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Nucleic acid induced protein aggregation and its role in biology and pathology

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1. ABSTRACT

Neurodegenerative diseases are characterized by the deposition in a variety of tissues of specific proteins as aggregated species that share a distinctive fibrillar or amorphous structure. Although amyloid inclusions (deposits) are predominantly proteinaceous, careful examination of diseases tissues has revealed the presence of a significant quantity of other species, such as nucleic acids and/or polysaccharide species, associated with the inclusions. Recently, both DNA and RNA have been shown to be able to stimulate formation of fibrillar or amorphous aggregates *in vitro* by alpha-synuclein, tau protein and prion proteins, and to act as a template for accelerating the aggregation of copper/zinc superoxide dismutase. Although the specificity and nature of interactions between disease-linked proteins and nucleic acids are controversial, the sites of interactions involved should be the positively charged surface motifs on the proteins. This review will mainly highlight the important progress in studies on the nucleic acid-induced structural conversions and aggregation of the proteins linked to neurodegenerative diseases. Thereby, we attempt to understand biological and pathological implications of nucleic acid-induced protein aggregation.

2. INTRODUCTION

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson disease (PD), prion disease, and amyotrophic lateral sclerosis (ALS) are characterized by the deposition in a variety of tissues of specific proteins as aggregated species that share a distinctive fibrillar or amorphous structure (1−3). It has been postulated that environmental conditions enriched with aggregation materials could act as a risk factor for neurodegenerative diseases (4). For example, injection of the recombinant mouse prion protein in the form of amyloid-like fibrils has been reported to cause diseases in mice that express the prion protein (5), indicating the presence of environmental conditions that are able to induce protein aggregation *in vivo* and *in vitro*. Although amyloid inclusions (deposits) are predominantly proteinaceous, careful examination of diseases tissues has revealed the presence of a significant quantity of other species, such as nucleic acids and/or polysaccharide species, associated with the inclusions. In the brain tissues from victims of AD, nucleic acids have been detected in neurofibrillary tangles, intracellular inclusions primarily composed of the tau protein, as well as in senile plaques composed of the amyloid-beta peptides (A-beta) (6). Moreover, it has been shown that RNA is able

to stimulate the aggregation of tau protein (7, 8) and to induce the conversion of cellular prion protein (PrP^{C}) to its infectious scrapie form (PrP^{Sc}) prone to aggregation (9), while DNA can promote formation of fibrillar or amorphous aggregates *in vitro* by alpha-synuclein and prion proteins (10−12). We have also indicated that DNA is a template for accelerating the aggregation of copper/zinc superoxide dismutase (SOD1) (13). On the other hand, significant interactions *in vitro* occur between amyloid fibrils and nucleic acids and their relevant species (14). Furthermore, it has been observed that DNA can bind strongly after cell lysis to the amyloid-like Curli fibrils of *E. coli (*15), and ATP has been found to promote the formation of fibrils of A-beta peptide and amylin (16, 17).

However, nucleic acids are not the only species found to be associated with amyloid inclusions. The polysaccharide species have also been shown to be associated with the proteinaceous inclusions. The polysaccharides belong to the glycosaminoglycan family and are long unbranched chains of repeating disaccharide units. Among these species, heparan sulfate is the most common, being found in a variety of amyloid disorders including AD, type II diabetes, light chain amyloidosis, and the prion related diseases (18−20). While direct binding of heparan sulfate or heparin, the hypersulfated and negatively charged form of heparan sulfate, to soluble amyloid precursor proteins has been established in many cases, the ability of these polymers to promote fibrillogenesis has been investigated for only a few peptides or proteins, such as the A-beta peptides (21, 22), tau protein (7, 23), alpha-synuclein (24), and beta2-microglobulin (25).

The majority of negatively charged species that have been shown so far to be associated with the proteinaceous inclusions in the tissues of patients with amyloid diseases are ubiquitous polyanions including nucleic acid and polysaccharides. Although the degree of specificity and the nature of interactions involved are controversial, the sites of interactions with the polyanionic species should be the positively charged surface motifs on the disease-linked proteins. This review will mainly focus on the important progress in studies on the nucleic acid-induced structural conversions and aggregation of the proteins linked to prion disease, PD, ALS and AD, as well as the interactions of the proteins with nucleic acids. The disease-linked proteins are termed disease proteins here. We also attempt to highlight the forces accelerating aggregation of disease proteins in the presence of nucleic acids, and to understand biological and pathological implications of nucleic acid-induced protein aggregation.

3. NUCLEIC ACID SEQUESTRATION TO PATHOLOGICAL TISSUES

Although the polypeptide composition in proteinaceous aggregates and/or inclusions has been characterized extensively within neurodegenerative disease brain, few data exist on the nonproteinaceous components of pathological lesions of related neurodegenerative disorders. The localization of cytoplasmic RNA species to neurofibrillary tangles (NFTs) and senile plaques (SPs) was demonstrated within the AD and Down's syndrome (DS) brain by acridine orange (AO) histochemistry (26). AO histofluorescence measurements, in combination with immunohistochemistry and thioflavin S (THS) staining, allowed for the selective delineation of cytoplasmic RNA species in pathological lesions from several neurodegenerative disorders, providing insight into the non-proteinaceous components of these lesions (6). RNA appears to bind to the insoluble filaments of NFTs and SPs, (for example, hyperphosphorylated and paired helical filament tau protein (PHF-tau) in NFTs, and dystrophic neurites and A-beta-containing fibrils in SPs), and this may have functional sequelae for the accumulation and/or deposition of these inclusions. The *in vitro* studies have shown that the RNA binding facilitates the aggregation of recombinant tau protein into PHFs (7, 8).

To establish whether the entrapment of RNA is a common feature of lesions found in other progressive neurodegenerative disorders or not, Ginsberg *et al.* used both AO histochemistry alone and in combination with immunohistochemistry and THS staining to probe brain tissues obtained from patients with the following diagnoses: AD (to further assess AO localization to NFTs and SPs by confocal microscopy), ALS/parkinsonism-dementia complex of Guam (PDC), corticobasal degeneration (CBD), diffuse Lewy body disease (DLBD), normal age-matched controls (CTR), multiple system atrophy (MSA), PD, Pick's disease (PiD), progressive supranuclear palsy (PSP), and Shy-Drager syndrome (S-D) (6). The assays were performed on tissue sections from selected brain regions for each neurodegenerative disorder based upon the cellular and regional localization of the neuropathology. For example, the hippocampal formation of AD patients was assessed due to the high abundance of NFTs and SPs as well as Hirano bodies (HBs) in this region (6, 27, 28), whereas Lewy bodies (LBs) were assessed within the anterior cingulate cortex of DLBD patients and substantia nigra of PD patients (29, 30). The data show that although NFTs and neuritic SPs contain AO labeling in all of the neurodegenerative disorders evaluated here, other lesions including LBs and HBs demonstrated little evidence of RNA localization. These observations demonstrate the selective localization of RNA species to distinct pathological lesions of neurodegenerative diseases and may have implications for the design of future studies of the molecular mechanisms leading to the formation of these pathological structures and their role in the onset and progression of human neurodegenerative diseases (6).

TAR-DNA-binding protein-43 (TDP-43) is a relatively unknown RNA-binding protein and predominantly located, under normal conditions, in the nucleus, where it was reported to regulate transcription, alternative splicing, and mRNA stability (31). *In vivo* and *in vitro* experiments have showed that TDP-43 interacts with the 3′-UTR of hNFL mRNA, and overexpression of TDP-43 in cell lines stabilizes hNFL transcript (32). The RNA-binding domain of TDP-43 resides close to the N-terminus. Since 2006, the protein was reported to be present in inclusions within the neurons and/or glial cells of a range of neurodegenerative diseases, including AD, ALS,

and frontotemporal lobar degeneration (FTLD) with ubiquitin-positive, tau- and alpha-synuclein-negative inclusions (33−37). One common characteristic of the ubiquitinated inclusions (UBIs), mostly cytoplasmic, in the neurons and/or glial cells in the affected CNS regions of FTLD, sporadic ALS and non-SOD1 familial ALS patients is the presence of poly-ubiquitinated TDP-43, phosphorylated TDP-43 and C-terminal fragment (s) of TDP-43, all of which are undetectable in extracts of the normal cells prepared under the same conditions. In fact, the range of occurrence of the TDP-43-positive inclusions among the neurodegenerative diseases is wider than initially thought. More recent immunohistochemical studies have also identified TDP-43-positive inclusions, albeit with much lower frequencies, in the brains of some cases of AD (38, 39), CBD, dementia with Lewy bodies (DLB) (40, 41), Guam-PDC (42), Guam-ALS (43) and even hippocampalsclerosis (HpScl) (44). Although variations in the composition of the different forms of TDP-43 polypeptides were found in the inclusions of these different neurodegenerative diseases, these differences probably resulted from the disturbance of certain common cellular pathway (s) in combination with the different clinical and pathological spectra of these diseases. Another interesting question is related to the different cellular locations of the TDP-43-containing aggregates in the different neurodegenerative diseases featuring TDP-43-positive UBIs. For instance, the ubiquitinated and TDP-43-positive inclusions of subtype 4 FTLD are mostly intranuclear, whereas in other subtypes of FTLD, as well as in ALS, these inclusions are mainly located in the cytoplasm (45, 46). It is not yet clear whether the abnormal distribution of TDP-43 and its derivatives in the different subcellular compartments resulted from malfunctioning of the cytoplasmic processing pathways or the nuclear import and export mechanisms in the diseased cells. In addition, whether the TDP-43-positive inclusions contain the TDP-43 bound nucleic acid molecules or not remains to be investigated.

4. A BRIEF SURVEY OF AGGREGATION OF DISEASE PROTEINS

A broad range of human diseases arises from the failure of a specific peptide or protein to adopt, or remain in, its native functional conformational state. The largest group of these diseases is associated with the conversion of specific peptides or proteins from their soluble functional state ultimately into highly organized aggregation. These structures are generally described as amyloid fibrils or plaques when they accumulate extracellularly, whereas the term “intracellular inclusions” has been suggested as appropriate when fibrils morphologically and structurally related to extracellular amyloid form inside the cell (1). It is also becoming clear that aggregation species with amyloid characteristics can serve a number of biological functions in living organisms, provided they form under controlled conditions. Perhaps, the most fascinating of these functions lies in the ability of such structures to serve as transmissible genetic traits distinct from DNA genes.

The proteins found as intractable aggregates under pathological conditions do not share any obvious sequence identity or structural homology to each other. Considerable heterogeneity also exists as to secondary structure composition or chain length. Interestingly, some amyloid deposits *in vivo* and fibrils generated *in vitro* have both been found to include higher-order assemblies (47, 48). On the other hand, the polymorphism of protein aggregates is very remarkable. Even before the molecular structures of amyloid fibrils began to emerge, it was clear that significant morphological variation can exist between different aggregates formed from the same peptide or protein (49, 50). Evidence is now accumulating that such variations in morphology is linked to heterogeneity in molecular structure, i.e., in the structural positioning of the polypeptide chains within the fibrils.

Comparison of the information about the structural properties of various aggregation systems can give us a number of tentative conclusions about their similarities and differences. Different aggregates clearly have many properties in common, including the canonical cross-beta structure and the frequent presence of repetitive hydrophobic or polar interactions along the fibrillar axis. The ubiquitous presence of a cross-beta structure strongly supports the view that the physicochemical properties of the polypeptide chain are the major determinants of the fibrillar structure in each case. Moreover, several of the proposed structures, despite very different sequences of their component polypeptides, suggest that the core region is composed of two to four sheets that interact closely with each other. An interesting feature of these sheets is that they appear to be much less twisted than expected from the analysis of the short arrays of beta-strands that form beta-sheets in globular protein structures (1). Nevertheless, it is clear that there are significant differences in detail attributable to the influence of the side chains on the structures adopted by the various systems. These appear to include the lengths of the beta-strands and whether they are arranged in a parallel or antiparallel arrangement within each sheet, and the number of beta-sheets in the protofilament.

The structures that will normally be adopted in the aggregates will be the lowest in free energy and the most kinetically accessible. What is clear, therefore, is that the interactions of the various side chains with each other and with environmental conditions are crucial in determining the variations in the aggregate architecture even though the main-chain interactions determine the overall framework within which these variations can occur. In other words, the interactions and environmental conditions involving the side chains in a given sequence can tip the balance between the alternative arrangements of a polypeptide chain in its aggregation structure. Such a situation contrasts with that pertaining to the native structures of the highly selected protein molecules, which are able to fold to unique structures that are significantly more stable for a given sequence than any alternatives (1).

The full elucidation of the aggregation process of a protein requires the identification of all the

conformational states and oligomeric structures adopted by the polypeptide chain during the process and the determination of the thermodynamics and kinetics of all the conformational changes that link these different species. This implies characterizing each of the intermediates in molecular detail and identifying the residues or regions of the sequence that results in the various aggregation steps. The identification and characterization of oligomers preceding the formation of well defined aggregates is of particular interest, both because of an increasing awareness that these species are likely to play a critical role in the pathogenesis of protein deposition diseases and because of their likely role in the mechanism of aggregate formation (1).

The protein oligomers prior to appearance of amyloid fibrils have been termed protofibrils, a structured aggregate (51, 52). Protofibrils from A-beta can bind Congo red (CR) and thioflavin T (THT), contain an extensive beta-sheet structure (53), and, in the form of the smaller spherical species, are made up of ~ 20 molecules (54). Analogous spherical and chain-like protofibrillar structures have been observed for many other systems including alpha-synuclein (55). Both the 40 and 42 residue forms of A-beta have been shown to exist as soluble oligomers in rapid equilibrium with the corresponding monomeric forms. These oligomers preceding fibril and protofibril formation appear to be composed of 2−4 and 5−6 molecules for A-beta1−40 and A-beta1−42, respectively, and circular dichroism (CD) measurements suggest that they are relatively disorganized (56). Interest in these low-molecular-weight oligomers has been particularly intense as species of this type have also been detected in the brains of AD patients (57) and in the lysates and conditioned media of cultured cells expressing the A-beta protein precursor (58).

It is widely established that amyloid fibril formation has many characteristics of a “nucleated growth” mechanism. The time course of the conversion of a peptide or protein into its fibrillar form (measured by THT fluorescence, light scattering, or other techniques) typically includes a lag phase that is followed by a rapid exponential growth phase (59, 60). The lag phase is assumed to be the time required for “nuclei” to form. Once a nucleus is formed, aggregate growth is thought to proceed rapidly by further association of either monomers or oligomers with the nucleus. It has been shown that changes under experimental conditions, or in certain types of mutations, can also reduce or eliminate the length of the lag phase, again assumed to result from a situation wherein nucleation is no longer rate limiting (61). The absence of a lag phase, therefore, does not necessarily imply that a nucleated growth mechanism is not operating, but it may simply be that the time required for aggregate growth is sufficiently short relative to the nucleation process and that the latter is no longer the slowest step in the conversion of a soluble protein into the amyloid state. Although fibrils do not appear to a significant extent during the lag phase, it is increasingly clear that this stage in aggregate formation is an important event in which a variety of oligomers form, including beta-sheet-rich species that provide nuclei for the formation of mature aggregate.

Aggregation of globular proteins including SOD1 can occur both via partial unfolding and via formation of native-like oligomers. It is generally believed that globular proteins need to unfold, at least partially, to aggregate into amyloid fibrils (62, 63). It is clear that globular proteins have an increased propensity to aggregate under conditions that promote their partial unfolding, such as high temperature, low pH, mutation, molecular crowding, neutralization of charges, complexation of metal ions, oxidative damage to proteins or moderate concentrations of organic solvents (13, 64−70). There is clear evidence that destabilization of the native structure, resulting in an increase in the population of nonnative states, is the primary mechanism through which natural mutations mediate their pathogenicity (71, 72). However, recent observations have suggested that in some cases the major conformational change associated with amyloid aggregation may not take place until after the initial aggregation step. Formation of amyloid aggregates by insulin at low pH, e. g., is preceded by an oligomerization step in which a native-like content of alpha-helical structure is almost completely retained, and aggregates with a morphology reminiscent of amyloid protofibrils and with a high content of beta-structure appear only later in the process (73). The different features between the aggregation processes reveal that polypeptide chains can adopt a multitude of conformational states and interconvert between them on a wide range of timescales. Despite their apparent differences, there are in fact substantial similarities between the fundamental mechanism of aggregation described here for folded proteins and that of natively unfolded systems, such as A-beta. In both cases, the polypeptide molecules assemble first into species that can have characteristics far from those of the final aggregates but similar to those of the precursor structures, whether natively unfolded or natively folded. The initial oligomers then transform into species that are not yet fibrillar in their morphologies but have other properties characteristic of amyloid-like structures, notably beta-sheet structure and binding to CR and THT. Clearly, fully or partially unfolded states of globular proteins are generally more susceptible to aggregation than the native states. Nevertheless, in some situations, particularly those close to physiological, the much higher populations of the latter can result in a consequence that they could play an important role in initiating an aggregation process that might be significant on the very slow timescales of the amyloid disorders (1).

5. INTERACTION BETWEEN DISEASE PROTEINS AND NUCLEIC ACIDS

Proteins interact with nucleic acids to form nucleic acid-protein complexes that mediate hosts of important cellular processes. Nucleic acid-protein complexes are at heart of many processes that include DNA duplication, recombination and repair, gene transcription and its regulation, pathology, and viral infection. Although the interactions between proteins and nucleic acids are

dynamic, and the mechanisms of formation of nucleic acid-protein complexes are different and complex, there is a restricted known set of the structure-function relationships involved. Protein binding of DNA/RNA sequences involves a wide range of contacts including nonpolar, direct, and indirect (water-mediated) hydrogen bonding, hydrophobic and π-π stacking interactions with nucleic acid bases, and direct (for example, electrostatic and van der Waals forces) and water-mediated contacts with the sugar-phosphate backbone of the nucleic acid. Up to date, there are no data that support a simple general mode for protein-nucleic acid recognitions, because the structural motifs including helix-turn-helix and zinc finger modulus contains alpha-helices, beta-sheets and loops. Therefore, the association of a protein molecule with a nucleic acid molecule may be sequence-specific, and also non-sequence-specific.

The interactions between disease proteins susceptible to aggregation and nucleic acids have been reported so far only for a few cases. The question of which nucleic acids (single- or double-stranded RNA or DNA) can serve as the natural partners of disease proteins, and indeed what might be the cellular function of the resulting complex, are targets for future research.

It is well-known that the primary function of SOD1 is to maintain the steady-state concentration of the superoxide anion as an antioxidant enzyme. It has been found that SOD1 involves ALS development, and causes motor neuron death through an as-yet unidentified molecular mechanism (74, 75). The concomitant presence of SOD1, nucleic acids both in the cytosol raises the possibility of formation of different assemblies between them. In fact, the stable union of SOD1 and DNA has been demonstrated *in vitro* by the affinity determination of SOD1 for DNA performed with UV-vis absorption titration of calf thymus DNA (ctDNA) with SOD1 and quenching of intrinsic fluorescence by ctDNA. The data show that the apparent affinity (K_d) of SOD1 for DNA is ~ 10 μM at pH 7.4 and 0.5 μM at pH 3.6, respectively, indicating that the affinity is dependent on the solution pH *in vitro* (13, 76). The calculation of electrostatic potential has revealed some striking positively charged regions over SOD1 surfaces under neutral conditions (77), suggesting that SOD1 may be a potential DNA-binding enzyme, and the presence of positively charged residue side chain-containing peptide segments could meet the requirement for DNA binding. The nonspecifically electrostatic interaction may occur between the positively charged residues over SOD1 surfaces and the negatively charged DNA backbone. Low pH can result in an increase in the net positive charge on SOD1, strengthening the binding of SOD1 to DNA. The SOD1 bound to DNA shows a nucleolytic activity similar to DNase I in the presence of added divalent metal ions (76, 78).

PrP is a conserved protein in vertebrates, but its function remains to be identified. Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases associated with the accumulation of a protease-resistant form of PrP (79). PrP can interact with a number of molecules including large nucleic acids and possibly with retroviral RNA (12, 80, 81). It has been shown that nanomolar affinities are observed for some complexes with DNA or RNA (82) and binding of nucleic acids to PrP^C can compete with binding of anilinenaphthalene sulfonate compounds (83). On the other hand, it has been shown that prions able to infect wild-type hamsters can be formed from a minimal set of components including native PrP^C, copurified lipid and poly (A) RNA molecules (84). A study has also suggested that in a mouse model the scrapie infectious process can be accelerated by MuLV replication (85). Therefore, the intriguing nucleic acid-binding features of PrP have attracted many research groups' attention (11). The interaction of PrP with retroviral RNA *in vitro* results in the formation of nucleoprotein complexes that appear to be similar to retroviral nucleocapsid-DNA complexes. Also, PrP can be recovered in association with retroviral particles (86, 87). Moreover, *ex vivo* experiments identified PrP^{Sc} bound to chromatin in the nuclei of scrapie-infected cells (88). These findings prompted Gabus and Adler *et al.* to investigate in details the interactions of human and ovine PrP with viral nucleic acids and compare these interactions with that of the retroviral nucleocapsid proteins (89−91). Gabus *et al.* used the well-characterized nucleocapsid protein HIV NCp7 and genomic RNA sequence necessary for the synthesis of both viral DNA strands by reverse transcriptase. The results showed the recombinant human PrP closely resembles NCp7 with respect to binding viral DNA with the formation of nucleoprotein complexes *in vitro*. The DNA-binding property of human PrP appear to map to the N-terminal fragment comprising residues 23 to 144, whereas the C-terminal domain is inactive. These findings suggest that PrP could be involved in nucleic acid metabolism *in vivo (*89, 90). On the other hand, Adler *et al.* demonstrated the formation of highly stable nucleoprotein complexes resistant to both proteinase K and ribonuclease A between a small, highly structured RNA and human recombinant PrP or PrP^C from various cell extracts and mammalian brain homogenates under physiological conditions (e.g. 10% bovine calf serum, neutral pH, nanomolar concentrations of RNA and PrP) (91). This RNA might be used to develop molecular biology assays for the screening of compounds associated with PrP structural transformation or for drugs that inhibit this transformation process.

A promising class of compounds with potentials as research tools and the diagnosis or therapy of neurodegenerative diseases are DNA or RNA aptamers or ligands, small structured polynucleotide sequences that can be isolated by *in vitro* selection from randomized oligonucleotide libraries and bind to various disease proteins and their aggregates at the various stages. On the one hand, some RNA aptamers have been generated against the disease-associated conformation of PrP (92) and inhibit conversion *in vitro* of monomer PrP to disease associated PrP^{Sc} (93). Mercey *et al.* has demonstrated the fast and reversible nature of PrP interactions with RNA aptamers containing specific sequence patterns (94). Moreover, the 2′-fluoro-RNA aptamers were observed to have more than 10-fold higher affinity for PrP^{Sc} than for recombinant PrP^C

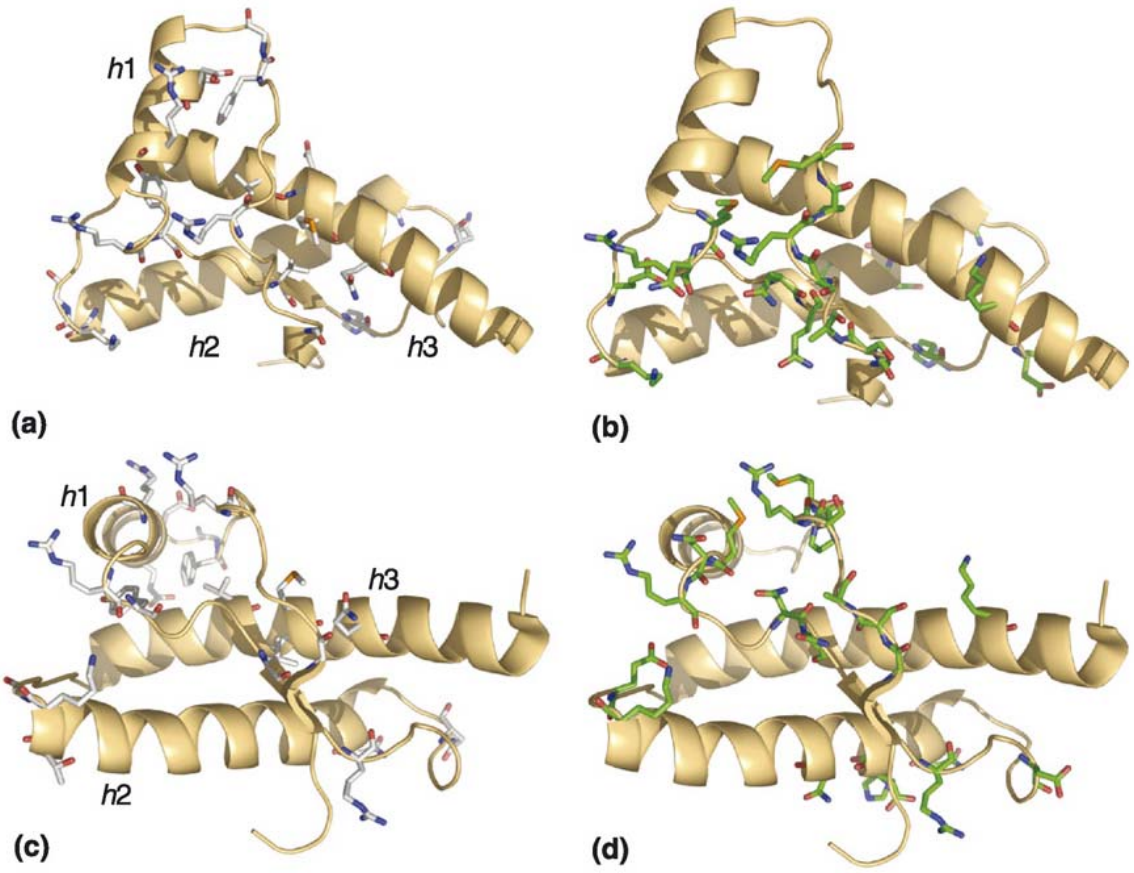

Figure 1. A model proposed for DNA binding of PrP using NMR measurements and predictive analysis (11). Insights into the most probable nucleic acid-binding sites in the PrP have been recently achieved using NMR chemical shift perturbation (101) and computational predictive analysis (102). The figure represents two topological views (upper and lower panels, rotated 90^{o}) of mapped DNA-binding regions of prion protein. (a,c) PrP NMR chemical shift changes induced by DNA binding (101). (b,d) Computer-aided predictive analysis of DNA binding sites in PrP (102). h1, h2 and h3 are the three helices in PrP^{C}. (Reproduced with permission from, ref 11)

and to inhibit the accumulation of PrP^{Sc} in a near physiological cell-free conversion assay (95). The degenerate phosphorothioate oligonucleotides with a potent anti-scrapie activity were also developed (96). Immunization experiments by Kaiser-Schulz *et al.* indicated that both prophylactic and therapeutic immunization can be achieved using the complex of recombinant tandem PrP with CpG oligonucleotide (97). On the other hand, DNA aptamers that bind to PrP^{C} but not to PrP^{Sc} show the sequence or structure-dependent selectivity (93, 98). For example, the interactions of single-stranded DNA thioaptamers with PrP occur on sequence-specific and non-specific binding sites on the PrP (99). The disease-associated DNA-PrP complexes in a wide variety of species and disease phenotypes were found to be specific targets of anti-DNA antibodies and a well established DNA-binding protein (100).

Until recently, although no high-resolution structural data had been obtained for disease proteins complexed to nucleic acids, the low-resolution structure of PrP in the complex with an 18 bp double-stranded DNA, derived from small-angle X-ray scattering (SAXS) and NMR measurements was reported (Figure 1) (11, 101). The full-length mouse recombinant PrP binds to this DNA sequence with an affinity in the nanomolar range at physiological ionic strength. SAXS studies indicate that the C-terminal globular domain of PrP is important in the formation of the complex, and NMR HSQC spectra reveal changes that are clustered in two major regions: one in the disordered N-terminal portion of PrP and the other in the C-terminal globular domain. Recently, an *in silico* approach (102) yielded a prediction of the PrP-DNA binding site consistent with the NMR chemical shift perturbation data (101) and supported the idea that the PrP globular domain recognizes DNA mainly through the helix 1 (see Figure 1). Clearly, higher-resolution structural studies on PrP complexed to nucleic acids are required as the structural description of a PrP-DNA/RNA complex is an important pre-requisite for understanding how oligonucleotides bind PrP and for the design of anti-prion compounds based on nucleic acids.

Nandi *et al.* has shown that binding of PrP can

induces conformational changes in nucleic acid structures (103). The fluorescence resonance energy transfer (FRET) experiments performed with oligonucleotides covalently labeled by the energy donor fluorescein and the acceptor rhodamine moieties and the thermal stability measurements of unlabeled oligonucleotides indicated appearance of significant bending and structural destabilization of the oligonucleotides caused by the prion protein (103). The truncated globular prion protein 121–231 fragment neither induces FRET effect on the oligonucleotides nor destabilizes their structures, indicating that the N-terminal segment of the prion protein is essential for the DNA bending process. Equilibrium binding and kinetics of FRET show that the protein binding to the oligonucleotides and their bending occur simultaneously. The DNA structural changes observed in the presence of the prion protein are similar to those caused by proteins involved in initiation and regulation for protein synthesis. In addition, the prion protein also facilitates DNA strand transfer and acts as a DNA chaperone which is modulated by the N-terminal unstructured basic segment of the protein (89).

Binding of other disease proteins to nucleic acids has been reported rarely. High-affinity RNA aptamers against A-beta40 were isolated from a combinatorial library of ~10^{15} different molecules by systematic evolution of ligands by exponential enrichment (104). The binding of the RNA to amyloid fibrils with 29–48 nM of K_d was confirmed by electron microscopy. The single-stranded RNA molecules have also been found to specifically target to amyloid-like fibrils formed *in vitro* from beta2-microglobulin, the amyloid fibril protein associated with dialysis-related amyloidosis (105).

6. NUCLEIC ACID-INDUCED CONFORMATIONAL CONVERSION OF DISEASE PROTEINS

One of the more interesting characteristics about the interactions between PrP and nucleic acids is the finding that some nucleic acids can stimulate the formation or conversion of PrP conformation (9). In fact, several research groups have clearly demonstrated that the interactions of PrP with DNA or RNA can lead to conformational changes both in the protein and in the nucleic acid molecules (82, 91). In 2001, Silva *et al.* has demonstrated that the high-affinity binding of some small DNAs to recombinant murine PrP (rPrP23–231) converted it from the alpha-helical conformation (cellular isoform) into the soluble beta-sheet isoform, leading them to first propose the hypothesis of nucleic acid-catalyzed PrP conversion (82). Accordingly, host nucleic acids might catalyze the conversion between PrP^C and PrP^{Sc} by acting as a scaffold and thereby making the protein-protein interactions more likely (106). Alternatively, the formation of DNA-PrP complexes could lower the free energy barrier of conversions between PrP^C and PrP^{Sc}, probably by rearrangement of some structural elements on the protein without necessarily passing through the unfolded state. Both nucleic acid binding and conversion occur simultaneously with a decrease in hydration (101, 107, 108), which might explain the bypass of the unfolded state. These mutual coupled rearrangements are typical of nucleic acid chaperones (109). However, Nandi *et al.* also indicated a partial unfolding of PrP fragment in the presence of nucleic acid by thermal denaturation study, and suggested that nucleic acid catalyzes unfolding of PrP helix 1 followed by a nucleation-dependent polymerization of the protein to amyloid (110).

A model has been proposed for nucleic acid-induced prion propagation (Figure 2) (11). The model shows how a nucleic acid molecule can act as a catalyst and/or molecular chaperone for the PrP^C to PrP^{Sc} conversion in a process that does not rely on the encoded genetic information within the nucleic acids (82), and consistent with *in vitro* biophysical studies (12, 82, 108, 110), as well as with the described *infra* fact that polyanions including nucleic acids increase the level of PrP^{res} amplification *in vitro (*9, 84, 111). However, the precise mechanism behind the conversion is still not understood, but the highly and negatively charged environment around the nucleic acids (as in the case of glycosaminoglycans) could contribute to the conversion.

Deleault *et al.* reported that stoichiometric transformation of PrP^C to protease-resistant PrP^{Sc}-like proteins (PrP^{res}, a fraction of the disease-associated PrP^{Sc} that is partially resistant to proteolysis) *in vitro* requires specific RNA molecules (9). Notably, although mammalian single-stranded RNA preparations stimulate *in vitro* amplification of PrP^{res}, double-stranded DNA, RNA:DNA hybrids and RNA preparations from invertebrate species do not, confirmed by treatments with RNase, DNase and the restriction enzyme *Eco*R I. They provided compelling evidence for the PrP-host derived nucleic acid hypothesis in prion replication using the protein misfolding cyclic amplification (PMCA) technique (84). PMCA involves the amplification of PrP^{res} from a small PrP^{Sc} fraction mixed with PrP^C until no more original PrP^{Sc} units are found in the infectious material (112). Using this approach, the authors showed with a preparation containing only native PrP^C and co-purified lipid molecules that successful PMCA propagation of PrP^{Sc} molecules in a purified system required accessory polyanion molecules (synthetic RNA). Moreover, the *de novo* formation of PrP^{Sc} molecules from these defined molecules in the absence of pre-existing prions was observed and inoculation of purified, *in vitro*-generated PrP^{Sc} molecules into hamsters caused disease (84). This work shows that infectious prions can be generated from a minimal set of components including native PrP^C molecules, co-purified lipid molecules, and a synthetic NA, stressing the possible role of nucleic acids in PrP^{Sc} generation. The results corroborate the hypothesis that endogenous polyanions (such as nucleic acids) can affect the rate of prion propagation by acting as scaffolds or surfaces that facilitate the interaction between PrP^C and PrP^{Sc} molecules. The findings suggest that host-encoded stimulatory RNA molecules may have a role in the pathogenesis of prion disease, and also provide a practical approach to improve the sensitivity of diagnostic techniques based on PrP^{res} amplification.

The reports on nucleic acid-induced structural conversion of other proteins linked to diseases are rare so

far. While stable complexes were observed to be formed between SOD1 and DNA, the secondary structures in the SOD1 are not significantly altered, as indicated by CD measurements (13). However, the hydrophobic assays with 8-anilino-1-naphthalene-sulfonic acid (ANS) show that the hydrophobicity of SOD1 is significantly altered upon binding to DNA, suggesting that the tertiary structure of SOD1 is altered to a certain extent.

7. NNULEIC ACID-INDUCED AGGREGATION OF PRION PROTEINS

For TSEs, It has been demonstrated previously that the abnormal forms of PrP vary and are poorly defined structurally, but are most commonly aggregated and more protease-resistant (113). Cellular PrP is a neuronal membrane glycoprotein whose function has not been fully understood. They have been given various names that emphasize associations with disease (for example, PrP^{TSE}), infectivity (PrP^{Sc}), toxicity (PrP^{tox}) and relative protease-resistance (PrP^{res}). In general, PrP^{res} is used to refer to a PrP^{Sc}-like aggregated state that is partially resistant to destruction by protein-digesting enzymes and has the ability to convert the normal, protease-sensitive form of the protein (PrP^{sen}) into PrP^{res} (114).

Although the conversions of PrP^{C} into a wide range of PrP^{res} aggregation states have been suggested to underlie the infection of TSEs (115), the relative pathogenesis with PrP^{res} aggregates remains unclear. Considerable experimental evidence implicates propagation of PrP^{res} and TSE infectivity unlikely rely on replication of a virus or foreign nucleic acid, supporting the 'prion hypothesis' that prion protein is the main agent that causes TSEs (116, 117). Experimental evidence obtained so far fall short of proving that the agent is composed solely of PrP^{res}. Nevertheless, it has been proposed that other host biological macromolecules, such as cellular adhesion molecules, nucleic acids, basal membrane molecules and sulfated glycans, are crucial for prion propagation, either as components or cofactors in the conformational conversion of PrP^{C} to PrP^{res} (11, 82, 111, 118−120). Therefore, we attempt to discuss the role of nucleic acids during the formation of PrP^{res} aggregates, and try to clarify the importance of nucleic acids both in propagation of PrP^{res} and in the occurrence of TSEs.

The most compelling evidence supporting nucleic acid-induced aggregation of PrPs comes from studies of the interaction between PrPs and nucleic acids *in vitro*, indicating that some nucleic acids can induce the formation of PrP^{res} aggregates. Nandi *et al.* first observed a phenomenon that nucleic acids can induce aggregation of recombinant PrP, and demonstrated that in the presence of DNA the murine recombinant prion protein polymerizes to linear amyloid like fibers (121). Up to now, increasing data show that different types of PrPs can aggregate in the presence of nucleic acids in their different forms under solution conditions where PrPs alone do not aggregate. The nucleic acid-induced PrP aggregates have been observed to have different morphologies. For instance, the incubation of murine recombinant PrP^{C} with different forms of nucleic acids, such as double-stranded circular and linearized DNAs, single-stranded DNAs, double and single-stranded RNAs, can result in the formation of aggregates of various morphologies (12, 110, 121, 122). In the initial phase of nucleic acid-induced aggregation of murine recombinant PrP^{C}, the globules of ~2–5 nm in diameter were observed, and after long time incubation of murine recombinant PrP^{C} with different nucleic acids, the various morphologies of linear amyloids and spherical structures are obtained (12). Furthermore, human recombinant PrP^{C} was found to be able to bind to nucleic acids *in vitro*, and the presence of nucleic acids can also induce the aggregation of human recombinant PrP^{C} (103, 123, 124). Although the precise mechanism of nucleic acid-induced aggregation of PrPs is still not completely understood, it has been proposed that the charge neutralization and hydrophobic association occurred between PrPs and nucleic acids, and structural changes in PrPs and nucleic acids caused by their interactions could contribute to the PrP^{res} aggregate accumulation (Figure 2) (83, 101, 121). Consequently, these observations lead the authors to propose that, during the process of PrP-nucleic acid interactions, the conversion of PrP into anomalous structures are important for making PrP^{res} aggregates and predominantly responsible for the formation and stabilization of PrP-nucleic acid aggregates resembling *in vivo* scrapie associated fibrils from infected hamster and Creutzfeldt-Jacob disease (CJD) brains (125).

Nucleic acids might act as a catalyst for the nucleic acid-induced aggregation of PrPs for prion propagation (11). This model is consistent with both *in vitro* and *in vivo* biophysical studies. On the one hand, nucleic acids has been shown to play a role in increasing the level of protease-resistant PrP^{res} aggregate accumulation *in vitro*, leading Silva *et al.* to conclude that the functional activity of the PrP-nucleic acid complexes is associated with prion propagation, as well as nucleic acids might play a cofactor role in prion disease *(*12, 110, 121, 122, 124). On the other hand, Cordeiro *et al.* proposes that nucleic acids can modulate the formation of PrP^{res} aggregate, i. e., nucleic acids would act not only as a guardian of the PrP^{res} conformation to prevent PrP^{res} from propagation, but also may catalyze the PrP^{res} conversion and aggregation if a threshold level is exceeded (11, 82). Moreover, Deleault *et al.* observed the PrP^{res} aggregate accumulated in the nuclei of prion-infected cells independently of proteasome activity, demonstrating that PrP is associated with chromatin *in vivo (*88). In conclusion, all these results support the hypothesis that endogenous nucleic acids may affect the rate of prion propagation by inducing the formation and accumulation of PrP^{res} aggregates during the pathological process of prion disease (11).

8. NUCLEIC ACID-INDUCED AGGREGATION OF ALPHA-SYNUCLEIN

Parkinson's disease is one of the most frequent neurodegenerative disorders affecting brain stem extrapyramidal neurons (for example, dopaminergic substantia nigra neurons) of middle-age individuals (126). It is characterized by muscle rigidity, bradykinesia and resting tremor, which respond well to levodopa treatment.

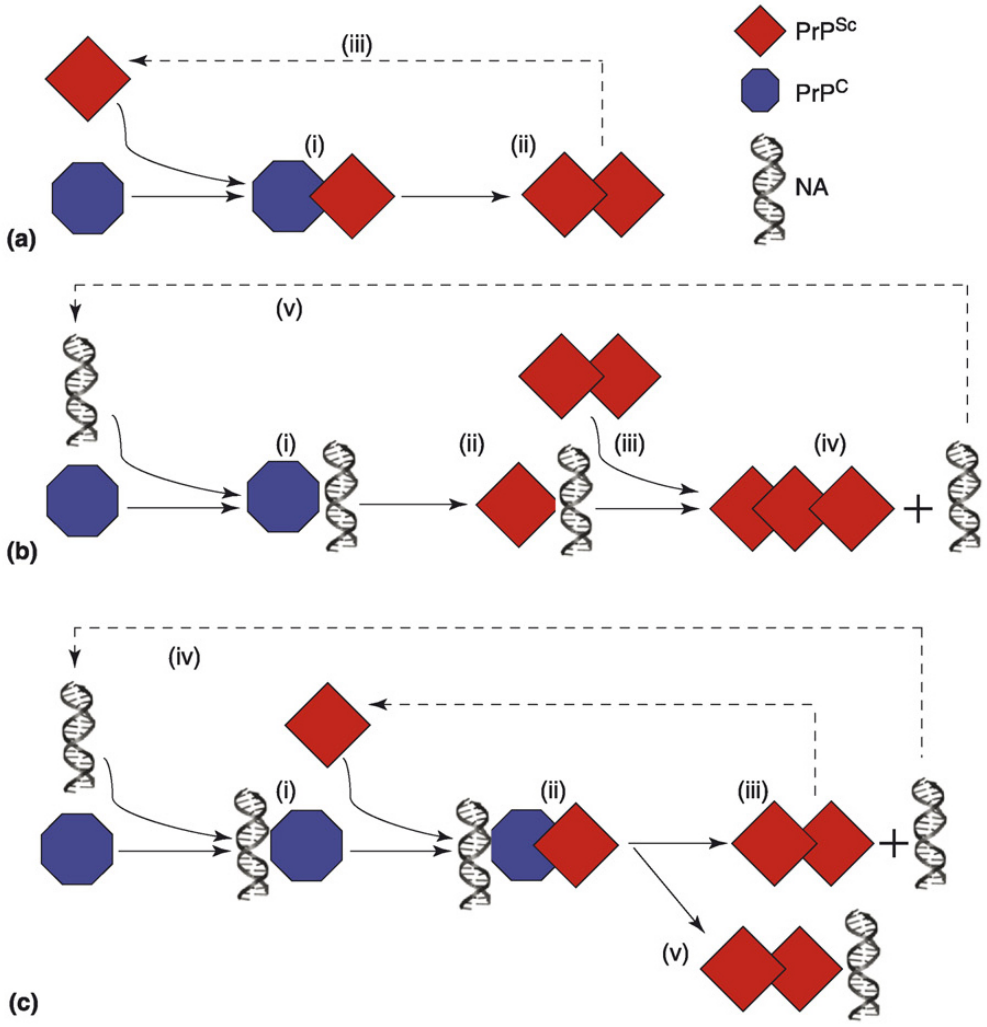

Figure 2. Models proposed for nucleic acid-mediated prion propagation. The models depicte how a nucleic acid (gray) can act as a catalyst and/or molecular chaperone for the PrP^{C} (blue) to PrP^{Sc} (red) conversion (11). (a) The template-assisted refolding model proposed for PrP^{C} conversion to PrP^{Sc}. PrP^{Sc} (exogenous PrP^{Sc} or generated from spontaneous conversion of PrP^{C} into PrP^{Sc}) interacts directly with PrP^{C} (i) and helps the latter to assume the misfolded, PrP^{Sc}-like conformation (ii). Formed PrP^{Sc} is further able to catalyze conversion of more PrP^{C} into the infectious species (iii). (b) Binding of nucleic acid by PrP^{C} (i) induces the acquisition of a PrP^{Sc}-like, beta-sheet-rich conformation (ii). Following the addition of PrP^{Sc} oligomers (iii), an aggregate is formed and the nucleic acid is released from the complex (iv). It is then able to catalyze another conversion event (v). (c) Formation of the PrP^{C}-nucleic acid complex (i) would act as a scaffold for binding of PrP^{Sc} to the protein part of the complex (ii), leading to conversion of the innocuous, nucleic acid-bound form into a scrapie-like conformation, giving rise into the net conversion of more PrP^{Sc} (iii). The nucleic acid could be released from the complex, after PrP^{Sc} formation (iii) being able to further convert PrP^{C} into PrP^{Sc} (iv), or could be irreversibly incorporated into the infectious aggregated particle (v). In the *in vitro* studies, the molar ratio between PrP and nucleic acid dictates the extent of aggregation, so that an apparently irreversible process in the direction of PrP aggregation predominates in a high PrP to nucleic acid ratio. It must be stressed that there are no experimental data to date that compare affinities of PrP^{C} and PrP^{Sc} towards the same nucleic acid. Therefore, the models are tentative. (Reproduced with permission from, ref 11)

The pathological hallmarks of PD are the formation of filamentous inclusions (deposits) known as LBs (127). alpha-Synuclein belongs to the family of "natively unfolded" proteins, exhibiting a random-coil secondary structure in aqueous solution (128). Human alpha-synuclein is a 140 amino acid residue protein, and three distinct regions can be identified in its amino acid sequence. The N-terminal part (residues 1–60) is amphipathic, the central region (residues 61–95) is highly hydrophobic and the C-terminal region (residues 96–140) is very acidic (129, 130). According to its structure, physical properties and interacting partners, several hypotheses have been proposed for the normal function of alpha-synuclein. The observation that alpha-synuclein can exist in three (possibly four) thermodynamic states, i.e., random coil, molten globule, or ordered form, supports the

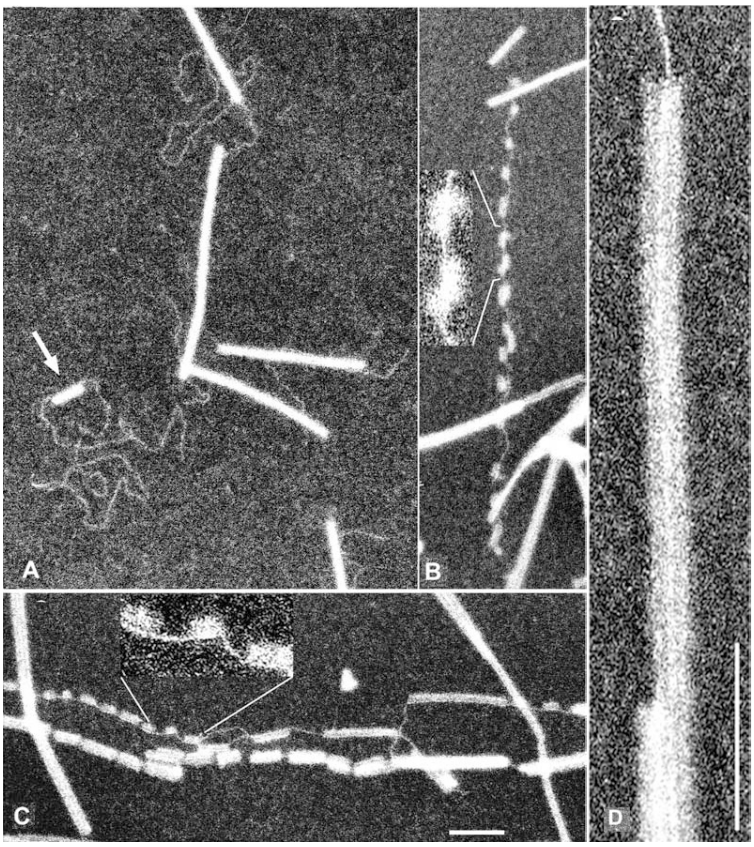

Figure 3. Assembly of wild-type alpha-synuclein into fibrils in the presence of linear DNA (10). (A-C) Electrom microscope images of various types of DNA–fibril complexes formed after the incubation of pPGM1/ScaI DNA (30 μg/ml) with alpha-synuclein (100 μM) at 37 degrees centigrade for several (three to five) days. (D) Electrom microscope images of DNA–fibril complexes formed after the incubation of pPGM1/ScaI DNA (30 mg/mL) with A53T mutant at 37 degrees centigrade for several (three to five) days, the concentration of protein was 100 mM. Images were acquired in an angular dark-field mode. The arrow on A indicates the complex used for length measurements. The scale bar represents 100 nm. (Reproduced with permission from, ref 10)

hypotheses that it plays an important role in molecular recognition (131).

Increasing evidence implicates wild type (WT) alpha-synuclein, its disease-related mutants and some fragments of the WT protein form aggregates *in vitro*, supporting the hypothesis that alpha-synuclein-linked aggregates are implicated in the pathogenesis of PD (130, 132−134). In general, the *in vitro* aggregates are similar to those isolated from disease-affected brains, and they exhibit typical amyloid-like fibrillar morphology (55, 132). Obviously, the aggregation of alpha-synuclein was accompanied by changes in the secondary structures from an unfolded random coil to an antiparallel beta sheet (55, 132, 133, 135). The aggregated states of alpha-synuclein exhibit a great morphological diversity, and the solution conditions appear important for the polymorphism of alpha-synuclein aggregates, presumably because of its the structural flexibility (136, 137). Thus, the pronounced sensitivity of alpha-synuclein aggregation rate and morphology to solution conditions may have critical implications for the pathology of PD. Besides the alpha-synuclein mutations and solution conditions, other reaction conditions such as pH and temperature can also influence the aggregation of alpha-synuclein. Moreover, it has been proposed that other host biological macromolecules, such as heparin and glycosaminoglycans (24), metal cations (136, 137), pesticides (138), anionic species including nucleic acid, fatty acids, and detergents (139), and polyamines (for example, putrescine, spermine, spermidine) (140, 141), can modulate the aggregation of alpha-synuclein, either as components or as cofactors in the interaction with alpha-synuclein.

Jovin *et al.* provided a line of evidence for the interaction of alpha-synuclein with nucleic acids, and proposed that DNA may act as a template and/or nucleation catalyst that facilitates assembly of alpha-synuclein into fibrils (10). They found that double-stranded DNAs, either linear or supercoiled, can interact with WT alpha-synuclein, leading to a significant stimulation of alpha-synuclein assembly into mature fibrils. Electron microscopy reveals that DNA forms various types of complexes upon association with the fibrils at their surface without distortion of the double-helical structure (Figure 3), and the morphology of the DNA-induced fibrils are similar

to the mature fibrils assembled from WT alpha-synuclein in the absence of DNA. The appearance of the structures shown in Figure 3D, in which long individual fibrils were associated side-by-side in a process mediated by the DNA, may be indicative of a preferential position of positive and negative charges on the fibril surface. The association of DNA with the fibrils is relatively strong, conferring upon the DNA a resistance to digestion by restriction endonucleases. According to these results, a plausible scenario is proposed for the DNA stimulatory effect. In solution, protein molecules, presented mainly as monomers, interact electrostatically with DNA phosphate groups. DNA interacts most likely with the positively charged lysine side-chains located primarily in the N-terminal part and the central region of the protein, whereas DNA interaction with the negatively charged C terminus is unlikely. The electrostatic interactions potentially (i) lead to the formation of nonsequence-specific complexes with DNA, and (ii) increase the local concentration of proteins. Both effects, acting in a concerted manner, dramatically increase the probability of dimer formation, the critical nucleation structure needed for fibril growth. In fact, it has been demonstrated previously that alpha-synuclein aggregation is compatible with a nucleation-polymerization model, characterized by an initial lag phase reflecting nucleation and a subsequent growth phase culminating in a steady state (142). Therefore, a possible explanation suggested for nucleic acids is that both electrostatic interactions between alpha-synuclein and nucleic acid molecules and the resulted structural conversions of alpha-synuclein can shorten the lag time and increase growth rate of nucleic acid-induced aggregation of alpha-synuclein. Furthermore, the observation of both interactions between alpha-synuclein and histones (143) and the result of DNA-induced alpha-synuclein aggregation (10) lead the researchers to propose that alpha-synuclein might interact with chromatin in the karyon and this interaction might influence the gene expression. In conclusion, these experimental evidences support the hypothesis that endogenous nucleic acids (also likely including other biological molecules) can induce the aggregation of alpha-synuclein, and then arouse the occurrence of PD.

Currently, the significance of nucleic acid-induced alpha-synuclein aggregation is unclear. However, it is tempting to postulate that alpha-synuclein mutations are toxic simply by accelerating the aggregation of the protein into protofibrils, fibrils and, finally, their deposition in LBs. To sum up, the importance of aggregates for protein deposition diseases has been demonstrated clearly for misfolding diseases (144), therefore, the nucleic acid-induced aggregation of alpha-synuclein into fibrillar structures also may have critical implications for PD.

9. DNA-INDUCED AGGREGATION OF Cu/Zn SUPEROXIDE DISMUTASE

ALS (also known as Lou Gehrig's disease and motor neuron disease) is one of the most common adult neurodegenerative diseases with unknown causes. The hallmark of ALS is the selective gradual degeneration of motor neurons in the cortex, brainstem, and spinal cord (145). Although most cases are classed as sporadic ALS, 10% of cases are inherited (known as familial ALS) (146). Approximately one-fifth of familial ALS cases are associated with inherited mutations in *SOD1*, the gene that locates on chromosome 21 and encodes human SOD1 (146).

Currently, two hypotheses have been proposed to explain the toxicity of ALS mutant SOD1 proteins: the oxidative damage hypothesis and the aggregation hypothesis (147, 148). The oxidative damage hypothesis proposes that ALS mutant SOD1 proteins catalyze reactions with hydrogen peroxide or peroxynitrite that damage cellular components critical for viability of the affected cells (149). Notably, some experimental evidence has shown that oxidative damage to WT SOD1 proteins can lead to the formation of SOD1 protein-containing abnormal aggregates (150, 151). The oligomerization hypothesis proposes that the conformational changes in mutant SOD1 proteins induce the formation of proteinaceous aggregates. This hypothesis can be supported by the observations that proteinaceous fibrils or insoluble inclusions rich in mutant SOD1 protein, ubiquitin, and neurofilament proteins have been found in motor neurons, the neuropil and astrocytes from ALS patients and ALS-SOD1 transgenic mice (75, 152, 153). According to the oligomerization hypothesis, the toxicity mechanism can be suggested as follows: (1) mutant SOD1 proteins are or become misfolded and consequently oligomerize into increasingly high molecular-weight species; (2) during this stage, mutant SOD1 aggregates can bind with other host biological macromolecules, such as other essential proteins, lipid, and nucleic acids; and (3) ultimately, these different biological molecules associate with each other and end up in large fibrils or insoluble inclusions. Moreover, it has been reported that unsaturated fatty acids induce cytotoxic aggregate formation of ALS-linked SOD1 mutants (154). Therefore, the oligomerized or aggregated proteins might be, at some stage in their formation, selectively toxic to motor neurons (149).

Although whether or not the proteinaceous aggregates rich in SOD1 contain nucleic acids has not been reported so far in the *in vivo* assays, we have found that single- and double-stranded DNAs are a template for accelerating the aggregation of SOD1 in an *in vitro* test (13). Firstly, we have examined the aggregation behavior of SOD1 in the presence of DNA under acidic conditions that facilitate protein aggregation. Using the low pH conditions which could mimic the effect of mutations and reflect the practical process occurred under physiological conditions to a high extent and is common to study the aggregation of SOD1 and other proteins (12, 13), several forms of double-stranded DNA were tested to trigger SOD1 aggregation by light scattering, single- and double-fluorescence imaging with dyes, atomic force microscopy, and direct observations under visible light. The data reveal that DNA acts as a template for accelerating the formation of SOD1 aggregates and is incorporated into SOD1 aggregates (Figure 4). The spherical and ellipsoidal SOD1 aggregates were characterized in both hydrated and dried states and have morphology similar to those identified in the diseased neurons. Light scattering experiments indicated that the aggregation first undergoes a rapid phase where the

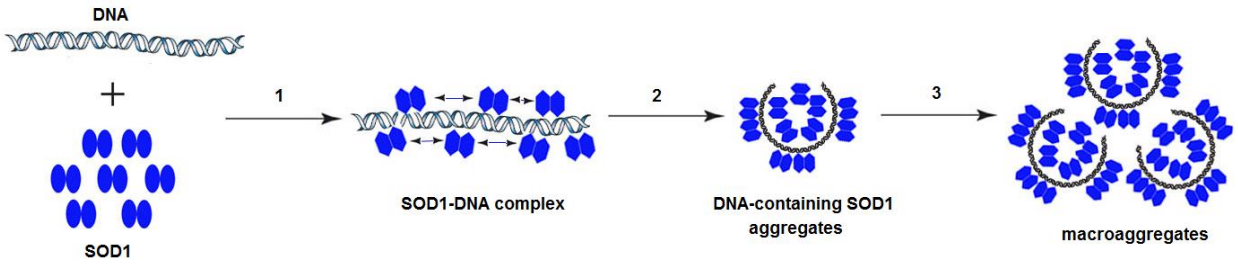

Figure 4. A proposed model of SOD1 aggregation upon addition of DNA (13). There are three key steps for the possible pathways of DNA-induced SOD1 aggregation. First, SOD1 which is converted into an aggregation-prone form under the reaction conditions can bind with DNA via electrostatic interactions. Then, the SOD1 molecules can assemble along DNA double strands and result in the formation of small granular aggregates by hydrophobic interactions. Finally, the granular aggregates can self-cluster into a macroaggregate by hydrophobic interactions.

aggregates with average diameters of 40−80 nm rapidly form in < 2 min, and then passes through a slow phase where the average diameters of aggregates were increased to at least 200−260 nm in 2 h. All forms of DNAs tested can lead to the aggregation of SOD1 at nanomolar levels. The association of SOD1 with DNA, driven by electrostatic interactions between both, can restrict the orientation of SOD1 molecules and increase a SOD1 population along DNA strands. This facilitates the hydrophobic interactions between SOD1 molecules, as indicated by hydrophobic probe binding and chemical denaturant treatment experiments. Demonstration of the DNA accelerated aggregation of SOD1 might establish a possible role of DNA in the pathogenesis of some diseases because of the ubiquitous expression of SOD1 and the coexistence of SOD1 and DNA in the crowded molecular environment of a cell.

Then, in order to support the conclusion that DNA is a template for accelerating the *in vitro* aggregation of SOD1, we sought to explore the aggregation of WT SOD1 exposed to physiologically relevant environments containing ascorbate and DNA under neutral conditions (155). The results showed that the oxidation of SOD1 is dependent on the presence of ascorbate regardless of the addition of DNA. The isoelectric point (p*I*) was increased by ~1 pH unit in the oxidized SOD1 proteins compared to that of the WT proteins. The effects of ascorbate and DNA doses demonstrated that the aggregation is determinated by both the degree of SOD1 oxidation and the presence of DNA. Moreover, we found a clear relationship between the oxidation, enrichment, increased hydrophobicity, and aggregation of SOD1 in the presence of DNA. The crucial step in aggregation is neutralization of the positive charges on some SOD1 surfaces by DNA binding. This study is crucial for understanding molecular forces driving the protein aggregation.

Furthermore, a morphological study on DNA-induced SOD1 aggregates, performed under both the acidic conditions and the neutral conditions similar to physiological state (156), has indicated that the polymorphism of SOD1-DNA aggregates is well modulated by the concentrations, shapes, and structures of the double-stranded DNA templates tested. Electron microscopy imaging shows that the nanometer- and micrometer-scale aggregates formed by SOD1 and DNA fall into three classes: aggregate monomers, oligomeric aggregates, and macroaggregates. The aggregate monomers observed at given DNA doses exhibit a polymorphism that is markedly corresponded to the coiled shapes of linear DNA and structures of plasmid DNA (Figure 5). This aggregation process is driven by both electrostatic interactions between SOD1 and DNA and SOD1 intermolecular hydrophobic interactions. During the process of DNA-induced aggregation of SOD1, the electrostatic and hydrophobic forces first drive the formation of a large number of aggregate monomers; then, the conversion of aggregate monomers through either a direct pathway or oligomeric aggregates into the macroaggregates occurs via the SOD1 hydrophobicity. In addition, we have found that the DNAs are simultaneously condensed into nanometer- and micrometer-scale particles with a specific morphology during SOD1 aggregation, revealing that the SOD1 aggregation and DNA condensation are two concurrent phenomena.

According to our studies mentioned above, a model is proposed to explain the possible pathways of SOD1 aggregation, as shown in Figure 5. The formation of SOD1-DNA complexes with the poorly defined stoichiometry via the electrostatic forces leads to SOD1 enrichment around the DNA double strands. Obviously, this enrichment facilitates the intermolecular contacts and hydrophobic interactions of SOD1. The plausible scenario under neutral conditions also includes the oxidative damage of SOD1 and DNA in addition to these reaction steps.

In summary, the investigations on nucleic acid-induced SOD1 might be in favor of understanding the molecular forces driving SOD1 aggregation, and provide a plausible mechanism for the formation of SOD1-containing aggregates in ALS. Moreover, it also can support the hypothesis that this abnormal interaction with nucleic acids might be a gain of function of SOD1.

10. NUCLEIC ACID-INDUCED AGGREGATION OF OTHER PROTEINS

Alzheimer disease is the most studied and common neurodegenerative disorder (157). More and more

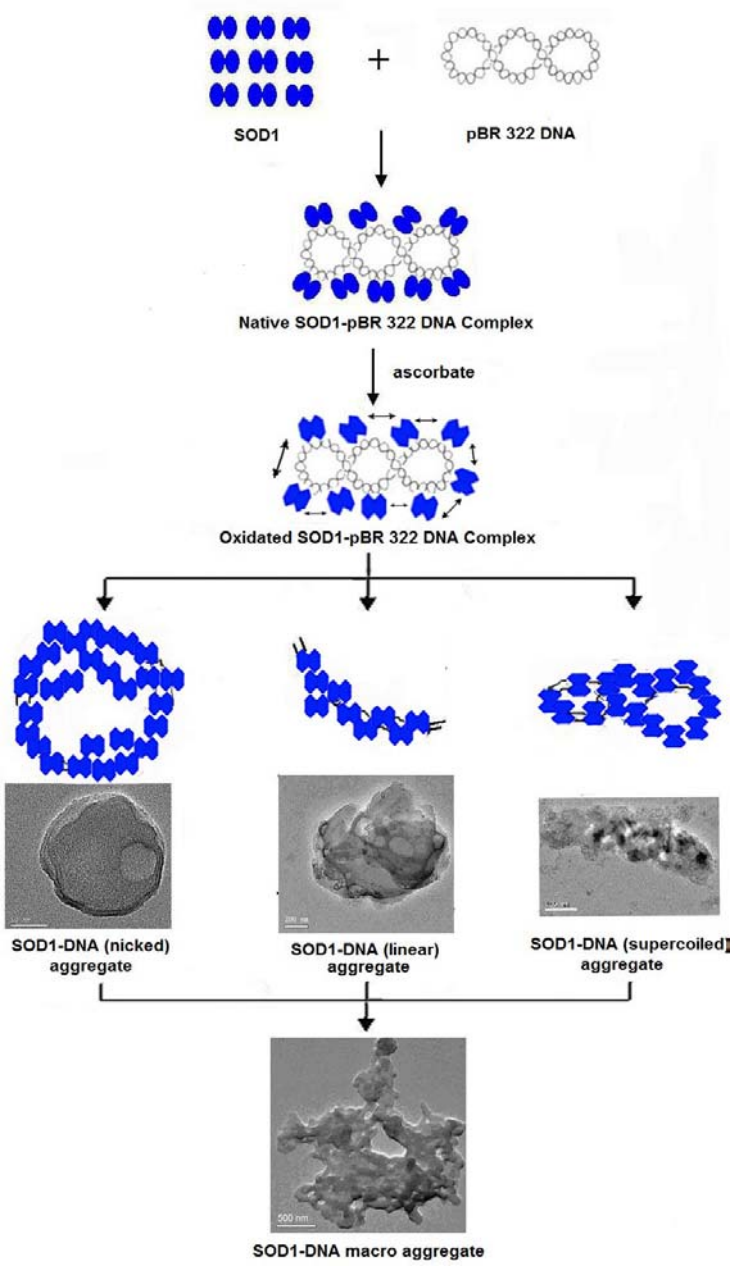

Figure 5. A proposed model of DNA-templated SOD1 aggregation under neutral conditions containing ascorbate (156). The key steps are common to the circular DNA templates. The natural SOD1 dimers (depicted as pairs of ellipsoids) are converted into an aggregation-prone form (depicted as pairs of polygons) owing to either low pH or the appearance of oxidants before or after binding to DNA. The SOD1 binds DNA in a nonspecific manner, but in a specific orientation via the electrostatic interactions between them. The binding leads to the tight arrangement of SOD1 around the DNA double strands and increase in its local concentration. Thereby, the hydrophobic interactions readily take place between the SOD1 molecules bound to the same DNA molecule. If the hydrophobic interactions are weak, the resulting aggregate monomers have a spherical or ellipsoidal shape with or without cavities. If the hydrophobic interactions are sufficient to overcome the tension within a DNA molecule, the resulting aggregate monomers are amorphous. The hydrophobic interactions between the SOD1 molecules bound to the neighboring DNA molecules drive the formation of SOD1 oligomeric aggregates. The SOD1 macroaggregates are assembled from the aggregate monomers or assembled via the oligomeric aggregates. This DNA-templated SOD1 aggregation driven by both electrostatic and hydrophobic interactions, indeed, is a SOD1-induced DNA condensation. Under neutral conditions containing ascorbate, the DNA-templated SOD1 aggregation also includes the oxidative cleavage of DNA in addition to these reaction steps. Therefore, three templates (supercoiled, nicked and linear DNA) exist simultaneously in the reactions containing ascorbate.

compelling evidence firmly supports the conclusion that the deposition of aggregates of peptides or proteins in specific organs or tissues is a key event in the pathogenesis of AD (158). Experimental data on various transgenic models of AD indicate that the abnormal deposition of two types of proteins, A-beta and the microtubule-associated protein tau, is the hallmark of brains afflicted with AD (159−161). In the brains of individuals affected with AD, amyloid plaques and neurofibrillary tangles are both observed. Amyloid plaques appear to consist primarily of an aberrantly proteolyzed form of the amyloid precursor protein (APP, a larger type I transmembrane protein) and neurofibrillary tangles consist of paired helical filaments (PHF) that contain the tau protein as a major constituent. Thus, the formation of abnormal aggregates, composed of A-beta, tau, or both of them (maybe include other cellar biological macromolecules), is predominantly responsible for the pathology of AD (2, 162). A-beta is a peptide of 39–43 amino acid that is derived through sequential proteolysis by the aspartyl protease beta-secretase and presenilin-dependent gamma-secretase cleavage of APP (163), and is the most common peptides found in amyloid plaques in the brains of AD patients (164). Microtubule-associated protein tau, a family of closely related phosphoproteins, promotes microtubule assembly and has been implicated in the development of axonal morphology. PHF is a kind of unit fibril of neurofibrillary tangles which are formed in the neurons affected with AD, and tau protein is the major component of PHF. These filaments are apparently composed of two intertwined strands of 10–20 nm diameter, giving a half-periodicity of about 75−80 nm.

According to previous studies, A-beta can exist as monomers, oligomers, fibrils, and insoluble aggregates. It has been reported that DNA could readily associate with the aggregated forms of the A-beta1–40 and A-beta25–35, giving rise to a shift in the electrophoretic mobility of DNA. DNA from different sources such as l phage, *Escherichia coli* plasmid, and human gene showed similar results. However, the electrophoretic mobility of RNA was shifted by the monomeric A-beta1–40 and A-beta25–35, as well as by the aggregated A-beta1–40 and A-beta25–35. The association of DNA with the aggregated A-beta could occur at pH 4–9. These results indicate that the DNA mobility shift assay is useful for kinetic study of A-beta aggregation as well as for testing of agents that might modulate A-beta aggregation (165).

The various aggregation species of A-beta, which may possess different degrees of neurotoxicity, are predominantly responsible for A-beta aggregation in the pathogenesis of AD (166−168). However, the exact mechanism of A-beta neurotoxicity is not clear. It has been demonstrated that the formation of A-beta aggregates can be influenced by polyanionic compounds such as Congo red, glycosaminoglycans, and proteoglycans (169−171), and can associate with other host biological macromolecules such as lipid, ATP, and nucleic acids (16, 172−175). It was previously observed that nucleic acids can bind A-beta fibrils with varying affinities, suggesting that nucleic acids can be used as diagnostic or therapeutic tools (104, 105). In these studies, the potential utility of nucleic acids has been indicated as recognition agent of A-beta aggregates. For instance, specific high-affinity RNA aptamers can be used to target specifically to amyloid-like fibrils formed from A-beta40 and some other amyloid fibrils generated *in vitro* or isolated from *ex vivo* sources (104, 105). Besides RNA aptamers, DNA has also been found to associate preferentially with the aggregated A-beta peptides to nonaggregated forms (165). On the other hand, experimental evidence shows that the interactions between A-beta and DNA can induced DNA condensation in a time-dependent manner, suggesting that A-beta may be folded in a predominantly beta-sheet secondary structure, which is promoted by the presence of DNA, and DNA can serve as a condensation nucleus which increases the propensity of A-beta to aggregate with DNA during incubation (174). Therefore, the interactions of A-beta and nucleic acids might play a role in the pathogenesis of AD (6, 174−176).

It has been proposed that tau protein becomes abnormally phosphorylated and self-associates through the microtubule-binding domain to form PHF, and all six brain tau isoforms in an abnormally phosphorylated state promote PHF assembly in the pathogenesis of AD (177−179). Other than abnormal phosphorylation-induced aggregation of tau protein into PHF (180), some biomolecules, such as sulphated glycosaminoglycans (such as heparin or heparan sulfate) and nucleic acids, can also stimulate the aggregation of tau protein into AD-linked PHF (8, 23, 181). As a factor in the cytoplasm, the various RNA species have been proposed to be responsible for inducing tau protein aggregation on the base of the experimental evidence that the aggregation of recombinant tau protein into AD-linked PHF can be strongly enhanced by RNA *in vitro*, likely because the interactions between tau protein and RNA can overcome its aggregation barrier (8). Consequently, the nucleic acid-induced aggregation of tau protein might also play a role in the development of neurofibrillary pathology in AD.

RepA, the replication initiator protein of *Pseudomonas* pPS10 plasmid, is made of two winged-helix (WH) domains (182). RepA dimers undergo a structural transformation upon binding to origin DNA sequences (iterons), resulting in monomerization and alpha-helix into beta-strand conversion. It has been shown that the interaction of short double-stranded DNA oligonucleotides, including iteron or operator RepA targets, with the isolated WH1 domain promotes the assembly of different nanostructures (183). These range from irregular aggregates to amyloid spheroids and fibers. Their intrinsic order inversely correlates with the extent of the transformation induced by each DNA sequence on RepA. However, DNA is not a constituent of the assembled fibers, in agreement with the protein-only principle for amyloid structure. Thus, the RepA-WH1 domain on DNA binding mimics the behavior of the mammalian prion protein. RepA amyloid assemblies could have a role in the negative regulation of plasmid replication. This result underlines the potential of specific nucleic acid sequences in promoting protein amyloidogenesis at nearly physiological conditions.

11. BIOLOGICAL AND PATHOLOGICAL RELEVANCES

The interaction of nucleic acids with disease

proteins and their aggregates appears, from the evidence described above, to be a general phenomenon, rather than just a feature of a number of disease proteins. This phenomenon is extremely likely to contribute significantly to the promotion of amyloid fibril formation *in vivo* and to the stabilization of the resulting aggregates, leading to alterations of cellular functions. In the light of the discussions above concerning nucleic acid-induced aggregation of proteins, on the one hand, interactions with nucleic acids can strengthen the intermolecular hydrophobic contacts of unfolding proteins, resulting in the significant acceleration of protein aggregation. On the other hand, the aggregation of misfolding proteins *in vivo* might be expected to increase dramatically the strength of the electrostatic interactions of the protein molecules with free anions and, to an even greater extent, with nucleic acid anions. Such an effect could have major consequences for a cellular function, e.g., easy sequestration of nucleic acid molecules into protein aggregates.

Nucleic acids are relatively abundant biological polyanions that bind to positively charged misfolding protein or aggregates and thus be compromised in their functions. Under normal conditions, cellular polyanions such as nucleic acids are usually found complexes with other specific species of opposite net charge (e.g., histones, Mg^{2+} and Na^{+}). Therefore, first, most notably, because DNA is the source of genetic information and a potential target of misfolded protein-induced cytotoxicity, the aggregation with DNA could have profound effects on viability and genetic stability, likely leading to destruction of DNA expression. Then, the formation of large aggregates bearing a high net charge could shift the association of at least some polyanions from their physiological target to these misfolded species, causing cellular dysfunction and generating more aggregates. An extremely interesting case is that coprecipitation with polyglutamine aggregates has been found to cause a depletion of essential proteins and hence to impair cellular functions (184). Furthermore, a high net charge could allow aggregates to interact more effectively with the phospholipids of the plasma membrane, resulting in disruption of phospholipid metabolism (185). Finally, an interesting implication in the studies on nucleic acid-induced protein aggregation is that the formation of DNA-accelerated proteinaceous deposits might promote depletion of essential proteins for normal cellular functions.

Since the polymorphism of aggregates generated from some including SOD1 can be manipulated by DNA, which might provide the reasonable basis of effective approaches for avoiding formation of the morphology-specific and toxic protein aggregates. A large number of compounds seem to act by altering the course of aggregation, rather than by blocking it. Usually, some compounds stimulate very rapid formation of alternative aggregates that seems to be typical spherical oligomers or protofibrils and then stabilize the aggregated products against further morphology changes (186). Increasing evidence has indicated that certain morphologies of protein aggregates are more toxic than others (187, 188). Therefore, if the nucleic acid-accelerated aggregation of proteins in solution can be borne out by further *in vivo* tests, there are significant therapeutic implications. Recently, since there is clear evidence to support the conclusion that the inclusions may be protective and that earlier aggregation oligomers may be more toxic than larger inclusions (189), the formation of DNA-accelerated protein inclusions would be become a potential pathway to avoid the accumulation of toxic protein oligomers.

12. CONCLUSIONS

In conclusion, the observations summarized above indicate that the nucleic acids tested so far can induce the aggrgation of disease proteins *in vitro*. This induced aggregation of proteins, *in vitro* and likely *in vivo,* may be drived by the electrostatic interactions with nucleic acid anions. The modulation of protein aggregation by nonproteinaceous components such as nucleic acids could be one of the many factors that are responsible for the late onset of diseases related to protein misfolding. Nucleic acid-induced aggregation of proteins may have implications for the design of future studies of the molecular mechanisms leading to the formation of these pathological aggregates and their role in the onset and progression of human neurodegenerative diseases. Moreover, understanding the molecular basis for the interactions between endogenous nucleic acids and misfolded neuronal proteins may open up new therapeutic avenues for the treatment of a broad range of neurodegenerative diseases. In addition, although whether or not the cytotoxic effect of amyloid aggregates results from their interactions with biological polyanions including nucleic acids, and whether or not the resulted fibrillar amyloid structures might have a role in the origin of life remain to be further investigated, the progress presented here emphasize that such interactions can be highly significant in modulating the functions of living systems.

13. ACKNOWLEDGEMENTS

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14. REFERENCES

1. Chiti, F., & C. M. Dobson: Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75, 333−366 (2006)

2. Lansbury, P. T., & H. A. Lashuel: A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature* 443, 774−779 (2006)

3. Winklhofer, K. F., J. Tatzelt, & C. Haass: The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. *The EMBO J* 27, 336−349 (2008)

4. Lundmark, K., G. T. Westermark, A. Olsen, & P. Westermark: Protein fibrils in nature can enhance amyloid

protein A amyloidosis in mice: Cross-seeding as a disease mechanism. *Proc Natl Acad Sci USA* 102, 6098−6102 (2005)

5. Legname, G., I. V. Baskakov, H. O. Nguyen, D. Riesner, F. E. Cohen, S. J. DeArmond, & S. B. Prusiner: Synthetic mammalian prions. *Science* 305, 673−676 (2004)

6. Ginsberg, S. D., J. E. Galvin, T.-S. Chiu, V. M.-Y. Lee, E. Masliah, & J. Q. Trojanowski: RNA sequestration to pathological lesions of neurodegenerative diseases. *Acta Neuropathol* 96, 487−494 (1998)

7. Hasegawa, M., R. A. Crowther, R. Jakes, & M. Goedert: Alzheimer-like changes in microtubule-associated protein Tau induced by sulfated glycosaminoglycans. Inhibition of microtubule binding, stimulation of phosphorylation, and filament assembly depend on the degree of sulfation. *J Biol Chem* 272, 33118−33124 (1997)

8. Kampers, T., P. Friedhoff, J. Biernat, E.-M. Mandelkow, & E. Mandelkow: RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. *FEBS Letters* 399, 344−349 (1996)

9. Deleault, N. R., R. W. Lucassen, & S. Supattapone: RNA molecules stimulate prion protein conversion. *Nature* 425, 717−720 (2003)

10. Cherny, D., W. Hoyer, V. Subramaniam, & T. M. Jovin: Double-stranded DNA stimulates the fibrillation of alpha-synuclein *in vitro* and is associated with the mature fibrils: an electron microscopy study. *J Mol Biol* 344, 929−938 (2004)

11. Silva, J. L., L. M. T.R. Lima, D. Foguel, & Y. Cordeiro: Intriguing nucleic-acid-binding features of mammalian prion protein. *Trends Biochem Sci* 33, 132−140 (2008)

12. Nandi, P. K., & J.-C. Nicole: Nucleic acid and prion protein interaction produces spherical amyloids which can function *in vivo* as coats of spongiform encephalopathy agent. *J Mol Biol* 344, 827−837 (2004)

13. Jiang, W., Y. Han, R. Zhou, L. Zhang, & C. Liu: DNA is a template for accelerating the aggregation of copper, zinc superoxide dismutase. *Biochemistry* 46, 5911−5923 (2007)

14. Calamai, M., J. R. Kumita, J. Mifsud, C. Parrini, M. Ramazzotti, G. Ramponi, N. Taddei, F. Chiti, & C. M. Dobson: Nature and significance of the interactions between amyloid fibrils and biological polyelectrolytes. *Biochemistry* 45, 12806−12815 (2006)

15. Cherny, I., L. Rockah, O. Levy-Nissenbaum, U. Gophna, E. Z. Ron, & E. Gazit: The formation of Escherichia coli curli amyloid fibrils is mediated by prion-like peptide repeats. *J Mol Biol* 352, 245−252 (2005)

16. Exley, C.: ATP-promoted amyloidosis of an amyloid beta peptide. *NeuroReport* 8, 3411−3414 (1997)

17. Exley, C., & O. V. Korchazhkina: Promotion of formation of amyloid fibrils by aluminium adenosine triphosphate (AlATP). *J Inorg Biochem* 84, 215−224 (2001)

18. Diaz-Nido, J., F. Wandosell, & J. Avila: Glycosaminoglycans and beta-amyloid, prion and tau peptides in neurodegenerative diseases. *Peptides* 23, 1323−1332 (2002)

19. van Horssen, J., P. Wesseling, L. P. van den Heuvel, R. M. de Waal, & M. M. Verbeek: Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. *Lancet Neurol* 2, 482−492 (2003)

20. Ancsin, J. B.: Amyloidogenesis: historical and modern observations point to heparan sulfate proteoglycans as a major culprit. *Amyloid* 10, 67−79 (2003)

21. Castillo, G. M., C. Ngo, J. Cummings, T. N. Wright, & A. D. Snow: Perlecan binds to the beta-amyloid proteins (A-beta) of Alzheimer's disease, accelerates A-beta fibril formation, and maintains A-beta fibril stability. *J Neurochem* 69, 2452−2465 (1997)

22. McLaurin, J., T. Franklin, X. Zhang, J. Deng, & P. E. Fraser: Interactions of Alzheimer amyloid-beta peptides with glycosaminoglycans effects on fibril nucleation and growth. *Eur J Biochem* 266, 1101−1110 (1999)

23. Goedert, M., R. Jakes, M. G. Spillantini, M. Hasegawa, M. J. Smith, & R. A. Crowther: Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature* 383, 550−553 (1996)

24. Cohlberg, J. A., J. Li, V. N. Uversky, & A. L. Fink: Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from alpha-synuclein *in vitro*. *Biochemistry* 41, 1502−1511 (2002)

25. Yamaguchi, I., H. Suda, N. Tsuzuike, K. Seto, M. Seki, Y. Yamaguchi, K. Hasegawa, N. Takahashi, S. Yamamoto, F. Gejyo, & H. Naiki: Glycosaminoglycan and proteoglycan inhibit the depolymerization of beta2-microglobulin amyloid fibrils *in vitro*. *Kidney Int* 64, 1080−1088 (2003)

26. Ginsberg, S. D., P. B. Crino, VM-Y Lee, J. H. Eberwine, & J. Q. Trojanowski: Sequestration of RNA in Alzheimer's disease neurofibrillary tangles and senile plaques. *Ann Neurol* 41, 200−209 (1997)

27. Gibson, P., & B. E. Tomlinson: The numbers of Hirano bodies in the hippocampus of normal and demented subjects with Alzheimer's disease. *J Neurol Sci* 33, 199−206 (1977)

28. Hirano, A., N. Malamud, T. S. Elizan, & L. T. Kurland: Amyotrophic lateral sclerosis and Parkinsonism-dementia complex on Guam. *Arch Neurol* 15, 35−51 (1966)

29. Trojanowski, J. Q., & VM-Y Lee: Phosphorylation of neuronal cytoskeletal proteins in Alzheimer's disease and Lewy body dementia. *Ann NY Acad Sci* 747, 92−109 (1994)

30. Spillantini, M. G., M. L. Schmidt, VM-Y Lee, J. Q. Trojanowski, R. Jakes, & M. Goedert: alpha-Synuclein in Lewy bodies. *Nature* 388, 839−840 (1997)

31. Ayala, Y. M., T. Misteli, & F. E. Baralle: TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc Natl Acad Sci USA* 105, 3785−3789 (2008)

32. Strong, M. J., K. Volkening, R. Hammond, W. Yang, W. Strong, C. Leystra-Lantz, & C. Shoesmith: TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. *Mol Cell Neurosci* 35, 320−327 (2007)

33. Neumann, M., D. M. Sampathu, L. K. Kwong, A. C. Truax, M. C. Micsenyi, T. T. Chou, J. Bruce, & V. M. Lee: Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130−133 (2006)

34. Sreedharan, J., I. P. Blair, V. B. Tripathi, X. Hu, C. Vance, B. Rogelj, S. Ackerley, J. C. Durnall, & C. E. Shaw: TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668−1672 (2008)

35. Arai, T., M. Hasegawa, H. Akiyama, K. Ikeda, T. Nonaka, H. Mori, D. Mann, K. Tsuchiya, M. Yoshida, Y. Hashizume, & T. Oda: TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351, 602−611 (2006)

36. Wang, I. F., L. S. Wu, & C. K. Shen: TDP-43: an emerging new player in neurodegenerative diseases. *Trends Mol Med* 14, 479−485 (2008)

37. Ule, J.: Ribonucleoprotein complexes in neurologic diseases. *Curr Opin Neurosci* 18, DOI 10. 1016, In press (2008)

38. Amador-Ortiz, C., W. L. Lin, Z. Ahmed, D. Personett, P. Davies, R. Duara, N. R. Graff-Radford, M. L. Hutton, & D. W. Dickson: TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* 61, 435−445 (2007)

39. Uryu, K., H. Nakashima-Yasuda, M. S. Forman, L. K. Kwong, C. M. Clark, M. Grossman, B. L. Miller, H. A. Kretzschmar, V. M. Lee, J. Q. Trojanowski, & M. Neumann: Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. *J Neuropathol Exp Neurol* 67, 555−564 (2008)

40. Higashi, S., E. Iseki, R. Yamamoto, M. Minegishi, H. Hino, K. Fujisawa, T. Togo, O. Katsuse, H. Uchikado, Y. Furukawa, K. Kosaka, & H. Arai: Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. *Brain Res* 1184, 284−294 (2007)

41. Nakashima-Yasuda, H., K. Uryu, J. Robinson, S. X. Xie, H. Hurtig, J. E. Duda, S. E. Arnold, & J. Q. Trojanowski: Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol* 114, 221−229 (2007)

42. Hasegawa, M., T. Arai, H. Akiyama, T. Nonaka, H. Mori, T. Hashimoto, M. Yamazaki, & K. Oyanagi: TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain* 130, 1386−1394 (2007)

43. Geser, F., M. J. Winton, L. K. Kwong, Y. Xu, S. X. Xie, L. M. Igaz, R. M. Garruto, D. P. Perl, D. Galasko, V. M. Lee, & J. Q. Trojanowski: Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol* 115, 133−145 (2008)

44. Probst, A., K. I. Taylor, & M. Tolnay: Hippocampal sclerosis dementia: a reappraisal. *Acta Neuropathol* 114, 335−345 (2007)

45. Cairns, N. J., M. Neumann, E. H. Bigio, I. E. Holm, D. Troost, K. J. Hatanpaa, C. Foong, & I. R. Mackenzie: TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 171, 227−240 (2007)

46. Davidson, Y., T. Kelley, I. R. Mackenzie, S. Pickering-Brown, D. Du Plessis, D. Neary, J. S. Snowden, & D. M. Mann: Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol* 113, 521−533 (2007)

47. Jin, L. W., K. A. Claborn, M. Kurimoto, M. A. Geday, I. Maezawa, F. Sohraby, M. Estrada, W. Kaminksy, & B. Kahr: Imaging linear birefringence and dichroism in cerebral amyloid pathologies. *Proc Natl Acad Sci USA* 100, 15294−15298 (2003)

48. Krebs, M. R. H., C. E. MacPhee, A. F. Miller, L. E. Dunlop, C. M. Dobson, & A. M. Donald: The formation of spherulites by amyloid fibrils of bovine insulin. *Proc Natl Acad Sci USA* 101, 14420−14424 (2004)

49. Jimenez, J. L., J. I. Guijarro, E. Orlova, J. Zurdo, C. M. Dobson, M. Sunde, & H. R. Saibil: Cryo-electron microscopy structure of an SH3 amyloid fibril and model of the molecular packing. *EMBO J* 18, 815−821 (1999)

50. Jimenez, J. L., E. J. Nettleton, M. Bouchard, C. V. Robinson, C. M. Dobson, & H. R. Saibil: The protofilament structure of insulin amyloid fibrils. *Proc Natl Acad Sci USA* 99, 9196−9201 (2002)

51. Harper, J. D., S. S. Wong, C. M. Lieber, & P. T. Jr. Lansbury: Observation of metastable A-beta amyloid protofibrils by atomic force microscopy. *Chem Biol* 4, 119−125 (1997)

52. Walsh, D. M., A. Lomakin, G. B. Benedek, M. M. Condron, & D. B. Teplow: Amyloid beta-protein fibrillogenesis. Detection of a protofibrillar intermediate. *J Biol Chem* 272, 22364−22372 (1997)

53. Walsh, D. M., D. M. Hartley, Y. Kusumoto, Y. Fezoui, M. M. Condron, A. Lomakin, G. B. Benedek, D. J. Selkoe, & D. B. Teplow: Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J Biol Chem* 274, 25945−25952 (1999)

54. Kayed, R., Y. Sokolov, B. Edmonds, T. M. McIntire, S. C. Milton, J. E. Hall, & C. G. Glabe: Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *J Biol Chem* 279, 46363−46366 (2004)

55. Conway, K. A., J. D. Harper, & P. T. Jr. Lansbury: Fibrils formed *in vitro* from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* 39, 2552−2563 (2000)

56. Bitan, G., M. D. Kirkitadze, A. Lomakin, S. S. Vollers, G. B. Benedek, & D. B. Teplow: Amyloid beta-protein (A-beta) assembly: A-beta 40 and A-beta 42 oligomerize through distinct pathways. *Proc Natl Acad Sci USA* 100, 330−335 (2003)

57. Roher, A. E., M. O. Chaney, Y. M. Kuo, S. D. Webster, W. B. Stine, & M. R. Emmerling: Morphology and toxicity of A-beta (1−42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J Biol Chem* 271, 20631−20635 (1996)

58. Podlisny, M. B., B. L. Ostaszewski, S. L. Squazzo, E. H. Koo, R. E. Rydell, D. B. Teplow, & D. J. Selkoe: Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *J Biol Chem* 270, 9564−9570 (1995)

59. Serio, T. R., A. G. Cashikar, A. S. Kowal, G. J. Sawicki, J. J. Moslehi, L. Serpell, M. F. Arnsdorf, & S. L. Lindquist: Nucleated conformational conversion and the replication of conformational information by a prion determinant. *Science* 289, 1317−1321 (2000)

60. Pedersen, J. S., G. Christensen, & D. E. Otzen: Modulation of S6 fibrillation by unfolding rates and gatekeeper residues. *J Mol Biol* 341, 575−588 (2004)

61. Fezoui, Y., & D. B. Teplow: Kinetic studies of amyloid beta-protein fibril assembly. Differential effects of alpha-helix stabilization. *J Biol Chem* 277, 36948−36954 (2002)

62. Kelly, J.W.: The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. *Curr Opin Struct Biol* 8, 101−106 (1998)

63. Dobson, C. M.: Protein misfolding, evolution and disease. *Trends Biochem Sci* 24, 329−332 (1999)

64. Gosal, W. S., I. J. Morten, E. W. Hewitt, D. A. Smith, N. H. Thomson, & S. E. Radford: Competing pathways determine fibril morphology in the self-assembly of beta2-microglobulin into amyloid. *J Mol Biol* 351, 850−864 (2005)

65. Guijarro, J. I., M. Sunde, J. A. Jones, I. D. Campbell, & C. M. Dobson: Amyloid fibril formation by an SH3 domain. *Proc Natl Acad Sci USA* 95, 4224−4228 (1998)

66. Chiti, F., N. Taddei, M. Bucciantini, P. White, G. Ramponi, & C. M. Dobson: Mutational analysis of the propensity for amyloid formation by a globular protein. *EMBO J* 19, 1441−1449 (2000)

67. Ferrao-Gonzales, A. D., S. O. Souto, J. L. Silva, & D. Foguel: The preaggregated state of an amyloidogenic protein: hydrostatic pressure converts native transthyretin into the amyloidogenic state. *Proc Natl Acad Sci USA* 97, 6445−6450 (2000)

68. Calabrese, M. F., C. M. Eakin, J. M. Wang, & A. D. Miranker: A regulatable switch mediates self-association in an immunoglobulin fold. *Nat Struct Biol* 15, 965−971 (2008)

69. Frederick, K. B., D. Sept, & E. M. De La Cruz: Effects of solution crowding on actin polymerization reveal the energetic basis for nucleotide-dependent filament stability. *J Mol Biol* 378, 540−550 (2008)

70. Gaggelli, E., H. Kozlowski, D. Valensin, & G. Valensin: Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). *Chem Rev* 106, 1995−2044 (2006)

71. Canet, D., A. M. Last, P. Tito, M. Sunde, A. Spencer, D. B. Archer, C. Redfield, C. V. Robinson, & C. M. Dobson: Local cooperativity in the unfolding of an amyloidogenic variant of human lysozyme. *Nat Struct Biol* 9, 308−315 (2002)

72. Hammarstrom, P., X. Jiang, A. R. Hurshman, E. T. Powers, & J. W. Kelly: Sequence-dependent denaturation energetics: A major determinant in amyloid disease diversity. *Proc Natl Acad Sci USA* 99, 16427−16432 (2002)

73. Bouchard, M., J. Zurdo, E. J. Nettleton, C. M. Dobson, & C. V. Robinson: Formation of insulin amyloid fibrils followed by FTIR simultaneously with CD and electron microscopy. *Protein Sci* 9, 1960−1967 (2000)

74. Valentine, J. S., P. A. Doucette, & S. Z. Potter: Copperzinc superoxide dismutase and amyotrophic lateral sclerosis. *Annu Rev Biochem* 74, 563−593 (2005)

75. Bruijn, L. I., T. M. Miller, & D. W. Cleveland: Unraveling the mechanisms involved in motor neuron degeneration in ALS. *Annu Rev Neurosci* 27, 723−749 (2004)

76. Jiang, W., T. Shen, Y. Han, Q. Pan, & C. Liu: Divalent-metal-dependent nucleolytic activity of Cu, Zn superoxide dismutase. *J Biol Inorg Chem* 11, 835−848 (2006)

77. Getzoff, E. D., J. A. Tainer, P. K. Weiner, P. A. Kollman, J. S. Richardson, & D. C. Richardson: Electrostatic recognition between superoxide and copper, zinc superoxide dismutase. *Nature* 306, 287−290 (1983)

78. Jiang, W., Y. Han, Q. Pan, T. Shen, & C. Liu: Roles of exogenous divalent metals in the nucleolytic activity of Cu,Zn superoxide dismutase. *J Inorg Biochem* 101, 667−677 (2007)

79. Prusiner, S. B., M. R. Scott, S. J. DeArmond, & F. E. Cohen: Prion protein biology. *Cell* 93, 337−348 (1998)

80. Akowitz, A., T. Sklaviadis, & L. Manuelidis: Endogenous viral complexes with long RNA cosediment with the agent of CJD. *Nucl Acids Res* 22, 1101−1107 (1994)

81. Sklaviadis, T., A. Akowitz, E. E. Manuelidis, & L. Manuelidis: Nucleic acid binding proteins in highly purified Creutzfeldt-Jakob disease preparations. *Proc Natl Acad Sci USA,* 90, 5713−5717 (1993)

82. Cordeiro, Y., F. Machado, L. Juliano, M. A. Juliano, R. R. Brentani, D. Foguel, & J. L. Silva: DNA converts cellular prion protein into the beta-sheet conformation and inhibits prion peptide aggregation. *J Biol Chem* 276, 49400−49409 (2001)

83. Cordeiro, Y., L. M. T. R. Lima, M. P. B. Gomes, D. Foguel, & J. L. Silva: Modulation of prion protein oligomerization, aggregation, and beta-sheet conversion by 4,4'-dianilino-1,1'-binaphthyl-5,5'-sulfonate (bis-ANS). *J Biol Chem* 279, 5346−5352 (2004)

84. Deleault, N. R., B. T. Harris, J. R. Rees, & S. Supattapone: Formation of native prions from minimal components *in vitro*. *Proc Natl Acad Sci USA* 104, 9741−9746 (2007)

85. Carp, R., H. Meeker, V. Caruso, & E. Sersen: Scrapie strain-specific interactions with endogenous murine leukemia virus. *J Gen Virol* 80, 5−10 (1999)

86. Murdoch, G., T. Sklaviadis, E. Manuelidis, & L. Manuelidis: Potential retroviral RNAs in Creutzfeldt-Jakob disease. *J Virol* 64, 1477−1486 (1990)

87. Manuelidis, L., T. Sklaviadis, A. Akowitz, & W. Fritch: Viral particles are required for infection in neurodegenerative Creutzfeldt-Jakob disease. *Proc Natl Acad Sci USA* 23, 5124−5128 (1995)

88. Mangé, A., C. Crozet, S. Lehmann, & F. Béranger: Scrapie-like prion protein is translocated to the nuclei of infected cells independently of proteasome inhibition and interacts with chromatin. *J Cell Sci* 117, 2411−2416 (2004)

89. Gabus, C., S. Auxilien, C. Péchoux, D. Dormont, W. Swietnicki, M. Morillas, W. Surewicz, P. Nandi, & J. L. Darlix: The prion protein has DNA strand transfer properties similar to retroviral nucleocapsid protein. *J Mol Biol* 307, 1011−1021 (2001)

90. Gabus, C., E. Derrington, P. Leblanc, J. Chnaiderman, D. Dormont, W. Swietnicki, M. Morillas, W. K. Surewicz, D. Marc, P. Nandi, & J. L. Darlix: The prion protein has RNA binding and chaperoning properties characteristic of nucleocapsid protein NCP7 of HIV-1. *J Biol Chem* 276, 19301−19309 (2001)

91. Adler, V., B. Zeiler, V. Kryukov, R. Kascsak, R. Rubenstein, & A. Grossman: Small, highly structured RNAs participate in the conversion of human recombinant PrP (Sen) to PrP (Res) *in vitro*. *J Mol Biol* 332, 47−57 (2003)

92. Weiss, S., D. Proske, M. Neumann, M. H. Groschup, H. A. Kretzschmar, M. Famulok, & E. L. Winnacker: RNA aptamers specifically interact with the prion protein PrP. *J Virol* 71, 8790−8797 (1997)

93. Sayer, N. M., M. Cubin, A. Rhie, M., Bullock A. Tahiri-Alaoui, & W. James: Structural determinants of conformationally selective, prion-binding aptamers. *J Biol Chem* 279, 13102−13109 (2004)

94. Mercey, R., I. Lantier, M. C. Maurel, J. Grosclaude, F. Lantier, & D. Marc: Fast, reversible interaction of prion protein with RNA aptamers containing specific sequence patterns. *Arch Virol* 151, 2197−2214 (2006)

95. Rhie, A., L. Kirby, N. Sayer, R. Wellesley, P. Disterer, I. Sylvester, A. Gill, J. Hope, W. James, & A. Tahiri-Alaoui: characterization of 2'-fluoro-RNA aptamers that bind preferentially to disease-associated conformations of prion protein and inhibit conversion. *J Biol Chem* 278, 39697−39705 (2003)

96. Kocisko, D. A., A. Vaillant, K. S. Lee, K. M. Arnold, N. Bertholet, R. E. Race, E. A. Olsen, J. M. Juteau, & B. Caughey: Potent antiscrapie activities of degenerate phosphorothioate oligonucleotides. *Antimicrob Agents Chemother* 50, 1034−1044 (2006)

97. Kaiser-Schulz, G., A. Heit, L. Quintanilla-Martinez, F. Hammerschmidt, S. Hess, L. Jennen, H. Rezaei, H. Wagner, & H. M. Schätzl: Polylactide-coglycolide microspheres coencapsulating recombinant tandem prion protein with CpG oligonucleotide break self-

tolerance to prion protein in wild-type mice and induce CD4 and CD8 T cell responses. *J Immunol* 179, 2797−2807 (2007)

98. Takemura, K., P. Wang, I. Vorberg, W. Surewicz, S. A. Priola, A. Kanthasamy, R. Pottathil, S. G. Chen, & S. Sreevatsan: DNA aptamers that bind to PrP (C) and not PrP (Sc) show sequence and structure specificity. *Exp Biol Med* 231, 204−214 (2006)

99. King, D. J., J. G. Safar, G. Legname, & S. B. Prusiner: Thioaptamer interactions with prion proteins: sequence-specific and non-specific binding sites. *J Mol Biol* 369, 1001−1014 (2007)

100. Zou, W. Q., J. Zheng, D. M. Gray, P. Gambetti, & S. G. Chen: Antibody to DNA detects scrapie but not normal prion protein. *Proc Natl Acad Sci USA* 101, 1380−1385 (2004)

101. Lima, L. M. T. R., Y. Cordeiro, L. W. Tinoco, A. F. Marques, C. L. P. Oliveira, S. Sampath, R. Kodali, G. Choi, D. Foguel, I. Torriani, B. Caughey, & J. L. Silva: Structural insights into the interaction between prion protein and nucleic acid. *Biochemistry* 45, 9180−9187 (2006)

102. Tjong, H., & H. X. Zhou: DISPLAR: an accurate method for predicting DNA-binding sites on protein surfaces. *Nucleic Acids Res* 35, 1465−1477 (2007)

103. Bera, A., A-C. Roche, & P. K. Nandi: Bending and unwinding of nucleic acid by prion protein. *Biochemistry* 46, 1320−1328 (2007)

104. Ylera, F., R. Lurz, V. A. Erdmann, & J. P. Furste: Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. *Biochem Biophys Res Commun* 290, 1583−1588 (2002)

105. Bunka, D. H. J., B. J. Mantle, I. J. Morten, G. A. Tennent, S. E. Radford, & P. G. Stockley: Production and characterization of RNA aptamers specific for amyloid fibril epitopes. *J Biol Chem* 282, 34500−34509 (2007)

106. Cordeiro, Y., & J. L. Silva: The hypothesis of the catalytic action of nucleic acid on the conversion of prion protein. *Protein Pept Lett* 12, 251−255 (2005)

107. Cordeiro, Y., J. Kraineva, R. Ravindra, L. M. Lima, M. P. Gomes, D. Foguel, R. Winter, & J. L. Silva: Hydration and packing effects on prion folding and beta-sheet conversion. High pressure spectroscopy and pressure perturbation calorimetry studies. *J Biol Chem* 279, 32354−32359 (2004)

108. Cordeiro, Y., J. Kraineva, M. P. Gomes, M. H. Lopes, V. R. Martins, L. M. Lima, D. Foguel, R. Winter, & J. L. Silva: The amino-terminal PrP domain is crucial to modulate prion misfolding and aggregation. *Biophys J* 89, 2667−2676 (2005)

109. Tompa, P., & P. Csermely: The role of structural disorder in the function of RNA and protein chaperones. *FASEB J* 18, 1169−1175 (2004)

110. Nandi, P. K., E. Leclerc, J.-C. Nicole, & M. Takahashi: DNA-induced partial unfolding of prion protein leads to its polymerisation to amyloid. *J Mol Biol* 322, 153−161 (2002)

111. Deleault, N. R., J. C. Geoghegan, K. Nishina, R. Kascsak, R. A. Williamson, & S. Supattapone: Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J Biol Chem* 280, 26873−26879 (2005)

112. Saborio, G. P., B. Permanne, & C. Soto: Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* 411, 810−813 (2001)

113. Kaytor, M. D., & S. T. Warren: Aberrant protein deposition and neurological disease. *J Biol Chem* 274, 37507−37510 (1999)

114. Kocisko, D. A., J. H. Come, S. A. Priola, B. Chesebro, G. J. Raymond, P. T. Lansbury, & B. Caughey: Cell-free formation of protease-resistant prion protein. *Nature* 370, 471−474 (1994)

115. Caughey, B., G. J. Raymond, D. A. Kocisko, & P. T. Jr. Lansbury: Scrapie infectivity correlates with converting activity, protease resistance, and aggregation of scrapie-associated prion protein in guanidine denaturation studies. *J Virol* 71, 4107−4110 (1997)

116. Prusiner, S. B.: Novel proteinaceous infectious particles cause scrapie. *Science* 216, 136−144 (1982)

117. Prusiner, S. B.: Prions. *Proc Natl Acad Sci USA* 95, 13363−13383 (1998)

118. Caughey, B., & G. S. Baron: Prions and their partners in crime. *Nature* 443, 803−810 (2006)

119. Caughey, B., K. Brown, G. J. Raymond, G. E. Katzenstein, & W. Thresher: Binding of the protease-sensitive form of PrP (prion protein) to sulfated glycosaminoglycan and congo red. *J Virol* 68, 2135−2141 (1994)

120. Horonchik, L., S. Tzaban, O. Ben-Zaken, Y. Yedidia, A. Rouvinski, D. Papy-Garcia, D. Barritault, I. Vlodavsky, & A. Taraboulos: Heparan sulfate is a cellular receptor for purified infectious prions. *J Biol Chem* 280, 17062−17067 (2005)

121. Nandi, P. K., & E. Leclerc: Polymerization of murine recombinant prion protein in nucleic acid solution. *Arch Virol* 144, 1751−1763 (1999)

122. Nandi, P. K., & P-Y. Sizaret: Murine recombinant prion protein induces ordered aggregation of linear nucleic

acids to condensed globular structures. *Arch Virol* 146, 327−345 (2001)

123. Nandi, P. K.: Interaction of prion peptide HuPrP106-126 with nucleic acid. *Arch Virol* 142, 2537−2545 (1997)

124. Nandi, P. K.: Polymerization of human prion peptide HuPrP 106−126 to amyloid in nucleic acid solution. *Arch Virol* 143, 1251−1263 (1998)

125. Narang, H. K., D. M. Asher, & D. C. Gajdusek: Evidence that DNA is present in abnormal tubulofilamentous structures found in scrapie. *Proc Natl Acad Sci USA* 85, 3575−3579 (1988)

126. Rijk, M. C. de, C. Tzourio, M. M. B. Breteler, J. F. Dartigues, L. Amaducci, & S. Lopez-Pousa: Prevalence of parkinsonism and Parkinson's disease in europe: the EUROPARKINSON collaborative study. European community concerted action on the epidemiology of Parkinson's disease. *J Neurol Neurosurg Psychiatry* 62, 10−15 (1997)

127. Pollanen, M. S., D. W. Dickson, & C. Bergeron: Pathology and biology of the Lewy body. *J Neuropathol Exp Neurol* 52, 183−191 (1993)

128. Weinreb, P. H., W. Zhen, A. W. Poon, K. A. Conway, & P. T. Jr. Lansbury: NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* 35, 13709−13715 (1996)

129. Narayanan, V., & S. Scarlata: Membrane binding and self-association of alpha-synucleins. *Biochemistry* 40, 9927−9934 (2001)

130. Giasson, B. I., I. V. Murray, J. Q. Trojanowski, & V. M. Lee: A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. *J Biol Chem* 276, 2380−2386 (2001)

131. Uversky, V. N.: Natively unfolded proteins: a point where biology waits for physics. *Protein Sci* 11, 739−756 (2002)

132. Serpell, L. C., J. Berriman, R. Jakes, M. Goedert, & R. A. Crowther: Fiber diffraction of synthetic alpha-synuclein filaments shows amyloid-like cross-beta conformation. *Proc Natl Acad Sci USA* 97, 4897−4902 (2000)

133. Conway, K. A., S. J. Lee, J. C. Rochet, T. T. Ding, R. E. Williamson, & P. T. Jr. Lansbury: Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc Natl Acad Sci USA* 97, 571−576 (2000)

134. Li, J., V. N. Uversky, & A. L. Fink: Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human alpha-synuclein. *Biochemistry* 40, 11604−11613 (2001)

135. Der-Sarkissian, A., C. C. Jao, J. Chen, & R. Langen: Structural organization of alpha-synuclein fibrils studied by site-directed spin labeling. *J Biol Chem* 278, 37530−37535 (2003)

136. Uversky, V. N., J. Li, & A. L. Fink: Metaltriggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular link between Parkinson's disease and heavy metal exposure. *J Biol Chem* 276, 44284−44296 (2001)

137. Hoyer, W., T. Antony, D. Cherny, G. Heim, T. M. Jovin, & V. Subramaniam: Dependence of alpha-synuclein aggregate morphology on solution conditions. *J Mol Biol* 322, 383-393 (2002)

138. Manning-Bog, A. B., A. L. McCormack, J. Li, V. N. Uversky, A. L. Fink, & D. A. Di Monte: The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem* 277, 1641−1644 (2002)

139. Necula, M., C. N. Chirita, & J. Kuret: Rapid anionic micelle-mediated alpha-synuclein fibrillization *in vitro*. *J Biol Chem* 278, 46674−46680 (2003)

140. Antony, T., W. Hoyer, D. Cherny, G. Heim, T. M. Jovin, & V. Subramaniam: Cellular polyamines promote the aggregation of alpha-synuclein. *J Biol Chem* 278, 3235−3240 (2003)

141. Goers, J., V. N. Uversky, & A. L. Fink: Polycation-induced oligomerization and accelerated fibrillation of human alpha-synuclein *in vitro*. *Protein Sci* 12, 702−707 (2003)

142. Wood, S. J., J. Wypych, S. Steavenson, J. C. Louis, M. Citron, & A. L. Biere: alpha-Synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease. *J Biol Chem* 274, 19509−19512 (1999)

143. Goers, J., A. B. Manning-Bog, A. L. McCormack, I. S. Millett, S. Doniach, & D. A. Di Monte: Nuclear localization of alpha-synuclein and its interaction with histones. *Biochemistry* 42, 8465−8471 (2003)

144. Buxbaum, J.: Mechanisms of disease: monoclonal immunoglobulin deposition. Amyloidosis, light chain deposition disease, and light and heavy chain deposition disease. *Hematol Oncol Clin North Am* 6, 323−346 (1992)

145. Rowland, L. P., & N. A. Shneider: Amyotrophic lateral sclerosis. *N Engl J Med* 344, 1688−1700 (2001)

146. Pasinelli, P., & R. H. Brown: Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat Rev Neurosci* 7, 710−723 (2006)

147. Valentine, J. S.: Do oxidatively modified proteins cause ALS? *Free Radical Biol Med* 33, 1314−1320, (2002)

148. Cleveland, D. W., & J. D. Rothstein: From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* 2, 806−819 (2001)

149. Valentine, J. S., & P. J. Hart: Misfolded CuZnSOD and amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 100, 3617−3622 (2003)

150. Rakhit, R., J. R. Lepock, L. H. Kondejewski, N. R. Cashman, & A. Chakrabartty: Monomeric Cu,Zn-superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial amyotrophic lateral sclerosis. *J Biol Chem* 279, 15499−15504 (2004)

151. Rakhit, R., P. Cunningham, A. Furtos-Matei, S. Dahan, X. F. Qi, J. P. Crow, N. R. Cashman, L. H. Kondejewski, & A. Chakrabartty: Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. *J Biol Chem* 277, 47551−47556 (2002)

152. Jonsson, P. A.: Disulphide-reduced superoxide dismutase-1 in CNS of transgenic amyotrophic lateral sclerosis models. *Brain* 129, 451−464 (2006)

153. Wang, J., G. Xu, & D. R. Borchelt: High molecular weight complexes of mutant superoxide dismutase 1: age-dependent and tissue-specific accumulation. *Neurobiol Dis* 9, 139−148 (2002)

154. Kim, Y. J., R. Nakatomi, T. Akagi, T. Hashikawa, & R. Takahashi: Unsaturated fatty acids induce cytotoxic aggregate formation of amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutants. *J Biol Chem* 280, 21515−21521 (2005)

155. Yin, J., W. Jiang, L. Liu, X. Huang, & C. Liu: DNA-triggered aggregation of copper, zinc superoxide dismutase in the presence of ascorbate. Unpublished results.

156. Jiang, W., B. Zhang, J. Yin, L. Liu, L. Wang, & C. Liu: Polymorphism of the SOD1-DNA aggregation species can be modulated by DNA. *Biopolymers* 89, 1154−1169 (2008)

157. Hebert, L. E., P. A. Scherr, J. L. Bienias, D. A. Bennett, & D. A. Evans: Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* 60, 1119−1122 (2003)

158. Huff, M. E., W. E. Balch, & J. W. Kelly: Pathological and functional amyloid formation orchestrated by the secretory pathway. Curr Opin Struct Biol 13, 674−682 (2003)

159. Oddo, S., A. Caccamo, L. Tran, M. P. Lambert, C. G. Glabe, W. L. Klein, & F. M. LaFerla: Temporal profile of amyloid-beta (A-beta) oligomerization in an in vivo model of Alzheimer disease. J Biol Chem 281, 1599−1604 (2006)

160. Lewis, J., D. W. Dickson, W. L. Lin, L. Chisholm, A. Corral, G. Jones, S. H. Yen, N. Sahara, L. Skipper, D. Yager, C. Eckman, J. Hardy, M. Hutton, & E. McGowan: Enhanced neurofibrillary degeneration in transgenic mice expressing mutant Tau and APP. Science 293, 1487−1491 (2001)

161. Oddo, S., L. Billings, J. P. Kesslak, D. H. Cribbs, & F. M. LaFerla: A-beta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43, 321−332 (2004)

162. Blurton-Jones, M., & F. M. Laferla: Pathways by which A-beta facilitates tau pathology. Curr Alzheimer Res 3, 437−448 (2006)

163. Vassar, R., & M. Citron: A-beta-generating enzymes: recent advances in beta- and gamma-secretase research. *Neuron* 27, 419−422 (2000)

164. Gravina, S. A., L. Ho, C. B. Eckman, K. E. Long, L. Jr. Otvos, L. H. Younkin, N. Suzuki, & S. G. Younkin: Amyloid beta protein (A-beta) in Alzheimer's disease brain**.** *J Biol Chem* 270, 7013−7016 (1995)

165. Ahn, B. W., D. U. Song, Y. D. Jung, K. O. Chay, M. A Chung, S. Y. Yang, & B. A. Shin: Detection of beta-amyloid peptide aggregation using DNA electrophoresis. *Anal Biochem* 284, 401−405 (2000)

166. Walsh, D. M., I. Klyubin, J. V. Fadeeva, M. J. Rowan, & D. J. Selkoe: Amyloid-beta oligomers: their production, toxicity and therapeutic inhibition. *Biochem Soc Trans* 30, 552−557 (2002)

167. Dahlgren, K. N., A. M. Manelli, Jr. W. B. Stine, L. K. Baker, G. A. Krafft, & M. J. LaDu: Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J Biol Chem* 277, 32046−32053 (2002)

168. Chromy, B. A., R. J. Nowak, M. P. Lambert, K. L. Viola, L. Chang, P. T. Velasco, B. W. Jones, S. J. Fernandez, P. N. Lacor, P. Horowitz, C. E. Finch, G. A. Krafft, & W. L. Klein: Self-assembly of A-beta (1–42) into globular neurotoxins. *Biochemistry* 42, 12749−12760 (2003)

169. Pollack, S. J., I. I. J. Sadler, S. R. Hawtin, V. J. Tailor, & M. S. Shearman: Sulfated glycosaminoglycans and dyes attenuate the neurotoxic effects of beta-amyloid in rat PC12 cells. *Neurosci Lett* 184, 113−116 (1995)

170. Iversen, L. L., R. J. Mortishire-Smith, S. J. Pollack, & M. S. Shearman: The toxicity *in vitro* of beta-amyloid protein. *Biochem J* 311, 1−16 (1995)

171. Lorenzo, A., & B. A. Yankner: beta-Amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. *Proc Natl Acad Sci USA* 91, 12243−12247 (1994)

172. Liu, L., H. Komatsu, I. V. J. Murray, & P. H. Axelsen: Promotion of amyloid beta protein misfolding and

fibrillogenesis by a lipid oxidation product. *J Mol Biol* 377, 1236−1250 (2008)

173. Evans, C. G., S. Wisen, & J. E. Gestwicki: Heat shock proteins 70 and 90 inhibit early stages of amyloid beta (1–42) aggregation *in vitro*. *J Biol Chem* 281, 33182−33191 (2006)

174. Yu, H. J., J. S. Ren, & X. G. Qu: Time-dependent DNA condensation induced by amyloid beta-peptide. *Biophys J* 92, 185−191 (2007)

175. Hegde, M. L., S. Anitha, & K. S. Latha: First evidence for helical transitions in supercoiled DNA by amyloid beta peptide (1–42) and aluminum-A new insight in understanding Alzheimer's disease. *J Mol Neurosci* 22, 19−31 (2003)

176. Anitha, S., K. S. R. Jagannatha, K. S. Latha, & M. A. Viswamitra: First evidence to show the topological change of DNA from B-DNA to Z-DNA conformation in the hippocampus of Alzheimer's brain. *Neuro Mol Med* 2, 289−298 (2002)

177. Lee, V. M.-Y., B. J. Balin, L. Otvos, & J. Q. Trojanowski: A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* 251, 675−678 (1991)

178. Wille, H., G. Drewes, J. Biernat, E. M. Mandelkow, & E. Mandelkow: Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau *in vitro*. *J Cell Biol* 118, 573−584 (1992)

179. Goedert, M., M. G. Spillantini, N. J. Cairns, & R. A. Crowther: Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 8, 159−168 (1992)

180. Avila, J.: Tau phosphorylation and aggregation in Alzheimer's disease pathology. *FEBS Letters* 580, 2922−2927 (2006)

181. Perez, M., J. M. Valpuesta, M. Medina, E. M. Degarcini, & J. Avila: Polymerization of tau into filaments in the presence of heparin: the minimal sequence required for tau-tau interaction. *J Neurochem* 67, 1183−1190 (1996)

182. Giraldo, R., & M. E. Fernandez-Tresguerres: Twenty years of the pPS10 replicon: insights on the molecular mechanism for the activation of DNA replication in iteron-containing bacterial plasmids. *Plasmid* 52, 69−83 (2004)

183. Giraldo, R.: Defined DNA sequences promote the assembly of a bacterial protein into distinct amyloid nanostructures. *Proc Natl Acad Sci USA* 104, 17388−17393 (2007)

184. Nucifora, F. C. Jr., M. Sasaki, M. F. Peters, H. Huang, J. K. Cooper, M. Yamada, H. Takahashi, S. Tsuji, J. Troncoso, V. L. Dawson, T. M. Dawson, & C. A. Ross: Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* 291, 2423−2428 (2001)

185. Berman, D. E., C. Dall'Armi, S. V. Voronov, L. B. McIntire, H. Zhang, A. Z. Moore, A. Staniszewski, O. Arancio, T. W. Kim, & G. Di Paolo: Oligomeric amyloid-beta peptide disrupts phosphatidylinositol-4,5-bisphosphate metabolism. *Nat Neurosci* 11, 547−554 (2008)

186. Rousseau, F., J. Schymkowitz, & L. Serrano: Protein aggregation and amyloidosis: confusion of the kinds? *Curr Opin Struct Biol* 16, 118−126 (2006)

187. Petkova, A. T., R. D. Leapman, Z. Guo, W. M. Yau, M. P. Mattson, & R. Tycko: Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* 307, 262−265 (2005)

188. Yoshiike, Y., T. Akagi, & A. Takashima: Surface structure of amyloid-beta fibrils contributes to cytotoxicity. *Biochemistry* 46, 9805−9812 (2007)

189. Haass, C., & D. J. Selkoe: Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 8, 101−112 (2007)

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