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Prionoid Proteins in the Central Nervous System: Both Sides of the Coin

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Abstract

Prionoid proteins are those proteins which can have different conformations, one of which can self-perpetuate and spread within an organism, but not between individuals. Traditionally, such proteins have been related to pathological conditions, such as various neurodegenerative diseases. Nonetheless, increasing evidence has been found that prionoid proteins may also perform functional roles when adopting the aggregated conformation, such as the ability to maintain long-term memory. However, the stimuli inducing the conformational switch of these proteins are still unknown, but various possibilities will be mentioned in this paper. Altogether, the study of pathological and functional prionoids is extremely important in order to understand their different aggregation process. Furthermore, acquiring a deeper knowledge may be of special interest with regard to the development of treatments both for memory deficiency diseases and neurodegenerative diseases.

Contents

1. Introduction: What is a prion?

The definition of *Proteinaceous Infectious Particles* (Prions) was initially proposed in 1982 by Stanley Prusiner after the discovery of the Prion Protein (PrP), which is described as a protein capable of self-replicating and forming persistent accumulations. The PrP is indeed responsible for causing transmissible spongiform encephalopathies, a set of neurogenerative disorders such as scrapie in animals and kuru or Creutzfeld-Jacob disease in humans. However, this definition has greatly expanded over the last years, as some prion characteristics are shared with other several proteins, broadening the concept of prion to prion-like proteins.[1,2,3,4,5]

In the present project, further attention will be placed on prion-like proteins and their particular features in comparison to prions, in order to understand the differences between them. Furthermore, two prion-like proteins of the central nervous system (CNS), α-synuclein and CPEB, will be described due to their relevance for the mentioned tissue. Moreover, they provide an opposite perspective of prion-like proteins, useful for the proper comprehension of both human pathology and physiology.

1.1. Structure

Proteins are initially synthesized as linear chains of attached amino acids, which then fold into tridimensional or even more complex conformational structures (see Figure 1A). Sometimes, the different structures are obtained due to post-translational modifications (PTM), such as SUMOylation or phosphorylation. Once these proteins have reached a specific structure, their particular functions are performed. Consequently, this allows a wide range of possible conformations for the very same protein, which can then lead not only to different functions but also to disease phenotypes.[6,7]

Indeed, prions are able to self-template in a range of different conformations, each of one is called a strain. The strains, despite having the same polypeptide sequence, provide the proteins with distinct biological functions. In fact, these strains can arouse even without the aid of PTM, as the protein-only hypothesis supports,[2,6] which suggests that such flexibility in protein conformation is regulated exclusively by the primary amino acid sequence of the prion.

The most common structure adopted by prions is the amyloid form, which consists of an aggregation of β-sheet rich fibrils (Figure 1B). Usually, as in the PrP protein,

there is a conformational misfolding in which the proteins turn from a majoritarian α-helix conformation into a β-sheet structure. Therefore, while the first conformation was functional, the transformation into the amyloid structure causes the persistent aggregation of dysfunctional proteins, leading to disease. Such aggregation has been proposed to spread due to a seeded nucleation process, a mechanism that involves a first switch of a protein to a β-sheet structure, which would then act as a seed and trigger the same conversion in other correctly folded monomers of the same protein. Then, a progressive growth of the aggregate happens while the β-sheet regions of the newly misfolded proteins interact among them (Figure 1B). However, it is important to remark that not all amyloids act as prions, although most prions are amyloidogenic proteins.[8,9,10]

In terms of primary structure, most prions have flexible intrinsic disordered regions (IDR), which are regions that do not adopt a specific tertiary structure. Therefore, IDRs are in part responsible for the characteristic conformational flexibility of prions. Sometimes, prions can be intrinsically disordered proteins, which implies that the whole protein is disordered and, in consequence, does not form amyloids either.[6,7,11]

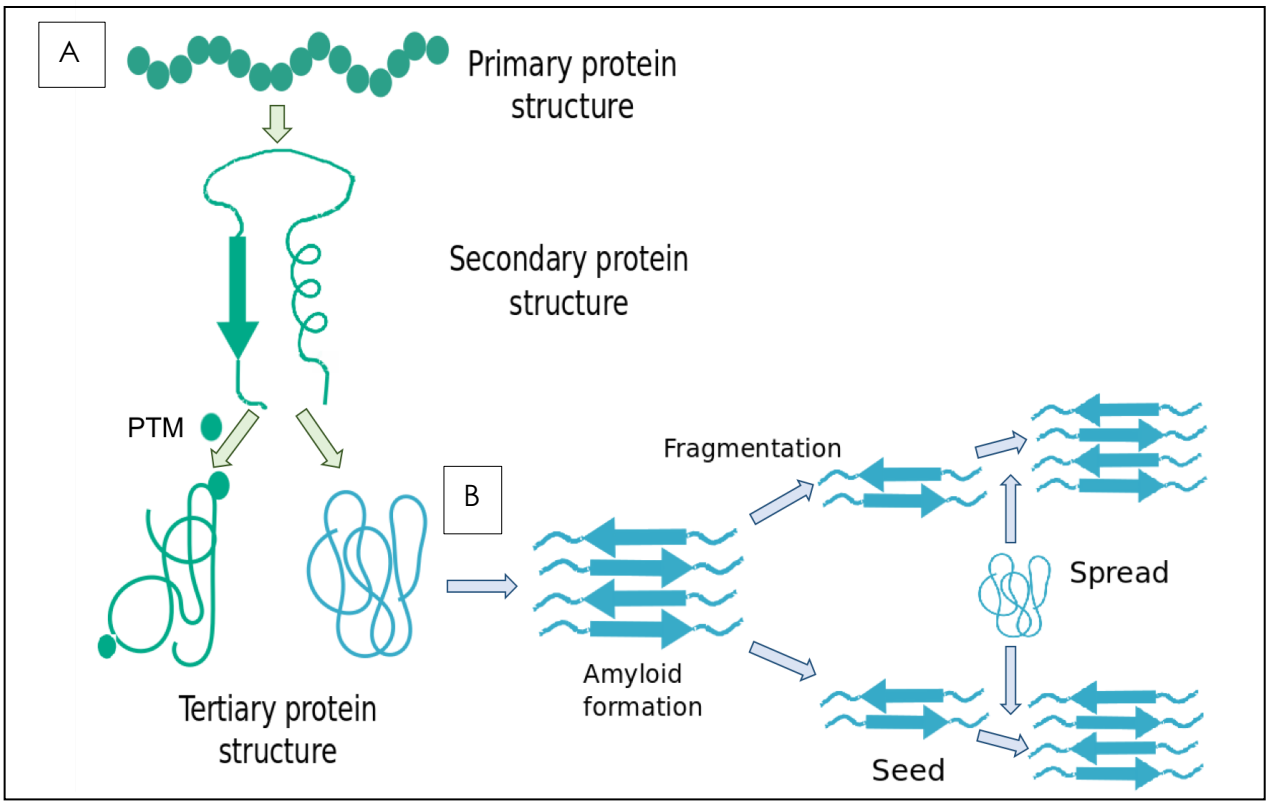

Figure 1. Process of formation of a prion or prion-like protein. A) Formation of a protein with different strains, with and without post-translational modifications (PTM). B) Formation of an amyloid structure and its spread through the seeded nucleation mechanism. *Figure designed by the author*.

Two other regions are also commonly found in prions. First, the compositionally biased regions (CBR), which means that a concrete region has a different proportion of residues than the expected by the average. Second, the low complexity regions (LCR), which refers to those regions with an enrichment in certain amino acids. In this case, prions usually have either N-/Q- enriched regions or hydrophobic rich domains. These regions have been suggested to be involved in the already mentioned conformational switch, as they allow the change to a β-strand conformation and, therefore, the formation and aggregation of the amyloid form. However, if these N/Q-rich regions are not present, IDR sequences can induce the aggregation in the same way. Besides, N-/Q- rich domains may also be important in DNA and RNA-binding or in protein interactions. [8,12]

Nonetheless, some of the newly identified prions in bacteria and fungi neither had CBR regions nor formed amyloid structures, although they shared IDR.[6] Accordingly, it is important to consider all the structural features above mentioned, and not only the capacity of forming amyloids, for the identification of more prions and prion-like proteins.

1.2. Transmission

The most striking characteristic of prions is their ability to spread through cells, tissues and, most important, between individuals. Thus, prions are considered infectious, as they are capable of invading and self-perpetuating in a susceptible host, regardless whether they cause disease or not. [10,13]

Prions' capacity of spreading is related to the already mentioned seeded nucleation mechanism coupled with the ability of prions to overcome proteostasis processes, so the aberrant forms cannot be deleted.[6,14] In consequence, as prion aggregates are constantly growing, they are susceptible to fragmentation. Then, if those fragments are transported to other cells, they can act again as seeds and induce the oligomerization of inner monomers, provoking the cells' infection.[8,13,15] Nonetheless, the infection would only propagate if the recipient host (say, cell, tissue or individual) also expresses the precursor of the prion protein.[6,10] Remarkably, this transmission is independent of nucleic acids, which was an unprecedented discovery.[13]

To summarize, the basic features of prions are their ability to self-propagate, say, trigger their own change to an aggregated form; and also the capacity of spreading within susceptible hosts.

Disease-related prionoid[14]		Functional prionoid	
Amyloid β[14]	Alzheimer's disease	**CPEB[18]**	Long term memory maintenance
α-synuclein[14]	Parkinson's disease	**MAVS[19]**	Immunological system
Huntingtin exon 1 [14]	Huntington disease	**Lumenal fragment Pmel 17[20]**	Melanin formation
p53 [21]	Cancer	**RIP1/RIP3 complex[22]**	TNF-induced programmed necrosis

Table 1. Examples of both disease and functional prionoids. In the left column, the name of the protein is written. In the right, the process or disease related to the prionoid form of the protein.

2. Prionoid proteins

2.1. Introduction

Prionoid proteins (prionoids from now on), also called prion-like proteins, are proteins which, despite sharing some of the prions' structural features and their spreading capacity and mechanism, are not transmissible between individuals. Nonetheless, prionoids can be confined in the cell in which they appear or can migrate within cells or tissues.[6,10,12,13] Various disease-related proteins can be included in this definition, such as α-synuclein and amyloid β, whose amyloidogenic form is related to Parkinson and Alzheimer disease respectively.[8,16]

Moreover, recent studies suggest that prionoid proteins are more common than originally thought, as various proteins with regulated, physiological functions have been discovered to share prions' properties. Thus, those proteins which acquire or change their function once the aggregated state is achieved are known as functional prionoids. Currently, the knowledge of functional prionoids is scarce but constantly raising, being all of them involved in cell-regulated functions, in stark contrast to the unregulated, irreversible oligomerization related to prions and pathological prionoids.[16,17] In Table 1, examples of both disease-related and functional prionoids in the animal kingdom are mentioned, although in the present project the focus of attention will be over α-synuclein and CPEB.

2.2. α-synuclein: Parkinson's Disease

2.2.1. Epidemiology

An example in which prionoids are involved in are synucleopathies. One of the most prevalent synucleopathies is Parkinson's Disease (PD), which has had an increasing prevalence over the last years, affecting 1% of the population over 60

years. Therefore, PD is the second more common neurodegenerative disease, after Alzheimer's. Although the majority of PD cases are idiopathic, there is an important genetic risk, mostly induced by sporadic causes. However, around 10% of these genetic cases are familiar forms, usually involving mutations in the α-synuclein coding gene, SNCA. One of the most common hereditary forms of PD, in fact, is the SNCA missense mutation A53T. [13,23,24,25,26]

2.2.2. Characteristics of α-synuclein

The α-synuclein protein is part of the synuclein family, together with β and γ-synuclein. The α-synuclein form has 140 amino acids and it is highly expressed in the brain, although it can also be found in other tissues. However, its biological function has not been determined yet. Otherwise, this protein shows a tendency of forming oligomers *in vivo*, so when monomers turn to a β-sheet conformation, they form insoluble, neurotoxic fibrils, similarly to prions.[27]

Accordingly, it would be logical to look for some of the already mentioned prions' characteristics in the structure of α-synuclein. Indeed, there are similarities, as α-synuclein has a central CBR, formed by 12 hydrophobic amino acids, which is essential for its oligomerization.[23]

2.2.3. Pathology

The typical hallmark of PD are Lewy bodies, caused by the accumulation of α-synuclein. When analyzing Lewy bodies, 90% of the α-synuclein forming such aggregates was discovered to be phosphorylated on its serine 129, so this raised the question if PTM may play a role in the oligomerization process. However, *Karampetsou et al.*[28] demonstrated that, although the phosphorylation of α-synuclein accelerates the pathology, it is not essential for the formation of oligomers (Figure 2). Nonetheless, the effect of such phosphorylation is critical for PD, as it:

- Increases the aggregation capacity of α-synuclein.

- Reduces the innate immune system response, diminishing macrophages infiltration and modifying the production of certain cytokines, such as TNFα and IL-10.

- Induces a higher uptake of extracellular α-synuclein by neurons.

Moreover, aggregated α-synuclein has been suggested to spread within healthy neurons, especially among those which form neural circuits. Such neurons are in tight contact, so the aberrant form of α-synuclein is transmitted both by exocytosis and endocytosis. Another point to bear in

mind is that, after some time, neurons affected by α-synuclein aggregation may die and the aggregates would then be released to the medium, being susceptible of reuptake by nearby cells. Under these circumstances, the seeded-nucleation mechanism is also applicable, as after the first oligomer is endocytated it would cause the aggregation of endogenous α-synuclein too (Figure 2).[13,27,29] Furthermore, this is consistent with the Braak hypothesis, which suggests that the progression of PD takes place within neural circuits and expands progressively to further regions, mainly affecting dopaminergic neurons.[13,16,30] Nonetheless, if the target areas do not express the α-synuclein protein, as demonstrated with exogenous injections of α-synuclein in null α-synuclein mice, the aggregates are unable to form.[13,28,29]

In fact, α-synuclein aggregates have a great potential of spreading, as various studies suggest. On the one hand, it was demonstrated by intraglosally and intraperitoneal α-synuclein injections that an invasion of the whole nervous system, including the spinal cord, happens even after a distal administration.[24] On the other hand, if α-synuclein is injected in certain areas of the brain, the aggregates spread throughout the CNS, even to the contralateral side of the injection,[28,29] which seems controversial to Braak hypothesis as these areas are not

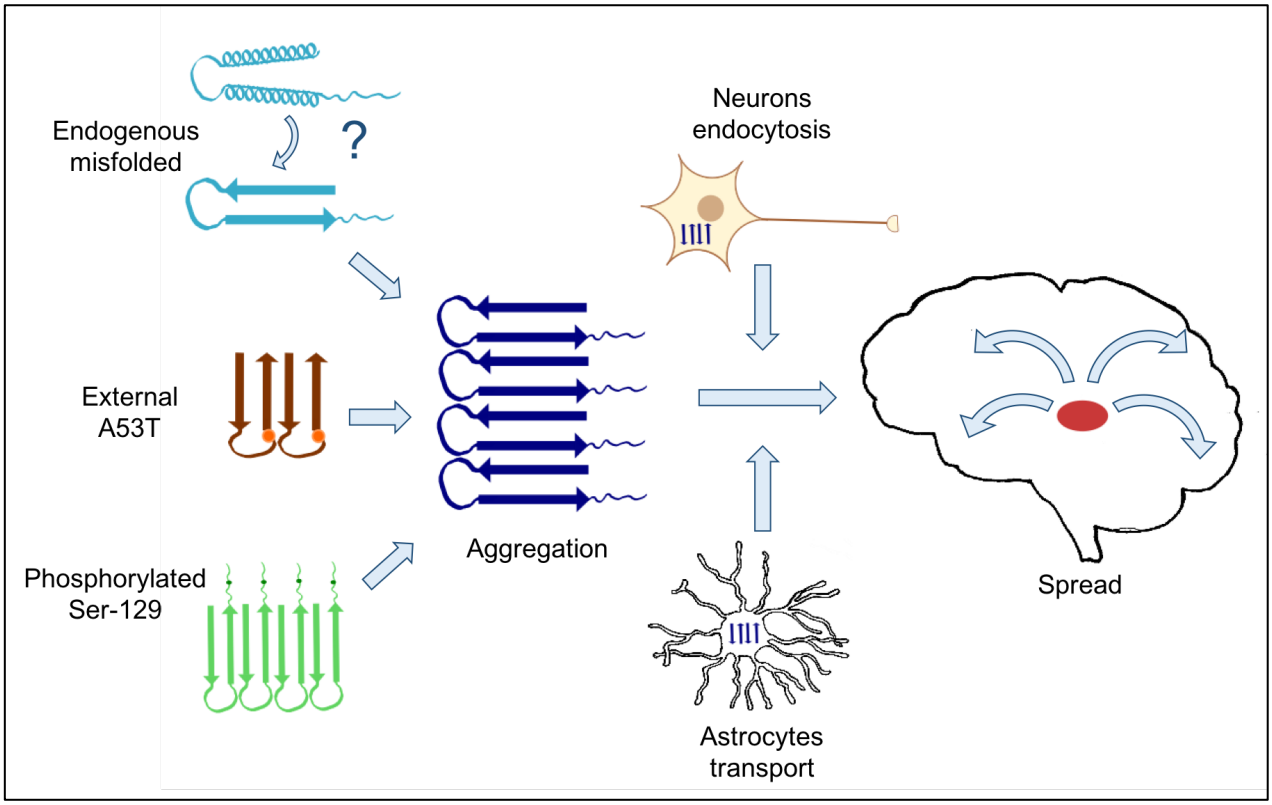

Figure 2. Different factors involved in PD pathology. On the top, the misfolded form of α-synuclein appears after an unknown stimulus. Then, the external A53T form present in food and the Ser-129 phosphorylated α-synuclein may also participate in further aggregation. Finally, both neurons endocytosis and astrocytes transport of aggregated α-synuclein participate in spreading the pathology throughout the brain. *Figure designed by the author.*

interconnected. A hypothesis that may explain this wide, fast spread of the pathology is that aggregates could be "transported" by some cellular components, such as astrocytes (Figure 2). Indeed, astrocytes engulf α-synuclein in order to degrade it, but instead they could act as a vehicle and transport the prionoid to other regions, even to unconnected ones.[29,31]

Despite all the experiments mentioned until here were carried using external injections of α-synuclein, neither the origin of the disease itself nor what triggers the transformation of the protein has been determined yet. Nevertheless, a recent paper has suggested a possible source of income of α-synuclein for humans: food. This fact is consistent with the experiments above mentioned, as human α-synuclein is able to aggregate in mice[31,32] and misfolded human α-synuclein is also capable of triggering the pathogenic process in primates,[33] despite some differences in the sequence. Then, taking into account that the mutated A53T α-synuclein seems to be present in meat products, food could be a potential source of aberrant α-synuclein. A53T, in fact, has already been mentioned for its ease of aggregation in the familiar form of PD (Figure 2). Accordingly, these ingested forms could seed in the human gut, as seen in Figure 3, and then spread through the vagal nerve until the CNS. Nonetheless, many other risk factors like genetic predisposition or inflammation

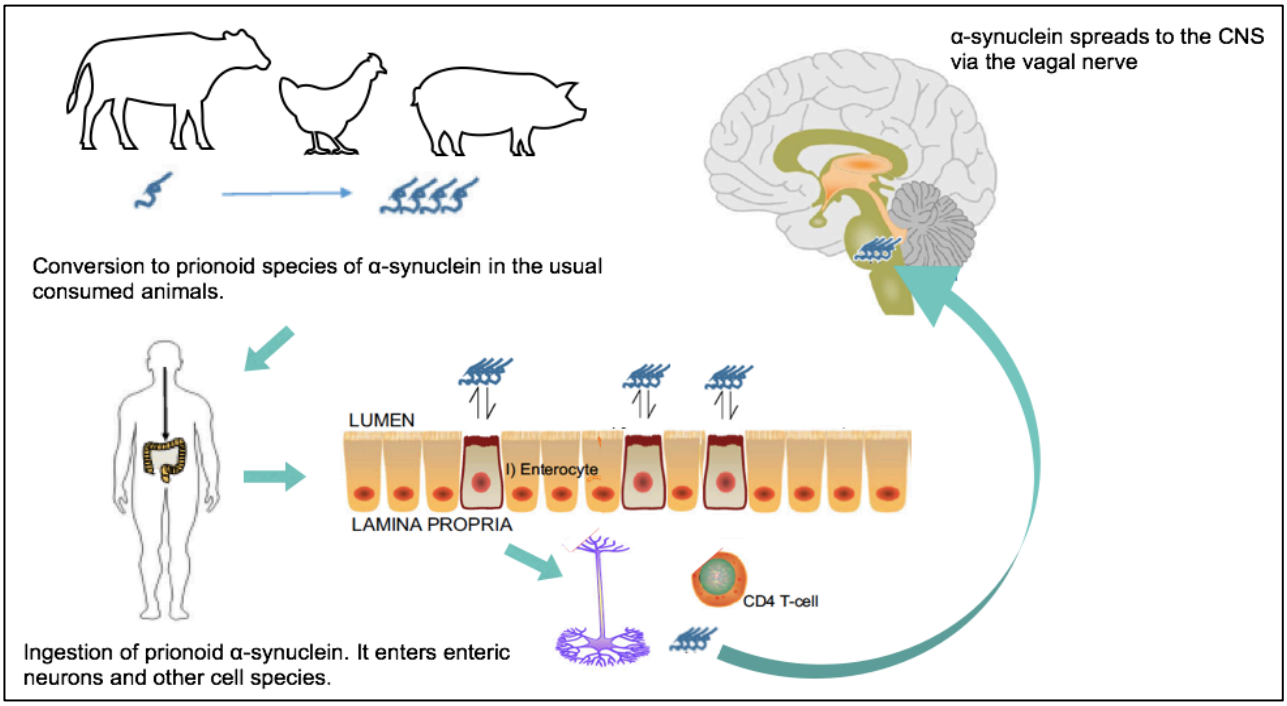

Figure 3. Vertebrate species ingestion can be a source of prionoid α-synuclein. The ingested form can seed in the lumen and infect enteric neurons. Hence, prionoid forms would be able to propagate until the CNS via the vagal nerve. *Modified of Killinger et al*.[27]

diseases could interfere with dietary factors to induce PD, so the ingestion of aberrant α-synuclein *per se* is not the cause of the disease. However, there are groups which totally discard this hypothesis due to lack of solid evidence.[27,29] All in all, although misfolded α-synuclein ingestion seem to be a plausible cause of PD in humans, further studies should be performed in this area.

2.3. CPEB

2.3.1. The role of CPEB in Long-Term Memory

Long-Term Memory (LTM) comprehension is of vital importance to understand the essential role of CPEB. Memory can be divided in two types: short-term memory, which is retained from seconds to minutes; and long-term memory, which can last from days, weeks or for a lifetime. Then, LTM relies on long-lasting changes in gene expression, which would then cause a structural and functional plasticity adaptation in synapses. But, taking into account that proteins have relatively short half-lives due to cell natural turnover, how can synaptic changes last for such a long amount of time?

Changes in gene expression can happen at different levels, although two phases are of great importance for the present purpose: new genes transcription, for which activation of transcription in the cell nucleus is necessary; and translation of dormant mRNA, which happens locally in the activated synapses. Already in 1998, Tompa and Friedrich announced the "prion theory of memory",[34] stating that LTM maintenance could be dependent on prion proteins, due to their self-sustained and persistent state. Although the first process is out of the scope of this project, CPEB protein has been proposed as a regulator of mRNAs translation, inducing it when adopting the functional prionoid conformation.[12,34,35,36]

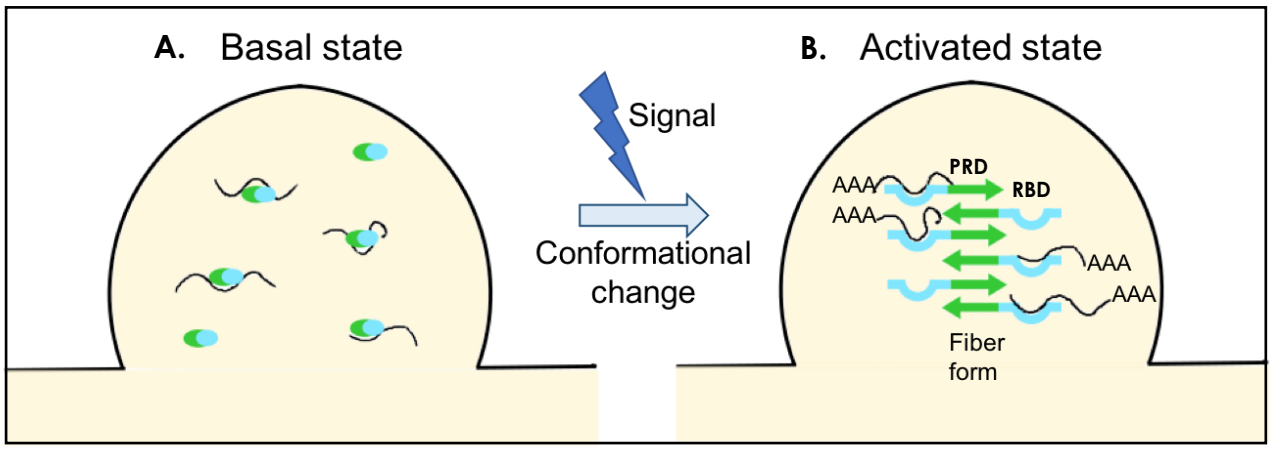

Figure 4. A) In a basal state, CPEB protein forms monomers which can bind certain mRNAs, avoiding their translation by inhibiting their polyadenilation (dormant mRNA). In response to a specific stimulus, CPEB conformation changes and B) adopts a fiber structure, with the prion domain (PRD) forming the core fiber and the RNA-binding domain (RBD) being exposed to the cytoplasm. RBDs can then anchor target mRNAs and their polyadenylation is induced at a time. *Figure designed by the author.*

CPEB corresponds to *Cytoplasmic Polyadenilation Element Binding protein*, which means that CPEB binds the 3' UTR CPE region of certain mRNAs in order to induce their polyadenylation and subsequent translation. The neuronal isoform of CPEB is confined to synapses, where it acts as a repressor of certain mRNA in the basal, monomeric state (Figure 4A).[34] However, after a concrete, specie-dependent signal, local CPEB is activated and turns into its self-sustained, multimeric form, inducing then the translation of bound mRNAs (Figure 4B). With this mechanism, several targets can be translated at the same time in the specific location where they are needed, inducing long-lasting synaptic changes.[12,17,36,37] Accordingly, CPEB activation should exclusively happen in the synapses where the synaptic modulation is necessary. Hence, the CPEB fiber must be retained in the stimulated region, without spreading neither between neurons nor synapses.[3,36]

Different forms of CPEB protein exist, although in the present project only three types will be mentioned: *Aplysia* CPEB, *Drosophila* Orb2 and mammalian CPEB3. Despite all of them share the same function; their regulation, mechanism of action and targets are different depending on the studied specie, as detailed below.

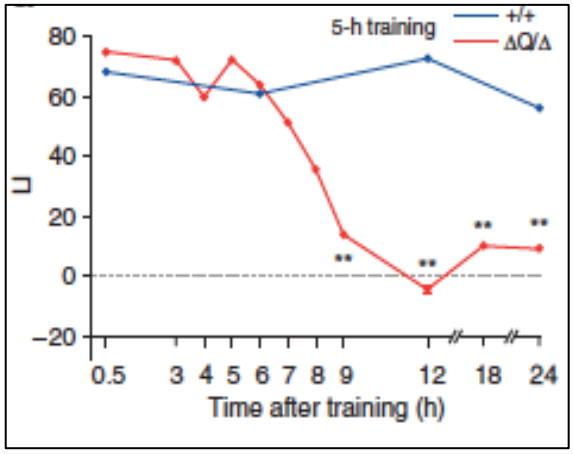

Figure 5. According to courtship conditioning studies, memory is lost beyond 9 hours in *Drosophila* Orb2 mutants (red line) in comparison to wild type (blue line).
** P < 0.0001.[38]

Various experiments have been performed in order to prove the importance of CPEB in LTM. Among all of them, the behavioral test based on courtship conditioning in *Drosophila melanogaster* is one of the most recurrent, as it shows that a male exposed to a non-receptive female acquires the capacity of discriminating between unreceptive and receptive females. Therefore, normal trained males develop LTM and can "remember" when to perform or suppress the courtship activity, depending on the type of female. In *Drosophila* Orb2 mutants, which cannot form the prionoid aggregation, memory was lost 9 hours after the first training (Figure 5), demonstrating the importance of CPEB for LTM.[38] Otherwise, if CPEB aggregation is prevented by adding a specific antibody against it, LTM would not be stabilized more than 72 hours.[36]

2.3.2. *Aplysia* CPEB

The first discovered CPEB, *Aplysia* CPEB (ApCPEB), has a 48% Q/P-rich region in its N-terminal domain, a C-terminal region with a RNA-binding domain and some coiled-coil helices between them (Figure 6A).[35,39]

In this case, the coiled-coil helices have been suggested to regulate the oligomerization of this protein, as they are accessible to environmental regulations, in sharp contrast to pathological prionoids.[3,35,37,39] Specifically, serotonin (5-HT) synaptic stimulation is the signal which induces ApCPEB switch to a β-sheet-enriched amyloid structure.[35,36] Then, as seen in Figure 7A, ApCPEB forms a fiber structure and binds its target mRNAs. In this point, ApCPEB facilitates the polyadenylation of several mRNAs at the same time, allowing a rapid increase in the LTM-related proteins concentration.[35]

The mechanism by which 5-HT induces the oligomerization of CPEB was unknown until 2015, when it was proposed that 5-HT signal downregulates certain miRNAs, such as miR-22 and miR-124, both expressed in the nervous system. In fact, miR-22 induces the degradation of ApCPEB mRNA by binding its 3' UTR region, as shown in Figure 6B. Accordingly, the inhibition of miR-22 results in an increase of ApCPEB concentration, allowing its aggregation in the multimeric, active form.[40] Indeed, this was the first evidence that 5-HT controls a whole set of non-coding RNAs, which at the same time regulate the translation of LTM-related proteins. However, more mechanisms could also be involved in ApCPEB activation.

ApCPEB reversible multimerization is another important difference in comparison to pathological prionoids, again probably by the coiled-coil helices. Such helices are not included in the fiber axis and, hence, are accessible even in the multimeric conformation.[35] Furthermore, it has been proposed that heat-shock proteins like HSP104 are able to remodel and reverse the active state of ApCPEB to its basal conformation, allowing the de-potentiation of synapses.[36]

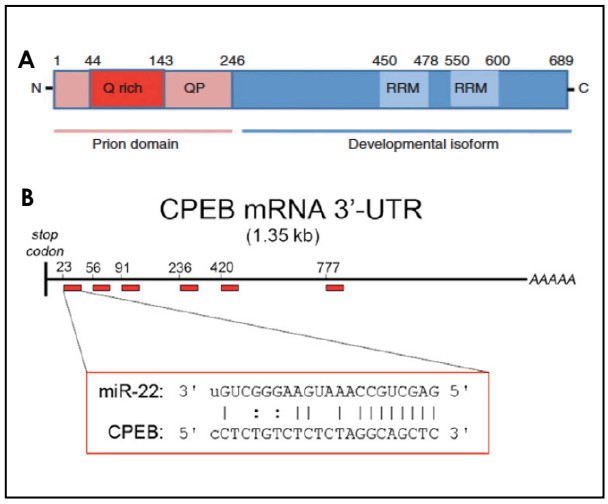

Figure 6. Schematic representation of the neuronal isoform of *Aplysia* CPEB. A) Structure of *Aplysia* CPEB, where the red is the prion domain and the blue, the RNA-binding domain.[35] B) Scheme of miR-22 binding to the 3' UTR region of ApCPEB mRNA. Red lines indicate possible binding sites. The homology of the sequence regarding the first homologous segment is expressed in the square below.[40]

2.3.3. *Drosophila* Orb2

Drosophila homologue of ApCPEB is called Orb2, member of the CPEB2 family, which has two different isoforms: Orb2A and Orb2B. These isoforms, although they differ in the composition before the prion domain, also share a Q-rich region in the N-terminal domain; while in the C-terminal region a RNA recognition motif and a zinc finger are found. Therefore, Orb2 also regulates local mRNA translation.[38,41,42]

However, certain differences between ApCPEB and Orb2 can be found. Firstly, it is worth to mention that the multimerization of Orb2 is regulated in response to octopamine, dopamine or tyramine, not by serotonin.[41,43] Secondly, it seems that Orb2A, whose concentration is low and specially localized in synapses, is the form which induces the oligomerization of Orb2B, while this one is widely distributed and binds strongly to target mRNAs.[3,41,42] In third place, Orb2 activation depends on its phosphorylation by Tob-LimK complex in response to tyramine, which stabilizes the protein concentration of Orb2A and then triggers its seeded nucleation.[43]

Furthermore, although the N-terminal domain of Orb2A is essential for the formation of the fiber core, its Q-rich region is excluded of it. In fact, such region has been proposed as a stabilizer or regulator of the multimeric form, but its function is still elusive. On the opposite, Orb2B is indispensable for RNA binding, so both Orb2 forms have complementary functions (Figure 7B).[41,44]

Recently, another regulatory point has been suggested for Orb2, as it seems that the amyloid formation is also dependent on the latter part of the N-terminal domain, which has metal binding capacities. Orb2 oligomerization, indeed, is increased in presence of several metals, which suggest that not only neurotransmissors but also metals, such as Zn^{2+} -whose concentration increases during memory processes-, may induce the prionoid change in Orb2 proteins.[45]

2.3.4. Mammalian CPEB3

Although four CPEB forms have been described in vertebrates, only CPEB3 can be considered an homologous of ApCPEB, as it also presents the two different states and performs the same biological function. Accordingly, such form has been extensively studied in mice, even though it is also present in humans.[46]

The particular regulation of CPEB3 implies such protein being SUMOylated in its basal state; while its monoubiquitination, which increases after synaptic stimulation, leads to its aggregation. Moreover, as seen in

Figure 7C, the switch between basal and active conformations is promoted by dopamine, glutamate and glycine.[12,47,48,49] However, if further modifications or processes are involved in this regulation or how CPEB3 returns to its basal state is still to be discovered.[49]

Furthermore, the prion domain of CPEB3 has three different parts: a PRD1, which contains the Q/N-rich domain, a PRD2 and an in-between regulatory region. Such regulatory domain, in fact, interacts with actin filaments when adopting the prionoid form (Figure 7C). Through this interaction, CPEB3 is concentrated in certain synaptic regions and, hence, its aggregation rate is increased.[46]

3. Discussion

As a consequence of broadening the concept of prions, the discovery of prionoid proteins has been possible, whose transmission is restricted within individuals and can be both pathological or functional. This is an important point to consider when differentiating prions and prionoids, as despite both can be infectious, only prionoids may perform signal-regulated biological functions in the amyloid form. Such functional prionoids, although already proven in yeast, are a recent discovery in eukaryotic organisms.[6]

One of the most important disease-related prionoids is α-synuclein, which causes PD. However, the severe and fast outcome of

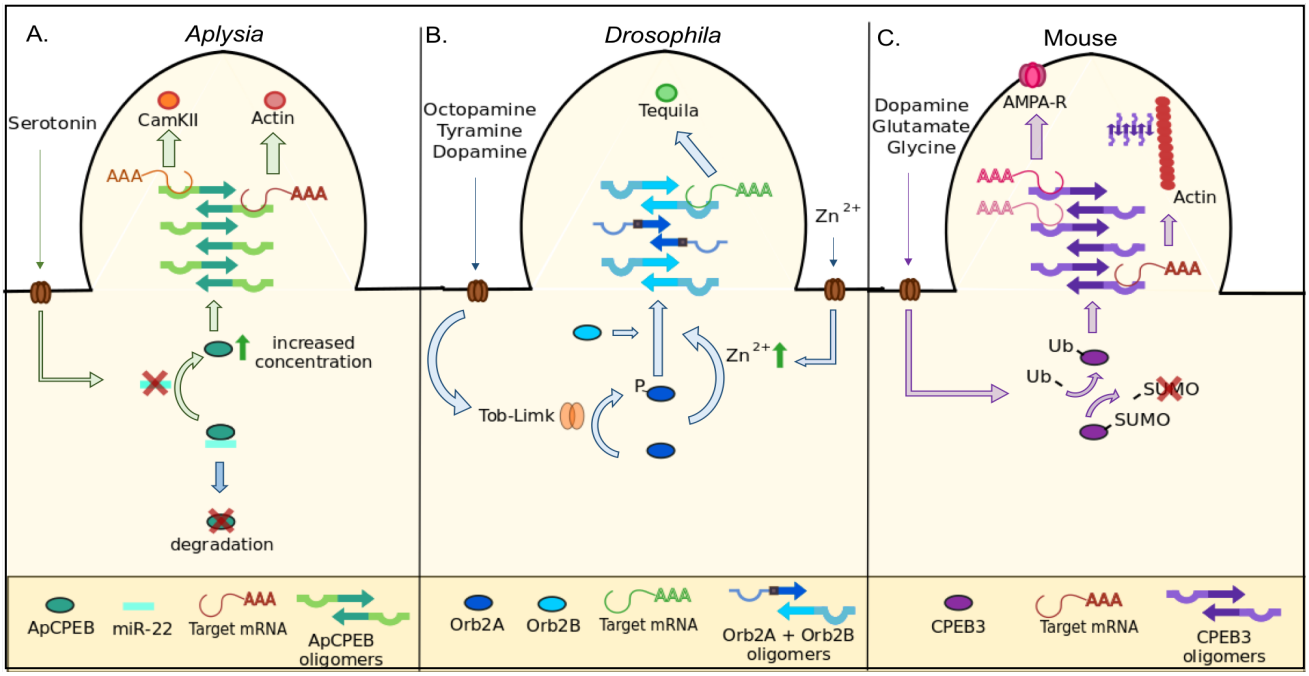

Figure 7. Differences among specie-specific CPEBs. A) If ApCPEB is bound to miR-22, it is degraded. On the contrary, under serotonin stimulus, miR-22 is degraded and ApCPEB concentration increases, inducing its aggregation in the amyloidogenic form. Then, certain targets are translated, such as CamKII[34] and actin[37]. B) In response to extracellular signaling, the Tob-LimK complex is activated. Consequently, Orb2A is phosphorylated and stabilized. Hence, Orb2A forms the core of the fiber axis, initiating the seeded nucleation process and recruiting Orb2B monomers. Otherwise, exposition to certain metals can also induce the same conformational change. In this case, different targets are translated, like Tequila[42]. C) CPEB3 is SUMOylated in its basal state. Under specific stimuli, the SUMO group is exchanged for a monoubiquitin. Such monoubiquitination induces CPEB3 aggregation and the translation of targets such as GluR1-GluR2 (subunits of AMPA receptor) and actin.[46] Furthermore, the oligomeric form of CPEB3 is transported to specific regions due to its interaction with actin filaments. *Figure designed by the author*.

PD is the consequence of a combination of various factors. First, the phosphorylation of α-synuclein, which enhances its aggregation. Secondly, the possibility that astrocytes and other cells may act as transporters of the pathological form within the brain, although further investigation should be performed in this field. Accordingly, the amyloid conformation does not cause the pathology exclusively by itself, but it requires its combination with other factors. This is also related to the fact that the ingestion of misfolded forms of α-synuclein seem to trigger PD in human beings, but solid evidence is still missing. Besides, available treatments for PD are quite inefficient, with several side effects and poor results, as they do not treat the real focus of the pathology.[50] Hence, should it be considered that Parkinson's Disease is not being addressed with the most suitable strategy?

Another important protein in the CNS is CPEB, both in its monomeric and oligomeric form, which is essential for LTM. Despite certain peculiarities in each specie, this functional prionoid is always related to synaptic plasticity. This fact also suggests that functional prionoids have been conserved along evolution. Then, their deeper study may not only provide further comprehension of various biological functions but also help in the development of suitable treatments for pathological prionoids.

To summarize, prionoid proteins are far more common than previously thought, and their wide comprehension is of vital importance both for pathological and physiological conditions. By studying them, it could be possible to discover:

1. Which events trigger the pathological prionoids formation and identify which processes are involved in their expansion, in order to manipulate them and avoid neurodegenerative diseases.

2. Whether the artificial recruitment of functional prionoids could anyway induce their biological function as, for example, for stabilizing memories when treating memory deficiency diseases.

4. Conclusions

Hitherto, the main facts related to both functional and pathological prionoids have been exposed. As a result, conclusions are:

- Prions and prionoids share the main structural characteristics and the spreading mechanism, despite the latter not being transmissible between individuals.

- Prionoid proteins can be both pathological or functional. As opposite to pathological prionoids, functional ones are regulated by cell and environmental signaling. Furthermore, functional prionoids' aggregation can be reversed.

- The disease-related prionoid α-synuclein reaches a severe pathological state due to the combination of its aggregation with other processes.

- The functional prionoid CPEB plays an important role in LTM, a conserved function along species. However, its regulation and targets may differ among the different homologous proteins of each specie.

- Prionoids' deep study could be extremely useful for the development of new treatments, such as in:
    - Treating neurodegenerative diseases in a more efficient manner. For example, treating PD by avoiding amyloid formation may be also interfering in functional prionoids' aggregation.

    - Treating memory deficiency diseases by exogenously triggering the oligomerization of functional prionoids, like CPEB.

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