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REVIEW ESSAY

Prospects & Overviews

Amyloid fibrils act as a reservoir of soluble oligomers, the main culprits in protein deposition diseases

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Abstract

Amyloid fibril formation plays a central role in the pathogenesis of a number of neurodegenerative diseases, including Alzheimer and Parkinson diseases. Transient prefibrillar oligomers forming during the aggregation process, exhibiting a small size and a large hydrophobic surface, can aberrantly interact with a number of molecular targets on neurons, including the lipid bilayer of plasma membranes, resulting in a fatal outcome for the cells. By contrast, the mature fibrils, despite presenting generally a high hydrophobic surface, are endowed with a low diffusion rate and poorly penetrate the interior of the lipid bilayer. However, increasing evidence shows that both intracellular α-synuclein fibrils, as well and as extracellular amyloid-β and β2-microglobulin fibrils, can release oligomers over time that quickly diffuse to reach the membrane of the neighboring cells. The persistent leakage of harmful oligomers from fibrils triggers an ongoing cascade of events resulting in a sustained injury to neurons and glia and also provides aggregates with the ability to cross biological membranes and diffuse between cells or cellular compartments.

KEYWORDS
AD, α-synuclein, amyloid-β peptide, neurodegeneration, PD, protein misfolding, toxic oligomers

INTRODUCTION: WHICH AGGREGATE SPECIES ARE MORE CYTOTOXIC?

The formation of intracellular/extracellular protein filamentous aggregates, of which the archetypal examples are amyloid fibrils, is the hallmark of several human diseases and disorders, some of them among the most common and debilitating medical conditions in the modern world, including Alzheimer's and Parkinson's diseases (AD and PD, respectively).[1] Such fibrils are thread-like structures with a diameter of 7–13 nm, typically composed of several protofilaments 2–7 nm wide with a common and amyloid-specific motif, termed cross-β structure in which the β-strands are perpendicular to the long fibril axis and the β-sheets are parallel.[2,3] Most of the experimental evidence points to protein aggregation as a gain-of-toxic-function process that plays a central role in the pathogenesis of neurodegenerative diseases.[1]

The study of the mechanism of protein aggregation has recently achieved significant advances by the development of accurate experimental and mathematical methods to analyze the kinetics of amyloid fibril formation.[4] It has long been recognized that the process underlying the conversion of a normally soluble peptide or protein into an aggregated state involves nucleation and growth steps.[5] In particular, when the quantity of fibrils formed during an aggregation reaction is measured as a function of time, sigmoidal kinetics are frequently observed, reflecting three distinct phases, generally described as a lag phase, an exponential phase, and a plateau phase. In this process, monomers convert into nuclei through a process that takes place

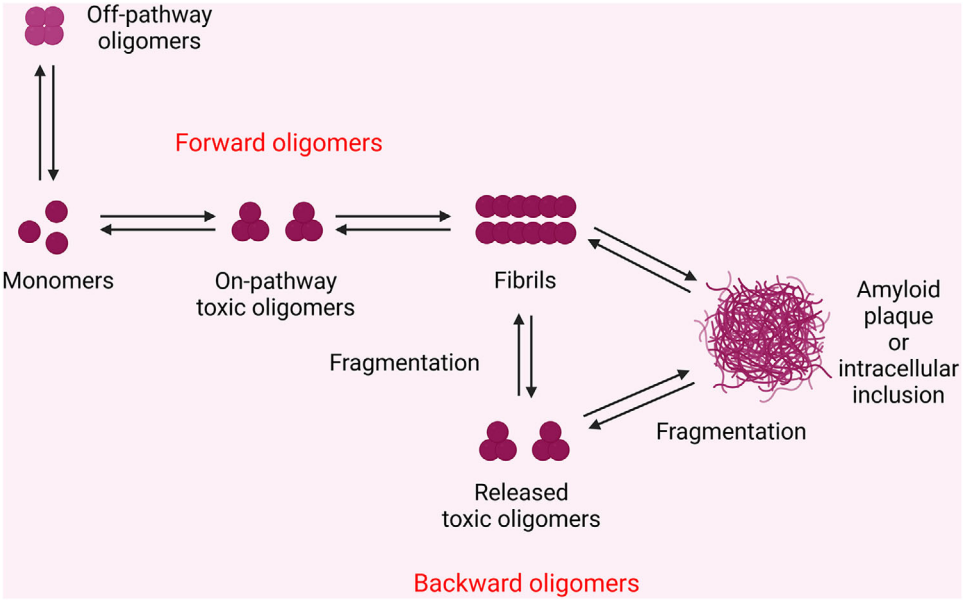

FIGURE 1 A generic scheme for the aggregation of amyloidogenic peptides and proteins. Monomeric proteins can undergo misfolding and aggregation, with the formation of forward oligomers and fibrils, that can accumulate in amyloid plaques. The mature fibrils fragmentate, generating highly diffusible backward toxic oligomers (created with BioRender.com).

early in the lag phase and termed primary nucleation. Nuclei can be monomers, dimers, oligomers, or even large aggregates, depending on the protein and on the aggregation conditions, as widely described in the literature. Fibrils then grow from these nuclei through the addition of monomers (Figure 1). As the population of fibrils becomes sufficiently high, which occurs early in the process and often well within the lag phase, new nuclei form on, and catalyzed by, the surface of the fibrils, through a process known as secondary nucleation. Fibril fragmentation can also occur as a secondary process to generate new fibril ends. Fibrils then grow from both primary nuclei, secondary nuclei and newly generated fibril ends resulting from fragmentation, giving rise to the exponential growth, until an equilibrium or plateau is reached.[5] Importantly, there are no clear temporary boundaries between the multiple microscopic steps of the process, and multiple protein species and microsteps can co-exist in time during the various macroscopically visible phases of aggregation.[5,6] A number of studies on the formation of amyloid fibrils by different protein are in agreement with either a nucleated polymerization mechanism or a nucleated conformational conversion model, depending on conditions and protein of analysis.[1,6–9] In particular, the nucleated polymerization mechanism consists in fibril growth from β-sheet containing nuclei through the addition of monomers.[1] By contrast, in the nucleated conformational conversion, monomers convert rapidly into misfolded aggregates devoid of any amyloid structure, thus in the absence of the structural characteristics needed to further grow into mature fibrils; such initial aggregates then undergo a structural reorganization to generate amyloid-like oligomers acting as nuclei, on which other disorganized oligomers achieve the amyloid conformation through a templating or induced-fit mechanism at the aggregate ends, leading eventually to fibril formation.[1,9]

An increasing number of studies attempt to identify the aggregate species causing neurodegeneration in vivo,[10,11] but limited information on these species has been obtained so far, due to the inherent challenges to isolate suitable quantities of pure samples required for accurate structural analysis, their intrinsically transient nature, and heterogenous structure. These characteristic features typically lead to the presence of very low fractions of on-pathway oligomers, that are aggregation intermediates *en-route* to fibril formation and, therefore, forming fibrils without dissociation, as compared to monomers, fibrils and other protein species at any time during the self-assembly reaction.[6] Moreover, heterogeneous samples are generally collected, with several problems in assigning whether the isolated toxic aggregates correspond to the entire oligomeric population or to specific structures within large structural distributions of oligomeric species. Basically, the size, in conjunction with the fraction of β-sheet content, was commonly used to identify the nature of such aggregated species.[1] Considerable effort has been expended, and is still ongoing, to quantify and selectively detect amyloidogenic oligomers in biological samples, determining their significance in neurodegenerative conditions. A detailed review of all the strategies aimed at quantifying pathologically relevant proteins is provided by Jin et al.[12] Current research also attempts to generate biomolecular tools to target oligomeric conformations directly associated with neurotoxicity: a number of molecules targeting amyloid aggregates have been developed, such as conformation-sensitive fluorescent probes, antibodies, and aptamers.[13–17] However, our diagnostic ability for these oligomeric species has still many limitations for a reliable utilization and for resulting into therapeutic applications.

Recently, we have found that misfolded protein oligomers by α-synuclein (αS) causing neurotoxicity are not only those species that

form directly from the assembly of monomers during the aggregation process, but also those released by mature fibrils after they are formed (Figure 1). In particular, we have compared the cellular effects induced by αS oligomers with those induced by the monomeric protein and fibrillar species of different size when extracellularly added to neuronal cells.[18] Although the β-sheet containing oligomers are the most toxic species and induce cellular stress immediately after addition to the cell culture medium, at longer times of exposure fibrillar aggregates also become toxic as a result of the release of structurally similar and β-sheet containing oligomers, while unstructured monomers were not found to induce any cellular damage at any time.[18,19] Both "forward oligomers" generated directly, or "backward oligomers" released from fibrils (Figure 1), were found to trigger the same cascade of cellular events that initiates upon interaction with the cell membrane.[18,19]

In this review, we extend this concept by showing three different proteins/peptides that support the common behavior of amyloid fibrils to dissociate into soluble and harmful oligomers. Our analysis underlies the dynamic and reversible nature of protein aggregates responsible for protein deposition diseases and may reveal new strategies to prevent the harmful biological events associated with protein aggregation.

α-SYNUCLEIN FIBRILS RELEASE OLIGOMERS ABLE TO CROSS BIOLOGICAL MEMBRANES OF NEURONAL CELLS

α-Synucleinopathies are a heterogeneous group of neurodegenerative disorders featured by the abnormal intracellular accumulation of αS inclusions both in neurons and glia, and associated with a spectrum of clinical conditions, including movement disorders, like PD, PD dementia (PDD), and dementia with Lewy body (DLB), as well as autonomic dysfunctional diseases, like pure autonomic failure (PAF) and multiple system atrophy (MSA). Specifically, neuronal inclusions are present in PD, PDD, DLB, and PAF, whereas glial deposits are characteristics of MSA.[1,20] αS, and a range of other proteins of particular interest in the context of protein deposition disorders, are largely unstructured in solution and behave like natively unfolded or intrinsically disordered proteins, although they can fold into more well-defined structures after interaction with specific binding partners.[21] In neuronal cells, there is a dynamic equilibrium between the soluble unfolded state assumed by cytosolic αS and the membrane-bound one, in which a partial α-helix secondary structure is assumed upon binding to lipid bilayers, such as those of synaptic vesicles involved in neurotransmitter release. The aggregation of these proteins may occur as a side-effect of the lack of their natural partners, which act as chaperones in physiological conditions.[22]

Increasing experimental evidence indicates that soluble αS oligomers are the most dangerous species, playing a crucial role in neurodegeneration.[6,10,20,23–25] They have been reported to interact aberrantly with neuronal membranes, thus causing their disruption and, as a consequence, ionic dyshomeostasis in the cell.[18,26] Such conformers were also found to transduce neurotoxic signals and to be uptaken by the cells through the interaction with several membrane protein receptors, such as the cellular prion protein (PrP^C).[27] Other studies have, however, shown that αS fibrils can be similarly or even more toxic than oligomeric forms[28–30] and can initiate and spread neurodegeneration in the brain.[29,31,32] For most of these studies the effects in cells or in mice were evaluated upon cell medium addition of pre-formed protein species generated in vitro, or after their injection into rodent brains.[32–34] Nevertheless, an in vivo quantitative assessment of the neurotoxic strength of the different αS aggregates is actually still missing.

Previous reports described a detailed characterization of αS oligomers that were stabilized, further purified in vitro and characterized.[25,35,36] Two species in particular were identified, both having a diameter of 4–5 nm and a globular morphology: the first were nontoxic, contained a disordered secondary structure, did not bind ANS or ThT, and were negative to the conformation-sensitive A11 antibody; the second were toxic, contained significant β-sheet structure, had ANS binding and weak ThT binding, and were positive to the A11 antibody.[6] The two species were named type A* and B*, respectively, to establish a clear link with the transient oligomeric species formed during the aggregation process that had been previously characterized, named type A and B, respectively.[6] Indeed, the structural and biological features of such stabilized αS oligomers termed A*/B* were found to resemble those previously observed to accumulate during the process of αS amyloid formation termed A/B.[6,35–37] Consistently, distinct types of oligomers, resulting from different aggregation pathways in vitro, possess marked structural differences responsible for different toxicities, also in the case of the $A\beta_{42}$ peptide.[38,39] Interestingly, such differences were also revealed for Aβ oligomers present in the brain of AD mice, which can be ascribed to two classes (type 1 and type 2), based on their temporal, spatial, and structural relationships to amyloid fibrils. Specifically, A11-positive type 1 oligomers possess a significant neurotoxic potential, whereas OC-positive type 2 close to amyloid plaques shows a limited capability to induce cognitive impairment.[40]

Using confocal microscopy and conformation-sensitive antibodies that are specific for well-defined soluble prefibrillar oligomers, we recently found that cultured neuronal cells and neurons exposed to culture medium containing fibrils pre-formed in vitro showed A11-positive type B* αS oligomers penetrated into the cytosolic compartment.[18,19] The release of oligomers from the fibrillar species was relatively slow, with rates and overall kinetic traces that closely matched those of toxicity for fibrils.[18,19] Using stimulated emission depletion (STED) microscopy as a super-resolution microscopy in primary rat cortical neurons coupled with the human specific antibody that recognized only the exogenous human form of αS, we provided further evidence for the oligomeric, rather than fibrillar, morphology of the αS species released from extracellular fibrils upon interaction with the cell membrane and internalized into the cytosol (Figure 2). When a z-stack analysis was used to perform the 3D reconstruction, the release of globular oligomeric species from fluorescently labeled fibrils was also evident inside the cells in the absence of any antibody.[18] Such oligomeric species could also contribute to the pathogenesis

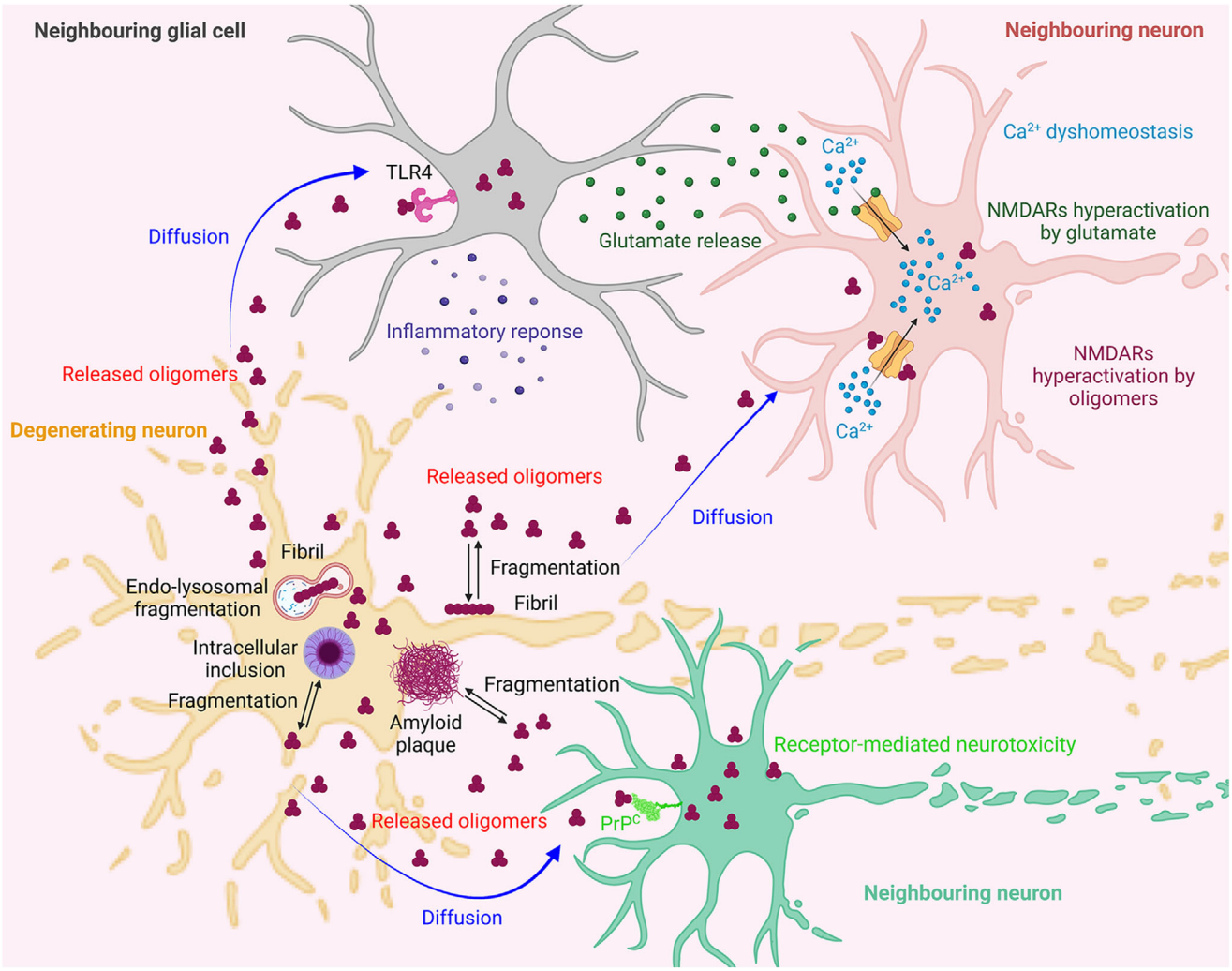

FIGURE 2 The release of toxic oligomers from mature fibrils and plaques induces cellular dysfunction and neurodegeneration. Depending on specific environmental conditions, such as the low pH of the endo-lysosomal compartment, or upon the interaction with the cellular membrane, fibrils and plaques can fragmentate into harmful oligomeric conformers, that further diffuse to neighboring cells and induce their dysfunction, thus exacerbating the pathology (created with BioRender.com).

via a neuron-to-neuron spreading by their direct cell-to-cell transfer or by generating new fibrils, following their neuronal uptake.[31] In particular, receptor-mediated endocytosis has been described as the main cell-to-cell propagation mechanism of αS oligomers.[41,42] Furthermore, a transmembrane seeding of αS aggregation induced by synthetic αS oligomers has been observed in neuronal cultured cells, thus contributing to pathology propagation.[43] Released αS aggregates can be transferred to neighboring neurons, where they induce the formation of inclusion bodies, but also to astroglia, where a pro-inflammatory response occurs.[31,44,45] In particular, astrocytes were reported to engulf large amounts of αS oligomers, and such conformers evoked mitochondrial abnormalities leading to cellular degeneration and death.[46,47] More recently, oligomeric αS was reported to interact aberrantly with astrocytic membranes, inducing a massive rise in intracellular Ca^{2+} levels responsible for glutamate release, that activated in turn extra synaptic NMDARs present in neurons, ultimately leading to synapse loss.[48] In the same work, a direct activation of NMDARs by αS oligomers has also been described[48] (Figure 2).

Collectively, our evidence on the release of αS oligomers from mature fibrils is perfectly in line with the observations by Pountney and coworkers, who described the release of soluble, highly stable, and potentially toxic annular αS oligomers from glial cytoplasmic inclusions arising from brain tissues of MSA patients.[49] In particular, such annular conformers were observed upon the interaction of pathological αS aggregates with detergent micelles, potentially mimicking the interaction with neurotransmitter vesicles in vivo.[49] They are also in agreement with observations of the dissociation of αS fibrils into soluble αS species under conditions close to physiological in vitro, in a non-cellular context[6]; importantly, a considerable portion of the oligomeric conformers formed in the early phases of the disaggregation experiments revealed Förster Resonance Energy Transfer (FRET) efficiencies that were very similar to those of type-B oligomers.[6]

Consistently, rapidly formed immature fibrils, as opposed to consolidated mature fibers, were reported to disintegrate and release oligomers that bound to native plasma membrane sheets.[50] The disaggregation of intracellular αS fibrils by chaperones in vitro has also been reported, giving rise to both monomeric and oligomeric conformers with heterogeneous toxic capabilities and seeding propensities.[51] Interestingly, the possibility that αS fibrils degradation by lysosomal proteases could induce the release of smaller seeding-prone assemblies has also been speculated.[52] Accordingly, in vitro exposure of αS fibrils to mildly acidic pH values, similar to those present in the lysosomes, was reported to increase fibrillar fragmentation.[53] Not surprisingly, pH variations have been reported to destabilize also the structure of mature fibrils of Microcin E492 (Mcc), a low molecular weight bacteriocin, resulting in the release of soluble and highly toxic oligomeric species.[54]

Interestingly, an in vitro investigation conducted by Kumar and collaborators suggested the possibility that monomeric αS at high concentrations, similar to those present in the cellular milieu in PD conditions, could undergo a liquid-to-gel phase transition, acting in turn as a reservoir of toxic oligomers.[55] Altogether, these pieces of evidence indicate that highly stable αS fibrils could potentially act as efficient kidnappers of toxic oligomeric conformers, whereas less stable strains can release harmful species into the surrounding environment. Thus, the definition of the degradation pathways undergone by distinct αS fibril polymorphs inside neurons will be of fundamental importance for the design of novel therapeutic approaches targeting α-synucleinopathies.

A HALO OF Aβ OLIGOMERS SURROUNDING SENILE PLAQUES CAUSES SYNAPTIC LOSS AND NEURODEGENERATION

According to the amyloid hypothesis, the accumulation of large aggregates in the extracellular space of the brain cortex and hippocampus, mainly composed of fibrillar forms of the amyloid-β (Aβ) peptide and referred to as amyloid plaques, is the central event in the pathogenesis of AD,[56,57] which is a chronic and fatal neurodegenerative condition affecting over 35 million people worldwide and representing the most common cause of dementia (source: Alzheimer's Association). The Aβ peptide is 40- or 42-residue long and is produced from the amyloid precursor protein (APP) following the action of β- and γ-secretases.[57] Similarly to αS, it is intrinsically disordered.

Until 25 years ago it was generally assumed that these deposits were directly responsible for the cognitive impairment typical of AD. In 1998, Lambert and coworkers discovered that fibril-free, in vitro prepared Aβ oligomers, which they termed amyloid-derived diffusible ligands (ADDLs), were potent neurotoxins, capable of inhibiting functional synaptic plasticity and long-term potentiation in rat hippocampal slices, and to ultimately cause neuronal cell death in mouse organotypic hippocampal slices.[58] In the following year, another type of Aβ oligomers identified independently and named protofibrils were found to be toxic to rat primary cortical neurons, as assessed by the MTT reduction assay,[59] and also using the LDH release and neuronal imaging and by monitoring the excitatory postsynaptic currents (EPSCs), action potential and membrane depolarization using whole-cell patch-clamp on mixed rat primary cortical cultures.[60] These important findings led to the conceptualization of the oligomer hypothesis to explain the role of the Aβ peptide in AD. According to this hypothesis, oligomers disrupt synaptic plasticity at the very early stages of the pathology, finally culminating in neurodegeneration and cell death at the later stages of dementia.[61] The proposition that Aβ oligomers can be the most harmful neurotoxic species driving neurodegeneration in AD is actually supported by an enormous number of papers corroborating their prominent role.[62–72] Collectively, oligomeric levels in the brain correlate better with the degree of dementia with respect to the simple presence of senile plaques.[73] Importantly, the molecular mechanisms of Aβ aggregation lead to the formation of a plethora of soluble oligomeric intermediates possessing considerable different biophysical, structural, and biological characteristics.[74,75] Specifically, smaller $A\beta_{42}$ oligomers are considered to be the most potent at inducing neurotoxicity, whereas larger soluble conformers were reported to be the most effective in causing an inflammatory response consisting in the production of pro-inflammatory cytokines from astrocytes and microglia.[75,76] A controversy in the field has recently arisen by a Matthew Schrag's report to NIH about apparently altered or duplicated images in dozens of journal articles, involving a specific type of Aβ oligomers called Aβ*56.[77] Although this report questions the validity of one specific type of oligomers, many other papers on Aβ oligomers are not affected and remain solid, leading to the opinion shared by many renown neuroscientists that the broader amyloid hypothesis and the associated Aβ oligomer hypothesis remain viable.[77]

Despite considerable efforts, the precise mechanisms by which Aβ oligomers cause neurodegeneration are not fully understood; in particular, it is not completely clear how such species target specific neuronal populations. Experimental evidence demonstrated that toxicity is not an inherent feature of a particular type of misfolded protein oligomers, but rather arises from a complex interplay between the structural and physicochemical characteristics of both oligomers and cells.[39,78] Indeed, nontoxic oligomers can become toxic and vice versa following an alteration of membrane lipid composition.[39] The lipid composition of the membrane seems to be crucial in the modulation of the cellular response to toxic species,[79–83] thus explaining the variable vulnerability to the same oligomers of different cell types.[78,84] Other lines of evidence pointed out to the involvement of peculiar cell surface proteins as Aβ oligomers receptors, potentially able to trigger a range of downstream signaling pathways responsible for synaptic loss, memory impairment, and neuronal death.[85] Among them, PrP^C is actually considered to be the most eminent in transducing the toxic activity of Aβ oligomers.[85–87] Moreover, the dimerization of PrP^C seems to be critical for its trophic activity. Thus, modulating PrP^C receptor activity was recently proposed as a potential approach to abrogate Aβ oligomer toxicity. In particular, using an aptamer-induced dimerization (AID) strategy, Liu and coworkers enforced PrP^C dimerization and modulated its neurotrophic signaling.[88] In addition, the interaction with microglial cells of soluble forms of Aβ was reported to take

place through the toll-like receptor 4 (TLR4), resulting in the production of inflammatory cytokines, which in turn contributed to AD-linked neurodegeneration (Figure 2).[76,89]

In the "oligomer-centric" context, Martins and coworkers investigated the possible influence of the biological lipids associated with amyloid deposits, such as sphingolipids and gangliosides, on the stability of Aβ fibrils.[90] These mature fibrils were substantially harmless to cultured neurons. However, upon incubation of the fibrils with a range of lipid preparations, all the obtained Aβ fibril mixtures appeared to be highly toxic when added to the cell medium of primary hippocampal neurons, to cause memory impairment and diffuse rapidly through the brain into areas relevant to AD in mice.[90] The species responsible for these neurotoxic effects were found to be Aβ protofibrils rather than Aβ monomers and the so-called "backward protofibrils" presented very similar morphological, structural, biochemical, and biological characteristics as those of "forward protofibrils," formed along the aggregation process of the monomeric protein, both in cultured neurons and in a mouse model.[90]

Consistently, an elegant work conducted by Koffie and collegues demonstrated that the release of Aβ oligomers from senile plaques in the mouse brain is the primary element responsible for synaptic loss (Figure 2), an early and widely recognized cardinal event in the pathogenesis of AD.[91] This proposition was based on the evidence that senile plaques contain and are surrounded by a halo of Aβ oligomers, aberrantly interacting with synapses, and that the observed synaptic loss was proportional to the quantity of oligomers released from such plaques.[91] In particular, the density of excitatory synapses monitored by post-synaptic density protein 95 (PSD95) was found to be dramatically decreased within the halo and to increase linearly with the distance from the amyloid plaque.[91] Accordingly, a halo of medium and small non-fibrillar Aβ entities surrounding the dense core of senile plaques has been observed in postmortem human brain tissue from AD patients by using super-resolution techniques, such as Array Tomography and STED microscopy.[92] Sanchez-Varo et al. recently corroborated again the location of Aβ oligomers at the plaque periphery, bordering mature fibrils, both in an amyloidogenic APP/PS1 transgenic mouse model and in the brain of AD patients.[93] Collectively, these data show that senile plaques may act as deleterious reservoirs of oligomeric Aβ that cause synaptic dysfunction, spine loss, and ultimately neuronal toxicity and loss.

β_2-MICROGLOBULIN FIBRILS DISASSEMBLE INTO TOXIC SPHERICAL OLIGOMERS AT ACIDIC PH

In a very different context distinct from neurodegeneration, amyloid fibrils of β_2-microglobulin (β_2m) accumulate systemically in the extracellular space of tissues other than the brain in patients undergoing long-term dialysis, causing dialysis-related amyloidosis (DRA), characterized by arthritis, inflammation, and pain at joints and massive bone damage.[94] β_2m is the non-covalently bound light chain of the major histocompatibility complex class I (MHC-I) in blood nucleated cells, it is an all-β folded protein and plays an important role in the cellular immunity, by chaperoning the assembly of the complex for antigen presentation.[95,96] Following MHC-I dissociation, β_2m circulates as a folded plasmatic monomeric protein, that is, then filtered by and metabolized in the kidney. Dialysis membranes have a poor ability to filter the protein and this is why its serological levels dramatically rise in people affected by renal failure and dialysis, thus resulting in amyloid deposition of β_2m.[97]

Different studies investigated the very early stages of β_2m fibril formation, with the aim to define the structural and biological properties of the initial oligomeric conformers populating the aggregation pathway.[98,99] A catalytic role for the divalent metal copper for initiating oligomer formation has been reported in vitro under near-physiological conditions (pH 7.4, 37°C, and 150 mM ionic strength),[100,101] even if it is not necessary for maintaining the stability of mature fibrils.[102] Not surprisingly, very high plasmatic copper concentrations have been revealed in hemodialyzed subjects. The cytotoxicity of β_2m oligomers has been reported initially in human neuroblastoma SH-SY5Y cells using the MTT reduction assay.[103]

Following the initial observation that a higher unfolding propensity of β_2m occurs at mildly acidic pH,[104] it has also been reported that β_2m fibrils easily disassemble by reducing the pH: a reduction from 7.4 to 6.4 (slightly similar to that observed during the endocytic trafficking) promotes fibrillar disassembly in toxic spherical oligomers able to disrupt synthetic membranes and potentially contributing to cellular dysfunction.[105] Consistently, the cytotoxic activity of β_2m fibrils has been previously associated to their endocytic uptake.[106] Taken together, these lines of evidence support the notion that fibrillar amyloid conformers of β_2m are not only a harmless terminal aggregation product, but, depending on specific environmental conditions, can be a reservoir of harmful oligomers. Actually, the precise role of the cellular degradation pathways in modulating fibril stability is not completely understood, so it will be critical to characterize their role in the protection or exacerbation of the disease.

FIBRIL DISASSEMBLY: A DOUBLE-EDGED SWORD IN THE THERAPY OF NEURODEGENERATIVE DISEASES

The disassembly and clearance of amyloid deposits is often considered to be a putative disease-modifying treatment for neurodegenerative diseases.[107,108] Specifically, a promising approach for PD therapy consists in increasing the metabolism of αS aggregates by modulating lysosomal and/or autophagic functionality.[108] However, it is crucial to define the αS species produced by such degradation pathways: unbalanced lysosomal enzymatic processes can produce non-canonical αS proteoforms, which are endowed with a high aggregation propensity and increased seeding capacity, thus acting as potentially more toxic than the initial aggregates.[109]

Disruption of mature fibrils and solubilization of amyloidogenic deposits is also suggested to be a possible therapeutic approach also for AD.[110] However, targeting fibrils with specific antibodies,

TABLE 1 Overview of the oligomeric conformers released by amyloid mature fibrils and plaques

Peptide or protein name	Associated disease(s)	Released oligomers
α-Synuclein (αS)	Parkinson's disease (PD), Parkinson's disease with dementia (PDD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA)	Toxic oligomers from mature fibrils[18,19] Annular oligomers from glial inclusions[49] Toxic oligomers in vitro[6] Toxic oligomers by chaperones[51] Seeding-prone assemblies from fibrils at acidic pH[52,53]
Amyloid-β (Aβ) peptide	Alzheimer's disease (AD)	Toxic oligomers upon lipid exposure[90] Toxic oligomers from senile plaques[91–93]
β_2-microglobulin (β_2m)	Dialysis-related amyloidosis (DRA)	Toxic oligomers from mature fibrils[105]

especially with those raised against the N-terminal of Aβ, is not only a late intervention, but such disaggregating antibodies can be also responsible for a substantial increase in the concentration of oligomers in the brain, thus exacerbating neurotoxicity.[111,112] As a consequence, removal of deposits or plaques from the brain of affected people does not appear to be the safest and most effective therapeutic strategy, because of the challenge in keeping the disaggregation process and the ensemble of released soluble aggregates under control. By contrast, targeting neurotoxic oligomeric forms of αS or Aβ seems to be a more promising treatment in neurodegenerative disorders. To this aim, antibodies recognizing conformational epitopes present in oligomers arising from different peptides or proteins would likely be the most exciting therapeutical approach for protein misfolding diseases.

CONCLUSIONS AND OUTLOOK

The disassembly of pre-formed amyloid fibrils into toxic oligomeric conformers emerges as a common feature of fibrillar species formed by amyloidogenic proteins associated with different pathologies (Table 1). Mounting evidence obtained with αS, Aβ, and β_2m, and possibly other systems in the future, suggests that mature fibrils and their further assemblies as senile plaques or Lewy bodies are not inert, but act as major reservoirs of soluble toxic oligomers. Depending on local environmental parameters, such as pH, abnormal changes in lipid metabolism, interaction with membranes, chaperones, or other factors, apparently harmless fibrillar aggregates can rapidly release "backward oligomers," morphologically, structurally and biologically similar to the "forward oligomers" generated at the very early phases of the aggregation process (Figure 1).

In conclusion, small molecules able to prevent the release or leakage of oligomers from mature amyloid fibrils may represent a valid therapeutic strategy against protein deposition diseases, which add to the strategy that is being pursued by over two decades to prevent the formation of toxic oligomers from their corresponding monomers during the aggregation process, such as aminosterols,[113–115] or to interact directly with such species and neutralize them, for example, through the use of conformation-sensitive antibodies.[37,116,117] A proper understanding of the fibril-to-oligomer release process will be of considerable interest for the design of novel therapeutic approaches against amyloidosis.

AUTHOR CONTRIBUTIONS

Conceptualization, *writing – original draft*, *writing – reviewing and editing*, *visualization*: Alessandra Bigi. *Conceptualization*, *writing – original draft*, *writing – reviewing and editing*: Roberta Cascella. *Conceptualization*, *writing – reviewing and editing*: Fabrizio Chiti. *Conceptualization*, *writing – original draft*, *writing – reviewing and editing*, *supervision*: Cristina Cecchi.

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NOTE

The graphical abstract was created with BioRender.com.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated.

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