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REVIEW

Amyloids and prions in the light of evolution

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Abstract
Functional amyloids have been identified in a wide variety of organisms including bacteria, fungi, plants, and vertebrates. Intracellular and extracellular amyloid fibrils of different proteins perform storage, protective, structural, and regulatory functions. The structural organization of amyloid fibrils determines their unique physical and biochemical properties. The formation of these fibrillar structures can provide adaptive advantages that are picked up by natural selection. Despite the great interest in functional and pathological amyloids, questions about the conservatism of the amyloid properties of proteins and the regularities in the appearance of these fibrillar structures in evolution remain almost unexplored. Using bioinformatics approaches and summarizing the data published previously, we have shown that amyloid fibrils performing similar functions in different organisms have been arising repeatedly and independently in the course of evolution. On the other hand, we show that the amyloid properties of a number of bacterial and eukaryotic proteins are evolutionarily conserved. We also discuss the role of protein-based inheritance in the evolution of microorganisms. Considering that missense mutations and the emergence of prions cause the same consequences, we propose the concept that the formation of prions, similarly to mutations, generally causes a negative effect, although it can also lead to adaptations in rare cases. In general, our analysis revealed certain patterns in the emergence and spread of amyloid fibrillar structures in the course of evolution.

Keywords Prions · Amyloids · Bacteria · Yeast · Higher eukaryotes · Evolution

Introduction

Amyloids are protein fibrils with the cross-β structure that can be found in representatives of all domains of life, from bacteria to mammals. They are present inside cells, in the intercellular space, in outer shells, and even in some structures secreted into the environment. Functional amyloids are involved in the regulation of vital processes, while pathological amyloids are associated with the development of incurable socially significant diseases.

The structural organization and properties of amyloids need to be reviewed briefly before proceeding to the discussion of the functional significance and role of amyloids in the evolution. Amyloid fibrils are formed by intermolecular hydrogen bonds between carboxyl and amino groups of amino acid residues of protein molecules (Sunde et al. 1997). Amyloidogenic potential of amino acid sequences depends on the position of amino acid residues, their steric features, as well as the charge and size of the radical (Al-Garawi et al. 2017). Amyloidogenic sequences usually include several cross-β chains which contain from eight to several dozens amino acid residues. Apart from the presence of potentially amyloidogenic regions, the ability of a protein to form amyloid fibrils is determined by its production rate, modifications, and interactions with its functional partners. Under stress, an abnormal increase in the level of protein production or its modification can lead to the formation of pathological amyloid fibrils and cause a cytotoxic effect (Galkin and Sysoev 2021).

The three main types of amyloid fibril structure are: parallel in-register, antiparallel, and β-helix amyloid structure (Fig. 1) (Makin and Serpell 2005). In the case of a parallel in-register structure, the same sequences of protein bind to each other in the same orientation and are perpendicular to the main axis of the protofibril. In the case of antiparallel folding, amyloidogenic sequences are oriented opposite to

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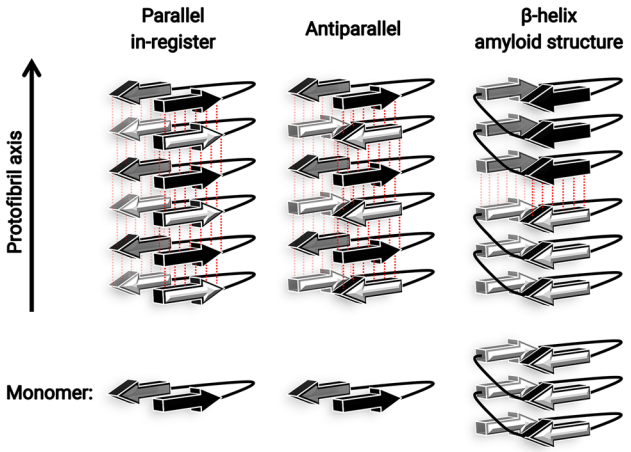

Fig. 1 Types of amyloid structure. Red dotted lines indicate intermolecular hydrogen bonds

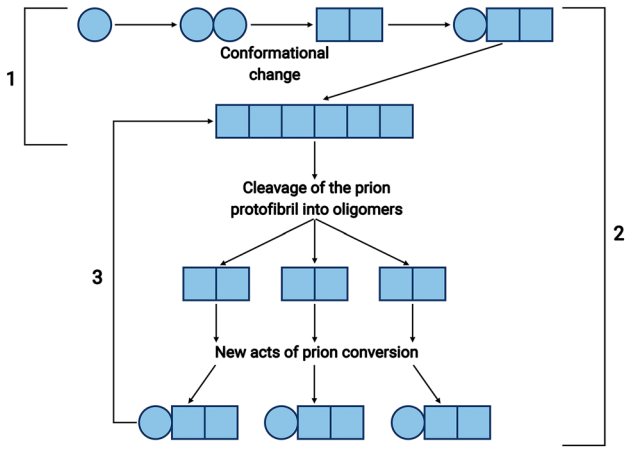

Fig. 2 Similarities and differences between amyloidogenesis and prionogenesis. 1 amyloidogenesis, 2 prionogenesis, 3 cycle of prion conversion

each other and perpendicular to the main axis of the protofibril. In "β-helix amyloid structure" structures, protein monomers are helical, and the upper turn of one monomer is connected by hydrogen bonds to the lower turn of another molecule. Several amyloid protofibrils usually bind laterally and coalesce into fibrils that can fold into large amyloid aggregates. Hydrogen bonds are weak, but a large number of such ordered bonds hidden inside the fibril can provide the latter with amazing mechanical strength and resistance to various agents. For example, pathological amyloid fibrils of mammalian Scrapie Prion Protein (PrP^{Sc}) are resistant to boiling, some autoclaving modes, and treatment with proteinase K (Prusiner et al. 1998; Sakudo et al. 2011). At the same time, the resistance of different amyloids to various factors varies greatly. In the context of the general properties of amyloid fibrils, their strength, compactness, and high resistance to treatment with ionic detergents are worth noting. Due to the peculiarities of their structural organization and their specific properties, amyloid fibrils are a unique material that is used in evolution to form vital structures in pro- and eukaryotes.

Prions, or proteinaceous infectious particles, are a special type of amyloids (Prusiner 1984; Wickner et al. 2008). The similarities and differences between prions and non-infectious amyloids are shown in Fig. 2. Monomers of amyloidogenic proteins can interact and form oligomers with the cross-β structure. These oligomers are a conformational matrix attaching new monomers and promoting protofibril growth. Unlike other amyloids, prion fibrils are cleaved into oligomers, which contributes to their reproduction. Chaperone Hsp104 and some other chaperones act as molecular scissors that cleave prion fibrils into oligomers in yeast cells (Chernoff et al. 1995; Kushnirov and Ter-Avanesyan 1998; Borchsenius et al. 2001). In addition, a number of other chaperones regulate the cleavage, growth, and stability of yeast prion fibrils (Chernova et al. 2017). In the mammalian brain, the cleavage of pathological prion fibrils is most likely carried out by αB-cristallin (Sun et al. 2008; Naeimi and Serio 2022). Prion oligomers attach protein monomers again promoting protofibril growth, and the prion conversion cycle closes (Fig. 2). Reproduction of this cycle ensures constant propagation of prion fibrils. In mammals, the amyloid particles of the Prion Protein spread throughout the brain, killing neurons and causing an incurable neurodegenerative disease (Prusiner 1984). In unicellular organisms, such as yeast *Saccharomyces cerevisiae*, prion particles are passed down across a series of cell generations and are inherited (Wickner et al. 2007a). Protein inactivation in the composition of prion fibrils leads to a change in the manifestation of the related trait and can be detected through phenotypic changes. This type of inheritance is not associated with any mutations in the DNA sequence.

The problem of the origin and spread of amyloid and prions in evolution remains almost unexplored. Meanwhile, as T. Dobzhansky noted, "Nothing in biology makes sense except in the light of evolution" (Dobzhansky 1973). In this review, we consider amyloids as a special form of protein fibrils that can provide adaptive advantages, become fixed in evolution, or cause cytotoxicity and death of an organism in some cases. We analyzed the conservatism of the amyloid properties of the most interesting functional amyloids and identified some examples of independent occurrence of amyloids with the same functions in different organisms. We also discuss the possible role of protein-based inheritance in the evolution of microorganisms.

Bacterial amyloids

So far functional amyloids have been identified in major bacterial phyla, such as Proteobacteria, Actinobacteria and Firmicutes (Larsen et al. 2007, 2008; Dueholm et al. 2012). Among the characterized bacterial amyloids, community behavior regulation by quorum-sensing or antimicrobial activity and biofilm formation are highly widespread (Van Gerven et al. 2018). In both cases, amyloid fibrils are excreted from the cell and function in the extracellular space.

A biofilm is a self-assembled, well-organized community of one or more bacterial species attached to a substrate. Bacteria form an extracellular matrix within biofilms which consists of exopolysaccharides, extracellular DNA, proteins, and lipids (Flemming and Wingender 2010). Biofilms, also known as microbial cities, are the dominant mode of microbial life in many natural, medical, and industrial environments as they protect the bacteria against both abiotic (UV radiation, desiccation, extreme conditions, such as high or low pH level or temperature, mechanical stress, etc.) and biotic (through increased resistance to antimicrobials, protozoan grazers, and host defense systems) stresses (Davies 2003; Hall-Stoodley and Stoodley 2009; Flemming and Wingender 2010; Flemming et al. 2016). It is not surprising that many bacterial species use amyloid fibrils, with their elasticity and high mechanical strength, as a structural scaffold for biofilms.

The first and currently best-described functional bacterial amyloid was discovered in *Salmonella typhimurium* and *Escherichia coli*. Grund and Weber described a new class of extracellular filaments in *S. typhimurium*, identifying them as thin, twisted fimbriae (Grund and Weber 1988). Later such coiled surface structures were called "curli" (Olsén et al. 1989). Extracellular assembly of curli filaments is regulated by seven genes encoded in two operons, *csgDEFG* and *csgBAC*. The filaments are formed by CsgA protein whose extracellular assembly into a fibril is nucleated by a cell-surface-associated CsgB (Chapman et al. 2002). Other proteins encoded by curli-specific genes are required for regulation of fibril biogenesis, pore formation in the outer membrane, secretion, and localization of CsgA monomers. CsgA amyloid fibrils enable attachment of bacterial cells to surfaces, including plant cells, stainless steel, glass, and plastics, and serve as important virulence factors in interaction with a wide range of host proteins (Akbey and Andreasen 2022).

CsgA orthologues were later identified in other members of Enterobacteriales: *Escherichia/Shigella*, *Salmonella*, *Citrobacter* and *Enterobacter* (Collinson et al. 1993; Römling et al. 1998; Zogaj et al. 2003). Further bioinformatic analysis has shown that the curli system is much more widespread phylogenetically than it had been initially assumed, and spans at least four phyla: Proteobacteria, Bacteroidetes, Firmicutes and Thermodesulfobacteria (Dueholm et al. 2012). Among bacterial classes, the composition and amounts of auxiliary regulatory proteins Csg may vary. However, the CsgA protein is an indispensable component of all discovered curli systems as the main structural element of them. *E. coli* CsgA monomers are composed of five incomplete repeats (minimalistic repeat region: $X_6QXGX_2NX_{10}$). Each of these repeats includes a strand–loop–strand motif (Blanco et al. 2012). CsgA orthologues have been identified in at least 50 representatives of various bacterial phyla (Dueholm et al. 2012). Among them, CsgA orthologues show a high degree of diversity in the size and organization of repeats that can vary from 4 to 22 repeat motifs.

Many of the bacteria containing CsgA orthologues are difficult to culture. Hence the analysis of amyloid properties of CsgA orthologues by staining them with amyloid-specific dyes in vivo or ex vivo becomes a challenging task. However, some bioinformatic algorithms make it possible to evaluate the amyloidogenicity of a protein in silico. We analyzed the presence of potentially amyloidogenic regions in 50 CsgA sequences in different bacterial species using the publicly available ArchCandy-1.0 algorithm. This tool analyzes the propensity of an amino acid sequence to form the strand-loop-strand motifs called β-arches by estimating the steady state energy of a hypothetical β-arch. The authors of the algorithm proposed a threshold of "amyloidogenicity" of 0.578 ± 0.019. According to the results they reported, this value allows distinguishing between amyloidogenic and non-amyloidogenic sequences and minimizes the number of false positive results as much as possible (Ahmed et al. 2015). We have shown that all 50 analyzed sequences contain potentially amyloidogenic regions with the threshold of "amyloidogenicity" value higher than 0.578 ± 0.019. Besides, some predicted amyloidogenic regions in the analyzed CsgA sequences correspond to the amino acid core $QXGX_2N$. In an earlier study of the *E. coli* CsgA using a comprehensive alanine scan mutagenesis screen, it was shown that conserved Gln and Asn residues in the $X_6QXGX_2NX_{10}$ repeats were critical for amyloid assembly (Wang and Chapman 2008). Thereby the presence of the $QXGX_2N$ amino acid core in the 50 analyzed sequences of CsgA orthologues indicates a high potential for amyloidogenicity of these proteins, although this requires experimental confirmation with an analysis of the fibril's nature.

The ecology of bacteria containing CsgA orthologues with potentially amyloidogenic regions according to the ArchCandy algorithm is quite diverse (Fig. 3). CsgA amyloidogenicity was predicted in an extreme halophile (*Salinibacter*), within several genera of soil bacteria (*Spirosoma*, *Flavobacterium*, *Agrobacterium*, *Rhizobium*), and marine

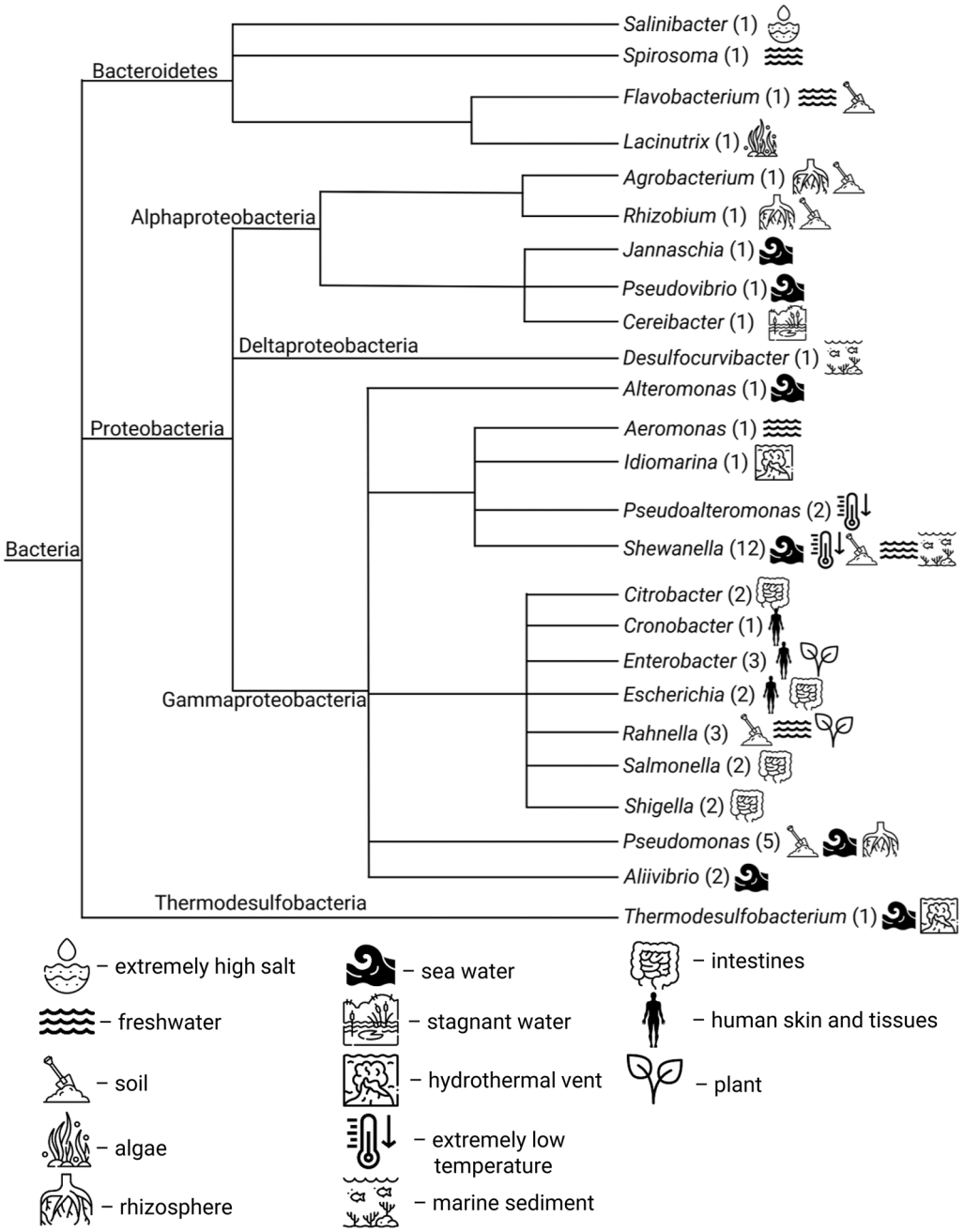

Fig. 3 Spread of potentially amyloidogenic CsgA properties across the phylogenetic tree of Bacteria. The number of species of a particular genus is indicated in brackets. Ecological niches of bacteria are deciphered in the legend

bacteria (*Lacinutrix*, *Jannaschia*, *Alteromonas*, and *Pseudoalteromonas*). Among the discovered representatives of the phyla, photosynthetic (*Rhodobacter*) and obligate anaerobic bacteria (*Desulfovibrio*) are also found. The diversity of habitats and related substrates (animal intestines, manure, sea soil, algal thallus) for bacteria producing the amyloidogenic CsgA protein suggests that amyloid fibrils may be a versatile biofilm scaffold. It is quite possible that the structural variability of CsgA orthologues provides additional survival benefits in extreme environmental and pathogenic conditions.

In addition to CsgA fibrils, *Pseudomonas* sp. contain amyloid fibrils of the FapC protein in the biofilm (Larsen et al. 2007; Dueholm et al. 2012). Being an opportunistic human pathogen, *Pseudomonas* sp. form strong biofilms that protect them from environmental stresses, prevent phagocytosis, and thereby enable them to colonize and exhibit long-term persistence (Moradali et al. 2017). In *Pseudomonas*

sp., the operon of 6 *fap* genes (*fapA-F*) is responsible for biosynthesis and excretion of biofilm amyloid fibrils (Dueholm et al. 2010). FapC forms the main component of the Fap amyloid fibrils; FapB functions as a nucleator protein and, together with FapE, can also be included in the fibril as smaller fractions (Dueholm et al. 2013; Herbst et al. 2015). FapD is a periplasmic cysteine protease required for secretion of FapC, and FapF forms a trimer of gated β-barrel channels in the outer membrane required for secretion of the fiber monomers (Rouse et al. 2016).

Bacillus subtilis biofilms can be present in three types: a pellicle biofilm formed at the air–liquid interface, a colony biofilm formed at the air–solid interface, and a submerged surface-attached biofilm (Branda et al. 2001; Hamon and Lazazzera 2001; Sanchez-Vizuete et al. 2015). TasA was shown to be an amyloid component of *B. subtilis* biofilms, which was confirmed by staining with amyloid-specific dyes both in vitro and in vivo (Romero et al. 2010). It is worth noting that TasA only aggregates under acidic conditions or on hydrophobic surfaces (Chai et al. 2013). TapA has the accessory function of binding TasA to the peptidoglycan in the cell wall, as well as nucleating the TasA assembly, which resembles the nucleator function of CsgB or FabB in curli and Fap fibrils, respectively (Chu et al. 2006; Romero et al. 2011). SipW is a membrane-bound protease that processes both TasA and TapA (Terra et al. 2012).

Despite their similar functions in biofilm formation, CsgA, FapC, and TasA proteins feature a low level of identity. In pairwise alignment of amino acid sequences using ClustalW, the percentage of identical amino acid residues was 8.0%, 11.1%, and 12.9% for CsgA-TasA, CsgA-FapC, and FapC-TasA pairs, respectively. Based on the generally accepted criterion for evaluating the homology of two sequences by their "percent identity" values (two sequences are homologous if they are more than 30% identical over their entire lengths) (Pearson 2013), and on the OrthoDB database (https://www.orthodb.org/), we can conclude that CsgA, FapC, and TasA proteins are not orthologues. Thus, there was an independent and multiple occurrence of amyloid fibrils of bacterial biofilm in the course of evolution which is a prime example of convergence. These data show that amyloid fibrils in bacterial biofilms could either arise independently or be inherited from ancestral forms.

The role of extracellular bacterial amyloids is not limited to biofilm scaffold formation. For adhesion, interaction with other pro- and eukaryotic cells, invasion into the cells of the host organism, and conjugation, bacteria form filamentous protein structures called pili, which differ in morphology, composition, and functions. Pili of *Mycobacterium tuberculosis* are known to be formed with MTP protein amyloid fibrils (Alteri et al. 2007).

An example of an amyloid with antimicrobial activity is Microcin E492 (MccE492), which is excreted by *Klebsiella pneumoniae* cells in a non-toxic monomeric form in the exponential phase of growth, and then, in the extracellular space, these monomers assemble into antibacterial oligomers. It is assumed that Mcc toxicity is due precisely to the oligomeric conformation of the protein capable of forming ion channels on the cell membrane of niche-occupying bacteria in a receptor-mediated manner, which leads to a rapid depolarization and eventual permeabilization of the cell membrane (Shahnawaz and Soto 2012). As the pH and salt concentration levels change, toxic oligomers assemble into non-toxic amyloid fibrils. The transition of oligomers to the fibrillar state is reversible and regulated by environmental conditions (Lagos et al. 2009; Shahnawaz and Soto 2012). MccE492 is not the only example of a pH-dependent amyloid toxin. So, listeriolysin O (LLO) of *Listeria monocytogenes* allows it to escape phagolysosome engulfment. It is secreted in a monomer form, and oligomerizes to form cytolytic pores in the phagolysosome upon contact with biological membranes at an acidic pH. Then, at the neutral pH found in the host cell cytosol, LLO spontaneously aggregates and inactivates in an amyloid form (Bavdek et al. 2012; Álvarez-Mena et al. 2020). The HpaG protein of *Xanthomonas axonopodis* is also capable of inducing cytotoxicity. In contrast to the examples discussed above, HpaG amyloid fibrils, rather than oligomers, demonstrate cytotoxicity. This protein belongs to harpins, a group of heat-stable, glycine-rich proteins produced by plant pathogenic bacteria that cause a hypersensitive response (HR). Although the specific mechanism of HR induction by HpaG is not known exactly, harpins, like Mcc and LLO, are believed to destabilize plant cell membranes by depolarizing them (Oh et al. 2007). Using the OrthoDB database (https://www.orthodb.org/), we have shown that MTP, Mcc, LLO, and HpaG proteins are not orthologues, and bear no resemblance to other bacterial amyloids.

The presented data allow us to conclude that amyloid extracellular fibrils arose many times and independently during the evolution of bacteria. At the same time, the amyloid properties of the CsgA protein involved in biofilm formation are highly conserved and likely provide adaptive benefits.

"Good" and "bad" fungal prions in terms of evolution

Yeast prions inheritance

A number of proteins with prion properties have been characterized in the unicellular fungus *S. cerevisiae*. Prions are proteins that convert between structurally distinct states, of which one or more is transmissible (Alberti et al. 2009). Yeast cells divide by budding, and prion particles present in the cytoplasm can be stably transmitted to daughter

cells. Yeast chaperones cleave prion fibrils into oligomers, which contributes to their efficient transfer to daughter cells and reproduction of the prion conversion cycle (Chernoff et al. 1995; Kushnirov and Ter-Avanesyan 1998; Borchsenius et al. 2001). The frequencies of spontaneous elimination of some prion variants are 10^{-7}, which corresponds to the frequencies of reversions of point mutations (Cox et al. 2003). Thus, the prion state of the protein in yeast is inherited. The discovery of prions in microorganisms provided a background for the concept of "protein-based inheritance". According to this concept, prion formation triggers the emergence of heritable DNA-independent traits in microorganisms. Prions of microorganisms are conformational templates that encode information at the protein level (Wickner et al. 1999). This second layer of information is superimposed over the information encoded in the gene and causes the heritable change of protein conformation and activity (Galkin 2017). The consequences of prion formation manifest similarly to mutations in the corresponding gene (Wickner et al. 2007b).

Yeast prions are inherited as dominant non-Mendelian factors (Wickner et al. 1999). When prion-containing and prionless haploids are crossed, the resulting diploid cell obtains prion particles. The same protein can form different prion variants or "prion strains" (Derkatch et al. 1996; Schlumpberger et al. 2001; Bradley et al. 2002). For example, prion strains of [*PSI*$^+$] (prion form of Sup35p) differ in the level of nonsense suppression they induce, in the size of the amyloid aggregates of the Sup35 protein, and in the stability of the inheritance of the prion phenotype (Derkatch et al. 1996; Kochneva-Pervukhova et al. 2001). These differences in prion variants are determined by amino acid residues in the amyloidogenic sequence which are involved in the formation of cross-β structures. Some prion variants are unstable and are eliminated during mitotic and meiotic divisions, while others are stably inherited through a series of cell generations. Since prion formation leads to heritable changes, prion strains can be considered as "prion alleles" (Tikhodeyev et al. 2017). When yeast cells carrying different strains of prion are crossed, only the one of them remains in the cells. This is an example of the dominant uptake of one prion variant by another one. In addition to the classical prion inheritance of traits, the phenomenon of "polyprion inheritance" mediated by the interaction of various prions present in the yeast cell was described (Nizhnikov et al. 2016; Galkin 2017). As it has been shown, two yeast proteins that normally do not interact can demonstrate genetic interaction in the prion form: one of them is the key determinant of the suppression of nonsense mutations, and the other one enhances this effect. By analogy with monogenic and polygenic inheritance, in the context of the prion concept, it is possible to distinguish "monoprionic" and "polyprionic" inheritance.

Yeast prions in evolution

Some time ago, a heated discussion broke out about whether the appearance of a prion in unicellular organisms results in adaptive advantages or whether the prion form of protein is a pathology. Susan Lindquist searched for prion forms of Sup35 and Rnq1 proteins (prion factors [*PSI*$^+$] and [*PIN*$^+$], respectively) in a large number of natural strains of S. cerevisiae. [*PSI*$^+$] was found in 1.4% of strains and [*PIN*$^+$] in 6.2% of the strains analyzed (Halfmann et al. 2012). The authors showed that some variants of [*PSI*$^+$] somehow improve adhesion of yeast cells to agar surfaces. Prion elimination led to the loss of adhesion. Some other prion variants did not manifest this property. In addition, [*MOT3*$^+$] (the prion form of the Mot3 protein) causes acid and fluconazole resistance in some strains (Halfmann et al. 2012). All these data show that prions can contribute to adaptive advantages under certain conditions. However, we cannot conclude that the presence of these prions is always beneficial for yeast cells. Natural yeast strains exist in changing environmental conditions, and inherited changes that are adaptive under some conditions may be detrimental under other conditions.

Theoretically, it can be assumed that the emergence and elimination of prions as a result of changes in environmental conditions can provide adaptive advantages. Environmental factors contributing to prion induction in a small proportion of cells have indeed been described. The [*MOT3*$^+$] prion is induced by ethanol and eliminated by hypoxia, the conditions that occur sequentially in the natural respiro-fermentative cycles of yeast populations (Holmes et al. 2013). However, ethanol-dependent induction occurs in a very small proportion of cells. It can also be noted that the very unstable [*LSB*$^+$] prion is induced at an extremely low frequency in response to heat shock (Ali et al. 2014). An effective factor for prion induction in nature has not been described yet.

In contrast to the works describing the adaptive properties of prions, Reed Wickner showed that some variants of the [*PSI*$^+$] prion kill yeast cells (McGlinchey et al. 2011). Sup35 functions as a translation termination factor, and its complete inactivation is lethal for yeast cells (Zhouravleva et al. 1995). The emergence of multiple variants of the [*PSI*$^+$] prion leads only to a partial inactivation of Sup35, which ensures the viability of yeast cells. However, as it turned out, some [*PSI*$^+$] variants are incompatible with yeast viability. It is worth noting that these data do not prove that yeast prions are always pathological.

Since prions can be stably inherited in unicellular organisms, they can be considered as an evolutionary factor. As mentioned above, the consequences of prion formation are similar to the consequences of a mutation in the corresponding gene. In both cases, inherited changes of protein conformation and functional activity occur (Chernoff 2001; Wickner et al. 2007b). We have to point out that most mutations

causing amino acid substitutions are neutral since they do not disrupt the functional activity of the protein, while prion conversion always leads to protein inactivation, and in some cases can contribute to the emergence of a new function. It is obvious that inactivation of the protein in the composition of prion fibrils should mainly cause a negative effect and be eliminated by natural selection. In those rare cases where protein inactivation does not significantly affect viability, prion formation is a neutral event. The emergence of a prion that provides benefits and is picked up by natural selection can be seen as an exceptional event. This is possible if the acquisition of a new function in the composition of prion aggregates is more important than the loss of the main function of the protein.

The [Het-s] prion of *Podospora anserina* can be considered a potentially beneficial prion. HET-s is a protein of the *P. anserina* fungus responsible for vegetative incompatibility regulation (Coustou et al. 1997). Two alleles encoding this protein have been described: *het-s* and *het-S*. The protein in *het-s* strains can exist in two forms: as a soluble, inactive [Het-s*] form or as an active prion [Het-s] form. In *het-S* strains, this protein always exists only in the soluble form called [HET-S]. The fusion of fungal hyphae containing prion [Het-s] and non-prion [HET-S] forms of this protein leads to programmed cell death. Although the [Het-s] prion is non-toxic, it includes the [HET-S] isoform in the composition of prion aggregates, which provokes disruption of the membrane structure and hyphae death (Seuring et al. 2012). Apparently, this is the way the presence of the prion form of the HET-s protein prevents the fusion of hyphae of strains with different alleles of this gene and transmission of infections and parasites from one strain to another (Bégueret et al. 1994; Pearson et al. 2009; Czárán et al. 2014). Thus, the [Het-s] prion may contribute to isolation and speciation of *P. anserina* strains. It is unclear whether this event is beneficial, yet it may lead to gradual isolation of strains with different alleles. Hence protein-based inheritance may play a role at least in microevolutionary events.

Functional amyloids of eukaryotes

A number of amyloid-like proteins have been characterized in the yeast cell wall (Kalebina et al. 2008; Otoo et al. 2008; Ramsook et al. 2010; Ryzhova et al. 2018; Sergeeva et al. 2019). Some of them perform protective functions, and some play a role in the regulation of cell adhesion. It was shown that the amyloid core β-aggregation sequence in Als5p, which plays a role in cell adhesion, is highly conserved (Otoo et al. 2008; Lipke et al. 2017). Functional amyloids have also been found in higher eukaryotes. They can be divided into several functional groups: regulatory, storage, protective, and structural. Despite active research in this area, the evolutionary patterns of the emergence and spread of functional amyloids in eukaryotes have not been studied experimentally. The only exception is the study of the evolutionary conservatism of the amyloid properties of the FXR1 protein in vertebrate cortical neurons (Velizhanina and Galkin 2022). This protein binds various mRNA molecules, including TNF-alpha mRNA, and prevents their translation (Garnon et al. 2005; Xu et al. 2011; George et al. 2021). We have shown that FXR1 functions in rat brain neurons and in human neuroblastoma cells in an amyloid form (Sopova et al. 2019). RNA molecules interacting with amyloid particles of this protein are resistant to treatment with RNase A. We have recently shown that FXR1 is present in an amyloid form in the brains of not only mammals but also of amphibians, reptiles, and birds (Velizhanina and Galkin 2022). The amyloidogenic region of this protein is highly conserved in mammals and jawed vertebrates. These data suggest that the formation of amyloid fibrils of FXR1 in neurons provides adaptive benefits.

The RNA-binding protein Orb2 of *Drosophila melanogaster* also functions in neurons in an amyloid form (Hervás et al. 2020). Orb2 belongs to the CPEB (cytoplasmic polyadenylation element-binding) family of conserved regulatory mRNA-binding proteins that play an important role in the formation and maintenance of long-term memory in various animal species, from *Aplysia californica* gastropod to mammals (Si et al. 2010; Krüttner et al. 2012; Fioriti et al. 2015; Hervás et al. 2021). Some fragments of the California sea hare, mouse, and human CPEB family proteins are capable of forming amyloid fibrils in vitro (Stephan et al. 2015; Hervás et al. 2021; Flores et al. 2022). However, clear evidence of amyloid properties in vivo has only been obtained for the Orb2 protein of the fruit fly. Hence, discussing the evolutionary conservatism of the amyloid properties of CPEB family proteins appears to be premature.

PMEL17 is another amyloid protein that regulates vital functions and is worth considering. PMEL17 is primarily expressed in melanocytes and is involved in the biogenesis of melanosomes, which are specialized organelles responsible for the synthesis and storage of melanin (Watt et al. 2013). In 2006, Fowler et al. demonstrated that the Mα fragment of *Bos taurus* PMEL17, presented in the retinal pigment epithelium and choroid layers of bovine eyes, forms amyloid fibrils (Fowler et al. 2006). These fibrils serve as a mechanical scaffold for the polymerization of melanin molecules in melanosomes, contributing to the normal adaptive response to ultraviolet radiation. Some initial studies showed that the RPT (RePeaT) region located in the C-terminus of the Mα fragment is responsible for the amyloidogenic properties of PMEL17 (Hoashi et al. 2006; McGlinchey et al. 2009; McGlinchey and Lee 2018; Dean and Lee 2019). RPT is a region of about 6–11 repeats that is conserved across vertebrate species (Dean and Lee 2020). However, later

studies suggest that another region called CAF (core amyloid fragment) is an amyloidogenic component of the Mα fragment of PMEL17 (Hee et al. 2017). For instance, CAF fragments have been shown to form amyloid fibrils, and these fibrils are reported to be capable of seeding the formation of fibrils from other Mα fragment subdomains (Hee et al. 2017). Through application of various bioinformatics algorithms, potentially amyloidogenic consensus sequences were found in the CAF region in different species, from fish to humans (Hee et al. 2017). Notably, this consensus included aromatic amino acids that were demonstrated to be essential for in vitro aggregation. These data suggest that the amyloid properties of PMEL17 may be conserved in the evolution of vertebrates.

A number of proteins form protective amyloid fibrils. They include proteins of the chorion of insect eggs, of the vitelline envelope of fish eggs, and of mammalian oocyte *zona pellucida* (Iconomidou et al. 2011; Podrabsky et al. 2001; Egge et al. 2015). For example, silkworm chorion proteins of A and B families are known to have an extremely conserved central region containing glycine-rich hexapeptide tandem repeats, and presumably be prone to amyloid aggregation (Iconomidou et al. 2011). These proteins form a dense network around the oocyte and the developing embryo to protect them from various external influences. Recently amyloid fibrils of an unidentified protein were shown to be part of specific structures of the shell of *Drosophila melanogaster* eggs, such as the micropyle, dorsal appendages, and pillars (Siniukova et al. 2020). Using the OrthoDB database (https://www.orthodb.org/), we showed that silkworm and fruit fly chorionic proteins are not orthologues. Hence it can be concluded that amyloid fibrils in the egg shell of silkworms and fruit flies arose independently of each other during the evolution of insects.

The presence of amyloids in fish egg and mammalian oocyte shells is also noteworthy. Some unidentified amyloids were shown to be part of the egg shells of the teleost fish *Austrofundulus limnaeus* (Podrabsky et al. 2001). Under dehydration, the number of β-layers in the egg shells of these fish increases, which leads to vitrification of the perivitelline space allowing the eggs to survive for a long time despite extreme conditions (Podrabsky et al. 2001). Specific proteins functioning in the egg shell of teleost fish in the amyloid form have not been identified; however, it is known that the main components of shells of fish eggs are homologous to ZP (*zona pellucida*) proteins of mammalian oocytes (Litscher and Wassarman 2007). It is also known that mouse *zona pellucida* contains structures that bind in vivo amyloid-specific antibodies OC and A11, as well as amyloid-specific dyes (Egge et al. 2015; Pimentel et al. 2019). All three mouse ZP proteins (ZP1, ZP2, and ZP3) are assumed to be involved in the formation of a dense network of fibrils that creates a barrier against various external factors, including proteases and hydrolases (Mondéjar et al. 2013). In humans, the ZP family includes four proteins: ZP1, ZP2, ZP3, and ZP4, whose amyloid-like properties have been demonstrated in vitro (Louros et al. 2013, 2016). Interestingly, each stage of embryonic development correlates with a certain intensity of amyloid-specific immunostaining in mouse and human oocytes (Pimentel et al. 2019). In addition, the AMYLPRED2 approach has demonstrated that the mouse ZP3 protein and its orthologues in other vertebrates contain potentially amyloidogenic sequences (Egge et al. 2015). Based on these results, it can be speculated that the ZP family proteins in fish, mice, humans, and probably other vertebrates, form functional amyloid fibrils.

A number of hormones of the mouse endocrine and neuroendocrine systems are stored in secretory granules in the amyloid form (Maji et al. 2009). Colocalization between Thioflavin S and amyloid-specific conformational-dependent antibodies with ACTH, β-endorphin, prolactin, growth hormone, oxytocin, and vasopressin hormones was demonstrated in the mouse pituitary gland. When secreted, these hormonal peptides and proteins lose their cross-β conformation and function in a monomeric form. It has also been shown that a number of hormonal peptides from various mammalian species (human, mouse, rat, and ovine) can form amyloid fibrils in vitro (Maji et al. 2009). It is quite possible that the storage of hormonal peptides in an amyloid form is adaptive, and that this feature was fixed in the course of the evolution of mammals. As an interesting parallel, it can be noted that the amyloid properties of vicilin, a major storage protein in the seeds of the pea *Pisum sativum*, were demonstrated in vitro and in vivo (Antonets et al. 2020). It is assumed that the compact amyloid packing of vicilin is responsible for extended storage of seeds of leguminous plants and their resistance to adverse factors. During the germination of pea seeds, vicilin amyloid aggregates rapidly cleave into monomers under the effect of an uncharacterized factor. Vicilin contains two functional amyloidogenic domains, Cupin-1.1 and Cupin-1.2, which form β-barrels. The amyloidogenicity of seed storage proteins containing ancient β-barrels was bioinformatically predicted for most land plant species, which may indicate a high degree of conservatism of this function (Antonets and Nizhnikov 2017).

Based on the results of bioinformatics analysis, it can be assumed that the amyloid properties of a number of eukaryotic proteins are evolutionarily conservative. At the same time, it should be taken into account that bioinformatics data are predictive in nature, and all modern algorithms perform reliably only with short peptides yet not with full-length proteins. For example, the AMYLPRED2 approach predicts the presence of potentially amyloidogenic sequences in many proteins, even in those that do not form amyloid fibrils in vitro or in vivo. However, as has been shown the amyloid

properties of the RNA-binding protein FXR1 in brain neurons are conservative in the evolution of vertebrates, and some eukaryotic amyloids that perform similar functions arose independently of each other.

Vertebrate cytotoxic amyloids

The emergence of pathological amyloids in terms of evolution is hardly worth being treated as a paradox. Firstly, it should be noted that the formation of pathological amyloids associated with neurodegenerative or other incurable diseases is observed in elderly humans or animals in most cases. For example, the rate of sporadic forms of Alzheimer's disease, Parkinson's disease, and cardiac amyloidosis in young people is insignificant, yet it grows exponentially with age (Reeve et al. 2014; Brunjes et al. 2016; Xia et al. 2018). Various defense systems developed during evolution perform efficiently in young individuals but may be switched off at the end of reproductive age. In the elderly, an abnormal accumulation or modification of amyloidogenic proteins leading to the formation of cytotoxic oligomers and aggregates can be observed. However, cytotoxic amyloid aggregates are not always pathological. A striking example confirming this thesis is the accumulation of Aβ peptide in the brain of the Pacific salmon (Maldonado et al. 2000). The Pacific salmon return from the sea into freshwater to spawn. Hormonal changes in males and females provoke morphological changes, and tissue degeneration at the final stage of life. After spawning, the adult fish die and become a nutrition base for the next generation. In the degenerating brain of these fish, plaques of Aβ peptide were detected during and after spawning (Maldonado et al. 2000). Obviously, the accumulation of cytotoxic amyloid aggregates and brain degeneration are a programmed final stage of the life cycle of adult fish rather than a pathology in this case. This program of life cycle regulation developed in the process of evolution to promote the survival of this species. The RIP1 and RIP3 proteins can be considered another example of programmed formation of cytotoxic amyloid aggregates. RIP1 and RIP3 kinases play a key role in TNF-induced programmed necrosis (Vandenabeele et al. 2010). At least in cell culture, RIP1/RIP3 form a functional amyloid signaling complex required for programmed necrosis (Li et al. 2012). RIP1/RIP3 fibril formation is a crucial step for necrosome assembly. It should be noted that apart from pathological cell death cases, programmed necrosis occurs in normal development and during adult tissue homeostasis (Vandenabeele et al. 2010). This is another example of how the assembly of amyloid cytotoxic fibrils can be involved in the regulation of vital processes.

Sporadic pathological amyloidoses are not limited to aging and can occur against the background of chronic diseases caused by infections, trauma, cancer, and other types of stress (Galkin and Sysoev 2021). Such pathologies either increase the rate of amyloidogenic protein production, or cause their modification, or disrupt their interaction with functional partners. For example, chronic infections cause a thousandfold increase in the production of the serum amyloid A protein (Eriksen et al. 1993). Multiple myeloma causes malignant multiplication of B cells, which naturally leads to overproduction and amyloidogenesis of immunoglobulin light and heavy chains (Picken 2007). The relationship to stress exposure has been shown for almost all sporadic amyloidoses (Galkin and Sysoev 2021). Hence we can conclude that sporadic amyloidoses are part of pathological cascades that the defense systems developed in the course of evolution are unable to deal with.

From an evolutionary point of view, it is also worth considering mammalian prion diseases associated with the penetration into the body of foreign amyloid particles of PrP^{Sc}. Many mammalian species are susceptible to prion infections, and this susceptibility can be a serious threat to some farmed and wild animal species. Amyloid infectious particles of PrP^{Sc} are not destroyed by the enzymes of the gastrointestinal tract and penetrate the follicular dendritic cells of the lymphatic system (Heikenwalder et al. 2007). Moreover, foreign PrP^{Sc} fibrils cause conversion of the host PrP protein which is further transported to the brain and causes irreversible neurodegeneration. These data strongly evidenced that the mechanism of this pathological infectious disease is evolutionarily conservative. The reason for the evolutionary conservatism of prion infections is still unknown, and we can only assume that it represents a retribution for certain functional features of this protein. The normal PrP^{C} (cellular) isoform anchors on the neuronal membrane by a glycosylphosphatidylinositol (GPI) moiety and functions as a receptor (Laurén et al. 2009). This receptor is involved in the regulation of cellular cohesion and ion transport and binds many different proteins (Biasini et al. 2012; Miranzadeh Mahabadi and Taghibiglou 2020). Most interestingly, the PrP^{C} receptor on the membrane of neurons binds amyloid fibrils of any protein: oligomers of Aβ peptide, PrP^{Sc}, the yeast prion Sup35, or artificially obtained amyloids (Laurén et al. 2009; Resenberger et al. 2011). This interaction causes pathological signaling and kills neurons. PrP^{C} ability to bind amyloids may be a side property of this receptor that normally interacts with one of its functional partners to form an intermolecular β-structure. This is one of the possible hypotheses explaining the evolutionary conservatism of PrP amyloid properties.

Thus, pathological mammalian amyloids are out of evolutionary control, whereas some cytotoxic amyloids are not

pathological and are responsible for programmed cell death control.

Conclusion

In this review, we consider amyloids as one of the possible forms of protein folding. In the course of evolution, many organisms began to use amyloid fibrils to protect cells or regulate vital processes. Surprisingly, despite the great interest in the study of amyloids, the conservatism of the amyloid properties of proteins and the regularities in the appearance of these fibrillar structures in evolution remain almost unexplored.

Our analysis showed that amyloid fibrils performing the same functions repeatedly and independently arose in the course of evolution in various organisms. Firstly, this is related to proteins in the composition of biofilm in phylogenetically distant bacterial species. The amyloid proteins that form the amyloid fibrils of the biofilm in different bacteria species are not orthologues. Independent emergence of amyloid fibrils forming the amyloid scaffold of biofilms in different bacterial species can be considered as a striking example of convergent evolution. Obviously, amyloid fibrils are an ideal material for the formation of such structures, and their appearance can be considered the pattern of evolution.

On the other hand, the amyloid properties of a number of bacterial and eukaryotic proteins are evolutionarily conserved. Experimental evidence of the evolutionary conservatism of amyloid properties has so far been obtained only for the FXR1 protein. This RNA-binding protein functions in amyloid form in the cerebral neurons of amphibians, reptiles, birds, and mammals (Velizhanina and Galkin 2022). Bioinformatics analysis has shown that the CsgA protein forming the biofilm matrix and containing amyloidogenic repeats is found in numerous phylogenetically distant bacterial species in addition to *E. coli* (Fig. 3). Conserved amyloidogenic sequences can also be found in the PMEL17 and ZP proteins in representatives of all major classes of vertebrates. All these examples allow us to conclude that the emergence of amyloid fibrils can provide adaptive advantages in a number of cases and is picked up by natural selection. Using the example of bacterial amyloids, we presented a scheme reflecting both the parallelism of their origin and the evolutionary conservatism of amyloid properties (Fig. 4).

The discovery of functional amyloids has proved that the presence of cross-β structures per se is not a factor of cytotoxicity. Obviously, the cytotoxicity of pathological amyloids is associated with their ability to interact with certain receptors or other proteins that provokes cell death. The occurrence of pathological amyloids is a consequence of chronic stress (Galkin and Sysoev 2021), and it quite frequently occurs in individuals of advanced age. In this context, the appearance of pathological amyloids associated with the development of various diseases is not controlled by natural selection.

In this review, we also considered the possible role of prions in the evolution of unicellular organisms. A change in protein conformation as a result of prion conversion causes an inherited change in the trait without alteration the DNA sequence. The protein in the prion fibril is inactivated or acquires new functions. Thus, such heritable changes should

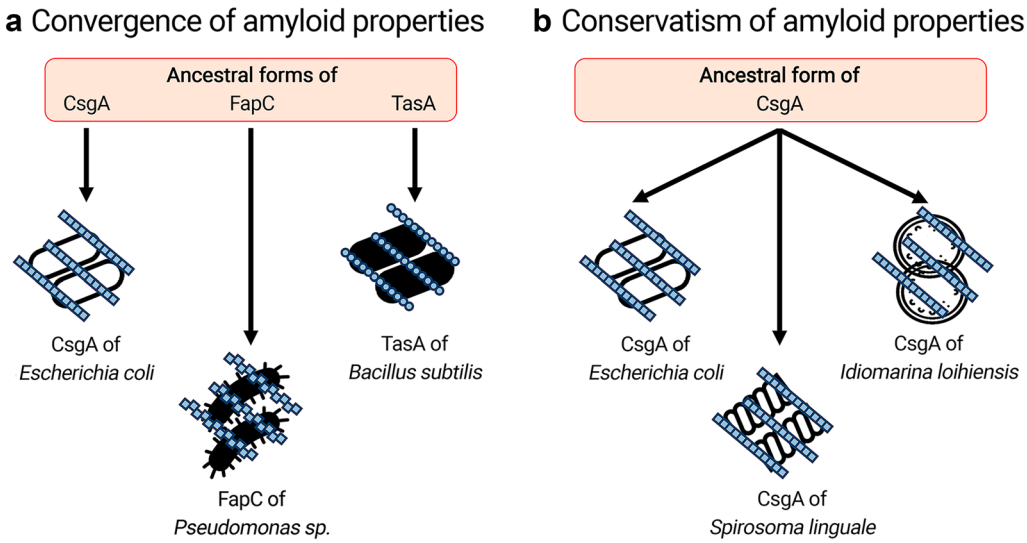

Fig. 4 Patterns of emergence and spread of amyloids in the evolution of Bacteria. **a** The independent emergence of various amyloid proteins that are a part of bacterial biofilms. **b** Conservatism of amyloid properties of CsgA proteins within Bacteria. Amyloid fibrils of various amyloid proteins are marked in blue. Cells of bacteria of different species are indicated as white or black figures of various shapes

in most cases be eliminated by natural selection, but in rare cases they may provide adaptive advantages.

Generally, our analysis shows that the emergence of prions and amyloids should not be treated as a paradox but rather as a natural event due to protein ability to form fibrillar cross-β structures. In a number of cases, the emergence of amyloid fibrils with their unique structural properties provides adaptive advantages and is picked up by natural selection.

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Declarations

Conflict of interest The authors declare no conflict of interests.

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