Disentangling the Amyloid Pathways: A Mechanistic Approach to Etiology

Maja Malmberg[1,2], Tarja Malm[3], Oskar Gustafsson[4], Anthony Wright[4], Samir El Andaloussi[4,5,6], Anders Linden[7,8] and Kariem Ezzat[4*]

[1]Section of Virology, Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Box 7028, 750 07 Uppsala, Sweden

[2]SLU Global Bioinformatics Centre, Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7023, 750 07 Uppsala, Sweden

[3]A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland.

[4]Department of Laboratory Medicine, Clinical Research Center, Karolinska Institutet, Stockholm, Sweden.

[5]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.

[6]Evox Therapeutics Limited, Oxford Science Park, Oxford, UK.

[7]Unit for Lung and Airway Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

[8]Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden.

*Correspondence should be addressed to: kariem.ezzat.ahmed@ki.se

Abstract

Amyloids are fibrillar protein aggregates that are associated with diseases such as Alzheimer's disease, Parkinson's disease, type II diabetes and Creutzfeldt–Jakob disease. The process of amyloid aggregation involves three pathological protein transformations; from natively-folded conformation to the cross-β conformation, from biophysically soluble to insoluble, and from biologically functional to non-functional. While amyloids share a similar cross-β conformation, the biophysical transformation can either take place spontaneously via a homogeneous nucleation mechanism (HON) or catalytically on an exogenous surface via a heterogeneous nucleation mechanism (HEN). Here, we postulate that the different nucleation pathways can serve as a mechanistic basis for an etiological classification of amyloidopathies, where hereditary forms generally follow the HON pathway, while sporadic forms follow surface-induced (including microbially-induced) HEN pathways. Furthermore, the conformational and biophysical amyloid transformation results in loss-of-function (LOF) of the original natively-folded and soluble protein. This LOF can, at least initially, be the mechanism of amyloid toxicity even before amyloid accumulation reaches toxic levels. By highlighting the important role of non-protein species in amyloid formation and LOF mechanisms of toxicity, we propose a generalized mechanistic framework that could help better understand the diverse etiology of amyloid diseases and offer new opportunities for therapeutic interventions including replacement therapies.

Introduction

The term amyloid refers to a particular conformational state of proteins where they transform from being soluble and natively-folded into insoluble aggregates of fibrillar nature. More than 35 peptides and proteins are known to form amyloids in different human diseases [1]. Nearly all the proteins that form amyloids have biological functions in their normal, natively-folded state. Some proteins such as antibodies, lipoproteins and serum amyloid A (SAA) lead to systemic amyloidosis including light-chain amyloidosis, Apo-AI amyloidosis and AA amyloidosis, respectively [1]. On the other hand, some proteins accumulate in specific organs leading to localized amyloid pathology. These amyloidopathies include thyroid medullary carcinoma, pulmonary alveolar proteinosis and atrial amyloidosis resulting from the amyloid accumulation of calcitonin, surfactant protein C and atrial natriuretic factor, respectively [1]. Localized amyloidopathies also include type II diabetes and neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD). Type II diabetes is characterized by the amyloid accumulation of the peptide hormone islet amyloid polypeptide (IAPP), while AD and PD are characterized by the accumulation of the amyloid beta (Aβ) and alpha syncuclein (α-syn) peptides, respectively [2]. Moreover, AD and other neurodegenerative disease such as frontal temporal dementia with Parkinsonism and Pick's disease involve amyloid aggregates of the microtubule-associated protein tau [3]. In addition, the amyloid aggregation of the infamous tumor suppressor transcription factor p53 is involved in many cancers [4]. While some amyloids were shown to have beneficial biological function, for example acting as storage for peptide hormones in secretory granules [5], the vast majority of amyloids are pathological. This explains the existence of several biological protective mechanisms that ensure that proteins are correctly folded such as the presence of chaperones, or degraded when incorrectly folded via processes such as autophagy, ubiquitin–proteasome mediated degradation and the unfolded protein response [6]. Moreover, specific sequence patterns that tend to easily form amyloids, such as alternating hydrophilic-hydrophobic stretches, appear to have been selected against during evolution [7].

Etiology of amyloidopathies

A small proportion of amyloidopathies is of genetic hereditary origin; however, the majority of amyloid diseases are sporadic [1]. Hereditary forms of amyloidopathies are caused by mutations in the genes encoding the amyloidogenic proteins. Such mutations usually facilitate the aggregation of the amyloidogenic proteins leading to early onset of the disease [8,9]. For sporadic forms, a small subset of amyloidopathies, termed transmissible spongiform encephalopathies, are caused by infectious protein particles called prions [10]. Prions transfer from one organism to another inducing neurodegeneration in the recipient host in diseases such as Creutzfeldt–Jakob disease and Kuru. For the vast majority of other

sporadic forms, the causes are not clear. However, several environmental factors are known to increase disease risk, including infections [11], lipid dysregulation [12], pollution [13] and traumatic brain injury [14].

Amyloid structure

The term amyloid describes a unique class of protein conformation, where proteins adopt an elongated fibrillar morphology. While this is a characteristic feature of pathological protein aggregates, it has been demonstrated that even normal nonpathogenic proteins can be forced to adopt the amyloid conformation under certain denaturing conditions [15]. This led to the "generic hypothesis" suggesting that amyloid formation originates from the fundamental properties of proteins, based on the ability of backbone groups to form hydrogen bonds and the ability of side-chain groups to interact via hydrophobic and van der Waals interactions [16]. To obtain their characteristic morphology, amyloids share a similar core cross-β conformation [17]. In this conformation, the protein molecules are arranged in the form of two oppositely stacked β-sheets, excluding the water molecules in-between and interdigitating their side chains forming a dry steric zipper [2]. Such an elongated cross-β spine constitutes the basic amyloid fibrillar subunit, the protofilament (Fig. 1). Apart from the fixed cross-β conformation, protein stacking in the spine can come in a variety of forms. For example, the cross-β architecture can consist of one folded molecule or two separate molecules and the β-sheets can stack in parallel, anti-parallel, face-to-face or face-to-back orientations [17]. Moreover, the protofilament length varies depending on the number strands forming each sheet. Once protofilaments are formed, they can associate in a variety of ways leading to different superstructural polymorphs. These polymorphs include flat fibrillar structures with varied number of horizontally-stacked protofilaments, which can evolve to amyloid crystals, or different twisted ribbon structures (of single or multiple intertwined protofilaments), which can further evolve into nanotubes [18]. Such superstructural polymorphism depends on many factors including the protein side chain arrangements, the nucleation mechanism (see below) and environmental conditions such as pH and ion concentration [19,20]. In addition, this biochemical structural transformation is accompanied by a biophysical phase transformation that is dominated by nucleation-growth kinetics as described below.
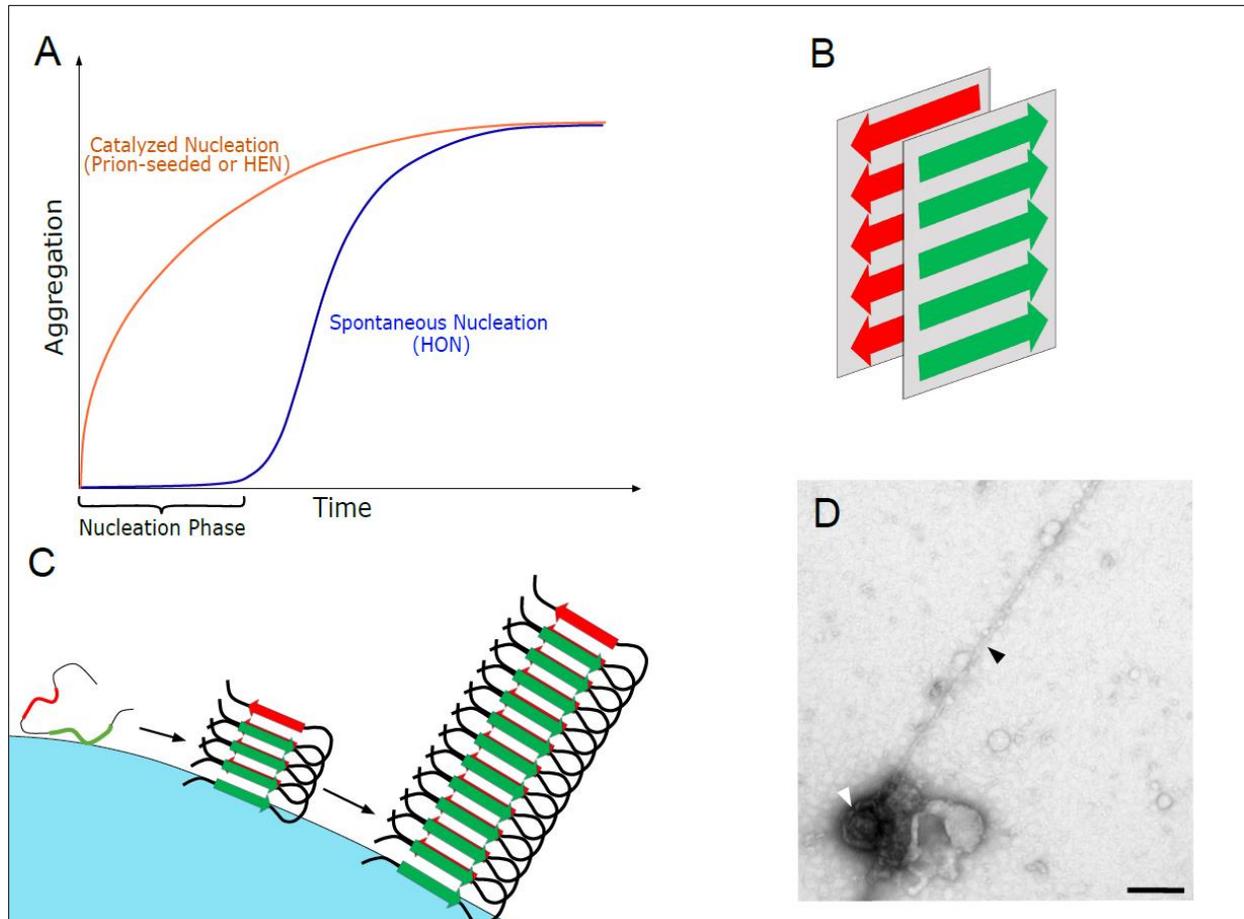
Figure 1. (A) A schematic representation of the kinetics of amyloid aggregation with the rate-limiting nucleation step that can be bypassed either by adding a preformed seed (prion) or by surface catalysis via HEN. **(B)** A schematic representation of the cross-β conformation, which is the core conformation of amyloids where two β-sheets are stacked opposite to each other forming the protofilament with the characteristic elongated amyloid morphology. While the cross-β conformation remains constant, variable β-sheet orientations or protofilament associations lead to different amyloid polymorphs. **(C)** A schematic representation of heterogeneous nucleation (HEN) where an exogenous surface catalyze amyloid nucleation via binding, concentrating and inducing conformational changes in the bound peptides/proteins, which facilitate amyloid transformation. **(D)** A negatively stained transmission electron microscopy picture demonstrating direct interaction between the surface of a virus (HSV-1) and a growing amyloid protofilament of Aβ 1-42 peptide (unpublished image from the viral catalyzed nucleation experiments performed in Ezzat et al. 2019, where HSV-1 was incubated with 50 µM Aβ 1-42 for 100 min. at 37 ˚C). In the same publication, we demonstrated that HSV-1 accelerated amyloid aggregation *in-vitro* and *in-vivo*. The viral particle is indicated by a white arrow and the protofilament with a black arrow, bar = 200nm.

Amyloid nucleation mechanisms

Phase transformation is the process involving transitioning from one state of matter to another, such as liquid to solid or gas to liquid or gas to solid transformations. These transformations are very common in nature, including phenomena such as crystallization and amyloid aggregation (liquid to solid transformation), rain precipitation (gas to liquid transformation) and planet formation (gas to solid transformation) [21,22]. That is why both the thermodynamics and kinetics of phase transformation have been widely studied. Thermodynamically, the process involves the transition from a less stable (higher free energy) to a more stable (lower free energy) phase under specified conditions [18]. Kinetically, the mechanism of phase transformation involves two steps that are well described by the classical nucleation theory [21]. Initially, a rate-limiting nucleation step takes place, where an energy barrier needs to be overcome to create the initial molecular assembly (nucleus) of the new phase. Once the nucleus is formed, this is followed by a growth step where the system rapidly transforms into the new phase. The nucleation-growth mechanism accurately describes the kinetics of amyloid formation as studied experimentally, with the distinctive sigmoidal kinetics curve involving a nucleation lag phase followed by a rapid growth or elongation phase [6] (Figure 1).

While nucleus formation is necessary, it is both thermodynamically unfavorable and rare, as it depends on the unlikely event of the spontaneous formation of a stable nucleus of the new phase within the bulk of the transforming phase [2,23]. In the case of amyloid aggregation, this involves the spontaneous conformational change of the protein followed by spontaneous association of protein molecules into cross-β sheet rich nucleus. This pathway to nucleation is called homogenous nucleation (HON) [24], and with all the protective mechanisms that are in place to maintain proper protein folding (see above), it is not surprising that proteins do not normally reach the amyloid state. In contrast to the slow and rare HON pathway, catalyzed nucleation pathways exist that are faster and more common. One catalytic pathway is called seeding, where adding a preformed nucleus (seed) enables the system to completely bypass the nucleation step and move directly to the growth or elongation step [2]. The other important catalytic pathway is the heterogeneous nucleation mechanism (HEN), where an exogenous surface catalyzes the nucleation process [24,25]. In HEN, the surface lowers the energy barrier to nucleation and acts a scaffold that facilitates nucleus formation via binding, concentrating and enabling conformational changes in the bound proteins [26,27] (Fig. 1). Like seeding, HEN usually eradicates the lag phase completely from the kinetics. In this regard, many biological and non-biological surfaces have been shown to be capable of HEN, including microbial surfaces [28,29], lipid vesicles [30] and nanoparticles [31]. Many polymer surfaces such as glycosaminoglycans (GAGs) [32] and nucleic acids [33] have also been shown to accelerate amyloid aggregation. In addition, the growing fibril surface itself can serve as a site for HEN, in a phenomenon

termed secondary nucleation [34]. However, the exact properties of a particular surface that mediate HEN remain poorly understood. Furthermore, the concentration of proteins in intracellular droplets that form via a liquid-liquid phase separation (LLPS) process can sometimes lead to amyloid formation [35]. Interestingly, low-complexity RNA sequences (CU/AG repeats) have been shown to induce amyloid aggregation within phase-separated liquid droplets, which can have implications for understanding amyloid aggregation in tri/hexa-nucleotide repeat expansion disorders such as Huntington's disease, spinocerebellar ataxias and amyotrophic lateral sclerosis (ALS) [36]. Very recently, Yuan C. et al have demonstrated that the interfaces created by LLPS can act as sites of HEN for amyloids [37].

A mechanistic approach to etiology

Amyloid aggregation is a process of pathological protein transformation at three levels, a biochemical conformational transformation, a biophysical phase transformation and a biological functional transformation. At the biochemical structural level, amyloids share a similar cross-β conformation across different pathologies and different polymorphs. At the biophysical level however, there are distinct nucleation-dependent pathways to amyloid formation that are well-defined in thermodynamic and kinetic terms. The nucleation barrier is what separates the soluble and insoluble states of a protein; and thus, the pathways to nucleation are the decisive mechanisms in the biophysical transformation process. The nucleation barrier dictates whether a protein would spontaneously form an amyloid via HON or whether it requires a catalytic event, which can be a preformed seed or an exogenous surface (HEN). In addition, together with other environmental factors such as pH and ion concentration, it affects the final polymorphic superstructure [38].

Here, we propose that different nucleation pathways could also serve as the mechanistic basis for an etiological classification of amyloid pathologies (Fig. 2). In this framework, HON is facilitated by mutations that render the protein more prone for spontaneous self-assembly; and hence, is expected to be the dominant mechanism in hereditary amyloidopathies. However, in the normal state, the protein retains its native structure with all the protective mechanisms that prevent proteins from going down the amyloid pathway. In this case, pathogenic catalytic mechanisms are required for the pathological transformation. This can take place via seeding by a preformed amyloid fragment (prion) whose source can be the same organism due to amyloid fragmentation leading to prion propagation, or a different organism resulting from a prion infection [39]. Alternatively, nucleation can be induced by aberrant surfaces that catalyze HEN. Such aberrant surfaces can either be of endogenous origin due to a membrane or lipid pathology, or from an exogenous source such as microbes or nanoparticulate pollutants. These HEN catalysts can be responsible for the non-hereditary sporadic disease forms.
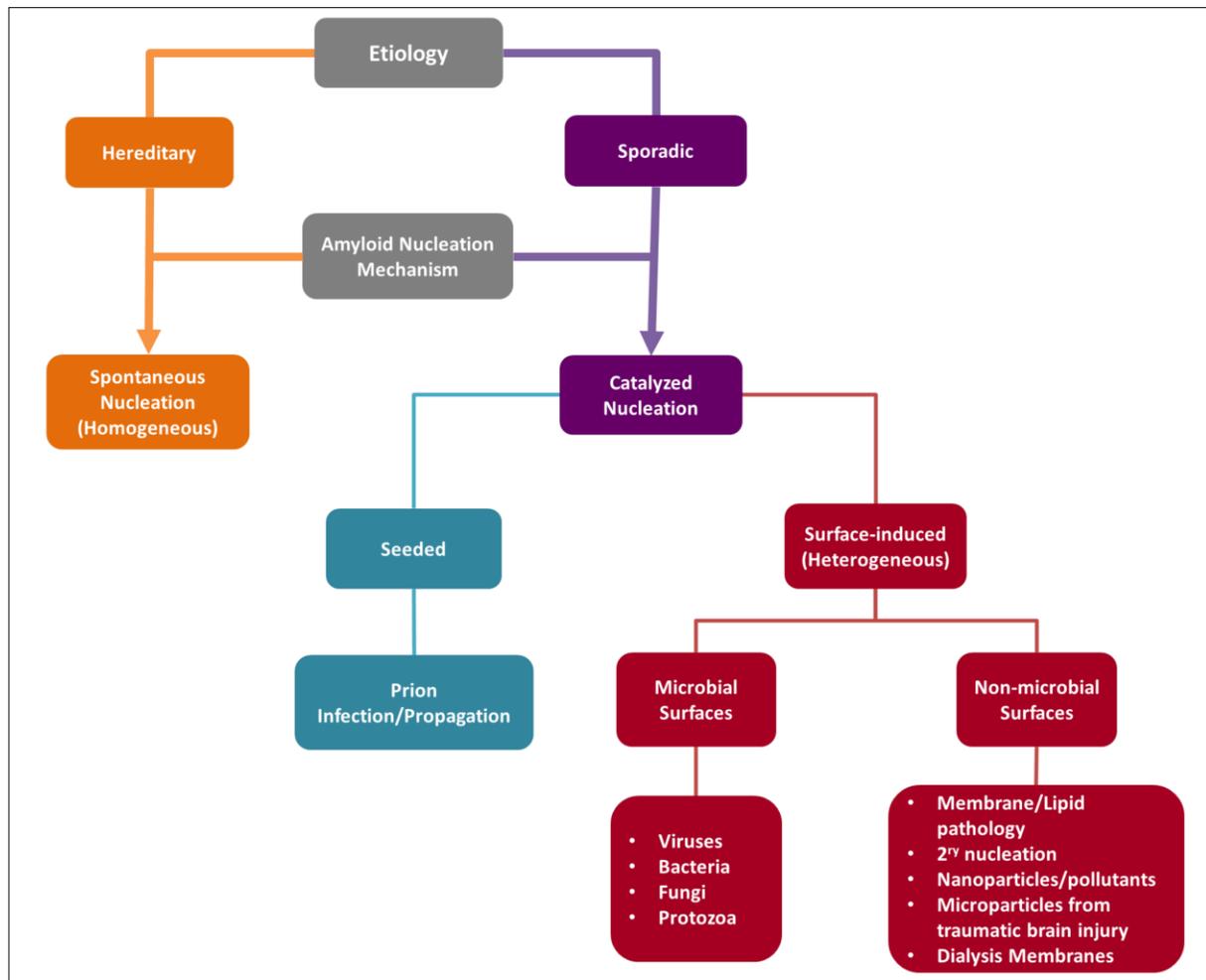
Figure 2. Classification of different amyloid etiologies in relation to the nucleation mechanisms where hereditary forms are usually caused by genetic mutations that facilitate HON, while sporadic forms are mainly catalyzed via prions or aberrant surfaces which can be of microbial or non-microbial origin.

While non-protein factors such as exogenous surfaces have usually been considered "co-factors" to a "protein-only" driven process [40], we emphasize that they are independent causal factors as they promote a distinct and important pathway of amyloid aggregation; HEN. Being thermodynamically more favorable, HEN is more likely to be the prevalent pathway of amyloid aggregation *in-vivo* in the absence of genetic mutations that can facilitate HON. In this regard, microbes such as viruses and bacteria, which are capable of invading and reproducing in tissues, can be potent mediators of HEN in sporadic amyloidopathies. We have recently shown that viruses such as respiratory syncytial virus (RSV) and herpes simplex virus type 1 (HSV-1) are able to induce amyloid formation by catalyzing HEN of IAPP and Aβ, respectively [28]. In vivo, HSV-1 intracranial infection in an AD animal model resulted in amyloid accumulation within 48 hours post-infection [28]. Similar observations were demonstrated for other

pathogens such as bacteria and fungi [41,42]. This shows that microbes are potent HEN inducers of amyloid aggregation. On the other hand, aberrant membranes may come from endogenous sources. These can be the result of lipid dysregulation involving lipoproteins such as apoE4, which is a known risk factor for AD [43], or membrane components such as cholesterol, gangliosides and GAGs [32,44]. Furthermore, membrane fragment microparticles from brain injury [45] can potentially act as catalytic surfaces for HEN mediated amyloid aggregation in traumatic brain injury. Moreover, as has been reported for the amyloid aggregation of insulin [46], synthetic membranes can act as sites for HEN mediated aggregation of some plasma proteins such as β2 microglobulin in dialysis-related amyloidosis [47].

It can also be postulated that in some cases HON and HEN mechanisms can overlap, where mutations that would facilitate spontaneous amyloid aggregation via HON can also render the protein more vulnerable for surface-catalyzed amyloid transformation via HEN pathways. Moreover, HEN mechanisms could lead to distinctive amyloid superstructural polymorphs based on the properties of the catalyzing surface, such as size and composition. Virus-induced amyloid aggregation, for example, can be expected to result in particularly deformed polymorphs due to HEN occurring on an acutely curved nanosurface. Crystalline deformation has been demonstrated before when crystallization takes place on a curved surface [48,49]. In the case of amyloids, horizontal stacking of protofilaments will be limited by the surface curvature. This, together with the possible existence of multiple nucleation sites on the same viral particle would lead to distinct polymorphic features that can act as histopathological hallmarks for viral-induced amyloidopathies and can help trace back the etiology. Moreover, the conformational and phase transformations would result in pathogenic functional transformations that are described in the next section.

Gain or loss of function?

From a functional point-of-view, it has been difficult to correlate the pathogenicity of amyloids with particular structural features [2,50]. Here we postulate that while the gain-of-function (GOF) type of toxicity increases with increased amyloid accumulation in a tissue (especially in systemic forms of amyloidosis), a loss-of-function (LOF) type of toxicity likely constitutes the initial cytotoxic mechanism. Nearly all amyloid-forming proteins have known functions in their native folded state. Since any protein needs to adopt an appropriate conformation in order to perform its function, protein unfolding into the cross-β conformation accompanied by phase transformation into solid fibrils generally abolishes the native function of the protein. Proteins such as lipoproteins, antibodies and IAPP (also called amylin) are not able to perform their homeostatic, immunological or hormonal functions in their pathological amyloid forms in Apo-AI amyloidosis, light-chain amyloidosis and diabetes, respectively [51–53]. The same applies

to Aβ, prion protein (PrP) and α-syn, which are the most studied in the context of neurodegenerative disorders. Soluble Aβ was shown to be important for synaptic plasticity and memory [54], soluble PrP on the other hand is involved in myelin maintenance and cellular proliferation processes [55], while α-syn is important for the regulation of neurotransmission and response to cellular stress [56]. Thus, it is expected that these original functions will be lost even before amyloid accumulation reaches substantial toxic GOF levels, and that LOF can at least initially be the neurodegenerative mechanism, as has been suggested previously [56,57].

Box 1. In further support for the importance of LOF diseases mechanisms, in many disease models of amyloid diseases, the knockout animals deficient in the pathogenic protein display disease phenotypes in absence of plaques or aggregates.

- α-synuclein [58–61]
- Amyloid Precursor Protein (APP) [62–68]
- TDP-53 [69–72]
- Tau [73–76]
- Superoxide dismutase 1 (SOD-1) [77]*
- PrP [78–81]
- IAPP [82,83]
- P53 [84–87]

*A comprehensive review of the evidence of LOF toxicity of SOD-1 from animal models and clinical data.

The importance of LOF is further supported by the fact that in many knockout transgenic models of amyloid diseases, the animals display disease phenotypes in the absence of the amyloidogenic protein and its aggregated forms (Box. 1). Moreover, in AD for example, it has been repeatedly demonstrated that there is not always a correlation between the plaque load and disease severity. This has been shown in animal models [88] and in healthy subjects with significant plaque load without significant cognitive impairment [89]. One way to explain this paradox within a GOF framework has been to postulate that toxicity comes from a not-very-well-defined species called the amyloid oligomers [90]. However, an additional explanation can be the fact that proteins that lose their native conformation will instantly lose their function even if they do not become particularly more toxic, and that such LOF contributes to neuronal degeneration. This is further illustrated by neuronal phenotypes in knockout animal models of several different amyloid pathologies (Box. 1).

Furthermore, it has been recently shown that amyloid fragments (seeds/prions) can propagate pathology from one region to another within the same organism in diseases such as AD and PD [91,92]. In this case, seeds/prions will induce LOF phase transformation when they encounter a new protein pool. The same can be true for HEN induced by microbes, where the ability of a pathogen to infect a particular area would lead to LOF amyloid transformation in that area. This may explain why in some neurodegenerative diseases the spread of the pathology follows the anatomical connections, which are the same routes for both amyloid fragment and microbial propagation [93,94]. The LOF framework might also explain the failure of therapeutic approaches aiming only to reduce the amyloid forming proteins and open new directions in treatment that include restoring protein homeostasis via replacement therapy with functional, non-aggregating forms of the protein [95]. Indeed, synthetic IAPP (amylin) analogues such as pramlintide are clinically used as replacement therapy in diabetes [53]. Furthermore, overexpression of soluble amyloid precursor protein alpha (APPsα) has been shown to restore synaptic plasticity, reduce soluble Aβ and plaque load and rescue spatial memory in an AD mouse model with preexisting pathology and amyloidosis [96]. This demonstrates that replacement therapy within a LOF framework is a promising approach; one that can be extended to other amyloidopathies.

Phase transformation or replication?

The amyloid aggregation phenomenon, especially in the context of prions, is sometimes referred to as a process of protein "self-replication" that is dominated by a "protein-only" species leading to different prion "strains" that possess different pathogenic potentials [50]. Here, we would like to argue that the phenomenon of amyloid aggregation is better described in physical terms rather than biological terms that imply information preservation and transfer via replication and strain diversity. Amyloid aggregation is a process of nucleation-dependent phase transformation that is very common in nature similar to crystal growth or rain precipitation in non-biological systems. Moreover, other normal biological processes such as biomineralization of hard tissues [97] and the assembly of actin or tubulin [98,99] are also dominated by nucleation-dependent mechanisms. However, in these normal biological processes, protein subunits assemble in their native rather than unfolded cross-β conformation. While many of these non-organic and organic phenomena share similar features with amyloids such as self-assembly, repeated patterns and superstructural polymorphism, in none of these cases is the process referred to as "self-replication" in the biological sense of the word. Moreover, polymorphic heterogeneity is dependent on the nucleation mechanism and environmental factors (such as pH and temperature); factors that are not encoded in the core molecular conformation; and hence, cannot be faithfully replicated. Importantly, nucleation reactions take place via HEN, where no information is transferred from the catalyzing surface to the growing fiber, while still affecting superstructural polymorphism. This lack of information preservation or transfer

indicates that the amyloid/prion phenomenon cannot be compared to the nucleic acids in terms of biological replication; which in the case of nucleic acids, is dominated by well-controlled mechanisms and machinery that ensure preservation and faithful replication of the genetic information.

We are aware that the "protein-only" hypothesis of protein "self-replication" was initially introduced to distinguish amyloids from viral infections based on the absence of nucleic acids within amyloids [100]. Despite the historical importance of such distinction, it does not imply that the amyloid phenomenon should always be understood within the bounds of this historical dichotomy. Structural and biophysical studies of amyloids in recent years have uncovered important details about the common structural features of amyloids and the different physical pathways of their aggregation (see above). Many of these new advances do not fit easily within the "protein-only" paradigm. This is particularly apparent in relation to HEN phenomena, which can be mediated by viral and other microbial surfaces; the very species the "protein-only" hypothesis was supposed to exclude from the pathology. HEN also clearly demonstrates the lack of information transfer during the amyloid aggregation process. That is why a new synthesis of the available data was necessary to accommodate for these findings. In that sense, we think that the nucleation-based classification of amyloid pathologies that we describe here does offer a more accurate and inclusive attempt to describe the multifactorial nature of amyloid aggregation using a well-defined physical framework.

One advantage of this physical classification is that it provides a mechanistic explanation of phenomena that are currently unaccounted for within the "protein-only" paradigm, including the sporadic forms of the disease. It allows the integration of risk factors (such as lipid pathology, infections and pollution) into the core of the pathogenesis via a well-defined mechanism. Furthermore, by highlighting the common physical foundations of the amyloid aggregation process, it becomes much easier to find correlations and common mechanisms between different amyloid pathologies that have been studied separately in isolated disease contexts. This creates a logical framework where data from different diseases can be integrated into a more general understanding. One outcome of such general understanding is that HEN and LOF mechanisms assume a more clear and prominent role in disease etiology and pathophysiology, opening new opportunities for novel diagnostic and therapeutic modalities. This is particularly important at a time where the failure of previous therapeutic interventions calls for new ways to understand amyloid pathologies.

In relation to novel diagnostics, HEN pathways are expected to contribute to amyloid polymorphism (see above), which can help in the differential histopathological diagnosis by relating particular amyloid polymorphs to certain HEN interactions. This may enable the development of new therapeutic

interventions to specifically target these interactions, or preventive measures such as vaccines targeting specific microbes involved in HEN-mediated amyloid induction. In addition, highlighting the LOF angle of the pathology can lead to new treatments that aim to restore the original protein functions via different replacement therapy approaches.

Conclusions: *Pathological Nucleation and Solidification of Soluble Proteins*

In this perspective article, we link amyloid etiologies to the physical mechanisms of nucleation. We point out that familial mutations facilitate spontaneous aggregation, leading to HON dominated mechanisms. The sporadic forms on the other hand may rely more on catalytic nucleation mechanisms, either via prion seeding or HEN. HEN mechanisms, in turn, can be mediated by a plethora of aberrant membranes, among which microbial membranes such as viruses and bacteria may be of critical importance, due to their ability to invade and replicate in various tissues. Moreover, we think that amyloids are more precisely described in physical terms similar to other organic and non-organic phase transformations, rather than in biological terms that invoke self-replication and biological strains. Furthermore, biochemical and biophysical amyloid transformations may lead to LOF toxicity due to loss of solubility and native protein conformation, even if the resulting aggregates are not particularly more toxic. Such a generalized framework for a mechanistic-based understanding can open new avenues for the exploration of new measures to diagnose, prevent and treat amyloidopathies.

References

1.   Chiti, F. & Dobson, C. M. Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. *Annu. Rev. Biochem.* **86**, 27–68 (2017).

2.   Eisenberg, D. & Jucker, M. The amyloid state of proteins in human diseases. *Cell* **148**, 1188–1203 (2012).

3.   Gendron, T. F. The role of tau in neurodegeneration. *Mol. Neurodegener.* **4**, 1–19 (2009).

4.   Navalkar, A. *et al.* Prion-like p53 Amyloids in Cancer. (2019). doi:10.1021/acs.biochem.9b00796

5.   Jackson, M. P. & Hewitt, E. W. Why are functional amyloids non-toxic in humans? *Biomolecules* **7**, 1–13 (2017).

6.   Knowles, T. P. J., Vendruscolo, M. & Dobson, C. M. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* **15**, 384–96 (2014).

7.   Broome, B. M. & Hecht, M. H. Nature disfavors sequences of alternating polar and non-polar amino acids: Implications for amyloidogenesis. *J. Mol. Biol.* **296**, 961–968 (2000).

8.   Chiti, F. *et al.* Studies of the aggregation of mutant proteins in vitro provide insights into the genetics of amyloid diseases. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 16419–16426 (2002).

9.   Kim, W. & Hecht, M. H. Mutations Enhance the Aggregation Propensity of the Alzheimer's Aβ Peptide. *J. Mol. Biol.* **377**, 565–574 (2008).

10.   Cobb, N. J. & Surewicz, W. K. Prion diseases and their biochemical mechanisms. *Biochemistry* **48**, 2574–2585 (2009).

11.   Itzhaki, R. F. *et al.* Microbes and Alzheimer's disease. *J. Alzheimer's Dis.* **51**, 979–984 (2016).

12.   Mesa-Herrera, Taoro-González, Valdés-Baizabal, Diaz & Marín. Lipid and Lipid Raft Alteration in Aging and Neurodegenerative Diseases: A Window for the Development of New Biomarkers. *Int. J. Mol. Sci.* **20**, 3810 (2019).

13.   Kilian, J. & Kitazawa, M. The emerging risk of exposure to air pollution on cognitive decline and Alzheimer's disease – Evidence from epidemiological and animal studies. *Biomed. J.* **41**, 141–162 (2018).

14.   Johnson, V. E., Stewart, W. & Smith, D. H. Traumatic brain injury and amyloid-β pathology: A link to alzheimer's disease? *Nat. Rev. Neurosci.* (2010). doi:10.1038/nrn2808

15.   Fändrich, M., Fletcher, M. A. & Dobson, C. M. Amyloid fibrils from muscle myoglobin. *Nature* **410**, 165–166 (2001).

16.   Auer, S., Dobson, C. M. & Vendruscolo, M. Characterization of the nucleation barriers for protein aggregation and amyloid formation. *HFSP J.* **1**, 137–146 (2007).

17.   Eisenberg, D. S. & Sawaya, M. R. Structural Studies of Amyloid Proteins at the Molecular Level. *Annu. Rev. Biochem.* **86**, null (2017).

18.   Mezzenga, R. & Adamcik, J. The Amyloid Polymorphism in the Protein Folding and Aggregation Energy Landscape. *Angew. Chemie* (2018). doi:10.1002/ange.201713416

19.   Tycko, R. & Wickner, R. B. Molecular structures of amyloid and prion fibrils: Consensus versus controversy. *Acc. Chem. Res.* **46**, 1487–1496 (2013).

20.   Morel, B., Varela, L., Azuaga, A. I. & Conejero-Lara, F. Environmental conditions affect the kinetics of nucleation of amyloid fibrils and determine their morphology. *Biophys. J.* **99**, 3801–3810 (2010).

21.   Karthika, S., Radhakrishnan, T. K. & Kalaichelvi, P. A Review of Classical and Nonclassical Nucleation Theories. *Cryst. Growth Des.* **16**, 6663–6681 (2016).

22.   Ros, K., Johansen, A., Riipinen, I. & Schlesinger, D. The effect of nucleation on icy pebble growth in protoplanetary discs. (2019).

23.   Vekilov, P. G. Nucleation. *Cryst. Growth Des.* **10**, 5007–5019 (2010).

24.   Buell, A. K. *The Nucleation of Protein Aggregates - From Crystals to Amyloid Fibrils*. *International Review of Cell and Molecular Biology* **329**, (Elsevier Inc., 2017).

25.   Srivastava, A. K. *et al.* β-Amyloid Aggregation and Heterogeneous Nucleation. *Protein Sci.* pro.3674 (2019). doi:10.1002/pro.3674

26.   Auer, S., Trovato, A. & Vendruscolo, M. A Condensation-Ordering Mechanism in Nanoparticle-Catalyzed Peptide Aggregation. *PLoS Comput. Biol.* **c**, 1–7 (2009).

27.   John, T. *et al.* Impact of nanoparticles on amyloid peptide and protein aggregation: A review with a focus on gold nanoparticles. *Nanoscale* **10**, 20894–20913 (2018).

28.   Ezzat, K. *et al.* The viral protein corona directs viral pathogenesis and amyloid aggregation. *Nat. Commun.* **10**, 2331 (2019).

29.   Bantle, C. M. *et al.* Infection with mosquito-borne alphavirus induces selective loss of dopaminergic neurons , neuroin fl ammation and widespread protein aggregation. *npj Park. Dis.* 1–15 (2019). doi:10.1038/s41531-019-0090-8

30.   Habchi, J. *et al.* Cholesterol catalyses Aβ42 aggregation through a heterogeneous nucleation pathway in the presence of lipid membranes. *Nat. Chem.* (2018). doi:10.1038/s41557-018-0031-x

31.   Linse, S. *et al.* Nucleation of protein fibrillation by nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 8691–8696 (2007).

32.   Iannuzzi, C., Irace, G. & Sirangelo, I. The effect of glycosaminoglycans (GAGs) on amyloid aggregation and toxicity. *Molecules* **20**, 2510–2528 (2015).

33.   Liu, C. & Zhang, Y. *Nucleic acid-mediated protein aggregation and assembly*. *Advances in Protein Chemistry and Structural Biology* **84**, (Elsevier Inc., 2011).

34.   Tö, M. *et al.* Secondary nucleation in amyloid formation. (2018). doi:10.1039/c8cc02204f

35.   Brangwynne, C. P. Liquid phase condensation in cell physiology and disease. **4382**, (2017).

36.   Wang, M. *et al.* Stress-Induced Low Complexity RNA Activates Physiological Amyloidogenesis. *Cell Rep.* **24**, 1713-1721.e4 (2018).

37.   Yuan, C. *et al.* Nucleation and Growth of Amino Acid and Peptide Supramolecular Polymers through Liquid – Liquid Phase Separation. *Angew. Chem. Int. Ed. Engl.* **100190**, 2–10 (2019).

38.   Srivastava, A. K. *et al.* β-Amyloid aggregation and heterogeneous nucleation. *Protein Sci.* (2019). doi:10.1002/pro.3674

39.   Jucker, M. & Walker, L. C. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature* **501**, 45–51 (2013).

40.   Ma, J. & Wang, F. Prion disease and the 'protein only hypothesis'. *Essays Biochem.* **56**, 181–191 (2014).

41.   Kumar, D. K. V. *et al.* Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimers disease. *Sci. Transl. Med.* **8**, 34072–34072 (2016).

42.   Dominy, S. S. *et al.* Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* **5**, eaau3333 (2019).

43.   Potter, H. & Wisniewski, T. Apolipoprotein E: essential catalyst of the Alzheimer amyloid cascade. *Int. J. Alzheimers. Dis.* **2012**, 489428 (2012).

44.   Penke, B. *et al.* The Role of Lipids and Membranes in the Pathogenesis of Alzheimer's Disease: A Comprehensive View. *Curr. Alzheimer Res.* **15**, 1191–1212 (2018).

45.   Zhao, Z. *et al.* Cellular microparticles and pathophysiology of traumatic brain injury. *Protein Cell* **8**, 801–810 (2017).

46.   Nayak, A., Dutta, A. K. & Belfort, G. Surface-enhanced nucleation of insulin amyloid fibrillation. *Biochem. Biophys. Res. Commun.* **369**, 303–307 (2008).

47.   Scarpioni, R. *et al.* Dialysis-related amyloidosis: Challenges and solutions. *International Journal*

of Nephrology and Renovascular Disease (2016). doi:10.2147/IJNRD.S84784

48.   Meng, G., Paulose, J., Nelson, D. R. & Manoharan, V. N. Elastic instability of a crystal growing on a curved surface. *Science (80-.).* **343**, 634–637 (2014).

49.   Gómez, L. R., García, N. A., Vitelli, V., Lorenzana, J. & Vega, D. A. Phase nucleation in curved space. *Nat. Commun.* **6**, (2015).

50.   Collinge, J. Mammalian prions and their wider relevance in neurodegenerative diseases. *Nature* **539**, 217–226 (2016).

51.   Lu, C. *et al.* Apolipoprotein A-1-related amyloidosis 2 case reports and review of the literature. *Med. (United States)* **96**, (2017).

52.   Muchtar, E., Buadi, F. K., Dispenzieri, A. & Gertz, M. A. Immunoglobulin light-chain amyloidosis: From basics to new developments in diagnosis, prognosis and therapy. *Acta Haematol.* **135**, 172–190 (2016).

53.   Hieronymus, L. & Griffin, S. Role of Amylin in Type 1 and Type 2 Diabetes. *Diabetes Educ.* **41**, 47S-56S (2015).

54.   Parihar, M. S. & Brewer, G. J. Amyloid-β as a modulator of synaptic plasticity. *J. Alzheimer's Dis.* **22**, 741–763 (2010).

55.   Castle, A. R. & Gill, A. C. Physiological functions of the cellular prion protein. *Front. Mol. Biosci.* **4**, 1–25 (2017).

56.   Benskey, M. J., Perez, R. G. & Manfredsson, F. P. The contribution of alpha synuclein to neuronal survival and function - Implications for Parkinson's disease. *J. Neurochem.* **137**, 331–359 (2016).

57.   Kepp, K. P. Alzheimer's disease due to loss of function: A new synthesis of the available data. *Prog. Neurobiol.* **143**, 36–60 (2016).

58.   Greten-Harrison, B. *et al.* αβγ-Synuclein triple knockout mice revealage-dependent neuronal dysfunction. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 19573–19578 (2010).

59.   Chandra, S., Gallardo, G., Fernández-Chacón, R., Schlüter, O. M. & Südhof, T. C. α-Synuclein cooperates with CSPα in preventing neurodegeneration. *Cell* **123**, 383–396 (2005).

60.   Gorbatyuk, O. S. *et al.* In vivo RNAi-mediated α-synuclein silencing induces nigrostriatal

degeneration. *Mol. Ther.* **18**, 1450–1457 (2010).

61.   Collier, T. J., Eugene Redmond, D., Steece-Collier, K., Lipton, J. W. & Manfredsson, F. P. Is alpha-synuclein loss-of-function a contributor to parkinsonian pathology? Evidence from non-human primates. *Front. Neurosci.* **10**, 1–7 (2016).

62.   Liu, A. *et al.* Regulation of Neurotransmitter Release by Amyloid Precursor Protein Through Synapsin Phosphorylation. *Neurochem. Res.* **44**, 683–691 (2019).

63.   Martinsson, I. *et al.* APP depletion alters selective pre- and post-synaptic proteins. *Mol. Cell. Neurosci.* **95**, 86–95 (2019).

64.   Senechal, Y., Kelly, P. H., Cryan, J. F., Natt, F. & Dev, K. K. Amyloid precursor protein knockdown by siRNA impairs spontaneous alternation in adult mice. *J. Neurochem.* **102**, 1928–1940 (2007).

65.   Southam, K. A., Stennard, F., Pavez, C. & Small, D. H. Knockout of Amyloid β Protein Precursor (APP) Expression Alters Synaptogenesis, Neurite Branching and Axonal Morphology of Hippocampal Neurons. *Neurochem. Res.* **44**, 1346–1355 (2019).

66.   Truong, P. H., Ciccotosto, G. D. & Cappai, R. Analysis of Motor Function in Amyloid Precursor-Like Protein 2 Knockout Mice: The Effects of Ageing and Sex. *Neurochem. Res.* **44**, 1356–1366 (2019).

67.   Wang, H., Megill, A., He, K., Kirkwood, A. & Lee, H. K. Consequences of inhibiting amyloid precursor protein processing enzymes on synaptic function and plasticity. *Neural Plast.* **2012**, (2012).

68.   Tyan, S. H. *et al.* Amyloid precursor protein (APP) regulates synaptic structure and function. *Mol. Cell. Neurosci.* **51**, 43–52 (2012).

69.   Prpar Mihevc, S., Baralle, M., Buratti, E. & Rogelj, B. TDP-43 aggregation mirrors TDP-43 knockdown, affecting the expression levels of a common set of proteins. *Sci. Rep.* **6**, 1–9 (2016).

70.   Vanden Broeck, L., Callaerts, P. & Dermaut, B. TDP-43-mediated neurodegeneration: Towards a loss-of-function hypothesis? *Trends Mol. Med.* **20**, 66–71 (2014).

71.   Yang, C. *et al.* Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 1121–1129 (2014).

72.   Kabashi, E. *et al.* Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo. *Hum. Mol. Genet.* **19**, 671–683 (2009).

73.   Biundo, F., Del Prete, D., Zhang, H., Arancio, O. & D'Adamio, L. A role for tau in learning, memory and synaptic plasticity. *Sci. Rep.* **8**, 1–13 (2018).

74.   Kimura, T. *et al.* Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos. Trans. R. Soc. B Biol. Sci.* **369**, (2014).

75.   Velazquez, R. *et al.* Acute tau knockdown in the hippocampus of adult mice causes learning and memory deficits. *Aging Cell* **17**, 1–12 (2018).

76.   Lei, P. *et al.* Motor and cognitive deficits in aged tau knockout mice in two background strains. *Mol. Neurodegener.* **9**, 1–12 (2014).

77.   Saccon, R. A., Bunton-Stasyshyn, R. K. A., Fisher, E. M. C. & Fratta, P. Is SOD1 loss of function involved in amyotrophic lateral sclerosis? *Brain* **136**, 2342–2358 (2013).

78.   Sakaguchi, S. *et al.* Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature* **380**, 528–531 (1996).

79.   Criado, J. R. *et al.* Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. *Neurobiol. Dis.* **19**, 255–265 (2005).

80.   Bremer, J. *et al.* Axonal prion protein is required for peripheral myelin maintenance. *Nat. Neurosci.* **13**, 310–318 (2010).

81.   Küffer, A. *et al.* The prion protein is an agonistic ligand of the G protein-coupled receptor Adgrg6. *Nature* **536**, 464–468 (2016).

82.   Mulder, H., Gebre-Medhin, S., Betsholtz, C., Sundler, F. & Ahrén, B. Islet amyloid polypeptide (amylin)-deficient mice develop a more severe form of alloxan-induced diabetes. *Am. J. Physiol. - Endocrinol. Metab.* (2000). doi:10.1152/ajpendo.2000.278.4.e684

83.   Gebre-Medhin, S. *et al.* Increased insulin secretion and glucose tolerance in mice lacking islet amyloid polypeptide (amylin). *Biochem. Biophys. Res. Commun.* (1998). doi:10.1006/bbrc.1998.9308

84.   Lasagna-Reeves, C. A. *et al.* Dual role of p53 amyloid formation in cancer; loss of function and gain of toxicity. *Biochem. Biophys. Res. Commun.* **430**, 963–968 (2013).

85.   Ghosh, S. *et al.* P53 amyloid formation leading to its loss of function: Implications in cancer pathogenesis. *Cell Death Differ.* **24**, 1784–1798 (2017).

86.   Ano Bom, A. P. D. *et al.* Mutant p53 aggregates into prion-like amyloid oligomers and fibrils: Implications for cancer. *J. Biol. Chem.* **287**, 28152–28162 (2012).

87.   Donehower, L. A. *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215–221 (1992).

88.   Hsia, A. Y. *et al.* Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3228–3233 (1999).

89.   Aizenstein, H. J. *et al.* Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch. Neurol.* **65**, 1509–1517 (2008).

90.   Selkoe, D. J. Alzheimer's disease is a synaptic failure. *Science (80-.).* **298**, 789–791 (2002).

91.   Brundin, P., Melki, R. & Kopito, R. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat. Rev. Mol. Cell Biol.* **11**, 301–7 (2010).

92.   Steiner, J. a, Angot, E. & Brundin, P. A deadly spread: cellular mechanisms of α-synuclein transfer. *Cell Death Differ.* **18**, 1425–33 (2011).

93.   Brettschneider, J., Del Tredici, K., Lee, V. M.-Y. & Trojanowski, J. Q. Spreading of pathology in neurodegenerative diseases: a focus on human studies. *Nat. Rev. Neurosci.* **16**, 109–20 (2015).

94.   Hawkes, C. H., Del Tredici, K. & Braak, H. Parkinson's disease: A dual-hit hypothesis. *Neuropathol. Appl. Neurobiol.* **33**, 599–614 (2007).

95.   Mockett, B. G., Richter, M., Abraham, W. C. & Mülle, U. C. Therapeutic potential of secreted amyloid precursor protein APPsα. *Front. Mol. Neurosci.* **10**, 1–14 (2017).

96.   Fol, R. *et al.* Viral gene transfer of APPsα rescues synaptic failure in an Alzheimer's disease mouse model. *Acta Neuropathol.* **131**, 247–266 (2016).

97.   Veis, A. & Dorvee, J. R. Biomineralization mechanisms: A new paradigm for crystal nucleation in organic matrices. *Calcif. Tissue Int.* **93**, 307–315 (2013).

98.   Firat-Karalar, E. N. & Welch, M. D. New mechanisms and functions of actin nucleation. *Curr. Opin. Cell Biol.* **23**, 4–13 (2011).

99.   Job, D., Valiron, O. & Oakley, B. Microtubule nucleation. *Curr. Opin. Cell Biol.* **15**, 111–117 (2003).

100.   Zabel, M. D. & Reid, C. A brief history of prions. *Pathog. Dis.* **73**, 1–8 (2015).