Review

Role of Different Alpha-Synuclein Strains in Synucleinopathies, Similarities with other Neurodegenerative Diseases

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Abstract. Misfolded protein aggregates are the hallmark of several neurodegenerative diseases in humans. The main protein constituent of these aggregates and the regions within the brain that are affected differ from one neurodegenerative disorder to another. A plethora of reports suggest that distinct diseases have in common the ability of protein aggregates to spread and amplify within the central nervous system. This review summarizes briefly what is known about the nature of the protein aggregates that are infectious and the reason they are toxic to cells. The chameleon property of polypeptides which aggregation into distinct high-molecular weight assemblies is associated to different diseases, in particular, that of alpha-synuclein which aggregation is the hallmark of distinct synucleinopathies, is discussed. Finally, strategies targeting the formation and propagation of structurally distinct alpha-synuclein assemblies associated to different synucleinopathies are presented and their therapeutic and diagnostic potential is discussed.

Keywords: Neurodegenerative diseases, Alzheimer, Parkinson, multiple system atrophy, dementia with lewy bodies, huntington, prion, protein misfolding, ageing

INTRODUCTION

Age-related dementias constitute a major health problem associated to increased life expectancy. These progressive diseases affect increasing number of people (presently 45 million people world wide, expected to reach 76 millions in 2030). These devastating diseases have high societal costs and lead, directly or indirectly, to a dramatic loss in life expectancy and quality of life. They represent therefore priorities for medical research and a threat to ageing societies. These diseases have in common the aggregation after misfolding and/or incorrect elimination of distinct polypeptides. The misfolded or incorrectly cleared proteins form aggregates that accumulate with time. The latter damage the cell, disrupt important neuronal structures and are therefore toxic. Most important however is their ability to amplify by recruiting the soluble form of homologous polypeptides and to propagate from one cell to another. Thus, through their 'template-driven misfolding' of similar proteins over time, e.g. amplification, spread from one cell from the central nervous system to another, e.g. transmissibility and accumulation within the central nervous system, misfolded protein aggregates create a self-sustaining, exponential vicious circle leading to disease.

PROTEIN MISFOLDING AND DISEASE

Within our cells, nascent proteins fold spontaneously or following interaction with molecular

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chaperones in order to be functional [1]. At any given time a fraction of newly synthesized polypeptides populate non-native conformers [2–4], with a sub-fraction corresponding to mis-processed peptides (for example Aβ after APP cleavage by β and γ secretases instead of the α and γ secretase product p3) or polypeptides where translation errors occurred. A fraction of these conformers is given another chance to fold correctly after full/partial unfolding by molecular chaperones [1] while another, never reaches the native state and is cleared or exported outside the cell. Thus, by opposing to protein misfolding, unfolding and aggregation, molecular chaperones, play a central role in the maintenance of normal cellular proteostasis [5]. Upon cellular stress the amounts of misfolded/unfolded proteins and/or mis-processed and mutant polypeptides increase [6]. This triggers heat shock response via the activation of the heat shock transcription factor 1 leading to partial correction of misfolding/unfolding and incorrect processing [5, 7, 8]. As sustained activation of the heat shock response is detrimental [9], normal cellular proteostasis is imbalanced with saturation of proteostatic networks [1, 5, 6]. As a consequence, misfolded proteins aggregate into intra- or extracellular protein deposits, depending on whether they are within the cytosol of in the extracellular milieu. It is widely accepted that the Ubiquitin Proteasome System degrades efficiently misfolded/unfolded or incorrectly processed proteins as long as the monomeric and aggregated forms are in dynamic equilibrium [10]. In contrast, high molecular weight irreversible assemblies are cleared by autophagy [11]. When the latter process fails either because of defects in autophagosome formation, transport, fusion with lysosomes or recognition of the substrate, protein aggregates accumulate with deleterious consequences [11].

Misfolded proteins aggregation can cause both loss of function and gain of pathologic function [12–14]. This is the consequence of either i - misfolded protein aggregates-mediated permeabilization of plasma membrane and/or membranous compartments, ii - perturbation in membrane protein dynamics and distribution they may cause, iii - the formation of novel pathogenic signaling platforms they may mediate, iv - the trapping of significant amounts of molecular chaperones and other partner proteins within the aggregates and v - the ability of the misfolded protein aggregates to recruit the soluble form of the proteins they are formed of and amplify.

Protein aggregates made of fibrillar material, in some cases straight as needles, in others curved and twisted, are the hallmarks of a variety of diseases affecting the central nervous system, in particular, Alzheimer's (AD), Parkinson's (PD), Huntington's (HD) and Creutzfeldt-Jacob (CJD) diseases. The number of deposits within the affected tissues correlates most often with disease severity [15–17]. As the probability of occurrence of events such as i - misfolding and aggregation events of newly synthesized polypeptide chains, ii - unfolding of native polypeptide chains following a variety of stresses, iii - errors during translation and iv - incorrect processing and elimination of proteins targeted to degradation, is dependent on time, misfolding diseases are associated to ageing [18].

SPREAD OF MISFOLDED PROTEIN AGGREGATES

Until recently, the spread and transmission of disease via misfolded protein aggregates was thought to be restricted to the prion protein (PrP) [19]. A wide range of recent experimental evidence suggest that other protein aggregates that are the hallmarks of major neurodegenerative diseases spread in a prion-like manner [20, 21]. Thus, misfolded protein aggregates propagation and amplification appears to be a widespread phenomenon in neurodegenerative diseases as opposed to a curiosity limited to a unique protein: PrP. Indications for such a process originated from the work of Heiko Braak and co-workers who described distinctive patterns of pathological changes over time throughout the brain for the major neurodegenerative diseases in man. Indeed, based on autopsy cases, Braak and co-workers noticed that protein aggregates associated to pathology initiate in circumscribed areas of the brain specific to each disease and progress in a topographically predictable manner following anatomical connections [15–17]. These observations led them to establish a disease progression scale and to hypothesize that neurotropic pathogens were spreading through defined pathways to interconnected regions within the central nervous system. As for PrP, these pathogens were first thought to be of viral nature.

The first evidence for the transmission of misfolded protein aggregates not involving PrP in man came from the observation that Lewy bodies in PD brains contaminate grafted fetal mesencephalic progenitor neurons decades after transplantation [22, 23]. Additional evidence came from *in vivo* and *in vitro* experiments. Injection of brain extracts containing amyloid plaques, which main constituent is the aggregated form of the amyloid β-peptide (Aβ), neurofibrillary tangles, that are mainly made of the microtubule associated protein

Tau, and Lewy bodies, which main constituent is the protein alpha-synuclein (α-syn), into the brain of model animal induced lesions characteristic of AD and PD [24–34]. No such lesions where observed when control brain extracts lacking protein aggregates were injected. The induced lesions, that were initially confined to the injected brain region, propagated to neighboring and/or axonally connected areas over time, suggesting directed spreading and amplification through neuronal transport processes [32–35]. The intraperitoneal injection of brain extracts containing amyloid plaques and neurofibrillary tangles also induced cerebral lesions characteristic of AD (Aβ and Tau deposits) [36, 37]. Similarly, the injection of brain extracts and Lewy bodies enriched fractions from patients developing PD into the brain of non-human primates, wild-type and model mice, induced several months after injection the formation of lesions characteristic of PD [31–35]. Finally, Lewy bodies of human nature appeared in rodents neural grafts implanted in the brains of mice expressing human α-syn that develop Lewy bodies over time [30] and aggregated huntingtin (Htt) with a polyQ stretch length associated to HD and huntingtin N-terminal moiety (Htt-Exon1) were shown to move between cultured cells, in organotypic slice cultures of a Htt mouse model and between host and grafted tissues [38–40].

Luckily, we can reproduce in test tubes the aggregation of Aβ, Tau, α-syn, Htt-Exon1 with a polyQ stretch length associated to HD, and Cu/Zn superoxide dismutase 1 (SOD1) point mutants associated to motor neuron death in familial cases of amyotrophic lateral sclerosis (ALS). Pathologic Htt-Exon1, only in its fibrillar form, binds to cultured cells, is internalized through yet an unknown mechanism, and induces the aggregation of sub-pathologic Htt-Exon1 within the cytosol of the recipient cells [38, 41]. α-syn mega-Dalton protein assemblies were also shown to be taken up [30] and to trigger the aggregation of endogenous cytosolic α-syn in cultured primary cortical neurons and in neuronal cell lines [42–45]. They were shown to be transported anterogradely and retrogradely following their uptake in primary neurons and to be transmitted to secondary neurons [46]. *In vitro* generated Tau assemblies were also shown to bind and enter cells and to transmit a misfolded state to intracellular Tau [44, 47–49]. Finally, mutant SOD1 aggregates made *in vitro* were shown to penetrate inside neurons by macropinocytosis and induce the aggregation of soluble mutant SOD1 in the cytoplasm [50].

Tau and α-syn assemblies made *in vitro* were also inoculated to transgenic model mice. They were taken up by neurons and glial cells and shown to transfer from neurons to oligodendrocytes [51]. Fibrillar assemblies induced several months after injection the formation of lesions characteristic of PD and AD [32, 37].

THE NATURE OF THE TOXIC PROTEIN ASSEMBLIES

Under physiological pH, salt and temperature conditions, Aβ, Tau, α-syn, Htt-Exon1 with a pathologic polyQ stretch and mutant SOD1, self-associate into assemblies that can elongate in a cooperative manner by incorporation of monomers. Growth is unlimited as the incorporation of a monomer creates a new binding site for another monomer (Fig. 1). Metastable, prefibrillar oligomeric intermediates, considered as the precursors of mature fibrils, are populated prior or concomitant to fibrils formation [52, 53]. In some cases, it is the binding of ligands to aggregation prone polypeptides or their modifications that yield oligomeric species that are off-assembly pathway as for example upon the interaction of α-syn with dopamine [54]. The nature of the assemblies that exhibit the highest toxicity and/or seeding capacity is subject to intense debate. Fibrillar assemblies made under physiological conditions have been shown to be the only entities capable of recruiting the soluble form of homolog proteins *in vitro*, in cell cultures and *in vivo*, *i.e.* with seeding capacities, and a body of evidence demonstrates their cytotoxic potential [30, 33, 38, 41, 43–45, 48-50, 55–58]. Nonetheless, they are considered as storage, inert form of misfolded proteins by those who consider the oligomers that form during the early stages of assembly as toxic [59–63]. Toxicity in the latter case is thought to be the consequence of impairment of membrane integrity and permeability [59, 64, 65]. The conflict is certainly due in some cases to non-physiological assembly conditions such as assembly after polypeptide dehydration, under high or low pH, at very low ionic strength (e.g. distilled water), extreme salt conditions and temperatures that are suitable for extremophiles. It is also and mostly due to i - comparison of the properties of protein particles at different concentrations, ii - difficulties in defining what is precisely an oligomer and iii- oligomers - fibrils inter-conversion. At a given concentration of monomeric Aβ, Tau, α-syn, Htt-Exon1 with a pathologic polyQ stretch or mutant SOD1 the concentrations of on-assembly pathway oligomers and fibrils diverge by several orders of magnitude as the molecular masses, number of constituting molecules

and sizes of oligomeric and fibrillar species differ very significantly. Indeed, oligomeric species are made of less than 10 to 50 monomeric proteins at most while fibrils that are over 0.1 μm in length are made of thousands of polypeptide chains. Thus, it is critical to compare the toxicity of the different assemblies at identical particle concentrations as cells interact with the particles as a whole, not the precursor monomeric protein within the assemblies. Measurement of the molecular weight of defined on-assembly pathway oligomeric assemblies and fibrils with homogeneous length allowed calculating the particle concentration of assemblies at any given concentration of precursor monomeric polypeptide. When the relative toxicities of monomeric, oligomeric, and fibrillar assemblies were compared, the fibrillar assemblies, with homolog polypeptide seeding and spreading propensity, were found most toxic [66]. As fibrils and oligomers have distinct molecular weights they are frequently separated by centrifugation. However as the solutions are often viscous, a significant amount of short fibrils may remain in the supernatant. It is therefore important to bear in mind that one cannot exclude that observations derived from such fractionation procedures are due to the presence of short fibrils. Finally as fibrils and precursor oligomeric species are in equilibrium [67] one needs to keep in mind that oligomers can form fibrils in the time course of the experiment either in solution or when associated to the cell membranes as their concentrations increase when moving from a three- (test tube) to a bi- (cell membrane) dimensional space. Similarly, while fibrils most often exhibit some resistance to detergents [68], one cannot exclude their possible disassembly by cellular factors [69].

WHY PROTEIN ASSEMBLIES ARE TOXIC?

The toxicity of fibrillar and oligomeric assemblies is in part the consequence of their binding to and permeabilization of the cell membrane [70]. The binding of assemblies, besides inducing lipid rafts and the monosialoganglioside GM1 clustering [71], certainly impinge membrane curvature [72], thus further affecting cell survival. Furthermore, the lateral diffusion of assemblies in the plane of the plasma membrane together or independently of membranous components such as receptors, channels, adhesion molecules, integrins certainly contributes to the redistribution and/or coalescence of protein receptors at the surface of the cell [73]. Misfolded protein assemblies may even selectively recruit membranous proteins thus creating

novel signaling platform with undesirable properties, thus contributing to cell apoptosis. Once internalized, they appear to leak out of the endosomal-lysosomal compartments as fibrils in particular reach the cytosol and seed the aggregation of their soluble cytosolic counterparts [38, 42, 45, 47]. By crossing these membranous compartments, through yet an unknown mechanism, they may cause leakage of the lysosomal content or rupture of lysozomes, with cathepsin B-dependent increase in reactive oxygen species within the cytosol, thus triggering pathogenesis [74]. The latter may account for the mitochondrial dysfunction and inflammation described in synucleinopathies [75]. Upon reaching the cytosol, exogenous misfolded protein assemblies certainly imbalance proteostasis following the titration of the cytosolic molecular chaperones and/or saturation of the clearance machinery of the cell leading to additional deleterious effects [5, 76]. Finally, toxicity may also be the consequence of the seeding capacity of the protein assemblies, e.g. the recruitment of fibrils soluble counterpart and exhaustion of the functional form of the protein.

As exogenous Aβ, Tau, α-syn etc... assemblies bind and penetrate cells, amplify by recruiting their endogenous soluble counterparts through a seeding process and propagate to other cells they can be considered as infectious [77] in accord with the modifications that have been proposed to Koch's postulates [78].

THE NOTION OF INFECTIOUS PROTEINS STRAINS

The observation that the aggregation of non-mutant PrP give rise to distinct diseases (Creutzfeldt Jakob's disease (CJD), new variant CJD (vCJD) and Kuru in man, classical and Nor98 scrapie in sheep, classical and atypical (H-BSE and L-BSE) bovine spongiform encephalopathies (BSE)) that are distinguishable by the disease incubation time, lesions type in the brain and proteolytic cleavage patterns has led to the notion that PrP can adopt different conformations into the mega-Dalton assemblies associated to disease. Indeed, each conformation an amino acid stretch adopts can establish a defined set of inter-molecular interactions. As a consequence alternative packing is possible yielding structurally unlike assemblies that expose distinct surfaces (Fig. 1). The lateral surfaces of these different assemblies rule their ability to interact with receptors and lipids at the surface of the cells and protein partners within the cell. They dictate functional properties such as toxicity. The surfaces at the tips of structurally
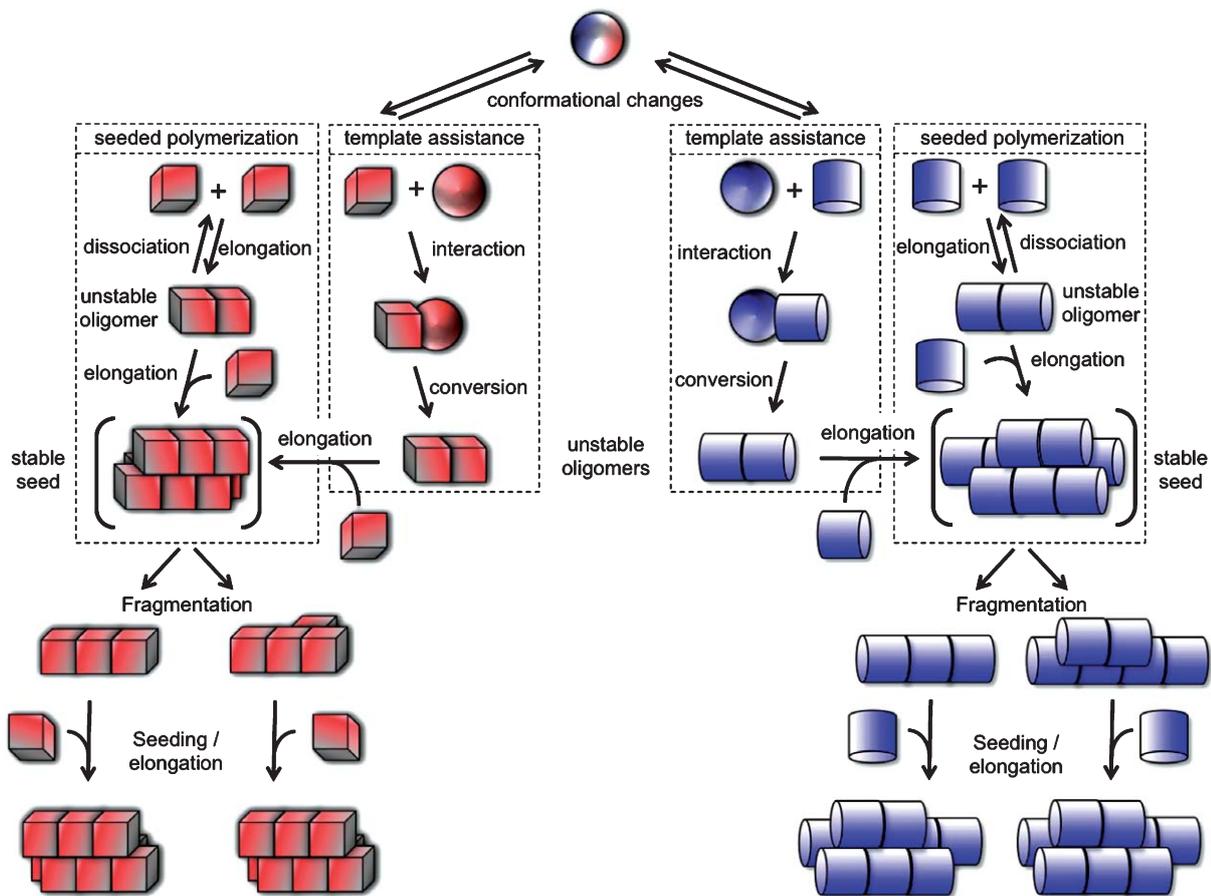
Fig. 1. Protein misfolding and aggregation into infectious fibrillar assemblies. Native or natively unfolded polypeptide chain (sphere) undergo conformational changes that lead to distinct abnormal (cube or cylinder) forms. The rates depend on the propensity of the polypeptide to populate any given (cube or cylinder) conformation. The abnormal forms are short lived either because they are unstable or sensitive to clearance. According to the "template assistance" model, the abnormal folding intermediates interact with the native form of the infectious protein and convert it into abnormal forms. The distinct abnormal forms of the polypeptide have the ability to interact transiently with like conformers and establish longitudinal or lateral interactions following the "seeded polymerization" model. The oligomeric species are unstable and dissociate because the inter-molecular interactions do not outweigh the entropic cost of binding. Once longitudinal and lateral interactions have been established between abnormal forms of the polypeptide, distinct stable seeds are formed (in brackets). These seeds grow indefinitely from one or both ends by incorporation of like molecules, yielding fibrils of distinct physical properties. The different fibrils can break into smaller fragments because, amongst other things, of Brownian movement. The rate of breakage depends on the number of bonds established between the molecules. Each resulting fragment acts as seed.

distinct assemblies determine their growth rate. These distinct conformations are indeed imprinted to the newly incorporated PrP upon assemblies growth either through a templating process or the selective recruitment of defined PrP conformers that can establish intermolecular interactions with the well defined assemblies tips thus leading to the faithful amplification of distinct prion strains.

In a manner similar to PrP, the deposition of α-syn within the central nervous system is not only tightly associated to PD but also to distinct pathological phenotypes: multiple system atrophy (MSA) and dementia with Lewy bodies (DLB). These diseases are grouped under the banner "synucleinopathies". Besides being intimately linked to AD, the aggregation of Tau also leads to distinct tauopathies: argyrophilic grain disease, tangle-only dementia, Pick disease, progressive supra-nuclear palsy and cortico-basal degeneration. The involvement of the deposits made of the same protein in diseases exhibiting different phenotypic traits can be accounted for either by the existence of distinct i - α-syn and Tau strains, ii - areas within the central nervous system affected by neurodegeneration or iii - neuroinflammatory responses, given that neuroinflammation has been repeatedly linked to neurodegeneration [76]. Indeed, mounting evidence

from epidemiological, postmortem, brain imaging and animal studies indicate that neuroinflammatory processes are tightly linked to neurodegeneration [79–82]. Nonetheless, it is still unclear whether the inflammatory process is involved in disease etiology or a secondary consequence of neurodegeneration and whether neuroinflammation affects to different extents distinct brain regions.

STRAINS OF α-SYN, Aβ AND TAU

Differences in the biochemical properties of aggregated α-syn in different genetic backgrounds in humans have been reported suggesting that α-syn aggregates may be unlike [83]. The existence of α-syn strains was established upon the generation of pure α-syn fibrillar polymorphs under stringent and tighly controlled assembly conditions *in vitro*. Distinct fibrillar polymorphs made of wild-type, full-length α-syn, exhibited different shapes in the electron microscope, limited proteolytic and X-ray fiber diffraction patterns and were distinguishable by conformational antibodies. Assessment of their intrinsic structures by solid-state nuclear magnetic resonance (NMR) revealed that although they have high beta-sheet content, α-syn molecules within the fibrillar polymorphs are packed in distinct manner. As a consequence they recruit soluble α-syn with different efficacy, elongate at distinguishable rates and must have different surface properties. Functional studies revealed that

the distinct polymorphs bind and permeabilize cells to different extents; they exhibit different toxicities, efficacy of recruitment of cellular α-syn and persistence/propagation propensity. They were also shown to imprint their distinct intrinsic structures to the recruited cellular α-syn [45] (Fig. 2).

The finding that distinct α-syn strains bind to cells and are taken up with different efficiencies suggest that they may exhibit different tropism for cells from the central nervous system, in other words, bind with different affinities to neurons, glial cells or astrocytes. By targeting different cells from the central nervous system or circuits, they may be at the origin of distinct synucleinopathies. As distinct strains differ by their surface properties they also may trigger the inflammatory response to different extents. Finally, the progression of distinct synucleinopathies may also reflect the different seeding propensity, resistance to clearance and persistence in cell cultures of distinct α-syn strains.

Baffling evidence for the existence of α-syn strains *in vivo* were brought by the groups of S. Prusiner, E. Bezard and M. Vila who demonstrated that semi-purified aggregated α-syn from patients developing MSA or PD faithfully led to MSA or PD-like neuropathological disorders in recipient animals [34, 84].

Additional evidence for the existence of putative α-syn strains came from the lesion profiles observed after injection of either newly formed α-syn fibrils or repeatedly seeded α-syn fibrils and the differential
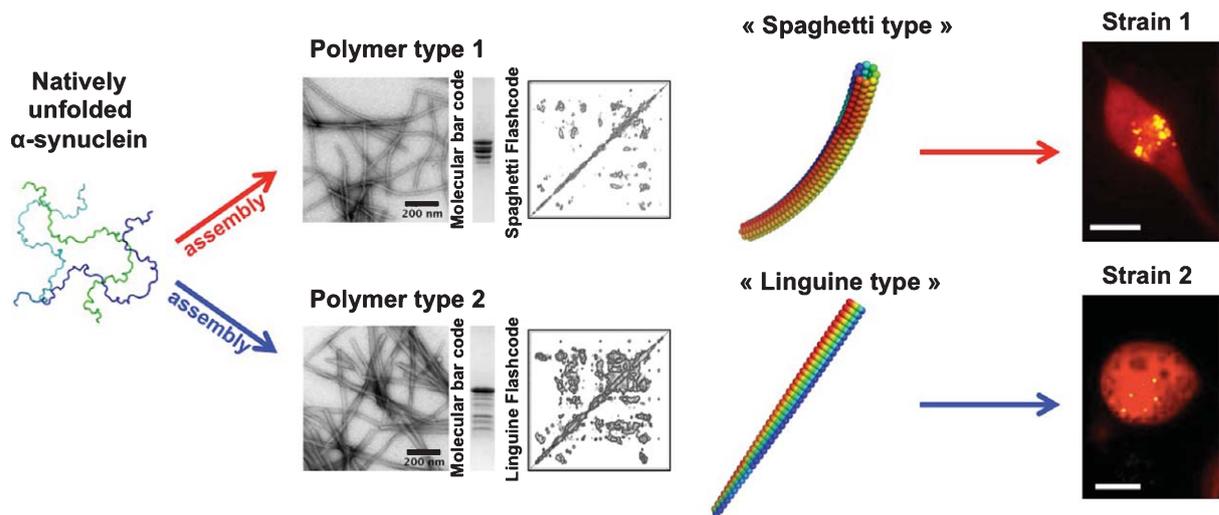

Fig. 2. Alpha-synuclein strains. Natively unfolded α-syn (3 different conformations are presented), assemble into polymers that look unlike in the electron microscope. One type of polymers has the shape of spaghetti, while another type has the shape of a flat linguine. The two assemblies have distinct limited proteolysis degradation patterns (molecular bar codes) and solid-state NMR spectra (structural fingerprint). The two kinds of polymers made of the same protein have different functional properties (toxicity, binding propensity to cells, seeding capacities and persistence). They imprint their intrinsic structure to the soluble α-syn they recruit in cells. They behave therefore as two distinct strains.

induction of Tau aggregation by the two kinds of assemblies [85]. It also came from comparing the proteinase K degradation profiles of sarkosyl-insoluble protein fractions isolated from patients diagnosed PD or PD and AD because of abundant tau neurofibrillary tangles and senile plaque [85]. While one cannot rule out that the differences in the degradation profile of aggregated α-syn are due to the abundance of tau neurofibrillary tangles and A-beta these findings remain striking [85].

Recently, amyloid plaques extracted from the brain of two patients developing AD, with distinct neuropathology and clinical history, were used to seed the aggregation of Aβ *in vitro*. The resulting fibrils exhibited somewhat different shapes as judged by electron microscopy observations. They also yielded distinct solid-state NMR spectra [86]. Although Aβ aggregation in control brain extracts from healthy aged individuals and patients developing unrelated neurodegenerative diseases was not assessed, these observations suggest that Aβ within the initial seeds recruit soluble Aβ *in vitro* and imprint their structures to the synthetic, isotopically labeled, Aβ. Further evidence for the existence of Aβ strains came from the use of tightly controlled assembly conditions *in vitro* of synthetic Aβ1–40 and 1–42 [87]. Starting from the observation that synthetic fibrillar Aβ1–40 recruits when injected into the brain of recipient animals both Aβ1–40 and Aβ1–42 while synthetic fibrillar Aβ1–42 induce the aggregation of Aβ1–42, it was shown that slightly different assembly conditions lead synthetic fibrillar Aβ1–42 to stably acquire the seeding properties characteristic of fibrillar Aβ1–40.

Evidence for the existence of Tau strains have been brought through the use of truncated Tau. When exposed to the fibrillar form of truncated Tau, cells expressing the same truncated Tau fused to a reporter fluorescent protein develop truncated Tau clusters of different shapes. The pattern of these clusters is stably inherited over generations and transmissible to naïve cells. When lysates from cells exhibiting distinct clusters were injected into the brain of recipient model animals, they induced distinguishable neuropathological patterns that could be stably propagated in mice. Finally, brain homogenate from patients developing different tauopathies induced the clustering of reporter Tau in cultured cell lines. Reporter Tau inclusion morphology and limited degradation patterns revealed homogeneous for most pathological cases with exceptions suggesting that a number of patients may suffer from more than one Tau strain [49].

PROPAGATION OF Aβ, TAU AND α-SYN STRAINS AND DISEASE MODIFYING STRATEGIES

Symptomatic treatments are the only therapeutic tools available for patients developing AD and PD. In PD, for example drugs aiming at restoring dopamine levels, such as L-DOPA, dopamine agonists, MAO-B and COMT inhibitors, although powerful, lose efficiency and occasion side effects with time [88–90]. Invasive interventions, such as deep-brain stimulation, are implemented when drugs fail to relieve the patients. They are used when neurodegeneration has become sufficiently severe to induce a clinical phenotype. The discovery that Aβ, Tau and α-syn fibrillar assemblies transit between cells within the central nervous system and amplify by recruitment of their soluble counterparts in recipient cells, thus contributing to disease progression, suggests that pathogenesis starts many years before the first symptoms become apparent. Targeting early enough protein assemblies involved in disease formation and propagation may therefore represent a novel and effective preventive therapeutic avenue. Thus, in compliment to the classical approaches aimed at either inhibiting the initial aggregation of proteins or favoring their clearance by boosting the level of molecular chaperones within the cells and/or the ubiquitin-proteasome clearance machinery, or both, therapeutic strategies targeting disease-associated protein assemblies propagation and seeding capacities become highly relevant.

Small molecules such as the polyphenols rottlerin, curcumin or Epigallocatechin-3-gallate have been shown to either interfere with the aggregation of α-syn, stimulate proteostasis, activate the ubiquitin-proteasome clearance machinery or counteract the neuro-inflammatory response associated to α-syn aggregation [91–93]. A novel class of small molecules that repair proteostasis networks specifically through HSF-1, FOXO and Nrf-2 and restore proteome balance has been identified [94]. These molecules have a promising therapeutic potential in a variety of protein conformational diseases as they enhance cell stress pathways and chaperone activity [95]. They protect therefore the cells in a generic manner against misfolding and/or aggregation and the resulting pleiotropic damages. In addition, strategies aimed at protecting and/or stimulating neuronal cells through the use of neurotrophic factors such as brain-derived neurotrophic factor [96] or clearing the polypeptides associated to neurodegeneration prior to their aggregation/deposition through immunotherapy

are explored [97, 98]. Finally, neural stem cell-based treatments for neurodegenerative diseases are considered [99].

In the case of PD, innovative strategies aimed at interfering specifically with α-syn assemblies propagation and seeding capacities have not yet been developed to a stage where they can be used for therapeutic purposes. The key step in this process is the docking of α-syn assemblies to the cell. One can interfere with this process either by i - perturbing the binding capacity of extrinsic membrane proteins that interact with α-syn assemblies or ii - changing the surface properties of α-syn assemblies so that they become incapable of binding to their receptors. As no specific receptors of α-syn assemblies have been identified so far, the strategy consisting of rendering those receptors unavailable for binding α-syn assemblies is impossible. Furthermore changing the binding capacities of these receptors may cause undesirable pleiotropic effects. Designing α-syn assemblies binders that change their surface properties in such a way that they either become unable to bind the cell surface or incapable of elongating by recruitment of the endogenous protein because their ends are capped appears more promising. Two classes of binders, molecular chaperones and antibodies, have been shown to interfere with α-syn assemblies uptake by cells and/or toxicity [100, 101]. These molecules cross the blood-brain barrier with difficulty because of their size. Thus, identifying within these molecules what is necessary and sufficient for binding is critical for the design of smaller molecules with therapeutic potential. The interaction interfaces between the molecular chaperone Hsc70 client protein binding site and α-syn have been identified through the use of chemical cross-linkers and mass spectrometry [102, 103]. The same strategy can be employed to identify paratopes within antibodies exhibiting high affinity for α-syn. Peptides reproducing Hsc70 or anti-α-syn paratopes that bind with the highest affinity α-syn assemblies represent potential therapeutic candidates.

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