obvious differences between pathological and functional amyloid assembly. Functional amyloid formation is highly controlled and usually depends on the presence of more than one protein in the same cell/location. The formation of pathological amyloids is very slow compared with the nucleated fibrillisation of functional amyloids. The fibrillisation of some functional amyloids can be reversible. Examples are peptide hormone granules and Balbiani bodies that can be dismembered when dissolution is needed. When hormone granules are secreted from cells functional peptide hormones are released [66]. This shows amyloid folds in a completely new light, as an ancient fold able to supporting various tasks, which can only occasionally form as a result of pathological protein misfolding.

In the present review we also focused on polymers based on death-fold domains (table 1). Death-fold signalosomes, such as MAVS and ASC filaments, are structurally very different from amyloid prions, as they are composed of filaments of polymerised death-fold domains [87, 155]. At first glance, it is like comparing apples and oranges. Specific segments of the death-fold domain adapt upon polymerisation, yet this is far from the prion protein conformational change from an α-helical to a predominantly β-sheet secondary structure. In contrast to loss-of-function yeast prions, signalosomes are gain-of-function polymers, as they signal to enzymes, which are activated by proximity-driven oligomerisation [149]. The loss of ability to polymerise correlates with the loss of signalling activity [83, 87, 153, 154]. However, oligomerisation-prone death-fold domains can replace prion domains in yeast screening. Additionally, signal-induced oligomerisation is initiated by a nucleator (e.g., RIG-I-like receptor, NLRP3, NAIP/NLRC4) and upon nucleus formation proceeds very fast. This is similar to functional amyloid formation. The major characteristic of death-fold signalosomes placing them into the prionoid group is the ability to transfer and initiate filament formation in the recipient cells [80, 88, 89]. Signalosomes are vital for normal innate immune response and efficient defence against pathogens, but they also perpetuate inflammation in autoinflammatory syndromes and in common diseases where innate immunity is activated through recognition of danger-associated molecular patterns. Prion-like or prionoid protein behaviour has been observed in a variety of situations, both vital and detrimental, and seems to serve as a way of information transfer beyond a disease-causing role.

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Appendix 1: Glossary

Term	Definition
ASC	Apoptosis-associated speck-like protein containing a CARD domain is adaptor protein, containing pyrin and CARD domain. It forms inflammasomes downstream of activated NLRs.
Inflammasome(s)	Cytosolic multiprotein complexes for activation of inflammatory caspases.
MAVS	Mitochondrial antiviral signalling protein is an adaptor protein, which upon binding to viral RNA-activated RLRs polymerizes, activates cytosolic kinases, which in turn activate transcription factors to induce antiviral signaling.
NLRs	Nucleotide-binding domain and leucine-rich repeat domain-containing receptors are cytosolic pattern recognition receptors of pathogen-associated molecular patterns and danger-associated molecular patterns. Some of the members of this family of proteins form inflammasomes.
Prion	Proteinaceous infectious particle lacking coding nucleic acids, the causative agent of prion diseases.
Prionoid	Prion-like protein, a protein with a prion-like propagation (and cell-to-cell transmission)
PrP^{c}	Cellular prion protein, the normal version of prion protein
PrP^{Sc}	Abnormal isoform of prion protein resulting from conformational transition of the cellular prion protein.
RLRs	Rig-I-like receptors are cytosolic pattern recognition receptors detecting viral RNA.