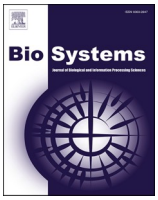
BioSystems 210 (2021) 104542

Contents lists available at ScienceDirect

BioSystems

journal homepage: www.elsevier.com/locate/biosystems



Biosemiotics comprehension of PrP code and prion disease

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ARTICLE INFO

Keywords:
Protein misfolding
Neurodegenerative diseases
Prion biosemiotics
Biosocial

ABSTRACT

Prions or PrP^{Sc} (prion protein, Scrapie isoform) are proteins with an aberrant three-dimensional conformation that present the ability to alter the three-dimensional structure of natively folded PrP^{C} (prion protein, cellular isoform) inducing its abnormal folding, giving raise to neurological diseases known as Transmissible spongiforms encephalopathies (TSEs) or prion diseases. In this work, through a biosemiotic study, we will analyze the molecular code of meanings that are known in the molecular pathway of PrP^{C} and how it is altered in prion diseases. This biosemiotic code presents a socio-semiotic correlate in organisms that could be unraveled with the ultimate goal of understanding the code of signs that mediates the process. Finally, we will study recent works that indicate possible relationships in the code between prion proteins and other proteins such as the tau protein and alpha-synuclein to evaluate if it is possible that there is a semiotic expansion of the PrP code and prion diseases in the meaning recently expounded by Prusiner, winner of the Nobel Prize for describing these unusual pathological processes.

1. Introduction

In 1982, Stanley Prusiner defined prions as proteinaceous infectious particles and as the only responsible of Transmissible Spongiform Encephalopathies (TSE). Since this controversial theory was postulated, the knowledge about these particles has increased notably and nowadays there are many neurodegenerative diseases, such as synucleinopathies and tauopathies, characterized by the presence of endogenous misfolded protein aggregates that could share important features with TSE causing prions. Apart from disease-causing proteins prone to misfold into self-replicating amyloids, other proteins have been described able to acquire an amyloidogenic structure, which are not related to disease. These proteins that utilize misfolding as a mean to regulate their function or activity, are known as functional amyloids and have been found in evolutionarily distant species such as bacteria (Giraldo et al., 2016), fungi (Wickner et al., 2015), gastropods (Heinrich and Lindquist, 2011) and mammals (Hou et al., 2011). As the molecular mechanisms underlying misfolding and accumulation of such amyloidogenic proteins are discovered, the line separating infectious or disease-causing prions from non-infectious or functional amyloids is getting blurred (Eraña, 2018). Therefore, it may be possible that all of them share a general common biological code. The first evidence of the existence of functional amyloids with commonalities with prions arises in 1994 from the discovery of the so-called yeast prions (Wickner, 1994). These proteins showed the capacity to be transmitted cell-to-cell and induce their conformation to natively folded counterparts, what led the researchers to include them under the same category as TSE-causing prions due to their similar autocatalytic replication mechanism.

Prion diseases are a set of transmissible neurodegenerative pathologies, that can occur sporadically, be inherited or acquired through dietary or iatrogenic exposure to prions. Human cellular prion protein (PrP^{C}) is a glycoprotein of 253 amino acids with an 85–90% homology with other mammalian PrP, presents a GPI anchor, and two N-glycosylation sites (Parchi et al., 2011; Baral et al., 2019). Upon misfolding through poorly characterized mechanisms, the cellular prion protein acquires an aberrant three-dimensional structure called prion or PrP^{Sc} (from scrapie, the disease in sheep), becoming aggregation prone, gaining the ability to induce this conformation to the native protein, and becoming neurotoxic, which in turn leads to the development of TSE. Among these illnesses, Creutzfeldt-Jakob diseases (CJD) is the most common type of prionopathy in humans. In fact, CJD can be further classified into sporadic CJD (sCJD), familial or genetically determined

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https://doi.org/10.1016/j.biosystems.2021.104542
Received 22 January 2021; Received in revised form 1 September 2021; Accepted 7 September 2021
Available online 10 September 2021

CJD, iatrogenic CJD (iCJD) which arise from infections due to medical procedures, and the variant CJD (vCJD) which differs from the others in its zoonotic nature and is acquired through ingestion of prion-infected cattle meat. The first one accounts for the 85–90% of all human CJD cases, and the second around 10% of the total, while iCJD and vCJD are almost eradicated nowadays (Pascuzzo et al., 2021; Yarus, 2011).

Genetic CJD arise due to mutations in the PrP^C encoding gene (*PRNP*). This gene is located on the short arm of chromosome 20 in humans and entails two exons and one intron of 13 kbp. Currently, around 70 variants of this gene have been described (Jones and Mead, 2020). However, most of gCJD cases are due to mutations E200K, V210I and V180I (Takada et al., 2017). Importantly, apart from disease-associated mutations, another polymorphism in *PRNP*, the presence of valine or methionine at codon 129, has been reported to exert a great influence on the pathological process. In fact, these variants determine the age of clinical onset and clinical duration in a subset of inherited prion disorders (IPDs) and alters the risk and clinical duration for sCJD and iatrogenic CJD (Jones and Mead, 2020). However, apart from the influence of the PrP^C amino acid from the host, the most important determinant of the disease genotype in TSE is the three-dimensional structure of the PrP^{Sc}. The fact that prions can show distinct biochemical and biological properties due to differences in their conformations is known as the strain phenomenon, in reminiscence of the viral strains, and is one of the most intriguing properties of prions. These structural differences enable them to present different features such as a determinate host range or tropism for specific brain areas (Stein and True, 2014). This in turn, demonstrates that the structure of a protein encodes biological information in absence of genetic variation, since prions with identical amino acid sequences can show strikingly different properties. We have therefore considered it important to analyze the biology of the prion code and to study how this code helps to understand biological phenomena related to the activity of these misfolded proteins and their effects on social behaviour in humans.

The key role of PrP^C in TSE was undoubtedly demonstrated through deletion of *PRNP* in mice, which resulted in complete resistance to prion infection, while restoration of *PRNP* gene also restored the susceptibility to prion infection (Priola, 2018). However, although expression of host PrP^C is an utter requirement for the infection, it is not sufficient determinant for a cell to be susceptible to prion infection, as demonstrated by cell lines which express PrP^C but can remain refractory to infection (Oelschlegel et al., 2015; Priola, 2018).

CJD results in a rapidly evolving neurodegenerative disease characterized by neuronal loss, spongiform degeneration and astrogliosis. Likewise, deposits of the misfolded form of the PrP are observed in the brain of these patients, what led to the finding that amyloidogenic proteins could be responsible of these disorders and ultimately to the definition of proteinaceous infectious particles by Prusiner. However, despite the knowledge gathered about prions and the disease they cause during the last decades, the molecular mechanisms leading to the misfolding of PrP^C into PrP^{Sc} remain unsolved, impeding further understanding of the causes of non-inherited and acquired prion diseases. As a result, existing knowledge is somewhat fragmentary and limits the possibilities for a complete biosemiotics analysis. However, in human prion diseases, the relationship between the biological code and its social correlate are clearer and could be analysed from the perspective of biosemiotics.

However, TSE-causing prions are not the only proteins that present the ability to misfold and encode information through their 3D structure. As mentioned before one of the most notable examples are yeast prions, which are not pathogenic and do not completely fit under the definition of prions understood as proteinaceous pathogens. Either way, yeast prions are a major line of research to comprehend the biological code of information transmission through protein misfolding. In this paper we perform a theoretical investigation to understand the basic elements of the biological code (according to code biology theory) that could operate around these proteinaceous particles which infectious or not, seem to be able to encode and transmit biological information in their three-dimensional conformation.

2. Code biology

Before going into further details, it is convenient to indicate that the code biology proposal is based on the idea that in living organisms we can find a multitude of biological codes. They contain meanings of great importance and will allow (in one way or another) the codification of different structures that will result in distinct effects. In our case, we will focus on analysing the biology of prions from the perspective of code biology. According to this viewpoint, it can be affirmed that living systems (also the molecular systems) are semiotic entities in the sense that they operate under the triadic structure of *sign*, *code*, and *meaning* (Barbieri, 2003; Faria, 2008). Furthermore, Barbieri (2008a) explained that all semiotic system is configurated by a tetralogy: *signs*, *meaning*, *code*, and *codemaker*. According to this, a semiotic system is always made of two worlds. On one hand is the world of objects named *signs* and on the other, the world of objects that represent *meanings* (Barbieri, 2008a). These two worlds are related through *codes*, also named *conventions*. Therefore, *codes* will be of major relevance in semiotic systems. In fact, the code has been defined as a mapping between objects of two worlds that is executed by objects of a third world: *the adaptors*. In fact, adaptors provide some meanings to molecular structures because they can cause modifications in pre-existing molecules. However, we are not particularly interested on adaptors in this work because, although they can help in the process of mediation between different biological worlds, they are not of major importance in terms of prion biosemiosis. To comprehend the code of prions and its biosemiosis, is necessary to introduce a fourth element in this structure: *the codemakers* (Barbieri, 2008c). Barbieri (2008a) clearly explains that the codemakers are the agents of semiosis (for this reason we focus our interest in these molecules), while signs and meanings are its instruments.

In biological systems, signs and meanings exists at the molecular and biochemical level, in turn the signs and meanings of our cultural world transcend the molecular reality (Barbieri, 2008b). In other words, biological systems contain and generate signs and meanings that can be detected and that shape life itself (biological and biosocial). However, the cultural world is made up of shared signs and meanings that operate in a different way, since it is not usually affected, in a direct way, by biological systems. If we exemplify this in prions, we can indicate that these biological structures have clear biosocial effects (neurodegenerations). However, whether this biosocial effect derives in cultural effects and, therefore, whether they have implications in the collective way of understanding the world is not within the scope of this manuscript, as it would be require a thorough social analysis on the cultural impact of these diseases.

Thus, we will focus on one of the major problems on code biology, that is to determine if there are codemaker-dependent entities, taking into account that mental signs and mental meanings do not exists without these *codemakers* and include outside a codemaking phenomenon (Barbieri, 2008a, 2008b). As we will see below, there is a certain evolutionary correlate in prions. Therefore, by unraveling part of this evolutionary phenomenon and exploring its linkage with prions, we will be able to approach the determination of the elements (codemakers) that generate the prion code and propose that it could have originated at the dawn of life on Earth.

The biological code operates as a structure made of genes, proteins and ribosoids, according to three major elements of the biochemical cell typology: genotype, phenotype and ribotype (Barbieri, 1985). This time we are particularly interested on the ribotype. This cellular subsystem refers to the set of ribosoids (proteins, enzymes, and RNAs that act as a molecular machinery implicated in gene and protein synthesis), and thus, this hypothesis could be closely related with the *RNA World hypothesis*. The RNA World theory postulates the existence of an RNA-based ecosystem as the origin of life, given that RNA could be able

to make copies of itself as well as showing enzymatic activity (ribozymes), which would on time evolve to a DNA, RNA and protein-based replication machinery. In fact, studies on the origin of the primitive biological systems, consider the development of transference RNA which would be the necessary adaptors to link the DNA and protein worlds, as the critical event in the transition from an RNA world to a ribonucleoprotein world (Farias and José, 2020). In turn, the *autocatalytic anabolist theory* considers that nucleic acids and replication are part of the evolution, so there must have been a primordial mechanism of evolution independent of nucleic acids based on the autocatalytic metabolic reproduction (Wächtershäuser, 2006).

In this primitive world, the molecules named by Barbieri as ribosoids, would not need catalytic processing or an accompanying nucleic acid. Then, these molecules could spontaneously copy themselves and generate a new code (*codemakers*). For that, Barbieri seems to support the second hypothesis mentioned: the anabolist theory. However, we do not intend to discuss these hypotheses and which of them is the closest to reality, but only to establish a framework for our semiotic analysis regarding amyloidogenic proteins like prions. In any case, the idea of ribosoids refers to the presence of molecules with primitive characteristics in terms of information coding that could be linked to the capacity for transmission and autocatalysis. As we shall see later, prions may have certain characteristics that are close to those of ancestral ribosoids, theoretically proposed by the ribotype theory. It is not intended to claim that prions are ribosoids. What we want to show is that prions present some characteristics that would also be present in those molecules of which Barbieri spoke: the ribosoids.

Also, Barbieri (2008b) affirms that if genotype is the pillar of heredity, and the phenotype is the support of metabolism, then ribotype is the codemaker pillar of the cell. The biological and informational characteristics of codemakers can be tracked to assess if there could exist molecules with similar behaviours inside cells. In this sense, and due to the primitive nature of codemakers, it would be possible to affirm that those molecules with the capacity to alter the code of a previous molecule are codemakers. Barbieri (2012, 2015) talked about *copymakers* and *codemakers* inside its ribotype theory. Copymakers are molecules with the ability to copy themselves and, because of that, are able to transmit information. Codemakers, in turn, are very relevant in the biological evolution because these molecules generate meanings from copymaker molecules. This typology helps to elucidate the biological codes involved in prion-mediated phenomena. In fact, as we will show below, prions use both codes to transmit themselves and to alter nerve cell processes. Hence, in the present case, it is not easy to delimit whether prions are copymakers or codemakers. This is because these misfolded proteins copy their own information and alter pre-existing information in the cell.

Once introduced the biochemical basis of prion replication or propagation and the main concepts of the semiotic systems, we can now anticipate that prions may operate as copymakers by generating copies of themselves, and as codemakers by generating new biological codes or alterations to previous ones. In addition, we hypothesised that prions also have effects by generating neuronal code-breaking mechanisms. In this sense, alterations in these proteins lead to neurodegeneration and have a strong impact on the affected individuals and their families.

3. The prionic replicator code

Once the general characteristics of the code biology are understood, we will now delve, more specifically, into the theoretical basis of the prion code. To do so, we will study molecules that could potentially function as codemakers, which are the fundamental basis of the prion code. In this sense, we will focus on showing the fundamental theoretical elements that allow us to identify the prion code as a replicative code.

Weiss et al. (2016) consider that the last universal common ancestor (named LUCA, or progenote) is a main model to study early evolution and life's origin. This progenote possessed a membrane, DNA, the basic molecular machines for copying nucleic acid, and a functional ribosome, among other elements. Yarus (2011) postulated that LUCA would need molecular self-replicators and the first one was named as the Initial Darwinian Ancestor (IDA). In other words, the self-replication mechanism is a primordial element in the first steps of life on Earth.

We have seen that there are theories that indicate that nucleic acids were essential in the origin of life. Other theories speak that it is really catabolism. What seems evident is that at the dawn of life on Earth there were molecules with the capacity for self-replication. In this sense, it is feasible to consider that molecules that have the capacity to bind to other molecules and induce their conversion into structural replicates of themselves could have been of great evolutionary importance. Different molecules have been proposed to operate as the first replicator such as protein or peptides alone (i. e. thiol-rich peptides or amyloids, inspired in the understanding of prions), nucleic acid alone (what is mainly represented by the RNA World theory) and a combination of both or nucleopeptide replicators (Piette and Heddle, 2020). This idea allows us to conceptually relate the prion activity as replicator with another replicator proposed by Dawkins in different works and allows us to hypothesize that the misfolded prion protein could be functionally close to the world of the ribosoids mentioned above. Obviously, we are not claiming that PrP is a ribosoid, but we intend to propose that prions could be located, biosemiotically, within that biological code. In this regard, it is also interesting to consider the theory proposing that amyloid folding lies within the origin of protein folding (Greenwald and Riek 2012). This could indicate the existence of another code in which amyloids would have been pivotal, not for their self-propagating ability but because they could help shed light on a common code for protein folding.

Wills (2001) indicate that the most elementary form of chemical autocatalysis is represented by equation A+B→2A. This equation also describes the prion replication mechanism in mammals, in yeast and fungi. Now, according to Wills, we could say that prions are part of an elementary mechanism of catalysis, so it seems plausible to say that they are molecules with ancestral reminiscences and therefore, it could be possible to consider prions as primitive replicators.

According to Richard Dawkins there are molecules that can be classified as *replicators* and *vehicles* (1976, 1982a). Later, Dawkins (1982b) explained that replicators are molecules with the capacity to make copies of themselves (i.e., genes). Hull et al. (2001) affirmed that replicators contain iteration possibilities and information. This is to say that the own structure of these *replicators* contains information and thus, this structure can be a code of the information. Dawkins (1976, 1982a) also defined *vehicles*, entities that are generated by codification of replicators, while the replicators can also modify vehicles. Furthermore, these entities interact with the environment. The conceptual determination made by Dawkins raises certain difficulties in determining what a prion is. In this sense, prions could be indifferently replicators and vehicles (especially in relation to those that are ingested). For this reason, we believe that the concepts proposed by Barbieri are more heuristic, which, as we have said, are those of copymakers and codemakers.

In this sense Szathmáry (2000) consider prions as molecular phenotypic replicators. Now, PrP^{Sc} could be understood as a codemaker because it shows the capacity to misfold PrP^{C} and convert this non-infective protein into an infective, self-replicating entity. Bearing this in mind, PrP^{C} could be conceptually expressed as a vehicle of code insert in this protein through making copies of itself (copymaker). Also, in the last decade different papers have provided convincing evidence that cellular molecules of non-protein nature including RNAs and lipids could assist prion replication (Katorcha et al., 2018).

Prion proteins therefore, seem to show a biological code reminiscent of ancestral proteins with analogies to some other proteins found in fungi and bacteria. This could imply the existence of an evolutionary *continuum* between different organisms. This possible continuum is reflected in the common molecular mechanisms shared by a series of proteins that have been often termed prion-like proteins. In all cases,

from bacteria to mammalian cells, proteins able to adopt an alternative, normally β-sheet enriched three-dimensional structure have been described, able to self-replicate through the induction of such structure in native counterparts and of cell-to-cell migration. These altered proteins acquire novel functions with respect to their natively folded counterparts. Furthermore, differences in such newly acquired properties have been observed, based on slightly different structural arrangements, showing that different information can be encoded and transmitted through distinct arrangements of protein structure. The fact that these closely related molecular mechanisms have been observed in strikingly evolutionarily distant organisms, as well as this phenomenon not being related just to disease, but also to specific cellular functions, argues in favor of a possible common origin or the existence of a primitive biological code based on structural rearrangement for replication and transmission of information (Eraña, 2018).

We know that the purpose of the ancestral biological systems was not the synthesis of specific proteins because they could not evaluate the *future* benefits of such proteins. What they could evaluate, however, were the *immediate* benefits of ribosomal machines that were increasingly efficient in producing their statistical proteins and it was for this reason that evolution systematically decreased the ambiguity of the ancestral genetic code (Barbieri, 2019). According to this sentence, it could be possible to consider the prionic code as a reminiscent of that ancestral biological system in which the ambiguity had not been yet decreased.

Replicator's characteristics of PrP^{Sc} and other amyloidogenic proteins conducted Maury (2009) to propose the existence of an 'amyloid world'. In this prebiotic model of the world different prebiotic informational entities could have emerged and thus, Maury's model is in line with the biological code proposal of Marcello Barbieri. The model of Maury has been supported by Li et al. (2010) who showed that prions could also be subjected to Darwinian evolution. According to their research, prions are subject to mutation (evidenced by heritable changes of their phenotypic properties) and to selective amplification (documented by the rise of distinct populations of prions in different environments). Maury (2018) has recently explained that a distinctive feature of amyloid formation is that the same peptide monomer can generate functionally and structurally different amyloid conformers. These conformers could propagate and make new copies of themselves. Hence, the proposed replication system could adapt to even small changes in the external environment, being consistent with the evolutionary process. Maury's hypothesis is related to Barbieri's work and suggests that the existence of proteins with prion-like behaviour could be related to these copymakers and codemakers present in this ancestral world. It should be recalled that these codemakers were fundamental to affirm the existence of a possible biological code.

Furthermore, Wickner (2016) proposed that although prion variants are propagated with certain constancy of their structure, changes in it, and in turn in their properties, could also occur under selection pressure in mammals and yeast. Some of these selection mechanisms could be crossing a species barrier, in which prions are exposed to PrP^{C} with different amino acid sequence and likely distinct compatibility with the pathogen, or administration of a drug that can block the propagation of specific structural arrangements (Li et al., 2010; Wickner, 2016). However, variant properties can change, and a mixture of variants can be segregated during propagation, even under nonselective conditions. In agreement with this, Wickner (2016) and others such as Collinge and Clarke (2007), who originally proposed this idea, defend the existence of prions as a "prion cloud" composed of slightly different conformers which would be responsible of the final phenotype as a whole. This model is intended to show that prions (at least yeast prions, although it seems that this model can be extended to TSE-causing prions) are not a uniform structure, but they have an array of related self-propagating amyloid structures (Bateman and Wickner, 2013). Therefore, and according to the "prion cloud" model, there is a basic primary code (the one related to the amyloid structure) that seems to be maintained throughout the evolutionary process. This, as we have already said, also bears some relation to an ancestral protein behaviour, reminiscent to that identified in the ribosoid code but not the same.

The different theoretical approaches shown, allow us to affirm that it is plausible to consider the existence of a prion biological code or even, an amyloid biological code. The fundamental characteristics of this code, as we shall see in the following section, are found in different organisms, potentially expanding the amyloid code under analysis to other proteins than TSE-causing prions.

4. Prions in non-human organisms

Proteins able to form amyloids can be found in many organisms such as bacteria, fungi, yeasts and, of course, higher vertebrates. Amyloids are insoluble aggregates of proteins, characterized by a cross-β sheet quaternary structure in which molecules in a β-strand conformation are stacked along a filament axis. These properties are shared by a variety of amyloid proteins that can aggregate in different ways, depending on the specific protein and the organism in which it is expressed. And while some of them are associated to pathologies, functional amyloids are also present in a wide variety of organisms and fulfil several relevant cellular functions (Hervás et al., 2021). Among others, these functional amyloids can form biofilms in bacteria or assist monolayer formation at a surface. In yeast, the HET-s prion from *Podospora anserina* participates in the heterokaryon compatibility of neighbouring colonies and in other cases, the aggregation is also used as a mechanism to "suppress" the function of the soluble or natively folded isoform of the protein (e.g., Cdc19), occurring for instance when the yeasts are in stress and generate several granules to increase their survival opportunities (Otzen and Riek, 2019). Moreover, the molecular mechanisms that lead to the biological function of HET-s prion have been studied in detail, revealing that the prion folding domain or prion motif of this protein participates in a signal transduction process through crosstalk with other homologous domains from other proteins. This indicates that such conformational crosstalk between proteins with amyloid forming domains could be a common molecular mechanism spread throughout several organisms, setting proteins with amyloidogenic domains in a context of wider biological significance that goes from functional amyloids to disease-causing amyloids (Riek and Saupe, 2016; Chiti and Dobson, 2017).

The cytoplasmic polyadenylation element-binding (CPEB) is another interesting example of a functional amyloid. The amyloid-like properties of this protein described in *Aplysia*, and thus named ApCPEB, has a prion-like domain (PLD) and presents a similar process of aggregation to that of TSE-causing prions and yeast prions, which has led a proposal that this molecule associated with formation of memory is a prion (Si et al., 2010; Glanzman, 2013). CPEB aggregation is due to the prion-like domain of the protein, which shares physicochemical properties with domains detected in well characterized amyloidogenic proteins. However, this domain varies in its sequences across species and in fact, there are similitudes between Aplysia CPEB (ApCPEB) PLD and the Drosophila ortholog, Orb2 PLD (Hervás et al., 2021).

The *Orb2* locus is another interesting example that encodes six closely related protein isoforms, of which two isoforms named Orb2A and Orb2B structural change and self-propagation properties (Majumdar et al., 2012). In the adult brain of *Drosophila*, the Orb2A protein is expressed at a low level. However, it is very important for the oligomerization of Orb2. In fact, the Orb2A form generate oligomers more easily than Orb2B. Actually, a mutation in Orb2A blocking its oligomerization affects to the persistence of memory and the existence of an Orb2A prion-like domain is sufficient for long-term memory formation (White-Grindley et al., 2014).

These latter prion-like proteins are mRNA-binding translation regulators, which newly related this protein with the ribotype code. However, although the possibility of positing that the existence of a common prion code is supported by the concomitances described, there is still the problem of species with low susceptibility to infection, which pose a

challenge for the establishment and implementation of a prion code theory. We say this because the discovery of these potential exceptions to a common prion code, in which PrP^{C} would be always able misfold to an alternative isoform, implies the need to know more about the basic elements of this biological code and the evolutionary alterations that allow certain organisms to present these low susceptibility, as they may defy the understanding of the prion code or pose exceptions to it. In fact, Vidal et al. (2020) showed that domestic dogs are resistant to infection because of the presence of aspartic and gutamic acid at position 163 of their PrP.

In any case, despite their common features, the enormous differences on amino acid sequences of all these amyloid forming proteins hinders the definition of molecular determinants of amyloid folding making difficult to stablish the fundaments of the amyloid code (Hecht et al., 2004). Given that the common feature of amyloids is their three-dimensional structure upon misfolding, likely structural determinants will be defined as a starting point to decipher the amyloid code. Nonetheless, the matter could be still more complex than we can even imagine, since it has been shown that some amyloidogenic proteins can sequester many other proteins with essential functions in their aggregate-formation pathway (Olzscha et al., 2011).

The different examples we have presented support the idea of a biological code based on the replicative character in amyloid proteins. As we have seen before, these proteins have the capacity to change to make copies of themselves and to adjust (according to Maury's hypothesis) to environmental characteristics. These supports also, to some extent, the possibility that, years ago, molecules with similar characters to those now found in species such as *Aplysia*, *Podospora*, among others, could have existed.

5. Prionic neural code

In order to understand prion replication, Prusiner (1982) proposed what is called the "protein only" hypothesis. This approach considered for the first time that TSE could be caused exclusively by misfolding of PrP^{C} into PrP^{Sc}, in the absence of nucleic acids that could explain the transmission of information resulting in a neurodegenerative pathology. In the light of this theory, understanding the structure of PrP^{C} and its conversion to PrP^{Sc} is particularly important to define the biological code that underlies the pathological process initiated by this protein.

PrP^{C} presents two clearly distinct regions, the N-terminal portion of the prion protein is unstructured, and it consists of a long and flexible tail. On the other side, the C-terminal domain, also known as the globular domain, contains three α-helices and a short, two-strand β-pleated sheet (Mabbott, 2017). Moreover, this protein is expressed most abundantly in the outer membrane of nervous cells where it is bound through a glycosylphosphatidylinositol (GPI) anchor. GPI has a major relevance in cell signalling transduction and initiating different gene expression cascades in response to external stimuli and in fact, in prion diseases, GPI-anchored PrP^{C} is thought to mediate prion caused neurotoxicity, while this moiety is not essential for PrP^{C} misfolding and conversion into the self-replicative, infectious isoform PrP^{Sc} (Priola, 2018).

The misfolding event by which the globular domain of PrP^{C} is converted into a β-sheet-rich isoform is completely unknown at a molecular level but this new structure shows neurotoxicity, relative resistance to proteinase digestion, and is accumulates in affected tissues in the form of insoluble aggregates. The exact three-dimensional structure of PrP^{Sc} has been long sought, since its structure encodes the biological properties acquired by prions and is the part of this semiotic code that needs to be unraveled in order to decipher the information transmission mechanism or code underlying prion disorders. Fortunately, the first high-resolution three-dimensional structure of a mammalian prion has been recently published, showing a parallel in-register β-sheet structure, and bringing biologists a step closer to understand this unusual information encoding mechanism (Kraus et al., 2021).

We have already mentioned that prion diseases show certain analogy to other diseases such as Alzheimer's Disease, Parkinson's Disease and Huntington, among other protein-misfolding related neurodegenerative disorders. They are neurodegenerative disorders in which conformational change and accumulation of amyloidogenic proteins occurs. Moreover, it has recently been proven that these proteins can self-replicate in a manner similar to the prion protein and thus, present characteristics of replicators. In fact, Tau protein, β-amyloid and α-synuclein appear to be capable also of cell-to-cell dissemination, and could be considered as infective proteins inside a single organism. Moreover, slightly different protein conformations causing distinct disease phenotypes were also described for some of these disorders, suggesting the existence of strains as in the case of TSE-causing prions. And finally, the latest structural data shows a similar arrangement for TSE causing prions and other misfolded proteins such as Aβ peptide and α-synuclein Kraus et al., (2021), making it even more plausible that a common code could be shared by all these proteins. Due to these similarities some of the disorders associated with misfolded proteins have been considered as prion-like diseases: Alzheimer's disease, Parkinson's disease, Frontotemporal dementia, Amyotrophic lateral sclerosis and Huntington's disease, which could all be caused by prions, a term that is being expanded from TSE-causing prions to a wider family of proteins sharing mechanisms and possibly semiotic codes.

Barbieri (2014) explains that from Code Biology three worlds can be defined. World 1 in which organic semiosis operates, with coding as its mechanism. World 2 in which animal semiosis functions being its mechanisms coding and interpretation. And World 3, in which human semiosis operates with its mechanisms of coding, interpretation and language. According to this description, prions could be understood as biosociological adaptors between a part of the molecular world and another part of the neural world, and also between World 1 and World 3, since as it has previously discussed, prions have similar characteristics to codemakers. We are not advocating the existence of a universal amyloid code that can explain the relationship between these worlds. What we intend to indicate is that this prion code allows us to understand differential elements produced by TSEs. In fact, the sociological dimension of prions, seen as adaptors, derives from the social impact generated by the pathological effects of misfolded PrP, with devastating effects in the daily life of affected people and in their families (Coca et al., 2019).

The manifestations of the different TSEs are variable. Some have a very rapid development of neurodegeneration (3–6 months in humans), while others manifest more slowly. This, together with the usually late diagnostic, leads to a great uncertainty for the closest relatives. In addition, while from the social perspective, some affected people identify prion diseases (namely Creutzfeld-Jakob disease) with Alzheimer's disease (Coca et al., 2019), there are specific social disturbances associated with prions that do not affect other neurodegenerative disorders. Such as the fear of infection which adds a social stigma, and the rapidity of disease progression, which forces the close relatives to adapt continuously to changing circumstances and hinders the access to social care due to long bureaucratic processes. This gives us the idea that these diseases also have certain concomitants in the social sphere and operate in a similar way in the world through a neurodegenerative process.

It is also worth considering, when analysing prions through the scope of biosemiotics, that one of the main ideas included in the code theory developed by Barbieri (2003, 2011, 2015, 2019), is that there has been a neural code at the origin of the conscious mind. According to this theory, that intends to shed some light on the origin of mind along evolution, one of the phases in its development was that named *major transition*. As stated by Barbieri (2019), the origin of the neural code is a true biological revolution because is a major transition that transformed the unconscious brain of the ancestral animals into the feeling brain of the modern animals (including humans). The result was the origin of *subjectivity*, the origin of *first-person* experiences, in short, the origin of the conscious *mind* (Barbieri, 2019). Without going into further details of the neural coding theory, we would like to highlight how the effects of conformational alteration of PrP generally break this code.

Going back to the biosociological dimension of the prion code, Coca et al. (2019) showed in a social study that the rapid course of the neurodegeneration that leads to the death of those affected, forces a rapid acceptance of the consequences of the disease. However, there is a certain lack of biomedical knowledge which limits the possibilities of information for the families, affecting to an effective social and health care. Furthermore, the biological process of the disease caused by the misfolding of PrP leads to a break in the neural code, resulting in neurodegeneration which in turn, causes a social disturbance, not only to those affected by the disease, but also to their closest relatives.

Research on this disease, from the perspective of code biology, has great epistemological virtues since it allows us to dig deeper into the knowledge of the pathology from a biosocial perspective. In this sense, it is worth remembering that these diseases alter codes that go beyond the merely biological. For this reason, we believe that the approach proposed herein, that intends to analyze prions from a biosemiotics perspective, opens the door to increase our understanding of these devastating disorders from a fresh viewpoint.

6. Conclusion

In this article we have made an approach to the biology of the prion code. Thanks to it, we have seen that this code is not limited to prion diseases. In fact, it has been shown that there are similarities in different proteins (called amyloids) that allow us to suggest the existence of a common biological code between them. Obviously, this code cannot be the same as the genetic code, since the related proteins have "infective" capacity and alter other proteins by misfolding them.

Delving into this prion biological code is important to understand the alterations that these proteins generate in organisms such as humans. We know that prion diseases are neurodegenerative diseases, but a better understanding of the biological code could help us to better understand the biosocial effects of this type of disease.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

References

Baral, P.K., Yin, J., Aguzzi, A., James, M.N.G., 2019. Transition of the prion protein from a structured cellular form (PrP^{C}) to the infectious scrapie agent (PrP^{Sc}). Protein Sci. 28, 2055–2063. https://doi.org/10.1002/pro.3735.

Barbieri, M., 1985. The Semantic Theory of Evolution. Harwood Academic Publishers, London-New York.

Barbieri, M., 2003. The Organic Codes. An Introduction to Semantic Biology. Cambridge University Press, Cambridge.

Barbieri, M., 2008a. Life is semiosis. Cosmos History 4 (1–2), 29–52.

Barbieri, M., 2008b. Is the cell a semiotic system? In: Barbieri, M. (Ed.), Introduction to Biosemiotics. The New Biological Synthesis. Springer, Dordrecht, pp. 179–207.

Barbieri, M., 2008c. Biosemiotics: a new understanding of life. Naturwissenschaften 95, 577–599. https://doi.org/10.1007/s00114-008-0368-x.

Barbieri, M., 2011. Origin and evolution of the brain. Biosemiotics 4, 369–399. https://doi.org/10.1007/s12304-011-9125-1.

Barbieri, M., 2012. Code biology – a new science of life. Biosemiotics 5, 411–437. https://doi.org/10.1007/s12304-012-9147-3.

Barbieri, M., 2014. Introduction to code biology. Biosemiotics 7, 167–179. https://doi.org/10.1007/s12304-014-9212-1.

Barbieri, M., 2015. Code Biology. A New Science of Life. Springer, Dordrecht.

Barbieri, M., 2019. A general model on the origin of biological codes. Biosystems 181, 11–19. https://doi.org/10.1016/j.biosystems.2019.04.010.

Bateman, D.A., Wickner, R.B., 2013. The [PSI+] prion exists as a dynamic cloud of variants. PLoS Genet. 9 (1), e1003257 https://doi.org/10.1371/journal.pgen.1003257.

Chiti, F., Dobson, C.M., 2017. Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade. Annu. Rev. Biochem. 86, 27–68. https://doi.org/10.1146/annurev-biochem-061516-045115.

Coca, J.R., Sanz-Molina, L., Eraña, H., y Castilla, J., 2019. Análisis cualitativo del impacto social y familiar de las encefalopatías espongiformes transmisibles humanas. Rev. Neurol. *69*/6, 242–249. https://doi.org/10.33588/rn.6906.2019122.

Collinge, J., Clarke, A.R., 2007. A general model of prion strains and their pathogenicity. Science 318, 930–936.

Dawkins, R., 1976. The Selfish Gene. Oxford University Press, New York.

Dawkins, R., 1982a. Replicators and vehicles. In: Current Problems in Sociobiology, King's College Sociobiology Group. Cambridge University Press, Cambridge, pp. 45–64.

Dawkins, R., 1982b. The Extended Phenotype: the Gene as the Unit of Selection. Freeman, Oxford; San Francisco.

Eraña, H., 2018. The Prion 2018 round tables (II): aβ, tau, α-synuclein… are they prions, prion-like proteins, or what? Prion 13 (/1), 41–45. https://doi.org/10.1080/19336896.2019.1569451.

Faria, M., 2008. RNA as code makers: a biosemiotic view of RNAi and cell immunity. In: Barbieri, M. (Ed.), Introduction to Biosemiotics. The New Biological Synthesis. Springer, Dordrecht, pp. 347–364.

Farias, S.T., José, M.V., 2020. Transfer RNA: the molecular demiurge in the origin of biological systems. Prog. Biophys. Mol. Biol. 153, 28–34. https://doi.org/10.1016/j.pbiomolbio.2020.02.006.

Giraldo, R., Fernández, C., Moreno-del Álamo, M., Molina-García, L., Revilla-García, A., Sánchez-Martínez, M.C., Giménez-Abián, J.F., Moreno-Díaz de la Espina, S., 2016. RepA-WH1 prionoid: clues from bacteria on factors governing phase transitions in amyloidogenesis. Prion 10 (1), 41–49. https://doi.org/10.1080/19336896.2015.1129479.

Glanzman, D.L., 2013. Synaptic mechanisms of induction and maintenance of long-term sensitization memory in Aplysia. In: Menzel, R., Benjamin, P.R. (Eds.), Handbook of Behavioral Neuroscience. Invertebrate Learning and Memory, vol. 22, pp. 206–220. https://doi.org/10.1016/B978-0-12-415823-8.00017-4.

Greenwald, J., Riek, R., 2012. On the possible amyloid origin of protein folds. J. Mol. Biol. 421 (4–5) https://doi.org/10.1016/j.jmb.2012.04.015.

Hecht, M.H., Das, A., Go, A., Bradley, L.H., Wei, Y., 2004. De novo proteins from designed combinatorial libraries. Protein Sci.: Publ. Protein Soc. 13 (7), 1711–1723. https://doi.org/10.1110/ps.04690804.

Heinrich, S.U., Lindquist, S., 2011. Protein-only mechanism induces self-perpetuating changes in the activity of neuronal Aplysia cytoplasmic polyadenylation element binding protein (CPEB). Proc. Natl. Acad. Sci. Unit. States Am. 108 (7), 2999–3004. https://doi.org/10.1073/pnas.1019368108.

Hervás, R., del Carmen Fernández-Ramírez, M., Galera-Prat, A., Suzuki, M., Nagai, Y., Bruix, M., Menéndez, M., Laurents, D.V., Carrión-Vázquez, M., 2021. Divergent CPEB prion-like domains reveal different assembly mechanisms for a generic amyloid-like fold. BMC Biol. 19, 43. https://doi.org/10.1186/s12915-021-00967-9.

Hou, F., Sun, L., Zheng, H., Skaug, B., Jiang, Q.X., Chen, Z.J., 2011. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. Cell 146 (3), 448–461. https://doi.org/10.1016/j.cell.2011.06.041.

Hull, D.L., Landman, R.E., Glenn, S.S., 2001. A general account of selection: biology, immunology, and behavior. Behav. Brain Sci. 24, 511–573.

Jones, E., Mead, S., 2020. Genetic risk factors for Creutzfeldt-Jakob disease. Neurobiol. Dis. 142, 104973. https://doi.org/10.1016/j.nbd.2020.104973.

Katorcha, E., Gonzalez-Montalban, N., Makarava, N., Kovacs, G.G., Baskakov, I.V., 2018. Prion replication environment defines the fate of prion strain adaptation. PLoS Pathog. 14 (6), e1007093 https://doi.org/10.1371/journal.ppat.1007093.

Kraus, A., Hoyt, F., Schwartz, C.L., Hansen, B., Hughson, A.G., Artikis, E., Race, B., Caughey, B., 2021. Structure of an infectious mammalian prion. bioRxiv. https://doi.org/10.1101/2021.02.14.431014, 2021.02.14.431014.

Li, J., Browning, S., Mahal, S.P., Oelschlegel, A.M., Weissmann, C., 2010. Darwinian evolution of prions in cell culture. Science 327 (5967), 869–872. https://doi.org/10.1126/science.1183218.

Mabbott, N.A., 2017. How do PrPSc prions spread between host species, and within hosts? Pathogens 6, 60. https://doi.org/10.3390/pathogens6040060.

Majumdar, A., Colón, W., White-Grindley, E., Jiang, H., Ren, F., Khan, M., Li, L., Man-Lik, E., Kannan, K., Guo, F., Unruh, J., Slaughter, B., Si, K., 2012. Critical role of amyloid-like oligomers of Drosophila Orb2 in the persistence of memory. Cell 48 (3), 515–529. https://doi.org/10.1016/j.cell.2012.01.004.

Maury, C.P., 2009. Self-propagating beta-sheet polypeptide structures as prebiotic informational molecular entities: the amyloid world. Orig. Life Evol. Biosph. : J. Int. Soc. Study Origin Life 39 (2), 141–150. https://doi.org/10.1007/s11084-009-9165-6.

Maury, C., 2018. Amyloid and the origin of life: self-replicating catalytic amyloids as prebiotic informational and protometabolic entities. Cell. Mol. Life Sci.: CMLS 75 (9), 1499–1507. https://doi.org/10.1007/s00018-018-2797-9.

Oelschlegel, A.M., Geissen, M., Lenk, M., Riebe, R., Angermann, M., Schatzl, H., Groschup, M.H., 2015. A bovine cell line that can Be infected by natural sheep scrapie prions. PloS One 10 (1), e0117154. https://doi.org/10.1371/journal.pone.0117154.

Olzscha, H., Schermann, S.M., Woerner, A.C., Pinkert, S., Hecht, M.H., Tartaglia, G.G., Vendruscolo, M., Hayer-Hartl, M., Hartl, F.U., Vabulas, R.M., 2011. Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. Cell 144 (1), 67–78. https://doi.org/10.1016/j.cell.2010.11.050.

Otzen, D., Riek, R., 2019. Functional amyloids. Cold Spring Harbor Perspect. Biol. 11 (12), a033860 https://doi.org/10.1101/cshperspect.a033860.

Parchi, P., Strammiello, R., Giese, A., Kretzschmar, H., 2011. Phenotypic variability of sporadic human prion disease and its molecular basis: past, present, and future. Acta Neuropathol. 121, 91–112. https://doi.org/10.1007/s00401-010-0779-6.

Pascuzzo, Riccardo, Oxtoby, Neil P, Young, Alexandra L, Blevins, Janis, Castelli, Gianmarco, Garbarino, Sara, Cohen, Mark L, et al., 2021. Prion propagation estimated from brain diffusion MRI is subtype dependent in sporadic Creutzfeldt-Jakob disease. Acta neuropathologica 140 (2), 169–181. https://doi.org/10.1007/s00401-020-02168-0.

Piette, B., Heddle, J., 2020. A peptide-nucleic acid replicator origin for life. Trends Ecol. Evol. 35 (5), 397–406. https://doi.org/10.1016/j.tree.2020.01.001.

Priola, S., 2018. Cell biology of prion infection. Handb. Clin. Neurol. 153, 45–68. https://doi.org/10.1016/B978-0-444-63945-5.00003-9.

Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. Science 216, 136–144.

Riek, R., Saupe, S.J., 2016. The HET-S/s prion motif in the control of programmed cell death. Cold Spring Harbor Perspect. Biol. 8 (9), a023515 https://doi.org/10.1101/cshperspect.a023515.

Si, K., Choi, Y.B., White-Grindley, E., Majumdar, A., Kandel, E.R., 2010. Aplysia CPEB can form prion-like multimers in sensory neurons that contribute to long-term facilitation. Cell 140 (3), 421–435. https://doi.org/10.1016/j.cell.2010.01.008.

Stein, K.C., True, H.L., 2014. Prion strains and amyloid polymorphism influence phenotypic variation. PLoS Pathog. 10 (9), e1004328 https://doi.org/10.1371/journal.ppat.1004328.

Szathmáry, E., 2000. The evolution of replicators. Phil. Trans. Biol. Sci. 355, 1403. https://doi.org/10.1098/rstb.2000.0730.

Takada, L.T., Kim, M.O., Cleveland, R.W., Wong, K., Forner, S.A., Illán, I., Fong, J.C., Geschwind, M.D., 2017. Genetic prion disease: experience of a rapidly progressive dementia center in the United States and a review of the literature. Am. J. Med. Genet. Part B, Neuropsychiatric Genetics 174 (1), 36–69. https://doi.org/10.1002/ajmg.b.32505.

Vidal, E., Fernández-Borges, N., Eraña, H., Parra, B., Pintado, B., Sánchez-Martín, M.A., Charco, J.M., Ordóñez, M., Pérez-Castro, M.A., Pumarola, M., Mathiason, C.K., Mayoral, T., Castilla, J., 2020. Dogs are resistant to prion infection, due to the presence of aspartic or glutamic acid at position 163 of their prion protein. Faseb. J. 34 (3), 3969–3982. https://doi.org/10.1096/fj.201902646R.

Wächtershäuser, G., 2006. Origin of life: RNA world versus autocatalytic anabolism. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30741-9_11.

Weiss, M., Sousa, F., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., Martin, W.F., 2016. The physiology and habitat of the last universal common ancestor. Nat. Microbiol. 1, 16116 https://doi.org/10.1038/nmicrobiol.2016.116.

White-Grindley, E., Li, L., Mohammad Khan, R., Ren, F., Saraf, A., Florens, L., Si, K., 2014. Contribution of Orb2A stability in regulated amyloid-like oligomerization of Drosophila Orb2. PLoS Biol. 12 (2), e1001786 https://doi.org/10.1371/journal.pbio.1001786.

Wickner, R.B., 1994. [URE3] as an altered URE2 protein: evidence for a prion analog in Saccharomyces cerevisiae. Science (New York, N.Y.) 264 (5158), 566–569. https://doi.org/10.1126/science.7909170.

Wickner, R.B., 2016. Yeast and fungal prions. Cold Spring Harbor Perspect. Biol. 8 (9), a023531 https://doi.org/10.1101/cshperspect.a023531.

Wickner, R.B., Shewmaker, F.P., Bateman, D.A., Edskes, H.K., Gorkovskiy, A., Dayani, Y., Bezsonov, E.E., 2015. Yeast prions: structure, biology, and prion-handling systems. Microbiol. Mol. Biol. Rev.: MMBR (Microbiol. Mol. Biol. Rev.) 79 (1), 1–17. https://doi.org/10.1128/MMBR.00041-14.

Wills, P.R., 2001. Autocatalysis, information and coding. Biosystems 60, 49–57.

Yarus, Michael, 2011. Getting Past the RNA World: The Initial Darwinian Ancestor. Cold Spring Harbor Perspectives in Biology 3 (4), a003590. https://doi.org/10.1101/2Fcshperspect.a003590.